We prepared the hydrochloride salt of *nitrara*-C (1) by acidifying a solution of 1 in MeOH with concentrated hydrochloric acid until the pH of the solution was ~1.0. The resulting solid was isolated and crystallized from a mixture of 1:1 absolute ethanol/96% ethanol: yield 65%; mp >170 °C dec; UV λ_{max} (0.1 N HCl) 276 nm (ε 13280); UV (0.1 N NaOH) λ_{max} 270 nm (ε 9290); UV (96% ethanol) λ_{max} 273 nm (ε 8730); UV (H_2O) λ_{max} 270 and 236 nm (ε 9446 and 7305); ^1H NMR (Me_2SO-d_6 + D_2O) δ 5.44 (dd, J = 5.6 Hz, 1, H-2′), 5.89–5.98 (two superimposing d, J = 7.6 and 5.6 Hz, 2, H-5 and H-1′), 7.82 (d, J = 7.6 Hz, 1, H-6); IR (KBr) 3250, 3070, 1715, 1680, 1655, 1285, 1090, 1040, 870 cm^{-1}; $[\alpha]^{23}_D$ +84.9 ± 0.5° (c 1.0, H_2O). Anal. ($C_9H_{13}ClN_4O_7$) C, H, Cl, N.

Cell-Kill in Vivo. Female B6D2F_1 mice were inoculated ip with 1×10^5 murine L1210 leukemic cells on day 0. Five days later, leukemic cells were washed from the ip compartments of untreated mice, and base-line counts were made. At that time, other mice were treated with the drug; control mice received saline. Twenty-four hours later, leukemic cells were washed from the ip compartments of mice. The number of cells from the treated and control mice was determined and expressed as the percentage of the base-line counts.

Acknowledgment. The authors are indebted to M. C. Connelly, H. A. Pasha, and R. Williams for technical assistance. We thank also T.-S. Lee for performing the regression analysis. This investigation was supported by the American–Lebanese–Syrian Associated Charities (ALSAC), Grants CA-21765 and CA-21677 from the NIH, and Grant CH-52C from the American Cancer Society.

Registry No. 1, 83831-64-5; 1·HCl, 83831-65-6; 3, 50896-84-9; 4, 13241-19-5; 5, 83831-66-7.

Synthesis of an *N*-Aminopyrazinonium Analogue of Cytidine[1]

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Received June 14, 1982

An N-aminated pyrazine analogue of cytidine, in which the pyrimidine N(3) ring nitrogen and C(4) amino group were replaced by a C-amino and an N-amino function, respectively, was prepared as a potential deaminase-resistant cytidine antimetabolite. The nucleoside 1,2-diamino-4-β-D-ribofuranosylpyrazin-2-onium chloride (6) was a mild cytostatic agent but was neither a substrate for nor an inhibitor of mouse kidney cytidine deaminase. It ionized with a lower pK_a than expected. The anion did not undergo the dimerization usually observed with *N*-imino heterocyclic ylides but underwent hydrolysis of the 2-amino group to yield a 1-aminopyrazine-2,3-dione nucleoside.

A recurring problem with the use of amino-substituted purine and pyrimidine nucleoside antimetabolites as cancer chemotherapeutic agents is their rapid deamination in vivo to inactive derivatives.[2-4] The concomitant administration of deaminase inhibitors, such as tetrahydrouridine for cytidine deaminase[5,6] and *erythro*-9-(2-hydroxy-3-nonyl)-adenine[7,8] or 2′-deoxycoformycin[8-10] for adenosine deaminase, has been one approach to alleviating this problem with arabinosylcytosine (*ara*-C) and arabinosyladenine (*ara*-A), respectively. An alternative approach is the synthesis of biosteres that retain chemotherapeutic activity but resist deamination. 2-Fluoro-*ara*-A,[11,12] carbocyclic *ara*-A,[13] and 2′-amino-*ara*-C[14] are examples of such derivatives. We have investigated the potential of a novel variation of the latter approach. On the premise that an *N*-amino group might be deaminase resistant, we prepared an analogue of cytidine in which the 3-nitrogen has been replaced by an amino substituent, and an *N*-amine function has been substituted for the enzymatically labile 4-amino group.

Chemical Studies. 2-Hydroxy-3-carboxamidopyrazine (1, Scheme I), was prepared by condensation of glyoxal sodium bisulfite and aminomalonamide by an improved procedure that avoids the heavy contamination of salt encountered with the reported[15] synthesis of 1. This was converted to 2-amino-3-hydroxypyrazine (2),[15] which was then reacted with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose in the presence of $SnCl_4$[16] to afford the tribenzoyl derivative of 1-β-D-ribofuranosyl-3-aminopyrazin-2-one (3) as an oil. Removal of the benzoyl groups (CH_3OH/NH_3) from purified 3 afforded the nucleoside 4 in an 80% yield for the two steps. The position of ribosylation was indicated by the presence of carbonyl absorption (1600 cm^{-1}) in the IR, the absence of an NH resonance in the NMR spectrum, and the similarity of the neutral UV-absorption spectrum to that of the neutral form, rather than the anion, of 2. The β configuration at the anomeric C-1′ position of 4 was indicated by a Δδ of 0.21 ppm between the two methyl groups of the isopropylidene derivative.[17,18]

(1) This investigation was supported in part by funds from the Public Health Service (Research Grants CA-08748, CA-23622, and CA-27569), National Cancer Institute, DHEW, and Grant CH-193 from the American Cancer Society.
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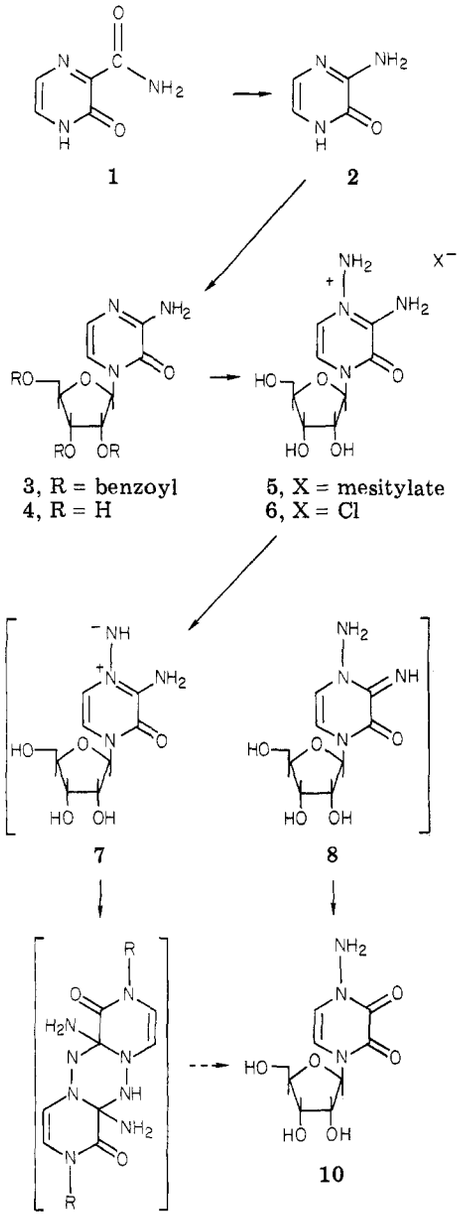
0022-2623/83/1826-0283$01.50/0

Scheme I



9, R = β-D-ribofuranosyl

N-Amination of aromatic heterocyclic amines has been accomplished with a number of reagents.[19,20] We found that **4** was not reactive with *O*-2,4-dinitrophenoxyamine,[21,22] but the reaction of **4** with *O*-mesitylenesulfonylhydroxylamine[23,24] afforded the mesitylate salt of 1,2-diamino-4-β-D-ribofuranosylpyrazin-2-one (**5**) in 77% yield. This was converted to the chloride salt **6** by ion-exchange chromatography. The structure of the *N*-amino nucleoside salts, **5** and **6**, was assigned from the elemental analyses and the presence in the NMR spectra of bands for the ribosyl and pyrazine ring hydrogens and two quite different amino groups. One amino resonance of **6** (δ 6.81) was shifted slightly downfield from that of the C-amino group of **4** (δ 6.66), while the second amino signal was substantially further downfield (δ 9.37), consistent with the presence of an aromatic *N*-amino substituent. N-Amination was also indicated by the large downfield shift (over 30 ppm) of the pyrazine ring hydrogens of **6**, relative to those of **4**.[25]

The amino substituent of N-aminated heterocycles can ionize in weak base to afford highly reactive *N*-imino ylides.[20,25] In the absence of added dienophiles, such ylides self-condense to form dimers.[20,25] The *N*-aminopyrazinonium nucleoside **6** exhibited an ionization pK_a of 8.8 ± 0.1. However, **6** has two amine functions, either of which could conceivably ionize to afford the ylide **7** or the *N*-amino imine **8**. The relatively slight shift of the UV absorption maximum accompanying ionization of **6** (313 to 322 nm), compared to that produced by ionization of *N*-aminopyridinium to its ylide (251 to 323 nm),[25] and the much lower pK_a of **6** than that of the *N*-aminopyridinium ion (11.2)[26] suggest that **8** is more likely to be the greater contributor. Once formed, the basic species, **7** or **8**, gradually reacted irreversibly to afford a new product with UV absorption (λ_{max} 310 nm) nearly identical with that of the neutral form of **6**. That product showed no UV spectral changes with changes in pH.

Dimerization of the ylide **7** to a structure such as **9** would accord with the known properties of *N*-imino heterocyclic ylides. However, the physical data were not consistent with the formation of dimer **9**. Chemical-ionization mass spectral analysis with isobutane or isobutane/ethylenediamine as a carrier indicated a mass of 259 for the parent ion. This was confirmed by negative ion chemical-ionization mass spectrometry with hydroxide ion. A mass of 259 would correspond to the loss of one amine function and the addition of one oxygen atom ($C_9H_{13}N_3O_6$) to the nucleoside **6**. The NMR spectrum of the product exhibited bands for the ribosyl moiety, the two pyrazine hydrogens and one amino group. The signal of the amino substituent was present at a much higher field (δ 5.94) than that of the C-amino function of **4** or **6**. Hydrolysis of an *N*-imine to an *N*-oxide function would be without precedent, and the product did not give a positive $FeCl_3$ test. These data indicated that the amine function lost under basic conditions must be the C-amino group of **6**. Replacement of the 2-amino substituent of **6** with a carbonyl function would afford 1-amino-4-β-D-ribofuranosylpyrazine-2,3-dione (**10**). This structure satisfied all criteria for the NMR, UV, mass spectral, and elemental analyses. In confirmation, **10** exhibited multiple strong bands in the IR spectrum in the region associated with carbonyl absorption (1680, 1640, 1600 cm^{-1}). The dione **10** could conceivably arise either by direct hydrolysis of the 2-amino function of **8** or by rapid hydrolysis of the dimer **9**. An attempt to prepare **10** or its 1-deamino derivative by diazotization of **6** gave no reaction. This was unexpected, since deamination of *N*-amino heterocycles by diazotization is well known.[20] The anomalous behavior of the anion of **6** represents the first example of an *N*-amino heterocyclic anion undergoing hydrolysis of an adjacent functional group, rather than dimerization. At pH 7.4, about 4% of **6** is present as the anion. At that pH and 37 °C, the half-life for the conversion of 2.5×10^{-4} M **6** to **10** was 2.6 h.

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Biological Studies. The amino nucleoside **6** showed moderate activity as a cytotoxic agent, but it exhibited a delayed response in inhibiting cell growth. There was little inhibition of growth of L1210 cells in culture during the first 48 h of exposure. However, after 72 h of exposure there was 25% inhibition at 5×10^{-5} M, 20% at 1×10^{-5} M, and 15% at 5×10^{-6} M. The 48-h lag time for inhibition to be seen was unanticipated. Whether inhibition is due to **6**, the ylide **7**, the imine **8**, or the dione **10** is not clear, although the time lag for inhibition to occur suggests that **10** is more likely to be the cytotoxic agent.

Neither the *N*-amino nucleoside **6** nor its hydrolysis product **10** was a substrate for or an inhibitor of mouse kidney cytidine deaminase at a concentration (1 mM) approximately five times the K_m (0.18 mM) of the enzyme. These data indicate that **6** and **10** bind poorly, if at all, to the deaminase. The presence of a functional group at the position corresponding to N-3 of cytidine, i.e., the 2-amino group of **6** and the 2-carbonyl function of **10**, must interfere with binding, despite the basic nature of the 2-amino substituent of **6**. The importance of the 3-nitrogen of cytidine for binding and reaction of the deaminase has been noted.[27,28] A better evaluation of the ability of an *N*-amine function to substitute for the C-amino group of cytidine will require a closer structural analogue of the pyrimidine moiety.

Experimental Section

NMR spectra were determined in $(CD_3)_2SO$ with Me_4Si as an internal standard with a JEOL PFT-100 NMR spectrometer. UV spectra and ϵ values were determined with a Cary 15 recording spectrophotometer. IR spectra were determined with a Perkin-Elmer Infracord spectrophotometer with KBr disks. Melting points were determined with a Mel-Temp Apparatus and were uncorrected. Microanalyses were performed by Spang Microanalytical Laboratory, Eagle Harbor, MI. We determined the $t_{1/2}$ for the conversion of **6** to **8** by monitoring the change in UV spectra of a reacting solution of 2.5×10^{-4} M **6** that was maintained at 37 °C. The pseudo-first-order rate constant was then calculated from a plot of ln $(OD_\infty - OD_0/OD_\infty - OD_t)$ vs. time by a least-squares analysis. The plot was linear over the entire course of reaction; $t_{1/2}$ was then calculated from the rate constant. The pK_a of **6** was determined spectrophotometrically from isosbestic spectra with 0.01 M buffers.[29]

[5-^{3}H]Cytidine (28 Ci/mmol) was purchased from ICN Pharmaceuticals, Inc., Irvine, CA, and purified by descending paper chromatography (Whatman 3MM) with the solvent system isopropyl alcohol/concentrated HCl/H_2O (68:17:16, v/v). The R_f value of [5-^{3}H]cytidine was 0.49. 3,4,5,6-Tetrahydrouridine was supplied by the Drug Research and Development Branch, National Cancer Institute, Bethesda, MD.

2-Hydroxy-3-carboxamidopyrazine (1). A solution containing glyoxal sodium bisulfite hemihydrate (32 g, 0.12 mol) and aminomalonamide[30] (11.5 g, 0.098 mol) dissolved in 80 mL of H_2O was stirred at 80 °C for 3 h and then made basic with 58% NH_4OH. Then 30% H_2O_2 (25 mL, 0.25 mol) was added dropwise with rapid stirring to the warm solution. The solution was chilled, and the resulting yellow precipitate was collected, washed with acetone, and air-dried to give 2.1 g of **1** containing a trace of unreacted aminomalonamide. The filtrate was reduced in volume under vacuum to 80 mL and chilled. The resulting precipitate of fine yellow needles was collected, washed with a small volume of cold H_2O and acetone, and air-dried to yield **1** (9.4 g): total yield 11.4 g (84%). Further reduction in volume of the filtrate and chilling afforded only salt: UV λ_{max} at pH 2, 230, 346 nm; at pH 7, 235, 346 nm; at pH 12, 237, 347 nm; NMR (Me_2SO-d_6) δ 13.27 (s, 1, exch, NH), 11.24 (s, 1, exch, NH), 8.71 (s, 1, exch, NH), 8.09 (d, 1, J = 2.8 Hz, CH), 7.92 (d, 1, J = 2.8 Hz, CH).

2-Amino-3-hydroxypyrazine (2). A crude sample of **1** was reacted with NaOBr, as reported,[15] to afford **2** (95%): UV λ_{max} at pH 2, 233, 315, 323, 342 (sh) nm; at pH 7, 240, 315, 340 (sh) nm; at pH 12, 240, 317 nm; NMR (Me_2SO-d_6) δ 11.52 (s, 1, exch, NH), 6.62 (d, 1, J = 4.6 Hz, CH), 6.53 (d, 1, J = 4.6 Hz, CH).

1-β-D-Ribofuranosyl-3-aminopyrazin-2(1*H*)-one (4). 2-Amino-3-hydroxypyrazine (**2**; 3.96 g, 35 mmol) was heated under reflux with 20 mL of hexamethyldisilazane until a clear solution was obtained. The solvent was evaporated under vacuum, and the residue was dissolved in 20 mL of dry $ClCH_2CH_2Cl$; then 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (15.1 g, 30 mmol) and $SnCl_4$ (3.8 mL, 8.6 g, 33 mmol) were added, and the solution was allowed to stir under anhydrous conditions at 25 °C. Progress of the reaction was monitored by TLC (silica gel, EtOAc; R_f of **2** 0.38; R_f of **3** 0.8). After 3 h, 150 mL of CH_2Cl_2 and 200 mL of saturated $NaHCO_3$ were added, and the mixture was stirred for 30 min and then filtered through Celite. The organic layer of the filtrate was separated, washed with H_2O, and dried over Na_2SO_4. Removal of the solvent yielded an oil (17.6 g) containing primarily product **3** with traces of both starting materials. The oil was dissolved in a minimum volume of hot EtOH. Upon chilling the solution, a gummy precipitate of **3** formed. This process was repeated until a single component, the tribenzoyl nucleoside **3**, could be visualized on TLC: yield 14.6 g (87%); NMR (Me_2SO-d_6) δ 8.0–7.38 (m, 15, C_6H_5), 6.97 (d, 1, J = 4.9 Hz, CH), 6.70 (d, 1, J = 4.9 Hz, CH), 6.31 (d, 1, $C_{1'}$ H), 6.01–5.98 (m, 2, $C_{2'}$ H and $C_{3'}$ H), 4.84–4.75 (m, 3, $C_{4'}$ H and $C_{5'}$ H_2).

We debenzoylated the protected nucleoside **3** by allowing a solution of **3** in NH_3/CH_3OH to stand overnight at 25 °C, evaporating the solvent under vacuum, and partitioning the residue between 350 mL of H_2O and 100 mL of EtOAc. The aqueous phase was extracted three times with EtOAc (100 mL), and the combined EtOAc fractions were evaporated to dryness under vacuum. The residue was recrystallized from CH_3OH–EtOAc to afford **4**: 5.88 g (92%); mp 171 °C; R_f (CH_3OH; silica gel) 0.64; IR 3400 (s), 1600 (s) cm^{-1}; NMR (Me_2SO-d_6) δ 7.08 (d, 1, J = 4.9 Hz, CH), 6.69 (d, 1, J = 4.9 Hz, CH), 6.66 (s, 2, exch, NH_2), 6.04 (d, 1, J = 4.0 Hz, $C_{1'}$ H), 5.40 (d, 1, exch, OH), 5.12–5.03 (m, 2, exch, 2 OH), 4.06–3.91 (m, 3, $C_{2'}$, $C_{3'}$, and $C_{4'}$ H), 3.65–3.60 (m, 2, $C_{5'}$ H_2); UV λ_{max} at pH 3, 235 nm (ϵ 7200), 318 (8400), 328 sh (8200), 345 sh (5300); at pH 7, 211 nm (ϵ 7700), 243 (7700), 320 (8500).

Anal. Calcd for $C_9H_{13}N_3O_5$: C, 44.44; H, 5.38; N, 17.28. Found: C, 44.00; H, 5.40; N, 16.86.

We prepared the isopropylidene derivative of **4** by dissolving a 100-mg sample of **4** in 100 mL of dry acetone and 8 mL of 2,2-dimethoxypropane containing a small quantity of *p*-toluenesulfonic acid. The solution was allowed to stir overnight; TLC (silica gel; CH_2Cl_2–CH_3OH, 1:1) indicated that the reaction was complete. We neutralized the solution by adding a saturated aqueous solution of $NaHCO_3$. The solvents were removed under reduced pressure, and the solid residue was extracted with CH_2Cl_2. The solution was reduced in volume and applied to a 1000-μm silica gel preparation TLC plate (10 cm^2), which was developed in CH_2Cl_2–CH_3OH, 9:1. The band of R_f 0.38 exhibited a UV spectrum identical with that of **4**. Elution with CH_3OH and removal of the solvent under reduced pressure afforded a pale yellow oil: NMR δ 6.95 (d, 1, J = 4.5 Hz, CH), 6.71 (s, 2, NH_2), 6.70 (d, 1, J = 4.6 Hz, CH), 6.03 (d, 1, J = 2.1 Hz, $C_{1'}$ H), 5.07 (m, 1, exch, OH), 4.91–4.72 (m, 2, $C_{2'}$ H, $C_{3'}$ H), 4.09–4.12 (m, 1, $C_{4'}$ H), 3.59 (m, 2, $C_{5'}$ H), 1.50 (s, 3, CH_3), 1.29 (s, 3, CH_3).

1,2-Diamino-4-β-D-ribofuranosylpyrazin-2-onium Mesitylenesulfonate (5). A solution of 1.92 g (7.9 mmol) of 3-amino-2-oxo-1-ribosylpyrazine (**4**) in 50 mL of $HCONH_2$ was chilled to 0 °C and then 1.72 g (8.0 mmol) *O*-mesitylenesulfonylhydroxylamine[24] was added and the solution was allowed to react at 25 °C overnight. TLC [(silica gel; CH_3OH) R_f of $RONH_2$ 0.9; R_f of **4** 0.63; R_f of **5** 0.25] indicated the reaction was complete. Ether (100 mL) was added to precipitate the product, **5**. This was collected, washed with ether, and air-dried: yield 2.80 g (77%); NMR (Me_2SO-d_6) δ 9.34 (s, 2, exch, N-NH_2), 7.44 (d, 1, J = 6.1 Hz, CH), 6.96 (d, 1, J = 6.1 Hz, CH), 6.74 (s, 2, mesityl CH), 5.88 (d, 1, J = 2.8 Hz, $C_{1'}$ H); UV λ_{max} at pH 3, 223 nm (ϵ 18 300), 315 (8500), 325 sh (8300); at pH 11 (unstable)

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225 nm (15 000), 246 (9850), 323 (7200), 335 sh (6600).

A sample for analysis was recrystallized from CH_3OH–EtOAc and dried over P_2O_5 at 65 °C under vacuum for 3 h, mp 185 °C.

Anal. Calcd for $C_{18}H_{26}N_4O_8S$: C, 47.15; H, 5.72; N, 12.21. Found: C, 47.13; H, 5.71; N, 12.19.

1,2-Diamino-4-β-D-ribofuranosylpyrazin-2-onium Chloride (6) An aqueous solution of 200 mg of the mesitylate **5** was applied to an AG-1 [Cl^-] column (28 × 250 nm) which was then eluted with H_2O to obtain the chloride salt **6**. Removal of the solvent under reduced pressure and crystallization of the residue from CH_3OH (c) afforded **6**: yield 110 mg (100%); mp >180 °C dec; R_f (silica gel; CH_3OH) 0.30; NMR (Me_2SO-d_6) δ 9.3 (s, 2, exch, NNH_2), 7.4 (d, 1, J = 6.4 Hz, CH), 7.0 (d, 1, J = 6.4 Hz, CH), 6.87 (s, 2, exch, CNH_2), 5.88 (d, 1, J = 2.8 Hz, $C_{1'}$ H), 5.56 (d, 1, exch, OH), 5.29–5.24 (m, 1, exch, OH), 3.96 (m, 3, $C_{2'}$, $C_{3'}$, $C_{4'}$ H), 3.66 (s, 2, $C_{5'}$ H_2); UV λ_{max} at pH 3, 220 nm (ε 9100), 240 sh (6200), 313 (8200), 325 sh (5100); at pH 11, 247 nm (ε 8900), 322 (6800), 335 sh (6400), 350 sh (4000).

A sample for elemental analysis was dried under vacuum at 65 °C for 3 h over P_2O_5.

Anal. Calcd for $C_9H_{15}N_4O_5Cl$: C, 36.68; H, 5.13; N, 19.01. Found: C, 36.46; H, 5.05; N, 18.65.

1-Amino-4-β-D-ribofuranosylpyrazine-2,3-dione (10). A solution of 200 mg (0.82 mmol) of the *N*-aminopyrazinium nucleoside **6** in 4 mL of 0.1 M phosphate buffer (pH 7.4) was incubated in a sealed flask at 37 °C overnight. The dark brown reaction mixture was reduced to dryness under reduced pressure. The residue was dissolved in CH_3OH, and the solution was applied to a 20 × 180 mm dry silica gel column; CH_2Cl_2–CH_3OH (1:1) eluted one component, and CH_3OH removed a small amount of unreacted **6**. The first fraction was recrystallized from CH_3OH–EtOAc: yield 100 mg (47%); mp >120 °C grad dec; IR 3400 (s), 2980 (m), 1680 (s), 1640 (s), 1600 (s) cm^{-1}; NMR (Me_2SO-d_6) δ 6.90 (d, 1, J = 6.4 Hz, CH), 6.65 (d, 1, J = 6.4 Hz, CH), 5.94 (s, 2, exch, NH_2), 5.90 (d, 1, J = 3.7 Hz, $C_{1'}$ H), 5.45 (d, 1, J = 4.3, exch, OH), 5.17–5.07 (m, 2, exch, 2 OH), 3.98–3.89 (m, 3, $C_{2'}$, $C_{3'}$, $C_{4'}$ H), 3.60 (m, $C_{5'}$ H_2); UV λ_{max} at pH 7, 219 nm (ε 9000), 240 sh (5100), 310 (6700), 323 sh (6100), 338 sh (3300).

A sample dried over P_2O_5 at 120 °C under vacuum melted and then resolidified to yellow crystals. This did not alter the NMR spectrum of the sample.

Anal. Calcd for $C_9H_{13}N_3O_6 \cdot 0.5H_2O$: C, 41.34; H, 5.10; N, 16.07. Found: C, 41.26; H, 5.04; N, 16.11.

Cytotoxicity Studies. Cytotoxicity was determined by the inhibition of L1210 murine leukemia cell growth in culture. Cells in the logarithmic phase of growth were harvested, resuspended in RPMI 1640 medium (Grand Island Biochemical Co., Grand Island, NY), 10% with respect to fetal bovine serum (Flow Laboratories, Rockville, MD), and dispensed into 16 × 125 mm culture tubes (final volume: 5.0 mL). A starting density of 17.5 × 10^4 cells/mL permitted 6–7 population doublings during 72 h. A solution (5 × 10^{-3} M) of compound was prepared in phosphate-buffered saline, pH 7.4, and filter was sterilized with a 0.22 μM Swinnex filter (Millipore Corp., Bedford, MA). Following filtration, serial dilutions were made with medium and serum before the analogue was added to the culture tubes (final concentration range: 5 × 10^{-5} to 1 × 10^{-6} M). The growth at 37 °C in control (no analogue) and drug-treated cultures was monitored every 24 h with a Model ZBI Coulter Counter (Coulter Electronics, Hialeah, FL).

Cytidine Deaminase Studies. Cytidine deaminase (cytidine aminohydrolase, EC 3.5.4.5) purified from BD_2F_1 mouse kidney was assayed both spectrophotometrically and chromatographically by separation of substrate and product on an ion-exchange column.[27] AG-50-X-8 cation-exchange resin (Bio-Rad) was used to separate labeled compounds, as described.[31] For the present studies, reaction solutions containing 0.15 mM substrate and 10 μg (0.2 unit/mg) of enzyme in a final volume of 0.4 mL of 10 mM (pH 7.6) Tris-HCl buffer containing 5 mM dithiothreitol were incubated at 37 °C for 30 min.

Acknowledgment. The authors thank Marvin J. Olsen for determining the NMR spectra. The mass spectra were performed at the Mass Spectrometric Biotechnology Resource at the Rockefeller University, New York, NY, which is supported in part by Grant RR-862-01 from the Division of Research Facilities and Resources, NIH.

Registry No. **1**, 55321-99-8; **2**, 43029-19-2; **3**, 83831-20-3; **4**, 83831-21-4; **4** (isopropylidene deriv), 83831-22-5; **5**, 83831-24-7; **6**, 83831-25-8; **10**, 83831-26-9; glyoxal sodium bisulfite, 517-21-5; aminomalonamide, 62009-47-6; 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose, 6974-32-9.

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Syntheses and Antitumor Activity of 2-Deoxyribofuranosides of 3-Deazaguanine

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Synthesis of 2-deoxyribofuranosides of 3-deazaguanine (IX–XII) has been achieved by a base-catalyzed ring closure of appropriate 2-deoxyribofuranosides of methyl 5(4)-(cyanomethyl)imidazole-4(5)-carboxalate (IV–VII). The separation of isomers and anomers were accomplished by column chromatography and HPLC. The site of glycosidic linkage and the anomeric configurations were established on the basis of C-13 and proton magnetic resonance spectroscopy, as well as UV absorption characteristics. Preliminary results of the antitumor activity of these derivatives, in vitro and in vivo, are described.

The synthesis of 3-deazaguanine[1,2] [6-aminoimidazo[4,5-*c*]pyridin-4(5*H*)-one (DG)] its ribosides[1,3] [6-amino-1-β-D-ribofuranosylimidazo[4,5-*c*]pyridin-4(5*H*)-one (9-DGR) and 6-amino-3-β-D-ribofuranosylimidazo[4,5-*c*]-pyridin-4(5*H*)-one (7-DGR)], arabinosides[4] [6-amino-1-β-D-arabinofuranosylimidazo[4,5-*c*]pyridin-4(5*H*)-one (9-araDG) and 6-amino-3-β-D-arabinofuranosylimidazo[4,5-*c*]-pyridin-4(5*H*)-one (7-araDG)], and related derivatives[5] have been described. DG and 9-DGR have been reported to exhibit a broad spectrum antiviral activity against both RNA and DNA viruses, in vitro.[5b,6] DG was also active against influenza types A and B and parainfluenza type I virus infection in mice.[5b,6] DG inhibited the growth of

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