Inhibition of Hog Liver Folylpolyglutamate Synthetase by 5-Substituted 5,8-Dideaza Analogues of Folic Acid Bearing a Terminal L-Ornithine Residue

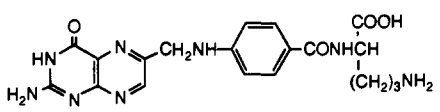
John B. Hynes,*,† Shyam K. Singh,† Oliver Fetzer,† and Barry Shane‡

Department of Pharmaceutical Sciences, Medical University of South Carolina, Charleston, South Carolina 29425, and Department of Nutritional Sciences, University of California, Berkeley, California 94720

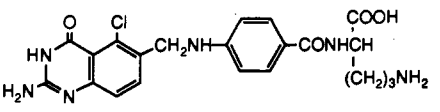
Received May 11, 1992

Five new N^{α}-(5,8-dideazapteroyl)-L-ornithines have been prepared using multistep synthetic sequences. These include N^{α}-[5-(trifluoromethyl)-5,8-dideazapteroyl]-L-ornithine, **3**, as well as N^{α}-[5-(trifluoromethyl)-5,8-dideazaisopteroyl]-L-ornithine, **4**, and its 5-fluoro and 5-chloro analogues. Both of the compounds containing a 5-(trifluoromethyl) group (**3** and **4**) were found to be excellent inhibitors of homogeneous hog liver folylpolyglutamate synthetase, having K_i values in the same range as N^{α}-(5-chloro-5,8-dideazapteroyl)-L-ornithine, **2**, (~10 nM). However, the bridge-reversed isomer of **2** was 60-fold less inhibitory than **2**.

A variety of derivatives of folic acid in which the terminal L-glutamate moiety is replaced by an L-ornithine residue have been found to be effective inhibitors of mammalian folylpolyglutamate synthetase (FPGS). For example, N^{α}-pteroyl-L-ornithine, **1**, had a K_i of 5.9 μM toward hog liver FPGS, while reduction to its 5,6,7,8-tetrahydro derivative resulted in a 30-fold enhancement of inhibitory potency.[1,2] The most potent L-ornithine modification of this type having a 2-amino-3,4-dihydro-4-oxo configuration in the pyrimidine nucleus was N^{α}-(5-chloro-5,8-dideazapteroyl)-L-ornithine, **2**, (K_i = 8.3 nM).[3]



1



2

The structurally related derivative N^{α}-(5,8-dideazapteroyl)-L-ornithine was reported to have K_i values in the 0.15 μM range toward human FPGS from CCRF-CEM and K562 leukemia cell lines,[4] suggesting that the presence of the 5-chlorine substituent can enhance inhibitory potency by nearly 20-fold. In an effort to determine the influence of other hydrophobic substituents located at position 5 upon inhibitory activity, five new L-ornithine derivatives containing trifluoromethyl, fluorine, or chlorine located at position 5 were prepared. The structures and K_i values for these compounds are presented in Table I, which also contains the kinetic constants for the corresponding L-glutamate modifications.

Chemistry. The preparation of N^{α}-[5-(trifluoromethyl)-5,8-dideazapteroyl]-L-ornithine, **3**, was facilitated by the recent description of the synthesis of 2-amino-6-cyano-3,4-dihydro-4-oxo-5-(trifluoromethyl)quinazoline, **8**[5] (Scheme I). This nitrile was condensed reductively with *tert*-butyl 4-aminobenzoate, **9**,[6] in the presence of Raney nickel to yield *tert*-butyl 5-(trifluoromethyl)-5,8-dideazapteroate, **10**, in 52.5% yield. Compound **10** was deesterified using trifluoroacetic acid to give 5-(trifluoromethyl)-5,8-dideazapteroic acid, **11**. Compound **11** was converted to its 10-(trifluoroacetyl) derivative, which was not fully characterized due to the lability of the trifluoroacetyl group. Standard peptide bond formation to N^{δ}-(*tert*-butyloxycarbonyl)-L-ornithine followed by treatment with ammonium hydroxide gave the N^{δ}-blocked derivative, **12**, which was converted to the target molecule **3** in the presence of trifluoroacetic acid.

The synthesis of N^{α}-[5-(trifluoromethyl)-5,8-dideazaisopteroyl]-L-ornithine, **4**, was conducted as shown in Scheme II. The key intermediate 2,6-diamino-3,4-dihydro-4-oxo-5-(trifluoromethyl)quinazoline, **13a**, was resynthesized as described previously.[5] It was alkylated with methyl 4-(bromomethyl)benzoate in the presence of $CaCO_3$ to yield methyl 5-(trifluoromethyl)-5,8-dideazaisopteroate, **14a**. Saponification in dilute base gave 5-(trifluoromethyl)-5,8-dideazaisopteroic acid, **15a**, which was then converted to the 9-(trifluoroacetyl) derivative using trifluoroacetic anhydride. Conventional peptide bond formation to N^{δ}-(*tert*-butyloxycarbonyl)-L-ornithine followed by deprotection first in base and then in trifluoroacetic acid yielded the target compound **4**. The corresponding 5-chloro derivative, N^{α}-(5-chloro-5,8-dideazaisopteroyl)-L-ornithine, **5**, was obtained in low yield in an analogous fashion as shown in Scheme II. A sample of 5-chloro-2,4,6-triaminoquinazoline, which was prepared according to the literature methods,[7,8] was hydrolyzed under acidic

† Medical University of South Carolina.
‡ University of California, Berkeley.

(1) Cichowicz, D. J.; Cook, J. D.; George, S.; Shane, B. Hog Liver Folylpolyglutamate Synthetase: Substrate Specificity and Regulation. In *Proceedings of the Second Workshop on Folyl and Antifolylpolyglutamates*: Goldman, I. D., Ed.; Praeger: New York, 1985; pp 7-13.

(2) George, S.; Cichowicz, D. J.; Shane, B. Mammalian Folylpoly-γ-glutamate Synthetase. 3. Specificity for Folate Analogues. *Biochemistry* **1987**, *26*, 522-529.

(3) Patil, S. A.; Shane, B.; Freisheim, J. H.; Singh, S. K.; Hynes, J. B. Inhibition of Mammalian Folylpolyglutamate Synthetase and Human Dihydrofolate Reductase by 5,8-Dideaza Analogues of Folic Acid and Aminopterin Bearing a Terminal L-Ornithine. *J. Med. Chem.* **1989**, *32*, 1559-1565.

(4) McGuire, J. J.; Bolanowska, W. E.; Piper, J. R. Structural Specificity of Inhibition of Human Folylpolyglutamate Synthetase by Ornithine-Containing Folate Analogues. *Biochem. Pharmacol.* **1988**, *37*, 3931-3939.

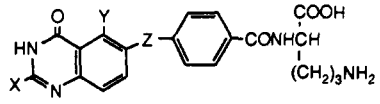
(5) Singh, S. K.; Govindan, M.; Hynes, J. B. Synthesis of 5-Trifluoromethyl-5,8-dideazafolic Acid and 5-Trifluoromethyl-5,8-dideazaisofolic Acid. *J. Heterocycl. Chem.* **1990**, *27*, 2101-2105.

(6) Taylor, E. C.; Fletcher, S. R.; Sabb, A. L. A Convenient Synthesis of *t*-Butyl *p*-Aminobenzoate. *Synth. Commun.* **1984**, *14*, 921-924.

(7) Davoll, J.; Johnson, A. M. Quinazoline Analogues of Folic Acid. *J. Chem. Soc. (C)* **1970**, 997-1002.

0022-2623/92/1835-4078$03.00/0

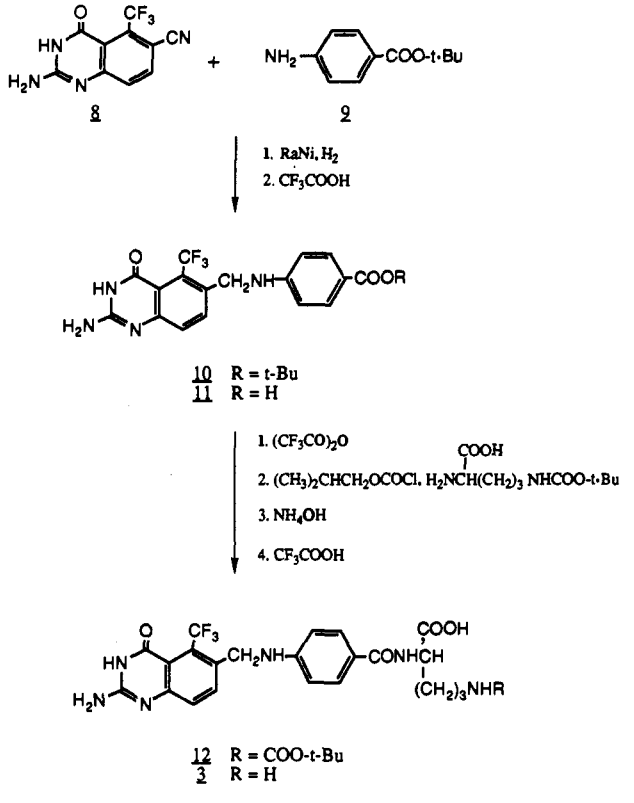
Table I. Comparison of the Kinetic Constants of 5,8-Dideazapteroyl-L-ornithine Derivatives with Their L-Glutamate Counterparts for Homogeneous Hog Liver Folypolyglutamate Synthetase[a]



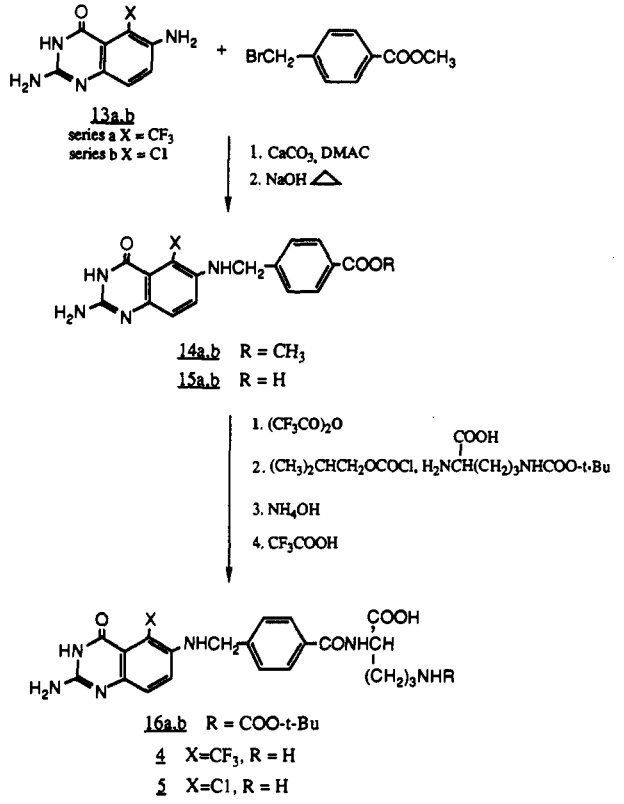
compd	X	Y	Z	K_i, μM	K_m, μM[b]	V_{max}, μmol/h per mg[b]	V_{max}/K_m[b,c]
3	NH_2	CF_3	CH_2NH	0.0088	1.8	35	121
4	NH_2	CF_3	$NHCH_2$	0.011	3.5	66	118
5	NH_2	Cl	$NHCH_2$	0.5	3.5[d]	57[d]	102[d]
2	NH_2	Cl	CH_2NH	0.0083[e]	0.3[e]	84[e]	1750[e]
6	NH_2	F	$NHCH_2$	0.9	6.7	69	64
7	CH_3	F	$NHCH_2$	0.15	4.8	68	88

[a] Standard error of the mean, K_m < ±20%, V_{max} < ±10%. [b] Kinetic constants obtained for the corresponding L-glutamate derivative. [c] Relative to results for (6*S*)-H_4PteGlu normalized to 100. [d] Reported previously; cf. ref 12. [e] Reported previously; cf. ref 3.

Scheme I. Synthetic Route to N^{α}-[5-(Trifluoromethyl)-5,8-dideazapteroyl]-L-ornithine



Scheme II. Synthetic Route to N^{α}-(5-Substituted-5,8-dideazaisopteroyl)-L-ornithines



conditions to give 5-chloro-2,6-diamino-3,4-dihydro-4-oxoquinazoline, **13b**, in excellent yield.

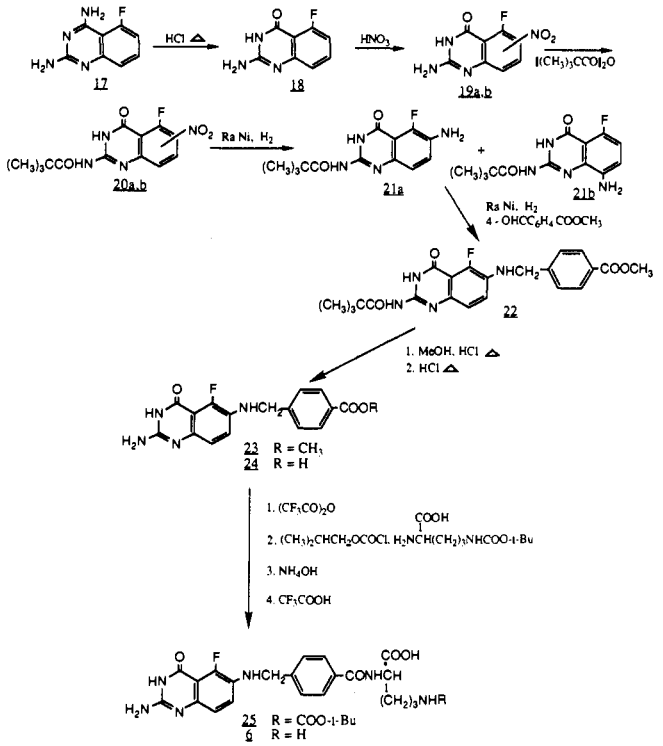
A more laborious approach was required for the synthesis of N^{α}-(5-fluoro-5,8-dideazaisopteroyl)-L-ornithine, **6**, as shown in Scheme III. The 2,4-diamino-5-fluoroquinazoline, **17**, was prepared in a large quantity according to the method developed earlier in this laboratory.[8] Acid-catalyzed hydrolysis gave 2-amino-3,4-dihydro-5-fluoro-4-oxoquinazoline, **18**, in excellent yield. The nitration of **18** with nitric acid gave a mixture of the six and eight nitro isomers **19a,b** in a 4:1 ratio. The nitration of 2,4-diamino-5-fluoroquinazoline was found to give similar results.[9] Resolution of these isomers was not achieved due to their insolubility in a variety of solvents as well as the lability of the fluorine atom in even weakly basic media. Therefore, in order to improve solubility, the mixture was acylated using pivalic anhydride to yield a mixture of **20a,b**.[10] Next, the **20a,b** mixture was hydrogenated in the presence of Raney nickel in acetic acid and neutralization to pH 6.5 caused the selective precipitation of the 6-amino isomer, **21a**. Compound **21a** was reductively condensed with methyl 4-formylbenzoate in the presence of Raney nickel to afford methyl 5-fluoro-2-pivaloyl-5,8-dideazaisopteroate, **22**, in low yield. The protecting groups were removed in a stepwise fashion yielding first methyl 5-fluoro-5,8-dideazaisopteroate, **23**, and finally, 5-fluoro-5,8-dideazaisopteroic acid, **24**. Compound **24** was trifluoroacetylated and then coupled to N^{δ}-(*tert*-butyloxycarbonyl)-L-ornithine using isobutyl chloroformate as the condensing agent. This material obtained could not be purified due to the lability of the trifluoroacetyl group and was, therefore, treated with ammonium hydroxide to give the blocked

(8) Hynes, J. B.; Pathak, A.; Panos, C. H.; Okeke, C. C. Direct Synthesis of 2,4-Diaminoquinazolines from 2-Fluorobenzonitriles. *J. Heterocycl. Chem.* **1988**, *25*, 1173–1177.

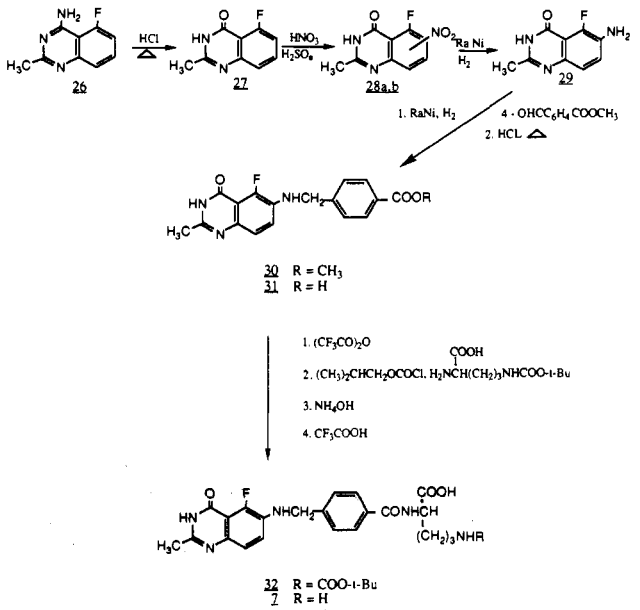
(9) Tomažič, A.; Hynes, J. B.; Gale, G. R.; Freisheim, J. H. Synthesis and Preliminary Biological Evaluation of 5-Fluoro-5,8-dideazaisoaminopterin. *J. Heterocycl. Chem.* **1990**, *27*, 2081–2084.

(10) Acharya, S. P.; Hynes, J. B. A New Synthetic Route to Quinazoline Analogues of Folic Acid. *J. Heterocycl. Chem.* **1975**, *12*, 1283–1286.

Scheme III. Synthetic Route to N^{α}-(5-Fluoro-5,8-dideazaisopteroyl)-L-ornithine



Scheme IV. Synthetic Route to N^{α}-(2-Desamino-2-methyl-5-fluoro-5,8-dideazaisopteroyl)-L-ornithine



ornithine derivative, **25**. The target compound, **6**, was then obtained by reacting **25** with anhydrous trifluoroacetic acid.

The final new L-ornithine derivative, N^{α}-(2-desamino-2-methyl-5-fluoro-5,8-dideazaisopteroyl)-L-ornithine, **7**, was obtained as shown in Scheme IV. 4-Amino-5-fluoro-2-methylquinazoline, **26**, was obtained as recently reported[11] and converted by hydrolysis to 3,4-dihydro-5-fluoro-2-methyl-4-oxoquinazoline, **27**, in excellent yield. Nitration of **27** using a mixture of nitric and sulfuric acids gave the 6- and 8-nitro isomers, **28a,b**, in a ratio of 45:55. This mixture was hydrogenated in the presence of Raney nickel and after column chromatography on silica gel a pure sample of 6-amino-3,4-dihydro-5-fluoro-2-methyl-4-oxoquinazoline, **29**, was obtained. Compound **29** was condensed reductively to methyl 4-formylbenzoate to give methyl 2-desamino-2-methyl-5-fluoro-5,8-dideazaisopteroate, **30**, which was hydrolyzed under acidic conditions to afford 2-desamino-2-methyl-5-fluoro-5,8-dideazaisopteroic acid, **31**. Analogous chemistry to that described above was used to convert **31** to **32** and then to **7**.

Biological Results and Discussion

Each of the new L-ornithine derivatives, **3–7**, was evaluated as an inhibitor of homogeneous hog liver FPGS and the results are presented in Table I. The kinetic constants for the structurally analogous L-glutamates are also included. The values for **2** and its corresponding L-glutamate derivative are presented for reference purposes.

Previous studies have indicated a reasonable correlation between the K_m or V_{max}/K_m for a folate or folate analogue containing an L-glutamate residue and the K_i for the corresponding L-ornithine modification toward FPGS.[3,4] In general, a similar trend was seen in the current study although compounds **3** and **4** are an exception to this relationship. Both of these compounds (**3** and **4**) are excellent inhibitors of FPGS, having K_i values similar to that of **2**, the most effective 4-oxo inhibitor of FPGS thus far reported. However, 5-(trifluoromethyl)-5,8-dideazafolic acid and 5-(trifluoromethyl)-5,8-dideazaisofolic acid have K_m values which are approximately 6- and 10-fold larger than that of the L-glutamate form of **2**. It should also be noted that the bridge-reversed 5-(trifluoromethyl) compound **4** is nearly as inhibitory as its normal-bridged isomer **3**, while for the corresponding 5-chloro isomers **5** is approximately 60-fold less inhibitory than **2**. This latter relationship was expected as the L-glutamate corresponding to **2** is a far better substrate for FPGS than is the L-glutamate counterpart of **5**.[3,12]

N^{α}-(5-Fluoro-5,8-dideazaisopteroyl)-L-ornithine, **6**, is a modest inhibitor of FPGS, while its 5-chloro counterpart, **5**, is slightly more potent. Its 2-desamino-2-methyl modification, **7**, increases binding 6-fold. Although this enhancement of binding would not have been predicted solely on the kinetic constants of the structurally equivalent L-glutamates, it does follow the trend relating more effective substrates with more effective inhibitors, as the glutamate form of **7** is a somewhat more effective substrate than **6**. We have previously shown that the 2-desamino-2-methyl modification of 5,8-dideazaisofolic acid increases its substrate effectiveness about 2–3-fold.[13]

It appears, therefore, that the relative affinity of an ornithine derivative for FPGS can, in many cases, be approximately predicted from the kinetic constants for the equivalent L-glutamate analogue but that there are significant exceptions to this relationship. The reason

(11) Hynes, J. B.; Tomažič, A.; Parrish, C. A.; Fetzer, O. S. Further Studies on the Synthesis of Quinazolines from 2-Fluorobenzonitriles. *J. Heterocycl. Chem.* **1991**, *28*, 1357–1363.

(12) Cichowicz, D. J.; Hynes, J. B.; Shane, B. Substrate Specificity of Mammalian Folylpoly-γ-glutamate Synthetase for 5,8-Dideazafolates and 5,8-Dideaza Analogues of Aminopterin. *Biochem. Biophys. Acta* **1988**, *957*, 363–369.

(13) Hagan, R. L.; Duch, D. S.; Smith, G. K.; Hanlon, M. H.; Shane, B.; Freisheim, J. H.; Hynes, J. B. Studies on the Mechanism of Antitumor Action of 2-Desamino-2-methyl-5,8-dideazaisofolic Acid. *Biochem. Pharmacol.* **1991**, *41*, 781–787.

for these exceptions, and even why ornithine derivatives are effective inhibitors of FPGS, will have to await a greater understanding of the mechanism of binding of substrates and inhibitors to FPGS.

Experimental Section

Melting points were determined on a Mel-temp apparatus and are uncorrected. Elemental analyses were performed by Atlantic Microlabs, Inc., Norcross, GA. Analytical samples gave combustion values for C, H, and N within ±0.4% of the theoretical values unless otherwise indicated. Solvation due to H_2O was confirmed by the presence of a broad peak centered at approximately 3.4 ppm in the ^1H NMR spectrum which was transformed into a sharp singlet (DOH) by the addition of D_2O. The presence of CF_3COOH was confirmed by ^{19}F NMR for compounds which contain CF_3COOH in the empirical formula. All intermediates were free of significant impurities by TLC on silica gel (Eastman 13181). All acids were checked for purity by TLC on cellulose (Eastman 13254). Column chromatographic separations were performed on Baker silica gel (60–200 mesh). Fractions homogeneous by TLC were pooled, evaporated to dryness under reduced pressure, and dried under vacuum at 50–65 °C over P_2O_5. High-resolution ^1H and ^{19}F NMR spectra were acquired either on a Varian VXR-400 or a Bruker AM-300. NMR values for ^1H chemical shifts are presented in parts per million downfield from Me_4Si as the internal standard, and the relative peak areas given to the nearest whole number. The ^{19}F chemical shifts are presented in parts per million relative to $CFCl_3$ as the internal standard unless stated otherwise. Positive (M + 1) and negative (M − 1) ion FAB spectra were obtained on a VG 70SQ Mass Spectrometer at the Chemistry Department, University of South Carolina, Columbia, SC, by Dr. Michael Walla. N^5-(*tert*-butyloxycarbonyl)-L-ornithine was purchased from Bachem, Inc., Torrance, CA. Anhydrous DMF was obtained from Aldrich Chemical Co., Milwaukee, WI. The synthetic methods for preparing 5-(trifluoromethyl)-5,8-dideazafolic acid and 5-(trifluoromethyl)-5,8-dideazaisofolic acid were recently reported.[5] The chemistry leading to the formation of 5-fluoro-5,8-dideazaisofolic acid and 2-desamino-2-methyl-5-fluoro-5,8-dideazaisofolic acid will be described in a forthcoming communication from this laboratory.

Hog liver FPGS was purified to homogeneity as described previously.[14] The specific activity of the purified enzyme with (6*S*)-tetrahydrofolate as the substrate was 123 units/mg of protein at saturating substrate concentrations. One unit equals 1 μmol of $H_4PteGlu_2$ formed/h. Enzyme activity was measured by the incorporation of [^{14}C]glutamate into products using unlabeled folate or folate analogue as the substrate. The assay conditions used were the same as those described previously.[3]

***tert*-Butyl 5-(Trifluoromethyl)-5,8-dideazapteroate (10).** A mixture of 2-amino-6-cyano-3,4-dihydro-4-oxo-5-(trifluoromethyl)quinazoline, **8** (5) (1.45 g, 5.70 mmol), and *tert*-butyl *p*-aminobenzoate, **9** (6) (1.22 g, 6.30 mmol), in 180 mL of 70% glacial AcOH was stirred at ambient temperature for 15 min. The resulting dark yellow solution was then treated with Raney nickel (0.90 g damp) and the mixture hydrogenated in a Parr shaker apparatus until hydrogen uptake ceased (45 h). Charcoal was added and the reaction mixture was filtered through Celite and then basified to pH 8.5 with 30% NH_4OH. After refrigeration, the precipitated cream-colored solid was collected by filtration, washed with H_2O (10 mL) and dried under vacuum at 70 °C overnight to afford 1.69 g of crude product. It was purified on a silica gel column (2.5 × 38 cm) packed in $CHCl_3$ and eluted with 2% MeOH in $CHCl_3$ to afford 1.3 g (52.5%) of product: mp >175 °C dec (with preliminary softening); ^1H NMR (Me_2SO-d_6) δ 1.48 [s, 9, $C(CH_3)_3$], 4.47 (app d, 2, CH_2NH), 6.49 (br s, 2, NH_2), 6.53 (d, 2, 3′, 5′, J_o = 8.96 Hz), 7.09 (t, 1, CH_2NH, J = 5.80 Hz), 7.36 (d, 1, 8-H, J_o = 8.76 Hz), 7.60 (d, 2, 2′, 6′, J_o = 8.80 Hz), 7.62 (d, 1, 7-H, J_o = 8.40 Hz); ^{19}F NMR (Me_2SO-d_6) δ −50.30 (s, CF_3); FAB/MS *m/e* 435 (M + 1). Anal. ($C_{21}H_{21}F_3N_4O$·0.5H_2O) C, H, N.

5-(Trifluoromethyl)-5,8-dideazapteroic Acid (11). A solution of **10** (1.0 g, 2.30 mmol) in CF_3COOH (35 mL) was stirred at ambient temperature for 3 h. The solution was clarified by filtration and the solvent removed under reduced pressure with the help of added portions of Et_2O. The residue was then dissolved in 0.05 N NaOH (20 mL) and filtered and the filtrate was acidified with 1 N HCl to pH 5 to precipitate a cream-colored solid. After refrigeration, the product was collected by filtration, washed with H_2O (5 mL), and then dried under vacuum at 65 °C overnight to yield 0.75 g (86%) of white product. The analytical sample was obtained by purification on a cellulose column by elution with 5% NH_4HCO_3. Appropriate fractions were pooled and acidified to pH 5 with 2 N HCl to precipitate the product. The solid was isolated by filtration, washed with H_2O and then Et_2O, and dried under vacuum at 65 °C overnight to afford **11**: mp >190 °C dec; ^1H NMR (Me_2SO-d_6) δ 4.48 (s, 2, CH_2NH), 6.54 (d, 2, 3′, 5′, J_o = 8.80 Hz), 6.67 (br s, 2 NH_2), 7.09 (t, 1, CH_2NH, J = 5.78 Hz), 7.40 (d, 1, 8-H, J_o = 8.76 Hz), 7.65 (d, 2, 2′, 6′, J_o = 8.92 Hz), 7.66 (d, 1, 7-H, J_o = 8.80 Hz); ^{19}F NMR (Me_2SO-d_6) δ −50.57 (s, CF_3); FAB/MS *m/e* 377 (M − 1). Anal. ($C_{17}H_{13}F_3N_4O_3$·2.5H_2O) C, H, N.

***N*^{5}-(*tert*-Butyloxycarbonyl)-*N*^{2}-[5-(trifluoromethyl)-5,8-dideazapteroyl]-L-ornithine (12).** A sample of **11** (0.70 g, 1.65 mmol) (redried under vacuum at 70 °C over P_2O_5 for 18 h just prior to use) in $(CF_3CO)_2O$ (90 mL) was stirred in a N_2 atmosphere for 48 h. The reaction mixture was evaporated to dryness under reduced pressure with the help of added portions of EtOH. The resultant white residue was dried under vacuum at 65 °C for 18 h to yield 0.58 g (78%). The ^1H and ^{19}F NMR were in accordance with the 10-(trifluoroacetyl) derivative and the negative ion FAB/MS showed a peak of *m/e* 473 corresponding to (M − 1). This sample could not be purified due to the lability of the CF_3CO group and was used in the next step in this condition.

To a stirred solution of this intermediate (0.55 g, 1.16 mmol) in anhydrous DMF (50 mL) at 0 °C was added Et_3N (0.235 g, 2.32 mmol) followed by *i*-BuOCOCl (0.238 g, 1.74 mmol). The solution was stirred at 0 °C under N_2 for 1 h at which time N^5-(*tert*-butyloxycarbonyl)-L-ornithine (0.403 g, 1.74 mmol) was added. Stirring was continued at 0 °C for 4 h to give a light yellow solution, which was stirred for an additional 18 h at ambient temperature. The solvent was removed under reduced pressure with the help of added portions of EtOH. Next, the residue was dissolved in 10% NH_4OH (50 mL) and stirred at ambient temperature for 1.5 h, after which the solution was clarified by adding EtOH (30 mL) and stirred for another 0.5 h. The solvent was removed under reduced pressure and the resultant residue was triturated with cold H_2O (20 mL) to give a white solid which was collected by filtration and dried at 60 °C under vacuum for 18 h. This material was purified in two batches on a silica gel column (1.9 × 17 cm) packed in $CHCl_3$ and eluted with $CHCl_3$–MeOH–NH_4OH 7:2.5:0.5. The resulting solid was dried under vacuum over P_2O_5 at 65 °C for 18 h to give 0.45 g of white solid: mp >185 °C dec; ^1H NMR (Me_2SO-d_6) δ 1.35 [s, 9, $C(CH_3)_3$], 1.37–1.45 (m, 2, γ-CH_2), 1.59–1.80 (m, 2, β-CH_2), 2.89 (q, 2, δ-CH_2), 4.19 (m, 1, α-CH), 4.47 (app d, 2, CH_2NH), 6.53 (d, 2, 3′, 5′, J_o = 8.92 Hz), 6.66 (br s, 2, NH_2), 6.76 (t, 1, orn-NH or 10-NH, J = 5.60 Hz), 6.88 (t, 1, orn-NH or 10-NH, J = 5.88 Hz), 7.35 (d, 1, H_8, J_o = 8.76 Hz), 7.60 (d, 2, 2′, 6′, J_o = 8.80 Hz), 7.64 (d, 1, H_7, J_o = 8.76 Hz), 7.89 (d, 1, CONH, J = 7.32 Hz); ^{19}F NMR (Me_2SO-d_6) δ −50.38 (s, CF_3); FAB/MS *m/e* 591 (M − 1). Anal. ($C_{27}H_{31}F_3N_6O_6$·0.75CF_3COONH_4·2H_2O) C, H, N.

***N*^{2}-[5-(Trifluoromethyl)-5,8-dideazapteroyl]-L-ornithine (3).** A solution of **12** (0.20 g, 0.275 mmol) in CF_3COOH (10 mL) was stirred at ambient temperature for 2 h. The CF_3COOH was removed under reduced pressure and the residue triturated with EtOH (3 × 15 mL) and then Et_2O (3 × 15 mL). After drying, the solid was dissolved in 10% NH_4OH (15 mL) and the solution was stirred at ambient temperature for 1 h to give a white suspension. The suspension was evaporated to dryness under vacuum with the help of added portions of EtOH. The resultant white residue was triturated with H_2O (10 mL) and refrigerated for 18 h. The solid was collected by filtration, washed with Et_2O, and dried under vacuum at 65 °C for 18 h to afford 0.13 g (88%) of white solid: mp >250 °C dec; UV λ_{max} 204 nm (ε 3.32 × 10^4), 234 (ε 4.03 × 10^4), 290 (ε 1.93 × 10^4); ^1H NMR (Me_2SO-d_6) δ 1.54–1.62 (m, 2, γ-CH_2), 1.70–1.86 (m, 2, β-CH_2),

(14) Cichowicz, D. J.; Shane, B.; Mammalian Folylpoly-γ-glutamate Synthetase. 1. Purification and General Properties. *Biochemistry* **1987**, *26*, 504–512.

2.76 (t, 2, δ-CH_2, J = 7.36 Hz), 4.03–4.08 (m, 1, α-CH), 4.46 (s, 2, CH_2NH), 6.54 (d, 2, 3′, 5′, J_o = 8.76 Hz), 6.73 (s, 2, NH_2), 6.87 (t, 1, CH_2NH, J = 5.96 Hz), 7.35 (d, 1, H_8, J_o = 8.56 Hz), 7.55 (d, 2, 2′, 6′, J_o = 8.76 Hz), 7.63 (d, 1, H_7, J_o = 8.76 Hz), 7.60–7.67 (br s, 2, NH_2), 8.12 (d, 1, CONH, J = 7.30 Hz); ^{19}F NMR (Me_2SO-d_6) δ −50.36 ppm (s, CF_3); FAB/MS m/e 493 (M + 1) and 491 (M − 1). Anal. ($C_{22}H_{23}F_3N_6O_4$·0.25CF_3COOH·H_2O) C, H, N.

Methyl 5-(Trifluoromethyl)-5,8-dideazaisopteroate (14a). A mixture of 2,6-diamino-3,4-dihydro-4-oxo-5-(trifluoromethyl)-quinazoline, **13a** (5) (2.49 g, 10.20 mmol), methyl 4-bromomethylbenzoate (2.20 g, 9.60 mmol), and powdered $CaCO_3$ (2.04 g, 20.40 mmol) in DMAC (50 mL) was stirred at 60 °C under N_2 for 20 h. The reaction mixture was cooled to ambient temperature and filtered, and the filtrate was evaporated to dryness under reduced pressure. The resultant yellow residue was treated with H_2O (75 mL) and the pH of the suspension was raised to 8.5 by dropwise addition of 30% NH_4OH. The suspension was stirred at ambient temperature for 30 min and then at 0 °C for 30 min. The solid was collected by filtration, washed with H_2O and Et_2O, and dried under vacuum at 75 °C overnight to afford 2.35 g (63%) of product homogenous by TLC. The analytical sample was obtained by purification on a silica gel column (1.9 × 17 cm) packed in $CHCl_3$ and eluted with 2% MeOH in $CHCl_3$ to afford a white solid: mp >200 °C dec (with preliminary softening); ^{1}H NMR (Me_2SO-d_6) δ 3.82 (s, 3, CH_3), 4.54 (d, 2, NHCH_2, J = 5.80 Hz), 6.09 (s, 2, NH_2), 6.54 (app m, 1, NHCH$_2$) 6.95 (d, 1, H_7 or H_8, J_o = 9.16 Hz), 7.14 (d, 1, H_7 or H_8, J_o = 9.24 Hz), 7.46 (d, 2, 3′, 5′, J_o = 8.36 Hz), 7.91 (d, 2, 2′, 6′, J_o = 8.32 Hz), 10.80 (s, 1, 3-NH); ^{19}F NMR (Me_2SO-d_6) δ −50.56 (s, CF_3); FAB/MS m/e 393 (M + 1). Anal. ($C_{18}H_{15}F_3N_4O_3$) C, H, N.

5-(Trifluoromethyl)-5,8-dideazaisopteroic Acid (15a). A suspension of **14a** (2.35 g, 5.99 mmol) in 0.5 N NaOH (100 mL) was stirred and heated at 95 °C for 18 h. The resultant dark red solution was cooled to ambient temperature, filtered, and acidified to pH 5 with 2 N HCl. The yellow suspension was stirred at 0 °C for 1 h. The solid was collected by filtration, washed with H_2O, Me_2CO, and Et_2O, and dried at 75 °C under vacuum for 18 h to yield 1.65 g (73%) of the product. This material was purified by cellulose chromatography using a 1.5 × 20 cm column packed in 2.5% NH_4HCO_3 and eluted with 5% NH_4HCO_3. Appropriate fractions were pooled, acidified to pH 5 with 2 N HCl, and refrigerated to produce a yellow precipitate, which was isolated by filtration, washed with H_2O and Et_2O, and dried under vacuum at 75 °C for 18 h. There was obtained 1.0 g (44%) of **15a**: mp >310 °C dec; ^{1}H NMR (Me_2SO-d_6) δ 4.46 (s, 2, NHCH_2) 5.60–5.82 (br s, 1, NHCH$_2$), 6.10 (br s, 2, NH_2), 6.88 (d, 1, H_7 or H_8, J_o = 8.92 Hz), 7.02 (d, 1, H_7 or H_8, J_o = 8.80 Hz), 7.42 (d, 2, 3′, 5′, J_o = 7.84 Hz), 7.87 (d, 2, 2′, 6′, J_o = 7.92 Hz); ^{19}F NMR (Me_2SO-d_6) δ −50.50 (s, CF_3); FAB/MS m/e 377 (M − 1). Anal. ($C_{17}H_{13}F_3N_4O_3$·3H_2O) C, H, N.

N^5-(*tert*-Butyloxycarbonyl)-N^α-[5-(trifluoromethyl)-5,8-dideazaisopteroyl]-L-ornithine (16a). A suspension of **15a** (0.86 g, 1.99 mmol) in $(CF_3CO)_2O$ (120 mL) was stirred in a N_2 atmosphere for 48 h. The reaction mixture was filtered to remove a small amount of insoluble material and the clear yellow filtrate was evaporated to dryness under reduced pressure with the help of added portions of EtOH. The yellow solid obtained was dried at 65 °C under vacuum for 18 h to yield 0.35 g (37%). The ^{1}H and ^{19}F NMR were consistent with the 9-(trifluoroacetyl) derivative; however, this material too was unstable to be purified and it was used in the next step in this condition.

To a stirred solution of the trifluoroacetyl derivative (0.092 g, 0.193 mmol) in anhydrous DMF (8.5 mL) at 0 °C was added Et_3N (0.039 g, 0.386 mmol) followed by *i*-BuOCOCl (0.0396 g, 0.29 mmol). The solution was stirred at 0 °C under N_2 for 1 h at which time N^5-(*tert*-butyloxycarbonyl)-L-ornithine (0.067 g, 0.29 mmol) was added. Stirring was continued at 0 °C for 4.5 h and an additional 18 h at ambient temperature. The solvent was removed under reduced pressure with the help of added portions of EtOH. The residue was dissolved in 10% NH_4OH (8.5 mL) and stirred at ambient temperature for 1 h after which EtOH (5 mL) was added and the solution stirred for another 0.5 h. The solvent was removed under reduced pressure and the resultant residue triturated with cold H_2O (3.5 mL). The cream-colored product was collected by filtration and dried at 60 °C under vacuum for 18 h. This sample was purified on a silica gel column (1.9 × 17 cm) packed in $CHCl_3$ and eluted with $CHCl_3$–MeOH–NH_4OH 7:2.5:0.5 to afford 0.060 g (52%) of cream-colored solid: mp >180 °C dec; ^{1}H NMR (Me_2SO-d_6) δ 1.36 (s, 9, C(CH_3)$_3$), 1.37–1.46 (m, 2, γ-CH_2), 1.62–1.85 (m, 2, β-CH_2), 2.90 (app q, 2, δ-CH_2), 4.23 (m, 1, α-CH), 4.34 (app d, 2, NHCH_2), 5.99 (br s, 2, NH_2), 6.32 (app t, 1, 9-NH or orn-NH), 6.77 (t, 1, 9-NH or orn-NH, J = 5.32 Hz), 6.88 (app d, 1, H_7 or H_8), 7.00 (app d, 1, H_7 or H_8), 7.44 (d, 2, 3′, 5′, J_o = 8.36 Hz), 7.80 (d, 2, 2′, 6′, J_o = 8.28 Hz), 8.36 (d, 1, CONH, J = 8.04 Hz); ^{19}F NMR (Me_2SO-d_6) δ −50.43 (s, CF_3); FAB/MS m/e 593 (M + 1). Anal. ($C_{27}H_{31}F_3N_6O_6$·H_2O) C, H, N.

N^α-[5-(Trifluoromethyl)-5,8-dideazaisopteroyl]-L-ornithine (4). A solution of 0.045 g (0.074 mmol) of **16a** in CF_3COOH (5 mL) was stirred at ambient temperature for 2 h. The CF_3COOH was removed under reduced pressure with the help of added portions of EtOH. Next, the residue was treated with 10% NH_4OH (3.5 mL) and the suspension stirred at ambient temperature for 1 h. The suspension was then evaporated to dryness at reduced pressure with the help of added EtOH. The residue was triturated with H_2O (3 mL) and cooled at 0 °C for 3 h. The solid was collected by filtration, washed with Et_2O, and dried under vacuum at 60 °C for 18 h to yield 0.026 (65%) of cream-colored product: mp >290 °C dec (with preliminary darkening); UV λ_{max} 204 nm (ε 2.91 × 10^4), 236 (ε 3.92 × 10^4), 288 (ε 1.86 × 10^4); ^{1}H NMR (Me_2SO-d_6) δ 1.40–1.51 (m, 2, γ-CH_2), 1.64–1.86 (m, 2, β-CH_2), 2.94 (app q, 2, δ-CH_2), 4.26 (m, 1, α-CH), 4.35 (app d, 2, NHCH_2), 6.02 (br s, 2, NH_2), 6.76 (t, 1, NHCH$_2$), 6.89 (app d, 1, H_7 or H_8), 7.01 (app d, 1, H_7 or H_8), 7.45 (d, 2, 3′, 5′, J_o = 8.52 Hz), 7.81 (d, 2, 2′, 6′, J_o = 8.36 Hz), 8.32 (d, 1, CONH, J = 7.12 Hz), 8.38 (br s, 2, NH_2); ^{19}F NMR (Me_2SO-d_6) δ −50.48 (s, CF_3); FAB/MS m/e 493 (M + 1). Anal. ($C_{22}H_{23}F_3N_6O_4$·0.25CF_3COOH·H_2O) C, H, N.

5-Chloro-2,6-diamino-3,4-dihydro-4-oxoquinazoline (13b). A 10.00-g (43.9 mmol) sample of 5-chloro-2,4,6-triaminoquinazoline (7) was stirred under N_2 at 105 °C in a mixture of 2 N HCl (125 mL) and 2-methoxyethanol (125 mL) for 12 h. The product was precipitated by basification to pH 9.0 at 0 °C with 30% NH_4OH, collected by filtration, washed with ice-cold H_2O and finally with Et_2O, and dried under vacuum at 85 °C to afford 8.38 g (91%) of a light brown powder: mp 292–294 °C dec (forms needles at 269–270 °C); ^{1}H NMR (Me_2SO-d_6) δ 5.21 (s, 2, 6-NH_2), 6.04 (s, 2, 2-NH_2), 6.97 (d, 1, H_8, $J_{7,8}$ = 8.82 Hz), 7.12 (d, 1, H_7, $J_{7,8}$ = 8.82 Hz), 10.79 (br s, 1, 3-NH); FAB/MS m/e 211 (M + 1). Anal. ($C_8H_7ClN_4O$·0.25H_2O) C, H, N.

Methyl 5-Chloro-5,8-dideazaisopteroate (14b). A mixture of 3.48 g (16.5 mmol) of **13b**, 3.44 g (15.0 mmol) of methyl 4-(bromomethyl)benzoate, and 3.00 g (30.0 mmol) of $CaCO_3$ was stirred at 60 °C in DMAC (150 mL) under N_2 for 24 h. The insoluble material was removed by filtration and the filtrate evaporated under reduced pressure with the help of added EtOH. The residue was triturated with EtOH, filtered, washed with Et_2O, and dried under vacuum at 50 °C over P_2O_5 to afford 5.01 g (93% crude yield) of a light gray powder, mp 263–267 °C, which was employed in the following step without further purification.

5-Chloro-5,8-dideazaisopteroic Acid (15b). A 2.00-g (5.57 mmol) sample of crude **14b** was stirred in a mixture of MeOH (100 mL) and 2 N NaOH (10 mL) at 55 °C for 18 h. Next, MeOH was removed under reduced pressure and the resultant solution acidified with 2 N HCl to pH 2.20 at 0 °C. The solid was isolated by filtration, washed with 10% citric acid, H_2O, Me_2CO, and Et_2O, and dried under vacuum at 75 °C over P_2O_5 to afford 1.48 g (72% overall crude yield) of a pale gray powder. A 0.50-g sample was recrystallized from MeOH–DMSO yielding 0.234 g of pure **15b** (34% overall yield): mp 310–311 °C (lit.[15] mp 278–280 °C dec as an HCl salt); ^{1}H NMR (Me_2SO-d_6) δ 4.52 (d, 2, NHCH_2, J = 5.02 Hz), 6.09 (s, 2, NH_2), 6.19 (br t, 1, NHCH$_2$), 6.87 (d, 1, H_8, $J_{7,8}$ = 8.90 Hz), 6.96 (d, 1, H_7, $J_{7,8}$ = 8.77 Hz), 7.43 (d, 2, 3′, 5′, J_o = 7.79 Hz), 7.88 (d, 2, 2′, 6′, J_o = 7.81 Hz); FAB/MS m/e 343 (M − 1). Anal. ($C_{16}H_{13}ClN_4O_3$·0.5H_2O) C, H, N.

N^5-(*tert*-Butyloxycarbonyl)-N^α-(5-chloro-5,8-dideazaisopteroyl)-L-ornithine (16b). A 0.234-g (0.678 mmol) sample of 5-chloro-5,8-dideazaisopteroic acid, **15b**, was stirred in 20 mL

(15) Hynes, J. B.; Kumar, A.; Tomažič, A.; Washtien, W. L. Synthesis of 5-Chloro-5,8-dideaza Analogues of Folic Acid and Aminopterin Targeted for Colon Adenocarcinoma. *J. Med. Chem.* **1987**, *30*, 1515–1519.

of $(CF_3CO)_2O$ at ambient temperature under N_2 for 23 h. The solvent was removed under vacuum with the help of added portions of EtOH, Me_2CO, and Et_2O and dried under vacuum at 60 °C over P_2O_5 for 1 h.

The crude trifluoroacetyl compound was dissolved in a solution of 20 mL of DMF, 0.189 mL (1.36 mmol) of Et_3N, and 0.133 mL (1.02 mmol) of *i*-BuOCOCl at 0 °C. After 1 h, 0.237 g (1.02 mmol) of N^5-(*tert*-butyloxycarbonyl)-L-ornithine was added to the reaction mixture at 0 °C and it was left stirring under N_2 for 14 h with gradual warming to ambient temperature. The mixture was spin evaporated with the help of added portions of Me_2CO and Et_2O. The residue was triturated with H_2O, collected by filtration, washed with H_2O, and air-dried. This material was dissolved in 20 mL of 10% NH_4OH and stirred for 3 h at ambient temperature. The solvent was removed under reduced pressure and the residue dried under vacuum at 50 °C over P_2O_5. It was applied to a silica gel column (40 × 1.25 cm) and eluted with $CHCl_3$–MeOH–NH_4OH 7:2.5:0.5 to afford 0.203 g of a tan powder which was still impure as indicated by NMR and TLC ($CHCl_3$–MeOH 1:1). A 0.143-g sample of this material was then purified on a silica gel column (13.5 × 1.25 cm), protected from light, using a stepwise gradient of $CHCl_3$–MeOH from 9:1 to 7:3. There was obtained 0.121 g (18%) of light yellow solid: mp 250–255 °C dec; ^{1}H NMR (Me_2SO-d_6) δ 1.34 [s, 9, $C(CH_3)_3$], 1.42 (m, 2, γ-CH_2), 1.65–1.81 (m, 2, β-CH_2), 2.89 (m, 2, δ-CH_2), 4.18 (m, 1, α-CH), 4.41 (m, 2, NHCH_2), 5.41 (m, 1, NHCH_2), 6.77 (br s, 2, 2-NH_2), 6.98–7.12 (1, NHCOO superimposed on H_7 and H_8), 7.00 (d, 1, H_8, $J_{7,8}$ = 8.74 Hz), 7.08 (d, 1, H_7, $J_{7,8}$ = 8.62 Hz), 7.32 (d, 2, 3′, 5′, J_o = 8.08 Hz), 7.78 (d, 2, 2′, 6′, J_o = 7.86 Hz), 8.00 (d, 1, CONH). Anal. ($C_{26}H_{31}ClN_6O_6$·1.5 CF_3COOH·0.5H_2O) C, H, N.

N^{α}-(5-Chloro-5,8-dideazaisopteroyl)-L-ornithine (5). A 0.0514-g (0.0704 mmol) sample of **16b** was stirred in 2 mL of CF_3COOH at ambient temperature for 1.25 h. The solvent removed under reduced pressure with the help of added portions of EtOH, Me_2CO, and Et_2O. The resultant solid was collected, washed with Et_2O, and dried under vacuum at 60 °C to give 0.055 g (68%) of a tan powder; mp 113–117 °C dec; UV λ_{max} 204 nm (ε 3.56 × 10⁴), 236 (ε 4.56 × 10⁴), 326 (ε 3.79 × 10³); ^{1}H NMR (Me_2SO-d_6) δ 1.63 (m, 2, γ-CH_2), 1.77–1.88 (m, 2, β-CH_2), 2.82 (br m, 2, δ-CH_2), 4.39–4.43 (m, 3, α-CH, NHCH_2), 5.44 (br s, 1, NHCH_2), 6.91 (br s, 2, 2-NH_2), 6.97–7.30 (br t, 1, $^+$NH, $J_{H,N}$ = 51.1 Hz), 7.05 (d, 1, H_8, $J_{7,8}$ = 8.85 Hz), 7.15 (d, 1, H_7, $J_{7,8}$ = 8.50 Hz), 7.36 (d, 2, 3′, 5′, J_o = 8.17 Hz), 7.69 (br s, 2, CH_2NH_2), 7.84 (d, 2, 2′, 6′, J_o = 8.14 Hz), 8.67 (d, 1, CONH, J = 8.20 Hz). No molecular ion was detected under positive or negative ion FAB conditions. The structurally analogous L-glutamate also failed to give a pseudo molecular ion.[15] Anal. ($C_{21}H_{23}ClN_6O_4$·3.5CF_3-COOH·H_2O) C, H; N: calcd, 9.59; found, 8.75.

2-Amino-3,4-dihydro-5-fluoro-4-oxoquinazoline (18). A 30.00-g (168 mmol) sample of 2,4-diamino-5-fluoroquinazoline, **17**, (8) was stirred at 104 °C in a mixture of 400 mL of 2 N HCl and 400 mL of 2-methoxyethanol for 8 h. The product was precipitated by basification to pH 8.5–9.0 at 10 °C with 30% NH_4OH, collected by filtration, and washed with ice-cold H_2O before drying in vacuo at 100 °C to yield 28.95 g (96%) of a white powder: mp 344–350 °C dec (with preliminary darkening); ^{1}H NMR (90 MHz, Me_2SO-d_6) δ 6.63–7.02 (m, 4, H_6, H_8 and NH_2), 7.48 (q, 1, H_7); ^{19}F NMR (90 MHz, Me_2SO-d_6) δ −112.4 (s); FAB/MS *m/e* 180 (M + 1). Anal. ($C_8H_6FN_3O$) C, H, N.

2-Amino-3,4-dihydro-5-fluoro-6-nitro-4-oxoquinazoline (19a) and 2-Amino-3,4-dihydro-5-fluoro-8-nitro-4-oxoquinazoline (19b). A 10.00-g (55.8 mmol) sample of **18** was dissolved in 100 mL of 90% HNO_3 at 0 °C. After 2.5 h, the product was precipitated by careful adjustment of the pH to 3.5–4.0 at 0 °C with 30% NH_4OH. The solid was collected by filtration and washed with ice-cold H_2O before drying in vacuo at 100 °C to yield 12.30 g (98%) of a light yellow powder: mp >400 °C dec; ^{1}H NMR (Me_2SO-d_6) δ 6.93 (dd, 1, H_6, $J_{6,7}$ = 8.87 Hz, $J_{6,F}$ = 10.43 Hz, 8-nitro isomer), 7.08 (dd, 1, H_8, $J_{7,8}$ = 9.31 Hz, $J_{8,F}$ = 1.00 Hz, 6-nitro isomer), 7.30 (br s, NH_2), 8.08 (dd, 1, H_7, $J_{6,7}$ = 8.84 Hz, $J_{7,F}$ = 5.05 Hz, 8-nitro isomer), 8.23 (dd, 1, H_7, $J_{7,8}$ = 9.28 Hz, $J_{7,F}$ = 8.19 Hz, 6-nitro isomer), ratio of 6-nitro:8-nitro isomers by integration = 78:22; ^{19}F NMR (Me_2SO-d_6, CF_3COOH internal standard) δ −27.83 (br s, 8-nitro isomer), −40.11 (d, $J_{7,F}$ = 8.05 Hz, 6-nitro isomer), ratio of 6-nitro:8-nitro isomers by integration = 80:20; FAB/MS *m/e* 225 (M + 1).

3,4-Dihydro-5-fluoro-6-nitro-4-oxo-2-(pivaloylamino)-quinazoline (20a). A mixture of 4.00 g (17.8 mmol) of **19a** and **19b** was stirred under N_2 in 10 mL of $[(CH_3)_3CCO]_2O$ at 115 °C for 12 h. The cooled reaction mixture was diluted with CH_2Cl_2 and filtered. The filtrate was spin evaporated and dried under vacuum at 100 °C to give 4.78 g of crude product. In order to obtain a pure sample of the title compound **20a**, 1.61 g of this material was chromatographed on a silica gel column (43 × 2 cm), using a stepwise gradient of CH_2Cl_2 to CH_2Cl_2–MeOH 98:02. Fractions that contained the 6-nitro isomer were pooled, evaporated, and dried under vacuum at 100 °C to give 1.15 g of a yellow powder. A 0.966-g sample of the product was recrystallized from EtOH and dried under vacuum at 75 °C to give 0.565 g (32% overall) of bright yellow needles: mp 231–232 °C (with preliminary softening); ^{1}H NMR (Me_2SO-d_6) δ 1.26 [s, 9, $C(CH_3)_3$], 7.34 (dd, 1, H_8, $J_{7,8}$ = 9.21 Hz, $J_{8,F}$ = 1.15 Hz), 8.37 (dd, 1, H_7, $J_{7,8}$ = 9.04 Hz, $J_{7,F}$ = 8.18 Hz), 11.86 (br s, 1, CONH); ^{19}F NMR (Me_2SO-d_6, CF_3COOH internal standard) δ −39.88 (d, $J_{7,F}$ = 7.56 Hz); EI/MS *m/e* 308 (M^+). Anal. ($C_{13}H_{13}FN_4O_4$) C, H, N.

6-Amino-3,4-dihydro-5-fluoro-4-oxo-2-(pivaloylamino)-quinazoline (21a) and 8-Amino-3,4-dihydro-5-fluoro-4-oxo-2-(pivaloylamino)quinazoline (21b). A 5.00-g (16.2 mmol) mixture of the 6-nitro compound **20a** and its corresponding 8-nitro isomer, **20b**, was dissolved in AcOH (800 mL) and reduced under H_2 in the presence of Raney nickel until H_2 uptake ceased. The catalyst was removed by filtration, the filtrate concentrated to 100 mL, and the pH adjusted to 6.5 at 0 °C with 30% NH_4OH, which selectively precipitated the 6-amino isomer **21a**. The product was separated by filtration, washed with ice-cold H_2O, and dried under vacuum over P_2O_5 at 70 °C to give 2.60 g of **21a**. The filtrate was extracted with three 80-mL portions of EtOAc, and the combined extracts were washed twice with H_2O, dried over $MgSO_4$, spin evaporated, and dried under vacuum at 45 °C to give 1.40 g of crude product. This was separated on a silica gel column (43 × 2 cm), using a stepwise gradient of $CHCl_3$ to $CHCl_3$–MeOH 98:02. Fractions homogeneous by TLC ($CHCl_3$–MeOH 98:2) consisting of **21a** (R_f = 0.48) and **21b** (R_f = 0.58) were pooled, spin evaporated, and dried under vacuum at 50 °C to give 0.67 g (total yield: 70%) of pure **21a** (mp 205–207 °C) and 0.28 g (6%) of pure **21b** (mp 231–235 °C). Analytical samples were recrystallized from H_2O–DMF.

Analytical data for 6-amino-3,4-dihydro-5-fluoro-4-oxo-2-(pivaloylamino)quinazoline, **21a**: ^{1}H NMR (Me_2SO-d_6) δ 1.23 [s, 9, $C(CH_3)_3$], 5.36 (s, 2, 6-NH_2), 7.11 (dd, 1, H_8, $J_{7,8}$ = 8.77 Hz, $J_{8,F}$ = 0.28 Hz), 7.24 (t, 1, H_7, J = 8.77 Hz), 10.81 (s, 1, 3-NH), 11.90 (s, 1, CONH); ^{19}F NMR (Me_2SO-d_6) δ −59.99 (d, $J_{7,F}$ = 8.74 Hz); FAB/MS *m/e* 279 (M + 1). Anal. ($C_{13}H_{15}FN_4O_2$) C, H, N.

Analytical data for 8-amino-3,4-dihydro-5-fluoro-4-oxo-2-(pivaloylamino)quinazoline, **21b**: ^{1}H NMR (Me_2SO-d_6) δ 1.26 [s, 9, $C(CH_3)_3$], 5.29 (s, 2, 8-NH_2), 6.84–6.99 (m, 2, H_6 and H_7), 10.77 (br s, 1, 3-NH), 12.00 (br s, 1, CONH); ^{19}F NMR (Me_2SO-d_6) δ −52.24 (dd, $J_{6,F}$ = 10.24 Hz, $J_{7,F}$ = 5.79 Hz); FAB/MS *m/e* 279 (M + 1). Anal. ($C_{13}H_{15}FN_4O_2$) C, H, N.

Methyl 5-Fluoro-2-pivaloyl-5,8-dideazaisopteroate (22). A mixture of 0.574 g (3.50 mmol) of methyl 4-formylbenzoate and 0.973 g (3.50 mmol) of **21a**, in 100 mL of 70% AcOH was hydrogenated in the presence of Raney nickel until H_2 uptake ceased. The catalyst was removed by filtration and the filtrate concentrated under vacuum to approximately 5 mL. Upon the addition of H_2O and adjustment to pH 7.0 at 0 °C with 30% NH_4OH, an orange suspension formed. The precipitate was isolated by filtration, washed with ice-cold H_2O, and dried under vacuum at 60 °C over P_2O_5. After washing with hexanes, the product was dried under vacuum at 60 °C over P_2O_5 to afford 1.05 g of a tan powder. This material was purified on a silica gel column (40 × 2 cm) using a stepwise gradient from $CHCl_3$ to $CHCl_3$–MeOH 99.5:0.5 to afford 0.44 g (30%) of a yellow powder. The analytical sample was recrystallized from MeOH–H_2O: mp 202–204 °C; ^{1}H NMR (Me_2SO-d_6) δ 1.22 (s, 9, $C(CH_3)_3$), 3.82 (s, 3, CH_3), 4.50 (d, 2, NHCH_2, J = 5.86 Hz), 6.60 (br m, 1, NHCH_2), 6.99–7.11 (q, 2, H_7 and H_8), 7.50 (d, 2, 3′, 5′, J_o = 8.22 Hz), 7.91 (d, 2, 2′, 6′, J_o = 8.25 Hz), 10.82 (br s, 1, 3-NH), 11.91 (s, 1, CONH);

^{19}F NMR (Me_2SO-d_6) δ −58.55 (d, $J_{7,F}$ = 7.46 Hz); FAB/MS *m/e* 427 (M + 1). Anal. ($C_{22}H_{23}FN_4O_4$) C, H, N.

Methyl 5-Fluoro-5,8-dideazaisopteroate (23). A 0.42-g (0.99 mmol) sample of methyl 5-fluoro-2-pivaloyl-5,8-dideazaisopteroate, **22**, was stirred under N_2 at 60 °C in a solution of 15 mL of MeOH and 25 mL of 2 N HCl for 5 h. The suspension was evaporated, diluted with H_2O, adjusted to pH 7.5 at 0 °C with 30% NH_4OH, and readjusted to pH 3.5 with 2 N HCl to effect precipitation. The precipitate was collected by filtration, washed with ice-cold H_2O, Me_2CO, Et_2O, and hexanes, and dried under vacuum at 60 °C over P_2O_5 to yield 0.23 g (68%) of a pale yellow-green powder: mp 312–314 °C dec; ^{1}H NMR (Me_2SO-d_6) δ 3.82 (s, 3, CH_3), 4.45 (br s, 2, NHCH_2), 6.37 (br s, 1, NHCH$_2$), 6.87–6.98 (q, 2, H_7 and H_8), 7.00 (br s, 2, NH_2), 7.48 (d, 2, 3′, 5′, J_o = 8.24 Hz), 7.90 (d, 2, 2′, 6′, J_o = 8.23 Hz); ^{19}F NMR (Me_2SO-d_6) δ −58.41 (d, $J_{7,F}$ = 8.28 Hz); FAB/MS *m/e* 343 (M + 1). Anal. ($C_{17}H_{15}FN_4O_3$·0.85H_2O) C, H, N.

5-Fluoro-5,8-dideazaisopteroic Acid (24). A 0.222-g (0.648 mmol) sample of **23** was stirred in 2.6 N HCl under N_2 at 75 °C for 12 h. The reaction mixture was poured onto crushed ice and the pH adjusted to 3.5–4.0 at 0 °C with 30% NH_4OH. The product was isolated by filtration, washed with ice-cold H_2O, Me_2CO, Et_2O, and hexanes, and dried under vacuum at 50 °C over P_2O_5 to yield 0.192 g (90%) of a gray-green powder. The analytical sample was recrystallized from DMSO-MeOH: mp >400 °C; ^{1}H NMR (Me_2SO-d_6) δ 4.43 (s, 2, NHCH_2), 6.11 (br s, 1, NHCH$_2$), 6.19 (br s, 2, NH_2), 6.80 (d, 1, H_8, $J_{7,8}$ = 9.02 Hz), 6.90 (dd, 1, H_7, $J_{7,8}$ = 8.68 Hz, $J_{7,F}$ = 8.44 Hz), 7.46 (d, 2, 3′, 5′, J_o = 7.95 Hz), 7.88 (d, 2, 2′, 6′, J_o = 7.91 Hz); ^{19}F NMR (Me_2SO-d_6) δ −58.79 (d, $J_{7,F}$ = 8.13 Hz); FAB/MS *m/e* 327 (M − 1). Anal. ($C_{16}H_{13}FN_4O_3$·0.5H_2O) C, H, N.

N^5-(*tert*-Butyloxycarbonyl)-N^α-(5-fluoro-5,8-dideazaisopteroyl)-L-ornithine (25). A 0.158-g (0.482 mmol) sample of 5-fluoro-5,8-dideazaisopteroic acid, **24**, was stirred in 5 mL of $(CF_3CO)_2O$ at ambient temperature under N_2 for 28 h. The solvent was removed under reduced pressure with the help of added portions of Et_2O, H_2O, and Me_2CO and residue dried under vacuum at 60 °C over P_2O_5 for 2.5 h to afford the trifluoroacetyl derivative as a pink solid. Next, the powder was dissolved in a solution of 15 mL of DMF, 0.134 mL (0.964 mmol) of Et_3N, and 0.0943 mL (0.723 mmol) of *i*-BuOCOCl at 0 °C. After 0.75 h, 0.168 g (0.723 mmol) of N^5-(*tert*-butyloxycarbonyl)-L-ornithine was added to the reaction mixture at 0 °C, and stirring was continued for 14 h while being warmed to ambient temperature. The mixture was spin evaporated with the help of added portions of H_2O and Et_2O. The solid was collected, washed with H_2O, and dried. This material was dissolved in 30 mL of 10% NH_4OH at pH 10.0 and stirred for 2.5 h at ambient temperature. The solvent was removed under reduced pressure, and the residue was dried under vacuum at 60 °C over P_2O_5. This material was purified on a silica gel column (27 × 1.40 cm) using $CHCl_3$–MeOH–NH_4OH 7:2.5:0.5 to afford 0.104 g (35%) of a tan powder: mp 230–235 °C dec (foamed at 200 °C); ^{1}H NMR (Me_2SO-d_6) δ 1.35 [s, 9, C(CH_3)$_3$], 1.46 (m, 2, γ-CH_2), 1.66–1.81 (m, 2, β-CH_2), 2.92 (q, 2, δ-CH_2), 4.32 (m, 1, α-CH), 4.40 (d, 2, NHCH_2, J = 6.50 Hz), 6.08 (br s, 1, NHCH$_2$), 6.11 (br s, 2, 2-NH_2), 6.78 (d, 2, H_8 and orn-NH, $J_{7,8}$ = 8.50 Hz), 6.90 (dd, 1, H_7, $J_{7,8}$ = 8.67 Hz, $J_{7,F}$ = 8.81 Hz), 7.43 (d, 2, 3′, 5′, J_o = 8.15 Hz), 7.80 (d, 2, 2′, 6′, J_o = 8.24 Hz), 8.49 (d, 1, CONH, J = 7.64 Hz); ^{19}F NMR (Me_2SO-d_6) δ −58.83 (d, $J_{7,F}$ = 8.32 Hz); FAB/MS *m/e* 541 (M − 1). Anal. ($C_{26}H_{31}FN_6O_6$·0.33CF_3COOH·1.8H_2O) C, H, N.

N^α-(5-Fluoro-5,8-dideazaisopteroyl)-L-ornithine (6). A 0.0845-g (0.156 mmol) sample of **25** was stirred in CF_3COOH (3 mL) at ambient temperature for 4 h. The solvent was removed under reduced pressure, and the residue was dissolved in Me_2CO and spin evaporated two times and then triturated with Et_2O. The material was dried under vacuum at 75 °C over P_2O_5 to give 0.0806 g of a light brown powder. In order to remove residual CF_3COOH, a 0.0614-g sample was stirred in 3 mL of EtOH for 2 h and then entrained successively with Me_2CO and Et_2O. The residue was filtered and washed with Et_2O and dried under vacuum at 50 °C over P_2O_5 to afford 0.0487 g (72%) of a tan powder: mp 200–203 °C dec; UV λ_{max} 204 nm (ϵ 2.76 × 10^4), 240 (ϵ 3.79 × 10^4), 274 (ϵ 1.48 × 10^4), 356 (ϵ 2.92 × 10^3); ^{1}H NMR (Me_2SO-d_6) δ 1.62 (m, 2, γ-CH_2), 1.78–1.89 (m, 2, β-CH_2), 2.79 (br m, 2, δ-CH_2), 4.42 (m, 3, α-CH and NHCH_2), 6.18 (br s, 1, NHCH$_2$), 6.50 (br m, 2-NH_2), 6.81 (d, 1, H_8, $J_{7,8}$ = 9.08 Hz), 6.93 (dd, 1, H_7, $J_{7,8}$ = 8.78 Hz, $J_{7,F}$ = 8.4 Hz), 6.97–7.31 (br t, 1, $^+$NH, $J_{H,N}$ = 49.8 Hz), 7.45 (d, 2, 3′, 5′, J_o = 8.0 Hz), 7.68 (br s, 2, CH_2NH_2), 7.82 (d, 2, 2′, 6′, J_o = 7.66 Hz), 8.58 (d, 1, CONH, J = 7.9 Hz); ^{19}F NMR (Me_2SO-d_6) δ −58.61 (d, $J_{7,F}$ = 6.99 Hz); FAB/MS *m/e* 443 (M + 1). Anal. ($C_{21}H_{23}FN_6O_4$·1.5CF_3COOH·1.5H_2O) C, H, N.

3,4-Dihydro-5-fluoro-2-methyl-4-oxoquinazoline (27). A 15.00-g (84.7 mmol) sample of 4-amino-5-fluoro-2-methylquinazoline, **26**, (11) was stirred under N_2 in a mixture of 2-methoxyethanol (120 mL) and 37% HCl (20 mL) at 100 °C for 15 h. The product was precipitated by basification to pH 8.0 at 0 °C with 30% NH_4OH. The suspension was concentrated under reduced pressure, diluted with H_2O, and readjusted to pH 8.0. The product was collected by filtration, washed with ice-cold H_2O and hexanes, and dried under vacuum at 65 °C over P_2O_5 to give 13.63 g (90%) of a light tan powder. The analytical sample was recrystallized from EtOAc–DMF: mp 268–270 °C; ^{1}H NMR (Me_2SO-d_6) δ 2.32 (s, 3, CH_3), 7.18 (m, 1, H_7, $J_{6,7}$ = 8.16 Hz, $J_{7,8}$ = 8.16 Hz, $J_{7,F}$ = 5.53 Hz), 7.37 (d, 1, H_8, $J_{7,8}$ = 8.23 Hz), 7.73 (m, 1, H_6, $J_{6,7}$ = 8.19 Hz, $J_{6,F}$ = 10.67 Hz), 12.24 (br s, 1, 3-NH); ^{19}F NMR (Me_2SO-d_6) δ −35.53 (q, $J_{6,F}$ = 11.06 Hz, $J_{7,F}$ = 5.64 Hz); EI/MS *m/e* 178 (M^+). Anal. ($C_9H_7FN_2O$) C, H, N.

3,4-Dihydro-5-fluoro-2-methyl-6-nitro-4-oxoquinazoline (28a) and 3,4-Dihydro-5-fluoro-2-methyl-8-nitro-4-oxoquinazoline (28b). A 13.29-g (74.6 mmol) sample of **27** was stirred in a mixture of 50 mL of 90% HNO_3 and 50 mL of 95–98% H_2SO_4 for 13 h, starting at 0 °C and slowly warming to ambient temperature. The reaction mixture was poured onto crushed ice and the product precipitated by neutralization to pH 3.5–4.0 with 30% NH_4OH at 0 °C. It was collected by filtration, washed with ice-cold H_2O, Et_2O, and hexanes, and dried under vacuum at 60 °C over P_2O_5 to give 15.97 g (96%) of a yellow powder. Finally, it was recrystallized from EtOAc–DMF: mp 246–248 °C; ^{1}H NMR (Me_2SO-d_6) δ 2.34 (s, 3, CH_3, 6-nitro isomer), 2.38 (s, 3, CH_3, 8-nitro isomer), 7.36 (dd, 1, H_6, $J_{6,F}$ = 10.26 Hz, $J_{6,7}$ = 8.95 Hz, 8-nitro isomer), 7.49 (dd, 1, H_8, $J_{7,8}$ = 9.17 Hz, $J_{8,F}$ = 1.26 Hz, 6-nitro isomer), 8.27 (dd, 1, H_7, $J_{6,7}$ = 8.83 Hz, $J_{7,F}$ = 4.82 Hz, 8-nitro isomer), 8.38 (dd, 1, H_7, $J_{7,8}$ = 9.10 Hz, $J_{7,F}$ = 7.95 Hz, 6-nitro isomer), 12.68 (br s, 2 [1 per isomer], 3-NH); ^{19}F NMR (Me_2SO-d_6) δ −24.49 (q, $J_{6,F}$ = 10.95 Hz, $J_{7,F}$ = 4.98 Hz, 8-nitro isomer), −40.29 (d, $J_{7,F}$ = 8.34 Hz, 6-nitro isomer); according to ^{19}F NMR the ratio of 6-nitro:8-nitro isomers = 45:55; EI/MS *m/e* 223 (M^+). Anal. ($C_9H_6FN_3O_3$) C, H, N.

6-Amino-3,4-dihydro-5-fluoro-2-methyl-4-oxoquinazoline (29). A 10.01-g (44.9 mmol) sample of the mixture of **28a** and **28b**, prepared as described above, was hydrogenated in the presence of Raney nickel in 2-methoxyethanol (500 mL) until H_2 uptake ceased. The catalyst was removed by filtration, the solvent evaporated under reduced pressure, and the residue triturated twice with Me_2CO and once with Et_2O to give a light brown powder that was dried under vacuum at 60 °C over P_2O_5 to yield 8.20 g of the crude product. A 4.01-g sample of this material was purified on a silica gel column (65 × 2.5 cm), using a stepwise gradient of $CHCl_3$ to $CHCl_3$–MeOH 97:3 to yield 1.24 g (66% based on the percentage of the 6-nitro isomer present) of a beige powder. The analytical sample was recrystallized from EtOAc–DMF: mp 304–306 °C; ^{1}H NMR (Me_2SO-d_6) δ 2.24 (s, 3, CH_3), 5.36 (s, 2, 6-NH_2), 7.18 (m, 2, H_7 and H_8), 11.85 (br s, 1, 3-NH); ^{19}F NMR (Me_2SO-d_6) δ −60.51 (d, $J_{7,F}$ = 7.99 Hz); EI/MS *m/e* 193 (M^+). Anal. ($C_9H_8FN_3O$) C, H, N.

Methyl 2-Desamino-2-methyl-5-fluoro-5,8-dideazaisopteroate (30). A mixture of 0.82 g (5.00 mmol) methyl 4-formylbenzoate and 0.965 g (5.00 mmol) of **29** in 100 mL of 70% AcOH was hydrogenated in the presence of Raney nickel until H_2 uptake ceased. The catalyst was removed by filtration and the filtrate spin evaporated to give a yellow solid, which was dried under vacuum at 60 °C over P_2O_5 to give 1.75 g of the yellow-green solid. This material was purified on a silica gel column (28 × 3 cm), using a stepwise gradient from $CHCl_3$ to $CHCl_3$–MeOH 97:3 to afford 0.515 g (30%) of a cream-colored powder: mp 289–291 °C; ^{1}H NMR (Me_2SO-d_6) δ 2.22 (s, 3, 2-CH_3), 3.82 (s, 3, $COOCH_3$), 4.50 (d, 2, NHCH_2, J = 6.18 Hz), 6.59 (br m, 1, NHCH$_2$), 7.01 (dd, 1, H_7, $J_{7,8}$ = 8.76 Hz, $J_{7,F}$ = 8.66 Hz), 7.14 (d, 1, H_8, $J_{7,8}$ = 8.91 Hz), 7.50 (d, 2, 3′, 5′, J_o = 8.27 Hz), 7.91 (d,

2, 2′, 6′, J_o = 8.28 Hz); ^{19}F NMR (Me_2SO-d_6) δ −59.08 (d, $J_{7,F}$ = 8.79 Hz); FAB/MS *m/e* 342 (M + 1). Anal. ($C_{18}H_{16}FN_3O_3$) C, H, N.

2-Desamino-2-methyl-5-fluoro-5,8-dideazaisopteroic Acid (31). A 0.472-g (1.38 mmol) sample of **30** was stirred in 2 N HCl (50 mL) at 75 °C for 15 h and then in 60 mL 3.5 N HCl at 80 °C for an additional 16 h. The reaction mixture was adjusted to pH 3.5–4.0 at 0 °C with 30% NH_4OH. The precipitate was isolated by filtration, washed with ice-cold H_2O and Et_2O, dried under N_2 at 60 °C, washed with Et_2O, and finally dried under vacuum at 60 °C over P_2O_5 to afford 0.417 g (93%) of a cream-colored product: mp 308–311 °C dec; ^{1}H NMR (Me_2SO-d_6) δ 2.22 (s, 3, 2-CH_3), 4.49 (d, 2, NHCH_2, J = 5.71 Hz), 6.58 (br m, 1, NHCH$_2$), 7.02 (dd, 1, H_7, $J_{7,8}$ = 8.80 Hz, $J_{7,F}$ = 8.66 Hz), 7.14 (d, 1, H_8, $J_{7,8}$ = 8.92 Hz), 7.47 (d, 2, 3′, 5′, J_o = 8.09 Hz), 7.89 (d, 2, 2′, 6′, J_o = 8.10 Hz), 11.88 (br s, 1, 3-NH), 12.86 (br s, 1, COOH); ^{19}F NMR (Me_2SO-d_6) δ −59.11 (d, $J_{7,F}$ = 8.61 Hz); FAB/MS *m/e* 326 (M − 1). Anal. ($C_{17}H_{14}FN_3O_3$·0.2H_2O) C, H, N.

***N*^{5}-(*tert*-Butyloxycarbonyl)-*N*$^\alpha$-(2-desamino-2-methyl-5-fluoro-5,8-dideazaisopteroyl)-L-ornithine (32).** A 0.20-g (0.612 mmol) sample of **31** was stirred in 25 mL of $(CF_3CO)_2O$ at ambient temperature under N_2 for 29 h. The solvent was removed under reduced pressure with the help of added portions of EtOH and Et_2O and dried under vacuum at 45 °C over P_2O_5 to afford the trifluoroacetyl derivative of **31**, which was used for coupling without further purification. This was dissolved in a solution of 20 mL of DMF, 0.169 mL (1.22 mmol) of Et_3N, and 0.120 mL (0.918 mmol) of *i*-BuOCOCl at 0 °C. After 0.33 h of stirring, 0.213 g (0.918 mmol) of *N*^{5}-(*tert*-butyloxycarbonyl)-L-ornithine was added to the reaction mixture at 0 °C and stirring under N_2 was continued for 22 h, during which time warming to ambient temperature occurred. The mixture was spin evaporated with the help of added portions of H_2O and EtOH. Next, the solid was triturated with H_2O, collected by filtration, washed with H_2O, and dried under vacuum at 45 °C over P_2O_5. This material was dissolved in 15 mL of 10% NH_4OH at pH 10.0 and the solution stirred for 6 h at ambient temperature. The solvent was removed under reduced pressure, the residue dried under vacuum at 45 °C over P_2O_5, and purified on a silica gel column (21 × 2 cm) using $CHCl_3$–MeOH–NH_4OH 7:2.5:0.5 to afford 0.114 g (34%) of a cream-colored powder: mp 175–177 °C dec; ^{1}H NMR (Me_2SO-d_6) δ 1.35 [s, 9, C(CH_3)$_3$], 1.46 (m, 2, γ-CH_2), 1.76 (m, 2, β-CH_2), 2.22 (s, 3, CH_3), 2.92 (q, 2, δ-CH_2), 4.32 (br m, 1, α-CH), 4.48 (d, 2, NHCH_2, J = 6.00 Hz), 6.56 (br m, 1, NHCH$_2$ or NHCOO), 6.80 (br m, 1, NHCH$_2$ or NHCOO), 7.01 (dd, 1, H_7, $J_{7,8}$ = 8.89 Hz, $J_{7,F}$ = 8.58 Hz), 7.13 (d, 1, H_8, $J_{7,8}$ = 8.82 Hz), 7.45 (d, 2, 3′, 5′, J_o = 7.89 Hz), 7.81 (d, 2, 2′, 6′, J_o = 7.81 Hz), 8.51 (d, 1, CONH, J = 8.03 Hz), 11.87 (s, 1, 3-NH), 12.55 (br m, COOH); ^{19}F NMR (Me_2SO-d_6) δ −59.11 (d, $J_{7,F}$ = 8.49 Hz); FAB/MS *m/e* 540 (M − 1). Anal. ($C_{27}H_{32}FN_5O_6$·0.05CF_3COOH·1.75H_2O) C, H, N.

***N*$^\alpha$-(2-Desamino-2-methyl-5-fluoro-5,8-dideazaisopteroyl)-L-ornithine (7).** A 0.0848-g (0.157 mmol) sample of **32** was stirred in 3 mL of CF_3COOH at ambient temperature for 3 h. The solvent was removed under reduced pressure with the help of added portions of EtOH, Me_2CO, and Et_2O. The resulting solid was separated by filtration, washed with hexanes, and dried under vacuum at 50 °C over P_2O_5 to give 0.0874 g (84%) of a white powder: mp 229–231 °C dec; UV λ_{max} 204 nm (ε 3.18 × 10^4), 238 (ε 4.02 × 10^4), 294 (ε 1.81 × 10^4); ^{1}H NMR (Me_2SO-d_6) δ 1.62 (m, 2, γ-CH_2), 1.78–1.89 (m, 2, β-CH_2), 2.23 (s, 3, CH_3), 2.78 (m, 2, δ-CH_2), 4.40 (br m, 1, α-CH), 4.48 (br m, 2, NHCH_2), 6.57 (br m, 1, NHCH$_2$), 6.96–7.31 (br t, 1, $^+$NH, $J_{H,N}$ = 51.1 Hz), 7.02 (dd, 1, H_7, $J_{7,F}$ = 8.82 Hz, $J_{7,8}$ = 8.74 Hz), 7.14 (d, 1, H_8, $J_{7,8}$ = 8.74 Hz), 7.46 (d, 2, 3′, 5′, J_o = 7.93 Hz), 7.68 (br m, 2, CH_2NH_2), 7.82 (d, 2, 2′, 6′, J_o = 7.67 Hz), 8.59 (d, 1, CONH, J = 8.15 Hz), 11.90 (br s, 1, 3-NH), 12.7 (br s, COOH); ^{19}F NMR (Me_2SO-d_6) δ −59.06 (d, $J_{7,F}$ = 8.34 Hz); FAB/MS *m/e* 440 (M − 1), 442 (M + 1). Anal. ($C_{22}H_{24}FN_5O_4$·1.75CF_3COOH·H_2O) C, H, N.

Acknowledgment. This investigation was supported in part by PHS Grant CA-41991 (B.S.) and a graduate assistantship from the College of Graduate Studies, Medical University of South Carolina (O.F.). We thank the Davison Chemical Division, W. R. Grace and Co. for a gift of Raney 30.

Registry No. **3**, 143745-07-7; **4**, 143745-08-8; **5**, 143745-09-9; **6**, 143745-10-2; **7**, 143745-11-3; **8**, 133116-88-8; **9**, 18144-47-3; **10**, 143745-12-4; **11**, 143745-13-5; **11** (10-trifluoroacetyl derivative), 143745-37-3; **12**, 143745-14-6; **13a**, 133116-87-7; **13b**, 143745-30-6; **14a**, 143745-15-7; **14b**, 143745-31-7; **15a**, 143745-16-8; **15a** (9-trifluoroacetyl derivative), 143745-38-4; **15b**, 143745-32-8; **16a**, 143745-17-9; **16b**, 143745-33-9; **17**, 119584-70-2; **18**, 142465-05-2; **19a**, 142465-06-3; **19b**, 142465-04-1; **20a**, 143745-18-0; **20b**, 143745-34-0; **21a**, 143745-19-1; **21b**, 143745-35-1; **22**, 143745-20-4; **23**, 143745-21-5; **24**, 143745-22-6; **25**, 143745-23-7; **26**, 137553-47-0; **27**, 143745-24-8; **28a**, 143745-25-9; **28b**, 143745-36-2; **29**, 143745-26-0; **30**, 143745-27-1; **31**, 143745-28-2; **32**, 143745-29-3; FPGS, 63363-84-8; H-Orn(Boc)-OH, 13650-49-2; 4-Br$CH_2C_6H_4$COOMe, 2417-72-3; 4-OHCC_6H_4COOMe, 1571-08-0; $(Me_3CCO)_2O$, 1538-75-6.