Development of Highly Potent Inhibitors of Ras Farnesyltransferase Possessing Cellular and *in Vivo* Activity

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Analogs of CVFM (a known nonsubstrate farnesyltransferase (FT) inhibitor derived from a CA_1A_2X sequence where C is cysteine, A is an aliphatic residue, and X is any residue) were prepared where phenylalanine was replaced by (*Z*)-dehydrophenylalanine, 2-aminoindan-2-carboxylate, 1,2,3,4-tetrahydroisoquinoline-3-carboxylate (Tic), and indoline-2-carboxylate. The greatest improvement in FT inhibitory potency was observed for the Tic derivative (IC_{50} = 1 nM); however, this compound was ineffective in blocking oncogenic Ras-induced transformation of NIH-3T3 fibroblast cells. A compound was prepared in which both the Cys-Val methyleneamine isostere and the Tic replacement were incorporated. This derivative inhibited FT with an IC_{50} of 0.6 nM and inhibited anchorage-independent growth of stably transformed NIH-3T3 fibroblast cells by 50% at 5 μM. Replacing the A_1 side chain of this derivative with a *tert*-butyl group and replacing the X position with glutamine led to a derivative with an IC_{50} of 2.8 nM and an EC_{50} of 0.19 μM, a 26-fold improvement over (*S**,*R**)-*N*-[[2-[*N*-(2-amino-3-mercaptopropyl)-L-valyl]-1,2,3,4-tetrahydro-3-isoquinolinyl]carbonyl]-L-methionine. This derivative, (*S**,*R**)-*N*-[[2-[*N*-(2-amino-3-mercaptopropyl)-L-*tert*-leucyl]-1,2,3,4-tetrahydro-3-isoquinolinyl]-carbonyl]-L-glutamine, was evaluated *in vivo* along with (*S**,*R**)-*N*-[[2-[*N*-(2-amino-3-mercaptopropyl)-L-*tert*-leucyl]-1,2,3,4-tetrahydro-3-isoquinolinyl]carbonyl]-L-methionine methyl ester for antitumor activity in an athymic mouse model implanted ip with *H-ras*-transformed rat-1 tumor cells. When administered by injection twice a day at 45 mg/kg for 11 consecutive days, both compounds showed prolonged survival time (T/C = 142−145%), thus demonstrating efficacy against *ras* oncogene-containing tumors *in vivo*.

Introduction

ras genes encode a family of GTP-binding proteins ($p21^{ras}$) known to play a major role in controlling cell growth and differentiation.[1] Cycling between the active GTP-bound form and inactive GDP-bound form, $p21^{ras}$ acts as a regulatory switch in cell signaling. Upstream elements associated with transmembrane receptors facilitate GTP binding by $p21^{ras}$ thus propagating an extracellular stimulus downstream to intracellular and ultimately intranuclear targets. In normal cell signaling, inactivation of $p21^{ras}$ occurs through hydrolysis of bound GTP to GDP by the protein's intrinsic GTPase activity coupled with strong stimulation by GTPase-activating protein (GAP). Specific mutations in the *ras* gene impair GTPase activity and response to GAP of $p21^{ras}$, rendering it constituitively active and resulting in uncontrolled cell growth and morphologic transformation of normal cells. Mutated *ras* genes have been found in 15% of all human carcinomas, with much higher occurrences in pancreatic adenocarcinomas (90%) and human colon tumors (50%).[1]

Ras proteins must be membrane associated[2,3] to function, and post-translational modification is critical to this localization. A conserved CA_1A_2X sequence (where C is cysteine, A is an aliphatic residue, and X is any residue) at the C-terminus of $p21^{ras}$ triggers a series of post-translational processing events.[4] The initial step, when X = Met or Ser, involves farnesylation of Cys^{186} by the enzyme farnesyltransferase (FT). Subsequently, the three amino acids distal to the prenyl cysteine are removed by proteolysis.[5] Finally, the C-terminal carboxyl is capped by methylation. Other modifications such as palmitoylation and phosphorylation occur in certain $p21^{ras}$ proteins.[6,7] The initial prenylation appears to be a prerequisite for all subsequent modifications which enable $p21^{ras}$ to localize to the plasma membrane.[8]

Inhibiting the S-farnesylation of $p21^{ras}$ may block the growth of Ras-mediated tumors, and much effort has been directed toward the design of inhibitors of FT.[9−20] Our efforts have focused on the design of inhibitors based on the CA_1A_2X tetrapeptide sequence.[12] As shown by others, the presence of an aromatic amino acid in the A_2 position, as in CVFM (compound **1**), is a major determinant for nonsubstrate tetrapeptide inhibitor activity.[19] Nonsubstrate inhibitors are not subject to inactivation through prenylation and are therefore well suited as FT inhibitor leads. The distance between the aromatic ring and the amide backbone is critical for preventing farnesylation of these inhibitors,[12] suggesting a defined binding site may exist for the aromatic ring. We and others have observed that replacing selected amide bonds with a reduced amide isostere in

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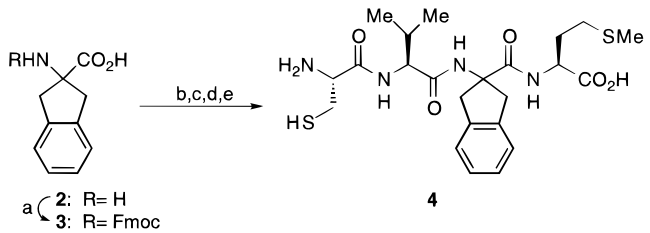
Scheme 1[a]



[a] (a) Fmoc-OSu, 10% Na_2CO_3, 1,4-dioxane, °C; (b) Bop/HOBt, Met-Wang resin followed by piperidine; (c) Fmoc-Val-OH, HOBt, Bop followed by piperidine; (d) Boc-Cys(S-Tr)-OH, Bop, HOBt, DIEA; (e) TFA, Et_3SiH.

a CAAX-based FT inhibitor leads to significant improvement in inhibitory potency and whole cell activity.[9,11,12,20] This isosteric replacement prevents proteolytic degradation, thereby improving the stability of peptide-based inhibitors. Herein, we report the development of CA_1A_2X-based inhibitors of FT where the aromatic ring at the designated A_2 position is conformationally restricted. These inhibitors are cell-permeable in nonprodrug form and are efficacious *in vivo*.

Chemistry

The unnatural amino acid **2** was prepared via a Strecker synthesis starting from indanone as described previously.[21] Following Fmoc protection of **2**, **3** was coupled to Met-Wang resin using standard coupling techniques. Fmoc deprotection of the resin-bound dipeptide using piperidine was followed by assembly of the remaining residues using solid phase peptide synthesis techniques. The cysteine residue was added as *N*-[(*tert*-butyloxy)carbonyl]-*S*-(triphenylmethyl)-L-cysteine (Boc-Cys(S-Tr)-OH) which allowed both resin cleavage and protecting group removal on treatment with trifluoroacetic acid (TFA). This approach afforded **4** in five steps (Scheme 1).

To prepare a tetrapeptide containing dehydrophenylalanine at the A_2 position, ring opening of oxazolone **5**[22] with methionine methyl ester (Met-OMe) afforded **6**. Boc deprotection of **6** using TFA followed by coupling of Boc-Cys(S-Tr)-OH and protecting group removal gave **7** (Scheme 2). Compound **14** was prepared by amide bond coupling of commercially available (*S*)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid to Met-OMe using standard coupling techniques followed by acidic cleavage of the Boc group. Of the amide bond-coupling methods attempted, bis(2-oxo-3-oxazolidinyl)phosphinic chloride (Bop-Cl) was superior in providing **10** from *N*-[(*tert*-butyloxy)carbonyl]-L-valine (Boc-Val) and **9** (>98% yield). Following Boc group removal of **10**, **11** was coupled with Boc-Cys(S-Tr)-OH and protecting groups were removed to afford **14** (Scheme 3). To provide the indoline derivative **21**, **15** was coupled to Boc-Val using Bop-Cl. After saponification and coupling to L-methionine *tert*-butyl ester (Met-OtBu) to give **18**, selective removal of the Boc group using formic acid followed by coupling of **19** with Boc-Cys(S-Tr)-OH and subsequent protecting group removal afforded **21** (Scheme 4). Scheme 5 shows a representative synthesis of compounds containing the reduced amide bond isostere. Aldehyde **22** was prepared via formation and reduction of the *N*,*O*-dimethylhydroxamate.[23] Reductive coupling of **22** with **11** followed by protecting group removal afforded **25**. Other derivatives in this series containing the reduced amide bond isostere are described in the Experimental Section. Compounds **34** and **35** were prepared as a mixture of diastereomers starting from racemic 1,2,3,6-tetrahydropyridine-2-carboxylic acid[24] utilizing the approach outlined for compound **21**. The isomers were separated by HPLC in the final purification step. Compound **36** was prepared starting from 3-amino-2-naphthoic acid utilizing the approach outlined for compound **25**. Modifications of the methionine (X) position were accomplished by utilizing a convergent approach (outlined in Scheme 6) in which **22** was converted to the partially protected tripeptide intermediate **30** followed by coupling to the desired amino acid ester and protecting group removal. Derivatives modified at the A_1 position were prepared as described for **25**.

Results and Discussion

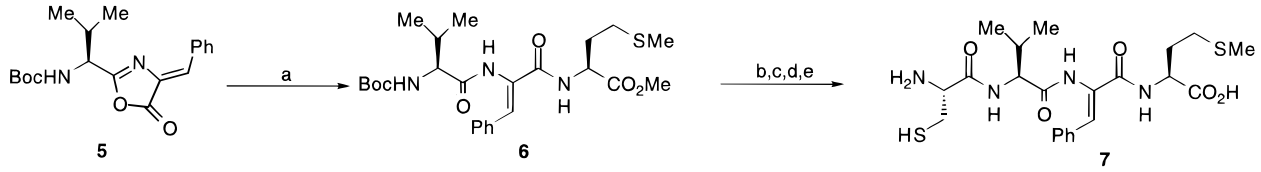
Starting with **1** as the lead, we initially determined if conformationally constraining the aromatic ring at the A_2 position could lead to improved FT inhibition. Thus, the compounds prepared in this study were initially screened as inhibitors of FT in an *in vitro* assay using recombinant $p21^{H\text{-}ras}$ protein and FT isolated from porcine brain.[25]

As shown in Table 1, orienting the aromatic ring perpendicular to the backbone (compound **4**) is strongly disfavored, leading to a 100-fold reduction in enzyme inhibitory potency compared to **1** (IC_{50} = 37 nM). Structures containing the A_2 aromatic ring oriented parallel to the amide backbone (**7** and **21**) are slightly less potent than **1**. By far, the greatest improvement in inhibitory activity was obtained by replacing Phe with (*S*)-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (Tic) (**14**), leading to a 37-fold enhancement in potency. The aromatic ring of Tic and the appended side chains are clearly adapting a highly favorable orientation for interaction with the enzyme. Others have found that Tic is a suitable replacement for Phe in CA_1A_2X-based FT inhibitors.[10,18] This observation demonstrates that alkylation of the amine at the A_2 position is not detrimental to inhibitory potency provided the proper orientation of the aromatic ring is maintained. Prior studies demonstrated that N-methylation at the A_2 amine of **1** leads to a 10-fold reduction in inhibitory potency,[12] conceivably because the aromatic ring is less able to orient in a favorable position.

Having identified **14** as an important lead, compounds **25** and **31**−**33** were prepared to examine the effects of amide bond modifications on FT inhibition. If tolerated, such modifications were expected to lead to improved whole cell and *in vivo* activity by stabilizing the peptide bonds to enzyme-mediated proteolysis. As found for other CAAX-based inhibitors, incorporating the methyleneamine amide bond isostere either maintains or slightly improves FT inhibitory potency (**31** and **33**) except when the methyleneamine is situated between Tic and Met (**32**).[11,12] These results and the improvement in whole cell activity that was realized (vide infra) led us to substitute a methyleneamine in place of the C−A_1 amide bond (i.e., **25**) in the remainder of our structure−activity development.

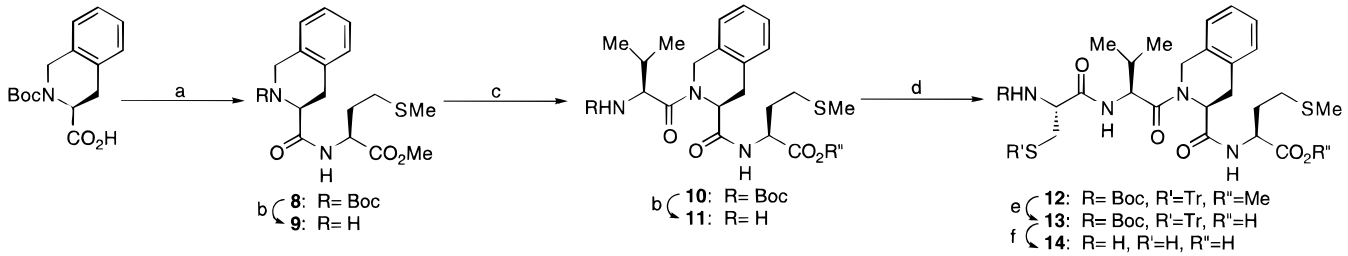
In an effort to understand the role of the Tic heterocyclic ring, 1,2,3,6-tetrahydropyridine-2-carboxylic acid stereoisomers **34** and **35** were prepared and determined

Scheme 2[a]



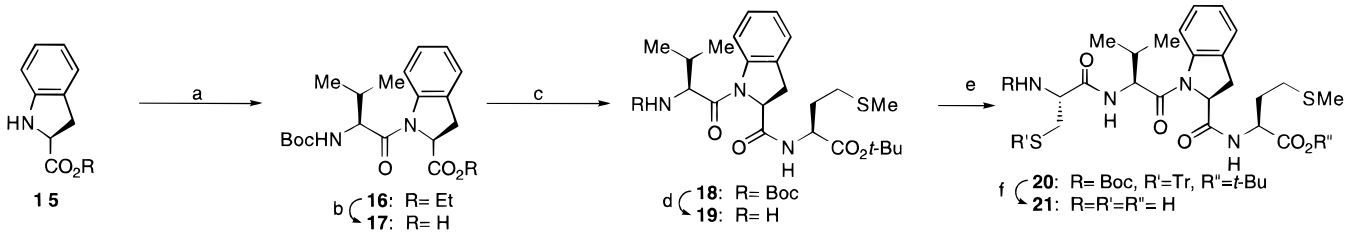
[a] (a) Met-OMe/THF; (b) TFA/CH_2Cl_2; (c) Boc-Cys(S-Tr)-OH, Bop, HOBt, DIEA, THF; (d) LiOH, MeOH, THF; (e) TFA, Et_3SiH.

Scheme 3[a]



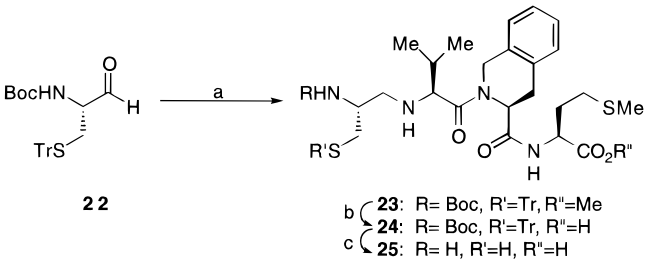
[a] (a) Met-OMe, EDC, HOBt, DIEA, NMP, CH_2Cl_2; (b) 4 N HCl/dioxane; (c) Boc-Val-OH, Bop-Cl, DIEA, CH_2Cl_2; (d) Boc-Cys(S-Tr)-OH, EDC, HOBt, DMF; (e) LiOH, MeOH, THF; (d) TFA, Et_3SiH.

Scheme 4[a]



[a] (a) Bop-Cl, NMM, Boc-Val-OH, CH_2Cl_2; (b) LiOH, MeOH, THF; (c) Bop, NMM, Met-OtBu, CH_2Cl_2; (d) Et_3SiH, HCO_2H; (e) Boc-Cys(S-Tr)-OH, Bop, NMM, CH_2Cl_2; (f) Et_3SiH, TFA.

Scheme 5[a]



[a] (a) 11, $NaBH_3CN$, AcOH, MeOH; (b) NaOH, MeOH; (c) TFA, CH_2Cl_2, Et_3SiH.

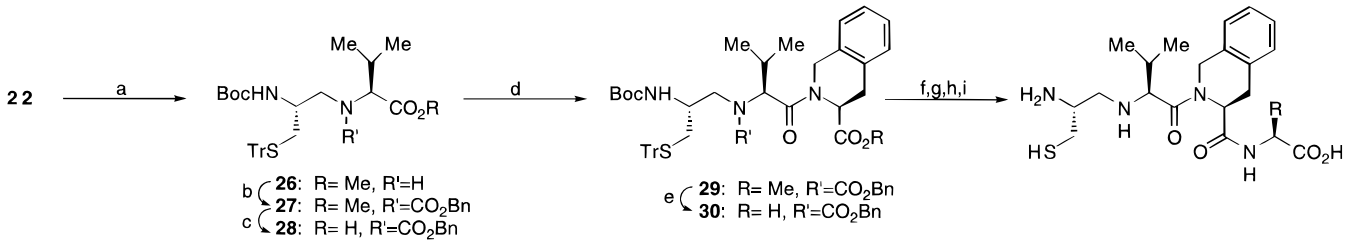
to be significantly less active than **25** against FT. Hence, the main function of the heterocyclic ring of Tic appears to be in anchoring the aromatic ring in an optimal binding orientation rather than orienting the rest of the molecule in a favorable bioactive conformation. Replacing Tic with 3-amino-2-naphthoic acid (**36**) leads to a significant reduction in inhibitory activity, suggesting the existence of a well-defined inhibitor-binding pocket.

To determine if aromatic ring alterations affect non-substrate character, several inhibitors were tested using a known substrate farnesylation assay (data not shown).[19] None of these compounds are substrates, suggesting that they probably bind in a manner similar to that of **1**. In addition, the selectivity of inhibitors for FT over geranylgeranyltransferase 1 (GGT1) is an important issue, since these enzymes share a common subunit and catalyze similar reactions. The three compounds in Table 1 (**1**, **25**, and **33**) tested against both enzymes are highly selective for FT over GGT1 with the greatest selectivity found for **33** (25 times better than **1**).

The whole cell activity profile of FT inhibitors was evaluated utilizing two assays. The *ras* transformation inhibition assay (RTI) measures the ability of FT inhibitors to inhibit the transformation of normal NIH-3T3 cells transfected with oncogenic *H-ras* DNA.[14] The cells were visually evaluated for percent inhibition of transformation and gross toxicity at 10 and 100 μM concentrations of inhibitors (Table 3). In a second assay, compounds were evaluated for their ability to inhibit the anchorage-independent growth in soft agar (SAG assay) of NIH-3T3 cells stably transformed with oncogenic *H-ras*.[15] The inhibitor was added to the growth medium every 2 days for an 8 day duration. The number of colonies >0.1 mm in size was measured at different concentrations of inhibitor and an EC_{50} value determined (Table 3).

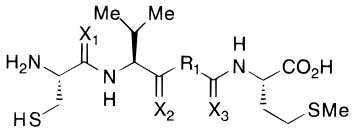
Compound **14** is not active at 100 μM in the RTI assay probably because of proteolytic cleavage, as has been shown by others for a similar compound.[11] Of the compounds tested in the Tic series, **25** and **33** display the best whole cell activity. Both are capable of inhibiting *ras* transformation in whole cells by 80% at 10 μM, and both inhibit the soft agar growth of *H-ras*-transformed cells with an EC_{50} of 5 μM. Compound **25** was evaluated for inhibition of Ras processing in H-Ras-transformed cells. Following treatment for 24 h, unprocessed precursor Ras proteins accumulated and localized to the cytosolic fraction. Inhibition of Ras processing was dose dependent with an IC_{50} value of 5

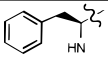
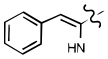
Scheme 6[a]



[a] (a) HCl·Val-OMe, $NaBH_3CN$, MeOH; (b) Cbz-Cl, DIEA, CH_2Cl_2; (c) 2 N LiOH; (d) HCl·Tic-OMe, Bop-Cl, DIEA, CH_2Cl_2; (e) 1 N LiOH, THF, MeOH; (f) $H_2NCHRCO_2Me$, Bop, DIEA, DMF; (g) 1 N LiOH, THF; (h) TFA, Et_3SiH; (i) TFA, thioanisole, 1,2-ethanedithiol, TMSBr.

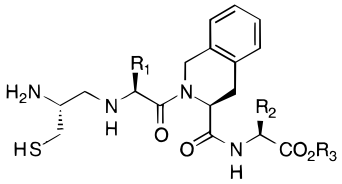
Table 1. FT *in Vitro* Activity of Derivatives Constrained at the A_2 Position



#	X_1	X_2	X_3	R_1	FT IC_{50}(nM)[a]	GGT1 IC_{50}(nM)[a]
1	O	O	O		37 ± 3.1	8100±1900
4	O	O	O		2900 ± 300	-
7	O	O	O		160 ± 30	-
14	O	O	O		1.0 ± 0.38	-
21	O	O	O		120 ± 44	-
25	H,H	O	O		0.60 ± 0.04	110±17
31	O	H,H	O		0.37 ± 0.09	-
32	O	O	H,H		5300 ±1200	-
33	H,H	H,H	O		0.75±0.27	4100 ± 580
34	H,H	O	O		270 ± 58	-
35	H,H	O	O		3900 ± 1800	-
36	H,H	O	O		8200(n=1)	-

[a] $n = 3$ unless otherwise indicated.

Table 2. *In Vitro* Inhibition of FT Inhibitors Modified at the A_1 and X Positions



			IC_{50} (nM)[a]		
no.	R_1	R_2	R_3	FT	GGT1
25	iPr	$-CH_2CH_2SCH_3$	H	0.60 ± 0.040	110 ± 17
37	Me	$-CH_2CH_2SCH_3$	H	45 ± 3.0	
38	Et	$-CH_2CH_2SCH_3$	H	14 ± 2.3	
41	iPr	n-butyl	H	770 ± 190	1.3 ± 0.5
42	iPr	$-CH_2CONH_2$	H	1100 ± 540	
43	iPr	$-CH_2CH_2SO_2NH_2$	H	90 ± 11	
44	iPr	$-CH_2CH_2SO_2CH_3$	H	0.92 ± 0.020	400 ± 12
45	iPr	$-CH_2CH_2CONH_2$	H	4.4 ± 0.55	1000 ± 240
46	tBu	$-CH_2CH_2CONH_2$	H	2.8 ± 0.070	1400 ± 370
47	tBu	$-CH_2CH_2CON(CH_3)_2$	H	1.8 ± 0.060	
48	tBu	$-CH_2CH_2OCH_3$	H	1.4 ± 0.50	22 ± 1.5
49	tBu	$-CH_2CH_2SCH_3$	H	0.57 ± 0.11	4.8 ± 1.6
50	tBu	$-CH_2CH_2SO_2CH_3$	H	0.46 ± 0.080	14 ± 1.1
51	tBu	$-CH_2CH_2SCH_3$	CH_3	85 ± 35	200 ± 23

[a] $n = 3$ unless otherwise indicated.

Table 3. Whole Cell Profile of FT Inhibitors

	RTI inhibition (%)		
no.	100 μM	10 μM	SAG EC_{50} (μM)
25	100	>80	5.0
31	70	0.0	
33	100	80	5.0
37	NT	NT	15
38	NT	NT	15
41	80	20	26
42	NT	NT	
43	75	15	8.3
44			1.0
45	100	90	2.0
46	100	100	0.19
47			1.8
48			2.6
49	100	90	0.5
50			0.57
51	100	100	0.41

μM. This inhibition of *ras* processing correlates well with the EC_{50} for SAG inhibition. Although both compounds show activity in whole cells, the EC_{50}/IC_{50} ratios are high (>6000) suggesting that **25** and **33** are poorly permeable. In an effort to address these issues, we chose to optimize both the X and A_1 positions.

Modifying the methionine side chain to norleucine (Nle) (**41**) leads to a large drop in FT inhibitory potency and an increase in GGT1 inhibitory potency, resulting in a highly selective GGT1 inhibitor. This is an intriguing result considering that Nle is often used as a chemically stable isosteric replacement for Met.[26] Although both are unbranched hydrophobic residues, they are clearly not interchangeable at the active site of either FT or GGT1.[27] Perhaps differences in flexibility or polarizability account for these differences.[26] The preference for Nle by GGT1 is also surprising, considering that GGT1 primarily recognizes CAAX sequences containing branched aliphatic side chains at the X position.[28] Another interesting finding is that replacement of the hydrophobic Met residue with a variety of polar side chains leads to analogs which retain substantial FT inhibitory potency. Methyl ether (**48**)-, carboxamide (**45**)-, and sulfone (**44**)-containing side chains provide potent inhibitors, while a sulfonamide (**43**) leads to a moderately potent inhibitor. The difference in FT inhibitory potency between **42** and **45**

Table 4. Effect of FT Inhibitors on Mice Bearing ip Rat-1 Tumors[a]

compd	dose (mg/kg/injection)	schedule	MST	%T/C
46	45	2qd×11	18.5	154
51	45	2qd×11	17.0	142
control			12.0	100
1/10 control			16.0	133

[a] 10^6 cells implanted on day 0 (except 10^5 cells in 1/10 control); treatments began on day 1.

indicates that the polar carboxamide is an effective replacement for the methionine sulfide. The Gln analog **45** is slightly more potent than **25** in whole cell assays, despite its lower potency versus FT. The sulfone analog **44**, while equipotent to **25** versus FT, also shows improved SAG activity over **25**.

At the A_1 position, the Ala analog **37** is a modest inhibitor. Increasing the number of methyl groups leads to increased potency (i.e., **37** < **38** < **25** = **49**). Although the isopropyl (**25**) and *tert*-butyl (**49**) side chains were equipotent as FT inhibitors, the *tert*-butyl side chain led to substantial improvement in cell activity**.** For example, **49** is 10-fold more potent than **25** in the SAG assay, even though it is equipotent as an FT inhibitor. The reason for the increased cell potency of these analogs is uncertain, although conformational effects of the *tert*-butyl side chain may play some undefined role. Increased hydrophobicity may also be a contributing factor.

Combining the two effects which independently lead to increased cell activity, namely, a *tert*-butyl side chain at A_1 and a polar X side chain, provides analog **46**, which displays an EC_{50} in soft agar of 0.19 μM (25-fold more potent than **25**). The very low EC_{50}/IC_{50} ratio for **46** (70-fold) suggests that this tetrapeptide is reasonably membrane permeable, despite the presence of an unesterified carboxyl group. These compounds showed no cytotoxicity up to 100 μM, indicating that the SAG activity observed is from cellular penetration of FT inhibitors and not due to general cytotoxicity (data not shown).

Two FT inhibitors were evaluated *in vivo* for their antitumor activity in athymic mice implanted ip with *H-ras*-transformed rat-1 tumor cells (Table 4). When administered ip twice a day at 45 mg/kg/injection for 11 consecutive days, both **46** and **51** (the methyl ester of **49**) were active, as demonstrated by prolonged survival time (T/C = 142−154%, T/C ≥ 125% considered an active result; see experimental for detailed description). Lower doses of either compound (15 mg/kg/injection) did not produce an active result. The efficacy of **51** at 45 mg/kg/injection using this animal model was confirmed by subsequent investigations with 2qd×14 (T/C = 180) and 2qd×7 (T/C = 140) treatment schedules.

In summary, we have designed potent FT inhibitors capable of penetrating cells and reversing morphological transformation at submicromolar concentrations. Optimizing the orientation of the side chain aromatic ring at the A_2 position leads to a significant improvement in inhibitory potency. Whole cell activity is improved by replacing amide bonds with the methyleneamine isostere and modifying the A_1 and X positions. As shown in one example, the observed effects in whole cells can be correlated with inhibition of *ras* processing. Evaluation of two compounds in an athymic mouse model against rat-1 tumor cells indicates that these derivatives are efficacious against *ras* oncogene-containing tumors *in vivo*.

Experimental Section

Ras Biological Assays. Enzymes. FT was isolated from pig brain as described by Manne et al.[25] and further purified at least 2000-fold relative to the initial crude pig brain cytosol. GGT1 was purified 400-fold from porcine brain tissue. Each of the final enzyme preparations is free of the other enzyme.

Protein Preparations. $p21^{ras}$ proteins, from bacteria carrying expression vectors coding for *v-H-ras* gene sequences, were purified as described[28] to ≥80% purity. $p21^{ras}$ CVLL proteins were purified as described[29] to ≥80% purity from an expression vector[30] kindly provided by Dr. Channing Der, University of North Carolina at Chapel Hill.

Farnesyltransferase Assay. FT assays were run in 96-well dishes in a reaction volume of 20 μL as described in detail earlier.[14] Briefly, the reaction mixtures contained 1 μM [^{3}H]-FPP (NEN DuPont), 7 μM p21 H-Ras, 25 mM $MgCl_2$, 10 mM DTT, 100 mM Hepes (pH 7.4), and serial dilutions of inhibitors usually ranging from 360 μM to 0.02 nM. Reactions were started by adding sufficient enzyme to produce 1−2 pmol of [^{3}H]FPP incorporation in 1 h in the control wells. Following incubation at 37 °C for 1 h, the reactions were stopped and the samples processed as described.[14] Dose−response curves for inhibitors used triplicate estimates at each drug concentration, and the IC_{50} estimations were made from percent control versus log drug concentration plots. Unless otherwise indicated, each compound was tested at least twice.

Geranylgeranyltransferase (GGT1) Inhibition Assay. To determine the IC_{50} values for inhibition, the GGT1 assay was also adapted to 96-well microtiter plate format as described.[14] Briefly, 12 μM $p21^{ras}$ CVLL, 0.5 μM [^{3}H]GGPP (19.3 Ci/mmol; NEN), 10 mM dithiothreitol (DTT), 5 mM $MgCl_2$, 5 μM $ZnCl_2$, 100 mM Hepes, pH 7.4, and partially purified GGT1 were incubated in a total volume of 20 μL at 37 °C for 1 h, and samples were processed as described for the FT assay. All assays were carried out in triplicate. Unless otherwise indicated, each compound was tested at least twice.

***ras* Transformation Inhibition (RTI) Assay.** The whole cell activity of FT inhibitors was evaluated by a *ras* transformation inhibiton (RTI) assay. The RTI assay is based on transformation of mouse NIH-3T3 cells by oncogenic *ras* DNA transfection. NIH-3T3 cells (2.5×10^4/35 mm well) were seeded and allowed to attach overnight. The cells were transfected with linearized plasmid carrying oncogenic *H-ras*[31] using the calcium phosphate precipitation technique. One day following the transfection, cells were washed and inhibitors added at the indicated concentrations into the medium. Inhibitors were replenished every 48 h for 8 days along with the change of medium, for a total of four treatments. The degree of transformation was scored after 14 days. In the absence of an inhibitor, transfected cells grow aggressively and initially give the appearance of abnormal "foci" surrounded by normal cells. In the presence of a cell-permeable inhibitor, either the number of foci is reduced or transformation is completely inhibited. The percent inhibition and the gross cytotoxicity of inhibitors were evaluated visually by light microscopy.

Anchorage-Independent Growth in Soft Agar Suspension. Anchorage-independent growth was measured in soft agar suspension as described earlier.[15] Briefly, single cell suspensions of *H-ras*-transformed NIH-3T3 cells (44−911 cells) were obtained, mixed into a top soft agar mixture (0.3%), and laid on a 0.6% bottom agar layer. Initially, inhibitors at the indicated concentrations were incorporated into the soft agar mixture. FT inhibitors were replenished every 2 days for 8 days by overlaying 100 μL of 20-fold concentrated stock in PBS on the surface of the top agar layer. All the assays were carried out in parallel duplicate wells. After 14 day growth, colonies larger than 0.1 mm size in diameter were counted.

***In Vivo* Studies.** Athymic Balb/c-background female mice, 18−22 g, were purchased from Harlan Sprague−Dawley (Indianapolis, IN). Rat-1 (*H-ras*-transformed rat fibroblast

line) tumors were sourced from *in vitro* propagated cells. A detailed description of the general assay and evaluation methods has been reported.[32] Briefly, experiments were initiated by the implantation, ip, of 1×10^6 cells, except in control groups given titrated cell inocula. Group sizes consisted of six mice in treatment groups and eight mice in untreated control groups. All experiments began on day 1 post-tumor implant. Compounds **46** and **51** were dissolved in sterile water and injected ip within 1 h of dissolution. Both compounds were administered ip, usually twice a day (see below) for 11 consecutive days (2qd×11) at varying dose levels. Additionally, **51** was evaluated at 45 mg/kg/injection, ip, twice daily for 7 and 14 days (2qd×7 and 2qd×14, respectively). Twice a day injections were given 6−8 h apart on weekdays; on weekends, a single injection was given at twice the indicated dose (i.e., the total intended daily amount of compound was given all at once, not as a split dosage as was done on weekdays). Therapeutic results are presented in terms of increases in life span reflected by the relative median survival time (MST) of treated (T) versus control (C) groups (%T/C values) and any long-term survivors. The activity criterion for increased life span was a T/C of ≥125%. At the highest twice daily dose level tested, 45 mg/kg/injection, both compounds produced an active result. Control mice had a MST of 12 days, and the mice treated with compound **46** or **51** had MST's of 18.5 and 17.0 days, respectively. Thus, these therapies resulted in %T/C values of 154% and 142%, respectively.

Chemistry. General. Partially protected amino acids were obtained from Bachem California, Novabiochem, Sigma, and Schweizerhall Inc. Other reagents were obtained from Aldrich. THF was distilled from Na/benzophenone, and methylene chloride was distilled from CaH_2 prior to use. IR spectra were recorded on a Mattson Sirius 100 spectrometer. Proton NMR (^{1}H-NMR) and carbon NMR (^{13}C-NMR) spectra were obtained on Jeol GSX 400 or 270 MHz spectrometers and are reported in parts per million (ppm) relative to tetramethylsilane as the internal standard. Fast atom bombardment mass spectrometry (FAB-MS) was conducted on a JEOL lSX 102 instrument; chemical ionization mass spectra (CI-MS) were obtained using NH_3 desorption chemical ionization (scan rate 50−900 μm/1.6 s; dynode voltage = 1000 (V) on a Finnigan TSQ 4600 mass spectrometer. High-resolution mass measurements were made using a JEOL HX-110 double-focusing mass spectrometer at a resolution of $M/\Delta M = 10\,000$ equipped with a fast atom bombardment (FAB) ionization source. The FAB matrix used was 50/50 thioglycerol/glycerol. Poly(ethylene glycol)s were used as the internal calibrant. Analytical and preparative HPLC were performed on YMC columns (YMC S-3 ODS 4.6 × 150 mm, S-5 ODS 4.6 × 250 mm, and YMC S-10 30 × 500 mm) with methanol/water and acetonitrile/water gradients containing 0.1% TFA or 0.2% H_3PO_4. Thin-layer chromatography (TLC) analyses of reactions were run on silica gel 50 F_{254} plates (Merck). Flash chromatography was run on 230−400 mesh silica gel 60 (EM Science). Unless otherwise noted, solutions were dried using $MgSO_4$.

[*N*-(Fluorenylmethoxycarbonyl)amino]-2,3-dihydro-1*H*-indene-2-carboxylic Acid (3). A solution of *N*-[(9-fluorenylmethoxycarbonyl)oxy]succinimide (4.5 g, 14 mmol) in 1,4-dioxane (50 mL) at 0 °C was added all at once to a solution of **2**[21] (2.0 g, 12 mmol) in 10% sodium carbonate solution (70 mL). The mixture was stirred at 0 °C for 2 h and for an additional 20 h at room temperature. The mixture was concentrated to remove 1,4-dioxane, and the remaining aqueous solution was washed with ether (3 × 100 mL), acidified to pH 2, and extracted with ethyl acetate (3 × 100 mL). The combined ethyl acetate extract was washed with brine (100 mL), dried, and concentrated to yield **3** as a white solid (3.8 g, 81%): R_f 0.5 (CH_2Cl_2:CH_3OH:CH_3CO_2H, 85:10:5); MS $(M + H)^+$ 400; ^{1}H-NMR (DMSO-d_6, 270 MHz) δ 12.58 (1H, br s), 7.92 (1H, s), 7.86 (2H, d, J = 7.6 Hz), 7.68 (2H, d, J = 7.0 Hz), 7.40 (2H, t), 7.30 (2H, t), 7.16 (4H, m), 4.27 (2H, d, J = 6.4 Hz), 3.45 (2H, d, J = 16.4 Hz), 3.22 (2H, d, J = 17.1 Hz).

***N*-[[2-[(*N*-L-Cysteinyl-L-valyl)amino]-2,3-dihydro-1*H*-inden-2-yl]carbonyl]-L-methionine Trifluoroacetate (4).** Compound **4** was assembled by the stepwise solid phase method on a Milligen Biosearch Model 9600 peptide synthesizer using the Fmoc chemistry program supplied with the instrument. Starting with Fmoc-methionine-substituted *p*-alkoxybenzyl alcohol resin (Wang, polystyrene, 1% divinylbenzene copolymer, 1.0 g, 0.31 mmol; Bachem), amino acids (including **3**) were coupled as their N^{α}-Fmoc derivatives with the exception of the final residue, cysteine, which was blocked as the N-Boc(S-Trt) derivative. Fmoc groups were removed at each cycle by a 10 min treatment with 30% piperidine/35% DMF/35% toluene. Each amino acid derivative was coupled (1 h, room temperature), in 6−7-fold molar excess, using 1 equiv of (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (Bop) and 1 equiv of hydroxybenzotriazole (HOBt) in DMF/CH_2Cl_2 mixtures. The dried, protected peptidyl-resin weighed 1.13 g (weight gain, 90% of theory). Simultaneous deprotection and cleavage of the peptide from the resin (0.4 g) was performed by stirring TFA (9 mL, 120 mmol), 1,2-ethanedithiol (1.0 mL), dimethyl sulfide (0.5 mL), phenol (0.7 g), and thioanisole (0.25 mL) for 1.5 h. The resin was filtered and washed with TFA (2 mL). The combined filtrate was concentrated, and cold ether was added to solidify the oily residue. The solution was filtered and the solid was washed with ether, dissolved in a small amount of methanol and 0.1% TFA/water, and purified by preparative HPLC (YMC S-5 ODS 22 × 250 mm column eluting over 40 min with 25−35% B in A (A, 90% aqueous acetonitile containing 0.1% TFA; B, 10% aqueous acetonitrile containing 0.1% TFA), flow rate 9 mL/min, UV monitored at 220 nm). The appropriate fractions were pooled, concentrated and lyophilized to yield **4** as a white fluffy powder (16 mg, 20%): MS $(M + H)^+$ 511; mp 129−130 °C; $[\alpha]^{25}_D = -18.3°$ (c 0.5, MeOH); ^{1}H-NMR (DMSO J, 270 MHz) δ 7.63 (1H, m), 7.12 (4H, m), 4.52 (1H, m), 3.98 (2H, m), 3.42−3.13 (2H, m), 2.90 (2H, m), 2.44 (2H, m), 2.08 (1H, m), 2.00 (3H, s), 1.92 (1H, m), 1.80 (1H, m), 0.70−0.80 (6H, m). Anal. ($C_{23}H_{34}N_4O_5S_2 \cdot 2C_2HF_3O_2 \cdot H_2O$) C, H, N.

***N*-[2-[[(1,1-Dimethylethoxy)carbonyl]-L-valyl]-1-oxo-3-phenyl-2-propenyl]-L-methionine Methyl Ester (6).** A solution of L-methionine methyl ester hydrochloride (950 mg, 5.8 mmol) in THF (20 mL) was added dropwise to an ice-cooled solution of oxazolinone **5**[22] (2.1 g, 5.8 mmol) in THF (20 mL). The mixture was allowed to equilibrate to room temperature, and stirring was continued for 72 h. The solution was concentrated *in vacuo* and the residue dissolved in ethyl acetate. The organic phase was washed successively with 5% aqueous citric acid, half-saturated aqueous $NaHCO_3$, and brine followed by drying and concentration to give 2.25 g (77%) of **6** as an oil: accurate mass measurement $(M + H)^+$ calcd for $C_{25}H_{38}N_3O_6S$ 508.2481, found 508.2470 (Δ_{ppm} = 2.3); ^{1}H-NMR ($CHCl_3$, 270 MHz) δ 7.31−7.4 (5H, m), 7.26 (1H, s), 7.00−7.10 (1H, br s), 4.82 (1H, m), 4.0 (1H, m), 3.78 (3H, s), 2.57−2.63 (2H, m), 2.20−2.40 (2H, m), 2.05−2.16 (5H, s over m), 1.50−1.76 (1H, br s), 1.45 (9H, s), 0.95−1.03 (6H, m).

***N*-[2-(*N*-L-Cysteinyl-L-valyl)-1-oxo-3-phenyl-2-propenyl]-L-methionine Trifluoroacetate (7).** Intermediate **6** (1.0 g, 2.0 mmol) was treated with TFA:CH_2Cl_2 (1:1, 15 mL) at 4 °C for 1 h followed by concentration under vacuum. Ethyl ether was added, and the resulting precipitate was filtered, washed with ethyl ether, and dried. The free base was generated by dissolving the residue in a solution of half-saturated aqueous $NaHCO_3$ and extracting the free amine using ethyl acetate. The organic layer was dried and concentrated, and the residue was dissolved in THF (5 mL) and added to an ice-cooled solution of Boc-Cys(S-Tr)-OH (2.8 g, 6.0 mmol), Bop (2.7 g, 6.0 mmol), HOBt (810 mg, 6.0 mmol), and diisopropylethylamine (1.0 mL, 6.0 mmol) in THF (60 mL). The reaction mixture was stirred under nitrogen for 16 h followed by concentration *in vacuo*. The residue was dissolved in ethyl acetate and washed with 5% citric acid, half-saturated $NaHCO_3$, and brine, dried, and concentrated *in vacuo*. Purification on flash silica gel (4 × 18 cm column) using 25−40% ethyl acetate in hexane provided 1.1 g (64%) of ester. A solution of LiOH (130 mg, 3.2 mmol) in 2 mL of H_2O and 10 mL of methanol was added to the above ester (1.0 g, 1.2 mmol) in 10 mL of THF, and the mixture was stirred for 16 h. The base was neutralized with 3.5 mmol of citric acid (dissolved in H_2O), the solution was

concentrated under vacuum, and the residue was dissolved in ethyl acetate. The organic solution was washed with saturated aqueous NaCl, dried, and concentrated *in vacuo* to give 920 mg of the acid (95%). To a solution of the acid in TFA: methylene chloride (1:1, 15 mL) was added triethylsilane (0.2 mL), and the solution was stirred for 2.6 h. The solvents were removed *in vacuo*, and the oily residue was washed with hexanes, dissolved in 1:1 methanol:water containing 0.1% TFA, and purified by preparative HPLC (YMC ODS column (22 × 250 mm, 5 μm, 120 Å); solvent A, 0.1% TFA in water; solvent B, 0.1% TFA in acetonitrile; 5−50% B over 40 min; flow rate 9 mL/min; UV monitored at 220 nm). The appropriate fractions were collected, concentrated, and lyophilized to yield 24 mg of **7** as a white fluffy powder: MS $(M + H)^+$ 497; $[\alpha]^{20}_D = +90°$ (*c* 0.5, methanol); ^{1}H-NMR (D_2O, 270 MHz) δ 7.34−7.47 (5H, m), 4.55−4.58 (1H, dd, *J* = 5 Hz), 4.29−4.30 (1H, t, J = 6 Hz), 4.21−4.23 (1H, t, *J* = 6 Hz), 2.93−3.02 (2H, m), 2.49−2.59 (2H, m), 2.00−2.02 (5H, s over m), 0.85−0.93 (6H, dd, *J* = 7, 26 Hz). Anal. ($C_{22}H_{33}N_4O_5S_2 \cdot C_2HF_3O_2$) C, H, N, F, S.

(*R)-*N*-[[2-[(1,1-Dimethylethoxy)carbonyl]-1,2,3,4-tetrahydro-3-isoquinolinyl]carbonyl]-L-methionine Methyl Ester (8).** A solution of N^{α}-Boc-L-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (Boc-L-Tic) (2.0 g, 7.2 mmol) and methionine methyl ester hydrochloride (1.4 g, 7.2 mmol) was stirred in 5:15 NMP:CH_2Cl_2 at 4 °C. Diisopropylethylamine (1.2 mL, 7.2 mmol) was added followed by HOBt (970 mg, 7.2 mmol). The reaction mixture was stirred for 5 min; then 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDCI; 1.4 g, 7.2 mmol) was added. The reaction mixture stirred at room temperature, under nitrogen for 16 h, followed by partitioning between CH_2Cl_2 and saturated aqueous NaCl. The organic phase was washed successively with 5% citric acid, half-saturated $NaHCO_3$, and brine, dried, filtered, and concentrated *in vacuo* to give 2.6 g (86%) of **8** as a white solid: accurate mass measurement $(M + H)^+$ calcd for $C_{21}H_{31}N_2O_5S$ 423.1954, found 423.1968 ($\Delta_{ppm} = 3.1$); ^{1}H-NMR ($CDCl_3$, 270 MHz) δ 7.22−7.26 (4H, br m), 4.52−4.57 (4H, br m), 3.69 (3H, s), 3.36−3.41 (2H, t, *J* = 7Hz), 2.38 (2H, t, *J* = 7 Hz), 1.93−2.02 (5H, s over m), 1.51 (9H, br s).

(*R)-*N*-[(1,2,3,4-Tetrahydro-3-isoquinolinyl)carbonyl]-L-methionine Methyl Ester (9).** A solution of **8** (2.0 g, 4.7 mmol) in CH_2Cl_2 (2 mL), dimethyl sulfide (0.6 mL), and 4 N HCl in dioxane (10 mL) was stirred for 1 h under nitrogen. The mixture was concentrated, and diethyl ether was added. The product precipitated and was filtered, washed with diethyl ether, and dried under vacuum to give 1.6 g (99%) of **9** as a glassy white solid which was used directly in the next step.

(*R)-*N*-[[2-[*N*-[(1,1-Dimethylethoxy)carbonyl]-L-valyl]-1,2,3,4-tetrahydro-3-isoquinolinyl]carbonyl]-L-methionine Methyl Ester (10).** To a solution of **9** in CH_2Cl_2 at 0 °C (20 mL) was added Boc-L-valine (2.1 g, 9.5 mmol), Bop-Cl (1.2 g, 4.7 mmol), and *N*,*N*-diisopropylethylamine (1.6 mL, 9.5 mmol). The mixture was stirred for 16 h at 0 °C. Additional Bop-Cl (1.2 g, 4.7 mmol) and *N*,*N*-diisopropylethylamine (0.80 mL, 4.7 mmol) were added, and the mixture was stirred for an additional 8 h at 0 °C. The mixture was concentrated and chromatographed (flash silica gel, 4.1 × 20 cm, 1:1 ethyl acetate:hexane) to give **10** as a clear oil (2.4 g, 97%): accurate mass measurement $(M + H)^+$ calcd for $C_{26}H_{40}N_3O_6S$ 522.2638, found 522.2623 ($\Delta_{ppm} = 2.6$); ^{1}H-NMR ($CDCl_3$) δ 7.20−7.13 (4H, m), 5.3−4.7 (3H, m), 4.7−4.5 (2H, m), 4.4−4.2 (1H, m), 3.64−3.59 (3H, 2s), 3.35−3.15 (2H, d, *J* = 7 Hz), 3.05−2.90 (2H, d, *J* = 7 Hz), 2.22−1.89 (7H, m), 1.34 (9H, s), 1.02−0.9 (6H, m).

(*R)-*N*-[(2-L-Valyl-1,2,3,4-tetrahydro-3-isoquinolinyl)carbonyl]-L-methionine Methyl Ester (11).** A solution of **10** (1.2 g, 2.3 mmol) in CH_2Cl_2 (3 mL), 4 N HCl/dioxane (10 mL), and 0.5 mL of dimethyl sulfide was stirred for 1 h followed by concentration under vacuum. Diethyl ether was added, and the precipitated product was filtered, washed with diethyl ether and dried under vacuum to give 1.1 g (99%) of **11** as a white solid: MS $(M + H)^+$ 422; this was used without purification.

(*R*,S)-*N*-[[2-[*N*-[[2-[[(1,1-Dimethylethoxy)carbonyl]-amino]-3-[(triphenylmethyl)thio]propyl]carbonyl]-L-valyl]-1,2,3,4-tetrahydro-3-isoquinolinyl]carbonyl]-L-methionine Methyl Ester (12).** To a cooled solution of **11** (1.1 g, 2.3 mmol) in DMF (30 mL) was added Boc-Cys(S-Tr)-OH (3.2 g, 6.9 mmol), Bop (3.1 g, 6.9 mmol), HOBt (940 mg, 6.9 mmol), and diisopropylethylamine (1.2 mL, 6.9 mmol). The mixture was stirred for 16 h under nitrogen. The solvent was removed *in vacuo* and the residue dissolved in ethyl acetate. The organic solution was washed successively with 5% aqueous citric acid, half-saturated aqueous $NaHCO_3$, and brine. The extract was dried and concentrated. The residue was chromatographed (flash silica gel, 5 × 16 cm, 25−40% ethyl acetate in hexane) to give 1.34 g (67%) of **12** as a yellow viscous oil: accurate mass measurement $(M + H)^+$ calcd for $C_{48}H_{59}N4O_7S_2$ 867.3825, found 867.3843 ($\Delta_{ppm} = 2.1$); ^{1}H-NMR ($CDCl_3$, 270 MHz) δ 8.10−7.90 (1H, d, J = 5 Hz), 7.45−7.17 (19H, m), 6.80−6.70 (1H, br d, *J* = 5 Hz), 6.55−6.45 (1H, d, *J* = 5 Hz), 5.20−4.10 (6H, m), 3.70, 3.57 (3H, 2s, rotamers), 3.25−2.85 (2H, m), 2.80−2.40 (2H, m), 2.13−1.94 (5H, m), 1.98−1.95 (3H, 2s, rotamers), 1.43, 1.40 (9H, 2s, rotamers), 1.06−0.92 (6H, ddd, *J* = 5, 5, 20 Hz).

(*R*,S)-*N*-[[2-[*N*-[[2-[[(1,1-Dimethylethoxy)carbonyl]-amino]-3-[(triphenylmethyl)thio]propyl]carbonyl]-L-valyl]-1,2,3,4-tetrahydro-3-isoquinolinyl]carbonyl]-L-methionine (13).** A solution of **12** (1.3 g, 1.6 mmol) and 1 M LiOH (3.1 mL, 3.1 mmol) in methanol (20 mL) and THF (10 mL) was stirred for 3 h. Citric acid (3.1 mmol) was added, and the solution was concentrated, diluted with water (25 mL), and washed with ethyl acetate. The ethyl acetate layer was washed with saturated aqueous NaCl solution and dried, and the solvent was removed *in vacuo* to give 650 mg (0.76 mmol, 50%) of **13** as a glassy solid: accurate mass measurement $(M + Na)^+$ calculated for $C_{47}H_{56}N_4O_7S_2Na$ 875.3489, found 875.3513 ($\Delta_{ppm} = 2.8$); ^{1}H-NMR ($CO(CD_3)_2$, 270 MHz) δ 7.5−6.9 (19 H, m), 6.2−5.9 (1H, m), 4.9−4.7 (2H, m), 4.3−4.7 (1H, m), 3.9−3.7 (2H, m), 3.0−2.8 (2H, m), 2.7−2.3 (2H, m), 2.2−2.1 (2H, m), 2.0−1.7 (2H, m), 1.3 (9H, s), 0.9−0.6 (6H, m).

(*R*,S)-*N*-[[2-[*N*-[(2-Amino-3-thiopropyl)carbonyl]-L-valyl]-1,2,3,4-tetrahydro-3-isoquinolinyl]carbonyl]-L-methionine Trifluoroacetate (14).** A solution of **13** (330 mg, 0.38 mmol) in 15 mL of 1:1 TFA:CH_2Cl_2 (15 mL) was stirred at room temperature for 2.6 h. The solvents were removed *in vacuo*, and the oily residue was washed with hexanes, dissolved in 1:1 methanol:water containing 0.1% TFA, and purified by preparative HPLC (YMC C-18 column (22 × 250 mm, 5 μm, 120 Å) using a gradient of 25−60% B in A over 60 min (*A* = 0.05% TFA in H_2O; B = 0.05% TFA in CH_3CN)) to give, after lyophilization, 120 mg (49%) of **14** as a white fluffy powder: MS $(M + H)^+$ 511; ^{1}H-NMR (D_2O, 270 MHz) δ 7.21−7.30 (4H, m), 4.8−5.1 (2H, d, *J* = 42 Hz), 4.80−4.84 (1H, m), 4.64−4.65 (1H, m), 4.32−4.34 (1H, t, *J* = 4 Hz), 4.19−4.23 (1H, t, *J* = 5 Hz), 2.91−3.22 (4H, m), 2.30−2.32 (2H, m), 2.20−2.29 (2H, m), 1.98 (3H, d, *J* = 1 Hz), 1.81−1.85 (2H, m), 0.91−1.00 (6H, dd, *J* = 6, 28 Hz). Anal. ($C_{23}H_{34}N_4O_5S_2 \cdot C_2HF_3O_2 \cdot H_2O$) C, H, N, F, S.

(*S*)-1-[*N*-[(1,1-Dimethylethoxy)carbonyl]-L-valyl]-2,3-dihydro-1*H*-indole-2-carboxylic Acid Ethyl Ester (16). To a solution of Bop-Cl (2.5 g, 10 mmol), indoline-2-carboxylic acid ethyl ester (**15**) (1.2 g, 5 mmol), and NMM (2.2 mL, 20 mmol) in CH_2Cl_2 (20 mL) was added a solution of Boc-Val-OH (2.2 g, 10 mmol) in CH_2Cl_2 (30 mL) containing DMF (1 mL) at 0 °C under argon. The reaction was allowed to warm to 5 °C over 2 h, and stirring was continued for 20 h at 5 °C. The mixture was washed with 1 N HCl (30 mL) and 10% LiCl (30 mL), dried, filtered, and concentrated. The oil obtained was purified by flash chromatography eluting with 10% acetone in hexane to afford **16** contaminated with **15**. The oil was dissolved in diethyl ether (30 mL), and the solution was washed with 1 N HCl (3 × 20 mL), dried, and concentrated to afford pure ester **16** (880 mg, 47%) as an oil. An additional 200 mg (17%) of **16** was recovered from the acidic fraction: TLC R_f 0.33 (20% acetone in hexane); MS $(M + H)^+$ 391; ^{1}H-NMR ($CDCl_3$, 270 MHz) δ 8.3 (1H, d, *J* = 6.0 Hz), 7.2−7.0 (3H, m), 5.5−5.4 (1H, m), 5.1−4.95 (1H, m), 4.3-4.1 (2H, m), 3.65−3.25 (2H, m), 2.25−2.2 (1H, m), 1.4 (9H, s), 1.25 (3H, t, *J* = 7.0 Hz), 1.21 (1.5H, d, *J* = 7.0 Hz, rotamers), 1.17 (1.5H, d, *J* = 7.0 Hz), 1.01 (1.5H, d, *J* = 7.0 Hz), 0.94 (1.5H, d, *J* = 7.0 Hz); ^{13}C-NMR ($CDCl_3$, 67.8 MHz) δ 171.2, 171.0, 170.7, 155.9, 155.3,

142.2, 130.9, 128.72, 128.1, 127.8, 125.7, 125.0, 124.3, 123.8, 122.3, 120.5, 117.3. 113.7, 111.9, 108.3, 79.5, 79.1, 77.2, 62.3, 61.3, 60.5, 60.3, 57.5, 56.4, 33.3, 32.6, 31.0, 30.2, 28.2, 19.6, 19.4, 17.2, 15.6, 14.3, 13.9, 13.8.

(*S*)-1-[*N*-[(1,1-Dimethylethoxy)carbonyl]-L-valyl]-2,3-dihydro-1*H*-indole-2-carboxylic Acid (17). To a solution of **16** (840 mg, 2.2 mmol) in THF (9 mL) was added 1 N LiOH (3 mL, 3 mmol) and sufficient methanol (0.3 mL) for a homogeneous solution. After 3 h, 1 N HCl (5 mL) was added and the mixture was extracted with ethyl acetate (2 × 5 mL). The organic extracts were combined, dried, filtered, and concentrated to afford acid **17** (650 mg, 83%): MS $(M + H)^+$ 363; ^{1}H-NMR ($CDCl_3$, 270 MHz) δ 8.27 (1H, d, J = 8.0 Hz), 7.3−7.0 (3H, m), 5.72 (1H, d, J = 9.4 Hz), 5.15−5.05 (1H, m), 4.7−4.60 (1H, m), 3.6−3.4 (2H, m), 2.1−2.0 (1H, m), 1.4, 1.3 (9H, 2s, 1:3 ratio), 1.05 (3H, d, J = 7.0 Hz), 0.92 (3H, d, J = 7.0 Hz). Anal. ($C_{28}H_{43}N_3O_6S$) C, H, N.

(*S*)-*N*-[[1-[*N*-[(1,1-Dimethylethoxy)carbonyl]-L-valyl]-2,3-dihydro-1*H*-indol-2-yl]carbonyl]-L-methionine Methyl Ester (18). To a solution of **17** (300 mg, 0.83 mmol) in CH_2Cl_2 (4 mL) at 0 °C under argon were added sequentially) HCl·Met-O-tBu (242 mg, 1.0 mmol), NMM (0.24 mL, 2.2 mmol), and Bop (442 mg, 1.0 mmol). The mixture was allowed to warm to room temperature over 2 h, stirred overnight, and then washed with 1 N HCl (5 mL) and saturated $NaHCO_3$ (5 mL). The two aqueous washes were extracted separately with $CHCl_3$ (5 mL each). The organic extracts were combined, dried, and concentrated. Purification by silica gel column chromatography eluting with 30% ethyl acetate in hexane afforded **18** (340 mg, 74%): TLC R_f 0.19 (30% ethyl acetate in hexane); mp 79−81 °C; MS $(M + H)^+$ 550; ^{1}H-NMR ($CDCl_3$, 270 MHz) δ 8.1 (0.5 H, d, J = 2.0 Hz, rotamers), 7.9 (0.5H, d, J = 2.0 Hz), 7.25 (1H, d, J = 9.0 Hz), 7.15 (2H, d, J = 15 Hz), 5.45 (1H, d, J = 2 Hz), 5.3−5.15 (1H, m), 5.1−4.9 (1H, m), 4.6−4.4 (1H, m), 4.3−4.2 (1H, m), 3.6−3.3.3 (2H, m), 2.4−1.8 (5H, m), 1.98 and 1.87 (3H, 2s, rotamers), 1.43 (9H, s), 1.2−0.95 (6H, m).

(*S*)-*N*-[(1-L-Valyl-2,3-dihydro-1*H*-indol-2-yl)carbonyl]-L-methionine Methyl Ester (19). To a solution of **18** (130 mg, 0.24 mmol) in formic acid (98%, 0.5 mL) at room temperature under argon was added triethylsilane (0.08 mL), and the progress of the reaction was monitored by TLC analysis. After 9 h, saturated aqueous $NaHCO_3$ (5 mL) was added and the mixture was extracted with $CHCl_3$ (2 × 5 mL). The organic extracts were combined, dried, filtered, and concentrated to afford free amine **19** (100 mg, 92%): MS $(M + H)^+$ 450; ^{1}H-NMR ($CDCl_3$, 270 MHz) δ 8.3 (1H, d, J = 2.0 Hz, rotamers), 7.25 (2H, m), 7.15 (1H, m), 5.0 (1H, d, J = 2 Hz), 5.45 (1H, m), 3.7−3.1 (4H, m), 2.4−2.2 (2H, m), 2.1−1.8 (7H, m), 1.45 (9H, s), 1.01 (3H, d, J = 7.0 Hz), 0.93 (3H, dd, J = 8.0 Hz); ^{13}C-NMR ($CDCl_3$, 67.8 MHz) δ 175.3, 171.2, 170.4, 141.9, 129.4, 127.8, 124.6, 117.8, 82.7, 62.0, 59.2, 52.2, 34.2, 32.2, 30.5, 29.8, 29.6, 27.9, 19.9, 17.4, 15.2, 6.7, 6.3.

(*R*,S)-*N*-[[2-[*N*-[[2-[(1,1-Dimethylethoxy)carbonyl]-amino]-3-[(triphenylmethyl)thio]propyl]carbonyl]-L-valyl]-2,3-dihydro-1*H*-indol-2-yl]carbonyl]-L-methionine (20).** To a solution of **19** (100 mg, 0.22 mmol) in CH_2Cl_2 (1 mL) at 0 °C under argon were added sequentially Boc-Cys(S-Tr)-OH (120 mg, 0.26 mmol), NMM (0.033 mL, 0.3 mmol), and Bop (120 mg, 0.26 mmol). The reaction mixture was allowed to warm to room temperature over 2 h and then stirred for 16 h. The reaction mixture was diluted with CH_2Cl_2 (5 mL) and washed sequentially with 1 N HCl, saturated aqueous $NaHCO_3$, and brine (5 mL each). Purification by flash silica gel column chromatography eluting with 30% ethyl acetate in hexanes afforded **20** (160 mg, 81%): MS $(M + H)^+$ 895; ^{1}H-NMR ($CDCl_3$, 270 MHz) δ 8.1−7.9 (1H, br m), 7.5−7.0 (18H, m), 5.0 (1H, d, J = 2 Hz), 5.3−5.1 (1H, m), 5.0−4.6 (1H, m), 4.5−4.2 (1H, m), 4.1−3.8 (1H, m), 3.6−3.2 (2H, m), 2.8−2.7 (1H, m), 2.6−2.2 (2H, m), 2.1−1.9 (7H), 1.43 (18H, s), 1.2−0.9 (6H, m).

(*S*)-*N*-[[1-(*N*-L-Cysteinyl-L-valyl)-2,3-dihydro-1*H*-indol-2-yl]carbonyl]-L-methionine Trifluoroacetate (21). To solution of **20** (140 mg, 0.16 mmol) in CH_2Cl_2 (1.5 mL) at room temperature under argon were added triethylsilane (0.2 mL, 1.25 mmol) and TFA (0.5 mL). After 2 h, the volatiles were removed and the residue was purified by preparative HPLC (YMC C-18 column (30 × 500 mm, 10 μm, 120 Å) using a gradient of 10−90% B in A over 60 min (A = 0.1% TFA in H_2O; B = 0.1% TFA in CH_3CN)) to give, after lyophilization, **21** (56 mg, 52%): mp 72−74 °C; MS $(M + H)^+$ 497; $[\alpha]_D$ = −49.8° (*c* 0.49, methanol); ^{1}H-NMR (CD_3OD, 400 MHz) δ 8.6, 8.1 (1H, 2d, 1:3 ratio, J = 9.0 Hz), 7.3−6.9 (3H, m), 5.0 (1H, m), 4.7−4.5 (2H, m), 4.0 (1H, m), 3.6−3.5 (1H, m), 3.3−3.2 (1H, m), 2.95−2.8 (2H, m), 2.8−2.6 (2H, m), 2.2−1.9 (3H), 2.0 (3H, s), 0.95 (3H, d, J = 7.0 Hz), 0.92 (3H, d, J = 7.0 Hz); IR (KBr) 1674, 2969 cm^{-1}. Anal. ($C_{22}H_{32}N_4O_5S_2 \cdot 1.3CHF_3O_2 \cdot 1.8 \cdot H_2O$) C, H, N, F, S.

(*R*,S)-*N*-[[2-[*N*-[2-[[(1,1-Dimethylethoxy)carbonyl]amino]-3-[(triphenylmethyl)thio]propyl]-L-valyl]-1,2,3,4-tetrahydro-3-isoquinolinyl]carbonyl]-L-methionine Methyl Ester (23).** A solution of **11** (460 mg, 1.0 mmol) and **22** (0.55 g, 1.2 mmol)[12] in dry methanol (15 mL) containing 0.5 g of 3 Å molecular sieves was stirred at room temperature for 0.5 h. Glacial acetic acid (0.4 mL) was added followed by portionwise addition of $NaBH_3CN$ (126 mg, 2.0 mmol) over 30 min under nitrogen. Additional compound **22** (0.28 g, 0.6 mmol) was added, and the mixture was stirred for 16 h. On cooling the mixture to 0 °C, saturated $NaHCO_3$ (100 mL) was slowly added. The product was then extracted into ethyl acetate (60 mL). The ethyl acetate layer was washed with water (100 mL) and brine (100 mL), dried, concentrated, and chromatographed (silica gel, 4.1 × 15 cm, 1:1 ethyl acetate:hexane) to yield 514 mg of **23** as a clear oil (67%): MS $(M + H)^+$ 853; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.10−7.90 (1H, m), 7.45−7.17 (19 H, m), 6.80−6.70 (1H, m), 6.55−6.45 (1H, m), 5.20−4.10 (6H, m), 3.70, 3.57 (3H, 2s, rotamers), 3.25−2.85 (2H, m), 2.80−2.40 (2H, m), 2.13−1.94 (5H, m), 1.98−1.95 (3H, 2s, rotamers), 1.43, 1.40 (9H, 2s, rotamers), 1.06−0.92 (6H, m).

(*R*,S)-*N*-[[2-[*N*-[2-[[(1,1-Dimethylethoxy)carbonyl]amino]-3-[(triphenylmethyl)thio]propyl]-L-valyl]-1,2,3,4-tetrahydro-3-isoquinolinyl]carbonyl]-L-methionine (24).** A solution of **23** (300 mg, 0.35 mmol) in methanol (10 mL), THF (5 mL), and 1 M LiOH (0.4 mmol, 0.4 mL) was stirred for 24 h. The mixture was neutralized by addition of 1 N HCl (0.4 mL, 0.4 mmol) and concentrated. The residue was redissolved in water (30 mL), and the solution was washed with ethyl acetate. The organic layer was washed with brine (50 mL), dried, filtered, and concentrated to yield 290 mg (99%) of **24** as a white solid: MS $(M + H)^+$ 840.

(*R*,S)-*N*-[[2-[*N*-(2-Amino-3-thiopropyl)-L-valyl]-1,2,3,4-tetrahydro-3-isoquinolinyl]carbonyl]-L-methionine Trifluoroacetate (25).** A solution of **24** (120 mg, 0.14 mmol) in CH_2Cl_2 (5 mL), trifluoroacetic acid (5 mL), and triethylsilane (0.2 mL) was stirred for 1 h at room temperature. The mixture was concentrated, redissolved in methylene chloride (50 mL), and concentrated. The crude solid was washed with hexanes, dissolved in 2 mL of 0.1% aqueous TFA containing 20% methanol, and purified by preparative HPLC (YMC C-18 column, 2.2 × 25 cm, 5 μm, 120 Å; solvent A, 0.1% TFA in water; solvent B, 0.1% TFA in acetonitrile; 25−35% B in 40 min, flow rate 9 mL/min; UV monitored at 220 nm) to yield, after lyophilization, 35 mg of **25** (33%) as a white fluffy solid: mp 103−104 °C; $[\alpha]^{25}_D$ = −44.2° (*c* 0.5, CH_3OH); MS $(M + H)^+$ 497; ^{1}H-NMR (CD_3OD) δ 7.17 (4H, m), 4.72 (4H, m), 4.48 (1H, m), 4.29 (1H, m), 3.92 (1H, m), 3.25 (2H, m), 2.69 (2H, m), 2.43 (1H, m), 2.12 (3H, m), 1.95 (3H, 2s), 1.83 (2H, m), 0.98 (6H, m). Anal. ($C_{23}H_{36}N_4O_4S_2 \cdot 0.65 \cdot CHF_3O_2 \cdot 0.93H_2O$) C, H, N, S, F.

Compounds **31**−**33**, **36**−**38**, and **49** were prepared using amide bond-coupling and reductive amination procedures described for the preparation of **25** starting with the appropriate amino acid or aldehyde. Final products were purified by reverse phase HPLC as described for **25**.

(*R*,R)-*N*-[[2-[2-(L-Cysteinylamino)-3-methylbutyl]-1,2,3,4-tetrahydro-3-isoquinolinyl]carbonyl]-L-methionine Trifluoroacetate (31):** mp 112−113 °C; $[\alpha]^{25}_D$ = −55.7° (*c* 0.5, CH_3OH); MS $(M + H)^+$ 497; ^{1}H-NMR (CD_3OD, 270 MHz) δ 7.28 (4H, m), 4.57 (2H, m), 4.39 (1H, m), 4.22 (2H, m), 4.11 (1H, m), 3.14 (4H, br m), 2.55 (2H, m), 2.21 (2H, m), 2.10 (3H, s), 1.97 (3H, m), 0.98 (6H, m). Anal. ($C_{23}H_{36}N_4O_4S_2 \cdot 2.20 \cdot CHF_3O_2 \cdot 0.80H_2O$) C, H, N.

(*S*)-*N*-[(*N*-L-Cysteinyl-L-valyl)-1,2,3,4-tetrahydro-3-isoquinolinyl]-L-methionine Trifluoroacetate (32): mp 129−130 °C; $[\alpha]^{25}_D = +110°$ (*c* 0.1, CH_3OH); MS $(M + H)^+$ 495; ^{1}H-NMR (CD_3OD, 400 MHz) δ 7.74 (1H, m), 7.66 (1H, m), 7.44 (1H, m), 7.33 (1H, m), 5.01 (1H, m), 4.68 (1H, m), 4.28 (2H, m), 4.14 (1H, m), 3.99 (1H, m), 3.73 (1H, m), 3.45 (1H, m), 2.94 (1H, m), 2.81 (1H, m), 2.54 (2H, m), 2.00 (3H, s), 2.02 (1H, m), 1.91 (2H, m), 0.94 (6H, m); ^{13}C-NMR (CD_3OD, 100 MHz) δ 174.1, 172.7, 160.84, 160.43, 160.03, 159.6, 116.50, 113.6, 61.03, 53.50, 47.4, 44.4, 41.5, 39.7, 33.4, 29.2, 26.1, 24.3, 23.0, 21.2; IR 3434, 2928, 2556, 1680, 1576, 1429, 1204, 1138, 837, 801, 723 cm^{-1}. Anal. ($C_{22}H_{32}N_4O_5S_2 \cdot 2.03CHF_3O_2 \cdot 1.8H_2O$) C, H, N.

(*R,*R**)-*N*-[[2-[2-[(2-Amino-3-thiopropyl)amino]-3-methylbutyl]-1,2,3,4-tetrahydro-3-isoquinolinyl]carbonyl]-L-methionine Trifluoroacetate (33):** mp 72−73 °C; $[\alpha]^{25}_D = -30.0°$ (*c* 0.18, CH_3OH); MS $(M + H)^+$ 483; ^{1}H-NMR (CD_3OD, 270 MHz) δ 7.28 (4H, m), 4.68 (1H, m), 4.19 (1H, m), 3.66 (1H, m), 3.42 (2H, m), 3.24 (2H, m), 3.03 (2H, m), 2.94 (2H, d, $J = 4.28$ Hz), 2.60 (2H, m), 2.20 (2H, m), 2.10 (3H, s), 2.07 (3H, m), 1.01 (3H, d, $J = 7.03$ Hz), 0.93 (3H, d, $J = 7.04$ Hz). Anal. ($C_{22}H_{32}N_4O_5S_2 \cdot 1.9CHF_3O_2 \cdot 1.5H_2O$) C, H, N, F.

(*R*)-*N*-[[3-[[*N*-(2-Amino-3-mercaptopropyl)-L-valyl]amino]-2-naphthalenyl]carbonyl]-L-methionine Trifloroacetate (36): mp 101−102 °C; $[\alpha]^{25}_D = -28.7°$ (*c* 0.15, CH_3OH); MS $(M + H)^+$ 507; ^{1}H-NMR (CD_3OD, 270 MHz) δ 8.80 (1H, s), 8.24 (1H, s), 7.80 (2H, m), 7.42 (2H, m), 4.74 (2H, m), 3.41 (1H, m), 3.05 (1H, m), 2.91 (1H, m), 2.81 (2H, m), 2.62 (2H, m), 2.17 (3H, m), 2.05 (3H, s), 0.97 (6H, m). Anal. ($C_{24}H_{34}N_4O_5S_2 \cdot 2.5 \cdot CHF_3O_2 \cdot 1.9H_2O$) C, H, N, F.

(*S,*R**)-*N*-[[2-[*N*-(2-Amino-3-mercaptopropyl)-L-alanyl]-1,2,3,4-tetrahydro-3-isoquinolinyl]carbonyl]-L-methionine Trifluoroacetate (37):** mp 89−90 °C; $[\alpha]^{25}_D = -32.1°$ (*c* 1.0, CH_3OH); MS $(M + H)^+$ 469; ^{1}H-NMR (CD_3OD, 270 MHz) δ 7.16 (4H, m), 4.71 (2H, m), 4.57 (2H, m), 4.43 (1H, m), 4.32 (1H, m), 3.98 (1H, m), 3.56 (1H, m), 3.33−3.02 (2H, m), 2.84 (2H, m), 2.37 (1H, m), 1.98 (2H, m), 1.89 (1H, m), 2.10−1.76 (3H, m), 1.57 (2H, m), 1.43 (1H, m). Anal. ($C_{21}H_{32}N_4O_4S_2 \cdot 1.9CHF_3O_2 \cdot 3.17H_2O$) C, H, N, F.

[*R-[*R**-(*S**)]]-*N*-[[2-[2-[(2-Amino-3-mercaptopropyl)-amino]-1-oxobutyl]-1,2,3,4-tetrahydro-3-isoquinolinyl]-carbonyl]-L-methionine Trifluoroacetate (38):** mp 85−95 °C; $[\alpha]_D = -31°$ (*c* 0.1, CH_3CH_2OH); IR (KBr) 3435, 2924, 1674, 1431 cm^{-1}; MS $(M + H)^+$ 483; ^{1}H-NMR (CD_3OD, 270 MHz) δ 7.25 (4H, m), 1.8−4.9 (20H, m), 1.97, 2.07 (3H, 2s, rotamers), 1.1 (6H, m). Anal. ($C_{22}H_{34}N_4O_4S_2 \cdot 1.9CHF_3O_2 \cdot 1.5H_2O$) C, H, N, S, F.

[*R*-(*R,*S**)]-*N*-[[2-[*N*-(2-Amino-3-mercaptopropyl)-3-methyl-L-valyl]-1,2,3,4-tetrahydro-3-isoquinolinyl]carbonyl]-L-methionine Trifluoroacetate (49):** mp 87−90 °C; MS $(M + H)^+$ 511; IR (KBr) 1676, 1204 cm^{-1}; $[\alpha]_D = -27°$ (*c* 0.46, CH_3OH); ^{1}H-NMR (CD_3OD, 400 MHz) δ 7.35−7.20 (4H, m), 5.0−4.55 (5H, m), 3.45−3.1 (2H, m), 2.90−2.45 (5H, m), 2.07, 2.03 (3H, 2 s, rotameric), 2.2−1.95 (2H, m), 1.12, 1.06 (9H, 2s). Anal. ($C_{24}H_{38}N_4O_4S_2 \cdot 1.9CHF_3O_2 \cdot 1.5H_2O$) C, H, N, S, F.

***N*-[[2-[[(1,1-Dimethylethoxy)carbonyl]amino]-3-[(triphenylmethyl)thio]propyl]carbonyl]-L-valine Methyl Ester (26).** Acetic acid (2.6 mL, 45 mmol) was added to a solution of **22** (20 g, 45 mmol) and L-valine methyl ester hydrochloride (9.0 g, 54 mmol) in methanol (50 mL). The mixture was stirred at room temperature for 30 min. $NaBH_3CN$ (2.8 g, 45 mmol) in THF (50 mL) was added dropwise, and the mixture was stirred at room temperature for 2 h. The reaction was quenched with $NaHCO_3$ (3.8 g, 45 mmol) in water (20 mL) and the mixture concentrated under vacuum. The residue was dissolved in 10% $NaHCO_3$ (10 mL) and extracted with CH_2Cl_2 (3 × 100 mL). The combined organic extracts were dried, filtered, and concentrated under vacuum. The residue was purified by flash chromatography (eluting with 9:1 hexane:acetone) to afford **26** (18 g, 70%) as a viscous oil: TLC R_f 0.25 (8:1 hexane:acetone, visualization by UV); MS $(M + H)^+$ 563; IR (CH_2Cl_2 film) 1491, 1715 cm^{-1}; $[\alpha]_D = -3.8°$ (*c* 1.3, CH_3OH); ^{1}H-NMR (CD_3OD) δ 0.86, 0.87 (d, 6H, $J = 7.04$, 6.45Hz), 1.43 (s, 9H), 1.80−1.82 (m, 1H), 2.29−2.31 (m, 2H), 2.53−2.70 (m, 2H), 2.89 (m, 1H), 3.60 (m, 1H), 3.66 (s, 3H), 7.16−7.40 (m, 15H); ^{13}C-NMR (CD_3OD) δ 19.1, 19.6, 28.8, 30.7, 32.6, 36.0, 51.6, 51.9, 52.3, 53.0, 67.6, 68.5, 80.1, 127.8, 128.9, 130.8, 146.2, 157.7, 169.0, 169.9, 171.2, 176.7, 202.9. Anal. ($C_{33}H_{42}N_2O_4S \cdot 0.17H_2O$) C, H, N.

(*R)-*N*-[[2-[[(1,1-Dimethylethoxy)carbonyl]amino]-3-[(triphenylmethyl)thio]propyl]carbonyl]-*N*-(benzyloxycarbonyl)-L-valine Methyl Ester (27).** Benzyl chloroformate (90%, 10% BzCl, 3.2 mL, 20 mmol) was added to a solution of **26** (3.8 g, 6.8 mmol) and DIEA (3.8 mL, 22 mmol) in CH_2Cl_2 (20 mL) at 0 °C. The mixture was warmed to room temperature and stirred for 16 h. The reaction mixture was diluted with CH_2Cl_2 (100 mL) and washed sequentially with 1 N HCl (2 × 100 mL) and 10% $NaHCO_3$ (2 × 100 mL). The organic layer was dried, filtered, and concentrated under vacuum. The residue was purified by flash chromatography (eluting with 8:1 hexane:acetone) to afford **27** (4.3 g, 92%) as a white solid: mp 48−52 °C; MS $(M - H)^-$ 695; ^{1}H-NMR (CD_3OD, 270 MHz) δ 0.73 (d, 3H, $J = 6.45$ Hz), 0.93 (d, 3H, $J = 6.45$ Hz), 1.42 (s, 9H), 2.27 (m, 3H), 3.14 (m, 6H), 4.97−5.10 (m, 3H), 7.14−7.36 (m, 20H); ^{13}C-NMR (CD_3OD) δ 19.6, 20.8, 28.8, 29.5, 29.9, 36.2, 50.6, 51.0, 51.8, 52.7, 65.2, 67.5, 68.8, 80.3, 127.8, 128.0, 128.2, 128.9, 129.2, 129.5, 130.7, 137.5, 146.1, 157.4, 158.1, 172.9; IR (KBr) 1709 cm^{-1}; $[\alpha]_D = -9.7°$ (*c* 1.5, CH_3OH). Anal. ($C_{40}H_{46}N_2O_6S$) C, H, N, S.

(*R)-*N*-[[2-[[[(1,1-Dimethylethoxy)carbonyl]amino]-3-[(triphenylmethyl)thio]propyl]carbonyl]-*N*-(benzyloxycarbonyl)-L-valine (28).** Lithium hydroxide (2 N, 1.5 mL, 3.0 mmol) was added dropwise to a solution of **27** (0.70 g, 1.0 mmol) in THF (5 mL) and methanol (3.5 mL). The homogeneous reaction mixture was warmed to room temperature and stirred for 16 h. The mixture was concentrated under vacuum, diluted with 1 N HCl (30 mL), and extracted with CH_2Cl_2 (3 × 50 mL). The combined organic extracts were dried, filtered, and concentrated under vacuum to afford **28** (0.68 g, 100%): mp 66−72 °C; TLC R_f 0.61 (9:1:0.05 chloroform:methanol:acetic acid, visualization by UV); MS $(M - H)^-$ 681; $[\alpha]_D = -0.2°$ (*c* 0.83, CH_3OH); ^{1}H-NMR (CD_3OD) δ 0.83 (m, 3H), 0.93 (m, 3H), 1.50 (m, 9H), 1.90−1.92 (m, 1H), 2.35−2.60 (m, 2H), 3.30−3.55 (m, 2H), 3.77−4.20 (m, 2H), 4.95−5.22 (m, 2H), 7.24−7.45 (m, 20H); ^{13}C-NMR (CD_3OD) δ 19.9, 21.1, 21.4, 28.8, 29.2, 29.5, 29.9, 36.2, 51.1, 51.6, 51.7, 52.0, 54.8, 61.8, 65.2, 67.7, 67.8, 68.7, 80.3, 127.7, 127.9, 128.2, 128.9, 129.1, 129.3, 129.5, 130.7, 137.4, 146.0, 157.4, 157.9, 158.4, 173.9, 178.2; IR (KBr) 1653, 1707 cm^{-1}. Anal. ($C_{40}H_{46}N_2O_6S$) C, H, N, S.

(*R)-*N*-[*N*-[2-[[(1,1-Dimethylethoxy)carbonyl]amino]-3-[(triphenylmethyl)thio]propyl]-*N*-(benzyloxycarbonyl)-L-valyl]-1,2,3,4-tetrahydroisoquinoline-2-carboxylic Acid Methyl Ester (29).** *N*,*N*-Diisopropylethylamine (20 mL, 110 mmol) was added to a solution of **28** (24 g, 35 mmol), Tic-OMe·HCl (9.7 g, 43 mmol), and Bop-Cl (11 g, 43 mmol) in CH_2Cl_2 (200 mL). The mixture was stirred at 0 °C for 16 h. The reaction was quenched with 1 N HCl (300 mL) and the mixture extracted with CH_2Cl_2 (3 × 100 mL). The combined organic extracts were washed with 10% $NaHCO_3$ (100 mL), dried, filtered, and concentrated under vacuum. The residue was purified by flash chromatography (eluting with 4:1 hexane:acetone) to afford **29** (22 g, 72%) as a white solid: mp 66−70 °C; TLC R_f 0.28 (4:1 hexane:acetone, visualization by UV); MS $(M + H)^+$ 856; $[\alpha]_D = +21.0°$ (*c* 1.0, CH_3OH); ^{1}H-NMR (CD_3OD, 400 MHz) δ 0.76−0.98 (m, 6H), 1.14−1.31 (m, 9H), 2.16−2.40 (m, 3H), 2.96−3.59 (m, 8H), 3.93 (m, 1H), 4.50−5.30 (m, 5H), 7.12−7.42 (m, 24H); ^{13}C-NMR (CD_3OD) δ 14.4, 18.7, 18.8, 19.6, 23.7, 28.4, 28.7, 29.5, 31.7, 32.2, 32.7, 36.4, 36.7, 36.9, 41.4, 45.0, 46.2, 46.5, 47.0, 47.4, 47.6, 50.2, 50.8, 52.7, 55.0, 62.2, 62.4, 65.2, 67.7, 69.2, 72.9, 80.4, 93.1, 127.3, 127.5, 127.8, 128.0, 128.1, 128.3, 128.9, 129.1, 129.2, 129.3, 129.6, 129.9, 130.3, 130.7, 133.9, 134.4, 137.4, 146.0, 157.3, 158.6, 172.8, 173.2, 219.3; IR (KBr) 1638, 1711 cm^{-1}. Anal. ($C_{51}H_{75}N_3O_7S$) C, H, N.

(*R)-*N*-[*N*-[2-[[(1,1-Dimethylethoxy)carbonyl]amino]-3-[(triphenylmethyl)thio]propyl]-*N*-(benzyloxycarbonyl)-L-valyl]-1,2,3,4-tetrahydroisoquinoline-2-carboxylic Acid (30).** Lithium hydroxide (1 N, 54 mL, 54 mmol) was added dropwise to a solution of **29** (13 g, 15 mmol) in THF (100 mL) and MeOH (50 mL). The homogeneous reaction mixture was warmed to room temperature and stirred for 16 h. The

mixture was concentrated under vacuum, diluted with 1 N HCl (100 mL), and extracted with CH_2Cl_2 (3 × 150 mL). The combined organic extracts were dried, filtered, and concentrated under vacuum to afford **30** (12 g, 100%) as a white solid: mp 88−94 °C; TLC R_f 0.52 (9:1:0.05 chloroform:methanol:acetic acid, visualization by UV); MS $(M - H)^-$ 840; $[\alpha]_D = +8.0°$ (*c* 1.68, CH_3OH); ^{1}H-NMR (CD_3OD, 270 MHz) δ 0.71−0.92 (m, 6H), 1.26−1.35 (m, 9H), 1.86 (m, 1H), 2.19−2.40 (m, 2H), 2.80−3.51 (m, 5H), 3.65−3.98 (m, 1H), 4.51−5.40 (m, 5H), 7.12−7.41 (m, 24H); ^{13}C-NMR (CD_3OD, 67.5 MHz) δ 18.7, 18.9, 19.6, 26.5, 28.7, 29.5, 31.8, 32.3, 36.6, 46.5, 47.0, 47.4, 50.8, 53.8, 54.4, 62.2, 62.3, 65.2, 67.6, 68.8, 69.2, 80.4, 127.2, 127.7, 127.9, 128.2, 128.5, 128.9, 129.1, 129.6, 129.8, 130.7, 133.4, 133.9, 134.2, 137.4, 146.0, 157.2, 158.6, 172.6, 173.1, 173.9; IR (KBr) 1638, 1711 cm^{-1}. Anal. ($C_{50}H_{55}N_3O_7S \cdot 0.37H_2O$) C, H, N, S.

(*S*,R)-*N*-[[2-[*N*-[2-[[(1,1-Dimethylethoxy)carbonyl]amino]-3-[(triphenylmethyl)thio]propyl]-*N*-(benzyloxycarbonyl)-L-valyl]-1,2,3,4-tetrahydro-3-isoquinolinyl]carbonyl]-L-norleucine Methyl Ester (39).** *N*,*N*-Diisopropylethylamine (0.86 mL, 5.0 mmol) was added to a solution of **30** (1.4 g, 1.7 mmol), norleucine methyl ester hydrochloride (0.3 g, 1.7 mmol), and Bop (0.73 g, 1.7 mmol) in CH_3CN (9 mL) and DMF (3 mL). The mixture was stirred for 16 h, the reaction quenched with 1 N HCl (50 mL), and the mixture extracted with ethyl acetate (3 × 60 mL). The combined organic extracts were washed with 10% $NaHCO_3$ (1 × 50 mL) and 10% LiCl (2 × 50 mL), dried, filtered, and concentrated under vacuum. The residue was purified by flash chromatography (eluting with 4:1 hexane:acetone) to afford **39** (1.3 g, 79%) as a white solid: mp 58−68 °C; TLC R_f 0.37 (4:1 hexane:acetone, visualization by UV); MS $(M + H)^+$ 969; $[\alpha]_D = -21.8°$ (*c* 1.35, CH_3OH); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.41−7.07 (m, 24H), 5.27−4.51 (m, 6H), 4.40−3.80 (m, 1H), 3.67, 3.65 (3H, 2s), 3.70−2.98 (m, 5H), 2.34−2.16 (m, 3H), 1.65−1.61 (m, 1H), 1.48−1.38 (m, 9H), 1.50−0.7 (m, 14H); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 172.5, 170.0, 157.2, 155.1, 144.6, 135.9, 133.8, 132.7, 129.5, 128.7, 128.4, 128.0, 127.9, 126.9, 125.8, 79.2, 68.0, 66.4, 61.0, 54.5, 52.1, 50.0, 46.3, 45.0, 35.6, 35.2, 32.0, 31.2, 28.3, 26.6, 22.1, 19.5, 19.2, 18.5; IR (KBr) 1696, 1653, 1499 cm^{-1}. Anal. ($C_{57}H_{68}N_4O_8S \cdot 0.27H_2O$) C, H, N, S.

(*S*,R)-*N*-[[2-[*N*-[2-[[(1,1-Dimethylethoxy)carbonyl]amino]-3-[(triphenylmethyl)thio]propyl]-*N*-(benzyloxycarbonyl)-L-valyl]-1,2,3,4-tetrahydro-3-isoquinolinyl]carbonyl]-L-norleucine (40).** Lithium hydroxide (2 N, 2.3 mL, 4.7 mmol) was added to a solution of **39** (1.1 g, 1.2 mmol) in THF (10 mL) at 0 °C, warmed to room temperature, and stirred for 4 h. The reaction mixture was concentrated under vacuum and dissolved in water (50 mL), the reaction quenched with 1 N HCl to pH of 4.0, and the mixture extracted with dichloromethane (3 × 50 mL). The combined organic extracts were dried, filtered, and concentrated under vacuum to afford **40** (1.1 g, 98%) as a hygroscopic solid: mp 86−94 °C; MS $(M + H)^+$ 955; $[\alpha]_D = -17°$ (*c* 1.15, CH_3OH); ^{1}H-NMR (CD_3OD, 400 MHz) δ 7.12−7.42 (24H, m), 4.59−5.31 (5H, m), 4.23−4.31 (1H, m), 3.70−3.85 (1H, m), 2.99−3.31 (5H, m), 2.02−2.23 (3H, m), 0.66−0.91, 1.04−1.71 (24H, m); ^{13}C-NMR (CD_3OD, 100 MHz) 175.2, 173.3, 172.6, 158.5, 158.0, 157.4, 146.0, 137.3, 135.2, 135.0, 130.7, 130.6, 129.9, 129.6, 129.1, 128.9, 128.2, 128.0, 127.7, 127.1, 80.4, 69.2, 68.8, 67.5, 62.6, 56.6, 56.4, 53.3, 50.5, 47.4, 46.7, 37.1, 36.8, 33.0, 32.6, 29.3, 29.1, 28.7, 28.3, 26.5, 23.3, 20.1,19.9, 18.6; IR (KBr) 1653, 1696, 2932 cm^{-1}. Anal. ($C_{56}H_{66}N_4O_8S \cdot 0.43H_2O$) C, H, N, S.

(*S*,R)-*N*-[[2-[(2-Amino-3-mercaptopropyl)-L-valyl]-1,2,3,4-tetrahydro-3-isoquinolinyl]carbonyl]-L-norleucine Trifluoroacetate (41).** Trifluoroacetic acid (5.3 mL, 68 mmol) was added dropwise to a solution of **40** (1.0 g, 1.05 mmol) and triethylsilane (1.7 mL, 11 mmol) in CH_2Cl_2 (10 mL) at 0 °C. The reaction mixture was warmed to room temperature and stirred for 0.75 h. The reaction mixture was concentrated under vacuum and the residue triturated with hexane (3 × 20 mL) and dried under vacuum. A solution at 0 °C of trifluoroacetic acid (18 mL, 220 mmol), thioanisole (1.2 mL, 11 mmol), and ethanedithiol (0.88 mL, 11 mmol) was added to the residue. Bromotrimethylsilane (0.96 mL, 7.3 mmol) was added at 0 °C, and the mixture was stirred for 1 h. The reaction mixture was diluted with diethyl ether (50 mL) and centrifuged and the solid triturated, discarding the liquid portion. Trituration was repeated with diethyl ether (2 × 50 mL), ethyl acetate (2 × 50 mL), and diethyl ether (1 × 50 mL). The solid was dried under vacuum and then purified by preparative HPLC (YMC S-10 ODS 30 × 500 mm, 220 nm, 28 mL/min, 10−90% aqueous methanol with 0.1% TFA). The appropriate fractions were concentrated under vacuum (at 35 °C), dissolved in water (20 mL), and lyophilized to afford **41** (0.25 g, 33%) as a white lyophilate: mp 80−90 °C; TLC R_f 0.69 (6:3:1 *n*-propanol:ammonium hydroxide:water, visualization by ceric ammonium sulfate); MS $(M + H)^+$ 479; $[\alpha]_D = -27.7°$ (*c* 0.43, CH_3OH); ^{1}H-NMR (CD_3OD, 400 MHz) δ 7.29−7.22 (4H, m), 4.89−4.51 (3H, m), 4.34−4.06 (2H, m), 3.77−2.75 (5H, m), 2.21−2.10 (1H, br m), 1.82−1.49 (4H, m), 1.31−0.83 (13H, m); IR (KBr) 1674, 3435 cm^{-1}. Anal. ($C_{24}H_{38}N_4O_4S \cdot CHF_3O_2 \cdot 1.29H_2O$) C, H, N, S, F.

Compounds **42**−**45** were prepared and purified in an analogous manner to **41** starting from **30** and the appropriate amino acid methyl ester.

[*R*-(*R,*S**)]-*N*^{2}[[2-[*N*-(2-Amino-3-mercaptopropyl)-L-valyl]-1,2,3,4-tetrahydro-3-isoquinolinyl]carbonyl]-L-asparagine Trifluoroacetate (42):** mp 95−110 °C; TLC R_f 0.50 (6:3:1 *n*-propanol:ammonium hydroxide:water, visualization by ceric ammonium sulfate); MS $(M + H)^+$ 480; $[\alpha]_D = -16.1°$ (*c* 0.54, CH_3OH); ^{1}H-NMR (CD_3OD) δ 7.24−7.22 (4H, m), 4.89−4.65 (4H, m), 3.51−3.08 (5H, m), 2.89−2.51 (5H, m), 2.20−1.9 (1H, br m), 1.02, 1.04, 1.15 (6H, dd, J = 6.84, 7.27 Hz); IR (KBr) 1204, 1674, 3424 cm^{-1}. Anal. ($C_{22}H_{33}N_5O_5S \cdot 1.8CHF_3O_2 \cdot 1.8H_2O$) C, H, N, S, F.

(*S*,R)-*N*-[[2-[*N*-(2-Amino-3-mercaptopropyl)-L-valyl]-1,2,3,4-tetrahydro-3-isoquinolinyl]carbonyl]-γ-(aminosulfonyl)-L-α-aminobutyric Acid Trifluoroacetate (43):** R_f 0.6 (6:3:1 nPrOH:NH_4OH:H_2O, visualized by UV, PMA); MS $(M + H)^+$ 530; $[\alpha]_D = -16.3°$ (*c* 0.19, CH_3OD); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.4−7.2 (4H, m), 4.9−4.4 (5H, m), 3.8−3.2 (5H, m), 2.9−2.2 (7H, m), 1.1 (6H, d, J = 8 Hz); IR (KBr) 3439, 1678, 1653, 1437, 1206, 1142, 723 cm^{-1}. Anal. ($C_{22}H_{35}N_5O_6S_2 \cdot 2.5CHF_3O_2 \cdot 0.75H_2O$) C, H, N.

(*S*,R)-*N*-[[2-[*N*-(2-Amino-3-mercaptopropyl)-L-valyl]-1,2,3,4-tetrahydro-3-isoquinolinyl]carbonyl]-3-[(methylsulfonyl)methyl]-L-alanine Trifluoroacetate (44):** mp 105−115 °C; TLC R_f = 0.52 (6:3:1 *n*-propanol:ammonium hydroxide:water, visualization by ceric ammonium sulfate); MS $(M + H)^+$ 529; $[\alpha]_D = -10.0°$ (*c* 0.12, CH_3OH); ^{1}H-NMR (CD_3OD, 400 MHz) δ 1.07−1.19 (m, 6H), 2.15 (br m, 2H), 2.25 (br m, 1H), 2.42 (br m, 2H), 2.80−3.02 (m, 5H), 2.92, 2.97 (s, 3H), 3.09−3.48 (m, 3H), 4.25 (m, 1H), 4.45−4.80 (m, 3H), 7.22−7.31 (m, 4H); IR (KBr) 2567, 1676, 1530, 1294 cm^{-1}. Anal. ($C_{23}H_{36}N_4O_6S_2 \cdot 2.21CHF_3O_2 \cdot 1.16H_2O$) C, H, N, S, F.

[*R*-(*R,*S**)]-*N*^{2}[[2-[*N*-(2-Amino-3-mercaptopropyl)-L-valyl]-1,2,3,4-tetrahydro-3-isoquinolinyl]carbonyl]-L-glutamine Trifluoracetate (45):** mp 65−75 °C; TLC R_f 0.69 (6:3:1 *n*-propanol:ammonium hydroxide:water, visualization by ceric ammonium sulfate); MS $(M + H)^+$ 494; $[\alpha]_D = -21.4°$ (*c* 0.69, CH_3OH); ^{1}H-NMR (400 MHz, CD_3OD) δ 7.33−7.22 (m, 4H), 4.64−4.91 (m, 3H), 4.38−4.23 (br m, 2H), 3.49−2.78 (m, 5H), 2.12−2.31 (m, 6H), 1.93 (br m, 1H), 1.07, 1.08, 1.09 (d, 6H, J = 7.6 Hz); IR (KBr) 1202, 1670, 2567 cm^{-1}. Anal. ($C_{23}H_{35}N_5O_5S \cdot 2.25CHF_3O_2 \cdot 1.63H_2O$) C, H, N, S, F.

***N*-[*N*-[(1,1-Dimethylethoxy)carbonyl]-L-valyl]-1,2,3,6-tetrahydropyridine-2-carboxylic Acid Methyl Ester (52).** Bop-Cl (2.2 g, 8.5 mmol) was added to a stirred solution of tetrahydropyridinecarboxylic acid methyl ester (1.0 g, 7.1 mmol), Boc-Val-OH (1.7 g, 7.8 mmol), and DIEA (3.0 g, 4.1 mL, 23 mmol) in DMF (25 mL) at 5 °C under argon. After stirring for 15 h at 5 °C, the mixture was partitioned between ethyl acetate (30 mL) and 10% HCl (10 mL). The aqueous layer was re-extracted (2 × 20 mL) with ethyl acetate. The combined organic extracts were washed sequentially with 10% HCl (2 × 10 mL), water (2 × 30 mL), saturated aqueous $NaHCO_3$ (3 × 10 mL), and brine (2 × 20 mL). The organic layer was dried, filtered, and concentrated to afford **52** (0.86 g, 36%) as a clear viscous oil: MS $(M + H)^+$ 341; ^{1}H-NMR (CD_3OD, 400 MHz) δ 5.9−5.1 (6H, m), 4.8−4.1 (3H, m), 2.9−2.5

(2H, m), 2.5−1.7 (8H, m), 1.5−1.4 (18H, m), 1.04−0.91 (6H, m). Anal. ($C_{17}H_{28}N_2O_5$·0.75H_2O) C, H, N.

(*R)-*N*-[[1-[*N*-[(1,1-Dimethylethoxy)carbonyl]-L-valyl]-1,2,3,6-tetrahydro-2-pyridinyl]carbonyl]-L-methionine *tert*-Butyl Ester (53).** To a solution of **52** (0.86 g, 2.5 mmol) in methanol (10 mL) at room temperature was added 1 N NaOH (2.8 mL, 2.8 mmol). The reaction mixture was stirred for 2 h at room temperature, concentrated, and diluted with 1 N HCl (4 mL)/ethyl acetate (10 mL). The aqueous layer was re-extracted with ethyl acetate (2 × 20 mL). The combined organic layers were washed with brine, dried, filtered, and concentrated to afford the free acid (0.80 g, 97%). To this acid (0.8 g, 2.7 mmol) was added Bop (1.3 g, 2.9 mmol), H-Met-O-tBu (0.65 g, 2.7 mmol), and *N*,*N*-diisopropylethylamine (0.66 mL, 3.7 mmol) in DMF (8 mL) at room temperature under argon. After stirring for 15 h, the reaction mixture was partitioned between ethyl acetate (20 mL) and 10% HCl (10 mL). The aqueous layer was extracted (2 × 20 mL) with ethyl acetate. The combined organic extracts were washed sequentially with 10% HCl (2 × 10 mL), water (2 × 20 mL), saturated aqueous $NaHCO_3$ (2 × 10 mL), and brine (2 × 20 mL). The organic layer was dried, filtered, and concentrated to afford **53** (0.87 g, 78%) as a yellow oil: accurate mass measurement $(M + H)^+$ calcd for $C_{25}H_{44}O_6N_3S$ 514.2927, found 514.2939 (Δ_{ppm} = 2.1); TLC R_f 0.57 (ethyl acetate, visualized by UV, PMA); ^{1}H-NMR (400 MHz) δ 5.9−5.5 (2H, m), 4.8−2.7 (4H, m), 2.5−2.3 (5H, m), 2.0−1.9 (6H, m), 1.5−1.4 (9H, m), 1.3−0.9 (6H, m).

***N*-[[1-[*N*-(*R*)-[2-[[(1,1-Dimethylethoxy)carbonyl]amino]-3-[(triphenylmethyl)thio]propyl]-L-valyl]-1,2,3,6-tetrahydro-2-pyridinyl]carbonyl]-L-methionine *tert*-Butyl Ester (54).** Triethylsilane (0.18 mL, 1.0 mmol) was added to a solution of **53** (0.26 g, 0.50 mmol) in formic acid (3 mL) at room temperature under argon. After stirring for 2 h, the reaction mixture was allowed to stand for 2 days at 4 °C. The reaction mixture was diluted with $NaHCO_3$ (saturated, to pH 8−9) and ethyl acetate (40 mL). The aqueous layer was extracted with ethyl acetate (2 × 30 mL). The combined organic extracts were washed with brine, dried, filtered, and concentrated to afford the amine (0.076 g, 36%). The product was used in the next step without purification. A solution of sodium cyanoborohydride (0.012 g, 0.20 mmol) in methanol (0.5 mL) was added dropwise to a stirred solution of the above amine (0.075 g, 0.16 mmol) and aldehyde **22** (0.11 g, 0.24 mmol) in acetic acid (0.76 mL) and methanol (0.5 mL) at 0 °C. The mixture was slowly warmed to room temperature and stirred for 15 h. The reaction mixture was diluted with $NaHCO_3$ at 0 °C and extracted with ethyl acetate (3 × 20 mL). The combined extracts were washed with brine, dried, filtered, and concentrated. The product was purified by flash chromatography eluting with 30−50% ethyl acetate/hexane to afford **54** (0.14 g, 97%) as a highly viscous oil: TLC R_f 0.27 (1:1 hexane:ethyl acetate, visualized by PMA); MS $(M + H)^+$ 845; ^{1}H-NMR (270 MHz) δ 7.5−7.2 (16H, m), 6.9 (1H, m), 6.8 (H, m), 5.9−5.6 (2H, m), 4.9−3.3 (8H, m), 2.8−2.1 (6H, m), 2.0−1.9 (3H, s), 1.5−1.4 (18H, m), 1.0−0.8 (6H, m).

***N*-[[1-[*N*-(*R*)-(2-Amino-3-mercaptopropyl)-L-valyl]-1,2,3,6-tetrahydro-2-pyridinyl]carbonyl]-L-methionine Trifluoroacetate (34).** Triethylsilane (0.05 mL, 0.037 g) was added to a solution of **54** (0.14 g, 0.16 mmol) and TFA (1.0 mL) in CH_2Cl_2 (1 mL). After stirring for 13 h, the reaction mixture was concentrated to afford a solid (0.6 g). A portion of this residue (0.2 g) was purified by prep HPLC (YMC S-10 ODS 30 × 500 mm column, eluting over 30 min with 0−90% aqueous methanol containing 0.1% TFA) to afford, after lyophilization, **34** (23 mg, 32%) as a white solid: accurate mass measurement $(M + H)^+$ calcd for $C_{19}H_{35}N_4O_4S_2$ 447.1200, found 447.2112 (Δ_{ppm} = 2.3); ^{1}H-NMR (400 MHz CD_3OD) δ 8.04−8.02 (H, d, J = 8 Hz), 5.8−5.7 (2H, m), 5.45−5.43 (H, d, J = 7 Hz), 4.5−3.6 (6H, m), 3.2−2.4 (12H, m), 2.09−2.05 (6H, m), 1.58−0.9 (6H, m, rotamers); IR (KBr) 2972, 1676, 1204, 1138 cm^{-1}.

***N*-[[1-[*N*-(*S*)-(2-Amino-3-mercaptopropyl]-L-valyl]-1,2,3,6-tetrahydro-2-pyridinyl]carbonyl]-L-methionine trifluoroacetate (35).** Compound **35** was obtained as a second component in the purification of **34**: yield 17 mg of white lyophilate; TLC R_f 0.16 (9:1:0.1 $CHCl_3$:CH_3OH:CH_3COOH, visualized by UV, PMA); IR (KBr) 2924, 1672, 1427, 1202, 1134 cm^{-1}; MS $(M + H)^+$ 447; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 8.09 (1H, m), 5.8−5.7 (2H, m), 5.46−5.45 (1H, m), 4.55 (1H, m), 4.18 (2H, m), 3.6 (1H, m), 3.03−2.4 (12H, m), 2.1−2.0 (6H, m), 1.02−1.00 (6H, m). Anal. ($C_{19}H_{34}N_4O_4S_2$·1.5CHF_3O_2·1.24H_2O) C, H, N.

(*R)-2-[*N*-(Benzyloxycarbonyl)-3-methyl-L-valyl]-1,2,3,4-tetrahydroisoquinoline-3-carboxylic Acid Methyl Ester (55).** To a solution of *N*-(benzyloxycarbonyl)-L-*tert*-leucine (5.3 g, 20 mmol) in CH_2Cl_2 (80 mL) at 0 °C under argon were sequentially added *N*,*N*-diisopropylethylamine (11 mL, 60 mmol), Bop-Cl (5.1 g, 20 mmol), and Tic-OMe·HCl (5.7 g, 25 mmol). The mixture was allowed to warm to 5 °C over 2 h and then stirred overnight (16 h). The mixture was washed with 1 N HCl, saturated aqueous $NaHCO_3$, and brine (50 mL each). The organic layer was dried, filtered, and concentrated to afford an oil. Purification by flash silica gel column chromatography eluting with 20% ethyl acetate in hexanes afforded **55** (4.0 g, 45%): TLC R_f 0.28 (30% ethyl acetate in hexanes, visualized by UV and ceric ammonium molybdate); MS $(M + H)^+$ 439; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.4−7.1 (9H, m), 5.6 (1H, d, J = 10 Hz), 5.3 (1H, t, J = 5 Hz), 5.06 (2H, dd, J = 12, 16 Hz), 5.1−5.0 (1H, m), 4.75 (1H, d, J = 10 Hz), 3.6, 3.5 (3H, 2s, rotamers), 3.2 (1H, dq, J = 5, 21 Hz), 1.1, 1.0 (9H, 2s); ^{13}C-NMR ($CDCl_3$, 100.4 MHz) δ 171.6, 171.3, 156.2, 136.2, 132.4, 132.2, 128.4, 128.1, 128.0, 127.4, 127.1, 126.2, 66.9, 57.2, 52.3, 52.2, 46.6, 36.3, 30.8, 26.3.

(*R)-2-[*N*-(Benzyloxycarbonyl)-3-methyl-L-valyl]-1,2,3,4-tetrahydroisoquinoline-3-carboxylic Acid Methyl Ester (56).** Sodium hydroxide (1 N, 13 mL, 13 mmol) was added in three portions to a solution of **55** (1.9 g, 4.3 mmol) in methanol (30 mL), and the resulting solution was stirred at room temperature for 2 h. The reaction mixture was concentrated under vacuum, dissolved in HCl (1 N, 70 mL), and extracted with CH_2Cl_2 (3 × 150 mL). The combined organic extracts were dried, filtered, and concentrated under vacuum to afford **56** (1.8 g, 100%) which was used without further purification: mp 78−88 °C; TLC R_f 0.32 (9:1 $CHCl_3$:methanol, visualization by UV); MS $(M + H)^+$ 425; $[\alpha]_D$ = +15.6° (*c* 0.9, CH_3OH); ^{1}H-NMR (CD_3OD, 400 MHz) δ 1.09 (s, 9H), 3.07−3.22 (m, 2H), 4.67−5.20 (m, 6H), 7.16−7.30 (m, 9H); ^{13}C-NMR (CD_3OD, 100.4 MHz) δ 26.8, 26.9, 31.8, 32.4, 36.8, 45.0, 47.7, 50.0, 54.0, 54.8, 56.7, 58.6, 58.8, 67.8, 127.1, 127.5, 127.8, 128.0, 128.3, 129.0, 129.4, 132.7, 133.2, 134.2, 138.0, 158.5, 173.3, 173.8, 173.9; IR (KBr) 3430, 1719, 1518 cm^{-1}. Anal. ($C_{24}H_{28}N_2O_5$) C, H, N.

(*R)-*N*-[[2-[*N*-(Benzyloxycarbony)-3-methyl-L-valyl]-1,2,3,4-tetrahydro-3-isoquinolinyl]carbonyl]-L-glutamine *tert*-Butyl Ester (57).** *N*,*N*-Diisopropylethylamine (1.1 mL, 6.3 mmol) was added to a solution of **56** (0.9 g, 2.1 mmol), L-glutamine *tert*-butyl ester hydrochloride (0.50 g, 2.1 mmol), and Bop (0.93 g, 2.1 mmol) in 3:1 CH_3CN:DMF (26 mL). The mixture was stirred at room temperature for 16 h. The reaction was quenched with HCl (1 N, 100 mL), the mixture was extracted with ethyl acetate (4 × 75 mL), and the combined organic extracts were washed with 10% LiCl (3 × 150 mL), dried, filtered, and concentrated under vacuum. The residue was purified by flash chromatography (eluting with 1:1 hexane:acetone) to afford **57** (1.1 g, 87%) as a white solid: mp 60−68 °C; TLC R_f 0.62 (2:1 acetone:hexane, visualization by UV); $[\alpha]_D$ = −37.2° (*c* 0.88, CH_3OH); MS $(M + H)^+$ 609; ^{1}H-NMR (CD_3OD, 400 MHz) δ 7.32−7.17 (m, 9H), 5.09−4.52 (m, 7H), 3.71, 3.58 (s, 3H), 2.95−2.30 (m, 4H), 2.93, 2.87 (s, 3H), 2.45−2.20 (m, 1H), 2.07−1.98 (m, 1H), 1.06, 1.02 (s, 9H); ^{13}C-NMR (CD_3OD, 100.4 MHz) δ 174.0, 173.1, 172.6, 138.0, 135.8, 135.5, 129.4, 129.2, 128.9, 128.6, 128.1, 128.0, 127.3, 126.9, 67.8, 61.5, 59.2, 58.9, 57.7, 57.3, 53.1, 52.5, 52.1, 51.8, 45.7, 40.7, 36.8, 36.3, 32.8, 27.1, 26.9, 26.1, 20.9, 14.4; IR (KBr) 1669, 1701, 1717, 3428 cm^{-1}. Anal. ($C_{33}H_{44}N_4O_7$·1.95H_2O) C, H, N.

(*R)-*N*-[[2-[3-Methyl-L-valyl]-1,2,3,4-tetrahydro-3-isoquinolinyl]carbonyl]-L-glutamine *tert*-Butyl Ester (58).** Palladium hydroxide on carbon (10%, 0.091 g) was added to a solution of **57** (0.91 g, 1.5 mmol) in THF (9.1 mL) and 1 N HCl (1.5 mL). A hydrogen balloon was attached to the

reaction, and the mixture was stirred for 3 h. The reaction mixture was filtered through Celite and concentrated under vacuum to afford **58** (0.77 g, 100%) which was used without further purification: mp foams and liberates gas above 128 °C; TLC R_f 0.77 (6:3:1 *n*-propanol:ammonium hydroxide:water, visualization by ninhydrin); MS $(M + H)^+$ 475; $[\alpha]_D = -35.9°$ (*c* 0.49, CH_3OH); ^{1}H-NMR (CD_3OD, 400 MHz) δ 7.45−7.27 (m, 4H), 5.00−4.60 (m, 3H), 4.35 (br m, 1H), 3.35−3.25 (m, 1H), 3.20−3.05 (m, 2 H), 2.35 (t, 2H, J = 7.04 Hz), 2.30−1.85 (m, 2H), 1.47, 1.46 (s, 9H), 1.19 (s, 9H); ^{13}C-NMR (CD_3OD, 100.4 MHz) δ 177.9, 172.1, 169.2, 135.7, 135.6, 129.2, 128.3, 128.2, 127, 83.1, 58.8, 58.0, 53.7, 36.0, 32.8, 32.6, 29.6, 28.9, 28.2, 26.8, 26.7; IR (KBr) 1370, 1663, 1728, 3416 cm^{-1}. Anal. ($C_{25}H_{39}N_4O_5Cl\cdot0.2CH_3OH\cdot0.2C_2H_4O$) C, H, N.

(*S,*R**)-*N*-[[2-[*N*-[2-[[(1,1-Dimethylethoxy)carbonyl]amino]-3-[(triphenylmethyl)thio]propyl]-3-methyl-L-valyl]-1,2,3,4-tetrahydro-3-isoquinolinyl]carbonyl]-L-glutamine *tert*-Butyl Ester (59).** Sodium cyanoborohydride (0.077 g, 1.2 mmol) was added portionwise to a solution of **22** (0.46 g, 0.91 mmol), **58** (0.61 g, 1.4 mmol), and acetic acid (0.135 mL) in methanol (4.5 mL), and the resulting solution was stirred at room temperature for 2.25 h. The reaction was quenched with water (100 mL) and the mixture extracted with CH_2Cl_2 (3 × 100 mL). The combined organic extracts were dried, filtered, and concentrated under vacuum. The residue was purified by flash chromatography (eluting with ethyl acetate) to afford **59** (0.55 g, 67%) as a gum: TLC R_f 0.41 (1:1 hexane:acetone, visualization by UV); MS $(M + H)^+$ 906; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 7.28−7.12 (m, 19H), 5.00 (m, 1H), 4.89, 4.84 (s, 2H), 4.56 (br m, 1H), 4.06 (m, 1H), 3.35 (br m, 1H), 3.09 (br m, 2H), 2.81 (m, 2 H), 2.40−2.10 (m, 2 H), 2.04 (t, 2 H, J = 7.62 Hz), 1.95−1.74 (m, 2 H), 1.35 (s, 9H), 1.33 (s, 9H), 0.92 (s, 9H); ^{13}C-NMR ($CDCl_3$, 100.4 MHz) δ 175.4, 171.6, 170.8, 144.5, 143.1, 133.9, 129.4, 127.9, 127.0, 126.6, 125.7, 82.6, 66.4, 60.4, 56.0, 51.9, 50.0, 49.7, 49.3, 49.0, 48.7, 47.2, 35.6, 34.2, 31.3, 29.0, 28.2, 27.8, 26.7, 20.9, 14.0; IR (KBr) 1491, 1628, 1676, 2976 cm^{-1}. Anal. ($C_{52}H_{67}N_5O_7S\cdot1.33H_2O$) C, H, N.

(*S,*R**)-N^2-[[2-[*N*-(2-Amino-3-mercaptopropyl)-3-methyl-L-valyl]-1,2,3,4-tetrahydro-3-isoquinolinyl]carbonyl]-L-glutamine Trifluoroacetate (46).** Trifluoroacetic acid (5 mL, 65 mmol) was added to a solution of **59** (0.49 g, 0.54 mmol) and triethylsilane (0.84 mL, 5.4 mmol) in CH_2Cl_2 (5 mL) at 0 °C, and the resulting solution was stirred at room temperature for 3 h. Chloroform (10 mL) was added, and the mixture was concentrated under vacuum. The residue was triturated with hexane and dried under vacuum. The residue was purified by preparative HPLC (EM S-12 Lichrosphere Select B, 50 × 250 mm, 220 nm, 40 mL/min, gradient 10−90% aqueous methanol with 0.1% TFA) to afford, after lyophilizing, **46** (0.28 g, 71%) as a white solid: mp decomposition above 97 °C; TLC R_f 0.55 (6:3:1 *n*-propanol:ammonium hydroxide:water, visualization by ceric ammonium sulfate); MS $(M + H)^+$ 508; $[\alpha]_D = -17.6°$ (*c* 0.33, CH_3OH); ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 7.17 (s, 4H), 5.02−4.85 (m, 3H), 4.59−4.52 (m, 1H), 4.20−4.18 (m, 1H), 3.34 (br m, 2H), 2.96 (br m, 2H), 2.72 (br m, 1H), 2.47−2.35 (m, 2H), 2.13−2.08 (m, 2H), 1.96−1.81 (m, 2H), 1.00 (s, 9H); IR (KBr) 1431, 1674, 2972, 3437 cm^{-1}. Anal. ($C_{28}H_{39}N_5O_9SF_6\cdot1.07H_2O$) C, H, N.

Compounds **47, 48**, and **50** were prepared and purified in a similar manner to **46** starting from **58** and the appropriate amino acid *tert*-butyl ester.

[*R*-(*R,*S**)]-N^2-[[2-[*N*-(2-Amino-3-mercaptopropyl)-3-methyl-L-valyl]-1,2,3,4-tetrahydro-3-isoquinolinyl]carbonyl]-N^5,N^5-dimethyl-L-glutamine Trifluoroacetate (47):** MS $(M + H)^+$ 536; $[\alpha]_D = -15°$ (*c* 0.22, CH_3OH); IR (KBr) 2969, 1680, 1638, 1427, 1206, 1138, 723 cm^{-1}. Anal. ($C_{26}H_{41}N_5O_5S\cdot2.83CF_3COOH$) C, H, N.

[*R*-(*R,*S**)]-*N*-[[2-[*N*-(2-Amino-3-mercaptopropyl)-3-methyl-L-valyl]-1,2,3,4-tetrahydro-3-isoquinolinyl]carbonyl]-*O*-methyl-L-homoserine Trifluoroacetate (48):** mp melts with decomposition at 85−95 °C; TLC R_f 0.41 (6:3:1 *n*-propanol:ammonium hydroxide:water, visualization by ceric ammonium sulfate); MS $(M + H)^+$ 495; $[\alpha]_D = -23.7°$ (*c* 0.27, CH_3OH); ^{1}H-NMR (DMSO-d_6, 125 °C, 400 MHz) δ 7.17 (s, 4H), 5.03 (t, 1H, J = 5.98 Hz), 4.90 (br m, 1H), 4.59 (br m, 1H), 4.25 (br m, 1H), 3.18 (s, 3H), 3.45−3.10 (m, 6H), 3.05−2.96 (m, 1H), 2.80−2.70 (m, 1H), 2.46−2.42 (m, 2H), 1.93−1.90 (m, 1H), 1.88−1.77 (m, 1H), 1.01 (s, 9H); IR (KBr) 1204, 1431, 1676, 2967 cm^{-1}. Anal. ($C_{28}H_{40}N_4O_9SF_6\cdot0.61H_2O$) C, H, N.

[3*S*-[2(*S),3*R**(*R**)]]-2-[[[2-[*N*-(2-Amino-3-mercaptopropyl)-3-methyl-L-valyl]-1,2,3,4-tetrahydro-3-isoquinolinyl]-carbonyl]amino]-4-(methylsulfonyl)butanoic Acid Trifluoroacetate (50):** mp 120−130 °C; TLC R_f 0.64 (6:3:1 *n*-propanol:ammonium hydroxide:water, visualization by ceric ammonium sulfate or UV); MS $(M + H)^+$ 543; $[\alpha]_D = -18.9°$ (*c* 0.62, CH_3OH); ^{1}H-NMR (DMSO-d_6, 125 °C, 400 MHz) δ 7.18 (s, 4H), 4.96−4.85 (m, 1H), 4.59−4.56 (m, 2H), 4.35−4.25 (m, 1H), 3.32 (br m, 1H), 3.20−3.12 (m, 3 H), 3.05−2.98 (m, 4H), 2.87 (s, 3H), 2.70 (br m, 1H), 2.47−2.43 (m, 1H), 2.18 (br m, 1H), 2.01 (br m, 1H), 1.01 (s, 9H); IR (KBr) 2971, 1678, 1433, 1204 cm^{-1}. Anal. ($C_{28}H_{40}N_4O_{10}S_2\cdot1.88H_2O$) C, H, N.

(*R)-*N*-[[2-[*N*-[[(1,1-Dimethylethoxy)carbonyl]amino]-3-methyl-L-valyl]-1,2,3,4-tetrahydro-3-isoquinolinyl]carbonyl]-L-methionine Methyl Ester (60).** To a solution of *N*-(*tert*-butyloxycarbonyl)-L-*tert*-leucine (0.4 g, 1.7 mmol) in CH_2Cl_2 (5 mL) at 0 °C under argon was added sequentially PyBroP (0.79 g, 1.7 mmol), *N*,*N*-diisopropylethylamine (0.72 mL, 5.1 mmol), and (dimethylamino)pyridine (22 mg, 0.2 mmol). After 5 min, **9** (0.6 g, 1.7 mmol) was added. The reaction mixture was allowed to warm to room temperature. After 3 h, the reaction mixture was washed successively with 1 N HCl, saturated aqueous $NaHCO_3$, and brine (5 mL each). Each aqueous layer was extracted with chloroform (5 mL). The organic layers were combined, dried, filtered, and concentrated. The residue was purified by flash silica gel column chromatography eluting with 30% ethyl acetate in hexanes to afford **60** (430 mg, 48%) as a viscous oil: MS $(M + H)^+$ 536; TLC R_f 0.41 (50% ethyl acetate in hexane, visualized by UV and ceric ammonium molybdate); ^{1}H-NMR ($CDCl_3$, 270 MHz) δ 7.25−7.1 (4H, m), 6.8 (1H, m), 5.44−5.4 (1H, m), 5.3−4.8 (2H, m), 4.7−4.6 (2H, m), 4.5−4.3 (1H, m), 3.7, 3.66 (3H, 2s, rotamers), 3.8−3.6 (1H, m), 3.3−3.2 (0.5H, m), 3.05−2.95 (0.5H, m), 2.3−2.2 (2H, m), 2.1−1.9 (2H, m), 2.0, 1.97 (3H, 2s, rotamers), 1.41 (9H, s), 1.07 (9H, s).

[*R*-(*R,*S**)]-*N*-[[2-[*N*-[2-[[(1,1-Dimethylethoxy)carbonyl]amino]-3-[(triphenylmethyl)thio]propyl]-3-methyl-L-valyl]-1,2,3,4-tetrahydro-3-isoquinolinyl]carbonyl]-L-methionine Methyl Ester (61).** Anhydrous HCl (4 N in dioxane, 1 mL, 4 mmol) and triethylsilane (0.24 mL, 1.5 mmol) were added to solid **60** (400 mg, 0.75 mmol) at room temperature under argon. After stirring for 2 h, the mixture was concentrated and the residue was triturated with diethyl ether and dried *in vacuo* to afford tLeu-Tic-Met-OMe (325 mg). To a solution of the tripeptide (310 mg, 0.66 mmol) in methanol (2 mL) was added a solution of **22** (0.3 g, 0.6 mmol) in methanol (1 mL) and acetic acid (0.1 mL). After 5 min, solid $NaCNBH_3$ (33 mg, 0.6 mmol) was added. After 1 h, **22** (0.3 g, 0.6 mmol) in methanol (1 mL) and $NaCNBH_3$ (67 mg, 1.2 mmol) were added. After 30 min, **22** (0.3 g, 0.6 mmol) in methanol (1 mL) was added. After another 1 h, the mixture was diluted with ethyl acetate (5 mL) and diethyl ether (5 mL) and washed with saturated aqueous $NaHCO_3$ (10 mL). The organic layer was washed with brine (10 mL), dried, filtered, and concentrated *in vacuo*. The residue was purified by flash column chromatography eluting with a step gradient of 30−50% ethyl acetate in hexane to afford the white foam **56** (540 mg, 95%): TLC R_f 0.27 (1:2, ethyl acetate in hexanes, visualized by UV and ceric ammonium molybdate); MS $(M + H)^+$ 867; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.4−7.1 (19H, m), 6.7 (1H, d, J = 7.0 Hz), 5.0−4.4 (6H, m), 3.7, 3.6 (3H, 2s), 3.7−3.5 (2H, m), 3.32 (1H, dd, J = 7, 14 Hz), 3.0 (1H, dd, J = 7, 14 Hz), 2.5−2.1 (2H, m), 2.0−1.7 (3H, m), 1.98, 1.85 (3H, 2s), 1.44, 1.35 (9H, 2s, 1:4 ratio, rotamers), 1.0, 0.93 (9H, 2s, 4:1 ratio, rotamers).

[*R*-(*R,*S**)]-*N*-[[2-[*N*-(2-Amino-3-mercaptopropyl)-3-methyl-L-valyl]-1,2,3,4-tetrahydro-3-isoquinolinyl]carbonyl]-L-methionine Methyl Ester Trifluoroacetate (51).** Triethylsilane (0.066 mL, 0.42 mmol) was added to a solution of **56** (0.18 g, 0.21 mmol) and TFA (0.4 mL) in dichloromethane (0.8 mL) at room temperature. After stirring for 3 h, the reaction mixture was concentrated to afford a solid which was purified by preparative HPLC (YMC S-10 ODS 30 × 500 mm

column, eluting with 30−90% aqueous methanol containing 0.1% TFA, gradient over 30 min) to afford, after lyophilization, **51** (0.036 g, 33%): $[\alpha]_D = -32°$ (*c* 0.25, MeOH): MS $(M + H)^+$ 525; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.19−7.13 (4H, m), 6.9 (1H, s), 4.8−4.4 (5H, m), 3.9−3.4 (7H, m), 3.1−2.3 (7H, m), 1.97−1.66 (7H, m), 1.07−1.06 (9H, s, rotamers); IR (KBr) 3435, 2924, 1437, 1682, 1636, 1208, 1134 cm^{-1}. Anal. ($C_{25}H_{40}N_4O_4S_2 \cdot 2.0CHF_3O_2 0.73H_2O$) C, H, N.

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