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Trichormamides C and D, Antiproliferative Cyclic Lipopeptides from the Cultured Freshwater Cyanobacterium cf. *Oscillatoria* sp. UIC 10045

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Abstract

Extract from the cultured freshwater cf. *Oscillatoria* sp. UIC 10045 showed antiproliferative activity against HT-29 cell line. Bioassay-guided fractionation led to the isolation of two new cyclic lipopeptides, named trichormamides C (**1**) and D (**2**). The planar structures were determined by combined analyses of HRESIMS, Q-TOF ESIMS/MS, and 1D and 2D NMR spectra. The absolute configurations of the amino acid residues were assigned by the advanced Marfey's analysis after partial and complete acid hydrolysis. Trichormamides C (**1**) is a cyclic undecapeptide and D (**2**) is a cyclic dodecapeptide, both containing a lipophilic β -aminodecanoic acid residue. Trichormamide C (**1**) displayed antiproliferative activities against HT-29 and MDA-MB-435 cancer cell lines with IC₅₀ values of 1.7 and 1.0 μ M, respectively, as well as anti-*Mycobacterium tuberculosis* activity with MIC value of 23.8 μ g/mL (17.3 μ M). Trichormamide D (**2**) was found to be less potent against both HT-29 and MDA-MB-435 cancer cell lines with IC₅₀ values of 11.5 and 11.7 μ M, respectively.

Graphical Abstract

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Supplementary data

Supplementary data (morphological and phylogenetic analysis of cf. *Oscillatoria* sp. UIC 10045; ¹H NMR, DEPTQ, COSY, TOCSY, HSQC, HMBC and ROESY spectra of trichormamides C (**1**) and D (**2**); the advanced Marfey's analysis of **1** and **2**; partial hydrolysis of **2**; Q-TOF ESIMS/MS spectra of **1** and **2**) associated with this article are available free of charge via the Internet.

Keywords

Cyanobacteria; *cf. Oscillatoria* sp; Cyclic lipopeptides; Antiproliferative activity; HT-29; MDA-MB-435

1. Introduction

Cyanobacteria are a rich source of novel natural products with diverse chemical scaffolds and a variety of biological activities.¹ Cyanobacteria of the genus *Oscillatoria* are particularly prolific producers of secondary metabolites. One class of secondary metabolites produced by cyanobacteria of this genus are polyketide-nonribosomal peptide (PK-NRP) hybrid macrolides, such as sanctolide A and palmyrolide A.^{2,3} However, a major class of secondary metabolites from the genus *Oscillatoria* are cyclic peptides, such as largamides, venturamides, and viridamides.^{4–6} Most of these cyclic peptides contain non-standard amino acid residues, and many were obtained from strains found in marine environments. These peptides exhibit a broad spectrum of biological activities, including protease inhibition, anti-parasitic, and antiproliferative activities.

In our continued search for biologically active natural products from cultured freshwater cyanobacteria, we found the extract of *cf. Oscillatoria* sp. UIC 10045 to possess antiproliferative activity against the human colon cancer cell line HT-29. Taxonomic identification, using morphological characterization and 16S rDNA gene sequencing, identified this strain to be a *cf. Oscillatoria* sp. (Supporting Information S1-S3). Herein we report the isolation, structure determination, and biological evaluation of two new cyclic lipopeptides, named trichormamides C (**1**) and D (**2**). They are structurally related to the trichormamides A and B, previously reported from the cultured freshwater cyanobacterium *Trichormus* sp. UIC 10339.⁷

2. Results and Discussion

The strain *cf. Oscillatoria* sp. UIC 10045 was obtained from a sample collected in Downers Grove, Illinois, in 2007, and cultured in Z media.⁸ The cell mass was harvested after eight weeks, freeze-dried, and extracted. The resulting extract was fractionated using Diaion HP-20ss resin with an increasing amount of isopropyl alcohol (IPA) in H₂O.⁹ The fraction eluting at 60% aqueous IPA exhibited antiproliferative activity against HT-29 cell line. LC-MS and ¹H NMR dereplication of the active fraction indicated the presence of two potentially new peptides with molecular weights of 1379 and 1257 Da. This fraction was subjected to reversed-phase HPLC to yield trichormamides C (**1**) and D (**2**).

Trichormamide C (**1**) was obtained as a white, amorphous powder. The molecular formula was determined as $C_{65}H_{114}N_{14}O_{18}$ by HRESIMS analysis (m/z 1379.8559 $[M + H]^+$). The signal distribution pattern observed in the 1H NMR spectrum indicated it to be a lipopeptide, with amide NH (δ_H 7.4 – 8.4), amino acid α -H (δ_H 4.0 – 4.7), highly overlapped aliphatic CH_2 (δ_H 1.1 – 1.3), and a group of doublet and triplet CH_3 signals (δ_H 0.7 – 1.1). The presence of 12 amino acid residues was determined by 2D NMR analysis. Analyses of the COSY and TOCSY spectra established the structures of eight standard amino acid residues: valine (Val), alanine (Ala), glutamine (Gln), asparagine (Asn), proline (Pro), leucine (Leu) and two threonines (Thr1, Thr2) (Figure 1 and Table 1). The presence of a 3-hydroxyleucine (3-OHLeu1) was deduced by a COSY correlation between the hydroxyl signal (δ_H 5.00) and the downfield methine H-3 (δ_H 3.47), the sequential COSY correlations between NH (δ_H 8.04)/ H-2 (δ_H 4.39)/ H-3 (δ_H 3.47)/ H-4 (δ_H 1.55)/ H₃-5 (δ_H 0.87), as well as the HMBC correlations from H₃-4-Me (δ_H 0.75) and H₃-5 (δ_H 0.87) to both C-3 (δ_C 76.8) and C-4 (δ_C 30.8). A second 3-hydroxyleucine (3-OHLeu2) was determined in a similar fashion (Table 1). The presence of an *N*-methylated isoleucine (NMeIle) was evident by the COSY and TOCSY correlations between H-2 (δ_H 4.68)/ H-3 (δ_H 1.89)/ H₃-3-Me (δ_H 0.75)/ H₂-4 (δ_H 1.27)/ H₃-5 (δ_H 0.76), and the HMBC correlation from H₃-N-Me (δ_H 3.00) to C-2 (δ_C 59.6). The lipophilic β -amino acid residue was identified as β -aminodecanoic acid (Ada) by the sequential COSY correlations between NH (δ_H 7.60)/ H-3 (δ_H 4.11)/ H₂-2 (δ_H 2.48; 2.51), and the COSY correlations between H-3 (δ_H 4.11) and H₂-4 (1.21; 1.33), combined with the sequential COSY correlations from H₂-4 (1.21; 1.33) to the largely overlapped CH_2 signals (H₂-5 – 9, δ_H 1.17 – 1.21), which correlate with five carbon signals in the HSQC spectrum (δ_C 22.1, 25.3, 28.6, 28.8 and 31.3), and finally to the CH_3 triplet H₃-10 (δ_H 0.87).

The amino acid sequence was assigned by analyses of the HMBC, selective HMBC, ROESY and ESIMS/MS spectra (Figures 1, 2 and Table 1). The selective HMBC spectrum was acquired to better resolve the carbonyl region (δ_C 166 – 176).¹⁰ The established 12 amino acid residues and 16 degrees of unsaturation deduced by the molecular formula suggested that **1** was a cyclic peptide. The HMBC correlations from Thr1-NH (δ_H 7.56) and Thr1 H-2 (δ_H 4.45) to Asn C-1 (δ_C 170.5), from Asn-NH (δ_H 8.34) to NMeIle C-1 (δ_C 169.8), from NMeIle *N*-Me (δ_H 3.00) and NMeIle H-2 (δ_H 4.68) to Gln C-1 (δ_C 172.4), and from Gln H-2 (δ_H 4.57) to 3-OHLeu2 C-1 (δ_C 170.8) suggested a partial sequence of Thr1-Asn-NMeIle-Gln-3-OHLeu2. This partial sequence was confirmed by ROESY correlations illustrated in Figure 1. Similarly, HMBC correlations from 3-OHLeu2-NH (δ_H 7.76) to Ala C-1 (δ_C 172.5), from Ala H-2 (δ_H 4.20) to 3-OHLeu1 C-1 (δ_C 171.5), from 3-OHLeu1-NH (δ_H 8.04) and 3-OHLeu1 H-2 (δ_H 4.39) to Val C-1 (δ_C 171.5), from Val-NH (δ_H 8.31) to Ada C-1 (δ_C 171.5), from Ada-NH (δ_H 7.60) to Thr2 C-1 (δ_C 168.7), from Thr2 H-2 (δ_H 4.03) to Leu C-1 (δ_H 172.0), and from Leu H-2 (δ_H 4.32) to Pro C-1 (δ_H 171.5) extended the partial sequence into a linear sequence Thr1-Asn-NMeIle-Gln-3-OHLeu2-Ala-3-OHLeu1-Val-Ada-Thr2-Leu-Pro confirmed by ROESY correlations, see Figure 1. Finally, ROESY correlations between Thr1 H-2 (δ_H 4.45) and Pro H₂-5 (δ_H 3.61; 3.66) closed the macrocycle and established the complete sequence as cyclo[Thr1-Asn-NMeIle-Gln-3-OHLeu2-Ala-3-OHLeu1-Val-Ada-Thr2-Leu-Pro]. In order to confirm the amino acid sequence determined by NMR experiments, a mass fragmentation analysis using a quadrupole-time-of-flight (Q-TOF) mass spectrometer was performed. The molecular ion $[M + H]^+$ 1379.9 was

fragmented and resulted in series of daughter ions illustrated in Figure 2. These ions were consistent with the amino acid sequence established by NMR analysis.

The absolute configurations of amino acid residues in **1** were determined by the advanced Marfey's method after acid hydrolysis.^{11,12} LC-MS comparison between L-FDLA and DL-FDLA derivatives of the acid hydrolysate of **1** assigned the L configurations for Val, Ala, Gln, Asn, Pro, Leu, and 3R configuration for Ada.¹³ The L-FDLA derivative of the acid hydrolysate of **1** was also compared with FDLA derivatives of authentic standards of Thr (L-Thr-L-FDLA, D-Thr-L-FDLA, L-allo-Thr-L-FDLA and D-allo-Thr-L-FDLA), NMeIle (L-NMeIle-L-FDLA, D-NMeIle-L-FDLA, L-allo-NMeIle-L-FDLA and D-allo-NMeIle-L-FDLA), 3-OHLeu ((2S, 3R)-3-OHLeu-L-FDLA, (2S, 3R)-3-OHLeu-D-FDLA, (2S, 3S)-3-OHLeu-L-FDLA and (2S, 3S)-3-OHLeu-D-FDLA). The stereoisomers of NMeIle and 3-OHLeu were synthesized as previously described.⁷ This allowed for assignment of L configurations for Thr1/2 and NMeIle, and 2R, 3S configurations for 3-OHLeu1/2. Therefore, the complete structure of trichormamide C (**1**) was determined as cyclo[L-Thr-D-Leu-L-Pro-L-Thr-D-Asn-L-NMeIle-L-Gln-(2R,3S)-3-OHLeu-L-Ala-(2R,3S)-3-OHLeu-L-Val-(3R)-Ada].

Trichormamide D (**2**) was obtained as a white, amorphous powder. The molecular formula of **2** was determined as C₆₄H₉₆N₁₂O₁₄ based on HRESIMS analysis (*m/z* 1257.7324 [M + H]⁺). Due to the broad NMR signals observed in DMSO-*d*₆, 1D and 2D NMR experiments of **2** were conducted using CD₃OH with signal suppression at 4.9 ppm. The ¹H NMR, COSY and TOCSY spectra of **2** were also acquired in CD₃OD to obtain proton coupling constants and COSY/TOCSY correlations for signals close to 4.9 ppm. The ¹H NMR spectrum of **2** exhibited a signal pattern characteristic of a lipopeptide. Two downfield doublets (δ_{H} 6.69, *J* = 8.3 Hz; δ_{H} 7.20, *J* = 8.3 Hz) indicated the presence of a *para*-substituted phenyl moiety. A group of overlapped aromatic signals (δ_{H} 7.28 – 7.30) integrated to five protons and correlated with three carbon signals (δ_{C} 128.1, 129.7 and 130.1) in the HSQC spectrum. These signals were attributed to a phenyl moiety. Analyses of the COSY and TOCSY spectra established the structures of nine standard amino acid residues: glutamine (Gln), proline (Pro), serine (Ser), tyrosine (Tyr), valine (Val), phenylalanine (Phe), glycine (Gly) and two leucines (Leu1/2) (Figure 1 and Table 2). The presence of a β -aminodecanoic acid (Ada) was deduced by the COSY, TOCSY and HSQC correlations similar as described for **1**. The presence of a 2,3-dehydro-2-aminobutyric acid (Dhb) was evident by the COSY correlation between H₃-4 (δ_{H} 1.82, d, *J* = 7.2 Hz) and the olefinic H-3 (δ_{H} 5.72, q, *J* = 7.2 Hz), the HMBC correlations from H₃-4 to both the olefinic C-3 (δ_{C} 122.5) and the quaternary C-2 (δ_{C} 131.9), as well as the HMBC correlations from NH (δ_{H} 10.79) to both C-1 (δ_{C} 168.8) and C-3 (δ_{C} 122.5).

The sequence of the 11 amino acid residues was established by analyses of the HMBC and ROESY correlations, and confirmed by Q-TOF ESIMS/MS fragmentation analysis (Figures 1, 3 and Table 2). HMBC correlations from Dhb-NH (δ_{H} 10.79) to Gln C-1 (δ_{C} 174.6), from Gln-NH (δ_{H} 7.14) to Ada C-1 (δ_{C} 172.0), from Ada-NH (δ_{H} 7.43) to Gly C-1 (δ_{C} 170.3), from Gly-NH (δ_{H} 9.01) and H₂-2 (δ_{H} 3.43; 4.09) to Leu2 C-1 (δ_{C} 175.7), from Leu2-NH (δ_{H} 8.57) to Phe C-1 (δ_{C} 174.6), from Phe-NH (δ_{H} 8.64) to Val C-1 (δ_{C} 173.8), from Val-NH (δ_{H} 6.63) to Leu1 C-1 (δ_{C} 174.1), from Leu1-NH (δ_{H} 7.30) to Tyr C-1 (δ_{C} 175.3), from Tyr-NH (δ_{H} 8.06) to Ser C-1 (δ_{C} 172.3), and from Ser-NH (δ_{H} 7.48) to Pro C-1 (δ_{C} 173.7).

indicated a linear sequence of Dhb-Gln-Ada-Gly-Leu2-Phe-Val-Leu1-Tyr-Ser-Pro. ROESY correlations demonstrated in Figure 1 confirmed this linear sequence. The planar structures of 11 amino acid residues and 23 degrees of unsaturation determined by the molecular formula indicated that **2** was a cyclic peptide. The ROESY correlations between Dhb H₃-4 (δ_{H} 1.82) and Pro H₂-4 (δ_{H} 1.96; 2.09) and H₂-5 (δ_{H} 3.63; 3.71) closed the ring and established the planar structure of **2** as cyclo[Dhb-Gln-Ada-Gly-Leu2-Phe-Val-Leu1-Tyr-Ser-Pro]. ESIMS/MS fragmentation analysis was performed to confirm the sequence assignment of **2**. The observed fragments, as shown in Figure 3, were in complete agreement with the amino acid sequence determined by NMR analysis.

The geometric configuration of Dhb was determined to be *E* based on the ROESY correlations between Dhb-NH (δ_{H} 10.79) and Dhb H-3 (δ_{H} 5.72), as well as the ROESY correlations between Dhb H₃-4 (δ_{H} 1.82) and Pro H₂-4 (δ_{H} 1.96; 2.09) and H₂-5 (δ_{H} 3.63; 3.71). Acid hydrolysis followed by the advanced Marfey's method was performed to assign the absolute configurations of amino acid residues in **2**. The residues of Gln, Pro, Ser, Val were determined as *L*, the residues of Tyr and Phe were assigned as *D*, and the β -amino acid residue Ada was determined as 3*R*. However, both *L*-leucine and *D*-leucine were observed in the hydrolysate, indicating that the two leucine residues in **2** had opposite configurations. A partial hydrolysis scheme was utilized to unambiguously assign the absolute configurations of the two leucine residues (Supporting Information S26). Trichormamide D (**2**) was hydrolyzed with 20% trifluoroacetic acid (TFA) under 110 °C for 4 hours, and subjected to HPLC analysis. A fraction containing a tetrapeptide fragment (Figure 4) was subjected to the advanced Marfey's analysis after complete acid hydrolysis. The fragment illustrated in Figure 4 contained only one leucine residue, which was assigned *D* configuration according to the advanced Marfey's analysis. Accordingly, the other leucine residue not contained in this fragment was assigned *L* configuration. Therefore, the complete structure of trichormamide D (**2**) was determined as cyclo[*E*-Dhb-*L*-Gln-(3*R*)-Ada-Gly-*L*-Leu-*D*-Phe-*L*-Val-*D*-Leu-*D*-Tyr-*L*-Ser-*L*-Pro].

Trichormamides are structurally related to laxaphycins, a series of cyclic lipopeptides initially obtained by Moore's group from the terrestrial cyanobacteria *Anabaena laxa*.¹⁴ Since the first report of laxaphycins, a variety of structurally similar compounds have been isolated from multiple sources of cyanobacteria (Table 3 and Supporting Information S27). This group of compounds can be divided into two subgroups based on their structural characteristics. Compounds in the Laxa A subgroup contain 11 amino acid residues and are characterized by the presence of a lipophilic β -amino acid residue (Ada or β -aminooctanoic acid (Aoa)). Compounds belonging to the Laxa B subgroup have 12 amino acid residues and also contain a lipophilic β -amino acid residue (Ada or Aoa). The amino acid sequence of compounds in the Laxa A subgroup can be summarized as follows: lipophilic β -amino acid residue, polar amino acid, Dhb or Ser, Pro or hydrolylated Pro, polar amino acid, aromatic amino acid, Leu followed by three nonpolar amino acid residues, and Gly (Figure 5). The sequence of peptides belonging to the Laxa B subgroup can be described as below: lipophilic β -amino acid residue, nonpolar residue, 3-OHLeu, Ala or Hse, Leu or hydroxylated Leu, Gln, *N*MeIle, polar residue, Thr, Pro or hydroxylated Pro, nonpolar residue, and Thr (Figure 5). These structural characteristics of laxaphycins and related

compounds revealed that the hydrophilicity or lipophilicity of amino acid residues at each position was strongly conserved, and that compounds in the Laxa A and B subgroups are both amphiphilic. This amphiphilic character of laxaphycins and related compounds may be associated with the cell membrane interacting activity commonly observed for lipopeptides.^{15–17}

The freshwater cyanobacterium strain UIC 10045 was identified as cf. *Oscillatoria* sp. based on morphological observation and phylogenetic analysis using a partial 16S rDNA gene sequence (1.2 Kb) (Supporting Information S3). A comparison of UIC 10045 to strains that produce structurally related peptides revealed a high degree of phylogenetic and geographic diversity. For example, hormothamnin A was obtained from the tropical marine species *Hormothamnion enteromorphoides*;¹⁸ laxaphycins were isolated from a marine species *Anabaena torulosa* and a terrestrial species *Anabaena laxa*;^{14,19,20} trichormamides A and B were isolated from a freshwater strain *Trichormus* sp.⁷ These strains all belong to the order of *Nostocales*. In contrast, lobocyclamides were isolated from a marine *Lyngbya confervoides*,¹³ and lyngbyacyclamides were isolated from a marine *Lyngbya* sp.²¹ These strains as well as UIC 10045 belong to the order of *Oscillatoriales*. The presence of these structurally related secondary metabolites in both orders *Nostocales* and *Oscillatoriales*, as well as their broad distributions among marine, terrestrial and freshwater environments, hints a possible important ecological function of this class of compounds.

Trichormamides C (1) and D (2) were evaluated for their antiproliferative activities in the human colon cancer cell line HT-29 and the human melanoma cell line MDA-MB-435. Trichormamide C (2) exhibited the similar level of antiproliferative activities as compared to structurally related trichormamide B, laxaphycins B, B2, B3, and lyngbyacyclamides A and B with IC₅₀ values of 1.7 and 1.0 μ M, respectively. A comparison of structural similarities between trichormamide C and structurally related compounds revealed that the amino acid residues of Ada, 3-OHLeu1, Gln, NMeIle and Thr1/2 may play a key role for their antiproliferative activities. Trichormamide D (2) exhibited the similar level of activities as compared to structurally related trichormamide A, with IC₅₀ values of 11.5 and 11.7 μ M, respectively. Trichormamides C (1) and D (2) were also evaluated for antibacterial activities against *Mycobacterium tuberculosis*, *Mycobacterium smegmatis*, *Staphylococcus aureus*, *Escherichia coli*, and antifungal activity against *Candida albicans*. Trichormamide C (1), showed moderate anti-*M. tuberculosis* activity with MIC value of 23.8 μ g/mL. No other activity was observed at the highest concentration tested (50 μ g/mL) for both Trichormamide C and D. Structurally related compounds, such as laxaphycins and lobocyclamides, have been reported to display synergistic activities against bacteria or cancer cell lines.^{13,19,20,22} However, no synergistic antiproliferative activities between trichormamides C (1) and D (2) were observed in either the HT-29 or MDA-MB-435 cancer cell lines. The limited supply of trichormamide D prevented us from acquiring statistically significant data in synergistic antimicrobial assays.

3. Conclusion

In summary, the assay guided chemical investigation of the cultured freshwater cyanobacterium cf. *Oscillatoria* sp. UIC 10045 led to the isolation of two new cyclic

lipopeptides, trichormamides C (**1**) and D (**2**). Taxonomical identification of UIC 10045 was achieved by a combination of morphological observation and phylogenetic analysis using the partial 16S rDNA gene sequence. The planar structures of **1** and **2** were elucidated by analyses of the HRESIMS, Q-TOF ESIMS/MS, as well as 1D and 2D NMR experiments. Stereoconfigurations of the amino acid residues were determined by the advanced Marfey's method incorporating a partial hydrolysis protocol and using the synthesized amino acid standards. Trichormamide C (**1**) is a cyclic lipododecapeptide, exhibiting antiproliferative activities against HT-29 and MDA-MB-435 human cancer cell lines with IC₅₀ values of 1.7 and 1.0 μ M, respectively, and anti-*M. tuberculosis* activity with MIC value of 23.8 μ g/mL (17.3 μ M). Trichormamide D (**2**) is a cyclic lipoundecapeptide and exhibits lower antiproliferative activities against both HT-29 and MDA-MB-435 cell lines as compared to **1**, with IC₅₀ values of 11.5 and 11.7 μ M, respectively.

4. Experimental section

4.1 General Experimental Procedures

Optical rotations were measured on a Perkin-Elmer 241 polarimeter. UV spectra were obtained on a Shimadzu UV spectrophotometer UV-1700. IR spectra were recorded on a Perkin-Elmer 577 IR spectrophotometer. 1D and 2D NMR spectra were acquired on a Bruker Avance DRX 600 MHz NMR spectrometer with a 5 mm CPTXI Z-gradient probe and a Bruker Avance II 900 MHz NMR spectrometer with a 5 mm ATM CPTCI Z-gradient probe. ¹H and ¹³C NMR chemical shifts were referenced to the DMSO-*d*₆ solvent signals (δ_{H} 2.50 and δ_{C} 39.51, respectively) and CD₃OD/CD₃OH solvent signals (δ_{H} 3.31 and δ_{C} 49.0, respectively). A mixing time of 60 ms was used for the TOCSY experiments and 200 ms for the ROESY experiment. The HMBC spectra were acquired with the ¹J_{CH} of 8 Hz, and the HSQC spectra were recorded with the ¹J_{CH} of 145 Hz. High-resolution ESI mass spectra and LC-MS data were obtained using a Shimadzu HPLC-IT-TOF spectrometer. Tandem mass analysis was conducted using a Waters Q-TOF mass spectrometer. The HPLC separations were performed using an Agilent 1100 series instrument with a diode array detector and a Waters instrument with Waters Delta 600 pumps and a Waters 2487 UV detector.

4.2 Biological Material

The unialgal strain cf. *Oscillatoria* sp. UIC 10045 was isolated from a sample collected in Downers Grove, Illinois, in 2007 (N 41°48'41", W 88°00'44") using micropipette isolation techniques.²³ The unialgal strain was cultured in 4 × 2 L of Z medium in 2.8 L Fernbach flasks with sterile aeration.⁸ Cultures were illuminated with fluorescent lamps at 1.03 klx with an 18/6 h light/dark cycle. Temperature of the culture room was maintained at 22 °C. After 8 weeks of growth, the biomass of UIC 10045 was harvested by centrifugation and lyophilized.

4.3 Taxonomic Identification

The initial taxonomic identification was performed by morphological characterization using a cultured UIC 10045 cyanobacterium. A Zeiss Axiostar Plus light microscope equipped with a Canon PowerShot A620 camera was used for the morphological observation. The

initial taxonomic designation of UIC 10045 was made according to the system by Komarek et al (Supporting Information S2).²⁴ Phylogenetic analysis was also performed to facilitate the taxonomic identification of UIC 10045. The genomic DNA of UIC 10045 was extracted, and a partial sequence of the 16S rDNA gene was PCR-amplified using established protocols.²⁵ The partial 16S rDNA gene sequence was deposited in the NCBI GenBank under the accession no. KF444211. Details of the phylogenetic analysis of UIC 10045 can be found in Supporting Information S3.

4.4 Extraction and Isolation

Lyophilized biomass (6.45 g) from 4×2 L cultures of UIC 10045 was extracted with CH_2Cl_2 - MeOH (1:1) and concentrated *in vacuo* to yield 1.09 g of crude extract. The extract was fractionated using Diaion HP-20SS resin with a gradient of IPA in H_2O to generate eight fractions (0, 20, 40, 60, 70, 80, 90, 100% aqueous IPA). The fraction eluting with 60% aqueous IPA (21.83 mg) exhibited significant antiproliferative activity against the MDA-MB-435 human melanoma cell line with 100% growth inhibition at $25 \mu\text{g/mL}$. Dereplication using LC-MS and ^1H NMR revealed the presence of two potentially new lipopeptides. Separation of these two new peptides was achieved using a semipreparative reversed-phase HPLC (Varian C_8 semipreparative column, 10×250 mm, 3 mL/min) with a linear gradient from 70% to 75% MeOH with H_2O over 30 min. Trichormamide C (**1**, 2.44 mg, 0.04% of dry weight) was eluted at 26.5 min, and trichormamide D (**2**, 1.32 mg, 0.02% of dry weight) was eluted at 29 min.

4.4.1 Trichormamide C (1)—White, amorphous powder; $[\alpha]_{\text{D}}^{22} -19.1$ (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ϵ) 243 (3.12) nm; IR (neat) ν_{max} 3304 (br), 2937, 2871, 2360, 2179, 1772, 1662, 1558, 1512, 1456, 1386, 1346, 1241, 1118 cm^{-1} ; ^1H and ^{13}C NMR see Table 1; HR-ESI-TOF-MS (+) m/z 1379.8559 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{65}\text{H}_{115}\text{N}_{14}\text{O}_{18}$, 1379.8514)

4.4.2 Trichormamide D (2)—White, amorphous powder; $[\alpha]_{\text{D}}^{22} -27.5$ (*c* 0.16, MeOH); UV (MeOH) λ_{max} (log ϵ) 240 (3.58), 278 (3.02) nm; IR (neat) ν_{max} 3326 (br), 2955, 2361, 2325, 2178, 2061, 2014, 1654, 1540, 1517, 1239, 1129 cm^{-1} ; ^1H and ^{13}C NMR see Table 2; HR-ESI-TOF-MS (+) m/z 1257.7324 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{64}\text{H}_{97}\text{N}_{12}\text{O}_{14}$, 1257.7274).

4.5 Advanced Marfey's Analysis

Approximately 0.3 mg of **1** and **2** was each hydrolyzed using 6 N HCl (1 mL) in separate Teflon-sealed pressure tubes for 20 h at 110 °C. The hydrolysate was dried *in vacuo* after cooling to room temperature, and resuspended in H_2O . This process was repeated three times to remove the remaining HCl. Each hydrolysate was separated into two equal portions for derivatization with either *L*- or *DL*-FDLA (1-fluoro-2,4-dinitrophenyl-5-leucinamide) using established protocols.⁷ LC-MS analysis was performed 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% CH_3CN in H_2O with 0.1% formic acid over 50 min. The absolute configurations of amino acid residues were assigned based on the retention times of *L*-FDLA and *DL*-FDLA derivatives of each amino acid in the extracted ion chromatograms (EIC). The absolute configurations of Gln and Asn residues were assigned based on the retention times

of Marfey's derivatives of glutamic acid (Glu) and aspartic acid (Asp) in the EIC. The retention times of each amino acid can be found in Supporting Information S13 and S25.

4.6 Partial Hydrolysis of Trichormamide D (2)

Approximately 1 mg of **2** was hydrolyzed using 500 μ L of 20% aqueous TFA for 4 hr at 110 $^{\circ}$ C and dried *in vacuo* after cooling. Traces of TFA were removed from the hydrolysate by repeated evaporation. The dry hydrolysate was redissolved in MeOH and analyzed by LC-MS. A linear tetrapeptide fragment Tyr-Leu-Val-Phe (Figure 4) was identified from the LC-MS data. The tetrapeptide was isolated from the hydrolysate using reversed-phase HPLC (Phenomenex Kinetex C₁₈, 250 $\times$ 4.6 mm, 5 μ m, 1.0 mL/min) with a linear gradient from 5% to 100% aqueous CH₃CN with 0.1% formic acid over 30 min. The structure of the tetrapeptide was confirmed by HRESIMS (+) m/z 541.3071 [M + H]⁺ (calcd for C₂₉H₄₁N₄O₆, 541.3026), Q-TOF ESIMS/MS, and the presence of Marfey's derivatives of all four expected amino acids (Supporting Information S26). This tetrapeptide was subjected to the advanced Marfey's analysis, and the absolute configuration of the leucine residue contained in this tetrapeptide was unambiguously assigned as D.

4.7 Antiproliferative Assay

Human colon cancer cell line HT-29 and human melanoma cell line MDA-MB-435 were purchased from American Type Culture Collection (ATCC). The antiproliferative assays against the HT-29 and MDA-MB-435 cancer cell lines were performed according to established protocols.²⁶

4.8 Antimicrobial and Antifungal Assays

The antimicrobial assays against *Mycobacterium tuberculosis*, *Mycobacterium smegmatis*, *Staphylococcus aureus*, *Escherichia coli*, and the antifungal assay against *Candida albicans* were conducted according to established protocols.²⁵

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Key COSY/TOCSY, HMBC and ROESY correlations of trichormamide C (**1**) and trichormamide D (**2**).

Figure 2.
Q-TOF MS/MS fragmentation of trichormamide C (**1**).

Figure 3.
Q-TOF MS/MS fragmentation of trichormamide D (**2**).

Figure 4.
Structure of the tetrapeptide fragment isolated from the partial hydrolysate of trichormamide D (2).

Figure 5.

Structure variance of laxaphycins and related compounds. The prevalence of amino acid residues is proportional to the size of font. (Ada: β -aminodecanoic acid; Aoa: β -aminooctanoic acid; Hse: homoserine; Dhb: 2,3-dehydro-2-aminobutyric acid; 4-OHPro: 4-hydroxyproline; 3-OHLeu: 3-hydroxyleucine; NMelle: *N*-methylisoleucine; 3-OHAsn: 3-hydroxyasparagine)

Table 1

NMR Spectroscopic Data of Trichormamide C (1) in DMSO- d_6

	position	δ_C^a , mult	δ_H^b , mult. (J in Hz)	COSY	HMBC	ROESY
Ada	1	171.5, C				
	2	39.9, CH ₂	2.48, m	3		3
			2.51, m			
	3	45.7, CH	4.11, m	2, 4, NH		2, 4, NH
	4	33.5, CH ₂	1.21, m	3		3
			1.33, m			
	5	25.3, ϵ CH ₂	1.21, m	overlapped		
	6	28.8, ϵ CH ₂	1.18, m	overlapped		
	7	28.6, ϵ CH ₂	1.17, m	overlapped		
	8	31.3, ϵ CH ₂	1.17, m	overlapped		
Val	9	22.1, CH ₂	1.17, m	10		
	10	13.9, CH ₃	0.87, overlapped	9	9	
	NH		7.60, d (8.0)	3	1 _{Thr2}	3, 2 _{Thr2}
	1	171.5, C				
	2	58.6, CH	4.16, t (7.2)	3, NH	1, 3	3, NH ₃ -OHLeu1
	3	29.6, CH	1.99, m	2, 3-Me, 4		2, 3-Me, 4
	3-Me	19.0, CH ₃	0.83, overlapped	3		3
	4	19.0, CH ₃	0.83, overlapped	3		3
	NH		8.31, d (7.2)	2	1 _{Ada}	
	3-OHLeu1					
3-OHLeu1	1	171.5, C				
	2	55.1, CH	4.39, d (8.7)	3, NH	1, 3, 1 _{Val} ^d	4-Me, NH _{Ala}
	3	76.8, CH	3.47, m	2, 4, 3-OH	4-Me, 5	4-Me, 5
	4	30.8, CH	1.55, m	3, 4-Me, 5	3, 4-Me, 5	
	4-Me	18.6, CH ₃	0.75, overlapped	4	3, 4	2, 3
	5	18.8, CH ₃	0.87, overlapped	4	3, 4	3

position	δ_C^a , mult	δ_H^b , mult. (J in Hz)	COSY	HMBC	ROESY
3-OH		5.00, d (6.5)	3		
NH		8.04, d (8.7)	2	1 _{Val}	2 _{Val}
Ala	1	172.5, C			
	2	49.4, CH	3, NH	1, 3, 1 _{3-OHLeu1} ^d	3, NH _{3-OHLeu2}
	3	17.8, CH ₃	2	1, 2	2
NH		8.00, br	2		2 _{3-OHLeu1}
3-OHLeu2	1	170.8, C			
	2	55.5, CH	3, NH	1, 3	4-Me, NH _{Gln}
	3	76.0, CH	2, 4, 3-OH	4-Me, 5	4-Me, 5
	4	30.0, CH	3, 4-Me, 5	3, 4-Me, 5	
	4-Me	18.9, CH ₃	4	3, 4	2, 3
5	18.9, CH ₃	0.89, overlapped	4	3, 4	3
3-OH		5.11, br	3		
NH		7.76, br	2	1 _{Ala} ^d	2 _{Ala}
Gln	1	172.4, C			
	2	48.9, CH	3, NH	1, 4, 1 _{3-OHLeu2} ^d	N-Me _{NMele}
	3	26.4, CH ₂	2, 4		
	4	30.5, CH ₂	3	2, 3, 5	
	5	174.5, C			
NH		7.75, br	2		2 _{3-OHLeu2}
NH ₂		6.98, s			
		7.41, s			
NMele	1	169.8, C			
	2	59.6, CH	3	1 _{Gln} ^d	3, NH _{Asn}
	3	32.0, CH	2, 3-Me, 4	2	2
	3-Me	15.2, CH ₃	0.75, overlapped	3	

	position	δ_C^a , mult	δ_H^b , mult. (<i>J</i> in Hz)	COSY	HMBC	ROESY
	4	23.9, CH ₂	1.27, m	3, 5		
	5	10.3, CH ₃	0.76, overlapped	4		
	N-Me	30.2, CH ₃	3.00, s		2, 1 _{Gln}	2 _{Gln}
	Asn					
	1	170.5, C				
	2	49.5, CH	4.66, q (7.4)	3, NH	1	NH _{Thr1}
	3	35.8, CH ₂	2.40, m	2	2, 4	
			2.51, m			
	4	171.6, C				
	NH		8.34, d (7.4)	2	1 _{NMele}	2 _{NMele}
	NH ₂		6.90, s			
			7.30, s			
Thr1	1	168.5, C				
	2	55.5, CH	4.45, t (7.2)	3, NH	1, 3, 1 _{Asn} ^d	4, 5 _{Pro}
	3	66.7, CH	3.86, m	2, 3-OH, 4		4
	4	18.9, CH ₃	1.05, d (6.2)	3	2, 3	2, 3
3-OH			5.04, br	3		
	NH		7.56, d (7.7)	2	1 _{Asn}	2 _{Asn}
Pro	1	171.5, C				
	2	59.6, CH	4.30, m	3	1, 4	3, 4, NH _{Leu}
	3	29.2, CH ₂	1.78, m	2, 4		2, 5
	4	24.3, CH ₂	2.02, m	3, 5		2
Leu	5	47.2, CH ₂	1.88, m			
			1.91, m			
			3.61, m	4		3, 2 _{Thr1}
			3.66, m			
Leu	1	172.0, C				
	2	51.6, CH	4.32, m	3, NH	1, 3, 1 _{Pro} ^d	3, NH _{Thr2}
	3	40.9, CH ₂	1.47, m	2, 4	1, 2, 4, 4-Me, 5	2
	4	24.2, CH	1.51, m	3, 4-Me, 5	3, 4-Me, 5	

position	δ_C^a , mult	δ_H^b , mult. (J in Hz)	COSY	HMBC	ROESY
4-Me	22.1, CH ₃	0.81, overlapped	4	3	
5	22.7, CH ₃	0.90, overlapped	4	3	
NH		7.91, br	2		2 _{Pro}
Thr2					
1	168.7, C				
2	58.6, CH	4.03, d (7.3)	3, NH	1, 3, 1 _{Leu} ^d	NH _{Ada}
3	66.1, CH	4.02, m	2, 3-OH, 4	4	4
4	19.8, CH ₃	0.99, d (6.0)	3	2, 3	3, 3-OH
3-OH		4.80, d (5.0)	3		4
NH		7.84, d (7.3)	2		2 _{Leu}

^a Carbon chemical shifts were assigned from DEPTQ spectrum recorded at 226 MHz.

^b Recorded at 600 MHz.

^c Carbon chemical shifts are interchangeable.

^d Assigned from the selective HMBC spectrum.

Table 2

NMR Spectroscopic Data of Trichormamide D (2) in CD₃OH

	position	δ_C^a , mult	δ_H^b , mult. (J in Hz)	COSY	HMBC	ROESY
Ada	1	172.0, C				
	2	42.0, CH ₂	1.77, m	3	1, 3	NH _{Gln}
			2.05, m			NH
	3	46.9, CH	4.46, m	2, 4, NH	2	
	4	36.3, CH ₂	1.52, m	3		NH
			1.59, m			NH
	5	30.5, ^c CH ₂	1.28, m	overlapped		
	6	30.5, ^c CH ₂	1.28, m	overlapped		
	7	27.3, ^c CH ₂	1.33, m	overlapped		
	8	33.1, CH ₂	1.33, m	overlapped		
Gln	9	23.8, CH ₂	1.31, m	10		
	10	14.5, CH ₃	0.91, t (7.1)	9	8, 9	
	NH		7.43, d (9.5)	3	3, 1 _{Gly}	2, 3, 4, 2 _{Gly}
	1	174.6, C				
	2	52.7, CH	4.55, dd (3.4, 10.0)	NH, 3	1, 3, 4	NH, NH _{Dhb}
	3	29.4, CH ₂	2.06, m	2, 4	1, 2, 4, 5	
			2.13, m			
	4	32.0, CH ₂	2.40, m	3	2, 3, 5	NH ₂
			2.45, m			
	5	177.9, C				
Dhb	NH		7.14, d (10.0)	2	2, 1 _{Ada}	2, 2 _{Ada}
	NH ₂		6.92, s		4, 5	4
			8.03, s			
	1	168.8, C				
	2	131.9, C				
	3	122.5, CH	5.72, q (7.2)	4	2, 4	4, NH

	position	δ_C^a , mult	δ_H^b , mult. (<i>J</i> in Hz)	COSY	HMBC	ROESY
Pro	4	12.9, CH ₃	1.82, d (7.2)	3	2, 3	3, 4 _{Pro} , 5 _{Pro}
	NH		10.79, s		1, 3, 1 _{Gln}	3, 2 _{Gln}
	1	173.7, C				
	2	62.7, CH	4.58, m	3	1, 3	3, NH _{Ser}
	3	30.9, CH ₂	2.04, m	2, 4		2, NH _{Ser}
			2.50, m			
	4	26.3, CH ₂	1.96, m	3, 5	3	5, 4 _{Dhb}
			2.09, m			
Ser	5	50.8, CH ₂	3.63, m	4	2, 4	4, 4 _{Dhb}
			3.71, m			
	1	172.3, C				
	2	56.3, CH	4.45, m	NH, 3	1, 3	3, NH _{Tyr}
Tyr	3	62.6, CH ₂	3.77, dd (3.7, 12.1)	2		2, NH
			3.99, dd (8.0, 12.1)			
	OH		nd ^d			
	NH		7.48, d (9.7)	2	2, 1 _{Pro}	3, 2 _{Pro} , 3 _{Pro}
	1	175.3, C				
	2	59.2, CH	4.30, m	NH, 3	1, 3, 4	5/9, NH, NH _{Leu1}
	3	37.5, CH ₂	2.96, dd (3.0, 13.7)	2	1, 2, 4, 5/9	5/9, NH, NH _{Leu1}
			3.08, dd (11.3, 13.7)			
	4	129.6, C				
	5/9	131.3, CH	7.20, d (8.3)	6/8	3, 4, 6/8, 7	2, 3, NH
	6/8	116.3, CH	6.69, d (8.3)	5/9	4, 5/9, 7	
	7	157.3, C				
Leu1	OH		nd ^d			
	NH		8.06, d (7.2)	2	2, 3, 1 _{Ser}	2, 3, 5/9, 2 _{Ser}
	1	174.1, C				
	2	53.3, CH	4.55, m	NH, 3	1, 3	3, 5
	3	40.3, CH ₂	1.11, m	2, 4	2, 4, 4-Me, 5	2

	position	δ_C^a , mult	δ_H^b , mult. (<i>J</i> in Hz)	COSY	HMBC	ROESY
Val			1.40, m			
	4	25.7, CH	1.59, m	3, 4-Me, 5	2, 3, 4-Me, 5	
	4-Me	23.8, CH ₃	0.85, d (6.5)	4	3, 4, 5	
	5	20.8, CH ₃	0.79, d (6.5)	4	3, 4, 4-Me	2
	NH		7.30, overlapped	2	2, 3, 1-Tyr	2-Tyr, 3-Tyr
Phe	1	173.8, C				
	2	57.8, CH	4.58, overlapped	NH, 3	1, 3, 3-Me	3, NH, NH _{Leu2}
	3	34.2, CH	1.77, m	2, 3-Me, 4		2, 3-Me, 4, NH _{Phe}
	3-Me	15.9, CH ₃	0.11, d (6.8)	3	2, 3, 4	3, 4
	4	19.9, CH ₃	0.71, d (6.8)	3	2, 3, 3-Me	3, 3-Me
Leu2	NH		6.63, d (9.9)	2	1-Leu1	2
	1	174.6, C				
	2	55.2, CH	4.90, m	NH, 3		NH, NH _{Leu2}
	3	40.3, CH ₂	2.82, dd (12.0, 13.7)	2	1, 2, 4, 5/9	NH, NH _{Leu2}
			3.30, dd (4.5, 13.7)			
Gly	4	138.4, C				
	5/9	130.1, CH	7.30, m	6/8, 7	3, 4, 6/8, 7	3
	6/8	129.7, CH	7.29, m	5/9, 7	4, 5/9, 7	
	7	128.1, CH	7.28, m	5/9, 6/8	5/9, 6/8	
	NH		8.64, d (8.7)	2	2, 3, 1-Val	2, 3, 2-Val, 3-Val
Leu1	1	175.7, C				
	2	55.3, CH	4.03, t (7.5)	NH, 3	1, 3	3, 5, NH, NH _{Gly}
	3	40.7, CH ₂	1.57, m	2, 4	1, 2, 4, 4-Me, 5	2, 4-Me, 5, NH
	4	25.9, CH	1.59, m	3, 4-Me, 5	2, 3, 4-Me, 5	NH
	4-Me	22.7, CH ₃	0.94, d (6.3)	4	3, 4, 5	3
Gly	5	22.9, CH ₃	0.99, d (6.3)	4	3, 4, 4-Me	2, 3
	NH		8.57, d (4.3)	2	2, 3, 1-Phe	2, 3, 4, 2-Phe, 3-Phe
	1	170.3, C				
	2	43.7, CH ₂	3.43, d (17.4)	NH	1, 1-Leu2	NH, NH _{Ala}

position	δ_C^a , mult	δ_H^b , mult. (<i>J</i> in Hz)	COSY	HMBC	ROESY
NH		4.09, d (17.4) 9.01, t (6.4)	2	1 _{Leu2}	2, 2 _{Leu2}

^a Carbon chemical shifts were assigned from DEPTQ spectrum recorded at 226 MHz.

^b Recorded at 600 MHz.

^c Carbon chemical shifts are interchangeable.

^d nd: not detected.

Table 3

Residue sequences of laxaphycins and structurally related compounds

compound name	residue											
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th
Laxa A Subgroup												
Laxaphycin A ^{14,19,20}	R-Aoa	L-Hse	E-Dhb	(2S,4R)-4-OHPPro	L-Hse	D-Phe	D-Leu	L-Ile	D- <i>allo</i> -Ile	L-Leu	Gly	
Laxaphycin E ¹⁴	Ada	Hse	E-Dhb	4-OHPPro	Hse	Phe	Leu	Ile	Ile	Leu	Gly	
Hormothamnin A ¹⁸	R-Aoa	L-Hse	Z-Dhb	(2S,4R)-4-OHPPro	L-Hse	D-Phe	D-Leu	L-Ile	D- <i>allo</i> -Ile	L-Leu	Gly	
Lobocyclamide A ¹³	R-Aoa	L-Ser	E-Dhb	(2S,4R)-4-OHPPro	L-Hse	D-Tyr	D-Leu	L-Ile	D- <i>allo</i> -Ile	L-Leu	Gly	
Trichormamide A ⁷	R-Ada	L-Ser	L-Ser	L-Pro	L-Ser	D-Tyr	D-Leu	L-Ile	L-Ile	L-Pro	Gly	
Trichormamide D	R-Ada	L-Gln	E-Dhb	L-Pro	L-Ser	D-Tyr	D-Leu	L-Val	D-Phe	L-Leu	Gly	
Laxa B Subgroup												
Laxaphycin B ^{14,19,20,27}	R-Ada	L-Val	(2R,3S)-3-OHLeu	L-Ala	(2R,3S)-3-OHLeu	L-Gln	L- <i>N</i> Melle	(2R,3R)-3-OHAsn	L-Thr	L-Pro	D-Leu	L-Thr
Laxaphycin B ²⁰	R-Ada	L-Val	(2R,3S)-3-OHLeu	L-Ala	D-Leu	L-Gln	L- <i>N</i> Melle	(2R,3R)-3-OHAsn	L-Thr	L-Pro	D-Leu	L-Thr
Laxaphycin B ^{3,20}	R-Ada	L-Val	(2R,3S)-3-OHLeu	L-Ala	(2R,3S)-3-OHLeu	L-Gln	L- <i>N</i> Melle	(2R,3R)-3-OHAsn	L-Thr	(2S,4R)-4-OHPPro	D-Leu	L-Thr
Laxaphycin D ¹⁴	Aoa	Val	3-OHLeu	Ala	3-OHLeu	Gln	<i>N</i> Melle	3-OHAsn	Thr	Pro	Leu	Thr
Lobocyclamide B ¹³	R-Ada	L-Val	(2R,3S)-3-OHLeu	L-Ala	(2R,3S)-3-OHLeu	D-Gln	L- <i>N</i> Melle	(2R,3R)-4-OHThr	L-Thr	(2S,4R)-4-OHPPro	D-Leu	L-Thr
Lobocyclamide C ¹³	R-Aoa	L-Val	(2R,3S)-3-OHLeu	L-Ala	(2R,3S)-3-OHLeu	D-Gln	L- <i>N</i> Melle	(2R,3R)-4-OHThr	L-Thr	(2S,4R)-4-OHPPro	D-Leu	L-Thr
Lyngbyacyclamide A ^{21,27}	R-Ada	L-Val	3-OHLeu	L-Hse	D-Leu	L-Gln	L- <i>N</i> Melle	(2R,3R)-3-OHAsn	L-Thr	L-Pro	D-Phe	L-Thr
Lyngbyacyclamide B ²¹	R-Ada	L-Val	3-OHLeu	L-Hse	D-Leu	L-Gln	L- <i>N</i> Melle	(2R,3R)-3-OHAsn	L-Thr	4-OHPPro	D-Phe	L-Thr
Trichormamide B ⁷	R-Ada	L-Ile	(2R,3S)-3-OHLeu	L-Hse	(2R,3S)-3-OHLeu	L-Gln	L- <i>N</i> Melle	D-Ser	L-Thr	L-Pro	D-Tyr	L-Thr
Trichormamide C	R-Ada	L-Val	(2R,3S)-3-OHLeu	L-Ala	(2R,3S)-3-OHLeu	L-Gln	L- <i>N</i> Melle	D-Asn	L-Thr	L-Pro	D-Leu	L-Thr