Tasipeptins A and B: New Cytotoxic Depsipeptides from the Marine Cyanobacterium *Symploca* sp.

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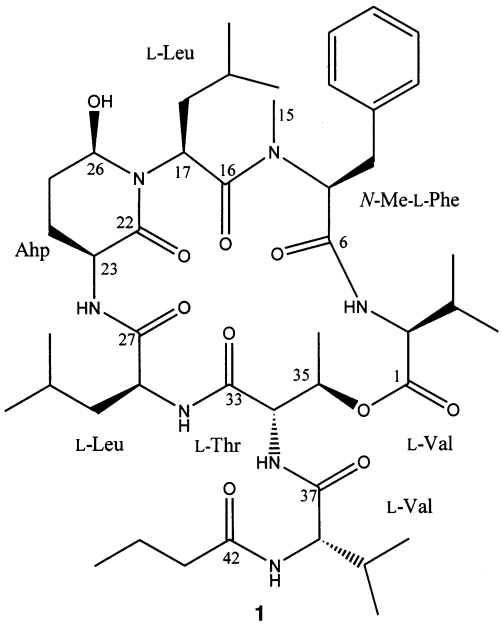
Received December 16, 2002

Two new depsipeptides have been isolated from a *Symploca* sp. collected in Palau. The gross structures of tasipeptins A (**1**) and B (**2**) were determined by standard spectroscopic techniques, and the absolute configuration of the amino acid units was determined by chiral HPLC. The relative stereochemistry of the 3-amino-6-hydroxy-2-piperidone (Ahp) moiety in both structures was determined by analysis of $^{2,3}J_{H,H}$ values. Oxidation with PCC and acid hydrolysis unmasked this latent glutamic acid moiety, allowing for elucidation of the total configuration of **1** and **2**. Tasipeptins A (**1**) and B (**2**) were cytotoxic toward KB cells with IC_{50} values of 0.93 and 0.82 μM, respectively.

Cyanobacteria are well-known sources of peptides and depsipeptides that display a variety of biological activities.[1] As part of a collaborative effort to discover new antitumor agents effective against solid and/or multidrug-resistant tumors, we began screening extracts of cyanobacteria collected in Micronesia.[2] The vast majority of these cyanobacteria belonged to the genus *Lyngbya*,[3] but among the samples collected in 1999 was a *Symploca* sp., the extracts of which displayed potent solid tumor selectivity.[4] Fewer reports have appeared in the literature on the chemistry and biological activity of this genus compared with *Lyngbya*;[5,6] however, recent investigations have hinted at the pharmaceutical potential of *Symploca*.[7−9]

Encouraged by these reports, a large collection of the cyanobacterium was made in the spring of 2000. From this *Symploca* sp., we have already described the isolation of tasiamide,[10] a cytotoxic acyclic peptide, and further examination of the aqueous extract has now led to the isolation and structure determination of the cyclic depsipeptides tasipeptins A (**1**) and B (**2**).



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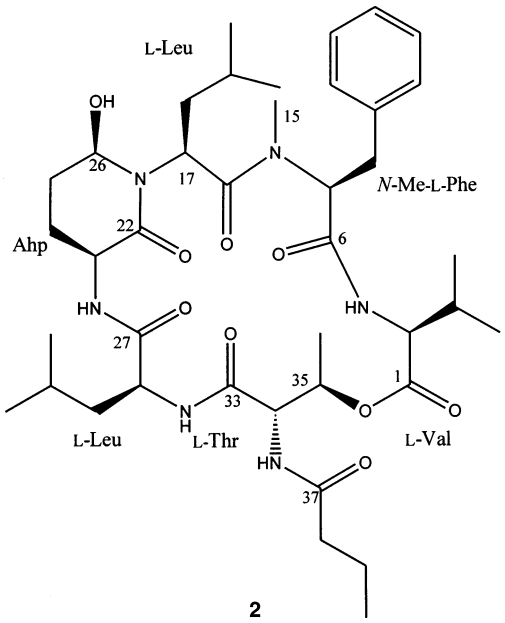
Results and Discussion

Solvent partitioning and gel permeation chromatography of the aqueous extract of *Symploca* sp. NIH304 yielded a series of cytotoxic fractions. One fraction after separation by repeated reversed-phase HPLC provided 4.3 and 2.2 mg of tasipeptins A (**1**) and B (**2**) in 0.24% and 0.12% yield based on the crude aqueous extract. Tasipeptins A (**1**) and B (**2**) were cytotoxic toward KB cells with IC_{50} values of 0.93 and 0.82 μM, respectively.

Tasipeptin A (**1**) was a colorless amorphous powder whose UV/vis spectrum showed end absorptions only. Examination of the ^{1}H and ^{13}C NMR spectra of **1** recorded in $CDCl_3$ indicated 14 sp^2 carbons, 13 methines, seven methylenes, and 11 methyl groups in accordance with a molecular weight of 892.5140 established by MADLI-TOF ($C_{45}H_{71}N_7O_{10}Na$, Δ 1.5 mmu). Based on chemical shifts, eight of the 14 sp^2 carbons were carbonyls (δ_C 174.0, 173.5, 172.5, 172.4, 171.2, 170.2, 169.6, and 168.8), and the remainder constituted a monosubstituted phenyl ring (δ_C 137.1, 129.2, 129.1, and 126.9). This accounted for a total of 12 of the 14 degrees of unsaturation implied by the molecular formula with the remaining two double bond equivalents in the form of rings. The thin-film IR spectrum of **1** suggested a depsipeptide with vibrations characteristic



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10.1021/np020582t CCC: $25.00
Published on Web 04/19/2003

of amides, esters, and hydroxyl groups at 1643, 1731, and 3291 cm^{-1}, respectively. These conclusions were corroborated by five exchangeable proton signals—one hydroxyl and four secondary amide proton signals—deduced by the lack of correlations in the HSQC spectrum.

One-dimensional TOCSY experiments on the secondary amide proton signals in **1** established the presence of one threonine, two leucine, and two valine residues. Irradiation of the α-proton signal at δ_H 5.40 (H-7) revealed a pair of geminal methylene proton signals with a −14.3 Hz coupling (H-8) that denoted an adjacent π system; namely, H-7 and H-8 were part of a phenylalanine unit. A TOCSY experiment on the alcohol proton signal (26-OH) indicated a modified amino acid residue with initial magnetization transfer to a proton signal at δ_H 5.14 (H-26) and then to a series of diastereotopic methylene protons. Irradiation of a secondary amide proton signal at δ_H 7.23 (23-NH), in a TOCSY experiment, showed strong correlations to these diastereotopic protons as well as a methine at δ_H 4.53 (H-23) and served to linked these spin systems. HMBC correlations to C-24 from H-25_{ax} and from this amide proton (23-NH) along with a COSY cross-peak from H-25_{ax} to H-26 established this unusual moiety as 3-amino-6-hydroxypiperidone (Ahp) found in previously identified *Symploca* metabolites.[9]

The remaining protons were assigned from the DQF-COSY spectrum that showed a methylene multiplet at δ_H 1.71 (H-44) with cross-peaks to a methyl triplet at δ_H 0.98 (H-45) and to a methylene triplet at δ_H 2.35 (H-43). This aliphatic chain was expanded into a butyric acid moiety by HMBC correlations between a carbonyl signal at 173.5 ppm and both methylenes.

The sequence of **1** was determined primarily by $^2J_{C,H}$ HMBC correlations between the 2° amide proton signals and the carbonyl carbons. Specifically cross-peaks between C-27/23-NH, C-33/28-NH, C-37/34-NH, C-42/38-NH, and C-6/2-NH established two partial fragments: Ahp-Leu-Thr-Val-butyrate and Val-Phe. The latter was expanded by HMBC cross-peaks between the lone *N*-methylamide proton signal (H-15) and the α-carbon of phenylalanine (C-7) and between H-15 and the carbonyl of the remaining leucine residue (C-16). Finally, $^3J_{C,H}$ correlations between H-17 and C-22 of the Ahp unit and between C-1 and H-35 unambiguously established the cyclic structure of **1**.

The stereochemistry of all the proteogenic and *N*-methylated amino acids was determined by chiral HPLC analysis of the acid hydrolysate of **1**, which contained diagnostic peaks for L-Thr, L-Val, L-Leu, and *N*-Me-L-Phe. PCC oxidation of **1** prior to acid hydrolysis and chiral HPLC analysis led to L-glutamic acid from the Ahp unit. The absolute stereochemistry of C-23 in the Ahp unit was therefore *S*. Analysis of the $^{2,3}J_{H,H}$ coupling constants, obtained through one-dimensional TOCSY and selective decoupling experiments, established the relative stereochemistry around the piperidone ring. Two large $^3J_{H,H}$ couplings to H-23 and the small $^3J_{H,H}$ couplings to H-26 indicated an axial and equatorial orientation, respectively, for these protons. Therefore the absolute configuration of the Ahp moiety was 23*S*, 26*R*.

The NMR signals of **2** were almost superimposable on those of **1**, and this suggested a minor variation in the gross structure. High-resolution mass spectrometry data of the optically active (−13° *c* 0.7, MeOH) amorphous powder established a molecular formula of $C_{40}H_{62}N_6O_9$ (MNa^+ 793.4454), indicating a smaller compound than **1**. Analysis of the one-dimensional TOCSY data established all the isolated spin systems and indicated one of the valine residues had been eliminated in **2**, which accounted for all the differences in the molecular formula. Once again HMBC correlations between the *N*-methylamide and 2° amide proton signals provided two fragments, Val-(*N*-Me-Phe)-Leu and Ahp-Leu-Thr-butyrate, that could be linked by HMBC cross-peaks (H-17/C-22, H-17/C-26) to form a linear chain. Finally, a HMBC correlation between C-1 and H-35 of threonine confirmed the cyclic nature of **2**. The absolute configuration was deduced in a manner analogous to **1**, leading us to conclude the same stereochemistry in the remaining units (2*S*, 7*S*, 17*S*, 23*S*, 26*R*, 28*S*, 34*S*, and 35*R*).

Tasipeptins A and B display characteristics typical of many cyanobacterial metabolites. Features such as *N*-methylation, the incorporation of polyketide units, and modified amino acids are biosynthetic signatures of cyanobacteria and probably serve to enhance the biological efficacy.[11] To date, the unusual Ahp unit has appeared in approximately 50 secondary metabolites isolated primarily from terrestrial and marine cyanobacteria (*Microcystis*,[12] *Oscillatoria*,[13] *Anabaena*,[14] *Nostoc*,[15] *Microchaete*,[16] *Scyonema*,[17] *Lyngbya*,[18] and *Symploca*[9] spp.), although this moiety has appeared in natural products from *Streptomyces resistomicificus*.[19] Surprisingly, regardless of the source, all known Ahp-containing metabolites share the same basic structure of a 19-membered ring constructed from six amino acids cyclized through the alcohol oxygen of threonine, or in one case 3-hydroxy-4-methylproline,[14] with variable side chains attached to the amino terminus of threonine. Also the sequence of the six amino acids that comprise the macrocycle is to a large extent conserved in the form of cyclo(L-Val/Ile-*N*-Me-L-Phe/Tyr[20]-X_1-(3*S*,6*R*)-Ahp-X_2-L-Thr) with X_1 a hydrophobic L-amino acid (Leu, Ile, Val, and Phe), although a few examples with Thr in this position have been reported.[16] The identity of X_2 appears to play a crucial role in determining the biological activity since compounds that possess a nonpolar amino acid in this position are often reported to be inhibitors of chymotrypsin, while those with polar residues generally inhibit trypsin but have little effect on chymotrypsin.[11,14,21−23] A crystal structure of a complex between trypsin and an Ahp-containing compound (A90720A; X_1 = L-Leu, X_2 = L-Arg) indeed showed that the guanadinium provided a number of key hydrogen bonds within the specificity pocket, while hydrogen bonds around the Ahp unit define the elliptical shape of A90720A.[24]

Experimental Section

General Experimental Procedures. The optical rotations were measured on a Jasco-DIP-700 polarimeter at the sodium D line (589 nm). The UV spectra were determined on a Hewlett-Packard 8453 spectrophotometer, and the IR spectra were recorded on a Perkin-Elmer 1600 FTIR instrument as a film on a NaCl disk. HRMALDI-MS data were recorded in the positive mode on a DE-STR spectrometer. The NMR spectra were recorded in $CDCl_3$ on a Varian Unity INOVA 500 operating at 500 and 125 MHz using the residual solvent signal as the internal reference. HPLC separations were performed on a Beckman 110B apparatus coupled to an Applied Biosystems 759A absorbance detector.

Biological Material. The cyanobacterium, designated NIH304, was collected at Short Drop-off in Palau during May of 2000. The organism was identified by V. J. Paul, and a voucher is maintained in formalin at the Smithsonian Marine Station, Fort Pierce, FL.

Extraction and Isolation. The freeze-dried cyanobacterium (250 g) was thrice extracted with 30% aqueous ethanol, and the extracts were combined and concentrated in vacuo. The crude extract was triturated with methanol, filtered, and

Table 1. NMR Spectral Data for Tasipeptin A (**1**) in $CDCl_3$

	C/H no.	δ_H[a] (J in Hz)	δ_C[b,c]	COSY[d]	HMBC[d,e]	ROESY
Val	1		172.5, s		3, 35	
	2	4.57, dd (7.8, 4.7)[f]	57.4, d	2-NH[f]	4, 5	
	2-NH	6.96, d (7.8)		2[f]		
	3	2.15, m	31.1, d	2[f]		
	4	0.92, d (6.6)	18.9, q	3		
	5	0.87, d (6.7)	18.1, q	3		15, 36
N-Me-Phe	6		169.6, s		2-NH, 7	
	7	5.40, dd (11.5, 2.5)	62.0, d	8	15	17
	8	3.45, dd (−14.3, 2.5)	34.0, t	7		
		2.91, dd (−14.3, 11.5)		7		
	9		137.1, s		7, 8	
	10/14	7.27, d (7.0)	129.1, d	11/13	8	
	11/13	7.18, t (7.0)	129.2, d	10/14, 12		
	12	7.22, t (7.0)	126.9, d	11/13		
	15	2.89, s	31.0, q		7	4
Leu	16		174.0, s		15, 17	
	17	4.84, dd (10.9, 4.2)	49.6, d	18		7
	18	1.68, m	37.4, t	17	17, 20, 21	26
		0.33, m		17		17
	19	0.97, m	24.2, d		20, 21	20, 21
	20	0.46, d (6.5)	21.6, q	19	21	17
	21	0.68, d (6.6)	23.6, q	19	20	
Ahp	22		170.2, s		17, 26	
	23	4.53, ddd (12.7, 8.0, 5.6)[f]	50.6, d	23-NH[f], 24	23-NH, 24_{ax}	
	23-NH	7.23, d (8.0)		23[f]		
	24_{ax}	2.43, qd (12.7, 2.2)	21.8, t	23, 25_{eq}	23-NH, 25_{ax}	24_{eq}, 25_{ax}, 23-NH
	24_{eq}	2.05, m				24_{ax}
	25_{eq}	2.04, m	29.5, t	26		26
	25_{ax}	1.83, td (13.5, 2.9)		24_{ax}, 26		26
	26	5.14, br d (5.0)	74.9, d	26-OH		18, 25, 26-OH
	26-OH	4.32, br d (5.0)		26		26
Leu	27		171.2, s		23-NH, 29a	
	28	4.48, br dd (9.4, 7.9)[f]	52.1, d	28-NH[f], 29a		
	28-NH	6.41, d (7.9)		28[f]	30	34
	29	1.91, ddd (−13.5, 9.4, 4.1)	38.8, t	28, 29b	28	
		1.57, m		28, 29a		
	30	1.53, m	24.7, d			
	31	0.90, d (6.1)	23.3, q	30	30, 32	
	32	0.83, d (6.6)	21.1, q	30	29, 30	
Thr	33		168.8, s		28-NH, 34, 35	
	34	4.74, d (9.2)	55.0, d	34-NH	35	28-NH, 35, 36
	34-NH	6.92, d (9.2)		34		38
	35	5.49, q (6.4)	71.6, d	36	1, 36	34, 36
	36	1.32, d (6.4)	18.3, q	35		4, 34, 35
Val	37		172.4, s		34, 34-NH	
	38	4.47, t (8.1)[f]	58.5, d	38-NH[f], 39		
	38-NH	6.36, d (8.1)		38[f]		43
	39	1.24, m	31.4, d	40, 41		
	40	0.96, d (7.4)	19.2, q	40	38	
	41	0.95, d (7.2)	18.1, q	40	38	
Butanoic	42		173.5, s		38-NH, 43, 44	
	43	2.35, t (7.1)	38.3, t	44	44, 45	38-NH, 44
	44	1.71, m	19.2, t	43, 45		43
	45	0.98, t (6.8)	13.3, q	44		38-NH

[a] Recorded at 500 MHz. [b] Recorded at 125 MHz. [c] Multiplicity deduced by HSQC. [d] Protons showing long-range correlation with indicated carbon. [e]Correlations were observed for $^nJ_{CH} = 7$ Hz. [f] Confirmed by 1D-TOCSY experiments on the appropriate 2° amide proton.

concentrated to afford approximately 2 g of extract from the organic layer. This mixture was chromatographed on a Sephadex LH-20 column (25 × 500 mm) and eluted with 5% MeOH in $CHCl_3$ (400 mL), 15% MeOH (700 mL), and pure MeOH (500 mL). The fractions eluting between 80 and 140 mL of 5% MeOH were combined and loaded on a C_{18} column and eluted with increasing amounts of aqueous MeCN. The 50% and 60% fractions were combined and subjected to reversed-phase HPLC (Ultracarb 5 ODS 30, 10 × 250 mm; flow rate 3 mL/min; detection at 220 nm) with 45% MeCN in H_2O to give tasipeptin B ($t_R = 47.6$ min) and tasipeptin A ($t_R = 56.3$ min). Both were subsequently repurified on a YMC-AQ column (10 × 250 mm; flow rate 2.5 mL/min; detection at 220 nm) with 50% aqueous MeCN to yield 2.2 mg ($t_R = 22.8$ min) of **2** and 4.3 mg ($t_R = 31.3$ min) of **1** in 0.12% and 0.24% yield, respectively, based on the crude aqueous extract.

Tasipeptin A (1): amorphous powder; $[\alpha]^{24}_D$ −23° (*c* 1.5, MeOH); UV (MeOH) λ_{max} (log ϵ) 202 (4.88) nm; IR (film) ν_{max} 3371, 3291, 1731, 1643, 1453 cm^{-1}; ^1H NMR, ^{13}C NMR, ^1H−^1H COSY, and HMBC data, see Table 1; HR-MALDI *m/z* [M + Na] $^+$ 892.5140 (calcd for $C_{45}H_{71}N_7O_{10}Na$ 892.5155).

Tasipeptin B (2): amorphous powder; $[\alpha]^{21}_D$ −13° (*c* 0.7, MeOH); UV (MeOH) λ_{max} (log ϵ) 202 (4.88) nm; IR (film) ν_{max} 3400, 3304, 1735, 1650, 1536, 1462, 1205 cm^{-1}; ^1H NMR, ^{13}C NMR, and HMBC data, see Table 2; MALDI *m/z* $[M + Na]^+$ 793; HR-MALDI *m/z* [M + Na] $^+$ 793.4454 (calcd for $C_{40}H_{62}N_6O_9$-Na 793.4475).

Absolute Stereochemistry. To 0.3 mg of **1** in 0.1 mL of CH_2Cl_2 was added 2 mL of dry CH_2Cl_2 containing 2 mg of pyridinium chlorochromate. The reaction was stirred overnight before being partitioned between methylene chloride and water. The organic layer was removed under a stream of N_2 and dissolved in 0.3 mL of 6 N HCl. The solution was refluxed at 118 °C for 18 h, and then the solvent was evaporated. The hydrolysate was analyzed by chiral HPLC, and the retention times were compared with authentic standards [Column

Table 2. NMR Spectral Data for Tasipeptin B (**2**) in $CDCl_3$

	C/H no.	δ_H[a] (*J* in Hz)	δ_C[b,c]	COSY	HMBC[d,e]	ROESY
Val	1		172.4, s		3, 35	
	2	4.57, dd (7.2, 4.7)[f]	57.3, d	2-NH[f]	3, 4, 5	
	2-NH	6.94, d (7.2)		2[f]		7, 15
	3	2.13, m	31.1, d	2[f]		
	4	0.89, d (6.6)	18.8, q		3	
	5	0.86, d (6.7)	18.1, q		3	
N-Me-Phe	6		169.5, s		2-NH, 7	
	7	5.41, dd (8.9, 2.5)	62.1, d	8	15	2-NH
	8	3.45, dd (−14.7, 2.5)	33.9, t	7		17
		2.90, dd (−14.7, 8.9)		7		
	9		137.1, s		8, 11/13	
	10/14	7.27, d (5.9)	129.0, d	11/13	8, 10/14	
	11/13	7.19, dd (6.0, 5.9)	129.3, d	10/14, 12	11/13, 12	
	12	7.22, t (6.0)	126.8, d	11/13		
	15	2.86, s	31.0, q		7	
Leu	16		174.0, s		15, 17	
	17	4.82, t (9.5)	48.5, d	18b		7
	18	1.69, m	37.4, t	18b	17, 20, 21	
		0.31, dt (10.4, 9.5)				
	19	0.99, m	24.2, d	18b	20, 21	
	20	0.46, d (6.5)	21.7, q	19		17
	21	0.69, d (6.6)	23.6, q	19		
Ahp	22		170.3, s		17, 26	
	23	4.55, ddd (11.6, 8.6, 3.5)[f]	50.6, d	23-NH[f], 24		
	23-NH	7.21, d (8.6)		23[f]		24_{ax}
	24_{ax}	2.45, qd (11.6, 2.2)	21.8, t	23[f], 25	23	NH-23, 24_{eq}, 25_{eq}
	24_{eq}	2.04, m		25		
	25_{eq}	2.01, m	29.5, t	24	23	
	25_{ax}	1.85, td (13.5, 2.9)		24[f]		26
	26	5.10, br d (5.0)	74.8, d	25, 26-OH	17	26
	26-OH	4.42, br d (5.0)		26		
Leu	27		171.4, s		23-NH	
	28	4.50, br dd (9.3, 8.3)[f]	51.9, d	28-NH[f]		23
	28-NH	6.65, d (8.3)		28		29b, 34, 35
	29	1.97, ddd (−14.1, 9.3, 4.4)	39.2, t	28[f]	31, 32	26
		1.57, m				26
	30	1.53, m	24.7, d			
	31	0.90, d (6.6)	23.2, q	30	29a	
	32	0.85, d (6.6)	21.3, q	30	29a	
Thr	33		169.3, s		28-NH, 34	
	34	4.85, d (9.2)	54.7, d	34-NH[f]	36	28-NH, 34-NH
	34-NH	6.75, d (9.2)		34		38
	35	5.50, q (6.4)	71.8, d	36	36	34
	36	1.30, d (6.4)	18.2, q	35		34
Butanoic	37		174.1, s		34-NH, 38, 39	
	38	2.35, t (7.1)	38.3, t	39	39, 40	
	39	1.71, m	19.2, t	38, 40	38	
	40	0.98, t (6.8)	13.3, q	39		

[a] Recorded at 500 MHz. [b] Recorded at 125 MHz. [c] Multiplicity deduced by HSQC. [d] Protons showing long-range correlation with indicated carbon. [e] Correlations were observed for $^nJ_{CH} = 7$ Hz. [f] Confirmed by 1D-TOCSY experiments on the appropriate 2° amide proton.

Chirex Phase 3126 (D) (4.6 × 250 mm), Phenomenex; flow rate 0.8 mL/min; detection at 254 nm; solvent 2 mM $CuSO_4$ for Thr; 2 mM $CuSO_4$−MeCN (95:5) for Val, Leu, and Glu; and 85:15 for *N*-Me-Phe]. The retention times (t_R, min) of the standards were L-Thr (12.0), L-allo-Thr (14.9), D-Thr (15.0), D-allo-Thr (20.0), L-Val (21.8), D-Val (30.2), L-Leu (61.5), D-Leu (80.2), L-Glu (83.3), D-Glu (93.4), *N*-Me-L-Phe (38.1), *N*-Me-D-Phe (40.6). The retention times of the components in the hydrolysate of **1** were L-Thr (12.0), L-Val (21.8), L-Leu (61.5), L-Glu (83.3) and *N*-Me-L-Phe (38.1). The hydrolysate of **2** after PCC oxidation as described above was found to contain L-Thr (12.0), L-Val (21.8), L-Leu (61.5), L-Glu (83.3) and *N*-Me-L-Phe (38.1).

Acknowledgment. We would like to thank the Republic of Palau for the marine research permit and the National Cancer Institute for providing support through NCNPDDG grant CA53001 and R01 grant CA12623. The upgrades of the spectrometers used in this research were funded by grants from the CRIF Program of the National Science Foundation (CHE9974921), the Elsa U. Pardee Foundation, and the Air Force Office of Scientific Research (F49620-01-1-0524). The cyanobacterium was collected by S. Belliveau, J. Biggs, I. Kuffner, B. Thacker, E. Cruz-Rivera, and H. Luesch. Bioassays were carried out by G. Tien at the University of Hawaii, Department of Chemistry. Mass spectral analyses were performed by the UCR Mass Spectrometry Facility, Department of Chemistry at the University of California at Riverside.

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NP020582T