Synthesis and Biological Evaluation of 8-Deazahomofolic Acid and Its Tetrahydro Derivative

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The syntheses of 8-deazahomofolic acid and its tetrahydro derivative, potential inhibitors of thymidylate synthase (TS) and other folate related enzymes, are described. Wittig condensation of 2-acetamido-6-formyl-4-pyrimidinol with the triphenylphosphine ylide **3** derived from *N*-acetyl-4-(*p*-carbethoxyanilino)-1-chloro-2-butanone, hydrogenation of the enone intermediate **5**, introduction of a 5-amino group via diazonium coupling, and reductive ring closure yielded ethyl N^{11}-acetyl-8-deazahomopteroate (**8**). Alkaline hydrolysis gave 8-deazahomopteroic acid, which was blocked as the 11-trifluoroacetyl derivative, coupled with diethyl L-glutamate, and the blocking groups saponified to afford 8-deazahomofolic acid (**12**). Hydrogenation of the glutamate diester intermediate and subsequent saponification yielded the tetrahydro-8-deazahomofolate (**14**). Growth inhibition of *Streptococcus faecium*, *Lactobacillus casei*, and L1210 cells in culture by the target compounds was modest. They were also weak inhibitors of thymidylate synthase, dihydrofolate reductase, glycinamide-ribonucleotide transformylase, and aminoimidazolecarboxamide ribonucleotide transformylase. In contrast, 8-deazafolate showed moderate inhibition of aminoimidazolecarboxamide ribonucleotide transformylase, suggesting that inhibition of this enzyme may be related to its cytotoxic action. Tetrahydro-8-deazahomofolate showed low substrate activity with thymidylate synthase.

The synthesis and antifolate activity of homofolic acid and its tetrahydro derivative were reported from our laboratories in 1964.[1,2] While the tetrahydro compound was found to be an inhibitor of the growth of folate dependent bacteria as well as an inhibitor of TS from *Escherichia coli*[1] and *Lactobacillus casei*,[3] growth of Sarcoma 180 cells was inhibited by a block in purine biosynthesis at the level of glycinamide ribonucleotide transformylase.[4] Although tetrahydrohomofolic acid showed promising antitumor activity,[5] efforts to develop the compound as a clinically useful drug were frustrated by its lack of oxidative stability in pharmaceutical formulations. The *N*-5-methyl analogue possessed similar antitumor activity and was found to be suitable for clinical investigations.[6] This compound has demonstrated antitumor activity in several experimental systems.

The 8-deaza series of homofolates offered the potential of being inhibitors of folate enzymes. If found to have useful antitumor activity, the tetrahydro derivatives would be expected to possess the required oxidative stability[7] for clinical usage. We describe herein the synthesis of 8-deazahomofolic acid (**12**) and its tetrahydro derivative **14** and their biological properties.

Chemistry

The synthesis of 8-deazahomofolic acid (**12**) was accomplished via the route outlined in Scheme I. The Wittig condensation of 2-acetamido-4-hydroxy-6-formylpyrimidine[7] (**4**) and the ylide (**3**)[8] derived from the triphenylphosphonium salt (**2**) of *N*-acetyl-4-(*p*-carbethoxyanilino)-1-chloro-2-butanone[2] (**1**) was the initial step in the process. The reaction to the enone (**5**) proceeded smoothly at room temperature in DMF, but required conversion of **4** in situ to the trimethylsilyl derivative. This was achieved by pretreatment of **4** with 1 equiv of bis(trimethylsilyl)-trifluoroacetamide before addition of the ylide **3**. The enone **5** was obtained in a 56% yield following chromatography on silica gel. Without trimethylsilylation, the condensation took place in only 10% yield and required temperatures of 100–120 °C to effect reaction, thus reflecting the need for solubilization of the aldehyde **4** to obtain a facile reaction. In an earlier paper[8] we indicated the lack of success with a keto phosphonate reagent derived from **1**.

The most suitable method for introduction of a nitrogen functionality at C-5 of the pyrimidine ring was coupling with a diazonium reagent. It was therefore necessary to saturate the olefinic group in **5** and to remove the 2-acetamido blocking group in order to provide sufficient activation at C-5. Hydrogenation of **5** over a rhodium catalyst in DMF readily afforded the dihydro acetamido ketone **6**. All attempts to hydrolyze the 2-acetyl group under mild alkaline conditions (including 0.1 N NH_4OH) resulted in fragmentation of the β-amido ketone side chain. However, selective deacetylation was easily accomplished by brief treatment of **6** with ethanolic HCl at reflux. After evaporation, the residue was suspended in aqueous DMF at 0–5 °C, and the mixture was adjusted to pH 8–9 by addition of Na_2CO_3. Benzenediazonium chloride was then added dropwise at 0 °C with occasional addition of 2 N NaOH to maintain the pH at 8–9. This procedure afforded the phenylazo ketone **7** as a yellow solid in 75% yield.

Compound **7** was hydrogenated over Pd black to cleave the phenylazo function and cause spontaneous ring closure to the ethyl N^{11}-acetyl-8-deazahomopteroate (**8**). The fully aromatic compound was the major product obtained as shown by TLC and probably was formed via air oxidation of the expected 7,8-dihydro intermediate during workup. Evidence for this mechanism is provided by the fact that

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(8) DeGraw, J.; Brown, V.; Kisliuk, R.; Gaumont, Y.; Sirotnak, F. *Chemistry and Biology of Pteridines*; Kisliuk, R. L., Brown, G. M., Eds.; Elsevier: North Holland, 1979; p 229. This is a preliminary communication on the synthetic process.

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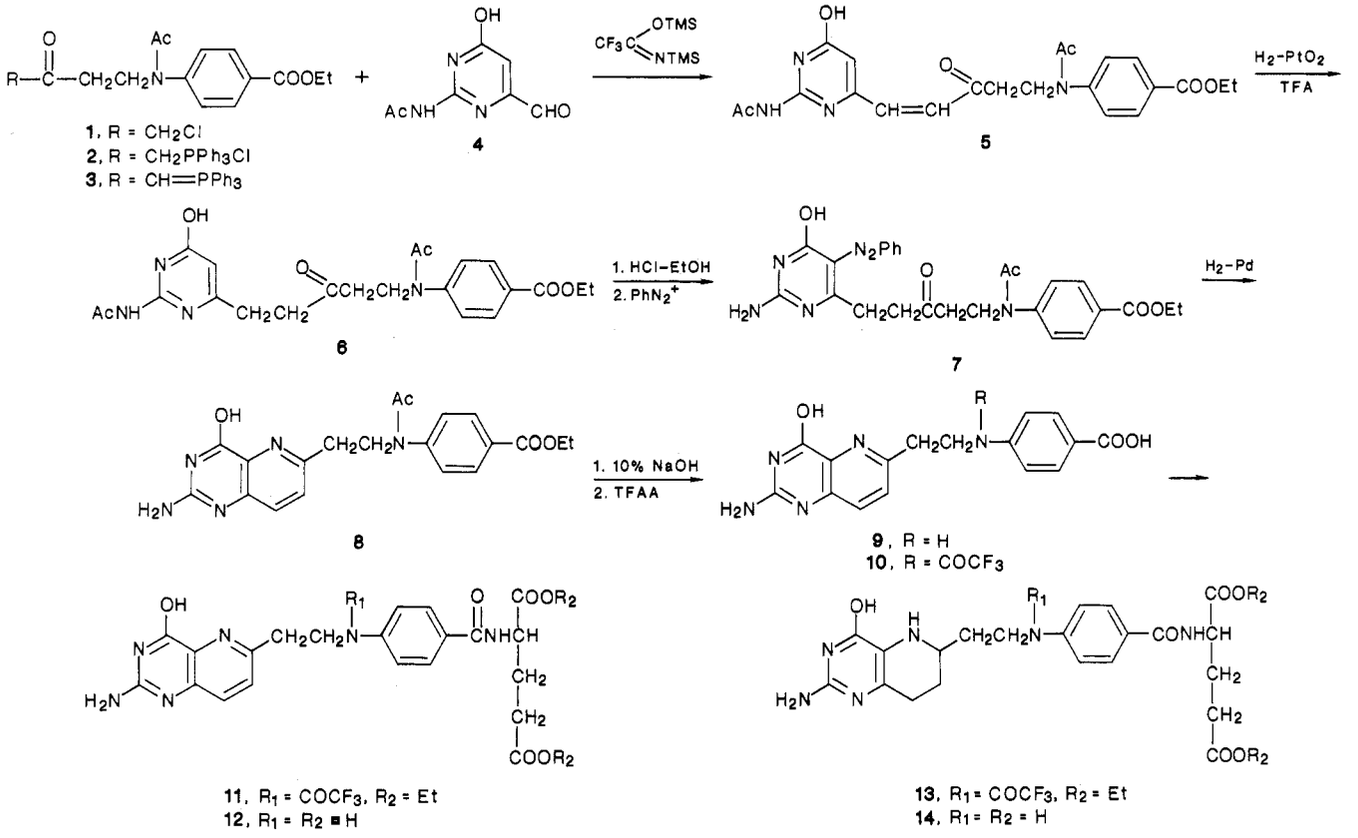
Scheme I



8 was isolated in 78% yield after chromatography. If the dihydro compound had undergone a redox disproportionation, an approximate 50:50 mixture of 8 and a tetrahydro form should have resulted. The tetrahydro compounds in this series are themselves stable to air or other oxidative media.

The blocked pteroate 8 was heated at 90–100 °C in 10% NaOH to effect hydrolysis to 8-deazahomopteroic acid (9), which crystallized as the disodium salt when the hydrolytic medium was refrigerated. The 11-NH group was blocked as the trifluoroacetyl derivative 10 by treatment with trifluoroacetic anhydride at reflux. Coupling of 10 with diethyl L-glutamate was conducted with activation of the benzoic carboxyl function as the mixed anhydride with isobutyl chloroformate. The resulting intermediate diester 11 was obtained as a noncrystalline solid following chromatographic purification. The material was saponified with 0.5 N NaOH at room temperature to yield 8-deazahomofolic acid (12).

The 8-deazatetrahydrohomofolate (14) was obtained via hydrogenation of the trifluoroacetyl diester 11 over PtO_2 in EtOH containing CF_3COOH. The intermediate tetrahydro diester 13 was then saponified with 0.5 N NaOH to give the diacid 14.

Biological Results

Compounds 12 and 14 were initially evaluated as inhibitors of growth in the folate dependent bacteria *S. faecium* and *L. casei* and their methotrexate (MTX) resistant strains. As shown in Table I, both compounds were only modest inhibitors for growth of the sensitive strains of the organisms when compared with the standard, MTX. Compounds 12 and 14 were almost equipotent against the sensitive strains, which is in sharp contrast to homofolic acid and tetrahydrohomofolic acid, the latter being much more inhibitory[1] (Table I). The antibacterial activity of tetrahydrohomofolic acid is due to the unnatural diastereoisomer at carbon-6.[9] Diastereoisomers of 14 were not resolved in the present study. 8-Deazafolic[10] acid was actually a considerably stronger inhibitor than the homo analogue, being 70 times more effective for *S. faecium* and 14 times more effective for *L. casei*. Compound 12 showed some activity against the MTX-resistant strains, being at least 3000 times more effective than MTX against MTX-resistant *L. casei*. However, the tetrahydro analogue 14 was inactive against MTX-resistant strains.

Bicyclic pyrimidine analogues of folic acid containing the 2-amino-4-hydroxy substitution pattern are usually poor inhibitors of dihydrofolate reductase (DHFR). Thus, it was not surprising that the compounds failed to inhibit the DHFR from *L. casei* (Table II). However, neither

Table I. Bacterial Growth Inhibition

	IC_{50}, nM[a]			
	S. faecium		*L. casei*	
compound	ATCC 8043	MTX resist.	ATCC 7649	MTX resist.
12[b]	21	1100	19	330
14[b]	15	>10^4	35	>10^4
homofolic acid[c,e]	2200		220	
tetrahydrohomofolic acid[c,e]	1.5		13	
8-deazafolic acid[d,e]	0.3	294	1.4	2036
tetrahydro-8-deazafolic acid[d,e]	1.6	1035	2.7	>4000
MTX	0.8	8000	0.05	>10^6

[a] Folate concentration was 1 ng/mL (see: DeGraw, J. I.; Kisliuk, R. L.; Gaumont, Y.; Baugh, C. M.; Nair, M. G. *J. Med. Chem.* **1974**, *17*, 552 for methods). [b] Did not support growth at concentrations up to 400 nM. [c] Data from ref 1. [d] Data from ref 10. [e] Data are historical and not normalized to MTX standards, intended only for rough comparisons.

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Table II. Enzyme Inhibition[a]

compound	IC_{50}, M			
	DHFR	TS	GAR[d] transformylase	AICAR[e] transformylase
12	2.2×10^{-4}	1.6×10^{-4}	$>4 \times 10^{-5}$	$>4 \times 10^{-5}$
14	2.3×10^{-4}	3.4×10^{-4}	$>2 \times 10^{-5}$	$>2 \times 10^{-5}$
tetrahydrohomofolic acid		2.0×10^{-6}[b]		
8-deazafolic acid	1.2×10^{-5}[c]	1.4×10^{-5}[c]	$>2 \times 10^{-5}$	6.4×10^{-6}
tetrahydro-8-deazafolic acid	5.5×10^{-5}[c]	7.5×10^{-5}[c]	$>2 \times 10^{-5}$	$>2 \times 10^{-5}$
MTX	1.6×10^{-8}	8.6×10^{-5}	$>2 \times 10^{-5}$	$>2 \times 10^{-5}$

[a] Enzymes isolated from *L. casei*. [b] Data from ref 1; enzyme derived from *E. coli*. [c] Data from ref 10. [d] Assayed as described (Smith, G. K.; Benkovic, P. A.; Benkovic, S. J. *Biochemistry* **1981**, *20*, 4034) except at 30 °C rather than 37 °C and Tris·HCl buffer, 50 mM, pH 7.5, rather than maleate buffer. These changes were suggested by Dr. John Mangum, Brigham Young University. [e] Assayed as described by Baggott, J. E.; Krumdieck, C. L. *Biochemistry* **1979**, *18*, 1036.

Table III. Growth Inhibition of L1210 Leukemia Cells

compound	growth inhibn: IC_{50}, M
12	8.75×10^{-5}
14	3.57×10^{-5}
8-deazafolic acid[a]	1.33×10^{-6}
MTX	3.90×10^{-9}

[a] Data from ref 7.

compound was very inhibitory toward thymidylate synthase, which is surprising in view of the activity of tetrahydrohomofolate toward this enzyme.[1] Furthermore, both **12** and **14** were poor inhibitors of glycinamide-ribonucleotide (GAR) transformylase and aminoimidazolecarboxamide ribonucleotide (AICAR) transformylase derived from *L. casei*. These results suggest that inhibition of these enzymes is not the basis of the modest growth inhibitory properties of **12** and **14**. It is noteworthy, however, that 8-deazafolate does show moderate inhibition of AICAR transformylase. This could be a significant factor in the bacterial growth inhibition caused by this compound as well as its antitumor activity in the L1210 system.[8] Tetrahydro-8-deazafolate, prepared as described,[10] did not inhibit GAR transformylase or AICAR transformylase (Table II).

Tetrahydro-8-deazahomofolate (**14**) shows low substrate activity for *L. casei* TS, which implies that N-8 is not required for thymidylate synthesis. The rate is 2% of that obtained with the standard tetrahydrofolate substrate. As the compound **14** used in this study is a mixture of diastereomers (racemic at C-6), only one-half would be expected to be a substrate for TS. This was found to be the case.

The target folate analogues were also tested for their ability to inhibit the growth of L1210 murine leukemia cells in culture as shown in Table III. Both compounds were ineffective as growth inhibitors, being about 4 logs less potent than MTX against these tumor cells.

Experimental Section

Microanalyses were performed by Galbraith Laboratories, Knoxville, TN. Mass spectra were obtained by using a LKB 9000 GC–MS instrument. HPLC analyses utilized C_{18} Novapak columns and elution with 25:75 CH_3OH–0.1 M NaH_2PO_4 at pH 6.7.

Thin-layer chromatographic analyses (TLC) were conducted on silica gel plates with CH_3OH–$CHCl_3$ (1:9) as eluent. The solvents dimethylformamide (DMF) and tetrahydrofuran (THF) were routinely dried over 4A molecular sieves before use. NMR spectra were taken on a Varian EM360A or JEOL FXL-90 spectrometer. UV spectra were run on a Perkin-Elmer 552 spectrophotometer.

1-(2′-Acetamido-4′-hydroxy-6′-pyrimidinyl)-5-(*N*-acetyl-*p*-carbethoxyanilino)pent-1-en-3-one (5). The aldehyde[7] **4** (9.43 g, 52 mmol) was suspended in 170 mL of DMF and treated with 13.8 mL (52 mmol) of bis(trimethylsilyl)trifluoroacetamide. The mixture became a clear solution after 1 h of stirring, and then 27.8 g (52 mmol) of the dry ylide **3**[8] in 170 mL of DMF was added to yield a red solution. After 65 h, the solvent was removed in vacuo (1 mm), and the residue was partitioned between 500 mL of $CHCl_3$ and 500 mL of H_2O. The $CHCl_3$ extract was filtered through Celite, dried over $MgSO_4$, and evaporated to leave 38 g of a dark gum. The gum was chromatographed on 250 g of Baker Flash silica gel with elution by EtOAc and EtOAc–Me_2CO (3:1). The product, 12.7 g (56%), was obtained as a yellow solid. An analytical sample, mp 158–159 °C, was obtained from benzene: UV_{max} (pH 13) 260, 326 nm; NMR ($CDCl_3$) δ 1.37 (3 H, t, CH_3), 1.90 (3 H, s, CH_3CON), 2.25 (3 H, s, CH_3CONH), 3.10 (2 H, m, $CH_2C{=}O$), 4.00 (2 H, m, CH_2NAc), 4.40 (2 H, q, OCH_2), 6.42 (1 H, s, 5′-H), 7.05 (2 H, d, CH=CH), 7.30 (2 H, d, 3,5-Ar H), 8.20 (2 H, d, 2,6-Ar H). Anal. Calcd for $C_{22}H_{24}N_4O_6$: C, H, N.

1-(2′-Acetamido-4′-hydroxy-6′-pyrimidinyl)-5-(*N*-acetyl-*p*-carbethoxyanilino)pentan-3-one (6). A mixture of 12.6 g of the enone **5**, 600 mg of 5% Rh/C and 300 mL of DMF was stirred under an atmosphere of hydrogen for 80 h. A second charge of catalyst was added, and hydrogenation was continued for another 45 h when TLC (MeOH–$CHCl_3$, 1:19) showed absence of starting material. The catalyst was removed by filtration, and the solvent was removed at 40 °C under 1 mm of pressure to leave an amber syrup. The crude material was chromtographed on 100 g of silica gel with preelution by $CHCl_3$, followed by elution of product (6.1 g, 48%) with MeOH–$CHCl_3$, 1:9. An analytical sample, mp 131–133 °C, was crystallized from EtOH: NMR ($CDCl_3$) δ 1.45 (3 H, t, CH_3), 1.95 (3 H, s, CH_3CON), 2.20 (3 H, s, CH_3CONH), 2.65 (2 H, m, CH_2CO), 2.80 (2 H, br s, CH_2CO), 3.00 (2 H, m, 6′-CH_2), 4.00 (2 H, m, CH_2NAc), 6.03 (1 H, s, 5′-H), 7.40 (2 H, d, 3,5-Ar H), 8.20 (2 H, d, 2,6-Ar H), 11.50 (NH). Anal. Calcd for $C_{22}N_{26}N_4O_6$: C, H, N.

1-[2′-Amino-4′-hydroxy-5′-(phenylazo)-6′-pyrimidinyl]-5-(*N*-acetyl-*p*-carbethoxyanilino)pentan-3-one (7). The ketone **6** (6.1 g, 13.8 mmol) in 100 mL of EtOH was treated with ethanolic HCl prepared from acetyl chloride (2.94 mL, 41 mmol) in 100 mL of EtOH. The mixture was stirred under reflux for 45 min and evaporated to leave a white foam that was dissolved in 280 mL of 50% DMF. The solution was cooled to 0 °C and treated with 2.9 g (27 mmol) of Na_2CO_3 followed by a benzene diazonium chloride solution prepared from aniline (2.18 g, 24 mmol), $NaNO_2$ (1.62 g, 35 mmol), and 7.9 mL of 6 N HCl. The pH was maintained around 8 by the periodic addition of 2 N NaOH. After the addition was completed, the mixture was stirred for another 30 min at 0 °C, during which time a yellow precipitate deposited. The solid was collected and washed with H_2O. Trituration with Et_2O afforded the product, 5.7 g (75%), as a yellow powder: UV_{max} (pH 13) 363 nm; NMR ($CDCl_3$) δ 1.35 (3 H, t, CH_3), 1.85 (3 H, s, CH_3CON), 2.80 (4 H, br m, CH_2COCH_2), 3.40 (2 H, m, 6′-CH_2), 3.95 (2 H, t, CH_2NAc), 4.35 (4 H, q, OCH_2), 7.25 (2 H, d, 3,5-Ar H), 7.40 (7 H, m, NH_2, C_6H_5), 8.10 (2 H, d, 2,6-Ar H).

Ethyl N^{11}-Acetyl-8-deazahomopteroate (8). The phenylazo compound **7** (5.7 g, 11.3 mmol) was hydrogenated at 1 atm over 280 mg of Pd black in 200 mL of DMF for 30 h. The catalyst was removed by filtration, and air was bubbled through the filtrate for 15 h. The solvent was removed in vacuo (40 °C, 1 mm) to yield 5.5 g of an orange gum. The crude material was chromatographed on 100 g of Baker Flash silica gel with preelution by $CHCl_3$ followed by $CHCl_3$–MeOH (9:1) to afford 3.52 g (78%) of

the product as a yellow foam. An analytical sample, mp 155–158 °C, was obtained from EtOH: UV_{max} (pH 13) 238 (ε 33 300), 265 sh (ε 13 600), 332 (ε 5100); NMR ($CDCl_3$) δ 1.35 (3 H, t, CH_3), 1.83 (3 H, s, CH_3CON), 3.05 (2 H, br s, C_9-CH_2), 3.95 (2 H, br s, C_{10}-CH_2), 4.35 (2 H, q, OCH_2), 7.25 (4 H, m, $C_{7,8}$-H, 3′,5′-Ar H), 7.50 (2 H, m, NH_2), 8.10 (2 H, d, 2′,6′-Ar H). Anal. Calcd for $C_{20}H_{21}N_5O_4 \cdot {}^3/_4H_2O$: C, H, N.

8-Deazahomopteroic Acid (9). The acetamido ester 8 (3.5 g, 8.9 mmol) was dissolved in 50 mL of 10% NaOH, and the solution was heated at 90 °C for 2.5 h. The solution was refrigerated for 15 h, and the precipitated sodium salt was collected by filtration. The solid was redissolved in 30 mL of H_2O, and the pH was adjusted to 4–5 with 1 N HCl to precipitate the product. The precipitate was collected, washed with H_2O, and dried to afford 1.32 g (46%): UV_{max} (pH 13) 240, 278, 330 (sh) nm; NMR (Me_2SO-d_6) δ 3.00 (2 H, m, C_9-CH_2), 6.53 (4 H, m, 3′,5′-Ar H, $C_{7,8}$-H), 7.49 (2 H, s, NH_2), 7.65 (2 H, d, 2′,6′-Ar H). Anal. Calcd for $C_{16}H_{15}N_5O_3 \cdot {}^1/_2H_2O$: C, H, N.

8-Deazahomofolic Acid (12). 8-Deazahomopteroic acid (9; 1.32 g, 4.1 mmol) was stirred at reflux with 25 mL of $(CF_3CO)_2O$ for 3 h. The solvent was removed in vacuo and the residue was thoroughly digested with 30 mL of ice water to afford a white solid, 1.70 g (100%). The infrared spectrum showed strong bands for CF_3 at 8.1–8.7 μ, indicative of the N^{11}-trifluoroacetyl intermediate **10**. The dried material was dissolved in 35 mL of DMF under argon. Triethylamine, 0.92 mL (6.6 mmol) was added, and the mixture was stirred for 15 min followed by the addition of isobutyl chloroformate (0.86 mL, 6.6 mmol). After the mixture was stirred for 1 h, 0.92 mL of Et_3N and 1.53 g (6.4 mmol) of diethyl L-glutamate hydrochloride were added sequentially. The mixture was stirred for 1 h at room temperature, and the entire addition sequence was repeated in the same manner and quantities. The resulting mixture was stirred for 18 h, and the solvent was removed in vacuo (40 °C, 0.5 mm). The residue was partitioned between 80 mL of $CHCl_3$ and 30 mL of H_2O. The $CHCl_3$ was separated, dried over $MgSO_4$, and evaporated to leave 3.2 g of an amber oil. The oil was chromatographed on 30 g of Baker Flash silica gel with preelution by $CHCl_3$ followed by $CHCl_3$–MeOH (19:1) to yield a yellow semisolid, 903 mg (34%) of the diester **11**: MS, *m/e* 606; UV_{max} (EtOH) 270, 330 nm.

The diester, 150 mg, was dissolved in 10 mL of 0.5 N NaOH, and the solution was kept at ambient temperature for 4 h. The pH was adjusted to 5 by addition of 2 N HCl, and the resulting precipitate was collected, washed with H_2O, and dried to give the product as a pale yellow solid, 77 mg (68%): UV_{max} (pH 13) 234 (ε 30 800), 278 (ε 25 700), 298 (ε 23 300), 340 (ε 7300) nm; NMR (Me_2SO-d_6) δ 2.05 (2 H, m, CH_2 of glutamate), 2.30 (2 H, m, CH_2COOH), 3.10 (2 H, m, C_9-CH_2), 4.35 (1 H, m, C*H*NH), 6.40 (2 H, m, $C_{7,8}$-H), 6.60 (2 H, d, 3′,5′-Ar H), 7.50 (2 H, s, NH_2), 7.67 (2 H, d, 2′,6′-Ar H), 8.10 (1 H, d, CONH). Anal. Calcd for $C_{21}H_{22}N_6O_6 \cdot 2H_2O$: C, H, N.

5,6,7,8-Tetrahydro-8-deazahomofolic Acid (14). A mixture of the diester intermediate **11** (200 mg, 0.33 mmol), CF_3CO_2H (0.026 mL, 0.35 mmol), 50 mg of PtO_2, and 10 mL of EtOH was stirred under 1 atm of H_2 for 20 h. TLC showed disappearance of the starting material. After removal of the catalyst, the filtrate was evaporated to leave 225 mg (100%) of a colorless gum: UV_{max} (pH 13) 250, 292 nm. A solution of the reduced ester **13** in 10 mL of 0.5 N NaOH was kept under an argon atmosphere for 3 h. HPLC analysis showed a single peak to be present. The solution was adjusted to pH 6 by addition of 2 N HCl. The precipitate was collected, washed with H_2O, and dried to yield the product as a white solid, 68 mg (54%): UV_{max} (pH 13) 250 nm (ε 12 700), 293 (ε 25 200); MS, *m/e* 458; NMR (Me_2SO-d_6) δ 1.80 (4 H, m, C_7 and C_9-CH_2), 2.05 (2 H, m, CH_2 of glutamate), 2.30 (2 H, m, CH_2COOH), 3.15 (5 H, m, C_{10}-CH_2, C_8-CH_2, C_6-H), 4.35 (1 H, m, C*H*, NH), 5.75 (2 H, s, NH_2), 6.20 (1 H, br s, NH), 6.55 (2 H, d, 3′,5′-Ar H), 7.65 (2 H, d, 2′,6′-Ar H), 8.08 (1 H, d, CONH). Anal. Calcd for $C_{21}H_{26}N_6O_6 \cdot 2{}^1/_4H_2O$: C, H, N.

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Registry No. **3**, 111113-65-6; **4**, 60656-63-5; **5**, 111113-66-7; **6**, 111113-67-8; **7**, 111113-68-9; **8**, 111113-69-0; **9**, 111113-70-3; **10**, 111113-71-4; **11**, 111113-72-5; **12**, 111113-73-6; **13** (diastereomer 1), 111113-74-7; **13** (diastereomer 2), 111113-76-9; **14** (diastereomer 1), 111113-75-8; **14** (diastereomer 2), 111113-77-0; H-Glu(OEt)-OEt·HCl, 1118-89-4; $PhNH_2$, 62-53-3; thymidylate synthase, 9031-61-2; dihydrofolate reductase, 9002-03-3; glycinamide–ribonucleotide transformylase, 9032-02-4; aminoimidazolecarboxamide ribonucleotide transformylase, 9032-03-5; 1-(2′-amino-4′-hydroxy-6′-pyrimidinyl)-5-(*N*-acetyl-*p*-carbethoxyanilino)pentan-3-one, 111113-78-1.