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Nitrosoureidonucleosides

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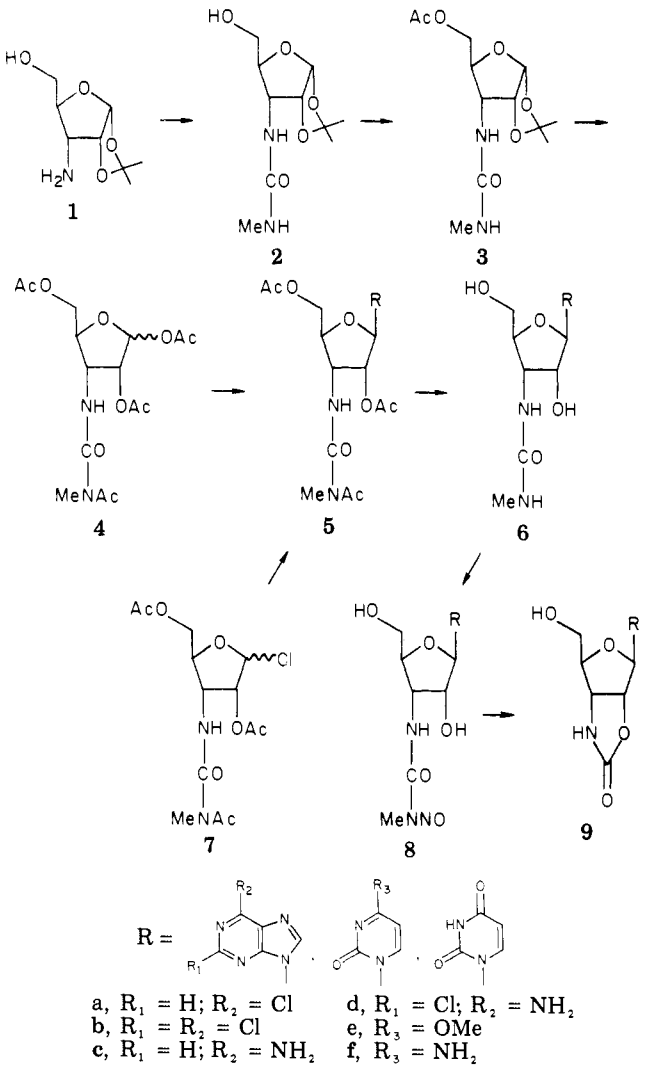
3-Deoxy-1,2-*O*-isopropylidine-3-(3-methylureido)-α-D-ribofuranose (**2**) was converted to 1,2,5-tri-*O*-acetyl-3-deoxy-3-(3-acetyl-3-methylureido)-D-ribofuranose (**4**) and the corresponding glycosyl chloride (**7**). These sugars were converted to the 3-deoxy-3-(3-methylureido)-β-D-ribofuranosyl derivatives of adenine (**6c**), 2-chloroadenine (**6d**), cytosine (**6f**), and uracil (**6g**). Nitrosation of these nucleosides gave the corresponding 3-methyl-3-nitrosoureidonucleosides **8c,d,f,g**. 5′-Amino-5′-deoxyadenosine (**10a**), 5′-amino-5′-deoxyuridine (**10b**), and 5′-amino-5′-deoxycytidine (**10c**) were all converted to the corresponding 5′-(methylureido)-5′-deoxynucleosides **15a–c** by reaction with methyl isocyanate. Nitrosation of these compounds gave the methylnitrosoureidonucleosides **16a–c**. These nitrosoureas, potential active-site-directed irreversible enzyme inhibitors, showed little cytotoxicity or activity against leukemia L1210 in vivo.

Attempts to prepare a purine or pyrimidine containing a chemically reactive function appear to date back to the work of Huber in 1956 on the synthesis of the "adenine mustard" [*N,N*-bis(2-chloroethyl)adenine].[1,2] Later, others were more successful in preparing related structures designed to combine irreversibly with the active site of purine-metabolizing enzymes.[3,4] The activity of the nitrosoureas against experimental animal neoplasms[5] then led to the preparation of purines with side chains containing a nitrosoureido function, one of which showed moderately good activity against leukemia L1210.[6]

These results and our interest in nucleosides caused us to undertake the synthesis of some nucleosides containing a nitrosoureido function in the carbohydrate moiety. Since our interest is in obtaining nucleosides whose activity might be due to their in vivo breakdown to an isocyanate capable of carbamoylating a basic center at the active site[7,8] of a nucleoside- or nucleotide-metabolizing enzyme, we chose the methylnitrosoureido group rather than the 2-chloroethylnitrosoureido group,[9] the activity of which would be more likely to be due to generation of the biologically potent 2-chloroethyldiazo hydroxide.[7,8,11-13] The methylnitrosoureas, on the other hand, show relatively low alkylating potential.[7] We chose to position the latent isocyanate moiety at the 3′ and 5′ positions of the nucleosides. Since coformycin, a nucleoside, is known to be a good inhibitor not only of adenosine deaminase but also of adenylate deaminase,[14] the 3′-deoxy-3′-methylnitrosoureidonucleosides themselves might inhibit nucleotide-metabolizing enzymes or they might be phosphorylated to the corresponding nucleotides, which in turn might be enzyme inhibitors. The 5′-[(methylnitroso)ureido]nucleosides, on the other hand, cannot be phosphorylated but might inhibit nucleotide-metabolizing enzymes by carbamoylating the basic center, usually a guanidinium group, known to be necessary for the binding of the phosphate moiety of nucleotides to the active site of the nucleotide-metabolizing enzymes.[15]

The most reasonable approach to the synthesis of nucleosides containing a 3′-(nitrosoureido) function appeared to be the synthesis of the appropriate ureido sugar for attachment to the desired purine or pyrimidine. Reaction of 3-amino-1,2-*O*-isopropylidine-α-D-ribofuranose (**1**)[16] with methyl isocyanate in ether proceeded in high yield to give

Scheme I



3-deoxy-1,2-*O*-isopropylidene-3-(3-methylureido)-α-D-ribofuranose (**2**) (Scheme I).[17] Acetylation of this compound with acetic anhydride in pyridine took place at the 5-hydroxyl only to give 5-*O*-acetyl-3-deoxy-1,2-*O*-iso-

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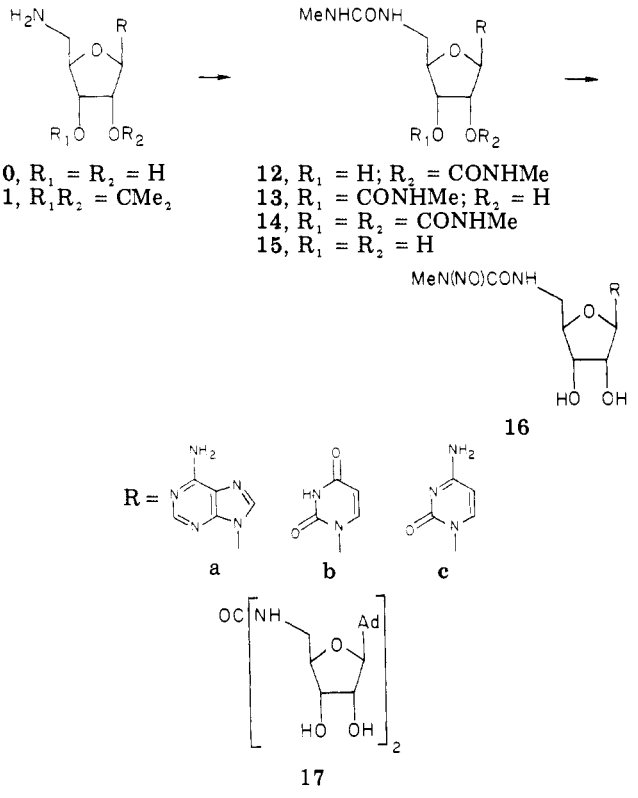
Scheme II



10, $R_1 = R_2 = H$
11, $R_1R_2 = CMe_2$
12, $R_1 = H$; $R_2 = CONHMe$
13, $R_1 = CONHMe$; $R_2 = H$
14, $R_1 = R_2 = CONHMe$
15, $R_1 = R_2 = H$
16
17

propylidene-3-(3-methylureido)-α-D-ribofuranose (**3**), which on acetolysis with acetic acid, acetic anhydride, and sulfuric acid gave 1,2,5-tri-*O*-acetyl-3-deoxy-3-(3-acetyl-3-methylureido)-D-ribofuranose (**4**), acetylation occurring on the terminal nitrogen of the methylureido group as well as on the hydroxyls of the sugar. A 50% yield of crystalline β anomer was obtained along with the syrupy α anomer (24%). Reaction of the chloro sugar **7**, prepared by the reaction of **4** and ethereal HCl, with 6-chloropurine in nitromethane containing mercuric cyanide gave a 54% yield of the β-nucleoside **5a**. Since the coupling constant of the H_1' of **5a** is 1.7 Hz, the anomeric configuration must be β.[18] Fusion of **4** with 6-chloropurine, in the absence of catalyst, gave 3′-deoxy-3′-(3-methylureido)adenosine (**6c**). Fusion of **4** with 2,6-dichloropurine also proceeded well without catalyst to give an 81% yield of β-nucleoside **5b**. That no epimerization of this ribofuranose (**4**) to the arabinofuranose occurred in these fusion reactions is somewhat surprising in view of the results obtained with 3′-acetamido-3′-deoxyribofuranoses.[19,20]

Reaction of 2,5-di-*O*-acetyl-3-(3-acetyl-3-methylureido)-3-deoxy-α-β-D-ribofuranosyl chloride (**7**) with 2,4-dimethoxypyrimidine gave the expected blocked nucleoside **5e**, which was purified by chromatography on thick silica gel plates. Treatment of **5e** with methanolic ammonia gave 1-(3-deoxy-3-methylureido-β-D-ribofuranosyl)cytosine (**6f**), purified chromatographically and identified by ^1H NMR. The methoxy group of **5e** was cleaved with HCl in $CHCl_3$ to give 1-[2,5-di-*O*-acetyl-3-(3-acetyl-3-methylureido)-3-deoxy-β-D-ribofuranosyl]uracil (**5g**), which was deacetylated with sodium methoxide to the desired 1-(3-deoxy-3-methylureido-β-D-ribofuranosyl)uracil (**6g**) isolated as its sodium salt. Since 5′-amino-5′-deoxynucleosides of both purines[21] and pyrimidines[22,23] have been prepared by displacement of the 5′-(tosyloxy) group from the intact nucleosides, we used this approach to the 5′-deoxy-5′-(methylureido)nucleosides. Reaction of 5′-amino-5′-deoxyadenosine (**10a**), prepared from 2′,3′-*O*-isopropylideneadenosine (**11a**) by the method of Jahn,[21] with methyl isocyanate gave the desired 5′-deoxy-5′-(3-methylureido)adenosine (**15a**) (Scheme II). Reaction of 5′-amino-5′-deoxyuridine (**10b**)[22] with methyl isocyanate was sluggish, and, when an excess of isocyanate was used, a mixture was formed that was resolved into two components by chromatography on silica gel plates. One was identified as a mixture of 5′-deoxy-2′-*O*-[(methylamino)carbonyl]-5′-(3-methylureido)uridine (**12b**) and its 3′ isomer **13b** (42% combined yield); the ratio of **12b** to **13b** according to ^1H NMR spectral data was 3:1. The other component was identified as 5′-deoxy-2′,3′-*O*-bis[(methylamino)carbonyl]-5′-(3-methylureido)uridine (**14b**; 16%). These compounds could be converted to the desired 5′-deoxy-5′-(methylureido)uridine (**15b**) by refluxing methanolic sodium methoxide. This problem was avoided in the preparation of 5′-deoxy-5′-(methylureido)cytidine (**15c**) by using 1 equiv of isocyanate.

Table I. Cytotoxicity to H.Ep-2 Cells and Activity against Leukemia L1210

no.	ED_{50}, μM[a]	life span T/C, %[b]
6c	>60	nt
6d	>50	nt
6f	>60	nt
8c	>60	123 (100)
8d	>50	nt
8f	>50	110 (25)
8g	>60	122 (100)
15a	>60	98 (200)
16a	40	106 (200)
16b	>50	nt
16c	>50	nt

[a] The concentration required to inhibit the growth of treated cells to 50% of untreated controls. [b] Dose in mg/kg given on day 1 only shown in parentheses; nt = not treated.

All of the methylureido nucleosides, except the cytidines **6f** and **15c**, were successfully nitrosated by the addition of solid sodium nitrite to a solution of them in dilute acetic acid, although, if the nitroso compound did not crystallize from solution, purification became something of a problem because of instability. Attempted nitrosation of **6f** and **15c** under these conditions was incomplete and gave an intractable mixture, but these compounds could be nitrosated by passing N_2O_3 gas into an aqueous solution of them. Compound **8f** crystallized as the nitrate salt, but **16c** remained in solution and, again because of instability in the reaction mixture, could only be isolated in low yield by precipitation by the addition of ethanol and ether. In all cases, the position of nitrosation was established by the coalescence of the methyl ^1H NMR signal from a doublet (due to NH splitting) to a singlet, indicating loss of the proton from that NH. In addition, heating aqueous solutions of **8c**, **8d**, and **8g** resulted in intramolecular cyclization to the urethanes **9c**, **9d**, and **9g** [isolation of the cyclic urethane from **8f** was not attempted, whereas the 5′-deoxy-5′-(3-methyl-3-nitrosoureido)adenosine gave the urea **17**].

Biologic Activity. Only one of the nucleosides was cytotoxic to H.Ep-2 cells in culture[24] (Table I). Only compound **8c** and **8g** showed slight activity against leukemia L1210[25] (Table I). This lack of biologic activity may be due to instability—the 3′-deoxy-3′-(3-methyl-3-nitrosoureido)nucleosides undergo intramolecular cyclization readily (vide supra). Lin et al.[10] found that 5′-(3-methyl-3-nitrosoureido)thymidine was only slightly toxic to L1210 cells in culture (ED_{50} = 95 μM) but that the 3′ isomer was significantly toxic (ED_{50} = 1 μM). Since the ED_{50} of 3′-amino-3′-deoxythymidine is also ca. 1 μM,[26] this

latter activity may simply be due to decomposition of the nitrosourea to the amino compound (via the isocyanate).[27]

Experimental Section

All evaporations were carried out in vacuo with a rotary evaporator. Analytical samples were normally dried in vacuo over P_2O_5 at room temperature for 16 h. Analtech precoated (250 μm) silica gel G(F) plates were used for TLC analyses; the spots were detected by irradiation with a Mineralight and by charring after spraying with saturated $(NH_4)_2SO_4$. Compounds containing amino groups were also detected with ninhydrin spray, and those containing the methylnitrosoureido function with the Greiss reagent. All analytical samples were essentially TLC homogeneous. Melting points were determined with a Mel-Temp apparatus and are not corrected. The UV absorption spectra were determined in 0.1 N HCl (pH 1), pH 7 buffer, and 0.1 N NaOH (pH 13) with a Cary 17 spectrophotometer; the maxima are reported in nm ($\epsilon \times 10^{-3}$). The integrated ^1H NMR spectra were determined with a Varian XL-100-15 spectrometer in Me_2SO-d_6 (unless otherwise specified) with tetramethylsilane as an internal reference: chemical shifts (δ) quoted in the case of multiplets are measured from the approximate center. The mass spectral data was obtained with a Varian MAT 311A mass spectrometer in the electron-impact (EI) or field-desorption (FD) mode. The high-pressure liquid chromatographic analysis was carried out with a Waters Associates ALC-242 chromatograph with an M-6000 pump and equipped with a μBondapak C_{18} column (0.25 in. × 30 cm) using H_2O–CH_3CN, 9:1, as the solvent. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values.

3-Deoxy-1,2-*O*-isopropylidene-3-(3-methylureido)-α-D-ribofuranose (2).[15] The addition of methyl isocyanate (1.88 g, 33 mmol) to a suspension of 3-amino-3-deoxy-1,2-*O*-isopropylidene-α-D-ribofuranose[14] (1; 5.67 g, 30 mmol) in anhydrous ether (150 mL) caused partial solution and then immediate formation of a precipitate. The mixture was allowed to stand for 2 h cold before the solid was removed by filtration and recrystallized from ethyl acetate: yield 6.06 g (81%); mp 155–157 °C; ^1H NMR δ 1.3 and 1.48 (2 s, isopropylidene), 2.59 (d, J_{HCNH} = 5 Hz, CH_3NH), 3.75 (m, H_4), 3.35–4.0 (m, H_3 and 2 H_5), 4.55 (m, H_2), 4.65 (t, OH), 5.75 (d, $J_{1,2}$ = 3 Hz, H_1), 5.9 (d, J_{HCNH} = 8 Hz, C_3NH), 6.2 (m, MeN*H*). Anal. ($C_{10}H_{18}N_2O_5$) C, H, N.

5-*O*-Acetyl-3-(3-methylureido)-3-deoxy-1,2-*O*-isopropylidene-α-D-ribofuranose (3). A cold solution of 3-deoxy-1,2-*O*-isopropylidene-3-(3-methylureido)-α-D-ribofuranose (2; 6.50 g, 26.4 mmol) in pyridine (3.5 mL) containing acetic anhydride (5.2 mL) was allowed to warm to ambient temperature and stand overnight before it was poured on ice (600 mL), and the resulting mixture was extracted with $CHCl_3$ (500 mL). The $CHCl_3$ extract was washed with 1 N H_2SO_4 (2 × 200 mL), saturated $NaHCO_3$ (100 mL), and H_2O (2 × 200 mL) before it was dried over $MgSO_4$. Evaporation to dryness gave 6.06 g of a white solid (70%): mp 120–121 °C; ^1H NMR δ 1.28 and 1.46 (2 s, isopropylidene), 2.0 (s, acetyl), 2.56 (d, J_{HCNH} = 4.8 Hz, CH_3NH), 3.9 (m, H_3 and 2 H_5), 4.24 (m, H_4), 4.56 (t, H_2), 5.77 (d, $J_{1,2}$ = 4 Hz, H_1), 5.94 (d, C_3 NH), 6.08 (d, MeN*H*). Anal. ($C_{12}H_{20}N_2O_6$) C, H, N.

1,2,5-Tri-*O*-acetyl-3-(3-acetyl-3-methylureido)-3-deoxy-D-ribofuranose (4). A solution of 5-*O*-acetyl-3-(3-acetyl-3-methylureido)-3-deoxy-1,2-*O*-isopropylidene-α-D-ribofuranose (3; 5.80 g, 17.6 mmol) in a mixture of glacial acetic acid (75 mL), acetic anhydride (75 mL), and H_2SO_4 (4.2 mL) was kept at 0–3 °C for 3 days before NaOAc (85 g) was added slowly with cooling and then the mixture diluted with 1 L of H_2O. The aqueous solution was extracted with $CHCl_3$, which was washed (H_2O) and dried ($MgSO_4$). The residue from removal of the $CHCl_3$ in vacuo was dissolved in ether from which it crystallized: yield of β-anomer 3.29 g (50%); mp 110–111 °C; ^1H NMR ($CDCl_3$) δ 2.08, 2.10, 2.18, and 2.30 (4 s, acetyls), 3.3 (s, N-CH_3), 4.0–4.5 (m, H_4 and 2 H_5), 4.8 (m, H_3), 5.2 (d, $J_{2,3}$ = 5 Hz, H_2), 6.13 (s, H_1), 9.6 (d, NH). Anal. ($C_{15}H_{22}N_2O_9$) C, H, N.

The ether filtrate gave an oil (1.5 g, 23%), identified as the α-anomer: ^1H NMR ($CDCl_3$) δ 2.08, 2.12, 2.17, and 2.35 (4 s, acetyls), 3.3 (s, NCH_3), 4.2–4.6 (m, H_3, H_4, and 2 H_5), 5.3 (m, H_2), 6.45 (d, $J_{1,2}$ = 3 Hz, H_1), 9.9 (d, NH).

9-[2,5-Di-*O*-acetyl-3-(3-acetyl-3-methylureido)-3-deoxy-β-D-ribofuranosyl]-6-chloropurine (5a). A. A solution of 2,5-di-*O*-acetyl-3-(3-acetyl-3-methylureido)-3-deoxy-D-ribofuranosyl chloride (7; 2.67 mmol), 6-chloropurine (412 mg, 2.67 mmol), and mercuric cyanide (765 mg, 3.04 mmol) in nitromethane (100 mL) was refluxed for 5 h before it was evaporated to dryness in vacuo. A $CHCl_3$ solution of the residue was washed with H_2O and then dried over $MgSO_4$ before evaporation to give a yellow glass (1.05 g) which was used in the next step without further purification: ^1H NMR ($CDCl_3$) δ 2.1, 2.25, and 2.35 (3 s, acetyls), 3.3 (s, N-CH_3), 4.1–5.4 (m of m, $H_{3'}$, $H_{4'}$, and 2 $H_{5'}$), 5.75 (m, $H_{2'}$), 6.15 (d, $J_{1',2'}$ = 1.7 Hz, $H_{1'}$), 8.35 and 8.76 (H_2 and H_8), 9.8 (d, NH).

B. An intimate mixture of 6-chloropurine (154 mg, 1.0 mmol) and 2,5-di-*O*-acetyl-3-(3-acetyl-3-methylureido)-3-deoxy-β-D-ribofuranose (4; 374 mg, 1.0 mmol) was fused with stirring in vacuo at 140 °C, rising to 160 °C over a 10-min period. After 20 min at 160 °C, the melt was cooled and dissolved in $CHCl_3$ (50 mL). The $CHCl_3$ solution was washed with saturated $NaHCO_3$ and then H_2O (twice) and dried ($MgSO_4$) before evaporation to dryness. The ^1H NMR spectrum of this product indicated it to be a mixture, the principal component (ca. 80%) of which is identical with that prepared by method A.

9-[2,5-Di-*O*-acetyl-3-(3-acetyl-3-methylureido)-3-deoxy-β-D-ribofuranosyl]-2,6-dichloropurine (5b). An intimate mixture of 2,6-dichloropurine (129 mg, 1 mmol) and 2,5-di-*O*-acetyl-3-(3-acetyl-3-methylureido)-3-deoxy-β-D-ribofuranose (4; 374 mg, 1 mmol) was fused with stirring in vacuo at 140 °C for 3 h before it was cooled and dissolved in $CHCl_3$ (50 mL). After the $CHCl_3$ solution was washed with saturated $NaHCO_3$ and H_2O (twice) and then dried over $MgSO_4$, it was evaporated in vacuo to give a white glass (405 mg) which was used without further purification: ^1H NMR ($CDCl_3$) δ 2.1, 2.25, 2.35 (3 s, acetyls), 3.3 (s, N-CH_3), 4.4 (m, $H_{4'}$ and 2 $H_{5'}$), 5.2 (m, $H_{3'}$), 5.7 (m, $H_{2'}$), 6.15 (d, $J_{1',2'}$ = 2 Hz, $H_{1'}$), 8.35 (s, H_8), 9.8 (d, NH).

1-[2,5-Di-*O*-acetyl-3-deoxy-3-(3-acetyl-3-methylureido)-β-D-ribofuranosyl]-4-methoxy-2(1*H*)-pyrimidinone (5e). A solution of 2,4-dimethoxypyrimidine (495 mg, 3.53 mmol) and 2,5-di-*O*-acetyl-3-(3-acetyl-3-methylureido)-3-deoxy-D-ribofuranosyl chloride (7, 3.53 mmol) in dry acetonitrile containing Linde 4Å molecular seive (2 g) was refluxed for 6 h. Another 2 g of seive was added, and reflux continued for 4 h before the mixture was filtered and the filtrate evaporated in vacuo. The yellow syrup was purified by chromatography on silica gel plates (benzene–acetone, 3:1) and eluted with MeOH: yield 595 mg; ^1H NMR ($CDCl_3$) δ 2.15, 2.2, 2.3 (3 s, acetyls), 3.25 (s, N-CH_3), 3.9 (s, OCH_3), 4–4.5 (m, $H_{4'}$ and 2 $H_{5'}$), 4.7 (m, $H_{3'}$), 5.5 (d of d, $H_{2'}$), 5.9 (m, $H_{1'}$ and H_5), 7.8 (d, $J_{5,6}$ = 7 Hz, H_6), 9.65 (d, NH).

3'-Deoxy-3'-(3-methylureido)adenosine (6c). A solution of 9-[2,5-di-*O*-acetyl-3-(3-acetyl-3-methylureido)-3-deoxy-β-D-ribofuranosyl]-6-chloropurine (5a; 368 mg, 0.78 mmol) in EtOH–NH_3 (saturated at 0 °C) was held at 70 °C for 16 h before it was cooled and evaporated to dryness in vacuo. The gummy residue was purified by chromatography on silica gel plates ($CHCl_3$–MeOH, 3:1). The eluate precipitated from MeOH: yield 77 mg (29%); mp indefinite; UV λ_{max} ($\epsilon \times 10^{-3}$) 257 nm at pH 1 (14.0), 259 at pH 7 (14.5), 260 at pH 13 (14.6); ^1H NMR δ 2.6 (d, J_{HCNH} = 3 Hz, CH_3), 3.7 and 3.9 (2 m, $H_{4'}$ and 2 $H_{5'}$), 4.3 (m, $H_{3'}$), 4.45 (m, $H_{2'}$), 5.2 (br, OH), 5.95 (d, $J_{1',2'}$ = 2 Hz, $H_{1'}$), 6.2 (m, 2 NH), 7.3 (s, NH_2), 8.2 and 8.4 (H_2 and H_8). Anal. ($C_{12}H_{17}N_7O_4 \cdot 0.5CH_3OH$) C, H, N.

2-Chloro-3'-deoxy-3'-(3-methylureido)adenosine (6d). A solution of 9-[2,5-di-*O*-acetyl-3-(3-acetyl-3-methylureido)-3-deoxy-β-D-ribofuranosyl]-2,6-dichloropurine (5b; 350 mg, 0.69 mmol) in EtOH–NH_3 (saturated at 0 °C) was heated at 70 °C for 16 h before it was evaporated to dryness in vacuo. The residue was purified by chromatography (twice) on silica gel plates ($CHCl_3$–MeOH, 3:1). The product was eluted with MeOH but failed to crystallize from either MeOH or EtOH and was isolated as a white glass: yield 125 mg (46%); ^1H NMR δ 2.55 (d, J_{HCNH} = 4 Hz, CH_3), 3.7 and 3.9 (2 m, $H_{4'}$ and $H_{5'}$), 4.4 (m, $H_{2'}$ and $H_{3'}$), 5.05 (m, 5'-OH), 5.9 (d, $J_{1',2'}$ = 2 Hz, $H_{1'}$), 6.2 (m, 2 NH and 2'-OH), 7.8 (s, NH_2), 8.4 (s, H_8). Anal. ($C_{12}H_{16}N_7O_4Cl \cdot 0.75C_2H_5OH$) C, H, N.

3'-Deoxy-3'-(3-methylureido)cytidine (6f). A solution of 1-[2,5-di-*O*-acetyl-3-deoxy-3-(3-acetyl-3-methylureido)-β-D-ribofuranosyl]-5-methoxy-2(1*H*)-pyrimidinone (5e; 425 mg, 0.97 mmol) in MeOH–NH_3 (saturated at 0 °C) was heated at 100 °C

for 16 h before evaporation to dryness in vacuo. The product was purified by chromatography on silica gel plates ($CHCl_3$–MeOH, 3:1) and by conversion to its picrate salt: yield 258 mg; mp 215–217 °C. The picrate was converted back to the free nucleoside by treatment with Dowex 1-X8 (CO_3^{2-}) in aqueous MeOH. The product was obtained from MeOH as a white glass: yield 144 mg (50%); UV λ_{max} ($\epsilon \times 10^{-3}$) 280 nm at pH 1 (13.8), 272 at pH 7 and 13 (9.36); ^{1}H NMR, δ 2.55 (d, CH_3), 3.15 (MeOH), 3.3–4.1 (m, $H_{2'}$, $H_{3'}$, $H_{4'}$, 2 $H_{5'}$), 5.0 (br s, OH), 5.7 (s, $H_{1'}$), 5.74 (d, $J_{5,6}$ = 7 Hz, H_5), 6.1 (m, 2 NH), 7.1 (broad, NH_2), 7.95 (d, $J_{5,6}$ = 7 Hz, H_6). Anal. ($C_{11}H_{17}N_2O_3 \cdot 0.75CH_3OH$) C, H, N.

3′-Deoxy-3′-(3-methylureido)uridine (6g). A solution of 1-[2,5-di-*O*-acetyl-3-deoxy-3-(3-acetyl-3-methylureido)-β-D-ribofuranosyl]-4-methoxy-2(1*H*)-pyrimidinone (**5e**; 400 mg, 0.93 mmol) in $CHCl_3$ (100 mL, saturated at 0 °C with HCl) was held at room temperature for 16 h before evaporation to dryness in vacuo. A solution of the residue in 50 mL of 0.02 N methanolic sodium methoxide was refluxed for 0.5 h, treated with Amberlite IR-120 (H) resin, and then evaporated to dryness in vacuo. The residue was dissolved in EtOH and precipitated by the addition of EtOAc: yield 190 mg (59%); UV λ_{max} ($\epsilon \times 10^{-3}$) 263 nm at pH 1 and 7 (10.8), 263 at pH 13 (8.08); ^{1}H NMR δ 2.25 (d, CH_3), 3.4–4.2 (m, $H_{2'}$, $H_{3'}$, $H_{4'}$ and 2 $H_{5'}$), 5.6 (d, $J_{5,6}$ = 7 Hz, H_5), 5.75 (d, $J_{1',2'}$ = ~1 Hz, $H_{1'}$), 6.2 (m, 2 NH of ureido group), 7.95 (d, $J_{5,6}$ = 7 Hz, H_6). Anal. ($C_{11}H_{15}N_4O_6Na$) C, H, N.

2,5-Di-*O*-acetyl-3-(3-acetyl-3-methylureido)-3-deoxy-D-ribofuranosyl Chloride (7). A solution of 1,2,5-tri-*O*-acetyl-3-(3-acetyl-3-methylureido)-3-deoxy-D-ribofuranose (4; 997 mg, 2.67 mmol) in ether (50 mL, saturated at 0 °C with HCl) containing acetyl chloride was kept at 0–3 °C for 3 days before it was evaporated to dryness in vacuo. A solution of the residue in toluene was evaporated to dryness and the process was repeated before **7** was used in the next reaction. The ^{1}H NMR of this material contained the expected peaks and showed it to be a mixture of 1α:2β.

3′-Deoxy-3′-(3-methyl-3-nitrosoureido)adenosine (8c). To a solution of 3′-deoxy-3′-(3-methylureido)adenosine (**6c**; 77 mg, 0.23 mmol) in H_2O (3 mL) containing HOAc (0.2 mL) at 0–5 °C was added solid $NaNO_2$ (160 mg, 2.3 mmol). After the solution was allowed to warm to room temperature, a precipitate formed. After overnight refrigeration, the light-yellow crystalline solid was collected, washed (H_2O), and dried: yield 17 mg (22%); mp indefinite; ^{1}H NMR δ 3.65 (m, 2 $H_{5'}$), 3.7 (s, CH_3), 4.1 (m, $H_{4'}$), 4.5 (m, $H_{3'}$), 5.7 (d of d, $H_{2'}$), 6.25 (d, $J_{1',2'}$ = 3 Hz, $H_{1'}$), 7.3 (NH_2), 8.2 and 8.35 (2 s, H_2 and H_8). Anal. ($C_{12}H_{16}N_8O_5$) C, H, N.

An attempt to recrystallize this material from water gave a new compound (TLC) identified by its mass spectrum (EI) as the cyclic urethane **9c**: MS *m/e* 292 (M^+·), 275 [(M – OH)$^+$], 262 [(M – CH_2OH + H)$^+$], 164 [(B + CH_2O)$^+$], 136 [(B + 2)$^+$], 135 [(B + 1)+].

2-Chloro-3′-deoxy-3′-(3-methyl-3-nitrosoureido)adenosine (8d). To a solution of 2-chloro-3′-deoxy-3′-(3-methylureido)-adenosine (**6d**; 100 mg, 0.25 mmol) in H_2O (3.5 mL) containing HOAc (0.23 mL) at 0–5 °C was added solid $NaNO_2$ (193 mg, 2.8 mmol). After the solution was left standing overnight at 0–5 °C the precipitate that formed was removed by filtration, washed with water, and dried: yield 101 mg (99%); mp indefinite; UV λ_{max} ($\epsilon \times 10^{-3}$) 264 nm at pH 1 and 7 (17.8), 261 at pH 13 (15.3). Anal. ($C_{12}H_{15}ClN_8O \cdot 0.75H_2O$) C, H, N.

A water solution of a small sample of **8d** was heated to boiling for a few minutes to convert it to the cyclic urethane. TLC showed the disappearance of **8d** and the appearance of a single new spot. The product (**9d**) was purified by chromatography on a silica gel plate ($CHCl_3$–MeOH, 5:1) and eluted with MeOH: ^{1}H NMR δ 3.55 (d, $J_{4',5'}$ = 4 Hz, 2 $H_{5'}$), 4.1 (m, $H_{4'}$), 4.4 (m, $H_{3'}$), 5.6 (d of d, $H_{2'}$), 6.2 (d, $J_{1',2'}$ = 2 Hz, $H_{1'}$), 7.8 (br, NH_2), 8.45 (s, H_8).

3′-Deoxy-3′-(3-methyl-3-nitrosoureido)cytidine (8f). N_2O_3 gas was bubbled into a solution of 3′-deoxy-3′-(3-methylureido)cytidine (**6f**; 50 mg, 0.17 mmol) in H_2O (1 mL) at 0–5 °C. The precipitate that formed was collected by filtration, washed with H_2O, and dried: yield 55 mg (98%); UV λ_{max} ($\epsilon \times 10^{-3}$) 278 nm at pH 1 (15.7), 268 at pH 7 (12.2), 270 at pH 13 (9.36); ^{1}H NMR δ 3.1 (s, CH_3), 3.7 (m, 2 $H_{5'}$), 4.2 (m, $H_{2'}$, $H_{3'}$, $H_{4'}$), 5–5.6 (OH, H_2O, HNO_3), 5.75 (m, $H_{1'}$, H_5), 7.2 (br, NH_2), 8.0 (d, $J_{5,6}$ = 8 Hz, H_6), 8.3 (d, NH). Anal. ($C_{11}H_{16}N_6O_6 \cdot HNO_3 \cdot 0.5H_2O$) C, H, N.

3′-Deoxy-3′-(3-methyl-3-nitrosoureido)uridine (8g). To a solution of 3′-deoxy-3′-(3-methylureido)uridine (**6g**; 190 mg, 0.59 mmol) in 2 mL of H_2O containing 0.6 mL of HOAc at 0–5 °C was added slowly $NaNO_2$ (138 mg, 2 mmol). The precipitate that formed on the addition of concentrated HCl (0.16 mL, 2 mmol) was collected by filtration, washed with H_2O, and dried: yield 180 mg (59%). A solution of part of this material (28 mg) in H_2O (10 mL) was stirred with Amberlite IR-120 (H), filtered, and freeze-dried: yield 15 mg; mp indefinite; UV λ_{max} ($\epsilon \times 10^{-3}$) 261 nm at pH 1 (14.2), 260 at pH 7 (13.8); ^{1}H NMR δ 3.1 (s, CH_3), 3.7 (m, 2 $H_{5'}$), 4.2 (m, $H_{2'}$, $H_{4'}$), 4.4 (m, $H_{3'}$), 5.2 (br, OH), 5.75 (d, $J_{5,6}$ = 8 Hz, H_5), 5.83 (d, $J_{1',2'}$ = 3 Hz, $H_{1'}$), 6.0 (m, OH), 8.0 (d, $J_{5,6}$ = 8 Hz, H_6), 8.45 (d, $C_{3'}$ NH), 10.3 (m, N_3 H). Anal. ($C_{11}H_{15}N_5O_7 \cdot 0.6H_2O$) C, H, N.

A solution of a small sample of **8g** (15 mg) in H_2O was refluxed for 20 min. TLC showed complete conversion to a new compound isolated as a crystalline solid (8 mg). The mass spectrum (EI) showed it to be the cyclic urethane (**9g**): MS *m/e* 270 [(M + 1)$^+$], 269 (M^+), 238 [(M – CH_2OH)$^+$], 225 [(M – CO_2)$^+$], 210 [(M – $NHCO_2$)$^+$], 158 (sugar$^+$), 113 [(cytosine + 1)$^+$].

5′-Deoxy-5′-(3-methylureido)adenosine (15a). The addition of methyl isocyanate (0.02 mL) to a solution of 5′-amino-5′-deoxyadenosine[21] (**10a**; 107 mg, 0.3 mmol) in DMF (1 mL) gave a precipitate that dissolved on stirring. The addition of ether produced a gum, which was purified by chromatography on silica gel plates ($CHCl_3$–MeOH, 3:1). The product was eluted with and recrystallized from MeOH: yield 27 mg (28%); mp 230–231 °C; ^{1}H NMR δ 2.5 (d, CH_3), 3.3 (m, 2 $H_{5'}$), 3.9 (m, $H_{4'}$), 4.1(m, $H_{3'}$), 4.7 (m, $H_{2'}$), 5.2 and 5.4 (2 d, OH), 5.8 (q, MeNH), 5.87 (d, $J_{1',2'}$ = 6 Hz, $H_{1'}$), 6.2 (t, CH_2NH), 7.3 (s, NH_2), 8.2 and 8.3 (H_2 and H_8). Anal. ($C_{12}H_{17}N_7O_4 \cdot 0.2H_2O$) C, H, N.

5′-Deoxy-5′-(3-methylureido)uridine (15b). A solution of 5′-amino-5′-deoxyuridine[22] (**10b**; 1.8 g, 7.4 mmol) and methyl isocyanate (844 mg, 14.8 mmol) in DMF (30 mL) was allowed to stand at room temperature for 5 h, refrigerated overnight, and then filtered and evaporated to dryness in vacuo. The syrupy mixture was resolved by chromatography on silica gel plates ($CHCl_3$–MeOH, 3:1, developed twice). Two bands were eluted with MeOH. The slower traveling band yielded 1.1 g (42%) of a syrup, a mixture of **12b** and **13b**: ^{1}H NMR of mixture δ ~2.5 (CH_3's and Me_2SO-d_5), 3–4.5 ($H_{5'}$, $H_{4'}$, $H_{3'}$ of **12b** + $H_{2'}$ of **13b** + OH's + H_2O), 4.8–5.1 (m, $H_{2'}$ of **12b** + $H_{3'}$ of **13b**), 5.61 (d, H_5 of **12b**), 5.63 (d, H_5 of **13b**), 5.79 (d, $H_{1'}$ of **13b**, $J_{1',2'} \simeq$ 6 Hz), 5.87 (d, $H_{1'}$ of **12b**, $J_{1',2'} \simeq$ 6 Hz), 6.05, 6.3, and 7.15 (carbamoyl and ureido NH's), 7.72 (d, H_6, $J_{5,6}$ = 8 Hz). The faster traveling band gave 503 g (16%) of a syrup (**14b**): ^{1}H NMR (100% Me_2SO-d_6) δ 2.53, 2.59, 2.61 (3 s, 3 CH_3), 3.35 (m, 2 $H_{5'}$), 3.99 (m, $C_{4'}$), 5.1 (m, $C_{3'}$), 5.24 (m, $C_{3'}$), 5.7 (d, $J_{5,6}$ = 8 Hz, H_5), 5.9 (d, $J_{1',2'}$ = 5 Hz, $H_{1'}$), 5.9, 6.2, and 7.15 (3 m, 4 NH), 7.75 (d, $J_{5,6}$ = 8 Hz, H_6).

A solution of a mixture of **12b** and **13b** (1.1 g) in 0.2 N methanolic NaOMe (30 mL) was refluxed for 6 h before treating with Amberlite IR-120 (H) and then evaporating to dryness: yield 940 mg. A portion (100 mg) of this material was purified by chromatography on cellulose plates (BuOH-H_2O, 6:1) followed by crystallization from EtOH: yield 41 mg (41%); mp 193–194 °C; UV λ_{max} ($\epsilon \times 10^{-3}$) 262 at pH 1 and 7 (10.0), 262 at pH 13 (7.42); ^{1}H NMR δ 3.3 (m, 2 $H_{5'}$), 3.8 (m, $H_{3'}$ and $H_{4'}$), 4.05 (t, $H_{2'}$), 4.8 (br, NH and OH), 5.65 (d of d, J_{HNCCH} = 2 Hz, $J_{5,6}$ = 8 Hz, H_5), 5.75 (d, $J_{1',2'}$ = 4 Hz, $H_{1'}$), 7.65 (d, $J_{5,6}$ = 8 Hz, H_6), 10.3 (d, NH). Anal. ($C_{11}H_{16}N_4O_6$) C, H, N.

5′-Deoxy-5′-(methylureido)cytidine (15c). A solution of 5′-amino-5′-deoxycytidine[23] (**10c**; 234 mg, 0.97 mmol) and methyl isocyanate (55 mg, 0.97 mmol) in DMF (20 mL) was held at room temperature for 1 h before evaporation to dryness in vacuo to give a colorless glass that was crystallized from MeOH: yield 240 mg (83%); MS (FD) 322 [(M + Na)$^+$], 300 [(M + 1)$^+$], 282 [(M – OH)$^+$]; UV λ_{max} ($\epsilon \times 10^{-3}$) 279 nm at pH 1 (10.6), 234 (sh) at pH 7 and 13, 272 (6.56, 7.49); ^{1}H NMR δ 2.55 (d, CH_3), 3.3 (m, 2 $H_{5'}$), 3.8 (m, $H_{3'}$, $H_{4'}$), 3.9 (m, $H_{2'}$), 5.75 (d, $H_{1'}$ and H_5), 6.05 (d, MeN*H*), 6.3 (t, CH_2N*H*), 7.25 (NH_2), 7.65 (d, $J_{5,6}$ = 7 Hz, H_6). Since this material was shown by TLC to contain two trace impurities, a small portion (90 mg) of it in H_2O (10 mL) was treated with picric acid (166 mg) in H_2O (5 mL). The resulting picrate was recrystallized from H_2O for analysis: yield 60 mg; mp 165–175 °C. Anal. ($C_{17}H_{20}N_8O_{12}$) C, H, N.

5'-Deoxy-5'-(3-methyl-3-nitrosoureido)adenosine (16a). To a solution of 5'-deoxy-5'-(3-methylureido)adenosine (**15a**; 180 mg, 0.56 mmol) in H_2O (5 mL) containing HOAc (0.5 mL) at 0–5 °C was added sodium nitrite (306 mg, 5.6 mmol). The solid that formed was removed by filtration, washed with H_2O, and dried: yield 142 mg (72%); UV λ_{max} ($\epsilon \times 10^{-3}$) 226 nm at pH 1 and 7 (17.4), 258 at pH 13 (14.7); ^1H NMR δ 3.1 (s, CH_3), 3.7 (m, 2 $H_{5'}$), 4.2 (m, $H_{3'}$ and $H_{4'}$), 4.75 (t, $H_{2'}$), 5.9 (d, $J_{1',2'}$ = 5 Hz, $H_{1'}$), 7.3 (s, NH_2), 8.15 and 8.35 (2 s, H_2 and H_8), 9.0 (t, NH). Anal. ($C_{12}H_{16}N_8O_5 \cdot 0.25H_2O$) C, H, N.

A solution of **16a** (50 mg) in H_2O was refluxed for 1 h before cooling. The precipitate that formed (**17**) was collected and dried: yield 30 mg; MS (FD) 581 [$(M + Na)^+$], 559 [$(M + 1)^+$]; ^1H NMR δ 3.4 (m, CH_2), 3.9 (m, $H_{4'}$), 4.1 (m, $H_{3'}$), 4.7 (m, $H_{2'}$), 5.23 (d, $C_{3'}$ OH), 5.45 (d, $C_{2'}$ OH), 5.9 (d, $J_{1',2'}$ = 6 Hz, $H_{1'}$), 6.2 (t, NH), 7.3 (s, NH_2), 8.2 and 8.35 (H_2 and H_8).

5'-Deoxy-5'-(3-methyl-3-nitrosoureido)uridine (16b). To a solution of 5'-deoxy-5'-(3-methylureido)uridine (**15b**; 300 mg, 1 mmol) in H_2O (5 mL) containing HOAc (0.6 mL) at 0–5 °C was added slowly $NaNO_2$ (345 mg, 5 mmol). The product was isolated by chromatography on a cellulose column (BuOH–H_2O, 6:1) and crystallized from H_2O: yield 50 mg (15%); mp 231 °C dec; UV λ_{max} ($\epsilon \times 10^{-3}$) 257 nm at pH 1 and 7 (13.3), 262 at pH 13 (7.83). Anal. ($C_{11}H_{15}N_5O_7$) C, H, N.

5'-Deoxy-5'-(3-methyl-3-nitrosoureido)cytidine (16c). N_2O_3 was bubbled in short bursts into a solution of 5'-deoxy-5'-(3-methylureido)cytidine (**15c**; 100 mg, 0.34 mmol) in H_2O (2 mL) at 5–10 °C. The addition of EtOH (10 mL) and ether (30 mL) to the cold solution caused precipitation of a hygroscopic white solid (10 mg), shown by TLC (char, Greiss test) and high-pressure LC to be pure **16c**: MS (FD) 339 [$(M + 1)^+$]; ^1H NMR δ 3.1 (s, CH_3), 3.6 (m, 2 $H_{5'}$), 3.9 (m, $H_{4'}$), 4.15 (m, $H_{2'}$, $H_{3'}$), 5.7 (d, $J_{1',2'}$ = 3 Hz, $H_{1'}$), 6.05 (d, $J_{5,6}$ = 7 Hz, H_5), 8.05 (d, $J_{5,6}$ = 7 Hz, H_6), 8.9 (m, NH).

This compound (**16c**) on warming in MeOH decomposed to a new compound that traveled slower on TLC and high-pressure LC, failed to give a Greiss test or ninhydrin test, and gave a molecular ion of 300 (FD), indicating that it is 5'-deoxy-5'-[(methoxycarbonyl)amino]cytidine.

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Antiparasitic Agents. 3.[1] Synthesis and Anthelmintic Activities of Novel 2-Pyridinyl-5-isothiocyanatobenzimidazoles

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The preparation and anthelmintic activities of a series of 2-pyridinyl-5-isothiocyanatobenzimidazoles are described. In the primary oral mouse screen, six derivatives showed 100% taeniacidal activity at 0.2% in diet. The most active member in this series, **1c**, is potentially an effective gastrointestinal nematocide in sheep at 50 mg/kg po.

In connection with an ongoing program to synthesize novel anthelmintic agents, the preparation of several 2-pyridinyl-5-isothiocyanatobenzimidazoles was undertaken. It was felt that these benzimidazoles should be capable of forming chelates similar to thiabendazole,[2] as well as be electrophilic enough to interact with sulfhydryl, hydroxy, and amino groups, and it was rationalized that this combination of properties might result in anthelmintic activity superior to that of thiabendazole.

Several investigators have reported antiparasitic properties for substituted phenyl isothiocyanates.[3] Recent studies have shown 1,4-diisothiocyanatobenzene to be an

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