Lyngbyastatin 1 and Ibu-epilyngbyastatin 1: Synthesis, Stereochemistry, and NMR Line Broadening

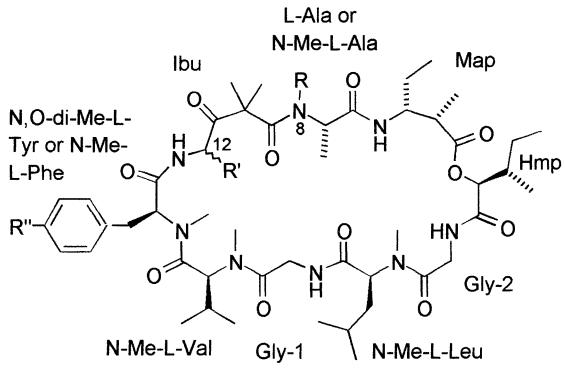
Ruoli Bai,[†] Robert B. Bates,*[,‡] Ernest Hamel,[†] Richard E. Moore,[§] Pichaya Nakkiew,[‡] George R. Pettit,[⊥] and Bilal A. Sufi[‡]

Screening Technologies Branch, Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute at Frederick, National Institutes of Health, Frederick, Maryland 21702, Department of Chemistry, University of Arizona, Tucson, Arizona 85721, Department of Chemistry, University of Hawaii, Manoa, Hawaii 96822, and Cancer Research Institute and Department of Chemistry, Arizona State University, Tempe, Arizona 85287-1604

Received March 20, 2002

The synthesis of a lyngbyastatin 1−Ibu-epilyngbyastatin 1 mixture combined with NMR and molecular modeling studies proved that natural lyngbyastatin 1 was only one Ibu epimer rather than a mixture of both and that the configuration of this epimer in the Ibu unit was *R*. The substance isolated with lyngbyastatin 1 was Ibu-epidolastatin 12. The extreme broadness in the proton NMR spectra of lyngbyastatin 1 and Ibu-epidolastatin 12 was exchange broadening due to rotation about the Ibu−Ala amide bond. It was a consequence of (1) a small energy difference between the cis and trans forms of this bond, (2) a substantial difference in conformation between these forms, and (3) a lowered barrier between them compared to most amide bonds (due to steric hindrance). The synthetic lyngbyastatin 1−Ibu-epilyngbyastatin 1 mixture had significant activities against cancer cells and in stimulating actin polymerization, but was less active than dolastatin 11 in all assays.

The isolation of the cytotoxic cyclic depsipeptides lyngbyastatin 1 (**4**, earlier assigned structure **3**), Ibu-epilyngbyastatin 1 (**3**, previously assigned structure **4**), dolastatin 12 (**5**),[1] and Ibu-epidolastatin 12 (**6**) was reported from a *Lyngbya majuscula/Schizothrix calcicola* assemblage and a *L. majuscula* strain collected near Guam.[2,3] The extreme broadness of the NMR peaks of these substances was attributed to the presence of both Ibu-epimers in unseparated mixtures. We now report the synthesis of a mixture of **3** and **4** and resulting data which show that the compounds isolated from *Lyngbya/Schizothrix* were lyngbyastatin 1 (**4**) and Ibu-epidolastatin 12 (**6**) and that the NMR line broadening is due to sterically lowered rotation barriers about the Ibu−Ala amide bonds with appreciable amounts of conformationally distinct cis and trans forms present at equilibrium. This steric hindrance, characteristic of *N*,*N*-dialkylamides with quaternary α-carbons, made the preparation of the Ibu-N-Me-Ala amide unit difficult, as described below.



	R	R'	R"	
1	H	α-Me (12*S*)	OMe	dolastatin 11
2	H	β-Me (12*R*)	OMe	Ibu-epidolastatin 11
3	Me	α-Me (12*S*)	OMe	Ibu-epilyngbyastatin 1
4	Me	β-Me (12*R*)	OMe	lyngbyastatin 1
5	Me	α-Me (12*S*)	H	dolastatin 12
6	Me	β-Me (12*R*)	H	Ibu-epidolastatin 12

Results and Discussion

Two unsuccessful strategies for making the critical Ibu-N-Me-L-Ala unit are shown in Figure 1. The first was to substitute L-Ala-OBn·HCl with N-Me-L-Ala-OBn·HCl (**7**) in the synthetic sequence used to make Boc-Ibu-L-Ala-OBn in the dolastatin 11 (**1**) synthesis,[4] hoping to obtain Boc-Ibu-N-Me-L-Ala-OBn (**11**). Benzyl ester **7**[5] was coupled to the *β*-keto acid from the hydrogenation of the *β*-keto ester **8**,[4] giving dipeptide **9** in 63% yield. However, attempts to dimethylate **9** with MeI/NaH failed, presumably for steric reasons; only monomethylation product **10** was obtained. A second approach to the Ibu-N-Me-L-Ala unit was by dimethylation of *β*-keto ester **8** to the gem-dimethylated ester **12**, followed by coupling with amine **7**. Attempts to dimethylate keto ester **8** using MeI/NaH did not give **12**. When DBU was used as the base, the first methyl group went to the right place but the second went on nitrogen, giving **13**. Again, the desired product was apparently not obtained for steric reasons.

In the successful route to the Ibu-N-Me-L-Ala unit (Figure 2), di-Boc-L-Ala (**14**)[6] was used instead of Boc-L-Ala to prevent N-methylation. Acid **14** was activated with CDI and allowed to react with $LiCH_2COOBn$, giving a 66% yield of *β*-keto ester **15**. Ester **15** was gem-dimethylated to ester **16** in 78% yield, using MeI/DBU in acetonitrile. Hydrogenolysis of benzyl ester **16**, followed immediately by coupling with amine salt **7** using BOP-Cl, gave a 93% yield of Boc_2-Ibu-N-Me-L-Ala-OBn (**17**). The Boc units of

* To whom correspondence should be addressed. Tel: (520)621-6317. Fax: (520)621-8407. E-mail: batesr@u.arizona.edu.
[†] National Cancer Institute.
[‡] University of Arizona.
[§] University of Hawaii.
[⊥] Arizona State University.

10.1021/np020117w CCC: $22.00
Published on Web 10/25/2002

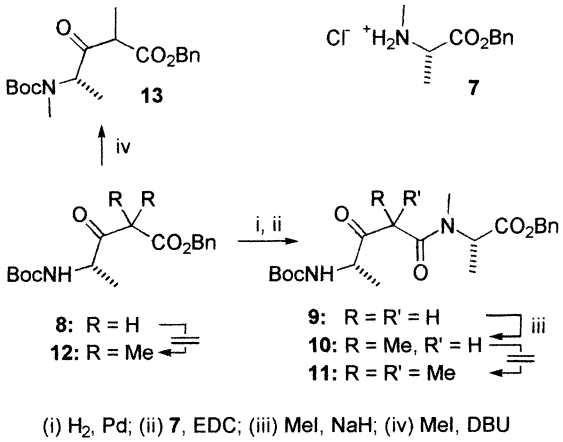


Figure 1. Unsuccessful strategies for synthesis of the Ibu-N-Me-L-Ala unit.

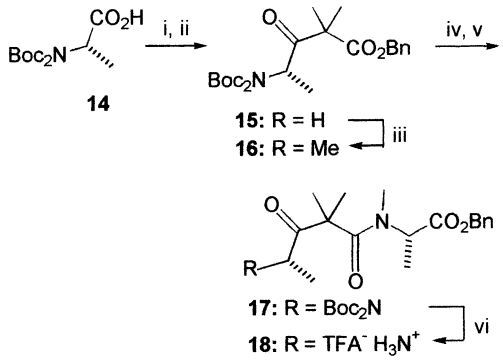


Figure 2. Successful route to the Ibu-N-Me-L-Ala unit.

Ibu-N-Me-L-Ala derivative **17** were removed with TFA to give TFA salt **18**, which was used in the synthesis of **3** and **4**.

Except for the use of di-Boc protection and the presence of the N-methyl group on the alanine, these procedures follow those used in the dolastatin 11 (**1**) synthesis, in which the *S* configuration was maintained in the Ibu unit throughout.[4] However, in the current synthesis, epimerization in the Ibu unit occurred, probably in the step in which DBU was used,[7] leading to equal amounts of Ibu epimers **3** and **4**.

When the route from dipeptide **18** to depsipeptide **4** analogous to that used for dolastatin 11 (**1**)[4] failed for unknown reasons, a synthesis was achieved by reversing the order of protecting group removal to that shown in Figure 3. The benzyl group of heptadepsipeptide **19** was removed by hydrogenolysis, yielding carboxylic acid **20** quantitatively. Nonadepsipeptide **21** was made in 79% yield by coupling TFA salt **18** with acid **20** using TBTU. The protecting groups were removed by hydrogenolysis and TFA. The final coupling using HBTU gave a 1:1 ratio of Ibu epimers **3** and **4** in 40% total yield after HPLC. Attempts to separate these epimers by HPLC failed since their retention times were so similar. However, it was clear from the NMR spectra (Figure 4) what compounds were present in the mixture, as described below.

One set of mostly very broad peaks matched those of natural lyngbyastatin 1, which we now assign as **4**. (The extensive degradative work on lyngbyastatin 1[2] had established the structure *except* for the configuration in the Ibu unit.) A second set of much sharper peaks matched

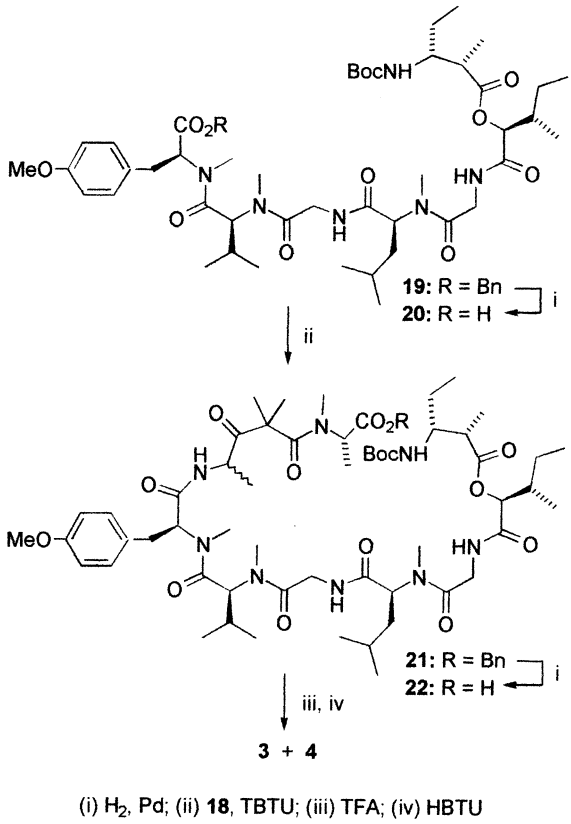


Figure 3. Synthesis of Ibu-epilyngbyastatin 1 (**3**) and lyngbyastatin 1 (**4**).

those of dolastatin 12 (**5**), except for the peaks due to the tyrosine side chain; these showed the presence of Ibu-epilyngbyastatin 1 (now **3**). The NMR spectral parameters of dolastatin 12 (**5**), previously unreported, were obtained with the aid of COSY and HETCOR spectra. The peaks of Ibu-epilyngbyastatin 1 (**3**) were then assigned by comparison with those of dolastatin 12 (**5**). The peaks of lyngbyastatin 1 (**4**) and Ibu-epidolastatin 12 (**6**)[2] remain largely unassigned due to their extreme broadness.

Three arguments that the configurations in the Ibu units of depsipeptides **3**−**6** are assigned correctly and not reversed are as follows: (a) dolastatins 11 (**1**) and 12 (**5**), found in the same organism, are more likely to share the same configuration at the Ibu center than not, and the configuration of dolastatin 11 (**1**) was shown to be *S* at the Ibu center by synthesis from *S*-alanine;[4] (b) the ^{1}H NMR spectrum (Table 1) of dolastatin 12 (**5**) is closer to that of dolastatin 11 (**1**) than to that of Ibu-epidolastatin 11 (**2**), especially noticeable in the valine and Hmp units;[8] (c) the NMR line-broadening considerations discussed next.

The severe line broadening in the proton NMR spectrum of lyngbyastatin 1 (**4**) and much less noticeable broadening for Ibu-epilyngbyastatin 1 (**3**) are related to the lowered rotation barrier about the amide bond in the Ibu-N-Me-Ala unit compared to most amides. For example, in *N*,*N*-dimethylamides, the energy barrier to rotation about the amide bond drops from 88 kJ/mol in *N*,*N*-dimethylformamide to 71 kJ/mol in *N*,*N*-dimethylacetamide, 67 kJ/mol in *N*,*N*-dimethylisobutyramide, and only 50 kJ/mol in *N*,*N*-dimethylpivalamide.[9] When the α-carbon is quaternary, as in *N*,*N*-dimethylpivalamide and the Ibu-N-Me-Ala units of depsipeptides **3**−**6**, the interconversion of cis and trans amides occurs at about the same rate as the NMR measurement and can result in broadness. In strong support of this interpretation, the broadened lines in the carbon

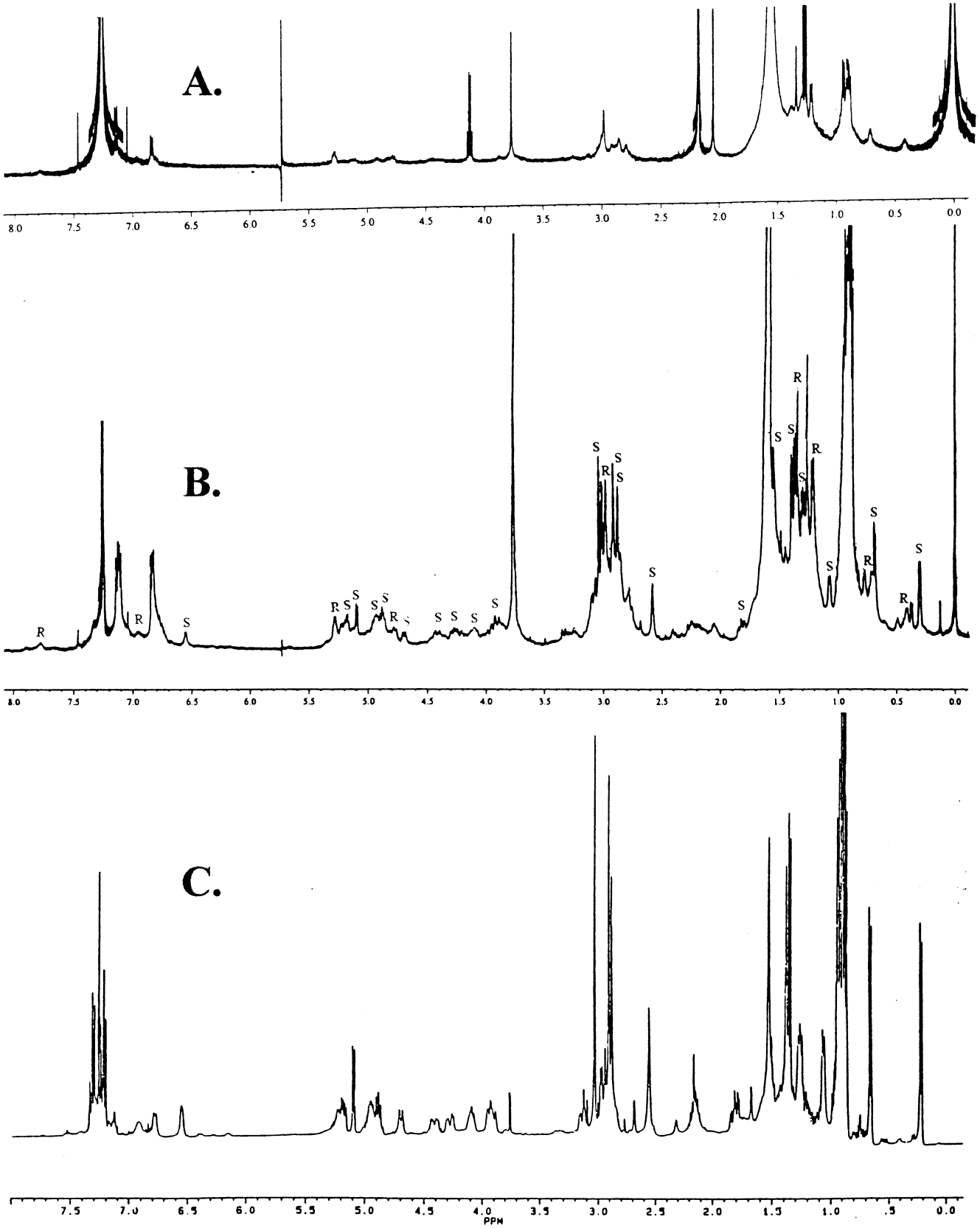


Figure 4. ^{1}H NMR spectra ($CDCl_3$) of *N*-methyl-L-alanine derivatives related to lyngbyastatin 1 (**4**). (A) Natural lyngbyastatin 1 (**4**, *R* configuration in Ibu unit, 500 MHz). (B) Synthetic mixture of lyngbyastatin 1 (**4**, *R* in Ibu unit) and Ibu-epilyngbyastatin 1 (**3**, *S* in Ibu unit, 500 MHz). (C) Natural dolastatin 12 (**5**, *S* configuration in Ibu unit, 400 MHz).

spectrum of dolastatin 12 (**5**), due to a lowered barrier about its Ibu-N-Me-L-Ala amide bond, are observed for carbons in its Map, Ala, Ibu, and Tyr units (Table 2).

That extreme broadness would be observed with the compounds that have *R* configurations in the Ibu unit (lyngbyastatin 1 (**4**) and Ibu-epidolastatin 12 (**6**)) and much less with compounds that have *S* configurations in the Ibu unit (Ibu-epilyngbyastatin 1 (**3**) and dolastatin 12 (**5**)) is supported by the molecular modeling studies described next, which suggest that at probe temperature depsipep-

Table 1. ^{1}H NMR Assignments of Dolastatin 11 (**1**), Ibu-epidolastatin 11 (**2**), Dolastatin 12 (**5**), Ibu-epidolastatin 12 (**6**), Lyngbyastatin 1 (**4**), and Ibu-epilyngbyastatin 1 (**3**)

	compound					
unit	**1**	**2**	**3**[a]	**4**[b]	**5**[a]	**6**[b]
Map						
α-CH	2.79qd(7,2.5)	2.75m	2.87m		2.87m	
β-CH_3	1.10d(7)	1.12d(7)	1.08m		1.09d(7)	
β-CH	4.47m		4.10m		4.13m	
γ-CH_2	1.46,1.56m				1.35,1.59m	
δ-CH_3	0.93t(7.5)	0.95t(7)	0.85−0.95		0.92t(7)	
NH	7.09d(10.5)	6.91d(10)			6.95br s	
Ala						
α-CH	4.44p(7)	4.61p(7)	5.23m		5.26q(7)	
β-CH_3	1.07d(7)	1.08d(7)	1.30d(6.5)	1.21d(7)	1.30d(7)	1.21d(7)
NH/NCH_3	7.78d(8.5)	7.52d(8)	2.58br s		2.59br s	
Ibu						
β-CH_3	1.44,1.49s	1.52,1.52s	1.40,1.54s	1.32s	1.42,1.56s	1.32s
γ-CH	4.91p(7)	4.98p(6.5)	4.89m		4.91p(7.5)	
δ-CH_3	1.13d(7)	1.22d(6.5)	1.38d(7)	1.21d(7)	1.39d(7)	1.19d(7)
NH	7.15d(9)	7.31d(8)			6.80d(7.5)	
Tyr						
α-CH	5.11dd(8.5,6.5)	5.04t(8)	4.94m		4.98m	
β-CH_2	2.82dd(14,8.5), 3.25dd(14,6.5)	2.78m, 3.27dd (13.5,8)			2.95m 3.17dd (13,4)	
δ-CH	7.14d(8.5)	7.14d(9)	7.12d(8)	7.14d(8)	7.23d(7.5)	7.25m
ϵ-CH	6.81d(8.5)	6.83d(9)	6.83d(8)	6.84d(8)	7.34t(7.5)	7.25m
ζ-CH					7.27t(7.5)	7.25m
OCH_3	3.75s	3.77s	3.77s	3.76br s		
NCH_3	2.96s	2.99s	2.92br s	2.75−3.10	2.94s	2.75−3.10
Val						
α-CH	4.78d(10.5)	4.89d(10.5)	4.69d(10)		4.72d(10.5)	
β-CH	2.23m	2.28m			2.16m	
γ-CH_3	0.37d(6.5), 0.74d(6.5)	0.57d(6.5), 0.81d(6.5)	0.29br s, 0.69br s	0.40br s, 0.70br s	0.24d(6.5), 0.69d(6.5)	0.34br s, 0.70br s
NCH_3	2.95s	2.93s	2.89br s	2.75−3.10	2.92s	2.75−3.10
Gly-1						
α-CH_2	3.60dd(18,2) 4.42dd(18,7.5)	3.65dd(18,2), 4.44dd(18,7.5)	3.94m, 4.40m		3.96m 4.44dd(18,7)	
NH	7.41dd(7.5,2)				7.30m	
Leu						
α-CH	5.37dd(11,5)	5.20dd(10,5.5)	5.19m		5.20dd(10.5,5)	
β-CH_2	1.62m,1.87ddd (13.5,11,4)	1.81ddd (13,10.5,4)	1.83ddd (14,10,4)		1.57m,1.85ddd (14,10,4)	
γ-CH	1.56m				1.45m	
δ-CH_3	0.92d(6.5), 0.98d(6.5)	0.89−0.94m	0.85−0.95	0.85−0.95	0.90d(7), 0.97d(6.5)	0.85−0.95
NCH_3	3.14s	3.10s	3.04br s	2.75−3.10	3.06s	2.75−3.10
Gly-2						
α-CH_2	3.58dd(16,4.5), 4.46dd(16,7)	3.71dd(16,4.5), 4.48dd(16,7)	3.92m, 4.27m		3.94m, 4.31dd(19,6)	
NH	7.35dd(7,4.5)	7.34dd(7,4.5)	6.55br s		6.59m	
Hmp						
α-CH	5.19d(3.5)	5.27d(3)	5.10d(5)		5.12d(5.5)	
β-CH	2.07m	2.07m			2.13m	
γ-CH_3	0.89d(7)	0.89d(7.5)	0.85−0.95	0.85−0.95	0.95d(6.5)	0.85−0.95
γ-CH_2	1.23,1.47m				1.23,1.53m	
δ-CH_3	0.88t(7.5)	0.88t(7.5)	0.85−0.95	0.85−0.95	0.92t(7)	0.85−0.95

[a] Some broadening of spectral lines in Ibu-Ala region. [b] All spectral lines very broad.

tides **4** and **6** have appreciable amounts of *trans*- and *cis*-Ibu-Ala-amide forms with very different conformations (and thus very different NMR shifts), while depsipeptides **3** and **5** have a predominant trans form accompanied by a cis form, which does not differ much in conformation (and thus NMR shifts) from the trans form except in the vicinity of the Ibu and Ala units. These calculations were done by first locking the Ibu-Ala amide bond in the trans configuration and then in the cis configuration. Table 3 shows the calculated energies of the lowest-energy cis and trans forms of lyngbyastatin 1 (**4**, *R* Ibu configuration, 4.4 kJ/mol difference) to be closer in energy than those of Ibu-epilyngbyastatin 1 (**3**, *S* Ibu configuration, 8.0 kJ/mol difference). The smaller energy difference between cis and trans forms for lyngbyastatin 1 (**3**) and resulting larger amounts of the minor form is one factor that should contribute to greater broadness in its NMR spectra.

Another factor that should make the spectra of lyngbyastatin 1 (**4**) much broader is that its lowest-energy cis and trans forms (**4**c and **4**t, respectively) have very different overall shapes, and thus they should have greater chemical shift differences between the cis and trans forms throughout the molecule. It should be noted that (except for conformation 5 of **4**t) all of the conformations in Table 3 share essentially the same conformation of the 30-membered ring as the other conformations in that column and differ only in side-chain rotations. These calculations of energy and shape differences between cis and trans forms thus both support the view that lyngbyastatin 1, with much broader NMR peaks, is **4**, and Ibu-epilyngbyastatin 1 is **3**.

This mixture of synthetic lyngbyastatin 1 (**4**) and its Ibu epimer (**3**) showed significant cytotoxicities against several human tumor cells and stimulated actin polymerization,[10]

Table 2. ^{13}C NMR Chemical Shifts for Dolastatins 11 (**1**) and 12 (**5**) in $CDCl_3$

	1	**5**		**1**	**5**		**1**	**5**
Map			**Tyr**			**Leu**		
C=O	172.6	173.5	C=O	168.0	167.7	C=O	171.7	171.6[b]
a-CH	42.4	42.5[a]	α-CH	61.1	61.2	α-CH	54.7	54.3
β-CH_3	9.9	11.5[a]	β-CH_2	34.7	35.3[a]	β-CH_2	38.1	37.6
β-CH	51.4	52.8[a]	γ-C	128.7	136.1	δ-CH	24.9	25.0
γ-CH_2	25.9	25.1[a]	δ-CH	130.4	129.1	δ^1-CH_3	24.4	21.7
δ-CH_3	10.9	11.2[a]	ϵ-CH	114.4	129.2	δ^2-CH_3	23.2	23.1
			ζ-CH	158.7	127.4	NCH_3	30.2	29.8
Ala			OCH_3	55.3				
C=O	172.8	172.2[ab]	NCH_3	29.4	29.3	**Gly-2**		
α-CH	48.3	52.6[a]				C=O	170.0	169.6[b]
β-CH_3	15.5	13.9[a]	**Val**			α-CH_2	40.7	41.5
NCH_3		31.9[a]	C=O	170.1	169.7[b]			
			α-CH	58.2	57.8	**Hmp**		
Ibu			β-CH	27.1	27.0	C=O	170.1	169.8[b]
C=O	171.9	170.8[ab]	γ^1-CH_3	18.4	18.3	α-CH	78.4	78.4
α−C	54.9	55.4[a]	γ^2-CH_3	18.5	18.4	β-CH	37.4	36.5
β^1-CH_3	21.6	21.5[a]	NCH_3	29.2	29.0	γ-CH_3	11.6	11.3
β^2-CH_3	22.0	25.2[a]				γ-CH_2	23.8	24.3
β-C=O	209.7	210.3	**Gly-1**			δ-CH_3	15.5	15.1
γ-CH	51.2	50.3	C=O	169.3	169.3			
δ-CH_3	19.2	19.6[a]	α-CH_2	41.1	41.5			

[a] Shows broadening. [b] May be interchanged.

Table 3. Calculated Energies (kJ/mol) of the Conformations within 10 kJ/mol of the Lowest-Energy Form for Lyngbyastatin 1 (**4**) and Ibu-epilyngbyastatin 1 (**3**) in Chloroform

	3 (*S* Ibu configuration)		**4** (*R* Ibu configuration)	
conformation	trans	cis	trans	cis
1	−596.8	−588.8	−580.3	−584.7
2	−593.8	−588.0	−579.7	−580.6
3			−577.8	−579.8
4			−576.2	−579.4
5			−575.6	

but the mixture was less active than dolastatin 11 (**1**) in all assays. The human cancer cell growth inhibitions (GI_{50}; μg/mL) of the mixture, probably largely due to the Ibu epimer **3** since Ibu-epidolastatin 11 (**2**) was much less active than dolastatin 11 (**1**),[8] were 0.031 and 0.24 against the NCI-H460 (lung) and DU-145 (prostate) cell lines, respectively, compared to 0.0013 and 0.22, respectively, for dolastatin 11 (**1**). For induction of actin polymerization, the mixture had an EC_{50} value of 40 ± 7 μM; cf. 42 ± 3 μM for phalloidin and 9.5 ± 0.7 μM for dolastatin 11 (**1**).[10]

In summary, a synthesis of a lyngbyastatin 1 (**4**)−Ibu-epilyngbyastatin 1 (**3**) mixture combined with NMR and molecular modeling studies proved that natural lyngbyastatin 1 (**4**) has the *R* configuration in the Ibu unit and is not accompanied by the *S* epimer as suggested earlier.[2] The natural substance isolated along with lyngbyastatin 1 (**4**)[2] was Ibu-epidolastatin 12 (**6**), unaccompanied by dolastatin 12 (**5**). The severe broadness in the proton NMR spectra of lyngbyastatin 1 (**4**) and Ibu-epidolastatin 12 (**6**) was exchange broadening from rotation about the Ibu−Ala amide bond; for such *N*,*N*-dialkylamides with quaternary α-carbons, when the cis−trans energy difference is small, considerable broadening should be expected for the parts of the molecule where their conformations differ significantly. The mixture of depsipeptides **3** and **4** displayed significant activities against human cancer cells and in stimulating actin hyperassembly, but was less active than dolastatin 11 (**1**).

Experimental Section

General Experimental Procedures. General procedures have been described previously.[4] Abbreviations used are BOP-Cl = bis(2-oxo-3-oxazolidinyl)phosphinic chloride, CDI = 1,1′-carbonyldiimidazole, DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene, EDC = 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride, HBTU = *O*-benzotriazol-1-yl-*N*,*N*,*N*′*N*′-tetramethyluronium hexafluorophosphate, Hmp = (2*S*,3*S*)-2-hydroxy-3-methylpentanoic acid, Ibu = (*S*)-4-amino-2,2-dimethyl-3-oxopentanoic acid, Map = (2*S*,3*R*)-3-amino-2-methylpentanoic acid, TBTU = *O*-benzotriazol-1-yl-*N*,*N*,*N*′*N*′-tetramethyluronium tetrafluoroborate, and TFA = trifluoroacetic acid.

N-Me-L-Ala-OBn·HCl (7). Prepared by the method of Coppola et al.:[5] ^1H NMR (DMSO-d_6) δ 1.44 (d, J = 7 Hz, 3H, β-Me), 2.55 (s, 3H, N-Me), 4.15 (q, J = 7 Hz, 1H, a-CH), 5.24 (s, 2H, $BnCH_2$), 7.39 (m, 5H, Ar).

Benzyl 4-(*N*,*N*-Di-Boc-amino)-3-oxopentanoate (15). A solution of **14** (1.0 g, 3.5 mmol) and 1,1′-carbonyldiimidazole (0.61 g, 3.8 mmol) in THF (5 mL) was stirred at 0 °C for 30 min and 25 °C for 2 h. To prepare LDA, BuLi (1.6 M in hexane, 6.5 mL, 10.4 mmol) was added dropwise to a solution of diisopropylamine (1.45 mL, 10.4 mmol) in THF (10 mL) at −78 °C. After warming to 0 °C for 15 min and recooling to −78 °C, benzyl acetate (1.5 mL, 10.4 mmol) was added and stirring was continued at −78 °C for 1.25 h. The acylimidazole prepared earlier was cannulated into this solution, stirring was continued at −78 °C for 15 min, and 1 N HCl (10 mL) was added. The mixture was warmed to 0 °C, acidified to pH 3 with citric acid, and extracted with EtOAc (3 × 100 mL). The combined organic phase was washed with 5% $NaHCO_3$ (2 × 100 mL) and brine (2 × 100 mL). Solvent evaporation and HPLC gave ester **15** (0.96 g, 66%): ^1H NMR δ 1.41 (d, J = 6.5 Hz, 3H, δ-Me), 1.49 (s, 18H, Boc), 3.52 and 3.59 (d, J = 16 Hz, 2H, α-CH_2), 4.85 (q, J = 6.5 Hz, 1H, γ-CH), 5.16 (s, 2H, $BnCH_2$), 7.34 (br s, 5H, Ar).

Benzyl 4-(*N*,*N*-Di-Boc-amino)-2,2-dimethyl-3-oxopentanoate (16). To a solution of ester **15** (0.22 g, 0.52 mmol) in CH_3CN (15 mL) were added DBU (0.155 mL, 1.03 mmol) and methyl iodide (0.16 mL, 1.03 mmol). The mixture was stirred at 25 °C for 16 h, the solvent was evaporated, and the residue was washed with water, giving methyl derivative **16** as a yellow oil (0.18 g, 78%): ^1H NMR δ 1.36 (d, J = 6.5 Hz, 3H, δ-Me), 1.40 and 1.44 (s, 6H, β-Me's), 1.49 (s, 18H, Boc), 5.07 (q, J = 6.5 Hz, 1H, γ-CH), 5.13 and 5.21 (d, J = 12.5 Hz, 2H, CH_2), 7.33 (br s, 5H, Ar).

***N*,*N*-Di-Boc-Ibu-N-Me-L-Ala-OBn (17).** A mixture of benzyl ester **16** (67 mg, 0.15 mmol), Pd/C (15 mg), and CH_2Cl_2 (5 mL) was stirred under H_2 (1 atm) for 3 h at −5 °C. After quickly filtering the mixture into a solution of amine salt **7** (37 mg, 0.16 mmol) in CH_2Cl_2 (10 mL) at 0 °C, bis(2-oxo-3-oxazolidinyl)phosphinic chloride (49 mg, 0.19 mmol) and diisopropylethylamine (67 μL, 0.38 mmol) were added. After stirring at 0 °C for 4 h and 25 °C for 16 h, solvent evaporation and HPLC gave dipeptide **17** as an oil (74 mg, 93%): ^1H NMR (mixture of rotamers) δ 1.38 (d, J = 7 Hz, 3H, Ibu-δ-Me), 1.44 (d, J = 6.5, 3H, Ala-Me), 1.41 and 1.45 (s, 6H, Ibu-β-Me's), 1.49 (s, 18H, Boc), 2.78 and 2.81 (s, 3H, NMe), 4.82 (q, J = 7 Hz, 1H, Ala-CH), 5.11 (q, J = 7 Hz, 1H, Ibu-CH), 5.13 and 5.16 (d, J = 16 Hz, 2H, $BnCH_2$), 7.34 (br s, 5H, Ar); FABMS m/z 535 $[M + 1]^+$.

Boc-Map-Hmp-Gly-N-Me-L-Leu-Gly-N-Me-L-Val-O,N-di-Me-L-Tyr-Ibu-N-Me-L-Ala-OBn (21). To dipeptide **17** (6.2 mg, 0.0012 mmol) was added TFA (15 mL), and the solvent was evaporated. To the residue was added EtOAc (10 mL), and the solvent was evaporated to give TFA salt **18** (6.4 mg, 100%). Heptadepsipeptide **19** (14 mg, 0.014 mmol)[4] was stirred with Pd/C (15 mg) in CH_2Cl_2 under H_2 (1 atm) for 2 h; catalyst filtration and solvent evaporation gave carboxylic acid **20** (12.4 mg, 100%). A solution of TFA salt **18** (6.4 mg, 0.0012 mmol), **20** (10.3 mg, 0.0012 mmol), diisopropylethylamine (8 μL, 0.046 mmol), and TBTU (22 mg, 0.069 mmol) in DMF (3 mL) was stirred for 20 h. Solvent evaporation and HPLC gave peptide **21** (11 mg, 79%): ^1H NMR δ 0.40 and 0.75 (m, 6H, Val-Me's), 1.42 (s, 9H, Boc), 2.40, 2.45, 2.68, 2.72, 2.91, 2.94, and 2.99 (s, 12H, NMe's), 3.72, 3.75, and 3.77 (s, 3H, OMe), 5.14 and 5.20 (d, J = 15 Hz, 2H, $BnCH_2$), 6.78 and 7.09 (m, 4H, Tyr-Ar), 7.34 (br s, 5H, Bn-Ar); FABMS m/z 1207 $[M + 1]^+$.

Boc-Map-Hmp-Gly-N-Me-L-Leu-Gly-N-Me-L-Val-O,N-di-Me-L-Tyr-Ibu-N-Me-L-Ala (22). A solution of benzyl ester **21** (11 mg, 0.0009 mmol) in CH_2Cl_2 was stirred with Pd/C (15 mg) under H_2 (1 atm) for 2 h. Removal of catalyst by filtration and solvent evaporation gave carboxylic acid **22** (10 mg, 99%): ^{1}H NMR δ 0.37 and 0.75 (d, $J = 7$ Hz, 6H, Val-Me's), 1.42 (s, 9H, Boc), 2.37, 2.68, 2.79, 2.92, 2.94, and 2.97 (s, 12H, NMe's), 3.76 and 3.77 (s, 3H, OMe), 6.78, 6.82, and 7.12 (m, 4H, Ar).

Lyngbyastatin 1 (4) and Ibu-epilyngbyastatin 1 (3). To acid **22** (10 mg, 0.009 mmol) was added TFA (10 mL) followed by solvent evaporation. EtOAc (5 mL) was added and evaporated. To the residual TFA salt (10 mg, 0.009 mmol) in DMF (3.5 mL) were added Et_3N (6 μL, 0.045 mmol) and HBTU (34 mg, 0.09 mmol). Stirring for 7 h, solvent evaporation, and HPLC gave lyngbyastatin 1 (**4**, 1.8 mg, 20%) admixed with Ibu-epilyngbyastatin 1 (**3**, 1.8 mg, 20%): ^{1}H NMR, Table 1 and Figure 4; FABMS *m*/*z* 999 $[M + 1]^+$.

Acknowledgment. For financial support, we thank the NIH (grant R01 CA78750), the Camille and Henry Dreyfus Foundation (Senior Scientist Mentor Initiative), the Research Corporation, Bristol-Myers Squibb Company, Aldrich Chemical Co., and the Government of Thailand (fellowship to P.N.).

Supporting Information Available: ^{1}H NMR spectra of **7**, **15**−**17**, **21**, and **22**; table of the 30-membered ring torsion angles for **3c**, **3t**, **4c**, and **4t**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Pettit, G. R.; Kamano, Y.; Kizu, H.; Dufresne, C.; Herald, C. L.; Bontems, R. J.; Schmidt, J. M.; Boettner, F. E.; Nieman, R. A. *Heterocycles* **1989**, *28*, 553−558.
(2) 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.
(3) "Ibu-epi-" is preferred to using a number, since dolastatins[1] and lyngbyastatins[2] have been numbered in different ways. Dolastatin numbering[1] is used herein.
(4) Bates, R. B.; Brusoe, K. G.; Burns, J. J.; Caldera, S.; Cui, W.; Gangwar, S.; Gramme, M. R.; McClure, K. J.; Rouen, G. P.; Schadow, H.; Stessman, C. C.; Taylor, S. R.; Vu, V. H.; Yarick, G. V.; Zhang, J.; Pettit, G. R.; Bontems, R. *J. Am. Chem. Soc.* **1997**, *119*, 2111−2113.
(5) Coppola, B. P.; Noe, M. C.; Schwartz, D. J.; Abdon, R. L., II; Trost, B. M. *Tetrahedron* **1994**, *50*, 93−116.
(6) Gunnarsson, K.; Ragnarsson, U. *Acta Chem. Scand.* **1990**, *44*, 944−951.
(7) Evans, P. A.; Roseman, J. D.; Garber, L. T. *J. Org. Chem.* **1996**, *61*, 4880−4881.
(8) Unpublished results from the laboratory of RBB.
(9) Jackman, L. M.; Cotton, F. A. *Dynamic Nuclear Magnetic Resonance Spectroscopy*; Academic: New York, 1975; pp 208−210.
(10) Bai, R.; Verdier-Pinard, P.; Gangwar, S.; Stessman, C. C.; McClure, K. J.; Sausville, E. A.; Pettit, G. R.; Bates, R. B.; Hamel, E. *Mol. Pharmacol.* **2001**, *59*, 462−469.

NP020117W