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Structure Elucidation |*Hot Paper*|

Total Synthesis and Complete Stereostructure of a Marine Macrolide Glycoside, (−)-Lyngbyaloside B

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Abstract: We have described in detail the total synthesis of both the proposed and correct structures of (−)-lyngbyaloside B, which facilitated the elucidation of the complete stereostructure of this natural product. Our study began with the total synthesis of 13-demethyllyngbyaloside B, in which an esterification/ring-closing metathesis (RCM) strategy was successfully used for the efficient construction of the macrocycle. We also established reliable methods for the introduction of the conjugated diene side chain and the L-rhamnose residue onto the macrocyclic framework. However, the esterification/RCM strategy proved ineffective for the parent natural product because of the difficulties in acylating the sterically encumbered C-13 tertiary alcohol; macrolactionization of a seco-acid was also extensively investigated under various conditions without success. We finally completed the total synthesis of the proposed structure of (−)-lyngbyaloside B by means of a macrolactonization that involves an acyl ketene as the reactive species. However, the NMR spectroscopic data of our synthetic material did not match those of the authentic material, which indicated that the proposed structure must be re-examined. Inspection of the NMR spectroscopic data of the natural product and molecular mechanics calculations led us to postulate that the configuration of the C-10, C-11, and C-13 stereogenic centers had been incorrectly assigned in the proposed structure. Finally, our revised structure of (−)-lyngbyaloside B was unambiguously verified through total synthesis.

Introduction

Marine natural products continue to attract the interest of the chemical and biological communities because they provide unique opportunities to develop therapeutics for intractable diseases as well as chemical probes for deciphering important biological questions.[1] Macrolide glycosides constitute a growing family of marine natural products with moderate to potent cytotoxic activity against human cancer cell lines. As exemplified by aurisides,[2] callipeltosides,[3] and dolastatin 19 (Figure 1),[4] these natural products were initially discovered from marine invertebrates, such as sea hares and sponges, but it is now speculated that these macrolide glycosides are actually the secondary metabolites of symbiotic microorganisms.

In 2002, Moore and co-workers described the isolation of (−)-lyngbyaloside B (proposed structure **1**) from the Palauan cyanobacterium *Lyngbya* sp., which closely resembled *Lyngbya bouillonii*.[5] The gross structure was determined on the basis of extensive 2D NMR analyses, and the relative configuration was assigned on the basis of conformational analyses by using *J* values and ROESY correlations. The absolute configuration could not be determined because of the limited availability of the natural product. Moore et al. reported that lyngbyaloside B displayed moderate cytotoxic activity against human oral epidermoid carcinoma KB cells ($IC_{50}=4.3$ μM) and considerably weaker effects on human colon adenocarcinoma LoVo cells ($IC_{50}\approx 15$ μM). Almost simultaneously, Gerwick et al. identified a moderately cytotoxic macrolide glycoside, (−)-lyngbouilloside (**2**), from the marine cyanobacterium *L. bouillonii*, which was collected off the north coast of Papua New Guinea.[6] Recently, Luesch and co-workers reported the isolation and structure characterization of (−)-18*E*-lyngbyaloside C (**3**) and (−)-18*Z*-lyngbyaloside C (**4**), the former of which showed moderate cytotoxic activity against human cervical adenocarcinoma HeLa cells.[7]

Considerable interest has been aroused in the total synthesis of these macrolide glycosides of cyanobacteria origin because of their structural complexity and cytotoxic activity.[8] Hoye and co-workers reported the synthesis of a model compound of lyngbyaloside B (**1**) through a macrocyclization that involved an acyl ketene as the reactive species (hereafter referred to as "acyl ketene macrocyclization").[8a,9] The Ley group described the synthesis of a protected aglycon of the proposed (−)-lyngbouilloside (**2**) by using an anion coupling and a ring-closing metathesis (RCM)[10] for the construction of the macrocycle.[8c] Importantly, Ley et al. noticed that the ^{1}H NMR spectrum of their synthetic material showed significant line broadening, whereas that of the authentic sample displayed a single set of sharp signals. Later, Cossy and co-workers successfully synthesized the aglycon of the proposed (−)-lyngbouilloside (**2**) by

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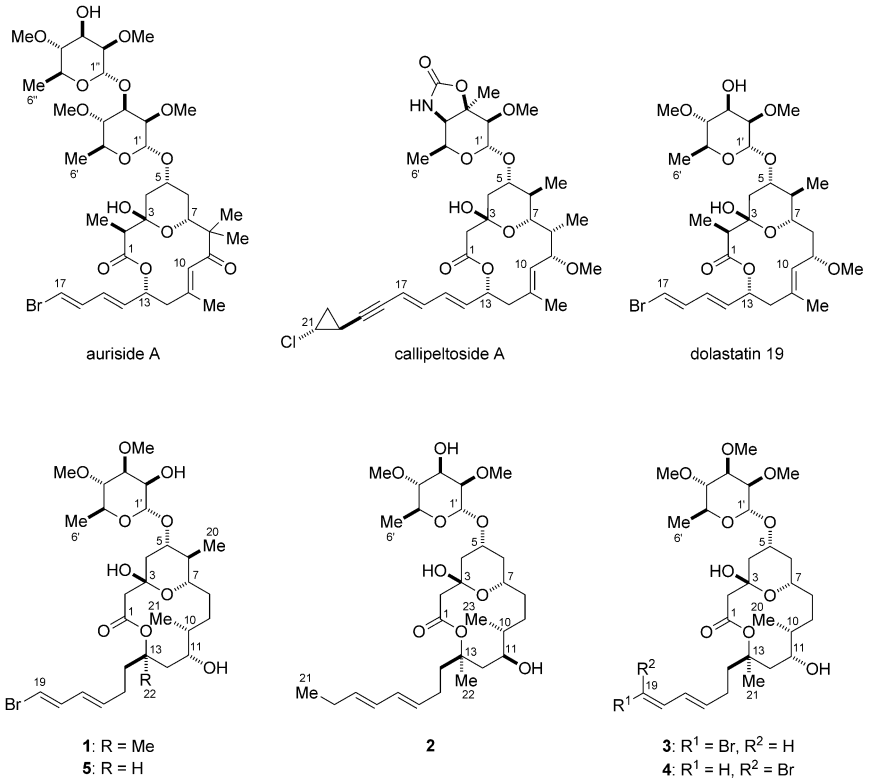


Figure 1. Structures of auriside A, callipeltoside A, and dolastatin 19, and the proposed structures of lyngbyaloside B (**1**), lyngbouilloside (**2**), 18*E*- and 18*Z*-lyngbyaloside C (**3** and **4**, respectively). 13-Demethyllyngbyaloside B (**5**) is a non-natural analogue of **1**.

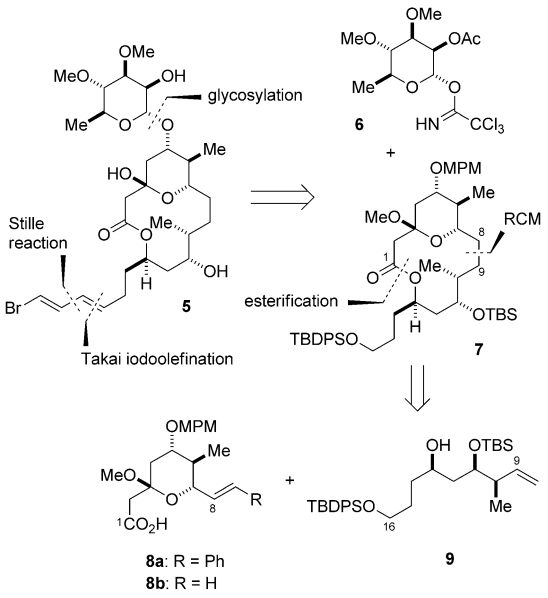
using an olefin cross-metathesis reaction[11] and an acyl ketene macrocyclization as the key transformations.[8d] The Cossy group reported that the NMR spectroscopic data of their synthetic aglycon were also not in agreement with those of the corresponding domain of natural lyngbouilloside. These studies highlighted the possibility that the proposed structure of lyngbouilloside (**2**) might have been erroneously determined; Cossy et al. speculated that the configuration of the C-11 stereogenic center of structure **2**, which is opposite to that of the proposed structure **1**, might have been incorrectly assigned. In any case, it is clear that total synthesis of these macrolide glycosides is required for the determination of their complete stereostructures. Herein, we describe in detail the first total synthesis of both the proposed and correct structures of (−)-lyngbyaloside B, which established the complete stereostructure of this natural product in an unambiguous manner.[12]

Results and Discussion

Total synthesis of (−)-13-demethyllyngbyaloside B

The initial target of this study was 13-demethyllyngbyaloside B (Figure 1, **5**) because this non-natural analogue represented a suitable target for preliminary investigations.[13] It was also anticipated that compound **5** would be useful for evaluating the impact of the C-13 methyl group on the solution-state conformation and biological activity of this compound.

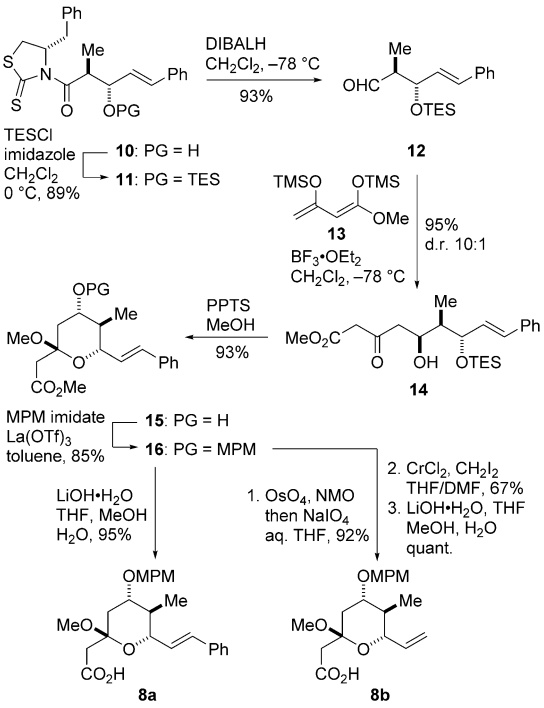
Our synthetic plan towards target **5** is summarized in Scheme 1. We envisaged that the 3,4-di-*O*-methyl-L-rhamnopyranoside moiety could be introduced to the aglycon at a late stage of the total synthesis by means of stereoselective glycosylation by using trichloroacetimidate **6** under Schmidt



Scheme 1. Synthetic plan towards **5**. MPM = *p*-methoxyphenylmethyl, RCM = ring-closing metathesis, TBDPS = *tert*-butyldiphenylsilyl, TBS = *tert*-butyldimethylsilyl.

conditions.[14] Construction of the conjugated diene side chain would be achieved through sequential Takai olefination[15] and Stille reaction.[16] These considerations led us to identify the macrolactone **7** as a precursor of target compound **5**. Based on our previous work on related macrolide natural products,[17] we envisioned that macrocycle **7** would be synthesized from the carboxylic acid **8a** or **8b** and the alcohol **9** by an esterification/RCM sequence.

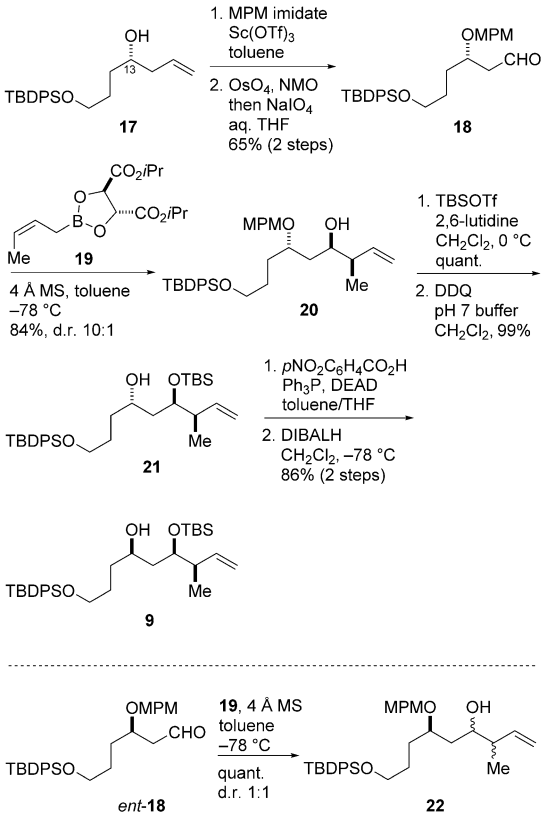
The synthesis of the carboxylic acids **8a**,**b** started with the silylation of the known alcohol **10**[18] to give the silyl ether **11** in 89% yield (Scheme 2). DIBALH reduction of the silyl ether **11** cleanly removed the chiral auxiliary to deliver the aldehyde **12** (93% yield), which was reacted with the dienol silyl ether **13**[19]



Scheme 2. Synthesis of carboxylic acids **8a** and **8b**. DIBALH = diisobutylaluminum hydride, NMO = *N*-methylmorpholine *N*-oxide, OTf = trifluoromethanesulfonate, PPTS = pyridinium *p*-toluenesulfonate, TES = triethylsilyl.

under Mukaiyama conditions (BF_3·OEt_2, CH_2Cl_2, −78 °C)[20,21] to afford the alcohol **14** in 95% yield (d.r. 10:1). The high diastereoselectivity that was observed for the vinylogous Mukaiyama aldol reaction could be ascribed to 1,2- and 1,3-asymmetric inductions that were brought about by the α-methyl and β-silyloxy groups.[22] Exposure of the alcohol **14** to PPTS in methanol resulted in the loss of the silyl group and concomitant methyl acetalization to provide the alcohol **15** in 93% yield. The relative configuration of the alcohol **15** was established on the basis of *J* values and an NOE enhancement.[23] Protection of the alcohol **15** by using MPMOC(=NH)CCl_3 (MPM imidate) and $La(OTf)_3$[24] led to the MPM ether **16** in 85% yield; subsequent hydrolysis under alkaline conditions furnished the carboxylic acid **8a** in 95% yield. Meanwhile, we also prepared the carboxylic acid **8b** from **16** by oxidative cleavage of the styryl group, methylenation of the resultant aldehyde under Takai conditions ($CrCl_2$, CH_2I_2, THF/DMF),[25] and hydrolysis of the methyl ester moiety.

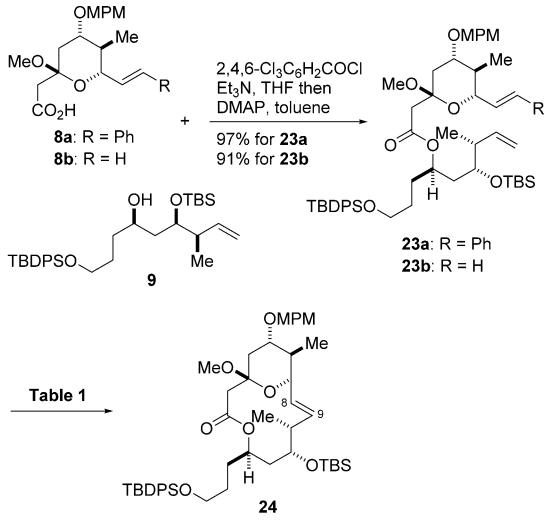
The synthesis of the alcohol **9** began with known homoallylic alcohol **17** (Scheme 3),[26] which was prepared in three steps from 1,4-butanediol. Protection of the alcohol **17** as its MPM ether followed by cleavage of the double bond gave the aldehyde **18** (65% yield over the two steps). Roush crotylation[27] of



Scheme 3. Synthesis of alcohol **9**. DDQ = 2,3-dichloro-5,6-dicyano-1,4-benzoquinone, DEAD = diethyl azodicarboxylate, MS = molecular sieves.

the aldehyde **18** by using chiral crotylboronate reagent **19** (4 Å MS, toluene, −78 °C) provided the alcohol **20** in 84% yield (d.r. 10:1); its absolute configuration was established by NMR analyses of suitable acetonide derivatives.[23] Silylation of the alcohol **20** (quant. yield) followed by removal of the MPM group (99% yield) led to the alcohol **21**. Finally, Mitsunobu inversion[28] of the alcohol **21** under standard conditions ($pNO_2C_6H_4CO_2H$, DEAD, Ph_3P) and DIBALH reduction of the resultant *p*-nitrobenzoate furnished the alcohol **9** (86% yield over two steps). Here, we note that we intentionally selected the alcohol **17** with "incorrect" configuration at the C-13 stereogenic center as the starting material. This was because Roush crotylation of aldehyde *ent*-**18** with crotylboronate **19** provided the corresponding crotylated product **22** as a 1:1 mixture of diastereoisomers, which suggests that these reactants are "mismatched".

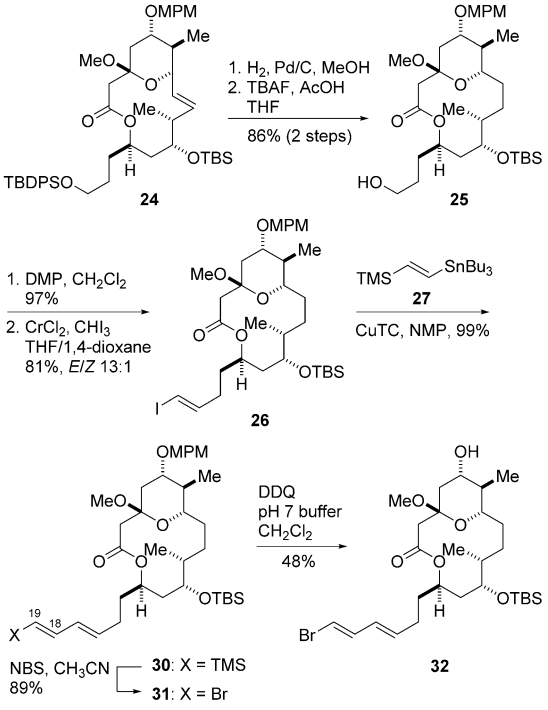
With the requisite fragments in hand, we proceeded to the construction of the macrocyclic backbone of compound **5**



Scheme 4. Synthesis of macrocycle **24**. DMAP = *N*,*N*-dimethylaminopyridine.

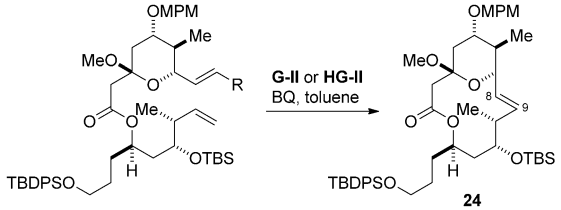
(Scheme 4). Esterification of the carboxylic acids **8a** and **8b** with the alcohol **9** under Yamaguchi conditions[29] afforded the dienes **23a** and **23b**, respectively. Our initial RCM experiments of **23a** by using the second-generation Grubbs (**G-II**)[30] or Hoveyda–Grubbs (**HG-II**)[31] catalyst turned out to be unproductive (Table 1, entries 1–3). These results could be ascribed to the low reactivity of the styryl group in olefin metathesis reactions;[17a,b] therefore, RCM of diene **23b** was next investigated under a series of reaction conditions (entries 4–6). By elevating the reaction temperature from 100 °C to 140 °C (bath temperature), we were able to improve the yield of the desired macrocycle **24** to 59% (entry 6). We completed the optimization of the reaction conditions by increasing the molar amount of 1,4-benzoquinone[32] (entries 7 and 8). The superior reactivity of the diene **23b** relative to its phenyl-substituted derivative **23a** was underscored by comparison of the results shown in entries 3 and 7. In all cases, macrocycle **24** was isolated as a single stereoisomer ($E/Z > 20:1$, $J_{H-8,H-9} = 15.5$ Hz) after purification by flash column chromatography on silica gel.

Our next task was the construction of the conjugated bromodiene side chain (Scheme 5). Hydrogenation of the unsaturated macrocycle **24** followed by selective deprotection of the TBDPS group[33] gave the alcohol **25** (86% yield over two steps). Dess–Martin oxidation[34] of the alcohol **25** (97% yield)



Scheme 5. Construction of the diene side chain. CuTC = copper thiophene-2-carboxylate, DMP = Dess–Martin periodinane, NBS = *N*-bromosuccinimide, NMP = *N*-methylpyrrolidone, TBAF = tetra-*n*-butylammonium fluoride, TMS = trimethylsilyl.

Table 1. RCM of dienes **23a** and **23b**.[a]

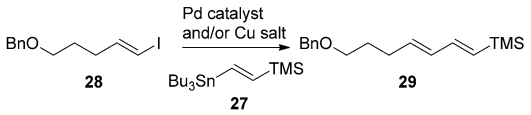


Entry	Diene	Catalyst (equiv)	BQ[b] [equiv]	Temp. [°C]	Yield
1	**23a**	**G-II** (0.3)	0.6	100	<5%
2	**23a**	**HG-II** (0.3)	0.6	100	<5%
3	**23a**	**HG-II** (0.1)	1.5	140	<5%
4	**23b**	**HG-II** (0.2)	0.4	100	22%
5	**23b**	**HG-II** (0.1)	0.2	120	33%
6	**23b**	**HG-II** (0.1)	0.2	140	59%
7	**23b**	**HG-II** (0.1)	1.5	140	81%
8[c]	**23b**	**HG-II** (0.1)	1.5	140	78%

[a] All the reactions were performed in degassed toluene (3 mM) overnight, unless otherwise noted. [b] BQ = 1,4-benzoquinone. [c] The reaction was performed in degassed toluene (1 mM).

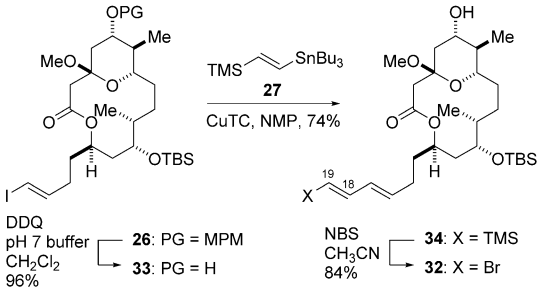
and ensuing Takai olefination ($CrCl_2$, CHI_3, 1,4-dioxane/THF) provided the vinyl iodide **26** in 81% yield (*E*/*Z* 13:1). The minor *Z*-isomer could be removed at this stage by flash column chromatography on silica gel. The Stille reaction of the vinyl iodide **26** with (2-trimethylsilylethenyl)tributylstannane (**27**)[35] proved more difficult than anticipated, possibly because of the low reactivity of the vinylstannane **27**. We screened a variety of reaction conditions by using the vinyl iodide **28** as a model compound and found the beneficial effect of CuTC[36] in this particular case (Table 2). Gratifyingly, CuTC-mediated coupling of the vinyl iodide **26** with the vinylstannane **27** in degassed NMP at room temperature afforded the conjugated diene **30** in 99% yield. Bromodesilylation of the diene **30** with NBS[37] gave the bromodiene **31**, which had undergone partial isomerization of the C-18=C-19 double bond geometry ($E/Z \approx 15:1$),[37] in 89% yield. Removal of the MPM group of the diene **31** by using DDQ did not proceed cleanly and gave the corresponding al-

Table 2. Stille-type reaction of vinyl iodide **28**[a] and vinylstannane **27**.[b]



Entry	Pd catalyst (equiv)	Cu salt (equiv)	**27** [equiv]	Yield
1	$[PdCl_2(PPh_3)_2]$ (0.1)	–	1.5	29%
2	$[PdCl_2(CH_3CN)_2]$ (0.1)	–	1.5	34%
3[c]	$[PdCl_2(CH_3CN)_2]$ (0.1)	–	1.5	35%
4	$[PdCl_2(CH_3CN)_2]$ (0.1)	CuI (1.0)	1.5	38%
5	$[PdCl_2(CH_3CN)_2]$ (0.1)	CuTC (3.8)	1.5	38%
6	$[PdCl_2(CH_3CN)_2]$ (0.1)	CuTC (7.6)	3.0	70%
7	–	CuTC (7.6)	3.0	83%

[a] Vinyl iodide **28** was used as a 10:1 mixture of *E*/*Z* isomers. [b] The reactions were performed in degassed DMF (entries 1–6) or NMP (entry 7) at room temperature. [c] iPr_2NEt (1.5 equiv) was used as additive.

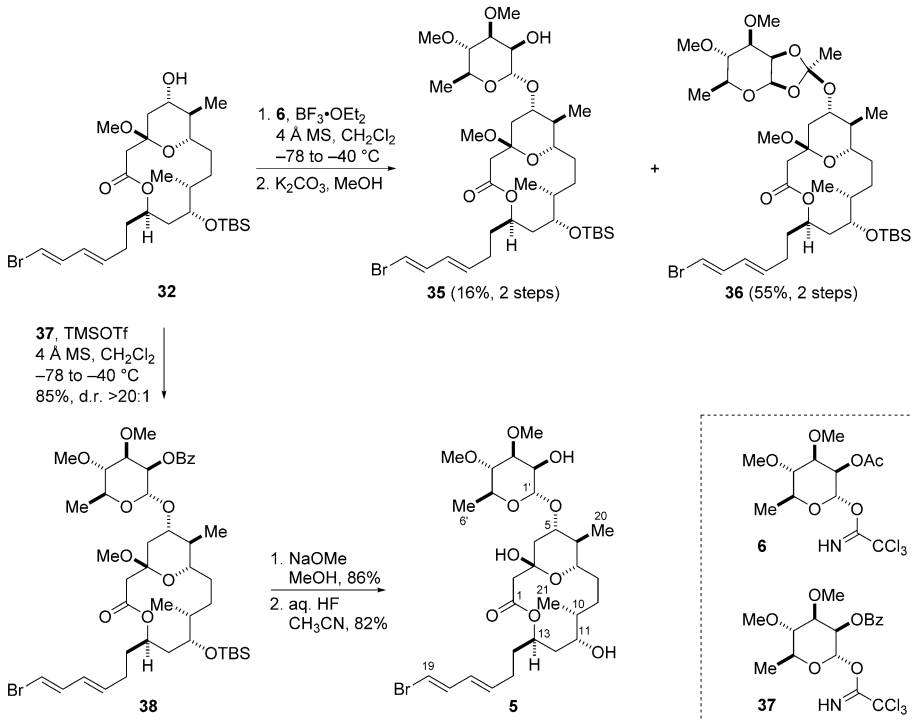


Scheme 6. Synthesis of aglycon **32**.

cohol **32** in only moderate yield; furthermore, treatment of the protected alcohol **31** with CAN resulted in decomposition of the material.

Therefore, we decided to remove the MPM group before construction of the sensitive conjugated diene side chain (Scheme 6). Treatment of the vinyl iodide **26** with DDQ led to the alcohol **33** (96% yield) without incident, which was coupled with the vinylstannane **27** under the influence of CuTC to provide the vinylsilane **34** (74% yield). Exposure of the vinylsilane **34** to NBS in acetonitrile afforded the aglycon **32** in 84% yield. As expected, the desilylation of intermediate **34** caused partial erosion of the geometry of the C-18=C-19 double bond,[37] as judged by ^1H NMR analysis. The minor impurities were removed by preparative reverse-phase HPLC after completion of the total synthesis.

Completion of the total synthesis of 13-demethyllyngbyaloside B (**5**) is shown in Scheme 7. Stereoselective glycosylation of the alcohol **32** with the 2-*O*-acetylated rhamnopyranosyl trichloroacetimidate derivative **6**[23] (10 mol% $BF_3{\cdot}OEt_2$, 4 Å MS, CH_2Cl_2, −78 to −40 °C) delivered, after treatment with K_2CO_3 in methanol, the desired glycosylated product **35** (16% yield over two steps) and the orthoester **36** (55% yield over two steps), the latter being the major product. The structure of the orthoester **36** was characterized on the basis of 2D NMR analyses.[23] It is known that 2-*O*-acetylated glycosyl donors are useful for controlling the stereochemical outcome of glycosylation through anchimeric assistance, although in some instances such donors potentially complicate the reaction by producing the corresponding orthoesters.[10,38] To circumvent the orthoester formation, we chose the 2-*O*-benzoylated rhamnopy-



Scheme 7. Completion of the total synthesis of 13-demethyllyngbyaloside B (**5**). Bz = benzoyl.

ranosyl trichloroacetimidate derivative **37**[23] as an alternative donor. Thus, glycosylation of the alcohol **32** with the glycosyl donor **37** (10 mol% TMSOTf, 4 Å MS, CH_2Cl_2, −78 to −40 °C) cleanly furnished the desired glycosylated product **38** in 85% yield with greater than 20:1 d.r. The use of $BF_3{\cdot}OEt_2$ as a Lewis acid gave a slightly lower yield of product **38** (68% yield). The stereochemical outcome of the glycosylation was confirmed by NOE experiments and the data were in accordance with those that were reported for the authentic material.[5] Finally, the benzoyl group was removed with NaOMe (86% yield), and the silyl ether and methyl acetal were cleaved with aqueous hydrofluoric acid in acetonitrile (82% yield), which afforded (−)-13-demethyllyngbyaloside B (**5**) ($[\alpha]_D^{24} = -46.1$ ($c = 0.5$ in $CHCl_3$)).

The ^1H and ^{13}C NMR signals of the non-natural derivative **5** were assigned on the basis of 2D NMR analyses and were compared with those of natural (−)-lyngbyaloside B; the ^{13}C NMR data are summarized in Table 3.[23] We were intrigued to find that significant chemical shift deviations were found not merely around the C-13 position but all over the macrocycle, whereas the NMR chemical shift values of the conjugated diene and L-rhamnopyranoside moieties of compound **5** were in good accordance with those of natural (−)-lyngbyaloside B. At this point, two possibilities arose from this unexpected result: first, the C-13 methyl group of the natural product has significant influence on the overall conformational property of the macrocycle, and second, the proposed structure **1** has been incorrectly assigned. Considering the caveats that had been independently described by Ley[8c] and Cossy[8d] on the proposed structure **2** of (−)-lyngbouilloside, the non-resemblance of the NMR spectroscopic data between derivative **5** and natural (−)-lyngbyaloside B implied possible misassignment of the proposed structure **1** and justified our efforts towards its total synthesis for structure validation.

Table 3. Comparison of the ^{13}C NMR spectroscopic data for (−)-13-demethyllyngbyaloside B (**5**) and the natural (−)-lyngbyaloside B.[a]

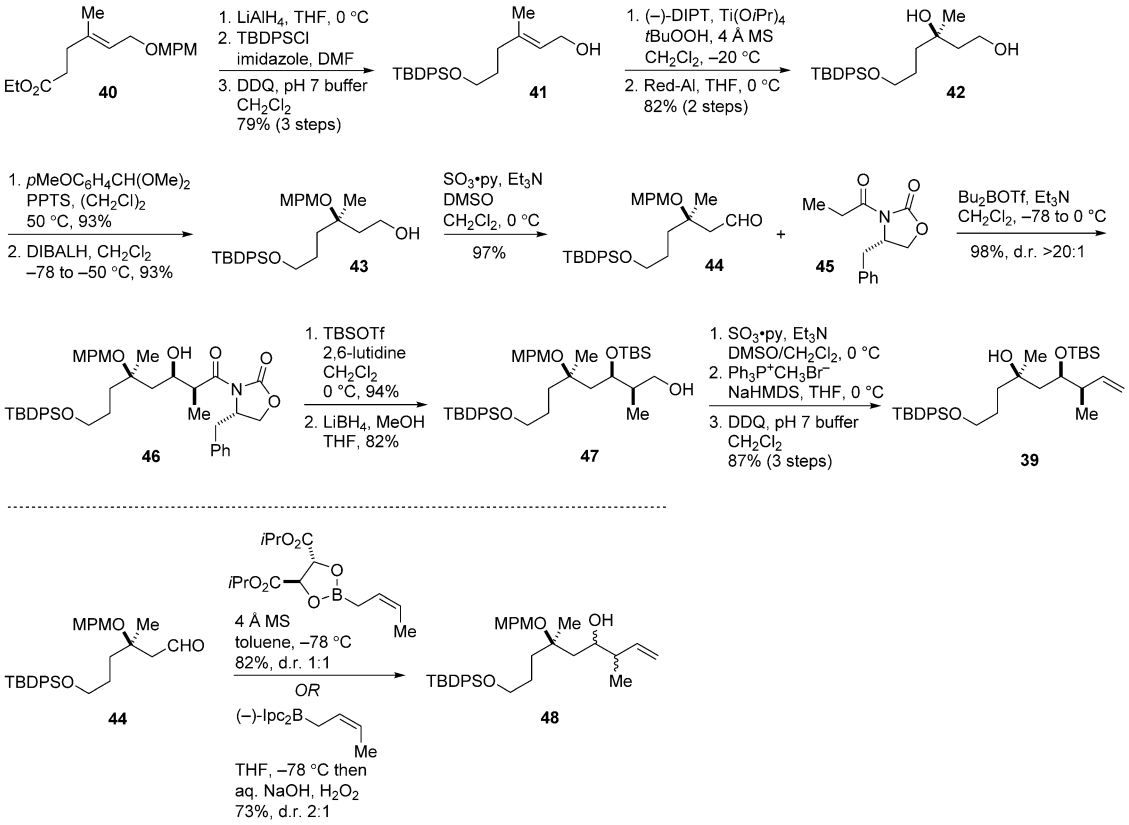
Position	δ_N [ppm]	δ_S [ppm]	$\Delta\delta$ [ppm]
1	172.5	169.1	+3.4
2	46.8	48.9	−1.9
3	96.1	96.8	−0.7
4	42.1	39.4	+2.7
5	79.1	79.1	0
6	41.5	38.5	+3.0
7	75.6	74.3	+1.3
8	28.1	26.2	+1.9
9	32.8	28.0	+4.8
10	36.9	32.0	+4.9
11	65.7	67.3	−1.6
12	44.1	38.2	+5.9
13	86.4	70.9	+15.5
14	38.6	34.6	+4.0
15	26.7	28.4	−1.7
16	135.6	134.6	+1.0
17	127.7	128.3	−0.6
18	137.5	137.4	+0.1
19	106.5	106.9	−0.4
20	13.6	12.7	+0.9
21	13.6	13.5	+0.1
22	23.4	N/A[b]	N/A[b]
1′	101.1	101.2	−0.1
2′	67.9	68.0	−0.1
3′	81.2	81.2	0
3′–OMe	57.4	57.5	−0.1
4′	81.8	81.8	0
4′-OMe	61.0	60.8	+0.2
5′	67.4	67.5	−0.1
6′	17.6	17.6	0

[a] The ^{13}C NMR spectra of natural (−)-lyngbyaloside B and synthetic (−)-13-demethyllyngbyaloside B (**5**) were collected in $CDCl_3$ at 125 MHz and 150 MHz, respectively. δ_N and δ_S are chemical shifts of the natural product and synthetic **5**. [b] N/A = not applicable.

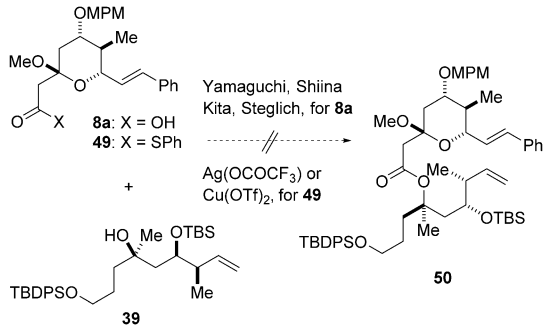
The first-generation approach toward the proposed structure 1 of (−)-lyngbyaloside B

Having completed the total synthesis of 13-demethyllyngbyaloside B (**5**), we were in a position to undertake the total synthesis of the proposed structure **1** of (−)-lyngbyaloside B, which we based on the esterification/RCM strategy. To this end, the tertiary alcohol **39** was synthesized from known ester **40**,[39] which was prepared in four steps from 3-methyl-2-buten-1-ol (Scheme 8). Reduction of the ester **40** with $LiAlH_4$, silylation of the resultant alcohol with TBDPSCl/imidazole, and deprotection of the MPM group by using DDQ gave the alcohol **41** in 79% yield over three steps. Sharpless asymmetric epoxidation of the alcohol **41** (e.r. 96:4)[40] followed by reduction of the derived epoxy alcohol with Red-Al[41] delivered the 1,3-diol **42** in 82% yield over the two steps. Acetalization of the diol **42** with pMeOC$_6$H$_4$CH(OMe)$_2$ and PPTS provided the corresponding *p*-methoxybenzylidene acetal (93% yield), which was treated with DIBALH[42] to give the alcohol **43** (93% yield). Oxidation of the alcohol **43** under Parikh–Doering conditions[43] led to the aldehyde **44** (97%). Evans *syn*-aldol reaction[44] of the aldehyde **44** with a boron enolate of **45** (Bu_2BOTf, Et_3N, CH_2Cl_2, −78–0 °C) afforded the alcohol **46** in 98% yield with greater than 20:1 diastereoselectivity. The absolute configuration of the alcohol **46** was confirmed by using a modified Mosher analysis[45] and NMR analyses on a suitable acetonide derivative.[23] After the alcohol **46** was silylated (94% yield), the resultant silyl ether was reduced with $LiBH_4$ in the presence of methanol to remove the chiral auxiliary, which gave rise to the alcohol **47** (82% yield). Oxidation of the alcohol **47** followed by Wittig methylenation and subsequent cleavage of the MPM ether delivered the alcohol **39** in 87% yield over the three steps. The synthesis of the alcohol **39** was somewhat circuitous because our attempts at asymmetric crotylation of the aldehyde **44** to directly obtain the alcohol **48** by using a Brown[46] or Roush[27] chiral crotylborane reagent only gave an inseparable mixture of diastereoisomers with essentially no diastereoselectivity, as judged by ^1H NMR spectroscopic analysis.

Unfortunately, we were unable to esterify the tertiary alcohol **39** with the carboxylic acid **8a** or its synthetic equivalent **49** (Scheme 9). Esterification under Yamaguchi, Shiina,[47] Kita,[48] or Steglich[49] conditions did not give the desired ester **50** at all; in all cases, unreacted alcohol **39** was recovered almost quantitatively. The use of the thioester **49** as an electrophile under the influence of $Ag(OCOCF_3)$ or $Cu(OTf)_2$[50] was also ineffective in this case. These unproductive results could be ascribed to



Scheme 8. Synthesis of alcohol **39**. DIPT = diisopropyl tartrate, HMDS = hexamethyldisilazide, py = pyridine, Red-Al = sodium bis(2-methoxyethoxy)aluminum hydride.



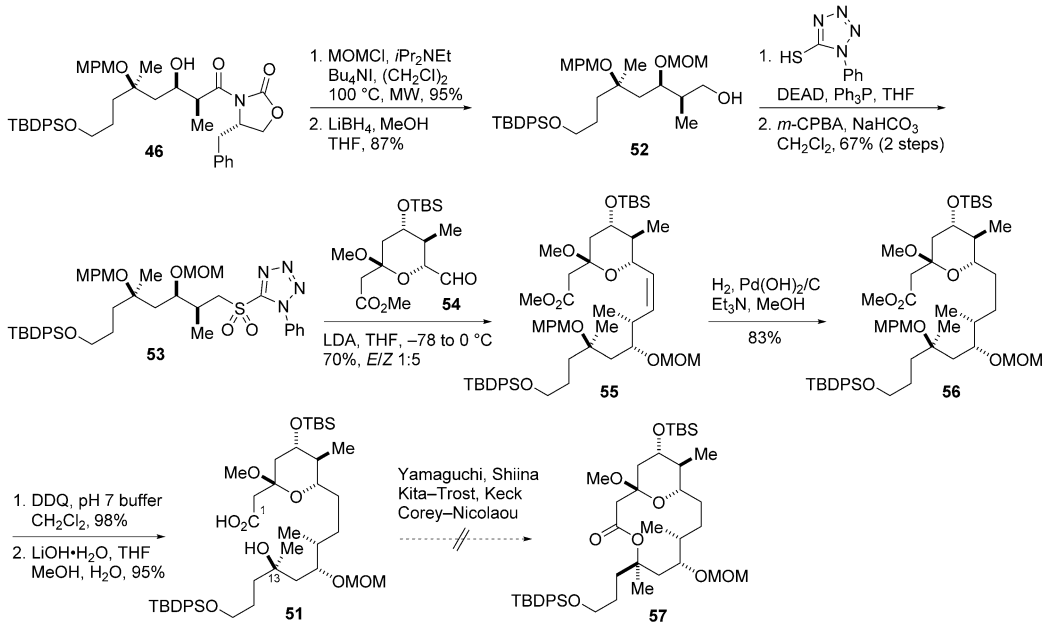
Scheme 9. Unproductive attempts at the acylation of tertiary alcohol **39**.

the extremely low reactivity of the sterically encumbered alcohol **39**, which surprisingly, proved to be completely unreactive even under standard acetylation conditions (Ac_2O, Et_3N, DMAP or AcCl, pyridine, DMAP).

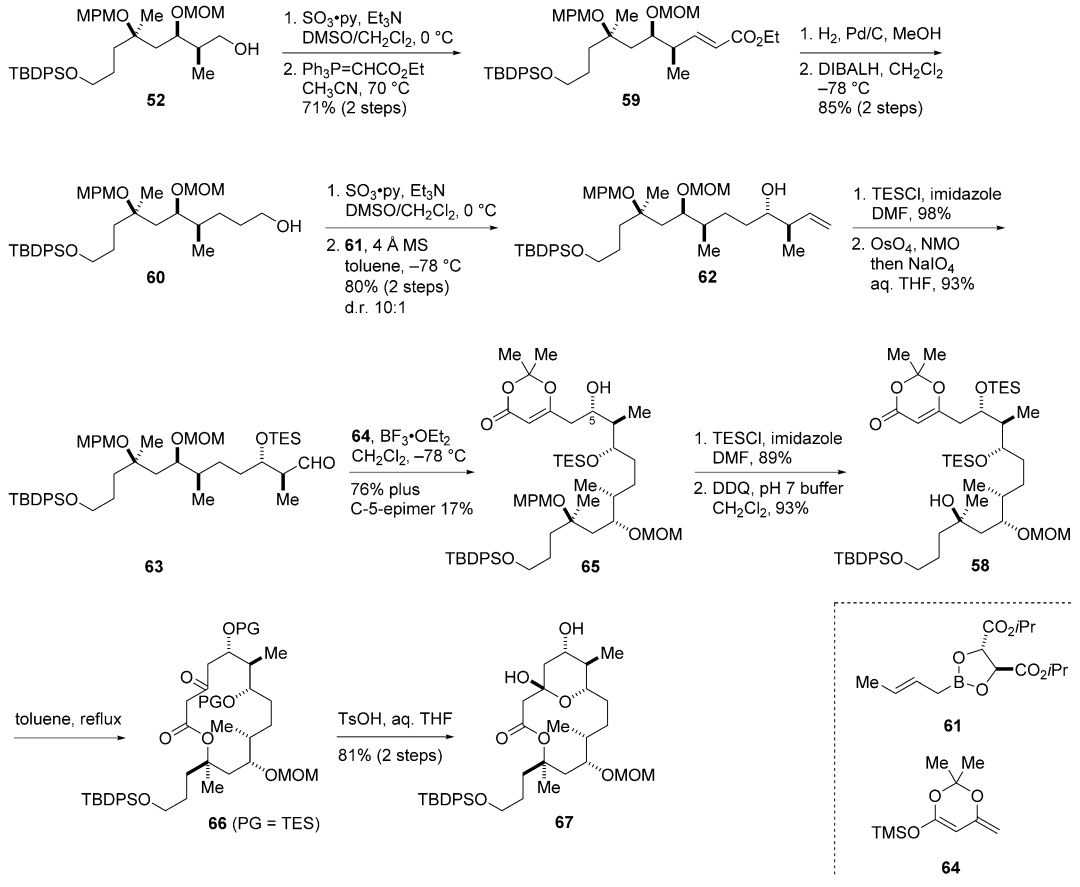
At this stage, we hypothesized that macrolactonization of the seco-acid **51** might overcome the inherent low reactivity of the C-13 tertiary hydroxy group if the precursor **51** adopts a conformation in which the reaction sites, i.e., C-1 and C-13 positions, are brought into close proximity (Scheme 10).[51] To test this idea, the seco-acid **51** was synthesized from the alcohol **46**: Protection of **46** as its MOM ether (95% yield) followed by reductive removal of the chiral auxiliary (87% yield) gave the alcohol **52**. Mitsunobu reaction of **52** with 1-phenyl-1*H*-tetrazole-5-thiol (DEAD, Ph_3P) and subsequent oxidation afforded the sulfone **53** (67% yield over two steps). Deprotonation of the sulfone **53** with LDA followed by addition of the aldehyde **54** (THF, −78 to 0 °C) led to the olefin **55** in 70% yield as an inconsequential mixture of *E*/*Z* isomers (*E*/*Z* 1:5).[52] Hydrogenation of the double bond within **55** (83% yield), cleavage of the MPM ether (98% yield), and alkaline hydrolysis of the methyl ester (95% yield) furnished the desired seco-acid **51**. However, in spite of our extensive efforts, macrolactonization of the seco-acid **51** was found to be completely unproductive; indeed, under Yamaguchi, Shiina, Keck,[53] Kita–Trost,[48,54] or Corey-Nicolaou[55] conditions, we did not observe any trace of the desired macrolactone **57**.

Owing to our inability to acylate the C-13 tertiary hydroxy group with activated anhydrides/esters, we were forced to reconsider our synthetic strategy for the macrolactone skeleton of target compound **1**. Hoye and co-workers[8a] have described the synthesis of a model derivative of compound **1** by means of an acyl ketene macrolactonization.[9] To test the feasibility of acyl ketene macrocyclization in the real system, we undertook the synthesis of the cyclization precursor, dioxinone **58** (Scheme 11). While this work was in progress, Cossy et al. reported the total synthesis of the nominal lyngbouilloside aglycon, in which acyl ketene macrocyclization was exploited for the construction of the macrolactone skeleton.[8d]

The synthesis of compound **58** started with oxidation of the alcohol **52** followed by Wittig reaction of the resultant alde-



Scheme 10. Unproductive attempts at the macrocyclization of seco-acid **51**. LDA = lithium diisopropylamide, *m*CPBA = *m*-chloroperoxybenzoic acid, MOM = methoxymethyl, MW = microwave.



Scheme 11. Synthesis of dioxinone **58** and its macrocyclization. Ts = *p*-toluenesulfonyl.

hyde to give the α,β-unsaturated ester **59** (71% yield over two steps). After hydrogenation of the double bond, the ester moiety was reduced with DIBALH to provide the alcohol **60** (85% yield over the two steps). Oxidation of the alcohol **60**

and subsequent Roush crotylation by using chiral crotyl boronate **61** (4 Å MS, toluene, −78 °C) delivered the alcohol **62** (80% yield over the two steps, d.r. > 10:1). At this stage, the configuration of the newly generated stereogenic centers was established by using NMR analyses of suitable derivatives.[23] Silylation of the alcohol **62** (TESCl, imidazole, 98% yield) was followed by oxidative cleavage of the terminal double bond to give the aldehyde **63** (93% yield), and its subsequent vinylogous Mukaiyama aldol reaction with the dienol silyl ether **64**[56] ($BF_3{\cdot}OEt_2$, CH_2Cl_2, −78 °C) led to the alcohol **65** and its C-5 epimer (76 and 17% yield, respectively). These diastereoisomers were separated by flash column chromatography on silica gel. After silylation of the alcohol **65** (TESCl, imidazole, 89% yield), the MPM group was cleaved with DDQ to afford the desired dioxinone **58** (93% yield).

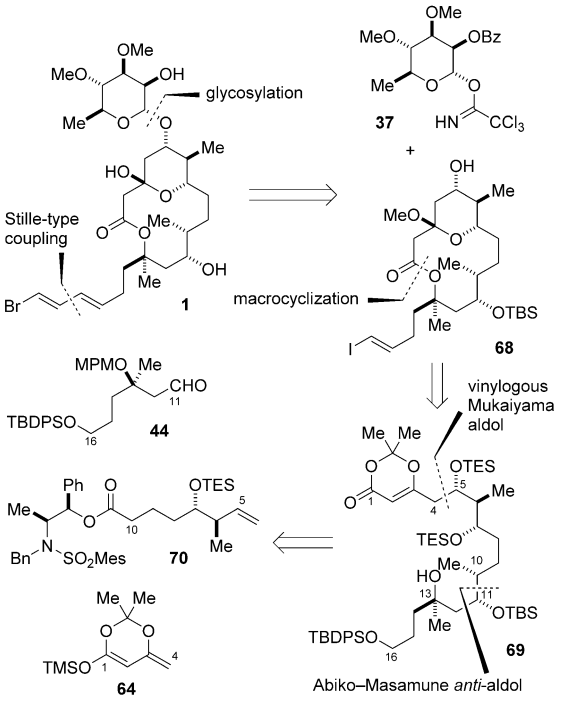
Gratifyingly, when a solution of compound **58** in toluene (0.3 mM) was heated to reflux, the cyclization proceeded to give the β-keto lactone **66**; this was treated with TsOH in THF/H_2O to cleave the TES groups and initiate simultaneous formation of the six-membered hemiacetal ring, which gave rise to the targeted macrolactone **67** in 81% yield over the two steps. The configuration of the C-5 stereogenic center was determined on the basis of *J* values at this stage.[23]

Thus, we have demonstrated that the 14-membered macrocyclic backbone of the proposed structure of lyngbyaloside B (**1**) could actually be synthesized by using acyl ketene macrocyclization. However, the synthesis of the cyclization precursor **58** required a number of transformations, and the overall synthetic efficiency was far from satisfactory. At this point, we decided to reconsider our synthetic plan towards target compound **1**.

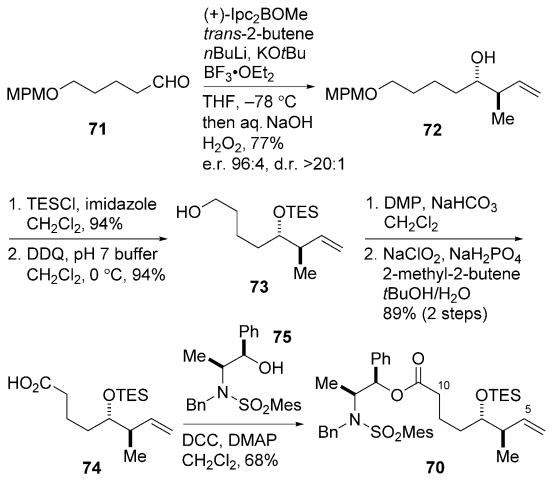
The second-generation approach towards the proposed structure 1 of (−)-lyngbyaloside B

Our second-generation synthetic plan is summarized in Scheme 12. The aglycon **68** would be accessible through an acyl ketene macrocyclization of the dioxinone **69**, which was to be synthesized in a convergent manner from three readily available fragments, the aldehyde **44**, the ester **70**, and the dienol silyl ether **64**,[56] by an Abiko–Masamune *anti*-aldol reaction[57] and a vinylogous Mukaiyama aldol reaction. Although the Abiko–Masamune *anti*-aldol reaction has been used to construct *anti*-propionate aldol motifs, we envisioned that it would also be useful as a means to assemble complex fragments with the simultaneous creation of two contiguous stereogenic centers.[58]

The synthesis of the ester **70** started with the Brown asymmetric crotylation of the known aldehyde **71**,[59] which gave the alcohol **72** in 77% yield (e.r. 96:4, d.r. > 20:1) (Scheme 13). The stereostructure of the alcohol **72** was corroborated by a modified Mosher analysis and NMR analyses of an acetonide derivative.[23] Silylation of the alcohol **72** with TESCl/imidazole (94% yield) followed by deprotection of the MPM group (94% yield) led to the alcohol **73**. A two-stage oxidation[60] of the alcohol **73** gave the corresponding carboxylic acid **74** (89% yield over the two steps), and subsequent esterification with known



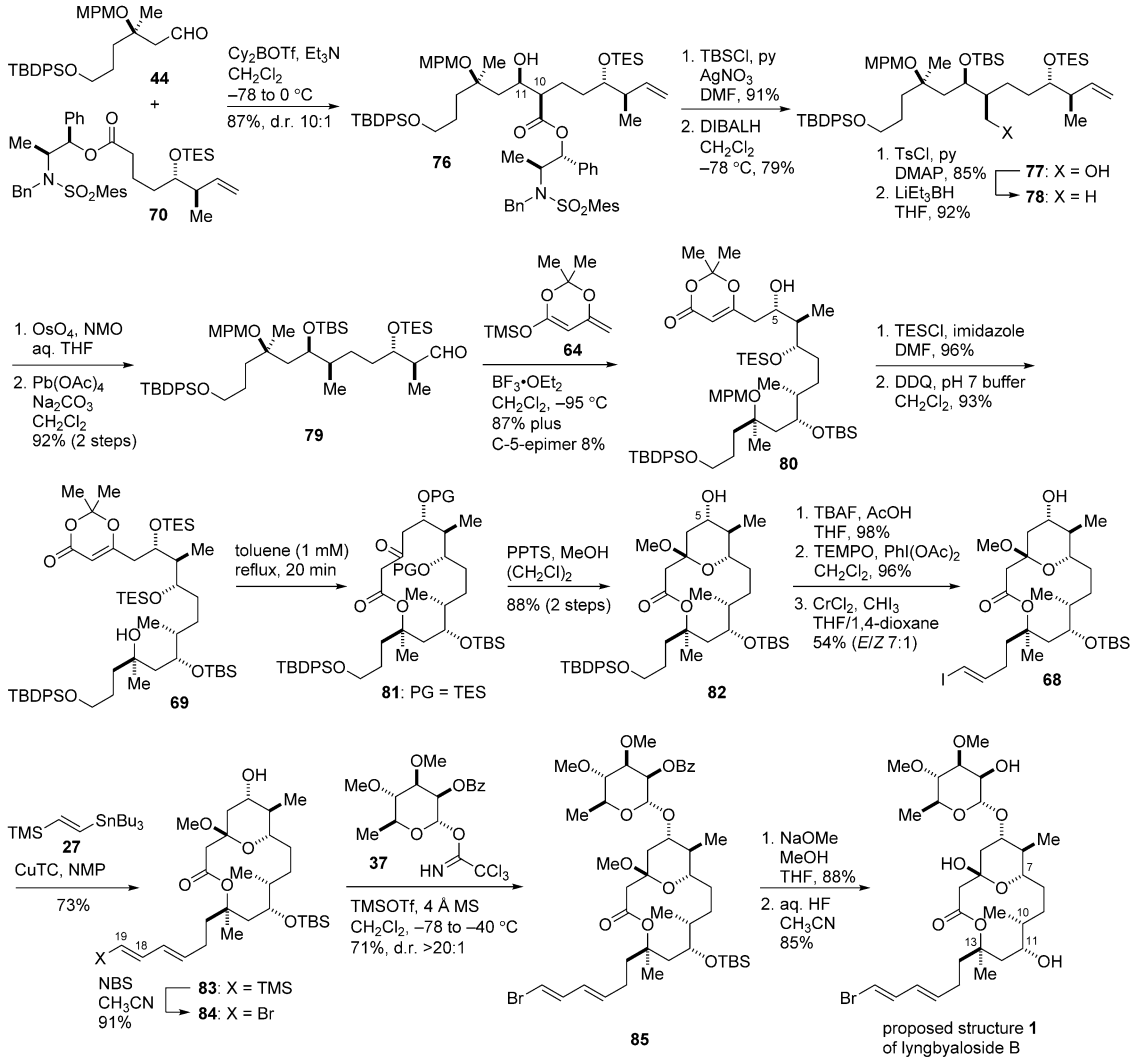
Scheme 12. Synthetic plan towards **68**.



Scheme 13. Synthesis of ester **70**. DCC = dicyclohexylcarbodiimide.

alcohol **75**[56] by using DCC/DMAP afforded the ester **70** (68% yield).

The synthesis of the cyclization precursor **69** and completion of the total synthesis of target compound **1** are summarized in Scheme 14. Abiko–Masamune *anti*-aldol reaction of the ester **70** and the aldehyde **44** was carried out under standard conditions (Cy_2BOTf, Et_3N, CH_2Cl_2, −78 to 0 °C) to provide the alcohol **76** in 87% yield with 10:1 d.r. The configurations of the newly generated C-10 and C-11 stereogenic centers of alcohol **76** were unambiguously determined on the basis of NMR analyses of suitable derivatives.[23] Silylation of the alcohol **76** with TBSCl in the presence of $AgNO_3$ and pyridine[61] (91% yield) fol-



Scheme 14. Synthesis of the cyclization precursor **69** and completion of the total synthesis of **1**. Cy = cyclohexyl, TEMPO = 2,2,6,6-tetramethylpiperidin-1-oxyl.

lowed by reductive removal of the superfluous chiral auxiliary gave rise to the alcohol **77** (79% yield). The alcohol **77** was deoxygenated by tosylation (85% yield) and $LiEt_3BH$ reduction[62] (92% yield) to deliver the olefin **78**. Dihydroxylation of the olefin **78** and subsequent cleavage of the resultant 1,2-diol with $Pb(OAc)_4$ gave the aldehyde **79** in 92% yield over the two steps, which was then reacted with the dienol silyl ether **64** under Mukaiyama conditions ($BF_3{\cdot}OEt_2$, CH_2Cl_2, $-95\,^{\circ}C$) to afford the alcohol **80** in 87% yield, along with its C-5 epimer in 8% yield. These diastereoisomers were separated by flash column chromatography on silica gel. Silylation of the alcohol **80** with TESCl/imidazole (96% yield) followed by removal of the MPM group (93% yield) led to the cyclization precursor **69**.

Macrocyclization of the alcohol **69** was carried out in refluxing toluene (1 mM) and gave rise to the β-keto lactone **81**,[63] which was treated with PPTS in $MeOH/(CH_2Cl)_2$ to remove the TES groups and induce a spontaneous acetalization to afford the methyl acetal **82** in 88% yield over the two steps. At this stage, the minor stereoisomer that originated from the Abiko–Masamune reaction was removed, and the configuration of the C-5 stereogenic center was determined on the basis of NOE experiments.[23]

Having constructed the macrocyclic backbone, we proceeded to complete the total synthesis of target compound **1**. The TBDPS group of compound **82** was selectively removed by using buffered TBAF to give a diol in 98% yield. The liberated primary hydroxy group was selectively oxidized with TEMPO/$PhI(OAc)_2$[64] (96% yield), and Takai olefination of the derived aldehyde afforded the vinyl iodide **68** in 54% yield as an inseparable mixture of *E*/*Z* isomers (*E*/*Z* 7:1). Stille-type reaction of the vinyl iodide **68** with the vinylstannane **27** by the action of CuTC in degassed NMP provided the vinylsilane **83** in 73% yield; its exposure to NBS in acetonitrile delivered the bromodiene **84** in 91% yield. As aforementioned, the bromodesilylation of compound **83** was accompanied by partial isomerization of the C-18=C-19 double bond; these minor stereoisomers were removed by preparative reverse-phase HPLC after completion of the total synthesis. Stereoselective glycosylation of the alcohol **84** with the trichloroacetimidate **37** under previously optimized conditions (10 mol% TMSOTf, 4 Å MS, CH_2Cl_2,

−78 to −40 °C) afforded the glycosylated product **85** in 71% yield (d.r. > 20:1). The α-glycosidic linkage of the rhamnopyranoside moiety of compound **85** was confirmed by NOE experiments.[23] Methanolysis of the benzoyl group of **85** (NaOMe, MeOH/THF, 88% yield) followed by removal of the TBS group and simultaneous cleavage of the methyl acetal (aq. HF, CH_3CN, 85% yield) furnished the proposed structure **1** of (−)-lyngbyaloside B. The synthetic material was purified by reverse-phase HPLC to remove minor stereoisomers prior to detailed spectroscopic characterization.

Revision of the original stereochemical assignment and establishment of the complete stereostructure

Unfortunately, the ^1H and ^{13}C NMR spectra of our synthetic compound **1** were obviously different from those of natural (−)-lyngyaloside B. The ^1H NMR spectrum of the authentic sample recorded in $CDCl_3$ showed a set of sharp signals, whereas the ^1H NMR spectrum of our sample **1** collected in $CDCl_3$ at room temperature displayed significant line broadening for the signals that correspond to the C-8–C-12 domain. Moreover, the ^{13}C NMR spectrum of compound **1** under the same conditions did not show any clear signals that account for the C-8–C-12 domain. These observations strongly suggested that, in solution, compound **1** actually exists as an ensemble of multiple conformers, which interconvert slowly within the NMR timescale, at least in $CDCl_3$ at room temperature.[65] Ley and co-workers have made a similar observation of their protected aglycon of (−)-lyngbouilloside (**2**), as noted above.[8c] In contrast to compound **1**, (−)-13-demethyllyngbyaloside B (**5**) did not show any broadening of signals in its ^1H and ^{13}C NMR spectra measured in $CDCl_3$ at room temperature. Therefore, it was reasonable to assume that the C-10, C-11, and C-13 stereogenic centers and/or substituents would be the important structural elements that together determine the conformational behavior of compound **1**.

After screening several deuterated solvents, we found that CD_3CN was the solvent of choice; the ^1H and ^{13}C NMR spectra of compound **1** measured in CD_3CN at room temperature displayed well-resolved, sharp signals. The gross structure of our synthetic analogue **1** was ascertained on the basis of 2D NMR analyses; the relative configuration has already been established by detailed NMR analyses of appropriate intermediates and their derivatives. Thus, it was evident that we have actually synthesized the proposed structure **1** of (−)-lyngbyaloside B and that the original stereochemical assignment made by Moore and co-workers requires reconsideration.

Moore et al. assigned the relative configuration of the six-membered hemiacetal and the L-rhamnopyranoside moieties in a solid manner on the basis of *J* values and ROE enhancements, but the relative configuration of the stereogenic centers along the macrocyclic backbone was deduced predominantly on the basis of ROE correlations. In fact, there was some ambiguity in the relative configurational assignment of the C-7/C-10 stereogenic centers because of signal overlap; therefore, we opted to further scrutinize the relative configuration of the C-7/C-10, C-10/C-11, and C-11/C-13 stereogenic centers with the aid of molecular mechanics (MM) calculations.

The relative configuration of the C-11/C-13 stereogenic centers could be assigned on the basis of the ROE correlations observed between the H-11 methine proton and the H_2-14 methylene protons of the natural product, which strongly suggests that the C-11 hydroxy group and the C-13 methyl group occupy the same face of the macrocycle.[5] Based on this premise, the correct configuration of the C-11 and C-13 stereogenic centers should be either (11*R*,13*R*) or (11*S*,13*S*). As the pro-

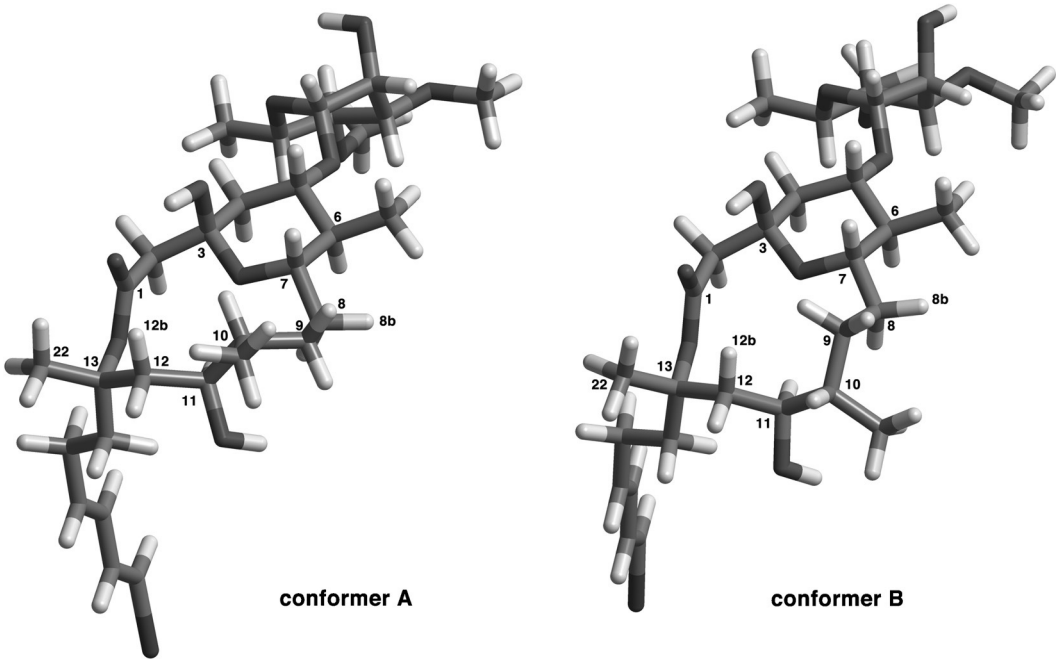
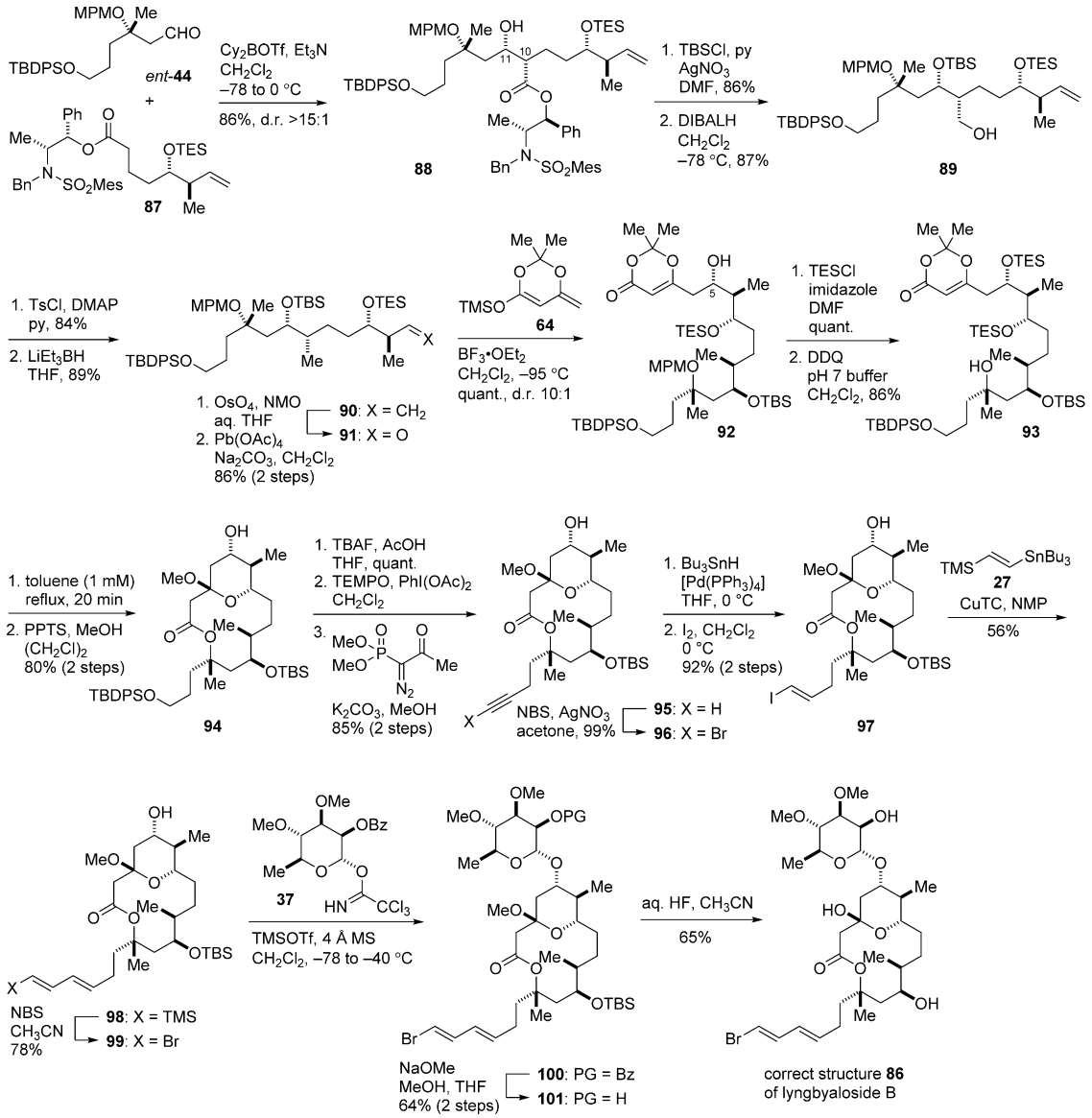


Figure 2. Two representative stable conformers of the (10*S*,11*S*,13*S*) isomer of (−)-lyngbyaloside B. The conformers were generated by MM calculations (MMFF94 s) and geometrically optimized at RB3LYP/6-31G* level of theory.

posed structure **1** has (10*R*,11*R*,13*R*) configuration, the possible stereoisomers for the correct structure of (−)-lyngbyaloside B could be narrowed down to the following three: (10*S*,11*R*,13*R*), (10*R*,11*S*,13*S*), or (10*S*,11*S*,13*S*).

Next, extensive conformational searches (MMFF94 s) were performed on the candidate stereoisomers. Not unexpectedly, MM calculations indicated that these stereoisomers show varying degrees of conformational flexibility with respect to the macrocyclic backbone, so that it would be difficult to draw definitive conclusions on the correct structure merely from MM-based conformational analyses. Nevertheless, it was deduced that the (10*S*,11*S*,13*S*) isomer most likely represents the correct structure of the natural product. As shown in Figure 2, conformational searches on the (10*S*,11*S*,13*S*) isomer found two representative conformers A and B.[66] It appears that the conformer A better fits the NMR characteristics of the authentic material, which included the splitting pattern and coupling constants of H-8b (dq, $J = 14.6$, ≈ 2 Hz), the weak coupling between H-10/H-11, and the ROESY correlation between Me-22/H-12b. Moreover, both of these conformers accommodate an H-bond between the C-1 carbonyl oxygen atom and the C-3 hemiacetal hydrogen atom, which likely accounts for the characteristic long-range "W-coupling" that is observed between the C-3–OH proton and the H-4 axial proton of the authentic material ($^4J_{H,H} = 2.4$ Hz).

Accordingly, we proceeded to verify the correct structure **86** of (−)-lyngbyaloside B through its total synthesis, as shown in Scheme 15. The synthesis started with the Abiko–Masamune *anti*-aldol reaction of the aldehyde *ent*-**44**[23] and the ester **87**,[23] which proceeded in essentially the same way as that described for compound **1**. Gratifyingly, it was found that the ^{1}H and ^{13}C NMR spectra of our synthetic analogue **86** were identical with those of natural lyngbyaloside B. Moreover, the specific rotation value of our synthetic **86** ($[\alpha]_D^{25} = -16.9$ ($c = 0.20$ in



Scheme 15. Total synthesis of the correct structure **86** of (−)-lyngbyaloside B.

$CHCl_3$)) was in close agreement with that of the authentic sample ($[\alpha]_D^{25} = -20$ ($c = 0.10$ in $CHCl_3$)). Thus, the complete stereostructure of (−)-lyngbyaloside B was undoubtedly established to be that shown by structure **86**.

Biological evaluation of synthetic (−)-lyngbyaloside B and related compounds

Evaluation of the antiproliferative activity of the proposed structure **1**, the correct structure **86** and its aglycon **102**, and (−)-13-demethyllyngbyaloside B (**5**) against a small panel of human cancer cell lines was carried out by using WST-8 assay (Figure 3).[67,68] The aglycon **102** was prepared from **99** by hydrolysis of the silyl ether and methyl acetal (Scheme 16). In contrast to what was reported by Moore et al., we were surprised to find that the correct structure **86** was almost inactive against KB cells and the proposed structure **1** was also essentially inactive. These compounds also did not show appreciable activity in human non-small cell lung adenocarcinoma A549 cells; however, moderate antiproliferative activity was observed

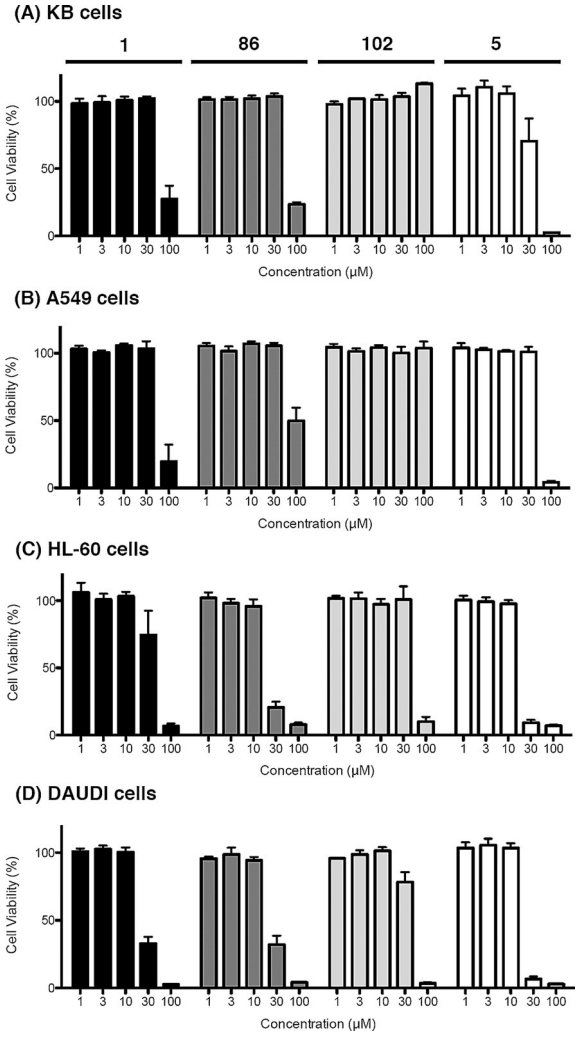
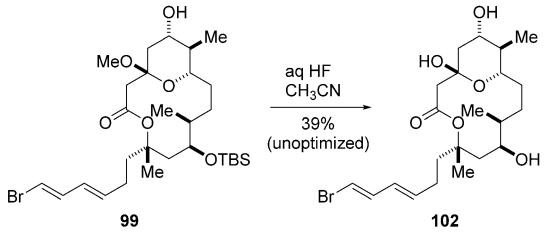


Figure 3. Antiproliferative activity of compounds **1**, **5**, **86**, and **102** against a small panel of human cancer cell lines ($n = 3$).



Scheme 16. Synthesis of aglycon **102**.

in human promyelocytic leukemia HL-60 cells and human Burkitt lymphoma DAUDI cells. Interestingly, 13-demethyllyngbyalsoside B (**5**) showed somewhat more potent activity than compounds **1** and **86**. Meanwhile, the aglycon **102** was considerably less active than the parent compound **86**.

At present, we are unable to address why the antiproliferative potency of our synthetic compound **86** was significantly lower than that which was reported in the isolation paper. However, our own data have implications in the structure–activity relationships of (−)-lyngbyaloside B: i) The absolute configuration of the stereogenic centers along the macrocyclic framework (C-10, C-11, and C-13) does not have appreciable impact on the antiproliferative potency (the proposed structure **1** versus the correct structure **86**), although it certainly affects the conformational behavior in solution; ii) omission of the C-13 methyl group was rather beneficial for improving the antiproliferative potency, at least in the case of the proposed structure **1**; iii) the L-rhamnopyranoside moiety plays an important role in exerting antiproliferative activity (the correct structure **86** versus the aglycon **102**).

Conclusion

We have described in detail our synthetic and structural studies on (−)-lyngbyaloside B, a marine macrolide glycoside, which culminated in the first total syntheses of the proposed and correct structures of this natural product (compounds **1** and **86**, respectively). Our initial investigations into the synthesis of (−)-13-demethyllyngbyaloside B (**5**), a non-natural analogue, exploited an RCM reaction for the construction of the macrocyclic skeleton. The bromodiene side chain was stereoselectively introduced by a Stille-type reaction that was mediated by CuTC. Stereoselective glycosylation was efficiently achieved under Schmidt conditions by using a 2-*O*-benzoylated L-rhamnopyranosyl trichloroacetimidate derivative. Thus, our total synthesis of compound **5** was achieved in 22 steps (longest linear sequence from 1,4-butanediol). Even though we have successfully established our synthetic path towards compound **5**, we soon realized that the synthesis of the proposed structure **1** of (−)-lyngbyaloside B was non-trivial because of our inability to acylate the sterically encumbered C-13 tertiary hydroxy group under standard esterification conditions. After extensive investigations, we were finally able to complete the total synthesis of target compound **1**, in which an Abiko–Masamune *anti*-aldol reaction and a vinylogous Mukaiyama aldol reaction were utilized to couple readily available fragments (**44**,

64, and **70**) and an acyl ketene macrolactonization was exploited to forge the macrocyclic backbone (32 linear steps from 3-methyl-2-buten-1-ol). However, comparison of the ^{1}H and ^{13}C NMR spectra of synthetic compound **1** with those of the authentic material indicated that the proposed structure **1** of (−)-lyngbyaloside B required correction. Re-investigation into the NMR spectroscopic data of the natural product, with the aid of molecular mechanics calculations, led us to determine that the configuration of the C-10, C-11, and C-13 stereogenic centers was incorrectly assigned in the initially proposed structure **1**. The correct structure **86** of (−)-lyngbyaloside B was finally verified in an unambiguous manner through total synthesis. In line with our conclusion, Taylor and co-workers very recently reported the total synthesis of (−)-18*Z*-lyngbyaloside C and revised the original stereochemical assignment of the proposed structure **4** at the same positions as **1** (C-10, C-11, and C-13).[8i] Therefore, we suggest that the originally assigned structure **2** of lyngbouilloside should also be reconsidered carefully. This work illuminates the importance of total synthesis in the establishment of the complete stereostructure of complex natural products.[69,70]

Acknowledgements

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Keywords: glycosides · macrocycles · natural products · structure elucidation · total synthesis

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