5.5, KCl 4.7, $MgSO_4$ 1.2, $NaH_2PO_4$ 1.3, $CaCl_2$ 2.5, and $NaHCO_3$ 25.0. The bath medium was bubbled with a gas mixture of 95% $O_2$–5% $CO_2$ and maintained at a temperature of 37 °C with a constant-temperature bath (Haake). The resting tension on the muscle segments was 1 g and isometric contractions were measured with a force-displacement transducer (Grass FT-03) connected to a polygraph (Grass Model 7C). An electric stimulus of supramaximal voltage (ca. 50 V) for 5 ms at 10 Hz was used. Tubocurarine chloride (Abbott) was added to the bath to obtain a concentration of 0.03 mg/mL. The preparation was allowed to stabilize for 30–60 min before experimentation started.

Due to the limited solubility of dantrolene sodium in aqueous solutions, increased drug concentrations were achieved by dissolving all drugs in $Me_2SO$. Stock solutions of the drugs containing either 3 or 6 mg/mL (where applicable) were diluted with $Me_2SO$ so that the addition of 0.07 (for the 6 mg/mL) or 0.13 mL (for the 3 mg/mL) to the bath would give final drug concentrations of 0.15, 1.5, 5, or 15 mg/L, and in the case of the 6 mg/mL concentrations the addition of 0.13 mL would give a final drug concentration of 30 mg/L. This bath concentration of $Me_2SO$, 0.52%, was found to be a no-effect level similar to that reported by Sams et al.[18] Higher concentrations of the drugs were achieved by increasing the concentration of $Me_2SO$ (up to 4%). Control experiments using $Me_2SO$ at comparable volumes were also conducted. The effects of solvent and the drugs were measured 5 min after addition to the bath; at this time a steady state of drug effect had been reached. Between doses of drugs or solvent the tissues were washed continuously for 1 min with the drug-free bathing medium. The effect measured was the percent decrease in the twitch response.

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# Antineoplastic Agents. 1. Synthesis and Antineoplastic Activities of Chloroethyl- and Methylnitrosourea Analogues of Thymidine

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A new class of chloroethyl- and methylnitrosourea analogues of thymidine, **5a,b**, **6**, **10**, and **11**, has been synthesized from the corresponding amino nucleosides, **2** and **7**. The 3′-chloroethyl and 3′-methyl derivatives, **10** and **11**, inhibited L1210 cell growth in culture ($ED_{50}$ = 1.5 and 1.0 μM, respectively) more effectively than 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) ($ED_{50}$ = 4 μM) and the 5′-nitrosourea analogues. Neither the alkylating nor the carbamoylating activities of these compounds correlated with their biological activity.

Important aspects of the clinical utility and pharmacology of the nitrosoureas as antineoplastic agents have been recently reviewed.[1] A current evaluation of the mechanism of action of these compounds cites the importance of the N and N′ substituents on their chemical and biological properties.[2] In particular, it has been suggested that therapeutic effectiveness might be maximized in drugs possessing high alkylating and low carbamoylating activities.[3] The D-glucopyranose derivatives, streptozotocin and chlorozotocin, support this hypothesis. They show reduced bone marrow toxicity[4,5] and reduced carbamoylation potential,[2] a critical finding since myelosuppression is the dose-limiting effect of the clinically useful nitrosoureas.[6,7]

The development of aminonucleosides in our laboratory as potential antiviral and antineoplastic agents[8-10] has afforded the opportunity to synthesize the corresponding nitrosourea analogues. Altered pharmacological properties, such as selective uptake or metabolism, and favorable changes in their chemical reactivity may increase the therapeutic value of these compounds over existing drugs. This report describes the synthesis, the alkylating and carbamoylating activities, and the cytotoxicity of several nitrosourea derivatives of 3′-amino and 5′-amino analogues of thymidine.

**Chemistry.** The synthesis of a new class of chloroethyl- and methylnitrosourea analogues of thymidine (**5**, **6**, **10**, and **11**) is outlined in Scheme I. The key intermediates, 5′-amino-5′-deoxythymidine (**2**)[11] and 3′-amino-3′-deoxythymidine (**7**),[12,13] were prepared according to the procedure reported by Horwitz and co-workers with minor modification. Compound **7** was isolated as the free base instead of the hydrochloride salt.[12] Compounds **2** and **7** were converted to the corresponding urea derivatives, **3**,

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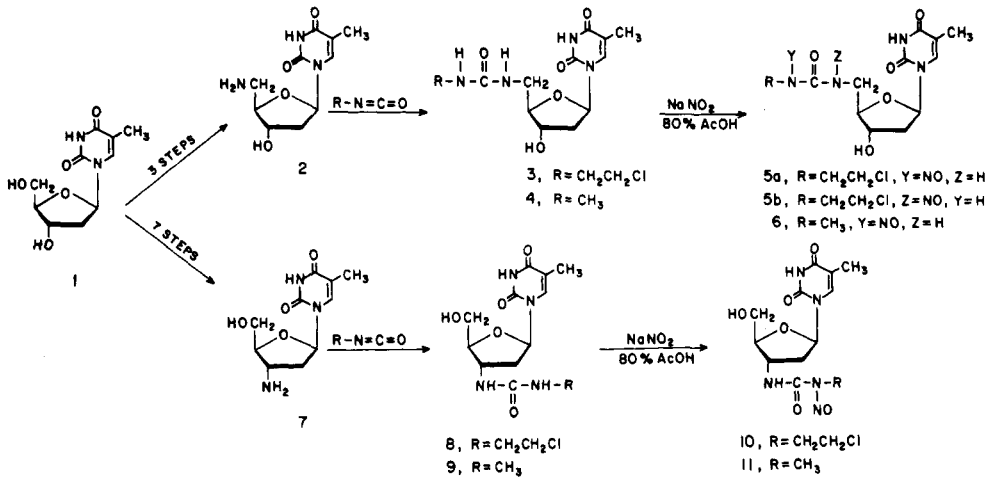
Scheme I




4, 8, and 9, by reacting with either chloroethyl or methyl isocyanate.[14,15] These urea derivatives were then allowed to react with sodium nitrite[14] in dilute acetic acid solution at 0 °C to afford the desired nitrosourea analogues of thymidine, **5**, **6**, **10**, and **11**. Compounds **5**, **6**, and **11** were isolated by crystallization from methanol or ethanol. Compound **10** was purified by preparative TLC using chloroform–ethanol (3:1 v/v) as the eluting solvent.

The NMR spectra of the nitrosourea analogues and several key intermediates provide useful information concerning the structure and identity of these compounds. The NMR spectrum of nitrosourea **5** suggests that the sample is a mixture of two isomers, **5a** and **5b**, in a ratio of 2:3 (estimated by NMR integral ratios). This is analogous to the findings reported by Johnston and coworkers[16] that nitrosation of 1-(2-bromoethyl)-3-(2-chloroethyl)urea gave a mixture of two isomers, 1-(2-bromoethyl)-3-(2-chloroethyl)-1-nitrosourea and 1-(2-chloroethyl)-3-(2-bromoethyl)-1-nitrosourea, in approximately equal parts (estimated by NMR integral ratios). In another case, nitrosation of 1-(2-chloroethyl)-3-phenylurea afforded an isomeric pair of 1-(2-chloroethyl)-1-nitroso-3-phenylurea and 3-(2-chloroethyl)-1-nitroso-1-phenylurea in a ratio of 3:2, respectively (estimated by NMR integral ratios).

Attempts to separate isomers **5a** and **5b** by TLC with various solvent systems (e.g., $CHCl_3$–EtOH, EtOAc–EtOH, *n*-BuOH–HOAc–$H_2O$) were unsuccessful. However, **5b** was isolated from the mother liquor and purified by repeated recrystallization from ethanol. The NMR spectroscopy data of **5b** are consistent with the assigned structure.

In the case of the other nitrosourea analogues of thymidine only one isomer, **6**, **10**, or **11**, was isolated.

Other nitrosourea analogues, of pyrimidine and purine ribo-, deoxyribo-, arabino-, xylo-, and lyxonucleosides have been or are in the process of being synthesized and their biological activities are under investigation. The results will be published elsewhere.

**Biological Activity.** The chemical reactivities and the cytotoxicity of the nucleoside nitrosoureas and 1,3-bis-(2-chloroethyl)-1-nitrosourea (BCNU) were compared. The procedures of Wheeler et al.[3] were used to determine alkylating and carbamoylating activities. The carbamoylation assay was modified such that the final reaction mixture of 150 μL contained 5 μmol of [$^{14}$C]lysine (specific activity = 200 μCi/mmol) and 5 μmol of the nitrosourea dissolved in 50 μL of dimethyl sulfoxide. The reaction products were separated by paper electrophoresis[17] and subsequently quantified by liquid scintillation spectrometry. Excessive impurities in [$^{14}$C]lysine (Schwartz/Mann, sp act. = 312 mCi/mmol) were removed by electrophoresis, prior to running the assay. The effect of the compounds on the growth of L1210 cells in vitro was used as a measure of cytotoxicity. Suspension cultures were grown in Fischer's media supplemented with 10% horse serum at 37 °C. After addition of the nitrosoureas (72 h), cell numbers were determined in two independent samples, in duplicate, using a Coulter counter for each concentration tested. $ED_{50}$ values were estimated from dose–response curves compiled from at least three separate experiments and represent the drug concentration needed to inhibit cell growth by 50%.

Table I. Alkylating and Carbamoylating Activity and Cytotoxicity against L1210 Cells in Vitro

| Compd | Alkylating[a] activity | Carbamoylating[b] activity | $ED_{50}$, μM,[c] L1210 |
|---|---|---|---|
| BCNU[d] | 1.0 | 1.0 | 4 |
| **5** | 0.47 | 1.6 | 6.6 |
| **5b** | 0.06 | 1.6 | 4.2 |
| **6** | 0.05 | 1.0 | 95 |
| **10** | 2.1 | 0.9 | 1.5 |
| **11** | 0.05 | 0.9 | 1 |

[a] The alkylating activity of all compounds is compared to BCNU (see ref 3); for BCNU $\Delta A_{540}/120' = 1.2$. At least three determinations were made for each compound and the standard error was less than 10% of the mean in all cases. [b] The carbamoylating activity of all compounds is compared to BCNU; for BCNU 36% of [$^{14}$C]lysine was present as products other than unreacted lysine. Each compound was assayed in duplicate with differences of less than 10%. [c] The correlation coefficients ($r$) of the linear regression curves from which the $ED_{50}$ values were estimated were equal to or greater than 0.9 in all cases. [d] BCNU is 1,3-bis(2-chloroethyl)-1-nitrosourea.

All of the compounds tested were cytotoxic as shown in Table I; however, activity was influenced by the position of the nitrosourea function on the nucleoside. The 3′ derivatives, **10** and **11**, were more active than the corresponding 5′ analogues, **5**, **5b**, and **6**. The 5′-chloroethyl compounds inhibited cell growth better than the 5′-methylnitrosourea, but *no* such difference was evident for the 3′ derivatives. Both **10** and **11** were quite potent, with $ED_{50}$ values about three times less than that of BCNU (Table I). No clear relationship was detected between the alkylating or carbamoylating activities and cellular toxicity in these experiments. The low alkylating activity of **5b**, an effective inhibitor of cell growth, may reflect the reduced ability of the carbonium ion which is generated to react with 4-(*p*-nitrobenzyl)pyridine, the reagent used in

the assay. The expected carbonium ion would be attached to a rather bulky thymidine group and steric hinderance may be a limiting factor. An intramolecular reaction in the pyrimidine moiety may also be involved.

Preliminary evidence indicates that the nucleoside portion of these nitrosoureas may enhance their in vivo antitumor activity. Further studies of the effectiveness of these new agents in animal tumor model systems are under investigation.

## Experimental Section

Melting points were determined with a Thomas-Hoover Unimelt apparatus and are not corrected. The UV spectra were recorded on a Beckman Model-25 spectrophotometer and the IR spectra on a Perkin-Elmer 257 instrument. $^1$H NMR spectra were recorded at 270 MHz on a Bruker 270 HX spectrometer in $Me_2SO$-$d_6$ solution ($\delta$ relative to $Me_4Si$, 0.00 ppm). TLC and preparative TLC were performed on Eastman 6060 precoated silica gel sheets with fluorescent indicator and on Analtech silica gel GF plates. Elemental analyses were carried out by Baron Consulting Co., Analytical Services, Orange, Conn. Where analyses are indicated only by symbols of the elements, the analytical results obtained for those elements were within ±0.4% of the theoretical values.

**5′-[3-(2-Chloroethylureido)]-5′-deoxythymidine (3).** 2-Chloroethyl isocyanate (0.58 g, 5.5 mmol) was added slowly to a solution of 5′-amino-5′-deoxythymidine[11] (**2**, 1.21 g, 5.02 mmol) in 60 mL of MeOH–$H_2O$ (2:1) at 0 °C. Upon addition of the isocyanate, white crystals precipitated out immediately. The reaction mixture was stirred at 0 °C for 1 h and the product was collected by filtration, washed with cold MeOH and ether, and dried to yield 1.63 g (94%) of analytical sample: mp 215–216 °C dec; TLC $R_f$ 0.46 ($CHCl_3$–EtOH, 4:1); NMR $\delta$ 11.33 (br s, 1 H, NH-3), 6.26 (t, 1 H, $J$ = 6.2 Hz, NH-5′), 6.24 (t, 1 H, $J$ = 5.7 Hz, NHR), 7.50 (s, 1 H, H-6), 6.15 (dd, 1 H, $J_{1'2'}$ = 7.5 Hz, $J_{1'2''}$ = 6.2 Hz, H-1′), 5.32 (d, 1 H, $J$ = 4.4 Hz, OH-3′), 4.11 (m, 1 H, H-3′), 3.71 (m, 1 H, H-4′), 3.57 (m, 2 H, $CH_2N$), 3.26 (m, 2 H, $CH_2Cl$), 3.31, 3.15 (m, 1 H each, H-5′), 2.07 (m, 2 H, H-2′), 1.81 (s, 3 H, $CH_3$-5). Anal. ($C_{13}H_{19}ClN_4O_5$) C, H, N.

**5′-[3-(2-Chloroethyl)nitrosoureido]-5′-deoxythymidine (5).** Sodium nitrite (0.31 g, 4.6 mmol) was added slowly to an ice-cooled solution of **3** (1.16 g, 3.35 mmol) in 50 mL of 50% aqueous acetic acid. The reaction mixture was stirred at 0 °C for 6 h, treated with AG 50W-X8 ($H^+$) resin (5 g, Bio-Rad Lab.), and then stirred for another hour at the same temperature. After removal of the resin by filtration, the filtrate was evaporated to dryness in vacuo below 35 °C. The residue was crystallized twice from MeOH to give 0.84 g (67%) of fine pale yellow crystals: mp 147–148 °C dec; TLC $R_f$ 0.69 ($CHCl_3$–EtOH, 4:1); UV $\lambda_{max}$ (EtOH) 265 nm ($\epsilon$ 13 200); UV $\lambda_{min}$ (EtOH) 234 nm. The NMR spectrum of this sample indicates that it is a mixture of two isomers (**5a** and **5b**) in a ratio of ~2:3 which was estimated by NMR integral ratios based on the 5-$CH_3$ resonance signal (singlet, $\delta$ 1.79 for **5a** and $\delta$ 1.86 for **5b**) and the 3′-OH resonance signal (doublet, $\delta$ 5.36 for **5a** and $\delta$ 5.43 for **5b**). Anal. ($C_{13}H_{18}ClN_5O_6$) C, H, N.

Attempts to separate **5a** and **5b** from the isomeric mixture by TLC using various solvent systems (e.g., $CHCl_3$–EtOH, EtOAc–EtOH, *n*-BuOH–AcOH–$H_2O$) were unsuccessful. However, isomer **5b** crystallized out from the concentrated mother liquor upon cooling at 3 °C overnight and was purified by fractional recrystallization from EtOH: mp 160–161 °C; TLC $R_f$ 0.69 ($CHCl_3$–EtOH, 4:1); UV $\lambda_{max}$ (EtOH) 265 nm ($\epsilon$ 12 900); UV $\lambda_{min}$ (EtOH) 234 nm; NMR $\delta$ 11.32 (br s, 1 H, NH-3), 8.99 (t, 1 H, $J$ = 6.2 Hz, NHR), 7.51 (s, 1 H, H-6), 6.10 (dd, 1 H, $J$ = 8.4 and 5.8 Hz, H-1′), 5.43 (d, 1 H, $J$ = 4.0 Hz, OH-3′), 4.14 (m, 3 H, H-3′ and $CH_2N$), 4.12 (m, 1 H, H-5″), 3.94 (dd, 1 H, $J_{gem}$ = 13.7 Hz, $J_{5',4'}$ = 4.9 Hz, H-5′), 3.82 (m, 1 H, H-4′), 3.75 (m, 2 H, $CH_2Cl$), 2.10 (m, 2 H, H-2′), 1.86 (s, 3 H, $CH_3$-5). Anal. ($C_{13}H_{18}ClN_5O_6$) C, H, N.

**5′-(3-Methylureido)-5′-deoxythymidine (4).** Methyl isocyanate (0.63 g, 11 mmol) was added to a solution of 5′-amino-5′-deoxythymidine[11] (**2**, 2.40 g, 9.95 mmol) in MeOH–$H_2O$ (2.5:1). The reaction mixture was kept at 0 °C with stirring for 1 h, allowed to warm up to room temperature, and then clarified by filtration. The filtrate was evaporated to dryness under reduced pressure at a temperature not exceeding 35 °C, and the residue was then crystallized from MeOH at 3 °C overnight to give 2.14 g (72%) of white fine needles: mp 238–239 °C; NMR $\delta$ 11.32 (s, 1 H, NH-3), 7.49 (s, 1 H, H-6), 6.14 (t, 1 H, $J$ = 7.1 Hz, H-1′), 6.06 (t, 1 H, $J$ = 6.2 Hz, NH-5′), 5.79 (q, 1 H, $J$ = 4.0 Hz, $CH_3NH$), 5.29 (d, 1 H, $J$ = 4.0 Hz, OH-3′), 4.12 (m, 1 H, H-3′), 3.71 (m, 1 H, H-4′), 3.31, 3.14 (m, 1 H each, H-5′), 2.55 (d, 3 H, $J$ = 4.0 Hz, $CH_3N$), 2.07 (m, 2 H, H-2′), 1.81 (s, 3 H, $CH_3$-5). Anal. ($C_{12}H_{18}N_4O_5$) C, H, N.

**5′-(3-Methyl-3-nitrosoureido)-5′-deoxythymidine (6).** Sodium nitrite (0.31 g, 4.6 mmol) was added slowly to a solution of **4** (1.00 g, 3.35 mmol) in 50% aqueous acetic acid at 0 °C. The reaction mixture was kept at 0 °C with stirring for 3 h, during which time crystals started to precipitate out. The product was collected by filtration, washed with water, cooled MeOH, and ether, and dried. The filtrate was concentrated and an additional fraction of crystals was obtained. The combined product was recrystallized from MeOH to yield 0.77 g (70%) of white fine needles: mp 159–160 °C dec; UV $\lambda_{max}$ (EtOH) 264 nm ($\epsilon$ 11 000); UV $\lambda_{min}$ (EtOH) 235 nm; NMR $\delta$ 11.32 (s, 1 H, NH-3), 8.92 (t, 1 H, $J$ = 6.2 Hz, NH-5′), 7.53 (s, 1 H, H-6), 6.16 (dd, 1 H, $J$ = 8.0 and 7.5 Hz, H-1′), 5.35 (d, 1 H, $J$ = 4.0 Hz, OH-3′), 4.25 (br s, 1 H, H-3′), 3.94 (m, 1 H, H-4′), 3.53 (dd, 2 H, $J$ = 6.2 and 5.3 Hz, H-5′), 3.10 (s, 3 H, $CH_3N$), 2.09 (m, 2 H, H-2′), 1.79 (s, 3 H, $CH_3$-5). Anal. ($C_{12}H_{17}N_5O_6$) C, H, N.

**3′-Amino-3′-deoxythymidine (7).** A solution of 3′-azido-3′-deoxythymidine[12] (6.10 g, 22.8 mmol) in 100 mL of EtOH was hydrogenated under 50 psi of hydrogen pressure at room temperature for 5 h in the presence of 10% palladium on charcoal (0.7 g). The catalyst was removed by filtration and the filtrate was evaporated to dryness. The residue was dissolved in a minimum amount of $H_2O$ and the pH of the aqueous solution was adjusted to 3 with hydrochloric acid. The solution was then applied directly to a column (2 × 24 cm) of AG 50W-X8 ($H^+$) resin and washed thoroughly with $H_2O$ (2 L), and the adsorbed product was eluted with 200 mL of 1 N $NH_4OH$ solution. The solvent was evaporated under reduced pressure and the residue was crystallized from EtOH to afford 3.69 g (67%) of product: mp 160–161 °C; UV $\lambda_{max}$ (0.1 N HCl) 266 nm ($\epsilon$ 9190); UV $\lambda_{min}$ (0.1 N HCl) 234 nm ($\epsilon$ 2250); UV $\lambda_{max}$ (0.1 N NaOH) 268 nm ($\epsilon$ 7180); UV $\lambda_{min}$ (0.1 N NaOH) 246 nm ($\epsilon$ 4240) [lit.[13] UV $\lambda_{max}$ (0.1 N HCl) 265 nm ($\epsilon$ 9400); UV $\lambda_{min}$ (0.1 N HCl) 233 nm ($\epsilon$ 2300); UV $\lambda_{max}$ (0.1 N NaOH) 266.5 nm ($\epsilon$ 7400); UV $\lambda_{min}$ (0.1 N NaOH) 244 nm ($\epsilon$ 4400)].

**3′-[3-(2-Chloroethylureido)]-3′-deoxythymidine (8).** 2-Chloroethyl isocyanate (0.89 g, 8.4 mmol) was added slowly to a solution of **7** (1.69 g, 7.00 mmol) in 35 mL of MeOH at 0 °C. The reaction mixture was then stirred at room temperature for 3 h. The solvent was evaporated in vacuo to give a residue which was used for the next step without further purification.

**3′-[3-(2-Chloroethyl)-3-nitrosoureido]-3′-deoxythymidine (10).** To a solution of **8** (as a residue) in 20 mL of 50% aqueous acetic acid, sodium nitrite (0.97 g, 14 mmol) was added slowly. The reaction mixture was stirred at 0 °C for 5 h and then treated with AG 50W-X8 ($H^+$) resin (6.5 g) to remove $Na^+$. The resin-containing mixture was stirred for an additional 30 min and then filtered. The filtrate was evaporated to dryness under reduced pressure below 35 °C, and the residue was dissolved in a minimum amount of EtOH. Upon the addition of ether to the alcoholic solution, a pale yellow solid precipitated out. The solid was collected by filtration and dried to give 1.83 g of crude product which was dissolved in a minimum amount of EtOH–DMF (2:1) and applied directly to 12 preparative Analtech silica gel GF plates. The plates were developed with a $CHCl_3$–EtOH (3:1) solvent system. The desired pale yellow band ($R_f$ 0.70) was scratched off the plates, and the product was extracted with $CHCl_3$–EtOH (1:1, 3 × 150 mL). The silica gel was removed by filtration, and then the filtrate was concentrated to a small volume (~20 mL). Ether was added to the solution and pale yellow crystals precipitated out. The product was isolated by filtration, washed with ether, and dried to yield 1.12 g (43% based on **7**) of an analytical pure sample. The compound effervesced above 95 °C and decomposed at 120 °C: UV $\lambda_{max}$ (EtOH) 266 nm ($\epsilon$ 12 400); UV $\lambda_{min}$ (EtOH) 235 nm; NMR $\delta$ 11.31 (br s, 1 H, NH-3), 9.18 (d, 1 H, $J$ = 8.0 Hz, NH-3′), 7.80 (s, 1 H, H-6), 6.27 (t, 1 H, $J$ = 6.6 Hz, H-1′), 5.13 (t, 1 H, $J$ = 4.9 Hz, OH-5′), 4.56 (t, 1 H, $J$ = 5.3 Hz,

H-3′), 4.10 (t, 2 H, $J$ = 6.2 Hz, $CH_2N$), 3.98 (br s, 1 H, H-4′), 3.63 (t, 5 H, $J$ = 6.2 Hz, $CH_2$-5′ and $CH_2Cl$), 2.34 (m, 2 H, H-2′), 1.79 (s, 3 H, $CH_3$-5). Anal. ($C_{13}H_{18}ClN_5O_6$) C, H, N.

**3′-(3-Methylureido)-3′-deoxythymidine (9).** Methyl isocyanate (0.13 g, 2.2 mmol) was added slowly to a solution of **7** (0.48 g, 2.0 mmol) in 15 mL of MeOH. The solution was stirred at 0 °C for 1 h, during which period white crystals precipitated out. The product was collected by filtration, washed with cooled MeOH, and dried to give 0.54 g (90%). The compound softened at 114 °C and effervesced at 120 °C: TLC $R_f$ 0.38 ($CHCl_3$–EtOH, 4:1); NMR $\delta$ 11.29 (s, 1 H, NH-3), 7.75 (s, 1 H, H-6), 6.39 (d, 1 H, $J$ = 7.5 Hz, NH-3′), 6.13 (t, 1 H, $J$ = 6.6 Hz, H-1′), 5.74 (q, 1 H, $J$ = 4.4 Hz, $CH_3NH$), 5.07 (t, 1 H, $J$ = 4.4, OH-5′), 4.15 (m, 1 H, H-3′), 3.71 (m, 1 H, H-4′), 3.64 (dd, 1 H, $J_{gem}$ = 12.4 Hz, H-5″), 3.53 (dd, 1 H, $J_{gem}$ = 12.4 Hz, $J_{5',OH\text{-}5'}$ = 4.4 Hz, H-5′), 2.55 (d, 3 H, $J$ = 4.4 Hz, $CH_3N$), 2.12 (m, 2 H, H-2′), 1.78 (s, 3 H, $CH_3$-5). Anal. ($C_{12}H_{18}N_4O_5$) C, H, N.

**3′-(3-Methyl-3-nitrosoureido)-3′-deoxythymidine (11).** Sodium nitrite (0.15 g, 2.2 mmol) was added slowly to an ice-cooled solution of **9** (0.48 g, 1.6 mmol) in 15 mL of 80% aqueous acetic acid. The reaction mixture was stirred at 0 °C for 5 h and then treated with AG 50W-X8 ($H^+$) resin (3 g). The mixture was stirred for another 30 min and then filtered. The filtrate was evaporated to dryness in vacuo below 35 °C to yield a residue which was crystallized from EtOH. The product was isolated by filtration, washed with cooled EtOH and ether, and dried to afford 0.28 g (53%) of pale yellow crystals. Upon heating the compound changed into a white crystalline mass around 135 °C and then decomposed at 159 °C: UV $\lambda_{max}$ (EtOH) 265 nm ($\epsilon$ 12 000); UV $\lambda_{min}$ (EtOH) 238 nm; IR (KBr) $\nu_{max}$ 1528 $cm^{-1}$ with shoulder at 1510 $cm^{-1}$ (NH bending and C–NO stretching); NMR $\delta$ 11.31 (s, 1 H, NH-3), 9.14 (d, 1 H, $J$ = 8.0 Hz, NH-3′), 7.81 (s, 1 H, H-6), 6.26 (t, 1 H, $J$ = 6.6 Hz, H-1′), 5.11 (t, 1 H, $J$ = 4.9 Hz, OH-5′), 4.57 (m, 1 H, H-3′), 3.99 (m, 1 H, H-4′), 3.69 (m, 2 H, H-5′), 3.09 (s, 3 H, $CH_3N$), 2.34 (m, 2 H, H-2′), 1.80 (s, 3 H, $CH_3$-5). Anal. ($C_{12}H_{17}N_5O_6$) H; C: calcd, 44.04; found, 42.94. N: calcd, 21.40; found, 20.91.

Probably due to the thermal instability of the compound, a satisfactory elemental analysis has not been obtained. However, the UV, IR, and NMR spectroscopic data are consistent with the assigned structure, and TLC indicated homogeneity in three solvent systems: (1) $R_f$ 0.78 ($CHCl_3$–EtOH, 4:1), (2) $R_f$ 0.88 (AcOEt–EtOH, 4:1), (3) $R_f$ 0.25 ($CHCl_3$–$i$-PrOH, 6:1).

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# Modifications of Primaquine as Antimalarials. 2. 5-Phenylthio and 5-Anilino Derivatives of Primaquine

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A number of 5-phenylthio and 5-anilino derivatives of primaquine have been prepared which are less toxic but less active than primaquine itself in murine and monkey antimalarial screens. It is apparent that the toxicity of primaquine can be diminished by introduction at position 5 of phenylthio, anilino, or phenoxy groups. However, the best hope for concomitant retention of high activity would seem to reside with the phenoxy moieties.

The first paper[1] in this series described a group of 5-phenoxyprimaquines. Concurrently with the phenoxy derivatives, as part of an accelerated program which precluded the wait for antimalarial screening data, we prepared the 5-phenylthio and 5-anilino analogues discussed in the present paper.

**Chemistry.** The preparative route (Scheme I) was similar to that described in paper 1 and its various stages are tabulated in Table I and exemplified in the Experimental Section.

**Biology.** Table II compares primaquine with some of its 5-phenylthio and 5-anilino derivatives in the radical curative antimalarial screen (*Plasmodium cynomolgi*, rhesus monkey). All of the phenylthio primaquines (**11a–h**) were curative. However, the best of these (**11c** and **11g**), with cures at 1 mg/kg, were less active than

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