A Total Synthesis of (−)-Hemiasterlin Using *N*-Bts Methodology

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A total synthesis of (−)-hemiasterlin has been accomplished in nine steps from **25**[8] (>35% yield overall). An improved enantiocontrolled route to the tetramethyltryptophan subunit **32** was developed using an asymmetric Strecker synthesis (five steps, 50% yield from **25**), and the dipeptide **22** was prepared in seven steps, 37% yield from valinol. The synthesis exploits the high reactivity of a Bts-protected amino acid chloride in the difficult peptide coupling of sterically hindered amino acid residues **18** and **20** to form **21** (70%, recrystallized) and also uses *N*-Bts intermediates for the high-yielding *N*-methylations of **14** and **31**. In addition, the Bts-protected di-*tert*-butyl *N*-acylimidodicarbonate **33** is shown to undergo efficient coupling with **22** to form **34** (97% in the coupling step; 79% over the activation; coupling sequence from **32**).

A family of cytotoxic and antimitotic tripeptides has recently been isolated from marine sponges,[1,2] including hemiasterlin (**1**),[2a−d] hemiasterlins A−C (**2**, **3**, **4**),[2b,d] milnamide A (**5**),[1] and criamides A and B (**6** and **7**) (Figure 1).[2b] Structural assignments based on NMR and degradation studies are supported by X-ray diffraction analysis of hemiasterlin methyl ester,[3] and the amino acid subunits in **1**−**4** as well as **6** and **7** are known to have the L configuration. The hemiasterlins and analogues display potent in vivo and in vitro activity as cytotoxins and mitosis inhibitors.[1,2b] Despite the relatively simple structures, the hemiasterlins are more potent in both cytotoxic and antimitotic activity than other microtubule agents such as taxol, vinblastine, and nocodazole.[4] A comparison with previously reported antimitotic peptides such as cryptophycin 1 and dolastatins 10 and 15 indicates that the hemiasterlins are more potent than dolastatin 15, equipotent with cryptophycin 1, but slightly less potent than dolastatin 10.[5a] Among the hemiasterlin family, hemiasterlin (**1**) is the most potent cytotoxic agent while hemiasterlin C (**4**) is the least potent derivative.[2d] The cytotoxic and antimitotic activities of the hemiasterin family have been shown to arise from their ability to bind to tubulin and to disrupt spindle microtubule dynamics by producing abnormal mitotic spindles at low concentrations, while at high concentrations the hemiasterlins cause microtubule depolymerization.[4,5b] Hemiasterlin has potent in vitro cytotoxicity against murine leukemia P388 (IC_{50} 4.57 × 10^{-5} mg/mL), and there are preliminary indications of in vitro cytotoxicity for several analogues.[6,7]

So far, one total synthesis of (−)-hemiasterlin has been reported (Scheme 1).[8] Andersen et al. described the preparation of a protected tetramethyltryptophan **8** (15 steps from indole-3-acetic acid) and an enoate **9** (four steps from *N*-Boc-*N*-methylvaline), followed by a difficult coupling sequence. In the initial report, the *N*-Boc-protected dipeptide ethyl ester **11** was obtained in 22% yield from **9** and (*S*)-*N*-Boc-*tert*-leucine (**10**) using PyBroP/DMAP conditions. A subsequent patent reported a 62% yield of **11** from the reaction of **9** with the mixed pivaloyl

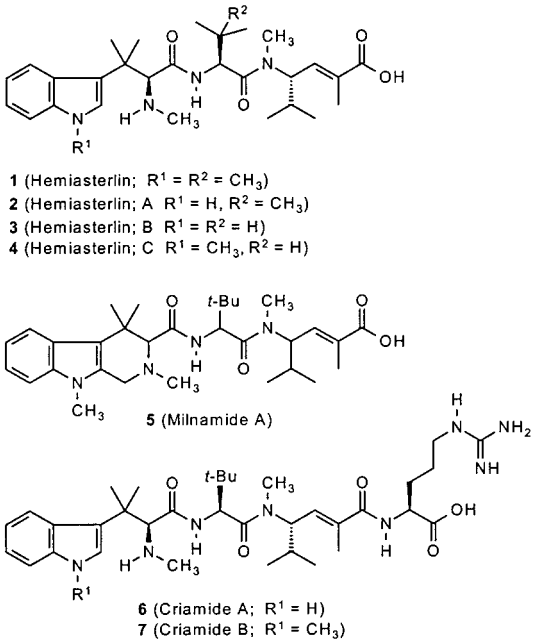

Figure 1. The hemiasterlins and related cytotoxic peptides.

(1) Crews, P.; Farias, J. J.; Emrich, R.; Keifer, P. A. *J. Org. Chem.* **1994**, *59*, 2932.

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(7) Andersen, R. J.; Piers, E.; Nieman, J.; Coleman, J. E.; Roberge, M. WO Patent 99,32509, 1999.

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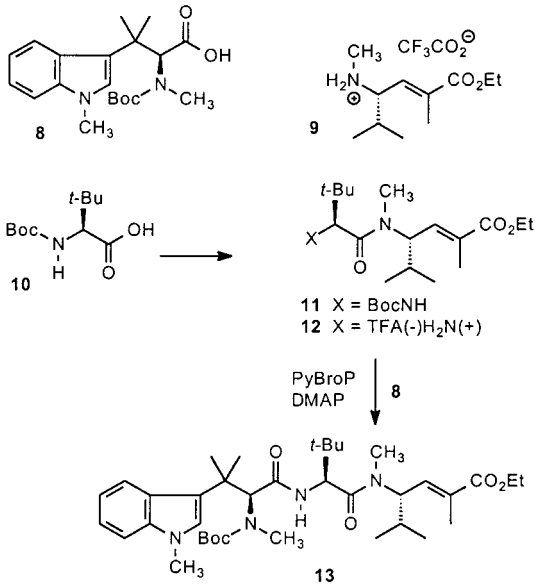
Scheme 1



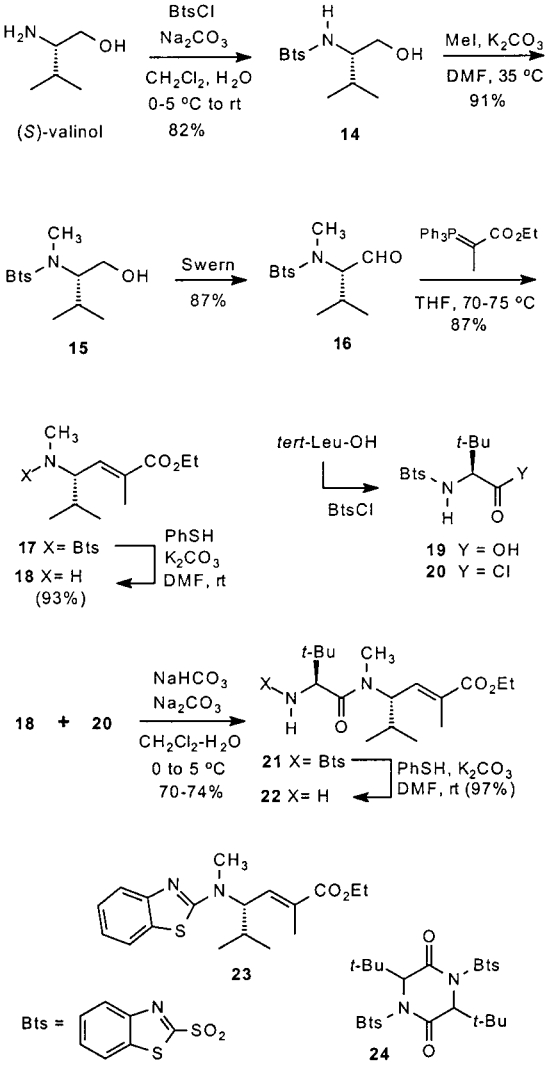
anhydride of **10**.[7] The final coupling to form the tripeptide **13** was achieved in 52% yield by reaction of **8** with **12**. These results prompted efforts in our laboratory to explore alternatives using *N*-benzothiazol-2-sulfonyl (Bts) protected amino acid derivatives[9] as described below. We hoped to develop a shorter synthesis of an enantiomerically pure tryptophan derivative that could be used in place of **8** and to improve the efficiency of peptide bond forming steps.

Results

Prior work in our laboratory used highly reactive Bts-protected amino acid chlorides to prepare hindered peptides derived from *N*-methylamino acid subunits.[9,10] Peptides were obtained in excellent yield and purity, and without detectable racemization of sensitive amino acid subunits.[9] Due to the high acidity of the Bts-NH proton, *N*-methylation of Bts-protected amino esters or peptides was easily achieved using a combination of iodomethane and a simple inorganic base.[10] We were therefore interested to evaluate the potential of Bts-protected intermediates for relevant steps in hemiasterlin synthesis using an approach that parallels the Andersen route.

Our work began with the synthesis of unsaturated ester **18** from commercially available (*S*)-valinol as summarized in Scheme 2. The Bts-protected (*S*)-valinol (**14**) was prepared in 82% yield from BtsCl and (*S*)-valinol using biphasic conditions (CH_2Cl_2/water; 1.02 mol % of Na_2CO_3 as base). A similar reaction under homogeneous conditions (CH_2Cl_2, pyridine) gave **14** in low yield (16%) along with several byproducts. Exposure of **14** to excess Na_2CO_3 under the biphasic conditions also gave side products.[11] However, sulfonamide **14** was sufficiently stable to allow *N*-methylation under mild conditions. Thus, reaction with iodomethane in the presence of K_2CO_3 in DMF (4 h at 35 °C) gave **15** in 91% yield.

Scheme 2



Oxidation of **15** using either Dess−Martin periodinane[12] or Swern conditions[13] (oxalyl chloride/DMSO/Et_3N) provided the corresponding aldehyde **16** in good yield (>90%) after optimization of the workup procedure and flash column chromatography. However, purification by preparative TLC (ca. 60 min time scale) gave racemic **16** regardless of the oxidation procedure. Furthermore, racemic **16** was obtained if the triethylamine used for Swern oxidation was not carefully removed by successive washes with aqueous ammonium chloride.[14] With suitable precautions taken, the Swern procedure could be used to prepare **16** in 97% yield (95% ee). Moreover, the optical purity of the aldehyde **16** could be improved to 99.4% ee after a single recrystallization from ether/hexane (87% isolated based on **15**). The unsaturated ester **17** was then obtained from **16** as a 26:1 mixture of *E*:*Z* isomers using an excess of the ester-stabilized Wittig reagent $Ph_3P{=}CH(Me)CO_2Et$. The optimized conditions required treatment of the aldehyde **16** with 2 equiv of the Wittig reagent in refluxing THF (3 h). This procedure afforded the pure ester **17** in 87% yield after chromato-

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(11) Exposure of **14** to excess sodium carbonate resulted in two products by NMR assay, one of which has NMR characteristics as expected for the *O*-(benzothiazol-2-yl) derivative of valinol.
(12) Dess, D. B.; Martin, J. C. *J. Org. Chem.* **1983**, *48*, 4155. Dess, D. B.; Martin, J. C. *J. Am. Chem. Soc.* **1991**, *113*, 7277.
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graphic separation of isomers, or 96% of the 26:1 *E*:*Z* mixture. Eventually, it was found that the mixture could be used in place of purified **17** because crystallization of a subsequent product (**21**) allowed efficient removal of the minor (*Z*) isomer.

In previous work in this laboratory, Bts deprotection was achieved with a variety of reducing agents, including H_3PO_2, Zn/HOAc, $NaBH_4$, and $PhSH/K_2CO_3$.[9,10] Deprotection using H_3PO_2 is limited to relatively simple peptides because incomplete consumption of starting material is observed as complexity increases. The $NaBH_4$ method works well, but only if the amino group lacks an acidic sulfonamide N−H hydrogen. We therefore used the $PhSH/K_2CO_3$/DMF conditions developed by Fukuyama for deprotection of *o*-nitrobenzene-sulfonamides.[15,10] This procedure converted **17** to the free amino ester **18** in 93% yield and with >95% purity after simple acid/base extraction. Enantiomeric purity was determined by HPLC assay of the *N*-benzoyl derivative of **18** (96.6% ee). Since **14** with >99.5% ee had been used as the starting material for the five-step sequence to **18**, measurable racemization had occurred. However, the subsequent steps provide opportunities for removing the minor (<2%) enantiomer via diastereomer separation at the dipeptide or tripeptide stages.

In preparation for amide bond formation, **19** was made by the reaction of BtsCl and (*S*)-*tert*-leucine (heterogeneous conditions; suspension of BtsCl in aqueous NaOH). It was important to maintain the reaction pH between 10 and 10.5 using a pH meter and slow addition of aqueous NaOH as needed to neutralize the HCl byproduct. The use of excess BtsCl (1.6 equiv) was necessary due to competing hydrolysis of BtsCl in the aqueous medium. Under these optimized conditions, the Bts-protected (*S*)-*tert*-leucine **19** was obtained in good yield (81%). Further conversion to the acid chloride **20** was carried out by routine thionyl chloride treatment,[9,10] and the crude **20** after removal of volatiles was used in the next step.

Coupling of the amino ester **18** with **20** (1.5 equiv) was performed under biphasic conditions ($NaHCO_3-Na_2CO_3$, $CH_2Cl_2-H_2O$) and furnished the corresponding dipeptide **21** in 86% yield. Minor byproducts were also obtained, including **23** (5% based on **18**), and the diketopiperazine **24** (11% based on **19**). Compound **23** appears to arise from the nucleophilic attack of **18** at the electrophilic *ipso* carbon of the benzothiazole ring, while **24** is probably derived from a competing self-condensation of the *N*-Bts-(*S*)-*tert*-leucine acid chloride. Analogous sidereactions were not detected in our prior study, suggesting that the somewhat slower *N*-acylation expected for the hindered *tert*-leucine derivative **20** may be responsible for the formation of **23** and **24**. In any event, **21** was obtained efficiently and could be purified by recrystallization (57% overall from **16**). The resulting material was deprotected using $PhSH/K_2CO_3$ in DMF to give **22** (97%), and HPLC assay of the *N*-benzoyl derivative established a 99.7:0.3 diastereomer ratio. Since **22** was formed with excellent purity corresponding closely to the estimated purity of **21** based on NMR assay, the steps from **17** were repeated using the 26:1 *E*:*Z* mixture obtained from the Horner−Emmons step. In this case, **21** was obtained in 61% overall yield from **16**, and the NMR spectrum was identical to that of material prepared from the purified *E*-isomer **17**. Thus, crystallization of **21** easily removes the minor stereoisomers formed as contaminants in earlier steps of the synthesis.

The last stages of the synthesis require acylation of **22** with a tetramethyltryptophan derivative, analogous to Andersen's intermediate **8**.[8] Our plan was to exploit asymmetric Strecker methodology using stoichiometric chiral agents[16−18] or chiral catalysts [19−24] to prepare Bts-protected analogues of **8** from the aldehyde **25** (Scheme 3).[8] Chiral amino nitriles **26** and **27** were prepared following a literature analogy[16,17] via the imine generated with (*R*)-2-phenylglycinol. The original method was modified by replacing the easily hydrolyzed TMSCN reagent with the more stable Bu_3SnCN and by using scandium triflate as the catalyst to improve reactivity.[25] The product was obtained as an 8:1 mixture of diastereomers (94%) assigned the structures **26** and **27** according to literature precedents with simpler substrates.[16,17] The 8:1 diastereoselectivity was not a major concern because the diastereomers were separable by chromatography to give an 81% yield of **26**. However, it became

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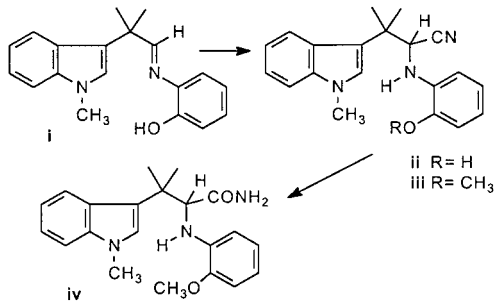
(24) The catalytic asymmetric Strecker synthesis using a chiral zirconium catalyst described by Kobayashi and co-workers[22] was tested. Treatment of the preformed imine **i** with the preformed chiral zirconium catalyst ($Zr(O\text{-}tBu)_4$/(*S*)-6-Br-BINOL/(*S*)-3-Br-BINOL) and Bu_3SnCN at −65 °C to 0 °C for 12 h afforded the amino nitrile **ii** in greater than 80% yield. Unfortunately, the asymmetric induction was fairly low (77:23 er, HPLC assay). *O*-Methylation of **ii** with MeI/K_2CO_3 in acetone (>90%), followed by hydrolysis of nitrile **iii** using H_2O_2/NaOH and the phase transfer catalyst $n\text{-}Bu_4NHSO_4$, yielded the corresponding amide **iv** (54%). Attempted removal of the *N*-aryl substituent using ceric ammonium nitrate led to decomposition of amide **iv**, apparently due to the sensitive indole moiety.



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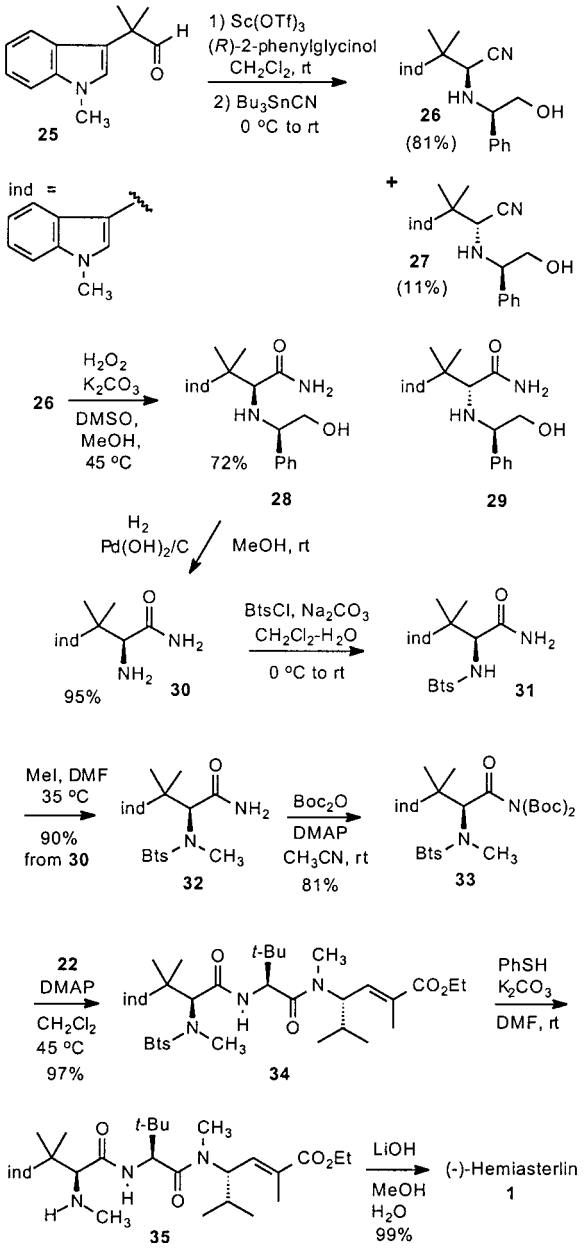
Scheme 3



clear that hydrolysis of the nitrile to the acid in the presence of a tryptophan subunit would be a difficult problem.

Efforts to remove the chiral auxiliary from the major Strecker product **26** under oxidative conditions (i.e., $Pb(OAc)_4$,[16] H_5IO_6,[26a] $NaIO_4$,[26b,c] and $NaIO_4/RuCl_3$) were unsuccessful due to extensive decomposition of the substrate. Furthermore, attempts to convert the nitrile to a carboxylate derivative failed under a variety of conditions.[27−30] We therefore opted to prepare the corresponding amide **28**. It has been shown that the nitrile group of a Strecker adduct can be hydrolyzed to the amide with $H_2O_2/NaOH/n\text{-}Bu_4NHSO_4$.[22,31] However, treatment of the amino nitrile (*S*,*R*)-**26** with this reagent combination gave no more than 40% of **28** along with extensive decomposition. Alternative procedures[32−34] were investigated, and a promising result was obtained under the conditions reported by Katritzky ($H_2O_2/K_2CO_3 \cdot 1.5H_2O$/DMSO).[33] Even though complete conversion was not achieved, hydrolysis was clean and there was little decomposition (39% yield of **28** isolated with 56% recovery of **26**). Further optimization improved the yield of **28** to 72% by using methanol as the solvent, together with 10% of DMSO. If desired, the hydrolysis procedure could also be carried out with a mixture of **26** and **27**. The resulting mixture of amide diastereomers **28** and **29** was somewhat easier to separate (flash column chromatography) than were the nitrile diastereomers. Thus, a 6:1 mixture of **26** and **27** was treated with $H_2O_2/K_2CO_3 \cdot 1.5H_2O$ in 10:1 MeOH:DMSO (9 h, 45 °C). After isomer separation, **28** was obtained in 52% yield based on the mixture of **26** and **27**.

The chiral auxiliary was cleaved from **28** by hydrogenolysis over $Pd(OH)_2$ to yield amino amide **30** in excellent yield (95%).[35] Treatment of **30** with BtsCl under biphasic conditions (CH_2Cl_2 in aqueous Na_2CO_3) gave **31** and methylation with excess MeI/K_2CO_3 in DMF proceeded uneventfully to afford the *N*-Bts-*N*-methyl amide **32** (90% over two steps). Several options had been considered for hydrolytic cleavage of the amide to an analogue of Andersen's acid **8**, but we were intrigued by the possibility that activation of the amide might allow peptide coupling without a hydrolysis step. Davidsen et al. have reported that amides react with Boc_2O/DMAP to produce the isolable di-*tert*-butyl *N*-acylimidodicarbonates, and that these products are sufficiently reactive to acylate amines.[36,37] Thus, **32** was converted into the bis-Boc derivative **33** (81% isolated) by reaction with Boc_2O/DMAP in CH_3CN. The activated amide **33** was then treated with 1.2 equiv of the amino ester **22** in

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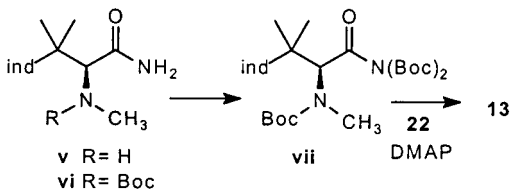
(37) We have briefly evaluated the feasibility of using the Davidsen coupling technique for the synthesis of Andersen's intermediate **13**. Thus, **32** was deprotected to **v** followed by treatment with Boc_2O to afford **vi**. More Boc_2O was added (3 equiv), together with DMAP in dichloromethane, resulting in **vii**, ca. 40% overall yield. Upon refluxing **vii** with 1.1 equiv of **22** in dichloromethane in the presence of DMAP (ca. 0.3 equiv), **13** was formed in 96% yield. Thus, efficient coupling does not require *N*-Bts protection. However, the activation procedure from **vi** is more difficult than from the Bts analogue **32**.



refluxing CH_2Cl_2 in the presence of DMAP (18 h), resulting in the formation of tripeptide **34** in excellent yield (97%). Subsequent Bts cleavage with $PhSH/K_2CO_3$ in DMF proceeded readily to furnish the free amino ester **35**, and hydrolysis with LiOH[8] in aqueous methanol afforded (−)-hemiasterlin (99% over two steps). The crude synthetic (−)-hemiasterlin displayed spectroscopic features (^1H, ^{13}C NMR) indistinguishable from those reported for natural (−)-hemiasterlin by Kashman et al.[2a] However, the synthetic (−)-hemiasterlin was observed to crystallize from MeOH/hexane. This gave analytically pure, colorless crystals with $[\alpha]^{23}_D$ values of −114.7 to −118.9 (*c* 0.08 or 0.07, respectively; MeOH), somewhat higher than the values reported previously for the natural (noncrystalline) material isolated by chromatography: $[\alpha]^{23}_D$= −95 (*c* 0.06, MeOH)[2a] and −76 (*c* 0.07, MeOH).[2b]

Summary

A total synthesis of (−)-hemiasterlin has been accomplished in nine steps from **25**[8] (>35% yield overall). An improved enantiocontrolled route to the tetramethyltryptophan subunit **32** was developed using an asymmetric Strecker synthesis (five steps, 50% yield from **25**) and the dipeptide **22** was prepared in seven steps, 37% yield from valinol. The corresponding intermediates in the Andersen synthesis are **8** (10 steps, 22% from **25**)[8] and **12** (six steps, 23% from *N*-Boc-MeVal).[7,8] Our synthesis exploits the high reactivity of a Bts-protected amino acid chloride in the difficult peptide coupling of sterically hindered amino acid residues **18** and **20** to form **21** (70%, recrystallized) and also uses *N*-Bts intermediates for the high yielding *N*-methylations of **14** and **31**. In addition, the Bts-protected di-*tert*-butyl *N*-acylimidodicarbonate **33** is shown to undergo efficient coupling with **22** to form **34** (97% in the coupling step; 79% over the activation; coupling sequence from **32**).

The principal advantage of the *N*-Bts derivatives over the *N*(-*o*-nitrophenylsulfonyl) ("nosylate") analogues is their crystallinity. All *N*-Bts amino acids and esters prepared to date in our laboratory have been solids that are easy to purify without chromatography. Crystallinity is less common with the *N*-nosyl amino acids, although the leucine and phenylalanine derivatives are reported to crystallize after chromatography.[15c] In other respects, the potential utility of the *N*-Bts and *N*-nosyl amino acids is comparable, although there are some differences in relative reactivity and deprotection profiles.[9,10,38] In the present application, high enantiomeric and diastereomeric purity was achieved by exploiting the crystallinity of key intermediates **16** and **21** and was established by rigorous HPLC assay at several stages. On the basis of the optical rotation data, the quality of the synthetic (−)-hemiasterlin is improved compared to the material from natural sources.

(38) As expected from earlier reports,[15] qualitative comparisons in our laboratory confirm that *N*-nosyl amino acids and peptides can be methylated efficiently using the same conditions as for *N*-Bts amino acid derivatives.[10] The derived *N*-nosyl amino acid chlorides form more slowly with thionyl chloride, but their reactivity for *N*-acylation is comparable to that of the Bts analogues and is sufficient for peptide synthesis. The most significant differences in terms of reactivity have been encountered under reductive deprotection conditions. The *N*-Bts derivatives are easily cleaved with Zn/HOAc[9] or (in the case of *N*-methyl amino acids) sodium borohydride[10] The latter procedure does not cleave the *N*-nosyl group, while Zn/HOAc reduces the nitro substituent, but not the *N*-sulfonyl linkage.

Experimental Section

General. All reactions were performed under N_2 atmosphere unless otherwise noted. Solvents and reagents were purified as follows: tetrahydrofuran (THF), dichloromethane (CH_2Cl_2), and ether were purified using an Anhydrous Engineering solvent purification system using column packed with A-2 alumina; acetonitrile (CH_3CN) was distilled from P_2O_5; toluene, *i*-Pr_2NEt, and Et_3N were distilled from CaH_2; methanol was distilled from magnesium; anhydrous dimethylformamide (DMF) was obtained form Aldrich. Flash column chromatography was performed with 230−400 mesh EM silica gel 60. Preparative TLC was performed with Whatman silica gel 60 PF_{254} glass plate (20 × 20 × 0.1 cm). Analytical TLC was performed with EM silica gel 60 PF_{254} glass plate with a 0.25 mm layer of silica gel.

***N*-Bts-(*S*)-Valinol (14).** To a stirred 0−5 °C suspension mixture of (*S*)-valinol (0.52 g, 5.0 mmol) and Na_2CO_3 (0.54 g, 5.1 mmol) in CH_2Cl_2 (10 mL) and H_2O (10 mL) was added solid BtsCl[10] (1.2 g, 5.1 mmol) in one portion. The reaction mixture was allowed to warm to room temperature over 12 h and then poured into H_2O (20 mL) and 10% MeOH in CH_2Cl_2 (20 mL). The layers were separated, and the aqueous phase was extracted with 10% MeOH in CH_2Cl_2 (4 × 20 mL). The combined organic extracts were washed with 1% aqueous HCl, brine, dried (Na_2SO_4), and concentrated (aspirator). The residue was purified by column chromatography on silica gel (15 × 3.5 cm, 3:2 to 1:1 hexane/EtOAc eluent) to afford *N*-Bts-(*S*)-valinol (**14**) as a colorless solid (1.23 g, 82%): analytical TLC on silica gel, 3:2 hexane/EtOAc, R_f = 0.21; analytical HPLC, CHIRALCEL AS (90% hex/IPA, 1 mL/min, P = 203 psi) t_R = 18.65 min; >99.75:0.25 er. Pure material was obtained by crystallization from CH_2Cl_2/MeOH/hexane, mp 162−163 °C. Molecular ion (M + H) calcd for $C_{12}H_{17}N_2O_3S_2$: 301.06806; found (CI, NH_3) *m/e* = 301.0679, error = 1 ppm; base peak = 301 amu; $[\alpha]^{25}_D$ +18.0 (*c* 1.0, acetone); IR (neat, cm^{-1}) 3381, O−H; 3250, N−H; 400 MHz NMR ($CDCl_3$, ppm) δ 8.11−8.09 (1H, m), 7.98−7.96 (1H, m), 7.62−7.54 (2H, m), 5.32 (1H, d, J = 9.2 Hz), 3.73−3.65 (2H, m), 3.58−3.52 (1H, m), 3.19 (1H, dd, J = 6.8, 6.0 Hz), 2.00−1.88 (1H, m), 0.98 (6H, d, J = 7.2 Hz). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ 167.9, 151.4, 136.2, 127.7, 127.6, 124.7, 122.2, 62.8, 62.7, 30.3, 19.2, 18.6.

N-Bts-*N*-Methyl-(*S*)-valinol (15). To a stirred suspension of *crystallized N*-Bts-(*S*)-valinol (**14**) (>99.7:0.3 er) (0.90 g, 3.0 mmol) and K_2CO_3 (2.07 g, 15.0 mmol) in dry DMF (6 mL) was added excess iodomethane (2.8 mL, 45.0 mmol) in one portion at room temperature. The reaction mixture was brought to 35 °C for 4 h and was monitored by TLC. After complete consumption of starting material (TLC analysis), the mixture was diluted with EtOAc and H_2O. The aqueous layer was separated and extracted with EtOAc. The combined organic layer was washed with H_2O, brine, dried (Na_2SO_4), and concentrated (aspirator). The residue was purified by column chromatography on silica gel (15 × 2.7 cm, 3:1 to 3:2 hexane/EtOAc eluent) to give *N*-Bts-*N*-methyl-(*S*)-valinol (**15**) (0.86 g, 91%): analytical TLC on silica gel, 3:2 hexane/EtOAc, R_f = 0.28; analytical HPLC, CHIRALCEL OJ (90% hex/IPA, 1 mL/min, P = 304 psi) t_R = 21.53 min; 99.7:0.3 er. Pure material was obtained by crystallization from CH_2Cl_2/hexane, mp 94.5−95.5 °C. Molecular ion (M + H) calcd for $C_{13}H_{19}N_2O_3S_2$: 315.08371; found (CI, NH_3) *m/e*= 315.0851, error= 4 ppm; base peak= 136 amu; $[\alpha]^{25}_D$ −12.3 (*c* 1.0, $CHCl_3$); IR (neat, cm^{-1}) 3416, O−H; 400 MHz NMR ($CDCl_3$, ppm) δ 8.14−8.11 (1H, m), 7.98−7.96 (1H, m), 7.63−7.54 (2H, m), 4.29 (1H, dd, J = 8.6, 3.6 Hz), 3.98 (1H, ddd, J = 10.0, 9.9, 3.6 Hz), 3.88 (1H, ddd, J = 12.4, 8.6, 3.6 Hz), 3.74 (1H, ddd, J = 12.4, 9.9, 3.6 Hz), 2.91 (3H, s), 1.88−1.76 (1H, m), 1.05 (3H, d, J = 6.6 Hz), 1.04 (3H, d, J = 6.6 Hz). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ 167.5, 151.2, 136.3, 127.6, 127.5, 124.7, 122.2, 68.1, 60.8, 29.6, 27.9, 20.2, 20.0.

***N*-Bts-*N*-Methyl-(*S*)-valinal (16).** To a stirred −78 °C solution of oxalyl chloride (0.26 mL, 3.0 mmol) in dry CH_2Cl_2 (12 mL) was added a solution of DMSO (0.40 mL, 5.5 mmol) in dry CH_2Cl_2 (12 mL) over 10 min. The solution was stirred

for 20 min, and noncrystallized *N*-Bts-*N*-methyl-(*S*)-valinol (**15**) (0.81 g, 2.5 mmol) (99.7:0.3 er) in dry CH_2Cl_2 (16 mL) was added. The mixture was stirred for 30 min followed by the addition of triethylamine (1.7 mL, 12.5 mmol). After 10 min at −78 °C, the mixture was warmed to 0 °C for 5 min and then was poured into water. The aqueous layer was separated and extracted with CH_2Cl_2. The combined CH_2Cl_2 extracts were washed with half-saturated aqueous NH_4Cl, H_2O, brine, and dried (Na_2SO_4), followed by evaporation (aspirator). After standing under high vacuum for 1−2 h, the residue was purified by column chromatography on silica gel (15 × 2.7 cm, 8:1 hexane/acetone eluent) to afford *N*-Bts-*N*-methyl-(*S*)-valinal (**16**) (0.78 g, 97%, 97.4:2.6 er): analytical TLC on silica gel, 4:1 hexane/acetone R_f = 0.24. Pure material (0.68 g, 87% based on **15**) was obtained by a single recrystallization from ether/hexane and was used in the next step: mp 76−77 °C; analytical HPLC, CHIRALCEL OJ (90 hex/IPA, 1 mL/min, *P* = 304 psi) t_R = 43.08 min; minor isomer (confirmed using the racemic mixture), t_R = 24.68 min, 99.73:0.27 er. Molecular ion (M + H) calcd for $C_{13}H_{17}N_2O_3S_2$: 313.06806; found (DCI, NH_3) *m/e* = 313.0687, error = 2 ppm; base peak= 283 amu; $[\alpha]^{25}_D$ −75.4 (*c* 1.2, $CHCl_3$); IR (neat, cm^{-1}) 1722, C=O; 400 MHz NMR ($CDCl_3$, ppm) δ 9.69 (1H, s), 8.18−8.15 (1H, m), 7.99−7.96 (1H, m), 7.63−7.54 (2H, m), 4.39 (1H, d, *J* = 10.0 Hz), 3.02 (3H, s), 2.28−2.15 (1H, m), 1.16 (3H, d, *J* = 6.6 Hz), 1.01 (3H, d, *J* = 6.6 Hz). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ 197.8, 165.0, 152.4, 136.3, 127.6, 127.4, 125.2, 122.1, 71.1, 31.8, 26.6, 19.8, 19.7.

(2*E*,4*S*)-4-[(Benzothiazole-2-sulfonyl)methylamino]-2,5-dimethylhex-2-enoic Acid Ethyl Ester (17). To a 25 mL round-bottomed flask charged with crystallized *N*-Bts-*N*-methyl-(*S*)-valinal (**16**) (99.7:0.3 er) (0.164 g, 0.5 mmol) and [(1-ethoxycarbonyl)ethylidene]triphenylphosphorane (0.37 g, 1.0 mmol) was added dry THF (10 mL). The mixture was refluxed for 3 h. After cooling, water and EtOAc were added. The aqueous layer was separated and was extracted with EtOAc. The combined organic extracts were washed with brine, dried (Na_2SO_4), and concentrated (aspirator). The residue was purified by column chromatography on silica gel (15 × 2 cm, 10:1 hexane/acetone eluent) to give the adduct as a mixture of *E*/*Z* isomers (*E*:*Z* = 26:1 by NMR assay) (0.19 g, 96% yield of the mixture). If desired (see next step), the isomers could be separated by preparative TLC on silica gel (20 × 20 × 0.1 cm, 3:1 hexane/ether eluent, two elutions). The lower R_f zone was the desired (*E*)-**17** (0.173 g, 87%): analytical TLC on silica gel, 2:1 hexane/ether, R_f = 0.27. Molecular ion calcd for $C_{18}H_{24}N_2O_4S_2$: 396.11780; found (EI) *m/e* = 396.1193, error = 4 ppm; base peak = 353 amu; 98.29:1.71 er after Bts-deprotection followed by *N*-benzoylation and HPLC assay; $[\alpha]^{25}_D$ +52.3 (*c* 1.15, $CHCl_3$); IR (neat, cm^{-1}) 1710, C=O; 1633, C=C; 400 MHz NMR ($CDCl_3$, ppm) δ 8.11−8.09 (1H, m), 7.94−7.91 (1H, m), 7.58−7.49 (2H, m), 6.41 (1H, dq, *J* = 10.6, 1.6 Hz), 4.43 (1H, dd, *J* = 10.6, 10.6 Hz), 3.91 (1H, AB quartet of q, *J* = 10.9, 6.8 Hz), 3.86 (1H, AB quartet of q, *J* = 10.9, 7.2 Hz), 3.10 (3H, s), 1.91−1.82 (1H, m), 1.86 (3H, d, *J* = 1.6 Hz), 1.08 (3H, dd, *J* = 7.2, 6.8 Hz), 1.05 (3H, d, *J* = 6.4 Hz), 0.85 (3H, d, *J* = 6.4 Hz). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ 167.1, 165.4, 152.4, 136.0, 134.1, 131.7, 127.3, 127.1, 125.0, 121.9, 62.0, 60.6, 30.1, 29.9, 19.4, 19.1, 14.0, 13.6. The higher R_f zone was the *Z*-isomer (*Z*)-**17** (0.007 g, 4%): analytical TLC on silica gel, 2:1 hexane/ether, R_f = 0.30; IR (neat, cm^{-1}) 1710, C=O; 1648, C=C; 400 MHz NMR ($CDCl_3$, ppm) δ 8.15−8.13 (1H, m), 7.94−7.92 (1H, m), 7.59−7.50 (2H, m), 5.66 (1H, dq, *J* = 10.6, 1.6 Hz), 5.29 (1H, dd, *J* = 10.6, 10.6 Hz), 4.23 (1H, AB quartet of q, *J* = 10.8, 7.2 Hz), 4.16 (1H, AB quartet of q, *J* = 10.8, 7.2 Hz), 3.09 (3H, s), 1.85−1.76 (1H, m), 1.53 (3H, d, *J* = 1.6 Hz), 1.33 (3H, dd, *J* = 7.2, 7.2 Hz), 1.27 (3H, d, *J* = 6.6 Hz), 0.89 (3H, d, *J* = 6.6 Hz). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ 166.7, 166.0, 152.6, 136.1, 134.3, 131.2, 127.2, 127.1, 125.0, 121.9, 61.3, 60.8, 30.2, 30.1, 20.8, 19.6, 19.1, 14.1.

(2*E*,4*S*)-4-Methylamino-2,5-dimethylhex-2-enoic Acid Ethyl Ester (18). To a stirred suspension of chromatographically purified (*E*)-**17** (87% yield material in prior step; 0.173 g, 0.43 mmol) and K_2CO_3 (0.24 g. 1.72 mmol) in dry DMF (2 mL) under N_2 was added benzenethiol (0.13 mL, 1.3 mmol) in one portion at room temperature. The suspension was vigorously stirred and was monitored by TLC assay. After conversion was complete (30 min), the mixture was diluted with ether and H_2O. The aqueous layer was separated and was extracted with ether, and the combined ether extracts were washed with H_2O. The organic phase was extracted with 1% aqueous hydrochloric acid. The combined aqueous hydrochloric acid extracts were washed with ether and neutralized with saturated aqueous $NaHCO_3$. The resulting aqueous phase was extracted with HPLC grade CH_2Cl_2. The combined CH_2Cl_2 layer was washed with brine, dried (Na_2SO_4), and concentrated (aspirator) to provide **18** (0.08 g, 93%; >98% (*E*) isomer). Alternatively, the 26:1 *E*:*Z* mixture (0.19 g) from the prior step (96% recovery) could be used in the same procedure to give **18** (0.086 g) contaminated by the *Z* isomer. This material could be used in the next step to give isomerically pure **21** after crystallization as described in the alternative preparation of **21** (see below). Characterization of (*E*)-**18**: analytical TLC on silica gel, 4.5:1:0.5 *n*-BuOH/H_2O/HOAc, R_f = 0.43. Molecular ion calcd for $C_{11}H_{21}NO_2$: 199.15720; found (EI) *m/e* = 199.1564, error = 4 ppm; base peak = 156 amu; 98.29:1.71 er after *N*-benzoylation and HPLC assay; $[\alpha]^{25}_D$ +27.1 (*c* 1.2, $CHCl_3$); IR (neat, cm^{-1}) 3312, N−H; 1708, C=O; 1648, C=C; 400 MHz NMR ($CDCl_3$, ppm) δ 6.51 (1H, dq, *J* = 10.2, 1.2 Hz), 4.21 (1H, AB quartet of q, *J* = 10.8, 7.0 Hz), 4.19 (1H, AB quartet of q, *J* = 10.8, 7.0 Hz), 3.08 (1H, dd, *J* = 10.2, 6.4 Hz), 2.33 (3H, s), 1.88 (3H, d, *J* = 1.2 Hz), 1.81−1.67 (1H, m), 1.30 (3H, dd, *J* = 7.0, 7.0 Hz), 1.22−1.06 (1H, br), 0.95 (3H, d, *J* = 6.8 Hz), 0.89 (3H, d, *J* = 6.8 Hz). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ 168.0, 142.8, 129.9, 63.6, 60.6, 34.5, 32.8, 19.3, 18.4, 14.2, 13.1.

Assay of 18: (2*E*,4*S*)-4-(Benzoylmethylamino)-2,5-dimethylhex-2-enoic Acid Ethyl Ester. To a solution of **18** (7 mg, 0.034 mmol) in dry CH_2Cl_2 (1 mL) were added *i*-Pr_2-NEt (9 μL, 0.051 mmol) and benzoyl chloride (5 μL, 0.041 mmol). After stirring under N_2 at room temperature for 1 h, the reaction mixture was diluted with CH_2Cl_2 and dilute citric acid. The aqueous phase was extracted with CH_2Cl_2. The combined CH_2Cl_2 extracts were washed with saturated aqueous $NaHCO_3$, H_2O, and brine. The resulting solution was then dried (Na_2SO_4) and concentrated (aspirator). The residue was purified by preparative TLC on silica gel (20 × 20 × 0.1 cm, 2:1 hexane/ether eluent) to furnish the title compound (9.3 mg, 91%): analytical TLC on silica gel, 4:1 hexane/EtOAc, R_f = 0.17; analytical HPLC, CHIRALCEL OD (98.5% hex/IPA, 1 mL/min, *P* = 261 psi), t_R = 20.45 min (minor isomer: t_R = 24.09 min); 98.29:1.71 er; IR (neat, cm^{-1}) 1710, C=O; 1633, C=O; 400 MHz NMR ($CDCl_3$, ppm) δ 7.39−7.29 (5H, m), 6.78−6.72 (1H, br), 5.19 (0.6H, dd, *J* = 10.2, 10.2 Hz), 4.23 (2H, q, *J* = 7.0 Hz), 4.04 (0.4H, dd, *J* = 10.2, 10.2 Hz), 3.06 (1.2H, s), 2.78 (1.8H, s), 2.03 (1.8H, s), 2.05−1.85 (1H, br), 1.42 (1.2H, s), 1.33 (3H, t, *J* = 7.0 Hz), 1.04 (1.8H, d, *J* = 6.4 Hz), 0.97 (1.8H, d, *J* = 6.4 Hz), 0.95 (1.2H, d, *J* = 6.4 Hz), 0.72 (1.2H, d, *J* = 6.4 Hz). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ 172.5, 171.4, 167.9, 167.7, 138.2, 136.8, 135.8, 132.9, 129.8, 129.3, 128.6, 128.4, 126.7, 126.6, 63.2, 61.0, 60.8, 56.4, 32.4, 30.6, 29.9, 27.6, 19.5, 19.4, 19.2, 18.9, 14.2, 13.6, 13.4.

***N*-Bts-(*S*)-*tert*-Leucine (19).** (*S*)-*tert*-Leucine (0.42 g, 3.2 mmol) was dissolved in 0.25 M aqueous NaOH (11 mL, 2.8 mmol) at 10 °C. Solid BtsCl[10] (1.10 g, 4.7 mmol) was added in one portion, and the suspension was stirred for 10 h at 10 °C. The reaction was monitored using a pH meter and was maintained at pH = 10−10.5 by addition of 1.3 M aqueous NaOH (total 4 mL, 5.2 mmol) as needed. The cloudy solution was extracted with ether to remove organic soluble materials. The aqueous phase was then acidified to pH 1 with concentrated HCl and was extracted with EtOAc. The combined EtOAc extracts were washed with H_2O and brine, dried (Na_2SO_4), and concentrated (aspirator). The residue was purified by crystallization from EtOAc/hexane to give *N*-Bts-(*S*)-*tert*-leucine (**19**) as a colorless solid (0.85 g, 81%): analytical TLC on silica gel, 20:10:0.25 EtOAc/hexane/HOAc, R_f = 0.53. Pure material was obtained by crystallization from EtOAc/hexane, mp 175−177 °C. Molecular ion (M + H) for

$C_{13}H_{17}N_2O_4S_2$: 329.06297; found (CI, NH_3) 329.0626, error = 1 ppm; base peak = 136 amu; $[\alpha]^{25}_D$ +70.0 (*c* 1.1, $CHCl_3$); IR (neat, cm^{-1}) 3273, CO_2H; 1710, C=O; 1633, C=C; 400 MHz NMR ($CDCl_3$, ppm) δ 11.25 (1H, br s), 7.97−7.93 (1H, m), 7.91−7.86 (1H, m), 7.50−7.44 (2H, m), 6.00 (1H, d, J = 9.2 Hz), 4.05 (1H, d, J = 9.2 Hz), 1.00 (9H, s). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ 174.8, 165.8, 151.8, 136.0, 127.7, 127.5, 124.8, 122.1, 65.2, 34.8, 26.4.

(2*E*,4*S*,2′*S*)-4-{[2′-[(Benzothiazole-2-sulfonyl)amino]-3′,3′-dimethylbutyryl]methylamino}-2,5-dimethylhex-2-enoic acid ethyl ester (21). To a stirred solution of *N*-Bts-(*S*)-*tert*-leucine (**19**) (0.20 g, 0.6 mmol) in dry CH_2Cl_2 (2.5 mL) under N_2 was added $SOCl_2$ (0.13 mL, 1.8 mmol) at room temperature, and the reaction was brought to reflux for 2 h. After cooling, the dichloromethane was removed (aspirator). The resulting residue was dissolved in dry toluene (3.0 mL), and the toluene and residual $SOCl_2$ were evaporated to give the acid chloride **20** which was used without further purification in the next step. Two batches of **18** that differ in isomer purity were tested in the coupling step. The procedure using material of the highest purity was subjected to detailed separation procedures to isolate and characterize the side products as follows. To a vigorously stirred mixture of the amino ester **18** (0.08 g, 0.4 mmol, >98% (*E*) isomer), $NaHCO_3$ (0.11 g, 1.28 mmol) and Na_2CO_3 (0.09 g, 0.8 mmol) in 1:1 CH_2Cl_2 (2 mL) and H_2O (2 mL) at 0−5 °C was added **20** in dry CH_2Cl_2 (3 mL) via cannula over 1 min. The reaction mixture was maintained at 0−5 °C for an additional 10 min, and the reaction mixture was diluted with H_2O. The aqueous phase was separated and extracted with CH_2Cl_2. The combined CH_2Cl_2 extracts were washed with aqueous 1% HCl and brine and dried (Na_2SO_4) followed by evaporation (aspirator). The residue was purified by column chromatography on silica gel (15 × 2 cm, 3:1 hexane/acetone eluent). The first fraction was a mixture of two compounds which was further purified by preparative TLC on silica gel (20 × 20 × 0.1 cm, 5:1 hexane/EtOAc eluent, 3 elutions). The higher R_f zone was (2*E*,4*S*)-4-[(benzothiazol-2-yl)methylamino]-2,5-dimethylhex-2-enoic acid ethyl ester (**23**) (0.007 g, 5%): analytical TLC on silica gel, 4:1 hexane/EtOAc, R_f = 0.40. Molecular ion calcd for $C_{18}H_{24}N_2O_2S$: 332.15590; found (EI) *m/e* = 332.1554, error = 2 ppm; base peak = 289 amu; IR (neat, cm^{-1}) 1710, C=O; 1652, C=C; 400 MHz NMR ($CDCl_3$, ppm) δ 7.60−7.53 (2H, m), 7.30−7.26 (1H, m), 7.07−7.03 (1H, m), 6.75 (1H, dq, J = 9.6, 1.6 Hz), 4.62 (1H, br dd, J = 9.6, 9.6 Hz), 4.20 (2H, q, J = 7.2 Hz), 3.06 (3H, s), 2.12−2.03 (1H, m), 1.99 (3H, d, J = 1.6 Hz), 1.30 (3H, t, J = 7.2 Hz), 1.00 (3H, d, J = 6.8 Hz), 0.97 (3H, d, J = 6.8 Hz). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ 168.8, 167.8, 153.0, 137.0, 132.4, 130.5, 125.9, 120.9, 120.5, 118.9, 64.1, 60.9, 33.0, 31.0, 19.34, 19.31, 14.2, 13.9. The lower R_f zone was *N*-Bts-(*S*)-*tert*-leucine diketopiperazine (**24**) (0.021 g, 11% based on total *N*-Bts-*tert*-leucine (0.6 mmol) used in the coupling): analytical TLC on silica gel, 4:1 hexane/EtOAc, R_f = 0.34. LRMS: no parent ion for $C_{26}H_{28}N_4O_6S_4$; found (EI) *m/e* = 247 (M-373, $C_{11}H_{21}NO_3S$); base peak = 190 amu; IR (neat, cm^{-1}) 1733, C=O; 400 MHz NMR ($CDCl_3$, ppm) δ 7.86−7.84 (2H, m), 7.40−7.38 (2H, m), 7.34−7.29 (2H, m), 7.24−7.20 (2H, m), 4.19 (2H, s), 1.15 (18H, s). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ 175.7, 163.6, 131.0, 127.5, 127.0, 125.6, 123.3, 113.5, 84.9, 35.9, 26.3.

The second fraction from column chromatography was the desired dipeptide **21** (0.175 g, 86%, 98.51:1.49 dr): analytical TLC on silica gel, 3:1 hexane/EtOAc, R_f = 0.23. Pure material (0.143 g, 70% yield based on **18**) was obtained by a single recrystallization from ether/hexane, mp 120−121 °C; analytical HPLC, CHIRALCEL OD (99:1:1 hex/IPA/HOAc, 1 mL/min, P = 261 psi) t_R = 31.46 min (minor diastereomer t_R = 21.86 min, confirmed using racemic **18**); 99.43:0.57 dr. Molecular ion calcd for $C_{24}H_{35}N_3O_5S_2$: 509.20180; found (EI) *m/e* = 509.2015, error = 1 ppm; base peak = 156 amu; $[\alpha]^{25}_D$ −12.2 (*c* 1.5, $CHCl_3$); IR (neat, cm^{-1}) 3192, N−H; 1702, C=O; 1625, C=C; 400 MHz NMR ($CDCl_3$, ppm) δ 8.13−8.11 (1H, m), 7.96−7.94 (1H, m), 7.62−7.53 (2H, m), 6.54 (1H, dq, J = 10.2, 1.4 Hz), 6.08 (1H, d, J = 9.0 Hz), 4.86 (1H, dd, J = 10.2, 10.2 Hz), 4.49 (1H, d, J = 9.0 Hz), 4.18 (2H, q, J = 7.0 Hz), 2.95 (3H, s), 1.83 (3H, d, J = 1.4 Hz), 1.60−1.50 (1H, m), 1.28 (3H, t, J = 7.0 Hz), 0.99 (9H, s), 0.64 (3H, d, J = 6.6 Hz), −0.10 (3H, d, J = 6.6 Hz). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ 170.3, 167.5, 166.3, 152.5, 137.4, 136.4, 132.6, 127.6, 127.4, 124.9, 122.2, 60.9, 60.2, 56.7, 36.6, 31.0, 29.3, 26.4, 19.0, 17.7, 14.2, 13.8.

The last fraction from column chromatography was tentatively identified as benzothiazole-2-sulfonamide (0.026 g, 20% based on total *N*-Bts-*tert*-leucine (0.6 mmol) used in the coupling): analytical TLC on silica gel, 3:2 hexane/EtOAc, R_f = 0.42. Pure material was obtained by crystallization from ethyl acetate/hexane, mp 178−179 °C (lit. mp 177 °C);[39] IR (neat, cm^{-1}) 3300, N−H; 1351, SO_2; 1162, SO_2; 400 MHz NMR (CD_3CN, ppm) δ 8.18−8.10 (2H, m), 7.69−7.59 (2H, m), 6.34 (2H, br s). ^{13}C NMR (100 MHz, CD_3CN, ppm) δ 153.6, 137.6, 129.0, 128.9, 125.9, 124.1. The origin of this byproduct was not investigated.

A second coupling experiment was performed using the 26:1 *E*:*Z* mixture of **18** described under the procedures for preparation of **17** and **18**. Thus, 0.086 g of the deprotected amino ester **18** (26:1 *E*:*Z*) was subjected to the same coupling procedure with **20** as described above. Column chromatography as before gave 0.18 g of the product fraction (side products were not collected or assayed in this case), and one crystallization from ether/hexane gave 0.16 g of **21**. The NMR spectrum of this material was indistinguishable from **21** prepared starting from purified **17** (>98% (*E*) isomer). The overall yield of **21** (>99% *E*) based on **16** is 57% if the major isomer (E)-**17** is separated by chromatography prior to coupling, or 61% based on **16** using the 26:1 mixture through the deprotection and coupling steps. Therefore, isomer separation is not necessary at the stage of **17** and is not recommended.

(2*E*,4*S*,2′*S*)-4-[(2′-Amino-3′,3′-dimethylbutyryl)methylamino]-2,5-dimethylhex-2-enoic Acid Ethyl Ester (22). To a stirred suspension of crystallized **21** (0.138 g, 0.27 mmol) and K_2CO_3 (0.15 g, 1.08 mmol) in dry DMF (1.3 mL) under N_2 was added benzenethiol (83 μL, 0.81 mmol) in one portion at room temperature. The suspension was vigorously stirred and was monitored by TLC. After conversion was complete (30 min), the reaction mixture was diluted with ether and H_2O. The aqueous layer was separated and was extracted with ether. The combined ether extracts were washed successively with H_2O. The organic phase was extracted with 1% hydrochloric acid, and the combined aqueous hydrochloric acid extracts were washed with ether and neutralized with $NaHCO_3$. The resulting aqueous phase was extracted with HPLC grade CH_2Cl_2, and the combined CH_2Cl_2 layer was washed with brine, dried (Na_2SO_4), and concentrated (aspirator) to provide **22** (0.0815 g, 97%, >95% pure by NMR assay): analytical TLC on silica gel, 4.5:0.5:1 *n*-BuOH/HOAc/H_2O, R_f = 0.49. Molecular ion (M + H) calcd for $C_{17}H_{33}N_2O_3$: 313.24911; found (CI, NH_3) *m/e* = 313.2498, error = 2 ppm; base peak = 313 amu; >99.7:0.3 dr after, *N*-benzoylation and HPLC assay; $[\alpha]^{25}_D$ −88.0 (*c* 1.58, $CHCl_3$); IR (neat, cm^{-1}) 3381, N−H; 1710, C=O; 1633, C=C; 400 MHz NMR ($CDCl_3$, ppm) δ 6.66 (1H, dq, J = 10.8, 1.2 Hz), 5.17 (1H, dd, J = 10.8, 10.8 Hz), 4.21 (2H, q, J = 7.2 Hz), 3.54−3.40 (1H, br), 2.91 (3H, s), 1.96−1.87 (1H, m), 1.92 (3H, d, J = 1.2 Hz), 1.54−1.40 (2H, br), 1.31 (3H, t, J = 7.2 Hz), 0.94 (9H, s), 0.89 (6H, d, J = 6.8 Hz). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ 174.6, 167.8, 138.6, 132.5, 60.8, 58.1, 55.7, 35.4, 30.8, 30.0, 26.7, 26.3, 19.4, 18.9, 14.2, 13.8.

Assay of 22: (2*E*,4*S*,2′*S*)-4-[(2′-Benzoylamino-3,3-dimethylbutyryl)methylamino]-2,5-dimethylhex-2-enoic Acid Ethyl Ester. To a solution of **22** (15.8 mg, 0.05 mmol) in dry CH_2Cl_2 (1 mL) were added *i*-Pr_2NEt (13 μL, 0.075 mmol) and benzoyl chloride (7 μL, 0.06 mmol). After stirring under N_2 at room temperature for 1 h, the reaction mixture was diluted with CH_2Cl_2 and dilute citric acid. The aqueous phase was extracted with CH_2Cl_2. The combined CH_2Cl_2 extracts were washed with saturated aqueous $NaHCO_3$, H_2O, and brine. The resulting solution was then dried (Na_2SO_4) and

(39) (a) Roblin, R. O., Jr.; Clapp, J. W. *J. Am. Chem. Soc.* **1950**, *72*, 1275. (b) Stanovnik, B.; Tišler, M. *Arch. Pharm.* **1965**, *298*, 357.

concentrated (aspirator). The residue was purified by preparative TLC on silica gel (20 × 20 × 0.1 cm, 4:1 hexane/EtOAc eluent) to give the title compound (20 mg, 98%): analytical TLC on silica gel, 1:1 hexane/ether, $R_f = 0.25$; analytical HPLC, CHIRALCEL AD (98.5:1.5:0.5 hex/IPA/HOAc, 1 mL/min, P = 232 psi), $t_R = 19.59$ min (minor diastereomer, $t_R = 30.54$ min) >99.7:0.3 dr; IR (neat, cm^{-1}) 3428, N−H; 1710, C═O; 1633, C═C; 400 MHz NMR ($CDCl_3$, ppm) δ 7.79−7.54 (2H, m), 7.53−7.49 (1H, m), 7.46−7.42 (2H, m), 6.87 (1H, d, $J = 9.6$ Hz), 6.66 (1H, dq, $J = 9.8$, 1.6 Hz), 5.12 (1H, dd, $J = 9.8$, 9.8 Hz), 5.09 (1H, d, $J = 9.6$ Hz), 4.22 (2H, q, $J = 6.8$ Hz), 3.05 (3H, s), 1.94−1.86 (1H, m), 1.93 (3H, d, $J = 1.6$ Hz), 1.32 (3H, t, J = 6.8 Hz), 1.04 (9H, s), 0.88 (3H, d, $J = 6.6$ Hz), 0.79 (3H, d, J = 6.6 Hz). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ 171.7, 167.7, 167.1, 138.2, 134.3, 132.7, 131.6, 128.6, 127.0, 60.9, 56.2, 55.1, 36.0, 31.1, 29.9, 26.6, 19.4, 18.7, 14.2, 13.8.

***N*-[(1′*R*)-2′-Hydroxy-1′-phenylethyl]-(2*S*)-2-amino-3-methyl-3-(1-methylindol-3-yl)butyronitrile [(*S, R*)-26] and *N*-[(1′*R*)-2′-Hydroxy-1′-phenylethyl]-(2*R*)-2-amino-3-methyl-3-(1-methylindol-3-yl)butyronitrile (27).** To a stirred room-temperature solution of aldehyde **25**[8] (0.43 g, 2.13 mmol) in dry CH_2Cl_2 (10 mL) were added (*R*)-2-phenylglycinol (0.44 g, 3.2 mmol) and $Sc(OTf)_3$ (0.1 g, 0.2 mmol). After 1 h, the reaction mixture was cooled to 0−5 °C, and tributyltin cyanide (1.01 g, 3.2 mmol) was added. The mixture was allowed to warm to room temperature over 20 h before being diluted with saturated aqueous $NaHCO_3$. The aqueous layer was separated and was extracted with CH_2Cl_2. The combined CH_2Cl_2 extracts were washed with H_2O and brine, dried (Na_2SO_4), and concentrated (aspirator). The residue was purified by column chromatography on silica gel (15 × 2 cm, 2:1 hexane/EtOAc eluent) to give product as a mixture of diastereomers. The diastereomers were separated by preparative TLC on silica gel (20 × 20 × 0.1 cm, 30:1 CH_2Cl_2/MeOH eluent, two elutions). The lower R_f zone was **26** (0.6 g, 81%): analytical TLC on silica gel, 20:1 CH_2Cl_2/MeOH, $R_f = 0.51$; analytical HPLC, CHIRALCEL OD (90% hex/IPA, 1 mL/min, P = 290 psi) $t_R = 9.86$ min. Molecular ion (M + H) calcd for $C_{22}H_{26}N_3O$: 348.20759; found (CI, NH_3) $m/e = 348.2074$, error = 1 ppm; base peak = 172 amu; $[\alpha]^{25}_D$ −126.2 (c 1.31, $CHCl_3$); IR (neat, cm^{-1}) 3455, O−H; 3343, N−H; 400 MHz NMR ($CDCl_3$, ppm) δ 7.36−7.34 (1H, m), 7.31−7.17 (5H, m), 7.08−7.06 (2H, m), 6.96−6.92 (1H, m), 6.95 (1H, s), 3.99 (1H, dd, $J = 8.8$, 4.0 Hz), 3.76 (3H, s), 3.74 (1H, s), 3.68 (1H, ddd, $J = 10.8$, 6.0, 4.0 Hz), 3.46 (1H, ddd, $J = 10.8$, 8.8, 6.0 Hz), 2.11−2.04 (1H, br), 1.84 (1H, dd, $J = 6.0$, 6.0 Hz), 1.61 (3H, s), 1.59 (3H, s). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ 138.2, 137.7, 128.7, 128.0, 127.5, 127.2, 125.6, 121.5, 120.8, 119.6, 118.9, 117.7, 109.6, 67.2, 63.0, 57.8, 38.5, 32.8, 25.6, 25.1. The higher R_f zone was **27** (0.085 g, 11%): analytical TLC on silica gel, 20:1 CH_2Cl_2/MeOH, R_f = 0.61; analytical HPLC, CHIRALCEL OD (90% hex/IPA, 1 mL/min, P = 290 psi) $t_R = 19.24$ min; $[\alpha]^{25}_D$ −25.2 (c 1.1, $CHCl_3$); IR (neat, cm^{-1}) 3474, O−H; 3358, N−H; 400 MHz NMR ($CDCl_3$, ppm) δ 7.74−7.72 (1H, m), 7.33−7.22 (7H, m), 7.13−7.09 (1H, m), 6.98 (1H, s), 4.06 (1H, br s), 3.76 (3H, s), 3.67 (1H, dd, $J = 6.8$, 4.4 Hz), 3.58 (1H, ddd, $J = 11.2$, 5.6, 4.4 Hz), 3.50 (1H, ddd, $J = 11.2$, 6.8, 6.8 Hz), 1.83−1.78 (1H, br), 1.67−1.63 (1H, br), 1.66 (3H, s), 1.64 (3H, s). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ 139.7, 137.7, 128.6, 128.0, 127.33, 127.28, 125.6, 121.7, 120.5, 119.9, 119.1, 117.5, 109.8, 65.6, 63.8, 58.6, 39.2, 32.8, 25.8, 24.8.

***N*-[(1′*R*)-2′-Hydroxy-1′-phenylethyl]-(2*S*)-2-amino-3-methyl-3-(1-methylindol-3-yl)butyramide (28).** From purified **26**: To a stirred suspension of **26** (0.053 g, 0.15 mmol) and K_2CO_3A1.5 H_2O (0.013 g, 0.075 mmol) in MeOH (0.5 mL) were added DMSO (43 μL, 0.6 mmol) and 30% aqueous H_2O_2 (68 μL, 0.6 mmol), respectively. The reaction mixture was brought to 45 °C for 6 h, and then water and EtOAc were added. The aqueous layer was separated and was extracted with EtOAc. The combined EtOAc extracts were washed successively with H_2O and brine, dried (Na_2SO_4), and concentrated (aspirator). The residue was purified by preparative TLC on silica gel (20 × 20 × 0.1 cm, 20:1 CH_2Cl_2/MeOH eluent) to furnish **28** (0.0392 g, 72%): analytical TLC on silica gel, 20:1 CH_2Cl_2/MeOH, $R_f = 0.22$; analytical HPLC, CHIRALCEL OJ (92% hex/IPA, 1 mL/min, P=290 psi) $t_R = 17.33$ min. Pure material was obtained by crystallization from CH_2Cl_2/hexane, mp 167−168 °C. Molecular ion (M + H) calcd for $C_{22}H_{28}N_3O_2$: 366.21815; found (CI, CH_4) $m/e = 366.2184$, error = 1 ppm; base peak = 79 amu; $[\alpha]^{25}_D$ −91.0 (c 1.0, $CHCl_3$); IR (neat, cm^{-1}) 3416, O−H; 3266, N−H; 1652, C═O; 400 MHz NMR ($CDCl_3$, ppm) δ 7.28−7.24 (2H, m), 7.18−7.10 (2H, m), 7.00−6.97 (2H, m), 6.85−6.81 (2H, m), 6.73−6.71 (2H, m), 6.14 (1H, br s), 5.52 (1H, br s), 3.75 (3H, s), 3.63−3.56 (2H, m), 3.53−3.50 (1H, m), 3.50−3.44 (1H, m), 2.37 (1H, dd, $J = 6.0$, 6.0 Hz), 2.14−2.06 (1H, br), 1.51 (3H, s), 1.49 (3H, s). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ 175.5, 139.2, 137.7, 128.2, 127.4, 127.2, 126.5, 125.5, 121.5, 120.9, 120.7, 119.0, 109.4, 67.2, 66.5, 63.3, 38.1, 32.7, 25.5, 24.6.

From a mixture of **26** and **27**: The 6:1 mixture of **26** and **27** (0.125 g, 0.36 mmol) was stirred with K_2CO_3A1.5 H_2O (0.030 g, 0.18 mmol) in MeOH (1 mL). DMSO (0.1 mL, 1.44 mmol) and 30% aqueous H_2O_2 (0.17 mL, 1.44 mmol) as described above, and the same workup was used to give the crude product after solvent evaporation. The residue was purified by column chromatography on silica gel (15 × 2 cm, 25:1 to 20:1 CH_2Cl_2/MeOH eluent) to furnish **28**. Diastereomer **29** (0.0115 g) was eluted first, followed by the desired **28** (0.0689 g, 52%, 63% based on recovered starting material).

***N*-[(1′*R*)-2′-Hydroxy-1′-phenylethyl]-(2*R*)-2-amino-3-methyl-3-(1-methylindol-3-yl)butyramide (29).** To a stirred suspension of **27** (0.0407 g, 0.11 mmol) and K_2CO_3A1.5 H_2O (0.011 g, 0.06 mmol) in DMSO (0.5 mL) cooled in water bath (ca. 20 °C) was added dropwise 30% aqueous H_2O_2 (0.35 mL). After stirring for 36 h, the mixture was worked up as described for **28**. Preparative TLC on silica gel (20 × 20 × 0.1 cm, 25:1 CH_2Cl_2/MeOH eluent) gave **29** (0.019 g, 48%): analytical TLC on silica gel, 20:1 CH_2Cl_2/MeOH, $R_f = 0.26$; analytical HPLC, CHIRALCEL OJ (92% hex/IPA, 1 mL/min, P = 290 psi) t_R = 25.00 min. Pure material was obtained by crystallization from ether/CH_2Cl_2, mp 164−165 °C; $[\alpha]^{25}_D$ −71.3 (c 1.01, $CHCl_3$); IR (neat, cm^{-1}) 3350, O−H; 400 MHz NMR ($CDCl_3$, ppm) δ 7.85−7.83 (1H, m), 7.34−7.12 (8H, m), 6.92 (1H, s), 5.96 (1H, br s), 5.02 (1H, br s), 3.79 (1H, d, $J = 4.0$ Hz), 3.77 (3H, s), 3.51−3.36 (3H, m), 2.38 (1H, dd, $J = 7.2$, 4.0 Hz), 1.57 (3H, s), 1.53 (3H, s), 1.14 (1H, dd, $J = 6.4$, 6.4 Hz). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ 175.5, 141.0, 137.9, 128.4, 127.4, 127.3, 127.0, 125.2, 121.9, 120.8, 120.5, 119.1, 109.9, 67.1, 64.8, 63.9, 38.9, 32.8, 25.4, 24.6

(2*S*)-2-Amino-3-methyl-3-(1-methylindol-3-yl)butyramide (30). To a stoppered 10 mL round-bottomed flask, charged with noncrystallized **28** (0.163 g, 0.44 mmol) and 20%$Pd(OH)_2$ on carbon (Pearlman's catalyst) (0.20 g) was added dry MeOH (4.0 mL). The reaction flask was stoppered with a septum secured by wire. The reaction mixture was successively degassed (aspirator) and backfilled with hydrogen gas (balloon) (three times). The mixture was then stirred at room temperature for 12 h under hydrogen (balloon). Catalyst was removed by filtration through a Celite pad and washed with 10:1 CH_2Cl_2/MeOH. Solvents were removed by aspirator to provide crude material which was purified by column chromatography on silica gel (15 × 1.5 cm, 20:1 to 10:1 CH_2Cl_2/MeOH eluent) to provide **30** (0.10 g, 95%): analytical TLC on silica gel, 10:1 CH_2Cl_2/MeOH, $R_f = 0.32$. Pure material was obtained by crystallization from MeOH/CH_2Cl_2, mp 175−176 °C. Molecular ion calcd for $C_{14}H_{19}N_3O$: 245.15290; found $m/e = 245.1520$, error = 4 ppm; base peak = 172 amu; $[\alpha]^{25}_D$ +45.4 (c 1.19, $CHCl_3$); IR (neat, cm^{-1}) 3339, N−H; 3289, N−H; 1679, C═O; 400 MHz NMR ($CDCl_3$, ppm) δ 7.87−7.85 (1H, m), 7.33−7.31 (1H, m), 7.26−7.22 (1H, m), 7.13−7.09 (1H, m), 6.89 (1H, s), 6.56 (1H, br s), 5.56 (1H, br s), 4.06 (1H, s), 3.76 (3H, s), 1.60 (3H, s), 1.45 (3H, s), 1.30 (2H, s). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ 176.3, 138.0, 126.8, 125.6, 121.7, 120.9, 118.9, 109.6, 61.8, 38.8, 32.7, 26.1, 23.2.

(2*S*)-2-[(Benzothiazole-2-sulfonyl)amino]-3-methyl-3-(1-methylindol-3-yl)butyramide (31). To a stirred 0−5 °C solution of noncrystallized **30** (0.14 g, 0.58 mmol) and BtsCl (0.15 g, 0.64 mmol) in CH_2Cl_2 (6 mL), was added a solution of Na_2CO_3 (0.26 g, 2.5 mmol) in water (6 mL) at 0−5 °C. The resulting suspension was allowed to warm to room tempera-

ture and was stirred for 2 h. The mixture was diluted with H_2O and 10:1 CH_2Cl_2/MeOH (more MeOH was added as needed). The aqueous phase was extracted with 10:1 CH_2Cl_2/MeOH, the combined organic layer was washed with brine, dried (Na_2SO_4), and concentrated (aspirator) to give a crude product as an off-white solid with low solubility in organic solvents (>90% pure by TLC assay): analytical TLC on silica gel, 20:1 CH_2Cl_2/MeOH, R_f = 0.50. The crude **31** was used without further purification in the following *N*-methylation reaction.

(2*S*)-2-[(Benzothiazole-2-sulfonyl)methylamino]-3-methyl-3-(1-methylindol-3-yl)butyramide (32). To a stirred suspension of crude **31** from the previous step and K_2CO_3 (0.4 g, 2.9 mmol) in dry DMF (4 mL) was added iodomethane (1.1 mL, 17.4 mmol) in one portion at room temperature. The reaction mixture was brought to 35 °C for 12 h. The mixture was diluted with H_2O and EtOAc, the aqueous layer was separated and was extracted with EtOAc, and the combined organic layer was washed with water and brine, dried (Na_2SO_4), and concentrated (aspirator). The crude residue was purified by column chromatography on silica gel (15 × 2 cm, 3:2 to 2:3 hexane/EtOAc eluent) to provide **32** (0.25 g, 90% over two steps): analytical TLC on silica gel, 2:3 hexane/EtOAc, R_f = 0.30. Molecular ion calcd for $C_{22}H_{24}N_4O_3S_2$: 456.12910; found (EI) *m*/*e*= 456.1305, error = 3 ppm; base peak = 172 amu; $[\alpha]^{25}_D$ +170.4 (*c* 1.3, $CHCl_3$); IR (neat, cm^{-1}) 3447, N−H; 1687, C=O; 400 MHz NMR ($CDCl_3$, ppm) δ 8.07−8.05 (1H, m), 8.02−7.99 (1H, m), 7.86−7.83 (1H, m), 7.45−7.35 (2H, m), 7.29−7.18 (3H, m), 6.85 (1H, s), 5.39 (1H, s), 5.23 (1H, br s), 4.98 (1H, br s), 3.69 (3H, s), 3.27 (3H, s), 1.66 (3H, s), 1.64 (3H, s). ^{13}C NMR (100 MHz, $CDCl_3$, ppm), δ 179.7, 169.9, 164.3, 152.2, 137.7, 136.2, 127.4, 127.3, 127.0, 125.3, 124.9, 122.0, 121.7, 121.0, 120.4, 119.6, 109.7, 65.2, 39.4, 34.5, 32.8, 28.0, 24.2.

***N*,*N*-Di(*tert*-butoxycarbonyl)-(2*S*)-2-[(benzothiazole-2-sulfonyl)methylamino]-3-methyl-3-(1-methylindol-3-yl)-butyramide (33).** To a solution of **32** (52 mg, 0.12 mmol) and DMAP (3 mg, 0.023 mmol) in dry CH_3CN (0.5 mL) was added di-*tert*-butyl dicarbonate (Boc_2O) (78 μL, 0.34 mmol) at room temperature. The reaction mixture was further stirred at room temperature for 3 h and then concentrated (aspirator). The residue was purified by preparative TLC on silica gel (20 × 20 × 0.1 cm, 5:1 hexane/EtOAc eluent) to provide **33** (59 mg, 81%): analytical TLC on silica gel, 4:1 hexane/EtOAc, R_f = 0.28. Molecular ion (M + Na) calcd for $C_{32}H_{40}N_4O_7NaS_2$: 679.22361; found (EI, Na) *m*/*e* = 679.2260, error = 3 ppm; base peak = 172 amu; $[\alpha]^{25}_D$ +44.5 (*c* 1.1, $CHCl_3$); IR (neat, cm^{-1}) 1778, C=O; 1752, C=O; 1702, C=O; 400 MHz NMR ($CDCl_3$, ppm) δ 8.17−8.15 (1H, m), 7.91−7.89 (1H, m), 7.84−7.81 (1H, m), 7.49−7.40 (2H, m), 7.13−7.03 (2H, m), 7.02−7.00 (1H, m), 6.82 (1H, s), 6.59 (1H, s), 3.60 (3H, s), 3.16 (3H, s), 1.72 (3H, s), 1.71 (3H, s), 1.23 (18H, s). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ 169.9, 165.1, 151.7, 149.0, 137.3, 136.3, 127.8, 127.0, 126.7, 126.4, 125.5, 122.3, 121.7, 121.0, 118.9, 118.5, 108.9, 84.4, 62.3, 40.6, 34.9, 32.6, 27.3, 27.1, 26.8.

(2*E*,4*S*,2′*S*,2″*S*)-4-({2′-[2′<bold>′-[(Benzothiazole-2-sulfonyl)methylamino]-3″-methyl-3″-(1-methylindol-3-yl)-butyrylamino]-3′,3′-dimethylbutyryl}methylamino)-2,5-dimethylhex-2-enoic Acid Ethyl Ester (34). To a room-temperature solution of the di-*tert*-butyl *N*-acylimidodicarbonate **33** (67 mg, 0.102 mmol) in dry CH_2Cl_2 (0.5 mL) was added a solution of the free amino dipeptide **22** (38 mg, 0.12 mmol) in dry CH_2Cl_2 (0.5 mL). The resulting mixture was added a catalytic amount of DMAP (3 mg, 0.02 mmol) and was brought to reflux (45 °C) for 18 h. After being cooled to room temperature, the reaction was diluted with 1% aqueous hydrochloric acid, and the aqueous layer was extracted with CH_2Cl_2. The combined CH_2Cl_2 extracts were washed with brine, dried (Na_2SO_4), and concentrated (aspirator). The residue was purified by preparative TLC on silica gel (20 × 20 × 0.1 cm, 3:2 hexane/EtOAc eluent) to give Bts-protected tripeptide **34** (0.075 g, 97%): analytical TLC on silica gel, 3:2 hexane/EtOAc, R_f = 0.39. Pure material was obtained by crystallization from ether/hexane, mp 202−203 °C. Molecular ion (M + H) calcd for $C_{39}H_{54}N_5O_6S_2$: 752.35155; found (FAB, Na) *m*/*e* = 752.3522, error = 1 ppm; base peak = 172 amu; $[\alpha]^{25}_D$ +50.5 (*c* 1.06, $CHCl_3$); IR (neat, cm^{-1}) 3366, N−H; 1714, C=O; 1675, C=O; 400 MHz NMR ($CDCl_3$, ppm) δ 8.23−8.21 (1H, m), 8.10−8.07 (1H, m), 7.88−7.85 (1H, m), 7.53−7.45 (2H, m), 7.33−7.25 (3H, m), 7.07 (1H, s), 6.57 (1H, dq, *J* = 10.2, 1.6 Hz), 5.98 (1H, d, *J* = 8.8 Hz), 5.64 (1H, s), 4.97 (1H, dd, *J* = 10.2, 10.2 Hz), 4.17 (2H, q, *J* = 7.2 Hz), 4.14 (1H, d, *J* = 8.8 Hz), 3.76 (3H, s), 3.36 (3H, s), 2.69 (3H, s), 1.84 (3H, d, *J* = 1.6 Hz), 1.84−1.78 (1H, m), 1.70 (3H, s), 1.50 (3H, s), 1.28 (3H, t, *J* = 7.2 Hz), 0.86 (3H, d, *J* = 6.8 Hz), 0.78 (3H, d, *J* = 6.8 Hz), 0.38 (9H, s). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ 170.8, 167.9, 167.7, 164.3, 152.6, 138.7, 138.1, 136.5, 132.2, 127.6, 126.9, 126.7, 125.3, 124.7, 122.1, 121.8, 121.1, 120.6, 119.9, 109.6, 65.8, 60.7, 56.2, 54.7, 39.4, 35.2, 34.3, 32.6, 31.1, 29.8, 28.3, 25.7, 24.1, 19.4, 19.0, 14.2, 13.7.

(2*E*,4*S*,2′*S*,2″*S*)-4-({3′,3′-Dimethyl-2′-[3″-methyl-2″-methylamino-3″-(1-methylindol-3-yl)butyrylamino]-butyryl}methylamino)-2,5-dimethylhex-2-enoic Acid Ethyl Ester (35). To a stirred suspension of noncrystallized Bts-protected tripeptide **34** (32 mg, 0.042 mmol) and K_2CO_3 (23 mg, 0.16 mmol) in dry DMF (0.4 mL) under N_2 was added benzenethiol (13 μL, 0.12 mmol) in one portion at room temperature. The suspension was vigorously stirred and was monitored by TLC. After complete consumption of starting material (30 min), the reaction mixture was diluted with H_2O and ether. The aqueous layer was separated and was extracted with ether, and the combined ether extracts were washed with H_2O and extracted with 1% aqueous hydrochloric acid. The combined aqueous hydrochloric acid extracts were washed with ether and neutralized with saturated aqueous $NaHCO_3$. The resulting aqueous phase was extracted with HPLC grade CH_2Cl_2. The combined CH_2Cl_2 layer was washed with brine, dried (Na_2SO_4), and concentrated (aspirator) to furnish **35** (23.5 mg, 99%, >95% pure): analytical TLC on silica gel, 20:1 CH_2Cl_2/MeOH, R_f = 0.34. Pure material was obtained by crystallization from ether/hexane, mp 142−143 °C. Molecular ion (M + H) calcd for $C_{32}H_{51}N_4O_4$: 555.39103; found (Fab, Na) *m*/*e*= 555.3906, error= 1 ppm; base peak= 172 amu; $[\alpha]^{25}_D$ −132.5 (*c* 1.52, $CHCl_3$), $[\alpha]^{25}_D$ −106 (*c* 0.44, MeOH); IR (neat, cm^{-1}) 3354, N−H; 3331, N−H; 1710, C=O; 400 MHz NMR ($CDCl_3$, ppm) δ 7.94 (1H, d, *J* = 9.8 Hz), 7.92−7.90 (1H, m), 7.32−7.30 (1H, m), 7.25−7.21 (1H, m), 7.10−7.06 (1H, m), 6.87 (1H, s), 6.66 (1H, dq, *J* = 10.2, 1.6 Hz), 5.11 (1H, dd, *J* = 10.2, 10.2 Hz), 4.89 (1H, d, *J* = 9.8 Hz), 4.20 (2H, q, *J* = 7.2 Hz), 3.76 (3H, s), 3.56 (3H, s), 3.07 (3H, s), 2.01 (3H, s), 1.94−1.84 (1H, m), 1.91 (3H, d, *J* = 1.6 Hz), 1.60 (3H, s), 1.44 (3H, s), 1.31 (3H, t, *J* = 7.2 Hz), 1.14 (1H, br s), 1.02 (9H, s), 0.85 (3H, d, *J* = 6.4 Hz), 0.80 (3H, d, *J* = 6.4 Hz). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ 172.8, 171.7, 167.8, 138.6, 138.0, 132.5, 126.6, 125.6, 121.6, 121.04, 120.96, 118.8, 109.5, 72.7, 60.8, 56.0, 54.5, 38.5, 35.9, 34.9, 32.6, 31.1, 29.9, 27.7, 26.7, 23.4, 19.4, 18.9, 14.2, 13.8.

(−)-Hemiasterlin (1). To a stirred room-temperature solution of noncrystallized (−)-hemiasterlin ethyl ester (**35**) (24.9 mg, 0.045 mmol) in methanol (1 mL) were added H_2O (0.4 mL) and 1.0 M aqueous LiOH (0.4 mL, 0.4 mmol) to give a cloudy mixture that became homogeneous after stirring 12 h at room temperature. TLC assay indicated complete consumption of the ester. Methanol was removed by aspirator, and the residue was diluted with H_2O and ether. The basic aqueous phase was washed with ether and was neutralized with pH 7 buffer solution, and the resulting aqueous phase was extracted with CH_2Cl_2. The combined dichloromethane extracts were washed with brine, dried (Na_2SO_4), concentrated (aspirator), and the residue was purified by preparative TLC on silica gel (20 × 20 × 0.1 cm, 10:1 CH_2Cl_2/MeOH eluent) to furnish (−)-hemiasterlin (**1**) as a glassy solid (22.5 mg, 95%): analytical TLC on silica gel, 10:1 CH_2Cl_2/MeOH, R_f = 0.35; $[\alpha]^{25}_D$ −84.4 (*c* 0.49, MeOH), $[\alpha]^{25}_D$ −91.3 (*c* 0.046, MeOH) or $[\alpha]^{25}_D$ −137.4 (*c* 0.46, $CHCl_3$) [lit. $[\alpha]^{25}_D$ −95 (*c* 0.06, MeOH),[2a] $[\alpha]^{25}_D$ −76 (*c* 0.07, MeOH)[2b]]. Pure material was obtained by crystallization from MeOH/hexane, mp 120−130 °C; analysis calcd: C, 68.39; H, 8.82; N, 10.64, found: C, 68.03; H, 8.84; N, 10.64; $[\alpha]^{23}_D$ −114.7 (*c* 0.08, MeOH) and −118.9 (*c* 0.07, MeOH); IR (neat, cm^{-1}) 3331, N−H;1633, C=O; 400 MHz NMR ($CDCl_3$, ppm) δ 7.93 (1H, d, *J* = 10.0 Hz), 7.92−7.88 (1H, m), 7.35−

7.20 (1H, m), 7.25−7.20 (1H, m), 7.11−7.07 (1H, m), 7.00−5.50 (1H, br), 6.87 (1H, s), 6.73 (1H, dq, J = 9.8, 1.6 Hz), 5.14 (1H, dd, J = 9.8, 9.8 Hz), 4.90 (1H, d, J = 10.0 Hz), 3.76 (3H, s), 3.58 (1H, s), 3.08 (3H, s), 2.02 (3H, s), 1.94−1−84 (1H, m), 1.93 (3H, d, J = 1.6 Hz), 1.60 (3H, s), 1.44 (3H, s), 1.01 (9H, s), 0.86 (3H, d, J = 6.6 Hz), 0.81 (3H, d, J = 6.6 Hz). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ 172.8, 172.0, 171.7, 141.0, 138.0, 131.7, 126.7, 125.5, 121.6, 121.0, 120.7, 118.8, 109.5, 72.5, 56.0, 54.5, 38.4, 35.8, 34.9, 32.7, 31.1, 29.8, 27.7, 26.6, 23.3, 19.3, 18.9, 13.5.

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Supporting Information Available: NMR spectra of new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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