JOURNAL OF NATURAL PRODUCTS

Article

pubs.acs.org/jnp

Trichormamides A and B with Antiproliferative Activity from the Cultured Freshwater Cyanobacterium *Trichormus* sp. UIC 10339

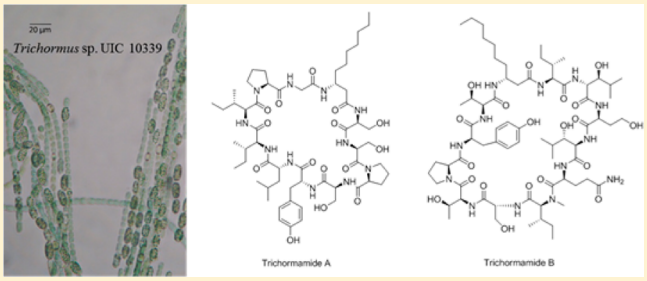
Shangwen Luo,[†] Aleksej Krunic,[†] Hahk-Soo Kang,[†] Wei-Lun Chen,[†] John L. Woodard,[‡] James R. Fuchs,[‡] Steven M. Swanson,[†] and Jimmy Orjala*[,†]

[†]Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, 833 S. Wood Street, Chicago, Illinois 60612, United States

[‡]Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, Columbus, Ohio 43210, United States

Supporting Information

ABSTRACT: Two new cyclic lipopeptides, trichormamides A (**1**) and B (**2**), were isolated from the cultured freshwater cyanobacterium *Trichormus* sp. UIC 10339. The strain was obtained from a sample collected in Raven Lake in Northern Wisconsin. The planar structures of trichormamides A (**1**) and B (**2**) were determined using a combination of spectroscopic analyses including HRESIMS and 1D and 2D NMR experiments. The absolute configurations of the amino acid residues were assigned by the advanced Marfey's method after acid hydrolysis. Trichormamide A (**1**) is a cyclic undecapeptide containing two D-amino acid residues (D-Tyr and D-Leu) and one β-amino acid residue (β-aminodecanoic acid). Trichormamide B (**2**) is a cyclic dodecapeptide characterized by the presence of four nonstandard α-amino acid residues (homoserine, *N*-methylisoleucine, and two 3-hydroxyleucines) and one β-amino acid residue (β-aminodecanoic acid). Trichormamide B (**2**) was cytotoxic against MDA-MB-435 and HT-29 cancer cell lines with IC_{50} values of 0.8 and 1.5 μM, respectively.



Many structurally diverse lipopeptides, characterized by the presence of a lipid tail connected to a linear or cyclic oligopeptide, have been discovered from bacteria and fungi to date.[1] As a class, lipopeptides are known to possess a wide range of biological activities including antimicrobial, antiproliferative, immunosuppressant, and surfactant properties.[2−4] Studies into the modes of action of lipopeptides such as daptomycin, syringomycin, and syringopeptin revealed that the lipid tail plays a key role in their interactions with plasma membranes, which leads to ion pump activation, causing membrane depolarization and eventually gives rise to cell death.[5−7] The cyclic forms of lipopeptides are thought to possess restricted conformational freedom and improved stability compared to their linearized forms. These characteristics are important in the interactions with relevant biological targets.[1,8] There has been a considerable interest in the discovery, biosynthesis, and mechanisms of action studies of cyclic lipopeptides for their potential pharmaceutical applications.[9]

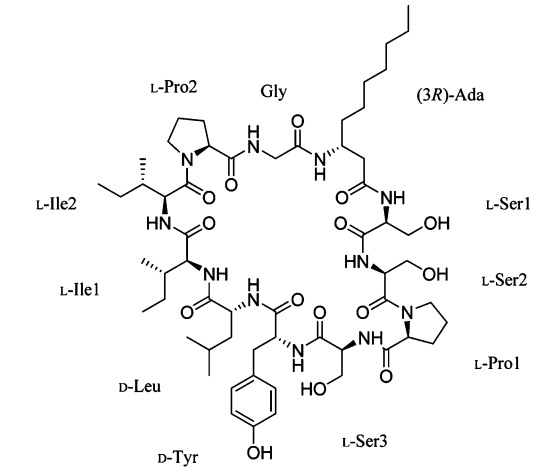
Cyanobacteria are prolific producers of cyclic lipopeptides, and many structurally diverse cyclic lipopeptides have been reported. Their activities include antimicrobial, antiproliferative, antitumor, 20S proteasome inhibitory, and allelopathic activities.[10−18] A common feature of the lipid portion of cyanobacterial cyclic lipopeptides is the presence of a β-amino or a β-hydroxy group, which is incorporated into the cyclic peptide backbone. The α position of the lipid unit is either hydroxylated, methylated, or left as a methylene. The chain of the lipid unit often shows modifications such as methylation, hydroxylation, carbonylation, and chlorination.

In our continuing search for antiproliferative natural products from cultured freshwater cyanobacteria, we evaluated the antiproliferative extract from *Trichormus* sp. UIC 10339. Morphological characterization and phylogenetic analysis using a partial 16S rDNA gene sequence identified this strain to be a *Trichormus* sp. (Supporting Information S1−S3). Herein we report the isolation, structure elucidation, and biological activity of two new cyclic lipopeptides, named trichormamides A (**1**) and B (**2**), each containing a β-aminodecanoic acid unit as part of their cyclic backbones.

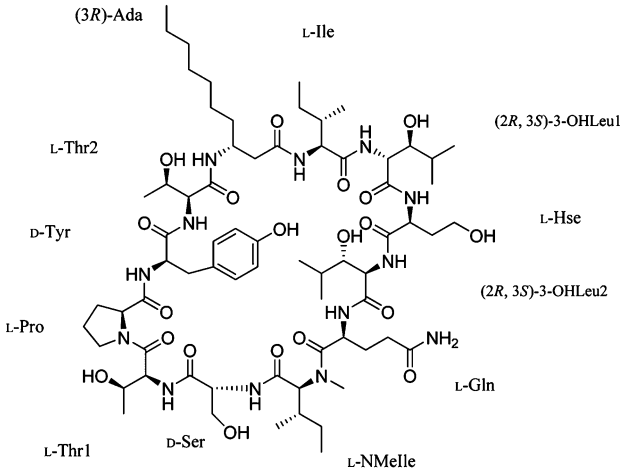
RESULTS AND DISCUSSION

Trichormus sp. UIC 10339 was obtained from a sample collected from Raven Lake in Northern Wisconsin in August 2010 and cultured in Z media. It was harvested after 8 weeks of growth.[19] The lyophilized cells were extracted with CH_2Cl_2 and MeOH (1:1) and dried *in vacuo*. This extract showed antiproliferative activity against the human colon cancer cell line HT-29 and was subjected to a bioassay-guided

Received: April 23, 2014
Published: August 4, 2014



Trichormamide A (**1**)



Trichormamide B (**2**)

fractionation scheme using Diaion vacuum liquid chromatography. The fraction eluting with 60% isopropyl alcohol (IPA) displayed antiproliferative activity. LC/MS and ^{1}H NMR dereplication indicated the presence of two potentially new peptides with molecular weights of 1184 and 1446 Da. Subsequent reversed-phase HPLC separations yielded trichormamides A (**1**) and B (**2**).

Trichormamide A (**1**) was obtained as a white, amorphous powder. The molecular formula of **1** was deduced as $C_{58}H_{93}N_{11}O_{15}$ based on HRESIMS analysis (*m*/*z* 1184.6978 $[M + H]^+$). The ^{1}H NMR spectrum of **1** in DMSO-d_6 showed broad signals. The ^{1}H NMR spectra of **1** in CD_3OH with suppression at 4.9 ppm exhibited sharper peaks, but the resonances close to 4.9 ppm were also suppressed. Therefore, ^{1}H NMR, COSY, and TOCSY experiments were conducted in both CD_3OD (without signal suppression, but NH signals absent) and CD_3OH (suppressed at 4.9 ppm, but NH signals present), respectively, to obtain both NH signals and clear signals close to 4.9 ppm (Supporting Information S4−S12). The values of chemical shifts were reported using 1D and 2D NMR spectra obtained in CD_3OH. The ^{1}H NMR spectrum of **1** exhibited a signal pattern characteristic of a lipopeptide, including exchangeable amide signals (δ_H 6.7−8.9), signals typical of α-protons (δ_H 4.1−5.0), aliphatic methylene signals (δ_H 1.1−1.4), and several doublet and triplet methyl signals (δ_H 0.7−1.0). The two downfield doublets (δ_H 7.25, J = 8.7 Hz; δ_H 6.71, J = 8.7) were attributed to a *para*-substituted phenyl moiety. Analysis of COSY and TOCSY correlations of **1** revealed the presence of 11 amino acid residues, including 10 standard α-amino acid residues: three serines (Ser1, Ser2, Ser3), two prolines (Pro1, Pro2), two isoleucines (Ile1, Ile2), tyrosine (Tyr), leucine (Leu), and glycine (Gly). Sequential COSY correlations between NH (δ_H 7.44), methine H-3 (δ_H 4.36), and methylene H_2-2 (δ_H 1.59; 2.06), as well as COSY correlations between methine H-3 (δ_H 4.36) and methylene H_2-4 (δ_H 1.50; 1.58), suggested the presence of a β-amino acid residue. Sequential COSY correlations from H_2-4 (δ_H 1.50; 1.58) to the region of highly overlapped methylene signals (H_2-5−9, δ_H 1.28−1.33), which corresponded to five carbons in the HSQC spectrum (δ_C 32.9, 30.3, 30.2, 27.2, and 23.6), and finally to the methyl triplet H-10 (δ_H 0.86) identified this β-amino acid residue as β-aminodecanoic acid (Ada).

Assignment of the amino acid sequence was carried out by a combination of HMBC, ROESY, and ESIMS/MS analysis (Figure 1). HMBC correlations from Ile2-NH (δ_H 8.84) to Ile1 C-1 (δ_C 174.1), from Ile1-NH (δ_H 6.95) to Leu C-1 (δ_C 173.6),

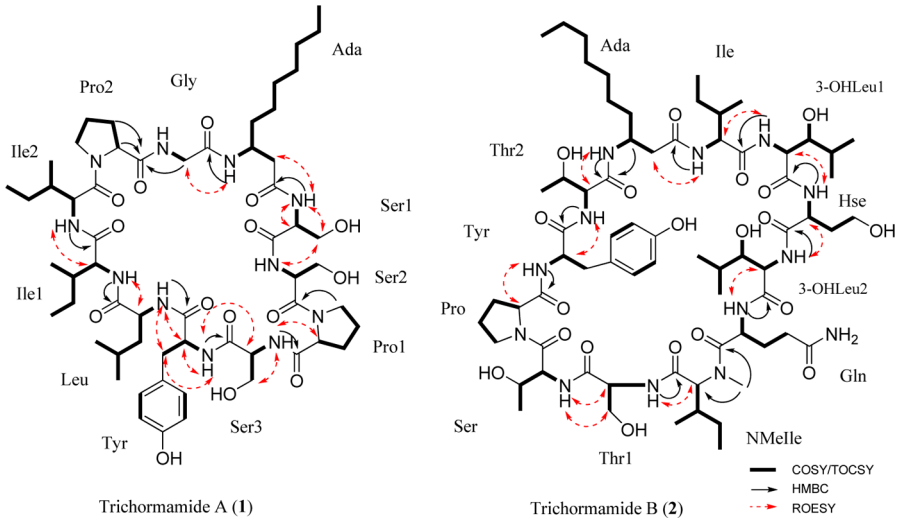


Figure 1. Key 2D NMR correlations of trichormamide A (**1**) and trichormamide B (**2**).

dx.doi.org/10.1021/np5003548 | *J. Nat. Prod.* 2014, 77, 1871−1880

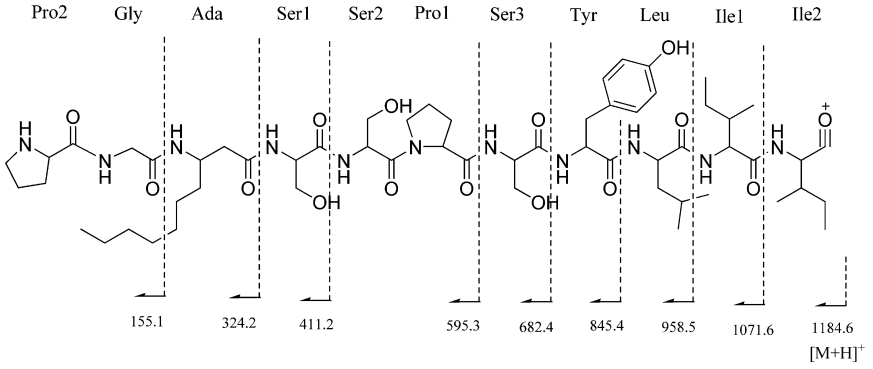


Figure 2. ESIMS/MS fragmentation of trichormamide A (**1**).

from Leu-NH (δ_H 7.71) to Tyr C-1 (δ_C 175.1), from Tyr-NH (δ_H 7.30) to Ser3 C-1 (δ_C 173.0), and from Ser3-NH (δ_H 7.57) to Pro1 C-1 (δ_C 172.2) suggested a partial sequence of Ile2-Ile1-Leu-Tyr-Ser3-Pro1. HMBC correlation from Pro1 H_2-5 (δ_H 3.60; 3.88) to Ser2 C-1 (δ_C 174.3) extended this sequence to Ile2-Ile1-Leu-Tyr-Ser3-Pro1-Ser2. Similarly, the HMBC correlations from Ser1-NH (δ_H 6.79) to Ada C-1 (δ_C 172.2), from Ada-NH (δ_H 7.44) to Gly C-1 (δ_C 170.4), and from Gly-NH (δ_H 8.75) to Pro2 C-1 (δ_C 176.1) established the partial sequence of Ser1-Ada-Gly-Pro2. This partial sequence was confirmed by ROESY correlations between Ser1-NH (δ_H 6.79) and Ada H_2-2 (δ_H 1.59; 2.06) and between Ada-NH (δ_H 7.44) and Gly H_2-2 (δ_H 3.42; 3.97). A ROESY correlation between Ser2-NH (δ_H 7.88) and Ser1 H_2-3 (δ_H 4.00; 4.27) linked the two partial sequences together into Ile2-Ile1-Leu-Tyr-Ser3-Pro1-Ser2-Ser1-Ada-Gly-Pro2. The 18 degrees of unsaturation and the molecular formula suggested that **1** was a cyclic peptide. The chemical shifts of Ile2 C-1 (δ_C 173.8), C-2 (δ_C 52.1) and Pro2 C-2 (δ_C 64.5), C-5 (δ_C 49.5), H_2-5 (δ_H 4.07; 4.13) suggested the presence of an amide moiety at Ile2 C-1 and Pro2 N.[16,17] Therefore, the cyclic undecapeptide ring was closed between Ile2 and Pro2. Mass fragmentation analysis using a quadrupole-time-of-flight (qTOF) tandem mass spectrometer confirmed the sequence of amino acid residues determined by NMR analysis. The parent ion $[M + H]^+$ 1184.6 was fragmented using a 40 eV collision energy. As expected, protonation and breakage of the proline amide bond is a highly favored process, and the macro ring of **1** opened between Ile2 and Pro2, forming a linear acylium ion.[20] The acylium ion was continuously fragmented to generate fragment ions as illustrated in Figure 2. The ESIMS/MS fragments were in complete agreement with the structure determined by NMR studies, and the planar structure of **1** was determined as cyclo[Ile2-Ile1-Leu-Tyr-Ser3-Pro1-Ser2-Ser1-Ada-Gly-Pro2].

The absolute configurations of the amino acids were determined using acid hydrolysis followed by the advanced Marfey's method (Supporting Information S14).[21−23] The chromatographic comparison between Marfey's derivatives of the hydrolysate of **1** and appropriate amino acid standards assigned the L configurations for Ser1/2/3 and Pro1/2 and the D configuration for Tyr. The absolute configuration of Ada was determined as 3*R* by comparison of the elution order of hydrolysate Ada L- and D-FDLA derivatives with those reported in the literature.[24] The stereoisomers of leucine and isoleucine standards were analyzed by Marfey's method, and retention times were compared with Marfey's derivatives of hydrolysate of **1**. As leucine and isoleucine isomers have identical molecular weight and very similar retention behavior in HPLC, a rigorous co-injection scheme was utilized to assign Leu as D and Ile1/2 as L (2*S*, 3*S*) (Supporting Information S14). The final structure of **1** was determined as cyclo[L-Ile−L-Ile−D-Leu−D-Tyr−L-Ser−L-Pro−L-Ser−L-Ser−(3*R*)-Ada−Gly−L-Pro].

Trichormamide B (**2**) was obtained as a white, amorphous powder. The molecular formula was determined as $C_{69}H_{115}N_{13}O_{20}$ (*m*/*z* 1446.8508 $[M + H]^+$) by HRESIMS analysis. The ^{1}H NMR spectrum of **2** in DMSO-d_6 (Table 2) also exhibited signals characteristic of a lipopeptide. Signal doubling was observed for some of the signals in both the ^{1}H NMR and DEPTQ spectra, indicating the presence of two conformers of **2** in solution (integration ratio 2:1 in DMSO-d_6, according to integration of the phenyl protons from tyrosine). This phenomenon has been reported for cyclic peptides with one or more *N*-methylated amino acid residues.[24−27] An elevated-temperature NMR experiment was attempted to generate one average set of signals. However, the coalescence temperature of **2** was higher than the highest operating temperature permitted by a cryogenically cooled probe (T = 328 K), and no signal coalescence was observed. Nevertheless, the elevated-temperature NMR experiment showed improved peak shapes and better resolved coupling patterns as compared to the room-temperature experiments. Thus, the structure elucidation of **2** was performed using the signals from the major conformer at elevated temperature (T = 328 K). Combined analysis of the COSY and TOCSY spectra identified the structures of seven standard amino acids: isoleucine (Ile), glutamine (Glu), serine (Ser), proline (Pro), tyrosine (Tyr), and two threonines (Thr1 and Thr2) (Figure 1 and Table 2). An *N*-methylated isoleucine (NMeIle) was deduced by TOCSY and the HMBC correlation observed from *N*-Me (δ_H 3.00) to NMeIle C-2 (δ_C 59.8). The presence of homoserine (Hse) was evident by sequential COSY correlations from NH (δ_H 8.01) to Hse H_2-4 (δ_H 3.42; 3.50). The downfield chemical shifts of the Hse C-4 methine (δ_C 57.3) and H_2-4 (δ_H 3.42; 3.50) indicated the attachment of a hydroxy group to C-4. A broad hydroxy signal (δ_H 5.05) with a COSY correlation to a downfield methine (δ_H 3.52), as well as sequential COSY correlations between NH (δ_H 8.16)/H-2 (δ_H 4.39)/H-3 (δ_H 3.52)/H-4 (δ_H 1.61)/H_3-5 (δ_H 0.93), combined with HMBC correlations from 4-Me (δ_H 0.81) and H_3-5 (δ_H 0.93) to both C-3 (δ_C 76.3) and C-4 (δ_C 30.5), indicated the presence of a 3-hydroxyleucine (3-OHLeu1). An additional 3-hydroxyleucine (3-OHLeu2) was identified using a similar approach (Table 2). The presence of a β-aminodecanoic acid was suggested from COSY, TOCSY, and HSQC correlations as described for trichormamide A (**1**).

The sequence of the 12 amino acid residues was established by analysis of the HMBC and ROESY spectra (Figure 1 and

dx.doi.org/10.1021/np5003548 | J. Nat. Prod. 2014, 77, 1871−1880

Table 1. NMR Spectroscopic Data of Trichormamide A (1) in CD_3OH, T = 300 K

	position	δ_C,[a] mult	δ_H,[b] mult. (J in Hz)	COSY	HMBC	ROESY
Ada	1	172.2, C				
	2	42.8, CH_2	1.59, m	3	1	NH_{Ser}
			2.06, m			
	3	47.7, CH	4.36, m	2, 4, NH	4, 5	
	4	35.9, CH_2	1.50, m	3	1, 3	
			1.58, m			
	5	32.9,[c] CH_2	1.28, m	overlapped		
	6	30.3,[c] CH_2	1.29, m	overlapped		
	7	30.2,[c] CH_2	1.32, m	overlapped		
	8	27.2,[c] CH_2	1.33, m	overlapped		
	9	23.6, CH_2	1.31, m	10		
	10	12.1, CH_3	0.86, t (7.4)	9	9	
	NH		7.44, d (9.9)	3	3, 1_{Gly}	2_{Gly}
Ser1	1	175.5, C				
	2	53.5, CH	4.94, dd (6.2, 3.0)	NH, 3		
	3	63.1, CH_2	4.00, t (9.7)	2	1, 2	NH, NH_{Ser2}
			4.27, m			
	OH		nd[d]			
	NH		6.79, d (7.6)	2	2, 3, 1_{Ada}	3, 2_{Ada}
Ser2	1	174.3, C				
	2	59.4, CH	4.34, m	NH, 3	1	
	3	61.9, CH_2	3.91, m	2	1, 2	
	OH		nd[d]			
	NH		7.88, br	2		3_{Ser}
Pro1	1	172.2, C				
	2	62.4, CH	4.13, m	3	3	NH_{Ser3}
	3	30.1, CH_2	1.95, m	2, 4	4	
			2.14, m			
	4	26.2, CH_2	2.21, m	3, 5	3	
			2.28, m			
	5	48.6, CH_2	3.60, m	4	3, 1_{Ser2}	
			3.88, m			
Ser3	1	173.0, C				
	2	55.5, CH	4.51, m	NH, 3	1	NH_{Tyr}
	3	62.4, CH_2	3.78, m	2	1, 2	NH
			4.13, m			
	OH		nd[d]			
	NH		7.57, d (9.8)	2	2, 1_{Pro}	3, 2_{Pro}
Tyr	1	175.1, C				
	2	59.9, CH	4.25, m	NH, 3	1, 3	NH_{Leu}
	3	37.7, CH_2	3.06, dd (13.9, 3.2)	2	1, 2	NH, NH_{Leu}
			2.98, dd (13.9, 10.8)			
	4	129.2, C				
	5/9	131.2, CH	7.25, d (8.7)	6/8	3, 4, 6/8, 7	
	6/8	116.4, CH	6.71, d (8.7)	5/9	4, 5/9, 7	
	7	157.4, C				
	OH		nd[d]			
	NH		7.30, d (5.7)	2	2, 1_{Ser3}	3, 2_{Ser3}
Leu	1	173.6, C				
	2	52.9, CH	4.53, m	NH, 3	1, 3, 4	NH_{Ile}
	3	40.1, CH_2	1.12, m	2, 4	1, 2, 4, 4-Me, 5	
			1.45, m			
	4	26.0, CH	1.73, m	3, 4-Me, 5	4-Me, 5	
	4-Me	23.6, CH_3	1.00, d (6.5)	4	3, 4, 5	
	5	20.6, CH_3	0.84, d (6.3)	4	3, 4, 4-Me	
	NH		7.71, d (8.7)	2	2, 3, 1_{Tyr}	2_{Tyr}, 3_{Tyr}
Ile1	1	174.1, C				
	2	58.1, CH	4.70, d (3.7)	NH, 3	1	NH_{Ile2}
	3	41.6, CH	1.92, m	2, 3-Me, 4		
	3-Me	16.2, CH_3	0.91, d (6.3)	3	2, 3, 4	
	4	24.1, CH_2	1.19, m	3, 5	5	

dx.doi.org/10.1021/np5003548 | J. Nat. Prod. 2014, 77, 1871−1880

Table 1. continued

	position	δ_C,[a] mult	δ_H,[b] mult. (J in Hz)	COSY	HMBC	ROESY
			1.28, m			
	5	14.3, CH_3	0.89, t (10.2)	4	3, 4	
	NH		6.95, d (9.8)	2	1_{Leu}	2_{Leu}
Ile2	1	173.8, C				
	2	52.1, CH	4.65, dd (12.2, 2.4)	NH, 3	1, 3-Me, 4	
	3	26.0, CH	1.44, m	2, 3-Me, 4		
	3-Me	20.5, CH_3	0.96, d (6.5)	3		
	4	40.5, CH_2	1.43, m	3, 5	5	
			1.73, m			
	5	23.6, CH_3	0.85, t m	4	3, 4	
	NH		8.84, d (7.0)	2	2, 1_{Ile}	2_{Ile}
Pro2	1	176.1, C				
	2	64.5, CH	4.31, m	3	1	
	3	30.6, CH_2	2.11, m	2, 4	1, 4	
			2.46, m			
	4	26.2, CH_2	2.00, m	3, 5	3	
			2.06, m			
	5	49.5, CH_2	4.07, m	4	3	
			4.13, m			
Gly	1	170.4, C				
	2	43.9, CH_2	3.42, d (17.3)	NH	1, 1_{Pro2}	NH_{Ada}
			3.97, d (17.3)			
	NH		8.75, br	2		

[a]Carbon chemical shifts were assigned from the DEPTQ spectrum recorded at 226 MHz. [b]Recorded at 600 MHz. [c]Carbon chemical shifts are interchangeable. [d]nd: not detected.

Table 2). HMBC correlations from 3-OHLeu1-NH (δ_H 8.01) to Ile C-1 (δ_C 170.8), from Ile-NH (δ_H 8.16) to Ada C-1 (δ_C 171.2), from Ada-NH (δ_H 7.52) and Ada H-3 (δ_H 4.08) to Thr2 C-1 (δ_C 169.6), from Thr2-NH (δ_H 7.60) to Tyr C-1 (δ_C 170.8), and from Tyr-NH (δ_H 7.97) to Pro C-1 (δ_C 171.5) indicated a partial sequence of 3-OHLeu1-Ile-Ada-Thr2-Tyr-Pro. ROESY correlations between 3-OHLeu1-NH (δ_H 8.01) and Ile H-2 (δ_H 4.19), between Ile-NH (δ_H 8.16) and Ada H_2-2 (δ_H 2.31; 2.49), between Ada-NH (δ_H 7.52) and Thr2 H-2 (δ_H 4.05), between Thr2-NH (δ_H 7.60) and Tyr H-2 (δ_H 4.45), and between Tyr-NH (δ_H 7.97) and Pro H-2 (δ_H 4.33) confirmed this partial sequence. Similarly, a partial sequence of Ser-NMeIle-Gln-3-OHLeu2-Hse was established by the HMBC correlations from Ser-NH (δ_H 7.83) to NMeIle C-1 (δ_C 169.8), from NMeIle N-Me (δ_H 3.00) to Gln C-1 (δ_C 172.3), from Gln-NH (δ_H 7.93) to 3-OHLeu2 C-1 (δ_C 171.3), and from 3-OHLeu2-NH (δ_H 7.76) to Hse C-1 (δ_C 171.3). This partial sequence was also confirmed by the ROESY correlations between Ser-NH (δ_H 7.83) and NMeIle H-2 (δ_H 4.73), between Gln-NH (δ_H 7.93) and 3-OHLeu2 H-2 (δ_H 4.32), and between 3-OHLeu2-NH (δ_H 7.76) to Hse H-2 (δ_H 4.35). The ROESY correlations between Thr1-NH (δ_H 7.51) and Ser H-2 (δ_H 4.33) and between Thr1-NH (δ_H 7.51) and Ser H_2-3 (δ_H 3.60) further expanded this partial sequence to Thr1-Ser-NMeIle-Gln-3-OHLeu2-Hse. An HMBC correlation from Hse-NH (δ_H 7.95) to 3-OHLeu1 C-1 (δ_C 172.3), together with a ROESY correlation between Hse-NH (δ_H 7.95) and 3-OHLeu1 H-2 (δ_H 4.39), connected those two partial sequences together into Thr1-Ser-NMeIle-Gln-3-OHLeu2-Hse-3-OHLeu1-Ile-Ada-Thr2-Tyr-Pro. The molecular formula and degree of unsaturation indicated the cyclic nature of **2**. The chemical shifts of Thr1 C-1 (δ_C 168.7) and C-2 (δ_C 55.6) suggested the presence of an amide moiety at Thr1 C-1.[16] Similarly, the chemical shifts of Pro C-2 (δ_C 59.5), C-5 (δ_C 47.0), and H_2-5 (δ_H 3.61; 3.67) also indicated that Pro N was part of an amide bond.[17] Therefore, the cyclic dodecapeptide ring was closed between Thr1 and Pro. Mass fragmentation analysis confirmed the sequence assignment of **2** (Figure 3). Protonation on the amide nitrogen at Pro generated amide bond cleavage to form a linear acylium ion, which produced series of fragments (Figure 3). These observed fragments were consistent with the amino acid sequence determined by NMR analysis, and the planar structure of **2** was assigned as cyclo[Thr1-Ser-NMeIle-Gln-3-OHLeu2-Hse-3-OHLeu1-Ile-Ada-Thr2-Tyr-Pro].

Acid hydrolysis of trichormamide B (**2**) followed by Marfey's derivatization with L- and D-FDLA and LC/MS analysis assigned the L configurations for Gln, Pro, Ile (2*S*, 3*S*), Thr1/2 (2*S*, 3*R*), and the nonproteinogenic amino acid Hse, D configurations for Tyr and Ser, and 3*R* configuration for Ada. The four stereoisomers of NMeIle were synthesized according to the literature.[28,29] On the basis of these standards, the absolute configuration of NMeIle was determined to be L (2*S*, 3*S*). The 2*S*, 3*R* and 2*S*, 3*S* isomers of 3-hydroxyleucine were synthesized following Bonnard's procedure[16] and were derivatized with L- and D-FDLA respectively. Because enantiomers exhibit identical retention behavior under non-chiral HPLC conditions, the (2*S*,3*R*)-D-FDLA derivative shows the same retention time as (2*R*,3*S*)-L-FDLA, and the (2*S*,3*S*)-D-FDLA derivative shows the same retention time as (2*R*,3*R*)-L-FDLA (detailed explanations can be found in Supporting Information S26). By comparing the above four retention times with retention times of 3-OHLeu1 and 3-OHLeu2 L-FDLA derivatives, both 3-OHLeu1 and 3-OHLeu2 were assigned as 2*R*, 3*S*. The complete structure of **2** was determined as cyclo[L-Thr−D-Ser−L-NMeIle−L-Gln−(2*R*,3*S*)-3-OHLeu−L-Hse−(2*R*,3*S*)-3-OHLeu−L-Ile−(3*R*)-Ada−L-Thr−D-Tyr−L-Pro].

Trichormamide A (**1**) is a cyclic undecapeptide characterized by the presence of β-aminodecanoic acid and two D-amino acid

dx.doi.org/10.1021/np5003548 | J. Nat. Prod. 2014, 77, 1871−1880

Table 2. NMR Spectroscopic Data of Trichormamide B (2) in DMSO-d_6, T = 328 K

	position	δ_C,[a] mult	δ_H,[b] mult. (J in Hz)	COSY	HMBC	ROESY
Ada	1	171.2, C				
	2	40.2, CH_2	2.31, m	3	1	3, NH_{Ile}
			2.49, m			
	3	46.0, CH	4.08, m	2, 4, NH	1	2, 4
	4	33.4, CH_2	1.36, m	3		3
			1.44, m			
	5	28.8,[c] CH_2	1.22, m	overlapped		
	6	28.4,[c] CH_2	1.22, m	overlapped		
	7	25.1,[c] CH_2	1.21, m	overlapped		
	8	31.0, CH_2	1.22, m	overlapped		
	9	21.8, CH_2	1.24, m	10		
	10	13.6, CH_3	0.85, t (7.2)	9	8, 9	
	NH		7.52, d (7.2)	3	1_{Thr2}	2_{Thr2}
Ile	1	170.8, C				
	2	57.6, CH	4.19, m	NH, 3	1, 3, 4	3
	3	35.7, CH	1.80, m	2, 3-Me, 4		2
	3-Me	15.1, CH_3	0.92, d (7.0)	3	2, 3, 4	
	4	24.4, CH_2	1.16, m	3, 5		
			1.47, m			
	5	10.2, CH_3	0.91, m	4	3, 4	
	NH		8.16, d (7.4)	2	1_{Ada}	2_{Ada}
3-OHLeu1	1	172.3, C				
	2	55.3, CH	4.39, m	NH, 3	1, 4	3
	3	76.3, CH	3.52, m	2, 3-OH, 4		2, 4-Me, 5
	4	30.5, CH	1.61, m	3, 4-Me, 5		
	4-Me	18.9, CH_3	0.81, d (6.5)	4	3, 4, 5	3
	5	18.5, CH_3	0.93, d (6.2)	4	3, 4, 4-Me	3
	3-OH		5.05, s	3		
	NH		8.01, d (7.4)	2	1_{Ile}	2_{Ile}
Hse	1	171.3, C				
	2	50.9, CH	4.35, m	NH, 3	1	3
	3	34.3, CH_2	1.83, m	2, 4		2
			1.89, m			
	4	57.3, CH_2	3.42, m	3		
			3.50, m			
	OH		nd[d]			
	NH		7.95, d (9.6)	2	1_{OHLeu}	2_{OHLeu}
3-OHLeu2	1	171.3, C				
	2	55.6, CH	4.32, m	NH, 3	1	3
	3	75.9, CH	3.47, m	2, 3-OH, 4		2, 4-Me, 5
	4	29.7, CH	1.58, m	3, 4-Me, 5	4-Me, 5	
	4-Me	18.8, CH_3	0.90, d (6.4)	4	3, 4, 5	3
	5	18.5, CH_3	0.78, d (6.8)	4	3, 4, 4-Me	3
	3-OH		4.93, s	3		
	NH		7.76, d (9.1)	2	1_{Hse}	
Gln	1	172.3, C				
	2	49.3, CH	4.59, m	NH, 3		3, 4, $N\text{-}Me_{NMeIle}$
	3	26.1, CH_2	1.75, m	2, 4		2
			1.92, m			
	4	30.6, CH_2	2.12, m	3	5	2
			2.15, m			
	5	174.4, C				
	NH		7.93, d (9.1)	2	1_{OHLeu2}	2_{OHLeu2}
	NH_2		6.76, s			
			7.17, s			
NMeIle	1	169.8, C				
	2	59.8, CH	4.73, d (11.0)	3	1, 3	N-Me
	3	31.5, CH	1.95, m	2, 3-Me, 4		N-Me
	3-Me	15.1, CH_3	0.83, m	3	2, 3, 4	
	4	23.8, CH_2	1.31, m	3, 5		
	5	10.6, CH_3	0.84, m	4	3, 4	

dx.doi.org/10.1021/np5003548 | J. Nat. Prod. 2014, 77, 1871−1880

Table 2. continued

	position	δ_C,[a] mult	δ_H,[b] mult. (*J* in Hz)	COSY	HMBC	ROESY
	N-Me	30.0, CH_3	3.00, s		2, 1_{Gln}	2, 3, 2_{Gln}
Ser	1	170.6, C				
	2	54.9, CH	4.33, m	NH, 3	1	
	3	60.8, CH_2	3.60, m	2	1	
	OH		nd[d]			
	NH		7.83, d (6.6)	2	1_{NMeIle}	2_{NMeIle}
Thr1	1	168.7, C				
	2	55.6, CH	4.42, dd (8.5, 3.4)	NH, 3		
	3	66.2, CH	3.93, m	2, 4		
	4	18.8, CH_3	1.08, d (5.9)	3	2, 3	
	3-OH		nd[d]			
	NH		7.51, d (7.2)	2		2_{Ser}, 3_{Ser}
Pro	1	171.5, C				
	2	59.5, CH	4.33, m	3	1	
	3	28.6, CH_2	1.78, m	2, 4		
			1.93, m			
	4	24.0, CH_2	1.78, m	3, 5		
	5	47.0, CH_2	3.61, m	4		
			3.67, m			
Tyr	1	170.8, C				
	2	54.5, CH	4.45, m	NH, 3		3
	3	36.6, CH_2	2.72, m	2	1, 2, 5/9	2
			2.92, m			
	4	127.4, C				
	5/9	129.8, CH	6.99, d (8.3)	6/8	3, 5/9, 7	
	6/8	114.7, CH	6.63, d (8.3)	5/9	4, 6/8, 7	
	7	155.6, C				
	OH		nd[d]			
	NH		7.97, d (8.6)	2	1_{Pro}	2_{Pro}
Thr2	1	169.6, C				
	2	58.0, CH	4.05, m	NH, 3	1, 3	
	3	66.0, CH	3.97, m	2, 4		
	4	19.2, CH_3	0.87, d (6.6)	3	2, 3	
	3-OH		nd[d]			
	NH		7.60, d (8.1)	2	1_{Tyr}	2_{Tyr}

[a]Carbon chemical shifts were assigned from the DEPTQ spectrum recorded at 226 MHz. [b]Recorded at 600 MHz. [c]Carbon chemical shifts are interchangeable. [d]nd: not detected.

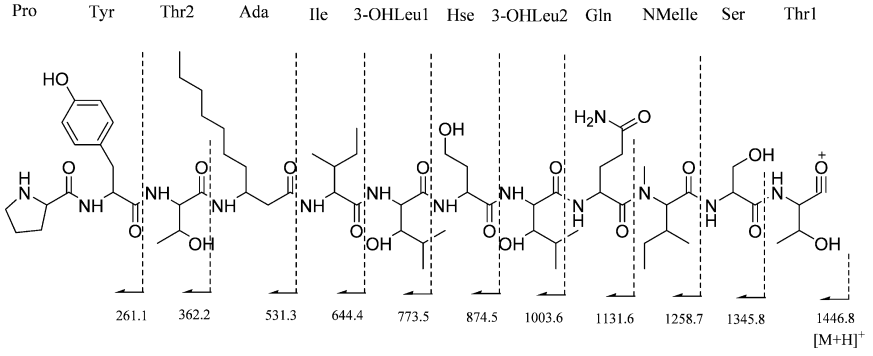


Figure 3. ESIMS/MS fragmentation of trichormamide B (**2**).

residues (D-Tyr and D-Leu). Trichormamide A (**1**) is a lipid-containing compound and displays amphiphilic character with half of the amino acid sequence being hydrophobic (Ada-Gly-Pro2-Ile2-Ile1-Leu) and the other half of the sequence hydrophilic (Tyr-Ser3-Pro1-Ser2-Ser1). The amphiphilic nature of lipopeptides has been found to be strongly correlated with their plasma membrane interactive potential.[5−7] Trichormamide A (**1**) is structurally related to hormothamnin A, lobocyclamide A, and laxaphycin A with conserved amino acid residues of Leu, Ile1, Ile2, and Gly.[14−16] The amino acid residues homoserine, 4-hydroxyproline, α,β-didehydro-α-aminobutyric acid, and β-aminooctanoic acid in the east portion of the three related compounds were replaced by structurally related residues of Ser, Pro, Ser, and Ada in trichormamide A (**1**) (Supporting Information S27). Trichormamide B (**2**) is a cyclic dodecapeptide characterized by the presence of a β-

dx.doi.org/10.1021/np5003548 | J. Nat. Prod. 2014, 77, 1871−1880

amino acid residue (β-aminodecanoic acid), two D-amino acid residues (D-Tyr and D-Ser), and four nonstandard amino acid residues (3-OHLeu1, Hse, 3-OHLeu2, NMeIle). Trichormamide B (**2**) is also an amphiphilic molecule containing a hydrophobic Ada residue and seven hydroxy groups. Trichormamide B (**2**) is structurally related to the cyclic dodecapeptides lobocyclamides B and C, laxaphycins B, B2, and B3, and lyngbyacyclamides A and B.[15−17] A comparison of **2** to these structures revealed that the presence and location of 3-OHLeu1, Gln, NMeIle, Thr1, and Thr2 are conserved in all these peptides, indicating these amino acid residues may be crucial for the biological functions of these molecules.

Trichormamides A (**1**) and B (**2**) were evaluated for their antiproliferative activities against the human melanoma cell line MDA-MB-435 and the human colon cancer cell line HT-29. Trichormamide B (**2**) displayed significant cytotoxic activity against MDA-MB-435 and HT-29 cell lines with IC_{50} values of 0.8 and 1.5 μM, respectively. Trichormamide A (**1**) was less active than **2** and showed IC_{50} values of 9.9 and 16.9 μM against MDA-MB-435 and HT-29, respectively. Compounds structurally related to **1** and **2** were reported to have 2−3-fold increased synergistic activities in antimicrobial and cytotoxicity assays.[15,16,26,30] However, the limited supply of **1** stopped us from obtaining statistically significant data in synergistic assays.

In summary, we have obtained two new cyclic lipopeptides, trichormamides A (**1**) and B (**2**), each containing the β-amino acid residue β-aminodecanoic acid, from the cultured freshwater cyanobacterium *Trichormus* sp. UIC 10339. Their planar structures were determined by analysis of the HRESIMS, MS/MS, and 1D and 2D NMR experiments. The stereoconfigurations of the amino acid residues were assigned by the advanced Marfey's method using synthesized amino acid standards. Compounds **1** and **2** are not analogues, but both exhibited amphiphilic properties. Trichormamide B (**2**) was found to be more active against MDA-MB-435 and HT-29 human cancer cell lines than trichormamide A (**1**), with IC_{50} values of 0.8 and 1.5 μM, respectively.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a PerkinElmer 241 polarimeter at 22 °C in MeOH. UV spectra were recorded on a Shimadzu UV spectrophotometer UV2401. IR spectra were acquired on a PerkinElmer 577 IR spectrophotometer. 1D and 2D NMR spectra including ^{1}H NMR, COSY, TOCSY, HSQC, HMBC, and ROESY were obtained on a Bruker Avance DRX 600 MHz spectrometer. A Bruker AVII 900 MHz NMR spectrometer was used to acquire the DEPTQ spectra. ^{1}H and ^{13}C NMR chemical shifts were referenced to the DMSO-d_6 (δ_H 2.50 and δ_C 39.51, respectively) and CD_3OD/CD_3OH (δ_H 3.31 and δ_C 49.0, respectively) residual solvent signals. The TOCSY experiments were conducted using a 60 ms mixing time. The ROESY spectra were acquired with a 200 ms mixing time. The HMBC spectrum was recorded with an average $^3J_{CH}$ of 8 Hz, and the HSQC spectrum was recorded with an average $^1J_{CH}$ of 145 Hz. High-resolution ESI mass spectra and LC-MS data were obtained using a Shimadzu HPLC-IT-TOF spectrometer. The HPLC experiments were performed using an Agilent 1100 series instrument equipped with a diode array detector, and a Waters instrument equipped with a Waters 600 pump and a Waters 2487 detector.

Biological Material. *Trichormus* sp. UIC 10339 was isolated from a sample collected at Raven Lake, Wisconsin, in 2010 (N 45°58.7′, W 89°51.5′°). The unialgal (not axenic) strain UIC 10339 was obtained through micropipette isolation techniques.[31] The strain was cultured in 8 × 2 L of Z medium in 2.8 L Fernbach flasks with sterile air aeration.[19] Cultures were illuminated with fluorescent lamps at 1.03 klx with an 18/6 h light/dark cycle. The temperature of the culture room was maintained at 22 °C. The biomass of UIC 10339 was harvested after 8 weeks of growth by centrifugation and freeze-dried.

Morphological Identification. Initial taxonomic identification was performed using a cultured UIC 10339 cyanobacterium. Morphological observation was performed using a Zeiss Axiostar Plus light microscope equipped with a Canon PowerShot A620 camera. The following characters were utilized for identification: structure of colony, morphology of thallus, size, shape, and motility of trichomes, shape of vegetative cells, presence or absence of mucilage or sheath, presence and arrangement of heterocytes, presence and relative size of akinetes, and morphology of the end cell (Supporting Information S1, S2). The morphological characterization and initial taxonomical designation of UIC 10339 were made according to the system by Komárek et al.[32]

DNA Extraction, 16S rDNA PCR Amplification, and Sequencing. Prior to DNA extraction, 8 mL of a static culture of *Trichormus* sp. UIC 10339 was combined with 1.5 mL of lysozyme buffer and 0.5 mL of 20 mg/mL lysozyme stock solution and incubated at 35 °C for 1 h. After incubation, the cell mass was centrifuged and transferred to a 2 mL microcentrifuge tube. A Wizard Genomic DNA purification kit (Promega) was used to extract the genomic DNA. A partial sequence of the 16S rDNA gene was PCR-amplified using the cyanobacteria-specific primers 106F and 1509R.[33] For a total volume of 25 μL, the reaction mixture contained DNA (1 μL, approximately 300 ng), GoTaq reaction buffer (5 μL, 5×), dNTP mix (0.5 μL, 10 mM), upstream and downstream primer (1 μL each, 10 μM), GoTaq DNA polymerase (0.25 μL, 2.5 u/μL), and H_2O (16.25 μL). The reaction was performed in a Bio-Rad C1000 thermal cycler with the following reaction program: initial denaturation for 30 s at 98 °C, 35 amplification cycles of 10 s at 98 °C, 30 s at 53 °C, 30 s at 72 °C, and a final extension for 10 min at 72 °C. PCR products were purified using a MinElute PCR purification kit (Qiagen) and sequenced using the cyanobacteria-specific primers 106F, 359F, and 1509R.[33] The resulting 16S rDNA gene sequence was deposited in the NCBI GenBank under the accession no. KF444210.

Extraction and Isolation. Lyophilized biomass (6.14 g) from eight 2 L cultures of UIC 10339 was extracted with 1:1 CH_2Cl_2/MeOH three times and concentrated *in vacuo* to yield 650.9 mg of extract, which was fractionated using Diaion HP-20SS resin with an H_2O/IPA step gradient (1:0, 4:1, 3:2, 2:3, 3:7, 1:4, 1:9, 0:1 v/v) to yield eight fractions. The fraction eluting with H_2O/IPA (2:3 v/v) (58.8 mg) displayed significant antiproliferative activity against the human melanoma cell line MDA-MB-435 with 100% growth inhibition at a concentration of 25 μg/mL. LC-MS combined with ^{1}H NMR dereplication of this fraction revealed the presence of new lipopeptides. The fraction was subjected to semipreparative reversed-phase HPLC (Varian C_8 semipreparative column, 10 × 250 mm, 3 mL/min) with a linear gradient from 80% to 100% MeOH with H_2O over 20 min, and the subfraction eluting from 9 to 10 min was collected and dried *in vacuo*. ^{1}H NMR and MS flow injection analysis revealed that this subfraction consisted of two compounds. The subfraction was subjected to semipreparative reversed-phase HPLC (Varian C_{18} semipreparative column, 10 × 250 mm, 3 mL/min) using isocratic 50% aqueous CH_3CN. Trichormamide A (**1**, 0.8 mg, 0.01% of dry weight) was eluted at 8 min, and trichormamide B (**2**, 3.7 mg, 0.06% of dry weight) was eluted at 5 min.

*Trichormamide A (**1**):* white, amorphous powder; $[\alpha]_D^{22}$ +20 (*c* 0.06, MeOH); UV (MeOH) λ_{max} (log ε) 240 (3.07), 278 (2.95) nm; IR (neat) ν_{max} 3312 (br), 2984, 2942, 2831, 1449 cm^{-1}; ^{1}H and ^{13}C NMR see Table 1; HR-ESI-TOF-MS (+) *m*/*z* 1184.6978 $[M + H]^+$ (calcd for $C_{58}H_{94}N_{11}O_{15}$, 1184.6931).

*Trichormamide B (**2**):* white, amorphous powder; $[\alpha]_D^{22}$ −25.9 (*c* 0.32, MeOH); UV (MeOH) λ_{max} (log ε) 240 (3.16), 278 (2.97) nm; IR (neat) ν_{max} 3317 (br), 2943, 2831, 1449, 1414, 1114 cm^{-1}; ^{1}H and ^{13}C NMR see Table 2; HR-ESI-TOF-MS (+) *m*/*z* 1446.8508 $[M + H]^+$ (calcd for $C_{69}H_{116}N_{13}O_{20}$, 1446.8460).

Synthesis of L-*N*-Methylisoleucine, D-*N*-Methylisoleucine, L-*allo*-*N*-Methylisoleucine, and D-*allo*-*N*-Methylisoleucine (Based on Shendage's and Malkov's Methods[28,29]). The amino group of

L-Ile was Boc-protected using a previously published protocol.[28] The methylation of *N*Boc-L-Ile was carried out using Malkov's method.[29] The Boc-protected NMeIle was subjected to acid hydrolysis using the general peptide hydrolysis scheme as described below, to yield L-*N*-methylisoleucine (73.11 mg), which was analyzed by the advanced Marfey's method. Although citric acid from the workup was observed as a minor impurity in the ^{1}H NMR spectrum of L-*N*-methylisoleucine, it did not interfere with the advanced Marfey's analysis. D-*N*-Methylisoleucine, L-*allo*-*N*-methylisoleucine, and D-*allo*-*N*-methylisoleucine were synthesized using the same protocol with D-Ile, L-*allo*-Ile, and D-*allo*-Ile as starting compounds. All were analyzed using the advanced Marfey's method as described for the peptide hydrolysate. L-*N*-Methylisoleucine: $[\alpha]_D^{22}$ +17.6 (*c* 0.39, MeOH); ^{1}H NMR data (DMSO-d_6, 400 MHz) δ 3.84 (1H, d, *J* = 3.0 Hz), 2.59 (3H, s), 1.95 (1H, m), 1.50 (1H, m), 1.32 (1H, m), 0.91 (3H, d, *J* = 8.5 Hz), 0.90 (3H, t, *J* = 7.1 Hz); DEPTQ data (DMSO-d_6, 400 MHz) δ 169.5, 64.6, 35.3, 32.5, 26.4, 14.5, 12.0. NMR spectra of D-Ile, L-*allo*-Ile, and D-*allo*-Ile can be found in Supporting Information S24.

Synthesis of (2*S*,3*R*)-3-Hydroxyleucine and (2*S*,3*S*)-3-Hydroxyleucine. These compounds were synthesized using the procedures by Bonnard et al.[16] We obtained 148.0 mg of major precursor (*R*)-1-((2*S*,5*R*)-5-isopropyl-3,6-dimethoxy-2,5-dihydropyrazin-2-yl)-2-methylpropan-1-ol and 54.5 mg of minor precursor (*S*)-1-((2*S*,5*R*)-5-isopropyl-3,6-dimethoxy-2,5-dihydropyrazin-2-yl)-2-methylpropan-1-ol. The hydroxyleucine precursors were hydrolyzed following the general peptide acid hydrolysis process as described below. The major precursor gave a mixture of (2*S*,3*R*)-3-hydroxyleucine and D-valine, and the minor precursor gave a mixture of (2*S*,3*S*)-3-hydroxyleucine and D-valine. These two mixtures were derivatized with L-FDLA and D-FDLA, respectively, and the resulting four derivatization mixtures were analyzed using the general LC-MS method as described for peptide hydrolysate analysis. ^{1}H NMR data of major precursor ($CDCl_3$, 300 MHz): δ 4.08 (1H, dd, *J* = 3.2 Hz, 2.9 Hz), 3.98 (1H, dd, *J* = 3.6 Hz, 3.4 Hz), 3.71 (3H, s), 3.67 (3H, s), 3.61 (1H, br), 2.24 (1H, m), 1.99, (1H, m), 1.69 (1H, br), 1.03 (3H, d, *J* = 6.9 Hz), 1.02 (3H, d, *J* = 6.9 Hz), 0.97 (3H, d, *J* = 6.9 Hz), 0.70 (3H, d, *J* = 6.9 Hz). ^{1}H NMR data of minor precursor ($CDCl_3$, 300 MHz): δ 4.17 (1H, dd, *J* = 4.3 Hz, 4.0 Hz), 3.98 (1H, dd, *J* = 3.7 Hz, 3.6 Hz), 3.71 (3H, s), 3.70 (3H, s), 3.65 (1H, br), 2.27 (1H, m), 1.82, (1H, m), 1.68 (1H, br), 1.04 (3H, d, *J* = 6.9 Hz), 0.90 (3H, d, *J* = 6.9 Hz), 0.89 (3H, d, *J* = 6.9 Hz), 0.69 (3H, d, *J* = 6.9 Hz).

Acid Hydrolysis of Trichormamides A (1) and B (2). Approximately 0.3 mg of **1** and **2** was hydrolyzed with 1 mL of 6 N HCl for 20 h at 110 °C in separate Teflon-sealed Ace pressure tubes. The cooled hydrolysate mixtures were evaporated to dryness, and traces of HCl were removed from the reaction mixtures by repeated evaporation.

Absolute Configurations of Amino Acids by the Advanced Marfey's Analysis. L- and D-FDLA (1-fluoro-2,4-dinitrophenyl-5-leucinamide) were purchased from TCI America. Each acid hydrolysate (approximately 0.3 mg) was separated into two equal portions for derivatization with either L-FDLA or D-FDLA. Each portion was dissolved in 110 μL of acetone and 50 μL of H_2O, followed by mixing with 20 μL of 1 N $NaHCO_3$. Finally, 20 μL of L-FDLA or D-FDLA (10 mg/mL in acetone) was added, and the mixtures were heated to 40 °C for 1 h. The reaction mixtures were then cooled to room temperature, and 20 μL of 1 N HCl was added to quench the reaction. The cooled reaction mixtures were evaporated to dryness and redissolved in 300 μL of CH_3CN. LC-MS analysis was carried out using a reversed-phase column (Phenomenex Kinetex C_{18}, 250 × 4.6 mm, 5 μm, 1.0 mL/min) with a linear gradient from 25% to 65% aqueous CH_3CN containing 0.1% formic acid over 50 min. The selective ion chromatograms of L-FDLA and D-FDLA derivatives of each amino acid were compared for the assignment of amino acid configurations. Detailed reports of retention times of each amino acid can be found in Supporting Information S14 and S23.

Antiproliferative Assay. Cytotoxicity assays against the MDA-MB-435 and HT-29 cancer cell lines were conducted according to established protocols.[34]

■ ASSOCIATED CONTENT

Supporting Information

Supplementary data (morphological and phylogenetic analysis of *Trichormus* sp. UIC 10339; ^{1}H NMR, DEPTQ, COSY, TOCSY, HSQC, HMBC, and ROESY spectra of **1** and **2**; Marfey's and advanced Marfey's analysis of **1** and **2**; MS/MS spectra of **1** and **2**) associated with this article are available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel: +1-312-996-5583. Fax: +1-312-996-7107. E-mail: orjala@uic.edu.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This research was supported by grant PO1 CA125066 from NCI/NIH. The 900 MHz NMR spectrometer was purchased by NIH grant GM068944 to Dr. Peter G. W. Gettins. We thank Dr. B. E. Ramirez for providing access to NMR spectrometers at the UIC Center for Structural Biology (CSB). We thank Dr. G. E. Chlipala for assisting in the taxonomic identification and phylogenetic analysis. We thank Dr. D. Nikolic and R. Davis for their aid in qTOF instrument operating. We thank Dr. G. R. Thacher and R. Xiong for assisting with NMeIle synthesis.

■ REFERENCES

(1) Raaijmakers, J. M.; de Bruijn, I.; Nybroe, O.; Ongena, M. *FEMS Microbiol. Rev.* **2010**, *34*, 1037−1062.
(2) Wrasidlo, W.; Mielgo, A.; Torres, V. A.; Barbero, S.; Stoletov, K.; Suyama, T. L.; Klemke, R. L.; Gerwick, W. H.; Carson, D. A.; Stupack, D. G. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 2313−2318.
(3) Vilhena, C.; Bettencourt, A. *Mini-Rev. Med. Chem.* **2012**, *12*, 202−209.
(4) Cameotra, S. S.; Makkar, R. S. *Curr. Opin. Microbiol.* **2004**, *7*, 262−266.
(5) Bidwai, A. P.; Takemoto, J. Y. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 6755−6759.
(6) Digiorgio, D.; Camoni, L.; Ballio, A. *Physiol. Plant.* **1994**, *91*, 741−746.
(7) Baltz, R. H. *Curr. Opin. Chem. Biol.* **2009**, *13*, 144−151.
(8) Sieber, S. A.; Marahiel, M. A. *Chem. Rev.* **2005**, *105*, 715−738.
(9) Pirri, G.; Giuliani, A.; Nicoletto, S. F.; Pizzuto, L.; Rinaldi, A. C. *Cent. Eur. J. Biol.* **2009**, *4*, 258−273.
(10) Mehner, C.; Muller, D.; Krick, A.; Kehraus, S.; Loser, R.; Guetschow, M.; Maier, A.; Fiebig, H. H.; Brun, R.; Konig, G. M. *Eur. J. Org. Chem.* **2008**, 1732−1739.
(11) Kang, H. S.; Krunic, A.; Shen, Q.; Swanson, S. M.; Orjala, J. *J. Nat. Prod.* **2011**, *74*, 1597−1605.
(12) Kang, H. S.; Sturdy, M.; Krunic, A.; Kim, H.; Shen, Q.; Swanson, S. M.; Orjala, J. *Biorg. Med. Chem.* **2012**, *20*, 6134−6143.
(13) Krunic, A.; Vallat, A.; Mo, S. Y.; Lantvit, D. D.; Swanson, S. M.; Orjala, J. *J. Nat. Prod.* **2010**, *73*, 1927−1932.
(14) Gerwick, W. H.; Jiang, Z. D.; Agarwal, S. K.; Farmer, B. T. *Tetrahedron* **1992**, *48*, 2313−2324.
(15) MacMillan, J. B.; Ernst-Russell, M. A.; de Ropp, J. S.; Molinski, T. F. *J. Org. Chem.* **2002**, *67*, 8210−8215.
(16) Bonnard, I.; Rolland, M.; Salmon, J. M.; Debiton, E.; Barthomeuf, C.; Banaigs, B. *J. Med. Chem.* **2007**, *50*, 1266−1279.
(17) Maru, N.; Ohno, O.; Uemura, D. *Tetrahedron Lett.* **2010**, *51*, 6384−6387.
(18) Leao, P. N.; Pereira, A. R.; Liu, W. T.; Ng, J.; Pevzner, P. A.; Dorrestein, P. C.; Konig, G. M.; Vasconcelosa, V. M.; Gerwick, W. H. *Proc. Natl. Acad. Sci. U.S.A.* **2010**, *107*, 11183−11188.

(19) Falch, B. S.; Konig, G. M.; Wright, A. D.; Sticher, O.; Angerhofer, C. K.; Pezzuto, J. M.; Bachmann, H. *Planta Med.* **1995**, *61*, 321−328.
(20) Eckart, K.; Schwarz, H.; Tomer, K. B.; Gross, M. L. *J. Am. Chem. Soc.* **1985**, *107*, 6765−6769.
(21) Harada, K.; Fujii, K.; Mayumi, T.; Hibino, Y.; Suzuki, M.; Ikai, Y.; Oka, H. *Tetrahedron Lett.* **1995**, *36*, 1515−1518.
(22) Fujii, K.; Ikai, Y.; Mayumi, T.; Oka, H.; Suzuki, M.; Harada, K. *Anal. Chem.* **1997**, *69*, 3346−3352.
(23) Fujii, K.; Ikai, Y.; Oka, H.; Suzuki, M.; Harada, K. *Anal. Chem.* **1997**, *69*, 5146−5151.
(24) MacMillan, J. B.; Ernst-Russell, M. A.; de Ropp, J. S.; Molinski, T. F. *J. Org. Chem.* **2002**, *67*, 8210−8215.
(25) Frankmolle, W. P.; Knubel, G.; Moore, R. E.; Patterson, G. M. *J. Antibiot.* **1992**, *45*, 1458−1466.
(26) Bonnard, I.; Rolland, M.; Francisco, C.; Banaigs, B. *Lett. Pept. Sci.* **1997**, *4*, 289−292.
(27) Kang, H. S.; Krunic, A.; Orjala, J. *J. Nat. Prod.* **2012**, *75*, 807−811.
(28) Shendage, D. M.; Frohlich, R.; Haufe, G. *Org. Lett.* **2004**, *6*, 3675−3678.
(29) Malkov, A. V.; Vrankova, K.; Cerny, M.; Kocovsky, P. *J. Org. Chem.* **2009**, *74*, 8425−8427.
(30) Frankmolle, W. P.; Larsen, L. K.; Caplan, F. R.; Patterson, G. M. L.; Knubel, G.; Levine, I. A.; Moore, R. E. *J. Antibiot.* **1992**, *45*, 1451−1457.
(31) Stein-Taylor, J. R. *Handbook of Phycological Methods: Culture Methods and Growth Measurements*. University Press, 1980.
(32) Komárek, J.; Komárková, J.; Kling, H., 4 - Filamentous Cyanobacteria. In *Freshwater Algae of North America*; John, D. W.; Robert, G. S., Eds.; Academic Press: Burlington, 2003; pp 117−196.
(33) Nubel, U.; GarciaPichel, F.; Muyzer, G. *Appl. Environ. Microbiol.* **1997**, *63*, 3327−3332.
(34) Ayers, S.; Graf, T. N.; Adcock, A. F.; Kroll, D. J.; Matthew, S.; Carcache de Blanco, E. J.; Shen, Q.; Swanson, S. M.; Wani, M. C.; Pearce, C. J.; Oberlies, N. H. *J. Nat. Prod.* **2011**, *74*, 1126−1131.