extracted with CH_2Cl_2, which was dried (Na_2SO_4) and concentrated to a residue of 58.5 g. The oil was distilled, and 41 g (49%) of **N-(4,6-dimethyl-2-pyridyl)-N-(4-acetoxybutyl)acetamide (17)**, bp 172–174 °C (1 mm) was obtained.

NaOH (1 N, 150 mL) was added to a solution of 41 g (0.147 mol) of the ester, **17**, in 150 mL of EtOH, and the mixture was stirred for 1 h. The EtOH was removed by vacuum concentration, and the residual aqueous solution was made alkaline with Na_2CO_3. The oily product was extracted with CH_2Cl_2, and the extracts were dried (Na_2SO_4) and concentrated to yield 35.9 (~100%) of **18** as an oil.

N-(4,6-Dimethyl-2-pyridyl)-N-(3-formylpropyl)acetamide (19). The alcohol, **18** (24.8 g, 0.106 mol), in 100 mL of CH_2Cl_2 was added to a suspension of 8.62 g (0.04 mol) of pyridinium chlorochromate in 200 mL of CH_2Cl_2 under N_2. After 2 h, 250 mL of saturated Na_2CO_3 was added, the layers were separated, and the aqueous layer was extracted with CH_2Cl_2. The combined CH_2Cl_2 extracts were washed with brine and then stirred with 15 g of Florosil. The mixture was filtered, and the filtrate was dried (Na_2SO_4) and concentrated to obtain 23.7 g (95%) of **19** as an oil.

N-(4,6-Dimethyl-2-pyridyl)-N-[4-(diisopropylamino)butyl]acetamide (20). The reductive amination of **19** (11.7 g, 0.05 mol) was performed as described for the preparation of **16**. Concentration of the solvent extract from the reaction workup yielded 14.7 g (92%) of **20** as an oil.

N,N-Dimethyl-N'-[4-(diisopropylamino)butyl]-N'-(4,6-dimethyl-2-pyridyl)urea (22). The acetamide **20** was deacetylated by acid hydrolysis as described for the preparation of **7g** from **6g**. The oil was distilled to give a 50% yield of **4,6-dimethyl-2-[[4-(diisopropylamino)butyl]amino]pyridine (21)**, bp 142–148 °C (0.7 mm), from **20**. Carbamoylation of **21** was performed essentially in the manner described for the preparation of **8g**, and the **22** base was distilled to give a 61% yield.

N-(5-Chloro-4,6-dimethyl-2-pyridyl)acetamide (5p). N-Chlorosuccinimide (32.8 g, 0.247 mol) was added to a stirred solution of 37.0 g (0.225 mol) of N-(4,6-dimethyl-2-pyridyl)acetamide (**5g**) in 500 mL of CCl_4 under N_2. The mixture was heated under reflux for 3.5 h and then cooled to 0 °C. The crystalline **5p** and insoluble succinimide byproduct were collected. The succinimide was removed by washing the mixed solid with H_2O, leaving 35.8 g (80%) of **5p**, mp 221–223 °C, after recrystallization from ethanol.

Biological Methods. Antisecretory studies were performed in unanesthetized female beagle dogs with a chronic gastric fistula. Compounds were administered in 50 mL of aqueous (1%) methylcellulose directly into the stomach through the fistula cannula (referred to herein as oral administration) 60 min prior to administration of the gastric stimulant. Gastric output was collected continuously by gravity drainage through the fistula cannula for three 30-min periods after administration of a maximal secretory dose of gastrin tetrapeptide (64 μg/kg sc) or histamine dihydrochloride [64 μg (base)/kg sc)]. Output volume (milliliters) was measured to the nearest 0.1 mL, and acid concentration (milliequivalents per liter) was determined by titration of an aliquot to pH 7 with 0.01 N NaOH; acid output (milliequivalents) was calculated as the product of output volume and acid concentration. Data were expressed as the percent change in the gastric secretion parameter relative to a placebo trial in the same animal. ED_{50} and confidence limits determined for the period of maximal output (0–30 min) were calculated by analysis of variance according to Finney.[9] Dogs used for the detailed study of **8g** had an esophagostomy which was occluded during the experiment because preliminary trials indicated that salivary secretions evoked by **8g** could interfere with the gastric analysis. Esophagostomy does not affect gastric secretory results.

Acknowledgment. The authors express their appreciation to Mr. K. B. Streeter and Mr. Y. C. Lee and their associates for microanalytical determinations, to Mr. W. R. McGaughran and Ms. J. S. Murphy for spectroscopic data, to Ms. P. G. Cook, Ms. C. A. Wesley, Ms. B. L. Westrick, and Ms. S. R. Wiese for expert technical assistance with in vitro and in vivo evaluations, and Ms. T. H. Brunner for manuscript preparation.

Registry No. 4, 23510-18-1; **5g**, 5407-88-5; **5p**, 84369-60-8; **6g**, 84369-57-3; **7c**, 84369-44-8; **7e**, 63763-27-9; **7f**, 75308-69-9; **7g**, 63763-73-5; **7h**, 23826-74-6; **7i**, 24573-36-2; **7j**, 75308-61-1; **7k**, 75329-50-9; **7l**, 75308-62-2; **7m**, 75398-05-9; **7n**, 75329-45-2; **7o**, 75308-59-7; **7p**, 84369-61-9; **8a**, 84369-62-0; **8b**, 63763-60-0; **8b** (base), 75308-30-4; **8c**, 63764-42-1; **8c** (base), 84369-65-3; **8d**, 63764-43-2; **8d** (base), 81523-89-9; **8e**, 63763-62-2; **8e** (base), 63763-75-7; **8f**, 75308-70-2; **8g**, 63763-54-2; **8g** (base), 75308-65-5; **8h**, 84369-63-1; **8i**, 63764-52-3; **8i** (base), 75329-86-1; **8j**, 63764-40-9; **8k**, 75329-51-0; **8k** (base), 75329-92-9; **8l**, 63764-41-0; **8m**, 63764-50-1; **8m** (base), 75329-89-4; **8n**, 75329-46-3; **8o**, 63764-39-6; **8o** (base), 75329-87-2; **8p**, 63764-44-3; **8p** (base), 75308-66-6; **10**, 63763-52-0; **11**, 84369-45-9; **11** (base), 81523-91-3; **12**, 63763-74-6; **12** (base), 81523-90-2; **13**, 84369-46-0; **14**, 84369-47-1; **15**, 84369-48-2; **16**, 84369-49-3; **16** (base), 84369-64-2; **17**, 84369-50-6; **18**, 84369-51-7; **19**, 84369-52-8; **20**, 84369-53-9; **21**, 84369-54-0; **22**, 84369-55-1; **23**, 84369-56-2; **23** (O-methylisourea), 84369-58-4; **26** (isomer 1), 75308-58-6; **26** (isomer 2), 84369-59-5; **28**, 75329-43-0; **29**, 75329-44-1; 2-amino-4,6-dimethylpyridine, 5407-87-4; 3,3-diethoxypropyl chloride, 35573-93-4; 2,6-dichloropyridine, 2402-78-0; 3-cyano-2,6-dichloro-4-methylpyridine, 875-35-4; 2-chloro-4-nitropyridine N-oxide, 14432-16-7; 2-(diisopropylamino)ethyl chloride hydrochloride, 4261-68-1; dimethylcarbamoyl chloride, 79-44-7; 2-(diisopropylamino)ethylamine, 121-05-1; diisopropylamine, 108-18-9; 4-chlorobutyl acetate, 6962-92-1; pyridinium chlorochromate, 26299-14-9.

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Synthesis and Antineoplastic Activity of 3'-Azido and 3'-Amino Analogues of Pyrimidine Deoxyribonucleoside

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Several new 3'-azido and 3'-amino nucleosides (**8**, **9**, **12**, and **13**) have been synthesized and their biological activities evaluated. Among them, 3'-amino-2',3'-dideoxycytidine (**13**) was found to exhibit potent cytotoxic activity against both L1210 and S-180 cells in vitro with an ID_{50} of 0.7 and 4.0 μM, respectively. Furthermore, **13** has also shown antitumor activiy against L1210 tumor bearing mice with a T/C × 100 value of 283.

Modification of the sugar moiety of nucleosides may produce marked changes in their spectrum of biological activity and degree of selective toxicity, as well as in their chemical and physical properties. Baker et al.[1] first re-

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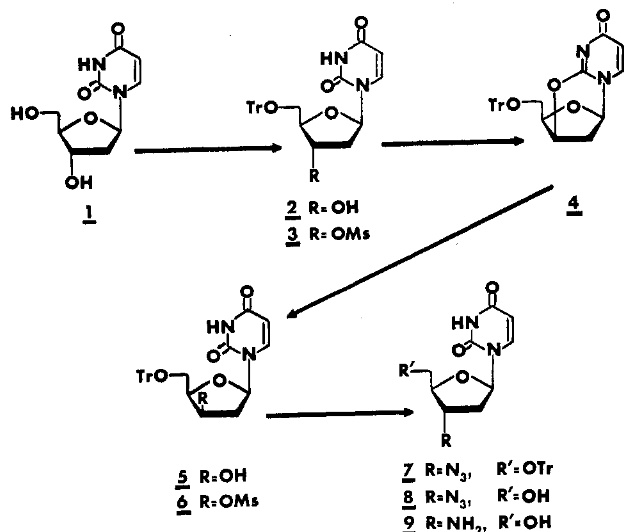
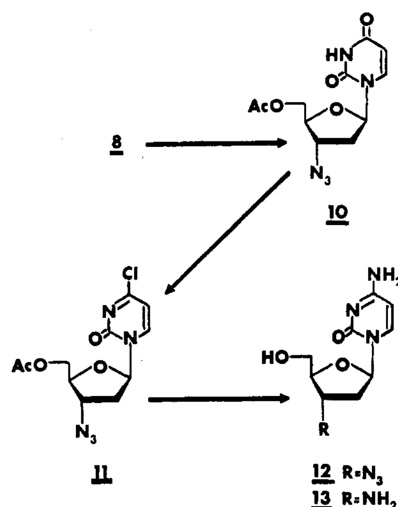
Scheme I



Scheme II



ported that amino nucleosides possess biological activity. The activity of puromycin against a mammary adenocarcinoma and *Trypanosoma equiperdum* in mice is believed to be due to the in vivo enzymatic formation of N^6,N^6-dimethyl-3′-aminoadenosine. The 3′-amino analogue of thymidine was first synthesized by Miller and Fox[2] and by Horwitz et al.,[3] and the 5′-amino analogue of thymidine was synthesized by Horwitz et al.[4] However, their biological potential had not been extensively investigated. Recently, it has been found that substitution of thymidine and its analogues at the 3′- or the 5′-position with an amino function yields compounds with striking differences in biological activity.[5-8] For example, 5′-amino-5′-deoxythymidine and 5-iodo-5′-amino-2′,5′-dideoxyuridine (AIU) inhibit herpes simplex type 1 replication in the absence of detectable host cell toxicity.[6-8] Conversely, 3′-amino-3′-deoxythymidine is a potent inhibitor of the replication of both murine sarcoma 180 and L1210 leukemia cells in vitro.[5] However, the 3′,5′-diamino analogue is devoid of both activities.[5] Although the biochemical basis of the antineoplastic activity produced by 3′-amino-3′-deoxythymidine is unknown, the findings of Fisher et al.[9] suggest that interference with DNA synthesis is involved. By modification of the sugar moiety of the 3′-position in the 2′-deoxycytidine molecule we hope to generate new compounds that may possess antitumor activity while being resistant to deamination by cytidine–deoxycytidine deaminase. Recently, 3′-amino-2′,3′-dideoxycytidine (13), a 3′-amino analogue of 2′-deoxycytidine, has been synthesized in this laboratory. This new compound has excellent water solubility (~0.5 g/mL at 23 °C) and exerts a potent inhibitory effect against L1210 leukemia both in culture and in tumor bearing mice (T/C × 100 = 283). These findings illustrate that modification of the deoxyribose moiety at the 3′-position of thymidine and 2′-deoxycytidine with an amino function yields compounds with significant anticancer activity, whereas the parent compounds are inactive.

The antitumor efficacy of *ara*-C and its various derivatives is diminished by their susceptibility to deamination.[10-12] However, our preliminary data[13] indicated that the 3′-amino analogue of 2′-deoxycytidine (13) was resistant to deamination by partially purified cytidine–deoxycytidine deaminase from HeLa S_3 cells.

In addition, the 3′-amino function in these nucleoside analogues provides a handle for the direct coupling of the sugar moiety in the nucleosides to a Carboxyhexylsepharose matrix[14] through a 3′-amido linkage. This will form the basis for construction of an affinity column that may be used to purify enzymes involved in nucleoside metabolism.

Chemistry. The synthesis of several new 3′-azido and 3′-amino nucleosides (8, 12, 9, and 13) is outlined in Schemes I and II.

Compound 5 was prepared from 1 essentially by the method of Horwitz et al.[15,16] The analytical sample of 3 was obtained as tan fine needles by crystallization from EtOH–Et_2O at −20 °C and was satisfactorily characterized by both NMR and elemental analysis. Pfitzner and Moffatt[17] and Horwitz et al.[15] have independently reported the synthesis of 3. However, in both cases, 3 was not obtained in crystalline form, and no acceptable elemental analysis was reported. Mesylation of 5 afforded the sulfonate 6. Compound 6 was then treated with lithium azide in *N*,*N*-dimethylformamide, followed by detritylation by refluxing with 80% aqueous acetic acid, to yield 3′-azido-2′,3′-dideoxyuridine (8, 3′-N_3-UdR). Catalytic hydrogenation of 8 in the presence of 10% palladium on charcoal in ethanol gave 3′-amino-2′,3′-dideoxyuridine (9). Compounds 8 and 9 are key intermediates for the synthesis of

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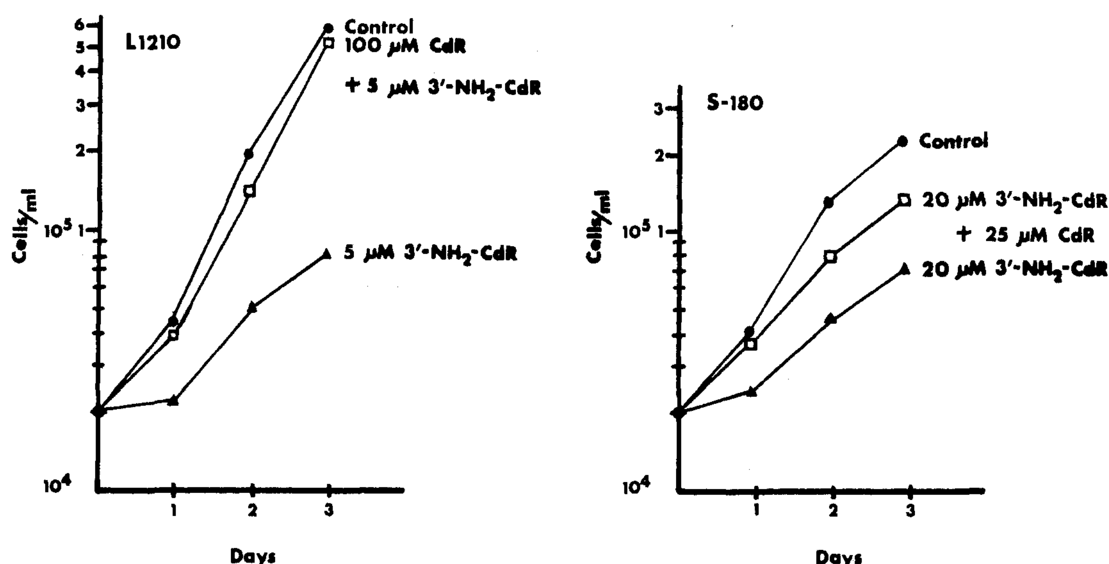

Figure 1. The effect of CdR on the cytotoxicity of 3'-NH_2-CdR (13) against L1210 and S-180 cells in vitro.

Table I. Effect of Various 3'-Azido and 3'-Amino Nucleosides on the Replication of L1210 and S-180 Cells in Vitro

compd	ID_{50},[a] μM	
	L1210	S-180
8 (3'-N_3-UdR)	inactive	
9 (3'-NH_2-UdR)	18	50
12 (3'-N_3-CdR)	inactive	
13 (3'-NH_2-CdR)	0.7	

[a] ID_{50} values were determined from plots of mean cell counts after 72 h. Assays were carried out in triplicate with appropriate controls.

various 5-substituted 3'-azido and 3'-amino nucleoside analogues. Acetylation of **8** (3'-N_3-UdR) with acetic anhydride in pyridine at 4 °C yielded 5'-*O*-acetyl-3'-azido-2',3'-dideoxyuridine (**10**). Reaction of **10** with thionyl chloride and *N*,*N*-dimethylformamide in absolute chloroform at reflux temperature for 6 h afforded the corresponding 4-chloro derivative **11**, which was then converted to 3'-azido-2',3'-dideoxycytidine (**12**, 3'-N_3-CdR) by treatment with saturated methanolic ammonia at room temperature for 6 days. Hydrogenation of **12** with 10% palladium on charcoal as a catalyst gave 3'-amino-2',3'-dideoxycytidine (**13**, 3'-NH_2-CdR).

Biological Evaluation. The effect of these new 3'-azido and 3'-amino nucleosides (**8**, **12**, **9**, and **13**) on the replication of L1210 leukemia cells in culture was investigated. Compounds **13** (3'-NH_2-CdR) and **9** (3'-NH_2-UdR) were also screened against S-180 neoplastic cells in vitro. 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 or S-180 neoplastic cells by 50%. The results are shown in Table I.

Among these compounds, 3'-amino-2',3'-dideoxycytidine (**13**) has shown potent anticancer activity against both L1210 and S-180 cells in vitro (ID_{50} = 0.7 and 4 μM, respectively). More significantly, the cytotoxicity of **13** was found to be *uniquely prevented* by 2'-deoxycytidine and *not* by other pyrimidine deoxyribo- and ribonucleosides. The preventive effect of 2'-deoxycytidine on the cytotoxicity of 3'-NH_2-CdR (**13**) against L1210 cells was dose dependent. At a 5 μM concentration of **13**, the addition of 25 and 100 μM 2'-deoxycytidine prevented the cytotoxicity of **13** against L1210 cells by 35 and 100%, respectively. However, the addition of 25 μM 2'-deoxycytidine to 20 μM **13** prevented the cytotoxicity of **13** against S-180 cells by 27%. These experimental results are illustrated in Figure 1.

Compounds **9** (3'-NH_2-UdR) and **13** (3'-NH_2-CdR) were also screened against CDF_1 female mice bearing L1210 leukemia according to NCI protocol. Three groups of mice with four mice in each group were inoculated with 1 × 10^5 L1210 murine leukemia cells. Starting 24 h after inoculation, the first group of mice was injected with saline as the vehicle control, the second group with 160 mg/kg of **13** (3'-NH_2-CdR), and the third group with 160 mg/kg of **9** (3'-NH_2-UdR) in saline twice a day for 3 days. All control mice died by day 9. The mice treated with compound **9** (3'-NH_2-UdR) died at days 9 and 10, whereas in the 3'-NH_2-CdR (**13**) treated group, one mouse died at day 21, the second at day 24, the third at day 29, and the last one at day 31, yielding a T/C × 100 value of 283.

The preliminary experimental data above indicates that 3'-NH_2-CdR (**13**) exhibited significant anticancer activity against L1210 leukemia in vivo. However, 3'-NH_2-UdR (**9**) was devoid of any anticancer activity in this experiment.

To determine the effect that 3'-NH_2-CdR (**13**) exerts on RNA, DNA, or protein biosynthesis, we grew log-phase L1210 cells in the presence or absence of drug for 2.5 h. At that time either [2-^{14}C]uridine, [2-^{14}C]thymidine, or [U-^{14}C]protein hydrolysate was added, and the amount of the particular radiolabel incorporated was determined.[9] As illustrated in Figure 2, no significant effect was observed on uridine or amino acid incorporation. However, in the presence of 2.5 μM **13**, radiolabeled thymidine incorporation was inhibited by more than 50% when compared to that of the non-drug-treated cells.

This suggests that neither RNA nor protein biosynthesis is altered when cells are exposed to **13**. Furthermore, under these conditions the presence of the drug apparently has no effect on nucleoside or amino acid uptake, since the amount of radiolabeled uridine and amino acid incorporated was the same in both control and drug-treated cells. The most significant finding was a consistent decrease in radiolabeled thymidine incorporation into acid-precipitable material, suggesting that either DNA biosynthesis was altered or that an increased pool of dTTP was responsible for dilution of the radiolabeled dTTP. In any event, normal deoxyribonucleoside metabolism is greatly perturbed under conditions where ribonucleoside metabolism

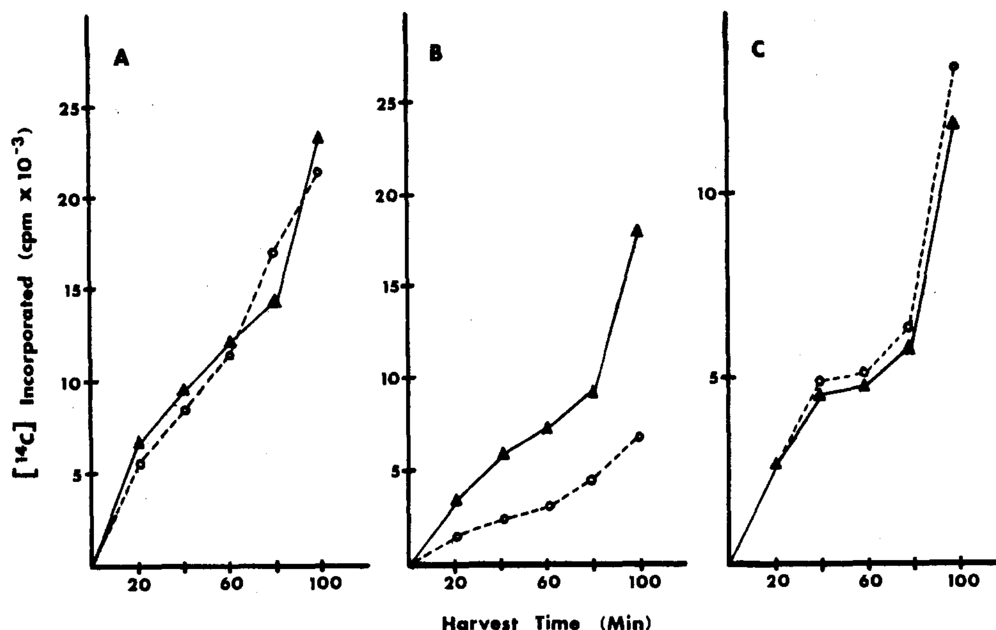

Figure 2. Effect of 3'-NH_2-CdR (**13**) on the biosynthesis of macromolecules of cultured L1210 cells. L1210 cells (2.5×10^5/mL) were incubated in the absence (▲) or presence of (○) of 2.5 μM 3'-NH_2-CdR. After 2.5 h of drug exposure, either (A) 2.6 μM [2-^{14}C]UR (0.15 μCi/mL), (B) 10.7 μM [2-^{14}C]TdR (0.6 μCi/mL), or (C) [U-^{14}C]protein hydrolysate (1 μCi/mL) was added to the medium. Cells were harvested every 20 min, and the amount of radiolabel incorporated into acid precipitable material was determined.

and protein biosynthesis are not significantly affected. It should be emphasized that this effect was observed within 2.5 h of the exposure of **13** to cells.

Experimental Section

Melting points were taken on a Thomas-Hoover Unimelt apparatus and are not corrected. Thin-layer chromatography was performed on EM silica gel 60 F_{254} sheets (0.2 mm). IR spectra were recorded on the Perkin-Elmer-21 spectrophotometer. 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 with Me_4Si as internal reference. 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'-*O*-Methanesulfonyl-5'-*O*-trityl-2'-deoxyuridine (3). Compound **2** (59 g, 0.13 mol) was dissolved at room temperature in 120 mL of pyridine. The solution was put into an ice bath, and 29 mL of methanesulfonyl chloride (0.34 mol) was added dropwise. The resulting solution was allowed to stir at 4 °C for 24 h. The reaction mixture was added in three-portions at a slow flow rate into a Waring commercial blender containing a mixture of ice–water (2:3). The pea-green solid was collected by filtration, washed twice with 2 L of water, and dried to yield 58 g (93%). An analytical sample was obtained by recrystallization of **3** from EtOH–Et_2O at −20 °C. The compound started to soften at 85 °C and melted at 106–108 °C: R_f 0.62 ($CHCl_3$–EtOH, 10:1); NMR (Me_2SO-d_6) δ 2.32–2.70 (m, 2, 2'-H_2), 3.25 (s, 3, CH_3SO_2O), 3.10–3.48 (m, 2, 5'-H_2), 4.21 (m, 1, 4'-H), 5.40 (m, 1, 3'-H), 5.48 (d, 1, 5-H), 6.20 (t, 1, 1'-H), 7.15–7.60 (m, 15, trityl), 7.60 (d, 1, 6-H), 11.35 (br s, 1, 3-NH, D_2O exchangeable). Anal. ($C_{29}H_{28}N_2O_7S$) C, H, N.

1-[2-Deoxy-5-*O*-(triphenylmethyl)-β-D-*threo*-pentofuranosyl]uracil (5). A solution of **3** (45 g, 0.08 mol) in 450 mL of absolute EtOH, 150 mL of 1 N NaOH, and 200 mL of H_2O was refluxed for 4 h at 130 °C (oil bath temperature). The solution was transferred to a 4-L beaker and diluted with about 500 mL of water and ice. While vigorously stirring, the solution was acidified to pH 3 with concentrated HCl. After stirring for 20 min, the precipitate was filtered, washed in 4 L of water, filtered again, and partially recrystallized in hot alcohol to yield 34 g (88%). Compound **5** was further purified by recrystallizing twice from boiling EtOH to give amber-color, plate-like crystals: mp 230–231 °C (lit.[16] 229–230 °C); R_f 0.55 ($CHCl_3$–EtOH, 10:1); UV (MeOH), λ_{max} 260 nm (ε 9920), λ_{min} 240 nm; NMR (Me_2SO-d_6) δ 2.39–2.79 (m, 2, 2'-H), 3.21–3.55 (m, 2, 5'-H), 3.90–4.40 (m, 2, 3'-H and 4'-H), 5.29 (br d, 1, 3'-OH, D_2O exchangeable), 5.58 (d, 1, 5-H), 6.16 (br d, 1, 1'-H), 7.10–7.62 (m, 15, trityl), 7.78 (d, 1, 6-H), 11.18 (br s, 1, 3-NH, D_2O exchangeable). Anal. ($C_{28}H_{26}N_2O_5$) C, H, N.

1-[2-Deoxy-3-*O*-methanesulfonyl-5-*O*-(triphenylmethyl)-β-D-*threo*-pentofuranosyl]uracil (6). Compound **5** (26.0 g, 0.054 mol) was dissolved in 100 mL of pyridine and then placed into an ice bath. Methanesulfonyl chloride (14.5 mL, 0.187 mol) was slowly added dropwise. After stirring at room temperature for 24 h, the solution was blended in a mixture of ice–water (2:3) by using a Waring commercial blender. The tan solid was collected by filtration, washed with 4 L of water, and filtered, and the filtrate was dried to afford 26 g (91%). The product was purified by silica gel column chromatography (EM 70–230 mesh, 4 × 60 cm, $CHCl_3$–EtOH, 8:1) to yield pale-yellow, find needles. Compound **6** softened above 140 °C and melted at 152–155 °C: R_f 0.52 ($CHCl_3$–EtOH, 8:1); UV (MeOH) λ_{max} 260 nm (ε 9180), λ_{min} 240 nm; NMR (Me_2SO-d_6) δ 2.30–2.81 (m, 2, 2'-H), 3.10 (s, 3, CH_3SO_2O), 3.18–3.52 (m, 2, 5'-H), 4.37 (m, 1, 4'-H), 5.32 (m, 1, 3'-H), 5.58 (d, 1, 5-H), 6.15 (m, 1, 1'-H), 7.10–7.68 (m, 16, trityl and 6-H), 11.42 (br s, 1, 3-NH, D_2O exchangeable). Anal. ($C_{29}H_{28}N_2O_7S$) C, H, N.

3'-Azido-5'-*O*-trityl-2',3'-dideoxyuridine (7). A mixture containing **6** (26. g, 47.8 mmol) and lithium azide (7.0 g, 143 mmol) in 60 mL of DMF was heated at 85 °C for 2 h. The solution was allowed to cool before being added, in two portions, to a blender containing ice-water. The filtered crystals were washed in 1 L of water and filtered, and the filtrate was dried to yield 22 g (86%). The crude product was purified by recrystallization twice from EtOH to give pale yellow needles: mp 175–176 °C; R_f 0.59 ($CHCl_3$–EtOH, 10:1); IR (KBr) 4.75 μm (azido); UV (MeOH) λ_{max} 260 nm (ε 9970), λ_{min} 240 nm; NMR (Me_2SO-d_6) δ 2.44 (br t, 2, 2'-H), 3.06–3.48 (m, 2, 5'-H), 3.88 (m, 1, 4'-H), 4.46 (m, 1, 3'-H), 5.44 (d, 1, 5-H), 6.09 (t, 1, 1'-H), 7.10–7.56 (m, 15, trityl), 7.68 (d, 1, 6-H), 11.29 (br s, 1, 3-NH, D_2O exchangeable). Anal. ($C_{28}H_{25}N_5O_4$) C, H, N.

3'-Azido-2',3'-dideoxyuridine (8). A suspension of **7** (13 g, 26 mmol) in 50 mL of 80% acetic acid was refluxed at 110 °C for 20 min. The solution was permitted to slowly cool to room temperature, at which time some trityl alcohol precipitated out. Water, 10 mL, was added to aid in the precipitation, and the mixture was filtered. The filtrate was clarified by stirring with charcoal (activated powder, Mallinckrodt) for 20 min. After two filtrations, the filtrate was concentrated under reduced pressure (35 °C) until it yielded tan crystals. The product was filtered,

and the filtrate was continuously concentrated until it no longer produced the solid. After washing with EtOH and Et_2O and drying in the oven, 4.6 g (70%) of 7 was collected: mp 161–163 °C dec; R_f 0.18 ($CHCl_3$–EtOH, 10:1); IR (KBr) 4.80 μm (azido); UV (0.01 N HCl) λ_{max} 260 nm (ϵ 10760), λ_{min} 232 nm; UV (0.01 N NaOH) λ_{max} 260 nm (ϵ 8470), λ_{min} 240 nm; NMR (Me_2SO-d_6) δ 1.81–2.06 (m, 2 H, 2′-H), 2.96–3.19 (m, 2, 5′-H), 3.38–3.42 (m, 1, 4′-H), 3.91–3.97 (m, 1, 3′-H), 4.77 (t, 1, 5′-OH, D_2O exchangeable), 5.20 (d, 1, 5′-H), 5.63 (t, 1, 1′-H), 7.40 (d, 1, 6-H), 10.89 (br s, 1, 3-NH, D_2O exchangeable). Anal. ($C_9H_{11}N_5O_4$) C, H, N.

3′-Amino-2′,3-dideoxyuridine (9). A solution of 8 (2.8 g, 10.9 mmol) in 150 mL of EtOH was hydrogenated under 50 psi for 2 h in the presence of 10% palladium on charcoal (0.5 g). At the end of the reduction, Norite was added to the mixture and stirred for 30 min. After two filtrations, the filtrate was concentrated to a glass (35 °C). The residue was crystallized from EtOH–Et_2O to yield 2.1 g (86%) of a fine tan powder, which softened around 156 °C and decomposed at 163 °C: ninhydrin test positive; UV (0.01 N HCl) λ_{max} 260 nm (ϵ 9060), λ_{min} 229 nm; UV (0.01 N NaOH) λ_{max} 260 nm (ϵ 7450), λ_{min} 240 nm; NMR (Me_2SO-d_6) δ 1.95–2.12 (m, 2 H, 2′-H), 3.32–3.39 (m, 3, 4′-H, and 3′-NH_2, D_2O exchangeable), 3.52–3.65 (m, 3, 3′-H and 5′-H), 4.80 (br s, 1, 5′-OH, D_2O exchangeable), 5.59 (d, 1, 5-H), 6.05 (t, 1, 1′-H), 7.90 (d, 1, 6-H). Anal. ($C_9H_{13}N_3O_4$) C, H, N.

3′-Azido-5′-*O*-acetyl-2′,3′-dideoxyuridine (10). Acetic anhydride (19 mL, 0.20 mol) was added dropwise to a solution of 8 (5.0 g, 19.8 mmol) in 40 mL of pyridine at 0 °C. After stirring at 4 °C for 24 h, the reaction mixture was quenched by slowly adding 15 mL of water (ice bath). The solvents were removed under reduced pressure (35 °C), leaving a thick syrup. This residue was then dissolved in 50 mL of $CHCl_3$ and washed with water (4 times), saturated $NaHCO_3$ solution (2 times), and water again (2 times). The $CHCl_3$ solution was clarified with charcoal (activated powder, Mallinkckrodt). After filtration, the solution was dried with anhydrous $MgSO_4$. After the solution was filtered, the filtrate was concentrated (35 °C) and dried under reduced pressure overnight to yield 4.2 g (71%) of crude product, which was purified by passing through a silica gel column (EM 70–230 mesh, 4 × 60 cm, Me_2CO–CH_2Cl_2, 3:7). The fractions containing 10 were collected (R_f 0.36), and the combined solution was evaporated to dryness in vacuo to give a colorless glassy syrup. Attempts to crystallize 10 from various solvents were unsuccessful. However, an analytical sample was obtained by further drying the syrup under reduced pressure (0.1 mmHg) for 3 days: IR (film) 4.80 μm (azido); UV (MeOH), λ_{max} 260 nm (ϵ 9190), λ_{min} 230 nm; NMR ($CDCl_3$) δ 2.12 (s, 3, CH_3CO_2), 2.49 (br t, 2, 2′-H), 3.92–4.53 (m, 4, 3′-H, 4′-H, and 5′-H), 5.74 (d, 1, 5-H), 6.01 (t, 1, 1′-H), 7.46 (d, 1, 6-H), 10.18 (br s, 1, 3-NH, D_2O exchangeable). Anal. ($C_{11}H_{13}N_5O_5$) C, H, N.

5′-*O*-Acetyl-3′-azido-2′,3′-dideoxy-4-chlorouridine (11). The glassy residue of 10 (4.2 g, 14.0 mmol) was dissolved in 60 mL of absolute $CHCl_3$, to which 9.5 mL of thionyl chloride and 0.6 mL of dry DMF were added. The reaction mixture was stirred under reflux for 6 h at 90 °C, after which it was concentrated (35 °C) to dryness. This residue was coevaporated (5 times) with 80-mL portions of toluene. Following the final concentration, the residue was put under reduced pressure (40 °C) for 30 min. The product was used immediately for the next reaction.

3′-Azido-2′,3′-dideoxycytidine (12). Compound 11 was dissolved in 100 mL of absolute MeOH saturated with NH_3 at 0 °C. The solution was allowed to stir at room temperature in a pressure bottle for 6 days. After the elapsed time, the reaction mixture was cooled to 0 °C before opening. The solvent was evaporated (30 °C) under reduced pressure to yield a glassy residue, which was dissolved in 150 mL of MeOH and clarified with charcoal (activated powder, Mallinckrodt). After the solution was filtered, the filtrate was reduced to a small volume. This concentrated solution was then applied directly to a column (3.5 × 94 cm) packed with EM silica gel 60, eluting with MeOH–$CHCl_3$ (2:3). The desired fractions with an R_f of 0.65 were collected and concentrated. The product was further purified on a Waters Prep LC/System 500A, with a 500/silica cartridge, with a $CHCl_3$–MeOH (4:1) solvent system. The fractions (R_f 0.65) were collected and concentrated to about 50 mL and clarified with charcoal (activated powder, Mallinckrodt). After filtering, the solution was evaporated to yield 1.1 g (31%) of a yellow glass. The analytically pure sample was obtained by crystallization from EtOH–Et_2O. The compound softened at 92 °C, effervesced around 140 °C, and melted at 160–162 °C: R_f 0.65 (MeOH–$CHCl_3$, 2:3); IR (film), 4.80 μm (azido); UV (0.01 N HCl) λ_{max} 278 nm (ϵ 13460), λ_{min} 238 nm; UV (0.01 N NaOH) λ_{max} 268 nm (ϵ 11070), λ_{min} 246 nm; NMR (Me_2SO-d_6) δ 2.17–2.34 (m, 2, 2-H), 3.58–3.62 (m, 2, 5′-H), 3.82–3.86 (m, 1, 4′-H), 4.30–4.37 (m, 1, 3′-H), 5.17 (t, 1, 5′-OH, D_2O exchangeable), 5.72 (d, 1, 5-H), 6.07 (t, 1, 1′-H), 7.16 (br d, 2, 4-NH_2, D_2O exchangeable), 7.78 (d, 1, 6-H). Anal. ($C_9H_{12}N_6O_3$) C, H, N.

3′-Amino-2′,3′-dideoxycytidine (13). A solution of 12 (1.11 g, 4.40 mmol) in 100 mL of MeOH was hydrogenated under 50 psi for 2 h in the presence of 10% palladium on charcoal (0.5 g). After filtration, the filtrate was concentrated to give a glassy residue. White crystals (0.65 g, 65%) were achieved by dissolving the residue in a minimal amount of MeOH and then slowly adding $CHCl_3$ and Et_2O. The compound began to darken in color at 184 °C until it melted at 207 °C: ninhydrin test positive; UV (0.01 N HCl) λ_{max} 277 nm (ϵ 11790), λ_{min} 238 nm; UV (0.01 N NaOH) λ_{max} 268 nm (ϵ 7820), λ_{min} 246 nm; NMR (Me_2SO-d_6) δ 1.90–2.08 (m, 2, 2′-H), 3.27–3.34 (m, 3, 4′-H and 3′-NH_2, D_2O exchangeable), 3.51–3.66 (m, 3, 3′-H and 5′-H), 4.94 (br s, 1, 5′-OH, D_2O exchangeable), 5.69 (d, 1, 5-H), 6.04 (t, 1, 1′-H), 7.06 (br d, 2, 4-NH_2, D_2O exchangeable), 7.87 (d, 1, 6-H). Anal. ($C_9H_{14}N_4O_3$) C, H, N.

Biological Test Procedures. Mouse L1210 and S-180 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 and S-180 cells is approximately 12 and 18 h, respectively. 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 test compounds, was determined after 24, 48, and 72 h of growth.

Transplantation of L1210 ascites cells was carried out by withdrawing peritoneal fluid from donor CDF_1 mice bearing 7-day growths. The suspension was centrifuged for 2 min (1600*g*), the supernatant peritoneal fluid was decanted, and a 10-fold dilution with isotonic saline was made. The cell number was determined with a Coulter particle counter, and the cell population was adjusted to 10^6 cells/mL. One-tenth milliliter of the resulting cell suspension (containing approximately 10^5 cells) was injected intraperitoneally into each animal. Drugs were administered by intraperitoneal injection, beginning 24 h after tumor implantation, twice daily for 3 consecutive days. The test compounds were injected as a solution in isotonic saline. All drugs were administered intraperitoneally in a volume of 0.25 mL. For any one experiment, animals were distributed into groups of four mice of comparable weight and maintained throughout the course of the experiment on Purina Laboratory Chow pellets and water "ad libitum". Controls given injections of a comparable volume of vehicle were included in each experiment. Mice were weighed during the course of the experiments, and the percentage change in body weight from onset to termination of therapy was used as an indication of drug toxicity. Determination of the sensitivity of ascitic neoplasms to these agents were based on the prolongation of survival time afforded by the drug treatments.

In the experiments of the effect of 13 on the biosynthesis of macromolecules of cultured L1210 cells, [2-^{14}C]uridine (58 mCi/mmol) and [2-^{14}C]thymidine (56 mCi/mmol) were obtained from Moravek Biochemicals, Inc. [U-^{14}C]Protein hydrolysate (57 mCi/milliatom) was obtained from Amersham Corp.

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