Nitrosoureido Nucleosides as Potential Inhibitors of Nucleotide Biosynthesis

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Several nitrosoureido nucleosides (**3a**, **3b**, **5a**, **7a**, **7c**, and **10a**) designed as inhibitors of enzymes that metabolize pyrimidine nucleotides have been prepared and their chemical and biological properties studied. The methylnitrosoureas **3a** and **3b** were not significantly cytotoxic to H.Ep.-2 and L1210 cells in vitro but showed moderate activity in the P388 mouse leukemia screen (79% ILS for **3a** and 56% ILS for **3b**). The (chloroethyl)nitrosoureas **7a** and **7c** inhibited proliferation of L1210 cells, were cytotoxic to H.Ep.-2 cells, and demonstrated good activity against P388 in vivo (135% ILS with one 30-day survivor for **7a** and 191% ILS with two 30-day survivors for **7c**). Overnight exposure of L1210 cells to **7a** and **7c** resulted in cell enlargement accompanied by cell lysis. Macromolecular synthesis in enlarged cells, particularly RNA and protein synthesis, was markedly increased relative to that in untreated control cells. The half-lives of each of the nitrosoureas in pH 7 buffer was determined and compared with biological activity.

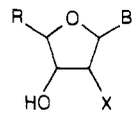
Extensive research in our laboratory has been directed toward the synthesis of nucleosides with reactive groups attached to C-5′ which can act as irreversible inhibitors of enzymes that act on the corresponding nucleotides.[1-7] The rationale for this work has been described in detail in a previous paper.[1] The nitrosoureido group has an advantage over other reactive substituents in that it can break down in solution into two highly reactive components, a diazo hydroxide and an isocyanate.[8-10] Either of these products if generated at an enzyme binding site could effectively destroy enzyme activity through alkylation or carbamoylation of a nucleophilic group on the enzyme.

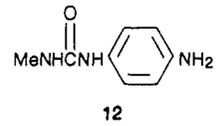
Previous papers from this laboratory have reported the synthesis and biological evaluation of nucleosides containing methyl- and cyclohexylnitrosoureido groups attached at C-3′ or C-5′ either directly or through spacers between C-5′ and the nitrosourea.[1,2,9] In this paper, the synthesis and evaluation of phenyl-, methyl-, (2-chloroethyl)-, and ethylnitrosoureas attached through a variety of spacer groups to the 5′-position of pyrimidine nucleosides are described.

The structures of nucleosides **3**, **7**, and **10** were designed so that, under physiological conditions, decomposition would occur to give an isocyanate group attached either directly or through a spacer to the nucleoside. The other product of the decomposition would be methane-, chloroethane-, or ethanediazohydroxide. The nucleoside **5** in contrast would be expected to decompose to give phenyl isocyanate and a diazohydroxide attached to the nucleoside.

N-(4-Aminophenyl)-*N*′-methylurea (**12**), required for the synthesis of **2a** and **2b**, was prepared by reaction of 1,4-phenylenediamine with 1 equiv of methyl isocyanate in chloroform. The ribofuranuronic acids **1** (**a** and **b**)[11] prepared by catalytic oxidation of thymidine and uridine were coupled with **12** in the presence of diphenylphosphoryl azide and triethylamine to give the ribofuranuronamides **2a** and **2b** in 85% and 95% yields, respectively. Nitrosation of **2a** and **2b** with sodium nitrite in 98% formic acid gave an 83% yield of **3a** and a 97% yield of **3b**. Evidence that nitrosation occurred on the urea nitrogen adjacent to the methyl group was obtained by comparison of the ^{1}H NMR spectra of **2** and **3** (**a** and **b**). The nitrosoureas showed a downfield shift of about 0.55 ppm for the *N*-methyl resonance resulting from anisotropy of the adjacent CONNO group.[12]

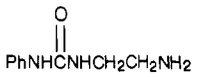
In a similar synthesis, **1a** was coupled with *N*-(2-aminoethyl)-*N*′-phenylurea[13] (**13**) to give a 42% yield of the ribofuranuronamide **4a**, which was nitrosated as above to give a quantitative yield of the nitrosourea **5a**. The position of the nitroso group in **5a** can be deduced from an examination of the ^{1}H NMR spectrum of **4a**, which showed a triplet at 6.21 ppm for the urea NH attached to the ethylene group and a triplet at 8.33 ppm for the amide NH. Assignment of the 6.21 ppm triplet to the urea NH is based on a comparison with the ^{1}H NMR spectrum of **13** in Me_2SO-d_6, which gives a triplet at 6.24 ppm for the





12

1: R = CO_2H
2: R = $MeNHCONHC_6H_4NHCO$
3: R = $MeN(NO)CONHC_6H_4NHCO$
4: R = $PhNHCONHCH_2CH_2NHCO$
5: R = $PhNHCON(NO)CH_2CH_2NHCO$
6: R = H_2NCH_2
7: R = $ClCH_2CH_2N(NO)CONHCH_2$
8: R = $ClCH_2CH_2NHCON(NO)CH_2$
9: R = $ClCH_2CH_2NHCONHCH_2$
10: R = $EtN(NO)CONHCH_2$
11: R = $OCNCH_2$



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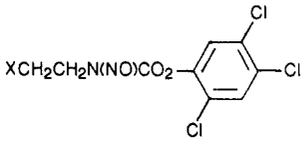

14: X = Cl
15: X = H

a: B = thymine, X = H; **b**: B = uracil, X = OH; **c**: B = uracil, X = H;

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Table I. Stability and Biological Activity of Nitrosoureido Nucleosides

compd	$T_{1/2}$,[a] h	I_{50},[b] μM		P388	
		H.Ep.-2	L1210	dose,[c] mg/kg	% ILS[d]
3a	0.4	46	>90	200	79
3b	2.0	33	>90	100	56
5a	0.7	90	>90	200	14
7a	6.0	10	5	25	135[e]
7c	4.0	14	12	25	191[f]
10a	19.0	>87	>87	200	0

[a] Half-life in pH 7 buffer determined by HPLC; the methodology is described in ref 1. [b] I_{50} is the concentration that produces 50% inhibition of proliferation of L1210 cells in suspension culture in 48 h[21] or 50% inhibition of colony formation of H.Ep.-2 cells exposed to drug over a 12-day period relative to growth in the controls.[20] [c] Single daily intraperitoneal dose on days 1–5 after implant of 10^6 P388 cells. [d] Percent increase in lifespan relative to untreated controls. [e] One 30-day survivor. [f] Two 30-day survivors.

urea NH attached to the ethylamine. Upon nitrosation of **4a**, the triplet at 6.21 ppm disappears and the single peak for phenyl NH is shifted downfield 2 ppm due to the effect of the attached CONNO group.[12] The finding that nitrosation occurred on a urea nitrogen in preference to the amide nitrogen is consistent with the known difficulty of nitrosation of amides.[1,14]

5′-*O*-Tosylthymidine[15] was converted via the 5′-azide to 5′-amino-5′-deoxythymidine[16] (**6a**). The reaction of **6a** with 2,4,5-trichlorophenyl *N*-(2-chloroethyl)-*N*-nitrosocarbamate[17] (**14**) in DMAC gave a 90% yield of the (chloroethyl)nitrosourea **7a**. In a similar synthesis, 2′-deoxy-5′-*O*-tosyluridine was converted to 5′-amino-2′,5′-dideoxyuridine[18] (**6c**), which was treated with **14** to give an 83% yield of (chloroethyl)nitrosourea **7c**. The *N*-nitroso-*N*-ethylcarbamate **15** was prepared by nitrosation of 2,4,5-trichlorophenyl ethylcarbamate[19] with nitrosyl chloride in pyridine and treated with **6a** in DMAC to give the ethylnitrosourea **10a**. This method[17] of nitrosourea synthesis gives only one isomer in contrast to direct nitrosation of unsymetrical *N*,*N*′-dialkylureas, which frequently leads to mixtures of both possible *N*-nitrosoureas.[20] A previously reported attempt to prepare **7a** by direct nitrosation of the urea **9a** gave a 2:3 mixture of **7a** and the isomeric nitrosourea **8a**.[12]

Stability Studies

Since the biological activity of nitrosoureas probably depends upon the generation of an isocyanate and a diazohydroxide under physiological conditions, a study was carried out to determine the relative stabilities of the nitrosoureas prepared and to compare the stabilities with their in vitro and in vivo anticancer activity (Table I). A series of previously prepared methylnitrosoureido and cyclohexylnitrosoureido nucleosides were biologically inactive and had half-lives in pH 7 buffer much longer than those of the active nitrosoureas *N*-methyl-*N*-nitrosourea (MNU), *N*-(2-chloroethyl)-*N*′-cyclohexyl-*N*-nitrosourea (CCNU), 2-[3-(2-chloroethyl)-3-nitrosoureido]-2-deoxy-D-glucopyranose (chlorozotocin), and *N*,*N*′-dicyclohexyl-*N*-nitrosourea (DCyNU), the half-lives of which range from 5.6 to 52.5 min.[1] We felt that synthesis of more reactive nitrosoureido nucleosides might lead to improved biological activity. The half-lives in pH 7 buffer as determined by HPLC are shown in Table I. Although the half-lives of compounds **3a**, **3b**, **5a**, **7a**, and **7c** (0.4–6 h) are less than those in the previous study[1] (8–127 h), the biological activity appears to be related more to the type of diazohydroxide produced rather than shorter half-life. The highly active (chloroethyl)nitrosoureas **7a** and **7c** have longer half-lives in pH 7 buffer than the less active and less stable methylnitrosoureas **3a** and **3b** and phenylnitrosourea **5a**. In addition, the active (chloroethyl)nitrosourea **7a** could generate chloroethanediazohydroxide as well as the isocyanate **11a**, either of which could react with nucleophilic groups at an enzyme binding site and produce the biological activity seen in Table I. Since **10a** would be expected to generate in solution the same isocyanate **11a**, its lack of biological activity could indicate that chloroethanediazohydroxide is responsible for the activity of **7a**, and the isocyanate **11a** does not play a significant role. An exact comparison cannot, however, be made because of the longer half-life of **10a**.

Biological Evaluation

Compounds **7a** and **7c** were cytotoxic to H.Ep.-2 cells[21] and inhibited the proliferation of L1210 cells in culture.[22] Compounds **3a** and **3b** inhibited colony formation of H. Ep.-2 cells but did not inhibit proliferation of L1210 cells (Table I). Effects on proliferation of L1210 cells during continuous exosure to **7a** were compared with effects of 2-h exposure to inhibitor followed by incubation of treated cells in drug-free medium. The concentration of **7a** required to produce 50% inhibition of proliferation (I_{50}) was the same (3 μg/mL) in both cases (data not shown). Similarly, H.Ep.-2 cell colony formation, a measure of cell viability, was comparably inhibited whether cells were continuously exposed to **7a** or whether they were exposed for a 2-h period and allowed to form colonies in drug-free medium. These results indicated that inhibitory effects were irreversible and were produced within 2 h.

The methylnitrosoureas **3a** and **3b** were moderately active (79% and 56% ILS) and the (chloroethyl)nitrosoureas **7a** and **7c** were very active (135% and 191% ILS) against P388 leukemia in vivo[23] (Table I). Treatment of tumor-bearing mice with compound **7a** resulted in one 30-day survivor at the highest nontoxic dose. Similar treatment with compound **7c** resulted in two 30-day survivors at each of the top two nontoxic doses. These compounds were examined in more detail with regard to their mode of action.

Biochemical Studies

Compounds **7a** and **7c** were examined for their effects on macromolecular synthesis in L1210 cells by means of published experimental procedures.[24] Synthesis of RNA, DNA, and protein were not inhibited by these agents in L1210 cells over a 4-h period. Exposure of L1210 cells to **7a** or **7c** for 18 h prior to addition of ^{3}H-labeled thymidine, uridine, or L-leucine resulted in marked increases in in-

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Table II. Effects of Nitrosoureido Compounds on Macromolecular Synthesis, Cell Size, and Cell Number in L1210 Cells in Culture

treatment		30-min incubation[a]					18-h incubation[a]				
		macromolecular synthesis (% of control)			cell size:[b]	cell no.:[c]	macromolecular synthesis (% of control)			cell size:[b]	cell no.:[c]
agent	concn, μM	RNA	DNA	Protein	peak setting	R value	RNA	DNA	protein	peak setting	R value
control	-	100	100	100	42.5	1.16	100	100	100	55	2.50
CCNU	100	101	46	54	65	1.0	246	131	314	85	0.42
	50	121	75	79	62.5	1.0	393	143	584	90	0.53
	25	106	88	81	67.5	1.02	282	139	399	90	0.48
control	-	100	100	100	57.5	1.11	100	100	100	55	2.48
7a	50	87	85	111	55	1.06	539	147	597	85	0.64
	25	88	98	99	57.5	1.08	374	171	455	80	0.92
	10	85	108	83	60	1.08	217	145	257	80	1.21
control	-	100	100	100	52.5	1.09	100	100	100	55	2.1
7c	50	87	98	96	60	1.06	554	189	658	82.5	0.54
	25	79	103	92	67.5	1.08	455	192	474	82.5	0.77
	10	80	100	95	65	1.09	276	152	254	75	0.99

[a] L1210 cells in suspension culture were incubated with inhibitor for 30 min or for 18 h prior to addition of [3H]dThd, [5-^{3}H]Urd, or [^{3}H]-L-Leu to the culture medium. Samples were taken for analysis of incorporation of radiolabeled substrates into DNA, RNA, and protein at 1-h intervals over a 4-h time period. Data presented are for the 4-h incubation period with labeled substrates. [b] Size distribution of cells within the population was determined with the Model ZH Coulter Counter. Values given are instrument settings at which the peak in cell size occurs. Higher values indicate larger cell size. [c] The number of cells present was quantitatively determined with the Coulter Counter. Cell numbers are expressed relative to the initial cell number (R = 1.0). R values < 1.0 indicate a decrease in cell number relative to the initial number; R values > 1.0 are a measure of cell proliferation.

corporation of these precursors into macromolecules. Effects of **7a** were compared with those of CCNU in L1210 cells preincubated with these inhibitors for 30 min or for 18 h prior to addition of radiolabeled substrates to the incubation medium (see Table II). Overnight incubation (18 h) of cells with each of these inhibitors resulted in significant enlargement of cells accompanied by cell lysis and a decrease in cell number relative to initial cell count. The remaining enlarged cells exhibited marked increases in the incorporation of [5-^{3}H]uridine into RNA and [4,5-^{3}H-L-leucine into protein over a 4-h period with much less pronounced increases in incorporation of [^{3}H]thymidine into DNA (Table II). Equimolar concentrations (50 μM) of CCNU and compounds **7a** and **7c** produced similar results after overnight incubation, but differences were seen in cells preincubated with these inhibitors for only 30 min prior to addition of radiolabeled substrates and subsequent analysis over a 4-h period of incorporation of these precursors into nucleic acids and protein. CCNU (100 μM) produced about 50% inhibition of DNA and protein synthesis with no evident inhibition of RNA synthesis. This effect was accompanied by cell enlargement over the 4-h incubation period, but cell lysis was not significant. Similar short-term incubation of **7a** or **7c** with L1210 cells did not produce significant inhibition of nucleic acid and protein synthesis and no significant increase in cell size was observed. Thus, CCNU soon produced moderate inhibition of DNA and protein synthesis with an increasing number of enlarged cells which, after overnight inhibition, resulted in extensive cell lysis. Similar effects were produced by BCNU in L1210 cells in vivo.[25] Compounds **7a** and **7c** did not produce early inhibition of nucleic acid and protein synthesis although overnight exposure of cells of **7a** and **7c** did result in cell lysis.

The observed marked increases in incorporation of precursors into RNA and protein after overnight incubation of cells with these inhibitors reflect the increase in the size of the cells. It should be noted that the increase in DNA synthesis, on a per cell basis, is much less pronounced. It appears that **7a** and **7c**, like CCNU, damage DNA, produce imbalances in nucleic acid and protein synthesis, and prevent cell division with disastrous consequences for the cell. We conclude that the long-term effects of **7a** and **7c** are comparable to those of CCNU.

Experimental Section

All evaporations were carried out in vacuo with a rotary evaporator or by short-path distillation into a dry ice-acetone cooled receiver under high vacuum. 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 the nitrosoureido function were also detected with the Greiss reagent. All analytical samples were homogeneous by TLC. Melting points were determined with a Mel-Temp apparatus unless otherwise specified. 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 nanometers ($\epsilon \times 10^{-3}$). The NMR spectra of compounds **3a**, **3b**, **4a**, **4b**, and **12** were determined with a Nicolet NMC NT-300NB spectrometer operating at 300.65 MHz in Me_2SO-d_6 with tetramethylsilane as an internal reference. NMR spectra of compounds **2a**, **2b**, **7a**, and **7c** were determined with a Varian XL-100-15 spectrometer operating at 100.1 MHz. Chemical shifts (δ, in ppm) quoted in the case of multiplets are measured from the approximate center. The mass spectra of **2b** and **12** were obtained with a Varian-MAT 311A mass spectrometer in the electron impact (EI) mode. The remaining mass spectra were obtained in the fast-atom-bombardment mode. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values.

1,2-Dideoxy-1-[3,4-dihydro-5-methyl-2,4-dioxo-1(2*H*)-pyrimidinyl]-*N*-[4-(3-methylureido)phenyl]-β-D-ribofuranuronamide (2a). To a cold (ice–methanol bath) solution of 1,2-dideoxy-1-[3,4-dihydro-5-methyl-2,4-dioxo-1(2*H*)-pyrimidinyl]-β-D-ribofuranuronic acid[11] (**1a**; 512 mg, 2.0 mmol) and **12** (396 mg, 4.2 mmol) in DMF (20 mL) was added diphenylphosphoryl azide (540 μL, 4.2 mmol) followed by triethylamine (340 μL, 4.2 mmol). The resulting solution was stirred at ambient temperature for 6 h and evaporated to dryness in vacuo. Trituration of the residue with methanol gave a crystalline solid: yield 685 mg (85%); mp 268–270 °C dec; MS m/z 404 $(M + 1)^+$.

The analytical sample was obtained by precipitation from DMF–MeOH: mp 270–271 °C dec; UV λ_{max} ($\epsilon \times 10^{-3}$) in pH 1, 260 (26.9), in pH 7, 261 (27.4), in pH 13, 261 (24.5); ^{1}H NMR δ 11.32 (br s, 1, H-3, exchanges with D_2O), 10.65 (s, 1, NH, exchanges with D_2O), 8.41 (s, 1, NH, exchanges with D_2O), 8.11 (s, 1, H-6), 7.54–7.29 (m, 4, aromatic CH's), 6.35 (dd, 1, H-1′, $J_{1',2'a}$ = 8 Hz, $J_{1',2'b}$ = 5 Hz), 5.94 (br q, 1, CH_3NH, J = 4 Hz, exchanges with

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D_2O), 5.64 (d, 1, 3′-OH, $J_{3',3'OH}$ = 4.4 Hz, exchanges with D_2O), 4.55–4.38 (m, 2, H-3′, H-4′), 2.62 (d, 3, CH_3NH), 2.34–2.12 (m, 2, H-2′$_a$, H-2′$_b$), 1.78 (br s, 3, 5-CH_3). Anal. ($C_{18}H_{21}N_5O_6$) C, H, N.

1-Deoxy-1-[3,4-dihydro-2,4-dioxo-1(2*H*)-pyrimidinyl]-*N*-[4-(3-methylureido)phenyl]-β-D-ribofuranuronamide (2b). To a cold (ice-methanol bath) solution of 1-deoxy-1-[3,4-dihydro-2,4-dioxo-1(2*H*)-pyrimidinyl]-β-D-ribofuranuronic acid[11] (**1b**; 258 mg, 1.0 mmol) and **12** (198 mg, 1.2 mmol) in DMF (10 mL) was added diphenylphosphoryl azide (270 μL, 1.2 mmol) followed by triethylamine (170 μL, 1.2 mmol). The resulting solution was stirred at ambient temperature for 16 h and evaporated to dryness in vacuo. The residue became a solid upon trituration with methanol: yield 365 mg (90%), mp 269–271 °C dec; MS (EI), *m/e* 405 (M^+).

The analytical sample was obtained by precipitation from DMF–MeOH: mp 279–281 °C dec; UV λ_{max} ($\epsilon \times 10^{-3}$) in pH 1, 258 (26.5), in pH 7, 258 (27.2), in pH 13, 257 (24.7); ^1H NMR δ 11.36 (d, 1, H-3, $J_{3,5}$ = 1.4 Hz, exchanges with D_2O), 10.19 (s, 1, NH, exchanges with D_2O), 8.44 (s, 1, NH, exchanges with D_2O), 8.34 (d, 1, H-6, $J_{5,6}$ = 10.2 Hz), 7.97 (s, 1, H of DMF), 7.56–7.30 (m, 4, aromatic CH's), [5.96 (q, CH_3NH) and 5.92 (d, H-1′, $J_{1',2'}$ = 5 Hz)] (2 H), 5.76 (dd, 1, H-5), [5.63 (d) and 5.60 (d)] (2 H, 2′-OH and 3′-OH, exchanges with D_2O), 4.49 (d, 1, H-4′, $J_{3',4'}$ = 2.6 Hz), 4.36–4.04 (m, 2, H-3′ and H-2′), 2.90 (s, CH_3 of DMF), 2.76 (s, CH_3 of DMF), 2.64 (d, 1, CH_3NH, J = 4.2 Hz). Anal. ($C_{17}H_{19}N_5O_7 \cdot 0.4H_2O \cdot 0.3Me_2NCHO$) C, H, N.

1,2-Dideoxy-1-[3,4-dihydro-5-methyl-2,4-dioxo-1(2*H*)-pyrimidinyl]-*N*-[4-(3-methyl-3-nitrosoureido)phenyl]-β-D-ribofuranuronamide (3a). To a cold (ice-MeOH bath) solution of **2a** (450 mg, 1.12 mmol) in 98% formic acid (50 mL) was added sodium nitrite (385 mg, 5.58 mmol). The resulting solution was stirred in the cold 1 h and evaporated to dryness in vacuo. Trituration of the residue with water gave a crystalline solid: yield 400 mg (83%); mp 203–205 °C dec; UV λ_{max} ($\epsilon \times 10^{-3}$) in pH 1, 257 (22.7), in pH 7, 257 (22.3), in pH 13, 262 (22.1), MS, *m/z* 433 $(M + 1)^+$; ^1H NMR δ 11.36 (s, 1, H-3, exchanges with D_2O), 10.65 (s, 1, NH, exchanges with D_2O), 10.27 (s, 1, NH, exchanges with D_2O), 8.11 (s, 1, H-6), 7.65 (m, 4, aromatic H's), 6.35 (dd, 1, H-1′, $J_{1',2'b}$ = 8.8 Hz, $J_{1',2'a}$ = 5.7 Hz), 5.73 (d, 1, 3′-OH, $J_{3',3'OH}$ = 4.2 Hz), 4.48 (br t, 1, H-3′), 4.42 (d, 1, H-4′, $J_{3',4'}$ = 1.3 Hz), 3.17 (s, 3, NCH_3), 2.35–2.26 (m, 1, H-2$_b$, $J_{2'b,3'}$ = 5.0 Hz, $J_{2'a,2'b}$ = 13.1 Hz), 2.18–2.12 (m, 1, H-2′$_a$, $J_{2'a,3'}$ = 1.4 Hz), 1.78 (br s, 3, 5-CH_3). Anal. ($C_{18}H_{20}N_6O_7$) C, H, N.

1-Deoxy-1-[3,4-dihydro-2,4-dioxo-1(2*H*)-pyrimidinyl]-*N*-[4-(3-methyl-3-nitrosoureido)phenyl]-β-D-ribofuranuronamide (3b). A suspension of **2b** (100 mg, 0.23 mmol) in 98% formic acid (10 mL) was heated gently to effect solution. The solution was chilled in an ice–methanol bath before addition of sodium nitrite (79 mg, 1.15 mmol). The reaction mixture was kept cold for 45 min and lyophilized. Trituration of the residue with water gave a solid: yield 97 mg (97%); mp indefinite; UV λ_{max} ($\epsilon \times 10^{-3}$) in pH 1, 256 (24.8), in pH 7, 256 (24.9), in pH 13, 259 (24.4); ^1H NMR δ 11.39 (d, 1, H-3, $J_{3,5}$ = 2.7 Hz, exchanges with D_2O), 10.65 (s, 1, NH, exchanges with D_2O), 10.39 (s, 1, NH, exchanges with D_2O), 8.31 (d, 1, H-6, $J_{5,6}$ = 8.1 Hz), 7.65 (m, 4, aromatic H's), 5.91 (d, 1, H-1′, $J_{1',2'}$ = 6.0 Hz), 5.75 (dd, 1, H-5), 5.69 (d, 1, 3′-OH, $J_{3',3'OH}$ = 4.9 Hz, exchanges with D_2O), 5.63 (d, 1, 2′-OH, $J_{2',2'OH}$ = 5.6 Hz, exchanges with D_2O), 4.49 (d, 1, H-4′, $J_{3',4'}$ = 2.8 Hz), 4.26 (ψq, 1, H-2′, $J_{2',3'}$ = 4.6 Hz), 4.15 (ψq, 1,H-3′), 3.17 (s, 3, CH_3). Anal. ($C_{17}H_{18}N_6O_8 \cdot 0.5\ H_2O$) C, H, N.

1,2-Dideoxy-1-[3,4-dihydro-2,4-dioxo-5-methyl-1(2*H*)-pyrimidinyl]-*N*-[2-(3-phenylureido)ethyl]-β-D-ribofuranuronamide (4a). To a cold (−10 °C) stirring solution of **1a** (512 mg, 1.0 mmol) and *N*-(2-aminoethyl)-*N*′-phenylurea[13] (**13**; 430 mg, 2.40 mmol) in DMF (20 mL) was added diphenylphosphoryl azide (660 mg, 2.40 mmol) followed by triethylamine (242 mg, 2.40 mmol). The resulting solution was allowed to warm to ambient temperature, stirred for 16 h, and evaporated to dryness in vacuo. The product, a syrup, was purified by chromatography on Brinkmann silica gel plates (2 mm) with $CHCl_3$–MeOH (3:1) as the developing solvent. The major band was extracted with MeOH. Removal of the MeOH in vacuo caused crystallization. The solid was recrystallized from MeOH–H_2O: yield 352 mg (42%); mp 234–235 °C dec; MS, *m/e* 418 $(M + 1)^+$; UV λ_{max} ($\epsilon \times 10^{-3}$) in pH 1, 240 (18.2), 265 (9.65), in pH 7, 240 (18.4), 265 (9.75), in pH 13, 236 (20.3), 265 (7.66); ^1H NMR δ 11.24 (br s, 1, H-3, exchanges with D_2O), 8.54 (s, 1, NHC_6H_5, exchanges with D_2O), 8.33 (br t, 1, CHCONH, exchanges with D_2O), 8.11 (q, 1, H-6, J = 1.0 Hz), 7.38 (d, 2, ortho H's of phenyl), 7.02 (t, 2, meta H's of phenyl), 6.38 (t, 1, para H of phenyl), 6.32 (dd, 1, H-1′, $J_{1',2'a}$ = 5,6 Hz, $J_{1',2'b}$ = 9.0 Hz), 6.21 (br t, 1, $CH_2NHCONH$, exchanges with D_2O), 5.61 (d, 1, 3′-OH, $J_{3',3'OH}$ = 3.8 Hz, exchanges with D_2O), 4.35 (m, 1, H-3′), 4.21 (d, 1, H-4′, $J_{3',4'}$ = 1.3 Hz), 3.20 (m, 4, CH_2CH_2), 2.16 (m, 1, H-2′$_b$, $J_{2'a,2'b}$ = 13.1 Hz, $J_{2'a,3'}$ = 5.1 Hz), 2.05 (m, 1, H-2′$_a$, $J_{2'a,3'}$ = 1.3 Hz), 1.78 (d, 1, CH_3). Anal. ($C_{19}H_{23}N_5O_6$) C, H, N.

1,2-Dideoxy-1-[3,4-dihydro-2,4-dioxo-5-methyl-1(2*H*)-pyrimidinyl]-*N*-[2-(1-nitroso-3-phenylureido)ethyl]-β-D-ribofuranuronamide (5a). A cold (0 °C) solution of **4a** (100 mg, 0.24 mmol) in 98% formic acid (1 mL, 98–100%) was treated portionwise with sodium nitrite (100 mg, 1.44 mmol) kept in the cold for 1 h, and poured directly onto a flash column of silica gel 60 (230–400 mesh, 25 g) and developed with $CHCl_3$–MeOH (97:3). The fractions containing product were pooled and evaporated to dryness in vacuo. An ethyl acetate solution of the residue was dried over $MgSO_4$ and evaporated to dryness in vacuo. The residue was a hygroscopic solid: yield 118 mg (100%); mp indefinite; MS, *m/z* 447 $(M + 1)^+$; UV λ_{max} ($\epsilon \times 10^{-3}$) in pH 1, 265 (14.1), in pH 7, 265 H-2′), in pH 13, 230 (21.0), 265 (8.89); ^1H NMR δ 11.31 (s, 1, H-3, exchanges with D_2O), 10.57 (s, 1, NHC_6H_5), 8.30 (t, 1, $NHCH_2$, J = 5.6 Hz), 7.99 (s, 1, H-6), 7.70 (d, 2, ortho H's of phenyl), 7.35 (t, 2, meta H's of phenyl), 7.13 (t, 1, para H of phenyl), 6.31 (dd, 1, H-1′, $J_{1',2'a}$ = 9.0, $J_{1',2'b}$ = 5.3), 5.58 (d, 1, 3′-OH, $J_{3',3'OH}$ = 3.8), 4.33 (m, 1, H-3′), 4.12 (d, 1, H-4′, $J_{3',4'}$ = 0.7), 4.03 (q, 2, CH_2 of EtOAc), 3.97 (m, 2, CH_2CH_2NNO), 3.24 (q, 2, CH_2CH_2NH), 2.12–1.97 (m, 2 H-2′), 1.99 (s, CH_3CO of EtOAc), 1.76 (s, 3, 5-CH_3), 1.18 (t, CH_3CH_2 of EtOAc). Anal. ($C_{19}H_{22}N_6O_7 \cdot 0.8\ H_2O \cdot 0.3$EtOAc) C, H, N.

5′-[[[(2-Chloroethyl)nitrosoamino]carbonyl]amino]-5′-deoxythymidine (7a). A solution of 5′-amino-5′-deoxythymidine[16] (**6a**; 210 mg, 0.871 mmol) in DMAC (15 mL) at 0 °C was treated with 2,4,5-trichlorophenyl *N*-(2-chloroethyl)-*N*-nitrosocarbamate[17] (**14**; 292 mg, 0.880 mmol), stirred at 25 °C for 30 min, and evaporated to dryness at 25 °C under high vacuum. The residue was triturated with ether (20 mL) to give a white powder, which was collected, washed with ether, and dried: yield 295 mg (90%); mp ca. 136 °C dec (Kofler-Heizbank); MS, *m/z* 376 $(M + 1)^+$; UV λ_{max} ($\epsilon \times 10^{-3}$) in pH 1, 262 (12.0), in pH 7, 262 (11.7), in pH 13, 265 (8.19); ^1H NMR δ 11.25 (s, 1, H-3, exchanges with D_2O), 8.88 (t, 1, NH), 7.52 (m, 1, H-6), 6.16 (t, 1, H-1′, $J_{1',2'}$ = 7.0 Hz), 5.32 (d, 1, 3′-OH, $J_{3',3'OH}$ = 4.0 Hz), [4.26 (m, H-3′), 4.11 (t, CH_2NNO), 3.99 (m, H-4′)] (4 H), 3.58 (m, 4, H-5′, CH_2Cl), 2.11 (m, 2, H-2′), 1.80 (s, 3, Me). Anal. ($C_{13}H_{18}ClN_5O_6$) C, H, N.

5′-[[[(2-Chloroethyl)nitrosoamino]carbonyl]amino]-2′,5′-dideoxyuridine (7c). A solution of **6c**[18] (200 mg, 0.881 mmol) in DMAC (13 mL) at 0 °C was treated with **14** (295 mg, 0.889 mmol), stirred at 25 °C for 30 min, and evaporated at 25 °C under high vacuum to a syrup. The syrup was triturated with ether (20 mL) to give a white powder, which was collected, washed well with ether, and dried: yield 271 mg (83%); mp ca. 136 °C (Kofler-Heizbank); elemental analysis indicated the presence of a trace of DMAC, which was confirmed by ^1H NMR; MS, *m/z* 362 $(M + 1)^+$; UV λ_{max} ($\epsilon \times 10^{-3}$) in pH 1, 257 (12.2), in pH 7, 257 (12.2), in pH 13, 260 (6.9); ^1H NMR δ 11.30 (s, 1, H-3), 8.89 (t, 1, NH), 7.70 (d, 1, H-6, $J_{5,6}$ = 7.9 Hz), 6.14 (ψt, 1, H-1′, $J_{1',2'}$ = 7.0 Hz), 5.60 (d, 1, H-5), 5.34 (br s, 1, 3′-OH), [4.25 (m, H-3′), 4.11 (t, CH_2NO), 3.95 (m, H-4′)] (4 H), [3.58 (t, CH_2Cl), 3.56 (m, H-5′)] (4 H), 2.14 (m, 2, H-2′), 2.95, 2.79, 1.96 (DMAC). Anal. ($C_{12}H_{16}ClN_5O_6 \cdot 0.18Me_2NAc$) C, H, N.

5′-Deoxy-5′-[[(ethylnitrosoamino)carbonyl]amino]thymidine (10a). A stirred solution of 2,4,5-trichlorophenyl ethylcarbamate[19] (4.89 g, 18.2 mmol) at −20 °C was treated dropwise with a cold solution of nitrosyl chloride (3.92 g, 59.9 mmol) in pyridine (15 mL), stirred at −20 °C for 30 min, and poured slowly into ice water (1.5 L) with stirring. The white crystalline 2,4,5-trichlorophenyl *N*-ethyl-*N*-nitrosocarbamate (**15**) was collected, washed with cold water, and dried: yield 4.8 g, mp 60 °C (Kofler-Heizbank). This product, which contained unnitrosated urea (^1H NMR), was used without further purification for the preparation of **12**. A solution of **6a** (241 mg, 1.00 mmol) in DMAC (15 mL) was cooled in an ice bath, treated with **15** (313

mg), stirred at 25 °C for 30 min, and evaporated to a syrup under high vacuum. The syrup was triturated with ether (20 mL) to give a solid, which was collected and extracted with $CHCl_3$ (6 × 100 mL). The $CHCl_3$ extract was evaporated to dryness and the residue triturated with ether, collected, and dried: yield 210 mg (62%); MS, m/z 342 $(M + 1)^+$; UV λ_{max} ($\epsilon \times 10^{-3}$) in pH 1, 263 (13.2), in pH 7, 262 (13.2), in pH 13, 266 (7.94); ^1H NMR δ 11.29 (s, 1, H-3), 8.82 (t, 1, NH), 7.51 (s, 1, H-6), 6.15 (ψq, 1, H-1', $J_{1',2a'}$ = 6.1, $J_{1',2b'}$ = 7.9), 5.33 (d, 1, 3'-OH), 4.24 (m, 1, H-3'), 3.93 (m, 1 H-4'), 3.77 (q, 2, CH_2NNO), 3.52 (t, 2, H-5'), 2.10 (m, 2, H-2'), 1.79 (s, 3, 5-Me), 0.91 (t, 3, CH_3CH_2). Anal. ($C_{13}H_{19}N_5O_6$) C, H, N.

***N*-(4-Aminophenyl)-*N'*-methylurea (12).** A solution of methyl isocyanate (5.7 g, 0.1 mol) in anhydrous chloroform (150 mL) was added dropwise to a stirred solution of 1,4-phenylenediamine (10.8 g, 0.1 mol) in anhydrous chloroform (350 mL). The resulting precipitate was collected and washed with chloroform: yield 12.5 g (75%); mp 146–148 °C.

The analytical sample was obtained from a similar reaction by recrystallization from chloroform: mp 147–148 °C; MS, m/z 165 (EI) (M^+); ^1H NMR δ 7.87 (s, 1, NH, exchanges with D_2O), 6.98 (d, 2, aromatic H ortho to NH), 6.45 (d, 2, aromatic H ortho to NH_2), 5.73 (q, 1 CH_3NH, J = 4.7 Hz), 4.65 (s, 2, NH_2, exchanges with D_2O), 2.59 (d, 3, CH_3, J = 4.7 Hz). Anal. ($C_8H_{11}N_3O$) C, H, N.

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Preparation and Antischistosomal and Antitumor Activity of Hycanthone and Some of Its Congeners. Evidence for the Mode of Action of Hycanthone

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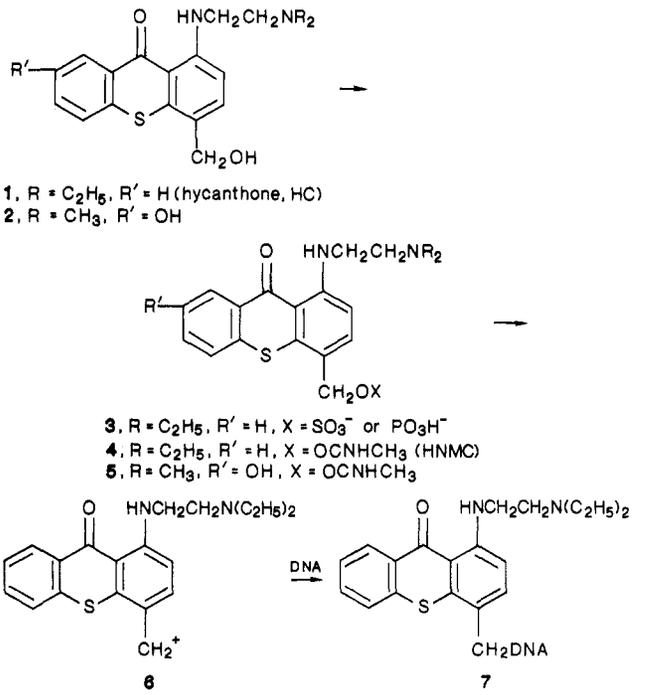
The synthesis of a series of esters of hycanthone (HC) and 7-hydroxyhycanthone, their antitumor activity, and their antischistosomal effects on HC-sensitive and HC-resistant schistosomes are reported. Binding studies using tritium-labeled HC and hycanthone *N*-methylcarbamate (HNMC) with calf thymus DNA provided evidence that HNMC but not HC alkylated the DNA. Tritiated HNMC also bound to the DNA of intact HeLa cells exposed to the drug while very little tritiated HC bound to DNA under the same conditions. The mechanism proposed previously[10] to account for the antischistosomal action of HC, namely, drug esterification followed by alkylation of DNA, applies also to the antitumor action of the drug as shown in Scheme I.

Hycanthone (HC) (1) is a schistosomicidal[1,2] and antitumor agent.[3] Its mutagenicity[4] proscribed its clinical use as a one-dose treatment for *Schistosoma mansoni* and *Schistosoma haemotobium* infections. Several years ago Hartman and Hulbert suggested a mechanism without any experimental support to account for the mutagenic properties of HC.[4] Elsewhere we proposed a slight variant of their mechanism to account for the antischistosomal action of hycanthone as shown in Scheme I.[5]

In this mechanism HC is enzymically esterified in the target cell to the ester **3**, which we propose is either a sulfate or phosphate. This ester, which now possesses a good leaving group, dissociates nonenzymically to give **6**, which alkylates DNA to give **7**. In this paper we describe the preparation and some biological properties of a group of esters derived from HC and one of its congeners and present experimental evidence that supports the view that the mechanism in Scheme I not only accounts for the schistosomicidal properties of HC but for its antitumor action as well.

Scheme I



Chemistry

The substituted carbamate esters **23–25** were prepared from HC and the appropriate substituted isocyanates. The methyl carbonate ester **26** was prepared by treatment of HC with methyl chloroformate. The 3,5-dinitrobenzoate **28** was a gift from the Sterling-Winthrop Research Institute. Since treatment of the known thioxanthenone **2** with CH_3NCO furnished the biscarbamate, the procedure shown in Scheme II was used to prepare the carbamate **5**.

Condensation of *p*-methoxybenzenethiol (8) with 2,6-dichlorobenzonitrile (**9**) gave the desired nitrile **10** accom-

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