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FULL PAPER

Leptolyngbyolides, Cytotoxic Macrolides from the Marine Cyanobacterium *Leptolyngbya* sp.: Isolation, Biological Activity, and Catalytic Asymmetric Total Synthesis

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Abstract: Four new macrolactones, leptolyngbyolides A–D, were isolated from the cyanobacterium *Leptolyngbya* sp. collected in Okinawa, Japan. The planar structure of leptolyngbyolides were determined by extensive NMR studies, although complete assignment of the absolute configuration awaited the catalytic asymmetric total synthesis of leptolyngbyolide C. The synthesis took advantage of the catalytic asymmetric thioamide-aldol reaction using copper (I) complexed with a chiral bidentate phosphine ligand to regulate two key stereochemistries of the molecule at the outset. The present total synthesis demonstrates the utility of this reaction for the construction of complex chemical entities. In addition to the total synthesis, this work reports that leptolyngbyolides depolymerize filamentous actin (F-actin) both *in vitro* and in cells. Detailed biological studies suggest the probable order of F-actin depolymerization and apoptosis caused by leptolyngbyolides.

Introduction

Marine natural products have attracted extraordinary attention from the scientific community due to their unusual and diverse structures, and intriguing biological activities. For example, swinholide A (**1**) was first reported by Kashman and Carmely as a constituent of extracts from the marine sponge *Theonella swinhoei*,[1] and later Kitagawa and co-workers unveiled its structure as a 44-membered macrolactone comprising two seco acids connected in a head-to-tail manner.[2] Swinholide A exhibits potent cytotoxicity against some cancer cell lines, which is believed to be due to its actin-binding activity.[3] Curiously, related natural products with a similar structure but clear differences in the ring substructure do not necessarily exhibit the same extent of activity.[4] The precise mode of interaction of this molecule with actin is now well understood at the atomic level based on their co-crystal structures.[5] In 2005, Gerwick and co-workers isolated related 44-membered marine macrolactones, ankaraholides produced by cyanobacteria samples collected in the Fijian and Madagascan seas that also affect the actin cytoskeleton.[6] Swinholide A (**1**) was also mentioned in their report as a secondary metabolite of this cyanobacteria. The fact that swinholide A was already isolated from several taxonomically different species of sponge suggests that macrolides of this class are products of symbiotic microorganisms. The presence of a related "monomeric" 22-membered cytotoxic marine macrolactone, scytophycins (**3**),[7] biosynthesized by another genus of cyanobacteria, corroborates this hypothesis. Accordingly, it is widely recognized that marine cyanobacteria are a promising source of pharmaceutical lead compounds belonging to relatively unexplored chemical entities.

In this report, we describe the chemistry and biology of novel 22-membered marine lactones, leptolyngbyolides A–D (**5a** – **5d**). Leptolyngbyolides were isolated from the extracts of cyanobacterium *Leptolyngbya* sp. collected in Okinawa, Japan; the structure is closely related to lobophorolide (**4**), which is also a 22-membered lactone isolated from the sea alga *Lobophora variegata*.[8] The gross structures of leptolyngbyolides were elucidated based on the extensive application of 2D NMR techniques; the stereochemistry, however, has remained uncharacterized. The absolute configuration of leptolyngbyolide C was unequivocally elucidated by total synthesis using catalytic asymmetric reactions developed in this laboratory as key stereo-defining transformations. Based on the structure similarity with related macrolides and observed changes in cell morphology caused by leptolyngbyolide A, we examined the actin-depolymerizing activities of leptolyngbyolides. In addition, we evaluated the effects of leptolyngbyolides on cancer-cell growth and apoptosis. This work sheds light on the biological mechanisms of leptolyngbyolides and suggests the probable order of F-actin depolymerization and apoptosis caused by this class of macrolactones.

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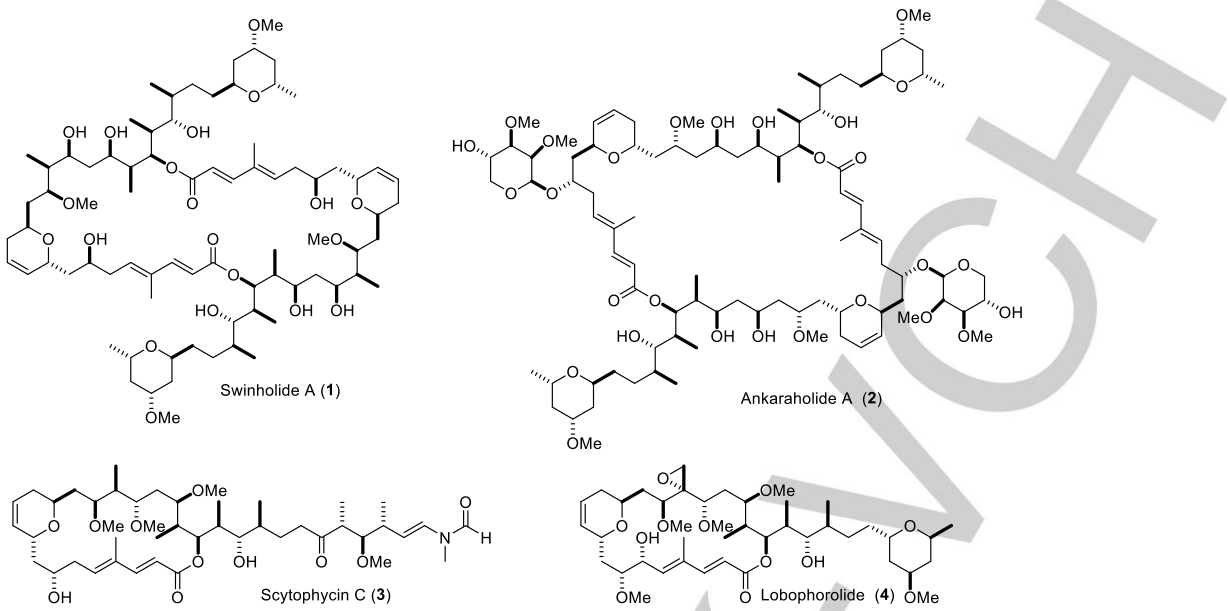

Figure 1. Structure of cytotoxic marine macrolactones structurally related to leptolyngbyolides.

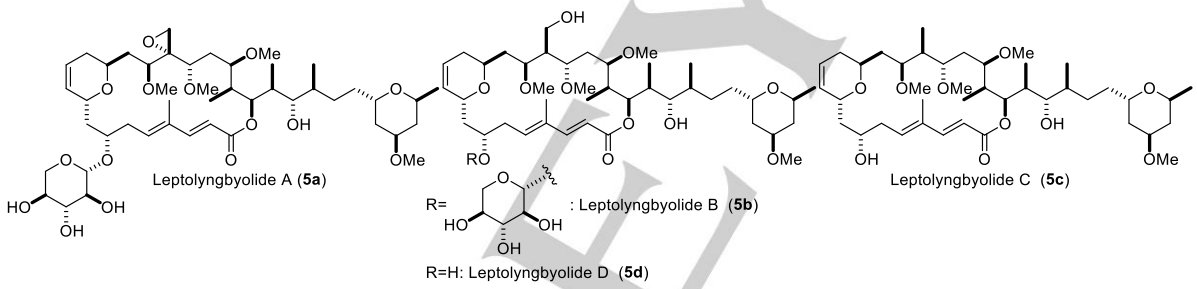

Figure 2. Structure of leptolyngbyolides (highly probable stereochemistry is shown for A, B, and D).

Results and Discussion

Isolation of leptolyngbyolides: Bioassay-guided fractionation of the extract of *Leptolyngbya* sp. led to isolation of four new macrolactones, leptolyngbyolides A–D (**5a**-**5d**). First, the marine cyanobacterium *Leptolyngbya* sp. was manually collected on the coast of Itoman City, Okinawa Prefecture, Japan, and stored at −30 ˚C until use. Approximately 2.1 kg (wet weight) of the frozen *Leptolyngbya* sp. was extracted with MeOH at room temperature for 2 months. The methanolic extract was filtered, and the filtrate was evaporated *in vacuo*. The residue was partitioned between AcOEt and H_2O. The material obtained from the organic layer was partitioned between 90% MeOH and hexane. Among these four fractions, the 90% MeOH fraction showed the most potent growth-inhibitory activity (IC_{50} < 0.1 μg/mL) against HeLa S_3 cells. Then, the aqueous MeOH fraction was applied to ODS column chromatography eluted with a series of MeOH/H_2O mixed solvent systems with varying ratios; 80% and absolute MeOH fractions potently inhibited the cell growth of HeLa S_3 cells. The fraction eluted with 80% methanol was subjected to HPLC [Cosmosil Cholester; 70% MeOH] to give leptolyngbyolide A (**5a**) (183 mg), leptolyngbyolide B (**5b**) (34.5 mg), and leptolyngbyolide D (**5d**) (2.6 mg), and [Cosmosil $5C_{18}$-MS-II; 75% MeCN] to give leptolyngbyolide C (**5c**) (12.1 mg).

Elucidation of the planar structures of leptolyngbyolides: The molecular formula of **5a** was deduced to be $C_{46}H_{76}O_{15}$ by high-resolution mass measurement (*m/z* 891.5096 calculated for $C_{46}H_{76}O_{15}Na$ $[M+Na]^+$ 891.5082). Subsequently, ^1H NMR, DEPT, and HMQC spectra showed that leptolyngbyolide A has four methyl groups substituted at the methine carbon, a vinylic methyl group, four *O*-methyl groups, three aliphatic methines, fifteen methine carbons adjacent to oxygen atoms, five methine carbons within olefin, and eleven methylene carbons. ^{13}C NMR revealed that this molecule consists of 46 carbons,

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including a carbonyl carbon (δ_C = 170.7 ppm) and six olefinic carbons (δ_C = 152.7, 139.8, 136.2, 131.4, 125.6, 116.7 ppm). Additionally, the ^{13}C NMR spectrum of **5a** showed resonances at δ 103.1 (CH), 74.1 (CH), 75.3 (CH), 71.9 (CH), and 63.8 (CH_2), which indicated the presence of a sugar moiety. Analysis of the COSY spectrum demonstrated the structures of four fragments as shown in Figure 3 as bold bonds. Detailed analysis of the HMBC spectrum allowed us to establish the connectivities of the four fragments and the locations of the *O*-methyl groups at C15, C17, C19, and C29. A 22-membered macrolactone ring of **5a** was identified based on an HMBC correlation between C1-O-C21 and supported by a deshielded chemical shift of an oxymethine proton at δ 5.23 (H21). Furthermore, the shielded oxymethylene carbon at δ 49.2 (C35) suggested the presence of an epoxide at C16, which was strongly supported by an unsaturation of **5a** and HMBC correlations between H35/C16 and H35/C17. The geometries of the two disubstituted olefins were determined to be 2*E* and 10*Z* based on their $^3J_{H\text{-}H}$ coupling constants, 15.8 Hz and 10.3 Hz, respectively. Additionally, the NOESY correlations between H2/H3, H3/H5, H6a/H33, and H6b/H33 led us to assign the geometry of the trisubstituted olefin at C4 as 4*E* (Figure 4), which was confirmed by the resonance in ^{13}C NMR at δ 12.7 (C33).[9] Overall, leptolyngbyolide A was found to be a glycosylated 22-membered macrolactone with an epoxide moiety within it.

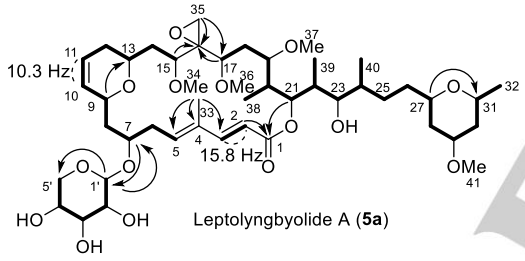

Figure 3. Planar structure of leptolyngbyolides (bold: COSY correlation, arrow: HMBC correlation (^{1}H to ^{13}C), dash: $^3J_{H\text{-}H}$ coupling constant of olefinic protons).

The structure of **5b**, **5c**, and **5d** was determined in a manner similar to the case of **5a**, which is described in Supporting Information. The high similarities of the ^{1}H and ^{13}C NMR spectra of **5d** with those of **5b** suggested that **5d** was an aglycon of **5b**; this assignment was confirmed by the analyses of COSY and HMBC spectra. To the best of our knowledge, most of the related swinholide-like macrolides have no sugar moiety except for ankaraholides including **2**. These structural features, together with the recent study on biosynthesis of this compound class,[7d] suggests that leptolyngbyolide C (**5c**), a methyl-branched analog, can be a biosynthetic precursor for leptolyngbyolides A (**5a**) and B (**5b**). Table S1 in Supporting Information summarizes the chemical shift data of ^{1}H and ^{13}C NMR for leptolyngbyolides A-D (**5a**-**5d**).

Relative stereochemistry of leptolyngbyolides: Due to the flexibility of the 22-membered macrocyclic ring and the highly overlapped ^{1}H NMR spectra of leptolyngbyolides, neither the NOESY experiments nor crystal structure analysis of leptolyngbyolides was successful in determining their relative structures. It is assumed that leptolyngbyolides have a relative stereochemistry identical to that of lobophorolide: the NOESY data for the present study did not contradict this assumption (Figure S1).

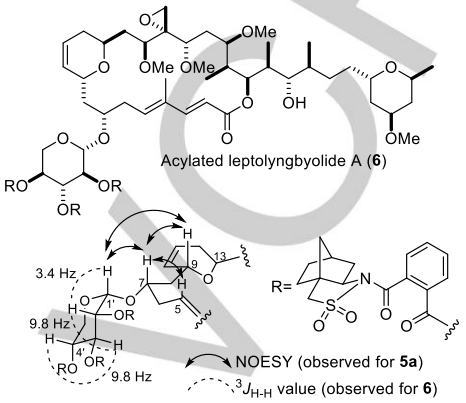

Figure 4. Stereochemistry of the sugar moiety of leptolyngbyolide A.

The NOESY spectrum of an acylated derivative **6**, which was originally prepared for the purpose of crystal structure analysis, enabled us to identify the sugar as a xylose (Figure 4). The relative stereochemistry of the xylose and aglycon was established by the NOESY correlations between H1'/H7 and H1'/H9. The stereochemistry of the THP ring system at the side chain moiety was examined by analyzing the ^{1}H NMR and NOESY spectra; the spectral data of leptolyngbyolide C (**5c**) were used due to the spectral complexity of **5a** in ^{1}H NMR. The NOESY correlation between H28b/H29, H29/H30b, and H30b/H31, together with the characteristic coupling constants between H29/H30a (12.6 Hz), H30a/H31 (10.3 Hz), and H30b/H31 (2.8 Hz), revealed the relative configuration of the THP ring shown in Figure 5.

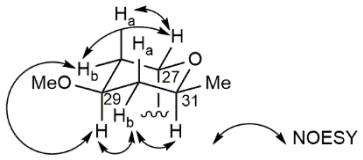

Figure 5. NOESY correlations observed for the THP moiety of leptolyngbyolide C.

Comparison of the ^{1}H and ^{13}C NMR spectral data of **5a** with those of **1**, **2**, and **4** strongly suggested that the side chain of **5a** had the same relative chemistry as that of the related swinholide-based compounds. As above, the spectral experiments gave us information on the relatively rigid and partial structures in leptolyngbyolides; however, their stereochemistries were not completely characterized before the total synthesis of **5c** was accomplished in this work (*vide infra*).

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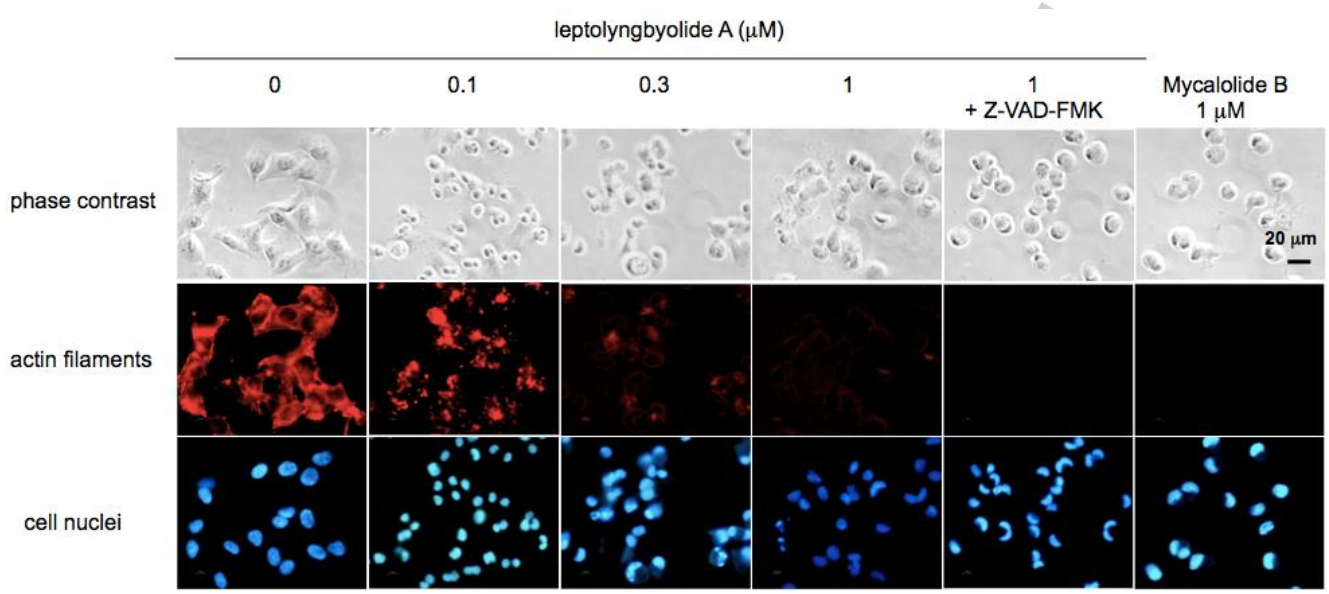

Figure 6. Effects of leptolyngbyolide A (**5a**) on F-actin of HeLa S_3 cells. HeLa S_3 cells were preincubated or not with 50 μM Z-VAD-FMK for 30 min, and then treated with **5a** or mycalolide B at various concentrations for 24 h. Cells were fixed and exposed to the microfilament-staining rhodamine-phalloidin (actin filaments are visualized as red) and to the DNA-reactive compound Hoechst33258 (cell nuclei are visualized as blue).

Biological activity of leptolyngbyolide: The related compounds, including **1**-**4**, exhibit potent growth-inhibitory activities against several cancer cell lines. The results of the MTT assay showed that **5a**-**5d** inhibited the cell growth of HeLa S_3 cells with IC_{50} values 0.099 to 0.64 μM (Table 1 and Figure S2). Comparison of the IC_{50} value of **5b** (0.16 μM) with that of **5d** (0.15 μM), which is an aglycon of **5b**, revealed that the xylose moiety is not essential for the growth-inhibitory activity of leptolyngbyolides. Furthermore, the results of the trypan blue dye exclusion assay and DNA ladder analyses showed that **5a**-**5d** induced apoptosis in HeLa S_3 cells (Figure S3).

Table 1. Growth inhibition against HeLaS_3 cells (IC_{50}) and depolymerization of F-actin (EC_{50}).

compound	growth-inhibitory activity (μM)	actin-depolymerizing activity (μM) [a]
5a	0.099	12.6
5b	0.16	11.6
5c	0.64	26.9
5d	0.15	21.5

Measurements were conducted in triplicate. Appendix Figures S2 and S7 present growth curves (for growth-inhibitory activities) and progress curves (for actin-depolymerizing activities), respectively.

[a] EC_{50} indicates the concentration required to depolymerize F-actin (3.0 μM) to 50% of its control amplitude.

Leptolyngbyolide A (**5a**) caused morphological changes in HeLa S_3 cells at 0.1 μM (Figures S4 and S5). As shown in Figure S4, the cell death induced by **5a** was completely blocked by Z-VAD-FMK, an irreversible and cell-permeable inhibitor of caspases. Caspases[10] are a group of death proteases that cause most of the visible morphological changes characteristic of apoptotic cell death.[11] The effect of **5a** on cellular morphology, however, was not blocked in the presence of Z-VAD-FMK. Thus, it was likely that the observed changes in cell morphology were not caused by apoptosis, but rather by the effect of **5a** on the cytoskeleton. In addition, previous studies reported that swinholide-based compounds, including **1**-**4,** targeted actin, a cytoskeleton protein that plays central roles in cell morphology, movement, and cytokinesis.[12] These actin-binding macrolides form a 1:1 or 2:1 of complex[12,13] with globular (G-) actin by binding to the barbed end of filamentous (F-) actin. Based on our observation and previous studies, we examined the effect of **5a**-**5d** *in vitro.* Fluorescent attenuation of the pyrenyl actin demonstrated that **5a** depolymerized F-actin over time in a concentration-dependent manner (Figure S6). Likewise, actin-depolymerizing activities of **5b**-**5d** were examined to evaluate the influences of the sugar moiety and substituent at C16 (Table 2 and Figure S7). Comparison of the IC_{50} value of **5b** (11.6 μM) with that of **5d** (12.6 μM) showed that the sugar moiety did not affect the actin-depolymerizing activity in the same way as the growth-inhibitory activity (Table 1). Among the four leptolyngbyolides, **5c** showed the weakest actin-depolymerizing activity with an IC_{50} value of 26.9 μM; interestingly, its growth-inhibitory potency was 5-fold weaker than that of the other analogs (Table 1). A previous study on synthetic analogs of aplyronine A, a potent actin-depolymerizing macrolide, indicated that there were strong correlations between actin-depolymerizing activity and growth-inhibitory activity.[14] Taken together, it is plausible that leptolyngbyolides **5a**-**5d** exhibited growth-inhibitory activities by targeting F-actin in HeLa S_3 cells. The effect of **5a** on F-actin in HeLa S_3 cells was then analyzed by fluorescence

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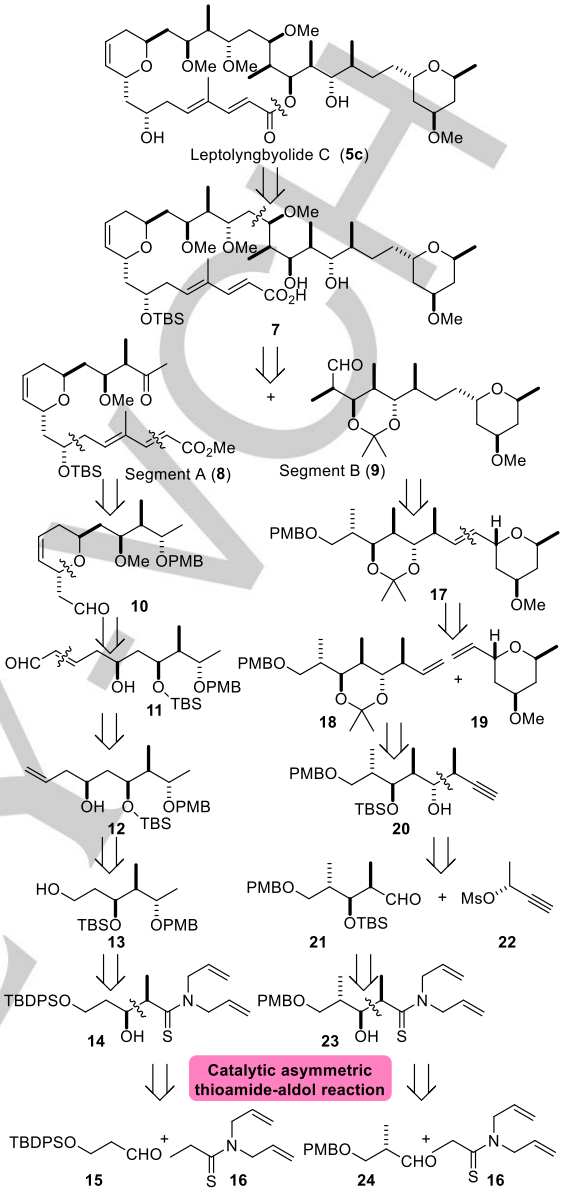
microscopy. As shown in Figure 6, **5a** led to the disruption and aggregation of F-actin in HeLa S_3 cells in a concentration-dependent manner. The F-actin disruption caused by **5a** was not suppressed by the presence of Z-VAD-FMK, which blocks apoptosis. This result suggests the probable order of action of leptolyngbyolides. Leptolyngbyolides are likely to target F-actin, disrupt the cytoskeleton in HeLa S_3 cells, and then induce apoptotic cell death.

Catalytic asymmetric total synthesis of leptolyngbyolide C: To evaluate the predicted stereochemistry, we performed a catalytic asymmetric total synthesis of leptolyngbyolide C. It should be also noted that macrocyclic compounds currently draw considerable attention of the medicinal chemistry community,[15] and so much efforts are being made for the synthetic studies on the molecules of this class.[16] Among the related natural products, swinholide A and its monomeric precursor preswinholide A have been extensively studied toward the total synthesis; monumental achievements by Paterson[17] and Nicolaou[18] for swinholide A as well as by Nakata for preswinholide A were disclosed in the 1990s.[19] Very recently, Krische and co-workers reported the concise total synthesis of swinholide A in almost half the steps of the precedents.[20] In the present total synthesis, a distinct strategy was used to install the appropriate stereochemistry at the initial stage of the fragment-synthesis; the catalytic asymmetric direct aldol reaction effected by Cu(I)-chiral bidentate phosphine ligand complex using thioamide as the prenucleophile developed by us was the key transformation.[21] In this process, a copper complex chemoselectively activates the thioamide by soft-soft interaction to facilitate deprotonation at the α-position in the presence of the more acidic enolizable aldehyde. The whole process of the reaction accompanies only the transfer of the α-proton to the hydroxy group of the aldol adduct wherein perfect atom economy is achieved; preactivation of the prenucleophile by derivatization, such as transformation into silylenolether, is not necessary to avoid emission of waste. The resultant thioamide moiety of the aldol products is a versatile functionality from which diverse substructures, including aldehydes, methylketones, primary alcohols, carboxylic acids, β-ketoester, amines, and amides, are accessible in a single operation.[21b] This catalytic asymmetric process was incorporated into the reaction schemes toward the total synthesis of biologically relevant compounds such as thuggacin B,[22] membrenones,[23] caprazamycin B,[24] and atorvastatin.[21c,d] Moreover, iterated use of this reaction facilitates the construction of the polypropionate backbone of natural products; propionate-derived thioamide gave the corresponding aldol adducts in good to excellent enantio- (up to > 99% ee) and *syn*-selectivity (up to > 20:1), after which the thioamide substructure was converted to a formyl group to serve in the subsequent thioamide-aldol reaction.



Scheme 1. Retrosynthesis of leptolyngbyolide C.

Scheme 1 illustrates our retrosynthetic plan: the macrocyclic core should be cyclized at the final stage of the synthesis, expecting regioselectivity between two unprotected hydroxy groups at C21 and C23 of **7**, which is preceded by the assembly of two segments (segment A, **8** and segment B, **9**) by aldol chemistry. The synthetic route to segment A (**8**) is similar to the scheme developed in the course of our formal total synthesis of scytophycin C.[25] The methyl ketone should be furnished by the oxidation of the corresponding secondary alcohol, and the diene part of this intermediate could be installed by the Horner-Wadsworth-Emmons reaction and vinylogous Mukaiyama aldol reaction from the aldehyde with the DHP ring system **10**. The cyclic ether structure should be formed via allylative cyclization[26] of δ-hydroxy enal **11**, which was expected to be prepared from cross metathesis with homoallylic alcohol **12**, a product of Krische allylation.[27] The

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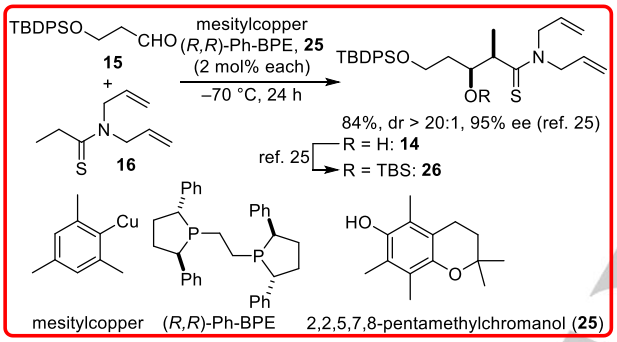
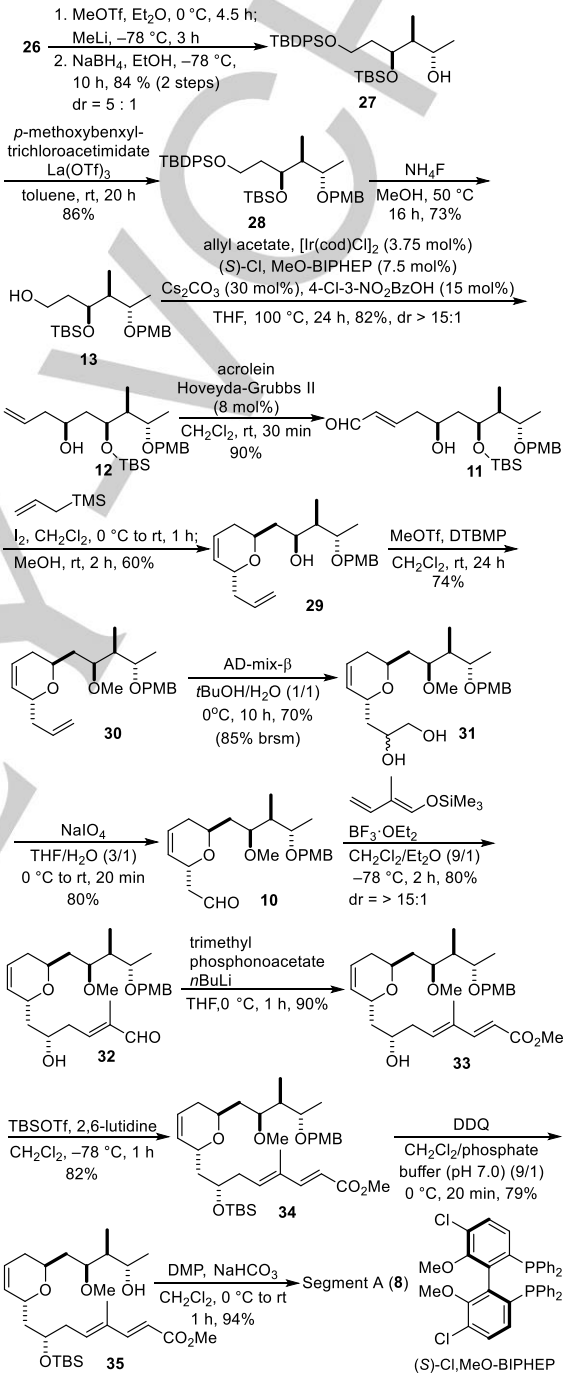
primary alcohol **13** should be readily obtained by sequential manipulation of the functional group from the product (**14**) of the initial catalytic asymmetric thioamide-aldol reaction of **15** and **16**, which we already optimized in our synthetic studies of thuggacin B[22] and scytophycin C.[25] Segment B (**9**) should be readily available by the hydrogenation of **17,** which can be prepared by cross metathesis between the two olefins **18** and **19**; the latter is reported in the literature.[28] The terminal double bond was furnished by partial reduction of the alkyne **20**, a product of the diastereoselective addition of allenylmetal species to aldehyde (**21**) generated from enantioenriched propargylic mesylate (**22**) catalyzed by Pd(0).[29] The aldehyde, **21**, was easily transformed from thioamide-aldol adduct **23** from α-chiral aldehyde (**24**) and propionate-derived thioamide, **16**.



Scheme 2. Catalytic asymmetric thioamide-aldol reaction.

The synthetic route of segment A was elaborated from the endeavor toward our formal total synthesis of the related marine natural product scytophycin C,[25] which also provided indispensable information during the synthesis of the substances of this class. The synthesis commenced with thioamide **26** in Scheme 3, which was prepared by silylation of **14**. It has already been reported that **14** could be obtained with excellent diastereo- (*syn*/*anti* = >20/1) and enantioselectivity (95% ee) by the catalytic asymmetric thioamide-aldol reaction developed in this laboratory using a second-generation catalyst system comprising a Cu(I)-bidentate chiral phosphine complex prepared from mesitylcopper and (*R,R*)-Ph-BPE, and 2,2,5,7,8-pentamethylchromanol (**25**) as an additive, as demonstrated in Scheme 2.

The thioamide moiety of **26** was readily transformed into methylketone in a one-pot protocol by way of an iminothioether intermediate, which accepts MeLi, followed by the hydrolysis upon work up. The resultant carbonyl group of the methyl ketone was converted to secondary alcohol **27** in 84% yield over 2 steps, followed by protection as PMB ether (**28**) under Lewis acidic conditions (86% yield). The reduction proceeded with reasonable diastereoselectivity (5:1) to a predominant Felkin-Anh product. In fact, the stereochemistry should not be meaningful as it is to be lost at the later stage. In reality, the major isomer was isolated for use during further synthetic study to simplify the characterization of the studies of the subsequent steps. Then, selective deprotection of the primary silyl ether was successfully achieved in 73% yield using NH_4F under gentle warming to lead to the substrate of the following Krische allylation (**13**).[26]



Scheme 3. Synthesis of the segment A.

A previous study on scytophycin C[25] revealed that Ir catalyst ligated with (*S*)-SEGPHOS and 4-cyano-3-nitrobenzoate (**S3**, Figure S8), which is commercially available, was not the correct choice for our case: the starting material **S2** (Figure S8)

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was mostly unchanged with 5 mol% of catalyst at 100 °C after either 24 h or 36 h to reach to a conversion as low as 16%-20%. Gratifyingly, the alternative system using Ir(III) and a chiral Cl, MeO-BIPHEP as the bidentate phosphine ligand, and the benzoic acid derivative with finely tuned substituents as depicted in Scheme 3 remarkably enhanced the reaction rate. Although 5 mol % of catalyst loading based on Ir produced an unsatisfactory yield of allylated product even after 48 h, 7.5 mol% was revealed to be enough to attain a 74% yield, even after a 24-h reaction. The optimized condition was applied to the present study using **13** as the substrate to give the desired hydrogenative allylated product **12** in good yield (82%) as well as excellent diastereoselectivity (>15:1). The succeeding cross metathesis with acrolein under 8 mol% of Hoveyda-Grubbs 2nd generation catalyst afforded the expected *trans*-enal **11** in 90% yield.

Next, we performed allylative cyclization following the procedure of Yadav and co-workers.[26,30] The cyclization condition employing $InBr_3$ suffered from undesired transformations such as trimerization and dimerization when 1,3-diol was used as the substrate (Figure S9). Changing the catalyst to $ZnBr_2$ and I_2, which are reported to be reliable alternatives,[31] had no beneficial effect. Even with a catalytic amount of $ZnBr_2$, decomposition followed after full consumption of the substrate. For these reasons, the present synthetic protocol requires TBS-protection at one of the secondary alcohols of **11**. In fact, the allylative cyclization easily proceeded with a catalytic amount of I_2 (25 mol%) and 3 equiv of allyltrimethylsilane in CH_2Cl_2 to afford **29** in a reasonable yield (60%) after pouring MeOH onto the reaction mixture and stirring for an additional 3 h, which favorably promoted the removal of the TBS group by HI that was generated during the course of the catalytic cycle. Use of CH_2Cl_2 in the first cyclization process was indispensable; the originally reported THF ended up with an unsatisfactory yield of less than 40%. Then, the unveiled hydroxy group was methylated to give **30** in 74% yield under standard conditions, which was followed by oxidative cleavage of the terminal olefin. A previous report on the synthesis of scytophycin C by Miyashita's group,[32] utilized the stepwise operation of osmium dihydroxylation and glycol cleavage mediated by $NaIO_4$. For the present synthesis, this condition afforded 57% of the diol with concomitant over-oxidation product, tetraol. OsO_4 reagent supported on PEM-polystyrene[33] slowed the reaction rate although the regioselectivity improved. Gratifyingly, AD-mix-β was found to be the reagent of choice to convert **30** to **31** in 70% yield with perfect regioselection, which preceded the oxidative cleavage of the diol to give aldehyde **10** (80%). Then, another key transformation in the synthesis of segment A, a vinylogous Mukaiyama aldol reaction between **10** and a silyldienol ether as used in Paterson's and Miyashita's syntheses[17c,32] smoothly (80%) afforded the corresponding adduct **32** together with no trace amounts of the undesired diastereomer (dr >15:1). Interestingly, the remote methyl group at the tip of the substrate highly influenced the diastereoselectivity; the lack of the methyl group substantially reduced the diastereomeric ratio to 9.3:1 (Scheme S1).

The subsequent Horner-Wadsworth-Emmons reaction proceeded uneventfully to add another olefin moiety with *E*-geometry exclusively (**33** in 90% yield). Finally, protecting group manipulations (silylation to give **33** in 82%, and removal of the PMB group to afford **35** in 79%) and Dess-Martin oxidation of the resulting secondary alcohol to the methylketone completed the synthesis of segment A, **8**, in 94%.

Scheme 4. Asymmetric thioamide-aldol reaction for the synthesis of segment B.

The synthesis of segment B was set out again with the asymmetric thioamide-aldol reaction demonstrated in Scheme 4. During the previous synthetic study to membrenones,[23] α-methylaldehydes were confirmed to be good substrates for diastereoselective thioamide-aldol reaction, which is also the case for **24**, an appropriate starting material for the present purpose to afford 10 times the desired diastereomer in amounts comparable to that of the minor undesired product.

The thioamide-aldol adduct **23** was protected as a TBS ether **36** (86%), which was reduced to the corresponding aldehyde **21** by treatment with $LiAlH(OtBu)_3$ to intermediary iminothioether in 76% yield (Scheme 5). For the first trial, the aldehyde was further reduced to an alcohol to which Krische allylation was applied.[27c] Even after thorough optimization of the reaction conditions, the desired product was obtained in no more than 15% yield, which led us to switch our strategy to diastereoselective allylation of aldehyde.

In the beginning, Roush crotylation was tested, but the undesired diastereoisomer predominated.[34] Disappointingly, the change in the protecting groups of the substrate never switched the selectivity, which led us to investigate alternative Roush's condition using $(Ipc)_2BH$ and allenylstannane reagent[35] to give the desired diastereomer as the major adduct although the selectivity was moderate (3.7:1). Unfortunately, the tedious purification procedure hampered its use for further reaction sequences. Finally, we found that the Marshall-Tamaru reaction[28] employing enantio-enriched propargylic mesylate **22** as the allenylindium source worked well; the nucleophilic species could be generated via an allenylpalladium intermediate. In the present case for aldehyde **21**, the homopropargylic alcohol product **20** was accessible in 75% yield with reasonable selectivity (5:1). Then, partial hydrogenation with poisoned palladium catalyst gave rise to the terminal olefin **37** (78%). Precision of the configuration was verified by analysis of the coupling constant of ^1H NMR of **S10** (Scheme S2), which was readily derived from **37** in a 3-step procedure.

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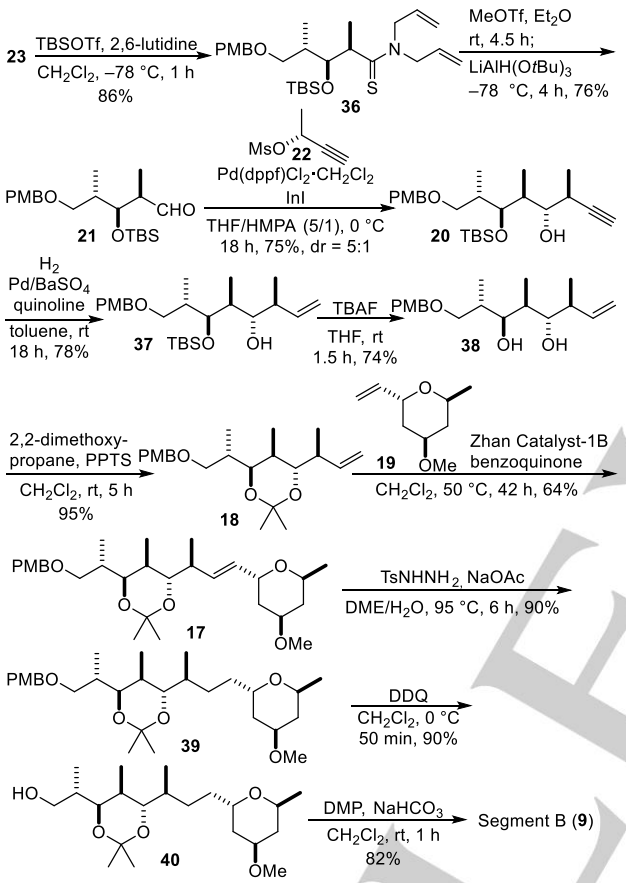
From here on, interconversion of the protective group to dimethylacetal (desilylation, **38** in 74%, acetalization in 95%) afforded the substrate of cross metathesis **18** to install the terminal THP part. Subsequently, metathesis effected by Zhan catalyst-1B with **19** as the counterpart olefin prepared according to the reported protocol[28] was carried out to construct the whole framework of segment B (**17** in 64% yield). A successive diimide reduction to afford **39** (90%) was followed by unmasking the PMB ether (**40** in 90%) and oxidation of the primary alcohol to the corresponding aldehyde achieved the asymmetric synthesis of segment B (**9**, 82%).



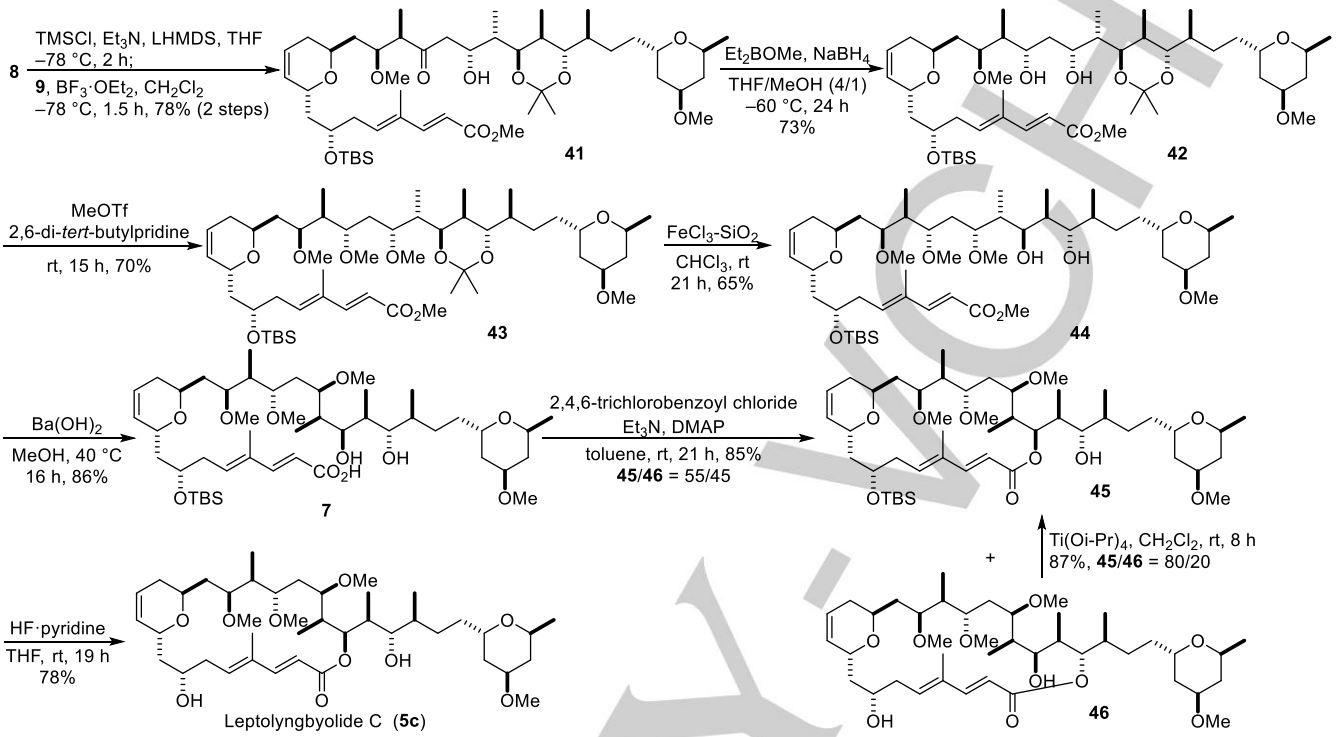
Scheme 5. Synthesis of segment B.

A key reaction in the endgame of the synthesis should be a coupling of the two segments by the aldol-approach; the Mukaiyama aldol reaction after the conversion of segment A to the silylenol ether, which was subjected to Lewis acidic conditions effected by $BF_3{\cdot}OEt_2$ to give the product **41** as a single isomer in 78% yield (Scheme 6). Then, the Narasaka-Prasad reduction,[36] a *syn*-selective protocol using β-hydroxyketone as the substrate, was successfully adopted to lead to the *syn*-diol **42** in 73% yield. The stereochemistry around this polyol portion was confirmed by analysis of the ^{13}C NMR chemical shift value of the corresponding bis-dimethylacetal derivative **S11** (Scheme S2).[37]

The diol system was methylated to give **43** (70%), which proceeded to the subsequent deacetalyzation (**44**, 65%) and hydrolysis of the methylester moiety to afford the substrate of macrolactonization (**7**, 86%). Then, the Yamaguchi protocol was applicable to this reaction (0.005 M) to result in the mixture of 22- and 24-membered isomers in good combined yield (85%);[38] 22-membered lactone slightly predominated (**45**:**46** = 55:45). Exposure of the undesired isomer **46** to Lewis acidic media ($Ti(O\textit{i}\text{-}Pr)_4$)[38,39] equilibrate **46** to **45** up to 80:20 (= **45**:**46**). Then, the final removal of the TBS group of **45** processed uneventfully (78%) to accomplish the catalytic asymmetric total synthesis of leptolyngbyolide C (**5c**). All the physicochemical data of the synthetic sample were identical to those of the naturally occurring compound to determine the absolute configuration of leptolyngbyolide C as shown in Figure 2.

Conclusions

Cyanobacteria are a reliable source of natural biological products. Here, we report the isolation of four cytotoxic macrolactones, leptolyngbyolides, that exhibit cytotoxicity against cancer cell lines and actin-depolymerizing activity. Detailed biological studies clarified that leptolyngbyolides depolymerize filamentous actin (F-actin) both *in vitro* and in cells, and suggested the probable order of F-actin depolymerization and apoptosis caused by leptolyngbyolides. Extensive NMR studies together with HRMS data revealed the planar structure of leptolyngbyolides. Characteristic coupling constants and NOESY experiments deciphered the relative stereochemistry of these natural products, and the catalytic asymmetric total synthesis of leptolyngbyolide C completely elucidated the absolute configuration of this molecule. The synthesis took advantage of the catalytic asymmetric thioamide-aldol reaction developed by our group using copper (I) complexed with chiral bidentate phosphine ligand; the catalyst selectively activates the thioamide substrate by soft-soft interaction to facilitate deprotonation of the α-proton, even in the presence of an aldehyde substrate with higher acidic protons. The whole process proceeds only with the migration of protons: perfect atom economy is fulfilled. We have already incorporated this method into several schemes of the total synthesis of biologically active compounds, including natural products, demonstrating the practicality of this reaction to produce chemical entities with complex structures. The present synthesis expands the repertoire of products produced by this reaction. Structure-activity relationship studies of substances that interfere with the behavior of actins as well as further synthetic endeavors toward the other three congeners, leptolyngbyolides A, B, and D, are underway, and will be reported in due course.



Scheme 6. Endgame of the catalytic asymmetric total synthesis of leptolyngbyolide C (**5c**).

Experimental Section

General: For isolation, purification, and structure determination of natural leptolyngbyolides, chemicals, and solvents except for the CH_2Cl_2 used for reactions, which was distilled over P_2O_5, were the best grade available and were used as received from commercial sources. Optical rotation was measured with a JASCO DIP-1000 polarimeter. CD spectra were measured with a JASCO J-720 W spectropolarimeter. ^{1}H NMR spectra were recorded on a JEOL JNM-ECA800 (800 MHz) or a JEOL JNM-ECX400 (400 MHz) instrument. Chemical shifts are reported as δ values in parts per million relative to the solvent signal (CHD_3OD: δ = 3.31 ppm, C_6HD_5: δ = 7.16 ppm for ^{1}H) and coupling constants are reported in hertz (Hz). The following abbreviations are used for spin multiplicity: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, and brd = broad. ^{13}C NMR spectra were recorded on a JEOL JNM-ECA800 (200 MHz), or JEOL JNM-ECA400 (100 MHz) instrument. Chemical shifts are reported as δ values in parts per million relative to the residual solvent signal (CD_3OD: δ = 49.0 ppm, C_6D_6: δ = 128.06 ppm for ^{13}C). Assignments of the ^{1}H NMR and ^{13}C NMR spectra were determined by H-H COSY, HMQC, HMBC, and DEPT experiments. ESI mass spectra were recorded on an LTC premier EX spectrometer (Waters). Both TLC analysis and preparative TLC were conducted on E. Merck precoated silica gel 60 F254. Nacalai Tesque ODS silica gel 75C_{18}-OPN was used for column chromatography.

Extraction and isolation of leptolyngbyolides: Approximately 2.1 kg (wet weight) of cyanobacterium *Leptolyngbya* sp. was extracted with MeOH (5 L) over 2 months. The extract was filtered, the filtrate was concentrated, and the resultant residue was partitioned between AcOEt (3 × 3 L) and H_2O (0.3 L). The material obtained from the organic layers was partitioned between 90% MeOH (3 × 3 L) and *n*-hexane. The aqueous MeOH fraction was partially purified by column chromatography on ODS using 40% MeOH, 60% MeOH, 80% MeOH, absolute MeOH, and $CHCl_3$/MeOH (1:1), successively. The fractions eluted with 80% MeOH were subjected to HPLC (Cosmosil Cholester (ϕ 20 × 250 mm); flow rate = 5 mL/min; detection at UV 269 nm; eluent 70% MeOH) to give leptolyngbyolide A (**5a**, 183 mg, t_R = 70.1 min), leptolyngbyolide B (**5b**, 34.5 mg, t_R = 60.7 min), and leptolyngbyolide D (**5d**, 2.4 mg, t_R = 83.8 min). Likewise, the fractions eluted with absolute MeOH were subjected to HPLC (Cosmosil 5C_{18}-MS-II (ϕ 20 × 250 mm); flow rate = 5 mL/min; detection at UV 269 nm; eluent 75% CH_3CN) to give leptolyngbyolide C (**5c**, 12.1 mg, t_R = 34.0 min).

Cell growth analysis: HeLa S_3 cells were cultured at 37 °C with 5% CO_2 in minimal essential medium (Nissui, Tokyo, Japan) supplemented with 10% heat-inactivated fetal bovine serum, 100 units/mL penicillin, 100 μg/mL streptomycin, 0.25 μg/mL amphotericin, 300 μg/mL L-glutamine, and 2.25 mg/mL $NaHCO_3$. HeLa S_3 cells were seeded at 4 × 10^3 cells/well in 96-well plates (Iwaki, Tokyo, Japan) and cultured overnight. Then, various concentrations of test compounds were added, and after incubation for 72 h, cell proliferation was measured with an MTT assay.

Actin-depolymerizing activity: The actin-depolymerizing activity of leptolyngbyolides were measured based on their ability to attenuate the fluorescence of pyrene-labeled (pyrenyl) actin, as previously described.[40] To the 3.0 μM solution of actin [2.7 μM of actin (Cytoskeleton, AKL95), 0.3 μM of pyrenyl-actin (Cytoskeleton, AP05)] in G-buffer [2 mM Tris-HCl (pH 8.0), 0.2 mM $CaCl_2$, 0.5 mM 2-mercaptoethanol, 0.2 mM ATP] was added a 0.15 M solution of $MgCl_2$ (1 mM of total). The mixture was then stirred at

rt for 1 h to polymerize the G-actin to F-actin. To a solution of F-actin was added various concentrations of test compounds with stirring. The time course of the depolymerization was continuously monitored by measuring the fluorescence of pyrenyl-actin with a JASCO CAF-110 intracellular ion analyzer at 25 °C at 365 nm excitation and 405 nm emission wavelengths. The IC_{50} values were the concentrations required to depolymerize F-actin to 50% of its control amplitude.

Fluorescence imaging: HeLa S_3 cells were treated with leptolyngbyolide A and a control compound, mycalolide B, for 24 h, then washed with PBS, and fixed with 4% formaldehyde solution at rt for 10 min. The cells were washed with PBS, then permeabilized with 0.1% Triton X-100 in PBS for 10 min. The cells were washed again with PBS and stained with 165 nM rhodamine-phalloidin at rt for 30 min. The cells were washed with PBS followed by treatment with 2.0 μg/mL of Hoechst 33258 in PBS at rt for 10 min. After washing with PBS, coverslips were mounted with aqueous glycerol (1:1), covered with glass coverslips, and sealed with nail polish. Fluorescence images were captured with an Eclipse E600 microscope. The cells were photographed through a UV filter at a magnification of × 400.

Catalytic asymmetric total synthesis of leptolyngbyolide C: See details in Supporting Information.

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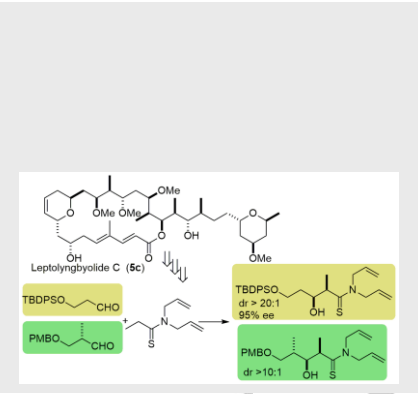

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