also contained 0.2 M lithium sulfate.

Incubation with Phenyl Isothiocyanate (PITC). To equal aliquots of purifed enzyme in 0.1 M phosphate buffer was added either phenyl isothiocyanate (enzyme modification grade, Pierce Chemical Company) or **1b** dissolved in Me_2SO to give a 10^{-5} M solution containing 2.5% Me_2SO. Controls contained Me_2SO instead of drug. Aqueous solutions of 10^{-4} M sorbinil were incubated 30 min prior to the addition of isothiocyanate. The isothiocyanate solutions were incubated for 10 min at 4 °C with stirring prior to gel filtration and assay as described above.

Timed Studies. To equal aliquots of purified enzyme in 0.1 M phosphate buffer was added either acid azide or acid isothiocyanate to give a 10^{-4} M aqueous solution of all drugs in a volume of 4.0 mL. Controls contained water instead of drug. Upon stirred incubation or photolysis from a flood lamp at a distance of 12 in., 0.75 mL-aliquots of enzyme solution were removed at intervals of 15 s to 30 min. These aliquots were immediately placed onto a 3.1-mL BioRad P-6DG desalting gel packed in disposable 0.8 × 4 cm polypropylene BioRad columns and then eluted with 1.0 mL of 0.1 M phosphate buffer, pH 6.2. Enzyme activity was spectrophotometrically monitored with 10 mM glyceraldehyde as described above in the presence of 0.2 M lithium sulfate.

All results were the average of 4–8 cuvette measurements on either a Guilford Response or Guilford 2400-2 spectrophotometer.

Registry No. **1b**, 103904-10-5; **1c**, 103884-86-2; **5**, 3027-38-1; **6**, 103884-82-8; **7**, 103884-83-9; **8**, 103884-84-0; **10**, 103884-85-1; Gly-OMe·HCl, 5680-79-5; 1,8-naphthalic acid anhydride, 81-84-5; aldose reductase, 9028-31-3.

9-(2-Deoxy-2-fluoro-β-D-arabinofuranosyl)guanine: A Metabolically Stable Cytotoxic Analogue of 2′-Deoxyguanosine

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The synthesis of 9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)guanine (**1b**) from 1,3-di-*O*-acetyl-5-*O*-benzoyl-2-deoxy-2-fluoro-D-arabinofuranose (**2a**) and 2,6-dichloropurine in six steps using an enzymatic deamination as the last step is reported. The target compound was found to be stable to purine nucleoside phosphorylase cleavage and was cytotoxic in two cell lines, one a T-cell line. Incubation of L1210 cells with **1b** results in an inhibition of DNA synthesis as judged by the reduced incorporation of labeled thymidine into DNA, while RNA and protein syntheses were unaffected.

The enhanced toxicity of certain deoxynucleosides toward T-cell lines relative to B-cell lines may result from the greater ability of these cells to accumulate toxic concentrations of the corresponding deoxynucleoside triphosphates.[1-4] Certain immunodeficiency diseases are associated with deficiencies of adenosine deaminase or purine nucleoside phosphorylase,[5-9] which result in increased pools of deoxynucleoside triphosphates. Recent reports indicate that 9-β-D-arabinofuranosylguanine (*ara*-G, **1a**), an analogue of 2′-deoxyguanosine, preferentially inhibits DNA synthesis in T- relative to B-lymphoblasts and is a selective inhibitor of T-lymphoblast growth.[10-14] *ara*-G is readily metabolized to the triphosphate level in various cell lines[10-15] and has been found to inhibit DNA polymerase α from L5178Y mouse lymphoma cells. In the presence of *ara*-G these cells die because of unbalanced growth.[15] *ara*-G has been reported to be resistant to purine nucleoside phosphorylase when tested against the isolated enzyme and in vitro,[10,14] but two reports indicate that it is degraded in vivo.[10,16]

Analogues with the basic properties of *ara*-G are attractive as potential chemotherapeutic agents for T-cell malignancies. We have now prepared another such analogue of 2′-deoxyguanosine, 9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)guanine (**1b**) and carried out an initial examination of its cytotoxicity and inhibitory effects. The known resistance of 9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)hypoxanthine to cleavage by purine nucleoside phosphorylase[17] made **1b** a particularly attractive target.

Chemistry. The synthesis of **1b** appeared to be best accomplished by the condensation of a 2-deoxy-2-fluoro-arabinose derivative with a purine. Starting with 1,3-di-*O*-acetyl-5-*O*-benzoyl-2-deoxy-2-fluoro-β-D-arabinose (**2a**),[18,19] we examined various approaches to achieve the

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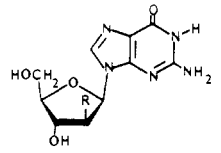
maximum amount of the 9β-isomer **4a**, using 2,6-dichloropurine (**3**) or a derivative of it. After conversion



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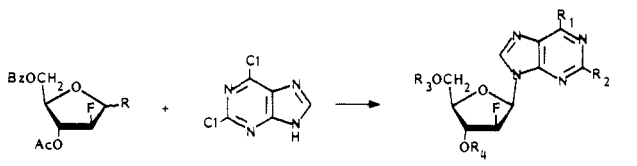
a) R = OH
b) R = F



2 **3** **4**

a) R = OAc
b) R = Br
c) R = Cl

a) $R_1 = R_2$ = Cl, R_3 = Bz, R_4 = Ac
b) α-isomer of **4a**
c) $R_1 = R_2 = N_3$, R_3 = Bz, R_4 = Ac
d) $R_1 = R_2 = NH_2$, R_3 = Bz, R_4 = Ac
e) $R_1 = R_2 = NH_2$, $R_3 = R_4$ = H

of **2a** to the bromo and chloro sugars (**2b** and **2c**), condensation was examined with the sodium salt of 2,6-dichloropurine as well as the trimethylsilyl derivative under a variety of conditions.[20] A fusion reaction between **2a** and **3** was also examined. None of these experiments provided an acceptable yield of **4a**. The method of choice proved to be a coupling of **3** with the bromo sugar **2b** in boiling 1,2-dichloroethane in the presence of 4A molecular sieves. This coupling gave **4a** as the major product, plus the 9α-isomer **4b**, as well as several other unidentified nucleoside products. The reaction succeeded equally well with the chloro sugar, but a much longer period of time was required to prepare chloro sugar **2c**. Because the ultraviolet absorption spectra of 9-substituted- and 7-substituted-2,6-dichloropurine derivatives are similar,[24] we had to ensure not only the anomeric configuration but also the substitution position. It is known that the 9- and 7-ethyl derivatives of 2-chloro-6-(dimethylamino)purine have widely different ultraviolet spectra (λ_{max} 277.5 nm and 296 nm, respectively, at pH 7).[25] Treatment of **4a** and **4b** with dimethylamine afforded only displacement of the 6-chlorine from both compounds. The UV spectra of these two compounds were identical, with a λ_{max} of 276 nm, confirming substitution at the 9-position. With regard to the anomeric configuration, it was not possible to use coupling constants with any certainty, as $J_{1',2'}$ was 2.7 Hz for **4a** and 1.3 Hz for **4b**. In the work delineating the

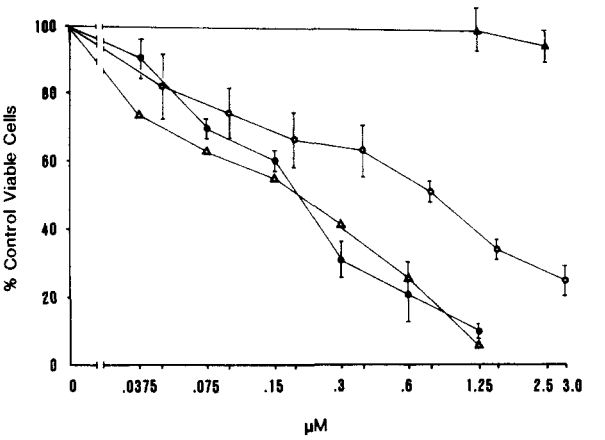


Figure 1. Growth curves for various cell lines in the presence of **1b**. The growth curves were performed as follows. Each cell line was suspended at a density of 1×10^5/mL in RPMI 1640 medium supplemented with 10% fetal calf serum, 2 mM L-glutamine, and increasing concentrations of the drug. After 3 days culture in an atmosphere of 95% air and 5% CO_2, viable cells were enumerated microscopically after the addition of 0.1% erythrocin B. The closed circles show the results with human CCRF-CEM T-cells, the closed triangles with a mutant CEM cell line that lacks deoxycytidine kinase, the open triangles with another mutant CEM line that lacks adenosine kinase, and the open circles with human Wi-L2 B-lymphoblasts. The data are expressed as percent control; the control value refers to cultures that did not receive any drug. The viable cell density in these cultures was approximately $(1-1.5) \times 10^6$/mL. Each point shows the mean ± SEM for the indicated concentration, except for the data with the adenosine kinase deficient mutant, which represents the average of duplicate points. The cell lines employed are described in ref 28.

synthesis of 9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-adenine,[26] in which some similar 2,6-dichloropurine nucleosides were prepared, it was reported that the H-8 signal for all β anomers was a doublet, while it was a singlet for the α anomers. The ^{1}H NMR spectra showed H-8 to be a doublet in **4a** and a singlet in **4b**, again strongly suggestive of the assigned configurations. This splitting of H-8 was seen in all of our β anomers, as well. A nuclear Overhauser experiment was also run on **4a**. Irradiation of the 5′-proton of **4a** produced an enhancement of both H-3′ and H-8, demonstrating the β nature of this compound. In addition, the ^{19}F NMR spectra of all of the β anomers showed a signal at around −197 to −199 ppm upfield from $CFCl_3$, while **4b** exhibited its resonance at −187.1 ppm. Later evidence for the β configuration came from the successful enzymatic deamination of **4e**, since 9-α-D-arabinofuranosyladenine is completely resistant to deamination.[27]

Treatment of **4a** with sodium azide gave the diazido compound **4c**, which was subjected to hydrogenolysis with palladium-on-charcoal under a hydrogen atmosphere, affording **4d** in 58% yield for the two steps. Deacylation of **4d** was achieved in 1 N NaOH to produce the diamino compound **4e** in 91% yield. Deamination of **4e** occurred rapidly with calf intestinal adenosine deaminase in water to produce the target structure **1b**.

Biological Results

Growth curves for various cell lines in the presence of **1b** are shown in Figure 1. Three of the cell lines are

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human CCRF-CEM T-cells, the wild type as well as a mutant CEM line deficient in adenosine kinase and one deficient in deoxycytidine kinase. The fourth cell line is human Wi-L2 B-lymphoblasts. A comparison of the wild-type T-cell line with the B-cell line shows that **1b** is more cytotoxic to the T-cell line ($IC_{50} \cong 0.2$ μM) than toward the B-cell line ($IC_{50} \cong 0.75$ μM). The IC_{50} for **1a** is reported to be 0.1 μM in the same cell line and 7.0 μM with MGL-8 B-lymphoblasts.[10] The CEM line deficient in adenosine kinase exhibits a curve similar to the wild-type line for **1b**, while **1b** has no effect on the CEM line deficient in deoxycytidine kinase. These results suggest that **1b** requires activation to exert its cytotoxic effects and that it is initially phosphorylated by deoxycytidine kinase. Incubation of **1b** with purine nucleoside phosphorylase (Sigma, bovine spleen) showed it to be neither a substrate nor an inhibitor of the enzyme under standard assay conditions.

Results of an examination of effects of **1b** on macromolecular synthesis in L1210 cells in culture (**1b** has an IC_{50} in the range of 8.5–17 μM) revealed that concentrations of 100, 50, and 25 μM produced 76, 62, and 12% inhibition of incorporation of [^{3}H]dThd into DNA. The highest concentration of **1b** (100 μM) did not inhibit incorporation of [5-^{3}H]Urd into RNA and produced only 17% inhibition of incorporation of [4,5-^{3}H]L-Leu into protein.

Experimental Section

Melting points were determined on a Mel-Temp apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Nicolet NMC 300NB spectrometer operating at 300.635 MHz (^{1}H) or 75.16 MHz (^{13}C). Chemical shifts are expressed in parts per million downfield from tetramethylsilane. ^{19}F NMR spectra were recorded on a Bruker CXP-200 spectrometer operating at 188.2 MHz (^{19}F). Chemical shifts are reported in parts per million upfield relative to $CFCl_3$ (δ 0.0). Ultraviolet absorption spectra were determined on a Cary 17 spectrophotometer by dissolving each compound in ethanol and diluting 10-fold with 0.1 N HCl, pH 7 buffer, and 0.1 N NaOH. Microanalyses were performed by Atlantic Microlab, Inc., Atlanta, GA, or the Molecular Spectroscopy Section of Southern Research Institute. Mass spectra were recorded on a Varian MAT 311A mass spectrometer in the fast atom bombardment (FAB) mode. Analytical results indicated by element symbols were within ±0.4% of the theoretical values. Where solvents are noted as part of the elemental analysis, they were seen in the ^{1}H NMR spectrum in the proper amounts. TLC was carried out on Analtech precoated (250 μm) silica gel (GF) plates.

2-Amino-9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-1,9-dihydro-6*H*-purin-6-one (1b). A solution of **4e** (150 mg, 0.53 mmol) in H_2O (20 mL) at room temperature was treated with adenosine deaminase type I (35 μL) (Sigma). After stirring for 3 h, a white precipitate began to form. The mixture was stirred an additional 16 h and chilled. The white solid was collected, washed with cold H_2O, and dried in vacuo at 56 °C to give crude **1b** (96 mg). This solid was allowed to crystallize at room temperature from 3 mL of hot H_2O. After chilling, the crystals were collected, washed with cold H_2O, and dried in vacuo at 56 °C for 16 h: yield, 87 mg (58%); mp 255 °C slight dec; TLC, 2:1 $CHCl_3$–MeOH + 1% concd NH_4OH, R_f = 0.30; MS (FAB), m/z 286 $(M + 1)^+$; UV λ_{max}, nm ($\epsilon \times 10^{-3}$) [pH 1] 280 (sh), 256 (13.7), [pH 7] 275 (sh), 252 (14.8), [pH 13] 261 (12.0); ^{1}H NMR (Me_2SO-d_6) δ 3.57 (m, 2, 5′-CH_2, $J_{5a',5b'}$ = 11.6 Hz), 3.81 (m, 1, H-4′, $J_{4,5a'}$ = 3.8 Hz, $J_{4,5b'}$ = 4.85 Hz), 4.37 (dm, 1, H-3′, $J_{3',4'}$ = 5.2 Hz, $J_{3',F}$ = 17.9 Hz), 5.07 (apparent t, 1, 5′-OH, $J_{5',OH}$ = 5.6 Hz), 5.10 (dm, 1, H-2′, $J_{2',3'}$ = 3.6 Hz, $J_{2',F}$ = 52.4 Hz), 5.94 (d, 1, 3′-OH, $J_{3',OH}$ = 4.6 Hz), 6.13 (dd, 1, H-1′, $J_{1',2'}$ = 4.2 Hz, $J_{1',F}$ = 16.0 Hz), 6.54 (br s, 2, 2-NH_2), 7.80 (d, 1, H-8, $J_{8,F}$ = 2.3 Hz), 10.68 (br 2, 1, 1-NH); ^{13}C NMR (Me_2SO-d_6) δ 60.34 (C-5′), 72.54 (d, C-3′, $J_{3',F}$ = 23.3 Hz), 81.10 (d, C-1′, $J_{1',F}$ = 16.9 Hz), 83.56 (C-4′), 95.02 (d, C-2′, $J_{2',F}$ = 191.5 Hz), 115.79 (C-5), 135.69 (d, C-8, $J_{8,F}$ = 4.5 Hz), 150.72 (C-4), 153.75 (C-2), 156.52 (C-6); ^{19}F NMR (Me_2SO-d_6) δ −197.79. Anal. ($C_{10}H_{12}FN_5O_4 \cdot 0.4H_2O$) C, H, N.

3-*O*-Acetyl-5-*O*-benzoyl-2-deoxy-2-fluoro-D-arabinofuranosyl Bromide (2b).[18] A solution of **2a** (4.7 g, 13.8 mmol) in 1 M HBr/CH_2Cl_2, prepared separately and added to **2a** at 0 °C, was allowed to stand at 5 °C for 24 h, at which time TLC analysis (2:1 cyclohexane–ethyl acetate) showed one major product. After removal of the solvent under reduced pressure, the residue was coevaporated several times with dry toluene to give crude **2b**, which was used directly in the next step.

2,6-Dichloro-9-(3-*O*-acetyl-5-*O*-benzoyl-2-deoxy-2-fluoro-β-D-arabinofuranosyl)-9*H*-purine (4a). To a solution of **2b** in dichloroethane (200 mL) were added 4A molecular sieves (100 mL) and 2,6-dichloropurine (2.61 g, 13.8 mmol). The resulting mixture was placed in an oil bath preheated to 100 °C and allowed to reflux for 16 h, at which time TLC (2:1 cyclohexane–ethyl acetate) indicated the absence of **2b** and the production of a series of nucleosides, with **4a** being the predominant product. After filtration and evaporation of solvent, the residue was dissolved in $CHCl_3$ and purified by flash chromatography (200 g of silica gel, 230–400 mesh, elution with 2:1 cyclohexane–ethyl acetate). Isolation of material from fractions containing the major product gave essentially pure **4a**, obtained as a foam, 2.05 g (32%). An analytical sample was obtained by crystallization from diethyl ether: mp 110–111 °C; MS, m/z 469 $(M + 1)^+$; ^{1}H NMR ($CDCl_3$) δ 2.22 (s, 3, CH_3), 4.46 (m, 1, H-4′), 4.74 (m, 2, 5′-CH_2, $J_{4',5'}$ = 4.7 Hz), 5.22 (br dd, 1, H-2′, $J_{2',F}$ = 49.9 Hz, $J_{2',3'}$ = 0.6 Hz), 5.53 (dd, 1, H-3′, $J_{3',4'}$ = 2.7 Hz, $J_{3',F}$ = 16.2 Hz), 6.54 (dd, 1, H-1′, $J_{1',F}$ = 21.9 Hz, $J_{1',2'}$ = 2.7 Hz), 7.45 (m, 2, meta phenyl protons), 7.59 (m, 1, para phenyl proton), 8.07 (m, 2, ortho phenyl protons), 8.35 (d, 1, H-8, $J_{8,F}$ = 3.1 Hz); ^{13}C NMR ($CDCl_3$) δ 20.63 (CH_3), 62.94 (C-5′), 76.00 (d, C-3′, $^2J_{3',F}$ = 30.1 Hz), 81.76 (C-4′), 83.81 (d, C-1′, $^2J_{1',F}$ = 16.8 Hz), 92.31 (d, C-2′, $^1J_{2',F}$ = 192.6 Hz), 128.54, 129.67, 130.56, 133.46 (phenyl ring), 129.20 (C-5), 144.98 (d, C-8, $^4J_{8,F}$ = 6.0 Hz), 152.12, 152.40, 153.21 (C-2,4,6), 166.03 (CO, benzoyl), 169.24 (CO, acetyl); ^{19}F NMR ($CDCl_3$) δ −198.88. Anal. ($C_{19}H_{15}Cl_2FN_4O_5$) C, H, N.

In one run the 9α-isomer **4b**, which was just slightly more polar than the 9β-isomer **4a**, was isolated and characterized spectrally: MS, m/z 469 $(M + 1)^+$; ^{1}H NMR ($CDCl_3$) δ 2.05 (s, 3, CH_3), 4.65 (br d, 2, 5′-CH_2), 4.86 (m, 1, H-4′), 5.58 (dm, 1, H-3′, $J_{3',F}$ = 17.2 Hz, $J_{3',4'}$ = 3.2 Hz), 5.78 (dm, 1, H-2′, $J_{2',3'}$ = 1.7 Hz, $J_{2',F}$ = 48.7 Hz), 6.52 (br d, 1, H-1′, $J_{1',2'}$ = 1.3 Hz, $J_{1',F}$ = 14.5 Hz), 7.48 (m, 2, meta phenyl protons), 7.61 (m, 1, para phenyl proton), 8.10 (m, 2, ortho phenyl protons), 8.27 (s, 1, H-8); ^{19}F NMR ($CDCl_3$) δ −187.10.

The 9α and 9β isomers gave the identical qualitative UV spectrum: λ_{max} (nm, EtOH) 230 (sh), 253, 273, 281 (sh). In order to provide additional support that these were 9-isomers rather than 7-isomers, the two compounds were treated with dimethylamine to produce the 2-chloro-6-(dimethylamino)purine derivatives. These two compounds also had identical qualitative UV spectra, λ_{max} (nm, EtOH) 220, 235 (sh), 276, and mass spectra, MS, m/z 478 $(M + 1)^+$. The UV spectra were similar to the UV spectrum of 6-(dimethylamino)-9-ethyl-9*H*-purine and distinct from the UV spectrum of 6-(dimethylamino)-7-ethyl-7*H*-purine. In addition, an NOE experiment on **4a** in which the 5′ protons were irradiated resulted in enhancement of the signals for H-3′ and H-8, also confirming the configuration.

2,6-Diazido-9-(3-*O*-acetyl-5-*O*-benzoyl-2-deoxy-2-fluoro-β-D-arabinofuranosyl)-9*H*-purine (4c). A solution of NaN_3 (529 mg, 8.05 mmol) in H_2O (2.6 mL) was added in one portion to a solution of **4a** (1.66 g, 3.5 mmol) in EtOH (166 mL). After heating at reflux for 30 min, the reaction was evaporated to dryness, dissolved in 100 mL of $CHCl_3$, washed with H_2O (4 × 55 mL), and dried over $MgSO_4$. After evaporation of the solvent, the residue in a small amount of $CHCl_3$ was purified by flash chromatography (100 g of silica gel, 230–400 mesh, packed and eluted with 2:1 cyclohexane–ethyl acetate). Appropriate fractions were combined to give pure **4c** as a white foam: yield, 1.22 g (72%). This material was used in the reduction step: TLC, 1:1 cyclohexane–ethyl acetate, R_f = 0.55; MS (FAB), m/z 483 (M +

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1)$^+$; ^{1}H NMR ($CDCl_3$) δ 2.20 (s, 3, CH_3), 4.42 (m, 1, H-4'), 4.70 (br d, 2, 5'-CH_2, $J_{4',5'}$ = 4.7 Hz), 5.17 (dd, 1, H-2', $J_{2',3'}$ = 0.3 Hz, $J_{2',F}$ = 49.8 Hz), 5.51 (dd, 1, H-3', $J_{3',4'}$ = 2.6 Hz, $J_{3',F}$ = 16.2 Hz), 6.48 (dd, 1, H-1', $J_{1',2'}$ = 2.6 Hz, $J_{1',F}$ = 22.3 Hz), 7.48 (m, 2, meta phenyl protons), 7.58 (m, 1, para phenyl proton), 8.07 (m, 2, ortho phenyl protons), 8.14 (d, 1, H-8, $J_{8,F}$ = 3.1 Hz); ^{19}F NMR ($CDCl_3$) δ -199.14.

9-(3-*O*-Acetyl-5-*O*-benzoyl-2-deoxy-2-fluoro-β-D-arabinofuranosyl)-9*H*-purine-2,6-diamine (4d). A solution of **4c** (1.22 g, 2.5 mmol) in EtOH (200 mL) was treated with Pd-C (5%, 165 mg) and hydrogenolyzed at atmospheric pressure for 5 h. The hydrogen atmosphere was changed after 2.5 h. The catalyst was removed by filtration and washed with $CHCl_3$ to dissolve any precipitated product. The combined filtrate and washings were evaporated to dryness, and the resulting residue was dissolved in boiling EtOH (80 mL). The crystals that formed on cooling were collected after chilling, washed with EtOH, and dried in vacuo at 56 °C for 6 h: yield, 865 mg (80%); mp 217-218 °C; TLC, 9:1 $CHCl_3$-MeOH, R_f = 0.50; exact mass, m/z (M + 1)$^+$ calcd 431.148, found 431.147; ^{1}H NMR (Me_2SO-d_6) δ 2.15 (s, 3, CH_3), 4.42 (m, 1, H-4'), 4.59, 4.70 (2 m, 2, 5'-CH_2), 5.47 (dm, 1, H-2', $J_{2',3'}$ = 1.8 Hz, $J_{2',F}$ = 55.5 Hz), 5.57 (dm, 1, H-3', $J_{3',4'}$ = 3.5 Hz, $J_{3',F}$ = 18.2 Hz), 5.92 (br s, 2, 2-NH_2), 6.25 (dd, 1, H-1', $J_{1',2'}$ = 3.5 Hz, $J_{1',F}$ = 20.9 Hz), 6.81 (br s, 2, 6-NH_2), 7.55 (m, 2, meta phenyl protons, 7.68 (m, 1, para phenyl proton), 7.69 (d, 1, H-8, $J_{8,F}$ = 3.0 Hz), 8.01 (m, 2, ortho phenyl protons); ^{19}F NMR ($CDCl_3$) δ -197.97. Anal. ($C_{19}H_{19}FN_6O_5$) C, H, N.

9-(2-Deoxy-2-fluoro-β-D-arabinofuranosyl)-9*H*-purine-2,6-diamine (4e). A suspension of **4d** (200 mg, 0.46 mmol) in 1:1 MeOH-H_2O (13 mL) was treated in one portion with 1 N NaOH (0.6 mL). The mixture was stirred at room temperature for 19 h. A TLC aliquot of the resulting solution showed complete reaction. After adjusting the pH to 5 with dilute acetic acid, the reaction was evaporated to dryness and the residue crystallized from boiling H_2O (5 mL), triturated with ether, and dried to afford a first crop of 105 mg: softens at 150 °C; mp 225 °C (with some decomposition). A second crop (15 mg) with identical melting behavior and of identical purity was obtained by reducing the volume: total yield, 120 mg (91%); exact mass, m/z (M + 1)$^+$ calcd 285.111, found 285.112; UV λ_{max} ($\epsilon \times 10^{-3}$) [pH 1] 1) 252 (12.2), 290 (10.4), [pH 7] 255 (10.1), 278 (10.4), [pH 13] 255 (9.9), 278 (10.6); ^{1}H NMR (Me_2SO-d_6) δ 3.63 (m, 2, 5'-CH_2), 3.81 (m, 1, H-4'), 4.40 (dm, 1, H-3', $J_{3',4'}$ = 4.6 Hz, $J_{3',F}$ = 18.9 Hz), 5.10 (br s, 1, 5'-OH), 5.09 (dm, 1, H-2', $J_{2',3'}$ = 3.4 Hz, $J_{2',F}$ = 52.6 Hz), 5.88 (br s, 2, 2-NH_2), 5.91 (d, 1, 3'-OH, $J_{3',3'\text{-OH}}$ = 4.0 Hz), 6.18 (dd, 1, H-1', $J_{1',2'}$ = 4.3 Hz, $J_{1',F}$ = 16.1 Hz), 6.75 (br s, 2, 6-NH_2), 7.80 (d, 1, H-8, $J_{8,F}$ = 2.3 Hz); ^{13}C NMR (Me_2SO-d_6) δ 60.27 (C-5'), 72.63 (C-3', $J_{3',F}$ = 23.6 Hz), 81.06 (C-1', $J_{1',F}$ = 17.0 Hz), 83.20 (C-4', $J_{4',F}$ = 4.5 Hz), 95.11 (C-2', $J_{2',F}$ = 191.2 Hz), 112.09 (C-5), 136.07 (C-8, $J_{8,F}$ = 3.7 Hz), 151.19 (C-4), 155.76 (C-6), 160.13 (C-2); ^{19}F NMR ($CDCl_3$) δ -197.40. Anal. ($C_{10}H_{13}FN_6O_3 \cdot 0.11HOAc \cdot 0.03Et_2O \cdot 0.4H_2O$) C, H, N.

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Registry No. **1b**, 103884-98-6; α-**2a**, 56632-79-2; β-**2a**, 56632-80-5; α-**2b**, 92283-83-5; β-**2b**, 93302-28-4; **3**, 5451-40-1; **4a**, 103884-99-7; **4b**, 103885-00-3; **4c**, 103885-01-4; **4d**, 103885-02-5; **4e**, 103884-97-5.

Comparison of the Chemical Reactivities and Antineoplastic Activities of α,β-, α,β'-, β,β'-, and α,α'-Bis[[[(2-propylamino)carbonyl]oxy]methyl]-Substituted Pyrroles[1]

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The bis[*N*-(2-propyl)carbamate] derivatives of 2,3-bis(hydroxymethyl)-4,5-diphenyl-1-methylpyrrole (**4a**), 3,4-bis(hydroxymethyl)-2,5-diphenyl-1-methylpyrrole (**4b**), 2,4-bis(hydroxymethyl)-3,5-diphenyl-1-methylpyrrole (**4c**), and 2,5-bis(hydroxymethyl)-3,4-diphenyl-1-methylpyrrole (**4d**) were synthesized and the reactivities of the four compounds were compared using the model nucleophile 4-(*p*-nitrobenzyl)pyridine (NBP). No significant correlation was seen between NBP reactivity and either toxicity or antineoplastic activity in this series. Three compounds **4a**, **4b**, and **4c** gave significant reproducible activity against P388 lymphocytic leukemia; the α,α'-bis(carbamate) **4d** was inactive. The α,β- and α,β'-bis(carbamates) **4a** and **4c** were evaluated against a panel of mouse tumor homografts and human tumor xenografts implanted under the kidney capsule of mice.

The class of antineoplastic agents broadly referred to as "acylated vinylogous carbinolamines" has been studied extensively over the past several years. Bis(carbamate) derivatives of bis-hydroxymethylated pyrroles,[2] pyrrolizines,[3] and other heterocycles[4] have been shown to possess significant antineoplastic activity in a number of different test systems.[5] The compounds that have been reported to date all have the hydroxymethyl moieties on adjacent carbon atoms; no study has been reported that evaluates the significance of different hydroxymethyl substitution patterns in the pyrrole skeleton.

This paper describes the synthesis and antitumor activity of four isomeric bis(hydroxymethyl)diphenyl-*N*-methylpyrrole bis(carbamates) that differ in the substi-

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