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Serinolamides and Lyngbyabellins from an *Okeania* sp. Cyanobacterium Collected from the Red Sea

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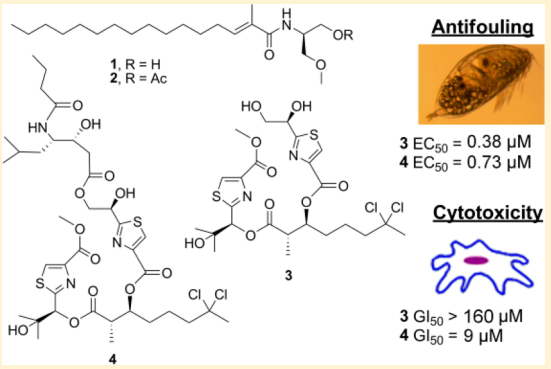
Supporting Information

ABSTRACT: NMR- and MS-guided fractionation of an extract of an *Okeania* sp. marine cyanobacterium, collected from the Red Sea, led to the isolation of four new metabolites, including serinolamides C (**1**) and D (**2**) and lyngbyabellins O (**3**) and P (**4**), together with the three known substances lyngbyabellins F (**5**) and G (**6**) and dolastatin 16 (**7**). The planar structures of the new compounds were determined using NMR and MS analyses. The absolute configurations of **1** and **2** were determined by Marfey's analysis of their hydrolysates. The absolute configuration of **3** was ascertained by chiral-phase chromatography of degradation products, while that of **4** was determined by comparison to **3** and **5**. The cytotoxic and antifouling activities of these compounds were evaluated using MCF7 breast cancer cells and *Amphibalanus amphitrite* larvae, respectively. Compounds **3**, **4**, and **7** exhibited strong antifouling activity, and **3** and **7** were not cytotoxic. A structure−activity relationship was observed for the cytotoxicity of the lyngbyabellins with the presence of a side chain (**4** is more active than **3**) leading to greater activity. For the antifouling activity, the acyclic form without a side chain (**3**) was the most active.



Cyanobacteria are a bountiful source of novel bioactive compounds.[1] *Okeania* gen. nov. was recently delineated from the genus *Lyngbya* as part of a phylogenetic study reported by Engene et al. in 2013.[2] Several compounds have subsequently been isolated from *O. hirsuta*, including malyngamide C[3] and bastimolide A,[4] as well as kurahyne B,[5] polycavernoside D,[6] janadolide,[7] odoamide,[8] and urumamide[9] from *Okeania* spp. Notably, all seven of these compounds have been reported to show cytotoxic effects toward various cancer cell lines.

As part of our ongoing research toward the isolation and identification of bioactive compounds from Saudi marine organisms,[10] we recently investigated the isolation of compounds from a dark brown filamentous cyanobacterium collected from the Red Sea, which was identified as an *Okeania* sp. Four new compounds were isolated, including serinolamides C (**1**) and D (**2**) and lyngbyabellins O (**3**) and P (**4**), together with the known compounds lyngbyabellins F (**5**) and G (**6**) and dolastatin 16 (**7**).[11−13] Serinolamides C and D were named with reference to the fatty acid amides serinolamides A and B, which were isolated from *Moorea producens*.[14,15] The antifouling and cytotoxic activities of these compounds were measured using the cyprids of the barnacle *Amphibalanus amphitrite* and MCF7 breast cancer cells, respectively.

RESULTS AND DISCUSSION

The *Okeania* sp. marine cyanobacterium was collected from the Red Sea and identified based on its tuft-forming morphology[2] and a phylogenetic analysis using 16S rRNA gene sequencing (Supporting Information S1, S2, and S3). The morphology and the presence of malyngamide C acetate, a metabolite commonly found in *Okeania hirsuta*[2] and confirmed by LC-

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Chart 1

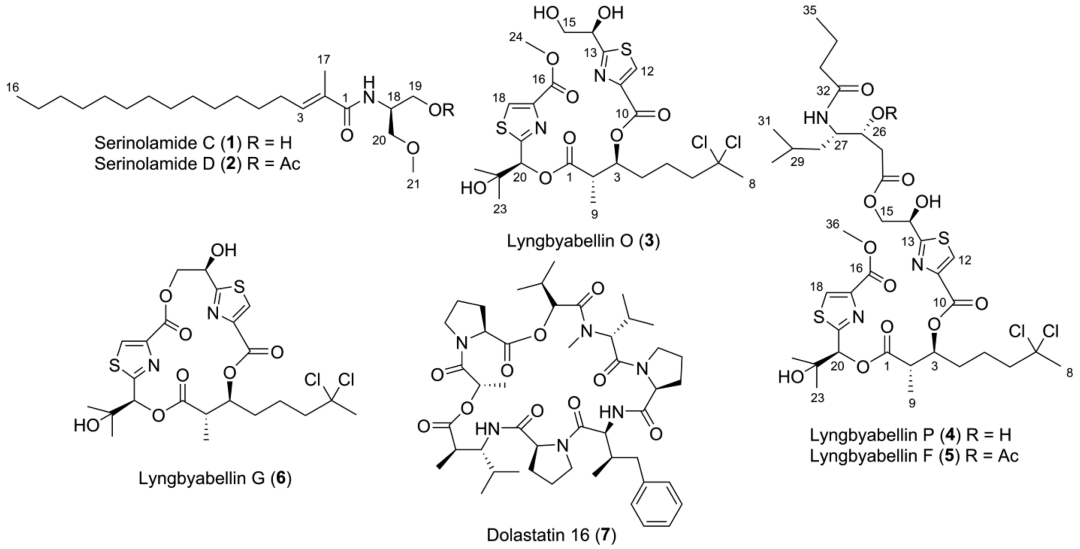

Table 1. NMR Spectroscopic Data (^{1}H 400 MHz, ^{13}C 100 MHz, $CDCl_3$) for Serinolamides C (1) and D (2)

	serinolamide C (1)			serinolamide D (2)		
position	δ_C, type	δ_H (J in Hz)	HMBC[a]	δ_C, type	δ_H (J in Hz)	HMBC[a]
1	169.9, C			169.2, C		
2	130.3, C			130.5, C		
3	137.4, CH	6.41, m		137.1, CH	6.36, m	
4	28.4, CH_2	2.14, ddd (7,7,7)		28.3, CH_2	2.14, ddd (7,7,7)	
5	28.7, CH_2	1.43, m		28.7, CH_2	1.42, m	
6–13	29.3–29.6, CH_2	1.26, m		29.3—29.6, CH_2	1.26, m	
14	31.9, CH_2	1.26, m		31.9, CH_2	1.26, m	
15	22.6, CH_2	1.26, m		22.6, CH_2	1.26, m	
16	14.0, CH_3	0.88, t (7.0)	14, 15	14.0, CH_3	0.88, t (6.4)	14, 15
17	12.6, CH_3	1.86, s	1, 2, 3	12.6, CH_3	1.84, s	1, 2, 3
18	50.8, CH	4.13, m		48.1, CH	4.40, m	
19	64.4, CH_2	3.71, dd (11.8, 4.2)		63.3, CH_2	4.14, dd (11.4, 6.0)	
		3.86, dd (11.4, 3.8)			4.28 (11.2, 6.4), dd	
20	73.3, CH_2	3.60, dd (9.6, 3.6)		71.1, CH_2	3.53, dd (9.8, 3.4)	
		3.63, dd (10.0, 3.6)				
21	59.3, CH_3	3.38, s	20	59.2, CH_3	3.37, s	20
22				171.3, C		
					3.46, dd (9.6, 4.8)	
23				20.8, CH_3	2.07, s	22
NH		6.41, m			6.11, d (7.6)	

[a]HMBC data optimized for 8 Hz are from proton(s) stated to the indicated carbon.

MS (Supporting Information S4), suggested that our strain was more closely related to *O. hirsuta* than *O. comitata*.[3]

The MeOH extract of this sample was partitioned between EtOAc and H_2O, and the aqueous fraction was further partitioned with *n*-BuOH. The EtOAc fraction was separated into nine subfractions by column chromatography over normal-phase (NP) silica gel. The fractionation process was monitored by LC-ESIMS analysis. The major compound in the EtOAc extract was determined to be the new compound serinolamide C (**1**), which was eluted from the column using a 2:8 (v/v) mixture of hexane/EtOAc. ESIMS analysis of this fraction showed an $[M + H]^+$ ion with an m/z value of 356.3165. Further purification of the fraction that eluted with a 6:4 (v/v) mixture of hexane/EtOAc by preparative HPLC led to the isolation of additional **1**, together with the related compound serinolamide D (**2**). Further purification of the fraction that eluted with 100% EtOAc by preparative HPLC gave two new compounds, lyngbyabellins O (**3**) and P (**4**), as well as three known compounds, lyngbyabellins F (**5**) and G (**6**) and dolastatin 16 (**7**), whose identities were confirmed by comparison with the chemical shifts reported in the literature[11−13] (Figures S26–S29).

The planar structure of **1** was elucidated using NMR and MS analyses. The molecular formula of **1** was determined to be $C_{21}H_{41}NO_3$ by ESITOFMS, indicating two degrees of unsaturation. ^{1}H and ^{13}C NMR analyses (Table 1) revealed the characteristic signals of an unsaturated acyl chain composed of eight methylene groups (δ_H 1.26, δ_C 29.3–29.6 ppm) and a terminal methyl group (δ_H 0.88, δ_C 14.0), as well as signals consistent with one *O*-methyl (δ_H 3.38, δ_C 59.3), one carbonyl (δ_C 169.9), one trisubstituted olefinic methyl (δ_H 1.86, δ_C

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Table 2. NMR Spectroscopic Data (^{1}H 600 MHz, ^{13}C 150 MHz, $CDCl_3$) for Lyngbyabellins O (3) and P (4)

	lyngbyabellin O (3)				lyngbyabellin P (4)			
position	δ_C, type	δ_H (J in Hz)	COSY	HMBC[a]	δ_C, type	δ_H (J in Hz)	COSY	HMBC[a]
1	171.0, C				171.0, C			
2	43.8, CH	3.00, dq (4.2, 6.6)	3, 9	1, 9	43.7, CH	3.03, dq (4.8, 7.2)	9	1
3	75.2, CH	5.43, dt (4.8, 12.0)	2, 4		75.0, CH	5.43, dt (7.8, 4.2)	4	
4	31.6, CH_2	1.81, m	3, 5	5	31.4, CH_2	1.82, m	3	
5	21.6, CH_2	1.81, m	4, 6		21.6, CH_2	1.79, m	6	
6	49.1, CH_2	2.18, 2.25, m	5	5	49.1, CH_2	2.18, 2.26, m	5	
7	90.1, C				90.1, C			
8	37.4, CH_3	2.12, s		6, 7	37.4, CH_3	2.12, s		6, 7
9	13.8, CH_3	1.28, d (6.6)		1, 2, 3	13.6, CH_3	1.29, d (6.6)		1, 3
10	166.4, C				160.9, C			
11	146.2, C				146.4, C			
12	129.0, CH	8.24, s		11, 13	129.0, CH	8.22, s		11, 13
13	179.7, C				173.0, C			
14	71.7, CH	5.09, br	15		70.0, CH	5.29, dd (3.6, 7.8)	15	
15	66.0, CH_2	4.02, brd (16.8)	14		67.8, CH_2	4.55, dd (11.4, 3.6)		
						4.59, dd (11.4, 7.8)	14	24
16	161.8, C				161.7, C			
17	146.1, C				146.2, C			
18	128.7, CH	8.17, s	20	17, 19	128.5, CH	8.17, s		17, 19
19	167.0, C				167.4, C			
20	78.3, CH	6.16, s	18	1, 19	78.4, CH	6.13, s		1, 19, 22, 23
21	72.2, C				72.0, C			
22	25.3, CH_3	1.18, s	23	20, 23	26.6, CH_3	1.38, s		20, 21, 23
23	26.9, CH_3	1.41, s	22	20, 21, 22	25.4, CH_3	1.19, s		20, 21, 11
24	52.6, CH_3	3.95, s		16	171.9, C			
25					37.5, CH_2	2.56, d (5.4)	26	24, 26, 27
26					71.8, CH	3.90, ddd (6.0, 6.0, 6.0)	25	
27					51.6, CH	4.07, m	27-NH, 28	
27-NH						5.52, d (8.4)	27	
28					39.0, CH_2	1.34, 1.50, m	27	
29					25.0, CH	1.63, m	30, 31	
30					23.5, CH_3	0.95, d (6.0)	29	28, 29, 31
31					21.5, CH_3	0.90, d (6.0)	29	28, 29, 30
32					174.3, C			
33					38.6, CH_2	2.20, m	34	32
34					19.2, CH_2	1.67, m	33, 35	32
35					13.7, CH_3	0.97, t (7.8)	34	34
36					52.5, CH_3	3.95, s		16

[a]HMBC optimized for 8 Hz are from proton(s) stated to the indicated carbon.

12.6), and one amide proton (δ_H 6.41), forming a fatty acid amide with an *O*-methylserinol.

Fatty acid amides are common metabolites of the genera *Moorea*,[14,15] *Lyngbya*,[16] and *Okeania*.[17] Moreover, two fatty acid amides bearing an *O*-methylserinol moiety have previously been isolated from *Moorea producens*,[14,15] namely, serinolamides A and B. HMBC analysis of **1** allowed for the assignment of the spin system formed by H-17, C-1, as well as the double bond formed by C-2 and C-3. The configuration of the double bond of **1** was determined based on the characteristic chemical shift of the C-17 methyl group. As the chemical shifts of α-carbons are dependent on the *cis*–*trans* isomerism of the double bond, the shift of C-17 (δ_C 12.6 ppm) indicated an *E*-configuration. Indeed, in *cis* alkenes, the α-carbons are shielded from their positions in the alkane, whereas in *trans* alkenes, they are deshielded.[18] The absolute configuration of the stereogenic center of the serinol moiety was determined by the synthesis of the (*S*)- and (*R*)-enantiomers of *O*-methylserinol, followed by the hydrolysis of **1** in conjunction with Marfey's analysis.[19] The retention time of the hydrolysate of **1** matched that of the synthetic (*S*)-*O*-methylserinol, leading to the assignment of C-18 in compound **1** as the *S*-configuration.

The molecular formula of serinolamide D (**2**) was determined to be $C_{23}H_{43}NO_4$ by ESITOFMS analysis, indicating three degrees of unsaturation. The ^{1}H NMR data for **2** were quite similar to those obtained for **1** (Table 1), except for the presence of an additional methyl singlet (δ_H 2.07), as well as a shielded shift for the amide proton (δ_H 6.11 instead of 6.41 for **1**) and deshielded shifts for H-19 (δ_H 4.14 and 4.28 instead of 3.71 and 3.86 for **1**) and H-18 (δ_H 4.40 instead of 4.13 for **1**). Moreover, the ^{13}C NMR spectrum of this compound revealed the presence of an extra carbonyl group (δ_C 171.3), as well as an extra methyl group (δ_C 20.8) compared with **1**. HMBC analysis (Figure S14) showed that the serinol was *O*-acetylated. The configuration of the double bond was also determined to be *E* based on the chemical shift of the C-17 methyl (δ_C 12.6). Marfey's analysis of the hydrolysate of **2** showed a peak with a retention consistent

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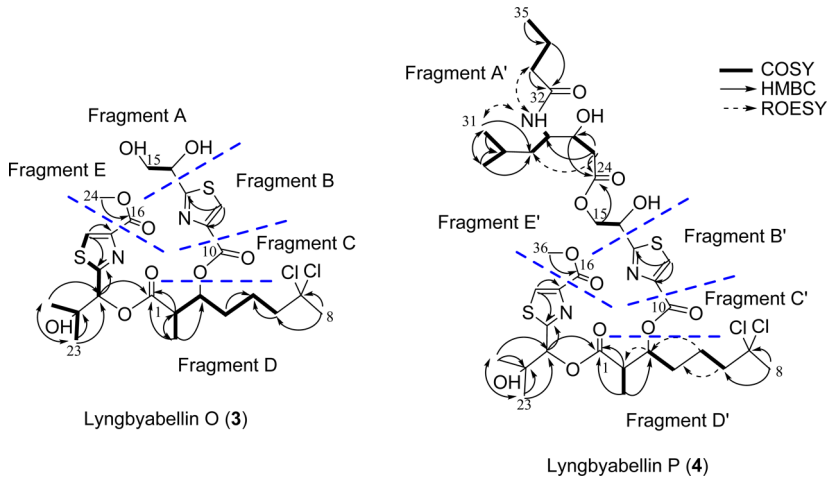

Figure 1. 2D NMR correlations for the partial structures of **3** (fragments A–E) and **4** (fragments A′–E′).

with synthetic (*S*)-*O*-methylserinol. In light of the *O*-acetylated serinol in **2**, the CIP rules led to the C-18 position being assigned as the *R*-configuration.

NMR and MS data analyses were used to determine the planar structures of **3** and **4**. ESITOFMS analysis revealed that the molecular formulas of **3** and **4** were $C_{24}H_{32}Cl_2N_2O_4S_2$ and $C_{36}H_{52}Cl_2N_3O_{12}S_2$ with nine and 11 degrees of unsaturation, respectively. Both of these compounds contained two chlorine atoms, as exemplified by the 9:6:1 isotope peaks observed in the mass spectra of **3** and **4** with *m*/*z* values of 627/629/631 and 854/856/858, respectively. The NMR data for **3** and **4** are summarized in Table 2.

The ^{1}H NMR spectrum of **3** (Table 2) showed two deshielded signals (δ_H 8.17 and 8.24), whereas the ^{13}C NMR spectrum contained signals consistent with eight sp^2 carbons (C-10 to C-13 and C-16 to C-19). Taken together, these data suggested the presence of two 2-alkylthiazole-4-carboxylates, which are typical of lyngbyabellins and dolabellin.[13] HMBC analysis (Figure 1) also confirmed the presence of two thiazole rings with correlations from H-12 to C-11 and C-13 and H-18 to C-17 and C-19. The remaining sp^2 carbon (C-1 δ_C 171.0) was assigned to the carbonyl group of a saturated ester. Between them, the two thiazole rings and the three carbonyl groups accounted for the nine degrees of unsaturation in **3**.

The ^{1}H NMR spectrum of **3** also showed a series of shielded, coupled resonance signals indicative of an aliphatic chain. A singlet corresponding to a methyl group at δ_H 2.12 was also detected, which showed a correlation to a methylene at δ_C 49.1 (C-6) and a dichlorinated carbon at δ_C 90.1 (C-7) by HMBC analysis, indicating an unusual *gem*-dichloro group. This group has been reported previously for lyngbyabellins A–K and M–N, as well as dolabellin and the hectochlorins.[13] HMBC and COSY analysis (Figure 1) indicated that this *gem*-dichloro group was attached to the aliphatic chain, with the C-9 methyl group attached to the C-2 methine.

Furthermore, HMBC analysis (Figure 1) revealed correlations between H-20 and C-1, as well as correlations with C-19 of one of the thiazole ring systems and two methyl groups (C-22 and C-23), both of which were attached to an oxygenated nonprotonated carbon (C-21) (forming fragment D). COSY and HMBC data (Figure 1) also revealed two partial structures: $-CH(OH)CH_2OH$ (fragment A) and a methoxy attached to a carbonyl group (fragment E).

Unfortunately, we did not observe any correlations connecting fragments A–E. However, based on ^{1}H and ^{13}C NMR data similar to lyngbyabellins and detailed consideration of the many different combinations, the proposed structure (Figure 1) was recognized as the best fit for the available data. The chemical shift of C-3 (δ_C 75.2) showed that this carbon should be attached to an oxygen atom. This proves that fragments C and D are attached. If C-17 of fragment D was attached to fragment A, it would be more deshielded, but attached to fragment E, its chemical shift corresponds to the one observed in the spectrum (Table 2). As the chemical shift of C-11 is almost identical to that of C-17, we can consider that it is also attached to a carboxyl, therefore to fragment C. The remaining fragment A is attached to fragment B at C-13.

Similar observations were recorded for **4**, except for the presence of an extra component corresponding to $C_{12}H_{22}NO_3$, which was determined to be attached to C-15 via an ester linkage based on an HMBC correlation from H-15 to C-24 (fragment A′) (Figure 1). HMBC analysis also showed that C-24 was attached to a methylene moiety (C-25), which was linked to an oxygenated methine (δ_C 71.8). A correlation was also observed between H-25 and a methine group at C-27 (δ_C 51.6) by HMBC analysis, whereas ROESY analysis revealed a correlation (Figure 1) to a methylene (C-28). A signal corresponding to an amide proton at δ_H 5.52 (27-NH) showed a COSY correlation to H-27 (Figure 1), which was expanded into a modified leucine unit (C-24 to C-31) based on 2D NMR correlations, in a similar manner to lyngbyabellins E, F, H, and I.[13] The 27-NH signal also showed a ROESY correlation to H-33 of an aliphatic chain (C-33 to C35), which was bonded to a carbonyl (C-32). Once again, no correlations were observed linking C-13 to C-14 (fragments A′ and B′), C-10 to C-11(fragments B′ and C′), C-3 to C-10 (fragments C′ and D′) or C-17 to C-16 (fragments D′ and E′). The linkages between the different fragments were therefore established using a similar logic to that applied to **3**.

The planar structure of **3** resembled that of lyngbyabellin G (**6**), which was also isolated from this *Okeania* sp. extract. The key difference between these two molecules is the open structure between C-15 and the ester attached to the second thiazole ring system. Likewise, the planar structure of compound **4** bears a striking resemblance to that of lyngbyabellin F (**5**), isolated from the same fraction, with the only major difference being an OH group at C-26 instead of an

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acetate group. Given that compounds **3** and **4** were both found in the same fraction as **5** and **6** and share similar structural features, it is highly likely that they also have the same configuration.

Identical ^{1}H and ^{13}C NMR data for **6** compared to the literature (the largest difference in ^{13}C chemical shifts is 0.028 ppm), together with similar specific rotation ($[\alpha]^{26}_{D}$ −28.6 for **6** and −26 reported for lyngbyabellin G), proved that **6** is the known lyngbyabellin G. Mild methanolysis converted **6** into **3**, so **3** has the same absolute configuration as **6**. Chiral-phase chromatography experiments supported this information. After ozonolysis and subsequent base hydrolysis, the obtained *α,β*-dihydroxyisovaleric acid (Dhiv) residue was methylated (CH_2N_2). MTPA derivatization with (*R*)-MTPA was conducted on both synthetic (*R*)- and (*S*)-Dhiv methyl esters and the ozonolyzed/hydrolyzed/methylated natural product **3**. Chiral-phase HPLC with comparison of the retention times of the diastereomeric synthesized (*R*)- and (*S*)-Dhiv methyl esters (14.6 and 16.5 min, respectively) established the absolute configuration of the *α,β*-dihydroxyisovaleric acid methyl ester (16.6 min) of **3** as the *S*-configuration at C-20. The ozonolyzed/hydrolyzed natural product **3** was confirmed to contain D-glyceric acid (3.8 min) by comparison of the retention times by ESI-LC-MS using a chiral-phase column of the commercially available L- and D-glyceric acids (3.5 and 3.8 min, respectively), giving an *R*-configuration at C-14. Identical to **6**, **3** has a 2*S*, 3*S* configuration.

Chemical shifts and the specific rotation of **5** ($[\alpha]^{26}_{D}$ −7.8 for **5** and −6.5 reported for lyngbyabellin F; the largest difference in ^{13}C chemical shifts is 0.015 ppm) were similar to those reported for lyngbyabellin F. Deacetylation ($NaOCH_3$) at C-26 of **5** led to the conversion into **4**. Degradation of **4** and **5** observed by ESI-TOF-MS analysis showed that both were converted into **3**, showing that these three compounds indeed share the same configuration. As a result, **4** has a 2*S*, 3*S*, 14*R*, 20*S*, 26*R*, and 27*S* configuration.

As observed for lyngbyabellin E that converted to lyngbyabellin G,[13] one ester linkage of **4** is apparently prone to methanolysis and a regioselective ester cleavage occurred at C-14, giving **3**, which can be considered as an artifact of **4** and also **6**, as mild methanolysis gave **3**. Conversion of **6** into **3** is possible by regioselective ester cleavage at C-16. This kind of conversion was observed for lyngbyabellin C, which was converted into homohydroxydolabellin.[20]

The isolated compounds, except **5**, were subjected to an antifouling assay using *Amphibalanus amphitrite* larvae. After an exposure time of 48 h, compounds **1** and **2** inhibited the settlement of the larvae at concentrations below 1 μg/mL. However, after an exposure time of 120 h at similar concentrations, barnacle larvae were able to settle. Although the two fatty acid amides **1** and **2** slowed the settlement process considerably, they were unable to inhibit it completely. A recovery test for **2** showed that after 7 days almost all of the exposed larvae settled regardless of the tested concentration. Compound **6** showed low activity (EC_{50} 7.4 μM), while **3** and **4** exhibited potent activity, with EC_{50} values of 0.38 and 0.73 μM, respectively. These results suggest that the larvae are more sensitive to the acyclic structure of **3** than the cyclic structure of **6**. The flexibility of the acyclic structure may facilitate interactions with a common target, but addition of a side chain decreases them, as observed with **4**. Compound **7**, which is well known as a powerful antifoulant,[21] exhibited high inhibition activity (EC_{50} 0.09 μM), and its activity remained intact even after 120 h. The observed antifouling activity of the *Okeania* sp. extract was therefore mainly attributed to compounds **3**, **4**, and **7** because the 100% EtOAc fraction from which they were isolated was the most active of all of the fractions tested.

The cytotoxicities of the isolated compounds were evaluated in MCF7 breast cancer cells. It is noteworthy that the two fatty acid amides **1** and **2** precipitated out of the medium, precluding their activities from being assessed. The GI_{50} values of compounds **3**, **4**, and **6** toward the MCF7 cells were >160, 9, and 120 μM, respectively. These differences in the cytotoxicity could most likely be attributed to the differences in their structures.[13] The side chain in compound **4** could play an important role in its superior cytotoxicity (GI_{50} 9 μM) over **3** and **6**, forming additional interactions with a common target, providing a different mode of action, or simply improving its permeability. Compound **6** appeared less active on MCF7 cells than on H460 and Neuro-2a cells (2.2 and 4.8 μM, respectively).[13] Its mechanism of action in MCF7 cells is probably different than in H460 and Neuro-2a cells or MCF7 cells, which are less sensitive to **6**.

The results of a previous study showed that **7**, isolated from the sea hare *Dolabella auricularia*, inhibited the growth of several human cancer cell lines (GI_{50} 0.0012−0.00096 μg/mL).[22] Surprisingly, a total synthesis of this cyclodepsipeptide revealed that synthetic **7** did not exhibit any discernible cytotoxicity.[23] The authors attributed this lack of activity to a change in the conformation of the synthetic compound or the presence of a chemically undetected (yet extremely active) compound in the sample isolated from the natural source. A new synthesis of **7** confirmed its inactivity.[24] In this study, it was confirmed that **7** is noncytotoxic but potently active toward barnacle larvae.

Okeania spp. appear to be a promising source of biologically active compounds based on the results obtained for the compounds isolated from the genus *Lyngbya* or *Moorea*. The cytotoxicity of the *Okeania* sp. extract in particular was attributed to the presence of several lyngbyabellins, which are well-known cytotoxic compounds. The antifouling activity of this extract was mainly attributed to the known dolastatin 16 (**7**) and the new lyngbyabellins O (**3**) and P (**4**).

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotation data were acquired on a HORIBA SEPA-300 polarimeter using a 0.1 dm cuvette with chloroform or methanol as the solvent. UV spectra were recorded on a JASCO MD-1510 multiwavelength UV detector. IR spectra were recorded on a JASCO FT/IR-4100 type A. The ^{1}H and ^{13}C NMR spectra for compounds **1**, **2**, and **6** were recorded on a JEOL 400 MHz spectrometer, whereas the 2D NMR spectra of **1** and **2** were recorded on a Bruker Avance 300 MHz. The ^{1}H, ^{13}C, and 2D NMR spectra for compounds **3** and **4** were recorded on a JEOL 600 MHz spectrometer. The ^{1}H NMR spectrum of **5** was recorded on a JEOL 400 MHz spectrometer, whereas its ^{13}C NMR spectrum was recorded on a Bruker AMX500. All of the NMR spectra were recorded in $CDCl_3$ (Cambridge Isotope Laboratories, Inc.) using the residual solvent peak ($CHCl_3$) as an internal reference (δ_H 7.27 and δ_C 77.00 ppm). LC-MS data were obtained on an Agilent 1100 Series HPLC system coupled with a Bruker Daltonics micrOTOF-HS mass spectrometer, which was configured for electrospray ionization (ESI). The HPLC system was equipped with a Cadenza CD-C18 column (2 × 150 mm, 3 μm, 0.2 mL/min, 25 °C). The HPLC system was operated under the following conditions: 0−20 min, linear gradient from 50% to 100% MeCN with 0.1% (v/v) formic acid in Milli-Q H_2O; 20−35 min, isocratic 100% MeCN with 0.1% (v/v) formic acid. Preparative HPLC purifications

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were conducted over a Cosmosil Cholester column (10 × 250 mm, 5 μm) at a flow rate of 3 mL/min, with UV detection at 210 nm.

Biological Material. The dark brown cyanobacterial sample S1501 (Figure S1) was collected by scuba diving at a depth of 10−15 m on the Algetah Alkabira reef near Jeddah, Saudi Arabia (N 21°41′23.98″; E 39°00′52.94″) in April 2015. Foreign particles were removed by hand, and the samples were squeezed by hand to remove any seawater before being stored in MeOH for transportation. A small portion of this material was also preserved in 10 mL of RNA*later* solution for genetic analysis. The sample was identified as an *Okeania* sp. by phylogenetic analysis (Figure S3) of its 16S rRNA gene sequence (GenBank KY889150). The morphological characteristics of this material (hair-like, dark brown color, cell width around 55 μm), as well as the detection of malyngamide C acetate by LC-MS analysis (Figure S4), suggested that S1501 was more closely related to *O. hirsuta* than *O. comitata*.[3] A voucher specimen (S1501) preserved in RNA*later* was deposited at Hokkaido University, Japan.

Gene Sequencing. The details of these procedures are reported in the Supporting Information.

Extraction and Isolation. A sample of the cyanobacterium was homogenized and extracted three times with MeOH. The dried MeOH extract (3.5 g) was partitioned between EtOAc and H_2O (1:1, v/v). The dried EtOAc fraction (2.8 g) was purified by NP-column chromatography over silica gel. A stepwise gradient composed of hexane and EtOAc (100:0, 9:1, 8:2, 6:4, 4:6, 2:8 v/v), followed by EtOAc and MeOH (100:0, 75:25, 0:100 v/v), was used to elute the column. LC-MS analysis revealed that the 2:8 (v/v) hexane/EtOAc fraction (118 mg) contained the main compound of the mother fraction, with an *m/z* value of 356.31. Isolation was performed using semipreparative RP-HPLC (gradient 0−30 min, 40−80% MeCN; 30−40 min, 80−100% MeCN). The fraction eluted at 40 min was further purified by RP-HPLC (gradient 0−40 min, 90−100% MeCN) to yield serinolamide C (**1**) (t_R 15.7 min, 12 mg). The fraction eluted with 6:4 (v/v) hexane/EtOAc (245 mg) was fractionated by RP-HPLC [gradient 0−50 min, 75−90% MeCN containing 0.05% (v/v) trifluoroacetic acid (TFA)]. The fraction eluted at 24.3 min was further purified by RP-HPLC (isocratic 90% MeCN containing 0.05% (v/v) TFA) to yield compound **1** (t_R 14.6 min, 10 mg) and another fraction (t_R 18.4 min, 10 mg). This fraction was further purified by RP-HPLC (Cosmosil MS-5C18-II 10 × 250 mm, 5 μm, 3 mL/min, UV detection at 210 nm, isocratic 87% MeCN) to give **1** (t_R 21.3 min, 1.7 mg) and serinolamide D (**2**) (t_R 30.3 min, 1.6 mg). The fraction eluted with 100% EtOAc (140 mg) was fractionated by RP-HPLC (gradient 0−30 min, 40−80% MeCN containing 0.05% (v/v) TFA) to yield fraction A (t_R 14.1 min, 3.4 mg), fraction B (t_R 16.4 min, 6.7 mg), fraction C (t_R 20.4 min, 4.4 mg), fraction D (t_R 21.8 min, 2.4 mg), and fraction E (t_R 22.1−25 min, 3.7 mg). Fraction A was further purified by RP-HPLC (Cosmosil Cholester 4.6 × 250 mm, 5 μm, 1 mL/min, UV detection at 210 nm, gradient 0−40 min, 40−70% MeCN containing 0.05% (v/v) TFA) to yield lyngbyabellin O (**3**) (t_R 9.6 min, 1.8 mg). Fraction B was further purified by RP-HPLC (gradient 0−40 min, 40−70% MeCN) to yield the known compound lynbyabellin G (**6**) (t_R 20.1 min, 3 mg). Fraction C was further purified by RP-HPLC (UV detection at 210 nm, gradient 0−40 min, 40−70% MeCN) to yield lyngbyabellin P (**4**) (t_R 27.5 min, 2 mg). Fraction D was further purified by RP-HPLC (Cosmosil Cholester 10 × 250 mm, 5 μm, 3 mL/min, gradient 0−60 min, 50−70% MeCN) to give the known compound lyngbyabellin F (**5**) (t_R 20.7 min, 1.1 mg). Fraction E was further purified by RP-HPLC (Cosmosil Cholester 4.6 × 250 mm, 5 μm, 1 mL/min, gradient 0−40 min, 60−80% MeCN containing 0.05% (v/v) TFA) to yield a subfraction (t_R 10.5 min, 7 mg), which was further purified by RP-HPLC (Cosmosil Cholester 4.6 × 250 mm, 5 μm, 1 mL/min, UV detection at 254 nm, gradient 0−40 min, 50−70% MeCN) to give the known compound dolastatin 16 (**7**) (t_R 22.5 min, 1.5 mg).

*Serinolamide C (**1**):* white, amorphous solid, $[\alpha]^{26}_D$ +4.9 (*c* 0.51, $CHCl_3$); UV (MeOH) λ_{max} 219 nm (log ε 3.42); IR (neat) λ_{max} 3305 (br), 2957, 2917, 2848, 1610, 1552, 1469, 1260 cm^{-1}; ^1H and ^{13}C NMR data, Table 1; ESITOFMS *m/z* 356.3165 $[M + H]^+$ (calcd for $C_{21}H_{42}NO_3$, 356.3159).

*Serinolamide D (**2**):* colorless, amorphous solid, $[\alpha]^{26}_D$ +14.3 (*c* 0.11, $CHCl_3$); UV (MeOH) λ_{max} 215 nm (log ε 4.01); IR (neat) λ_{max} 2966, 2924, 2852, 1741, 1625, 1531, 1463, 1260 cm^{-1}; ^1H and ^{13}C NMR data, Table 1; ESITOFMS *m/z* 398.3255 $[M + H]^+$ (calcd for $C_{23}H_{44}NO_4$, 398.3265).

*Lyngbyabellin O (**3**):* colorless, amorphous solid; $[\alpha]^{26}_D$ −9.5 (*c* 0.23, MeOH); UV (MeOH) λ_{max} 243 nm (log ε 4.76); ^1H and ^{13}C NMR data, Table 2; ESITOFMS *m/z* 627.1024 $[M + H]^+$ (calcd for $C_{24}H_{33}Cl_2N_2O_9S_2$, 627.0999).

*Lyngbyabellin P (**4**):* colorless, amorphous solid; ^1H and ^{13}C NMR data, Table 2; ESITOFMS *m/z* 854.2544 $[M + H]^+$ (calcd for $C_{36}H_{54}Cl_2N_3O_{12}S_2$, 854.2520).

Absolute Configurations of 1 and 2. The absolute configurations of **1** and **2** were determined by the synthesis of (*S*)- and (*R*)-*O*-methylserinol according to the method reported by Gao et al.[19] A small aliquot of **1** (0.1 mg) was hydrolyzed using 6 M HCl at 110 °C for 16 h. Compound **2** was hydrolyzed in a similar manner using 12 M HCl at 120 °C to convert its acetate group to an alcohol. The hydrolysates were dried *in vacuo* and subjected to Marfey's analysis, along with the two synthesized *O*-methylserinol enantiomers. The hydrolysates and derivatives were analyzed by RP-HPLC (Cosmosil 5C18-AR-II 4.6 × 250 mm, 5 μm, 1 mL/min, UV detection at 340 nm, gradient 0−45 min, 10−50% MeCN containing 0.05% (v/v) TFA). A comparison of the retention times (t_R) of the standard (21 and 31 min for (*R*)- and (*S*)-*O*-methylserinol, respectively) and the hydrolysates (31 min for **1** and **2**) led to the assignment of the stereogenic centers in compounds **1** and **2** as *S* and *R*, respectively.

Absolute Configurations of 3 at C-14 and C-20. The absolute configurations of **3** at C-14 and C-20 were determined by chiral-phase chromatography techniques. A small portion of **3** (0.6 mg) was dissolved in MeOH (2 mL) and ozonolyzed at −78 °C for 25 min. The solvent was removed under a stream of Ar, and the sample was hydrolyzed with 2 N NaOH at 90 °C for 7 h. To determine the relative configuration at C-14, the hydrolysate was analyzed by chiral-phase ESI-LC-MS (column Astec Chirobiotic TAG 2.1 × 250 mm; 0.2 mL/min; ESI negative low mode; 1:1 0.1% aqueous formic acid/1% NH_4OAc in MeOH; column oven: 20 °C), and it was found to contain D-glyceric acid (3.8 min) by comparison of the retention times of the commercially available L- and D-glyceric acids (3.5 and 3.8 min, respectively). The hydrolysate was then methylated (2 M CH_2N_2 for 10 min at 0 °C) to produce α,β-dihydroxyisovaleric (Dhiv) methyl esters. The standards *R*- and *S*-Dhiv methyl esters were synthesized by Sharpless asymmetric dihydroxylation of methyl 3,3-dimethyl acrylate using AD-mix-α and AD-mix-β, respectively.[25] The product obtained from **3** and the standards *R*- and *S*-Dhiv methyl esters were derivatized using (*R*)-MTPA. The samples were dissolved in CH_2Cl_2, mixed with (*R*)-MTPA (50 μL of a solution of 50 μL/mL of MeCN) and heated for 1 h at 70 °C. The reaction was quenched by addition of EtOH (100 μL) and heating for 15 min at 70 °C. After evaporation of the solvent, the samples were dissolved in MeOH and subjected to HPLC analysis (column Cosmosil 5C18-MS-II 4.6 × 250 mm, 5 μm, 1 mL/min, UV detection 254 nm, gradient 0−30 min, 20−30% MeOH). The retention time of the MTPA derivative from the hydrolysate matched that of the *S*-Dhiv methyl ester standard (16.5 min, instead of 14.6 min for the *R*-Dhiv methyl ester standard).

Mild Methanolysis of 6. A portion of **6** (0.4 mg) was dissolved in MeOH (100 μL), and 0.1 M HCl in MeOH was added (900 μL). The mixture was heated at 85 °C for 45 min. The reaction was quenched by evaporation of the solvent. The product was then dissolved in MeOH for ESI-LC-MS analysis. The results showed that **6** was converted into **3**.

Deacetylation of 5. Compound **5** (1 μmol) was dissolved in MeOH (100 μL), and 0.1 M $NaOCH_3$ in MeOH was added (1 μL). After 15 min at room temperature, the reaction was quenched by evaporation of the solvent. The product was then dissolved in EtOH for ESI-LC-MS analysis. The results showed that **5** was converted into **4**.

Antifouling Assay. An antifouling assay was performed on *Amphibalanus amphitrite* cypris according to the method described by Kitano et al.[26] Adult barnacles were procured from oyster farms in

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Lake Hamana, Japan, as well as a pier at Shimizu Bay, Shizuoka, Japan. All of these barnacles were kept in an aquarium at 20 °C, where they were fed brine shrimp *Artemia salina* nauplii. The barnacles were dried at room temperature overnight before being immersed in seawater, which lead to the release of I–II nauplii from the broods. These nauplii were cultured at 25 °C in 80% filtered (0.2 μm of pore filter) natural seawater (diluted with deionized water) containing 30 μg/L streptomycin and 20 mg/L penicillin G, where they were fed on the diatom *Chaetoceros gracilis* until they reached the cyprid stage within 5 days. The day that the newly transformed cyprids were collected was designated as day 0. The test samples and compounds were dissolved in EtOH. The antifouling assays were conducted in flat-bottomed 24-well polystyrene culture plates, and four wells containing six healthy cyprids were used for each concentration. After an exposure time of 48 h in the dark at 25 °C, the numbers of larvae that had attached or metamorphosed or did not settle were counted under a microscope. After an exposure time of 120 h, the number of dead cyprids was counted in the same way. Concerning the recovery test, floating larvae after an exposure time of 120 h were collected and put in fresh marine water without compound, and floating and settled larvae were counted daily for 7 days. For the initial screening of the extracts, the samples were tested at two different concentrations, namely, 10 and 100 μg/mL. Pure compounds were tested at six different concentrations in triplicate ($n = 3$), including 0.03, 0.1, 0.3, 1.0, 3.0, and 10 μg/mL. The antifouling activity of each compound was expressed as an EC_{50} value, which represents the concentration of compound required to reduce larval settlement to 50% of the control. Probit analysis was used to calculate the EC_{50} values.

Cytotoxicity Assay. The cytotoxicities of the compounds were determined according to the procedure reported by Lopez et al. (2016).[27]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.7b00449.

Figures S1–S29; procedure of 16S rRNA gene sequencing and phylogenetic analysis experiments; 1D and 2D NMR spectra for serinolamides C (**1**) and D (**2**) and lyngbyabellins O (**3**) and (**4**); 1D NMR for lyngbyabellins F (**5**) and G (**6**) (PDF)

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Notes

The authors declare no competing financial interest.

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