Incorporation of Nucleosides into RNA and DNA. Three milliliters of an exponentially growing cell suspension (4–5 × 10^5 cells/mL) was treated with the appropriate radioactive nucleoside for 1 h at 37 °C. Cells were harvested by centrifugation (1000*g*, 5 min) at 4 °C and washed with 5 mL of ice-cold phosphate-buffered saline. Cells were mixed with 10 volumes of ice-cold 5% TCA, the precipitate was collected by suction onto 2.4-cm Whatman GF/C glass-fiber filters, and the filters were washed twice with 2.5 mL of ice-cold 5% TCA. The filters were then placed into glass scintillation vials to which was added 0.5 mL of Protosol (New England Nuclear, Boston, MA). After incubation of the tightly covered vials at 68 °C for 30 min, 0.05 mL of glacial acetic acid was added, followed by 10 mL of scintillation fluid, and the vials were counted.

Metabolism of [6-^{3}H]NO_2dUrd. Exponentially growing L1210 cells were harvested and resuspended at a density of 2 × 10^6 cells/mL in 3 mL of fresh media containing [6-^{3}H]NO_2dUrd (20 Ci/mmol; final concentration 165 nM). The cells were maintained at 37 °C in a CO_2 incubator for 2 h, at which time the cells were pelleted at 4 °C (1000*g*, 5 min) and washed twice with ice-cold phosphate-buffered saline. The cell pellet was suspended in 0.3 mL of this buffer, and a cell homogenate was prepared by sonication of the cell suspension (Brownwill Biosonik, PI = 40, 20 s). Cell debris was removed by centrifugation (4 °C, 1000*g* for 5 min). An aliquot of cytosol (0.2 mL) was applied to a Sephadex G-25 column (0.7 × 13 cm) equilibrated at 4 °C with 20 mM sodium phosphate (pH 7.3) and 10 mM β-mercaptoethanol. Column fractions (0.4 mL) were collected, and aliquots were analyzed for radioactivity. A clean separation was obtained between radioactivity bound to macromolecules eluting in the void volume and low-molecular-weight metabolites (see text). The pooled high-molecular-weight fraction was heated at 65 °C for 20 min and analyzed by repassage through Sephadex G-25. Fractions were collected and analyzed for radioactivity by liquid scintillation counting. The low-molecular-weight fraction from each Sephadex G-25 chromatography was pooled and taken to dryness by lyophilization; the residue was dissolved in water and analyzed by HPLC with a Lichrosorb RP-18 column (4.6 × 250 mm) with 5 mM $(n\text{-Bu})_4N^+HSO_4^-$, 5 mM potassium phosphate, pH 7.1, 20% MeOH. The retention volumes of authentic standards in this system are: NO_2dUrd, 7 mL; NO_2Ura, 12 mL; NO_2dUMP, 19 mL.

Nucleoside Triphosphate Pools. Exponentially growing L1210 cells were treated with specified amounts of NO_2dUrd or FdUrd. At intervals of 1.5, 3, and 5 h, acid-soluble fractions were prepared and analyzed for ribo- and deoxyribonucleoside triphosphates as previously described.[15]

Antineoplastic Activity in Vivo. Female CDH_1 mice were inoculated intraperitoneally with 0.1 mL of a suspension of L1210 cells (10^6 cells/mL). The cells were taken from a 7-day ascites tumor-bearing animal and were diluted with normal saline. After all of the animals had been inoculated, they were divided into groups of five, such that each group had approximately the same mean weight. Drugs were suspended or dissolved in saline containing 5% ethanol and 1 drop of 20% aqueous Tween-80 per 12 mL at the concentration used for the highest doses. Lower doses were obtained by serial dilution with saline. Drugs (in 0.5 mL) were injected ip on days 1 to 6 after tumor implantation. Control animals received 0.5 mL of saline. Each group of animals was weighed on the day following the last injection (day 7), and the difference in weight from the day of implantation (day 0) was noted and used as a measure of toxicity.

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Synthesis and Biological Evaluation of 6-Ethynyluracil, a Thiol-Specific Alkylating Pyrimidine

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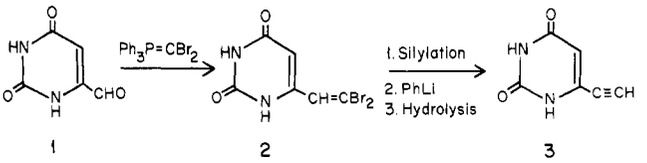
6-Ethynyluracil (**3**) was prepared by two different synthetic procedures. In one approach, 6-formyluracil was reacted with (dibromomethylene)triphenylphosphorane to give 6-(2,2-dibromovinyl)uracil (**2**), which was silylated and treated with phenyllithium to yield **3**. Alternatively, silylated 6-iodouracil was reacted with trimethylsilylacetylene in dry triethylamine in the presence of a palladium/copper catalyst to give 6-[(trimethylsilyl)ethynyl]uracil (**5**). Compound **5** was converted to **3** in refluxing methanol. At neutral pH, **3** reacted with thiols, such as glutathione, 2-mercaptoethanol, and L-cysteine, but did not react with glycine or L-lysine. This reaction was accompanied by a shift in the UV maximum of **3** from 286 nm to 321–325 nm. The reaction of **3** with 2-mercaptoethanol gave *cis*-6-[2-[(2-hydroxyethyl)-thio]vinyl]uracil as the predominant product. Compounds **2** and **3** inhibited the growth of leukemia L1210, B-16 melanoma, and lewis lung carcinoma cells at concentrations ranging from 1 × 10^{-6} to 2 × 10^{-5} M. As determined with L1210 cells, the inhibition of growth caused by **2** and **3** was not prevented by the natural pyrimidines, indicating that the agents do not act as antimetabolites.

Previous work in this laboratory on the development of new pyrimidine analogues and their nucleoside derivatives led to the synthesis of 5-ethynyluracil nucleosides,[1] which showed in vitro activity against leukemia L1210 cells at concentrations as low as 2 × 10^{-8} M. In a related effort, we have introduced the ethynyl group at the 6-position of uracil with the aim of generating an analogue of orotic acid. The synthesis and biological evaluation of 5-ethynyluracil nucleosides have also been carried out in various other laboratories,[2,3] and the preparation of 5-ethynylorotic acid

Scheme I



1 2 3

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Scheme II

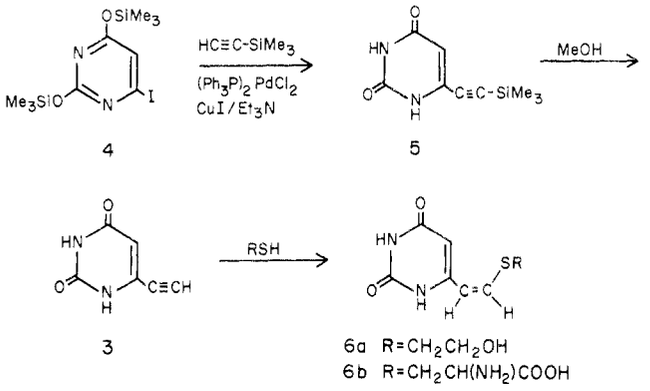

Table I. Effects of 6-Ethynyl- (3) and 6-(2,2-Dibromovinyl)uracil (2) on the in vitro Growth of Three Tumor Cell Lines

	concn, M, for 50% growth inhibn of		
no.	leukemia L1210	lewis lung	B16 melanoma
2	1×10^{-6}	8×10^{-6}	1×10^{-6}
3	7×10^{-6}	2×10^{-5}	1×10^{-5}

has recently been reported.[4] In this paper we describe two alternative procedures for preparing 6-ethynyluracil (3) and provide information on its biological activity and on its ability to interact with thiols. A preliminary report of some aspects of this paper has been given.[1c,5]

Chemistry. The initial synthesis of 3 was carried out as outlined in Scheme I. 6-Formyluracil (1), obtained by selenium dioxide oxidation of 6-methyluracil, was reacted with (dibromomethylene)triphenylphosphorane, followed by silylation and treatment with phenyllithium to give 3 in 22.5% overall yield.

A somewhat more rapid synthetic procedure (Scheme II) was subsequently applied, based on the approach by Edo et al.[6], who employed palladium/copper to catalyze the introduction of acetylenic functions into iodopyrimidines. 2,4-Bis[(trimethylsilyl)oxy]-6-iodopyrimidine (4), prepared by silylation of 6-iodouracil with hexamethyldisilazane, was reacted with trimethylsilylacetylene in anhydrous triethylamine in the presence of Pd/Cu catalyst to yield 6-[(trimethylsilyl)ethynyl]uracil (5). An increased amount of trimethylsilylacetylene and Pd/Cu and an increased proportion of copper were used, in analogy with conditions employed by Robins and Barr.[7] Compound 5, when heated in refluxing methanol, gave 3 in an overall yield of 65% (based on 6-iodouracil).

Since the cytotoxic effect of 3 to L1210 cells (Table I) was not diminished in the presence of natural pyrimidines, the possibility that the compound may alkylate biological nucleophiles was examined. Glutathione (GSH) was found to readily react with 3 in buffer at pH 7.5 or in water at neutral pH, causing a time-dependent shift in UV absorption maxima from 286 to 321 nm, with a shoulder occurring at 292 nm (Figure 1).

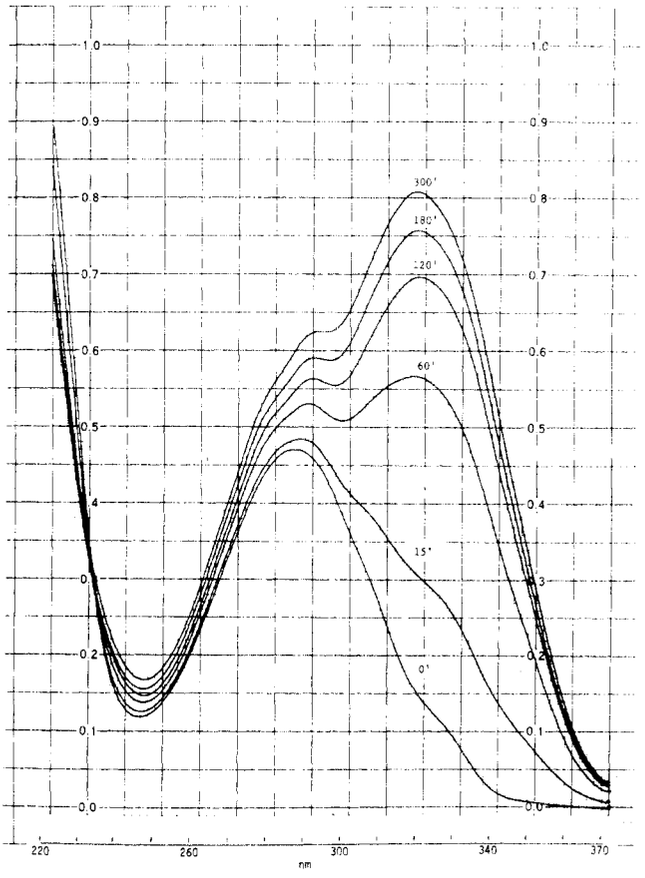

Figure 1. UV spectra of the reaction between 6-ethynyluracil (3) and glutathione as a function of time at 37 °C. The initial mixture contained 1.2×10^{-4} M 3 and 3×10^{-4} M reduced glutathione in 0.05 M phosphate buffer, pH 7.5.

To examine whether adducts are formed with other thiols, we reacted 2-mercaptoethanol and L-cysteine with 3 in water at room temperature. The product that precipitated from a solution of 2-mercaptoethanol (ME) and 3 displayed UV absorption maxima of 325 and 291 nm at pH 7.5. The NMR data suggested structure **6a** (Scheme II) for the predominant component, a minor amount (15–20%) of assumed trans isomer being present as well. Fractional crystallization from water gave analytically pure (cis) **6a**. The vinylic protons of **6a** displayed a coupling constant of 11.1 Hz, whereas the assumed trans isomer showed a coupling constant of 15.7 Hz for these protons. These values are consistent with cis and trans vinylic proton coupling.

The supernatant solution that remained after separation of crude **6a** was lyophilized, and NMR spectra of the residue indicated the presence of **6a**, its trans isomer, and oxidized mercaptoethanol in a 1:1:1 molar ratio.

L-Cysteine in a solution of 3 in water gave, after standing overnight at room temperature, a sparingly soluble precipitate that showed a λ_{max} of 321 nm with a shoulder at 290 nm in pH 7.5 buffer. The NMR spectrum displayed two sets of doublets that are suggested to correspond to **6b** (Scheme II) and its trans isomer. These doublets resemble in their chemical shifts and coupling constants the corresponding proton resonance signals of **6a** and its trans isomer. Upon attempted recrystallization of the precipitate from hot water, a change in its UV spectrum occurred, a

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new maximum appearing at 264 nm with diminution of the absorption at 321 nm. Under controlled conditions in buffer at pH 7.5 at 100 °C, the shift from 321 to 264 nm was complete in 1–2 min. An identical shift occurred when the precipitate was shaken with buffer at room temperature, the undissolved solid was filtered, and the filtrate was kept at room temperature overnight. The shift in the UV spectrum suggests loss of conjugation of the exocyclic double bond with the uracil ring, an assumption that is supported by the absence of vinylic protons in the NMR spectrum. Diminution of the absorption peak at 321–325 nm and appearance of a new peak at 267–270 nm also occurred when **6a** or the addition product of **3** and glutathione were heated in pH 7.5 buffer for 3 h. Whereas these spectral changes indicate loss of the double bond, the mechanism by which this process occurs is unclear.

Selectivity of the reaction for sulfhydryl groups is suggested by the observation that other amino acids, such as glycine or L-lysine, did not react with **3** at 37 °C and pH 7.5. This difference may result from the fact that near physiological pH the amino groups of these amino acids are extensively protonated.

Although the intermediate **2** exhibited biological activity, it did not show the same reactivity toward thiols as did **3**. In the presence of ME and phosphate buffer, pH 7.5 for 4 h at 37 °C, the UV spectrum of **2** did not differ significantly from that of the control under these conditions. Cysteine caused a marked diminution at pH 7.5 of the 291-nm maximum and a shift in the 258-nm maximum to 262 nm. The basis for these spectral changes remains undetermined.

Biological Evaluation. As shown in Table I, **2** and **3** are moderately effective inhibitors of the in vitro growth of three different tumor cell lines. To evaluate whether the agents act as antimetabolites, we added the natural pyrimidines, including orotate, and purines to the L1210 growth medium containing the drug. No reversal of inhibition was observed under these conditions, suggesting that the activity of the agents is not specifically directed against the pyrimidine or purine pathways. The fact that compound **3** had the same biological activity when prepared by two different methods eliminates contamination with traces of catalyst or other reactants as a source of such activity. To eliminate selenium contamination as a factor, compound **2** was silylated and distilled, and the distillate hydrolyzed back to **2**.

Experimental Section

Melting points were determined on either a Fisher-Johns or Mel-Temp melting point apparatus and are uncorrected. UV spectra were measured in 0.05 M potassium phosphate buffer, pH 7.50, on a Beckman Model 25 spectrophotometer. NMR spectra were taken on Varian EM-390 and XL-100 spectrometers, with Me_4Si as the internal standard. IR spectroscopy was performed on a Perkin-Elmer 197 spectrophotometer. Mass spectra were obtained on a Finnegan Model 4000 mass spectrometer. Thin-layer chromatography was carried out on silica gel 60 F_{254} (E. Merck) with solvent system A, CH_2Cl_2–Me_2CO (2:1), and on Polygram CEL 300 UV_{254} cellulose (Macherey–Nagel) with solvent system B, *n*-PrOH–H_2O (60:40). Dry column chromatography was performed using silica Woelm DCC (ICN 404526). Elemental analyses, obtained from Robertson Laboratory, Florham Park, NJ, were within 0.40% of theoretical values.

6-(2,2-Dibromovinyl)uracil (2). (Dibromomethylene)triphenylphosphorane was prepared by the method of Corey and Fuchs.[8] A mixture of triphenylphosphine (52.5 g, 0.200 mol), CBr_4 (66.3 g, 0.200 mol), and Zn dust (13.1 g, 0.200 mol) in dry CH_2Cl_2 (500 mL) was stirred at room temperature for 30 h. To this mixture was added 6-formyluracil[9] (1; 14.0 g, 0.100 mol) dissolved in a minimum amount of dry DMF. The mixture was stirred overnight at room temperature and filtered. The precipitate was suspended in $CHCl_3$ and filtered, yielding 11.2 g (38%) of **2**. Another 5.1 g (17%) of **2** was obtained from the combined filtrates after evaporation under reduced pressure, followed by suspending the thick residue in $CHCl_3$ prior to filtering. We prepared an analytical sample by dissolving **2** in hot MeOH–H_2O (5:1) and filtering, after treatment with charcoal, to yield crystals of **2**: mp 274–275 °C; NMR (Me_2SO-d_6) δ 5.83 (s, 1, C_5 H), 7.40 (s, 1, vinylic CH) 10.87 and 11.20 (m, 2, NH); UV (pH 7.5) λ_{max} 258 nm (ϵ 8600), 291 (8400). Anal. ($C_6H_4Br_2N_2O_2$) C, H, Br, N.

6-Ethynyluracil (3). Method A (Scheme I). Compound **2** (5.9 g, 0.020 mol) and $(NH_4)_2SO_4$ (0.100 g) were combined with 1,1,1,3,3,3-hexamethyldisilazane (HMDS; 20 mL) and dry toluene (200 mL), and the mixture was heated at reflux until the solid was dissolved. After cooling, the solution was evaporated under reduced pressure. Dry toluene was added to the resulting oil, followed by evaporation. This process was repeated three times. Dry THF (100 mL) was added and cooled in a dry ice–acetone bath, followed by the addition of phenyllithium (25 mL of a 2 M solution in cyclohexane–Et_2O, 70:30) through a rubber septum. After the solution was stirred for 1 h, the bath temperature was raised to 0 °C, and after an additional hour, MeOH (25 mL) was added, followed by H_2O (50 mL). The solution was neutralized with Dowex 50 (H^+) ion-exchange resin and filtered, and the filtrate was evaporated to dryness. Crystallization of the residue from MeOH yielded 1.1 g (41%) of **3**: mp > 300 °C; NMR (Me_2SO-d_6) δ 4.91 (s, 1, C≡CH), 5.77 (s, 1, C_5 H), 11.17 (m, 2, NH); UV (pH 7.5) λ_{max} 286 nm (ϵ 8200); MS, *m/e* (relative intensity) 136 (M^+, 100), 93 (87), 65 (16), 64 (13), 50 (19); IR (KBr) 2115 (C≡C) cm^{-1}. Anal. ($C_6H_4N_2O_2$) C, H, N.

Method B (Scheme II). A mixture of 6-iodouracil (505 mg, 2.12 mmol; Vega Biochemicals), HMDS (10 mL), and trimethylsilyl chloride (1.0 mL) was refluxed with stirring for 2 h. After cooling to room temperature, the mixture was concentrated under reduced pressure, and the residue was dissolved in dry toluene (5 mL). The solvent was removed in vacuo, and the process was repeated twice more. The residue was dissolved in dry Et_3N (10 mL) containing trimethylsilylacetylene (571 mg, 5.82 mmol), and CuI (33 mg) and bis(triphenylphosphine)palladium(II) chloride (33 mg) were added. The reaction flask was flushed with N_2 and stoppered, and the mixture was stirred at room temperature overnight. Absolute EtOH (3 mL) was added, and the mixture was stirred for 5 min. The solvents were removed in vacuo at room temperature, and the residue was mixed with Me_2CO (20 mL) and heated to boiling. After filtration through Celite Filter Aid, the residue was washed with Me_2CO, and the combined filtrates were concentrated at room temperature. TLC (A) of the residue showed a product at R_f 0.54 and the absence of 6-iodouracil. The product was stored overnight at −20 °C and was then mixed with hot EtOAc (40 mL) and filtered from a small amount of insoluble brown solid. The filtrate and EtOAc washings of the solid were combined and adsorbed onto dry column silica gel (10 g) using a rotary evaporator. The gel was then placed on top of a silica gel dry column (120 g, 2.5 × 40 cm) and eluted with EtOAc. The first 15.5 mL was discarded, and the next 98 mL was combined and concentrated to give a yellow solid (289 mg, 65%), shown by NMR and TLC to be predominantly **5** but containing a small amount of **3** (~10% or less), apparently formed by desilylation of **5** on silica gel: NMR (Me_2CO-d_6) δ 0.24 (s, 9, $SiMe_3$), 5.70 (s, 1, C_5H); MS, *m/e* (relative intensity) 208 (M^+, 31), 193 (100).

This mixture (277 mg) was dissolved in MeOH (20 mL) and refluxed with stirring until essentially all of **5** was converted to **3** (~9 h): the R_f values of **5** and **3** on TLC (A) are 0.54 and 0.31, respectively. Norit-A was added, and the solution refluxed with stirring for 5 min before filtering through Celite Filter Aid. Charcoal and Celite were washed with hot MeOH, and the combined filtrate and washings were concentrated to give 186 mg (65% yield based on 6-iodouracil) of light yellow powder of **3**. Crystallization of **3** from EtOH yielded 116 mg of a tan solid shown by UV, IR, NMR, MS, and elemental analysis to be identical with

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the product obtained by method A.

***cis*-6-[2-[(2-Hydroxyethyl)thio]vinyl]uracil (6a).** Compound **3** (82 mg, 0.60 mmol) was dissolved in hot H_2O (8 mL) and cooled to room temperature prior to the addition of 2-mercaptoethanol (0.042 mL, 0.60 mmol). The solution was stirred for 24 h at room temperature, additional 2-mercaptoethanol (0.01 mL) was added, and the mixture was stirred overnight. The precipitate was removed by centrifugation, washed with H_2O (2.5 mL), and dried. The light tan powder (99 mg, 77%) obtained was shown by NMR to consist of a mixture of cis/trans isomers approximately 5:1, mp 235–239 °C. Crystallization and recrystallization of 61 mg of the solid from hot H_2O yielded analytically pure **6a** (37 mg) as fine, light yellow needles: mp 245–246 °C; NMR (Me_2SO-d_6) δ 2.99 (t, 2, CH_2S, J = 6.0 Hz), 3.61 (q, 2, CH_2OH, J = 5.7 Hz), 4.93 (t, 1, OH, J = 5.3 Hz), 5.58 (s, 1, C_5 H), 5.92 (d, 1, vinylic CH, J = 11.1 Hz), 7.17 (d, 1, vinylic CH, J = 11.1 Hz), 10.67 and 10.86 (m, 2, NH). The quartet at δ 3.61 collapses to a triplet (J = 6.0 Hz) after D_2O exchange: UV (pH 7.5) λ_{max} 325 nm (ϵ 13 000), 291 (ϵ 9300); MS, m/e 214 (13, M^+), 169 (84), 126 (100), 98 (44). Anal. ($C_8H_{10}N_2O_3S$) C, H, N, S.

The combined supernatant and washings remaining after initial removal of the isomers was lyophilized, dissolved in MeOH, and concentrated to give a yellow solid (23 mg). By NMR, this material was indicated to consist of approximately a 1:1:1 mixture of the cis isomer (**6a**), trans isomer, and oxidized 2-mercaptoethanol: NMR (Me_2SO-d_6, trans isomer) δ 2.87 (m, CH_2S), 3.58 (m, CH_2OH), 4.80 (br m, OH), 5.42 (s, C_5 H), 6.00 (d, vinylic CH, J = 15.7 Hz), 7.61 (d, vinylic CH, J = 15.7 Hz), 10.72 (br m, NH).

Cysteine-6-Ethynyluracil Addition Product. L-Cysteine hydrochloride hydrate (113 mg, 0.64 mmol) was dissolved in H_2O (0.4 mL), neutralized with 1 N NaOH, and added to a solution of **3** (73 mg, 0.54 mmol) in H_2O (7 mL) at room temperature. The flask was flushed with N_2, and the mixture was stirred at room temperature overnight. TLC of the ensuing supernatant showed the absence of **3**. The precipitate was removed by centrifugation, washed with a 2.5- and a 1.5-mL aliquot of H_2O, and dried to give a light tan solid (113 mg), which appeared as a single spot on TLC (B). NMR indicated the presence of cis and trans isomers: NMR (Me_2SO-d_6) δ 5.50 (s, C_5 H, trans), 5.65 (s, C_5 H, cis), 5.98 (d, vinylic CH, cis, J = 11.2 Hz), 6.14 (d, vinylic CH, trans, J = 16.0 Hz), 7.25 (d, vinylic CH, cis, J = 11.2 Hz), 7.71 (d, vinylic CH, trans, J = 16.0 Hz); UV (pH 7.5) λ_{max} 321 nm, 290 (sh).

Biological Assay Procedures. Evaluation of the effect of the agents on the growth of leukemia L1210 was carried out by previously described procedures.[10] B-16 melanoma and lewis lung carcinoma were grown in monolayer cultures in RPMI 1640 medium containing 5% heat-inactivated calf serum and 20 mM Hepes buffer. The initial innoculum of 2.5×10^4 cells was incubated at 37 °C for 1 day before addition of drug, at which time the number of cells had approximately doubled. After 3 days of further incubation, the growth medium was poured off, the cells were rinsed twice with saline, and protein was determined by the Lowry procedure.[11] The lewis lung carcinoma cells were scraped from the disks with a rubber policeman and washed twice with saline with centrifugation for 3 min at 1500 rpm, and Lowry protein determinations were performed on the washed pellets.

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Synthesis of 4-Amino-1-β-D-ribofuranosyl-1*H*-pyrrolo[2,3-*b*]pyridine (1-Deazatubercidin) as a Potential Antitumor Agent

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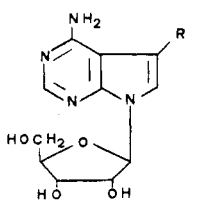
The synthesis of 4-amino-1-β-D-ribofuranosyl-1*H*-pyrrolo[2,3-*b*]pyridine, a deaza analogue of the antitumor antibiotic tubercidin, starting from 1*H*-pyrrolo[2,3-*b*]pyridine (7-azaindole), is described. It was evaluated against L1210 and S-180 cells in culture and found to be inactive.

Several natural nucleosides produced by different streptomyces species are derivatives of pyrrolo[2,3-*d*]pyrimidine. Among these, tubercidin (7-deazaadenosine) (**1a**), toyocamycin (**1b**), and sangivamycin (**1c**) have been



1 a, R = H
b, R = CN
c, R = $CONH_2$

particularly studied.[1,2] Biological tests performed with such compounds have pointed out their antitumor, antibacterical, and antiviral activities. On the other hand, much effort has been made to synthesize deaza-, dideaza-, and trideazaadenosine analogues as potential chemotherapeutic agents.[3-7]

In this paper we report the synthesis of 1,7-dideazaadenosine (1-deazatubercidin; **15**), the first example of dideazaadenosine containing only one nitrogen atom in each of the two heterocyclic rings.

Chemistry. The synthesis of 4-amino-1-β-ribofuranosyl-1*H*-pyrrolo[2,3-*b*]pyridine was carried out by the sequence shown in the Scheme I.

4-Amino-2,3-dihydro-1*H*-pyrrolo[2,3-*b*]pyridine (**8**), a key intermediate in our synthesis, was prepared by hy-

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