# Symplostatin 2: A Dolastatin 13 Analogue from the Marine Cyanobacterium *Symploca hydnoides*

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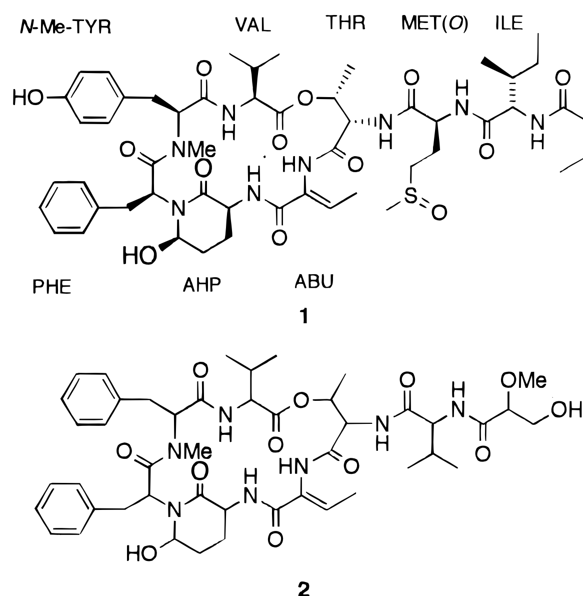
An analogue of dolastatin 13 (**2**) has been isolated from a marine cyanobacterium, *Symploca hydnoides*, collected near Guam. This new cyclic depsipeptide contains a L-methionine sulfoxide residue; however, the sulfoxide exists as both *R*- and *S*-forms, resulting in the doubling of several signals in the $^1$H and $^{13}$C NMR spectra. Structure elucidation required extensive application of 2-D NMR techniques such as COSY, HMQC, HMBC, and ROESY. The trivial name symplostatin 2 (**1**) has been assigned to the new metabolite and its isolation from *S. hydnoides* further supports the proposal that many compounds originally isolated from the sea hare *Dolabella auricularia* are most probably of cyanobacterial origin.

Marine cyanobacteria continue to be prolific sources of new biologically active compounds. As an example, *Lyngbya majuscula* produces a large variety of secondary metabolites, including the majusculamides,[1] curacins,[2] malyngamides,[3] lyngbyatoxins,[4] and microcolins.[5] We recently reported the isolation of C-15 epimers of the cytotoxic depsipeptide, dolastatin 12, and the related peptolide, lyngbyastatin 1, from certain strains of *L. majuscula* and assemblages of *L. majuscula* and *Schizothrix calcicola*.[6] This finding implied that many of the metabolites isolated from the sea hare *Dolabella auricularia,* a known generalist herbivore[7] and the original source of the dolastatins,[8] are of cyanobacterial origin. Investigations on a Guamanian variety of the cyanobacterium *Symploca hydnoides* led us to the discovery of a new solid tumor selective cytotoxic analogue of dolastatin 10, symplostatin 1.[9] The same blue-green algal collection afforded cyclic depsipeptide **1**, possessing a structure that was highly analogous to that of dolastatin 13 (**2**).[10] We report in this paper the isolation and structure elucidation of symplostatin 2 (**1**).




Liquid−liquid partition of an organic extract of *S. hydnoides*, followed by successive chromatographic steps on silica gel and Sephadex LH-20, afforded compound **1** as a white glassy oil. The $^1$H and $^{13}$C NMR spectra of compound **1** in MeOH at 50 °C showed signals that were indicative of a depsipeptide (Table 1). Analysis of these spectra in conjunction with COSY, HMQC, and HMBC experiments allowed the identification of isoleucine, *N*-methyltyrosine, phenylalanine, threonine, and valine residues. The presence of residues derived from 3-amino-6-hydroxy-2-piperidone (AHP) and from 2-amino-2-butenoic acid (ABU) were also established. AHP has been found in several freshwater cyanobacterial-derived depsipeptides, such as the nostopeptins,[11] micropeptins,[12] oscillapeptins,[13] A90720A,[14] and also in dolastatin 13 (**2**), which was first isolated from *D. auricularia*.[10] Despite extensive signal overlap and doubling in the upfield region of the NMR spectra, the presence of a residue derived from butanoic acid could also be discerned. A methionine sulfoxide unit was also present, as shown by the molecular formula (HRFABMS *m*/*z* [M + Na]$^+$ 1073.5023 for $C_{52}H_{74}N_8O_{13}S$ + Na), a methyl singlet at $\delta$ 2.59 in the $^1$H NMR spectrum, and two doubled signals at $\delta$ 38.3/38.6 and $\delta$ 50.9/51.1 in the $^{13}$C NMR spectrum. This doubling of signals was observed for almost all of the methionine sulfoxide carbons (see Table 1) and indicated the presence of both *R* and *S* sulfoxides.[15] One of the signals in the $^{13}$C NMR spectrum (see Table 1) was tripled and another one was quadrupled, presumably due to conformational differences around the sulfoxide. The presence of two conformers in addition to *R* and *S* sulfoxides was supported by the presence of two methyl singlets of lower intensity at 2.58 and 2.61 in the $^1$H NMR spectrum which integrated together with the singlet at 2.59 for one methyl group (S-Me). FABMS analysis indicated a small [M + Na + 16]$^+$ peak (10% relative to the [M + Na]$^+$ peak) which could, at least in principle, indicate the presence of the sulfone. The intensity of the $^{13}$C NMR signals, however, clearly suggests that the number of signals is due to conformers. The sulfoxide is most probably an artifact formed by oxidation of the methionine-containing natural product during isolation.[15]

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**Table 1.** NMR Spectral Data for Symplostatin 2 (**1**) in $CD_3OH$ at 50 °C (400 MHz)

| C/H no. | | $\delta_H$ ($J$ in Hz) | $\delta_C$[a] | $^1H-^1H$ COSY | HMBC[b] |
|---|---|---|---|---|---|
| VAL | 1 | – | 175.7, s | | H-2, H-3 |
| | 2 | 4.31, br t (7.8) | 60.2, d | H-3, NH | H-3, H-4a/b |
| | 3 | 2.07, m | 31.7, d | H-2, H-4a/b | H-4a/b |
| | 4a | 0.94 | 19.5, q | H-3 | |
| | 4b | 0.94 | 19.7, q | H-3 | H-3 |
| | NH | 8.08, br s | – | H-2 | |
| *N*-Me-TYR | 1 | – | 172.5, s | | H-2 |
| | 2 | 5.00, m | 63.2, d | H-3 | N-Me |
| | 3 | 2.78, m | 34.4, t | H-2 | |
| | 4 | – | 129.3, s | | H-3, H-6/8 |
| | 5/9 | 7.08, d (8.2) | 131.8, d | H-6/8 | H-3, H-5/9 |
| | 6/8 | 6.79, d (8.2) | 116.9, d | H-5/9 | H-6/8 |
| | 7 | – | 157.8, s | | H-6/8 |
| | N-Me | 2.87, s | 31.6, q | | |
| PHE | 1 | – | 173.3, s | | H-2, TYR N-Me |
| | 2 | 4.95, m | 52.8, d | H-3 | H-2 |
| | 3 | 2.15, 2.94 | 36.3, t | H-2 | H-2 |
| | 4 | – | 137.7, s | | H-3, H-6/8 |
| | 5/9 | 6.86, br d (8.2) | 130.6, d | H-6/8 | H-3, H-5/9, H-7 |
| | 6/8 | 7.18 | 129.2, d | H-5/9 | H-6/8 |
| | 7 | 7.13 | 127.7, d | H-5/9, H-6/8 | H-5/9 |
| AHP | 2 | – | 171.04, 171.08, s | | H-3, H-6, PHE H-2 |
| | 3 | 4.02, m | 50.6, d | H-4, NH | |
| | 4 | 1.70, 2.51, m | 22.6, t | H-3 | |
| | 5 | 1.65, 1.82 | 30.9, t | H-6 | |
| | 6 | 5.20, s | 76.1, d | H-5 | PHE H-2 |
| | OH | | – | | |
| | NH | 7.52, d (8.8) | – | H-3 | |
| ABU | 1 | – | 166.2, s | | H-3, AHP H-2, AHP NH |
| | 2 | – | 130.86, 130.93, s | | H-4 |
| | 3 | 6.83, q (7.2) | 135.8, d | H-4 | H-4 |
| | 4 | 1.66, d (7.2) | 13.6, q | H-3 | H-3 |
| | NH | 9.00, br s | – | | |
| THR | 1 | – | 173.84, 173.90, s | | |
| | 2 | 4.78, m | 57.9, d | | H-3, H-4 |
| | 3 | 5.48, br s | 73.5, d | H-4 | H-4 |
| | 4 | 1.39, d (6.7) | 19.7, q | H-3 | |
| | NH | 8.14, br t (6.1) | – | | |
| MET(O) | 1 | – | 176.4, s | | THR NH |
| | 2 | 4.77, br q (6.7), 4.81, br q (6.7) | 53.7, 53.5, d | NH, H-3 | |
| | 3 | 2.20, 2.34 | 25.7, 25.9, 26.4, 26.6,[d] t | H-2 | H-2 |
| | 4 | 2.80, 2.92 | 50.9, 51.1, t | | H-2, H-5 |
| | 5 | 2.58, 2.59, 2.61[c] | 38.6, 38.3, 38.1,[d] q | | |
| | NH | 8.25, d (6.7) | – | H-2 | |
| ILE | 1 | – | 174.05, 174.07, s | | H-2, MET(O) NH |
| | 2 | 4.25, q (7.4) | 59.30, 59.34, d | NH | NH |
| | 3 | 1.84 | 38.09, 38.13, d | H-2 | H-2, H-4, 3-Me, H-5 |
| | 3-Me | 0.95 | 16.1, q | | H-3, H-4 |
| | 4 | 1.20, m, 1.56, m | 26.1, t | H-5 | H-2, H-3, H-5 |
| | 5 | 0.90, d (7.5) | 11.4, q | H-4 | H-4 |
| | NH | 7.82, dd (2.9, 8.0) | – | H-2 | |
| butanoic acid | 1 | – | 176.1, 176.4, s | | H-2, ILE NH |
| | 2 | 2.25, 2.27 br t (7.3) | 38.9, 36.9, t | H-3 | |
| | 3 | 1.62 | 20.3, t | H-2, H-4 | H-2 |
| | 4 | 0.94 | 14.1, q | H-3 | H-2, H-3 |

[a] Multiplicity deduced from DEPT spectroscopy. [b] Proton showing long-range correlation to indicated carbon. [c] Peak area in an approximate 1:2:1 ratio, indicates presence of conformers. [d] Indicates the presence of conformers.

The amino acid content of compound **1**, particularly the presence of the unusual AHP and ABU units, suggested a close structural relationship to dolastatin 13 (**2**). A cyclic core as shown in structure **1** was therefore proposed. Because of signal broadening in the NMR spectra, only a few of the amide proton to adjacent amino acid carbonyl carbon connectivities, which are generally needed for peptide sequencing by HMBC, were discernible. Nevertheless the following sequences (see Table 1) could be established from HMBC: *N*-Me-Tyr-Phe-AHP-ABU and Thr-Met(O)-Ile-butanoic acid. Only the placement of the valine residue remained. Again, because of signal broadening, a HMBC experiment performed in DMSO-$d_6$ failed to yield any additional connectivities for peptide sequencing. A ROESY experiment performed in the latter solvent, however, demonstrated a strong NOE between the *N*-Me-Tyr $H_\alpha$ ($\delta$ 4.89, br d, $J$ = 10.9 Hz, $\delta_c$ 60.7) and the valine NH (7.44, br s) and between the Thr $H_\alpha$ ($\delta$ 4.57, m, $\delta_c$ 55.8) and the ABU NH ($\delta$ 9.19, br s), thus confirming the gross structure as shown.

Next the absolute configurations of the amino acid units were determined. Prior to Marfey analysis, **1** was subjected to PCC oxidation.[16] This allowed the liberation of glutamic acid from AHP on subsequent acid hydrolysis. Marfey

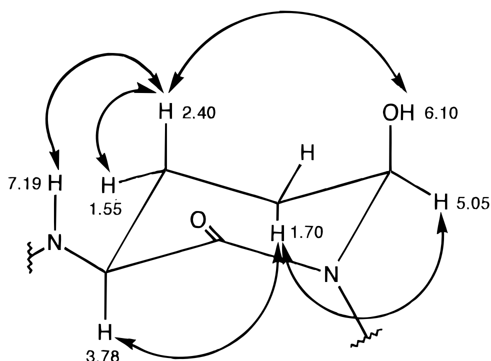


**Figure 1.** Selected ROESY correlations showing relative stereochemistry of the AHP residue in symplostatin 2(**1**).

analysis indicated that all the amino acids in **1** were L. L-Methionine was detected as its sulfone derivative. Marfey analysis on the nonoxidized substrate confirmed the presence of L-methionine sulfoxide. The absolute stereochemistries at the $\beta$-positions of the isoleucine and threonine residues were determined using chiral GC–MS, which unambiguosly established that they were L-isoleucine (2*S*,3*S*) and L-threonine (2*S*,3*R*) units and not *allo* units.

The detection of L-glutamic acid in the Marfey analysis of the oxidized substrate indicated that the stereochemistry for AHP at C-3 was *S*. The relative stereochemistry for the AHP residue had been deduced from ROESY correlations recorded in DMSO-$d_6$ (see Figure 1), particularly those between H-$4_{ax}$ ($\delta$ 2.40, $\delta_c$ 22.0) and NH ($\delta$ 7.19), H-$4_{ax}$ and OH ($\delta$ 6.10, br s), H-$5_{ax}$ ($\delta$ 1.70, $\delta_c$ 29.3) and H-3 ($\delta$ 3.78, m, $\delta_c$ 48.1), and H-$5_{ax}$ and H-6 ($\delta$ 5.05, s, $\delta_c$ 73.7). These data also established that the absolute stereochemistry at AHP C-6 had to be *R*. The (3*S*,6*R*) stereochemistry for the AHP residue of symplostatin 2 (**1**) proved to be identical to the one derived for the AHP residue in the micropeptins.[12]

A ROESY correlation, again recorded in DMSO-$d_6$, between the ABU NH ($\delta$ 9.19, br s) and the ABU methyl group ($\delta$ 1.48, d, $J$ = 6.6, $\delta_c$ 13.2) established the *Z*-geometry for the double bond in the ABU residue.

Compound **1** has been assigned the trivial name symplostatin 2 based upon its structural similarity to dolastatin 13 (**2**). It has been suggested that dolastatin 13 (**2**), isolated from *D. auricularia*, probably has a cyanobacterial dietary origin.[17] This proposal was based on its structural similarity with the nostopeptins,[11] micropeptins,[12] oscillapeptins,[13] and A90720A.[14] The latter cyclic depsipeptides have all been isolated from freshwater cyanobacteria, whereas symplostatin 2 (**1**) represents the first such analogue from a marine source. The sequestration of algal metabolites by sea hares is well-documented in the ecological literature.[7] Secondary metabolites of *L. majuscula*, for example, have previously been isolated from the sea hare *Stylocheilus longicauda*.[18] We recently reported the isolation of C-15 epimers of dolastatin 12 and a *N*-methylated analogue of dolastatin 11 from certain strains of *L. majuscula*.[6] The exceedingly small yields, including those of the potentially important anticancer drugs dolastatins 10 and 15, obtained from repeated recollections of *D. auricularia*,[8] indicate that all of the dolastatins are most probably of cyanobacterial origin.

## Experimental Section

**General Experimental Procedures.** $^1$H and $^{13}$C NMR spectra were recorded in $CD_3OH$ at 50 °C or DMSO-$d_6$ at 25 °C on a Varian 400 spectrometer operating at 400 and 100 MHz, respectively, using residual solvent signals as an internal reference. All 2-D NMR experiments were performed on the same spectrometer. HMQC experiments were optimized for $^1J_{CH}$ = 150 Hz, and HMBC experiments for $^nJ_{CH}$ = 6 Hz. HRFABMS were recorded on a VG ZAB2SE mass spectrometer in the positive mode.

**Extraction and Isolation.** Organism VP377 was *S. hydnoides* collected at the reef flat of Pago Bay, Guam, on April 8, 1996 (specimen preserved in formalin and deposited at UH). The freeze-dried organism was extracted with $CH_2Cl_2$/MeOH (1:1) to afford a lipophilic extract VP377L (4.34 g). VP377L was partitioned between hexanes and 80% aqueous MeOH, and the aqueous MeOH portion was evaporated and further partitioned between *n*-BuOH and $H_2O$. The *n*-BuOH fraction was subjected to silica gel chromatography. Elution was initiated with hexanes/$CH_2Cl_2$ (1:4) followed by $CH_2Cl_2$, then $CH_2Cl_2$ mixtures containing progressively increasing amounts of *i*-PrOH, and finally MeOH. The MeOH fraction (1.61 g) was chromatographed on Sephadex LH-20 (Sigma) with $CHCl_3$/MeOH (3:7). The first material eluted from the column (972 mg) was subjected to reversed-phase $C_{18}$ chromatography on YMC-ODS-A. Elution was initiated with 10% aqueous MeCN followed by $H_2O$ mixtures containing increasing amounts of MeCN and finally 100% MeCN. The column was then washed with MeOH followed by $CH_2Cl_2$. The 40% aqueous MeCN fraction was partitioned between $CH_2Cl_2$ and $H_2O$. Symplostatin 2 (**1**) (46.7 mg) precipitated from the $CH_2Cl_2$ fraction.

**Symplostatin 2 (1):** glassy oil; $[\alpha]_D$ −18.0° (*c* 1.2, MeOH); IR $\nu_{max}$ 3370, 1734, 1676, 1646, 1540, 1518, 1446, 1203, 1137 cm$^{-1}$; $^1$H NMR data, see Table 1; $^{13}$C NMR data, see Table 1; HRFABMS *m*/*z* [M + Na]$^+$ 1073.5023 (calcd for $C_{52}H_{74}N_8O_{13}S$ + Na, 1073.4994).

**Oxidation of Symplostatin 2 (1).** Symplostatin 2 (**1**) (1.0 mg) was dissolved in $CH_2Cl_2$ (1.0 mL), and PCC/$Al_2O_3$ (2.0 mg) was added. The reaction mixture was allowed to stand for 12 h at room temperature, after which it was washed with $H_2O$. The $CH_2Cl_2$ portion was dried under $N_2$, and the resulting oxidized material was subjected to Marfey analysis as described below.

**Absolute Configuration of Amino Acids in Symplostatin 2 (1).** The PCC-oxidized symplostatin 2 was heated at 108 °C in 6 N HCl for 18 h. The mixture was evaporated to dryness, and the residue was dissolved in $H_2O$ (50 $\mu$L) to which were added a 1% (w/v) solution of 1-fluoro-2,4-dinitrophenyl-5-L-alanine amide (Marfey's reagent) in acetone (100 $\mu$L) and 1 M $NaHCO_3$. After heating at 37 °C for 1 h, the reaction mixture was cooled, acidified with 2 N HCl (10 $\mu$L), and evaporated to dryness. The residue was then resuspended in DMSO/$H_2O$ (1:1) (2 mL) and aliquots were subjected to reversed-phase HPLC analysis [Econosphere (Alltech) $C_{18}$, 5 $\mu$M, 0.46 × 25.0 cm column, 1 mL/min flow rate; UV detection at 340 nm) using a MeCN in 50 mM $NH_4OAc$ linear gradient (10−50% MeCN over 60 min). The retention times ($t_R$, min) of the derivatized amino acids in the hydrolyzate matched those of L-Thr (17.1), L-Met-($O_2$) (21.1), L-Val (24.8), L-Ile (28.7), and L-Phe (31.8) but not those of D-Thr (23.0), D-Met($O_2$) (23.0), D-Ile (36.0), and D-Phe (37.4).

A second MeCN in 50 mM $NH_4OAc$ linear gradient (0−50% MeCN over 60 min) was also performed. Under these conditions the $t_R$ for L-Glu was 22.8 min, and its presence could be deduced from the Marfey profile. *N*,*O*-diMe-L-Tyr was incubated in 6 N HCl at 108 °C for 24 h. This gave, on Marfey analysis, two peaks at $t_R$ 30.8 and 32.8 min when eluting with the 10−50% MeCN in 50 mM $NH_4OAc$ linear gradient. The earliest eluting peak was attributed to *N*,*O*-diMe-L-Tyr, and the second to *N*-Me-L-Tyr. The latter peak was observed in the Marfey profile of symplostatin 2 (**1**). In the Marfey analysis of the unoxidized symplostatin 2 (**1**), the diastereomeric derivatives of L-Met(O) eluted at 18.4 and 18.6 min while the retention times for standard derivatives of D-Met(O) were found to be 21.4 and 21.8 min.

**Absolute Stereochemistry of the Isoleucine and Threonine Units in Symplostatin 2 Using Chiral GC−MS.** Symplostatin 2 (**1**) (1.0 mg) was dissolved in 6 N HCl (300 $\mu$L) and heated at 110 °C for 12 h. The solvent was removed under

vacuum and the dry hydrolyzate mixture was treated with a mixture of 300 $\mu$L of 2-propanol and 50 $\mu$L of acetyl chloride at 100 °C for 45 min. The excess reagent was evaporated at 115 °C under a stream of nitrogen, and the residue of isopropyl esters was treated with 50 $\mu$L of 1:1 $(CF_3CF_2CO)_2O/CH_2Cl_2$ at 100 °C for 15 min. After the mixture had cooled to ambient temperature, the excess reagent was evaporated with a stream of dry nitrogen, and the resulting mixture of isopropyl esters of *N*-(pentafluoropropionyl)amino acids was dissolved in 50 $\mu$L of MeOH for GC−MS analysis (Chirasil-Val column (Alltech), 25 m × 0.25 mm). The following conditions were used for GC: a 12-psi initial head pressure and a column temperature held at 35 for 4 min after injection of the sample, then increased from 35 to 100 °C at 2.0 K/min, then from 100 °C to 190 °C at 6.0 K/min, and finally held at 190 °C for 10 min. The same procedure was repeated for standard samples of l-Ile, L-*allo*-Ile, L-Thr, and L-*allo*-Thr. The retention times found were 22.96 min for the L-Ile derivative vs 22.15 min for the L-*allo*-Ile derivative, and 19.27 min for the L-Thr derivative vs 25.28 min for the L-*allo*-Thr derivative. The retention times for the derivatives of isoleucine and threonine originated from symplostatin 2 (**1**) were 22.96 and 19.27 min, respectively.

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## References and Notes

(1) (a) Marner, F.-J.; Moore, R. E.; Hirotsu, K.; Clardy, J. *J. Org. Chem.* **1977**, *42*, 2815−2819. (b) Carter, D. C.; Moore, R. E.; Mynderse, J. S.; Niemczura, W. P.; Todd, J. S. *J. Org. Chem.* **1984**, *49*, 236−241.
(2) (a) Gerwick, W. H.; Proteau, P. J.; Nagle, D. G.; Hamel, E.; Blokhin, A.; Slate, D. L. *J. Org. Chem.* **1994**, *59*, 1243−1246. (b) Nagle, D. G.; Geralds, R. S.; Yoo, H.-D.; Gerwick, W. H.; Kim, T.-S.; Nambu, M.; White, J. D. *Tetrahedron Lett.* **1995**, *36*, 1189−1192. (c) Yoo, H.-D.; Gerwick, W. H. *J. Nat. Prod.* **1995**, *58*, 1961−1965.
(3) (a) Mynderse, J. S.; Moore, R. E. *J. Org. Chem.* **1978**, *43*, 4359−4363. (b) Cardellina, J. H., II; Marner, F.-J.; Moore, R. E. *J. Am. Chem. Soc.* **1979**, *101*, 240−242.
(4) (a) Cardellina, J. H., II; Marner, F.-J.; Moore, R. E. *Science* **1979**, *204*, 193−195. (b) Aimi, N.; Odaka, H.; Sakai, S.-I.; Fujiki, H.; Suganuma, M.; Moore, R. E.; Patterson, G. M. L. *J. Nat. Prod.* **1990**, *53*, 1593−1596.
(5) Koehn, F. E.; Longley, R. E.; Reed, J. K. *J. Nat. Prod.* **1992**, *55*, 613−619.
(6) Harrigan, G. G.; Yoshida, W. Y.; Moore, R. E.; Nagle, D. G.; Park, P. U.; Biggs, J.; Paul, V. J., Mooberry, S. L.; Corbett, T. H.; Valeriote, F. A. *J. Nat. Prod.* **1998**, *61*, 1221−1225.
(7) Pennings, S. C.; Paul, V. J. *Marine Biol.* **1993**, *117*, 535−546.
(8) (a) Pettit, G. R. *J. Nat. Prod.* **1996**, *59*, 812−821. (b) Pettit, G. R. In *Progress in the Chemistry of Organic Natural Products*; Herz, W., Kirby, G. W., Moore, R. E., Steglich, W., Tamm, C., Eds.; Springer-Verlag: Berlin, 1997; Vol. 70, pp 1−79.
(9) Harrigan, G. G.; Luesch, H.; Yoshida, W. Y.; Moore, R. E.; Nagle, D. G.; Paul, V. J.; Mooberry, S. L.; Corbett, T. H.; Valeriote, F. A. *J. Nat. Prod.* **1998**, *61*, 1075−1077.
(10) Pettit, G. R.; Kamano, Y.; Herald, C. L.; Dufresne, C.; Cerny, R. L.; Herald, D. L.; Schmidt, J. M.; Kizu, H. *J. Am. Chem. Soc.* **1989**, *111*, 5015−5017.
(11) Okino, T.; Qi, S.; Matsuda, H.; Murakami, M.; Yamaguchi, K. *J. Nat. Prod.* **1997**, *60*, 158−161.
(12) Ishida, K.; Matsuda, H.; Murakami, M.; Yamaguchi, K. *J. Nat. Prod.* **1997**, *60*, 184−187.
(13) Shin, H. J.; Murakami, H.; Matsuda, H.; Ishida, K.; Yamaguchi, K. *Tetrahedron Lett.* **1995**, *36*, 5235−5238.
(14) Lee, A. Y.; Smitka, T. A.; Bonjouklian, R.; Clardy, J. *Chem. Biol.* **1994**, *1*, 113−117.
(15) Other cyanobacterial natural products (e.g. dendroamide C) containing a methionine sulfoxide residue also show this signal doubling and are probably artifacts. Ogini, J.; Moore, R. E.; Patterson, G. M. L.; Smith, C. D. *J. Nat. Prod.* **1996**, *59*, 581−586.
(16) Marfey, P. *Carlsberg Res. Commun.* **1984**, *49*, 591−596.
(17) Moore, R. E. *J. Ind. Microbiol.* **1996**, *16*, 134−143.
(18) (a) Kato, Y.; Scheuer, P. J. *J. Am. Chem. Soc.* **1974**, *96*, 2245−2246. (b) Moore, R. E.; Blackman, A. J.; Cheuk, C. E.; Mynderse, J. S.; Matsumoto, G. K.; Clardy, J.; Woodard, R. W.; Craig, J. C. *J. Org. Chem.* **1984**, *49*, 2484−2489.

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