CMC/T in poly(ethylene glycol) (PEG)], or with 5% acyclovir ointment (Zovirax, Burroughs Wellcome Co., Research Triangle, NC). One area on each animal was treated with PEG (placebo control), and one area was not treated (untreated control). Fifty microliters of the compound solution was applied at each treatment and spread over the infected site. Each drug was tested in five animals, with one area/animal for each compound.

For statistical analysis and interpretation of data, accumulative scores were collected from individual animals. Two separate response variables, the lesion formation stage and the healing period stage, were analyzed statistically. A high cumulative score for the healing stage indicates an infection with a short healing period. The response variable that measures the severity of the infection is equal to the highest sequential step reached in the formative stage.

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Registry No. **2**, 113852-37-2; **2**·Na, 120362-37-0; **3**, 117087-39-5; **4**, 17325-85-8; **5**, 120362-27-8; **6**, 120362-28-9; **7**, 120362-29-0; **8**, 120362-30-3; **9**, 117087-22-6; **10**, 120362-31-4; **11**, 120362-32-5; **12**, 120362-33-6; **13**, 120362-34-7; **14**, 120362-35-8; **15**, 120362-36-9; $TsOCH_2P(O)(OEt)_2$, 31618-90-3; $HOCH_2CH_2OCH_2P(O)(OEt)_2$, 116384-55-5; (*R*)-2,3-*O*-isopropylideneglycerol, 14347-78-5; (*R*)-1-*O*-benzyl-2,3-*O*-isopropylideneglycerol, 14347-83-2; cytosine, 71-30-7.

3,7-Dideazapurine Nucleosides. Synthesis and Antitumor Activity of 1-Deazatubercidin and 2-Chloro-2′-deoxy-3,7-dideazaadenosine

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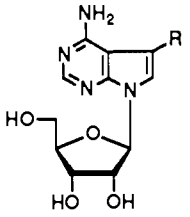
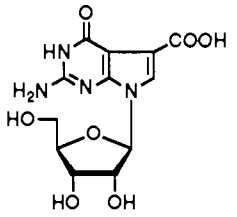
1-Deazatubercidin (**5**) has been synthesized by glycosylation of the anion of 4,6-dichloro-1*H*-pyrrolo[3,2-c]pyridine (**9**) with 1-chloro-2,3-*O*-isopropylidene-5-*O*-(*tert*-butyldimethylsilyl)-α-D-ribofuranose (**12**). The reaction gave a mixture of blocked nucleosides with β- and α-configuration (**13a** and **13b**). Deprotection of **13a** provided 4,6-dichloro-1-β-D-ribofuranosylpyrrolo[3,2-c]pyridine (**14**), which on treatment with hydrazine, followed by reduction of the resulting 4-hydrazino compound with Raney nickel, gave 4-amino-6-chloro-1-β-D-ribofuranosylpyrrolo[3,2-c]pyridine (**15**), 1-deazatubercidin, and a small quantity of 4,6-diamino-1-β-D-ribofuranosylpyrrolo[3,2-c]pyridine (**16**). Dehalogenation of **15** provided another route to **5**. 2-Chloro-2′-deoxy-3,7-dideazaadenosine (**6**) together with 2′-deoxy-3,7-dideazaadenosine (**18**) was obtained by hydrazinolysis of 4,6-dichloro-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)pyrrolo-[3,2-c]pyridine (**17**), followed by reduction of the resulting 4-hydrazino compound. Nucleosides **5**, **6**, **15**, and **18** are devoid of any significant antitumor activity in vitro. Compound **16** showed significant activity against P388 leukemia in cell culture.

After the discovery that several naturally occurring nucleoside antitumor antibiotics are derivatives of pyrrolo[2,3-*d*]pyrimidine, i.e., tubercidin (**1**), toyocamycin (**2**), sangivamycin (**3**), and cadeguomycin (**4**),[1] several structurally related deazapurine nucleosides have been synthesized. Among these all the four possible monodeazaadenosines, 1-deaza-,[2] 3-deaza-,[3] 9-deaza-,[4] and 7-deazaadenosine (tubercidin),[5] have been synthesized.



1, R = H
2, R = CN
3, R = $CONH_2$



4

1-Deaza- and 9-deazaadenosine have shown pronounced growth inhibitory activity against several mouse and human leukemic cell lines,[2,4] whereas 3-deazaadenosine is an antiviral agent.[6] Of the six possible dideazaadenosines only 1,3-dideaza-[7] and 1,7-dideazaadenosine (3-deazatubercidin)[8] have been synthesized. 3-Deazatubercidin was found inactive as antitumor agent against murine leukemia L1210 and murine sarcoma S-180 cells in culture.[8] This result suggests that the nitrogen atom at position 3 of the pyrimidine ring of tubercidin is important for antitumor activity. In order to verify whether also the nitrogen atom at position 1 is essential for antitumor activity, we decided to synthesize the 1-deaza analogue of tubercidin (**5**). We now report the synthesis of compound **5** and certain other pyrrolo[3,2-*c*]pyridine nucleosides including the 3,7-dideaza analogue of the antitumor and immunosuppressive agent 2-chloro-2′-deoxyadenosine.[9]

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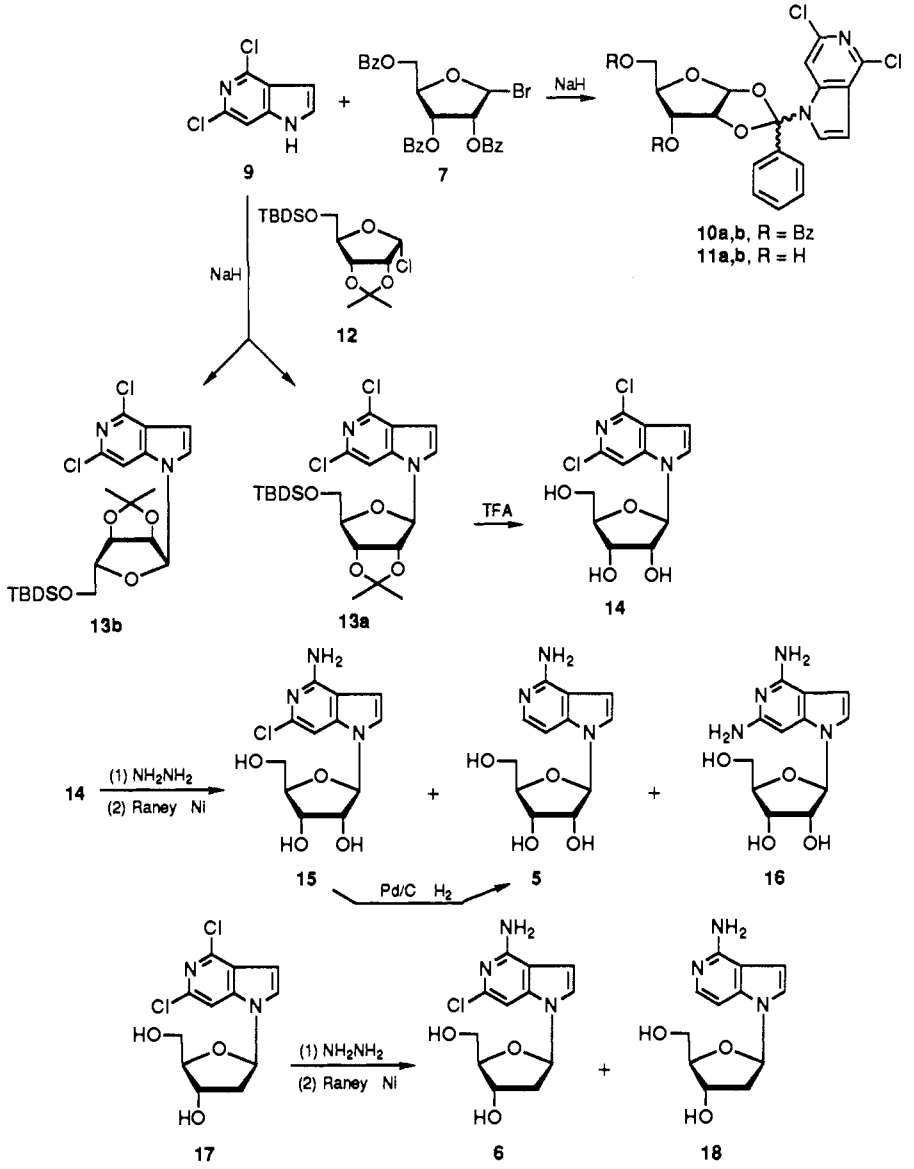
Scheme I



Chemistry

Several years ago Ducrocq and co-workers[10] attempted to synthesize 3,7-dideazaadenosine through the direct glycosylation of 4-(benzoylamino)-1*H*-pyrrolo[3,2-*c*]-pyridine with 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl bromide (**7**)[11] in acetonitrile in the presence of mercury(II) cyanide. This procedure gave after debenzoylation the N-5 ribonucleoside derivative rather than N-1 ribonucleoside isomer **5**. Recently, Robins and co-workers have developed a new glycosylation procedure for the direct preparation of 1-(2-deoxy-β-D-ribofuranosyl) derivatives of pyrrolo-[2,3-*d*]pyrimidine and pyrrolo[3,2-*c*]pyridine ring systems by the condensation of the requisite sodium salt of various halodeazapurines with **7** or 1-chloro-2-deoxy-3,5-di-*O*-*p*-toluoyl-α-D-*erythro*-pentofuranose (**8**).[12a,b]

Owing to the general applicability of the Robins's procedure, we first tried to synthesize 1-deazatubercidin by reacting freshly prepared **7** with the sodium salt of 4,6-dichloro-1*H*-pyrrolo[3,2-*c*]pyridine (4,6-dichloro-5-azaindole) (**9**)[13] in acetonitrile. Two nucleoside products (**10a** and **10b**) were formed and isolated in 43% and 18% yields, respectively, after silica gel column chromatography of the reaction mixture (Scheme I). The benzoyl blocking groups of **10a** and **10b** were removed with CH_3ONa/CH_3OH to give **11a** and **11b**. The ^1H NMR spectra and the elemental analyses of **11a** and **11b** indicated the presence of a phenyl type group; however, in the IR spectra carbonyl stretching bands were not detected. On the basis of this evidence, the structure 1,2-*O*-[phenyl(4,6-dichloro-5-azaindolyl)-methylidene]-α-D-ribofuranose was assigned to these compounds. The α-configuration was confirmed by proton NMR studies using the nuclear Overhauser effect (NOE). Indeed, selective irradiation of the $H_{1'}$ signal increased by 6% the intensity of the C-2′ proton signal of both isomers. So the two isomers appear to be epimers at the methylidene carbon atom. The formation of **10a** and **10b** was presumably due to the participation of the neighboring benzoyl group and resulted from the attack of the anion of **9** on both faces of the carbonyl carbon of the protection

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group rather than the C_1 carbon of the glycon. A similar compound was obtained by Robins and co-workers in the reaction of the sodium salt of pyrrole-2-carbonitrile with 8.[14]

In order to prepare the desired 1-deazatubercidin, 1-chloro-2,3-*O*-isopropylidene-5-*O*-(*tert*-butyldimethylsilyl)-α-D-ribofuranose (**12**)[15] was reacted with the sodium salt of **9** in anhydrous acetonitrile at room temperature. Two isomeric blocked nucleosides, which were separated on a silica gel column with benzene–ethyl acetate and identified as anomers of 4,6-dichloro-1-[2,3-*O*-isopropylidene-5-*O*-(*tert*-butyldimethylsilyl)-D-ribofuranosyl]pyrrolo[3,2-*c*]pyridine (**13a** and **13b**), were obtained. To the fast moving compound **13a** was assigned the β-configuration on the basis of ^{1}H NMR spectrum in DMSO-d_6, which exhibited the anomeric proton as a doublet with a chemical shift at higher magnetic field (δ 6.28) than that of the slower moving compound **13b** (δ 6.35), and with a small coupling constant ($J_{1',2'}$ = 3 Hz) which is within the acceptable limits for β-ribonucleosides.[16-18] The α-configuration of the slower moving compound **13b** was furthermore supported by the ^{1}H NMR spectrum, which revealed the difference between the chemical shift of the two methyl signals of the isopropylidene group to be 0.15 ppm (Δ = 0.02), a difference characteristic of the α-configuration.[19] Deprotection of **13a** with aqueous trifluoroacetic acid at room temperature gave 4,6-dichloro-1-β-D-ribofuranosylpyrrolo[3,2-*c*]pyridine (**14**) in 71% yield. Treatment of **14** with hydrazine hydrate, followed by reduction of the resulting 4-hydrazino compound with Raney nickel, gave a mixture of 4-amino-6-chloro-1-β-D-ribofuranosylpyrrolo[3,2-*c*]pyridine (**15**), 4-amino-1-β-D-ribofuranosylpyrrolo[3,2-*c*]pyridine (**5**), and a small amount of 4,6-diamino-1-β-D-ribofuranosylpyrrolo[3,2-*c*]pyridine (**16**). Compound **16** was produced through the reduction of the 4,6-dihydrazino compound presumably formed as a byproduct in the reaction of **14** with hydrazine. Dehalogenation of **15** with Pd/C in a hydrogen atmosphere readily provided another route to **5**.

In order to prepare the 3,7-dideaza analogue of 2-chloro-2′-deoxyadenosine, the already known 4,6-dichloro-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)pyrrolo[3,2-*c*]pyridine (**17**)[12] was used as starting material. Treatment of **17** with hydrazine hydrate, followed by reduction of the resulting 4-hydrazino compound with Raney nickel, gave 4-amino-6-chloro-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)pyrrolo[3,2-*c*]pyridine (**18**) and 4-amino-1-β-D-*erythro*-pentofuranosylpyrrolo[3,2-*c*]pyridine (**6**) in 16% and 44% yields, respectively.

Biological Evaluation

Compounds **5**, **6**, **15**, and **18** have been evaluated in vitro for their ability to inhibit the growth of murine leukemia P388 and L1210, human promyelocytic leukemia HL-60, and human epidermoid carcinoma KB and found to be inactive until a maximum tested concentration of 1×10^{-4} M. Compound **16** was found to be active in the same test only against murine leukemia P388 with an inhibitory dose 50 (ID_{50}) value of 7.2×10^{-6} M. In our testing system tubercidin was found to be active at lower concentrations on every tested cell line (ID_{50} values: 3.8×10^{-7} M on P388, 5.4×10^{-8} M on L1210, 1×10^{-7} M on HL-60, 5.2×10^{-8} M on KB). The inactivity of 1-deazatubercidin (**5**) and 3-deazatubercidin[8] indicates that the nitrogen atoms at positions 1 and 3 of the pyrimidine ring of tubercidin are both essential for in vitro antitumor activity.

Experimental Section

Melting points were taken on a Büchi apparatus and are uncorrected. Nuclear magnetic resonance (^{1}H NMR) spectra were determined at 300 MHz with a Varian VXR-300 spectrometer. The chemical shift values are expressed in δ values (parts per million) relative to tetramethylsilane as an internal standard. All exchangeable protons were confirmed by addition of D_2O. Ultraviolet spectra were recorded with a Carlo-Erba Spectracamp 601 spectrophotometer. Infrared spectra (IR in Nujol) were recorded with a Perkin-Elmer Model 297 spectrophotometer. Elemental analyses were determined on a Carlo-Erba Model 1106 analyzer. Thin-layer chromatography (TLC) was run on silica gel 60 F-254 plates (Merck). Silica gel 230–400 mesh (Merck) for flash column chromatography and silica gel 60 (Merck) for column chromatography were used.

Epimeric 3,5-Di-*O*-benzoyl-1,2-*O*-[phenyl(4,6-dichloro-5-azaindolyl)methylidene]-α-D-ribofuranoses (10a and 10b). To a stirred suspension of 4,6-dichloro-1*H*-pyrrolo[3,2-*c*]pyridine (**9**)[13] (1 g, 5.34 mmol) in dry CH_3CN (80 mL) was added NaH (80% in oil, 0.24 g, 10 mmol) in small portions. The reaction mixture was stirred at room temperature under nitrogen for 0.5 h. 1-Bromo-2,3,5-tri-*O*-benzoyl-D-ribofuranose[11] (**7**, 2.8 g, 5.34 mmol, in dry CH_3CN) was added, and the stirring was continued at room temperature for 1 h. Evaporation to dryness of the filtered reaction mixture gave an oil which was chromatographed on a silica gel column, eluted with cyclohexane–ethyl acetate (8:2) to give 1.46 g (43%) of **10a** as white crystals, mp 69–72 °C: IR ν 1728 (C=O) cm^{-1}; ^{1}H NMR (Me_2SO-d_6) δ 6.48 (d, 1, J = 4.0 Hz, $C_{1'}$-H), 6.78 (d, 1, J = 3.5 Hz, C_3-H), and 7.23–8.06 (m, 17, 3-Ph, C_2-H, and C_7-H). Anal. ($C_{33}H_{24}Cl_2N_2O_7$) C, H, N.

Further elution of the column with the same mixture of solvents gave 0.6 g (18%) of **10b** as a foam: IR ν 1728 (C=O) cm^{-1}; ^{1}H NMR (Me_2SO-d_6) δ 6.56 (d, 1, J = 4.1 Hz, $C_{1'}$-H), 6.83 (d, 1, J = 3.4 Hz, C_3H), and 7.26–8.07 (m, 17, 3-Ph, C_2-H, and C_7-H). Anal. ($C_{33}H_{24}Cl_2N_2O_7$) C, H, N.

Epimeric 1,2-*O*-[Phenyl(4,6-dichloro-5-azaindolyl)-methylidene]-α-D-ribofuranoses (11a and 11b). A solution of sodium methoxide (34 mg, 0.63 mmol) in dry methanol (15 mL) was added to a stirred suspension of **10a** (0.4 g, 0.63 mmol, in dry methanol). The reaction mixture was kept at room temperature for 3 h and then neutralized (pH 6) with Amberlite IRC-50 (H^+) resin (0.8 g). The resin was removed by filtration and washed with methanol, and the filtrate was evaporated to dryness. Addition of ethyl ether to residue gave 160 mg (60%) of **11a** as a pure white solid, mp 179–81 °C: IR ν 1595 (Ph) cm^{-1}; ^{1}H NMR (Me_2SO-d_6) δ 6.11 (d, 1, J = 4.1 Hz, $C_{1'}$-H), 6.78 (d, 1, J = 3.4 Hz, C_3-H), 7.34–7.50 (m, 6, Ph and C_7-H), and 7.89 (d, 1, J = 3.4 Hz, C_2-H). Anal. ($C_{19}H_{16}Cl_2N_2O_5$) C, H, N.

In similar way, starting from **10b**, the epimer **11b** was obtained by purification on a flash silica gel column. Elution with $CHCl_3$–MeOH–NH_4OH (96:3:1) yielded 80 mg (29%) of **11b** as a foam: IR ν 1595 (Ph) cm^{-1}; ^{1}H NMR (Me_2SO-d_6) δ 6.31 (d, 1, J = 4.1 Hz, $C_{1'}$-H), 6.76 (d, 1, J = 3.4 Hz, C_3-H), 7.15–7.40 (m, 5, Ph), 7.45 (s, 1, C_7-H), and 7.96 (d, 1, J = 3.4 Hz, C_2-H). Anal. ($C_{19}H_{16}Cl_2N_2O_5$) C, H, N.

Anomeric 4,6-Dichloro-1-[2,3-*O*-isopropylidene-5-*O*-(*tert*-butyldimethylsilyl)-D-ribofuranosyl]pyrrolo[3,2-*c*]-pyridines (13a and 13b). To a stirred suspension of **9** (2 g, 10.68 mmol) in dry CH_3CN (200 mL) was added NaH (60% in oil, 0.5 g, 20.8 mmol) in small portions. The reaction mixture was stirred at room temperature under nitrogen for 1 h. 1-Chloro-2,3-*O*-isopropylidene-5-*O*-(*tert*-butyldimethylsilyl)-α-D-ribofuranose[15] (3.44 g, 10.68 mmol, in dry THF) was added, and the stirring was continued at room temperature for 14 h. The reaction mixture

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was evaporated to dryness, and the residue was suspended in water (50 mL). The aqueous solution was extracted with ethyl acetate (3 × 70 mL), and the organic extract was dried over anhydrous Na_2SO_4. Evaporation of the filtrate to dryness gave a residue which was chromatographed on a silica gel column. Elution with benzene–ethyl acetate (97:3) yielded 0.64 g (13%) of **13a** as oil: ^{1}H NMR (Me_2SO-d_6) δ 0.07 (s, 6, 2 CH_3), 0.81 (s, 9, *tert*-butyl), 1.35 and 1.59 (2 s, 6, isopropylidene CH_3), 6.28 (d, 1, J = 3 Hz, $C_{1'}$-H), 6.72 (d, 1, J = 3.7 Hz, C_3-H), 7.82 (d, 1, J = 3.7 Hz, C_2-H), and 7.85 (s, 1, C_7-H). Anal. ($C_{21}H_{30}Cl_2N_2O_4Si$) C, H, N.

Further elution of the column with the same mixture of solvents gave 2.04 g (40%) of **13b** as an oil: ^{1}H NMR (Me_2SO-d_6) δ 0.11 (s, 6, 2 CH_3-H), 0.91 (s, 9, *tert*-butyl), 1.24 and 1.26 (2 s, 6, isopropylidene CH_3), 6.34 (d, 1, J = 4.2 Hz, $C_{1'}$-H), 6.67 (d, 1, J = 3.7 Hz, C_3-H), 7.67 (d, 1, J = 3.7 Hz, C_2-H), and 7.71 (s, 1, C_7-H). Anal. ($C_{21}H_{30}Cl_2N_2O_4Si$) C, H, N.

4,6-Dichloro-1-β-D-ribofuranosylpyrrolo[3,2-*c*]pyridine (14). To a solution of trifluoroacetic acid (1.86 mL) and H_2O (0.18 mL) was added 0.44 g (0.92 mmol) of **13a**; the mixture was stirred at room temperature for 40 min. The solvents were evaporated to dryness, and the residue was dissolved in methanol (20 mL) and again evaporated to dryness. This process was repeated three times to remove the last traces of trifluoroacetic acid. The crude product was chromatographed on a silica gel column by eluting with $CHCl_3$–MeOH–NH_4OH (89:10:1) to give 0.21 g (71%) of **14** as a white solid, mp 194–96 °C: UV (EtOH) λ_{max} 225 nm (ε 37 458), 278 (ε 4740); ^{1}H NMR (Me_2SO-d_6) δ 5.92 (d, 1, J = 6.1 Hz, $C_{1'}$-H), 6.70 (d, 1, J = 3.3 Hz, C_3-H), 7.90 (d, 1, J = 3.3 Hz, C_2-H), and 7.97 (s, 1, C_7-H). Anal. ($C_{12}H_{12}Cl_2N_2O_4$) C, H, N.

4-Amino-6-chloro-1-β-D-ribofuranosylpyrrolo[3,2-*c*]pyridine (15), 4,6-Diamino-1-β-D-ribofuranosylpyrrolo[3,2-*c*]pyridine (16), and 4-Amino-1-β-D-ribofuranosylpyrrolo[3,2-*c*]pyridine (1-Deazatubercidin) (5). A stirred mixture of **14** (0.5 g, 1.56 mmol) and hydrazine hydrate (20 mL) was refluxed under nitrogen for 1 h. After evaporation of the hydrazine to dryness, to the residue were added deoxygenated water (37 mL) and Raney nickel (3 g), and the mixture was refluxed for 1.5 h. The reaction mixture was filtered and washed three times with hot water and methanol. Evaporation to dryness gave a residue which was chromatographed on a silica gel column, eluted with $CHCl_3$–MeOH–NH_4OH (77:22:1). Evaporation of the first eluate gave 0.12 g (27%) of **15** as a viscous oil; UV (EtOH) λ_{max} 211 nm (ε 38 709), 277 (ε 13 145); ^{1}H NMR (Me_2SO-d_6) δ 6.3 (d, 1, J = 4.7 Hz, $C_{1'}$-H), 6.61 (d, 1, J = 3.5 Hz, C_3-H), 7.79 (d, 1, J = 3.5 Hz, C_2-H), and 7.81 (s, 1, C_7-H). Anal. ($C_{12}H_{14}ClN_3O_4$) C, H, N.

Further elution of the column with MeOH–NH_4OH (98:2) gave 9 mg (2%) of **16** as a viscous oil: UV (EtOH) λ_{max} 203.5 nm (ε 29 696), 281 (ε 10 746); ^{1}H NMR (Me_2SO-d_6) δ 4.92 (br s, 3, NH_2 and OH), 5.51 (d, 1, J = 6 Hz, $C_{1'}$-H), 5.73 (s, 1, C_7-H), 5.87 (s, 2, NH_2), 6.45 (d, 1, J = 3.3 Hz, C_3-H), and 7.0 (d, 1, J = 3.3, C_2-H). Anal. ($C_{12}H_{16}N_4O_4$) C, H, N.

Further elution of the column with the same mixture of solvents gave 37 mg (9%) of **5** as viscous oil: UV (EtOH) λ_{max} 206 nm (ε 27 374), 272 (ε 8110); ^{1}H NMR (Me_2SO-d_6) δ 5.72 (d, 1, J = 6 Hz, $C_{1'}$-H), 6.1 (s, 2, NH_2), 6.66 (d, 1, J = 3.4 Hz, C_3-H), 6.77 (d, 1, J = 6.2 Hz, C_7-H), 7.38 (d, 1, J = 3.4 Hz, C_2-H), and 7.56 (d, 1, J = 6.2 Hz, C_6-H). Anal. ($C_{12}H_{15}N_3O_4$) C, H, N.

Conversion of 4-Amino-6-chloro-1-β-D-ribofuranosylpyrrolo[3,2-*c*]pyridine (15) into 5. To a solution of **15** (0.2 g, 0.66 mmol) in 50% aqueous EtOH (25 mL) was added 1 N NaOH (0.5 mL), followed by 10% Pd/C (50 mg), and the reaction mixture was shaken with hydrogen at 20 psi for 2.5 h. After filtration, the solvent was evaporated to dryness and the residue obtained was chromatographed on a silica gel column by eluting with MeOH–NH_4OH (98:2) to give 143 mg (81%) of **5**.

4,6-Dichloro-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)pyrrolo[3,2-*c*]pyridine (17). This compound was prepared as described by Robins and co-workers[12] with modifications. 4,6-Dichloro-1-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)pyrrolo[3,2-c]pyridine (1 g, 1.85 mmol) in methanolic ammonia (60 mL, saturated at 0 °C) was set aside at room temperature for 24 h. The solvent was evaporated to dryness, and the residue was purified by flash chromatography over silica gel, eluting with $CHCl_3$–MeOH (95:5) to give 0.45 g (80%) of **17** (lit.[12] yield 71%).

4-Amino-6-chloro-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)pyrrolo[3,2-*c*]pyridine (6) and 4-Amino-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)pyrrolo[3,2-*c*]pyridine (18). A stirred mixture of **17** (0.55 g, 18.14 mmol) and hydrazine hydrate (20 mL) was refluxed under nitrogen for 1.5 h. Evaporation of the hydrazine to dryness gave a residue which was treated with deoxygenated water (40 mL) and Raney nickel (3.43 g). The reaction mixture was refluxed for 45 min, filtered, and evaporated to dryness. The residue was chromatographed on a silica gel column by eluting with $CHCl_3$–MeOH–NH_4OH (80:19:1). Evaporation of the first eluate gave 60 mg (12%) of **6** as a pure solid, mp 209–212 °C: UV (EtOH) λ_{max} 208 nm (ε 36 927), 277 (ε 12 817); ^{1}H NMR (Me_2SO-d_6) δ 6.18 (t, 1, J = 6.9 Hz, $C_{1'}$-H), 6.54 (s, 2, NH_2), 6.64 (d, 1, J = 3.5 Hz, C_3-H), 6.83 (s, 1, C_7-H), and 7.35 (d, 1, J = 3.5 Hz, C_2-H). Anal. ($C_{12}H_{14}ClN_3O_3$) C, H, N.

Further elution of the column with the same mixture of solvents gave 0.2 g (44%) of **18** as a solid which was crystallized from water, mp 202–203 °C dec: UV (EtOH) λ_{max} 205 nm (ε 31 316), 275 (ε 10 764); ^{1}H NMR (Me_2SO-d_6) δ 6.30 (t, 1, J = 6.9 Hz, $C_{1'}$-H), 6.89 (d, 1, J = 3.5 Hz, C_3-H), 7.09 (d, 1, J = 7.2 Hz, C_7-H), 7.40 (s, 2, NH_2), 7.56 (d, 1, J = 7.2 Hz, C_6-H), and 7.58 (d, 1, J = 3.5 Hz, C_2-H). Anal. ($C_{12}H_{15}N_3O_3$) C, H, N.

Antitumor Evaluation. The following cell lines were used: P388 murine lymphocytic leukemia, L1210 murine leukemia, HL-60 human promyelocytic leukemia, and KB human epidermoid carcinoma. Cell lines, maintained in vitro, in exponential growth, were cultured in RPMI-1640 medium supplemented with antibiotics (100 units/mL penicillin, 100 μg/mL streptomycin, 50 μg/mL gentamicin), 3 mM glutamine, 10 mM HEPES buffer, and 5% (for KB cell line) or 15% (for P388 and L1210 cell lines) heat-inactivated newborn calf serum or 10% (for HL-60 cell line) heat-inactivated fetal calf serum. In order to determine cell growth inhibition, an antimetabolic assay was performed. Compounds were solubilized in DMSO, and then water and culture medium were added; final concentration of DMSO (not more than 0.5%) had no cytotoxic effect in our testing system. Various concentrations of each compound were placed, in quadruplicate, in flat-bottomed microculture wells with tumor cell suspensions for 48 h at 37 °C. Cells were placed in aliquots of 0.2 mL at the following concentrations: P388, 10^5 cells/well; L1210, 5×10^4 cells/well; HL-60, 5×10^4 cells/well; KB, 5×10^3 cells/well. Antiproliferative activity was determined by adding to the cultured cells [^{125}I]-5-iodo-2′-deoxyuridine together with 5-fluoro-2′-deoxyuridine, for an additional 18 h. Harvesting was performed by a multiple suction filtration apparatus on a fiberglass filter.

Immediately before being harvested, KB cells were treated with 0.05% trypsin plus 0.02% EDTA. The filter paper was washed six times with 0.85% NaCl solution, and the paper disks containing the aspirated cells were read in a γ-scintillation counter. At each dose level of compounds tested, cell growth inhibition was expressed as a percentage of inhibition of radioisotope incorporation in the treated cultures with respect to the control cultures. The dose resulting in 50% inhibition of radioisotope incorporation (ID_{50}) was determined; the mean ID_{50} of at least three experiments was reported.

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