Synthesis and Antimitotic/Cytotoxic Activity of Hemiasterlin Analogues

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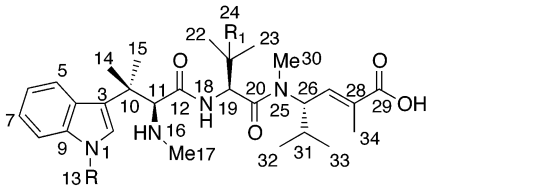
The antimitotic sponge tripeptide hemiasterlin (**1**) and a number of structural analogues have been synthesized and evaluated in cell-based assays for both cytotoxic and antimitotic activity in order to explore the SAR for this promising anticancer drug lead. One synthetic analogue, SPA110 (**8**), showed more potent in vitro cytotoxicty and antimitotic activity than the natural product hemiasterlin (**1**), and consequently it has been subjected to thorough preclinical evaluation and targeted for clinical evaluation. The details of the synthesis of hemiasterlin (**1**) and the analogues and a discussion of how their biological activities vary with their structures are presented in this paper.

A number of antimitotic drugs derived from natural products are important components of current cancer chemotherapy.[1,2] This group includes the taxanes[2a] paclitaxel (Taxol) and docetaxel and the vinca alkaloids[2b] vinblastine, vincristine, and vinorelbine. Many other antimitotic natural product chemotypes, represented by cryptophycin-52,[3] dolastatin-10,[4] halichondrin B,[1] epothilone A,[5] eleutherobin,[5,6] laulimalide,[5] and discodermolide,[5] are currently in preclinical evaluation and development or are already in clinical trials, illustrating that microtubule function remains an important target for natural products-based anticancer drug development.

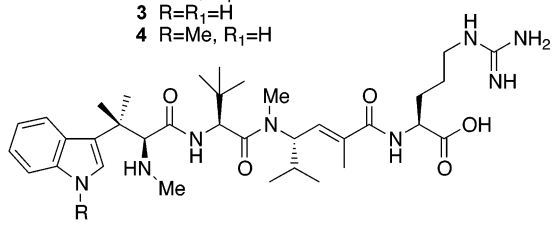
Hemiasterlin (**1**),[7−9] hemiasterlins A (**2**),[8] B (**3**),[8] and C (**4**),[10] criamides A (**5**) and B (**6**),[8] and milnamide A (**7**)[11] are members of a small family of cytotoxic tri- and tetrapeptides that have been isolated from the marine sponges *Hemiasterella minor*,[7] *Cymbastella* sp.,[8] *Auletta* sp.,[10,11] and *Siphonochalina* spp.[10] Characteristic structural features of these peptides are the presence of tri- or tetramethylated tryptophan, *tert*-leucine, and *N*-methylvinylogous valine residues. The hemiasterlins and criamides are antimitotic agents that inhibit the polymerization of tubulin.[12,13] Hemiasterlin is a more potent in vitro cytotoxin and antimitotic agent than either of the anticancer drugs Taxol or vincristine.[12] It is marginally less active than dolastatin-10 and approximately equal in activity to cryptophycin-1 in a standard tubulin polymerization assay,[13] and preliminary experiments have shown that it has promising in vivo activity.[8]

Relative to other known antimitotic agents,[1,5] the hemiasterlins possess an attractive combination of structural simplicity and potent antimitotic activity, making them ideal targets for synthetic modification. We have previously reported the total synthesis of hemiasterlin (**1**) using a general synthetic route to this family of peptides,[14] and subsequently a shorter synthesis of hemiasterlin[15a] and an alternate synthesis of the tetramethyltryptophan residue[15b] have been reported.

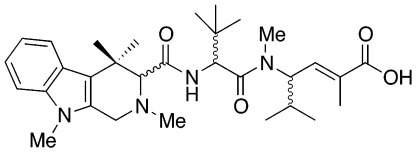
To further explore the potential of the hemiasterlins as anticancer drugs, we have undertaken the synthesis and biological evaluation of a number of analogues. The goals of the analogue program were (i) to define what portions of the structure are required for cytotoxic and antimitotic activity, (ii) to prepare more potent analogues, and (iii) to prepare analogues that are easier to synthesize. Reported below are the results of this study, which has identified the Synthetic Peptide Analogue-SPA110 (**8**)[16] as a promising candidate for further preclinical evaluation and development.



1 R=R$_1$= Me
2 R=H, R$_1$=Me
3 R=R$_1$=H
4 R=Me, R$_1$=H



5 R=H
6 R=Me



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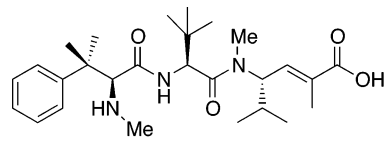

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Results and Discussion

Synthesis of Hemiasterlin and Analogues. The tripeptide structure of the hemiasterlins dictated that the most efficient approach to analogue preparation and exploration of the antimitotic SAR would involve independent modification of each of the three component amino acid

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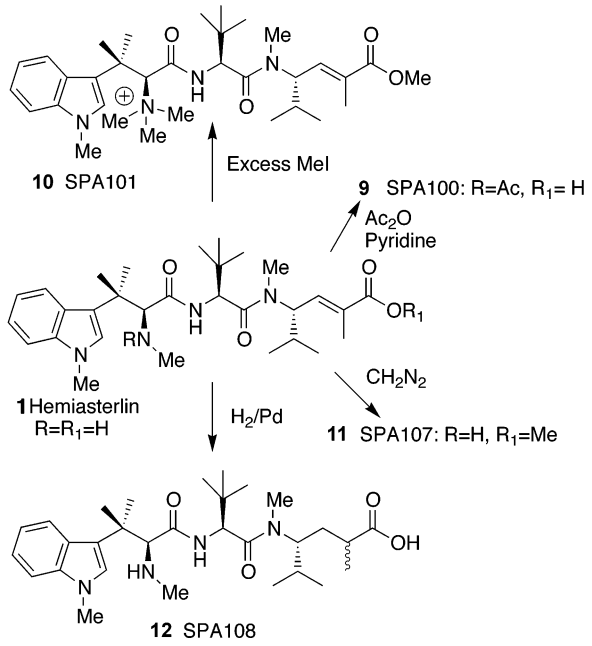
Scheme 1



residues. For the purposes of the following discussion, the amino acids have been designated amino acid A (*N-terminus*), amino acid B (*middle amino acid*), and amino acid C (*C-terminus*).

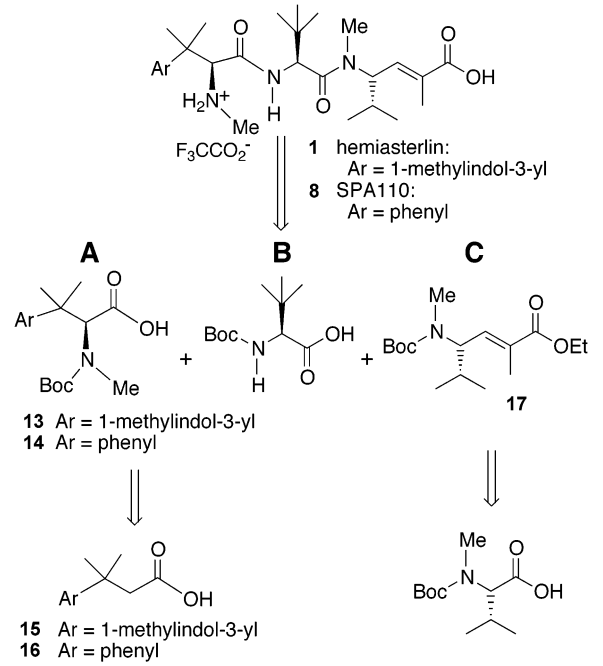
Our initial approach to obtaining SAR information focused on preparing a small number of functional group analogues by selective chemical modification of the amino acid residues A and C in the natural product hemiasterlin (**1**) as shown in Scheme 1. This set of transformations gave *N*-16-acetylhemiasterlin, SPA100 (**9**), *N*-16-trimethylhemiasterlin, SPA101 (**10**), hemiasterlin methyl ester, SPA107 (**11**), and an epimeric mixture of 27,28-dihydrohemiasterlins, SPA108 (**12**), all in good yield.

Subsequently, we used a total synthesis program to explore the importance of several structural features that distinguish the hemiasterlins from standard tripeptides. One striking feature of the hemiasterlins is the extensive methylation present at the β-carbon (C-10), α-amino (N-16 and N-25), and indole nitrogen (N-1) atoms of the core tripeptide. The methyl groups were thought to protect the tripeptide from proteolysis and perhaps also to establish a preferred conformation, roles that should be critical to biological activity. Another important feature of the hemiasterlin structures is the presence of the conserved homologated valine residue, and consequently a number of analogues that varied the functionality and skeleton of this amino acid were prepared. Finally, analogues with the indole ring replaced with simple alkyl groups or a phenyl group were prepared and tested to see if these more readily synthesizable analogues retained antimitotic activity.

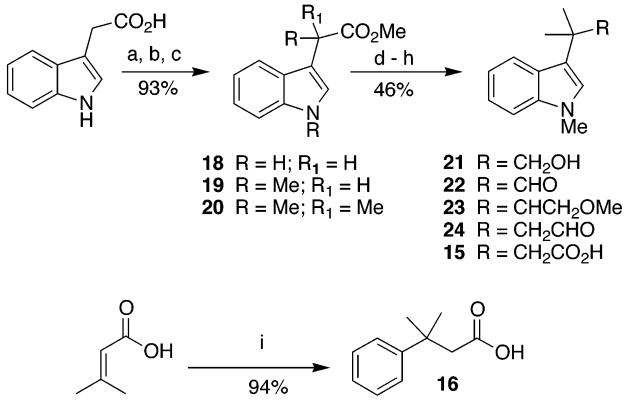
A detailed description of the synthesis of the natural product hemiasterlin (**1**) and the unnatural analogue SPA110 (**8**) is presented below. Only minor modifications of the routes to **1** and **8** were required to prepare all of the other Synthetic Peptide Analogues shown in Chart 1. A detailed description of the preparation of each derivative is provided in the Experimental Section.

The general synthetic approach to the tripeptides hemiasterlin (**1**) and SPA110 (**8**) is outlined in the retrosynthesis shown in Scheme 2. We envisioned that chemistry developed in the Evans' laboratory,[17] employing the electrophilic nitrogen source triisopropylphenylsulfonyl azide, could be used to introduce the α-amino functionality with the correct

Scheme 2



Scheme 3[a]

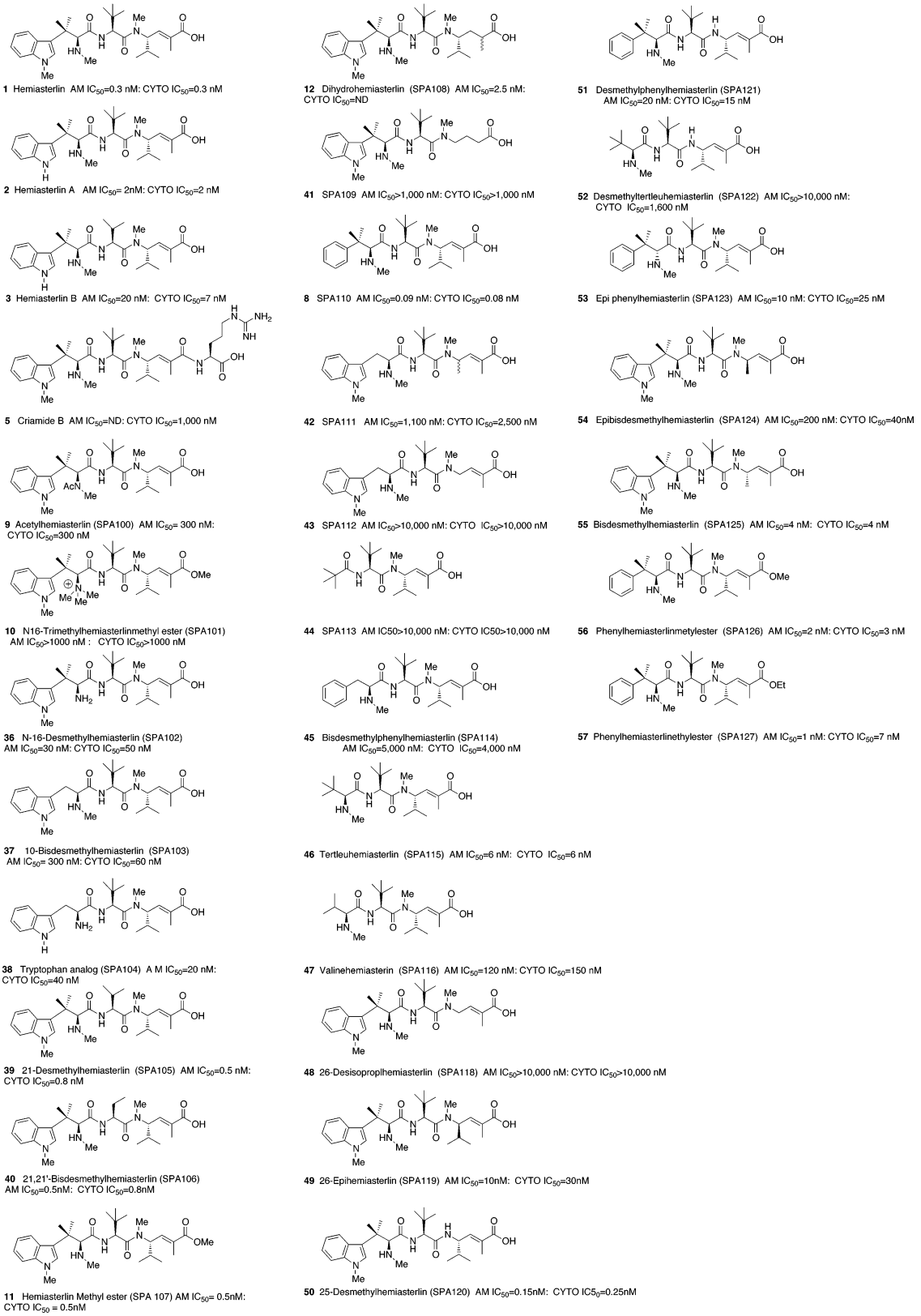


[a] (a) CH_2N_2, Et_2O; (b) KHMDS, MeI, THF; (c) KHMDS, MeI, THF; (d) DIBAL-H, THF, −78 to 0 °C; (e) TPAP, NMO, CH_2Cl_2; (f) Ph_3PCH_2OMeCl, KOt-Bu, THF; (g) TsOH, dioxane, H_2O; (h) $NaClO_2$, NaH_2PO_4, t-BuOH, H_2O; (i) $AlCl_3$, PhH, 65 °C.

configuration into the carbon framework **15** for hemiasterlin and **16** for SPA110 to generate the required protected amino acid A residues **13** and **14**. The *tert*-leucine amino acid B residue present in both hemiasterlin and SPA110 was commercially available as its *N*-Boc derivative. We envisioned that the protected amino acid C residue **17**, which is also required in the synthesis of both hemiasterlin (**1**) and SPA110 (**8**), could be produced by reaction between the *N*-Boc-protected amino aldehyde (*S*)-*N*-Boc-*N*-methylvalinal and a stabilized Wittig reagent. (*S*)-*N*-Boc-*N*-methylvalinal could in turn be obtained from reduction of the Weinreb amide of *N*-Boc-*N*-methylvaline.

The syntheses of the carbon skeletons for amino acid A of hemiasterlin and SPA110 are shown in Scheme 3. Indol-3-ylacetic acid was converted quantitatively to its methyl ester (**18**) with diazomethane. Repeated methylation of ester **18** provided first the dimethylated intermediate **19** and finally the required trimethyl derivative **20**. Homologation of **20** was accomplished via a straightforward sequence of reactions. Reduction of the ester **20** gave the primary alcohol **21**, which was oxidized with TPAP in the presence of NMO[18] to give the corresponding aldehyde **22**. Wittig olefination of aldehyde **22** with methoxymethylene-

Chart 1. Cytotoxicity and Antimitotic Activity of Natural Hemiasterlins and Criamides and Synthetic Peptide Analogues (SPA)



1 Hemiasterlin AM IC_{50}=0.3 nM: CYTO IC_{50}=0.3 nM

2 Hemiasterlin A AM IC_{50}= 2nM: CYTO IC_{50}=2 nM

3 Hemiasterlin B AM IC_{50}=20 nM: CYTO IC_{50}=7 nM

5 Criamide B AM IC_{50}=ND: CYTO IC_{50}=1,000 nM

9 Acetylhemiasterlin (SPA100) AM IC_{50}= 300 nM: CYTO IC_{50}=300 nM

10 N16-Trimethylhemiasterlinmethyl ester (SPA101) AM IC_{50}>1000 nM : CYTO IC_{50}>1000 nM

36 N-16-Desmethylhemiasterlin (SPA102) AM IC_{50}=30 nM: CYTO IC_{50}=50 nM

37 10-Bisdesmethylhemiasterlin (SPA103) AM IC_{50}= 300 nM: CYTO IC_{50}=60 nM

38 Tryptophan analog (SPA104) A M IC_{50}=20 nM: CYTO IC_{50}=40 nM

39 21-Desmethylhemiasterlin (SPA105) AM IC_{50}=0.5 nM: CYTO IC_{50}=0.8 nM

40 21,21'-Bisdesmethylhemiasterlin (SPA106) AM IC_{50}=0.5nM: CYTO IC_{50}=0.8nM

11 Hemiasterlin Methyl ester (SPA 107) AM IC_{50}= 0.5nM: CYTO IC_{50} = 0.5nM

12 Dihydrohemiasterlin (SPA108) AM IC_{50}=2.5 nM: CYTO IC_{50}=ND

41 SPA109 AM IC_{50}>1,000 nM: CYTO IC_{50}>1,000 nM

8 SPA110 AM IC_{50}=0.09 nM: CYTO IC_{50}=0.08 nM

42 SPA111 AM IC_{50}=1,100 nM: CYTO IC_{50}=2,500 nM

43 SPA112 AM IC_{50}>10,000 nM: CYTO IC_{50}>10,000 nM

44 SPA113 AM IC50>10,000 nM: CYTO IC50>10,000 nM

45 Bisdesmethylphenylhemiasterlin (SPA114) AM IC_{50}=5,000 nM: CYTO IC_{50}=4,000 nM

46 Tertleuhemiasterlin (SPA115) AM IC_{50}=6 nM: CYTO IC_{50}=6 nM

47 Valinehemiasterin (SPA116) AM IC_{50}=120 nM: CYTO IC_{50}=150 nM

48 26-Desisoproplhemiasterlin (SPA118) AM IC_{50}>10,000 nM: CYTO IC_{50}>10,000 nM

49 26-Epihemiasterlin (SPA119) AM IC_{50}=10nM: CYTO IC_{50}=30nM

50 25-Desmethylhemiasterlin (SPA120) AM IC_{50}=0.15nM: CYTO $IC5_0$=0.25nM

51 Desmethylphenylhemiasterlin (SPA121) AM IC_{50}=20 nM: CYTO IC_{50}=15 nM

52 Desmethyltertleuhemiasterlin (SPA122) AM IC_{50}>10,000 nM: CYTO IC_{50}=1,600 nM

53 Epi phenylhemiasterlin (SPA123) AM IC_{50}=10 nM: CYTO IC_{50}=25 nM

54 Epibisdesmethylhemiasterlin (SPA124) AM IC_{50}=200 nM: CYTO IC_{50}=40nM

55 Bisdesmethylhemiasterlin (SPA125) AM IC_{50}=4 nM: CYTO IC_{50}=4 nM

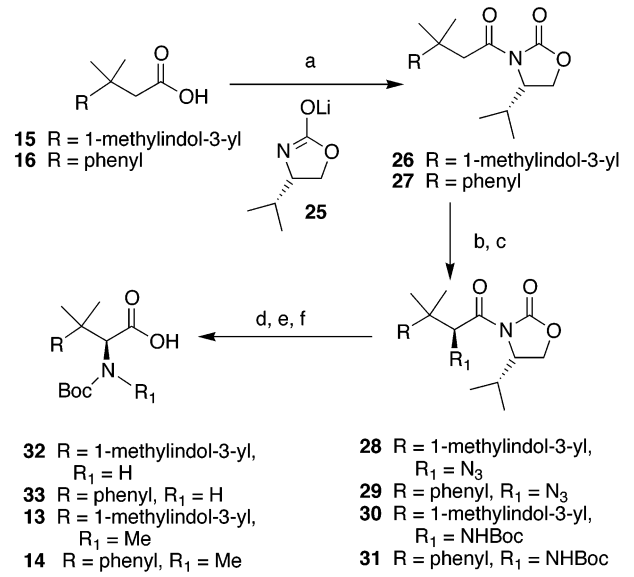
56 Phenylhemiasterlinmetylester (SPA126) AM IC_{50}=2 nM: CYTO IC_{50}=3 nM

57 Phenylhemiasterlinethylester (SPA127) AM IC_{50}=1 nM: CYTO IC_{50}=7 nM

triphenylphosphorane gave the alkenyl ether **23**, which was converted to the aldehyde **24** by acid hydrolysis. Oxidation of aldehyde **24** with sodium chlorite[19] gave the desired carboxylic acid **15**. The synthesis of 3-methyl-3-phenylbutanoic acid (**16**) was accomplished in a single step by heating 3-methyl-2-butenoic acid and aluminum chloride in benzene as shown in Scheme 3.[20]

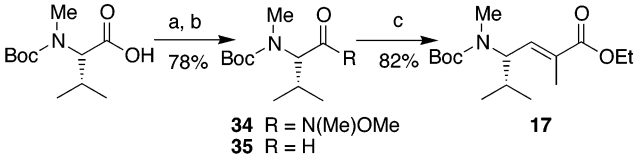
Stereoselective introduction of the α-stereogenic center of compounds **13** and **14** was acheived using Evans' oxazolidinone chemistry[17] as outlined in Scheme 4. Reaction of the acid (**15** or **16**) with pivaloyl chloride in the presence of triethylamine gave the corresponding mixed anhydride, which upon treatment with lithiated oxazolidinone **25** afforded compound **26** or **27**. Sequential treat-

Scheme 4[a]



[a] (a) (i) pivaloyl chloride, THF; (ii) **25**, THF, −78 ° C; (b) KHMDS, trisylN_3, THF, −78 °C; (c) H_2/Pd(C), Boc_2O, EtOAc or (i) $SnCl_2$, dioxane, H_2O; (ii) Boc_2O, $NaHCO_3$, dioxane, H_2O; (d) LiOH, H_2O_2, MeOH; (e) NaH, MeI, DMF; (f) LiOH, MeOH, H_2O.

Scheme 5[a]



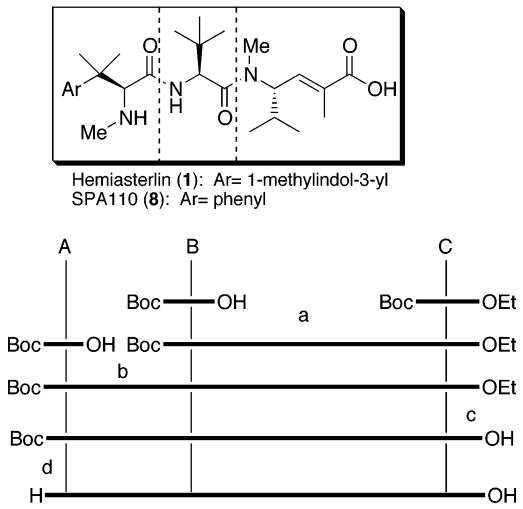
[a] (a) PyBOP, [H_2N(Me)OMe]Cl, DIEA, CH_2Cl_2; (b) LAH, THF; (c) $Ph_3PC(Me)CO_2Et$, CH_2Cl_2.

ment of **26** or **27** with base and trisylN_3[21] in THF produced the corresponding azides **28** and **29**. Reduction of the azides to the amines in the presence of di-*tert*-butyl dicarbonate afforded the Boc-protected amines **30** and **31**. Lithium hydroperoxide-promoted removal of the chiral auxiliary from **30** and **31** produced **32** and **33**, respectively.[22] Introduction of the *N*-methyl was accomplished by treatment of **32** or **33** with NaH and MeI in DMF, followed by hydrolysis of the resulting methyl ester to provide the desired Boc-protected tetramethyltryptophan **13** and trimethylphenylalanine **14** derivatives (42% and 85% yield, respectively, for the 6 steps in Scheme 4). All other amino acid A analogues used commercially available amino acids or their *N*-methylated counterparts (see Experimental Section for more detail).

Formation of compound **17**, a protected version of the amino acid C residue in **1** and **8**, is summarized in Scheme 5. Conversion of the commercially available (*S*)-*N*-Boc-*N*-methylvaline into the corresponding amide derivative **34**, followed by reduction of the latter substance with LAH, gave aldehyde **35**.[23] Reaction of **35** with [(1-ethoxycarbonyl)ethylidene]triphenylphosphorane in CH_2Cl_2 afforded, stereoselectively, the desired *E*-2-alkenoate **17**. This same synthetic sequence was also applied to *N*-Boc-*N*-methylglycine, *N*-Boc-*N*-methylalanine, and *N*-Boc-valine to prepare SPA118 (**48**), SPA124 (**54**), SPA125 (**55**), and SPA120 (**50**), respectively.

The coupling of the *N*-Boc-amino acids and deprotection to form the desired tripeptides is shown in Scheme 6. The formation of the *N*-Boc-B-C-OEt dipeptide was accomplished by removal of the *N*-Boc group of amino acid C with TFA in CH_2Cl_2 (1:1), forming the TFA salt, followed by

Scheme 6[a]



[a] (a) (i) TFA, CH_2Cl_2; (ii) HATU, HOAt, DIEA, CH_2Cl_2 or pivaloyl chloride, DIEA, THF, −78 °C to rt; (b) (i) TFA, CH_2Cl_2; (ii) PyBOP, DIEA, CH_2Cl_2; (c) LiOH, MeOH, H_2O; (d) TFA, CH_2Cl_2.

coupling with *N*-Boc-amino acid B as either the mixed anhydride or in the presence of the uronium coupling agent HATU. Formation of the protected tripeptide was achieved by treating the TFA salt of the dipeptide B-C-OEt with the *N*-Boc-protected amino acid A using PyBOP as the coupling agent. The tripeptide was deprotected by saponification of the ethyl ester followed by application of standard Boc removal conditions (i.e., 1:1 TFA/CH_2Cl_2). The resulting TFA salt was purified by reversed-phase HPLC using a Magnum ODS column with a H_2O/MeOH (with 0.05% TFA) mixture as the mobile phase. Hemiasterlin (**1**) (A = **13**; B = Boc-*tert*-LEU; C = **17**) and SPA110 (**8**) (A = **14**; B = Boc-*tert*-LEU; C = **17**) were obtained in 27 and 31% yield, respectively, for the 4 steps in Scheme 6. Characterization and biological testing were done on the TFA salts.

Antimitotic and Cytotoxic Activities of the Natural Hemiasterlins and Synthetic Peptide Analogues. Chart 1 shows the structures of the natural products and synthetic analogues that were evaluated for biological activity. Listed below each compound is its IC_{50} (nM) for antimitotic (AM) activity in a cell-based assay[24] that uses human mammary carcinoma MCF-7 cells expressing a dominant-negative mutant p53 tumor suppressor gene (MCF-7 mup53).[25] Also given is the IC_{50} (nM) for cytotoxicity (CYTO) against the same cell line measured using the MTT assay. Figure 1 shows a plot of the antimitotic IC_{50}'s versus the cytotoxic IC_{50}'s for all of the natural products and analogues that were tested.

The plot of IC_{50} for antimitotic activity versus IC_{50} for cytotoxic activity against MCF-7 mup53 cells (Figure 1) for each of the naturally occurring hemiasterlins and Synthetic Peptide Analogues illustrates a number of important features of the biological activity of these tripeptides. The most striking aspect of the data is the linear relation between antimitotic and cytotoxic activity over a wide range of structural variations and roughly 6 orders of magnitude in potency (0.1 to 10^5 nM). This strong correlation ($r = 0.958$) suggests that, in these cells, cytotoxicity is solely due to inhibition of tubulin function at mitosis, i.e., that hemiasterlin compounds are "pure" antimitotic agents.

A series of grid lines have been used in Figure 1 to arbitrarily divide the natural hemiasterlins and SPAs into seven groups based on their antimitotic and cytotoxic potencies (group I most active, IC_{50}'s ≤ 0.1 nM; group VII least active IC_{50}'s ≥ 10 000 nM). This grouping provides a

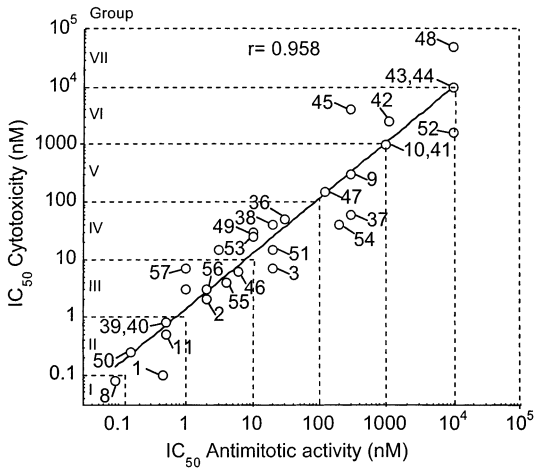


Figure 1. Plot of in vitro cytotoxicity (IC_{50} in nM) versus antimitotic activity (IC_{50} in nM) against human breast cancer MCF-7 mup53 cells for natural hemiasterlins, criamides, and synthetic analogues.

useful framework for discussing the SAR for this family of tripeptides. Hemiasterlin (**1**), the reference compound for the following discussion, is a group II compound.

If one first considers single modifications in the hemiasterlin structure that result in loss of activity, two changes, which each give compounds in categories VI and VII, stand out as being the most deleterious. The first is the conversion of the N-16 methylamine to a N-16 trimethylammonium ion (SPA101 (**10**)), and the second is the replacement of the isopropyl functionality at C-26 with a hydrogen atom (SPA118 (**48**)). The dramatic attenuation of activity resulting from these two changes indicates that the N-16 and C-26 functionalities are crucial to metabolic stability and/or tubulin binding. The sensitivity of N-16 is confirmed by the observation that the N-16 acetyl hemiasterlin derivative SPA100 (**9**) and N-16 desmethylhemiasterlin SPA102 (**36**) are category V and IV compounds, respectively, both being more than 2 orders of magnitude less active than hemiasterlin. The C-26-epimer of hemiasterlin SPA119 (**49**) is a category IV compound, the C-26 methyl analogue SPA125 (**55**) is a category III compound, and the epimeric C-26 methyl compound SPA124 (**54**) is a category V compound, illustrating that even a methyl group at C-26 is much better than no alkyl substitutent (SPA118, **48**) and that the L-configuration of an alkyl-substituted C-26 is also important.

Other changes in hemiasterlin that significantly reduce activity include removal of the Me-14 and Me-15 groups to give SPA103 (**37**), a group V compound, replacement of the C amino acid by *N*-methyl-4-aminobutanoic acid to give SPA109 (**41**), a group VI compound, and formation of a tetrapeptide by addition of L-arginine to the C terminus to give criamide B (**5**), a group V compound. These three changes confirm the sensitivity of residue C to alteration and also illustrate the importance of the β-dimethylation on residue A.

A number of modifications of hemiasterlin reduced activity 10- to 500-fold, to produce group III and IV compounds, which are still quite potent antimitotic agents (IC_{50}'s < 100 nM). Replacement of the methyl group on the indole nitrogen with a hydrogen converts hemiasterlin (**1**) to hemiasterlin A (**2**), a group III compound that is approximately an order of magnitude less active than **1**. Further reduction in the methylation of hemiasterlin A (**2**) resulting from conversion of the *tert*-LEU residue to a VAL residue, generates hemiasterlin B (**3**), a group IV compound, which reflects an additional loss in potency. Reduction of the $\Delta^{27,28}$ olefin to give the mixture of epimers SPA108 (**12**) reduces the potency by roughly an order of magnitude to give a group III compound, as does replacing the *N*-methylindole ring with a methyl group to give the *tert*-LEUhemiasterlin SPA115 (**46**). However, replacing the *N*-methylindole ring with a hydrogen atom to give VAL-hemiasterlin SPA116 (**47**) significantly reduced the potency to give a group V compound.

Another series of single-point changes was well tolerated, resulting in no significant loss in potency. These include the conversion of the B residue *tert*-LEU to VAL to give SPA105 (**39**), conversion of the *tert*-LEU to butyrine to give SPA106 (**40**), formation of the methyl ester to give SPA107 (**11**), and replacement of the N-25 methyl substituent with a proton to give SPA120 (**50**).

Only one change, the replacement of the *N*-methylindole ring by phenyl to give phenylhemiasterlin SPA110 (**8**), resulted in a group I compound that was approximately 3-fold more potent than the natural product hemiasterlin (**1**). Within the phenyl series, removal of the A residue β,β-dimethyl gave SPA114 (**45**), a group VI compound, removal of the C residue *N*-methyl substituent gave SPA121 (**51**), a group IV compound, epimerization of the A residue gave SPA123 (**53**), a group IV compound, and making the C residue methyl ester SPA126 (**56**) and ethyl ester SPA (**57**) gave group III compounds. It is interesting to note that sensitivity to removal of the A residue β,β-dimethyl groups was greater in phenylhemiasterlin SPA110 (**8**) than in hemiasterlin (**1**) and that removal of the C residue *N*-methyl group and formation of the C residue methyl ester led to significant reductions in activity in the phenyl series but no appreciable change in activity in the *N*-methylindole series. These observations illustrate that the effects of more than one change in the hemiasterlin structure are not simply additive. This is further illustrated by the observation that SPA104 (**38**), which has no methyl substituents on the A residue, is more potent than SPA103 (**37**), which is missing only the β,β-dimethyl group on the A residue. The decrease in potency observed for hemiasterlin A (**2**), which is missing only the indole *N*-methyl, and SPA102 (**36**), which is missing only the A residue α-amino *N*-methyl, seem to indicate that these methyl substituents are important for maximal activity. If the effects of methyl removal were simply additive, SPA 104 (**38**) should be much less active than SPA 103 (**37**), which is not observed.

In summary, the present study has identified four regions of hemiasterlin where single structural changes are possible without seriously compromising antimitotic activity: replacing the *N*-methylindole with phenyl and methyl groups, replacing the *tert*-Leu residue with valine or butyrine residues, removing the N-25 methyl substituent, and making the C residue methyl ester (Figure 2). It has also been shown that the C-10 methyl substituents, the C-11 methylamino group, and the C-26 isopropyl groups are extremely important structural elements for potent antimitotic activity.

On the basis of the results reported above, the phenyl analogue SPA110 (**8**) was selected for further preclinical testing and eventually targeted for clinical evaluation.[26] It is the only synthetic analogue prepared in this study[26b] that showed equal or greater potency than hemiasterlin (**1**) in the antimitotic and cytotoxic assays used to evaluate compounds. In addition, the synthesis of the amino acid A residue (i.e., **14**) in SPA110 (**8**) is significantly shorter and higher yielding than the synthesis of the amino acid A residue (i.e., **13**) in the *N*-methylindole analogues such as

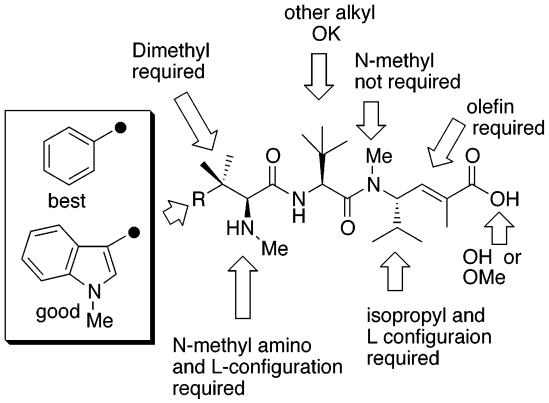


Figure 2. Structural requirements for optimal cytotoxicity (IC_{50} < 1 nM) and antimitotic activity (IC_{50} < 1 nM).

hemiasterlin (**1**), making for greater ease of preparation of SPA110 (**8**).

Experimental Section[33]

General Experimental Procedures. Solvents and reagents were purchased in anhydrous form or were purified by standard methods[27] when necessary. Tetrahydrofuran (THF) was distilled from sodium benzophenone ketyl, while methylene chloride (CH_2Cl_2) and benzene were distilled from calcium hydride. All three solvents (THF, CH_2Cl_2, and benzene) were distilled immediately before use. *N*,*N*-Dimethylformamide (DMF) and diisopropylethylamine (DIEA) were purchased as an anhydrous solvent in Sure/Seal bottles from the Aldrich Chemical Co.

All glassware, stir bars, Teflon cannulas, and metal syringe needles employed in anhydrous reactions were dried in an oven set at 120 °C for at least 2 h. Reaction vessels were cooled to room temperature under a stream of argon, while glass syringes and metal syringe needles were cooled in a desiccator containing Drierite. In most cases plastic, sterilized, nonpyrogenic syringes (FORTUNA, Einmalspritze type A) were used for the addition of anhydrous reagent and solvents. Moisture- or oxygen-sensitive reactions were performed under an argon atmosphere.

All melting point determinations were performed on a Fischer-Johns melting point apparatus. Infrared spectra were obtained on a Perkin-Elmer 1710 FTIR using a KBr pellet or $CHCl_3$ solution for solids or on NaCl plates for liquid samples. Nuclear magnetic resonance (NMR) spectra were obtained on a Bruker AC-200 or WH-400 for ^{1}H NMR and on either a Varian XL-300 (75 MHz) or Bruker AM-400 (100 MHz) for ^{13}C NMR. ^{1}H NMR spectra were recorded using $CDCl_3$, D_6-DMSO, or D_3COD as the solvent, and signal position (δ value) were measured relative to the signals for the solvent (δ 7.24 ($CHCl_3$), 2.49 (D_5-DMSO), and 3.30 (D_2HCOD)). ^{13}C NMR spectra were recorded using $CDCl_3$, D_6-DMSO, or D_3COD as the solvent and signal position (δ value) were measured relative to the signals for the solvent (i.e., δ 77.0, 39.5, and 49.0, respectively). Mass spectra were run by the UBC Mass Spectrometry Laboratory using a Kratos MS50 for EI, Kratos Concept IIH for LSIMS; DelsiNermag mode R-10-10C mass spectrometer (using NH_3 or NH_3 and CH_4 mixture) for DCI, and Kratos Concept IIHQ for FAB. Elemental analyses (C,H,N) were performed on a CarloErba CHN Model 1106 or on a Fison's EA Model 1108 elemental analyzer, by the UBC Microanalytical Laboratory. Optical rotations were run on a Perkin-Elmer 241MC polarimeter using the indicated solvent in a cell with a 1 cm path length.

General Procedure 1: *N*-Acyloxazolidinone Preparation.[17] The carboxylic acid (1 equiv) was dissolved in THF (12 mL/mmol acid) and cooled to −78 °C. Triethylamine (1.5 equiv) and pivaloyl chloride (1.1 equiv) were added to the reaction flask, producing a white solid. The resulting mixture was warmed to 0 °C for 1 h and then cooled back down to −78 °C. In a second flask *n*-butyllithium (1.6 M in hexanes, 1.9 equiv) was added dropwise with vigorous stirring to a solution of (4*S*)-(−)-4-isopropyl-2-oxazolidinone (2 equiv) at −78 °C in THF (5 mL/mmol oxazolidinone), producing a white precipitate. The resulting suspension of the lithiated oxazolidinone **25** was added via cannula to the reaction flask. Stirring was continued for 2 h, H_2O was added, and the reaction mixture was warmed to room temperature, whereupon it was extracted three times with diethyl ether. The combined organic layer was dried over magnesium sulfate and concentrated in vacuo. Purification of product was accomplished by silica gel column chromatography using a diethyl ether/petroleum ether mixture as the mobile phase.

General Procedure 2: α-Azido-*N*-acyloxazolidinone Preparation.[17] *N*-Acyloxazolidinone (1 equiv), dried under high vacuum for 0.5 h, was dissolved in THF (6 mL/mmol *N*-acyloxazolidinone) and cooled to −78 °C. Freshly titrated KHMDS (0.115 M in THF, 1.1 equiv) was added, and the resulting solution was stirred at −78 °C for 1 h. A solution of trisylN_3[21] (1.25 equiv) in THF (2.5 mL/mmol trisylN_3) at −78 °C was added rapidly via cannula, and after 2 min the orange-colored reaction mixture was treated with glacial acetic acid (4.6 equiv), warmed to 40 °C in a H_2O bath, and stirred for 1 h. To the light yellow mixture was added brine and H_2O, and the aqueous phase was extracted three times with diethyl ether. The combined organic layer was washed with a saturated sodium hydrogen carbonate solution, dried with magnesium sulfate, and concentrated in vacuo. Purification of the product was accomplished using silica gel column chromatography using a diethyl ether/petroleum ether mixture as the mobile phase.

General Procedure 3: Oxazolidinone Auxiliary Removal.[22] The oxazolidinone (1 equiv) was dissolved in a mixture of THF (12 mL/mmol of oxazolidinone) and H_2O (3 mL/mmol of oxazolidinone). This solution was cooled to 0 °C, and hydrogen peroxide (30% aqueous, 9 equiv) and lithium hydroxide (1.0 M, 3 equiv) were added. The resulting mixture was stirred at room temperature overnight (∼15 h). The excess peroxide was quenched by addition of sodium hydrogen sulfite (1.5 M, 18 equiv), and stirring was continued for 1 h. The aqueous phase was acidified with 1.0 M citric acid, and the mixture was extracted three times with either ethyl acetate or chloroform. The combined organic layer was dried over magnesium sulfate and concentrated in vacuo. Silica gel column chromatography was used to purify the resulting carboxylic acid using a mixture of diethyl ether/petroleum ether with 1% acetic acid as the mobile phase.

General Procedure 4: Conversion of an N-H to an N-Me. To a vigorously stirred solution of carboxylic acid (1 equiv) in dry DMF or THF (14 mL/mmol of carboxylic acid) at 0 °C was added sodium hydride (5 equiv) followed, after the bubbling had ceased, by methyl iodide (10 equiv), and the resulting gray suspension was allowed to warm to room temperature overnight (∼20 h). The excess sodium hydride was quenched by cautious addition of H_2O, and the mixture was acidified by dropwise addition of 1.0 M citric acid. The acidic mixture was extracted three times with either ethyl acetate or chloroform, and the combined organic layer was dried over magnesium sulfate and concentrated in vacuo.

If DMF was used as the solvent, saponification of the resulting methyl ester was typically required (see General Procedure 5).

General Procedure 5: Ester Saponification. The ester (1 equiv) was dissolved in methanol (24 mL/mmol ester). While stirring, first H_2O (8 mL/mmol ester) and then an aqueous solution of lithium hydoxide (1.0 M, 8 equiv) were added. The resulting mixture was stirred at either room temperature or 60 °C until TLC analysis showed no remaining starting material (usually overnight). The reaction was acidified by addition of a solution of citric acid (1.0 M) and extracted three times with chloroform. The organic layer was dried and the solvent removed, which yielded the carboxylic acid product. If needed, the product was purified by silica gel column chromatography using a diethyl ether/petroleum ether mixture with 1% acetic acid as the mobile phase.

General Procedure 6: Weinreb Amide Formation.[23] To a cold (0 °C) solution of *N*-Boc-amino acid (1 equiv), *N*,*O*-dimethylhydroxylamine hydrochloride (1.3 equiv), and PyBOP (1.05 equiv) in CH_2Cl_2 (1 mL/mmol of *N*-Boc-amino acid) was added DIEA (3 equiv). After 1 min the reaction mixture was warmed to room temperature, and stirring was continued for 1 h. If the pH value of the mixture was less than 7, the mixture could be treated with a few drops of DIEA to allow the reaction to go to completion. The mixture was poured into diethyl ether, and the resulting mixture was washed successively with 3 N hydrochloric acid (three times), saturated aqueous sodium hydrogen carbonate solution (three times), and saturated aqueous sodium chloride (three times). The organic layer was dried over magnesium sulfate and concentrated in vacuo. The crude Weinreb amide was purified by silica gel column chromatography using a diethyl ether/petroleum ether mixture as the mobile phase.

General Procedure 7: Weinreb Amide Reduction.[28] Lithium aluminum hydride (3 equiv) was added to a solution of Weinreb amide (1 equiv) in dry THF (1 mL/mmol Weinreb amide) at 0 °C, and the reaction mixture was stirred for 1 h. The mixture was poured into a stirring aqueous solution of potassium hydrogen sulfate (0.23 M, 3 equiv). Diethyl ether was added, the layers were separated, and the aqueous layer was extracted three times with diethyl ether. The organic layers were combined and washed sequentially with 3 N hydrochloric acid (three times), saturated aqueous sodium hydrogen carbonate solution (three times), and saturated aqueous sodium chloride (three times). The organic layer was dried with magnesium sulfate, and the solvent was evaporated to yield the crude *N*-Boc-aminoaldehyde. The *N*-Boc-aminoaldehyde was used without further purification.

General Procedure 8: δ-Amino Vinylogous Esters Formation.[29,30] To a solution of *N*-Boc-aminoaldehyde (1 equiv) in dry CH_2Cl_2 (1 mL/mmol *N*-Boc-aminoaldehyde) under an argon atmosphere at room temperature was added [(1-ethoxycarbonyl)ethylidene]triphenylphosphorane (1.5 equiv), and stirring was continued for 4 h. The reaction mixture was diluted with H_2O and extracted three times with diethyl ether. The combined organic layer was dried with magnesium sulfate and concentrated in vacuo. The crude oil was purified by silica gel column chromatography using a diethyl ether/petroleum ether mixture as the mobile phase. Only the *E*-2-alkenoate was obtained.

General Procedure 9: Trifluoacetic Acid-Promoted Cleavage of *N*-Boc Groups. The *N*-Boc-protected amine (1.0 equiv) was dissolved in CH_2Cl_2 (10 mL/mmol amine) and treated with TFA (10 mL/mmol amine) at room temperature for 0.5 h. Removal of the solvent in vacuo, followed by repeated rinsings of the remaining material with CH_2Cl_2 and evaporation of the remaining traces of solvent (done three times), afforded the TFA salt of the amine in quantitative yield. For coupling reactions the TFA salts were used without further purification.

General Procedure 10: Pivaloyl Chloride-Mediated Amide Bond Formation. To a cold (−78 °C) solution of carboxylic acid (1.1 equiv) in dry THF (1 mL/mmol of amino acid ester) under an argon atmosphere was added DIEA (1.5 equiv) and pivaloyl chloride (1.2 equiv). The resulting mixture was warmed to 0 °C for 1 h and then re-cooled to −78 °C. Diisopropylethylamine (2.2 equiv) was added to the reaction flask followed by the addition, via cannula, of the TFA salt of the amino acid ester (1.0 equiv, prepared by General Procedure 9 from the *N*-Boc-amino acid ester) in dry THF (0.5 mL/mmol of amino acid ester) at −78 °C. Stirring was continued for 1 h and then quenched by the addition of H_2O. The mixture was allowed to warm to room temperature and extracted three times with diethyl ether. The combined organic layer was dried over magnesium sulfate and concentrated in vacuo. The crude oil was purified by silica gel column chromatography using a diethyl ether/petroleum ether mixture as the mobile phase.

General Procedure 11: HATU-Mediated Amide Bond Formation.[31] To *N*-Boc-amino acid (1 equiv), HATU (1 equiv), and HOAt (1 equiv) was added CH_2Cl_2 (4 mL/mmol *N*-Boc-amino acid). To the suspension was added DIEA (1 equiv), and the reaction flask was cooled to 0 °C. After stirring at 0 °C for 5 min, to the reaction mixture was added, via cannula, the TFA salt of the aminoester (approximately 1 equiv, prepared from the *N*-Boc-aminoester by General Procedure 9) dissolved in CH_2Cl_2 (1.5 mL/mmol aminoester), followed by DIEA (2 equiv). The solution was kept at 0 °C for an additional 5 min, the ice bath was removed, and the reaction mixture was stirred at room temperature overnight (18 h). The reaction was acidified by addition of 1.0 M citric acid, and the resulting mixture was extracted three times with chloroform. The combined organic layer was dried over magnesium sulfate and concentrated. The crude oil was purified by silica gel chromatography using a diethyl ether/petroleum ether mixture as the mobile phase.

General Procedure 12: PyBOP-Mediated Amide Bond Formation.[32] To a cold (0 °C) solution of *N*-Boc-amino acid (1 equiv), ammonium salt (approximately 1 equiv, prepared from *N*-Boc-aminoester by General Procedure 9), and PyBOP (1.05 equiv) in CH_2Cl_2 (1 mL/mmol of *N*-Boc-amino acid) was added DIEA (3 equiv). After 5 min, the ice bath was removed and the reaction mixture was stirred at room temperature overnight (20 h). The reaction was acidified by the addition of 1.0 M citric acid and extracted three times with chloroform. The combined organic layer was dried with magnesium sulfate and concentrated in vacuo. Purification was accomplished using silica gel column chromatography with a diethyl ether/petroleum ether mixture as the mobile phase.

General Procedure 13: PyBroP-Mediated Amide Bond Formation.[32] To a solution (or suspension) of the *N*-Boc-amino acid (1 equiv) in CH_2Cl_2 (3 mL/mmol *N*-Boc-amino acid) was added DIEA (3 equiv), DMAP (0.6 equiv), and PyBroP (1 equiv). The solution was stirred for a few minutes, and then a solution of the TFA salt of the aminoester (approximately 1 equiv, prepared from *N*-Boc-aminoester by General Procedure 9) was added in CH_2Cl_2 (6 mL/mmol aminoester) via cannula addition. The reaction mixture was stirred at room temperature overnight (18 h). To the mixture was added H_2O, CH_2Cl_2, and a few drops of 10% aqueous HCl. The resulting biphasic solution was extracted three times with CH_2Cl_2. The combined organic layer was extracted with saturated aqueous sodium hydrogen carbonate (10 mL), dried over magnesium sulfate, and concentrated in vacuo. Purification was accomplished by silica gel column chromatography using a diethyl ether/petroleum ether mixture as the mobile phase.

Compounds. All compounds with a free amine were isolated, characterized, and tested in biological assays as their TFA salts.

Methyl Indol-3-ylacetate (18). To a stirred suspension of indol-3-ylacetic acid (4.60 g, 26.3 mmol) in diethyl ether (20 mL) at room temperature was added an ethereal solution of diazomethane dropwise until the yellow color of the diazomethane persisted in the reaction mixture, and TLC analysis showed complete consumption of starting material. Excess diazomethane was removed under a stream of argon and the remaining solvent removed in vacuo. The crude oil thus obtained was purified by silica gel column chromatography (1:1 diethyl ether/petroleum ether) to afford ester **18** as a colorless solid (4.97 g, quantitative yield): mp 47−48 °C; IR ($CHCl_3$ soln) 3409, 1729, 1621 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) 3.71 (s, 3H), 3.79 (s, 2H), 7.03 (s, 1H), 7.15 (t, 1H, $J = 7.8$ Hz), 7.20 (t, 1H, $J = 7.8$ Hz), 7.31 (d, 1H, $J = 7.8$ Hz), 7.63 (d, 1H, $J = 7.8$ Hz), 8.12 (bs, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) 31.9, 51.9, 108.1, 111.2, 118.7, 119.6, 122.1, 123.1, 127.1, 136.0, 172.6; HREIMS *m*/*z* 189.0786 (calcd for $C_{11}H_{11}NO_2$, 189.0789); *anal.* calcd for $C_{11}H_{11}NO_2$, C 69.83%; H 5.86%; N 7.40%; found C 69.47%; H 5.91%; N 7.50%.

Methyl 2-(1-Methylindol-3-yl)propanoate (19). To a stirred, cooled (0 °C) suspension of potassium hydride (1.82 g, 45.5 mmol) in dry THF (200 mL) under an argon atmosphere was added bis(trimethylsilyl)amine (12.5 mL, 59.2 mmol), and the resulting mixture was stirred at room temperature for 45 min to allow for the formation of the KHMDS. This mixture was then cooled to −78 °C, and a solution of ester **18** (2.8 g, 14.8 mmol) in THF (50 mL + 20 mL washings) was added via cannula. The reaction mixture was warmed to 0 °C and stirred

for 2 h before re-cooling to −78 °C. Freshly distilled methyl iodide (7.4 mL, 118 mmol) was added, the mixture allowed to warm to 0 °C, and stirring continued for 3 h. The reaction mixture was then placed in the freezer (−26 °C) for 16 h, after which time TLC analysis showed complete consumption of starting material. The reaction was quenched by addition of H_2O (200 mL) and then extracted with diethyl ether (3 × 200 mL), and the combined organic layer was washed with brine (200 mL), dried with magnesium sulfate, and concentrated in vacuo. The resulting crude oil was purified by silica gel column chromatography (3:7 diethyl ether/petroleum ether) to afford ester **19** as a viscous pale yellow oil (3.20 g, 99% yield): IR (neat) 1734, 1615, 1550 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) 1.61 (d, 3H, J = 7.1 Hz), 3.67 (s, 3H), 3.75 (s, 3H), 4.01 (q, 1H, J = 7.1 Hz), 7.00 (s, 1H), 7.13 (t, 1H, J = 7.8 Hz), 7.24 (t, 1H, J = 7.8 Hz), 7.30 (d, 1H, J = 7.8 Hz), 7.68 (d, 1H, J = 7.8 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) 18.0, 32.7, 36.7, 51.9, 109.2, 113.9, 119.0, 119.2, 121.7, 126.2, 126.7, 136.9, 175.6; HREIMS *m/z* 217.1101 (calcd for $C_{13}H_{15}NO_2$, 217.1102); *anal.* calcd for $C_{13}H_{15}NO_2$, C 71.87%; H 6.96%; N 6.45%; found C, 71.52%; H 6.80%; N 6.26%.

Methyl 2-Methyl-2-(1-methylindol-3-yl)propanoate (20). To a stirred, cooled (0 °C) suspension of potassium hydride (900 mg, 22.5 mmol) in dry THF (200 mL) under an argon atmosphere was added bis(trimethylsilyl)amine (6.3 mL, 29.4 mmol), and the resulting mixture was stirred at room temperature for 45 min to allow for formation of the KHMDS. This mixture was cooled to −78 °C, and a solution of ester **19** (3.2 g, 14.7 mmol) in THF (40 mL + 30 mL washings) was added via cannula. The reaction mixture was warmed to 0 °C and stirred for 2 h before re-cooling to −78 °C. Freshly distilled methyl iodide (5.5 mL, 88.5 mmol) was added, the mixture allowed to warm to 0 °C, and stirring continued for 3 h or until TLC analysis showed no remaining starting material. The reaction was quenched by addition of H_2O (150 mL) and then extracted with ether (3 × 150 mL). The combined organic layer was washed with brine (150 mL), dried with magnesium sulfate, and concentrated in vacuo. The resulting crude oil was purified by silica gel column chromatography (1:4 diethyl ether/petroleum ether) to afford the ester **20** as a colorless solid (3.18 g, 94% yield): mp 99−101 °C; IR ($CHCl_3$ soln) 1727, 1618, 1550 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) 1.70 (s, 6H), 3.64 (s, 3H), 3.75 (s, 3H), 6.94 (s, 1H), 7.10 (t, 1H, J = 7.9 Hz), 7.22 (t, 1H, J = 7.9 Hz), 7.29 (d, 1H, J = 7.9 Hz), 7.64 (d, 1H, J = 7.9 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) 26.3, 32.7, 41.9, 52.1, 109.3, 119.0, 119.1, 120.2, 121.5, 125.2, 125.9, 137.4, 177.6; HREIMS *m/z* 231.1257 (calcd for $C_{14}H_{17}NO_2$, 231.1259); *anal.* calcd for $C_{14}H_{17}NO_2$, C 72.70%; H 7.41%; N 6.06%; found, C 72.83%; H 7.44%; N 6.04%.

2-Methyl-2-(1-methylindol-3-yl)propanol (21). To a stirred, cooled (−78 °C) solution of ester **20** (3.18 g, 13.8 mmol) in dry diethyl ether (150 mL), and dichloromethane (40 mL), under an argon atmosphere was added DIBAL-H (34.4 mL, 1.0 M in hexanes, 34.4 mmol). The resulting solution was allowed to warm to 0 °C, and stirring was continued for 3 h. The reaction was quenched by addition of H_2O (30 mL) and allowed to warm to room temperature, whereupon Rochelles salt (70 mL, saturated aqueous) was added. The organic layer was separated, and the aqueous layer was extracted with ether (2 × 150 mL). The combined organic layer was washed with brine (150 mL), dried with magnesium sulfate, and concentrated in vacuo. The crude mixture was purified by silica gel column chromatography (1:1 diethyl ether/petroleum ether) to afford the alcohol **21** as a colorless solid (2.79 g, quantitative yield): mp 80−82 °C; IR ($CHCl_3$ soln) 3400 (br), 1614, 1545 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) 1.48 (s, 6H), 3.75 (s, 3H), 3.79 (s, 2H), 6.90 (s, 1H), 7.11 (t, 1H, J = 7.8 Hz), 7.22 (t, 1H, J = 7.8 Hz), 7.32 (d, 1H, J = 7.8 Hz), 7.78 (d, 1H, J = 7.8 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) 25.5, 32.7, 37.7, 71.6, 109.6, 118.8, 119.4, 121.0, 121.5, 126.1, 127.1, 137.9; HREIMS *m/z* 203.1305 (calcd for $C_{13}H_{17}NO$, 203.1310); *anal.* calcd for $C_{13}H_{17}NO$, C 76.81%; H 8.43%; N 6.895; found, C 76.89%; H 8.43%; N 6.70%.

2-Methyl-2-(1-methylindol-3-yl)propanal (22). To a mixture of alcohol **21** (2.79 g, 13.8 mmol), *N*-methylmorpholine *N*-oxide (2.9 g, 24.8 mmol), and 4 Å powdered molecular sieves (3 g) in dry dichloromethane (100 mL) under an argon atmosphere at room temperature was added solid TPAP (240 mg, 0.688 mmol) in one portion.[18] The resulting black mixture was stirred at the same temperature for 16 h, then filtered through Celite (to remove the molecular sieves), and concentrated in vacuo. The black oil thus obtained was purified by silica gel column chromatography (1:4 diethyl ether/petroleum ether) to afford the aldehyde **22** as a colorless solid (2.31 g, 84% yield): mp 61−63 °C; IR ($CHCl_3$ soln) 1718, 1610, 1542 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) 1.58 (s, 6H), 3.70 (s, 3H), 6.96 (s, 1H), 7.10 (t, 1H, J = 7.9 Hz), 7.24 (t, 1H, J = 7.9 Hz), 7.32 (d, 1H, J = 7.9 Hz), 7.56 (d, 1H, J = 7.9 Hz), 9.49 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) 21.9, 32.8, 46.5, 109.5, 115.0, 119.3, 120.1, 121.8, 126.1, 126.6, 137.6, 202.2; HREIMS *m/z* 201.1147 (calcd for $C_{13}H_{15}NO$, 201.1153); *anal.* calcd for $C_{13}H_{15}NO$, C 77.58%; H 7.51%; N 6.96%; found, C 77.42%; H 7.58%; N 6.83%.

(*E*/*Z*)-5-Methyl-5-(1-methylindol-3-yl)-2-oxahex-3-ene (23). To a stirred suspension of methoxymethyltriphenylphosphonium chloride (5.9 g, 17.2 mmol) in dry THF (120 mL) under an argon atmosphere at room temperature (H_2O bath, for cooling, in place) was added potassium *tert*-butoxide (1.87 g, 16.7 mmol) as a solid in one portion. The reaction mixture immediately turned a deep red color, the H_2O bath was removed, and stirring was continued for 1.5 h at room temperature. Aldehyde **22** (2.31 g, 11.5 mmol) in THF (20 mL + 20 mL washings) was added via cannula, and stirring was continued for a further 2 h. The reaction mixture was diluted with H_2O (100 mL) and extracted with ether (3 × 100 mL). The combined organic layer was washed with brine (100 mL), dried with magnesium sulfate, and concentrated in vacuo. The crude oil was purified by silica gel column chromatography (1:25 diethyl ether/petroleum ether) to afford the required enol ether **23** as a 40:60 mixture of *Z* and *E* isomers (2.67 g) and a trace of a higher R_f impurity (not separated). The integrity of this mixture was checked by ^1H NMR, and the mixture was taken on and used in the following step without further characterization: ^1H NMR (200 MHz, $CDCl_3$) 1.52 (s, 2.4H), 1.62 (s, 3.6H), 3.49 (s, 1.8H), 3.53 (s, 1.2H), 3.73 (s, 1.2H), 3.74 (s, 1.6H), 4.60 (d, 0.4H, J = 6.9 Hz), 5.13 (d, 0.6H, J = 12.7 Hz), 5.78 (d, 0.4H, J = 6.9 Hz), 6.32 (d, 0.6H, J = 12.7 Hz), 6.83 (s, 1H), 7.02−7.40 (m, 3H), 7.73−7.78 (m, 1H).

3-Methyl-3-(1-methylindol-3-yl)butanal (24). To a stirred solution of enol ether **23** (2.67 g, theoretical maximum 2.63 g, 11.5 mmol) in dioxane (120 mL) and H_2O (30 mL) at room temperature was added *p*-toluenesulfonic acid monohydrate (100 mg, 0.526 mmol), and the resulting mixture was heated to 60 °C for 16 h. The reaction mixture was then diluted with H_2O (150 mL) and extracted with ether (3 × 150 mL), and the combined organic layer was washed with sodium hydrogen carbonate (150 mL, saturated aqueous) and brine (150 mL), then dried with magnesium sulfate, and concentrated in vacuo. The crude oil was purified by silica gel column chromatography (1:4 diethyl ether/petroleum ether) to afford the desired aldehyde **24** as a colorless solid (1.94 g, 78% yield over two steps): mp 39−40 °C; IR ($CHCl_3$ soln) 1718, 1615, 1546 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) 1.55 (s, 6H), 2.83 (d, 2H, J = 3.1 Hz), 3.74 (s, 3H), 6.82 (s, 1H), 7.10 (t, 1H, J = 7.8 Hz), 7.24 (t, 1H, J = 7.8 Hz), 7.32 (d, 1H, J = 7.8 Hz), 7.56 (d, 1H, J = 7.8 Hz), 9.51 (t, 1H, J = 3.1 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) 29.2, 32.6, 33.6, 54.7, 109.6, 118.7, 120.7, 121.3, 121.4, 125.3, 125.6, 137.9, 204.1; HREIMS *m/z* 215.131 (calcd for $C_{14}H_{17}NO$, 215.1310); *anal.* calcd for $C_{14}H_{17}NO$, C 78.10%; H 7.96%; N 6.51%; found, C 78.22%; H 7.98%; N 6.41%.

3-Methyl-3-(1-methylindol-3-yl)butanoic acid (15). To a solution of aldehyde **24** (557 mg, 2.59 mmol) in *tert*-butyl alcohol (15 mL) at room temperature was added 2-methyl-2-butene (13.0 mL, 2.0 M in THF, 26.0 mmol), and the mixture was cooled to 0 °C. To this was added a solution of sodium hydrogen phosphate (1.42 g, 10.3 mmol) in H_2O (5 mL) followed by sodium chlorite (351 mg, 3.88 mmol) in H_2O (10 mL). The resulting solution was stirred for 20 min at the same temperature and then diluted with H_2O (30 mL), acidified to pH 1−2 with dilute hydrochloric acid, and extracted with ethyl acetate (3 × 50 mL). The combined organic layer was washed with

brine and concentrated in vacuo. The resulting crude mixture was purified by silica gel column chromatography (1:1 diethyl ether/petroleum ether + 1% acetic acid) to afford the acid **15** as a colorless solid (422 mg, 70%): mp 139−140 °C; IR ($CHCl_3$ soln) 3054, 2981(br), 1705, 1620, 1540 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) 1.63 (s, 6H), 2.88 (s, 2H), 3.74 (s, 3H), 6.87 (s, 1H), 7.10 (t, 1H, J = 8.0 Hz), 7.24 (t, 1H, J = 8.0 Hz), 7.32 (d, 1H, J = 8.0 Hz), 7.56 (d, 1H, J = 8.0 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) 28.3, 32.5, 34.0, 46.8, 109.5, 118.5, 120.7, 121.2, 122.4, 125.1, 125.7, 137.7, 178.4; HREIMS *m*/*z* 231.125 (calcd for $C_{14}H_{17}NO_2$, 231.1259); *anal*. calcd for $C_{14}H_{17}NO_2$, C 72.70%; H 7.41%; N 6.06%; found, C 72.74%; H 7.60%; N 5.85%.

3-Methyl-3-phenylbutanoic acid (16).[20] 3-Methyl-2-butenoic acid (5.10 g, 50.9 mmol) and $AlCl_3$ (20.4 g, 153 mmol) were placed in a one-neck round-bottomed flask. Benzene (50 mL) was added, which produced vigorous bubbling. Upon completion of the bubbling, a capped condenser (i.e., closed system) was attached, and the reaction mixture was stirred and placed in an oil bath at 65 °C. The pressure in the system was occasionally released. The progress of the reaction was followed by the loss of starting material by GC. If the reaction was not complete within 1 h, a small quantity of $AlCl_3$ was added and stirring was continued. To the solution was added diethyl ether, and the mixture was cooled to 0 °C. Slowly concentrated HCl and some H_2O were added until all the solid dissolved and the pH was less than 2. The aqueous layer was extracted with diethyl ether three times. The organic layer was concentrated to 150 mL and then was extracted with a saturated sodium hydrogen carbonate solution six times. The combined aqueous layer was acidified with concentrated HCl until the pH was less than 2. The acidic aqueous layer was extracted with diethyl ether three times, and the accumulated organic layer was dried with magnesium sulfate. The solution was filtered and the diethyl ether was removed in vacuo, producing a white solid (8.51 g, 47.7 mmol) in 94% yield, which did not need further purification. **16**: mp 55−56 °C; IR (KBr pellet) 3210−2100 (br), 1698, 1319, 696 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) 1.47 (s, 6H), 2.65 (s, 2H), 7.21 (t, 2H, J = 7.2 Hz), 7.32 (t, 2H, J = 7.2 Hz), 7.38 (d, 2H, J = 7.2 Hz), 10.45 (bs, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) 28.7, 36.9, 47.9, 125.3, 126.0, 128.1, 147.9, 178.2; HREIMS *m*/*z* 178.0995 (calcd for $C_{11}H_{14}O_2$, 178.0993); *anal*. calcd for $C_{11}H_{14}O_2$, C 74.13%; H 7.92%; found, C 73.88%; H 7.99%.

(4*S*)-3-(3-Methyl-3-(1-methylindol-3-yl)-1-oxobutyl)-4-isopropyl-2-oxazolidinone (26). 26 was prepared according to General Procedure 1 with the following: carboxylic acid **15** (477 mg, 2.06 mmol); THF (25 mL); triethylamine (0.43 mL, 3.09 mmol); pivaloyl chloride (0.28 mL, 2.27 mmol); *n*-butyllithium (2.68 mL, 1.52 M in hexanes, 4.03 mmol); (4*S*)-(−)-4-isopropyl-2-oxazolidinone (514 mg, 4.13 mmol); and THF (20 mL). The crude product (yellow oil) was purified by silica gel column chromatography (2:3 diethyl ether/petroleum ether) to afford the desired compound **26** as a colorless solid (697 mg, 99% yield): mp 118−119 °C; $[\alpha]_D^{20}$ +61.1 (*c* 0.90, $CHCl_3$); IR ($CHCl_3$ soln) 1777, 1693, 1615, 1540 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) 0.67 (d, 3H, J = 6.9 Hz), 0.77 (d, 3H, J = 6.9 Hz), 1.59 (s, 3H), 1.61 (s, 3H), 2.14 (m, 1H), 3.48 (s, 2H), 3.71 (s, 3H), 3.71 (bt, 1H, J = 9.0 Hz), 3.97 (dd, 1H, J = 2.7 and 9.0 Hz), 4.18 (m, 1H), 6.86 (s, 1H), 7.07 (t, 1H, J = 8.0 Hz), 7.16 (t, 1H, J = 8.0 Hz), 7.24 (d, 1H, J = 8.0 Hz), 7.82 (d, 1H, J = 8.0 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) 14.5, 17.9, 28.5, 28.7, 29.6, 32.6, 35.0, 45.4, 58.5, 62.9, 109.3, 118.5, 121.0, 122.1, 125.6, 125.9, 137.5, 154.0, 171.5; HREIMS *m*/*z* 342.1938 (calcd for $C_{20}H_{26}N_2O_3$, 342.1943); *anal*. calcd for $C_{20}H_{26}N_2O_3$, C 70.15%; H 7.65%; N 8.185%; found, C 70.39%; H 7.81%; N 8.18%.

(4*S*)-3-(3-Methyl-1-oxo-3-phenylbutyl)-4-isopropyl-2-oxazolidinone (27). 27 was prepared according to General Procedure 1 with the following: 3-methyl-3-phenylbutanoic acid (**16**, 1.24 g, 6.95 mmol); THF (85 mL); triethylamine (1.45 mL, 10.4 mmol); pivaloyl chloride (0.942 mL, 7.65 mmol); *n*-butyllithium (8.47 mL, 1.6 M in hexanes, 13.6 mmol); (4*S*)-(−)-4-isopropyl-2-oxazolidinone (1.80 g, 13.9 mmol); and THF (68 mL). The product was purified by radial chromatography (4 mm plate, 3:7 diethyl ether/petroleum ether) affording compound **27** as a clear, colorless oil in 94% yield (1.84 g, 6.35 mmol): $[\alpha]_D^{25}$ +69.5 (*c* 1.16, $CHCl_3$); IR (neat) 2965, 1779, 1703, 1365, 1206 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) 0.71 (d, 3H, J = 6.9 Hz), 0.79 (d, 3H, J = 7.1 Hz), 1.47 (s, 3H), 1.48 (s, 3H), 2.12−2.16 (m, 1H), 3.30−3.38 (m, 2H), 4.00 (t, 1H, J = 9.0 Hz), 4.05 (dd, 1H, J = 2.8 and 9.0 Hz), 4.18−4.22 (m, 1H), 7.16 (t, 1H, J = 7.3 Hz), 7.28 (t, 2H, J = 7.3 Hz), 7.38 (d, 2H, J = 7.3 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) 14.5, 17.8, 28.3, 29.0, 29.3, 37.6, 46.6, 58.3, 62.9, 125.4, 125.7,127.9, 147.8, 153.9, 170.9; HREIMS *m*/*z* 289.1675 (calcd for $C_{17}H_{23}NO_3$, 289.1677); *anal*. calcd for $C_{17}H_{23}NO_3$, C 70.56%; H 8.01%; N 4.84%; found, C 70.60%; H 8.05%; N 4.82%.

(4*S*)-3-((2*S*)-2-Azido-3-methyl-3-(1-methylindol-3-yl)-1-oxobutyl)-4-isopropyl-2-oxazolidinone (28). 28 was prepared according to General Procedure 2 with the following: *N*-acyloxazolidinone **26** (303 mg, 0.886 mmol); THF (5 mL); KHMDS (2.87 mL, 0.34 M in THF, 0.975 mmol); trisylN_3 (342 mg, 1.11 mmol); THF (3 mL); and glacial acetic acid (0.26 mL, 4.55 mmol). The resulting crude oil was purified by silica gel column chromatography (3:7 diethyl ether/petroleum ether) to afford a mixture of the desired compound **28** and a small amount of triisopropylbenzenesulfonylamine (302 mg total, estimated 236 mg of desired compound, approximately 70%). A small amount of the compound, for purposes of characterization, was further purified by application to another column of silica gel (TLC grade silica, 3:7 diethyl ether/petroleum ether): $[\alpha]_D^{20}$ +95.9 (*c* 0.90, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$) 0.70 (d, 3H, J = 6.9 Hz), 0.76 (d, 3H, J = 6.9 Hz), 1.62 (s, 3H), 1.66 (s, 3H), 2.16 (m, 1H), 3.66−3.73 (m, 6H), 5.66 (s, 1H), 6.94 (s, 1H), 7.06 (t, 1H, J = 8.0 Hz), 7.16 (t, 1H, J = 8.0 Hz), 7.26 (d, 1H, J = 8.0 Hz), 7.75 (d, 1H, J = 8.0 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) 14.9, 17.9, 22.7, 27.7, 28.7, 32.8, 41.3, 59.6, 63.3, 66.0, 109.4, 117.7, 119.0, 121.0, 121.5, 126.0, 128.2, 137.1, 153.3, 169.5; HREIMS *m*/*z* 383.1950 (calcd for $C_{20}H_{25}N_5O_3$, 383.1957).

(4*S*)-3-((2*S*)-2-Azido-3-methyl-1-oxo-3-phenylbutyl)-4-isopropyl-2-oxazolidinone (29). 29 was prepared according to General Procedure 2 with the following: *N*-acyloxazolidinone **27** (347 mg, 1.20 mmol); THF (6.8 mL); KHMDS (5.4 mL, 0.267 M in THF, 1.44 mmol); trisylN_3 (460 mg, 1.50 mmol); THF (4.1 mL); and glacial acetic acid (0.316 mL, 5.52 mmol). The product was purified by radial chromatography (4 mm plate, 3:7 diethyl ether/petroleum ether, sample was loaded with diethyl ether), affording azide **29** as a colorless oil (388 mg, 1.17 mmol) in 98% yield: $[\alpha]_D^{25}$ +121.5 (*c* 1.1, $CHCl_3$); IR (neat) 2103, 1780, 1702, 1386, 1201 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) 0.79 (d, 3H, J = 6.9 Hz), 0.83 (d, 3H, J = 7.0 Hz), 1.52 (s, 3H), 1.54 (s, 3H), 2.26−2.31 (m, 1H), 3.56 (t, 1H, J = 8.7 Hz), 3.85−3.89 (m, 1H), 3.95 (dd, 1H, J = 8.7 and 2.2 Hz), 5.64 (s, 1H), 7.23 (t, 1H, J = 7.2 Hz), 7.31 (t, 2H, J = 7.2 Hz), 7.39 (d, 2H, J = 7.2 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) 14.6, 17.9, 22.1, 26.5, 28.5, 43.7, 59.1, 63.4, 66.4, 126.5, 126.9, 128.2, 144.1, 153.3, 168.7; HRCIMS *m*/*z* 331.1769 (calcd for $C_{17}H_{23}N_4O_3$ (M + H), 331.1770); *anal*. calcd for $C_{17}H_{22}N_4O_3$, C 61.80%; H 6.71%; N 16.96%; found, C 61.34%; H 6.52%; N 16.43%.

(4*S*)-3-((2*S*)-2-*tert*-Butoxycarbonylamino-3-methyl-3-(1-methylindol-3-yl)-1-oxobutyl)-4-isopropyl-2-oxazolidinone (30). To a suspension of tin(II) chloride (349 mg, 1.84 mmol) in dioxane (6 mL) and H_2O (3 mL) at room temperature under an argon atmosphere was added a solution of azide **28** (235 mg, 0.614 mmol) in dioxane (3 mL). The resulting mixture was stirred at room temperature for 36 h, and then a suspension of di-*tert*-butyl dicarbonate (675 mg, 3.09 mmol) and sodium hydrogen carbonate (258 mg, 3.07 mmol) in H_2O (4 mL) and dioxane (2 mL) was added and stirring was continued for a further 24 h. The reaction mixture was acidified with 1.0 M sodium hydrogen sulfate, diluted further with H_2O (30 mL), and then extracted with ethyl acetate (3 × 40 mL). The combined organic layer was washed with brine (40 mL), dried over magnesium sulfate, and concentrated in vacuo. The crude mixture was purified by silica gel column chromatography (2:3 diethyl ether/petroleum ether) to afford the desired compound **30** as a colorless crystalline solid (234 mg, 83% yield): mp 204−206 °C; $[\alpha]_D^{20}$ +87.0 (*c* 1.8, $CHCl_3$); IR ($CHCl_3$ soln) 3445, 1784, 1693, 1605, 1594, 1520 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) 0.70 (d, 3H, J = 6.9 Hz), 0.73 (d, 3H,

J = 6.9 Hz), 1.40 (s, 9H), 1.53 (s, 3H), 1.59 (s, 3H), 2.07−2.09 (m, 1H), 3.66−3.60 (m, 3H), 3.72 (bs, 3H), 5.38 (bs, 1H), 6.05 (bs, 1H), 6.98 (s, 1H), 7.02 (t, 1H, J = 7.8 Hz), 7.16 (t, 1H, J = 7.8 Hz), 7.24 (d, 1H, J = 7.8 Hz), 7.72 (d, 1H, J = 7.8 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) 15.0, 18.0, 22.7, 28.0, 28.3, 28.8, 32.8, 39.9, 58.0, 59.7, 63.1, 79.7, 109.3, 117.8, 118.8, 121.1, 121.3, 126.5, 128.8, 136.9, 153.0, 155.3, 173.0; HREIMS m/z 457.2583 (calcd for $C_{25}H_{35}N_3O_5$, 457.2576); *anal.* calcd for $C_{25}H_{35}N_3O_5$, C 65.62%; H 7.71%; N 9.18%; found, C 65.31%; H 7.76%; N 9.05%.

(4*S*)-3-((2*S*)-2-*tert*-Butoxycarbonylamino-3-methyl-1-oxo-3-phenylbutyl)-4-isopropyl-2-oxazolidinone (31). Azide **29** (879 mg, 2.66 mmol), 10% palladium on carbon (587 mg), and di-*tert*-butyl dicarbonate (1.28 g, 5.85 mmol) were placed in a 100 mL flask. Ethyl acetate (50 mL) was added, and the resulting black suspension was stirred at room temperature. The mixture was flushed with argon, then with hydrogen, and was stirred under a hydrogen balloon for 4 h. The reaction mixture was filtered through silica gel, and the collected material was washed with ethyl acetate. The combined filtrate was concentrated in vacuo, and the crude mixture was purified by silica gel column chromatography (3:7 diethyl ether/petroleum ether) to afford compound **31**, a viscous colorless oil, in 95% yield (1.02 g, 2.52 mmol): $[\alpha]_D^{24}$ +118.4 (*c* 0.94, $CHCl_3$); IR (neat) 3453, 1784, 1698, 1369, 1201, 1169 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) 0.76 (d, 3H, J = 6.9 Hz), 0.80 (d, 3H, J = 7.0 Hz), 1.41 (s, 9H, H-24), 2.22−2.26 (m, 1H), 3.45 (t, 1H, J = 8.4 Hz), 3.79−3.82 (m, 1H), 3.89 (d, 1H, J = 1.9 and 8.4 Hz), 5.11 (bs, 1H), 6.12 (d, 1H, J = 9.9 Hz), 7.21 (t, 1H, J = 7.4 Hz), 7.29 (t, 2H, J = 7.4 Hz), 7.40 (d, 2H, J = 7.4 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) 14.5, 17.8, 22.4, 25.9, 28.1, 28.4, 42.2, 57.6, 58.8, 63.0, 79.6, 126.5, 127.8, 144.3, 152.8, 154.9, 172.3; HRCIMS m/z 405.2388 (calcd for $C_{22}H_{33}N_2O_5$ (M + H), 405.2390); *anal.* calcd for $C_{22}H_{32}N_2O_5$, C 65.32%; H 7.97%; N 6.93%; found, C 64.93%; H 7.83%; N 6.77%.

(*S*)-*N*$^\alpha$-*tert*-Butoxycarbonyl-*N*^{1},*β*,*β*-trimethyltryptophan (32). 32 was prepared according to General Procedure 3 with the following: oxazolidinone **30** (179 mg, 0.392 mmol); THF (4 mL); H_2O (1 mL); hydrogen peroxide (0.4 mL, 30% aqueous, 3.53 mmol); lithium hydroxide (1.2 mL, 1.0 M, 1.17 mmol); and sodium hydrogen sulfite (4.0 mL, 1.5 M, 5.88 mmol). The product was purified by silica gel column chromatography (1:1 diethyl ether/pet ether with 1% acetic acid), which afforded 129.2 mg of the acid **32** (95% yield). The structural integrity of this compound was checked by 400 MHz ^{1}H NMR spectrometry and taken on to the following reaction without further purification or analysis: ^{1}H NMR (400 MHz, $CDCl_3$) 1.38 (s, 9H), 1.51 (s, 3H), 1.54 (s, 3H), 3.71 (s, 3H), 4.80 (bs, 1H), 5.08 (bs, 1H), 6.83 (s, 1H), 7.07 (t, 1H, J = 7.8 Hz), 7.19 (t, 1H, J = 7.8 Hz), 7.26 (d, 1H, J = 7.8 Hz), 7.82 (bd, 1H, J = 7.8 Hz).

(*S*)-*N*-*tert*-Butoxycarbonyl-*β*,*β*-dimethylphenylalanine (33). 33 was prepared according to General Procedure 3 with the following: oxazolidinone **31** (373 mg, 0.923 mmol); THF (10.5 mL); H_2O (2.6 mL); hydrogen peroxide (0.942 mL, 30% aqueous, 8.31 mmol); lithium hydroxide (2.77 mL, 1.0 M, 2.77 mmol); and sodium hydrogen sulfite (10.5 mL, 1.5 M, 15.8 mmol). The product was purified by silica gel column chromatography (1:1 diethyl ether/petroleum ether with 1% acetic acid), which afforded 266 mg (0.909 mmol, 98% yield) of acid **33** as a viscous oil: $[\alpha]_D^{23}$ +22.3 (*c* 1.12, $CHCl_3$); IR (neat) 3350−2400 (br), 1713, 1660, 1397, 1369, 1165 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$, mixture of rotamers) 1.31−1.52 (m, 15H), 4.32 (bs, 0.3H), 4.55 (bd, 0.6H, J = 9.1 Hz), 4.99 (bd, 0.6H, J = 9.1 Hz), 6.12 (bs, 0.3H), 7.21 (t, 1H, J = 7.6 Hz), 7.30 (t, 2H, J = 7.6 Hz), 7.37 (d, 2H, J = 7.6 Hz); ^{13}C NMR (75 MHz, $CDCl_3$, mixture of rotamers) 23.5, 25.1, 25.5, 26.1, 27.9, 28.2, 40.8, 41.4, 61.8, 63.6, 80.0, 81.5, 126.3, 126.5, 128.1, 144.5, 145.0, 155.4, 156.1, 175.9; HRCIMS m/z 294.1706 (calcd for $C_{16}H_{24}NO_4$ (M + H), 294.1705).

(*S*)-*N*$^\alpha$-*tert*-Butoxycarbonyl-*N*$^\alpha$,*N*^{1},*β*,*β*-tetramethyltryptophan (13). 13 was prepared according to General Procedure 4 with the following: carboxylic acid **32** (95 mg, 0.275 mmol); DMF (2 mL); sodium hydride (66 mg, 2.75 mmol); and methyl iodide (0.171 mL, 2.75 mmol). For the saponification of the resulting methyl ester General Procedure 5 (at 60 °C) was used with the following quantities: methyl ester (80.0 mg, 0.214 mmol); methanol (6 mL); H_2O (1.5 mL); and lithium hydroxide (1.7 mL, 1.0 M, 1.7 mmol). The crude oil was purified by silica gel column chromatography (1:1 diethyl ether/petroleum ether with 1% acetic acid) to afford the desired acid **13** as a pale yellow oil (75.7 mg, 76% yield for the 2 steps): $[\alpha]_D^{20}$ −45.6 (*c* 0.8, $CHCl_3$); IR ($CHCl_3$ soln) 3200 (br), 1690, 1546 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) 1.43 (s, 9H), 1.54 (s, 3H), 1.64 (s, 3H), 2.68 (s, 3H), 3.71 (s, 3H), 5.40 (s, 1H), 6.85 (s, 1H), 7.00−7.25 (m, 3H), 7.85 (bd, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) (25.8, 26.4), (26.5, 26.9), 28.3, 32.7, (34.1, 34.3), (39.2, 39.4), (64.7, 64.8), (81.0, 80.2), (109.3, 109.6), (118.5, 118.9), (120.4, 120.5), 120.8, (121.1, 121.3), (125.3, 125.8), (126.6, 126.9), 137.5, (155.9, 156.9), 175.0; HREIMS m/z 360.2054 (calcd for $C_{20}H_{28}N_2O_4$, 360.2049); *anal.* calcd for $C_{20}H_{28}N_2O_4$, C 66.64%; H 7.38%; found, C 66.42%; H 7.75%.

(*S*)-*N*-*tert*-Butoxycarbonyl-*N*,*β*,*β*-trimethylphenylalanine (14). 14 was prepared according to General Procedure 4 with the following: carboxylic acid **33** (64.8 mg, 0.221 mmol); DMF (2 mL); sodium hydride (26.5 mg, 1.11 mmol); and methyl iodide (0.165 mL, 2.65 mmol). For the saponification of the resulting methyl ester General Procedure 5 (at 60 °C) was used with the following quantities: methanol (6 mL); H_2O (1.5 mL); and lithium hydroxide (1.7 mL, 1.0 M, 1.7 mmol). Compound **14** was obtained in 67.1 mg (0.218 mmol, 99% yield for 2 steps) as a colorless viscous oil. No further purification was needed in this case; however, if needed, the product could be purified further by silica gel column chromatography (1:2 diethyl ether/petroleum ether with 1% acetic acid): $[\alpha]_D^{28}$ −13.1 (*c* 6.96, $CHCl_3$); IR (neat) 3490−2840 (br), 1665, 1153 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$, mixture of rotamers) 1.39−1.49 (m, 12H), 1.55 (s, 3H), 2.62 (s, 1.95H), 2.75 (s, 1.05H), 4.93 (bs, 0.33H), 5.17 (bs, 0.66H), 7.18 (t, 1H, J = 7.2 Hz), 7.28 (t, 2H, J = 7.6 Hz), 7.37 (d, 1.3H, J = 7.6 Hz), 7.41 (d, 1.3H, J = 7.6 Hz); ^{13}C NMR (75 MHz, $CDCl_3$, mixture of rotamers) 26.1, 26.4, 26.8, 27.5, 28.2, 33.9, 42.1,42.3, 66.3 (b), 67.0 (b), 80.4, 80.9, 126.2, 126.4, 128.0, 128.1, 146.3, 146.4, 155.6, 156.8, 175.3, 175.4; HREIMS m/z 307.1792 (calcd for $C_{17}H_{25}NO_4$, 307.1783).

(*S*)-*N*$^\alpha$-*tert*-Butoxycarbonyl-*N*$^\alpha$,*N*^{1}-dimethyltryptophan (58). (*S*)-*N*$^\alpha$-Boc-tryptophan (600 mg, 1.9 mmol) and methyl iodide (618 μL, 9.85 mmol) were dissolved in dry THF (4 mL), and the solution was cooled to 0 °C under an argon atmosphere. A 60% dispersion of sodium hydride in mineral oil (236 mg, 5.9 mmol) was added cautiously with gentle stirring, the reaction mixture was warmed to room temperature, and stirring was continued for 16 h. Ethyl acetate (20 mL) was then added, followed by the dropwise addition of H_2O (15 mL). The solution was evaporated to dryness, and the oily residue partitioned between ether (20 mL) and H_2O (50 mL). The ether layer was washed with saturated sodium hydrogen carbonate solution (20 mL), and the combined aqueous layer was acidified to pH 3 with citric acid. The product was extracted with ethyl acetate (3 × 25 mL), and the organic extracts were washed with H_2O (2 × 25 mL), 5% aqueous sodium thiosulfate solution (2 × 25 mL), and H_2O (25 mL), dried over magnesium sulfate, and evaporated to yield 621 mg (94% yield) of **58** as a pale yellow oil: $[\alpha]_D^{25}$ −9.1 (*c* 4.6, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) 1.23 (s, 6H), 1.47 (s, 3H), 2.76 (s, 1H), 2.86 (s, 2H), 3.19 (m, 1H), 3.49 (m, 1H), 3.72 (s, 3H), 4.87 (dd, 0.6H, J = 5 and 10 Hz), 5.11 (dd, 0.4H, J = 3 and 5 Hz), 6.89 (s, 0.6H), 6.96 (s, 0.4H), 7.15 (m, 3H) 7.29 (s, 1H), 7.64 (d, 1H, J = 6 Hz); ^{13}C NMR (50 MHz, $CDCl_3$) 24.9, 27.8, 28.2, 31.6, 31.9, 32.4, 59.0, 59.9, 80.5, 102.6, 118.4, 118.9, 121.6, 126.9, 127.5, 136.9, 155.4, 155.3, 176.4; HRCIMS m/z 333.1813 (calcd for $C_{18}H_{25}N_2O_4$ (M + H), 333.1814).

(*S*)-*N*$^\alpha$-*tert*-Butoxycarbonyl-*N*$^\alpha$-methylvalin-*N*-methoxy-*N*-methylamide (34). 34 was prepared according to General Procedure 6 with the following: *N*-Boc-*N*-methylvaline (5.0 g, 21.6 mmol); *N*,*O*-dimethylhydroxylamine hydrochloride (2.8 g, 28 mmol); PyBOP (11.2 g, 22 mmol); CH_2Cl_2 (22 mL); and DIEA (8.4 mL, 75 mmol). The crude product was purified by silica gel column chromatography of the crude product (1:3 diethyl ether/petroleum ether), which afforded **34** (4.46 g, 75% yield) as a colorless oil: $[\alpha]_D^{25}$ +128.3 (*c* 2.9, $CHCl_3$); ^{1}H NMR

(200 MHz, $CDCl_3$) 0.84 (d, 4H, J = 6.6 Hz), 0.85 (d, 2H, J = 6.6 Hz), 1.41 (s, 6H), 1.44 (s, 3H), 2.15−2.30 (m, 1H), 2.75 (s, 1H), 2.78 (s, 2H), 3.10 (bs, 3H), 3.64 (s, 1H), 3.68 (s, 2H), 4.66 (d, 0.4H, J = 10.0 Hz), 4.95 (d, 0.6H, J = 10.0 Hz); HRCIMS m/z 275.1971 (calcd for $C_{13}H_{27}N_2O_4$ (M + H), 275.1970).

(*S*)-*N*-*tert*-Butoxycarbonyl-*N*-methylvalinal (35). This compound was prepared using General Procedure 7 with the following: lithium aluminum hydride (875 mg, 23 mmol); (*S*)-N^{α}-Boc-N^{α}-methylvalin-*N*-methoxy-*N*-methylamide (**34**) (2.0 g, 7.7 mmol); THF (8 mL); and aqueous potassium hydrogen sulfate (100 mL, 0.23 M, 23 mmol). A yield of 1.52 g (92% yield) of crude aldehyde **35** was obtained. Aldehyde **35** was used without further purification: $[\alpha]_D^{25}$ −104.2 (*c* 5.5, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) 0.73 (d, 3H, J = 6.9 Hz), 0.91 (d, 3H, J = 6.9 Hz), 1.27 (s, 9H), 2.02−2.15 (m, 1H), 2.63 (s, 2H), 2.72 (s, 1H), 3.44 (d, 0.5H, J = 9.5 Hz), 3.86 (d, 0.5H, J = 9 Hz), 9.45 (s, 1H); HRCIMS m/z 216.1599 (calcd for $C_{11}H_{22}NO_3$ (M + H), 216.1599).

Ethyl (2*E*,4*S*)-*N*-*tert*-Butoxycarbonyl-*N*-methyl-4-amino-2,5-dimethylhex-2-enoate (17). This compound was prepared according to General Procedure 8 with the following: *N*-Boc-aminoaldehyde **35** (1.75 g, 8.7 mmol); CH_2Cl_2 (9.0 mL); and [(1-ethoxycarbonyl)ethylidene]triphenylphosphorane (4.19 g, 11.3 mmol). The crude oil was purified by silica gel column chromatography (2:3 diethyl ether/petroleum ether) to afford the required *E*-2-alkenoate **17** as a colorless oil (2.13 g, 82% yield): $[\alpha]_D^{25}$ +61.1 (*c* 9.1, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) 0.74 (d, 3H, J = 6 Hz), 0.79 (d, 3H, J = 6 Hz), 1.17 (t, 3H, J = 7 Hz), 1.34 (s, 9H), 1.72 (m, 1H), 1.78 (s, 3H), 2.60 (bs, 3H), 4.08 (q, 2H, J = 7 Hz), 4.15−4.20 (m, 0.5H), 4.21−4.32 (m, 0.5H), 6.54 (d, 1H, J = 8 Hz); HRCIMS m/z 300.2175 (calcd for $C_{16}H_{30}NO_4$ (M + H), 300.2175).

Ethyl (*S*)-*N*-*tert*-Butoxycarbonyl-*tert*-leucyl-(2*E*,4*S*)-*N*-methyl-4-amino-2,5-dimethylhex-2-enoate (59). Following General Procedure 10, dipeptide **59** was prepared with the following: *N*-Boc-*tert*-leucine[34] (156 mg, 0.52 mmol); pivaloyl chloride (64 μL, 0.52 mmol); DIEA (99 μL, 0.57 mmol); TFA salt of ethyl (2*E*,4*S*)-*N*-methyl-4-amino-2,5-dimethylhex-2-enoate (crude from General Procedure 9 with 110 mg (0.47 mmol) of **17**); DIEA (198 μL, 1.14 mmol); and THF (7 mL). Purification of the crude product by silica gel column chromatography (1:5 diethyl ether/petroleum ether) afforded 121 mg of **59** (62% yield). Alternatively compound **59** could be prepared in similar yield from the same starting materials by application of General Procedure 11: $[\alpha]_D^{25}$ −76.9 (*c* 2.43, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) 0.76 (d, 3H, J = 6 Hz), 0.80 (d, 3H, J = 6 Hz), 0.88 (s, 9H), 1.22 (t, 3H, J = 7 Hz), 1.33 (s, 9H), 1.79−1.89 (m, 1H), 1.83 (s, 3H), 2.91 (s, 3H), 4.12 (q, 2H, J = 7 Hz), 4.35 (d, 1H, J = 10 Hz), 5.03 (t, 1H, J = 10 Hz), 5.14 (d, 1H, J = 10 Hz), 6.57 (d, 1H, J = 8 Hz); HRCIMS m/z 413.3011 (calcd for $C_{22}H_{41}N_2O_5$ (M + H), 413.3015).

Ethyl (*S*)-*N*-*tert*-Butoxycarbonylvalyl-(2*E*,4*S*)-*N*-methyl-4-amino-2,5-dimethylhex-2-enoate (60). Following the General Procedure 10, ester **60** was prepared with the following: *N*-Boc-valine (100 mg, 0.46 mmol); DIEA (109 μL, 0.63 mmol); pivaloyl chloride (56 μL, 0.46 mmol); DIEA (160 μL, 0.92 mmol); TFA salt of ethyl (2*E*,4*S*)-*N*-methyl-4-amino-2,5-dimethylhex-2-enoate (crude from General Procedure 9 with 126 mg (0.42 mmol) of **17**); and THF (7 mL). Purification of the crude product by silica gel column chromatography (3:7 diethyl ether/petroleum ether) afforded 121 mg of **60** (69% yield). IR ($CHCl_3$): 2970, 1705, 1637, 1410 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) 0.82 (d, 3H, J = 6.8 Hz), 0.85 (d, 3H, J = 6.8 Hz), 0.86 (d, 6H, J = 6.8 Hz), 1.25 (t, 3H, J = 6.8 Hz), 1.38 (s, 9H), 1.82−1.94 (m, 2H), 1.84 (d, 3H, J = 1.5 Hz), 2.90 (s, 3H), 4.16 (q, 2H, J = 7.3 Hz), 4.31 (dd, 1H, J = 6.8 and 9.5 Hz), 5.00 (dd, 1H, J = 9.5 and 10.4 Hz), 5.16 (d, 1H, J = 9.3 Hz), 6.60 (dd, 1H, J = 1.5 and 9.3 Hz); ^{13}C NMR (50 MHz, $CDCl_3$) 11.5, 13.6, 14.2, 17.5, 18.7, 19.4, 19.5, 28.6, 29.9, 30.3, 30.9, 55.4, 56.3, 60.8, 79.4, 132.7, 138.3, 156.0, 167.7, 172.6; HRCIMS m/z 413.3017 (calcd for $C_{22}H_{41}N_2O_5$ (M + H), 413.3015).

Ethyl (*S*)-*N*-*tert*-Butoxycarbonyl-2-aminobutanoyl-(2*E*,4*S*)-*N*-methyl-4-amino-2,5-dimethylhex-2-enoate (61). Following the General Procedure 10, ester **61** was prepared with the following: (*S*)-*N*-Boc-2-aminobutanoic acid (93.5 mg, 0.46 mmol); DIEA (109 μL, 0.63 mmol); pivaloyl chloride (60 μL, 0.50 mmol); DIEA (160 μL, 0.92 mmol); TFA salt of ethyl (2*E*,4*S*)-*N*-methyl-4-amino-2,5-dimethylhex-2-enoate (crude from General Procedure 9 with 126 mg (0.42 mmol) of **17**); and THF (7 mL). Purification of the crude product by silica gel column chromatography (2:3 diethyl ether/petroleum ether) afforded 105 mg of **61** (63% yield): IR ($CHCl_3$) 2974, 1705, 1639, 1496 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) 0.82−0.91 (m, 9H), 1.27 (t, 3H, J = 7.1 Hz), 1.39 (s, 9H), 1.81−1.89 (m, 1H), 1.82 (d, 3H, J = 1.5 Hz), 1.91−1.95 (m, 1H), 2.88 (s, 3H), 4.17 (q, 2H, J = 7.1 Hz), 4.44−4.52 (m, 1H), 4.99 (dd, 1H, J = 9.3 and 10.6 Hz), 5.27 (d, 1H, J = 9.0 Hz), 6.61 (dd, 1H, J = 1.5 and 9.0 Hz); ^{13}C NMR (50 MHz, $CDCl_3$) 9.6, 11.5, 13.5, 14.1, 18.7, 19.4, 25.9, 28.7, 29.9, 51.6, 56.5, 60.8, 79.4, 132.8, 138.2, 167.7, 172.5; HRCIMS m/z 399.2855 (calcd for $C_{21}H_{39}N_2O_5$ (M + H), 399.2858).

Methyl (*S*)-*N*-*tert*-Butoxycarbonyl-*tert*-leucyl-*N*-methyl-4-aminobutanoate (62). Following the General Procedure 10, ester **62** was prepared with the following: *N*-Boc-*tert*-leucine[34] (106 mg, 0.42 mmol); DIEA (109 μL, 0.63 mmol); pivaloyl chloride (60 μL, 0.50 mmol); DIEA (160 μL, 0.92 mmol); methyl *N*-methyl-4-aminobutanoate hydrochloride (71 mg, 0.42 mmol); and THF (7 mL). Purification of the crude product by silica gel column chromatography (1:4 diethyl ether/petroleum ether) afforded 98 mg of **62** (68% yield): ^{1}H NMR (200 MHz, $CDCl_3$) 0.89 (s, 3H), 0.93 (s, 6H), 1.36 (s, 9H), 1.83−1.89 (m, 2H), 2.25 (t, 2H, J = 7.6 Hz), 2.86 (s, 1H), 3.06 (s, 2H), 3.11−3.50 (m, 2H), 4.42 (dd, 1H, J = 7.5 and 9.8 Hz), 5.27 (d, 1H, J = 9.8 Hz); ^{13}C NMR (50 MHz, $CDCl_3$) 22.2, 26.3, 28.2, 35.4, 36.3, 47.2, 49.7, 51.5, 55.6, 79.3, 171.9, 173.3; HRCIMS m/z 345.2386 (calcd for $C_{17}H_{33}N_2O_5$ (M + H), 345.2389).

Ethyl (*S*)-*N*-*tert*-Butoxycarbonyl-*tert*-leucyl-(2*E*)-*N*-methyl-4-amino-2-methylbut-2-enoate (63). Compound **63** was made by General Procedure 11 using the following: *N*-Boc-*tert*-leucine[34] (0.248 g, 1.07 mmol); HATU (0.406 g, 1.07 mmol); HOAt (0.146 g, 1.07 mmol); CH_2Cl_2 (1.5 mL); TMP (0.141 mL, 1.07 mmol, used instead of DIEA); TFA salt of ethyl (2*E*)-*N*-methyl-4-amino-2-methylbut-2-enoate (1.03 mmol, prepared from *N*-Bocglycine by General Procedures 4, 6−9 (15% yield to end of General Procedure 8)); CH_2Cl_2 (2.0 mL); and TMP (0.282 mL, 2.14 mmol, used instead of DIEA). The crude product was purified by silica gel column chromatography (1:1 diethyl ether/petroleum ether), affording 0.267 g (0.72 mmol, 70% yield) of **63** as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$, mixture of rotamers) 0.96 (s, 2.25H), 0.97 (s, 6.75H), 1.26 (t, 3H, J = 7.1 Hz), 1.40 (s, 2.25H), 1.41 (s, 6.75H), 1.88 (s, 2.25H), 1.90 (d, 0.75H, J = 1.3 Hz), 2.90 (s, 0.75H), 3.09 (s, 2.25H), 4.07−4.25 (m, 5H), 4.44 (d, 0.25H, J = 9.9 Hz); 4.49 (d, 0.75H, J = 9.8 Hz), 5.27 (d, 0.75H, J = 10.0 Hz), 6.56 (dt, 0.75H, J = 1.3 and 6.6 Hz), 6.59−6.63 (m, 0.25H); HREIMS m/z 370.24599 (calcd for $C_{19}H_{34}N_2O_5$, 370.24677).

Ethyl (*S*)-*N*-*tert*-Butoxycarbonyl-*tert*-leucyl-(2*E*,4*S*)-*N*-methyl-4-amino-2-methylpent-2-enoate (64). Compound **64** was made by General Procedure 11 using the following: *N*-Boc-*tert*-leucine[34] (43.4 mg, 0.188 mmol); HATU (71.2 mg, 0.188 mmol); HOAt (25.5 mg, 0.188 mmol); CH_2Cl_2 (1.0 mL); DIEA (0.0327 mL, 0.188 mmol); TFA salt of ethyl (2*E*,4*S*)-*N*-methyl-4-amino-2-methylpent-2-enoate (0.169 mmol, prepared from *N*-Boc-*N*-methylalanine by General Procedures 6−9 (7% yield to end of General Procedure 8)); CH_2Cl_2 (1.5 mL); and DIEA (0.0653 mL, 0.376 mmol). The crude product was purified by radial column chromatography (1 mm plate, 3:7 diethyl ether/petroleum ether, loaded with chloroform) affording 39.8 mg (0.104 mmol, 61% yield) of **64** as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$, major rotamer) 0.93 (s, 9H), 1.21 (d, 3H, J = 6.8 Hz), 1.27 (t, 3H, J = 7.2 Hz), 1.40 (s, 9H), 1.85 (d, 3H, J = 1.5 Hz), 2.97 (s, 3H), 4.17 (q, 2H, J = 7.2 Hz), 4.42 (d, 1H, J = 9.8 Hz), 5.26 (bd, 1H, J = 9.1 Hz), 5.46−5.53 (m, 1H), 6.59 (dd, 1H, J = 1.5 and 8.6 Hz); HREIMS m/z 384.2625 (calcd for $C_{20}H_{36}N_2O_5$, 384.2624).

Ethyl (*S*)-*N*-*tert*-Butoxycarbonyl-*tert*-leucyl-(2*E*,4*S*)-4-amino-2,5-dimethylhex-2-enoate (65). Compound **65** was made by General Procedure 12 using the following: *N*-Boc-*tert*-leucine[34] (0.205 g, 0.887 mmol); PyBOP (0.461 g, 0.887

mmol); TFA salt of ethyl (2*E*,4*S*)-4-amino-2,5-dimethylhex-2-enoate (0.822 mmol, prepared from *N*-Bocvaline by General Procedures 6−9 (69% yield to end of General Procedure 8)); CH_2Cl_2 (1.5 mL); and DIEA (0.464 mL, 2.66 mmol). The crude product was purified by radial column chromatography (2 mm plate, 3:7 diethyl ether/petroleum ether, loaded with chloroform), affording 0.328 g (0.822 mmol, quantitative yield) of a colorless oil. **65**: ^{1}H NMR (400 MHz, $CDCl_3$) 0.88 (d, 3H, J = 6.8 Hz), 0.91 (d, 3H, J = 6.8 Hz), 0.95 (3, 9H), 1.27 (t, 3H, J = 14.3 Hz), 1.40 (s, 9H), 1.78 (octet, 1H, J = 6.8 Hz), 1.91 (d, 3H, J = 1.1 Hz), 3.72 (bd, 1H, J = 9.7 Hz), 4.17 (q, 2H, J = 7.1 Hz), 4.55 (bq, 1H, J = 8.5 Hz), 5.16 (bs, 1H), 5.67 (bs, 1H), 6.47 (d, 1H, J = 9.7 Hz); HREIMS *m*/*z* 398.2778 (calcd for $C_{21}H_{38}N_2O_5$, 398.2782).

***N*^{16}-*tert*-Butoxycarbonylhemiasterlin Ethyl Ester (66).** Following general procedure 13, ester **66** was prepared with the following: (*S*)-*N*$^{\alpha}$-Boc-*N*$^{\alpha}$,*N*^{1},*β*,*β*-tetramethyltryptophan 13 (50 mg, 0.14 mmol); DIEA (72 *μ*L, 0.41 mmol); DMAP (10 mg, 0.08 mmol); PyBroP (65 mg, 0.14 mmol); TFA salt of ethyl (*S*)-*tert*-leucyl-(2*E*,4*S*)-*N*-methyl-4-amino-2,5-dimethylhex-2-enoate (crude from General Procedure 9 with 74 mg (0.18 mmol) of **59**); and CH_2Cl_2 (2 mL). Purification of the crude product by silica gel column chromatography (3:7 diethyl ether/petroleum ether) afforded 48 mg of **66** (53% yield): ^{1}H NMR (400 MHz, $CDCl_3$, mixture of rotamers) 0.42 (s, 6H), 0.46 (s, 3H), 0.82 (t, 3H, J = 6.7 Hz), 0.86 (d, 3H, J = 6.4 Hz), 1.26 (t, 3H, J = 7.3 Hz), 1.40 (s, 1H), 1.42 (s, 1H), 1.49 (s, 6H), 1.51 (s, 2H), 1.52 (s, 3H), 1.54 (s, 2H), 1.84 (d, 3H, J = 1.2 Hz), 1.80−1.90 (m, 1H), 2.92 (s, 3H), 2.99 (s, 3H), 3.72 (s, 2H), 3.73 (s, 1H), 4.09 (q, 0.3H, J = 7.0 Hz), 4.16 (q, 0.7H, J = 7.0 Hz), 4.37 (d, 0.7H, J = 8.6 Hz), 4.45 (d, 0.3H, J = 8.8 Hz), 5.02 (t, 1H, J = 10.4 Hz), 5.64 (s, 0.3H), 5.97 (s, 0.7H), 6.12 (d, 0.3H, J = 8.6 Hz), 6.18 (d, 0.7H, J = 8.5 Hz), 6.60 (dd, 1H, J = 1.2 and 9.0 Hz), 7.05 (s, 0.3H), 7.06 (s, 0.7H), 7.16−7.30 (m, 3H), 7.95 (d, 0.3H, J = 8.2 Hz), 8.27 (d, 0.7H, J = 7.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$, mixture of rotamers) 13.8, 14.2, 18.8, 19.4, 24.1, 24.3, 25.9, 27.7, 28.4, 28.6, 29.7, 30.1, 30.3, 31.1, 32.6, 32.7, 33.8, 34.1, 34.4, 34.7, 39.5, 39.6, 54.8, 55.2, 55.9, 60.8, 63.0, 64.2, 77.2, 79.5, 80.6, 109.3, 109.7, 118.8, 119.7, 120.7, 121.4, 121.6, 121.9, 121.9, 122.1, 124.9, 125.0, 126.6, 126.8, 132.4, 132.5, 138.0, 138.8, 139.0, 156.3, 157.3, 167.7, 169.7, 170.4, 171.3, 171.4; HRCIMS *m*/*z* 655.4423 (calcd for $C_{37}H_{59}N_4O_6$ (M + H), 655.4434); *anal.* calcd for $C_{37}H_{58}N_4O_6$, C 67.86%; H 8.93%; N 8.56%; found, C 67.98%; H 8.70%; N 8.34%.

***N*^{16}-*tert*-Butoxycarbonyl-*N*^{16}-desmethylhemiasterlin Ethyl Ester (67).** Following the general procedure 13, ester **67** was prepared with the following: (*S*)-*N*$^{\alpha}$-Boc-*N*^{1},*β*,*β*-trimethyltryptophan **32** (30 mg, 0.08 mmol); DIEA (45 *μ*L, 0.26 mmol); DMAP (6.2 mg, 0.05 mmol); PyBroP (40 mg, 0.08 mmol); TFA salt of ethyl (*S*)-*tert*-leucyl-(2*E*,4*S*)-*N*-methyl-4-amino-2,5-dimethylhex-2-enoate (crude from General Procedure 9 with 32 mg (0.08 mmol) of **59**); and CH_2Cl_2 (2 mL). Purification of the crude product by silica gel column chromatography (2:3 diethyl ether/petroleum ether) afforded 21 mg of **67** (42% yield): ^{1}H NMR (200 MHz, $CDCl_3$) 0.41 (s, 9H), 0.85 (d, 3H, J = 6.6 Hz), 0.86 (d, 3H, J = 6.6 Hz), 1.14 (s, 3H), 1.26 (t, 3H, J = 7.1 Hz), 1.46 (s, 9H), 1.79−1.90 (m, 1H), 1.84 (d, 3H, J = 1.5 Hz), 2.95 (s, 3H), 3.76 (s, 3H), 4.16 (q, 1H, J = 7.1 Hz), 4.98 (t, 1H, J = 10.2 Hz), 5.12 (d, 1H, J = 8.3 Hz), 5.39 (d, 1H, J = 8.3 Hz), 5.69 (d, 1H, J = 8.1 Hz), 6.61 (dd, 1H, J = 1.5 and 9.5 Hz), 6.99 (s, 1H), 7.11−7.31 (m, 3H), 8.13 (d, 1H, J = 8.1 Hz); HRCIMS *m*/*z* 641.4280 (calcd for $C_{36}H_{57}N_4O_6$ (M + H), 641.4278).

***N*^{16}-*tert*-Butoxycarbonyl-11,11′-bisdesmethylhemiasterlin Ethyl Ester (68).** Following the general procedure 13, ester **68** was prepared with the following: (*S*)-*N*$^{\alpha}$-Boc-*N*$^{\alpha}$,*N*^{1}-dimethyltryptophan **58** (44 mg, 0.13 mmol); DIEA (70 *μ*L, 0.40 mmol); DMAP (10 mg, 0.08 mmol); PyBroP (62 mg, 0.13 mmol); TFA salt of ethyl (*S*)-*tert*-leucyl-(2*E*,4*S*)-*N*-methyl-4-amino-2,5-dimethylhex-2-enoate (crude from General Procedure 9 with 50 mg (0.12 mmol) of **59**); and CH_2Cl_2 (2 mL). Purification of the crude product by silica gel column chromatography (1:1 diethyl ether/petroleum ether) afforded 45 mg (54% yield) of **68**: ^{1}H NMR (200 MHz, $CDCl_3$) 0.67−0.89 (m, 6H), 0.91 (s, 9H), 1.13 (s, 1H), 1.15 (s, 1H), 1.21 (s, 4H), 1.22 (s, 1H), 1.28 (t, 3H, J = 7.1 Hz), 1.40 (s, 2H), 1.82−1.92 (m, 1H), 1.86 (d, 3H, J = 1.2 Hz), 2.72 (s, 1H), 2.80 (s, 2H), 2.88 (s, 1H), 2.96 (s, 2H), 3.01−3.2 (m, 1H), 3.26−3.48 (m, 1H), 3.67 (s, 3H), 4.17 (q, 2H, J = 7.3 Hz), 4.79 (t, 0.6H, J = 9.0 Hz), 4.86−5.09 (m, 1.5H), 6.60 (d, 1H, J = 9.0 Hz), 6.81 (bs, 1H), 7.02−7.25 (m, 3H), 7.55 (m, 1H); HRCIMS *m*/*z* 627.4138 (calcd for $C_{36}H_{57}N_4O_6$ (M + H), 627.4121).

Ethyl (*S*)-*N*$^{\alpha}$-*tert*-Butoxycarbonyltryptophanyl-(*S*)-*tert*-leucyl-(2*E*,4*S*)-*N*-methyl-4-amino-2,5-dimethylhex-2-enoate (69). Following the general procedure 13, ester **69** was prepared with the following: *N*$^{\alpha}$-Boc-tryptophan (40 mg, 0.13 mmol); DIEA (70 *μ*L, 0.40 mmol); DMAP (10 mg, 0.08 mmol); PyBrop (62 mg, 0.13 mmol); TFA salt of ethyl (*S*)-*tert*-leucine-(2*E*,4*S*)-*N*-methyl-4-amino-2,5-dimethylhex-2-enoate (crude from General Procedure 9 with 50 mg (0.12 mmol) of **59**); and CH_2Cl_2 (2 mL). Purification of the crude product by silica gel column chromatography (1:1 diethyl ether/petroleum ether) afforded 43 mg (59% yield) of **69**: ^{1}H NMR (200 MHz, $CDCl_3$) 0.76 (d, 3H, J = 6.6 Hz), 0.83 (d, 3H, J = 6.6 Hz), 0.84 (s, 9H), 1.27 (t, 3H, J = 7.1 Hz), 1.37 (s, 9H), 1.82−1.90 (m, 1H), 1.86 (d, 3H, J = 1.5 Hz), 2.93 (s, 3H), 3.17 (d, 1H, J = 6.8 Hz), 4.17 (q, 2H, J = 7.3 Hz), 4.42 (q, 1H, J = 7.1 Hz), 7.75 (d, 1H, J = 9.5 Hz), 5.00 (t, 1H, J = 9.5 Hz), 5.10 (d, 0.5H, J = 7.8 Hz), 6.62 (dd, 1H, J = 1.5 and 9.5 Hz), 7.04−7.20 (m, 4H), 7.31 (dd, 1H, J = 1.2 and 6.7 Hz), 7.67 (d, 1H, J = 7.1 Hz), 8.3 (s, 1H); HRCIMS *m*/*z* 599.3806 (calcd for $C_{33}H_{51}N_4O_6$ (M + H), 599.3808).

***N*^{16}-*tert*-Butoxycarbonyl-21-desmethylhemiasterlin Ethyl Ester (70).** Following the general procedure 13, ester **70** was prepared with the following: (*S*)-*N*$^{\alpha}$-Boc-*N*$^{\alpha}$,*N*^{1},*β*,*β*-tetramethyltryptophan **13** (5 mg, 0.014 mmol); DIEA (7.2 *μ*L, 0.041 mmol); DMAP (1 mg, 0.008 mmol); PyBrop (7.1 mg, 0.014 mmol); TFA salt of ethyl (*S*)-valyl-(2*E*,4*S*)-*N*-methyl-4-amino-2,5-dimethylhex-2-enoate (crude from General Procedure 9 with 6.8 mg (0.016 mmol) of **60**); and CH_2Cl_2 (1 mL). Purification of the crude product by silica gel column chromatography (3:7 diethyl ether/petroleum ether) afforded 4.4 mg (51% yield) of **70**: ^{1}H NMR (400 MHz, $CDCl_3$) 0.22 (d, 1H, J = 6.7 Hz), 0.30 (d, 0.5H, J = 6.7 Hz), 0.54 (d, 1H, J = 6.7 Hz), 0.59 (d, 0.5H, J = 6.7 Hz), 0.69−0.94 (m, 9H), 1.23 (s, 9H), 1.27 (t, 3H, J = 7.3 Hz), 1.48 (s, 2H), 1.50 (s, 2H), 1.57 (s, 1H), 1.60 (s, 1H), 1.82 (s, 2H), 1.87 (d, 1H, J = 1.5 Hz), 1.95−2.08 (m, 1H), 2.81 (s, 2H), 2.83 (s, 1H), 2.96 (s, 2H), 2.97 (s, 1H), 3.45 (q, 0.8H, J = 7.0 Hz), 3.74 (s, 3H), 4.13−4.21 (m, 1.5H), 4.32 (t, 0.6H, J = 7.0 Hz), 4.41 (t, 0.4H, J = 7.0 Hz), 4.76 (dd, 0.6H, J = 6.0 and 10.0 Hz), 4.93−5.00 (m, 1.5H), 5.53 (bs, 0.4H), 5.83 (s, 0.6H), 6.12 (d, 0.5H, J = 7.9 Hz), 6.57−6.63 (m, 1H), 7.02 (s, 0.4H), 7.05 (s, 0.6H), 7.14−7.29 (m, 3H), 7.91 (d, 0.4H, J = 8.2 Hz), 8.19 (d, 0.6H, J = 8.2 Hz); HRCIMS *m*/*z* 641.4284 (calcd for $C_{36}H_{57}N_4O_6$ (M + H), 641.4278).

***N*^{16}-*tert*-Butoxycarbonyl-21,21′-bisdesmethylhemiasterlin Ethyl Ester (71).** Following the general procedure 13, ester **71** was prepared with the following: (*S*)-*N*$^{\alpha}$-Boc-*N*$^{\alpha}$,*N*^{1},*β*,*β*-tetramethyltryptophan **13** (5 mg, 0.014 mmol); DIEA (7.1 *μ*L, 0.41 mmol); DMAP (1 mg, 0.008 mmol); PyBrop (7.1 mg, 0.014 mmol); TFA salt of ethyl (*S*)-2-aminobutanoyl-(2*E*,4*S*)-*N*-methyl-4-amino-2,5-dimethylhex-2-enoate (crude from General Procedure 9 with 6.2 mg (0.016 mmol) of **61**); and CH_2Cl_2 (1 mL). Purification of the crude product by silica gel column chromatography (3:7 diethyl ether/petroleum ether) afforded 4.0 mg (46% yield) of **71**: ^{1}H NMR (400 MHz, $CDCl_3$) 0.41 (t, 2H, J = 7.3 Hz), 0.47 (t, 1H, J = 7.3 Hz), 0.77 (d, 3H, J = 6.5 Hz), 0.86 (d, 3H, J = 6.7 Hz), 1.18 (t, 1H, J = 7.0 Hz), 1.27 (t, 2H, J = 7.0 Hz), 1.47 (s, 9H), 1.51 (s, 1H), 1.59 (s, 4H), 1.61 (s, 1H), 1.79−1.85 (m, 1H), 1.80 (s, 3H), 2.74 (s, 2H), 2.77 (s, 1H), 2.97 (s, 2H), 2.99 (s, 1H), 3.45 (q, 0.7H, J = 7.0 Hz), 3.74 (s, 3H), 4.16 (q, 1.3H, J = 7.0 Hz), 4.45 (q, 0.6H, J = 6.5 Hz), 4.52 (q, 0.4H, J = 6.5 Hz), 4.91 (t, 1H, J = 9.3 Hz), 5.43 (bs, 0.6H), 5.73 (s, 0.6H), 6.10 (d, 0.4H, J = 7.6 Hz), 6.16 (d, 0.6H, J = 8.8 Hz), 6.58 (d, 1H, J = 7.6 Hz), 6.99 (s, 0.4H), 7.01 (s, 0.6H), 7.12−7.28 (m, 3H), 7.89 (d, 0.4H, J = 8.2 Hz), 8.14 (d, 0.6H, J = 7.6 Hz); HRCIMS *m*/*z* 627.4138 (calcd for $C_{35}H_{55}N_4O_6$ (M + H), 627.4121).

Methyl (*S*)-*N*$^{\alpha}$-*tert*-Butoxycarbonyl-*N*$^{\alpha}$,*N*^{1},*β*,*β*-tetramethyltryptophanyl-(*S*)-*tert*-leucyl-*N*-methyl-4-amino-

butanoate (72). Following the general procedure 13, ester **72** was prepared with the following: (*S*)-N^{α}-Boc-N^{α},N^{1},*β*,*β*-tetramethyltryptophan **13** (10 mg, 0.028 mmol); DIEA (14.5 *μ*L, 0.083 mmol); DMAP (2 mg 0.016 mmol); PyBroP (13 mg, 0.028 mmol); TFA salt of methyl (*S*)-*tert*-leucyl-*N*-methyl-4-aminobutanoate (crude from General Procedure 9 with 12.5 mg (0.036 mmol) of **62**); and CH_2Cl_2 (1 mL). Purification of the crude product by silica gel column chromatography (2:3 diethyl ether/petroleum ether) afforded 6.1 mg (37% yield) of **72**: ^{1}H NMR (400 MHz, $CDCl_3$) 0.49 (d, 3H, J = 6.5 Hz), 0.54 (d, 3H, J = 6.5 Hz), 0.82−0.85 (m, 1H), 1.43 (s, 1H), 1.44 (s, 1H), 1.49 (s, 9H), 1.51 (s, 1H), 1.54 (s, 1H), 1.57 (s, 1H), 1.63 (s, 1H), 1.76−1.78 (m, 1H), 2.24−2.30 (m, 1H), 3.01 (s, 2H), 3.07 (s, 1H), 3.22−3.32 (m, 1H), 3.33−3.42 (m, 1H), 3.65 (s, 3H), 3.72 (s, 1H), 3.73 (s, 2H), 4.39 (d, 0.5H, J = 8.8 Hz), 4.48 (d, 0.2H, J = 9.1 Hz), 4.55 (d, 0.2H, J = 9.7 Hz), 4.60 (d, 0.1H, J = 9.5 Hz), 5.52 (s, 0.1H), 5.57 (bs, 0.2H), 5.82 (s, 0.2H), 5.88 (s, 0.5H), 5.99 (d, 0.3H, J = 8.8 Hz), 6.06 (d, 0.5H, J = 8.5 Hz), 6.22 (d, 0.2H, J = 8.2 Hz), 6.84−7.08 (m, 1.3H), 7.12−7.29 (m, 3H), 7.91−7.94 (m. 0.3H), 8.15−8.20 (m, 0.3H), 8.23 (d, 0.4H, J = 7.3 Hz); HRCIMS *m*/*z* 587.3803 (calcd for $C_{32}H_{51}N_4O_6$ (M + H), 587.3808).

Ethyl (*S*)-*N*-*tert*-Butoxycarbonyl-*N*,*β*,*β*-trimethylphenylalanyl-(*S*)-*tert*-leucyl-(2*E*,4*S*)-*N*-methyl-4-amino-2,5-dimethylhex-2-enoate (73). For the formation of compound **73**, General Procedure 12 was employed with the following: (*S*)-*N*-*tert*-butoxycarbonyl-*N*,*β*,*β*-trimethylphenylalanine (37.6 mg, 0.122 mmol); TFA salt of ethyl (*S*)-*tert*-leucyl-(2*E*,4*S*)-*N*-methyl-4-amino-2,5-dimethylhex-2-enoate (crude for General Procedure 9 with 46.4 mg (0.113 mmol) of **60**); PyBOP (63.6 mg, 0.122 mmol); CH_2Cl_2 (1.5 mL); and DIEA (0.059 mL, 0.338 mmol). The product was purified by silica gel column chromatography (1:1 diethyl ether/petroleum ether), affording the protected tripeptide **73** as a clear colorless oil in 52% yield (35.2 mg, 0.0585 mmol): $[\alpha]_D^{27}$ −85.6 (*c* 1.04, $CHCl_3$); IR (neat) 3411, 1719−1620 (br), 1499,1481, 1391, 1368, 1327, 1280, 1248, 1154 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$, mixture of rotamers) 0.72−0.81 (m, 12H), 0.85 (d, 3H, J = 6.5 Hz), 1.27 (t, 3H, J = 7.1 Hz), 1.36 (s, 3H), 1.42 (s, 9H), 1.51 (s, 3H), 1.78−1.86 (m, 1H), 1.85 (s, 3H), 2.86−2.90 (m, 6H), 4.17 (q, 2H, J = 7.1 Hz), 4.58 (bd, 0.7H, J = 9.2 Hz), 4.66 (bd, 0.3H, J = 9.2 Hz), 4.87 (bs, 0.3H), 5.01−5.05 (m, 1H), 5.27 (bs, 0.7H), 5.98−6.05 (m, 1H), 6.60 (dd, 1H, J = 1.4 and 9.2 Hz), 7.17 (t, 1H, J = 7.5 Hz), 7.30 (bt, 2H, J = 7.5 Hz), 7.40 (bd, 0.6 H, J = 7.5 Hz), 7.52 (bd, 1.4H, J = 7.5 Hz); ^{13}C NMR (75 MHz, $CDCl_3$, major rotamer) 13.8, 14.2, 18.8, 19.4, 24.9, 26.3, 27.7, 28.3, 30.0, 30.9, 33.6, 34.6, 42.4, 54.8, 55.8, 60.8, 65.2, 80.0, 126.3, 126.5, 128.5, 132.6, 138.7, 147.2, 157.0, 167.7, 169.6, 171.1; HREIMS *m*/*z* 601.4115 (calcd for $C_{34}H_{55}N_3O_6$, 601.4090).

Ethyl (*S*)-*N*-*tert*-Butoxycarbonyl-*N*-methyl-*tert*-leucyl-(*S*)-*tert*-leucyl-(2*E*,4*S*)-*N*-methyl-4-amino-2,5-dimethylhex-2-enoate (74). For the formation of compound **74** General Procedure 12 was employed with the following: (*S*)-*N*-Boc-*N*-methyl-*tert*-leucine (74.3 mg, 0.303 mmol); TFA salt of ethyl (*S*)-*tert*-leucyl-(2*E*,4*S*)-*N*-methyl-4-amino-2,5-dimethylhex-2-enoate (crude from General Procedure 9 with 0.100 mg (0.244 mmol) of **60**); PyBOP (158 mg, 0.303 mmol); CH_2Cl_2 (1.5 mL); and DIEA (0.158 mL, 0.909 mmol). The product was purified by radial chromatography (1 mm plate, 1:2 diethyl ether/petroleum ether, loaded with chloroform), affording the protected tripeptide **74** in 95% yield (0.125 g, 0.231 mmol) as a clear colorless oil, which solidified upon standing: mp 93−96 °C; $[\alpha]_D^{25}$ −126.6 (*c* 2.6, $CHCl_3$); IR (KBr pellet) 2964, 1718, 1685, 1645, 1482, 1367, 1248, 1176, 1147 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$, mixture of rotamers) 0.78 (d, 3H, J = 6.6 Hz), 0.85 (d, 3H, J = 6.5 Hz), 0.92 (s, 9H), 1.02 (s, 9H), 1.28 (t, 3H, J = 7.1 Hz), 1.44 (s, 9H), 1.87 (s, 3H), 1.81−1.90 (m, 1H), 2.93 (s, 3H), 2.97 (s, 3H), 4.18 (q, 2H, J = 7.1 Hz), 4.24 (bs, 0.4H), 4.46 (bs, 0.6H), 4.77 (bd, 1H, J = 9.0 Hz), 5.07 (t, 1H, J = 9.6 Hz), 6.23 (bs, 0.4H), 6.37 (bd, 0.6H, J = 9.0 Hz), 6.61 (dd, 1H, J = 1.3 and 9.6 Hz); ^{13}C NMR (75 MHz, $CDCl_3$, major rotamer) 13.7, 14.1, 18.5, 19.3, 26.3, 27.6, 28.3, 30.0, 30.9, 32.5, 32.9, 34.9, 53.9, 55.7, 60.7, 64.4, 79.9, 132.7, 138.5, 156.9, 167.6, 169.8, 171.2; HRCIMS *m*/*z* 539.3913 (calcd for $C_{29}H_{53}N_3O_6$ (M + H), 539.3934).

(−)-Hemiasterlin (1). Following General Procedures 5 and 9, hemiasterlin (**1**) was prepared with the following: N^{16}-Boc-hemiasterlin ethyl ester **70** (10 mg, 0.015 mmol); lithium hydroxide (122 *μ*L, 1.0 M, 0.12 mmol); CH_2Cl_2 (1 mL); and TFA (1 mL). Purification of the crude product by reversed-phase HPLC (1:1 H_2O/MeOH with 0.05% TFA) afforded 6.5 mg of an amorphous white solid (**1**, 83% yield): $[\alpha]_D^{25}$ −76 (*c* 0.7, MeOH); UV (MeOH) λ_{max} (ϵ) 216 (15400), 273 nm (1600); IR (neat) 3412, 2962, 1650, 1635 cm^{-1}; ^{1}H NMR (400 MHz, D_6-DMSO) 0.78 (d, 3H, J = 7 Hz), 0.80 (d, 3H, J = 7 Hz), 0.99 (s, 9H), 1.37 (s, 3H), 1.40 (s, 3H), 1.80 (s, 3H), 1.99−2.08 (m, 1H), 2.22 (bs, 3H), 3.02 (s, 3H), 3.75 (s, 3H), 4.41 (d, 1H, J = 10.3 Hz), 4.85 (d, 1H, J = 9.4 Hz), 4.93 (t, 1H, J = 10.1 Hz), 6.66 (dd, 1H, J = 1.1 and 10 Hz), 7.08 (t, 1H, J = 8 Hz), 7.17 (s, 1H), 7.20 (t, 1H, J = 8 Hz), 7.34 (bs, 1H), 7.45 (d, 1H, J = 8 Hz), 8.11 (d, 1H, J = 8 Hz), 8.85 (bs, 1H), 8.89 (d, 1H, J = 8.4 Hz); ^{13}C NMR (100 MHz, D_6-DMSO) 13.5, 18.9, 19.3, 22.6, 26.3, 27.0, 28.7, 31.1, 32.4, 33.4, 34.6, 37.5, 55.6, 56.2, 67.5, 110.0, 116.5, 118.4, 120.6, 121.1, 125.0, 128.7, 131.6, 137.7, 138.3, 166.0, 168.5, 170.1; HRFABMS *m*/*z* 527.3609 (calcd for $C_{30}H_{47}N_4O_4$ (M + H), 527.3597).

(*S*)-*N*,*β*,*β*-Trimethylphenylalanyl-(*S*)-*tert*-leucyl-(2*E*,4*S*)-*N*-methyl-4-amino-2,5-dimethylhex-2-enoic acid (SPA110, 8). The protecting groups were removed by General Procedures 5 and 9 with the following: ethyl ester **73** (25.7 mg, 0.0427 mmol); MeOH (3 mL); H_2O (1 mL); lithium hydroxide (1.0 M aqueous, 1 mL); CH_2Cl_2 (2 mL); and TFA (2 mL). HPLC purification of the crude product using a Magnum reversed-phase ODS column (45:55 H_2O/MeOH with 0.05% TFA) afforded the tripeptide **8** as a white powder (24.1 mg, 0.0410 mmol) in 96% yield. Alternatively, purification could be accomplished by lyophilization: mp 135−137 °C; $[\alpha]_D^{22}$ −21.7 (*c* 0.23, MeOH); IR (KBr pellet) 3600−2800 (br), 1678, 1204 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) 0.88 (d, 3H, J = 6.5 Hz), 0.89 (d, 3H, J = 6.1 Hz), 1.05 (s, 9H), 1.37 (s, 3H), 1.46 (s, 3H), 1.90 (s, 3H), 1.99−2.08 (m, 1H), 2.49 (s, 3H), 3.13 (s, 3H), 4.34 (s, 1H), 4.91 (s, 1H), 5.04 (t, 1H, J = 10.1 Hz), 6.76 (d, 1H, J = 9.1 Hz), 7.34 (t, 1H, J = 7.6 Hz), 7.44 (t, 2H, J = 7.6 Hz), 7.53 (d, 2H, J = 7.6 Hz); ^{13}C NMR (75 MHz, D_3COD) 14.2, 19.8 (2×), 21.5, 26.9, 29.8, 30.8, 31.9, 34.2, 36.0, 42.1, 57.8, 58.5, 71.3, 127.4, 128.9, 130.4, 133.7, 139.7, 145.0, 167.2, 170.9, 172.3; HREIMS *m*/*z* 474.3327 (calcd for $C_{27}H_{44}N_3O_4$, 474.3331).

Hemiasterlin Methyl Ester (SPA107, 11). To a stirred suspension of hemiasterlin (**1**, 5.0 mg, 0.009 mmol) in ether (1 mL) at room temperature was added an ethereal solution of diazomethane dropwise until the yellow color of the diazomethane persisted in the reaction mixture, and TLC analysis showed complete consumption of starting material. Excess diazomethane was removed under a stream of argon and the remaining solvent removed in vacuo to afford ester **11** as a crystalline solid (5 mg, 100% yield). Recrystallization of **11** from 1:3 acetone/hexanes solution afforded clear, colorless rodlike crystals:[9] ^{1}H NMR (500 MHz, $CDCl_3$) 0.78 (d, 3H, J = 7 Hz), 0.86 (d, 3H, J = 7 Hz), 0.96 (s, 9H), 1.45 (s, 3H), 1.57 (s, 3H), 1.89 (s, 3H), 1.98−2.05 (m, 1H), 3.03 (s, 3H), 3.59 (s, 1H), 3.73 (s, 3H), 3.74 (s, 3H), 4.84 (m, 1H), 5.08 (t, 1H, J = 9.5 Hz), 6.64 (d, 1H, J = 9.5 Hz), 7.07 (t, 1H, J = 8 Hz), 7.12 (t, 1H, J = 8 Hz), 7.20 (d, 1H, J = 8 Hz), 7.29 (s, 1H), 7.86 (m, 1H); HRFABMS *m*/*z* 541.3755 (calcd for $C_{31}H_{49}N_4O_4$ (M + H), 541.3753).

27,28-Dihydrohemiasterlin (SPA108, 12). Hemiasterlin (**1**, 3 mg) was stirred overnight in a sealed Erlenmeyer flask with a positive pressure of hydrogen with ethanol (2 mL) and Pd/C catalyst (∼1.0 mg). Celite (50 mg) was added and the resulting slurry filtered. Excess solvents were removed in vacuo, and the crude material was purified by reversed-phase HPLC (1:1 H_2O/MeOH with 0.05% TFA) to afford 2 mg of **12** (67% yield): ^{1}H NMR (500 MHz, D_3COD) 0.80 (d, 3H, J = 6.4 Hz), 1.01 (d, 3H, J = 6.4 Hz), 1.10 (s, 9H), 1.19 (d, 3H, J = 7.1 Hz), 1.25−1.32 (m, 2H), 1.45 (s, 3H), 1.52 (s, 3H), 2.01−2.09 (m, 2H), 2.43 (s, 3H), 3.05 (s, 3H), 3.79 (s, 3H), 4.45 (s, 1H), 5.03 (s, 1H), 5.30−5.36 (m, 1H), 7.12 (s, 1H), 7.13 (t, 1H, J = 8.1 Hz), 7.24 (t, 1H, J = 8.1 Hz), 7.42 (d, 1H, J = 8.1 Hz), 8.07 (d, 1H, J = 8.1 Hz); HRFABMS *m*/*z* 529.3759 (calcd for $C_{30}H_{49}N_4O_4$ (M + H), 529.3753).

***N*^{16}-Acetylhemiasterlin (SPA100, 9).** Hemiasterlin (**1**, 5 mg) was stirred overnight under a nitrogen atmosphere with pyridine (0.5 mL) and acetic anhydride (0.5 mL). Excess reagents were removed in vacuo, and the crude material was purified by reversed-phase HPLC (3:7 H_2O/MeOH with 0.05% TFA) to afford 4 mg of **9** (74% yield): ^{1}H NMR (500 MHz, D_6-DMSO, mixture of rotamers) 0.40 (s, 9H), 0.74 (d, 3H, J = 8.4 Hz), 0.79 (d, 3H, J = 8 Hz), 1.29 (s, 1.5H), 1.47 (s, 1.5 H), 1.57 (s, 1H), 1.61 (s, 2H), 1.73 (s, 2 H), 1.78 (s, 1H), 1.81−1.97 (m, 1H), 2.08 (s, 3 H), 2.87 (s, 2H), 2.89 (s, 1H), 2.97 (s, 1H), 3.04 (s, 2H), 3.70 (s, 1H), 3.73 (s, 2H), 4.33 (d, 1H, J = 10.6 Hz), 4.75 (d, 0.5H, J = 11.4 Hz), 4.86−4.94 (m, 1.5H), 6.26 (s, 0.4H), 6.29 (d, 0.6H, J = 11.1 Hz), 6.60 (dd, 0.6H, J = 11.8, 1.5 Hz), 6.64 (d, 0.4H, J = 11.4 Hz), 6.98−7.06 (m, 1H), 7.10−7.19 (m, 1H), 7.23 (s, 0.6H), 7.35 (d, 0.4H, J = 10.3 Hz), 7.42 (d, 0.6H, J = 10.7 Hz), 7.96 (d, 0.4H, J = 10.3 Hz), 8.15 (d, 0.6H, J = 10.3 Hz), 8.48 (d, 0.4H, J = 11.1 Hz); HRFABMS m/z 569.3675 (calcd for $C_{32}H_{49}N_4O_5$ (M + H), 569.3702).

***N*^{16}-Permethylhemiasterlin Methyl Ester (SPA101, 10).** Hemiasterlin (**1**, 5 mg, 0.009 mmol) was stirred overnight under a nitrogen atmosphere with potassium hydrogen carbonate (∼2 mg) and methyl iodide (50 μL, 0.3 mmol) in DMF (2 mL). Excess reagents were removed in vacuo, and the crude product was purified by reversed-phase HPLC (2:1 H_2O/MeOH with 0.05% TFA), which afforded 3.5 mg of **10** (64% yield): ^{1}H NMR (500 MHz, D_6-DMSO) 0.77 (d, 3H, J = 6.7 Hz), 0.78 (d, 3H, J = 6.7 Hz), 1.00 (s, 9H), 1.37 (s, 3H), 1.72 (s, 3H), 1.83 (s, 3H), 2.01−2.06 (m, 1H), 2.90 (s, 9H), 3.04 (s, 3H), 3.66 (s, 3H), 3.76 (s, 3H), 4.74 (d, 1H, J = 7.6 Hz), 4.87 (s, 1H), 4.92 (t, 1H, J = 10 Hz), 6.70 (dd, 1H, J = 1.5, 9.7 Hz), 7.14 (t, 1H, J = 7.6 Hz), 7.22 (t, 1H, J = 7.6 Hz), 7.39 (s, 1H), 7.46 (d, 1H, J = 7.6 Hz), 8.05 (d, 1H, J = 7.6 Hz), 8.83 (d, 1H, J = 7.3 Hz); HRFABMS m/z 569.4068 (calcd for $C_{33}H_{53}N_4O_4$ (M + H), 569.4066).

***N*^{16}-Desmethylhemiasterlin (SPA102, 36).** Following the General Procedures 5 and 9, N^{16}-desmethylhemiasterlin (**36**) was prepared with the following: N^{16}-Boc-N^{16}-desmethylhemiasterlin ethyl ester **67** (10 mg, 0.015 mmol); lithium hydroxide (122 μL, 1.0 M, 0.12 mmol); CH_2Cl_2 (1 mL); and TFA (1 mL). Purification of the crude product by reversed-phase HPLC (1:1 H_2O/MeOH with 0.05% TFA) afforded 5.3 mg (69% yield) of **36**: ^{1}H NMR (400 MHz, CD_3OD) 0.85 (s, 9H), 0.90 (d, 3H, J = 6.4 Hz), 0.91 (d, 3H, J = 6.4 Hz), 1.46 (s, 3H), 1.54 (s, 3H), 1.89 (d, 3H, J = 1.3 Hz), 1.99−2.06 (m, 1H), 3.01 (s, 3H), 3.80 (s, 3H), 4.64 (s, 1H), 4.72 (s, 1H), 5.03 (t, 1H, J = 9.7 Hz), 6.76 (dd, 1H, J = 1.3 and 9.7 Hz), 7.13 (t, 1H, J = 8.1 Hz), 7.14 (s, 1H), 7.24 (t, 1H, J = 8.1 Hz), 7.42 (d, 1H, J = 8.1 Hz), 7.99 (d, 1H, J = 8.1 Hz); ^{13}C NMR (100 MHz, CD_3OD): 14.1, 19.8, 19.9, 24.8, 24.9, 26.6, 30.8, 32.0, 32.9, 35.6, 38.7, 57.5, 58.7, 60.4, 111.3, 118.0, 120.4, 121.0, 123.2, 125.9, 129.5, 133.4, 139.8, 168.4, 170.9, 172.4; HRFABMS m/z 513.3443 (calcd for $C_{29}H_{45}N_4O_4$ (M + H), 513.3440).

11,11′-Bisdesmethylhemiasterlin (SPA103, 37). Following the General Procedures 5 and 9, 11,11′-bisdesmethylhemiasterlin (**37**) was prepared with the following: N^{16}-Boc-11,11′-bisdesmethylhemiasterlin ethyl ester **68** (10 mg, 0.015 mmol); lithium hydroxide (122 μL, 1.0 M, 0.12 mmol); CH_2Cl_2 (1 mL); and TFA (1 mL). Purification of the crude product by reversed-phase HPLC (1:1 H_2O/MeOH with 0.05% TFA) afforded 6 mg (80% yield) of **37**: ^{1}H NMR (400 MHz, CD_3OD) 0.77 (d, 1H, J = 6.9 Hz), 0.86 (d, 1H, J = 6.9 Hz), 1.00 (s, 9H), 1.87−1.96 (m, 1H), 0.88 (s, 3H), 2.59 (s, 3H), 3.05 (s, 3H), 3.15 (dd, 1H, J = 15.3, 8 Hz), 3.34 (dd, 1H, J = 15.3, 6.1 Hz), 3.78 (s, 3H), 4.26 (dd, 1H, J = 6.1 and 6.5 Hz), 4.85 (s, 1H), 5.03 (t, 1H, J = 10.3 Hz), 6.73 (dd, 1H, J = 1.2 and 9.5 Hz), 7.10 (t, 1H, J = 8 Hz), 7.13 (s, 1H), 7.20 (t, 1H, J = 8 Hz), 7.36 (d, 1H, J = 8 Hz), 7.65 (d, 1H, J = 8 Hz); HRFABMS m/z 499.3280 (calcd for $C_{28}H_{43}N_4O_4$ (M + H), 499.3284).

(*S*)-Tryptophanyl-(*S*)-*tert*-leucyl-(2*E*,4*S*)-*N*-methyl-4-amino-2,5-dimethylhex-2-enoic acid (SPA104, 38). Following the General Procedures 5 and 9, (*S*)-tryptophanyl-(*S*)-*tert*-leucyl-(2*E*,4*S*)-*N*-methyl-4-amino-2,5-dimethylhex-2-enoic acid (**38**) was prepared with the following: ethyl (*S*)-N^{α}-Boc-tryptophanyl-(*S*)-*tert*-leucyl-(2*E*,4*S*)-*N*-methyl-4-amino-2,5-dimethylhex-2-enoate **69** (10 mg, 0.017 mmol); lithium hydroxide (136 μL, 1.0 M, 0.14 mmol); CH_2Cl_2 (1 mL); and TFA (1 mL). Purification of the crude product by reversed-phase HPLC (1:1 H_2O/MeOH with 0.05% TFA) afforded 5.3 mg (66% yield) of **38**: ^{1}H NMR (400 MHz, $CDCl_3$) 0.84 (d, 3H, J = 6.5 Hz), 0.88 (d, 3H, J = 6.5 Hz), 1.01 (s, 9H), 1.89 (d, 3H, J = 1.5 Hz), 1.93−2.00 (m, 1H), 3.02−3.11 (m, 1H), 3.06 (s, 3H), 3.36 (dd, 1H, J = 5 and 14.9 Hz), 4.32 (dd, 1H, J = 5.3 and 9.5 Hz), 4.85 (s, 1H), 5.05 (t, 3H, J = 9.9 Hz), 6.75 (dd, 3H, J = 1.5 and 9.9 Hz), 7.07 (t, 1H, J = 8 Hz), 7.14 (t, 1H, J = 8 Hz), 7.22 (s, 1H), 7.38 (d, 1H, J = 8 Hz), 7.73 (t, 1H, J = 8 Hz); HRFABMS m/z 471.2982 (calcd for $C_{26}H_{39}N_4O_4$ (M + H), 471.2971).

(*S*)-N^{α},N^1,*β*,*β*-Tetramethyltryptophanyl-(*S*)-*tert*-leucyl-*N*-methyl-4-aminobutanoic Acid (SPA109, 41). Following General Procedures 5 and 9, (*S*)-N^{α},N^1,*β*,*β*-tetramethyltryptophanyl-(*S*)-*tert*-leucyl-*N*-methyl-4-aminobutanoic acid (**41**) was prepared with the following: methyl (*S*)-N^{α},N^1,*β*,*β*-tetramethyltryptophanyl-(*S*)-*tert*-leucyl-*N*-methyl-4-aminobutanoate **72** (6 mg, 0.010 mmol); lithium hydroxide (82 μL, 1.0 M, 0.08 mmol); CH_2Cl_2 (1 mL); and TFA (1 mL). Purification of the crude product by reversed-phase HPLC (2:3 H_2O/MeOH with 0.05% TFA) afforded 2.2 mg (45% yield) of **41**: ^{1}H NMR (500 MHz, CD_3OD) 1.04 (s, 9H), 1.46−1.52 (m, 2H), 1.49 (s, 1H), 1.57 (s, 3H), 1.80−1.83 (m, 2H), 2.27−0.35 (m, 2H), 2.45 (s, 3H), 3.19 (s, 3H), 3.79 (s, 3H), 4.45 (s, 1H), 4.89 (s, 1H), 7.12−7.16 (m, 2H), 7.23 (t, 1H, J = 8 Hz), 7.41 (d, 1H, J = 8 Hz), 8.01 (d, 1H, J = 8 Hz); HRFABMS m/z 473.3134 (calcd for $C_{26}H_{41}N_4O_4$ (M + H), 473.3127).

21-Desmethylhemiasterlin (SPA105, 39). Following General Procedures 5 and 9, 21-desmethylhemiasterlin (**39**) was prepared with the following: N^{16}-Boc-21-desmethylhemiasterlin ethyl ester **70** (4.4 mg, 0.007 mmol); lithium hydroxide (56 μL, 1.0 M, 0.056 mmol); CH_2Cl_2 (1 mL); and TFA (1 mL). Purification of the crude product by reversed-phase HPLC (1:1 H_2O/MeOH with 0.05% TFA) afforded 2.5 mg (69% yield) of **39**: ^{1}H NMR (500 MHz, CD_3OD) 0.85−0.92 (m, 6H), 0.96 (d, 3H, J = 6.4 Hz), 1.04 (d, 3H, J = 6.4 Hz), 1.44 (s, 3H), 1.45 (s, 3H), 1.58 (s, 3H), 1.91−1.96 (m, 1H), 2.01−2.08 (m, 1H), 2.40 (s, 3H), 3.13 (s, 3H), 3.08 (s, 3H), 4.36 (s, 1H), 4.77 (m, 1H), 4.99−5.02 (m, 1H), 6.71−6.78 (m, 1H), 7.08−7.14 (m, 1H), 7.24 (t, 1H, J = 8 Hz), 7.41 (d, 1H, J = 8 Hz), 8.03 (d, 1H, J = 8 Hz); HRFABMS m/z 513.3449 (calcd for $C_{29}H_{45}N_4O_4$ (M + H), 513.3440).

21,21′-Bisdesmethylhemiasterlin (SPA106, 40). Following General Procedures 5 and 9, 21,21′-bisdesmethylhemiasterlin (**40**) was prepared with the following: N^{16}-Boc-21,21′-bisdesmethylhemiasterlin ethyl ester **71** (4 mg, 0.006 mmol); lithium hydroxide (48 μL, 1.0 M, 0.05 mmol); CH_2Cl_2 (1 mL); and TFA (1 mL). Purification of the crude product by reversed-phase HPLC (1:1 H_2O/MeOH with 0.05% TFA) afforded 2.3 mg (76% yield) of **40**: ^{1}H NMR (500 MHz, CD_3OD) 0.91 (d, 6H, J = 6.4 Hz,), 1.02 (t, 3H, J = 7.5 Hz), 1.45 (s, 3H), 1.64 (s, 3H), 1.72−1.78 (m, 1H), 1.79−1.91 (bs, 4H), 1.93−2.05 (m, 1H), 2.40 (s, 3H), 3.08 (s, 3H), 3.80 (s, 3H), 4.35 (s, 1H), 4.90−5.02 (m, 2H), 6.72−6.78 (m, 1H), 7.11−7.15 (m, 2H), 7.24 (d, 1H, J = 8 Hz), 7.42 (t, 1H, J = 8 Hz), 8.00 (d, 1H, J = 8 Hz); HRFABMS m/z 499.3298 (calcd for $C_{28}H_{43}N_4O_4$ (M + H), 499.3284).

(*R*)-*N*,*β*,*β*-Trimethylphenylalanyl-(*S*)-*tert*-leucyl-(2*E*,4*S*)-*N*-methyl-4-amino-2,5-dimethylhex-2-enoic Acid (SPA123, 53). The Boc-protected ethyl ester of **53** was isolated from impure fractions of **73** by normal-phase HPLC separation with a Magnum column (1:4 ethyl acetate/hexanes). The protecting groups were removed by General Procedures 5 and 9 using the following: *N*-Boc-protected ethyl ester of **53** (65.5 mg, 0.109 mmol); MeOH (3 mL); H_2O (0.871 mL); lithium hydroxide (0.871 mL, 1.0 M, 0.871 mmol); CH_2Cl_2 (2 mL); and TFA (2 mL). Purification of the crude product was accomplished by lyophilization, which produced 64.1 mg (0.109 mmol, quantitative yield) of **53** as a white fluffy powder: mp 132−136 °C; $[\alpha]_D^{22}$ −96.7 (*c* 2.4, MeOH); IR (KBr pellet) 3550−2700 (br), 1680, 1204 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) 0.85−0.92 (m, 15H), 1.47 (s, 3H), 1.53 (s, 3H), 1.87 (d, 3H, J = 1.4 Hz), 1.97−2.03 (m, 1H), 2.47 (s, 3H), 3.10 (s, 3H), 4.32 (s, 1H), 4.54 (d, 1H, J = 7.5 Hz), 5.02 (t, 1H, J = 9.9 Hz), 6.74 (dd, 1H, J = 1.4

and 9.9 Hz), 7.30 (t, 1H, $J = 7.5$ Hz), 7.42 (t, 2H, $J = 7.5$ Hz), 7.50 (d, 2H, $J = 7.5$ Hz), 8.13 (d, 0.8H, $J = 7.5$ Hz); ^{13}C NMR (100 MHz, D_3COD) 14.1, 19.6, 19.7, 24.5, 26.6, 27.0, 30.8, 31.8, 34.0, 35.0, 41.5, 58.5, 58.6, 71.3, 127.4, 128.8, 130.2, 133.6, 139.7, 145.0, 167.7, 170.8, 173.0; HRFABMS *m*/*z* 474.3332 (calcd for $C_{27}H_{44}N_3O_4$ (M + H), 474.3331).

(*S*)-*N*-Methylphenylalanyl-(*S*)-*tert*-leucyl-(2*E*,4*S*)-*N*-methyl-4-amino-2,5-dimethylhex-2-enoic Acid (SPA114, 45). Compound **45** was formed by General Procedures 13, 5, and 9. The following was used for General Procedure 13: *N*-Boc-*N*-methylphenylalanine (77.4 mg, 0.252 mmol, prepared from *N*-Boc-phenylalanine by General Procedure 4 using THF as the solvent); CH_2Cl_2 (1 mL); DIEA (0.155 mL, 0.893 mmol); DMAP (not used); PyBroP (139 mg, 0.298 mmol); TFA salt of ethyl (*S*)-*tert*-leucyl-(2*E*,4*S*)-*N*-methyl-4-amino-2,5-dimethylhex-2-enoate (crude from General Procedure 9 with 69.1 mg (0.168 mmol) of **60**); and CH_2Cl_2 (1.5 mL). General Procedure 5 was done with the following: *N*-Boc-protected ethyl ester of **45** (82.0 mg, 0.143 mmol); methanol (3 mL); H_2O (1 mL); and lithium hydroxide (1.14 mL, 1.0 M, 1.14 mmol). General Procedure 9 was done with the following: *N*-Boc-protected **45** (52.2 mg, 0.0956 mmol); CH_2Cl_2 (1.5 mL); and TFA (1.5 mL). The crude product was purified by reversed-phase HPLC using a Magnum column (45:55 H_2O/MeOH with 0.05% TFA), which yielded 44.9 mg (0.0802 mmol, 71% yield for 3 steps) of **45** as a white solid: mp 134−136 °C; $[\alpha]_D^{23}$ −52.5 (*c* 0.53, MeOH); IR (KBr pellet) 3566−2906 (br), 1684, 1205, 1140 cm^{-1}; ^{1}H NMR (400 MHz, D_3COD) 0.85 (d, 3H, $J = 6.5$ Hz), 0.89 (d, 3H, $J = 6.6$ Hz), 1.02 (s, 9H), 1.88 (s, 3H), 1.96−2.09 (m, 1H), 2.59 (s, 3H), 3.00 (dd, 1H, $J = 7.6$ and 14.6 Hz), 3.07 (s, 3H), 3.22 (dd, 1H, $J = 5.6$ and 14.6 Hz), 4.24 (dd, 1H, $J = 5.6$ and 7.6 Hz), 4.86 (s, 1H), 5.03 (t, 1H, $J = 9.8$ Hz), 6.74 (d, 1H, $J = 9.8$ Hz), 7.27 (d, 2H, $J = 6.9$ Hz), 7.30−7.37 (m, 3H); ^{13}C NMR (100 MHz, D_3COD) 14.1, 19.7, 19.8, 26.9, 30.9, 31.8, 32.9, 36.2, 37.9, 57.6, 58.3, 63.5, 129.1, 130.3, 130.5, 133.7, 135.0, 139.7, 168.4, 170.8, 172.4; HREIMS *m*/*z* 446.3019 (calcd for $C_{25}H_{40}N_3O_4$, 446.3018).

Pivaloyl-(*S*)-*tert*-leucyl-(2*E*,4*S*)-*N*-methyl-4-amino-2,5-dimethylhex-2-enoic Acid (SPA113, 44). The ethyl ester **75** of the acid **44** was obtained as a byproduct while attempting to use General Procedure 10 to couple the TFA salt of ethyl (*S*)-*tert*-leucyl-(2*E*,4*S*)-*N*-methyl-4-amino-2,5-dimethylhex-2-enoate (produced after application of General Procedure 9 to **60**) to *N*-Boc-*N*,*β*,*β*-trimethylphenylalanine. The ethyl ester **75** was cleaved using General Procedure 5 with the following: ethyl ester **75** (63.4 mg, 0.160 mmol); MeOH (3 mL); H_2O (1 mL); and lithium hydroxide (1.28 mL, 1.0 M, 1.28 mmol). The colorless oil of **44** obtained did not need further purification (56.4 mg, 0.153 mmol, 96% yield): IR (neat) 3440−2700 (br), 1703, 1631, 1482, 1236 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) 0.76 (d, 3H, $J = 6.6$ Hz), 0.84 (d, 3H, $J = 6.6$ Hz), 0.93 (s, 9H), 1.16 (s, 9H), 1.85−1.92 (m, 1H), 1.88 (s, 3H), 2.97 (s, 3H), 4.82 (d, 1H, $J = 9.4$ Hz), 5.08 (t, 1H, $J = 9.2$ Hz), 6.44 (d, 1H, $J = 9.4$ Hz), 6.73 (d, 1H, $J = 9.4$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$) 13.5, 18.5, 19.4, 26.4, 27.4, 29.9, 31.0, 35.3, 38.8, 54.4, 56.1, 132.1, 140.4, 171.8, 172.0, 178.4; HREIMS *m*/*z* 368.2664 (calcd for $C_{20}H_{36}N_2O_4$, 368.2675).

(*S*)-*N*-Methyl-*tert*-leucyl-(*S*)-*tert*-leucyl-(2*E*,4*S*)-*N*-methyl-4-amino-2,5-dimethylhex-2-enoic Acid (SPA115, 46). Following General Procedures 5 and 9, compound **46** was prepared using the following: ethyl (*S*)-*N*-*tert*-butoxycarbonyl-*N*-methyl-*tert*-leucyl-(*S*)-*tert*-leucyl-(2*E*,4*S*)-*N*-methyl-4-amino-2,5-dimethylhex-2-enoate, **74** (62.2 mg, 0.115 mmol); MeOH (3 mL); H_2O (1 mL); lithium hydroxide (0.922 mL, 1.0 M, 0.922 mmol); CH_2Cl_2 (2 mL); and TFA (2 mL). Purification was accomplished by HPLC using a Magnum ODS column (45:55 H_2O/MeOH with 0.05% TFA), producing 51.0 mg (0.0970 mmol, 84% yield for the two steps) of **46** as a white solid: mp 157−159 °C; $[\alpha]_D^{23}$ −66.0 (*c* 0.53, MeOH); IR (KBr pellet) 3580−2800 (br), 1675, 1651, 1204, 1138 cm^{-1}; ^{1}H NMR (400 MHz, D_3COD) 0.83 (d, 3H, $J = 6.6$ Hz), 0.88 (d, 3H, $J = 6.6$ Hz), 1.02 (s, 9H), 1.06 (s, 9H), 1.88 (d, 3H, $J = 1.1$ Hz), 1.97−2.05 (m, 1H), 2.63 (s, 3H), 3.09 (s, 3H), 3.71 (s, 1H), 4.88 (s, 1H), 5.02 (t, 1H, $J = 9.9$ Hz), 6.74 (dd, 1H, $J = 1.1$ and 9.9 Hz); ^{13}C NMR (75 MHz, D_3COD) 14.2, 19.7, 19.8, 26.9, 30.8, 31.9, 33.9, 35.0, 36.0, 57.4, 58.3, 71.5, 133.7, 139.7, 167.6, 170.8, 172.2; HREIMS *m*/*z* 354.2387 (calcd for $C_{18}H_{32}N_3O_4$, 354.2392).

(*S*)-*N*-Methylvalyl-(*S*)-*tert*-leucyl-(2*E*,4*S*)-*N*-methyl-4-amino-2,5-dimethylhex-2-enoic Acid (SPA116, 47). Compound **47** was produced using General Procedures 13, 5, and 9 on the following: *N*-Boc-*N*-methylvaline (24.3 mg, 0.105 mmol); CH_2Cl_2 (1 mL); DIEA (0.055 mL, 0.315 mmol); DMAP (not used); PyBroP (49.0 mg, 0.105 mmol); TFA salt of ethyl (*S*)-*tert*-leucyl-(2*E*,4*S*)-*N*-methyl-4-amino-2,5-dimethylhex-2-enoate (crude from General Procedure 9 with 33.1 mg (0.0804 mmol) of **60**); CH_2Cl_2 (1 mL); MeOH (3 mL); H_2O (1 mL); lithium hydroxide (1.0 mL, 1.0 M, 1.0 mmol); CH_2Cl_2 (1.5 mL); and TFA (1.5 mL). The crude product was purified by lyophilization, which produced 20.9 mg (0.0408 mmol, 51% yield for the three steps) of **47** as a white, fluffy powder: mp 132−134 °C; $[\alpha]_D^{25}$ −65.9 (*c* 0.64, MeOH); IR (KBr pellet) 3560−2680 (br), 1675, 1204, 1138 cm^{-1}; ^{1}H NMR (400 MHz, D_3COD) 0.83 (d, 3H, $J = 6.6$ Hz), 0.88 (d, 3H, $J = 6.6$ Hz), 0.99 (d, 3H, $J = 6.9$ Hz), 1.02 (s, 9H), 1.04 (d, 3H, $J = 6.9$ Hz), 1.88 (d, 3H, $J = 1.4$ Hz), 1.96−2.05 (m, 1H), 2.12−2.19 (m, 1H), 2.64 (s, 3H), 3.08 (s, 3H), 3.78 (d, 1H, $J = 5.5$ Hz), 4.87 (s, 1H), 5.03 (t, 1H, $J = 9.9$ Hz), 6.74 (dd, 1H, $J = 1.4$ and 9.9 Hz); ^{13}C NMR (75 MHz, D_3COD) 14.2, 18.4, 18.6, 19.6, 19.8, 26.9, 30.8, 31.6, 31.8, 33.1, 35.9, 57.4, 58.2, 67.9, 133.7, 139.7, 167.8, 170.8, 172.5; HRCIMS *m*/*z* 398.3016 (calcd for $C_{21}H_{40}N_3O_4$ (M + H), 398.3018).

(*S*)-*N*$^{\alpha}$,*N*^{1},*β*,*β*-Tetramethyltryptophanyl-(*S*)-*tert*-leucyl-(2*E*)-*N*-methyl-4-amino-2-methylbut-2-enoic Acid (SPA118, 48). Compound **48** was formed by General Procedures 12, 5, and 9. The following was used for General Procedure 12: *N*$^{\alpha}$-Boc-*N*$^{\alpha}$,*N*^{1},*β*,*β*-tetramethyltryptophan **13** (51.0 mg, 0.142 mmol); PyBOP (74 mg, 0.142 mmol); TFA salt of ethyl (*S*)-*tert*-leucyl-(2*E*)-*N*-methyl-4-amino-2-methylbut-2-enoate (crude from General Procedure 9 with 51.5 mg (0.139 mmol) of **63**); CH_2Cl_2 (1.5 mL); and TMP (0.056 mL, 0.425 mmol, used instead of DIEA). General Procedure 5 was done with the following: *N*-Boc-protected ethyl ester of **48** (50.3 mg, 0.0821 mmol); MeOH (3 mL); H_2O (1 mL); and lithium hydroxide (1.0 mL, 1.0 M, 1.0 mmol). General Procedure 9 was done with the following: *N*-Boc-protected **48** (22.4 mg, 0.0383 mmol); CH_2Cl_2 (1.0 mL); and TFA (1.5 mL). The crude product was purified by lyophilization, yielding 20.8 mg (0.0347 mmol, 25% yield for the three steps) of a very light yellow solid. **48**: mp 135−137 °C; $[\alpha]_D^{25}$ +38.3 (*c* 0.24, MeOH); IR (KBr pellet) 3622−2836 (br), 1675, 1203, 1138 cm^{-1}; ^{1}H NMR (400 MHz, D_3COD, mixture of rotamers) 1.02 (s, 3H), 1.04 (s, 6H), 1.49−1.57 (m, 6H), 1.90 (d, 2H, $J = 1.2$ Hz), 1.97 (s, 1H), 2.48 (s, 3H), 3.80 (s, 3H), 4.07 (dd, 0.67H, $J = 6.7$ and 16.8 Hz), 4.21−4.32 (m, 1H), 4.47 (s, 1H), 4.91 (s, 0.33H), 4.92 (s, 0.67H), 6.60 (dt, 0.67H, $J = 1.2$ and 6.7 Hz), 6.68 (t, 0.33H, $J = 6.4$ Hz), 7.12−7.16 (m, 2H), 7.24 (t, 1H, $J = 7.6$ Hz), 7.43 (d, 1H, $J = 8.1$ Hz), 8.03 (d, 0.67H, $J = 8.0$ Hz), 8.23 (d, 0.33H, $J \approx 8.0$ Hz); ^{13}C NMR (75 MHz, D_3COD) 12.7, 24.0, 26.9, 27.1, 33.0, 34.2, 35.8, 37.3, 39.4, 47.4, 57.3, 70.1, 111.2, 117.6, 120.4, 121.1, 123.0, 126.3, 129.4, 132.1, 137.5, 139.8, 167.3, 170.7, 172.7; HRCIMS *m*/*z* 485.3121 (calcd for $C_{27}H_{41}N_4O_4$ (M + H), 485.3127).

(*S*)-*N*$^{\alpha}$,*N*^{1},*β*,*β*-Tetramethyltryptophanyl-(*S*)-*tert*-leucyl-(2*E*,4*R*)-4-amino-2,5-dimethylhex-2-enoic Acid (SPA119, 49). The *N*-Boc-protected ethyl ester of compound **49** was an impurity isolated during radial column chromatographic purification (1 mm plate, 1:1 diethyl ether/petroleum ether, sample loaded with chloroform) of the coupled product for (*S*)-*N*$^{\alpha}$-Boc-*N*$^{\alpha}$,*N*^{1},*β*,*β*-tetramethyltryptophan **13** and the TFA salt of ethyl (*S*)-*tert*-leucyl-(2*E*,4*S*)-4-amino-2,5-dimethylhex-2-enoate. The protecting groups were removed by applying General Procedures 5 and 9 on the following: *N*-Boc-protected ethyl ester of **49** (34.9 mg, 0.0545 mmol); MeOH (1.5 mL); H_2O (0.5 mL); lithium hydroxide (0.5 mL, 1.0 M, 0.500 mmol); CH_2Cl_2 (1.0 mL); and TFA (1.25 mL). The crude product was purified using HPLC with a Magnum ODS column (37.5:62.5 H_2O/MeOH with 0.05% TFA), producing 7.3 mg (0.0116 mmol, 21% yield for the deprotection steps) of a light orange solid. **49**: mp 146−148 °C; $[\alpha]_D^{24}$ +7.1 (*c* 0.14, MeOH); IR (KBr pellet) 3600−2840 (br), 1675, 1204, 1139 cm^{-1}; ^{1}H NMR (400

MHz, D_3COD) 0.93 (d, 3H, J = 6.8 Hz), 1.01 (d, 3H, J = 6.6 Hz), 1.07 (s, 9H), 1.42 (s, 3H), 1.49 (s, 3H), 1.88 (d, 3H, J = 1.4 Hz), 1.82−1.91 (m,1H), 2.42 (s, 3H), 3.79 (s, 3H), 4.43−4.71 (m, 3H), 6.59 (dd, 1H, J = 1.4 and 9.9 Hz), 7.09 (s, 1H), 7.13 (t, 1H, J = 7.6 Hz), 7.23 (t, 1H, J = 7.6 Hz), 7.41 (d, 1H, J = 7.6 Hz), 8.03 (d, 1H, J = 7.6 Hz), 8.38 (d, 1H, J = 11.3 Hz), 8.41 (d, 1H, J = 8.7 Hz); ^{13}C NMR (100 MHz, D_3COD) 13.4, 19.2, 19.6, 23.3, 27.2, 27.5, 33.0, 33.2, 34.3, 34.9, 39.4, 54.9, 63.2, 70.3, 11.2, 117.6, 120.5, 121.1, 123.1, 126.3, 129.4, 131.4, 140.0, 141.0, 167.0, 171.2, 171.3; HRCIMS m/z 513.3430 (calcd for $C_{29}H_{45}N_4O_4$ (M + H), 513.3443).

(*S*)-*N*$^{\alpha}$,*N*^{1},*β*,*β*-Tetramethyltryptophanyl-(*S*)-*tert*-leucyl-(2*E*,4*S*)-4-amino-2,5-dimethylhex-2-enoic Acid (SPA120, 50). For the formation of compound **50** General Procedures 12, 5, and 9 were used with the following: (*S*)-*N*$^{\alpha}$-Boc-*N*$^{\alpha}$,*N*^{1},*β*,*β*-tetramethyltryptophan (19.0 mg, 0.0527 mmol); PyBOP (27.4 mg, 0.0527 mmol); TFA salt of ethyl (*S*)-*tert*-leucyl-(2*E*,4*S*)-4-amino-2,5-dimethylhex-2-enoate (crude from General Procedure 9 with 23.2 mg (0.0582 mmol) of **65**); CH_2Cl_2 (1.5 mL); DIEA (27.5 μL, 0.158 mmol); MeOH (3 mL); H_2O (1 mL); lithium hydroxide (1 mL, 1.0 M, 1.0 mmol); CH_2Cl_2 (1 mL); and TFA (1 mL). Purification of the crude product by HPLC using a Magnum ODS column (40:60 H_2O/MeOH with 0.05% TFA) afforded 20.6 mg (0.0329 mmol, 62% yield for the three steps) of a light orange solid. **50**: mp 142−144 °C; $[\alpha]_D^{25}$ +4.2 (*c* 0.42, MeOH); IR (KBr pellet) 3600−2850 (br), 1680, 1203, 1138 cm^{-1}; ^{1}H NMR (400 MHz, D_3COD) 0.91 (d, 3H, J = 6.8 Hz), 0.96 (d, 3H, J = 8.8 Hz), 1.01 (s, 9H), 1.48 (s, 3H), 1.58 (s, 3H), 1.80−1.89 (m, 1H), 1.91 (d, 3H, J = 1.1 Hz), 2.44 (s, 3H), 3.80 (s, 3H), 4.42−4.48 (m, 3H), 6.62 (d, 1H, J = 10.0 Hz), 7.13 (s, 1H), 7.12−7.15 (m, 1H), 7.24 (t, 1H, J = 7.8 Hz), 7.42 (d, 1H, J = 7.8 Hz), 8.06 (d, 1H, J = 7.8 Hz), 8.36 (d, 1H, J = 9.0 Hz), 8.40 (d, 1H, J = 8.3 Hz); ^{13}C NMR (100 MHz, D_3COD) 13.5, 19.2, 19.4, 23.4, 27.1, 27.5, 33.0, 33.7, 34.4, 35.2, 39.4, 54.5, 63.1, 70.3, 111.2, 117.5, 120.5, 121.1, 123.1, 126.3, 129.5, 131.9, 140.0, 140.9, 167.0, 171.0 (2×); HRCIMS m/z 513.3438 (calcd for $C_{29}H_{45}N_4O_4$ (M + H), 513.3443).

(*S*)-*N*,*β*,*β*-Trimethylphenylalanyl-(*S*)-*tert*-leucyl-(2*E*,4*S*)-4-amino-2,5-dimethylhex-2-enoic Acid (SPA121, 51). Formation of **51** was accomplished by application of General Procedures 12, 5, and 9 with the following: (*S*)-*N*-*tert*-butoxycarbonyl-*N*,*β*,*β*-trimethylphenylalanine (45.5 mg, 0.148 mmol); PyBOP (77.0 mg, 0.148 mmol); TFA salt of ethyl (*S*)-*tert*-leucyl-(2*E*,4*S*)-4-amino-2,5-dimethylhex-2-enoate (crude from General Procedure 9 wtih 62.5 mg (0.157 mmol) of **65**); CH_2Cl_2 (1.8 mL); DIEA (77.3 μL, 0.444 mmol); MeOH (2.4 mL); H_2O (0.8 mL); lithium hydroxide (0.80 mL, 1.0 M, 0.80 mmol); CH_2Cl_2 (2 mL); and TFA (2.25 mL). The product was purified by lyophilization, which produced 46.3 mg (0.0807 mmol, 55% yield for the three steps) of a white powder. **51**: mp 158−161 °C; $[\alpha]_D^{22}$ −1.6 (*c* 0.64, MeOH); IR (KBr pellet) 3575−2650 (br), 1673, 1204, 1141 cm^{-1}; ^{1}H NMR (400 MHz, D_3COD) 0.90 (d, 3H, J = 6.7 Hz), 0.97 (d, 3H, J = 6.7 Hz), 1.01 (s, 9H), 1.39 (s, 3H), 1.49 (s, 3H), 1.79−1.88 (m, 1H), 1.90 (d, 3H, J = 1.4 Hz), 2.50 (s, 3H), 4.33 (s, 1H), 4.38 (d, 1H, J = 8.9 Hz), 4.47 (t, 1H, J = 9.0 Hz), 6.60 (dd, 1H, J = 1.4 and 9.0 Hz), 7.33 (t, 1H, J = 7.6 Hz), 7.43 (t, 2H, J = 7.6 Hz), 7.52 (d, 2H, J = 7.6 Hz), 8.29 (d, 1H, J = 8.9 Hz), 8.33 (d, 1H, J = 8.8 Hz); ^{13}C NMR (100 MHz, D_3COD) 13.4, 19.2, 19.4, 22.3, 27.1, 29.3, 33.6, 34.2, 35.3, 42.0, 54.4, 62.9, 71.6, 127.4, 128.8, 130.3, 131.3, 140.8, 144.8, 166.9, 170.9, 171.1; HRFABMS m/z 460.3172 (calcd for $C_{26}H_{42}N_3O_4$ (M + H), 460.3177).

(*S*)-*N*-Methyl-*tert*-leucyl-(*S*)-*tert*-leucyl-(2*E*,4*S*)-4-amino-2,5-dimethylhex-2-enoic Acid (SPA122, 52). Formation of **52** was accomplished by application of General Procedures 12, 5, and 9 with the following: (*S*)-*N*-Boc-*N*-methyl-*tert*-leucine (65.1 mg, 0.265 mmol); PyBOP (138 mg, 0.265 mmol); TFA salt of ethyl (*S*)-*tert*-leucyl-(2*E*,4*S*)-4-amino-2,5-dimethylhex-2-enoate (crude from General Procedure 9 with 120 mg (0.300 mmol) of **65**); CH_2Cl_2 (1.5 mL); DIEA (0.139 mL, 0.796 mmol); MeOH (3.5 mL); H_2O (1.2 mL); lithium hydroxide (1.2 mL, 1.0 M, 1.2 mmol); CH_2Cl_2 (2 mL); and TFA (2.25 mL). The product was purified by lyophilization, which produced 42.5 mg (0.0831 mmol, 31% yield for the three steps) of **52** as a white powder: mp 215−219 °C; $[\alpha]_D^{22}$ −32.1 (*c* 0.28, MeOH); IR (KBr pellet) 3640−2750 (br), 1672, 1204, 1142 cm^{-1}; ^{1}H NMR (400 MHz, D_3COD) 0.88 (d, 3H, J = 6.8 Hz), 0.92 (d, 3H, J = 6.7 Hz), 0.99 (s, 9H), 1.07 (s, 9H), 1.76−1.83 (m, 1H), 1.89 (d, 3H, J = 1.4 Hz), 2.64 (s, 3H), 3.72 (s, 1H), 4.36 (s, 0.3H), 4.36 (d, 0.7 H, J = 9.2 Hz), 4.46 (q, 1H, J = 9.0 Hz), 6.59 (dd, 1H, J = 1.4 and 10.0 Hz), 8.22 (bd, 0.7H, J = 9.2 Hz), 8.32 (bd, 1H, J = 9.0 Hz); ^{13}C NMR (100 MHz, D_3COD) 13.4, 19.2, 19.3, 26.9, 27.1, 33.7, 33.9, 34.9, 35.3, 54.4, 62.6, 71.8, 131.3, 140.8, 167.5, 170.9, 171.1; HRFABMS m/z 398.3030 (calcd for $C_{21}H_{40}N_3O_4$ (M + H), 398.3021).

(*S*)-*N*$^{\alpha}$,*N*^{1},*β*,*β*-Tetramethyltryptophanyl-(*S*)-*tert*-leucyl-(2*E*,4*R*)-*N*-methyl-4-amino-2-methylpent-2-enoic Acid (SPA124, 54). The *N*-Boc-protected ethyl ester of compound **54** was an impurity isolated during HPLC purification, using a normal-phase Magnum column (1:2 ethyl acetate/hexanes) of the coupled product for (*S*)-*N*$^{\alpha}$-Boc-*N*$^{\alpha}$,*N*^{1},*β*,*β*-tetramethyltryptophan and the TFA salt of ethyl (*S*)-*tert*-leucyl-(2*E*,4*S*)-*N*-methyl-4-amino-2-methylpent-2-enoate. The protecting groups were removed by applying General Procedures 5 and 9 on the following: *N*-Boc-protected ethyl ester of **54** (12.5 mg, 0.0199 mmol); MeOH (3 mL); H_2O (1 mL); lithium hydroxide (1 mL, 1.0 M, 1 mmol); CH_2Cl_2 (1.0 mL); and TFA (1.1 mL). The crude product was purified using HPLC with a Magnum ODS column (45:55 H_2O/MeOH with 0.05% TFA), producing 7.8 mg (0.0127 mmol, 64% yield for the deprotection steps) of a light yellow solid. **54**: mp 132−134 °C; $[\alpha]_D^{23}$ +27.8 (*c* 0.18, MeOH); IR (KBr pellet) 3400−2840 (br), 1681, 1205, 1138 cm^{-1}; ^{1}H NMR (400 MHz, D_3COD) 1.07 (s, 9H), 1.30 (d, 3H, J = 6.9 Hz), 1.43 (s, 3H), 1.51 (s, 3H), 1.85 (d, 3H, J = 1.4 Hz), 2.44 (s, 3H), 3.14 (s, 3H), 3.79 (s, 3H), 4.48 (s, 1H), 4.93 (s, 1H), 5.48−5.54 (m, 1H), 6.69 (dd, 1H, J = 1.4 and 8.4 Hz), 7.11 (s, 1H), 7.14 (t, 1H, J = 7.6 Hz), 7.24 (t, 1H, J = 7.6 Hz), 7.42 (d, 1H, J = 7.6 Hz), 8.03 (d, 1H, J = 7.6 Hz); ^{13}C NMR (100 MHz, D_3COD) 13.5, 18.1, 23.5, 27.0, 27.3, 31.6, 33.0, 34.4, 35.9, 39.4, 57.3, 70.0, 70.1, 111.2, 117.5, 120.5, 121.0, 123.1, 125.7, 127.8, 129.5, 139.7, 140.5, 167.2, 169.4, 171.9; HRFABMS m/z 499.3283 (calcd for $C_{28}H_{43}N_4O_4$ (M + H), 499.3286).

(*S*)-*N*$^{\alpha}$,*N*^{1},*β*,*β*-Tetramethyltryptophanyl-(*S*)-*tert*-leucyl-(2*E*,4*S*)-*N*-methyl-4-amino-2-methylpent-2-enoic Acid (SPA125, 55). For the formation of compound **55** General Procedures 12, 5, and 9 were used. For General Procedure 12 the following was used: (*S*)-*N*$^{\alpha}$-Boc-*N*$^{\alpha}$,*N*^{1},*β*,*β*-tetramethyltryptophan (42.5 mg, 0.118 mmol); PyBOP (61.3 mg, 0.118 mmol); TFA salt of ethyl (*S*)-*tert*-leucyl-(2*E*,4*S*)-*N*-methyl-4-amino-2-methylpent-2-enoate (crude from General Procedure 9 with 39.8 mg (0.103 mmol) of **64**); CH_2Cl_2 (1.0 mL); and TMP (46.8 μL, 0.354 mmol, used instead of DIEA). For General Procedures 5 the following was used: *N*-Boc-protected ethyl ester of **55** (48.1 mg, 0.0890 mmol); MeOH (3 mL); H_2O (1 mL); and lithium hydroxide (1 mL, 1.0 M, 1.0 mmol). For General Procedure 9 (5 min reaction time) the following was used: *N*-Boc-protected **55** (40.1 mg, 0.0670 mmol); CH_2Cl_2 (1 mL); and TFA (1 mL). Purification of the crude product by HPLC using a Magnum ODS column (45:55 H_2O/MeOH with 0.05% TFA) afforded 37.8 mg (0.0617 mmol, 60% yield for the three steps) of a light yellow powder. **55**: mp 151−154 °C; $[\alpha]_D^{22}$ +1.9 (*c* 0.54, MeOH); IR (KBr pellet) 3640−2800 (br), 1684, 1654, 1204, 1140 cm^{-1}; ^{1}H NMR (400 MHz, D_3COD, major rotamer) 1.04 (s, 9H), 1.28 (d, 3H, J = 6.8 Hz), 1.48 (s, 3H), 1.57 (s, 3H), 1.86 (d, 3H, J = 1.4 Hz), 2.46 (s, 3H), 3.13 (s, 3H), 3.80 (s, 3H), 4.50 (s, 1H), 4.91 (d, 1H, J = 8.3 Hz), 5.34−5.42 (m, 1H), 6.76 (dd, 1H, J = 1.4 and 8.5 Hz), 7.10−7.15 (m, 2H), 7.24 (t, 1H, J = 7.2 Hz), 7.42 (d, 1H, J = 8.1 Hz), 8.04 (d, 1H, J = 8.1 Hz), 8.39 (d, 1H, J = 8.3 Hz); ^{13}C NMR (100 MHz, D_3COD, major rotamer) 13.5, 17.8, 23.7, 26.9, 27.3, 32.2, 33.0, 34.4, 35.8, 39.4, 53.2, 57.9, 70.1, 111.2, 117.5, 120.4, 121.1, 123.1, 126.3, 129.5, 132.4, 139.9, 140.7, 167.2, 170.8, 171.8; HRFABMS m/z 499.3284 (calcd for $C_{28}H_{43}N_4O_4$ (M + H), 499.3287).

Methyl (*S*)-*N*,*β*,*β*-Trimethylphenylalanyl-(*S*)-*tert*-leucyl-(2*E*,4*S*)-*N*-methyl-4-amino-2,5-dimethylhex-2-enoate (SPA126, 56). For the formation of compound **56**, first General Procedure 5 was employed on ethyl (*S*)-*N*-*tert*-butoxycarbonyl-*N*,*β*,*β*-trimethylphenylalanyl-(*S*)-*tert*-leucyl-(2*E*,4*S*)-*N*-methyl-4-amino-2,5-dimethylhex-2-enoate, **73**. The resulting carboxylic

acid (14.8 mg, 0.0258 mmol) was converted to its methyl ester by treatment with an ethereal solution of diazomethane until a yellow color persisted. The solution was blown down with argon to remove the excess diazomethane and dried in vacuo. The Boc group was then removed by subjecting the crude methyl ester to General Procedure 9. The crude ammonium salt was purified by silica gel column chromatography (20:1 CH_2Cl_2/MeOH with 0.5% TFA), producing 11.5 mg of **56** (0.0191 mmol, 74% yield over the last 2 steps): ^{1}H NMR (400 MHz, D_3COD) 0.88 (d, 6H, J = 6.5 Hz), 1.04 (s, 6H), 1.29 (s, 3H), 1.37 (s, 3H), 1.37 (s, 3H), 1.46 (s, 3H), 1.92 (d, 3H, J = 1.4 Hz), 2.00−2.11 (m, 1H), 2.49 (s, 3H), 3.12 (s, 3H), 3.74 (s, 3H), 4.35 (s, 1H), 4.90 (d, 1H, J = 8.2 Hz), 5.05 (t, 1H, J = 10.0 Hz), 6.75 (dd, 1H, J = 1.4 and 10.0 Hz), 7.34 (t, 1H, J = 7.5 Hz), 7.44 (t, 2H, J = 7.5 Hz), 7.53 (d, 2H, J = 7.5 Hz), 8.41 (d, 1H, J = 8.2 Hz); HRFABMS m/z 488.3481 (calcd for $C_{28}H_{46}N_3O_4$ (M + H), 488.3498).

Ethyl (*S*)-*N*,*β*,*β*-Trimethylphenylalanyl-(*S*)-*tert*-leucyl-(2*E*,4*S*)-*N*-methyl-4-amino-2,5-dimethylhex-2-enoate (SPA127, 57). For the formation of compound **57**, General Procedure 9 was employed with the following: ethyl (*S*)-*N*-*tert*-butoxycarbonyl-*N*,*β*,*β*-trimethylphenylalanyl-(*S*)-*tert*-leucyl-(2*E*,4*S*)-*N*-methyl-4-amino-2,5-dimethylhex-2-enoate, **73** (24.2 mg, 0.0402 mmol); CH_2Cl_2 (1.5 mL); and TFA (1.5 mL). The product was purified by silica gel column chromatography (20:1 CH_2Cl_2/MeOH with 0.5% TFA), producing 24.1 mg of **57** (0.0391 mmol, 97% yield): ^{1}H NMR (400 MHz, D3COD) 0.88 (d, 6H, J = 6.6 Hz), 1.00 (s, 3H), 1.04 (s, 6H), 1.28 (t, 3H, J = 7.1 Hz), 1.36 (s, 3H), 1.46 (s, 3H), 1.90 (d, 3H, J = 1.4 Hz), 2.00−2.11 (m, 1H), 2.49 (s, 3H), 3.12 (s, 3H), 4.19 (q, 2H, J = 7.1 Hz), 4.35 (s, 1H), 4.90 (d, 1H, J = 8.2 Hz), 5.04 (t, 1H, J = 9.7 Hz), 6.74 (dd, 1H, J = 1.4 and 9.7 Hz), 7.33 (t, 1H,J = 7.4 Hz), 7.44 (t, 2H, J = 7.4 Hz), 7.53 (d, 2H, J = 7.4 Hz), 8.41 (d, 1H, J = 8.2 Hz); HRFABMS m/z 502.3635 (calcd for $C_{29}H_{48}N_3O_4$ (M + H), 502.3645).

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(34) *N*-Boc-*tert*-leucine was purchased from Advanced ChemTech.

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