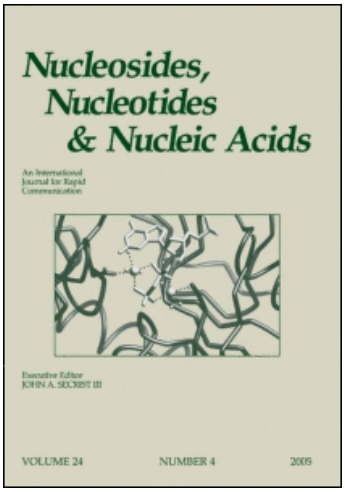

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Synthesis of Tubercidin, 6-Chlorotubercidin and Related Nucleosides

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SYNTHESIS OF TUBERCIDIN, 6-CHLOROTUBERCIDIN AND RELATED NUCLEOSIDES

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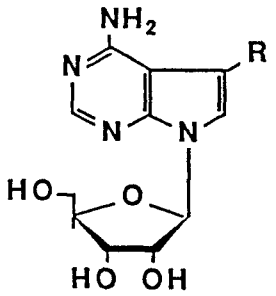
ABSTRACT. Tubercidin (7-deazaadenosine, **1a**) and several 6-chlorotubercidin derivatives were synthesized including 4-amino-6-chloro-7-β-D-ribofuranosylpyrrolo[2,3-d]pyrimidine-3′,5′-cyclic phosphate **9**. Isolation of a side product found in the glycosylation step of the reaction sequence proved to be the N-1 ribosyl-attached isomer as shown by X-ray diffraction analysis. All derivatives were tested for in vitro antiviral and antitumor activity.

Pyrrolo[2,3-d]pyrimidine nucleosides exhibit a broad spectrum of biological activity.[1] Tubercidin, **1a**, has demonstrated high cytotoxicity and has been shown to interfere with numerous cellular processes. Similar in structure to adenosine, tubercidin is rapidly anabolized by adenosine kinase to the 5′-monophosphate and later to higher nucleotide forms which inhibit various steps in adenosine metabolism. These metabolites inhibit various enzymes including adenosine kinase, adenosine phosphoribosyl transferase, and nucleoside phosphorylases.[1-3] Because the general cytotoxicity of tubercidin precludes its clinical use, considerable effort has been expended to make derivatives which would show a greater selectivity in their biological response. Changes in the carbohydrate portion of the molecule have resulted in an increase in the antiviral therapeutic index as seen in the arabinosyl[4] and xylofuranosyl[5] derivatives. Various substitutions at the C-5 position



a; R = H
b; R = CN
c; R = C(O)NH$_2$

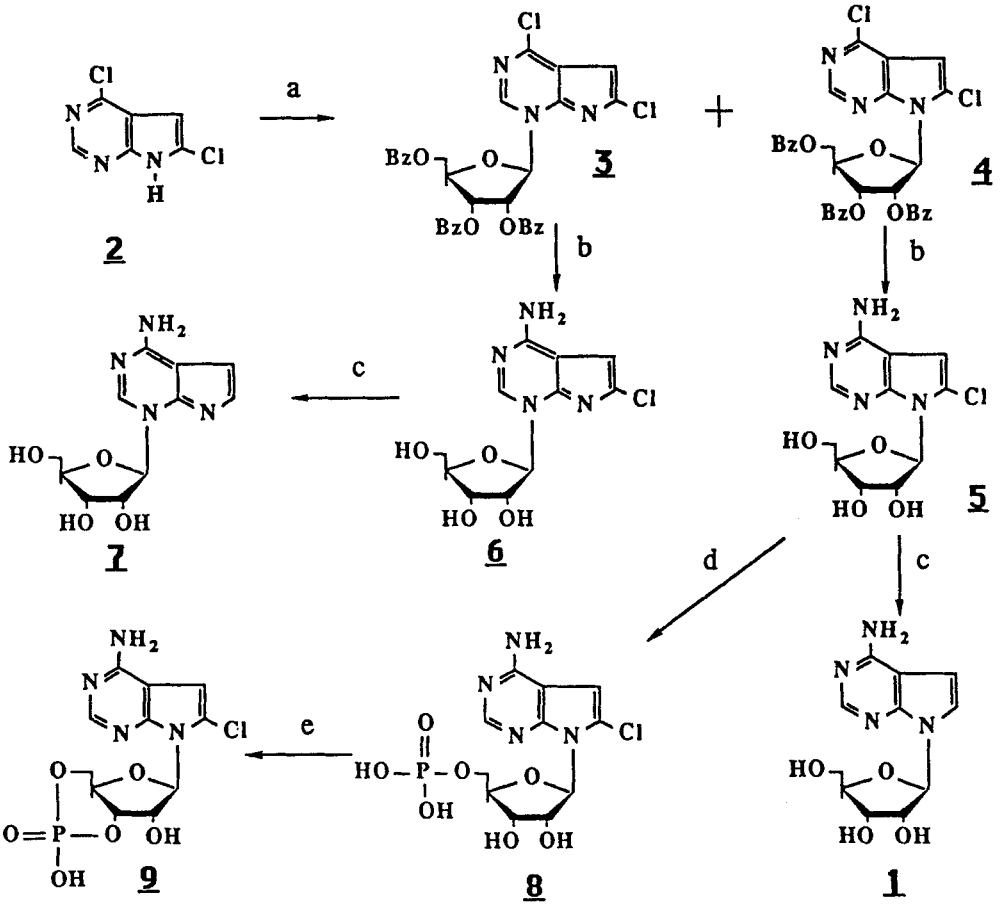
1a-c

of the heterocyclic moiety have effected a broadened profile of activity as shown by the naturally-occurring analogs toyocamycin, **1b**, and sangivamycin, **1c**.[2,3] Several synthetic C-5 alkylated derivatives of tubercidin have exhibited reduced *in vitro* cytotoxicity with a retention of antiviral potency.[6,7]

The C-5 chloro-substituted derivative of tubercidin has shown an increase of selectivity in its antiviral and antitumor response relative to the parent **1a**.[6,8] In an effort to build upon the broadened profile of activity of the halogen-substituted tubercidin analogs, we sought to synthesize 6-chlorotubercidin, **5**, and more specifically, the cyclic nucleotide analog **9**, a structural isomer of the potent antitumor agent, 8-chloro cyclic AMP. Cyclic AMP has been implicated as a regulatory agent in cell growth and differentiation.[9] Recent studies have shown potent growth inhibition of breast and colon cancer cell lines upon treatment with certain cAMP analogs.[10,11] The most potent inhibitors were those which were substituted at C-8 (e.g. 8-chloro-cAMP). These analogs were shown to selectively activate cAMP-dependent protein kinase type II as well as cause significant reduction in the level of p-21 *ras* protein.[12] To determine if we would observe similar site-selective tumor inhibition, the "7-deaza" analog of 8-chloro-cAMP, or 4-amino-6-chloro-7-β-D-ribofuranosylpyrrolo[2,3-d]pyrimidine-3',5'-cyclic monophosphate, **9**, was synthesized and evaluated for biological activity.

Although the synthesis of 6-chlorotubercidin was reported earlier by our laboratory,[13] we sought another method which would give an increased yield of the nucleoside and thus enable further transformation

to the cyclic nucleotide. Bergstrom, et al.[14] reported that the bromination of tubercidin with NBS in potassium acetate buffer gave good yields



(a) 1-Bromo-2,3,5-tri-O-benzoyl-D-ribofuranose, NaH ; (b) NH_3/MeOH; (c) Pd/C, H_2; (d) $POCl_3$, trimethylphosphate; (e) DCC, pyridine.

of 6-bromotubercidin. Since the analogous procedure using NCS failed to give the 6-chloro-substituted congener, it therefore seemed propitious in our work to start with a 6-chloro-substituted pyrrolo[2,3-d]pyrimidine. Utilizing a simple procedure developed in our laboratory,[13,15] 4,6-dichloropyrrolo[2,3-d]pyrimidine (2) was glycosylated with 1-bromo-2,3,5-tri-O-benzoyl-D-ribofuranose and sodium hydride in DMF to give a 66% yield of 4,6-dichloro-7-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine (4). A minor product (14% yield) was also observed which could be separated from the major isomer by flash column chroma-

tography. Treatment of the blocked nucleoside mixture with methanolic ammonia afforded deprotection of the sugar moiety with concomitant amination of the nucleobase to give a mixture of deblocked nucleosides 5 and 6 which could be separated by fractional crystallization. The synthesis of the major isomer, 4-amino-6-chloro-7-β-D-ribofuranosyl-pyrrolo[2,3-d]pyrimidine (6-chlorotubercidin), 5, represented a marked improvement over the earlier reported procedure.[13] Hydrogenolysis of 5 in the presence of palladium on carbon gave a product which possessed the same physicochemical characteristics as tubercidin (1a). The product obtained upon treatment of the minor isomer, 3, with methanolic ammonia was assumed to be the N-1 ribosyl-attached nucleoside. Earlier glycosylation studies[13,15] also reported minor side products which were assumed to be the N-1 ribosides. To verify the site of ribosyl attachment (at N-1 or N-3) we at first utilized UV absorption and ^{13}C data. The UV spectrum of the major isomer, 4, showed a 5 nm hypsochromic shift relative to that of 2 which would be expected of a compound alkylated at N-7. A 16 nm bathochromic shift, however, was observed for the minor isomer 3 which suggested that perhaps N-3 was the site of glycosylation.[16,17] Using two-dimensional (^{13}C-^{1}H, XHCORR) NMR analysis, the peak assignment for each of the carbon atoms of 5 and 6 was facilitated and these were compared to the ^{13}C spectrum of tubercidin.[18] The ^{13}C spectrum of 5 proved to be quite similar to that of tubercidin with δ differences of $< \pm 1.6$ ppm for each of the signals. The spectrum of 6, however, showed substantial shifts (4.0 - 10.6 ppm) for all signals except C-4 and C-4a. Since the signals for ring carbons which are proximate to the site of ribosyl attachment show relatively greater shift values[18], the absence of shift of the C-4 carbon led us to believe that the site of glycosylation was not at N-3 but at the N-1 nitrogen. The structure of 6 was unequivocally proven to be the N-1 isomer by single-crystal X-ray diffraction analysis (see Figure 1). Compound 6 was dehalogenated with palladium on carbon in the presence of hydrogen to give 7, a positional isomer of tubercidin. This isomer proved to be relatively unstable in aqueous solution at room temperature ($t_{1/2} < 5$ days). Modifying the procedure of Yoshikawa and coworkers[19], 6-chlorotubercidin (5) was phosphorylated using $POCl_3$ in trimethyl phosphate to give the 5′-monophosphate, 8, which was cyclized under high dilution conditions using DCC and pyridine to give the 3′,5′-cyclic phosphate, 9, in 61% yield.

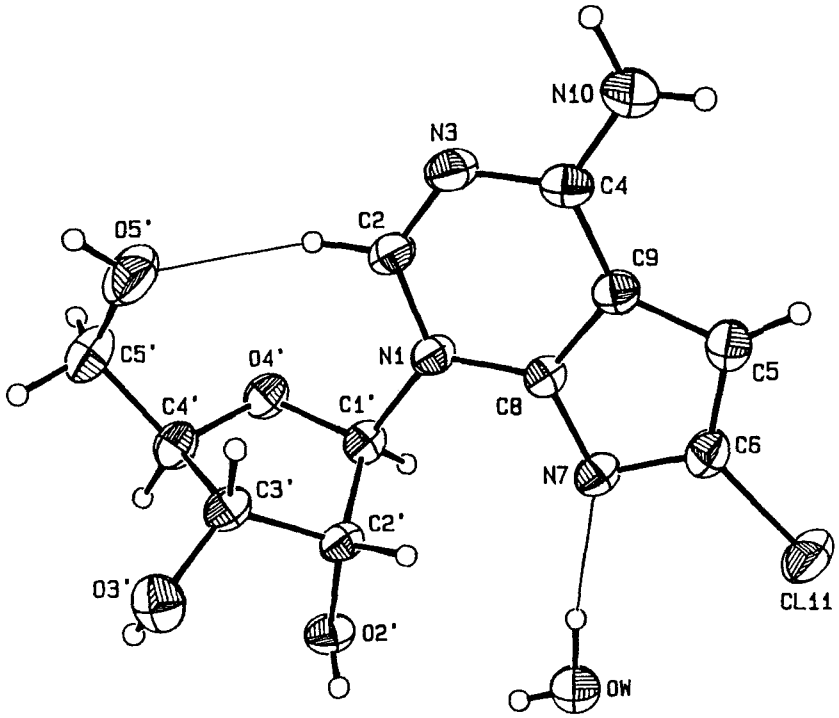


FIGURE 1. ORTEPII drawing of **6** illustrating atom labeling. Thermal ellipsoids are drawn at the 50% probability level.

X-RAY CRYSTALLOGRAPHY: A crystal of **6** (0.45x0.07x0.055 mm) was obtained from a slowly cooled solution of 1% ethanol in water and mounted on an Enraf-Nonius CAD4 diffractometer equipped with graphite-monochromated CuKα radiation. Compound **6** crystallizes in the monoclinic space group $P2_1$ (No. 4) with Z=2. Cell parameters determined from a least-squares fit of the setting angles of 25 centered reflections ($51.8<2\theta<59.3°$) are $\underline{a}$ = 10.159(2), $\underline{b}$ = 4.8062(14), $\underline{c}$ = 13.954(7) Å, β = 99.30(2)°, $\underline{V}$ = 672.4(4) $Å^3$. A total of 2936 reflections with $2\theta \leq 152°$ were measured by ω-2θ scans at various scan speeds with scan widths determined as 1.0 + 0.15tanθ (in degrees). The data were corrected for stability (factor range: 1.000-1.011), absorption (μ=28.241 cm^{-1}; factor range: 0.455-0.867), Lorentz and polarization effects and merged to a unique set of 2731 reflections (R_{int} = 0.039). Cell parameters and data were measured at 295 K.

TABLE 1. Positional and equivalent isotropic thermal parameters for non-hydrogen atoms in **6**.

Atom	x/a	y/b	z/c	U_{eq} [a]
N1	.7085(2)	.3956	.7916(2)	.0268(6)
C2	.8357(3)	.4164(9)	.8366(2)	.0326(8)
N3	.8783(2)	.5878(8)	.9085(2)	.0344(8)
C4	.7903(3)	.7617(9)	.9419(2)	.0315(8)
C5	.5363(3)	.8992(10)	.9116(2)	.0330(8)
C6	.4413(3)	.7869(9)	.8415(2)	.0311(8)
N7	.4854(2)	.5799(8)	.7854(2)	.0291(7)
C8	.6149(3)	.5650(8)	.8235(2)	.0255(7)
C9	.6543(3)	.7503(9)	.9006(2)	.0288(8)
N10	.8378(3)	.9369(9)	1.0127(2)	.0428(10)
CL11	.27555(7)	.8836(6)	.81847(6)	.0470(3)
C1′	.6667(3)	.2031(8)	.7087(2)	.0262(8)
C2′	.6352(2)	.3647(9)	.6141(2)	.0264(7)
C3′	.7697(3)	.3580(8)	.5786(2)	.0280(7)
C4′	.8252(3)	.0742(9)	.6113(2)	.0290(8)
C5′	.9758(3)	.0541(9)	.6298(3)	.0411(11)
O2′	.5375(2)	.2129(8)	.5501(2)	.0328(6)
O3′	.7629(2)	.4075(8)	.4781(2)	.0382(7)
O4′	.7711(2)	.0163(7)	.69944(15)	.0300(6)
O5′	1.0301(2)	.2727(9)	.6924(2)	.0536(9)
OW	.2787(2)	.4179(8)	.6326(2)	.0397(7)

[a] $U_{eq} = 1/3\Sigma_i\Sigma_j\ U_{ij}\ a_i^*\ a_j^*\ A_{ij}$, where A_{ij} is the dot product of the i^{th} and j^{th} direct-space unit-cell vectors.

All non-hydrogen atoms including the oxygen of the solvent were obtained with SHELXS86.[20] All hydrogen atoms were located in an electron density difference map at R = 0.057 as peaks of 0.49-0.88 $e\text{Å}^{-3}$. All atomic positional parameters, anisotropic thermal parameters for non-hydrogen atoms and isotropic thermal parameters for hydrogen atoms (except for the y-coordinate of N1 which was fixed to define the origin) were refined by full-matrix least-squares (SHELX76).[21] An extinction parameter refined to a value of $1.3(3)\times10^{-6}$. After the final cycle, the maximum shift/error was 0.0008, R = 0.0409, wR = 0.0513 and S = 1.546 for 250 variables and 2321 reflections having $F \geq 4\sigma_F$. Residual density in the final difference map was in the range $-0.45 \leq \Delta\rho \leq 0.47\ e\text{Å}^{-3}$. The

TABLE 2. Bond lengths (Å) and bond angles (°) in 6.

1	2	3	1 - 2	1-2-3
C2	N1	C8	1.345(3)	117.8(2)
C8	N1	C1′	1.379(4)	119.5(2)
C1′	N1	C2	1.489(4)	122.7(2)
N3	C2	N1	1.317(5)	125.1(3)
C4	N3	C2	1.360(5)	119.5(2)
C9	C4	N10	1.409(4)	122.9(3)
C9	C4	N3		119.5(3)
N10	C4	N3	1.328(5)	117.6(3)
C6	C5	C9	1.369(4)	103.5(3)
N7	C6	CL11	1.384(5)	118.9(2)
N7	C6	C5		115.6(3)
CL11	C6	C5	1.726(3)	125.5(3)
C8	N7	C6	1.339(3)	101.6(3)
C9	C8	N1	1.405(5)	119.7(2)
C9	C8	N7		114.2(3)
N1	C8	N7		126.0(3)
C4	C9	C5		136.3(3)
C4	C9	C8		118.5(3)
C5	C9	C8	1.425(5)	105.1(2)
C2′	C1′	O4′	1.520(4)	107.6(2)
C2′	C1′	N1		110.6(3)
O4′	C1′	N1	1.411(4)	110.0(2)
C3′	C2′	O2′	1.527(4)	110.8(2)
C3′	C2′	C1′		101.4(2)
O2′	C2′	C1′	1.424(4)	108.1(3)
C4′	C3′	O3′	1.518(5)	114.0(3)
C4′	C3′	C2′		103.3(3)
O3′	C3′	C2′	1.413(4)	114.5(2)
C5′	C4′	O4′	1.513(4)	110.7(2)
C5′	C4′	C3′		115.2(3)
O4′	C4′	C3′	1.452(4)	104.7(3)
O5′	C5′	C4′	1.419(5)	109.8(3)
C1′	O4′	C4′		110.4(3)

function minimized was $\Sigma w(|F_o|-|F_c|)^2$ where $w^{-1} = \sigma_F^2 + 0.0004F^2$. Data were reduced to structure factors using SDP-Plus.[22] Scattering factors and anomalous dispersion corrections were taken from the International Tables for X-ray Crystallography[23] except for hydrogen which were taken from Stewart, Davidson and Simpson.[24] Least-squares planes were calculated with the program PLANES.[25] Parameters for non-hydrogen atoms are listed in Table 1; bond lengths and bond angles are given in Table 2.

TABLE 3. Hydrogen bonding in 6.

D - H ··· A			Symmetry of A relative to D	d(D···A) (Å)	d(H···A) (Å)	∠(D-H··A) (°)
C2	H2	O5′	x, y, z	3.117(4)	2.26(3)	147.(2)
N10	H10A	N3	2.0-x,0.5+y,2.0-z	3.005(4)	2.08(4)	164.(4)
N10	H10B	CL11	1.0-x,0.5+y,2.0-z	3.516(4)	2.76(4)	152.(4)
O2′	HO2′	O2′	1.0-x,0.5+y,1.0-z	2.823(4)	2.10(4)	156.(4)
O3′	HO3′	OW	1.0-x,y-0.5,1.0-z	2.894(5)	2.10(4)	166.(5)
O5′	HO5′	OW	1.0+x, y, z	2.871(4)	1.98(5)	168.(4)
OW	HOWA	N7	x, y, z	2.849(4)	1.97(5)	168.(4)
OW	HOWB	O3′	1.0-x,y-0.5,1.0-z	2.894(5)	2.30(6)	138.(6)

Figure 1 is an ORTEPII[26] illustration of the molecule showing atom labeling and confirming the N-1 attachment of the ribose moiety and the β-anomeric configuration. The effect of the N-1 attachment of the ribosyl moiety on the geometry is seen in the comparison of the present study to the studies of tubercidin[27] and 2′-deoxytubercidin.[28] The C2-N3 bond is significantly shorter (1.317 Å compared to an average of 1.339 Å) while the N1-C8 (N1-C7a) bond is longer (1.379 Å compared to a mean of 1.345 Å) resulting in an enhanced double bond character of the C2-N3 bond. The same effect is observed in the structures of adenosine[29] and 3-isoadenosine.[30,31]

The heterocycle is nearly planar [rms deviation: 0.0179(12) Å]. The dihedral angle between the pyrimidine plane [rms deviation of plane atoms: 0.007(2) Å] and the pyrrole plane [rmsd: 0.002(2) Å] is 2.6(2)°. Isoadenosine exhibits an interplanar angle of 2.5° while the dihedral angles of the tubercidin compounds range from 0.35-1.80°.

The glycosidic bond is long [1.489(4) Å] similar to the length observed in isoadenosine (1.488[30] and 1.493 Å[31]). In contrast, the glycosyl bond length range is 1.438-1.466 Å in adenosine[29] and the tubercidins.[27,28] The glycosidic torsion angle (χ_{CN}) is 11.8(4)° corresponding to the anti conformation. The bond lengths and angles are normal in the sugar portion of the molecule. The side chain is gg with torsion angles of -67.5(4) and 51.0(4)° involving O4′ and C3′, respectively. The ring torsion angles, θ_0 through θ_4, are 35.0, -30.7, 13.7,

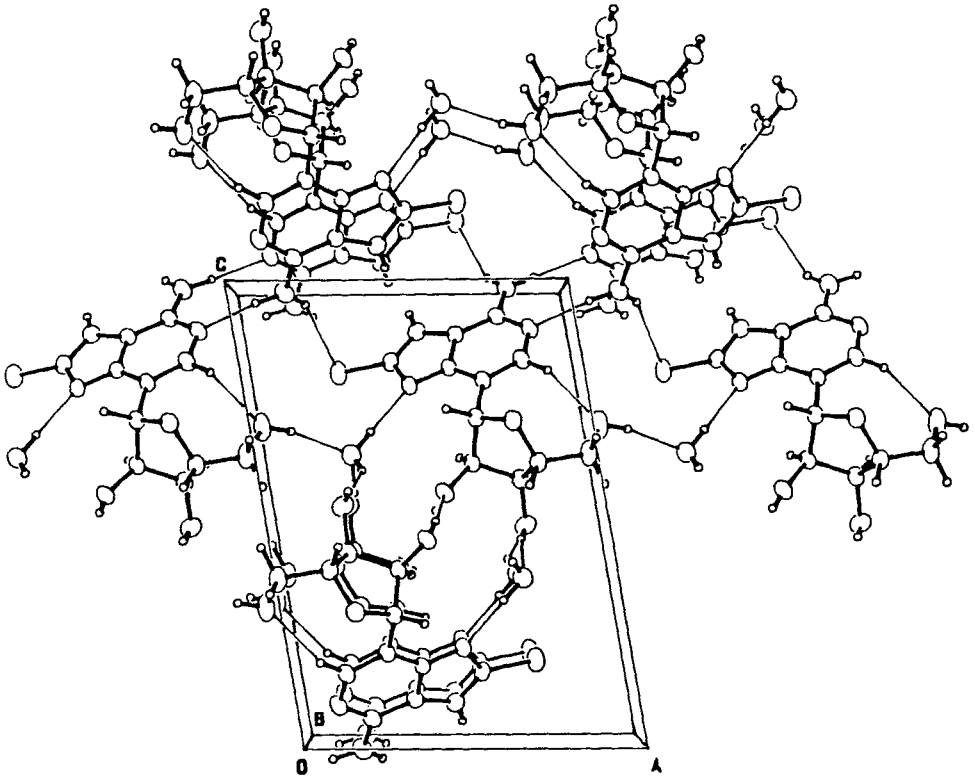


FIGURE 2. Packing diagram illustrating the hydrogen bonding environment of a single molecule.

9.1 and -27.7° resulting in the C_3, endo-C_2, exo (form $^3T_2$) conformation. The pseudorotation angle and puckering amplitude are P = 4.0° and τ_m = 35.1°, both of which fall into normal ranges.[32] Isoadenosine has a similar conformation (C_3, endo)[30,31] but the tubercidins are distinctly different (C_2, endo for tubercidin[27] and C_3, exo for the 2'-deoxy analogs[28]).

The hydrogen bonding is illustrated in the packing diagram (Figure 2) and tabulated in Table 3. Figure 1 also illustrates the possible C-H··O intramolecular hydrogen bond. This type of hydrogen bond has been observed in the nucleoside 1-(5-nitro-2,4-dioxopyrimidinyl)-β-D-ribofuranuronic acid monohydrate [d(H··O)=2.080(6) Å][33] and 1-methylthymine [d(H··O) = 2.045(3) Å].[34] All hydroxyl and amino hydrogens are involved in hydrogen bonding interactions.

TABLE 4. Comparative In Vitro Cytotoxicity Of Tubercidin Analogs.

Compound No.	ID_{50} μM[a]		
	L1210	WI-L2	LOVO
1a	0.020	0.005	0.027
5	ia	33.1	50.0
6	ia	ia	60%
7	ia	ia	ia
8	79%	79%	100
9	61%	ia	73%
8-Cl-cAMP	2.2	1.8	2.9

[a] 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. The notation "ia" denotes inactivity at a drug concentration below 100 μM. A percent (%) value indicates percent cell growth inhibition at the highest drug concentration tested (100 μM).

BIOLOGICAL EVALUATION: All of the deblocked nucleoside/nucleotides were tested for inhibition of virus-induced cytopathogenicity following previously established procedures.[35] In vitro antiviral activity was assayed using the following viral strain/cell line systems: Herpes Simplex virus type 1 (MS/MA104), Adenovirus type 2 (Adenoid 6/HeLa), Parainfluenza virus type 3 (C243/MA104), Rhinovirus type 1A (2060/HeLa), Visna virus (1513/sheep coroid plexus cells), Semliki Forest virus (original/Vero) and Influenza A virus (Chile/MDCK). None of the compounds tested showed appreciable antiviral activity (ID_{50} >320 μM). The compounds were screened for their in vitro cytotoxic effect by MTT assay methods[36] against the following cancer cell lines: mouse leukemia (L1210), human lymphoblast (WI-L2), and human colon carcinoma (LOVO). The results are summarized in Table 4. In contrast to the increase in cytotoxicity observed with 5-chlorotubercidin[6] relative to 1a, the 6-chloro substituted analog, 5, lost essentially all activity in L1210 and showed only moderate activity in the WI-L2 and LOVO lines. The nucleosides with ribosyl attachment at N-1 showed no cytotoxicity. The

5'-monophosphate, **8**, and 3',5'-cyclic nucleotide, **9**, showed a slight increase in L1210 activity when compared to **5** but showed no activity in the other cell lines. In comparison with 8-Cl-cAMP, the "7-deaza" analog, **9**, demonstrated a substantial loss of *in vitro* activity. Studies are now being pursued to determine the *in vivo* antitumor efficacy of these compounds in models sensitive to cAMP-dependent protein kinase inhibition.[10-12]

EXPERIMENTAL

Melting points (uncorrected) were determined in a Haake-Buchler capillary melting point apparatus. Elemental analyses were performed by Robertson Laboratory, Florham Park, NJ. Thin-layer chromatography (TLC) was conducted on plates of silica gel 60 F-254 (EM Reagents). Silica gel (E. Merck; 230-400 mesh) was used for flash column chromatography. All solvents used were reagent grade. Ion-exchange chromatography was performed using DEAE cellulose (DE 52, Whatman). A semi-preparative C-18 column (2.5 x 30 cm, 10μ particle size; Rainin) was used for reversed-phase HPLC purifications (Delta Prep 3000, Waters Corp.). Nucleoside components were detected on TLC by UV light, and with 7% H_2SO_4 in MeOH spray followed by heating. Evaporations were conducted under diminished pressure with the bath temperature below 30°C. Infrared (IR) and ultraviolet (UV) spectra were recorded with Perkin-Elmer 1420 and Beckman DU-50 spectrophotometers, respectively. Nuclear magnetic resonance (^{1}H NMR) and (^{13}C NMR) spectra were determined at 300.135 and at 75.469 MHz, respectively, with an IBM NR/300AF spectrometer. The chemical shift values are expressed in δ values (parts per million) relative to DMSO as an internal standard. The presence of H_2O as indicated by elemental analysis was verified by ^{1}H NMR spectroscopy.

4,6-Dichloro-1-(2,3,5-tri-O-benzoylribofuranosyl)pyrrolo[2,3-d]-pyrimidine (3) and 4,6-dichloro-7-(2,3,5-tri-O-benzoylribofuranosyl)pyrrolo[2,3-d]pyrimidine (4). 4,6-Dichloropyrrolo[2,3-d]pyrimidine[15] (**2**, 9.4 g, 50.0 mmol) was dissolved in refluxing dry acetonitrile (1.5 L) with exclusion of moisture. After cooling to 50°C, sodium hydride (2.20 g, 55.0 mmol, 60% oil dispersion) was carefully added and the mixture was allowed to cool to room temperature. After 1 hour, 1-bromo-2,3,5-

tri-O-benzoyl-D-ribofuranose[37] (27.8 g, 55.0 mmol) in 100 mL of acetonitrile was added. Immediately a fine precipitate formed and the reaction mixture turned yellow. After stirring 16 h at room temperature the reaction contents were evaporated to dryness. The residue was flash chromatographed (dichloromethane) to give 25.5 g (81%) of crude nucleoside product consisting of a mixture of 3 and 4. Note: The two products 3 and 4 can be further separated by flash chromatography (hexane-ethyl acetate, 85:15). In one instance, a small (~100 mg) portion purified by this method gave 80 mg (66% yield from 2) of the N-7 isomer 4 and 18 mg (14%) of the corresponding N-1 isomer 3. For 3: UV λ_{max} (CH_3CN) 230 nm, 304; ^1H NMR ($CDCl_3$) δ 6.12 - 6.21 (m, 2, $C_{2',3'}\underline{H}$), 6.60 (s, 1, $C_5\underline{H}$), 6.87 (d, 1, J = 4.3 Hz, $C_1,\underline{H}$), 7.33 - 8.08 (3m, 15, benzoyl), 8.59 (s, 1, $C_2\underline{H}$), and other sugar protons. For 4 (oil): UV λ_{max} (CH_3CN) 230 nm, 280; ^1H NMR ($CDCl_3$) δ 6.43 - 6.47 (m, 2, $C_{2',3'}\underline{H}$), 6.63 (s, 1, $C_5\underline{H}$), 6.70 (dd, 1, J = 3.9, 6.3 Hz, $C_1,\underline{H}$), 7.33 - 8.07 (3m, 15, benzoyl), 8.51 (s, 1, $C_2\underline{H}$), and other sugar protons.

4-Amino-6-chloro-7-β-D-ribofuranosylpyrrolo[2,3-d]pyrimidine (5). A mixture of blocked nucleosides 3 and 4 (25.5 g, 40.3 mmol) and methanol (300 mL) were placed in a cooled bomb (dry ice-acetone bath) through which anhydrous ammonia was bubbled until saturated. The bomb was sealed and heated in an oil bath at 110°C for 16 h. The contents were evaporated to dryness and the residue flash chromatographed with dichloromethane-methanol (95:5) to give 11.7 g of crude product. Recrystallization from water gave 9.1 g (75%) of 5 as clear plates melting at 177-178°C.(lit[13] 182°C), UV λ_{max} (pH 1) 225 nm (ε 12,900), 272 (10,100) (lit: 226 nm (17,500), 273 (13,700)); UV λ_{max} (pH 7) 211 nm (ε 9,200), 273 (5,400); UV λ_{max} (pH 11) 273 nm (ε 6,600) (lit: pH 7 and 11: 274 nm (14,100); ^1H NMR (DMSO-$\underline{d}_6$) δ 5.86 (d, 1, J = 6.8 Hz, $C_1,\underline{H}$), 6.70 (s, 1, $C_5\underline{H}$), 7.28 (br s, 2, $N\underline{H}_2$, exchanged with D_2O), 8.05 (s, 1, $C_2\underline{H}$) (lit: δ 8.24, $C_2\underline{H}$) and other sugar protons. ^{13}C NMR (DMSO-$\underline{d}_6$): δ 98.83 ($\underline{C}$-5), 102.6 ($\underline{C}$-4a), 121.2 ($\underline{C}$-6), 149.0 ($\underline{C}$-7a), 151.7 ($\underline{C}$-2), 156.8 ($\underline{C}$-4); Anal. Calcd for $C_{11}H_{13}N_4O_4Cl$: C, 43.93; H, 4.36; N, 18.63; Cl, 11.79. Found: C, 43.89; H, 4.25; N, 18.50; Cl, 11.49.

4-Amino-6-chloro-1-β-D-ribofuranosylpyrrolo[2,3-d]pyrimidine (6). A solid crystalline product formed from the mother liquor of compound 5

(above) which, after recrystallization from 1% ethanol in water, gave 1.3 g (9% from 2) of 6 as colorless needles; mp 143-145°C; UV λ_{max} (pH 1) 219 nm (ε 20,800), 293 (11,700); UV λ_{max} (pH 7) 219 nm (ε 25,400), 250 (6,900), 282 (9,900), 316 (6,900); UV λ_{max} (pH 11) 216 nm (ε sh 27,000), 249 (7,200), 282 (10,100), 313 (7,000); ^{1}H NMR (DMSO-$\underline{d}_6$) δ 5.77 (d, 1, J = 6.1 Hz, C_1,$\underline{H}$), 6.42 (s, 1, $C_5\underline{H}$), 7.95 (br s, 2, $N\underline{H}_2$, exchanged with D_2O), 8.49 (s, 1, $C_2\underline{H}$) and other sugar protons. ^{13}C NMR (DMSO-$\underline{d}_6$): 95.83 ($\underline{C}$-5), 104.8 ($\underline{C}$-4a), 133.4 ($\underline{C}$-6), 142.2 ($\underline{C}$-7a), 142.6 ($\underline{C}$-2), 155.9 ($\underline{C}$-4); Anal. Calcd for $C_{11}H_{13}N_4O_4Cl$: C, 43.93; H, 4.36; N, 18.63; Cl, 11.79. Found: C, 43.91; H, 4.47; N, 18.41; Cl, 11.64.

4-Amino-7-β-D-ribofuranosylpyrrolo[2,3-d]pyrimidine (tubercidin, 1a). Compound 5 (93 mg, 0.3 mmole) and Pd on carbon (10%, 30 mg) were combined in water (25 mL) and ethanol (25 mL) and the resulting mixture shaken under H_2 pressure (34 psi initially) for 6 hours. The mixture was filtered through a glass microfiber filter pad and lyophilized to yield a product (75 mg, 90%) which matched the physicochemical characteristics of tubercidin as reported in the literature.[13,38] mp 244-247°C (Lit.[13] 246-248°C) UV λ_{max} (pH 1) 226 nm (ε 25,000), 271 (14,600); UV λ_{max} (pH 7, 11) 271 nm (ε 11,900); ^{1}H NMR (DMSO-$\underline{d}_6$) δ 6.0 (d, 1, J = 6.1 Hz, C_1,$\underline{H}$), 6.7 (d, 1, J = 3.4 Hz, $C_5\underline{H}$), 7.42 (d, 1, J = 3.4 Hz, $C_6\underline{H}$), 7.5 (br s, 2, $N\underline{H}_2$, exchanged with D_2O), 8.1 (s, 1, $C_2\underline{H}$) and other sugar protons.

4-Amino-1-β-D-ribofuranosylpyrrolo[2,3-d]pyrimidine (7). Compound 6 (250 mg, 0.83 mmole) and Pd/C (5%, 100 mg) were suspended in water and shaken under an atmosphere of hydrogen (35 psi) for 5 h. The reaction mixture was filtered and the filtrate lyophilized to give 180 mg (81%) of 7. Purification by reverse-phase HPLC utilizing H_2O/MeOH (95:5) as eluent followed by evaporation of the solvent gave a white ppt. mp. 122-124°; UV λ_{max} (pH 1) 216 nm (ε 22,200), 272 (sh 8,700), 291 (10,000); UV λ_{max} (pH 7) 216 nm (ε 22,600), 272 (8,700), 292 (ε 10,000); UV λ_{max} (pH 11) 220 nm (ε 29,800), 246 (7,500), 281 (10,400), 310 (sh 5,100); ^{1}H NMR (DMSO-$\underline{d}_6$) δ 6.0 (d, 1, J = 5.8 Hz, C_1,$\underline{H}$), 6.93 (d, 1, J = 3.48 Hz, $C_5\underline{H}$), 7.38 (d, 1, J = 3.33 Hz, $C_6\underline{H}$), 8.85 (s, 1, $C_2\underline{H}$), 8.9, 9.2 (2 br s, 2, $N\underline{H}_2$, exchanged with D_2O) and other sugar protons. Anal. Calcd for $C_{11}H_{14}N_4O_4 \cdot HCl \cdot 3/4H_2O$: C, 41.78; H, 5.26; N, 17.72; Cl 11.21. Found: C, 41.81; H, 5.02; N, 18.12; Cl, 11.14.

4-Amino-6-chloro-7-(β-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine-5′-monophosphate (8). Nucleoside 5 (7.68 g, 25.6 mmol) was dissolved in trimethyl phosphate (75 mL) and cooled in an ice bath. Phosphorus oxychloride (2.6 mL, 28.0 mmol) was added dropwise and the reaction was stirred under anhydrous conditions. After 2½ h, additional phosphorus oxychloride (2.6 mL) was added maintaining the reaction temperature at 4°C. After two more hours the solution was neutralized with saturated sodium bicarbonate (pH 7) and stored at 0°C. After 4 h the pH was again adjusted to pH 7 and stored cold (5°C) overnight. The resulting precipitate was filtered and dried to yield 3.8 g. Additional product was obtained by reducing the volume, cooling, filtering the precipitate and drying to give an additional 2.5 g. Purification by ion exchange chromatography (DEAE cellulose, eluted with a linear gradient of 0.0 to 0.5 M NH_4HCO_3, 2L-2L) followed by multiple lyophilizations gave a white solid which darkened at 180°C (gas evolution began at 140°C). UV λ_{max} (pH 1) 224 nm (ε 13,000), 272 (10,200); UV λ_{max} (pH 7) 211 nm (ε 17,400), 272 (11,300); UV λ_{max} (pH 11) 272 nm (ε 11,500); ^1H NMR (DMSO-d_6) δ 5.90 (d, 1, J = 5.5 Hz, C_1,H), 6.69 (s, 1, C_5H), 7.16 (br s, 2, NH_2, exchanged with D_2O), 8.09 (s, 1, C_2H) and other sugar protons. Anal. Calcd for $C_{11}H_{14}N_4O_7PCl \cdot NH_3 \cdot 1/2H_2O$: C, 32.48; H, 4.46; N, 17.22; P, 7.62; Cl, 8.72. Found: C, 32.44; H, 4.42; N, 17.21; P, 7.73; Cl, 8.71.

4-Amino-6-chloro-7-β-D-ribofuranosylpyrrolo[2,3-d]pyrimidine-3′,5′-cyclic phosphate (9). Monophosphate 8 (3.25 g, 8.5 mmol) was dissolved in pyridine (150 mL) and water (100 mL). Morpholino dicyclohexylcarbodiimide was then added with stirring to effect a clear solution. The reaction mixture was evaporated to dryness and co-evaporated with pyridine (2 x 50 mL) and the resulting white foam placed under high vacuum for 4 h. To a solution of dicyclohexylcarbodiimide (8.8 g, 42.5 mmol) in pyridine (1 L) heated under reflux the above foam in pyridine (200 mL) was added dropwise through the condenser. After 2 h at reflux the heating mantle was removed, water (200 mL) was added and the mixture stirred overnight. The resulting suspension was filtered and the filtrate evaporated to dryness. The residue was dissolved in a minimum amount of 0.5 M ammonium bicarbonate and placed upon an ion exchange column (DEAE cellulose) and eluted with a linear gradient of 0.0 M to 0.25 M NH_4HCO_3 (2L-2L). The fractions containing product were pooled, and lyophilized to yield 1.9 g (61%) of 9 as the

ammonium salt. The sodium salt form was prepared by dissolving the compound in 5% sodium carbonate followed by reverse phase (C-18) preparative HPLC (water as eluent) to give, after lyophilization, a white powder; m.p. darkens >260°C; UV λ_{max} (pH 1) 222 nm (ε 17,000), 270 (13,500); UV λ_{max} (pH 7) 211 nm (ε 21,500), 270 (13,700); UV λ_{max} (pH 11) 270 nm (ε 13,800); ^{1}H NMR (DMSO-d_6) δ 5.87 (s, 1, C_1,H), 6.83 (s, 1, C_5H), 7.75 (br s, 2, NH_2, exchanged with D_2O), 8.21 (s, 1, C_2H) and other sugar protons. Anal. Calcd for $C_{11}H_{11}N_4O_6PClNa \cdot 3H_2O$: C, 30.11; H, 3.90; N, 12.77; Cl, 8.08. Found: C, 29.71; H, 3.53; N, 12.44; Cl, 7.81.

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