Novel 5-Desmethylene Analogues of 5,10-Dideaza-5,6,7,8-tetrahydrofolic Acid as Potential Anticancer Agents

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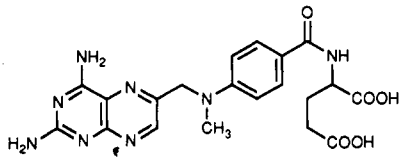
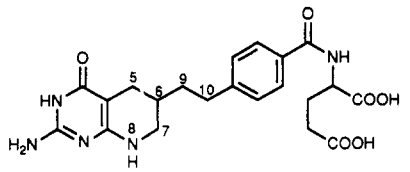
The synthesis and biological activity of novel 5-desmethylene analogues of 5,10-dideaza-5,6,7,8-tetrahydrofolic acid (DDATHF), a potent antitumor agent presently undergoing clinical trials, are described. These compounds are representative of a new series of optically pure analogues of DDATHF.

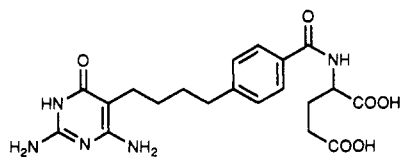
In the treatment of cancer, one of the primary programs available to the clinician is chemotherapy. Methotrexate (MTX, 1), which represents a class of chemotherapeutic agents known as folate antimetabolites, was first prepared in 1947[1] and has in the intervening years has been the most widely used chemotherapeutic agent either alone or in combination therapy in the treatment of a wide variety of tumors.[2] MTX exerts its cytostatic effects in large part by inhibition of dihydrofolate reductase (DHFR), an essential enzyme in the biosynthesis of purines and pyrimidines and therefore DNA.[3] However, severe side effects are normally associated with MTX therapy, and as a consequence an intensive search has been underway for more selective and less toxic antitumor agents. 5,10-Dideaza-5,6,7,8-tetrahydrofolic acid (DDATHF, Lometrexol, 2) was first described by us in 1985 and came from a search



Methotrexate (MTX, 1)



DDATHF, 2



7-DM-DDATHF, 3

for new antifolates which would be active at sites other than DHFR.[4] DDATHF exhibits its cytostatic effects by inhibition of glycinamide ribonucleotide formyltransferase (GARFT), a folate-requiring enzyme which catalyses the first of two one-carbon transfers in purine biosynthesis.[5] The resulting inhibition of de novo purine biosynthesis provides an effective and novel mechanism of cytotostatic activity and defines a new class of antifolates with remarkable antitumor activity.[6] Biochemical determinants of selectivity and resistance established the importance of polyglutamation for the action of DDATHF,[7] whose transport mechanism has been shown to be the same as that of MTX.[7] DDATHF possesses potent antitumor activity against a broad spectrum of solid tumors and is fully active against tumors which are resistant to DHFR inhibitors for reasons (such as overproduction of DHFR, or DHFR mutation to a low MTX affinity form) other than defects in active transport.[7] Protocols utilizing the B-16 melanoma cell line have demonstrated that DDATHF completely inhibits tumor growth at 6.25 mg/kg per day for 10 days without evidence of host toxicity up to 100 mg/kg per day.[5] DDATHF possesses structural features which allow it to act as a uniquely effective folate antimetabolite. The 2-amino-4-oxopyrimidine as well as the fully reduced pyridine ring ensure lack of inhibition of DHFR, and absence of nitrogens at the 5- and 10-positions precludes its participation as a cofactor in any one-carbon transfer reaction in folate metabolism. Retention of the *p*-benzoylglutamate moiety allows conversion to polyglutamates which are believed to be the biologically active form(s) of DDATHF. In addition, substitution of carbon for nitrogen at position 5 allows for increased chemical stability of DDATHF over tetrahydrofolic acid derivatives.

As a result of the remarkable therapeutic potential of DDATHF (now in phase II clinical trials), we have undertaken the synthesis of a broad variety of analogues in an attempt to optimize antitumor activity. Since the C-6 carbon of DDATHF is chiral, DDATHF as originally prepared (by catalytic reduction of the pyridine ring) consisted of two diastereomers, each of which exhibited slightly different intrinsic in vivo and in vitro properties. In an effort to examine the relationship between C-6 chirality and antitumor activity, we recently prepared 7-desmethylene DDATHF (7-DM-DDATHF, 3), which lacks the chiral center at C-6.[8] This analogue showed in vitro and in vivo activity comparable to that of DDATHF

(1) Seeger, D. R.; Smith, J. M., Jr.; Hultquist, M. E. *J. Am. Chem. Soc.* **1947**, *69*, 2567.

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(3) For extensive reviews of the chemistry of folic acid, aminopterin, methotrexate and analogs as antitumor agents see: (a) Palmer, D. C.; Skotnicki, J. S.; Taylor, E. C. In *Progress in Medicinal Chemistry*; Ellis, G. P., West, G. B., Eds.; Elsevier Science Publishers: Holland, 1988; Vol. 25, p 85. (b) Rosowsky, A. In *Progress in Medicinal Chemistry*; Ellis, G. P., West, G. B., Eds.; Elsevier Science Publishers: Holland, 1989; Vol. 26, p 1.

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(5) (a) Beardsley, G. P.; Taylor, E. C.; Shih, C.; Poore, G. A.; Grindey, G. B.; Moroson, B. A.; Moran, R. G. *Proc. Am. Assoc. Cancer Res.* **1986**, *27*, 259. (b) Beardsley, G. P.; Moroson, B. A.; Taylor, E. C.; Moran, R. G. *J. Biol. Chem.* **1989**, *264*, 328.

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(7) Pizzorno, G.; Moroson, B. A.; Cashmore, A. R.; Taylor, E. C.; Beardsley, G. P. *Proc. Am. Assoc. Cancer Res.* **1988**, *29*, 281.

(8) Taylor, E. C.; Harrington, P. M.; Shih, C. *Heterocycles* **1989**, *28*, 1169.

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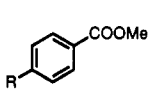
Chart I



4, CH_2CH_2CHO

5, $CH_2CH_2CH_2OH$

6, $CH_2CH_2CH_2OMs$

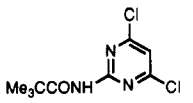
7, $CH_2CH_2CH_2CH_2OMs$

8, $CH_2CH_2CH_2N_3$

9, $CH_2CH_2CH_2CH_2N_3$

10, $CH_2CH_2CH_2NH_2$

11, $CH_2CH_2CH_2CH_2NH_2$



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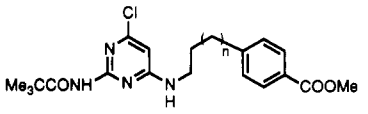
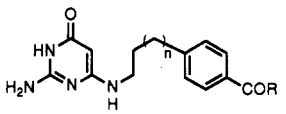


13, n = 1 14, n = 2



15, n = 1, R = OH

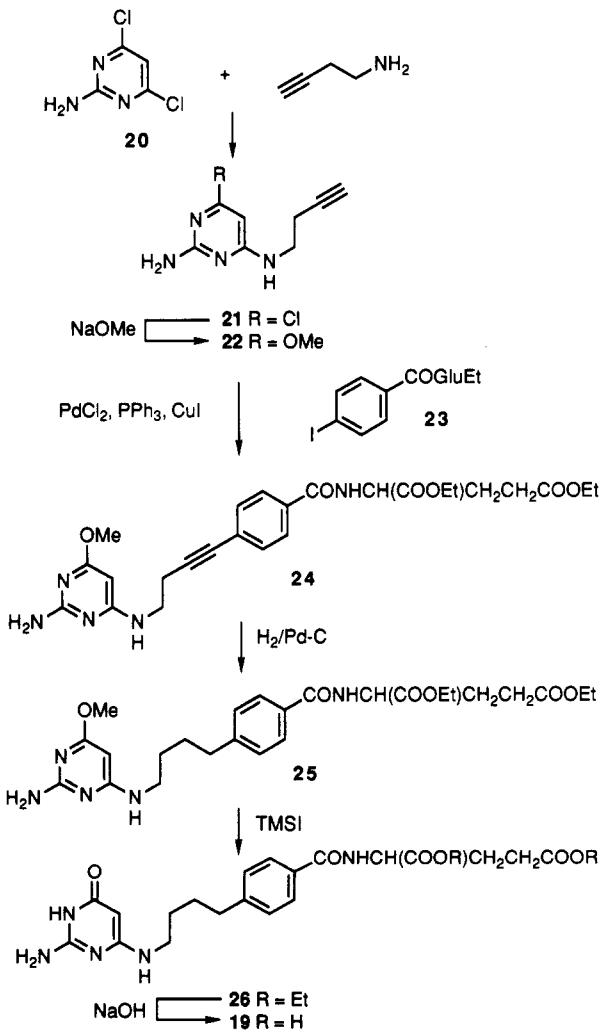
16, n = 2, R = OH

17, n = 1, R = NHCH(COOMe)CH_2CH_2COOMe

18, n = 1, R = NHCH(COOH)CH_2CH_2COOH

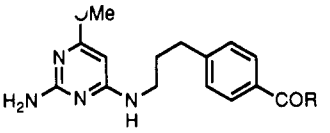
19, n = 2, R = NHCH(COOH)CH_2CH_2COOH

Chart II



itself and in addition proved to be orally effective. Encouraged by these results, we have now prepared several DDATHF analogues in which the chiral center at C-6 has been eliminated by deletion of the methylene group at position 5. Our target compounds were selected to provide us with data on a range of compounds in which (i) the length and rigidity of the tether were varied and (ii) the lactam functionality in the pyrimidine ring was replaced by imino ether or imidoyl chloride functionalities.

Chart III



27, R = OH

28, R = NHCH(COOMe)CH_2CH_2COOMe

29, R = NHCH(COOH)CH_2CH_2COOH

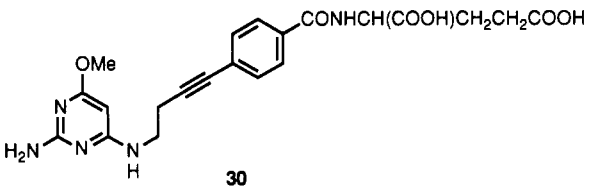


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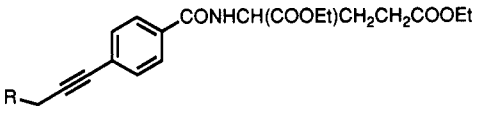
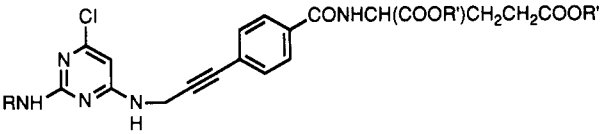
Chart IV



31, R = OH 32, R = OMs 33, R = N_3 34, R = NH_2



35, R = Me_3CCO, R' = Et

36, R = R' = H

For our synthesis of open-chain analogues **18** and **19**, we required mesylates **6** and **7** (Chart I). Methyl 4-iodobenzoate underwent palladium-catalyzed coupling with allyl alcohol[9] to give aldehyde **4**.[10] Sodium borohydride reduction and reaction with methanesulfonyl chloride furnished the required mesylate **6**. The synthesis of **7** has been described previously.[8] Reaction of mesylates **6** and **7** with sodium azide followed by a Staudinger reaction[11] gave amines **10** and **11**, which underwent coupling with 2-(pivaloylamino)-4,6-dichloropyrimidine (**12**)[12] to give the corresponding substituted pyrimidines **13** and **14**. Hydrolysis followed by glutamate coupling gave the dimethyl L-glutamate derivatives which were hydrolyzed to provide the desired target materials.

An alternative approach to compound **19** is shown in Chart II. In this case, palladium-catalyzed coupling of acetylene **22** with diethyl *N*-(4-iodobenzoyl)-L-glutamate[13]

(9) (a) Melpolder, J. B.; Heck, R. F. *J. Org. Chem.* **1976**, *41*, 265. (b) Taylor, E. C.; Szulc, Z. Unpublished results.

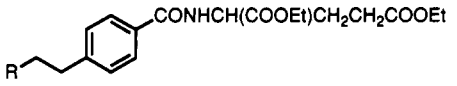
(10) Varma, R. K.; Gordon, E. M. U.S. Patent Appl. 4711900 A 1987; *Chem. Abstr.* **1987**, *109*, 37618c.

(11) Knouzi, N.; Vaultier, M.; Carrié, R. *Bull. Soc. Chim. Fr.* **1985**, 815.

(12) Taylor, E. C.; Vigneswaran, M. Unpublished results.

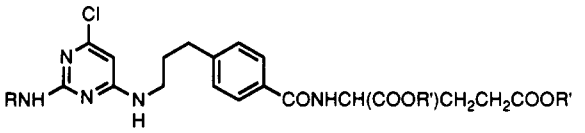
(13) Taylor, E. C.; Wong, G. S. K. *J. Org. Chem.* **1989**, *54*, 3618.

Chart V

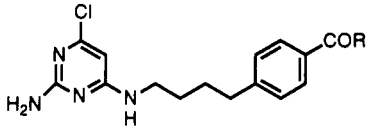


37, R = CHO **38**, R = CH_2OH **39**, R = CH_2OMs

40, R = CH_2N_3 **41**, R = CH_2NH_2

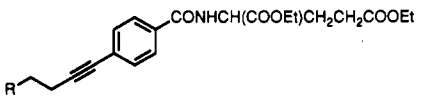


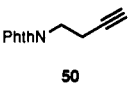
42, R = Me_3CCO, R' = Et **43**, R = R' = H



44, R = OH **45**, R = NHCH(COOH)CH_2CH_2COOH

Chart VI





50

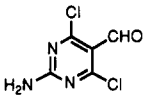
46, R = OH

47, R = OMs

48, R = N_3

49, R = NH_2

51, R = NPhth



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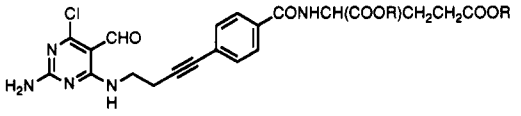


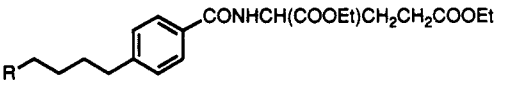
53, R = Et **54**, R = H

proceeded in poor yield, and the overall yield of **19** by this route was much lower.

In order to probe the requirement for the 4-oxo group in the pyrimidine ring, we prepared compounds **29** and **30** in which the lactam functionality is replaced by a lactim ether (Chart III). Treatment of chloropyrimidine **13** with methanolic sodium hydroxide furnished methoxypyrimidine **27** which underwent glutamate coupling and hydrolysis to furnish **29**. Compound **30** was obtained by hydrolysis of diester **24**.

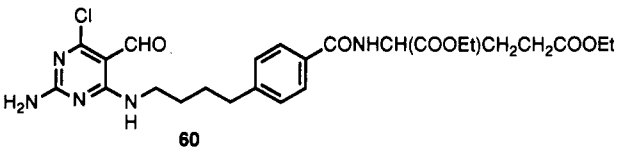
Syntheses of analogues in which the 4-oxo functionality is replaced by a chloro substituent are outlined in Charts IV and V. Glutamate **23** underwent palladium-catalyzed coupling with propargyl alcohol to give alcohol **31**. Mesylation, reaction with sodium azide, and a Staudinger reaction provided amine **34** which was condensed with **12** to give chloropyrimidine **35**. Basic hydrolysis furnished target compound **36**. Preparation of analogue **43** with a saturated tether necessitated the synthesis of alcohol **38**. This was accomplished conveniently in two steps: palladium-catalyzed coupling of glutamate **23** with allyl alcohol gave aldehyde **37** which was reduced to the alcohol with sodium borohydride. Alcohol **38** was then converted to

Chart VII

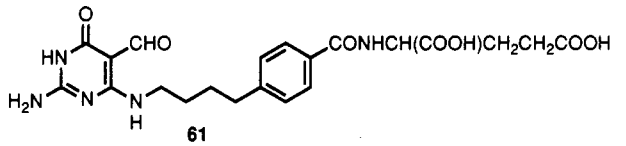


55, R = OMs **56**, R = N_3 **57**, R = NH_2

58, R = H **59**, R = OAc



60



61

Table I. Inhibition of L1210 Cell Growth in Culture[a]

compd	IC_{50} (μg/mL)	compd	IC_{50} (μg/mL)
3	0.132	**36**	>20
18	0.9	**43**	>20
19	2.3	**45**	>20
29	>20	**54**	17.4
30	>20	**61**	>20

[a] Data due to Dr. G. B. Grindey, Eli Lilly & Co.

target compound **43** by the sequence of reactions described above for the unsaturated alcohol **31**. We had already in ester **14** an advanced intermediate in hand for the preparation of our other chloropyrimidine target **45**. Acidic hydrolysis of benzoate **14** to benzoic acid **44** followed by glutamate coupling and ester hydrolysis furnished **45** in good yield.

The effect of formyl substitution in the pyrimidine ring was probed through compounds **54** and **61** (Charts VI and VII). Amine **49** was synthesized by two different methods. The first route started from alcohol **46**[14] and followed the same course as the reactions already described. The second route utilized a palladium-catalyzed C–C coupling reaction of glutamate **23** with acetylene **50**[15] followed by cleavage of the phthalimide protecting group in the resulting coupled product by treatment with methylamine[16] to give the unsaturated amine **49**. The corresponding saturated amine (**57**) was obtained by a Staudinger reaction on azide **56** which was prepared in the usual manner. We had initially planned to synthesize amine **57** by catalytic hydrogenation of the unsaturated azide **48**. However, we found it impossible to obtain good yields of **57** by this approach: in alcoholic solvents, low yields of **57** were obtained, whereas in acetic acid, the unexpected products **58** and **59** were isolated. Amines **49** and **57** underwent coupling with 2-amino-4,6-dichloro-5-formylpyrimidine (**52**)[17] to give esters **53** and **60** which were subjected to alkaline hydrolysis to provide compounds **54** and **61**.

Biological Activity. The above new folate analogues were examined for their ability to inhibit the growth of L1210 cells in culture. The results of these studies are

(14) Harrington, P. M. Ph.D. Thesis, Princeton University, 1989, p 86.
(15) Hoffmann, K.-J.; Stenberg, P.; Ljunggren, C.; Svensson, U.; Nilsson, J.; Eriksson, O.; Hartkoorn, A.; Lundén, R. *J. Med. Chem.* **1975**, *18*, 278.
(16) Wolfe, S.; Hasan, S. K. *Can. J. Chem.* **1970**, *48*, 3572.
(17) Bell, L.; McGuire, H. M.; Freeman, G. A. *J. Heterocycl. Chem.* **1983**, *20*, 41.

shown in Table I; none of these new analogues exhibited significant cytostatic activity.

Experimental Section

Methyl 4-(2-Formylethyl)benzoate (4). A mixture of methyl 4-iodobenzoate (4 g, 15.27 mmol), allyl alcohol (1.56 mL, 22.90 mmol), $NaHCO_3$ (3.21 g, 38.18 mmol), $Pd(OAc)_2$ (103 mg, 0.46 mmol), $(n\text{-}Bu)_4NBr$ (4.92 g, 15.27 mmol), and 4-Å sieves (3 g) in DMF (50 mL) was stirred at 25 °C for 72 h. The reaction mixture was filtered through Celite and the filtrate poured onto water and extracted with ether (7 × 100 mL). The combined extracts were dried over anhydrous Na_2SO_4 and concentrated in vacuo. Flash chromatography[18] (silica gel, 30% ether–hexanes) gave 2.67 g (91%) of **4** as a clear oil: ^1H NMR ($CDCl_3$, 300 MHz) δ 2.78 (t, 2 H, J = 7 Hz), 2.96 (t, 2 H, J = 7 Hz), 3.88 (s, 3 H), 7.25 and 7.94 (AA'BB', 4 H), 9.79 (s, 1 H); EIMS m/e 192 (M^+), 161 (base), 150, 133, 105, 91, 77; HRMS calcd for $C_{11}H_{12}O_3$ 192.0783, found 192.0802.

Methyl 4-(3-Hydroxypropyl)benzoate (5). Sodium borohydride (1.51 g, 39.69 mmol) was added in portions to a solution of aldehyde **4** (5.08 g, 26.46 mmol) in CH_3OH (100 mL) at 0 °C, and the resulting solution was stirred at 25 °C for 1 h. The reaction mixture was poured onto water and extracted with EtOAc (4 × 100 mL). The combined extracts were dried over anhydrous Na_2SO_4 and concentrated in vacuo. Flash chromatography (silica gel, 50% ether–hexanes) gave 4.94 g (96%) of **5** as a clear oil: ^1H NMR ($CDCl_3$, 300 MHz) δ 1.91 (m, 2 H, J = 7 Hz), 2.77 (t, 2 H, J = 7 Hz), 3.68 (t, 2 H, J = 7 Hz), 3.90 (s, 3 H), 7.27 and 7.96 (AA'BB', 4 H); EIMS m/e 194 (M^+), 176, 145 (base), 117, 91, 77; HRMS calcd for $C_{11}H_{14}O_3$ 194.0939, found 194.0962. Anal. Calcd for $C_{11}H_{14}O_3$: C, 68.02; H, 7.27. Found: C, 67.98; H, 7.46.

Methyl 4-[3-(Methanesulfonyloxy)propyl]benzoate (6). To a solution of alcohol **5** (1.34 g, 6.91 mmol) in THF (30 mL) at 0 °C was added TEA (1.45 mL, 10.37 mmol) followed by MsCl (0.63 mL, 8.29 mmol), and the resulting solution was allowed to stir at 25 °C for 3 h. The reaction mixture was poured onto water and extracted with CH_2Cl_2 (3 × 100 mL). The combined extracts were dried over anhydrous Na_2SO_4 and concentrated in vacuo. Flash chromatography (silica gel, 50% ether–hexanes) gave 1.85 g (97%) of **6** as a pale yellow solid: mp 42–45 °C; ^1H NMR ($CDCl_3$, 300 MHz) δ 2.11 (m, 2 H, J = 7 Hz), 2.82 (t, 2 H, J = 7 Hz), 3.00 (s, 3 H), 3.90 (s, 3 H), 4.23 (t, 2 H, J = 7 Hz), 7.26 and 7.96 (AA'BB', 4 H); EIMS m/e 272 (M^+), 212, 176, 145 (base), 115, 91; HRMS calcd for $C_{12}H_{16}O_5S$ 272.0714, found 272.0725. Anal. Calcd for $C_{12}H_{16}O_5S$: C, 52.93; H, 5.92. Found: C, 52.74; H, 6.10.

Methyl 4-(3-Azidopropyl)benzoate (8). Sodium azide (5.07 g, 77.79 mmol) was added to a solution of mesylate **6** (7.07 g, 25.99 mmol) in DMF (50 mL) at 25 °C, and the resulting solution was stirred at 25 °C for 18 h. The reaction mixture was poured onto water and extracted with CH_2Cl_2 (2 × 100 mL). The combined extracts were dried over anhydrous Na_2SO_4 and concentrated in vacuo. Flash chromatography (silica gel, 50% ether–hexanes) gave 5.28 g (93%) of **8** as a pale yellow oil: ^1H NMR ($CDCl_3$, 300 MHz) δ 1.92 (m, 2 H, J = 7 Hz), 2.76 (t, 2 H, J = 7 Hz), 3.29 (t, 2 H, J = 7 Hz), 3.90 (s, 3 H), 7.25 and 7.97 (AA'BB', 4 H). Anal. Calcd for $C_{11}H_{13}N_3O_2$: C, 60.26; H, 5.98; N, 19.17. Found: C, 60.01; H, 5.89; N, 19.09.

Methyl 4-(4-Azidobutyl)benzoate (9). Azide **9** was prepared in 85% yield from mesylate **7**[8] by the procedure described for the preparation of **8**: ^1H NMR ($CDCl_3$, 270 MHz) δ 1.57–1.74 (m, 4 H), 2.68 (t, 2 H, J = 6.9 Hz), 3.26 (t, 2 H, J = 6.6 Hz), 3.88 (s, 3 H), 7.23 and 7.96 (AA'BB', 4 H); ^{13}C NMR ($CDCl_3$, 67.9 MHz) δ 27.9, 28.2, 35.2, 51.0, 51.7, 127.9, 128.2, 129.6, 147.2, 166.8; EIMS m/e 204 ($M^+ - N_2 - H$), 176, 172 (base), 162, 149, 131; HRMS calcd for $C_{12}H_{14}NO_2$ ($M^+ - N_2 - H$) 204.1025, found 204.1022. Anal. Calcd for $C_{12}H_{15}N_3O_2$: C, 61.79; H, 6.48; N, 18.01. Found: C, 61.64; H, 6.50; N, 17.74.

Methyl 4-(3-Aminopropyl)benzoate (10). To a solution of azide **8** (2.06 g, 9.41 mmol) in THF (18 mL) at 25 °C was added water (0.25 mL, 14.12 mmol) and Ph_3P (2.47 g, 9.41 mmol), and the resulting reaction mixture was stirred at 25 °C for 18 h. The reaction mixture was poured onto 10% HCl and extracted with ether (3 × 100 mL). The organic layer was discarded. The aqueous layer was made basic (pH = 9) with 10% NaOH and extacted with CH_2Cl_2 (4 × 100 mL). The combined extracts were dried over anhydrous Na_2SO_4 and concentrated in vacuo to give 1.78 g (98%) of **10** as a pale yellow oil: ^1H NMR ($CDCl_3$, 300 MHz) δ 1.20 (br s, 1 H), 1.73 (m, 2 H, J = 7 Hz), 2.70 (app q, 4 H), 3.87 (s, 3 H) 7.23 and 7.93 (AA'BB', 4 H); EIMS m/e 193 (M^+), 176 (base), 161, 148, 116, 91, 77; HRMS calcd for $C_{11}H_{15}NO_2$ 193.1103, found 193.1110.

Methyl 4-(4-Aminobutyl)benzoate (11). Amine **11** was prepared in 95% yield from azide **9** by the procedure described for the preparation of **10**: mp 120–122 °C; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.42–1.47 (m, 2 H), 1.50–1.63 (m, 2 H), 1.66 (br s, 2 H), 2.50–2.69 (m, 4 H), 3.85 (s, 3 H), 7.20 and 7.92 (AA'BB', 4 H); ^{13}C NMR ($CDCl_3$, 75.6 MHz) δ 27.8, 32.6, 35.2, 41.4, 51.4, 127.2, 127.9, 129.1, 147.5, 166.5; EIMS m/e 207 (M^+), 192, 172, 162, 150 (base), 131; HRMS calcd for $C_{12}H_{17}NO_2$ 207.1259, found 207.1258.

Methyl 4-[3-[[6-Chloro-2-(pivaloylamino)pyrimidin-4-yl]amino]propyl]benzoate (13). Triethylamine (1.4 mL, 10.16 mmol) was added to a solution of amine **10** (1.78 g, 9.24 mmol) and pyrimidine **12**[12] (2.74 g, 11.09 mmol) in THF (20 mL) at 25 °C, and the resulting reaction mixture was stirred at 25 °C for 2 h. The reaction mixture was poured onto water and extracted with CH_2Cl_2 (3 × 100 mL). The combined extracts were dried over anhydrous Na_2SO_4 and concentrated in vacuo. Flash chromatography (silica gel, 50% ether–hexanes) gave 3.46 g (93%) of **13** as a white foam: ^1H NMR ($CDCl_3$, 300 MHz) δ 1.26 (s, 9 H), 1.94 (m, 2 H), 2.7 (m, 4 H), 3.88 (s, 3 H), 6.00 (s, 1 H), 7.20 and 7.93 (AA'BB', 4 H), 7.82 (s, 1 H); EIMS m/e 404 (M^+), 284, 256, 242 (base), 213, 171, 158, 145, 97, 85; HRMS calcd for $C_{20}H_{25}ClN_4O_3$ 404.1615, found 404.1601.

Methyl 4-[3-[[6-Chloro-2-(pivaloylamino)pyrimidin-4-yl]amino]butyl]benzoate (14). Ester **14** was prepared in 61% yield from amine **11** by the procedure described for the preparation of **13**: ^1H NMR ($CDCl_3$, 300 MHz) δ 1.29 (s, 9 H), 1.50–1.80 (m, 4 H), 2.68 (t, 2 H, J = 7.2 Hz), 3.91 (s, 3 H), 6.15 (br s, 2 H), 7.22 and 7.95 (AA'BB', 5 H); ^{13}C NMR ($CDCl_3$, 67.9 MHz) δ 20.6, 26.9, 27.7, 28.3, 35.0, 39.8, 51.5, 127.5, 128.0, 129.3, 147.1, 156.5, 163.9, 166.6, 170.6, 175.3; EIMS m/e 418 (M^+), 329, 269, 255 (base) 228; HRMS calcd for $C_{21}H_{27}ClN_4O_3$ 418.1772, found 418.1761.

4-[3-[(2-Amino-1,6-dihydro-6-oxopyrimidin-4-yl)amino]propyl]benzoic Acid (15). A slurry of ester **13** (450 mg, 1.1 mmol) in 3 N NaOH (5 mL) was allowed to reflux for 20 h. The reaction mixture was cooled, acidified with excess glacial HOAc and filtered. The residue was washed with ether (30 mL) followed by water (50 mL) and dried in vacuo to provide 317 mg (95%) of **15** as a white solid: mp >300 °C; ^1H NMR (DMSO-d_6, 300 MHz) δ 1.95 (m, 2 H), 2.3 (m, 2 H), 3.2 (m, 2 H), 5.65 (s, 1 H), 6.10 (br s, 1 H) 6.40 (br s, 2 H) 7.30 and 7.85 (AA'BB', 4 H), 9.65 (br s, 1 H), 12.65 (br s, 1 H); LRFABMS m/e 289 (MH^+), 177, 155, 119 (base), 103; HRFABMS calcd for $C_{14}H_{17}N_4O_3$ 289.1301 (MH^+), found 289.1309.

4-[3-[(2-Amino-1,6-dihydro-6-oxopyrimidin-4-yl)amino]butyl]benzoic Acid (16). Acid **16** was prepared as a white solid in 64% yield from ester **14** by the procedure described above for the preparation of **15**: mp >300 °C; ^1H NMR (DMSO-d_6, 300 MHz) δ 1.44–1.57 (m, 4 H), 2.63 (br s, 2 H), 3.03 (br s, 2 H), 4.40 (s, 1 H), 6.20 (br s, 2 H), 6.35 (br s, 1 H), 7.29 and 7.83 (AA'BB', 4 H), 9.84 (br s, 1 H), 12.60 (br s, 1 H); HRFABMS calcd for $C_{15}H_{19}N_4O_3$ (MH^+) 303.1457, found 303.1440.

Dimethyl *N*-[4-[3-[(2-Amino-1,6-dihydro-6-oxopyrimidin-4-yl)amino]propyl]benzoyl]-L-glutamate (17). To a stirred slurry of acid **15** (300 mg, 1.04 mmol) in DMF (4 mL) at 25 °C was added *N*-methylmorpholine (NMM) (0.14 mL, 1.25 mmol) followed by 2-chloro-4,6-dimethoxy-1,3,5-triazine[19] (201 mg, 1.15 mmol), and the resulting reaction mixture was stirred at 25 °C for 40 min. NMM (0.14 mL, 1.25 mmol) was added to the solution followed by dimethyl L-glutamate hydrochloride (285 mg, 1.35 mmol), and the resulting reaction mixture was allowed to stir at 25 °C for 24 h. The reaction mixture was concentrated in vacuo and the residue taken up $CHCl_3$ (50 mL). The $CHCl_3$ layer was washed with 5% $NaHCO_3$, dried over anhydrous Na_2SO_4, and

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concentrated in vacuo. Flash chromatography (silica gel, 7% CH_3OH–CH_2Cl_2) gave 256 mg (55%) of **17** as a clear foam: ^1H NMR ($CDCl_3$, 300 MHz) δ 1.85 (m, 2 H) 2.1 (m, 1 H), 2.15 (m, 1 H), 2.45 (m, 2 H), 2.60 (m, 2 H), 3.0 (m, 2 H), 3.6 (s, 3 H), 3.75 (s, 3 H), 4.75 (br s, 1 H), 4.80 (m, 1 H), 5.3 (s, 1 H), 5.5 (br s, 1 H), 6.2 (br s, 1 H), 7.05 and 7.70 (AA'BB', 4 H), 7.6 (d, 1 H, J = 7 Hz); EIMS m/e 445 (M^+), 353, 285, 207, 167, 154 (base) 140, 111, 98, 84, 69; HRMS calcd for $C_{21}H_{27}N_5O_6$ 445.1961, found 445.1970.

***N*-[4-[3-[(2-Amino-1,6-dihydro-6-oxopyrimidin-4-yl)amino]propyl]benzoyl]-L-glutamic Acid (18).** A solution of glutamate **17** (106 mg, 0.24 mmol) in 1 N NaOH (2.5 mL) was allowed to stir at 25 °C for 24 h. The reaction mixture was acidified with excess glacial HOAc and filtered. The residue was washed with ether (20 mL) followed by water (100 mL) and dried in vacuo to provide 82 mg (82%) of **18** as a white solid: mp 205–208 °C; ^1H NMR (DMSO-d_6, 300 MHz) δ 1.75 (m, 2 H), 1.95 (m, 1 H), 2.0 (m, 1 H), 2.30 (m, 2 H), 2.65 (m, 2 H), 3.00 (m, 2 H), 4.40 (m, 1 H), 4.40 (s, 1 H), 6.10 (br s, 2 H), 6.40 (br s, 1 H), 7.30 and 7.80 (AA'BB', 4 H), 8.50 (d, 1 H, J = 7 Hz), 9.65 (br s, 1 H), 12.40 (br s, 2 H); HRFABMS calcd for $C_{19}H_{24}N_5O_6$ (MH^+) 418.1727, found 418.1744.

***N*-[4-[4-[(2-Amino-1,6-dihydro-6-oxopyrimidin-4-yl)amino]butyl]benzoyl]-L-glutamic Acid (19). From 16.** Compound **16** was coupled with dimethyl L-glutamate by the procedure described above for the preparation of **17**. The crude diester was hydrolyzed by the procedure described above for the preparation of **18** to give **19** in 32% overall yield: mp >200 °C; ^1H NMR (DMSO-d_6, 300 MHz) δ 1.40–1.61 (m, 4 H), 1.86–1.97 (m, 1 H), 1.99–2.10 (m, 1 H), 2.31 (t, 2 H, J = 7.4 Hz), 2.61 (t, 2 H, J = 7.3 Hz), 3.33 (m, 2 H), 6.09 (br s, 2 H), 6.34 (br s, 1 H), 7.26 and 7.76 (AA'BB', 4 H), 8.48 (d, 1 H, J = 7.6 Hz), 9.84 (br s, 1 H), 12.36 (br s, 2 H); HRFABMS calcd for $C_{20}H_{28}N_5O_6$ (MH^+) 432.1883, found 432.1882.

From 26. Diester **26** was hydrolyzed by the procedure described above for the preparation of **18** to give **19** in 40% yield.

2-Amino-4-(3-butynylamino)-6-chloropyrimidine (21). A solution of **20** (8.20 g, 50 mmol), 3-butynylamine (3.45 g, 50 mmol) and NEt_3 (5.06 g, 50 mmol) was heated under reflux in EtOH (100 mL) for 13 h. The reaction mixture was filtered and the filtrate concentrated in vacuo. The residue was taken up in H_2O (300 mL) and extracted with $CHCl_3$ (2 × 300 mL). The combined extracts were dried over anhydrous $MgSO_4$ and concentrated in vacuo. The residue upon recrystallization from H_2O gave 5.93 g (60%) of **21** as white needles: mp 74–76 °C; ^1H NMR ($CDCl_3$, 300 MHz) δ 2.06 (t, 1 H, J = 2.5 Hz), 2.51 (dt, 2 H, J = 7.0, 2.5 Hz), 3.49 (m, 2 H), 4.93 (br s, 2 H), 5.10 (br s, 1 H), 5.84 (s, 1 H); EIMS m/e 196 (M^+), 195, 157 (base), 105; HRMS calcd for $C_8H_9ClN_4$ 196.0516, found 196.0510. Anal. Calcd for $C_8H_9ClN_4$: C, 48.87; H, 4.61; N, 28.49; Cl, 18.03. Found: C, 49.14; H, 4.66; N, 28.21; Cl, 18.20.

2-Amino-4-(3-butynylamino)-6-methoxypyrimidine (22). NaOMe (2.50 g, 46 mmol) was added to a solution of **21** (4.07 g, 21 mmol) in MeOH (200 mL). The reaction mixture was heated at reflux for 24 h. An additional portion of NaOMe (2.60 g, 48 mmol) was added, and the solution was heated at reflux for 48 h, filtered to remove NaCl, concentrated to about 80 mL, and heated in a sealed tube at 140–146 °C for 4 h. The reaction mixture was filtered and the filtrate was concentrated in vacuo. Flash chromatography (silica gel, 2% MeOH–CH_2Cl_2) gave 2.17 g (54%) of **22** as an oil: ^1H NMR ($CDCl_3$, 300 MHz) δ 2.03 (t, H, J = 2.5 Hz), 2.48 (dt, 2 H, J = 6.5, 2.5 Hz), 3.40 (app q, 2 H, J = 6.5 Hz), 3.83 (s, 3 H), 4.79 (br s, 2 H), 4.95 (br s, 1 H), 5.18 (s, 1 H); EIMS m/e 192 (M^+), 177, 153 (base), 140; HRMS calcd for $C_9H_{12}N_4O$ 192.1011, found 192.1004.

Diethyl *N*-[4-[4-[(2-Amino-6-methoxypyrimidin-4-yl)amino]but-1-ynyl]benzoyl]-L-glutamate (24). A mixture of **22** (2.70 g, 14.05 mmol), **23**[13] (6.09 g, 14.05 mmol), $PdCl_2$ (55 mg, 0.31 mmol), CuI (120 mg, 0.63 mmol), PPh_3 (167 mg, 0.64 mol), and NEt_3 (5 mL) was heated under reflux in CH_3CN (100 mL) for 12 h. The reaction mixture was concentrated in vacuo. The residue taken up in $CHCl_3$ (200 mL) and washed with H_2O (3 × 200 mL). The $CHCl_3$ solution was dried over anhydrous $MgSO_4$ and concentrated in vacuo. Flash chromatography (silica gel, 7% MeOH–CH_2Cl_2) gave 2.07 g (30%) of **24** as an oil: ^1H NMR ($CDCl_3$, 300 MHz) δ 1.24 (t, 3 H, J = 7.2 Hz), 1.32 (t, 3 H, J = 7.2 Hz), 2.12–2.22 (m, 1 H), 2.30–2.42 (m, 1 H), 2.46–2.57 (m, 2 H), 2.73 (t, 2 H, J = 6.8 Hz), 3.49 (m, 2 H), 3.84 (s, 3 H), 4.11 (q, 2 H, J = 7.2 Hz), 4.26 (q, 2 H, J = 7.2 Hz), 4.76–4.82 (m, 3 H), 5.15 (br s, 1 H), 5.27 (s, 1 H), 7.14 (d, 1 H, J = 7.4 Hz), 7.46 and 7.76 (AA'BB', 4 H); ^{13}C NMR ($CDCl_3$, 67.9 MHz) δ 13.9, 20.2, 26.9, 30.4, 40.3, 52.3, 53.1, 60.6, 61.5, 77.2, 81.4, 89.7, 126.8, 126.9, 131.5, 132.6, 162.4, 164.8, 166.5, 171.3, 171.9, 173.0; EIMS m/e 497 (M^+), 452, 295, 266, 191, 153 (base); HRMS calcd for $C_{25}H_{31}N_5O_6$ 497.2274, found 497.2256.

Diethyl *N*-[4-[4-[(2-Amino-6-methoxypyrimidin-4-yl)amino]butyl]benzoyl]-L-glutamate (25). To a solution of 0.50 g (1 mmol) of **24** in CF_3COOH (50 mL) was added 10% Pd–C (200 mg). The mixture was hydrogenated on a Parr shaker for 4 h at 50 psi. The catalyst was filtered off through Celite and the filtrate concentrated in vacuo. The residue was taken up in $CHCl_3$ (70 mL) and washed with water (2 × 50 mL). The $CHCl_3$ solution was dried over anhydrous $MgSO_4$ and concentrated in vacuo to give 340 mg (68%) of **25** as a yellow gum: ^1H NMR ($CDCl_3$, 300 MHz) δ 1.24 (t, 3 H, J = 7.1 Hz), 1.32 (t, 3 H, J = 7.1 Hz), 1.56–1.74 (m, 4 H), 2.11–2.21 (m, 1 H), 2.30–2.41 (m, 1 H), 2.45–2.56 (m, 2 H), 2.69 (t, 2 H, J = 7.2 Hz), 3.19 (m, 2 H), 3.83 (s, 3 H), 4.13 (q, 2 H, J = 7.1 Hz), 4.25 (q, 2 H, J = 7.1 Hz), 4.73 (br s, 3 H), 4.77–4.83 (m, 1 H), 5.13 (s, 1 H), 7.08 (d, 1 H, J = 7.5 Hz), 7.24 and 7.75 (AA'BB', 4 H); EIMS m/e 501 (M^+), 456, 299, 202, 181, 153 (base); HRMS calcd for $C_{25}H_{35}N_5O_6$ 501.2587, found 501.2616.

Diethyl *N*-[4-[4-[(2-Amino-1,6-dihydro-6-oxopyrimidin-4-yl)amino]butyl]benzoyl]-L-glutamate (26). To a solution of **25** (210 mg, 0.42 mmol) in $CHCl_3$ (50 mL) was added dropwise Me_3SiI (0.6 mL, 4.2 mmol), and the resulting reaction mixture was heated under reflux for 2 h. The reaction mixture was quenched with MeOH (100 mL) and concentrated in vacuo. Flash chromatography (silica gel, 14% MeOH–CH_2Cl_2) gave 170 mg (83%) of **26** as an orange glass: ^1H NMR ($CDCl_3$, 300 MHz) δ 1.25 (t, 3 H, J = 7.2 Hz), 1.33 (t, 3 H, J = 7.2 Hz), 1.51–1.76 (m, 4 H), 2.13–2.23 (m, 1 H), 2.29–2.37 (m, 1 H), 2.39–2.57 (m, 2 H), 2.67–2.74 (m, 2 H), 3.42–3.50 (m, 2 H), 4.14 (q, 2 H, J = 7.2 Hz), 4.25 (q, 2 H, J = 7.2 Hz), 4.75–4.82 (m, 1 H), 5.13–5.17 (m, 1 H), 5.95 (br s, 2 H), 7.11 (d, 1 H, J = 7.6 Hz), 7.24 and 7.76 (AA'BB', 4 H), 11.20 (br s, 1 H); EIMS m/e 487, 285 (M^+, base) 213, 202; HRMS calcd for $C_{24}H_{33}N_5O_6$ 487.2431, found 487.2439.

4-[3-[(2-Amino-6-methoxypyrimidin-4-yl)amino]propyl]benzoic Acid (27). A solution of ester **13** (1.08 g, 2.67 mmol) in 3 N NaOH (10 mL) and CH_3OH (2 mL) was heated to reflux for 20 h. The reaction mixture was cooled, acidified with excess glacial HOAc, and filtered. The residue was washed with ether (10 mL) and water (100 mL) and dried in vacuo to provide 0.70 g (87%) of **27** as a white solid: mp 194–196 °C; ^1H NMR (DMSO-d_6, 300 MHz) δ 1.75 (m, 2 H), 2.65 (t, 2 H, J = 7 Hz), 5.00 (s, 1 H), 5.89 (br s, 2 H), 6.60 (br t, 1 H), 7.30 and 7.82 (AA'BB', 4 H), 12.65 (br s, 1 H); EIMS m/e 302 (M^+), 166, 154 (base), 138, 129, 125, 111, 97, 81, 71; HRMS calcd for $C_{15}H_{18}N_4O_3$ 302.1379, found 302.1394.

Dimethyl *N*-[4-[3-[(2-Amino-6-methoxypyrimidin-4-yl)amino]propyl]benzoyl]-L-glutamate (28). Diester **28** was prepared as a clear foam in 68% yield from acid **27** by the procedure described above for the preparation of **17**: ^1H NMR ($CDCl_3$, 300 MHz) δ 1.98 (m, 2 H), 2.15 (m, 1 H), 2.35 (m, 1 H), 2.55 (m, 2 H), 2.65 (m, 2 H), 3.2 (m, 2 H), 3.63 s, 3 H), 3.76 (s, 3 H), 3.78 (s, 3 H), 4.8 (br s, 2 H), 4.8 (m, 1 H), 4.95 (br t, 1 H), 5.07 (s, 2 H), 7.2 and 7.65 (AA'BB', 4 H), 7.25 (br s, 1 H); EIMS m/e 459 (M^+), 428, 400, 285, 167, 154 (base), 140, 125, 98; HRMS calcd for $C_{22}H_{29}N_5O_6$ 459.2118, found 459.2092.

***N*-[4-[3-[(2-Amino-6-methoxypyrimidin-4-yl)amino]propyl]benzoyl]-L-glutamic Acid (29).** Diacid **29** was prepared in 64% yield from diester **28** by the procedure described above for the preparation of **18**: mp 186–188 °C; ^1H NMR (DMSO-d_6, 300 MHz) δ 1.75 (m, 2 H), 1.95 (m, 1 H), 2.05 (m, 1 H), 2.35 (m, 2 H), 2.65 (m, 2 H), 3.15 (m, 2 H), 3.65 (s, 3 H), 4.40 (m, 1 H), 5.01 (s, 1 H), 5.87 (br s, 2 H), 6.56 (br t, 1 H), 7.29 and 7.78 (AA'BB', 4 H), 8.47 (d, 1 H, J = 7 Hz); LRFABMS m/e 432 (M^+ + H), 330, 309, 279, 155, 119 (base), 103; HRFABMS calcd for $C_{20}H_{26}N_5O_6$ 432.1883 (MH^+), found 432.1859.

***N*-[4-[4-[(2-Amino-6-methoxypyrimidin-4-yl)amino]but-1-ynyl]benzoyl]-L-glutamic Acid (30).** Diacid **30** was prepared in 40% yield from diester **24** by the procedure described for the

preparation of 18: mp 170 °C dec; ^{1}H NMR (DMSO-d_6, 270 MHz) δ 2.04–2.28 (m, 2 H), 2.46–2.55 (m, 2 H), 2.76–2.90 (m, 2 H), 3.55–3.60 (m, 2 H), 3.82 (s, 3 H), 4.48–4.54 (m, 1 H), 5.26 (s, 1 H), 6.15 (br s, 1 H), 7.61 and 8.00 (AA'BB', 4 H), 8.82 (d, 1 H, J = 7.6 Hz), 12.60 (br s, 2 H); HRFABMS calcd for $C_{21}H_{24}N_5O_6$ (MH^+) 442.1727, found 442.1741.

Diethyl *N*-[4-(3-Hydroxy-1-propynyl)benzoyl]-L-glutamate (31). A mixture of **23** (10 g, 23.09 mmol), CuI (73 mg, 0.38 mmol), PPh_3 (100 mg, 0.38 mmol), $PdCl_2$ (33 mg, 0.19 mmol), and propargyl alcohol (2.22 mL, 38.16 mmol) in $HNEt_2$ (75 mL) was allowed to stir at 25 °C for 48 h. The reaction mixture was concentrated in vacuo. Flash chromatography (silica gel, ether) gave 7.64 g (92%) of **31** as a yellow oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.24 (t, 3 H, J = 7 Hz), 1.32 (t, 3 H, J = 7 Hz), 1.80 (br s, 1 H), 2.20 (m, 1 H), 2.4 (m, 1 H), 2.55 (m, 2 H), 4.05 (q, 2 H, J = 7 Hz), 4.25 (q, 2 H, J = 7 Hz), 4.52 (d, 2 H, J = 5 Hz), 4.80 (m, 1 H), 7.20 (d, 1 H, J = 5 Hz), 7.47 and 7.79 (AA'BB', 4 H); EIMS m/e 361 (M^+), 287, 257, 202, 158 (base), 129, 112, 84; HRMS calcd for $C_{19}H_{23}NO_6$ 361.1525, found 361.1554.

Diethyl *N*-[4-[3-(Methanesulfonyloxy)-1-propynyl]benzoyl]-L-glutamate (32). Mesylate **32** was prepared in 91% yield from alcohol **31** by the procedure described above for the preparation of **6**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.24 (t, 3 H, J = 7 Hz), 1.32 (t, 3 H, J = 7 Hz), 2.20 (m, 1 H), 2.40 (m, 1 H), 2.55 (m, 2 H), 3.14 (s, 3 H), 4.05 (q, 2 H, J = 7 Hz), 4.25 (q, 2 H, J = 7 Hz), 4.80 (m, 1 H), 5.07 (s, 2 H), 7.20 (d, 1 H, J = 7 Hz), 7.47 and 7.79 (AA'BB', 4 H); EIMS m/e 439 (M^+), 394, 366, 344, 237 (base), 202, 159, 142, 112, 84; HRMS calcd for $C_{20}H_{25}NO_8S$ 439.1301, found 439.1282.

Diethyl *N*-[4-(3-Azido-1-propynyl)benzoyl]-L-glutamate (33). Azide **33** was prepared in 80% yield from mesylate **32** by the procedure described above for the preparation of **8**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.24 (t, 3 H, J = 7 Hz), 1.32 (t, 3 H, J = 7 Hz), 2.20 (m, 1 H), 2.40 (m, 1 H), 2.55 (m, 2 H), 4.05 (q, 2 H, J = 7 Hz), 4.17 (s, 2 H), 4.25 (q, 2 H, J = 7 Hz), 4.80 (m, 1 H), 7.20 (d, 1 H, J = 7 Hz), 7.47 and 7.79 (AA'BB', 4 H); EIMS m/e 386 (M^+), 358, 312, 285, 239, 202, 156 (base), 112, 84; HRMS calcd for $C_{19}H_{22}N_4O_5$ 386.1590, found 386.1589.

Diethyl *N*-[4-(3-Amino-1-propynyl)benzoyl]-L-glutamate (34). Amine **34** was prepared in 61% yield from azide **33** by the procedure described above for the preparation of **10**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.24 (t, 3 H, J = 7 Hz), 1.32 (t, 3 H, J = 7 Hz), 2.20 (m, 1 H), 2.40 (m, 1 H), 2.55 (m, 2 H), 3.65 (br s, 2 H), 4.05 (q, 2 H, J = 7 Hz), 4.25 (q, 2 H, J = 7 Hz), 4.80 (m, 1 H), 5.15 (s, 2 H), 7.30 (d, 1 H, J = 7 Hz), 7.37 and 7.71 (AA'BB', 4 H); EIMS m/e 360 (M^+), 287, 202, 158 (base), 112, 77; HRMS calcd for $C_{19}H_{24}N_2O_5$ 360.1685, found 360.1666.

Diethyl *N*-[4-[3-[[2-(Pivaloylamino)-6-chloropyrimidin-4-yl]amino]-1-propynyl]benzoyl]-L-glutamate (35). Diester **35** was prepared in 90% yield from amine **34** by the procedure described above for the preparation of **13**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.24 (t, 3 H, J = 7 Hz), 1.32 (t, 3 H, J = 7 Hz), 1.35 (s, 9 H), 1.80 (s, 2 H), 2.20 (m, 1 H), 2.35 (m, 1 H), 2.55 (m, 2 H), 4.05 (q, 2 H, J = 7 Hz), 4.25 (q, 2 H, J = 7 Hz), 4.40 (br s, 1 H), 4.80 (m, 1 H), 6.25 (s, 1 H), 7.20 (d, 1 H, J = 7 Hz), 7.45 and 7.80 (AA'BB', 4 H), 7.91 (br s, 1 H); EIMS m/e 570 (M^+ – 2H), 513, 514, 487, 485, 370, 368, 312, 310, 285, 283, 257, 255, 246, 128, 84; HRMS calcd for $C_{28}H_{35}ClN_5O_6$ 572.2275, found 572.2248.

***N*-[4-[3-[(2-Amino-6-chloropyrimidin-4-yl)amino]-1-propynyl]benzoyl]-L-glutamic Acid (36).** Diacid **36** was prepared in 45% yield from diester **35** by the procedure described above for the preparation of **18**: mp 138–140 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 2.00 (m, 2 H), 2.2 (m, 2 H), 4.40 (m, 3 H), 5.90 (s, 1 H), 6.6 (br s, 2 H), 7.50 and 7.90 (AA'BB', 4 H), 7.60 (br s, 1 H), 8.60 (br s, 1 H), 12.50 (br s, 2 H); LRFABMS, m/e 432 (M^+), 287, 285, 229, 267, 155, 119 (base); HRFABMS calcd for $C_{19}H_{18}ClN_5O_5$ 432.1075, found 432.1069.

Diethyl *N*-[4-(2-Formylethyl)benzoyl]-L-glutamate (37). Aldehyde **37** was prepared in 74% yield from **23**[13] by the procedure described above for the preparation of **4**: mp 75–76 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.24 (t, 3 H, J = 7 Hz), 1.28 (t, 3 H, J = 7 Hz), 2.15 (m, 1 H), 2.35 (m, 1 H), 2.55 (m, 2 H), 2.83 (t, 2 H, J = 7 Hz), 3.03 (t, 2 H, J = 7 Hz), 4.15 (q, 2 H, J = 7 Hz), 4.27 (q, 2 H, J = 7 Hz), 4.80 (m, 1 H), 7.05 (d, 1 H, J = 7 Hz), 7.30 and 7.78 (AA'BB', 4 H), 9.85 (s, 1 H); EIMS m/e 363 (M^+), 290, 202, 161 (base), 131, 112, 91, 84; HRMS calcd for $C_{19}H_{25}NO_6$ 363.1682, found 363.1667. Anal. Calcd for $C_{19}H_{25}NO_6$: C, 62.80; H, 6.93; N, 3.85. Found: C, 62.81; H, 6.98; N, 4.00.

Diethyl *N*-[4-(3-Hydroxypropyl)benzoyl]-L-glutamate (38). Alcohol **38** was prepared in 93% yield by the procedure described above for the preparation of **5**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.20 (t, 3 H, J = 7 Hz), 1.35 (t, 3 H, J = 7 Hz), 1.95 (t, 2 H, J = 7 Hz), 2.15 (m, 1 H), 2.35 (m, 1 H), 2.55 (m, 2 H), 2.80 (t, 3 H, J = 7 Hz), 3.80 (br s, 2 H), 4.05 (q, 2 H, J = 7 Hz), 4.20 (q, 2 H, J = 7 Hz), 4.80 (m, 1 H), 7.05 (d, 1 H, J = 7 Hz), 7.25 and 7.74 (AA'BB', 4 H); EIMS, m/e 365 (M^+), 292, 202, 163 (base), 145, 112, 91; HRMS calcd for $C_{19}H_{27}NO_6$ 365.1838, found 365.1817.

Diethyl *N*-[4-[3-(Methanesulfonyloxy)propyl]benzoyl]-L-glutamate (39). Mesylate **39** was prepared in 94% yield by the procedure described above for the preparation of **6**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.20 (t, 3 H, J = 7 Hz), 1.35 (t, 3 H, J = 7 Hz), 2.10 (m, 3 H), 2.35 (m, 1 H), 2.55 (m, 2 H), 2.80 (t, 2 H, J = 7 Hz), 3.02 (s, 3 H), 4.15 (m, 4 H), 4.20 (m, 2 H), 4.80 (m, 1 H), 7.10 (d, 1 H, J = 7 Hz), 7.25 and 7.80 (AA'BB', 4 H); EIMS m/e 443 (M^+), 370, 241, 202, 161, 145, 131, 112, 91; HRMS calcd for $C_{20}H_{29}NO_8S$ 443.1614, found 443.1595.

Diethyl *N*-[4-(3-Azidopropyl)benzoyl]-L-glutamate (40). Azide **40** was prepared in 93% yield by the procedure described above for the preparation of **8**: ^{1}H NMR ($CDCl_3$), 300 MHz), δ 1.15 (t, 3 H, J = 7 Hz), 1.25 (t, 3 H, J = 7 Hz), 1.85 (m, 2 H), 2.05 (m, 1 H), 2.15 (m, 2 H), 2.4 (m, 2 H), 2.65 (t, 2 H, J = 7 Hz), 3.20 (t, 2 H, J = 7 Hz), 4.0 (q, 2 H, J = 7 Hz), 4.15 (q, 2 H, J = 7 Hz), 4.75 (m, 1 H), 7.15 and 7.75 (AA'BB', 4 H), 7.30 (d, 1 H, J = 7 Hz); EIMS m/e 390 (M^+), 362, 335, 317, 289, 202, 188, 160 (base), 131, 112, 90; HRMS calcd for $C_{19}H_{26}N_4O_5$ 390.1903, found 390.1912.

Diethyl *N*-[4-(3-Aminopropyl)benzoyl]-L-glutamate (41). Amine **41** was prepared in 95% yield by the procedure described above for the preparation of **10**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.20 (t, 3 H, J = 7 Hz), 1.35 (t, 3 H, J = 7 Hz), 1.80 (m, 2 H), 2.10 (m, 1 H), 2.35 (m, 1 H), 2.45 (m, 2 H), 2.70 (m, 4 H), 3.2 (br s, 2 H), 4.05 (q, 2 H, J = 7 Hz), 4.25 (q, 4 H, J = 7 Hz), 4.80 (m, 1 H), 7.20 (m, 1 H), 7.25 and 7.75 (AA'BB', 4 H); EIMS m/e 364 (M^+), 334, 291, 274, 202, 162 (base), 145, 130, 112, 91; HRMS calcd for $C_{19}H_{28}N_2O_5$ 364.1998, found 364.1986.

Diethyl *N*-[4-[3-[[2-(Pivaloylamino)-6-chloropyrimidin-4-yl]amino]propyl]benzoyl]-L-glutamate (42). Diester **42** was prepared in 82% yield by the procedure described above for the preparation of **13**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.2 (m, 6 H), 1.31 (s, 9 H), 1.95 (m, 4 H), 2.2 (m, 1 H), 2.35 (m, 1 H), 2.55 (m, 2 H), 2.8 (m, 2 H), 3.2 (br s, 1 H), 4.15 (q, 2 H, J = 7 Hz), 4.25 (q, 2 H, J = 7 Hz), 4.80 (m, 1 H), 6.00 (s, 1 H), 7.15 (d, 1 H, J = 7 Hz), 7.25 and 7.75 (AA'BB', 4 H), 7.90 (br s, 1 H); EIMS m/e 575 (M^+), 518, 315, 242 (base), 158, 98, 84; HRMS calcd for $C_{28}H_{38}ClN_5O_6$ 575.2511, found 575.2524.

***N*-[4-[3-[(2-Amino-6-chloropyrimidin-4-yl)amino]propyl]benzoyl]-L-glutamic Acid (43).** Diacid **43** was prepared in 62% yield from diester **42** by the procedure described above for the preparation of **18**: mp 198–200 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.00 (m, 2 H), 2.2 (m, 1 H) 2.35 (m, 1 H), 2.55 (br s, 2 H), 2.60 (m, 2 H), 2.95 (t, 2 H, J = 7 Hz), 4.65 (m, 1 H), 6.00 (s, 1 H), 6.65 (br s, 2 H), 7.40 (br s, 1 H), 7.6 and 8.10 (AA'BB', 4 H), 8.80 (d, 1 H, J = 7 Hz), 12.65 (br s, 1 H); LRFABMS m/e 436 (M^+), 311, 309, 167, 157, 155, 135, 119 (base); HRFABMS calcd for $C_{19}H_{22}ClN_5O_5$ 436.1388, found 436.1384.

4-[4-[(6-Chloro-2-aminopyrimidin-4-yl)amino]butyl]benzoic Acid (44). A solution of **14** (1.20 g, 2.86 mmol) in 6 M HCl (50 mL) was heated under reflux for 3 h. Solid $NaHCO_3$ was added to the cooled solution to bring the pH to 5, and the precipitated solid was filtered, washed with water, and dried in vacuo to give 650 mg (71%) of **44** as a white solid: mp 133–135 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.44–1.65 (m, 4 H), 2.62 (t, 2 H, J = 7.3 Hz), 3.10–3.35 (br s, 1 H), 5.69 (s, 1 H), 6.37 (br s, 2 H), 7.14 (br s, 1 H), 7.28 and 7.82 (AA'BB', 4 H), 12.72 (br s, 1 H); EIMS, m/e 320 (M^+), 185, 171, 157 (base), 144; HRMS calcd for $C_{15}H_{17}ClN_4O_2$ 320.1040, found 320.1041.

***N*-[4-[4-[(6-Chloro-2-aminopyrimidin-4-yl)amino]butyl]benzoyl]-L-glutamic Acid (45).** Diacid **45** was prepared in 56% yield from **44** by the procedure described above for the preparation of **19** from **16**: mp 112–114 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.42–1.49 (m, 2 H), 1.53–1.62 (m, 2 H), 1.87–1.97 (m, 1 H), 2.00–2.11 (m, 1 H), 2.31 (t, 2 H, J = 7.3 Hz), 2.62 (t, 2 H, J = 7.2

Hz), 3.22 (br s, 2 H), 4.31–4.38 (m, 1 H), 5.72 (s, 1 H), 6.45 (br s, 2 H), 7.25 (m, 3 H), 7.76 (d, 2 H, J = 8.0 Hz), 8.50 (d, 1 H, J = 7.7 Hz), 12.40 (br s, 2 H); HRFABMS calcd for $C_{20}H_{25}ClN_5O_5$ (MH^+) 450.1544, found 450.1544.

Diethyl *N*-[4-[4-(Methanesulfonyloxy)-1-butynyl]benzoyl]-L-glutamate (47). Mesylate **47** was prepared in 99% yield from alcohol **46**[8] by the procedure described above for the preparation of **6**: mp 82–84 °C; ^1H NMR ($CDCl_3$, 300 MHz), δ 1.23 (t, 3 H, J = 7.2 Hz), 1.31 (t, 3 H, J = 7.2 Hz), 2.11–2.19 (m, 1 H), 2.28–2.38 (m, 1 H), 2.44–2.52 (m, 2 H), 2.92 (t, 2 H, J = 6.7 Hz), 3.09 (s, 3 H), 4.10 (q, 2 H, J = 7.2 Hz), 4.25 (q, 2 H, J = 7.2 Hz), 4.41 (t, 2 H, J = 6.7 Hz), 4.74–4.80 (m, 1 H), 7.13 (d, 1 H, J = 7.3 Hz), 7.47 and 7.77 (AA'BB', 4 H); ^{13}C NMR ($CDCl_3$, 67.9 MHz) δ 14.1, 20.8, 27.2, 30.5, 37.8, 52.5, 60.8, 61.8, 67.1, 82.2, 86.6, 126.5, 127.1, 131.8, 133.2, 166.3, 171.9, 173.3; EIMS m/e 453 (M^+), 251 (base), 202, 155; HRMS calcd for $C_{21}H_{27}NO_8S$: 453.1457, found 453.1432. Anal. Calcd for $C_{21}H_{27}NO_8S$: C, 55.62; H. 6.00; N, 3.09; S, 7.07. Found: C, 55.41; H, 6.10; N, 3.28; S, 7.35.

Diethyl *N*-[4-(4-Azido-1-butynyl)benzoyl]-L-glutamate (48). Azide **48** was prepared in 84% yield from mesylate **47** by the procedure described above for the preparation of **8**: mp 35–37 °C; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.24 (t, 3 H, J = 7.0 Hz), 1.32 (t, 3 H, J = 7.0 Hz), 2.12–2.22 (m, 1 H), 2.30–2.39 (m, 1 H), 2.45–2.56 (m, 2 H), 2.76 (t, 2 H, J = 6.7 Hz), 3.51 (t, 2 H, J = 6.7 Hz), 4.12 (q, 2 H, J = 7.0 Hz), 4.26 (q, 2 H, J = 7.0 Hz), 4.77–4.82 (m, 1 H), 7.09 (d, 1 H, J = 7.3 Hz), 7.49 and 7.77 (AA'BB', 4 H); ^{13}C NMR ($CDCl_3$, 67.9 MHz) δ 13.8, 20.4, 26.8, 30.2, 49.5, 52.2, 60.4, 61.3, 8.16, 88.3, 126.4, 126.8, 131.3, 132.7, 166.2, 171.6, 172.8; EIMS m/e 400 (M^+), 372, 326, 202, 198, 170 (base); HRMS calcd for $C_{20}H_{24}N_4O_5$ 400.1747, found 400.1760. Anal. Calcd for $C_{20}H_{24}N_4O_5$: C, 59.99; H, 6.04; N, 13.99. Found: C, 60.25; H, 6.05; N, 14.09.

Diethyl *N*-[4-(4-Amino-1-butynyl)benzoyl]-L-glutamate (49). From 48. To a solution of **48** (1.25 g, 3.12 mmol) in CH_2Cl_2 (100 mL) was added PPh_3 (862 mg, 3.29 mmol) and the resulting reaction mixture was heated at reflux overnight. Water (50 mL) was added, and the resulting solution was stirred at 25 °C for 20 h. The CH_2Cl_2 layer was separated, washed with water (50 mL), dried over anhydrous $MgSO_4$, and concentrated in vacuo. Flash chromatography (silica gel, $CH_2Cl_2/NEt_3/MeOH$ [92:5:3]) gave 870 mg (74%) of **49** as an oil: ^1H NMR ($CDCl_3$, 300 MHz) δ 1.21 (t, 3 H, J = 7.1 Hz), 1.28 (t, 3 H, J = 7.1 Hz), 1.60 (br s, 2 H), 2.10–2.20 (m, 1 H), 2.23–2.35 (m, 1 H), 2.42–2.52 (m, 2 H), 2.56 (t, 2 H, J = 6.0 Hz), 2.90 (t, 2 H, J = 6.2 Hz), 4.09 (q, 2 H, J = 7.1 Hz), 4.20 (q, 2 H, J = 7.1 Hz), 4.73–4.78 (m, 1 H), 7.43 and 7.79 (AA'BB', 5 H); ^{13}C NMR ($CDCl_3$, 67.9 MHz) δ 13.7, 24.0, 26.5, 30.1, 40.6, 52.0, 60.2, 61.0, 89.0, 90.3, 126.8, 131.1, 132.3, 166.3, 171.5, 172.6; EIMS m/e 374 (M^+), 345, 301, 172, 142 (base); HRMS calcd for $C_{20}H_{26}N_2O_5$ 374.1842, found 374.1834.

From 51. To a solution of **51** (4.21 g, 8.34 mmol) in EtOH (100 mL) and $CHCl_3$ (100 mL) under N_2 was added 40% aqueous $MeNH_2$ (2.75 g, 35.42 mmol), and the resulting reaction mixture was stirred at 25 °C for 18 h and then concentrated in vacuo. Flash chromatography (silica gel, $CH_2Cl_2/NEt_3/MeOH$ [92:5:3]) gave 2.34 g (75%) of **49**.

Diethyl *N*-[4-(4-Phthalimidobut-1-ynyl)benzoyl]-L-glutamate (51). A mixture of 1.09 g (5.45 mmol) of **50**,[10] 2.36 g (5.45 mmol) of **23**, 60 mg (0.27 mmol) of $Pd(OAc)_2$, 50 mg (0.26 mmol) of CuI, 145 mg (0.55 mmol) of PPh_3, 5 mL of NEt_3, and 10 mL of CH_3CN was heated under reflux for 24 h. The solvent was removed to give a brown solid which was filtered through a short pad of silica gel (eluting with 3% $MeOH/CH_2Cl_2$) to give 2.41 g (4.78 mmol, 92%) of **51**. The analytical sample was recrystallized from $AcOH/H_2O$: mp 145–146 °C; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.22 (t, 3 H, J = 7.1 Hz), 1.30 (t, 3 H, J = 7.1 Hz), 2.10–2.20 (m, 1 H), 2.26–2.36 (m, 1 H), 2.38–2.54 (m, 2 H), 2.85 (t, 2 H, J = 6.9 Hz), 3.98 (t, 2 H, J = 7.0 Hz), 4.10 (q, 2 H, J = 7.1 Hz), 4.24 (q, 2 H, J = 7.1 Hz), 4.75–4.80 (m, 1 H), 7.00 (d, 1 H, J = 7.5 Hz), 7.39 (d, 2 H, J = 8.0 Hz), 7.70–7.75 (m, 4 H), 7.90 (m, 2 H); EIMS m/e 504 (M^+), 431, 302 (base), 202, 160; HRMS calcd for $C_{28}H_{28}N_2O_7$ 504.1897, found, 504.1896. Anal. Calcd for $C_{28}H_{28}N_2O_7$: C, 66.66; N, 5.59; N, 5.55. Found: C, 66.46; H, 5.46; N, 5.55.

Diethyl *N*-[4-[4-[(2-Amino-6-chloro-5-formylpyrimidin-4-yl)amino]-1-butynyl]benzoyl]-L-glutamate (53). A mixture of amine **49** (670 mg, 1.79 mmol), pyrimidine **52**[17] (343 mg, 1.79 mmol), and NEt_3 (183 mg, 1.81 mmol) was heated under reflux in MeOH (80 mL) for 3 h. The reaction mixture was concentrated in vacuo, and the residue taken up in $CHCl_3$ (100 mL) and washed with H_2O (2 × 50 mL). The $CHCl_3$ solution was dried over anhydrous $MgSO_4$ and concentrated in vacuo. Flash column chromatography (silica gel, 65% EtOAc–hexanes) gave 660 mg (70%) of **53** as a white powder: mp 153–155 °C; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.23 (t, 3 H, J = 7.1 Hz), 1.31 (t, 3 H, J = 7.1 Hz), 2.13–2.18 (m, 1 H), 2.30–2.40 (m, 1 H), 2.43–2.52 (m, 2 H), 2.75 (t, 2 H, J = 6.8 Hz), 3.74 (m, 2 H), 4.11 (q, 2 H, J = 7.1 Hz), 4.25 (q, 2 H, J = 7.1 Hz), 4.78 (m, 1 H), 5.50 (br s, 2 H), 7.06 (d, 1 H, J = 7.4 Hz), 7.50 and 7.76 (AA'BB', 4 H), 9.54 (br s, 1 H), 10.11 (s, 1 H); ^{13}C NMR ($CDCl_3$, 67.9 MHz) δ 14.1, 20.1, 27.2, 30.5, 39.4, 52.5, 60.8, 61.7, 81.9, 94.1, 102.8, 126.9, 127.0, 131.8, 132.8, 162.3, 162.7, 166.4, 171.9, 173.2, 188.8; EIMS m/e 529 (M^+), 327, 223 (base), 185, 155, 141; HRMS calcd for $C_{25}H_{28}ClN_5O_6$ 529.1728, found 529.1739. Anal. Calcd for $C_{25}H_{28}ClN_5O_6$: C, 56.66; H, 5.33; N, 13.21; Cl, 6.69. Found: C, 56.48; H, 5.20; N, 13.08; Cl, 6.97.

***N*-[4-[4-[(2-Amino-1,6-dihydro-5-formyl-6-oxopyrimidin-4-yl)amino]but-1-ynyl]benzoyl]-L-glutamic Acid (54).** Compound **54** was prepared in 41% yield from **53** by the procedure described for the preparation of **18**: mp 157–162 °C dec; ^1H NMR (DMSO-d_6) δ 2.04–2.26 (m, 2 H), 2.49 (t, 2 H, J = 7.3 Hz), 2.88 (t, 2 H, J = 7.0 Hz), 3.78–3.86 (m, 2 H), 4.47–4.55 (m, 1 H), 7.66 and 8.01 (AA'BB', 4 H), 8.76 (d, 1 H, J = 7.6 Hz), 9.81 (s, 1 H), 10.07 (br s, 2 H), 10.88 (br s, 1 H), 12.50 (br s, 2 H); FABMS calcd for $C_{21}H_{22}N_5O_7$ (MH^+) 456.1519, found 456.1510.

Diethyl *N*-[4-(4-Azidobutyl)benzoyl]-L-glutamate (56). Azide **56** was prepared from mesylate **55**[8] in 72% yield by the procedure described above for the preparation of **8**: mp 54–55 °C; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.22 (t, 3 H, J = 7.1 Hz), 1.31 (t, 3 H, J = 7.1 Hz), 1.59–1.75 (m, 4 H), 2.12–2.17 (m, 1 H), 2.28–2.40 (m, 2 H), 2.43–2.55 (m, 1 H), 2.70 (t, 2 H, J = 7.3 Hz), 3.30 (t, 2 H, J = 6.5 Hz), 4.11 (q, 2 H, J = 7.1 Hz), 4.23 (q, 2 H, J = 7.1 Hz), 4.77–4.82 (m, 1 H), 7.06 (d, 1 H, J = 7.5 Hz), 7.24 and 7.76 (AA'BB', 4 H); ^{13}C NMR ($CDCl_3$, 75.6 MHz) δ 13.8, 26.9, 27.8, 28.1, 30.2, 34.9, 37.3, 50.9, 52.0, 60.4, 61.3, 127.0, 128.2, 131.2, 145.7, 166.7, 171.7, 172.8; EIMS m/e 404 (M^+), 375, 359, 331 (base), 202; HRMS calcd for $C_{20}H_{28}N_4O_5$ 404.2060, found 404.2057. Anal. Calcd for $C_{20}H_{28}N_4O_5$: C, 59.39; H, 6.98; N, 13.85. Found: C, 59.52; H, 6.78; N, 13.60.

Diethyl *N*-[4-(4-Aminobutyl)benzoyl]-L-glutamate (57). Amine **57** was prepared from azide **56** in 95% yield by the procedure described above for the preparation of **10**: ^1H NMR ($CDCl_3$, 300 MHz) δ 1.22 (t, 3 H, J = 7.1 Hz), 1.30 (t, 3 H, J = 7.1 Hz), 1.52–1.70 (m, 4 H), 2.12–2.20 (m, 1 H), 2.27–2.42 (m, 1 H), 2.43–2.55 (m, 2 H), 2.67 (t, 2 H, J = 7.3 Hz), 2.77 (m, 2 H), 3.60 (br s, 2 H), 4.11 (q, 2 H, J = 7.1 Hz), 4.23 (q, 2 H, J = 7.1 Hz), 4.75–4.80 (m, 1 H), 7.08 (d, 1 H, J = 7.6 Hz), 7.24 and 7.73 (AA'BB', 4 H); ^{13}C NMR ($CDCl_3$, 75.6 MHz) δ 14.1, 27.1, 28.2, 30.5, 35.3, 40.9, 46.1, 52.3, 60.8, 61.6, 127.3, 128.5, 131.2, 146.3, 167.2, 172.1, 173.2; EIMS m/e 378 (M^+), 321, 204, 176 (base); HRMS calcd for $C_{20}H_{30}N_2O_5$ 378.2155, found 378.2154.

Diethyl *N*-[4-(4-Acetoxybutyl)benzoyl]-L-glutamate (59) and Diethyl *N*-(4-Butylbenzoyl)-L-glutamate (58). A solution of azide **48** (2.98 g, 7.44 mmol) in AcOH (50 mL) was hydrogenated over 300 mg of 10% Pd–C on a Parr shaker for 10 h at 50 psi. The reaction mixture was filtered through Celite and the filtrate concentrated in vacuo. Flash chromatography (silica gel, 40% EtOAc–hexanes) gave 290 mg (11%) of **58** as white needles [mp 53–55 °C; ^1H NMR ($CDCl_3$, 300 MHz) δ 0.95 (t, 3 H, J = 7.3 Hz), 1.24 (t, 3 H, J = 7.2 Hz), 1.30–1.40 (m, 5 H), 1.57–1.63 (m, 2 H), 2.11–2.19 (m, 1 H), 2.31–2.39 (m, 1 H), 2.44–2.54 (m, 2 H), 2.68 (t, 2 H, J = 7.6 Hz), 4.12 (q, 2 H, J = 7.2 Hz), 4.26 (q, 2 H, J = 7.2 Hz), 4.81 (m, 1 H), 6.99 (d, 1 H, J = 5.3 Hz), 7.27 and 7.75 (AA'BB', 4 H); ^{13}C NMR ($CDCl_3$, 67.9 MHz) δ 13.8, 14.1, 14.1, 22.2, 27.5, 30.5, 33.2, 35.5, 52.3, 60.7, 61.6, 127.1, 128.6, 131.3, 147.2, 167.1, 172.0, 173.1; EIMS m/e 363 (M^+), 318, 290, 202, 161 (base); HRMS calcd for $C_{20}H_{29}NO_5$ 363.2046, found 363.2029. Anal. Calcd for $C_{20}H_{29}NO_5$: C, 66.09; H, 8.04; N, 3.85. Found: C, 66.36; H, 7.81; N, 3.92] and 1.35 g (43%) of **59** as a liquid: ^1H NMR ($CDCl_3$, 300 MHz) δ 1.22 (t, 3 H, J = 7.0 Hz), 1.30 (t, 3 H, J = 7.0 Hz), 1.60–1.80 (m, 4 H), 2.04 (s, 3 H), 2.06–2.20 (m, 1 H), 2.26–2.36 (m, 1 H), 2.38–2.57 (m, 2 H), 2.69 (t, 2 H, J = 6.7 Hz), 4.05–4.15 (m, 4 H), 4.23 (q, 2 H, J = 7.2 Hz), 4.80 (m, 1 H), 7.11 (d, 1 H, J = 7.3 Hz), 7.24 and 7.75 (AA'BB', 4 H); ^{13}C NMR ($CDCl_3$, 67.9 MHz) δ 14.0, 20.8, 27.1, 27.3, 28.0, 30.4, 35.1, 52.2,

60.6, 61.5, 64.0, 127.1, 128.4, 131.2, 146.1, 166.9, 171.0, 171.9, 173.0; EIMS m/e 421 (M^+), 348, 219 (base), 202, 159; HRMS calcd for $C_{22}H_{31}NO_7$ 421.2101, found 421.2104. Anal. Calcd for $C_{22}H_{31}NO_7$: C, 62.69; H, 7.41; N, 3.32. Found: C, 62.91; H, 7.27; N, 3.37.

Diethyl *N*-[4-[4-[(2-Amino-6-chloro-5-formylpyrimidin-4-yl)amino]butyl]benzoyl]-L-glutamate (60). Diester **60** was prepared in 71% yield from amine **57** by the procedure described above for the preparation of **53**: mp 113–115 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.22 (t, 3 H, J = 7.1 Hz), 1.30 (t, 3 H, J = 7.1 Hz), 1.56–1.76 (m, 4 H), 2.08–2.21 (m, 1 H), 2.27–2.34 (m, 1 H), 2.40–2.53 (m, 2 H), 2.67 (t, 2 H, J = 6.8 Hz), 3.39–3.48 (m, 2 H), 4.11 (q, 2 H, J = 7.1 Hz), 4.23 (q, 2 H, J = 7.1 Hz), 4.76–4.83 (m, 1 H), 5.80 (br s, 1 H), 7.15–7.23 (m, 3 H), 7.73 (d, 2 H, J = 8 Hz), 9.23 (t, 1 H, J = 5.5 Hz), 10.04 (s, 1 H); ^{13}C NMR ($CDCl_3$, 75.6 MHz) δ 14.0, 27.1, 28.0, 28.3, 30.4, 35.1, 40.1, 52.2, 60.6, 61.5, 102.2, 127.1, 128.4, 131.3, 146.0, 162.1, 162.3, 166.3, 167.1, 172.1, 173.0, 188.5; EIMS m/e 533 (M^+), 505, 460, 331 (base), 302, 185; HRMS calcd for $C_{25}H_{32}ClN_5O_6$ 533.2041, found 533.2017. Anal. Calcd for $C_{25}H_{32}ClN_5O_6$: C, 56.23; H, 6.04; N, 13.11; Cl, 6.64. Found: C, 56.03; H, 5.79; N, 12.88; Cl, 6.77.

***N*-[4-[4-[(2-Amino-1,6-dihydro-5-formyl-6-oxopyrimidin-4-yl)amino]butyl]benzoyl]-L-glutamic Acid (61).** Diacid **61** was prepared in 25% yield from diester **60** by the procedure described above for the preparation of **18**: mp 222–224 °C; ^{1}H NMR (DMSO-d_6, 270 MHz) δ 1.67–1.75 (m, 4 H), 2.03–2.26 (m, 2 H), 2.46–2.52 (m, 2 H), 2.64 (s, 2 H), 2.81 (t, 2 H, J = 6.9 Hz), 3.58 (m, 2 H), 4.49–4.57 (m, 1 H), 7.44 and 7.94 (AA'BB', 4 H), 8.67 (d, 1 H, J = 7.6 Hz), 9.77 (s, 1 H), 9.88 (t, 1 H, J = 5.5 Hz), 10.40 (s, 1 H), 12.52 (br s, 2 H); HRFABMS calcd for $C_{21}H_{26}N_5O_7$ (MH^+) 460.1832, found 460.1834. Anal. Calcd for $C_{21}H_{25}N_5O_7$: C, 54.90; H, 5.48; N, 15.24. Found: C, 55.14; H, 5.40; N, 15.04.

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Supplementary Material Available: ^{1}H NMR spectra for compounds **4**, **10**, **11**, **13–19**, **22**, **24–34**, **38–45**, **49**, **54**, and **57** and IR spectral data for compounds **4–6**, **8–11**, **13**, **14**, **16–19**, **21**, **22**, **24–45**, **47–49**, **51**, **53**, **54**, and **56–61** (42 pages). Ordering information is given on any current masthead page.

Triplex Formation of an Oligonucleotide Containing 2′-*O*-Methylpseudoisocytidine with a DNA Duplex at Neutral pH

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The synthesis of the hexadecanucleotide 5′TTTT1TTTT111111T3′ (**1-16mer**) containing 2-amino-5-(2-*O*-methyl-β-D-ribofuranosyl)-4(1*H*)-pyrimidinone (2′-*O*-methylpseudoisocytidine or **1**) is described. Triplex formation of **1-16mer** with a deoxyribonucleotide duplex 5′d(ACCAAAAGAAAAGGGGGGACCA)3′–5′d-(TGGTCCCCCCTTTTCTTTTGGT)3′ (**duplex-22**) which contains the "polypurine tract" found in the genome of human T-cell leukaemia (lymphotropic) virus (HTLV-III) was studied by thermal denaturation and circular dichroism (CD) spectra in aqueous solution at neutral pH. The "polypurine tract" contains a homoguanine cluster consisting of 6 deoxyguanine residues. The results indicate that **1-16mer** and **duplex-22** formed a triplex at neutral condition (0.01 M Na cacodylate, 0.5 M NaCl, 5 mM $MgCl_2$, pH 7.2. T_m = 20 °C). In contrast, a hexadecadeoxynucleotide, 5′TTTTMTTTTMMMMMMT3′ (**M-16mer**), containing 5-methyl-2′-deoxycytidine (**M**) did not form a stable triplex with **duplex-22** at the same condition (T_m < 0 °C). The CD mixing titration indicated that the triplex was formed with 1:1 (duplex:third strand) molecular stoichiometry.

Introduction

The formation of triple-stranded nucleic acid helices (triplex) has been studied for more than three decades.[1-3] In recent years, studies of sequence-specific triplex formation of short synthetic oligonucleotides (or their analogues) are of prime interest because the approach is applicable to biological and biochemical studies such as site specific cleavage of DNA,[4-7] inhibition of DNA–protein binding,[8-10] and inhibition of gene expression.[11]

The most common motif is that each case triad of a triplex consists of two pyrimidine residues from two homopyrimidine strands and a purine residue from a homopurine strand. The third strand (i.e., the second pyrimidine strand) is located in the major groove of a duplex consisting of Watson–Crick base pairing, and its sugar–phosphate backbone polarity is parallel to that of the purine strand.[4,12-16] Thymines or cytosines in the third

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