# Isolation, Structure Determination, and Biological Activity of Lyngbyabellin A from the Marine Cyanobacterium *Lyngbya majuscula*

Hendrik Luesch,[†] Wesley Y. Yoshida,[†] Richard E. Moore,*[,†] Valerie J. Paul,*[,‡] and Susan L. Mooberry[§]

*Department of Chemistry, University of Hawaii at Manoa, Honolulu, Hawaii 96822, University of Guam Marine Laboratory, UOG Station, Mangilao, Guam 96923, and Cancer Research Center of Hawaii, 1236 Lauhala Street, Honolulu, Hawaii 96813*

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Lyngbyabellin A (**1**), a significantly cytotoxic compound with unusual structural features, was isolated from a Guamanian strain of the marine cyanobacterium *Lyngbya majuscula*. This novel peptolide is structurally related to dolabellin (**2**) in that both depsipeptides bear a dichlorinated $\beta$-hydroxy acid and two functionalized thiazole carboxylic acid units. Its gross structure has been elucidated by spectral analysis, including 2D NMR techniques. The absolute stereochemistry of **1** was determined by chiral HPLC analysis of hydrolysis products and by characterization of the degradation products methyl 7,7-dichloro-3-hydroxy-2,2-dimethyloctanoate (**3**) and the corresponding acid **4**. The total structure was further supported by molecular modeling studies. The isolation of **1** from *L. majuscula* once more supports the proposal that many compounds originally isolated from the sea hare *Dolabella auricularia* are of cyanobacterial origin. Lyngbyabellin A (**1**) was shown to be a potent disrupter of the cellular microfilament network.

Terrestrial and marine cyanobacteria are producers of numerous bioactive compounds such as the cryptophycins[1] and curacins.[2] In our ongoing program to explore these organisms as sources for novel anticancer agents, our most recent investigations have led to the finding of epimeric dolastatin 12[3] and several dolastatin analogues in certain field-collected marine cyanobacteria belonging to the Oscillatoriaceae.[3,4] Among them is symplostatin 1,[4a] structurally almost identical to dolastatin 10,[5] one of the most potent antineoplastic compounds known to date and currently in clinical trials. Our discoveries indicated a likely cyanobacterial origin for the dolastatins,[6] which were first isolated from the sea hare *Dolabella auricularia*, a known generalist herbivore.[7] This paper describes lyngbyabellin A (**1**), a new cytotoxin that is closely related to another compound originally isolated from *D. auricularia*, dolabellin (**2**).[8] Peptolide **1** has been isolated from several recollections of a lyngbyastatin 2-producing strain of *Lyngbya majuscula* Harvey ex Gomont (UOG strain VP417) found on Finger's Reef, Guam.[4c] Lipophilic extracts of the various collections of VP417 showed remarkable cytotoxicity due to the presence of several active components. Bioassay-guided fractionation by solvent partition and successive chromatographic steps afforded the new cytotoxin **1** as a colorless crystalline solid.

## Results and Discussion

**Gross Structure.** HRFABMS analysis established the molecular formula for **1** as $C_{29}H_{40}Cl_2N_4O_7S_2$. The observed isotope peaks at *m/z* 691/693/695, in an approximately 5:4:1 ratio, in the HRFABMS are consistent with the presence of two chlorine atoms in the molecule. The two chlorine atoms were suspected to be geminal to each other because the $^{13}C$ NMR spectrum (Table 1) displayed a signal at $\delta$ 90.0 for a quaternary carbon (C-7), which showed a HMBC correlation to a methyl singlet resonating at $\delta_H$ 2.05 (H-8). The chemical shift for the methyl carbon was consistent with the partial structure $CCl_2CH_3$ ($\delta_{C-8}$ 37.1). Further (mainly HMBC) analysis (Table 1) allowed us to expand the structure to a 7,7-dichloro-3-acyloxy-2,2-dimethyloctanoate residue. Chemical shifts indicated that C-3 was clearly oxygenated ($\delta_{C-3}$ 78.1, $\delta_{H-3}$ 5.31), and the low-field resonance for H-3 suggested that an acyloxy group was at this position.

Two low-field signals in the $^1H$ NMR spectrum at $\delta$ 8.09 and 8.23, as well as eight $sp^2$ carbon resonances at $\delta$ 126.5, 127.9, 146.8, 148.1, 161.0, 161.5, 164.6, and 168.5, were indicative of two 2-alkylthiazole-4-carboxylic acid units (C-11 to C-14 and C-22 to C-25). The signal for H-24 ($\delta$ 8.23) of one heteroaromatic ring appeared as a doublet showing long-range coupling ($J$ = 0.8 Hz) to a signal at $\delta$ 6.13. The coupling partner at $\delta$ 6.13 was found to be a methine proton (H-26) on an oxygenated $sp^3$ carbon (C-26, $\delta$ 77.1). The extremely low-field chemical shift for H-26 could be ascribed to the deshielding anisotropic effects of the adjacent heterocycle and the acyloxy group at this position. Furthermore, C-26 was connected to a quaternary oxygenated carbon (C-27, $\delta$ 71.8), inasmuch as two methyl singlets ($\delta_H$ 1.24 and 1.38 for H-28 and H-29, respectively) showed HMBC correlation to both oxygenated carbons (C-26, C-27). A deuterium exchange experiment (1:1 mixture of MeOH-$d_3$ and MeOH-$d_4$ as solvent) established that C-27 bore a hydroxyl group, as depicted in structure **1**, and hence was indeed oxygenated and not, for example, chlorinated. The doubling of the signal for C-27 in the $^{13}C$ NMR spectrum clearly confirmed the presence of the hydroxyl group on this carbon. NMR analysis further revealed the presence of a glycine moiety and an isoleucine-derived unit. Two HMBC experiments optimized for different $^{13}C-^1H$ coupling constants provided proof for all of the linkages leading to the cyclic gross structure shown for **1** (Table 1).

The IR spectrum shows a strong absorption at 1725 $cm^{-1}$ and an even more intense band centered at 1659 $cm^{-1}$, confirming the presence of both ester and amide functionalities in the molecule. The broad band at 3403 $cm^{-1}$ further confirmed the presence of a hydroxyl group.

Characteristic for both **1** and **2** is the presence of a dichlorinated $\beta$-hydroxy acid unit, which is additionally

* To whom correspondence should be addressed. (R.E.M.) Tel.: (808) 956-7232. Fax: (808) 956-5908. E-mail: moore@gold.chem.hawaii.edu. (V.J.P.) Tel.: (671) 735-2186. Fax: (671) 734-6767. E-mail: vpaul@uog9.uog.edu.
[†] University of Hawaii at Manoa.
[‡] University of Guam Marine Laboratory.
[§] Cancer Research Center of Hawaii.

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**Table 1.** NMR Spectral Data for Lyngbyabellin A (**1**) in $CDCl_3$

| C/H no. | $\delta_H$[a] (*J* in Hz) | $\delta_C$[b] | $^1H-^1H$ COSY[c] | HMBC[a,d,e] |
|---|---|---|---|---|
| 1 | | 173.1, s | | H-3, H-9, H-10, H-26 |
| 2 | | 46.6, s | | H-3, H-9, H-10 |
| 3 | 5.31, dd (10.6, 2.3) | 78.1, d | H-4 | H-9, H-10 |
| 4 | 1.33, m, 1.72, m | 29.4, t | H-3, H-5 | H-3 |
| 5 | 1.60, m | 22.3, t | H-4, H-6 | H-3, H-6 |
| 6 | 2.00, m, 2.22, ddd (−14.2, 10.8, 5.2) | 49.2, t | H-5 | H-8 |
| 7 | | 90.0, s | | H-6, H-8 |
| 8 | 2.05, s | 37.1, q | | H-6 |
| 9 | 1.31, s | 24.1, q | | H-10 |
| 10 | 1.36, s | 20.4, q | | H-3, H-9 |
| 11 | | 161.5, s | | H-3, H-13[f] |
| 12 | | 146.8, s | | H-13 |
| 13 | 8.09, s | 127.9, d | | |
| 14 | | 168.5, s | | H-13, H-15 |
| 15 | 5.24, dd (9.0, 6.8) | 55.0, d | 15-NH, H-16 | H-17, H-19 |
| 15-NH | 7.27, d (9.0) | | H-15 | |
| 16 | 1.97, m | 40.0, d | H-17, H-19 | H-15, H-17, H-18, H-19 |
| 17 | 1.13, m, 1.50, m | 25.4, t | H-16, H-18 | H-15, H-18, H-19 |
| 18 | 0.90, t (7.3) | 11.2, q | H-17 | H-17 |
| 19 | 0.75, d (6.6) | 14.9, q | H-16 | H-15, H-17 |
| 20 | | 168.2, s | | 15-NH, H-21 |
| 21 | 3.70, dd (−17.0, 4.3)<br>4.70, dd (−17.0, 9.2) | 42.9, t | 21-NH | |
| 21-NH | 7.97, dd (9.2, 4.3) | | | |
| 22 | | 161.0, s | | H-21, 21-NH, H-24[f] |
| 23 | | 148.1, s | | H-24 |
| 24 | 8.23, d (0.8) | 126.5, d | | |
| 25 | | 164.6, s | | H-24, H-26 |
| 26 | 6.13, d (0.8) | 77.1, d | | H-28, H-29 |
| 27 | | 71.8, s | | H-28, H-29 |
| 28 | 1.24, s | 25.8, q | | H-29 |
| 29 | 1.38, s | 27.0, q | | H-26, H-28 |

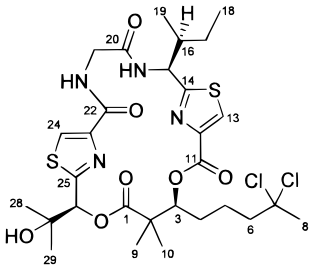
[a] Recorded at 500 MHz. [b] Recorded at 125 MHz. [c] Recorded at 400 MHz. [d] Protons showing long-range correlation with indicated carbon. [e] If not indicated otherwise, correlations were observed after optimization for $^nJ_{CH} = 7$ Hz. [f] Correlation observed after optimization for $^nJ_{CH} = 5$ Hz.

methylated in the new metabolite **1**. The compounds are structurally related to mirabimide E, which contains a tetrachlorinated *β*-hydroxy acid unit.[9] α,*β*-Dihydroxyisovaleric acid is a building block in **1**, whereas an α-hydroxyisovaleric acid-derived unit is present in **2**. Both acids are biosynthetically related, inasmuch as α,*β*-dihydroxyisovaleric acid is a known intermediate in the biosynthesis of valine and ketoisovaleric acid from which α-hydroxyisovaleric acid is derived.[10] Dolabellin (**2**) was the first example of a natural product containing a thiazole hydroxy acid residue; lyngbyabellin A (**1**) represents another one. The structural modifications, including the presence of two amino acid units (glycine and isoleucine), hydroxylation of C-27, and its cyclic nature make **1** interesting and distinctive. However, the structural analogy between lyngbyabellin A (**1**) and dolabellin (**2**) strongly suggests a cyanobacterial origin for **2**, which is consistent with our previous findings about the origin of many dolastatins.

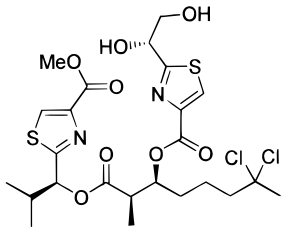
**Absolute Stereochemistry.** The absolute stereochemistry could be established by analysis of degradation products. First, **1** was subjected to ozonolysis followed by acid hydrolysis, and the product mixture was analyzed by HPLC on two chiral columns [Chirex phase 3126 (D) and CHIRALPAK MA(+)]. This procedure allowed the detection of glycine and L-isoleucine and clearly eliminated the three other isoleucine isomers from further consideration. The configurations at C-15 and C-16 in **1** are, therefore, 15*S*, 16*S*. (*S*)- and/or (*R*)-α,*β*-dihydroxyisovaleric acid could not be detected in the acid hydrolyzate by comparison with the retention times of synthetic standards.[11] It appeared that the α,*β*-dihydroxy acid was unstable under the conditions of acid hydrolysis and had probably dehydrated and decarboxylated. However, ozonolysis and subsequent base hydrolysis at 90 °C afforded (*S*)-α,*β*-dihydroxyisovaleric acid, without any noticeable racemization, as demonstrated by chiral HPLC analysis [CHIRALPAK MA(+)].[12] Thus, C-26 in peptolide **1** has the *S* configuration.

The remaining stereocenter at C-3 was determined after methanolysis of **1**. Attempts to convert one of the products, methylester **3**, to Mosher esters by reaction with (*R*)- and (*S*)-MTPA-Cl failed; only starting material could be recovered. Compound **3** was subsequently saponified to hydroxy acid **4**, and its optical rotation was compared with the optical rotation of the known (*S*)-3-hydroxy-2,2-dimethyloctanoic acid.[14] Because the two chlorine substituents in **4** were fairly remote from the stereocenter, we did not expect them to greatly influence the optical rotation.[15] The $[\alpha]_D$ values of both compounds were found to be levorotatory and similar in magnitude in methanol.[16] This meant that C-3 in **1** has the 3*S* configuration.

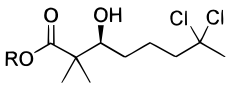
The CD spectrum of **1** displayed a weak negative Cotton effect (CE, $\Sigma\Delta\epsilon$ 6.8) near the UV absorption maximum at 236 nm, which is indicative for exciton coupling[17] between the thiazole carboxylate residues. However, the weakness of this interaction suggests that the electric transition moments of the aromatic chromophores might almost be parallel to each other. Molecular modeling studies were carried out to confirm whether the proposed structure for **1** was in agreement with the CD spectrum. The geometry-optimized starting structure was subjected to quenched dynamics [high-temperature molecular dynamics (MD) and energy minimization] to allow the molecule to escape local energy minimum wells and to sample larger conformational space during the MD trajectory.[18] As expected, many local energy minima were found;



**1**



**2**



**3** R = Me

**4** R = H

however, all of the lower-energy conformers exhibited negative chirality. Convergence to a conformational family that was estimated to have no CE, or a slightly negative one, occurred toward the end of the 10-ps simulation (Figure 1). Solvent effects are not expected to change the situation significantly. The calculations are overall consistent with the experimental observation.

**Biological Activity.** Lyngbyabellin A (**1**) exhibits moderate cytotoxicity against KB cells (a human nasopharyngeal carcinoma cell line) and LoVo cells (a human colon adenocarcinoma cell line), with $IC_{50}$ values of 0.03 $\mu$g/mL and 0.50 $\mu$g/mL, respectively. In vivo trials revealed that lyngbyabellin A (**1**) is toxic to mice. The lethal dose varied from 2.4 to 8.0 mg/kg. At sublethal doses (i.e., 1.2−1.5 mg/kg), there was no antitumor activity against the murine colon adenocarcinoma C38 or the mammary adenocarcinoma M16.

The cytoskeletal-disrupting effects of **1** were tested in A-10 cells. Lyngbyabellin A (**1**), at concentrations of 0.01−5.0 $\mu$g/mL, disrupted the cellular microfilament network in A-10 cells (Figure 2). Consistent with these effects, compound **1** caused the normally fibroblastic A-10 cells to exhibit neuron-like gross cellular morphology with small central areas and multiple long processes extending to the periphery. Additionally, at the higher concentrations tested, many cells contained two nuclei, consistent with the inhibition of cytokinesis that often occurs after disruption of the microfilament network. Apoptosis and the formation of apoptotic bodies were evident at the 0.01-$\mu$g/mL concentration.

The cytoskeletal-disrupting effects of lyngbyabellin A (**1**) were specific for microfilaments, as there was no evidence of microtubule disruption. Furthermore, **1** did not inhibit topoisomerase I at 5 and 10 $\mu$g/mL.

## Experimental Section

**General Experimental Procedures.** $^1$H and $^{13}$C NMR spectra were recorded in $CDCl_3$ at 400/500 MHz and 100/125 MHz, respectively, using residual solvent signals as internal references. The HMQC experiments were optimized for $^1J_{CH}$ = 140 Hz, and the HMBC experiments for $^nJ_{CH}$ = 7 or 5 Hz. Molecular modeling studies were performed using HyperChem 5.0 software produced by Hypercube, Inc.

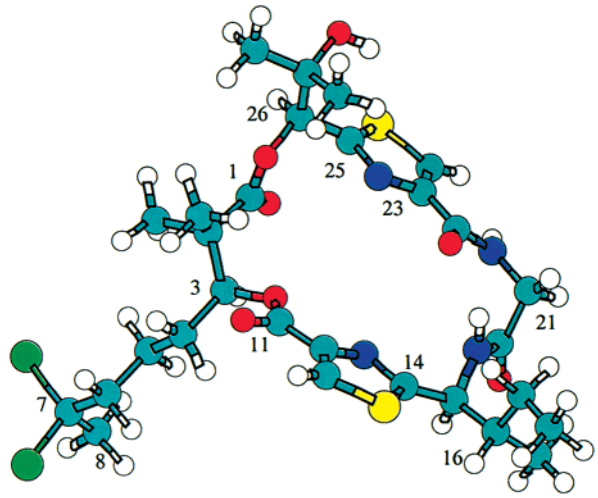


**Figure 1.** Stereostructure of the lowest-energy conformer of lyngbyabellin A (**1**) found during quenched dynamics simulations.

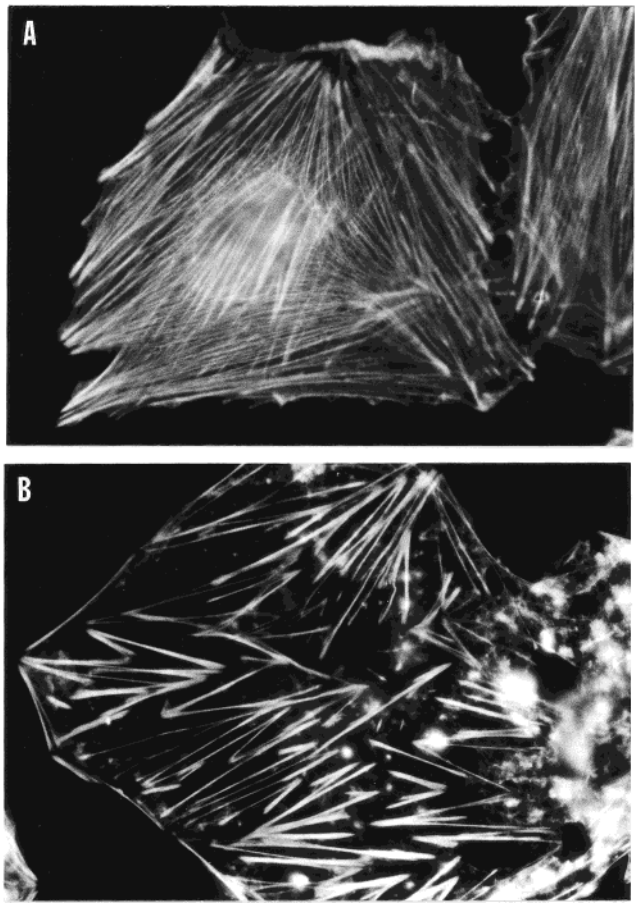


**Figure 2.** Microfilaments, composed of filamentous actin, were visualized in A-10 cells with rhodamine-phalloidin. The normal, extensive filamentous actin network is seen in the control, vehicle-treated cells in panel A. Disruption of the actin network is seen in cells treated with lyngbyabellin A (**1**) (0.05 $\mu$g/mL) in panel B.

**Biological Material.** Cyanobacterium VP417 was collected at Finger's Reef, Apra Harbor, Guam, and identified as a strain of *L. majuscula*. It was first collected on August 4, 1997, and a specimen preserved in formalin has been deposited at the University of Hawaii. Lyngbyabellin A-affording recollections of VP417 were made on November 14, 1997, as well as on January 19, March 5, and May 28, 1999. The isolation of

dolastatin G and dolastatin 11 analogues from the two collections made in 1997 has already been reported.[4c]

**Extraction and Isolation.** Fractionation of the cytotoxic organic extract of the recollection of VP417 made on November 14, 1997, has previously been described.[4c] Lyngbyabellin A (**1**) (2.2 mg) was isolated from the less cytotoxic fraction eluting from Si gel with 8% *i*-PrOH in $CH_2Cl_2$ (25.1 mg) after semipreparative reversed-phase HPLC (Ultracarb, 5 ODS 30, 250 × 10 mm, 3 mL/min; UV detection at 220 nm) using an isocratic system of 80% aqueous MeCN ($t_R$ 9.2 min.).

The freeze-dried recollection of VP417 made in January 1999, was extracted with $CH_2Cl_2$−EtOAc−MeOH (1:1:1). This lipophilic extract (6.31 g) was partitioned between hexanes and 80% aqueous MeOH. The solvent-evaporated methanolic phase (1.73 g) was chromatographed on Si gel, eluting initially with $CH_2Cl_2$, followed by $CH_2Cl_2$ solutions containing progressively increasing amounts of *i*-PrOH, and finally with MeOH. The fraction eluting with 8% *i*-PrOH (14.3 mg) was subjected to semipreparative reversed-phase HPLC as described above (flow rate, 2 mL/min) to afford **1** (5.6 mg, $t_R$ 14.0 min).

The material recollected in March and May 1999, was extracted separately (yielding 0.72 and 0.96 g), partitioned, and chromatographed on Si gel in the same way as described above. The fractions eluting with 8% *i*-PrOH (13.0 mg and 19.5 mg) were then subjected to semipreparative reversed-phase HPLC (Econosil $C_{18}$, 10 $\mu$m, 10 × 250 mm, 2.5 mL/min; PDA detection from 210 to 540 nm) using a MeCN−$H_2O$ linear gradient (40−100% over 50 min and then 100% MeCN for 20 min). Lyngbyabellin A (**1**) eluted at $t_R$ 28.3 min (2.2 mg and 3.2 mg, respectively).

**Lyngbyabellin A (1):** colorless, crystalline solid (isooctane−$CH_2Cl_2$), mp 150−152 °C; $[\alpha]^{27}_D$ −74° (*c* 0.50, $CHCl_3$); UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 202 (4.48), 236 (4.08) nm; CD (MeOH) $\Delta\epsilon_{208}$ −36.2, $\Delta\epsilon_{234}$ +1.7, $\Delta\epsilon_{242}$ −5.1; IR (film) $\nu_{max}$ 3403 (br), 3110, 2963, 2923, 2862, 1725, 1659, 1540, 1490, 1377, 1320, 1236, 1140, 1095, 965, 734, 695 $cm^{-1}$; $^1H$ NMR, $^{13}C$ NMR, $^1H$−$^1H$ COSY, and HMBC data, see Table 1; FABMS *m*/*z* 691/693/695 (100:80:23, $[M + H]^+$ ion cluster), HRFABMS *m*/*z* $[M + H]^+$ 691.1791 (calcd for $C_{29}H_{41}35Cl_2N_4O_7S_2$, 691.1794).

**Ozonolysis and Acid Hydrolysis.** Lyngbyabellin A (**1**) (0.3 mg) was dissolved in 3 mL of $CH_2Cl_2$ and subjected to ozonolysis at −78 °C. The solvent was evaporated and the residue suspended in 6 N HCl (0.5 mL) and incubated at 110 °C for 12 h. The product mixture was concentrated to dryness and analyzed by chiral HPLC [column, Chirex phase 3126 (D) (4.6 × 250 mm), Phenomenex; solvent, 2 mM $CuSO_4$−MeCN (95:5); flow rate, 1.0 mL/min; detection at 254 nm]. Glycine and isoleucine eluted at $t_R$ 5.5 and 36.6 min, respectively. The retention times ($t_R$, min) of the authentic amino acids were: Gly (5.5), L-Ile (36.6), D-Ile (48.1), L-*allo*-Ile (30.9), and D-*allo*-Ile (39.6), indicating the presence of L-Ile in the hydrolyzate. This result was confirmed by co-injection and by HPLC analysis on a different chiral column [column, CHIRALPAK MA(+) (4.6 × 50 mm), Daicel Chemical Industries, Ltd.; solvent, 2 mM $CuSO_4$; flow rate, 1.0 mL/min; detection at 230 nm]. L-Ile eluted after $t_R$ 17.5 min, corresponding to the retention time of the authentic standard [$t_R$ (min) of other standards: D-Ile (9.1), L-*allo*-Ile (12.8), D-*allo*-Ile (7.1)].

**Ozonolysis and Base Hydrolysis.** Lyngbyabellin A (**1**) (0.3 mg) was ozonized as described above. The residue after evaporation of the solvent was dissolved in 1.5 mL of a mixture of 2 N KOH (aqueous solution) and MeOH (2:1). The mixture was heated at 90 °C for 6 h, acidified with HCl, and dried. The water-soluble part was subjected to chiral HPLC analysis [CHIRALPAK MA(+)] using 2 mM $CuSO_4$−MeCN (95:5) as the solvent system and otherwise the conditions employed above. A new peak in the HPLC profile was assigned to (*S*)-α,*β*-dihydroxyisovaleric acid ($t_R$ 15.1 min) by comparison with the retention times of synthetic standards [(*R*)-α,*β*-dihydroxyisovaleric acid ($t_R$ 10.0 min)] and co-injection. D-*allo*-Ile and L-Ile eluted at $t_R$ 6.0 and 9.0 min, respectively, under these conditions. The presence of L-Ile and D-*allo*-Ile was confirmed on the Chirex phase 3126 (D) column (conditions and $t_R$, see above: Ozonolysis and Acid Hydrolysis).[12] α,*β*-Dihydroxyisovaleric acid peaks could not be detected on the latter column.

**Methanolysis of 1.** A 6.0-mg (8.7 $\mu$mol) sample of compound **1** was dissolved in 0.5 mL of MeOH, and the solution was added to 0.5 mL of a 0.5-M solution of NaOMe in MeOH. The mixture was stirred at room temperature for 22 h. The solution was dried under $N_2$, 1 mL of $H_2O$ added, and the cloudy solution acidified to pH 4 with 1 N HCl. The aqueous phase was extracted with EtOAc (3 × 2 mL). The organic extracts were combined, and the solvent was evaporated. The crude product mixture was applied to a $SiO_2$ Sep Pak, and methylester **3** (1.9 mg, 81%) was eluted with hexanes−EtOAc (2:1).

**Methyl (*S*)-7,7-dichloro-3-hydroxy-2,2-dimethyloctanoate (3):** colorless oil; $R_f$ = 0.59 (2:1 hexane−EtOAc); $[\alpha]^{27}_D$ −10° (*c* 0.13, MeOH); $^1H$ NMR (500 MHz, $CDCl_3$) $\delta$ 1.18 (3H, s), 1.21 (3H, s), 1.36 (1H, m), 1.50 (1H, m), 1.72 (1H, m), 1.97 (1H, m), 2.15 (3H, s), 2.24 (2H, m), 2.55 (1H, br s), 3.64 (1H, dd, *J* = 10.5, 1.9 Hz), 3.71 (3H, s).

**Base Hydrolysis of 3.** To 1 mL of a mixture of 0.5 N KOH−MeOH (1:1) was added 1.0 mg (3.7 $\mu$mol) of methylester **3**. The solution was stirred for 24 h, the MeOH evaporated under $N_2$, and the basic solution washed with EtOAc (1 × 0.5 mL). After acidification, the aqueous phase was extracted with EtOAc (3 × 1 mL), and the combined organic extracts were evaporated to yield the hydroxy acid **4** (0.6 mg, 63%).

**(*S*)-7,7-Dichloro-3-hydroxy-2,2-dimethyloctanoic acid (4):** colorless oil; $[\alpha]^{27}_D$ −30° (*c* 0.20, MeOH); $^1H$ NMR (500 MHz, $CDCl_3$) $\delta$ 1.21 (3H, s), 1.26 (3H, s), 1.42 (1H, m), 1.50−1.80 (3H, m), 1.99 (1H, m), 2.16 (3H, s), 2.24 (1H, m), 3.68 (1H, dd, *J* = 10.7, 1.7 Hz).

**Molecular Modeling.** Molecular mechanics (MM) and molecular dynamics (MD) calculations were carried out in vacuo. Modeling involved a minimization−molecular dynamics−minimization protocol. MM+ energy minimizations were performed with a conjugate gradient algorithm (Polak-Ribiere) using an rms gradient of less than 0.01 kcal/mol·Å as termination condition. For the high-temperature molecular dynamics, the molecule was heated for 1 ps to 1000 K and then subjected to a 10-ps MD simulation at this temperature. After each picosecond of simulation time, data sets were collected and minimized.

**Microfilament-Disrupting Activity.** Lyngbyabellin A (**1**) was tested for microfilament-disrupting activity using rhodamine−phalloidin. A-10 cells, smooth muscle cells with an easily visualized cytoskeleton, were grown on glass coverslips in Basal Medium Eagle containing 10% fetal calf serum. The cells were treated with various concentrations of **1** or vehicle for 24 h and then fixed with 3% paraformaldehyde for 20 min, permeabilized with 0.2% Triton X-100 for 2 min, and chemically reduced with sodium borohydride (1 mg/mL in phosphate-buffered saline) three times for 5 min each. The microfilaments in the fixed cells were stained for 45 min with 100 nM TRITC−phalloidin in phosphate-buffered saline. The cells were then washed, the chromatin stained with 4,6-diamidino-2-phenylindole, the coverslips mounted on microscope slides, and finally examined using a Zeiss Axioplan fluorescence microscope.

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**Supporting Information Available:** Copies of $^1$H and $^{13}$C NMR spectra of **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

## References and Notes


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