Synthesis and Tumor Uptake of 5-Halo-1-(2′-fluoro-2′-deoxy-β-D-ribofuranosyl)[2-^{14}C]uracils

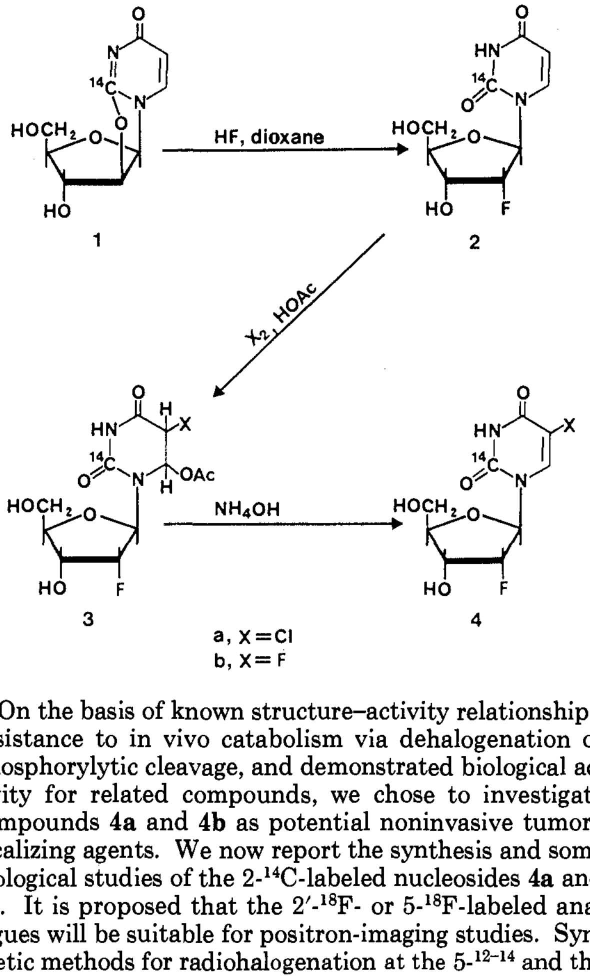
John R. Mercer, Edward E. Knaus, and Leonard I. Wiebe*

Faculty of Pharmacy and Pharmaceutical Sciences, The University of Alberta, Edmonton, Alberta, Canada T6G 2N8.
Received September 15, 1986

A synthesis of 5-chloro- and 5-fluoro-1-(2′-fluoro-2′-deoxy-β-D-ribofuranosyl)uracil (4a and 4b) and their 2-^{14}C analogues has been developed. The tissue distribution of these radiolabeled compounds in BDF$_1$ mice bearing Lewis lung tumors has been investigated. Compounds **4a** and **4b** undergo rapid blood clearance and urinary excretion. Selective retention of radioactivity was observed in tumor tissue, spleen, and intestine and with compound **4b** also in the bone. Maximum tumor to blood ratios of 4.2 for the 5-chloro compound **4a** and 10.3 for the 5-fluoro compound **4b** were observed at 4 h. These compounds were resistant to phosphorylytic cleavage and dehalogenation as indicated by the metabolic products observed in the urine and the absence of radioactivity in the liver. The interaction of **4b** with the mouse erythrocyte transporter system was compared with physiological nucleosides in respect to ability to effect zero-trans influx of thymidine. The results show a competitive inhibition between **4b** and the natural nucleoside. Evidence is presented for the direct metabolic defluorination of 5-fluorouracil to form uracil.

Many pyrimidine nucleoside analogues are selectively incorporated into a variety of experimental tumors. For example, 5-fluorouracil and 5-fluoro-2′-deoxyuridine are taken up selectively by animal tumor cells, such as the Lewis lung carcinoma in BDF$_1$ mice,[1,2] Ehrlich ascites tumor in ICR mice,[1,2] L-1210 leukemia cells in culture,[3] and the human breast carcinoma cell line MCF-7.[4,5] Recently several fluorinated pyrimidine nucleoside analogues, labeled with the positron-emitting radionuclide ^{18}F, have been used successfully in imaging studies with experimental tumors.[6] The presence of a fluorine or chlorine substituent in the ribo or arabino configuration at the 2′-position of pyrimidine nucleosides confers biochemical stability since these analogues are less susceptible than the analogous natural nucleosides to phosphorylytic cleavage catalyzed by the enzyme pyrimidine phosphorylase.[7] Despite structural modification many of the pyrimidine nucleoside analogues are still transported across cell membranes by a nucleoside transporter as demonstrated by their influx into murine[8] and human[9] erythrocytes. A number of 5-halopyrimidine nucleosides that have been evaluated as tumor-localizing agents undergo rapid dehalogenation, either directly from the monophosphate nucleotides, catalyzed by the enzyme thymidylate synthetase (5-bromo and 5-iodo analogues),[10] or via a common mechanism after phosphorylytic cleavage to the free bases (5-chloro, 5-bromo, and 5-iodo analogues).[11] In contrast, the 5-fluoro and to a lesser extent the 5-chloro analogues are resistant to the dehalogenation processes.

Scheme I



On the basis of known structure–activity relationships, resistance to in vivo catabolism via dehalogenation or phosphorylytic cleavage, and demonstrated biological activity for related compounds, we chose to investigate compounds **4a** and **4b** as potential noninvasive tumor-localizing agents. We now report the synthesis and some biological studies of the 2-^{14}C-labeled nucleosides **4a** and **4b**. It is proposed that the 2′-^{18}F- or 5-^{18}F-labeled analogues will be suitable for positron-imaging studies. Synthetic methods for radiohalogenation at the 5-[12-14] and the 2′-positions[15] of pyrimidine nucleosides are well established.

---

(1) Abrams, D. N.; Knaus, E. E.; Wiebe, L. I. *Int. J. Appl. Radiat. Isotopes* **1979**, *6*, 97.
(2) Abrams, D. N.; Knaus, E. E.; Lentle, B. C.; Wiebe, L. I. *Int. J. Nucl. Med. Biol.* **1979**, *6*, 97.
(3) Roobol, G.; Dobbeleer, G. B. E.; Bernheim, J. L. *Br. J. Cancer* **1984**, *49*, 739.
(4) Kufe, D.; Major, P. *J. Biol. Chem.* **1981**, *256*, 9802.
(5) Major, P.; Egan, E.; Herrick, D.; Kufe, D. W. *Cancer Res.* **1982**, *42*, 3005.
(6) Abe, Y.; Fukuda, H.; Ishiwata, K.; Yoshioka, S.; Yamada, K.; Endo, S.; Kubota, K.; Sato, T.; Matsuzawa, T.; Takahashi, T.; Ido, T. *Eur. J. Nucl. Med.* **1983**, *8*, 258.
(7) Abrams, D. N.; Lee, Y. W.; Mercer, J. R.; Knaus, E. E.; Wiebe, L. I. *Br. J. Radiol.* **1985**, *59*, 263.
(8) Gati, W. P.; Misra, H. K.; Knaus, E. E.; Wiebe, L. I. *Biochem. Pharmacol.* **1984**, *33*, 3325.
(9) Gati, W. P.; Knaus, E. E.; Wiebe, L. I. *Mol. Pharmacol.* **1983**, *23*, 146.
(10) Garrett, C.; Wataya, Y.; Santi, D. V. *Biochemistry* **1979**, *18*, 2798.
(11) Wasternac, C. *Pharmacol. Ther.* **1980**, *8*, 629.
(12) Crawford, E.; Friedkin, M.; Fowler, J.; Gallagher, B.; MacGregor, R.; Wolf, A.; Wodinsky, I.; Golden, A. *J. Nucl. Med.* **1978**, *19*, 702.
(13) Shuie, C.-Y.; Wolf, A. P.; Friedkin, M. *J. Labeled Compd. Radiopharm.* **1984**, *21*, 865.
(14) Abrams, D. N.; Knaus, E. E.; McQuarrie, S. A.; Wiebe, L. I. *The Chemistry of Radiopharmaceuticals*; Heindel, N. D., Burns, H. D., Honda, T., Brady, L. W., Eds.; Masson: New York, 1978; p 205.
(15) Abrams, D. N.; Mercer, J. R.; Knaus, E. E.; Wiebe, L. I. *Int. J. Appl. Radiat. Isotopes* **1985**, *36*, 233.

Table I. Tissue Distribution in BDF$_1$ Female Mice after Intravenous Injection of 5-Chloro-1-(2′-fluoro-2′-deoxy-β-D-ribofuranosyl)[2-^{14}C]uracil (**4a**)

	time, h						
organ	0.25	0.50	1	2	4	8	24
blood	3.54 ± 0.3[a]	1.82 ± 0.07	0.58 ± 0.01	0.18 ± 0.02	0.06 ± 0.002	0.03 ± 0.002	0.01 ± 0.001
	1.4[b]	1.0	1.0	0.5	0.2	0.4	0.4
spleen	2.12 ± 0.02	1.08 ± 0.02	0.48 ± 0.06	0.34 ± 0.03	0.17 ± 0.03	0.15 ± 0.02	0.06 ± 0.01
	0.83	0.60	0.83	0.99	0.74	2.1	2.0
stomach	1.41 ± 0.14	0.75 ± 0.11	0.27 ± 0.02	0.31 ± 0.04	0.07 ± 0.03	0.05 ± 0.005	0.01 ± 0.003
	0.55	0.41	0.47	0.90	0.29	0.69	0.49
git[c]	1.91 ± 0.15	1.02 ± 0.18	0.38 ± 0.04	0.17 ± 0.02	0.06 ± 0.01	0.04 ± 0.006	0.02 ± 0.004
	0.75	0.57	0.67	0.51	0.26	0.58	0.72
kidney	36.2 ± 2.2	23.2 ± 1.7	7.28 ± 0.58	1.60 ± 0.14	0.33 ± 0.03	0.09 ± 0.02	0.03 ± 0.004
	14.3	12.9	12.9	4.7	1.5	1.2	1.2
skin	2.24 ± 0.71	1.24 ± 0.31	0.36 ± 0.13	0.13 ± 0.07	0.04 ± 0.003	0.02 ± 0.008	0.01 ± 0.002
	0.85	0.69	0.63	0.37	0.15	0.33	0.32
muscle	1.48 ± 0.04	0.78 ± 0.08	0.27 ± 0.04	0.15 ± 0.02	0.05 ± 0.01	0.03 ± 0.001	0.01 ± 0.002
	0.58	0.43	0.49	0.44	0.19	0.38	0.42
bone	0.77 ± 0.11	0.41 ± 0.06	0.17 ± 0.03	0.08 ± 0.05	0.04 ± 0.02	0.02 ± 0.002	0.01 ± 0.002
	0.31	0.23	0.31	0.25	0.15	0.30	0.51
lung	2.73 ± 0.26	1.41 ± 0.14	0.43 ± 0.07	0.14 ± 0.01	0.06 ± 0.008	0.03 ± 0.004	0.01 ± 0.003
	1.1	0.78	0.73	0.42	0.25	0.46	0.50
heart	3.07 ± 0.35	1.56 ± 0.16	0.53 ± 0.02	0.17 ± 0.02	0.06 ± 0.01	0.03 ± 0.004	0.02 ± 0.003
	1.2	0.86	0.94	0.51	0.26	0.46	0.54
liver	2.39 ± 0.19	1.43 ± 0.17	0.47 ± 0.09	0.15 ± 0.02	0.05 ± 0.01	0.03 ± 0.005	0.02 ± 0.003
	0.93	0.80	0.86	0.44	0.23	0.45	0.54
tumor	2.62 ± 0.5	1.82 ± 0.2	0.60 ± 0.12	0.34 ± 0.04	0.23 ± 0.03	0.07 ± 0.01	0.03 ± 0.004
	0.74[d]	1.0	1.0	2.0	4.2	2.5	2.3

[a] The numbers represent the mean ± standard deviation for percent of injected dose per gram of wet tissue for six animals. [b] Organ to tumor ratio. [c] Section of intestine. [d] Tumor to blood ratio.

Table II. Tissue Distribution in BDF$_1$ Female Mice after Intravenous Injection of 5-Fluoro-1-(2′-fluoro-2′-deoxy-β-D-ribofuranosyl)[2-^{14}C]uracil (**4b**)

	time, h						
organ	0.25	0.50	1	2	4	8	24
blood	3.49 ± 0.02[a]	1.59 ± 0.15	0.54 ± 0.08	0.06 ± 0.004	0.02 ± 0.001	0.01 ± 0.001	0.01 ± 0.001
	0.7[b]	0.4	0.2	0.1	0.1	0.1	0.4
spleen	8.72 ± 1.0	3.77 ± 0.13	1.44 ± 0.15	0.29 ± 0.03	0.18 ± 0.04	0.09 ± 0.01	0.03 ± 0.003
	1.8	1.0	0.7	0.6	1.1	1.6	2.0
stomach	2.18 ± 0.08	0.96 ± 0.06	0.38 ± 0.04	0.07 ± 0.02	0.02 ± 0.007	0.01 ± 0.005	0.005 ± 0.001
	0.5	0.3	0.2	0.1	0.1	0.2	0.3
git[c]	3.35 ± 1.59	1.59 ± 0.18	0.71 ± 0.10	0.11 ± 0.03	0.04 ± 0.01	0.02 ± 0.004	0.01 ± 0.002
	0.7	0.4	0.3	0.2	0.3	0.4	0.8
kidney	47.4 ± 6.3	21.9 ± 2.6	8.21 ± 1.5	0.84 ± 0.17	0.08 ± 0.009	0.02 ± 0.004	0.01 ± 0.003
	10.0	5.7	3.7	1.8	0.5	0.5	0.8
skin	3.30 ± 1.2	1.18 ± 0.12	0.43 ± 0.15	0.06 ± 0.01	0.11 ± 0.17	0.01 ± 0.002	0.004 ± 0.001
	0.7	0.3	0.2	0.1	0.6	0.1	0.2
muscle	2.48 ± 0.18	1.06 ± 0.07	0.37 ± 0.04	0.06 ± 0.001	0.02 ± 0.003	0.01 ± 0.002	0.004 ± 0.001
	0.5	0.3	0.2	0.1	0.1	0.3	0.3
bone	1.69 ± 0.27	0.85 ± 0.32	0.35 ± 0.06	0.11 ± 0.04	0.07 ± 0.03	0.03 ± 0.005	0.02 ± 0.002
	0.3	0.2	0.2	0.3	0.4	0.6	0.9
lung	3.37 ± 0.32	1.43 ± 0.11	0.51 ± 0.07	0.07 ± 0.005	0.02 ± 0.001	0.01 ± 0.002	0.005 ± 0.001
	0.7	0.4	0.2	0.2	0.1	0.2	0.3
heart	3.80 ± 0.19	1.71 ± 0.13	0.58 ± 0.06	0.08 ± 0.006	0.02 ± 0.002	0.009 ± 0.002	0.005 ± 0
	0.8	0.4	0.3	0.2	0.1	0.2	0.3
liver	3.37 ± 0.23	1.54 ± 0.20	0.53 ± 0.10	0.08 ± 0.01	0.02 ± 0.004	0.01 ± 0.003	0.005 ± 0.001
	0.7	0.4	0.2	0.2	0.2	0.2	0.3
tumor	5.26 ± 1.5	3.88 ± 0.6	2.24 ± 0.37	0.49 ± 0.07	0.16 ± 0.02	0.05 ± 0.009	0.02 ± 0.002
	1.5[d]	2.4	4.2	7.7	10.3	7.6	2.6

[a] The numbers represent the mean ± standard deviation for percent of injected dose per gram of wet tissue for six animals. [b] Organ to tumor ratio. [c] Section of intestine. [d] Tumor to blood ratio.

Results and Discussion

Chemistry. The versatile intermediate 2,2′-anhydro-1-β-D-arabinofuranosyluracil (**1**) was synthesized by the method of Verheyden et al.[16] and its 2-^{14}C analogue was synthesized from Ba[^{14}C]CO$_3$ as described previously.[17] Unlabeled 1-(2′-fluoro-2′-deoxy-β-D-ribofuranosyl)uracil (**2**) was prepared by the reaction of **1** with HF in anhydrous dioxane,[15,18] while [2-^{14}C]-**2** was obtained in 25% yield by using a modified procedure (Scheme I). The 2′,5-dihalo compounds **4a** and **4b** were synthesized in unlabeled and 2-^{14}C-labeled form by the electrophilic addition reactions of **2** with dilute solutions of the appropriate halogen in acetic acid. TLC analysis of the crude reaction mixtures often indicated an additional major product, 5-halo-6-*O*-acetyl-5,6-dihydro-1-(2′-fluoro-2′-deoxy-β-D-ribofuranosyl)uracil (**3**) characterized by its chromatographic and chemical behavior and by analogy to observations recorded in related syntheses.[19-21] Compounds **3a** and **3b**

---

(16) Verheyden, J. P. H.; Wagner, D.; Moffatt, J. G. *J. Org. Chem.* 1971, *36*, 250.

(17) Giziewicz, J.; Gati, L. J.; Knaus, E. E.; Mercer, J. R.; Flanagan, R. J.; Wiebe, L. I. *Int. J. Appl. Radiat. Isotopes* **1985**, *36*, 227.

(18) Codington, J. F.; Doerr, I. L.; Fox, J. J. *J. Org. Chem.* **1964**, *29*, 558.

were readily converted to **4a** and **4b** by treatment with aqueous or methanolic ammonia.

Biological Distribution Studies. The biological distributions of [2-^{14}C]-**4a** and [2-^{14}C]-**4b** were determined following injection of 23–193 kBq of the compound in normal saline via the tail vein into female BDF$_1$ mice bearing an implanted Lewis lung tumor. The differential tissue distributions of **4a** and **4b** in animals sacrificed at appropriate time intervals are shown in Tables I and II.

Blood levels measured at 15 min, 30 min, and 1 h were 3.54%, 1.82%, and 0.58%, respectively, of the injected dose per gram for the 5-chloro compound **4a** (Table I) and 3.49%, 1.59%, and 0.54%, respectively, for the 5-fluoro compound **4b** (Table II). Rapid blood clearance is indicated since less than 1% of the injected dose remained in the blood of mice at 1 h after injection of **4a** and **4b**. The kidney and urinary bladder contents showed very high activity during the first hour, indicating that urinary excretion was the main route of elimination. The major radioactive constituent present in the urine at times up to 4 h was unmetabolized **4a** and **4b**. Although the blood ^{14}C activity levels measured for **4a** and **4b** were similar over the first hour, some difference was noted between the compounds in their activity levels in a number of tissues. With the chloro compound **4a** only the kidney showed an activity level greater than 1% of the injected dose per gram at 1 h after injection. Tumor tissue showed the next highest concentration of activity at 0.60%. The 1-h tumor concentration of ^{14}C activity for the fluoro compound **4b** was 2.24% of the dose per gram followed by the spleen, intestine, and heart at 1.44%, 0.71%, and 0.58%, respectively.

A number of the tissues examined showed selective ^{14}C activity uptake. The results (Tables I and II) indicate that selective retention of ^{14}C activity occurred for both **4a** and **4b** in spleen, tumor, stomach, and intestine and in the bone for the 5-fluoro compound. The tumor to blood ratios for **4a** and **4b** were of particular interest, reaching maximum values of 4.2 and 10.3, respectively, at 4 h. For the 5-fluoro compound **4b**, only the spleen had an organ to tumor ratio greater than 1.0 at 4 h. A second group of tissues including liver, heart, and lung showed levels of ^{14}C activity comparable to blood levels.

These data suggest that the 5-chloro and 5-fluoro compounds **4a** and **4b** are transported freely into most tissues and that the equilibrium between intracellular and extracellular concentrations of the nucleoside is rapidly achieved. A number of tissues having a population of cells with a high mitotic index, such as intestine, spleen (a site of hematopoietic activity in the mouse), tumor, and bone, accumulated activity against the concentration gradient and demonstrated a relative increase in activity when compared to blood and other organs.

Interaction of 4b with the Mouse Erythrocyte Nucleoside Transporter. The methods used to determine the transport behavior of **4b** have been described previously.[8,9] Competition studies, in which the effect of **4b** on the zero-trans influx of [6-^{3}H]thymidine into mouse erythrocytes was compared with thymidine, were analyzed according to the method of Dixon.[22] The value for inhibitor constant (K_i) obtained in the experiment was 0.21 (±0.019). This value is somewhat larger than values obtained with physiological nucleosides such as thymidine and deoxyuridine (0.09 ± 0.001 and 0.11 ± 0.02, respectively)[8] and is interpreted as indicating that **4b** binds to a nucleoside transporter although with somewhat less affinity than the natural nucleosides. This observation of zero-trans influx suggests that **4b** is carried across the cell membrane by a nucleoside transporter.

Table III. Radioactive Constituents of Urine at Various Time Intervals after Injection of 5-Fluoro-1-(2′-fluoro-2′-deoxy-β-D-ribofuranosyl)[2-^{14}C]uracil (**4b**) into Female BDF$_1$ Mice Bearing a Lewis Lung Tumor

	percent of total urine radioactivity				total urine activity[a]	
time, h	4b	5-fluorouracil	uracil	other	interval, h	% of dose
0.5	90.7	2.1	1.4	5.0	0–0.5	68
2	88.5	2.8	3.3	5.4	0.5–2	30
4	63.4	16.3	7.3	23.0	2–4	0.8
8	52.4	4.5	4.5	38.6	4–8	0.2

[a] The total urine activity excreted in each time interval was estimated from the measurement of loss of tissue activity.

Elimination and Metabolism. The identity and relative concentration of the urinary metabolites for **4a** and **4b** were determined by reverse-phase high-pressure liquid chromatography (HPLC) measurements on urine collected at time of sacrifice. Analysis of urine at times up to 4 h after administration of the 5-chloro nucleoside **4a** revealed that greater than 95% of the ^{14}C activity was due to unmetabolized compound. Small amounts of activity detected in 4-h urine were present as [^{14}C]uracil (2.0%) and 5-chloro[^{14}C]uracil (2.1%). The presence of these minor components is consistent with the expected route for catabolism of nucleoside analogues via phosphorylytic cleavage followed by dehalogenation of the resulting 5-halopyrimidine.[11] Unmetabolized [^{14}C]-**4b** was the major radioactive constituent in urine at times up to 8 h after injection of [^{14}C]-**4b** into BDF$_1$ mice as indicated in Table III. An estimated 90% of the injected dose of **4b** is eliminated in the urine as unmetabolized compound. A more complex mixture of metabolic products was observed for the 5-fluoro compound **4b** relative to the 5-chloro compound **4a**. 5-Fluorouracil and uracil were detected as metabolic products of **4b** at all time periods and accounted for 2.3% and 2.0%, respectively, of the injected dose. Unidentified metabolic products accounted for 5.3% of the injected dose, with these products increasing in proportion at the longer time periods.

The detection of [^{14}C]uracil as a urinary metabolite of **4b** was unexpected. Until recently, catabolism of 5-fluorouracil and its nucleosides was believed to proceed via 5-fluoro-5,6-dihydrouracil, α-fluoro-β-ureidopropionic acid, and α-fluoro-β-alanine[11] without defluorination. In vivo defluorination has, however, been suggested to explain the uptake of ^{18}F by the bone in experimental animals after the injection of 5-^{18}F-labeled fluoropyrimidine nucleosides and bases.[6] Recently F$^-$ anion has also been detected in a ^{19}F NMR study of the urine of human patients after injection of 5′-deoxy-5-fluorodeoxyuridine.[23] The fluorine anion was proposed to arise from α-fluoro-β-alanine. The present study provided the first evidence that direct defluorination from 5-fluorouracil may occur in a manner analogous to the dehalogenation that occurs with 5-chloro, -bromo, and -iodouracil.[10,11] The detection of 5-fluorouracil

---

(19) Vine, E. N.; Young, D.; Vine, W. H.; Wolf, W. *Int. J. Appl. Radiat. Isotopes* **1979**, *30*, 401.

(20) Cech, D.; Holý, A. *Collect. Czech. Chem. Commun.* **1976**, *41*, 3335.

(21) Kobayashi, Y.; Kumadaki, I.; Nakazato, A. *Tetrahedron Lett.* **1980**, *21*, 4605.

(22) Dixon, M. *Biochem. J.* **1953**, *55*, 170.

(23) Malet-Martino, M. C.; Martino, R.; Lopez, A.; Beteille, J. P.; Bon, M.; Bernadou, J.; Armand, J. P. *Biochem. Pharmacol.* **1985**, *34*, 429.

and the absence of 2′-fluoro-2′-deoxyuridine in the urine after injection of **4b** suggests a metabolic defluorination from the free base rather than from the intact nucleoside.

Discussion. Although the animal studies were designed primarily to evaluate compounds **4a** and **4b** as potential agents for use in noninvasive diagnostic oncology, it is possible to make a number of general observations relating to their in vivo behavior and biochemistry. These compounds demonstrated rapid blood clearance, were eliminated primarily in unmetabolized form via the urine (Table III), and demonstrated resistance to phosphorylytic cleavage catalyzed by pyrimidine phosphorylase. These observations are consistent with the behavior observed for a number of other pyrimidine nucleoside analogues modified at the 2′- and 3′-positions.[24-27]

Compounds 4a and **4b** appeared to freely permeate most tissues, suggesting that they are good substrates for a pyrimidine nucleoside transport system. The transport studies with compound **4b** in mouse erythrocytes confirmed the affinity of this modified nucleoside for a pyrimidine nucleoside transporter. This compound demonstrated effective competition with physiological nucleosides in transport across all cell membranes. A number of tissues showed activity levels similar to blood levels, indicating that the concentration gradient was a sufficient driving force to allow movement of the compounds across the cell membrane. Transport of nucleosides and nucleoside analogues into mammalian cells occurs via a rapid and nonconcentrative mechanism that is dependent upon the relative concentration of substrate on either side of the cell membrane and on the affinity of the substrate for a transporter required to move it across the membrane.[28] It is proposed that unmetabolized nucleosides 4a and 4b were the species transported. A second group of tissues showed retention of activity in excess of blood concentration. This suggests metabolic trapping of 4a and 4b through cellular processes such as phosphorylation. Metabolic trapping is supported by the observation of enhanced activity uptake in tissues with a high mitotic index (spleen, tumor, and intestine). Cellular thymidine kinase activity is known to be elevated in rapidly proliferating cells.[29]

The actual biochemical involvement of the nucleosides within the cell appeared to involve a reversible process. Although the relative concentration of the radioactivity in some tissues remained high, the absolute concentration dropped as the blood concentrations of the injected nucleosides 4a and 4b decreased. Those tissues containing an activity concentration greater than blood concentration may be considered to have an intracellular activity component due to freely permeating unaltered nucleoside and a second intracellular component due to metabolically trapped nucleoside.

These results are consistent with a model in which the nucleoside is reversibly incorporated into the tumor's cellular nucleotide pool via phosphorylation by the uridine or thymidine kinases. The persistence of activity in the spleen and tumor, and the increase in unidentified radioactive constituents in the urine at 4 and 8 h, suggested that **4a** and more particularly **4b** were metabolically trapped within the cells, possibly as constituents of DNA and RNA pools.

The relative utility of radiopharmaceuticals as imaging agents has been assessed by using the "tumor index" (TI).[30] This value is the product of tumor selectivity, as measured by the tumor to blood ratio, and tumor activity, as measured by the percent of the injected dose per gram of tumor tissue. The TI value for compound **4b** had a maximum value of about 9.4 between 0.5 and 1.0 h. In comparison, 5-[^{18}F]fluoro-2′-deoxyuridine ([^{18}F]FUdR), which was successfully used to obtain positron emission tomographic images of a rabbit tumor,[6] had a maximum TI of about 6.9 at 2 h when the tissue distribution of [^{18}F]FUdR was determined in tumor-bearing rats. In contrast to [^{18}F]FUdR, **4b** demonstrated a low hepatic uptake. Presumably the catabolic processes leading to deposition of ^{18}F activity in the liver after injection of [^{18}F]FUdR are less active with [^{14}C]-**4b**. The lower liver activity demonstrated by **4b** should allow improved imaging of tumors in the abdominal cavity and **4b** may prove to be a suitable agent for hepatic tumor imaging. Furthermore, higher blood levels and improved tumor uptake of **4a** and **4b** might be achieved by altering the route or the rate of administration.

Experimental Section

Chemicals and reagents used were of reagent grade. Reference compounds for high-performance liquid chromatography (HPLC) analysis were obtained from commercial suppliers or synthesized in our laboratories by using established procedures. Fluorine and chlorine gas were obtained from Matheson Gas Products and were of the highest quality routinely available. The fluorine was manipulated via a manifold system using components approved for fluorine service. ^{14}C-labeled compounds were derived from Ba[^{14}C]CO$_3$ purchased from Atomic Energy of Canada Ltd. The measured specific activity of 1.89 GBq/mmol for Ba[^{14}C]CO$_3$ was maintained in all the synthetic products. Melting points (mp) were determined on a Büchi capilliary apparatus and are uncorrected. NMR spectra were determined on a Brucker AM 300 spectrometer using deuteriated dimethyl sulfoxide (Me_2SO-d_6) as solvent with Me_4Si as in internal reference. Mass spectra (MS) were determined on an AEI MS 50 mass spectrometer. High-resolution mass spectra (HRMS) were used in lieu of combustion analysis for determining the elemental composition. Tissue samples containing ^{14}C-labeled compounds were combusted in a H. J. Harvey Instrument Corp. "Biological Oxidizer" Model OX 300. The [^{14}C]CO$_2$ produced by combustion was trapped in Harvey [^{14}C]-Cocktail and counted by liquid scintillation (LSC) with either a Beckman LS 9000 or a Searle Mark III counter. Samples from sources other than combustion were counted in Aquasol II (New England Nuclear). HPLC analyses were carried out with a Waters system consisting of a Model 860 automated gradient controller, Models 510 and M-45 solvent pumps, Model U6K injector, and Model 480LC ultraviolet detector at 256 nm. Separations were performed with a Whatman Partisil M9 10/25 ODS reverse-phase column (column A) or a Waters C-18 Radial-PAK reverse-phase column (column B). Thin-layer chromatography (TLC) was performed on Whatman MK6F silica gel microslides. Activity histograms of compound mixtures were obtained by scraping the silica from developed plates and counting the fractions by liquid scintillation (TLC–LSC).

2,2′-Anhydro-1-β-D-arabinofuranosyluracil (1). The title compound was obtained in 82% yield after recrystallization from ethanol by the literature procedure;[16] mp 238–239.5 °C (lit.[16] mp 238–244 °C); exact mass calcd. for $C_9H_{10}N_2O_5$ 226.0589, found (HRMS) 226.0591 (M$^+$, 45%).

1-(2′-Fluoro-2′-deoxy-β-D-ribofuranosyl)uracil (2). The title compound was prepared according to the literature procedure[15,18] and purified by column chromatography (2% to 10% MeOH in

---

(24) Cook, A. F.; Holman, M. J.; Kramer, M. J. *J. Med. Chem.* 1980, *23*, 852.

(25) Cook, A. F.; Holman, M. J.; Kramer, M. J.; Trown, P. W. *J. Med. Chem.* 1979, *22*, 1330.

(26) Coderre, J. A.; Santi, D. V.; Matsuda, A.; Watanabe, K. A.; Fox, J. J. *J. Med. Chem.* 1983, *26*, 1149.

(27) Ajmera, S.; Bapat, A. R.; Danenberg, K.; Danenberg, P. V. *J. Med. Chem.* 1984, *27*, 11.

(28) Plagemann, P. G. W.; Wohlhueter, R. M. *Current Topics in Membranes and Transport*; Bronner, F., Kleinzeller, A., Eds.; Academic: New York, 1980; p 226.

(29) Bresnic, E.; Williams, S. S. *Biochem. Pharmacol.* 1967, *16*, 503.

(30) Emrich, D.; Mühlen, A.; Willgeroth, F.; Lammach, A. *Acta Radiol.: Ther., Phys., Biol.* 1972, *11*, 566.

$CHCl_3$ gradient, silica gel) to give a 41% yield of **2** as fine needles; mp 149–150 °C (lit.[31] mp 150–151 °C); exact mass calcd for $C_9H_{11}N_2O_5F$ 246.0652, found (HRMS) 246.0649 (M^+, 2.7%).

5-Chloro-1-(2′-fluoro-2′-deoxy-β-D-ribofuranosyl)uracil (4a). A solution of chlorine in acetic acid (1.5 mL, 0.85 M, 1.28 mmol) was added to a solution of **2** (0.201 g, 0.82 mmol) in 25 mL of acetic acid. The reaction mixture was stirred for 10 min at room temperature and then the acetic acid was removed in vacuo below 40 °C. The residue was treated twice with ethanol and the ethanol evaporated to remove residual acetic acid. The dry residue was treated with 0.5 mL of concentrated NH_4OH in 20 mL of methanol with stirring for 18 h to convert the dihydro intermediate **3a** to the required product **4a**. The crude reaction mixture was purified by elution from a column of silica gel (15 g, 1.6 cm × 16 cm, 0% to 5% MeOH in CH_2Cl_2 gradient elution). The product **4a** was obtained in 52% yield as a colorless gum that resisted crystallization; ^1H NMR (Me_2SO-d_6) δ 11.90 [1 H, s, N(3)-H], 8.52 [1 H, s, C(6)-H], 5.92 [1 H, d, *J*(1′,5) = 16.5 Hz, C(1′)-H], 5.68 [1 H, d, *J*(OH,3′) = 6.5 Hz, C(3′)-OH], 5.48 [1 H, t, *J*(OH,5′) = 4.5 Hz, C(5′)-OH], 5.09 [1 H, dd, *J*(2′,F) = 54, *J*(2′,3′) = 4.1 Hz, C(2′)-H], 4.14–4.32 [1 H, complex m, becomes ddd after D_2O exchange, *J*(3′,F) = 23.5, *J*(3′,4′) = 8.5, *J*(3′,2′) = 4.1 Hz, C(3′)-H], 3.96 [1 H, br d, *J*(4′,3′) = 8.5 Hz, C(4′)-H], 3.89 and 3.87 [2 H, ddd, *J*(gem) = 12.5, *J*(5′,OH) = 4.5, *J*(5′,4′) = 2.1 Hz, becomes dd on D_2O exchange, C(5′)-H], signals at 11.90, 5.68, and 5.48 disappear after D_2O exchange; exact mass calcd for $C_9H_{10}N_2O_5F^{37}Cl$ 282.0232, found (HRMS) 282.0230 (M^+, 0.42%); exact mass calcd for $C_9H_{10}N_2O_5F^{35}Cl$ 280.0262, found (HRMS) 280.0256 (M^+, 1.27%).

5-Fluoro-1-(2′-fluoro-2′-deoxy-β-D-ribofuranosyl)uracil (4b). A fluorination solution was prepared by bubbling nitrogen-diluted fluorine (2.5% F_2) through 100 mL of acetic acid contained in a round-bottom flask at room temperature. The fluorine concentration was determined by treating an aliquot of this solution with excess KI and titrating the I_2 liberated with standard thiosulfate. The freshly prepared solution of F_2 in acetic acid (60 mL, 0.0185 M, 1.11 mmol) was added to **2** (0.160 g, 0.66 mmol) in a 250-mL flask. After the mixture was stirred for 10 min at room temperature, the acetic acid was removed in vacuo at 50 °C. The residue was redissolved in ethanol twice and the solvent evaporated to remove residual acetic acid. The resulting yellow foam contained the title compound **4b** and a second major component believed to be **3b**. The dihydro adduct **3b** was converted to **4b** by stirring for 18 h with 1 mL of concentrated NH_4OH in 25 mL of CH_3OH. Purification by silica gel column chromatography (15 g, 1.5 cm × 15 cm, 5% CH_3OH in CH_2Cl_2) yielded **4b** as a white foam (163 mg, 0.62 mmol, 94%), which resisted all attempts at crystallization. HPLC analysis (column B, 5% CH_3OH in H_2O) indicated this material to be >98% pure; ^1H NMR (Me_2SO-d_6) δ 11.70 [1 H, br s, N(3)-H], 8.38 [1 H, d, *J*(6,F) = 8.4 Hz, C(6)-H], 5.90 [1 H, d, *J*(1′,F) = 16.5 Hz, C(1′)-H], 5.68 [1 H, d, *J*(OH,3′) = 5.7 Hz, C(3′)-OH], 5.45 [1 H, br s, C(5′)-OH], 5.05 [1 H, dd, *J*(2′,F) = 53, *J*(2′,3′) = 4.0 Hz, C(2′)-H], 4.20–4.32 [1 H, complex, becomes ddd after D_2O exchange, *J*(3′,F) = 23.4, *J*(3′,4′) = 8.5, *J*(3′,2′) = 4.0 Hz, C(3′)-H], 3.92 [1 H, br d, *J*(4′,3′) = 8.5 Hz, C(4′)-H], 3.82 and 3.65 [2 H, complex d, *J*(gem) = 12.6 Hz, becomes dd on D_2O exchange, *J*(gem) = 12.6, *J*(5′,4′) = 2.0 Hz, C(5′)-H], signals at 11.70, 5.68, and 5.45 disappear after D_2O exchange; exact mass calcd for $C_9H_{10}N_2O_5F_2$ 264.0557, found (HRMS) 264.0555 (M^+, 9.2%).

2,2′-Anhydro-1-β-D-arabinofuranosyl[2-^{14}C]uracil ([2-^{14}C]-1). This compound was prepared in 24.6% chemical and radiochemical yield from Ba[^{14}C]CO_3 by the literature procedure;[17] mp 246.5–248 °C (lit.[16] mp for nonradioactive compound 238–244 °C).

1-(2′-Fluoro-2′-deoxy-β-D-ribofuranosyl)[2-^{14}C]uracil ([2-^{14}C]-2). A sample of [2-^{14}C]-**1** (12.81 mg, 51.7 μmol, 96.2 MBq) in a 3-mL Teflon reaction vial was dried for 18 h in vacuo over P_2O_5. A solution of anhydrous HF in dioxane was prepared by condensing HF gas in a sealed Teflon vial containing 3 mL of carefully dried and freshly distilled dioxane. The resulting solution contained 250 mg/mL of HF. This hydrogen fluoride solution (1 mL, 250 mg HF, 12.5 mmol) was injected into the Teflon vial containing [2-^{14}C]-**1** through a Teflon-backed septum. The sealed vial was heated at 115 °C (bath temperature) for 18 h. The cooled solution and a 5-mL wash with water were transferred to a 30-mL Teflon vial. This solution was treated with solid $CaCO_3$ until no further evolution of gas was noted. The aqueous layer was separated from the solid by centrifugation, evaporated to dryness in vacuo at 45 °C, and dissolved in ethanol and the solvent was evaporated. Analysis of this crude product by HPLC showed the expected product [2-^{14}C]-**2** as the major component with a retention time of 13.8 min with [2-^{14}C]-**1** at 3.9 min and uracil at 4.2 min as well as several unidentified minor compounds (column B, 2% CH_3OH in H_2O, 1 mL/min). The identity of the HPLC peaks were confirmed with authentic nonradioactive compounds as reference materials. The crude material was purified by preparative HPLC (column A, 2% CH_3OH in H_2O, 3 mL/min). The product after chromatography had chemical and radiochemical purity of 98%. The total activity of the isolated product was 26.6 MBq (25.4% radiochemical yield).

5-Chloro-1-(2′-fluoro-2′-deoxy-β-D-ribofuranosyl)[2-^{14}C]uracil ([2-^{14}C]-4a). A freshly prepared solution of Cl_2 in HOAc (40 μL, 13.6 μmol, 2.4 equiv) was added to a solution of [2-^{14}C]-**2** (1.39 mg, 5.60 μmol, 10.42 MBq) in 40 μL of HOAc in a 1-mL reaction vial. After 20 min at room temperature, the solvent was blown off with dry N_2 and the residue was treated with two drops of NH_4OH in 0.5 mL of CH_3OH at 50 °C for 10 min. The solvent was again blown off and the residue was examined by TLC. A single product was visible and combined TLC–LSC indicated that greater than 90% of the plate activity corresponded to [2-^{14}C]-**4a**. The product was purified by HPLC (column A, 2% CH_3OH in H_2O, 3 mL/min) to yield [2-^{14}C]-**4a** in 99% chemical and radiochemical purity with no trace of starting [2-^{14}C]-**2**. This product was identical (TLC, HPLC) with an authentic nonradioactive sample of **4a**. The product had an activity of 8.03 MBq (77% radiochemical yield).

5-Fluoro-1-(2′-fluoro-2′-deoxy-β-D-ribofuranosyl)[2-^{14}C]uracil ([2-^{14}C]-4b). A solution of F_2 in HOAc (1 mL, 9.4 μmol of F_2) was added to [2-^{14}C]-**2** (1.4 mg. 5.97 μmol, 11.1 MBq) in a 25-mL flask. After 20 min at room temperature, the solvent was removed in vacuo at 40 °C. The residue was dissolved in ethanol (5 mL) and again evaporated to dryness. The pale yellow gum was treated with 100 μL of NH_4OH in 1 mL of CH_3OH for 10 min at 40 °C and again reduced to dryness in vacuo. A TLC chromatogram showed complete conversion to [2-^{14}C]-**4b** and combined TLC–LSC indicated a 91.7% radiochemical yield of the crude product. A portion of this product was purified by HPLC (column A, 5% CH_3OH in H_2O, 3 mL/min) to give 6.44 MBq of [2-^{14}C]-**4b** with a radiochemical purity >99% and no trace of the starting compound [2-^{14}C]-**2**. This material was identical (TLC, HPLC) with authentic unlabeled **4b**.

Tissue Distribution Studies. The Lewis lung tumor was transplanted and maintained as described previously,[32] with female BDF_1 mice (18–20 g). Radiolabeled compounds were stored as a dry film in sterile multidose vials and were reconstituted with normal saline just prior to use. Compounds were injected via the tail vein. Animals were maintained with food and water ad lib and sacrificed by asphyxiation with CO_2 and exsanguination via cardiac puncture. An upper weight limit of about 200 mg for tissue sample combustion required the use of tissue aliquots with some organs. Tissues were weighed wet into paper combustion cups (Packard Instrument Co.) and dried under a heat lamp prior to combustion.

Transport Studies. Transport of compound **4b** was determined with mouse erythrocytes exposed to various concentrations of extracellular **4b** and [6-^{3}H]thymidine. The concentration of [6-^{3}H]thymidine in the extracellular fluid at various times after this exposure was determined by counting aliquots by liquid scintillation counting. These procedures and the interpretation of results have been described in detail elsewhere.[8,9]

HPLC Analysis of Urinary Metabolites. Urine collected at the time of sacrifice was stored frozen until required. The urine was thawed, filtered through a 0.45-μm filter, spiked with a series

---

(31) Codington, J. F.; Doerr, I. L.; Praag, D. V.; Bendich, A.; Fox, J. J. *J. Am. Chem. Soc.* **1961**, *83*, 5030.

(32) Lee, Y. W.; Abrams, D. N.; Wiebe, L. I.; Knaus, E. E. *Int. J. Nucl. Med. Biol.* 1984, *11*, 262.

J. Med. Chem. 1987, 30, 675–678

of nonradioactive reference compounds and injected directly for HPLC analysis. The reference compounds were those that are possible products arising from simple metabolic or degradative modification of the test compounds **4a** and **4b**. The reference mixture contained, in addition to **4a** and **4b**, **2**, uracil, uridine, 2′-deoxyuridine, the 5-halouracil, and the 5-halouridine. The reference mixture containing 5-chloro compounds was resolved with 2% CH_3OH in H_2O at a flow rate of 1 mL/min (column B) and the 5-fluoro compounds similarly with 4% CH_3OH in H_2O. The radioactive components of the urine were identified by superimposing the activity histogram, obtained by liquid scintillation counting of the column eluate, with the UV trace of the spiked urine. Assignments were confirmed by exact coincidence of UV and radioactivity chromatograms under varying HPLC conditions.

Acknowledgment. We are grateful to Li Xu for performing the transport study on compound **4b**. We thank the Medical Research Council of Canada (Grant No. MT-5965) for financial support of this research. One of the authors (J.R.M.) is indebted to the Alberta Heritage Foundation for Medical Research for financial assistance in the form of a studentship.

Registry No. 1, 3736-77-4; [2-^{14}C]-1, 99285-47-9; 2, 784-71-4; [2-^{14}C]-**2**, 99277-84-6; **4a**, 55612-15-2; [2-^{14}C]-**4a**, 106711-36-8; **4b**, 72-84-4; [2-^{14}C]-**4b**, 106711-37-9.

Folate Analogues as Inhibitors of Thymidylate Synthase

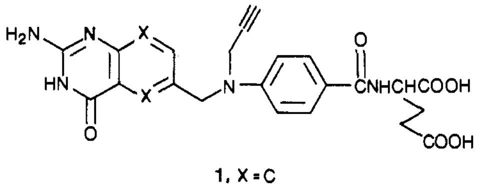
Diana I. Brixner,[†] Takamori Ueda,[‡] Yung-Chi Cheng,[‡] John B. Hynes,[§] and Arthur D. Broom*[†]

Department of Medicinal Chemistry, College of Pharmacy, University of Utah, Salt Lake City, Utah 84112, Department of Pharmacology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27514, and Department of Pharmaceutical Sciences, University of South Carolina, Charleston, South Carolina 29401. *Received August 11, 1986*

Recent demonstrations that deazafolate analogues may act as potent inhibitors of thymidylate synthase (TS) provided a firm rationale for the synthesis of N^{10}-propargyl derivatives of 8-deazafolate (**3**) and 8-deazaaminopterin (**4**). A complete assignment of the ^{1}H NMR spectra of these compounds was made possible through application of 2D (COSY) techniques at 200 MHz. Data describing the inhibition of TS derived from human leukemia (K562) cells are presented. IC_{50} values of 2.25 and 1.26 μM were determined for 8-deaza-10-propargylfolate and 8-deaza-10-propargylaminopterin, respectively. Comparison of the data for various folate analogues reveals a striking dependence of TS inhibitory potency upon the number of nitrogens in the folate pyrazine ring.

A number of folate analogues have been studied for their ability to inhibit thymidylate synthase (TS), an enzyme that catalyzes the C-methylation reaction of 2′-deoxyuridylate (dUMP) to provide 2′-deoxythymidylate (dTMP).[1-6] This one-carbon transfer reaction is critical to cell division, since it provides the sole de novo source of dTMP, an essential building block for DNA synthesis.[7] Hence, TS has long been considered a key target enzyme in the design and synthesis of antitumor agents.[7-9]

The most impressive activity demonstrated to data has been associated with N^{10}-propargyl-5,8-dideazafolate (PDDF, **1**) with K_i values reported as about 1 nM for the murine leukemia L1210 enzyme[3] and 20 nM for the human enzyme derived from either HeLa or KB cells.[10] Surprisingly, N^{10}-propargylfolate (**2**) itself was a poor inhibitor of the bacterial enzyme, having an IC_{50} of 3.9 μM against the *L. casei* enzyme,[6] very similar to the value of 5.5 μM against TS from K562 cells found in this study. N^{10}-Propargylaminopterin has also been prepared and was a very poor inhibitor of the enzyme (IC_{50} = 20 μM).[5] The preparation and evaluation of the 8-deaza analogue **3** became, therefore, of considerable interest; the 4-amino congener **4** was prepared for comparison purposes.

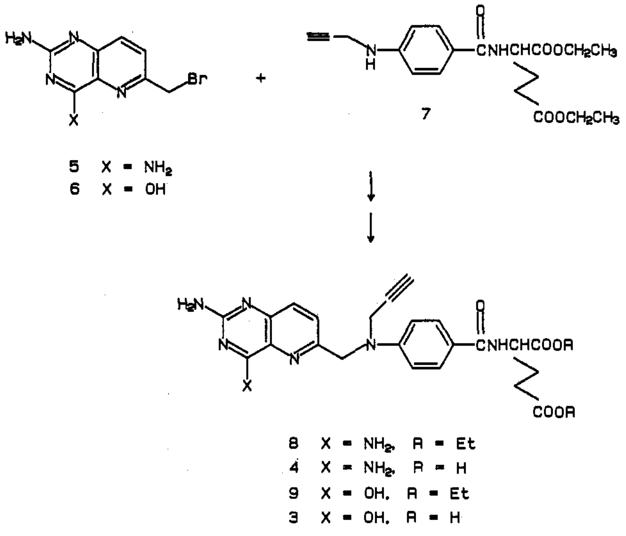


1, X=C
2, X=N

Chemistry

Previously described procedures[11,12] were used to prepare 6-(bromomethyl)-2,4-diamino- (**5**) and 2-amino-6-(bromomethyl)-4-oxopyrido[3,2-*d*]pyrimidine (**6**). Diethyl *N*-[4-(propargylamino)benzoyl]glutamate (**7**) was prepared

Scheme I



[†] University of Utah.

[‡] University of North Carolina at Chapel Hill.

[§] University of South Carolina.

(1) Szeto, D. W.; Cheng, Y.-C.; Rosowsky, A.; Yu. C.-S.; Modest, E. J.; Piper, J. R.; Temple, C., Jr.; Elliott, R. D.; Rose, J. D.; Montgomery, J. A. *Biochem. Pharmacol.* **1979**, *28*, 2633.

(2) Scanlon, K. J.; Moroson, B. A.; Bertino, J. R.; Hynes, J. B. *Mol. Pharmacol.* **1979**, *16*, 261.

(3) Jones, T. R.; Calvert, A. H.; Jackman, A. L.; Brown, S. J.; Jones, M.; Harrap, K. R. *Eur. J. Cancer* **1981**, *17*, 11.

(4) Fernandes, D. J.; Bertino, J. R.; Hynes, J. B. *Cancer Res.* **1983**, *43*, 1117.

(5) Piper, J. R.; McCaleb, G. S.; Montgomery, J. A.; Kisliuk, R. L.; Gaumont, Y.; Sirotnak, F. M. *J. Med. Chem.* **1982**, *25*, 877.

(6) Ghazala, M.; Nair, M. G.; Toghiyani, R. R.; Kisliuk, R. L.; Gaumont, Y.; Kalman, T. I. *J. Med. Chem.* **1986**, *29*, 1263.

(7) Santi, D. V.; Dannenberg, P. V. In *Folates and Pterins*: Blakley, R. L., Benkovic, S. J., Eds.; Wiley: New York, 1984; p 345.

(8) Cohen, S. S.; Barner, H. D. *Proc. Natl. Acad. Sci. U.S.A.* **1954**, *46*, 885.

0022-2623/87/1830-0675$01.50/0