°C; ^{1}H NMR (Me_2SO-d_6) δ 5.4 (d, 1, $J_{1',2'} \leq 1.5$ Hz, $C_{1'}$ H), 1.3 and 1.46 (2 s, 3 and 3, isopropylidene methyls, difference in chemical shifts of methyl groups = 0.16 ppm). Anal. ($C_{10}H_{14}N_2O_7$) C, H, N.

2-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-1,2,4-oxadiazole-3,5-dione (17). A mixture of **12** (1.02 g, 10 mmol), 1-*O*-acetyl-2-deoxy-3,5-di-*O*-*p*-toluoyl-D-*erythro*-pentofuranose[21] (4.13 g, 10 mmol), and a catalytic amount of bis(*p*-nitrophenyl) phosphate was finely powdered and heated in a pear-shaped flask at 125 °C (bath temperature). When the mixture melted to a clear syrup, the vacuum from a water aspirator was applied and the heating continued for an additional 30 min. The reaction mixture was cooled to room temperature, and the thick syrup was dissolved in $CHCl_3$ (200 mL) and washed with water saturated with NaCl. The $CHCl_3$ portion was separated, dried ($MgSO_4$), and evaporated in vacuo. The crude residue was passed through a column (3.5 × 36 cm) packed with silica gel in $CHCl_3$. Elution with 25% ethyl acetate in $CHCl_3$ provided the chromatographically pure 2-(deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)-1,2,4-oxadiazole-3,5-dione (**16**), which was crystallized from benzene and cyclohexane: mp 149–151 °C (softens at 142 °C); ^{1}H NMR ($CDCl_3$) δ 8.5 (br s, 1, NH, exchangeable with D_2O), 7.82 and 7.15 (2 m, 4 and 4, toluene ring protons), 6.02 (t, 1, $C_{1'}$ H), 2.35 (s, 6, methyls), and other sugar protons. Compound **16** was dissolved in methanol (50 mL), adjusted to pH 8.5 with NaOMe, and allowed to stand at room temperature for 48 h. The solent was evaporated in vacuo, and the residue was taken in water (25 mL), which was extracted with $CHCl_3$ (15 mL × 2). The water portion was passed through a column of AG 50W-X8, 20–50 mesh, H^+ form (15 mL). The column was washed with additional water (30 mL). The water fractions were combined, washed with $CHCl_3$ (45 mL), and lyophilized to provide a residue, which was crystallized from acetone and ethyl ether to provide 1.3 g (63%) of **17**: mp 140–141 °C; IR (KBr) 1805 and 1730 (>C=O); ^{1}H NMR (Me_2SO-d_6) δ 5.8 (t, 1, $J_{H_{1'},H_{2'}} = 6.7$ Hz, peak width 13.4 Hz, $C_{1'}$ H), 4.08 and 3.6 (2 m, 1 and 1, $C_{3'}$ H and $C_{4'}$ H), 3.3 (m, 2, $C_{5'}$ H_2), 2.1 (m, 2, $C_{2'}$ H_2). Anal. ($C_7H_{10}N_2O_6$) C, H, N.

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Synthesis and Antitumor Activity of an Acyclonucleoside Derivative of 5-Fluorouracil

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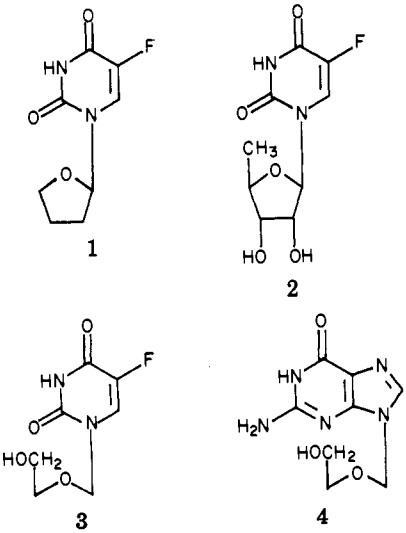
The pyrimidine acyclonucleoside 5-fluoro-1-[(2-hydroxyethoxy)methyl]uracil (**3**) was synthesized as part of a program aimed at the development of new 5-fluorouracil derivatives with fewer side effects and a broader margin of safety. Condensation of 5-fluoro-2,4-bis[(trimethylsilyl)oxy]pyrimidine with 2-acetoxyethyl acetoxymethyl ether (**6**) in the presence of $SnCl_4$ afforded the acetate ester **7**, which on deprotection with NaOMe gave **3** in 50–60% overall yield. The 5-bromo and 5-iodo analogues **10** and **11**, respectively, were obtained similarly. Reaction of 5-fluoro-4-(methylthio)-2-[(trimethylsilyl)oxy]pyrimidine with 2-acetoxyethyl acetoxymethyl ether and $SnCl_4$, followed by ammonolysis, yielded 5-fluoro-1-[(2-hydroxyethoxy)methyl]cytosine (**12**). Deamination of **12** with nitrous acid produced **3**, thereby confirming that alkylation of 5-fluoro-2,4-bis[(trimethylsilyl)oxy]pyrimidine had occurred at N^1. The ID_{50} of **3** against L1210 mouse leukemia cells in culture was 1.7×10^{-5} M, as compared with 1×10^{-6} M for FU. The 5-fluorocytosine analogue **12** was inactive at up to 1×10^{-4} M, and the other halogenated derivatives **10** and **11** had no effect even at 1×10^{-3} M. When **3** was given ip in water to P388 leukemic mice at 400 mg/kg (b.i.d. × 4) or 240 mg/kg (q.d. 1–9), a 75% increase in survival was observed relative to untreated controls, and there was no evidence of any host toxicity.

Novel prodrug derivatives of 5-fluorouracil (FU) possessing a broader spectrum of antitumor activity and fewer toxic side effects than FU have been sought diligently in a number of laboratories. One such derivative which has received attention in recent years is 1-(2-tetrahydrofuranyl)-5-fluorouracil (**1**, Ftorafur).[1] In addition to several clinical studies,[2] the human and animal pharmacology of this compound has been extensively investigated,[3] and several improved methods of chemical synthesis have been developed.[4] Another FU prodrug that has aroused interest more recently is 5-fluoro-5′-deoxyuridine (**2**, 5-DFUR).[5] This compound is reported to be therapeutically superior to both Ftorafur and 5-fluoro-2′-deoxyuridine (FUDR) against several murine tumors, including P388 and L1210 leukemias, Lewis lung carcinoma, and Crocker sarcoma S180.[6,7] Among the reported advantages of the



(1) S. A. Hiller, R. A. Zhuk, and M. Ya. Lidak, *Dokl. Akad. Nauk SSSR*, **176**, 332 (1967).
(2) M. Valdivieso, G. P. Bodey, J. A. Gottlieb, and E. J. Freireich, *Cancer Res.*, **36**, 1821 (1976), and earlier references cited.
(3) J. A. Benvenuto, J. G. Liehr, T. Winkler, D. Farquhar, R. M. Caprioli, and T. L. Loo, *Cancer Res.*, **39**, 3199 (1979), and earlier references cited.
(4) M. Yasumoto, I. Yamawaki, T. Marunaka, and S. Hashimoto, *J. Med. Chem.*, **21**, 738 (1978), and earlier references cited.
(5) A. F. Cook, M. J. Holman, M. J. Kramer, and P. W. Trown, *J. Med. Chem.*, **22**, 1330 (1979).

0022-2623/81/1824-1177$01.25/0

5′-deoxy derivative are that it is well-absorbed orally and that there is a notable absence of bone marrow or gastrointestinal toxicity.[6] In addition, the immunosuppressive effects of 5-DFUR are reported to be markedly lower than those of FU or Ftorafur at equiactive doses.[8a] Selective antitumor action appears to be related to the fact that the enzyme uridine phosphorylase, which converts 5-DFUR to FU and 5-deoxyribose 1-phosphate, is present in higher amount in tumor cells than in cells derived from normal tissues.[7] This differential bioactivation mechanism is supported by radioactive drug distribution studies in rodents showing that FU levels in sensitive organs such as liver, spleen, and small intestine are much smaller after 5-DFUR treatment than after a comparable dose of FU or Ftorafur.[8b]

In this paper we report the synthesis of 5-fluoro-1-[(2-hydroxyethoxy)methyl]uracil (3) and several related compounds as candidate antitumor agents.[9] The impetus for this work came from the recent success of 9-[(2-hydroxyethoxy)methyl]guanine (acyclovir, 4) as an antiherpetic[10-12] and the recent report by Fox[13] that certain pyrimidine nucleoside analogues known until now primilary for their antiviral activity are also toxic to *virally transformed neoplastic cells*. The antiviral activity of the guanine derivative 4 stems from the fact that viral, but not mammalian, thymidine kinase happens to accept this compound as a substrate and converts it into a monophosphate ester. Further phosphorylation yields a triphosphate derivative which interferes with DNA synthesis in the infected cell. In a potentially significant extrapolation of this argument, it is possible that certain types of tumor cells are synthesizing virally coded thymidine kinase in addition to the enzyme coded by their own DNA and that these cells could phosphorylate an acyclic nucleoside ("acyclonucleoside") analogue such as 3 and thereby bring about their own destruction. In addition to this rationale, we also considered the possibility that 3 might serve as a substrate for a viral, but not mammalian, pyrimidine nucleoside phosphorylase or else that 3 might be cleaved by simple chemical hydrolysis of the glycoside bond to give FU. According to this view, compound 3 would serve as a prodrug of FU and thereby have a pharmacologic mode of action similar to that of Ftorafur (1) or 5-DFUR (2). The present method of preparation of 1-[(2-hydroxyethoxy)methyl] derivatives of FU and other halogenated uracils is rapid and economical, does not seem to present serious scaling-up problems,[14] and is clearly adaptable to the synthesis of other pyrimidine acyclonucleosides for biological evaluation of their antitumor and/or antiviral properties.

The bis(trimethylsilyl) derivative 5 of FU was condensed with 2-acetoxyethyl acetoxymethyl ether (6) in the presence of 0.2 molar equiv of stannic chloride[15] to obtain the acetate ester 7 (53% yield). Deprotection with sodium methoxide then gave the desired product 3 (83% yield). TLC analysis of the crude coupling product indicated the presence of a small amount of less polar material which was probably N^1,N^3-disubstituted, but there was no evidence of any isomeric N^3-substituted derivative. Since none of the putative N^1,N^3-disubstituted compound could be isolated after workup and chromatography, it is assumed that this produce undergoes facile cleavage to 3. Similar instability has been reported for N^1,N^3-disubstituted 2-tetrahydrofuranyl derivatives of FU.[4] Replacement of FU by 5-bromouracil and 5-iodouracil in the foregoing reaction sequence afforded the acetate esters 8 and 9, and deacylation led to the alcohols 10 and 11, respectively. Overall yields were 50–60% for the two steps. Once again, TLC analysis indicated that alkylation occurred at only one of the nitrogens, presumably N^1. Infrared, ultraviolet, and NMR spectra of the products were consistent with these structure assignments. The infrared spectra of the acetate esters displayed prominent peaks at 1750 cm^{-1} (ester C=O) which were absent in the corresponding alcohols. Compound 3 showed an ultraviolet absorption maximum at 266 nm (EtOH solution), which was shifted to 276 nm in the bromo analogue 10 and to 280 nm in the iodo analogue 11. Each compound showed only a single vinylic proton signal (C_6 H of the uracil moiety) in the NMR spectrum. In 3 this signal was split into a doublet [τ 2.0 (J = 7 Hz)] by H–F coupling, whereas 10 (τ 1.8) and 11 (τ 1.7) each gave only a singlet.

Direct chemical evidence for N^1-substitution in the reaction of FU was furnished by the isolation of 3 from 5-fluoro-1-[(2-hydroxyethoxy)methyl]cytosine (12) upon treatment with nitrous acid. The amine 12 was prepared unambiguously from 5-fluoro-4-(methylthio)pyrimidin-2-(1*H*)-one by trimethylsilylation followed by condensation with the diacetate 6 in the presence of 0.6 molar equiv of stannic chloride to give the N^1-alkylated product 13 (84% yield). Simultaneous deacylation and replacement of the methylthio group by amino was effected in 76% yield by heating for 18 h in methanolic ammonia. Compound 13 showed ultraviolet absorption maxima (EtOH solution) at 275 and 310 nm, with amination resulting in the expected hypsochromic shift to 242 and 280 nm. The NMR spectrum of 12 (Me_2SO-d_6 solution) showed a doublet [τ 2.1 (J = 6 Hz)] whose upfield shift relative to 3 was indicative of a slight shielding effect by the 4-amino substituent counteracting the influence of the adjacent fluorine atom. The fact that the product of deamination of 12 was identical in every respect with the compound obtained directly from FU furnished unequivocal proof that substitution of

(6) W. Bollag and H. R. Hartmann, *Eur. J. Cancer*, **16**, 427 (1980).
(7) H. Ishitsuka, M. Miwa, K. Takemoto, K. Fukuoka, A. Itoga, and H. Murayama, *Gann*, 71, 112 (1980).
(8) (a) Y. Ohta, K. Sueki, K. Kitta, K. Takemoto, H. Ishitsuka, and Y. Yagi, *Gann*, 71, 190 (1980); (b) S. Suzuki, Y. Hongo, H. Fukuzawa, S. Ishikara, and H. Shimizu, *ibid.*, 71, 238 (1980).
(9) For a preliminary account of this work, see A. Rosowsky and S.-H. Kim, "Abstracts of Papers", 181st National Meeting of the American Chemical Society, Atlanta, GA, Mar 29–Apr 3, 1981, American Chemical Society, Washington, DC, 1981, Abstr MEDI 34. Following completion of the work, we became aware of another study in which compounds 3, 10, and 11 were synthesized via an alternative approach [J. L. Kelley, J. E. Kelsey, W. R. Hall, M. P. Krochmal, and H. J. Schaeffer, *ibid.*, Abstr MEDI 33, and *J. Med. Chem.*, **24**, 753 (1981)].
(10) H. J. Schaeffer, L. Beauchamp, P. de Miranda, G. B. Elion, D. J. Bauer, and P. Collins, *Nature (London)*, **272**, 583 (1978).
(11) J. A. Fyfe, P. M. Keller, P. A. Furman, R. L. Miller, and G. B. Elion, *J. Biol. Chem.*, **253**, 8721 (1978).
(12) H. J. Schaeffer, "Abstracts of Papers", 2nd Chemical Congress of the North American Continent, San Francisco, CA, Aug 25–29, 1980, American Chemical Society, Washington, DC, 1980, Abstr MEDI 3.
(13) J. J. Fox, in ref 12, Abstr MEDI 2.
(14) A different method of synthesis was described recently in which alkylation is accomplished by adding the trimethylsilyl derivative of 2-hydroxyethoxymethyl iodide to the sodium salt of the N-heterocyclic base [J. R. Barrio, J. D. Bryant, and G. E. Keyser, *J. Med. Chem.*, **23**, 572 (1980)]. 4-(Methylthio)pyrimidin-2(1*H*)-one was converted to its 1-[(2-hydroxyethoxy)methyl] derivative in this fashion, and the latter was aminated to form 1-[(2-hydroxyethoxy)methyl]cytosine. We have also used this route to prepare compound 3 from FU but have found it to be much less convenient, especially for reactions scaled up to several grams, than the procedure reported here.
(15) U. Niedballa and H. Vorbrüggen, *Angew. Chem., Int. Ed. Engl.*, **9**, 461 (1970).

Table I. Antitumor Activity of 5-Fluoro-1-[(2-hydroxyethoxy)methyl]uracil (3) against P388 Leukemia in Mice[a]

expt	compd	dose, g/kg	schedule	7-day wt change, %	median survival, days	% ILS
1	none			+15	13	
	FU	20	q.d. 1–4	−5	23	+76
		40		−29	10	−24 (toxic)
	3	50	q.d. 1–4	+7	14	+7
		100		+6	15	+15
		200		+6	17	+30
2	none			+17	12	
	FU	10	b.i.d. × 4	+9	19	+58
		20		−12	12	0 (toxic)
	3	50	b.i.d. × 4	+5	16	+33
		100		+4	17	+42
		200		+4	20	+67
		400		0	21	+75
	FU	10	q.d. 1–9	−2	21	+75
		20		−23	12	0 (toxic)
	3	60	q.d. 1–9	+8	16	+33
		120		0	18	+50
		240		0	21	+75

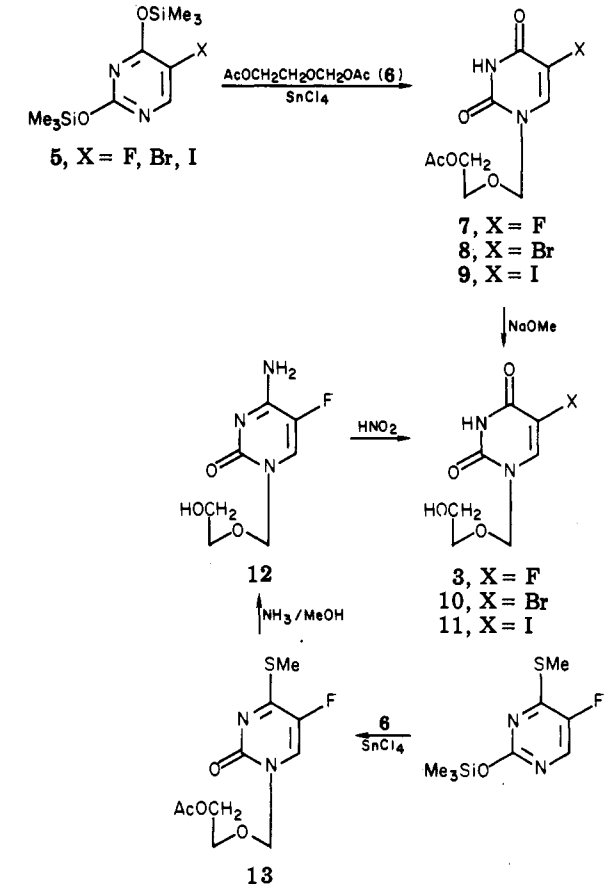
[a] Groups of five male B6D2F$_1$J mice were inoculated ip with 10^6 P388 cells on day 0, and drugs were injected ip in water on the indicated schedules. Animals were weighed on days 1 and 7, and the weight change was expressed as a percentage. ILS = increase in life span.

the uracil derivative had occurred regioselectively at N^1 as shown in Scheme I.

Biological Activity. Compounds reported in this paper were tested as inhibitors of the growth of L1210 mouse leukemia cells in culture. Compound **3** was found to have an ID_{50} of 1.7×10^{-5} M as compared with 1×10^{-6} M for FU. The 5-fluorocytosine analogue **12** had no effect even at 1×10^{-4} M and is therefore presumably not deaminated to **3** intracellularly or in the incubation medium. Phosphorolysis of **12** would give 5-fluorocytosine, which is known to be inactive. The other 5-halogenated derivatives **10** and **11** were likewise inactive at concentrations of up to 1×10^{-3} M. The cytoxicity of **3** could not be due to contamination by FU, since HPLC analysis showed less than 0.5% FU in the bioassay specimen.

In vivo antitumor evaluation was carried out with compound **3** against P388 leukemia in mice on three different schedules (q.d. 1–4, b.i.d. × 4, and q.d. 1–9). Only a marginal 30% increase in life span was observed at 200 mg/kg (q.d. 1–4), and dose escalation to as much as 1200 mg/kg did not increase activity (data not shown). However, when **3** was given twice daily × 4 at 400 mg/kg or once-daily × 9 at 240 mg/kg, a 75% increase in life span was achieved, with no evidence of any toxicity as judged by lack of weight loss. By comparison, 10 mg/kg of FU on the b.i.d. × 4 schedule gave a 58% ILS, with a doubling of the dose to 20 mg/kg being toxic. On the q.d. 1–9 schedule, 10 mg/kg of FU gave a 75% ILS, but again a doubling of the dose was highly toxic. The results of this in vivo experiment indicate that **3** is a well-tolerated substance that can bring about increases in survival comparable to FU on at least two dose schedules. Of notable interest was the finding that the dose–response curve for **3** is obviously much less steep than the one for FU. This is characteristic of many prodrugs and usually signifies an increase in the therapeutic index and a broader margin of safety. Further studies on the mechanism of action and scope of antitumor activity of this compound are planned.

Scheme I



Experimental Section

Infrared spectra were obtained on a Perkin-Elmer Model 137B double-beam recording spectrophotometer, and ultraviolet spectra were recorded on a Cary Model 15 instrument. NMR spectra were determined by means of a Varian T60A instrument with tetramethylsilane as the reference. TLC was performed on Eastman 13181 silica gel sheets containing a fluorescent indicator, and spots were visualized under ultraviolet light at 254 nm. Dry-column chromatography was carried out with Woelm activity grade III/30 mm silica gel (ICN Nutritional Biochemicals, Cleveland, OH). Conventional column chromatography was done on Baker 3405 silica gel (60–200 mesh). Melting points were measured in Pyrex capillary tubes in a Mel-Temp apparatus (Laboratory Devices, Inc., Cambridge, MA) and are not corrected. Microchemical analyses were performed by Galbraith Laboratories, Knoxville, TN, and were within ±0.4% of the calculated values. Analytical samples all gave single TLC spots.

2-Acetoxyethyl Acetoxymethyl Ether (6). A mixture of dioxolane (70 mL, 1.0 mol) and acetic anhydride (95 mL, 1.0 mol) was cooled to 0 °C, and concentrated H_2SO_4 (0.6 mL, 0.01 mol)

was added slowly with stirring. Gas evolution occurred immediately. The solution was allowed to come to room temperature, stirred overnight, and then poured into ice-cold $NaHCO_3$ (30 g, as a saturated solution). Extraction with $CHCl_3$ (300 mL), washing with saturated $NaHCO_3$, drying over anhydrous Na_2SO_4, and solvent evaporation yielded a liquid which was vacuum distilled. Following removal of some low-boiling material consisting mainly of acetic acid and acetic anhydride, the product (130 g, 74% yield) was obtained as a colorless liquid: bp 120–130 °C (30 mmHg) [lit.[16] bp 114–116 °C (10 mmHg)]; NMR ($CDCl_3$) τ 7.95 (s, 6 H, $COCH_3$), 6.0 (A_2B_2 pattern, 4 H, OCH_2CH_2O), 4.7 (s, 2 H, OCH_2O). The product was used without additional purification for the next step.

1-[(2-Acetoxyethoxy)methyl]-5-fluorouracil (7). *N,O*-Bis(trimethylsilyl)acetamide (6 mL, 0.024 mol) was added dropwise under nitrogen to a stirred mixture of 5-fluorouracil (1.3 g, 0.01 mol) and **6** (2.7 g, 0.015 mol) in CH_2Cl_2 (25 mL). After 3 h of stirring at room temperture, the clear solution was cooled to 0 °C and $SnCl_4$ (0.2 mL, 0.002 mol) was added. The mixture was then warmed to room temperature, left to stir overnight, and finally poured slowly into a mixture of cold saturated aqueous $NaHCO_3$ (50 mL) and $CHCl_3$ (100 mL). The resulting emulsion was separated by filtration through Celite, the aqueous layer was extracted further with EtOAc (3 × 50 mL), and the combined organic layers were dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. Trituration of the remaining oily residue with ether afforded the product as colorless crystals (1.0 g, 53% yield): mp 138–144 °C; TLC (silica gel, 4:1 $CHCl_3$–EtOH) R_f 0.84; NMR (Me_2SO-d_6) τ 8.0 (s, CH_3CO), 6.1 (A_2B_2 pattern, $AcOCH_2CH_2O$), 5.0 (s, NCH_2O), 1.9 (d, J = 7 Hz, C_6 H). Anal. ($C_9H_{11}N_2O_5F$) C, H, N, F.

1-[(2-Acetoxyethoxy)methyl]-5-fluoro-4-(methylthio)pyrimidin-2(1*H*)-one (13). *N,O*-Bis(trimethylsilyl)acetamide (1.5 mL, 0.006 mol) was added to a mixture of 5-fluoro-4-(methylthio)pyrimidin-2(1*H*)-one (0.8 g, 0.0075 mol)[17] and 2-acetoxyethyl acetoxymethyl ether (1.4 g, 0.0075 mol) in CH_2CL_2 (20 mL). After 3 h at room temperature, the clear solution was cooled to 0 °C, $SnCl_4$ (0.28 mL, 0.003 mol) was added, and the mixture was left to stir at room temperature overnight and finally poured into cold saturated aqueous $NaHCO_3$ (50 mL). Extraction with $CHCl_3$ (3 × 50 mL), drying, and rotary evaporation left a pale yellow oil, which solidified on trituration with petroleum ether (bp 30–60 °C). Chromatography on a silica gel dry column (2.3 × 27 cm), with 4:1 $CHCl_3$–acetone as the eluent, gave 1.2 g (84% yield) of the product as an oil, which crystallized slowly on standing: mp 116–122 °C; TLC (silica gel, 4:1 $CHCl_3$–acetone) R_f 0.73; IR (KBr) 1750 (ester C=O), 1650 (amide C=O) cm^{-1}; UV λ_{max} (95% EtOH) 275 nm, 310; NMR ($CDCl_3$) τ 7.9 (s, CH_3CO), 7.4 (s, CH_2S), 6.0 (A_2B_2 pattern, $AcOCH_2CH_2O$), 4.74 (s, NCH_2O), 2.62 (d, J = 6 Hz, C_6 H). Anal. ($C_{10}H_{13}N_2O_4FS$) C, H, N, F, S.

5-Fluoro-1-[(2-hydroxyethoxy)methyl]cytosine (12). A solution of **13** (0.9 g, 0.33 mmol) in MeOH (100 mL) presaturated at 0 °C with ammonia for 15 min was heated at 115 °C (bath temperature) for 18 h in a glass pressure bottle. After cooling, the contents of the bottle were transferred to a rotary evaporator, and the solvent and residual ammonia were removed. The remaining solid was passed through a silica gel dry column (1.7 × 21 cm) with 4:1 $CHCl_3$–EtOH as the eluent. Appropriate TLC-homogeneous fractions were pooled and evaporated to dryness, and the resultant solid was triturated with petroleum ether (bp 30–60 °C): yield 0.5 g (76%); mp 155–163 °C; TLC (silica gel, 4:1 $CHCl_3$–EtOH) R_f 0.16; UV λ_{max} (95% EtOH) 242 nm, 280; IR (KBr) 1680 (C=O) cm^{-1}; NMR (Me_2SO-d_6) τ 6.5 (s, CH_2CH_2), 5.0 (s, NCH_2O), 2.4 (br s, NH_2), 2.1 (d, J = 7 Hz, C_6 H). Anal. ($C_7H_{11}N_3O_3F$) C, H, N, F.

5-Fluoro-1-[(2-hydroxyethoxy)methyl]uracil (3). A. Alkylation of 5-Fluorouracil with 1-(Iodomethoxy)-3-[(trimethylsilyl)oxy]ethane. Thoroughly dried 5-fluorouracil (0.65 g, 0.005 mol) was added to dry DMF (15 mL) in a flask fitted with a rubber septum and nitrogen inlet, and the disodium salt was generated by adding 50% NaH in mineral oil dispersion (0.72 g, 0.015 mol). The mixture was then flushed with nitrogen and cooled to −63 °C with the aid of a dry ice/$CHCl_3$ bath. In a separate flask, a solution of dry dioloxane (0.8 mL, 0.011 mol) in cyclohexene (2 mL) was likewise cooled to −78 °C (dry ice/acetone) and treated with trimethylsilyl iodide (1.6 mL, 0.011 mol). After 10 min, the second solution was taken up into a dry glass syringe and quickly transferred, while cold, to the flask containing the 5-fluorouracil salt. The mixture was allowed to come slowly to room temperature (3 h) and stirred overnight. The solvent was evaporated under reduced pressure, the pale yellow residue was triturated with 4:1 $CHCl_3$–EtOH (300 mL), the insoluble portion was dissolved in water (pH 6.0), and the pH was adjusted to 4.0 with glacial AcOH. Lyophilization gave a solid which was purified further by dry column chomatography on silica gel (75 g) with 9:1 $CHCl_3$–EtOH as the eluent. Two fractions were collected: 0.3 g of an approximately 1:1 mixture of unchanged 5-fluorouracil and the desired product: yield 0.116 g of pure product (total yield ~0.27 g, 26%); mp 146–153 °C; TLC (silica gel, 85:15 $CHCl_3$–EtOH) R_f 0.40; UV λ_{max} (95% EtOH) 266 nm; NMR (Me_2SO-d_6) τ 6.65 (s, CH_2CH_2), 5.0 (s, NCH_2O), 2.0 (d, J = 7 Hz, C_6 H). Anal. ($C_7H_9N_2O_4F$) C, H, N, F.

B. Hydrolysis of 1-[(2-Acetoxyethoxy)methyl]-5-fluorouracil. To a solution of **7** (0.40 g, 0.016 mol) in MeOH (10 mL) was added 2 mL of 1 N NaOMe in MeOH. After 2 h at room temperature, the pH was adjusted to 4.0 with 1 N HCl, and the solvent was evaporated under reduced pressure to obtain a solid, which was purified by dry-column chromatography on silica gel (50 g) with 9:1 $CHCl_3$–EtOH as the eluent. Appropriate TLC-homogeneous fractions were pooled and evaporated, and the resultant solid was triturated with ether, suction filtered, and dried: yield 0.27 g (83%); mp 148–154 °C; TLC (silica gel, 85:15 $CHCl_3$-EtOH) R_f 0.40. Infrared, ultraviolet, and NMR spectra of this product and the compound obtained in the preceding experiment were identical.

C. Hydrolysis of 5-Fluoro-1-[(2-hydroxyethoxy)methyl]cytosine. A solution of **12** (25 mg, 0.12 mmol) in 2 mL of 50% aqueous AcOH was cooled to 0 °C, and a tenfold excess of solid $NaNO_2$ was added in small portions with stirring. After being left to stand at room temperature for 4 h, the solution was freeze-dried, and the residue was applied onto a silica gel dry column (1.7 × 17.5 cm) which was eluted with 9:1 $CHCl_3$–EtOH. The product (~100% yield) possessed the same TLC and spectral properties as the one obtained in the preceding two experiments.

1-[(2-Acetoxyethoxy)methyl]-5-bromouracil (8). *N,O*-Bis(trimethylsilyl)acetamide (1.5 mL, 0.006 mol) was added dropwise under nitrogen to a stirred mixture of 5-bromouracil (0.95 g, 0.005 mol) and **6** (1.3 g, 0.0075 mol) in dry CH_2Cl_2 (30 mL). After overnight stirring at room temperature, another portion of *N,O*-bis(trimethylsilyl)acetamide (0.8 mL, 0.0032 mol) was added in order to bring about complete dissolution (3 h). The reaction mixture was cooled to 0 °C and $SnCl_4$ (0.3 mL, 0.003 mol) was added. After being warmed to room temperature, the mixture was left overnight and then poured into ice-cold saturated aqueous $NaHCO_3$ (50 mL) and $CHCl_3$ (50 mL). The layers were separated, and the aqueous layer was extracted with $CHCl_3$ (3 × 100 mL) and ethyl acetate (2 × 100 mL). The combined organic layers were dried over Na_2SO_4 and evaporated under reduced pressure to give an oily residue, which was triturated with petroleum ether (bp 30–60 °C) before being applied onto a silica gel column (2 × 26 cm). Elution with 19:1 $CHCl_3$–EtOH, pooling of appropriate TLC-homogeneous fractions, and solvent evaporation yielded a pale pink solid (0.88 g, 57%): mp 100–114 °C; TLC (silica gel, 19:1 $CHCl_3$–EtOH) R_f 0.71; NMR (Me_2SO-d_6) τ 8.0 (s, CH_3CO), 6.1 (m, A_2B_2 pattern, $AcOCH_2CH_2O$), 4.9 (s, NHC_2O), 1.7 (s, C_6H). Anal. ($C_9H_{11}N_2O_5Br$) C, H, N, Br.

5-Bromo-1-[(2-hydroxyethoxy)methyl]uracil (10). Sodium methoxide (1 N, 3 mL) was added to a suspension of **8** (0.62 g, 0.002 mol) in MeOH (10 mL), and the mixture was stirred at room temperature for 2 h. The pH was then adjusted to 4.0 with 1 N HCl, and the solvent was evaporated. Dry-column chromatography on silica gel (50 g) with 9:1 $CHCl_3$–EtOH as the eluent gave the desired product as a colorless granular powder (0.55 g, ~100% yield): mp 150–151 °C; TLC (silica gel, 19:1 $CHCl_3$-EtOH) R_f 0.17; UV λ_{max} (95% EtOH) 276 nm; NMR (Me_2SO-d_6) τ 6.45 (s, CH_2CH_2), 5.0 (s, NCH_2O), 1.8 (s, 1 H, C_6 H). Anal. ($C_7H_9N_2O_4Br$) C, H, N, Br.

(16) M. Senkus, *J. Am. Chem. Soc.*, 68, 734 (1946).
(17) V. Uchytilova, A. Holy, D. Cech, and J. Gut., *Collect. Czech. Chem. Commun.*, 40, 2347 (1975).

1-[(2-Acetoxy)methyl]-5-iodouracil (9). *N,O*-Bis(trimethylsilyl)acetamide (6 mL, 0.024 mol) was added to a mixture of 5-iodouracil (2.4 g, 0.01 mol) and **6** (4 g, 0.015 mol) in dry CH_2Cl_2 (30 mL). After overnight stirring *in the dark*, the mixture was cooled in an ice bath, $SnCl_4$ (0.6 mL, 0.024 mol) was added, and the temperature was allowed to come to 25 °C overnight. After the usual workup, the crude oily product was passed through a silica gel column (50 g), using 2:1 $CHCl_3$–acetone as the eluent. Appropriate TLC-homogeneous fractions were pooled and evaporated to give the product as a pale pink glass (2.5 g, 75%), which solidified on standing: mp 110–113 °C; TLC (silica gel, 2:1 $CHCl_3$–acetone) R_f 0.53; NMR (Me_2SO-d_6) τ 7.9 (s, CH_3CO), 6.0 (A_2B_2 pattern, $AcOCH_2CH_2O$), 4.9 (s, OCH_2N), 2.3 (s, C_6 H). Anal. ($C_9H_{11}N_2O_5I$) C, H, N.

1-[(2-Hydroxyethoxy)methyl]-5-iodouracil (11). Sodium hydroxide (1 N, 15 mL) was added to an ice-cold solution of **9** (2.4 g, 0.0068 mol) in MeOH (50 mL), and the mixture was stirred at room temperature for 2 h. Cooling was resumed, and 1 N HCl was added carefully until the pH came to 4.0. Solvent evaporation under reduced pressure and dry-column chromatography of the residue on silica gel (50 g) with 9:1 $CHCl_3$–EtOH as the eluent afforded the product as a colorless powder (1.6 g, 76% yield): mp 165–175 °C; TLC (siliica gel, 9:1 $CHCl_3$–EtOH) R_f 0.33; UV λ_{max} (95% EtOH) 280 nm; NMR (Me_2SO-d_6) τ 4.5 (s, CH_2CH_2), 4.9 (NCH_2O), 1.7 (s, C_6 H). Anal. ($C_7H_9N_2O_4I$) C, H, N, I.

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N*-N*-S* Tridentate Ligand System as Potential Antitumor Agents

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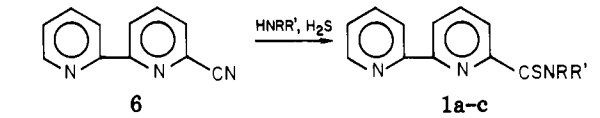
Compounds containing an N*-N*-S* tridentate ligand were synthesized and tested for antitumor activity against P-388 lymphocytic leukemia in mice. Of these, only 2,2′-bipyridyl-6-carbothioamide (**1a**) showed antitumor activity at relatively high dosage levels. Compound **1a** was also evaluated against L-1210 and S180 cells in culture and found to have significant activity.

Several α-N-heterocyclic carboxaldehyde thiosemicarbazones and their iron and copper complexes have been tested for antitumor activity.[1-6] These agents inhibit ribonucleoside diphosphate reductase, an obligatory enzyme in the pathway of synthesis of precursors of DNA. These thiosemicarbazones coordinate with ferrous and ferric ions through the N*-N*-S* tridentate ligand to give iron complexes. The inhibition occurs because the thiosemicarbazones chelate the iron necessary for the enzyme or because the iron complexes block the enzyme.[7,8]

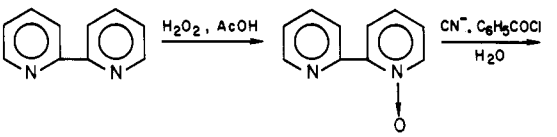
In order to extend the knowledge of the structure–activity relationships of these antitumor agents, a series of new compounds containing the N*-N*-S* tridentate ligand system has been synthesized. These derivatives can be divided into three groups (Chart I). The first group (a) includes thioamides of 2,2′-bipyridyl and of 1,10-phenanthroline. The second group (b) includes iminomethyl derivatives of 6-pyridine-2-carbothioamide. With these compounds only the second one of the two nitrogen atoms of the ligand belongs to a heterocyclic ring. The third group (c) includes imino derivatives of thiosemicarbazones of α,α-dicarbonyl compounds. In this case, none of the two sp^2 hybridized nitrogen atoms of the ligand belongs to any heterocyclic ring.

Scheme I



6 1a-c

Scheme II



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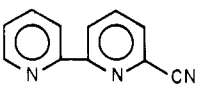

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Chemistry. In the first group, the 1,10-phenanthroline-2-carbothioamide (**2**) is a compound previously described and studied as a tridentate ligand of iron(II).[9] The 2,2′-bipyridyl-6-carbothioamides **1a–c** were synthesized from 2,2′-bipyridyl-6-carbonitrile (**6**) (Scheme I).

The 2,2′-bipyridyl-6-carbonitrile (**6**) was prepared by reacting 2,2′-bipyridyl 1-oxide (**5**) with sodium cyanide and benzoyl chloride in water; **5** was synthesized by oxidizing 2,2′-bipyridyl with H_2O_2 in AcOH (Scheme II). This method gave a higher total yield and used fewer steps than the one described by Case.[10]

In the second group, the compounds **3a–e** and **11** were synthesized from 2-cyano-6-(acetoxymethyl)pyridine (**7**)

(1) E. J. Blanz, Jr., F. A. French, J. R. Doamaral, and D. A. French, *J. Med. Chem.*, **13**, 1124 (1970).
(2) F. A. French, E. J. Blanz, Jr., S. C. Shaddik, and R. W. Brockman, *J. Med. Chem.*, **17**, 172 (1974).
(3) I. Antonini, F. Claudi, G. Cristalli, P. Franchetti, M. Grifantini, and S. Martelli, *Eur. J. Med. Chem.*, **14**, 89 (1979).
(4) W. Antholine and D. H. Petering, *Proc. Am. Assoc. Cancer Res.*, **15**, 63 (1974).
(5) K. C. Agrawal, B. A. Booth, E. C. Moore, and A. C. Sartorelli, *Proc. Am. Assoc. Cancer Res.*, **15**, 289 (1974).
(6) W. E. Antholine, J. M. Knight, and D. H. Petering, *J. Med. Chem.*, **19**, 339 (1976).
(7) A. C. Sartorelli, K. C. Agrawal, and E. C. Moore, *Biochem. Pharmacol.*, **20**, 3119 (1971).
(8) F. A. French, E. J. Blanz, Jr., J. R. Doamaral, and D. A. French, *J. Med. Chem.*, **13**, 1117 (1970).
(9) H. A. Goodwin, F. E. Smith, E. König, and G. Ritter, *Aust. J. Chem.*, **26**, 521 (1973).
(10) F. H. Case, *J. Org. Chem.*, **31**, 2398 (1966).

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