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NOTE

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Malyngamide 3 and Cocosamides A and B from the Marine Cyanobacterium *Lyngbya majuscula* from Cocos Lagoon, Guam

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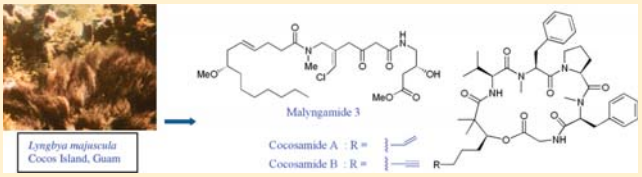
Supporting Information

ABSTRACT: Malyngamide 3 (**1**) and cocosamides A (**2**) and B (**3**) were isolated from the lipophilic extract of a collection of *Lyngbya majuscula* from Cocos Lagoon, Guam. The planar structures of compounds **1**−**3** were determined by spectroscopic methods. The absolute configuration of **1** was determined by modified Mosher's method, NOESY data, and comparison with lyngbic acid (**4**). The absolute configurations of **2** and **3** were assigned by enantioselective HPLC analysis and comparison with the closely related compound pitipeptolide A (**5**). Compounds **1**−**3** showed weak cytotoxicity against MCF7 breast cancer and HT-29 colon cancer cells.



Marine cyanobacteria of the genus *Lyngbya* are a prolific source of chemically diverse bioactive secondary metabolites.[1] Malyngamides are small amides first discovered in the late 1970s and early 1980s from *L. majuscula* by Richard E. Moore's research group.[2−6] There are now over 30 known examples of malyngamides, and the majority are reported from cyanobacteria.[7] Recently, William Gerwick's group reported the newest addition, malyngamide 2, isolated from *L. sordida* collected from Papua New Guinea.[8] Malyngamides are characterized by a fatty acid side chain, which is most commonly 7*S*-methoxytetradec-4(*E*)-enoic acid (lyngbic acid). The other part of the malyngamides usually encloses a cyclic unit. In one notable example (malyngamide J) the cyclic ketone has a pendant 2,4-dimethoxyxylose.[9] Malyngamides O and P are the only examples[10] of acyclic molecules in this series. Malyngamide 3 (**1**) described here is the next example of an acyclic malyngamide.

Cyclic depsipeptides containing a unique 2,2-dimethyl-3-hydroxy-7-octynoic acid (Dhoya), 2,2-dimethyl-3-hydroxy-7-octenoic acid (Dhoea), or 2,2-dimethyl-3-hydroxyoctanoic acid (Dhoaa) were first reported by Scheuer's group from a marine mollusk.[11,12] Subsequently, Richard Moore's[13] and William Gerwick's[14] groups have isolated several of these unique cyclic depsipeptides from *L. majuscula*. Here, we report the isolation, structure determination, and biological activity determination of two new cyclic depsipeptides that possess these distinctive moieties (Dhoea/Dhoya), namely, cocosamides A (**2**) and B (**3**), from *L. majuscula* collected from Cocos Lagoon, Guam. Interestingly, this is the first report of cyclic depsipeptides in this series with one ester linkage, while other related compounds reported thus far with these unique acids have two or more ester linkages.[11−14]

The sample of the marine cyanobacterium *L. majuscula* was collected from a patch reef near Cocos Island, Guam, in February 2001. The freeze-dried material was extracted with a mixture of EtOAc−MeOH (1:1) to afford a lipophilic extract, which was subsequently partitioned between EtOAc and H_2O. The EtOAc-soluble portion was repeatedly fractionated by SiO_2 chromatography followed by reversed-phase C18 HPLC to give three new compounds, malyngamide 3 (**1**) and cocosamides A (**2**) and B (**3**), in addition to the known compounds malyngamide A,[4] malyngamide B,[3] and an unresolved mixture of majusculamides A and B.[15]

Malyngamide 3 (**1**) was obtained as a colorless, amorphous powder. The molecular formula $C_{28}H_{47}ClN_2O_7$ was determined from HRESIMS data. Its infrared spectrum contained absorption due to an amide proton at 3320 cm^{-1}, an ester carbonyl at 1725 cm^{-1}, and an amide carbonyl at 1636 cm^{-1}. The ^1H and ^{13}C NMR spectra (Table 1) showed signature signals for the presence of the characteristic 7-methoxytetradec-4(*E*)-enoic acid moiety, suggesting compound **1** to be an analogue of the malyngamides.

Following the interpretation of DQF COSY and edited HSQC experiments, the ^1H and ^{13}C NMR signals were assignable to three partial structures, C-1 to C-4, C-8 to C-10, and C-2′ to C-14′, and an isolated C-6 methylene group. In addition, the ^1H, ^{13}C, and edited HSQC spectra indicated the presence of signals for two *O*-Me groups (C-12, δ_H 3.68, δ_C 51.9 and C-15′, δ_H 3.30, δ_C 56.5), one *N*-Me (C-13, δ_H 2.93, δ_C 35.5), and four carbonyl groups (C-5, δ_C 202.2; C-7, δ_C 166.6; C-11, δ_C 172.4; and C-1′, δ_C 174.0). The chemical shift values for C-2 (δ_C 131.4, C) and C-3 (δ_H 6.08, s; δ_C 120.7, CH) indicated the presence of a chloromethylene moiety in the C-1 to C-4 partial structure as in other malyngamides[4,5,16] and accounted for the chlorine atom in the molecular formula. HMBC correlations (Table 1) from H-3 (δ_H 6.08) to C-1 (δ_C 45.9), C-2 (δ_C 131.4), and C-4 (δ_C 46.8) confirmed the position of the chloromethylene moiety. Similarly,

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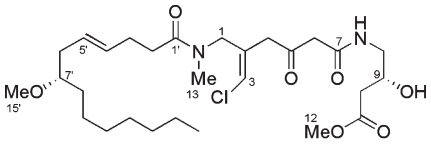
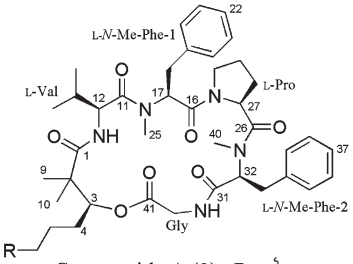
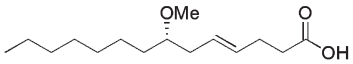


Malyngamide 3 (**1**)

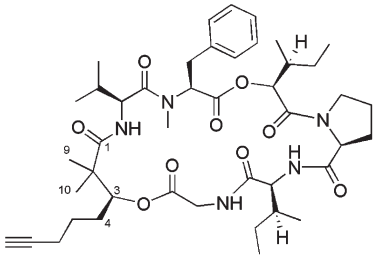


Cocosamide A (**2**) : R = 

Cocosamide B (**3**) : R =



Lyngbic acid (**4**)



Pitipeptolide A (**5**)

HMBC correlations from H-4a and H-4b (δ_H 3.20, 3.15) to C-5 (δ_C 202.2) and H_2-6 (δ_H 3.39) to the C-5 and C-7 (δ_C 166.6) carbonyl groups extended the carbon chain to the amide carbonyl group. The HMBC correlations of N-H (δ_H 7.26) to C-7 and C-8 (δ_C 45.2), H-9 (δ_H 4.17) to C-8, C-10 (δ_C 38.9), and C-11 (δ_C 172.4), and H_3-12 (δ_H 3.68) to the C-11 carbonyl group established the planar structure for the right-hand end of the molecule. The HMBC correlations from H_3-13 (δ_H 2.93) to the C-1′ carbonyl (δ_C 174.0) and to C-1 connected the fatty acid chain to the right-hand end of the molecule via an amide linkage, resembling other malyngamides. An *E* configuration was assigned for the C-4′/C-5′ olefin on the basis of the coupling constant (15.7 Hz).[17] The NOESY spectrum of **1** did not show any cross-peaks between H-3 and H_2-1 nor between H-3 and H_3-13. However, the presence of a strong cross-peak between H-3 (δ_H 6.08) and H-4b (δ_H 3.15) established a *Z* configuration for the chloromethylene moiety in **1** as in isomalyngamides A and B.[17] In order to determine the configuration at C-7′, compound **1** was hydrolyzed under basic conditions to give lyngbic acid (**4**). The observed specific rotation of **4** ($[\alpha]^{25}_D$ −12) was comparable to the reported value for 7(*S*)-methoxytetradec-4(*E*)-enoic acid ($[\alpha]^{20}_D$ −12.6)[16] and, thus, established a 7(*S*) configuration at the C-7′ position in **1**. The absolute configuration at C-9 of **1** was determined by the modified Mosher's method.[18] Compound **1** was converted to (*S*)- and (*R*)-MTPA esters. The $\Delta\delta$ (= $\delta_S - \delta_R$) values of (*S*)- and (*R*)-MTPA esters (−0.078 for Ha-8; −0.031 for Hb-8; +0.028 for H_2-10; +0.072 for CH_3-12) revealed the *R* configuration at C-9. These data confirmed the structure **1** for malyngamide 3.

Cocosamides A (**2**) and B (**3**) were obtained as white solids. The molecular weights of **2** and **3** differ by two mass units on the basis of HRESI/TOFMS analysis. The ^{1}H and ^{13}C NMR spectra were indicative of depsipeptides (Table 2).

Following the interpretation of DQF COSY, edited HSQC, and HMBC experiments, the ^{1}H and ^{13}C NMR signals of **2** and **3** were assignable to six partial structures, which accounted for all atoms in both molecules. These partial structures consisted of the amino acids valine, proline, glycine, and two *N*-Me-phenylalanines, besides 2,2-dimethyl-3-hydroxy-7-octenoic acid (Dhoea) in **2** and 2,2-dimethyl-3-hydroxy-7-octynoic acid (Dhoya) in **3**. The ^{1}H NMR spectra showed the presence of three olefinic protons (δ_H 5.76, H-7; δ_H 4.96, 5.04, H-8a, b) in the spectrum of **2**, while no olefinic protons were seen in the spectrum of **3**, which instead revealed a characteristic acetylenic proton (δ_H 1.96, t, *J* = 2.7 Hz, H-8). The ^{13}C spectrum of **2** indicated olefinic signals (δ_C 138.0, CH, C-7; δ_C 115.2, CH_2, C-8), while the ^{13}C spectrum of **3** indicated acetylenic signals (δ_C 83.6, C, C-7; δ_C 69.2, CH, C-8). These data together with other data presented in Table 2 confirmed the presence of a 2,2-dimethyl-3-hydroxy-7-octenoic acid in **2** and 2,2-dimethyl-3-hydroxy-7-octynoic acid in **3**, respectively. The residue sequences for **2** and **3** were determined from HMBC data, which showed linkages Val-NH to C-1, Me-25 to C-11, CH_2-30 to C-16, Me-40 to C-26, Gly-NH to C-31, and H-3 to C-41, and these connections were confirmed by NOESY correlations. These data established the residue sequences as 1,6-anhydro[Dhoea-Val-*N*-Me-Phe(1)-Pro-*N*-Me-Phe(2)-Gly] for **2** and 1,6-anhydro[Dhoya-Val-*N*-Me-Phe(1)-Pro-*N*-Me-Phe(2)-Gly] for **3**. The absolute configurations of the amino acids were determined by enantioselective HPLC analysis of the acid hydrolysates of **2** and **3**. The analysis revealed L-configurations for valine, proline, and both *N*-Me-phenylalanines in compounds **2** and **3**. Because we have isolated only small quantities of **2** and **3**, the configuration at C-3 of the hydroxy acids (Dhoea and Dhoya) was investigated by comparison of NOE data with pitipeptolide A (**5**).[13] Pitipeptolide A (**5**) is a cyclic depsipeptide that has a (*S*)-Dhoya moiety, which is connected to L-valine and glycine, forming amide and ester linkages similar to compounds **2** and **3**. The NOE data for **5** were not previously reported;[13] therefore, we used **5** that we isolated from another *Lyngbya* sample for NOE comparison studies. The ^{1}H NMR spectrum, HRMS, and specific rotation data for this sample matched those reported in the literature.[13,19] The NOESY spectrum of **5** showed a strong correlation between H-3 (δ_H 4.94) and H_3-10 (δ_H 1.15) and another correlation between H-4a (δ_H 1.80) and H_3-10. Similarly, strong correlations were observed between H-4b (δ_H 1.58) and H_3-9 (δ_H 1.29) and methyl H_3-9 and L-Val-NH (δ_H 6.08). There was no correlation seen between H-3 and H_3-9. These data clearly indicated that in **5** the methine (H-3) is closer to methyl H_3-10 and away from methyl H_3-9. Cocosamides A (**2**) and B (**3**) showed the same patterns of NOE correlations for H-3, H_3-9, and H_3-10. These data suggested a 3*S* configuration at C-3 in compounds **2** and **3**.

Compounds **1**−**3** were tested for antiproliferative activity against MCF7 breast cancer and HT-29 colon cancer cells and found to be weakly active. Malyngamide 3 (**1**) showed cytotoxic

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Table 1. NMR Spectroscopic Data for Malyngamide 3 (1) in $CDCl_3$ (^1H 600 MHz, ^{13}C 150 MHz)

position	δ_C mult.	δ_H (J in Hz)	COSY[a]	HMBC	NOESY[b]
1a	45.9, CH_2	4.24, d (15.1)		2, 3, 4, 1′	13
1b		4.17, d (15.1)	3	2, 3, 4, 1′	13
2	131.4, C				
3	120.7, CH	6.08, s	1, 4	1, 2, 4	4b
4a	46.8, CH_2	3.20, d (17.1)	3	1, 2, 3, 5	3, 6
4b		3.15, d (17.1)	3	1, 2, 3, 5	1b, 3, 6
5	202.2, C				
6	49.7, CH_2	3.39, s		5, 7	4, NH
7	166.6, C				
8a	45.2, CH_2	3.52, ddd (14.2, 7.0, 5.4)	NH, 9	7, 9, 10	9, NH
8b		3.21, ddd (14.2, 9.0, 5.4)	NH, 9	7, 9, 10	9, NH
9	67.2, CH	4.17, m	8, 10	8, 10, 11	8a, 8b, 10
10	38.9, CH_2	2.50, d (6.9)	9	8, 9, 11	8a, 8b, 9
11	172.4, C				
12	51.9, CH_3	3.68, s		11	
13	35.5, CH_3	2.93, s		1, 1′	1a, 1b, 2′
N-H		7.26, t (5.4)	8	7, 8	6, 8a, 8b
1′	174.0, C				
2′	33.1, CH_2	2.35, m	3′	1′, 3′, 4′	13, 4′
3′	28.1, CH_2	2.28, m	2′, 4′	1′, 2′, 4′, 5	5′
4′	130.8, CH	5.43, dt (15.7, 3.5)		3′, 5′	2′, 6′
5′	127.6, CH	5.49, dt (15.7, 3.5)		3′, 4′, 6′	3′, 7′, 15′
6′	36.4, CH_2	2.17, m	5, 7	4′, 5′, 7′, 8′	4′, 7′
7′	80.8, CH	3.14, m	6, 8	5′, 9′, 15′	5′, 6′, 15′
8′	33.4, CH_2	1.41, m	7, 9	6′, 7′, 9′, 10′	
9′	25.4, CH_2	1.32, m	8, 10	8′, 10′	
		1.26, m			
10′	29.4, CH_2	1.27, m			
11′	29.9, CH_2	1.27, m			
12′	31.9, CH_2	1.27, m			
13′	22.7, CH_2	1.27, m			
14′	14.2, CH_3	0.86, t (6.9)	13	12′, 13′	
15′	56.5, CH_3	3.30, s		7′	5′, 7′

[a] $^1H-^1H$ COSY correlations are from proton(s) stated to the indicated proton(s). [b] NOESY correlations are from proton(s) stated to the indicated proton(s).

activity against MCF7 and HT-29 cells with IC_{50} values of 29 and 48 μM, respectively. This is an about 10-fold weaker activity than reported for the closely related analogue malyngamide O.[10] Cocosamides A (**2**) and B (**3**) showed cytotoxic activity against HT-29 cells with IC_{50} values of 24 and 11 μM, respectively. MCF7 cells were slightly less susceptible to both compounds, with IC_{50} values of 30 μM for **2** and 39 μM for **3**. The closely related pitipeptolides A and B exert similar activity against cancer cells.[13]

EXPERIMENTAL SECTION

General Experimental Procedures. The optical rotations were recorded on a Perkin-Elmer model 343 polarimeter. UV spectrophotometric data were acquired on a Hitachi U-3010 spectrophotometer. IR spectroscopic data were obtained on a Bruker Vector 22 FT-IR spectrometer. NMR data were collected on a JEOL ECA-600 spectrometer operating at 600.17 MHz for ^1H and 150.9 MHz for ^{13}C. The edited-HSQC experiment was optimized for J_{CH} = 140 Hz, and the HMBC spectrum was optimized for $^{2/3}J_{CH}$ = 8 Hz. ^1H NMR chemical shifts (referenced to residual $CHCl_3$ observed at δ 7.25) were assigned using a combination of data from 2D DQF COSY and multiplicity-edited HSQC experiments. Similarly, ^{13}C NMR chemical shifts (referenced to $CDCl_3$ observed at δ 77.0) were assigned on the basis of multiplicity-edited HSQC experiments. The HRMS data were obtained using an Agilent 6210 LC-TOF mass spectrometer equipped with an APCI/ESI multimode ion source detector at the Mass Spectrometer Facility at the University of California, Riverside, CA. Silica gel 60 (EMD Chemicals, Inc. 230−400 mesh) was used for column chromatography. All solvents used were of HPLC grade (Fisher Scientific).

Collection, Extraction, and Isolation. The sample of cyanobacterial assemblage of *Lyngbya majuscula* for this study was collected in February 2001 from a patch reef near Cocos Island, Guam. This was a collection of ECO 27, first collected in March 1999. This chemotype of *L. majuscula* grew during winter months on Guam (January−March) and consistently produced malyngamides A and B and majusculamides A and B. The samples were identified by one of us (V.J.P.) based on morphological characteristics of the genus, and a voucher specimen (VP-ECO 27) is maintained at the Smithsonian Marine Station, Fort Pierce,

Table 2. NMR Spectroscopic Data for Cocosamides A (2) and B (3) in $CDCl_3$ (^1H 600 MHz, ^{13}C 150 MHz)

		cocosamide A (**2**)				cocosamide B (**3**)	
unit	position	δ_C mult.	δ_H (J in Hz)	HMBC[a]	NOESY[b]	δ_C mult.	δ_H (J in Hz)
Dhoea[c]/Dhoya[d]	1	176.5, C				176.5, C	
	2	48.9, C				46.3, C	
	3	77.8, CH	5.19, dd (11.0, 2.1)	1, 9, 41	4a, 4b, 10	77.3, CH	5.20, br. d (11.0)
	4a	27.9, CH_2	1.54, m	5	3, 10	27.6, CH_2	1.75, m
	4b		1.48, m	5	3, 9		1.58, m
	5	25.0, CH_2	1.34, m	7	3, 6	24.6, CH_2	1.51, m
	6	33.3, CH_2	2.06, m		5, 7	18.1, CH_2	2.21, m
	7	138.0, CH	5.76, ddt (17.0, 11.6, 6.9)	6	6, 8a	83.6, C	
	8a	115.2, CH_2	4.96, dd (11.6, 3.4)	6	7	69.2, CH	1.96, t (2.7)
	8b		5.04, dd (17.0, 3.4)	6			
	9	17.5, CH_3	1.25, s	1, 3, 10	4a, 4b, NH (Val)	17.6, CH_3	1.28, s
	10	23.5, CH_3	1.17, s	1, 3, 9	3, 4a, 9	23.5, CH_3	1.20, s
Val	11	172.2, C				172.3, C	
	12	55.5, CH	4.33, dd (7.7, 7.5)	1, 11, 14, 15	13, NH (Val), 25	55.6, CH	4.32, dd (7.7, 7.5)
	13	30.5, CH	1.91, m	12	12, 14, 15	30.6, CH	1.93, m
	14	19.1, CH_3	0.97, d (6.9)	12, 13, 15	12, 13, 15	19.2, CH_3	0.97, d (6.9)
	15	18.7, CH_3	0.88, d (6.9)	12, 13, 14	12, 13, 14	18.8, CH_3	0.90, d (6.9)
	NH		5.82, d (7.5)	1, 12	9, 12, 13, 14		5.83, d (7.6)
N-Me-Phe-1	16	168.9, C				169.0, C	
	17	54.2, CH	5.08, dd (12.4, 3.9)		18a, 18b, 25, 27	54.3, CH	5.03, br d (12.4)
	18a	37.7, CH_2	3.18, dd (12.4, 12.3)	16, 19, 20/24	17, 20/24, 25	37.8, CH_2	3.18, dd (12.4, 12.3)
	18b		3.02, dd (12.4, 3.9)	16, 19, 20/24	17, 20/24, 25		3.02, dd (12.4, 3.9)
	19	137.6, C				137.6, C	
	20/24	129.8, CH	7.41, d (7.6, 2.7)	18	17, 18, 21/23	129.9, CH	7.40, d (7.6, 2.7)
	21/23	128.5, CH	7.24, m	19	20/24, 22	128.6, CH	7.24, m
	22	127.0, CH	7.18, m	20/24	21/23	127.0, CH	7.18, m
	25	32.3, CH_3	3.58, s	11, 17	12, 17, 18a	32.3, CH_3	3.58, s
Pro	26	171.5, C				171.6, C	
	27	56.0, CH	3.08, dd (8.2, 1.8)	28, 29, 30	17, 28a, 32	56.1, CH	3.06, dd (8.2, 1.8)
	28a	29.8, CH_2	0.46, m	26	27, 28b, 29b	29.9, CH_2	0.47, m
	28b		−0.18, m		28a, 32		−0.19, m
	29a	21.9, CH_2	1.31, m		28b, 29b, 30a	22.0, CH_2	1.31, m
	29b		1.21, m		28a, 29a, 30b		1.21, m
	30a	46.2, CH_2	3.38, m	16	29a, 30b	46.0, CH_2	3.37, m
	30b		3.22, m	16	29b, 30a		3.21, m
N-Me-Phe-2	31	169.4, C				169.5, C	
	32	63.5, CH	3.93, dd (9.7, 3.5)	31, 33, 40	27, 33, NH (Gly)	63.6, CH	3.94, dd (9.7, 3.5)
	33a	34.9, CH_2	3.68, dd (12.0, 3.5)	32, 34, 35/39	32, 33b, 35/39	35.0, CH_2	3.68, dd (12.0, 3.5)
	33b		2.75, dd (12.0, 9.7)	32, 34, 35/39	32, 33a, 35/39		2.75, dd (12.0, 9.7)
	34	138.1, C				138.2, C	
	35/39	129.4, CH	7.05, d (7.6)	33, 37	33, 36/38	129.5, CH	7.05, d (7.6)
	36/38	128.8, CH	7.23, m	34	35/39, 37	128.9, CH	7.23, m
	37	127.0, CH	7.22, m	35, 39	36/38	127.1, CH	7.22, m
	40	31.0, CH_3	2.82, s			31.0, CH_3	2.82, s
Gly	41	168.3, C				168.4, C	
	42a	41.9, CH_2	4.80, dd (16.8, 8.9)	41	42b, NH (Gly)	41.9, CH_2	4.78, dd (16.8, 8.9)
	42b		3.66, dd (16.8, 1.0)	41	42a, NH (Gly)		3.68, dd (16.8, 1.0)
	NH		8.91, dd (8.9, 1.0)	31	32, 40, 42b		8.91, dd (8.3, 1.0)

[a] HMBC correlations, optimized for $^{2/3}J_{CH} = 8$ Hz, are from proton(s) stated to the indicated carbon. [b] NOESY correlations are from proton(s) stated to the indicated proton(s). [c] Dhoea moiety in cocosamide A. [d] Dhoya moiety in cocosamide B.

FL. The freeze-dried material (328 g) was extracted with EtOAc–MeOH (1:1). This lipophilic extract was partitioned between EtOAc and H_2O, and the aqueous portion subsequently partitioned between *n*-BuOH and H_2O. Concentration of these extracts furnished 9.56 g

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(2.9%) of EtOAc-soluble fraction and 2.24 g (0.6%) of BuOH-soluble material. The EtOAc-soluble fraction (9.56 g) was chromatographed on a column of SiO_2 (100 g) using a hexanes−EtOAc step gradient system followed by an EtOAc−MeOH step gradient to give 13 subfractions. The combined subfractions 7 to 9 (2.0 g), eluting with hexanes−75% EtOAc, was further chromatographed on a Si-column (100 g) using a hexanes−EtOAc step gradient system to give eight subfractions. Subfraction 6 (36 mg), eluting with hexanes−25% EtOAc, was further purified by reversed-phase HPLC (semiprep 250 × 10 mm, 5 μm, RP-18, flow 3.0 mL/min) using 15% H_2O−MeOH to give 3 mg of impure cocosamide B, 20 mg of majusculamides A and B, 4.0 mg of malyngamide 3 (**1**, yield, 0.001% dry wt), and 1.4 mg of cocosamide A (**2**, yield, 0.0004% dry wt). The impure cocosamide B fraction was further separated by reversed-phase HPLC using 30% H_2O−MeOH to give 2.0 mg of cocosamide B (**3**, yield 0.0006% dry wt).

Malyngamide 3 (1): colorless, amorphous powder; $[\alpha]^{25}_D$ −10.1 (*c* 0.36, MeOH); UV (MeOH) λ_{max} (log ε) 205 (4.19), 274 (3.40) nm; IR (film) ν_{max} 3320, 2932, 1725, 1636 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1, assignments were made by interpretation of 2D DQF COSY, edited-HSQC, HMBC, and NOESY data; HRESI/TOFMS *m/z* 559.3146 $[M + H]^+$ (calcd for $C_{28}H_{48}{}^{35}ClN_2O_7$, 559.3145).

Cocosamide A (2): white solid; $[\alpha]^{25}_D$ −77.7 (*c* 0.12, MeOH); UV (MeOH) λ_{max} (log ε) 208 (4.36), 260 (3.26) nm; IR (film) ν_{max} 3330, 2920, 1665, 1634, 1527, 1197 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2, assignments were made by interpretation of 2D DQF COSY, edited-HSQC, HMBC, and NOESY data; HRESI/TOFMS *m/z* 744.4330 $[M + H]^+$ (calcd for $C_{42}H_{58}N_5O_7$, 744.4331).

Cocosamide B (3): white solid; $[\alpha]^{25}_D$ −103 (*c* 0.18, MeOH); UV (MeOH) λ_{max} (log ε) 208 (4.54), 260 (3.35) nm; IR (film) ν_{max} 3416, 2950, 1665, 1634, 1541, 1032 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2, assignments were made by interpretation of 2D DQF COSY, edited-HSQC, HMBC, and NOESY data; HRESI/TOFMS *m/z* 742.4186 $[M + H]^+$ (calcd for $C_{42}H_{56}N_5O_7$, 742.4174).

Preparation of (*R*)-MTPA and (*S*)-MTPA Esters of 1. Compound **1** (1.0 mg) was dissolved in $CHCl_3$ (50 μL), and pyridine (50 μL) and a catalytic amount of 4-DMAP were added. The solution was treated with *S*(+)-MTPA chloride (1.0 μL) and stirred at room temperature for 12 h. The reaction was terminated with the addition of MeOH (200 μL), and the solvent was evaporated to give the (*R*)-MTPA ester of **1**. Similarly, the (*S*)-MTPA ester of **1** was prepared with *R*(−)-MTPA chloride using the same procedure. Both esters were subjected to HPLC (semiprep 250 × 10 mm, 5 μm, SiO_2, flow 3.0 mL/min) using EtOAc−3% MeOH to yield the pure (*R*)-MTPA ester of **1** (0.4 mg) and (*S*)-MTPA esters of **1** (0.3 mg).

***R*-MTPA ester of 1:** ^1H NMR δ (only key resonances are listed) 5.921 (1H, t, *J* = 5.4 Hz, NH), 5.292 (1H, m, H-9), 3.584 (3H, s, *O*Me-12), 3.536 (2H, s, H_2-6), 2.624 (2H, d, *J* = 6.9 Hz, H_2-10); ESIMS *m/z* 777.4 $[M + H]^+$; HRESI/TOFMS *m/z* 777.3404 $[M + H]^+$ (calcd for $C_{38}H_{57}{}^{35}ClF_3N_2O_9$, 777.3422).

***S*-MTPA ester of 1:** ^1H NMR δ (only key resonances are listed) 5.732 (1H, t, *J* = 5.4 Hz, NH), 5.292 (1H, m, H-9), 3.656 (3H, s, OMe-12), 3.535 (2H, s, H_2-6), 2.652 (2H, d, *J* = 6.9 Hz, H_2-10); ESIMS *m/z* 777.4 $[M + H]^+$; HRESI/TOFMS *m/z* 777.3443 $[M + H]^+$ (calcd for $C_{38}H_{57}{}^{35}ClF_3N_2O_9$, 777.3422).

Base Hydrolysis of Malyngamide 3. Compound **1** (1.9 mg) was dissolved in a 0.5 mL solution of 10% KOH in 80% aqueous EtOH and refluxed for 12 h. The hydrolysate was concentrated in vacuo and partitioned between H_2O and CH_2Cl_2. The H_2O layer was separated, acidified, and extracted with CH_2Cl_2 to yield lyngbic acid (**5**, 0.4 mg): colorless oil; $[\alpha]^{25}_D$ −12 (*c* 0.04, $CHCl_3$) [lit. −12.6 (*c* 0.8, MeOH)[16]]; ^1H NMR (600 MHz, $CDCl_3$) δ 5.48 (2H, m), 3.31 (3H, s, *O*Me), 3.15 (1H, quin, *J* = 5.5 Hz), 2.42 (2H, t, *J* = 7.5 Hz), 2.34 (2H, m), 2.18 (2H, m), 1.43 (2H, m), 1.27 (10H, m), 0.87 (3H, t, *J* = 7.0 Hz); HRESI/TOFMS *m/z* 257.2113 $[M + H]^+$ (calcd for $C_{15}H_{29}O_3$, 257.2114).

Acid Hydrolysis and Enantioselective HPLC Analysis. Compounds **2** and **3** (0.1 mg each) were suspended in 6 N HCl (0.3 mL) and heated at 115 °C for 18 h in two sealed tubes. The hydrolysates were concentrated to dryness. The residues were reconstituted in 0.3 mL of H_2O and analyzed by enantioselective HPLC, comparing the retention times with those of authentic standards [Phenomenex Chirex (D) penicillamine, 4.6 × 250 mm, 5 μm]; solvent mixtures of 2.0 mM $CuSO_4$−CH_3CN (85:15 or 90:10); detection at 254 nm. Using 2.0 mM $CuSO_4$−CH_3CN (90:10) with a flow rate of 0.8 mL/min, the retention times (t_R, min) for authentic standards were L-Pro (10.0) and D-Pro (19.6), and with a flow rate of 1.0 mL/min the retention times (t_R, min) for authentic standards were L-Val (17.0) and D-Val (22.9). Using 2.0 mM $CuSO_4$−CH_3CN (85:15) with a flow rate of 1.0 mL/min, the retention times (t_R, min) for authentic standards were *N*-Me-L-Phe (34.2) and *N*-Me-D-Phe (36.6). The retention times (and respective HPLC conditions) of the amino acids in the hydrolysates of **2** and **3** were (min) 10.0 (90:10, 0.8 mL/min), 17.0 (90:10, 1.0 mL/min), and 34.2 (85:15, 1.0 mL/min), indicating the presence of L-Pro, L-Val, and *N*-Me-L-Phe.

Cell Viability Assays. Cells were propagated and maintained in DMEM (Invitrogen) supplemented with 10% FBS (Hyclone) at 37 °C in humidified air and 5% CO_2. Cells were seeded in 96-well plates (MCF7 10 500 cells/well; HT-29 13 000 cells/well). After 24 h, cells were treated with various concentrations of the test compound or solvent control (1% EtOH). After 48 h of incubation, cell viability was measured using MTT according to the manufacturer's instructions (Promega). Paclitaxel was used as a positive control; IC_{50} values were 7 and 6 nM in HT-29 and MCF7 cell lines, respectively. Experiments were done in duplicate. IC_{50} values were determined using nonlinear regression in GraphPad Prism.

■ ASSOCIATED CONTENT

Supporting Information. ^1H, ^{13}C, and 2D NOESY NMR spectra in $CDCl_3$ for malyngamide 3 (**1**) and cocosamide A (**2**). ^1H, ^{13}C, COSY, HMBC, and 2D NOESY NMR spectra in $CDCl_3$ for cocosamide B (**3**). ^1H and 2D NOESY NMR spectra in $CDCl_3$ for pitipeptolide A (**5**). This material is available free of charge via the Internet at http://pubs.acs.org.

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