unrestricted intake during the 3-h feeding period. Food eaten on the test (drug) day was compared with the amount consumed on the immediately preceding (control) day. Test compounds or a placebo dose of 1% methylcellulose were administered by gavage 30 min preceding presentation of the test meal. No cat was given a test compound more often than once weekly. The compounds were suspended in 1% methylcellulose prior to administration. Each compound was tested at two dose levels (0.0312 and 0.25 mg/kg) using ten cats per dose level. The compounds (±)-4, (+)-4, and (−)-4 were tested, at both doses, on three separate days.

The determination of ED_{50} and $ED_{1.5}$ was based on the dose–response relationships calculated by the regression of response on log dose in accordance with the method of Finney.[7]

Acknowledgment. Excellent technical assistance was provided by Audrey A. Morris, Terry A. Welch, Jodie C. McGuffin, Ruby Ballantine, Patricia Bunting, and Lars Flataker. Dr. W. Randall and his associates provided the analytical data, and Dr. N. Bohidar performed statistical analyses.

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1,2,4-Triazole Amino Nucleosides. 1-β-D-3′-Amino-3′-deoxyribofuranosyl-1,2,4-triazole-3-carboxamide and Related Nucleosides

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Received June 3, 1977

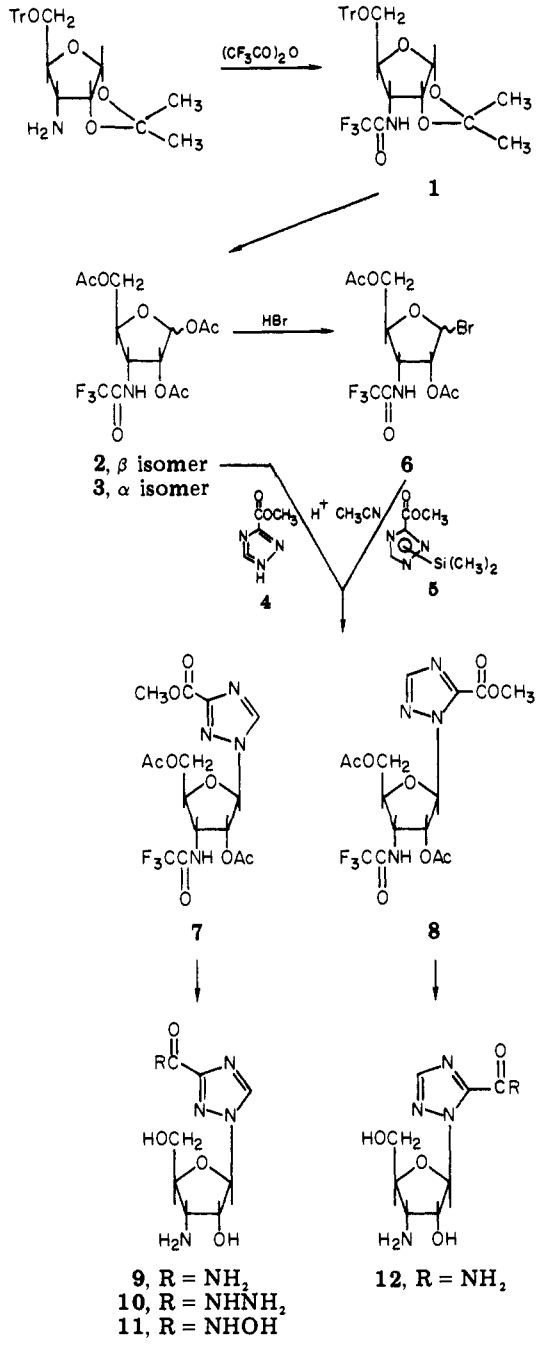
The synthesis of 1-β-D-3′-amino-3′-deoxyribofuranosyl-1,2,4-triazole-3-carboxamide—the 3′-amino analogue of ribavirin—and five related nucleoside analogues is described. Each analogue exhibited LD_{50} concentrations > 100 μg/mL against P-388 mouse lymphoid leukemia cells in tissue culture. Antiviral testing indicated that none of the compounds exhibited significant activity.

The antitumor activities of 6-dimethylamino-9-(3′-amino-3′-deoxy-β-D-ribofuranosyl)purine (puromycin aminonucleoside) and 3′-amino-3′-deoxyadenosine have generated considerable interest in the biological properties of amino nucleosides.[1] The broad-spectrum antiviral activity of 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide[2-4] (ribavirin) has stimulated a great deal of effort into the synthesis of five-membered heterocyclic nucleosides. As part of a program involving the design of chemotherapeutic nucleosides,[5] it seemed likely that the hybridization of these two nucleoside classes into one molecule should provide a new class of biologically active compounds.

Chemistry. Preparation of the appropriately blocked amino sugar was accomplished as outlined in Scheme I. The amino function of 1,2-*O*-isopropylidene-3-amino-3-deoxy-5-*O*-trityl-α-D-ribofuranose[6] was protected with the trifluoroacetyl group by treating with trifluoroacetic anhydride in pyridine. Acetolysis of **1** gave a mixture of 1,2,5-tri-*O*-acetyl-3-trifluoroacetamido-3-deoxy-β-D-ribofuranose (**2**, 51%) and the corresponding α isomer **3** (40%). The isomers were separated by crystallization of the β isomer, followed by chromatography of the filtrate to obtain the α isomer. The anomeric configurations of the sugars were determined from the anomeric proton signals in the ^1H NMR spectrum: δ 6.06 (s, β anomer), 7.16 (d, $J_{1,2}$ = 2.0 Hz, α anomer). The acid-catalyzed fusion of methyl 1,2,4-triazole-3-carboxylate[7] and **2** at 150–155 °C provided a 9:1 mixture of the two isomeric nucleosides **7** and **8** in 80% yield. The blocked nucleosides were easily separated by chromatography over silica gel. Alternatively, the condensation of the trimethylsilyl derivative **5** with 3-trifluoroacetamido-3-deoxy-2,5-di-*O*-acetylribofuranosyl bromide (**6**) in acetonitrile gave **7** and **8** in a ≃1:1 ratio. Treatment of the blocked nucleosides, **7** and **8**, with methanolic ammonia provided 1-β-D-3′-amino-3′-deoxyribofuranosyl-1,2,4-triazole-3-carboxamide (**9**)—the 3′-amino analogue of ribavirin—and the corresponding 5-carboxamide isomer **12**. The assignments of the glycosylation sites in the 3- and 5-substituted 1,2,4-triazoles were accomplished with ^1H NMR. The aromatic proton of 1,3-disubstituted 1,2,4-triazoles occurs downfield from the aromatic proton of the 1,5-disubstituted triazoles.[8] In addition, a striking difference in the ^1H NMR spectra is a large downfield shift of the anomeric proton of the 5-substituted 1,2,4-triazole nucleosides relative to the corresponding 3-substituted isomers.[8] The observed chemical shifts of δ 8.83 and 7.93 for the aromatic protons of **9** and **12**, respectively, are consistent with the predicted values. In addition, the anomeric proton of **9** occurs at higher field (δ 5.86) than the anomeric proton of **12** (δ 6.58) as predicted.[9] The 3-carboxyhydrazide **10** was prepared by reacting **7** with hydrazine overnight, and the corresponding 3-carbohydroxamic acid **11** was readily obtained with hydroxylamine.

Condensation of 3-cyano-1,2,4-triazole[10] with **2** by the fusion method provided 1-(3′-trifluoroacetamido-3′-deoxy-2′,5′-di-*O*-acetyl-β-D-ribofuranosyl)-3-cyano-1,2,4-

Scheme I

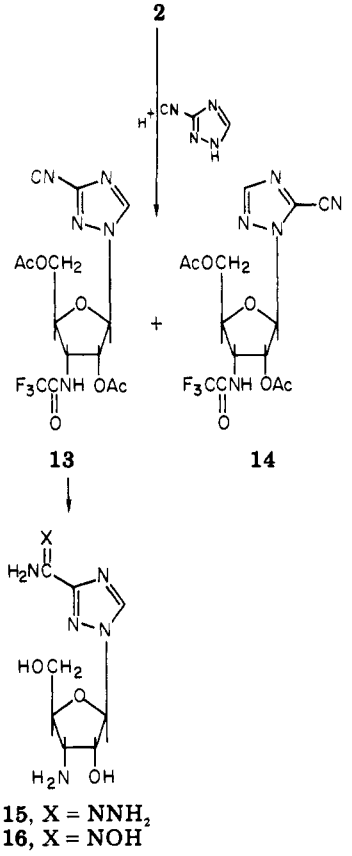


triazole (**13**) as the major product (70%) and the 1,5-disubstituted isomer **14** as a minor product (4%) (Scheme II). The ^1H NMR spectra of **13** and **14** were used for structural assignments. The unblocked nucleosides **15** and **16** were prepared by the addition of hydrazine or hydroxylamine to the cyano-substituted triazole nucleoside **13**. The assignments of β configuration for compounds **9** and **12–14** were established from the ^1H NMR signals of the anomeric protons as described previously.[9,11,12]

Biological Testing. The cytotoxicities of 1,2,4-triazole amino nucleosides, **9–12, 15, and 16**, were evaluated by growing P-388 mouse lymphoid leukemia cells in the presence of the nucleoside analogues using a method previously described.[13] Each of the analogues exhibited LD_{50} concentrations > 100 μg/mL.

The above nucleoside analogues were examined for in vitro antiviral activity against two representative DNA-containing animal viruses by the quantitative determination of their ability to inhibit virus-induced cytopathogenic effects in infected cultures. The viruses employed in these assays were Herpes simplex virus type 1 (strain HF) and vaccinia virus (strain Lederle Chorioallantoic). The procedure employed was a modification of the method of Ehrlich et al.[14] previously described by Sidwell et al.[15] The results indicate that none of the 1,2,4-triazole amino nucleosides exhibit significant antiviral activity.

Scheme II



Experimental Section

Melting points were determined on a Thomas-Hoover apparatus and are uncorrected. All evaporations were carried out under reduced pressure using a rotary evaporator with a bath temperature of <35 °C. Optical rotations were determined with a Perkin-Elmer 141 polarimeter, ^1H NMR with a Varian T-60 spectrometer using tetramethylsilane as an internal standard, IR with a Perkin-Elmer 237B spectrophotometer, and UV with a Beckman Model 25 spectrophotometer. Thin-layer chromatography was carried out on silica gel GF 250 or G 250 (Anal-Tech) plates and visualized either with UV light or developed in iodine. Microanalyses were performed by M-H-W Laboratories, Garden City, Mich., and are within ±0.4% of the calculated values.

1,2-*O*-Isopropylidene-3-trifluoroacetamido-3-deoxy-5-*O*-trityl-α-D-ribofuranose (1). To an ice-cooled and stirred solution of 1,2-*O*-isopropylidene-3-deoxy-3-amino-5-*O*-trityl-α-D-ribofuranose[6] (21.55 g, 0.05 mol) in anhydrous pyridine (150 mL) was added excess trifluoroacetic anhydride (22 mL, 0.155 mol) dropwise while maintaining the temperature at 0–3 °C. The resulting dark brown solution was stirred at room temperature for 16 h and then poured into ice cold water (600 mL). The light-brown gummy solid obtained was extracted with $CHCl_3$ (2 × 100 mL), washed with H_2O (3 × 200 mL), and dried (Na_2SO_4). The $CHCl_3$ solution was concentrated to a small volume and then applied to a column of silica gel (40 × 3.5 cm). The column was first washed with Skelly B (250 mL) and eluted with $CHCl_3$ (500 mL). Evaporation of the $CHCl_3$ fraction provided **1** as a light yellow solid (21.5 g, 81.5%). An analytical sample was prepared by crystallization once from ether–petroleum ether (bp 30–60 °C): mp 140–141 °C; $[\alpha]^{20}_D$ +40.4° (*c* 1, $CHCl_3$); IR (KBr) 3425 (NH), 1730 (C=O), 1540, 1490, and 1450 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.28

(m, 15, aromatic protons), 6.4 (d, J = 8 Hz, 1, NH), 5.83 (d, $J_{1,2}$ = 3.5 Hz, 1, C_1-H), 4.6 (t, 1, C_2-H), 4.26 (m, 1, C_3-H), 3.81 (m, 1, C_4-H), 3.33 (m, 2, C_5-H_2), 1.51 and 1.33 (2 s, 6, 2 × CH_3). Anal. ($C_{29}H_{28}F_3NO_5$) C, H, N.

1,2,5-Tri-*O*-acetyl-3-trifluoroacetamido-3-deoxy-β-D-ribofuranose (2) and 1,2,5-Tri-*O*-acetyl-3-trifluoroacetamido-3-deoxy-α-D-ribofuranose (3). To a solution of acetic anhydride (90 mL), acetic acid (90 mL), and concentrated sulfuric acid (7 mL) at 0 °C was added 10 g (19 mmol) of **1** with stirring while the temperature was maintained below 5 °C. The resulting dark brown solution was stirred at 0 °C for 8 h and at room temperature for 18 h. It was then poured over ice-cold 10% sodium acetate (700 mL) and stirred for 0.5 h at ambient temperature. The precipitated triphenylcarbinol was removed by filtration and the light yellow filtrate was extracted with $CHCl_3$ (10 × 100 mL), washed succesively with saturated $NaHCO_3$ solution (6 × 100 mL) and saturated NaCl solution (2 × 200 mL), and dried (Na_2SO_4). Evaporation of the solvent gave a light yellow oil which was crystallized from ether and gave the β-sugar **2** (3.2 g) (R_f 0.6, 10% MeOH–$CHCl_3$) as a white crystalline solid: mp 108–109 °C; $[\alpha]^{20}_D$ +16.8° (*c* 4.0, $CHCl_3$); IR (KBr) 3425 (NH), 1748, 1737, and 1700 cm^{-1} (CO); ^1H NMR ($CDCl_3$) δ 6.57 (br d, 1, NH), 6.06 (s, 1, C_1-H) 5.15 (d, $J_{2,3}$ = 5.0 Hz, 1, C_2-H), 4.76 (m, 1, C_3-H), 4.20 (m, 3, C_4-H and C_5-H_2), 2.13, 2.10, and 2.05 (3 s, 9, 3 × OAc). The mother liquor which contained mainly the α-sugar was chromatographed on a column of silica gel (100 g). Elution with $CHCl_3$ provided the α-sugar **3** (2.8 g, 39.8%, R_f 0.83, 10% MeOH–$CHCl_3$) as a light yellow oil: IR (neat) 3325, 1730 cm^{-1} (br); ^1H NMR ($CDCl_3$) δ 7.16 (d, J = 2.0 Hz, 1, C_1-H), 2.08 (m, 9, 3 × OAc). Further elution with up to 3% MeOH–$CHCl_3$ provided additional β-sugar **2**, 310 mg (total yield, 51%). Anal. (**2**, $C_{13}H_{16}F_3NO_8$) C, H, N.

1-(3′-Trifluoroacetamido-3′-deoxy-2′,5′-di-*O*-acetyl-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxylic Acid Methyl Ester (7) and 1-(3′-Trifluoroacetamido-3′-deoxy-2′,5′-di-*O*-acetyl-β-D-ribofuranosyl)-1,2,4-triazole-5-carboxylic Acid Methyl Ester (8). Method 1. A mixture of 1,2,5-tri-*O*-acetyl-3-trifluoroacetamido-3-deoxy-β-D-ribofuranose (**2**) (1.0 g, 2.30 mmol) and methyl 1,2,4-triazole-3-carboxylate[7] (376 mg, 2.36 mmol) was heated in an oil bath maintained at 130 °C until the sugar had melted. Bis(*p*-nitrophenyl) hydrogen phosphate (~40 mg) was added and heating at 150–155 °C was continued under reduced pressure for 20 min. The residue was dissolved in $CHCl_3$ and applied to a silica gel (100 g) column. Elution with 2% MeOH–$CHCl_3$ provided **8** (92 mg, 8%) as a syrup: R_f 0.55 (10% CH_3OH–$CHCl_3$); ^1H NMR ($CDCl_3$) δ 8.0 (s, 1, C_3-H), 7.18 (d, J = 8 Hz, 1, NH), 6.91 (s, 1, C_1-H), 3.96 (s, 3, OCH_3), 2.16 and 1.98 (2 s, 6, 2 × OAc). Anal. ($C_{15}H_{17}F_3N_4O_8$) C, H, N. Continued elution with 3% MeOH–$CHCl_3$ furnished **7** (836 mg, 70.5%): mp 68–70 °C; R_f 0.47 (10% CH_3OH–$CHCl_3$); ^1H NMR ($CDCl_3$) δ 8.40 (s, 1, C_5-H), 7.38 (d, J = 8 Hz, 1, NH), 6.06 (s, 1, C_1-H), 3.96 (s, 3, OCH_3), 2.16 and 2.03 (2 s, 6, 2 × OAc). Anal. ($C_{15}H_{17}F_3N_4O_8$) C, H, N.

Method 2. A solution of trimethylsilyl derivative of methyl 1,2,4-triazolecarboxylate (prepared by refluxing 753 mg, 5.92 mmol, of the triazole with excess of hexamethyl disilazane for 24 h and evaporating the excess hexamethyldisilazane under reduced pressure)[9] and 2,5-di-*O*-acetyl-3-trifluoroacetamido-3-deoxy-D-ribofuranosyl bromide (from 2.0 g, 5.39 mmol, of **2**) in dry CH_3CN (75 mL) was stirred at room temperature for 3 days. Solvent was removed under reduced pressure and the residue was dissolved in $CHCl_3$ and applied to a silica gel (100 g) column. Elution with 2% MeOH–$CHCl_3$ provided **8** (1.00 g, 41.3%) as a first product and **7** (1.2 g, 49.6%) in subsequent fractions.

1-β-D-3′-Amino-3′-deoxyribofuranosyl-1,2,4-triazole-3-carboxamide (9). A solution of **7** (500 mg, 1.14 mmol) in MeOH (50 mL) saturated at 0 °C with NH_3 was kept in a sealed flask at room temperature for 72 h. The solvent was removed under reduced pressure, the residue was treated with MeOH (5 mL), and a white crystalline product which separated was collected by filtration. Crystallization from ethanol gave pure **9** (198 mg, 71.5%): mp 177–178 °C; $[\alpha]^{20}_D$ −6.8° (*c* 1.0, H_2O); IR (KBr) 3050–3500 (br, OH, NH_2), 1710 cm^{-1} (C=O); ^1H NMR (Me_2SO-d_6) δ 8.83 (s, 1, 5-H), 7.75, 7.57 (2 s, br, 2, $CONH_2$), 5.86 (s, $W_{1/2}$ = 2 Hz, 1, C_1′-H), 4.85 (br, 1, C_2′-OH), 4.06 (dd, $W_{1/2}$ = 2 Hz, $J_{2',3'}$ = 5 Hz, 1, C_2′-H), 4.06–3.1 (m, 5, C_3′-H, C_4′-H, C_5′-H_2, and C_5′-OH); mass spectrum (20 eV, 200 °C) *m/e* (rel intensity ≥ 0.5) 244 (1.0, M + 1), 226 (0.6, $M^+ - H_2O$), 213 (0.6, M − HCHO − OH), 155 (35.9, B^+H–CH=CHOH), 132 (6.2 sugar), 113 (79.7 BH_2^+), 112 (26.6 BH^+), 85 (13.2 BH^+–HCN), 69 (84.5, B^+H_2–$CONH_2$). Anal. ($C_8H_{13}N_5O_4$) C, H, N.

1-β-D-3′-Amino-3′-deoxyribofuranosyl-1,2,4-triazole-5-carboxamide (12). A solution of **8** (360 mg, 0.82 mmol) in CH_3OH saturated with NH_3 at 0 °C was kept in a sealed pressure flask at room temperature for 72 h. The solvent was removed under reduced pressure and the residue was treated with MeOH (5 mL). A white crystalline product separated and was collected by filtration. Crystallization from ethanol gave **12** (105 mg, 52.6%): mp 193–194 °C; $[\alpha]^{20}_D$ −36.8° (*c* 1, H_2O); IR (KBr) 3100–3500 (br, OH, NH_2), 1720 cm^{-1} (sh, C=O); ^1H NMR (Me_2SO-d_6) δ 7.93 (s, 1, 3-H), 6.58 (s, 1, C_1′-H). Anal. ($C_8H_{13}N_5O_4$) C, H, N.

1-β-D-3′-Amino-3′-deoxyribofuranosyl-1,2,4-triazole 3-Carboxyhydrazide (10). Hydrazine (95%, 0.5 mL) was added to a solution of **7** (500 mg, 1.14 mmol) in EtOH (20 mL) and reaction mixture was stirred at room temperature for 40 h. The solid that separated was collected by filtration. Crystallization gave **10** (190 mg, 64.6%): mp 198–200 °C; $[\alpha]^{20}_D$ −3.8° (*c* 1.0, H_2O). Anal. ($C_8H_{14}N_6O_4$) C, H, N.

1-β-D-3′-Amino-3′-deoxyribofuranosyl-1,2,4-triazole-3-carbohydroxamic Acid (11). A solution of **7** (500 mg, 1.14 mmol) and excess of NH_2OH (1.71 g, from 3.4 g of NH_2OH·HCl and 3.5 g of KOH) in ethanol (100 mL) was refluxed with stirring for 2 h. The solvent was removed and the product **11** crystallized from aqueous ethanol (162 mg, 54%): mp 208–209 °C dec; $[\alpha]^{20}_D$ −3.9° (*c* 1.0, H_2O). Anal. ($C_8H_{13}N_5O_5$) C, H, N.

1-(3′-Trifluoroacetamido-3′-deoxy-2′,5′-di-*O*-acetyl-β-D-ribofuranosyl)-3-cyano-1,2,4-triazole (13) and 1-(3′-Trifluoroacetamido-3′-deoxy-2′,5′-di-*O*-acetyl)-5-cyano-1,2,4-triazole (14). A mixture of **2** (2.0 g, 5.39 mmol) and 3-cyano-1,2,4-triazole[9] (576 mg, 5.42 mmol) was heated with stirring in an oil bath at 130 °C. Immediately a melt was obtained and to this melt was added a catalytic amount of bis(*p*-nitrophenyl) hydrogen phosphate (~45 mg) and heating was continued at 165–170 °C under reduced pressure for 15–20 min. The residue was dissolved in $CHCl_3$ and chromatographed on a column of silica gel (30 × 3.5 cm). Elution with 2% MeOH–$CHCl_3$ provided an oil (290 mg). The oil was further purified by chromatography on 2-mm silica gel plates (Brinkman) developed with 10% MeOH–$CHCl_3$. The desired product was extracted with 20% MeOH–$CHCl_3$ and evaporation of the solvent provided **14** (90 mg, 4%) as a oil: ^1H NMR ($CDCl_3$) δ 8.21 (s, 1, 5 H), 6.4 (s, 1, C_1′-H), 5.38 (d, J = 5.0 Hz, 1, C_2′-H), 5.13 (m, 1, C_3′-H), 4.30 (m, 3, C_4′-H and C_5′-H_2), 2.21 and 2.05 (2 s, 6, 2 × OAc). Anal. ($C_{14}H_{14}F_3N_5O_6$) C, H, N.

Continued elution of the silica gel column with 3% MeOH–$CHCl_3$ gave a white crystalline product (1.6 g, 69.5%). Recrystallization from $CHCl_3$–ether gave pure **13**: mp 147–148 °C; IR (KBr) 3300 (NH), 2250 (CN) 1748, 1735 (sh), 1720 (sh, CO), 1210 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.47 (s, 1, 5-H), 6.7 (d, J = 8 Hz, 1, NH), 5.97 (s, 1, C_1′-H), 5.38 (d, J = 5.0 Hz, 1, C_2′-H), 5.13 (m, 1, C_3′-H), 4.30 (m, 3, C_4′-H and C_5′-H_2), 2.21 and 2.05 (2 s, 6, 2 × OAc). Anal. ($C_{14}H_{14}F_3N_5O_6$) C, H, N.

1-β-D-3′-Amino-3′-deoxyribofuranosyl-1,2,4-triazole-3-carboxamidrazone (15). Hydrazine (95%, 0.5 mL) was added to a solution of **13** (500 mg, 1.23 mmol) in ethanol (20 mL) and the reaction mixture was stirred at room temperature for 48 h. A solid material separated and was collected and crystallized from aqueous ethanol and gave pure **15**: 198 mg (62.4%); mp 165–166 °C dec; $[\alpha]^{20}_D$ −2.3° (*c* 1.0, H_2O). Anal. ($C_8H_{15}N_7O_3$) C, H, N.

1-β-D-3′-Amino-3′-deoxyribofuranosyl-1,2,4-triazole-3-carboxamidoxine (16). A solution of **13** (500 mg, 1.23 mmol) and excess NH_2OH (800 mg) in ethanol (50 mL) was refluxed for 2 h. More hydroxylamine (800 mg) was added and the clear reaction mixture was refluxed for another 1 h and then concentrated to about 3 mL. The solution was then cooled overnight in a refrigerator and a white crystalline product which separated was crystallized from water and yielded **16**: 255 mg (80%); mp 218–219 °C dec. Anal. ($C_8H_{14}N_6O_4$) C, H, N.

Acknowledgment. We are grateful to Drs. W. M. Shannon and G. Arnett of Southern Research Institute for antiviral testing. We also wish to thank Jay Brownell for

performing the tissue culture experiments. This investigation was supported by Grant No. CA 16623 and Career Development Award CA 2525A from the National Cancer Institute, Department of Health, Education and Welfare.

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Aminobenzoic Acid Diuretics. 9.[1] 3,4-Disubstituted 5-Acylaminobenzoic Acids and Related Compounds

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A number of 3,4-disubstituted 5-acylamino-, 5-alkylamino-, and 5-ureidobenzoic acids corresponding to previously described 3,4-disubstituted 5-sulfamoylbenzoic acid diuretics were prepared and screened for their diuretic properties in dogs. The tabulated results reveal that several 3,4-disubstituted 5-formamido and 5-acetamidobenzoic acids possess considerable diuretic potency demonstrating that a 5-sulfamoyl or 5-methylsulfonyl substituent is not a necessity for potent diuretic activity of 3,4-disubstituted benzoic acids. 4-Benzoyl-3-benzyloxy-5-formamidobenzoic acid, one of the most potent compounds of the present series, is approximately one-tenth as potent as bumetanide. The dose response and diuretic pattern indicate high-ceiling diuretic activity and suggest a mode of action similar to that of bumetanide.

In the preceding paper[1] of this series we reported that substitution of the sulfamoyl group by the sterically similar methylsulfonyl group in various 3,4-disubstituted 5-sulfamoylbenzoic acid diuretics resulted in diuretically active compounds. Furthermore, we observed that the 5-methylthio and the 5-methylsulfinyl analogues of the highly active 3-benzylamino-4-phenoxy-5-methylsulfonylbenzoic acid still exhibit significant diuretic activity. In continuation of our investigation dealing with potential substitutes for the sulfamoyl group in benzoic acid diuretics, the present paper deals with the synthesis and diuretic properties of several 3,4-disubstituted benzoic acids carrying an acylamino, a ureido, or an alkylamino substituent in the 5 position.

Chemistry. The 3,4-disubstituted 5-acylaminobenzoic acids **26**, **27**, **31–46**, and **48–53** (Table I) were provided by acylation of the corresponding aminobenzoic acids **10–25** (Scheme I). 4-Benzoyl-3-benzyloxy-5-(α-dimethylaminoacetamido)benzoic acid (**47**, Table I) was prepared from the 5-(α-chloroacetamido)benzoic acid **44**. The 5-ureidobenzoic acids **28–30** were obtained from the corresponding aminobenzoic acid **10** by reaction with isocyanic acid or alkyl isocyanate. The latter reaction performed with 5-amino-4-benzoyl-3-benzyloxybenzoic acid (**19**) resulted in the 7-carboxy-2(3*H*)-quinazolinone derivatives **54–57** (Scheme I, Table II) by in situ cyclodehydration of the N′-unsubstituted and N′-alkylated 4-benzoyl-3-benzyloxy-5-ureidobenzoic acids. The starting aminobenzoic acids **10–25** have been described as intermediates in the synthesis of the corresponding 3,4-disubstituted 5-sulfamoylbenzoic acid diuretics[2,3] or have been made available as outlined in Scheme I.

The early finding that **41** possesses potent diuretic activity prompted the preparation of the corresponding 5-*N*-alkylformamido- and 5-monoalkylaminobenzoic acids **60–63** (Table III) as shown in Scheme II. Methylation of the 5-aminobenzoic acid **19** and subsequent saponification resulted in 5-dimethylamino-4-benzoyl-3-benzyloxybenzoic acid (**64**).

Diuretic Effect and Structure–Activity Relationship. The title compounds were screened for their diuretic properties in dogs. For the 3,4-disubstituted 5-acylamino- and 5-ureidobenzoic acids **26–53**, the results are presented in Table I and compared with those of 3-*n*-butylamino-4-phenoxy-5-sulfamoylbenzoic acid (bumetanide) and 4-benzoyl-3-benzyloxy-5-sulfamoylbenzoic acid. The urinary volume and electrolyte excretion reveal that many compounds of this series exhibit considerable diuretic activity and demonstrate that the highest potency is obtained within the 3-substituted 5-formamido- and 5-acetamido-4-benzoylbenzoic acids. For 4-benzoyl-3-benzyloxy-5-formamidobenzoic acid (**41**), one of the most potent compounds, the level of potency after intravenous application is approximately one-tenth that of bumetanide. The onset of diuresis was observed within the first hour after injection and became, except for higher dosage, negligible after 3 h. The dose response and diuretic pattern of **41** indicate high-ceiling diuretic activity and suggest that substitution of the sulfamoyl group by the formamido group in 3,4-disubstituted 5-sulfamoylbenzoic acids may not influence the mode of diuretic action.

With **41**, several variants of the 5-substituent were investigated (**42–47**, Table I; **60–64**, Table III). Except for the 5-acetamidobenzoic acid **42**, exchange of the form-