SYNTHESIS OF SOME CONFORMATIONALLY-CONSTRAINED GLUTAMATE MIMICS OF N-{5-[2-(2-AMINO-3,4-DIHYDRO-4-OXO-5,6,7,8-TETRAHYDROPYRIDO[2,3-d]PYRIMIDIN-6-YL)ETHYL]THIEN-2-YLCARBONYL]-L-GLUTAMIC ACID (LY254155)

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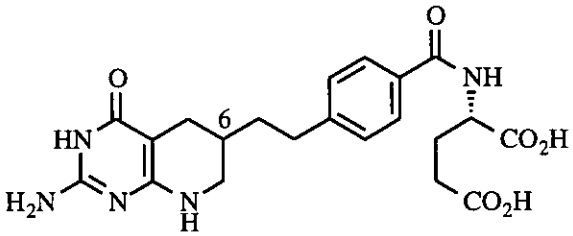
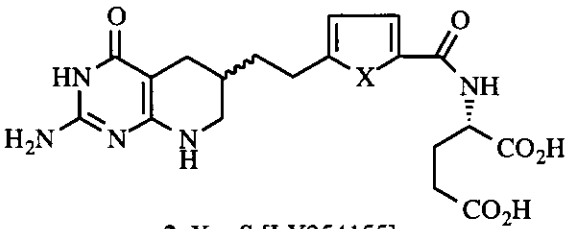
Abstract - Several new analogues of the active antitumor agent N-{5-[2-(2-amino-3,4-dihydro-4-oxo-5,6,7,8-tetrahydropyrido[2,3-d]pyrimidin-6-yl)ethyl]thien-2-ylcarbonyl]-L-glutamic acid (LY254155) have been prepared in which the glutamate moiety has been replaced with conformationally-constrained azetidine and cyclopropane mimics. None of these new analogues exhibited significant cell growth inhibitory activity.

The discovery of 5,10-dideaza-5,6,7,8-tetrahydrofolic acid (1) [DDATHF, 6(RS); Lometrexol, 6(R)] is an important development in the search for new folate-based antitumor agents.[1] This compound possesses potent and broad spectrum antitumor activity as a consequence of its profound inhibitory effect on glycinamide ribonucleotide formyltransferase (GAR TFase), and hence on the biosynthesis of GTP, and thus ATP and other purine nucleotides.[2] However, this inhibition requires initial intracellular polyglutamylation by the enzyme folylpolyglutamyl synthetase (FPGS);[3] polyglutamylated (1) is more than 100 times more potent as an inhibitor of GAR FTase than the monoglutamate prodrug. As a consequence, inevitable disadvantages are associated with **1** which include (a) lack of activity in tumors with low levels of FPGS, (b) susceptibility to the development of resistance due to overexpression of the gene for FPGS synthesis, and/or increased γ-glutamyl hydrolase activity, and (c) toxicity towards normal cells as a result of significant intracellular accumulation due to the large size and highly charged nature of the polyglutamates of **1**. We have therefore been interested in the development of analogues of **1** which might not require polyglutamylation for effective binding to GAR FTase. The recently prepared thiophene and furan analogues of **1** (**2**, LY254155; **3**, LY222306), in which a 2',5'-thiophene or 2',5'-furan ring has replaced the central benzene ring of **1**, provide encouraging precedents for this objective, since both **2** and **3** have been shown to inhibit GAR TFase without the necessity for prior polyglutamylation.[4] In the present report we describe the synthesis of several analogues of **2** in which the glutamic acid moiety has been replaced by azetidine and cyclopropane mimics in order to explore the consequences of conformational constraints[5] in the glutamate moiety on cell growth inhibition.

Synthesis of the azetidine-*cis*-2,4-dicarboxylic acid analog (**10a**) is outlined in **Scheme 1**. 5-Bromothiophene-2-carboxylic acid (**4**)[6] was condensed with dimethyl azetidine-*cis*-2,4-dicarboxylate



1 [6(R,S) DDATHF; 6(R) Lometrexol]



2, X = S [LY254155]

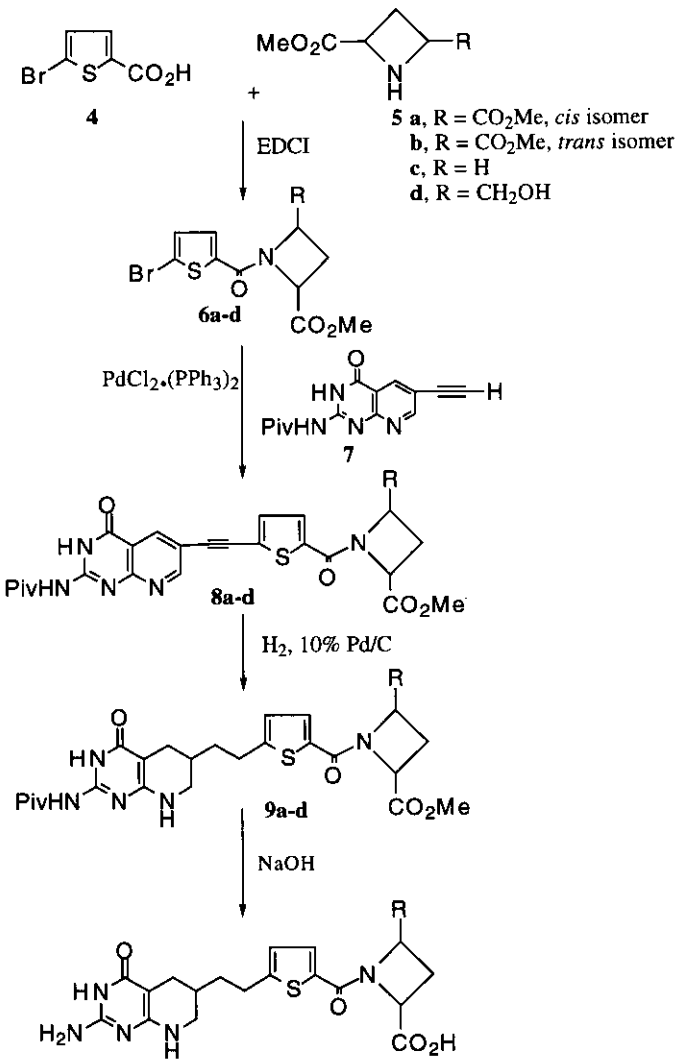
3, X = O [LY222306]

(**5a**)[7] using 1-[3-(dimethylamino)propy]-3-ethylcarbodiimide hydrochloride as the coupling agent.[8] The resulting amide (**6a**) was then subjected to palladium-catalyzed coupling with 2-pivaloyl-6-ethynyl-5-deazapterin (**7**)[9] to give **8a**. Catalytic hydrogenation of both the ethynyl linkage and the pyridine ring led to **9a**, which was deprotected by careful saponification with 1N aqueous NaOH to give **10a**. The corresponding azetidine-*trans*-2,4-dicarboxylic acid analog (**10b**), as well as the azetidine monocarboxylic acid analog (**10c**), were prepared by analogous series of reactions starting with the corresponding azetidine derivatives.

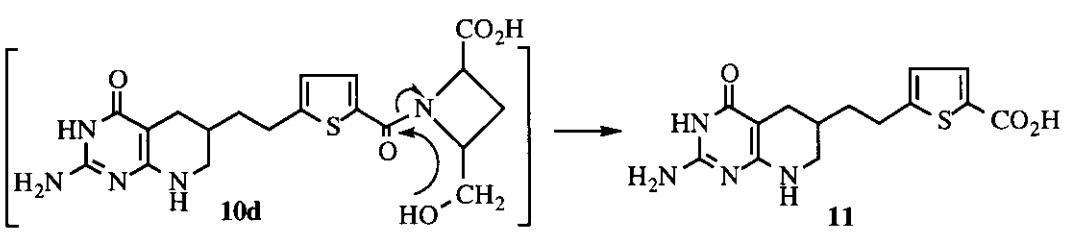
A potentially intriguing target compound in this series was **10d**, an analogue of **10a** in which one of the azetidinecarboxylic acid substituents has been reduced to a primary alcohol. Reduction of the diester (**6a**) with lithium borohydride yielded **6d**, which was then coupled with **7** as described above. Catalytic hydrogenation gave **9d**, which upon saponification led to **11**[10] as the only isolated product. Conversion of the primary alcohol functionality in **8d** into its THP derivative, followed by reduction and deprotection, first with base and then with acid, also led disappointingly to **11**. This acid clearly is formed in both cases as a consequence of an unexpectedly facile intramolecular acyl transfer (**Scheme 2**).

Two cyclopropane-derived analogues of **2** were also prepared for evaluation as antitumor agents. Since the cyclopropane ring is potentially susceptible to reductive cleavage, a different strategy was employed for their synthesis. Coupling of **11** with methyl *trans*-α-(2-carbomethoxycyclopropyl)glycinate hydrochloride (**12**)[11] using 6-chloro-2,4-dimethoxy-1,3,5-triazine as the coupling reagent[12] yielded **13**, which was then deprotected with dilute base to give target compound (**14**) (**Scheme 3**). Analogous methodology was employed to prepare the analog (**17**) encorporating 1-aminocyclopropane-1-carboxylic acid (**Acc**, **15**)[13] as a glutamic acid mimic.

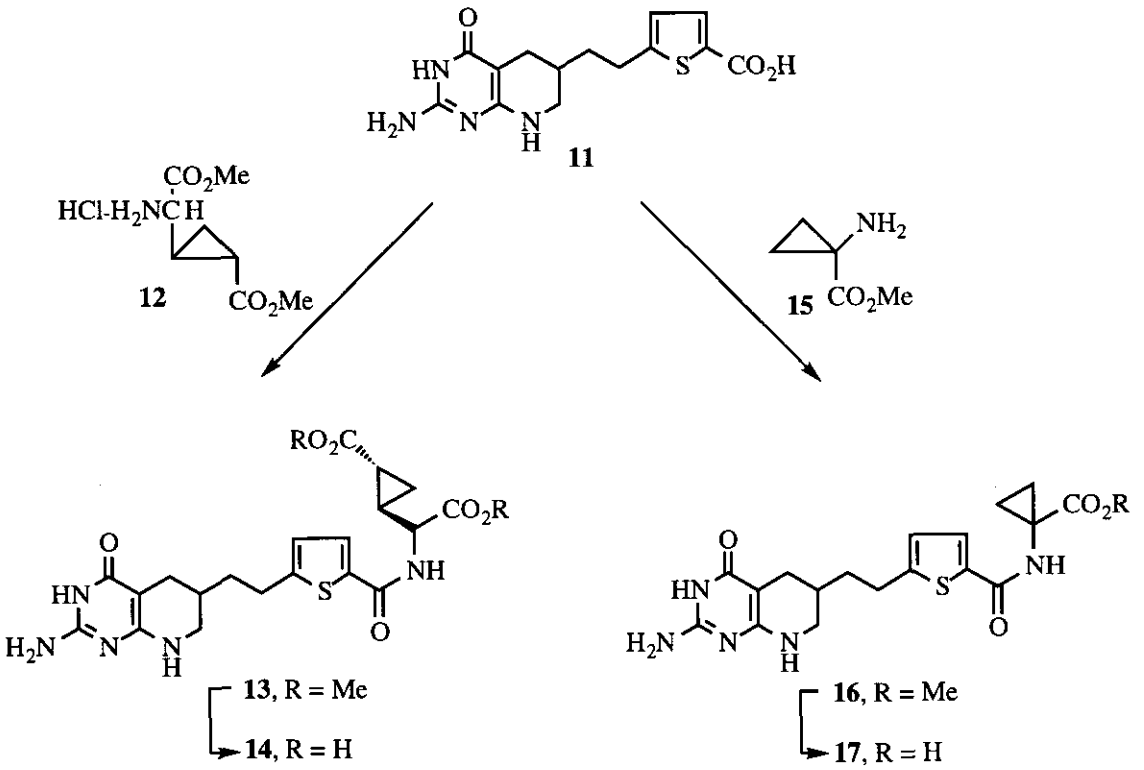
Scheme 1



Scheme 2



Scheme 3



Cell culture assays indicated that none of the above conformationally-constrained glutamate analogues of **1** exhibited significant cell growth inhibition against CCRF-CEM cells *in vitro*. The significance of these results with respect to active site binding parameters, and further details on the biological activity of these new analogues, will be presented separately.

EXPERIMENTAL

General. ^{1}H and ^{13}C nmr data were obtained with a General Electric QE-300 MHz instrument. ir spectra were determined on a Nicolet FT-ir instrument. Melting points are uncorrected. High resolution mass spectral data were determined at Princeton University on AEI MS-902 and Kratos MS50TC spectrometers. FAB mass spectra, elemental analyses and biological evaluations were provided by Eli Lilly and Co., Indianapolis, Indiana.

N-(5-Bromothien-2-ylcarbonyl)-*cis*-2,4-dicarbomethoxyazetidine (6a): A mixture of 5 - bromothiophene-2-carboxylic acid[6] (1.04 g, 5 mmol), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide[8]

(1.15 g, 6 mmol), and dimethyl azetidine-*cis*-2,4-dicarboxylate[7] (**5a**) (1.38 g, 8 mmol) was stirred in CH_2Cl_2 (150 ml) under argon. Triethylamine (0.81 g, 8 mmol) was added dropwise and the mixture was stirred overnight. The mixture was then washed with 0.05 N HCl, saturated $NaHCO_3$, and water. The resulting solution was dried with $MgSO_4$ and concentrated *in vacuo*. Purification by silica gel column chromatography with 30-50% EtOAc/hexanes as eluent gave 0.75 g (41%) of **6a** as a colorless oil which crystallized upon trituration with hexanes: mp 85-87 °C; ir (neat) 1746, 1619 cm^{-1}; ^1H nmr ($CDCl_3$) δ 7.17 (d, *J* = 4.1 Hz, 1 H), 7.00 (d, *J* = 4.1 Hz, 1 H), 4.95 (dd, *J*= 9.7, 5.3 Hz, 2 H), 3.80 (s, 6 H), 3.03 (dt, *J*= 12.0, 9.7 Hz, 1 H), 2.49 (dt, *J*= 12.0, 5.3 Hz, 1 H); ^{13}C nmr ($CDCl_3$) δ 169.7, 161.4, 136.8, 130.4, 130.1, 118.6, 59.3, 52.5, 25.5; HRms calcd for $C_{12}H_{12}NO_5BrS$ (M^+): 360.9620, 362.9600, found: 360.9625, 362.9615 ; Anal. Calcd for $C_{12}H_{12}NO_5BrS$: C, 39.79; H, 3.34, N, 3.87. Found: C, 40.01; H, 3.35; N, 3.86.

N-{5-[2-(2-Pivaloylamino-3,4-dihydro-4-oxopyrido[2,3-d]pyrimidin-6-yl)ethynyl]thien-2-ylcarbonyl}-*cis*-2,4-dicarbomethoxyazetidine (8a): A mixture of **6a** (0.181 g, 0.5 mmol), 2-pivaloyl-6-ethynyl-5-deazapterin[9] (**7**) (0.163 g, 0.6 mmol), bis(triphenylphosphine)palladium(II) chloride (0.035 g, 0.05 mmol), and triethylamine (0.51 g, 5 mmol) in 30 ml of acetonitrile was refluxed overnight under argon. After cooling to room temperature, the reaction mixture was filtered, and the filtrate was concentrated and purified by silica gel chromatography using 2 - 5 % MeOH/CH_2Cl_2 as eluent to give 0.19 g (69%) of **8a** as a pale yellow solid: mp 228-230 °C; ir (KBr) 1745, 1720, 1682, 1625 cm^{-1}; ^1H nmr ($CDCl_3$) δ 12.08 (br s, 1 H), 8.93 (br s, 1 H), 8.57 (d, *J* = 2.3 Hz, 1 H), 8.37 (br s, 1 H), 7.38 (d, *J* = 4.0 Hz, 1 H), 7.22 (d, *J* = 4.0 Hz, 1 H), 5.00 (dd, *J* = 9.7, 5.3 Hz, 2 H), 3.82 (s, 6 H), 3.06 (dt, *J* = 11.8, 9.7 Hz, 1 H), 2.52 (dt, *J* = 11.8, 5.3 Hz, 1 H), 1.32 (s, 9 H); HRms calcd for $C_{26}H_{25}N_5O_7S$ (M^+) : 551.1474, found: 551.1477. Anal. Calcd for $C_{26}H_{25}N_5O_7S$: C, 56.62; H, 4.57, N,12.70. Found: C, 56.92; H, 4.47; N, 12.43.

N-{5-[2-(2-Pivaloylamino-3,4-dihydro-4-oxo-5,6,7,8-tetrahydropyrido[2,3-d]pyrimidin-6-yl)ethyl]thien-2-ylcarbonyl}-*cis*-2,4-dicarbomethoxyazetidine (9a): A mixture of **8a** (0.080 g, 0.15 mmol) and 10% Pd/C (0.16 g) in 150 ml of methanol/CH_2Cl_2 (1:1) was pressurized with 50 psi of H_2 and heated to 50 °C. After overnight shaking at 50 °C, the mixture was filtered through a short pad of silica gel to remove catalyst, and the filtrate was concentrated and purified by preparative tlc (20x20, Uniplate™ Silica Gel GF) with 4% MeOH/CH_2Cl_2 as the developing solvent to yield 0.064 g (79%) of **9a** as a white solid: mp 208-210 °C; ir (KBr) 1752, 1739, 1640 cm^{-1}; ^1H nmr ($CDCl_3$) δ 11.30 (br s, 1 H), 8.07 (br s, 1 H), 7.35 (br s, 1 H), 6.84 (d, *J* = 3.7 Hz, 1 H), 5.07 (dd, *J* = 9.3, 5.0 Hz, 2 H), 4.90 (br s, 1 H), 3.90 (s, 6 H), 3.40-2.80 (m, 6 H), 2.58 (dt, *J* = 12.1, 5.0 Hz, 1 H), 2.30-1.70 (m, 4 H), 1.37 (s, 9 H); HRms calcd for $C_{26}H_{33}N_5O_7S$ (M^+): 559.2100, found: 559.2109. Anal. Calcd for $C_{26}H_{33}N_5O_7S$: C, 55.80; H, 5.94, N, 12.51. Found: C, 55.58; H, 5.84; N, 12.71.

N-{5-[2-(2-Amino-3,4-dihydro-4-oxo-5,6,7,8-tetrahydropyrido[2,3-d]pyrimidin-6-yl)ethyl]thien-2-ylcarbonyl}-*cis*-2,4-dicarboxyazetidine (10a): Method A. The suspension of the diester (**9a**) (0.045 g, 0.08 mmol) in 2 ml of 1 N aqueous NaOH was stirred at room temperature for 3 days. Acidification with

1N aqueous HCl followed by filtration gave the diacid (**10a**) (0.025 g, 70%) as an off-white solid: mp 232 °C (dec.); ir (KBr) 3653-2140, 1760-1500 cm^{-1}; ^1H nmr (DMSO-d_6) δ 9.90 (br s, 1H), 7.25 (d, J = 3.7 Hz, 1 H), 6.91 (d, J = 3.7 Hz, 1 H), 6.37 (br s, 1 H), 6.09 (br s, 2 H), 4.89 (br s, 2 H), 3.3-1.4 (m, 11 H); FABHRms calcd for $C_{19}H_{22}N_5O_6S$ (MH^+): 448.1291, found: 448.1281. Anal. Calcd for $C_{19}H_{21}N_5O_6S \cdot 1.32\ H_2O$: C, 48.43; H, 5.06, N, 14.86. Found: C, 48.82; H, 4.84; N, 14.46.

Method B. A 100 ml round-bottomed flask, equipped with a magnetic stirrer and gas inlet, was charged with 5-[2-(2-amino-3,4-dihydro-4-oxo-5,6,7,8-tetrahydropyrido[2,3-d]pyrimidin-6-yl)ethyl]thiophene-2-car-boxylic acid (**11**)[10] (0.91 g, 2.8 mmol), 6-chloro-2,4-dimethoxy-1,3,5-triazine (0.61 g, 3.5 mmol), N-methylmorpholine (1.01g, 10 mmol), and 30 ml of anhydrous DMF. The mixture was stirred at 0 °C under an argon atmosphere for 2 h. To the resulting homogeneous solution was added 0.90 g (5.2 mmol) of dimethyl azetidine-*cis*-2,4-dicarboxylate. The mixture was stirred at 0 °C for 1 h and then at room temperature for 14 h, the solvent was removed under reduced pressure, and the residue was purified by silica gel column chromatography with 10-50% MeOH/CH_2Cl_2 as eluent to give 0.25 g (19%) of **N-{5-[2-(2-amino-3,4-dihydro-4-oxo-5,6,7,8-tetrahydropyrido[2,3-d]pyrimidin-6-yl)ethyl]thien-2-ylcarbonyl}-*cis*-2,4-dicarbomethoxyazetidine** as a white solid: mp 220-223 °C; ir (KBr) 3520-2300, 1770-1560 cm^{-1}; ^1H nmr (DMSO-d_6) δ 9.71 (br s, 1H), 7.20 (d, J = 3.6 Hz, 1 H), 6.90 (d, J = 3.6 Hz, 1 H), 6.26 (br s, 1 H), 5.95 (br s, 2 H), 5.02 (br s, 2 H), 3.68 (s, 6 H), 3.6-2.7 (m, 6 H), 2.5-2.2 (m, 2 H), 1.9-1.5 (m, 3 H); FABHRms calcd for $C_{21}H_{26}N_5O_6S$ (MH^+): 476.1604, found: 476.1606. Anal. Calcd for $C_{21}H_{25}N_5O_6S \cdot H_2O$: C, 51.10; H, 5.51, N, 14.19. Found: C, 51.35; H, 5.32; N, 13.84.

A suspension of above diester (0.10 g) in 10 ml of 1 N aqueous NaOH was stirred at room temperature for 18 h. Acidification with acetic acid followed by filtration gave the diacid (**10a**) (0.053 g, 59%) as an off-white solid whose nmr spectrum was identical with that of the material obtained by **Method A**.

N-(5-Bromothien-2-ylcarbonyl)-*trans*-2,4-dicarbomethoxyazetidine (6b): A mixture of 5-bromothiophene-2-carboxylic acid (0.73 g, 3.5 mmol), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (0.96 g, 5 mmol), and dimethyl azetidine-*trans*-2,4-dicarboxylate (0.87 g, 5 mmol) was stirred in CH_2Cl_2 (150 ml) under argon overnight. The mixture was then washed with 0.05 N HCl, saturated $NaHCO_3$, and water. The resulting solution was dried with $MgSO_4$ and concentrated *in vacuo*. Purification by silica gel column chromatography with 50% EtOAc/hexanes as the eluent gave 0.48 g (38%) of product as a colorless solid: mp 74-75 °C; ir (KBr) 1731, 1611 cm^{-1}; ^1H nmr (DMSO-d_6) δ 7.31 (d, J = 4.3 Hz, 1 H), 7.28 (d, J = 4.3 Hz, 1 H), 5.48 (m, 1 H), 4.83 (m, 1 H), 3.68 (s, 3 H), 3.61 (s, 3 H), 2.8-2.5 (m, 2 H); ^1H nmr ($CDCl_3$) δ 7.12 (d, J= 4.0 Hz, 1 H), 7.0 (d, J= 4.0 Hz, 1 H), 5.05 (br s, 2 H), 3.73 (m, 6 H), 2.9-2.3 (m, 2 H); HRms calcd for $C_{12}H_{12}NO_5BrS$ (M^+): 360.9620, 362.9600, found: 360.9625, 362.9604. Anal. Calcd for $C_{12}H_{12}NO_5BrS$: C, 39.79; H, 3.34, N, 3.87. Found: C, 39.90; H, 3.29; N, 3.90.

N-(5-Bromothien-2-ylcarbonyl)-2-carbomethoxyazetidine (6c): To a stirred solution of L-azetidine-2-carboxylic acid (1 g, 10 mmol) in 10 ml of methanol, chilled to 0 °C, $SOCl_2$ (2 ml) was added. The reaction mixture was heated under reflux for 6 h and then the solvent was removed *in vacuo*. The residual material was redissolved in CH_2Cl_2 under argon at 0 °C and mixed with 5-bromothiophene-2-

carboxylic acid (2.07g, 10 mmol) and 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide (1.91 g, 10 mmol). Triethylamine (3.03 g, 30 mmol) was added dropwise and the mixture was allowed to warm to room temperature. After being stirred for one day, the solution was washed with 0.05 N HCl followed by saturated $NaHCO_3$, and dried with $MgSO_4$. The concentrated residue was purified by silica gel column chromatography with 30-50% EtOAc/hexanes as eluent to give 1.42 g (47%) of **6c** as a white solid: mp 119-120 °C; ir (KBr) 1732 cm^{-1}; ^1H nmr ($CDCl_3$) δ 7.22 (d, J = 4.0 Hz, 1 H), 7.03 (d, J = 4.0 Hz, 1 H), 5.00-4.88 (m, 1 H), 4.50 (m, 1 H), 4.30 (m, 1 H), 3.77 (s, 3 H), 2.7-2.5 (m, 1 H), 2.45 - 2.28 (m, 1 H); HRms calcd for $C_{10}H_{10}NO_3BrS$ (M^+): 302.9565, 304.9546, found: 302.9573, 304.9553. Anal. Calcd for $C_{10}H_{10}NO_3BrS$: C, 39.49; H, 3.31, N, 4.61. Found: C, 39.42; H, 3.26; N, 4.53.

N-{5-[(2-Pivaloylamino-3,4-dihydro-4-oxopyrido[2,3-d]pyrimidin-6-yl)-ethynyl]thien-2-ylcarbonyl}-*trans*-2,4-dicarbomethoxyazetidine (8b): A mixture of **6b** (0.26 g, 0.72 mmol), 2-pivaloyl-6-ethynyl-5-deazapterin (**7**) (0.39 g, 1.44 mmol), bis(triphenylphosphine)palladium(II) chloride (0.05 g, 0.07 mmol), and triethylamine (0.72 g, 7.2 mmol) in 60 ml of acetonitrile was heated under argon overnight, then cooled to room temperature, filtered, the filtrate concentrate, and the residual material purified by silica gel chromatography using 1 - 3 % MeOH/CH_2Cl_2 as eluent to give 0.30 g (76%) of **8b** as a pale yellow solid: mp 218 °C (decomp.); ir (KBr) 3350-2360, 1739, 1689, 1633 cm^{-1}; ^1H nmr ($CDCl_3$) δ 12.05 (br s, 1 H), 8.93 (br s, 1 H), 8.57 (d, J = 1.3 Hz, 1 H), 8.35 (br s, 1 H), 7.33 (d, J = 5.0 Hz, 1 H), 7.23 (d, J = 5.0 Hz, 1 H), 5.3-4.95 (m, 2 H), 3.9-3.7 (m, 6 H), 2.9-2.4 (m, 2 H), 1.32 (s, 9 H); HRms calcd for $C_{26}H_{25}N_5O_7S$ (M^+): 551.1474, found: 551.1455. Anal. Calcd for $C_{26}H_{25}N_5O_7S$: C, 56.62; H, 4.57, N, 12.70. Found: C, 56.37; H, 4.64; N, 12.49.

N-{5-[2-(2-Pivaloylamino-3,4-dihydro-4-oxopyrido[2,3-d]pyrimidin-6-yl)ethynyl]thien-2-ylcarbonyl}-2-carbomethoxyazetidine (8c): A mixture of **6c** (0.608 g, 2 mmol), 2-pivaloyl-6-ethynyl-5-deazapterin (**7**) (1.08 g, 4 mmol), bis(triphenylphosphine)palladium(II) chloride (0.070 g, 0.10 mmol), and triethylamine (1.01 g, 10 mmol) in 50 ml of acetonitrile was heated under argon overnight and then cooled to room temperature. It was filtered, the filtrate was concentrated, and the residual material was purified by silica gel chromatography using 1-5 % MeOH/CH_2Cl_2 as eluent to give 0.94 g (95%) of **8c** as a pale yellowish solid: mp 198-200 °C; ir (KBr) 1744, 1671, 1618 cm^{-1}; ^1H nmr (DMSO-d_6) δ 12.35 (br s, 1H), 11.55 (br s, 1 H), 8.98 (d, J = 2.1 Hz, 1 H), 8.49 (d, J = 2.1 Hz, 1 H), 7.51 (m, 2 H), 5.0-4.85 (m, 1 H), 4.63-4.35 (m, 2 H), 3.32 (s, 3 H), 2.8-2.6 (m, 1 H), 2.35-2.2 (m, 1 H), 1.26 (s, 9 H); HRms calcd for $C_{24}H_{23}N_5O_5S$ (M^+): 493.1420, found: 493.1422. Anal. Calcd for $C_{24}H_{23}N_5O_5S$: C, 58.41; H, 4.70, N, 14.19. Found: C, 58.63; H, 4.59; N, 13.95.

N-{5-[2-(2-Pivaloylamino-3,4-dihydro-4-oxo-5,6,7,8-tetrahydropyrido[2,3-d]pyrimidin-6-yl)ethyl]thien-2-ylcarbonyl}-*trans*-2,4-dicarbomethoxyazetidine (9b): A mixture of **8b** (0.13 g, 0.24 mmol) and 10% Pd/C (0.50 g) in 150 ml of methanol/CH_2Cl_2 (1:1) was pressurized with 50 psi H_2, heated to 50 °C, and shaken overnight. The catalyst was then removed by filtering through a short pad of silica gel. The filtrate was concentrated and purified by silica gel chromatography using 1 - 3 % MeOH/CH_2Cl_2 as eluent to give 0.10 g (77%) of **9b** as a white solid: mp 178-181 °C; ir (KBr) 3323-

3006, 1739, 1647 cm^{-1}; ^1H nmr ($CDCl_3$) δ 11.2 (br s, 1 H), 7.84 (br s, 1 H), 7.23 (d, *J* = 4.0 Hz, 1 H), 6.75 (d, *J* = 4.0 Hz, 1 H), 5.3-4.6 (m, 3 H), 3.9-3.7 (m, 6 H), 3.4-1.6 (m, 11H), 1.28 (s, 9 H); HRms calcd for $C_{26}H_{33}N_5O_7S$ (M^+): 559.2100, found: 559.2103. Anal. Calcd for $C_{26}H_{33}N_5O_7S$: C, 55.80; H, 5.94, N, 12.51. Found: C, 55.89; H, 6.00; N, 12.60.

N-{5-[2-(2-Pivaloylamino-3,4-dihydro-4-oxo-5,6,7,8-tetrahydropyrido[2,3-d]pyrimidin-6-yl)ethyl]thien-2-ylcarbonyl}-2-carbomethoxyazetidine (9c): A mixture of **8c** (0.5 g, 1.01 mmol) and 10% Pd/C (1.0 g) in 150 ml of methanol and 20 ml of CH_2Cl_2 was pressurized with 50 psi H_2, heated to 50 °C and shaken overnight. It was then cooled to room temperature and the catalyst removed by filtration through a short pad of silica gel. The filtrate was concentrated and purified by silica gel chromatography with 1% MeOH/CH_2Cl_2 as eluent to yield 0.34 g (67%) of **9c** as a white solid: mp 236-238 °C; ir (KBr) 3460-2791, 1729, 1633 cm^{-1}; ^1H nmr (DMSO-d_6) δ 11.21 (br s, 1H), 10.59 (br s, 1 H), 7.35 (br s, 1 H), 6.94 (d, *J* = 3.0 Hz, 1 H), 6.47 (d, *J* = 3.0 Hz, 1 H), 4.9-4.75 (m, 1 H), 4.5-4.3 (m, 2 H), 3.66 (s, 3 H), 3.3-3.2 (m, 2 H), 3.0-2.8 (m, 3 H), 2.7-2.5 (m, 1 H), 2.35-2.15 (m, 1 H), 2.0-1.85 (m, 1 H), 1.8-1.55 (m, 3 H), 1.19 (s, 9 H); HRms calcd for $C_{24}H_{31}N_5O_5S$ (M^+): 501.2046, found: 501.2049. Anal. Calcd for $C_{24}H_{31}N_5O_5S$: C, 57.47; H, 6.23, N, 13.96. Found: C, 57.60; H, 6.41; N, 14.03.

N-{5-[2-(2-Amino-3,4-dihydro-4-oxo-5,6,7,8-tetrahydropyrido[2,3-d]pyrimidin-6-yl)ethyl]thien-2-ylcarbonyl}-*trans*-2,4-dicarboxyazetidine (10b): A suspension of **9b** (0.045 g, 0.8 mmol) in 2.5 ml of 0.4 N aqueous NaOH was stirred at room temperature for 3 days. Acidification with 1 N HCl followed by filtration gave the diacid (**10b**) (0.019 g, 53%) as an white solid: mp 180 °C (decomp.); ir (KBr) 3600-2360, 1720-1480 cm^{-1}; ^1H nmr (DMSO-d_6) δ 9.70 (br s, 1H), 7.29 (m, 1 H), 6.89 (d, *J* = 3.6 Hz, 1 H), 6.29 (br s, 1 H), 5.93 (br s, 2 H), 5.2 (dd, *J* = 15.3, 5.0 Hz, 1 H), 4.7 (dd, *J* = 15.3, 8.3 Hz, 1 H), 3.2-1.1 (m, 11 H); FABHRms Calcd for $C_{19}H_{22}N_5O_6S$ (MH^+): 448.1291, found: 448.1285. Anal. Calcd for $C_{19}H_{21}N_5O_6S \cdot H_2O$: C, 49.02; H, 4.98, N, 15.05. Found: C, 49.22; H, 5.04; N, 14.72.

N-{5-[2-(2-Amino-3,4-dihydro-4-oxo-5,6,7,8-tetrahydropyrido[2,3-d]pyrimidin-6-yl)ethyl]thien-2-ylcarbonyl}-2-carboxyazetidine (10c): A suspension of **9c** (0.23 g, 0.046 mmol) in 0.5 N NaOH (10 ml, 5.0 mmol) was stirred at room temperature for 3 days. The resulting clear solution was then acidified with 1 N HCl and the solid that formed was collected by filtration, washed with water, and dried *in vacuo* to yield 0.07 g (38%) of **10c** as an off-white solid: mp 150 °C (decomp.); ir (KBr) 3580-3048, 1740-1492 cm^{-1}; ^1H nmr (DMSO-d_6) δ 9.7 (br s, 1H), 7.3 (br s, 1 H), 6.91 (br s, 1 H), 6.25 (br s, 1 H), 5.91 (br s, 2 H), 4.8-4.55 (m, 1 H), 4.55-4.2 (m, 2 H), 3.3-1.1 (m, 11 H); FABHRms calcd for $C_{18}H_{22}N_5O_4S$ (MH^+): 404.1393, found: 404.1368. Anal. Calcd for $C_{18}H_{21}N_5O_4S \cdot 0.16NaCl$: C, 52.37; H, 5.13, N, 16.96. Found: C, 52.75; H, 5.13; N, 16.55.

N-(5-Bromothien-2-ylcarbonyl)-*cis*-2-carbomethoxy-4-hydroxymethylazetidine (6d): Lithium borohydride (0.33 g, 15 mmol) was added in three portions over 1.5 h to a stirred solution of **6a** (1.10 g, 3.0 mmol) in methanol (100 ml) at 0 °C, the resulting solution was stirred at 0 °C for 30 min and CH_2Cl_2 (200 ml) was added. The resulting solution was washed with water and dried over $MgSO_4$. The

concentrated residue was purified by silica gel chromatography using 50-100 % EtOAc/hexanes as eluent to give 0.51 g (51%) of **6d** as a colorless oil. Ir (CCl_4) 3600-3130, 1740 cm^{-1}; ^1H nmr ($CDCl_3$) δ 7.12 (d, J = 4.0 Hz, 1 H), 7.0 (d, J = 4.0 Hz, 1 H), 5.03 (dd, J = 9.6, 5.3 Hz, 1 H), 4.71 (m, 1 H), 4.09 (br s, 1 H), 3.86 (m, 2 H), 3.77 (s, 3 H), 2.81 (ddd, J = 11.8, 9.2, 9.2 Hz, 1 H), 2.15 (ddd, J = 11.8, 5.0, 5.0 Hz, 1 H); ^{13}C nmr ($CDCl_3$) δ 170.8, 162.5, 137.1, 130.7, 130.3, 119.2, 64.6, 64.0, 61.0, 52.9, 23.7; HRms calcd for $C_{11}H_{12}NO_4BrS$ (M^+): 332.9671, 334.9651, found: 332.9677, 334.9660. Anal. Calcd for $C_{11}H_{12}NO_4BrS$: C, 39.54; H, 3.62, N, 4.19. Found: C, 39.66; H, 3.59; N, 4.26.

N-{5-[2-(2-Pivaloylamino-3,4-dihydro-4-oxopyrido[2,3-d]pyrimidin-6-yl)ethynyl]thien-2-ylcarbonyl}-*cis*-2-carbomethoxy-4-hydroxymethylazetidine (8d): A mixture of **6 d** (0.33 g, 1.0 mmol), 2-pivaloyl-6-ethynyl-5-deazapterin (**7**) (0.54 g, 2 mmol), bis(triphenylphosphine)palladium(II) chloride (0.070 g, 0.1 mmol), and triethylamine (1.01 g, 10 mmol) in 30 ml of acetonitrile was refluxed under argon overnight, cooled to room temperature, and filtered. The filtrate was concentrated under reduced pressure, and the residual material purified by silica gel chromatography using 1-5 % MeOH/CH_2Cl_2 as eluent to give 0.41 g (77%) of **8d** as a pale yellowish solid: mp 145-148 °C; ir (KBr) 3534-3000, 1745, 1675, 1633 cm^{-1}; ^1H nmr ($CDCl_3$) δ 12.1 (br s, 1 H), 9.03 (br s, 1 H), 8.56 (d, J = 2.3 Hz, 1 H), 8.46 (br s, 1 H), 7.33 (d, J = 4.0 Hz, 1 H), 7.22 (d, J = 4.0 Hz, 1 H), 5.08 (dd, J = 9.2, 5.3 Hz, 1 H), 4.75 (m, 1 H), 4.12 (br s, 1 H), 3.9 (m, 2 H), 3.79 (s, 3 H), 2.85 (ddd, J= 11.5, 9.4, 9.4 Hz, 1 H), 2.25-2.1 (m, 1 H), 1.23 (s, 9 H); ^{13}C nmr ($CDCl_3$) δ 180.9, 170.9, 162.9, 160.2, 157.9, 149.8, 138.4, 136.9, 132.8, 130.3, 127.4, 116.4, 114.8, 91.4, 85.5, 64.7, 64.1, 60.4, 53.0, 40.5, 26.8, 23.8; HRms calcd for $C_{25}H_{25}N_5O_6S$ (M^+): 523.1525, found: 523.1528. Anal. Calcd for $C_{25}H_{25}N_5O_6S \cdot 0.8\ H_2O$: C, 55.81; H, 4.98, N, 13.02. Found: C, 55.76; H, 4.62; N, 12.96.

N-{5-[2-(2-Pivaloylamino-3,4-dihydro-4-oxopyrido[2,3-d]pyrimidin-6-yl)ethynyl]thien-2-ylcarbonyl}-*cis*-2-carbomethoxy-4-tetrahydropyranyloxymethylazetidine (8 *cis*, R = CH_2OTHP): To a stirred solution of **8d** (0.118 g, 0.22 mmol) in CH_2Cl_2, 1,4-dihydro-2*H*-pyran (0.064 g, 0.67 mmol) and pyridium *p*-toluenesulfonate (0.168 g, 0.67 mmol) were added and the mixture was stirred one day. The solution was then washed with aqueous 10% $NaHCO_3$ and dried over $MgSO_4$. The concentrated residue was purified by silica gel chromatography using 1-5 % MeOH/CH_2Cl_2 as eluent to give 0.040 g (29%) of product as a pale yellowish solid: ^1H nmr (mixture of diasteromers, $CDCl_3$) δ 12.05 (br s, 1 H), 8.91 (br s, 1 H), 8.55 (m, 1 H), 8.45 (br s, 1 H), 7.5-7.3 (m, 1 H), 7.25-7.1 (m, 1 H), 5.1-4.5 (m, 3 H), 4.2-3.65 (m, 7 H), 3.55-3.45 (m, 1 H), 3.0-2.8 (m, 1 H), 2.4-2.2 (m, 1 H), 2.0-1.4 (m, 5 H), 1.32 (s, 9 H); HRms calcd for $C_{30}H_{33}N_5O_6S$: (M^+): 607.2100, found: 607.2105.

N-{5-[2-(2-Pivaloylamino-3,4-dihydro-4-oxo-5,6,7,8-tetrahydropyrido[2,3-d]pyrimidin-6-yl)ethyl]-thien-2-ylcarbonyl}-*cis*-2-carbomethoxy-4-hydroxymethylazetidine (9d): A mixture of **8d** (0.060 g) and 10% Pd/C (0.12 g) in 150 ml of methanol/CH_2Cl_2 (1:1) was pressurized with 50 psi H_2, heated to 50 °C, and shaken overnight. The catalyst was removed by filtration through a short pad of silica gel, and the filtrated concentrated and purified by preparative tlc (20x20, Uniplate™ Silica Gel GF) with 6% MeOH/CH_2Cl_2 as the developing solvent to yield 0.027 g (45%) of **9d** as a white solid: 174-176 °C; ir

(KBr) 3591-3316, 1739, 1654, 1563 cm^{-1}; ^1H nmr ($CDCl_3$) δ 11.28 (br s, 1 H), 9.32 (br s, 1 H), 7.06 (d, *J* = 3.6 Hz, 1 H), 6.75 (d, *J* = 3.6 Hz, 1 H), 6.4-6.2 (m, 1 H), 5.2-5.0 (m, 1 H), 4.85-4.72 (m, 1 H), 4.15-4.0 (m, 1 H), 3.75 (m, 5 H), 3.4-3.3 (m, 1 H), 3.15-2.75 (m, 3 H), 2.2-1.6 (m, 7 H), 1.24 (s, 9 H); HRms calcd for $C_{25}H_{33}N_5O_6S$ (M^+): 531.2151, found: 531.2151. Anal. Calcd for $C_{25}H_{33}N_5O_6S$: C, 56.48; H, 6.26, N, 13.17. Found: C, 56.36; H, 6.14; N, 13.06.

N-{5-[2-(2-Pivaloylamino-3,4-dihydro-4-oxo-5,6,7,8-tetrahydropyrido[2,3-d]pyrimidin-6-yl)ethyl]-thien-2-ylcarbonyl}-*cis*-2-carbomethoxy-4-tetrahydropyranyloxymethylazetidine (9 *cis*, R = CH_2OTHP): A mixture of **8** (*cis*, R = CH_2OTHP) (0.040 g) and 10% Pd/C (0.15 g) in 100 ml of $MeOH/CH_2Cl_2$ (1:1) was pressurized with 50 psi H_2, heated to 50 °C, and shaken overnight. The catalyst was removed by filtration through a short pad of silica gel, and the filtrate was concentrated and purified by preparative tlc (20x20, Uniplate™ Silica Gel GF) with 4% $MeOH/CH_2Cl_2$ as the developing solvent to yield 0.036 g (89%) of **9** (*cis*, R = CH_2OTHP) as a white solid: ^1H Nmr (mixture of diasteromers, $CDCl_3$) δ 11.27 (br s, 1 H), 9.15 (m, 1 H), 7.08 (m, 1 H), 6.76 (m, 1 H), 6.05 (m, 1 H), 5.5-4.5 (m, 4 H), 4.2-2.6 (m, 12 H), 2.2-1.4 (m, 10 H), 1.25 (s, 9 H); HRms calcd for $C_{30}H_{41}N_5O_6S$ (M^+): 615.2726, found: 615.2727.

5-[2-(2-Amino-3,4-dihydro-4-oxo-5,6,7,8-tetrahydropyrido[2,3-d]pyrimidin-6-yl)ethyl]thiophene-2-carboxylic Acid (11):[10] A suspension of the ester (**9d**) (0.025 g) in 2 ml of 1 N aqueous NaOH was stirred at room temperature for 3 days. Acidification with 1 N HCl followed by filtration gave **11** (0.015 g, *99%*) as an white solid, identical in every respect with authentic material. *Saponification of* **9** (*cis*, R = CH_2OTHP) with 0.13 N NaOH for three days, followed by acidification with HOAc, also led only to **11**.

Methyl N-{5-[2-(2-Amino-3,4-dihydro-4-oxo-5,6,7,8-tetrahydropyrido[2,3-d]pyrimidin-6-yl)ethyl]-thien-2-ylcarbonyl}-*trans*-2-carbomethoxycyclopropylglycinate (13): A 100 ml round-bottomed flask, equipped with a magnetic stirrer and gas inlet, was charged with **11** (0.64 g, 2 mmol), 6-chloro-2,4-dimethoxy-1,3,5-triazine (0.70 g, 4 mmol), N-methylmorpholine (0.51 g, 5 mmol), and 40 ml of anhydrous DMF. The mixture was stirred at room temperature under an argon atmosphere for 3 h. To the resulting homogeneous solution was added 0.45 g (4.0 mmol) of methyl trans-α-(2-carbomethoxycyclopropyl)glycinate hydrochloride[11] (**12**) and 0.51 g (5 mmol) of N-methylmorpholine. The mixture was stirred for an additional 14 h, the solvent was removed under reduced pressure, and the residual material was purified by silica gel column chromatography with 5-50% $MeOH/CH_2Cl_2$ as eluent to give 0.34 g (35%) of **13** as a white solid: mp 125-128 °C; ir (KBr) 3600-2360, 1740-1540 cm^{-1}; ^1H nmr (DMSO-d_6, major isomer) δ 9.73 (br s, 1 H), 8.93 (d, *J* = 7.0 Hz, 1H), 7.7 (d, *J* = 3.6 Hz, 1 H), 6.9 (d, *J* = 3.6 Hz, 1 H), 6.26 (br s, 1 H), 5.95 (br s, 2 H), 4.0-3.85 (m, 1 H), 3.61 (s, 3 H), 3.6 (s, 3 H), 3.25-3.0 (m, 1 H), 3.0-2.6 (m, 3 H), 2.5-2.3 (m, 1 H), 1.95-1.5 (m, 6 H), 1.25-1.0 (m, 2 H); Anal. Calcd for $C_{22}H_{27}N_5O_6S$: C, 53.98; H, 5.56, N, 14.31, S, 6.55. Found: C, 53.69; H, 5.28; N, 14.41, S, 6.32.

N-{5-[2-(2-Amino-3,4-dihydro-4-oxo-5,6,7,8-tetrahydropyrido[2,3-d]pyrimidin-6-yl)ethyl]thien-2-ylcarbonyl}-*trans*-2-carboxycyclopropylglycine (14): A suspension of **13** (0.20 g) in 0.3 N NaOH (7

ml) and THF (10 ml) was stirred at room temperature for 1 day. The organic solvent was removed by evaporation under reduced pressure, the remaining aqueous solution was acidified with 0.5 N HCl, and the solid that separated was collected by filtration, washed with water, and dried *in vacuo* to yield 0.055 g (27%) of **14** as an off-white solid: mp 220 °C (decomp.); ir (KBr) 3640-2360, 1750-1400 cm^{-1}; ^1H nmr (major isomer, DMSO-d_6) δ 8.8 (d, *J* = 7.2 Hz, 1H), 7.71 (d, *J* = 3.8 Hz, 1 H), 6.9 (d, *J* = 3.7 Hz, 1 H), 6.35 (br s, 1 H), 6.09 (br s, 2 H), 3.89 (t, *J* = 7.9 Hz, 1 H), 3.5-2.7 (m, 3 H), 2.6-2.4 (m, 1 H), 2.0-1.5 (m, 7 H), 1.2-0.9 (m, 2 H); FABHRms calcd for $C_{20}H_{24}N_5O_6S$ (MH^+): 462.1447, found: 462.1419. Anal. Calcd for $C_{20}H_{23}N_5O_6S \cdot 0.35$ NaCl: C, 49.84; H, 4.80, N, 14.51, S, 6.65. Found: C, 49.97; H, 4.94; N, 14.73, S, 6.32.

Methyl N-{5-[2-(2-Amino-3,4-dihydro-4-oxo-5,6,7,8-tetrahydropyrido[2,3-d]pyrimidin-6-yl)ethyl]-thien-2-ylcarbonyl}-1-aminocyclopropane-1-carboxylate (16): A 100 ml round-bottomed flask, equipped with a magnetic stirrer and gas inlet, was charged with **11** (0.96 g, 3 mmol), 6-chloro-2,4-dimethoxy-1,3,5-triazine (0.88 g, 5 mmol), N-methylmorpholine (0.51 g, 5 mmol), and 30 ml of anhydrous DMF. The mixture was stirred at 0 °C under an argon atmosphere for 2 h. To the resulting homogeneous solution was added 0.69 g (5.0 mmol) of methyl 1-aminocyclopropane-1-carboxylate hydrochloride.[13] The mixture was stirred at 0 °C for 1 h and at room temperature for 14 h, the solvent was removed under reduced pressure, and the residual material was purified by silica gel column chromatography with 2-5% MeOH/CH_2Cl_2 as eluent to give 0.44 g (35%) of **16** as a white solid: mp 150 °C (decomp.); ir (KBr) 3620-2360, 1731, 1647 cm^{-1}; ^1H nmr (DMSO-d_6) δ 9.76 (br s, 1 H), 9.0 (br s, 1 H), 7.6 (d, *J* = 4.0 Hz, 1 H), 6.93 (d, *J* = 4.0 Hz, 1 H), 6.3 (br s, 1 H), 6.0 (br s, 2 H), 3.63 (s, 3 H), 3.3-3.15 (m, 1 H), 3.0-2.7 (m, 2 H), 2.5-2.4 (m, 1 H), 1.92-1.8 (m, 1 H), 1.75-1.6 (m, 3 H), 1.5-1.4 (m, 1 H), 1.35-1.22 (m, 2 H), 1.2-1.1 (m, 2 H); FABHRms calcd for $C_{19}H_{24}N_5O_4S$ (MH^+): 418.1549, found: 418.1564. Anal. Calcd for $C_{19}H_{23}N_5O_4S$: C, 54.66; H, 5.55, N, 16.78. Found: C, 54.95; H, 5.74; N, 16.51.

N-{5-[2-(2-Amino-3,4-dihydro-4-oxo-5,6,7,8-tetrahydropyrido[2,3-d]pyrimidin-6-yl)ethyl]thien-2-ylcarbonyl}-1-aminocyclopropane-1-carboxylic Acid (17): A suspension of **16** (0.20 g) in 0.5 N NaOH (15 ml) was stirred at room temperature for 1 day. The resulting clear solution was then acidified with 1 N HCl and the solid that formed was collected by filtration, washed with water, and dried *in vacuo* to yield 0.12 g (62%) of **17** as an off-white solid: mp >250 °C; ir (KBr) 3560-2990, 1760-1550 cm^{-1}; ^1H nmr (DMSO-d_6) δ 9.7 (br s, 1 H), 8.85 (br s, 1 H), 7.56 (d, *J* = 3.7 Hz, 1 H), 6.87 (d, *J* = 3.7 Hz, 1 H), 6.24 (br s, 1 H), 5.92 (br s, 2 H), 3.3-3.1 (m, 1 H), 3.0-2.7 (m, 2 H), 2.55-2.4 (m, 1 H), 1.9-1.7 (m, 1 H), 1.7-1.5 (m, 3 H), 1.5-1.4 (m, 2 H), 1.2-1.0 (m, 2 H); FABHRms Calcd for $C_{18}H_{22}N_5O_4S$ (MH^+): 404.1393, found: 404.1372. Anal. Calcd for $C_{18}H_{21}N_5O_4S \cdot 0.4$ NaCl: C, 50.65; H, 4.95, N, 16.40. Found: C, 50.35; H, 5.09; N, 16.19.

ACKNOWLEDGEMENT

We are indebted to Eli Lilly & Company, Indianapolis, IN for support of this work, and for the *in vitro* cell culture assays.

REFERENCES

1. For a recent summary with leading references, see E. C. Taylor, *Adv. Exp. Med. Biol.*, 1993, **338**, 387.

2. G. P. Beardsley, B. A. Moroson, E. C. Taylor, and R. G. Moran, *J. Biol. Chem.*, 1989, **264**, 328.

3. (a) G. Pizzorno, J. A. Sokoloski, A. R. Cashmore, B. A. Moroson, A. D. Cross, and G. P. Beardsley, *Mol. Pharmacol.*, 1991, **39**, 85. (b) G. Pizzorno, O. Russello, A. R. Cashmore, B. A. Moroson, A. D. Cross, M. Coronello, and G. P. Beardsley, *Proc. Am. Assoc. Cancer Res.*, 1990, **31**, 339.

4. L. L. Habeck, T. A. Leitner, K. A. Shackelford, L. S. Gossett, R. M. Schultz, S. L. Andis, C. Shih, G. B. Grindey, and L. G. Mendelsohn, *Cancer Res.*, 1994, **54**, 1021.

5. For leading references to the use of conformationally-constrained α-amino acids as bioactive agents and for conformational probes of active sites, see (a) J. Gante, *Angew. Chem. Int. Ed. Engl.*, 1994, **33**, 1699; (b) R. M. J. Liskamp, *Recl. Trav. Chim. Pays-Bas*, 1994, **1**, 113; (c) A. Gainnis and T. Kolter, *Angew. Chem. Int. Ed. Engl.*, 1993, **32**, 1244; (d) D. Mendel, J. Ellman, and P. G. Schultz, *J. Am. Chem. Soc.*, 1993, **115**, 4359. (e) K. Burgess, W.-K. Ho, and D. Moye-Sherman, *Synlett.*, 1944, 575; (f) J. Salaün and M. S. Baird, *Current Medicinal Chemistry*, 1995, Shimamoto and Y. Ohfune, *J. Med. Chem.*, 1996, **39**, 407.

6. C. Shih, L. S. Gossett, J. F. Worzalla, S. M. Rinzel, G., B. Grindey, P. M. Harrington, and E. C. Taylor, *J. Med. Chem.*, 1992, **35**, 1109.

7. A. P. Kozikowski, W. Tuckmantel, I. J. Reynolds, and J. T. Wroblewski, *J. Med. Chem.*, 1993, **33**, 1561.

8. S. J. Hudson, E. C. Bigham, D. S. Duch, G. K. Smith, and R. Ferone, *J. Med. Chem.*, 1994, **37**, 2112.

9. E. C. Taylor and G. S. K. Wong, *J. Org. Chem.*, 1989, **54**, 3618.

10. Obtained from Dr. Chuan Shih, The Lilly Research Laboratories, Eli Lilly & Company, Indianapolis, Indiana 46285.

11. A. Rosowsky, R. A. Forsch, R. G. Moran, and J. H. Freisheim, *Pteridines*, 1990, **2**, 133.

12. Z. J. Kaminski, *Tetrahedron Lett.*, 1985, **26**, 2901.

13. C. Mapelli, G. Newton, C. E. Ringold, and C. H. Stammer, *Int. J. Peptide Protein Res.*, 1987, **30**, 498.

Received, 6th August, 1996