A FACILE ROUTE TO "OPEN CHAIN" ANALOGUES OF DDATHF☆

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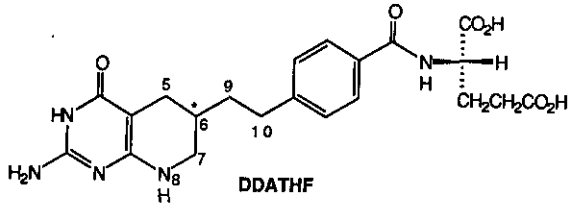
Abstract – N-{4-[4-(2,4-Diamino-6(1H)-oxopyrimidin-5-yl)butyl]benzoyl}-L-glutamic acid (7-DM-DDATHF, **18**) is a representative of a new series of achiral analogues of the potent anticancer agent 5,10-dideaza-5,6,7,8-tetrahydrofolic acid (DDATHF). Members of this "open chain" pyrimidine series, **18**, **19**, and **20**, were synthesized via guanidine cyclization of **6**, **7**, and **8** to give the pyrimidines **9**, **10**, and **11**. Ester hydrolysis, glutamate coupling and final saponification yielded the target compounds.

5,10-Dideaza-5,6,7,8-tetrahydrofolic acid (DDATHF) is a folate antimetabolite with a novel mechanism of action (inhibition of glycinamide ribonucleotide transformylase, GAR TFase, in the purine de novo biosynthetic pathway). It exhibits potent and broad spectrum antitumor activity in experimental animals with transplantable murine or human-derived solid tumors.[1-10] DDATHF was originally synthesized as a 1:1 mixture of two diastereomers differing in chirality at carbon-6 (dL + lL) and was later separated into each of the pure diastereomers by a unique fractional crystallization process.[7] It was then discovered that the two chemically distinct diastereomers exhibited different intrinsic biological properties both in vitro and in vivo. The B diastereomer of DDATHF (LY 264618) absolute configuration not yet determined) is currently under development as an anticancer agent for human neoplastic diseases.

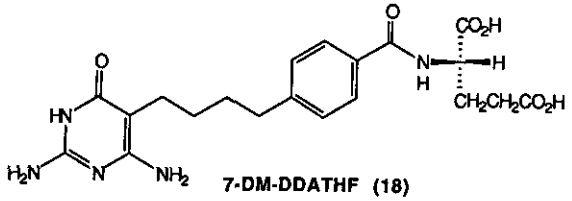
The fractional crystallization procedure currently employed for DDATHF represents the first successful large scale diastereomer separation in the tetrahydrofolate field. However, this tedious process appears not to be applicable to other DDATHF analogues. There is clearly a need for the development of either a chiral synthesis of 6-substituted 5-deaza-5,6,7,8-tetrahydropterins, or of DDATHF analogues possessing similar biological profiles but lacking the asymmetric center at position 6. Herein we report a novel class of folate antimetabolites of the latter type as part of our continuing structure-activity studies on DDATHF.

Removal of the methylene group at position 7 in DDATHF removes the chiral center at position 6 and gives what we term an "open-chain" or des-methylene analogue (7-DM-DDATHF), which is a single enantiomer. The lead compound in this "open-chain" series is N-

☆ *Dedicated with affection and respect to Sir Derek H. R. Barton on the occasion of his 70th birthday*



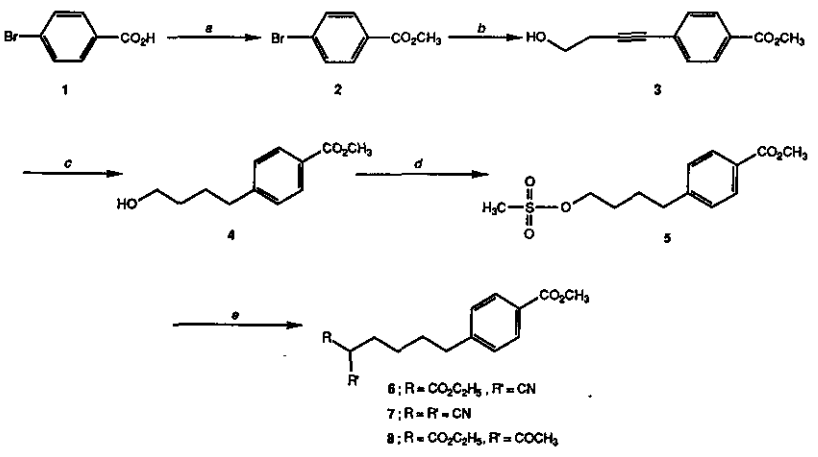
DDATHF



7-DM-DDATHF (18)

{4-[4-(2,4-diamino-6(1H)-oxopyrimidin-5-yl)butyl]benzoyl}-L-glutamic acid (**18**). A key step in the synthesis of **18**, and the analogues **19** and **20** (see Scheme II), involved guanidine cyclization of **6-8** to give the corresponding 2-amino-5-substituted pyrimidines **9-11**. Compounds **6-8** were prepared as depicted in Scheme I. 4-Bromobenzoic acid (**1**)

SCHEME I



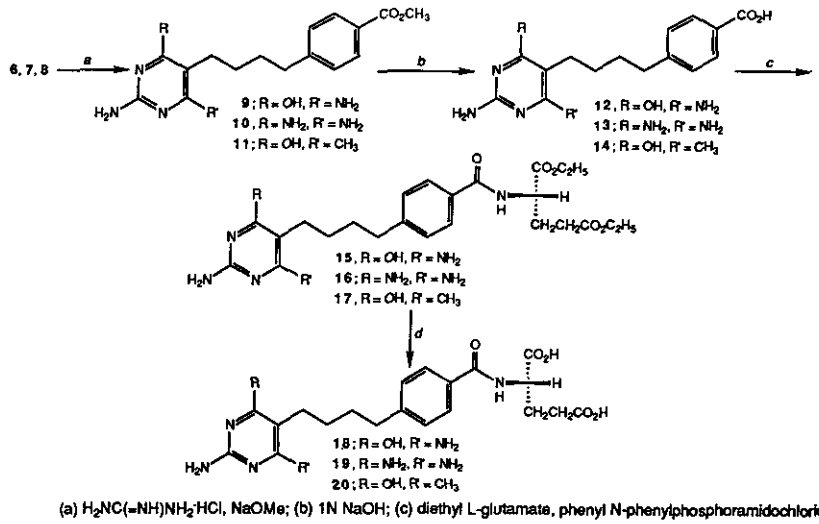
(a) MeOH, H_2SO_4; (b) 3-butyn-1-ol, $(Ph_3P)_2PdCl_2$, Cu(I), $HNEt_2$; (c) H_2, Pd/C; (d) Et_3N, MsCl; (e) NaCH(R)R'

was converted into its methyl ester **2** in quantitative yield under the Fisher esterification conditions. Palladium ($PdCl_2/PPh_3$)-catalyzed coupling[11] of **2** with 3-butyn-1-ol in diethylamine gave **3** in 76% yield. Hydrogenation of the acetylenic triple bond using 5% palladium-on-carbon in ethanol gave the saturated alcohol **4** in quantitative yield.[12] Mesylate **5** was then prepared (also in quantitative yield) from **4** and mesyl

chloride/triethylamine. Alkylation of the anions of ethyl cyanoacetate, malononitrile, and ethyl acetoacetate with **5** proceeded smoothly to give **6-8** in 66%, 76%, and 81% yields respectively.

The critical guanidine cyclization step was accomplished by generating salt-free guanidine in sodium methoxide/methanol (the mixture was filtered before use) and adding it to compounds **6**, **7**, or **8** in dimethylformamide (Scheme II). Addition of aqueous acid to the resulting reaction mixtures led to separation of the 5-substituted pyrimidines **9**, **10**, and **11** in 61%, 75%, and 70% yields respectively.

SCHEME II



(a) $H_2NC(=NH)NH_2$·HCl, NaOMe; (b) 1N NaOH; (c) diethyl L-glutamate, phenyl N-phenylphosphoramidochloridate, N-methylmorpholine; (d) 1N NaOH.

Formation of the requisite peptide bond to give **18-20** was achieved by hydrolysis of the methyl esters **9-11** with 1.0 N sodium hydroxide followed by acidification with glacial acetic acid to afford the free acids **12-14** in 71%, 82%, and 100% yields respectively. The L-glutamate moiety was then introduced using phenyl N-phenylphosphoramidochloridate as the coupling agent and N-methylpyrrolidone as the reaction solvent.[1] Isolated yields of the glutamate derivatives **15-17** were 47%, 45%, and 59% respectively. Final saponification with 1.0 N sodium hydroxide followed by acidification with glacial acetic acid[13] gave the final desired products **18-20** in 68%, 69% and 47% yields respectively.

This straightforward synthetic sequence provides a facile route to open-chain analogues of DDATHF and allows substantial flexibility in the nature of the heterocyclic left-hand ring. The biological activity of compounds in this "open-chain" series of DDATHF analogues will be presented elsewhere.

EXPERIMENTAL

General. Melting points were determined in open capillary tubes using a Thomas-Hoover apparatus and are uncorrected. Ir spectra were recorded on a Perkin-Elmer 1320 instrument

and are reported in cm^{-1}. ^1H Nmr data were obtained with a General Electric QE300 MHz instrument and chemical shifts are reported in ppm using residual solvent as an internal standard. Mass spectral data were obtained by Dr. Dorothy Little on AEI MS-902 and Kratos MS50TC spectrometers. Elemental analyses were performed by Eli Lilly and Co., Indianapolis, Indiana. Column chromatography was performed on Merck silica gel 60 (240-400 mesh). Tlc analyses were carried out on Bakerflex IB2-F plates utilizing Uv visualization. Commercial reagents were utilized without further purification. Anhydrous solvents were distilled before use.

Methyl 4-Bromobenzoate (2).

A 2 l round-bottomed flask, equipped with a magnetic stirrer, reflux condenser, heating mantle, and drying tube, was charged with 100 g of 4-bromobenzoic acid (**1**), 1 l of methanol, and 10 g of sulfuric acid. The reaction mixture was refluxed with stirring and exclusion of moisture for 12 h. After cooling to room temperature, methanol was removed under reduced pressure, and the resulting residue was extracted with methylene chloride. The organic extract was washed with water (3x), brine, dried (sodium sulfate), filtered, and concentrated under reduced pressure. The residue was recrystallized from methanol to give 105 g (98%) of pure **2** as a white solid whose physical properties were identical with those reported: mp 83-84 °C (lit.[14] mp 81 °C).

Methyl 4-(4-Hydroxy-1-butynyl)benzoate (3).

A literature procedure for coupling of aryl halides to terminal acetylenes was followed.[11] A 500 ml round-bottomed flask, equipped with a magnetic stirrer and gas inlet, was charged with a mixture of 0.082 g (0.005 eq.) of palladium chloride and 0.244 g (0.01 eq) of triphenylphosphine, and solution of 20.00 g (1.0 eq.) of **2** in 250 ml of diethylamine under a nitrogen atmosphere. To the stirred mixture was added 0.178 g (0.01 eq.) of copper (I) iodide and 6.52 g (1.0 eq.) of 3-butyn-1-ol, and the reaction mixture was stirred under a nitrogen atmosphere at room temperature for 18 h. Diethylamine was removed under reduced pressure and water was added to the residue. The mixture was extracted with benzene. The benzene extract was passed over a short silica pad to remove the catalyst and concentrated under reduced pressure. The residue was then recrystallized from benzene-hexanes to give 14.40 g (75.8%) of pure **3** as white flakes: mp 95.5-96.0 °C; ir (KBr) 3310, 2955, 1718, 1604, 1433, 1275, 1177, 1108, 1040, 955, 852, and 769 cm^{-1}; ^1H nmr ($CDCl_3$) δ 7.98 (d, J=8.3 Hz, 2H, Ar), 7.49 (d, J=8.3 Hz, 2H, Ar), 3.93 (s, 3H, CH_3), 3.87 (m, 2H, $C\underline{H_2}OH$), 2.74 (t, J=6.2 Hz, 2H, CH_2), 1.88 (m, 1H, OH). Anal. Calcd for $C_{12}H_{12}O_3$: C, 70.57; H, 5.92. Found: C, 70.36; H, 5.68.

Methyl 4-(4-Hydroxybutyl)benzoate (4).

A Parr flask was charged with 2.55 g of **3** in 200 ml of ethanol. Then 0.26 g (10% wt eq.) of 5% palladium-on-charcoal was added with 25 ml of ethanol. Hydrogenation was carried out at 50 psi of hydrogen for 12 h. The reaction mixture was filtered through a silica gel pad which was washed with ethanol. The filtrate was concentrated under reduced pressure to give 2.60 g (quantitative) of pure **4** as a clear oil: ir (film) 3390, 2965, 2920, 2850, 1705, 1605, 1568, 1520, 1500, 1410, 1387, 1362, 1308, 1286, 1250, 1160, 1055, 1013, 843, 755, and 695 cm^{-1}; ^1H nmr ($CDCl_3$) δ 7.95 (d, J=8.1 Hz, 2H, Ar), 7.25 (d, J=8.1 Hz, 2H, Ar), 3.89 (s, 3H, CH_3), 3.65 (t, J=6.3 Hz, 2H, $C\underline{H_2}OH$), 2.69 (t, J=7.5 Hz, 2H, benzyl), 1.66 (m, 4H, 2° aliphatic). Anal. Calcd for $C_{12}H_{16}O_3$: C, 69.21; H, 7.74. Found: C, 68.97; H, 7.92.

Methyl 4-[(4-Methylsulfonyloxy)butyl]benzoate (5).
A 2 l three-necked round-bottomed flask, equipped with an overhead stirrer, reflux condenser, pressure equalizing addition funnel, gas inlet, and an ice bath, was charged with 65.07 g (1.0 eq.) of **4** and 33.20 g (1.05 eq.) of triethylamine in 350 ml of anhydrous ethyl ether. The solution was stirred under nitrogen, brought to 0 °C, and 37.58 g (1.05 eq.) of mesyl chloride added dropwise. A precipitate immediately began to form, and the mildly exothermic reaction mixture was brought gradually to room temperature. After 4 h, 400 ml of water was added, bringing the precipitated salts into solution. The organic layer was separated, washed with water, dried (magnesium sulfate), and concentrated under reduced pressure to give pure **5** as a low melting white solid in quantitative yield: mp 52-53 °C; ir (KBr) 3010, 2930, 2850, 1700, 1603, 1432, 1334, 1323, 1165, 1104, 937, 822, and 700 cm^{-1}; ^1H nmr ($CDCl_3$) δ 7.92 (d, J=8.2 Hz, 2H, Ar), 7.22 (d, J=8.2 Hz, 2H, Ar), 4.18-4.22 (m, 2H, CH_2OMs), 3.86 (s, 3H, OCH_3), 2.96 (s, 3H, SO_2CH_3), 2.65-2.68 (m, 2H, benzyl), 1.72-1.74 (m, 4H, 2° aliphatic). Anal. Calcd for $C_{13}H_{18}SO_5$: C, 54.53; H, 6.34; S, 11.20. Found: C, 54.79; H, 6.46; S, 11.17.

Methyl 4-(5-Carboethoxy-5-cyanopentyl)benzoate (6).
A 1 l three-neck round-bottomed flask, equipped with a magnetic stirrer, reflux condenser, addition funnel, and gas inlet, was torched dry, cooled to room temperature, and charged with 10.30 g (1.1 eq.) of 80% NaH in anhydrous tetrahydrofuran. The mineral oil was removed by washing twice with anhydrous tetrahydrofuran, and 400 ml of anhydrous tetrahydrofuran were then added. This mixture was brought to 0 °C on an ice bath and a *solution of 35.29 g (1.0 eq.) of ethyl cyanoacetate in 50 ml of tetrahydrofuran was added* dropwise under a nitrogen atmosphere. The mixture was stirred vigorously while warming to room temperature until gas evolution (hydrogen) was no longer observed from the salt solution. To this mixture was added a solution of 89.33 g (1.0 eq.) of **5** in 100 ml of tetrahydrofuran. The resulting mixture was stirred at room temperature under a nitrogen atmosphere for 12 h. Thin layer chromatography indicated the reaction to be incomplete, and the reaction mixture was then refluxed for 6 h. After cooling to room temperature, solvent was removed under reduced pressure and diethyl ether was added to the residue. The organic extract was washed with water, brine, dried (magnesium sulfate), filtered, and concentrated under reduced pressure to give a purple oil. Purification was carried out by column chromatography, eluting with 20% ethyl acetate/hexanes. Fractions homogeneous by thin layer chromatography for the major component were combined and concentrated under reduced pressure to give 62.08 g (65.6%) of pure **6** as a clear oil; ir (film) 2915, 2860, 2255, 1730, 1609, 1571, 1432, 1413, 1368, 1320, 1178, 1105, 1019, 962, 853, 759, and 602 cm^{-1}; ^1H nmr ($CDCl_3$) δ 7.95 (d, J=8.1 Hz, 2H, Ar), 7.24 (d, J=8.1 Hz, 2H, Ar), 4.25 (q, J=7.2 Hz, 2H, $C\underline{H}_2CH_3$), 3.90 (s, 3H, CO_2CH_3), 3.46 (t, J=6.8 Hz, 1H, CHCN), 2.69 (t, J=7.5 Hz, 2H, benzyl), 1.93-2.00 (m, 2H, $C\underline{H}_2CH$), 1.54-1.72 (m, 4H, 2° aliphatic), 1.30 (t, J=7.3 Hz, 3H, $CH_2C\underline{H}_3$). Anal. Calcd for $C_{17}H_{21}NO_4$: C, 67.31; H, 6.98; N, 4.62. Found: C, 67.10; H, 6.96; N, 4.33.

Methyl 4-(5,5-Dicyanopentyl)benzoate (7).
This compound was prepared from 3.12 g (1.1 eq.) of 80% NaH, 6.24 g (1.0 eq.) of malononitrile, and 27.06 g (1.0 eq.) of **5** as described previously for the preparation of **6**; yield 18.46 g (76.2%) of **7** as a yellow oil; ir (film) 3026, 2953, 2943, 1717, 1611, 1437,

1310, 1285, 1230, 1225, 1222, 1180, 1114, and 1020 cm^{-1}; ^1H nmr ($CDCl_3$) δ 7.93 (d, J=8.1 Hz, 2H, Ar), 7.21 (d, J=8.1 Hz, 2H, Ar), 3.86 (s, 3H, CO_2CH_3), 3.80 (t, J=6.8 Hz, 1H, CH), 2.68 (t, J=7.2 Hz, 2H, benzyl), 1.99-2.04 (m, 2H, CH_2CH), 1.62-1.75 (m, 4H, 2° aliphatic). Anal. Calcd for $C_{15}H_{16}N_2O_2$: C, 70.29; H, 6.29; N, 10.93. Found: C, 70.50; H, 6.48; N, 10.69.

Methyl 4-(5-Ethoxycarbonyl-6-oxoheptyl)benzoate (8).
This compound was prepared from 1.54 g (1.1 eq.) of 60% NaH, 4.45 mL (1.0 eq.) of ethyl acetoacetate, and 10.00 g (1.0 eq.) of **5** as described previously for the preparation of **6**; yield 4.07 g (81.2%, recovered 5.55 g of **5**) of **8** as a clear oil: ir (film) 3019, 2941, 1715, 1437, 1310, 1284, 1245, 1236, 1232, 1227, 1225, 1207, 1194, 1180, 1114, and 1021 cm^{-1}; ^1H nmr ($CDCl_3$) δ 7.93 (d, J=8.2 Hz, 2H, Ar), 7.22 (d, J=8.2 Hz, 2H, Ar), 4.17 (q, J=7.1 Hz, 2H, CH_2CH_3), 3.89 (s, 3H, CO_2CH_3), 3.38 (t, J=7.3 Hz, 1H, CH), 2.65 (t, J=7.6 Hz, 2H, benzyl), 2.21 (s, 3H, $COCH_3$), 1.82-1.91 (m, 2H, CH_2CH), 1.60-1.70 (m, 2H, 2° aliphatic), 1.26-1.36 (m, 2H, 2° aliphatic), 1.25 (t, J=7.2 Hz, 3H, CH_2CH_3). Anal. Calcd for $C_{18}H_{24}O_5$: C, 67.48; H, 7.55; Found: C, 67.36; H, 7.50.

Methyl 4-[4-(2,4-Diamino-6(1H)-oxopyrimidin-5-yl)butyl]benzoate (9).
A 100 ml round-bottomed flask, equipped with a magnetic stirrer and gas inlet, was charged with 0.71 g (1.05 eq.) of guanidine hydrochloride, 0.40 g (1.05 eq.) of sodium methoxide, and 20 ml of anhydrous methanol. This mixture was stirred under a nitrogen atmosphere for 0.5 h, the precipitated sodium chloride removed by filtration, and the filtrate concentrated under reduced pressure. To the resulting solution of guanidine free base was added 2.15 g (1.0 eq.) of **6** in 20 ml of anhydrous dimethylformamide. The reaction mixture was stirred under a nitrogen atmosphere with mild warming for 12 h. Thin layer chromatography indicated the absence of **6**. The reaction mixture was then poured over very dilute sulfuric acid, and the solid which separated was collected by filtration, washed with water, diethyl ether, and dried to give 1.37 g (61%) of pure **9** as a tan solid; mp 203-205 °C; ir (KBr) 3495, 3380, 2925, 2850, 1685, 1600, 1490, 1430, 1359, 1282, 1174, 1111, 1015, and 754 cm^{-1}; ^1H nmr (DMSO-d_6) δ 9.74 (bs, 1H, (3)-NH), 7.83 (d, J=8.1 Hz, 2H, Ar), 7.31 (d, J=8.1 Hz, 2H, Ar), 5.87 (bs, 2H, (2)-NH_2), 5.60 (bs, 2H, (6)-NH_2), 3.80 (s, 3H, CH_3), 2.62 (t, J=7.6 Hz, 2H, (5)-CH_2), 2.15 (t, J=7.2 Hz, 2H, benzyl), 1.51-1.55 (m, 2H, 2° aliphatic), 1.26-1.28 (m, 2H, 2° aliphatic). Anal. Calcd for $C_{16}H_{20}N_4O_3$: C, 60.75; H, 6.37; N, 17.71. Found: C, 61.05; H, 6.45; N, 17.46.

Methyl 4-[4-(2,4,6-Triaminopyrimidin-5-yl)butyl]benzoate (10).
This compound was prepared from 5.70 g (1.05 eq.) of guanidine hydrochloride, 3.22 g (1.05 eq.) of sodium methoxide, and 14.57 g (1.0 eq.) of **7** as described above for the preparation of **9**; yield 13.43 g (74.9%) of **10** as a tan solid: mp 228-231 °C; ir (KBr) 3366, 1692, 1648, 1630, and 1290 cm^{-1}; ^1H nmr (DMSO-d_6) δ 7.84 (d, J=8.1 Hz, 2H, Ar), 7.31 (d, J=8.1 Hz, 2H, Ar), 7.02 (bs, 2H, (2)-NH_2), 6.92 (bs, 4H, (4)-NH_2, (6)-NH_2), 3.80 (s, 3H, CH_3), 2.56-2.65 (m, 2H, (5)-CH_2), 2.24 (t, J=7.2 Hz, 2H, benzyl), 1.56-1.63 (m, 2H, 2° aliphatic), 1.26-1.31 (m, 2H, 2° aliphatic); hrms calcd for $C_{16}H_{21}N_5O_2$ (M^+) m/z 315.1695, found 315.1681; other ions at 284, 225, 192, 149, 138, 96, 78.

Methyl 4-[4-(2-Amino-4-methyl-6(1H)-oxopyrimidin-5-yl)butyl]benzoate (11).
This compound was prepared from 1.33 g (1.0 eq.) of guanidine hydrochloride, 0.32 g (1.1 eq.) of sodium metal in methanol, and 4.05 g (1.0 eq.) of **8** as described above for the preparation of **9**; yield 2.80 g (70.2%) of **11** as a white solid: mp 155-157 °C; ir (KBr) 1716, 1655, 1609, 1435, and 1285 cm^{-1}; ^1H nmr (DMSO-d_6) δ 7.83 (d, J=8.1 Hz, 2H, Ar), 7.32 (d, J=8.1 Hz, 2H, Ar), 6.17 (bs, 2H, NH_2), 3.80 (s, 3H, CH_3), 2.64 (t, J=7.5 Hz, 2H, (5)-CH_2), 2.26 (t, J=7.5 Hz, 2H, benzyl), 1.98 (s, 3H, CH_3), 1.49-1.58 (m, 2H, 2° aliphatic), 1.29-1.38 (m, 2H, 2° aliphatic). Anal. Calcd for $C_{17}H_{21}N_3O_3$: C, 64.74; H, 6.71; N, 13.32. Found: C, 64.51; H, 6.86; N, 13.07.

4-[4-(2,4-Diamino-6(1H)-oxopyrimidin-5-yl)butyl]benzoic Acid (12).
A 100 ml round-bottomed flask equipped with a magnetic stirrer was charged with 0.91 g of **9** in 40 ml of 1N sodium hydroxide. The reaction mixture was stirred at room temperature under mild heat for 18 h, filtered, and the filtrate acidified with glacial acetic acid. The resulting precipitate was collected by centrifugation with fresh water several times, and it was then transferred to a round-bottomed flask with methanol. The mixture was concentrated under reduced pressure to give 0.62 g (71.3%) of pure **12** as a yellow microcrystalline solid: mp 293-294 °C; ir (KBr) 3460, 3345, 3140, 2920, 2845, 1575, 1435, 1368, 1247, 1170, and 744 cm^{-1}; ^1H nmr (DMSO-d_6) δ 9.78 (bs, 1H, (3)-NH), 7.80 (d, J=8.0 Hz, 2H, Ar), 7.26 (d, J=8.0 Hz, 2H, Ar), 5.88 (bs, 2H, (2)-NH_2), 5.59 (bs, 2H, (6)-NH_2), 2.61 (t, J=7.4 Hz, 2H, (5)-CH_2), 2.15 (t, J=7.2 Hz, 2H, benzyl), 1.51-1.56 (m, 2H, 2° aliphatic), 1.27-1.31 (m, 2H, 2° aliphatic). Anal. Calcd for $C_{15}H_{18}N_4O_3$: C, 59.59; H, 6.00; N, 18.53. Found: C, 59.79; H, 5.77; N, 18.30.

4-[4-(2,4,6-Triaminopyrimidin-5-yl)butyl]benzoic Acid (13).
This compound was prepared from 13.10 g of **10** as described above for the preparation of **12**; yield 10.28 g (82.1%) of **13** as a tan powder: mp 268-270 °C; ir (KBr) 3362, 3356, 3352, 3350, 1645, 1612, and 1388 cm^{-1}; ^1H nmr (DMSO-d_6) δ 7.77 (d, J=8.0 Hz, 2H, Ar), 7.14 (d, J=8.0 Hz, 2H, Ar), 5.89 (bs, 4H, (4)-NH_2, (6)-NH_2), 5.83 (bs, 2H, (2)-NH_2), 2.58 (t, J=7.4 Hz, 2H, (5)-CH_2), 2.21 (t, J=7.2 Hz, 2H, benzyl), 1.55-1.62 (m, 2H, 2° aliphatic), 1.27-1.34 (m, 2H, 2° aliphatic); hrms calcd for $C_{15}H_{19}N_5O_2$ (M^+) m/z 301.1538, found 301.1534; other ions at 149, 137, 125, 107, 96, 91, 78, 69.

4-[4-(2-Amino-4-methyl-6(1H)-oxopyrimidin-5-yl)butyl]benzoic Acid (14).
This compound was prepared from 2.44 g of **11** as described previously for the preparation of **12**; yield 2.33 g (quantitative) of **14** as a white powder: mp 308-309 °C; ir (KBr) 2931, 1700, 1696, 1675, 1610, and 1508 cm^{-1}; ^1H nmr (DMSO-d_6) δ 7.74 (d, J=8.1 Hz, 2H, Ar), 7.23 (d, J=8.1 Hz, 2H, Ar), 6.36 (bs, 2H, NH_2), 2.63 (t, J=7.4 Hz, 2H, (5)-CH_2), 2.27 (t, J=7.4 Hz, 2H, benzyl), 2.00 (s, 3H, CH_3), 1.51-1.59 (m, 2H, 2° aliphatic), 1.28-1.37 (m, 2H, 2° aliphatic); mass: m/z 302 (FD).

Diethyl N-[4-[4-(2,4-Diamino-6(1H)-oxopyrimidin-5-yl)butyl]benzoyl]-L-glutamate (15). A literature procedure for glutamate coupling was followed.[3] A 100 ml round-bottomed flask, equipped with a magnetic stirrer and gas inlet, was charged with 0.47 g (1.0 eq.) of **12**, 0.62 g (1.5 eq.) of phenyl N-phenylphosphoramidochloridate, 0.79 g (5.0 eq.) of N-methylmorpholine, and 50 ml of anhydrous N-methylpyrrolidone. The mixture was stirred at

room temperature under a nitrogen atmosphere for 1 h. To the resulting homogeneous solution was added 0.75 g (2.0 eq.) of diethyl L-glutamate hydrochloride and stirring under nitrogen was continued for 24 h. The solvent was removed by vacuum distillation and chloroform was added to the residue. The chloroform solution was washed with water, the organic extracts combined, dried (magnesium sulfate), filtered, and concentrated under reduced pressure. Purification was carried out by flash chromatography, eluting with 10% methanol/chloroform. Fractions homogeneous by thin layer chromatography for the major component were combined and concentrated under reduced pressure to give 0.36 g (47.4%) of **15** as a pale yellow solid: mp 75-76 °C; ir (KBr) 3320, 2970, 2915, 1845, 1720, 1580, 1529, 1427, 1368, 1326, 1192, 1093, 1021, 849, and 750 cm^{-1}; ^1H nmr ($CDCl_3$) δ 11.58 (bs, 1H, (3)-NH), 7.68 (d, J=7.9 Hz, 2H, Ar), 7.33 (d, J=7.9 Hz, 1H, NH), 7.17 (d, J=7.9 Hz, 2H, Ar), 5.54 (bs, 2H, (2)-NH_2), 4.76-4.82 (m, 1H, CH), 4.71 (bs, 2H, (6)-NH_2), 4.23 (q, J=7.11 Hz, 2H, CO_2CH_2), 4.12 (q, J=6.5 Hz, 2H, CO_2CH_2), 2.15-2.68 (m, 8H, 2° aliphatic), 1.57-1.66 (m, 2H, 2° aliphatic), 1.35-1.45 (m, 2H, 2° aliphatic), 1.30 (t, J=7.1 Hz, 3H, CH_3), 1.22 (t, J=7.1 Hz, 3H, CH_3). Anal. Calcd for $C_{24}H_{33}N_5O_6$: C, 59.12; H, 6.82; N, 14.36. Found: C, 59.08; H, 6.79; N, 14.11.

Diethyl N-{4-[4-(2,4,6-Triaminopyrimidin-5-yl)butyl]benzoyl}-L-glutamate (16).
This compound was prepared from 1.50 g (1.0 eq.) of **13**, 2.00 g (1.5 eq.) of phenyl N-phenylphosphoramidochloridate, 2.52 g (5.0 eq.) of N-methylmorpholine, and 2.39 g (2.0 eq.) of diethyl L-glutamate hydrochloride as described above for the preparation of **15**; yield 1.08 g (45%) of **16** as a pale yellow microcrystalline solid: mp 83-85 °C; ir (KBr) 1696, 1657, 1654, 1606, 1576, 1525, 1496, 1478, 1447, 1435, 1414, 1396, 1377, 1349, 1332, 1231, 1224, 1223, 1219, 1216, 1210, 1206, 1200, 1188, 1095, 1066, 1048, 1033, 1031, 1019, and 855 cm^{-1}; ^1H nmr ($CDCl_3$) δ 7.68 (d, J=8.0 Hz, 2H, Ar), 7.19 (d, J=8.2 Hz, 1H, NH), 7.16 (d, J=8.0 Hz, 2H, Ar), 4.74-4.80 (m, 1H, CH), 4.64 (bs, 4H, (4)-NH_2, (6)-NH_2), 4.20 (q, J=7.1 Hz, 2H, CO_2CH_2), 4.08 (q, J=7.0 Hz, 2H, CO_2CH_2), 2.08-2.65 (m, 8H, 2° aliphatic), 1.59-1.65 (m, 2H, 2° aliphatic), 1.42-1.48 (m, 2H, 2° aliphatic), 1.27 (t, J=7.1 Hz, 3H, CH_3), 1.19 (t, J=7.1 Hz, 3H, CH_3); hrms calcd for $C_{24}H_{34}N_6O_5$ (M^+) 486.2590, found m/z 486.2595; other ions at 441, 413, 359, 284, 267, 236, 188, 166.

Diethyl N-{4-[4-(2-Amino-4-methyl-6(1H)-oxopyrimidin-5-yl)butyl]benzoyl}-L-glutamate (17).
This compound was prepared from 0.20 g (1.0 eq.) of **14**, 0.27 g (1.5 eq.) of phenyl N-phenylphosphoramidochloridate, 0.34 g (5.0 eq.) of N-methylmorpholine, and 0.32 g (2.0 eq.) of diethyl L-glutamate hydrochloride as described above for the preparation of **15**; yield 0.19 g (59.4%) of **17** as a pale yellow microcrystalline solid: mp 62-64 °C; ir (KBr) 3019, 1731, 1653, 1611, 1526, 1496, 1226, and 1205 cm^{-1}; ^1H nmr ($CDCl_3$) δ 7.70 (d, J=8.0 Hz, 2H, Ar), 7.18-7.28 (m, 3H, Ar, NH), 6.78 (bs, 2H, NH_2), 4.73-4.81 (m, 1H, CH), 4.24 (q, J=7.1 Hz, 2H, CO_2CH_2), 4.12 (q, J=7.1 Hz, 2H, CO_2CH_2), 2.10-2.72 (m, 8H, 2° aliphatic), 2.12 (s, 3H, CH_3), 1.62-1.71 (m, 2H, 2° aliphatic), 1.42-1.53 (m, 2H, 2° aliphatic), 1.31 (t, J=7.1 Hz, 3H, CH_3), 1.23 (t, J=7.1 Hz, 3H, CH_3). Anal. Calcd for $C_{25}H_{34}N_4O_7$: C, 59.75; H, 6.82; N, 11.15. Found: C, 59.52; H, 6.69; N, 10.88.

N-{4-[4-(2,4-Diamino-6(1H)-oxopyrimidin-5-yl)butyl]benzoyl}-L-glutamic Acid (**18**).
A 100 ml round-bottomed flask equipped with a magnetic stirrer was charged with 0.50 g of **15** in 40 ml of 1N sodium hydroxide. The reaction mixture was stirred at room temperature for 72 h.[13] The solution was neutralized with hydrochloric acid and the resulting precipitate collected by filtration, washed with water, and dried to give 0.30 g (68%) of **18** as a white microcrystalline solid: mp 169-171 °C; ir (KBr) 3340, 3200, 2920, 2860, 1600, 1380, 1170, and 755 cm^{-1}; ^1H nmr (dTFA, DMSO-d_6) δ 7.30 (d, J=8.7 Hz, 2H, Ar), 6.90 (d, J=8.7 Hz, 2H, Ar), 4.56-4.61 (m, 1H, CH), 2.26-2.38 (m, 4H, 2° aliphatic), 1.93-2.14 (m, 4H, 2° aliphatic) 1.25-1.34 (m, 2H, 2° aliphatic), 1.05-1.17 (m, 2H, 2° aliphatic). Anal. Calcd for $C_{20}H_{25}N_5O_6$: C, 55.68; H, 5.84; N, 16.23. Found: C, 55.47; H, 5.94; N, 16.54.

N-{4-[4-(2,4,6-Triaminopyrimidin-5-yl)butyl]benzoyl}-L-glutamic Acid (**19**).
This compound was prepared from 0.36 g of **16** as described previously for the preparation of **18**; yield 0.22 g (69%) of **19** as a white microcrystalline solid: mp 186-190 °C; ir (KBr) 3345, 3342, 1711, 1637, 1539, and 1218 cm^{-1}; ^1H nmr (dTFA, DMSO-d_6) δ 7.29 (d, J=8.2 Hz, 2H, Ar), 6.89 (d, J=8.2 Hz, 2H, Ar), 4.56-4.61 (m, 1H, CH), 2.25-2.38 (m, 4H, 2° aliphatic), 1.91-2.15 (m, 4H, 2° aliphatic), 1.27-1.37 (m, 2H, 2° aliphatic), 1.10-1.19 (m, 2H, 2° aliphatic); mass: m/z 431 (FAB).

N-{4-[4-(2-Amino-4-methyl-6(1H)-oxopyrimidin-5-yl)butyl]benzoyl}-L-glutamic Acid (**20**).
This compound was prepared from 0.17 g of **17** as described above for the preparation of **18**; yield 0.07 g (46.7%) of **20** as a white microcrystalline solid: mp 232-235 °C; ir (KBr) 1701, 1669, 1665, 1661, 1660, 1616, 1612, and 1540 cm^{-1}; ^1H nmr (DMSO-d_6) δ 8.48 (d, J=7.7 Hz, 1H, NH), 7.76 (d, J=7.9 Hz, 2H, Ar), 7.26 (d, J=7.9 Hz, 2H, Ar), 6.69 (bs, 2H, NH_2), 4.33-4.40 (m, 1H, CH), 2.62 (t, J=7.2 Hz, 2H, (5)-CH_2), 2.22-2.37 (m, 4H, 2° aliphatic), 2.03 (s, 3H, CH_3), 1.89-1.96 (m, 2H, 2° aliphatic), 1.52-1.58 (m, 2H, 2° aliphatic), 1.30-1.36 (m, 2H, 2° aliphatic); mass: m/z 431 (FAB).

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