

**FUSED PYRIMIDINES. PART 5. PYRIMIDO[4,5-*d*]PYRIMIDINE
ANALOGUES OF FOLIC ACID**

Thomas J. Delia*, Markus Baumann, and Amy Bunker

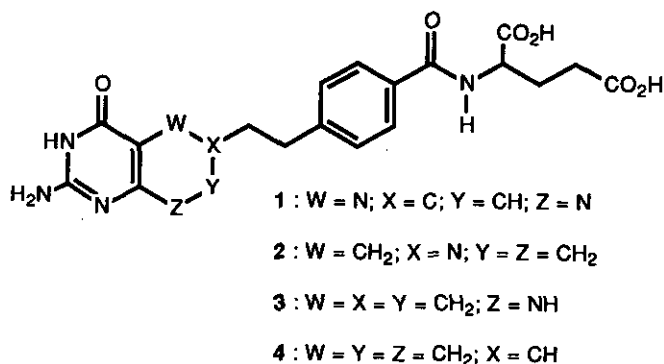
Malcolm H. Filson Laboratories, Department of Chemistry,
Central Michigan University, Mt. Pleasant, MI 48859, U.S.A.

Abstract- *Pyrimido[4,5-*d*]pyrimidine analogues of folic acid have been prepared and tested for antitumor activity. Using Mannich reaction conditions, 2,4,6-triaminopyrimidine (12) or 2,4-diamino-6-oxopyrimidine (13) was treated with formaldehyde and either diethyl *N*-[4-(2-aminoethyl)benzoyl]-L-glutamate (18) or diethyl *N*-[4-(3-aminopropyl)benzoyl]-L-glutamate (24). The corresponding diester products (19, 20 and 25, 26) were converted to the diacids (5, 6 and 7, 8) by treatment with aqueous ethanolic sodium hydroxide. Compounds (5, 6, and 7) were screened against CCRF-CEM leukemic cells and found to be significantly less active than DDATHF, one of the most active compounds for this system.*

The synthesis of new structures that may have biological activity as antitumor agents continues to be an important goal. The folate pathway has proven to be a fruitful target for many of these

synthetic attempts. Many alterations to the folic acid structure(1) have been accomplished that have affected nearly all parts of the molecule.^{1,2}

Because of a continuing interest in fused pyrimidine ring systems, our attention was drawn to the pyrazine portion of the folic acid structure. Much of the recent interest in this ring has focused on



removing one or both of the ring nitrogen atoms and replacing them with carbon atoms. In the most recent example³ removal of one nitrogen and relocation of the other has led to the inactive pyridopyrimidine analog(2). The partially reduced pyridopyrimidine derivative (3)⁴, as well as the fully aromatic compound have also been described.⁵ Both nitrogen atoms of the pyrazine ring have been eliminated to give the tetrahydroquinazoline analog (4)⁶ and the fully aromatic quinazoline.⁷ Two other related examples involve the replacement of N-8 by the oxygen atom in the tetrahydrofolate structure⁸ and the addition of a nitrogen atom at position 7 of folic acid.⁹

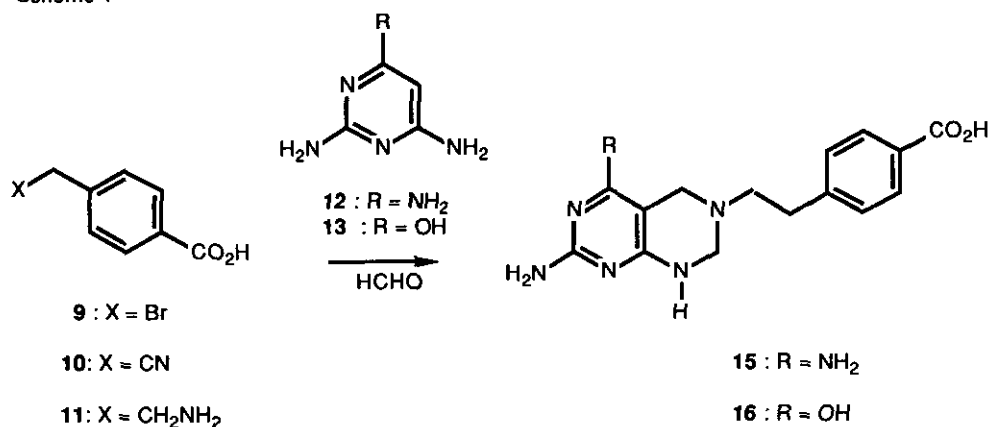
Until now no examples of simple rearrangement of the nitrogen atoms of the pyrazine ring to a different configuration have been reported. Pyrimidopyrimidines and pyrimidopyrazines represent classes of heterocyclic rings that remain to be explored. This paper describes the synthesis of some members of a new class of antifolate compounds, pyrimido[4,5-*d*]pyrimidine derivatives(5 - 8).¹⁰

We had previously prepared a number of 6-substituted pyrimido[4,5-*d*]pyrimidines through a Mannich reaction of 2,4,6-triaminopyrimidine and a series of halogenated benzyl- and phenethylamines.¹¹

This method seemed suitable to us for the incorporation of a more complex amine, and so we began an examination of the possibility of constructing pyrimido[4,5-d]pyrimidines with folic acid side chains. We chose to focus on those compounds with either two or three methylene groups as spacers between the pyrimido[4,5-d]pyrimidine and phenyl rings. Based on our previous experience and the known structure-activity relationships compounds (5 - 8) were selected as appropriate target molecules.

Two approaches were contemplated for the synthesis of the target molecules. One could incorporate a benzoic acid derivative *via* the Mannich reaction and subsequently couple the glutamic acid moiety or, alternatively, couple the benzoic acid derivative to the glutamate prior to performing the Mannich reaction. Initially we wanted to test the methodology by condensing 4-(2-aminoethyl)benzoic acid (11) and formaldehyde with both 2,4,6-triaminopyrimidine (12) and 2,4-diamino-6-oxopyrimidine (13) (Scheme 1). It was necessary to find a suitable synthesis of the amine (11). The original preparation of this amine¹² proved to be tedious. An alternative approach involved the conversion of 4-toluic acid first to the benzyl bromide (9)¹³ and then by treatment with sodium

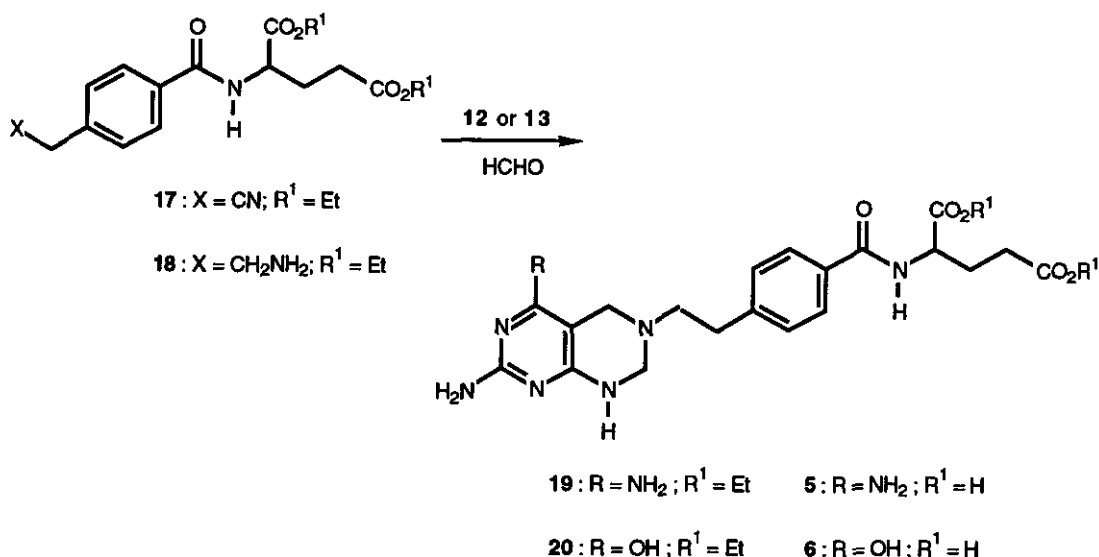
Scheme 1



cyanide to the cyano compound (10).¹⁴ Considerable difficulty was encountered in the reduction of 10 to the corresponding amine (11), presumably due to unwanted further reactions of the amine. Taylor and Hamby¹⁵ independently solved this problem by using concentrated sulfuric acid in the reduction medium with hydrogen in the presence of Pd-C. Treatment of amine (11) with either 12 or 13 and aqueous formaldehyde in aqueous methanol led to the formation of the corresponding acids (15) or (16) in approximately 50% yield, respectively. It had been our intention to couple these acids with diethyl glutamate, but we found the acid to be quite insoluble in most solvents that would be compatible with the coupling reaction.

Even though we were concerned that incorporating the glutamate portion of the molecule into the amine prior to condensation might lead to serious problems, we directed all of our efforts at this second approach (Scheme 2). The treatment of 10 with thionyl chloride, followed by diethyl L-glutamate, provided the desired compound (17). Again, the method of Taylor and Hamby resulted

Scheme 2

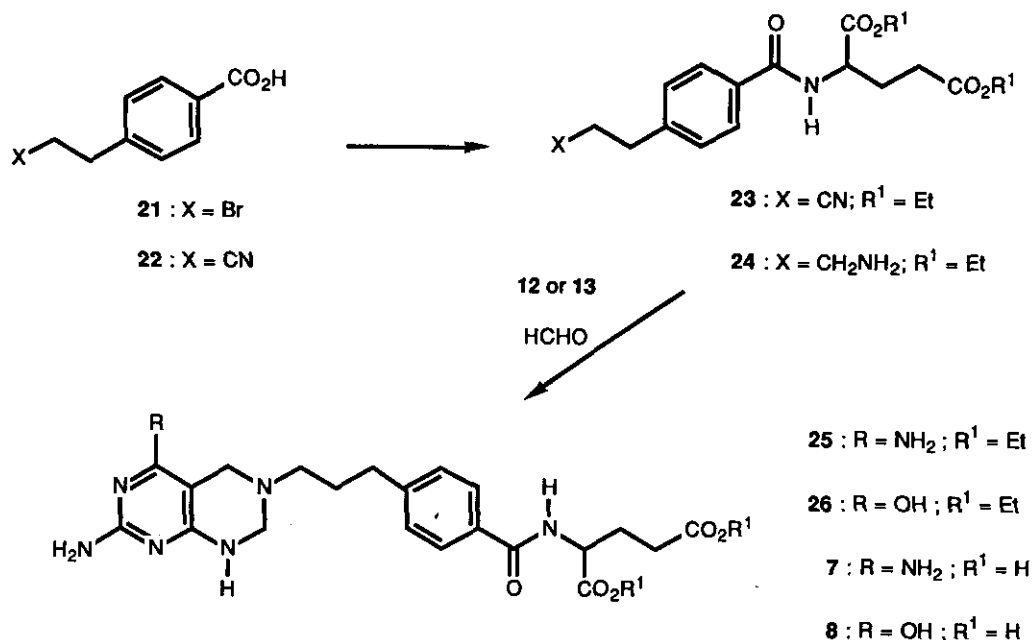


in the corresponding amine (**18**) in good yield. This amine with aqueous formaldehyde and either **12** or **13** gave the esters (**19**) and (**20**) in poor yields, 16% and 23%, respectively. The reaction has not been optimized and it has been observed that the products are formed rapidly with an increase in the side reactions the longer the reaction was allowed to proceed. The difficulty here may lie in the additional sites of active hydrogen at which the Mannich reaction could occur, especially on the glutamate portion of the molecule. This was suggested by the observation of a number of minor products shown by tlc. Nevertheless, the simplicity of the reaction made this a worthwhile process. Considerable effort was made to obtain esters (**19**) and (**20**) in very pure state before continuing because of the expectation that the corresponding acids would be extremely insoluble. Despite repeated efforts to obtain **19** in analytically pure form no satisfactory elemental analysis was achieved. This is not uncommon for diesters of other related compounds. Nevertheless, suitable spectroscopic data were obtained which supported the assigned structure.

Hydrolysis of the diesters (**19**, **20**) occurred under mild conditions by stirring in aqueous ethanolic sodium hydroxide at room temperature overnight. Purification of the diacids was accomplished by repeated precipitation and washing in aqueous solution. The yields of the diacids were 43% and 45%, respectively for **5** and **6**.

The preparation of the compounds with three methylene groups as spacers was accomplished in a similar manner (Scheme 3). The known 4-(2-bromoethyl)benzoic acid (**21**)¹⁶ was converted into the cyano compound (**22**) in 71% yield. Treatment of **22** with thionyl chloride followed by diethyl L-glutamate provided the diester (**23**) in 66% yield. Hydrogenation over Pd-C in ethanol containing concentrated sulfuric acid afforded the requisite amine (**24**) in 59% yield. This amine, with aqueous formaldehyde and either **12** or **13**, resulted in the corresponding esters (**25**) and (**26**) in 37% and 34%, respectively. Hydrolysis of these esters was accomplished in the same manner as before and

Scheme 3



gave the diacids (7) and (8) in 43% and 36%, respectively.

The compounds prepared in this study were examined for growth inhibition of CCRF-CEM leukemic cells in culture. All three pyrimido[4,5-*d*]pyrimidine derivatives tested are poor inhibitors of CCRF-CEM leukemic cells.

EXPERIMENTAL SECTION

Melting points are uncorrected and were determined in open capillary tubes using a Thomas-Hoover apparatus. Purity of products was determined by hplc and nmr. Mass spectra were measured on a Hewlett Packard 5995A GC/MS instrument, using a direct insertion probe. The ¹H nmr spectra were recorded either on a Bruker NR80 or a General Electric QE300 instrument in Me₂SO-*d*₆ with

TMS as the internal standard. All values are reported in ppm relative to TMS. Relative integrals of peak areas are in agreement with the assigned structures. Elemental analyses were performed by Galbraith Laboratories, Knoxville, TN. Column chromatographic purifications were done with silica gel (Mallinckrodt, 60 - 200 mesh for gravity and Fisher Scientific, 230 - 425 mesh for flash chromatography).

4-(2-Cyanoethyl)benzoic Acid. 22. A solution of NaCN (30.3 g, 0.60 mol) in H₂O (85 ml) was added during 10 min to a stirred suspension of **21**¹⁶ (57.9 g, 0.25 mol) in MeOH (300 ml). The mixture was refluxed for 7 h and, after cooling, acidified with 3 N HCl to cause precipitation of **22**. The collected solid was dried and recrystallized from CHCl₃ to give 31.5 g (71%) of an off-white product. Further recrystallization from benzene/hexane gave an analytical sample as small colorless needles: mp 185-7°C; ms (EI), m/z 175, M⁺; ¹H nmr (300 MHz) 2.9 and 3.0 (2t, CH₂CH₂CN), 7.4 and 7.9 (AB spin system, C₆H₄). Anal. Calcd for C₁₀H₉NO₂: C, 68.56; H, 5.18; N, 8.00. Found: C, 68.22; H, 5.11; N, 7.87.

Diethyl N-[4-(2-Cyanoethyl)benzoyl]-L-glutamate. 23. A mixture of **22** (8.9 g, 0.05 mol), excess thionyl chloride (10 ml), DMF (0.1 ml), and benzene (150 ml) was stirred and heated to reflux for 2 h. The solvent was removed, and the residue coevaporated with benzene (2 X 50 ml). Then CH₂Cl₂ (100 ml) and diethyl L-glutamate hydrochloride (12.2 g, 0.05 mol) were added to the residue. The resulting solution was cooled in an ice bath and triethylamine (10.1 g, 0.10 mol) in CH₂Cl₂ (50 ml) was added dropwise during one h. After addition was complete, the mixture was stirred at room temperature overnight, and CH₂Cl₂ (200 ml) was added to dissolve suspended solids. The organic solution was washed with 0.5 N HCl (2X100 ml), with a saturated solution of NaHCO₃ (2X100 ml),

dried over MgSO_4 , filtered and evaporated to dryness. The crude product was recrystallized from EtOAc/hexane to give **23** in 66% yield (12.2 g) as a colorless solid; mp 92-94°C; ms (EI), m/z 360, M^+ ; ^1H nmr (300 MHz) 1.1 and 1.2 (2t, 2 CH_2CH_3), 2.0 and 2.1 (2m, CHCH_2CH_2 , nonequivalent), 2.4 (t, CHCH_2CH_2), 2.8 and 2.9 (2t, $\text{CH}_2\text{CH}_2\text{CN}$), 4.0 and 4.1 (2q, 2 CH_2CH_3), 4.4 (m, CHCH_2CH_2), 7.4 and 7.8 (AB spin system, C_6H_4), 8.7 (d, CONH). Anal. Calcd for $\text{C}_{19}\text{H}_{24}\text{N}_2\text{O}_5 \cdot 0.2 \text{ H}_2\text{O}$: C, 62.69; H, 6.76; N, 7.70. Found: C, 62.81; H, 6.62; N, 7.53.

Diethyl N-[4-(3-Aminopropyl)benzoyl]-L-glutamate. 24. Hydrogenation of **23** (6.1 g, 17 mmol) in absolute EtOH (75 ml), containing conc. H_2SO_4 (3 ml) and 10% Pd/C (1.0 g) was carried out in a Parr shaker with H_2 pressure at 3.6 kg/cm² (51 psi). After 16 h the catalyst was removed by filtration through Celite; the filtrate was neutralized with saturated NaHCO_3 solution. The EtOH was removed in vacuo and the product extracted with EtOAc; the organic phase was dried over Na_2SO_4 and evaporated. The crude product was chromatographed on silica gel (30 g) under N_2 pressure (flash technique) with CH_2Cl_2 -MeOH (9:1) as eluent. The combined fractions containing the product gave, after evaporation of the solvent, 3.6 g (59%) **24** as an oil. An analytical sample was obtained by dissolving a portion of the product in a small volume of CH_2Cl_2 and adding ethereal HCl to provide a white solid; ^1H nmr (300 MHz) 1.1 and 1.2 (2t, 2 CH_2CH_3), 1.7 (m, $\text{CH}_2\text{CH}_2\text{CH}_2$), 2.0 and 2.1 (2m, CHCH_2CH_2 , nonequivalent), 2.4 (t, CHCH_2CH_2), 2.7 (m, $\text{CH}_2\text{CH}_2\text{CH}_2$), 4.0 and 4.1 (2q, 2 CH_2CH_3), 4.4 (m, CHCH_2CH_2), 7.3 and 7.8 (AB spin system, C_6H_4), 8.0 (s, NH_3^+), 8.7 (d, CONH). Anal. Calcd for $\text{C}_{19}\text{H}_{28}\text{N}_2\text{O}_5 \cdot \text{HCl}$: C, 56.92; H, 7.29; N, 6.99. Found: C, 56.61; H, 7.48; N, 6.82.

4-[2-(2,4-Diamino-5,6,7,8-tetrahydropyrimido[4,5-d]pyrimidin-6-yl)ethyl]benzoic Acid. 15. A mixture of **11**¹⁵ (2.15 g, 10 mmol), NaHCO_3 (0.84 g, 10 mmol), and 37% HCHO (1 ml, 12.5 mmol)

in H₂O-MeOH (50 ml, 1:1) was stirred at room temperature for one h. To the resulting solution was then added **12** (0.63 g, 5 mmol) and stirred further for 18 h. The precipitate was filtered, washed with MeOH, and dried at 60°C in vacuo to leave 0.90 g (54%) **12**. The analytical sample was prepared by recrystallization from Me₂SO/MeOH; ¹H nmr (80MHz): 2.8 (m, CH₂CH₂), 3.5 (s, 5-CH₂), 4.0 (s, 7-CH₂), 6.2 (s, 2-NH₂), 6.7 (s, 8-NH), 7.2-8.0 (AB spin system, C₆H₄). Anal. Calcd for C₁₅H₁₈N₅O₂·H₂O: C, 54.21; H, 6.07; N, 25.29. Found: C, 54.29; H, 6.29; N, 25.18.

4-[2-(2-Amino-5,6,7,8-tetrahydro-4-oxo(3H)-pyrimido[4,5-d]pyrimidin-6-yl)ethyl]benzoic Acid.

16. The product **16** was prepared in 53% yield by the same procedure described for **15**, using **13**. The filtered product was redissolved in 0.1 N NaOH and acidified with 1 N HCl. The precipitate was filtered, washed with H₂O, and dried to provide a sample for analysis; ¹H nmr (80 MHz): 2.8 (m, CH₂CH₂), 3.4 (s, 5-CH₂), 3.9 (s, 7-CH₂), 6.1 (s, NH₂), 6.6 (s, 8-NH), 7.3-8.0 (AB spin system, C₆H₄). Anal. Calcd for C₁₅H₁₇N₅O₃·0.35 H₂O: C, 56.02; H, 5.55; N, 21.77. Found: C, 56.29; H, 5.61; N, 21.35.

Diethyl N-[4-[2-(2,4-Diamino-5,6,7,8-tetrahydropyrimido[4,5-d]pyrimidin-6-yl)ethyl]benzoyl]-L-glutamate. 19. A mixture of **18** (5.6 g, 16 mmol) and 37% HCHO (1.6 ml, 20 mmol) in MeOH (100 ml) was stirred at room temperature for 30 min. After which **12** (1.0 g, 8 mmol) was added and the reaction mixture stirred overnight. The solvent was removed with a rotary evaporator. The residue was dissolved in a minimum of CHCl₃-MeOH (9:1) and chromatographed on a silica gel column with the same eluent. The oily product obtained, after removal of the solvent, was dissolved in CHCl₃ and treated with hexane to give **19** (16 %) as a white powder; ¹H nmr (300 MHz) 1.1 and 1.2 (2t, 2 CH₂CH₃), 2.0 and 2.1 (2m, CHCH₂CH₂, nonequivalent), 2.4 (t, CHCH₂CH₂), 2.6 and 2.8 (2t,

CH_2CH_2), 3.4 (s, 5- CH_2), 3.9 (s, 7- CH_2), 4.0 and 4.1 (2q, 2 CH_2CH_3), 4.4 (m, CHCH_2CH_2), 5.2 and 5.5 (2s, 2 NH_2), 6.2 (s, 8-NH), 7.3 and 7.8 (AB spin system, C_6H_4), 8.6 (d, CONH).

Diethyl N-[4-[2-(2-Amino-5,6,7,8-tetrahydro-4-oxo(3H)-pyrimido[4,5-d]pyrimidin-6-yl)ethyl]benzoyl]-L-glutamate. 20. 20 was prepared according to the method described above for 19, utilizing 13 (1.3 g, 10 mmol) in 23 % yield; ^1H nmr (300 MHz) 1.1 and 1.2 (2t, 2 CH_2CH_3), 2.0 and 2.1 (2m, CHCH_2CH_2 , nonequivalent), 2.4 (t, CHCH_2CH_2), 2.6 and 2.8 (2t, CH_2CH_2), 3.4 (s, 5- CH_2 , overlapping with water peak), 3.9 (s, 7- CH_2), 4.0 and 4.1 (2q, 2 CH_2CH_3), 4.4 (m, CHCH_2CH_2), 6.1 (s, NH_2), 6.5 (s, 8-NH), 7.3 and 7.8 (AB system, C_6H_4), 8.6 (d, CONH). Anal. Calcd for $\text{C}_{24}\text{H}_{32}\text{N}_6\text{O}_6 \cdot 0.50 \text{ H}_2\text{O}$: C, 56.17; H, 6.56; N, 16.38. Found: C, 56.28; H, 6.63; N, 16.31.

Diethyl N-[4-[3-(2,4-Diamino-5,6,7,8-tetrahydropyrimido[4,5-d]pyrimidin-6-yl)propyl]benzoyl]-L-glutamate. 25. A solution of 24 (1.20 g, 3.3 mmol) and 37% HCHO (0.53 ml, 6.6 mmol) in MeOH (40 ml) was stirred for 15 min at room temperature, then 12 (0.41 g, 3.3 mmol) was added. After stirring for 15 min, the solvent was removed, the residue dissolved in a minimum volume of CH_2Cl_2 , applied on a silica gel column (30 g), and eluted by CH_2Cl_2 -MeOH (9:1) under N_2 pressure (flash technique). Pooling and evaporation of the appropriate fractions gave 25 (0.88 g, 49 %) as an oil, that gave a foam-like solid when dried in vacuum; ^1H nmr (300 MHz) 1.1 and 1.2 (2t, 2 CH_2CH_3), 1.8 (m, $\text{CH}_2\text{CH}_2\text{CH}_2$), 2.0 and 2.1 (2m, CHCH_2CH_2 , nonequivalent), 2.4 (t, CHCH_2CH_2), 2.4 and 2.7 (2t, $\text{CH}_2\text{CH}_2\text{CH}_2$), 3.2 (s, 5- CH_2), 3.8 (s, 7- CH_2), 4.0 and 4.1 (2q, 2 CH_2CH_3), 4.4 (m, CHCH_2CH_2), 5.3 and 5.5 (2s, 2 NH_2), 6.3 (s, 8-NH), 7.3 and 7.8 (AB spin system, C_6H_4), 8.6 (d, CONH). Anal. Calcd for $\text{C}_{25}\text{H}_{35}\text{N}_7\text{O}_5 \cdot 1.6 \text{ H}_2\text{O}$: C, 55.36; H, 7.10; N, 18.08. Found: C, 55.76; H, 6.99; N, 17.70.

Diethyl N-[4-[3-(2-Amino-5,6,7,8-tetrahydro-4-oxo(3H)-pyrimido[4,5-d]pyrimidin-6-yl)propyl]benzoyl]-L-glutamate. 26. The reaction and purification was carried out as described for 25 except that 13 (1.09 g, 3 mmol) was added portionwise during 20 min. The yield of 26 was 0.55 g (34%); ^1H nmr (300 MHz) 1.1 and 1.2 (2t, 2 CH_2CH_3), 1.7 (m, $\text{CH}_2\text{CH}_2\text{CH}_2$), 2.0 and 2.1 (2m, CHCH_2CH_2 , nonequivalent), 2.4 (t, CHCH_2CH_2), 2.4 and 2.7 (2t, $\text{CH}_2\text{CH}_2\text{CH}_2$), 3.2 (s, 5- CH_2), 3.9 (s, 7- CH_2), 4.0 and 4.1 (2q, 2 CH_2CH_3), 4.4 (m, CHCH_2CH_2), 6.0 (s, NH_2), 6.5 (s, 8-NH), 7.3 and 7.8 (AB spin system, C_6H_4), 8.6 (d, CONH). Anal. Calcd for $\text{C}_{25}\text{H}_{34}\text{N}_6\text{O}_6 \cdot 1.1\text{H}_2\text{O}$: C, 56.19; H, 6.83; N, 15.73. Found: C, 55.90; H, 6.81; N, 16.05.

Hydrolysis To Antifolate Diacids 5-8. General Method. The appropriate diester (19, 20, 25, or 26) was suspended in $\text{EtOH-H}_2\text{O}$ (1:4; 20 ml/mmol), treated with 1 M NaOH (5 ml/mmol), and stirred at room temperature for 16 h. Filtration into a centrifuge tube and acidification with 1 M HCl to pH 6 gave a precipitate which was collected by centrifugation. The product was subjected to three cycles of aqueous suspension, centrifugation, and decantation to give a colorless powder after drying in vacuum at room temperature.

N-[4-[2-(2,4-Diamino-5,6,7,8-tetrahydropyrimido[4,5-d]pyrimidin-6-yl)ethyl]benzoyl]-L-glutamic Acid. 5. Yield: 43%; ^1H nmr (300 MHz) 1.9 and 2.0 (2m, CHCH_2CH_2 , nonequivalent), 2.3 (t, CHCH_2CH_2), 2.7 and 2.8 (2t, CH_2CH_2), 3.4 (s, 5- CH_2 , overlapping with H_2O peak), 3.9 (s, 7- CH_2), 4.3 (m, CHCH_2CH_2), 5.7 and 5.8 (2s, 2 NH_2), 6.6 (s, 8-NH), 7.3 and 7.8 (AB spin system, C_6H_4), 8.4 (d, CONH). Anal. Calcd for $\text{C}_{20}\text{H}_{25}\text{N}_7\text{O}_5 \cdot 2.2\text{H}_2\text{O}$: C, 49.73; H, 6.13; N, 20.30. Found: C, 49.45; H, 6.12; N, 20.52.

N-[4-[2-(2-Amino-5,6,7,8-tetrahydro-4-oxo(3H)-pyrimido[4,5-d]pyrimidin-6-yl)ethyl]benzoyl]-L-glutamic Acid. 6. Yield: 45%; ^1H nmr (300 MHz) 1.9 and 2.0 (2m, CHCH_2CH_2 , nonequivalent), 2.3 (t, CHCH_2CH_2), 2.6 and 2.8 (2t, CH_2CH_2), 3.4 (s, 5- CH_2), 3.9 (s, 7- CH_2), 4.4 (m, CHCH_2CH_2), 6.1 (s, NH_2), 6.5 (s, 8-NH), 7.3 and 7.8 (AB spin system, C_6H_4), 8.5 (d, CONH). Anal. Calcd for $\text{C}_{20}\text{H}_{24}\text{N}_6\text{O}_6 \cdot 0.6 \text{ H}_2\text{O}$: C, 52.77; H, 5.58; N, 18.46. Found: C, 53.14; H, 5.71; N, 18.03.

N-[4-[3-(2,4-Diamino-5,6,7,8-tetrahydropyrimido[4,5-d]pyrimidin-6-yl)propyl]benzoyl]-L-glutamic Acid. 7. Yield: 43%; ^1H nmr (300 MHz) 1.8 (m, $\text{CH}_2\text{CH}_2\text{CH}_2$), 2.0 and 2.1 (2m, CHCH_2CH_2 , nonequivalent), 2.4 (t, CHCH_2CH_2), 2.3 and 2.7 (2t, $\text{CH}_2\text{CH}_2\text{CH}_2$), 3.4 (s, 5- CH_2), 3.9 (s, 7- CH_2), 4.3 (m, CHCH_2CH_2), 6.2 and 6.4 (2s, 2 NH_2), 7.0 (s, 8-NH), 7.3 and 7.8 (AB spin system, C_6H_4), 8.5 (d, CONH). Anal. Calcd for $\text{C}_{21}\text{H}_{27}\text{N}_7\text{O}_5 \cdot 1.7 \text{ H}_2\text{O}$: C, 51.67; H, 6.28; N, 20.09. Found: C, 52.09; H, 6.60; N, 19.56.

N-[4-[2-(2-Amino-5,6,7,8-tetrahydro-4-oxo(3H)-pyrimido[4,5-d]pyrimidin-6-yl)propyl]benzoyl]-L-glutamic Acid. 8. Yield: 36%; ^1H nmr (300 MHz) 1.7 (m, $\text{CH}_2\text{CH}_2\text{CH}_2$), 1.9 and 2.0 (2m, CHCH_2CH_2 , nonequivalent), 2.4 (t, CHCH_2CH_2), 2.3 and 2.6 (2t, $\text{CH}_2\text{CH}_2\text{CH}_2$), 3.3 (s, 5- CH_2 , overlapping with H_2O peak), 3.8 (s, 7- CH_2), 4.4 (m, CHCH_2CH_2), 6.0 (s, NH_2), 6.5 (s, 8-NH), 7.3 and 7.8 (AB spin system, C_6H_4), 8.5 (d, CONH). Anal. Calcd for $\text{C}_{21}\text{H}_{26}\text{N}_6\text{O}_6 \cdot 2.2 \text{ H}_2\text{O}$: C, 50.64; H, 6.15; N, 16.87. Found: C, 50.22; H, 5.75; N, 17.45.

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