Tumonoic Acids, Novel Metabolites from a Cyanobacterial Assemblage of *Lyngbya majuscula* and *Schizothrix calcicola*

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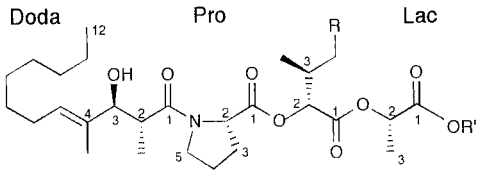
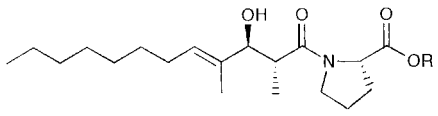
Five new metabolites have been isolated from a lyngbyastatin 1- and dolastatin 12-producing assemblage of *Lyngbya majuscula* and *Schizothrix calcicola* collected at Tumon Bay, Guam. Structure elucidation employed 2D NMR techniques and chemical derivatization. These compounds have been assigned the trivial names tumonoic acids A (**2**), B (**1**), and C (**5**); methyl tumonoate A (**3**), and methyl tumonoate B (**4**). Compounds **1** and **4** were also found in a lyngbyastatin 1-producing strain of *L. majuscula* from Guam.

The marine cyanobacterium (blue-green alga) *Lyngbya majuscula* Gomont (Oscillatoriaceae) has proven to be a prolific source of bioactive and structurally diverse secondary metabolites. These include the majusculamides,[1] curacins,[2] malyngamides,[3] lyngbyatoxins,[4] and microcolins.[5] We have recently reported the discovery of the cytotoxic cyclic depsipeptide, dolastatin 12, and a related compound, lyngbyastatin 1, along with the 15-*epi* analogues, from collections of certain *L. majuscula* strains and assemblages of *L. majuscula* and *Schizothrix calcicola* (Ag.) Gomont (Oscillatoriaceae) found in Guam.[6,7] A chemical investigation of these cyanobacteria has led to the isolation and identification of five novel acyclic depsipeptides (**1**−**5**).



1 Ahmp R = CH_3 R' = H
4 Ahmp R = CH_3 R' = CH_3
5 Hiv R = H R' = H



2 R = H
3 R = CH_3

Results and Discussion

The molecular formula of tumonoic acid B (**1**), the most abundant metabolite, was determined to be $C_{28}H_{47}NO_8$ based on HREIMS data, which indicated six degrees of unsaturation. The IR spectrum gave a prominent band at 1736 cm^{-1} and a less intense, broad jagged band centered at 1620 cm^{-1}, suggesting the presence of ester and amide groups in the molecule. $^1H-^1H$ COSY analysis, supported by ^1H and ^{13}C NMR chemical shift information, established the presence of spin systems attributed to 2-hydroxypropanoic (lactic) acid (Lac), 2-hydroxy-3-methylpentanoic (isoleucic) acid (Ahmp),[8] 2,4-dimethyl-3-hydroxydodec-4-enoic acid (Doda), and proline (Pro) residues. The presence of these substructures was further supported by HMQC and HMBC correlations as shown in Table 1. More importantly, the HMBC correlations established the sequential relationships of the individual spin systems and permitted the assignment of the gross structure for **1** as depicted in Figure 1.

Hydrolysis of **1** in 6 N HCl followed by Marfey analysis[9] showed that the configuration of the proline residue was L. To ascertain the absolute stereochemistry of the lactic and isoleucic acid units, the individual acids were isolated from the acid hydrolyzate of **1** by C_{18} reversed-phase HPLC. The CD spectrum of the lactic acid displayed a positive Cotton effect at 210 nm, indicative of the 2*S* configuration.[10] The negative CD curve for the isolated isoleucic acid fragment indicated that the absolute stereochemistry at C-2 was 2*R*.[10] Comparison of its ^1H and ^{13}C NMR spectra with those of authentic samples of D-isoleucic acid and D-*allo*-isoleucic acid rigorously established its identity as the allo isomer. The absolute configuration of the other stereocenter in this residue was, therefore, 3*S*.

The ^1H and ^{13}C NMR spectra of tumonoic acid A (**2**) lacked the signals corresponding to the Lac and Ahmp residues found in **1**. The HRFABMS indicated a molecular formula of $C_{19}H_{33}NO_4$, and the IR spectrum contained a less intense band at 1724 cm^{-1} compared with the one observed in the IR spectrum of **1**. The presence of a L-proline residue was confirmed by Marfey analysis. Basic hydrolysis of **1** yielded a compound that was chromatographically and spectroscopically identical (IR, MS, ^1H and ^{13}C NMR) to **2**. In the ^1H NMR spectrum of **2**, irradiation of the olefinic H-5 of **2** resulted in NOE enhancements for H-6 and Me-2, but not for Me-4. Furthermore, irradiation of Me-4 resulted in NOE enhancements for H-2, H-6, and Me-2, but not for H-5. The double-bond geometry in **1** and **2** was, therefore, *E*.

The presence of the characteristic *threo* pentet[11] (δ 2.78, J = 7.7 Hz, H-2) in the ^1H NMR spectrum suggested that the methyl substituent on C-2 and the hydroxyl group on C-3 of the alkenoic acid residue were anti to each other.

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Table 1. NMR Spectral Data for Compound 1 in $CDCl_3$

C/H no.		^1H (δ, J inHz)	^{13}C[a]	$^1H-^1H$ COSY	HMBC[b]
Doda	1		175.0, s		Me-2, H-2, H-3
	2	2.78, br p (7.5)	41.0, d	Me-2, H-3	Me-2, H-3
	Me-2	1.03, d (7.0)	14.4, q	H-2	H-2, H-3
	3	4.11, d (7.2)	80.1, d	H-2	Me-2, H-2, Me-4, H-5
	4		133.9, s		H-2, H-3, H-6
	Me-4	1.60, s	11.1, q	H-5	H-3, H-5
	5	5.43, br t (7.1)	129.7, d	Me-4, H-6	H-3, Me-4, H-6
	6	2.01, m	27.6, t	H-5	H-5
	7	1.26−1.4	29.17,[c] t		H-5
	8	1.26−1.4	29.20,[c] t		
	9	1.26−1.4	29.3,[c] t		
	10	1.27	31.1, t		H-11, H-12
	11	1.27	22.6, t		H-12
	12	0.87, t (6.8)	14.0, q		
Pro	1		171.7, s		H-2, Ahmp-H-2
	2	4.58, dd (3.6, 8.0)	59.0, d	H-3	
	3	2.01, m, 2.14, m	29.4,[c] t	H-2, H-4	H-2, H-5
	4	2.01, m, 2.14, m	24.7, t	H-3, H-5	H-2, H-4
	5	3.67, m, 3.71, m	47.2, t	H-4	
Ahmp	1		169.1, s		H-2, Lac-H-2
	2	5.12, d (3.3)	75.1, d	H-3	Me-3
	3	2.0 m	36.6, d	H-2	H-2, Me-3, H-5
	Me-3	0.96, d (7.0)	14.1, q		H-2
	4	1.25, m, 1.40, m	25.7, t,		H-2, Me-3, H-5
	5	0.91, t (6.8)	11.5, q		H-4
Lac	1		172.9, s		H-2, H-3
	2	5.14, q (7.0)	69.9, d	H-3	H-3
	3	1.50, d (7.0)	16.9, q	H-2	H-2

[a] Multiplicity deduced from DEPT spectroscopy. [b] Protons showing long-range correlations with indicated carbon. [c] Chemical shifts with same superscript within a given column are interchangeable.

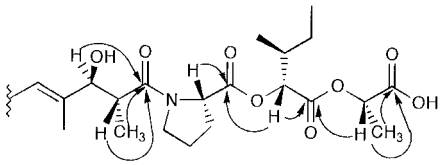

Figure 1. Selected HMBC correlations for compound **1**.

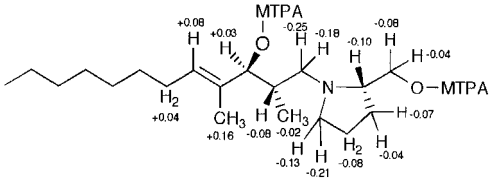

Figure 2. $\Delta\delta$ ($\delta_S - \delta_R$) values for the Mosher diesters of the reduction product of **2** with $LiAlH_4$.

Unfortunately, Mosher analysis of **2** could not be carried out directly to solve the absolute stereochemistry, as the secondary alcohol group appeared to be too sterically hindered for MTPA ester formation. After reduction of **2** with lithium aluminum hydride, however, the resulting aminodiol gave the desired Mosher esters. Actually, an MTPA diester was formed, but the MTPA esterification of the primary alcohol group did not interfere with the stereochemical interpretation given, and the $\Delta\delta$ values shown in Figure 2 indicated that the absolute configuration at C-3 was *S*. The absolute stereochemistry of the Doda unit in **2** was, therefore, 2*R*,3*S*.

Methyl tumonoate A (**3**) and **2** exhibited virtually identical IR, ^1H and ^{13}C NMR, and DEPT spectra. The NMR data for **3**, however, clearly showed the presence of a methoxy functionality, and its ^1H chemical shift and coupling (HMBC) to C-1 of the proline unit were consistent with a methyl ester group. Because HRFABMS analysis indicated a molecular formula of $C_{20}H_{35}NO_4$, **3** was the methyl ester of **2**.

Similarly, methyl tumonoate B (**4**) and **1** exhibited virtually identical IR, ^1H and ^{13}C NMR, and DEPT spectra, but, again, the data clearly showed that **4** possessed a methyl ester group. This was further supported by HMBC data and HREIMS analysis, which indicated that **4** had the molecular formula $C_{29}H_{49}NO_8$. Compound **4** was, therefore, the methyl ester of **1**.

The IR spectra of tumonoic acid C (**5**) and **1** were similar. The ^1H NMR spectra were also similar; however, the one for **5** differed primarily in showing three methyl doublet signals at δ 0.99, 1.00, and 1.04, whereas the one for **1** showed two methyl doublet signals at δ 0.96 and 1.03 and a triplet signal at δ 0.91. This suggested that **5** contained a 2-hydroxy-3-methylbutanoic (2-hydroxy-isovaleric) acid (Hiv) residue in lieu of the Ahmp residue found in **1**. Further confirmation was provided by the HRFABMS, which established the molecular formula as $C_{27}H_{45}NO_8$, one methylene group less than the molecular formula for **1**. The ^{13}C NMR and DEPT spectra also supported the presence of an Hiv residue as shown in Table 3. The absolute stereochemistries of the hydroxy acid units in **5** appeared to be the same as the ones for **1** based on similar ^1H and ^{13}C NMR data.

Compounds **1**−**5** are new noncytotoxic acyclic depsipeptides from *L. majuscula* and assemblages of *L. majuscula* and *S. calcicola*, although the methyl esters (**3** and **4**) could be isolation artifacts. Their trivial names are based on the site of collection of the first cyanobacterial assemblage found to contain these compounds. Their possible ecological role as antifeedants is currently being investigated.

Experimental Section

General Experimental Procedures. Unless otherwise stated, ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ or CD_3CN on a 9.4 T spectrometer (Varian) operating at 400 and 100 MHz, respectively, using residual solvent signals as internal references. All 2D NMR experiments were performed on the same spectrometer. HMQC and HMBC experiments were optimized for $^1J_{CH} = 150$ Hz and $^nJ_{CH} = 6$ Hz, respectively. CD spectra were recorded on a JASCO J-600 spectropolarimeter.

Extraction and Isolation. Fractionation of the cytotoxic lipophilic extract (VP337L, 11.64 g) of an assemblage of *L. majuscula* and *S. calcicola* (VP337) collected at Tumon Bay, Guam, in September 1995, and the isolation of lyngbyastatin 1 mixed with its 15-*epi* analogue (150 mg) and dolastatin 12 mixed with its 15-*epi* analogue (167 mg) has previously been described.[6,12] Tumonoic acid B (**1**) (272 mg) was isolated from the cytotoxic 60% aqueous CH_3CN fraction (from flash reversed-phase chromatography of VP337L) and separated from the cytotoxins by semipreparative reversed-phase HPLC (Econosil C_{18}, 10 μM, 1.0 × 25.0 cm, 2 mL/min) using a linear gradient of CH_3CN in 0.1% HCOOH (20−100% over 20 min and then 100% CH_3CN for 20 min), with lyngbyastatin 1, dolastatin 12, and **1** eluting at t_R 24.2, 24.8, and 28.2 min, respectively. The 80% aqueous CH_3CN fraction was also subjected to semipreparative reversed-phase C_{18} chromatography using a 65% CH_3CN in 0.1% HCOOH isocratic system to afford **3** (38.7 mg, t_R 19.6 min) and additional **1** (33.3 mg, t_R 22.1 min).

Fractionation of the more polar extract (VP337A, 1.0 g) has also been previously reported.[6] Subjection of the 65% aqueous CH_3CN fraction to semipreparative reversed-phase HPLC using the linear gradient elution system described above gave **2** (47.0 mg, t_R 23.2 min), **5** (9.8 mg, t_R 26.8 min), **1** (148.1 mg, t_R 28.2 min), and **4** (12.0 mg, t_R 32.4 min).

L. majuscula (VP216) was collected from Piti Bomb Hole, Guam, in February 1995. The freeze-dried organism was

Table 2. ^{1}H NMR Spectral Data for Compounds **2**, **3**, and **5** in $CDCl_3$ and **4** in CD_3CN (δ_H, *J* in Hz)

C/H no.		2	3	4	5
Doda	2	2.79, p (7.1)	2.73, p (7.2)	2.79, p (7.1)	2.79, p (7.2)
	Me-2	0.94, d (7.1)	0.99, d (7.0)	0.930, d (7.1)	0.99, d (7.2)
	3	4.17, d (6.6)	4.05, d (7.1)	3.94, dd (4.8, 7.1)	4.13, d (8.2)
	OH-3			3.30, br d (4.8)	
	Me-4	1.62, s	1.59, s	1.57, s	1.62, s
	5	5.46, br t (6.7)	5.41, br t (7.1)	5.38, br t (7.1)	5.45, br t (7.1)
	6	2.04, m	2.00, m	2.01, m	2.04, m
	7	1.27−1.36	1.25−1.35	1.26−1.36	1.27−1.36
	8	1.27−1.36	1.25−1.35	1.26−1.36	1.27−1.36
	9	1.27−1.36	1.25−1.35	1.26−1.36	1.27−1.36
	10	1.27	1.25	1.28	1.27
	11	1.27	1.25	1.28	1.27
	12	0.88, t (6.8)	0.87, t (6.8)	0.87, t (7.1)	0.88, t (6.7)
Pro	2	4.61, dd (2.8, 7.3)	4.50, m	4.38, dd (4.1,8.7)	4.59, dd (4.3, 8.2)
	3	2.04, m	2.00, m, 2.18,m	2.01, m, 2.20, m	2.04, m
	4	2.04, m	2.00, m	1.94, m, 2.08, m	2.04, m
	5	3.71, m, 3.61, m	3.64, m	3.64, m	3.75, m, 3.68, m
Hiv	2				4.93, d (4.4)
	3				2.1−2.3, m
	4a				1.00, d (6.3)
	4b				1.04, d (7.0)
Ahmp	2			5.00, d (3.3)	
	3			1.96, m	
	Me-3			0.927, d (7.1)	
	4			1.24, m, 1.38, m	
	5			0.86, t (7.5)	
Lac	2			5.06, q (7.1)	5.20, q (6.9)
	3			1.43, d (7.1)	1.52, d (6.7)
	$-OCH_3$		3.72, s	3.68, s	

extracted with CH_2Cl_2 to give 1.14 g of lipophilic extract, VP216L. This material was fractionated on a Varian Mega Bond Elut Si gel column, eluting initially with hexanes followed by mixtures of progressively increasing amounts of EtOAc in hexanes. A fraction eluting at 15% EtOAc in hexanes was rechromatographed on the same column, eluting initially with CH_2Cl_2 followed by mixtures of increasing amounts of MeOH in CH_2Cl_2. The 2% MeOH fraction afforded **1** (10.0 mg) along with lyngbyastatin 1. A fraction eluting at 20% EtOAc in hexanes from the original Bond Elut Si gel chromatography was also rechromatographed on the same column, eluting initially with CH_2Cl_2 followed by CH_2Cl_2 mixtures containing increasing amounts of MeOH. The 6% MeOH fraction was subjected to reversed-phase C_{18} semipreparative HPLC using the linear gradient elution system described above to give **4** (9.6 mg).

Tumonoic acid B (1): pale yellow oil, $[\alpha]_D$ −14° (*c* 1.6, $CHCl_3$); IR (film) ν_{max} 3437, 1736, 1620 cm^{-1}; ^{1}H NMR, ^{13}C NMR, ^{1}H−^{1}H COSY, and HMBC data, see Table 1; HREIMS *m*/*z* $[M]^+$ 525.3380 (calcd for $C_{28}H_{47}NO_8$, 525.3302) 507 (25), 426 (35), 357 (100).

Tumonoic acid A (2): pale yellow oil, $[\alpha]_D$ −79° (*c* 1.1, $CHCl_3$); IR (film) ν_{max} 3417, 1724, 1618 cm^{-1}; ^{1}H NMR data, see Table 2; ^{13}C NMR data, see Table 3; HRFABMS *m*/*z* $[M + H]^+$ 340.2471 (calcd for $C_{19}H_{34}NO_4$, 340.2488) 322 (100), 114 (30).

Methyl tumonoate A (3): pale yellow oil, $[\alpha]_D$ −51° (*c* 1.3, $CHCl_3$); IR (film) ν_{max} 3420, 1748, 1627 cm^{-1}; ^{1}H NMR data, see Table 2; ^{13}C NMR data, see Table 3; HRFABMS *m*/*z* $[M + H]^+$ 354.2633 (calcd for $C_{20}H_{36}NO_4$, 354.2644) 336 (100), 128 (40).

Methyl tumonoate B (4): pale yellow oil, $[\alpha]_D$ −76° (*c* 1.0, $CHCl_3$); IR (film) ν_{max} 3421, 1748, 1636 cm^{-1}; ^{1}H NMR data, see Table 2; ^{13}C NMR data, see Table 3; HREIMS *m*/*z* $[M]^+$ 539.3519 (calcd for $C_{29}H_{49}NO_8$, 539.3458).

Tumonoic acid C (5): pale yellow oil, $[\alpha]_D$ −35° (*c* 1.2, $CHCl_3$); IR (film) ν_{max} 3432, 1744, 1622 cm^{-1}; ^{1}H NMR data, see Table 2; ^{13}C NMR data, see Table 3; HRFABMS *m*/*z* $[M + Na]^+$ 534.3038 (calcd for $C_{27}H_{45}NO_8$ + Na, 534.3043).

Absolute Configuration of the Proline Residue. Solutions of compounds **1**, **2**, **4**, and **5** (1 mg) in 6 N HCl were each heated at 108 °C for 18 h and then concentrated to dryness. The residues were each dissolved in H_2O (50 μL), and to the resulting mixtures were added a 1% (w/v) solution of 1-fluoro-2,4-dinitrophenyl-5-L-alanine amide (Marfey's reagent) in Me_2CO (100 μL) and 1 M $NaHCO_3$. After heating at 37 °C for 1 h, the reaction mixture was cooled, acidified with 2 N HCl (10 μL), and evaporated to dryness. The resulting products were then resuspended in DMSO−H_2O (1:1) (2 mL), and aliquots were subjected to reversed-phase HPLC analysis [Econosphere (Alltech) C_{18}, 5 μM, 0.46 × 15.0 cm, 2 mL/min; detection at 340 nm] using a CH_3CN−50 mM NH_4OAc linear gradient (10−50% over 60 min). The t_R values for Marfey derivatives of authentic D- and L-proline were 12.2 and 8.8 min, respectively. The Marfey derivatives from compounds **1**, **2**, **4**, and **5** all showed t_R values of 8.8 min.

Acid Hydrolysis of 1 and Isolation of Lactic and Isoleucic Acid. A solution of compound **1** (64 mg) in 6 N HCl (5 mL) was heated at 108 °C for 18 h. The reaction mixture was evaporated to dryness, and the residue was subjected to semipreparative reversed-phase HPLC [Econosil (Alltech) C_{18}, 10 μM, 1.0 × 25.0 cm, 2 mL/min; detection at 220 nm] using a CH_3CN−0.1% HCOOH linear gradient (0−100% over 60 min after a 5 min period at 0% CH_3CN) for elution. This afforded L-lactic acid (3.2 mg), which was collected between 8.0 and 22.0 min [CD (0.01 N HCl) $[\theta]_{210}$ +1009; ^{1}H NMR (D_2O, 300 MHz) δ 1.36 (3H, d, J = 6.8 Hz, H-3), 4.31 (1H, q, J = 6.8 Hz, H-2)], and D-*allo*-isoleucic acid (6.5 mg) at t_R 29.2 min [CD (0.01 N HCl) $[\theta]_{209}$ −2890; ^{1}H NMR (D_2O) δ 0.94 (3H, d, J = 6.9 Hz, CH_3-3), 1.00 (3H, t, J = 7.4 Hz, H-5), 1.37 (1H, m, H-4), 1.52 (1H, m, H-4), 1.93 (1H, ~hd, J ca. 7.0, 3.3 Hz, H-3), 4.37 (1H, d, J = 3.1 Hz, H-2); ^{13}C NMR (D_2O) δ 12.3 (q, C-5), 14.2 (q, CH_3-3), 26.7 (t, C-4), 39.2 (d, C-3), 74.2 (d, C-2), 179.5 (s, C-1)].

Basic Hydrolysis of 1. Compound **1** (27.5 mg) was placed in 0.5 N NaOH−MeOH (1:1) for 18 h at room temperature. The reaction mixture was dried under N_2 to remove MeOH, acidified by the addition of HCl and extracted with EtOAc. Semipreparative C_{18} reversed-phase HPLC using a CH_3CN−0.1% HCOOH linear gradient (20−100% over 20 min and then 100% CH_3CN for 20 min) afforded 1.5 mg of unhydrolyzed **1** and 15.1 mg of a compound with spectral characteristics (HRFABMS, ^{1}H NMR, IR, $[\alpha]_D$) identical to **2**.

Table 3. ^{13}C NMR Spectral Data for Compounds **2**, **3**, and **5** in $CDCl_3$ and **4** in CD_3CN (δ_C[a])

C/H no.		2	3	4	5
Doda	1	177.5, s	175.0, s	175.4, s	175.0, s
	2	41.3, d	41.1, d	41.5, d	41.0, d
	Me-2	14.3, q	14.1, q	15.2, q	14.4, q
	3	80.3, d	80.0, d	81.1, d	80.2, d
	4	133.7, s	134.1, s	136.2, s	133.8, s
	Me-4	10.9, q	11.4, q	11.4, q	11.1, q
	5	130.2, d	129.2, d	129.1, d	129.8, s
	6	27.58,[b] t	27.6, t	28.1, t	27.6, t
	7	29.1,[c] t	29.1,[b] t	30.1,[b] t	29.1,[b] t
	8	29.3,[c] t	29.2,[b] t	29.1,[b] t	29.2,[b] t
	9	29.6,[c] t	29.3,[b] t	30.2,[b] t	29.3,[b] t
	10	31.8, t	31.8, t	32.6, t	31.8, t
	11	22.6, t	22.6, t	23.3, t	22.6, t
	12	14.1, q	14.4, q	14.3, q	14.1, q
Pro	1	173.0, s	172.7, s	172.8, s	172.6, s
	2	59.7, d	58.5, d	59.9, d	58.9, d
	3	27.63,[b] t	29.4,[b] t	29.9,[b] t	29.4,[b] t
	4	24.7, t	24.8, t	25.4, t	24.7, t
	5	47.8, t	47.0, t	48.0, t	47.2, t
Hiv	1				168.6, s
	2				76.8, d
	3				30.1, d
	4a				16.8, q
	4b				18.5, q
Ahmp	1			170.2, s	
	2			75.4, d	
	3			37.6, d	
	Me-3			14.4, q	
	4			26.4, t	
	5			11.8, q	
Lac	1			171.6, s	171.8, s
	2			70.4, d	69.9, d
	3			17.2, q	17.1, q
	$-OCH_3$		52.2, q	52.9, q	

[a] Multiplicity deduced from DEPT spectroscopy. [b,c] Chemical shifts bearing the same superscript within a given column are interchangeable.

Reduction of 2 with $LiAlH_4$ and Mosher Analysis of the Aminodiol Product. Compound **2** (14 mg) was dissolved in 3 mL of Et_2O and the solution added dropwise to a solution of $LiAlH_4$ (30 mg) in 3 mL of Et_2O. The mixture was heated for 5 h and, after cooling, excess reagent was decomposed by carefully adding wet solvent. The precipitate of $Al(OH)_3$ was filtered off, washed with Et_2O, and KOH was added to the filtrate until pH 14 of the aqueous phase. The phases were separated, and the aqueous phase was extracted with 3 × 15 mL of Et_2O. The combined organic phases were washed with 10 mL of H_2O, and evaporation of the solvent yielded the expected aminodiol as a colorless oil (8.8 mg).

A solution of the aminodiol (1.0 mg), 10 μL of (*R*)-MTPA-Cl, 200 μL of dry Et_3N, and 2.0 mg of DMAP in 1 mL of CH_2Cl_2 was stirred for 18 h. Saturated $NaHCO_3$ (2 mL) and EtOAc (1 mL) were then added, and the mixture was stirred for an additional 30 min to destroy excess MTPA-Cl. The phases were separated, and the aqueous phase was extracted with 3 × 2 mL of EtOAc. The organic phases were combined, washed with 10 mL of brine, and the solvent evaporated. The yellow oily residue was applied to a Si gel column, and elution with hexane−EtOAc (10:1) afforded the (*S*)-MTPA diester as a colorless oil: ^1H NMR ($CDCl_3$, 500 MHz) δ 0.74 (3H, d, J = 6.6 Hz, $CHCH_3$), 0.88 (3H, t, J = 7.0 Hz, CH_2CH_3), 1.20−1.38 (10H, m, $CH_3CH_2CH_2CH_2CH_2CH_2$), 1.56 (3H, s, CCH_3), 1.57 (1H, m, NCH_2CH_2CHH'), 1.59 (2H, m, NCH_2CH_2), 1.77 (2H, m, $NCHH'CH_2CHH'$), 1.90 (1H, m, $CHCH_3$), 2.02 (2H, m, C=CHCH_2), 2.08 (1H, dd, J = 11.4, 2.3 Hz, $CHCHH'N$), 2.24 (1H, t, J = 11.4 Hz, $CHCHH'N$), 2.50 (1H, m, NCH), 2.91 (1H, m, $NCHH'CH_2$), 3.52 (3H, s, OCH_3), 3.54 (3H, s, OCH_3), 4.11 (1H, dd, J = 10.9, 4.6 Hz, $CHH'OMTPA$), 4.21 (1H, J = 11.0, 6.2 Hz, $CHH'OMTPA$), 4.99 (1H, d, J = 9.6 Hz, $CHOMTPA$), 5.56 (1H, br t, J = 6.8 Hz, C=CH), 7.32−7.55 (10H, m, ArH); HRFABMS m/z $[M + H]^+$ 744.3685 (calcd for $C_{39}H_{52}F_6NO_6$, 744.3699).

Using the same procedure with (*S*)-MTPA-Cl, the (*R*)-MTPA diester was obtained as a colorless oil: ^1H NMR ($CDCl_3$, 500 MHz) δ 0.76 (3H, d, J = 6.6 Hz, $CHCH_3$), 0.88 (3H, t, J = 7.0 Hz, CH_2CH_3), 1.20−1.36 (10H, m, $CH_3CH_2CH_2CH_2CH_2CH_2$), 1.40 (3H, s, CCH_3), 1.61 (1H, m, NCH_2CH_2CHH'), 1.67 (2H, m, NCH_2CH_2), 1.84 (1H, m, NCH_2CH_2CHH'), 1.98 (4H, m, $CHCH_3$, C=CHCH_2, $NCHH'CH_2$), 2.33 (1H, dd, J = 11.5, 2.7 Hz, $CHCHH'N$), 2.42 (1H, t, J = 11.4 Hz, $CHCHH'N$), 2.60 (1H, m, NCH), 3.04 (1H, m, $NCHH'CH_2$), 3.52 (3H, s, OCH_3), 3.55 (3H, s, OCH_3), 4.15 (1H, dd, J = 11.1, 3.8 Hz, $CHH'OMTPA$), 4.29 (1H, dd, J = 11.1, 5.7 Hz, $CHH'OMTPA$), 4.96 (1H, d, J = 9.4 Hz, $CHOMTPA$), 5.48 (1H, br t, J = 7.0 Hz, C=CH), 7.34−7.57 (10H, m, ArH); HRFABMS m/z $[M + H]^+$ 744.3670 (calcd for $C_{39}H_{52}F_6NO_6$, 744.3699).

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References and Notes

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(7) Lyngbyastatin 1 and dolastatin 12 were isolated as inseparable mixtures with their C-15 epimers.
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(12) To date we have not been able to separate these mixtures by HPLC.

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