paring 3b: yield 89%; mp 260–262 °C (dec). Anal. ($C_{10}H_{12}N_3Cl_3$) C, H, N.

Pharmacological Testing. Rabbits were killed by injecting air into the marginal ear veins, and thoraic aorta were isolated in a petri dish containing physiological salt solution. Aorta strips 4 cm × 2.5 mm wide were prepared as described by Furchgott and Bhadrakom.[18] The tissues were mounted in a 10-mL jacketed tissue bath containing oxygenated (95% O_2 and 5% CO_2) physiological salt solution at 37 °C. The resting tension of 4 g was maintained and the drug-induced changes in the tension were recorded via a transducer on a polygraph. After an hour of tissue equilibrium, the strip was exposed to 10^{-6} M phenylephrine, and the tissue was thoroughly washed. Subsequently, the cumulative dose–response of (−)-phenylephrine was obtained in the presence and in the absence of antagonist, which was incubated for 60 min. Either single or multiple concentrations of antagonists were used to calculate the dissociation constant as described by Furchgott.[19] When phentolamine was used as a blocker, the dissociation constant was 1.5×10^{-8} M.

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Synthesis and Antineoplastic Activity of a Novel Series of Phosphoramide Mustard Analogues of Pyrimidine Deoxyribonucleosides[1]

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A novel series of cyclophosphamide derivatives of pyrimidine deoxyribonucleosides (**6–9**) has been synthesized from the corresponding amino nucleosides. Our preliminary findings have shown that three of these cyclophosphamide nucleoside analogues, **6**, **7**, and **9**, have potent inhibitory effects on the replication of L1210 cells in vitro (ED_{50} = $1.2–1.5 \times 10^{-5}$ M). Since cyclophosphamide (cytoxan) has no cytotoxicity under these conditions, our findings indicate that these novel phosphamide derivatives have unusual biological properties which may include a unique mode of activation.

The synthesis of phosphoramide mustards as latent alkylating agents that might be selectively "activated" in tumors by enzymatic (hydrolytic) release of nornitrogen mustard represents one of the earliest design strategies in cancer chemotherapy.[3] Hundreds of candidate compounds belonging to this structural class have been screened[4–9] and cyclophosphamide,[4] 2-[bis(2-chloroethyl)amino]tetrahydro-2*H*-1,3,2-oxazaphosphorine 2-oxide, has uniquely emerged as the one which exhibits clinical effectiveness against a relatively wide spectrum of human cancers.[10] Cyclophosphamide requires activation by hepatic microsomal mixed function oxidases, and in animals its rate of activation was shown to be increased by barbiturate induction of these enzymes and decreased by inhibition of these enzymes.[11]

Although many phosphoramide mustard derivatives have been synthesized and screened, the previous synthesis of 3′,5′-[[bis(2-chloroethyl)amino]phosphoryl] mustard nucleoside analogues has not been reported. Our concept to combine a nucleoside moiety and a phosphoramide mustard functionality into *one molecule* has evolved a new series of phosphoramide mustard nucleoside analogues, which may, by virtue of affecting specific metabolic pathways, have unique and desirable pharmacologic properties with good clinical potential as antineoplastic agents.

The addition of a nucleoside moiety to the phosphoramide mustard molecule provides a versatile handle for pharmacologic manipulation. For example, attaching an acyl group (acetyl or longer chain acyl) to the pyrimidine (cytosine) or purine (adenine, guanine) base will increase the lipophilicity of the compound. Such modification may increase transport across membranes and thereby favorably affect the pharmacologic and biological properties.

A possible way to modulate the distribution of biologically active compounds is by using selective moieties, such as nucleosides, sugars, amino acids, etc., which are actively transported in the body by a specific transport mechanism. Ascites tumor cells, for instance, are reported to have a high capacity for uptake of amino acids, sugars, and nucleosides. Therefore, joining the phosphoramide mustard group with nucleosides may lead to compounds which would fit the "latency" and "carrier" principles with hopefully preferential distribution into tumor cells. *ara*-C, for example, is a nucleoside of great clinical utility in therapy of certain neoplasms. Recently, we have syn-

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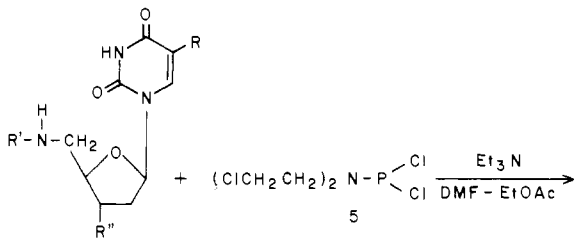
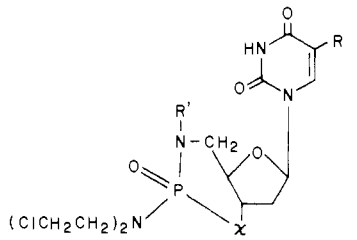
Scheme I



1. R = CH_3, R' = H, R" = OH
2. R = CH_3, R' = CH_3, R" = OH
3. R = CH_3, R' = H, R" = NH_2
4. R = I, R' = H, R" = OH



6. R = CH_3, R' = H, X = O
7. R = CH_3, R' = CH_3, X = O
8. R = CH_3, R' = H, X = NH
9. R = I, R' = H, X = O

thesized a new series of novel 5'- and 3'-(chloroethyl)- and -methylnitrosourea analogues of thymidine[12] which appear in our preliminary experiments to be very promising anticancer agents.

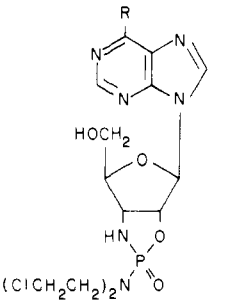
Our preliminary findings have indicated that several cyclophosphamide nucleoside analogues have potent cytotoxic activity against L1210 cells in culture. On the contrary, cyclophosphamide (cytoxan) has *no* cytotoxicity in vitro. These findings illustrate unusual biological properties of this new series of nucleoside cyclophosphamide analogues and suggest an additional or completely different mode of activation.

Chemistry. The synthesis of a new class of cyclophosphamide analogues of thymidine (6–8) and 5-iodo-2'-deoxyuridine (9) is outlined in Scheme I. The key intermediate, 5'-amino-5'-deoxythymidine (1), was prepared according to the method reported by Horwitz and co-workers[13] with modification. 5'-(Methylamino)-5'-deoxythymidine (2), 2',5'-diamino-2',5'-dideoxythymidine, and 5-iodo-5'-amino-2',5'-dideoxyuridine (4) were synthesized by the procedures developed in this laboratory.[14-16] Bis(2-chloroethyl)phosphoramidic dichloride (5) was prepared by the method reported by the Romania Ministry of Petroleum and Chemical Industry in a British patent.[17] Compounds 1–4 were converted to the corresponding nucleoside cyclophosphamide analogues 6–9 by reacting with bis(2-chloroethyl)phosphoramidic dichloride (5) in DMF–EtOAc in the presence of triethylamine at room temperature. Compounds 6–9 were isolated by crystallization from ethanol–ether.

Table I. Effect of Various Nucleoside Cyclophosphamide Analogues on the Replication of L1210 Cells in Vitro

compd	$ID_{50} \times 10^{-5}$, M
cyclophosphamide	inactive
6	1.2
7	1.5
8	inactive
9	1.4

Earlier Fujiwara et al.[18] reported the synthesis of an isomeric mixture of 2',3'-[[bis(2-chloroethyl)amino]-phosphoryl]-*N*,*N*-dimethyl-3'-amino-3'-deoxyadenosine (10) in 51% yield by treatment of the corresponding 3'-



10 a, b, R = $N(CH_3)_2$
11 a, b, R = NH_2

amino nucleoside with bis(2-chloroethyl)phosphoramidic dichloride in DMF. Recently, Okruszek and Verkade[19] described the synthesis and isolation of the isomers of 2',3'-[[bis(2-chloroethyl)amino]phosphoryl]-3'-amino-3'-deoxyadenosine (11), which are isomeric at phosphorus, from 3'-amino-3'-deoxyadenosine and bis(2-chloroethyl)-phosphoramidic dichloride in $(EtO)_3PO$.

In this report, compounds 6–9 were isolated as isomeric mixtures and no attempt was made to separate the isomers.

Biology. The effect of these nucleoside cyclophosphamide analogues and cyclophosphamide on the replication of a neoplastic cell line in culture was investigated. Mouse L1210 cells were maintained as suspension cultures in Fischer's medium supplemented with 10% horse serum at 37 °C in a humidified atmosphere of 5% CO_2–95% air. Under these conditions the generation time for L1210 cells is approximately 18 h. Each compound, at the given concentration, was added to L1210 cells (2×10^4 cells/mL) which were in their exponential phase of growth. The increase in cell number of the drug-free culture (control), as well as that of the cultures supplemented with the tested compounds, was determined after 24, 48, and 72 h of growth.

The ID_{50} values were estimated from dose–response curves compiled from at least two independent experiments and represent the drug concentration required to inhibit replication of L1210 neoplastic cells by 50% (Table I).

All of the compounds tested were cytotoxic in a dose-dependent manner, except cyclophosphamide and 8 which has a -NH- linkage instead of an oxygen at the 3' position in the deoxyribose portion as shown in Table I. Thus the mode of the linkage of a nucleoside with the cyclo-

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phosphamide moiety affects the biological property of the resultant molecule. ID_{50} values were determined from plots of mean cell counts after 72 h. Assays were carried out in triplicate, with appropriate controls.

The above findings are of interest in that they represent a novel series of nucleoside cyclophosphamide analogues, with good biological activity; however, whether they have unique and desirable pharmacologic properties in vivo remains to be determined.

Experimental Section

Melting points were taken on a Thomas-Hoover Unimelt apparatus and are not corrected. The UV spectra were recorded on a Beckman-25 spectrophotometer, and the NMR spectra were taken on a Bruker 270 HX spectrometer at 270 MHz (Me_4Si). The elemental analyses were carried out by Baron Consulting Co., Analytical Services, Orange, CT. 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.

3′,5′-[[Bis(2-chloroethyl)amino]phosphoryl]-5′-amino-5′-deoxythymidine (6). A solution of bis(2-chloroethyl)-phosphoramidic dichloride (0.54 g, 2.07 mmol) in 5 mL of ethyl acetate was added to a magnetically stirred mixture of 5′-amino-5′-deoxythymidine (1; 0.50 g, 2.07 mmol) and triethylamine (0.42 g, 4.15 mmol) in 15 mL of DMF. The reaction mixture was stirred for 48 h at room temperature. The insoluble material was removed by filtration and the filtrate was concentrated under reduced pressure. The residue was crystallized from 95% EtOH–Et_2O to yield 0.42 g (42%) of product. The compound effervesced above 120 °C: UV (EtOH) λ_{max} 267 nm (ϵ 7820), λ_{min} 240 nm; NMR (Me_2SO-d_6) δ 1.81 (s, 3, C-5 CH_3), 2.10 (m, 2, C-2′ H), 3.15–3.35 (m, 6, C-5′ H, C-CH_2-N), 3.69–3.80 (m, 5, C-4′ H, Cl-CH_2-C), 4.30 (m, 1, C-3′ H), 6.13 (m, 1, C-1′ H), 7.54 (s, 1, C-6 H), 9.41 (br s, 1, 5′-NH), 11.34 (br s, 1, 3-NH). Anal. ($C_{14}H_{20}Cl_2N_4O_5P \cdot 0.5H_2O$) C, H, N.

3′,5′-[[Bis(2-chloroethyl)amino]phosphoryl]-5′-(methylamino)-5′-deoxythymidine (7). A solution of bis(2-chloroethyl)phosphoramidic dichloride (1.02 g, 3.92 mmol) in 10 mL of ethyl acetate was reacted with 5′-(methylamino)-5′-deoxythymidine (2; 1.00 g, 3.92 mmol) in DMF (15 mL) and triethylamine (0.79 g, 7.84 mmol) according to the method described above to give 0.79 (46%) of 7. Compound 7 started to soften at 105 °C and effervesced above 130 °C: UV (EtOH) λ_{max} 266 nm (ϵ 8680), λ_{min} 238 nm; NMR (Me_2SO-d_6) δ 1.81 (s, 3, C-5 CH_3), 2.07 (m, 2, C-2′ H), 2.50 (s, 3, C-5′ N-CH_3), 3.16–3.33 (m, 6, C-5′ H, C-CH_2-N), 3.64–3.73 (m, 5, C-4′ H, Cl-CH_2-C), 4.13 (m, 1, C-3′ H), 6.20 (m, 1, C-1′ H), 7.63 (s, 1, C-6 H), 11.33 (br s, 1, 3-NH). Anal. ($C_{15}H_{22}Cl_2N_4O_5P$) C, H, N.

3′,5′-[[Bis(2-chloroethyl)amino]phosphoryl]-3′,5′-diamino-3′,5′-dideoxythymidine (8). A solution of bis(2-chloroethyl)phosphoramidic dichloride (0.32 g, 1.25 mmol) in ethyl acetate (10 mL) was added to a solution of 3′,5′-diamino-3′,5′-dideoxythymidine (3; 0.30 g, 1.25 mmol) and triethylamine (0.25 g, 2.50 mmol) in 10 mL of DMF. The reaction mixture was stirred for 48 h at room temperature. Compound 8 was isolated in the same manner as mentioned previously to afford 0.22 g (41%): mp 220 °C (dec); UV (EtOH) λ_{max} 267 nm (ϵ 8960), λ_{min} 234 nm; NMR (Me_2SO-d_6) δ 1.81 (s, 3, C-5 CH_3), 2.15 (m, 2, C-2′ H), 3.33–3.49 (m, 7, C-4′ H, C-5′ H, C-CH_2-N), 3.63–3.69 (m, 5, C-3′ H, Cl-CH_2-C), 4.33 (s, 1, C-5′ NH), 4.60 (s, 1, C-3′ NH), 6.19 (t, 1, C-1′ H, $J_{1',2'a}$ = 3.98 Hz, $J_{1',2'b}$ = 6.19 Hz), 7.45 (s, 1, C-6 H), 11.30 (s, 1, 3-NH). Anal. ($C_{14}H_{21}Cl_2N_5O_4P$) C, H, N.

3′,5′-[[Bis(2-chloroethyl)amino]phosphoryl]-5′-amino-2′,5′-dideoxy-5-iodouridine (9). A solution of bis(2-chloroethyl)phosphoramidic dichloride (0.74 g, 2.83 mmol) in 15 mL of ethyl acetate was added to a suspension of 5-iodo-5′-amino-2′,5′-dideoxyuridine (4; 1.00 g, 2.83 mmol) in DMF (30 mL) and triethylamine (0.57 g, 5.66 mmol). The reaction mixture was stirred at room temperature for 6 days. The insoluble solid was removed by filtration. Ether was added to the filtrate until it turned cloudy. The solution was then kept at −20 °C for several days, during which time the compound crystallized out. The product was collected by filtration, washed with ether, and dried to afford 0.79 g (52%). Compound 9 softened above 140 °C and decomposed at 189 °C: UV (EtOH) λ_{max} 283 nm (ϵ 7470), λ_{min} 245 nm; NMR (Me_2SO-d_6) δ 2.11 (m, 1, C-2′ H_a), 2.35 (m, 1, C-2′ H_b), 3.61–3.82 (m, 11, C-4′ H, C-5′ H, C-CH_2-N, Cl-CH_2-C), 4.30 (m, 1, C-3′ H), 6.04 (t, 1, C-1′ H, $J_{1',2'a}$ = 6.63 Hz, $J_{1',2'b}$ = 7.08 Hz), 8.00 (s, 1, C-6 H), 9.75 (br s, 1, C-5′ NH), 11.73 (br s 1, 3-NH). Anal. ($C_{13}H_{17}Cl_2IN_4O_5P$) N; C: calcd, 29.02; found, 29.72. H: calcd, 3.18; found, 3.95.

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2-Methylanthraquinone Derivatives as Potential Bioreductive Alkylating Agents

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Hypoxic cells of solid tumors are an obstacle to effective cancer therapy. Since hypoxic cells remote from the tumor blood supply may have a greater capacity for reductive reactions than well-oxygenated cells, we have prepared a series of anthraquinone prodrugs which may be capable of generating a reactive quinonemethide species following enzymatic reduction to the hydroquinone and loss of the substituent on the methylene group in the 2 position. The synthesized 2-methyl-substituted anthraquinone derivatives have first half-wave reduction potentials of −0.52 to −0.56 V at pH 7.0, which are the lowest oxidation–reduction potentials of quinone bioreductive alkylating agents synthesized by this laboratory to date. Tests of the cytotoxicity of these agents to oxygenated and chronically hypoxic EMT6 tumor cells in culture demonstrated that 2-(hydroxymethyl)anthraquinone, 2-[(*N*-methylcarbamyl)-methyl]anthraquinone, 2-[[(*p*-toluenesulfonyl)oxy]methyl]anthraquinone, and 2-(methoxymethyl)anthraquinone were significantly more toxic to hypoxic cells than to their normally aerated counterparts. The findings demonstrate differences between various leaving groups in the 2 position for the expression of differential cytotoxicity.

Significant advances toward the cure of human cancer by chemotherapy have been achieved primarily with cytotoxic agents directed toward proliferating cells. Thus, certain rapidly growing cancers, such as acute lymphocytic leukemia and Hodgkin's disease, can respond dramatically to existing chemotherapy, and cure of both localized and disseminated malignancies are not uncommon. The more slow-growing solid tumors which represent the majority

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