μg/mL Neutral red was added 2 days after infection, and the results were read the following day. Cytosine nucleoside deaminase was partially purified from human liver, obtained at autopsy, and assayed ("after autopsy") according to the procedures of Wentworth and Wolfenden.[35] The analogues and the substrate 2′-deoxycytidine were added to the assay mixtures at 10^{-4} M concentrations.

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Carbocyclic Analogues of 5-Fluorouracil Nucleosides

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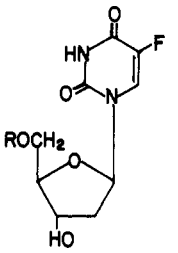
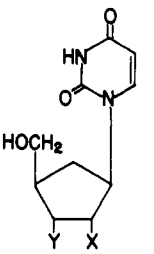
The carbocyclic analogues of 5-fluoro-2′-deoxyuridine (5-FdUrd, 1), 5-fluorouridine, and 5-fluoro-3′-deoxyuridine were prepared by fluorination of the uracil nucleoside analogues with elemental fluorine. The 5-FdUrd analogue (C-5-F-2′-dUrd, 6) was enzymatically phosphorylated to the analogue of 5-FdUrd 5′-phosphate and inhibited the incorporation of 2′-deoxyuridine into DNA of murine colon 26 tumor cells and L-1210 cells in culture. Biochemical studies also indicated that C-5-F-2′-dUrd (6) was a less potent inhibitor of DNA synthesis in tumor cells than was 5-FdUrd (1). C-5-F-2′-dUrd was cytotoxic (ED_{50} = 2.5 mcg/mL) to L-1210 cells in culture; the other two analogues were less cytotoxic. C-5-F-2′-dUrd was inactive—or, at best, borderline active—in tests against P-388 leukemia in vivo.

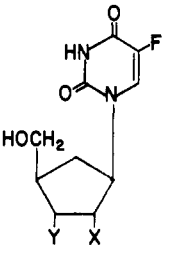
5-Fluorouracil (5-FUra)[1] is an important clinical anticancer drug, particularly in the treatment of gastrointestinal and mammary tumors.[2-6] 5-FUra proceeds through the same anabolic and catabolic steps as does uracil.[2,7] It may be transformed enzymatically to 5-fluorouridine[8,9] (5-FUrd), to 5-fluoro-2′-deoxyuridine[9] (5-FdUrd, 1), or (by reaction with 5-phosphoribosyl-1-pyrophosphate) to 5-fluorouridine 5′-phosphate[10,11] (5-FUMP)[1]. The nucleosides, 5-FUrd and 5-FdUrd (1), may, in turn, be converted by kinases to the nucleotides, 5-FUMP[7-9] and 5-fluoro-2′-deoxyuridine 5′-phosphate[9] (5-FdUMP, 2), and 5-FUMP may be phosphorylated further to the di- and triphosphate.[7] 5-FdUMP (2) is a potent inhibitor of thymidylate synthetase,[12] and 5-FUra, after metabolism to 5-FUTP, is incorporated into RNA;[13] however, 5-FUra and its nucleosides and nucleotides act at several biochemical loci and produce a multiplicity of biological effects.[2] Furthermore, the nucleotides may be converted to nucleosides by the action of phosphatases, and the nucleosides are subject to the action of phosphorylases, which cleave them to 5-FUra.[7,14] The result of these biochemical interconversions among 5-FUra, 5-FUrd, 5-FdUrd, and the nucleotides is that administration of any one of these derivatives may affect the sites of action of all of them. Thus, the anticipated effectiveness of 5-FdUrd (1), an immediate precursor of the thymidylate synthetase in-



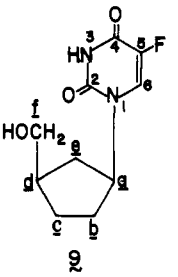
1: R = H
2: R = PO(OH)2



3: X = H, Y = OH
4: X = OH, Y = H
5: X = Y = OH



6: X = H, Y = OH
7: X = OH, Y = H
8: X = Y = OH



9

Letter g-f specify positions for NMR assignments.

(1) The abbreviations used here for 5-fluorouracil and its nucleosides were recommended by the IUPAC–IUB Commission on Biochemical Nomenclature, *J. Biol. Chem.*, 245, 5171 (1970). The nucleotide abbreviations are those commonly used (ref 2).
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hibitor, is muted by the partial conversion to 5-FUra, which is rapidly catabolized or incorporated into RNA. Since carbocyclic analogues of nucleosides lack the glycosidic bond of true nucleosides, they are not subject to the action of phosphorylases; consequently, carbocyclic analogues cannot revert in vivo to 5-FUra. Therefore, it is reasonable to anticipate that the carbocyclic analogues of 5-FUrd and 5-FdUrd will be more selective in their sites of action than are the true nucleosides and that the 5-FdUrd analogue might be more effective than 5-FdUrd. These considerations led us to synthesize and study the carbocyclic analogues of 5-FUra nucleosides.

5-Fluoropyrimidines have been synthesized by constructing the pyrimidine ring from acyclic precursors[15] or by fluorinating preformed pyrimidines with trifluoromethyl hypofluorite[16-18] or with elemental fluorine.[17,19,20] We chose the latter method for the preparation of the carbocyclic analogues (6–8) of 5-FdUrd, 5-fluoro-3′-deoxyuridine (5-F-3′-dUrd), and 5-FUrd from the carbocyclic analogues[21] (3–5) of uracil nucleosides. Thus, the carbocyclic analogue (6) of 5-FdUrd was obtained by treating the carbocyclic analogue (3) of 2′-deoxyuridine in acetic acid with a fluorine–nitrogen mixture (5:95), dehydrofluorinating the crude addition product[20] with aqueous ammonia, and purifying crude 6 by preparative TLC. Yields from 3 were 50–60%. The 5-F-3′-dUrd analogue (7) and the 5-FUrd analogue (8) were prepared similarly.

The ultraviolet maxima of these 5-FUra derivatives (6–8) showed a small bathochromic shift (4–6 nm) from the maxima of the 5-FdUrd and 5-FUrd spectra.[22] Mass spectra were consistent with the structures of 6–8, including prominent fragment peaks due to the base moiety (5-FUra) plus C_2H_4 and the base moiety plus one proton and two protons. These peaks are typical of carbocyclic analogues of nucleosides. The proton NMR spectra of 6 and the carbocyclic analogue of thymidine[23] are very similar, except for the splitting and the downfield shift of the pyrimidine CH and, of course, the absence of a methyl signal in the spectrum of 6. The appearance of the signals due to the protons at *a* and *c* (9) of these two 2′-deoxyribonucleoside analogues are remarkably similar.

Biological and Biochemical Results

The carbocyclic analogue (6) of 5-FdUrd, designated C-5-F-2′-dUrd, inhibited the proliferation of L-1210 cells in culture, ED_{50} = 2.5 mcg/mL. The 5-F-3′-dUrd analogue (7) was moderately inhibitory, ED_{50} = 19 mcg/mL, but the 5-FUrd analogue (8) was inhibitory only at higher concentrations, ED_{50} = 100 mcg/mL.

A cell-free enzyme preparation from L-1210 leukemia cells phosphorylated C-5-F-2′-dUrd and 5-FdUrd to the corresponding 5′-monophosphate derivatives. The conversion of nucleoside to nucleotide was followed by anion-exchange HPLC analysis of enzyme reaction mixtures. Comparative rates of phosphorylation were 6 pmol/min for 6 and 35 pmol/min for 5-FdUrd (1). Enzymatic phosphorylation of [6-^{3}H]dUrd[24] (0.08 mM) was decreased 50% by 6 at a concentration of 0.6 mM. As anticipated, phosphorolysis of 6 to 5-FUra by enzyme preparations from L-1210 cells was not observed under conditions that readily converted 5-FdUrd to FUra; 6 did not inhibit phosphorolysis of 5-FdUrd.

As expected, C-5-F-2′-dUrd did not inhibit the incorporation of [*methyl*-$^3H_3$]thymidine[24] into DNA of L-1210 cells in culture at a concentration of 10 mcg/mL, whereas the incorporation of [6-^{3}H]dUrd into DNA was inhibited to 25% of control values in the same experiment. In murine colon tumor 26 cells in culture, 6 (1.0 mcg/mL) produced 50% inhibition of incorporation of [6-^{3}H]dUrd into DNA; higher concentrations (10 mcg/mL) had no effect on the incorporation of [*methyl*-$^3H_3$]thymidine into DNA. FdUrd inhibited the incorporation of [6-^{3}H]dUrd into DNA of L-1210 cells and colon tumor cells in culture at much lower concentrations (0.001 mcg/mL). When L-1210 cells in culture were exposed to 6 for 15 min and were then grown in medium that did not contain 6, incorporation of [6-^{3}H]dUrd into DNA was not inhibited. This result suggests that 6 is a reversible inhibitor of DNA synthesis. In a similar experiment with 5-FdUrd, inhibition of [6-^{3}H]dUrd incorporation into DNA was sustained even after removal of inhibitor from the growth medium. The results of these incorporation studies indicate that 6 inhibits DNA synthesis in tumor cells in culture but that it is a much less potent inhibitor than is 5-FdUrd.

The 5-FdUrd analogue (6) did not increase the life span of mice bearing P-388 or L-1210 leukemia (Table I) at doses at which 5-FdUrd produces significant increases.[25] At a dose of 400 (mg/kg)/day, there was evidence (% T/C and death pattern) of a slight increase in survival time of mice with P-388 leukemia, but a higher dose did not produce a significant increase in life span. The weight-change differences did not indicate toxicity in any of these tests. Compounds 6–8 were not active in vitro against herpes virus type 1 (strain 377) in rabbit kidney cells, and 6 and 8 showed no activity against influenza virus, type Ao/PR/8/34, in Madin–Darby canine kidney cells.

Experimental Section

General Methods. Melting temperatures were determined in capillary tubes heated in a Mel-Temp apparatus. Ultraviolet spectra (UV) were recorded with a Cary Model 17 spectrophotometer, and maxima are reported in nanometers. Solutions for ultraviolet determinations were prepared by diluting an aliquot of an ethanol solution with 0.1 N hydrochloric acid, phosphate buffer (pH 7), or 0.1 N sodium hydroxide; absorption maxima of these solutions are reported as being determined in 0.1 N HCl,

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(24) [6-^{3}H]dUrd is 2′-deoxyuridine labeled with tritium at position 6 of the pyrimidine ring. [*methyl*-$^3H_3$]Thymidine is thymidine with a completely tritiated methyl group at position 5 of the pyrimidine ring.
(25) In tests of 5-FdUrd against leukemias P-388 and L-1210, reported values of T/C were as follows: P-388, T/C = 176% at a dose of 75 mg/kg, 3 times/week; L-1210, T/C = 148% at a dose of 75 mg/kg daily.[26] In addition, when 5-FdUrd was administered q.d. 1–9 at doses of 50–75 mg/kg vs. P-388 leukemia, T/C ratios in several tests were 148–190%,[27] and in several tests of 5-FdUrd vs. L-1210 leukemia, q.d. 1–9 at 72 mg/kg, the T/C ratios were 131–158%.[27]
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(27) F. M. Schabel, Jr., Southern Research Institute, private communication.

Table I. Tests of Carbocyclic Analogues of 5-Fluorouracil Nucleosides vs. P-388 Leukemia in Vivo[a]

compd	ip dose, (mg/kg)/day	deaths on days 1-5	wt change:[b] T - C, g	median life span, days: T/C	life span ratio: T/C, %
C-5-F-2′-dUrd (6)	50, q.d. 1-9	0/5	+1.0	10.5/10.9	96
	100, q.d. 1-9	0/6	+0.6	11.4/11.3	100
	200, q.d. 1-5	0/6	+0.8	12.0/11.9	100
	400, q.d. 1-5	0/6	-0.3	14.3/11.9	120
	600, q.d. 1-5	0/6	-0.7	13.1/12.0	109
	L-1210: 100, q.d. 1-9[c]	0/6	-0.5	8.3/8.2	101
C-5-FUrd (8)	50, q.d. 1-9	0/6	+0.9	11.1/10.9	101

[a] Mice were implanted intraperitoneally with 10^6 P-388 cells on day 0. Treatment was begun by intraperitoneal inoculation on day 1 and continued through day 9 or day 5. T = treated mice; C = control mice. [b] The weight change is the average change in weight of treated mice on day 5 minus the average change in weight of control mice on the same day. [c] L-1210 leukemia in mice; 10^5 cells implanted intraperitoneally on day 0.

at pH 7, or in 0.1 N NaOH, respectively. Mass spectral data (MS) were taken from low-resolution spectra determined at 70 eV with a Varian MAT Model 311A spectrometer equipped with a combination electron-impact, field-ionization, and field-desorption ion source. The peaks listed are those due to the molecular ion (M), those attributable to the loss of certain fragments from the molecular ion (M - fragment), and some other prominent peaks. Nuclear magnetic resonance spectra were determined with a Varian Model XL-100-15 spectrometer operating at 100.1 MHz for proton (^{1}H NMR) and at 25.2 MHz for carbon-13 (^{13}C NMR) spectra. The internal standard was $(CH_3)_4Si$; s = singlet, d = doublet, m = multiplet. Thin-layer chromatography (TLC) was performed on plates of silica gel,[28] and developed plates were examined by UV light (254 nm). Other pertinent information (amount applied, developing solvent) are given parenthetically at the appropriate places in the experimental procedures.

(±)-5-Fluoro-1-[(1α,3β,4α)-3-hydroxy-4-(hydroxymethyl)-cyclopentyl]-2,4(1*H*,3*H*)-pyrimidinedione (6). A fluorine–nitrogen mixture[29] containing 5% fluorine was bubbled into a solution of 224 mg of **3** and 150 mL of acetic acid for about 10 min. (A drop of the reaction solution placed on a TLC plate of silica gel and examined with a UV lamp emitting principally at 254 nm showed that little, or none, of **3** remained.) The reaction solution was concentrated to dryness in vacuo, and a solution of the residue in 15 N aqueous ammonia (60 mL) was boiled under reflux for 0.5 h and concentrated in vacuo to dryness. A solution of the brown residue in methanol was applied to a layer of silica gel on a preparative TLC plate.[28] The chromatogram was developed twice in $CHCl_3$–CH_3OH (4:1), the major band was divided into upper (faster) and lower regions, and the two portions of the major band were extracted separately with hot methanol. The extract of the lower region was concentrated to dryness, the residue was extracted with ethanol, the mixture was filtered, and the ethanol filtrate was concentrated to dryness in vacuo. The residue was dried further in vacuo at 64 °C for 2 h: yield, 122 mg (50%); TLC, 1 spot with tailing (20 mcg, 4:1 $CHCl_3$–CH_3OH); HPLC, 99.9% (reverse phase, 97:3 H_2O–acetonitrile, isocratic, monitored at 254 nm); UV max 274 nm (ε 8900) in 0.1 N HCl, 275 (ε 8500) at pH 7, 272 (ε 7000) in 0.1 N NaOH; MS[30] (direct-probe temperature, 20 °C), *m/e* 245 (M + 1), 244 (M), 227 (M - OH), 226 (M - H_2O), 214, 207 (M - OH - HF), 200, 195 (M - H_2O - CH_2OH), 187, 185, 157 (P + C_2H_4), 152, 149, 131 (P + 2H), 130 (P + H), 114; ^{1}H NMR[31] (Me_2SO-d_6, 6 mg/0.16 mL in a microcell) δ 1–1.6 and 1.6–2.3 (multiplets, CH_2 at *b* and *e* and CH at *d*), 3.46 (m, CH_2OH), 3.98 (m, CH at *c*), 4.96 (m, CH at *a*), ~6 (broad, OH, NH, H_2O), 8.03 (d, pyrimidine CH); ^{13}C NMR[31] (Me_2SO-d_6, 6 mg/0.16 mL in a microcell) δ 32.24 (*e*), 48.93 (*b* or *d*), 54.10 (*a*), 62.78 (*f*), 71.44 (*c*), 126.48 (pyrimidine C6, $^2J_{CF}$ = 33.0 Hz), 140.03 (pyrimidine C5, $^1J_{CF}$ = 230.1 Hz), 150.14 (pyrimidine C2), 157.46 (pyrimidine C4, $^2J_{CF}$ = 25.6 Hz). Either the peaks due to carbon atoms *b* and *d* are coincident or one of them is obscured by the solvent peaks. Anal. ($C_{10}H_{13}FN_2O_4 \cdot {}^2/_3H_2O$) C, H, N.

The upper region of the major band on the preparative TLC plate was extracted in the same way as was the lower region and furnished additional **6** (23 mg). This fraction contained a slight amount of an impurity that moved faster than **6** on an analytical TLC plate. The average yield from three other experiments was 58%. During each of these experiments, the major band from a preparative TLC plate was extracted in a Soxhlet extractor with methanol, and the residue from the methanol extract was extracted batchwise with ethanol.

(±)-5-Fluoro-1-[(1α,2β,4α)-2-hydroxy-4-(hydroxymethyl)-cyclopentyl]-2,4(1*H*,3*H*)-pyrimidinedione (7). Compound **7** was prepared by the procedure described for **6** and was dried at 64 °C for 2 h: yields 40–50%; mp 180–182 °C; TLC, 1 spot with tailing (5:1 $CHCl_3$–CH_3OH); HPLC, 98–99.5% (reverse phase, 97:3 H_2O–acetonitrile, isocratic, monitored at 254 nm); UV max 273 nm in 0.1 N HCl, 275 at pH 7, 272 in 0.1 N NaOH; MS[30] (direct-probe temperature, 20 °C), *m/e* 245 (M + 1), 244 (M), 226 (M - H_2O), 216 (M - CO), 198, 195 (M - H_2O - CH_2OH), 157 (P + C_2H_4), 131 (P + 2H), 114. Anal. ($C_{10}H_{13}FN_2O_4 \cdot 1.25H_2O$) C, H, N.

(±)-1-[(1α,2β,3β,4α)-2,3-Dihydroxy-4-(hydroxymethyl)-cyclopentyl]-5-fluoro-2,4(1*H*,3*H*)-pyrimidinedione (8). Compound **8** was prepared by a procedure similar to that described for the preparation of **6**, except that the reaction-mixture residue obtained after fluorination of **5** was treated with triethylamine. The crude product was purified by preparative TLC in 3:1 $CHCl_3$–CH_3OH. The pure product, which is very hygroscopic, was dried at 110 °C for 2 h: TLC, 1 spot (60 mcg, 3:1 $CHCl_3$–CH_3OH); HPLC, 99.6% (reverse phase, 97:3, H_2O-acetonitrile, isocratic, monitored at 254 nm); UV max 274 nm (ε 8400) in 0.1 N HCl, 275 (ε 8200) at pH 7, 273 (ε 6700) in 0.1 N NaOH; MS[30] (direct-probe temperature, 150 °C), *m/e* 261 (M + 1), 260 (M), 243 (M - OH), 242 (M - H_2O), 213, 211 (M - H_2O - CH_2OH), 185, 183, 173, 172, 157 (P + C_2H_4), 131 (P + 2H), 130 (P + H), 114, 113. Special precautions were required to prevent absorption of moisture during the weighing of specimens for UV and microanalytical determinations. Anal. ($C_{10}H_{13}FN_2O_5$) C, H, N.

Biochemical Methods. Examination of the effects of 5-FdUrd and of C-5-F-2′-dUrd on proliferation of L-1210 leukemia cells in suspension culture and on DNA synthesis in L-1210 cells and in murine colon tumor 26 cells in culture were conducted according to published procedures.[32,33] Enzymatic conversion of 5-FdUR and of C-5-F-2′-dUrd to the corresponding monophosphates was accomplished by a dialyzed, soluble, supernatant enzyme preparation from L-1210 cells using the reaction conditions described by Ives et al.[34] Nucleosides and nucleotides were separated by

(28) Analytical TLC was performed with plates of silica gel GF. [Precoated thin-layer chromatography plates (fluorescent), 250-μm in thickness, were purchased from Analtech Inc., Blue Hen Industrial Park, Newark, DE 19711.] Preparative TLC was performed with precoated plates of silica gel (Whatman PLK5F, 20 × 20 cm, 1 mm in thickness, Whatman Inc., Clifton, NJ 07014).

(29) 5% Fluorine–95% nitrogen; purchased from Matheson Division, East Rutherford, NJ 07073.

(30) P = 5-fluorouracilyl moiety ($C_4H_2FN_2O_2$).

(31) Positions *a–f* are shown in structure **9**.

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means of anion-exchange HPLC as described in published work from this laboratory.[35] Nucleosides were not retained on a Whatman Partisil-10 SAX HPLC column, whereas the 5'-monophosphates of 5-FdUrd and C-5-F-2'-dUrd were retained for 7 min on a linear gradient (40 min) of $NH_4H_2PO_4$ (5 mM, pH 2.8, to 750 mM, pH 3.7) at a flow rate of 2 mL/min. The dUMP analogues were separated from other UV-absorbing components of the enzyme reaction mixture, primarily adenine nucleotides, so that quantitation of nucleotide formation was possible.

Enzymatic phosphorylation of [6-^{3}H]dUrd by enzyme preparations from L-1210 cells was examined by the kinase assay method used above,[34] and effects of the deoxyuridine analogues on such phosphorylation was determined. Enzymatic phosphorolysis of deoxyuridine, 5-FdUrd, and C-5-F-2'-dUrd in 0.05 M sodium phosphate buffer, pH 7.2, was examined by means of reverse-phase HPLC on a Waters C_{18} μBondapak column using 5 mM $NH_4H_2PO_4$, pH 2.5, as an isocratic eluant (0.4 mL/min). The retention times (min) for uracil (9), 5-FUra (9.4), dUrd (15), 5-FdUrd (16.6), and C-5-F-2'-dUrd (18) were such that the enzymatic conversion of nucleoside to base could be followed by means of a UV detector (254 nm). Deoxyuridine and 5-FdUrd were readily phosphorylyzed to Ura and to 5-FUra, respectively, but conversion of C-5-F-2'-dUrd to 5-FUra was not detectable.

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Synthesis of 5,11-Methenyltetrahydrohomofolate and Its Antifolate Activity in Vitro

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The synthesis of 5,11-methenyltetrahydrohomofolate was accomplished by treatment of tetrahydrohomofolate (H_4homofolate) with triethyl orthoformate in glacial acetic acid. This compound is a homofolate analogue of 5,10-methenyltetrahydrofolate which serves as one precursor to the 10-formyl one-carbon donor for the first transformylation in de novo purine biosynthesis, namely, the conversion of glycinamide ribonucleotide (GAR) to *N*-formylglycinamide ribonucleotide (FGAR), catalyzed by the enzyme glycinamide ribonucleotide transformylase (EC 2.1.2.2). The analogue proved to retard the rate of formation of formylglycinamide ribonucleotide apparently by inhibiting the rate of synthesis of 10-formyltetrahydrofolate, the actual cofactor for the transformylase enzyme, from 5,10-methenyltetrahydrofolate. Its inhibition of the enzyme, 5,10-methenyltetrahydrofolate cyclohydrolase (EC 3.5.4.9), was competitive against (+)-L-5,10-methenyltetrahydrofolate, with a K_i = 41 μM. This derivative of homofolate may be responsible for its inhibition of purine biosynthesis in Sarcoma 180 cells.

Homofolate has been found to be a potent inhibitor of *N*-formylglycinamide ribonucleotide (FGAR) biosynthesis in Sarcoma 180 cells.[1] However, maximal expression of inhibition required prior incubation of the cells with homofolate for 24 h. Under these conditions, it was found that 20–40 μM homofolate inhibited the incorporation of [2-^{14}C]glycine into FGAR by 20–90%. Coadministration of 5-formyl-H_4folate (0.01 and 0.1 mM) and homofolate (1–200 μM) resulted in competitive protection against inhibition for FGAR biosynthesis, while administration of 5-formyl-H_4folate (0.1–10 mM) subsequent to a 24-h incubation with homofolate (10 μM) resulted in only a slight reversal of inhibition of FGAR biosynthesis.[1] These results suggested that a metabolite of homofolate was the actual inhibitor and that the enzyme GAR transformylase was the target of inhibition in this cell line. Reduced homofolates, such as H_2homofolate and H_4homofolate, appeared not to be solely responsible for the observed inhibition, since a cell subline containing 300 times more dihydrofolate reductase responded similarly.[1]

A conceivable metabolite of homofolate capable of inhibiting GAR transformylase would be the homofolate analogue of (+)L-5,10-methenyl-H_4folate that might block the synthesis of 10-formyltetrahydrofolate through inhibition of the cyclohydrolase activity of the trifunctional enzyme. The latter is one of four enzymes, along with the two tetrahydrofolate-requiring transformylases and serine transhydroxymethylase, that comprise a possible complex of enzymes involved in de novo purine biosynthesis.[2] We recently have shown that the 10-formyl rather than the 5,10-methenyl species is the actual cofactor for the GAR transformylase from the chicken liver enzyme.[3] This report describes the synthesis of 5,10-methenyl-H_4homofolate and its effect on FGAR synthesis utilizing 5,10-methenyl-H_4folate as the assay substrate for the enzyme complex in vitro.

Chemistry. The synthesis of 5,11-methenyl-H_4homofolate was accomplished, in moderate yield, through reaction of H_4homofolate with triethyl orthoformate in glacial acetic acid. H_4homofolate was obtained from homofolate in two steps by reduction with dithionite to H_2homofolate,[4] followed by dihydrofolate reductase cata-

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