Hydroxamic Acid-Based Bisubstrate Analog Inhibitors of Ras Farnesyl Protein Transferase

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The rational design, synthesis, and activity of novel, hydroxamic acid-based, collective bisubstrate analog inhibitors of farnesyl protein transferase (FPT) is described. This class of compounds differ structurally from the conventional FPT inhibitors by being non-sulfhydryl and by being bisubstrate based rather than peptide or FPP derived inhibitors. Whereas replacement of the sulfhydryl group of tetrapeptide CVLS ($I_{50} = 1\ \mu$M) by an *N*-methylhydroxamic acid had a deleterious effect (**10**, $I_{50} > 360\ \mu$M), moderate inhibition was realized with **16** ($I_{50} = 42.5\ \mu$M), a bisubstrate analog involving anchorage of farnesyl and tripeptide groups by a hydroxamic acid-embedded linker. Starting from **16**, a 1 order of magnitude improvement in *in vitro* potency was obtained by optimization of the linker (**20**, $I_{50} = 4.35\ \mu$M). An additional 13-fold enhancement was achieved by substituting the tripeptide moiety VLS in **20** by VVM (**23**, $I_{50} = 0.33\ \mu$M). The dependence of these inhibitors on their peptide and farnesyl subunits is suggestive of their bisubstrate nature. Compound **23** ($I_{50} = 0.33\ \mu$M) is 2 orders of magnitude better in activity compared to the initial lead **16** ($I_{50} = 42.5\ \mu$M) and is effective in blocking prenylation of protein in whole cells including p21^{ras}.

Introduction

Uncontrolled cellular proliferation and differentiation is the primary mechanism for cancer. This event has now been recognized to be triggered by two main pathways, namely, activation of cellular protooncogenes and/or inactivation of tumor-suppressor genes.[1] The most common and potent members in these two categories are ras oncogenes[2] and p53 tumor supressor genes,[3] respectively, as evident by their frequent detection in a wide variety of human tumors. Intervention of the biological pathway leading to the oncogenic activity of the corresponding oncoproteins forms the basis for rational design of novel and specific "anti-cancer" agents. To date, blockade or attenuation of the ras pathway has received most attention from the medicinal community in their search for antitumor drugs.[4] A prerequisite to the transforming activity of the cytosolic ras proteins is their localization to the plasma membrane, an event that is carried out by a well-defined sequence of post-translational modifications.[5] The first and obligatory step in this cascade is farnesylation of a cysteine residue of the CAAX motif present at the C-terminal of ras.[6] This consensus sequence wherein C is cysteine, A is an aliphatic amino acid, and X is preferably serine or methionine is present in various proteins subject to post-translational prenylation.[7] Following prenylation, the protein is acted upon by a protease which cleaves the carboxyl terminal tripeptide fragment AAX and forms the protein bearing an S-farnesylated cysteine at the C-terminus.[8] Carboxymethylation of the free carboxyl group of cysteine then completes the conversion of pro-p21^{ras} to the hydrophobic c-p21^{ras}.[9] The protein can now become anchored to the membrane and function as a transforming, mature protein.[10] In some but not all ras proteins, further modification occurs in the form of palmitoylation of one of the upstream cysteine residues.[11]

One approach for blocking the oncogenic ras activity would be to use specific inhibitors to interrupt the activity of any one or more of the enzymes catalyzing these post-translational modifications.[4] However, farnesylation is the only true mandatory step in this cascade, since proteolysis and carboxymethylation have been shown to be nonobligatory for ras cell transforming activity.[12] Not surprisingly, most attention has been focused toward the design and synthesis of inhibitors of farnesyl protein transferase (FPT), the enzyme catalyzing farnesylation of p21^{ras}.[13] Either of the two reacting substrates, namely, the ras protein or the prenyl group donor farnesyl pyrophosphate (FPP), can form the basis for design of such inhibitors.[14] Thus, examples of both peptide-[15] and FPP-based[16] inhibitors of FPT have been reported. The CAAX tetrapeptide sequence renders enough specifity for recognition by the enzyme, and various peptidomimetic variants of this motif have recently emerged as very novel and potent inhibitors of FPT.[17] With the exception of the recently reported imidazole zinc chelator-based compounds, a mandatory feature of almost all peptide-based inhibitors with good potency has been the presence of a cysteine-like free mercaptan group.[15,17] FPP analogs bear the advantage of being small sized and non-peptidic, but face the challenge of reducing the overall charge of pyrophosphate surrogates in order to confer adequate cell permeability to such molecules.[16] Recently, we reported on a third class of FPT inhibitors which differ from the conventional inhibitors in two important aspects.[18] First, these are collective bisubstrate analog inhibitors, and second, the sulfhydryl group has been replaced by a phosphinyl or carboxylic acid pharmacophore. Besides imparting structural novelty, these features may display a different degree of selectivity and specificity profile from the conventional CAAX- or FPP-based inhibitors in the intervention of these ubiquitous

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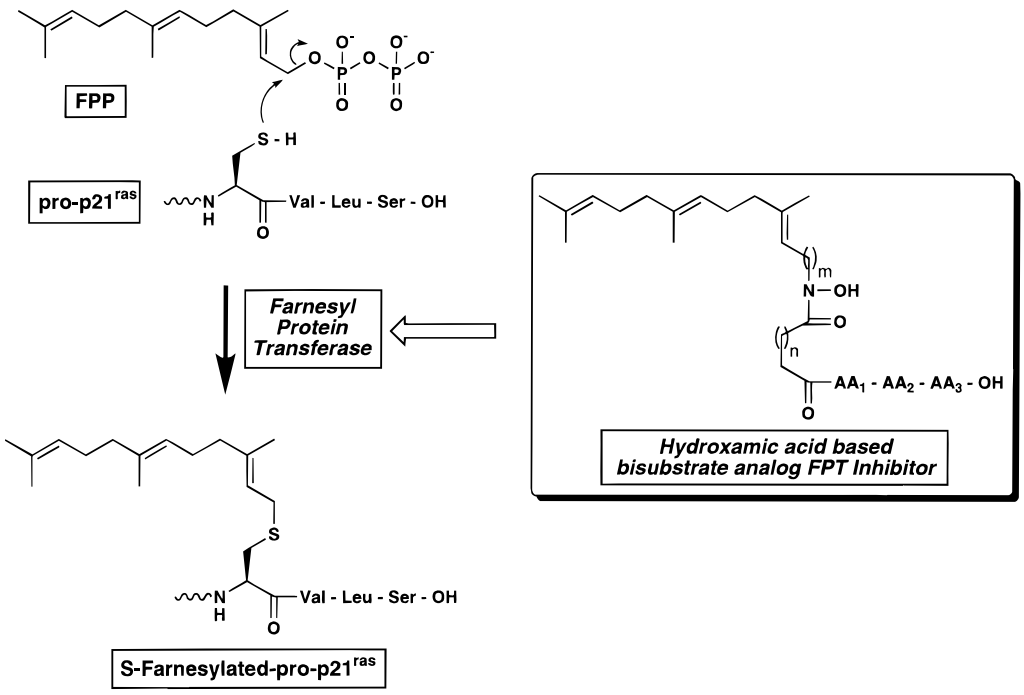


Figure 1.

post-translational pathways.[19] Non-sulfhydryl inhibitors would also bypass toxicological and metabolic issues that may be specifically associated with a mercaptan moiety.[20] In this study, we discuss our results on a new class of hydroxamic acid pharmacophore-based, non-sulfhydryl bisubstrate analog inhibitors of FPT.

Inhibitor Design. The functional equivalence of hydroxamate and mercaptan moieties in terms of metal chelation is well precedented, especially in the area of metalloproteases.[21] Thus, the hydroxamic group is expected to benefit from ionic and/or metal chelation interactions prevailing at the active site of FPT during the farnesylation reaction. A key feature in bisubstrate design is the assembly and interconnection of critical binding components of both the reacting partners (FPP and *ras*) in a chemically and biologically stable form. An adequate degree of information about the enzyme FPT and its catalytic machinery is reported in the literature,[16a,22] and this can serve as a guideline in this design process. Thus, the farnesyl group of FPP and the tripeptide groups (AAX) of the C-terminal CAAX motif are anchored together via a hydroxamic acid-bearing linker (Figure 1). Since an N-terminal amino group of simple CAAX type tetrapeptides is not a critical requirement for activity, it was deleted in the bisustrate inhibitor design.[20] By introducing the hydroxamate group as a linear component of the linker instead of a branched functional group, the issue of additional chirality in that region of the molecule is also avoided. These features led to substantial simplicity in the syntheses of these molecules.

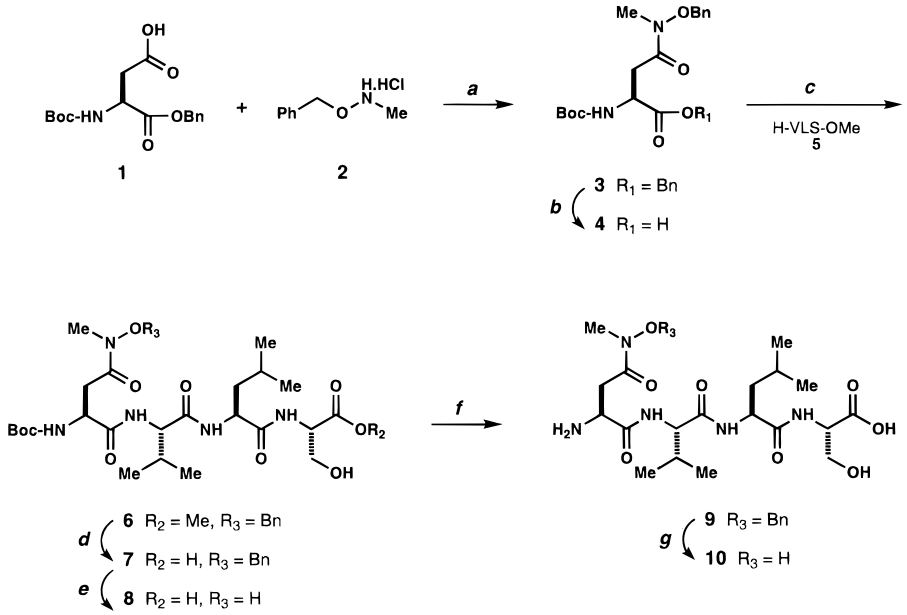
Chemistry

A successful preparation of the simple *N*-methyl-substituted hydroxamic acids **7**−**10** is outlined in Scheme 1.[23]

Thus, *N*-methylbenzyloxyamine **2** was prepared according to literature procedure[24] and coupled with aspartic acid derivative **1** to afford intermediate **3** (95%). Hydrolysis of **3** (90%) followed by coupling with tripeptide H-Val-Leu-Ser-OMe **5** yielded tetrapeptide **6** (68%), which contained the entire framework of the final target molecules. Base hydrolysis with NaOH gave 72% of the desired compound **7**, but it was also accompanied by 20% of the free aspartic acid derivative resulting from cleavage of hydroxamic functionality from **7**. Additionally, **7** was also accompanied by a substantial amount of an epimeric contaminant (ca. 20%, most probably at the serine center) as revealed by HPLC. This prompted us to try out milder, epimerization-free conditions recently highlighted in the literature.[25] Indeed, when hydrolysis was performed with Na_2CO_3, production of aspartic acid byproduct was not observed, and the chirality of desired **7** was essentially intact (95%). Hydrogenolysis of **7** gave **8** (97%), the Boc-protected tetrapeptide with an *N*-methyl substituent on the hydroxamic acid side chain. Alternatively, the sodium salt of **7** was converted to the free acid and then treated with anhydrous HCl to to yield **9** (75%), which was hydrogenolyzed to the hydroxamic acid **10** (95%).

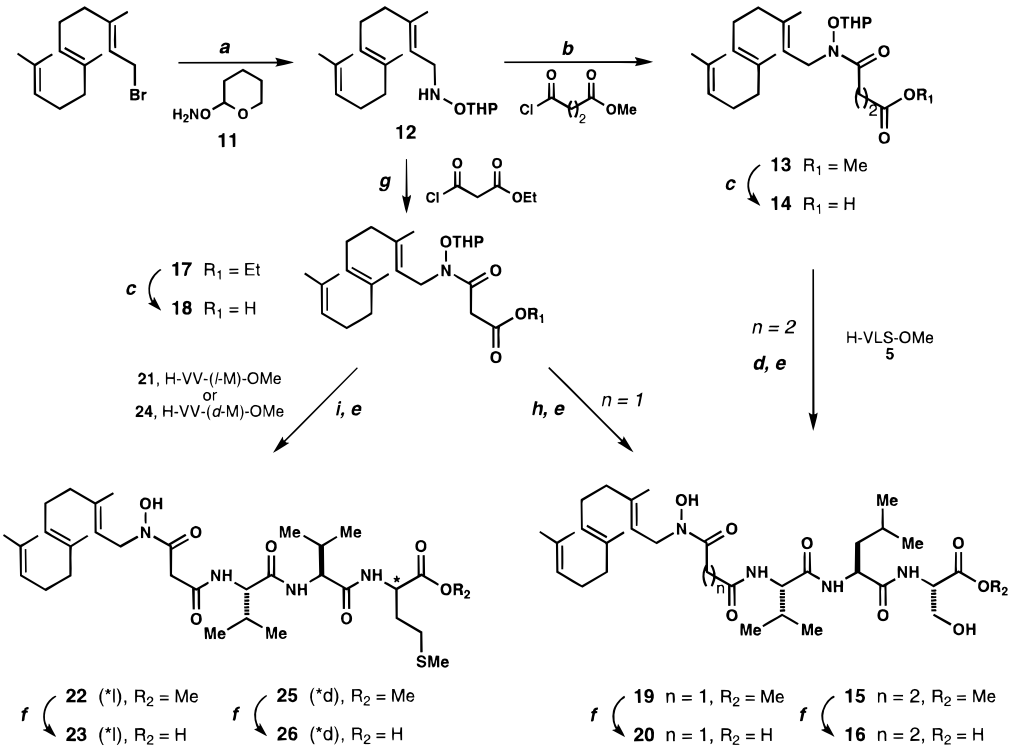
Preparation of the hydroxamic bisubstrate analogs is outlined in Scheme 2 and commences with alkylation of farnesyl bromide with NH_2−OTHP **11** to obtain the THP-protected alkoxyamine intermediate **12** in moderate yields (49%).[26] Acylation of **12** with 3-carbomethoxypropionyl chloride was uneventful (85%). Hydrolysis of the methyl ester **13** and coupling of resulting acid **14** with **5** using EDC/HOBt[27] gave the fully assembled bisubstrate intermediate in 58% overall yield for two steps. Removal of the THP protecting group in the presence of a farnesyl chain was a questionable step in this synthetic sequence. Fortunately it was removed with *p*-TsOH to give **15** in moderate yields (40%), which was subjected to basic hydrolysis with 1 N NaOH to provide **16** in 57% yield. Similarly, acylation of **12** with ethylmalonyl chloride gave **17** in 82% yield which was efficiently hydrolyzed to the acid **18** (78%). However, EDC/HOBt coupling of **18** with tripeptide **5** proceeded in modest yields (20%) and was accompanied by substantial amounts of unreacted **18** (30%). The carboxylic

Scheme 1[a]



[a] Reagents: (a) CDI, iPr_2NEt, 95%; (b) 1 N NaOH, MeOH, 90%; (c) EDC, HOBT, iPr_2NEt, H-VLS-OCH_3 **5**, 68%; (d) Na_2CO_3 (1.0), 2:1 MeOH/H_2O, 95%; (e) H_2, 10% Pd/C, MeOH, 97%; (f) anhydrous HCl/dioxane, EtOAc, 75%; (g) H_2, 10% Pd/C, MeOH, 95%.

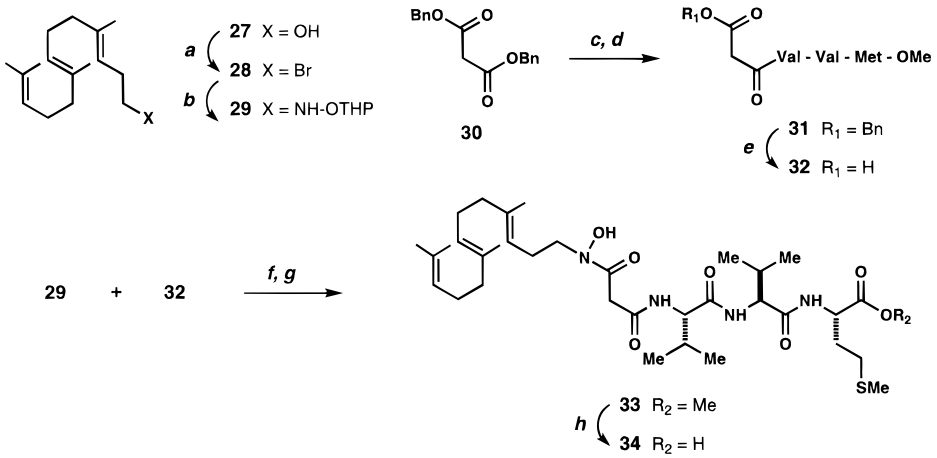
Scheme 2[a]



[a] Reagents: (a) NH_2OTHP, THF, 49%; (b) iPr_2NEt, THF, $ClCO(CH_2Me)$, 85%; (c) 1 N NaOH, MeOH, 100% for **14**, 78% for **18**; (d) EDC, HOBT, iPr_2NEt, H-VLS-OCH_3 **5**, 58%; (e) pTsOH, MeOH, 40% for **15**, 54% for **19**, 50% for **22**; (f) 1 N NaOH, MeOH, 57% for **16**, 66% for **20**, 57% for **23**, 43% for **26** from **18**; (g) iPr_2NEt, THF, $ClCOCH_2CO_2Et$, 82%; (h) EDC, HOBT, iPr_2NEt, H-VLS-OCH_3 **5**, 20%; (i) EDC, HOBT, iPr_2NEt, H-VVM-OCH_3 **21**, 22% for **22**, 57% for **25**.

acid group of a malonic monester monoacid like **18** is deactivated because of the electron-withdrawing nature of the adjacent ester moiety and can be expected to be less reactive compared to normal acids. Treatment of the coupled product with *p*-TsOH gave **19** (54%), which was hydrolyzed to the hydroxamic bisubstrate **20** (66%). Having realized inferior coupling yields in the malonyl series with EDC/HOBT, we utilized the BOP[27] reagent during preparation of the VVM analog **23**, but observed only a marginal improvement upon BOP-mediated coupling of acid **18** with tripeptide H-Val-Val-Met-OMe **21** (32%). The rest of the sequence leading to preparation of VVM analog **23** was uneventful. BOP-coupling did, however, result in substantial improvement during reaction of **18** with D-methionine bearing tripeptide H-Val-Val-(D-Met)-OMe **24** (57%). Treatment of the coupled product with *p*-TsOH gave **25** which was isolated but not purified, since in previous instances,

Scheme 3[a]



[a] Reagents: (a) (i) TsCl, pyridine, DMAP, CH_2Cl_2, 70%; (ii) LiBr, THF, 72%; (b) H_2NOTHP, K_2CO_3, DMF, 31%; (c) KOH, BzOH, 95%; (d) BOP, iPr_2NEt, HCl·H-VVM-OCH_3 **21**, CH_3CN/DMF (1:1), 79%; (e) $Pd(OH)_2$, H_2, iPr_2NEt, DMF/H_2O (4:1), 88%; (f) BOP, iPr_2NEt, CH_3CN/DMF (9:4), 64%; (g) *p*-TsOH, THF, 32%; (h) 1 N NaOH, dioxane/CH_3OH/H_2O (6:2:1), 79%.

isolation of similar intermediates (e.g. **15**, **19**, and **22**) had led to moderate overall yields due to low recovery from silica gel columns. Hydrolysis of the ester **25** with 1 N sodium hydroxide in dioxane and purification of the final product on CHP-20P gave **26** in 43% yield for two steps.

Synthesis of the homofarnesyl analog of **23** is shown in Scheme 3. Here, we opted for an alternate route that is more convergent with respect to farnesyl group replacements. This also circumvented the problem of low yields observed previously in the coupling of malonyl residues to the tripeptides. Thus, homofarnesol **27** was prepared according to literature procedure[28] and converted to its bromide by tosylation of **27** (70%) followed by treatment with LiBr (72%). Upon reaction of homofarnesyl bromide **28** with H_2NOTHP, the desired alkoxyamine **29** (31%) was accompanied by formation of the N-dialkylated side product (42%). In a separate step, dibenzyl malonate was selectively saponified to its monoester (95%)[29] and coupled with **21** to provide the tripeptide **31** (77%). It is noteworthy that hydrogenolysis of the benzyl protecting group in methionine-bearing intermediate **31** with palladium hydroxide proceeded smoothly to give the acid **32** (88%). Coupling of intermediates **29** and **32** with BOP (64%), followed by the usual deprotection with *p*-TsOH (32%) and hydrolysis (79%), gave the desired analog **34**.

Biology and Discussion

It is becoming increasingly clear that intervention of farnesylation of ras proteins may be an effective way of attenuating the cell proliferation process and that FPT inhibitors are likely to emerge as novel, rationally designed, antitumor agents.[7−18] In regular peptide-based inhibitors, the sulfhydryl group is critically required for biological activity.[15,17] We have initiated a program based on novel replacements for the thiol group with functionalities that may benefit from binding interactions normally enjoyed by a sulfhydryl moiety.[18] Ideally, such a group will not act as a substrate but rather compete with the substrate and function as a competitive inhibitor for the enzyme. An appropriately substituted hydroxamic acid group seemed to meet the above mentioned requirements. It is a good metal ion chelator, and the acidity of its NH and/or OH proton is very similar to that of an SH group ($pK_a = 10$). This led to our attempts at discovering hydroxamic acid-based bisubstrate analogs specifically designed as inhibitors of FPT.

Structure−Activity Relationship (SAR) of FPT Inhibitors. Our efforts in the project commenced with the preparation of *N*-methylhydroxamic acid analogs **8** and **10**. These baseline compounds were found to be devoid of any significant activity (I_{50} >360 μM) (Table 1), suggesting the inability of a simple hydroxamic moiety in itself to derive appreciable binding and/or the need for additional recognition sites in these molecules. A benzyl group may be able to benefit from hydrophobic interactions in the lipid binding domain of the enzyme that may normally be occupied by the prenyl moiety of FPP during the farnesylation event. A similar rational was recently applied and found to be partly successful in the area of phenol-based FPT inhibitors.[18d] This line of reasoning prompted the testing of penultimate *O*-benzylhydroxamic ether precursors **7** and **9** for inhibitory potency, but these were also found to be inactive (I_{50} >360 μM).

Table 1. Summary of *in Vitro* Activity of Inhibitors in the Ras Farnesyl Protein Transferase Assay[a]

compd no.	I_{50}[b] (μM)	compd no.	I_{50}[b] (μM)
7	>360	**16**	42.5 ± 2.5
8	>360	**20**	4.3 ± 2.7
9	>360	**23**	0.33 ± 0.17
10	>360	**26**	30.5 ± 10.5
		34	5.6 ± 2.3

[a] See the general Experimental Section for a description of the methods for determining the IC_{50} values of these inhibitors. [b] Values are the mean ± SE for two individual estimates of the I_{50}.

At this stage, attention was diverted to the preparation of bisusbtrate analog inhibitors involving the embodiment of a full farnesyl group on the hydroxamic ether functionality of these molecules. This reasoning was based to some extent on our initial success with carboxylic and phosphonic acid-based bisubstrate inhibitors of FPT.[18a−c] The first bisubstrate analog **16** was a moderately active FPT inhibitor ($I_{50} = 42$ μM), thereby validating the hypothesis of collective bisubstrate hydroxamic acid based inhibition of FPT. Next, the possibility of enhancing the *in vitro* potency of this class

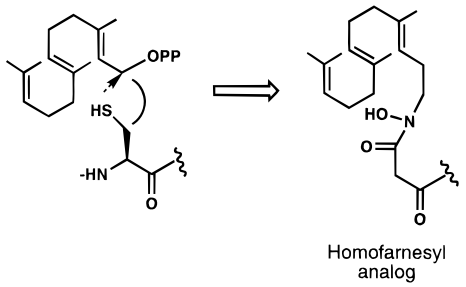


Figure 2.

of compounds was investigated. For this purpose, optimization of three structural components—the linker, the peptide, and the farnesyl group—of these compounds was undertaken. To explore the optimal length of the linker connecting the farnesyl and peptide cosubstrates, the malonyl analog **20** was prepared. A 10-fold improvement in activity over **16** was obtained by shortening the linker by one methylene unit (**20**, $I_{50} = 4.3\ \mu$M). Since **16** and **20** bear the VLS tripeptide sequence, there was good opportunity for activity enhancement by replacement with a VVM sequence. Such a substitution has led to 1 order of magnitude improvement in activity in the case of simple CAAX-based inhibitors.[15,17] It was gratifying to realize that compound **23**, the VVM analog of **20**, did register a 13-fold improvement in activity ($I_{50} = 0.33\ \mu$M). The dependency of these inhibitors on the nature of their peptide moiety was further evidenced by the 100-fold inferior activity of **26** ($I_{50} = 30\ \mu$M), the D-methionine analog of **23**. Having identified the adequate linker and tripeptide for these inhibitors as exemplified by **23**, we next worked at altering the farnesyl group in these molecules. We reasoned that the transition state of the enzymatic reaction involving attack of the cysteine thiol group on the allylic, pyrophosphate (PP)-bearing carbon atom of FPP may be mimicked more closely if the hydrophobic side chain of an inhibitor like **23** had an additional methylene unit (Figure 2).

This led to the preparation of the homofarnesyl analog **34**. Unfortunately, **34** was found to be more than 1 order of magnitude less potent ($I_{50} = 5.5\ \mu$M) than the parent molecule **23**. This suggests that the extra carbon atom is most likely forcing the farnesyl group of **34** in an orientation unfavorable for adequate binding at the active site.

A farnesyl group is a critical component for the potency of these inhibitors, as evidenced by inactivity of non-farnesylated analogs **8** and **10** ($I_{50} > 360\ \mu$M). Additionally, the SAR with respect to alterations in the tripeptide portion of these farnesylated compounds parallels the trend observed for simple CAAX peptide-based inhibitors. Such dependency and sensitivity on the farnesyl and peptide moieties of the molecule is an indirect illustration of the bisubstrate nature of this class of FPT inhibitors.

Bisubstrate analog **23**, the best FPT inhibitor for this series of compounds [I_{50}(FPT) = 0.33 μM), was evaluated against the related enzyme geranylgeranyl transferase and found to be 20-fold less active [I_{50}(GGT-1) = 6.2 μM). Additional modifications are probably required to achieve better levels of selectivity that have been typically observed with other classes of bisubstrate FPT inhibitors.[18b]

Protein Prenylation Studies. The effect of some of these inhibitors on prenylation of cellular proteins in general, and specifically on $p21^{ras}$, was examined in H-ras-transformed NIH-3T3 cells. Treatment of cells with [^{3}H]mevalonolactone results in the radiolabeling of prenylated cellular proteins. Autoradiography of SDS gels following electrophoresis of proteins from radiolabeled cells consistantly indicated prenylated protiens of 70, 50, 46, 35, 26−21, and 18 kDa with the majority of labeling at the 21−26 kDa region. Treatment with the hydroxamic bisubstate **23** ($IC_{50} = 0.33\ \mu$M on the isolated enzyme) partly prevented radiolabeling of some proteins at 10 μg/mL (14 μM) and more completely inhibited the labeling of the same proteins at 100 μg/mL (140 μM; see Figure 3A).

In contrast the less active analog **16** ($IC_{50} = 42\ \mu$M on the isolated enzyme) failed to inhibit whole cell prenylation even up to 100 μg/mL (see Figure 3B). Interestingly inhibition of the radiolabeling of cellular protiens by **23** was not apparent for the proteins in the 21−26 kDa range. These findings are consistent with the effects recently reported for a series of benzodiazepine peptidomimetic farnesyl transferase inhibitors that inhibited farnesylation of a number of higher molecular weight protiens in the whole cell but not the large number of small G protiens that are geranylgerylated.[13] To look more precisely at protien farnesylation, p21 ras was immunoprecipitated from the radiolabeled cell lysates and the level of prenylation of ras examined specifically. This indicated a reduced level of p21 ras prenylation at 100 μM (see Figure 4).

Whole-Cell Studies. The best hydroxamate bisubstrate analog **23** was evaluated in our previously described *ras* transformation inhibition (RTI) assay.[16c] Essentially, NIH-3T3 cells transfected with oncogenic H-ras DNA were treated with compound **23** 24 h after transfection, and the effect on transformation was evaluated after 14 days. A 20% inhibition was observed at 100 μM, and no inhibition could be observed at 10 μM concentration of **23**.

Conclusions

The rational design, synthesis, and activity of novel, hydroxamic acid-based bisubstrate analog inhibitors of FPT is described. This adds to the list of recently described non-sulfhydryl inhibitors for the enzyme, namely, the carboxylic acids,[18a] phosphinyl acids,[18b,c] and phenols.[18d] Whereas replacement of the sulfhydryl group of tetrapeptide CVLS ($I_{50} = 1\ \mu$M) by an *N*-methylhydroxamic acid was unsuccessful (**10**, $I_{50} > 360\ \mu$M), moderate inhibition was realized with **16** ($I_{50} = 42.5\ \mu$M), a bisubstrate analog involving anchorage of farnesyl and tripeptide groups by a hydroxamic acid embedded linker. Starting from **16**, a 1 order of magnitude improvement in *in vitro* potency was obtained by optimization of the linker (**20**, $I_{50} = 4.35\ \mu$M). An additional 13-fold enhancement was realized by substituting the tripeptide moiety VLS in **20** by VVM (**23**, $I_{50} = 0.33\ \mu$M). The dependence of these inhibitors on their peptide and farnesyl subunits is suggestive of their bisubstrate nature. Compound **23** is 2 orders of magnitude better in activity compared to the initial lead **16** and is effective in blocking prenylation of protein in whole cells including $p21^{ras}$.

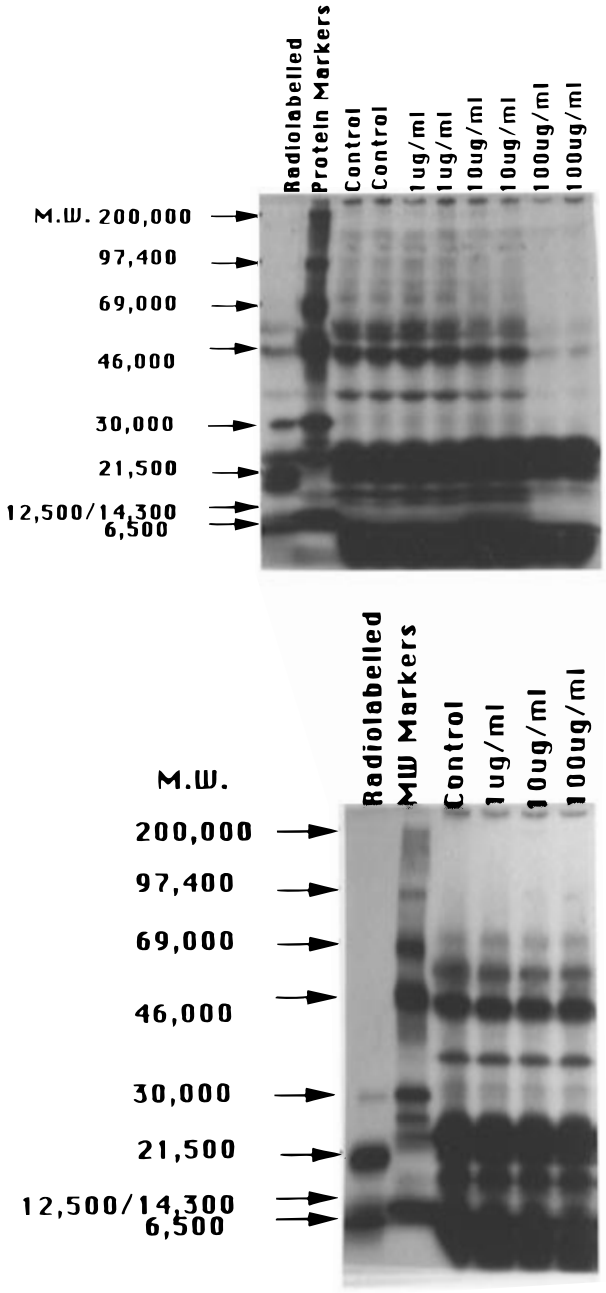


Figure 3. Autoradiographs of 10/20% SDS polyacrylamide gels of H-ras transfected NIH3T3 cell lysates following radiolabeling of prenylated proteins by treatment with [^{3}H]mevalonolactone in the presence of increasing concentrations of the potent hydroxamic bisubstrate **23**, (A, top) or the less active diastereomer bisubstrate **16** (B, bottom).. See the general Experimental Section for details of methods.

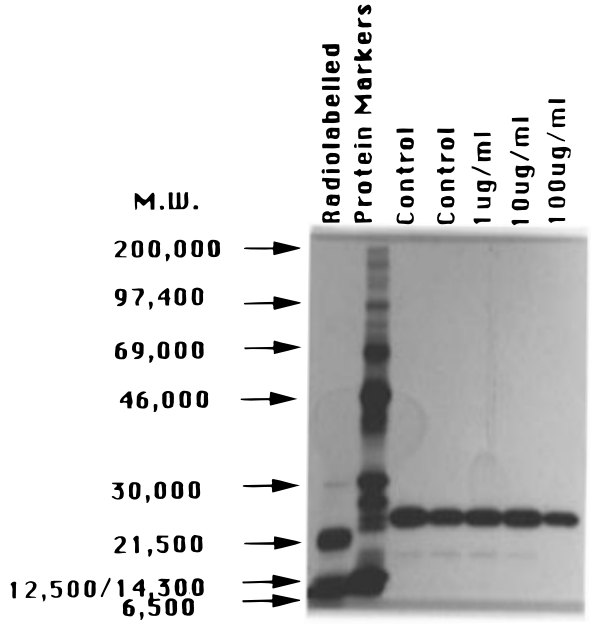


Figure 4. Autoradiograph of a 10/20% SDS−polyacrylamide gel of immunoprecipites of p21 ras from the lysates of cell shown in Figure 3A. The bisubstrate **23** has partially inhibited prenylation of p21 ras at 100 μg/mL. See the general Experimental Section for details of methods.

Experimental Section

General. All reactions were carried out under a positive pressure of dry argon, unless otherwise specified. Tetrahydrofuran (THF) and diethyl ether were distilled from sodium or potassium benzophenone ketyl prior to use. Acetonitrile, benzene, dichloromethane, diisopropylamine, hexane, methanol, pyridine, and toluene were distilled from calcium hydride prior to use.

TLC was performed using EM Science (E. Merck) 5- × 10-cm plates precoated with silica gel 60 F_{254} (0.25 mm thickness), and the spots were visualized by any of the following: UV, iodine, phosphomolymdic acid (PMA), ceric ammonium sulfate, anisaldehyde, vanillin, or Rydons stain. EM Science's silica gel 60 (230−400 mesh ASTM) was used for flash chromatography. A ratio of 25−100:1 silica gel/crude product by weight and a nitrogen pressure of 5−25 psi was normally employed for flash columns. Reverse phase chromatographic purification of final compounds was carried out using CHP20P gel, a 75−150 μm polystyrene−divinyl benzene copolymer purchased from Mitsubishi Chemical Industries. Analytical HPLC was performed using two Shimadzu LC-6A pumps with an SCL-6B system controller and a C-R4AX chromatopac, and an SPD-6AV UV−vis spectrophotometric detector. HPLC columns were commercially available from either Whatman or YMC Corp.

Melting points were determined on an electrothermal Thomas Hoover capillary melting point apparatus and are uncorrected. NMR spectra were recorded on one of the following instruments: JOEL GX-400 operating at 400 (^{1}H) or 100 MHz (^{13}C), JOEL FX-270 operating at 270 (^{1}H) or 67.8 (^{13}C) MHz, and JOEL FX-60Q operating at 15 MHz (^{13}C). Chemical shifts are reported in parts per million (ppm) downfield from tetramethylsilane (TMS), and coupling constants (J) are in hertz (Hz). IR spectra were recorded on a Mattson Sirius 100 FT-IR spectrophotometer, and the absorption maxima are reported in cm^{-1}. Mass spectra were recorded on a Finnigan MAT TSQ-4600 mass spectrometer (chemical ionization, CI) or a VG-ZAB-2F mass spectrometer (fast atom bombardment, FAB). High-resolution mass spectra (HRMS) were determined using peak-matching techniques versus PEG standards on a VG-ZAB-2F spectrometer. Optical rotations were measured using a Perkin-Elmer model 241 polarimeter and a 10 cm path length optical cell. Microanalysis results were adjusted to obtain the best fit assuming nonstoichiometric hydration.

Enzyme Inhibition Studies. Farnesyl protein transferase was isolated from pig brain as described by Manne et al.[13a] and further purified by a 30−55% ammonium sulfate precipitation and subsequent FPLC using a DE52 column with a linear 0−400 mM NaCl gradient, Hydoxylapatite column with a 10−110 mM potassium phosphate gradient, and Mono Q column with a 0−1000 mM NaCl gradient. Fractions containing farnesyl protein transferase were identified on the basis of enzyme activity using the assay described below. Active fractions were combined and dialyzed overnight into 20 mM Tris-HCl, pH 7.4 (for DE52 and Mono Q), or 10m M potassium phosphate, pH 7.6 containing 100 mM NaCl (for hydroxlapatite). All dialysis buffers contained 1 mM DTT, 5% glycerol, 0.1−0.25 mM EDTA, 0.1−0.25 mM EGTA, 1 mM benzamidine, and 10 μg/mL soybean trypsin inhibitor and for the DE52 and Mono Q dialysis buffers, 1 μg/mL leupeptin and 0.25−0.5 mM

PMSF. Enzyme activity was purified approximately 2000-fold relative to the initial crude pig brain cytosol. The PXCR expression vector containing H-ras and bacterial strain PR13Q were kindly provided by Dr. Larry Feig (Farnsworth, C. L.; Marshall, M. S.; Gibbs, J. B.; Stacey, D. W.; Feig, L. A. Preferential inhibition of the oncogenic form of Ras by mutations in the GAP binding/"effector" domain. *Cell* **1991**, *64*, 625−633). Recombinant p21 H-ras was express in the *Escherichia coli* strain PR13Q and processed as described by Farnsworth et al.[28] Following processing and ammonium sulfate precipitation, the pellet was resuspended in 50 mM Tris-HCl, pH 7.5, 20 mM NaCl, 1 mM DTT, 1 mM PMSF, 1 mM benzamidine, 10 μg/mL soybean trypsin inhibitor, 10 μM E64, and 1 μM pepstatin and dialyzed overnight. The recombinant p21 H-ras was then partially purified by FPLC using a DE52 column and a linear NaCl gradient from 20 to 320 mM. Fractions containing the p21 H-ras were visualized by coomassie blue-stained SDS−polyacrylamide gels and assayed for substrate capacity using the farnesylation assay described below. p21 H-ras with a purity of >60% was obtained with this single-column purification. Additional processing often led to a more pure protein with a reduced capacity to be farnesylated (assumed to be a consequence of the carboxyl terminus cleavage described in Farnsworth et al (Farnsworth, C. L.; Marshall, M. S.; Gibbs, J. B.; Stacey, D. W.; Feig, L. A. Preferential inhibition of the oncogenic form of Ras by mutations in the GAP binding/"effector" domain. *Cell* 1991, *64*, 625−633)). Farnesyl protein transferase assays were run in 96-well dishes in a reaction volume of 20 μL. The final reaction mixture contained 1 μM [^{3}H] FPP (NEN Dupont), 7 μM p21 H-ras, 25 mM $MgCl_2$, 10 mM DTT, 100 mM HEPES, 7.4, and serial dilutions of inhibitor usually ranging from 360 to 0.02 μM. Reactions were started by adding sufficient enzyme to produce approximately 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 by adding 90 μL of 4% SDS followed by 90 μL of 30% TCA. Plates were incubated overnight at 4 °C, and then the precipitates were transferred to Millipore multiscreen filtration 96-well plates with 0.65 PVDF membranes. Following filtration using the multiscreen vacuum manifold, the wells were washed once with 200 μL of 4% SDS/6% TCA and five times with 200 μL of 6% TCA. Following removal of the bottom seal, excess washing fluid was blotted and the plates were allowed to dry before the filters were punched into 4 mL vials using the multiscreen punch. After incubation at 60−70 °C with 300 μL of Solvable (NEN Dupont), 3 mL of Formula 989 (Dupont) scintillation fluid was added and radioactivity determined by scintillation counting. 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. Each compound was tested at least twice.

GGT-1 inhibition assays were carried out as described[16c] except that recombinant GST-CIIL protein was used as the geranylgeranyl acceptor substrate.

Cell Prenylation Assays. NIH-3T3 cells (1.5×10^6) transformed with H-ras (kindly provided by Dr. R. Weinburg, Whitehead Ins., Cambridge, MA) were plated in 6-well dishes in 3 mL of 10% DMEM media and allowed to attach for 3−4 h. The media was removed, and 0.7 mL of DMEM containing 100 μCi/mL of [^{3}H]mevalonolactone (NEN Dupont) and 25 μM lovastatin was added. Following overnight incubation the media was removed and discarded and the wells were rinsed and incubated with trypsin. The cells were harvested by tapping the plate and rinsing with fresh media. After centrifugation the pellet was washed and transferred to a microfuge tube and centrifuged again. The pellet was resuspended in 35 μL of 1x PBS and 35 μL of loading buffer (10 mL of Seprasol (Integrated Separation Systems), 100 μL of B-mercaptoethanol, and 0.2 g of SDS). Samples were boiled for 10 min, and 20 μL samples were loaded on a 10−20% SDS−PAGE (Integrated Separation Systems). Low- and mid-range radiolabeled molecular weight markers (Amersham) were also run with all gels. After electrophoresis the gels were soaked in Entensify (NEN Dupont), dried under vacuum, and exposed to film at −76 °C for 5 days. Prenylation of P21 ras in cells was examined by incubating 2×106 cells as with [^{3}H]-mevalonolactone as described above. After overnight incubation cells were washed with 1 mL of cold PBS and then incubated for 10 min with 0.7 mL of cold PBSTDS (PBS containing 1% triton-X-100, 0.5% sodium deoxycholate, 0.1% SDS, and 0.2% sodium azide). This was transferred to a microfuge tube together with a 0.8 mL of PBSTDS rinse of the well. Cells were lysed by sonication and microfuged at 15 000 rpm for 1 h at 4 °C. To 1 mL of cell lysate were added 15 μL of goat anti rat IgG/ protein-A agarose complex (Oncogene Sciences) and 10 μL of ras antibody Y13−259 (Oncogene Sciences), and the mixture was incubated at 4 °C overnight. The immunoprecipitate was collected by microfuge at 2500 rpm at 4 °C for 15 min. Following removal of the supernatant, the pellet was washed 4 times with 1 mL of cold PBSTDS. The pellet was resuspended in 30 μL of loading lysis buffer and boiled for 10 min. Samples were processed as for whole cell lysates described above.

Whole-Cell Assay. The ras transformation inhibition (RTI) assay that measures whole-cell activity and cytotoxicity of FPT inhibitors has been described before in detail by us.[16c]

Preparation of 3. CDI (1.16 g, 7.15 mmol) was added in one portion to a 0 °C solution of acid **1** (2.1 g, 6.5 mmol) in THF (20 mL). After 15 min at 0 °C and 30 min at room temperature, it was recooled to 0 °C, and amine **2** (1.128 g, 6.5 mmol) was added in one portion. iPr_2NEt (1.245 mL, 7.15 mmol) was added to this suspension, and the mixture was stirred with gradual warming to room temperature. After 4h at room temperature, the reaction mixture was concentrated by rotary evaporation to remove THF. The residue was taken up in EtOAc (200 mL) and washed sequentially with 10% aqueous HCl (2×200 mL), saturated aqueous $NaHCO_3$, and saturated aqueous NaCl (1×100 mL), dried, and concentrated to provide **3** (2.722 g). TLC $R_f = 0.28$ (2:1 hexane/EtOAc, visualized by PMA); MS $(M + H)^+$ 443; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.42 (s, 9 H, Boc), 2.88 (d, 1 H, $J = 14$), 3.14 (s, 3 H, NMe), 3.16 (d, 1 H, $J = 14$), 4.75 (s, 2 H), 5.16 (app q, 2 H, $J = 12.4$), 5.73 (d, 1 H, $J = 8$), 7.25−7.37 (m, 10 H): ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.2 (Me_3C), 34.23 (CH_2CO), 34.99 (NMe), 49.8 ((CH), 67.0 ($PhCH_2OC(O)$), 76.2 ($PhCH_2ON(Me)$), 79.7 (Me_3C), 128.0, 128.1, 128.3, 128.6, 128.8, 129.0, 129.2, 134.0, 135.5, 155.6 (Boc C=O), 171.4, 172.1. Anal. ($C_{24}H_{30}N_2O_{6)}$ C, H, N.

Preparation of 4. NaOH (1 N, 10 mL, 10 mmol) was added in one portion to a 0 °C solution of ester **3** (2.652 g, 6.0 mmol) in MeOH (24 mL). The reaction mixture was stirred with gradual warming to room temperature, and TLC revealed total disappearance of starting material after 2 h. The reaction mixture was concentrated on the rotary evaporator to remove MeOH. The residue was suspended in water (25 mL) and extracted with hexane (50 mL) and ethyl acetate (2×50 mL), and the organic extracts were discarded. EtOAc (50 mL) was added to the aqueous solution and acidified to pH = 2.0 with 1 N HCl, and the two layers were separated. The acidic solution was reextracted with EtOAc (2×5 mL). The combined organic extracts were dried and concentrated *in vacuo* to provide **4** as a white solid (1.894 g, 89.6%): mp 135−137 °C: TLC $R_f = 0.33$ (90:20:2.5:1.0 $CHCl_3$/MeOH/H_2O/AcOH); MS $(M + H)^+$ 353; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.45 (s, 9 H, Boc), 2.75 (dd, 1 H, $J = 7$, 17), 3.25 (s, 3 H, NMe), 3.24−3.33 (m, 1 H), 4.53, (br s, 1 H), 4.89 (s, 2 H), 5.76 (d, 1 H, $J = 7$), 7.39 (s, 5 H). Anal. ($C_{17}H_{24}N_2O_{6)}$ C, H, N.

Preparation of 6. iPr_2NEt (453 uL, 2.6 mmol) and HOBt (337 mg, 2.2 mmol) were sequentially added to a 0 °C solution of acid **4** (704 mg, 2.0 mmol) and HCl·H-VLS-OMe **5** (735 mg, 2.0 mmol) in THF (6 mL). After 5 min at 0 °C, EDC (403 mg, 2.1 mmol) was added followed by DMF (2 mL). The reaction mixture was stirred overnight with gradual warming to room temperature. The next day (total reaction time = 12 h), the reaction mixture was concentrated by rotary evaporation to remove THF, and the residue was taken up in EtOAc (200 mL). The organic solution was washed sequentially with 10% aqueous LiCl (2×75 mL), phosphate buffer (pH = 4, 2×75 mL), saturated aqueous $NaHCO_3$ (2×75 mL), and saturated aqueous NaCl (1×75 mL). Drying and concentration provided the crude product (1.377 g) which was purified by silica gel

chromatography, eluting with 7:3 hexane/acetone to yield pure **6** (896 mg, 67.4%). mp 173−175 °C; TLC R_f = 0.11 (2:1 hexane/acetone); MS: $(M + H)^+$ 666; ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.89−1.25 (m, 12 H), 1.44 (s, 9 H), 1.5−1.9 (m, 3 H), 2.5 (m, 1 H), 2.8−3.0 (m, 2 H), 3.17 (s, 3 H), 3.75 (s, 3 H), 3.75−4.0 (m, 2 H), 4.18−4.70 (m, 4 H), 4.87 (s, 2 H), 5.35 (d, 1 H, J = 7), 6.8 (m, 1 H), 7.22 (d, 1 H, J = 7), 7.28 (d, 1 H, J = 7), 7.39 (s, 5 H); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 17.4, 19.6, 21.5, 23.7, 25.1, 28.5, 29.2, 33.8, 34.1, 39.3, 51.5, 51.6, 52.7, 55.5, 60.4, 63.2, 76.6, 81.1, 129.1, 129.5, 129.6, 134.3, 156.1, 170.8, 171.3, 172.2, 172.5, 173.6. Anal. ($C_{32}H_{51}N_5O_{10}$) C, H, N.

Preparation of 7. Na_2CO_3 (23 mg, 0.217 mmol) was added in one portion to a 0 °C solution of ester **6** (134 mg, o.2 mmol) in MeOH/H_2O (2:1, 3 mL). The reaction mixture was stirred with gradual warming to room temperature, and TLC revealed total disappearance of starting material after 4 h. The reaction mixture was concentrated by rotary evaporation to remove MeOH, and the residue was chromatographed on a CHP20 resin eluting first with H_2O and then with 60% aqueous MeOH. The appropriate fractions were combined and concentrated, millipore-filtered, and lyophilized to obtain pure **7** (125 mg, 93%): mp 135−142 °C; TLC R_f = 0.13 (90:20:2.5:1.0 $CHCl_3$/MeOH/H_2O/AcOH, visualized by Rydons stain); $[\alpha]_D$ = −38.3° (c = 0.58, MeOH); ^{1}H NMR (400 MHz, CD_3OD) δ 0.89−1.01 (m, 12 H), 1.44 (s, 9 H), 1.65−1.73 (m, 3 H), 2.28 (m, 1 H), 2.8−3.1 (m, 2 H), 3.19 (s, 3 H), 3.72−3.85 (m, 2 H), 4.15−4.55 (m, 4 H), 4.95 (s, 2 H), 7.37−7.45 (m, 5 H). HRMS $(M + H)^+$ calculated 652.3522, found 652.3518. Anal. ($C_{31}H_{48}N_5O_{10}$-Na·1.8 H_2O) C, H, N.

Preparation of 8. 10% Pd/C (20 mg) was added in one portion to a solution of hydroxamic ether **7** (75 mg, 0.111 mmol) in MeOH (6 mL). The reaction mixture was stirred under an atmosphere of hydrogen (balloon) for 1 h at room temperature, at which stage TLC revealed completion of reaction. The reaction mixture was filtered through Celite, and the catalyst was washed with MeOH (3 × 10 mL). The combined filtrate was concentrated by rotary evaporation, and the residue was dissolved in water (3 mL), millipore-filtered, and lyophilized to obtain pure **8** (63 mg, 97%): mp 180−185 °C; TLC R_f = 0.63 (4:1:1 BuOH/AcOH/H_2O, visualized by Rydons stain); $[\alpha]_D$ = −43.3° (c = 0.45, MeOH); ^{1}H NMR (400 MHz, CD_3OD) δ 0.85−1.05 (m, 12 H), 1.44 (s, 9 H), 1.6−1.8 (m, 2 H), 2.15−2.30 (m, 1 H), 2.85−3.02 (m, 2 H), 3.17 (s, 3 H), 3.79 (m, 2 H), 4.15−4.30 (m, 2 H), 4.37−4.55 (m, 2 H); HRMS calculated for $(M + Na)^+$ calculated 584.2907, found 584.2881. Anal. ($C_{24}H_{43}N_5O_{10}Na$·1.2H_2O, 1.0 MeOH) C, H, N.

Preparation of 9. Anhydrous HCl in dioxane (Aldrich, 4 M, 0.25 mL, 1mmol) was added to a 0 °C solution of **7** (116 mg, 0.178 mmol) in EtOAc (1 mL). After 15 min at 0 °C and 3 h at room temperature, the reaction was judged to be complete by TLC. It was concentrated by rotary evaporation to remove the solvents, and the residue was triturated with EtOAc. The precipitated solid was washed with Et_2O and dried *in vacuo* to give **9** (82 mg, 75.4%): TLC R_f = 0.39 (4:1:1 BuOH/AcOH/H_2O, visualized by Rydons stain); MS $(M + H)^+$ 552; $[\alpha]_D$ = −26.3° (c = 0.65, MeOH); ^{1}H NMR (400 MHz, CD_3-OD) δ 0.88 (d, 3 H, J = 5.8), 0.93 (d, 3 H, J = 5.8), 0.98 (d, 3 H, J = 7.0), 1.02 (d, 3 H, J = 6.5), 1.5−1.8 (m, 2 H), 2.10−2.28 (m, 1 H), 2.95−3.4 (m, 2 H), 3.25 (s, 3 H), 3.75−4.0 (m, 2 H), 4.15−4.60 (m, 4 H), 4.95 (s, 2 H), 7.41 (m, 5 H). Anal. ($C_{26}H_{42}N_5O_8Cl$·0.8H_2O, 0.1 dioxane) C, H, N.

Preparation of 10. 10% Pd/C (25 mg) was added in one portion to a solution of hydroxamic ether **9** (176 mg, 0.3 mmol) in MeOH (3 mL). The reaction mixture was stirred under an atmosphere of hydrogen (balloon) for 3 h at room temperature, at which stage TLC revealed completion of reaction. It was filtered through Celite, and the catalyst was washed with MeOH (3 × 10 mL). The combined filtrate was concentrated by rotary evaporation to remove MeOH, and the residue was chromatographed on a CHP20 resin by stepwise gradient elution with 0%, 25%, and 60% aqueous MeOH. The appropriate fractions were combined and concentrated, millipore-filtered, and lyophilized to obtain pure **10** (138 mg, 95%); mp 175−190 °C; TLC R_f = 0.15 (4:1:1 BuOH/AcOH/H_2O, visualized by Rydons stain); ^{1}H NMR (400 MHz, CD_3OD) δ 0.98 (d, 3 H, J = 6.5), 1.02 (d, 3 H, J = 6.5), 1.06 (d, 3 H, J = 6.0), 1.08 (d, 3 H, J = 6.0), 1.68−1.85 (m, 2 H), 2.25 (m, 1 H), 3.22 (m, 2 H), 3.30 (s, 3 H), 3.85−4.05 (m, 2 H), 4.25−4.60 (m, 4 H), 8.05 (d, 1 H, J = 7), 8.37 (d, 1 H, J = 8). HRMS $(M + H)^+$ calculated 462.2564, found 462.2557. Anal. ($C_{19}H_{35}N_5O_8$·1.2H_2O, 1.0 MeOH) C, H, N.

Preparation of 11. To a solution of *N*-hydroxyphthalimide (20 g, 0.2123 mol, 1 equiv) in THF (350 mL) were added dihyropyran (17.0 mL, 0.187 mol, 1 equiv) and *p*-TsOH·H_2O monohydrate (400 mg) under N_2. Additional dihydropyran (5.6 mL, 0.062 mol, 0.3 equiv) was added after 8 and 24 h. The reaction mixture was filtered and concentrated using a Vigreux column to 150 mL. Then dihydropyran (11.2 mL, 0.125 mol, 0.6 equiv) and *p*-toluenesulfonic acid monohydrate (800 mg) were added. After being stirred for 16 h, the reaction mixture was concentrated *in vacuo*. The residue was dissolved in ethyl acetate (400 mL) and washed with saturated $NaHCO_3$ (3 × 150 mL), dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was triturated with hexane (3 × 200 mL) to give *O*-tetrahydropyranylhydroxyphthalimide (23.9 g, 78%): mp >210 °C; TLC R_f = 0.81 (3:7 hexane/EtOAc, visualized by UV, 254 mm); MS $(M + H)^+$ 248; IR (KBr) 1788, 1738 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.70−2.22 (m, 6 H), 3.32 (m, 1 H), 4.52 (m, 1 H), 5.42 (m, 1 H), 7.71−7.84 (m, 4 H). ^{13}C NMR MHz ($CDCl_3$) δ 18.2, 25.5, 28.3, 62.9, 103.7, 124.0, 129.8, 134.9, 163.0. To a solution of this intermediate (23.64 g, 0.095 mol, 1 equiv) in toluene (60 mL) was added methylhydrazine (5.59 mL, 0.105 mol, 1.1 equiv). After being heated at 80 °C for 1 h, the solution was cooled and the solvent removed *in vacuo*. The residue was triturated with ether (20 mL) and filtered. The solid was dissolved in hexane, treated with charcoal, filtered through Celite, and concentrated to 50 mL. The crystals were filtered and dried to give **11** (5.542 g, 50%): mp 33−38 °C; TLC R_f = 0.67 (1:4 hexane/EtOAc, visualized by ceric ammonium sulfate); MS $(M + H)^+$ 118; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.53−1.78 (m, 6 H), 3.57 (m, 1 H), 3.91 (m, 1 H), 4.71 (m, 1 H), 5.49 (br s, 2 H); ^{13}C 100 MHz ($CDCl_3$) δ 19.6, 25.3, 28.8, 62.5, 102.5. Anal. ($C_5H_{11}NO_2$) C, H, N.

Preparation of 12. To a solution of NH_2OTHP (3.28 g, 28 mmol, 1.5 equiv) in dimethylformamide (50 mL) at 0 °C under N_2 was added farnesyl bromide **11** (5.1 mL, 18.7 mmol, 1.0 equiv) in dimethylformamide (15 mL) and potassium carbonate (10.3 g, 75 mmol, 4 equiv). After being stirred for 20 h at room temperature, the reaction mixture was filtered, and the precipitate was washed with ethyl acetate (30 mL). The organic layers were concentrated *in vacuo* to 25 mL and then diluted with ethyl acetate (70 mL) and washed with 10% lithium chloride (3 × 30 mL). The organic layer was dried over sodium sulfate, filtered, and concentrated *in vacuo*. The residue was purified on Merck silica gel (400 mL), eluting with hexane/ethyl acetate (6:1) to give dialkylated material [2.32 g, 24%, R_f = 0.55 (1:1 hexane/EtOAc)] and **12** [2.93 g, 47%, R_f = 0.93 (1:1 hexane/EtOAc]: MS: $(M + H)^+$ 526; ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.59 (s, 6 H), 1.67 (s, 6 H), 1.54−1.74 (m, 6 H), 2.02 (br m, 8 H), 3.60 (m, 3 H), 3.93 (m, 1 H), 4.81−5.30 (m, 4 H); ^{13}C NMR (68 MHz, $CDCl_3$) δ 16.0, 16.4, 17.8, 20.2, 25.4, 25.7, 26.4, 26.7, 29.3, 50.0, 63.1, 101.4, 118.9, 123.7, 123.9, 124.4, 131.2, 135.2, 140.2.

Preparation of 13. To a solution of **12** (385 mg, 1.2 mmol, 1 equiv) and DIPEA (730 mL, 4.2 mmol, 3.5 equiv) in THF (2 mL) was added 3-carbomethoxypropionyl chloride (295 mL, 2.4 mmol, 2.0 equiv) at 0 °C. After the reaction mixture was stirred for 0.5 h at 0 °C and 2 h at room temperature, it was diluted with ethyl acetate (50 mL) and washed with saturated sodium bicarbonate. The aqueous layer was reextracted with ethyl acetate (3 × 50 mL). The organic extracts were combined, dried over sodium sulfate, filtered, and concentrated *in vacuo*. The residue was purified on Merck silica gel (100 mL) eluting with hexane/acetone (20:1) to give **13** (435 mg, 85%): TLC R_f = 0.6 (4:1 hexane/acetone visualized by ceric ammonium sulfate); IR (CH_2Cl_2 film) 1742, 1670, 1437 cm^{-1}; HRMS $(M + H)^+$ calculated 436.3063, found 436.3072 for $C_{25}H_{42}O_5N$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.59 (s, 6 H), 1.67 (s, 3 H), 1.68 (s, 3 H), 1.60−1.83 (m, 6 H), 1.94−2.17 (m, 8 H), 2.54−2.88 (m, 4 H), 3.60 (m, 1 H), 3.67 (s, 3 H), 3.99 (m, 1 H), 4.22 (dd, 1 H, J = 6.84, J = 15.81), 4.45 (dd, 1 H, J = 6.24, 15.81), 4.95−5.30 (m, 4 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.0,

16.4, 17.7, 19.8, 25.0, 25.7, 26.4, 26.5, 26.8, 28.1, 28.6, 28.9, 29.2, 39.6, 39.7, 46.8, 51.6, 63.7, 104.5, 118.9, 123.9, 124.4, 131.1, 135.2, 139.2, 139.4, 173.4, 173.7.

Preparation of 14. To a solution of **13** (435 mg, 1.0 mmol, 1 equiv) in methanol/water (1:1, 10 mL) was added 1 N NaOH (1.4 mL, 1.4 mmol, 1.4 equiv) at 0 °C. After 2 h at room temperature, additional 1 N NaOH (0.2 mL, 0.2 mmol, 0.2 equiv) was added since the TLC (hexane/acetone, 4:1) indicated that some unreacted starting material remained. After an additional 2 h, the reaction was judged to be complete by TLC. Methanol was removed *in vacuo*, and the aqueous layer was extracted with ethyl acetate (15 mL). The organic layer was discarded, and the aqueous solution was acidified with 1 N HCl (pH = 3) in the presence on ethyl acetate (50 mL). After the two layers were separated, the aqueous solution was reextracted with ethyl acetate (2 × 20 mL). The combined organic layers were dried over sodium sulfate, filtered, and concentrated *in vacuo* to give **14** (480 mg, 100%): TLC R_f = 0.0 (4:1 hexane/EtOAc visualized by ceric ammonium sulfate); MS $(M + H)^+$ 422; HRMS $(M + H)^+$ calculated 422.2906, found 422.2888 for $C_{24}H_{40}O_5N$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.60 (s, 6 H), 1.67 (s, 3 H), 1.69 (s, 3 H), 1.60−1.81 (m, 6 H), 1.94−2.10 (m, 8 H), 2.58−2.89 (m, 4 H), 3.59 (m, 1 H), 4.0 (m, 1 H), 4.24 (dd, 1 H, J = 6.84, 15.81), 4.45 (dd, 1 H, J = 6.42, 15.81), 5.00−5.30 (m, 4 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.0, 16.4, 17.7, 19.8, 24.9, 25.7, 26.4, 26.8, 28.0, 28.8, 29.1, 39.6, 39.7, 46.8, 63.7, 104.4, 118.6, 119.4, 123.9, 124.3, 131.2, 135.2, 135.5, 139.5, 139.6, 173.9, 177.9.

Preparation of 15. To a solution of **14** (480 mg, 1.0 mmol, 1 equiv) in THF (50 mL) were added EDC (219 mg, 1.14 mmol, 1.14 equiv) and HOBT (174 mg, 1.14 mmol, 1.14 equiv). After the mixture was stirred for 30 min, HCl-H-VLS-OCH_3 (419 mg, 1.14 mmol, 1.14 equiv) and DIPEA (198 mL, 1.14 mmol, 1.14 equiv) were added. After the mixture was stirred for 24 h at room temperature, the solvents were removed and the residue was dissolved in ethyl acetate (50 mL). The organic layer was washed sequentially with saturated sodium bicarbonate (50 mL), 10% sodium bisulfate (50 mL) and saturated sodium chloride (3 × 50 mL). The organic layer was dried over sodium sulfate, filtered, and concentrated *in vacuo*. The residue was purified on Merck silica gel (100 mL), eluting with hexane/ethyl acetate (1:3 to 0:100) to afford the coupled intermediate (427 mg, 58%): TLC R_f = 0.5 (EtOAc, visualized by ceric ammonium sulfate); MS $(M + H)^+$ 735; ^{1}H NMR (270 MHz ($CDCl_3$) δ 0.89, 0.93, 0.98, 1.04 (d,d,d,d, 12 H, J = 6.45, 5.86, 7.03, 7.03), 1.60 (s, 6 H), 1.66 (s, 3 H), 1.67 (s, 3 H), 1.60−1.90 (m, 9 H), 2.04 (m, 9 H), 2.45−2.94 (m, 4 H), 3.57 (m, 1 H), 3.72 (s, 3 H), 3.90−4.88 (m, 9 H), 5.09−5.22 (m, 3 H), 6.81 (m, 1 H), 7.21−7.68 (m, 2 H); ^{13}C 68MHz ($CDCl_3$) δ 16.0, 16.4, 16.5, 17.2, 17.7, 19.5, 19.8, 20.2, 21.0, 23.4, 25.7, 26.4, 26.8, 28.5, 28.8, 28.9, 29.1, 30.7, 31.0, 38.8, 39.0, 39.5, 39.7, 46.8, 51.3, 51.4, 52.3, 55.3, 55.5, 60.4, 63.0, 63.1, 63.9, 64.4, 103.7, 104.7, 118.1, 118.2, 123.7, 124.3, 131.3, 135.3, 139.7, 139.9, 170,4, 170.6, 171.1, 172.3, 172.4, 174.4, 175.6.

To a solution of above intermediate (377 mg, 0.514 mmol, 1 equiv) in methanol (4 mL) under N_2 was added *p*-toluenesulfonic acid monohydrate (107 mg, 0.565 mmol, 1.1 equiv). After the mixture was stirred 4 h at room temperature, the solvents were removed, and the residue was dissolved in ethyl acetate (40 mL). The organic layer was washed sequentially with 10% sodium bicarbonate (40 mL) and saturated sodium chloride (20 mL), dried over sodium sulfate, filtered, and concentrated *in vacuo*. The residue was purified on Merck silica gel (50 mL) eluting with hexane/acetone (4:1 to 1:1) to afford **15** (121 mg, 40%): TLC R_f = 0.63 (hexane/acetone, 1:1, visualized by ceric ammonium sulfate); MS $(M + H)^+$ 651; HRMS $(M + H)^+$ calculated 651.4333, found 651.4347 for $C_{34}H_{59}O_8N_4$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.95 (m, 12 H), 1.60 (s, 6 H), 1.68 (s, 6 H), 1.60−1.68 (m, 3 H), 2.05 (br m, 9 H), 2.52−2.95 (m, 4 H), 3.75 (s, 3 H), 3.90−4.75 (m, 7 H), 5.02−5.31 (m, 3 H), 7.32−8.10 (m, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.0, 16.4, 17.7, 18.2, 19.2, 19.3, 19.4, 21.9, 22.0, 22.7, 22.9, 24.8, 25.7, 26.5, 26.7, 28.1, 29.3, 30.2, 305, 30,7, 30.8, 39.6, 39.7, 40.2, 45.9, 51.6, 51.8, 51.9, 52.6, 55.1, 59.0, 59.5, 62.2, 62.7, 62.9, 117.2, 117.8, 123.6, 123.8, 124.3, 131.3, 135.3, 135.5, 140.6, 170.4, 170.6, 170.9, 172.0, 172.2, 172.4, 172.8, 173.7, 174.3.

Preparation of 16. To a solution of **15** (134 mg, 0.206 mmol, 1 equiv) in methanol/water (1:1, 3 mL) at 0 °C was added 1 N sodium hydroxide (289 mL, 0.289 mmol, 1.4 equiv). After the mixture was stirred at 0 °C for 2 h and room temperature for 2 h, TLC (hexane:acetone, 1:1) indicated that some **15** remained. Additional 1 N NaOH (100 mL, 100 mmol, 0.5 equiv) was added and after 1 more h TLC indicated the reaction was complete. Methanol was removed *in vacuo*, and the residue was purified on CHP-20P, eluting with water/acetonitrile (100:0 to 20:80), to afford **16** (78 mg, 57%): mp 120−121 °C; TLC R_f = 0.26 (9:1:0.05 $CHCl_3/CH_3OH$/HOAc visualized by ceric ammonium sulfate); MS $(M + Na)^+$ 659; $[\alpha]_D = -21.48°$ (c = 0.27, H_2O); ^{1}H NMR (400 MHz (D_2O) δ 1.05 (br m, 12 H), 1.60 (s, 3 H), 1.61 (s, 3 H), 1.68 (s, 3 H), 1.73 (s, 3 H), 1.60-1.73 (m, 3 H), 1.99−2.30 (9 H), 2.66−2.92 (m, 4 H), 3.93−4.56 (m, 7 H), 5.13−5.32 (m, 3 H); ^{13}C 100 MHz (D_2O) δ 17.6, 17.9, 19.1, 20.5, 22.5, 24.8, 26.3, 27.2, 28.3, 28.5, 29.9, 31.5, 32.3, 41.3, 41.5, 47.7, 53.9, 58.9, 61.6, 64.2, 119.8, 125.9, 126.3, 132.3, 136.4, 141.9, 174.9, 175.1, 175.2, 177.6, 177.8. Anal. ($C_{33}H_{55}N_4O_8Na\cdot1.25H_2O$) C, H, N.

Preparation of 17. To a solution of **12** (405 mg, 1.26 mmol, 1 equiv) in tetrahydrofuran (2 mL) were added DIPEA (657 mL, 3.78 mmol, 3 equiv) and ethylmalonyl chloride (322 mL, 2.52 mmol, 2 equiv) at 0 °C under N_2. After the mixture was stirred for 30 min at 0 °C and 2 h at room temperature, the reaction mixture was diluted with ethyl acetate (50 mL) and washed with saturated sodium bicarbonate (30 mL). The aqueous solution was reextracted with ethyl acetate (2 × 30 mL). The organic layers were combined, washed with saturated sodium chloride (10 mL), dried over sodium sulfate, filtered, and concentrated. The residue was purified on Merck silica gel (100 mL), eluting with hexane/ethyl acetate (8:1) to afford **17** (423 mg, 82%): TLC R_f = 0.4 (4:1hexane/EtOAc visualization by ceric ammonium sulfate); HRMS $(M + H)^+$ calculated 436.3063, found 436.3076 for $C_{25}H_{42}O_5N$; ^{1}H NMR (400 MHz ($CDCl_3$) δ 1.28 (t, 3 H, J = 6.84), 1.60 (s, 6 H), 1.68 (s, 3 H), 1.69 (s, 3 H), 1.57−1.76 (m, 6 H), 1.97−2.18 (m, 8 H), 3.50 (dd, 2 H, J = 5.82), 3.58 (m, 1 H), 4.0 (m, 1 H), 4.19 (q, 2 H, J = 6.84), 4.20−4.48 (m, 2 H), 4.92−5.32 (m, 4 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.1, 16.0, 17.7, 19.9, 24.8, 25.7, 26.4, 27.2, 29.1, 39.6, 39.7, 41.4, 46.4, 61.1, 63.9, 104.5, 118.4, 124.0, 124.3, 131.2, 135.3, 139.6, 167.5.

Preparation of 18. To a solution of **17** (423 mg, 1.03 mmol, 1 equiv) in ethanol/water (10 mL, 2:1) was added 1 N sodium hydroxide (1.44 mL, 1.44 mmol, 1.2 equiv). After being stirred for 2 h, the reaction mixture was concentrated to ca. 4 mL, diluted with water (20 mL), and extracted with ethyl acetate (30 mL). The organic layer was discarded, and the aqueous layer was acidified with 1 N hydrochloric acid (pH = 2) in the presence of ethyl acetate (50 mL). The layers were separated, and the aqueous solution was reextracted with ethyl acetate (3 × 50 mL). The organic extracts were combined, dried over sodium sulfate, filtered, and concentrated *in vacuo* to give **18** (316 mg, 78%): TLC R_f = 0.41 (9:1:0.05 $CHCl_3/CH_3OH$/HOAc visualized by ceric ammonium sulfate); MS $(M + H)^+$ 408; HRMS $(M + H)^+$ calculated 408.2750, found 408.2763 for $C_{23}H_{38}O_5N$; ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.59 (s, 6 H), 1.68 (s, 3 H), 1.70 (s, 3 H), 1.60−2.18 (m, 14 H), 3.42−3.95 (m, 4 H), 4.36 (d, 2 H, J = 7.01), 4.91−5.29 (m, 4 H); ^{13}C NMR (68 MHz, $CDCl_3$) δ 16.0, 16.4, 17.7, 20.2, 24.7, 25.7, 26.3, 26.7, 29.1, 45.9, 64.7, 104.2, 117.3, 123.6, 124.3, 131.3, 135.4, 140.9, 169.5, 170.7.

Preparation of 19. To a solution of **18** (316 mg, 0.78 mmol, 1 equiv) in THF (40 mL) at room temperature under N_2 was added HOBT•H_2O (119 mg, 0.78 mmol, 1 equiv) and EDC (149 mg, 0.78 mmol, 1 equiv). The reaction mixture was stirred at room temperature for 1 h after which HCl·H-VLS-OCH_3 **5** (287 mg, 0.78 mmol, 1 equiv) and DIPEA (136 μL, 0.78 mmol, 1 equiv) were added, and the reaction mixture was stirred for an additional 20 h. The solvents were removed *in vacuo*, and the residue was dissolved in ethyl acetate (50 mL) and washed sequentially with saturated sodium bicarbonate (35 mL), 10% sodium bisulfate (35 mL), and saturated sodium chloride (35

mL). The organic layer was dried over sodium sulfate, filtered, and concentrated *in vacuo*. The residue was purified on Merck silica gel (30 mL) and eluted with ethyl acetate/hexane (3:1 to 100:0) to give the coupled intermediate (113 mg, 20%): TLC $R_f = 0.45$ (EtOAc, visualized by ceric ammonium sulfate); MS $(M + H)^+$ 721; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.95 (m, 12 H), 1.60 (s, 6 H), 1.67 (s, 3 H), 1.70 (s, 3 H), 1.60−2.33 (m, 18 H), 3.55 (m, 3 H), 3.73 (s, 3 H), 3.97 (m, 2 H), 4.25−4.75 (m, 6 H), 4.91−5.27 (m, 4 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.0, 16.4, 17.6, 17.7, 19.4, 20.2, 21.3, 23.2, 24.7, 24.8, 25.7, 26.3, 26.4, 26.7, 29.1, 29.4, 29.5, 37.5, 39.5, 39.6, 39.7, 40.4, 46.0, 51.7, 52.4, 55.2, 55.3, 60.3, 60.4, 62.2, 62.6, 64.7, 67.9, 104.2, 117.5, 117.7, 117.8, 123.6, 123.7, 124.3, 131.3, 135.4, 140.5, 140.6, 140.8, 169.0, 160.2, 170.6, 171.3, 172.4, 172.5.

To a solution of of the above intermediate (113 mg, 0.157 mmol, 1 equiv) in THF (1 mL) at room temperature under N_2 was added *p*-toluenesulfonic acid monohydrate (33 mg, 0.173 mmol, 1.1 equiv). After the mixture was stirred at room temperature for 3 h, more *p*-toluenesulfonic acid monohydrate (16 mg, 0.086 mmol, 0.55 equiv) was added. After the mixture was stirred an additional 2 h, the solvents were removed *in vacuo*, and the residue was dissolved in ethyl acetate (20 mL) and washed sequentially with saturated sodium bicarbonate (10 mL) and saturated sodium chloride (10 mL). The organic solution was dried over sodium sulfate, filtered, and concentrated *in vacuo*. The residue was purified on Merck silica gel (14 mL), eluting with hexane/acetone (4:1 to 1:4) to give **19** (54 mg, 54%): TLC $R_f = 0.40$ (EtOAc, visualized by ceric ammonium sulfate); ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.80−1.05 (m, 12 H), 1.60 (s, 6 H), 1.67 (s, 3 H), 1.69 (s, 3 H), 1.60−2.30 (m, 12 H), 3.72 (s, 3 H), 3.5−4.5 (m, 9 H), 5.02−5.3 (m, 3 H), 7.60−8.00 (m, 3 H); ^{13}C NMR (68 MHz, $CDCl_3$) δ 16.0, 16.4, 17.6, 17.7, 19.3, 21.1, 23.2, 24.8, 25.7, 26.4, 26.8, 29.3, 39.7, 40.1, 41.1, 41.3, 46.0, 52.0, 52.5, 53.9, 55.1, 60.9, 62.4, 69.6, 117.2, 123.7, 124.3, 131.3, 135.4, 141..3, 168.1, 170.5, 170.8, 171.1, 171.9, 173,2

Preparation of 20. To a solution of **19** (54 mg, 0.085 mmol, 1 equiv) in 2:1 methanol/water (1 mL) at 0 °C was added 1 N sodium hydroxide (125 μL, 0.125 mmol, 1.4 equiv). After the mixture was stirred for 1 h, TLC indicated completion of the reaction. The solvents were removed, and the residue was purified on CHP-20P (10 mL) eluting with acetonitrile/water (1:9 to 8:2). Appropriate fractions were combined and lyophilized to give **20** (39 mg, 66%): mp 136−139 °C; TLC $R_f =$ 0.15 (9:1:0.05 $CHCl_3$/CH_3OH/HOAc visualized by ceric ammonium sulfate); MS $(M + H)^+$ 623; $[\alpha]_D = -19.6°$ ($c = 1.0$, H_2O); ^{1}H NMR (400 MHz, D_2O) δ 0.90−1.11 (m, 12 H), 1.58 (s, 6 H), 1.65 (s, 3 H), 1.71 (s, 3 H), 1.58−1.71 (m, 3 H), 1.96−2.04 (m, 9 H), 3.87−4.51 (m, 9 H), 5.09−5.32 (m, 3 H); ^{13}C NMR (68 MHz, D_2O) δ 1.6.7, 17.2, 18.2, 18.4, 19.8, 21.7, 23.9, 26.4, 27.5, 27.7, 30.3, 40.7, 47.2, 52.3, 58.2, 60.7, 62.4, 117.2, 125.2, 125.5, 131.3, 135.4, 141.3, 168.1, 170.5, 175.1, 176.2. HRMS $(M + Na)^+$ calculated 645.3839, found 645.3841. Anal. ($C_{32}H_{53}N_4O_8Na \cdot 0.75H_2O$) C, H, N.

Preparation of 22. To a solution of **18** (415 mg, 1.02 mmol, 1.0 equiv) in dimethylformamide (12 mL) was added H-VVM-OCH_3 **21** (368 mg, 1.02 mmol, 1.0 equiv), BOP (451 mg, 1.02 mmol, 1.0 equiv), and iPr_2NEt (522 μL, 3.06 mmol, 3.0 equiv). After the reaction mixture was stirred for 20 h at room temperature, it was concentrated *in vacuo*. The residue was purified on Merck silica gel (100 mL), eluting with hexane/ethyl acetate (1:1 to 1:2) to give the coupled product (247 mg, 32%): TLC $R_f = 0.62$ (ethyl acetate, visualized by ceric ammonium sulfate); MS $(M + H)^+$ 751; HRMS $(M + H)^+$ calculated 751.4679, found 751.4614 for $C_{39}H_{67}O_8N_4S$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.95 (m, 12 H), 1.59 (s, 6 H), 1.67 (s, 6 H), 2.0 (s, 3 H), 1.60−2.25 (m, 18 H), 2.48 (m, 2 H), 3.72 (s, 3 H), 3.42−4.00 (m, 4 H), 4.22−4.71 (m, 5 H), 4.97−5.29 (m, 4 H), 7.53 (br m, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 15.3, 16.0, 16.4, 17.7, 18.2, 19.2, 19.4, 199, 24.9, 25.7, 26.4, 26.7, 30.1, 30.4, 30.5, 30,8, 31.2, 39.6, 39.7, 40.2, 46.2, 51.5, 52.3, 58.6, 59.0, 64.1, 104.5, 118.3, 118.4, 123.8, 124.3, 131.2, 135.3, 139.8, 140.0, 167.3, 170.1, 171.4, 172.2.

To a solution of above intermediate (247 mg, 0.329 mmol, 1 equiv) in tetrahydrofuran (2 mL) was added p-toluenesulfonic acid monohydrate (81 mg, 0.428 mmol 1.3 equiv) under N_2. After the mixture was stirred for 3 h, additional *p*-toluenesulfonic acid monohydrate (42 mg, 0.214 mmol, 0.65 equiv) was added. After the mixture was stirred an additional 4 h, the solvents were removed *in vacuo* and the residue was dissolved in ethyl acetate (40 mL) and washed sequentially with 10% sodium bicarbonate (10 mL) and saturated sodium chloride (10 mL). The organic solution was dried over sodium sulfate, filtered, and concentrated *in vacuo*. The residue was purified on Merck silica gel (30 mL), eluting with hexane/acetone (2:1) to give **22** (110 mg, 50%): TLC $R_f = 0.48$ (1:1 hexane/acetone visualized by ceric ammonium sulfate); MS $(M + H)^+$ 667; HRMS $(M + H)^+$ calculated 667.4104, found 667.4082 for $C_{34}H_{59}O_7N_4S$; ^{1}H NMR (270 MHz, $CDCl_3$/CD_3OD, 10:1) δ 0.92 (m, 12 H), 1.59 (s, 6 H), 1.67 (s, 3 H), 1.68 (s, 3 H), 2.07 (s, 3 H), 1.90−2.22 (m, 12 H), 2.51 (br m, 2 H), 3.57 (br m, 2 H), 3.73 (s, 3 H), 4.20−4.65 (m, 5 H), 5.10−5.27 (m, 3 H); ^{13}C 68 MHZ ($CDCl_3$:CD_3OD 10:1) δ 15.2, 160, 16.4, 17.7, 18.4, 18.5, 19.1, 19.2, 25.7, 26.5, 26.8, 29.2, 30.0, 30.6, 31.1, 39.7, 45.8, 51.4, 51.5, 52.5, 53.8, 53.9, 58.8, 59.2, 117.4, 123.8, 124.4, 131.4, 135.4, 141.1, 168.7, 171.7, 172.3.

Preparation of 23. To a solution of **22** (110 mg, 0.165 mmol, 1 equiv) in dioxane/methanol/water (3.5 mL, 4:2:1) was added 1 N NaOH (231 μL, 0.231 mmol, 1.4 equiv). After the mixture was stirred for 6 h, the solvents were removed *in vacuo*. The residue was purified on CHP-20P (15 mL), eluting with water/acetonitrile (0:100 to 100:0) to give **23** after lyophilization (63 mg, 57%): mp 135−139 °C; TLC $R_f = 0.67$ ($CHCl_3$/CH_3OH/HOAc, 5:1:0.05, visualized by ceric ammonium sulfate); MS $(M + H)^+$ 653; $[\alpha]_D = -27.8°$ ($c = 0.78$, H_2O); ^{1}H NMR (400 MHz (CD_3OD) δ 0.96 (m, 12 H), 1.60 (s, 6 H), 1.66 (s, 3 H), 1.71 (s, 3 H), 2.05 (s, 3 H), 1.88−2.21 (m, 12 H), 2.50 (m, 2 H), 4.18−4.30 (m, 5 H), 5.10−5.32 (m, 3 H); ^{13}C NMR (68 MHz, CD_3OD) δ 15.3, 16.1, 16.5, 17..8, 18.3, 18.9, 19.9, 25.9, 27.4, 27.8, 31.1, 31.6, 24.1, 40.7, 40.8, 47.2, 48.0, 48.4, 48.6, 55.7, 60.3, 60.7, 119.3, 125.1, 125.4, 136.2, 141.4, 172.6, 173.8, 177.7. HRMS $(M + Na)^+$ calculated 675.3767, found 675.3759. Anal. ($C_{33}H_{53}N_4O_7SNa \cdot 2.65H_2O$) C, H, N.

Preparation of HCl·H-Val-Val-(D-Met)-OMe (24): HCl·H-(D-Met)-OMe. To a suspension of D-methionine (5g, 0.335 mmol, 1 equiv) in 2,2-dimethoxypropane (330 mL) was added 11.7 M hydrochloric acid (33 mL). After the mixture was stirred for 16 h, the solvents were removed *in vacuo*. The residue was dissolved in methanol (50 mL) and diluted with ether (400 mL), and the resulting precipitate was filtered and washed with ether (50 mL). The precipitate was redissolved in methanol (30 mL) and slowly precipitated with ether (200 mL). The precipitate was filtered, washed with ether (40 mL), and dried *in vacuo* to give the HCl salt of H-(D-Met)-OMe (5.44 g, 84%): mp 147−148 °C; TLC $R_f = 0.48$ (9:1:0.05 chloroform/methanol/acetic acid visualized by ceric ammonium sulfate); IR (KBr) 1748 cm^{-1}; MS $(M + H)^+$ 164; $[\alpha]_D = -23.58°$ ($c =$ 0.85, CH_3OH); ^{1}H NMR (270 MHz, CD_3OD) δ 2.21 (s, 3 H), 2.31 (m, 2 H), 2.76 (t, 2 H, $J = 7.03$), 3.95 (s, 3 H), 4.32 (t, 1 H, $J = 6.45$); ^{13}C NMR (68 MHz, CD_3OD) δ 14.9, 30.0, 30.7, 52.7, 53.7, 170.7.

Boc-Val-(D-Met)-OMe. To a suspension of D-methionine methyl ester (5 g, 25 mmol, 1 equiv), Boc-valine (5.51 g, 25 mmol, 1 equiv), and HOBT (3.88 g, 25 mmol, 1 equiv) in THF/DMF (160 mL, 3:1) was added iPr_2NEt (4.85 mL, 28 mmol, 1.1 equiv). The suspension was cooled to 0 °C, and EDC (4.87 g, 25 mmol, 1 equiv) was added. The ice bath was removed, and after being stirred for 1 h, the reaction mixture was a clear solution. The solution was stirred for 16 h, and the reaction mixture was concentrated to ~40 mL. The concentrate was diluted with ethyl acetate (200 mL) and washed with saturated sodium bicarbonate (100 mL), phosphate buffer (pH = 4, 100 mL), and 10% lithium chloride (3 × 100 mL), dried over sodium sulfate, filtered, and concentrated *in vacuo*. The residue was triturated with petroleum ether (50 mL) to give Boc-Val-(D-Met)-OMe (7.72 g, 84%): mp 94−95 °C; TLC $R_f = 0.91$ (4:1:1 butanol/acetic acid/water visualized by ceric ammonium sulfate); IR (KBr) 1750 cm^{-1}; MS $(M + H)^+$ 363; ^{1}H NMR (400 MHz ($CDCl_3$) δ 0.91 (d, 3 H, $J = 6.84$), 0.98 (d, 3 H, $J = 6.84$), 1.45 (s, 9 H), 2.01 (m, 1 H, $J = 7.26$), 2.09 (s, 3 H), 2.82 (m, 2 H), 2.49 (t, 2 H, $J = 7.26$), 3.75 (s, 3 H), 4.0 (m, 1 H), 4.71 (dd, 1 H, $J = 7.26$), 5.0 (m, 1 H), 6.81 (d, 1 H, $J = 7.69$); ^{13}C NMR

(100 MHz, $CDCl_3$) δ 15.4, 17.5, 19.4, 28.3, 29.9, 30.6, 31.5, 51.4, 52.5, 59.9, 80.0, 155.8, 171.5, 172.2. Anal. ($C_{16}H_{30}N_2O_5S$) C, H, N.

Boc-Val-Val-(D-Met)-OMe. To a solution of 1.56 M hydrochloric acid (60 mL, in acetic acid) and dimethyl sulfide (6 mL) at 0 °C under N_2 was added Boc-Val-(D-Met)-OMe (7.72 g, 21 mmol). The ice bath was removed, and after 0.5 h the reaction mixture was concentrated *in vacuo*. The residue was triturated with ether (3 × 100 mL) and petroleum ether (100 mL). The slightly tacky solid was dissolved in acetone (100 mL) and concentrated *in vacuo* to give the hydrochloride salt (5.84 g, 92%): TLC R_f = 0.38 (9:1:0.05 chloroform/methanol/acetic acid visualized by ceric ammonium sulfate); HRMS calculated 263.1429, found 263.1433 for $C_{11}H_{23}O_3N_2S$.

To a solution of Boc-Val-OH (3.35 g, 15.4 mmol, 1 equiv) and HOBT (2.35 g, 15.4 mmol 1 equiv) in THF/DMF (190 mL, 5:3) was added EDC (2.96 g, 15.4 mmol, 1 equiv) at 0 °C under N_2. The ice bath was removed, and the reaction mixture was stirred for 30 min. The reaction mixture was recooled to 0 °C, and HCl·H-Val-Val-(D-Met)-OMe prepared above (4.59 g, 15.4 mmol, 1 equiv) was added. Then the ice bath was removed, and iPr_2NEt (3.57 mL, 20.0 mmol, 1.3 equiv) was added in four portions over 4 h. After the reaction mixture was stirred for 36 h, the solvents were concentrated to ca. $^1/_3$ volume. The concentrate was diluted with ethyl acetate (250 mL) and washed with saturated sodium bicarbonate (100 mL), phosphate buffer (100 mL, pH = 4), and 10% lithium chloride (3 × 100 mL). The oragnic layer was dried over sodium sulfate, filtered, and concentrated *in vacuo*. The residue was triturated with ether (50 mL) and filtered to give Boc-Val-Val-(D-Met)-OMe (5.73 g, 82%): TLC R_f = 0.92 (butanol/acetic acid/water, 4:1:1, visualized by ceric ammonium sulfate); mp 157−158 °C; IR (KBr) 1740, 1694, 1643, 1522 cm^{-1}; MS $(M + H)^+$ 462; $[\alpha]_D = -16.25°$ (c = 0.80, CH_3OH); ^1H NMR (400 MHz, $CDCl_3$) δ 0.97 (m, 12 H), 1.45 (s, 9 H), 2.09 (s, 3 H), 2.01−2.60 (m, 6 H), 3.71 (s, 3 H), 3.91 (dd, 1 H, J = 6.0, 6.4), 4.35 (m, 1 H), 4.67 (m, 1 H), 5.18 (m, 1 H), 6.66 (m, 1 H), 7.20 (m, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 15.5, 17.3, 17.8, 19.4, 19.5, 28.3, 30.2, 31.4, 51.6, 52.3, 58.4, 60.9, 156.4, 171.1, 171.8, 172.1. Anal. ($C_{21}H_{39}N_3O_6S$) C, H, N.

HCl·H-Val-Val-(D-Met)-OMe (24). A solution of Boc-Val-Val-(D-Met)-OMe (2 g, 4.34 mmol) in 1.56 M HCl in acetic acid (20 mL) and dimethyl sulfide (2 mL) under argon was stirred for 30 min while being warmed from 0 °C to room temperature. The solvents were removed *in vacuo*, and the residue was triturated with ether (3 × 25 mL). The residue was filtered and washed with ether (2 × 20 mL) to give **24** (1.65 g, 96%): mp >210 °C; TLC R_f = 0.10 (chloroform/methanol/acetic acid, 9:1:0.05, visualized by ceric ammonium sulfate); IR (KBr) 1748, 1649, 1553 cm^{-1}; MS $(M + Na)^+$ 675; $[\alpha]_D = -24.39°$ (c = 0.52, CH_3OH); ^1H NMR (400 MHz, CD_3OD) δ 1.00 (m, 12 H), 2.07 (s, 3 H), 1.94−2.24 (m, 4 H), 2.42−2.63 (m, 2 H), 3.71 (s, 3 H), 3.80 (d, 1 H, J = 5.56), 4.26 (d, 1 H, J = 8.12), 4.54 (m, 1 H); ^{13}C NMR (100 MHz, CD_3OD) δ 15.1, 17.9, 18l.9, 19.0, 25.0, 31.2, 31.5, 31.7, 32.0, 52.7, 52.8, 59.5, 60.5, 169.4, 173.4, 173.5. Anal. ($C_{16}H_{32}N_3O_4SCl·0.23H_2O$) C, H, N.

Preparation of 25. To a solution of HCl·H-Val-Val-D-Met-OMe (**24**) (562 mg, 1.55 mmol, 1.03 equiv) in DMF (25 mL) were added sequentially **18** (614 mg, 1.50 mmol, 1 equiv) in DMF (5 mL), BOP (667 mg, 1.50 mmol, 1 equiv), and iPr_2NEt (262 mL, 1.50 mmol, 1 equiv). After being stirred for 6 h, the reaction mixture was diluted with ethyl acetate (300 mL) and washed sequentially with saturated sodium bicarbonate (150 mL), phospate buffer (pH = 4, 100 mL), and 10% lithium chloride (3 × 150 mL). The organic solution was dried over sodium sulfate, filtered, and concentrated *in vacuo*. The residue was purified on Merck silica gel (400 mL), eluting with hexane/acetone (4:1 to 3:1) to give the coupled product (639 mg, 57%): mp 147−150 °C; TLC R_f = 0.22 (hexane/acetone, 2:1 visualized by ceric ammonium sulfate); IR (KBr) 1738, 1636 cm^{-1}; MS $(M + H)^+$ 751; $[\alpha]_D = -8.99°$ (c = 1.00, CH_3OH); ^1H NMR (400 MHz, $CDCl_3$) δ 0.97 (m, 12 H), 1.60 (s, 6 H), 1.68 (s, 3 H), 1.70 (s, 3 H), 2.12 (s, 3 H), 1.60−2.6 (m, 20 H), 3.55−3.71 (m, 3 H), 3.70 (s, 3 H), 3.91−4.65 (m, 6 H), 4.90−5.30 (m, 4 H), 7.05 (m, 2 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 15.5, 16.0, 16.4, 17.3, 17.4, 17.7, 19.5, 19.6, 20.1, 24.8, 25.7, 26.4, 26.7, 29.1, 29.4, 29.5, 29.6, 29.7, 30.2, 31.4, 39.4, 39.6, 39.7, 46.0, 51.5, 51.6, 52.2, 58.5, 60.0, 60.1, 64.5, 104.3, 104.4, 117.8, 123.7, 124.3, 131.3, 135.5, 140.5, 140.6, 168.1, 168.3, 171.2, 171.3, 172.2, 172.3. Anal. ($C_{39}H_{66}N_4O_8S·0.17H_2O$) C, H, N.

To a solution of the above intermediate (445 mg, 0.593 mmol, 1 equiv) in tetrahydrofuran (6 mL) was added p-toluenesulfonic acid monohydrate (113 mg, 0.593 mmol, 1 equiv). After being stirred for 6 h, the reaction mixture was concentrated *in vacuo*, and the residue was dissolved in ethyl acetate (300 mL). The organic solution was washed with saturated sodium bicarbonate (100 mL) and saturated sodium chloride (100 mL), dried over sodium sulfate, filtered, and concentrated *in vacuo* to give **25** (386 mg, 0.593 mmol): TLC R_f = 0.47 (9:1 chloroform/methanol visualized by ceric ammonium sulfate); IR (KBr) 1742 cm^{-1}; MS $(M + H)^+$ 667; HRMS calculated 667.4104, found 667.4116 for $C_{34}H_{59}O_7N_4S$; ^1H NMR (400 MHz, $CDCl_3/CD_3OD$, 10:1) δ 0.99 (m, 12 H), 1.60 (s, 6 H), 1.67 (s, 3 H), 1.70 (s, 3 H), 2.09 (s, 3 H), 1.95−2.57 (m, 14 H), 3.55 (q, 2 H, J = 14.1), 3.71 (s, 3 H), 4.23−4.62 (m, 5 H), 5.11−5.30 (3 H); ^{13}H 100 MHz ($CDCl_3/CD_3OD$, 10:1) δ 15.23, 16.1, 16.4, 17.8, 18.1, 18.3, 19.4, 19.5, 19.6, 25.8, 26.8, 27.1, 29.5, 30.2, 30.5, 30.8, 31.2, 37.8, 40.1, 40.7, 46.4, 51.9, 52.5, 52.7, 59.3, 59.6, 60.0, 60.2, 117.8, 118.9, 124.2, 124.3, 124.7, 131.6, 135.6, 135.7, 141.2, 141.5, 167.7, 168.8, 169.8, 172.5, 172.7, 172.8.

Preparation of 26. To a solution of **25** (180 mg, 0.270 mmol, 1 equiv) in dioxane (4 mL) was added 1 N sodium hydroxide (324 mL, 0.324 mmol, 1.2 equiv). After being stirred for 6 h, the reaction mixture was cool to −40 °C for 16 h. The reaction mixture was warmed to room temperature, and water (5 mL) was added. The solution was concentrated to ∼3 mL and purified on CHP-20P (40 mL), eluting with water/acetonitrile (100:0 to 0:100) to give **26** (lyophilized, 78 mg, 43%): mp 122−130 °C; TLC R_f = 0.50 (chloroform/methanol/acetic acid, 9:1:0.05, visualized by ceric ammonium sulfate); IR (KBr) 1634 cm^{-1}; $[\alpha]_D = -27.54°$ (c = 0.43, CH_3OH); ^1H NMR (400 MHz, CD_3OD) δ 0.97 (m, 12 H), 1.60 (s, 6 H), 1.66 (s, 3 H), 1.71 (s,3 H), 2.05 (s, 3 H), 1.96−2.49 (m, 14 H), 3.35 (s, 2 H, partially exchanged with deuterium), 4.26 (m, 5 H), 5.10-5.30 (m, 3 H); ^{13}C NMR (100 MHz, CD_3OD) δ 15.5, 16.3, 16.8, 18.0 18.9, 20.1, 20.2, 26.1, 27.6, 28.0, 31.5, 31.7, 34.1, 40.9, 41.0, 47.3, 55.9, 60.8, 61.0, 119.4, 125.3, 125.6, 132.3, 136.4, 141.6, 169.2, 170.7, 172.8, 174.1, 178.2. HRMS $(M + Na)^+$ calculated 675.3767, found 675.3716. Anal. ($C_{33}H_{53}N_4O_7SNa·1.51H_2O$) C, H, N.

Preparation of 28. To a solution of homofarnesol **27** (600 mg, 2.54 mmol, 1 equiv) in dichloromethane (30 mL) was added pyridine (1.02 mL, 12.7 mmol, 5.0 equiv), DMAP (62 mg, 0.508 mmol, 0.2 equiv), and tosyl chloride (1.06 g, 5.59 mmol, 2.2 equiv) under N_2. Additional tosyl chloride (200 mg, 0.4 equiv) was added after 20 and 28 h. After the mixture was stirred a total of 44 h, the reaction was quenched with saturated aqueous sodium bicarbonate (15 mL). This was stirred vigorously for 45 min, and the layers were separated. The water layer was extracted with dichloromethane (2 × 15 mL). The organic layers were combined and washed with 5% copper sulfate (15 mL) and water (15 mL), dried over magnesium sulfate, filtered, and concentrated *in vacuo*. The residue was purified on Merck silica (100 mL), eluting with hexane/ethyl acetate (85:15) to give homofarnesyl tosylate (840 mg, 86%): TLC R_f = 0.33 (95:5 hexane/EtOAc, visualized by ceric ammonium sulfate); MS $(M + NH_4)^+$ 408; ^1H NMR (270, $CDCl_3$) δ 1.56 (s, 3 H), 1.58 (s, 3 H), 1.60 (s, 3 H), 1.68 (s, 3 H), 2.02 (br m, 8 H), 2.34 (dd, 2 H, J = 7.04), 2.44 (s, 3 H), 3.97 (d of d, 2 H, J = 7.04), 4.92−5.13 (m, 3 H), 7.33 (d, 2 H, J = 8.6), 7.79 (d, 2 H, J = 8.60); ^{13}C NMR (68 MHz ($CDCl_3$) δ 15.9, 16.1, 17.6, 21.5, 26.3, 26.6, 27.7, 39.5, 69.9, 117.3, 123.7, 124.2, 127.8, 129.7, 131.2, 133.2, 135.1, 139.3, 144.5.

To a solution of of the above tosylate intermediate (2.25 g, 57.7 mol, 1 equiv) in tetrahydrofuran (40 mL) was added lithium bromide (5.6 g, 64.5 mmol, 1.1 equiv) under N_2. After the mixture was refluxed for 4 h, saturated ammonium chloride (40 mL) was added, and the solution was extracted with ether (3 × 100 mL). The organic extracts were combined, washed with saturated sodium bicarbonate (100 mL), dried over magnesium sulfate, filtered, and concentrated *in vacuo*.

The residue was purified on Merck silica gel (300 mL) eluting with hexane to give **28** (1.24g, 72%): TLC R_f = 0.23 (95:5 hexane/EtOAc, visualized by ceric ammonium sulfate); MS (M + H)$^+$ 299; IR ($CHCl_3$ film) 1446 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.59(s, 6 H), 1.63 (s, 3 H), 1.67 (s, 3 H), 1.90−2.18 (m, 8 H), 2.55 (dd, 2 H, J = 7.62), 3.31 (dd, 2 H, J = 7.63), 5.12 (br m, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 15.9, 16.1, 17.6, 25.6, 26.3, 26.6, 31.6, 32.5, 39.5, 39.6, 120.8, 123.8, 124.3, 131.0, 135.0, 138.3. Anal. ($C_{16}H_{27}Br\cdot0.40H_2O$) C, H, N.

Preparation of 29. To a solution of homofarnesyl bromide **28** (900 mg, 3 mmol, 1 equiv) in dimethylformamide (7 mL) at 0 °C under N_2 were added NH_2OTHP (527 mg, 4.5 mmol, 1.5 equiv) and potassium carbonate (1.65 g, 12 mmol, 4 equiv). After being stirred for 4 days at room temperature, the reaction was filtered, diluted with ethyl acetate (75 mL), and washed with 10% lithium chloride (4 × 70 mL), dried over sodium sulfate, and concentrated *in vacuo*. The residue was purified on Merck silica gel (100 mL), eluting with hexane/ethyl acetate (9:1) to give unreacted **28** (382 mg, 42%) and the desired **29** (316 mg, 31%): TLC R_f = 0.36 (hexane/ethyl acetate, 4:1, visualized by ceric ammonium sulfate); MS (M + H)$^+$ 336; IR (neat) 1441 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.59 (s, 6 H), 1.63 (s, 3 H), 1.67 (s, 3 H), 1.45−2.33 (m, 16 H), 2.98 (m, 2 H), 3.56 (m, 1 H), 3.91 (m, 1 H), 4.80 (m, 1 H), 5.12 (br m, 3 H), 5.72 (m, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.01, 16.1, 17.7, 20.2, 25.4, 25.7, 26.0, 26.6, 26.8, 29.3, 39.7, 39.8, 52.1, 52.3, 63.0, 101.4, 121.2, 124.1, 124.4, 131.1, 135.0, 137.6. Anal. ($C_{21}H_{37}NO_2$) C, H, N.

Preparation of 31. KOH (6.5 g, 0.1 mol, 1 equiv) in benzyl alcohol (125 mL) was added dropwise to a solution of dibenzyl malonate (24.98 mL, 0.1 mmol, 1 equiv) in benzyl alcohol (250 mL). After being stirred for 3 h, the reaction mixture was diluted with water (500 mL) and extracted with ether (1.5 l and 2 × 200 mL). The organic extracts were discarded, and the aqueous layer was acidified with 6 N HCl (pH = 2.3) in the presence of ether (200 mL). The layers were separated, and the aqueous solution was reextracted with ether (2 × 300 mL). The organic extracts were combined, dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give the monoester monoacid (18.57 g, 95%): TLC R_f = 0.34 (4:1hexane/EtOAc visualized by ceric ammonium sulfate); MS (M + NH_4)$^+$ 212; ^{1}H NMR (270 MHz, $CDCl_3$) δ 3.47 (s, 2 H), 5.19 (s, 3 H), 7.35 (s, 5 H); ^{13}C NMR (68 MHz, $CDCl_3$) δ 40.9, 67.6, 128.4, 128.6, 128.6, 166.4, 171.8.

To a solution of H-Val-Val-Met-OMe (**21**) (3.26 g, 8.19 mmol, 1 equiv) in acetonitrile/dimethylformamide (1:1, 54 mL) at 0 °C under N_2 was added the above monoacid intermediate (1.59 g, 8.19 mmol, 1 equiv), iPr_2NEt (2.84 mL, 16.38 mmol, 2 equiv), and BOP (3.62 g, 8.19 mmol, 1 equiv). The reaction mixture was stirred for 20 h at room temperature, and the precipitate (A) was filtered (filtrate B). The precipitate A was dissolved in chloroform (80 mL) and was washed sequentially with 0.02 N hydrochloric acid (40 mL), saturated sodium bicarbonate (40 mL), and 10% lithium chloride (2 × 40 mL), dried over sodium sulfate, filtered, and concentrated *in vacuo* to give **31** (2.40 g).

The filtrate B was concentrated to 9 mL, diluted with ethyl acetate (150 mL), washed with the same solvents as above, dried over sodim sulfate, filtered, and concentrated *in vacuo*. The residue was triturated with hexane (50 mL) and ether (50 mL), filtered, and dried *in vacuo* to give additional **31** (1.1 g) which was combined with the material from above to give 3.5 g (79%) overall yield: TLC R_f = 0.6 (9:1:0.05 $CHCl_3$/MeOH/HOAc visualized by ceric ammonium sulfate); MS (M + H)$^+$ 538; IR (KBr) 1642 cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.85−0.98 (m, 12 H), 2.01 (s, 3 H), 1.95−2.15 (m, 4 H), 2.45 (m, 2 H), 3.46 (dd, 2 H, J = 16.42), 3.67 (s, 3 H), 4.42−4.75 (m, 3 H), 5.11 (s, 2 H), 7.3 (m, 5 H); ^{13}C NMR (68 MHz, $CDCl_3$) δ 15.2, 18.7, 18.9, 19.1, 30.1, 30.8, 31.9, 42.3, 51.37, 52.2, 58.4, 58.6, 66.9, 128.2, 128.5, 135.5, 165.7, 168.2, 171.5, 172.0, 172.2. Anal. ($C_{26}H_{39}N_3O_7S\cdot0.64H_2O$) C, H, N.

Preparation of 32. To a solution of **31** (3.3 g, 6.14 mmol, 1 equiv) in dimethylformamide/water (4:1, 165 mL) were added iPr_2NEt (9.61 mL, 55.3 mmol, 9 equiv) and palladium hydroxide on carbon (350 mg). The reaction mixture was stirred under an H_2 atmosphere for 16 h, filtered through Celite, and concentrated *in vacuo*. The residue was dissolved in methanol/chloroform and filtered through Celite and 0.2 mm filter paper. The material was concentrated *in vacuo* and triturated with ether to give **32** (2.4 g, 88% yield).: TLC R_f = 0.3 (9:1:0.05 $CHCl_3$/MeOH/HOAc visualized by ceric ammonium sulfate); MS (M + H)$^+$ 448; IR (KBr) 1746, 1640 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$/CD_3OD, 10:1) δ 0.93−0.97 (m, 12 H), 2.09 (s, 3 H), 1.93−2.17 (m, 4 H), 2.5 (m, 2 H), 3.37 (s, 2 H), 3.74 (s, 3 H), 4.20 (dd, 1 H, J = 8.55), 4.29 (d, 1 H J = 6.42), 4.62 (m, 1 H), 7.81 (d, 1 H, J = 8.55), 7.98 (d, 1 H, J = 7.69); ^{13}C NMR (100 MHz, $CDCl_3$/CD_3OD, 10:1) δ 15.2, 18.1, 18.3, 19.2, 19.3, 30.2, 30.7, 30.9, 31.2, 41.3, 51.6, 51.7, 52.5, 59.1, 59.3, 167.8, 170.9, 172.1, 172.2, 172.6. Anal. ($C_{19}H_{33}N_3O_7S\cdot0.14H_2O$) C, H, N.

Preparation of 33. To a solution of **29** (445 mg, 1.33 mmol, 1 equiv) in acetonitrile/dimethylformamide (9:4, 13 mL) under N_2 were added **32** (594 mg, 1.33 mmol, 1 equiv) and diisopropylethylamine (462 mL, 2.66 mmol, 2 equiv). After the solution was cooled to 0 °C, BOP (588 mg, 1.33 mmol, 1 equiv) was added. The reaction mixture was warmed to room temperature and stirred for 16 h. More acetonitrile (5 mL) was added, and the reaction mixture was stirred for 6 h. After the solvents were removed *in vacuo*, the residue was dissolved in ethyl acetate (150 mL) and washed sequentially with saturated sodium bicarbonate (75 mL) and 10% lithium chloride (4 × 75 mL). The organic layer was dried over sodium sulfate, filtered, and concentrated *in vacuo*. The residue was purified on Merck silica gel (106 mL), eluting with hexane/acetone (4:1 to 3:1) to give the coupled intermediate (655 mg, 64%): mp 151−152 °C; TLC R_f = 0.23 (2:1 hexane/acetone visualized by ceric ammonium sulfate); MS (M + H)$^+$ 765; IR 1755, 1636 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$/CD_3OD, 10:1) δ 0.96 (m, 12 H), 1.60 (s, 6 H), 1.64 (s, 3 H), 1.67 (s, 3 H), 2.07and 2.08 (s, 3 H), 1.60−2.53 (m, 22 H), 3.72 and 3.73 (s, 3 H), 3.44−4.00 (m, 6 H), 4.25−4.61 (m, 3 H), 4.98−5.09 (m, 4 H); ^{13}C NMR (100 MHz, $CDCl_3$/CD_3OD, 10:1) δ 15.2, 16.1, 17.7, 18.0, 18,4, 19.4, 20.3, 25.2, 25.8, 26.0, 26.9, 27.1, 29.5, 30.4, 30.6, 30.8, 30.9, 31.3, 40.1, 48.5, 48.7, 48.9, 49.1, 49.4, 51.8, 52.5, 59.2, 59.6, 64.6, 104.8, 105.0, 120.3, 124.4, 124.7, 131.5, 135.4, 138.5, 168.6, 170.8, 172,4, 172.6. Anal. ($C_{40}H_{68}N_4O_8S\cdot0.16H_2O$) C, H, N.

To a solution of of this intermediate (640 mg, 0.835 mmol, 1 equiv) in tetrahydrofuran (8 mL) was added *p*-toluenesulfonic acid monohydrate (317 mg, 1.67 mmol, 2 equiv). After the mixture was stirred for 6 h, more p-toluenesulfonic acid monohydrate (158 mg, 0.835 mmol, 1 equiv) was added, and the reaction mixture was stirred an additional 3 h. The solvents were removed *in vacuo*, and the residue was dissolved in 100 mL of ethyl acetate and washed sequentially with 10% sodium bicarbonate (50 mL) and saturated sodium chloride (50 mL). The organic layer was dried over sodium sulfate, filtered, and concentrated *in vacuo*. The residue was purified on Merck silica gel (110 mL), eluting with hexane/acetone (4:1 to 1:2) to give **33** (183 mg, 32%): mp 185−186 °C dec; TLC R_f = 0.11 (hexane/acetone, 2:1, visualized by ceric ammonium sulfate); MS (M + H)$^+$ 681; IR (KBr) 1751, 1638 cm^{-1}; $[\alpha]_D$ = −40.6° (c = 0.20, CH_3OH); ^{1}H NMR (270 MHz, CD_3OD) δ 0.98 (m, 12 H), 1.54 (s, 6 H), 1.64 (s, 3 H), 1.66 (s, 3 H), 2.07 (s, 3 H), 1.96−2.54 (m, 16 H), 3.71 (s, 3 H), 3.42−3.71(m, 4 H), 4.23−4.55 (m, 3 H), 5.10 (br m, 3 H); ^{13}C NMR (100 MHz, CD_3OD) δ 15.3, 16.2, 17.8, 18.3, 18.8, 19.7, 25.9, 26.2, 27.3, 27.6, 30.8, 31.2, 31.3, 31.6, 40.6, 41.2, 48.0, 48.4, 48.7, 49.0, 49.3, 49.6, 49.9, 52.3, 52.4, 52.7, 60.0, 60.3, 120.9, 125.0, 125.2, 131.8, 135.7, 138.7, 169.6, 170.1, 173.2, 173.3, 173.4. Anal. ($C_{35}H_{60}N_4O_7S\cdot0.42H_2O$) C, H, N.

Preparation of 34. To a solution of **33** (113 mg, 0.166 mmol, 1 equiv) in dioxane/methanol/water (3:1:0.5, 4.5 mL) was added 1 N sodium hydroxide (0.232 mL, 0.232 mmol, 1.4 equiv). After the mixture was stirred for 16 h, the solvents were removed *in vacuo*, and the residue was purified on CHP-20P (15 mL), eluting with water/acetonitrile (100:0 to 0:100) to give **34** (90 mg, 79%): mp 169−171 °C; TLC R_f = 0.27 (9:1:0.05 $CHCl_3$/MeOH/HOAc, visualized by ceric ammonium sulfate); IR (KBr) 1572 cm^{-1}; $[\alpha]_D$ = −23.7° (c = 0.29, CH_3OH); ^{1}H NMR (270 MHz, CD_3OD) δ 0.86−0.90 (m, 12 H), 1.51 (s, 6 H), 1.56 (s, 3 H), 1.57 (s, 3 H), 1.96 (s, 3 H), 1.88−2.41 (m, 16 H), 3.49 (m, 2 H), 4.10 (d, 1 H, J = 7.63), 4.20 (m, 2 H),

4.8 (m, 2 H, D_2O also), 5.03 (br m, 3 H); ^{13}C NMR (68 MHz, CD_3OD) δ 15.3, 16.1, 16.2, 17.7, 18.4, 18.9, 19.9, 25.9, 26.4, 27.6, 27.8, 31.1, 34.1, 40.8, 48.1, 48.4, 48.7, 49.0, 49.3, 49.6, 49.9, 50.3, 55.7, 60.4, 60.7, 121.5, 125.4, 125.5, 132.1, 136.0, 138.8, 169.4, 170.1, 172.5, 173.7, 177.7. HRMS $(M + H)^+$ calculated 667.4105, found 667.4099. Anal. ($C_{34}H_{57}N_4O_7SNa \cdot 1.5H_2O$) C, H, N.

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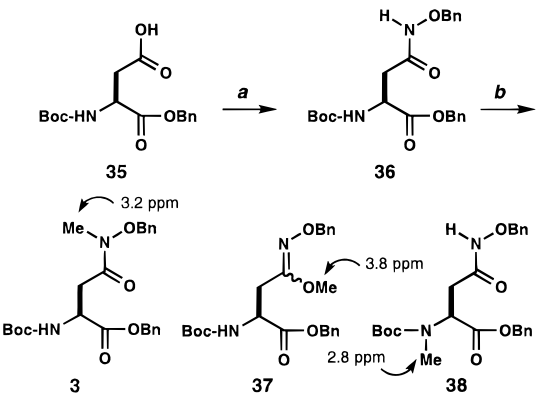
(23) Initially, an alternate method for preparation of N-methyl hydroxamic ether intermediate **3** was investigated. Unfortunately, alkylation of **36** was incomplete (<65%) and gave only moderate yields of **3** (<45%) under a variety of reaction conditions, alongwith O- and N-alkylated side products **37** and **38** respectively.



Reagents: a) CDI, NH_2-OBn b) base (K_2CO_3, DBU, NaH, etc.), MeI

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(26) The desired product **12** was accompanied by substantial amounts of N-dialkylation product (24%).

(27) Abbreviations: EDC, ethyl[3-(dimethylamino)propyl]carbodiimide hydrochloride; HOBt, hydroxybenzotriazole; BOP, (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate.

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