# Lyngbyastatin 2 and Norlyngbyastatin 2, Analogues of Dolastatin G and Nordolastatin G from the Marine Cyanobacterium *Lyngbya majuscula*

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Lyngbyastatin 2 (**1**) and norlyngbyastatin 2 (**2**), new cytotoxic analogues of dolastatin G (**3**) and nordolastatin G (**4**), respectively, have been isolated as constituents from a Guamanian variety of the marine cyanobacterium *Lyngbya majuscula*. Structure elucidation of these cyclic depsipeptides relied on extensive application of 2D NMR techniques. The finding of these new metabolites further supports the proposal that many compounds originally isolated from the sea hare *Dolabella auricularia* are most likely of cyanobacterial origin.

The dolastatins are remarkable cytotoxic compounds isolated from the Indian Ocean sea hare *Dolabella auricularia*[1] and Japanese specimens of this mollusk.[2] The exceedingly low yields of dolastatins suggested that *D. auricularia* is not the true producer of these compounds. Our previous findings of epimeric mixtures of dolastatin 12 and of lyngbyastatin 1 (a dolastatin 11 analogue) from certain strains of *Lyngbya majuscula* and also from assemblages of *L. majuscula* and *Schizothrix calcicola*[3] implied already that at least some metabolites isolated from *D. auricularia* (a known generalist herbivore)[4] are of cyanobacterial origin. The more recent discovery of the dolastatin 10 and dolastatin 13 analogues, symplostatin 1[5] and symplostatin 2,[6] respectively, from a Guamanian variety of the cyanobacterium *Symploca hydnoides* strongly supported the idea. Our ongoing investigations of cyanobacteria as sources of novel anticancer drugs have now led to more dolastatin analogues. Lyngbyastatin 2 (**1**) and norlyngbyastatin 2 (**2**), structurally highly similar to dolastatin G (**3**) and nordolastatin G (**4**),[2d] have been isolated from collections of *Lyngbya majuscula* Harvey ex Gomont (UOG strains VP417 and VP503) found on Finger's Reef, Guam.

Lipophilic extracts of VP417 and VP503 were cytotoxic, and the latter showed human (but not murine) solid tumor selective cytotoxicity ($Z_{H116} - Z_{L1210} = 350$) and tumor selective cytotoxicity in the Corbett assay.[7] Solvent partition of these extracts followed by Si gel chromatography and reversed-phase HPLC, afforded **1** and **2** as colorless oils. Compounds **1** and **2** exhibited cytotoxicity against KB cells (a human nasopharyngeal carcinoma cell line) with $IC_{50}$ values of 20 ng/mL and 930 ng/mL, and against LoVo cells (a human colon adenocarcinoma cell line) with $IC_{50}$ values of 14 ng/mL and 475 ng/mL, respectively.

NMR analysis was first carried out for **1** in $CDCl_3$. COSY and HMBC experiments enabled the identification of six amino acid units (2 × proline; 2 × *N*-methylvaline; *N*-methylisoleucine; *N*,*O*-dimethylserine) and two polyketide-type hydroxy acid units [3-methoxy-8-hydroxy-4,7-dimethylnon-2,4-dienoic acid (Nena) and 3,7-dihydroxy-2-methylnonanoic acid (Nana)]. The chemical shifts of C-42 ($\delta$ 74.1), C-49 ($\delta$ 73.7), and C-53 ($\delta$ 76.1) clearly demonstrated that these carbons are oxygenated. The lowfield shift for protons H-42 ($\delta$ 4.86) and H-53 ($\delta$ 4.84) suggested that acyloxy groups are at these positions, while the $^1H-^1H$ COSY correlation of H-49 ($\delta$ 3.65) to an exchangeable proton ($\delta$ 3.84) indicated the presence of a hydroxyl group. One of the nine carbon signals in the ester and amide region ($\delta$ 169.7) was ascribed to the oxygenated carbon in an enol ether functionality (C-37), unambiguously identified by observed HMBC correlation of C-37 with H-36,H-39 as well as with H-44. HMBC experiments, optimized for different $^{13}C-^1H$ long-range coupling constants ($^nJ_{CH} = 5, 7$ Hz), allowed sequencing of the identified units only in part. The following sequence (see Table 1) could be established: (*N*-Me-Val-2)−(*N*-Me-Ile)−Nena−Nana.

The ROESY spectrum provided the missing sequencing information. Cross-peaks between H-21 and H-23 enabled the expansion of the established sequence by the *N*,*O*-dimethylserine residue. Due to correlation between H-2 and H-7 as well as between H-11 and H-13, the other *N*-methylvaline unit was connected to proline units on both carboxy and amino termini: (Pro-1)−(*N*-Me-Val-1)−(Pro-2). A weak cross-peak between H-16 and H-18 established the linkage of these two partial structures. Because all NMR signals were accounted for and an acyloxy group should be located at C-53 (as mentioned above), the Nana unit had to be connected to Pro-1 via this oxygenated carbon, therefore leading to the cyclic gross structure as shown for **1**. The ROESY experiment also revealed that the configuration of both olefinic bonds in the Nena unit was *E* because it disclosed correlation between H-36 and H-44, H-40 and H-45, but not between H-39 and H-45.

Compound **1** differs from dolastatin G (**3**) only in bearing an ethyl instead of an isopropyl group on C-53. HRFABMS analysis established the molecular formula for **1** to be $C_{56}H_{94}N_6O_{13}$, one methylene unit smaller than the one for **3**. IR absorptions at 1734 and 1639 $cm^{-1}$ support the depsipeptide nature of **1**, and UV data correspond closely to the data for dolastatin G (**3**) as well.

The relative stereochemistry for **1** was determined by comparison of its $^1H$ and $^{13}C$ NMR spectral data in $C_6D_6$ (see Table 2) with the ones reported for dolastatin G (**3**),[2d] to which the correct stereochemistry was assigned, as clearly confirmed by total synthesis.[8] Matching proton and carbon chemical shifts as well as $^1H-^1H$ coupling constants indicated that both compounds have the same relative stereochemistry. The fact that C-52 is downfield-shifted in

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**Table 1.** NMR Spectral Data for Lyngbyastatin 2 (**1**) in $CDCl_3$

| unit, C/H no.[a] | | $\delta_H$ ($J$ in Hz) | $\delta_C$[b] | $^1H-^1H$ COSY | HMBC[c,d] | ROESY[e] |
|---|---|---|---|---|---|---|
| Pro-1 | 1 | | 170.7, s | H-2[f] | | |
| | 2 | 4.78, dd (7.8, 1.1) | 60.1, d | H-3 | | H-7 |
| | 3 | 2.15, m, 2.19, m | 31.5, t | H-2, H-4 | | |
| | 4 | 1.75, m, 1.95, m | 21.8, t | H-3, H-5 | | |
| | 5 | 3.57, m, 3.61, m | 45.6, t | H-4 | | |
| *N*-Me-Val-1 | 6 | | 169.1, s | | H-7 | |
| | 7 | 4.99, d (10.9) | 58.1, d | H-8 | H-9, H-10, H-11 | H-2 |
| | 8 | 2.31, m | 27.3, d | H-7, H-9, H-10 | H-7, H-9, H-10 | |
| | 9 | 0.84, d (6.7) | 19.0, q | H-8 | H-7, H-10 | |
| | 10 | 0.88, d (6.7) | 18.2, q | H-8 | H-7, H-9 | |
| | 11 | 3.11, s | 29.7, q | | H-7 | H-13 |
| Pro-2 | 12 | | 170.9, s | | H-7, H-11 | |
| | 13 | 4.71, dd (8.4, 1.7) | 58.1, d | H-14 | | H-11 |
| | 14 | 2.00, m, 2.07, m | 27.7, t | H-13, H-15 | | |
| | 15 | 1.85, m, 2.16, m | 24.1, t | H-14, H-16 | | |
| | 16 | 3.13, m, 3.54, m | 47.2, t | H-15 | | H-18 |
| *N*,*O*-diMe-Ser | 17 | | 166.8, s | | H-18 | |
| | 18 | 5.70, dd (10.6, 4.6) | 54.3, d | H-19 | H-19, H-21 | H-16 |
| | 19 | 3.64, dd (11.3, 4.6), 3.78, dd (11.3, 10.6) | 68.1, t | H-18 | H-20 | |
| | 20 | 3.28, s | 58.7, q | | H-19 | |
| | 21 | 2.82, s | 29.9, q | | H-18 | H-23 |
| *N*-Me-Val-2 | 22 | | 170.3, s | | H-21 | |
| | 23 | 5.14, d (10.5) | 58.5, d | H-24 | H-25, H-26, H-27 | H-21 |
| | 24 | 2.27, m | 26.9, d | H-23, H-25, H-26 | H-23, H-25, H-26 | |
| | 25 | 0.70, d (6.9) | 17.3, q | H-24 | H-26 | |
| | 26 | 0.83, d (6.5) | 20.0, q | H-24 | H-23, H-25 | |
| | 27 | 2.93, s | 30.4, q | | H-23 | |
| *N*-Me-Ile | 28 | | 170.1, s | | H-23, H-27, H-29 | |
| | 29 | 5.27, d (10.9) | 56.9, d | H-30 | H-33, H-34 | |
| | 30 | 2.12, m | 33.0, d | H-29, H-31, H-33 | H-29, H-32, H-33 | |
| | 31 | 1.02, m, 1.30, m | 24.0, t | H-30, H-32 | H-32, H-33 | |
| | 32 | 0.89, t (7.3) | 11.2, q | H-31 | | |
| | 33 | 0.79, d (6.5) | 15.7, q | H-30 | H-29 | |
| | 34 | 2.93, s | 31.1, q | | H-29 | H-36 |
| Nena | 35 | | 168.4, s | | H-29, H-34, H-36[f] | |
| | 36 | 5.07, s | 93.0, d | | | H-34, H-44, H-45 |
| | 37 | | 169.7, s | | H-36, H-44, H-45 | |
| | 38 | | 132.8, s | | H-36, H-45 | |
| | 39 | 5.45, m | 130.3, d | H-40 | H-45 | |
| | 40 | 1.83, m, 2.11, m | 31.0, t | H-39, H-41 | H-46 | H-45 |
| | 41 | 1.64, m | 37.9, d | H-40, H-46 | H-43, H-46 | |
| | 42 | 4.86, qd (6.2, 4.5) | 74.1, d | H-43 | H-43, H-46 | |
| | 43 | 1.15, d (6.2) | 14.8, q | H-42 | | |
| | 44 | 3.64, s | 55.4, q | | | H-36, H-45 |
| | 45 | 1.83, br s | 14.5, q | | | H-40, H-44 |
| | 46 | 0.93, d (6.7) | 13.5, q | H-41 | | |
| Nana | 47 | | 175.6, s | | H-42,[f] H-48, H-56 | |
| | 48 | 2.50, dq (10.0, 7.1) | 47.8, d | H-49, H-56 | H-56 | |
| | 49 | 3.65, m | 73.7, d | H-48, H-50, OH | H-56 | |
| | 50 | 1.55, m, 1.67, m | 35.0, t | H-49, H-50 | | |
| | 51 | 1.28, m, 1.68, m | 23.2, t | H-50, H-52 | | |
| | 52 | 1.68, m, 1.90, m | 33.9, t | H-51, H-53 | | |
| | 53 | 4.84, m | 76.1, d | H-52, H-54 | H-55 | |
| | 54 | 1.44, m, 1.73, m | 26.2, t | H-53, H-55 | H-55 | |
| | 55 | 0.84, t (7.4) | 8.9, q | H-54 | | |
| | 56 | 1.06, d (7.1) | 15.2, q | | | |
| | OH | 3.84, d (11.1) | | H-49 | | |

[a] In order to allow direct comparison with the data presented by Mutou et al.,[2d] their numbering system has been adopted. [b] Multiplicity deduced by DEPT spectroscopy. [c] Protons showing long-range correlation with indicated carbon. [d] If not indicated otherwise, correlations were observed after optimization for $^nJ_{CH} = 7$ Hz. [e] Only key cross-peaks are listed. [f] Correlation observed after optimization for $^nJ_{CH} = 5$ Hz.

**1** (by 2.1 ppm) and C-53 is upfield-shifted (by 2.3 ppm) compared with **3** can be rationalized due to the lack of the $\gamma$- and $\beta$-effect, respectively, exhibited by the extra methyl group in the Nana unit of **3**. The absolute configurations of all amino acid units in **1** were determined to be L (as in **3**) by chiral HPLC analysis of the mixture resulting from acid hydrolysis (6 N HCl at 110 °C, 24 h).[9] Furthermore, the nearly equal optical rotation proved that the absolute stereochemistry of **1** and dolastatin G (**3**) is identical for both compounds.[10]

Compound **2** showed the same NMR spectral features as **1** (see Table 2), except that its $^1H$ NMR spectrum lacked the singlets at $\delta$ 5.07 for H-36 and at $\delta$ 3.64 for H-44, and instead, an AB spin system for a methylene group ($\delta$ 3.75 and 3.79, $^2J_{CH} = 14.2$ Hz) has been observed. Distinctive in the $^{13}C$ NMR spectrum was the resonance at $\delta$ 194.5,

**Table 2.** NMR Spectral Data for Lyngbyastatin 2 (**1**) in $C_6D_6$ and Norlyngbyastatin 2 (**2**) in $CDCl_3$

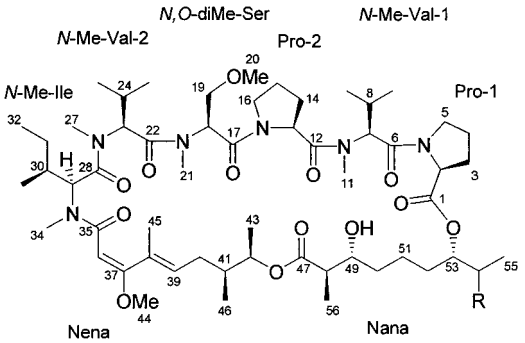
| | | **1** | | **2** | |
|---|---|---|---|---|---|
| unit, C/H no.[a] | | $\delta_H$ ($J$ in Hz) | $\delta_C$ | $\delta_H$ ($J$ in Hz) | $\delta_C$ |
| Pro-1 | 1 | | 171.0, s | | 170.7, s |
| | 2 | 4.66, br d (~8.5) | 60.1, d | 4.78, br d (7.2) | 60.1, d |
| | 3 | 1.53, m, 1.85, m | 31.62, t | 2.15, m, 2.19, m | 31.5, t |
| | 4 | 1.22, m, 1.45, m | 21.9, t | 1.75, m, 1.93, m | 21.8, t |
| | 5 | 3.46, br dt (12.2, 8.5)<br>3.68, ddd (12.2, 8.8, 2.1) | 46.1, t | 3.57, m, 3.61, m | 45.7, t |
| *N*-Me-Val-1 | 6 | | 169.2, s | | 169.0, s |
| | 7 | 5.18, d (11.3) | 58.2, d | 4.99, d (10.9) | 58.1, d |
| | 8 | 2.42, m | 27.9, d | 2.30, m | 27.3, d |
| | 9 | 0.76, d (6.6) | 19.0,q | 0.84, d (6.5) | 19.0, q |
| | 10 | 1.02, d (6.6) | 18.8, q | 0.88, d (6.7) | 18.2, q |
| | 11 | 3.05, s | 29.6, q | 3.10, s | 29.7, q |
| Pro-2 | 12 | | 171.2, s | | 170.8, s |
| | 13 | 4.66, br d (~8.5) | 58.4, d | 4.71, dd (8.6, 1.6) | 58.2, d |
| | 14 | 1.52, m, 1.85, m | 27.7, t | 1.99, m, 2.06, m | 27.7, t |
| | 15 | 1.64, m, 2.32, m | 24.3, t | 1.84, m, 2.16, m | 23.9, t |
| | 16 | 3.13, m<br>3.77, br t (8.8) | 47.2, t | 3.13, m, 3.53, m | 47.1, t |
| *N*,*O*-diMe-Ser | 17 | | 166.8, s | | 166.7, s |
| | 18 | 5.93, dd (10.8, 4.7) | 54.8, d | 5.69, dd (10.7, 4.4) | 54.4, d |
| | 19 | 3.63, dd (11.3, 4.7)<br>3.91, dd (11.3, 10.8) | 68.7, t | 3.64, dd (11.4, 4.4)<br>3.76, dd (11.4, 10.7) | 68.1, t |
| | 20 | 2.90, s | 58.3, q | 3.28, s | 58.8, q |
| | 21 | 2.89, s | 29.8, q | 2.81, s | 29.7, q |
| *N*-Me-Val-2 | 22 | | 170.7, s | | 170.1, s |
| | 23 | 5.41, d (10.4) | 59.0, d | 5.10, d (11.2) | 58.3, d |
| | 24 | 2.66, m | 27.4, d | 2.18, m | 26.8, d |
| | 25 | 0.92, d (6.6) | 17.8, q | 0.59, d (6.7) | 17.0, q |
| | 26 | 1.00, d (6.6) | 20.4, q | 0.82, d (6.3) | 19.9, q |
| | 27 | 3.29, s | 30.8, q | 2.82, s | 30.0, q |
| *N*-Me-Ile | 28 | | 170.4, s | | 169.4, s |
| | 29 | 5.60, d (10.8) | 57.0, d | 5.25, d (10.9) | 57.2, d |
| | 30 | 2.38, m | 33.3, d | 2.12, m | 32.4, d |
| | 31 | 1.15, m, 1.41, m | 24.5, t | 1.02, m, 1.30, m | 23.9, t |
| | 32 | 0.89, t (7.5) | 11.1, q | 0.89, t (6.7) | 10.9, q |
| | 33 | 0.83, d (6.6) | 15.9, q | 0.79, d (6.3) | 15.6, q |
| | 34 | 2.84, s | 30.9, q | 2.84, s | 30.8, q |
| Nena | 35 | | 167.9, s | | 168.2, s |
| | 36 | 4.88, s | 93.4, d | 3.75, d (14.2)<br>3.79, d (14.2) | 48.6, t |
| | 37 | | 170.2, s | | 194.5, s |
| | 38 | | 133.2, s | | 138.1, s |
| | 39 | 5.73, m | 130.3, d | 6.87, m | 145.3, d |
| | 40 | 1.84, m, 2.00, m | 31.6, t | 2.02, m, 2.33, m | 33.4, t |
| | 41 | 1.72, m | 38.5, d | 1.75, m | 38.1, d |
| | 42 | 4.99, qd (6.1, 4.5) | 74.2, d | 4.80, m | 74.0, d |
| | 43 | 1.10, d (6.1) | 14.7, q | 1.17, d (6.1) | 15.4, q |
| | 44 | 3.07, s | 54.9, q | | |
| | 45 | 1.95, br s | 14.9, q | 1.75, br s | 11.7, q |
| | 46 | 1.11, d (7.0) | 13.5, q | 0.90, d (7.0) | 13.9, q |
| Nana | 47 | | 175.5, s | | 175.7, s |
| | 48 | 2.74, dq (9.7, 7.1) | 48.5, d | 2.44, m | 47.9, d |
| | 49 | 4.12, m | 74.0, d | 3.68, m | 73.5, d |
| | 50 | 1.69, m, 1.78, m | 35.6, t | 1.55, m, 1.63, m | 34.9, t |
| | 51 | 1.40, m, 2.02, m | 23.8, t | 1.28, m, 1.68, m | 23.2, t |
| | 52 | 1.76, m, 2.27, m | 34.2, t | 1.65, m, 1.88, m | 33.8, t |
| | 53 | 5.06, m | 76.2, d | 4.83, m | 76.1, d |
| | 54 | 1.30, m, 1.57, m | 26.6, t | 1.45, m, 1.72, m | 26.2, t |
| | 55 | 0.79, t (7.3) | 9.1, q | 0.845, t (7.3) | 8.9, q |
| | 56 | 1.05, d (7.1) | 15.2, q | 1.06, d (7.0) | 15.1, q |
| | OH | 4.22, d (10.8) | | 3.88, d (10.2) | |

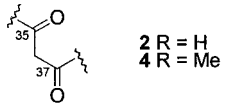
[a] In order to allow direct comparison with the data presented by Mutou et al.[2d] their numbering system has been adopted.

characteristic for a conjugated ketocarbonyl group carbon (C-37). The olefinic proton at 6.87 for H-39, indicative of a $\beta$-proton in an $\alpha,\beta$-unsaturated carbonyl system, is further evidence for the carbonyl group. Therefore, **1** was the enol ether of **2**, consistent with the HRFABMS. Conversion of **1** into **2** upon treatment with acidic $CDCl_3$ verified that the absolute stereochemistry of both compounds is the same.[11]

Cytotoxins **1** and **2** have been assigned the trivial names lyngbyastatin 2 and norlyngbyastatin 2 based on their structural analogy to dolastatin G (**3**) and nordolastatin G (**4**). The considerably lower $IC_{50}$ values for lyngbyastatin

2 (**1**) compared with the ones for norlyngbyastatin 2 (**2**) suggest that the enol ether functionality plays a role in the cytotoxicity. The isolation of these two secondary metabolites from a cyanobacterium contributes further to the accumulating evidence that cyanobacteria, which are known to be prolific sources of numerous bioactive agents,[12] are also the actual producers of the dolastatins originally isolated from the (herbivorous) sea hare *D. auricularia*.




Lyngbyastatin 2 (**1**) is toxic to mice at 3 mg/kg ($LD_{100}$). At sublethal doses (i.e., 1.8 mg/kg or lower) **1** was inactive in vivo against C38, a murine colon adenocarcinoma. The mode of action of **1** and **2** is unknown, but the two compounds are not inhibitors of microtubule or microfilament assembly, nor are they inhibitors of topoisomerase I.

## Experimental Section

**General Experimental Procedures.** $^1$H and $^{13}$C NMR spectra were recorded in $CDCl_3$ or $C_6D_6$ on a GE Omega 500 spectrometer operating at 500 and 125 MHz, respectively, using residual solvent signals as internal references. The 2D NMR experiments were performed on the same spectrometer. The HMQC experiments were optimized for $^1J_{CH} = 140$ Hz, and the HMBC experiments for $^nJ_{CH} = 7$ or 5 Hz. HRFABMS were recorded on a VG ZAB2SE mass spectrometer in the positive mode. IR spectra were recorded on a Perkin−Elmer 1600 Series FT-IR. UV spectra were obtained on a Hewlett−Packard 8453 spectrometer. Optical rotations were measured on a JASCO DIP-370 polarimeter.

**Biological Material.** Cyanobacterium VP417 was a *L. majuscula* strain collected at Finger's Reef, Apra Harbor, Guam, August 4, 1997 (specimen preserved in formalin and deposited at UH). Organism VP417 was recollected at the same site on November 14, 1997. An earlier collection of the same species of cyanobacterium from Finger's Reef, carried out in November 1993, was also investigated (VP503).

**Extraction and Isolation.** The freeze-dried organism VP417 was extracted with $CH_2Cl_2$−MeOH (1:1) to afford a lipophilic extract VP417L (1.06 g). VP417L was partitioned between hexanes and 80% aqueous MeOH. The bioactive MeOH fraction was chromatographed on Si gel (200−425 mesh) eluting with $CH_2Cl_2$ followed by $CH_2Cl_2$ solutions containing progressively increasing amounts of *i*-PrOH, and finally with MeOH. The mixture eluting with 6% *i*-PrOH in $CH_2Cl_2$ (23.2 mg) was subjected to 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 to afford norlyngbyastatin 2 (**2**) (0.6 mg, $t_R$ 24.2 min) and lyngbyastatin 2 (**1**) (1.2 mg, $t_R$ 33.8 min).[13]

The freeze-dried recollection of VP417 was extracted consecutively with $CH_2Cl_2$, $CH_2Cl_2$−MeOH (1:1), and EtOH. All fractions were cytotoxic. They were combined and partitioned between hexanes and 80% aqueous MeOH. The latter phase was dried and partitioned between $H_2O$ and *sec*-BuOH. The bioactive *sec*-BuOH portion (1.67 g) was fractionated by Si gel chromatography using the step gradient described above. The most cytotoxic portion eluting with 6% *i*-PrOH in $CH_2Cl_2$ (51.0 mg) was subjected to semipreparative reversed-phase HPLC as aforementioned to give further quantities of **1** (0.7 mg) and **2** (0.8 mg).[14]

Extraction of the freeze-dried organism VP503 with $CH_2Cl_2$−MeOH (1:1) afforded 588 mg of lipophilic extract (VP503L). This material was partitioned between hexanes and 80% aqueous MeOH, and the alcoholic phase was dried and partitioned between $H_2O$ and *n*-BuOH. Si gel chromatography of the *n*-BuOH portion, using the conditions described above, yielded a cytotoxic fraction that eluted with 8% *i*-PrOH in $CH_2Cl_2$ and gave 61.1 mg after evaporation of the solvent mixture. The residue was further purified by semipreparative reversed-phase HPLC similarly as described earlier (flow rate: 2 mL/min) to yield **2** (1.8 mg, $t_R$ 35.3 min) and **1** (7.7 mg, $t_R$ 49.5 min).

**Lyngbyastatin 2 (1):** colorless oil, $[\alpha]^{27}_D$ −218° (*c* 0.04, MeOH); UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 202 (4.61), 225 (sh) (4.28), 250 (sh) (3.94) nm; IR (film) $\nu_{max}$ 3455 (br), 1734, 1639, 1453, 1196, 1094 cm$^{-1}$; $^1$H NMR, $^{13}$C NMR, $^1$H−$^1$H COSY, and HMBC data ($CDCl_3$), see Table 1; $^1$H NMR, $^{13}$C NMR ($C_6D_6$), see Table 2; HRFABMS *m/z* $[M + H]^+$ 1059.6977 (calcd for $C_{56}H_{95}N_6O_{13}$, 1059.6957).

**Norlyngbyastatin 2 (2):** colorless oil; $[\alpha]^{27}_D$ −179° (*c* 0.05, MeOH); UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 202 (4.58), 227 (sh) (4.28), 290 (2.95) nm; IR (film) $\nu_{max}$ 3452 (br), 1736, 1638, 1450, 1195, 1103 cm$^{-1}$; $^1$H NMR, $^{13}$C NMR data, see Table 2; HRFABMS *m/z* $[M + H]^+$ 1045.6757 (calcd for $C_{55}H_{93}N_6O_{13}$, 1045.6801).

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## References and Notes

(1) (a) Pettit, G. R.; Kamano, Y.; Herald, C. L.; Fujii, Y.; Kizu, H.; Boyd, M. R.; Boettner, F. E.; Doubek, D. L.; Schmidt, J. M.; Chapuis, J.-C.; Michel, C. *Tetrahedron* **1993**, *49*, 9151−9170. (b) Pettit, G. R. In *Progress in the Chemistry of Organic Natural Products*; Herz, W., Kirby, G. W., Moore, R. E., Steglich, W., Tamm, C., Eds.; Springer-Verlag: New York, 1997; Vol. 70, pp 1−79. (c) Pettit, G. R.; Xu, J.-P.; Hogan, F.; Williams, M. D.; Doubek, D. L.; Schmidt, J. M.; Cerny, R. L.; Boyd, M. R. *J. Nat. Prod.* **1997**, *60*, 752−754. (d) Pettit, G. R., Xu, J.-P.; Hogan, F.; Cerny, R. L. *Heterocycles* **1998**, *47*, 491−496. (e) Pettit, G. R.; Xu, J.-P.; Williams, M. D.; Hogan, F.; Schmidt, J. M.; Cerny, R. L. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 827−832.

(2) (a) Sone, H.; Nemoto, T.; Ojika, M.; Yamada, K. *Tetrahedron Lett.* **1993**, *34*, 8445−8448. (b) Sone, H.; Nemoto, T.; Ishiwata, H.; Ojika, M.; Yamada, K. *Tetrahedron Lett.* **1993**, *34*, 8449−8452. (c) Ojika, M.; Nemoto, T.; Nakamura, M.; Yamada, K. *Tetrahedron Lett.* **1995**, *36*, 5057−5058. (d) Mutou, T.; Kondo, T.; Ojika, M.; Yamada, K. *J. Org. Chem.* **1996**, *61*, 6340−6345. (e) Sone, H.; Shibata, T.; Fujita, T.; Ojika, M.; Yamada, K. *J. Am. Chem. Soc.* **1996**, *118*, 1874−1880.

(3) Harrigan, G. G.; Yoshida, W. Y.; Moore, R. E.; Nagle, D. G.; Park, P. U.; Biggs, J.; Paul, V. J.; Mooberry, S. L.; Corbett, T. H.; Valeriote, F. A. *J. Nat. Prod.* **1998**, *61*, 1221−1225.

(4) (a) Pennings, S. C.; Paul, V. J. *Ecology* **1992**, *73*, 1606−1619. (b) Pennings, S. C.; Paul, V. J. *Mar. Biol.* **1993**, *117*, 535−546.

(5) Harrigan, G. G.; Luesch, H.; Yoshida, W. Y.; Moore, R. E.; Nagle, D. G.; Paul, V. J.; Mooberry, S. L.; Corbett, T. H.; Valeriote, F. A. *J. Nat. Prod.* **1998**, *61*, 1075−1077.

(6) Harrigan, G. G.; Luesch, H.; Yoshida, W. Y.; Moore, R. E.; Nagle, D. G.; Paul, V. J. *J. Nat. Prod.* **1999**, *62*, 655−658.
(7) (a) Corbett, T. H.; Valeriote, F. A.; Polin, L.; Panchapor, C.; Pugh, S.; White, K.; Lowichik, N.; Knight, J.; Bissery, M.-C.; Wozniak, A.; LoRusso, P.; Biernat, L.; Polin, D.; Knight, L.; Biggar, S.; Looney, D.; Demchik, L.; Jones, J.; Jones, L.; Blair, S.; Palmer, K.; Essenmacher, S.; Lisow, L.; Mattes, K. C.; Cavanaugh, P. F.; Rake, J. B.; Baker, L. In *Cytotoxic Anticancer Drugs: Models and Concepts for Drug Discovery and Development*, Valeriote, F. A., Corbett, T. H., Baker, L. H., Eds.; Kluwer Academic Publishers: Norwell, 1992; pp 35−87. (b) Valeriote, F. A.; Moore, R. E.; Patterson, G. M. L.; Paul, V. J.; Scheuer, P. J.; Corbett, T. In *Anticancer Drug Discovery and Development: Natural Products and New Molecular Models*, Valeriote, F. A., Corbett, T. H., and Baker, L. H., Eds.; Kluwer Academic Publishers: Norwell, 1994; pp 1−25.
(8) Mutou, T.; Kondo, T.; Shibata, T.; Ojika, M.; Kigoshi, H.; Yamada, K. *Tetrahedron Lett.* **1996**, *37*, 7299−7302.
(9) Conditions for the chiral HPLC analysis: column, Chirex phase 3126 (D) (4.6 × 250 mm), Phenomenex; solvent, 2 mM $CuSO_4$−MeCN (95:5); flow rate, 0.8 mL/min; detection at 254 nm. The retention times ($t_R$, min) of the authentic amino acids were: L-Pro (11.5); D-Pro (22.2); *N*-Me-L-Val (13.0); *N*-Me-D-Val (17.5); *N*,*O*-diMe-L-Ser (14.9); *N*,*O*-diMe-D-Ser (14.0); *N*-Me-L-Ile (26.7); *N*-Me-D-Ile (40.4); *N*-Me-L-*allo*-Ile (25.3); and *N*-Me-D-*allo*-Ile (39.1). The retention times of the amino acid components in the acid hydrolyzate were 11.5, 13.0, 14.9, and 26.7 min, indicating the presence of L-Pro; *N*-Me-L-Val; *N*,*O*-diMe-L-Ser; and *N*-Me-L-Ile, respectively.
(10) The contribution of the additional methyl group in **3** to the magnitude of the optical rotation is negligible, as expected. Reported[2d] for **3**, $[\alpha]^{25}_D$ −211° (*c* 0.40, MeOH), and for **4**, $[\alpha]^{25}_D$ −183° (*c* 0.11, MeOH).
(11) The product showed the same spectral characteristics as **2**.
(12) Moore, R. E. *J. Ind. Microbiol.* **1996**, *16*, 134−143.
(13) Epimeric lyngbyastatin 1[3] was the major cytotoxin in this extract. It mainly eluted with 20% *i*-PrOH in $CH_2Cl_2$ during normal-phase chromatography (64.7 mg), and the lesser part that eluted earlier with the new compounds could be separated from them by HPLC ($t_R$ 14.2 min, 2.1 mg).
(14) The activity of the extract was also due to other cytotoxins, and their structure elucidation is in progress.

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