# *N*-Arylalkyl Pseudopeptide Inhibitors of Farnesyltransferase

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Inhibitors of Ras protein farnesyltransferase are described which are reduced pseudopeptides related to the C-terminal tetrapeptide of the Ras protein that signals farnesylation. Reduction of the carbonyl groups linking the first three residues of the tetrapeptide leads to active inhibitors which are chemically unstable. Stability can be restored by alkylating the central amine of the tetrapeptide. Studies of the SAR of these alkylated pseudopeptides with concomitant modification of the side chain of the third residue led to 2(*S*)-(2(*S*)-{[2(*S*)-(2(*R*)-amino-3-mercaptopropylamino)-3(*S*)-methylpentyl]naphthalen-1-ylmethylamino}acetylamino)-4-methylsulfanylbutyric acid (**11**), a subnanomolar inhibitor. The methyl ester (**10**) of this compound exhibited submicromolar activity in the processing assay and selectively inhibited anchorage-independent growth of Rat1 cells transformed by v-*ras* at 2.5−5 $\mu$M.

The *ras* oncogene product Ras p21 is a key player in controlling cell proliferation. Mutations in Ras proteins can lead to unregulated cell growth and are implicated in many human cancers.[1] Ras is initially synthesized in vivo as an inactive cytosolic protein that requires a series of posttranslational modifications to bind to the cell membrane and express its biological activity. The key modification is *S*-farnesylation of a cysteine residue that is the fourth amino acid from the carboxy terminus of the protein. This transformation is catalyzed by farnesyl transferase (FTase) and uses farnesyl pyrophosphate (FPP) as cosubstrate.[2] The carboxy-terminal tetrapeptide of Ras and other farnesylated proteins, known as the CAAX box, is required for recognition by FTase. Furthermore, the CAAX tetrapeptide alone is able to serve as a FTase substrate.[3]

Several types of FTase inhibitors have been reported which mimic this tetrapeptide.[4] CAAX tetrapeptides themselves are inhibitors of protein farnesylation, while serving as alternate substrates for FTase. However, these peptides do not inhibit farnesylation in whole cells in culture due to their proteolytic lability. Recently we described a class of CAAX-like pseudotetrapeptides which contained reduced amide bonds between the cysteine and second amino acid and between the second and third amino acids.[5] The prototype, L-731,734 (**1**), inhibited the growth of *ras*-transformed cells in soft agar and completely inhibited Ras processing in intact cells at 100 mM.[6] While L-731,734 is itself a very weak inhibitor of FTase, intracellular hydrolysis of the homoserine lactone generates the corresponding acid **2**, a 20 nM inhibitor of the enzyme in vitro. Since the acid **2** was inactive in these cell-based assays, we concluded that temporarily masking the carboxylic acid as the lactone was required to impart adequate cell membrane permeability to observe antiproliferative activity in this series of CAAX analogues.

The poor activity of **1** in cell culture in relation to the intrinsic inhibitory potency of its corresponding acid (**2**) prompted us to explore the chemical stability of this compound. In pH 7.4 buffer, **1** readily cyclizes to the diketopiperazine **3** at 25 °C with first-order kinetics ($t_{1/2}$ = 20 h) (Figure 1). The diketopiperazine (**3**, FTase $IC_{50}$ = 5 $\mu$M) was 250-fold less active than **2** against FTase. Since the time scale of the growth assay in soft agar is 2 weeks with feeding twice weekly, the rate of destruction of **1** could clearly play a major role in limiting its activity in cell culture. In this paper we present the results of a test of the hypothesis that masking the central amine of compounds related to **1** through alkylation or acylation to prevent this cyclization would result in inhibitors that exhibit a better profile in cell culture with respect to their intrinsic potency. We were concerned that the intrinsic potency of these alkylated derivatives might be compromised since we had found that *N*-methylation of the tetrapeptide CVFM in any position led to weaker inhibitors.[7] This is consistent with recently published results.[8]

## Chemistry

The syntheses of **1** and **2** have been described previously.[5] *N*-Alkylated analogues were prepared by a series of reductive alkylations and deprotections as illustrated in Scheme 1 for the synthesis of **10** and **11**. All of the compounds (**12** through **26**) were synthesized in a similar manner by substituting the requisite aldehydes or amino acid intermediates.

**Structure−Activity Relationships: In Vitro Inhibition of FTase.** Compounds were characterized as inhibitors of FTase in vitro using either FTase purified from bovine brain or homogeneous human recombinant FTase, [$^3$H]FPP, and recombinant Ha-Ras protein.[9−11] 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 was reduced by 50% compared to an experiment in which no inhibitor was present. To more

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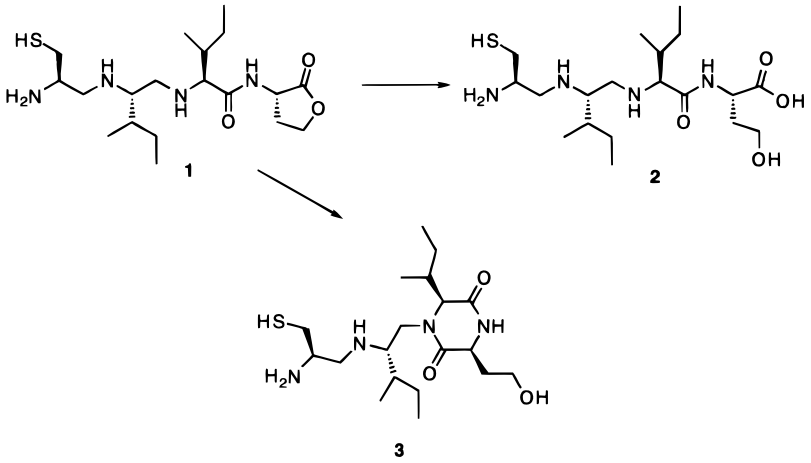
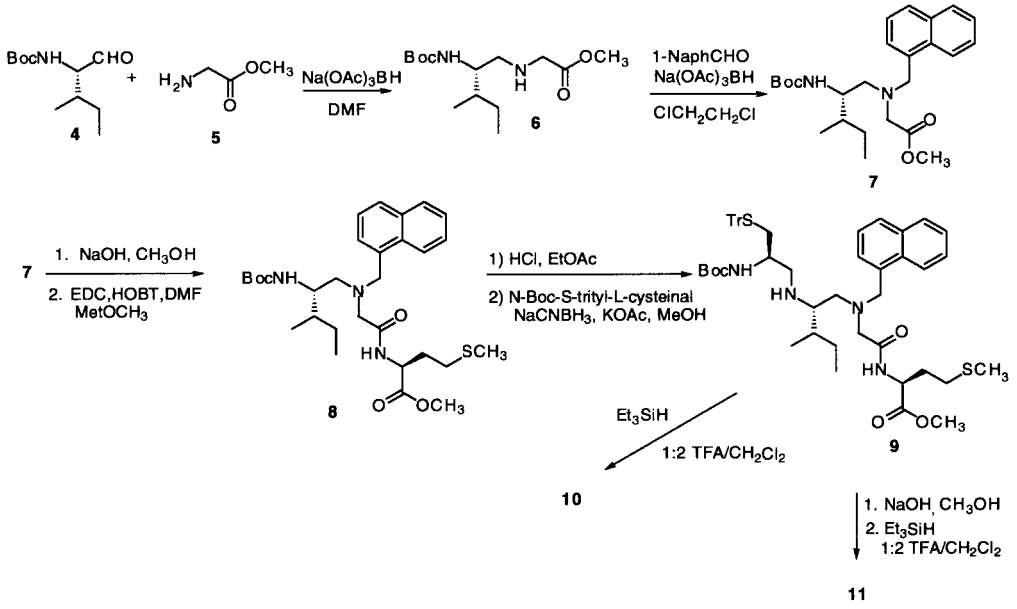


**Figure 1.**

**Scheme 1.** Representative Synthesis of a Farnesyltransferase Inhibitor



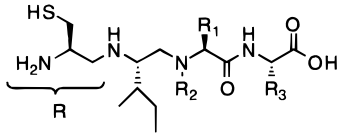
accurately determine the potency of the more active compounds, they were further tested in a similar protocol which contained less enzyme (10 pM). Representative compounds were evaluated as inhibitors of the closely related enzyme protein geranylgeranyl transferase (GGTase-1).[9,12] Details of these assays are found in the cited literature.

*N*-Methylation of **2** to give **12** led to a 4-fold loss in FTase activity (Table 1). Replacing the *sec*-butyl group of **1** with an *n*-propyl group gave **13** of equal potency. *N*-Methylation of **13** to give **14**, however, did not alter the inhibitory activity. This suggests that the reduction in potency upon methylation of **2** is due to a conformational bias imposed by the interaction of the *N*-methyl group and the *sec*-butyl group of the isoleucine residue. If that is the case, the effect must be peculiar to the inhibitor bearing the homoserine residue at the carboxy terminus of the inhibitor. In the nonmethylated series, replacement of the homoserine carboxy terminus with methionine in **2** gave **17** of comparable potency. *N*-Methylation of **17** to give **18** did not alter potency. It is possible that different bound conformations of the inhibitors are required to correctly present the hydrophilic side chain of homoserine as opposed to the hydrophobic methionine side chain, perhaps in unique binding sites.

Similar results were obtained in the CIFM series. We previously reported that replacing the *sec*-butyl group of **2** with benzyl provided a more potent inhibitor (**19**, $IC_{50} = 4.7$ nM).[5] *N*-Methylation of **19** to give **20** led to a 9-fold loss of activity. In the CIF−HSer series, the effect of *N*-methylation was much more dramatic, leading to a 64-fold loss in potency upon *N*-methylation of **15** to give **16**. Incorporating benzyl groups at both $R_1$ and $R_2$ (**21**) led to a 70-fold drop in FTase activity, indicating that only one aromatic binding site is available.

Removing the substituent at $R_1$ gave the glycine analogue (**22**), a potent FTase inhibitor ($IC_{50} = 1.2$ nM). This shifting of the amino acid side chain from the α-position to nitrogen has provided potent inhibitors. Recently, this same ploy has been reported to give potent inhibitors in the tetrapeptide CVFM series.[13] Replacement of benzyl with 1-naphthylmethyl **(11)** gave the most potent inhibitor in this series. All compounds tested were 10−1000-fold selective for FTase versus GGTase-1. No definitive trend of structure vs GGTase-1 activity could be established.

**Table 1.** FTase Inhibition and Selectivity of Pseudopeptides in Vitro[a]



| compd | $R_1$ | $R_2$ | $R_3$ | $IC_{50}$ (nM) FPTase[b] | $IC_{50}$ (nM) GGTase-1[c] |
|---|---|---|---|---|---|
| **2** | (*S*)CH(CH$_3$)C$_2$H$_5$ | H | CH$_2$CH$_2$OH | 20 ± 6 (3) | 45000 (1) |
| **11** | H | 1-naphthyl CH$_2$ | CH$_2$CH$_2$OH | 0.123* ± 0.057 (4) | 10 (1) |
| **12** | H | CH$_3$ | CH$_2$CH$_2$OH | 83$^+$ ± 15 (3) | 10000 (1) |
| **13** | CH$_2$CH$_2$CH$_3$ | H | CH$_2$CH$_2$OH | 27$^+$ (1) | 10000 (1) |
| **14** | CH$_2$CH$_2$CH$_3$ | CH$_3$ | CH$_2$CH$_2$OH | 21 (1) | 43000 (1) |
| **15** | CH$_2$Ph | H | CH$_2$CH$_2$OH | 26$^+$ ± 1 (3) | 10000 (1) |
| **16** | CH$_2$Ph | CH$_3$ | CH$_2$CH$_2$OH | 1600 (1) | nd |
| **17** | (*S*)CH(CH$_3$)C$_2$H$_5$ | H | CH$_2$CH$_2$SCH$_3$ | 30 (1) | nd |
| **18** | (*S*)CH(CH$_3$)C$_2$H$_5$ | CH$_3$ | CH$_2$CH$_2$SCH$_3$ | 25 ± 10 (5) | 4700 (1) |
| **19** | CH$_2$Ph | H | CH$_2$CH$_2$SCH$_3$ | 4.8 ± 0.4 | 5750 ± 50 (2) |
| **20** | CH$_2$Ph | CH$_3$ | CH$_2$CH$_2$SCH$_3$ | 42 (1) | nd |
| **21** | CH$_2$Ph | CH$_2$Ph | CH$_2$CH$_2$SCH$_3$ | 1400 (1) | nd |
| **22** | H | CH$_2$Ph | CH$_2$CH$_2$SCH$_3$ | 1.2* ± 0.8 (2) | 450 (1) |
| **23** | H, R = HS(CH$_2$)$_3$ | CH$_2$Ph | CH$_2$CH$_2$SCH$_3$ | 2.2* (1) | 39000 (1) |
| **24** | H, R = HS(CH$_2$)$_2$CO | CH$_2$Ph | CH$_2$CH$_2$SCH$_3$ | 110 (1) | nd |

[a] nd, not determined. [b] Concentration of compound required to inhibit 50% of FPTase-catalyzed incorporation of [$^3$H]FPP into recombinant human Ha-Ras protein by 50%. Those values marked with $^+$ were obtained using enzyme from bovine brain at a concentration of approximately 1 nM, and those marked with * used 10 pM enzyme concentration. [c] Inhibition of bovine type-I geranylgeranyltransferase.[8] Assay results are reported as concentration ± SEM for the number of determinations shown in parentheses. With one determination, the values are estimated to be reliable within 2-fold.

Deletion of the primary amino group of the cysteine residue has a deleterious effect on binding to FTase: compound **23** is at least 20-fold less potent an inhibitor than **11**. The mercaptopropionyl analogue of **23**, compound **24**, is even less potent ($IC_{50}$ = 110 nM). Thus, the presence of a basic group, preferably a primary amine, in the vicinity of the cysteine residue is an element critical for the binding of this family of inhibitors. In this sense, the SAR in the naphthylmethyl glycine series parallels the SAR that we and others observed in analogues of CVFM[5,14] and is distinct from the SAR of tetrapeptide analogues based on CVIM. Thus, it appears that the CVFM and naphthylmethyl glycine series bind to FTase in a conformation distinct from the nonaromatic inhibitors (i.e., reduced analogues of CVIM), with the former presenting the cysteine residue from a conformation that allows productive interaction between the cationic amine and a complementary binding element on the enzyme. Indeed, this observation has been exploited in the preparation of novel FTase inhibitors.[15]

## Inhibition of Ras Farnesylation in Cell Culture

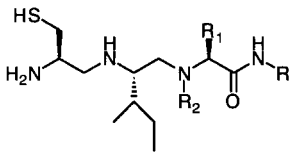
The cytotoxicity of the prodrug esters of the more active FTase inhibitors was assessed using a viable staining method with MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide). The cytotoxic endpoint is the highest compound concentration tolerated by NIH 3T3 cells in a 48-h assay. NIH 3T3 cells transformed by v-*ras* were used to evaluate the effect of the prodrug esters of the more active inhibitors on the posttranslational processing of Ras in intact cells. The cells were incubated in the presence of the indicated compound or solvent control for 24 h and were labeled with [$^{35}$S]methionine during the final 20 h. Ras was immunoprecipitated from detergent lysates of cell extracts, and the mobility of farnesylated versus nonfarnesylated protein was observed with SDS−polyacrylamide gel electrophoresis. The HMG-CoA reductase inhibitor lovastatin was used as a positive control. Lovastatin at 15 $\mu$M gave >90% inhibition of Ras processing.

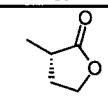
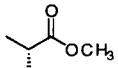
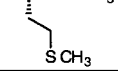
In addition, the most active ester in the processing assay was evaluated in a second cell assay which measured its ability to inhibit the anchorage-independent growth of Rat1 cells transformed by v-*ras* or v-*raf* oncogene. A minimum inhibitory concentration (MIC) required to achieve a reduction in size and number of colonies of transformed cells in soft agar relative to vehicle-treated controls was determined. The Raf-transformed cells, which do not require farnesylation for biological activity, were included to evaluate the specificity of these inhibitors for Ras-induced cell transformation. Details of these assays are described elsewhere.[6]

Although the *N*-methyl analogue **12** was 4-fold less active against FTase than **2**, its corresponding ester prodrug **25** was 4-fold more active than **1** in the processing assay (Table 2). In pH 7.4 buffer, at 25 °C, **25** remained intact with the hydrolyzed lactone **12** ($t_{1/2}$ = 70 h) as the only product. This suggests that our hypothesis was correct: masking the central amine of these molecules results in inhibitors that exhibit a better profile in cell culture with respect to their intrinsic potency.

Furthermore, the transposition of the arylalkyl group from the α-position of the amino acid residue in $A_2$ of the $CA_1A_2X$ box to its amino group, to give compounds such as **11** and **22**, leads to considerably more potent FTase inhibitors. The prodrug esters **10** and **26** of the most potent inhibitors **11** and **22**, respectively, exhibited submicromolar activity in the processing assay, 10-fold lower than their cytotoxic endpoints. Compound **10** also inhibited anchorage-independent growth of Rat1 cells

**Table 2.** Effect of FTase Inhibitors in Cell Culture[a]



| Compd | R | $R_1$ | $R_2$ | CTE[d] (μM) | $IC_{50}$ (μM) Ras processing[e] | soft agar MIC (μM)[f] ras | soft agar MIC (μM)[f] raf |
|---|---|---|---|---|---|---|---|
| **1** |  | $CH(CH_3)C_2H_5$ | H | >1000 | 100 | 1000 | >1000 |
| **25*** | | $CH(CH_3)C_2H_5$ | $CH_3$ | 500 | 25-50 | 500 | nd |
| **10** |  | H | 1-naphthyl $CH_2$ | 5 | 0.5 | 2.5-5 | >5 |
| **26** |  | H | $CH_2Ph$ | 10 | 0.1 | nd | nd |

[a] See footnote *a* of Table 1. [d] Highest nontoxic concentration for cultured NIH3T3 cells as assessed by MTT staining. [e] Inhibition of posttranslational processing of v-Ras protein in cultured NIH3T3 cells.[6] [f] Minimum inhibitory concentration (MIC) required to achieve a reduction in size and number of colonies of RAT1-v-*ras*- or RAT1-v-*raf*-transformed cells in soft agar relative to vehicle-treated control.[6]

transformed by v-*ras* with a MIC of 2.5−5 μM. This inhibition was selective for v-*ras* transformed cells since the v-*raf* transformed cells were not inhibited at 5 μM.

## Conclusion

CAAX-like pseudotetrapeptides with reduced amide linkages between cysteine and the second amino acid and between the second and third amino acids can be stabilized by alkylation of the central amine of the pseudotetrapeptide. Concomitant modification of the akyl group on the amine as well as the alkyl side chain of the third amino acid has led to a series of subnanomolar FTase inhibitors. The ester prodrugs of these potent inhibitors inhibit Ras processing at submicromolar levels and selectively inhibit anchorage-independent growth of Rat1 cells transformed by v-*ras*. These results suggest that the *N*-arylalkyl pseudopeptides represent a useful template for further development as cancer chemotherapeutics.

## 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 consisted of extraction with an organic solvent and washing as appropriate with saturated sodium bicarbonate solution 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. Preparative reverse phase HPLC was performed on a Waters 3000 instrument using either C-18 Vydac or PrepPak columns. Products and intermediates were characterized by 300-MHz $^1$H NMR. Final products were also characterized by mass spectrometry and combustion analyses. Observed values were within 0.4% of calculated values for the compound formulas shown. The following examples are illustrative of the procedures employed.

**1-[2(*S*)-(2(*R*)-Amino-3-mercaptopropylamino)-3(*S*)-methylpentyl]-6(*S*)-*sec*-butyl-3(*S*)-(2-hydroxyethyl)piperazine-2,5-dione (3).** 2-{[2-(2-Amino-3-mercaptopropylamino)-3-methylpentyl]amino}-3-methylpentanoic acid (2-oxotetrahydrofuran-3-yl)amide (**1**)[5] (0.020 g, 0.026 mmol) was dissolved in degassed 0.1 M $KHSO_4$ solution buffered to pH 7.36 with NaOH under Ar with stirring for 72 h. The reaction mixture was treated with dithiothreitol (0.008 g, 0.052 mmol) dissolved in MeOH (0.5 mL) and prepped on a RPLC using a VYDAC column eluting with 0.1%TFA/$H_2O$:0.1%TFA/$CH_3CN$ (95:5 to 5:95 gradient over 1 h). Like fractions were combined to give 0.0045 g (28%) of **3** after lyophilization: $^1$H NMR ($CD_3$-OD) $\delta$ 4.21−4.28 (m, 1H), 4.1−4.18 (m, 1H), 3.71 (d, 1H, $J$ = 6 Hz), 3.7−3.82 (m, 2H), 3.4−3.5 (m, 1H), 2.75−3.21 (m, 6H), 2.18−2.3 (m, 1H), 1.83−2.01 (m, 1H), 1.64−1.82 (m, 2H), 1.4−1.58 (m, 2H), 1.21−1.4 (m, 1H), 0.92−1.11 (m, 12H); MS (M + 1) 403.

**Preparation of 2(*S*)-(2(*S*)-{[2(*S*)-(2(*R*)-Amino-3-mercaptopropylamino)-3(*S*)-methylpentyl]naphthalen-1-ylmethylamino}acetylamino)-4-methylsulfanylbutyric Acid Methyl Ester (10).** Glycine methyl ester hydrochloride (**5**) (4.41 g, 0.035 mol) was dissolved in 1,2-dichloroethane (50 mL) and DMF (5 mL) and treated with 3A molecular sieves (10 g) and *N*-*tert*-butoxycarbonylisoleucinal (**4**) (6.3 g, 0.029 mol) with stirring at 0 °C. Sodium triacetoxyborohydride (9.27 g, 0.044 mol) was added, and the pH of the mixture was adjusted to 6 with $Et_3N$ (3 mL, 0.022 mol). After stirring for 18 h, the mixture was filtered, concentrated to a small volume, and partitioned between EtOAc and water. Standard workup gave 3.88 g (54%) of compound **6** after purification by chromatography ($SiO_2$, EtOAc:hexane, 1:3): $^1$H NMR ($CDCl_3$) $\delta$ 4.69 (m, 1H), 3.72 (s, 3H), 3.48−3.62 (m, 1H), 3.42 (ABq, 2H), 2.65 (d, 2H, $J$ = 6 Hz), 1.4−1.6 (m, 2H), 1.48 (s, 9H), 1.04−1.2 (m, 1H), 0.85−0.95 (m, 6H).

Compound **6** (2.00 g, 6.97 mmol) was dissolved in 1,2-dichloroethane (56 mL), and 3A molecular sieves were added followed by 1-naphthaldehyde (1.89 mL, 13.9 mmol) and sodium triacetoxyborohydride (6.65 g, 31.4 mmol). The mixture was stirred at ambient temperature for 16 h, filtered through glass fiber paper, and concentrated. The residue was partitioned between EtOAc and saturated $NaHCO_3$ (100 mL/25 mL). Standard workup gave 5.0 g of crude product which was purified by chromatography ($SiO_2$, 1:6 to 1:3 EtOAc:hexane) to give 3.8 g of compound **7**: $^1$H NMR ($CD_3OD$) $\delta$ 8.44−8.38 (d, 1H, $J$ = 6 Hz), 7.88−7.77 (m, 2H), 7.55−7.35 (m, 4H), 6.34−6.27 (m, 1H), 4.25 (ABq, 2H), 3.66 (s, 3H), 3.40−3.23 (m, 1H), 2.95−2.85 (dd, 1H, $J$ = 6, 15 Hz), 2.68−2.57 (dd, 1H, $J$ = 6, 15 Hz), 1.57−1.46 (m, 1H), 1.43 (s, 9H), 1.34−1.18 (m, 2H), 1.06−0.85 (m, 1H), 0.85−0.71 (m, 6H).

Compound **7** (2.61 g, 6.10 mmol) was dissolved in $CH_3OH$ (50 mL), and 1 N NaOH (24.4 mL, 24.4 mmol) was added. The mixture was stirred at ambient temperature for 4 h and concentrated. The resulting residue was dissolved in $H_2O$ (25 mL) and neutralized with 1 N HCl (24.4 mL). The aqueous layer was washed with EtOAc (3 × 50 mL). The organic layers

were combined, dried with $Na_2SO_4$, filtered, and concentrated to give 2.29 g of the corresponding acid: $^1$H NMR ($CD_3OD$) $\delta$ 8.48−8.39 (d, 1H, $J$ = 6 Hz), 8.03−7.91 (t, 2H, $J$ = 6 Hz) 7.75−7.48 (m, 4H), 5.00−4.93 (d, 1H, $J$ = 12 Hz), 4.78−4.66 (d, 1H, $J$ = 12 Hz), 3.80−3.58 (m, 3H), 3.49−3.40 (dd, 1H, $J$ = 3, 12 Hz), 3.09−2.98 (dd, 1H, $J$ = 3, 12 Hz), 1.42 (s, 9H), 1.37−1.28 (m, 2H), 1.80−1.00 (m, 1H), 0.94−0.78 (m, 6H).

This acid was dissolved in DMF (20 mL) and treated with HOBT (0.822 g, 6.08 mmol), EDC (1.17 g, 6.08 mmol), and methionine methyl ester hydrochloride (1.21 g, 6.08 mmol). The pH was adjusted to 7.5 with $Et_3N$ (1.7 mL, 12 mmol), and the mixture was stirred at ambient temperature for 24 h. The mixture was concentrated, and the residue was partitioned between EtOAc (50 mL) and saturated $NaHCO_3$ solution (25 mL). Standard workup gave 3.2 g of crude product which was purified by chromatography ($SiO_2$ eluting with 1:3 to 1:2 EtOAc:hexane) to give 2.82 g of compound **8**: $^1$H NMR ($CD_3OD$) $\delta$ 8.36−8.29 (d, 1H, $J$ = 6 Hz), 7.93−7.86 (d, 1H, $J$ = 6 Hz), 7.85−7.80 (d, 1H, $J$ = 6 Hz), 7.61−7.39 (m, 4H), 6.60−6.52 (m, 1H), 4.32−4.06 (m, 2H), 3.90−3.69 (m, 1H), 3.65 (s, 3H), 3.27−3.14 (m, 2H), 2.93−2.70 (m, 2H), 2.19−1.78 (m, 6H, 1.63−1.30 (m, 13H), 1.19−1.05 (m, 1H), 0.95−0.81 (m, 6H).

Compound **8** (2.82 g, 5.04 mmol) was dissolved in EtOAc (50 mL) and cooled to −25 °C. HCl was bubbled through the mixture until TLC (95:5 $CH_2Cl_2$:$CH_3OH$) indicated complete reaction. Nitrogen was bubbled through the mixture to remove excess HCl, and the mixture was then concentrated to give 2.68 g of the deprotected amine: $^1$H NMR ($CD_3OD$); d 8.34−8.28 (d, 1H, $J$ = 6 Hz), 8.00−7.92 (d, 2H, $J$ = 6 Hz), 7.83−7.71 (m, 1H), 7.68−7.49 (m, 3H), 4.76−4.55 (m, 4H), 3.84−3.75 (m, 2H), 3.71 (s, 3H), 3.59−3.70 (m, 1H), 3.21−3.00 (m, 2H), 2.57−2.38 (m, 3H), 2.17−2.04 (m, 4H), 1.97−1.81 (m, 1H), 1.63−1.50 (m, 1H), 1.39−1.20 (m, 1H), 1.19−1.00 (m, 1H), 0.95−0.79 (m, 6H).

This amine (0.200 g, 0.376 mmol) was dissolved in $CH_3OH$ (5 mL), treated with KOAc (0.074 g, 0.752 mmol), 3A molecular sieves (0.5 g), and *N*-(*tert*-butoxycarbonylamino)-*S*-triphenylmethylcysteine aldehyde[5] (0.219 g, 0.489 mmol) followed by $NaCNBH_3$ (0.038 g, 0.602 mmol), and stirred at ambient temperature for 18 h. The reaction mixture was filtered and partitioned between EtOAc (20 mL) and aqueous saturated $NaHCO_3$ solution. The organic layer was washed with brine and dried ($Na_2SO_4$). Filtration and concentration to dryness gave crude product which was chromatographed ($SiO_2$, EtOAc:hexane, 1:3 to 1:1) to give 0.178 g (53%) of compound **9**: $^1$H NMR ($CD_3OD$) $\delta$ 8.31 (d, 1H, $J$ = 9 Hz), 7.75−7.89 (m, 2H), 7.15−7.5 (m, 19H), 4.25−4.40 (m, 2H), 4.03 (d, 1H, $J$ = 12 Hz), 3.5−3.7 (m, 1H), 3.66 (s, 3H), 3.2−3.4 (m, 3H), 2.78−2.9 (m, 1H), 2.45−2.66 (m, 3H), 2.2−2.4 (m, 3H), 2.05 (s, 3H), 1.95 (s, 3H), 1.95−2.2 (m, 2H), 1.75−1.9 (m, 1H), 1.3−1.6 (m, 2H), 1.36 (s, 9H), 0.95−1.1 (m, 1H), 0.75−0.93 (m, 6H).

Compound **9** (0.178 g, 0.200 mmol) dissolved in $CH_2Cl_2$ (4 mL) and TFA (2 mL) at ambient temperature was treated with triethylsilane (0.128 mL, 0.800 mmol). After 2 h the reaction mixture was concentrated to dryness and partitioned between hexane and 0.1% TFA/$H_2O$, and the aqueous layer was chromatographed by preparative RP HPLC using a Waters PrepPak. Pure fractions were combined and lyophilized to give compound **10**: $^1$H NMR ($CD_3OD$) $\delta$ 8.29 (d, 1H, $J$ = 9 Hz), 8.0 (d, 2H, $J$ = 9 Hz), 7.5−7.75 (m, 4H), 4.6−4.8 (m, 2H), 4.2−4.4 (m, 1H), 3.74 (s, 3H), 3.6−4.0 (m, 3H), 2.95−3.4 (m, 3H), 2.5−2.85 (m, 8H), 2.08 (s, 3H), 1.92−2.23 (m, 2H), 1.6−1.9 (m, 2H), 1.1−1.36 (m, 2H), 0.78−0.92 (m, 6H), MS *m/e* 563 (M + 1). Anal. ($C_{28}H_{44}N_4O_3S_2\cdot 2CF_3CO_2H\cdot H_2O$) C, H, N.

Compound **9** (0.097 g, 0.109 mmol) was dissolved in $CH_3OH$ (5 mL), cooled to 0 °C, and treated with 1 N NaOH solution (0.436 mL, 0.436 mmol). After stirring for 5 h, the reaction was neutralized with 1 N HCl (0.436 mL, 0.436 mmol) and extracted with EtOAc (3 × 20 mL). The organic layers were combined, washed with brine, dried ($Na_2SO_4$), filtered, and concentrated to give the protected acid (0.071 g) (74%) which was used without further purification.

The acid (0.071 g, 0.081 mmol) dissolved in $CH_2Cl_2$ (2 mL) and TFA (1 mL) at ambient temperature was treated with triethylsilane (0.052 mL, 0.324 mmol). After 2 h the reaction mixture was concentrated to dryness and partitioned between hexane and 0.1%TFA/$H_2O$, and the aqueous layer was chromatographed by preparative RP HPLC using a Waters Prep-Pak. Pure fractions were combined and lyophilized to give 0.047 g (71%) of compound **11**: $^1$H NMR ($CD_3OD$) $\delta$ 8.3 (d, 1H, $J$ = 9 Hz), 7.99 (d, 2H, $J$ = 9 Hz), 7.5−7.65 (m, 4H), 4.48−4.8 (m, 2H), 4.2−4.36 (m, 1H), 3.55−3.95 (m, 2H), 2.82−3.55 (m, 3H), 2.45−2.82 (m, 8H), 2.10 (s, 3H), 2.14−2.28 (m, 1H), 1.6−2.1 (m, 4H), 1.08−1.35 (m, 2H), 0.75−0.9 (m, 6H). Anal. ($C_{27}H_{42}N_4O_3S_2\cdot 2.5\ CF_3CO_2H$) C, H, N.

**2(*S*)-(2(*S*)-{[2(*S*)-(3-Mercaptopropylamino)-3(*S*)-methylpentyl]naphthalen-1-ylmethylamino}acetylamino)-4-methylsulfanylbutyric Acid (23).** The amine hydrochloride (0.200 g, 0.376 mmol) from the deprotection of compound **8** described above was dissolved in $CH_3OH$ (10 mL), treated with KOAc (0.074 g, 0.752 mmol), 3A molecular sieves (0.5 g), and *S*-triphenylmethylpropionaldehyde (0.150 g, 0.451 mmol) followed by $NaCNBH_3$ (0.036 g, 0.564 mmol), and stirred at ambient temperature for 18 h. The reaction mixture was filtered and partitioned between EtOAc (20 mL) and aqueous saturated $NaHCO_3$ solution. The organic layer was washed with brine and dried ($Na_2SO_4$). Filtration and concentration to dryness gave crude product which was chromatographed ($SiO_2$, $CH_2Cl_2$:$CH_3OH$, 98:2 to 94:6) to give 0.230 g (79%) of fully protected **23**. Following the procedures described above, basic hydrolysis followed by detritylation (TFA/triethylsilane) gave compound **23** in 27% overall yield: $^1$H NMR ($CD_3OD$) $\delta$ 8.25 (d, 1H, $J$ = 9 Hz), 7.86−7.98 (m, 2H), 7.44−7.66 (m, 4H), 4.58−4.64 (m, 1H), 4.43 (d, 1H, $J$ = 12 Hz), 4.18 (d, 1H, $J$ = 12 Hz), 3.71 (d, 1H, $J$ = 13 Hz), 3.56 (d, 1H, $J$ = 13 Hz), 3.03 (dd, 1H, $J$ = 3, 13 Hz), 2.44−2.71 (m, 4H), 2.27−2.40 (m, 2H), 2.12−2.28 (m, 2H), 2.10 (s, 3H), 1.85−2.1 (m, 2H), 1.46−1.8 (m, 3H), 1.06−1.2 (m, 2H), 0.82 (d, 3H, $J$ = 6 Hz), 0.76 (t, 3H, $J$ = 6 Hz). Anal. ($C_{27}H_{41}N_3O_3S_2\cdot 3.75CF_3CO_2H$) C, H, N.

**2(*S*)-(2(*S*)-{[2(*S*)-(3-Mercaptopropionylamino)-3(*S*)-methylpentyl]naphthalen-1-ylmethylamino}acetylamino)-4-methylsulfanylbutyric Acid (24).** The amine hydrochloride (0.200 g, 0.376 mmol) from the deprotection of compound **8** described above was dissolved in DMF (5 mL) and treated with *S*-triphenylmethylpropionic acid (0.157 g, 0.451 mmol), EDC (0.086 g, 0.451 mmol), and HOBT (0.061 g, 0.451 mmol). The pH was adjusted to 8 with $Et_3N$ (0.180 mL, 1.3 mmol) and the reaction mixture stirred for 48 h at ambient temperature. Standard workup followed by flash chromatography ($SiO_2$, $CH_2Cl_2$:$CH_3OH$, 99:1 to 95:5) gave 0.230 g (77%) of the fully protected **24**. Following the procedures described above, basic hydrolysis followed by detritylation (TFA/triethylsilane) gave compound **24** in 48% overall yield: $^1$H NMR ($CD_3OD$) $\delta$ 8.36 (d, 1H, $J$ = 9 Hz), 7.94−8.05 (m, 2H), 7.48−7.79 (m, 4H), 4.41 4.9 (m, 2H), 3.71−4.2 (m, 3H), 3.4−3.7 (m, 1H), 3.05−3.3 (m, 1H), 2.1−2.9 (m, 6H), 2.02 (s, 3H), 1.75−2.1 (m, 3H), 1.3−1.65 (m, 2H), 1.05−1.3 (m, 1H), 0.7−1.0 (m, 6H). Anal. ($C_{27}H_{39}N_3O_4S_2\cdot CF_3CO_2H\cdot .75H_2O$) C, H, N.

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