Protein Structure-Based Design, Synthesis, and Biological Evaluation of 5-Thia-2,6-diamino-4(3*H*)-oxopyrimidines: Potent Inhibitors of Glycinamide Ribonucleotide Transformylase with Potent Cell Growth Inhibition

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The design, synthesis, biochemical, and biological evaluation of a novel series of 5-thia-2,6-diamino-4(3*H*)-oxopyrimidine inhibitors of glycinamide ribonucleotide transformylase (GART) are described. The compounds were designed using the X-ray crystal structure of human GART. The monocyclic 5-thiapyrimidinones were synthesized by coupling an alkyl thiol with 5-bromo-2,6-diamino-4(3*H*)-pyrimidinone, **20**. The bicyclic compounds were prepared in both racemic and diastereomerically pure forms using two distinct synthetic routes. The compounds were found to have human GART K_is ranging from 30 μM to 2 nM. The compounds inhibited the growth of both L1210 and CCRF-CEM cells in culture with potencies down to the low nanomolar range and were found to be selective for the de novo purine biosynthesis pathway. The most potent inhibitors had 2,5-disubstituted thiophene rings attached to the glutamate moiety. Placement of a methyl substituent at the 4-position of the thiophene ring to give compounds **10**, **18**, and **19** resulted in inhibitors with significantly decreased mFBP affinity.

Introduction

The design of small-molecule antagonists of folic acid, acting at a number of critical folate binding enzymes, has been an active area of drug discovery research for over 50 years and has resulted in the development of a number of clinically useful anticancer agents.[1] Only relatively recently however have the two folate-utilizing enzymes in the de novo purine biosynthesis pathway, glycinamide ribonucleotide transformylase (GART, EC 2.1.2.2) and aminoimidazole carboxamide ribonucleotide transformylase (AICART, EC.2.1.2.3), been recognized as useful targets in the search for new, potent, and selective antitumor agents.[1b]

The first of the folate dependent enzymes in the pathway, GART, catalyzes the formylation of the glycine nitrogen of glycinamide ribonucleotide **1** using *N*-10-formyltetrahydrofolic acid **2** as the cofactor.[2] Validation of GART as a legitimate anticancer target came in the mid 1980s with the discovery of the first potent and selective inhibitor, 5,10-dideaza-5,6,7,8-tetrahydrofolic acid (DDATHF, **3a**).[3] The C-6 *R* isomer of DDATHF **3b**,[4] referred to as Lometrexol, eventually entered clinical trials and demonstrated antitumor activity against a wide range of solid tumors.[5] Additional investigations have examined many of the structure−activity relationships of the DDATHF core.[6] The most potent compound reported to date in this class is the thiophene derivative **3c**.[7] In addition to the bicyclic pyrimidinones **3a**−**c**, a family of monocyclic pyrimidinones represented by the structures **4a**,**b** have also been reported to have selective GART inhibition.[8]

The antitumor activity of the previous GART inhibitors, and for that matter most glutamate containing antifolates, has been shown to be dependent upon three properties: enzyme inhibition, active cellular transport, and polyglutamation.[9] Previously, however, no structural information regarding the protein or the binding modes of the inhibitors was available to guide structure−activity studies. We, along with other investigators,[10] have recently reported the solution of the X-ray crystal structure of the *Escherichia coli* GART in both its apo and complexed form, and in addition, we have reported the cloning and expression of an active soluble form of the human enzyme.[11] Herein, we report the use of the X-ray crystal structure of both the *E. coli* and the human GART enzymes, complexed with a number of inhibitors, in the design of a class of 5-thiapyrimidinone inhibitors and the synthesis and biological evaluation of these inhibitors as antitumor agents.

Design

The starting point for our design effort was the X-ray crystal structure of *E. coli* GART complexed with the potent inhibitor 5-deaza-5,6,7,8-tetrahydrofolic acid (5dTHF).[10a] Analysis of the *E. coli* structure reveals that there are 20 residues that contribute to the folate binding pocket (this analysis excluded the glutamate binding region since this is at the solvent interface and all of the designs were expected to contain the glutamate moiety), and of these, 12 use at least part of their side chain to form the binding site. An overlap of the sequences of the human and the *E. coli* enzymes in this active site region revealed that all of the side chains that contribute to folate binding are identical in both enzymes except for the conservative change of residue 143 (*E. coli* numbering) from a Leu in *E. coli* to a Val in human, thus lending credence to the usefulness of the *E. coli* structure as a surrogate for the human enzyme in the initial designs.

Initial design work focused on the region of the active site surrounding the methylene group at the 5-position of 5dTHF. An overlap of the van der Waals surfaces of the inhibitor and the protein showed that this area of the active site is hydrophobic and is not completely filled by the CH_2 group. It was felt that a somewhat bulkier sulfur atom might better fill the available space. In addition, introducing the sulfur atom onto the pyrimi-

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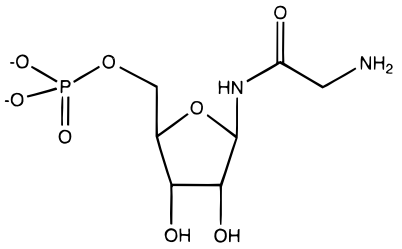
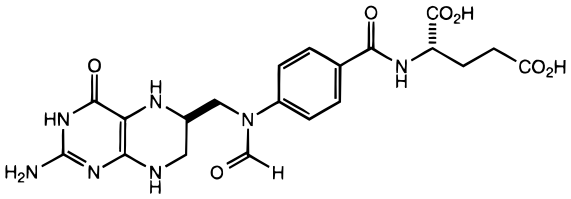


1



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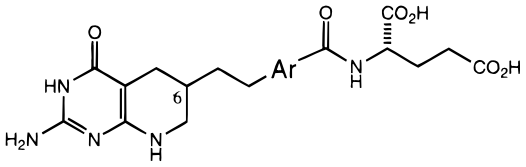
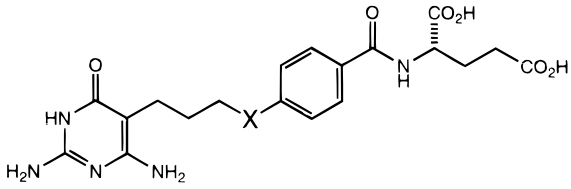


3a, C-6=R,S; Ar=1,4-Phenylene
3b, C-6=R; Ar=1,4-Phenylene
3c, C-6=R; Ar=2,5-Thienyl



4a, X=NH
4b, X=CH_2

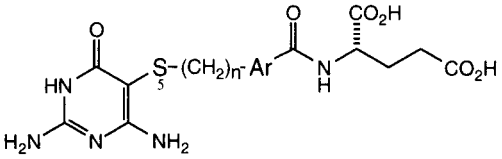
dinone ring might confer some synthetic advantage over the methylene group. A search of the chemical literature revealed that no glutamate-containing 5-thia antifolates had been reported.[12] To test this design hypothesis, the monocyclic 5-thiapyrimidinones **5**−**8** (see Table 1) were prepared and tested for human GART inhibition.[13] Compounds **5** and **7** (with the four atom linker) showed good GART inhibition (confirming that the sulfur was a suitable substitute for the CH_2), while the compounds **6** and **8** (with one less linker atom) were significantly less active.

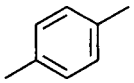
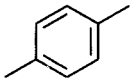
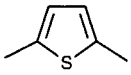
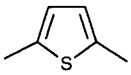
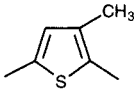
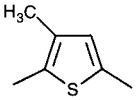
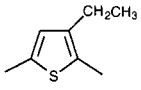
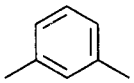
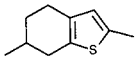
Crystals of human GART were grown in the presence of the substrate GAR **1** and compound **7**, and the X-ray structure of this complex was solved by the molecular replacement technique[14] using the coordinates of the previously solved *E. coli* enzyme[10a] and is shown in Figure 1. The structure shows that from the standpoint of the protein active site, the human enzyme is essentially identical to that of *E. coli*. In addition, with the exception of the additional water molecules in the human structure due to higher resolution and more extensive refinement, all of the hydrophilic interactions with the pyrimidine portion of **7** are identical to those made to 5dTHF in the *E. coli* structure.[10a] The only difference in the hydrophilic interactions between protein and ligand are seen at the protein solvent interface where the glutamate moiety is located. In contrast to the *E. coli* case, in the complex of **7** with the human enzyme, there is a pair of hydrogen bonds between the two oxygens of the α-carboxylate of the glutamate and one of the guanidine NH groups of the Arg 64 side chain and the backbone NH of Ile 91. All of the hydrophobic residues that make up the active site in the human protein are in essentially the same positions to that seen in *E. coli*.

After a detailed analysis of this structure, we drew the following conclusions: (1) Given that all of the available hydrogen-bonding groups on the pyrimidinone portion of the molecule are complemented by the protein, making changes in this region would likely not be productive, (2) since the α-carboxylate of the glutamate moiety appears to make a set of strong hydrogen bonds with the protein, and since the glutamate is critical for membrane transport and intracellular activity due to polyglutamation, making changes in this region would also not likely be productive, and (3) the most striking aspect of the structure is that the atoms of the inhibitor that link the sulfur at the 5-position of the pyrimidinone to the glutamate portion are flexible and do not optimally fill the available space. As a result, all of the designed derivatives were intended to investigate ways to more efficiently fill this region of the active site and to reduce the number of conformations each inhibitor has available.

As can be seen in Figure 1, there are hydrophobic residues Val 143 and Met 89 that make up the opening of the active site on both sides of the thiophene ring. It was felt that small hydrophobic substituents (such as methyl or ethyl) could be placed at either the 3- or the 4-position of the thiophene ring that could make favorable interactions with these residues. A methyl and an ethyl group were placed at the 3-position to give compounds **9** and **11**, and these two compounds showed small but measurable increases in GART inhibition. Compound **10**, with a methyl at the 4-position, was equipotent with the parent compound **7**. In an attempt to fill more of the space and to restrict the conformational flexibility of the linker, compound **13** was designed. As a result of the fact that compound **13** has a new chiral center, and that the S-CH_2 substituent can be situated in either a pseudoaxial or equatorial orientation, models of the four possible binding isomers were built and evaluated in the active site of the protein. The modeling study indicated that only two of the four isomers could fit into the active site without extensive overlap with the protein. Molecular mechanics calculations were performed in which these two isomers were minimized in the protein in order to try to predict the likely binding mode.[14] The calculations indicated that the energy of these two binding modes were similar with the pseudoaxial isomer being lower by 1−2 kcal/mol. Compound **13** was synthesized, and it was found to be equipotent with the parent compound **7** in terms of GART inhibition. The X-ray crystal structure of human GART, as a ternary complex with GAR **1** and compound **13**, was solved and is shown in Figure 2. Compound **13** is bound with the pyrimidinone and the α-carboxylate essentially overlapping the compound **7** structure, and the substituent on the 6-membered ring is in a

Table 1. Structure–Activity Data of Monocyclic 5-Thiapyrimidinones



no.	n	Ar	GARFT K_i, μM[a]	IC_{50}, μM L1210	IC_{50}, μM CCRF-CEM	IC_{50}, μM L1210-CI92	Hx Shift	FPGS Vm/Km	mFBP K_d, nM
5	3		0.038	0.105	0.045	5.0	>48	0.048	ND
6	2		30	1.6	0.66	12	>20	0.026	ND
7	3		0.035	0.031	0.032	18	>49	0.371	0.153
8	2		2	0.20	1.39	24	111	0.023	83
9	3		0.0085	0.25	0.048	45	234	0.089	0.38
10	3		0.032	0.075	0.061	43	>215	0.178	136
11	3		0.014	0.44	0.29	70	>543	Not a Substrate	ND
12	3		0.825	15.0	12	250	>73	NT	ND
13	1		0.030	0.036	0.010	16	13	0.152	0.57

[a]See experimental section for a detailed description of the assays.

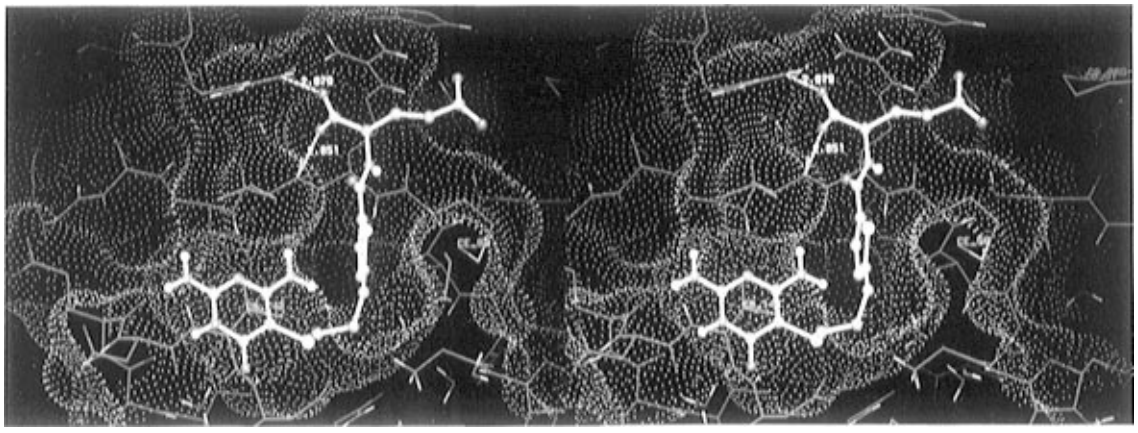


Figure 1. Stereodrawing showing the X-ray structure of human GART complexed with GAR **1** and compound **7**. The linker and the thiophene ring are shown exiting the active site flanked by the Val 143 side chain on the left and the Met 89 side chain on the right. The α-carboxyl of the glutamate moiety is shown hydrogen bonding to the Arg 64 side chain.

pseudoequatorial orientation. The bound isomer is one of the isomers that by modeling looked like it would have extensive overlap with the protein, and careful analysis shows that in this crystal structure, in order to relieve this unfavorable interaction, the ligand has pushed the side chain of Val 143 and its local peptide backbone back by as much as 1 Å from the position seen in the compound **7** structure. It would appear that the energy cost of moving this backbone segment is compensated by a more favorable hydrophobic interaction. In our experience, this type of large change in protein structure is difficult to anticipate and underscores the value of iterative structural information.

The final set of designed compounds were the bicyclic 5-thiapyrimidinones **14**–**19**. These were prepared to test the concept of ligand preorganization as with

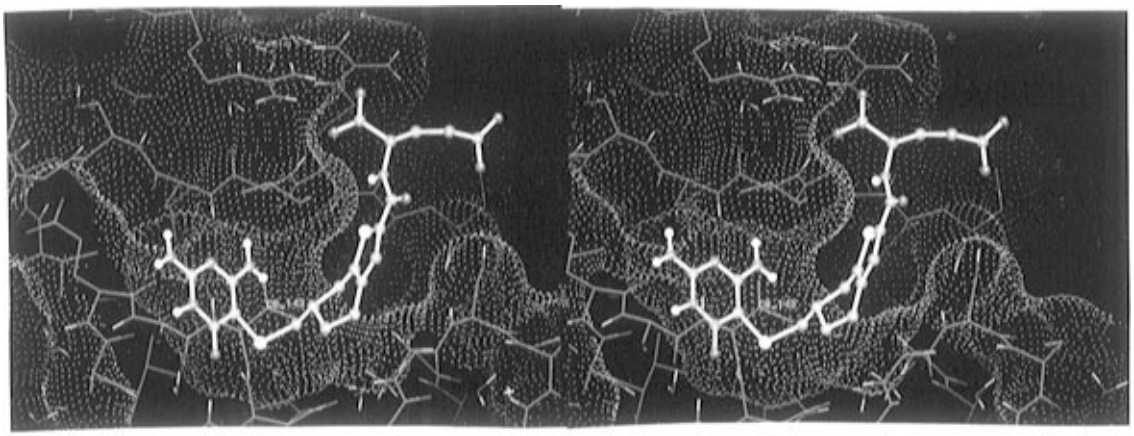


Figure 2. Stereodrawing showing the X-ray structure of human GART complexed with GAR **1** and compound **13**. The linker attached to the six-membered ring of the tetrahydrobenzthiophene is in an equatorial orientation. The Val 143 side chain has shifted to the left roughly 1 Å from that seen in the structure of compound **7**.

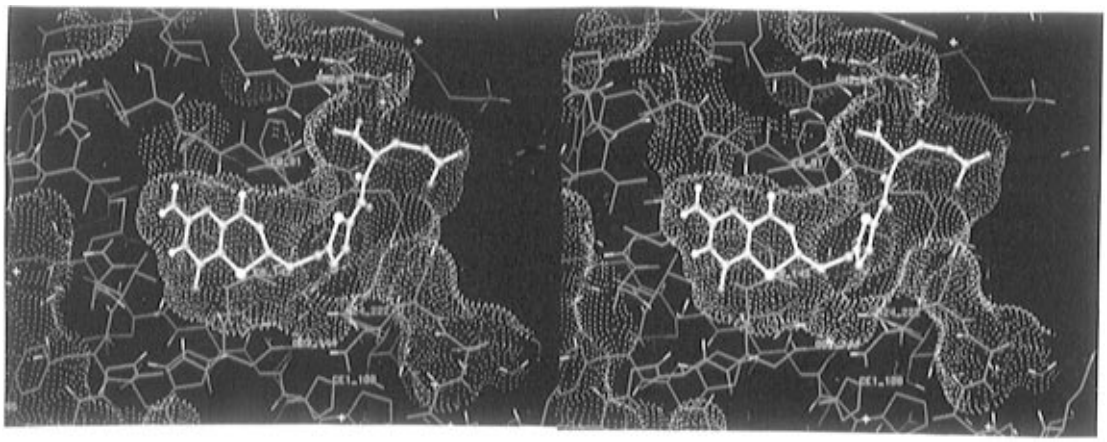
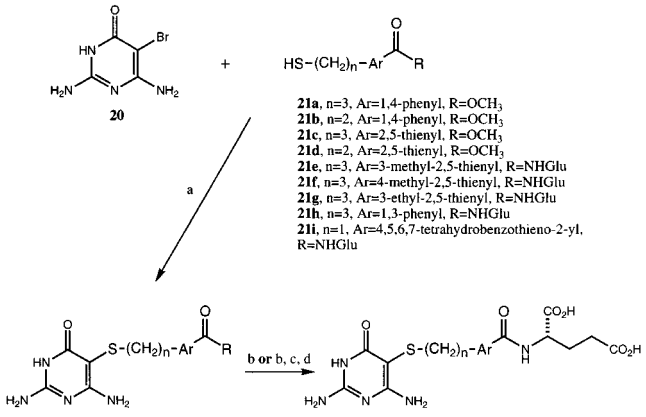


Figure 3. Stereodrawing showing the X-ray structure of human GART complexed with GAR **1** and compound **17**. The linker attached to the six-membered ring of the pyrimidothiazine is in an equatorial orientation. The substrate GAR **1** (residue 222) is on the lower right.

compound **13**. In addition, in previous GART inhibitor series,[3−8] the bicyclic compounds, as a rule, showed increased activity over the monocyclic pyrimidinones. The 2,5-thienyl compounds **16**−**19** showed tighter binding than did the 1,4-phenylene **14**. We believe this is due, at least in part, to the better orientation of the glutamate toward Arg 64 and Ile 91, and this is consistent with the 1,3-phenylene **15** having superior inhibition. The X-ray crystal structure of human GART, as a ternary complex with GAR **1** and compound **17**, was solved and is shown in Figure 3. The most significant observation from this structure is that the orientation of the C-6 *S* linker (which is analogous to the natural configuration) is equatorial. This is consistent with the lower GART inhibition of this compound when compared to the C-6 *R* isomer **16** because the methylene groups of the linker can no longer make a favorable interaction with the side chain of Val 143. In addition, because the linker now runs along the bottom portion of the active site, where the catalytic Asp 144 and His 108 residues reside, the nitrogen of the substrate GAR (residue 222) has been rotated from the previous structures by roughly 90°.[10a] This is presumably to avoid a steric clash, but the energetic consequences of this movement are unknown. Finally, as can be seen with compounds **18** and **19**, placement of a methyl group at the 4-position of the thiophene ring conferred no increased GART inhibition.

Scheme 1.[a] General Synthesis of Monocyclic 5-Thiapyrimidinones

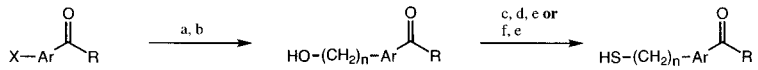


[a] (a) DIEA, DMF, Δ; (b) NaOH; (c) L-glutamate diethyl ester, coupling agent; (d) NaOH.

Chemistry

Shown in Scheme 1 is the general strategy for the synthesis of the monocyclic 5-thiapyrimidinones **5**−**13**.

Scheme 2.[a] General Synthesis of Thiols **21a**−**b**



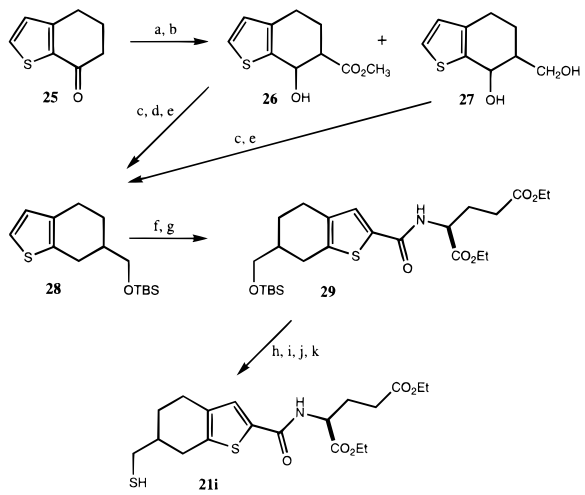
23a, X=I, Ar=1,4-phenyl, R=OCH_3
23b, X=Br, Ar=2,5-thienyl, R=OCH_3
23c, X=Br, Ar=3-methyl-2,5-thienyl, R=NHGlu
23d, X=Br, Ar=4-methyl-2,5-thienyl, R=NHGlu
23e, X=Br, Ar=3-ethyl-2,5-thienyl, R=NHGlu
23f, X=I, Ar=1,3-phenyl, R=NHGlu

24a, n=3, Ar=1,4-phenyl, R=OCH_3
24b, n=3, Ar=2,5-thienyl, R=OCH_3
24c, n=2, Ar=2,5-thienyl, R=OCH_3
24d, n=3, Ar=3-methyl-2,5-thienyl, R=NHGlu
24e, n=3, Ar=4-methyl-2,5-thienyl, R=NHGlu
24f, n=3, Ar=3-ethyl-2,5-thienyl, R=NHGlu
24g, n=3, Ar=1,3-phenyl, R=NHGlu

21a, n=3, Ar=1,4-phenyl, R=OCH_3
21b, n=2, Ar=1,4-phenyl, R=OCH_3
21c, n=3, Ar=2,5-thienyl, R=OCH_3
21d, n=2, Ar=2,5-thienyl, R=OCH_3
21e, n=3, Ar=3-methyl-2,5-thienyl, R=NHGlu
21f, n=3, Ar=4-methyl-2,5-thienyl, R=NHGlu
21g, n=3, Ar=3-ethyl-2,5-thienyl, R=NHGlu
21h, n=3, Ar=1,3-phenyl, R=NHGlu

[a] (a) Propargyl alcohol, $(Ph_3P)_2PdCl_2$, CuI, Et_2NH; (b) H_2, Pd/C, EtOH; (c) Ph_3P, CBr_4, CH_2Cl_2; (d) KSAc, acetone, Δ; (e) 1 N HCl, MeOH; (f) Ph_3P, DEAD, HSAc, THF.

Scheme 3.[a] Synthesis of Thiol **21i**



[a] (a) NaH, $(CH_3O)_2CO$, DMF; (b) $NaBH_4$, THF, MeOH; (c) Et_3SiH, $BF_3{\cdot}Et_2O$, CH_2Cl_2; (d) LAH, THF; (e) TBSCl, TEA, CH_2Cl_2; (f) BuLi, THF, CO_2; (g) L-glutamate diethyl ester, EDC, HOBT, DIEA, DMF; (h) TBAF, THF; (i) CBr_4, Ph_3P, CH_2Cl_2; (j) KSAc, acetone, Δ; (k) 0.5 N HCl, EtOH.

The key reaction in the sequence is the base-initiated coupling of the alkyl thiols **21a**−**i** with the 5-bromo-2,6-diamino-4(3*H*)-pyrimidinone (**20**)[15] to give the thioethers **22a**−**i**. The reaction proceeds under relatively mild conditions in yields ranging from 67% with the simple thiols like **21d** to 19% for the more complex thiol **21i** and is compatible with both the aromatic ester functionality and the diethyl glutamate. Displacements of this type have only been reported previously with aromatic thiols.[16]

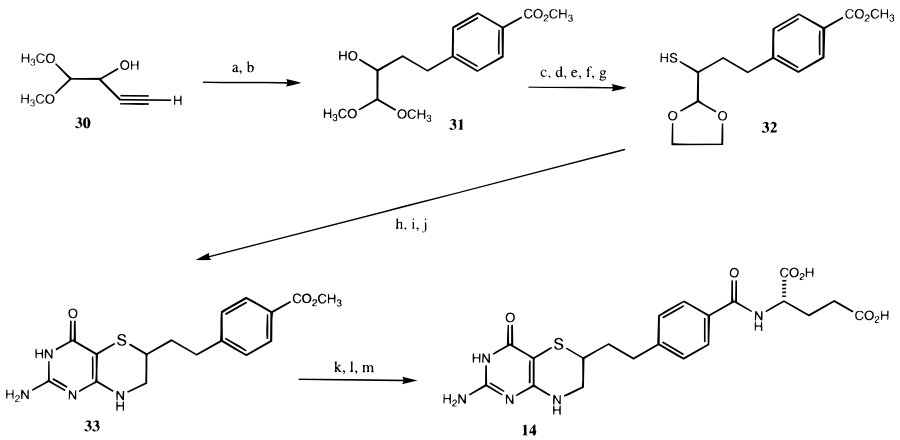
The thiols **21a**−**h** were prepared as shown in Scheme 2. The intermediate alcohols either were prepared by coupling of propargyl alcohol with an aromatic halide[8a] followed by reduction or were purchased. The thiols were generated from the intermediate thioacetates by acid hydrolysis. The thioacetates were prepared by either displacing the corresponding bromides or directly using thioacetic acid under Mitsunobu conditions.[17]

The synthesis of the thiol **21i** is shown in Scheme 3. The synthesis begins with the known ketone **25**[18] and proceeds by one-carbon homologation and deoxygenation. Once the alcohol was protected as a *tert*-butyldimethylsilyl ether, the carboxyl group was introduced by selective lithiation of the 5-position of the thiophene. Standard peptide coupling followed by introduction of the thiol went without event.

Two distinct synthetic strategies were developed to prepare the bicyclic 5-thiapyrimidinones **14**−**19**.[12] The first route, shown in Scheme 4, utilized the coupling of thiol **32** with the bromopyrimidinone **20** as the key reaction to prepare compound **14** as a mixture of diastereomers.[19] The synthesis began with the coupling of the lithium anion of TMS acetylene with glyoxal dimethyl acetal followed by the palladium-catalyzed coupling of the resulting terminal acetylene with methyl 4-iodobenzoate.[8a] Subsequent reduction of the alkyne gave compound **31**. Initially, we felt that conversion of the secondary alcohol to a small leaving group would allow for introduction of the sulfur atom by simple displacement, and as a result, the mesylate of compound **31** was prepared. In the event, we were not able to find any nucleophile that would displace the mesylate group even under forcing conditions. One possible explanation for this unexpected result is the proximity of the two adjacent oxygen atoms of the dimethyl acetal which precludes any positive charge from building up during the transition state making the activation barrier too high. The solution to the problem was to hydrolyze the acetal to the aldehyde, displace the mesylate with a protected sulfur atom, and reintroduce the acetal in the form of a dioxolane group. Removal of the sulfur protecting group[20] provided the thiol **32**. Base-initiated coupling followed by removal of the acetal under acidic conditions provided the aldehyde which spontaneously cyclized to a diastereomeric mixture of hemiaminals.[8b] The reduction of the hemiaminal to give the thiazine ring containing compound **33** proved difficult. Eventually it was found that sodium cyanoborohydride in the presence of $BF_3{\cdot}Et_2O$ could be used successfully.[21] The synthesis was completed as described for the earlier compounds.

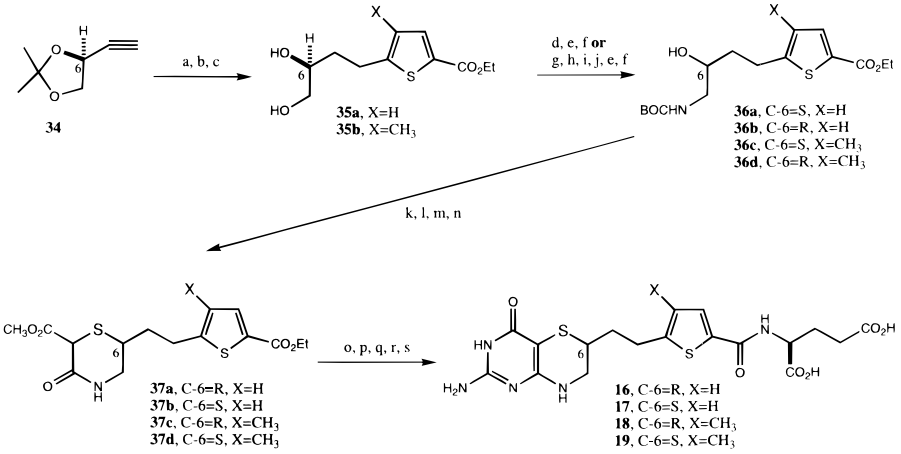
An alternate synthetic route, shown in Scheme 5, was used to prepare the optically pure thiophene derivatives **16**−**19**. This strategy, which is based in part on work described for 5-thiapterin ring systems by Benkovic,[22] has as the key reaction the guanidine cyclization of the lactams **37a**−**d** to produce the bicyclic pyrimidothiazine ring system. The chiral centers for both the C-6(*R*) and C-6(*S*) isomers (using folic acid numbering) were obtained from the known optically pure acetylene **34**[23] prepared from D-(*R*)-glyceraldehyde acetonide.[24] The synthesis began with the palladium-catalyzed coupling of the optically pure form of the acetylene **34** with both 2-bromothiophene-5-carboxylic acid ethyl ester[8c] and the 3-methyl derivative[25] followed by reduction and acid hydrolysis to afford the diol intermediates **35a**,**b**. For the C-6(*R*) compounds, the nitrogen atom at the 8-position was introduced by monotosylation of the primary alcohol followed by displacement with sodium azide. For the C-6(*S*) compounds, the hydroxy group was inverted by protecting the primary alcohol as a silyl ether, mesylating the secondary alcohol, removing the protecting group, and forming the inverted epoxide with

Scheme 4.[a] Synthesis of Pyrimidothiazine **14**



[a] (a) Methyl 4-iodobenzoate, $(Ph_3P)_2PdCl_2$, CuI, Et_2NH; (b) H_2, 5% Pd/C, EtOH; (c) MsCl, Et_3N, CH_2Cl_2; (d) TFA, H_2O, $CHCl_3$; (e) (4-methoxyphenyl)methanethiol, DIEA, DMF; (f) ethylene glycol, PPTS; (g) $Hg(OAc)_2$, CH_2Cl_2, TFA; (h) **20**, DIEA, DMF, Δ; (i) 2 N HCl, THF, H_2O; (j) $BF_3{\cdot}Et_2O$, $NaBH_3CN$, THF; (k) NaOH; (l) L-glutamate diethyl ester, EDC, HOBT, DIEA, DMF; (m) NaOH.

Scheme 5.[a] Synthesis of Pyrimidothiazines **16**−**19**



[a] (a) Bromothiophene ethyl ester, $(Ph_3P)_2PdCl_2$, CuI, Et_3N, CH_3CN; (b) H_2, 5% Pd/C, EtOH; (c) TsOH, EtOH; (d) TsCl, Et_3N, CH_2Cl_2; (e) NaN_3, DMF or CH_3CN, Δ; (f) $(BOC)_2O$, H_2, 5% Pd/C, THF; (g) TBSCl, Et_3N, CH_2Cl_2; (h) MsCl, Et_3N, CH_2Cl_2; (i) TBAF, THF; (j) NaH, THF; (k) MsCl, Et_3N, CH_2Cl_2; (l) KSAc, acetone; (m) $(CH_3OCO)_2CHCl$, K_2CO_3, MeOH; (n) TFA, CH_2Cl_2; (o) $(CH_3)_3O{\cdot}BF_4$, CH_2Cl_2; (p) guanidine hydrochloride, NaOEt, EtOH; (q) NaOH; (r) L-glutamate diethyl ester, EDC, HOBT, DIEA, DMF; (s) NaOH.

sodium hydride. The epoxide was opened selectively at the less hindered primary position with sodium azide.[26] The azido alcohols were reduced in the presence of BOC anhydride to give the alcohols **36a**−**d**. The sulfur atom at the 5-position was introduced by the displacement of the corresponding mesylate with KSAc.[27] The acetyl protecting group was removed under basic conditions in the presence of dimethyl chloromalonate, and once the BOC protecting group was removed, the free amine spontaneously cyclized to the lactams **37a**−**d**. The lactam was alkylated with trimethyloxonium tetrafluoroborate, and the resultant lactim ether was treated with 3 equiv of free-base guanidine in refluxing ethanol to give the 2-amino-4(3*H*)-oxopyrimido[5,4-*b*][1,4]-thiazines. Hydrolysis of the ethyl esters, peptide coupling, and hydrolysis completed the synthesis of the glutamic acids **16**−**19**.

Biochemistry and Biology

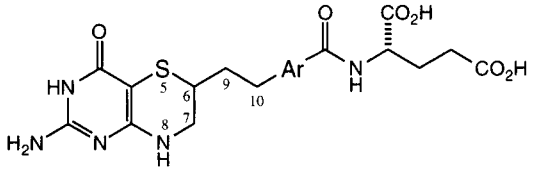
The compounds shown in Tables 1 and 2 were all evaluated for their inhibition of recombinant human GART using N^{10}-formyl-5,8-dideazafolate (FDDF) as the cofactor and were found to be competitive with respect to FDDF.[28] Results are reported as K_i values (in μM).[29]

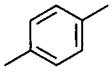
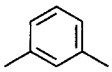
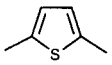
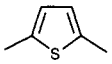
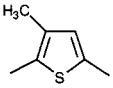
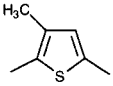
The compounds were evaluated for cell growth inhibition against three cell lines: L1210 murine leukemia;[30] CCRF-CEM,[30] a human lymphoblastic leukemia line of T-cell origin; and L1210/CI920, a cell line that was developed for resistance to Fostriecin which lacks the reduced folate carrier function.[31] Results are reported as IC_{50} values (in μM). As a measure of the selectivity of the compounds for the de novo purine biosynthesis pathway, cell growth inhibition protection experiments were performed with and without the addition of 100 μM hypoxanthine. The results are reported as the ratio of the IC_{50} with 100 μM hypoxanthine to the IC_{50} without hypoxanthine added. Larger numbers are indicative of increased specificity.

The ability of the inhibitors to serve as substrates for folyl-polyglutamate synthetase (FPGS) was measured using the enzyme prepared from rat liver.[35] The results are reported as the ratio of the velocity V_m divided by the K_m of the test compound. Larger numbers indicate that the compounds are better substrates.

Selected compounds were also measured for their ability to function as transport substrates for the human folate-binding protein (mFBP). The assay was a competitive binding assay in which membrane-associated mFBP was prepared from cultured KB cells[38] and the

Table 2. Structure−Activity Data of Bicyclic 5-Thiapyrimidinones



no.	C-6	Ar	GARFT K_i, μM[a]	IC_{50}, μM L1210	IC_{50}, μM CCRF-CEM	IC_{50}, μM L1210CI92	Hx Shift	FPGS Vm/Km	mFBP K_d, nM
14	R,S		0.035	0.048	0.029	3.2	41	0.075	0.002
15	R,S		0.0034	2.8	1.8	23.0	>86	Not a Substrate	ND
16	R		0.003	0.12	0.009	2.82	>1593	0.295	0.0005
17	S		0.028	0.004	0.0029	1.4	>67,585	0.150	0.0032
18	R		0.002	0.636	0.008	24.8	>213	Not a Substrate	0.95
19	S		0.015	0.055	0.010	57	>2757	0.142	0.69

[a]See experimental section for a detailed description of the assays.

individual compounds were allowed to compete against [^{3}H]folic acid. The results are reported as K_d values (in nM).

Discussion

The in vitro cell growth inhibition activity of these glutamate-containing GART inhibitors is largely dependent upon three measurable properties: GART enzyme inhibition, FPGS substrate activity, and the ability of the compounds to function as substrates for either one or both of folate transporters (the reduced folate transporter or the mFBP). In addition, although a more complicated situation to interpret, in vivo activity is also influenced by the same set of properties.[9]

As can be seen from Tables 1 and 2, the 5-thiapyrimidinones **5**−**19** have GART inhibition constants ranging from 30 μM to 2 nM. The bicyclic compounds **14**−**19** are, in general, more potent as enzyme inhibitors than are the monocyclic compounds **5**−**13**. We believe this is due in part to preorganization of the more rigid bicyclic compounds. Among the bicyclic compounds, the unnatural C-6(*R*) compounds **16** and **18** are 7−10 times more potent than the corresponding natural C-6(*S*) isomers **17** and **19**. This increased potency of the unnatural C-6(*R*) isomers is believed to occur because the linker connecting the thiazine ring to the aromatic thiophene ring on these compounds is oriented in a pseudoaxial conformation which positions the two methylene groups nicely against the side chain of Val 143. As shown in Figure 3, for the natural C-6(*S*) isomer compound **17**, the linker is in a pseudoequatorial conformation which does not allow this compound to make the same favorable interactions.

The compounds **5**−**19** were tested for their cell growth inhibition and were found to have IC_{50} values ranging from the low micromolar to the low nanomolar range. Again, as was the case with GART enzyme inhibition, the bicyclic compounds in Table 2 showed superior activity over the monocyclic compounds in Table 1. The one exception is compound **15** which showed poor cell growth inhibition. The most potent compound in the 5-thiapyrimidinone series is compound **17** with cell growth inhibition IC_{50}s of 4 nM against both L1210 and CCRF-CEM cell lines. As described below, the cell growth inhibition activity is a composite of a number factors.

Hypoxanthine shift experiments showed that the cell growth inhibition for all the compounds was due to a block in the de novo purine biosynthesis pathway. In particular, compounds **16**−**19** were very selective in this regard with protection ratios of over 1500.

Folyl polyglutamate synthetase (FPGS)[35] is the intracellular enzyme that extends the glutamate chain on monoglutamated cofactors by sequentially adding glutamate moieties to the γ-carboxylic acid as a mechanism for enhancing the binding to target enzymes and retaining the cofactors inside the cell. Many of the known folate analogues also function as substrates for this enzyme which serves to trap the inhibitors inside the cells and build them to high concentrations. As a result, we tested compounds **5**−**19** for their ability to serve as substrates for the readily available rat enzyme, and as can be seen from Tables 1 and 2, a number of the compounds are good substrates. In general, the compounds that are good substrates are also potent inhibitors of cell growth, whereas poor substrates like compounds **11**, **15**, and **18** tend to also have poor cell growth inhibition activity.

The relative activity of individual compounds **5**−**19** against the L1210/CI920 cell line versus L1210 is an indirect measure of the dependence on the reduced folate carrier. As evidenced by the observed poor cell growth inhibition against the L1210/CI920 cell line, all the compounds are dependent to some extent upon the

reduced folate carrier for in vitro activity. This is an important observation since a number of human tumors are known to have the reduced folate carrier.[39]

The membrane folate binding protein (mFBP) is a high-affinity low-flux transporter which has a preference for folic acid.[39] One of the unique properties of the benchmark GART inhibitor, DDATHF, is the fact that it is transported by both the reduced folate transporter and the mFBP into cells.[40] Investigators have shown that concurrent folic acid treatment in both low folate animal models and in humans, which is believed to block the mFBP, can attenuate the toxicity of DDATHF.[41] As a result, the compounds **5**−**19** were tested for their binding affinity to the mFBP. All of the compounds have at least moderate affinity for the mFBP, and compounds **14**, **16**, and **17** are very tight binders to the mFBP with K_d values in the picomolar range. In addition, a relationship between the substitution pattern on the aromatic ring and the mFBP affinity was discovered. That is, a simple replacement of the hydrogen at the 4-position of the thiophene ring with a methyl group decreases mFBP binding by a factor of 889, 1900, and 215 for the three pairs of compounds **7** and **10**, **16** and **18**, and **17** and **19**, respectively, without significantly affecting enzyme inhibition. In addition, compound **19** is still a good substrate for FPGS and retains much of the cell growth inhibition of the parent compound **17**.[42]

Conclusion

We have described a series of 5-thiapyrimidinone GART inhibitors that were designed using the X-ray crystal structure of human GART complexed with a number of inhibitors. The most potent compounds have inhibition constants in the low nanomolar range. A synthetic route to the monocyclic 5-thiapyrimidinone compounds **5**−**13** was developed in which the key reaction was the coupling of an alkyl thiol with 5-bromo-2,6-diamino-4(3*H*)-pyrimidinone (**20**). The bicyclic compounds **14**−**19** were prepared using two distinct synthetic routes which generated both the racemic products **14** and **15** and the diastereomerically pure compounds **16**−**19**. A number of the compounds were found to have cell growth IC_{50} values in the low nanomolar range, and all compounds, as evidenced by hypoxanthine protection, were found to selectively inhibit cell growth by blocking the de novo purine biosynthesis pathway. Placement of a methyl substituent at the 4-position of the thiophene ring of compounds **7**, **16**, and **17** gave inhibitors with significantly decreased mFBP affinity. From this series of 5-thiapyrimidinone GART inhibitors, compound **17** has shown excellent in vivo antitumor activity[43] and has been chosen for clinical evaluation.

Experimental Section

^{1}H NMR spectra were determined using a General Electric QE-300 spectrometer operating at a field strength of 300 MHz. Chemical shifts are reported in parts per million (δ) and setting the references such that in $CDCl_3$ the $CHCl_3$ is at 7.26 ppm and in DMSO-d_6 the DMSO is at 2.49 ppm. Standard and peak multiplicities are designated as follows: s, singlet; d, doublet; dd, doublet of doublets; t, triplet; brs, broad singlet; brd, broad doublet; br, broad signal; m, multiplet. Mass spectra were determined at the Scripps Reaseach Institute Mass Spectrometry Center. Infrared absorption spectra were taken on a MIDAC Corporation FTIR. Elemental microanalyses were performed by Atlantic Microlab Inc., Norcross, GA, or MHW Laboratories, Phoenix, AZ, and gave results for the elements stated with ±0.4% of the theoretical values. *N*,*N*-Dimethylformamide (DMF) was dried over activated (250 °C) 4-Å molecular sieves. Tetrahydrofuran (THF) was distilled from sodium benzophenone ketyl under nitrogen. Et_2O refers to diethyl ether, DIEA refers to diisopropylethylamine, TBSCl refers to *tert*-butyldimethylsilyl chloride, TEA refers to triethylamine, HOBT refers to 1-hydroxybenzotriazole hydrate, EDC refers to 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride and DMAP refers to 4-(dimethylamino)pyridine. Flash chromatography was performed using silica gel 60 (Merck Art 9385). Thin layer chomatograph (TLC) were performed on precoated sheets of silica 60 F_{254} (Merck Art 5719). Melting points were determined on a Mel-TeMp apparatus and are uncorrected. mFBP refers to human folate-binding protein; FPGS, folyl-polyglutamate synthetase; BSA, bovine serum albumin; FDDF, *N* ^{10}-formyl-5,8-dideazafolate; HEPES, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid; PMSF, phenylmethanesulfonyl fluoride; ϵ_{294}, extinction coeficient at 294 nm; K_i, inhibition constant; $K_{i,app}$, apparent inhibition constant; K_d, equilibrium disociation constant; K_m, Michaelis constant; V_{max}, maximal velocity.

Biochemical Assays. GART activity was measured using a modification of the method of Young.[28] Reaction mixtures contained the catalytic domain of the human GART,[11] 20 μM α,β-GAR, 10 or 20 μM FDDF, a variable concentration of GART inhibitor, 50 mM HEPES-KOH, pH 7.5, and 50 mM KCl. The reaction was initiated with the addition of enzyme and followed by monitoring the increase in absorbance at 294 nm at 20 °C ($\epsilon_{294} = 18.9\ mM^{-1}\ cm^{-1}$). GART inhibition constants (K_i) were determined from the dependence of the steady-state catalytic rate upon inhibitor and substrate concentration. The type of inhibition observed was determined to be competitive with respect to FDDF by the dependence of the apparent K_i ($K_{i,app}$) on the concentration of FDDF and was shown to be described by $K_{i,app} = K_i + (K_i/K_m)[FDDF]$. The Michaelis constant for FDDF, K_m, was determined independently by the dependence of the catalytic rate upon FDDF concentration and shown to be 0.6 μM. Data for both the K_m and K_i determinations were fitted by nonlinear methods to the Michaelis equation or the Michaelis equation for competitive inhibition as appropriate. Data resulting from tight-binding inhibition was analyzed, and K_i values were determined by fitting the data to the tight-binding equation of Morrison[29] by nonlinear methods.

The ability of GART inhibitors to serve as substrates for FPGS was measured using the enzyme prepared from rat liver. FPGS was partially purified from rat liver essentially by the method of Moran and Colman.[35] Sprague−Dawley rats were killed by CO_2 inhalation. The liver was removed and perfused though the portal vein with 20 mL of ice-cold 20 mM HEPES, pH 7.4; 250 mM sucrose; 50 mM 2-mercaptoethanol. The tissue was kept at 4 °C or on ice for the remainder of the procedure. Livers were rinsed in fresh perfusion buffer, weighed, and then blended in 2 times the wet-weight volume of fresh perfusion buffer containing 0.5 mM PMSF; 50 μg/mL soybean trypsin inhibitor; and 0.5 mM EDTA until smooth. The homogenate was filtered though cheesecloth and the filtrate centrifuged at 130000*g* for 60 min. The resulting supernatant was filtered through fine glass wool to remove suspended fats, and ATP was added to 2.5 mM. Protein was precipitated with 30% saturated ammonium sulfate and centrifuged at 30000*g* for 15 min. The resulting pellet was resuspended in a small volume of 20 mM Tris-Cl, pH 7.4, 50 mM 2-mercaptoethanol, 5 mM $MgCl_2$, 5 mM ATP, and 25 μg/mL soybean trypsin inhibitor and then centrifuged as before. Suspended fats were removed from the supernatant, and proteins were precipitated with 50% saturated ammonium sulfate and then centrifuged as before to obtain the pellet. Protein pellets precipitated in this manner were stable, when stored at −70 °C, for more than 4 months. Since FPGS is quite unstable in solution, a frozen FPGS pellet was resuspended for assay as described below and used immediately. The pellet was thawed and resuspended in a minimal amount of buffer (20 mM Tris-Cl, pH 7.4, 50 mM 2-mercaptoethanol, 5 mM $MgCl_2$, 5 mM ATP, and 25 μg/mL soybean trypsin inhibitor)

and centrifuged to remove residual solids. The supernatant was desalted by passage though a Sephadex G25 column equilibrated in 20 mM Tris, pH 7.4, 50 mM 2-mercaptoethanol, and 5 mM $MgCl_2$. Fractions containing protein were pooled and brought to 5 mM ATP. Protein concentrations were determined by the Bradford method with BSA as a protein standard.[36] Typical protein concentrations were 3 mg/mL. The typical assay mixture contained 0.7 mg of partially purified FPGS protein along with 200 mM Tris-Cl, pH 8.5, a variable concentration of GART inhibitor or folic acid, 5 mM ATP, 10 mM $MgCl_2$, 30 mM KCl, 50 mM 2-mercaptoethanol, 1 mM glutamic acid, and 2 μCi [^{3}H]glutamic acid in a volume of 0.5 mL. Reactions were initiated with the addition of enzyme and incubated for 60 min at 37 °C. The reaction was then quenched with 0.5 mL of charcoal solution and placed on ice to complete product absorption. (Preparation of the charcoal solution and the important purification of [^{3}H]glutamic acid was accomplished as described previously.[37]) The charcoal was separated from the reaction mixture by centrifugation and the resulting pellet was washed four times with 1 mL of 10 mM glutamate, pH 6.8, and 10 mM 2-mercaptoethanol by repeated resuspension and centrifugation. The products are finally eluted from the charcoal with 1 mL of ethanolic ammonia (3 M NH_4OH; 60% ethanol) by resuspension and centrifugation. The radioactivity in 1 mL of eluent supernatant containing the tritiated product was measured by scintillation counting after the addition of 1 mL of water. The kinetic parameters K_m and V_{max} were determined by nonlinear fitting of the substrate dependence of product formation to the Michaelis equation.

The dissociation constant (K_d) mFBP was determined for GART inhibitors with a competitive binding assay using membrane-associated FBP prepared from cultured KB cells.[38] Adherent KB cells were scraped from flasks, washed once in ice-cold PBS, and centrifuged at 5000*g* for 5 min at 4 °C. KB cell pellets were stored at −70 °C, and mFBP activity was stable for at least 6 months. Cell pellets containing approximately 2×10^8 cells were resuspended in 10 mL of suspension buffer (KH_2PO_4−KOH, pH 7.4:10 mM EDTA:10 mM 2-mercaptoethanol), sonicated briefly to complete cell lysis, and centrifuged at 12000*g* for 10 min at 4 °C. The pellet was stripped of endogenous bound folate by resuspension in 20 mL of acidic buffer (50 mM KH_2PO_4−KOH, pH 3.5:10 mM EDTA: 10 mM 2-mercaptoethanol) and centrifuged as before. The pellet was then resuspended in 20 mL of the suspension buffer at pH 7.4 and centrifuged as before. The pellet was resuspended in 5 mL of suspension buffer at pH 7.4 lacking EDTA. Protein content was quantitated using the Bradford method with BSA as a protein standard.[36] Typical yields for this procedure were 4−5 mg of total membrane protein per 2×10^8 cells. This final suspension was used as a source of membrane-associated human FBP. In this competitive binding assay, competitor ligand was allowed to compete against [^{3}H]folic acid for binding to mFBP. Reactions mixtures contained 50−100 μg of cell membrane protein containing 3−6 pmol (3−6 nM) of FBP, 17.25 pmol of [^{3}H]folic acid (17.25 nM, 0.5 μCi), and various concentrations of competitor ligand, in 1 mL of 50 mM KH_2PO_4−KOH, pH 7.4:10 mM 2-mercaptoethanol. Binding reactions were performed at 25 °C. Because of the rapid binding and very slow release of bound [^{3}H]folic acid, the competitor ligand was prebound for 30 min in the absence of [^{3}H]folic acid. [^{3}H]Folic acid was then added, and the mixtures were allowed to equilibrate for 2.5 h. The full reaction mixtures were drawn though nitrocellulose filters under vacuum to trap the cell membranes with bound [^{3}H]-folic acid. The trapped membranes were then washed four times with 1 mL of reaction buffer. The amount of bound [^{3}H]-folic acid was measured by scintillation counting of the nitrocellulose membrane. The data obtained were nonlinearly fitted as described above. The mFBP K_d for [^{3}H]folic acid, used to calculate the competitor K_d, was obtained by direct titration of mFBP with [^{3}H]folate and subsequent nonlinear fitting of the data to a tight-binding K_d equation and found to be 60 ± 14 pM.

Measurement of Tissue Culture IC_{50}'s. The L1210 and CCRF-CEM cell lines were obtained from the American Type Culture Collection. The L1210/CI920 cell line was developed for resistance to Fostriecin[31] and lacks reduced folate carrier function. L1210 cells were grown in RPMI-1640 medium[32] and supplemented with 5% dialyzed fetal calf serum.[33] The CEM and L1210/CI920 cell lines were grown in RPMI medium supplemented with 10% dialyzed fetal calf serum. All cultures were maintained at 37 °C, 5% air−CO_2 in a humidified incubator. Cell growth inhibition was measured by a modification of the method of Mosmann.[34] Mid-log phase cells of each cell line were diluted to 18 500 cells/mL in fresh RPMI-1640 growth medium supplemented with dialyzed fetal calf serum and then aliquoted into columns 2−12 of 96-well microtiter plates. Column 1 was filled with the same volume, 135 mL, of fresh medium, without cells, for use as a blank. The plates were then placed in a 37 °C, 5% air−CO_2 incubator. After 4 h, plates were removed from the incubator followed by addition of drug solution, 15 mL/well in binary dilutions, to columns 12 to 4. Wells containing test compound were prepared in quadruplicate on each plate. To the wells in columns 1 and 2 of the plates was added 15 mL of media, without test compound. Column 3 received 15 μL of drug diluent. The cells were then returned to the incubator for 72 (L1210 and CI920 cell lines) or 120 h (CEM cell line). For reversal experiments media was supplemented with 100 μM hypoxanthine. After drug exposure, 50 mL of 0.8 mg/mL of (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) dissolved in tissue culture medium was added to each well of all plates after which cells were returned to the incubator. After 4 h all plates were removed from the incubator and centrifuged at 1200 rpm for 7 min. Media was siphoned off, and 150 mL of DMSO was added to each well of all plates. Plates were then mixed at slow speed on a vortex mixer for 1 h in the dark at room temperature. The extent of metabolized MTT was measured spectrophotometrically at 540 nm on a Molecular Devices Vmax kinetic microplate reader. The concentration of drug required to reduce cell growth by 50% as measured by MTT metabolism was determined by interpolation between the OD (minus blank) immediately above and below 50% of control OD (minus blank).

Molecular Mechanics Calculations on Compound 13. These calculations were performed using the batchmin tool within the program Macromodel version 3.5.[44] Models of the pseudoequatorial and pseudoaxial orientations of compound **13** were built, and charges were generated using MOPAC 6.[45] The ligands were each then placed in the active site of the enzyme by overlaying the pyrimidine and glutamate portions on top of the crystal structure of compound **5**, and the substructure was minimized using the AMBER forcefield[46] with the implicit solvent model.[47]

Crystallography on Compounds 7, 13, and 17.[50] The GART domain (residues 808−1010) of the trifunctional human GARS-AIRS-GART enzyme was purified as described previously.[11] Following purification, GART was concentrated to 20 mg/mL in a buffer containing 25 mM Tris pH 7.0 and 1 mM DTT. Crystallization was done by hanging-drop vapor diffusion, mixing the protein and reservoir solution (38−44% MPD, 0.1 M Hepes, pH 7.2−7.6) in a 1:1 ratio, and equilibrating at 13 °C. Crystals would typically grow within 3 days and measure 0.2 × 0.25 × 0.3 mm.

X-ray diffraction data were collected from ternary complex crystals of GART, GAR **1** and inhibitor at 4 °C using a San Diego Multiwire Systems 2-panel area detector and a Rigaku AFC-6R monochomatic Cu Kα X-ray source and goniostat (Table 3). The space group was determined to be $P3_221$, with the cell constants shown below. The crystal structures of both compound **7** and **13** complexes were solved by molecular replacement using MERLOT.[14a] The search model consisted of residues 1−209 from an *E. coli* GART ternary complex structure (Protein Data Bank accession number 1cde). The highest peak in the cross rotation function[14b] was used in 3-dimensional translation functions,[14c] in search of Harker vectors. The top peak in all five searches (i.e. from one molecule to each of the five symmetry related molecules) produced a consistent set of vectors that positioned the model. After initial refinement with XPLOR,[49] density was seen for the substrate GAR **1** and the inhibitor. The final structures

Table 3. Summary of X-ray Data and Refinement for Compounds **7**, **13**, and **17**

	7	**13**	**17**
resolution (Å)	8.5−2.6	8.4−2.4	7.7−2.5
cell (*a*, Å)	76.8	76.8	77.0
cell (*c*, Å)	101.5	101.5	101.9
R_{merge} (%)[a]	9.0	6.1	6.8
total refls	51851	53387	38594
unique refls	10684	13534	11545
R factor (%)[b]	17.5	17.9	15.5
no. solvent	50	64	67
bond dev (Å)[c]	0.017	0.017	0.016
angle dev (deg)[c]	3.0	3.1	3.0

[a] R_{merge}: $100 \times \sum_h\sum_i|I(h)i - \langle I(h)\rangle|/\sum_h\sum_i I(h)i$ where $I(h)i$ is the ith measurement of reflection h and $I(h)i$ is the mean intensity from N measurements of reflection h. [b] *R* factor: $\sum||F_o| - |F_c||/\sum|F_o|$. [c] Average deviation from ideal values.

were obtained by manual model building in $2F_o - F_c$ and $F_o - F_c$ electron density maps followed by further refinement with XPLOR (Table 3).

General Method for Acetylene Coupling to Aryl Halides. 4-(3-Hydroxyprop-1-ynyl)benzoic Acid Methyl Ester. A mixture of methyl 4-iodobenzoate **23a** (9.00 g, 34.4 mmol), cuprous iodide (65 mg, 0.34 mmol), propargyl alcohol (1.93 g, 34.4 mmol), and bis(triphenylphosphine)palladium chloride (121 mg, 0.17 mmol) in diethylamine (90 mL) was stirred under an argon atmosphere overnight at room temperature. After the solvent was removed in vacuo, the residue was diluted with water (200 mL) and then extracted with C_6H_6 (3 × 100 mL) and EtOAc (75 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated to give a brown solid which was purified by flash chromatography with hexane/EtOAc (2:1) to give 5.44 g (83%) of the product as a yellow solid: mp 81−82 °C; ^{1}H NMR ($CDCI_3$) δ 3.92 (3H, s), 4.52 (2H, s), 7.49 (2H, d, J = 8.3 Hz), 7.98 (2H, d, J = 8.3 Hz). Anal. ($C_{11}H_{10}O_3$) C, H.

5-(3-Hydroxyprop-1-ynyl)thiophene-2-carboxylic acid methyl ester: yellow solid; mp 66−68 °C; ^{1}H NMR ($CDCl_3$) δ 1.84 (1H, broad), 3.88 (3H, s), 4.52 (2H, broad), 7.15 (1H, d, J = 3.9 Hz), 7.64 (1H, d, J = 3.9 Hz). Anal. ($C_9H_8O_3S$) C, H, S.

2(*S*)-[[[5-(3-Hydroxyprop-1-ynyl)-3-methylthiophene-2-yl]carbonyl]amino]pentanedioic acid diethyl ester: yellow oil; IR (neat) 3366, 2982, 2250, 1738, 1640, 1545, 1516, 1445, 1377 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.24 (3H, t, J = 7.1 Hz), 1.30 (3H, t, J = 7.1 Hz), 1.80 (1H, broad t), 2.04−2.46 (4H, m), 2.47 (3H, s), 4.12 (2H, q, J = 7.0 Hz), 4.24 (2H, q, J = 7.1 Hz), 4.50 (2H, d, J = 5.2 Hz), 4.73 (1H, m), 6.63 (1H, d, J = 7.3 Hz), 6.96 (1H, s). Anal. ($C_{18}H_{23}NO_6S$) C, H, N, S.

2(*S*)-[[[5-(3-Hydroxyprop-1-ynyl)-4-methylthiophene-2-yl]carbonyl]amino]pentanedioic acid diethyl ester: yellow oil; IR (neat) 3329, 2980, 2222, 1738, 1634, 1557, 1532, 1447 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.23 (3H, t, J = 7.1 Hz), 1.29 (3H, t, J = 7.2 Hz), 2.04 (3H, s), 2.07−2.50 (4H, m), 4.14 (2H, q, J = 7.1 Hz), 4.24 (2H, q, J = 7.1 Hz), 4.54 (2H, s), 4.71 (1H, m), 6.88 (1H, d, J = 7.4 Hz), 7.27 (1H, s). Anal. ($C_{18}H_{23}NO_6S$) C, H, N, S.

2(*S*)-[[[5-(3-Hydroxyprop-1-ynyl)-3-ethylthiophene-2-yl]carbonyl]amino]pentanedioic acid diethyl ester: brown oil; IR (neat) 3364 (broad), 2980, 2224, 1732, 1638, 1543, 1516, 1206 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.25 (2 overlapping triplets, 6H), 1.30 (t, 3H, J = 7.1 Hz), 2.12 (m, 1H), 2.25−2.54 (m, 3H), 2.90 (q, 2H, J = 7.5 Hz), 4.12 (q, 2H, J = 7.1 Hz), 4.23 (q, 2H, J = 7.1 Hz), 4.50 (s, 2H), 4.73 (m, 1H), 6.61 (d, 1H, J = 7.3 Hz), 7.05 (s, 1H). Anal. ($C_{19}H_{25}NO_6S$) C, H, N, S.

2(*S*)-[[3-(3-Hydroxyprop-1-ynyl)benzoyl]amino]pentanedioic acid diethyl ester: orange oil; ^{1}H NMR ($CDCl_3$) δ 1.22 (3H, t, J = 7.0 Hz), 1.30 (3H, t, J = 7.1 Hz), 2.19−2.04 (1H, m), 2.39−2.26 (1H, m), 2.56−2.41 (2H, m), 4.11 (2H, q, J = 7.0 Hz), 4.24 (2H, q, J = 7.1 Hz), 4.49 (2H, s), 4.81 (1H, ddd, J = 4.8, 7.6, 12.5 Hz), 7.11 (1H, d, J = 7.6 Hz), 7.38 (1H, t, J = 7.7 Hz), 7.53 (1H, d, J = 7.7 Hz), 7.78 (1H, d, J = 7.7 Hz), 7.86 (1H, s). Anal. ($C_{19}H_{23}NO_6 \cdot 0.2H_2O$) C, H, N.

4-(3-Hydroxy-4,4-dimethoxybut-1-ynyl)benzoic acid methyl ester: from alkyne **30**; orange oil; IR (neat) 3451 (broad), 2953, 2838, 1717, 1607, 1437, 1310, 1283 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.46 (brs, 1H), 3.55 (s, 3H), 3.56 (s, 3H), 3.92 (s, 3H), 4.45 (d, 1H, J = 5.4 Hz), 4.60 (d, 1H, J = 5.3 Hz), 7.52 (d, 2H, J = 8.3 Hz), 7.98 (d, 2H, J = 8.3 Hz). Anal. ($C_{14}H_{16}O_5$) C, H.

3-(3-Hydroxy-4,4-dimethoxybut-1-ynyl)benzoic acid methyl ester: from alkyne **30**; yellow oil; ^{1}H NMR ($CDCl_3$) δ 3.55 (3H, s), 3.56 (3H, s), 3.92 (3H, s), 4.44 (1H, d, J = 5.4 Hz), 4.59 (1H, d, J = 5.4 Hz), 7.39 (1H, t, J = 7.8 Hz), 7.64 (1H, dt, J = 7.8, 1.5 Hz), 7.98 (1H, dt, J = 7.8, 1.5 Hz), 8.14 (1H, t, J = 1.5 Hz). Anal. ($C_{14}H_{16}O_5 \cdot 0.3H_2O$) C, H.

5-[(2,2-Dimethyl[1,3]dioxolan-4(*S*)-yl)ethynyl]thiophene-2-carboxylic acid ethyl ester: from 4(*S*)-ethynyl-2,2-dimethyl-[1,3]dioxolane, **34**;[23] amber oil; $[\alpha]_{589}$ +36.6° (c = 0.88, MeOH); IR (neat) 2986, 2226, 1715, 1451, 1255, 1223 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.34 (t, 3H, J = 7.0 Hz), 1.42 (s, 3H), 1.53 (s, 1H), 4.03 (dd, 1H, J = 6.2, 1.8 Hz), 4.34 (dd, 1H, J = 6.4, 1.6 Hz), 4.34 (q, 2H, J = 7.1 Hz), 4.95 (dd, 1H, J = 6.4, 0 Hz), 7.16 (d, 1H, J = 3.9 Hz), 7.63 (d, 1H, J = 3.9 Hz). Anal. ($C_{14}H_{16}O_4S$) C, H, S.

5-[(2,2-Dimethyl[1,3]dioxolan-4(*S*)-yl)ethynyl]-4-methylthiophene-2-carboxylic acid ethyl ester: starting with 4(*S*)-ethynyl-2,2-dimethyl[1,3]dioxolane (**34**) and 2-bromo-3-methylthiophene-5-carboxylic acid ethyl ester;[25] orange oil; $[\alpha]_{589}$ +36.7° (c = 1.04, MeOH); IR (neat) 2986, 2936, 2222, 1711, 1441, 1283, 1244 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.36 (t, 3H, J = 7.0 Hz), 1.42 (s, 3H), 1.53 (s, 3H), 2.29 (s, 3H), 4.04 (dd, 1H, J = 6.2, 1.8 Hz), 4.26 (dd, 1H, J = 6.2, 1.8 Hz), 4.32 (q, 2H, J = 7.0 Hz), 4.99 (t, 1H, J = 6.0 Hz), 7.50 (s, 1H). Anal. ($C_{15}H_{18}O_4S$) C, H, S.

General Procedure for Hydrogenation of Coupled Acetylenes. 4-(3-Hydroxypropyl)benzoic Acid Methyl Ester (24a). A mixture of methyl 4-(3-hydroxypropynyl)-benzoate (3.0 g, 15.8 mmol) and 5% Pd/C (0.3 g, 10% wt equiv) in ethanol (200 mL) was shaken in a Parr flask under 45 psi of H_2 for 3.5 h. The crude reaction mixture was filtered though a pad of Celite, and the filtrate was concentrated to give a green oil which was purified by flash chromatography with hexane/EtOAc (2:1) to give 2.9 g (95%) of the product as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 1.91 (2H, tt, J = 6.4, 7.5 Hz), 2.77 (2H, t, J = 7.5 Hz), 3.68 (2H, t, J = 6.4 Hz), 3.90 (3H, s), 7.27 (2H, d, J = 8.3 Hz), 7.96 (2H, d, J = 8.3 Hz). Anal. ($C_{11}H_{14}O_3 \cdot 0.3H_2O$) C, H.

2(*S*)-[[[5-(3-Hydroxypropyl)-3-methylthiophene-2-yl]-carbonyl]amino]pentanedioic acid diethyl ester (24d): colorless oil; IR (neat) 3354, 2980, 2930, 1732, 1634, 1514, 1447, 1377 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.23 (3H, t, J = 7.1 Hz), 1.30 (3H, t, J = 7.1 Hz), 1.93 (2H, quintet, J = 7.2 Hz), 2.08−2.46 (4H, m), 2.47 (3H, s), 2.88 (2H, t, J = 7.6 Hz), 3.70 (2H, t, J = 6.3 Hz), 4.11 (2H, q, J = 7.1 Hz), 4.23 (2H, q, J = 7.1 Hz), 4.75 (1H, m), 6.47 (1H, d, J = 7.3 Hz), 6.62 (1H, s). Anal. ($C_{18}H_{27}NO_6S$) C, H, N, S.

2(*S*)-[[[5-(3-Hydroxypropyl)-4-methylthiophene-2-yl]-carbonyl]amino]pentanedioic acid diethyl ester (24e): colorless oil; IR (neat) 3337, 2980, 2938, 1738, 1632, 1560, 1530, 1449 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.23 (3H, t, J = 7.2 Hz), 1.30 (3H, t, J = 7.1 Hz), 1.89 ((2H, quintet, J = 7.2 Hz), 2.06−2.49 (4H, m) 2.16 (3H, s), 2.85 (2H, t, J = 7.5 Hz), 3.70 (2H, t, J = 6.3 Hz), 4.11 (2H, q, J = 7.1 Hz), 4.23 (2H, q, J = 7.2 Hz), 4.74 (1H, m), 6.67 (1H, d, J = 7.6 Hz), 7.26 (1H, s). Anal. ($C_{18}H_{27}NO_6S$) C, H, N, S.

2(*S*)-[[[5-(3-Hydroxypropyl)-3-ethylthiophene-2-yl]carbonyl]amino]pentanedioic acid diethyl ester (24f): colorless oil; IR (neat) 3374 (broad), 2973, 2936, 1738, 1634, 1514, 1454, 1206 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.23 (2 overlapping triplets, 6H), 1.30 (t, 3H, J = 7.1 Hz), 1.93 (m, 2H), 2.10 (m, 1H), 2.35 (m, 1H), 2.43 (m, 2H), 2.90 (t, 2H, J = 7.7 Hz), 2.92 (t, 2H, J = 7.8 Hz), 3.72 (t, 2H, J = 6.6 Hz), 4.12 (q, 2H, J = 7.1 Hz), 4.23 (q, 2H, J = 7.1 Hz), 4.74 (m, 1H), 6.45 (d, 1H, J = 7.4 Hz), 6.70 (s, 1H). Anal. ($C_{19}H_{29}NO_6S$) C, H, N, S.

2(*S*)-[[3-(3-Hydroxypropyl)benzoyl]amino]pentanedioic acid diethyl ester (24g): colorless oil; ^{1}H NMR ($CDCl_3$) δ 1.22 (3H, t, J = 7.1 Hz), 7.67 (1H, s), 1.31 (3H, t, J = 7.1 Hz), 1.91 (2H, tt, J = 6.3, 7.4 Hz), 2.19−2.08 (1H, m), 2.38−2.26 (1H, m), 2.58−2.40 (2H, m), 2.77 (2H, t, J = 7.4 Hz), 3.67 (2H, t, J = 6.3 Hz), 4.11 (2H, q, J = 7.1 Hz), 4.24 (2H, q, J =

7.1 Hz), 4.79 (1H, ddd, J = 4.7, 7.7, 12.4 Hz), 7.04 (1H, d, J = 7.7 Hz), 7.37−7.34 (2H, m), 7.64−7.60 (1H, m). Anal. ($C_{19}H_{27}NO_6$) C, H, N.

4-(3-Hydroxy-4,4-dimethoxybutyl)benzoic acid methyl ester (31): yellow oil; IR (neat) 3495 (broad), 2953, 1721, 1611, 1437, 1283 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.72−1.93 (m, 2H), 2.75−2.93 (m, 2H), 3.39 (s, 3H), 3.44 (s, 3H), 3.58 (m, 1H), 3.90 (s, 3H), 4.13 (d, 1H, J = 6.1 Hz), 7.29 (d, 2H, J = 8.1 Hz), 7.95 (d, 2H, J = 8.2 Hz). Anal. ($C_{14}H_{20}O_5 \cdot 0.20H_2O$) C, H.

3-(3-Hydroxy-4,4-dimethoxybutyl)benzoic acid methyl ester: yellow oil; ^{1}H NMR ($CDCl_3$) δ 1.74 (1H, dddd, J = 5.0, 9.3, 9.7, 17.0 Hz), 1.91 (1H, dddd, J = 3.2, 7.0, 10.0, 17.0 Hz), 2.75 (1H, ddd, J = 7.0, 9.7, 13.8 Hz), 2.93 (1H, ddd, J = 5.0, 10.0, 13.8 Hz), 3.39 (3H, s), 3.45 (3H, s), 3.59 (1H, ddd, J = 3.2, 6.1, 9.3 Hz), 3.91 (3H, s), 4.14 (1H, d, J = 6.1 Hz), 7.34 (1H, t, J = 7.6 Hz), 7.42 (1H, dt, J = 7.6, 1.4 Hz), 7.86 (1H, dt, J = 7.6, 1.4 Hz), 7.91 (1H, t, J = 1.4 Hz). Anal. ($C_{14}H_{20}O_5 \cdot 0.2H_2O$) C, H.

5-[2-(2,2-Dimethyl[1,3]dioxolan-4(*S*)-yl)ethyl]thiophene-2-carboxylic acid ethyl ester: colorless oil; $[\alpha]_{589}$ −12.1° (c = 0.78, MeOH); IR (neat) 2984, 2938, 2874, 1709, 1462, 1263 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.36 (t, 3H, J = 7.0 Hz), 1.37 (s, 3H), 1.43 (s, 3H), 1.94 (m, 2H), 2.96 (m, 2H), 3.55 (t, 1H, J = 7.3 Hz), 4.04 (dd, 1H, J = 6.1, 1.5 Hz), 4.12 (m, 1H), 4.32 (q, 2H, J = 7.0 Hz), 6.82 (d, 1H, J = 3.7 Hz), 7.63 (d, 1H, J = 4.0 Hz). Anal. ($C_{14}H_{20}O_4S$) C, H, S.

5-[2-(2,2-Dimethyl[1,3]dioxolan-4(*S*)-yl)ethyl]-4-methylthiophene-2-carboxylic acid ethyl ester: colorless oil; $[\alpha]_{589}$ −12.6° (c = 0.94, MeOH); IR (neat) 2984, 2936, 2872, 1707, 1456, 1371, 1246 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.35 (t, 3H, J = 7.0 Hz), 1.36 (s, 3H), 1.43 (s, 3H), 1.88 (m, 2H), 2.16 (s, 3H), 2.80−2.91 (m, 2H), 3.55 (dd, 1H, J = 6.6, 0.7 Hz), 4.03 (dd, 1H, J = 5.9, 1.8 Hz), 4.12 (m, 1H), 4.31 (q, 2H, J = 7.0 Hz), 7.50 (s, 1H). Anal. ($C_{15}H_{22}O_4S$) C, H, S.

General Procedure for the Preparation of Alkyl Bromides. 4-(3-Bromopropyl)benzoic Acid Methyl Ester. A solution of Ph_3P (5.67 g, 21.6 mmol) in CH_2Cl_2 (25 mL) was added dropwise to a solution of methyl 4-(3-hydroxypropyl)-benzoate (3.49 g, 18 mmol) and CBr_4 (7.16 g, 21.6 mmol) in CH_2Cl_2 (40 mL) at 0 °C. The resultant reaction mixture was stirred for 30 min at 0 °C and then overnight at room temperature. The solvent was removed, and the residue obtained was purified by flash chromatography with hexane/EtOAc (9:1) to give 4.38 g (95%) of the product as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 2.18 (2H, tt, J = 6.5, 7.4 Hz), 2.84 (2H, t, J = 7.4 Hz), 3.39 (2H, t, J = 6.5 Hz), 3.91 (3H, s), 7.27 (2H, d, J = 8.2 Hz), 7.97 (2H, d, J = 8.2 Hz). Anal. ($C_{11}H_{13}O_2Br$) C, H, Br.

5-(3-Bromopropyl)thiophene-2-carboxylic acid methyl ester: yellow oil; ^{1}H NMR ($CDCl_3$) δ 2.22 (2H, tt, J = 6.4, 7.2 Hz), 3.03 (2H, t, J = 7.2 Hz), 3.43 (2H, t, J = 6.4 Hz), 3.86 (3H, s), 6.85 (1H, d, J = 3.7 Hz). 7.64 (1H, d, J = 3.7 Hz). Anal. ($C_9H_{11}O_2SBr$) C, H, S, Br.

5-(2-Bromoethyl)thiophene-2-carboxylic acid methyl ester: yellow oil; ^{1}H NMR ($CDCl_3$) δ 3.38 (2H, t, J = 7.1 Hz), 3.58 (2H, t, J = 7.1 Hz), 3.87 (3H, s), 6.89 (1H, d, J = 3.8 Hz), 7.66 (1H, d, J = 3.8 Hz). Anal. ($C_8H_9O_2SBr$) C, H, S, Br.

2(*S*)-[[3-(3-Bromoprop-1-yl)benzoyl]amino]pentanedioic acid diethyl ester: colorless oil; ^{1}H NMR ($CDCl_3$) δ 1.23 (3H, t, J = 7.1 Hz), 1.31 (3H, t, J = 7.1 Hz), 2.19−2.08 (3H, m), 2.38−2.21 (1H, m), 2.58−2.40 (2H, m), 2.84 (2H, t, J = 7.4 Hz), 3.39 (2H, t, J = 6.5 Hz), 4.12 (2H, q, J = 7.1 Hz), 4.24 (2H, q, J = 7.1 Hz), 4.79 (1H, ddd, J = 4.7, 7.6, 12.5 Hz), 7.03 (1H, d, J = 7.6 Hz), 7.39−7.34 (2H, m), 7.66−7.62 (1H, m), 7.68 (1H, s). Anal. ($C_{19}H_{26}NO_5Br$) C, H, N, Br.

2(*S*)-[[[6-(Bromomethyl)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-2-yl]carbonyl]amino]pentanedioic acid diethyl ester: colorless gum; ^{1}H NMR ($CDCl_3$) δ 1.23 (3H, t, J = 7.2 Hz), 1.29 (3H, t, J = 7.2 Hz), 1.67−1.53 (1H, m), 2.79−2.03 (9H, m), 3.04 (1H, dd, J = 5.0, 16.8 Hz), 3.49−3.41 (2H, m), 4.10 (2H, q, J = 7.2 Hz), 4.23 (2H, q, J = 7.2 Hz), 4.73 (1H, ddd, J = 4.8, 7.6, 12.6 Hz), 6.73 (1H, d, J = 7.6 Hz), 7.23 (1H, s). Anal. ($C_{19}H_{26}NO_5SBr$) C, H, N, S, Br.

General Procedures for the Formation of Thioacetyl Compounds. Method A. 4-[3-(Acetylsulfanyl)propyl]-benzoic Acid Methyl Ester. Method A. A mixture of methyl 4-(3-bromopropyl)benzoate (1.29 g, 5.0 mmol) and KSAc (1.14 g, 10 mmol) in acetone (40 mL) was heated at reflux for 40 min. After being cooled to room temperature, the reaction mixture was filtered and the filtrate was concentrated. The residue was partitioned between Et_2O and water (25 mL each). The layers were separated, and the aqueous phase was extracted with Et_2O (25 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated to give a red oil which was purified by flash chromatography with hexane/EtOAc (9:1) to give 1.22 g (97%) of the product as an amber oil: ^{1}H NMR ($CDCl_3$) δ 1.91 (2H, tt, J = 7.2, 7.7 Hz), 2.34 (3H, s), 2.74 (2H, t, J = 7.7 Hz), 2.88 (2H, t, J = 7.2 Hz), 3.90 (3H, s), 7.24 (2H, d, J = 8.3 Hz), 7.95 (2H, d, J = 8.3 Hz). Anal. ($C_{13}H_{16}O_3S$) C, H, S.

4-[2-(Acetylsulfanyl)ethyl]benzoic Acid Methyl Ester. Method A: yellow solid; mp 68−69 °C; ^{1}H NMR ($CDCl_3$) δ 2.33 (3H, s), 2.92 (2H, t, J = 7.3 Hz), 3.13 (2H, t, J = 7.3 Hz), 3.91 (3H, s), 7.29 (2H, d, J = 8.2 Hz), 7.97 (2H, d, J = 8.2 Hz). Anal. ($C_{12}H_{14}O_3S$) C, H, S.

5-[3-(Acetylsulfanyl)propyl]thiophene-2-carboxylic Acid Methyl Ester. Method A: yellow oil; ^{1}H NMR ($CDCl_3$) δ 1.97 (2H, pentet, J = 7.3 Hz), 2.34 (3H, s), 2.91 (4H, t, J = 7.3 Hz), 3.86 (3H, s), 6.81 (1H, d, J = 3.7 Hz), 7.63 (1H, d, J = 3.7 Hz). Anal. ($C_{11}H_{14}O_3S_2$) C, H, S.

5-[2-(Acetylsulfanyl)ethyl]thiophene-2-carboxylic Acid Methyl Ester. Method A: yellow solid; mp 67−68 °C; ^{1}H NMR ($CDCl_3$) δ 2.35 (3H, s), 3.19−3.06 (4H, m), 3.86 (3H, s), 6.85 (1H, d, J = 3.7 Hz), 7.64 (1H, d, J = 3.7 Hz). Anal. ($C_{10}H_{12}O_3S_2$) C, H, S.

2(*S*)-[[3-[3-(Acetylsulfanyl)propyl]benzoyl]amino]pentanedioic Acid Diethyl Ester. Method A: orange solid; mp 83−85 °C; ^{1}H NMR ($CDCl_3$) δ 1.22 (3H, t, J = 7.1 Hz), 1.30 (2H, t, J = 7.1 Hz), 1.93 (2H, tt, J = 7.3, 7.6 Hz), 2.20−2.08 (1H, m), 2.34 (3H, s), 2.38−2.26 (1H, m), 2.55−2.40 (2H, m), 2.74 (2H, t, J = 7.6 Hz), 2.88 (2H, t, J = 7.3 Hz), 4.11 (2H, q, J = 7.1 Hz), 4.24 (2H, q, J = 7.1 Hz), 4.79 (1H, ddd, J = 4.8, 7.8, 12.6 Hz), 7.06 (1H, d, J = 7.8 Hz), 7.38−7.30 (2H, m), 7.65−7.61 (2H, m). Anal. ($C_{21}H_{29}NO_6S$) C, H, N, S.

2(*S*)-[[[6-[(Acetylsulfanyl)methyl]-4,5,6,7-tetrahydrobenzo[*b*]thiophene-2-yl]carbonyl]amino]pentanedioic Acid Diethyl Ester. Method A: orange oil; ^{1}H NMR ($CDCl_3$) δ 1.23 (3H, t, J = 7.1 Hz), 1.29 (3H, t, J = 7.1 Hz), 1.57−1.46 (1H, m), 2.15−1.96 (3H, m), 2.35−2.22 (1H, m), 2.36 (3H, s), 2.74−2.38 (5H, m), 3.02−2.92 (3H, m), 4.11 (2H, q, J = 7.1 Hz), 4.22 (2H, q, J = 7.1 Hz), 4.72 (1H, ddd, J = 4.8, 7.6, 12.6 Hz), 6.69 (1H, d, J = 7.6 Hz), 7.21 (1H, s). Anal. ($C_{21}H_{29}NO_6S_2 \cdot 0.5H_2O$) C, H, N, S.

Method B. 2(*S*)-[[[5-[3-(Acetylsulfanyl)propyl]-3-methylthiophene-2-yl]carbonyl]amino]pentanedioic Acid Diethyl Ester. To a stirred solution of triphenylphosphine (210 mg, 0.79 mmol) and diethyl azodicarboxylate (126 μL, 0.79 mmol) in THF (5 mL) at 0 °C was added dropwise a solution of the above alcohol (154 mg, 0.39 mmol) and thioacetic acid (57 μL, 0.79 mmol) in THF (5 mL) over a 15 min period. After 1 h at 0 °C, the solvent was removed in vacuo. The crude residue was flash chromatographed with CH_2Cl_2/EtOAc (20:1) to give 118 mg (67%) of the desired thioacetate as a colorless oil: IR (neat) 3366, 2980, 2936, 1736, 1692, 1649, 1510, 1447, 1377 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.23 (3H, t, J = 7.2 Hz), 1.30 (3H, t, J = 7.1 Hz), 1.95 (2H, pentet, J = 7.3 Hz), 2.04−2.48 (4H, m), 2.34 (3H, s), 2.47 (3H, s), 2.83 (2H, t, J = 7.4 Hz), 2.91 (2H, t, J = 7.2 Hz), 4.11 (2H, q, J = 7.1 Hz), 4.23 (2H, q, J = 7.1 Hz), 4.75 (1H, m), 6.47 (1H, d, J = 7.4 Hz), 6.61 (1H, s).

2(*S*)-[[[5-[3-(Acetylsulfanyl)propyl]-4-methylthiophene-2-yl]carbonyl]amino]pentanedioic Acid Diethyl Ester. Method B: yellow oil; IR (neat) 3337, 2982, 2938, 1736, 1694, 1634, 1526, 1449, 1206 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.20 (3H, t, J = 7.1 Hz), 1.30 (3H, t, J = 7.1 Hz), 1.91 (2H, pentet, J = 7.4 Hz), 2.08−2.49 (4H, m), 2.15 (3H, s), 2.34 (3H, s), 2.80 (2H, t, J = 7.4 Hz), 2.91 (2H, t, J = 7.2 Hz), 4.13 (2H, q, J = 7.1 Hz), 4.24 (2H, q, J = 7.2 Hz), 4.74 (1H, m), 6.67 (1H, d, J = 7.6 Hz), 7.26 (1H, s). Anal. ($C_{20}H_{29}NO_6S_2$) C, H, N, S.

2(*S*)-[[[5-[3-(Acetylsulfanyl)propyl]-3-ethylthiophene-2-yl]carbonyl]amino]pentanedioic Acid Diethyl Ester. Method B: yellow oil; ^{1}H NMR ($CDCl_3$) δ 1.24 (2 overlapping

triplets, 6H), 1.30 (t, 3H, J = 7.1 Hz), 1.96 (m, 2H), 2.26−2.56 (m, 3H), 2.35 (5, 3H), 2.85 (t, 2H, J = 7.4 Hz), 2.92 (m, 4H), 4.12 (q, 2H, J = 7.1 Hz), 4.23 (q, 2H, J = 7.1 Hz), 4.74 (m, 1H), 6.45 (d, 1H, J = 7.4 Hz), 6.68 (s, 1H).

Method C. 5-[3(*R*)-(Acetylsulfanyl)-4-[(*tert*-butoxycarbonyl)amino]butyl]thiophene-2-carboxylic Acid Ethyl Ester. To a stirred solution of 5-[3(*S*)-[(methylsulfonyl)oxy]-4-[(*tert*-butoxycarbonyl)amino]butyl]thiophene-2-carboxylic acid ethyl ester (38.36 g, 91.0 mmol) in acetone (650 mL) was added KSAc (41.57 g, 364 mmol). After 14 h, the starting mesylate had disappeared by TLC and the reaction mixture was a red-brown color and thick with precipitate. The crude reaction was filtered, washing the precipitate with acetone and Et_2O until the filter cake was beige colored. The filtrate was concentrated in vacuo to a volume of 500 mL, diluted with EtOAc, washed with brine, dried ($MgSO_4$), and concentrated. The crude oil was purified by flash chromatography on 250 g of silica gel, eluting hexanes/EtOAc (1:1) to remove baseline colored impurities yielding 39.77 g (109%) of the thioacetate as a red oil which was used without further purification: $[\alpha]_{589}$ +7.6° (c = 0.66, MeOH); IR (neat) 3376, 2978, 2932, 1712, 1684, 1520, 1462, 1263 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.35 (t, 3H, J = 7.0 Hz), 1.44 (s, 9H), 1.90 (m, 1H), 2.04 (m, 1H), 2.36 (s, 3H), 2.95 (m, 2H), 3.36 (m, 2H), 3.60 (m, 1H), 4.32 (q, 2H, J = 7.0 Hz), 4.78 (1H, br t), 6.78 (d, 1H, J = 3.7 Hz), 7.61 (d, 1H, J = 3.7 Hz). Anal. ($C_{18}H_{27}NO_5S_2$) C, H, N, S.

5-[3(*S*)-(Acetylsulfanyl)-4-[(*tert*-butoxycarbonyl)amino]-butyl]thiophene-2-carboxylic Acid Ethyl Ester. Method C: orange oil; $[\alpha]_{589}$ −4.9° (c = 0.61, MeOH); IR (neat) 3374, 2978, 2932, 1715, 1695, 1518, 1460, 1264 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.35 (t, 3H, J = 7.0 Hz), 1.44 (s, 9H), 1.90 (m, 1H), 2.03 (m, 1H), 2.36 (s, 3H), 2.95 (m, 2H), 3.35 (m, 2H), 3.59 (m, 1H), 4.31 (2H, q, J = 7.0 Hz), 4.74 (br t, 1H), 6.79 (d, 1H, J = 3.7 Hz), 7.62 (d, 1H, J = 3.7 Hz). Anal. ($C_{18}H_{27}NO_5S_2$) C, H, N, S.

5-[3(*R*)-(Acetylsulfanyl)-4-[(*tert*-butoxycarbonyl)amino]butyl]-4-methylthiophene-2-carboxylic Acid Ethyl Ester. Method C: orange oil; $[\alpha]_{589}$ −2.8° (c = 0.78, MeOH); IR (neat) 3376, 2978, 2932, 1699, 1516, 1454, 1250 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.34 (t, 3H, J = 7.0 Hz), 1.44 (s, 9H), 1.80−2.04 (m, 2H), 2.13 (s, 3H), 2.37 (s, 3H), 2.85 (m, 2H), 3.38 (m, 2H), 3.60 (m, 1H), 4.30 (q, 2H, J = 7.0 Hz), 4.88 (br t, 1H), 7.48 (s, 1H). Anal. ($C_{19}H_{29}NO_5S_2$) C, H, N, S.

5-[3(*S*)-(Acetylsulfanyl)-4-[(*tert*-butoxycarbonyl)amino]-butyl]-4-methylthiophene-2-carboxylic Acid Ethyl Ester. Method C: orange oil; $[\alpha]_{589}$ +2.8° (c = 0.93, MeOH); ^1H NMR ($CDCl_3$) δ 1.34 (t, 3H, J = 7.0 Hz), 1.44 (s, 9H), 1.78−1.89 (m, 1H), 1.93−2.04 (m, 1H), 2.13 (s, 3H), 2.37 (s, 3H), 2.75−2.94 (m, 2H), 3.26−3.45 (m, 2H), 3.56−3.64 (m, 1H), 4.30 (q, 2H, J = 7.0 Hz), 4.75 (broad, 1H), 7.48 (s, 1H). Anal. ($C_{19}H_{29}NO_5S_2$) C, H, N, S.

General Procedure for the Preparation of Thiols 21a−i. 4-(3-Mercaptopropyl)benzoic Acid Methyl Ester (21a). A solution of 4-[3-(acetylsulfanyl)propyl]benzoic acid methyl ester (1.01 g, 4 mmol) in 1 N HCl in MeOH (15 mL) was heated at reflux for 2 h. The reaction mixture was diluted with water (10 mL), the MeOH was removed in vacuo, and the aqueous residue was extracted with Et_2O (2 × 25 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated to provide 818 mg (97%) of the product as a yellow oil: ^1H NMR ($CDCl_3$) δ 1.37 (1H, t, J = 7.9 Hz), 2.00 (2H, m), 2.57−2.50 (2H, m), 2.79 (2H, t, J = 7.6 Hz), 3.90 (3H, s), 7.25 (2H, d, J = 8.2 Hz), 7.96 (2H, d, J = 8.2 Hz). Anal. ($C_{11}H_{14}O_2S$) C, H, S.

4-(2-Mercaptoethyl)benzoic acid methyl ester (21b): yellow oil; ^1H NMR ($CDCl_3$) δ 1.37 (1H, t, J = 7.9 Hz), 2.81 (2H, dt, J = 7.9, 7.3 Hz), 2.98 (2H, t, J = 7.3 Hz), 3.91 (3H, s), 7.27 (2H, d, J = 8.2 Hz), 7.98 (2H, d, J = 8.2 Hz). Anal. ($C_{10}H_{12}O_2S$) C, H, S.

5-(3-Mercaptopropyl)thiophene-2-carboxylic acid methyl ester (21c): orange oil; ^1H NMR ($CDCl_3$) δ 1.36 (1H, t, J = 8.0 Hz), 1.99 (2H, tt, J = 7.1, 7.4 Hz), 2.57 (2H, dt, J = 8.0, 7.1 Hz), 2.98 (2H, t, J = 7.4 Hz), 3.86 (3H, s), 6.81 (1H, d, J = 3.7 Hz), 7.63 (1H, d, J = 3.7 Hz). Anal. ($C_9H_{12}O_2S_2$) C, H, S.

5-(2-Mercaptoethyl)thiophene-2-carboxylic acid methyl ester (21d): yellow oil; ^1H NMR ($CDCl_3$) δ 1.50 (1H, t, J = 8.2 Hz), 2.83 (2H, dt, J = 8.2, 7.1 Hz), 3.14 (2H, t, J = 7.1 Hz), 3.87 (3H, s), 6.86 (1H, d, J = 3.7 Hz), 7.66 (1H, d, J = 3.7 Hz). Anal. ($C_8H_{10}O_2S_2$) C, H, S.

2(*S*)-[[[5-(3-Mercaptopropyl)-3-methylthiophene-2-yl]-carbonyl]amino]pentanedioic acid diethyl ester (21e): used as a crude oil.

2(*S*)-[[[5-(3-Mercaptopropyl)-4-methylthiophene-2-yl]-carbonyl]amino]pentanedioic acid diethyl ester (21f): used as a crude oil.

2(*S*)-[[[5-(3-Mercaptopropyl)-3-ethylthiophene-2-yl]carbonyl]amino]pentanedioic acid diethyl ester (21g): used as a crude oil.

2(*S*)-[[3-(3-Mercaptopropyl)benzoyl]amino]pentanedioic acid diethyl ester (21h): orange oil; ^1H NMR ($CDCl_3$) δ 1.22 (3H, t, J = 7.1 Hz), 1.31 (3H, t, J = 7.1 Hz), 1.37 (1H, t, J = 7.9 Hz), 1.95 (2H, pentet, J = 7.4 Hz), 2.20−2.08 (1H, m), 2.40−2.26 (1H, m), 2.45 (2H, t, J = 6.9 Hz), 2.54 (2H, dt, J = 7.9, 7.4 Hz), 2.78 (2H, t, J = 7.4 Hz), 4.11 (2H, q, J = 7.1 Hz), 4.24 (2H, q, J = 7.1 Hz), 4.79 (1H, ddd, J = 4.8, 7.7, 12.5 Hz), 7.02 (1H, d, J = 7.7 Hz), 7.40−7.32 (2H, m), 7.62 (1H, dt, J = 6.5, 2.0 Hz), 7.66 (1H, brs). Anal. ($C_{19}H_{27}NO_5S$) C, H, N, S.

2(*S*)-[[[6-(Mercaptomethyl)-4,5,6,7-tetrahydrobenzo[*b*]-thiophene-2-yl]carbonyl]amino]pentanedioic acid diethyl ester (21i): orange oil; ^1H NMR ($CDCl_3$) δ 1.23 (3H, t, J = 7.1 Hz), 1.30 (3H, t, J = 7.1 Hz), 1.38 (1H, t, J = 8.3 Hz), 1.59−1.48 (1H, m), 2.15−1.92 (3H, m), 2.77−2.23 (8H, m), 3.04 (1H, dd, J = 5.0, 16.6 Hz), 4.12 (2H, q, J = 7.1 Hz), 4.23 (2H, q, J = 7.1 Hz), 4.73 (1H, ddd, J = 4.8, 7.6, 12.6 Hz), 6.69 (1H, d, J = 7.6 Hz), 7.23 (1H, s). Anal. ($C_{19}H_{27}NO_5S_2 \cdot 0.25H_2O$) C, H, N, S.

General Procedure for the Preparation of Sulfides. 4-[3-[(2,4-Diamino-6-oxo-1,6-dihydropyrimidin-5-yl)sulfanyl]propyl]benzoic Acid Methyl Ester (22a). Argon was bubbled through a slurry of 5-bromo-2,6-diamino-4(3*H*)-pyrimidinone (**20**) (1.01 g, 4.9 mmol) in DMF (15 mL). To this slurry was added a solution of thiol **21a** (1.11 g, 5.3 mmol) in DMF (10 mL) and DIEA (1.8 mL, 10.3 mmol). The resultant reaction mixture was heated at 100 °C for 30 min and then poured over ice (100 g).[13] The precipitate that formed was collected by filtration and washed with water (2 × 30 mL) and then with Et_2O (2 × 30 mL) to provide 711 mg (43%) of the product as an off-white powder: mp 248−251 °C dec; ^1H NMR (DMSO-d_6) δ 1.70 (2H, tt, J = 7.0, 7.2 Hz), 2.42 (2H, t, J = 7.0 Hz), 2.76 (2H, t, J = 7.2 Hz), 3.82 (3H, s), 6.28 (4H, broad), 7.33 (2H, d, J = 8.2 Hz), 7.84 (2H, d, J = 8.2 Hz), 9.95 (1H, s). Anal. ($C_{15}H_{18}N_4O_3S \cdot 0.3H_2O$) C, H, N, S.

4-[2-[(2,4-Diamino-6-oxo-1,6-dihydropyrimidin-5-yl)-sulfanyl]ethyl]benzoic acid methyl ester (22b): white solid; mp 286−288 °C dec; ^1H NMR (DMSO-d_6) δ 2.70 (2H, t, J = 7.3 Hz), 2.81 (2H, t, J = 7.3 Hz), 3.82 (3H, s), 6.33 (4H, broad), 7.36 (2H, d, J = 8.2 Hz), 7.85 (2H, d, J = 8.2 Hz), 9.98 (1H, brs). Anal. ($C_{14}H_{16}N_4O_3S$) C, H, N, S.

5-[3-[(2,4-Diamino-6-oxo-1,6-dihydropyrimidin-5-yl)-sulfanyl]propyl]thiophene-2-carboxylic acid methyl ester (22c): off-white solid; mp 196 °C dec; ^1H NMR (DMSO-d_6) δ 1.74 (2H, tt, J = 6.8, 7.5 Hz), 2.45 (2H, t, J = 6.8 Hz), 2.97 (2H, t, J = 7.5 Hz), 3.77 (3H, s), 6.29 (4H, broad), 6.93 (1H, d, J = 3.8 Hz), 7.61 (1H, d, J = 3.8 Hz), 9.96 (1H, brs). Anal. ($C_{13}H_{16}N_4O_3S_2$) C, H, N, S.

5-[2-[(2,4-Diamino-6-oxo-1,6-dihydropyrimidin-5-yl)-sulfanyl]ethyl]thiophene-2-carboxylic acid methyl ester (22d): off-white solid; mp 228−229 °C; ^1H NMR (DMSO-d_6) δ 2.72 (2H, t, J = 7.1 Hz), 2.98 (2H, t, J = 7.1 Hz), 3.77 (3H, s), 6.33 (4H, brs), 7.02 (1H, d, J = 3.6 Hz), 7.62 (1H, d, J = 3.6 Hz), 9.98 (1H, s). Anal. ($C_{12}H_{14}N_4O_3S_2$) C, H, N, S.

2(*S*)-[[[5-[3-[(2,4-Diamino-6-oxo-1,6-dihydropyrimidin-5-yl)sulfanyl]propyl]-3-methylthiophene-2-yl]carbonyl]-amino]pentanedioic acid diethyl ester (22e): white solid; mp 164−165 °C; IR (KBr) 3329, 2930, 1734, 1636, 1597, 1518, 1441 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.16 (3H, t, J = 7.1 Hz), 1.18 (3H, t, J = 7.0 Hz), 1.71 (2H, m), 1.90−2.10 (2H, m), 2.32 (3H, s) 2.40 (4H, m), 2.86 (2H, t, J = 7.4 Hz), 4.07 (4H, m), 4.32 (1H, m), 6.30 (4H, brs), 6.67 (1H, s), 8.13 (1H, d, J = 7.5 Hz), 9.95 (1H, s). Anal. ($C_{22}H_{31}N_5O_6S_2 \cdot 1.0H_2O$) C, H, N, S.

2(*S*)-[[[5-[3-[(2,4-Diamino-6-oxo-1,6-dihydropyrimidin-5-yl)sulfanyl]propyl]-4-methylthiophene-2-yl]carbonyl]-amino]pentanedioic acid diethyl ester (22f): white solid; mp 159−160 °C; IR (KBr) 3324, 2980, 1734, 1657, 1632, 1603, 1466, 1206 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.15 (3H, t, J = 7.1 Hz), 1.17 (3H, t, J = 7.1 Hz), 1.68 (2H, m), 2.01 (2H, m), 2.11 (3H, s), 2.43 (4H, m), 2.85 (2H, t, J = 7.3 Hz), 4.04 (2H, q, J = 7.1 Hz), 4.12 (2H, q, J = 7.1 Hz), 4.34 (1H, m), 6.31 (4H, brs), 7.55 (1H, s), 8.52 (1H, d, J = 7.5 Hz), 9.95 (1H, s). Anal. ($C_{24}H_{31}N_5O_6S$) C, H, N, S.

2(*S*)-[[[5-[3-[(2,4-Diamino-6-oxo-1,6-dihydropyrimidin-5-yl)sulfanyl]propyl]-3-ethylthiophene-2-yl]carbonyl]amino]pentanedioic acid diethyl ester (22g): light yellow solid; mp 166.6 °C dec; IR (KBr) 3324 (broad), 1734, 1638, 1601, 1443, 1206 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.09 (t, 3H, J = 7.5 Hz), 1.17 (2 overlapping triplets, 6H), 1.74 (m, 2H), 1.91−2.07 (m, 2H), 2.39 (t, 2H, J = 7.5 Hz), 2.47 (t, 2H, partially obscured by DMSO), 2.76 (q, 2H, J = 7.5 Hz), 2.88 (t, 2H, J = 7.4 Hz), 4.03 (q, 2H, J = 7.1 Hz), 4.10 (q, 2H, J = 7.1 Hz), 4.31 (m, 1H), 6.32 (brs, 4H), 6.75 (s, 1H), 8.19 (d, 1H, J = 7.5 Hz), 9.95 (s, 1H). Anal. ($C_{23}H_{33}N_5O_6S_2$) C, H, N, S.

2(*S*)-[[3-[3-[(2,4-Diamino-6-oxo-1,6-dihydropyrimidin-5-yl)sulfanyl]propyl]benzoyl]amino]pentanedioic acid diethyl ester (22h): yellow amorphous powder; ^1H NMR (DMSO-d_6) δ 1.15 (3H, t, J = 7.1 Hz), 1.18 (3H, t, J = 7.1 Hz), 1.72 (2H, tt, J = 7.0, 7.5 Hz), 2.16−1.95 (2H, m), 2.43 (2H, t, J = 7.4 Hz), 2.44 (2H, t, J = 7.0 Hz), 2.73 (2H, t, J = 7.5 Hz), 4.03 (2H, q, J = 7.1 Hz), 4.10 (2H, q, J = 7.1 Hz), 4.41 (1H, ddd, J = 5.5, 7.3, 9.4 Hz), 6.28 (4H, brs), 7.37−7.32 (2H, m), 7.69−7.64 (2H, m), 8.68 (1H, d, J = 7.3 Hz), 9.94 (1H, s). Anal. ($C_{23}H_{31}N_5O_6S$) C, H, N, S.

2-[[[6-[[(2,4-Diamino-6-oxo-1,6-dihydropyrimidin-5-yl)-sulfanyl]methyl]-4,5,6,7-tetrahydrobenzo[*b*]thiophene-2-yl]carbonyl]amino]pentanedioic acid diethyl ester (22i): yellow solid; mp 122−128 °C; ^1H NMR (DMSO-d_6) δ 1.15 (3H, t, J = 7.1 Hz), 1.17 (3H, t, J = 7.1 Hz), 1.48−1.37 (1H, m), 1.83−1.73 (1H, m), 2.09−1.89 (3H, m), 2.40 (2H, t, J = 7.4 Hz), 2.66−2.43 (5H, m), 3.07 (1H, dd, J = 4.4, 16.6 Hz), 4.03 (2H, q, J = 7.1 Hz), 4.08 (2H, q, J = 7.1 Hz), 4.34 (1H, ddd, J = 5.3, 7.5, 9.4 Hz), 6.28 (4H, brs), 7.54 (1H, s), 8.53 (1H, d, J = 7.5 Hz), 9.92 (1H, s). Anal. ($C_{23}H_{31}N_5O_6S_2$) C, H, N, S.

4-[3-[(2,4-Diamino-6-oxo-1,6-dihydropyrimidin-5-yl)-sulfanyl]-3-[1,3]dioxolan-2-ylpropyl]benzoic acid methyl ester: off-white solid; mp 206−208 °C dec; IR (KBr) 3439, 3341, 3154, 1701, 1636, 1591, 1470, 1447, 1287 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.64 (m, 1H), 1.84 (m, 1H), 2.58 (m, 1H), 2.79 (m, 1H), 3.19 (m, 1H), 3.82 (s, 3H), 3.85 (m, 4H), 4.83 (d, 1H, J = 4.3 Hz), 6.33 (brs, 4H), 7.33 (d, 2H, J = 8.1 Hz), 7.83 (d, 2H, J = 8.1 Hz), 10.03 (s, 1H). Anal. ($C_{18}H_{22}N_4O_5S$) C, H, N, S.

General Procedures for the Hydrolysis of Methyl and Diethyl Glutamate Esters. 4-[3-[(2,4-Diamino-6-oxo-1,6-dihydropyrimidin-5-yl)sulfanyl]propyl]benzoic Acid. A suspension of ester **22a** (669 mg, 2 mmol) in a 1 N NaOH solution (30 mL) was stirred overnight at room temperature and then filtered. The filtrate was acidified to ∼pH 5 by addition of AcOH (∼3 mL). The precipitate that formed was collected by filtration and washed with water (3 × 5 mL) to give 589 mg (92%) of the product as an off-white powder: mp 262−263 °C; ^1H NMR (DMSO-d_6) δ 1.70 (2H, tt, J = 7.0, 7.2 Hz), 2.43 (2H, t, J = 7.0 Hz), 2.74 (2H, t, J = 7.2 Hz), 6.29 (4H, broad), 7.29 (2H, d, J = 8.1 Hz), 7.82 (2H, d, J = 8.1 Hz), 9.97 (1H, broad), 12.72 (1H, broad). Anal. ($C_{14}H_{16}N_4O_3S$) C, H, N, S.

2(*S*)-[[4-[3-[(2,4-Diamino-6-oxo-1,6-dihydropyrimidin-5-yl)sulfanyl]propyl]benzoyl]amino]pentanedioic Acid (5). A solution of the glutamate diethyl ester (192 mg, 0.4 mmol) in a 1 N NaOH solution (15 mL) was stirred at room temperature for 70 h. After the pH was adjusted to ∼3−4 with 6 N HCl, the precipitate that formed was collected by filtration and washed with water (3 × 10 mL) to give 147 mg (86%) of the product as a white solid: mp 205−206 °C; ^1H NMR (DMSO-d_6) δ 1.69 (2H, tt, J = 6.9, 7.2 Hz), 1.99−1.89 (1H, m), 2.11−2.01 (1H, m), 2.34 (2H, t, J = 7.9 Hz), 2.43 (2H, t, J = 6.9 Hz), 2.73 (2H, t, J = 7.2 Hz), 4.37 (1H, ddd, J = 4.8, 7.7, 9.7 Hz), 6.29 (4H, brs), 7.27 (2H, d, J = 8.2 Hz), 7.76 (2H, d, J = 8.2 Hz), 8.51 (1H, d, J = 7.7 Hz), 9.96 (1H, broad), 12.34 (2H, broad). Anal. ($C_{19}H_{23}N_5O_6S$) C, H, N, S.

4-[3-[(2,4-Diamino-6-oxo-1,6-dihydropyrimidin-5-yl)-sulfanyl]ethyl]benzoic acid: white solid; mp 291−292 °C; ^1H NMR (DMSO-d_6) δ 2.69 (2H, t, J = 7.3 Hz), 2.80 (2H, t, J = 7.3 Hz), 6.31 (4H, broad), 7.32 (2H, d, J = 8.2 Hz), 7.82 (2H, d, J = 8.2 Hz), 9.98 (1H, brs), 12.78 (1H, brs). Anal. ($C_{13}H_{14}N_4O_3S\cdot0.5H_2O$) C, H, N, S.

2(*S*)-[[4-[2-[(2,4-Diamino-6-oxo-1,6-dihydropyrimidin-5-yl)sulfanyl]ethyl]benzoyl]amino]pentanedioic acid (6): white solid; mp 177−181 °C; ^1H NMR (DMSO-d_6) δ 1.89−2.09 (2H, m), 2.33 (2H, t, J = 7.3 Hz), 2.69 (2H, t, J = 6.8 Hz), 2.78 (2H, t, J = 6.8 Hz), 4.35 (1H, ddd, J = 5.3, 7.6, 13.0 Hz), 6.33 (4H, broad), 7.30 (2H, d, J = 8.1 Hz), 7.77 (2H, d, J = 8.1 Hz), 8.49 (1H, d, J = 7.6 Hz), 10.01 (1H, brs). Anal. ($C_{18}H_{21}N_5O_6S\cdot1.05H_2O$) C, H, N, S.

5-[3-[(2,4-Diamino-6-oxo-1,6-dihydropyrimidin-5-yl)-sulfanyl]propyl]thiophene-2-carboxylic acid: yellow powder; mp 254 °C dec; ^1H NMR (DMSO-d_6) δ 1.73 (2H, tt, J = 6.9, 7.4 Hz), 2.46 (2H, t, J = 6.9 Hz), 2.95 (2H, t, J = 7.4 Hz), 6.30 (4H, brs), 6.89 (1H, d, J = 3.7 Hz), 7.53 (1H, d, J = 3.7 Hz), 10.02 (1H, broad), 12.86 (1H, broad). Anal. ($C_{12}H_{14}N_4O_3S_2\cdot0.5H_2O$) C, H, N, S.

2(*S*)-[[[5-[3-[(2,4-Diamino-6-oxo-1,6-dihydropyrimidin-5-yl)sulfanyl]propyl]thiophene-2-yl]carbonyl]amino]pentanedioic acid (7): yellow powder; mp 171−173 °C; ^1H NMR (DMSO-d_6) δ 1.73 (2H, tt, J = 6.8, 7.2 Hz), 1.95−1.83 (1H, m), 2.11−2.00 (1H, m), 2.32 (2H, t, J = 7.3 Hz), 2.46 (2H, t, J = 6.8 Hz), 2.92 (2H, t, J = 7.2 Hz), 4.36−4.28 (1H, m), 6.39 (4H, broad), 6.85 (1H, d, J = 3.6 Hz), 7.66 (1H, d, J = 3.6 Hz), 8.49 (1H, d, J = 7.7 Hz), 10.09 (1H, broad), 12.41 (2H, broad). Anal. ($C_{17}H_{21}N_5O_6S_2\cdot0.3H_2O$) C, H, N, S.

5-[2-[(2,4-Diamino-6-oxo-1,6-dihydropyrimidin-5-yl)-sulfanyl]ethyl]thiophene-2-carboxylic acid: yellow powder; mp 273 °C dec; ^1H NMR (DMSO-d_6) δ 2.72 (2H, t, J = 7.3 Hz), 2.97 (2H, t, J = 7.3 Hz), 6.34 (4H, brs), 6.97 (1H, d, J = 3.7 Hz), 7.53 (1H, d, J = 3.7 Hz), 9.99 (1H, brs), 12.86 (1H, broad). Anal. ($C_{11}H_{12}N_4O_3S_2$) C, H, N, S.

2(*S*)-[[5-[2-[(2,4-Diamino-6-oxo-1,6-dihydropyrimidin-5-yl)sulfanyl]ethyl]thiophene-2-yl]carbonyl]amino]pentanedioic acid (8): off-white powder; mp 228−230 °C dec; ^1H NMR (DMSO-d_6) δ 1.95−1.85 (1H, m), 2.12−2.00 (1H, m), 2.32 (2H, t, J = 7.1 Hz), 2.70 (2H, t, J = 7.3 Hz), 2.94 (2H, t, J = 7.3 Hz), 4.36−4.28 (1H, m), 6.36 (4H, brs), 6.94 (1H, d, J = 3.3 Hz), 7.67 (1H, d, J = 3.3 Hz), 8.51 (1H, d, J = 7.7 Hz), 10.02 (1H, brs), 12.41 (2H, broad). Anal. ($C_{16}H_{19}N_5O_6S_2$) C, H, N, S.

2(*S*)-[[[5-[3-[(2,4-Diamino-6-oxo-1,6-dihydropyrimidin-5-yl)sulfanyl]propyl]-3-methylthiophene-2-yl]carbonyl]-amino]pentanedioic acid (9): white solid; mp 217−220 °C; IR (KBr) 3341, 3200, 2922, 1709, 1620, 1516, 1468, 1263 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.71 (2H, pentet, J = 7.2 Hz), 1.74−2.05 (2H, m), 2.31 (2H, t, J = 7.5 Hz), 2.33 (3H, s), 2.46 (2H, t, J = 6.9 Hz), 2.86 (2H, t, J = 6.9 Hz), 4.28 (1H, m), 6.33 (4H, brs), 6.66 (1H, s), 7.96 (1H, d, J = 7.7 Hz), 9.96 (1H, brs). Anal. ($C_{18}H_{23}N_5O_6S_2\cdot1.3H_2O$) C, H, N, S.

2(*S*)-[[[5-[3-[(2,4-Diamino-6-oxo-1,6-dihydropyrimidin-5-yl)sulfanyl]propyl]-4-methylthiophene-2-yl]carbonyl]-amino]pentanedioic acid (10): white solid; mp 154−158 °C; IR (KBr) 3322, 3179, 2922, 1705, 1632, 1564, 1445 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.68 (2H, m), 1.92−2.01 (2H, m), 2.10 (3H, s), 2.31 (2H, t, J = 7.4 Hz), 2.47 (2H, t, J = 7.4 Hz), 2.82 (2H, t, J = 7.4 Hz), 4.29 (1H, m), 6.29 (4H, brs), 7.53 (1H, s), 8.36 (1H, d, J = 7.7 Hz), 9.90 (1H, brs). Anal. ($C_{18}H_{23}N_5O_6S_2\cdot0.4H_2O$) C, H, N, S.

2(*S*)-[[[5-[3-[(2,4-Diamino-6-oxo-1,6-dihydropyrimidin-5-yl)sulfanyl]propyl]-3-ethylthiophene-2-yl]carbonyl]amino]pentanedioic acid (11): off-white solid; mp 137 °C dec; IR (KBr) 3337 (broad), 1709, 1640, 1601, 1514, 1451 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.10 (t, 3H, J = 7.5 Hz), 1.72 (m, 2H), 1.95 (m, 2H), 2.30 (t, 2H, J = 7.3 Hz), 2.46 (t, 2H, partially obscured by DMSO), 2.77 (q, 2H, J = 7.5 Hz), 2.87 (t, 2H, J = 7.4 Hz), 4.26 (m, 1H), 6.26 (brs, 4H), 6.74 (s, 1H), 7.98 (d, 1H, J = 7.4 Hz), 10.03 (brs, 1H), 12.50 (brs, 2H). Anal. ($C_{19}H_{25}N_5O_6S_2\cdot1.7H_2O$) C, H, N, S.

2(*S*)-[[3-[3-[(2,4-Diamino-6-oxo-1,6-dihydropyrimidin-5-yl)sulfanyl]propyl]benzoyl]amino]pentanedioic acid (12): yellow solid; mp 207−209 °C dec; ^{1}H NMR (DMSO-d_6) δ 1.72 (2H, tt, J = 7.0, 7.5 Hz), 2.00−1.90 (1H, m), 2.14−2.02 (1H, m), 2.34 (2H, t, J = 7.4 Hz), 2.44 (2H, t, J = 7.0 Hz), 2.73 (2H, t, J = 7.5 Hz), 4.38 (1H, ddd, J = 5.1, 7.7, 9.5 Hz), 6.30 (4H, brs), 7.36−7.31 (2H, m), 7.70−7.63 (2H, m), 8.54 (1H, d, J = 7.7 Hz), 10.00 (1H, broad), 12.41 (2H, broad). Anal. ($C_{19}H_{23}N_5O_6S\cdot0.5H_2O$) C, H, N, S.

2(*S*)-[[[6-[[(2,4-Diamino-6-oxo-1,6-dihydropyrimidin-5-yl)sulfanyl]methyl]-4,5,6,7-tetrahydrobenzo[*b*]thiophene-2-yl]carbonyl]amino]pentanedioic acid (13): white solid; mp 227−230 °C dec; ^{1}H NMR (DMSO-d_6) δ 1.46−1.37 (1H, m), 2.10−1.77 (4H, m), 2.31 (2H, t, J = 7.3 Hz), 2.66−2.36 (5H, m), 3.07 (1H, dd, J = 4.3, 16.6 Hz), 4.30 (1H, ddd, J = 4.6, 7.8, 12.6 Hz), 6.44 (4H, brs), 7.53 (1H, s), 8.42 (1H, d, J = 7.8 Hz), 10.08 (1H, broad), 12.38 (2H, broad). Anal. ($C_{19}H_{23}N_5O_6S_2\cdot0.7H_2O$) C, H, N, S.

4-[2-(2-Amino-4-oxo-4,6,7,8-tetrahydro-3*H*-pyrimido-[5,4-*b*][1,4]thiazin-6-yl)ethyl]benzoic acid: white solid; mp >310 °C dec; IR (KBr) 3285, 3086, 2928, 1698, 1642, 1611, 1576, 1449, 1348 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.72 (m, 1H), 1.89 (m, 1H), 2.78 (m, 3H), 3.20 (m, 1H), 3.48 (m, 1H), 6.07 (s, 2H), 6.68 (s, 1H), 7.33 (d, 2H, J = 8.1 Hz), 7.85 (d, 2H, J = 8.1 Hz), 10.11 (s, 1H), 12.77 (brs, 1H). Anal. ($C_{15}H_{16}N_4O_3S\cdot1.20H_2O$) C, H, N, S.

2(*S*)-[[4-[2-(2-Amino-4-oxo-4,6,7,8-tetrahydro-3*H*-pyrimido[5,4-*b*][1,4]thiazin-6-yl)ethyl]benzoyl]amino]pentanedioic acid (14): off-white solid; mp 188−190 °C; IR (KBr) 3348 (broad), 2930, 1717, 1642, 1539, 1505, 1348 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.71 (m, 1H), 1.92 (m, 2H), 2.08 (m, 1H), 2.34 (t, 2H, J = 7.4 Hz), 2.79 (m, 3H), 3.20 (m, 1H), 3.55 (m, 1H), 4.38 (m, 1H), 6.07 (s, 2H), 6.68 (s, 1H), 7.31 (d, 2H, J = 8.1 Hz), 7.80 (d, 2H, J = 8.2 Hz), 8.53 (d, 1H, J = 7.7 Hz), 10.11 (s, 1H), 12.40 (brs, 2H). Anal. ($C_{20}H_{23}N_5O_6S\cdot1.5H_2O$) C, H, N, S.

3-[2-(2-Amino-4-oxo-4,6,7,8-tetrahydro-3*H*-pyrimido-[5,4-*b*][1,4]thiazin-6-yl)ethyl]benzoic acid: beige powder; mp 253 °C dec; ^{1}H NMR (DMSO-d_6) δ 1.75−1.63 (1H, m), 1.94−1.82 (1H, m), 2.88−2.70 (3H, m), 3.25−3.16 (1H, m), 3.57−3.48 (1H, m), 6.02 (2H, s), 6.66 (1H, s), 7.48−7.35 (2H, m), 7.78−7.72 (2H, m), 10.07 (1H, brs), 12.83 (1H, brs). Anal. ($C_{15}H_{16}N_4O_3S\cdot0.5H_2O$) C, H, N, S.

2(*S*)-[[3-[2-(2-Amino-4-oxo-4,6,7,8-tetrahydro-3*H*-pyrimido[5,4-*b*][1,4]thiazin-6-yl)ethyl]benzoyl]amino]pentanedioic acid (15): off-white soild; mp 188−189 °C dec; ^{1}H NMR (DMSO-d_6) δ 1.77−1.67 (1H, m), 1.98−1.83 (2H, m), 2.11−2.02 (1H, m), 2.34 (2H, t, J = 7.3 Hz), 2.87−2.72 (3H, m), 3.25−3.17 (1H, m), 3.58−3.51 (1H, m), 4.38 (1H, ddd, J = 5.3, 7.6, 12.8 Hz), 6.02 (2H, s), 6.67 (1H, s), 7.44−7.34 (2H, m), 7.77−7.65 (2H, m), 8.56 (1H, d, J = 7.6 Hz), 10.07 (1H, brs), 12.42 (2H, broad). Anal. ($C_{20}H_{23}N_5O_6S\cdot1.1H_2O$) C, H, N, S.

5-[2-(2-Amino-4-oxo-4,6,7,8-tetrahydro-3*H*-pyrimido-[5,4-*b*][1,4]thiazin-6(*R*)-yl)ethyl]thiophene-2-carboxylic acid: off-white solid; mp 283−285 °C dec; [α]$_{589}$ +71.0° (c = 0.60, 1 N NaOH); IR (KBr) 3256 (broad) 2942, 1707, 1641, 1612, 1464, 1364 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.72 (m, 1H), 1.89 (m, 1H), 2.81−3.04 (m, 2H), 3.16−52 (m, 3H, partially obscured by H_2O), 6.08 (s, 2H), 6.68 (s, 1H), 6.92 (d, 1H, J = 4.0 Hz), 7.52 (d, 1H, J = 3.7 Hz), 10.12 (s, 1H), 12.80 (brs, 1H). Anal. ($C_{13}H_{14}N_4O_3S_2\cdot0.60H_2O$) C, H, N, S.

2(*S*)-[[[5-[2-(2-Amino-4-oxo-4,6,7,8-tetrahydro-3*H*-pyrimido[5,4-*b*][1,4]thiazin-6(*R*)-yl)ethyl]thiophene-2-yl]carbonyl]amino]pentanedioic acid (16): off-white solid; mp 191−194 °C foams; [α]$_{589}$ +61.9° (c = 0.65, 1 N NaOH); IR (KBr) 3389, 3235, 3086, 2924, 1701, 1624, 1545, 1340 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.70−2.04 (m, 4H), 2.29 (t, 2H, J = 7.3 Hz), 2.90 (m, 2H), 3.13−3.53 (m, 3H, partially obscured by H_2O), 4.29 (m, 1H), 6.30 (s, 2H), 6.77 (s, 1H), 6.89 (d, 1H, J = 3.7 Hz), 7.66 (d, 1H, J = 3.7 Hz), 8.50 (d, 1H, J = 8.1 Hz), 10.30 (brs, 1H). Anal. ($C_{18}H_{21}N_5O_6S_2\cdot1.8H_2O$) C, H, N, S.

5-[2-(2-Amino-4-oxo-4,6,7,8-tetrahydro-3*H*-pyrimido-[5,4-*b*][1,4]thiazin-6(*S*)-yl)ethyl]thiophene-2-carboxylic acid: off-white solid; mp 258−261 °C dec; [α]$_{589}$ −81.3° (c = 0.63, 1 N NaOH); IR (KBr) 3254 (broad), 2918, 1692, 1635, 1458, 1352 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.72 (m, 1H), 1.89 (m, 1H), 2.80−3.03 (m, 2H), 3.16−3.52 (m, 3H, partially obscured by H_2O), 6.09 (s, 2H), 6.80 (s, 1H), 6.92 (d, 1H, J = 3.7 Hz), 7.52 (d, 1H, J = 3.7 Hz), 10.20 (s, 1H), 12.80 (brs, 1H); HMS calcd for $C_{13}H_{14}N_4O_3S_s$ (M + Na$^+$) 361.0405, found 361.0390.

2(*S*)-[[[5-[2-(2-Amino-4-oxo-4,6,7,8-tetrahydro-3*H*-pyrimido[5,4-*b*][1,4]thiazin-6(*S*)-yl)ethyl]thiophene-2-yl]carbonyl]amino]pentanedioic acid (17): off-white solid; mp 220 °C dec; [α]$_{589}$ −57.1° (c = 0.61, 1 N NaOH); IR (KBr) 3353, 3094, 2926, 1711, 1641, 1605, 1559, 1454, 1400, 1333, 1279 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.70−2.05 (m, 4H), 2.29 (t, 2H, J = 7.4 Hz), 2.87 (m, 2H), 3.15−3.48 (m, 3H, partially obscured by H_2O), 4.29 (m, 1H), 6.03 (s, 2H), 6.66 (s, 1H), 6.89 (d, 1H, J = 3.7 Hz), 7.65 (d, 1H, J = 3.7 Hz), 8.50 (d, 1H, J = 7.7 Hz), 10.05 (s, 1H), 12.50 (brs, 2H). Anal. ($C_{18}H_{21}N_5O_6S_2\cdot1.4H_2O$) C, H, N, S.

5-[2-(2-Amino-4-oxo-4,6,7,8-tetrahydro-3*H*-pyrimido-[5,4-*b*][1,4]thiazin-6(*R*)-yl)ethyl]-4-methylthiophene-2-carboxylic acid: off-white solid; mp 253 °C dec; [α]$_{589}$ +60.7° (c = 0.29, 1 N NaOH); IR (KBr) 3339 (broad), 2922, 1641, 1539, 1451, 1346, 1269 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.64 (m, 1H), 1.82 (m, 1H), 2.09 (s, 3H), 2.78−2.94 (m, 2H), 3.10−3.55 (m, 3H, partially obscured by H_2O), 6.10 (s, 2H), 6.69 (s, 1H), 7.42 (s, 1H), 10.15 (s, 1H), 12.75 (brs, 1H); HMS calcd for $C_{14}H_{16}N_4O_3S_2$ (M + Na$^+$) 375.0562, found 375.0575.

2(*S*)-[[[5-[2-(2-Amino-4-oxo-4,6,7,8-tetrahydro-3*H*-pyrimido[5,4-*b*][1,4]thiazin-6(*R*)-yl)ethyl]-4-methylthiophene-2-yl]carbonyl]amino]pentanedioic acid (18): off-white solid; mp 210 °C dec; [α]$_{589}$ +64.4° (c = 0.45, 1 N NaOH); IR (KBr) 3341 (broad), 2928, 1701, 1638, 1536, 1449, 1340 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.78−2.05 (m, 4H), 2.10 (s, 3H), 2.28 (t, 2H, J = 7.0 Hz), 2.82 (m, 2H), 3.45 (m, 3H, partially obscured by H_2O), 4.25 (m, 1H), 5.98 (s, 2H), 6.65 (s, 1H), 7.54 (s, 1H), 8.38 (d, 1H, J = 7.7 Hz), 10.05 (s, 1H), 12.5 (brs, 2H). Anal. ($C_{19}H_{23}N_5O_6S_2\cdot0.7H_2O$) C, H, N, S.

5-[2-(2-Amino-4(3*H*)-oxo-5,6,7,8-tetrahydropyrimido-[5,6-*b*][1,4]thiazin-6(*S*)-yl)ethyl]-4-methylthiophene-2-carboxylic acid: [α]$_{589}$ −77.9° (c = 0.58, 1 N NaOH); ^{1}H NMR (DMSO-d_6) δ 1.62−1.73 (m, 1H), 1.79−1.92 (m, 1H), 2.12 (s, 3H), 2.81−2.98 (m, 3H), 3.16−3.26 (m, 1H), 3.50−3.58 (m, 1H), 6.21 (brs, 2H), 6.76 (brs, 1H), 7.45 (s, 1H), 10.24 (broad, 1H), 12.76 (broad, 1H). Anal. ($C_{14}H_{16}N_4O_3S_2\cdot1.4H_2O$) C, H, N, S.

2(*S*)-[[[5-[2-(2-Amino-4-oxo-4,6,7,8-tetrahydro-3*H*-pyrimido[5,4-*b*][1,4]thiazin-6(*S*)-yl)ethyl]-4-methylthiophene-2-yl]carbonyl]amino]pentanedioic acid (19): [α]$_{589}$ −36.8° (c = 0.57, 1 N NaOH); ^{1}H NMR (DMSO-d_6) δ 1.61−1.72 (m, 1H), 1.76−1.92 (m, 2H), 1.99−2.08 (m, 1H), 2.12 (s, 3H), 2.31 (t, 2H, J = 7.0 Hz), 2.79−2.94 (m, 3H), 3.17−3.28 (m, 1H), 3.49−3.56 (m, 1H), 4.30 (ddd, 1H, J = 5.7, 7.7, 9.8 Hz), 6.08 (s, 2H), 6.70 (s, 1H), 7.58 (s, 1H), 8.44 (d, 1H, J = 7.7 Hz), 10.12 (brs, 1H), 12.43 (broad, 2H). Anal. ($C_{19}H_{23}N_5O_6S_2\cdot0.75H_2O$) C, H, N, S.

General Procedures for the Coupling of L-Glutamic Acid Diethyl Ester. Method A. 2(*S*)-[[4-[3-[(2,4-Diamino-6-oxo-1,6-dihydropyrimidin-5-yl)sulfanyl]propyl]benzoyl]-amino]pentanedioic Acid Diethyl Ester. A solution of 4-[3-[(2,6-diamino-4(3*H*)-oxopyrimidin-5-yl)sulfanyl]propyl]-benzoic acid (577 mg, 1.8 mmol), 4-methylmorpholine (l mL, 9.1 mmol), and phenyl *N*-phenylphosphoramidochloridate (723 mg, 2.7 mmol) in 1-methyl-2-pyrrolidinone (40 mL) was stirred for 1 h prior to addition of of L-glutamic acid diethyl ester hydrochloride (863 mg, 3.6 mmol). The reaction mixture was stirred overnight at room temperature. After removal of the solvent in vacuo, the residue was partitioned between $CHCl_3$ (30 mL) and water (30 mL). The layers were separated, and the aqueous phase was extracted with $CHCl_3$ (30 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated to give a yellow gum which was purified by flash chromatography with 5% MeOH in EtOAc to give 212 mg (23%) of the product as a white solid: mp 78−81 °C; ^{1}H NMR ($CDCl_3$) δ 1.21 (3H, t, J = 7.1 Hz), 1.29 (3H, t, J = 7.1 Hz), 1.83 (2H, tt, J = 7.0, 7.3 Hz), 2.21−2.09 (2H, m), 2.57−2.25 (4H, m), 2.64 (2H, t, J = 7.3 Hz), 4.09 (2H, q, J = 7.1 Hz), 4.21 (2H, q, J = 7.1 Hz), 4.78 (1H, ddd, J = 5.0, 8.0, 12.8 Hz), 5.84 (2H, brs), 6.64 (2H, brs), 7.16 (2H, d, J = 8.1 Hz), 7.28

(1H, d, J = 8.0 Hz), 7.67 (2H, d, J = 8.1 Hz). Anal. ($C_{23}H_{31}N_5O_6S$) C, H, N, S.

2(*S*)-[[4-[2-[(2,4-Diamino-6-oxo-1,6-dihydropyrimidin-5-yl)sulfanyl]ethyl]benzoyl]amino]pentanedioic Acid Diethyl Ester. Method A: white solid; mp 105−107 °C; ^{1}H NMR (acetone-d_6) δ 1.18 (3H, t, J = 7.1 Hz), 1.23 (3H, t, J = 7.1 Hz), 2.15−2.05 (1H, m), 2.29−2.17 (1H, m), 2.50 (2H, t, J = 7.3 Hz), 2.93−2.78 (4H, m), 4.07 (2H, q, J = 7.1 Hz), 4.15 (2H, q, J = 7.1 Hz), 4.65 (1H, ddd, J = 5.3, 8.0, 13.3 Hz), 6.11 (2H, s), 6.62 (2H, s), 7.31 (2H, d, J = 7.8 Hz), 7.80 (2H, d, J = 7.8 Hz), 7.87 (1H, d, J = 8.0 Hz), 10.72 (1H, s). Anal. ($C_{22}H_{29}N_5O_6S$) C, H, N, S.

2(*S*)-[[[5-[2-[(2,4-Diamino-6-oxo-1,6-dihydropyrimidin-5-yl)sulfanyl]ethyl]thiophene-2-yl]carbonyl]amino]pentanedioic Acid Diethyl Ester. Method A: yellow solid; mp 95−96 °C; ^{1}H NMR (DMSO-d_6) δ 1.15 (3H, t, J = 7.1 Hz), 1.17 (3H, t, J = 7.1 Hz), 2.00−1.91 (1H, m), 2.13−2.01 (1H, m), 2.40 (2H, t, J = 7.4 Hz), 2.70 (2H, t, J = 7.3 Hz), 2.94 (2H, t, J = 7.3 Hz), 4.03 (2H, q, J = 7.1 Hz), 4.09 (2H, q, J = 7.1 Hz), 4.40−4.33 (1H, m), 6.32 (4H, brs), 6.94 (1H, d, J = 3.0 Hz), 7.67 (1H, d, J = 3.0 Hz), 8.61 (1H, d, J = 7.5 Hz), 9.98 (1H, s). Anal. ($C_{20}H_{27}N_5O_6S_2\cdot0.5H_2O$) C, H, N, S.

2(*S*)-[[[5-[3-[(2,4-Diamino-6-oxo-1,6-dihydropyrimidin-5-yl)sulfanyl]propyl]thiophene-2-yl]carbonyl]amino]pentanedioic Acid Diethyl Ester. Method A: off-white solid; mp 136−138 °C; ^{1}H NMR ($CDCl_3$) δ 1.21 (3H, t, J = 7.1 Hz), 1.29 (3H, t, J = 7.1 Hz), 1.89 (2H, quintet, J = 7.2 Hz), 2.18−2.08 (1H, m), 2.34−2.22 (1H, m), 2.45 (2H, t, J = 7.2 Hz), 2.55 (2H, t, J = 7.0 Hz), 2.89 (2H, t, J = 7.2 Hz), 4.10 (2H, q, J = 7.1 Hz), 4.22 (2H, q, J = 7.1 Hz), 4.72 (1H, ddd, J = 4.8, 7.8, 12.6 Hz), 5.55 (2H, broad), 6.29 (2H, broad), 6.72 (1H, d, J = 3.7 Hz), 7.02 (1H, d, J = 7.8 Hz), 7.36 (1H, d, J = 3.7 Hz), 11.24 (1H, brs). Anal. ($C_{21}H_{29}N_5O_6S_2$) C, H, N, S.

Method B. 2(*S*)-[[(5-Bromo-3-methylthiophene-2-yl]-carbonyl]amino]pentanedioic Acid Diethyl Ester (23c). To a stirred solution of of 5-bromo-3-methylthiophene-2-carboxylic acid[48] (10.86 g, 49.1 mmol), HOBT (6.97 g, 51.6 mmol), DIEA (9.0 mL, 51.7 mmol), and L-glutamic acid diethyl ester hydrochloride (12.36 g, 51.6 mmol) in DMF (70 mL) was added EDC (9.89 g, 51.6 mmol). The reaction mixture was stirred under argon for 18 h, poured into H_2O, and extracted with ethyl acetate. The organic layer was washed sequentially with 0.5 N HCl, saturated $NaHCO_3$, and brine, dried ($MgSO_4$), and then concentrated in vacuo. This residue was purified by flash chromatography, eluting with CH_2Cl_2−EtOAc (20:1) to give 19.70 g (99%) of the desired product as a colorless oil: IR (neat) 3329, 2982, 1738, 1651,1545, 1514, 1417, 1377, 1258, 1206 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.24 (3H, t, J = 7.1 Hz), 1.30 (3H, t, J = 7.1 Hz), 2.04−2.45 (4H, m), 2.48 (3H, s), 4.12 (2H, q, J = 7.1 Hz), 4.23 (2H, q, J = 7.1 Hz), 4.71 (1H, ddd, J = 12.3, 7.2, 4.8 Hz), 6.56 (1H, d, J = 7.3 Hz), 6.87 (1H, s). Anal. ($C_{15}H_{20}BrNO_5S$) C, H, Br, N, S.

2(*S*)-[[(5-Bromo-4-methylthiophene-2-yl)carbonyl]amino]pentanedioic Acid Diethyl Ester (23d). Method B: yellow oil; IR (neat) 3339, 2984, 1738, 1634, 1562, 1527, 1425, 1209 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.24 (3H, t, J = 7.1 Hz), 1.30 (3H, t, J = 7.1 Hz), 2.04−2.52 (4H, m), 2.20 (3H, s), 4.12 (2H, q, J = 7.3 Hz), 4.23 (2H, q, J = 7.3 Hz), 4.70 (1H, ddd, J = 12.3, 7.7, 4.8 Hz), 6.84 (1H, d, J = 7.4 Hz), 7.22 (1H, s). Anal. ($C_{15}H_{20}BrNO_5S$) C, H, Br, N, S.

2(*S*)-[[(5-Bromo-3-ethylthiophene-2-yl)carbonyl]amino]-pentanedioic Acid Diethyl Ester (23e). Method B: yellow oil; IR (neat) 3324, 2978, 2936, 1738, 1651, 1543, 1512, 1204 cm $^{-1}$; ^{1}H NMR ($CDCl_3$) δ 1.22−1.33 (3 overlapping triplets, 9H), 2.11 (m, 1H), 2.30 (m, 1H), 2.44 (m, 2H), 2.90 (q, 2H, J = 7.5 Hz), 4.12 (q, 2H, J = 7.1 Hz), 4.23 (q, 2H, J = 7.1 Hz), 4.71 (m, 1H), 6.54 (d, 1H, J = 7.2 Hz), 6.94 (s, 1H). Anal. ($C_{16}H_{22}$-$BrNO_5S$) C, H, Br, N, S.

2(*S*)-[(3-Iodobenzoyl)amino]pentanedioic Acid Diethyl Ester (23f). Method B: white solid; mp 65−66 °C; ^{1}H NMR ($CDCl_3$) δ 1.23 (3H, t, J = 7.1 Hz), 1.30 (3H, t, J = 7.1 Hz), 2.19−2.06 (1H, m), 2.36−2.24 (1H, m), 2.56−2.39 (2H, m), 4.12 (2H, q, J = 7.1 Hz), 4.23 (2H, q, J = 7.1 Hz), 4.75 (1H, ddd, J = 4.8, 7.4, 12.5 Hz), 7.09 (1H, d, J = 7.4 Hz), 7.17 (1H, d, J = 7.8 Hz), 7.76 (1H, dd, J = 1.7, 7.8 Hz), 7.83 (1H, dd, J = 1.7, 7.8 Hz), 8.15 (1H, t, J = 1.7 Hz). Anal. ($C_{16}H_{20}NO_5I$) C, H, N, I.

2(*S*)-[[4-[2-(2-Amino-4-oxo-4,6,7,8-tetrahydro-3*H*-pyrimido[5,4-*b*][1,4]thiazin-6-yl)ethyl]benzoyl]amino]pentanedioic Acid Diethyl Ester. Method B: orange solid; mp 132−136 °C; IR (KBr) 3333, 1732, 1645, 1572, 1535, 1449, 1343, 1203 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.15 (t, 3H, J = 7.3 Hz), 1.17 (t, 3H, J = 7.3 Hz), 1.72 (m, 1H), 1.88−2.10 (m, 3H), 2.42 (t, 2H, J = 7.4 Hz), 2.79 (m, 3H), 3.22 (m, 1H), 3.50 (m, 1H), 4.02 (q, 2H, J = 7.3, 14.5 Hz), 4.09 (q, 2H, J = 7.2, 14.3 Hz), 4.41 (m, 1H), 6.21 (s, 2H), 6.74 (s, 1H), 7.32 (d, 2H, J = 8.0 Hz), 7.80 (d, 2H, J = 8.0 Hz), 8.64 (d, 1H, J = 7.4 Hz), 10.24 (s, 1H). Anal. ($C_{24}H_{31}N_5O_6S$) C, H, N, S.

2(*S*)-[[3-[2-(2-Amino-4-oxo-4,6,7,8-tetrahydro-3*H*-pyrimido[5,4-*b*][1,4]thiazin-6-yl)ethyl]benzoyl]amino]pentanedioic Acid Diethyl Ester. Method B: white solid; mp 69−72 °C; ^{1}H NMR (DMSO-d_6) δ 1.18 (3H, t, J = 7.1 Hz), 1.23 (3H, t, J = 7.1 Hz), 1.89−1.79 (1H, m), 2.00−1.92 (1H, m), 2.11−2.05 (1H, m), 2.29−2.18 (1H, m), 2.50 (2H, t, J = 7.6 Hz), 2.97−2.77 (3H, m), 3.44−3.36 (1H, m), 3.76−3.70 (1H, m), 4.07 (2H, q, J = 7.1 Hz), 4.15 (2H, q, J = 7.1 Hz), 4.66 (1H, ddd, J = 5.1, 7.7, 13.0 Hz), 6.07 (2H, s), 6.12 (1H, s), 7.44−7.33 (2H, m), 7.73 (1H, d, J = 7.7 Hz), 7.82 (1H, s), 8.01−7.95 (1H, m), 10.43 (1H, brs). Anal. ($C_{24}H_{31}N_5O_6S\cdot0.5H_2O$) C, H, N, S.

2(*S*)-[[[5-[2-(2-Amino-4-oxo-4,6,7,8-tetrahydro-3*H*-pyrimido[5,4-*b*][1,4]thiazin-6(*R*)-yl)ethyl]thiophene-2-yl]carbonyl]amino]pentanedioic Acid Diethyl Ester. Method B: white solid; mp 108−112 °C; $[\alpha]_{589}$ +35.0° (c = 0.68, DMSO); IR (KBr) 3343 (broad) 2930, 1732, 1634, 1545, 1450, 1344, 1207 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.13 (t, 3H, J = 7.0 Hz), 1.14 (t, 3H, J = 7.0 Hz), 1.91 (m, 4H), 2.37 (t, 2H, J = 7.3 Hz), 2.89 (m, 2H), 3.23 (m, 2H), 3.49 (m, 1H), 4.00 (q, 2H, J = 7.0 Hz), 4.06 (q, 2H, J = 7.0 Hz), 4.34 (m, 1H), 6.01 (s, 2H), 6.63 (s, 1H), 6.90 (d, 1H, J = 3.7 Hz), 7.66 (d, 1H, J = 3.7 Hz), 8.60 (d, 1H, J = 7.3 Hz), 10.07 (s, 1H). Anal. ($C_{22}H_{29}$-$N_5O_6S_2\cdot1.0H_2O$) C, H, N, S.

2(*S*)-[[[5-[2-(2-Amino-4-oxo-4,6,7,8-tetrahydro-3*H*-pyrimido[5,4-*b*][1,4]thiazin-6(*S*)-yl)ethyl]thiophene-2-yl]carbonyl]amino]pentanedioic Acid Diethyl Ester. Method B: off-white solid; mp 105−110 °C (with foaming); $[\alpha]_{589}$ −55.5° (c = 0.53, DMSO); IR (Br) 3345, 2930, 1734, 1653, 1636, 1541, 1456, 1345 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.12 (t, 3H, J = 7.0 Hz), 1.14 (t, 3H, J = 7.0 Hz), 1.75−2.03 (m, 4H), 2.37 (t, 2H, J = 7.4 Hz), 2.88 (m, 2H), 3.17 (m, 2H), 3.47 (m, 1H), 4.01 (q, 2H, J = 7.0 Hz), 4.07 (q, 2H, J = 7.0 Hz), 4.34 (m, 1H), 6.00 (s, 2H), 6.64 (s, 1H), 6.89 (d, 1H, J = 3.7 Hz), 7.65 (d, 1H, J = 3.7 Hz), 8.60 (d, 1H, J = 7.3 Hz), 10.05 (s, 1H). Anal. ($C_{22}H_{29}N_5O_6S_2\cdot1.0H_2O$) C, H, N, S.

2(*S*)-[[[5-[2-(2-Amino-4-oxo-4,6,7,8-tetrahydro-3*H*-pyrimido[5,4-*b*][1,4]thiazin-6(*R*)-yl)ethyl]-4-methylthiophene-2-yl]carbonyl]amino]pentanedioic Acid Diethyl Ester. Method B: off-white solid; mp 124 °C (with foaming); $[\alpha]_{589}$ +33.6° (c = 0.50, DMSO); IR (KBr) 2996, 2860, 1734, 1653, 1636, 1559, 1456, 1206 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.11 (t, 3H, J = 7.0 Hz), 1.15 (t, 3H, J = 7.0 Hz), 1.75−2.04 (m, 4H), 2.10 (s, 3H), 2.37 (t, 2H, J = 7.4 Hz), 2.85 (m, 2H), 3.12 (m, 2H), 3.50 (m, 1H), 4.00 (q, 2H, J = 7.0 Hz), 4.06 (q, 2H, J = 7.0 Hz), 4.31 (m, 1H), 6.01 (s, 2H), 6.65 (s, 1H), 7.55 (s, 1H), 8.53 (d, 1H, J = 7.7 Hz), 10.08 (s, 1H); HRMS calcd for $C_{23}H_{31}N_5O_6S_2$ (M + Cs^+) 670.0770, found 670.0742. Anal. ($C_{23}H_{31}N_5O_6S_2$) C, H, N, S.

2(*S*)-[[[5-[2-(2-Amino-4-oxo-4,6,7,8-tetrahydro-3*H*-pyrimido[5,4-*b*][1,4]thiazin-6(*S*)-yl)ethyl]-4-methylthiophene-2-yl]carbonyl]amino]pentanedioic Acid Diethyl Ester. Method B: amorphous solid; $[\alpha]_{589}$ −54.1° (c = 0.61, DMSO); ^{1}H NMR (DMSO-d_6) δ 1.15 (t, 3H, J = 7.1 Hz), 1.17 (t, 3H, J = 7.1 Hz), 1.61−1.72 (m, 1H), 1.77−2.07 (m, 3H), 2.13 (s, 3H), 2.40 (t, 2H, J = 7.5 Hz), 2.77−2.94 (m, 3H), 3.18−3.28 (m, 1H), 3.50−3.56 (m, 1H), 4.03 (q, 2H, J = 7.1 Hz), 4.08 (q, 2H, J = 7.1 Hz), 4.34 (ddd, 1H, J = 5.4, 7.7, 9.6 Hz), 6.02 (s, 2H), 6.67 (s, 1H), 7.58 (s, 1H), 8.55 (d, 1H, J = 7.7 Hz), 10.06 (s, 1H). Anal. ($C_{23}H_{31}N_5O_6S_2\cdot0.5H_2O$) C, H, N, S.

General Procedure for the Formation of a TBS Ether. *tert*-Butyldimethyl(2-thiophene-2-ylethoxy)silane. To a

solution of TBSCl (26.38 g, 0.18 mol), TEA (25 mL, 0.18 mol), and DMAP (300 mg, 2.5 mmol) in CH_2Cl_2 (200 mL) under an argon atmosphere at −5 °C was added, dropwise, 2-(2-thienyl)-ethanol (18 mL, 0.16 mol). The resultant mixture was stirred at 0 °C for 30 min and then at room temperature for 18 h. The crude mixture was poured into water (300 mL), and the layers were separated. The organic phase was washed with 0.5 N HCl (200 mL) and then with brine (200 mL), dried (Na_2SO_4), and concentrated. The crude residue was purified by flash chromatography with hexane/EtOAc (95:5) to give 38.73 g (99%) of the product as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 0.03 (6H, s), 0.89 (9H, s), 3.03 (2H, t, J = 6.7 Hz), 3.82 (2H, t, J = 6.7 Hz), 6.83 (1H, d, J = 3.3 Hz), 6.92 (1H, dd, J = 3.3, 5.1 Hz), 7.13 (1H, d, J = 5.1 Hz). Anal. ($C_{12}H_{22}OSSi$) C, H, S.

***tert*-Butyldimethyl(4,5,6,7-tetrahydrobenzo[*b*]thiophene-6-ylmethoxy)silane (28):** colorless oil; ^{1}H NMR ($CDCl_3$) δ 0.06 (6H, s), 0.91 (9H, s), 1.53−1.41 (1H, m), 2.03−1.92 (2H, m), 2.65−2.42 (2H, m), 2.76−2.68 (1H, m), 2.89 (1H, dd, J = 5.2, 16.2 Hz), 3.60 (2H, d, J = 6.2 Hz), 6.75 (1H, d, J = 5.1 Hz), 7.05 (1H, d, J = 5.1 Hz). Anal. ($C_{15}H_{26}OSSi$) C, H, S.

5-[4-[(*tert*-Butyldimethylsilanyl)oxy]-3(*S*)-hydroxybutyl]thiophene-2-carboxylic acid ethyl ester: colorless oil; $[\alpha]_{589}$ −26.6° (c = 0.82, MeOH); IR (neat) 3486 (broad) 2953, 2930, 2859, 1709, 1462, 1259 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.07 (s, 6H), 0.90 (s, 9H), 1.36 (t, 3H, J = 7.2 Hz), 1.78 (m, 2H), 2.96 (m, 2H), 3.43 (m, 1H), 3.64 (m, 2H), 4.32 (q, 2H, J = 7.0 Hz), 6.82 (d, 1H, J = 3.7 Hz), 7.62 (d 1H, J = 4.0 Hz). Anal. ($C_{17}H_{30}O_4SSi$) C, H, S.

5-[4-[(*tert*-Butyldimethylsilyl)oxy]-3(*S*)-hydroxybutyl]-4-methylthiophene-2-carboxylic acid ethyl ester: colorless oil; $[\alpha]_{589}$ −23.5° (c = 1.19, MeOH); ^{1}H NMR ($CDCl_3$) δ 0.07 (s, 6H), 0.90 (s, 9H), 1.35 (t, 3H, J = 7.1 Hz), 1.61−1.81 (m, 2H), 2.17 (s, 3H), 2.79−3.01 (m, 2H), 3.43 (dd, 1H, J = 7.0, 9.6 Hz), 3.63 (dd, 1H, J = 3.3, 9.6 Hz), 3.66−3.71 (m, 1H), 4.31 (q, 2H, J = 7.1 Hz), 7.50 (s, 1H). Anal. ($C_{18}H_{32}O_4SSi$) C, H, S.

4-(2-Bromoethyl)benzoic Acid Methyl Ester. This was prepared using 4-(2-bromoethyl)benzoic and CH_2N_2: yellow oil; ^{1}H NMR ($CDCl_3$) δ 3.23 (2H, t, J = 7.4 Hz), 3.59 (2H, t, J = 7.4 Hz), 3.92 (3H, s), 7.30 (2H, d, J = 8.3 Hz), 8.01 (2H, d, J = 8.3 Hz). Anal. ($C_{10}H_{11}O_2Br$) C, H, Br.

5-Bromothiophene-2-carboxylic Acid Methyl Ester (23b). This was prepared using CH_2N_2 from 5-bromothiophene-2-carboxylate: yellow solid; mp 58−59 °C; ^{1}H NMR ($CDCl_3$) δ 3.87 (3H, s), 7.07 (1H, d, J = 4.0 Hz), 7.55 (1H, d, J = 4.0 Hz). Anal. ($C_6H_5O_2SBr$) C, H, S, Br.

5-(3-Hydroxypropyl)thiophene-2-carboxylic Acid Methyl Ester (24b). To a solution of the above alkyne (7.41 g, 38 mmol) in THF (140 mL) was added 2,4,6-triisopropylbenzenesulfonyl hydrazide (96.13 g, 0.32 mol) in four portions at 90 min intervals. After the reaction had been heated at reflux for a total of 6.5 h, the solvent was removed and the residue was partitioned between 0.5 N NaOH (700 mL) and Et_2O (500 mL). The layers were separated, and the aqueous phase was extracted with Et_2O (250 mL). The combined organic extracts were washed with 0.5 N NaOH (2 × 150 mL), dried (Na_2SO_4), and concentrated to give an oil which was purified by flash chromatography with hexane/EtOAc (2:1) to give 3.67 g (48%) of the product as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 1.96 (2H, tt, J = 6.2, 7.6 Hz), 2.96 (2H, t, J = 7.6 Hz), 3.71 (2H, t, J = 6.2 Hz), 3.86 (3H, s), 6.82 (1H, d, J = 3.8 Hz), 7.64 (1H, d, J = 3.8 Hz). Anal. ($C_9H_{12}O_3S$) C, H, S.

5-(2-Hydroxyethyl)thiophene-2-carboxylic Acid Methyl Ester (24c). To a solution of 2-[2-[(*tert*-butyldimethylsilyl)-oxy]ethyl]thiophene (36.16 g, 0.15 mol) in THF (350 mL) under argon at −75 °C was added a 1.6 M solution of *n*-butyllithium in hexane (140 mL, 0.22 mol). After the mixture was stirred for 1 h at −70 °C, dry CO_2 was then bubbled though this solution, for 40 min at −60 °C, and then for 60 min at −5 °C and an additional 75 min while warming to room temperature. The crude reaction mixture was poured into a mixture of saturated NH_4Cl (600 mL) and ice (600 g) and extracted with Et_2O (300 mL) and then with EtOAc (2 × 300 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated to give 47 g a yellow solid which was used without further purification. The above product (47 g) was dissolved in MeOH (1 L) containing concentrated H_2SO_4 (10 mL), and this solution was heated under refluxed for 18 h. After the solvent was removed by concentration, the residue was partitioned between saturated $NaHCO_3$ (300 mL) and Et_2O (300 mL). The layers were separated and the aqueous phase extracted with EtOAc (2 × 300 mL). The combined organic extracts were dried (Na_2SO_4). The crude residue was purified by flash chromatography with hexane/EtOAc (2:1) to give 12.31 g (44%) of the product as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 3.09 (2H, t, J = 6.2 Hz), 3.86 (3H, s), 3.89 (2H, t, J = 6.2 Hz), 6.88 (1H, d, J = 3.7 Hz), 7.66 (1H, d, J = 3.7 Hz). Anal. ($C_8H_{10}O_3S$) C, H, S.

5-Bromo-3-ethylthiophene-2-carboxylic Acid Methyl Ester. To a stirred solution of 3-ethylthiophene (10.55 g, 94 mmol) and DMF (9.5 mL, 123 mmol) at 0 °C was added $POCl_3$ (11.0 mL, 123 mmol). When the addition was complete, the reaction mixture was heated at 70 °C for about 30 min, cooled to room temperature, poured into H_2O, and extracted with Et_2O. The aqueous layers were made basic with 1 N aqueous NaOH and extracted with Et_2O until free from any UV active compound by TLC. The combined Et_2O layers were washed once with saturated $NaHCO_3$ solution, dried ($MgSO_4$), and concentrated to 13.16 g (quantitative) of an orange oil as a mixture of 3-ethyl 2-carboxaldehyde and 4-ethyl 2-carboxaldehyde in a 5:1 ratio. To this crude mixture dissolved in HOAc was added 5.03 mL (103 mmol) of bromine. After 44 h at 45 °C, the mixture was cautiously poured into saturated $NaHCO_3$ solution and extracted with EtOAc. The combined organic layers were washed with brine solution, dried ($MgSO_4$), and concentrated to give 17.71 g of a crude mixture of bromo carboxaldehydes which were subjected to oxidation conditions without purification. To a stirred solution of silver nitrate (27.46 g, 162 mmol) in H_2O (350 mL) was added a solution of NaOH (12.93 g, 323 mmol) in H_2O (50 mL). The resulting thick brown slurry was stirred at room temperature while the crude aldehyde was added neat dropwise. After 3 h at room temperature, the reaction mixture was filtered washing the filter cake with 2 N NaOH. The filtrate was acidified with 12 N HCl and cooled, and the precipitate was collected, washed with H_2O, and dried. The residue was slurried in hexanes, filtered, and dried to give 6.27 g (28% overall, from 3-ethylthiophene) of 5-bromo-3-ethylthiophene-2-carboxylic acid as a light gray solid: IR (KBr) 2961 (broad), 1674, 1651, 1423, 1279 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.23 (t, 3H, J = 7.5 Hz), 2.99 (q, 2H, J = 7.5 Hz), 6.70 (s, 1H).

7-Oxo-4,5,6,7-tetrahydrobenzo[*b*]thiophene-6-carboxylic Acid Methyl Ester. A solution of 7-oxo-4,5,6,7-tetrahydrobenzothiophene (**25**) (1.37 g, 9 mmol) in DMF (10 mL) was added dropwise, under an argon atmosphere, to a suspension of NaH (800 mg, 20 mmol) in DMF (6 mL). The resultant, purple solution was stirred at ambient temperature for 15 min and then cooled to 0 °C prior to the dropwise addition of dimethyl carbonate (5.35 g, 59 mmol). The resultant reaction mixture was stirred at ambient temperature for 90 min, then poured into water (150 mL), and extracted with Et_2O (3 × 50 mL) and EtOAc (50 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated. The crude residue was purified by flash chromatography with hexane/EtOAc (4:1) to give 1.28 g (68%) of the product as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 2.43−2.33 (1H, m), 2.62−2.50 (1H, m), 2.87 (1H, ddd, J = 4.9, 8.2, 17.1 Hz), 3.05 (1H, ddd, J = 4.9, 6.5, 17.1 Hz), 3.61 (1H, dd, J = 4.8, 9.0 Hz), 3.77 (3H, s), 6.97 (1H, d, J = 4.8 Hz), 7.66 (1H, d, J = 4.8 Hz). Anal. ($C_{10}H_{10}O_3S$) C, H, S.

7-Hydroxy-4,5,6,7-tetrahydrobenzo[*b*]thiophene-6-carboxylic Acid Methyl Ester (26) and 6-(Hydroxymethyl)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-7-ol (27). To a solution of methyl 7-oxo-4,5,6,7-tetrahydrobenzothiophene-6-carboxylate (2.57 g, 12.2 mmol) in THF (15 mL) and MeOH (10 mL) at 0 °C was added, portionwise, $NaBH_4$ (465 mg, 12.2 mmol). The resultant reaction mixture was stirred for 2 h, gradually warming to 15 °C, and then poured into saturated NH_4Cl (30 mL). The layers were separated, and the aqueous phase was extracted with EtOAc (30 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated. The crude residue was purified by flash chromatography with hexane/EtOAc (3:2) to give two separate products. The faster-

eluting product was a yellow oil, 0.91 g (35%). The following analyses indicated that this product was compound **26**: ^{1}H NMR ($CDCl_3$) δ 2.38−2.05 (2H, m), 2.90−2.57 (3H, m), 3.09 (1H, broad), 3.78 (3H, s), 5.23−5.16 (1H, m), 6.78 (1H, d, J = 5.1 Hz), 7.25 (1H, d, J = 5.1 Hz). Anal. ($C_{10}H_{12}O_3S$) C, H, S. The slower-eluting product was a milky-white gum, 1.28 g (57%). The following analyses indicated that this second product was diol **27**: ^{1}H NMR ($CDCl_3$) δ 1.63−1.48 (1H, m), 1.77−1.71 (1H, m), 1.91−1.80 (1H, m), 2.07−1.94 (1H, m), 2.86−2.54 (3H, m), 3.96−3.79 (2H, m), 5.03−4.85 (1H, m), 6.79 (1H, d, J = 5.1 Hz), 7.24 (1H, d, J = 5.1 Hz). Anal. ($C_9H_{12}O_2S$) C, H, S.

4,5,6,7-Tetrahydrobenzo[*b*]thiophene-6-carboxylic Acid Methyl Ester. To a solution of methyl 7-hydroxy-4,5,6,7-tetrahydrobenzothiophene-6-carboxylate (459 mg, 2.2 mmol) and Et_3SiH (510 mg, 4.4 mmol) in CH_2Cl_2 (10 mL), under argon at −5 °C, was added $BF_3{\cdot}Et_2O$ (0.55, 4.5 mmol). The resultant reaction mixture was stirred for 3 h, gradually warming to 15 °C, and then poured into saturated $NaHCO_3$ (30 mL). After addition of K_2CO_3 (∼1 g), the layers were separated and the aqueous phase was extracted with CH_2Cl_2 (10 mL) and Et_2O (2 × 15 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated. The crude residue was purified by flash chromatography with hexane/EtOAc (95:5) to give 300 mg (71%) of the product as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.94−1.81 (1H, m), 2.27−2.18 (1H, m), 2.85−2.59 (3H, m), 3.11−2.94 (2H, m), 3.73 (3H, s), 6.75 (1H, d, J = 5.1 Hz), 7.07 (1H, d, J = 5.1 Hz). Anal. ($C_{10}H_{12}O_2S$) C, H, S.

4,5,6,7-Tetrahydrobenzo[*b*]thiophene-6-yl)methanol. Method A. A solution of methyl 4,5,6,7-tetrahydrobenzothiophene-6-carboxylate (209 mg, 1.1 mmol) in THF (6 mL) was added to a slurry of $LiAlH_4$ (50 mg, 1.3 mmol) in THF (3 mL). The resultant reaction mixture was heated at reflux for 3 h. After being cooled to room temperature, the crude reaction mixture was diluted with saturated NH_4Cl (20 mL). The layers were separated, and the aqueous phase was extracted with Et_2O (10 mL) and then with EtOAc (2 × 10 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated to give the 167 mg (93%) of product as a colorless oil.

Method B. To a solution of 988 mg (5.4 mmol) of 7-hydroxy-6-(hydroxymethyl)-4,5,6,7-tetrahydrobenzothiophene and 1.31 g (11.3 mmol) of Et_3SiH in CH_2Cl_2 (25 mL), under argon at −5 °C, was added 1.62 g (11.3 mmol) of $BF_3{\cdot}Et_2O$. The resultant reaction mixture was stirred for 3 h, gradually warming to 10 °C, and then poured into saturated $NaHCO_3$ (50 mL). After addition of K_2CO_3 (1.5 g), the layers were separated and the aqueous phase was extracted with Et_2O (2 × 40 mL). The combined organic extracts were dried ($MgSO_4$), and the crude residue was purified by flash chromatography with hexane/EtOAc (2:1) to give 593 mg (66%) of the product as a colorless oil. The following analyses indicated that the product was 6-(hydroxymethyl)-5,5,6,7-tetrahydrobenzothiophene: ^{1}H NMR ($CDCl_3$) δ 1.54−1.46 (1H, m), 2.11−1.96 (2H, m), 2.68−2.46 (2H, m), 2.79−2.71 (1H, m), 2.94 (1H, dd, J = 5.2, 16.2 Hz), 3.66 (2H, d, J = 6.4 Hz), 6.76 (1H, d, J = 5.1 Hz), 7.06 (1H, d, J = 5.1 Hz). Anal. ($C_9H_{12}OS$) C, H, S.

2(*S*)-[[[6-[[(*tert*-Butyldimethylsilanyl)oxy]methyl]-4,5,6,7-tetrahydrobenzo[*b*]thiophene-2-yl]carbonyl]amino]pentanedioic Acid Diethyl Ester (29). To a solution of 6-[[(*tert*-butyldimethylsilyl)oxy]methyl]-4,5,6,7-tetrahydrobenzothiophene (7.69 g, 27.2 mmol) in THF (100 mL), under argon at −70 °C, was added a 2.5 M solution of *n*-butyllithium in hexane (12 mL, 30 mmol). The resultant reaction mixture was stirred for an additional 40 min at −70 °C and then at −10 °C for 45 min while dry CO_2 was bubbled though the solution. The crude reaction mixture was subsequently poured into saturated NH_4Cl (300 mL). The layers were separated, and the aqueous phase was extracted with Et_2O (2 × 150 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated to give 8.24 g of a yellow solid. This intermediate was employed in the subsequent reaction without any further purification. The glutamate was coupled using method B described above to give 6.63 g (48%) of the product as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 0.06 (6H, s), 0.90 (9H, s), 1.23 (3H, t, J = 7.1 Hz), 1.29 (3H, t, J = 7.1 Hz), 1.56−1.42 (1H, m), 2.15−1.91 (3H, m), 2.75−2.23 (6H, m), 2.89 (1H, dd, J = 5.1, 16.7 Hz), 3.59 (2H, d, J = 6.2 Hz), 4.11 (2H, q, J = 7.1 Hz), 4.22 (2H, q, J = 7.1 Hz), 4.73 (1H, ddd, J = 4.8, 7.7, 12.6 Hz), 6.67 (1H, d, J = 7.7 Hz), 7.23 (1H, s). Anal. ($C_{25}H_{41}NO_6SSi$) C, H, N, S.

2(*S*)-[[[6-(Hydroxymethyl)-4,5,6,7-tetrahydrobenzo[*b*]-thiophene-2-yl]carbonyl]amino]pentanedioic Acid Diethyl Ester. This material was prepared using the procedure described for 5-(2-hydroxyethyl)thiophene-2-carboxylic acid methyl ester: yellow gum; ^{1}H NMR ($CDCl_3$) δ 1.23 (3H, t, J = 7.1 Hz), 1.29 (3H, t, J = 7.1 Hz), 1.57−1.44 (1H, m), 2.15−1.97 (3H, m), 2.77−2.22 (6H, m), 2.94 (1H, dd, J = 5.0, 16.9 Hz), 3.66 (2H, d, J = 6.3 Hz), 4.11 (2H, q, J = 7.1 Hz), 4.22 (2H, q, J = 7.1 Hz), 4.73 (1H, ddd, J = 4.8, 7.6, 12.6 Hz), 6.70 (1H, d, J = 7.6 Hz), 7.23 (1H, s). Anal. ($C_{19}H_{27}NO_6S{\cdot}0.5H_2O$) C, H, N, S.

1,1-Dimethoxybut-3-yn-2-ol (30). To a stirred solution of TMS acetylene (1.037 g, 10.55 mmol) in dry THF (50 mL) under argon at −78 °C was added dropwise 1.6 M *n*-butyllithium (6.6 mL). After 10 min at −78 °C, a solution of glyoxal dimethyl acetal (1.21 g, 10.46 mmol) in THF (5 mL) was added dropwise. After 1 h at −78 °C, the reaction was quenched with H_2O (1 mL), and the mixture was allowed to warm to room temperature, diluted with EtOAc, and washed with brine. The aqueous layer was reextracted with EtOAc, and the combined organic layers were dried ($MgSO_4$) and concentrated to give 785 mg of the crude addition product as a yellow oil. The crude product was dissolved in THF, and to it was added 5.8 mL of 1.0 M tetrabutylammonium fluoride in THF. After the mixture was heated for 1 h at 50 °C, the volatiles were evaporated and the residue was flash chromatographed on silica with CH_2Cl_2−EtOAc (9:1) to give 412 mg (60% overall) of the alkyne−alcohol **30** as a colorless oil: IR (neat) 3441 (broad), 3277, 2944, 2839, 1636, 1450 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.42 (bs, 1H), 2.49 (s, 1H), 3.51 (s, 3H), 3.53 (s, 3H), 4.36 (bs, 2H). Anal. ($C_6H_{10}O_3{\cdot}0.35H_2O$) C, H.

4-[3-[(Methylsulfonyl)oxy]-4,4-dimethoxybutyl]benzoic Acid Methyl Ester. To a stirred solution of alcohol **31** (206 mg, 0.77 mmol) and TEA (0.16 mL, 1.15 mmol) in CH_2Cl_2 (5 mL) at 0 °C was added methanesulfonyl chloride (0.07 mL, 0.85 mmol). After 20 min at 0 °C, another 0.02 mL more methanesulfonyl chloride was added. After 30 min more, the reaction mixture was poured into saturated $NaHCO_3$ and extracted with 2 × CH_2Cl_2. The combined organic layers were dried ($MgSO_4$). This material was sufficiently pure for use in the next step. An analytical sample was obtained by flash chromatography on silica, eluting with CH_2Cl_2/EtOAc (20:1) to give the mesylate as a colorless oil: IR (neat) 2949, 2839, 1719, 1611, 1437, 1352, 1283 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.04 (m, 2H), 2.77−2.91 (m, 2H), 3.09 (s, 3H), 3.41 (s, 3H), 3.45 (s, 3H), 3.90 (s, 3H), 4.38 (d, 1H, J = 5.5 Hz), 4.64 (m, 1H), 7.29 (d, 2H, J = 8.2 Hz), 7.96 (d, 2H, J = 8.2 Hz). Anal. ($C_{15}H_{22}O_7S$) C, H, S.

4-[3-[(Methylsulfonyl)oxy]-4-oxobutyl]benzoic Acid Methyl Ester. To a stirred solution of the crude dimethyl acetal mesylate from above (600 mg, 1.73 mmol) in $CHCl_3$ (5 mL) at 0 °C was added H_2O (1 mL) and trifluoroacetic acid (1 mL). The reaction was warmed to room temperature and then heated under reflux for 24 h. The cooled reaction mixture was diluted with EtOAc, washed sequentially with saturated NaCl, saturated $NaHCO_3$, and then again with saturated NaCl, and dried ($MgSO_4$), and the volatiles were removed in vacuo. This material was used immediately in the next step: ^{1}H NMR ($CDCl_3$) δ 2.20 (m, 2H), 2.85 (m, 2H), 3.17 (s, 3H), 3.91 (s, 3H), 4.95 (dd, 1H, J = 4.2, 8.4 Hz), 7.29 (d, 2H, J = 8.1 Hz), 7.99 (d, 2H, J = 8.2 Hz), 9.59 (s, 1H).

4-[3-[(4-Methoxybenzyl)thio]-4-oxobutyl]benzoic Acid Methyl Ester. To a stirred solution of the mesylate aldehyde prepared above (686 mg, 2.28 mmol) and DIEA (0.40 mL, 2.29 mmol) in DMF was added 4-methoxy-α-toluenethiol (0.48 mL, 3.44 mmol). After 3 h at room temperature, the reaction mixture was poured into 0.5 N HCl and extracted with EtOAc. The combined organic layers were washed with brine, dried ($MgSO_4$), and concentrated in vacuo. The material obtained was sufficiently pure to use without further purification in the next step: IR (KBr) 2930, 1715, 1703, 1611, 1512, 1282, 1244

cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.80−2.16 (m, 2H), 2.75 (m, 2H), 2.99 (m, 1H), 3.53 (AB, 2H, J = 13.4 Hz), 3.81 (s, 3H), 3.90 (s, 3H), 6.83 (d, 2H, J = 8.5 Hz), 7.12 (d, 2H, J = 8.2 Hz), 7.19 (d, 2H, J = 8.6 Hz), 7.90 (d, 2H, J = 8.2 Hz), 9.27 (d, 1H, J = 4.2 Hz).

4-[3-[1,3]Dioxolan-2-yl-3-[(4-methoxybenzyl)sulfanyl]-propyl]benzoic Acid Methyl Ester. A flask containing the above aldehyde (301 mg, 0.84 mmol), ethylene glycol (94 μL, 1.68 mmol), and pyridinium *p*-toluenesulfonate (42 mg, 0.17 mmol) in C_6H_6 (30 mL) was heated at reflux, removing the generated water with a Dean−Stark trap. After 3 h, the reaction mixture was poured into brine and extracted twice with EtOAc. The combined organic layers were dried ($MgSO_4$), and the crude residue was flash chromatographed on silica eluting hexanes−EtOAc (5:1) to give an 84% overall of the title compound from the mesylate dimethyl acetal: IR (neat) 2949, 2886, 1721, 1611, 1510, 1435, 1279, 1248 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.67 (m, 1H), 1.99 (m, 1H), 2.57 (m, 2H), 2.84 (m, 1H), 3.72−4.03 (m, 6H), 3.81 (s, 3H), 3.90 (s, 3H), 4.97 (d, 1H, J = 4.6 Hz), 6.83 (d, 2H, J = 8.6 Hz), 7.09 (d, 2H, J = 8.1 Hz), 7.23 (d, 2H, J = 8.5 Hz), 7.88 (d, 2H, J = 8.1 Hz). Anal. ($C_{22}H_{26}O_5S$) C, H, S.

4-(3-[1,3]Dioxolan-2-yl-3-mercaptopropyl)benzoic Acid Methyl Ester (32). A stirred solution of the above benzyl thioether (5.70 g, 14.16 mmol) and mercuric acetate (5.42 g, 17.00 mmol) in CH_2Cl_2 cooled to 0 °C was added dropwise to trifluoroacetic acid (5 mL). After 3 h at 0 °C, hydrogen sulfide saturated MeOH was added, and stirring was continued at 0 °C for 20 min. The reaction mixture was poured into brine and extracted with CH_2Cl_2. The combined organic layers were dried ($MgSO_4$) and concentrated at reduced pressure. The crude residue was partially suspended in MeOH, and to this was added 1.07 g (28.28 mmol) of $NaBH_4$ in portions. After about 30 min, the reaction mixture was poured into EtOAc and 0.5 N HCl, and the layers were separated. The metallic mercury that formed in the reaction was washed with EtOAc. The combined organic layers were washed with brine and dried ($MgSO_4$), and the crude residue was flash chomatographed on silica, eluting with hexanes−EtOAc (4:1) to give 1.99 g (50%) of the thiol as a yellow oil: IR (neat) 2951, 2886, 1719, 1611, 1435, 1281 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.64 (d, 1H, J = 7.8 Hz), 1.74 (m, 1H), 2.15 (m, 1H), 2.80 (m, 2H), 3.00 (m, 1H), 3.90 (s, 3H), 3.97 (m, 4H), 4.91 (d, 1H, J = 4.0 Hz), 7.28 (d, 2H, J = 8.1 Hz), 7.95 (d, 2H, J = 8.1 Hz). Anal. ($C_{14}H_{18}O_4S$) C, H.

4-[2-(2-Amino-7-hydroxy-4-oxo-4,6,7,8-tetrahydro-3*H*-pyrimido[5,4-*b*][1,4]thiazin-6-yl)ethyl]benzoic Acid Methyl Ester. A stirred suspension of the above dioxolane (1.20 g, 2.85 mmol) and 2 N HCl (4 mL) in THF (20 mL) was heated under reflux for 2.5 h. The homogeneous solution was poured slowly into saturated $NaHCO_3$, and the precipitate which formed was collected. The filtrate was extracted with EtOAc. A precipitate (58 mg) formed between layers, and it was collected and combined with the first precipitate. The EtOAc layer was dried ($MgSO_4$) and the solvent removed in vacuo. The residue (42 mg) was also combined with the original precipitate to give 984 mg (98%) of the carbinolamine as an orange solid: mp 213−216 °C; IR (KBr) 3351, 3441, 1705, 1638, 1609, 1557, 1470, 1289 cm^{-1}; ^{1}H NMR as a single pair of diastereomers (DMSO-d_6) δ 1.39 and 1.96 (m, m, 1H), 1.70 (m, 1H), 2.56−2.89 (m, 3H), 3.82 (s, 3H), 4.71 and 4.84 (m, m, 1H), 5.37 and 5.40 (d, d, 1H, J = 6.6 Hz), 6.06 (s, 2H), 7.20 (d, 1H, J = 4.5 Hz), 7.31 and 7.36 (d, d, 2H, J = 8.1 Hz), 7.86 and 7.88 (d, d, 2H, J = 8.0 Hz), 10.16 and 10.19 (s, s, 1H). Anal. ($C_{16}H_{18}N_4O_4S\cdot1.7H_2O$) C, H, N, S.

4-[2-(2-Amino-4(3*H*)-oxo-5,6,7,8-tetrahydro[5,6-*b*][1,4]-thiazin-6-yl)ethyl]benzoic Acid Methyl Ester (33). To a 0 °C suspension of the carbinolamine from above (1.126 g, 3.1 mmol) in THF was added boron trifluoride etherate (2.3 mL, 18.64 mmol). When the addition was complete, $NaCNBH_3$ (0.586 g, 9.32 mmol) was added in portions over 5 min. After an additional 30 min, ammonia-saturated MeOH (5 mL) was added, the reaction mixture was diluted with EtOAc and washed with brine. The organic layer was dried ($MgSO_4$), and the crude residue was flash chromatographed on silica, eluting with CH_2Cl_2/MeOH (9:1) to give 542 mg (50%) of the dehydrated product as an orange solid: mp 245−246 °C dec; IR (KBr) 3358, 2936, 1721, 1644, 1595, 1537, 1447, 1346, 1281 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.72 (m, 1H), 1.90 (m, 1H), 2.80 (m, 3H), 3.22 (m, 1H), 3.52 (m, 1H), 3.82 (s, 3H), 6.00 (s, 2H), 6.65 (s, 1H), 7.37 (d, 2H, J = 8.1 Hz), 7.87 (d, 2H, J = 8.1 Hz), 10.05 (s, 1H). Anal. ($C_{16}H_{18}N_4O_3S$) C, H, N, S.

3-[3-[(Methylsulfonyl)oxy]-4,4-dimethoxybutyl]benzoic Acid Methyl Ester. This compound was prepared according to the general procedure described for 4-[3-[(methylsulfonyl)-oxy]-4,4-dimethoxybutyl]benzoic acid methyl ester to give 20.69 g (99%) of a yellow oil: ^{1}H NMR ($CDCl_3$) δ 2.13−1.96 (2H, m), 2.77 (1H, ddd, J = 6.6, 10.0 14.0 Hz), 2.90 (1H, ddd, J = 6.0, 10.1, 14.0 Hz), 3.09 (3H, s), 3.42 (3H, s), 3.45 (3H, s), 3.91 (3H, s), 4.38 (1H, d, J = 5.4 Hz), 4.66 (1H, ddd, J = 3.8, 5.4, 8.8 Hz), 7.36 (1H, t, J = 7.5 Hz), 7.42 (1H, dt, J = 7.5, 1.4 Hz), 7.90−7.85 (2H, m). Anal. ($C_{15}H_{22}O_7S$) C, H, S.

3-[3-[(Methylsulfonyl)oxy]-4-oxobutyl]benzoic Acid Methyl Ester. This compound was prepared according to the general procedure described for 4-[3-[(methylsulfonyl)oxy]-4-oxobutyl]benzoic acid methyl ester to give 15.42 g (87%) of a yellow oil: ^{1}H NMR ($CDCl_3$) δ 2.33−2.09 (2H, m), 2.90−2.83 (1H, m), 3.15−3.08 (1H, m), 3.18 (3H, s), 3.92 (3H, s), 4.95 (1H, dd, J = 4.3, 8.5 Hz), 7.44−7.36 (2H, m), 7.93−7.86 (2H, m), 9.59 (1H, s). Anal. ($C_{13}H_{16}O_6S\cdot0.6H_2O$) C, H, S.

3-[3-[(4-Methoxybenzyl)sulfanyl]-4-oxobutyl]benzoic Acid Methyl Ester. This compound was prepared according to the general procedure described for 4-[3-[(4-methoxybenzyl)-sulfanyl]-4-oxobutyl]benzoic acid methyl ester to give 7.88 g (93%) of a yellow oil: ^{1}H NMR ($CDCl_3$) δ 1.95−1.83 (1H, m), 2.16−2.04 (1H, m), 2.84−2.65 (2H, m), 3.00 (1H, ddd, J = 4.3, 7.4, 11.7 Hz), 3.49 (1H, d, J = 13.3 Hz), 3.59 (1H, d, J = 13.3 Hz), 3.80 (3H, s), 3.91 (3H, s), 6.83 (2H, d, J = 8.6 Hz), 7.19 (2H, d, J = 8.6 Hz), 7.35−7.28 (2H, m), 7.88−7.82 (2H, m), 9.22 (1H, d, J = 4.3 Hz). Anal. ($C_{20}H_{22}O_4S$) C, H, S.

3-[3-[1,3]Dioxolan-2-yl-3-[(4-methoxybenzyl)sulfanyl]-propyl]benzoic Acid Methyl Ester. This compound was prepared according to the general procedure described for 4-[3-[1,3]dioxolan-2-yl-3-[(4-methoxybenzyl)sulfanyl]propyl]-benzoic acid methyl ester to give 10.68 g (95%) of a yellow oil: ^{1}H NMR ($CDCl_3$) δ 1.75−1.61 (1H, m), 2.07−1.95 (1H, m), 2.64−2.54 (2H, m), 2.89 (1H, ddd, J = 4.4, 9.5, 14.2 Hz), 3.80 (3H, s), 3.90 (3H, s), 4.05−3.75 (6H, m), 4.98 (1H, d, J = 4.4 Hz), 6.83 (2H, d, J = 8.7 Hz), 7.24 (2H, d, J = 8.7 Hz), 7.31−7.26 (2H, m), 7.86−7.82 (2H, m). Anal. ($C_{22}H_{26}O_5S$) C, H, S.

3-(3-[1,3]Dioxolan-2-yl-3-mercaptopropyl)benzoic Acid Methyl Ester. This compound was prepared according to the general procedure described for 4-(3-[1,3]dioxolan-2-yl-3-mer-captopropyl)benzoic acid methyl ester to give 3.58 g (47%) of a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.65 (1H, d, J = 7.8 Hz), 1.81−1.67 (1H, m), 2.22−2.07 (1H, m), 2.91−2.73 (2H, m), 3.05−2.96 (1H, m), 3.91 (3H, s), 4.04−3.92 (4H, m), 4.92 (1H, d, J = 4.1 Hz), 7.43−7.31 (2H, m), 7.90−7.84 (2H, m). Anal. ($C_{14}H_{18}O_4S$) C, H, S.

3-[3-[(2,4-Diamino-6-oxo-1,6-dihydropyrimidin-5-yl)-sulfanyl]-3-[1,3]dioxolan-2-ylpropyl]benzoic Acid Methyl Ester. This compound was prepared according to the general procedure described for 4-[3-[(2,4-diamino-6-oxo-1,6-dihydro-pyrimidin-5-yl)sulfanyl]-3-[1,3]dioxolan-2-ylpropyl]benzoic acid methyl ester to give 3.12 g (55%) of a white solid: mp 146−148 °C; ^{1}H NMR (DMSO-d_6) δ 1.67−1.56 (1H, m), 1.91−1.79 (1H, m), 2.57 (1H, ddd, J = 3.8, 8.0, 9.6 Hz), 2.79 (1H, ddd, J = 6.7, 9.6, 13.6 Hz), 3.12 (1H, ddd, J = 4.4, 9.2, 14.1 Hz), 3.81−3.74 (2H, m), 3.83 (3H, s), 3.91−3.84 (2H, m), 4.83 (1H, d, J = 4.4 Hz), 6.33 (4H, broad), 7.38 (1H, t, J = 7.7 Hz), 7.48 (1H, d, J = 7.7 Hz), 7.76−7.72 (2H, m), 10.00 (1H, s). Anal. ($C_{18}H_{22}N_4O_5S\cdot0.6H_2O$) C, H, N, S.

3-[2-(2-Amino-7-hydroxy-4-oxo-4,6,7,8-tetrahydro-3*H*-pyrimido[5,4-*b*][1,4]thiazin-6-yl)ethyl]benzoic Acid Methyl Ester. This compound was prepared according to the general procedure described for 4-[2-(2-amino-7-hydroxy-4-oxo-4,6,7,8-tetrahydro-3*H*-pyrimido[5,4-*b*][1,4]thiazin-6-yl)ethyl]-benzoic acid methyl ester to give 1.23 g (48%) of an orange solid: mp 105−108 °C; ^{1}H NMR (DMSO-d_6) δ 1.55−1.35 (1H, m), 1.78−1.60 (1H, m), 2.88−2.54 (3H, m), 3.83 (3H, s), 4.74−4.69 (1H, m), 5.38 (1H, d, J = 6.6 Hz), 5.41, 6.06 (2H, s), 7.21 (1H, d, J = 4.8 Hz), 7.52−7.39 (2H, m), 7.80−7.74 (2H, m), 10.20 (1H, s). Anal. ($C_{16}H_{18}N_4O_4S\cdot0.5H_2O$) C, H, N, S.

Methyl 3-[2-(2-Amino-4(3*H*)-oxo-5,6,7,8-tetrahydropyrimido[5,6-*b*][1,4]thiazin-6-yl)ethyl]benzoate. This compound was prepared according to the general procedure described for methyl 4-[2-(2-amino-4(3*H*)-oxo-5,6,7,8-tetrahydro[5,6-*b*][1,4]thiazin-6-yl)ethyl]benzoate to give 308 mg (46%) of an orange solid: mp 120 °C dec; ^{1}H NMR (DMSO-d_6) δ 1.75−1.64 (1H, m), 1.94−1.82 (1H, m), 2.89−2.71 (3H, m), 3.23−3.16 (1H, m), 3.56−3.38 (1H, m), 3.83 (3H, s), 6.01 (2H, s), 6.66 (1H. s), 7.53−7.41 (2H, m), 7.80−7.76 (2H, m), 10.06 (1H, s). Anal. ($C_{16}H_{18}N_4O_3S\cdot0.7H_2O$) C, H, N, S.

5-(3(*S*),4-Dihydroxybutyl)thiophene-2-carboxylic Acid Ethyl Ester (35a). To a stirred solution of 5-[2-(2,2-dimethyl-[1,3]dioxolan-4(*S*)-yl)ethyl]thiophene-2-carboxylic acid ethyl ester (65.98 g, 232 mmol) in EtOH (300 mL) was added *p*-toluenesulfonic acid monohydrate (17.65 g, 93 mmol). The reaction mixture was heated at 65 °C for 3 h, concentrated in vacuo, and redissolved in EtOH, and heating was continued. This procedure was repeated until starting material had disappeared by TLC. The crude reaction was diluted with EtOAc, washed with saturated $NaHCO_3$ and then with saturated NaCl, dried ($MgSO_4$), and concentrated in vacuo. The residue was recrystallized from Et_2O/hexanes, yielding 49.90 g of the desired diol. The mother liquors were flash chomatographed on silica gel, eluting with 30−100% EtOAc in CH_2Cl_2 to give an additional 5.81 g of product. The combined yield was 55.71 g (98%): $[\alpha]_{589}$ −28.7° (c = 0.82, MeOH); IR (neat) 3393 (broad), 2936, 1705, 1462, 1287 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.36 (t, 3H, J = 7.2 Hz), 1.7 (brs, 2H), 1.76 (m, 2H), 2.99 (m, 2H), 3.48 (dd, 1H, J = 7.4, 3.5 Hz), 3.68 (dd, 1H, J = 7.6, 3.2 Hz), 3.77 (m, 1H), 4.32 (q, 2H, J = 7.2 Hz), 6.82 (d, 1H, J = 3.9 Hz), 7.63 (d, 1H, J = 3.7 Hz). Anal. ($C_{11}H_{16}O_4S$) C, H, S.

5-[3(*S*)-Hydroxy-4-[[*p*-tolylmethyl)sulfonyl]oxy]butyl]-thiophene-2-carboxylic Acid Ethyl Ester. To an ice-cold solution of diol **35a** (45.42 g, 186 mmol) and TEA (31.1 mL, 223 mmol) in CH_2Cl_2 (400 mL) was added *p*-toluenesulfonyl chloride (38.99 g, 204 mmol). The ice bath was removed, the reaction mixture was stirred at room temperature for 18 h and poured into 0.5 N HCl, and the layers were separated. The aqueous layer was reextracted with CH_2Cl_2. The combined organic layers were washed with brine and dried ($MgSO_4$). The crude residue was purified by flash chromatography on silica gel, eluting with 4−100% EtOAc in CH_2Cl_2 until product eluted and then 0−10% MeOH in EtOAc to elute the starting diol. In this manner there were recovered 7.43 g of diol **35a** and 49.23 g (66%) of the monotosylate as a lightly colored oil which solidified on standing: $[\alpha]_{589}$ −8.2° (c = 0.74, MeOH); IR (neat) 3507 (broad) 2947, 1705, 1462, 1362, 1287 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.36 (t, 3H, J = 6.9 Hz), 1.78 (m, 2H), 2.46 (s, 3H), 2.95 (m, 2H), 3.90 (m, 2H), 4.03 (m, 1H), 4.32 (q, 2H, J = 7.0 Hz), 6.78 (d, 1H, J = 3.7 Hz), 7.36 (d, 2H, J = 8.2 Hz), 7.61 (d, 1H, J = 3.7 Hz), 7.79 (d, 2H, J = 8.2 Hz). Anal. ($C_{18}H_{22}O_6S_2$) C, H, S.

5-(4-Azido-3(*S*)-hydroxybutyl)thiophene-2-carboxylic Acid Ethyl Ester. To a stirred solution of the monotosylate prepared above (42.94 g, 108 mmol) in DMF (250 mL) was added NaN_3 (31.52 g, 485 mmol). The solution was heated under argon for 4 h at 80 °C. The cooled reaction mixture was poured into 600 mL of brine, and the oil was separated. The aqueous solution was extracted three times with Et_2O. The Et_2O extracts were combined with the oil, washed once with brine, dried ($MgSO_4$), and concentrated to yield 33.0 g of crude azide which was sufficiently pure to use in the next step. An analytical sample was obtained by flash chromatography on silica gel eluting CH_2Cl_2/EtOAc (20:1) to yield a colorless oil: $[\alpha]_{589}$ −18.8° (c = 0.84, MeOH); IR (neat) 3445 (broad), 2926, 2099, 1705, 1539, 1460, 1281 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.36 (t, 3H, J = 7.1 Hz), 1.85 (m, 2H), 2.99 (m, 2H), 3.35 (m, 2H), 3.79 (m, 1H), 4.32 (q, 2H, J = 7.1 Hz), 6.82 (d, 1H, J = 3.6 Hz), 7.63 (d, 1H, J = 4.0 Hz). Anal. ($C_{11}H_{15}N_3O_3S_2$) C, H, N, S.

5-[4-[(*tert*-Butoxycarbonyl)amino]-3(*S*)-hydroxybutyl]-thiophene-2-carboxylic Acid Ethyl Ester (36a). A Parr flask containing the azide prepared above (32.70 g, 121 mmol), di-*tert*-butyl dicarbonate (27.83 g, 127 mmol), 5% Pd/C (4.5 g), and THF (300 mL) was shaken under 30 psi of H_2. The exothermic reaction was kept below 45 psi. After 2 h, the crude mixture was filtered though a pad of Celite and the filtrate concentrated. The residue was purified by flash chromatography on silica gel, eluting with 6−50% EtOAc in CH_2Cl_2 to give 35.04 g (84%) of the desired product as a colorless oil: $[\alpha]_{589}$ −24.8° (c = 0.86, MeOH); IR (neat) 3391 (broad), 2980, 1723, 1674, 1537, 1462, 1368, 1283 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.36 (t, 3H, J = 7.0 Hz), 1.45 (s, 9H), 1.81 (m, 2H), 2.94−3.07 (m, 3H), 3.26 (m, 1H), 3.75 (m, 1H), 4.32 (q, 2H, J = 7.1 Hz), 4.9 (brs, 1H), 6.82 (d, 1H, J = 3.7 Hz), 7.62 (d, 1H, J = 4.0 Hz). Anal. ($C_{16}H_{25}NO_5S$) C, H, N, S.

5-[4-[(*tert*-Butoxycarbonyl)amino]-3(*S*)-[(methylsulfonyl)oxy]butyl]thiophene-2-carboxylic Acid Ethyl Ester. To an ice-cold solution of the alcohol **36a** (34.34 g, 100 mmol) and TEA (20.9 mL, 150 mmol) in CH_2Cl_2 (250 mL) was added methanesulfonyl chloride (9.3 mL, 120 mmol). After 30 min the reaction mixture was poured into 0.5 N HCl, and the layers were separated. The organic layer was washed with saturated $NaHCO_3$ and then with brine, dried ($MgSO_4$), and concentrated to give 38.36 g (91%) of the crude mesylate as a yellow oil, which solidified on standing. This material was used without further purification: ^{1}H NMR ($CDCl_3$) δ 1.36 (t, 3H, J = 7.0 Hz), 1.44 (s, 9H), 2.09 (m, 2H), 3.00 (m, 2H), 3.08 (s, 3H), 3.34−3.56 (m, 2H), 4.32 (q, 2H, J = 7.0 Hz), 4.79 (m, 1H), 4.95 (br t, 1H), 6.82 (d, 1H, J = 3.7 Hz), 7.62 (d, 1H, J = 3.8 Hz).

2-[[1-[[(*tert*-Butoxycarbonyl)amino]methyl]-3(*R*)-[5-(ethoxycarbonyl)thiophene-2-yl]propyl]thio]malonic Acid Dimethyl Ester. To an ice-cold stirred solution of the crude thiolacetate from above (39.77 g, 99.0 mmol) and dimethyl chloromalonate (13.91 mL, 109.0 mmol) in MeOH (350 mL) was added K_2CO_3 (27.38 g, 198.1 mmol). After 3 h at 0 °C, the reaction mixture was stirred at room temperature for 2 h. After the mixture was poured into H_2O and extracted with EtOAc (3×), the combined organic layers were washed with brine, dried ($MgSO_4$), and concentrated in vacuo to yield, instead of the desired malonate, 49.80 g of the crude disulfide. To a stirred solution of this disulfide (11.44 g, 15.96 mmol) in EtOH under argon was added $NaBH_4$ (1.81 g, 47.85 mmol). After 4 h, the reaction was quenched with 0.5 N HCl, and the mixture was diluted with EtOAc and washed with more 0.5 N HCl. The organic layer was washed twice with brine, dried ($MgSO_4$), and concentrated in vacuo to yield 10.88 g of the crude thiol/disulfide mixture, which was dissolved in degassed MeOH. To this stirred solution were added dimethyl chloromalonate (5.79 mL, 45.36 mmol) and potassium carbonate (8.36 g, 60.49 mmol). After 30 min of stirring under argon, the reaction mixture was diluted with EtOAc, washed twice with brine, dried ($MgSO_4$), and concentrated. The residue was purified by flash chromatography on silica gel eluting hexanes/EtOAc (3:1) to give 4.20 g of disulfide which was recycled and 6.96 g (45%) of the desired malonate as a light yellow oil: $[\alpha]_{589}$ +31.9° (c = 0.64, MeOH); IR (neat) 3397, 2978, 2938, 1759, 1715, 1505, 1454, 1275 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.36 (t, 3H, J = 7.0 Hz), 1.46 (s, 9H), 1.86 (m, 1H), 2.03 (m, 1H), 3.04 (m, 3H), 3.31 (m, 2H), 3.81 (s, 6H), 4.24 (s, 1H), 4.34 (q, 2H, J = 7.0 Hz), 5.13 (brs, 1H), 6.83 (d, 1H, J = 3.7 Hz), 7.64 (d, 1H, J = 3.7 Hz). Anal. ($C_{21}H_{31}NO_8S_2$) C, H, N, S.

6-[2-[5-(Ethoxycarbonyl)thiophene-2-yl]ethyl]-3(*R*)-oxothiomorpholine-2-carboxylic Acid Ethyl Ester (37a). To an ice-cold solution of the malonate prepared above (29.18 g, 59.60 mmol) in CH_2Cl_2 (225 mL) was added trifluoroacetic acid (35 mL). After 1 h at 0 °C, the reaction mixture was diluted with CH_2Cl_2 and continuously washed with saturated $NaHCO_3$, making sure the aqueous layer was alkaline. The organic layer was washed with brine, dried ($MgSO_4$), and concentrated. The crude amine was dissolved in MeOH and stirred for 2 h at room temperature. The crude lactam was flash chromatographed on silica gel, eluting with CH_2Cl_2/EtOAc (2:1) to give 17.74 g (83%) of the desired product as an amber oil: $[\alpha]_{589}$ +30.9° (c = 0.92, MeOH); IR (neat) 3314, 3242, 2951, 1732, 1660, 1462, 1294 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.36 (t, 3H, J = 7.0 Hz), 1.94 (m, 2H), 2.95−3.25 (m, 2H), 3.42−3.67 (m, 3H), 3.79 and 3.82 (s, s, 3H), 4.14 and 4.25 (s, s, 1H), 4.32 (q, 2H, J = 7.0 Hz), 6.23 (m, 1H), 6.82 (d, 1H, J = 3.7 Hz), 7.63 (d, 1H, J = 3.7 Hz). Anal. ($C_{15}H_{19}NO_5S_2$) C, H, N, S.

6-[2-[5-(Ethoxycarbonyl)thiophene-2-yl]ethyl]-3(*R*)-methoxy-5,6-dihydro-2*H*-[1,4]thiazine-2-carboxylic Acid Ethyl Ester. To a stirred solution of lactam **37a** (11.8 g, 33.01 mmol) in CH_2Cl_2 (70 mL) was added trimethyloxonium tetrafluoroborate (6.835 g, 46.21 mmol). After 1 h of stirring under argon at room temperature all the starting lactam was consumed. The reaction mixture was cooled, and 50% aqueous K_2CO_3 was added until the pH was alkaline. The KBF_4 was filtered off, and the aqueous layer was extracted with CH_2Cl_2. The combined organic layers were washed with brine, dried (Na_2SO_4), and concentrated to give 11.55 g (94%) of the crude lactim ether which was used without further purifiction.

5-[2-(2-Amino-4-oxo-4,6,7,8-tetrahydro-3*H*-pyrimido-[5,4-*b*]thiazin-6(*R*)-yl)ethyl]thiophene-2-carboxylic Acid Ethyl Ester. A solution of sodium ethoxide was prepared by dissolving sodium metal (0.845 g, 36.75 mmol) in degassed absolute ethanol under argon. To this solution was added guanidine hydrochloride (3.63 g, 38.00 mmol). After 15 min of stirring, a solution of the lactim ether prepared above (4.55 g, 12.25 mmol) in degassed absolute EtOH was added, and the mixture was heated at reflux for 1 h. The cooled reaction was neutralized with 0.5 N HCl, diluted with EtOAc, and extracted repeatedly. The combined EtOAc layers were washed with brine, dried ($MgSO_4$), and concentrated. The solid residue was slurried in hot EtOH, cooled, and filtered to give 1.75 g (33%) of the desired product as a light yellow solid: mp 166 °C foams; $[\alpha]_{589}$ +57.7° (*c* = 0.62, DMSO); IR (KBr) 3349 (broad) 2926, 1701, 1640, 1603, 1537, 1458, 1344, 1285 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.23 (t, 3H, *J* = 7.0 Hz), 1.80 (m, 2H), 2.82−3.00 (m, 2H), 3.15−3.52 (m, 3H), 4.21 (q, 2H, *J* = 7.0 Hz), 6.00 (s, 2H), 6.64 (s, 1H), 6.95 (d, 1H, *J* = 3.7 Hz), 7.59 (d, 1H, *J* = 3.7 Hz), 10.04 (s, 1H); HMS calcd for $C_{15}H_{18}N_4O_3S_2$ ($M + Na^+$) 389.0718, found 389.0731.

5-[4-[(*tert*-Butyldimethylsilanyl)oxy]-3(*S*)-[(methylsulfonyl)oxy]butyl]thiophene-2-carboxylic Acid Ethyl Ester. To an ice-cold stirred solution of the mono TBS alcohol prepared above (23.19 g, 64.68 mmol) and TEA (13.5 mL, 96.86 mmol) in CH_2Cl_2 (200 mL) was added methanesulfonyl chloride (6.0 mL, 77.52 mmol). After 30 min at 0 °C, the reaction was warmed to room temperature. After an additional 30 min, the reaction mixture was diluted with CH_2Cl_2 and washed sequentially with 0.5 N HCl, saturated $NaHCO_3$, and brine. The organic layer was dried ($MgSO_4$) and the solvent removed to give 26.61 g (94%) of the desired mesylate as a colorless oil which was used without further purification: ^1H NMR ($CDCl_3$) δ 0.11 (s, 3H), 0.13 (s, 3H), 0.92 (s, 9H), 1.39 (t, 3H, *J* = 7.0 Hz), 2.08 (m, 2H), 3.03 (m, 2H), 3.10 (s, 3H), 3.80 (m, 2H), 4.36 (q, 2H, *J* = 7.0 Hz), 4.75 (m, 1H), 6.88 (d, 1H, *J* = 3.7 Hz), 7.66 (d 1H, *J* = 3.7 Hz).

5-[4-Hydroxy-3(*S*)-[(methylsulfonyl)oxy]butyl]thiophene-2-carboxylic Acid Ethyl Ester. To a stirred solution of the above mesylate (26.56 g, 60.83 mmol) in THF (200 mL) was added a 1.0 M solution of tetrabutylammonium fluoride in THF (67 mL, 67.00 mmol). After 1.5 h of stirring at room temperature, the reaction mixture was diluted with EtOAc and washed with 0.5 N HCl. The aqueous layer was reextracted with EtOAc. The combined organic layers were washed twice with brine and dried ($MgSO_4$), and the crude residue was flash chromatographed on silica gel, eluting with 0−20% EtOAc in CH_2Cl_2 to give 12.465 g (64%) of the desired alcohol as a colorless oil which solidified on standing: $[\alpha]_{589}$ −4.3° (*c* = 0.78, MeOH); IR (neat) 3520, 2982, 2940, 1705, 1684, 1462, 1345, 1287 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.36 (t, 3H, *J* = 7.0 Hz), 2.09 (m, 2H), 3.01 (m, 2H), 3.12 (s, 3H), 3.80 (m, 2H), 4.32 (q, 2H, *J* = 7.0 Hz), 4.81 (m, 1H), 6.86 (d, 1H, *J* = 3.7 Hz), 7.63 (d, 1H, *J* = 3.7 Hz). Anal. ($C_{12}H_{18}O_6S_2$) C, H, S.

5-[2(*R*)-Oxiranylethyl]thiophene-2-carboxylic Acid Ethyl Ester. To an ice-cold stirred solution of the mesylate alcohol prepared above (559 mg, 1.73 mmol) in THF (10 mL) was added a 60% dispersion of NaH in mineral oil (76 mg, 1.90 mmol). After 1 h, the ice bath was removed, and the reaction mixture was stirred at room temperature. After 18 h, additional 60% dispersion NaH (35 mg, 0.88 mmol) was added. After 4 days more, the reaction mixture was diluted with EtOAc, washed with 0.5 N HCl and then with brine, and dried ($MgSO_4$), and the crude residue was flash chomatographed on silica gel, eluting with CH_2Cl_2 to give 322 mg (82%) of the desired epoxide as a colorless oil: $[\alpha]_{589}$ +19.6° (*c* = 1.14, MeOH); IR (neat) 2984, 1707, 1460, 1283, 1262 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.36 (t, 3H, *J* = 7.0 Hz), 1.80−2.05 (m, 2H), 2.51 (dd, 1H, *J* = 4.8, 2.6 Hz), 2.78 (dd, 1H, *J* = 4.8, 4.1 Hz), 2.99 (m, 3H), 4.32 (q, 2H, *J* = 7.0 Hz), 6.82 (d, 1H, *J* = 3.7 Hz), 7.63 (d, 1H, *J* = 3.7 Hz). Anal. ($C_{11}H_{14}O_3S$) C, H, S.

5-(4-Azido-3(*R*)-hydroxybutyl)thiophene-2-carboxylic Acid Ethyl Ester. To a stirred solution of the above epoxide (200 mg, 0.88 mmol) in CH_3CN (10 mL) was added magnesium perchlorate (296 mg, 1.33 mmol). After 10 min, the reaction mixture became homogeneous and NaN_3 (144 mg, 2.21 mmol) was added. The reaction mixture was heated at 75 °C. After 18 h, NaN_3 (25 mg, 0.39 mmol) was added, and heating was continued for 18 h more. The cooled reaction mixture was diluted with EtOAc and washed with brine. The aqueous layer was reextracted with EtOAc. The combined organic layers were washed again with brine and dried ($MgSO_4$), and the crude residue was flash chromatographed on silica gel, eluting with 20−25% EtOAc in hexanes to give 187 mg (79%) of the azide as a colorless oil: $[\alpha]_{589}$ +18.9° (*c* = 0.62, MeOH); IR (neat) 3462 (broad) 2982, 2936, 2101, 1705, 1682, 1462, 1269 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.36 (t, 3H, *J* = 7.0 Hz), 1.85 (m, 2H), 2.99 (m, 2H), 3.30 (m, 2H), 3.79 (m, 1H), 4.32 (q, 2H, *J* = 7.0 Hz), 6.82 (d, 1H, *J* = 3.7 Hz), 7.63 (d, 1H, *J* = 3.7 Hz). Anal. ($C_{11}H_{15}N_3O_3S$) C, H, N, S.

5-[4-[(*tert*-Butoxycarbonyl)amino]-3(*R*)-hydroxybutyl]-thiophene-2-carboxylic Acid Ethyl Ester (36b). This compound was prepared as described for compound **36a**: colorless oil; $[\alpha]_{589}$ +26.3° (*c* = 0.76, MeOH); IR (neat) 3378 (broad) 2980, 2934, 1715, 1682, 1516, 1462, 1294 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.33 (t, 3H, *J* = 7.0 Hz), 1.44 (s, 9H), 1.79 (m, 2H), 2.22 (brs, 1H), 3.04 (m, 3H), 3.25 (m, 1H), 3.73 (m, 1H), 4.32 (q, 2H, *J* = 7.0 Hz), 6.81 (d, 1H, *J* = 3.7 Hz), 7.62 (d, 1H, *J* = 3.7 Hz). Anal. ($C_{16}H_{25}NO_5S$) C, H, N, S.

5-[4-[(*tert*-Butoxycarbonyl)amino]-3(*R*)-[(methylsulfonyl)oxy]butyl]thiophene-2-carboxylic Acid Ethyl Ester. Starting with alcohol **36b**, the title compound was prepared in 94% crude yield according to the general procedure described for 5-[4[(-*tert*-butoxycarbonyl)amino]-3(*S*)-[(methylsulfonyl)oxy]butyl]thiophene-2-carboxylic acid ethyl ester: ^1H NMR ($CDCl_3$) δ 1.36 (t, 3H, *J* = 7.0 Hz), 1.45 (s, 9H), 2.07 (m, 2H), 3.00 (m, 2H), 3.06 (s, 3H), 3.37−3.50 (m, 2H), 4.32 (q, 2H, *J* = 7.0 Hz), 4.78 (m, 1H), 4.91 (br t, 1H), 6.84 (d, 1H, *J* = 4.0 Hz), 7.62 (d, 1H, *J* = 4.0 Hz).

2-[[1-[[(*tert*-Butoxycarbonyl)amino]methyl]-3(*S*)-[5-(ethoxycarbonyl)thiophene-2-yl]propyl]thio]malonic Acid Dimethyl Ester. To a stirred solution of the thioacetate prepared above (8.44 g, 21.0 mmol) and dimethyl chloromalonate (3.22 mL, 25.2 mmol) in degassed MeOH (75 mL) at 0 °C was added K_2CO_3 (5.81 g, 42.0 mmol). The reaction mixture, under an Ar atmosphere, was allowed to stir for 1 h at 0 °C and then for 1.5 h at room temperature, poured into brine, and extracted twice with EtOAc. The combined organic layer was washed twice more with brine solution and dried ($MgSO_4$). The crude residue was purified by flash chromatography on silica gel, eluting with hexanes/EtOAc (3:1) to give 9.678 g (94%) of the desired malonate as a yellow oil: $[\alpha]_{589}$ −32.8° (*c* = 0.67, MeOH); IR (neat) 3395 (broad), 2978, 1755, 1715, 1699, 1462, 1261 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.36 (t, 3H, *J* = 7.0 Hz), 1.44 (s, 9H), 1.86 (m, 1H), 2.05 (m, 1H), 3.02 (m, 3H), 3.31 (m, 2H), 3.79 (s, 6H), 4.23 (s, 1H), 4.33 (q, 2H, *J* = 7.0 Hz), 6.81 (d, 1H, *J* = 3.7 Hz), 7.62 (d, 1H, *J* = 3.7 Hz). Anal. ($C_{21}H_{31}NO_8S_2$) C, H, N, S.

6-[2-[5-(Ethoxycarbonyl)thiophene-2-yl]ethyl]-3(*S*)-oxothiomorpholine-2-carboxylic Acid Ethyl Ester (37b). Starting with the malonate prepared above, compound **37b** was prepared in 86% yield according to the general procedure described for compound **37a**: $[\alpha]_{589}$ −30.5° (*c* = 1.01, MeOH); IR (neat) 3320, 3229, 2951, 1738, 1703, 1669, 1460, 1281 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.36 (t, 3H, *J* = 7.0 Hz), 1.91−2.04 (m, 2H), 2.95−3.25 (m, 2H), 3.42−3.65 (m, 3H), 3.79 and 3.82 (s, s, 3H), 4.13 and 4.25 (s, s, 1H), 4.32 (q, 2H, *J* = 7.0 Hz), 6.26 (m, 1H), 6.82 (d, 1H, *J* = 3.7 Hz), 7.64 (d, 1H, *J* = 3.7 Hz). Anal. ($C_{15}H_{19}NO_5S_2$) C, H, N, S.

6-[2-[5-(Ethoxycarbonyl)thiophene-2-yl]ethyl]-3(*S*)-methoxy-5,6-dihydro-2*H*-[1,4]thiazine-2-carboxylic Acid Ethyl Ester. Starting with lactam **37b**, the title compound was prepared in quantitative crude yield according to the general procedure described for compound 6-[2-[5-(ethoxycarbonyl)thiophene-2-yl]ethyl]-3(*R*)-methoxy-5,6-dihydro-2*H*-[1,4]-thiazine-2-carboxylic acid ethyl ester and was used in the next step without purification.

5-[2-(2-Amino-4-oxo-4,6,7,8-tetrahydro-3*H*-pyrimido-[5,4-*b*]thiazin-6(*S*)-yl)ethyl]thiophene-2-carboxylic Acid Ethyl Ester. Starting with the lactim ether prepared above, the title compound was prepared in 45% yield according to the general procedure described for compound 5-[2-(2-amino-4-oxo-4,6,7,8-tetrahydro-3*H*-pyrimido[5,4-*b*]thiazin-6(*R*)-yl)ethyl]thiophene-2-carboxylic acid ethyl ester: mp 181−184 °C; $[\alpha]_{589}$ −59.7° (*c* = 0.38, DMSO); IR (KBr) 3335, 2926, 1705, 1632, 1593, 1456, 1335, 1260 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.23 (t, 3H, *J* = 7.0 Hz), 1.72 (m, 1H), 1.88 (m, 1H), 2.81−3.00 (m, 2H), 3.16−3.52 (m, 3H), 4.22 (q, 2H, *J* = 7.0 Hz), 6.00 (s, 2H), 6.65 (s, 1H), 6.96 (d, 1H, *J* = 3.7 Hz), 7.60 (d, 1H, *J* = 3.7 Hz), 10.04 (s, 1H). Anal. ($C_{15}H_{18}N_4O_3S_2 \cdot 0.4H_2O \cdot 0.5EtOH$) C, H, N, S.

5-(3(*S*),4-Dihydroxybutyl)-4-methylthiophene-2-carboxylic Acid Ethyl Ester (35b). Starting with the dioxolane prepared above, the title compound was prepared in 92% yield according to the general procedure described for compound **35a**: $[\alpha]_{589}$ −24.3° (*c* = 0.61, MeOH); IR (KBr) 3264 (broad), 2924, 1707, 1458, 1447, 1260 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.35 (t, 3H, *J* = 7.0 Hz), 1.76 (m, 2H), 2.17 (s, 3H), 2.89 (m, 2H), 3.48 (dd, 1H, *J* = 7.4, 3.3 Hz), 3.68 (dd, 1H, *J* = 7.7, 3.3 Hz), 3.75 (m, 1H), 4.30 (q, 2H, *J* = 7.0 Hz), 7.50 (s, 1H). Anal. ($C_{12}H_{18}O_4S$) C, H, S.

5-(3(*S*)-Hydroxy-4-[[(*p*-tolylmethyl)sulfonyl]oxy]butyl)-4-methylthiophene-2-carboxylic Acid Ethyl Ester. Starting with diol **35b**, the title compound was prepared in 60% yield according to the general procedure described for compound 5-[3(*S*)-hydroxy-4-[[(*p*-tolylmethyl)sulfonyl]oxy]butyl]-thiophene-2-carboxylic acid ethyl ester: $[\alpha]_{589}$ −5.7° (*c* = 0.74, MeOH); IR (neat) 3491 (broad), 2982, 2928, 1703, 1449, 1360, 1250 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.35 (t, 3H, *J* = 7.0 Hz), 1.71 (m, 2H), 2.13 (s, 3H), 2.46 (s, 3H), 2.85 (m, 2H), 3.88−4.03 (m, 3H), 4.31 (q, 2H, *J* = 7.0 Hz), 7.36 (d, 2H, *J* = 8.1 Hz), 7.48 (s, 1H), 7.79 (d, 2H, *J* = 8.4 Hz). Anal. ($C_{19}H_{24}O_6S_2$) C, H, S.

5-(4-Azido-3(*S*)-hydroxybutyl)-4-methylthiophene-2-carboxylic Acid Ethyl Ester. Starting with the tosylate prepared above, the title compound was prepared in 96% crude yield according to the general procedure described for compound 5-(4-azido-3(*S*)-hydroxybutyl)thiophene-2-carboxylic acid ethyl ester and used without purification: ^1H NMR ($CDCl_3$) δ 1.36 (t, 3H, *J* = 7.0 Hz), 1.80 (m, 2H), 2.16 (s, 3H), 2.83−2.96 (m, 2H), 3.29 (dd, 1H, *J* = 12.1, 7.4 Hz), 3.40 (dd, 1H, *J* = 12.1, 3.7 Hz), 3.78 (m, 1H), 4.30 (q, 2H, *J* = 7.0 Hz), 7.50 (s, 1H).

5-[4-[(*tert*-Butoxycarbonyl)amino]-3(*S*)-hydroxybutyl]-4-methylthiophene-2-carboxylic Acid Ethyl Ester (36c). Starting with the azide prepared above, compound **36c** was prepared in 89% yield according to the general procedure described for compound **36a**: $[\alpha]_{589}$ −21.7° (*c* = 0.71, MeOH); IR (neat) 3385 (broad), 2978, 2932, 1715, 1682, 1520, 1454, 1254 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.35 (t, 3H, *J* = 7.0 Hz), 1.44 (s, 9H), 1.75 (m, 2H), 2.16 (s, 3H), 2.80−2.94 (m, 2H), 3.08 (dd, 1H, *J* = 14.3, 7.4 Hz), 3.28 (dd, 1H, *J* = 14.3, 3.0 Hz), 3.74 (m, 1H), 4.30 (q, 2H, *J* = 7.0 Hz), 7.49 (s, 1H). Anal. ($C_{17}H_{27}NO_5S$) C, H, N, S.

5-[4-[(*tert*-Butoxycarbonyl)amino]-3(*S*)-[(methylsulfonyl)oxy]butyl]-4-methylthiophene-2-carboxylic Acid Ethyl Ester. Starting with alcohol **36c**, the title compound was prepared in 94% yield according to the general procedure described for compound 5-[4-[(*tert*-butoxycarbonyl)amino]-3(*S*)-[(methylsulfonyl)oxy]butyl]thiophene-2-carboxylic acid ethyl ester: mp 75−76 °C; $[\alpha]_{589}$ +7.8° (*c* = 0.60, MeOH); IR (KBr) 3362, 2982, 1699, 1680, 1530, 1350, 1278 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.35 (t, 3H, *J* = 7.0 Hz), 1.45 (s, 9H), 2.01 (m, 2H), 2.16 (s, 3H), 2.89 (m, 2H), 3.07 (s, 3H), 3.30−3.48 (m, 2H), 4.31 (q, 2H, *J* = 7.0 Hz), 4.80 (m, 1H), 4.95 (br t, 1H), 7.50 (s, 1H). Anal. ($C_{18}H_{29}NO_7S_2$) C, H, N, S.

2-[[1-[[(*tert*-Butoxycarbonyl)amino]methyl]-3(*R*)-[5-(ethoxycarbonyl)-3-methylthiophene-2-yl]propyl]thio]-malonic Acid Dimethyl Ester. Starting with the thioacetate prepared above, the title compound was prepared in 90% yield according to the general procedure described for compound 2-[[1-[[(*tert*-butoxycarbonyl)amino]methyl]-3(*S*)-[5-(ethoxycarbonyl)thiophene-2-yl]propyl]sulfanyl]malonic acid dimethyl ester: $[\alpha]_{589}$ +22.7° (*c* = 0.67, MeOH); IR (neat) 3397, 2978, 1755, 1714, 1514, 1454, 1250 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.34 (t, 3H, *J* = 7.0 Hz), 1.44 (s, 9H), 1.92 (m, 2H), 2.15 (s, 3H), 2.95−3.40 (m, 5H), 3.80 and 3.82 (s, s, 6H), 4.23 (s, 1H), 4.31 (q, 2H, *J* = 7.0 Hz), 5.18 (brs, 1H), 7.49 (s, 1H). Anal. ($C_{22}H_{33}NO_8S$) C, H, N, S.

6-[2-[5-(Ethoxycarbonyl)-3-methylthiophene-2-yl]ethyl]-3(*R*)-oxothiomorpholine-2-carboxylic Acid Methyl Ester (37c). Starting with the malonate prepared above, compound **37c** was prepared in 87% yield according to the general procedure described for compound **37a**: mp 72−73 °C; $[\alpha]_{589}$ +19.1° (*c* = 0.67, MeOH); IR (thin film) 2954, 1738, 1694, 1651, 1464, 1296 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.35 (t, 3H, *J* = 7.0 Hz), 1.90 (m, 2H), 2.17 (s, 3H), 2.88 (m, 2H), 3.21−3.62 (m, 3H), 3.80 and 3.82 (s, s, 3H), 4.14 and 4.25 (s, s, 1H), 4.31 (q, 2H, *J* = 7.0 Hz), 6.22 (m, 1H), 7.50 (s, 1H). Anal. ($C_{16}H_{21}NO_5S_2$) C, H, N, S.

5-[2-(2-Amino-4-oxo-4,6,7,8-tetrahydro-3*H*-pyrimido-[5,4-*b*]thiazin-6(*R*)-yl)ethyl]-4-methylthiophene-2-carboxylic Acid Ethyl Ester. Starting with the lactam prepared above, the title compound was prepared in 27% yield according to the general procedure described for compound 5-[2-(2-amino-4-oxo-4,6,7,8-tetrahydro-3*H*-pyrimido[5,4-*b*]thiazin-6(*R*)-yl)ethyl]thiophene-2-carboxylic acid ethyl ester: mp 120−140 °C dec; $[\alpha]_{589}$ +57.1° (*c* = 0.67, DMSO); IR (KBr) 3337, 2926, 1701, 1653, 1635, 1599, 1449, 1250 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.22 (t, 3H, *J* = 7.0 Hz), 1.60−1.75 (m, 1H), 1.80−1.92 (m, 1H), 2.10 (s, 3H), 2.86 (m, 2H), 3.12−3.52 (m, 3H), 4.20 (q, 2H, *J* = 7.0 Hz), 6.02 (s, 2H), 6.65 (s, 1H), 7.50 (s, 1H), 10.08 (s, 1H); HMS calcd for $C_{16}H_{20}N_4O_3S_2$ (M + H^+) 381.1055, found 381.1067. Anal. ($C_{16}H_{20}N_4O_3S_2 \cdot 0.8H_2O$) C, H, N, S.

5-[4-[(*tert*-Butyldimethylsilyl)oxy]-3(*S*)-[(methylsulfonyl)oxy]butyl]-4-methylthiophene-2-carboxylic Acid Ethyl Ester. Starting with the alcohol prepared above, the title compound was prepared in 84% yield according to the general procedure described for compound 5-[4-[(*tert*-butyldimethylsilyl)oxy]-3(*R*)-[(methylsulfonyl)oxy]butyl]-4-methylthiophene-2-carboxylic acid ethyl ester: $[\alpha]_{589}$ +3.9° (*c* = 1.48, MeOH); ^1H NMR ($CDCl_3$) δ 0.09 (s, 6H), 0.90 (s, 9H), 1.35 (t, 3H, *J* = 7.0 Hz), 1.97−2.05 (m, 2H), 2.16 (s, 3H), 2.82−3.01 (m, 2H), 3.07 (s, 3H), 3.68−3.82 (m, 2H), 4.31 (q, 2H, *J* = 7.0 Hz), 4.69−4.77 (m, 1H), 7.50 (s, 1H). Anal. ($C_{19}H_{34}O_6S_2Si$) C, H S.

5-[4-Hydroxy-3(*S*)-[(methylsulfonyl)oxy]butyl]-4-methylthiophene-2-carboxylic Acid Ethyl Ester. Starting with the silyl ether prepared above, the title compound was prepared in 39% yield according to the general procedure described for compound 5-[4-hydroxy-3(*R*)-[(methylsulfonyl)-oxy]butyl]-4-methylthiophene-2-carboxylic acid ethyl ester: $[\alpha]_{589}$ +14.2° (*c* = 0.97, MeOH); ^1H NMR ($CDCl_3$) δ 1.35 (t, 3H, *J* = 7.0 Hz), 1.96−2.12 (m, 2H), 2.17 (s, 3H), 2.85−2.94 (m, 2H), 3.12 (s, 3H), 3.77 (dd, 1H, *J* = 6.6, 12.5 Hz), 3.86 (dd, 1H, *J* = 2.9, 12.5 Hz), 4.31 (q, 2H, *J* = 7.0 Hz), 4.78−4.87 (m, 1H), 7.50 (s, 1H). Anal. ($C_{13}H_{20}O_6S_2$) C, H, S.

4-Methyl-5-(2(*R*)-oxiranylethyl)thiophene-2-carboxylic Acid Ethyl Ester. Starting with the mesylate prepared above, the title compound was prepared in 70% yield according to the general procedure described for compound 5-[3(*S*),4-epoxybutyl]-4-methylthiophene-2-carboxylic acid ethyl ester: $[\alpha]_{589}$ +23.5° (*c* = 0.80, MeOH); ^1H NMR ($CDCl_3$) δ 1.35 (t, 3H, *J* = 7.1 Hz), 1.74−1.86 (m, 1H), 1.89−2.01 (m, 1H), 2.17 (s, 3H), 2.51 (dd, 1H, *J* = 2.6, 14.8 Hz), 2.78 (dd, 1H, *J* = 4.0, 14.8 Hz), 2.86−3.00 (m, 3H), 4.31 (q, 2H, *J* = 7.1 Hz), 7.50 (s, 1H). Anal. ($C_{12}H_{16}O_3S$) C, H, S.

5-[4-Azido-3(*R*)-hydroxybutyl]-4-methylthiophene-2-carboxylic Acid Ethyl Ester. Starting with the epoxide prepared above, the title compound was prepared in 79% yield according to the general procedure described for compound 5-[4-azido-3(*S*)-hydroxybutyl]-4-methylthiophene-2-carboxyl-

ic acid ethyl ester: $[\alpha]_{589}$ +11.0° (c = 0.58, MeOH); ^{1}H NMR ($CDCl_3$) δ 1.35 (t, 3H, J = 7.2 Hz), 1.75−1.84 (m, 2H), 2.17 (s, 3H), 2.80−3.00 (m, 2H), 3.29 (dd, 1H, J = 7.0, 12.3 Hz), 3.42 (dd, 1H, J = 3.1, 12.3 Hz), 3.75−3.83 (m, 1H), 4.31 (q, 2H, J = 7.2 Hz), 7.50 (s, 1H). Anal. ($C_{12}H_{17}N_3O_3S$) C, H, S.

5-[4-[(*tert*-Butoxycarbonyl)amino]-3(*R*)-hydroxybutyl]-4-methylthiophene-2-carboxylic Acid Ethyl Ester (36d). Starting with the azide prepared above, the title compound was prepared in 96% yield according to the general procedure described for compound **36a**: $[\alpha]_{589}$ +22.6° (c = 0.66, MeOH); ^{1}H NMR ($CDCl_3$) δ 1.35 (t, 3H, J = 7.2 Hz), 1.45 (s, 9H), 1.72−1.81 (m, 2H), 2.16 (s, 3H), 2.78−2.99 (m, 2H), 3.09 (dd, 1H, J = 7.4, 14.3 Hz), 3.29 (dd, 1H, J = 2.9, 14.3 Hz), 3.70−3.78 (m, 1H), 4.31 (q, 2H, J = 7.2 Hz), 7.50 (s, 1H). Anal. ($C_{17}H_{27}NO_5S$) C, H, N, S.

5-[4-[(*tert*-Butoxycarbonyl)amino]-3(*R*)-[(methylsulfonyl)oxy]butyl]-4-methylthiophene-2-carboxylic Acid Ethyl Ester. Starting with the alcohol prepared above, the title compound was prepared in 93% yield according to the general procedure described for compound 5-[4-[(*tert*-butoxycarbonyl)amino]-3(*S*)-[(methylsulfonyl)oxy]butyl]thiophene-2-carboxylic acid ethyl ester: $[\alpha]_{589}$ −7.8° (c = 0.98, MeOH); ^{1}H NMR ($CDCl_3$) δ 1.35 (t, 3H, J = 7.2 Hz), 1.45 (s, 9H), 1.98−2.06 (m, 2H), 2.16 (s, 3H), 2.86−2.92 (m, 2H), 3.06 (s, 3H), 3.34−3.43 (m, 2H), 4.31 (q, 2H, J = 7.2 Hz), 4.77−4.84 (m, 1H), 4.92 (broad, 1H), 7.50 (s, 1H). Anal. ($C_{18}H_{29}NO_7S_2$) C, H, N, S.

2-[[1-[(*tert*-Butoxycarbonyl)amino]-4-[5-(ethoxycarbonyl)-3-methylthien-2-yl]but-2(*S*)-yl]thio]malonic Acid Diethyl Ester. Starting with the thioacetate prepared above, the title compound was prepared in 88% yield according to the general procedure described for compound 2-[[1-[(*tert*-butoxycarbonyl)amino]-4-[5-(ethoxycarbonyl)thien-2-yl]but-2(*S*)-yl]thio]malonic acid diethyl ester: $[\alpha]_{589}$ −23.7° (c = 0.68, MeOH); ^{1}H NMR ($CDCl_3$) δ 1.34 (t, 3H, J = 7.1 Hz), 1.44 (s, 9H), 1.75−1.86 (m, 1H), 1.90−1.99 (m, 1H), 2.15 (s, 3H), 2.85−2.97 (m, 2H), 3.00−3.07 (m, 1H), 3.23−3.39 (m, 2H), 3.80 (s, 6H), 3.87 (s, 1H), 4.31 (q, 2H, J = 7.1 Hz), 5.12 (broad, 1H), 7.49 (s, 1H). Anal. ($C_{22}H_{33}NO_8S_2$) C, H, N, S.

6(*S*)-[2-[5-(Ethoxycarbonyl)-3-methylthien-2-yl]ethyl]-3-oxo-1,4-thiazine-2-carboxylic Acid Methyl Ester (37d). Starting with the malonate prepared above, compound **37d** was prepared in 88% yield according to the general procedure described for compound **37a**: $[\alpha]_{589}$ −26.2° (c = 0.81, MeOH); ^{1}H NMR ($CDCl_3$) δ 1.35 (t, 3H, J = 7.1 Hz), 1.83−2.03 (m, 2H), 2.16 (s, 3H), 2.83−2.97 (m, 2H), 3.39−3.67 (m, 3H), 3.80, 3.82 (s, 3H), 4.14, 4.25 (s, 1H), 4.31 (q, 2H, J = 7.1 Hz), 6.29 (broad, 1H), 7.50 (s, 1H). Anal. ($C_{16}H_{21}NO_5S_2$) C, H, N, S.

5-[2-(2-Amino-4(3*H*)-oxo-5,6,7,8-tetrahydropyrimido-[5,6-*b*][1,4]thiazin-6(*S*)-yl)ethyl]-4-methylthiophene-2-carboxylic Acid Ethyl Ester. Starting with lactam prepared above, the title compound was prepared in 27% yield according to the general procedure described for compound 5-[2-(2-amino-4(3*H*)-oxo-5,6,7,8-tetrahydropyrimido[5,6-*b*][1,4]thiazin-6(*R*)-yl)ethyl]thiophene-2-carboxylic acid ethyl ester: $[\alpha]_{589}$ −41.5° (c = 0.66, DMSO); ^{1}H NMR (DMSO-d_6) δ 1.25 (t, 3H, J = 7.0 Hz), 1.62−1.73 (m, 1H), 1.80−1.91 (m, 1H), 2.13 (s, 3H), 2.79−2.97 (m, 3H), 3.19−3.26 (m, 1H), 3.47−3.55 (m, 1H), 4.22 (q, 2H, J = 7.0 Hz), 6.05 (s, 2H), 6.68 (s, 1H), 7.53 (s, 1H), 10.10 (s, 1H); HMS calcd for $C_{16}H_{20}N_4O_3S_2$ (M + Na^+) 403.0875, found 403.0859.

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