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Lyngbyabellins K–N from Two Palmyra Atoll Collections of the Marine Cyanobacterium *Moorea bouillonii*

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Dedicated to the memory of Ernesto Fattorusso

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Five lipopeptides of the lyngbyabellin structure class, four cyclic (**1**–**3** and **5**) and one linear (**4**), were isolated from the extracts of two collections of filamentous marine cyanobacteria obtained from the Palmyra Atoll in the Central Pacific Ocean. Their planar structures and absolute configurations were elucidated through a combination of spectroscopic and chromatographic analyses as well as chemical synthesis of fragments. In addition to structural features typical of the lyngbyabellins, such as two thiazole rings and a chlorinated 2-methyloctanoate residue, these new compounds possess several unique aspects. Of note, metabolites **2** and **3** possess rare monochlorination on the 3-acyloxy-2-methyloctanoate residue, whereas lyngbyabellin N (**5**) has an unusual *N*,*N*-dimethylvaline terminus. Lyngbyabellin N also possesses a leucine statine residue and shows strong cytotoxic activity against the HCT116 colon cancer cell line (IC_{50} = 40.9 ± 3.3 nM).

Introduction

Over the last 20 years, marine cyanobacteria have emerged as exceptionally prolific producers of biologically active secondary metabolites, rivaling the metabolic richness of the actinobacteria.[1] Because they lack other more visible defense mechanisms, such as a hardened exterior or a cryptic habitat, and have an overall macroscopic structure, it is thought that cyanobacteria derive value from the biosynthesis of these structurally intriguing secondary metabolites for their chemical defense.[2] The genus *Moorea* (formally *Lyngbya* spp) is one of the chemically most prolific and has yielded important metabolites such as the apratoxins,[3] antillatoxin A,[4] lyngbyatoxin A,[5] curacin A,[6] barbamide,[7] the jamaicamides,[8] and the malyngamides.[9] In general, these structurally diverse metabolites exhibit a range of interesting biological activities, such as anticancer,[10] antifeedant,[11] molluscicidal,[12] anti-inflammatory,[13] and neuromodulatory.[14] The lyngbyabellins are another family of metabolites produced by *Moorea* sp. that are non-ribosomal peptide synthetase/polyketide synthase (NRPS/PKS) derived peptides and have a recognizable architecture composed of thiazole rings, hydroxy acid residues, and an acyl group with distinctive chlorination at the penultimate carbon atom.[15] Several of the lyngbyabellins are reported to exhibit moderate to potent cytotoxicity to various cancer cell lines and to exert this activity through interference with the actin system.[15]

In the present work, a number of filamentous marine cyanobacteria were collected from the Palmyra Atoll (approximately 1000 miles SSW of Hawaii) in 2008 and 2009, and their extracts were evaluated in several biological assays. A few of the reduced complexity fractions from two extracts, both subsequently identified as *Moorea bouillonii*, were found to be highly cytotoxic to H-460 human lung cancer cells in vitro [52% survival at 30 μg/mL (fraction F from the 2009 collection), and 20% survival at 3 μg/mL (fraction H from the 2008 collection)], and these were chosen for further investigation. Bioassay-guided fractionation of these extracts yielded five new peptides, lyngbyabellin K–N (**1**–**5**), and these were structurally fully defined by spectrochemical methods. Additionally, the known metabolites

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apratoxins F and G were also isolated from these fractions and accounted for the majority of the remarkable cytotoxicity of the crude fractions.

Results and Discussion

Collection and Isolation of Lyngbyabellins K–N (1–5)

A collection of tube-like mats of the filamentous marine cyanobacterium *M. bouillonii* (PAL 8/3/09-1) was obtained by SCUBA diving in the north lagoon at Strawn Island, Palmyra Atoll, USA, in August 2009. The ethanol-preserved collection was subsequently repeatedly extracted with CH_2Cl_2/CH_3OH (2:1) and then fractionated by silica-gel vacuum column chromatography (VLC) to produce nine fractions (A–I). Fraction F possessed moderate anticancer activity against H-460 human lung cancer cells (52% survival at 30 μg/mL). Thus, this fraction was further purified by reverse-phase (RP) HPLC to afford lyngbyabellins K (**1**, 10 mg, 0.26%) and M (**4**, 2 mg, 0.05%) as well as mixtures of two epimers of lyngbyabellin L (**2** and **3**). This last mixture was subjected to chiral HPLC to ultimately yield pure lyngbyabellin L (**2**, 0.7 mg, 0.018%) and 7-*epi*-lyngbyabellin L (**3**, 0.6 mg, 0.015%).

An additional sample of *M. bouillonii* (PAL 8/16/08-3) was collected by SCUBA diving in 2008 from reefs 9–15 m deep surrounding Palmyra Atoll. This ethanol-preserved material was repeatedly extracted (CH_2Cl_2/CH_3OH, 2:1) and fractionated by using normal-phase VLC to yield nine fractions. Three polar fractions (100% EtOAc, 25% EtOAc/CH_3OH, and 75% EtOAc/CH_3OH) were strongly cytotoxic to H-460 cancer cells (20% survival at 3 μg/mL) and were thus fractionated with reverse-phase solid-phase extraction (RP-SPE) followed by preparative thin-layer chromatography (prepTLC) to yield 4.3 mg of highly purified lyngbyabellin N (**5**) as a pale yellow oil. These same fractions also contained several known apratoxins, and these compounds were responsible for the majority of the cytotoxicity.[3]

Structures of Lyngbyabellins K–N (1–3, 5) and 7-*epi*-Lyngbyabellin L (4)

Lyngbyabellin K (**1**), a pale yellow oil, showed an ion cluster at m/z = 578.99/580.96/582.96 in a ratio of 100:85:25 by low-resolution (LR) ESIMS, indicating the presence of two chlorine atoms in the molecule. The molecular formula of **1** was determined to be $C_{23}H_{28}Cl_2N_2O_7S_2$ by interpretation of high-resolution (HR) ESITOFMS data (m/z [M + Na]$^+$ = 601.0609). The IR spectrum of **1** displayed absorption bands at 3421, 1737, and 1616 cm^{-1}, indicating the presence of hydroxy, ester, and amide functionalities, respectively. The ^{13}C NMR spectra of **1** in $CDCl_3$ showed five downfield-shifted signals of quaternary carbon atoms (δ = 174.3, 169.8, 169.1, 161.0, and 160.2 ppm) that made double bonds with O or N and two carbon–carbon double bonds (δ = 146.4, 145.8, 128.9, and 128.5 ppm), accounting for seven of the ten degrees of unsaturation.

Two downfield-shifted singlets of protons at δ = 8.13 and 8.17 ppm with attached carbon atoms (signals at δ = 128.9 and 128.5 ppm) were attributed to two 2,4-disubstituted thiazole rings, accounting for two more degrees of unsaturation. The final degree of unsaturation was thus deduced to constitute an overall macrocyclic structure for **1**.

The two thiazole ring structures were confirmed by HMBC correlations from 12-H (δ = 8.13 ppm) to C-11 (δ = 145.8 ppm) and C-13 (δ = 169.8 ppm), and 18-H (δ = 8.17 ppm) to C-17 (δ = 146.4 ppm) and C-19 (δ = 169.1 ppm) (Table 1). The HMBC correlations from 12-H and 18-H of the two thiazole rings to carbonyl carbon atoms C-10 (δ = 160.2 ppm) and C-16 (δ = 161.0 ppm) indicated that carboxylic acid derivatives were directly attached to the 4-position of each of thiazole ring. In addition, COSY correlations between 14-OH (δ = 5.73 ppm), 14-H (δ = 5.39 ppm), and 15-Ha (δ = 4.74 ppm) and 15-Hb (δ = 4.65 ppm), as well as HMBC correlations from 15-Ha and 15-Hb to C-13 and C-16, established that a 1,2-dihydroxyethyl moiety formed a linkage between the two thiazole-4-carboxylate groups (Figure 1).

Further inspection of the ^1H NMR spectrum of **1** revealed a series of upfield and highly coupled resonances reflective of an aliphatic chain. Additionally, a downfield methyl singlet at δ = 2.12 (8-H_3) showed HMBC correlations to the signal of a quaternary carbon atom at δ = 90.4 ppm (C-7) as well as to a signal of a methylene carbon atom at δ = 49.5 ppm (C-6). The chemical shift of C-7 (δ = 90.4 ppm) was indicative of a *gem*-dichloro substituent, as observed in dolabellin,[16] hectochlorin,[17] and the lyngbyabellins,[15] and thus, accounted for the two chlorine atoms in the molecular formula of **1**. This moiety was extended to include an additional six carbon atoms (C-1 to C-5, and C-9, see Table 1) by integrated reasoning of COSY, HSQC, and HMBC data and identified this moiety as 7,7-dichloro-3-acyloxy-2-methyloctanoate (DCAMO). Additional HMBC correlations from 3-H of the DCAMO residue to a carbonyl carbon, C-10, of the first thiazole-4-carboxylate unit, allowed connection between these atoms through an ester bond.

Sequential COSY correlations between adjacent methine protons 20-H (δ = 5.53 ppm) and 21-H (δ = 2.24 ppm) to both doublet methyl groups 22-H_3 and 23-H_3, along with HMBC correlations from these two methyl groups back to C-20 and C-21, defined the side chain of a 2-hydroxyisovaleric acid (HIVA) residue. The HMBC correlations from 20-H to C-1 (δ = 174.3 ppm) and C-19 (δ = 169.1 ppm) supported the position of this HIVA-derived residue between C-19 and C-1, as shown in Figure 1, and completed the assignment of all atoms from the molecular formula of lyngbyabellin K (**1**). Additionally, from the above discussion, the sequence of residues in the macrocyclic ring was defined as DCAMO–HIVA–thiazole-1-carboxylate–glyceric acid–thiazole-2-carboxylate–DCAMO.

To define the absolute configuration at each stereocenter, lyngbyabellin K (**1**) was crystallized from CH_3CN. A needle-shaped crystal (monoclinic, 0.21 × 0.11 × 0.05 mm) was subjected to single-crystal X-ray diffraction analysis. Re-

Table 1. NMR spectral data for lyngbyabellin K–M (**1**–**4**) in $CDCl_3$ at 500 (^1H) and 125 MHz (^{13}C).

Position	**1**				**2**		**3**		**4**	
	δ_C [ppm]	δ_H multiplicity (*J*) [ppm] [Hz]	COSY	HMBC	δ_C [ppm]	δ_H [ppm]	δ_C [ppm]	δ_H [ppm]	δ_C [ppm]	δ_H [ppm]
1	174.3				174.6		174.6		174.4	
2	43.2	3.18 dt (9.4, 7.3)	3, 9	1, 3, 9	42.7	3.31	42.6	3.31	44.4	2.84
3	75.1	5.36 br. s	2, 4a, 4b	1, 10	75.6	5.33	75.4	5.34	75.4	3.80
3-OH										5.51
4a	30.7	1.92 m	3, 4b, 5		29.9	1.91	30.3	1.95	34.4	1.93
4b		1.73 m	3, 4a, 5	3, 5, 6		1.72		1.67		
5a	20.5	1.75 m	4, 6a, 6b	3, 4, 6, 7	20.5	1.61	21.0	1.63	21.5	1.86
5b						1.55		1.52		
6a	49.5	2.30 m	5, 6b	4, 5, 7, 8	40.1	1.77	40.3	1.77	50.0	2.24
6b		2.15 m	5, 6a	4, 5, 7, 8		1.66		1.66		
7	90.4				58.2	4.02	58.5	3.98	90.8	
8	37.6	2.12 s		6, 7	25.5	1.49	25.4	1.49	37.7	2.16
9	15.3	1.30 d (7.3)	2	1, 2, 3	15.3	1.30	15.1	1.30	15.0	1.26
10	160.2				160.0		160.4		161.8	
11	145.8				145.7		145.9		147.6	
12	128.5	8.13 s		10, 11, 13	128.3	8.15	128.2	8.14	128.2	8.16
13	169.8				169.7		169.2		171.2	
14	70.1	5.39 br. s	14-OH, 15a, 15b		70.1	5.35	70.0	5.33	70.30	5.41
14-OH		5.73 br. s	14			6.25		6.24		6.68
15a	69.1	4.74 dd (11.3, 2.3)	14	13, 16	69.9	4.76	69.4	4.76	70.26	4.95
15b		4.65 dd (11.3, 3.0)	14	13, 14, 16		4.58		4.57		4.30
16	161.0				160.8		160.7		161.7	
17	146.4				146.1		145.5		145.9	
18	128.9	8.17 s		16, 17, 19	128.9	8.16	128.9	8.16	129.2	8.30
19	169.1				169.9		169.7		170.5	
20	77.5	5.53 d (6.6)	21	1, 19, 21, 22, 23	77.6	5.51	77.4	5.51	77.5	5.99
21	33.6	2.37 m	20, 22, 23	19, 20, 22, 23	34.3	2.30	34.8	2.32	34.4	2.33
22	19.3	0.99 d (6.5)	21	20, 21, 23	19.2	1.04	19.0	1.03	19.5	1.13
23	17.9	1.07 d (6.5)	21	20, 21, 22	17.4	1.06	17.3	1.07	16.8	0.98
24									61.8	4.42
25									14.7	1.41

sulting from the presence of heavy atoms (chlorine and sulfur) in compound **1**, the absolute configuration could be deduced as (2*S*,3*S*,14*R*,20*S*) [final $R(F^2) = –0.02(2)$, see the Supporting Information].

Lyngbyabellin L (**2**) and 7-*epi*-lyngbyabellin L (**3**) showed essentially identical sodiated parent ion clusters by HR ESI-TOF MS at *m*/*z* = 567.1000 and 569.0980 in a ratio of 78:32, indicating that both possessed the same molecular formula of $C_{23}H_{28}ClN_2O_7S_2$, containing a single chlorine atom each. The ^1H and ^{13}C NMR spectra of **2** possessed several resonances similar to those of **1** (Table 1); thus confirming the presence of two extended 2,4-disubstituted thiazole units, an HIVA unit, and a 1,2-dihydroxyethyl moiety (Figure 1). However, in the ^1H and ^{13}C NMR spectra of **2**, a new doublet methyl signal appeared at δ = 1.49 ppm (8-H_3) and a downfield-shifted methine signal was observed at δ = 4.02 (7-H) and 58.2 ppm (C-7). These new features were accompanied by several missing resonances relative to **1**, namely, the distinctive methyl group singlet at δ = 2.12 ppm (8-H_3) and the signal of the quaternary *gem*-dichloro carbon atom at δ = 90.4 ppm (C-7). Interpretation of these data in combination with the altered molecular formula suggested that **2** was a monochloro species at C-7. The spectroscopic and 2D NMR linkage data as well as the HR ESITOFMS results for compound **3** were almost identical to those of **2**, and thus, compound **3** was deduced to be stereoisomeric with compound **2** at C-7.

Because the planar structures of **2** and **3** are closely related to that of **1**, except for the alterations at C-7, similar ^{13}C NMR shifts, optical rotations, and circular dichroism (CD) absorption measurements for all three compounds indicate that they are of the same enantiomeric series at all comparable centers (see the Supporting Information). Therefore, the absolute configurations of C-2, C-3, C-14, and C-20 of **2** and **3** were assigned to be the same as those for lyngbyabellin K (**1**), namely, (2*S*,3*S*,14*R*,20*S*).

Several different approaches were employed to determine the absolute configuration of C-7 in lyngbyabellin L (**2**) and its C-7 epimer (**3**). First, a detailed analysis of the ^1H NMR spectroscopic data for compound **2**, using a DQF-COSY experiment, revealed all of the vicinal ^1H–^1H coupling constants in the 7-chloro-3-acyloxy-2-methyloctanoate (CAMO) residue (Figure 2a). The large vicinal coupling constants (> 7 Hz) of 2-H–3-H and 3-H–4-Hb in **2** indicated that these protons were *anti* to one another. Conversely, the relatively small vicinal coupling constant of 3-H–4-Ha (*J* = 3 Hz) showed that they were in a *gauche* relationship. NOESY correlations between 2-H/9-H_3–4-Ha indicated 4-Ha and 4-Hb were *pro-S* and *pro-R*, respectively. The large vicinal coupling constants between 4-Ha and 5-Hb, as well as the NOESY correlation between 5-Ha and 3-H, allowed assignment of 5-Ha and 5-Hb as *pro-S* and *pro-R*, respectively. Similarly, a large vicinal coupling constant observed between 5-Ha and 6-Hb and NOESY

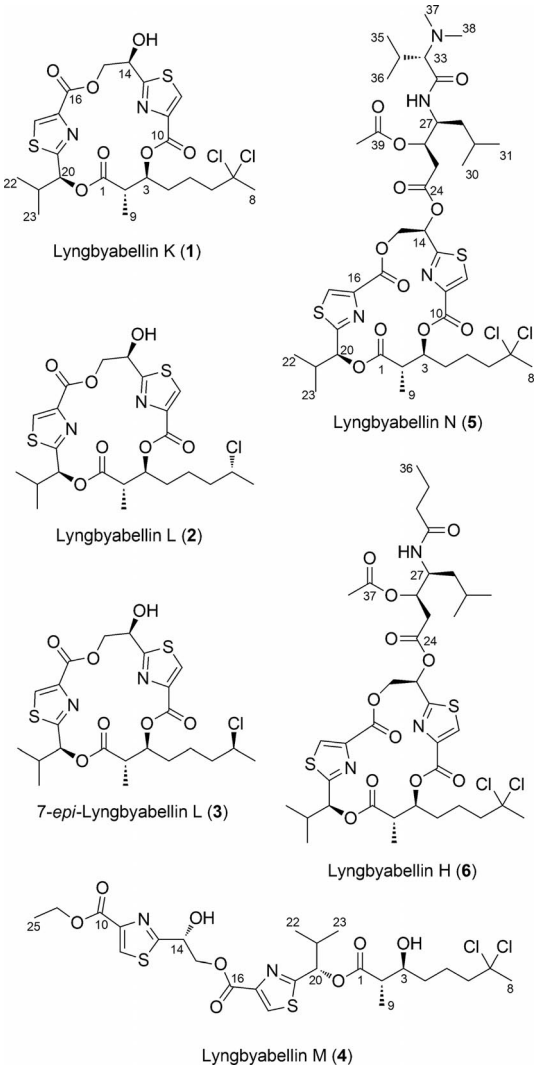

Figure 1. Structures of lyngbyabellin K–N (**1–5**) and lyngbyabellin H (**6**).

correlation between 4-Hb and 6-Hb indicated 6-Ha and 6-Hb to be *pro-R* and *pro-S*. Finally, a large coupling constant between 6-Ha and 7-H, small vicinal coupling constant between 6-Hb and 7-H, and NOESY correlations between 7-H with 5-Ha and 5-Hb and 8-H_3 with 6-Ha and 6-Hb, revealed that the absolute configuration at C-7 of **2** was (*R*). In a similar fashion, a detailed coupling constant analysis for the DCAMO residue of **3** revealed that the absolute configuration at C-7 was (*S*) (Figure 2a).

Finally, the prochirality of each proton attached to C-4, C-5, and C-6, as well as the absolute configuration at C-7 in **2**, was confirmed by *J*-based configurational analysis, as shown in Figure 2c.[18] A large homonuclear coupling constant, as measured by a DQF-COSY experiment, between 2-H and 3-H ($^3J_{2\text{-H},3\text{-H}}$ = 11.0 Hz) indicated an *anti* relationship between these protons. A HETLOC experiment was used to measure a large heteronuclear coupling constant between 2-H and C-3 ($^2J_{2\text{-H,C-3}}$ = –6.4 Hz), and in conjunction with NOESY correlations between 9-H_3 and 3-H/4-Ha led to the assignment of the relative stereochemistry of C-12–C-13 as the *erythro* rotamer B-3. Heteronuclear coupling constants between C-3/4-Ha ($^2J_{\text{C-3,4-Ha}}$ = 0 Hz) and C-3/4-Hb ($^2J_{\text{C-3,4-Hb}}$ = –5.9 Hz) also indicated that 4-Ha and the oxygen substituent at C-3 were in an *anti* relationship, whereas 4-Hb and the C-3 oxygen atom were *gauche*. The small heteronuclear coupling constants between 4-Ha/C-6, 5-Ha/C-3, 5-Hb/C-3 ($^3J_{\text{4-Ha,C-6}}$ = 1.5, $^3J_{\text{C-3,5-Ha}}$ = 1.0, $^3J_{\text{C-3,5-Hb}}$ = 0.8 Hz) revealed that the paired protons and carbon atoms were *gauche* to one another (Figure 2b). Additional small heteronuclear coupling constants between 5-Ha/C-7 and 5-Hb/C-7 ($^3J_{\text{5-Ha,C-7}}$ = 2.1, $^3J_{\text{5-Hb,C-7}}$ = 1.7 Hz) confirmed that C-7 was *gauche* to both 5-Ha and 5-Hb. Further small heteronuclear coupling constants between 7-H/C-5, 6-Ha/C-8, and 6-Hb/C-8 indicated that these paired atoms were in *gauche* relationships. Thus, based on this *J*-based configuration analysis and the absolute configuration of C-2 and C-3, the absolute configuration at C-7 in **2** was deduced to be (*R*); this was consistent with previous analyses of homonuclear coupling constants and NOESY correlations. These assignments were supported by a reversal in chemical shifts for the *pro-R* and *pro-S* protons attached to C-5 in **2** (δ = 1.55 and 1.61 ppm) relative to those in compound **3** (δ = 1.63 and 1.52 ppm), which we interpret to reflect deshielding effects to these nonequivalent protons from the chlorine atom at C-7 and oxygen atom at C-3[19] (Figure 2c).

The LR ESIMS results for lyngbyabellin M (**4**) showed a parent ion cluster at *m*/*z* = 624.95/626.95/628.93 in the ratio of 100:80:20, indicating the presence of two chlorine atoms, similar to metabolite **1**. The molecular formula of **4** was determined to be $C_{25}H_{34}N_2O_8Cl_2S_2$ by HR ESITOFMS: *m*/*z* $[M + Na]^+$ = 647.1029 (calcd. for $C_{25}H_{34}Cl_2N_2O_8S_2Na$ 647.1026). The ^1H and ^{13}C NMR spectra of **4** showed many features similar to those of **1**. However, the 3-H signal in lyngbyabellin K (**1**) was at δ = 5.36 ppm, whereas the signal of this proton in **4** was observed at δ = 3.80 ppm. The latter proton in **4** showed a unique COSY correlation with an additional exchangeable proton whose signal was found at δ = 5.51 ppm. Furthermore, in lyngbyabellin M a new methylene quartet (24-H_2) and methyl triplet (25-H_3) were observed at δ = 4.42 and 1.41 ppm, respectively, and these were connected to one another by COSY. An HMBC correlation from 24-H_2 to C-10 confirmed that the ester linkage between C-3 and C-10 of **1** had been cleaved and the carboxylic acid at C-10 in **4** was esterified with an ethyl moiety. Owing to the similarities in ^{13}C NMR shifts at most carbon positions and similar optical rotations, we propose that lyngbyabellin M (**4**) is of the same enantiomeric series as lyngbyabellin K (**1**).

The relative configuration at C-2 and C-3 of the DCAMO residue in lyngbyabellin M (**4**) was assigned as *erythro* by comparing $^3J_{\text{2-H,3-H}}$ of **4** (6.2 Hz) to the coupling constants of model diastereomers of methyl 3-hydroxy-2-methyloctanoate ($^3J_{\text{2-H,3-H}}$ for the *erythro* isomer 6.3 Hz; $^3J_{\text{2-H,3-H}}$ for the *threo* isomer = 3.6 Hz).[16] The absolute configuration at these two centers in compound **4** were revealed by NMR spectroscopic analyses of the di-Mosher esters produced by esterification of the C-3 and C-14 hy-

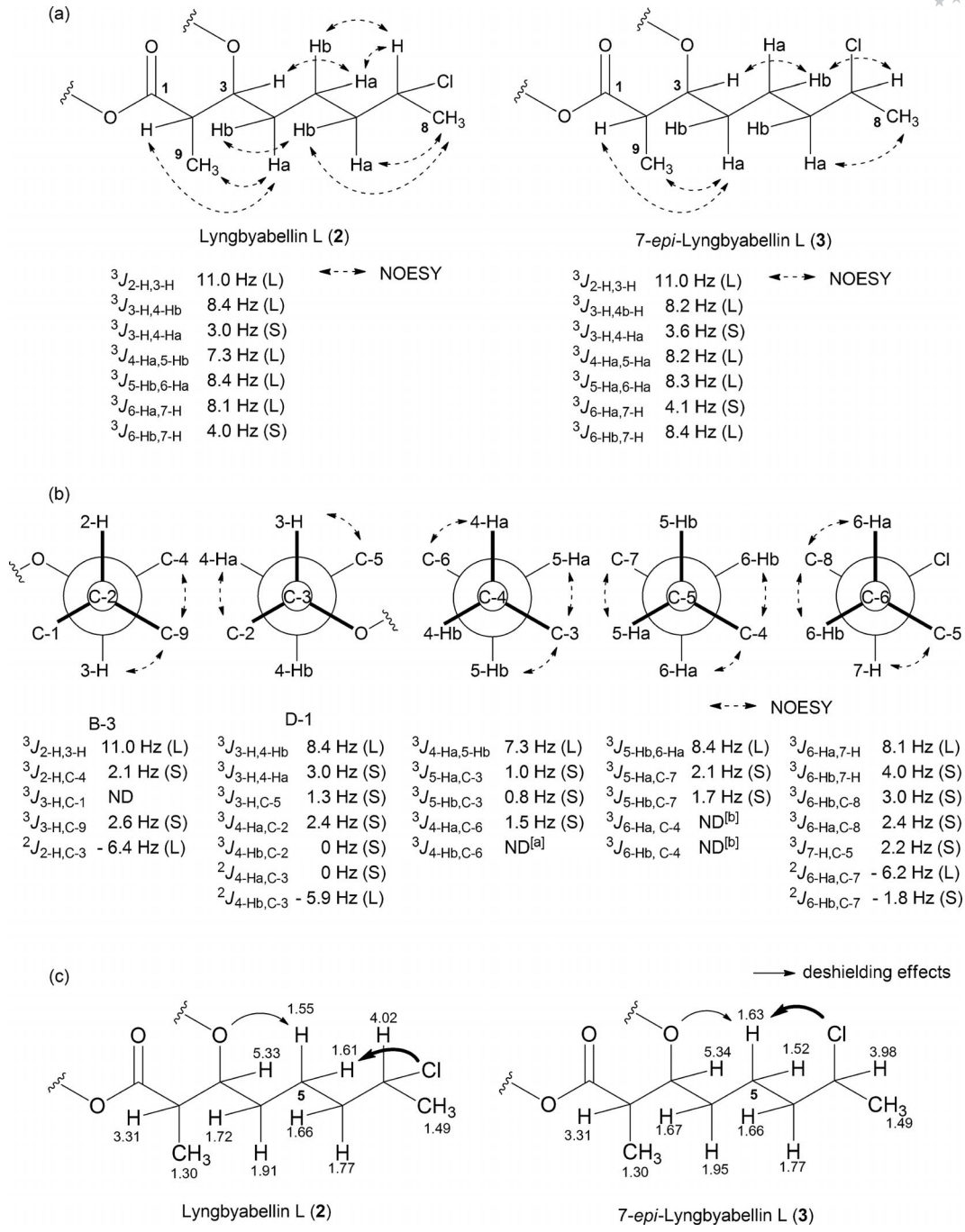

Figure 2. Approaches to the determination of the relative configuration of the C-7 stereocenter in the DCAMO residue of lyngbyabellin L (**2**) and 7-*epi*-lyngbyabellin L (**3**): (a) homonuclear coupling constant and NOE-based determination of relative configuration, (b) *J*-based configuration analysis on lyngbyabellin L (**2**), and (c) deshielding effects to inequivalent protons from the chlorine atom at C-7 and oxygen atom at C-3 of lyngbyabellin L (**2**) and 7-*epi*-lyngbyabellin L (**3**). [a] The coupling constant is not observed due to overlap. [b] The coupling constant is not detected due to a weak signal.

droxy groups. Calculation of $\Delta\delta_{S\text{-}R}$ values for protons near C-3 and C-14 allowed assignment of the absolute configuration as (3*S*) and (14*R*), respectively (Figure 3). Finally, the absolute configuration of the HIVA residue in **4** was determined to be L by GC–MS analysis of the methyl ester derivative following acid hydrolysis and comparison with standards, and thus, the complete absolute configuration of **4** was determined to be (2*S*,3*S*,14*R*,20*S*).

The HR ESITOFMS results for lyngbyabellin N (**5**) showed a parent ion cluster nearly identical to those of the other lyngbyabellin analogues with an $[M + H]^+$ ion at *m*/*z* = 905.2997/907.2977/909.2950 (calcd. for $C_{40}H_{59}Cl_2N_4O_{11}S_2$ 905.2993) in a ratio of 100:80:20, indicating the presence of two chlorine atoms and 13 degrees of unsaturation; 3 more than in compound **1**. The ^1H and ^{13}C NMR spectra of **5** again showed features similar to those of **1**; however, there

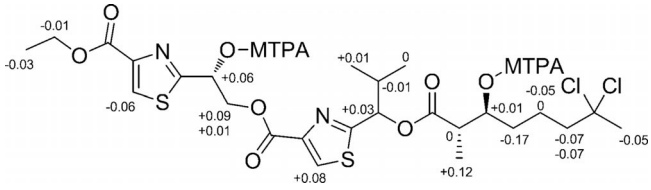

Figure 3. $\Delta\delta_{S-R}$ values of the di-Mosher esters of lyngbyabellin M (**4**).

was a significant increase in the number of new signals, suggesting the addition of several more residues. In the ^{1}H NMR spectrum, the broad singlet at δ = 5.73 ppm was no longer present; thus suggesting that **5** was similar to other known lyngbyabellins, which contain attached residues esterified to C-14.[15] Furthermore, there were three additional downfield methyl groups (δ = 2.77, 2.72, and 1.91 ppm) and two amide protons (δ = 9.38 and 8.81 ppm). From the ^{13}C NMR spectra, there were an additional three ester/amide carbonyl groups (δ = 165.8, 168.9, and 169.4 ppm), accounting for the remaining degrees of unsaturation. COSY and HMBC correlations established the presence of an *N*,*N*-dimethylvaline (DiMeVal) residue and an *O*-acetylated leucine statine. HMBC correlations from 14-H (δ = 6.27 ppm) to C-24 (δ = 168.9 ppm) and 27-NH (δ = 8.81 ppm) to C-32 (δ = 165.8 ppm) completed the planar structure of **5**, as depicted in Figure 1.

The planar structure of lyngbyabellin N (**5**) is closely related to that of lyngbyabellin H (**6**), except for the replacement of the polyketide portion with a DiMeVal residue. A comparison of optical rotations and carbon chemical shifts (macrocyclic lactone portion) strongly supported the assumption that the absolute configurations of the macrocyclic lactone in **5** and **6** were identical, and thus, we assigned the stereoconfiguration of lyngbyabellin N as (2*S*,3*S*,14*R*,20*S*). Further support of this configuration was obtained by a comparison of the CD absorption curve of **5** with those of **1**, **2**, and **3**. All of these CD curves were nearly identical, confirming that **5** has the (2*S*,3*S*,14*R*,20*S*) configuration.

The absolute configuration of the leucine statine in **5** was determined by LC–MS analysis of the acid hydrolysate appropriately derivatized with Marfey's reagent (D-FDAA). The four standards, (3*S*,4*S*)-statine (Sta), (3*R*,4*S*)-Sta, (3*S*,4*R*)-Sta, and (3*R*,4*R*)-Sta, were all synthesized from their corresponding amino acids (L- and D-leucine, respectively), and according to literature procedures, converted into their respective *N*-benzyl-protected aldehydes.[20] Each standard was then treated with *tert*-butyl 2-bromoacetate in the presence of *n*BuLi to yield a mixture of diastereomeric protected leucine statines. However, these diastereomers were inseparable by HPLC and were thus converted into *tert*-butyloxycarbonyl (Boc) protected analogues, which were readily purified by RP HPLC.[21] Hydrolysis, followed by derivatization with Marfey's reagent, yielded the four standards, which each possessed a distinct retention time in LC–MS [(3*R*,4*R*)-Sta-D-FDAA (78.2 min), (3*S*,4*R*)-Sta-D-FDAA (80.9 min), (3*S*,4*S*)-Sta-D-FDAA (92.2 min), and (3*R*,4*S*)-Sta-D-FDAA (93.1 min)]. From the retention time of the natural product statine derivative (93.29 min), it was clear that this residue was of the (3*R*,4*S*) configuration.

The absolute configuration of the DiMeVal residue in compound **5** was determined by comparing the chiral GC–MS retention time of the methylated residue liberated by acid hydrolysis with authentic standards. The two standards, L-*N*,*N*-DiMeVal and D-*N*,*N*-DiMeVal, were synthesized from L- and D-Val, respectively, according to literature procedures.[22] The two standards each possessed distinctly different retention times in GC–MS [L-DiMeVal (63.7 min) and D-DiMeVal (64.2 min)]. The methylated residue from the acid hydrolysate gave a single peak at 63.8 min; thus indicating the L configuration.

The lyngbyabellin family of compounds are known to exhibit moderate to potent cytotoxicity against a number of different cancer cell types through the promotion of actin polymerization.[15] Thus, after completion of the structural analysis, compounds **1**–**5** were evaluated in an H-460 human lung carcinoma cell cytotoxicity assay. Compound **5** showed strong yet variable cytotoxicity (IC_{50} = 0.0048–1.8 μM, perhaps due to solubility problems), whereas compounds **1**–**4** were inactive. However, in the HCT116 colon cancer cell line, reproducible IC_{50} values were obtained for lyngbyabellin N (40.9 ± 3.3 nM),[23] confirming the potent cytotoxic effect of this new member of the lyngbyabellin class of compounds and suggesting that the side chain of lyngbyabellin N is an essential structural feature for this potent activity. However, this trend is not entirely consistent within this structure class, because other lyngbyabellin analogues without the side chain exhibit sub-micromolar activity against HT29 and HeLa cells.[15]

It is interesting to note the increasing structural complexity in the lyngbyabellin family of metabolites, with that of lyngbyabelling N (**5**) being the most intricate to date. While it has the recognizable core of the lyngbyabellins, the side chain and DiMeVal terminus resemble those of the dolastatin 10 and coibacin A families of metabolites and, in this regard, it has a hybrid structure between these cyanobacterial natural product classes. Additionally, from a biosynthetic logic perspective, these more complex lyngbyabellins are perplexing, because they possess two logical points for the initiation of molecule construction: the polyketide chain represents one such point and DiMeVal the second. Thus, these complex lyngbyabellins may indeed represent the hybridization and cojoining of two natural product structure classes. An additional point of interest is the occurrence of monochlorinated carbon atoms in the polyketide section (compounds **2** and **3**). Previously, the Walsh group[24]and ourselves[25] independently showed that the monochloro species was not encountered in the process of chlorination of the methyl group of barbamide (only the di- and trichloro species are formed). Hence, the occurrence of a monochloromethylene moiety in these two metabolites suggests the functioning of a radical halogenase with differing catalytic properties from any of the currently characterized or partially characterized halogenases. It is still unclear whether lyngbyabellin M (**4**) is an extraction artefact or a

naturally occurring compound. Whereas lyngbyabellin M (**4**) has an ethyl ester at C-10, previously reported linear lyngbyabellins have a methyl ester at C-16. The position of the ethyl ester in **4** is consistent with the proposed biosynthetic pathway of the lyngbyabellins, as predicted from our knowledge of hectochlorin biosynthesis.[26] It has been shown that methyl esters of hybrid polyketides/non-ribosomal peptides are produced by an unusual *S*-adenosyl-L-methionine (SAM) dependent methyltransferase in myxobacteria.[27] Methyl and ethyl esters have been found as naturally occurring compounds in cyanobacterial extracts,[28] and thus, may be formed by pathway termination reactions similar to those in myxobacteria.

Conclusions

A chemical investigation of anticancer-active extracts of *M. bouillonii* collected in the Palmyra Atoll led to the isolation of the five new lyngbyabellins **1**–**5**. Their planar structures and absolute configurations were determined by a combination of various spectroscopy, chromatography and synthetic chemistry techniques. While compounds **1**–**4** were inactive in the H-460 cytotoxicity assay, compound **5** exhibited strong yet variable cytotoxicity (IC_{50} = 0.0048–1.8 μM). We do not understand the basis for this variable level of activity; however, it most likely relates to solubility issues in the assay buffer. These new lyngbyabellin metabolites possess several unique structural features, a monochloromethylene group and two conceptual points of chain initiation, which reflect unique biosynthetic reactions not yet characterized or understood.

Experimental Section

General Experimental Procedures: Optical rotations were measured with a JASCO P-2000 polarimeter; CD spectra were recorded in EtOH by using a JASCO J-810 spectropolarimeter. UV and IR spectra were recorded with a Beckman Coulter DU800 spectrophotometer and a Nicolet ThermoElectron Nicolet IR100 FTIR spectrometer as KBr plates, respectively. NMR spectra were recorded with chloroform as the internal standard (δ_C = 77.2 ppm; δ_H = 7.26 ppm) with a Varian 500 MHz spectrometer (500 and 125 MHz for ^1H and ^{13}C NMR, respectively) or a Bruker 600 MHz spectrometer (600 and 150 MHz for ^1H and ^{13}C NMR, respectively) equipped with a 1.7 mm MicroCryoProbe. For the HETLOC experiments, 128 scans (4 K) and 512 experiments were obtained with zero-filling at *F*1 to 4 K. A mixing time of 60–80 ms was used. HR ESI mass spectra were obtained with an Agilent 6230 ESI-TOF mass spectrometer. X-ray diffraction data was obtained by using state-of-the-art Bruker single-crystal diffractometers with CCD detectors. HPLC was carried out by using a Waters 515 pump system with a Waters 996 PDA detector. Acid hydrolysis was performed by using a Biotage (Initiator) microwave reactor equipped with high-pressure vessels.

Cyanobacterial Collections and Taxonomic Identification: The lyngbyabellins K–M producing cyanobacterium (collection code: PAL 8/3/09-1) was collected by SCUBA diving in the north of lagoon at Strawn Island, Palmyra Atoll, USA, in August 2008 (5° 53′ 55.76′′ N, 162° 05′ 0.092′′ W). The lyngbyabellin N producing cyanobacterium (collection code: PAL 8/16/08-3) was collected by SCUBA diving on reefs 9–15 m deep around the Palmyra Atoll, USA. The environmental samples were stored in EtOH/H_2O (1:1) at –20 °C, while genetic materials were preserved in RNA stabilization solution at –20 °C (RNAlater, Ambion Inc.). Morphological characterization was performed by using an Olympus IX51 epifluorescent microscope (100×) equipped with an Olympus U-CMAD3 camera. Taxonomic identification of cyanobacterial specimens was performed in accordance with current phycological systems.[29,30]

Isolation of Lyngbyabellins K, L, and M and 7-*epi*-Lyngbyabellin L (1–4): The cyanobacterial tissue (PAL 8/3/09-1, morphologically identified as *M. bouillonii*) was repetitively extracted with 2:1 CH_2Cl_2/CH_3OH to afford 3.9 g of crude extract. A portion of the extract (3.2 g) was fractionated by silica gel VLC with a stepwise gradient solvent system of increasing polarity starting from 10% EtOAc in hexanes to 100% CH_3OH to produce nine fractions (A–I). The fraction eluting with 80% EtOAc in hexane (fraction F) was subsequently separated by RP HPLC (Phenomenex Fusion RP 4 μ, 250×10 mm, 65% CH_3CN/H_2O at 3 mL/min) to give pure lyngbyabellin K (**1**, 10 mg, 0.26%), lyngbyabellin M (**4**, 2 mg, 0.05%), and a mixture of lyngbyabellin L and 7-*epi*-lyngbyabellin L. The mixtures were subjected to chiral HPLC (Chiral-AGP, 150×4.0 mm, 5 μm, UV: 210 nm), and lyngbyabellin L (**2**, 0.7 mg, 0.018%) and 7-*epi*-lyngbyabellin L (**3**, 0.6 mg, 0.015%) were eluted with 13% CH_3CN in H_2O.

Isolation of Lyngbyabellin N (5): 1 L of cyanobacterial tissue (previously identified as *M. bouillonii*)[3] was repetitively extracted with 2:1 CH_2Cl_2/CH_3OH to afford 4.2 g of crude extract. The extract was fractionated by silica gel VLC with a stepwise gradient solvent system of increasing polarity starting from 10% EtOAc in hexanes to 100% CH_3OH to produce nine fractions (A–I). The fraction eluting with 75% EtOAc in CH_3OH (fraction H) was subsequently separated by using 5 g RP SPE with a stepwise gradient solvent system decreasing in polarity starting from 55% CH_3CN in H_2O to 100% CH_2Cl_2 to produce five fractions (1–5). The fraction eluting with 70% CH_3CN in H_2O (fraction 3) was further separated by prepTLC, with an isocratic solvent system of 100% EtOAc, to yield pure lyngbyabellin N (**5**, 5.4 mg, 0.12%).

Lyngbyabellin K (1): Pale yellow oil. $[\alpha]_D^{25}$ = –28.0 (*c* = 0.5, MeOH). UV (MeOH): λ_{max} = 236 nm (log ε = 3.76). CD (MeOH): λ_{max} (Δε) = 211 (+1.11), 227 (–1.45), 238 (–0.58), 245 (–0.69), 265 nm (+0.28). IR (KBr): $\tilde{\nu}_{max}$ = 3421, 3127, 2965, 2935, 2880, 1737, 1616, 1481, 1379, 1320, 1211, 1163, 1096 cm^{-1}. ^1H, ^{13}C and 2D NMR: Table 1. HR ESIMS: *m*/*z*[M + Na]$^+$ = 601.0609 (calcd. for $C_{23}H_{28}Cl_2N_2O_7S_2Na$ 601.0607, Δ +0.2 mmu).

Lyngbyabellin L (2): Pale yellow oil. $[\alpha]_D^{25}$ = –33.0 (*c* = 0.5, MeOH). UV (MeOH): λ_{max} = 236 nm (log ε = 3.69). CD (MeOH) λ_{max} (Δε) = 210 (+0.19), 225 (–1.98), 239 (–0.70), 245 (–0.74), 265 nm (+0.17). IR (KBr): $\tilde{\nu}_{max}$ = 3440, 3116, 2962, 2921, 1738, 1480, 1367, 1320, 1211, 1100 cm^{-1}. ^1H, ^{13}C and 2D NMR: Table 1 and Supporting Information. HR ESIMS: *m*/*z*[M + Na]$^+$ = 567.1000 (calcd. for $C_{23}H_{29}ClN_2O_7S_2Na$ 567.0997, Δ +0.3 mmu).

7-*epi*-Lyngbyabellin L (3): Pale yellow oil. $[\alpha]_D^{25}$ = –34.8 (*c* = 0.3, MeOH). UV (MeOH): λ_{max} = 236 nm (log ε = 3.59). CD (MeOH) λ_{max} (Δε) = 210 (+0.25), 225 (–1.42), 239 (–0.51), 245 (–0.52), 265 nm (+0.12). IR (KBr): $\tilde{\nu}_{max}$ = 3731, 3623, 2962, 2920, 2846, 1737, 1458, 1368, 1319, 1211, 1101 cm^{-1}. ^1H, ^{13}C and 2D NMR: Table 1 and Supporting Information. HR ESIMS: *m*/*z*[M + Na]$^+$ = 567.1000 (calcd. for $C_{23}H_{29}ClN_2O_7S_2Na$ 567.0997, Δ +0.3 mmu).

Lyngbyabellin M (4): Pale yellow oil. $[\alpha]_D^{25}$ = –4.5 (*c* = 0.5, MeOH). UV (MeOH): λ_{max} = 234 nm (log ε = 3.71). IR (KBr): $\tilde{\nu}_{max}$ = 3377, 2963, 2926, 2853, 1730, 1644, 1465, 1334, 1224, 1175 cm^{-1}. ^1H, ^{13}C, and 2D NMR: Table 1 and Supporting Information. HR ESIMS: *m*/*z*[M + Na]$^+$ 647.1029 (calcd. for $C_{25}H_{34}Cl_2N_2O_8S_2Na$ 647.1026, Δ +0.3 mmu).

Lyngbyabellin N (5): Pale yellow oil. $[\alpha]_D^{27}$ = –24.0 (*c* = 1.05, MeOH). UV (MeOH): λ_{max} = 202 (log ε = 4.36), 235 nm (log ε = 3.99). CD (MeOH): λ_{max} ($\Delta\varepsilon$) = 212 (–0.64), 224 (–1.60), 237 (–0.27), 248 (–1.36), 268 nm (+0.03). IR (KBr): $\tilde{\nu}_{max}$ = 3436, 2961, 2933, 1742, 1677, 1467, 1372, 1321, 1233, 1166, 1097, 1037 cm^{-1}. ^1H, ^{13}C, and 2D NMR: Table 2. HR ESIMS: *m*/*z*[M + H]$^+$ = 905.2997 (calcd. for $C_{40}H_{59}Cl_2N_4O_{11}S_2$ 905.2993, Δ +0.4 mmu).

Table 2. NMR spectral data for lyngbyabellin N (**5**) in [D$_6$]DMSO at 500 (^1H) and 125 MHz (^{13}C).

Position	δ_C [ppm]	δ_H multiplicity (*J*) [ppm] [Hz]	COSY	HMBC
1	173.2			
2	42.9	2.84 dd (9.4, 6.8)	3, 9	1, 3, 9
3	74.5	5.13 m	2, 4a, 4b	
4a	30.0	1.80 m	3	3. 6
4b		1.69 m	3	3, 6
5a	29.0	1.24 m	6	4
6a	48.3	2.27 m	4a, 4b, 5	7, 8
6b		2.20 m	4a, 4b, 5	7, 8
7	92.1			
8	37.0	2.09 s		6, 7
9	14.6	1.16 d (7.1)	2	1, 2, 4
10	159.4			
11	145.4			
12	129.7	8.44 s		10, 11, 13
13	165.5			
14	70.2	6.27 t (6.1)	15	15
15a	63.6	4.81 dd (11.2, 5.2)	14	14
15b		4.57 dd (11.7, 6.8)	14	14
16	160.2			
17	145.0			
18	130.2	8.46 s		17, 19
19	167.2			
20	76.2	5.55 d (8.1)	21	1, 19, 21, 22, 23
21	31.9	2.24 m	20, 22, 23	20, 23
22	18.4	0.80 d (6.5)	21	20, 21, 23
23	17.9	1.00 d (6.5)	21	20, 21, 22
24	168.9			
25a	34.0	2.91 m	26	24
25b		2.70 m	26	24, 26
26	71.6	5.12 m	25a, 25b, 27	
27	47.9	4.35 t (4.4)	26, 28a, 28b, *N*H-1	
28a	37.5	1.41 m	29	29, 31
28b		1.23 m	29	29, 31
29	24.4	1.55 m	28, 30, 31	
30	24.0	0.90 d (6.4)	29	28, 29, 31
31	20.8	0.81 d (6.2)	29	28, 29, 31
27-NH		8.81 d (8.7)	27	
32	165.8			
33	71.8	3.63 t (6.7)	34	32, 34, 35, 36
34	26.3	2.31 m	33, 35, 36	32, 33, 35, 36
35	19.5	1.07 d (6.8)	34	33, 34, 36
36	16.5	0.94 d (6.6)	34	33, 34, 35
37	41.5	2.72 d (4.0)		33, 38
38	41.0	2.77 d (4.2)		33, 37
33-NH		9.38 br. s		
39	169.4			
40	20.8	1.91 s		39

X-ray Crystallography: Compound **1** (8 mg), dissolved in CH_3CN (400 μL), was transferred into a tube and the tube was placed in a vial. The vial was sealed and monitored for crystal growth over a month, then colorless needle-shaped crystals were observed.

Crystal Data for Lyngbyabellin K (1): Monoclinic, space group $P2_1/c$, unit cell dimensions *a* = 10.6419(8) Å, *b* = 5.8776(5) Å, *c* = 23.859(2) Å, α = 90°, β = 101.928(6)°, γ = 90°, *V* = 1460.1(2) Å^3, *Z* = 2, $\rho_{calcd.}$ = 1.411 g cm^{-3}, crystal dimensions 0.21 × 0.11 × 0.05 mm. A total of 7218 reflections were collected covering the indices –12 ≤ *h* ≤ 12, –6 ≤ *k* ≤ 6, –27 ≤ *l* ≤ 10. 4362 reflections were symmetry-independent, with R_{int} = 0.0358. Final $R(F^2)$ = –0.02(2). Relevant data collection parameters and results of structural refinement for this structural determination are given in the Supporting Information. CCDC-882823 [lyngbyabellin K (**1**)] contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Ozonolysis, Acid Hydrolysis, and Chiral GC–MS: A portion (100 μg) of **4** was dissolved in CH_2Cl_2 (500 μL) at −78 °C, and O_3 was bubbled through the sample for 5 min. The pale blue solution was dried under N_2 (g). The products were resuspended in 6 N HCl (200 μL) and allowed to react at 110 °C for 2 h. The acid hydrolysates were dried under N_2 (g), treated with an excess of CH_2N_2 at room temperature for 30 min, and dried under N_2 (g). The products were dissolved in CH_2Cl_2 and injected into chiral GC–MS (Chirasil-Val, Agilent Technologies J&W Scientific, 30 m × 0.25 mm) under the following conditions: the initial oven temperature was 32 °C, kept for 15 min, followed by a ramp from 32 to 60 °C at a rate of 10 °C/min, followed by another ramp to 200 °C, at a rate of 15 °C/min and kept at 200 °C for 5 min. The retention time of products resulting from the acid hydrolysate of **4** matched the synthetic (2*S*)-HIVA standard [9.7 min; (2*R*)-HIVA: 10.2 min]. Synthetic standards of (2*S*)-HIVA and (2*R*)-HIVA were methylated with an excess of CH_2N_2, dried under N_2 (g), resuspended in CH_2Cl_2, then analyzed with chiral GC–MS. The retention time of products resulting from the acid hydrolysate of **4** matched the synthetic (2*S*)-HIVA standard [9.7 min; (2*R*)-HIVA: 10.2 min].

Ozonolysis and Acid Hydrolysis of Lyngbyabellin N (5): A portion (1 mg) of **5** was dissolved in CH_2Cl_2 (1 mL) at –78 °C, and O_3 was bubbled through the sample for 10 min. The pale blue solution was dried under N_2 (g). The products were resuspended in 6 N HCl (500 μL) and allowed to react at 160 °C in a microwave reactor for 5 min.

Modified Marfey's Analysis to Determine the Configuration of the Statine Units: An aliquot (ca. 500 μg) of the acid hydrolysate was dried under N_2 (g) and dissolved in 1 M sodium hydrogen carbonate (1 mL), and 1% D-FDAA (12 μL) in acetone was added. The solution was maintained at 40 °C for 90 min, at which time the reaction was quenched by the addition of CH_3CN, and 10 μL of the solution was analyzed by LC–ESIMS. The Marfey's derivatives of the hydrolysate and standards were analyzed by RP HPLC by using a Phenomenex Luna 5 μ C_{18} column (4.6 × 250 mm). The HPLC conditions began with 10% CH_3CN/H_2O acidified with 0.1% formic acid (FA) followed by a gradient profile to 50% CH_3CN/H_2O acidified with 0.1% FA over 85 min at a flow of 0.4 mL/min, monitoring from 200 to 600 nm. The retention times of the authentic acid D-FDAA derivatives were 78.2, 80.9, 92.2, and 93.1 min for (3*R*,4*R*)-, (3*S*,4*R*)-, (3*S*,4*S*)-, and (3*R*,4*S*)-Sta-D-FDAA, respectively; the hydrolysate product gave a peak with a retention time of 93.3 min, indicating an absolute configuration of (3*R*,4*S*). Correspondingly, (3*S*,4*S*)-, (3*S*,4*R*)-, (3*R*,4*R*)-, and (3*R*,4*S*)-statine were

synthesized from L- (1 g) and D-leucine (1 g), respectively, according to a procedure reported by Reetz et al. to yield the benzyl-protected aldehyde.[20] An aliquot (3.38 mmol) of the benzyl-protected aldehydes dissolved in tetrahydrofuran (15 mL) was then added to a solution of *tert*-butyl acetate (3.72 mmol) and freshly prepared lithium diisoproplyamine (5.06 mmol). The reaction mixture was warmed to –40 °C for 1 h and then quenched with $NaHCO_3$ (85 mL), filtered, and the aqueous layer was separated and washed with Et_2O (2× 20 mL). The organic layer was then washed with brine and dried with $NaSO_4$ and filtered to yield diastereomeric *tert*-butyl 4-(dibenzylamino)-3-hydroxy-6-methylheptanoate. The mixture of diastereomers were inseparable; thus, the method outlined by Andrés et al. was used, where the benzyl-protected amine was converted into the Boc-protected derivative allowing for purification of a small amount of each of the diastereomers.[21] Each of the diastereomers were identified by a comparison of ^{1}H NMR spectra of each of the pure standards to the known compounds reported in the literature.[21] An aliquot (2 mg) of each protected statine was treated with 6 N HCl (1 mL) and heated to 160 °C in a microwave reactor for 5 min. Each of the hydrolysate products was dried with N_2 (g) and then treated with Marfey's reagent as mentioned above to yield the four Marfey-derived statine standards.

Preparation and GC–MS Analysis of HIVA: An aliquot (ca. 500 μg) of the hydrolysate was treated with an excess of CH_2N_2 at room temperature for 30 min and dried under N_2 (g). Correspondingly, L- and D-HIVA were synthesized as described above. The products were dissolved in CH_2Cl_2 and injected into a chiral GC–MS instrument with a Chiralsil-Val column (Agilent Technologies J&W Scientific, 30 m × 0.25 mm) under the following conditions: the initial oven temperature was 32 °C, kept for 15 min, followed by a ramp from 32 to 60 °C at a rate of 10 °C/min, followed by another ramp to 200 °C, at a rate of 15 °C/min and kept at 200 °C for 5 min. The retention time of products resulting from the acid hydrolysate of **4** matched those of the synthetic (2*S*)-HIVA standard [9.7 min; (2*R*)-HIVA, 10.2 min].

Preparation and GC–MS Analysis of DiMeVal: The methylated hydrolysate product of **5** was analyzed by chiral GC–MS using a Cyclosil B column (Agilent Technologies J&W Scientific, 30 m × 0.25 mm) under the following conditions: the initial oven temperature was 34 °C and kept for 68 min, followed by a ramp from 34 to 100 °C at a rate of 30 °C/min and kept at 100 °C for 5 min. Synthetic standards of (2*S*)- and (2*R*)-DiMeVal were first methylated by dissolving each starting material (10 mg) in H_2O (433 μL), followed by the addition of formaldehyde (27 μL) and 10% Pd/C (10.4 mg). The mixture was then treated with H_2 (g) for 16 h. After 16 h, the reaction mixtures were brought to a boil and then concentrated in vacuo. Each of the synthetic standards were then treated with CH_2N_2 for 5 min and then dried with N_2 (g), resuspended in CH_2Cl_2, then analyzed by chiral GC–MS. The retention time of products resulting from the acid hydrolysate of **5** matched that of the authentic (2*S*)-DiMeVal standard [63.7 min; (2*R*)-DiMeVal, 64.2 min].

Preparation of the α-Methoxy-α-(trifluoromethyl)phenylacetic Acid (MTPA) Ester of Lyngbyabellin M: Duplicate samples of compound **4** (0.5 mg) were dried and dissolved in anhydrous pyridine (1 mL), and a catalytic amount of 4-(dimethylamino)pyridine was added. Separately and into each vial, 15 μL of (*R*)-MTPA chloride and 15 μL of (*S*)-MTPA chloride were added. The reaction vials were stored at 40 °C with stirring for 48 h. The acylation products were purified by using RP HPLC (Phenomenex Jupiter 5 μ C_{18}, 4.6 × 250 mm, 85% CH_3OH/H_2O at 1 mL/min). The *m*/*z* values of the two diastereomeric MTPA derivatives of compound **4** were observed by ESIMS, and the ^{1}H NMR chemical shift was assigned by COSY.

3,14-Di-(*S*)-MTPA Ester of Lyngbyabellin M (6): Pale yellow amorphous solid. ^{1}H NMR ($CDCl_3$, 600 MHz): δ = 8.16 (s, 1 H, 12-H), 7.96 (s, 1 H, 13-H), 6.78 (dd, *J* = 7.6, 2.9 Hz, 1 H, 14-H), 5.98 (d, *J* = 5.4 Hz, 1 H, 20-H), 5.48 (td, *J* = 7.6, 3.4 Hz, 1 H, 3-H), 5.07 (dd, *J* = 12.3, 2.9 Hz, 1 H, 15-Ha), 4.79 (dd, *J* = 12.3, 7.6 Hz, 1 H, 15-Hb), 4.43 (q, *J* = 7.1 Hz, 2 H, 24-H_2), 3.05 (dq, *J* = 7.6, 7.2 Hz, 1 H, 2-H), 2.41 (m, 1 H, 21-H), 2.12 (m, 1 H, 6-Ha), 2.07 (m, 1 H, 6-Hb), 2.06 (s, 3 H, 8-H_3), 1.73 (m, 1 H, 5-Ha), 1.68 (m, 1 H, 5-Hb), 1.59 (m, 2 H, 4-H_2), 1.41 (t, *J* = 7.1 Hz, 3 H, 25-H_3), 1.28 (d, *J* = 7.2 Hz, 3 H, 9-H_3), 0.96 (d, *J* = 6.8 Hz, 3 H, 22-H_3), 0.95 (d, *J* = 6.8 Hz, 3 H, 23-H_3) ppm. LR ESIMS: *m*/*z* = 1057.02 $[M + H]^+$, 1079.12 $[M + Na]^+$.

3,14-Di-(*R*)-MTPA Ester of Lyngbyabellin M (7): Pale yellow amorphous solid. ^{1}H NMR ($CDCl_3$, 600 MHz): δ = 8.22 (s, 1 H, 12-H), 7.88 (s, 1 H, 18-H), 6.72 (dd, *J* = 7.1, 3.1 Hz, 1 H, 14-H), 5.95 (d, *J* = 5.4 Hz, 1 H, 20-H), 5.47 (m, 1 H, 3-H), 4.98 (dd, *J* = 12.3, 3.1 Hz, 1 H, 15-Ha), 4.78 (dd, *J* = 12.3, 7.1 Hz, 1 H, 15-Hb), 4.44 (q, *J* = 7.1 Hz, 2 H, 24-H_2), 3.05 (m, 1 H, 2-H), 2.42 (m, 1 H, 21-H), 2.19 (m, 1 H, 6-Ha), 2.14 (m, 1 H, 6-Hb), 2.11 (s, 3 H, 8-H_3), 1.78 (m, 1 H, 5-Ha), 1.68 (m, 1 H, 5-Hb), 1.76 (m, 2 H, 4-H_2), 1.42 (t, *J* = 7.1 Hz, 3 H, 25-H_3), 1.16 (d, *J* = 7.2 Hz, 1 H, 9-H), 0.95 (d, *J* = 6.8 Hz, 6 H, 22-H_3, 23-H_3) ppm. LR ESIMS: *m*/*z* = 1056.99 $[M + H]^+$, 1079.07.58 $[M + Na]^+$.

Cytotoxicity Assay: H-460 cells were added to 96-well plates at 3.33×10^4 cells/mL of Roswell Park Memorial Institute (RPMI) 1640 medium with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. The cells, in a volume of 180 μL per well, were incubated overnight (37 °C, 5% CO_2) to allow recovery before treatment with test compounds. Compounds were dissolved in DMSO to a stock concentration of 10 mg/mL. Working solutions of the compounds were made in RPMI 1640 medium without FBS, with a volume of 20 μL added to each well to give a final compound concentration of either 30 or 3 μg/mL. An equal volume of RPMI 1640 medium without FBS was added to wells designated as negative controls for each plate. Plates were incubated for approximately 48 h before being stained with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). By using a ThermoElectron Multiskan Ascent plate reader, plates were read at 570 and 630 nm. Concentration response graphs were generated by using GraphPad Prism (GraphPad Software Inc., San Diego, CA).

Supporting Information (see footnote on the first page of this article): ^{1}H NMR, ^{13}C NMR, COSY, gHSQC, and HMBC spectra in $CDCl_3$ for lyngbyabellin K and M (**1** and **4**). ^{1}H NMR spectra in $CDCl_3$ for the Mosher derivatives of lyngbyabellin M (**4**). ^{1}H NMR, ^{13}C NMR, gHSQC, COSY, HMBC, DQF-COSY, NOESY, and HETLOC spectra in $CDCl_3$ for lyngbyabellin L (**2**) and 7-*epi*-lyngbyabellin L (**3**). ^{1}H NMR, ^{13}C NMR, gHSQC, COSY, HMBC, and TOCSY spectra in [D_6]DMSO for lyngbyabellin N (**5**). ^{1}H NMR and ^{13}C NMR in $CDCl_3$ for lyngbyabellin N (**5**). ORTEP representation of lyngbyabellin K (**1**) crystal, and crystal data. CD spectra for lyngbyabellin K, L, N (**1**, **2**, and **5**) and 7-*epi*-lyngbyabellin L (**3**).

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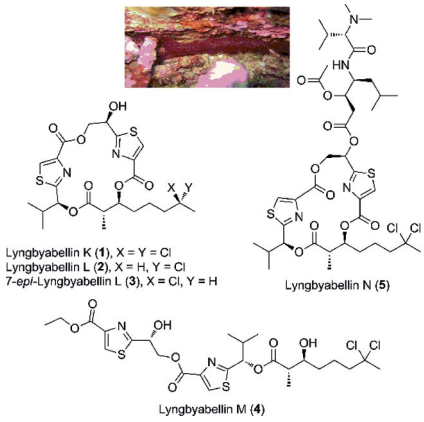
Cytotoxic Lipopeptides

Two independent collections of the marine cyanobacterium *Moorea bouillonii* led to the isolation of five lipopeptides of the lyngbyabellin structure class. Their structures were elucidated by various spectroscopy, synthesis, and chromatography techniques. Lyngbyabellin N showed strong cytotoxic activity against the HCT116 colon cancer cell line (IC_{50} = 40.9 ± 3.3 nM).



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Lyngbyabellins K–N from Two Palmyra Atoll Collections of the Marine Cyanobacterium *Moorea bouillonii*

Keywords: Natural products / Peptides / Structure elucidation / Biological activity

