# Synthesis and Biological Activity of Acyclic Analogues of 5,10-Dideaza-5,6,7,8-tetrahydrofolic Acid

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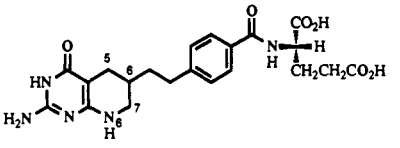
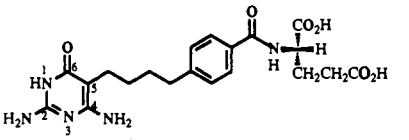
The synthesis and biological evaluation of a number of analogues of *N*-[4-[4-(2,4-diamino-1,6-dihydro-6-oxo-5-pyrimidyl)butyl]benzoyl]-L-glutamic acid (**2**) (7-DM-DDATHF), an acyclic modification of the novel folate antimetabolite 5,10-dideazatetrahydrofolic acid (DDATHF), are described. The synthetic procedure utilized previously for the synthesis of **2**, **15**, and **16** was extended to the preparation of analogues modified in the benzoyl region with thiophene and methylene groups replacing the benzene ring (compounds **27a–c**) and in the glutamate region with aspartic acid and phenylalanine replacing L-glutamic acid (compounds **36**, **37**). The 2-amino-4,6-dioxo derivative **33** was obtained from intermediate **30** via a palladium-catalyzed carbon–carbon coupling reaction with diethyl (4-iodobenzoyl)-L-glutamate, followed by reduction and removal of protecting groups with base. Cell culture cytotoxicity studies of all of the above acyclic analogues of DDATHF against CCRF-CEM human lymphoblastic leukemic cells gave $IC_{50}$s ranging from 0.042 to >48 μM. Inhibition and cell culture reversal studies against isolated enzymes suggest the mode of action of these compounds. Compound **2** was only 3-fold less inhibitory toward glycinamide ribonucleotide formyltransferase (GARFT, isolated from L1210 leukemic cells) than DDATHF itself. These acyclic analogues were less efficient substrates for the enzyme folylpolyglutamate synthetase (FPGS) compared with their bicyclic counterparts. Moderate antitumor activity was observed for compound **2** against 6C3HED lymphosarcoma and C3H mammary adenocarcinoma in vivo.

5,10-Dideazatetrahydrofolic acid (DDATHF, **1**)[1] has demonstrated remarkable antitumor activity against a broad spectrum of murine solid tumors and human tumor xenografts which are refractory towards conventional antifolate (methotrexate, MTX) therapy.[2,3] DDATHF is a poor inhibitor against the two classical targets for antifolates, namely dihydrofolate reductase (EC 1.5.1.3) and thymidylate synthase (EC 2.1.1.45).[1] By contrast, its main locus of action is the enzyme glycinamide ribonucleotide formyltransferase (GARFT, EC 2.1.2.1),[2b,4,5] which mediates the first formyl transfer reaction in purine de novo biosynthesis. DDATHF, as the first specific and potent inhibitor of GARFT ($K_i$ = 0.12 μM),[6,7] is the prototype of a new class of folate antagonists with potential clinical utility. The 6*R* diastereomer of DDATHF (Lometrexol, LY264618) is currently in phase II clinical evaluation as an antitumor agent.[8,9]



DDATHF, **1**



7-Desmethylene-DDATHF, **2**

DDATHF is a structural analogue of tetrahydrofolic acid (THF) with carbon replacing the nitrogen atoms at both positions 5 and 10. The asymmetric center at position 6 of the tetrahydropyridopyrimidine ring in DDATHF posed a special problem when this compound was initially synthesized and developed.[1,2c,10] The two diastereomers of DDATHF were separated by a novel fractional crystallization process through a crystalline *d*-10-camphorsulfonate

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(2) (a) Beardsley, G. P.; Taylor, E. C.; Shih, C.; Poore, G. A.; Grindey, G. B.; Moran, R. G. A New Class of Antifolates. 5,10-Dideazatetrahydrofolic Acid (DDATHF), An Inhibitor of GAR Transformylase With Broad In Vivo Activity. *Proc. Amer. Assoc. Cancer Res.* **1986**, *27*, 259. (b) Beardsley, G. P.; Taylor, E. C.; Grindey, G. B.; Moran, R. G. Deaza Derivatives of Tetrahydrofolic Acid. A New Class of Folate Antimetabolites. *Chemistry and Biology of Pteridines*; Cooper, B. A., Whitehead, V. M., Eds.; Walter de Gruyter: Berlin, 1986; pp 953–957. (c) Taylor, E. C. New Pathways from Pteridines. Design and Synthesis of A New Class of Potent and Selective Antitumor Agents. *J. Heterocycl. Chem.* **1989**, *27*, 1–12.

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(4) Moran, R. G.; Taylor, E. C.; Beardsley, G. P. 5,10-Dideaza-5,6,7,8-Tetrahydrofolic Acid (DDATHF), A Potent Antifolate Inhibitory To *De Novo* Purine Synthesis. *Proc. Amer. Assoc. Cancer Res.* **1985**, *26*, 231.

(5) Beardsley, G. P.; Moroson, B. A.; Taylor, E. C.; Moran, R. G. Synthesis and Structure-Activity Relationship Studies of 5,10-Dideazatetrahydrofolic Acid (DDATHF). *J. Biol. Chem.* **1989**, *264*, 328–332.

(6) Baldwin, S. W.; Tse, A.; Grindey, G. B.; Taylor, E. C.; Rosowsky, A.; Shih, C.; Moran, R. G. Inhibition of Glycinamide Ribonucleotide Formyltransferase (GRT) by 5,10-Dideazatetrahydrofolate (DDATHF) Analogs. *Proc. Amer. Assoc. Cancer Res.* **1990**, *31*, 341.

(7) Baldwin, S. W.; Tse, A.; Gossett, L. S.; Taylor, E. C.; Rosowsky, A.; Shih, C.; Moran, R. G. Structural Features of 5,10-Dideaza-5,6,7,8-tetrahydrofolate That Determine Inhibition of Mammalian Glycinamide Ribonucleotide Formyltransferase. *Biochemistry* **1991**, *30*, 1997–2006.

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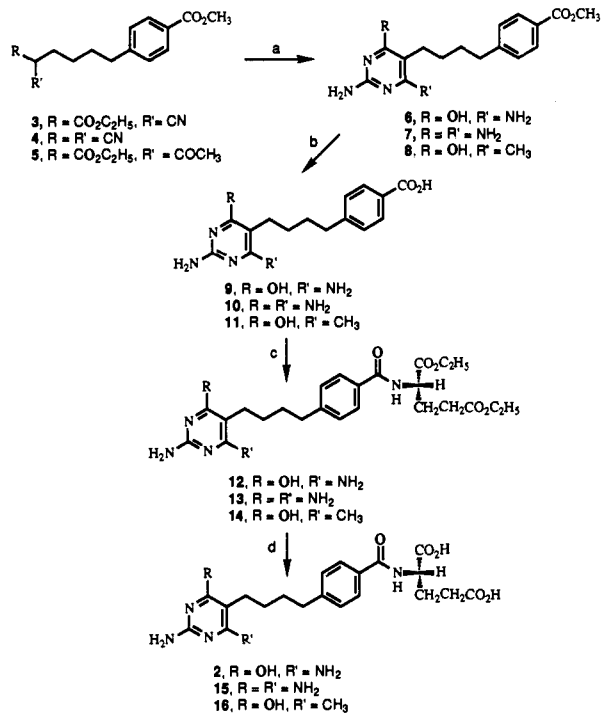
**Scheme I**[a]



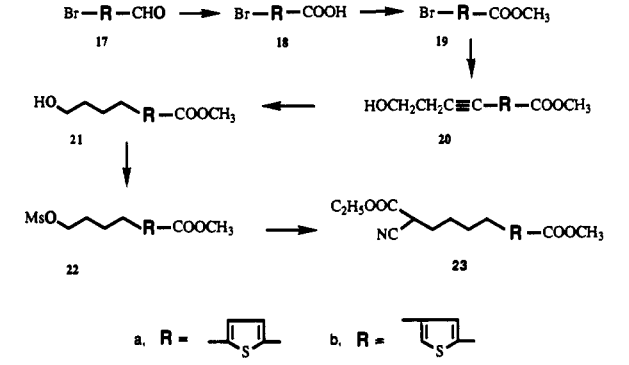

[a] (a) $H_2NC(=NH)NH_2$·HCl, NaOMe, MeOH; (b) 1 N NaOH, MeOH; (c) diethyl L-glutamate hydrochloride, phenyl *N*-phenylphosphoramidochloridate, *N*-methylmorpholine; (d) 1 N NaOH.

diethyl ester salt intermediate.[11] However, the ability to incorporate this separation only at a late stage of the synthesis, the relatively low efficiency of the process, and the limited success experienced with other analogues in the series have impeded the general applicability of this crystallization procedure. Two alternative approaches for controlling the C-6 chirality problem have subsequently been considered: (a) development of a chiral synthesis for the tetrahydropyridopyrimidine nucleus,[12] or (b) *removal* of the chiral center through structural simplification. Our previously described synthesis of *N*-[4-[4-(2,4-diamino-1,6-dihydro-6-oxo-5-pyrimidyl)butyl]benzoyl]-L-glutamic acid (**2**) (7-DM-DDATHF) was a direct outcome of the latter approach.[13] We have now prepared a number of related folate antimetabolites, all of which lack the C-7 methylene unit of the DDATHF structure and therefore exist as single enantiomers. The synthesis and biological activity of these additional "open chain" DDATHF analogues are reported herein.

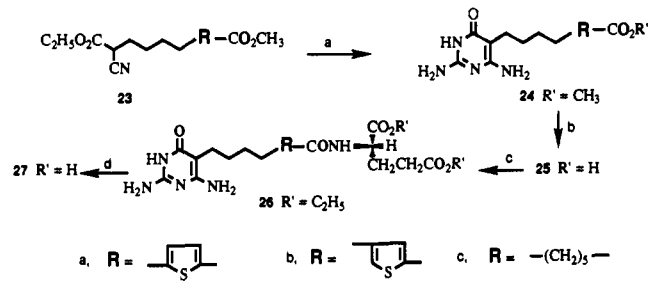
## Chemistry

The "open chain" DDATHF analogues **2**, **15**, and **16** were previously prepared by alkylation of ethyl cyanoacetate, malononitrile, and ethyl acetoacetate with methyl 4-[4′-(mesyloxy)butyl]benzoate to give **3–5** (see Scheme I), and the products were then cyclized to the pyrimidines **6–8**

**Scheme II**




**Scheme III**[a]




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

with guanidine in DMF at room temperature. Saponification of the methyl benzoate functionality followed by coupling of the resulting benzoic acid derivatives **9–11** with diethyl L-glutamate, using phenyl *N*-phenylphosphoramidochloridate as the coupling agent, *N*-methylmorpholine as the base, and *N*-methyl-2-pyrrolidinone as the solvent, gave the diethyl glutamate derivatives **12–14**. Hydrolysis in 1.0 N NaOH followed by acidification with glacial acetic acid then gave the desired acyclic DDATHF analogues **2**, **15**, and **16**.

We have now prepared a new series of analogues in which the 1′,4′-phenylene moiety of the above open-chain DDATHF analogues has been replaced by a thiophene ring. The requisite precursors for the pyrimidine annulation reaction were prepared as depicted in Scheme II; a series of synthetic transformations similar to those shown in Scheme I then gave the thiophene analogues **27a** and **27b** (Scheme III). Analogous chemistry led to the analogue with a bridge region consisting only of a straight chain of methylene groups (compound **27c**). In these cases, however, guanidine cyclization to the 2-amino-5-substituted-pyrimidines **24a–c** proceeded in poor yield.

Numerous attempts to effect coupling of diethyl L-glutamate with **9** (R = R′ = OH, Scheme I) were unsuccessful. For that reason, an alternate strategy was developed for the preparation of target compound **33**. Diethyl malonate was alkylated with 4-(mesyloxy)-1-butyne, and the resulting alkylated diethyl malonate derivative **28** was cyclized with guanidine to give the terminal acetylene **29**. This was pivaloylated with pivalic anhydride in pyridine containing DMAP, and the resulting (more soluble) 2-pivaloyl derivative **30** successfully coupled with diethyl (4-iodobenzoyl)-L-glutamate in the presence of $PdCl_2$.[2c,10] The resulting fully protected intermediate **31** was reduced catalytically to **32**, which was then saponified to the 2-amino-4,6-dioxo analogue **33** (Scheme IV).

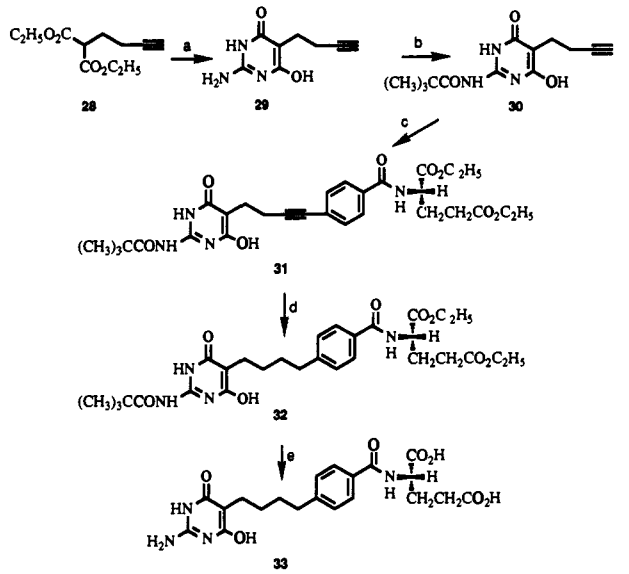
In order to investigate the biological significance of the glutamate portion of the above folate analogues on biological activity, two derivatives with amino acids different

(11) Moran, R. G.; Baldwin, S. W.; Taylor, E. C.; Shih, C. The 6S- and 6R-Diastereomers of 5,10-Dideaza-5,6,7,8-tetrahydrofolate Are Equiactive Inhibitors of de Novo Purine Synthesis. *J. Biol. Chem.* **1989**, *264*, 21047–21051.

(12) For a stereospecific synthesis of DDATHF diastereomers, see: Barnett, C. J.; Wilson, T. M. Asymmetric Synthesis and Absolute Configuration of 5,10-Dideaza-5,6,7,8-tetrahydropteroic Acid and 5,10-Dideaza-5,6,7,8-tetrahydrofolic Acid (DDATHF). *Tetrahedron Lett.* **1989**, *30*, 6291–6294.

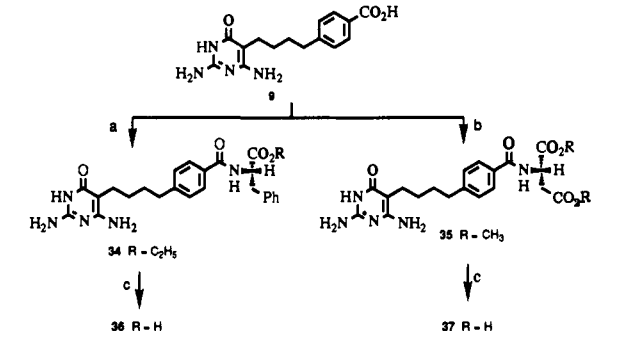
(13) Taylor, E. C.; Harrington, P. M.; Shih, C. A Facile Route to "Open Chain" Analogues of DDATHF. *Heterocycles* **1989**, *28*, 1169–1178.

**Scheme IV**[a]



[a] (a) $H_2NC(=NH)NH_2$·HCl, NaOH, MeOH; (b) $(Piv)_2O$, pyridine, 4-DMAP; (c) diethyl (4-iodobenzoyl)-L-glutamate, $(PPh_3)_2PdCl_2$, CuI, $Et_3N$, $CH_3CN$; (d) $H_2$, 5% Pd/C, EtOH; (e) 1 N NaOH.

**Scheme V**[a]



[a] (a) L-Phenylalanine ethyl ester, phenyl *N*-phenylphosphoramidochloridate, *N*-methylmorpholine, NMP; (b) dimethyl DL-aspartate hydrochloride, phenyl *N*-phenylphosphoramidochloridate, *N*-methylmorpholine, NMP; (c) 1 N NaOH.

from L-glutamic acid were prepared through intermediate **9** (Scheme V). Condensation of **9** with L-phenylalanine ethyl ester and with dimethyl DL-aspartate, again utilizing phenyl *N*-phenylphosphoramidochloridate as the coupling agent, gave the penultimate intermediates **34** and **35** which were smoothly saponified to the acyclic analogues **36** and **37**.

## Biological Results and Discussion

The various acyclic analogues of DDATHF prepared in this study (compounds **2**, **15**, **16**, **27a–c**, **33**, **36**, and **37**) were first evaluated against human lymphoblastic leukemic cells (CCRF-CEM) in culture. The $IC_{50}$s of the above compounds are listed in Table I.

The lead compound in the series, 7-DM-DDATHF, **2**, was found to be ca. 8-fold ($IC_{50}$ = 0.132 μM) less inhibitory against CCRF-CEM leukemic cells than DDATHF ($IC_{50}$ = 0.016 μM). Cell culture reversal studies of 7-DM-DDATHF (Table II) revealed that it had a similar mechanism of action as does DDATHF; thus, thymidine (5 μM) did not protect the cells from the cytotoxic effects of 7-DM-DDATHF. In contrast, however, hypoxanthine (100 μM) and aminoimidazolecarboxamide (AICA, 300 μM) were each able to reverse the cytotoxicity of **2** at all dose levels examined. These results indicate that the cytotoxicity of **2** is due mainly to inhibition of purine de novo biosynthesis. Studies against GARFT isolated from L1210 cells showed that this flexible monocyclic pyrimidine analogue was surprisingly only 3-fold ($K_i$ = 0.38 μM) less inhibitory than was (6*RS*)-DDATHF. Removal of the methylene group at position 7 from DDATHF thus affected only slightly the binding efficacy of the resulting acyclic analogue toward GARFT. As with DDATHF itself, compound **2** proved to be only very weakly inhibitory against other folate-dependent enzymes.[14] By contrast, the 4-methyl (compound **16**) and 4-OH (compound **33**) analogues of **2** exhibited much weaker growth inhibitory activity toward CCRF-CEM leukemic cells. It thus appears that the group at position 4 of the pyrimidine ring plays a significant role in the cellular cytotoxicity of this series of antifolates. Similar structure–activity relationships were observed previously for bicyclic tetrahydropyridine DDATHF analogues; the nitrogen atom at position 8 was shown to be critical both for enzyme inhibitory activity as well as for cellular cytotoxicity.[6,7,15] The presence of a hydrogen bond donor (the NH group at position 8 in bicyclic DDATHF analogues, and the amino group at position 4 of these acyclic derivatives) supports the idea that critical hydrogen bonding interactions involving these NH or $NH_2$ groupings may exist with the active site residues of GARFT. The 2,4,6-triaminopyrimidine analogue **15** was more cytotoxic ($IC_{50}$ = 0.018 μg/mL) against CEM cells than was compound **2**, but reversal studies indicated that it was most likely a DHFR inhibitor rather than an inhibitor of de novo purine biosynthesis. Thus, neither thymidine, hypoxanthine, nor AICA alone reversed the cytotoxic effect of **15**; thymidine

**Table I.** Cell Culture Cytotoxicity of 7-DM-DDATHF (2) and Analogues

| compound | CCRF-CEM $IC_{50}$ (μM)[a] | compound | CCRF-CEM $IC_{50}$ (μM)[a] |
|---|---|---|---|
| 7-DM-DDATHF, **2** | 0.132 | **33** | 39.8 |
| **15** | 0.042 | **36** | >44.5[b] |
| **16** | 2.33 | **37** | >48.0[b] |
| **27a** | 0.059 | DDATHF[c] | 0.016 |
| **27b** | 0.142 | 5-DACTHF[d] | 0.198 |
| **27c** | >47.1[b] | | |

[a] $IC_{50}$ = concentration which causes 50% inhibition of the growth of the control value. [b] No inhibition at the specified concentration. [c] Mixture of diastereomers at C-6. [d] This compound, *N*-[4-[[3-(2,4-diamino-1,6-dihydro-6-oxo-5-pyrimidinyl)propyl]-amino]benzoyl]-L-glutamic acid, was reported recently by Kelly et al. (ref 17).

**Table II.** Reversal of Growth Inhibition of 7-DM-DDATHF (2) and 5-DACTHF with Hypoxanthine, Thymidine, and AICA (against CCRF-CEM Cells)

| | % of inhibition by | |
|---|---|---|
| additions to medium | **2** (0.46 μM) | 5-DACTHF[17] (0.46 μM) |
| control | 80 | 75 |
| thymidine (5 μM) | 80 | 77 |
| hypoxanthine (100 μM) | 0 | 0 |
| AICA (300 μM) | 0 | 55 |

(14) Russello, O.; Cashmore, A. R.; Moroson, B. A.; Wildman, D. E.; Taylor, E. C.; Beardsley, G. P. Dideazatetrahydrofolates: Novel Potent Inhibitors of the de novo Purine Synthesis. *Proc. Am. Assoc. Cancer Res.* **1990**, *31*, 343.

(15) Rosowsky, A.; Forsch, R.; Moran, R. G. (6R,6S)-5,8,10-Trideaza-5,6,7,8-tetrahydrofolate and (6R,6S)-5,8,10-Trideaza-5,6,7,8-tetrahydropteroyl-L-ornithine as Potential Antifolates and Antitumor Agents. *J. Med. Chem.* **1989**, *32*, 709–715.

plus hypoxanthine, however, completely reversed its cytotoxicity at all dose levels (data not shown).

The cytotoxic activity of 7-DM-DDATHF was effectively retained upon replacement of the bridging phenyl group by a bioisosteric thiophene ring. Thus, compound **27a** was 2-fold more inhibitory than **2**, and both thiophene analogues **27a** and **27b** were shown to be cytotoxic to CEM cells by the same purine synthesis inhibition mechanism as compound **2** itself (determined by reversal studies; data not shown).[16] On the other hand, replacement of the bridging phenyl or thiophene rings by methylene groups, or replacement of the glutamate moiety by phenylalanine or aspartic acid, led to inactive compounds. The fact that cytotoxicity requires the presence of the terminal glutamate grouping is consistent with analogous findings with bicyclic DDATHF analogues and is presumably due to the necessity for intracellular polyglutamation by the enzyme folylpolyglutamate synthetase.

A close structural analogue of **2** in which the benzylic methylene group is replaced by an NH unit (5-DACTHF) has recently been reported.[17] This agent is cytotoxic to CEM cells with a potency similar to that of 7-DM-DDATHF, although differences between the activities of the two compounds exist. For example, AICA was less efficient in protecting cells from the cytotoxicity effects of 5-DACTHF (Table II), and it appears that, at least in CCRF-CEM cells, a second inhibitory site (possibly AICAR formyltransferase) exists for 5-DACTHF when this agent is present at higher concentrations.

Recent studies have shown that polyglutamated derivatives of DDATHF are much more potent inhibitors of GARFT than is DDATHF itself. The pentaglutamate of DDATHF, for example, was ca. 100-fold more potent than the parent monoglutamate in inhibiting GARFT isolated both from murine and from human sources.[7,11,18] These results, in addition to observations that DDATHF is an excellent substrate for the enzyme folylpolyglutamate synthetase (FPGS)[1,2b,5] and is extensively metabolized into the polyglutamated form in vivo,[19] suggested that polyglutamation of DDATHF is central to its cytotoxicity. It was found that compounds **2**, **27a**, and **15** were all utilized as moderate substrates for mouse liver FPGS, but that compound **16** was >10-fold poorer as a substrate than these compounds for this enzyme, and was ~100-fold poorer as a substrate than DDATHF.[20] Presumably, this explains the low inhibition of tumor cell growth by compound **16**.

Table III. In Vivo Antitumor Activity of 7-DM-DDATHF

| tumor type | dose (mg/kg) | % inhibition | toxicity/ total |
|---|---|---|---|
| 6C3HED lymphosarcoma[a] | 400 | 100 | 2/10 |
| | 200 | 92 | 1/10 |
| | 100 | 66 | 0/9 |
| | 50 | 44 | 0/10 |
| C3H mammary adenocarcinoma[b] | 400 | 78 | 2/10 |
| | 200 | 36 | 0/10 |
| | 100 | 37 | 1/10 |
| | 50 | 17 | 2/10 |

[a] Compound was given ip twice daily for 8 days. [b] Compound was given ip once daily for 10 days.

Polyglutamates of **2** with additional glutamic acid moieties attached to its γ-carboxy terminus were prepared according to the procedure of Pawelczak et al.[21] and tested against GARFT isolated from L1210 cells. As anticipated, these polyglutamate conjugates were found to be much more potent inhibitors of isolated GARFT than was the monoglutamate itself, with a $K_i$ for the pentaglutamate 50-fold lower than that of the parent compound.[20] Hence, polyglutamation greatly increases the activity of the acyclic analogues against GARFT, as has also been found to be the case for DDATHF[7] and 5-DACTHF.[17]

In vivo evaluation of compound **2** in mice with transplantable murine solid tumors (6C3HED lymphosarcoma and C3H mammary adenocarcinoma) led to the results summarized in Table III. On a twice daily × 8 (ip) schedule, moderate antitumor activity was observed against 6C3HED lymphosarcoma, and similar activity was observed when **2** was administered orally (twice daily × 8).[22] Lower activity was shown against C3H mammary adenocarcinoma tumors.

## Summary

Removal of the 7-methylene bridge between C-6 and N-8 of DDATHF has generated a novel "open-chain" pyrimidine antifolate, 7-DM-DDATHF (**2**), as a single enantiomer. This compound, which is clearly less rigid than DDATHF itself, surprisingly is a potent inhibitor of GARFT; removal of the annulated tetrahydropyridine ring of DDATHF thus has a minimal effect (3-fold) on binding efficacy to GARFT. This result, taken together with previous work which showed that replacement of the NH grouping at position 8 of DDATHF by a methylene group resulted in a 100-fold increase in $K_i$ against GARFT,[7,15] emphasizes the importance for tight substrate–GARFT binding of a hydrogen bond between the N-8 hydrogen of DDATHF (or the $NH_2$ grouping at C-4 in the acyclic series) with an active-site residue on GARFT. An intact glutamic acid moiety in these open-chain analogues is also mandatory for cytotoxicity. Modifications in the bridge and methylene regions are tolerated, although the decreased cell culture and in vivo antitumor activity of these latter analogues may be due to their decreased substrate activity for the enzyme folylpolyglutamate synthetase (FPGS).

(16) Substitution of the 1′,4′-phenylene of DDATHF with bioisosteric thiophenes also increases the cytotoxicity of DDATHF; see: (a) Shih, C.; Grindey, G. B.; Moran, R. G.; Gossett, L. S.; Worzalla, S. M.; Rinzel, S. M.; Taylor, E. C.; Harrington, P. M. New Analogs of 5,10-Dideazatetrahydrofolic Acid (DDATHF) With Modification in the Phenyl Region. *Proc. Amer. Assoc. Cancer Res.* **1989**, *30*, 479. (b) Shih, C.; Taylor, E. C.; Grindey, G. B.; Moran, R. G.; Gossett, L. S.; Harrington, P. M. *Chemistry and Biology of Pteridines*; Synthesis and Structure-Activity Relationship Studies of 5,10-Dideazatetrahydrofolic Acid (DDATHF). Curtius, H.-Ch., Ghisla, S., Blau, N., Eds.; Walter de Gruyter: Berlin, 1989; pp 1035–1038.

(17) Kelly, J. L.; McLean, E. W.; Cohn, N. K.; Edelstein, M. P.; Duch, D. S.; Smith, G. K.; Hanlon, M. H.; Ferone, R. Synthesis and Biological Activity of an Acyclic Analogue of 5,6,7,8-Tetrahydrofolic Acid, N-[4-[[3-(2,4-Diamino-1,6-dihydro-6-oxo-5-pyrimidinyl)propyl]amino]benzoyl]-L-glutamic Acid. *J. Med. Chem.* **1990**, *33*, 561–567.

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(19) Wildman, D. E.; Cross, A. D.; Shih, C.; Beardsley, G. P. Comparative Metabolism of 5,10-Dideaza-5,6,7,8-tetrahydrofolate (DDATHF) Diastereomers in CCRF-CEM Cells. *Proc. Am. Assoc. Cancer Res.* **1989**, *30*, 474.

(20) Moran, R. G., unpublished results.

(21) Pawelczak, K.; Jones, T. R.; Kempny, M.; Jackman, A. L.; Newell, D. R.; Krzyzanowski, L.; Rzeszotarska, B. Quinazoline Anitfolates Inhibiting Thymidylate Synthase: Synthesis of Four Oligo (L-Y-glutamyl) Conjugates of $N^{10}$-Propargyl-5,8-dideazafolic Acid and Their Enzyme Inhibition. *J. Med. Chem.* **1989**, *32*, 160–165.

(22) No toxic deaths and similar antitumor efficacy were observed when compound **2** was administered orally.

## Experimental Section

**General Methods.** Melting points are uncorrected and were determined in open capillary tubes using a Thomas-Hoover apparatus for temperatures below 250 °C and a Meltemp or electrothermal apparatus for temperatures above 250 °C. Infrared spectra were determined with a Nicolet DX-10 FTIR spectrometer. $^1$H NMR data were obtained with a General Electric QE300 MHz instrument using residual solvent as an internal standard, and chemical shifts are reported in ppm downfield from TMS. Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, qn = quintet, m = multiplet, and b = broad), coupling constant (Hz), integration, and assignment. Mass spectra were recorded on a Consolidated Electrodynamics Corp. 21-110 for EI spectra, on a Varian Mat 731 for FD spectra, or on a Zab 3F-VG Analytical for FAB spectra for determination of exact mass. Elemental analyses were done either on a Perkin-Elmer 240 elemental analyzer or on a Control Equipment Corp. 240-XA and are within 0.4% of the theoretical values. Flash chromatography was performed on EM Reagents silica gel 60 (230–400 mesh). Preparative high-performance liquid chromatography (HPLC) was performed on a Waters Associates PrepLC/System 500 liquid chromatograph equipped with a refractive index detector and using two PrepPak 500 silica gel cartridges (5 × 30 cm). Analytical thin-layer chromatography (TLC) was performed with Merck 0.25-mm silica gel 60-F plates utilizing UV visualization.

**Materials.** Commercial reagents were utilized without further purification. Anhydrous solvents were distilled before use.

**Cell Culture Study.** CCRF-CEM cells, a human leukemic cell line,[23] were grown as previously described.[24] Dose–response curves were generated for various compounds to determine the concentration required for 50% inhibition of growth ($IC_{50}$). Cluster plates were prepared in duplicate with the compound at various concentrations. Test solutions were made initially in DMSO at a concentration of 4 mg/mL and further diluted with solvent to the desired concentration. Cells in Roswell Park Memorial Institute 1640 media supplemented with 10% dialyzed fetal bovine serum, 16 mM HEPES, and 8 mM MOPS buffers were added to the well at a final concentration of $4.8 \times 10^4$ cells/well in a total volume of 2.0 mL. After 72 h of incubation (95% air, 5% $CO_2$), cell numbers were determined on a ZBI coulter counter. Cell number for indicated controls at the end of incubation is usually (4–6) × $10^5$ cells/well.

**In Vivo Antitumor Activity.** For the various solid tumors, 1–2 mm$^3$ tumor fragments were implanted sc by trocar in the axillary region of syngeneic mice (C3H). Treatment was initiated 24 h after tumor implantation. One day after the final dose, the inhibition of tumor growth was determined by comparing the tumor volume of the treated group to that of controls. Tumor volume ($V$) was calculated by measuring the tumor width ($W$) and length ($L$) and using the equation $V = L(W^2)/2$. For all studies, the compounds were suspended in 2.5% Emulphor in 0.9% saline and administered ip or as a suspension in 5% Emulphor by oral gavage.

**5-Bromothiophene-2-carboxylic Acid (18a).** To a stirred solution of 10.00 g (1.0 equiv) of 2-bromothiophene-5-carboxaldehyde (purchased from Aldrich) in 100 mL of acetone under nitrogen at 5 °C was added dropwise 30.0 mL (1.5 equiv) of 2.67 M Jones reagent (chromic acid/sulfuric acid/water). Stirring was continued at 5 °C for 30 min followed by stirring at room temperature for 2 h. Methanol was added to the reaction mixture to destroy any excess oxidant, and the solids were filtered off. The filtrate was concentrated in vacuo, and the residue was partitioned between ether and water. The ether extract was washed with water (2×) and brine, dried ($MgSO_4$), filtered, and concentrated under reduced pressure. Trituration of the residue in hexanes and filtration then gave 10.72 g (99%) of pure **18a** as a pale yellow solid: mp 136–137 °C; IR (KBr) 1692, 1664, 1532, 1433, 1403, 1327, 1277, 1108, 983, 816 cm$^{-1}$; $^1$H NMR (DMSO-$d_6$) $\delta$ 7.55 (d, $J$ = 4.3 Hz, 1 H, Ar), 7.33 (d, $J$ = 4.3 Hz, 1 H, Ar); MS $m/e$ 207 ($M^+$). Anal. ($C_5H_3O_2SBr$) C, H, S, Br.

**4-Bromothiophene-2-carboxylic acid (18b)** was prepared in 75% yield from 4-bromothiophene-2-carboxaldehyde (purchased from Aldrich) as described above: mp 98–99 °C; IR (KBr) 3114, 3034, 3025, 2981, 1688, 1523, 1422, 1345, 1297, 1268, 1221, 1185, 874, 828 cm$^{-1}$; $^1$H NMR ($CDCl_3$) $\delta$ 7.67 (s, 1 H, Ar), 7.43 (s, 1 H, Ar); MS $m/e$ 207 ($M^+$). Anal. ($C_5H_3O_2SBr$) C, H, S, Br.

**Methyl 5-Bromothiophene-2-carboxylate (19a).** A mixture of 10.66 g of **18a**, 100 mL of MeOH, and 1 g of $H_2SO_4$ was heated under reflux with stirring and exclusion of moisture for 12 h and cooled to room temperature, the MeOH was removed under reduced pressure, and the resulting residue was extracted with $CH_2Cl_2$. The organic extract was washed with water (3×), saturated $NaHCO_3$, and brine, dried ($Na_2SO_4$), filtered, and concentrated under reduced pressure to give 11.33 g (>99%) of pure **19a** as a white solid: mp 61–62 °C; IR (KBr) 3020, 1716, 1534, 1439, 1415, 1332, 1294, 1259, 1213, 1096 cm$^{-1}$; $^1$H NMR ($CDCl_3$) $\delta$ 7.56 (d, $J$ = 4.3 Hz, 1 H, Ar), 7.09 (d, $J$ = 4.3 Hz, 1 H, Ar), 3.88 (s, 3 H, $CH_3$); MS $m/e$ 221 ($M^+$). Anal. ($C_6H_5O_2SBr$) C, H.

**Methyl 4-bromothiophene-2-carboxylate (19b)** was prepared in 67% yield as an orange liquid from **18b** as described above for the preparation of **19a**: IR (film) 1716, 1517, 1438, 1404, 1353, 1344, 1291, 1257, 1225, 1218, 1184, 1101, 1067, 872, 830 cm$^{-1}$; $^1$H NMR ($CDCl_3$) $\delta$ 7.67 (s, 1 H, Ar), 7.43 (s, 1 H, Ar), 3.87 (s, 3 H, $CH_3$); MS $m/e$ 221 ($M^+$). Anal. ($C_6H_5O_2SBr$) C, H.

**Methyl 5-(4-Hydroxy-1-butynyl)thiophene-2-carboxylate (20a).** A mixture of 4.5 mg (0.005 equiv) of $PdCl_2$, 13.4 mg (0.01 equiv) of triphenylphosphine, 9.8 mg (0.01 equiv) of CuI, 11.33 g of **19a**, 25.93 g of $Et_3N$, and 3.59 g of 1-butyn-4-ol in 200 mL of MeCN was refluxed under nitrogen with stirring for 18 h and cooled to room temperature, and the volatiles were removed under reduced pressure. The residue was partitioned between $CHCl_3$ and water, and the organic extract was washed with water (2×) and brine, dried ($Na_2SO_4$), filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography, eluting with 1:1 hexane/EtOAc. Fractions homogeneous to TLC for the major component were combined and concentrated under reduced pressure to give 0.12 g (68%) of **20a** as a dark red oil: IR (film) 3020, 1710, 1452, 1348, 1293, 1267, 1225, 1101, 1051, 1044 cm$^{-1}$; $^1$H NMR ($CDCl_3$) $\delta$ 7.63 (d, $J$ = 3.7 Hz, 1 H, Ar), 7.10 (d, $J$ = 3.7 Hz, 1 H, Ar), 3.81–3.93 (m, 5 H, $CH_3$, $CH_2OH$), 2.73 (t, $J$ = 6.2 Hz, 2 H, $ArCH_2$), 2.05 (bs, 1 H, OH); MS $m/e$ 210 ($M^+$). Anal. ($C_{10}H_{10}O_3S$) C, H, S.

**Methyl 4-(4-hydroxy-1-butynyl)thiophene-2-carboxylate (20b)** was prepared in 67% yield as a yellow oil from **19b** as described above for the preparation of **20a**: IR (film) 3025, 3021, 3015, 1713, 1446, 1293, 1261, 1231, 1228, 1225, 1222, 1214, 1192, 1080, 1053 cm$^{-1}$; $^1$H NMR ($CDCl_3$) $\delta$ 7.72 (s, 1 H, Ar), 7.53 (s, 1 H, Ar), 3.87 (s, 3 H, $CH_3$), 3.79 (t, $J$ = 6.2 Hz, 2 H, $CH_2OH$), 2.65 (t, $J$ = 6.3 Hz, 2 H, $ArCH_2$), 1.87 (bs, 1 H, OH); MS $m/e$ 210 ($M^+$). Anal. ($C_{10}H_{10}O_3S$) C, H, S.

**Methyl 5-(4-Hydroxybutyl)thiophene-2-carboxylate (21a).** A solution of 6.50 g of **20a** in 200 mL of ethanol containing 3.25 g (50% wt equiv) of 5% Pd/C was hydrogenated in a Parr vessel at 50 psi of hydrogen for 6 h. The reaction mixture was filtered through a pad of Celite which was washed with EtOH. Concentration of the filtrate under reduced pressure then gave 4.35 g (67%) of pure **21a** as a yellow liquid: IR (film) 1707, 1462, 1297, 1272, 1224, 1100 cm$^{-1}$; $^1$H NMR ($CDCl_3$) $\delta$ 7.62 (d, $J$ = 3.7 Hz, 1 H, Ar), 6.80 (d, $J$ = 3.7 Hz, 1 H, Ar), 3.85 (s, 3 H, $CH_3$), 3.67 (t, $J$ = 6.3 Hz, 2 H, $CH_2OH$), 2.87 (t, $J$ = 7.4 Hz, 2 H, $ArCH_2$), 1.73–1.81 (m, 2 H, $CH_2$), 1.61–1.68 (m, 3 H, $CH_2$, OH); MS $m/e$ 214 ($M^+$). Anal. ($C_{10}H_{14}O_3S$) C, H, S.

**Methyl 4-(4-hydroxybutyl)thiophene-2-carboxylate (21b)** was prepared in 82% yield as a clear oil from **20b** as described above for the preparation of **21a**: IR (film) 1710, 1444, 1293, 1260, 1225 cm$^{-1}$; $^1$H NMR ($CDCl_3$) $\delta$ 7.59 (s, 1 H, Ar), 7.12 (s, 1 H, Ar), 3.81 (s, 3 H, $CH_3$), 3.60 (t, $J$ = 6.3 Hz, 2 H, $CH_2OH$), 2.59 (t, $J$ = 7.4 Hz, 2 H, $ArCH_2$), 2.10 (bs, 1 H, OH), 1.60–1.68 (m, 2 H, $CH_2$), 1.51–1.58 (m, 2 H, $CH_2$); MS $m/e$ 214 ($M^+$). Anal. ($C_{10}H_{14}O_3S$) C, H, S.

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(24) Grindey, G. B.; Wang, M. C.; Kinahan, J. Thymidine Induced Perturbations in Ribonucleoside and Deoxyribonucleoside Triphosphate Pools in Human Leukemic CCRF-CEM Cells. *Mol. Pharmacol.* **1979**, *16*, 601–614.

**Methyl 5-[4-(Mesyloxy)butyl]thiophene-2-carboxylate (22a).** To a mixture of 3.93 g (1.0 equiv) of **21a** and 1.95 g (1.05 equiv) of $Et_3N$ in 125 mL of anhydrous $Et_2O$ at 0 °C was added dropwise 2.21 g (1.05 equiv) of mesyl chloride. A precipitate immediately began to form, and the mildly exothermic reaction mixture was brought gradually to room temperature. After 4 h, 100 mL of water was added, bringing the precipitated salts into solution. The organic layer was separated, washed with water, dried ($MgSO_4$), and concentrated under reduced pressure. The residue was purified by flash chromatography, eluting with 30% EtOAc/hexanes. Fractions homogeneous by TLC for the major component were combined and concentrated under reduced pressure to give 5.10 g (95%) of **22a** as a clear oil (0.19 g of **21a** were recovered): IR (film) 1708, 1463, 1359, 1341, 1296, 1276, 1225, 1220, 1216, 1215, 1174, 1100, 972, 936 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 7.63 (d, $J$ = 3.7 Hz, 1 H, Ar), 6.81 (d, $J$ = 3.7 Hz, 1 H, Ar), 4.25 (t, $J$ = 5.6 Hz, 2 H, $CH_2OSO_2$), 3.86 (s, 3 H, $CH_3$), 3.01 (s, 3 H, $OSO_2CH_3$), 2.90 (t, $J$ = 6.6 Hz, 2 H, $ArCH_2$), 1.80–1.86 (m, 4 H, $CH_2CH_2$); MS $m/e$ 292 ($M^+$). Anal. ($C_{11}H_{16}O_5S_2$) C, H, S.

**Methyl 4-[4-(mesyloxy)butyl]thiophene-2-carboxylate (22b)** was prepared in 93% yield as a yellow oil from **21b** as described above for the preparation of **22a**: IR (film) 3019, 1710, 1445, 1359, 1339, 1260, 1206, 1175, 972, 937 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 7.60 (s, 1 H, Ar), 7.15 (s, 1 H, Ar), 4.21 (t, $J$ = 5.6 Hz, 2 H, $CH_2OSO_2$), 3.84 (s, 3 H, $CH_3$), 2.98 (s, 3 H, $OSO_2CH_3$), 2.64 (t, $J$ = 6.7 Hz, 2 H, $ArCH_2$), 1.69–1.75 (m, 4 H, $CH_2CH_2$); MS $m/e$ 292 ($M^+$). Anal. ($C_{11}H_{16}O_5S_2$) C, H, S.

**Methyl 5-[5-(Ethoxycarbonyl)-5-cyanopentyl]thiophene-2-carboxylate (23a).** A dry 250-mL flask was charged with 0.74 g (1.1 equiv) of 60% NaH in mineral oil, the mineral oil was removed by washing twice with anhydrous THF, and 100 mL of anhydrous THF was added. The flask was cooled to 0 °C in an ice bath, and an anhydrous THF solution of 1.90 g (1.0 equiv) of ethyl cyanoacetate was added dropwise under nitrogen. There was a moderate evolution of hydrogen. The mixture was stirred vigorously while warming to room temperature until gas evolution ceased, and to this mixture was added dropwise an anhydrous THF solution of 4.92 g (1.0 equiv) of **22a**. The resulting mixture was refluxed with stirring under nitrogen for 24 h. After cooling to room temperature, solvent was removed under reduced pressure, and $Et_2O$ was added. The organic extracts were washed with water and brine, dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography, eluting with 30% EtOAc/hexanes. Fractions homogeneous by thin-layer chromatography for the major component were combined and concentrated under reduced pressure to give 2.01 g (39%) of pure **23a** as a clear oil (2.33 g of **22a** were recovered): IR (film) 3020, 1745, 1708, 1464, 1296, 1270, 1225, 1223, 1220, 1213, 1207, 1100 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 7.63 (d, $J$ = 7.3 Hz, 1 H, Ar), 6.79 (d, $J$ = 7.3 Hz, 1 H, Ar), 4.21–4.30 (m, 2 H, $CO_2CH_2$), 3.86 (s, 3 H, $CH_3$), 3.50 (t, $J$ = 6.9 Hz, 1 H, CHCN), 2.86 (t, $J$ = 7.4 Hz, 2 H, $ArCH_2$), 1.94–2.17 (m, 2 H, $CH_2$), 1.55–1.80 (m, 4 H, $CH_2CH_2$), 1.26–1.35 (m, 3 H, $CO_2CH_2CH_3$); MS $m/e$ 309 ($M^+$). Anal. ($C_{15}H_{19}NO_4S$) C, H, N, S.

**Methyl 4-[5-(Ethoxycarbonyl)-5-cyanopentyl]thiophene-2-carboxylate (23b).** This compound was prepared in 48% yield from **22b** as described above for the preparation of **23a**: IR (film) 1746, 1711, 1444, 1260, 1225, 1220, 1207 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 7.60 (s, 1 H, Ar), 7.15 (s, 1 H, Ar), 4.23 (q, $J$ = 7.2 Hz, 2 H, $CO_2CH_2$), 3.85 (s, 3 H, $CH_3$), 3.47 (t, $J$ = 6.9 Hz, 1 H, CHCN), 2.62 (t, $J$ = 7.5 Hz, 2 H, $ArCH_2$), 1.95 (q, $J$ = 7.3 Hz, 2 H, $CH_2CHCN$), 1.62–1.69 (m, 2 H, $CH_2$), 1.48–1.47 (m, 2 H, $CH_2$), 1.27–1.33 (m, 3 H, $CO_2CH_2CH_3$); MS $m/e$ 309 ($M^+$). Anal. ($C_{15}H_{19}NO_4S$) C, H, N, S.

**Methyl 4-[4-(2,4-Diamino-1,6-dihydro-6-oxo-5-pyrimidinyl)butyl]thiophene-2-carboxylate (24b).** To a freshly prepared solution of sodium methoxide [from 0.15 g (6.5 mmol) of sodium and 25 mL of anhydrous MeOH] was added 0.60 g (6.5 mmol) of guanidine hydrochloride, the mixture was heated to 40 °C for 0.5 h, and 1.78 g (5.76 mmol) of **23b** in 25 mL of anhydrous MeOH was added. The reaction mixture was refluxed with stirring under nitrogen for 24 h. After cooling to room temperature, the volatiles were removed under reduced pressure and the residue was triturated in $Et_2O$. The solid was collected by vacuum filtration and washed with water. Purification was carried out by flash column chromatography, eluting with 10% MeOH/$CHCl_3$. Fractions homogeneous by TLC for the major component were combined and concentrated under reduced pressure to give 0.48 g (25%) of **24b** as white powder: mp 185–186 °C; IR (KBr) 1696, 1672, 1622, 1602, 1442, 1430, 1385, 1259 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$) $\delta$ 9.68 (bs, 1 H, (3)-NH), 7.60 (s, 1 H, Ar), 7.51 (s, 1 H, Ar), 5.84 (bs, 2 H, (2)-$NH_2$), 5.59 (bs, 2 H, (4)-$NH_2$), 3.70 (s, 3 H, $CH_3$), 2.56 (t, $J$ = 7.2 Hz, 2 H, (5)-$CH_2$), 2.14 (t, $J$ = 7.4 Hz, 2 H, $ArCH_2$), 1.52 (m, 2 H, $CH_2$), 1.25 (m, 2 H, $CH_2$); MS $m/e$ 322 ($M^+$). Anal. ($C_{14}H_{18}N_4O_3S$) C, H, N, S.

**Methyl 5-[4-(2,4-Diamino-1,6-dihydro-6-oxo-5-pyrimidinyl)butyl]thiophene-2-carboxylate (24a).** This compound was prepared from 0.08 g (3.56 mmol) of sodium metal, 0.34 g (3.56 mmol) of guanidine hydrochloride, and 1.00 g (3.32 mmol) of **23a** as described above for the preparation of **24b**; yield 0.32 g (30%) of **24a** as a pale yellow solid after flash column chromatography eluting with 10% MeOH/$CHCl_3$: mp 180–181 °C; IR (KBr) 3391, 1709, 1689, 1618, 1602, 1460, 1447, 1436, 1426, 1304, 1297, 1271, 1101 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$) $\delta$ 9.66 (bs, 1 H, (3)-NH), 7.58 (d, $J$ = 3.7 Hz, 1 H, Ar), 6.89 (d, $J$ = 3.7 Hz, 1 H, Ar), 5.82 (bs, 2 H, (2)-$NH_2$), 5.58 (bs, 2 H, (4)-$NH_2$), 3.73 (s, 3 H, $CH_3$), 2.79 (t, $J$ = 7.6 Hz, 2 H, (5)-$CH_2$), 2.13 (t, $J$ = 7.3 Hz, 2 H, $ArCH_2$), 1.55 (m, 2 H, $CH_2$), 1.31 (m, 2 H, $CH_2$); MS $m/e$ 322 ($M^+$). Anal. ($C_{14}H_{18}N_4O_3S$) C, H, N, S.

**Methyl 11-Cyano-11-(ethoxycarbonyl)undecanoate (23c).** This compound was prepared from 9.50 g (33.9 mmol) of methyl 10-[(methylsulfonyl)oxy]decanoate, 4.02 g (33.9 mmol) of ethyl cyanoacetate, and 1.12 g (37.3 mmol) of 80% sodium hydride as described above for **23a** and yielded 8.80 g (87%) of **23c** as an orange oil: IR (film) 3021, 1719, 1612, 1437, 1375, 1312, 1285, 1234, 1220, 1216, 1210, 1193, 1181, 1158, 1114, 1021 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 3.97 (t, $J$ = 7.2 Hz, 2 H, $CO_2CH_2$), 3.63 (s, 3 H, $CO_2CH_3$), 3.44 (m, 1 H, CHCN), 2.25 (m, 2 H, $CH_2CO_2CH_3$), 1.83 (m, 2 H, $CH_2$), 1.43–1.48 (m, 4 H, $CH_2CH_2$), 1.17–1.31 (bs, 13 H, secondary aliphatic $CH_2$, $CO_2CH_2CH_3$); MS $m/e$ 271 ($M^+$ - CN). Anal. ($C_{16}H_{27}NO_4$) C, H, N.

**Methyl 10-(2,4-Diamino-1,6-dihydro-6-oxo-5-pyrimidinyl)decanoate (24c).** This compound was prepared from 3.22 g (33.0 mmol) of guanidine hydrochloride, 0.76 g (33.0 mmol) of sodium methoxide, and 8.22 g (31.5 mmol) of **23c** as described above for the preparation of **24a**; yield 1.99 g (23%) of **24c** as a white solid after a standard workup with $CHCl_3$ and gravity column chromatography eluting with 10% MeOH/$CHCl_3$: mp 134–135 °C; IR (KBr) 3393, 2926, 1708, 1605, 1441, 1370 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$) $\delta$ 9.77 (bs, 1 H, (3)-NH), 5.86 (bs, 2 H, (2)-$NH_2$), 5.55 (bs, 2 H, (4)-$NH_2$), 3.54 (s, 3 H, $CO_2CH_3$), 2.25 (t, $J$ = 7.2 Hz, 2 H, $CH_2CO_2CH_3$), 2.09 (m, 2 H, (5)-$CH_2$), 1.47–1.53 (m, 4 H, secondary aliphatic $CH_2$), 1.20 (bs, 10 H, secondary aliphatic $CH_2$); MS-HR $M^+$ at $m/e$ 310.1999 (calcd 310.2005). Anal. ($C_{15}H_{26}N_4O_3$) C, H; N: calcd, 18.05; found, 17.31.

**4-[4-(2,4-Diamino-1,6-dihydro-6-oxo-5-pyrimidinyl)butyl]thiophene-2-carboxylic Acid (25b).** This compound was prepared from 0.25 g (0.78 mmol) of **24b** and 30 mL of 1.0 N NaOH as described previously for the preparation of **9**;[13] yield 0.21 g (91%) of **25b** as a white powder: mp 258–259 °C; IR (KBr) 2540, 2538, 2534, 2520, 2509, 1696, 1653, 1617, 1547, 1461, 1406, 1377, 1366, 1298, 1280 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$) $\delta$ 7.53 (s, 1 H, Ar), 7.45 (s, 1 H, Ar), 6.67 (bs, 2 H, (2)-$NH_2$), 6.14 (bs, 2 H, (4)-$NH_2$), 2.55 (t, $J$ = 7.3 Hz, 2 H, (5)-$CH_2$), 2.17 (t, $J$ = 7.3 Hz, 2 H, $ArCH_2$), 1.50 (m, 2 H, secondary aliphatic $CH_2$), 1.30 (m, 2 H, secondary aliphatic $CH_2$); MS $m/e$ 308 ($M^+$). Anal. ($C_{13}H_{16}N_4O_3S$) C, H, N, S.

**5-[4-(2,4-Diamino-1,6-dihydro-6-oxo-5-pyrimidinyl)butyl]thiophene-2-carboxylic Acid (25a).** This compound was prepared from 0.80 g (2.48 mmol) of **24a** and 30 mL of 1.0 N NaOH as described previously for the preparation of **9**;[13] yield 0.76 g (quantitative) of **25a** as a white solid: mp 258–259 °C; IR (KBr) 3185, 2947, 1695, 1664, 1600, 1536, 1461 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$) $\delta$ 7.51 (d, $J$ = 3.7 Hz, 1 H, Ar), 6.85 (d, $J$ = 3.7 Hz, 1 H, Ar), 7.49 (bs, 2 H, (2)-$NH_2$), 6.73 (bs, 2 H, (4)-$NH_2$), 2.77 (t, $J$ = 7.4 Hz, 2 H, (5)-$CH_2$), 2.19 (t, $J$ = 7.0 Hz, 2 H, $ArCH_2$), 1.55 (m, 2 H, secondary aliphatic $CH_2$), 1.34 (m, 2 H, secondary aliphatic $CH_2$); MS $m/e$ 308 ($M^+$). Anal. ($C_{13}H_{16}N_4O_3S$) C, H, N, S.

**10-(2,4-Diamino-1,6-dihydro-6-oxo-5-pyrimidinyl)decanoic Acid (25c).** This compound was prepared from 0.68 g (2.19 mmol) of **24c** as described previously for the preparation of **9**;[13] yield

0.42 g (65%) of **25c** as a pale yellow solid: mp 213–216 °C; IR (KBr) 2924, 2849, 1719, 1684, 1628, 1599, 1446 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$) $\delta$ 5.85 (bs, 2 H, (2)-$NH_2$), 5.55 (bs, 2 H, (6)-$NH_2$), 2.15 (t, $J$ = 7.3 Hz, 2 H, $CH_2CO_2H$), 2.08 (m, 2 H, (5)-$CH_2$), 1.42–1.48 (m, 4 H, secondary aliphatic $CH_2$), 1.21 (bs, 10 H, secondary aliphatic $CH_2$); MS-HR $M^+$ at $m/e$ 296.1850 (calcd 296.1848).

**Diethyl *N*-[4-[4-(2,4-Diamino-1,6-dihydro-6-oxo-5-pyrimidinyl)butyl]-2-thienyl]-L-glutamate (26b).** This compound was prepared from 0.15 g (0.49 mmol) of **25b**, 0.20 g (0.73 mmol) of phenyl *N*-phenylphosphoramidochloridate, 0.25 g (2.5 mmol) of *N*-methylmorpholine, and 0.23 g (0.98 mmol) of diethyl L-glutamate hydrochloride as described previously for the preparation of **12**;[13] yield 0.13 g (54%) of **26b** as a pale yellow powder after flash column chromatography eluting with 10% MeOH/$CHCl_3$: mp 94–96 °C; IR (KBr) 3368, 3363, 3361, 3360, 3352, 3349, 3346, 3343, 1735, 1600, 1552, 1525, 1499, 1447, 1428, 1371, 1206, 1180 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 7.94 (s, 1 H, Ar), 7.85 (d, $J$ = 7.8 Hz, 1 H, NH), 7.08 (s, 1 H, Ar), 5.11 (bs, 2 H, (2)-$NH_2$), 4.70 (m, 1 H, CH), 4.55 (bs, 2 H, (4)-$NH_2$), 4.23 (q, $J$ = 7.2 Hz, 2 H, $CO_2CH_2$), 4.15 (q, $J$ = 7.2 Hz, 2 H, $CO_2CH_2$), 2.69 (m, 2 H, (5)-$CH_2$), 2.59 (m, 2 H, $ArCH_2$), 2.23–2.44 (m, 4 H, secondary aliphatic $CH_2$), 1.64 (m, 2 H, secondary aliphatic $CH_2$), 1.47 (m, 2 H, secondary aliphatic $CH_2$), 1.25–1.32 (m, 6 H, 2 $CH_3$); MS $m/e$ 493 ($M^+$). Anal. ($C_{22}H_{31}N_5O_6S$) C, H, N, S.

**Diethyl *N*-[5-[4-(2,4-Diamino-1,6-dihydro-6-oxo-5-pyrimidinyl)butyl]-2-thienyl]-L-glutamate (26a).** This compound was prepared from 0.09 g (0.29 mmol) of **25a**, 0.12 g (0.44 mmol) of phenyl *N*-phenylphosphoramidochloridate, 0.15 g (1.5 mmol) of *N*-methylmorpholine, and 0.14 g (0.58 mmol) of diethyl L-glutamate hydrochloride as described previously for the preparation of **12**;[13] yield 0.10 g (72%) of **26a** as a pale yellow microcrystalline solid after flash column chromatography eluting with 10% MeOH/$CHCl_3$: mp 96–98 °C; IR (KBr) 3366, 3325, 1732, 1605, 1530, 1500, 1441, 1210, 1180 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 7.34 (d, $J$ = 3.4 Hz, 1 H, Ar), 6.66 (d, $J$ = 3.4 Hz, 1 H, Ar), 7.19 (d, $J$ = 7.5 Hz, 1 H, NH), 5.70 (bs, 2 H, (2)-$NH_2$), 4.80 (bs, 2 H, (4)-$NH_2$), 4.69 (m, 1 H, CH), 4.19 (q, $J$ = 7.1 Hz, 2 H, $CO_2CH_2$), 4.08 (q, $J$ = 7.1 Hz, 2 H, $CO_2CH_2$), 2.75 (m, 2 H, (5)-$CH_2$), 2.44 (m, 2 H, $ArCH_2$), 2.06–2.31 (m, 4 H, secondary aliphatic $CH_2$), 1.63 (m, 2 H, secondary aliphatic $CH_2$), 1.42 (m, 2 H, secondary aliphatic $CH_2$), 1.17–1.29 (m, 6 H, 2 $CH_3$); MS $m/e$ 493 ($M^+$). Anal. ($C_{22}H_{31}N_5O_6S$) C, H, N, S.

**Diethyl *N*-[10-(2,4-Diamino-1,6-dihydro-6-oxo-5-pyrimidinyl)decanoyl]-L-glutamate (26c).** This compound was prepared from 0.39 g (1.32 mmol) of **25c**, 0.53 g (1.98 mmol) of phenyl *N*-phenylphosphoramidochloridate, 0.67 g (6.60 mmol) of *N*-methylmorpholine, and 0.63 g (2.64 mmol) of diethyl L-glutamate hydrochloride as described previously for the preparation of **12**;[13] yield 0.44 g (70%) of **26c** as a pale yellow microcrystalline solid: mp 102–104 °C; IR (KBr) 3400, 1729, 1625, 1055, 998 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 6.90 (d, $J$ = 8.0 Hz, 1 H, NH), 6.03 (bs, 2 H, (2)-$NH_2$), 4.88 (bs, 2 H, (4)-$NH_2$), 4.62 (m, 1 H, CH), 4.23 (q, $J$ = 7.2 Hz, 2 H, $CO_2CH_2$), 4.13 (q, $J$ = 7.2 Hz, 2 H, $CO_2CH_2$), 2.01–2.46 (m, 6 H, secondary aliphatic $CH_2$), 1.60 (t, $J$ = 6.3 Hz, 2 H, (5)-$CH_2$), 1.21–1.48 (m, 20 H, secondary aliphatic $CH_2$, 2 $CH_3$); MS-HR $M^+$ at $m/e$ 481.2902 (calcd 481.2900).

***N*-[5-[4-(2,4-Diamino-1,6-dihydro-6-oxo-5-pyrimidinyl)butyl]-2-thienyl]-L-glutamic Acid (27a).** This compound was prepared from 0.022 g (0.04 mmol) of **26a** and 20 mL of 1.0 N NaOH as described previously for the preparation of **2**;[13] yield 0.014 g (72%) of **27a** as a white powder: mp 253–256 °C; IR (KBr) 1706, 1653, 1642, 1635, 1631, 1627, 1624, 1547 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$) $\delta$ 9.68 (bs, 1 H, (3)-NH), 8.45 (d, $J$ = 7.1 Hz, 1 H, NH), 7.64 (d, $J$ = 3.6 Hz, 1 H, Ar), 6.83 (d, $J$ = 3.6 Hz, 1 H, Ar), 5.86 (bs, 2 H, (2)-$NH_2$), 5.60 (bs, 2 H, (6)-$NH_2$), 4.30 (m, 1 H, CH), 4.09 (m, 2 H, secondary aliphatic $CH_2$), 2.76 (t, $J$ = 7.3 Hz, 2 H, (5)-$CH_2$), 2.30 (t, $J$ = 7.7 Hz, 2 H, $ArCH_2$), 2.15 (t, $J$ = 6.8 Hz, 2 H, secondary aliphatic $CH_2$), 1.54 (m, 2 H, secondary aliphatic $CH_2$), 1.32 (m, 2 H, secondary aliphatic $CH_2$); MS (FAB) $m/e$ 438 ($M^+$ + 1). Anal. ($C_{18}H_{23}N_5O_6S$) C, H, N, S.

***N*-[4-[4-(2,4-Diamino-1,6-dihydro-6-oxo-5-pyrimidinyl)butyl]-2-thienyl]-L-glutamic Acid (27b).** This compound was prepared from 0.055 g (0.11 mmol) of **26b** and 5.0 mL of 1.0 N NaOH as described previously for the preparation of **2**;[13] yield 0.030 g (76%) of **27b** as a white solid: mp 235–236 °C; IR (KBr) 3184, 1712, 1620, 1414, 1271, 721, 553 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$) $\delta$ 7.72 (s, 1 H, Ar), 7.25 (s, 1 H, Ar), 5.80 (bs, 2 H, (2)-$NH_2$), 5.60 (bs, 2 H, (4)-$NH_2$), 4.32 (m, 1 H, CH), 2.10–2.39 (m, 4 H, secondary aliphatic $CH_2$), 1.89–2.12 (m, 4 H, secondary aliphatic $CH_2$), 1.03–1.46 (m, 4 H, secondary aliphatic $CH_2$); MS (FAB) $m/e$ 438 ($M^+$ + 1). Anal. ($C_{18}H_{23}N_5O_6S$) C, H, N, S.

***N*-[10-(2,4-Diamino-1,6-dihydro-6-oxo-5-pyrimidinyl)decanoyl]-L-glutamic Acid (27c).** This compound was prepared from 0.06 g (0.12 mmol) of **26c** as described previously for the preparation of **2**;[13] yield 0.02 g (40%) of **27c** as a white microcrystalline solid: mp >270 °C; IR (KBr) 3415, 2919, 1715, 1636, 1046, 898 $cm^{-1}$; $^1H$ NMR (dTFA, DMSO-$d_6$) $\delta$ 4.38 (m, 1 H, $CHCO_2H$), 2.25 (m, 2 H, secondary aliphatic $CH_2$), 1.96–2.16 (m, 4 H, secondary aliphatic $CH_2$), 1.78 (m, 2 H, secondary aliphatic $CH_2$), 1.30 (m, 2 H, secondary aliphatic $CH_2$), 0.80–1.16 (m, 12 H, secondary aliphatic $CH_2$); MS-HR $M^+$ at $m/e$ 425.4882 (calcd 425.4888).

**2-Amino-1,6-dihydro-4-hydroxy-6-oxo-5-(3-butynyl)pyrimidine (29).** This compound was prepared from 5.01 g (51.4 mmol) of guanidine hydrochloride, 2.83 g (52.4 mmol) of sodium methoxide, and 10.11 g (46.7 mmol) of ethyl 2-(ethoxycarbonyl)-5-hexynoate as described previously for the preparation of **6**;[13] yield 2.88 g (36%) of **29** as a white powder: mp >300 °C; IR (KBr) 3379, 3246, 3236, 1694, 1666, 1637, 1616, 1562, 1422, 1344, 1337 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$) $\delta$ 6.50 (bs, 2 H, $NH_2$), 2.62 (t, $J$ = 2.5 Hz, 1 H, acetylenic), 2.30 (m, 2 H, secondary aliphatic $CH_2$), 2.10 (m, 2 H, secondary aliphatic $CH_2$); MS-HR $M^+$ at $m/e$ 179.1796 (calcd 179.1798).

**5-(3-Butynyl)-1,6-dihydro-4-hydroxy-6-oxo-2-(pivaloylamino)pyrimidine (30).** A mixture of 0.22 g (1.23 mmol) of **29**, 0.44 g (2.37 mmol) of pivalic anhydride, and 0.66 g of pyridine was heated at 140 °C with stirring under nitrogen for 8 h. The suspension was cooled, and diethyl ether was added. A pale yellow solid was collected by vacuum filtration, and purification of the solid was carried out by flash column chromatography, eluting with 10% MeOH/$CHCl_3$. Fractions homogeneous by TLC for the major component were combined and concentrated under reduced pressure to give 0.24 g (75%) of pure **30** as a pale yellow solid: mp 211–214 °C; IR (KBr) 3109, 2974, 1643, 1628, 1589, 1507, 1480, 1464, 1440, 1431, 1404, 1394, 1374, 1344, 1319, 1297, 1275, 1231, 1185, 1146, 1098 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$) $\delta$ 2.64 (t, $J$ = 2.2 Hz, 1 H, acetylenic), 2.42 (t, $J$ = 8.0 Hz, 2 H, secondary aliphatic $CH_2$), 2.18 (t, $J$ = 7.0 Hz, 2 H, secondary aliphatic $CH_2$), 1.16 (s, 9 H, *tert*-butyl); MS $m/e$ 263 ($M^+$). Anal. ($C_{13}H_{17}N_3O_3$) C, H, N.

**Diethyl *N*-[4-[4-[1,6-Dihydro-4-hydroxy-6-oxo-2-(pivaloylamino)-5-pyrimidinyl]butynyl]benzoyl]-L-glutamate (31).** To a stirred mixture of 0.0010 g (0.06 mmol) of $PdCl_2$ and 0.0030 g (0.11 mmol) of triphenylphosphine in 20 mL of MeCN under nitrogen was added 0.30 g (1.14 mmol) of **30**, 0.49 g (1.14 mmol) of diethyl *N*-(4-iodobenzoyl)-L-glutamate, 0.58 g (5.70 mmol) of $Et_3N$, and 0.0022 g (0.11 mmol) of CuI. The reaction mixture was refluxed with stirring under nitrogen for 12 h. After cooling to room temperature, the volatiles were removed under reduced pressure and the residue was purified by flash column chromatography, eluting with 1:1 EtOAc/hexanes followed by a second chromatography, eluting with 10% MeOH/$CHCl_3$. Fractions homogeneous by TLC for the major component were combined and concentrated under reduced pressure to give 0.24 g (36%) of **31** as a white solid: mp 205–207 °C; IR (KBr) 3278, 2978, 1740, 1642, 1581, 1538, 1388, 1319, 1241, 1175, 1142 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 8.10 (bs, 1 H, (3)-NH), 7.80, 7.30 (AA'BB', 4 H, Ar), 7.06 (d, $J$ = 7.4 Hz, 1 H, benzoyl-NH), 6.42 (bs, 1 H, OH), 4.78 (m, 1 H, CH), 4.23 (q, $J$ = 7.3 Hz, 2 H, $CO_2CH_2$), 4.08 (q, $J$ = 7.3 Hz, 2 H, $CO_2CH_2$), 2.79 (t, $J$ = 6.6 Hz, 2 H, secondary aliphatic), 2.57 (t, $J$ = 6.8 Hz, 2 H, secondary aliphatic $CH_2$), 2.12–2.55 (m, 4 H, secondary aliphatic $CH_2$), 1.29 (s, 9 H, *tert*-butyl), 1.16–1.33 (m, 6 H, 2 $CH_3$). MS $m/e$ 568 ($M^+$). Anal. ($C_{29}H_{36}N_4O_8$) C, H; N: calcd, 9.86; found, 9.25.

**Diethyl *N*-[4-[4-[1,6-Dihydro-4-hydroxy-6-oxo-2-(pivaloylamino)-5-pyrimidinyl]butyl]benzoyl]-L-glutamate (32).** This compound was prepared from 0.12 g (0.21 mmol) of **31** and 0.12 g of 5% Pd/C in MeOH with hydrogen (atmospheric pressure); yield 0.06 g (50%) of **32** as a white solid after flash column chromatography eluting with 5% MeOH/$CHCl_3$: mp 227–230 °C; IR (KBr) 3345, 1737, 1642, 1501, 1210, 1086, 1024, 931, 754 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 7.72, 7.22 (AA'BB', 4 H, Ar), 7.18 (d,

$J$ = 7.9 Hz, NH), 4.74 (m, 1 H, CH), 4.22 (q, $J$ = 6.9 Hz, 2 H, $CO_2CH_2$), 4.10 (q, $J$ = 6.9 Hz, 2 H, $CO_2CH_2$), 2.68 (t, $J$ = 9.1 Hz, 2 H, secondary aliphatic $CH_2$), 2.46 (t, $J$ = 6.7 Hz, 2 H, secondary aliphatic $CH_2$), 2.10–2.38 (m, 4 H, secondary aliphatic $CH_2$), 1.22 (s, 9 H, *tert*-butyl), 1.13–1.34 (m, 6 H, 2 $CH_3$); MS-HR $M^+$ at $m/e$ 572.2842 (calcd 572.2846).

***N*-[4-[4-(1,6-Dihydro-4-hydroxy-6-oxo-2-amino-5-pyrimidinyl)butyl]benzoyl]-L-glutamic Acid (33).** This compound was prepared from 0.011 g (0.19 mmol) of **32** and 5 mL of 1.0 N NaOH as described previously for the preparation of **2**;[13] yield 0.006 g (76%) of **33** as a white solid: mp >270 °C; IR (KBr) 3400, 1710, 1624, 1498, 1074 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$) $\delta$ 7.72, 7.25 (AA'BB', 4 H, Ar), 4.32 (m, 1 H, CH), 2.10–2.39 (m, 4 H, secondary aliphatic $CH_2$), 1.89–2.12 (m, 4 H, secondary aliphatic $CH_2$), 1.03–1.46 (m, 4 H, secondary aliphatic $CH_2$); MS $m/e$ 432 ($M^+$). Anal. ($C_{20}H_{24}N_4O_7$) C, H, N.

**Ethyl *N*-[4-[4-(2,4-Diamino-1,6-dihydro-6-oxo-5-pyrimidinyl)butyl]benzoyl]-L-phenylalaninate (34).** This compound was prepared from 0.20 g (0.67 mmol) of **9**,[13] 0.27 g (1.0 mmol) of phenyl *N*-phenylphosphoramidochloridate, 0.33 g (3.30 mmol) of *N*-methylmorpholine, and 0.30 g (1.34 mmol) of ethyl L-phenylalanine hydrochloride as described previously for the preparation of **12**;[13] yield 0.16 g (50%) of **34** as a pale yellow microcrystalline solid after flash column chromatography eluting with 5% MeOH/$CHCl_3$: mp 95–97 °C; IR (KBr) 3346, 1735, 1615, 1557, 1491, 1455, 1203, 1072, 931 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 7.62 (AA'BB', 2 H, Ar), 7.13–7.28 (m, 5 H, Ar), 6.95 (m, 2 H, Ar), 6.72 (m, 1 H, NH), 5.05 (m, 1 H, CH), 4.80 (bs, 4 H, 2 $NH_2$), 4.21 (q, $J$ = 7.1 Hz, 2 H, $CO_2CH_2$), 3.25 (m, 2 H, Ph$CH_2$CH), 2.54 (t, $J$ = 7.2 Hz, 2 H, (5)-$CH_2$), 2.19 (m, 2 H, benzylic $CH_2$), 1.49 (m, 2 H, secondary aliphatic $CH_2$), 1.19–1.38 (m, 5 H, secondary aliphatic $CH_2$, $CH_3$); MS $m/e$ 477 ($M^+$). Anal. ($C_{26}H_{31}N_5O_4$) C, H, N.

**Dimethyl *N*-[4-[4-(2,4-Diamino-1,6-dihydro-6-oxo-5-pyrimidinyl)butyl]benzoyl]-DL-aspartate (35).** This compound was prepared from 0.20 g (0.67 mmol) of **9**, 0.27 g (1.0 mmol) of phenyl *N*-phenylphosphoramidochloridate, 0.33 g (3.30 mmol) of *N*-methylmorpholine, and 0.26 g (1.34 mmol) of dimethyl DL-aspartate hydrochloride as described previously for the preparation of **12**;[13] yield 0.12 g (41%) of **35** as a white microcrystalline solid after flash column chromatography eluting with 10% MeOH/$CHCl_3$: mp 99–101 °C; IR (KBr) 3320, 3298, 3291, 3191, 3186, 3928, 3008, 2975, 2956, 2935, 2907, 2858, 1741, 1638, 1611, 1574, 1524, 1494, 1440, 1427, 1370, 1348, 1298, 1280, 1230, 1225, 1219, 1218, 1215, 1212, 1205, 1202, 1183, 1048, 997 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 7.63 (AA'BB', 2 H, Ar), 7.00–7.12 (m, 3 H, Ar, NH), 5.70 (bs, 2 H, (2)-$NH_2$), 5.06 (m, 1 H, CH), 4.84 (bs, 2 H, (4)-$NH_2$), 3.77 (s, 3 H, $CH_3$), 3.68 (s, 3 H, $CH_3$), 2.99 (m, 2 H, $CH_2CO_2CH_3$), 2.56 (m, 2 H, (5)-$CH_2$), 2.23 (m, 2 H, benzylic $CH_2$), 1.53 (m, 2 H, secondary aliphatic $CH_2$), 1.35 (m, 2 H, secondary aliphatic $CH_2$); MS $m/e$ 445 ($M^+$). Anal. ($C_{21}H_{27}N_5O_6$) C, H, N.

***N*-[4-[4-(2,4-Diamino-1,6-dihydro-6-oxo-5-pyrimidinyl)butyl]benzoyl]-L-phenylalanine (36).** This compound was prepared from 0.06 g (0.13 mmol) of **34** and 5.0 mL of 1.0 N NaOH as described previously for the preparation of **2**;[13] yield 0.05 g (83%) of **36** as a white solid: mp 240–243 °C; IR (KBr) 1702, 1640, 1613, 1569, 1531, 1497, 1442, 1401 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$) $\delta$ 8.56 (d, $J$ = 8.1 Hz, 1 H, NH), 7.65 (AA'BB', 2 H, Ar), 7.03–7.30 (m, 7 H, Ar), 5.88 (bs, 2 H, (2)-$NH_2$), 5.59 (bs, 2 H, (4)-$NH_2$), 4.55 (m, 1 H, CH), 3.06 (m, 2 H, Ph$CH_2$CH), 2.57 (t, $J$ = 7.6 Hz, 2 H, (5)-$CH_2$), 2.14 (t, $J$ = 7.0 Hz, 2 H, benzylic $CH_2$), 1.51 (m, 2 H, secondary aliphatic $CH_2$), 1.28 (m, 2 H, secondary aliphatic $CH_2$); MS $m/e$ 449 ($M^+$). Anal. ($C_{24}H_{27}N_5O_4$) C, H, N.

***N*-[4-[4-(2,4-Diamino-1,6-dihydro-6-oxo-5-pyrimidinyl)butyl]benzoyl]-DL-aspartic Acid (37).** This compound was prepared from 0.12 g (0.27 mmol) of **35** and 10 mL of 1.0 N NaOH as described previously for the preparation of **2**;[13] yield 0.08 g (73%) of **37** as a white solid: mp 265–270 °C; IR (KBr) 1705, 1644, 1641, 1567, 1540, 1499, 1403 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$) $\delta$ 8.56 (d, $J$ = 8.1 Hz, 1 H, NH), 7.72, 7.25 (AA'BB', 4 H, Ar), 5.85 (bs, 2 H, (2)-$NH_2$), 5.58 (bs, 2 H, (4)-$NH_2$), 4.66 (m, 1 H, CH), 2.57–2.82 (m, 4 H, $CH_2CO_2CH_3$, (5)-$CH_2$), 2.15 (t, $J$ = 7.1 Hz, 2 H, benzylic $CH_2$), 1.52 (m, 2 H, secondary aliphatic $CH_2$), 1.29 (m, 2 H, secondary aliphatic $CH_2$); MS $m/e$ 417 ($M^+$). Anal. ($C_{19}H_{23}N_5O_6$) C, H, N.

**Polyglutamates of Compound 2.** Following is a representative procedure for the preparation of 7-DM-DDATHF tetraglutamate (2 + $Glu_3$). In this procedure, 2-chloro-4,6-dimethoxytriazine was used as the condensing agent instead of isobutyl chloroformate. A mixture of 0.096 g (0.32 mmol) of **9**, 2 mL of anhydrous DMF, 0.035 mL (0.32 mmol) of 4-methylmorpholine, and 0.056 g (0.32 mmol) of 2-chloro-4,6-dimethoxytriazine was stirred for 30 min at room temperature under nitrogen, and then 0.26 g (0.32 mmol) of penta-*tert*-butyl *N*-[*N*-[*N*-[*N*-(benzyloxycarbonyl)-L-glutamyl]-L-glutamyl]-L-glutamyl]-L-glutamate was added, and the reaction mixture was stirred at room temperature for 3 h. The DMF was removed in vacuo, and the residue was dissolved in 30 mL of $CH_2Cl_2$, washed twice with 5% $NaHCO_3$, dried over $Na_2SO_4$, and concentrated in vacuo. This crude residue was then purified using flash chromatography on silica gel, eluting with 7% MeOH/$CHCl_3$ to give 0.12 g (34%) of protected 7-DM-DDATHF-tetraglutamate as a white solid: mp 128–131 °C; IR (KBr) 1155, 1252, 1369, 1541, 1571, 1625, 1646, 1734 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 1.31–1.49 (m, 45 H), 1.66–1.74 (m, 4 H), 1.83–1.96 (m, 4 H), 2.08–2.45 (m, 12 H), 2.46–2.72 (m, 3 H), 2.75–2.87 (m, 1 H), 4.42–4.51 (m, 4 H), 4.62–4.70 (m, 2 H), 4.96 (br s, 2 H), 7.24 (d, $J$ = 7.9 Hz, 2 H), 7.44–7.52 (m, 2 H), 7.70 (d, $J$ = 8.2 Hz, 2 H), 8.10–8.15 (m, 1 H); MS $m/e$ 1099 ($M^+$). This protected polyglutamate of **2** (0.097 g, 0.088 mmol) was then dissolved in 2 mL of 90% $HCO_2H$ and stirred at room temperature for 40 h. The solvent was removed in vacuo, and the residue was triturated in ether, filtered, and dried to give 0.070 g (97%) of 7-DM-DDATHF-tetraglutamate as a white solid: mp 190 °C (foam); IR (KBr) 1221, 1409, 1504, 1541, 1645, 1717 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$) $\delta$ 1.28–1.32 (m, 3 H), 1.36 (s, 1 H), 1.48–1.60 (m, 4 H), 1.65–1.80 (m, 4 H), 2.06–2.26 (m, 6 H), 2.48–2.58 (m, 2 H), 4.12–4.20 (m, 3 H), 4.30–4.38 (m, 1 H), 5.62 (s, 2 H), 5.90 (s, 2 H), 7.25 (d, $J$ = 7.8 Hz, 2 H), 7.77 (d, $J$ = 7.6, 2 H), 8.08–8.18 (m, 4 H), 8.50–8.58 (m, 1 H); MS $m/e$ 818 ($M^+$).

**Registry No.** **2**, 124656-55-9; **9**, 125402-87-1; **15**, 125402-91-7; **16**, 125402-92-8; **17a**, 4701-17-1; **17b**, 18791-75-8; **18a**, 7311-63-9; **18b**, 16694-18-1; **19a**, 62224-19-5; **19b**, 62224-16-2; **20a**, 137793-36-3; **20b**, 130073-85-7; **21a**, 76865-50-4; **21b**, 130073-86-8; **22a**, 130073-78-8; **22b**, 130073-77-7; **23a**, 130073-80-2; **23b**, 130073-79-9; **23c**, 137793-37-4; **24a**, 130073-82-4; **24b**, 130073-81-3; **24c**, 137793-38-5; **25a**, 130073-84-6; **25b**, 130073-83-5; **25c**, 137793-39-6; **26a**, 137793-40-9; **26b**, 130073-76-6; **26c**, 137793-41-0; **27a**, 130073-75-5; **27b**, 130073-74-4; **27c**, 137793-42-1; **28**, 117500-15-9; **29**, 137793-43-2; **30**, 137793-44-3; **31**, 137793-45-4; **32**, 137793-46-5; **33**, 137793-47-6; **34**, 137793-48-7; **35**, 137793-49-8; **36**, 137793-50-1; **37**, 137793-51-2; ethyl cyanoacetate, 105-56-6; guanidine hydrochloride, 50-01-1; diethyl L-glutamate hydrochloride, 1118-89-4; pivalic anhydride, 1538-75-6; diethyl *N*-(4-iodobenzyl)glutamate, 116387-21-4; phenylalanine hydrochloride, 17585-69-2; dimethyl DL-aspartate hydrochloride, 14358-33-9.