Replacement of the 1′,4′-Phenylene Region in 5,10-Dideaza-5,6,7,8-tetrahydrofolic Acid (DDATHF) by 4,5,6,7-Tetrahydrobenzo[*c*]thiophene and 4,5,6,7-Tetrahydroisobenzofuran Nuclei

Edward C. Taylor* and James E. Dowling

Department of Chemistry, Princeton University, Princeton, New Jersey 08544

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Two new analogs of DDATHF, in which the 1′,4′-phenylene unit is replaced by 4,5,6,7-tetrahydrobenzo[*c*]thiophene and 4,5,6,7-tetrahydroisobenzofuran nuclei, have been prepared and evaluated for *in vitro* cytotoxicity, GARFT inhibition, and FPGS affinity as potential antitumor agents.

Introduction

In the *de novo* purine biosynthetic pathway, glycinamide ribonucleotide formyltransferase (GARFT) catalyzes the transformylation of 5′-phosphoribosyl glycinamide (*β*-GAR) to 5′-phosphoribosyl formylglycinamide, a reaction which requires 10-formyl-5,6,7,8-tetrahydrofolate as a cofactor.[1] The cytotoxic[2] effects of the tetrahydrofolic acid analog 5,10-dideaza-5,6,7,8-tetrahydrofolic acid (DDATHF, **1**) arise from potent and selective inhibition of GARFT.[3] DDATHF exhibits pronounced *in vitro* activity toward a variety of solid murine and human xenograft tumors against which the clinically-employed dihydrofolate reductase (DHFR) inhibitor methotrexate (MTX) has little or no activity.[4] Currently, the (6*R*) diastereomer of **1** (lometrexol, LTX)[5] is being evaluated in Phase II clinical trials in which coadministration with folic acid has been shown to ameliorate the delayed, cumulative toxicity observed in earlier studies conducted with the drug alone.[6] Tight-binding inhibition of GARFT requires prior modification of DDATHF by folylpolyglutamate synthetase (FPGS) which is responsible for the intracellular conversion of folates to their *γ*-polyglutamyl derivatives.[7] Some cancer cell lines which exhibit resistance to DDATHF are unable to accumulate polyglutamylated derivatives, a result that has been attributed to reduced levels of FPGS activity and which implicates the polyglutamyl forms as the active intracellular metabolites.[8] Acquired resistance to DDATHF has also been noted in cell lines which exhibit an increase in *γ*-glutamyl hydrolase (*γ*-GH) activity and which possess impaired reduced folate transport mechanisms.[9] Recently, two antifolates differing from DDATHF in the replacement of the 1′,4′-phenylene unit by a 2′,5′-thiophene ring (**2**, $k_i = 2.1$ nm) and a 2′,5′-furan ring (**3**, $k_i = 0.77$ nm) were found to possess inhibitory activity against the recombinant human monofunctional enzyme (hGARFT)[10] without the need for further glutamylation.[11]

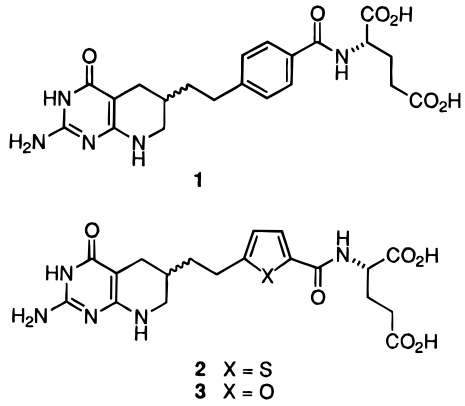

Figure 1.

As an extension of our earlier structure−activity relationship (SAR) studies, we have initiated a molecular modeling approach to aid in the design of new inhibitors of GARFT based on **2** and **3**. Visual inspection[12] of the hGARFT crystallographic ternary complex with **2** and *β*-GAR revealed the existence of a large cavity in the region which surrounds the 2′,5′-thienyl substitutent. The presence of such a conspicuous unoccupied volume suggested that substrate mimics which bear a larger or a bicyclic ring system in place of the commonly-employed monocyclic units might be readily accommodated. A similar hydrophobic cavity was observed in a molecular modeling study conducted on analogs in the MTX series using bacterial DHFR obtained from *Lactobacillus casei*.[13] Subsequent replacement of the 1,4-phenylene moiety of MTX with naphthalene-derived rings was found to have a favorable effect on the transport of such derivatives into

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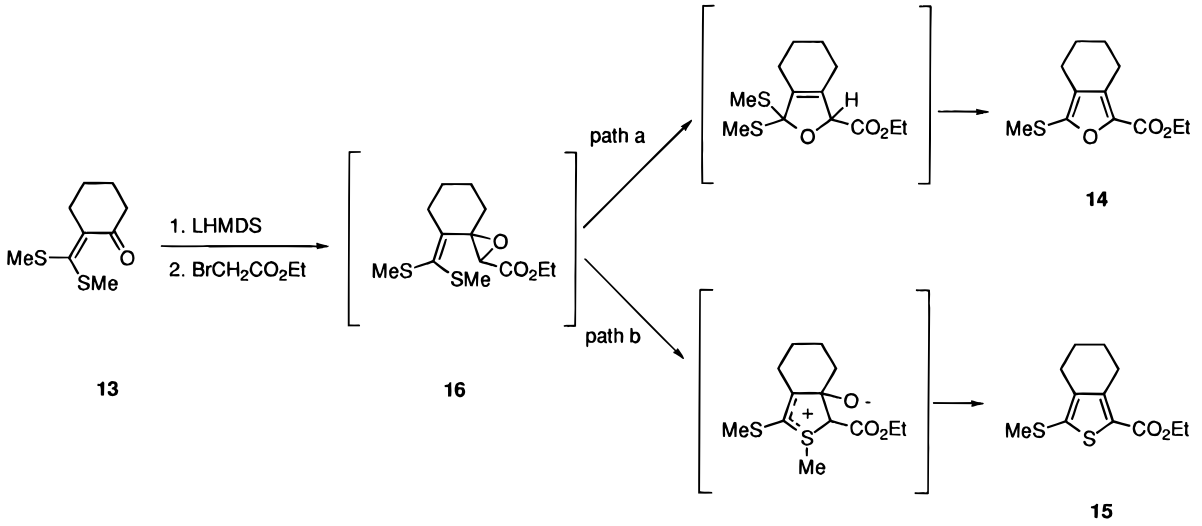
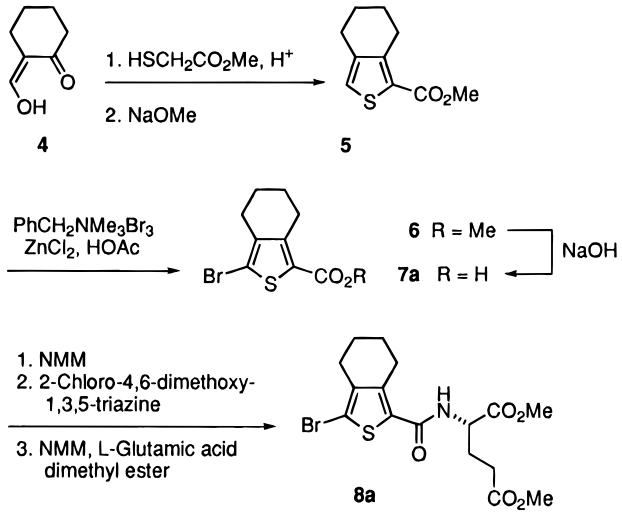
Scheme 1



L1210 cells, with the greatest enhancement in influx efficiency observed for the 5,6,7,8-tetrahydronaphthoyl derivative. Given that both DDATHF and MTX utilize the reduced folate carrier protein (RFC),[14] a similar improvement in the rate of cellular uptake was conceivable for derivatives which had been modified by fusion of a fully-reduced six-membered ring onto the bridging 2′,5′-thienyl or 2′,5′-furanyl ring of **2** and **3**. Since it is well recognized that antifolate cytotoxicity is a consequence, *inter alia*, of enzyme inhibition, transport efficiency, and, usually, the extent of intracellular polyglutamation, it is reasonable to expect that analogs which exhibit high affinity for GARFT yet are more readily internalized should exhibit improved cytotoxicity. In this paper we report the synthesis of two new analogs of **1** in which the 1′,4′-phenylene feature has been replaced by 4,5,6,7-tetrahydrobenzo[*c*]thiophene-1′,3′-diyl and -isobenzofuran-1′,3′-diyl nuclei. It was anticipated that due to the spacious hydrophobic cavity in the target enzyme, the larger substrates would retain the high binding affinity for GARFT exhibited by **2** and **3** while having the potential for improved rates of internalization via the RFC. Although this type of structural modification was found to have a deleterious effect on the affinity for FPGS of the naphthoyl-derived MTX analogues[13] such that the extent of intracellular polyglutamation was reduced relative to the benzoyl analogues, manifestation of this characteristic in the proposed analogs of **1** may be of lesser concern since it has been established that polyglutamylation of **2** and **3** is not a requirement for tight-binding inhibition of hGARFT.[11]

Results and Discussion

Our synthetic strategy relies principally upon a convergent, palladium-mediated approach to the preparation of 6-substituted deazapterins initially employed in the synthesis of **1**[15] and subsequently in the preparation of numerous analogs. Acid-catalyzed condensation of 2-formylcyclohexanone[16] (**4**) with methyl thioglycolate followed by aromatization with freshly-prepared sodium methoxide provided methyl 4,5,6,7-tetrahydrobenzo[*c*]-thiophene-2-carboxylate (**5**).[17] Treatment of **5** with benzyltrimethylammonium tribromide[18] and $ZnCl_2$ in acetic acid gave the nuclear bromination product **6** in 85% yield. Conversion to carboxylic acid **7a** by saponification with 1 N NaOH followed by 2-chloro-4,6-dimethoxy-1,3,5-triazine-mediated[19] amino acid coupling (84%) afforded the L-glutamic acid amide protected as its dimethyl ester (**8a**).



Palladium-catalyzed coupling of **8a** with excess 2-pivaloyl-6-ethynyl-5-deazapterin[15] (**9**) gave the acetylene **10a** in 65% yield. Hydrogenation using 2 wt equiv of 10% palladium-on-carbon in a mixture of methanol and dichloromethane at 50 °C proceeded in 95% yield to give the penultimate intermediate **11a**. Subsequent simultaneous deprotection of the exocyclic amino group and glutamic acid side chain with 1 N NaOH provided the desired material **12a** in 75% yield (30% overall for the six-step sequence beginning with **5**).

The synthesis of the 4,5,6,7-tetrahydroisobenzofuran derivative **12b** relies upon initial assembly of the furan ring by treatment of 2-[bis(methylthio)methylene]cyclohexanone[20] (**13**) with LHMDS and ethyl bromoacetate to

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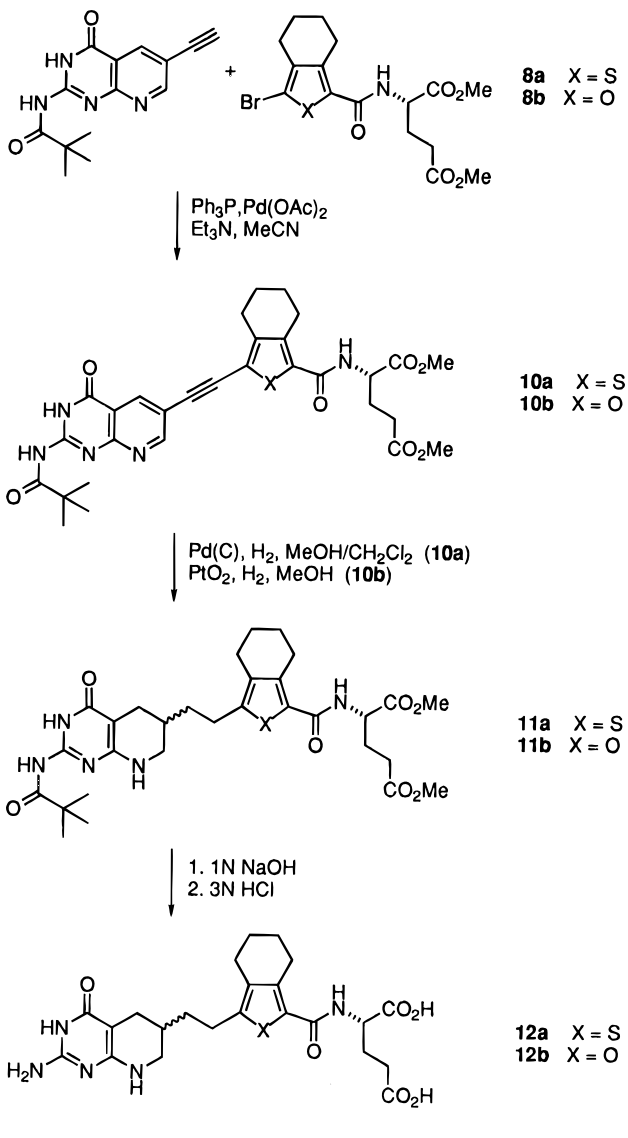
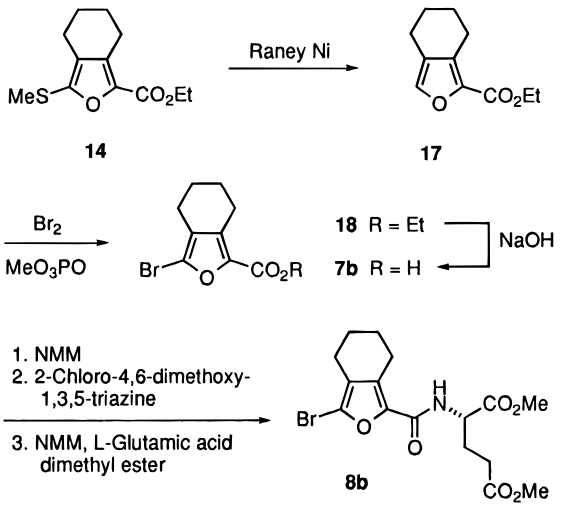


give the methylthio derivative **14** (Scheme 1, path a).[21] In our hands, **14** was consistently obtained in low yields and was accompanied by the corresponding 4,5,6,7-tetrahydrobenzo[*c*]thiophene **15**. The contaminant **15** is presumed to result from sulfur-promoted ring opening of the Darzen's reaction product, common intermediate **16**, followed by demethylation and aromatization (Scheme 1, path b). A similar mechanism has been invoked to rationalize the formation of thiophenes from α-oxoketene dithioacetals under Simmons−Smith conditions.[22] Formation of **15** was more extensive when the reaction temperature was maintained at −78 °C for extended periods (3 h); brief exposure (0.5 h) to the same conditions resulted in formation of **14** as the major product (26%).

Attempted bromination of **17**, obtained by Raney nickel desulfurization of **14**, using the previously-employed conditions or bromine alone, gave complex mixtures. Conversion to the bromo derivative **18**, in 50% yield, was finally achieved with bromine in trimethyl phosphate in the dark to minimize the formation of additional products which resulted from apparent benzylic bromination.

In the same fashion as described for the 4,5,6,7-tetrahydrobenzo[*c*]thiophene derivative, **18** was converted to glutamate **8b** via the intermediacy of acid **7b**. Subsequent coupling with excess **9** gave acetylene **10b** which, unlike its counterpart **10a**, resisted all efforts to separate it from an aromatic impurity. To overcome this minor difficulty, the crude material was catalytically reduced using PtO_2 to give **11b** which was then deprotected to afford **12b** in 61% yield (20% overall from **15**).



The results of *in vitro* cell culture screening of **12a** and **12b** against human T-cell derived lymphoblastic leukemia (CCRF-CEM) cells indicated that the new compounds were modest cytotoxic agents, with IC_{50} values of 0.19 and 1.6 mM, respectively. Reversal of the cytotoxicity of **12a** and **12b** could be effected by the addition of hypoxanthine (100 μM) but not by the addition of thymidine (5 μM), indicating that the locus of activity resides in the purine *de novo* biosynthetic pathway. Subsequent evaluation of **12a** and **12b** against murine trifunctional GARFT revealed the two analogs to be competitive inhibitors with K_i's of 25.4 and 8.7 nM, respectively, although, surprisingly, neither was found to have any activity against hog liver FPGS at drug concentrations up to 300 μM in the presence of 2 μg of enzyme. In the absence of additional data it cannot be determined if the diminished cytotoxicity of **12a** and **12b** relative to **2** and **3** (IC_{50} (CCRF-CEM) = 1.3 and 15.2 nM, respectively) is a consequence of reduced affinity of the analogs for the RFC or if attainment of a useful level of cytotoxicity requires that some degree of polyglutamation be achieved even for antifolates which exhibit tight binding inhibition of GARFT in their monoglutamated forms.

Experimental Section

General Methods. NMR spectra were recorded on a JEOL GSX 270 FT or a Varian INOVA 500 spectrometer. Proton and carbon chemical shifts are reported in parts per million (ppm) and are referenced to internal solvent. Multiplicities are abbreviated in the following manner: singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), and broadened (br). Coupling constants were determined by first-order approximation. High and low resolution mass spectra were obtained on a Kratos MS50 spectrometer. Analytical thin-layer chromatography (TLC) was conducted using precoated silica gel 60 plates with a fluorescent indicator. Radial chromatography was performed with the aid of the Chromatotron, a product of Harrison Research Inc. All reactions were carried out under an argon atmosphere with dry, freshly-distilled solvents under anhydrous conditions unless otherwise noted. All reagents obtained from commercial suppliers were used without further purification unless otherwise stated.

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Methyl 4,5,6,7-Tetrahydrobenzo[*c*]thiophene-1-carboxylate (5). To a magnetically stirred, neat mixture of 2-oxocyclohexanecarboxaldehyde (3.6 g, 28 mmol) and methyl thioglycolate (6.0 g, 56 mmol) was added 3 drops of concentrated H_2SO_4. The resulting yellow solution was stirred at rt for 12 h, diluted with 25 mL of ice−water, and extracted with CH_2Cl_2 (25 mL). The aqueous phase was reextracted with an additional 25 mL portion of CH_2Cl_2, and the combined organic phases were washed with 50 mL of a saturated aqueous NaCl solution and dried over Na_2SO_4. Removal of the drying agent by filtration followed by evaporation *in vacuo* gave a viscous yellow oil which was dissolved in 25 mL of MeOH and added dropwise over 1 h to a freshly-prepared solution of NaOMe (from 1.7 g, 2.5 equiv of sodium metal) in 100 mL of MeOH. The deep orange solution was allowed to stir overnight (12 h), concentrated to one-quarter volume *in vacuo*, and partitioned between CH_2Cl_2 (50 mL) and water (50 mL). The aqueous phase was reextracted with an additional 25 mL portion of CH_2Cl_2, and the combined organic phases were washed with 50 mL of a saturated aqueous NaCl solution and dried over Na_2SO_4. The drying agent was removed by filtration and the filtrate concentrated *in vacuo* to afford a yellow oil which was purified by chromatography on silica using 5% EtOAc in hexanes as eluent to give 1.5 g (27%) of a clear, colorless liquid. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.73 (m, 4 H), 2.68 (t, 2 H, J = 6.2 Hz), 3.02 (t, 2 H, J = 6.2 Hz), 3.83 (s, 3 H), 7.05 (s, 1 H); ^{13}C NMR ($CDCl_3$, 75.6 MHz) 22.5, 22.5, 26.1, 26.6, 51.2, 125.1, 125.1, 139.8, 146.4, 162.9; IR (NaCl) 3088, 2935, 2848, 1698, 1538 cm^{-1}; MS *m/e* (relative intensity) 196 (99), 181 (15), 165 (55), 137 (92); HRMS calcd for $C_{10}H_{12}O_2S$: 196.0557, found: 196.0557.

Methyl 3-Bromo-4,5,6,7-tetrahydrobenzo[*c*]thiophene-1-carboxylate (6). To a solution of **5** (0.40 g, 2.0 mmol) in 5 mL of HOAc was added benzyl trimethylammonium tribromide (0.84 g, 2.1 mmol) followed by zinc chloride (1.1 g, 8.0 mmol). The resulting orange suspension was stirred at rt until a fine precipitate had developed and a pale yellow coloration persisted (5 h). The reaction mixture was partitioned between CH_2Cl_2 (25 mL) and water (25 mL). The organic phase was washed with an aqueous NaCl solution (25 mL), dried over Na_2SO_4, filtered, and evaporated. The resulting solid residue was purified by radial chromatography on a 2 mm plate using 5% EtOAc in hexanes as eluent to give a white solid (0.47 g, 85%) which was recrystallized from hexanes, mp 85−86 °C. ^{1}H NMR ($CDCl_3$, 270 MHz) δ 1.55 (m, 4 H), 2.35 (br t, 2 H, J = 6.1 Hz), 2.82 (br t, 2 H, J = 6.1 Hz), 3.65 (s, 3 H); ^{13}C NMR ($CDCl_3$, 75.6 MHz) δ 23.3, 23.4, 27.3, 28.1, 52.8, 117.1, 126.7, 141.0, 148.3, 163.4; IR (KBr) 2929, 2851, 1675, 1534 cm^{-1}; MS *m/e* (relative intensity) 276 (88), 274 (92), 261 (88), 259 (90), 245 (45), 243 (66), 217 (27), 215 (36); HRMS calcd for $C_{10}H_{11}BrO_2S$: 273.9663, found: 273.9663. Anal. Calcd for $C_{10}H_{11}BrO_2S$: C, 43.65; H, 4.03. Found: C, 43.94; H, 4.05.

3-Bromo-4,5,6,7-tetrahydrobenzo[*c*]thiophene-1-carboxylic Acid (7a). A suspension of **6** (300 mg, 1.09 mmol) in 6 mL of 1 N NaOH was heated at 80 °C for 6 h. The resulting clear solution was cooled with the aid of an ice bath and carefully acidified using a 3 M HCl solution. The milky white suspension was extracted with EtOAc (2 × 10 mL), and the combined organic phases were dried over $MgSO_4$, filtered, and evaporated to afford a white solid (255 mg, 90%), mp 220 °C dec. ^{1}H NMR ($CDCl_3$, 270 MHz) δ 1.65 (m, 4 H), 2.47 (br t, 2 H, J = 6.1 Hz), 2.95 (br t, 2 H, J = 6.1 Hz); IR (KBr) 1661 cm^{-1}; MS *m/e* (relative intensity) 262 (100) 260 (96) 215 (69) 217 (71); HRMS calcd for $C_9H_9BrO_2S$: 259.9507, found: 259.9507. Anal. Calcd for $C_9H_9BrO_2S$: C, 41.40; H, 3.47. Found: C, 41.33; H, 3.52.

Dimethyl *N*-(3-Bromo-4,5,6,7-tetrahydrobenzo[*c*]thienoyl)-L-glutamate (8a). To a solution of **7** (90 mg, 0.34 mmol) in 5 mL of MeCN and 5 mL of THF at rt was added 4-methylmorpholine (NMM, 45 μL, 0.41 mmol, 1.2 equiv) neat, via syringe. After 10 min, 2-chloro-4,6-dimethoxy-1,3,5-triazine (66 mg, 0.37 mmol, 1.1 equiv) was added in one portion, and the resulting mixture was stirred for 1 h. An additional portion of NMM (1.2 equiv) was added followed by L-glutamic acid dimethyl ester hydrochloride (106 mg, 0.41 mmol). After stirring for 4 h, the mixture was partitioned between CH_2Cl_2 (15 mL) and water (15 mL). The aqueous phase was extracted with CH_2Cl_2 (15 mL), and the combined organic extracts were washed with an aqueous NaCl solution (15 mL), dried over Na_2SO_4, filtered, and evaporated *in vacuo*. The resulting oily residue was purified by radial chromatography (2 mm plate) using 20% EtOAc in hexanes as eluent to give 120 mg (84%) of a clear, colorless oil. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.73 (m, 4 H), 2.09 (m, 1 H), 2.28 (m, 1 H), 2.37−2.51 (m, 4 H), 2.94 (m, 2 H), 3.64 (s, 3 H), 3.76 (s, 3 H), 4.73 (dt, 1 H, J = 5.1, 7.2 Hz), 6.50 (d, 1 H, J = 7.2 Hz); ^{13}C NMR ($CDCl_3$, 75.6 MHz) δ 21.9, 22.3, 26.0, 26.7, 27.0, 29.8, 51.6, 51.9, 52.4, 113.0, 130.3, 139.6, 141.6, 161.6, 172.0, 173.0; IR (NaCl) 3309, 2936, 1731, 1632 cm^{-1}; MS *m/e* (relative intensity) 419 (6), 417 (16), 246 (14), 245 (92), 244 (100), 243 (95), 242 (94); HRMS calcd for $C_{16}H_{20}BrNO_5S$: 417.0245, found: 417.0252. Anal. Calcd for $C_{16}H_{20}BrNO_5S$: C, 45.94; H, 4.82; N, 3.35; S, 7.66. Found: C, 46.17; H, 4.79; N, 3.32; S, 7.92.

Dimethyl *N*-[3-[[2-(Pivaloylamino)-4-oxo-3,4-pyrido[2,3-*d*]pyrimidin-6-yl]ethynyl]-4,5,6,7-tetrahydrobenzo[*c*]thienoyl]-L-glutamate (10a). A mixture of **9** (210 mg, 0.77 mmol), $Pd(OAc)_2$ (3.8 mg, 4 mol %), tri-*o*-tolylphosphine (9.4 mg, 8 mol %), Et_3N (117 mg, 1.16 mmol), and **8a** (162 mg, 0.39 mmol) in 10 mL of MeCN was heated at reflux under an argon atmosphere for 24 h. At this point an additional 53 mg of **9** was added, and the reaction was allowed to continue for 24 h. After cooling to rt, the solvent was removed *in vacuo*, and the resulting black residue was passed through a short column of silica gel using 5% MeOH in CH_2Cl_2 as eluent. Further purification by radial chromatography on a 2 mm plate using 80% EtOAc in hexanes as eluent afforded the coupled product as a pale yellow solid (141 mg, 60%). ^{1}H NMR ($CDCl_3$, 270 MHz) δ 1.33 (s, 9 H), 1.76 (m, 4 H), 2.24 (m, 2 H), 2.47 (m, 2 H), 2.76 (m, 2 H), 2.95 (m, 2 H), 3.65 (s, 3 H), 3.77 (s, 3 H), 4.76 (dt, 1 H, J = 5.1, 7.2 Hz), 6.75 (d, 1 H, J = 7.3 Hz), 8.53 (d, 1 H, J = 2.3 Hz), 8.56 (br s, 1 H), 8.87 (s, 1 H), 12.12 (br s, 1 H); MS *m/e* (relative intensity) 607 (21), 564 (50), 550 (20), 432 (100), 389 (51), 375 (50); HRMS calcd for $C_{30}H_{33}N_5O_7S$: 607.2111, found: 607.2100.

Dimethyl *N*-[3-[2-[2-(Pivaloylamino)-4-oxo-3,4,5,6,7,8-hexahydropyrido[2,3-*d*]pyrimidin-6-yl]ethyl]-4,5,6,7-tetrahydrobenzo[*c*]thienoyl]-L-glutamate (11a). A mixture of **10a** (220 mg, 0.36 mmol) and 10% Pd/C (440 mg, 2 wt eq) in 25 mL of MeOH and 2 mL of CH_2Cl_2 was hydrogenated at 48 psi and 50 °C for 24 h. The mixture was filtered through Celite with the aid of additional MeOH, and the filtrate was concentrated *in vacuo* to afford a pale yellow oil which was purified by radial chromatography, using 5% MeOH in CH_2Cl_2 as eluent, to give a white solid (210 mg, 95%), mp 178−180 °C dec.^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.28 (s, 9 H), 1.58−1.72 (m, 6 H), 1.90 (m, 1 H), 2.13 (m, 2 H), 2.33 (m, 1 H), 2.47 (m, 2 H), 2.57 (br t, 2H, J = 6.1 Hz), 2.80 (m, 2 H), 3.01 (m, 4 H), 3.39 (d, 1H, J = 11.9 Hz), 3.62 (s, 3 H), 3.74 (s, 3 H), 4.76 (dt, 1 H, J = 5.1, 7.3 Hz), 5.11 (s, 1 H), 6.44 (d, 1 H, J = 7.3 Hz), 8.47 (s, 1 H), 11.25 (s, 1 H); ^{13}C NMR ($CDCl_3$, 75.6 MHz) δ 22.8, 23.1, 25.3, 25.4, 25.7, 27.2 27.4, 27.8, 30.3, 30.8, 34.2, 40.4, 46.3, 52.1, 52.1, 52.8, 89.8, 126.2, 136.2, 141.8, 143.2, 148.4, 158.2, 160.7, 163.0, 172.8, 173.5, 179.9. MS *m/e* (relative intensity) 615 (34), 583 (7), 441 (25), 440 (13), 413 (40), 412 (33), 250 (90), 249 (100); HRMS calcd for $C_{30}H_{41}N_5O_7S$: 615.2726, found: 615.2718.

***N*-[3-[2-(2-Amino-4-oxo-3,4,5,6,7,8-hexahydropyrido[2,3-*d*]pyrimidin-6-yl)ethyl]-4,5,6,7-tetrahydrobenzo[*c*]thienoyl]-L-glutamic Acid (12a).** A suspension of **11a** (245 mg, 0.400 mmol) in 2 mL of 1 N NaOH was stirred at rt for 48 h. Acidification with 3 M HCl gave a yellow gum which was stirred overnight. The resulting pale yellow solid was collected, washed with water, and dried to afford the glutamic acid derivative (150 mg, 75%), mp 195−200 °C dec. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 1.52−1.66 (m, 6 H), 1.85−1.91 (m, 2 H), 2.05 (m, 1 H), 2.31 (m, 2 H), 2.50 (m, 2 H, obscured by residual solvent peak), 2.54 (m, 2 H), 2.75 (m, 2 H), 2.81−2.93 (m, 3 H), 3.24 (m, 1 H), 4.31 (dt, 1 H, J = 5.1, 7.3 Hz), 6.49 (s, 2 H), 6.61 (s, 1 H), 7.76 (d, 1 H, J = 7.8 Hz), 12.50 (br s, 1 H). HRMS (FAB) calcd for $C_{23}H_{30}N_5O_6S$: 504.1917 (MH^+), found: 504.1924.

Ethyl 3-(Methylthio)-4,5,6,7-tetrahydroisobenzofuran-

1-carboxylate (14) and Ethyl 3-(Methylthio)-4,5,6,7-tetrahydrobenzo[*c*]thiophene-1-carboxylate (15). A solution of LHMDS was prepared by the addition of *n*-BuLi (3.8 mL of a 2.5 M solution in hexanes) to a solution of HMDS (1.51 g, 9.4 mmol) in 10 mL of THF at 0 °C. The solution was then cooled to −78 °C and stirred at this temperature for 0.5 h. Ethyl bromoacetate (1.05 mL, 9.4 mmol) in 7 mL of THF was added over a period of 10 min. After the reaction mixture had been allowed to stir for 0.5 h, a solution of 2-[bis(methylthio)-methylene]cyclohexanone (950 mg, 4.7 mmol) in 10 mL of THF was added over 15 min. The reaction mixture was maintained at −78 °C for 3 h, allowed to stir at rt for 12 h, and partitioned between water (50 mL) and ether (50 mL). The aqueous phase was extracted with ether (25 mL), and the combined organic phases were then washed with brine (25 mL) and dried over $MgSO_4$. Filtration and evaporation *in vacuo* gave 1.8 g of a red oil which was purified by chromatography on silica using 5% EtOAc in hexanes as eluent. Further purification by radial chromatography (2 mm plate), using 2% EtOAc in hexanes as eluent afforded **14** (0.20 g, 0.83 mmol, 18%) and **15** (0.20 g, 0.78 mmol, 16%), mp 41 °C (from hexanes). Spectral and analytical data for **15** are as follows: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.35 (t, 3 H, J = 7.3 Hz), 1.74 (m, 4 H), 2.51 (s, 3 H), 2.55 (m, 2 H), 2.99 (m, 2 H), 4.30 (q, 2 H, J = 7.3, 14.6 Hz); ^{13}C NMR ($CDCl_3$, 75.6 MHz) δ 14.6, 19.1, 22.7, 22.8, 25.7, 27.3, 60.6, 124.9, 138.9, 139.2, 147.5, 162.4; MS *m/e* (relative intensity) 256 (100), 227 (87), 211 (31), 183 (28), 136 (55); HRMS calcd for $C_{12}H_{16}O_2S_2$: 256.0592. Found: 256.0598. Anal. Calcd for $C_{12}H_{16}O_2S_2$: C, 56.22; H, 6.29; S, 25.01. Found: C, 56.00; H, 6.20; S, 24.87.

Ethyl 3-Bromo-4,5,6,7-tetrahydroisobenzofuran-1-carboxylate (18). Ethyl 4,5,6,7-tetrahydroisobenzofuran-1-carboxylate[21] (425 mg, 2.20 mmol) was dissolved in 12 mL of 1,2-dichloroethane, and the reaction flask was then shrouded with aluminum foil. After extinguishing the hood lights, bromine (2.2 mL of a 1.0 M solution in trimethyl phosphate) was added via syringe over 10 min. The resulting solution was stirred at rt for 20 min. The reaction mixture was partitioned between CH_2Cl_2 (15 mL) and ice−water (15 mL). The organic phase was washed with 15 mL of a 20% $Na_2S_2O_3$ aqueous solution, dried over Na_2SO_4, filtered, and evaporated *in vacuo* to give a clear yellow oil. Purification of the crude material by radial chromatography (2 mm plate) using 2% EtOAc in hexanes gave 269 mg (50%) of a white powder, mp 89−90 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.28 (t, 3 H, J = 7.3 Hz), 1.63 (m, 4 H), 2.30 (m, 2 H), 2.70 (m, 2 H), 4.24 (q, 2 H, J = 7.3 , 14.3 Hz); ^{13}C NMR ($CDCl_3$, 75.6 MHz) δ 14.2, 20.2, 21.8, 21.9, 22.0, 60.3, 122.9, 123.8, 134.0, 140.1, 158.3; IR (KBr) 1710, 1520, cm^{-1}; MS *m/e* (relative intensity) 274 (21), 272 (22), 246 (28), 245 (33), 244 (29), 243 (32), 229 (13), 227 (20), 78 (100); HRMS calcd for $C_{11}H_{13}BrO_3$: 272.0048, found: 272.0036. Anal. Calcd for $C_{11}H_{13}BrO_3$: C, 48.37; H, 4.80; Br, 29.26. Found: C, 48.49; H, 4.82; Br, 29.07.

3-Bromo-4,5,6,7-tetrahydroisobenzofuran-1-carboxylic Acid (7b). A suspension of **18** (200 mg, 0.73 mmol) in 6 mL of 1 N NaOH was heated at 80 °C for 6 h. The resulting clear solution was cooled with an ice bath and carefully acidified using a 3 M HCl solution. The milky white suspension was extracted with EtOAc (2 × 10 mL), and the combined organic phases were then dried over $MgSO_4$, filtered and evaporated to afford a white solid (160 mg, 89%), mp 145−150 °C dec. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.57 (m, 4 H), 2.36 (m 2 H), 2.84 (m, 2 H); IR (KBr) 1661, 1597, cm^{-1}; MS *m/e* (relative intensity) 246 (77) 245 (24) 244 (78) 243 (17); HRMS calcd for $C_9H_9BrO_3$: 243.9736. Found: 243.9742. Anal. Calcd for $C_9H_9BrO_3$: C, 44.11; H, 3.70. Found: C, 43.80; H, 3.55.

Dimethyl N-(3-Bromo-4,5,6,7-tetrahydroisobenzofuroyl)-L-glutamate (8b). Acid **7b** (89 mg, 0.36 mmol) was dissolved in 10 mL of THF, and NMM (47 μL, 0.43 mmol) was added neat, via syringe. After stirring the solution for 10 min, 2-chloro-4,6-dimethoxy-1,3,5-triazine (69 mg, 0.40 mmol) was added in one portion, and the resulting mixture was allowed to stir for 40 min as a fine white precipitate deposited. Additional NMM (47 μL, 0.43 mmol) and L-glutamic acid dimethyl ester hydrochloride (91 mg, 0.43 mmol) were added sequentially, and after 2 h the reaction mixture was partitioned between EtOAc (15 mL) and water (15 mL). The organic phase was dried over $MgSO_4$, filtered, and evaporated *in vacuo* to give a clear oil. Purification by radial chromatography (2 mm plate), using 25% EtOAc in hexanes as eluent, provided 120 mg (83%) of a clear, colorless oil which darkened upon prolonged exposure to air. ^{1}H NMR ($CDCl_3$, 270 MHz) δ 1.47 (m, 4 H), 1.81−2.26 (m, 6 H), 2.60 (m, 2 H), 3.44 (s, 3 H), 3.54 (s, 3 H), 4.53 (dt, 1 H, J = 4.9, 8.1 Hz), 6.70 (d, 1 H, J = 8.1 Hz); ^{13}C NMR ($CDCl_3$, 75.6 MHz) δ 20.6, 22.1, 22.2, 22.3, 27.7, 30.3, 51.1, 51.8, 52.6, 120.5, 124.4, 132.0, 142.1, 158.3, 172.2, 173.1; IR (NaCl) 1731, 1647, 1541 cm^{-1}; MS *m/e* (relative intensity) 403 (8), 401 (8); Anal. Calcd for $C_{16}H_{20}BrNO_6$: C, 47.78; H, 5.01; N, 3.48. Found: C, 47.36; H, 4.86; N, 3.05.

Dimethyl *N*-[3-[[2-(Pivaloylamino)-4-oxo-3,4-dihydropyrido[2,3-*d*]pyrimidin-6-yl]ethynyl]-4,5,6,7-tetrahydroisobenzofuroyl]-L-glutamate (10b). A mixture of **9** (270 mg, 1.0 mmol), $Pd(OAc)_2$ (4.5 mg, 4 mol %), tri-*o*-tolylphosphine (12 mg, 8 mol%), Et_3N (103 mg, 1.0 mmol), and **8b** (205 mg, 0.51 mmol) in 10 mL of MeCN was heated at reflux under an argon atmosphere for 24 h. After cooling to rt, the solvent was removed *in vacuo*, and the resulting black residue was passed through a short column of silica gel using 5% MeOH in CH_2Cl_2 as eluent. Further purification by radial chromatography on a 2 mm plate using 80% EtOAc in hexanes as eluent afforded the coupled product as a pale yellow solid (195 mg, 65%) which was used directly in the next step. ^{1}H NMR ($CDCl_3$, 270 MHz) δ 1.33 (s, 9 H), 1.76 (m, 4 H), 2.24 (m, 2 H), 2.47 (m, 2 H), 2.76 (m, 2 H), 2.95 (m, 2 H), 3.65 (s, 3 H), 3.77 (s, 3 H), 4.76 (dt, 1 H, J = 4.9, 8.2 Hz), 6.75 (d, 1 H, J = 7.9 Hz), 8.53 (d, 1 H, J = 2.3 Hz), 8.56 (br s, 1 H), 8.87 (s, 1 H), 12.12 (br s, 1 H).

Dimethyl *N*-[3-[2-[2-(Pivaloylamino)-4-oxo-3,4,5,6,7,8-hexahydropyrido[2,3-*d*]pyrimidin-6-yl]ethyl]-4,5,6,7-tetrahydroisobenzofuroyl]-L-glutamate (11b). A mixture of **10b** (195 mg, 0.33 mmol) and PtO_2 (4 mg) in 25 mL of MeOH and 2 mL of CH_2Cl_2 was hydrogenated at 48 psi for 24 h. The mixture was filtered through Celite with the aid of additional MeOH, and the filtrate was concentrated *in vacuo* to afford a pale yellow solid. Purification by radial chromatography (2 mm plate), using 5% MeOH in CH_2Cl_2 as eluent, afforded a white solid (188 mg, 95%) .^{1}H NMR ($CDCl_3$, 270 MHz) δ 1.27 (s, 9 H), 1.66 (m, 4 H), 1.62−1.80 (m, 2 H), 1.92 (m, 1 H), 2.11 (m, 2 H), 2.28 (m, 1 H), 2.39 (m, 2 H), 2.45 (m, 2 H), 2.62−2.77 (m, 2 H), 2.79 (m, 3 H), 2.97 (t, 1 H, J = 10.2 Hz), 3.35 (m, 1 H), 3.63 (s, 3 H), 3.75 (s, 3 H), 4.76 (dt, 1 H, J = 5.1, 8.0 Hz), 4.84 (s, 1 H), 6.70 (d, 1 H, J = 8.0 Hz), 8.09 (s, 1 H), 11.27 (s, 1 H); HRMS calcd for $C_{30}H_{41}N_5O_8$: 599.2954. Found: 599.2933.

***N*-[3-[2-(2-Amino-4-oxo-3,4,5,6,7,8-hexahydropyrido-[2,3-*d*]pyrimidin-6-yl)ethyl]-4,5,6,7-tetrahydroisobenzofuroyl]-L-glutamic Acid (12b).** A suspension of **11b** (35 mg, 58 μmol) in 1 mL of 1 N NaOH was stirred at rt for 48 h. Acidification with 3 M HCl gave a pale yellow solid which was collected and dried (17 mg, 61%). ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.65 (m, 6 H), 2.00 (m, 3 H), 2.34 (m, 2 H), 2.46 (m, 2 H), 2.72−2.87 (m, 7 H), 3.25 (m, 1 H, partially obscured by residual water peak), 4.35 (dt, 1 H, J = 5.0, 8.0 Hz), 5.99 (s, 1 H), 6.33 (br s, 2 H), 7.92 (d, 1H, J = 8.1 Hz), 9.71 (br s,1 H), 12.55 (br s, 1 H). HRMS (FAB) calcd for $C_{23}H_{30}N_5O_7$: 488.2145 (MH^+). Found: 488.2140.

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Supporting Information Available: ^{1}H NMR spectra for compounds **10a**, **11a**, **11b**, **12a**, and **12b** (5 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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