on the β-helix transition of poly-L-lysine in sodium dodecyl sulfate solution. *Biopolymers* **14**: 1841–1846, 1975.
17. Sarker PK and Doty P, The optical rotatory properties of the β-configuration in polypeptides and proteins. *Proc Natl Acad Sci USA* **55**: 981–989, 1966.
18. Watanabe S and Saito T, A CD study of the role of metal ions in the conformation of poly(L-Lysine). *Biopolymers* **26**: 625–632, 1987.
19. Davidson B and Fasman GD, The conformational transitions of uncharged poly-L-lysine. α Helix–random coil–β structure. *Biochemistry* **6**: 1616–1629, 1967.

Biochemical Pharmacology, Vol. 42, No. 12, pp. 2400–2403, 1991.
Printed in Great Britain.

0006-2952/91 $3.00 + 0.00

Biochemical and biological properties of methotrexate analogs containing D-glutamic acid or D-erythro,threo-4-fluoroglutamic acid

(*Received* 6 *May* 1991; *accepted* 30 *July* 1991)

Structural modification of existing antifolates may create new agents with altered therapeutic effects [1]. For example, substitution of an amino acid analog for L-glutamate (Glu) in classical antifolates may alter enzyme inhibition, transport properties, or the ability to form poly-γ-glutamate metabolites [1]. Often, the amino acid analog chosen is only or most readily available as the D,L-racemate. Thus, the D,L-racemate may be used to synthesize the analog first; if interesting biological results are obtained, the analog containing the L-enantiomer may be prepared. It is generally assumed in studies using a D,L-racemate that the D-enantiomer-containing analog is inactive and does not interfere with effects of the L-enantiomer-containing species. In the case of methotrexate (MTX*), this assumption has been validated only for D-MTX compared to L-MTX [2].

We previously studied D,L-*e,t*-γ-fluoroMTX (4-amino-10-methylpteroyl-D,L-*erythro,threo*-4-fluoroGlu; D,L-*e,t*-FMTX), an MTX analog in which L-Glu is replaced by D,L-*erythro,threo*-4-fluoroGlu, and its constituent diastereomers D,L-*e*-FMTX and D,L-*t*-FMTX [3–5]. Based on published studies of D-MTX [2], we assumed that the D-enantiomer-containing species were essentially inactive. However, we remained concerned about the remote possibility that fluorine substitution might alter enantiomeric specificity in our test systems. To address this concern, we enzymatically prepared D-*e,t*-FMTX and studied its activity. We included D-MTX in these studies to expand the data base on this contaminant found in clinical MTX preparations [2].

Materials and Methods

L-MTX was a gift of Lederle (Pearl River, NY). D,L- and D-MTX were from Aldrich Chemicals (Milwaukee, WI). 4-Amino-10-methylpteroyl[γ-(1*H*-tetrazolyl- 5-yl)-L-α-amino butyric acid] [6] was a gift of Dr. T. Kalman (SUNY, Buffalo, NY). 4-Amino-10-methylpteroyl-D,L-(3-hydroxy-Glu) and 4-amino-10-methylpteroyl-D,L-(4-methylene-Glu) [7] were gifts of Dr. M. G. Nair (University of South Alabama, Mobile). Other chemicals were reagent grade or higher.

D-*e,t*-FMTX was prepared by exhaustive digestion of D,L-*e,t*-FMTX [3] with carboxypeptidase G_2 (CPG_2), which specifically releases L-amino acids from pteroates (*vide infra*). D,L-*e,t*-FMTX (20 μmol) was hydrolyzed (37°) by 40 I.U. of CPG_2 in 25 mM Tris–Cl, pH 7.3 and 0.1 mM $ZnCl_2$ (200 mL). After no further absorbance change at 320 nm was observed (t = 15 min), incubation was continued for 30 min. Based on ΔA_{320} and the $E_{320,\,pH\,7.3}$ for production of 4-amino-10-methylpteroate [8], 49% of the substrate was hydrolyzed. The resulting solution was chromatographed in two portions on DE-52 (0.7 × 21 cm; Whatman, Clifton, NJ) equilibrated at 4° with 50 mM NH_4HCO_3, pH 8.0. After loading and washing with 70 mL of initial buffer, each column was eluted with a linear gradient (500 mL total) from 50 to 200 mM NH_4HCO_3, pH 8.0. 4-Amino-10-methylpteroate, identified by its UV spectrum at pH 13 and HPLC retention time [9], was well resolved from D-*e,t*-FMTX. Fractions containing material with a UV spectrum and HPLC retention time similar to D,L-*e,t*-FMTX were lyophilized. Exhaustive CPG_2 digestion of this material showed it contained <4% of the L-isomer (D-*e,t*-FMTX does not inhibit CPG_2; *vide infra*).

Radiochemicals. L-[3′,5′,7′,-^{3}H]MTX (20 Ci/mmol) and [5-^{3}H]dUrd (22 Ci/mmol) were from Moravek Biochemicals (Brea, CA). The purity of L-[^{3}H]MTX was assessed by HPLC [4].

Enzymes and assays. CPG_2 was purified [10] from *Escherichia coli* harboring a plasmid containing the *Pseudomonas* CPG_2 cDNA [11] and assayed as described [8], except that 100 μM L-MTX was used. Dihydrofolate reductase (DHFR; EC 1.5.1.3) was partially purified from CCRF-CEM cells and assayed as described [6]. Drug concentrations inhibiting DHFR activity (1.6×10^{-3} I.U.) by 50% (IC_{50}) were determined as described [6]. L-[^{3}H]-MTX uptake by CCRF-CEM cells was measured as described [4].

Cell culture. Human T-lymphoblastic CCRF-CEM [12] and sublines MTX resistant via decreased transport [13] or DHFR increase [14] were cultured in RPMI 1640 containing 10% horse serum (GIBCO) and additions as indicated [4]. Cell outgrowth inhibition and drug concentration inhibiting cell growth by 50% (EC_{50}) were determined as described [6]. CCRF-CEM cells used as a DHFR source and to determine EC_{50} were *Mycoplasma* free (Gen-Probe Inc., San Diego, CA). Studies on thymidylate (dTMP) biosynthesis and inhibition of [^{3}H]MTX uptake were completed within 11 days and 2 months, respectively, of this negative test; testing 10 months later showed contamination in all lines. D-*e,t*-FMTX was depleted prior to this discovery so the studies could not be repeated. However, since cells grew normally during the studies

* Abbreviations: MTX, methotrexate; D,L-*e,t*-γ-fluoro-MTX (D,L-*e,t*-FMTX), 4-amino-10-methylpteroyl-D,L-*erythro,threo*-4-fluoroGlu; CPG_2, carboxypeptidase G_2; DHFR, dihydrofolate reductase; and dTMP, thymidylate.

Table 1. Inhibition of CCRF-CEM DHFR and cell outgrowth by MTX analogs containing enantiomers and analogs of L-Glu.

Compound	DHFR inhibition		Growth inhibition
	IC_{50} (nM)	Slope*	EC_{50} (nM)
L-MTX	0.62 ± 0.06	1.49 ± 0.16	16
D,L-MTX	1.15	1.35	29
D-MTX	5.6	0.84	535
D,L-*e,t*-FMTX	1.25	1.25	73
D-*e,t*-FMTX	5.2	0.96	690

Inhibition of DHFR was determined as described in Materials and Methods. Values are averages of duplicate determinations, except for L-MTX which is the mean ± SD (N = 3). Inhibition of outgrowth of CCRF-CEM cells was measured over 120 hr; drug was present throughout the growth period. Results of the outgrowth studies are averages of duplicate values.

* Slope of linear regression of a plot of log $[v/(V_{control} - v)]$ vs log [Inhibitor] for each concentration–inhibition curve; the slope quantitates the sigmoidicity of the curve [17].

presented and control values in each case were similar to those we previously reported with pure cultures [5, 6], we believe the cells in the reported studies were not contaminated.

Biosynthesis of dTMP. De novo synthesis of dTMP was measured by incubating cells with [5-^{3}H]dUrd and measuring release of ^3H_2O as a function of time [6].

Results and Discussion

CPG_2 specificity. CPG_2 is believed to be similar to CPG_1 which specifically hydrolyzes C-terminal L-Glu, L-Asp, and L-Gln from oligopeptides, N-acylated amino acids, folates, and folate analogs [2, 8]. Stereospecificity of CPG_2 was verified by showing that, at 100 μM, D-MTX was hydrolyzed at <0.2% the rate of L-MTX; the low rate measured may result from L-MTX contamination since limit digestion of D-MTX indicated the presence of ≤2% L-MTX. Also, over 10–50 μM L-MTX, the hydrolysis rate was the same for L-MTX or D,L-MTX and the E_{320} for the reaction with D,L-MTX was ≈50% that with L-MTX (3900 vs 7660 cm^{-1} M^{-1}). Quantitation of 20 μM L-MTX based on CPG_2-catalyzed hydrolysis was unaffected by the presence of 100 μM D-MTX or 40 μM D-*e,t*-FMTX. Thus, similar to CPG_1 [8], CPG_2 was specific for L-Glu and D-Glu did not inhibit its action [2].

4-Amino-10-methylpteroyl[γ-(1*H*-tetrazolyl-5-yl)-L-α-aminobutyric acid], 4-amino-10-methylpteroyl-D,L-(3-hydroxy-Glu), and 4-amino-10-methylpteroyl-D,L-(4-methylene-Glu) were hydrolyzed 100, 50, and 0%, respectively, by CPG_2. These data (not shown) suggested that some Glu analogs could be released in an apparently L-enantiomer-specific manner, but demonstrated sensitivity of CPG_2 towards γ-substituents. Thus if D,L-*e,t*-FMTX were a substrate, only the L-enantiomer might be hydrolyzed.

D,L-*e,t*-FMTX was resistant to CPG_2 digestion compared to D,L-MTX. At 200 μM, D,L-*e,t*-FMTX was 7- to 9-fold less active than D,L-MTX ($K_{m,L\text{-}MTX} = 8$ μM) as a substrate (not shown) suggesting a lower V_{max} for CPG_2 with 4-fluoroGlu-containing species. Limit digestion was effected, however, by increasing the CPG_2 level; the E_{320} for hydrolysis of D,L-*e,t*-FMTX was the same as for D,L-MTX and thus 50% of the starting material was hydrolyzed. This was consistent with L-*erythro* and L-*threo* isomers both being hydrolyzed. The slower hydrolysis rate of D,L-*e,t*-FMTX by CPG_2 is reminiscent of the decreased sensitivity of other 4-fluoroGlu-containing antifolates to γ-glutamyl hydrolase activity [15]; scissile peptide bonds in proximity to fluorine may thus generally be less susceptible to enzymatic hydrolysis.

DHFR inhibition. DHFR is the primary target of MTX and its analogs [16]. All MTX analogs tested here inhibited CCRF-CEM DHFR (Table 1). The IC_{50} for D,L-MTX was about twice that for L-MTX. D-MTX and D-*e,t*-FMTX had IC_{50} values 4-fold higher than the corresponding D,L-mixtures. In addition to higher IC_{50} values, slopes of linear transformations of the inhibition curves [17] were lowest for the D-enantiomers (Table 1), further indicating weaker interaction with DHFR. Previous work also showed that D-MTX was weaker than L-MTX as an inhibitor of human and murine DHFR based on IC_{50} values [2]. Slopes of inhibition curves were not reported in that study.

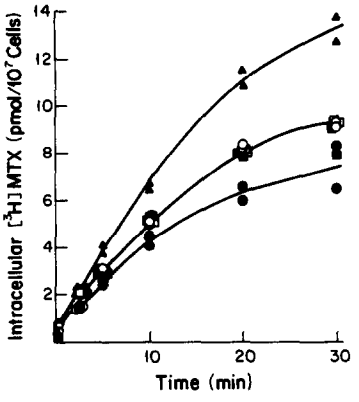

Fig. 1. Inhibition of initial uptake and accumulation of [^{3}H]MTX in CCRF-CEM cells by MTX analogs containing enantiomers and analogs of L-Glu. Cells were added to incubation tubes already containing sufficient [^{3}H]MTX (0.75 μCi/mL; 760 cpm/pmol) and compound of interest to give final concentrations of 1 μM [^{3}H]MTX and: solvent control (▲); 4 μM D,L-MTX (●); 20 μM D-MTX (○); 8 μM D,L-*e,t*-FMTX (■); or 40 μM D-*e,t*-FMTX (□). This experiment was repeated with similar results.

[^{3}H]*MTX uptake inhibition.* MTX and FMTX isomers share transport systems in H35 hepatoma [3] and CCRF-CEM cells [4]. Preliminary studies showed that 4 μM D,L-MTX was equivalent to 2 μM L-MTX in decreasing the initial velocity (v_i) of transport and accumulation at 30 min of 1 μM L-[^{3}H]MTX. D-MTX at ≤10 μM had no effect on v_i and caused only a slight decrease in accumulation at 30 min (not shown); 20 μM D-MTX affected both v_i and accumulation at 30 min, but the effect was less than that of 4 μM D,L-MTX (Fig. 1). Effects of D-MTX on MTX

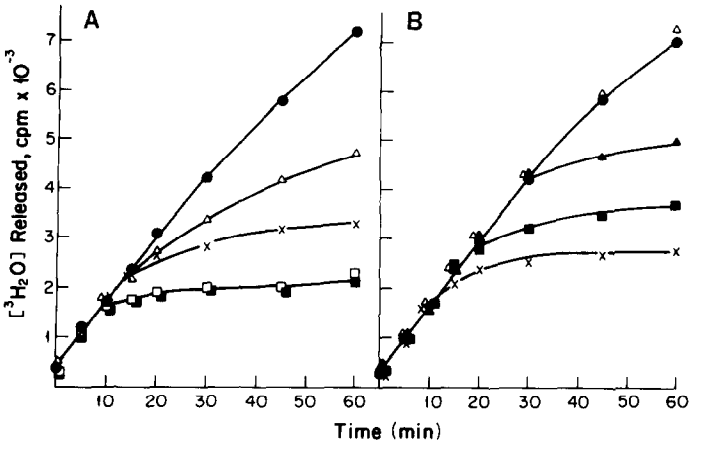

Fig. 2. Inhibition of thymidylate biosynthesis in CCRF-CEM cells by MTX analogs containing enantiomers and analogs of L-Glu. Cells were added to incubation tubes already containing [5-^{3}H]dUrd (1 μCi/mL) to give a final concentration of 45 nM; the compound of interest was present simultaneously to give the final indicated concentration. [5-^{3}H]dUrd metabolism was measured as described in Materials and Methods. Panel A: Solvent control (●); 0.5 μM L-MTX (×); 1 μM L-MTX (□); 2 μM D,L-MTX (■); or 10 μM D-MTX (△). Panel B: Solvent control (●); 0.5 μM L-MTX (×); 2 μM D,L-*e,t*-FMTX (■); 2.8 μM D-*e,t*-FMTX (△); or 10 μM D-*e,t*-FMTX (▲). This experiment was repeated with similar results.

transport were not examined previously [2]. D,L-*e,t*-FMTX at 8 μM inhibited L-[^{3}H]MTX uptake; 40 μM D-*e,t*-FMTX was required to achieve the same level of inhibition (Fig. 1). Thus, D-MTX and D-*e,t*-FMTX were much weaker than the corresponding L-enantiomers as inhibitors of L-[^{3}H]-MTX uptake. Assuming that inhibitory potency reflects affinity for the carrier and that other routes of transport are not used at higher efficiency, these data suggest that D-enantiomers are poorly transported.

Uptake measurements. An attempt was made to use CPG_2 hydrolysis to assess the enantiomeric composition of intracellular radiolabel following exposure of CCRF-CEM cells to L-[^{3}H]MTX or D,L-*erythro*-[^{3}H]FMTX. L-[^{3}H]MTX and D,L-*erythro*-[^{3}H]FMTX [4] were hydrolyzed by CPG_2 to yield ≥96% and 45% [^{3}H]4-amino-10-methylpteroate, respectively, indicating that the approach was feasible. The extensive sample processing required in cell studies, however, resulted in significant conversion of radiolabel to unidentifiable products even in control samples; thus intracellular drug could not be studied.

Inhibition of dTMP biosynthesis. dTMP biosynthesis was measured by conversion of [5-^{3}H]dUrd to dTMP in intact cells. Inhibition by L-MTX was concentration-dependent and 2 μM D,L-MTX was equivalent to 1 μM L-MTX (Fig. 2A). D-MTX at 10 μM inhibited dTMP biosynthesis, but was less potent than 0.5 μM L-MTX; 2 μM D-MTX was no different from control (not shown). Inhibition of dTMP biosynthesis by D-MTX was not studied previously [2]. D,L-*e,t*-FMTX at 2 μM took longer to initiate inhibition of dTMP biosynthesis than did 0.5 μM L-MTX (Fig. 2B). D-*e,t*-FMTX at 2.8 μM was not different from control; 10 μM D-*e,t*-FMTX eventually caused inhibition but took much longer than 2 μM D,L-*e,t*-FMTX. Thus, D-MTX and D-*e,t*-FMTX were weaker inhibitors of dTMP biosynthesis than were the corresponding L-enantiomers.

Outgrowth inhibition. Using a 120-hr exposure period, L-MTX was about 2- and 33-fold more effective than D,L-MTX and D-MTX, respectively, as an inhibitor of CCRF-CEM cell growth (Table 1). These results are similar to earlier studies on L1210 cells using unpurified D-MTX, but dissimilar to earlier results with CCRF-CEM cells where the EC_{50} for purified D-MTX was >1000 nM [2]. It is doubtful that trace contamination of our D-MTX by L-MTX (≤2%, above) accounts for the difference in potency found in the two studies. A likely source for the difference is the conditions used to assess growth inhibition. Earlier studies used initial densities of 5×10^4 cells/mL and allowed 3 generations of growth [2], while our initial density was 1×10^4 cells/mL and allowed 5–6 generations. Slower uptake of D-MTX and weaker inhibition of DHFR may mean that a longer exposure time is required for the effects to be evident (cf. Fig. 2). D-*e,t*-FMTX was also a much less potent inhibitor than D,L-*e,t*-FMTX of CCRF-CEM cell growth (Table 1).

At 92–97% growth inhibition, effects of each compound could be reversed by the simultaneous presence of 10^{-7} M leucovorin (not shown). In addition, in one experiment, CCRF-CEM sublines resistant to MTX because of reduced transport or increased DHFR were cross-resistant to D-MTX and D-*e,t*-FMTX. These results indicate that each drug was acting as an antifolate. Similar protection and cross-resistance experiments were not reported previously with D-MTX [2].

Data presented here indicate that MTX analogs containing D-Glu or D-*erythro,threo*-4-fluoroGlu exert growth inhibitory effects by mechanisms similar to MTX, but are markedly less potent. Decreased potency of both D-species appears to be a result of decreased uptake and weaker DHFR inhibition. Inability of D-enantiomer-containing analogs to form polyglutamate derivatives [18] probably contributed little to decreased potency here since polyglutamylation is not essential under continuous exposure conditions [3]. Previously [2], only weaker DHFR inhibition was recognized as a factor in the decreased potency of D-MTX. The present results thus provide further evidence that D-MTX occurring [2] as a contaminant in MTX should not be of major concern in its clinical use. These results also validate the use of MTX analogs containing D,L-amino acids. Specifically, the results validate our earlier assumption that D-*e,t*-FMTX is essentially inactive compared to L-*e,t*-FMTX in the biological systems employed. Further, the results demonstrate that 4-fluoro-substitution in Glu does not alter stereospecificity in the folate-dependent systems examined.

These results are also of significance in terms of the future use of D,L-*e,t*-FMTX *in vivo*. Plasma clearance of D-MTX is as rapid as that of L-MTX [2]; thus, if D,L-MTX was used, plasma ratios of D-MTX:L-MTX should not rise to a value where the D-isomer could interfere with the action of L-MTX. This observation and the similarity in properties of MTX and FMTX suggest that D-*e,t*-FMTX and L-*e,t*-FMTX should be cleared with similar kinetics. This, coupled with the low potency of the D-isomers, indicates that use of mixed isomers D,L-*e,t*-FMTX *in vivo* should not have significant therapeutic disadvantages.

In summary, analogs of MTX (4-amino-10-methylpteroyl-L-Glu) containing D-Glu (D-MTX) or D-*erythro,threo*-4-fluoroGlu (D-*e,t*-FMTX) were characterized. D-MTX and D-*e,t*-FMTX were >98 and >96% enantiomerically pure, respectively, by enzymatic assay. D-MTX and D-*e,t*-FMTX were less potent inhibitors of DHFR, [^{3}H]MTX uptake, and folate-mediated dTMP biosynthesis than the L-enantiomer-containing species. These properties were reflected in their decreased cytoxicity for CCRF-CEM cells compared to the L-enantiomer-containing species. These results indicate that MTX analogs containing D-enantiomers of Glu or Glu analogs are less active than the L-enantiomer-containing counterparts at each key step in the mechanism of MTX and these decreased activities combine to produce lower overall biological activity.

Acknowledgements—This research was supported in part by Grants CA43500 (J.J.M), CA24538 (J.J.M), and CA28097 (J.K.C.) from the National Cancer Institute, Department of Health and Human Services. R.F.S. receives support from the U.K. Cancer Research Campaign under Grant SP1391. D.M.F. was supported by Training Grant CA09072.

JOHN J. MCGUIRE*†
WANDA E. BOLANOWSKA*
JAMES K. COWARD‡
ROGER F. SHERWOOD§
CYNTHIA A. RUSSELL*
DONNA M. FELSCHOW*

* *Grace Cancer Drug Center Roswell Park Memorial Institute Buffalo, NY 14263 U.S.A.*

‡ *Departments of Chemistry and Medicinal Chemistry University of Michigan Ann Arbor, MI 48109, U.S.A.*

§ *Division of Biotechnology PHLS Centre for Applied Microbiology and Research Salisbury, Wilts SP4 OJG, U.K.*

REFERENCES

1. Rosowsky A, Chemistry and biological activity of antifolates. *Prog Med Chem* **26**: 1–252, 1989.
2. Cramer SM, Schornagel JH, Kalghatgi KK, Bertino JR and Horvath C, Occurrence and significance of D-methotrexate as a contaminant of commercial methotrexate. *Cancer Res* **44**: 1843–1846, 1984.
3. Galivan J, Inglese J, McGuire JJ, Nimec Z and Coward JK, γ-Fluoromethotrexate: synthesis and biological activity of a potent inhibitor of dihydrofolate reductase with greatly diminished ability to form poly-γ-glutamates. *Proc Natl Acad Sci USA* **82**: 2598–2602, 1985.
4. McGuire, JJ, Graber M, Licato N, Vincenz C, Coward JK, Nimec Z and Galivan J, Biochemical and growth inhibitory effects of the *erythro* and *threo* isomers of γ-fluoromethotrexate, a methotrexate analogue defective in polyglutamylation. *Cancer Res* **49**: 4517–4525, 1989.
5. McGuire JJ, Haile WH and Coward JK, Interaction of *erythro*- and *threo*-γ-fluoromethotrexate with human leukemia cell dihydrofolate reductase. *Biochem Pharmacol* **38**: 4321–4325, 1989.
6. McGuire JJ, Russell CA, Bolanowska WE, Freitag CM, Jones CS and Kalman TI, Biochemical and growth inhibition studies of methotrexate and aminopterin analogues containing a tetrazole ring in place of the γ-carboxyl group. *Cancer Res* **50**: 1726–1731 1990.
7. Abraham A, McGuire JJ, Galivan J, Kisliuk RL, Gaumont Y and Nair MG, Folate analogues 34: Synthesis and antitumor activity of non-polyglutamylatable inhibitors of dihydrofolate reductase. *J Med Chem* **34**: 222–227, 1991.
8. McCullough JL, Chabner BA and Bertino JR, Purification and properties of carboxypeptidase G_1. *J Biol Chem* **246**: 7207–7213, 1971.
9. McGuire JJ, Mini E, Hsieh P and Bertino JR, Role of methotrexate polyglutamates in methotrexate- and sequential methotrexate-5-fluorouracil-mediated cell kill. *Cancer Res* **45**: 6395–6400, 1985.
10. Sherwood RF, Melton RG, Alwan SM and Hughes P, Purification and properties of carboxypeptidase G_2 from *Pseudomonas* sp. strain RS-216. Use of a novel triazine dye affinity method. *Eur J Biochem* **148**: 447–453, 1985.
11. Minton MP, Atkinson T and Sherwood RF, Molecular cloning of the *Pseudomonas* CPG_2 gene and its expression in *E. coli* and *Pseudomonas putida*. *J Bacteriol* **156**: 1222–1227, 1983.
12. Foley GF, Lazurus H, Farber S, Uzman BG, Boone BA and McCarthy RE, Continuous culture of lymphoblasts from peripheral blood of a child with acute leukemia. *Cancer* **18**: 522–529 1965.
13. Rosowsky A, Lazarus H, Yuan GC, Beltz WR, Mangini L, Abelson HT, Modest EJ and Frei E III, Effects of methotrexate esters and other lipophilic antifolates on methotrexate-resistant human leukemic lymphoblasts. *Biochem Pharmacol* **29**: 648–652, 1980.
14. Mini E, Srimatkandada S, Medina WD, Moroson BA, Carman MD and Bertino JR, Molecular and karyological analysis of methotrexate-resistant and -sensitive human leukemic CCRF-CEM cells. *Cancer Res* **45**: 317–325, 1985.
15. Licato NJ, Nimec Z, Galivan J, McGuire JJ and Coward JK, The synthesis of 4-deoxy-4-amino-10-methylpteroyl-(4-fluoroglutamyl)-γ-glutamate, an unusual substrate for folylpoly-γ-glutamate synthetase and γ-glutamyl hydrolase. *J Med Chem* **33**: 1022–1027, 1990.
16. Bertino JR, Methotrexate: Molecular pharmacology. In: *Cancer and Chemotherapy* (Eds. Crooke ST and Prestayko AW), pp. 311–322. Academic Press, New York, 1981.
17. Chou T-C and Talalay P, Quantitative analysis of dose-effect relationships: The combined effects of multiple drugs or enzyme inhibitors. *Adv Enzyme Regul* **22**: 27–55, 1984.
18. McGuire JJ, Hsieh P, Coward JK and Bertino JR, Enzymatic synthesis of folylpolyglutamates. Characterization of the reaction and its products. *J Biol Chem* **255**: 5776–5788, 1980.

† Corresponding author: John J. McGuire, Ph.D., Grace Cancer Drug Center, Roswell Park Memorial Institute, 666 Elm St., Buffalo, NY 14263. Tel. (716) 845-8249; FAX (716) 845-8857.