# Bistratamides E−J, Modified Cyclic Hexapeptides from the Philippines Ascidian *Lissoclinum bistratum*

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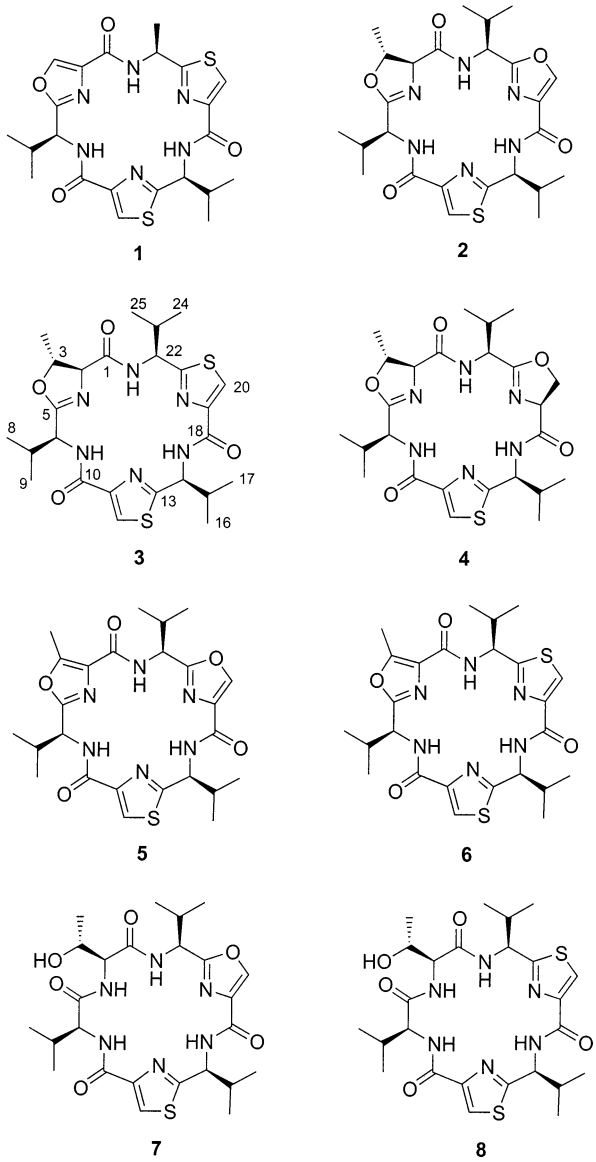
The Philippines ascidian *Lissoclinum bistratum* contained the known metabolites bistratamides C and D, together with six novel modified cyclic hexapeptides, bistratamides E−J. The structures of bistratamides E−J were elucidated by interpretation of spectral data, and the absolute stereochemistry of the constituent amino acids was determined after ozonolysis and acid-catalyzed hydrolysis. Bistratamides E−J were moderately cytotoxic in the HCT-116 cell line assay.

Ascidians of the genera *Lissoclinum* and *Didemnum* are prolific producers of cyclic peptides,[1] many of which incorporate modified amino acid residues containing thiazole, oxazole, thiazoline, or oxazoline rings.[2−7] Similar structures have also been reported from cyanobacteria,[8,9] which led to the suggestion that the peptides were produced by symbiotic cyanobacteria.[2,3] This hypothesis has not yet been confirmed.[10] In 1989, Degnan et al. reported the isolation and identification of bistratamides A and B, two thiazoline- or thiazole-containing cyclic hexapeptides from a Great Barrier Reef specimen of *Lissoclinum bistratum*.[2] The same source yielded cycloxazoline, the structure of which was determined by X-ray analysis[5] and which was identical to westiellamide, a metabolite of the terrestrial cyanobacterium *Westiellopsis prolifica*.[9] In 1992, Ireland's group obtained bistratamides C (**1**) and D (**2**) from a specimen of *L. bistratum* from the northern Philippines.[4] We now report the isolation and structural elucidation of six novel cyclic peptides, bistratamides E−J (**3**−**7**), from the same ascidian collected in the southern Philippines.




## Results and Discussion

The specimen of *Lissoclinum bistratum* Sluiter was collected by hand using scuba at Tablas Island in the Philippines. The ethyl acetate-soluble portion of a methanolic extract was subjected to column chromatography on silica gel. Fractions that showed activity in the human colon tumor HCT-116 cell line assay were purified by reversed-phase HPLC to obtain bistratamides C (**1**),[4] D (**2**),[4] E (**3**), F (**4**), G (**5**), H (**6**), I (**7**), and J (**8**).

Bistratamide E (**3**) was obtained as a clear solid, $[\alpha]_D$ −31°. The molecular formula, $C_{25}H_{34}N_6O_4S_2$, was determined by high-resolution MALDI-FTMS mass measurement of the $[M + Na]^+$ ion at *m*/*z* 569.1971 (Δ −0.4 mmu). The infrared spectrum contained bands at 3390 $cm^{-1}$ (amide NH) and 1670 $cm^{-1}$ (amide). The modified hexapeptide nature of the molecule was suggested by the presence of six nitrogen atoms in the molecular formula and six signals between $\delta$ 158.5 and 169.0 in the $^{13}C$ NMR spectrum (Table 1). However, the presence of only three amide −*N*H signals in the $^1H$ NMR spectrum implied that three of the amino acid residues had been modified, as is the case for bistratamides C (**1**) and D (**2**).[4] Analysis of the $^1H$ NMR and COSY experiments indicated the presence of three valine residues. The $^1H$ NMR spectrum (Table 1) contained two signals at $\delta$ 8.35 (1H, s) and 8.38 (1H, s) that were assigned to the protons of two thiazole rings, the presence of which was confirmed by analysis of the HMBC experiment. The remaining $^1H$ and $^{13}C$ NMR signals were assigned to a methyloxazoline ring. These assignments accounted for 11 of the 12 unsaturation equivalents re-

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**Table 1.** $^{13}C$ and $^{1}H$ NMR Data for Bistratamides E (**3**) and F (**4**)

| | **3** | | | | **4** | | | |
|---|---|---|---|---|---|---|---|---|
| C# | $\delta_C$ | $\delta_H$ | mult., *J* (Hz) | HMBC | $\delta_C$ | $\delta_H$ | mult., *J* (Hz) | HMBC |
| 1 | 169.0 | | | | 169.4 | | | |
| 2 | 72.7 | 4.22 | dd, 9, 5 | C1, C3, C4 | 72.7 | 4.30 | dd, 8.5, 2 | C1, C3, C4 |
| 3 | 81.9 | 4.79 | m | | 81.7 | 4.81 | m | |
| 4 | 21.4 | 1.46 | 3H, d, 6 | C2, C3 | 21.4 | 1.47 | 3H, d, 6 | C2, C3 |
| 5 | 167.6 | | | | 167.2 | | | |
| 6 | 51.1 | 4.79 | m | | 51.3 | 4.81 | m | |
| 7 | 30.4 | 2.32 | m | | 30.8 | 2.32 | m | |
| 8 | 18.9 | 0.95 | 3H, d, 7 | | 16.0 | 0.89 | 3H, d, 7 | |
| 9 | 16.0 | 0.88 | 3H, d, 7 | | 15.9 | 0.79 | 3H, d, 7 | |
| NH | | 8.04 | d, 9.5 | C5, C6, C10 | | 7.90 | d, 9 | C5, C10 |
| 10 | 159.0 | | | | 158.7 | | | |
| 11 | 147.3 | | | | 147.6 | | | |
| 12 | 124.9 | 8.38 | s | C10, C11, C13 | 124.6 | 8.36 | s | C10, C11, C13 |
| 13 | 167.5 | | | | 167.0 | | | |
| 14 | 54.7 | 5.51 | dd, 8.5, 4.5 | C13, C18 | 54.8 | 5.28 | dd, 7.5, 4.5 | C13, C18 |
| 15 | 34.2 | 2.21 | m | C13, C17 | 33.7 | 2.11 | m | C13, C16 |
| 16 | 17.5 | 0.90 | 3H, d, 7 | | 17.3 | 0.81 | 3H, d, 6 | |
| 17 | 17.9 | 0.86 | 3H, d, 7 | | 18.5 | 0.75 | 3H, d, 6.5 | |
| NH | | 8.56 | d, 8.5 | | | 7.72 | d, 7.5 | |
| 18 | 158.5 | | | | 169.5 | | | |
| 19 | 147.8 | | | | 66.6 | 4.81 | m | |
| 20 | 124.7 | 8.36 | s | C18, C19, C21 | 72.3 | 4.81 | m | C18, C19 |
| | | | | | | 4.37 | t, 6.5 | |
| 21 | 167.9 | | | | 168.6 | | | |
| 22 | 53.9 | 5.29 | dd, 9, 5 | C21, C23 | 51.1 | 4.59 | br d, 9 | C21 |
| 23 | 34.3 | 2.04 | m | C21, C22 | 30.7 | 2.20 | m | |
| 24 | 18.2 | 0.84 | 3H, d, 7 | | 18.7 | 0.80 | 3H, d, 7 | |
| 25 | 17.4 | 0.84 | 3H, d, 7 | | 17.8 | 0.84 | 3H, d, 7 | |
| NH | | 7.78 | d, 9 | C22, C1 | | 7.63 | d, 9 | C22, C1 |

quired by the molecular formula, indicating that bistratamide E (**3**) is a cyclic peptide. The sequence of the cyclic peptide was determined by HMBC correlations from the amide protons to each of the modified amino acid entities. The amide proton at $\delta$ 8.04 showed a correlation to C-5 ($\delta$ 167.6) in the methyloxazoline ring and to C-11 ($\delta$ 147.3) in the adjacent thiazole ring. Similarly, the amide proton at $\delta$ 8.56 showed correlations to C-13 ($\delta$ 167.5) and C-18 ($\delta$ 158.5), and the amide proton at $\delta$ 7.78 showed a correlation to C-1 ($\delta$ 169.0). The absolute stereochemistry of bistratamide E was assigned by ozonolysis followed by acid hydrolysis, derivatization of the resulting amino acids with *o*-phenyldialdehyde (OPA) and *N*-acetylcysteine (NAC), and HPLC analysis to obtain peaks identical to those of the OPA/NAC derivatives of L-threonine and L-valine.[11]

Bistratamide F (**4**) was obtained as a pale yellow waxy solid, $[\alpha]_D$ +23.2°. The molecular formula, $C_{26}H_{36}N_6O_5S$, was determined by high-resolution mass measurement of the $[M + Na]^+$ ion at *m*/*z* 555.2365 ($\Delta$ +0.5 mmu). The $^{1}H$ NMR spectrum (Table 1) contained signals for three valine residues and a thiazole proton. Comparison of the $^{1}H$ and $^{13}C$ NMR spectra of **4** with those of **3** indicated the presence of a methyloxazoline ring. Since the molecular formula of **4** differs from that of **3** by replacement of one of the sulfur atoms by an oxygen atom and the addition of two hydrogens, it seemed likely that the third ring was an oxazoline ring. This proposal was confirmed by analysis of the HMBC data, which were also used to determine the sequence of amino acid units in the cyclic peptide. HMBC correlations were observed from the amide proton at $\delta$ 7.63 to C-21 ($\delta$ 168.6) and C-1 ($\delta$ 169.4), which was further correlated to signals in the methyloxazoline residue. Additional HMBC correlations from the amide −*N*H signal at $\delta$ 7.90 to C-5 ($\delta$ 167.2) and C-10 ($\delta$ 158.7) and from the −*N*H signal at $\delta$ 7.72 to C-13 and C-18 completed the sequence assignment. The absolute stereochemistry was determined using the OPA/NAC method described above, and the amino acids were found to be L-serine, L-threonine, and L-valine.

Bistratamide G (**5**) was obtained as a clear solid, $[\alpha]_D$ −73.8°. The molecular formula, $C_{25}H_{32}N_6O_5S$, was determined by high-resolution mass measurement of the $[M + Na]^+$ ion at *m*/*z* 551.2059 ($\Delta$ +1.2 mmu). Analysis of the $^{1}H$ NMR and COSY experiments established the presence of three valine residues, a thiazole ring ($\delta$ 8.34), and an oxazole ring ($\delta$ 8.78). These assignments were confirmed by interpretation of HMBC data. The signal at $\delta$ 8.78 (s, H-20) showed a correlation to C-21 ($\delta$ 162.9), which was correlated to the −*N*H signal at $\delta$ 8.30, which was in turn correlated to C-1 ($\delta$ 160.0). The methyl signal at $\delta$ 2.57 (3H, s, H-4) showed correlations to C-1 ($\delta$ 160.0), C-2 ($\delta$ 127.8), C-3 ($\delta$ 152.6), and C-5 ($\delta$ 160.2) to define the methylated oxazole ring. The thiazole proton signal at $\delta$ 8.34 (s, H-12) showed correlations to C-10 ($\delta$ 159.0) and C-13 ($\delta$ 168.0). The C-10 signal showed a correlation to the −*N*H signal at $\delta$ 8.46, and the H-6 signal at $\delta$ 5.08 showed a correlation to C-5 ($\delta$ 160.2). HMBC correlations from C-13 ($\delta$ 168.0) to H-14 ($\delta$ 5.38) to C-18 ($\delta$ 158.0) established the link between the thiazole and oxazole rings. The absolute stereochemistry of all three valine residues was shown to be L using the OPA/NAC method.

Bistratamide H (**6**) was obtained as a waxy solid, $[\alpha]_D$ −92.2°. The molecular formula, $C_{25}H_{32}N_6O_4S_2$, was determined by high-resolution mass measurement of the $[M + Na]^+$ ion at *m*/*z* 567.1813 ($\Delta$ −0.6 mmu). Analysis of the $^{1}H$ NMR and COSY experiments suggested the presence of three valine residues, two thiazole rings [$\delta$ 8.32 (H-20) and 8.34 (H-12)], and a methylated oxazole ring [$\delta$ 2.58 (3H, s, H-4)]. Analysis of the $^{13}C$ NMR spectra, which contained signals appropriate for two thiazole rings, showed that bistratamide H (**6**) differed from bistratamide G (**5**) by replacement of the oxazole ring by a thiazole ring. The $^{13}C$ NMR signal assignments were confirmed using the HMBC data, which also confirmed the linkages across the amide bonds. The absolute stereochemistry of the L-valine residues was determined using the OPA/NAC method.

Bistratamide I (**7**) was obtained as a white solid, $[\alpha]_D$ −122°. The molecular formula, $C_{25}H_{36}N_6O_6S$, was determined by high-resolution mass measurement of the $[M + Na]^+$ ion at *m/z* 571.2298 (Δ +1.1 mmu). The infrared spectrum was significantly different from those of bistratamides E−H (**3**−**6**) and contained a strong band at 3380 $cm^{-1}$ (br), which was assigned to a hydroxyl group. Analysis of the $^1H$ NMR and COSY NMR experiments established the presence of three valine residues, a thiazole ring [δ 8.30 (s, H-12)], an oxazole ring [δ 8.67 (s, H-20)], and a threonine unit that was defined as follows. The methyl signal at δ 1.10 (3H, d, *J* = 6 Hz, H-4) was coupled to a signal at 4.08 (1H, m, H-3) that was coupled to both the hydroxyl signal at 5.37 (1H, d) and the H-2 signal at 4.30 (1H, dd, *J* = 9.5, 2.5 Hz), which was in turn coupled to the −NH signal at 8.46 (1H, d, *J* = 9 Hz). The presence of the thiazole residue was confirmed by correlations in the HMBC spectrum from H-12 [δ 8.30 (1H, s)] to C-11 (δ 147.8) and C-13 (δ 168.9). The H-14 signal (δ 5.28) showed a correlation to C-13 (δ 168.9) and to C-18 (δ 159.0). The H-20 signal at δ 8.66 showed HMBC correlations to C-19 (δ 134.8) and C-21 (δ 162.8), establishing the oxazole residue. The H-22 signal at δ 5.04 was correlated to C-21 (δ 162.8) and C-1 (δ 169.7), and the H-6 signal at δ 4.35 showed a correlation to C-5 (δ 169.9). Ozonolysis of bistratamide I (**7**), followed by acid-catalyzed hydrolysis, gave L-valine and L-threonine.

Bistratamide J (**8**) was obtained as a white solid, $[\alpha]_D$ −25°. The molecular formula, $C_{25}H_{36}N_6O_5S_2$, was determined by high-resolution mass measurement of the $[M + Na]^+$ ion at *m/z* 587.2077 (Δ −0.4 mmu). The infrared spectrum again contained a strong band at 3380 $cm^{-1}$ (br) that was due to a hydroxyl group. Analysis of the $^1H$ and $^{13}C$ NMR and COSY data suggested that bistratamide J (**8**) differed from bistratamide I (**7**) by replacement of the oxazole ring by a thiazole ring. This assignment was fully supported by the HMBC data. Ozonolysis of bistratamide J (**8**), followed by acid-catalyzed hydrolysis, again gave L-valine and L-threonine.

Bistratamides E−J (**3**−**8**) showed weak to moderate activity against the human colon tumor (HCT-116) cell line ($IC_{50}$'s: **3**, 7.9 μg/mL; **4**, 28 μg/mL; **5**, 5 μg/mL; **6**, 1.7 μg/mL; **7**, 9 μg/mL; **8**, 1 μg/mL). The compounds containing two thiazole rings were more active than those containing a thiazole ring and an oxazole ring.

## Experimental Section

**General Experimental Procedures.** Optical rotations were recorded at 589 nm using a Rudolph III polarimeter. Infrared spectra were recorded on a Perkin-Elmer 1600 spectrophotometer. COSY, HSQC, and HMBC NMR spectra were recorded on a Varian Inova 300 MHz spectrometer, and $^1H$, $^{13}C$, and DEPT NMR spectra were recorded on a Varian Gemini 400 MHz spectrometer. High-resolution mass measurements were recorded on a MALDI-FTMS instrument at the Scripps Research Institute. All solvents were distilled prior to being used.

**Biological Material.** The specimen (collection # NCI 2875) of *Lissoclinum bistratum* Sluiter (Urochordata, Didemnidae) was collected by hand using scuba at Tablas Island, Philippines, in 1998 and was kept frozen from the day of collection until it was extracted. A voucher specimen is available from the corresponding author.

**Extraction and Purification.** The ascidian was lyophilized (91 g dry wt) before extraction with methanol (4 × 500 mL). The extracts were dried to obtain a dark green gum, which was partitioned between ethyl acetate and water. The organic extract (981 mg) was purified by silica gel chromatography (2.0 × 20 cm column, EM Science Kieselgel 60, stepwise gradient 20% ethyl acetate/hexanes to 100% ethyl acetate). Fractions active in the HCT-116 assay were further purified by reversed-phase HPLC (Dynamax C-18) using mixtures of methanol and water to obtain bistratamides C (**1**, 33.5 mg, 3.7 × $10^{-2}$ % dry wt),[4] D (**2**, 5.5 mg, 6.0 × $10^{-3}$ % dry wt),[4] E (**3**, 10.1 mg, 1.1 × $10^{-2}$ % dry wt), F (**4**, 4.1 mg, 4.5 × $10^{-3}$ % dry wt), G (**5**, 1.4 mg, 1.5 × $10^{-3}$ % dry wt), H (**6**, 1.6 mg, 1.8 × $10^{-3}$ % dry wt), I (**7**, 1.1 mg, 1.2 × $10^{-3}$ % dry wt), and J (**8**, 0.6 mg, 6.6 × $10^{-4}$ % dry wt).

**Bistratamide E (3):** clear glass; $[\alpha]_D$ −31.0° (*c* 1, MeOH); UV (MeOH) 237 nm (ε 3030); IR (neat) 3390, 2955, 1670, 1535, 1510, 1495 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$) see Table 1; $^{13}C$ NMR (100 MHz, DMSO-$d_6$) see Table 1; EIMS $[M + Na]^+$ *m/z* 569; HRMS (MALDI-FTMS) $[M + Na]^+$ *m/z* 569.1971 (calcd for $C_{25}H_{34}N_6NaO_4S_2$, 569.1975).

**Bistratamide F (4):** cream solid, $[\alpha]_D$ +23.2° (*c* 1, MeOH); UV(MeOH) 230 nm (sh); IR (neat) 3380, 2955, 1685, 1655, 1535, 1520, 1505 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$) see Table 1; $^{13}C$ NMR (100 MHz, DMSO-$d_6$) see Table 1; EIMS $[M + Na]^+$ *m/z* 555; HRMS (MALDI -FTMS) $[M + Na]^+$ *m/z* 555.2365 (calcd for $C_{26}H_{36}N_6NaO_5S$, 555.2360).

**Bistratamide G (5):** clear solid, $[\alpha]_D$ −73.8° (*c* 1, MeOH); UV (MeOH) 230 nm (sh); IR (neat) 3390, 1675, 1640, 1590, 1535, 1515 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$) δ 0.89 (3H, d, *J* = 7 Hz), 0.91 (3H, d, *J* = 7 Hz), 0.93 (3H, d, *J* = 7 Hz), 0.95 (3H, d, *J* = 7 Hz), 0.96 (3H, d, *J* = 7 Hz), 0.98 (3H, d, *J* = 7 Hz), 2.19 (1H, m, H15), 2.22 (1H, m, H7), 2.29 (1H, m, H23), 2.57 (3H, s, H4), 5.02 (1H, dd, *J* = 7.5, 4.5 Hz, H22), 5.08 (1H, dd, *J* = 9, 6 Hz, H6), 5.38 (1H, dd, *J* = 9, 6 Hz, H14), 8.32 (1H, d, *J* = 7.5 Hz, *N*(3)H), 8.33 (1H, d, *J* = 9 Hz, *N*(2)H), 8.34 (1H, s, H12), 8.46 (1H, d, *J* = 9 Hz, *N*(1)H), 8.78 (1H, s, H20); $^{13}C$ NMR (100 MHz, DMSO-$d_6$) δ 11.2 (C4), 18.1, 18.12, 18.17, 18.3, 18.4, 18.7, 32.6 (C23), 32.9 (C7), 34.6 (C15), 52.1 (C6), 52.6 (C22), 54.8 (C14), 125.0 (C12), 127.8 (C2), 134.2 (C19), 142.7 (C20), 147.6 (C11), 152.6 (C3), 158.1 (C18), 159.0 (C10), 160.0 (C1), 160.2 (C5), 162.9 (C21), 168.0 (C13); EIMS $[M + Na]^+$ *m/z* 551; HRMS (MALDI-FTMS) $[M + Na]^+$ *m/z* 551.2059 (calcd for $C_{25}H_{32}N_6NaO_5S$, 551.2047).

**Bistratamide H (6):** clear solid; $[\alpha]_D$ −92.9° (*c* 1, MeOH); UV (MeOH) 237 nm (sh); IR (neat) 3390, 2955, 1670, 1530, 1505, 1490 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$) δ 0.90 (3H, d, *J* = 7 Hz), 0.93 (3H, d, *J* = 7 Hz), 0.94 (3H, d, *J* = 7 Hz), 0.95 (3H, d, *J* = 7 Hz), 0.96 (3H, d, *J* = 7 Hz), 0.97 (3H, d, *J* = 7 Hz), 2.21 (3H, m, H7, H15, H23), 2.58 (3H, s, H4), 5.06 (1H, dd, *J* = 8.5, 5 Hz, H6), 5.34 (1H, dd, *J* = 8.5, 5.5 Hz, H14), 5.44 (1H, dd, *J* = 9.5, 6.5 Hz, H22), 8.32 (1H, s, H20), 8.34 (1H, s, H12), 8.35 (1H, d, *J* = 9.5 Hz, *N*(3)H), 8.48 (1H, d, *J* = 9 Hz, *N*(1)H), 8.52 (1H, d, *J* = 8.5 Hz, *N*(2)H); $^{13}C$ NMR (100 MHz, DMSO-$d_6$) δ 11.3 (C4), 18.0, 18.1, 18.2, 18.3, 18.5, 18.9, 32.8 (C7), 34.4 (C23), 34.5 (C15), 52.2 (C6), 54.5 (C22), 54.7 (C14), 124.5 (C20), 125.0 (C12), 127.6 (C2), 147.5 (C11), 148.0 (C19), 152.9 (C3), 158.7 (C18), 159.1 (C10), 159.4 (C1), 160.2 (C5), 168.1 (C13), 168.6 (C21); EIMS $[M + Na]^+$ *m/z* 567; HRMS (MALDI-FTMS) $[M + Na]^+$ *m/z* 567.1813 (calcd for $C_{25}H_{32}N_6NaO_4S_2$, 567.1819).

**Bistratamide I (7):** white solid; $[\alpha]_D$ −122° (*c* 0.5, MeOH); UV (MeOH) 243 nm (sh); $[\alpha]_D$ −122° (*c* 0.5, MeOH); IR (neat) 3380 (br), 3380, 3305, 2955, 1660, 1595, 1530 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$) δ 0.85 (3H, d, *J* = 7 Hz), 0.89 (3H, d, *J* = 7 Hz), 0.94 (3H, d, *J* = 7 Hz), 0.98 (3H, d, *J* = 7 Hz), 0.99 (3H, d, *J* = 7 Hz), 1.02 (3H, d, *J* = 7 Hz), 1.10 (3H, d, *J* = 6 Hz, H4), 2.13 (1H, m, H15), 2.16 (1H, m, H23), 2.21 (1H, m, H7), 4.08 (1H, m, H3), 4.30 (1H, dd, *J* = 9.5, 2.5 Hz, H2), 4.35 (1H, t, *J* = 10.5 Hz, H6), 5.04 (1H, dd, *J* = 9, 6 Hz, H22), 5.28 (1H, dd, *J* = 9, 6.5 Hz, H14), 5.37 (1H, d, *J* = 5 Hz, OH), 8.05 (1H, d, *J* = 9 Hz, *N*(4)H), 8.30 (1H, s, H12), 8.31 (1H, d, *J* = 9 Hz, *N*(3)H), 8.46 (1H, d, *J* = 8 Hz, *N*(1)H), 8.58 (1H, d, *J* = 10.5 Hz, *N*(2)H), 8.67 (1H, s, H20); $^{13}C$ NMR (100 MHz, DMSO-$d_6$) δ 18.2, 18.3, 18.4, 18.9, 19.3, 19.8, 21.1 (C4), 30.8 (C7), 33.1 (C23), 34.6 (C15), 52.1 (C22), 55.2 (C14), 59.1 (C2), 61.2 (C6), 67.2 (C3), 125.3 (C12), 134.8 (C19), 141.6 (C20), 147.8 (C11), 159.0 (C18), 159.3 (C10), 162.8 (C21), 169.0 (C13), 169.8 (C1), 170.0 (C5); EIMS $[M + Na]^+$ *m/z* 571; HRMS (MALDI-FTMS) $[M + Na]^+$ *m/z* 571.2298 (calcd for $C_{25}H_{36}N_6NaO_6S$, 571.2309).

**Bistratamide J (8):** white solid; $[\alpha]_D$ −25.0° (*c* 0.5, MeOH); UV (MeOH) 248 nm (sh); IR (neat) 3380 (br), 2965, 2905, 1660,

1535, 1490 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$) $\delta$ 0.80 (3H, d, $J = 7$ Hz), 0.89 (3H, d, $J = 7$ Hz), 0.98 (3H, d, $J = 7$ Hz), 1.02 (9H, d, $J = 7$ Hz), 1.09 (3H, d, $J = 6$ Hz, H4), 2.14 (2H, m, H15, H23), 2.22 (1H, m, H7), 4.11 (1H, m, H3), 4.31 (1H, dd, $J = 10$, 2 Hz, H2), 4.33 (1H, t, $J = 10.5$ Hz, H6), 5.19 (1H, t, $J = 9$ Hz, H22), 5.29 (1H, br s, OH), 5.32 (1H, dd, $J = 10$, 7 Hz, H14), 8.21 (2H, br m, *N*(4)H, *N*(3)H), 8.22 (1H, s, H20), 8.28 (1H, s, H12), 8.45 (1H, d, $J = 10.5$ Hz, *N*(1)H), 8.55 (1H, d, $J = 10.5$ Hz, *N*(2)H); $^{13}C$ NMR (100 MHz, DMSO-$d_6$) $\delta$ 18.6, 18.8, 18.9, 19.4 (2C), 19.8, 21.2 (C4), 30.8 (C7), 34.2 (C23), 34.6 (C15), 55.0 (C22), 55.3 (C14), 59.0 (C2), 61.3 (C6), 67.6 (C3), 123.9 (C20), 125.0 (C12), 148.0 (C11), 148.7 (C19), 159.4 (C10), 159.6 (C18), 169.0 (C13), 169.6 (C21), 169.7 (C1), 169.8 (C5); EIMS $[M + Na]^+$ *m*/*z* 587; HRMS (MALDI-FTMS) $[M + Na]^+$ *m*/*z* 587.2077 (calcd for $C_{25}H_{36}N_6NaO_5S_2$, 587.2081).

**Determination of Absolute Stereochemistry.** Bistratamides E (**3**), F (**4**), G (**5**), H (**6**), I (**7**), and J (**8**) (100 $\mu$g) were each dissolved in dry methylene chloride (3 mL), and a stream of ozone in oxygen was bubbled through each solution for 10 min, after which time the solvent was removed under vacuum. The tubes containing the ozonolysis products were then placed inside a larger test tube containing 6 N HCl (1 mL), and the tubes were sealed. The sealed tubes were heated at 150 °C for 21 h and cooled, and the resulting hydrolysate was dried under vacuum and derivatized with *o*-phthaldialdehyde/*N*-acetyl-L-cysteine (OPA/NAC) reagent.[11] In a typical procedure, the amino acid (20 $\mu$L of a 0.1 mg/mL solution) was mixed with OPA/NAC reagent (5 $\mu$L). Derivatization was allowed to occur for 1 min before quenching with NaOAc buffer (47.5 $\mu$L of a 50 mM solution, pH 5.2). A portion (50 $\mu$L) of each sample was analyzed by HPLC on a Phenomenex Synergi Hydro-$C_{18}$ column. The effluent was monitored with a Shimadzu RF-535 fluorescence HPLC monitor at an excitation wavelength of 340 nm and an emission wavelength of 450 nm. Bistratamide E (**3**) gave peaks at 16.9 and 25.1 min, bistratamide F (4) gave peaks at 14.2, 16.8, and 25.2 min, bistratamides G (**5**) and H (**6**) showed one peak at 25.1 min, and bistratamides I (**7**) and J (**8**) gave peaks at 16.8 and 25.1 min. The amino acid standards showed the following retention times: serine, 13.9 min (D) and 14.1 min (L); threonine, 15.9 min (D) and 16.8 min (L); valine, 24.9 min (L) and 26.9 min (D).

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**Supporting Information Available:** $^1H$ and $^{13}C$ NMR spectra of bistratamides E−J and the COSY, gHSQC, and HMBC spectra of bistratamide E. This material is available free of charge via the Internet at http://pubs.acs.org.

## References and Notes

(1) Faulkner, D. J. *Nat. Prod. Rep.* **2002**, *19*, 1−48, and previous reports in this series.
(2) Degnan, B. M.; Hawkins, C. J.; Lavin, M. F.; McCaffrey, E. J.; Parry, D. L.; Watters, D. J. *J. Med. Chem.* **1989**, *32*, 1354−1359.
(3) Degnan, B. M.; Hawkins, C. J.; Lavin, M. F.; McCaffrey, E. J.; Parry, D. L.; van den Brenk, A. L.; Watters, D. J. *J. Med. Chem.* **1989**, *32*, 1349−1354.
(4) Foster, M. P.; Concepción, G. P.; Caraan, G. B.; Ireland, C. M. *J. Org. Chem.* **1992**, *57*, 6671−6675.
(5) Hambley, T. W.; Hawkins, C. J.; Lavin, M. F.; van den Brenk, A.; Watters, D. J. *Tetrahedron* **1992**, *48*, 341−348.
(6) Hawkins, C. J.; Lavin, M. F.; Marshall, K. A.; van den Brenk, A. L.; Watters, D. J. *J. Med. Chem.* **1990**, *33*, 1634−1638.
(7) Carroll, A. R.; Feng, Y.; Coll, J. C.; Bowden B. F. *J. Nat. Prod.* **1996**, *61*, 4059−4061.
(8) Ishida, K.; Nagagawa, H.; Murakama, M. *J. Nat. Prod.* **2000**, *63*, 1315−1317.
(9) Prinsep, M. R.; Moore, R. E.; Levine, I. A.; Patterson, G. M. L. *J. Nat. Prod.* **1992**, *55*, 140−142.
(10) Salomon, C. E.; Faulkner, D. J. *J. Nat. Prod.* **2002**, *65*, 689−692.
(11) Zhao, M.; Bada, J. L. *J. Chrom. A* **1995**, *690*, 55−63.

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