Synthesis and Biological Evaluation of N^{α}-(5-Deaza-5,6,7,8-tetrahydropteroyl)-L-ornithine

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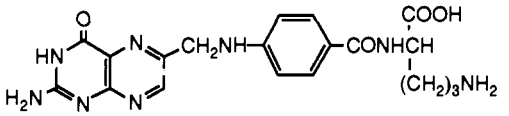
A novel folic acid analogue, N^{α}-(5-deaza-5,6,7,8-tetrahydropteroyl)-L-ornithine, **3**, was prepared via a multistep synthetic sequence. The key steps involved the conversion of 5-deazapteroic acid to its N^{10}-formyl derivative followed by catalytic hydrogenation of the pyridine ring and subsequent heating in dilute sodium hydroxide to afford the new 5-deaza-5,6,7,8-tetrahydropteroic acid. After trifluoroacetylation, this compound was coupled to N^{δ}-(*tert*-butyloxycarbonyl)-L-ornithine using conventional peptide bond forming conditions. Deprotection first in base and then in acid gave the title compound. Compound **3** was an effective inhibitor of hog liver folylpolyglutamate synthetase (K_{is}, estimated = 64 nM), and was shown to retard the formation of polyglutamates of a structurally related folic acid analogue in HCT-8 cells in vitro.

Several derivatives of folic acid in which the terminal L-glutamate moiety is replaced by L-ornithine have been shown 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 against hog liver FPGS, while reduction to its 5,6,7,8-tetrahydro derivative caused a 30-fold decrease in the K_i value.[1] The most potent inhibitor of FPGS reported thus far having a folic acid configuration in the pyrimidine nucleus was N^{α}-(5-chloro-5,8-dideazapteroyl)-L-ornithine, **2**, (K_i = 8.3 nM).[2] While **2** appeared to possess a significant degree of selectivity for FPGS versus dihydrofolate reductase (DHFR), Chinese hamster ovary cells made resistant to this analogue were found to overproduce DHFR and not FPGS.[3] It should be noted that **2** was found to be a reasonably effective inhibitor of human DHFR (I_{50} = 0.43 μM).[2]



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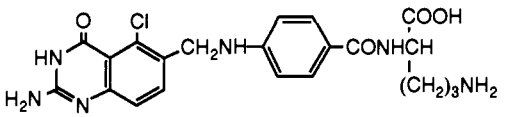

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It has been suggested that FPGS constitutes a potential target for inhibiting the survival of tumor cells. This contention is predicated upon numerous observations that the formation of poly-γ-L-glutamate derivatives of natural folates is essential for their effective retention in mammalian cells.[4-6] In order to test this hypothesis, we sought to identify a selective inhibitor of FPGS having the potential for effective uptake into target cells. The tital compound, N^{α}-(5-deaza-5,6,7,8-tetrahydropteroyl)-L-ornithine, **3**, was considered to be a logical candidate for the following reasons: (a) by virtue of being an analogue of structurally related 5-deaza-5,6,7,8-tetrahydrofolates, it was anticipated that **3** would be an exceptionally weak inhibitor of both DHFR as well as thymidylate synthase (TS).[7,8] (b) On the basis of earlier studies, there appears to be a reasonably good correlation between the K_m for an L-glutamate substrate of FPGS and the K_i for its structurally analogous L-ornithine inhibitor.[2,9] Therefore, **3** was expected to be a potent inhibitor of FPGS, since 5-deaza-5,6,7,8-tetrahydrofolic acid was reported to be an excellent substrate for mouse liver FPGS ($K_m \cong 1$ μM).[10] (c) It appears that oxidized folate derivatives possessing a

Scheme I. Synthetic Route to N^{α}-(5-Deaza-5,6,7,8-tetrahydropteroyl)-L-ornithine

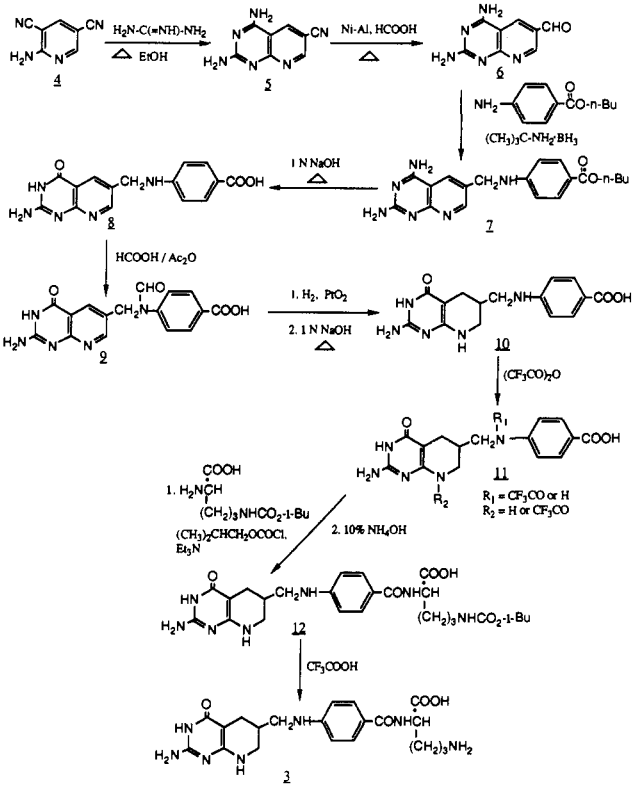

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Table I. Inhibition of Folate-Requiring Enzymes by 3

enzyme	source	I_{50} (μM)	K_i (μM) est
folylpolyglutamate synthetase	porcine	0.21 ± 0.03	0.064 ± 0.009
thymidylate synthase	human	470 ± 40	
dihydrofolate reductase	human	≅2[a]	
glycinamide ribonucleotide transformylase	porcine	4.8 ± 0.5	1.4 ± 0.2
5-aminoimidazole-4-carboxamide ribonucleotide transformylase	human	(16% inhibn at 100 μM)	

[a] DHFR inhibitory activity was partially separated from 3 by HPLC. DHFR inhibition by 3 was typical of that of a tight binding inhibitor.

terminal L-ornithine display little or no cytotoxic activity because they have difficulty gaining entry into target cells.[3,9] It should be noted in this regard that the related analogue, N^{α}-(5,8-dideazapteroyl)-L-ornithine, was shown to be essentially devoid of activity against two human leukemia cells lines.[9] The low cytotoxicity reported for the methotrexate (MTX) and aminopterin analogues bearing a terminal L-ornithine in place of an L-glutamyl residue has been attributed to poor cellular uptake.[11,12] By virtue of being a tetrahydrofolate analogue, it was hoped that 3 would be more readily taken up by target cells than 1 or 2.

Chemistry

The synthetic route employed for preparing N^{α}-(5-deaza-5,6,7,8-tetrahydropteroyl)-L-ornithine, 3, is depicted in Scheme I. The key intermediate, 2,4-diaminopyrido-[2,3-*d*]pyrimidine-6-carboxaldehyde, 6, was obtained according to the literature methods.[13] It was then subjected to reductive amination with *n*-butyl *p*-aminobenzoate in the presence of *tert*-butylamine–borane complex to afford *n*-butyl 4-amino-4-deoxy-5-deazapteroate, 7, in good yield. Basic hydrolysis of 7 then provided the 5-deazapteroic acid 8 in excellent yield. Attempts to reduce the pyridine ring of 8 were unsuccessful primarily due to the benzylic hydrogenolysis of the carbon–nitrogen bond. This is analogous to the observed benzylic cleavage of diethyl 2-acetyl-5-deazaglutamate.[10] Taylor's approach of reducing the pyridine ring after formylation or acetylation of the N^{10} position was then adopted.[10] Our initial effort to block the N^{10} position with the readily removable trifluoroacetyl group by refluxing 8 in trifluoroacetic anhydride failed, and only unchanged starting material was recovered. This is similar to the behavior of diethyl 2-pivaloyl-5-deazaglutamate toward acetic anhydride.[10] However, treatment of 8 with a mixture of acetic anhydride and formic acid at ambient temperature gave the 10-formyl derivative 9 in good yield. Intermediate 9 underwent catalytic reduction (PtO_2 in trifluoroacetic acid), without any detectable benzylic cleavage, to give 10-formyl-5,6,7,8-tetrahydropteroic acid, which upon deprotection with base gave the desired 5-deaza-5,6,7,8-tetrahydropteroic acid 10. It was then converted to the trifluoroacetyl derivative 11 using anhydrous trifluoroacetic anhydride. It is interesting to note that the tetrahydro compound 10 could be readily trifluoroacetylated, while this derivatization process was unsuccessful in the case of the unreduced derivative 8. This difference in the behavior is most probably due to the insolubility of 8 in trifluoroacetic anhydride as compared to the excellent solubility of 10. Removal of the N^{10}-formyl group in 10 and then blocking the N^{10} position with the easily deprotectable trifluoroacetyl group in 11 was carried out in order to avoid the harsh conditions required for formyl group removal. The compound 11 could not be purified by column chromatography as the trifluoroacetyl group was labile under the basic conditions required for elution. Instead it was used without further purification, in peptide bond formation with N^{δ}-(*tert*-butyloxycarbonyl)-L-ornithine using isobutyl chloroformate as the activating agent to afford the fully protected derivative of 3, which was deprotected, without isolation, by treatment with ammonium hydroxide to yield N^{δ}-(*tert*-butyloxycarbonyl)-N^{α}-(5-deaza-5,6,7,8-tetrahydropteroyl)-L-ornithine, 12. Removal of the *tert*-butyloxycarbonyl group from the N^{δ}-amino function of 12 with trifluoroacetic acid then gave the target compound 3 as a diastereomeric mixture, in respectable overall yield. Compound 3 was fully characterized by high resolution NMR, FAB, and elemental analysis and was free from

Table II. Effect of N^{α}-(5-Deaza-5,6,7,8-tetrahydropteroyl)-L-ornithine on Folate Pools in MCF-7 Cells in Vitro

	pmol/million cells[a]			
	control	50 μM	100 μM	1 μM MTX
5-CH_3-THF	0.7	0.6	0.6	0.1
5-CHO-THF	0.1	0.2	0.1	0.1
FA	0.0	0.0	0.0	0.1
DHF	0.2	0.2	0.2	1.6
THF	1.0	1.0	0.9	0.4
10-CHO-FA	0.1	0.2	0.1	0.3
10-CHO-DHF	0.2	0.3	0.3	0.6
10-CHO-THF	1.7	1.8	1.7	0.8
total folates	4.0	4.1	3.9	4.1

[a] Average of two separate cultures.

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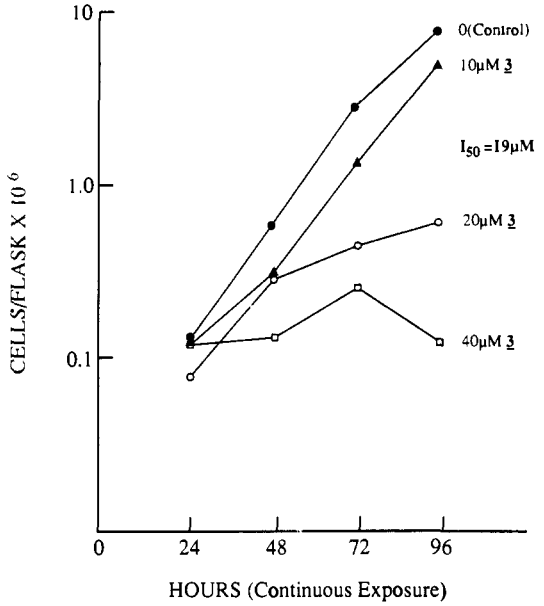

Figure 1. Inhibition of the growth of HCT-8 cells in vitro by compound **3**. The I_{50} value was calculated at 96 h.

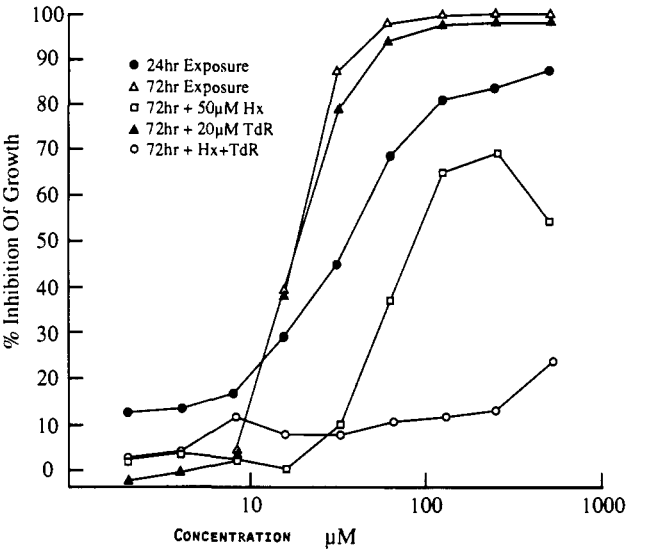

Figure 2. Reversal of the growth inhibition of HCT-8 cells by **3** using hypoxanthine (Hx), thymidine (TdR), or a combination of these normal metabolites.

impurities at the limits of detectability of these analytical methods.

Biological Evaluation

Compound **3** was evaluated as an inhibitor of five folate-requiring enzymes obtained from mammalian sources, and the results are presented in Table I. It was found to be a potent inhibitor of FPGS, having a K_{is} (est) of 64 nM. It was also a modest inhibitor of glycinamide ribonucleotide transformylase (GAR Tfase) (K_{is} (est) = 1.4 μM), but a very weak inhibitor of 5-aminoimidazole-4-carboxamide ribonucleotide transformylase (AICAR Tfase). As expected, **3** showed very little affinity for human TS. Surprisingly however, **3** was a moderately effective inhibitor of DHFR, a result which seemed inconsistent with values reported for structurally related analogues of 5,6,7,8-tetrahydrofolic acid. For example, 5,10-dideaza-5,6,7,8-tetrahydrofolic acid had a K_i versus the DHFR from L1210 leukemia cells which was greater than 1000.[13] Therefore, compound **3** was subjected to analysis by reverse-phase HPLC, and only one significant peak was observed. However, material obtained from the leading side of this peak contained no DHFR inhibitory activity, while that from a later trailing fraction did possess modest DHFR inhibiting activity.

The effect of **3** (not subjected to HPLC) upon folate pools in human breast adenocarcinoma cells (MCF-7) in vitro is shown in Table II. For comparison, the results obtained with the classical DHFR inhibitor, MTX, are also included. In this short exposure experiment (4 h) it will be seen that **3** caused very little change in the distribution of folates. In the case of MTX, however, the levels of tetrahydrofolate (THF), 5-methyltetrahydrofolate (5-CH_3-THF) and 10-formyltetrahydrofolate (10-CHO-THF) were reduced significantly and as expected, the dihydrofolate (DHF) and 10-formyldihydrofolate (10-CHO-DHF) levels expanded dramatically. These results suggest that the limited DHFR inhibitory activity present in the sample of compound **3** studied did not contribute to the effects of this compound in cells.

The I_{50} of **3** versus the growth of HCT-8 cells for 96 h was 19 μM (Figure 1), and 20 μM versus MCF-7 cells. In addition, both thymidine (TdR) and hypoxanthine (Hx) are required for the effective reversal of the cytotoxicity of **3** (>27-fold) (Figure 2). Finally, the effect of **3** upon the extent of polyglutamylation of 5-deazaacyclotetrahydrofolic acid (5-DACTHF) in HCT-8 cells was examined, and the results are presented in Figure 3. It will be seen that the distribution of polyglutamates of 5-DACTHF is shifted in favor of those having shorter chain length after a 24-h exposure to **3**. The total 5-DACTHF accumulated was reduced by ca. 61% by **3**, and the long-chain pools (Glu_6–Glu_8) were reduced by 77%.

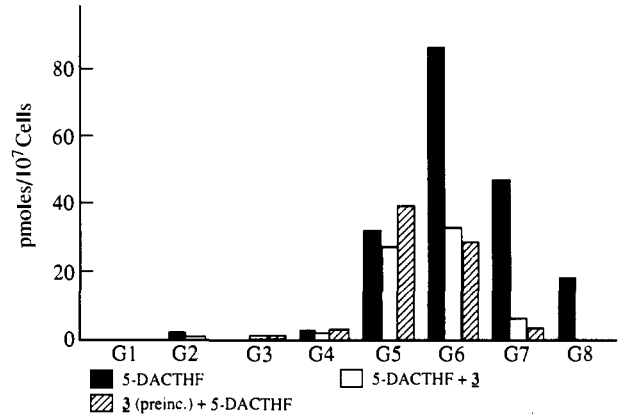

Figure 3. Effect of **3** at 20 μM upon the extent of polyglutamylation of 5-deazaacyclotetrahydrofolic acid (5-DACTHF) at 0.1 μM in HCT-8 cells incubated for 24 h. The open bars indicate simultaneous addition, while the slashed bars are for **3** added 24 h prior to 5-DACTHF.

Discussion

N^{α}-(5-Deaza-5,6,7,8-tetrahydropteroyl)-L-ornithine, **3**, has been synthesized and found to be a potent inhibitor of hog liver FPGS. Compound **3** had a modest level of cytotoxicity toward HCT-8 and MCF-7 cells in vitro and a combination of TdR and Hx was required for the effective reversal of its action in HCT-8 cells, consistent with FPGS being the primary target. The degree of polyglutamylation of the acyclic analogue of THF, 5-DACTHF, in HCT-8 cells was retarded somewhat by **3**. These results demonstrate that **3** was capable of entering tumor cells and suggest that it inhibited FPGS intracellularly. However, it is not entirely clear whether the cytotoxic action of **3** was due exclusively to the inhibition of FPGS, since **3** was also

found to be a reasonably effective inhibitor of GAR Tfase and the material studied presumably contained traces of a potent DHFR inhibitor.

Experimental Section

Melting points were determined on a Mel-temp apparatus and are uncorrected. Elemental analyses were performed by Atlantic Microlabs, Inc., Atlanta, GA. Analytical samples gave combustion values for C, H, and N within ±0.4% of the theoretical values. 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 compound 3, which contains CF_3COOH in the empirical formula. Compound 7 was free of significant impurities on TLC on silica gel (Eastman 13181). All acids were checked for purity by TLC on cellulose (Eastman 13254). Column chromatographic separations for 12 were performed on Baker silica gel (60–200 mesh). The UV spectra were determined on a Hewlett-Packard 8451A spectrophotometer in 0.1 N phosphate buffer, pH 7.0. High-resolution ^1H NMR spectra were acquired on a Varian VXR-400. NMR values for chemical shifts are presented in parts per million downfield from Me_4Si as the internal standard, and the relative peak areas were given to the nearest whole number. 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.

Homogeneous DHFR was obtained from human WIL2 cells as described earlier.[14] It was assayed spectrophotometrically at 340 nm using 45 μM dihydrofolate, 50 μM NADPH, and 0.10 M phosphate buffer, pH 7.0; [DHFR] = 2 nM by MTX titration. Thymidylate synthase was obtained from a SV 40-transformed human fibroblast cell line, which had been cloned in *Escherichia coli*.[15] The protein was purified to homogeneity by affinity chromatography.[16] It was assayed by the ^3H-release method described by Ferone and Roland.[17] Compound 3 was evaluated as an inhibitor of purified GAR Tfase according to the literature method.[8,18] Partially purified AICAR Tfase was obtained from MOLT-4 cells by a modification of the procedure of Mueller and Benkovic.[8,19] The continuous spectrophotometric assay was employed.[20] The growth inhibitory effect of 3 on HCT-8 cells was evaluated according to the literature method.[21] Hog liver FPGS was purified by a modification of the method of Cichowicz and Shane.[8,22] The enzyme was assayed by the charcoal absorption method,[23] with the KCl concentration being reduced to 20 mM. Aminopterin (50 μM) was employed as the standard substrate.

HCT-8 cells in logarithmic growth were incubated for 24 h with 100 nM [^{14}C]-5-DACTHF (8) alone, with simultaneous exposure to 20 μM of 3, or with 20 μM of 3 having been added 24 h prior to the addition of 100 nM [^{14}C]-5-DACTHF. The cultures were extracted and analyzed for polyglutamates of 5-DACTHF by HPLC as described previously.[24] MCF-7 cells were grown in a folate-free medium containing TdR and Hx. They were then transferred to a folate-free medium containing 5 nM [^3H]-(6*S*)-5-formyltetrahydrofolate and incubated for 120 h. Test compounds were then added and 4 h later, the cultures were harvested and the folate pools analyzed using a modification of the procedure recently described by Mullin et al.[25] Analysis of 3 by HPLC was performed using a Waters C_{18} Novapak radial compression column eluted at 1 mL/min with 10% CH_3CN in 126 mM NH_4OAc, pH 5.1. The main A_{280} peak (retention time 20 min) was collected in two fractions which were then assayed for DHFR inhibitory activity.

***n*-Butyl 4-Amino-4-deoxy-5-deazapteroate (7).** A suspension of 2,4-diaminopyrido[2,3-*d*]pyrimidine-6-carboxaldehyde, 6,[13] (2.50 g, 13.2 mmol) in glacial AcOH (250 mL) was warmed to give a dark yellow solution and cooled to room temperature. To this solution was added *n*-butyl *p*-aminobenzoate (2.85 g, 14.75 mmol), with stirring. This mixture was charged with 3-Å molecular sieves and stirred under N_2 for 18 h. Next, $(CH_3)_3CNH_2{\cdot}BH_3$ (0.43 g, 4.94 mmol) was added, and the solution was stirred at ambient temperature for 18 h and then warmed to 80 °C for an additional 18 h. The reaction mixture was cooled to room temperature, and another batch of $(CH_3)_3CNH_2{\cdot}BH_3$ (0.43 g, 4.94 mmol) was added and stirred for 2 h at ambient temeprature and 40 h at 70 °C. The reaction mixture was filtered, and the filtrate was evaporated to dryness under vacuum to afford a reddish oily material, which upon cooling and stirring with Me_2CO (250 mL) resulted in a light yellow solid. The product was isolated by filtration, washed with cold Me_2CO (100 mL), and dried under vacuum at 80 °C overnight to afford 2.55 g of solid. Another 0.75 g of product crystallized out of the Me_2CO filtrate. The total yield was 3.30 g (65%). This material was pure by NMR and was used in the preparation of 8 without further purification. A small sample was recrystallized from Me_2CO to give light yellow needles: mp 283–285 °C dec; ^1H NMR (Me_2SO-d_6) δ 0.92 (t, 3, CH_3, J = 7.28 Hz), 1.39 ((6), 2, γ-CH_2, J = 7.44 Hz), 1.64 ((5), 2, β-CH_2, J = 7.76 Hz), 4.16 (t, 2, OCH_2, J = 6.56 Hz), 4.30 (d, 2, CH_2NH, J = 5.08 Hz), 6.29 (s, 2, NH_2), 6.66 (d, 2, 3′, 5′, J_o = 8.80 Hz), 6.98 (t, 1, CH_2NH, J = 5.56 Hz), 7.48 (br s, 1, NH), 7.69 (d, 2, 2′, 6′, J_o = 8.72 Hz), 8.37 (d, 1, 5-H, J_m = 2.20 Hz), 8.63 (d, 1, 7-H, J_m = 2.32 Hz); FAB/MS 367 (M + 1). Anal. ($C_{19}H_{22}N_6O_2{\cdot}0.85H_2O$) C, H, N.

5-Deazapteroic Acid (8). A near solution of 7 (2.00 g, 5.24 mmol) in 200 mL of 0.5 N NaOH was purged with N_2 for 15 min and then stirred and refluxed for 8 h under a N_2 atmosphere. The reaction mixture was cooled to ambient temperature, clarified by filtration, and acidified to pH 6.0 by dropwise addition of glacial AcOH, with cooling, to effect precipitation. The yellow solid was collected by filtration, washed with H_2O, Me_2CO, and Et_2O, and then dried under vacuum at 80 °C overnight to afford 1.45 g (86%) of 8. An analytical sample was obtained by crystallization from DMF–H_2O: mp > 300 °C dec; ^1H NMR (Me_2SO-d_6) δ 4.38 (d,

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2, CH_2, J = 5.00 Hz), 6.61 (d, 2, 3′, 5′, J_o = 8.92 Hz), 6.92 (br s, 2, NH_2), 6.98 (t, 1, CH_2NH, J = 6.00 Hz), 7.65 (d, 2, 2′, 6′, J_o = 8.72 Hz), 8.17 (d, 1, 5-H, J_m = 2.56 Hz), 8.62 (d, 1, 7-H, J_m = 2.60 Hz); FAB/MS 310 (M − 1). Anal. ($C_{15}H_{13}N_5O_3 \cdot 0.5H_2O$) C, H, N.

10-Formyl-5-deazapteroic Acid (9). A 1.45 g (4.53 mmol) sample of 8 was dissolved in 50 mL of 98% HCOOH, and to this solution was added 25 mL of Ac_2O with stirring at room temperature. The reaction mixture was stirred at ambient temperature for 3 h and poured into 700 mL of cold Et_2O to effect precipitation of a light yellow solid. The precipitate was collected by filtration, washed with H_2O, Me_2CO, and Et_2O, and dried at 90 °C under vacuum to obtain 1.34 g (79%) of 9, which was recrystallized from DMF–H_2O: mp > 300 °C dec; ^{1}H NMR (Me_2SO-d_6) δ 5.15 (s, 1, CH_2), 6.95 (br s, 2, NH_2), 7.51 (d, 2, 3′, 5′, J_o = 8.52 Hz), 7.91 (d, 2, 2′, 6′, J_o = 8.56 Hz), 8.08 (d, 1, 5-H, J_m = 2.12 Hz), 8.52 (d, 1, 7-H, J_m = 2.20 Hz), 8.83 (s, 1, CHO); FAB/MS 338 (M − 1). Anal. ($C_{16}H_{13}N_5O_4 \cdot 2H_2O$) C, H, N.

5-Deaza-5,6,7,8-tetrahydropteroic Acid (10). To a solution of 9 (1.00 g, 2.66 mmol) in CF_3COOH (100 mL) was added PtO_2 (0.50 g), and the suspension was hydrogenated (45–55 psi) in a Parr apparatus for 3 h at 25 °C. The yellow solution was filtered through Celite, and the filtrate was evaporated under reduced pressure with the help of added portions of Et_2O. The residue was treated with 10% NH_4OH (15 mL), stirred for 30 min, and evaporated to dryness. Trituration with Me_2CO afforded a yellow solid, which was collected by filtration, washed with Me_2CO and Et_2O, and dried at 60 °C under vacuum for 18 h to afford 0.70 g (76%) of 5-deaza-10-formyl-5,6,7,8-tetrahydropteroic acid. Its ^{1}H NMR was consistent with this structure and FAB/MS showed the expected peak of m/e 344 corresponding to (M + 1). This sample was then dissolved in 1 N NaOH (25 mL) and heated to 45 °C with stirring under N_2 atmosphere for 15 h. The reaction mixture was treated with charcoal and filtered through Celite. The pH of the filtrate was adjusted to 4.5 by the dropwise addition of 2 N HCl with cooling and stirring to effect precipitation. The suspension was stirred in an ice bath for 2 h, and the yellow precipitate was isolated by filtration, washed with H_2O, Me_2CO, and Et_2O, and dried under vacuum at 60 °C overnight to afford 0.415 g of crude product. This material was purified by cellulose column chromatography using a 1.5 × 20 cm column packed in 5% NH_4HCO_3. A solution of 5% NH_4HCO_3 was used to elute the product. Appropriate fractions were pooled and acidified to pH 4.0 with 2 N HCl to produce a cream-colored precipitate which was isolated by filtration, washed with a small amount of H_2O, and dried under vacuum at 60 °C for 18 h to yield 0.375 g (58%) of pure 10; mp > 230 °C dec; UV λ_{max} 208 (ε 25.6 × 10^3), 220 (27.6 × 10^3), 280 (27.3 × 10^3); ^{1}H NMR (Me_2SO-d_6 + D_2O) δ 1.88–2.00 (m, 2), 2.42–2.50 (m, 1), 2.83–2.90 (m, 1), 2.97–3.08 (m, 2), 3.22–3.28 (m, 1), 6.58 (d, 2, 3′, 5′, J_o = 9.00 Hz) 7.64 (d, 2, 2′, 6′, J_o = 8.96 Hz); FAB/MS 316 (M + 1) and 314 (M − 1). Anal. ($C_{15}H_{17}N_5O_3 \cdot 1.5H_2O$) C, H, N.

***N*$^\delta$-(*tert*-Butyloxycarbonyl)-*N*$^\alpha$-(5-deaza-5,6,7,8-tetrahydropteroyl)-L-ornithine (12).** A sample of 10 (0.78 g, 2.28 mmol) (redried under vacuum at 65 °C over P_2O_5 for 18 h just prior to use) in $(CF_3CO)_2O$ (100 mL) was stirred in a N_2 atmosphere for 72 h. The reaction mixture was evaporated to dryness under reduced pressure with the help of added portions of EtOH. The residue was triturated with cold H_2O (10 mL), and the resultant dark cream-colored solid was isolated by filtration and dried under vacuum at 65 °C for 18 h to yield 0.69 g (73%) of the trifluoroacetyl derivative 11. Its ^{1}H and ^{19}F NMR were in accordance with the structure and positive ion and negative ion FAB/MS showed peaks of m/e at 412 and 410 corresponding to (M + 1) and (M − 1) respectively. This sample could not be purified by column chromatography due to the lability of the CF_3CO group and was used in this condition.

To a stirred solution of 11 (0.59 g, 1.44 mmol) in anhydrous DMF (40 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*$^\delta$-(*tert*-butyloxycarbonyl)-L-ornithine (0.403 g, 1.74 mmol) was added. Stirring was continued at 0 °C for 4 h to give a dark red 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 (100 mL) and stirred at ambient temperature for 1.5 h, after which the solution was clarified by adding EtOH (50 mL) and stirred for another 0.5 h. The solvent was removed under reduced pressure, and the resultant yellow residue was triturated with cold H_2O (40 mL), collected by filtration, and dried at 65 °C under vacuum overnight to obtain 0.675 g of 12, which 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. Fractions homogeneous by TLC were pooled and evaporated to dryness at reduced pressure. The residue was triturated with 3 × 15 mL of Et_2O, and the resulting solid was dried under vacuum over P_2O_5 at 65 °C for 18 h to give 0.37 g (46% yield from 11) of pure yellowish solid: mp > 230 °C dec (with preliminary softening); ^{1}H NMR (Me_2SO-d_6) δ 1.36 [s, 9, $C(CH_3)_3$], 1.35–1.44 ((5), 2, γ-CH_2), 1.59–1.81 (m, 2, β-CH_2), 1.88–2.00 (m, 2), 2.47–2.53 (m, 1), 2.83–2.93 (m, 3), 2.98–3.06 (m, 2), 3.24–3.30 (m, 1), 4.08–4.14 (m, 1, α-CH), 6.01 (br s, 2, NH_2), 6.27 (unresolved m, 1, 8- or 10-NH), 6.31 (t, 1, 8- or 10-NH, J = 5.56 Hz), 6.58 (d, 2, 3′, 5′, J_o = 8.76 Hz), 6.77 (t, 1, orn-NH, J = 5.56 Hz) 7.59 (d, 2, 2′, 6′, J_o = 8.76 Hz), 7.77 (d, 1, CONH, J = 6.88 Hz); FAB/MS m/e 530 (M + 1) and 528 (M − 1). Anal. $C_{25}H_{35}N_7O_6 \cdot 1.75H_2O$) C, H, N.

***N*$^\alpha$-(5-Deaza-5,6,7,8-tetrahydropteroyl)-L-ornithine (3).** A solution of 12 (0.10 g, 0.178 mmol) in CF_3COOH (5 mL) was stirred at ambient temperature for 2 h. The CF_3COOH was removed under reduced pressure, and the residue was triturated with EtOH (15 mL) and then 3 × 15 mL of Et_2O. After drying, the solid was dissolved in 10% NH_4OH (15 mL), and the solution was stirred at ambient temperature for 1 h and then evaporated to dryness under vacuum. The residual NH_3 was removed by treatment with 3 × 30 mL of H_2O and then 20 mL of EtOH. The residue was triturated with cold EtOH, collected by filtration, washed with EtOH and Et_2O, and dried under vacuum at 60 °C over P_2O_5 for 18 h to afford 0.055 g (58%) of yellowish solid: mp > 200 °C dec (with preliminary softening); UV λ_{max} 208 (ε 26.5 × 10^3), 220 (29.8 × 10^3), 282 (25.3 × 10^3); ^{1}H NMR (Me_2SO-d_6 + D_2O) δ 1.55–1.63 ((5), 2, γ-CH_2), 1.67–1.91 (m, 2, β-CH_2), 1.88–1.97 (m, 2), 2.43–2.50 (m, 1), 2.75–2.81 (t, 2, J = 7.64 Hz), 2.83–2.90 (m, 1), 2.99–3.02 (m, 2), 3.22–3.28 (m, 1), 4.18–4.23 (m, 1, α-CH), 6.58 (d, 2, 3′, 5′, J_o = 8.76 Hz), 7.61 (d, 2, 2′, 6′, J_o = 8.76 Hz); FAB/MS 430 (M + 1) and 428 (M − 1). Anal. ($C_{20}H_{27}N_7O_4 \cdot 0.75CF_3COOH \cdot H_2O$) C, H, N.

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