Pergamon

Tetrahedron Vol. 50, No. 38, pp. 11303-11314, 1994

0040-4020/94 $7.00+0.00

0040-4020(94)00691-1

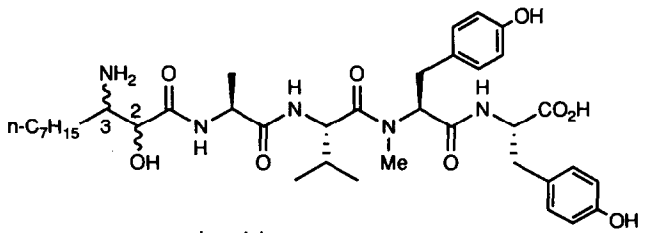
Total Synthesis of Microginin, an Angiotensin-Converting Enzyme Inhibitory Pentapeptide from the Blue-Green Alga *Microcystis aeruginosa*

Fumiyoshi Matsuura, Yasumasa Hamada, and Takayuki Shioiri*

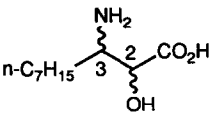
Faculty of Pharmaceutical Sciences, Nagoya City University
Tanabe-dori, Mizuho-ku, Nagoya 467, JAPAN

Abstract: Microginin, an angiotensin-converting enzyme inhibitory peptide isolated form the blue-green alga *Microcystis aeruginosa*, and its three diastereoisomers were efficiently synthesized, which unequivocally established the absolute stereostructure of microginin to be **1d**.

Microginin has been isolated from the cultured freshwater blue-green alga *Microcystis aeruginosa* (NIES-100) by Murakami and co-workers.[1] This pentapeptide has been reported to have an angiotensin-converting enzyme inhibitory action. The structure of microginin has been proposed as shown in **1**, in which the stereogenic center at the C-3 position of the N-terminal 3-amino-2-hydroxydecanoic acid (Ahda, **2**) has not been determined. We now report an efficient total synthesis of microginin and its congeners isomeric at the Ahda fragment, as shown in Fig. 1, which has unequivocally determined the absolute stereostructure of microginin to be **1d**.[2] Recent synthesis of Ahda by Davies and co-workers has reached the same conclusions as ours.[3]



microginins	C-2	C-3
proposed structure **1**	R	-
1a	R	R
1b	R	S
1c	S	S
revised structure **1d**	S	R

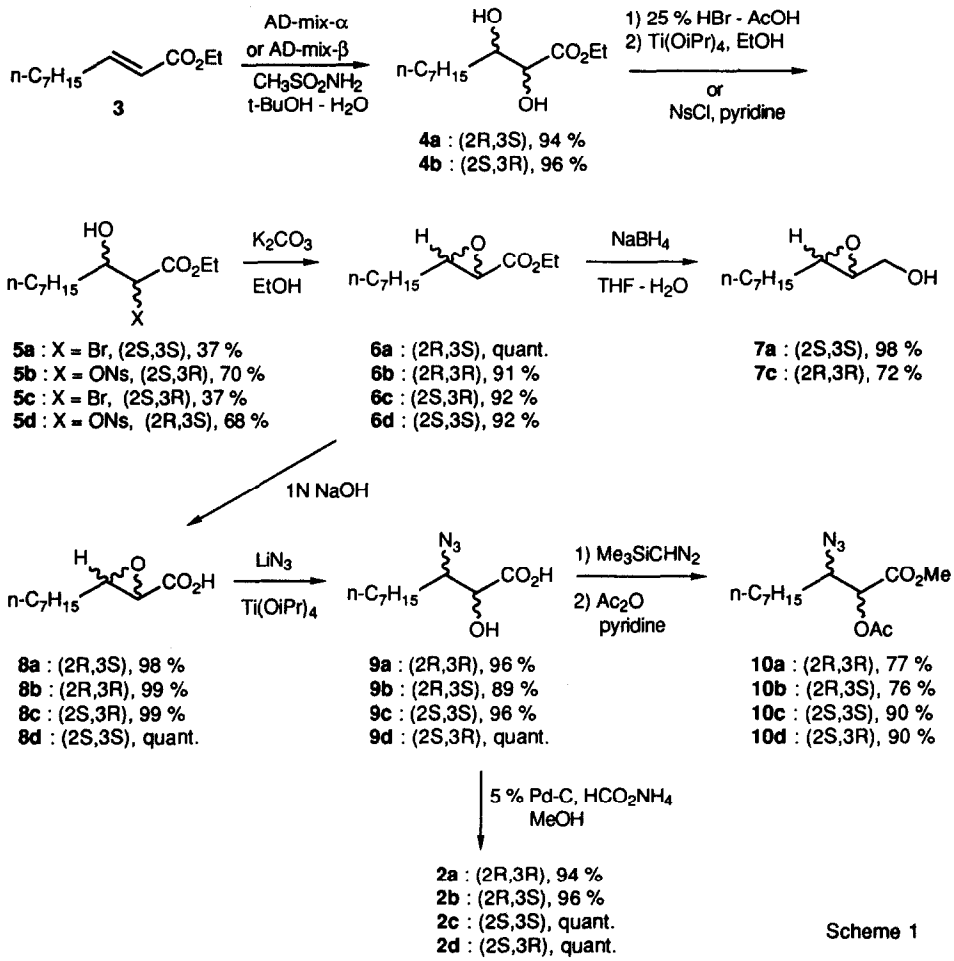


Ahda (2)

2a : (2R,3R)
2b : (2R,3S)
2c : (2S,3S)
2d : (2S,3R)

Fig. 1

The key feature for our synthesis of microginins is the stereodefined construction of the Ahda fragment, for which the stereoselective nucleophilic ring opening reaction of 2,3-epoxy acids[4] has been employed as a key step. We first synthesized both (2R,3R)[5]- and (2R,3S)[5]-microginins (**1a** and **1b**) having the proposed (2R)-configuration at the Ahda portion. First, ethyl (E)-2-decenoate (**3**) underwent the asymmetric dihydroxylation by use of AD-mix-α[6] to give the diol ester **4a**.[7] Treatment with hydrogen bromide in acetic acid followed by titanium isopropoxide afforded the bromohydrin **5a**, which was converted to the epoxy ester **6a** by use of potassium carbonate.[8] The absolute configuration of the epoxy ester **6a** was confirmed by comparison of the physical data of the corresponding epoxy alcohol **7a** with the reported values.[9] After saponification of the epoxy ester **6a**, the resulting epoxy acid **8a** smoothly underwent the nucleophilic ring opening with lithium azide in the presence of titanium isopropoxide[4] to give (2R,3R)-3-azido-2-hydroxydecanoic acid (**9a**), as shown in Scheme 1. The acid was further converted to the corresponding acetoxy methyl ester **10a** by treatment with trimethylsilyldiazomethane[10] followed by acetic anhydride. The IR and ^{1}H-NMR spectra of **10a** were consistent with the reported values of racemic **10a**,[4] confirming the stereochemistry of the azido hydroxy acid **9a**.



3

4a : (2R,3S), 94 %
4b : (2S,3R), 96 %

5a : X = Br, (2S,3S), 37 %
5b : X = ONs, (2S,3R), 70 %
5c : X = Br, (2S,3R), 37 %
5d : X = ONs, (2R,3S), 68 %

6a : (2R,3S), quant.
6b : (2R,3R), 91 %
6c : (2S,3R), 92 %
6d : (2S,3S), 92 %

7a : (2S,3S), 98 %
7c : (2R,3R), 72 %

8a : (2R,3S), 98 %
8b : (2R,3R), 99 %
8c : (2S,3R), 99 %
8d : (2S,3S), quant.

9a : (2R,3R), 96 %
9b : (2R,3S), 89 %
9c : (2S,3S), 96 %
9d : (2S,3R), quant.

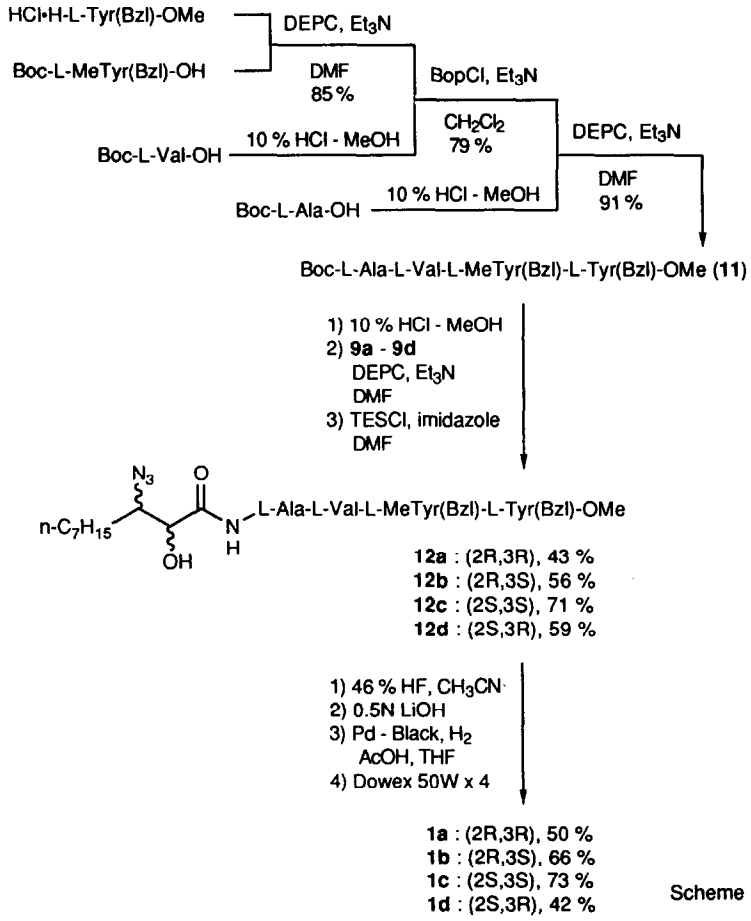
10a : (2R,3R), 77 %
10b : (2R,3S), 76 %
10c : (2S,3S), 90 %
10d : (2S,3R), 90 %

2a : (2R,3R), 94 %
2b : (2R,3S), 96 %
2c : (2S,3S), quant.
2d : (2S,3R), quant.

Scheme 1

Analogously, ethyl (2R,3R)-2,3-dihydroxydecanoate (**4b**) was obtained from ethyl (E)-2-decenoate (**3**) by use of AD-mix-β.[6] Treatment of **4b** with p-nitrobenzenesulfonyl (Ns) chloride afforded the α-Ns derivative which was converted to the epoxide **6b** by use of potassium carbonate, as outlined in Scheme 1. Hydrolysis of **6b**, followed by the nucleophilic ring opening as described above produced (2R,3S)-3-azido-2-hydroxydecanoic acid **9b**, which was further converted to the azido acetoxy methyl ester **10b**, an epimer of **10a**.

The construction of the whole carbon skeletons for (2R,3R)- and (2R,3S)-microginins (**1a** and **1b**) was carried out by stepwise elongation from the C-terminal HCl·H-L-Tyr(Bzl)-OMe.[11] Diethyl phosphorocyanidate (DEPC, $(C_2H_5O)_2P(O)CN$)[12] and 10% hydrogen chloride in methanol were mainly used for the coupling and deprotection of each Boc group, respectively, as summarized in Scheme 2. Bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BopCl)[13] was used for the attachment of Boc-L-Val-OH. Boc-L-Ala-L-Val-L-MeTyr(Bzl)-Tyr(Bzl)-OMe (**11**) thus obtained was treated with 10% HCl in methanol. The resulting N-terminal free tetrapeptide was coupled with the azido hydroxy acids **9a** and **9b**, respectively, followed by the protection of the hydroxyl group with chlorotriethylsilane (TESCl)[14] to give the fully protected microginins **12a** and **12b**. Successive treatment of **12a** with hydrofluoric acid, lithium hydroxide, and palladium black under hydrogen afforded (2R,3R)-microginin (**1a**) after purification on Dowex 50 W x 4 resin. Analogously, (2R,3S)-microginin (**1b**) was obtained from **9b**.



Scheme 2

The reported[1] optical rotation of natural microginin, $[\alpha]_D$ -80° (c 0.02, MeOH), however, differed from those of **1a**, $[\alpha]_D^{23}$ -68.0° (c 0.025, MeOH), and **1b**, $[\alpha]_D^{23}$ -12.2° (c 0.025, MeOH). Furthermore, the HPLC behavior of three microginins was different from each other, which has clearly indicated that the absolute stereostructure of the Ahda part of natural microginin is neither (2R,3R)- nor (2R,3S)-configuration.

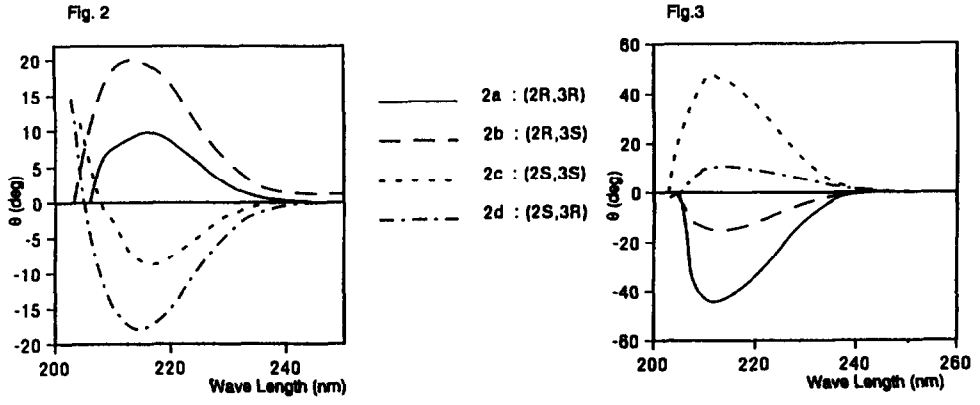
This unexpected result led us to synthesize two other possible diastereoisomers, (2S,3S)- and (2S,3R)-microginins (**1c** and **1d**), to clarify the absolute configuration of natural microginin. Thus, the diol ester **4a** was converted to the (2S,3S)-azido hydroxy acid **9c** via the (2S,3R)-epoxy ester **6c**, verified its absolute configuration by its conversion to **7c**,[9] while **4b** afforded (2S,3R)-azido hydroxy acid **9d** by the analogous way as described for the preparation of **9a** and **9b**.

After acidic removal of the Boc group from the tetrapeptide **11**, attachment of the Ahda fragment **9c**, the hydroxy protection, followed by sequential removal of all of the protecting groups afforded (2S,3S)-microginin (**1c**), as shown in Scheme 2. Analogously, (2S,3R)-microginin (**1d**) was obtained from **9d** and **11**.

Although the HPLC behavior and the optical rotation of (2S,3S)-microginin (**1c**), $[\alpha]_D^{23}$ -46.5° (c 0.062, MeOH), were different from those of natural microginin, (2S,3R)-microginin (**1d**) showed a completely identical behavior with the natural one on HPLC. Furthermore, the optical rotation of **1d**, $[\alpha]_D^{23}$ -78.8° (c 0.023, MeOH), was almost identical with that of natural microginin. These results clearly demonstrate that microginin has (2S,3R)-configuration at the Ahda portion. Thus, we could not only establish the absolute configuration of microginin, but also succeed in the total synthesis of microginin (**1d**) and its three diastereoisomers (**1a-c**).

The absolute configuration at the C-2 position of Ahda was originally proposed to be (R) by the analysis of the CD spectrum of Ahda,[1] showing a negative Cotton effect at 215 nm in MeOH. Since the absolute stereochemistry of the Ahda portion was assigned to be (2S,3R), we investigated the CD spectra of four isomers of Ahda, which were obtained by transfer-hydrogenation of the azido hydroxy acids with 5% Pd-C and ammonium formate, followed by purification on a Dowex column. As shown in Fig. 2 and 3, the Cotton effect of each CD spectrum depends on the solvent used. In a methanolic solution forming a zwitter ion, the positive Cotton effect was observed around 215 nm in both **2a** and **2b** having (2R)-configuration, while **2c** and **2d** having (2S)-configuration showed a negative Cotton effect at the same region, as shown in Fig. 2. On the contrary, in an acidic solution (1N HCl) which forms the non-ionized carboxylic acid, the sign of the Cotton effect is completely opposite as shown in Fig. 3. While **2a** and **2c** showed a negative Cotton effect, a positive Cotton effect was observed in **2b** and **2d**. Thus it should be taken care of solvent in the determination of the absolute configuration to use the CD spectra.

In conclusion, we have accomplished an efficient synthesis of microginin and its epimers, which unequivocally determined the absolute configuration of microginin. The CD spectral behavior of Ahda and its isomers was also discussed.



EXPERIMENTAL

Melting points were determined on a YAMATO MP-21 apparatus or a YANAGIMOTO micro melting point apparatus. Infrared spectra were measured with SHIMADZU FT IR-8100 spectrometer. ^{1}H-NMR spectra were recorded in $CDCl_3$, unless otherwise stated, on EX-270 spectrometer with tetramethylsilane or chloroform as an internal standard. Optical rotations were measured with a JASCO DIP-140 automatic polarimeter. CD spectra were measured with a JASCO J-720 spectropolarimeter. Silica gel (BW 200 or BW 820 MH) was used for column chromatography.

Ethyl (2R,3S)-2,3-dihydroxydecanoate (4a). To a stirred suspension of AD-mix-α (6.4 g) and $CH_3SO_2NH_2$ (390 mg, 4.00 mM) in t-BuOH (20 ml) and H_2O (20 ml) at 0°C was added ethyl (E)-2-decenoate (**3**) (789 mg, 4.00 mM). After being stirred at 0°C for 4 h, the mixture was warmed to 4°C and stirred for 20 h. The mixture was quenched with $Na_2S_2O_3$ (6 g) and extracted with CH_2Cl_2 (100 ml x 3). The combined organic extracts were dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by silica gel column chromatography (BW 820 MH, 40 g, hexane-EtOAc=3:1→2:1) to give the diol **4a** (870 mg, 94%) as a white solid: mp 42-43°C; $[\alpha]_D^{24}$ -11.7° (c 1.43, $CHCl_3$); IR ν_{max} (neat) 3389, 1732, 1715 cm^{-1}; ^{1}H NMR δ 0.88 (3H, t, J=6.9 Hz), 1.28-1.63 (15H, m), 1.88 (1H, d, J=8.9 Hz), 3.06 (1H, d, J=5.3 Hz), 3.84-3.93 (1H, m), 4.08 (1H, dd, J=2.1, 5.3 Hz), 4.29 (2H, q, J=6.9 Hz); Anal. calcd for $C_{12}H_{24}O_4$: C, 62.4; H, 10.41. Found: C, 61.92; H, 10.44.

Ethyl (2S,3S)-2-bromo-3-hydroxydecanoate (5a). The diol **4a** (384 mg, 1.65 mM) was dissolved in 25% HBr-AcOH (2 ml) and warmed to 45°C. After being stirred at 45°C for 2 h, the mixture was poured into saturated aqueous $NaHCO_3$. The mixture was extracted with CH_2Cl_2, dried over Na_2SO_4, and concentrated in vacuo to give the crude bromo acetate (531 mg) as a colorless oil: $[\alpha]_D^{24}$ -9.8° (c 0.74, $CHCl_3$); IR ν_{max} (neat) 1750 cm^{-1}; ^{1}H NMR δ 0.88 (3H, t, J=6.9 Hz), 1.27-1.43 (13H, m), 1.71-1.89 (2H, m), 2.06 (3H, s), 4.23 (2H, q, J=7.3 Hz), 4.37 (1H, d, J=7.3 Hz), 5.28 (1H, dt, J=3.3, 7.6 Hz). This crude acetate was dissolved in EtOH (5 ml) and $Ti(OiPr)_4$ (230 μl, 0.79 mM) was added. The mixture was heated to reflux with stiring for 5 h. The mixture was cooled to room temperature, quenched with 1M aqueous $KHSO_4$, and extracted with CH_2Cl_2. The organic extracts were dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by silica gel column chromatography (BW 820 MH, 20 g, hexane-Et_2O=4:1) to give the bromohydrin **5a** (180 mg, 37%) as a colorless oil: $[\alpha]_D^{24}$ -24.3° (c 0.98, $CHCl_3$); IR ν_{max} (neat) 3389, 1732 cm^{-1}; ^{1}H NMR δ 0.88 (3H, t, J=6.9 Hz), 1.18-1.83 (15H, m), 2.64-2.68 (1H, m), 3.95-4.04 (1H, m), 4.13 (1H, d, J=7.3 Hz), 4.26 (2H, q, J=6.9 Hz).

Ethyl (2R,3S)-2,3-epoxydecanoate (6a). To a stirred solution of **5a** (180 mg, 0.61 mM) in EtOH (5 ml) at 0°C was added K_2CO_3 (125 mg, 0.914 mM). After being stirred at room temperature for 2 h, the mixture was acidified with 1M $KHSO_4$ and extracted with CH_2Cl_2 (20 ml x 3). The combined organic extracts were dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by silica gel column chromatography (BW 200, 10 g, hexane-Et_2O=14:1) to give **6a** (130 mg, 99.5%) as a colorless oil: $[\alpha]_D^{24}$ -23.9° (c 1.23, $CHCl_3$); IR ν_{max} (neat) 1755,1738 cm^{-1}, ^{1}H NMR δ 0.88 (3H, t, J=6.9 Hz), 1.21-1.67 (15H, m), 3.12-3.17(1H, m), 3.20 (1H, d, J=2.0 Hz), 4.23 (2H, dq, J=3.3, 6.9 Hz); Anal. calcd for $C_{12}H_{22}O_3$: C, 67.26; H, 10.35. Found: C, 67.07; H, 10.22.

(2S,3S)-2,3-Epoxydecan-1-ol (7a). To a stirred solution of **6a** (47 mg, 0.219 mM) in THF (0.3 ml) and H_2O (0.3 ml) at 0°C was added a suspension of $NaBH_4$ (17 mg, 0.44 mM) in H_2O (0.7 ml). After being stirred at room temperature for 13 h, the mixture was quenched with 1M aqueous $KHSO_4$. The mixture was extracted with CH_2Cl_2 (30 ml x 3), dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (BW 820 MH, 5 g, hexane-Et_2O=2:1) to give **7a** (37 mg, 98%) as a white solid: mp 51-51.5°C; $[\alpha]_D^{24}$ -37.0° (c 0.61, $CHCl_3$); IR ν_{max} (KBr) 3325 cm^{-1}; ^{1}H NMR δ 0.88 (3H, t, J=6.9 Hz), 1.24-1.62 (13H, m), 2.90-2.94 (1H, m), 2.97 (1H, dd, J=2.3, 5.3 Hz), 3.63 (1H, dd, J=4.1, 12.5 Hz), 3.92 (1H, dd, J=2.3, 15.4 Hz). [Lit.[9] mp 51-52°C; $[\alpha]_D$ -31.5° (c 1.0, $CHCl_3$); IR ν_{max} 3600-3200 cm^{-1}; ^{1}H NMR δ 0.95 (3H, t), 1.25 (12H, m), 2.90 (3H, m), 4.65 (2H, m).]

(2R,3S)-2,3-Epoxydecanoic acid (8a). To a stirred solution of **6a** (60 mg, 0.28 mM) in EtOH (2 ml) at 0°C was added 1N NaOH (340 μl, 0.34 mM). After being stirred at 0°C for 1 h, the mixture was acidified by

1M aqueous $KHSO_4$ (10 ml) and extracted with CH_2Cl_2 (20 ml x 3). The organic extracts were dried over Na_2SO_4 and concentrated in vacuo to give **8a** (51 mg, 98%) as a white solid: $[\alpha]_D^{24}$ -16.2° (c 0.31, $CHCl_3$); IR ν_{max} (KBr) 3688-2598 (br), 1705 cm^{-1}; ^1H NMR δ 0.88 (3H, t, J=6.9 Hz), 1.28-1.68 (12H, m), 3.17 (1H, brs), 3.24 (1H, brs), 5.04-5.63 (1H, br). Anal. calcd for $C_{10}H_{18}O_3$: C, 64.49; H, 9.74. Found: C, 64.51; H, 9.92. [Lit.[5] ^1H NMR δ 0.80-1.0 (3H, brt), 1.1-1.8 (12H, m), 3.20 (1H, dt, J=1.7, 5 Hz), 3.27 (1H, d, J=1.7 Hz).]

(2R,3R)-3-Azido-2-hydroxydecanoic acid (9a). To a stirred solution of LiN_3 (30 mg, 0.61 mM) and $Ti(OiPr)_4$ (91 ml, 0.31 mM) in EtOH (1 ml) at room temperature under argon atmosphere was added a solution of the epoxy acid **8a** (38 mg, 0.2 mM) in EtOH (1 ml). After being stirred at room temperature for 20 h, the mixture was quenched with 1M $KHSO_4$, extracted with CH_2Cl_2 (30 ml x 3), dried over Na_2SO_4, and concentrated in vacuo to give **9a** (45 mg, 96%) as a pale yellow oil: $[\alpha]_D^{23}$ +33.2° (c 1.03, $CHCl_3$); IR ν_{max} (neat) 3710-2470 (br), 2109, 1732 cm^{-1}; ^1H NMR δ 0.89 (3H, t, J=6.9 Hz), 1.22-1.81 (12H, m), 3.60-3.65 (1H, m), 4.43 (1H, d, J=3.0 Hz), 3.93-4.43 (1H, br).

Ethyl (2S,3R)-2,3-dihydroxydecanoate (4b). The reaction was performed by the same procedure as that for **4a** by use of AD-mix-β (8.8 g), $CH_3SO_2NH_2$ (610 mg, 6.29 mM), t-BuOH (30 ml), H_2O (30 ml), and **3** (1.25 g, 6.29 mM). The diol **4b** was obtained as a white solid (1.4 g, 96%), which was recrystallized from Et_2O-hexane: mp 41-42°C; $[\alpha]_D^{24}$ +11.4° (c 0.57, $CHCl_3$); IR ν_{max} (KBr) 3355, 1732, 1715 cm^{-1}; ^1H NMR δ 0.88 (3H, t, J=6.9 Hz), 1.28-1.63 (15H, m), 1.86-1.98 (1H, br), 3.04-3.09 (1H, br), 3.86-3.92 (1H, m), 4.09 (1H, d,J=2.0 Hz), 4.30 (2H, q, J=6.9 Hz); Anal. calcd for $C_{12}H_{24}O_4$: C, 62.04; H, 10.41. Found: C, 61.77; H, 10.13.

Ethyl (2S,3R)-3-hydroxy-2-(4-nitrobenzenesulfonyloxy)decanoate (5b). To a stirred solution of **4b** (577 mg, 2.48 mM) in pyridine at 0°C was added NsCl (605 mg, 2.73 mM). After being stirred at 4°C for 20 h, the mixture was quenched with H_2O and treated with Et_2O. The ethereal solution was washed with 1M aqueous $KHSO_4$ (20 ml x 3) and saturated brine (20 ml x 1), dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (BW 200, 30 g, hexane-EtOAc=5:1) to give **5b** (722 mg, 70%) as a colorless oil: $[\alpha]_D^{24}$ +6.3° (c 1.81, $CHCl_3$); IR ν_{max} (neat) 3517, 1759 cm^{-1}; ^1H NMR δ 0.88 (3H, t, J=6.9 Hz), 1.20-1.59 (15H, m), 1.86 (1H, d, J=8.3 Hz), 4.03-4.12 (1H, m), 4.17 (2H, q, J=6.9 Hz), 4.99 (1H, d, J=3.0 Hz), 8.18 (2H, d, J=9.2 Hz), 8.40 (2H, d, J=9.2 Hz).

Ethyl (2R,3R)-2,3-epoxydecanoate (6b). To a stirred solution of **5b** (653 mg, 1.56 mM) in EtOH (10 ml) was added K_2CO_3 (540 mg, 3.91 mM). After being stirred at room temperature for 11 h, the mixture was acidified with 1M aqueous $KHSO_4$ (10 ml) and extracted with CH_2Cl_2 (30 ml x 3). The combined organic extracts were dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by silica gel column chromatography (BW 820 MH, 20 g, hexane-Et_2O=14:1→9:1) to give the cis-epoxy ester **6b** (305 mg, 91%) as a colorless oil: $[\alpha]_D^{24}$ +6.1° (c 1.08, $CHCl_3$); IR ν_{max} (neat) 1753, 1730 cm^{-1}; ^1H NMR δ 0.87 (3H, t, J=6.9 Hz), 1.27-1.75 (15H, m), 3.13-3.19 (1H, m), 3.51 (1H, d, J=4.6 Hz), 4.26 (2H, dq, J=1.3, 6.9 Hz); Anal. calcd for $C_{12}H_{22}O_3$: C, 67.26; H, 10.35. Found: C, 67.01, H, 10.45.

(2R,3R)-2,3-Epoxydecanoic acid (8b). The reaction was performed by the same procedure as that for **8a** by use of **6b** (69 mg, 0.322 mM), EtOH (2 ml), and 1N NaOH (420 ml, 0.42 mM) to give **8b** as a white solid (58 mg, 99%): mp 52-53°C; $[\alpha]_D^{24}$ +11.7° (c 0.83 $CHCl_3$); IR ν_{max} (KBr) 3517-2689 (br), 1728 cm^{-1}; ^1H NMR δ 0.88 (3H, t, J=6.9 Hz), 1.24-1.73 (12H, m), 3.22-3.28 (1H, m), 3.58 (1H, d, J=4.6 Hz), 6.03-7.20 (1H, br); Anal. calcd for $C_{10}H_{18}O_3$: C, 64.49; H, 9.74. Found: C, 64.34; H, 9.53.

(2R,3S)-3-Azido-2-hydroxydecanoic acid (9b). The reaction was performed by the same procedure as that for **9a** by use of LiN_3 (42 mg, 0.86 mM), $Ti(OiPr)_4$ (127 ml, 0.43 mM), and **8b** (53 mg, 0.29 mM) to give **9b** as a colorless oil (58 mg, 89%): $[\alpha]_D^{23}$ +24.6° (c 1.08, $CHCl_3$); IR ν_{max} (neat) 3688-2859 (br), 2114, 1732 cm^{-1}; ^1H NMR δ 0.89 (3H, t, J=6.9 Hz), 1.30-1.45 (10H, m), 1.78-1.91 (2H, m), 3.57-3.63 (1H, m), 4.29 (1H, d, J=2.0 Hz), 4.80-5.21 (1H, br).

Ethyl (2S,3R)-2-bromo-3-hydroxydecanoate (5c). The reaction was performed by the same procedure as that for **5a** by use of **4b** (1.64 g, 7.08 mM) and 25% HBr-AcOH (10 ml)to give the crude acetate (2.39 g) as a colorless oil: $[\alpha]_D^{24}$ +10.0° (c 0.85, $CHCl_3$); IR ν_{max} (neat) 1747, 1755 cm^{-1}; ^1H NMR δ 0.88

(3H, t, J=6.9 Hz), 1.26-1.32 (13H, m), 1.66-1.88 (2H, m), 2.06 (3H, s), 4.23 (2H, q, J=7.3 Hz), 4.37 (1H, d, J=7.6 Hz), 5.28 (1H, dt, J=3.3, 7.6 Hz). The crude acetate was treated with EtOH (20 ml) and $Ti(OiPr)_4$ (1.1 ml, 3.67 mM) to give **5c** (777 mg, 37%) as a colorless oil: $[\alpha]_D^{24}$ +23.4° (c 1.65, $CHCl_3$); IR ν_{max} (neat) 3475, 1740 cm^{-1}; ^1H NMR δ 0.88 (3H, t, J=6.9 Hz), 1.29-1.58 (14H, m), 1.77-1.88 (1H, m), 2.63 (1H, d, J=6.6 Hz), 3.95-4.05 (1H, m), 4.13 (1H, d, J=7.3 Hz), 4.26 (2H, q, J=6.9 Hz).

Ethyl (2S,3R)-2,3-epoxydecanoate (6c). The reaction was performed by the same procedure as that for **6a** by use of **5c** (777 mg, 2.63 mM), EtOH (10 ml), and K_2CO_3 (550 mg) to give **6c** (518 mg, 92%) as a colorless oil: $[\alpha]_D^{24}$ +25.0° (c 0.63, $CHCl_3$); IR ν_{max} (neat) 1754, 1738 cm^{-1}; ^1H NMR δ 0.88 (3H, t, J=6.9 Hz), 1.28-1.68 (15H, m), 3.13-3.17 (1H, m), 3.21 (1H, d, J=2.0 Hz), 4.24 (2H, dq, J=3.0, 6.9 Hz); Anal. calcd for $C_{12}H_{22}O_3$: C, 67.26; H, 10.35. Found: C, 67.52; H, 10.32.

(2S,3R)-2,3-Epoxydecanoic acid (8c). The reaction was performed by the same procedure as that for **8a** by use of **6c** (518 mg, 2.42 mM), EtOH (15 ml), and 1N NaOH (3.63 ml, 3.63 mM) to give **8c** (446 mg, 99%) as a white solid: $[\alpha]_D^{24}$ +16.8° (c 0.71, $CHCl_3$); IR ν_{max} (KBr) 3646-2541 (br), 1705 cm^{-1}, ^1H NMR δ 0.88 (3H, t, J=6.9 Hz), 1.23-1.70 (12H, m), 3.17-3.21 (1H, m), 3.26 (1H, d, J=2.0 Hz), 7.73-8.08 (1H, br); High mass calcd for $C_{10}H_{18}O_3$: 186.2532. Found: 186.1253.

(2R,3R)-2,3-Epoxydecan-1-ol (7c). The reaction was performed by the same procedure as that for **7a** by use of **6c** (47 mg, 0.219 mM). Purification on silica gel column chromatography (BW 820 MH, 5 g, hexane-Et_2O=2:1) afforded **7c** (27 mg, 72%) as a white solid: mp 48-49°C; $[\alpha]_D^{24}$ +36.0° (c 0.39, $CHCl_3$); IR ν_{max} (KBr) 3286 cm^{-1}; ^1H NMR δ 0.88 (3H, t, J=6.9 Hz), 1.28-1.62 (13H, m), 2.91-2.99 (2H, m), 3.63 (1H, dd, J=4.3, 12.5 Hz), 3.92 (1H, dd, J=2.3, 12.5 Hz). [Lit.[9] mp 48-49°C; $[\alpha]_D$ +37° (c 1.0 $CHCl_3$)].

(2S,3S)-3-Azido-2-hydroxydecanoic acid (9c). The reaction was performed by the same procedure as that for **9a** by use of **8c** (446 mg, 2.39 mM), LiN_3 (352 mg, 7.18 mM), $Ti(OiPr)_4$ (1.07 ml, 3.59 mM), and EtOH (10 ml) to give **9c** (527 mg, 96.0%) as a colorless oil: $[\alpha]_D^{23}$ -36.0° (c 1.06, $CHCl_3$); IR ν_{max} (neat) 3453-2598 (br), 2109, 1732 cm^{-1}; ^1H NMR δ 0.89 (3H, t, J=6.9 Hz), 1.20-1.57 (11H, m), 1.76 (1H, q, J=9.6 Hz), 3.63 (1H, dt, J=3.3, 9.9 Hz), 4.42 (1H, d, J=3.0 Hz), 5.75 (1H, br).

Ethyl (2R,3S)-3-hydroxy-2-(4-nitrobenzenesulfonyloxy)decanoate (5d). The reaction was performed by the same procedure as that for **5b** by use of **4a** (1.68 g, 7.23 mM), NsCl (1.76 g, 7.95 mM), and pyridine (18 ml). Purification on and silica gel column chromatography (BW 820 MH, 100 g, hexane-EtOAc=5:1) afforded **5d** (2.04 g, 68%) as a colorless oil: $[\alpha]_D^{24}$ -6.1° (c 0.97, $CHCl_3$); IR ν_{max} (neat) 3560, 1759, 1744 cm^{-1}; ^1H NMR δ 0.88 (3H, t, J=6.9 Hz), 1.20-1.60 (15H, m), 1.80 (1H, dd, J=4.3, 8.6 Hz), 4.04-4.12 (1H, m), 4.18 (2H, q, J=6.9 Hz), 4.99 (1H, d, J=3.0 Hz), 8.18 (2H, d, J=8.9 Hz), 8.41 (2H, d, J=8.9 Hz).

Ethyl (2S,3S)-2,3-epoxydecanoate (6d). The reaction was performed by the same procedure as that for **6b** by use of **5d** (2.04 g, 4.88 mM), K_2CO_3 (1.69 g, 12.19 mM), and EtOH (20 ml). Purification on silica gel column chromatography (BW 820 MH, 40 g, hexane-Et_2O=12:1) afforded **6d** (961 mg, 92%) as a colorless oil: $[\alpha]_D^{24}$ -5.7° (c 1.57, $CHCl_2$); IR ν_{max} (neat) 1755, 1732 cm^{-1}; ^1H NMR δ 0.88 (3H, t, J=6.9 Hz), 1.27-1.76 (15H, m), 3.14-3.20 (1H, m), 3.52 (1H, d, J=4.6 Hz), 4.26 (2H, dq, J=1.3, 6.9 Hz); Anal. calcd for $C_{12}H_{22}O_3$: C, 67.26; H, 10.35. Found: C, 67.47, H, 10.18.

(2S,3S)-2,3-epoxydecanoic acid (8d). The reaction was performed by the same procedure as that for **8a** by use of **6d** (516 mg, 2.41 mM), EtOH (15 ml), and 1N NaOH (3.61 ml, 3.61 mM) to give **8d** (480 mg, quant.) as a white solid: mp 56-56.5°C; $[\alpha]_D^{24}$ -12.0° (c 0.57, $CHCl_3$); IR ν_{max} (KBr) 3650-2687 (br), 1728 cm^{-1}: ^1H NMR δ 0.88 (3H, t, J=6.9 Hz), 1.28-1.73 (12H, m), 3.22-3.29 (1H, m), 3.59 (1H, d, J=4.6 Hz), 6.00-7.95 (1H, br); Anal. calcd for $C_{10}H_{18}O_3$: C, 64.49; H, 9.74. Found: C, 64.28; H, 9.48.

(2S,3R)-3-Azido-2-hydroxydecanoic acid (9d). The reaction was performed by the same procedure as that for **9a** by use of **8d** (480 mg, 2.41 mM), LiN_3 (353 mg, 722 mM), $Ti(OiPr)_4$, (1.07 ml, 3.61 mM), and EtOH (10 ml) to give **9d** (552 mg, quant) as a colorless oil: $[\alpha]_D^{23}$ -28.9° (c 1.30, $CHCl_3$); IR ν_{max} (neat) 3475-2598 (br), 2112, 1732 cm^{-1}; ^1H NMR δ 0.89 (3H, t, J=6.9 Hz), 1.25-1.48 (10H, m), 1.73-1.93 (2H, m), 3.57-3.63 (1H, m), 4.30 (1H, d, J=2.3 Hz), 5.27-6.36 (1H, br).

Methyl (2R,3R)-2-acetoxy-3-azidodecanoate (10a). To a stirred solution of **9a** (44 mg, 0.19 mM) in benzene (0.8 ml) and MeOH (0.2 ml) was added 0.87M $TMSCHN_2$ in hexane (400 µl, 0.35 mM). After being stirred at room temperature for 15 min, the solvent was removed in vacuo to give the crude ester (46 mg) as a pale yellow oil. This crude ester was dissolved in pyridine (1 ml) and Ac_2O (36 µl, 0.38 mM) was added. After being stirred at room temperature for 16 h, the mixture was treated with ether. The ethereal solution was washed with 1M aqueous $KHSO_4$ (20 ml x 3) and saturated aqueous $NaHCO_3$ (20 ml x 1), dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (BW 820 MH, 8 g, hexane-Et_2O=14:1) to give **10a** (42 mg, 77%) as a colorless oil: $[\alpha]_D^{25}$ +37.9° (c 0.79, $CHCl_3$); IR ν_{max} (neat) 2105, 1755 cm^{-1}; ^1H NMR δ 0.89 (3H, t, J=6.9 Hz), 1.29-1.79 (12H, m), 2.19 (3H, s), 3.65 (1H, dt, J=3.6, 9.9 Hz), 3.79 (3H, s), 5.20 (1H, d, J=3.6 Hz). [Lit.[4] for racemic **10a** : IR ν_{max} (neat) 2100, 1741 cm^{-1}; ^1H NMR δ 0.89 (3H, t, J=7 Hz), 1.1-1.8 (12H, m), 2.19 (3H, s), 3.66 (1H, m), 3.79 (3H, s), 5.20 (1H, d, J=3.5 Hz).]

Methyl (2R,3S)-2-acetoxy-3-azidodecanoate (10b). The reaction was performed by the same procedure as that for **10a** by use of **9b** (51 mg, 0.22 mM). The crude ester (62 mg) was treated with Ac_2O (42 µl, 0.44 mM), follwed by silica gel column chromatography (BW 820 MH, 8 g, hexane-Et_2O=14:1) to give **10b** (48 mg, 76%) as a colorless oil: $[\alpha]_D^{25}$ +25.3° (c 0.74, $CHCl_3$); IR ν_{max} (neat) 2110, 1755 cm^{-1}; ^1H NMR δ 0.89 (3H, t, J=6.9 Hz), 1.24-1.74 (12H, m), 2.21 (3H, s), 3.67-3.74 (1H, m), 3.80 (3H, s), 5.14 (1H, d, J=3.6 Hz). [Lit.[4] for racemic **10b** : ^1H NMR δ 0.89 (3H, t, J=7 Hz), 1.1-1.8 (12H, m), 2.22 (3H, s), 3.71 (1H, m), 3.80 (3H, s), 5.14 (1H, d, J=3.4 Hz).]

Methyl (2S,3S)-2-acetoxy-3-azidodecanoate (10c). The reaction was performed by the same procedure as that for **10a** by use of **9c** (51 mg, 0.22 mM). The crude ester (56 mg) obtained as a pale yellow oil was treated with Ac_2O (42 µl, 0.44 mM) and pyridine (0.5 ml) to give **10c** (57 mg, 90%) as a colorless oil: $[\alpha]_D^{25}$ -39.4° (c 0.89, $CHCl_3$); IR ν_{max} (neat) 2107, 1755 cm^{-1}; ^1H NMR δ 0.89 (3H, t, J=6.9Hz), 1.29-1.79 (12H, m), 2.19 (3H, t), 3.66 (1H, dt, J=3.6, 9.9Hz), 3.79 (3H, S), 5.20 (1H, d, J=3.6Hz).

Methyl (2S,3R)-2-acetoxy-3-azidodecanoate (10d). The reaction was performed by the same procedure as that for **10a** by use of **9d** (51 mg, 0.22 mM) to give a pale yellow oil (55 mg), which was acetylated with Ac_2O (50 ml, 0.53 mM) and pyridine (0.5 ml), giving **10d** (51 mg, 80%) as a colorless oil: $[\alpha]_D^{25}$ -27.6° (c 0.79, $CHCl_3$); IR ν_{max} (neat) 2110, 1755 cm^{-1}; ^1H NMR δ 0.89 (3H, t, J=6.9 Hz), 1.29-1.73 (12H, m), 2.21 (3H, s), 3.68-3.74 (1H, m), 3.80 (3H, s), 5.14 (1H, d, J=3.6 Hz).

(2R,3R)-3-Amino-2-hydroxydecanoic acid (2a). To a stirred suspension of **9a** (30 mg, 0.13 mM) and 5% Pd-C (30 mg) in MeOH (1 ml) at 0°C was added HCO_2NH_4 (33 mg, 0.52 mM). After being stirred at room temperature for 1 h, the mixture was filtered through the pad of celite and the filtrate was concentrated in vacuo. The residue was purified by ion-exchange resin (Dowex 50Wx4, 5 ml, 50% aqueous MeOH then 15% pyridine, 43% MeOH) to give **2a** (25 mg, 94%) as a white powder. An analytical sample was purified by Sephadex LH-20 (MeOH) followed by recrystallization (MeOH-Et_2O): mp 183-186°C (dec); $[\alpha]_D^{24}$ +34.7° (c 0.46, MeOH); IR ν_{max} (KBr) 3650-2363 (br), 1653 cm^{-1}; ^1H NMR (DMSO-d^6) δ 0.86 (3H, t, J=6.6 Hz), 1.25-1.68 (12H, m), 2.87 (1H, dd, J=3.3, 8.3 Hz), 33.9 (1H, d, J=7.9 Hz); Anal calcd for $C_{10}H_{21}NO_3 \cdot 0.2\ H_2O$: C, 58.06; H, 10.43; N, 6.77. Found: C, 58.30; H, 10.25; N, 6.67. [Lit.[4] $[\alpha]_D^{25}$ +3.4° (c 0.70, 1N HCl); ^1H NMR (DMSO-d^6) δ 0.86 (3H, t, J=6.9 Hz), 1.71-1.26 (1H, m), 3.35 (1H, d, J=8.1 Hz).]

(2R,3S)-3-Amino-2-hydroxydecanoic acid (2b). The reaction was performed by the same procedure as that for **2a** by use of **9b** (34 mg, 0.15 mM) and HCO_2NH_4 (37 mg, 0.59 mM) to give **2b** (29 mg, 96%) as a white solid, which was purified by Sephadex LH-20 (MeOH): mp 152-156°C (dec); $[\alpha]_D^{23}$ +9.0° (c 0.11, MeOH); IR ν_{max} (KBr) 3630-2360 (br), 1592 cm^{-1}; ^1H NMR (DMSO-d^6) δ 0.86 (3H, t, J=6.6 Hz), 1.25-1.58 (12H, m), 3.11-3.14 (1H, m), 3.56 (1H, d, J=3.3 Hz); FABMS (glycerin) m/z: 203 (M+1).

(2S,3S)-3-Amino-2-hydroxydecanoic acid (2c). The reaction was performed by the same procedure as that for **2a** by use of **9c** (30 mg, 0.13 mM), 5% Pd-C (20 mg), and HCO_2NH_4 (33 mg, 0.52 mM) to give **2c** (27 mg, quant) as a white solid. An analytical sample was purified by Sephadex LH-20 (MeOH) followed by recrystallization from MeOH-Et_2O: mp 189-193°C (dec); $[\alpha]_D^{24}$ -34.5° (c 0.47, MeOH); IR ν_{max} (KBr) 3645-2360 (br), 1630 cm^{-1}; ^1H NMR (DMSO-d^6) δ 0.86 (3H, t, J=6.6 Hz), 1.15-1.74 (12H, m), 2.86 (1H, dt, J=3.6, 8.3 Hz), 3.38 (1H, d, J=8.6 Hz); Anal. Calcd for $C_{10}H_{21}NO_3 \cdot 0.1\ H_2O$: C, 58.57; H, 10.42; N,

6.83. Found: C, 58.57; H, 10.34; N, 6.67.

(2S,3R)-3-Amino-2-hydroxydecanoic acid (2d). The reaction was performed by the same procedure as that for **2a** by use of **9d** (31 mg, 0.14 mM), 5% Pd-C (25 mg), and HCO_2NH_4 (34 mg, 0.54 mM) to give **2d** (29 mg, quant) as a white solid. An analytical sample was purified by Sephadex LH-20 (MeOH) followed by recrystallization from MeOH-Et_2O: mp 156-159°C (dec); $[\alpha]_D^{23}$ -8.8° (c 0.19, MeOH); IR ν_{max} (KBr) 3630-2370 (br), 1590 cm^{-1}; ^1H NMR (DMSO-d^6) δ 0.86 (3H, t, J=6.9 Hz), 1.17-1.60 (12H, m), 3.10-3.15 (1H, m), 3.56 (1H, brs); Anal. calcd for $C_{10}H_{21}NO_3 \cdot 0.3\ H_2O$: C, 57.56; H, 10.43; N, 6.71. Found: C, 57.24, H, 10.34; N, 6.43. [Lit.[3] $[\alpha]_D^{25}$ +5.4° (c 0.59, 1N HCl); ^1H NMR (DMSO-d^6) δ 0.86 (3H, t, J=6.8 Hz), 1.20-1.60 (12H, m), 3.10 (1H, br), 3.55 (1H, br).]

Boc-(S)-MeTyr(Bzl)-(S)-Tyr(Bzl)-OMe. To a cooled (0°C) solution of HCl·H-(S)-Tyr(Bzl)-OMe (2.02 g, 6.28 mM) and Boc-(S)-MeTyr(Bzl)-OH (2.42 g, 6.28 mM) in DMF (20 ml) were added DEPC (1.0 ml, 6.59 mM) and Et_3N (1.75 ml, 12.55 mM) successively. After being stirred at 0°C for 4 h, the mixture was allowed to warm to temperature and stirred for 14 h. The mixture was diluted with EtOAc, washed with 1M aqueous $KHSO_4$, H_2O, saturated aqueous $NaHCO_3$, H_2O, and saturated brine (each 50 ml x 1), dried over Na_2SO_4, and concentrated in vacuo. The residure was purified by silica gel column chromatography (BW 820 MH, 150 g, hexane-EtOAc=3:1→2:1) to give the dipeptide (3.48 g, 85%) as a colorless oil: $[\alpha]_D^{25}$ -42.4° (c 1.77, $CHCl_3$); IR ν_{max} (neat) 3415, 1744, 1682 cm^{-1}; ^1H NMR δ 1.27, 1.37 (9H, each s), 2.53, 2.59 (3H, each s), 2.77-3.25 (4H, m), 3.69, 3.72 (3H, each s), 4.71-4.88 (2H, m), 5.02 (4H, s), 6.26-6.29, 6.51-6.53 (1H, each br), 6.85-6.90 (4H, m), 6.99 (2H, d, J=8.3 Hz), 7.07 (2H, br), 7.32-7.43 (10H, m); FABMS (glycerin) m/z: 653 (M+1); Anal. calcd for $C_{39}H_{44}O_7$: C, 71.76; H, 6.79; N, 4.29. Found: C, 71.51; H, 6.84; N, 4.13.

Boc-(S)-Val-(S)-MeTyr(Bzl)-(S)-Tyr(Bzl)-OMe. Boc-(S)-MeTyr(Bzl)-(S)-Tyr(Bzl)-OMe (1.27 g, 1.94 mM) was dissolved in 10% HCl-MeOH (10 ml) at 0°C and stirred at room temperature for 1 h. The solvent was removed in vacuo. The residue was neutralized with saturated aqueous $NaHCO_3$ (50 ml), extracted with CH_2Cl_2 (50 ml x 3), dried over Na_2SO_4, and concentrated in vacuo to give the crude peptide (1.00 g) as a white solid. The crude peptide and Boc-(S)-Val-OH (526 mg, 2.42 mM) were dissolved in CH_2Cl_2 (3 ml) and cooled to 0°C. BopCl (640 mg, 2.52 mM) and Et_3N (703 μl, 5.04 mM) was successively added to the cooled mixture. After the mixture was stirred at 4°C for 36 h, Boc-(S)-Val-OH (316 mg, 1.45 mM), BopCl (394 mg, 1.55 mM), and Et_3N (324 μl, 2.33 mM) was successively added and the mixture was stirred for 36 h. The mixture was diluted with EtOAc, washed with 1M aqueous $KHSO_4$, H_2O, saturated aqueous $NaHCO_3$, H_2O, and saturated brine (each 50 ml x 1), dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (BW 200, 100 g, hexane-EtOAc=5:2→2:1) to give the tripeptide (1.14 g, 79%) as a colorless oil: $[\alpha]_D^{25}$ -70.3° (c 1.28, $CHCl_3$); IR ν_{max} (neat) 3432, 3346, 1746, 1709, 1678, 1613 cm^{-1}; ^1H NMR δ 0.83, 0.89 (3H, each d, J=6.9 Hz), 1.01, 1.07 (3H, each d, J=6.6 Hz and J=6.9 Hz), 1.42 (9H, s), 1.78-1.85 (0.5H, m), 2.04-2.26 (0.5H, m), 2.49 (1.5H, s), 2.71-3.34 (4H, m), 2.87 (1.5H, s), 3.66 (1.5H, s), 4.10 (0.5H, dd, J=4.8, 8.1 Hz), 4.32 (0.5H, dd, J=5.9, 9.6 Hz), 4.61 (0.5H, d, J=5.4, 8.7 Hz), 4.66-4.77 (1H, m), 5.00 (2H, s), 5.02 (2H, s), 5.02-5.08 (1H, m), 5.29 (0.5H, t, J=7.8 Hz), 6.31 (0.5H, d, J=7.6 Hz), 6.80-7.11 (8H, m), 7.27-7.44 (10H, m), 7.93 (0.5H, d, J=8.3 Hz); FABMS (glycerin) m/z: 752 (M+1), Anal. calcd for $C_{44}H_{53}N_3O_8$: C, 70.28; H, 7.10; N, 5.59. Found: C, 70.42; H, 7.31; N, 5.26.

Boc-(S)-Ala-(S)-Val-(S)-MeTyr(Bzl)-(S)-Tyr(Bzl)-OMe (11). The above tripeptide (766 mg, 1.02 mM) was dissolved in 10% HCl-MeOH (6 ml) at 0°C and stirred at room temperature for 4 h. The solvent was removed in vacuo to give the crude hydrochloride (756 mg). The crude hydrochloride and Boc-(S)-Ala-OH (193 mg, 1.02 mM) were dissolved in DMF (3.4 ml) and cooled to 0°C. DEPC (162 μl, 1.07 mM) and Et_3N (298 μl, 2.14 mM) were successively added to the cooled mixture. The mixture was stirred at 0°C for 4 h and allowed to warm to room temparature. After being stirred at room temparature for 8 h, the mixture was treated with EtOAc-benzene (4:1), washed with 1M aqueous $KHSO_4$, saturated aqueous $NaHCO_3$, H_2O, and saturated brine (each 30 ml x 1), dried over Na_2SO_4, and conceutrated in vacuo. The residue was purified by silica gel column chromatography (BW 200, 400 g, hexane-EtOAc=3:2) to give the tetrapeptide **11** (762 mg, 91%) as a

colorless oil: $[\alpha]_D^{25}$ -83.2° (c 1.02, $CHCl_3$); IR ν_{max} (neat) 3346, 1744, 1713, 1682, 1628 cm^{-1}, ^1H NMR δ 0.55 (1.5H, d, J=6.6 Hz), 0.73 (1.5H, d, J=6.6 Hz), 0.83 (3H, d, J=6.6 Hz), 0.87 (3H, d, J=6.9 Hz), 1.43 (9H, s), 1.84-1.92 (1H, m), 2.56 (1.5H, s), 2.72-3.33 (4H, m), 2.86 (1.5H, s), 3.66 (1.5H, s), 3.70 (1.5H, s), 4.06-4.17 (0.5H, m), 4.23-4.28 (0.5H, m), 4.62 (0.5H, dd, J=6.1, 9.1 Hz), 4.67-4.87 (2H, m), 5.00 (2H, s), 5.01 (1H, d, J=8.5 Hz), 5.02 (2H, s), 5.31 (0.5H, t, J=7.9 Hz), 6.36 (0.5H, d, J=7.6 Hz), 6.62 (0.5H, d, J=8.9 Hz), 6.81-7.09 (8.5H, m), 7.27-7.43 (10H, m), 7.95 (0.5H, d, J=7.7 Hz); FABMS (glycerin) m/z: 823 (M+1);

General Procedure for the Preparation of 3-Azido-2-triethylsiloxydecanoyl-(S)-Ala-(S)-Val-(S)-MeTyr(Bzl)-(S)-Tyr(Bzl)-OMe (12). The tetrapeptide **11** (0.30 mM) was dissolved in 10% HCl-MeOH (2 ml) at 0°C and stirred for 1 h. The solvent was removed in vacuo to give the crude hydrochloride. The crude hydrochloride and 3-azido-2-hydroxydecanoic acid (0.33 mM) were dissolved in DMF (1 ml) at 0°C, and DEPC (0.36 mM) and i-Pr_2NEt (0.72 mM) were successively added. After being stirred at 0°C for 1 h, the mixture was allowed to warm to room temperature and stirred for 15 h. The mixture was diluted with EtOAc-benzene (4:1), washed with 1M aqueous $KHSO_4$, H_2O, saturated aqueous $NaHCO_3$, H_2O, and saturated brine (each 10 ml x 1), dried over Na_2SO_4, and concentrated in vacuo to give the crude alcohol. The crude alcohol was dissolved in DMF (1 ml) at 0°C, and imidazole (1.00 mM) and TESCl (0.68 mM) were successively added. After being stirred at room temperature for 1 h, the mixture was treated with Et_2O, washed with 1M aqueous $KHSO_4$, H_2O, and saturated brine (each 10 ml x 1), dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (BW 200, hexane-benzene-EtOAc=3:1:2) to give **12** (43-71%) as a colorless oil.

(2R,3R)-3-Azido-2-triethylsiloxydecanoic-(S)-Ala-(S)-Val-(S)-MeTyr(Bzl)-(S)-Tyr-(Bzl)-OMe (12a): Yield, 43 %; $[\alpha]_D^{25}$ -36.7° (c 1.70, $CHCl_3$); IR ν_{max} (neat) 3584, 3325, 2103, 1746, 1682, 1661, 1651, 1626 cm^{-1}; ^1H NMR δ 0.53 (1.5H, d, J=6.9 Hz), 0.63-0.72 (7.5H, m), 0.81-0.67 (9H, m), 0.94-1.01 (9H, m), 1.24-1.43 (12H, m), 1.82-1.96 (1H, m), 2.47 (1.5H, s), 2.69-3.33 (4H, m), 2.86 (1.5H, s), 3.56-3.61 (1H, m), 3.66 (1.5H, s), 3.71 (1.5H, s), 4.20-4.23 (0.5H, m), 4.25 (0.5H, d, J=2.6 Hz), 4.28 (0.5H, d, J=2.3 Hz), 4.36-4.46 (1H, m), 4.62 (0.5H, dd, J=5.9, 8.9 Hz), 4.66-4.77 (1.5H, m), 5.00 (1H, s), 5.02 (1H, s), 5.03 (2H, s), 5.28 (0.5H, t, J=7.9 Hz), 6.32 (0.5H, d, J=7.6 Hz), 6.56 (0.5H, d, J=8.9 Hz), 6.82-7.10 (9.5H, m), 7.28-7.43 (10H, m), 7.91 (0.5H, d, J=8.2 Hz); Anal. calcd for $C_{58}H_{81}N_7O_9Si$: C, 66.45; H, 7.79; N, 9.35. Found: C, 66.59; H, 8.02; N, 8.97; FABMS (glycerin) m/z: 1048 (M+1);

(2R,3S)-3-Azido-2-triethylsiloxydecanoic-(S)-Ala-(S)-Val-(S)-MeTyr(Bzl)-(S)-Tyr-(Bzl)-OMe (12b): Yield, 56 %; $[\alpha]_D^{25}$ -30.9° (c 1.37, $CHCl_3$); IR ν_{max} (neat) 3586, 3409, 3310, 2019, 1748, 1688, 1682, 1651, 1634 cm^{-1}; ^1H NMR δ 0.53 (1.5H, d, J=6.6 Hz), 0.61-0.72 (7.5H, m), 0.82-0.90 (9H, m), 0.95-1.02 (9H, m), 1.21-1.52 (12H, m), 1.74-1.93 (1H, m), 2.47 (1.5H, s), 2.69-3.28 (4H, m), 2,87 (1.5H, s), 3.32-3.41 (1H, m), 3.65 (1.5H, s), 3.70 (1.5H, s), 4.14 (0.5H, d, J=2.3 Hz), 4.16 (0.5H, d, J=8.0 Hz), 4.21 (0.5H, t, J=7.1 Hz), 4.42-4.53 (1H, m), 4.59-4.78 (2H, m), 5.00 (1H, s), 5.02 (1H, s), 5.03 (1H, s), 5.29 (0.5H, t, J=7.8 Hz), 6.32 (0.5H, d, J=7.9 Hz), 6.63 (0.5H, d, J=8.9 Hz), 6.78 (0.5H, d, J=8.3 Hz), 6.83-7.11 (9H, m), 6.32 (0.5H, d, J=7.9 Hz), 6.63 (0.5H, d, J=8.9 Hz), 6.78 (0.5H, d, J=8.3 Hz), 6.83-7.11 (9H, m), 7.27-7.43 (10H, m), 7.93 (0.5H, d, J=7.9 Hz); Anal. calcd for $C_{58}H_{81}N_7O_9Si$: C, 66.45; H, 7.79; N, 9.35. Found: C, 66.30; H, 7.97; N, 8.99; FABMS (glycerin) m/z: 1048 (M+1);

(2S,3S)-3-Azido-2-triethylsiloxydecanoic-(S)-Ala-(S)-Val-(S)-MeTyr(Bzl)-(S)-Tyr(Bzl)-OMe (12c): Yield, 71 %; $[\alpha]_D^{23}$ -79.1° (c 1.14, $CHCl_3$); IR ν_{max} (neat) 3586, 3407, 3325, 2103, 1748, 1690, 1682, 1651, 1617 cm^{-1}; ^1H NMR δ 0.55 (1H, d, J=6.6 Hz), 0.61-0.72 (8H, m), 0.78-0.89 (9H, m), 0.93-1.00 (9H, m), 1.25-1.43 (12H, m), 1.81-1.93 (1H, m), 2.53 (1H, s), 2.73-3.33 (4H, m), 2.86 (2H, s), 3.57-3.64 (1H, m), 3.66 (2H, s), 3.70 (1H, s), 4.26 (0.3H, d, J=2.6 Hz), 4.28 (0.7H, d, J=2.6 Hz), 4.36-4.42 (1H, m), 4.61-4.74 (2.3H, m), 4.99 (1.3H, s), 5.03 (2.7H, s), 5.31 (0.7H, t, J=6.9 Hz), 6.38 (0.7H, d, J=7.3 Hz), 6.52 (0.7H, d, J=8.6 Hz), 6.78-7.09 (9.3H, m), 7.23-7.42 (10H, m), 7.78 (0.3H, d, J=7.9 Hz); Anal. calcd for $C_{58}H_{81}N_7O_9Si$: C, 66.45, H, 7.79; N, 9.35. Found: C, 66.16; H, 7.96; N, 9.05; FABMS (glycerin) m/z: 1048 (M+1).

(2S,3R)-3-Azido-2-triethylsiloxydecanoic-(S)-Ala-(S)-Vzl-(S)-MeTyr(Bzl)-(S)-Tyr(Bzl)-OMe (12d): Yield, 59 %; $[\alpha]_D^{23}$ -79.6° (c 1.20, $CHCl_3$); IR ν_{max} (neat) 3584, 3411, 3325, 2109, 1746, 1688, 1659, 1651, 1626 cm^{-1}; ^1H NMR d 0.56 (1H, d, J=6.6 Hz), 0.60-0.71 (8H, m), 0.79-0.91 (9H, m), 0.94-1.00 (9H, m),1.20-1.43 (12H, m), 1.84-1.91 (1H, m), 2.53 (1H, s), 2.73-3.28 (4H, m), 2.87 (2H, s), 3,32-3.42 (1H, m), 3.66 (2H, s), 3.70 (1H, s), 4.13 (1H, d, J=2.3 Hz), 4.27 (0.3H, t, J=6.4 Hz), 4.40 (1H, q, J=7.3 Hz), 4.46-4.74 (2H, m), 4.99 (1.3H, s), 5.03 (2.7H, s), 5.32 (0.7H, dd, J=7.6, 8.3 Hz), 6.43 (0.7H, d, J=7.6 Hz), 6.57 (0.7H, d, J=8.9 Hz), 6.83-7.10 (8.6H, m), 7.19 (0.7H, d, J=7.6 Hz), 7.30-7.43 (10H, m), 7.82 (0.3H, d, J=7.9 Hz); Anal. calcd for $C_{58}H_{81}N_7O_9Si$: C, 66.45, H, 7.79; N, 9.35. Found: C, 66.95; H, 8.10; N, 9.05; FABMS (glycerin) m/z: 1048 (M+1).

(2R,3R)-Microginin (1a). To a stirred solution of the pentapeptide **12a** (86 mg, 0.88 mM) in CH_3CN (0.5 ml) at 0°C was added 46% aqueous HF (30 µl, 0.82 mM). After being stirred at temperature for 2 h, the mixture was extracted with CH_2Cl_2. The organic extracts were dried over Na_2SO_4 and concentrated in vacuo to give the crude alcohol (81 mg) as a colorless oil. The crude alcohol was dissolved in THF (1 ml) at 0°C and 0.5N LiOH (250 µl, 0.12 mM) was added. After being stirred at room temperature for 2 h, the mixture was acidified with 1M aqueous $KHSO_4$, extracted with CH_2Cl_2 (20 ml x 3), dried over Na_2SO_4, and concentrated in vacuo to give the acid (79 mg) as a colorless oil. The acid was dissolved in THF (3 ml) and AcOH (1.5 ml), and Pd-black (30 mg) was added. After being stirred at room temperature under H_2 atmosphere for 20 h, the mixture was filtered though the pad of celite and concentrated in vacuo. The residue was purified by ion-exchange resin (Dowex 50W x 4, 10 ml, 50% aqueous MeOH then 10% pyridine, 45% MeOH) to give (2R,3R)-microginin (**1a**) (29 mg, 50%) as a pale yellow amorphous powder. An analytical sample was purified by HPLC (YMC-Pack R&D, 35% $CHCN_3$, 0.1% TFA): $[\alpha]_D^{23}$ -68.0° (c 0.025, MeOH); ^1H NMR (DMSO-d^6) δ 0.78 (6H, d, J=6.6 Hz), 0.85 (3H, d, J=6.6 Hz), 1.08 (3H, d, J=6.6 Hz), 1.12-1.52 (12H, m), 1.85-1.93 (1H, m), 2.59-3.07 (5H, m), 2.78 (3H, s), 4.17 (1H brs), 4.28-4.96 (3H, m), 5.24 (1H, dd, J=6.4, 8.7 Hz), 6.47-6.52 (1H, br), 6.58 (2H, d, J=8.3 Hz), 6.62 (2H, d, J=8.6 Hz), 6.94 (4H, d, J=6.9 Hz), 7.84-8.02 (4H, m), 8.04 (1H, d, J=7.9 Hz), 8.14 (1H, d, J=8.9 Hz), 9.10-9.25 (2H, br); HR FABMS calcd for $C_{37}H_{55}N_5O_9$: 713.8790. Found: 714.4064 (M+1).

(2R,3S)-Microginin (1b). The reaction was performed by the same procedure as that for **1a** by use of **12b** (125 mg, 0.12 mM), CH_3CN (1 ml), and 46% aqueous HF (50 ml, 1.34 mM). The crude alcohol (115 mg) was treated with $LiOH{\cdot}H_2O$ (73 mg, 0.174 mM) in MeOH (1 ml) and H_2O (0.3 ml) at 0°C. After being stirred at room temperature for 1 h, the mixture was acidified by 1M aqueous $KHSO_4$, extracted with CH_2Cl_2, dried over Na_2SO_4, and concentrated in vacuo to give the acid (117 mg) which was hydrogenated over Pd-black (25 mg) in THF (3 ml) and AcOH (1.5 ml), giving (2R,3S)-microginin (**1b**) (56 mg, 66%) as a pale yellow amorphous powder. An analytical sample was purified by HPLC (YMC-Pack R&D, 35% CH_3CN, 0.1% TFA): $[\alpha]_D^{23}$ -12.2° (c 0.025, MeOH); ^1H NMR (DMSO-d^6) δ 0.79 (3H, d, J= 5.9 Hz), 0.81 (3H, d, J=5.6 Hz), 0.86 (3H, t, J=6.3 Hz), 1.09 (3H, d, J=6.6 Hz), 1.17-1.57 (12H, m), 1.86-1.93 (1H, m), 2.61-3.18 (5H, m), 2.80 (3H, s), 4.07 (1H, brs), 4.29-4.44 (3H, m), 5.25 (1H, dd, J=6.0, 8.9 Hz), 6.58 (3H, d, J=8.3 Hz), 6.63 (2H, d, J=8.2 Hz), 6.94 (2H, d, J=8.3 Hz), 6.95 (2H, d, J=8.6 Hz), 7.75 (3H, brs), 7.93 (1H, d, J=7.3 Hz), 8.06 (1H, d, J=8.3 Hz), 8.23 (1H, d, J=9.2 Hz), 9.15 (1H, brs), 9.23 (1H, brs); HR FABMS calcd for $C_{37}H_{55}N_5O_9$: 713.8790. Found: 714.4086 (M+1).

(2S,3S)-Microginin (1c). The reactions were performed by the same procedure as those for **1a** by use of **12c** (114 mg, 0.11 mM), CH_3CN (1 ml), 46% aqueous HF (40 µl, 1.09 mM), THF (1.5 ml), 0.5 N LiOH (330 ml, 0.16 mM), THF (4 ml), AcOH (2 ml), and Pd-black (25 mg). (2S,3S)-Microginin (**1c**) was obtained as an amorphous powder (57 mg, 73%). An analytical sample was purified by HPLC (YMC-Pack R&D, 35% CH_3CN, 0.1% TFA): $[\alpha]_D^{23}$ -46.5° (c 0.062, MeOH); ^1H NMR (DMSO-d^6) δ 0.79 (6H, d, J=6.9 Hz), 0.85 (3H, t, J=5.8 Hz), 1.07 (3H, t, J=6.6 Hz), 1.13-1.46 (12H, m), 1.86-1.94 (1H, m), 2.63-3.20 (4H, m), 2.78 (3H, s), 3.22 (1H, dd, J=2.8, 4.1 Hz), 4.17 (1H, brs), 4.26-4.46 (3H, m), 5.23 (1H, dd, J=6.4, 8.7 Hz), 6.46-6.54 (1H, br), 6.58 (2H, d, J=8.6 Hz), 6.62 (2H, d, J=8.2 Hz), 6.94 (2H, d, J=8.6 Hz), 6.95 (2H, d, J=8.3 Hz), 7.83-7.99 (4H, m), 8.02 (1H, d, J=7.9 Hz), 8.14 (1H, d, J=8.9 Hz), 9.10-9.17 (1H, br), 9.21 (1H, brs); HR FABMS calcd for $C_{37}H_{55}N_5O_9$: 713.8790. Found: 714.4108 (M+1).

(2S,3R)-Microginin (1d). The reactions were performed by the same procedure as those for **1a** by use of **12d** (98 mg, 0.99 mM), CH_3CN (1 ml), 46% aqueous HF (70 ml, 1.87 mM), THF (1.5 ml), 0.5 N LiOH (280 ml, 0.14 mM), THF (4 ml), AcOH (2 ml), and Pd-black (25 mg). (2S,3R)-Microginin (**1d**) was obtained as a pale yellow amorphous powder (28 mg, 42%). An analytical sample was purified by HPLC (YMC-Pack R&D, 35% CH_3CN, 0.1% TFA): $[\alpha]_D^{23}$ -78.8° (c 0.022, MeOH); ^1H NMR (DMSO-d^6) δ 0.79 (3H, d, J=4.6 Hz), 0.80 (3H, t, J=6.0 Hz), 1.10 (3H, d, J=6.9 Hz), 1.18-1.53 (12H, m), 1.86-1.94 (1H, m), 2.63-3.10 (4H, m), 2.78 (3H, s), 3.21-3.29 (1H, m), 4.05 (1H, brs), 4.28-4.45 (3H, m), 5.23 (1H, dd, J=6.4, 8.4 Hz), 5.44-6.46 (1H, br), 6.58 (2H, d, J=8.6 Hz), 6.62 (2H, d, J=8.2 Hz), 6.94 (4H, d, J=6.9 Hz), 7.73 (3H, brs), 8.01 (1H, d, J=7.9 Hz), 8.05 (1H, d, J=7.9 Hz), 8.10 (1H, d, J=9.2 Hz), 9.14-9.19 (1H, br), 9.22 (1H, brs); HR FABMS calcd for $C_{37}H_{55}N_5O_9$: 713.8790. Found: 714.4075 (M+1). [Lit.[1] $[\alpha]_D$ -80° (c 0.02, MeOH); ^1H NMR δ 0.77 (d, J=6.0 Hz), 0.78 (d, J=6.0 Hz), 0.85 (t, J=6.6 Hz), 1.10 (d, J=6.9 Hz), 1.23-1.58 (m), 1.87 (dq, J=6.0, 7.8 Hz), 2.63 (dd, J=6.1, 14.5 Hz), 2.79 (m), 2.92 (dd, J=4.7, 14.3 Hz), 3.02 (dd, J=6.1, 14.5 Hz), 2.77 (s), 3.22 (m), 4.05 (brs), 4.32 (m), 4.33 (dd, J=6.9, 7.4 Hz), 4.41 (dd, J=7.8, 9.0 Hz), 5.21 (dd, J=6.1, 8.0 Hz), 6.48 (br), 6.58 (d, J=8.2 Hz), 6.62 (d, J=8.2 Hz), 6.92 (d, J=8.2 Hz), 6.93 (d, J=8.2 Hz), 7.76 (br), 7.96 (d, J=7.7 Hz), 7.99 (d, J=4.4 Hz), 8.05 (d, J=9.0 Hz), 9.15 (brs), 9.22 (brs).]

Acknowledgements: This work was supported in part by Grant-in-Aids from the Ministry of Education, Science and Culture, Japan, and the Japan Foundation for Optically Active Compounds. We are grateful to Professor M. Murakami for the gifts of natural microginin and their spectra.

REFERENCES AND NOTES

1. Okino, T., Matsuda, H.; Murakami, M.; Yamaguchi, K. *Tetrahedron Lett.* **1993**, *34*, 501.
2. Presented in part at the 114th Annual Meeting of the Pharmaceutical Society of Japan, Tokyo, 29-31 March, 1994, Abstracts, **2**, p.145.
3. Bunnage, M. E.; Burke, A. J.; Davies, S. G.; Goodwin, C. J. *Tetrahedron: Asymmetry* **1994**, *5*, 203.
4. Chong, J. M.; Sharpless, K. B. *J. Org. Chem.* **1985**, *50*, 1560.
5. The numbering is used following that of Ahda.
6. Sharpless, K. B.; Amberg, W.; Bennani, Y. L.; Crispino, G. A.; Hartung, J.; Jeong, K.-S.; Kwong, H.-L.; Morikawa, K.; Wang, Z.-M.; Xu, D.; Zhang, X.-L. *J. Org. Chem.* **1992**, *57*, 2768.
7. Enantiomeric excesses of **4a** and **4b** were more than 98% which was determined by analysis of methoxy protons in ^1H-NMR spectra of the corresponding di-MTPA esters, shown in Experimental. Cf. Gao, Y.; Hanson, R. M.; Klunder, J. M.; Ko, S. Y.; Masamune, H.; Sharpless, K. B. *J. Am. Chem. Soc.* **1987**, *109*, 5765.
8. Sharpless, K. B.; Fleming, P. R. *J. Org. Chem.* **1991**, *56*, 2869.
9. Thijs, L.; Waanders, P. P.; Stokkingreef, E. H. M.; Zwanenburg, B. *Recl. Trav. Chim. Pays-Bas*, **1986**, *105*, 332.
10. Hashimoto, N.; Aoyama, T.; Shioiri, T. *Chem. Pharm. Bull.* **1981**, *29*, 1475.
11. Symbols and abbrevitations are used in accordance with the recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature, *Eur. J. Biochem.* **1984**, *138*, 9.
12. Takuma, S.; Hamada, Y.; Shioiri, T. *Chem. Pharm. Bull.* **1982**, *30*, 3147 and references therein.
13. Tung, R. D.; Rich, D. H. *J. Am. Chem. Soc.* **1985**, *107*, 4342.
14. This work-up, though not essential, facilitates the purification of the peptides.

(*Received in UK* 14 *July* 1994; *accepted* 4 *August* 1994)