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Isolation and Structures of Theonezolides B and C from the Okinawan Marine Sponge *Theonella* sp.

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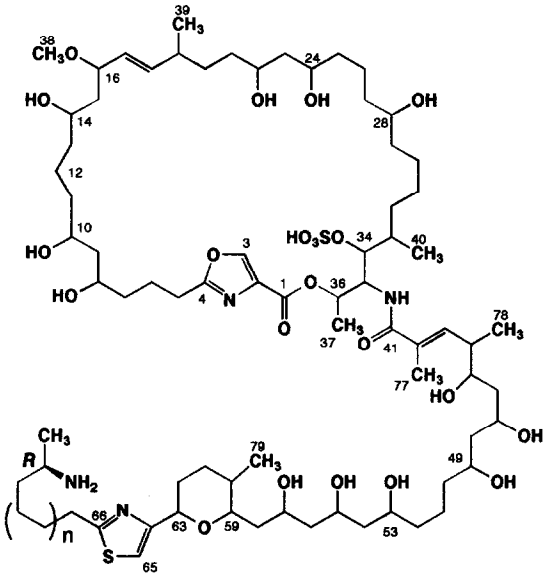
Abstract: Theonezolides B (**2**) and C (**3**), two new macrolides with various functionalities, have been isolated from the Okinawan marine sponge *Theonella* sp. and their structures elucidated on the basis of spectroscopic data as well as chemical degradation experiments. The absolute configurations of the terminal chiral center of theonezolides A (**1**), B (**2**), and C (**3**) were determined by synthesis of their ozonolysis products.

During our studies on bioactive substances from Okinawan marine organisms,[1] we recently isolated a novel macrolide, theonezolide A (**1**), from the Okinawan marine sponge *Theonella* sp.[2] Theonezolide A (**1**) belongs to a new class of polyketide natural products, consisting of two principal fatty acid chains with various functional groups. Further investigation on extracts of this sponge has now resulted in isolation of two new related macrolides, theonezolides B (**2**) and C (**3**), with relatively considerable abundance. Here we describe the isolation and structure elucidation of **2** and **3**. Theonezolides A (**1**), B (**2**), and C (**3**) possess a chiral center with a primary amino group at the terminal position, and this paper also deals with the determination of the absolute configurations of the terminal chiral centers of **1** ~ **3** on the basis of synthesis of one of their ozonolysis products (**8a**, **8b**, and **8c**).

The methanolic extract of the sponge *Theonella* sp., collected off Ie Islands, Okinawa, was partitioned between EtOAc and water. The aqueous phase was further extracted with *n*-BuOH. The *n*-BuOH-soluble fraction was subjected to silica gel column chromatography ($CHCl_3$/MeOH, 8:2) followed by gel filtration on Sephadex LH-20 (MeOH) and reversed-phase HPLC (ODS; 75% MeOH) to give theonezolides B (**2**, 0.01%, wet weight) and C (**3**, 0.01%).

Although the ^1H NMR spectra of theonezolides B (**2**) and C (**3**) appeared almost indistinguishable to that of theonezolide A (**1**), the negative FABMS spectra of **2** and **3** clearly showed the pseudomolecular ion peaks at *m/z* 1531 and 1587, respectively, being different from that of **1** (*m/z* 1559).[2] The HRFABMS data of **2** and **3** suggested the molecular formulas as $C_{77}H_{136}N_4O_{22}S_2$ and $C_{81}H_{144}N_4O_{22}S_2$, respectively, which implied that **2** has two less CH_2 units than **1** while **3** includes two more CH_2 units than **1**. The presence of a lactone linkage in theonezolide B (**2**) was inferred from the fact that a seco acid acetate [**4**, negative FABMS, *m/z* 2137 $(M - H)^-$] was obtained by treatment of **2** with NaOMe followed by acetylation (Ac_2O/pyridine). Since information on the differences of the structures of **1** ~ **3** was hardly obtained from comparison of their ^1H and ^{13}C NMR data (Table 1) including several types of 2D NMR experiments (^1H-^1H COSY, HSQC,[3] and HMBC[4]), theonezolides B (**2**) and C (**3**) were subjected to degradation experiments by ozonolysis, which was



1 n=3, **2** n=2, **3** n=4

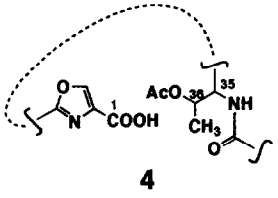


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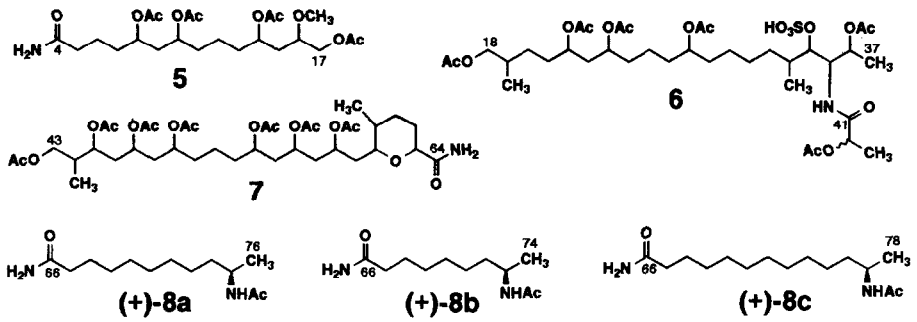
Table 1. ^{13}C NMR Data of Theonezolides A (1), B (2), and C (3) in DMSO-d_6[a]

position	1	2	3	position	1	2	3	position	1	2	3
1	159.9	159.9	159.9	28	69.7	69.7	69.7	55	65.9	65.8	65.8
2	132.5	132.5	132.5	29	37.2	37.3	37.3	56	45.1	45.2	45.2
3	145.3	145.3	145.4	30	25.4	25.5	25.5	57	66.4	66.3	66.2
4	165.0	165.0	165.0	31	26.7	26.8	26.8	58	40.3	40.2	40.2
5	27.2	27.2	27.2	32	32.9	32.9	32.9	59	77.4	77.4	77.3
6	22.4	22.4	22.4	33	34.6	34.6	34.6	60	29.2	29.2	29.1
7	36.3	36.3	36.3	34	81.9	81.9	81.9	61	30.5	30.5	30.5
8	68.5	68.5	68.5	35	51.8	51.7	51.7	62	25.9	25.9	25.9
9	44.1	44.1	44.1	36	70.5	70.5	70.5	63	76.5	76.5	76.5
10	69.0	68.9	68.9	37	17.4	17.4	17.4	64	157.3	157.3	157.3
11	37.4	37.5	37.5	38	54.8	54.8	54.8	65	113.2	113.3	113.3
12	21.3	21.4	21.3	39	21.3	21.3	21.3	66	170.0	170.0	170.0
13	37.8	37.9	37.9	40	15.3	15.4	15.4	67	32.6	32.5	32.6
14	66.9	66.8	66.8	41	168.2	168.2	168.1	68	29.4	29.3	29.4
15	43.2	43.2	43.2	42	129.7	129.7	129.7	69	28.3	28.3	28.4
16	79.8	79.8	79.8	43	138.9	139.0	139.0	70	28.6	28.2	28.8
17	128.9	128.9	128.9	44	39.2	39.1	39.1	71	28.5	24.6	28.7
18	139.9	140.0	140.0	45	70.5	70.5	70.4	72	28.6	34.1	28.8
19	36.2	36.2	36.2	46	42.8	42.9	42.9	73	24.6	46.8	28.6
20	32.1	32.1	32.1	47	64.1	64.0	64.0	74	43.1	18.2	28.8
21	35.0	35.0	35.0	48	45.6	45.7	45.7	75	46.9	12.8	24.7
22	68.8	68.8	68.8	49	66.8	66.8	66.8	76	18.2	15.7	34.2
23	44.3	44.4	44.4	50	38.2	38.2	38.2	77	12.8	11.6	46.8
24	69.2	69.2	69.2	51	21.6	21.6	21.6	78	15.6		18.3
25	37.5	37.5	37.5	52	38.1	38.1	38.1	79	11.6		12.8
26	21.4	21.4	21.4	53	66.6	66.6	66.5	80			15.7
27	37.7	37.7	37.7	54	45.0	45.0	45.0	81			11.6

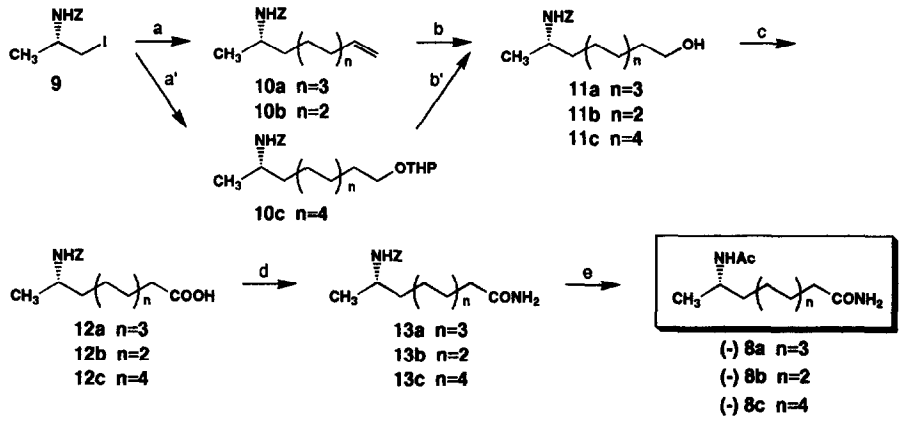
[a]Assignments of ^{13}C chemical shifts for 2 and 3 were mainly based on their 2D NMR data and comparison with the data of 1.



also carried out for theonezolide A (**1**).[2] Treatment of theonezolide B (**2**) with ozone followed by $NaBH_4$ reduction and acetylation afforded a complex mixture, from which four degradation products were isolated by careful HPLC separation. Three of the four products were revealed to be identical with compounds **5**, **6**, and **7**, which had been obtained by ozonolysis of **1**[2] and corresponded to C-4 ~ C-17, C-18 ~ C-37, and C-43 ~ C-64 moieties of **1**, respectively, on the basis of comparison of their ^1H NMR, FABMS, and optical rotation data. The fourth product **8b** obtained from **2** showed analogous ^1H NMR spectrum to that of **8a** (C-66 ~ C-76 moiety)[2] obtained from **1**, and EIMS data indicated that **8b** was a homologue of **8a** with two less sp^3 methylenes [**8b**: *m/z* 214 (M^+); **8a**: *m/z* 242 (M^+)]. Ozonolysis of theonezolide C (**3**) was also carried out by the same procedure described as above to afford three identical products (**5**, **6**, and **7**) and one homologous product **8c** [EIMS, *m/z* 270 (M^+)] with two more CH_2 groups than **8a**. From these results, the structures of theonezolides B and C were concluded to be **2** and **3**, respectively; viz., the numbers of sp^3 methylenes between the thiazole moiety (C-64 ~ C-66 position) and the terminal amino group of **1**, **2**, and **3** are 8, 6, and 10, respectively.

Theonezolides A ~ C (**1** ~ **3**) contain 23 chiral centers, among which we first chose the chiral center at the terminal position bearing the primary amino and secondary methyl groups (C-75 of **1**, C-73 of **2**, and C-77 of **3**) for the study of determination of the absolute stereochemistry. The fragments **8a**, **8b**, and **8c** retaining the terminal chiral centers of **1** ~ **3**, respectively, were prepared in optically active forms as follows (Scheme 1). The iodide **9** prepared from L-alaninol by the procedure described by Schlessinger,[5] was treated with Grignard reagent followed by hydroboration to give the alcohol (**11a**), which was converted via 2 steps into the amide (**13a**). Deprotection and acetylation of **13a** afforded *S*-(-)-enantiomer of **8a**, the ^1H NMR and EIMS spectra of which were identical with those of **8a** obtained by ozonolysis of **1**. Since the sign of optical rotation of synthetic (-)-**8a** ($[\alpha]_D$ -10°) was opposite to that of (+)-**8a** ($[\alpha]_D$ +12°) from natural specimen, the absolute configuration of C-75 position of theonezolide A (**1**) was established as *R*. By the similar procedures as shown in Scheme 1, *S*-(-)-enantiomers of **8b** and **8c** were prepared and their ^1H NMR and EIMS spectral data were completely superimposable to those of **8b** and **8c** derived from natural theonezolides B (**2**) and C (**3**). The signs of their optical rotations, however, were also different (synthetic: (-)-**8b**, $[\alpha]_D$ -8°; (-)-**8c**, $[\alpha]_D$ -12°; natural: (+)-**8b**, $[\alpha]_D$ +14°; (+)-**8c**, $[\alpha]_D$ +10°). Theonezolides B (**2**) and C (**3**) were therefore revealed to have 73*R*- and 77*R*-configurations, respectively.

Scheme 1. Synthesis of **8a**, **8b**, and **8c**.



(a) CH_2=$CH(CH_2)_6MgBr$ [or CH_2=$CH(CH_2)_4MgBr$], CuI, THF (a') $THPO(CH_2)_{10}MgBr$, CuI, THF
(b) BH_3-THF; H_2O_2-NaOH (b') TsOH, MeOH (c) PDC, DMF (d) 1) N,N'-Carbonyldiimidazole, THF, 2) NH_3
(e) 1) 10% Pd-C, H_2, MeOH 2) Ac_2O, Pyridine

Theonezolides B (**2**) and C (**3**) exhibited cytotoxicity against murine lymphoma L1210 and human epidermoid carcinoma KB cells in vitro (IC_{50} values, **2**: 5.6 and 11 μg/mL, respectively; **3**: 0.3 and 0.37 μg/mL, respectively). Further studies on defining the relative and absolute stereochemistry of chiral centers contained in the fragments **5**, **6**, and **7**, based on spectral and synthetic methods are currently under investigation.

Experimental Section

General Methods. Melting points were determined on a Yanaco MP-500D melting point apparatus and uncorrected. Optical rotations were measured on a JASCO DIP-370 digital polarimeter. UV and IR spectra were taken on a Shimadzu UV-220 and a JASCO IR Report-100 spectrometers, respectively. ^1H and ^{13}C NMR spectra were recorded on a JEOL GSX-400 and an EX-400 spectrometers in DMSO-d_6 and $CDCl_3$. FAB mass spectra were obtained on a JEOL HX-110 and an AX-500 spectrometers. EI mass spectra were obtained on a JEOL AX-500 spectrometer.

Isolation. The sponge *Theonella* sp. was collected off Ie Islands, Okinawa and kept frozen until used. The sponge (0.8 kg, wet weight) was extracted with MeOH (0.8 L x 3). After evaporation under reduced pressure, the residue (61 g) was partitioned between EtOAc (400 mL x 3) and 1M NaCl aqueous solution (400 mL), and the aqueous portion was subsequently extracted with *n*-BuOH (400 mL x 3). The *n*-BuOH soluble material (6.7 g) was subjected to a silica gel column with $CHCl_3$/MeOH (80:20) followed by a Sephadex LH-20 column (Pharmacia Fine Chemicals) with MeOH and reversed-phase HPLC (Develosil Lop ODS 24S, Nomura Chemical, 24 x 360 mm, 30 μm; flow rate, 6.0 mL/min; UV detection at 254 nm; eluent, 75% MeOH) to give theonezolide B (**2**, t_R 30.3 min, 96 mg, 0.01 % wet weight) and theonezolide C (**3**, t_R 81.1 min, 76 mg, 0.01 %).

Theonezolide B (2): a colorless solid; mp 125 °C; $[\alpha]_D^{28}$ -8.0° (*c* 1.5, MeOH); UV λ_{max} (MeOH) 211 nm (ε 22000); IR ν_{max} (KBr) 3400, 1720, 1620, 1220, and 1110 cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.84 (3H, d, *J* = 6.4 Hz), 0.92 (3H, d, *J* = 6.8 Hz), 0.93 (3H, d, *J* = 6.8 Hz), 0.96 (3H, d, *J* = 6.8 Hz), 1.14 (3H, d, *J* = 6.4 Hz), 1.23 (3H, d, *J* = 6.8 Hz), 1.77 (3H, s), 2.10 (1H, m), 2.37 (1H, m), 2.76 (2H, t, *J* = 7.1 Hz), 2.92 (2H, t, *J* = 7.6 Hz), 3.10 (3H, s), 3.13 (1H, m), 3.31 (1H, m), 3.37 (1H, m), 3.55 (4H, m), 3.62 (4H, m), 3.76 (1H, m), 3.78 (1H, m), 3.81 (1H, m), 3.87 (1H, m), 4.05 (1H, dd, *J* = 6.8, 3.9 Hz), 4.26 (1H, m), 4.42 (1H, m), 5.08 (1H, dd, *J* = 15.1, 8.3 Hz), 5.26 (1H, qd, *J* = 6.3, 2.9 Hz), 5.42 (1H, dd, *J* = 15.1, 8.3 Hz), 6.15 (1H, d, *J* = 9.3 Hz), 7.25 (1H, s), 7.98 (1H, d, *J* = 8.8 Hz), and 8.69 (1H, s); ^{13}C NMR (Table 1); FABMS (negative ion; triethanolamine as a matrix) *m/z* 1531 $(M - H)^-$; (positive ion; triethanolamine as a matrix) *m/z* 1533 $(M + H)^+$; HRFABMS *m/z* 1533.9230 [$(M + H)^+$, calcd for $C_{77}H_{137}N_4O_{22}S_2$, 1533.9167].

Theonezolide C (3): a colorless solid; mp 122 °C; $[\alpha]_D^{28}$ -7.5° (*c* 1.5, MeOH); UV λ_{max} (MeOH) 211 nm (ε 25000); IR ν_{max} (KBr) 3390, 1720, 1620, 1220, and 1110 cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.84 (3H, d, *J* = 6.4 Hz), 0.92 (3H, d, *J* = 6.8 Hz), 0.93 (3H, d, *J* = 6.8 Hz), 0.96 (3H, d, *J* = 6.8 Hz), 1.14 (3H, d, *J* = 6.4 Hz), 1.23 (3H, d, *J* = 6.8 Hz), 1.77 (3H, s), 2.10 (1H, m), 2.37 (1H, m), 2.76 (2H, t, *J* = 7.1 Hz), 2.92 (2H, t, *J* = 7.6 Hz), 3.10 (3H, s), 3.13 (1H, m), 3.31 (1H, m), 3.37 (1H, m), 3.55 (4H, m), 3.62 (4H, m), 3.76 (1H, m), 3.78 (1H, m), 3.81 (1H, m), 3.87 (1H, m), 4.05 (1H, dd, *J* = 6.8, 3.9 Hz), 4.26 (1H, m), 4.42 (1H, m), 5.08 (1H, dd, *J* = 15.1, 8.3 Hz), 5.26 (1H, qd, *J* = 6.3, 2.9 Hz), 5.42 (1H, dd, *J* = 15.1, 8.3 Hz), 6.15 (1H, d, *J* = 9.3 Hz), 7.25 (1H, s), 7.98 (1H, d, *J* = 8.8 Hz), and 8.69 (1H, s); ^{13}C NMR (Table 1); FABMS (negative ion; triethanolamine as a matrix) *m/z* 1587 $(M - H)^-$; (positive ion; triethanolamine as a matrix) *m/z* 1589 $(M + H)^+$; HRFABMS *m/z* 1589.9710 [$(M + H)^+$, calcd for $C_{81}H_{145}N_4O_{22}S_2$, 1589.9794].

Hydrolysis of Theonezolide B (2). To a solution of theonezolide B (2, 2.9 mg) in MeOH (0.4 mL), 28% NaOMe-MeOH (0.04 mL) was added and the mixture was stirred at room temperature for 1 h. After addition of Dowex 50 W x 8 (H^+ form) and filtration, the filtrate was evaporated under reduced pressure to give a residue, which was treated with acetic anhydride (1 mL) and pyridine (1 mL) at room temperature for 24 h. After evaporation, the residue was purified by silica gel column chromatography ($CHCl_3$/MeOH, 9:1) to give **4** (2.8 mg, 69 %): ^1H NMR (DMSO-d_6) δ 0.83 (3H, d, *J* = 6.8 Hz), 0.90 (6H, d, *J* = 6.4 Hz), 0.94 (3H, d, *J* = 6.8 Hz), 0.98 (3H, d, *J* = 6.4 Hz), 1.08 (3H, d, *J* = 6.4 Hz), 1.76 (3H, s), 1.88-2.03 (3H x 13, each s), 2.69 (1H, m), 2.73 (2H, m), 2.91 (2H, t, *J* = 7.6 Hz), 3.09 (3H, s), 3.49 (1H, dd, *J* = 14.2, 6.8 Hz), 3.64 (1H, m), 3.69 (1H, m), 3.92 (1H, dd, *J* = 8.3, 3.4 Hz), 4.12 (1H, m), 4.38 (1H, m), 4.78 (12H, m), 4.93 (1H, m), 5.13 (1H, dd, *J* = 15.6, 8.3 Hz), 5.15 (1H, m), 5.43 (1H, dd, *J* = 15.6, 7.8 Hz), 6.12 (1H, dd, *J* = 9.2, 1.0 Hz), 7.23 (1H, s), 7.58 (1H, d, *J* = 8.3 Hz), 8.07 (1H, d, *J* = 9.3 Hz), and 8.31 (1H, br s); FABMS (negative ion; triethanolamine as a matrix) *m/z* 2137 $(M - H)^-$.

Ozonolysis of Theonezolide B (2). Theonezolide B (2, 19.2 mg) was treated with acetic anhydride (2 mL) and pyridine (2 mL) at room temperature for 10 h. The solvents were evaporated under reduced pressure to afford the tridecaacetate (26.4 mg), which was dissolved in MeOH (1 mL) and was bubbled with O_3 at -78 °C for 10 min. After excess ozone was removed by a stream of argon, a solution of $NaBH_4$ (40 mg) in MeOH (0.5 mL) was added and the whole mixture was stirred at 0 °C for 1 h. After addition of 2 mL of 1 M potassium phosphate buffer (pH 7.0), the mixture was partitioned between EtOAc and brine. The organic layer was evaporated under reduced pressure and the residue was treated with acetic anhydride (1 mL) and pyridine (1 mL) at room temperature for 12 h. After evaporation under reduced

pressure, the residue was separated by silica gel column chromatography ($CHCl_3$/MeOH, 95:5 and 85:15) to give **6** (5.6 mg) and a mixture of other products, and the latter was purified by reversed-phase HPLC (Develosil ODS-5, 10 x 250 mm, 5 μm; flow rate, 2.5 mL/min; refractive index detection; eluent, 50 and 70% MeOH) to afford **5** (1.0 mg, t_R 7.6 min, 70% MeOH), **7** (0.4 mg, t_R 27.0 min, 70% MeOH), and **8b** (0.1 mg, t_R 9.1 min, 50% MeOH). Compounds **5**, **6**, and **7** were identified with those obtained from theonezolide A (**1**)[2] by ^1H NMR, FABMS, and optical rotation. **8b**: a colorless solid; $[\alpha]_D^{27}$ +14° (*c* 0.02, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.12 (3H, d, *J* = 6.4 Hz), 1.33 (6H, br s), 1.40 (2H, m), 1.64 (2H, m), 1.96 (3H, s), 2.22 (2H, m), 3.98 (1H, m), 5.22 (2H, br s), and 5.55 (1H, br s); EIMS m/z 214 M^+; HREIMS *m/z* 214.1687 (M^+, calcd for $C_{11}H_{22}N_2O_2$, 214.1681).

Ozonolysis of Theonezolide C (3). Theonezolide C (**3**, 25.6 mg) was acetylated and subjected to ozonolysis by the same procedures as above to afford **5** (1.6 mg), **6** (3.8 mg), **7** (1.3 mg), and **8c** (1.0 mg, t_R 10.7 min, 70% MeOH). Compounds **5**, **6**, and **7** were identified with those obtained from theonezolide A (**1**)[2] by ^1H NMR, FABMS, and optical rotation. **8c**: a colorless solid; $[\alpha]_D^{26}$ +10° (*c* 0.1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.11 (3H, d, *J* = 6.4 Hz), 1.26 (14H, br s), 1.39 (2H, m), 1.64 (2H, m), 1.96 (3H, s), 2.22 (2H, t, *J* = 7.6 Hz), 3.96 (1H, m), 5.20 (1H, br d, *J* = 8.3 Hz), 5.20 (1H, d, *J* = 8.3 Hz), and 5.46 (1H, br s); ^{13}C NMR ($CDCl_3$) δ 45.3d, 37.0t, 35.9t, 29.4t x 3, 29.3t, 29.2t x 2, 26.0t, 25.5t, 23.6q, and 21.0q; EIMS m/z 270 M^+; HREIMS *m/z* 270.2315 (M^+, calcd for $C_{15}H_{30}N_2O_2$, 270.2307).

10a. To a suspension of copper (I) iodide (1.15 g, 6 mmol) in anhydrous THF (15 mL) cooled at -78 °C under argon atmosphere, 1.0 M 1-octenyl magnesium bromide in THF solution (6 mL, 6 mmol) was added and the mixture was warmed to -30 °C for 5 min and then cooled to -78 °C. Then, **9** (638 mg, 2 mmol) in THF (3 mL) was added to the mixture, which was stirred at -30 °C for 6 h. After addition of saturated NH_4Cl the mixture was extracted with Et_2O. The organic layer was washed with brine, 5% NH_4OH, and brine, dried over $MgSO_4$. After evaporation of the solvent, the residue was purified by silica gel column chromatography (hexane/EtOAc, 8:1) to give **10a** (451 mg, 74 %): colorless needles; mp 55 °C; $[\alpha]_D^{26}$ +7° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.13 (3H, d, *J* = 6.8 Hz), 1.28 (8H, br s), 1.37 (4H, m), 2.03 (2H, m), 3.70 (1H, m), 4.50 (1H, br s), 4.93 (1H, m), 4.99 (1H, m), 5.09 (2H, s), 5.81 (1H, m), and 7.35 (5H, m); EIMS *m/z* 303 M^+; HREIMS *m/z* 303.2179 (M^+, calcd for $C_{19}H_{29}NO_2$, 303.2199).

11a. To a solution of **10a** (451 mg, 1.48 mmol) in anhydrous THF (5 mL) at 0 °C under argon atmosphere, 1.0 M borane-tetrahydrofuran complex in THF solution (3.6 mL, 3.6 mmol) was added and the mixture was stirred at room temperature for 16 h. After addition of 2N NaOH (2 mL) and 35% H_2O_2 (2 mL), the mixture was stirred at room temperature for 6 h and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$. After evaporation, the residue was purified by silica gel column chromatography (hexane/EtOAc, 2:1) to give **11a** (328 mg, 69 %): colorless needles; mp 54 °C; $[\alpha]_D^{26}$ +4° (*c* 0.5, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.13 (3H, d, *J* = 6.8 Hz), 1.28 (12H, m), 1.40 (2H, m), 1.56 (2H, m), 3.64 (2H, t, *J* = 6.6 Hz), 3.70 (1H, m), 4.52 (1H, d, *J* = 6.3 Hz), 5.09 (2H, s), and 7.35 (5H, m); EIMS *m/z* 321 M^+; HREIMS *m/z* 321.2278 (M^+, calcd for $C_{19}H_{31}NO_3$, 321.2304).

12a. To a solution of **11a** (328 mg, 1.02 mmol) in DMF (4 mL), PDC (1.33 g, 3.57 mmol) was added and the mixture was stirred at room temperature for 12 h. After extraction with Et_2O, the organic layer was washed with brine, dried over $MgSO_4$, and evaporated to give a residue, which was purified by silica gel column chromatography (hexane/EtOAc, 1:1) to give **12a** (205 mg, 60 %): colorless needles, mp 95 °C; $[\alpha]_D^{26}$

+8° (*c* 0.5, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.13 (3H, d, *J* = 6.4 Hz), 1.28 (10H, br s), 1.35 (2H, m), 1.63 (2H, m), 2.34 (2H, t, *J* = 7.3 Hz), 3.71 (1H, m), 4.53 (1H, d, *J* = 5.9 Hz), 5.09 (2H, s), and 7.35 (5H, m); EIMS *m/z* 335 M^+; HREIMS *m/z* 335.2120 (M^+, calcd for $C_{19}H_{29}NO_4$, 335.2097).

13a. To a solution of **12a** (172 mg, 0.51 mmol) in THF (10 mL), N, N'-carbonyldiimidazole (166 mg, 1.02 mmol)[6] was added and the mixture was stirred at 60 °C for 45 min. Then the mixture was bubbled with ammonia gas at 0 °C for 5 min and stirred at 0 °C for 30 min. After evaporation, the mixture was extracted with EtOAc, washed with water and brine, and dried over $MgSO_4$. Evaporation and purification by alumina column chromatography (MeOH) afforded **13a** (168 mg, 98 %): colorless needles; mp 142 °C; $[\alpha]_D^{28}$ +8° (*c* 0.2, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.13 (3H, d, *J* = 6.4 Hz), 1.27 (10H, br s), 1.39 (2H, br s), 1.62 (2H, m), 2.21 (2H, t, *J* = 7.6 Hz), 3.69 (1H, m), 4.65 (1H, d, *J* = 8.3 Hz), 5.09 (2H, s), 6.07 (2H, br s), and 7.35 (5H, m); EIMS *m/z* 334 M^+; HREIMS *m/z* 334.2245 (M^+, calcd for $C_{19}H_{30}N_2O_3$, 334.2256).

S-(-)-8a. A solution of **13a** (141 mg, 0.42 mmol) in MeOH (20 mL) containing 10 % Pd-C (141 mg) was stirred at room temperature under hydrogen atmosphere for 18 h. After removal of the catalyst by filtration, the solvent was evaporated under reduced pressure to afford amine (83.7 mg, 99 %), a part of which (74 mg, 0.37 mmol) was treated with acetic anhydride (2 mL) and pyridine (2 mL) at room temperature for 24 h. After evaporation under reduced pressure, the residue was purified by silica gel column chromatography ($CHCl_3$/MeOH, 9:1) to give **S-(-)-8a** (66.2 mg, 74 %): colorless needles; mp 146 °C; $[\alpha]_D^{23}$ -10° (*c* 0.2, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.11 (3H, d, *J* = 6.4 Hz), 1.29 (10H, br s), 1.40 (2H, m), 1.64 (2H, m), 1.96 (3H, s), 2.22 (2H, t, *J* = 7.8 Hz), 3.96 (1H, m), 5.21 (1H, br d, *J* = 6.8 Hz), 5.28 (1H, br s), and 5.51 (1H, br s); EIMS *m/z* 242 M^+; HREIMS *m/z* 242.2005 (M^+, calcd for $C_{13}H_{26}N_2O_2$, 242.1995).

Preparation of S-(-)-8b and S-(-)-8c. S-(-)-Enantiomers of **8b** and **8c** were prepared by the same procedures as those for **8a** (Scheme 1) except for the deprotection of tetrahydropyranyl group of **10c**.

10b: colorless needles; mp 44 °C; $[\alpha]_D^{28}$ +7° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.13 (3H, d, *J* = 6.4 Hz), 1.32 (4H, br s), 1.38 (4H, m), 2.03 (2H, m), 3.71 (1H, m), 4.52 (1H, br s), 4.93 (1H, m), 4.99 (1H, m), 5.09 (2H, s), 5.80 (1H, m), and 7.35 (5H, m); EIMS *m/z* 275 M^+; HREIMS *m/z* 275.1902 (M^+, calcd for $C_{17}H_{25}NO_2$, 275.1885); 72 % yield from **9**.

11b: colorless needles; mp 40 °C; $[\alpha]_D^{28}$ +9° (*c* 0.5, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.13 (3H, d, *J* = 6.3 Hz), 1.31 (8H, br s), 1.41 (2H, br s), 1.56 (2H, m), 3.63 (2H, t, *J* = 6.6 Hz), 3.70 (1H, m), 4.52 (1H, br s), 5.09 (2H, s), and 7.35 (5H, m); EIMS *m/z* 293 M^+; HREIMS *m/z* 293.2011 (M^+, calcd for $C_{17}H_{27}NO_3$, 293.1991); 75 % yield from **10b**.

12b: colorless needles; mp 85 °C; $[\alpha]_D^{28}$ +8° (*c* 0.5, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.13 (3H, d, *J* = 6.4 Hz), 1.32 (6H, br s), 1.40 (2H, br s), 1.62 (2H, m), 2.34 (2H, t, *J* = 7.6 Hz), 3.71 (1H, m), 4.53 (1H, br s), 5.09 (2H, s), and 7.35 (5H, m); EIMS *m/z* 307 M^+; HREIMS *m/z* 307.1769 (M^+, calcd for $C_{17}H_{25}NO_4$, 307.1784); 42 % yield from **11b**.

13b: colorless needles; mp 142 °C; $[\alpha]_D^{28}$ +8° (*c* 0.1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.13 (3H, d, *J* = 6.4 Hz), 1.33 (10H, br s), 1.41 (2H, br s), 1.63 (2H, m), 2.21 (2H, t, *J* = 7.6 Hz), 3.71 (1H, m), 4.52 (1H, d, *J* = 6.4 Hz), 5.08 (2H, s), 5.23 (1H, br s), 5.43 (1H, br s), and 7.35 (5H, m); EIMS *m/z* 306 M^+; HREIMS *m/z* 306.1949 (M^+, calcd for $C_{17}H_{26}N_2O_3$, 306.1943); 76 % yield from **12b**.

S-(-)-8b: colorless needles; mp 136 °C; $[\alpha]_D^{27}$ -8° (*c* 0.1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.12 (3H, d, *J* = 6.8 Hz), 1.33 (6H, br s), 1.41 (2H, m), 1.63 (2H, m), 1.96 (3H, s), 2.22 (2H, m), 3.98 (1H, m), 5.22

(1H, br d, J = 6.8 Hz), 5.29 (1H, br s), and 5.59 (1H, br s); EIMS *m/z* 214 M^+; HREIMS *m/z* 214.1659 (M^+, calcd for $C_{11}H_{22}N_2O_2$, 214.1681); 78 % yield from **13b**.

10c: colorless needles; mp 47 °C; $[\alpha]_D^{26}$ +0.4° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.13 (3H, d, J = 6.4 Hz), 1.26 (16H, br s), 1.58 (8H, m), 1.71 (1H, m), 1.83 (1H, m), 3.38 (1H, m), 3.50 (1H, m), 3.73 (2H, m), 3.87 (1H, m), 4.52 (1H, br d, J = 7.3 Hz), 4.57 (1H, m), 5.09 (2H, s), and 7.35 (5H, m); EIMS *m/z* 433 M^+; HREIMS *m/z* 433.3199 (M^+, calcd for $C_{26}H_{43}NO_4$, 433.3192); 62 % yield from **9**.

11c. To a solution of **10c** (530 mg, 1.22 mmol) in MeOH (5 mL), TsOH (21 mg, 0.122 mmol) was added and the mixture was stirred at room temperature for 1 h. After evaporation of the solvent , the residue was purified by silica gel column chromatography (hexane/EtOAc, 2:1) to give **11c** (300 mg, 70 %): colorless needles; mp 68 °C; $[\alpha]_D^{26}$ +4° (*c* 0.5, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.13 (3H, d, J = 6.8 Hz), 1.26 (8H, br s), 1.40 (2H, br s), 1.56 (2H, m), 3.64 (2H, dd, J = 12.2 and 6.8 Hz), 3.70 (1H, m), 4.52 (1H, br s), 5.09 (2H, s), and 7.35 (5H, m); EIMS *m/z* 349 M^+; HREIMS *m/z* 349.2605 (M^+, calcd for $C_{21}H_{35}NO_3$, 349.2617).

12c: colorless needles; mp 103 °C; $[\alpha]_D^{26}$ +5° (*c* 0.5, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.13 (3H, d, J = 6.8 Hz), 1.26 (6H, br s), 1.40 (2H, br s), 1.63 (2H, m), 2.34 (2H, t, J = 7.3 Hz), 3.71 (1H, m), 4.55 (1H, br d, J = 6.8 Hz), 5.09 (2H, s), and 7.35 (5H, m); EIMS *m/z* 363 M^+; HREIMS *m/z* 363.2417 (M^+, calcd for $C_{21}H_{33}NO_4$, 363.2409); 59 % yield from **11c**.

13c: colorless needles; mp 146 °C; $[\alpha]_D^{26}$ +8° (*c* 0.1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.13 (3H, d, J = 6.8 Hz), 1.25 (10H, br s), 1.40 (2H, br s), 1.62 (2H, m), 2.22 (2H, t, J = 7.6 Hz), 3.70 (1H, m), 4.56 (1H, d, J = 8.8 Hz), 5.09 (2H, s), 5.24 (1H, br s), 5.40 (1H, br s), and 7.35 (5H, m); EIMS *m/z* 362 M^+; HREIMS *m/z* 362.2547 (M^+, calcd for $C_{21}H_{34}N_2O_3$, 362.2569); 99 % yield from **12c**.

S-(-)-8c: colorless needles; mp 145 °C; $[\alpha]_D^{26}$ -12° (*c* 0.1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.12 (3H, d, J = 6.4 Hz), 1.26 (14H, br s), 1.40 (2H, m), 1.64 (2H, m), 1.96 (3H, s), 2.22 (2H, t, J = 7.6 Hz), 3.96 (1H, m), 5.21 (1H, br d, J = 6.8 Hz), 5.28 (1H, br s), and 5.48 (1H, br s); EIMS *m/z* 270 M^+; HREIMS *m/z* 270.2315 (M^+, calcd for $C_{15}H_{30}N_2O_2$, 270.2307); 78 % yield from **13c**.

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