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Structures of Amamistatins A and B, Novel Growth Inhibitors of Human Tumor Cell Lines from *Nocardia asteroides*

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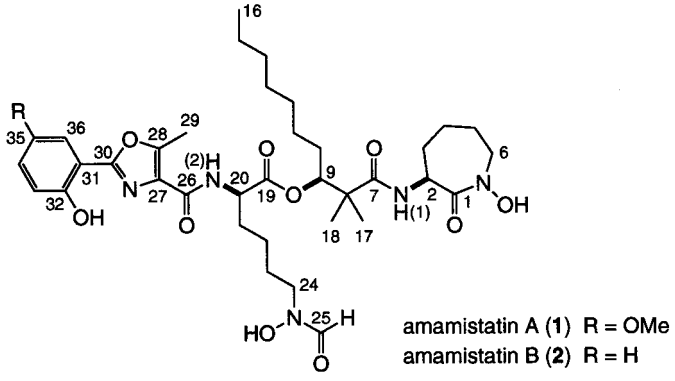
Abstract—Two new lipopeptides, amamistatins A and B, were isolated from *Nocardia asteroides* SCRC-A2359, and their absolute stereostructures were determined by spectroscopic and chemical analyses. Amamistatin A inhibited the growth of human tumor cell lines. © 2000 Elsevier Science Ltd. All rights reserved.

Introduction

In our screening for compounds derived from microorganisms that inhibited the growth of human tumor cell lines, amamistatins A (**1**) and B (**2**) were isolated from *Nocardia asteroides* SCRC-A2359, which was isolated from a soil sample collected at Amami Island in Kagoshima Prefecture, Japan. We have previously reported the isolation and structure of **1** and **2**.[1] The total synthesis of **1** has been already achieved by Shioiri and co-workers.[2] Recently, we also determined the absolute structure of **2**. We report here the detailed isolation and structure determination of **1** and **2**.



Results and Discussion

Structure of amamistatin A

Amamistatins were purified from the mycelial cake of culture broth through a series of processes: EtOAc extraction, silica gel column chromatography, and reversed-phase HPLC. The biological method for monitoring the biological active components was used cytotoxicity against mouse lymphocytic leukemia cells (P388). The molecular formula of **1** was determined to be $C_{37}H_{55}N_5O_{11}$ by HRFABMS [*m*/*z* 746.3981, calcd for $C_{37}H_{56}N_5O_{11}$ $(M+H)^+$ 746.3976]. The IR spectrum showed bands at 1740 and 1660 cm^{-1} that were assigned to ester and amide groups, respectively. A $FeCl_3$ test of **1** indicated the presence of a phenolic functionality.

The NMR data for **1** are summarized in Table 1. The ^1H NMR spectrum of **1** showed the presence of two amide NH groups (δ 8.93 and 7.48), three hydroxyl groups (δ 9.93, 9.77, and 9.50), a 1,2,4-trisubstituted benzene ring (δ 7.25, 7.04, and 7.00) and a methoxy group (δ 3.76). In the ^{13}C NMR and DEPT spectra, 37 carbon signals were observed, including five carbonyl carbons (δ 173.3, 171.6, 168.6, 161.6, and 160.8), nine aromatic carbons (δ 157.1, 152.4, 152.1, 150.2, 128.5, 120.0, 118.4, 110.1, and 109.3), one oxymethine carbon (δ 77.9), one methoxy carbon (δ 55.6), and four nitrogen-bearing carbons (δ 52.3, 52.0, 50.9, and 48.9). Duplicate signals due to a formyl group (δ_C 161.6, 157.0; δ_H 8.22, 7.88) were also observed. Due to restricted rotation about the formamide moiety, the NMR signals for several protons and carbons were doubled at 23°C, as shown in Table 1. The doubled NMR signals broadened at 60 and 90°C, and were superimposed at 120°C. The remaining carbon signals were assigned to four methyls, 12 methylenes, and one quaternary carbon.

A detailed analysis of the phase-sensitive DQF-COSY and HOHAHA spectra of **1** gave three partial structures, two lysine residues [NH(1)–C2–C6 and NH(2)–C20–C24] and C9–C16 (Fig. 1). In addition, the presence of a

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Table 1. NMR Data for amamistatin A (**1**) in DMSO-d_6 at 23°C

Position	^{1}H (ppm)	^{13}C (ppm)	Position	^{1}H (ppm)	^{13}C (ppm)
1		168.6[a]	21	1.88 m	29.9
2	4.40 br d (11.5)[b]	50.9	22	1.35 m	22.6
3	1.74 m, 1.39 m	30.0	23	1.56 m	26.4
4	1.78 m, 1.63 m	27.0	24	3.37 m [3.43 m][c]	48.9 [45.4][c]
5	1.66 m, 1.39 m	25.5	25	8.22 s [7.88 s][c]	161.6 [157.0][c]
6	3.87 dd (15.8, 11.7)	52.3	26		160.8
	3.47 dd (15.8, 3.8)		27		128.5
7		173.3	28		152.4
8		45.6	29	2.65 s	11.4
9	5.09 d (9.0)	77.9	30		157.1
10	1.43 m, 1.37 m	29.4	31		110.1
11	1.18 m, 1.12 m	25.6	32		150.2
12	1.15 m	28.5	33	7.00 d (9.0)	118.4
13	1.23 m	28.6	34	7.04 dd (9.0, 2.9)	120.0
14	1.15 m	31.1	35		152.1
15	1.23 m	22.0	36	7.25 d (2.9)	109.3
16	0.81 t (7.1)	13.9	37	3.76 s	55.6
17	1.09 s	19.7 [19.6][c]	C32–OH	9.93 br s	
18	1.04 s	22.9	OH	9.77 br s	
19		171.6	OH	9.50 br s [9.93 br s][c]	
20	4.49 m [4.47 m][c]	52.0	NH(1)	7.48 br d (6.2)	
			NH(2)	8.93 br d (8.2) [8.89 br d (8.2)][c]	

[a] Recorded at 150 MHz.
[b] Recorded at 600 MHz. Coupling constants (Hz) are in parentheses.
[c] Observed as doubled signals.

methyloxazole group in **1** was revealed by correlations in the HMBC spectrum, H-29/C27 and H-29/C28, as well as by characteristic NMR signals ($\delta_{H\text{-}29}$ 2.65, δ_{C27} 128.5, δ_{C28} 152.4, and δ_{C30} 157.1) that corresponded to those of the methyloxazole group in nostocyclamide.[3] The connections among the aromatic partial structural units were clarified by the HMBC correlations (H-37/C35, H-33/C31, C32-OH/C32, C32-OH/C31, and H-36/C30). HMBC correlations suggested the presence of a 2-aminocaprolactam structure (H-2/C1 and H-6/C1), a 2,2-dimethyl-3-hydroxydecanoic acid portion (H-9/C8, H-9/C7, H-18/C8, H-18/C9, and H-18/C17), and an *N*-formyllysine residue (H-20/C19, H-25/C24, and H-20/C26), which were connected by HMBC correlations, H-2/C7 and H-9/C19.

The locations of the two remaining hydroxyl groups in **1** were determined as follows. Hydrogenolysis (H_2, Pd/C, MeOH, 23°C) of **1** gave **3**, the molecular weight of which was 32 MS units (2×O) less than that of **1**. In addition, the ^{1}H NMR spectrum of **3** showed the presence of two more amide NH groups (δ 6.53, 6.04) than in **1**. This information indicated that **1** had two amide N–OH groups, which were degraded to amide NH groups by hydrogenolysis. Although a connection between C26 and C27 could not be established, such connectivity was obvious based on a consideration of the molecular formula of **1**. Thus, the gross structure of amamistatin A was clarified as shown in formula **1**. The chemical shifts of ^{13}C and ^{1}H NMR spectra of **1** were considerably in accord with those of formobactin[4] closely related to **1**, except positions 34, 35 and 36.

The absolute stereostructure of **1** was elucidated as follows (Scheme 1). Hydrogenolysis of **1** followed by methanolysis gave methyl ester **5** and alcohol **7**. Acidic hydrolysis of **5** and **7** (6 M HCl, 110°C, 24 h) followed by separation by reversed-phase HPLC gave lysines, respectively. The absolute configurations of the lysines in **5** and **7** were respectively determined to be D and L by a chiral HPLC analysis. The absolute stereochemistry of C9 in **7** was determined using a modified Mosher's method.[5] The alcohol **7** was transformed into (*S*)- and (*R*)-MTPA esters, **9a** and **9b**, the ^{1}H NMR signals of which were assigned based on the

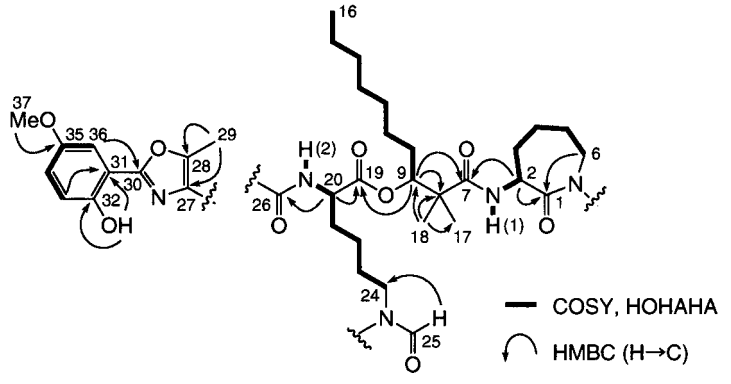
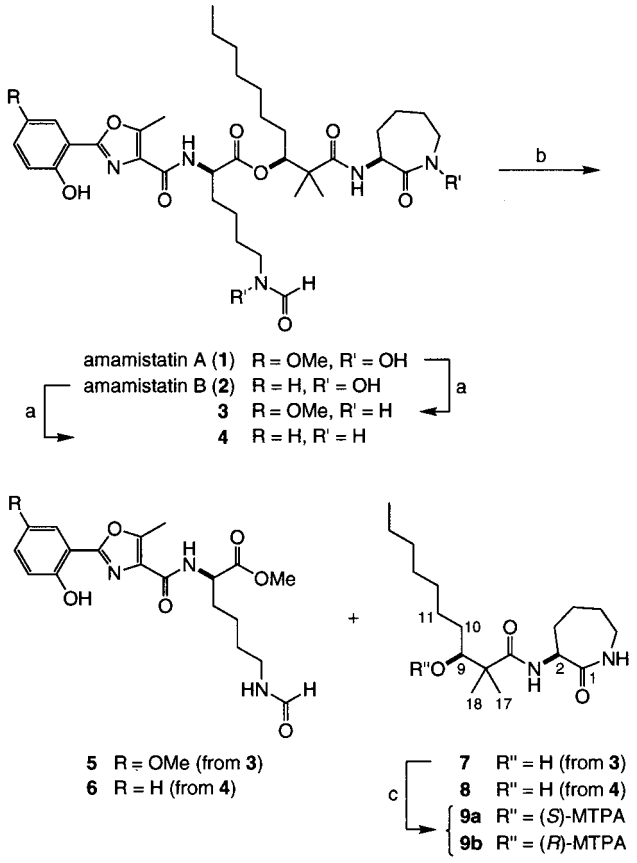


Figure 1. Partial structures of amamistatin A (**1**) based on phase-sensitive DQF-COSY and HOHAHA spectra and selected HMBC correlations.



Scheme 1. (a) H_2, Pd/C, MeOH; (b) NaOMe, MeOH; (c) (*R*)- or (*S*)-MTPACl, Py.

2D NMR spectra, and the $\Delta\delta$ values ($\delta_S-\delta_R$, ppm) were then calculated. The results established that the absolute stereochemistry of C9 in **7** was *S*. Thus, the absolute stereochemistry of amamistatin A was determined as depicted in formula **1**.

Structure of amamistatin B

The molecular formula of **2** was determined to be $C_{36}H_{53}N_5O_{10}$ by HRFABMS [*m/z* 716.3901, calcd for $C_{36}H_{54}N_5O_{10}$ $(M+H)^+$ 716.3871], which is 30 MS units (CH_2O) less than that of **1**. The NMR data for **2** are summarized in Table 2. The ^1H NMR spectrum of **2** showed the presence of a 1,2-disubstituted benzene ring (δ 7.85, 7.40, 7.03, and 6.99). In the ^{13}C NMR and DEPT spectra, 36 carbon signals were observed, including five carbonyl carbons (δ 177.3, 173.3, 171.1, 164.5, and 164.0), nine aromatic carbons (δ 160.3, 158.5, 154.9, 134.4, 130.3, 128.1, 121.4, 118.7, and 112.3), one oxymethine carbon (δ 80.9), and four nitrogen-bearing carbons (δ 54.6, 54.4, 53.5, and 51.5), while that of a methoxy carbon was not observed. The gross structure of **2** was determined by the same 2D NMR technique as described above for **1**. Thus, amamistatin B was shown to be the demethoxy derivative of **1**.

The absolute stereostructure of **2** was elucidated by a procedure similar to that for **1**. Hydrogenolysis of **2** followed by methanolysis gave methyl ester **6** and alcohol **8**. The ^1H NMR spectrum of **8** was identical to that of **7** derived from amamistatin A (**1**). Acidic hydrolysis of **6** and **8** (6 M HCl, 110°C, 24 h) followed by separation by reversed-phase HPLC gave lysines. The absolute configurations of the lysines in **6** and **8** were respectively determined to be D and L by a chiral HPLC analysis. Since both of the lysines from **7** and **8** were L, the absolute stereochemistry of C9 in **8** should be the same as that in **7**. Thus, the absolute stereochemistry of amamistatin B was determined as shown in formula **2**.

Biological activities

Amamistatins A (**1**) had anti-proliferative, but not cell-killing, effects against several kinds of human tumor cell lines. The IC_{50} values of **1** were 0.48, 0.56, and 0.24 μM

Table 2. NMR Data for amamistatin B (**2**) in CD_3OD at 23°C

Position	^1H (ppm)	^{13}C (ppm)	Position	^1H (ppm)	^{13}C (ppm)
1		171.1[a]	19		173.3 [173.2][b]
2	4.52 br d (10.3)[c]	53.5	20	4.67 dd (9.9, 5.1)	54.4 [54.5][b]
3	1.88 m, 1.55 m	32.3		[4.64 dd (9.9, 5.1)][b]	
4	1.91 m, 1.76 m	29.2	21	2.02 m, 1.93 m	32.2 [32.1][b]
5	1.76 m, 1.56 m	27.3	22	1.56 m, 1.45 m	24.7 [24.6][b]
6	3.92 dd (15.9, 11.8)	54.6 [54.5][b]	23	1.77 m, 1.70 m	28.1
	3.65 dd (15.9, 3.8)		24	3.52 t (6.2) [3.62 m, 3.54 m][b]	51.5 [47.7][b]
7		177.3	25	8.27 s [7.93 s][b]	164.5 [159.9][b]
8		48.0	26		164.0
9	5.17 dd (10.2, 2.1)	80.9 [80.8][b]	27		130.3
10	1.55 m, 1.48 m	31.6 [31.5][b]	28		154.9
11	1.28 m, 1.24 m	27.7	29	2.71 s	12.2
12	1.20 m	30.7	30		160.3
13	1.32 m	30.8	31		112.3
14	1.20 m	33.3	32		158.5
15	1.25 m	24.1	33	7.03 dd (8.3, 0.7)	118.7
16	0.84 dd (7.1, 6.1)	14.8	34	7.40 ddd (8.3, 7.3, 1.5)	134.4
17	1.18 s	22.2 [22.0][b]	35	6.99 ddd (7.9, 7.3, 0.7)	121.4
18	1.18 s	23.6 [23.3][b]	36	7.85 dd (7.9, 1.5)	128.1

[a] Recorded at 150 MHz.
[b] Observed as doubled signals.
[c] Recorded at 600 MHz. Coupling constants (Hz) are in parentheses.

against MCF-7 breast, A549 lung, and MKN45 stomach cancer cell lines, respectively. Amamistatins A (**1**) and B (**2**) exhibited potent cytotoxicity against mouse lymphocytic leukemia cells (P388), with IC_{50} values of 15 and 16 ng/mL, respectively. This result may be meant that the methoxy group of **1** is not essential for biological activity. The structures of **1** and **2** are closely related to those of formobactin[4] and nocobactin NA.[6] Mycobactins form extremely stable hexadentate iron (III) complexes by binding the iron with two hydroxamic acids and a 2-hydroxyphenyloxazoline moiety.[7] Therefore, it was presumed that the antitumor activities of amamistatins are based on their properties as siderophores. Further studies on the mode of action and the selectivity for tumor cells are currently in progress.

Conclusion

Two new lipopeptides, amamistatins A (**1**) and B (**2**), were isolated from *N. asteroides*. The absolute stereostructures of **1** and **2** were determined by spectroscopic and chemical analyses. Amamistatin A (**1**) inhibited the growth of human tumor cell lines such as MCF-7 breast, A549 lung, and MKN45 stomach cancer cell lines. Further biological studies of **1** and **2** are currently in progress.

Experimental

General aspects

Optical rotations were measured with a JASCO DIP-1000 polarimeter. NMR spectra were determined on a JEOL JNM-A400 [400 MHz (^{1}H)] or a JEOL JNM-A600 [600 MHz (^{1}H) and 150 MHz (^{13}C)] spectrometer. The ^{1}H and ^{13}C chemical shifts were referenced to the solvent peaks [(δ_H=3.31 and δ_C=49.5 in methanol-d_4), (δ_H=2.49 and δ_C=39.5 in DMSO-d_6), or (δ_H=7.26 in $CDCl_3$)]. Infrared (IR) spectra were measured with a JASCO FT/IR-230 spectrophotometer. UV/VIS spectra were recorded on a JASCO V-550 spectrophotometer. High-resolution mass spectra (HRMS) and FAB mass spectra (FAB-MS) were obtained on a JEOL JMS-LG2000 mass spectrometer. Column chromatography was performed on silica gel (Wako gel C-200). Reversed-phase high performance liquid chromatography (HPLC) was carried out on CAPCELL PAK C_{18} UG120 (Shiseido Co., Ltd), Develosil ODS-HG-5, Develosil Ph-5 (Nomura Chemical Co., Ltd), or a chiral column CROWNPAK CR (+) (Daicel Chemical Ind., Ltd). Preparative TLC was carried out using Merck precoated silica gel 60 F_{254} HPTLC plates. Absolute methanol was distilled from $Mg(OMe)_2$. Pyridine was distilled from calcium hydride.

Isolation

The producing organism, strain SCRC-A2359, was isolated from a soil sample collected at Amami Island in Kagoshima Prefecture, Japan. Isolate was analyzed by MIDI's system based on gas liquid chromatography analysis of fatty acids produced from organism.[8] A similarity index (SI) value of 0.667 with *N. asteroides* was obtained. This SI value is large enough to suggest that isolate SCRC-A2359 is a strain of *N. asteroides*. Strain SCRC-A2359 has been deposited in the National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology, Tsukuba, Japan, under the name SCRC-A2359 and accession number FERM P-16804.

N. asteroides SCRC-A2359 was added to medium (100 mL) containing 3% soluble starch, 1% glucose, 0.5% peptone, 2% bean-peptide, 0.5% yeast extract and 0.2% $CaCO_3$ at pH 7.0 in a 500 mL flask, and pre-cultured at 26.5°C and 160 rpm for 4 days. This pre-culture was added to 45 units of same flask and cultured under the same conditions for 35 days. The cultured broth (4.5 L) was centrifuged, and the mycelial cake was extracted with methanol (900 mL). The extract was concentrated to a volume of 50 mL by an evaporator, adjusted to pH 3 with hydrochloric acid and extracted with ethyl acetate. The organic layer was concentrated to give an oil (450 mg). The materials were dissolved with chloroform-methanol (2:1) and absorbed with silica gel (0.9 g, Wako gel C-200). This was applied to a silica gel column (260×12 mm, i.d.), washed with 450 mL of hexane–acetone (4:1) and eluted with 450 mL of hexane–acetone (3:2). The eluate was concentrated in vacuo to give an oil (281 mg). This oily material was separated by HPLC [CAPCELL PAK C_{18} UG120 (250×20 mm, i.d.); flow rate, 20 mL/min; detection, UV 210 nm; solvent, acetonitrile–0.1% aqueous phosphoric acid (3:2)]. The fractions at retention times 11.6 and 12.6 min were concentrated, and the aqueous residues were extracted with ethyl acetate. The extracts were concentrated to give amamistatin A (**1**) (21.8 mg) and amamistatin B (**2**) (6.2 mg) as brown oils, respectively.

Amamistatins A (1) and B (2). 1: $[\alpha]_D^{26}=-9.8$ (*c* 0.61, MeOH); UV (MeOH) λ_{max} 335 (ϵ 11,000), 272 (ϵ 22,000) nm; FT/IR ($CHCl_3$) 3340, 1740, 1660, 1500 cm^{-1}. **2:** $[\alpha]_D^{28}=-8.2$ (*c* 0.47, MeOH); UV (MeOH) λ_{max} 307 (ϵ 12,000), 266 (ϵ 22,000) nm; FT/IR ($CHCl_3$) 3350, 1740, 1665, 1510 cm^{-1}.

Hydrogenolysis of 1. To a solution of amamistatin A (**1**) (1.6 mg, 2.1 μmol) in methanol (0.3 mL) was added 10% palladium–carbon (3 mg). After being stirred at room temperature for 8 h under a hydrogen atmosphere, the reaction mixture was filtered through a pad of Celite, and the residue was washed with methanol. The filtrate and washings were combined and concentrated. The residual oil was purified by HPLC [Develosil ODS-HG-5 (250×10 mm, i.d.); flow rate, 2 mL/min; detection, UV 254 nm; solvent, methanol–H_2O (4:1)] to give **3** (t_R=43.7 min, 1.1 mg, 72%) as a colorless oil: FT/IR ($CHCl_3$) 3350, 1740, 1665, 1510 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) 10.23 (s, 1H) [10.05 (s, 1H)], 8.31 (br d, *J*=7.9 Hz, 1H) [8.16 (br d, *J*=8.8 Hz, 1H)], 8.19 (br s, 1H), 7.68 (br d, *J*=6.2 Hz, 1H) [7.90 (br d, *J*=5.3 Hz, 1H)], 7.30 (d, *J*=3.0 Hz, 1H), 6.98 (dd, *J*=8.9, 3.0 Hz, 1H), 6.95 (d, *J*=8.9 Hz, 1H), 6.53 (br t, *J*=6.3 Hz, 1H) [7.78 (br t, *J*=6.3 Hz, 1H)], 6.04 (m, 1H), 5.04 (dd, *J*=10.5, 2.5 Hz, 1H) [4.97 (dd, *J*=10.4, 2.6 Hz, 1H)], 4.71 (dd, *J*=15.4, 7.9 Hz, 1H) [4.75 (ddd, *J*=12.0, 8.8, 4.1 Hz, 1H)], 4.49 (ddd, *J*=12.0, 6.2, 1.6 Hz, 1H) [4.47 (ddd, *J*=11.7, 5.3, 1.6 Hz, 1H)], 3.84 (s, 3H) [3.83 (s, 3H)], 3.41 (m, 1H),

3.24 (m, 1H), 3.22 (m, 2H) [3.26 (m, 2H)], 2.74 (s, 3H), 1.98 (m, 2H) [2.05 (m, 2H)], 1.98 (m, 1H), 1.86 (m, 1H) [1.82 (m, 1H)], 1.74 (m, 1H), 1.73 (m, 1H), 1.71 (m, 1H) [1.79 (m, 1H)], 1.61 (m, 1H), 1.59 (m, 1H), 1.58 (m, 2H) [1.61 (m, 2H)], 1.54 (m, 1H), 1.37 (m, 2H) [1.41 (m, 2H)], 1.25 (m, 1H), 1.23 (m, 2H) [1.21 (m, 2H)], 1.22 (m, 2H), 1.18 (m, 2H), 1.16 (s, 3H), 1.15 (s, 3H) [1.14 (s, 3H)], 1.12 (m, 2H), 1.11 (m, 1H), 0.82 (t, J=7.1 Hz, 3H). The counterparts of the doubled signals in brackets; FAB-MS m/z 714 $[M+H]^+$.

Methanolysis of 3. Sodium methoxide solution was prepared by adding sodium metal (46 mg, 2.0 mmol) to absolute methanol (2 mL). To a solution of **3** (1.7 mg, 2.4 μmol) in absolute methanol (0.3 mL) was added sodium methoxide solution (0.3 mL) with stirring. After being stirred at room temperature for 4 h under a nitrogen atmosphere, amberlite IRC-50 (H^+ form, 150 mg) was added to the solution and stirred for 20 min. The solution was filtered through a cotton plug, and the residue was washed with methanol. The filtrate and washings were combined and concentrated. The residual oil was separated by HPLC [Develosil Ph-5 (250×20 mm, i.d.); flow rate, 5 mL/min; detection, UV 215 nm; solvent, gradation (30 min) methanol–H_2O (1:1→1:0)] to give methyl ester **5** (t_R=30.9 min, 0.9 mg, 72%) and alcohol **7** (t_R=32.9 min, 0.8 mg, quant.) as colorless oils, respectively. Methyl ester **5**: ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) 9.96 (s, 1H), 8.20 (d, J=1.3 Hz, 1H), 7.31 (m, 1H), 7.29 (d, J=2.8 Hz, 1H), 7.01 (m, 1H), 7.00 (m, 1H), 5.69 (br s, 1H), 4.72 (td, J=8.2, 4.7 Hz, 1H), 3.84 (s, 3H), 3.78 (s, 3H), 3.37 (m, 1H), 3.31 (m, 1H), 2.75 (s, 3H), 1.98 (m, 1H), 1.89 (m, 1H), 1.62 (m, 2H), 1.47 (m, 2H); FAB-MS m/z 420 $[M+H]^+$. Alcohol **7:** ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.25 (br d, J=6.1 Hz, 1H), 5.94 (br dd, J=5.9, 4.6 Hz, 1H), 4.50 (ddd, J=10.0, 6.1, 1.1 Hz, 1H), 3.59 (d, J=6.3 Hz, 1H), 3.46 (ddd, J=10.3, 6.3, 2.1 Hz, 1H), 3.33 (m, 1H), 3.27 (m, 1H), 2.04 (m, 1H), 2.04 (m, 1H), 1.88 (m, 1H), 1.80 (m, 1H), 1.49 (m, 1H), 1.41 (m, 1H), 1.31–1.25 (m, 10H), 1.25 (m, 2H), 1.24 (s, 3H), 1.18 (s, 3H), 0.87 (t, J=6.8 Hz, 3H); FAB-MS m/z 327 $[M+H]^+$.

Acidic hydrolysis of 5 and 7. A solution of methyl ester **5** (0.5 mg, 1.2 μmol) in 6 M hydrochloric acid (0.1 mL) was stirred at 110°C in a sealed tube for 1 day. The reaction mixture was concentrated under reduced pressure. The residue was separated by HPLC [Develosil ODS-HG-5 (250×4.6 mm, i.d.) ×2; flow rate, 1 mL/min; detection, UV 205 nm; solvent 0.05% aqueous TFA] to give lysine (t_R=5.6 min). The stereochemistry of lysine from **5** was determined to be D by chiral HPLC analysis [CROWNPAK CR (+) (150×4.0 mm, i.d.); flow rate, 0.4 mL/min; detection, UV 200 nm; solvent, 0.13% aqueous $HClO_4$]. The stereochemistry of lysine in alcohol **7** was determined to be L by the same procedure as described above. The retention times of D and L lysines were 5.4 and 5.9 min, respectively.

Esterification of alcohol 7. To a solution of alcohol **7** (0.2 mg, 0.61 μmol) in pyridine (0.1 mL) was added (−)-MTPA chloride (50 mg, 0.20 mmol). After being stirred at room temperature for 2 days, these reaction mixtures were concentrated under blowing nitrogen gas. The residual oil was separated by preparative TLC, R_f=0.44 [chloroform–methanol (49:1)] to give (*S*)-MTPA ester **9a**. By the same procedure as described above, (*R*)-MTPA ester **9b** was obtained by reacting alcohol **7** and (+)-MTPA chloride. **9a:** ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) 7.58 (m, 2H), 7.39 (m, 3H), 7.17 (m, 1H), 5.87 (m, 1H), 5.41 (t, J=6.0 Hz, 1H), 4.43 (dd, J=11.1, 6.3 Hz, 1H), 3.53 (s, 3H), 3.25 (m, 2H), 2.00 (m, 1H), 1.99 (m, 1H), 1.85 (m, 1H), 1.80 (m, 1H), 1.49 (m, 2H), 1.42 (m, 1H), 1.38 (m, 1H), 1.32–1.18 (m, 6H), 1.26 (m, 2H), 1.25 (s, 3H), 1.19 (m, 2H), 1.17 (s, 3H), 0.86 (t, J=7.0 Hz, 3H). **9b:** ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) 7.60 (m, 2H), 7.39 (m, 3H), 7.17 (m, 1H), 5.86 (m, 1H), 5.41 (t, J=6.3 Hz, 1H), 4.39 (dd, J=10.4, 6.0 Hz, 1H), 3.55 (s, 3H), 3.24 (m, 2H), 2.02 (m, 1H), 2.00 (m, 1H), 1.86 (m, 1H), 1.79 (m, 1H), 1.53 (m, 2H), 1.43 (m, 1H), 1.39 (m, 1H), 1.33–1.25 (m, 4H), 1.30 (m, 2H), 1.26 (m, 2H), 1.23 (m, 2H), 1.20 (s, 3H), 1.13 (s, 3H), 0.88 (m, 3H). The $\Delta\delta$ values ($\delta_S-\delta_R$) in ppm: −0.07 (H-11), −0.04 (H-10), 0 (H-9), +0.05 (H-17), +0.04 (H-18), +0.04 (H-2).

Hydrogenolysis of 2. The experimental procedure was the same as that described for compound **3**. **4** (A colorless oil): ^{1}H NMR (400 MHz, CD_3OD) δ (ppm) 8.17 (br s, 1H), 7.84 (dd, J=8.3, 1.5 Hz, 1H), 7.40 (dt, J=8.3, 8.3, 1.5 Hz, 1H), 7.04 (d, J=8.3 Hz, 1H), 6.99 (t, J=8.3 Hz, 1H), 5.11 (dd, J=10.2, 2.4 Hz, 1H), 4.64 (dd, J=9.5, 5.6 Hz, 1H), 4.48 (d, J=10.9 Hz, 1H), 3.82–3.45 (m, 3H), 3.28–3.12 (m, 3H), 2.71 (s, 3H), 2.01–1.85 (m, 4H), 1.80–1.70 (m, 2H), 1.63–1.38 (m, 6H), 1.34–1.17 (m, 10H), 1.16 (s, 6H), 0.83 (t, J=6.6 Hz, 3H); FAB-MS m/z 684 $[M+H]^+$.

Methanolysis of 4. The experimental procedure was the same as that described for compounds **5** and **7**. Methyl ester **6** (a colorless oil): ^{1}H NMR (400 MHz, CD_3OD) δ (ppm) 8.02 (s, 1H), 7.85 (dd, J=7.8, 1.5 Hz, 1H), 7.39 (ddd, J=8.3, 7.2, 1.5 Hz, 1H), 7.03 (dd, J=8.3, 1.2 Hz, 1H), 6.98 (ddd, J=7.8, 7.2, 1.2 Hz, 1H), 4.58 (dd, J=9.6, 5.5 Hz, 1H), 3.74 (s, 3H), 3.23 (m, 2H), 2.70 (s, 3H), 2.05–1.90 (m, 2H), 1.62–1.43 (m, 3H), 1.34 (m, 1H); FAB-MS m/z 390 $[M+H]^+$.

Acidic hydrolysis of 6 and 8. A solution of methyl ester **6** (50 μg, 0.1 μmol) in 6 M hydrochloric acid (0.1 mL) was stirred at 110°C in a sealed tube for 1 day. The reaction mixture was concentrated under reduced pressure. The residue was separated by HPLC [Develosil ODS-HG-5 (250×10 mm, i.d.); flow rate, 2 mL/min; detection, UV 205 nm; solvent 0.05% aqueous TFA] to give lysine (t_R=7.3 min). The stereochemistry of lysine from **6** was determined to be D by chiral HPLC analysis [CROWNPAK CR (+) (150×4.0 mm, i.d.); flow rate, 0.4 mL/min; detection, UV 200 nm; solvent, 0.13% aqueous $HClO_4$]. The stereochemistry of lysine in **8** was determined to be L by the procedure described above. The retention times of D and L lysines were 7.0 and 8.2 min, respectively.

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