Expedited Articles

Pseudopeptide Inhibitors of Ras Farnesyl-Protein Transferase

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Inhibitors of Ras farnesyl-protein transferase are described. These are reduced pseudopeptides related to the C-terminal tetrapeptide of the Ras protein that signals farnesylation. Deletion of the carbonyl groups between the first two residues of the tetrapeptides either preserves or improves activity, depending on the peptide sequence. The most potent *in vitro* enzyme inhibitor described (IC_{50} = 5 nM) is Cys[ΨCH_2NH]Ile[ΨCH_2NH]Phe-Met (**3**). To obtain compounds able to suppress Ras farnesylation in cell culture, further structural modification to include a homoserine lactone prodrug was required. Compound **18** (Cys[ΨCH_2NH]Ile[ΨCH_2NH]Ile-homoserine lactone) reduced the extent of Ras farnesylation by 50% in NIH3T3 fibroblasts in culture at a concentration of 50 μM. Structure–activity studies also led to **12** (Cys[ΨCH_2NH]Val-Ile-Leu), a potent and selective inhibitor of a related enzyme, the type-I geranylgeranyl protein transferase.

The oncogene product Ras p21 plays a key role in controlling cell proliferation.[1] Mutations in Ras proteins can lead to unregulated cell division and are found in a significant number of human cancers,[2] including approximately 50% of colon and 90% of pancreatic carcinomas. Thus, pharmacological methods to modulate Ras activity may have a role in cancer therapy. However, no direct methods for interfering with Ras function are known that would appear to have the potential for clinical use.

It has long been appreciated that Ras must associate with the plasma membrane in order to function, both in the normal sense and in its role in cell transformation. However, it was not until quite recently that the nature of the membrane anchor for the Ras protein was fully elucidated. Several posttranslational modifications of Ras are required to promote membrane association. The key modification, S-farnesylation[3] of a cysteine residue near the carboxy terminus of the protein, is catalyzed by the enzyme farnesyl-protein transferase (FPTase),[4] using farnesyl diphosphate (FPP) as cosubstrate. Subsequent events, which are dependent on prior farnesylation, involve removal of the carboxy-terminal tripeptide adjacent to the farnesylated cysteine and carboxy methylation of the now C-terminal farnesyl cysteine. It has been shown that mutation of the critical cysteine residue to serine prevents farnesylation and that the resulting cytosolic form of Ras is nonfunctional.[5] Therefore, indirect regulation of Ras function by inhibiting FPTase is an attractive approach to developing a new class of anticancer drug.[6]

Several types of FPTase inhibitors with good activity *in vitro* have been described. However, only recently have compounds been obtained that can inhibit Ras farnesylation in intact cells.[7,8] One class of inhibitors with good *in vitro* potency mimics the carboxy-terminal tetrapeptide of the Ras protein (the so-called CAAX motif) that is the signal sequence for farnesylation.[4,9] For example, simple CAAX tetrapeptides such as CIIM[10] and CIFM inhibit Ras FPTase with potency in the range of 10–100 nM. In many cases, this inhibition of Ras farnesylation is the result of the CAAX peptide serving as an alternative substrate for farnesylation. While simple CAAX analogs do not inhibit Ras processing *in vivo*, we have reported a structurally modified tetrapeptide, **18**, that inhibited Ras farnesylation in cells growing in culture.[8] Furthermore, **18** restored anchorage dependence to the growth of *ras*-transformed cells in soft agar, reversing one of the key features of the phenotype of these tumorigenic cells. In this paper we describe the synthesis of **18** and the structure–activity relationships of related pseudopeptide inhibitors.

Chemistry

Incubation of tetrapeptides such as CIFM with a cytosolic fraction derived from NIH3T3 mouse fibroblasts leads to their rapid destruction, apparently by an aminopeptidase activity.[11] On the basis of the premise that this rapid proteolytic degradation accounted for the lack of activity of ordinary tetrapeptides *in vivo*, the effect of incorporating reduced pseudopeptide isosteres[12] for the peptide bonds in these tetrapeptide sequences was explored. The methods used for the preparation of these FPTase inhibitors are exemplified in Scheme 1, which describes the synthesis of compound **18**. *N*-Boc-isoleucinal **19**[13] and isoleucine were reductively coupled using sodium triacetoxyborohydride in dimethylformamide providing the reduced dipeptide fragment **20**. The crude product was coupled with homoserine lactone under conventional solution-phase conditions to provide the pseudotripeptide **21**. The Boc group was cleaved from **21**, and the primary amino group was reductively coupled to *N*-Boc-*S*-trityl-cysteinal (**22**, prepared by reduction of the corresponding *N*-methoxy-*N*-methylamide) using sodium cyanoborohydride to provide **23**. Finally, cleavage of the Boc and trityl protecting groups was achieved with trifluoroacetic acid

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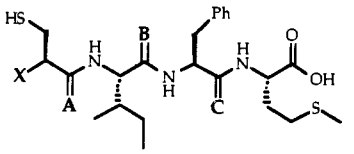
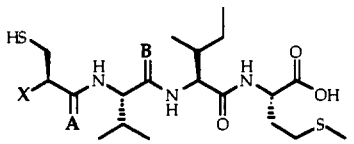
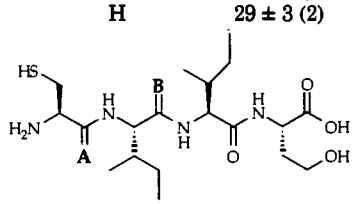
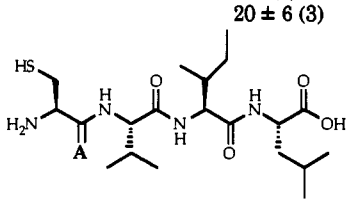
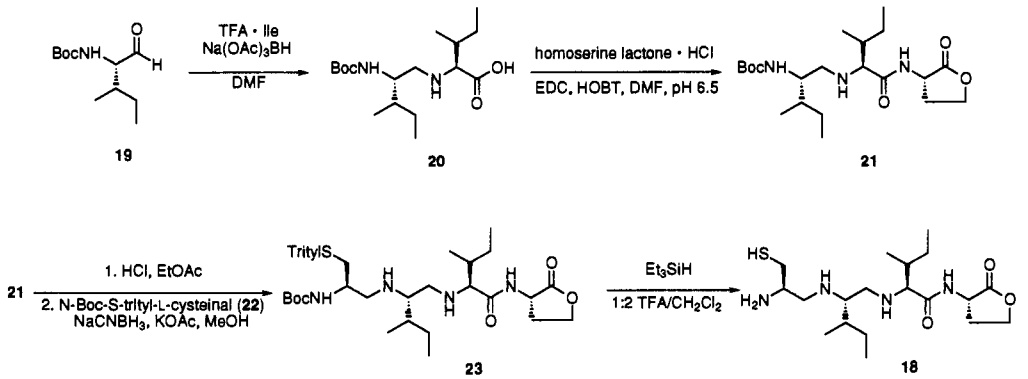
Table 1. Pseudopeptide Inhibitors of Farnesyl-Protein Transferase[a]

compd	A	B	C	X	IC_{50} (nM) FPTase[b]	IC_{50} (nM) GGPTase-I[c]	substrate[d]
							
CIFM	O	O	O	NH_2	27 ± 19 (3)	300000 (1)	n
1	H, H	O	O	NH_2	18 ± 4 (2)	310 ± 30 (2)	n
2	H, H	O	O	H	50 (1)	>10000 (1)	s
3	H, H	H, H	O	NH_2	4.8 ± 0.4 (2)	5750 ± 50 (2)	n
4	H, H	H, H	O	H	400 ± 30 (2)	>10000 (2)	n
5	H, H	O	H, H	NH_2	140 (1)	1100 (1)	nd
							
CVIM	O	O		NH_2	165 ± 23 (6)	49000 ± 9000 (5)	s
6	H, H	O		NH_2	7 ± 1 (3)	28 ± 1 (2)	s
7	H, H	O		H	29 ± 3 (2)	2200 (1)	s
							
CII-hS (8)	O	O			330 (1)	nd	nd
9	H, H	O			42 (1)	8000 (1)	s
10	O	H, H			150 (1)	nd	s
11	H, H	H, H			20 ± 6 (3)	45000 (1)	n
							
CVIL	O				16700 ± 3900 (3)	11300 ± 1200 (3)	nd
12	H, H				550 ± 150 (2)	1.9 ± 0.1 (2)	p

[a] nd, not determined. [b] Concentration of compound required to reduce the FPTase-catalyzed incorporation of [^{3}H]FPP into recombinant Ha-Ras protein by 50%. The assay protocol is described in ref 9 and used enzyme purified from bovine brain at a concentration of approximately 1 nM. Assay results are reported as concentration ± SEM for the number of determinations shown in parentheses. [c] Inhibition of bovine type-I geranylgeranyl transferase.[9] [d] The ability of compounds to serve as substrates for FPTase was determined using a TLC assay[9] for the formation of tritiated products from the reaction of the compound with [^{3}H]FPP in the presence of FPTase. Results are reported as s, substrate; p, poor substrate; n, nonsubstrate.

Scheme 1. Representative Synthesis of a Farnesyl-Protein Transferase Inhibitor



and triethylsilane. Lactone 18 was purified by reverse-phase HPLC and isolated as its trifluoroacetate salt by lyophilization. The sodium salt of hydroxy acid 11 was generated in methanol solution by alkaline hydrolysis of 18.[14] Ring cleavage was confirmed by HPLC and ^{1}H NMR. The syntheses of other compounds in this series were analogous to that of 18 and are described in the Experimental Section.

Structure–Activity Relationships: *In Vitro* Inhibition of FPTase. Compounds were initially characterized as inhibitors of Ras FPTase *in vitro* using an enzyme purified from bovine brain.[9,15] Compounds were incubated

with the enzyme, [^{3}H]FPP and recombinant Harvey-Ras protein in the presence of varying concentrations of inhibitor. Total protein was precipitated with acid, and protein-bound FPP was quantitated by scintillation counting. The activity of the compounds is reported as an IC_{50} value, the concentration at which radiolabel incorporation into Ras is reduced by 50% compared to an experiment in which no inhibitor is present. Several of these compounds were characterized further for their ability to serve as substrates for farnesylation. In this assay,[9] the compounds were incubated with [^{3}H]FPP and FPTase, and the reaction mixtures were assayed by thin-layer chromatography for the formation of tritiated products. Band intensities were assessed using CVIM (a good substrate) and CIFM (a nonsubstrate) as reference points to characterize compounds as substrates (s), non-substrates (n), or poorly farnesylated substrates (p). Finally, some of the compounds were evaluated with regard to their potency as inhibitors of a closely related enzyme, the type-I geranylgeranyl protein transferase (GGPTase-I).[9,16] Details of each of these assays are found in the cited literature. The results obtained for three related groups of reduced tetrapeptides are summarized below.

Among the many CAAX tetrapeptides that have been surveyed, Goldstein and co-workers found that CIFM was the most potent inhibitor of Ras farnesylation *in vitro*.[17a] Furthermore, CIFM has two other attributes that are potentially useful. First, CIFM, unlike many CAAX tetrapeptides, is not a substrate for farnesylation, thus precluding a pathway for metabolic inactivation of an inhibitor. Second, CIFM is quite selective with respect to inhibition of other prenyl-protein transferases, notably GGPTase-I, which recognizes very similar CAAX signal sequences.

Incorporation of a reduced peptide linkage between the first two residues of CIFM (affording **1**) had little effect on FPTase inhibitory activity. However, selectivity with respect to GGPTase-I was significantly eroded. In common with the parent peptide, compound **1** was not a substrate for farnesylation. Interestingly, deletion of the primary amino group (compound **2**) of the cysteine-derived residue led to a small decrease in affinity for FPTase but dramatically reduced affinity for GGPTase-I. Furthermore the desamino compound now served as a substrate for farnesylation. Such a reversion to substrate character was previously observed by the Brown and Goldstein group upon deletion of the amino group from the normal peptide CIFM.[17b]

Reduction of both the first and second amide carbonyls in CIFM afforded **3**, which was somewhat more potent than the parent peptide. Very good selectivity vs GGPTase-I was also observed. Again, the compound was not a substrate for farnesylation. The effect of deleting the amino-terminal nitrogen differs significantly in the doubly-reduced system, **3**, compared to compound **2**. Here, the desamino compound **4** was 80-fold less active as a farnesyl transferase inhibitor. In addition, deletion of the amine did not convert **4** into a substrate for farnesylation.

Although the primary mode of degradation of CAAX peptides in cytosol appeared to result from aminopeptidase activity, we also prepared compound **5** to stabilize the compound to degradation by carboxypeptidases. Unfortunately, incorporating the reduced peptide linkage between both the first and the last pairs of residues in the CIFM sequence resulted in a significant loss of FPTase inhibitory potency. This modification also eroded selectivity with respect to GGPTase-I.

Somewhat different results were obtained upon reduction of the amide bonds in the tetrapeptide CVIM. The parent peptide is a good inhibitor of Ras farnesylation and serves as an alternate substrate. This peptide is also >100-fold selective with respect to inhibition of GGPTase-I. Reduction of the amide linkage between cysteine and valine, affording **6**, significantly improved activity vs farnesyl transferase (~20-fold). On the other hand, *activity against GGPTase-I was improved approximately 2000-fold.* Thus, selectivity vs GGPTase-I was reduced to only 4-fold. Reduction had no effect on the ability of **6** to serve as a substrate for farnesylation. The deletion of the cysteine-derived primary amino group of **6** was also explored. As seen in the CIFM series, the desamino compound **7** was somewhat less active as an FPTase inhibitor, but selectivity vs GGPTase was restored to nearly 100-fold. This compound was a substrate for farnesylation.

The third family of pseudopeptide FPTase inhibitors are analogs of the naturally occurring CAAX motif CIIM, in which methionine is replaced by homoserine (hS) as the C-terminal residue. This substitution was anticipated to be well-tolerated in an FPTase inhibitor since serine-containing tetrapeptides (e.g., CVLS) are substrates for FPTase. In fact, the sodium salt of parent peptide CII-(hS) (**8**, prepared by in situ saponification of the lactone) was a fairly good FPTase inhibitor (IC_{50} = 330 nM), differing only 2-fold in potency from CVIM. Reduction of the amide linkage between the first two residues provided **9**, which was 8 times more potent than **8**. Compound **9** was 20-fold more selective with respect to inhibition of GGPTase-I. The presence of the reduced linkage between the second and third residues in **10** gave a 2-fold increase in activity compared to **8**. The gains in binding energy with the reduction of each of these amide bonds were independent and additive: compound **11**, in which both amide groups are reduced, was a 20 nM inhibitor. Furthermore, compound **11** is 3 orders of magnitude less active as an inhibitor of GGPTase-I. Standard kinetic analysis showed that **11** was competitive with respect to Ras (K_i = 20 ± 6 nM) and was noncompetitive with respect to FPP.

Amide bond reduction also affects the ability of analogs of **8** to serve as substrates for farnesylation. While the sodium salts of hydroxy acids **9** and **10** were substrates for FPTase, **11** was not farnesylated. This is a result similar to that obtained with the desamino analogs of CIFM: while the singly reduced compound **2** was farnesylated, the doubly reduced compound **4** was not. Thus, it appears that the nonsubstrate property can be conferred on CAAX analogs by simultaneous reduction of these two amide linkages, independent of peptide sequence. This may reflect the existence of interactions between the enzyme and these substrate backbone elements that are important in stabilizing the catalytic conformation of the protein.

A Potent and Selective Inhibitor of GGPTase-I. The high affinity of **6** for GGPTase-I was surprising, but this discovery offered an opportunity to obtain a selective inhibitor of GGPTase. Selectivity between FPTase and GGPTase-I has primarily been ascribed to the nature of the fourth residue of the CAAX motif, as reflected in the prenyl transferase selectivity of CVIM (FPTase/GGPTase-I = 300) and CVIL (FPTase/GGPTase-I = 0.7).[9] Although somewhat selective, CVIL is a weak inhibitor of GGPTase-I (IC_{50} = 11 300 nM). As we had observed with the peptide

Table 2. Properties of Selected FPTase Inhibitors and Their Prodrugs in Cell Culture

compd	FPTase inhibitor	prodrug	cytotoxic endpoint[a]	inhibition of Ras processing[b] dose (μM)	score[c]
1	1	none	>100	100	±
13	1	ethyl ester	10	10	±
14	1	benzyl ester	5	5	–
15	1	decyl ester	2	nd	nd
3	3	none	>100	100	+
16	3	methyl ester	1	1	±
9	9	none	>100	100	–
17	9	lactone	>100	100	+
11	11	none	>100	100	–
18	11	lactone	>1000	100	+++
lovastatin				15	+++

[a] Highest nontoxic concentration (μM) for cultured NIH3T3 cells as assessed by MTT staining. With the exception of 18, the highest concentration tested in this assay was 100 μM.[18] [b] Inhibition of posttranslational processing of v-Ras protein in cultured NIH3T3 cells.[7a,19] [c] Scoring system: +++, ≥90% inhibition; +, 30–60% inhibition; ±, 10–30% inhibition; –, no inhibition observed.

CVIM, reduction of the amide bond between cysteine and valine in CVIL led to a tremendous improvement in affinity for GGPTase. Excellent selectivity vs FPTase was also observed. Compound 12 is the first potent and selective GGPTase inhibitor to be described.

Inhibition of Ras Farnesylation in Cell Culture. The potential utility of Ras FPTase inhibitors in cancer therapy depends on their ability to penetrate the cell membrane and inhibit posttranslational processing of Ras *in vivo*, without being toxic to normal cells. The cytotoxicity of our compounds was assessed using a viable staining method with MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide).[18] The cytotoxic endpoint, shown in Table 2, is the highest compound concentration tolerated by NIH3T3 cells in a 48-h assay. To assess inhibition of Ras processing in cell culture we employed the method of DeClue *et al.*[19] NIH 3T3 cells expressing a viral Ha-*ras* gene were metabolically labeled with [^{35}S]methionine in the presence of a compound or solvent as control. Cells were lysed after 24 h, and the Ras protein was isolated by immunoprecipitation. The protein was subjected to denaturing gel electrophoresis and visualized by fluorography. Distinct bands for mature and unprocessed ras were observed, and the extent of inhibition was assessed by visual inspection of the band intensity. As a positive control, the HMG-CoA reductase inhibitor lovastatin was employed. Lovastatin at 15 μM gave ≥90% inhibition of Ras processing. The results of these assays are presented in Table 2.

When tested at 100 μM in cell culture, compounds 1 and 3 were not cytotoxic but only weakly inhibited Ras processing. On the basis of the hypothesis that the free carboxylate group in these molecules was a limiting factor for the membrane permeance of 1 and 3, esters of these compounds also were studied in cell culture. Compound 13, the ethyl ester of 1, was considerably more active in cells showing the same level of activity at 10 μM as was observed for the free acid at a 10-fold higher concentration. Unfortunately, 13 could not be tested at higher concentratiosn due to cytotoxicity. The benzyl (14) and decyl (15) esters of 1 were quite cytotoxic. Compound 14 did not inhibit Ras processing to any measurable extent at its cytotoxic endpoint. The methyl ester 16 of the potent inhibitor 3 was quite cytotoxic. However, Ras processing was inhibited to a measurable extent at the cytotoxic endpoint of 1 μM.

When tested at 100 μM for inhibition of Ras processing in cultured cells, the sodium salts of the homoserine-derived hydroxy acids 9 and 11 were inactive. However, somewhat more promising results were obtained in cell culture with the lactone forms 17 and 18 of these inhibitors. Lactone 17 was not cytotoxic at the highest concentration tested (100 μM) and inhibited Ras farnesylation approximately 30–60% at a concentration of 100 μM. Ras processing in intact cells was inhibited more efficiently by lactone 18 with an IC_{50} of 50 μM. Lactone 18 was remarkably nontoxic: cultured cells tolerated dosing with 1 mM concentrations. Given the relatively poor intrinsic FPTase inhibitory activity of 18 (IC_{50} = 280 nM),[8,20] it seems likely that the lactone serves as a membrane permeant prodrug for 11, which is formed by the action of intracellular esterases.

Conclusions

We have described the preparation of several pseudopeptide inhibitors of the Ras farnesyl transferase. While very good *in vitro* potency was attained, securing compounds able to inhibit farnesylation in intact cells proved more problematic. Deletion of two amide bonds in these CAAX analogs and introduction of a lactone prodrug for the C-terminal carboxylate has afforded compounds of adequate activity to explore some of the biological ramifications of inhibiting protein farnesylation in cultured cells. As reported elsewhere, compound 18, although of modest potency, blocks the growth of *ras*-transformed cells cultured in soft agar. Thus, 18 can reverse one of the key elements of the transformed phenotype, anchorage-independent growth.

Experimental Section

Solvents and reagents were obtained from commercial suppliers and were used as received. Simple peptides used as synthetic intermediates were prepared using standard solution-phase methods and are not described in detail. Reactions were generally conducted under an argon atmosphere using magnetic stirring. Standard workup referred to in the experimental procedures refers to dilution with an organic solvent, washing as appropriate with 10% citric acid, 10% sodium bicarbonate, and brine. The organic solutions were dried over sodium sulfate, and the solvent was removed on a rotary evaporator. Chromatography was performed on silica gel (230–400 mesh) at approximately 5 psig. Products and intermediates were characterized by 300-MHz ^{1}H NMR. Final products were also characterized by combustion analyses performed by Mr. J. Moreau of the Medicinal Chemistry Department. Observed values were within 0.4% of calculated values for the compound formulas shown.

***N*-Boc-*S*-tritylcysteinal (22).** The preparation of 22 was carried out by a modification of the method described in *Org. Synth.* **1988**, *67*, 69. *O,N*-Dimethylhydroxylamine hydrochloride (5.27 g, 54 mmol) was suspended in 50 mL of methylene chloride and cooled to −10 °C. *N*-Methylpiperidine (6.6 mL, 54 mmol) was added in a slow stream, maintaining $T \leq -2$ °C. This solution was stored at −10 °C. In a separate flask, *N*-Boc-*S*-tritylcysteine (25.0 g, 53.9 mmol) was dissolved in 250 mL of methylene chloride and cooled to −20 °C. *N*-Methylpiperidine (6.6 mL, 54 mmol) was added followed by isobutyl chloroformate (7.0 mL, 54 mmol), maintaining the temperature at −20 °C during addition. After 5 min the hydroxylamine solution prepared above was added in a single portion. The cooling bath was removed, and the solution was stirred overnight. Standard workup and chromatography (10–30% ethyl acetate in hexanes) gave the *N*-methoxy-*N*-methylamide as a white foam weighing 25.7 g (94%): ^{1}H NMR δ 7.45–7.18 (15H, m), 5.14 (1H, d), 4.75 (1H, br s), 3.67 (3H, s), 3.15 (3H, s), 2.57 (1H, dd), 2.38 (1H, dd), 1.45 (9H, s).

The amide prepared above was dissolved in 50 mL of anhydrous ether and added to a cold (−45 °C), mechanically stirred suspension of 2.32 g (61 mmol) of lithium aluminum hydride in 200 mL of ether, maintaining the temperature between −45 °C and −35 °C. After the addition was complete, the cooling bath was removed and the mixture was allowed to warm to 5 °C. The solution was cooled to −35 °C, and a solution of 12.7 g of potassium bisulfate in 35 mL of water was added slowly (gas evolution). The cooling bath was removed. After 1 h, Celite was added to aggregate the aluminum salts, and the mixture was filtered. The aluminum salts were washed with ether (2 × 100 mL). The combined ether solution was worked up in the standard way, yielding 23.7 g of **22** as a white foam, which was used without purification. ^{1}H NMR of this material was complex, probably due to the presence of aldehyde hydrate.

***N*-[2(*R*)-[(*tert*-Butoxycarbonyl)amino]-3-[(triphenylmethyl)thio]propyl]isoleucylphenylalanylmethionine Ethyl Ester (24).** A solution of isoleucylphenylalanylmethionine ethyl ester hydrochloride (285 mg, 0.60 mmol) in ethyl acetate was washed with 5% ammonium hydroxide to obtain the free base. This solution was washed with brine, dried over 3A molecular sieves, and filtered. To this solution was added 270 mg (0.67 mmol) of **22**, 0.58 g of 3A molecular sieves, and 300 μL of 1 M sodium cyanoborohydride in tetrahydrofuran (THF). After being stirred overnight the solution was filtered and washed with 5% ammonium hydroxide. Standard workup and chromatography (1% methanol in methylene chloride) gave 370 mg (74%) of **24**: ^{1}H NMR δ 7.5–7.1 (20H, m), 4.8 (1H, m), 4.6 (2H, m), 4.17 (2H, q), 2.05 (3H, s), 1.45 (9H, s), 1.27 (3H, t), 0.65 (6H, m).

***N*-(2(*R*)-Amino-3-mercaptopropyl)isoleucylphenylalanylmethionine Ethyl Ester (13).** A solution of 146 mg of **24** was prepared in 5 mL of 1:1 TFA/methylene chloride, and 72 μL of triethylsilane was added. After 45 min the solvent was evaporated, and the residue was triturated with 1:1 ether/hexane. The gummy solid was purified by preparative HPLC (Vydac C-18, 25–45% acetonitrile/0.1% TFA/water, 35-min gradient). The product (**13**), weighing 47 mg, was obtained by lyophilization: ^{1}H NMR (CD_3OD) δ 7.33 (4H, m), 7.25 (1H, m), 4.60 (1H, m), 4.19 (2H, q), 3.25 (2H, m), 2.10 (3H, s), 1.15 (3H, t), 0.85 (3H, t), 0.75 (3H, d); FAB MS *m/z* 527 (M + 1). Anal. ($C_{25}H_{42}N_4O_4S_2$·2.2TFA) C, H, N.

Esters **14** and **15** were synthesized as described for **13**, proceeding from the appropriate ester of methionine.

***N*-(2(*R*)-Amino-3-mercaptopropyl)isoleucylphenylalanylmethionine Benzyl Ester (14).** Anal. ($C_{30}H_{44}N_4O_4S_2$·1.6TFA) C, H, N.

***N*-(2(*R*)-Amino-3-mercaptopropyl)isoleucylphenylalanylmethionine Decyl Ester (15).** Anal. ($C_{33}H_{58}N_4O_4S_2$·1.7TFA), C, H, N.

***N*-(2(*R*)-Amino-3-mercaptopropyl)isoleucylphenylalanylmethionine (1).** A solution of 386 mg (0.45 mmol) of **24** in 10 mL of methanol was prepared, and 1.8 mL of 1 N LiOH was added. After 3 h, the solution was acidified to pH 4 with dilute hydrochloric acid. Methanol was removed *in vacuo*, and the semisolid residue was lyophilized. The resulting solid was triturated and ether, filtered, and dried. This solid was dissolved in 3 mL of 50% trifluoroacetic acid (TFA) methylene chloride, and 0.15 mL (0.9 mmol) of triethylsilane was added. After 1 h the solvent was evaporated, and the resulting material was triturated with ether. The product was further purified by HPLC (Waters PrepPak C-18, 5–40% acetonitrile/0.1% TFA/water, 35-min gradient). The lyophilized product (**1**) weighed 81 mg (28%): ^{1}H NMR (CD_3OD) δ 7.30 (4H, m), 7.21 (1H, m), 4.58 (1H, dd), 3.25 (1H, dd), 3.20 (1H, dd), 2.88 (2H, dd), 2.76 (1H, dd), 2.68–2.48 (5H, m), 2.18 (1H, m), 2.08 (3H, s), 1.98 (1H, m), 1.58–1.40 (2H, m), 1.06 (1H, m), 0.83 (3H, t); 0.70 (3H, d); FAB MS *m/z* 499 (M + 1). Anal. ($C_{23}H_{38}N_4O_4S_2$·2TFA·1.6H_2O) C, H, N.

***N*-[(2*S*)-[(*tert*-Butoxycarbonyl)amino]-3-methylpentyl]phenylalanylmethionine Methyl Ester (25).** *N*-(*tert*-Butoxycarbonyl)isoleucinal (**19**, 0.62 g, 2.9 mmol) and phenylalanylmethionine methyl ester hydrochloride (1.0 g, 2.9 mmol) were dissolved in dimethylformamide (DMF) (20 mL) at 0 °C with 3A molecular sieves (1 g). After 30 min sodium triacetoxyborohydride (0.738 g, 2.5 mmol) was added. The cooling bath was removed, and the mixture was stirred at 25 °C for 3 h. The reaction mixture was filtered. Standard workup and chromatography (CH_2Cl_2/MeOH, 98:2) gave 1.01 g (68%) of **25**: ^{1}H NMR (CD_3OD) δ 7.33–7.23 (5H, m), 4.66–4.60 (1H, m), 3.73 (3H, s), 3.55–3.46 (1H, m), 3.10–3.01 (1H, m), 2.87–2.69 (2H, m), 2.55–2.36 (3H, m), 2.09 (3H, s), 2.2–1.98 (1H, m), 1.46 (9H, s), 1.55–1.35 (1H, m), 1.19–1.04 (1H, m), 0.89 (3H, t, *J* = 7 Hz), 0.82 (3H, d, *J* = 7 Hz).

***N*-(2(*S*)-Amino-3-methylpentyl)phenylalanylmethionine Methyl Ester Hydrochloride (26).** Compound **25** (0.20 g, 0.39 mmol) was dissolved in EtOAc (10 mL) and cooled to −20 °C. HCl gas was bubbled into the solution for 30 min until TLC (EtOAc/hexane, 1:3) showed complete consumption of the starting material. Argon was bubbled into the solution for 15 min, and the solvent was removed in vacuo to give 0.19 g (100%) of **26**: ^{1}H NMR (CD_3OD) δ 7.42–7.3 (5H, m), 4.53–4.46 (1H, m), 4.22–4.13 (1H, m), 3.72 (3H, s), 3.57–3.48 (1H, m), 3.4–3.15 (3H, m), 2.51–2.4 (1H, m), 2.38–2.26 (1H, m), 2.06 (3H, s), 2.1–1.92 (2H, m), 1.88–1.75 (1H, m), 1.56–1.43 (1H, m), 1.35–1.19 (1H, m), 1.08–0.95 (6H, m).

***N*-[2(*S*)-[[(2*R*)-[(*tert*-Butoxycarbonyl)amino]-3-[(triphenylmethyl)thio]propyl]amino]-3(*S*)-methylpentyl]phenylalanylmethionine Methyl Ester (27).** To a solution of 0.19 g (0.39 mmol) of **26** in MeOH (4 mL) at ambient temperature were added 3A molecular sieves (0.3 g), KOAc (80 mg, 0.8 mmol), and **22** (0.183 g, 0.4 mmol). Sodium cyanoborohydride (0.38 g, 0.6 mmol) was added in one portion, and the mixture was stirred for 18 h. The reaction mixture was filtered through glass-fiber paper and given a standard workup. The residue was chromatographed (CH_2Cl_2/MeOH, 99:1 to 98:2) to give 0.25 g (77%) of **27**: ^{1}H NMR (CD_3OD) δ 7.52–7.13 (20H, m), 4.64–4.55 (1H, m), 3.69 (3H, s), 3.65–3.5 (1H, m), 3.35–3.24 (2H, m), 3.00 (1H, dd, *J* = 7, 13 Hz), 2.78 (1H, dd, *J* = 7, 13 Hz), 2.63 (1H, dd, *J* = 5, 12 Hz), 2.56–2.32 (6H, m), 2.31–2.2 (1H, m), 2.2–2.07 (1H, m), 2.07 (3H, s), 2.05–1.88 (1H, m), 1.54–1.3 (2H, m), 1.48 (9H, s), 1.19–1.02 (1H, m), 0.89 (3H, t, *J* = 7 Hz), 0.71 (3H, d, *J* = 7 Hz).

***N*-[2(*S*)-[(2(*R*)-Amino-3-mercaptopropyl)amino]-3(*S*)-methylpentyl]phenylalanylmethionine (3).** A solution of **27** (109 mg, 0.13 mmol) in a minimal volume of methanol was treatd with 0.52 mL of 1 N LiOH. Sufficient methanol was added to obtain a homogeneous solution. After 5 h, 0.52 mL of 1 N HCl was added and the solvent was evaporated *in vacuo*. The residue was taken up in ethyl acetate and worked up in the standard way. The residue was redissolved in 5 mL of 1:1 TFA/methylene chloride, and triethylsilane (41 μL, 0.26 mmol) was added. The mixture was stirred for 40 min, and the solvents were evaporated *in vacuo*. The residue was triturated with ether and purified by HPLC (Waters PrepPak C-18, 5–60% acetonitrile/0.1% TFA/water, 35-min gradient). Lyophilization gave 24 mg (24%) of **3** as its trifluoroacetate salt: ^{1}H NMR (CD_3OD) δ 7.30 (5H, m), 4.41 (1H, dd), 4.12 (1H, t), 3.22 (1H, m), 3.02 (2H, m), 2.70–2.90 (5H, m), 2.42 (1H, m), 2.28 (1H, m), 2.12 (1H, m), 2.05 (3H, s), 1.98 (1H, m), 1.78 (1H, m), 1.34 (1H, m), 1.22 (1H, m), 0.95 (3H, t), 0.84 (3H, d). Anal. ($C_{23}H_{40}N_4O_3S_2$·2.5TFA) C, H, N.

***N*-[2(*S*)-[(2(*R*)-Amino-3-mercaptopropyl)amino]-3(*S*)-methylpentyl]phenylalanylmethionine Methyl Ester (16).** A solution of 0.135 g of **27** (0.15 mmol) in CH_2Cl_2 (1.5 mL) was treated with TFA (0.75 mL) and triethylsilane (0.096 mL, 0.6 mmol) at ambient temperature for 1 h. The reaction mixture was concentrated to dryness and stirred with 0.1% TFA in H_2O. The insoluble triphenylmethane was removed by filtration, and the filtrate was purified by reverse-phase HPLC (Waters PrepPak C-18, 5–95% acetonitrile/0.1% TFA/water). After lyophilization, the hygroscopic trifluoroacetate salt of **27** was dissolved in methanol and treated with a slight excess of concentrated hydrochloric acid. Evaporation and ether trituration provided 0.038 g (41%) of the hydrochloride salt of **27**: mp 119–124 °C; ^{1}H NMR (CD_3OD) δ 7.42–7.29 (5H, m), 4.56–4.5 (1H, m), 4.16 (1H, t, *J* = 7 Hz), 3.70 (3H, s), 3.33–3.2 (3H, m), 3.11–2.98 (2H, m), 2.92–2.72 (5H, m), 2.51–2.4 (1H, m), 2.4–2.28 (1H, m), 2.2–1.9 (2H, m), 2.06 (3H, s), 1.89–1.75 (1H, m), 1.42–1.2 (2H, m), 0.98 (3H, t, *J* = 7 Hz), 0.87 (3H, d, *J* = 7 Hz). Anal. $C_{24}H_{42}N_4O_3S_2$·3HCl·0.75H_2O) C, H, N.

Compounds **2** and **4** were prepared using the routes described for **1** and **3**, respectively, substituting 3-[(triphenylmethyl)thio]propanal for the cysteine-derived aldehyde.

***N*-(3-Mercaptopropyl)isoleucylphenylalanylmethionine (2).** Anal. ($C_{23}H_{37}N_3O_4S_2$·1.4TFA) C, H, N.

N-[2(S)-[(3-Mercaptopropyl)amino]-3(S)-methylpentyl]-phenylalanylmethionine (4). Anal. ($C_{23}H_{39}N_3O_3S_2$·1.9TFA) C, H, N.

The procedures described for the synthesis of 1 were also used to prepare compounds 6 and 7 from the tripeptide valylisoleucylmethionine methyl ester.

N-(2(R)-Amino-3-mercaptopropyl)valylisoleucylmethionine (6). Anal. ($C_{19}H_{38}N_4O_4S_2$·2TFA) C, H, N.

N-(3-Mercaptopropyl)valylisoleucylmethionine (7). Anal. ($C_{19}H_{37}N_3O_4S_2$·TFA) C, H, N.

N-(3-Mercaptopropyl)valylisoleucylleucine (12). Compound 12 was prepared analogously from valylisoleucylleucine methyl ester. Anal. ($C_{20}H_{40}N_4O_4S$·2TFA) C, H, N.

N-[2(R)-[(tert-Butoxycarbonyl)amino]-3-[(triphenylmethyl)thio]propyl]isoleucine (28). Isoleucine (1.97 g, 0.015 mol) was suspended in EtOH (150 mL) with 22 (6.71 g, 0.015 mol) and 3A molecular sieves. Sodium cyanoborohydride (0.47 g, 0.0075 mol) was added, and the mixture was stirred at ambient temperature for 72 h. Filtration and concentration gave an oil, which was chromatographed (silica gel, CH_2Cl_2/MeOH, 95:5 to 9:1) to give 2.1 g of 28: mp 83–90 °C; ^{1}H NMR ($CDCl_3$) δ 7.41–7.19 (15H, m), 5.12–4.98 (1H, m), 3.70–3.58 (2H, m), 3.18 (1H, br s), 2.81–2.78 (2H, m), 2.60–2.32 (2H, m), 1.96–1.80 (1H, m), 1.40 (9H, s), 1.35–1.20 (1H, m), 0.93–0.84 (6H, m).

N-[2-(S)-[(tert-Butoxycarbonyl)amino]-3-phenylpropyl]-methionine Methyl Ester (29). N-(tert-Butoxycarbonyl)-phenylalaninal (2.5 g, 0.01 mol) and methionine methyl ester hydrochloride (2.0 g, 0.01 mol) were dissolved in MeOH (99 mL) with AcOH (1 mL) at ambient temperature under argon and treated with sodium cyanoborohydride (1.0 g, 0.015 mol) with stirring. After 2 h the mixture was worked up in the standard manner and chromatographed (hexane/EtOAc, 3:1) to give 2.9 g (73%) of 29 as a white solid: ^{1}H NMR ($CDCl_3$) δ 7.35–7.15 (5H, m), 4.8 (1H, br s), 4.0–3.8 (1H, m), 3.72 (3H, s), 3.6–3.45 (1H, m), 2.9–2.75 (3H, m), 2.7–2.5 (2H, m), 2.10 (3H, s), 2.05–1.85 (2H, m), 1.7–1.5 (1H, m), 1.40 (9H, s); FAB MS m/z 397 (M + 1).

N-(2(S)-Amino-3-phenylpropyl)methionine Methyl Ester (30). HCl gas was bubbled into a solution of 29 (0.20 g, 0.5 mmol) in EtOAc (10 mL) with stirring at −20 °C over 0.5 h. The solution was purged with argon for 0.5 h and concentrated to give 0.18 g (100%) of 30 as a white solid: ^{1}H NMR (d_6-DMSO) δ 8.62 (1H, br s), 7.45–7.3 (5H, m), 4.3–4.1 (1H, m), 3.69 (3H, s), 3.2–3.0 (2H, m), 2.9–2.8 (1H, m), 2.7–2.4 (2H, m), 2.15–2.0 (1H, m), 2.05 (3H, s).

N-[2-(R)-[(tert-Butoxycarbonyl)amino]-3-[(triphenylmethyl)thio]propyl]isoleucyl-N-[2(S)-amino-3-phenylpropyl]methionine Methyl Ester (31). Compound 28 (0.28 g, 0.5 mmol), dissolved in DMF (5 mL), was treated with 1-hydroxybenzotriazole (HOBT, 0.137 g, 1.0 mmol), 1-[3-(dimethylamino)-propyl]-3-ethylcarbodiimide hydrochloride (EDC, 0.1 g, 0.5 mmol), and 30 (0.182 g, 0.5 mmol). The pH was adjusted to 6.5–7.0 with N-methylmorpholine (0.4 mL), and the mixture was stirred at ambient temperature for 20 h. The mixture was concentrated, worked up, and chromatographed (EtOAc/hexane, 1:3) to give 0.33 g of 31. FAB MS m/z 841 (M + 1).

N-(2(R)-Amino-3-mercaptopropyl)isoleucyl-N-(2(S)-amino-3-phenylpropyl)methionine (5). The ester 31 (0.33 g, 0.4 mmol) was dissolved in MeOH (10 mL). A solution of LiOH (0.04 g, 1.6 mmol) in H_2O (5 mL) was added, and the mixture was stirred at ambient temperature for 31 h. The mixture was diluted with H_2O, filtered, and neutralized with 10% citric acid solution (5 mL) to precipitate a white solid product (0.28 g, 85%): FAB MS m/z 827 (M + 1).

The crude product (0.28 g, 0.34 mmol) was dissolved in CH_2Cl_2 (10 mL) with TFA (4 mL), and triethylsilane (0.2 mL) was added. The mixture was stirred at ambient temperature under argon for 2 h and concentrated, and the residue was triturated with Et_2O. The residue was purified by reverse-phase HPLC to give 45 mg of 5: ^{1}H NMR (D_2O) δ 7.44–7.30 (5H, m), 4.60–4.52 (1H, m), 3.86 (1H, t, J = 6.1 Hz), 3.53–3.46 (1H, m), 3.43 (1H, d, J = 5 Hz), 3.41–3.30 (2H, m), 3.19 (1H, dd, J = 4.5, 13.2 Hz), 2.88–2.71 (4H, m), 2.66 (2H, t, J = 7.4 Hz), 2.52 (1H, dd, J = 4.2, 13.5 Hz), 2.25–2.16 (2H, m), 2.14 (3H, s), 1.87–1.77 (1H, m), 1.42–1.30 (1H, m), 0.90 (3H, d, J = 6.9 Hz), 0.84 (3H, t, J = 7.2 Hz); FAB MS m/z 484 (M + 1). Anal. ($C_{23}H_{40}N_4O_3S_2$·2.8TFA) C, H, N.

Cysteinylisoleucylisoleucylhomoserine (8). This compound was prepared using standard solution phase methods beginning with homoserine lactone. The compound was characterized in the lactone form. Anal. ($C_{20}H_{40}N_4O_4S$·1.4TFA) C, H, N. The lactone was converted to the hydroxy acid 8 as described below in the synthesis of 9.

N-[2(R)-[(tert-Butoxycarbonyl)amino]-3-[(triphenylmethyl)thio]propyl]isoleucylisoleucylhomoserine Lactone (32). Compound 28 (300 mg, 0.53 mmol), dissolved in CH_2Cl_2 (10 mL) and EtOAc (10 mL), was treated with 3-hydroxy-1,2,3-benzotriazin-4(3H)-one (HOOBT, 96 mg, 0.59 mmol) and EDC (112 mg, 0.59 mmol) followed by isoleucylhomoserine lactone hydrochloride (0.147 g, 0.59 mmol). The pH was adjusted to 6.5 with N,N-diisopropylethylamine (0.10 mL, 0.58 mmol), and the mixture was stirred at ambient temperature for 24 h. Workup and chromatography (1:1 to 2:1 EtOAc/hexane) provided 160 mg (40%) of 32: ^{1}H NMR ($CDCl_3$) δ 7.68–7.54 (1H, m), 7.47–7.13 (15H, m), 6.88–6.73 (1H, m), 4.95–4.80 (1H, m), 4.51–4.36 (2H, m), 4.31–4.13 (2H, m), 3.62–3.42 (2H, m), 2.88–2.82 (1H, m), 2.71–1.77 (8H, m), 1.76–1.45 (2H, m), 1.43 (9H, s), 1.39–1.0 (2H, m), 0.99–0.81 (12H, m).

N-(2(R)-Amino-3-mercaptopropyl)isoleucylisoleucylhomoserine Lactone (17). Compound 32 (0.160 g, 0.21 mmol) was dissolved in CH_2Cl_2 (4 mL), and TFA (2 mL) and triethylsilane (0.135 mL, 0.84 mmol) were added. The mixture was stirred at ambient temperature for 1.5 h. Concentration and trituration of the residue with Et_2O gave 0.115 g (85%) of 17: ^{1}H NMR (d_6-DMSO) δ 8.66 (1H, d, J = 9 Hz), 8.49–8.28 (1H, m), 4.61 (1H, q, J = 9 Hz), 4.36 (1H, t, J = 9 Hz), 4.31–4.15 (2H, m), 3.50–3.34 (2H, m), 3.00–2.71 (4H, m), 2.45–2.30 (1H, m), 2.30–2.17 (1H, m), 1.85–1.40 (5H, m), 1.22–1.05 (2H, m), 0.97–0.74 (12H, m). Anal. ($C_{19}H_{36}N_4O_4S$·2TFA) C, H, N.

N-(2(R)-Amino-3-mercaptopropyl)isoleucylisoleucylhomoserine (9). Compound 17 was dissolved in MeOH (0.40 mL), and 1 N NaOH (0.013 mL, 0.013 mmol) was added to give a 10 mM solution of the sodium salt of hydroxy acid 9. Complete consumption of the lactone was observed by HPLC, and products corresponding to 9 and its disulfide were detected. Treatment of an aliquot of this mixture was dithiothreitol rapidly led to a single major species by HPLC (~90%). Hydrolysis was also carried out in an NMR tube to confirm the structure of the product: ^{1}H NMR (CD_3OD + NaOD/D_2O) δ 4.59–4.52 (1H, m) 4.30–4.15 (2H, m), 3.65–3.50 (2H, m), 2.91 (1H, d, J = 6 Hz), 2.75–2.40 (5H, m), 2.10–1.97 (1H, m), 1.96–1.80 (3H, m), 1.72–1.50 (2H, m), 0.99–0.82 (12H, m).

N-[2(S)-[(tert-Butoxycarbonyl)amino]-3-methylpentyl]-isoleucine (20). Isoleucine (3.66 g, 27.9 mmol) was ground into a fine powder and suspended in a mixture of MeOH (30 mL) and TFA (2.15 mL, 27.9 mmol). The mixture was stirred until all of the isoleucine had dissolved, and the solvent was evaporated. DMF (30 mL) was added to the resulting isoleucine trifluoroacetate salt followed by 3A molecular sieves and a solution of 19 (3.00 g, 14 mmol) in DMF (10 mL). The mixture was stirred for 5 min, treated with sodium triacetoxyborohydride (4.44 g, 20.9 mmol), and stirred at ambient temperature for 24 h. After filtration and concentration, standard workup gave 4.56 g (99%) of 20: ^{1}H NMR (CD_3OD) δ 4.01–3.97 (1H, d, J = 3 Hz), 3.78–3.69 (1H, m), 3.34–3.25 (1H, m), 3.09–3.02 (1H, m), 1.7–1.57 (2H, m), 1.56–1.33 (2H, m), 1.45 (9H, s), 1.2–1.12 (2H, m), 1.08–0.88 (12H, m).

N-[2-(S)-[(tert-Butoxycarbonyl)amino]-3-methylpentyl]-isoleucylhomoserine Lactone (21). Compound 20 (5.56 g, 16.85 mmol), dissolved in DMF (30 mL), was treated with HOBT (2.28 g, 16.8 mmol), EDC (4.41 g, 23.0 mmol), and homoserine lactone hydrochloride (2.11 g, 15.3 mmol). Triethylamine (4.0 mL, 29 mmol) was added, and the mixture was stirred at ambient temperature for 24 h. The solution was concentrated and worked up. The crude product was triturated with Et_2O/hexane to give 3.1 g (49%) of 21: ^{1}H NMR (CD_3OD) δ 4.74–4.64 (1H, t, J = 8 Hz), 4.50–4.41 (1H, dt, J = 2, 8 Hz), 4.37–4.25 (1H, m), 3.54–3.44 (1H, m), 2.95–2.88 (1H, d, J = 6 Hz), 2.76–2.67 (1H, dd, J = 5, 12 Hz), 2.60–2.25 (4H, m), 1.44 (9 H, s), 1.77–1.37 (3H, m), 1.28–1.07 (2H, m), 1.0–0.80 (12H, m).

N-(2(S)-Amino-3-methylpentyl)isoleucylhomoserine Lactone Hydrochloride (33). Compound 21 was dissolved in EtOAc (50 mL) and cooled to −25 °C. HCl was bubbled through the mixture until TLC (95:5 CH_2Cl_2/MeOH) indicated complete

reaction. Nitrogen was bubbled through the mixture to remove excess HCl, and the mixture was concentrated to give 2.90 g (100%) of **33**.

***N*-[2(*S*)-[[(*tert*-Butoxycarbonyl)-*S*-(triphenylmethyl)-cysteinyl]amino]-3-methylpentyl]isoleucylhomoserine Lactone (34).** *N*-(*tert*-Butoxycarbonyl)-*S*-(triphenylmethyl)cysteine (0.073 g, 0.157 mmol) was dissolved in DMF (5 mL). HOBT (0.021 g, 0.157 mmol) and EDC (0.030 g, 0.157 mmol) were added followed by **33** (0.055 g, 0.14 mmol). The pH was adjusted to 6.5 with Et_3N (0.022 mL, 0.16 mmol), and the mixture was stirred at ambient temperature for 24 h. The mixture was concentrated. Workup and chromatography (2–3% MeOH in methylene chloride) provided 0.080 g (74%) of **34**: ^1H NMR (CD_3OD) δ 7.49–7.19 (15H, m), 4.73–4.61 (1H, m), 4.49–4.39 (1H, m), 4.38–4.20 (1H, m), 4.18–4.05 (2H, m), 3.98–3.87 (1H, m), 3.84–3.72 (1H, m), 2.97–2.90 (1H, d, J = 4 Hz), 2.75–2.22 (6H, m), 1.45 (9H, s), 1.70–1.35 (2H, m), 1.18–1.04 (2H, m), 0.96–0.75 (12H, m).

***N*-[2(*S*)-(Cysteinylamino)-3-methylpentyl]isoleucylhomoserine (10).** Compound **34** (0.080 g, 0.11 mmol) was dissolved in CH_2Cl_2 (2 mL) and TFA (1 mL) and treated with triethylsilane (0.070 mL, 0.44 mmol). The mixture was stirred at ambient temperature for 2 h and concentrated, and the residue was triturated with Et_2O. The resulting crude product was purified by preparative HPLC (Waters PrepPak C-18 eluting with acetonitrile/0.1% TFA in H_2O) to give 0.050 g (71%) of the lactone of **10** after lyophilization: ^1H NMR (CD_3OD) δ 4.59–4.40 (2H, m), 4.35–4.22 (1H, m), 4.00–3.90 (1H, m), 3.70–3.55 (1H, m), 3.18–3.00 (2H, m), 2.62–2.3 (2H, m), 2.04–1.84 (1H, m), 1.83–1.30 (8H, m), 1.05–0.87 (12H, m). Anal. ($C_{19}H_{36}N_4O_4S$·2TFA·2.25H_2O) C, H, N.

The lactone was hydrolyzed to provide **10**, as described in the synthesis of **9**.

***N*-[2(*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-[(triphenylmethyl)thio]propyl]amino]-3-methylpentyl]isoleucylhomoserine Lactone (23).** Compound **33** (2.90 g, 7.51 mmol) was dissolved in MeOH (20 mL) and treated with 3A molecular sieves, KOAc (1.47 g, 15.0 mmol), **22** (6.71 g, 15.0 mmol), and sodium cyanoborohydride (0.708 g, 11.3 mmol). The mixture was stirred at ambient temperature for 24 h. Workup and chromatography (1–3% MeOH in methylene chloride) gave 2.51 g (45%) of **23**, 0.800 g of impure **23**, and 0.50 g of the corresponding homoserine methyl ester: ^1H NMR (CD_3OD) δ 7.45–7.18 (15H, m), 4.62–4.51 (1H, m), 4.48–4.36 (1H, m), 4.33–4.21 (1H, m), 3.65–3.52 (2H, m), 2.85–2.79 (1H, d, J = 6 Hz), 2.70–2.23 (9H, m), 1.70–1.49 (3H, m), 1.45 (9H, s), 1.28–1.09 (2H, m), 0.98–0.78 (12H, m).

***N*-[2(*R*)-[(2-Amino-3-mercaptopropyl)amino]-3-methylpentyl]isoleucylhomoserine Lactone (18).** Compound **23** (2.51 g, 3.37 mmol) was dissolved in CH_2Cl_2 (24 mL) with TFA (12 mL) and treated with triethylsilane (2.16 mL, 13.5 mmol). The mixture was stirred at ambient temperature for 2 h and concentrated, and the residue was partitioned between hexane and 0.1% TFA in H_2O (25 mL/75 mL). The aqueous layer was washed with hexane (3 × 25 mL), filtered, and lyophilized. The crude product was purified by preparative HPLC (Waters C-18 Prep Pak eluting with acetonitrile/0.1% TFA in H_2O) to give 0.5 g (20%) of the trifluoroacetate salt of **18** after lyophilization: ^1H NMR (CD_3OD) δ 4.56–4.45 (2H, m), 4.42–4.27 (1H, m), 3.75–3.70 (1H, d, J = 6 Hz), 3.45–3.37 (1H, m), 3.13–2.71 (7H, m), 2.59–2.45 (2H, m), 2.11–1.96 (1H, m), 1.87–1.76 (1H, m), 1.75–1.60 (1H, m), 1.39–1.21 (3H, m), 1.10–0.82 (12H, m). Anal. ($C_{19}H_{33}N_4O_3S$·3TFA·H_2O) C, H, N.

The corresponding sodium salt of the hydroxy acid **11** was prepared as described for **9**: ^1H NMR (CD_3OD + NaOD/D_2O) δ 4.65–4.55 (1H, m), 3.71–3.52 (3H, m), 2.87 (1H, d, J = 6 Hz), 2.82–2.32 (8H, m), 2.31–2.15 (1H, m), 2.14–2.00 (1H, m), 1.98–1.82 (1H, m), 1.74–1.55 (3H, m), 1.50–1.45 (1H, m), 1.32–1.10 (2H, m), 1.00–0.80 (12H, m).

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