

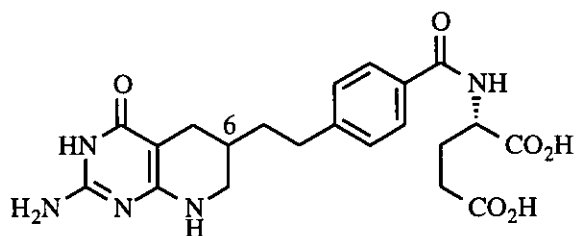
**SYNTHESIS OF SOME CONFORMATIONALLY-CONSTRAINED GLUTAMATE MIMICS OF *N*-{5-[2-(2-AMINO-3,4-DIHYDRO-4-OXO-5,6,7,8-TETRAHYDROPYRIDO[2,3-*d*]PYRIMIDIN-6-YL)ETHYL]THIEN-2-YLCARBONYL]-L-GLUTAMIC ACID (LY254155)}**

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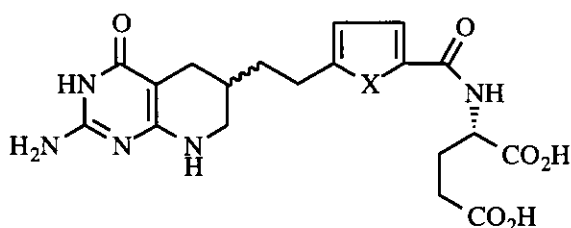
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**Abstract** - Several new analogues of the active antitumor agent *N*-{5-[2-(2-amino-3,4-dihydro-4-oxo-5,6,7,8-tetrahydropyrido[2,3-*d*]pyrimidin-6-yl)ethyl]thien-2-ylcarbonyl]-L-glutamic acid (LY254155) have been prepared in which the glutamate moiety 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.<sup>1</sup> 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.<sup>2</sup> However, this inhibition requires initial intracellular polyglutamylation by the enzyme folylpolyglutamyl synthetase (FPGS);<sup>3</sup> 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  $\gamma$ -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.<sup>4</sup> 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<sup>5</sup> 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**)<sup>6</sup> was condensed with dimethyl azetidine-*cis*-2,4-dicarboxylate



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



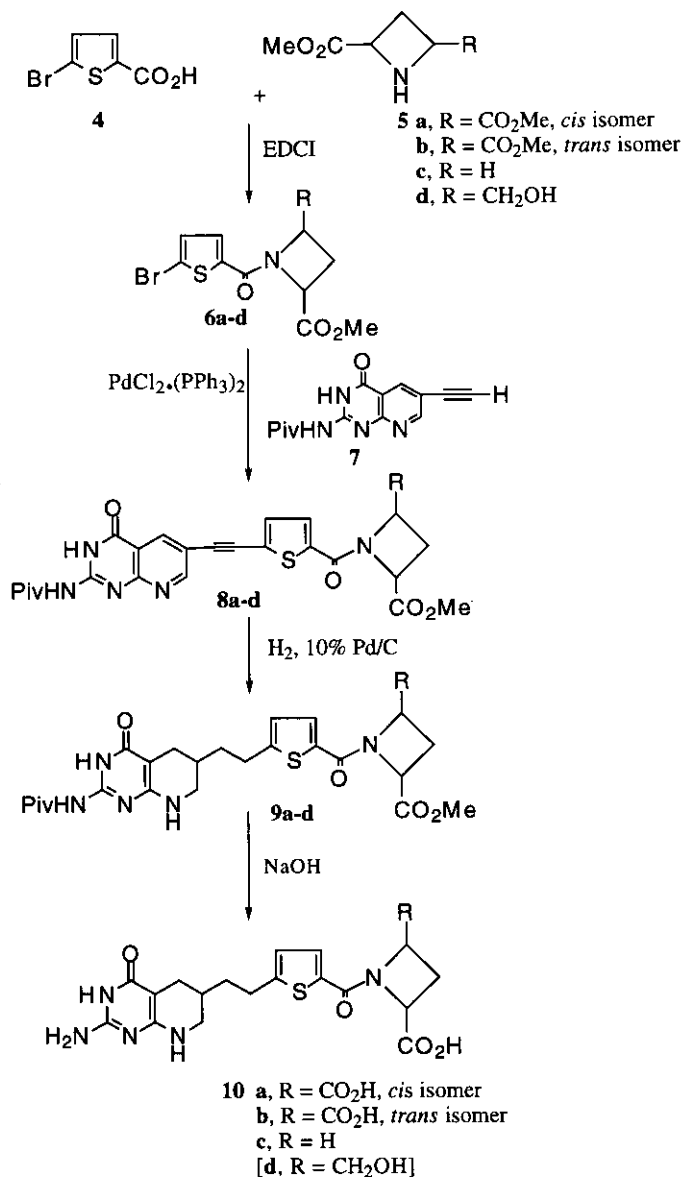
2, X = S [LY254155]

3, X = O [LY222306]

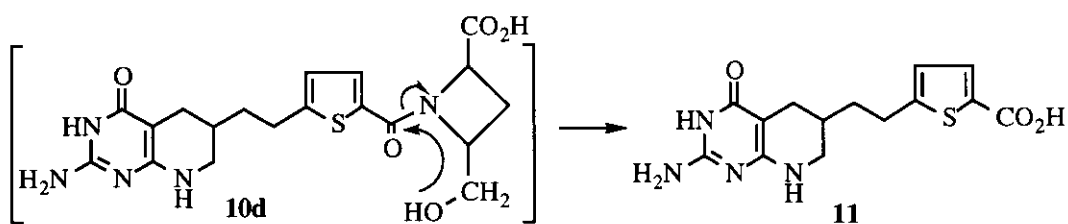
(5a)<sup>7</sup> using 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride as the coupling agent.<sup>8</sup> The resulting amide (6a) was then subjected to palladium-catalyzed coupling with 2-pivaloyl-6-ethynyl-5-deazapterin (7)<sup>9</sup> to give 8a. Catalytic hydrogenation of both the ethynyl 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 azetidinedicarboxylic 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<sup>10</sup> 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*- $\alpha$ -(2-carbomethoxycyclopropyl)glycinate hydrochloride (12)<sup>11</sup> using 6-chloro-2,4-dimethoxy-1,3,5-triazine as the coupling reagent<sup>12</sup> 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) incorporating 1-aminocyclopropane-1-carboxylic acid (Acc, 15)<sup>13</sup> as a glutamic acid mimic.

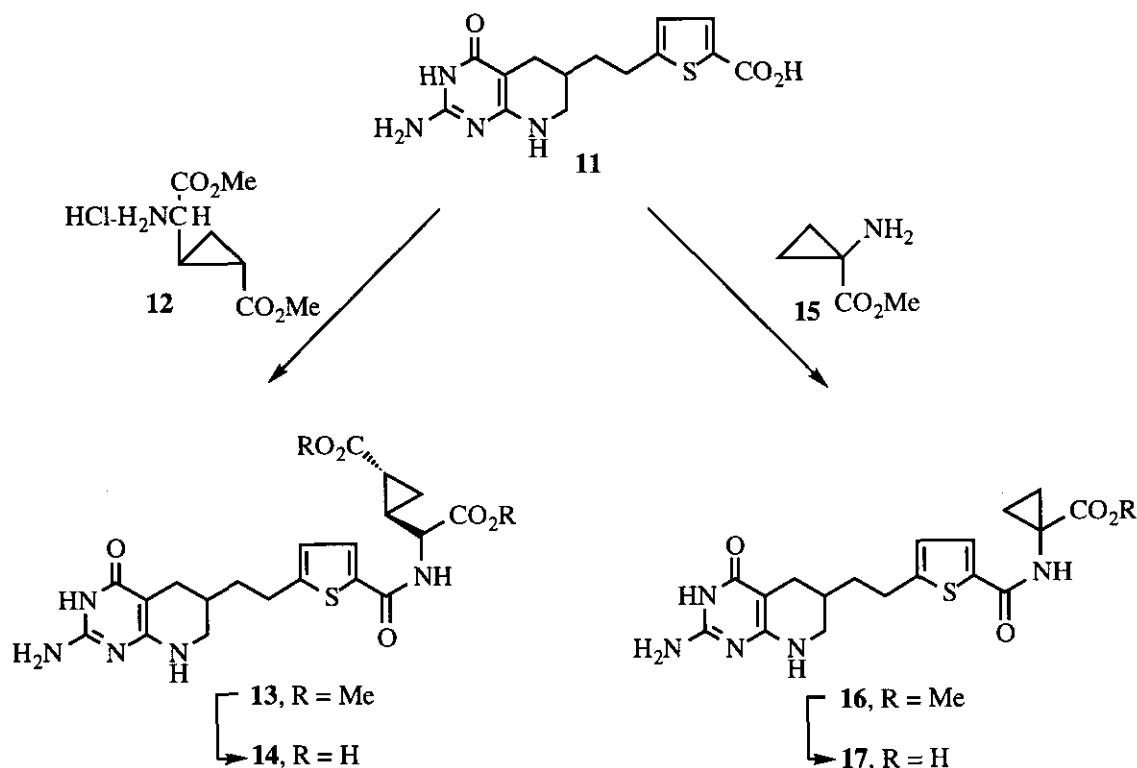
Scheme 1



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.** <sup>1</sup>H and <sup>13</sup>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-Bromothiophen-2-ylcarbonyl)-cis-2,4-dicarbomethoxyazetidide (6a):** A mixture of 5-bromothiophene-2-carboxylic acid<sup>6</sup> (1.04 g, 5 mmol), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide<sup>8</sup>

(1.15 g, 6 mmol), and dimethyl azetidine-*cis*-2,4-dicarboxylate<sup>7</sup> (**5a**) (1.38 g, 8 mmol) was stirred in CH<sub>2</sub>Cl<sub>2</sub> (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<sub>3</sub>, and water. The resulting solution was dried with MgSO<sub>4</sub> 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<sup>-1</sup>; <sup>1</sup>H nmr (CDCl<sub>3</sub>) 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); <sup>13</sup>C nmr (CDCl<sub>3</sub>) δ 169.7, 161.4, 136.8, 130.4, 130.1, 118.6, 59.3, 52.5, 25.5; HRms calcd for C<sub>12</sub>H<sub>12</sub>NO<sub>5</sub>BrS (M<sup>+</sup>): 360.9620, 362.9600, found: 360.9625, 362.9615; Anal. Calcd for C<sub>12</sub>H<sub>12</sub>NO<sub>5</sub>BrS: 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<sup>9</sup> (**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<sub>2</sub>Cl<sub>2</sub> 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<sup>-1</sup>; <sup>1</sup>H nmr (CDCl<sub>3</sub>) 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<sub>26</sub>H<sub>25</sub>N<sub>5</sub>O<sub>7</sub>S (M<sup>+</sup>): 551.1474, found: 551.1477. Anal. Calcd for C<sub>26</sub>H<sub>25</sub>N<sub>5</sub>O<sub>7</sub>S: 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<sub>2</sub>Cl<sub>2</sub> (1:1) was pressurized with 50 psi of H<sub>2</sub> 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, Uniplat<sup>TM</sup> Silica Gel GF) with 4% MeOH/CH<sub>2</sub>Cl<sub>2</sub> 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<sup>-1</sup>; <sup>1</sup>H nmr (CDCl<sub>3</sub>) 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<sub>26</sub>H<sub>33</sub>N<sub>5</sub>O<sub>7</sub>S (M<sup>+</sup>): 559.2100, found: 559.2109. Anal. Calcd for C<sub>26</sub>H<sub>33</sub>N<sub>5</sub>O<sub>7</sub>S: 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  $\text{cm}^{-1}$ ;  $^1\text{H}$  nmr (DMSO- $d_6$ )  $\delta$  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  $\text{C}_{19}\text{H}_{22}\text{N}_5\text{O}_6\text{S}$  ( $\text{MH}^+$ ): 448.1291, found: 448.1281. Anal. Calcd for  $\text{C}_{19}\text{H}_{21}\text{N}_5\text{O}_6\text{S} \cdot 1.32 \text{H}_2\text{O}$ : 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-carboxylic acid (**11**)<sup>10</sup> (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/ $\text{CH}_2\text{Cl}_2$  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  $\text{cm}^{-1}$ ;  $^1\text{H}$  nmr (DMSO- $d_6$ )  $\delta$  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  $\text{C}_{21}\text{H}_{26}\text{N}_5\text{O}_6\text{S}$  ( $\text{MH}^+$ ): 476.1604, found: 476.1606. Anal. Calcd for  $\text{C}_{21}\text{H}_{25}\text{N}_5\text{O}_6\text{S} \cdot \text{H}_2\text{O}$ : 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-Bromothiien-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  $\text{CH}_2\text{Cl}_2$  (150 ml) under argon overnight. The mixture was then washed with 0.05 N HCl, saturated  $\text{NaHCO}_3$ , and water. The resulting solution was dried with  $\text{MgSO}_4$  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  $\text{cm}^{-1}$ ;  $^1\text{H}$  nmr (DMSO- $d_6$ )  $\delta$  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);  $^1\text{H}$  nmr ( $\text{CDCl}_3$ )  $\delta$  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  $\text{C}_{12}\text{H}_{12}\text{NO}_5\text{BrS}$  ( $\text{M}^+$ ): 360.9620, 362.9600, found: 360.9625, 362.9604. Anal. Calcd for  $\text{C}_{12}\text{H}_{12}\text{NO}_5\text{BrS}$ : C, 39.79; H, 3.34, N, 3.87. Found: C, 39.90; H, 3.29; N, 3.90.

**N**-(5-Bromothiien-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,  $\text{SOCl}_2$  (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  $\text{CH}_2\text{Cl}_2$  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<sub>3</sub>, and dried with MgSO<sub>4</sub>. 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<sup>-1</sup>; <sup>1</sup>H nmr (CDCl<sub>3</sub>) 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<sub>10</sub>H<sub>10</sub>NO<sub>3</sub>BrS (M<sup>+</sup>): 302.9565, 304.9546, found: 302.9573, 304.9553. Anal. Calcd for C<sub>10</sub>H<sub>10</sub>NO<sub>3</sub>BrS: 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<sub>2</sub>Cl<sub>2</sub> 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<sup>-1</sup>; <sup>1</sup>H nmr (CDCl<sub>3</sub>) 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<sub>26</sub>H<sub>25</sub>N<sub>5</sub>O<sub>7</sub>S (M<sup>+</sup>): 551.1474, found: 551.1455. Anal. Calcd for C<sub>26</sub>H<sub>25</sub>N<sub>5</sub>O<sub>7</sub>S: 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<sub>2</sub>Cl<sub>2</sub> 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<sup>-1</sup>; <sup>1</sup>H nmr (DMSO-*d*<sub>6</sub>) 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<sub>24</sub>H<sub>23</sub>N<sub>5</sub>O<sub>5</sub>S (M<sup>+</sup>): 493.1420, found: 493.1422. Anal. Calcd for C<sub>24</sub>H<sub>23</sub>N<sub>5</sub>O<sub>5</sub>S: 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<sub>2</sub>Cl<sub>2</sub> (1:1) was pressurized with 50 psi H<sub>2</sub>, 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<sub>2</sub>Cl<sub>2</sub> as eluent to give 0.10 g (77%) of **9b** as a white solid: mp 178-181 °C; ir (KBr) 3323-

3006, 1739, 1647  $\text{cm}^{-1}$ ;  $^1\text{H}$  nmr ( $\text{CDCl}_3$ )  $\delta$  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  $\text{C}_{26}\text{H}_{33}\text{N}_5\text{O}_7\text{S}$  ( $\text{M}^+$ ): 559.2100, found: 559.2103. Anal. Calcd for  $\text{C}_{26}\text{H}_{33}\text{N}_5\text{O}_7\text{S}$ : 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  $\text{CH}_2\text{Cl}_2$  was pressurized with 50 psi  $\text{H}_2$ , heated to 50  $^\circ\text{C}$  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/ $\text{CH}_2\text{Cl}_2$  as eluent to yield 0.34 g (67%) of **9c** as a white solid: mp 236-238  $^\circ\text{C}$ ; ir (KBr) 3460-2791, 1729, 1633  $\text{cm}^{-1}$ ;  $^1\text{H}$  nmr ( $\text{DMSO}-d_6$ )  $\delta$  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  $\text{C}_{24}\text{H}_{31}\text{N}_5\text{O}_5\text{S}$  ( $\text{M}^+$ ): 501.2046, found: 501.2049. Anal. Calcd for  $\text{C}_{24}\text{H}_{31}\text{N}_5\text{O}_5\text{S}$ : 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 a white solid: mp 180  $^\circ\text{C}$  (decomp.); ir (KBr) 3600-2360, 1720-1480  $\text{cm}^{-1}$ ;  $^1\text{H}$  nmr ( $\text{DMSO}-d_6$ )  $\delta$  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  $\text{C}_{19}\text{H}_{22}\text{N}_5\text{O}_6\text{S}$  ( $\text{MH}^+$ ): 448.1291, found: 448.1285. Anal. Calcd for  $\text{C}_{19}\text{H}_{21}\text{N}_5\text{O}_6\text{S} \cdot \text{H}_2\text{O}$ : 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  $^\circ\text{C}$  (decomp.); ir (KBr) 3580-3048, 1740-1492  $\text{cm}^{-1}$ ;  $^1\text{H}$  nmr ( $\text{DMSO}-d_6$ )  $\delta$  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  $\text{C}_{18}\text{H}_{22}\text{N}_5\text{O}_4\text{S}$  ( $\text{MH}^+$ ): 404.1393, found: 404.1368. Anal. Calcd for  $\text{C}_{18}\text{H}_{21}\text{N}_5\text{O}_4\text{S} \cdot 0.16\text{NaCl}$ : C, 52.37; H, 5.13, N, 16.96. Found: C, 52.75; H, 5.13; N, 16.55.

**N-(5-Bromothiien-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  $^\circ\text{C}$ , the resulting solution was stirred at 0  $^\circ\text{C}$  for 30 min and  $\text{CH}_2\text{Cl}_2$  (200 ml) was added. The resulting solution was washed with water and dried over  $\text{MgSO}_4$ . 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<sub>4</sub>) 3600-3130, 1740 cm<sup>-1</sup>; <sup>1</sup>H nmr (CDCl<sub>3</sub>) 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); <sup>13</sup>C nmr (CDCl<sub>3</sub>) 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<sub>11</sub>H<sub>12</sub>NO<sub>4</sub>BrS (M<sup>+</sup>): 332.9671, 334.9651, found: 332.9677, 334.9660. Anal. Calcd for C<sub>11</sub>H<sub>12</sub>NO<sub>4</sub>BrS: 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-hydroxymethylazetidide (**8d**):** A mixture of **6d** (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<sub>2</sub>Cl<sub>2</sub> 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<sup>-1</sup>; <sup>1</sup>H nmr (CDCl<sub>3</sub>) 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); <sup>13</sup>C nmr (CDCl<sub>3</sub>) 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<sub>25</sub>H<sub>25</sub>N<sub>5</sub>O<sub>6</sub>S (M<sup>+</sup>): 523.1525, found: 523.1528. Anal. Calcd for C<sub>25</sub>H<sub>25</sub>N<sub>5</sub>O<sub>6</sub>S•0.8 H<sub>2</sub>O: 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-tetrahydropyranoxymethylazetidide (**8 cis**, R = CH<sub>2</sub>OTHP):** To a stirred solution of **8d** (0.118 g, 0.22 mmol) in CH<sub>2</sub>Cl<sub>2</sub>, 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<sub>3</sub> and dried over MgSO<sub>4</sub>. The concentrated residue was purified by silica gel chromatography using 1-5 % MeOH/CH<sub>2</sub>Cl<sub>2</sub> as eluent to give 0.040 g (29%) of product as a pale yellowish solid: <sup>1</sup>H nmr (mixture of diastereomers, CDCl<sub>3</sub>) 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<sub>30</sub>H<sub>33</sub>N<sub>5</sub>O<sub>6</sub>S: (M<sup>+</sup>): 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-hydroxymethylazetidide (**9d**):** A mixture of **8d** (0.060 g) and 10% Pd/C (0.12 g) in 150 ml of methanol/CH<sub>2</sub>Cl<sub>2</sub> (1:1) was pressurized with 50 psi H<sub>2</sub>, heated to 50 °C, and shaken overnight. The catalyst was removed by filtration through a short pad of silica gel, and the filtrate concentrated and purified by preparative tlc (20x20, Uniplat<sup>TM</sup> Silica Gel GF) with 6% MeOH/CH<sub>2</sub>Cl<sub>2</sub> 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  $\text{cm}^{-1}$ ;  $^1\text{H}$  nmr ( $\text{CDCl}_3$ )  $\delta$  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  $\text{C}_{25}\text{H}_{33}\text{N}_5\text{O}_6\text{S}$  ( $\text{M}^+$ ): 531.2151, found: 531.2151. Anal. Calcd for  $\text{C}_{25}\text{H}_{33}\text{N}_5\text{O}_6\text{S}$ : 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 =  $\text{CH}_2\text{OTHP}$ ):** A mixture of **8** (*cis*, R =  $\text{CH}_2\text{OTHP}$ ) (0.040 g) and 10% Pd/C (0.15 g) in 100 ml of  $\text{MeOH}/\text{CH}_2\text{Cl}_2$  (1:1) was pressurized with 50 psi  $\text{H}_2$ , heated to 50  $^\circ\text{C}$ , 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, Uniplat<sup>TM</sup> Silica Gel GF) with 4%  $\text{MeOH}/\text{CH}_2\text{Cl}_2$  as the developing solvent to yield 0.036 g (89%) of **9** (*cis*, R =  $\text{CH}_2\text{OTHP}$ ) as a white solid:  $^1\text{H}$  Nmr (mixture of diastereomers,  $\text{CDCl}_3$ )  $\delta$  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  $\text{C}_{30}\text{H}_{41}\text{N}_5\text{O}_6\text{S}$  ( $\text{M}^+$ ): 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):**<sup>10</sup> 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 a white solid, identical in every respect with authentic material. Saponification of **9** (*cis*, R =  $\text{CH}_2\text{OTHP}$ ) 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- $\alpha$ -(2-carbomethoxycyclopropyl)glycinate hydrochloride<sup>11</sup> (**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%  $\text{MeOH}/\text{CH}_2\text{Cl}_2$  as eluent to give 0.34 g (35%) of **13** as a white solid: mp 125-128  $^\circ\text{C}$ ; ir (KBr) 3600-2360, 1740-1540  $\text{cm}^{-1}$ ;  $^1\text{H}$  nmr ( $\text{DMSO}-d_6$ , major isomer)  $\delta$  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  $\text{C}_{22}\text{H}_{27}\text{N}_5\text{O}_6\text{S}$ : 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  $\text{cm}^{-1}$ ;  $^1\text{H}$  nmr (major isomer, DMSO- $d_6$ )  $\delta$  8.8 (d,  $J = 7.2$  Hz, 1H), 7.71 (d,  $J = 3.8$  Hz, 1H), 6.9 (d,  $J = 3.7$  Hz, 1H), 6.35 (br s, 1H), 6.09 (br s, 2H), 3.89 (t,  $J = 7.9$  Hz, 1H), 3.5-2.7 (m, 3H), 2.6-2.4 (m, 1H), 2.0-1.5 (m, 7H), 1.2-0.9 (m, 2H); FABHRms calcd for  $\text{C}_{20}\text{H}_{24}\text{N}_5\text{O}_6\text{S}$  ( $\text{MH}^+$ ): 462.1447, found: 462.1419. Anal. Calcd for  $\text{C}_{20}\text{H}_{23}\text{N}_5\text{O}_6\text{S} \cdot 0.35 \text{ NaCl}$ : 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.<sup>13</sup> 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/ $\text{CH}_2\text{Cl}_2$  as eluent to give 0.44 g (35%) of **16** as a white solid: mp 150 °C (decomp.); ir (KBr) 3620-2360, 1731, 1647  $\text{cm}^{-1}$ ;  $^1\text{H}$  nmr (DMSO- $d_6$ )  $\delta$  9.76 (br s, 1H), 9.0 (br s, 1H), 7.6 (d,  $J = 4.0$  Hz, 1H), 6.93 (d,  $J = 4.0$  Hz, 1H), 6.3 (br s, 1H), 6.0 (br s, 2H), 3.63 (s, 3H), 3.3-3.15 (m, 1H), 3.0-2.7 (m, 2H), 2.5-2.4 (m, 1H), 1.92-1.8 (m, 1H), 1.75-1.6 (m, 3H), 1.5-1.4 (m, 1H), 1.35-1.22 (m, 2H); 1.2-1.1 (m, 2H); FABHRms calcd for  $\text{C}_{19}\text{H}_{24}\text{N}_5\text{O}_4\text{S}$  ( $\text{MH}^+$ ): 418.1549, found: 418.1564. Anal. Calcd for  $\text{C}_{19}\text{H}_{23}\text{N}_5\text{O}_4\text{S}$ : 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  $\text{cm}^{-1}$ ;  $^1\text{H}$  nmr (DMSO- $d_6$ )  $\delta$  9.7 (br s, 1H), 8.85 (br s, 1H), 7.56 (d,  $J = 3.7$  Hz, 1H), 6.87 (d,  $J = 3.7$  Hz, 1H), 6.24 (br s, 1H), 5.92 (br s, 2H), 3.3-3.1 (m, 1H), 3.0-2.7 (m, 2H), 2.55-2.4 (m, 1H), 1.9-1.7 (m, 1H), 1.7-1.5 (m, 3H), 1.5-1.4 (m, 2H), 1.2-1.0 (m, 2H); FABHRms Calcd for  $\text{C}_{18}\text{H}_{22}\text{N}_5\text{O}_4\text{S}$  ( $\text{MH}^+$ ): 404.1393, found: 404.1372. Anal. Calcd for  $\text{C}_{18}\text{H}_{21}\text{N}_5\text{O}_4\text{S} \cdot 0.4 \text{ NaCl}$ : C, 50.65; H, 4.95, N, 16.40. Found: C, 50.35; H, 5.09; N, 16.19.

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