Isolation and Structure Determination of Lyngbyastatin 3, a Lyngbyastatin 1 Homologue from the Marine Cyanobacterium *Lyngbya majuscula*. Determination of the Configuration of the 4-Amino-2,2-dimethyl-3-oxopentanoic Acid Unit in Majusculamide C, Dolastatin 12, Lyngbyastatin 1, and Lyngbyastatin 3 from Cyanobacteria

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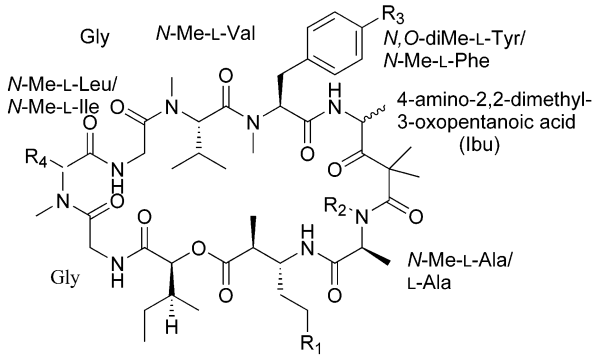
The structure of lyngbyastatin 3 (**1**), including the configurations of the two unusual amino acid residues, viz., the 3-amino-2-methylhexanoic acid (Amha) and 4-amino-2,2-dimethyl-3-oxopentanoic acid units (Ibu), has been established by chemical degradation. Analysis of the cyanobacterial samples of lyngbyastatin 3 (**1**), lyngbyastatin 1 (**2**), and dolastatin 12 (**3**) demonstrated that they are mixtures of Ibu epimers [*R* (major) and *S* (minor)], whereas the structurally related majusculamide C (**4**) is a single diastereomer having an *S*-Ibu unit.

Isolation of the same secondary metabolite from a variety of organisms that have a dietary or a symbiotic relationship can cloud the issue of the compound's true origin. For example, the dolastatins are a remarkable series of cytotoxic peptides and depsipeptides that were originally isolated from the mollusk *Dolabella auricularia*.[1] The most potent cytotoxin of the series, dolastatin 10, has been recently isolated from the cyanobacterium *Symploca* sp. in a yield several orders of magnitude greater than what the extract of the sea hare provided.[2,3] This dramatic difference suggested that the mollusk, a generalist herbivore, may be acquiring these cytotoxins from cyanobacteria through diet. The isolation of dolastatins 3, 12 (**3**), and 16 and a variety of dolastatin analogues from cyanobacteria lends further credibility to this proposal.[4−7]

In 1998, we reported the isolation and biological evaluation of samples of lyngbyastatin 1 (**2**) and dolastatin 12 (**3**) from a *Lyngbya majuscula*/*Schizothrix calcicola* assemblage.[5] The structure determinations of these cyanobacterial samples were hampered by the extensive signal doubling and broadening observed in the NMR spectra, a phenomenon not seen in the NMR spectra of majusculamide C (**4**)[7d] from cyanobacteria or the samples of dolastatin 11 (**5**)[8] and dolastatin 12 (**3**)[8] from the sea hare. On the basis of epimerization experiments on **4**, we concluded that the unusual spectral broadness observed in the cyanobacterial samples of **2** and **3** was due to these samples being mixtures of diastereomers arising from epimerization of the acid-sensitive Ibu unit. Synthetic studies have now established an *S* configuration for the Ibu unit of dolastatin 11[9] (**5**) and more recently have suggested that the Ibu units of the cyanobacterial metabolites **2** and **3** have an *R* configuration. In other words, *R*-Ibu-**3** had been isolated from the cyanobacteria, whereas *S*-Ibu-**3** had been isolated from a sea hare.[10]

We have investigated this problem further as a result of the structure elucidation of lyngbyastatin 3 (**1**), a new lyngbyastatin 1 (**2**) homologue from *Lyngbya majuscula* Harvey ex Gomont[11] (Oscillatoriaceae) strains collected in Guam. We report here the isolation and characterization of **1** along with the configuration of the 4-amino-2,2-dimethyl-3-oxopentanoic acid (Ibu) unit in **1**. In light of the result, we have also reexamined the configuration of the Ibu unit in our cyanobacterial samples of lyngbyastatin 1 (**2**), dolastatin 12 (**3**), and majusculamide C (**4**).



Lyngbyastatin 3	(**1**) R_1 = Me	R_2 = Me	R_3 = OMe	R_4 = *i*-Bu
Lyngbyastatin 1	(**2**) R_1 = H	R_2 = Me	R_3 = OMe	R_4 = *i*-Bu
Dolastatin 12	(**3**) R_1 = H	R_2 = Me	R_3 = H	R_4 = *i*-Bu
Majusculamide C	(**4**) R_1 = H	R_2 = H	R_3 = OMe	R_4 = *sec*-Bu
Dolastatin 11	(**5**) R_1 = H	R_2 = H	R_3 = OMe	R_4 = *i*-Bu

Results and Discussion

Lyngbyastatin 3 (**1**) was isolated by bioassay-guided fractionation of the weakly solid tumor selective[12] and highly cytotoxic (even at 1/100 dilution) extracts of NIH143, -154, and -199. The pure compound **1** had IC_{50} values of 32 and 400 nM against KB and LoVo cell lines, respectively, but when tested in vivo against colon adenocarcinoma #38 or mammary adenocarcinoma #16/C in mice, **1** was poorly tolerated and exhibited only marginal or nil antitumor activity.

Very few structural features could be elucidated from the spectral data of **1**.

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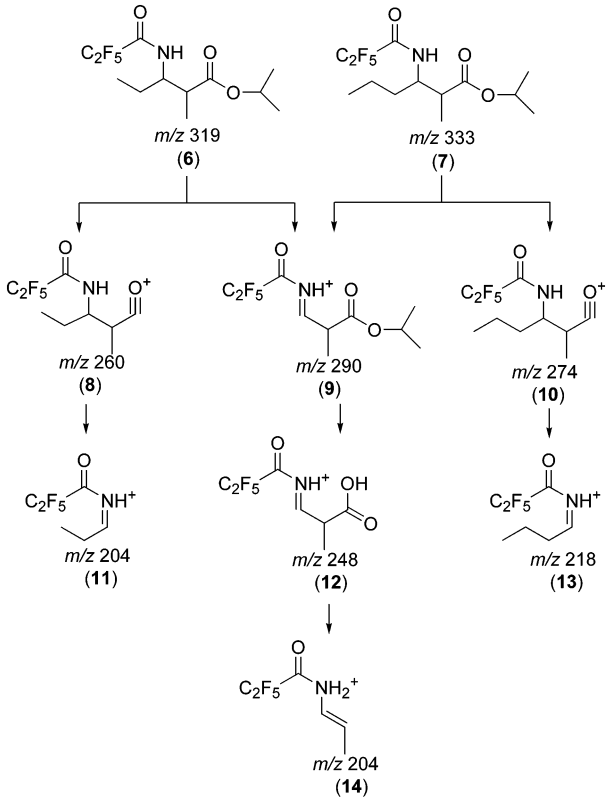


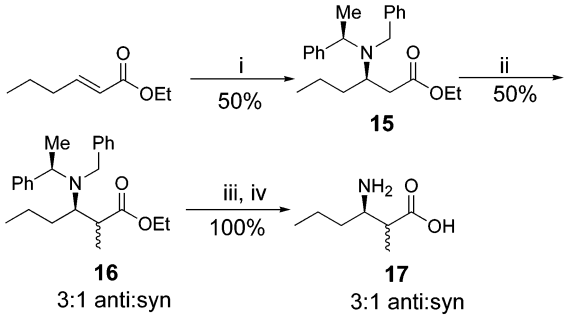
Figure 1. GC−MS fragmentations of the Amha unit (**7**) found in **1** and the synthetic MAP unit (**6**).

A series of 2D NMR and 1D TOCSY experiments were largely unsuccessful due to the extensive signal broadening and doubling in the ^{1}H and ^{13}C NMR spectra of the apparently chromatographically homogeneous material. Variable-temperature NMR experiments between 70 and −20 °C in a variety of solvents with and without LiCl[13] failed to alter the conformational ratio and ameliorate the situation. These experiments did indicate that **1** contained at least eight amino acids, based on four *N*-methylamide and four secondary amide proton signals.[14] Lyngbyastatin 3 (**1**) was likely a depsipeptide on the basis of the prominent vibrations at 1729 and 1637 cm^{-1} in the thin-film IR spectrum that indicated ester and amide functionalities, respectively.

These physical characteristics suggested that **1** was related to the known *L. majuscula* metabolite lyngbyastatin 1 (**2**). While the IR, UV, and ^{1}H NMR spectra of **1** and **2** were virtually identical, a comparison of the two samples by reversed-phase HPLC[15] indicated that **1** was more lipophilic than **2**. This difference in polarity was attributed to an extra methylene (δ_C 20.7) in **1** as revealed by a DEPT experiment in CD_3CN at 70 °C and confirmed by high-resolution mass spectrometry.

Analysis of the derivatized acid hydrolysate[16] of **1** by chiral GC−MS[17] established the location of the extra methylene. The GC−MS chromatogram of this mixture did not show a peak corresponding to the derivatized (2*S*,3*R*)-3-amino-2-methylpentanoic acid unit (**6**, *m*/*z* 319) found in **2**. A synthetic standard[18] of this MAP unit exhibited two predominant fragmentation pathways (Figure 1). In the first pathway, successive homolytic cleavage of the aliphatic side chain adjacent to the amide bond (**9**), McLafferty rearrangement (**12**), and decarboxylation (**14**) generated fragment ion peaks at *m*/*z* 290, 248, and 204, respectively. In the second pathway, cleavage around the ester linkage in **6** produced ion peaks at *m*/*z* 260 (**8**) and 204 (**11**)[19] where the side chain was still intact. Analysis of the hydrolysate revealed a derivative (**7**, *m*/*z* 333) in which all fragments derived from cleavage adjacent to the amide linkage were accounted for (**9**, **12**, and **14**). Two new ions, at *m*/*z* 274 (**10**) and 218 (**13**), established that the extra methylene was part of the aliphatic side chain of a 3-amino-2-methylhexanoic acid (Amha) unit, which has also been found in three other marine natural products, viz., malevamide B,[20] ulongamides A−F,[21] and kulokekahilide-1.[22]

Scheme 1. Synthesis of Amha Standards[a]



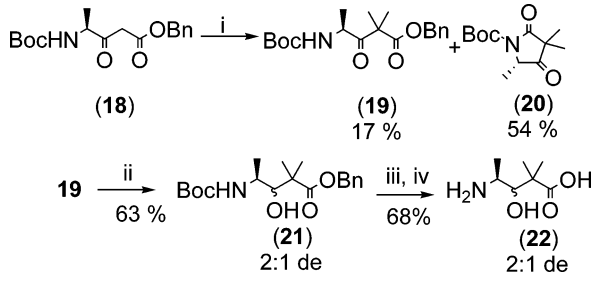
[a] (i) *n*-BuLi, THF, (*R*)-*N*-benzyl-*N*-methylbenzylamine; (ii) KHMDS, MeI; (iii) H_2, 20% Pd/C; (iv) 6 N HCl.

The stereochemistry of **1** was determined by a combination of chiral HPLC and Marfey analysis.[23] Comparison with authentic standards by chiral HPLC established the configuration of the α-amino and α-hydroxy acids in **1** as *N*,*O*-dimethyl-L-tyrosine, *N*-methyl-L-valine, *N*-methyl-L-leucine, *N*-methyl-L-alanine, and L-2-hydroxy-3-methylvaleric acid. Synthetic standards of the Amha unit were prepared following Davies' procedure for anti-α-alkyl-β-amino acids.[24] The Michael addition of (*R*)-(+)-*N*-benzyl-*N*-α-methylbenzylamine to ethyl hex-2(*E*)-enoate afforded the enantiomerically pure adduct **15** in 50% yield (Scheme 1). Treatment of **15** with potassium hexamethyldisilazane (KHMDS) and an excess of iodomethane gave **16**, which was then hydrogenated and hydrolyzed to afford a mixture of (2*S*,3*R*)- and (2*R*,3*R*)-**17**. Half of this mixture was then separated as their L-(1-fluoro-2,4-dinitrophenyl)-5-leucinamide (L-FDLA)[25] derivatives by HPLC. The configurations of these standards were determined on the basis of the ratio of the derivatives with the major peak being assigned as L-FDLA-(2*R*,3*R*)-**17** as expected from synthesis.[24] Derivatization of the remaining amount of **17** with DL-FDLA and subsequent HPLC separation produced standards equivalent to (2*R*,3*S*)- and (2*S*,3*S*)-**17**.[23,26,27] Comparison by LC-MS of these standards with the L-FDLA-derivatized hydrolysate established the 2*S*,3*R* configuration of the β-amino acid unit in **1**.

Next, we turned our attention to the configuration of the Ibu units in **1**−**4**. Synthetic standards of the reduced Ibu unit, 4-amino-2,2-dimethyl-3-hydroxypentanoic acid (Adhpa), were prepared as shown in Scheme 2. The β-ketoester (**18**), prepared by the 1,1′-carbonyldiimidazole activated coupling of *N*-Boc-L-Ala to the enolate of benzyl acetate, was dimethylated with NaH and MeI.[9,28] The gem-dimethyl adduct (**19**) was isolated in 17% yield by silica chromatography and eluted after the major product (53%), (4*S*)-*N*-Boc-3-oxo-2,2,4-trimethyl-γ-lactam (**20**). Reduction of **19** with $NaBH_4$ provided a mixture of epimeric alcohols (**21**),[29] which were deprotected to afford a mixture of (3*R*,4*S*)- and (3*S*,4*S*)-**22** (Adhpa). Derivatization with L- and DL-FDLA and HPLC analyses of these mixtures established the elution order of the standards.[30]

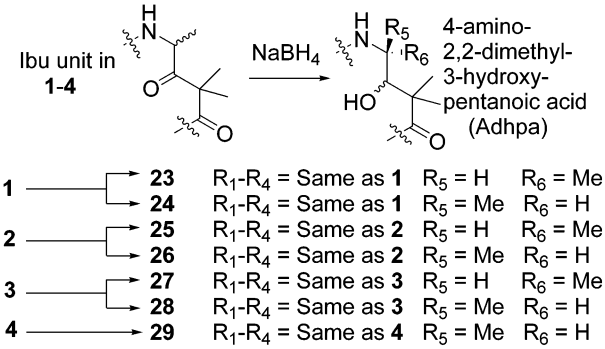
$NaBH_4$ reduction[31] of the natural products produced derivatives that could be degraded to liberate Adhpa (**22**),

Scheme 2. Synthesis of Adhpa Standards[a]



[a] (i) 2 equiv of NaH, 18 equiv of MeI; (ii) $NaBH_4$; (iii) H_2, Pd/C; (iv) TFA.

Scheme 3. Outcome of the $NaBH_4$ Reductions of **1**−**4**



from which the configurations of the Ibu units could be determined.[32] The reductions of **1**−**3** afforded two products apiece (**23**−**28**) after HPLC purification of each reaction mixture, while the reduction of **4** produced a single adduct (**29**) (Scheme 3). All of the reaction products were identified by HR-MS, and the major reduction products of lyngbyastatin 3 (**23**) and dolastatin 12 (**27**) were also characterized by NMR (Tables 2 and 3) to ensure that the gross structures of the original cyanobacterial metabolites were correct. In general, the ^{1}H NMR spectra of the major reduction products showed a methine doublet (J = 6.4 Hz) at approximately δ_H 3.35 coupled only to the alcohol proton (δ_H 5.43) and appeared to be a single compound. Conversely, the minor reduction products each appeared to be mixtures of two compounds, as the HSQC spectrum of each sample showed two protons at approximately δ_H 3.3 that correlated to two different carbons at approximately 80 ppm.

Acid hydrolysis of the reduction products of **1**−**4** and comparison with the Adhpa synthetic standards (**22**) by Marfey analysis established the configuration of the Ibu units in the cyanobacterial metabolites. As shown in Table 1, the reduction of the samples of **1**−**3** produced adducts that contained Adhpa units in which the configuration of C-4 was derived from both *R*- and *S*-Ibu, and in all three cases, the minor products (**24**, **26**, **28**) had the configuration of C-4 in Adhpa derived from *S*-Ibu. On the other hand, the reduction of majusculamide C (**4**) produced a sample (**29**) that contained an Adhpa unit derived from only *S*-Ibu. It should be noted that only those samples that had broad NMR spectra (**1**−**3**) produced reduction products corresponding to *both R*- and *S*-Ibu units.

It is unlikely these mixtures are artifacts of the reduction process. In a control experiment dolastatin 12 (**3**) was reduced in 100% MeOH-d_4 with $NaBH_4$ and the purified reaction products were analyzed by mass spectrometry. A comparison of the minor reduction products from the original and deuterium reduction experiments showed only an 8% increase in the signal intensity of the M +1 peak relative to the M^+ ion in the latter.[33] The magnitude of this deuterium incorporation suggests very little enolization occurs during the reduction and that (*S*)-Ibu-**3** is present in the original sample.

These results indicate that our cyanobacterial samples of dolastatin 12 and lyngbyastatin 1 and 3 are mixtures of two Ibu epimers. The configuration of the major Ibu epimer in each of the three mixtures is *R*. The relative amounts of the two epimers, as determined by HPLC analysis after borohydride reduction, was 4:1 for both lyngbyastatin 1 (**2**) and dolastatin 12 (**3**), but 2:1 for lyngbyastatin 3 (**1**). The samples of **2** and **3** had both been isolated from the same collection of *L. majuscula*, but the sample of lyngbyastatin 3 (**1**) had been obtained from several different collections of *L. majuscula* from Apra Harbor.[34] Since the collections were subjected to slightly different isolation procedures, this variation in the ratio of **1** might be due to some epimerization during the isolation. The plausibility of this event is supported by the molecular modeling of lyngbyastatin 1 (**2**) and Ibu-epi-**2** (data not shown), which suggests that the *S* configuration for the Ibu unit is thermodynamically favored.[10]

The broadness in the NMR spectra of the cyanobacterial samples of **1**, **2**, and **3** is most likely due to a combination of factors. The presence of a minor diastereomer obviously broadens the spectra considerably, but it has also been suggested that the *R*- and *S*-Ibu diastereomers probably have appreciably different amounts of *cis* and *trans* conformers around the (Ibu)−(*N*-Me-Ala) bond. Also in the case of the *R*-Ibu compounds, the overall shape of the *cis* and *trans* conformers differs significantly.[10] All of these factors likely contribute to the unusual degree of broadness in the NMR spectra.

It has been proposed that dolastatins 11 (**4**) and 12 (**3**) arise in the sea hare from feeding on cyanobacteria.[8] The isolation of another dolastatin analogue, lyngbyastatin 3 (**1**), from a cyanobacterium further supports this proposal. The stereochemical differences in the Ibu units of the compounds isolated from cyanobacteria and sea hares may be caused by epimerization of the *R* to the thermodynamically favorable *S*-Ibu configuration that occurred either in the digestive gut of the sea hare or perhaps during the isolation of the sea hare metabolites.[2] However, a simpler explanation is that the cyanobacterium eaten by the sea hare produces dolastatin 12 with only the (*S*)-Ibu unit, analogous to what is found in majusculamide C.[7b]

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Jasco-DIP-700 polarimeter at the sodium D line (589 nm). UV spectra were taken on a Hewlett-Packard 8453 spectrophotometer. The IR spectra were recorded on a Perkin-Elmer 1600 FTIR instrument as a film on a NaCl disk. FABMS and HRFABMS were recorded in the positive mode on a VG ZAB2SE spectrometer and MALDI on a DE-STR. The NMR spectra of **1**, **2**, and **3** were recorded in CD_3CN at 70 °C on a Varian Unity Inova 400wb operating at 400 and 100 MHz using the residual solvent signals as the internal reference, while the NMR of the reduction products were performed at 500 and 125 MHz at 15 °C on a Varian instrument. NMR analyses of the synthetic products were carried out at 300 MHz using a Varian spectrometer. HPLC separations were performed on a Beckman 110B apparatus coupled to an Applied Biosystems 759A absorbance detector. All synthetic yields are unoptimized. The following reagents were purchased from Sigma-Aldrich: 1,5-difluoro-2,4-dinitrobenzene, L- and DL-leucinamide hydrochloride, (*R*)- and (*S*)-*N*-benzyl-*N*-methylbenzylamine. Ethyl *trans*-2-hexanoate was purchased from Alfa Aesar.

Table 1. Stereochemistry of the Cyanobacterial Metabolites

cyanobacterial sample	broad NMR spectra	reduction product(s)	Ibu configuration determined[a]
lyngbyastatin 3 (**1**)	yes	**23** (major)	*R* (major) = lyngbyastatin 3
		24 (minor)	*S* (minor) = Ibu-epilyngbyastatin 3
lyngbyastatin 1 (**2**)	yes	**25** (major)	*R* (major) = lyngbyastatin 1
		26 (minor)	*S* (minor) = Ibu-epilyngbyastatin 1
dolastatin 12 (**3**)	yes	**27** (major)	*R* (major) = Ibu-epidolastatin 12
		28 (minor)	*S* (minor) = dolastatin 12
majusculamide C (**4**)	no	**29**	*S*

[a] This is the configuration at C-4 of Adhpa that was detected in the L-FDLA-derivatized hydrolysate of the reduction product.

Biological Material. The cyanobacteria were collected on the Tokai Maru shipwreck in Apra Harbor, Guam. The organisms were identified by V. J. Paul, and vouchers are maintained in formalin at the Smithsonian Marine Station, Fort Pierce, FL.

Extraction. The freeze-dried cyanobacterium (95.1 g) designated NIH199 was sequentially extracted with a 1:1 mixture of MeOH−CH_2Cl_2 and 30% aqueous ethanol to afford 1.29 and 4.60 g of lipophilic and aqueous extracts, respectively. NIH154 (72.4 g) and NIH143 (45.4 g) were treated in a similar manner to provide 12.4 and 5.4 g of the lipophilic extracts, respectively. The 30% aqueous EtOH extracts of NIH154 and NIH143 were not cytotoxic and therefore not fractionated.

Isolation of Lyngbyastatin 3 (1). Each extract was first partioned between hexane and 80% aqueous MeOH. The material in the polar layer was then subjected to a second partitioning with *n*-BuOH and water. The organic residue was separated on silica gel with CH_2Cl_2 and increasing amounts of *i*-PrOH. The biologically active fractions (5 and 8%) were separated on a prepacked Alltech C_{18} column (2000 mg) with increasing amounts of CH_3CN in H_2O. Final purification of the active fractions by RP-HPLC [Ultracarb ODS 30, 250 × 10 mm, 70% aqueous CH_3CN, flow rate 3 mL/min, detection at 220 nm] afforded pure **1** (t_R 18.5 min). NIH199 yielded 10.2 and 15.5 mg of **1** from the lipophilic and aqueous extracts, respectively, while NIH154 and NIH143 gave 49.1 and 48.8 mg of **1** from the extracts, respectively.

Extraction and Isolation of Lyngbyastatin 1 (2) and Dolastatin 12 (3). A 121.9 g sample of freeze-dried *Lyngbya majuscula* NIH 198 was extracted with a 1:1 mixture of ethyl acetate and methanol to give 2.5 g of lipophilic extract. The remaining cell mass was subsequently extracted with a 3:7 mixture of ethanol and water to afford 13.5 g of aqueous extract. Solvent partitioning of these two extracts as described for **1** and subsequent Si chromatography with increasing amount of *i*-PrOH in CH_2Cl_2 yielded two active fractions (5% and 8% *i*-PrOH). Final purification of these fractions [Ultracarb ODS 30, 250 × 10 mm, flow rate 3 mL/min, detection at 220 nm] was achieved using an isocratic system of 55% aqueous acetonitrile to afford samples of lyngbyastatin 1 (**2**) (t_R 40.4 min, 10.3 mg) and dolastatin 12 (**3**) (t_R 46.7 min, 20.1 mg) from the lipophilic extract. The aqueous extract was purified in the same manner to afford 3.6 mg of **2** and 6.8 mg of **3**.

The mixture of lyngbyastatin 3 (*R*-Ibu-**1**) and Ibu-epilyngbyastatin 3 (*S*-Ibu-**1**) was obtained as a colorless oil: $[\alpha]^{27}_D$ −62° (*c* 0.12, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 201 (4.23), 209 (3.98), 221 (3.87), 277 (3.45) nm; IR (film) ν_{max} 3386, 1729, 1637, 1513, 1466, 1248 cm^{-1}; FABMS *m*/*z* $[M + H]^+$ 1014, $[M + Na]^+$ 1036; HRFABMS *m*/*z* $[M + H]^+$ 1013.6337 (calcd for $C_{52}H_{82}N_8O_{12}$ 1013.6399, 5.8 mDa error).

The mixture of lyngbyastatin 1 (*R*-Ibu-**2**) and Ibu-epilyngbyastatin 1 (*S*-Ibu-**2**) was obtained as a clear glassy oil: $[\alpha]^{27}_D$ −17° (*c* 0.3, MeOH); UV (MeOH) λ_{max} (log ϵ) 236 (3.15), 277 (2.80), 284 (2.75) nm; IR (film) ν_{max} 3320, 1791, 1673, 1525, 1461, 1402, 1279 cm^{-1}; HRFABMS *m*/*z* 1021.5965 (calcd for $C_{51}H_{82}N_8O_{12}Na$ 1021.5944, 2.1 mDa error).

The mixture of dolastatin 12 (*S*-Ibu-**3**) and Ibu-epidolastatin 12 (*R*-Ibu-**3**) was obtained as a clear glassy oil: $[\alpha]^{27}_D$ −54° (*c* 0.7, MeOH); UV (MeOH) λ_{max} (log ϵ) 234 (2.70), 241 (3.00), 243 (3.66), 277 (2.24), 283 (2.24) nm; IR (film) ν_{max} 3316, 1791, 1672, 1525, 1461, 1402, 1278 cm^{-1}; HRFABMS *m*/*z* 991.5818 (calcd for $C_{50}H_{80}N_8O_{11}Na$ 991.5839, 2.1 mDa error).

Chiral GC−MS Analysis of Lyngbyastatin 3 (1). Lyngbyastatin 3 (**1**) (1.0 mg) was dissolved in 6 N HCl (0.3 mL) and heated to 118 °C for 18 h. The acid was removed under N_2, and the dry hydrolysate was treated at 100 °C for 45 min with a mixture of 0.3 mL of 2-propanol and 50 μL of acetyl chloride. The excess reagent was removed under N_2 and the residue treated at 100 °C for 15 min with 50 μL of a 1:1 solution of $(CF_3CF_2CO)_2O/CH_2Cl_2$. After the mixture had cooled to room temperature, the excess reagent was removed under a stream of N_2 and the resulting mixture of isopropyl esters and *N*-(pentafluoropropionyl) amino acids was dissolved in 50 μL of MeOH and analyzed by GC−MS [Chirasil-Val column, 25 μm × 0.25 m, Alltech; 12 psi initial pressure, temperature gradient of 40 to 120 °C with a heating rate of 3 °C/min]. (2*S*,3*R*)-3-Amino-2-methylpentanoic acid (**6**) had a t_R of 18.6 min: *m*/*z* (intensity) 319 (5), 290 (25), 260 (42), 248 (40), 230 (50), 204 (100). Lyngbyastatin 3 (**1**) showed 3-amino-2-methylhexanoic acid (**7**) with a t_R of 21.0 min: *m*/*z* (intensity) 333 (2), 290 (20), 274 (45), 248 (38), 230 (40), 218 (85), 204 (70), 176 (80).

Synthesis of 3-Amino-2-methylhexanoic Acid Standards: (3*R*,7*R*)-Ethyl-*N*-(α-methylbenzyl benzyl)-3-aminohexanoate (15). A solution of (*R*)-(+)-*N*-benzyl-*N*-α-methylbenzylamine (3.2 mmol) in THF (10 mL) was cooled to −78 °C prior to the slow addition of *n*-BuLi (3.0 mmol). The resulting pink solution was stirred for 30 min before the slow addition of ethyl *trans*-2-hexenoate (2 mmol) in 2 mL of THF. After stirring for 2 h at −78 °C, the reaction was quenched with saturated aqueous NH_4Cl. The solution was warmed to room temperature and partitioned between diethyl ether and water. Chromatography of the organic residue on silica with petroleum ether (bp 40−60 °C) and diethyl ether (20:1) resulted in 504 mg (50%) of **15**: ^{1}H NMR of **15** (300 MHz, $CDCl_3$) δ_H (integration, multiplicity; *J* in Hz) 7.33 (10, m), 3.91 (3, m), 3.86 (2, br s), 3.28 (1, m), 1.97 (2, d; 5.1), 1.54 (2, m), 1.25 (2, m), 1.09 (3, d; 7.1), 1.09 (3, t; 7.1), 0.88 (3, t; 7.2).

(2*S*R*,3*R*,7*R*)-Ethyl-*N*-(α-methylbenzyl benzyl)-3-amino-2-methylhexanoate (16). A solution of **15** (0.3 mmol) in 2 mL of THF was added dropwise to a solution of KHMDS (0.5 mmol) in 10 mL of THF. The reaction mixture was stirred for 1 h before the addition of neat iodomethane (5 mmol). The mixture was warmed to room temperature over 16 h and then partitioned between diethyl ether and water to afford the crude product (52 mg, 50%) as a 3:1 anti:syn mixture of C-2 diastereomers. The resulting mixture was used without further purification. ^{1}H NMR of the (2*R*,3*R*)-diastereomer as determined by 1D TOCSY experiments (300 MHz, $CDCl_3$): δ_H (position, multiplicity; *J* in Hz) 7.25 (Ar, m), 4.01 (OCH_2 and NC*H*, m), 3.87 and 3.74 ($PhCH_2$, AB system;14.6), 3.13 (NC*H*, m), 2.51 (MeC*H*, p; 7.0), 1.54 ($NCCH_2$, m), 1.38 ($MeCH_2$, m), 1.31 ($NCHCH_3$, d; 6.0), 1.19 (OCH_2CH_3, t; 6.2), 0.97 ($CHCH_3$, d; 7.0), 0.89 (CH_2CH_3, t; 6.7). ^{1}H NMR of the (2*S*,3*R*)-diastereomer as determined by 1D TOCSY experiments (300 MHz, $CDCl_3$): δ_H (position, multiplicity; *J* in Hz) 7.25 (Ar, m), 4.01 (OCH_2 and NC*H*, m), 3.87 and 3.74 ($PhCH_2$, AB system; 14.6), 2.84 (NC*H*, m), 2.48 (MeC*H*, p; 7.3), 1.54 ($NCCH_2$, m), 1.32 ($MeCH_2$, m), 1.31 ($NCHCH_3$, d; 6.0), 1.19 (OCH_2CH_3, t; 6.2), 0.95 ($CHCH_3$, d; 7.0), 0.85 (CH_2CH_3, t; 6.7).

(2*S*R*,3*R*)-3-Amino-2-methylhexanoic Acid Hydrochloride (17). To a solution of **16** and its C-2 epimer (25 mg total) in 6 mL of glacial acetic acid was added 14 mg of 20% Pd/C. After 16 h under approximately 5 atm of H_2, the mixture was

Table 2. NMR Spectral Data for **23** in $CDCl_3$

unit	C/H no.	δ_H (J in Hz)	δ_C	$^1H-^1H$ COSY	HMBC
Amha	1		173.6, s		2, 7, 48
	2	2.82, m	43.5, d	3, 7	
	3	3.89, m	52.5, d	2, 3-NH, 4	2
	3-NH	7.68, d (7.9)		3	
	4	1.38, m	24.2, t		6
		1.20, m		3	
	5	1.22, m	19.9, t		6
	6	0.86, t (6.4)	13.7, q		
	7	1.25, d (7.2)	14.9, q	2	
N-Me-Ala	8		170.9, s		9, 10
	9	5.14, q (6.9)	54.6, d	10	11
	10	1.38, d (6.9)	14.2, q	9	
	11	3.09, s	32.2, q		9
Adhpa	12		178.0, s		11, 14, 17, 18
	13		46.2, s		17, 18
	14	3.36, d (6.4)	81.3, d	14-OH	16, 17, 18
	14-OH	5.43, d (6.4)		14	
	15	4.24, dq (8.6, 6.7)	44.6, d	15-NH, 16	16
	15-NH	6.04, d (8.6)		15	
	16	1.04, d (6.7)	21.2, q	15	
	17	1.26, s	26.3, q		
	18	1.27, s	22.8, q		
N,*O*-diMe-Tyr	19		168.5, s		15, 20, 21
	20	4.78, dd (10.2, 4.5)	60.8, d	21	21, 29
	21	2.96, dd (−17.7, 10.2)	34.8, t	20	
		2.90, dd (−17.7, 4.5)		20	
	22		128.4, s		21
	23/27	7.12, d (8.2)	130.3, d	24/26	21, 23/27, 25
	24/26	6.81, d (8.2)	114.3, d	23/27	22, 24/26, 25
	25		158.7, s		28
	28	3.74, s	55.3, q		
	29	2.93, s	29.3, q		20
N-Me-Val	30		169.5, s		29, 31
	31	4.61, d (10.7)	58.4, d	32	33, 34
	32	2.16, m	26.8, d	31, 33, 34	31, 33, 34
	33	0.62, d (6.7)	18.0, q	32	34
	34	0.18, d (6.9)	18.2, q	32	33
	35	2.87, s	29.9, q		31
Gly	36		169.2, s		35, 37
	37	4.22, dd (−18.9, 7.2)	41.5, t	37_u, 37-NH	
		4.10, d (−18.9)		37_d	
	37-NH	7.09, d (7.2)		37_d	
N-Me-Leu	38		169.9, s		37, 39, 40
	39	5.32, dd (9.5, 4.5)	54.1, d	40	
	40	1.72, m	36.6, t	39, 41	42, 43
		1.66, m		39, 41	
	41	1.36, m	24.6, d	40	
	42	0.98, d (6.2)	23.3, q	41	
	43	0.88, d (7.3)	21.9, q	41	
	44	2.92, s	29.4, q		
Gly	45		169.0, s		44, 46
	46	4.46, dd (−18.1, 7.1)	41.2, t	46-NH	
		3.81, dd (−18.1, 1.7)		46-NH	
	46-NH	7.27, dd (7.1, 1.7)		46	
HMVA	47		168.9, s		46, 48
	48	5.15, d (4.3)	77.7, d	49	
	49	2.04, m	37.0, d	48, 50, 52	48
	50	1.58, m	30.5, t	49	48
		1.20, m		49	
	51	0.90, t (7.3)	11.4, q	50	
	52	0.95, d (6.2)	15.0, q	49	48

filtered through a pad of Celite and the solvent removed under N_2. Half of this sample was dissolved in 6 N HCl and refluxed for 18 h at 118 °C to yield 5.1 mg of **17** (100%). ^1H NMR of (2*R*,3*R*)- and (2*S*,3*R*)-**17** obtained by 1D TOCSY experiments was identical to literature data.[22]

Reduction of Natural Products: Dihydro-majusculamide C (29). A solution of 2 mg of **4** in 0.2 mL of methanol was added to 4 mg of $NaBH_4$. After stirring 25 min, another 4 mg aliquot of $NaBH_4$ was added and the solution stirred for another 25 min before quenching with 1 N HCl. The mixture was partitioned between ethyl acetate and water. The organic residue purified by C_{18} HPLC [Ultracarb ODS 30, 250 × 10 mm, 60% aqueous CH_3CN, flow rate 3 mL/min, detection at 220 nm] afforded a single product (**29**, t_R 39.5 min) with no sign of **4** (t_R 50.2 min): ^{13}C NMR ($CDCl_3$) δ_C 176.7, 173.4, 172.4, 170.50, 170.45, 170.0, 169.8, 169.7, 167.7, 158.6, 130.4, 128.7, 114.2, 80.3,[35] 77.9, 61.4, 61.0, 58.0, 55.3, 50.2, 47.5, 45.2, 44.4, 42.3, 41.3, 40.5, 37.5, 35.2, 29.9, 29.5, 29.3, 27.1, 26.8, 25.6, 24.7, 23.6, 22.4, 18.33, 18.26, 15.48, 15.44, 14.6, 13.8, 11.7, 10.9, 10.7, 8.9; HR-MALDI *m*/*z* 1009.5991 [calcd for $C_{50}H_{82}N_8O_{12}$-Na 1009.5944, 4.7 mDa error].

Dihydro-lyngbyastatin 3 (23, 24). A sample of **1** (6 mg) was reduced as described for **4** and purified by HPLC (62% aqueous CH_3CN) to afford the minor (**24**, t_R 31.3 min) and the major reduction products (**23**, t_R 37.9 min) in a ratio of 1:2. **23**: ^1H and ^{13}C NMR, see Table 2; HR-MALDI *m*/*z* 1037.6310

Table 3. NMR Spectral Data for **27** in $CDCl_3$

unit	C/H no.	δ_H (*J* in Hz)	δ_C	$^1H-^1H$ COSY	HMBC
Map	1		173.6, s		2, 3, 6
	2	2.89, m	42.5, d	3, 6	6
	3	3.74, m	54.4, d	2, 3-NH, 4	2, 4, 5
	3-NH	7.73, d (7.1)		3	
	4	1.66, m	21.6, t	3, 4_u, 5	2
		1.18, m		3, 4_d, 5	
	5	0.89, t (6.6)	11.5, q	4	4
	6	1.25, d (8.2)	14.9, q	2	2
N-Me-Ala	7		171.1, s		3, 8, 9
	8	5.18, q (6.9)	54.5, d	9	9, 10
	9	1.38, d (6.9)	14.2, q	8	
	10	3.08, s	32.2, q		
Adhpa	11		178.0, s		10, 13, 16, 17
	12		46.2, s		13, 16, 17
	13	3.35, d (5.6)	81.1, d	13-OH	15, 16, 17
	13-OH	5.44, d (5.6)		13	
	14	4.23, dq (8.1, 6.7)	44.6, d	14-NH, 15	15
	14-NH	6.05, d (8.1)		14	
	15	1.04, d (6.7)	21.3, q	14	14
	16	1.24, s	26.1, q		
	17	1.27, s	22.7, q		
N-Me-Phe	18		168.4, s		14, 19
	19	4.85, dd (10.4, 4.4)	60.8, d	20	20, 27
	20	3.05, dd (−14.3, 4.4)	34.8, t	19	19
		2.97, dd (−14.3, 10.4)		19	
	21		136.5, s		20, 23/25
	22/26	7.20, d (7.6)	129.3, d	23/25	
	23/25	7.26, t (7.6)	128.9, d	22/26, 24	
	24	7.20, d (7.6)	127.1, d	23/25	
	27	2.92, s	29.3, q		19
N-Me-Val	28		169.5, s		19, 27, 29
	29	4.60, d (10.4)	58.4, d	30	30, 31, 32
	30	2.12, m	26.8, d	29, 31, 32	29, 31, 32
	31	0.60, d (6.7)	18.0, q	30	
	32	0.09, d (6.4)	18.1, q	30	
	33	2.86, s	29.0, q		29
Gly	34		169.2, s		33, 35
	35	4.46, dd (−18.1, 7.1)	41.2, t	35_u	
		3.81, d (−18.1)		35_d	
	35-NH	7.23, d (7.1)		35_d	
N-Me-Leu	36		169.9, s		35, 37, 38
	37	5.32, dd (9.9, 4.2)	54.5, d	38	39, 42
	38	1.75, ddd (−14.6, 10.4, 4.2)	36.6, t	37, 38_u, 39	37
		1.61, ddd (−14.6, 9.9, 4.0)		37, 38_d, 39	
	39	1.34, m	24.6, d		37, 38
	40	0.93, d (6.6)	23.3, q		38
	41	0.86, d (6.4)	21.8, q		38
	42	2.91, s	29.3, q		
Gly	43		168.9, s		37, 42, 44
	44	4.19, d (−17.1)	41.4, t	44_u	
		3.99, dd (−17.1, 5.2)		44_d, 44-NH	
	44-NH	7.06, d (5.2)		44_u	
HMVA	45		169.1, s		44, 46
	46	5.15, d (4.7)	77.8, d	47	47, 48, 50
	47	2.04, m	36.9, d	46, 48, 50	
	48	1.49, m	24.2, t	47, 48_u	
		1.19, m		48_d	
	49	0.88, t (7.4)	11.3, q	48_d	48_u
	50	0.92, d (6.9)	15.0, q	47	48_d

[calcd for $C_{52}H_{86}N_8O_{12}Na$ 1037.6257, 5.3 mDa error]. **24**:[36] HR-MALDI *m/z* 1037.6273 [calcd for $C_{52}H_{86}N_8O_{12}Na$ 1037.6257, 1.6 mDa error].

Dihydro-lyngbyastatin 1 (25, 26). A sample of **2** (2 mg) was reduced as described for **4** and purified by HPLC (55% CH_3CN) to afford the minor (**26**, t_R 37.7 min) and major reduction products (**25**, t_R 45.0 min) in a 1:4 ratio. **25**:[36] HR-MALDI *m/z* 1023.6078 [calcd for $C_{51}H_{84}N_8O_{12}Na$ 1023.6101, 2.3 mDa error]. **26**:[36] HR-MALDI *m/z* 1023.6057 [calcd for $C_{51}H_{84}N_8O_{12}Na$ 1023.6101, 4.3 mDa error].

Dihydro-dolastatin 12 (27, 28). A sample of **3** (4 mg) was reduced as described for **4** and purified by HPLC (55% CH_3CN) to afford the minor (**28**, t_R 44.4 min) and the major reduction products (**27**, t_R 55.9 min) in a 1:4 ratio. **27**: ^1H NMR, ^{13}C NMR, $^1H-^1H$ COSY, and HMBC data, see Table 3; HR-MALDI *m/z* 993.5944 [calcd for $C_{50}H_{82}N_8O_{11}Na$ 993.5995, 5.1 mDa error]. **28**: ^{13}C NMR ($CDCl_3$) δ_C 178.0, 174.1, 170.7, 169.9, 169.1, 167.7, 137.3, 129.3, 128.9, 127.0, 80.9,[35] 78.0, 61.9, 58.8, 54.9, 46.4, 43.6, 41.4, 37.4, 36.7, 35.0, 32.7, 29.9, 29.6, 29.4, 27.1, 24.9, 24.3, 22.9, 22.1, 21.0, 18.6, 18.3, 15.8, 15.1, 14.6, 11.2; HR-MALDI *m/z* 993.6040 [calcd for $C_{50}H_{82}N_8O_{11}Na$ 993.5995, 4.5 mDa error].

Synthesis of (3*RS*,4*S*)-4-Amino-2,2-dimethyl-3-hydroxypentanoic Acid: (4*S*)-Benzyl *N*-Boc-4-amino-3-oxo-pentanoate (18). This reaction was carried out as previously described,[9] except the solution of LDA and benzyl acetate was stirred for only 5 min before the addition of the acyl imidazole. Purification as described[9] afforded **18** in a yield of 50%. ^1H NMR ($CDCl_3$) δ_H (integration, multiplicity; *J* in Hz): 7.38 (5,

s), 5.18 (2, s), 5.08 (1, d; 7.0), 4.36 (1, p; 7.0), 3.64 (1, d; −16.0), 3.57 (1, d; −16.0), 1.43 (9, s), 1.32 (3, d; 7.0).

(4*S*)-Benzyl *N*-Boc-4-amino-2,2-dimethyl-3-oxopentanoate (19). To a solution of 150 mg of **18** and 9 equiv of MeI in THF (5 mL) was added 1 equiv of NaH (65% dispersion). The solution was stirred for 1 h before the addition of one more equivalent of NaH and nine more equivalents of MeI. After 16 h, 100 mL of diethyl ether was added and the solution was sequentially partitioned with sodium thiosulfate and brine. The organic layer was dried over $MgSO_4$ and the solvent removed in vacuo. Purification over silica with 6:1 hexane−EtOAc yielded 60 mg (53%) of (4*S*)-*N*-Boc-2,2,4-trimethyl-3-oxo-γ-lactam first (**20**), followed by 33 mg of **19** (17%). ^{1}H NMR of **19** ($CDCl_3$): δ_H (integration, multiplicity; *J* in Hz) 7.33 (5, m), 5.15 (2, s), 4.89 (1, d; 7.9), 4.63 (1, p; 7.9), 1.59 (3, s), 1.44 (3, s), 1.41 (9, s), 1.18 (3, d; 7.9). Lactam **20**: ^{1}H NMR ($CDCl_3$) δ_H (integration, multiplicity; *J* in Hz) 4.35 (1, q; 6.9), 1.56 (9, s), 1.52 (3, d; 7.0), 1.30 (3, s), 1.28 (3, s).

(3*SR*,4*S*)-4-Amino-2,2-dimethyl-3-hydroxypentanoic Acid (22). A solution of **19** (33 mg) in methanol was added to 4 equiv of $NaBH_4$ at 0 °C. The mixture was stirred for 30 min, until it was negative to 2,4-dinitrophenylhydrazine, and then it was quenched with 1 N HCl. It was subsequently partitioned between EtOAc and brine, then concentrated to dryness to yield 20 mg (62%) of (3*SR*,4*S*)-benzyl *N*-Boc-4-amino-2,2-dimethyl-3-hydroxypentanoate (**21**). The benzyl group was removed by hydrogenation over 30% Pd/C in methanol at room temperature for 12 h. The suspension was filtrated through Celite and evaporated to dryness. The residue was dissolved in concentrated TFA and stirred for 15 min before removing the acid under a stream of nitrogen to afford 6.3 mg (69%) of **22**. The ^{1}H NMR of the major diastereomer in the mixture of **22**: (MeOH-d_4) δ_H (integration, multiplicity; *J* in Hz) 3.85 (1, d; 1.8), 3.51 (1, qd; 6.0, 1.8), 1.25 (3, s),[37] 1.24 (3, d; 6.0),[37] 1.18 (3, s). Minor diastereomer of **22**: δ_H (MeOH-d_4) 3.67 (0.7, d; 5.6), 3.39 (0.7, qd; 6.5, 5.6), 1.30 (2, s), 1.27 (2, s), 1.24 (2, d; 6.5).[37]

Absolute Stereochemistry of the Amino Acid-Derived Units: Chiral HPLC. The hydrolysate of **1** was analyzed by chiral HPLC, and the retention times were compared with authentic standards [Column Chirex Phase 3126 (D), 250 × 4.6 mm, Phenomenex, solvent 2 mM $CuSO_4$−MeCN (95:5) for Gly, *N*-Me-Ala, *N*-Me-Val, and *N*-Me-Leu; *N*,*O*-diMe-Tyr with 2 mM $CuSO_4$−MeCN (90:10), flow rate 0.8 mL/min, detection at 254 nm, except 2-hydroxy-3-methylvaleric acid, which was determined on a CHIRALPAK MA(+), 50 × 4.6 mm, Diacel Chemical Industries, Ltd., 2 mM $CuSO_4$−MeCN (85:15), flow rate 0.8 mL/min, detection at 254 nm]. The retention times of the standards were Gly (7.1 min), *N*-Me-L-Ala (9.0 min), *N*-Me-D-Ala (9.8 min), *N*-Me-L-Val (14.1 min), *N*-Me-D-Val (19.2 min), *N*-Me-L-Leu (52.1 min), *N*-Me-D-Leu (79.0 min), *N*,*O*-diMe-L-Tyr (81.5 min), *N*,*O*-diMe-D-Tyr (87.1 min), D-2-hydroxy-3-methylvaleric acid (40.1 min), D-allo-2-hydroxy-3-methylvaleric acid (34.1 min), L-allo-2-hydroxy-3-methylvaleric acid (52.0 min), and L-2-hydroxy-3-methylvaleric acid (66.2 min). The retention times of the amino acid components of the hydrolysate were Gly (7.1 min), *N*-Me-L-Ala (9.0 min), *N*-Me-L-Val (14.1 min), *N*-Me-L-Leu (52.1 min), L-2-hydroxy-3-methylvaleric acid (66.2 min), and *N*,*O*-diMe-L-Tyr (81.5 min). The identities of the peaks were confirmed by co-injection.

LC-MS Analysis of β-Amino Acids. Before the synthetic standards of the Amha unit were prepared, the configuration at C-3 of the Amha unit in **1** was determined by LC-MS. The sample of lyngbyastatin 3 (**1**) (1 mg) was hydrolyzed in 6 N HCl at 118 °C for 16 h, after which the sample was dried under N_2. The residue was twice dissolved in 25% aqueous triethylamine and evaporated. The hydrolysate was resuspended in 50 μL of water, and then 20 μL of 1 M $NaHCO_3$ and 50 μL of 1% FDLA (either the L- or DL-mixture as required) were added. The reaction was maintained at 80 °C for 3 min until the yellow solution turned orange. After cooling to room temperature 20 μL of 1 N HCl was added and the yellow solution was diluted with 0.8 mL of CH_3CN. The separations of the L- and DL-FDLA derivatives were performed on a Hydrobond-ODS [100 × 3.0 mm, a linear gradient of 20 to 80% CH_3CN with 0.01 M TFA over 50 min, flow rate 0.3 mL/min, detection at 342 nm]. The mass spectra were collected in the negative mode and had an ESI voltage of 4.6 kV with the auxiliary and sheath gas pressure set at 5 units and 70 psi, respectively, and the capillary heated to 200 °C. A mass range of *m*/*z* 438−440 was covered. Analysis of the L-FDLA-derivatized hydrolysate gave two peaks at 32.3 and 35.8 min. The former was identified as *N*-Me-L-Leu by co-injection of a derivatized standard, and the latter therefore was the derivatized Amha unit. Analysis of the DL-FDLA-derivatized hydrolysate gave four peaks which eluted at 30.6, 32.3, 35.3, and 35.8 min, which corresponded to (D-FDLA)-Amha, (D-FDLA)-(*N*-Me-L-Leu), (L-FDLA)-(*N*-Me-L-Leu), and (L-FDLA)-Amha, respectively. Since the D-FDLA-derivatized Amha unit eluted before the L-FDLA-derivatized Amha unit, based on the proposed separation mechanism of the FDLA derivatives, the absolute configuration of the Amha unit at C-3 was *R*.[23] The retention times of L-FDLA-derivatized *N*-Me-DL-Leu were 32.3 and 35.3 min for the L- and D-derivatized amino acid, respectively.

Advanced Marfey: β-Amino Acids. A portion of the sample (25 μL) prepared for the LC-MS analysis of the β-amino acids was analyzed by RP-HPLC [Bondclone 10 C_{18}, 300 × 7.8 mm, a linear gradient of 20 to 80% CH_3CN with 0.01 M TFA over 50 min, flow rate 3 mL/min, PDA detection]. The retention times of the L-FDLA-derivatized amino acids in the hydrolysate were Gly (21.5 min), *N*-Me-L-Ala (22.1 min), *N*-Me-L-Val (25.8 min), *N*,*O*-diMe-L-Tyr (26.5 min), *N*-Me-L-Leu (28.2 min), and (2*S*,3*R*)-**17** (31.2 min). The retention times of the D-FDLA-derivatized amino acids in the DL-FDLA-derivatized hydrolysate were Gly (21.5 min), *N*-Me-L-Ala (22.9 min), (2*S*,3*R*)-**17** (26.5 min), *N*-Me-L-Val (29.2 min), *N*,*O*-diMe-L-Tyr (30.3 min), and *N*-Me-L-Leu (30.7 min). The retention times of L-FDLA-derivatized standards were (2*R*,3*R*)-**17** (30.3 min) and (2*S*,3*R*)-**17** (31.3 min), while the D-FDLA-derivatized standards were detected at (2*S*,3*R*)-**17** (26.7 min) and (2*R*,3*R*)-**17** (27.2 min).

Marfey Analysis of the Reduction Products 23−29. The samples were hydrolyzed for 24 h as described for **1** and derivatized with L-FDLA before HPLC analyses [YMC-AQ ODS, 250 × 10 mm, a linear gradient of 40 to 50% aqueous CH_3CN with 0.1% TFA (pH 4.13) over 60 min, flow rate 2.5 mL/min, PDA detection]. The retention time of L-FDLA-(3*SR*,4*S*)-**22** was 20.3 min, while D-FDLA-(3*SR*,4*S*)-**22** eluted at 22.6 min. A peak corresponding to L-FDLA-(3*SR*,4*S*)-**22** (20.3) was found in **24**, **26**, **28**, and **29**, which was confirmed by co-injection of the appropriate standard and by comparison of the UV spectrum. The stereochemistry of Ibu units in the major reduction products **23**, **25**, and **27** was determined by LC-MS due to overlap in the HPLC trace.

LC-MS Analyses of the Reduction Products. The separations of the L- and DL-FDLA derivatives were performed on a Hydrobond-ODS [100 × 3 mm, a linear gradient of 40 to 50% CH_3CN with 0.25% acetic acid over 60 min, flow rate 0.3 mL/min, detection at 342 nm]. The mass spectra were collected in the positive mode and had an ESI voltage of 4.6 kV with the auxiliary and sheath gas pressure set at 5 units and 70 psi, respectively, and the capillary heated to 200 °C. A mass range of *m*/*z* 455.4−456.4 was covered. The retention time of L-FDLA-(3*SR*,4*S*)-**22** was 18.2 min. The DL-FDLA-**22** sample gave three peaks (4:3:1 ratio) at 18.2, 20.0, and 24.8 min. The presence of three peaks indicated that D-FDLA-(3*R*,4*S*)-**22** and D-FDLA-(3*S*,4*S*)-**22** were resolved under the LC-MS conditions. The elution order of these last two peaks was not conclusively established, since it was irrelevant to the analysis, but (3*R*,4*S*)-**22** is likely the major reduction product with a retention time of 20.0 min.[29] The L-FDLA-derivatized acid hydrolysate of the major reduction products **23**, **25**, and **27** contained a peak at 20.0 min with the correct mass for D-FDLA-**22** indicating an *R* configuration in the Ibu units of the natural products. The L-FDLA-derivatized acid hydrolysate of the minor reduction products (**24**, **26**) of lyngbyastatins 3 and 1 were also analyzed by LC-MS and provided peaks of the correct mass that coeluted with L-FDLA-(3*SR*,4*S*)-**22** (18.2), confirming the *S* configuration in these natural products.

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Supporting Information Available: 2D NMR spectra of **23**, **27**; ^{1}H and ^{13}C NMR spectra of **1**, **2**, **3**, **29**; ^{1}H NMR spectra of **24**, **25**, **26**, **28**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(27) The same elution order with respect to C-3 (3*S* elutes before 3*R*) was seen when synthetic MAP units were derivatized with the commercially available Marfey reagent (L-FDAA). In this case the C-2 diastereomers could not be separated though. See: Williams, D. E.; Burgoyne, D. L.; Rettig, J. J.; Andersen, R. J.; Faith-Afshar, Z. R.; Allen, T. M. *J. Nat. Prod.* **1993**, *56*, 545−551.
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(30) Under the HPLC analysis conditions, the C-3 diastereomers of **22** were not resolved; that is, only a single peak was detected when the mixture of L-FDLA-derivatized (3*R*,4*S*)- and (3*S*,4*S*)-**22** was injected. Likewise, when the D-FDLA-derivatized mixture was analyzed on the Bondclone C_{18} column the standards equivalent to (3*S*,4*R*)- and (3*R*,4*S*)-**22** appeared as a single peak, but when these samples were submitted for LC-MS analysis on a different C_{18} column, the standards equivalent to (3*S*,4*R*)- and (3*R*,4*S*)-**22** resolved into two peaks.
(31) In general the stereochemical outcome (Cram vs anti-Cram) of the reductions of the natural products was irrelevant to our analysis.
(32) We tried several of other strategies to determine the Ibu configuration before settling on reducing the ketone. These include Baeyer−Villiger oxidations of **1** or its methanolysis product with m-CPBA, peracetic acid, trifluoroperacetic acid, or hydrogen peroxide, a Beckmann rearrangement of an oxime derivative of the methanolysis product of **1**, and reductive deoxygenation with tosylhydrazine and sodium cyanoborohydride. If successful, the first two routes would have allowed the configuration of the Ibu unit to be deduced by the presence of L- or D-Ala after hydrolysis, while the third route would have allowed direct application of the advanced Marfey technique without synthetic standards.
(33) On average the ratio of the two signals was 61 and 53% for the minor reduction products obtained in MeOH-d_4 and MeOH, respectively. The theoretical calculated ratio of $(M + 1 + H)^+/(M + H)^+$ is 61% for $C_{50}H_{82}N_8O_{11}$.
(34) The samples of **1** from NIH199, -153, and -143 were combined before the stereochemistry of the Ibu unit was known. This precluded determining if each collection had the same ratio of epimers as might be expected if the diastereomers were artifacts of the isolation procedure and not due to biosynthetic differences in the strains.
(35) This signal is not present in the starting material.
(36) Too little material was isolated to characterize by ^{13}C NMR spectrum.
(37) These three signals overlapped significantly and had a combined integration of 8 relative to the other observed signals.

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