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Myriastramides A–C, new modified cyclic peptides from the Philippines marine sponge *Myriastra clavosa*

Karen L. Erickson,[a,†] Kirk R. Gustafson,[a,*] Dennis J. Milanowski,[a] Lewis K. Pannell,[b,‡] John R. Klose[c] and Michael R. Boyd[d]

[a]*Molecular Targets Development Program, Center for Cancer Research, National Cancer Institute, Building 1052, Room 121, Frederick, MD 21702-1201, USA*
[b]*Laboratory of Bioorganic Chemistry, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD 20892-0805, USA*
[c]*Analytical Chemistry Laboratory, SAIC-Frederick, Inc., NCI-Frederick, Frederick, MD 21702-1201, USA*
[d]*USA Cancer Research Institute, College of Medicine, University of South Alabama, 307 University Blvd, MSB 322, Mobile, AL 36688, USA*

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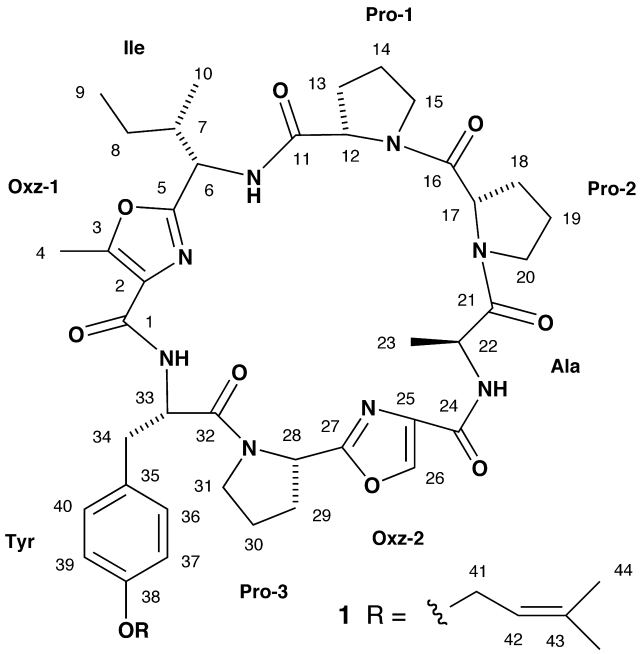
Abstract—Chemical investigation of the Philippines marine sponge *Myriastra clavosa* has resulted in the isolation of three new cyclic octapeptides, myriastramides A (**1**), B (**2**), and C (**3**). The structures of compounds **1**–**3** were assigned based on extensive NMR spectroscopic analyses and a series of degradation and derivatization studies.

Marine sponges have been a prolific source of structurally unique secondary metabolites,[1] whose biosynthetic origins can often be attributed to either polyketide or nonribosomal peptide synthase based pathways.[2–5] Previous chemical investigations of the sponge *Myriastra clavosa* (Order Astrophorida, Family Ancorinidae) have revealed three distinct structural classes of compounds that appear to be produced via polyketide biosynthesis. A collection of *M. clavosa* made in Palau provided clavosines A–C.[6] These highly functionalized cytotoxins are structurally related to the calyculins and they have been shown to inhibit serine/threonine protein phosphatases. More recently, Faulkner's laboratory had two reports detailing their work with a Philippines collection of *M. clavosa*. They described the isolation and structural elucidation of a homologous series of polymethoxydienes,[7] and two novel dimeric macrolide glycosides, clavosolides A and B.[8] The polymethoxydienes were reported to have moderate cytotoxic activity, while the clavosolides were not cytotoxic. Our interest in *M. clavosa* began when extracts of a Philippines sample of the sponge produced a distinctive pattern of differential cytotoxicity in the NCI's 60-cell line human antitumor screen.[9,10] Fractionation of the aqueous extract provided two new clavosolide macrolides, along with clavosolides A and B.[11] The clavosolides were not cytotoxic when tested against a select set of human tumor



Keywords: *Myriastra clavosa*; myriastramide A–C; marine sponge; cyclic peptide.
* Corresponding author. Tel.: +1-301-846-5391; fax: +1-301-846-6919; e-mail: manuscripts@mail.ncifcrf.gov
† On leave from the Carlson School of Chemistry and Biochemistry, Clark University, Worcester, MA 01610, USA.
‡ Present address: USA Cancer Research Institute, College of Medicine, University of South Alabama, Mobile, AL 36688, USA.

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cell lines, which was consistent with the results reported by Faulkner. In a continuation of our studies, we report herein the isolation and structural assignment of myriastramides A–C (**1**–**3**), three new modified cyclic octapeptides that were obtained from both the organic and aqueous extracts of the sponge. The myriastramides represent the first peptide metabolites isolated from *M. clavosa*.

The organic extract (4.1 g) was separated by vacuum liquid chromatography (VLC) on diol-bonded silica, gel

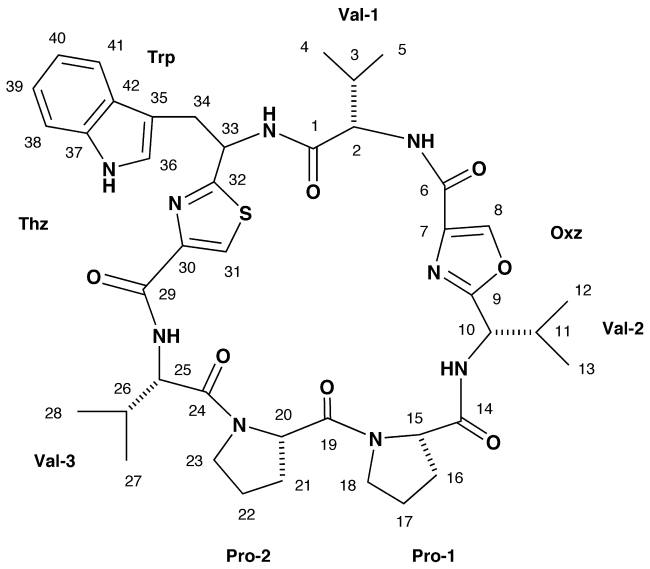
Table 1. NMR data for Myriastramide A (**1**) recorded in $CDCl_3$

Residue	Position	δ C[a] mult.[b]	δ H[c] mult. (*J* in Hz)	COSY	HMBC (H)
Oxz-1	1	160.30 s			4, NH (Tyr)
	2	127.43 s			4
	3	153.20 s			4
	4	11.13 q	2.20 s, 3H		
	5	158.80 s			6
Ile	6	51.29 d	5.13 dd (6.2, 9.9)	7, NH (Ile)	10
	7	35.56 d	2.33 m	6, 8a, 10	6, 9, 10
	8	24.28 t	1.10 m	7, 8b, 9	6, 9, 10
			1.72 m	7, 8a, 9	
	9	11.51 q	0.98 t (7.4), 3H	8a, 8b	
	10	16.71 q	1.09 d (6.6), 3H	7	
	NH		7.65 d (9.8)	6	
Pro-1	11	171.00 s			6, 12, 13b, NH (Ile)
	12	60.93 d	4.40 m	13a, 13b	13a
	13	32.13 t	2.18 m	12, 13b, 14a, 14b	
			2.53 m	12, 13a, 14a, 14b	
	14	22.32 t	1.78 m	13a, 13b, 14b, 15a, 15b	12
			1.96 m	13a, 13b, 14a, 15a, 15b	
	15	47.04 t	3.56 m	14a, 14b, 15b	12, 13b
			3.60 m	14a, 14b, 15a	
Pro-2	16	170.75 s			18a
	17	58.59 d	4.40 m	18a, 18b	
	18	28.35 t	1.81 m	17, 18b, 19a, 19b	17
			2.28 m	17, 18a, 19a, 19b	
	19	25.27 t	1.96 m	18a, 18b, 19b, 20a, 20b	17
			2.12 m	18a, 18b, 19a, 20a, 20b	
	20	47.89 t	3.64 m	19a, 19b, 20b	
			4.04 m	19a, 19b, 20a	
Ala	21	170.57 s			22, 23
	22	45.93 d	4.90 m	23, NH (Ala)	23, NH (Ala)
	23	18.02 q	1.33 d (6.9), 3H	22	22
	NH		6.82 d (8.5)	22	
Oxz-2	24	159.98 s			22, NH (Ala)
	25	136.00 s			26
	26	142.47 d	8.12 s		
	27	163.18 s			26, 28, 29
Pro-3	28	55.07 d	4.42 t (6.6)	29	
	29	32.19 t	1.90 m, 2H	28, 30	28
	30	22.16 t	1.74 m, 2H	29, 31a, 31b	28
	31	45.93 t	3.31 m	30, 31b	
			3.78 m	30, 31a	
Tyr	32	170.64 s			34a
	33	51.24 d	4.79 dt (5.0, 10.9)	34a, 34b, NH (Tyr)	34a, 34b
	34	39.41 t	2.84 dd (5.0, 13.0)	33, 34b	36,40
			3.07 t (11.8)	33, 34a	
	35	128.61 s			37, 39
	36, 40	130.23 d	7.14 d (8.4), 2H	37, 39	34a, 34b, 36, 40
	37, 39	114.77 d	6.83 d (8.4), 2H	36, 40	37, 39
	38	157.83 s			36, 37, 39, 40, 41
	NH (Try)		7.47 d (9.9)	33	
Prenyl	41	64.86 t	4.48 br d (6.9)	42, 44, 45	
	42	119.66 d	5.46 br t (6.5)	41, 44, 45	41, 44, 45
	43[d]	137.8			41, 44, 45
	44	18.21 q	1.73 br s, 3H	41, 42	45
	45	25.81 q	1.78 br s, 3H	41, 42	44

[a] Assignments made from HSQC correlations.
[b] Determined from the DEPT spectrum.
[c] With geminal protons, the smaller δ- value is given the 'a' designation, the larger δ- value is given the 'b' designation.
[d] Detected in the HMBC spectrum only.

permeation on Sephadex LH-20, and diol TLC to afford 1.3 mg of myriastramide A (**1**), 0.2 mg of myriastramide B (**2**), and 2.6 mg of myriastramide C (**3**). An additional 1.0 mg of **1** and 0.1 mg of **2** were obtained from the aqueous extract of the organism after sequential C_4 reversed-phase chromatography, Sephadex LH-20, diol VLC, and preparative TLC.

HRFABMS established the molecular formula of myriastramide A (**1**) as $C_{45}H_{58}N_8O_9$. The peptidic nature of the compound was indicated by IR absorption bands at 3498, 3402, 3313, 1642, and 1509 cm^{-1}. The gross structure of **1** was solved through extensive NMR spectral studies. Complete NMR data sets were collected in both $CDCl_3$ (Table 1) and CD_3OD (see Section 1) to circumvent signal overlap problems and facilitate spectral interpretations. Isoleucine, *O*-substituted tyrosine, alanine, three proline residues and a prenyl ether moiety were readily identified from the NMR data.



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The amino acid sequence of myriastramide A (**1**) was established by a combination of NOESY and HMBC data (Fig. 1). The NOESY spectrum ($CDCl_3$) established peptide links between Pro-1 and Ile Pro-3 and Tyr, and between Pro-2 and Ala. Complete resolution of the α-methine protons of the three proline moieties was achieved in CD_3OD, and a Pro-1 to Pro-2 peptide link could be inferred from a correlation between their respective α-methines (δ 4.57, H-12 and δ 4.44, H-17). NOESY data placed the prenyl group on the oxygen of the tyrosine residue and an HMBC correlation between the quaternary aromatic carbon at δ 157.83 and the oxymethylene protons at δ 4.48 (H_2-41) supported placement of the prenyl ether substituent at C-38. HMBC correlations between the Pro-1 carbonyl (δ 171.00) and both the NH (δ 7.65) and α-methine (H-6, δ 5.13) of Ile confirmed these two residues were linked. The molecular formula required 21 degrees of unsaturation, 18 of which could be accounted for by the residues described above, thus **1** contained three additional rings. The chemical shifts of the remaining sp^2 carbons suggested the presence of two oxazole rings, one with a methyl group at the oxazole

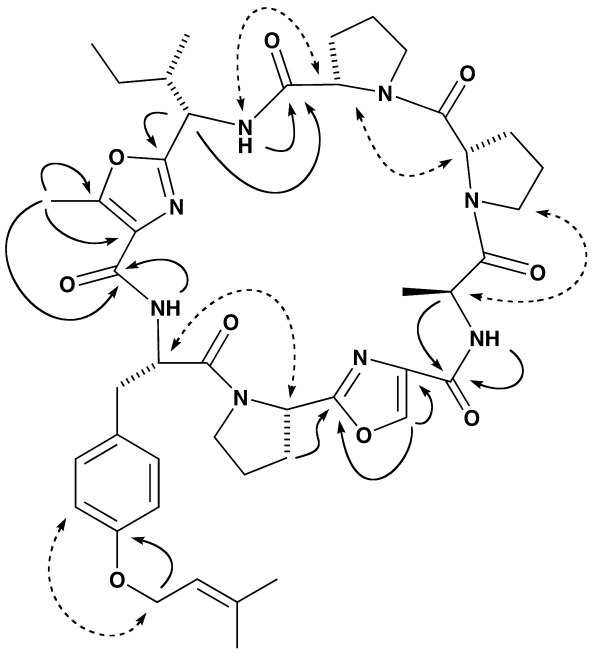

Figure 1. Key HMBC (→) and NOESY (◄---►) correlations for myriastramide A (**1**).

5-position (C-3 in **1**) and the other with a hydrogen at that position (C-26 in **1**).[12–15] The oxazole methyl protons (H_3-4, δ 2.20) had HMBC correlations with C-2 (δ 127.43) and C-3 (δ 153.20), while H-26 (δ 8.12) correlated with C-25 (δ 136.00) and C-27 (163.18). Oxz-1 incorporated the original carbonyl carbon of the isoleucine moiety, as evidenced by an HMBC correlation between C-5 (δ 158.80) and the Ile α-methine. The conjugated carbonyl at δ 160.30 (C-1) showed HMBC correlations with the Oxz-1 methyl protons and the Tyr NH (δ 7.47), which placed this oxazole ring between the Ile and Tyr residues. The H_3-4 to C-1 HMBC correlation represents a four-bond coupling, which is often observed in these systems.[14,15]

HMBC correlations from H-28 (δ 4.67) and H-29 (δ 2.01) to the Oxz-2 carbon at δ 165.03 (C-27) (CD_3OD) identified this carbon as the original carbonyl of Pro-3. Regardless of the NMR solvent or the magnitude of the $^nJ_{C-H}$ value that was used, no HMBC correlations could be detected between the oxazole proton (H-26) and the conjugated amide carbonyl δ 163.18 (C-24). Similar oxazole protons have very long relaxation times, and correlations with these protons are often not observed in HMBC experiments.[14] The linkage between C-24 and the alanine residue was provided by HMBC correlations with the Ala NH at δ 6.83 and α-methine (H-22) at δ 4.90. This peptide bond connected the two ends of the amino acid chain to generate the final cyclic structure of myriastramide A (**1**).

The absolute configurations of the amino acid residues in **1** were established by application of Marfey's method.[16] The peptide was hydrolyzed with 6N HCl and the resulting free amino acids were derivatized with *N*-(3-fluoro-4,6-dinitrophenyl)-L-alaninamide (FDAA). Under the acid hydrolysis conditions that were used with **1**, Oxz-1 and Oxz-2 opened to provide the adjoining amino acids, isoleucine and proline, respectively. LC-MS comparison of the FDAA derivatives

obtained from **1**, with similarly derivatized amino acid standards, established L-stereochemistry at the α- position for all of the residues. The four possible diastereomers of isoleucine were difficult to fully resolve by Marfey's analysis, so the isoleucine derived from hydrolysis of **1** was also analyzed by a ligand-exchange, chiral HPLC method.[17] This technique confirmed the presence of L-isoleucine (6*S*, 7*S* using the numbering scheme for **1**).

The structure of myriastramide B (**2**) was established largely by comparison of its spectral data with that of myriastramide A (**1**). The molecular formula of **2**, $C_{45}H_{57}ClN_8O_9$, revealed that a chlorine atom had replaced one of the hydrogens of **1**. Insufficient sample prohibited the acquisition of a comprehensive set of HMBC data for **2**, but comparison of the ^{1}H, COSY, TOCSY, NOESY, and HSQC spectral data with those of **1** permitted a structure assignment. The only significant differences in the NMR data appeared in the prenyl ether region and a comparison of carbon and hydrogen chemical shifts ($CDCl_3$) for this moiety is shown in Figure 2a. The vinyl methyl group and olefinic CH in **1** were replaced in **2** with an exomethylene group and a chloromethine. Confirmation was provided by MS fragmentation data (Fig. 2b), thus the structure of myriastramide B (**2**) was assigned as the chlorinated aryl ether derivative of **1**.

Myriastramide C (**3**) was structurally distinct from myriastramides A and B. It decomposed upon storage and was difficult to obtain in pure form due to its instability. HRFABMS established a molecular formula of $C_{42}H_{53}N_9O_7S$, indicating 21 degrees of unsaturation, and IR absorptions at 3512, 3387, 3306, 1649, and 1601 cm^{-1} suggested amide functionalities. Analysis of the NMR data (Table 2) identified a tryptophan, two proline residues, and three valine residues. Chemical shift values and HMBC correlations (Fig. 3) also established the presence of one thiazole and one oxazole ring.[11–14] The thiazole proton (H-31, δ 7.85) provided HMBC correlations with C-30 (δ 149.21) and C-32 (δ 169.51), while the oxazole proton (H-8, δ 7.96) correlated with C-7 (δ 134.80) and C-9 (δ 162.79). The thiazole moiety incorporated the original tryptophan carbonyl carbon (C-32, δ 169.5) as evidenced by HMBC correlations to H-33 (δ 5.56) and H_2-34 (δ 3.28 and 3.47). The thiazole proton (H-31) also displayed a 4-bond HMBC coupling to the tryptophan α-methine carbon.

The partial sequence Pro-1-Val-2-Oxz was established by HMBC correlations between H-10 (δ 5.22) and C-9 and the Val-2 NH (δ 8.12) and C-14 (δ 171.19). NOESY correlations between the α-methine of Val-3 (H-25 δ 4.76) and both the α-methine (δ 4.51) and δ-methylene protons (δ 3.81 and 4.03) of Pro-2 revealed the Val-3-Pro-2 linkage. Two conjugated carbonyl groups at δ 160.99 and 160.62 were assigned to the 4-positions of the oxazole and thiazole rings, respectively. Neither the Oxz proton (δ 7.96), nor the Thz proton (δ 7.85), displayed an HMBC correlation to either of the conjugated carbonyl carbons when the experiment was run with $^nJ_{C-H}$ set at 8.3 Hz. However, with $^nJ_{C-H}$ set at 5.3 Hz the thiazole proton correlated with the carbonyl carbon at δ 160.62, which placed this carbon on the thiazole ring, and by default placed the δ 160.99 carbonyl on the oxazole ring. The Val-1 NH (δ 7.11) displayed an HMBC correlation to the δ 160.99 carbon, therefore Val-1 and Oxz were linked. The δ 160.62 carbonyl displayed HMBC correlations with both the Val-3 NH (δ 7.21) and α-methine (δ 4.76, H-25) thereby linking these positions. The α- and β-methines (H-2, δ 4.15 and H-3, δ 2.46 of Val-1 showed NOESY correlations to the Trp NH at δ 7.21 which established a connection between Trp and the Val-1. The final connection that was needed to cyclize the structure and generate the last unsaturation equivalent, required joining the two proline residues as illustrated in **3**. Myriastramide C (**3**) was hydrolyzed and subjected to Marfey's analysis, as described above for **1**, which revealed only L-Val and L-Pro. Attempts to define the stereochemistry of the tryptophan residue were unsuccessful due to decomposition.

Myriastramides A (**1**) and B (**2**) are structurally related to

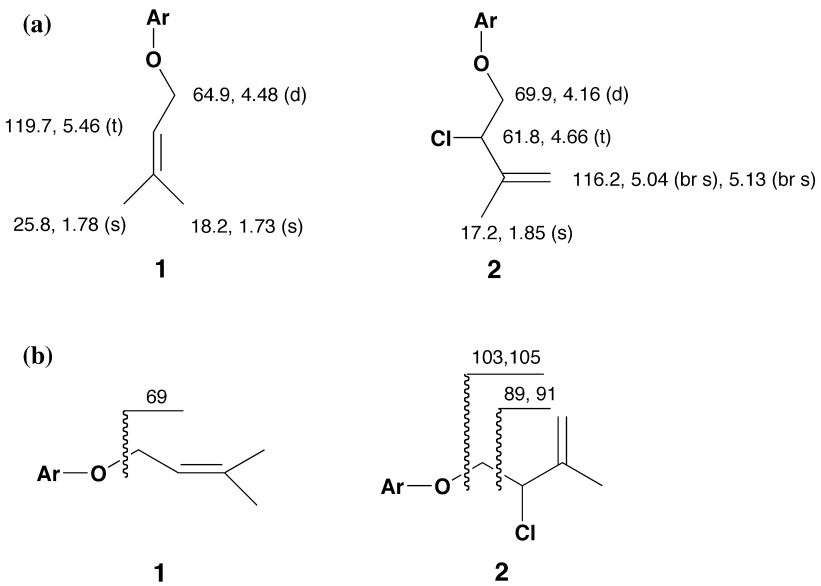

Figure 2. NMR data (a) and mass spectral fragmentation data (b) for the aryl ether substituent in myriastramide A (**1**) and B (**2**).

Table 2. NMR data for Myriastramide C (**3**) recorded in $CDCl_3$

Residue	Position	δ C[a] mult.[b]	δ H[c] mult. (*J* in Hz)	COSY	HMBC (H)
Val-1	1	170.19 s			2
	2	60.28 d	4.15 br t (5.9)	3, NH (Val-l)	4, 5, NH (Val-1)
	3	28.91 d	2.46 m	2, 4, 5	4, 5
	4	17.54 q	0.85 d (6.6), 3H	3	2, 3, 5
	5	19.47 q	0.93 d (6.9), 3H	3	3, 4
	NH		7.11 d (6.0)	2	
	6	160.99 s			2, NH (Val-1)
Oxz	7	134.80 s			8
	8	141.42 d	7.96 s		
	9	162.79 s			8, 10
Val-2	10	52.32 d	5.22 dd (5.8, 9.5)	11, NH (Val-2)	11, 12, 13
	11	29.62 d	2.76 m	10, 12, 13	10, 12, 13
	12	20.09 q	1.11 d (7.5), 3H	11	10, 11, 13
	13	17.63 q	1.12 d (7.5), 3H	11	10, 11, 12
	NH		8.12 d (9.1)	10	
Pro-1	14	171.19 s			10, 15, 16a, 16b, NH (Val-2)
	15	60.97 d	4.50 m	16a, 16b	16a, 17b
	16	31.23 t	2.07 m	15, 16b, 17a, 17b	15
			2.70 br dd (6.6, 12.5)	15, 16a, 17a	
	17	22.04 t	1.80 m	16a, 16b, 17b, 18a	15, 16a, 16b
			2.00 m	16a, 17a, 18b	
	18	46.56 t	3.56 m	17a, 18b	15, 16b
			3.62 m	17b, 18a	
Pro-2	19	170.74 s			21a, 21b
	20	58.52 d	4.51 m	21a, 21b	21a, 21b, 22a
	21	28.51 t	1.85 m	20, 21b, 22a	20, 22b
			2.25 m	20, 21a	
	22	24.93 t	2.00 m	21a, 22b, 23a, 23b	21a, 21b
			2.16 m	22a, 23a, 23b	
	23	48.03 t	3.81 m	22a, 22b, 23b	21a, 21b, 22a
			4.03 m	22a, 22b, 23a	
Val-3	24	170.19 s			20, 25, 26
	25	55.43 d	4.76 t (8.4)	26, NH (Val-3)	26, 27, 28, NH (Val-3)
	26	31.70 d	2.16 m	25, 27, 28	25, 27, 28
	27	19.43 q	1.06 d (7.0), 3H	26	25, 26, 28
	28	18.14 q	0.97 d (6.6), 3H	26	25, 27
	NH		7.21 d (9.5)	25	
	29	160.62 s			25, 31, NH (Val-3)
Thz	30	149.21 s			31
	31	124.14 d	7.85 s		
	32	169.51 s			31, 33, 34a, 34b
Trp	33	51.50 d	5.56 br q (8.0)	34a, 34b, NH (Trp)	31, 34a, 34b
	34	32.19 t	3.28 dd (8.1, 13.6)	33, 34b	
			3.47 br dd (6.6, 14.0)	33, 34a, 36	
	35	110.89 s			34a, 34b, 36, NH[d]
	36	122.80 d	6.95 br s	34b, NH[d]	34a, 34b
	NH[d]		8.06 br s	36	
	37	135.98 s			36, 39, 41, NH[d]
	38	111.13 d	7.29 d (8.1)	39	39, 40
	39	122.18 d	7.13 t (7.5)	38, 40	38, 41
	40	119.67 d	7.03 t (7.4)	39, 41	38
	41	118.37 d	7.48 d (8.1)	40	39
	42	127.49 s			36, 38, 40, NH[d]
	NH		7.21 d (9.5)	33	

[a] Assignments made from HSQC correlations.
[b] Determined from the DEPT spectrum.
[c] With geminal protons, the smaller δ- value is given the 'a' designation, the larger δ- value is given the 'b' designation.
[d] Indole NH.

the haliclonamides, oxazole containing cyclic octapeptides previously isolated from a Palau collection of the sponge *Haliclona* sp.[18] However, differences in the amino acid sequence, N-to-C linkage, and partial reduction of the methyloxazole ring in the haliclonamides differentiate these peptides from the myriastramides. Although the myriastramides were isolated from cytotoxic fractions of the *M. clavosa* extracts, they do not appear to be responsible for the observed cytotoxicity. Myriastramide A was tested for cytotoxicity against 10 different human tumor cell lines, but displayed no activity at a high test concentration of 10 μg/mL. Insufficient amounts of myriastramides B and C

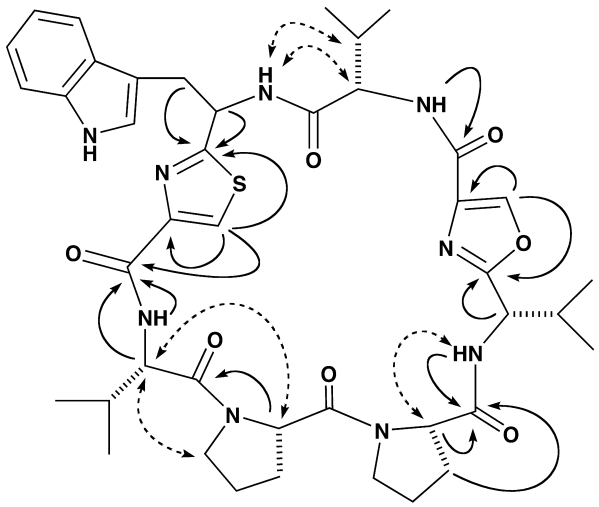

Figure 3. Key HMBC (⟶) and NOESY (◀- - -▶) correlations for myriastramide C (**3**).

were available for similar testing. Given that the clavosolides that have been tested were also noncytotoxic,[9,10] the origin of the bioactivity of the parent extracts remains elusive.

1. Experimental

1.1. General experimental procedures

^{1}H NMR spectra were obtained at 500 MHz and ^{13}C NMR spectra at 125 MHz on a Varian INOVA 500 spectrometer. Chemical shifts are reported in ppm relative to the residual nondeuterated solvent. Inverse-detected heteronuclear correlations were measured using HSQC (optimized for $^1J_{CH}$=140 Hz) and HMBC (optimized for $^nJ_{CH}$=5.3, 8.3 Hz) pulse sequences. Several HMBC data sets were acquired using a Varian 500 gHX Nano probe, with a 40 μL sample tube that was spun at 2200 rpm. Assignments for the ^{13}C resonances were based on HSQC and HMBC correlations. VLC was carried out with wide-pore C_4 and YMC 60 Å diol-bonded silica stationary phases. PTLC was done on EM HPTLC 10×10 cm glass-backed plates, 200 μm thickness. Optical rotations were measured with a Perkin–Elmer 241 polarimeter. Ultraviolet (UV) and infrared (IR) spectra were obtained on a Beckmann DU-640 and a Perkin–Elmer 1600 FTIR spectrometer, respectively. High-resolution FAB mass spectra were acquired on a JEOL SX102 mass spectrometer and CsI was added to the samples prior to analysis. Electrospray mass spectra were obtained on a Hewlett–Packard HP1100 integrated LC-MS system. Chiral HPLC was performed on a Waters 600E system with a Waters 990 photodiode array detector.

1.2. Animal materials

The sample (290 g, wet weight) of *Myriastra clavosa* (order Astrophorida, family Ancorinidae) was collected around Pamilican Island in the Bohol Sea, Philippines in 1997. The sponge was frozen shortly after collection and transported frozen to Frederick, MD where the extracts were prepared. The collection was made by the Coral Reef Research Foundation and taxonomic identification was made by Michelle Kelly. A voucher specimen (OCDN5139) for this collection is maintained at the Smithsonian Institution, Washington, DC.

1.3. Extraction and isolation

The organism was ground in a Waring blender with dry ice then percolated overnight with water. The solid sponge material was separated from the water solution by centrifugation and the clarified liquid was lyophilized to provide 75.3 g of aqueous extract. The marc was freeze-dried and re-extracted with a mixture of CH_2Cl_2–MeOH (1:1 v/v) at 25°C overnight. The crude organic extract was then concentrated to give 8.8 g. A 4.1 g sample of the organic extract was dissolved in 40 mL of CH_2Cl_2–MeOH with sonication and coated on 10 g of diol-bonded silica. The dried sample was then applied to a 30 g column of diol-bonded silica and batch-eluted with 500 mL aliquots of hexane, CH_2Cl_2, EtOAc, acetone, and MeOH. A second 2.0 g sample of the extract was processed in the same manner and the acetone eluants from both columns were combined to give 133 mg. This sample was then passed through a Sephadex LH-20 column with CH_2Cl_2–MeOH (1:1) to give 10 fractions. Fraction three (43 mg) was subjected to PTLC on diol-bonded silica with 4% MeOH–CH_2Cl_2 (2×) to give three fractions. Fraction 1 was myriastramide B (**2**), 0.2 mg (0.005%). Fraction 2 (3.7 mg), after PTLC on diol-bonded silica with 20% MeOH–CH_2Cl_2 (4×), afforded myriastramide A (**1**), 1.3 mg (0.03%). Myriastramide C (**3**), 2.6 mg (0.06%), was obtained from fraction 3 (4.6 mg) after PTLC on diol-bonded silica with 10% MeOH–C_6H_6 (3×).

An 18.7 g portion of the aqueous extract was subjected to VLC on a wide-pore (WP) C_4 column in three separate batches employing a sequence of five solvent systems: 100% H_2O, H_2O–MeOH (2:1), H_2O–MeOH (1:2), 100% MeOH, and MeOH–CH_2Cl_2 (1:1). The 1:2 H_2O–MeOH fraction, 121 mg, was suspended in CH_2Cl_2–MeOH (1:1), sonicated and then filtered. The filtrate was subjected to VLC on diol-bonded silica and the fraction eluting with 2% MeOH–CH_2Cl_2 was chromatographed on a C_{18}-TLC plate with 85% MeOH–H_2O (2×). Fraction 1 (1.7 mg), after C_{18}-HPLC (85% MeOH–H_2O), yielded a trace amount of impure **3**. Fraction 2 (5.0 mg) was subjected to sequential PTLC on C_{18} bonded silica with 85% MeOH–H_2O (2×) and on diol-bonded silica with 3% MeOH–CH_2Cl_2 (2×) to give 1.0 mg of **1** (0.005%) and 0.1 mg **2** (0.0005%).

1.3.1. Myriastramide A (1). $[\alpha]_D$=−115.4° (*c* 0.13, MeOH); UV λ_{max} (MeOH) 210 (ε 33992), 222 (sh, ε 28656), 275 (ε 14704) nm; IR ν_{max} (neat) 3498, 3402, 3313, 2966, 2929, 2878, 1642, 1598, 1509, 1447, 916, 835, 728 cm^{-1}; NMR data ($CDCl_3$) see Table 1; ^{1}H NMR (CD_3OD) δ 0.99 (t, *J*=7.3 Hz, H_3-9), 1.12 (d, *J*=6.9 Hz, H_3-10), 1.15 (m, H-8a), 1.29 (d, *J*=6.6 Hz, H_3-23), 1.74 (br s, H_3-44), 1.77 (br s, H_3-45), 1.78 (m, H_2-30), 1.78 (m, H-14a), 1.80 (m, H-8b), 1.85 (m, H-18a), 2.01 (m, H_2-29), 2.01 (m, H-19a), 2.01 (m, H-14b), 2.10 (m, H-19b), 2.21 (s, H_3-4), 2.27 (m, H-13a), 2.37 (m, H-7), 2.41 (m, H-13b), 2.42 (m, H-18b), 2.84 (dd, *J*=5.3, 12.8 Hz, H-34a), 3.00 (dd,

J=10.6, 12.8 Hz, H-34b), 3.34 (m, H-31a), 3.52 (m, H-15a), 3.57 (m, H-15b), 3.63 (m, H-20a), 3.73 (m, H-31b), 4.04 (m, H-20b), 4.44 (m, H-17), 4.51 (br d, J=6.6 Hz, H-41), 4.57 (bd, J=8.1 Hz, H-12), 4.67 (t, J=5.0 Hz, H-28), 4.81 (m, H-33), 4.88 (br quint., J=7.0 Hz, H-22), 5.14 (dd, J=5.9, 8.8 Hz, H-6), 5.43 (br t, J=6.5 Hz, H-42), 6.85 (d, J=8.4 Hz, H-37 and H-39), 7.16 (d, J=8.4 Hz, H-36 and H-40), 7.70 (Ala-NH),[a] 8.21 (d, J=9.5 Hz, Ile-NH), 8.33 (s, H-26). [a]Signal assigned from COSY data; ^{13}C NMR (CD_3OD) δ 11.41 (C-4), 11.60 (C-9), 17.02 (C-10), 17.75 (C-23), 18.19 (C-44), 23.08 (C-30), 23.20 (C-14), 25.45 (C-8), 25.85 (C-45), 26.15 (C-19), 29.29 (C-18), 32.81 (C-13), 33.02 (C-29), 36.59 (C-7), 40.04 (C-34), 47.35 (C-22 and C-31), 48.24 (C-15), 48.90 (C-20),[a] 52.39 (C-6), 52.48 (C-33), 56.80 (C-28), 60.19 (C-17), 62.37 (C-12), 65.85 (C-41), 115.98 (C-37 and C-39), 121.25 (C-42), 128.87 (C-2), 129.54 (C-35), 131.38 (C-36 and C-40), 137.02 (C-25), 138.60 (C-43), 144.73 (C-26), 154.92 (C-3), 159.46 (C-38), 160.60 (C-5), 161.65 (C-24),[b] 162.01 (C-1),[b] 165.03 (C-27), 172.38 (C-21), 172.54 (C-32), 172.72 (C-16),[c] 173.36 (C-11).[c] [a]Signal only observed in HSQC spectral data, [b,c]Assignments may be interchanged; HRFABMS $(M+Cs)^+$ m/z 987.3386, calcd for $C_{45}H_{58}N_8O_9Cs$, 987.3385.

1.3.2. Myriastramide B (2). $[\alpha]_D$=−90.0° (c 0.03, MeOH); UV λ_{max} (MeOH) 220 (ε 21512), 227 (ε 1317) ^{1}H, ^{13}C, COSY, TOCSY, NOESY, HSQC NMR data ($CDCl_3$) were virtually identical to those of **1** (Table 1) for C-1 through C-40. For C-41 to C-45, see Figure 2. The following NOESY correlations of the chlorinated prenyl group were observed (H/H): 41/37, 41/39, 41/42, 42/44b, 44a/45; HRFABMS $(M+Cs)^+$ m/z 1021.2994, calcd for $C_{45}H_{57}Cl^{35}N_8O_9Cs$, 1021.2996.

1.3.3. Myriastramide C (3). $[\alpha]_D$=−136.5° (c 0.26, MeOH); UV λ_{max} (MeOH) 210 (ε 47137), 282 (ε 5095), 289 (ε 4275) nm; IR ν_{max} (neat) 3512, 3387, 3306, 2974, 2929, 2885, 1649, 1601, 1539, 1443, 1104, 916, 827, 731 cm^{-1}; NMR data see Table 2; HRFABMS $(M+Cs)^+$ m/z 960.2843, calcd for $C_{42}H_{53}N_9O_7SCs$, 960.2848.

1.4. Determination of the absolute stereochemistry of Myriastramide A (1)

The peptide (1.0 mg) was first treated with O_3 in CH_2Cl_2, however, by TLC it did not appear to have reacted. An aliquot of the peptide (50–100 μg) was then dissolved in 6N HCl (constant boiling, 0.5 mL), degassed, and heated at 105–108°C for 16 h under vacuum. The solvent was removed under a stream of N_2, and the residue was washed with water and dried in vacuo. The hydrolysis product (25 μg) was dissolved in 15 μL of 6% TEA (in MeCN–H_2O, 1:1) and treated with 7.5 μL of 1% *N*-(3-fluoro-4,6-dinitrophenyl)-L-alaninamide (FDAA) in acetone at 40°C for 1 h. The reaction mixture was diluted with 45 μL of water and an aliquot applied to a C_3 column (Zorbax 300SB, 2.1×150 mm, 5 μm) and eluted with a linear gradient (0–50%) of MeCN in 5% CH_3CO_2H over 30 min (0.25 mL/min, 50°C). FDAA derivatized amino acids were detected by absorption at 340 nm and by MSD (positive ion, mass range 100–1000 Da) and compared with similarly derivatized commercially available amino acid standards (retention times in min shown in parentheses): L-Ala (18.05), D-Ala (21.24), L-Pro (18.98), D-Pro (20.36), L-Ile (25.00), L-*allo*-Ile (25.00), D-Ile (27.95), D-*allo*-Ile (27.95), L-Tyr (22.01), D-Tyr (24.10). The FDAA derivatized amino acids from the hydrolysate of **1** were analyzed as: L-Ala (18.29), L-Pro (19.18), L-Ile (25.16), L-Tyr (22.11).

Ligand exchange chiral HPLC analysis of underivatized amino acids was performed in a manner similar to previously published reports.[17] The peptide hydrolysis product (10 μg), or amino acid standards (10 μg), were dissolved in 10 μL ddH_2O and analyzed by chiral HPLC (column: Chirex 3126 [D-penicillamine], 4.6×250 mm, Phenomenex, Inc.; solvent: 2 mM $CuSO_4$ at a flow rate of 1 mL/min; UV detection at 254 nm). Amino acid standards with retention times (min) shown in parentheses: L-Ala (8.64), D-Ala (12.34), L-Ile (68.74), L-*allo*-Ile (58.15), D-Ile (122.94), D-*allo*-Ile (100.33), L-Pro (18.83), D-Pro (39.97). The amino acids from the hydrolysate of **1** were analyzed as: L-Ala (8.64), L-Ile (68.0), L-Pro (18.64).

1.5. Determination of the absolute stereochemistry of Myriastramide C (3)

A 50 μg aliquot of **3** was hydrolyzed, derivatized with FDAA, and analyzed in a manner similar to that described above for **1**. Amino acid standards with retention times (min) shown in parentheses were: L-Pro (20.04), D-Pro (21.26), L-Val (23.52), D-Val (26.57). The FDAA derivatized amino acids from the hydrolysate of **3** analyzed as: L-Pro (20.12), L-Val (23.55).

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