

Highly Efficient Biomimetic Total Synthesis and Structural Verification of Bistratamides E and J from *Lissoclinum bistratum*

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Abstract: The interesting biological activities of heterocycle-containing cyclic peptide-derived natural products, isolated from marine organisms over the past twenty years, have attracted the interest of many synthetic and natural products chemists. Bistratamides E–J, members of this class of natural products that were isolated very recently from *Lissoclinum bistratum*, exhibited cytotoxic activity against a human colon tumor (HCT-116) cell line. Here

we report the first total syntheses of bistratamides E (**1**) and J (**2**) in overall yields of 19 and 34 %, respectively. The thiazole substructures have been synthesized by oxidation of their corresponding thiazoline substructures, which were obtained from cysteine

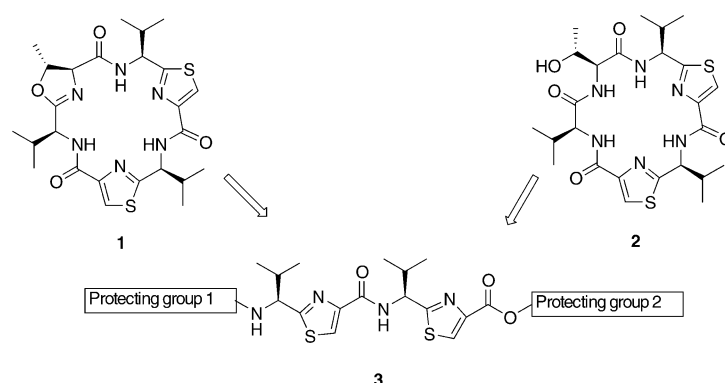
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containing peptides using a novel biomimetic approach wherein Val-Cys dipeptide units were converted to thiazolines by a bisphosphonium salt. The final macrocyclization was promoted efficiently using the combination of PyBOP and DMAP. This approach allows the use of readily available Fmoc-protected amino acids to make complex thiazole and oxazoline-containing natural products.

Introduction

Numerous thiazole and/or thiazoline-containing natural products have been isolated from marine organisms including ascidians (sea squirts) over the past twenty years.^[1] Their cytotoxic activities as well as their metal binding capacity has attracted the interest of several synthetic and natural product chemists.^[2] The thiazole–oxazoline and thiazole containing peptide-derived macrocycles, bistratamides E (**1**) and J (**2**), respectively, were very recently isolated from *Lissoclinum bistratum* in the southern Philippines (Scheme 1).^[3] They exhibited moderate cytotoxic activity against a human colon tumor (HCT-116) cell line. The antimicrobial, antitumor and the anti-drug resistance properties of members of this family of natural products warrant the synthetic efforts published thus far to prepare natural products related to bistratamides E and J.^[4–6]

Construction of the thiazoles in these peptide-derived macrocycles is central to their total synthesis. Commonly used methods for the preparation of thiazoles include 1) a modification of Hantzsch's procedure using thioamides as



Scheme 1. Retrosynthetic analysis for **1** and **2**.

intermediates,^[7] 2) a condensation reaction between cysteine esters and *N*-protected imino esters,^[8] and 3) the cyclodehydration of β -hydroxythioamides using either Mitsunobu conditions or the Burgess reagent.^[9–10] Thiazolines synthesized by the last two procedures are readily converted into thiazoles by oxidation. Recently, we reported that *N*-acylated cysteine substructures when treated with bis(triphenyl) oxodiphosphonium trifluoromethanesulfonate afford thiazolines efficiently.^[11] In this approach, thiazolines are formed by a nucleophilic attack of the cysteine thiol group on the phosphorus-activated amide carbonyl group of the preceding residue, followed by dehydration via phosphine oxide formation. The reaction proceeds in high yield with excellent chemo- and enantioselectivity without epimerization of the

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chiral center of the neighboring amino acid residue. Thiazole-containing peptides can be obtained by oxidation of the resulting thiazolines employing activated MnO_2 . Here, we report the total synthesis of bistratamides E and J in overall yields of 19 and 34 %, respectively.

Results and Discussion

A retrosynthetic analysis for the preparation of **1** and **2** depicts how both macrocycles can be synthesized from the common bis-thiazole intermediate **3**, derived from a tetrapeptide prepared from ordinary Fmoc-protected amino acids (Scheme 1).

The preparation of **8**, a specific analogue of **3** with allyl and Fmoc protecting groups on the C- and N-termini, commences with protecting the carboxylic acid of *N*-Fmoc-S-trityl-cysteine (**4**) as an allyl ester (Scheme 2). Fmoc depro-

tection of donor compound **7a** was prepared by removing the Fmoc protecting group of **7** using diethylamine. Thiazoles **7a** and **7b** were coupled utilizing HBTU, HOBT and DIEA delivering tetrapeptide-derived bisheterocycle **8** in 91 % yield.

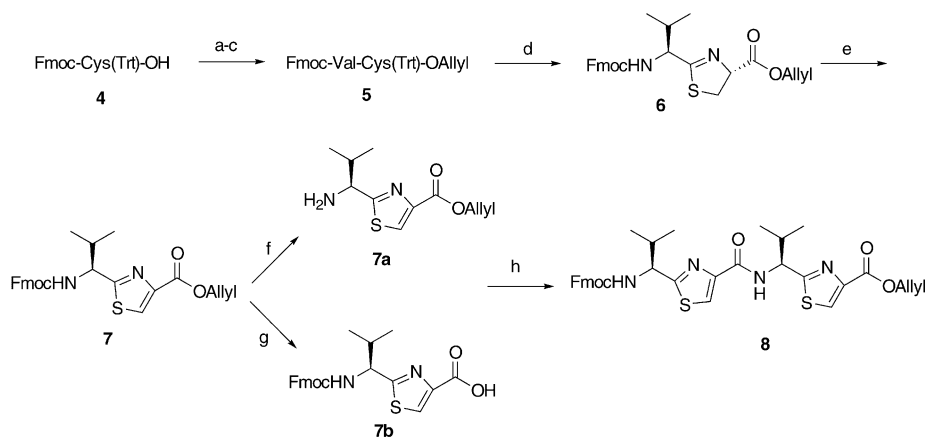
N-Fmoc deprotection and coupling of **8** to *N*-Fmoc-*O*-trityl-threonine afforded **9** (92 %) which was Fmoc deprotected and coupled with *N*-Fmoc-L-valine to give **10** (93 %), Scheme 3. Macrocycle precursor **11** can be obtained from hexapeptide **10** by removing the Fmoc and allyl groups as described above affording reactive termini. Several methods (DPPA,^[14] FDPP,^[15] PyBOP^[16]) were evaluated to cyclodehydrate **11** affording **12**. The combination of PyBOP and DMAP gave the highest yield (93 %), achieved by adding **11** [0.5 mmol in 30 mL $\text{CH}_2\text{Cl}_2/\text{DMF}$ 2:1 v/v] into a solution of PyBOP (1 mmol) and DMAP (1 mmol) in 120 mL $\text{CH}_2\text{Cl}_2/\text{DMF}$ (2:1 v/v) over 16 h with a syringe pump. The trityl group was removed from the threonine residue in **12** by treating the macrocycle with TFA (2 %) in CH_2Cl_2 affording

bistratamide J (**2**), a colorless solid. The ^1H and ^{13}C NMR spectra were identical to those reported by Perez and Faulkner.^[3] The structure of **2** was also verified by X-ray crystallography.^[17]

The final steps of the synthesis of bistratamide E (**1**) are depicted in Scheme 4. Hexapeptide **14** was synthesized from **8** after consecutive Fmoc deprotections and couplings with *N*-Fmoc-*O*-*allo*-threonine and *N*-Fmoc-L-valine. *Allo*-threonine was used to ensure the correct stereochemistry in a subsequent step because the amide oxygen inverts the activated side chain upon nucleophilic attack in the preparation of the oxazoline. Removal of the Fmoc and allyl protecting groups of **14** afforded the macrocycle precursor **15** in quantitative yield.

Cyclodehydration of **15** to **16** was achieved in 81 % yield by adding **15** [0.5 mmol in 30 mL $\text{CH}_2\text{Cl}_2/\text{DMF}$ 2:1 v/v] into a solution of PyBOP (1 mmol) and DMAP (1 mmol) in 120 mL $\text{CH}_2\text{Cl}_2/\text{DMF}$ 2:1 (v/v) over 16 h with a syringe pump. The Val-Thr substructure was then converted to an oxazoline using the Burgess reagent, providing bistratamide E (**1**) (63 %). The ^1H and ^{13}C NMR of bistratamide E (**1**) were identical to those reported by Perez and Faulkner.^[3]

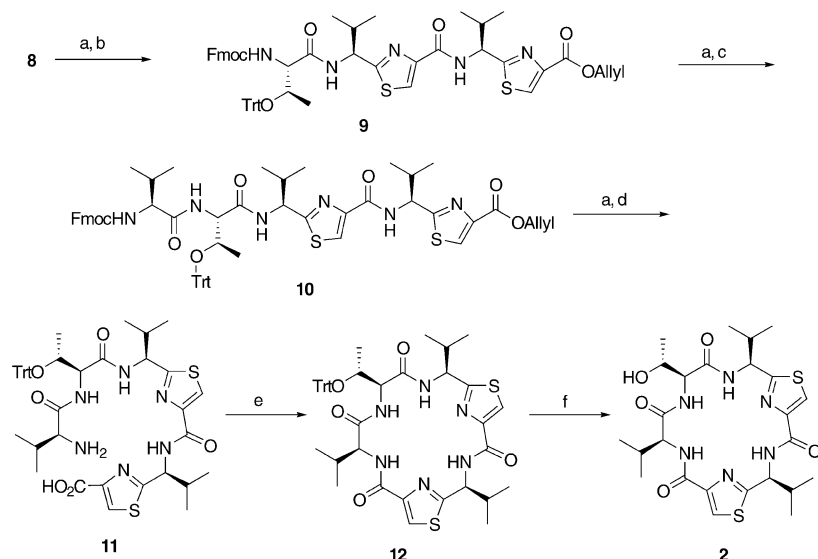
In summary, bistratamides E and J have been synthesized from cysteine containing peptides using a novel biomimetic approach wherein Val-Cys dipeptide substructures are converted to thiazolines by a bisphosphonium salt. This approach allows the use of readily available Fmoc-protected amino acids to make complex natural products in high yields with excellent regio and stereoselectivity.



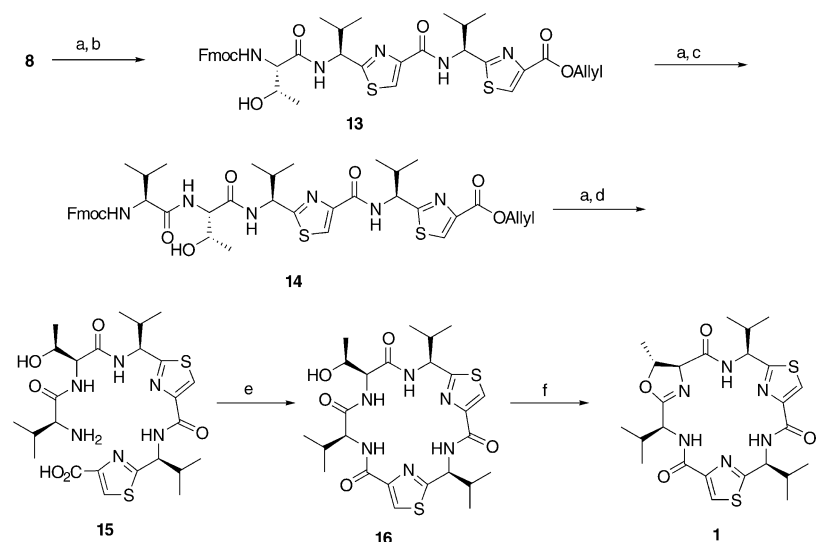
Scheme 2. Synthesis of tetrapeptide **8**. a) HOBt, HBTU, DIEA, allyl alcohol, DMF; b) DEA, CH_3CN ; c) HOBt, HBTU, DIEA, *N*-Fmoc-L-valine, DMF, 84 %; d) Ph_3PO , Ti_2O_3 , CH_2Cl_2 , -20°C , 89 %; e) activated MnO_2 , CH_2Cl_2 , 94 % (>96 % *ee*); f) DEA, CH_3CN ; g) $\text{Pd}(\text{OAc})_2$, PS-triphenylphosphine, PhSiH_3 , CH_2Cl_2 ; h) HOBt, HBTU, DIEA, DMF, 91 % for f)–h); DEA = *N,N*-diethylamine, DIEA = *N,N*-diisopropylethyl-

amine, DMF = *N,N*-dimethylformamide, HOBt = *N*-hydroxybenzotriazole, HBTU = 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate, PS-triphenylphosphine = polystyrene triphenylphosphine.

tection allows the resulting amine to be coupled with an active ester of *N*-Fmoc-L-valine to afford the fully protected dipeptide **5** (84 % for three steps). Bis(triphenyl)oxodiphosphonium trifluoromethanesulfonate^[12] was utilized to convert the trityl protected cysteine residue in **5** to a thiazoline yielding **6** (89 %). Thiazoline **6** was oxidized to a thiazole **7** employing activated manganese oxide (94 %; >96 % *ee*). The bis-thiazole **8** was prepared by a coupling between **7a** and **7b**, differentially protected thiazoles derived from **7**. The carboxylic acid compound **7b** was obtained by removing the allyl protecting group of **7** using a solid phase palladium catalyst (generated from $\text{Pd}(\text{OAc})_2$ and polymer-supported triphenylphosphine) in the presence of phenylsilane.^[13] The use of a solid phase catalyst greatly simplified workup of the reaction, as the product could be separated from the catalyst by filtration through silica gel. The amine



Scheme 3. The later steps of the synthesis affording **2**. a) DEA, CH₃CN; b) HOBT, HBTU, DIEA, *N*-Fmoc-*O*-trityl-L-threonine, DMF, 92%; c) HOBT, HBTU, DIEA, *N*-Fmoc-L-valine, DMF, 93%; d) Pd(OAc)₂, PS-triphenylphosphine, PhSiH₃, CH₂Cl₂; e) PyBOP, DMAP, CH₂Cl₂, DMF, 93%; f) TFA, PhSH, CH₂Cl₂, 96%; PyBOP = benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate, DMAP = 4-(*N,N*-dimethyl)pyridine, TFA = trifluoroacetic acid (other reagents defined in Scheme 2).



Scheme 4. The later steps of the synthesis affording **1**. a) DEA, CH₃CN; b) HOBT, HBTU, DIEA, *N*-Fmoc-*allo*-threonine, DMF, 95%; c) HOBT, HBTU, DIEA, *N*-Fmoc-L-valine, DMF, 86%; d) Pd(OAc)₂, PS-triphenylphosphine, PhSiH₃, CH₂Cl₂; e) PyBOP, DMAP, CH₂Cl₂, DMF, 81%; f) Burgess reagent, THF, reflux, 63%; Burgess reagent = (methoxycarbonylsulfamoyl)triethylammonium hydroxide, inner salt (other reagents defined in Schemes 2 and 3).

Experimental Section

General: Unless stated otherwise, all reactions were carried out in flame-dried glassware under a dry argon atmosphere. All solvents were purchased from Fisher and were dried prior to use. ¹H NMR spectra were measured at 600 MHz on a Bruker DRX spectrometer, and were referenced to internal TMS (0.0 ppm). ¹³C NMR spectra were performed at 150 MHz on a Bruker DRX-600 instrument and were referenced to CDCl₃. The chemical shift assignments for major diastereomers, not for minor diastereomers, were reported. Flash chromatography was performed on silica gel 60 (230–400 mesh, E. Merck no. 9385).

Synthesis of dipeptide 5: A solution of *N*^ε-Fmoc-Cys(S-trityl)-OH (5.86 g, 10 mmol) in DMF (50 mL) was treated with HOBT·H₂O (1.68 g, 11 mmol) and HBTU (4.17 g, 11 mmol) at 25°C. After 10 min, allyl alcohol (1.36 mL, 20 mmol) and DIEA (3.65 mL, 21 mmol) were added separately. The resulting mixture was stirred at 25°C overnight. The reaction mixture was concentrated and the residue was dissolved in EtOAc and washed with 10% aqueous NaHCO₃. The organic layer was dried over Na₂SO₄, filtered and concentrated.

Diethylamine (30 mL) was added to a solution of the above crude ester in CH₃CN (30 mL) and the resulting mixture was stirred at 25°C for 30 min to ensure complete removal of the Fmoc protecting group. After concentration in vacuo, the reaction mixture was azeotroped to dryness with CH₃CN (2×30 mL) and the residue was dissolved in DMF (40 mL). In another flask, a solution of *N*^ε-Fmoc-valine (3.73 g, 11 mmol) in DMF (40 mL) was treated with HOBT·H₂O (1.68 g, 11 mmol) and HBTU (4.17 g, 11 mmol). After 10 min, this mixture and DIEA (3.65 mL, 21 mmol) were sequentially added to the above free amino ester. After stirring at 25°C for 8 h. The reaction mixture was concentrated and the residue was dissolved in EtOAc and washed with 10% aqueous NaHCO₃. The organic layer was dried over Na₂SO₄, filtered and concentrated. The resulting crude product was purified by flash chromatography using a mixture of EtOAc/hexanes. Dipeptide **5** was obtained as a white foam (6.09 g, 84%). [α]_D²⁴ = +4.4 (*c* = 1.61 in CHCl₃); ¹H NMR (600 MHz, CDCl₃, 25°C, TMS): δ = 7.76 (d, *J* = 7.5 Hz, 2H), 7.59 (t, *J* = 7.0 Hz, 2H), 7.39 (m, 8H), 7.31–7.24 (m, 9H), 7.19 (dd, *J* = 7.5, 7.0 Hz, 2H), 6.06 (m, 1H), 5.86 (m, 1H), 5.40 (m, 1H), 5.30 (d, *J* = 16.6 Hz, 1H), 5.25 (d, *J* = 10.5 Hz, 1H), 4.61–4.56 (m, 3H), 4.44 (dd, *J* = 8.3, 7.2 Hz, 1H), 4.35 (dd, *J* = 10.1, 7.0 Hz, 1H), 4.22 (t, *J* = 7.0 Hz, 1H), 4.01 (m, 1H), 2.76 (dd, *J* = 12.3, 6.6 Hz, 1H), 2.08 (dd, *J* = 12.3, 3.1 Hz, 1H), 2.08 (m, 1H), 0.96 (d, *J* = 6.1 Hz, 3H), 0.91 (d, *J* = 6.6 Hz, 3H); ¹³C NMR (150 MHz, CDCl₃): δ = 172.7, 169.7, 156.2, 144.1, 143.9, 143.8, 141.3, 131.3, 129.4, 128.0, 127.7, 127.0, 126.9, 125.1, 119.9 (2C), 118.9, 67.0, 66.3, 59.7, 51.2,

47.2, 33.4, 31.6, 19.0, 17.5; HRMS (MALDI-FTMS): calcd for C₄₅H₄₄NaN₂O₅S: 747.2863, found: 747.2886 [*M*+Na]⁺.

Synthesis of compound 6: Trifluoromethanesulfonic anhydride (2.35 mL, 15 mmol) was added slowly at 0°C to a solution of triphenylphosphine oxide (8.35 g, 30 mmol) in dry CH₂Cl₂ (100 mL). The reaction mixture was stirred for 10 min at 0°C and then adjusted to –20°C using a brine-ice bath. Then a solution of **5** (7.25 g, 10 mmol) in CH₂Cl₂ (10 mL) was added. The reaction progress was monitored by TLC and completed in 2 h. The reaction mixture was quenched with 10% aqueous NaHCO₃. The aqueous layer was extracted with CH₂Cl₂ and the combined organic

layers were dried over Na_2SO_4 , filtered and concentrated. The resulting crude product was purified by flash chromatography. Compound **6** was obtained as a colorless oil (4.135 g, 89%). $[\alpha]_D^{25} = +11.8$ ($c=1.00$ in CHCl_3); $^1\text{H NMR}$ (600 MHz, CDCl_3 , 25 °C, TMS): $\delta = 7.76$ (d, $J=7.5$ Hz, 2H), 7.61 (dd, $J=9.7$, 8.3 Hz, 2H), 7.39 (t, $J=7.5$ Hz, 2H), 7.31 (t, $J=7.5$ Hz, 2H), 5.96–5.92 (m, 1H), 5.55 (d, $J=9.2$ Hz, 1H), 5.36 (t, $J=17.1$ Hz, 1H), 5.28 (d, $J=10.5$ Hz, 1H), 5.17 (dd, $J=8.8$, 8.3 Hz, 1H), 4.70 (br, 2H), 4.57 (dd, $J=9.2$, 4.8 Hz, 1H), 4.43–4.37 (m, 2H), 4.24 (t, $J=7.0$ Hz, 1H), 3.60–3.55 (m, 2H), 2.22–2.19 (m, 1H), 1.02 (d, $J=7.0$ Hz, 3H), 0.93 (d, $J=7.0$ Hz, 3H); $^{13}\text{C NMR}$ (150 MHz, CDCl_3): $\delta = 175.5$, 170.2, 156.1, 143.9, 143.7, 141.2, 131.4, 127.6, 127.0, 125.1, 119.9, 119.1, 77.4, 66.9, 66.2, 58.4, 47.2, 35.9, 32.3, 19.3, 16.6; HRMS (MALDI-FTMS): calcd for $\text{C}_{26}\text{H}_{28}\text{N}_2\text{O}_5\text{S}$: 465.1842, found: 465.1858 $[M+H]^+$.

Synthesis of compound 7: Activated MnO_2 (<5 micron, 85%, 7.16 g, 70 mmol) was added to a solution of **7** (3.25 g, 7 mmol) in CH_2Cl_2 (35 mL). The reaction mixture was stirred overnight at 25 °C, then filtered through a short silica gel and Celite column and washed with EtOAc. The organic solution was concentrated. The resulting crude product was purified by flash chromatography. Compound **7** was obtained as a white solid (3.04 g, 94%). M.p. 104–105 °C; $[\alpha]_D^{25} = -43.4$ ($c=0.58$ in CHCl_3); $^1\text{H NMR}$ (600 MHz, CDCl_3 , 25 °C, TMS): $\delta = 8.10$ (s, 1H), 7.76 (d, $J=7.0$ Hz, 2H), 7.59 (m, 2H), 7.39 (m, 2H), 7.30 (m, 2H), 6.03 (m, 1H), 5.59 (d, $J=8.8$ Hz, 1H), 5.41 (t, $J=17.1$ Hz, 1H), 5.30 (d, $J=10.1$ Hz, 1H), 4.94 (dd, $J=8.8$, 6.1 Hz, 1H), 4.85 (d, $J=5.3$ Hz, 2H), 4.44 (m, 2H), 4.22 (m, 1H), 2.43 (m, 1H), 0.96 (d, $J=6.6$ Hz, 3H), 0.94 (d, $J=7.0$ Hz, 3H); $^{13}\text{C NMR}$ (150 MHz, CDCl_3): $\delta = 172.2$, 160.8, 156.0, 147.0, 143.8, 143.7, 141.3, 131.8, 127.7, 127.2, 127.0, 125.0 (2C), 119.9, 118.9, 66.9, 65.9, 58.5, 47.2, 33.4, 19.4, 17.6; HRMS (MALDI-FTMS): calcd for $\text{C}_{26}\text{H}_{26}\text{N}_2\text{O}_5\text{S}$: 463.1686, found: 463.1668 $[M+H]^+$.

Synthesis of compound 8: $\text{Pd}(\text{OAc})_2$ (11.2 mg, 0.05 mmol) and PS-triphenylphosphine (252 mg, 1.59 mmol g^{-1} , 0.4 mmol, purchased from Argonaut Technologies) were added to a flask containing CH_2Cl_2 (20 mL). After stirring for 10 min, **7** (1.155 g, 2.5 mmol) and PhSiH_3 (0.61 mL, 5 mmol) were added separately. TLC showed that the starting material disappeared in 15 min. After removing the solvent, the residue was passed through a short silica gel column and eluted with $\text{CHCl}_3/\text{EtOH}$ 1:1. The carboxylic acid **7b** was used in next step without further purification.

In separate flask, the Fmoc group in **7** (1.063 g, 2.3 mmol) was removed using diethylamine as described in the synthesis of **5**. The resulting free amine **7a** was dissolved in DMF (10 mL), then, a solution of the above free carboxylic acid, HOBt· H_2O (383 mg, 2.5 mmol), HBTU (948 mg, 2.5 mmol) and DIEA (0.92 mL, 5.3 mmol) in DMF (10 mL) was added. The resulting mixture was stirred for 8 h. Regular workup and purification with flash chromatography using a mixture of EtOAc/hexanes gave **8** as a white foam (1.35 g, 91%). $[\alpha]_D^{25} = -16.9$ ($c=0.45$ in CHCl_3); $^1\text{H NMR}$ (600 MHz, CDCl_3 , 25 °C, TMS): $\delta = 8.08$ (s, 1H), 8.04 (s, 1H), 7.98 (d, $J=8.8$ Hz, 1H), 7.74 (d, $J=7.0$ Hz, 2H), 7.58 (dd, $J=16.2$, 7.0 Hz, 2H), 7.37 (m, 2H), 7.29–7.23 (m, 2H), 5.99 (m, 1H), 5.65 (m, 1H), 5.36 (d, $J=9.3$ Hz, 1H), 5.33 (d, $J=8.8$ Hz, 1H), 5.25 (d, $J=10.5$ Hz, 1H), 4.93 (dd, $J=8.3$, 6.1 Hz, 1H), 4.79 (m, 2H), 4.47 (d, $J=6.6$ Hz, 2H), 4.23 (m, 1H), 2.62 (m, 1H), 2.40 (m, 1H), 1.04 (d, $J=6.6$ Hz, 3H), 1.00 (d, $J=6.6$ Hz, 6H), 0.95 (d, $J=6.6$ Hz, 3H); $^{13}\text{C NMR}$ (150 MHz, CDCl_3): $\delta = 172.1$, 171.5, 160.7, 160.6, 156.0, 149.3, 146.9, 143.6 (2C), 141.2 (2C), 131.7, 127.6, 127.2, 127.0, 126.9, 124.9, 124.8, 123.5, 119.9, 118.7, 66.8, 65.8, 56.3, 47.1, 33.0 (2C), 19.6, 19.3, 18.0, 17.5; HRMS (MALDI-FTMS): calcd for $\text{C}_{34}\text{H}_{36}\text{NaN}_4\text{O}_5\text{S}_2$: 667.2019, found: 667.2046 $[M+Na]^+$.

Synthesis of compound 9: Deprotecting the Fmoc group in **8** and coupling with N^{α} -Fmoc-Thr(*O*-trityl)-OH followed the procedure described in synthesis of **5**. Compound **9** was obtained in 92% yield as a white foam. $[\alpha]_D^{25} = -13.9$ ($c=0.56$ in CHCl_3); $^1\text{H NMR}$ (600 MHz, CDCl_3 , 25 °C, TMS): $\delta = 8.15$ (s, 1H), 8.06 (s, 1H), 7.93 (d, $J=9.2$ Hz, 1H), 7.73 (d, $J=7.5$ Hz, 2H), 7.53 (m, 8H), 7.47 (d, $J=8.4$ Hz, 1H), 7.37–7.34 (m, 2H), 7.28–7.22 (m, 11H), 5.98 (m, 1H), 5.75 (br, 1H), 5.39–5.35 (m, 2H), 5.25 (d, $J=10.1$ Hz, 1H), 5.18 (dd, $J=18.3$, 6.6 Hz, 1H), 4.81 (d, $J=5.3$ Hz, 2H), 4.34 (m, 1H), 4.29 (dd, $J=9.7$, 7.9 Hz, 1H), 4.24 (dd, $J=10.1$, 7.5 Hz, 1H), 4.14–4.12 (m, 1H), 3.49 (br, 1H), 2.61 (m, 1H), 2.38 (m, 1H), 1.14 (m, 3H), 1.01–0.99 (m, 3H), 0.96 (d, $J=6.6$ Hz, 6H), 0.82 (d, $J=6.6$ Hz, 3H); $^{13}\text{C NMR}$ (150 MHz, CDCl_3): $\delta = 171.8$, 169.5, 160.8, 160.7, 155.0, 149.2, 147.0, 143.7 (2C), 143.6, 141.1, 128.6, 128.1, 127.6,

127.5, 127.2, 126.9 (2C), 125.0, 124.9, 123.8, 119.8 (2C), 118.7, 88.6, 69.8, 66.8, 65.8, 56.9, 56.5, 46.9, 32.9, 32.8, 19.6, 19.5, 18.0, 17.8, 16.4; HRMS (MALDI-FTMS): calcd for $\text{C}_{57}\text{H}_{57}\text{NaN}_5\text{O}_7\text{S}_2$: 1010.3591, found: 1010.3613 $[M+Na]^+$.

Synthesis of compound 10: Deprotecting the Fmoc group in **9** and coupling with N^{α} -Fmoc-valine followed the procedure described in synthesis of **5**. Compound **10** was obtained in 93% yield as a white foam. $[\alpha]_D^{25} = -19.9$ ($c=1.55$ in CHCl_3); $^1\text{H NMR}$ (600 MHz, CDCl_3 , 25 °C, TMS): $\delta = 8.11$ (s, 1H), 8.06 (s, 1H), 7.95 (d, $J=9.2$ Hz, 1H), 7.75 (d, $J=7.5$ Hz, 2H), 7.57–7.51 (m, 8H), 7.38 (m, 2H), 7.29–7.26 (m, 10H), 7.23 (dd, $J=14.0$, 6.6 Hz, 2H), 6.83 (m, 1H), 5.98 (m, 1H), 5.41 (d, $J=8.3$ Hz, 1H), 5.39–5.35 (m, 2H), 5.27–5.25 (m, 1H), 5.13 (dd, $J=8.3$, 6.6 Hz, 1H), 4.82 (d, $J=5.7$ Hz, 2H), 4.41 (dd, $J=10.1$, 6.6 Hz, 2H), 4.31 (dd, $J=10.5$, 7.0 Hz, 1H), 4.19 (m, 1H), 4.01 (m, 1H), 3.66 (m, 1H), 2.60 (m, 1H), 2.33 (m, 1H), 2.06 (m, 1H), 1.07 (d, $J=6.1$ Hz, 3H), 0.99 (d, $J=6.6$ Hz, 3H), 0.98 (d, $J=6.6$ Hz, 3H), 0.90 (d, $J=7.1$ Hz, 3H), 0.89 (d, $J=6.1$ Hz, 3H), 0.84 (d, $J=6.6$ Hz, 3H), 0.82 (d, $J=6.6$ Hz, 3H); $^{13}\text{C NMR}$ (150 MHz, CDCl_3): $\delta = 171.9$, 171.7, 170.3, 169.4, 160.9, 160.8, 156.3, 149.2, 147.0, 143.7, 143.6, 141.2, 131.8, 128.9, 128.7, 127.6, 127.5, 127.2, 127.0, 125.0, 123.8, 119.9 (2C), 118.8, 88.5, 69.2, 67.0, 65.9, 57.0, 56.6, 55.9, 47.1, 32.9 (2C), 31.2, 19.6, 19.5, 18.0 (2C), 17.5, 16.9; HRMS (MALDI-FTMS): calcd for $\text{C}_{62}\text{H}_{66}\text{NaN}_6\text{O}_8\text{S}_2$: 1109.4276, found: 1109.4293 $[M+Na]^+$.

Synthesis of compound 12: Deprotecting the Fmoc group in **10** (544 mg, 0.5 mmol) followed the procedure described in synthesis of **5**. The allyl group was removed using a palladium catalyst as described in synthesis of **8**. The resulting amino acid **11** was dissolved in $\text{CH}_2\text{Cl}_2/\text{DMF}$ (30 mL, 2:1 v/v). This solution was added into a flask containing PyBOP (520 mg, 1 mmol) and DMAP (122 mg, 1 mmol) in $\text{CH}_2\text{Cl}_2/\text{DMF}$ (120 mL, 2:1 v/v) over 16 h using a syringe pump. After the completion of addition, the mixture was stirred for 4 h. Regular workup and purification by flash chromatography gave **12** as a white foam (375 mg, 93%). $[\alpha]_D^{25} = -157.6$ ($c=0.50$ in CHCl_3); $^1\text{H NMR}$ (600 MHz, DMSO , 25 °C): $\delta = 8.38$ (d, $J=10.1$ Hz, 1H), 8.34 (s, 1H), 8.28 (d, $J=9.2$ Hz, 1H), 8.25 (s, 1H), 8.17 (d, $J=10.5$ Hz, 1H), 7.92 (d, $J=9.6$ Hz, 1H), 7.48 (d, $J=7.9$ Hz, 6H), 7.31 (dd, $J=7.9$, 7.5 Hz, 6H), 7.24 (dd, $J=7.5$, 7.0 Hz, 3H), 5.35 (dd, $J=9.7$, 7.0 Hz, 1H), 5.22 (dd, $J=9.2$, 8.8 Hz, 1H), 4.62 (dd, $J=10.1$, 3.1 Hz, 1H), 4.33 (t, $J=11.0$ Hz, 1H), 3.71 (m, 1H), 2.24 (m, 1H), 2.16–2.11 (m, 2H), 1.01 (d, $J=6.6$ Hz, 3H), 0.98 (d, $J=6.6$ Hz, 3H), 0.97 (d, $J=4.8$ Hz, 3H), 0.95 (d, $J=6.1$ Hz, 3H), 0.87 (d, $J=7.0$ Hz, 3H), 0.79 (d, $J=6.6$ Hz, 3H), 0.68 (d, $J=6.6$ Hz, 3H); $^{13}\text{C NMR}$ (150 MHz, DMSO): $\delta = 170.3$, 169.8, 169.6, 167.7, 159.8, 159.5, 149.0, 148.0, 144.6, 128.5, 127.7, 127.0, 125.9, 124.3, 57.9, 55.4, 55.2, 34.4, 33.9, 30.3, 19.6, 19.5, 19.4, 18.9, 18.6, 17.9; HRMS (MALDI-FTMS): calcd for $\text{C}_{44}\text{H}_{50}\text{NaN}_6\text{O}_5\text{S}_2$: 829.3176, found: 829.3190 $[M+Na]^+$.

Synthesis of bistratamide J (2): TFA (0.1 mL) and PhSH (21 μL , 0.2 mmol) were added to a flask containing **12** (161 mg, 0.2 mmol) in CH_2Cl_2 (5 mL). TLC showed that **12** disappeared in 5 min. After removing all of the solvents, the residue was purified by flash chromatography. Bistratamide **J (2)** was obtained as a colorless solid (108 mg, 96%). M.p. 165–167 °C; $[\alpha]_D^{25} = -134.9$ ($c=0.50$ in MeOH) $[\text{lit}^{[1]}]$; $[\alpha]_D = -25.0$ ($c=0.5$ in MeOH); $^1\text{H NMR}$ (600 MHz, DMSO , 25 °C): $\delta = 8.49$ (d, $J=10.5$ Hz, 1H), 8.43 (d, $J=9.7$ Hz, 1H), 8.30 (s, 1H), 8.24 (s, 1H), 8.13 (d, $J=9.7$ Hz, 1H), 8.08 (d, $J=10.1$ Hz, 1H), 5.33 (dd, $J=10.1$, 7.4 Hz, 1H), 5.22 (t, $J=4.8$ Hz, 1H), 5.20 (dd, $J=9.7$, 9.2 Hz, 1H), 4.35 (t, $J=11.0$ Hz, 1H), 4.32 (dd, $J=10.1$, 2.0 Hz, 1H), 4.14 (m, 1H), 2.22 (m, 1H), 2.16–2.11 (m, 2H), 1.11 (d, $J=6.6$ Hz, 3H), 1.05 (d, $J=6.1$ Hz, 3H), 1.04 (d, $J=5.8$ Hz, 3H), 1.03 (d, $J=6.6$ Hz, 3H), 0.99 (d, $J=6.6$ Hz, 3H), 0.91 (d, $J=6.6$ Hz, 3H), 0.81 (d, $J=6.6$ Hz, 3H); $^{13}\text{C NMR}$ (150 MHz, DMSO): $\delta = 170.1$ (2C), 169.4 (2C), 159.9, 159.7, 149.0, 148.3, 125.4, 124.2, 67.7, 61.3, 58.8, 55.4, 55.0, 34.7, 34.3, 30.8, 21.3, 19.8, 19.5, 19.4, 18.9, 18.8, 18.6; HRMS (MALDI-FTMS): calcd for $\text{C}_{25}\text{H}_{36}\text{NaN}_6\text{O}_5\text{S}_2$: 587.2081, found: 587.2062 $[M+Na]^+$.

Synthesis of compound 13: Deprotecting the Fmoc group in **8** and coupling with N^{α} -allo-Fmoc-threonine followed the procedure described in synthesis of **5**. Compound **13** was obtained in 95% yield. $[\alpha]_D^{25} = -41.0$ ($c=1.07$ in CHCl_3); $^1\text{H NMR}$ (600 MHz, CDCl_3 , 25 °C, TMS): $\delta = 8.28$ (d, $J=9.6$ Hz, 1H), 8.09 (s, 1H), 8.04 (s, 1H), 7.76 (d, $J=7.5$ Hz, 2H), 7.58 (d, $J=7.5$ Hz, 2H), 7.43 (d, $J=8.8$ Hz, 1H), 7.39 (t, $J=7.5$ Hz, 2H), 7.30 (t, $J=7.5$ Hz, 2H), 6.03–5.96 (m, 1H), 5.94 (d, $J=8.4$ Hz, 1H), 5.39–5.35 (m, 2H), 5.29–5.26 (m, 2H), 4.81 (d, $J=5.7$ Hz, 2H), 4.55 (d, $J=$

5.3 Hz, 1H), 4.42–4.35 (m, 2H), 4.25–4.20 (m, 2H), 4.08–4.06 (m, 1H), 2.48–2.44 (m, 1H), 2.35–2.32 (m, 1H), 1.31 (d, $J=6.1$ Hz, 3H), 1.05 (d, $J=7.0$ Hz, 3H), 1.00 (d, $J=7.0$ Hz, 3H), 0.99 (d, $J=6.6$ Hz, 3H), 0.93 (d, $J=7.0$ Hz, 3H); ^{13}C NMR (150 MHz, CDCl_3): δ = 171.2 (2C), 170.5, 160.8, 160.4, 156.5, 148.7, 146.6, 143.7, 143.6, 141.2, 131.6, 127.7, 127.2, 127.0, 125.0, 123.9, 120.0, 119.0, 69.1, 67.2, 66.0, 59.2, 56.5, 56.1, 47.0, 33.8, 33.5, 20.4, 19.5, 19.2, 18.2, 17.2; HRMS (MALDI-FTMS): calcd for $\text{C}_{38}\text{H}_{43}\text{N}_5\text{O}_7\text{S}_2$: 746.2677, found: 746.2677 $[M+H]^+$.

Synthesis of compound 14: Deprotecting the Fmoc group in **13** and coupling with N^t -Fmoc-valine followed the procedure described in synthesis of **5**. Compound **14** was obtained in 86% yield: $[\alpha]_D^{24}$ = -42.0 ($c=0.92$ in CHCl_3); ^1H NMR (600 MHz, CDCl_3 , 25°C, TMS): δ = 8.26 (d, $J=9.7$ Hz, 1H), 8.08 (s, 1H), 7.99 (s, 1H), 7.75 (d, $J=7.5$ Hz, 1H), 7.73 (d, $J=7.9$ Hz, 2H), 7.57–7.53 (m, 2H), 7.49 (d, $J=7.5$ Hz, 1H), 7.39–7.35 (m, 2H), 7.29–7.25 (m, 2H), 5.98 (m, 1H), 5.91 (d, $J=8.8$ Hz, 1H), 5.39–5.34 (m, 2H), 5.27–5.24 (m, 2H), 4.80 (d, $J=5.7$ Hz, 2H), 4.65 (d, $J=5.7$ Hz, 1H), 4.53 (dd, $J=7.5$, 7.0 Hz, 1H), 4.38 (dd, $J=10.1$, 8.9 Hz, 1H), 4.24 (q, $J=7.3$ Hz, 1H), 4.18 (dd, $J=7.5$, 7.0 Hz, 1H), 4.13–4.12 (m, 1H), 2.48–2.46 (m, 2H), 2.32–2.30 (m, 1H), 2.12–2.08 (m, 1H), 1.27 (d, $J=6.1$ Hz, 3H), 1.03 (d, $J=7.0$ Hz, 3H), 0.98–0.97 (m, 6H), 0.93 (d, $J=6.6$ Hz, 3H), 0.92 (d, $J=6.6$ Hz, 3H), 0.89 (d, $J=6.6$ Hz, 3H); ^{13}C NMR (150 MHz, CDCl_3): δ = 172.0, 171.3, 171.0, 170.7, 160.8, 160.4, 156.4, 148.7, 146.6, 143.7, 143.6, 141.2, 131.6, 127.6, 127.3, 127.0, 125.0, 123.8, 119.9, 118.9, 68.6, 67.0, 66.0, 60.1, 57.8, 56.6, 56.1, 47.0, 38.5, 33.6, 33.3, 31.5, 20.4, 19.5, 19.1, 18.2, 18.0, 17.1; HRMS (MALDI-FTMS): calcd for $\text{C}_{43}\text{H}_{52}\text{NaN}_6\text{O}_8\text{S}_2$: 867.3180, found: 867.3202 $[M+Na]^+$.

Synthesis of compound 16: Compound **16** was synthesized from **14** in 81% yield, following the procedure described in synthesis of **12**. $[\alpha]_D^{24}$ = -124.6 ($c=0.56$ in CHCl_3); ^1H NMR (600 MHz, CDCl_3 , 25°C, TMS): δ = 8.47 (d, $J=8.8$ Hz, 1H), 8.07 (s, 1H), 7.95 (d, $J=10.1$ Hz, 1H), 7.87 (d, $J=7.5$ Hz, 1H), 7.75 (s, 1H), 7.72 (d, $J=8.3$ Hz, 1H), 5.24 (dd, $J=8.8$, 5.7 Hz, 1H), 5.10 (t, $J=8.3$ Hz, 1H), 4.66–4.62 (m, 2H), 4.37 (br, 1H), 3.61 (t, $J=7.0$, 1H), 2.53–2.50 (m, 1H), 2.26–2.16 (m, 2H), 1.27 (d, $J=6.6$ Hz, 3H), 1.08 (d, $J=7.0$ Hz, 3H), 1.05 (d, $J=6.6$ Hz, 3H), 1.03 (d, $J=6.6$ Hz, 3H), 0.99 (d, $J=7.0$ Hz, 6H), 0.97 (d, $J=6.6$ Hz, 3H); ^{13}C NMR (150 MHz, CDCl_3): δ = 172.1, 170.3, 169.1, 168.9, 161.0, 160.0, 149.8, 147.8, 123.4, 123.3, 67.0, 62.2, 57.8, 56.1 (2C), 34.7, 34.4, 29.8, 20.2, 19.6, 19.1, 18.9, 18.6, 17.1; HRMS (MALDI-FTMS): calcd for $\text{C}_{25}\text{H}_{36}\text{NaN}_6\text{O}_5\text{S}_2$: 587.2081, found: 587.2059 $[M+Na]^+$.

Synthesis of bistratamide E (1): The Burgess reagent (48 mg, 97%, 0.2 mmol) was added to a solution of **16** (85 mg, 0.15 mmol) in THF (8 mL). After stirring at 25°C for 10 min, the mixture was refluxed for 2 h. Regular workup and purification with flash chromatography gave bistratamide **E (1)** as a white solid (52 mg, 63%). M.p. 90–96°C; $[\alpha]_D^{25}$ = -35.7 ($c=0.53$ in MeOH)^[3]; $[\alpha]_D$ = -31.0 ($c=1.0$ in MeOH)^[3]; ^1H NMR (600 MHz, DMSO, 25°C): δ = 8.57 (d, $J=8.3$ Hz, 1H), 8.39 (s, 1H), 8.36 (s, 1H), 8.05 (d, $J=9.7$ Hz, 1H), 7.79 (d, $J=8.8$ Hz, 1H), 5.52 (dd, $J=8.3$, 4.4 Hz, 1H), 5.30 (dd, $J=8.8$, 5.3 Hz, 1H), 4.83 (m, 1H), 4.78 (dq, $J=8.3$, 6.1 Hz, 1H), 4.24 (dd, $J=8.8$, 1.8 Hz, 1H), 2.33 (m, 1H), 2.22 (m, 1H), 2.05 (m, 1H), 1.47 (d, $J=6.1$ Hz, 3H), 0.95 (d, $J=7.0$ Hz, 3H), 0.90 (d, $J=7.0$ Hz, 3H), 0.89 (d, $J=6.6$ Hz, 3H), 0.86 (d, $J=6.1$ Hz, 3H), 0.85 (d, $J=6.6$ Hz, 6H); ^{13}C NMR (150 MHz, DMSO): δ = 169.4, 168.2, 168.0, 167.9, 159.4, 158.8, 148.1, 147.6, 125.2, 124.9, 82.0, 72.8, 54.8, 54.0, 51.2, 34.3, 34.2, 30.5, 21.4, 18.8, 18.1, 17.9, 17.5, 17.4, 16.0; HRMS (MALDI-FTMS): calcd for $\text{C}_{25}\text{H}_{34}\text{N}_6\text{O}_5\text{S}_2$: 547.2156, found: 547.2173 $[M+H]^+$.

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