Title	Triproamide and pemukainalides, cyclic depsipeptides from the marine cyanobacterium *Symploca hydnoides*
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7 **Triproamide and Pemukainalides, Cyclic Depsipeptides from the Marine Cyanobacterium**
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10 ***Symploca hydnoides***
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Dedicated to Dr. William H. Gerwick, University of California at San Diego, for his pioneering work on bioactive natural products.

ABSTRACT

A new cyclic depsipeptide, triproamide (**1**), containing the rare 4-phenylvaline (dolaphenvaline, Dpv) and a β-amino acid, dolamethylleucine (Dml), originally found in dolastatin 16, was isolated from the polar VLC-derived fraction of the extracts prepared from the marine cyanobacterium, *Symploca hydnoides*. Triproamide (**1**) was isolated along with the known molecule, kulokainalide-1 (**2**), as well as its two new analogues, pemukainalides A (**3**) and B (**4**). Their planar structures were elucidated based on extensive NMR and mass spectrometric data. The absolute and relative configurations of the compounds were determined utilizing a combination of Marfey's method, *J*-based configuration and chiral-phase HPLC analyses. Kulokainalide-1 (**2**) and pemukainalide A (**3**) exhibited cytotoxicity against the MOLT-4 leukemia cell line with IC_{50} values of 5.9 μM and 5.6 μM, respectively.

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Cyanobacteria are ubiquitous gram-negative photoautotrophic nitrogen-fixing prokaryotes, inhabiting both marine and terrestrial ecosystems and are a source of bioactive secondary metabolites encompassing diverse structures.[1] A majority of these natural products are nitrogen-containing and are products of biosynthetic pathways, such as the non-ribosomal peptide (NRP), hybrid-polyketide-NRP and the ribosomally synthesized and post translationally modified peptide (RiPP) pathways.[2]

Some opisthobranch mollusks are known to sequester these metabolites through their cyanobacterial diet and were thus initially perceived to be the true producers of these bioactive biomolecules.[3] A classic example would be the discovery of the potent antitumor agent, dolastatin 10, which was first isolated, albeit in low yield, from the sea hare *Dolabella auricularia* and subsequently isolated from a *Symploca* sp cyanobacterium.[4,5] Similarly, dolastatin 16,[6] another potent anticancer agent originally isolated from *D. auricularia*, was later reported from the marine cyanobacteria, *Lyngbya majuscula*, by Nogle et al.[7] and *Symploca* cf. *hydnoides* by Salvador et al.[8]

The last known report of a dolastatin 16 analogue dates back to nearly a decade ago when pitiprolamide,[9] a proline rich cyclic depsipeptide with the β-hydroxy acid unit, 2,2-dimethyl-3-hydroxyhexanoic-acid, was isolated from *Lyngbya majuscula* collected at Guam. In our quest to hunt for novel bioactive secondary metabolites from marine cyanobacteria, we isolated three new cyclic depsipeptides, triproamide (**1**), pemukainalides A (**3**) and B (**4**) as well as the known compound kulokainalide-1[10] (**2**) from the marine cyanobacterium, *Symploca hydnoides*. While compound **1** is a new analogue of dolastatin 16, compounds **3** and **4** are analogues of kulokainalide-

1 (**2**). Herein, we report the isolation, structural elucidation and biological evaluation of these new cyclic depsipeptides.

RESULTS AND DISCUSSION

Samples of the marine cyanobacterium, *Symploca hydnoides*, identified previously based on morphological and 16S rRNA analyses,[11] collected from Bintan Island, Indonesia, were extracted exhaustively with CH_2Cl_2:MeOH (2:1). The extracts were subjected to normal phase vacuum liquid chromatography (NP-VLC) resulting in nine fractions. The polar fraction, eluted with 10% MeOH in EtOAc, was further subjected to fractionation by reverse-phase solid phase extraction (RP-SPE) followed by a series of reverse-phase HPLC purifications to yield pure triproamide (**1**), pemukainalides A (**3**) and B (**4**), along with the known compound, kulokainalide-1 (**2**).

Triproamide (**1**)

Kulokainalide 1 (**2**)
Pemukainalide A (**3**)
Pemukainalide B (**4**)

Triproamide (**1**) gave a $[M+H]^+$ protonated molecule at *m/z* 907.5564 by HR-ESI-OrbitrapMS, consistent with the molecular formula $C_{49}H_{74}N_6O_{10}$ requiring 16 degrees of unsaturation. From the 1D NMR spectra, the presence of exchangeable proton doublets at δ_H 6.75 and δ_H 7.69, α protons with chemical shifts between δ_H 3.68-5.47 and eight carbonyl ^{13}C NMR signals (δ_C 167.8, 169.3, 169.7, 170.9, 171.0, 171.3, 172.4, 174.7) suggest **1** to be peptidic in nature. The ^{13}C NMR chemical shifts at δ_C 75.3 and δ_C 76.3 further implied oxygenation of two carbon atoms which led to the finding that **1** could be a depsipeptide. From ^{13}C NMR and DEPT spectroscopic data, the presence of aromatic methine carbons, including δ_C 129.6 × 2, δ_C 128.4 × 2, δ_C 126.2, and a single quaternary carbon (δ_C 140.6) were characteristic of a monosubstituted benzene. A combination of

1D and 2D NMR spectroscopic data led to the assignments of four regular amino acid units, namely 3 × Pro and one *N*-Me-Val, two 2-hydroxy isovaleric acid (Hiva) units, 2-amino-4-phenylisovaleric acid (= Dolaphenvaline, Dpv) and 2,4-dimethyl-3-aminopentanoic acid (= Dolamethylleucine, Dml) units (Table 1).

The partial structures of **1** were assembled utilizing the corresponding signals evident from HMBC spectrum, such as H-2 of *N*-Me-Val/C-1 of $Hiva^2$, H-2 of $Hiva^2$/C-1 of Dml, H-3 of Dml/C-1 of Pro^1, H-2 of Pro^1/C-1 of Dpv, *N*-H of Dpv/C-1 of Pro^2 and H-2 of Pro^2/C-1 of $Hiva^1$ and NOESY spectrum, including H-5a/5b of Pro^1 and H-2 of Dpv, H-2 of Pro^2 and H-2 of Dpv, H-2 of $Hiva^1$ and H-2 of Pro^2, H-5a/5b of Pro^3 and H-2 of *N*-Me-Val, H-2 of $Hiva^2$ and *N*-CH_3 of *N*-Me-Val, CH_3-7 of Dml and H-2 of $Hiva^2$, *N*-H of Dml and H-2 of Pro^1. This allowed the arrangement of the sequence as $Hiva^1$-Pro^2-Dpv-Pro^1-Dml-$Hiva^2$-*N*-Me-Val-Pro^3. An additional HMBC correlation between H-2 of $Hiva^1$ and C-1 of Pro^3 established the structure of **1** as a cyclic octadepsipeptide having 16 degrees of unsaturation (Figure 1). The geometries of the amide bonds at the Pro residues were determined based on the ^{13}C NMR chemical shift differences at the β and γ positions ($\Delta\delta_{\beta\text{-}\gamma}$).[12] The small (< 6 ppm) differences in chemical shifts of the β and γ chemical

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signals in Pro[1] to Pro[3] ($\Delta\delta_{\beta\text{-}\gamma}$ = 3.4, 5.9, 5.6 ppm, respectively) suggested *trans* geometries of the peptide bonds in all of the Pro units.

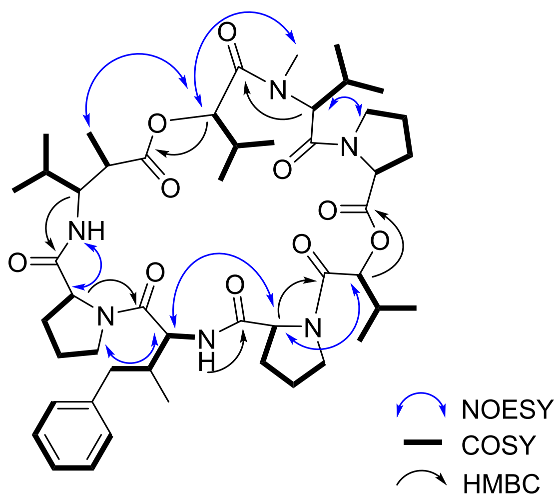

Figure 1. Selected NOESY, COSY and HMBC correlations of triproamide (**1**).

The absolute configurations of the regular amino acids in triproamide (**1**) were determined using Marfey's analysis.[13] Acid hydrolysis of **1** and subsequent derivatization with Marfey's reagent revealed the L configuration of the Pro and the D configuration of the *N*-Me-Val residues. The absolute configuration of the Hiva units were determined to be D by chiral-phase HPLC analysis. To determine the absolute configurations of the two unusual amino acids, Dml and Dpv, dolastatin 16, previously isolated in our lab as a potent antifouling compound and bearing the same amino acid units,[14] was used to compare the retention times of the Marfey's derivatized Dml and Dpv units in the two molecules. The absolute configuration of the isolated dolastatin 16 was previously

confirmed via total synthesis by Casalme and co-workers.[15] The resulting Marfey's derivatised hydrolysates, derived from the acid hydrolysis of both compounds, were then analysed on a LC-HRMS. While the Marfey's derivatised product of Dml was not detected in both compounds, there was a peak in the total ion chromatogram with mass corresponding to the Marfey's derivatised Dpv in dolastatin 16 and **1** having the same retention times. This observation indicated the likely identical absolute configuration of the Dpv unit in both molecules. Taken together, the absolute configuration of the C-2 and C-3 in Dpv of **1** were established to be 2*S* and 3*R*, respectively. A similar approach was previously demonstrated by Salvador et al. for the absolute stereochemistry of the Dpv unit in their isolated dolastatin 16, utilizing enantioselective HPLC-MS analysis of the DLA-derivatized acid hydrolysate in comparison with that of pitiprolamide.[8] For the relative configuration of the Dml unit in **1**, a direct comparison of the ^{1}H and ^{13}C NMR spectroscopic data with dolastatin 16 revealed almost identical chemical shifts pertaining to the Dml unit (refer to Supporting Information S42 for details). Furthermore, similar NOESY correlations observed for H-2 (e.g. H-2/H-3, H-2/H-4, H-2/H-7) and H-3 (e.g. H-3/H-2, H-3/H-4, H-3/H-7) and a similar coupling constant of $^{2}J_{H2\text{-}C7}$ = -6.4 Hz, calculated from the HSQC-HECADE[16] spectroscopic data, of the Dml unit in **1** and dolastatin 16 led us to conclude that they likely have the same

configuration. Due to the minute quantity of **1** being isolated, the resolution of the HSQC-HECADE spectrum was low and thus we were only able to calculate the coupling constant of one related signal. Consequently, only the relative configuration of the C-2 and C-3 in the Dml unit in **1** was assigned as 2*R*, 3*R*, respectively.

Triproamide (**1**) is the fourth analogue of dolastatin 16 that has been isolated along with other related compounds over the past two decades (Figure 2). The previous three analogues isolated include homodolastatin 16[17] isolated from a Kenyan *Lyngbya majuscula*, kulokekahilide-1[18] isolated from the cephalaspidean mollusk *Philinopsis speciosa* and pitiprolamide[8] isolated from a Guamanian *Lyngbya majuscula*. Analysis of the five structures revealed three conserved sequence residues of Pro-Dpv-Pro and triproamide (**1**) is the only compound that has two 2-hydroxy isovaleric acid units in its structure (Figure 2). Triproamide (**1**) is also one of the two dolastatin 16 analogues that has the unique dolamethyleucine residue retained. Compound **1** and dolastatin 16 have similar absolute configurations of the Pro, *N*-Me-Val and Hiva residues.

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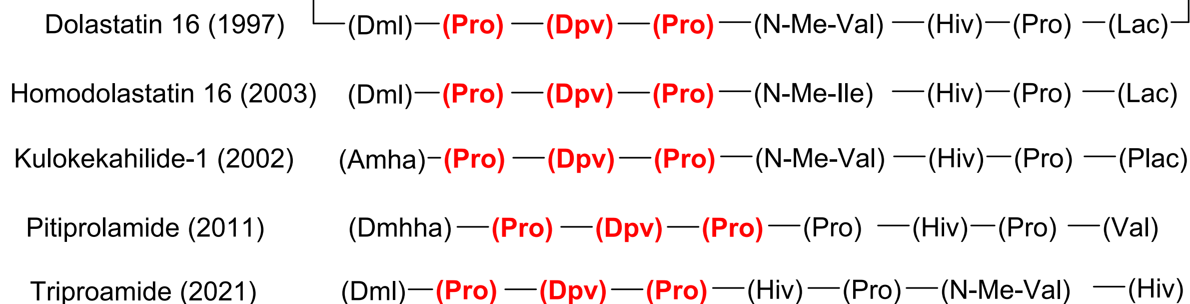

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16 **Figure 2.** Sequences of the residues in dolastatin 16, homodolastatin 16, kulokekahilide-1,
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19 pitiprolamide and triproamide (**1**). Conserved residues are highlighted in red and the year of
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Table 1. NMR Spectroscopic Data (400 MHz, 100 MHz, $CDCl_3$) for Triproamide (1)

Unit	Position	δ_C, type	δ_H (J in Hz)	COSY	Selected NOESY
Pro[1]	1	170.9, C			
	2	57.9, CH	4.56, d (7.4)	3, 4	2 of Dpv, *N*H of Dml, 3, 4
	3	25.1, CH_2	2.04, m	2, 4	
	4	21.7, CH_2	2.07-1.96, m	2, 3, 5	
	5a	46.4, CH_2	2.89, m	3, 4	
	5b		2.55, m	3, 4	
Dpv	1	171.3, C			
	2	50.7, CH	4.97, d (8.4)	*N*H, Pro[2]-4	5a & 5b of Pro[1] and 3, 4a, 4b, *N*H
	3	40.9, CH	2.54, m	4a, 4b	
	4a	41.0, CH_2	2.41, m	3, 4b	
	4b		1.76, m	3, 4a	
	5	140.6, C			
	6/10	129.6, CH	7.34, d (7.5)	7, 8, 9	
	7/9	128.4, CH	7.30, d (8.2)	6, 8, 10	
	8	126.2, CH	7.18, m	6, 7, 9, 10	
	11	15.1, CH_3	0.79, d (6.8)	3, 4	
	*N*H	-	6.75, d (8.8)	2	2
Pro[2]	1	172.4, C			
	2	61.3, CH	4.63, dd (9.0, 2.4)	3, 4	2 of Dpv, 2 of Hiva[1]
	3	30.8, CH_2	2.27-2.18, m	2, 4	
	4	24.9, CH_2	2.12-1.75, m	2, 3, 5	
	5a	47.6, CH_2	3.66, m	3, 4	
	5b		3.46, m	3, 4	
Hiva[1]	1	167.8, C			
	2	75.3, CH	4.86, d (7.4)	3	2 of Pro[2]
	3	25.6, CH	2.16, m	2.4.5	
	4	18.6, CH_3	1.00, d (6.7)	3	
	5	18.1, CH_3	1.01, d (6.5)	3	

Pro[3]	1	171.0, C			
	2	58.8, CH	4.55, d (7.4)	3, 4	
	3	30.7, CH_2	2.17, m	2, 4	
	4	25.1, CH_2	2.08-1.75, m	2, 3, 5	
	5a	45.9, CH_2	3.92, m	3, 4	2 of *N*-Me-Val
	5b		3.49, m	3, 4	2 of *N*-Me-Val
N-Me-Val	1	169.3, C			
	2	59.5, CH	5.17, d (10.2)	3	5a and 5b of Pro[3]
	3	30.5, CH	2.31, m	2,4,5	
	4	19.7, CH_3	1.09, d (6.9)	3	
	5	18.9, CH_3	1.00, d (6.7)	3	
	6	29.7, *N*-CH_3	3.08, s		2 of Hiva[2]
Hiva[2]	1	169.7, C			
	2	76.3, CH	5.47, d (3.1)	3	*N*-CH_3 of *N*-Me-Val, 7 of Dml
	3	28.3, CH	2.21, m	2,4,5	
	4	19.5, CH_3	0.92, d (6.5)	3	
	5	16.2, CH_3	1.05, d (6.7)	3	
Dml	1	174.7, C			
	2	38.8, CH	2.89, m	*N*H, 3	3, 4, 7
	3	56.3, CH	3.68, m	2,4	2, 4, 7
	4	32.2, CH	1.56, m	3,5,6	2, 3
	5	20.4, CH_3	0.87, d (7.2)	4	
	6	19.8, CH_3	1.08, d (7.3)	4	
	7	14.8, CH_3	1.00, d (7.1)	4	2 of Hiva[2]
	*N*H	-	7.69, d (10.2)	2	2 of Pro[1]

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HR-ESI-OrbitrapMS data of **2** established its molecular composition as $C_{48}H_{70}N_6O_{10}$ with the $[M+H]^+$ protonated molecule peak observed at *m/z* 891.5256. Subsequent comparison of the 1D NMR data with the published data established **2** as kulokainalide-1. Kulokainalide-1 contains a 2,2-dimethyl-3-hydroxy-7-octynoic acid (Dhoya) unit and is a kulolide related compound originally reported from the mollusk, *Philinopsis speciosa*, by Nakao et al.[10]

Pemukainalides A (**3**) and B (**4**) gave $[M+H]^+$ protonated molecules at *m/z* 893.5417 and 895.5574, which were two and four mass units higher than that of kulokainalide-1 (**2**), exhibiting molecular formulas of $C_{48}H_{72}N_6O_{10}$ and $C_{48}H_{74}N_6O_{10}$ respectively. Compounds **3** and **4** also adopted single conformers as judged by their respective NMR spectroscopic data. Comparison of the ^{1}H NMR spectra of compounds **2**–**4** showed nearly identical chemical shifts, suggesting the close relatedness of **3** and **4** with kulokainalide-1 (Supporting Information Figures S17, S22 and S29). Analysis of the 1D and 2D NMR spectra alongside the MS/MS fragmentation patterns for **2**–**4** revealed the identical sequence of amino acids and the identity of the units as 2 × Pro, 2 × Val, one each of *N*-Me-Val, Phe, Lac and Dhoya/Dhoea/Dhoaa (Supporting Information Figures S14 and S15). The main difference observed is in the Dhoya unit where in pemukainalide A (**3**), the acetylene carbon signals in **2** have been replaced with the more deshielded methine (δ_C 138.8, δ_H

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5.80) and methylene signals (δ_C 114.7, δ_H 4.94 and 5.01), which were characteristic of a monosubstituted olefinic functional group. These chemical shifts represent a double bond in pemukainalide A (**3**) compared to a triple bond in kulokainalide-1 (**2**) at the Dhoya moiety. Further analysis of the 1D and 2D NMR spectra of **3** led to the assignment of the unit as 2,2-dimethyl-3-hydroxy-7-octenoic acid (Dhoea) (Table 2).

^{1}H NMR data of pemukainalide B (**4**) revealed the absence of deshielded chemical shifts at δ_H 5.80, δ_H 5.01 and δ_H 4.94 which have been replaced by shielded methylene signals at δ_H 1.27 and δ_H 0.86. In the HSQC NMR spectrum, the shielded methylene signals corresponded to δ_C 22.3 and δ_C 13.9 insinuating that the terminal double bond in the Dhoea moiety of **3** is saturated to a single bond in **4**, resulting in a 2,2-dimethyl-3-hydroxy-7-octanoic acid (Dhoaa) unit instead (Table 2).

The absolute configurations of the α-amino acids in kulokainalide-1 (**2**) were determined using Marfey's analysis revealing the L-configurations of the Pro, Val, Phe residues and the D-configuration of the *N*-Me-Val unit and chiral-phase HPLC analysis was performed to determine the absolute configuration of the Lac unit as L-Lac, consistent with the reported kulokainalide-1 (**2**). The small $\Delta\delta_{\beta\text{-}\gamma}$ = 3.2 ppm (< 6 ppm) difference in chemical shifts of the β and γ chemical

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signals suggested a *trans* geometry in Pro[1] while the large $\Delta\delta_{\beta\text{-}\gamma}$ = 8.1 ppm difference suggested a *cis* geometry in Pro[2].[11]

Due to the minute quantities of **2**–**4** obtained, the configuration at C-3 of the Dhoya/Dhoea/Dhoaa moiety was investigated using NOE correlations instead. The C-3 of the Dhoya moiety in the reported kulokainalide-1 (**2**) has the *S*-configuration.[10] Because there was no former NOE data obtained for **2**, a comparison of its NOE data with that reported for the Dhoya-containing pitipeptolide A[20] and cocosamide B[21] was carried out to determine the configuration at C-3 of the Dhoya residue in the isolated **2**. The reported NOESY spectra of pitipeptolide A and cocosamide B showed strong correlations between H-3 (δ_H 4.94) and H_3-10 (δ_H 1.15), H-4a (δ_H 1.18) and H_3-10, H-4b (δ_H 1.58) and H_3-9 (δ_H 1.29) while there were no correlations reported between H-3 and H_3-9 (Figure 3). Similarly, the NOESY spectrum of kulokainalide-1 (**2**) revealed strong NOE correlations between H-3 (δ_H 5.29) and H_3-10 (δ_H 1.23), H-4a (δ_H 1.76) and H_3-10, H-4b (δ_H 1.42) and H_3-9 (δ_H 1.14). In addition, the ^{1}H NMR signal for H-3 (δ_H 5.29) is a doublet of doublet with vicinal coupling constants of $^3J_{H3\text{-}H4a}$ = 1.9 Hz and $^3J_{H3\text{-}H4b}$ = 10.8 Hz which also indicates a gauche and anti-conformations of H-3 to H-4a and H-4b, respectively. Further evidence from the absence of correlations observed between H-3 and H_3-9 as well as the negative specific

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rotation, $[\alpha]_D$ –55°, identical to the reported kulokainalide-1 (**2**) suggested the *S* configuration at C-3 of the Dhoya unit. The configurational analysis was not performed on **3** and **4** as they possess almost identical ^1H and ^{13}C NMR signals and specific rotations. As such, compounds **3** and **4**, having the same biosynthetic pathway as **2**, likely share the same absolute configurations of the amino acids and hydroxy acids.

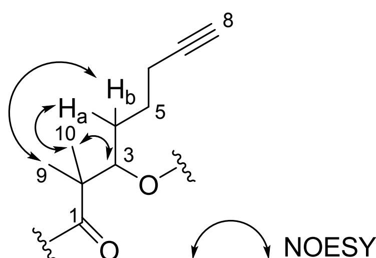

Figure 3. NOE correlations deduced from NOESY NMR spectra of the Dhoya unit in pitiprolamide A, cocosamide B and kulokainalide-1 (**2**).

Table 2. NMR Spectroscopic Data (400 MHz, 100 MHz, $CDCl_3$) for Pemukainalides A (3) and B (4)

Unit	Position	Pemukainalide A			Pemukainalide B		
		δC, type	δH (*J* in Hz)	COSY	δC, type	δH (*J* in Hz)	COSY
Pro[1]	1	170.2, C			170.2, C		
	2	58.6, CH	2.92, m	3a, 3b	58.5, CH	2.92, m	3a, 3b
	3a	28.2, CH_2	1.81, m	2, 3b	29.0, CH_2	1.81, m	2, 3b
	3b		1.65, m	2, 3a		1.64, m	2, 3a
	4a	25.0, CH_2	1.99, m	3, 4b	25.0, CH_2	1.99, m	3, 4b
	4b		1.81, m	3, 4a		1.81, m	3, 4a
	5a	47.2, CH_2	3.58, m	4, 5b	47.2, CH_2	3.59, m	4, 5b
	5b		3.55, m	4, 5a		3.56, m	4, 5a
Pro[2]	1	169.7, C			169.7, C		
	2	61.2, CH	3.89, d (7.2)	3	61.0, CH	3.87, d (7.5)	3
	3	30.2, CH_2	2.68, m	2, 4	30.2, CH_2	2.68, m	2, 4
	4a	22.1, CH_2	1.63, m	3, 4b	22.1, CH_2	1.63, m	3, 4b
	4b		1.50, m	3, 4a		1.50, m	3, 4a
	5a	46.6, CH_2	3.45, m	4, 5b	46.5, CH_2	3.46, m	4, 5b
	5b		3.41, m	4, 5a		3.42, m	4, 5a
Val[1]	1	169.8, C			169.1, C		
	2	55.6, CH	4.61, t (8.3)	*N*H, 3	55.6, CH	4.61, t (8.5)	*N*H, 3
	3	33.4, CH	2.04, m	2,4	33.3, CH	2.01, m	2,4
	4	19.5, CH_3	0.96, d (6.8)	5	19.5, CH_3	0.96, d (6.8)	5
	5	18.2, CH_3	0.86, d (7.0	4	18.2, CH_3	0.86, d (7.0)	4
	*N*H		9.08, d (7.3)	2 of Val[1]		9.07, d (7.4)	2 of Val[1]
Phe	1	170.9, C			170.9, C		
	2	54.0, CH	4.74, m	*N*H, 3a, 3b	53.7, CH	4.77, m	*N*H, 3a, 3b
	3a	34.0, CH_2	3.73, m	2	34.5, CH_2	3.74, m	2
	3b		3.17, dd (14.6, 5.2)	2		3.17, dd (14.5, 5.2)	2
	4	138.7, C			136.4, C		
	5	129.8, CH	7.28, m	6,7	128.8, CH	7.28, m	6,7

	6	127.9, CH	7.07, m	5,7	128.0, CH	7.08, m	5,7
	7	126.8, CH	7.21, m	5,6	126.8, CH	7.21, m	5,6
	8	127.9, CH	7.07, m	7,9	128.0, CH	7.08, m	7,9
	9	129.8, CH	7.28, m	7,8	128.8, CH	7.28, m	7,8
	*N*H		7.76, d (8.8)	2 of Phe		7.76, d (8.8)	2 of Phe
Dhoea/ Dhoaa	1	176.9, C			176.9, C		
	2	46.6, C			46.5, C		
	3	77.2, CH	5.27, dd (10.8, 1.9)	4	77.1, CH	5.26, dd (10.8, 1.9)	4
	4a	29.2, CH_2	1.75, m	3, 5a, 5b	29.4, CH_2	1.75, m	3, 5a, 5b
	4b		1.44, m			1.43, m	
	5a	24.4, CH_2	1.63, m	4	25.8, CH_2	1.61, m	4
	5b		1.42, m	4		1.42, m	4
	6	33.1, CH_2	2.13, m	5,7	31.4, CH_2	1.27, m	5,7
	7	138.8, CH	5.80, m	6,8	22.3, CH_2	1.27, m	6,8
	8	114.7, CH_2	4.94, m 5.01, m	4,7	13.9, CH_3	0.86, d (7.8)	4,7
	9	24.0, CH_3	1.17, s		23.9, CH_3	1.17, s	
	10	17.3, CH_3	1.22, s		17.3, CH_3	1.22, s	
Val[2]	1	175.2, C			175.2, C		
	2	55.4, CH	4.50, t (8.5)	*N*H, 3	54.9, C	4.54, m	*N*H, 3
	3	30.8, CH	2.06, m	2,4	30.6, CH	2.05, m	2,4
	4	18.2, CH_3	0.86, d (6.7)	3	18.2, CH_3	0.86, d (6.7)	3
	5	19.8, CH_3	0.93, d (6.8)	3	19.8, CH_3	0.93, d (6.8)	3
	*N*H		6.78, d (8.1)	2 of Val[2]		6.78, d (8.1)	2 of Val[2]
N-Me-Val	1	172.5, C			172.5, C		
	2	64.5, CH	4.48, d (11.6)	3	64.6, CH	4.48, d (11.6)	3
	3	27.2, CH	2.24, m	2,4	28.0, CH	2.33, m	2,4
	4	20.2, CH_3	1.06, d (6.5)	3	20.2, CH_3	1.06, d (6.5)	3
	5	19.5, CH_3	0.96, d (6.8)	3	19.5, CH_3	0.96, d (6.8)	3
	6	30.5, CH_3	3.29, s		30.4, CH_3	3.25, s	
Lac	1	169.7, C			169.7, C		
	2	69.4, CH	5.58, q (6.8)	3	69.4, CH	5.56, q (6.8)	3
	3	17.3, CH_3	1.58, d (6.8)	2	17.3, CH_3	1.58, d (6.8)	2

Compounds **1**–**4** along with dolastatin 16 were screened for their *in vitro* cytotoxicity assay based on MOLT-4 human leukemia cell line (Figure 4). Dolastatin 16 was the most cytotoxic among all the compounds with an IC_{50} value of 2.5 μM. Its analogue, triproamide (**1**) on the other hand, was not cytotoxic towards the MOLT-4 human leukemia cell line with an IC_{50} value of 23 μM. Triproamide (**1**) differs from dolastatin 16 by the replacement of Lac residue with a Hiva moiety as well as the different arrangement of the *N*-Me-Val unit in the sequence and these could be responsible for the significant change in IC_{50} value by about nine-fold. While homodolastatin 16 and kulokekahilide-1 are reported to be more cytotoxic, triproamide (**1**) ranks with pitiprolamide, which possessed weak cytotoxicity against HCT116 colon and MCF7 breast cancer cell lines.[17,18,20] A closer examination seems to suggest the specific arrangement of *N*-Me-Val/*N*-Me-Ile unit next to Pro unit might be an important structural feature that could possibly imbue cytotoxicity.

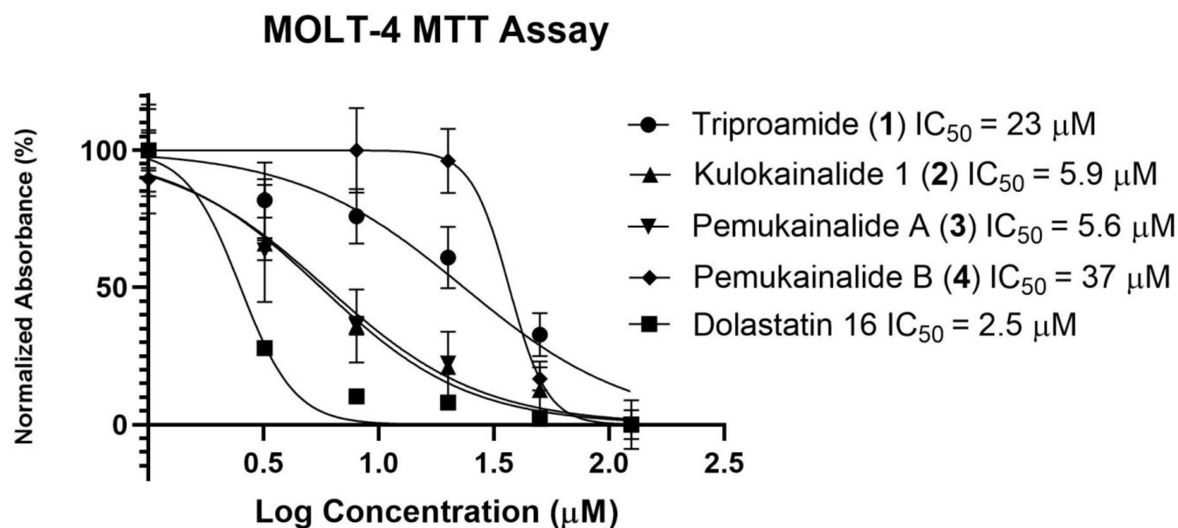

Figure 4. IC_{50} graphs of compounds **1**–**4** and dolastatin 16 for the MTT assay conducted on MOLT-4 leukemia cell line.

Pemukainalide A (**3**) and kulokainalide-1 (**2**) have similar cytotoxicity with IC_{50} values of 5.6 μM and 5.9 μM, respectively (Figure 4). Pemukainalide B (**4**) had the lowest IC_{50} against the MOLT-4 leukemia cell line with a value of 37 μM. It is interesting to observe the difference in cytotoxicity between the three compounds, especially the significant drop in IC_{50} value of pemukainalide B (**4**) having the saturated Dhoaa residue. Kulokainalide-1 (**2**) was found to possess moderate cytotoxicity against P388 mouse leukemia cell line in a prior study.[10]

Compounds **2**–**4** belong to the kulolide superfamily which is a large class of cyanobacterial molecules reported to possess varying degree of cytotoxic activities. These compounds include

yanucamides (brine shrimp cytotoxicity, LD_{50} = 5 ppm), [19] pitipeptolides (average IC_{50} value of about 2 μg/mL against LoVo cells), [20] cocosamides (MCF7 breast cancer cell line; average IC_{50} value of 35 μM), [21] wewakpeptins (LC_{50} values between 0.2–11 μM against the NCI-460 human lung tumor and neuro-2a mouse neuroblastoma cell lines), [22] trungapeptin A (no cytotoxicity against LoVo and KB cells at 10 μg/mL), [23] hantupeptins (IC_{50} values ranging from 0.2–32 μM against the MOLT-4 and MCF7 breast cancer cell lines), [24] veraguamides (e.g. veraguamide A with IC_{50} value of 141 nM against H460 human lung cancer cell line), [8,25] viequeamide A (IC_{50} value of 60 nM against H460 human lung cancer cell line), [26] kohamamides (moderate cytotoxicity towards HeLa and HL60 cell lines), [27] tiahuramides (average IC_{50} value of 10 μM towards SH-SY5Y human neuroblastoma cell line)[28] and trikoveramides (IC_{50} values of 9.3–49 μM towards MOLT-4 human leukemia cell line)[29]. In addition to their cytotoxicity activities, other biological properties, such as antibacterial (e.g. pitipeptolide F)[30] and antimalarial (e.g. kulolide-1)[31] activities have been reported, thus highlighting the potential of this class of compounds for further investigation as potential therapeutics.

A number of kulolide class of compounds (e.g. antanapeptins A–D[7]) have been reported to be isolated along with dolastatin 16, suggesting the presence of mixed polyketide synthase-

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nonribosomal peptide (PKS-NRP) biosynthetic pathways involved in the production of both classes of compounds. Herein, the discovery of three new cyanobacterial cyclic depsipeptides, including triproamide (**1**) and pemukainalides A (**3**) and B (**4**), has been described entailing the isolation, structure elucidation and biological activity evaluation of these molecules. This is also the first report of kulokainalide-1 (**2**) from a marine cyanobacterium since its discovery from the mollusk *Philinopsis speciosa*.[10]

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a Bellingham + Stanley Polarimeter while UV and IR spectra were measured on a PerkinElmer UV-Visible spectrophotometer and a PerkinElmer spectrum 100 FT-IR spectrophotometer, respectively. All NMR spectra were recorded in $CDCl_3$ on a 400 MHz Bruker NMR Spectrometer (400.13 MHz ^{1}H, 100.61 MHz ^{13}C) using residual solvent signals as internal references (referenced to residual $CDCl_3$ observed at δ_H 7.24 or δ_C 77.0). High resolution MS data and MS/MS data were acquired on Q Exactive Plus Hybrid Quadrupole-Orbitrap Mass Spectrometer (Thermo Fisher Scientific) equipped with a heated electronspray ionization (H-ESI) probe. Isolation, purification and absolute

configuration analysis of compounds **1**–**4** were conducted on a Shimadzu LC-8A preparative LC coupled to a Shimadzu SPD-M10A VP diode array detector.

Collection, Extraction and Isolation. The marine cyanobacterium, *Symploca hydnoides*,[11] was collected in September 2018 by hand from the intertidal shores of Trikora beach, Bintan Island and stored in 70% EtOH at –20 °C before workup. A voucher specimen, TLT/Tri/28Sept2018/001, is deposited at Natural Sciences and Science Education, Nanyang Technological University, Singapore. The sample (2.5 L, wet weight) was thawed and extracted exhaustively with 2:1 CH_2Cl_2/MeOH. After the solvent was evaporated *in vacuo*, 3.9 g of an organic extract was obtained. The extract was then fractionated using normal phase Si gel column chromatography based on a stepwise gradient (100% hexanes, 9:1 hexanes/EtOAc, 4:1 hexanes/EtOAc, 3:2 hexanes/EtOAc, 2:3 hexanes/EtOAc, 1:4 hexanes/EtOAc, 100% EtOAc, 9:1 EtOAc/MeOH and 8:2 EtOAc/MeOH). Fraction 8, eluted with 9:1 EtOAc/MeOH, was subjected to solid-phase fractionation on a Sep-Pak C_{18} cartridge (Phenomenex) using 100% MeOH to remove pigments. The resulting filtrate was further subjected to semipreparative HPLC separation (Shim-pak 5μm Phenyl Hexyl, 250 × 10mm, 75% MeOH/H_2O for 60 min at 3.0 mL/min, detected at 210 nm, 230 nm and 290 nm) to yield semi-pure triproamide (**1**), kulokainalide-1 (**2**), pemukainalide A (**3**) and

B (**4**). A final purification was achieved using semipreparative HPLC (Phenomenex Kinetex 5 μm C_{18}, 250 × 4.6 mm, 60% MeCN/H_2O) to yield pure triproamide (**1**, 0.7 mg, t_R = 9.21 min), kulokainalide-1 (**2**, 0.9 mg, t_R = 10.23 min), pemukainalide A (**3**, 0.5 mg, t_R = 10.47 min) and pemukainalide B (**4**, 0.5 mg, t_R = 12.01 min).

Triproamide (1): white amorphous solid; $[\alpha]_D^{20}$ –35° (*c* 0.03, MeOH); UV (MeOH) λ_{max} (log ε) 210 (4.42), 254 (1.35) nm; IR (Nujol) ν_{max} 3395, 3324, 2933, 2870, 2360, 1743, 1651, 1508, 1458, 1185, 753, 722 cm^{-1}; ^1H and ^{13}C NMR data ($CDCl_3$, 400.13 and 100.61 MHz, respectively), Table 1; HR-ESI-OrbitrapMS *m/z* 907.5564 $[M+H]^+$ (calcd for $C_{49}H_{75}N_6O_{10}$, 907.5539).

Kulokainalide-1 (2): white amorphous solid; $[\alpha]_D^{20}$ –55° (*c* 1.0, MeOH) [lit[10] –56° (*c* 1.0, MeOH)]; UV (MeOH) λ_{max} (log ε) 254 (3.25) nm; HR-ESI-OrbitrapMS *m/z* 891.5256 $[M+H]^+$ (calcd for $C_{48}H_{71}N_6O_{10}$, 891.5226).

Pemukainalide A (3): white amorphous solid; $[\alpha]_D^{20}$ –54° (*c* 0.03, MeOH); UV (MeOH) λ_{max} (log ε) 254 (3.21) nm; IR (Nujol) ν_{max} 3395, 2937, 2360, 1743, 1681, 1504, 1468, 1185 cm^{-1}; ^1H and

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4 ^{13}C NMR data ($CDCl_3$, 400.13 and 100.61 MHz, respectively), Table 2; HR-ESI-OrbitrapMS
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7 *m/z* 893.5417 $[M+H]^+$ (calcd for $C_{48}H_{73}N_6O_{10}$, 893.5382).
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11 *Pemukainalide B (4):* white amorphous solid; $[\alpha]_D^{20}$ –52° (*c* 0.03, MeOH); UV (MeOH) λ_{max} (log
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15 ε) 254 (3.24) nm; IR (Nujol) ν_{max} 3396, 2934, 2359, 1741, 1680, 1503, 1463, 1182 cm^{-1}; ^1H and
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18 ^{13}C NMR data ($CDCl_3$, 400.13 and 100.61 MHz, respectively), Table 2; HR-ESI-OrbitrapMS
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22 *m/z* 895.5574 $[M+H]^+$ (calcd for $C_{48}H_{75}N_6O_{10}$, 895.5539).
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26 **Marfey's Analysis of the Acid Hydrolysate of Triproamide (1) and Kulokainalide-1 (2).**
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29 Approximately 100 μg each of triproamide (**1**) and kulokainalide-1 (**2**) were separately hydrolysed
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33 with 0.5 mL of 6N HCl. The reaction mixture was placed in a sealed reaction vial purged with N_2
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36 gas at 105 °C for 16 h. Trace HCl was then removed *in vacuo* and the resulting hydrolysate was
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39 redissolved in 0.1 mL of H_2O. A 1% solution of L-FDAA (1-fluoro-2,4-dinitrophenyl-5-L-
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43 alaninamide) (100 μL) in acetone and 1N $NaHCO_3$ (20 μL) was added to the aqueous hydrolysate
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46 and the mixture subsequently was heated at 50 °C for 3 h. Once the resulting mixture was cooled
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50 to rt, it was quenched with 2N HCl (20 μL), then dried *in vacuo* and resuspended in 1:1 H_2O/MeCN
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53 for RP-HPLC analysis along with Marfey derivatized amino acid standards. Each HPLC analysis
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56 was carried out using a Phenomenex Kinetex C_{18} column (250 × 4.6 mm, 2.6 μm) and an isocratic
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elution at 40% MeCN – 60% 0.05 M trifluoroacetic acid with 1.0 mL/min flow rate. The retention times t_{RL}/t_{RD} in min of the L-DAA monoderivatized standards were: Pro (9.61/10.1), Val (13.3/14.7), *N*-Me-Val (17.3/21.5) and Phe (19.0/26.2). The derivatized hydrolysate peaks of **1** gave retention times at 9.49 min and 21.6 min, which corresponded to L-Pro and D-*N*-Me-Val, respectively. The derivatised hydrolysate peaks of **2** gave retention times at 9.61 min, 13.4 min, 19.3 min and 21.6 min which corresponded to L-Pro, L-Val, L-Phe and D-*N*-Me-Val, respectively.

Absolute Configuration of the 2-Hydroxy Isovaleric Acid Unit in Triproamide (1) and Lactic Acid Residue in Kulokainalide-1 (2). Approximately half of the hydrolysate of triproamide (**1**) and kulokainalide-1 (**2**) obtained previously during Marfey's analysis was concentrated to dryness and analysed by chiral-phase HPLC column (Phenomenex Chirex 3126 (D)-penicillamine, 50 × 4.6 mm), flow rate 1.0 mL/min, detection at 254 nm, with 1 mM $CuSO_4$/IPA (85:15). Hydroxyisovaleric acid in the hydrolysate of **1** eluted at t_R = 26.9 min corresponding to the retention time of an authentic standard of D-hydroxyisovaleric acid and therefore indicating an *R* configuration (t_R of L-hydroxyisovaleric acid = 21.9 min). Lactic acid in the hydrolysate of **2** eluted at t_R = 6.70 min corresponding to the retention time of an authentic standard of L-lactic acid and therefore indicating an *S* configuration (t_R of D-lactic acid = 7.01 min).

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Absolute Configuration of the 2-Amino-4-Phenylisovaleric acid (Dpv) Unit in Triproamide (1).

Acid hydrolysis of triproamide (**1**, 100 µg) and dolastatin 16 (100 µg) was achieved in 0.5 mL of 6N HCl placed in a sealed reaction vial purged with N_2 gas at 108 °C for 16 h. Trace HCl was then removed *in vacuo* and the resulting hydrolysate was redissolved in 0.1 mL of H_2O. A 1% solution of L-FDAA (100 µL) in acetone and 1N $NaHCO_3$ (20 µL) was added to the aqueous hydrolysate and the mixture subsequently was heated at 50 °C for 3 h. Once the resulting mixture was cooled to rt, it was quenched with 2N HCl (50 µL), then dried *in vacuo* and resuspended in 1:1 H_2O/MeCN for HR-LCMS analysis. The retention times and ESIMS protonated molecules ($t_{R(\mathrm{Dpv})}$ in min, *m/z* $[M+H]^+$) of the Marfey-derivatized Dpv in the hydrolysate of **1** (10.1 min, 446.2) and dolastatin 16 (10.1 min, 446.2) were observed at 10.1 min, *m/z* 446.2. This suggested both **1** and dolastatin 16 have identical stereochemistry of the Dpv unit, corresponding to 2*S*, 3*R* of the dolaphenvaline unit.[15]

MOLT-4 Cancer Cell Line Assay. Assessment of the cytotoxicity of compounds **1**–**4** and dolastatin 16 was carried out using the MTT bioassay based on the MOLT-4 (T lymphoblast; acute lymphoblastic leukemia), cancer cell line over a 3-day procedure. On the first day, each compound was prepared in a 96-well microtiter plate at 10 mM stock concentration dissolved in 100% DMSO, conducted in triplicate. The mixtures were then added with RPMI media, supplemented with fetal calf serum; and serial diluted to give concentrations of 125, 50, 20, 8, and 3.2 µM. To each of the concentration, 10 µL of the diluted compound was added with 70 µL of the cancer cells. The plate was incubated for 24 h in a 37 °C, 5% CO_2 incubator. On day 2, 20 µL of MTT solution were

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added to each of the wells and incubated for 3 h. Another 100 μL of lysing buffer was added to each well thereafter and incubated overnight. On day 3, the microtiter plate was measured at OD 570 nm and the results were tabulated, sigmoidal curves were generated and IC_{50} values were calculated, using the software GraphPad Prism 8.

ASSOCIATED CONTENT

Supporting Information. The Supporting Information is available free of charge at XXXX.

HRMS and HR MS/MS spectra of triproamide (**1**); ^{1}H, ^{13}C, DEPT 90, DEPT 135, COSY, HSQC, NOESY, HMBC, HSQC-HECADE NMR spectra of **1** in $CDCl_3$; ^{1}H, NOESY and HSQC-HECADE NMR spectra of dolastatin 16 in $CDCl_3$; HRMS and HR MS/MS spectra of kulokainalide-1 (**2**), pemukainalides A (**3**) and B (**4**); ^{1}H, ^{13}C, COSY, HSQC, HMBC and NOESY NMR spectra of **2** in $CDCl_3$; ^{1}H, ^{13}C, DEPT 135, DEPT 90, COSY, HSQC, HMBC NMR spectra of **3** in $CDCl_3$; ^{1}H, ^{13}C, DEPT 135, DEPT 90, COSY, HSQC, HMBC NMR spectra of **4** in $CDCl_3$; HPLC Chromatograms of Marfey's and chiral-phase HPLC analyses; Table showing ^{1}H and ^{13}C NMR comparison of Dml unit in triproamide (**1**) and dolastatin 16. (PDF)

Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Kumar, J.; Singh, D.; Tyagi, M. B.; Kumar, A. Cyanobacteria: Applications in Biotechnology. In *Cyanobacteria: From Basic Science to Applications*; Mishra, A. K., Tiwari, D. N., Rai, A. N., Eds.; Academic Press: London, **2019**; pp 327–346.

(2) Shah, S. A. A.; Akhter, N.; Auckloo, B. N.; Khan, I.; Lu, Y.; Wang, K.; Wu, B.; Guo, Y. W. Structural diversity, biological properties and applications of natural products from cyanobacteria. A review. *Mar. Drugs* **2017**, *15*.

(3) Pennings, S. C.; Paul, V. J. Sequestration of dietary secondary metabolites by three species of sea hares: location, specificity and dynamics. *Mar. Biol.* **1993**, *117*, 535–546.

(4) Pettit, G. R.; Kamano, Y.; Herald, C. L.; Tuinman, A. A.; Boettner, F. E.; Kizu, H.; Schmidt, J. M.; Baczynskyj, L.; Tomer, K. B.; Bontems, R. J. The isolation and structure of a remarkable marine animal antineoplastic constituent: dolastatin 10. *J. Am. Chem. Soc.* **1987**, *109*, 6883–6885.

(5) Luesch, H.; Moore, R. E.; Paul, V. J.; Mooberry, S. L.; Corbett, T. H. Isolation of dolastatin 10 from the marine cyanobacterium *Symploca* species VP642 and total stereochemistry and biological evaluation of its analogue symplostatin 1. *J. Nat. Prod.* **2001**, *64*, 907–910.

(6) Pettit, G. R.; Xu, J.-P.; Hogan, F.; Williams, M. D.; Doubek, D. L.; Schmidt, J. M.; Cerny, R. L.; Boyd, M. R. Isolation and structure of the human cancer cell growth inhibitory cyclodepsipeptide dolastatin 16. *J. Nat. Prod.* **1997**, *60*, 752–754.

(7) Nogle, L. M.; Gerwick, W. H. Isolation of four new cyclic depsipeptides, antanapeptins A−D, and dolastatin 16 from a Madagascan collection of *Lyngbya majuscula*. *J. Nat. Prod.* **2002**, *65*, 21–24.

(8) Salvador, L. A.; Biggs, J. S.; Paul, V. J.; Luesch, H. Veraguamides A−G, cyclic hexadepsipeptides from a dolastatin 16-producing cyanobacterium *Symploca* cf. *hydnoides* from Guam. *J. Nat. Prod.* **2011**, *74*, 917–927.

(9) Montaser, R.; Abboud, K. A.; Paul, V. J.; Luesch, H. Pitiprolamide, a proline-rich dolastatin 16 analogue from the marine cyanobacterium *Lyngbya majuscula* from Guam. *J. Nat. Prod.* **2011**, *74*, 109–112.

(10) Nakao, Y.; Yoshida, W. Y.; Szabo, C. M.; Baker, B. J.; Scheuer, P. J. More peptides and other diverse constituents of the marine mollusk *Philinopsis speciosa*. *J. Org. Chem.* **1998**, *63*, 3272–3280.

(11) Phyo, M. Y.; Ding, C. Y. G.; Goh, H. C.; Goh, J. X.; Ong, J. F. M.; Chan, S. H.; Yung, P. Y. M.; Candra, H.; Tan, L. T. Trikoramide A, a prenylated cyanobactin from the marine cyanobacterium *Symploca hydnoides*. *J. Nat. Prod.* **2019**, *82*, 3482–3488.

(12) Siemion, I. Z.; Wieland, T.; Pook, K. H. Influence of the distance of the proline carbonyl from the beta and gamma carbon on the ^{13}C chemical shifts. *Angew. Chem. Int. Ed. Engl.* **1975**, *14*, 702–703.

(13) Marfey, P. Determination of D-amino acids. II. Use of a bifunctional reagent, 1,5-difluoro-2,4-dinitrobenzene. *Carlsberg Res. Commun.* **1984**, *49*, 591.

(14) Tan, L. T.; Goh, B. P.; Tripathi, A.; Lim, M. G.; Dickinson, G. H.; Lee, S. S.; Teo, S. L. Natural antifoulants from the marine cyanobacterium *Lyngbya majuscula*. Biofouling **2010**, *26*, 685–695.

(15) Casalme, L. O.; Yamauchi, A.; Sato, A.; Petitbois, J. G.; Nogata, Y.; Yoshimura, E.; Okino, T.; Umezawa, T.; Matsuda, F. Total synthesis and biological activity of dolastatin 16. *Org. Biomol. Chem.* **2017**, *15*, 1140–1150.

(16) Koźmiński, W.; Nanz, D. HECADE: HMQC- and HSQC-based 2D NMR experiments for accurate and sensitive determination of heteronuclear coupling constants from E.COSY-type cross peaks. *J. Magn. Reson.* **1997**, *124*, 383–392.

(17) Davies-Coleman, M. T.; Dzeha, T. M.; Gray, C. A.; Hess, S.; Pannell, L. K.; Hendricks, D. T.; Arendse, C. E. Isolation of homodolastatin 16, a new cyclic depsipeptide from a Kenyan collection of *Lyngbya majuscula. J. Nat. Prod.* **2003**, *66* (5), 712–715.

(18) Kimura, J.; Takada, Y.; Inayoshi, T.; Nakao, Y.; Goetz, G.; Yoshida, W. Y.; Scheuer, P. J. Kulokekahilide-1, a cytotoxic depsipeptide from the cephalaspidean mollusk *Philinopsis speciosa. J. Org. Chem.* **2002**, *67*, 1760–1767.

(19) Sitachitta, N.; Williamson, R. T.; Gerwick, W. H. Yanucamides A and B, two new depsipeptides from an assemblage of the marine cyanobacteria *Lyngbya majuscula* and *Schizothrix* species. *J. Nat. Prod.* **2000**, *63*, 197–200.

(20) Luesch, H.; Pangilinan, R.; Yoshida, W. Y.; Moore, R. E.; Paul, V. J. Pitipeptolides A and B, new cyclodepsipeptides from the marine cyanobacterium *Lyngbya majuscula. J. Nat. Prod.* **2001**, *64*, 304–307.

(21) Gunasekera, S. P.; Owle, C. S.; Montaser, R.; Luesch, H.; Paul, V. J. Malyngamide 3 and cocosamides A and B from the marine cyanobacterium *Lyngbya majuscula* from Cocos Lagoon, Guam. *J. Nat. Prod.* **2011**, *74*, 871–876.

(22) Han, B.; Goeger, D.; Maier, C. S.; Gerwick, W. H. The wewakpeptins, cyclic depsipeptides from a Papua New Guinea collection of the marine cyanobacterium *Lyngbya semiplena. J. Org. Chem.* **2005**, *70*, 3133–3139.

(23) Bunyajetpong, S.; Yoshida, W. Y.; Sitachitta, N.; Kaya, K. Trungapeptins A−C, cyclodepsipeptides from the marine cyanobacterium *Lyngbya majuscula. J. Nat. Prod.* **2006**, *69*, 1539–1542.

(24) (a) Tripathi, A.; Puddick, J.; Prinsep, M. R.; Lee, P. P. F.; Tan, L. T. Hantupeptin A, a cytotoxic cyclic depsipeptide from a Singapore collection of *Lyngbya majuscula. J. Nat. Prod.* **2009**, *72*, 29–32. (b) Tripathi, A.; Puddick, J.; Prinsep, M. R.; Lee, P. P. F.; Tan, L. T.

Hantupeptins B and C, cytotoxic cyclodepsipeptides from the marine cyanobacterium *Lyngbya majuscula*. *Phytochemistry* **2010**, *71*, 307–311.

(25) Mevers, E.; Liu, W.-T.; Engene, N.; Mohimani, H.; Byrum, T.; Pevzner, P. A.; Dorrestein, P. C.; Spadafora, C.; Gerwick, W. H. Cytotoxic veraguamides, alkynyl bromide-containing cyclic depsipeptides from the marine cyanobacterium cf. *Oscillatoria margaritifera*. *J. Nat. Prod.* **2011**, *74*, 928–936.

(26) Boudreau, P. D.; Byrum, T.; Liu, W.-T.; Dorrestein, P. C.; Gerwick, W. H. Viequeamide A, a cytotoxic member of the kulolide superfamily of cyclic depsipeptides from a marine button cyanobacterium. *J. Nat. Prod.* **2012**, *75*, 1560–1570.

(27) Iwasaki, A.; Shiota, I.; Sumimoto, S.; Matsubara, T.; Sato, T.; Suenaga, K. Kohamamides A, B, and C, cyclic depsipeptides from an *Okeania* sp. marine cyanobacterium. *J. Nat. Prod.* **2017**, *80*, 1948–1952.

(28) Levert, A.; Alvariño, R.; Bornancin, L.; Abou Mansour, E.; Burja, A. M.; Genevière, A.-M.; Bonnard, I.; Alonso, E.; Botana, L.; Banaigs, B. Structures and activities of tiahuramides A–C, cyclic depsipeptides from a Tahitian collection of the marine cyanobacterium *Lyngbya majuscula*. *J. Nat. Prod.* **2018**, *81*, 1301–1310.

(29) Phyo, M. Y.; Katermeran, N. P.; Goh, J. X.; Tan, L. T. Trikoveramides A–C, cyclic depsipeptides from the marine cyanobacterium *Symploca hydnoides*. *Phytochemistry* **2021**, *190*, 112879.

(30) Montaser, R.; Paul, V. J.; Luesch, H. Pitipeptolides C–F, antimycobacterial cyclodepsipeptides from the marine cyanobacterium *Lyngbya majuscula* from Guam. *Phytochemistry* **2011**, *72*, 2068–2074.

(31) Goto, Y.; Kamihira, R.; Nakao, Y.; Nonaka, M.; Takano, R.; Xuan, X.; Kato, K. The efficacy of marine natural products against *Plasmodium falciparum*. *J. Parasitol.* **2021**, *107*, 284–288.

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