VOL. 52, NO. 2, FEB. 1999 THE JOURNAL OF ANTIBIOTICS pp. 150 — 159

First Total Synthesis of N-4909 and Its Diastereomer; A Stimulant of Apolipoprotein E Secretion in Human Hepatoma Hep G2 Cells

MAKOTO YANAI* and SHIGERU HIRAMOTO

1st Pharmaceutical Laboratory, Pharmaceutical Research Laboratories,
Nisshin Flour Milling Co., Ltd.,
5-3-1 Tsurugaoka, Ohi-machi, Iruma-gun, Saitama 356-8511, Japan
† Research Laboratories,
Nisshin Kyorin Pharmaceutical Co., Ltd.,
5-3-1 Tsurugaoka, Ohi-machi, Iruma-gun, Saitama 356-8511, Japan

(Received for publication October 8, 1998)

Both (*R*)- and (*S*)-3-hydroxy-13-methyltetradecanoic acids were prepared *via* a lipase-catalyzed enantioselective acylation. The total synthesis of N-4909 and its diastereomer were achieved by a coupling of either (*R*)- or (*S*)-3-hydroxy-13-methyltetradecanoic acid moiety with a hexapeptide moiety and by a cyclization with HATU (*O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate) and HOAt (1-hydroxy-7-azabenzotriazole) in a high dilution condition. The *R* configuration of 3-hydroxy-13-methyltetradecanoic acid was found to be important for stimulating the activity of apolipoprotein E secretion in human hepatoma Hep G2 cells.

N-4909 (**1a**), a stimulator of apolipoprotein E (apo E) secretion from human hepatoma Hep G2 cells, was isolated from the culture broth of *Bacillus* sp. No. 4691 by HIRAMOTO *et al.*[1] Apo E regulates plasma clearance of apolipoprotein B containing lipoproteins such as high density lipoprotein, intermediate density lipoprotein and low density lipoprotein (LDL). In Watanabe heritable hyperlipidemic rabbits, apo E prevented the progression of atherosclerosis.[2] In hyperlipidemic rabbits, plasma cholesterol levels were decreased markedly by intravenous injection.[3,4]

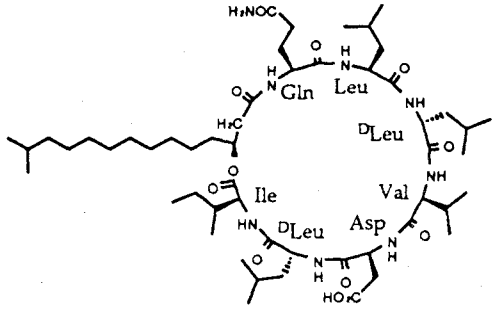
The structure of this compound was determined as shown in Fig.1. This compound had been found to be an inhibitor of acyl-CoA: cholesterol acyltransferase.[5]

In this paper, we report the first total synthesis of N-4909 and its diastereomer and also their effect on apo E secretion from Hep G2 cells.

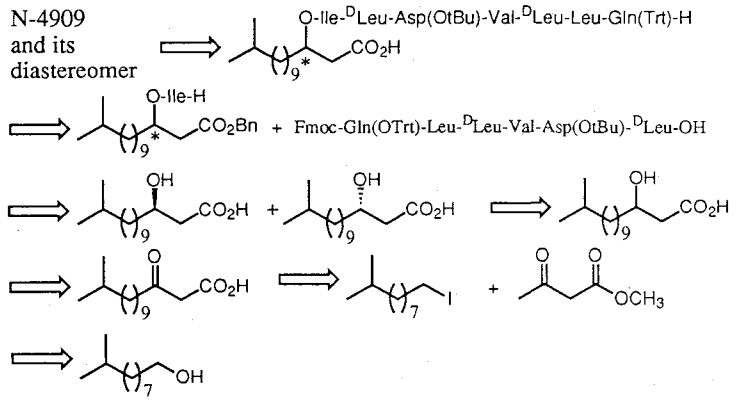
Chemistry

The synthetic strategy for N-4909 and its diastereomer is outlined retrosynthetically in Scheme 1. The cyclization reaction between a carboxyl group of the hydroxy fatty acid and an α-amino group of glutamine is conducted by the HATU-HOAt method in a high dilution condition. The heptapeptide moiety of the cyclization precursor is constructed from the hexapeptide and the amino ester. The hexapeptide portion is synthesized by a conventional method. The Fmoc group is used for protection of the α-amino function and later removed with diethylamine or piperidine. The carboxylic acid in the side chain of Asp is protected with *tert*-butyl ester and the carbamoyl in the side chain of Gln is protected with the Trt group. These protecting groups are removed by TFA after the ring formation. Each of the protected peptide fragments is prepared in a stepwise manner using the WSCI-HOBt method. The *C*-terminal of the fragments is protected with a benzyl ester which is removed before the

Fig. 1. Structure of N-4909.



Scheme 1. Retrosynthetic analysis of N-4909 and its diastereoisomer.



condensation.

The fatty acid (*R*)- or (*S*)-3-hydroxy-13-methyltetradecanoic acid is prepared *via* a lipase-catalyzed enantioselective acylation.[6] The racemic β-hydroxy carboxylic acid is obtained from the β-ketoester which is prepared from a dianion of methyl acetacetate and an alkyl iodide. The alkyl iodide is synthesized from the corresponding alcohol.

First, we attempted to construct 9-methyldecanol (**6**) (Scheme 2). 9-Methyl-7-oxo-1-decanoic acid (**4**) was obtained from 3-methylbutanoyl chloride (**2**) and 1-morpholinocyclohexene (**3**).[7] Wolff-Kishner reduction and lithium aluminum hydride reduction of this keto acid **4** gave the desired alcohol **6** in 41% yield from **2**. An alternative route to make this alcohol **6** was the following sequential reactions: the Wittig reaction of phosphonium salt of ethyl 6-bromocaproate (**13**) with isovaleraldehyde, lithium aluminum hydride reduction and hydrogenation.

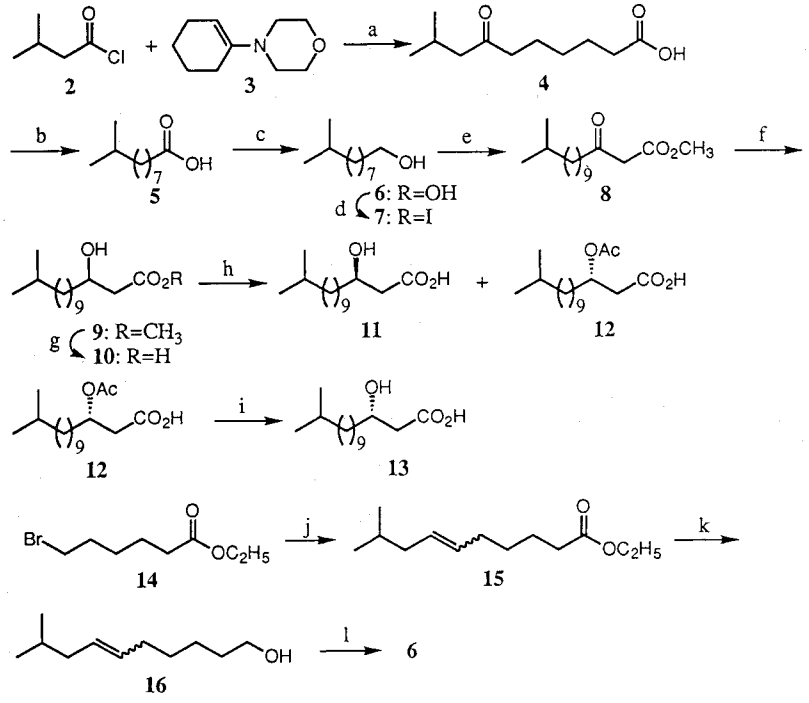
Second, we attempted to make racemic β-hydroxy carboxylic acid (**9**). Methyl 13-methyl-3-oxo-tetradecanoate (**8**) was synthesized by the γ-alkylation of the dianion of methyl acetoacetate with 1-iodo-9-methyldecane (**7**) obtained from **6** with iodine, PPh_3 and imidazole in tetrahydrofuran-hexamethylphosphoramide in 69% yield.[8] This compound **8** was converted to (±)-3-hydroxy-13-methyltetradecanoic acid (**10**) by sodium borohydride reduction and saponification.

Racemic β-hydroxy carboxylic acid **10** was treated with *Pseudomonas* lipase (lipase PS-30, Amano) in a mixture of vinyl acetate and THF in the presence of the polymerization inhibitor, di-*t*-butyl-*p*-cresol (BHT) at 65°C for 48 hours. (*R*)-3-Hydroxy-13-methyltetradecanoic acid (**11**) in a highly pure state ($[\alpha]_D$ −13.6° (*c* 1.02, $CHCl_3$), 98% e.e.; determined by NMR spectrum of the (*R*)-(+)-methyl-α-(trifluoromethyl)phenylacetyl ester) was obtained in 37% yield after recrystallization from *n*-hexane. The *O*-acetyl group of the S-rich acetylated product **12** was removed by alkaline hydrolysis. Fractional crystallization of the resulting S-rich hydroxy fatty acid **13** as (*S*)-(−)-α-methylbenzyl amine (MBA) salt for three times gave the optically highly pure (*S*)-3-hydroxy-13-methyltetradecanoic acid (**13**) ($[\alpha]_D$ +13.6° (*c* 1.05, $CHCl_3$), 93% e.e.; determined by NMR spectrum of the (*R*)-(+)-methyl-α-(trifluoromethyl)phenylacetyl ester) in 29% yield from **10**.

To yield Fmoc-Asp(OtBu)-D-Leu-Obzl (**17**), Fmoc-Asp(OtBu)-OH was coupled quantitatively with H-D-Leu-OBzl by the WSCI-HOBt method. A CH_2Cl_2 solution of Fmoc-Val-OH and H-Asp(OtBu)-D-Leu-OBzl (**18**), obtained by deblocking the dipeptide **17** with diethylamine, was treated with WSCI-HOBt to give Fmoc-Val-Asp(OtBu)-D-Leu-OBzl (**19**) quantitatively. After deprotection with diethylamine in 69% yield, the tripeptide **20** was coupled with Fmoc-D-Leu-OH, isolated and then purified by the usual manner to yield the tetrapeptide **21** in 87% yield. The peptide **21** was converted to Fmoc-Leu-D-Leu-Val-Asp(OtBu)-D-Leu-OBzl (**22**) by the deprotection of the peptide **21** with piperidine and the coupling with Fmoc-Leu-OH by the WSCI-HOBt method in 80% yield. The peptide **23** was deblocked with piperidine in 96% yield and then allowed to react with Fmoc-Gln(Trt)-OH to give Fmoc-Gln(Trt)-Leu-D-Leu-Val-Asp(OtBu)-D-Leu-OBzl (**25**) in 95% yield, which was hydrogenated to yield Fmoc-Gln(Trt)-Leu-D-Leu-Val-Asp(OtBu)-D-Leu-OH (**26**) in 83% yield.

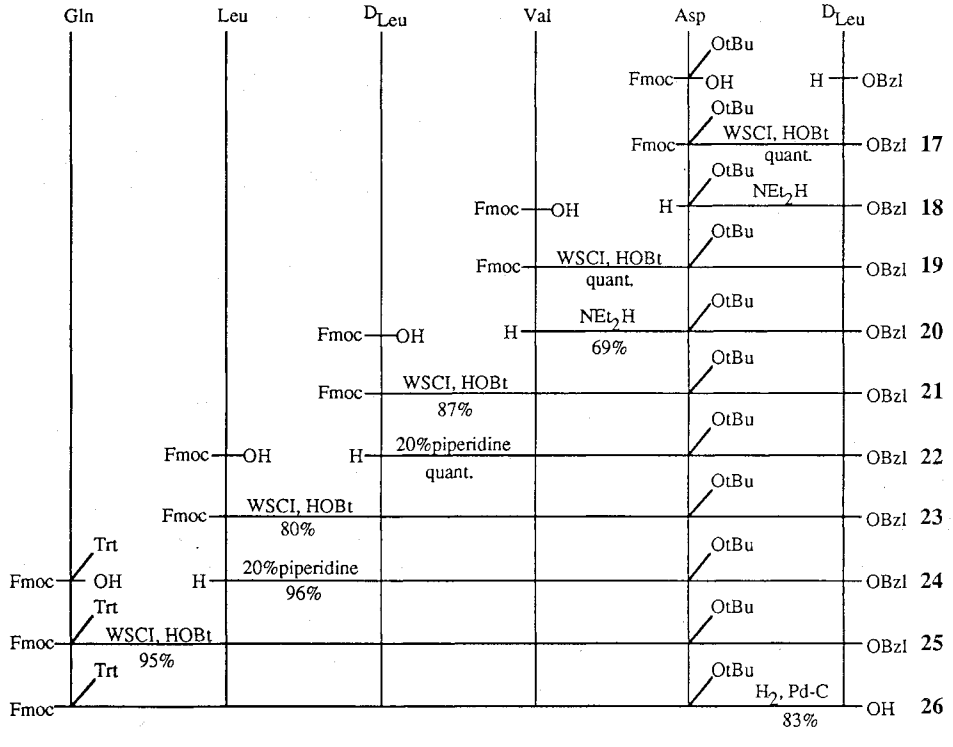
Optically active 13-methyl-(*S*) or (*R*)-3-hydroxytetradecanoic acid (**11** or **13**) was converted to the corresponding benzyl ester **27** using benzyl bromide.

Scheme 2. Synthesis of 13-methyl-(*S*)-3-hydroxytetradecanoic acid and its enantiomer.

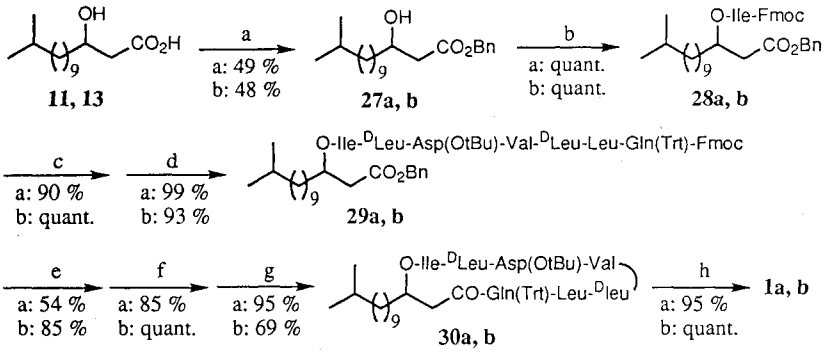


a: 1) TEA, 2) 20% HCl, b: NaOH, 76% for three steps, c: LAH, 74%, d: imidazole, PPh_3, I_2, e: methyl acetoacetate, NaH, *n*-BuLi/HMPA-THF, 69%, f: $NaBH_4$, 73%, g: KOH/MeOH - H_2O, 82%, h: Lipase PS-30, vinyl acetate, 65°C, 48 hours, i: 1) KOH/MeOH, 83%, 2) (*S*)-(−)-1-phenylethylamine/CH_3CN, recrystallization, three times, j: 1) PPh_3/toluene, 2) NaH/CH_3CN, $(CH_3)_2CHCH_2CHO$, k: LAH, 81% from **14**, l: 5% Pd-C, H_2.

Scheme 3. Synthesis of hexapeptide: Fmoc-Gln(Trt)-Leu-D-Leu-Val-Asp(OtBu)-D-Leu-OH.



Scheme 4. Synthesis of N-4909 and its diastereomer.



a: $PhCH_2Br$, NEt_3, b: Fmoc-Ile, DCC, DMAP, c: NEt_2H, d: **26**, WSCI, HOBt, e: 20% piperidine, f: 5% Pd-C, H_2, g: HATU, HOAt, DIPEA, h: TFA

Table 1. Effects of N-4909 (**1a**) and its diastereomer (**1b**) on the secretion of apo E by Hep G2 cells. (% of each control value)

(μM)	N-4909 (**1a**)	Its diastereomer (**1b**)
1.0	273	259
5.0	471	381

Benzyl 13-methyl-(*R*) or (*S*)-3-(Fmoc-Ile-O)-tetradecanoate (**28**) was obtained by the coupling between Fmoc-Ile-OH and benzyl 13-methyl- (*R*) or (*S*)-3-hydroxytetradecanoic acid (**27**) with DCC and DMAP. The N^{α}-deprotected product of the ester (**28**) was coupled with Fmoc-Gln(Trt)-Leu-D-Leu-Val-Asp(OtBu)-D-Leu-OH (**26**) by the WSCI-HOBt method to yield benzyl 13-methyl-(*R*) or (*S*)-3-(Fmoc-Gln(Trt)-Leu-D-Leu-Val-Asp(OtBu)-D-Leu-O)-tetradecanoate (**29**). Then the Fmoc group and the benzyl group were deprotected in the usual manner. Cyclization was achieved by a high dilution method in DMF with HATU and HOAt at room temperature. The protected cyclization product, cyclo{13-methyl- (*R*) or (*S*)-3-[Gln-(Trt)-Leu-D-Leu-Val-Asp(OtBu)-D-Leu-O]-tetradecanoate} (**30**), was obtained. The removal of the protecting group by TFA gave the product, N-4909 (**1a**) or its diastereomer (**1b**).

Results and Discussion

Table 1 shows the secreted level of apo E from Hep G2 cells with N-4909 (**1a**) and its diastereomer (**1b**). These results show that the effects of both enantiomer at 1.0 μM level are almost the same, but at a higher dose (5.0 μM) **1a** has a stronger effect than **1b**. Therefore, the (*R*) configuration of 3-hydroxy-13-methyltetradecanoic acid is important for showing the stronger effect on the secretion of apo E by Hep G2 cells. The effect of this configuration on other lipoproteins is now under investigation.

Experimental

General

Melting points were determined on a micro melting point apparatus and were uncorrected. Optical rotations were obtained on a JASCO DIP-370 digital polarimeter. ^{1}H NMR spectra were recorded at 400 MHz on a JEOL JNM-EX400 spectrometer. ESI-MS spectra were obtained on a Micromass Quattro II instrument.

Biological Activity

Effects of N-4909 (**1a**) and its diastereomer (**1b**) on the secretion of apolipoprotein E by Hep G2 cells were measured by the procedure described in the previous paper.[1)]

Reagents

Unless otherwise stated, all reagents and solvents were obtained commercially as reagent grade products and used without further purification.

Peptide Synthesis

The α-amino function of amino acids was protected by the Fmoc group. The β-carboxyl group of Asp was protected by the *tert*-Bu group. The carbamoyl group of

Gln was protected by the Trt group. The protecting group for fatty acids was Bzl for the carboxyl group.

9-Methy-7-oxo-1-decanoic acid (4)

To a solution of 1-morpholinocyclohexene (**3**) (25.0 g, 149 mmol) and triethylamine (15.1 g, 149 mmol) in $CHCl_3$ (75 ml) under Ar was added a solution of 3-methylbutanoyl chloride (**2**) (18.0 g, 149 mmol) in $CHCl_3$ (30 ml) at an ice cooled temperature over a period of 30 minutes. This reaction mixture was stirred at room temperature overnight. Then, 20% aq. HCl (75 ml) was added and the mixture was refluxed for 5 hours and cooled to room temperature. The separated $CHCl_3$ layer was rinsed with brine and dried over anhydrous Na_2SO_4. After removal of the solvent, the residure was refluxed in H_2O (30 ml) containing NaOH (18 g, 0.45 mol) for 1 hour. The resultant mixture was poured into ice water (150 ml) and extracted with $CHCl_3$ (2×75 ml). The combined organic layers were rinsed with brine and dried (Na_2SO_4). After removal of the solvent, the crude product was purified by chromatography on silica gel (150 g), eluting with *n*-Hex : AcOEt = 4 : 1, to yield the product (22.7 g, 76%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 10.4 (1H, br s, COO*H*), 2.39 (2H, t, J=7.3 Hz, $COCH_2$), 2.36 (2H, t, J=7.6 Hz, CH_2CO_2H), 2.27 (2H, d, J=6.8 Hz, $CHCH_2$), 2.08~2.16 (1H, m, $CH(CH_3)_2$), 1.64 (2H, quint., J=7.3 Hz, $CH_2CH_2CH_2$), 1.59 (2H, quint., J=7.2 Hz, $CH_2CH_2CH_2$), 1.30~1.38 (2H, m, $CH_2CH_2CH_2$), 0.91 (6H, d, J=6.8 Hz, $CH(CH_3)_2$).

9-Methyl-1-decanoic Acid (5)

A mixture of **4** (11.8 g, 58.9 mmol), $N_2H_4 \cdot H_2O$ (20.2 ml), KOH (3.31 g, 58.9 mmol) and diethylene glycol (60 ml) was refluxed for 8 hours. After cooling to room temperature, KOH (1.65 g, 29.5 mmol, 0.5 eq) and diethylene glycol (60 ml) were added to the mixture. This reaction mixture was refluxed for 15 hours and then poured into ice cooled water (1.6 liter). The resultant mixture was acidified with conc. HCl to pH 3 and cooled with an ice bath. The crystalline solid was collected, dissolved in $CHCl_3$ and then dried ($MgSO_4$). After removal of the solvent, the crude product was purified by chromatography on silica gel (60 g), eluting with $CHCl_3$, to yield the product (5.04 g, 46%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.00 (1H, br s, COO*H*), 2.35 (2H, t, J=7.6 Hz, CH_2CO_2H), 1.63 (2H, quint., J=7.1 Hz, $CH_2CH_2CO_2H$), 1.52 (1H, sept., J=6.7 Hz, $CH(CH_3)_2$), 1.22~1.37 (8H, m, CH_2), 1.11~1.17 (2H, m, CH_2), 0.86 (6H, d, J=6.8 Hz, $CH(CH_3)_2$).

9-Methyl-1-decanol (6)

To a suspension of LAH (0.60 g, 16 mmol) in Et_2O (40 ml) was added a solution of **5** (3.70 g, 19.9 mmol) in Et_2O (30 ml) over a period of 30 minutes. After refluxing for 30 minutes, this was cooled to room temperature. To this was added H_2O (0.6 ml), 15% aq. NaOH and H_2O (1.8 ml). The ether layer was dried (Na_2SO_4). After removal of the solvent, the crude product was purified by chromatography on silica gel (40 g), eluting with *n*-Hex : AcOEt = 4 : 1, to yield the product (2.53 g, 74%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.64 (2H, t, J=6.6 Hz, CH_2OH), 1.45~1.61 (3H, m, $CH_2H_2OH + CH(CH_3)_2$), 1.20~1.40 (11H, m, $CH_2 + CH_2OH$), 1.10~1.20 (2H, m, CH_2), 0.86 (6H, d, J=6.8 Hz, $CH(CH_3)_2$).

1-Iodo-9-methyldecane (7)

To a solution of **6** of benzene (1.5 liters) were added imidazole (28.6 g, 0.42 mol, 2.5 eq), triphenylphosphine (110 g, 0.42 mol, 2.5 eq) and iodine (85 g, 0.34 mol, 2 eq) at room temperature. This reaction mixture was stirred at room temperature for 3 hours and then rinsed with sat. aq. Na_2SO_4 and brine, and then dried ($MgSO_4$). After removal of the solvent at a bath temperature below 30°C, the formed crystals were filtered off. After removal of the solvent at a bath temperature below 30°C, the crude product was purified by chromatography on silica gel (200 g), eluting with *n*-Hex, to yield the product (36.1 g, 76%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.19 (2H, t, J=6.8 Hz, CH_2I), 1.82 (2H, quint., J=7.2 Hz, CH_2CH_2I), 1.46~1.56 (1H, m, $CH(CH_3)_2$), 1.20~1.40 (10H, m, CH_2), 1.10~1.20 (2H, m, CH_2), 0.86 (6H, d, J=6.3 Hz, $CH(CH_3)_2$).

Methyl 3-Oxo-13-methyltetradecanoate (8)

To a suspension of NaH (60%, 12.8 g, 0.32 mol, 2.5 eq) in THF (720 ml) and HMPA (86 ml) was added dropwise methyl acetoacetate (27.6 ml, 0.26 ml, 2.0 eq) at an ice cooled temperature. After stirring at 5°C for 10 minutes, *n*-BuLi in hexane (1.57 M, 178 ml, 0.28 mol, 2.2 eq) was added dropwise to this and stirred at 5°C for 10 minutes. To this added a solution of **7** (36.1 g, 0.13 mol) in THF (100 ml). This reaction mixture was stirred at room temperature for 1 hour and sat. aq. NH_4Cl was added to this. 1 M HCl aq was added to this solution to pH 2 and extracted with Et_2O. The combined organic layers were dried (Na_2SO_4). After removal of the solvent, the crude product was purified by chromatography on silica gel (300 g), eluting with *n*-Hex : AcOEt = 400 : 0~20, to yield the product (23.8 g, 69%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.74 (3H, s, CO_2CH_3), 3.45 (2H, s, $COCH_2CO_2H$), 2.53 (2H, t, J=7.6 Hz, CH_2CO), 1.55~1.63 (2H, m, CH_2CH_2CO), 1.46~1.55 (1H, m, $CH(CH_3)_2$), 1.20~1.35 (12H, m, CH_2), 1.10~1.20 (2H, m, CH_2), 0.86 (6H, d, J=6.8 Hz, $CH(CH_3)_2$).

Methyl 3-Hydroxy-13-methyltetradecanoate (9)

To a solution of $NaBH_4$ (16.0 g, 0.42 mol, 4.8 eq) in MeOH (300 ml) was added a solution of **8** (23.8 g, 88 mmol) in MeOH (100 ml) dropwise at −25~−30°C. This was stirred at this temperature for 2 hours and acidified to pH 2 with 1 N aq. HCl. This was extracted with AcOEt and the combined organic layers were rinsed with sat. aq. $NaHCO_3$ and brine, and then dried (Na_2SO_4). After removal of the solvent, the crude product was purified by chromatography on silica gel (200 g), eluting with *n*-Hex : AcOEt = 200 : 0~60, to yield the product (17.4 g, 73%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.97~4.03 (1H, m, $CHOH$), 3.72 (3H, s, CO_2CH_3), 2.86 (1H, d, J=3.9 Hz, $CHOH$), 2.52 (1H, dd, J=3.2, 17 Hz, $CH_2CO_2CH_3$), 2.41 (1H, dd, J=9.0, 17 Hz, $CH_2CO_2CH_3$), 1.35~1.60 (4H, m, CH_2), 1.20~1.35 (13H, m, CH_2), 1.10~1.20 (2H, m, CH_2), 0.86 (6H, d, J=6.4 Hz, $CH(CH_3)_2$).

(±)-3-Hydroxy-13-methyltetradecanoic Acid (**10**)

A solution of **9** (6.94 g, 25.5 mmol) in 1.3 M KOH (130 ml, MeOH : H_2O = 4 : 1) was stirred at room temperature overnight. This was acidified with dilute HCl to pH1 and saturated with NaCl. This was extracted with AcOEt and the combined organic layers were rinsed with brine and dried (Na_2SO_4). After removal of the solvent, the crude product was purified by recrystallization from *n*-Hexane to yield the product (5.40 g, 82%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.99~4.07 (1H, m, $CHOH$), 2.58 (1H, dd, J=3.4, 17 Hz, $CH_2CO_2CH_3$), 2.47 (1H, dd, J=8.8, 17 Hz, $CH_2CO_2CH_3$), 1.39~1.60 (4H, m, CH_2), 1.19~1.39 (13H, m, CH_2), 1.09~1.19 (2H, m, CH_2), 0.86 (6H, d, J=6.3 Hz, $CH(CH_3)_2$).

(*R*)-3-Hydroxy-13-methyltetradecanoic Acid (**11**)

To a solution of **10** (15.0 g, 58.0 mmol) and BHT (140 mg) in a mixture of vinyl acetate (140 ml) and THF (140 ml) was added lipase PS-30 (9.3 g). This reaction mixture was stirred at 65°C for 48 hours under the slow stream of N_2 gas. After filtration and evaporation of the solvent, the crude product was purified by chromatography on silica gel (100 g), eluting with $CHCl_3$: MeOH = 200 : 0~30, to yield the acetate **12** (10.3 g, 59%) and the product **11** which was recrystallized from *n*-hexane (5.49 g, 37%). MP 46.5~47.0°C. $[\alpha]_D^{25}$ −13.6° (*c* 1.02, $CHCl_3$).

NMR spectrum of **11** was identical with that of **10**. This was treated with diazomethane, subsequently by (*S*)-MTPA-Cl in pyridine to give (*S*)-MTPA ester.

(*S*)-3-Hydroxy-13-methyltetradecanoic Acid (**13**)

To a solution of **12** (10.3 g, 34.2 mmol) in methanol (60 ml) was added slowly a solution of KOH (3.84 g, 58.2 mmol, 1.7 eq) in methanol (57 ml) at an ice cooled tempetarure. After stirred at room temperature overnight this was poured into ice cooled water (400 ml). This was acidified to pH 4 with dilute aq. HCl and extracted with AcOEt. The combined organic layers were rinsed with H_2O and dried ($MgSO_4$). After removal of the solvent, the crude product was purified by recrystallization from *n*-hexane to yield the product (7.37 g, 83%). This was crystallized as (*S*)-(−)-1-phenylethylamine salt in CH_3CN three times. Treatment with 10% aq. citric acid and recrystallization from *n*-hexane gave **13**- (4.31 g, 29% from **10**). MP 47.0°C. $[\alpha]_D^{25}$ +13.6° (*c* 1.05, $CHCl_3$).

NMR spectrum of **13** was identical with that of **10**. This was treated with diazomethane, subsequently by (*S*)-MTPA-Cl in pyridine to give (*S*)-MTPA ester.

Ethyl 9-Methyl-6-decenoate (**14**)

A solution of ethyl 6-bromocaproate (**13**) (50 g, 0.22 mol) and triphenylphosphine (61.7 g, 0.24 mol) in toluene (200 ml) was refluxed for 16 hours. After removal of the solvent, the residue was dissolved in acetonitrile (200 ml) and isovaleraldehyde (24 ml, 0.22 mol) was added at room temperature. NaH (60%, 9.0 g, 0.22 mol) was added little by little to keep the reaction temperature within 25~35°C. After addition, the reaction mixture was stirred at room temperature for 3 days. After addition of water (110 ml), this was extracted with *n*-hexane three times. The combined organic layers were rinsed with water and dried ($MgSO_4$). After removal of the solvent, the crude product was cooled by an ice bath. The formed crystals were filtered off and the filtrate was concentrated to obtain the product which was used in the next reaction without further purification.

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.34~5.42 (2H, m, $CH=CH$), 4.12 (2H, q, J=7.2 Hz, $COCH_2CH_3$), 2.30 (2H, t, J=7.6 Hz, CH_2CO), 2.04 (2H, q, J=6.7 Hz, $CHCH_2CH_2$), 1.91 (2H, t, J=6.1 Hz, $CHCH_2CH$), 1.55~1.68 (3H, m, $CH_2+CH(CH_3)_2$), 1.34~1.42 (2H, m, CH_2), 1.25 (3H, t, J=7.3 Hz, CH_2CH_3), 0.89 (6H, d, J=6.4 Hz, $CH(CH_3)_2$).

9-Methyl-6-decen-1-ol (**15**)

To a solution of LAH (4.25 g, 0.11 mol) in Et_2O (420 ml) was added dropwise **14** at an ice-cooled temperature. After addition, this reaction mixture was stirred at room temperature for 4 hours. Then, water (4.3 ml), 15% aq. NaOH (4.3 ml) and water (13 ml) were added dropwise and then dried (Na_2SO_4). After removal of the solvent, the crude product was purified by chromatography on silica gel (200 g), eluting with *n*-Hex : AcOEt = 400 : 0 ~ 60, to yield the product (30.8 g, 81%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.34 ~ 5.44 (2H, m, $CH=CH$), 3.64 (2H, t, J=6.6 Hz, CH_2OH), 2.00 ~ 2.10 (2H, m, $CHCH_2CH_2$), 1.91 (2H, t, J=6.4 Hz, $CHCH_2CH$), 1.52 ~ 1.61 (3H, m, $CH_2+CH(CH_3)_2$), 1.30 ~ 1.42 (5H, m, CH_2+CH_2OH), 0.89 (6H, d, J=6.8 Hz, $CH(CH_3)_2$).

9-Methyl-1-decanol (**6**)

A suspension of **15** (30.8 g, 0.18 mol) and 5% Pd-C (2.00 g) in methanol (140 ml) was reacted at room temperature under a H_2 atmosphere (~2 kg/cm^2) for 3 hours. Filtration and removal of the solvent gave **6** quantitatively.

Fmoc-Asp(OtBu)-D-Leu-OBzl (**17**)

To a solution of Fmoc-D-Leu-OBzl (9.00 g, 20 mmol) in DMF (200 ml) was added NEt_2H (20 ml) and the solution was stirred at room temperature for 5 hours. Removal of the solvent gave the crude product which was used in the next reaction without further purification.

To a solution of this amine (20 mmol), Fmoc-Asp(OtBu) (9.05 g, 22 mmol, 1.1 eq) and HOBt·H_2O (3.37 g, 22 mmol, 1.1 eq) in CH_2Cl_2 (80 ml) was added WSCI·HCl (4.22 g, 22 mmol, 1.1 eq) at an ice cooled temperature. This reaction mixture was stirred at this temperature for 2 hours and then at room temperature overnight. After removal of solvent, the residue was taken up to AcOEt and 10% aq. citric acid. The separated organic layer was rinsed with H_2O, 5% aq. $NaHCO_3$ and H_2O, and then dried (Na_2SO_4). After removal of the solvent, the crude product was purified by recrystallization from Hex.-AcOEt to yield the product (8.36 g, 68%). MP 102 ~ 102.5°C. ESI-MS *m/z* 615 $(M+H)^+$. $[\alpha]_D^{25}$ +14.0° (*c* 1.00, $CHCl_3$).

Fmoc-Val-Asp(OtBu)-D-Leu-OBzl (**19**)

To a solution of **17** (6.14 g, 10 mmol) in DMF (100 ml) was added NEt_2H (10 ml) and the solution was stirred at room temperature for 3 hourrs. Removal of the solvent gave the crude product (**18**) which was used in the next reaction without further purification.

To a solution of **18** (10 mmol), Fmoc-Val (3.39 g, 10 mmol) and HOBt·H_2O (4.53 g, 10 mmol) in DMF (70 ml) was added WSCI·HCl (1.92 g, 10 mmol) at an ice cooled temperature. This reaction mixture was stirred at this temperature for 2 hours and then at room temperature overnight. After removal of solvent, the residue was taken up to AcOEt and 10% aq. citric acid. The separated organic layer was rinsed with H_2O, 5% aq. $NaHCO_3$ and H_2O, and then dried (Na_2SO_4). After removal of the solvent, the crude product was purified by recrystallization from $CHCl_3$-Et_2O to yield the product (6.19 g, 87%). MP 160 ~ 162°C. ESI-MS *m/z* 714 $(M+H)^+$. $[\alpha]_D^{25}$ −7.02° (*c* 1.00, $CHCl_3$).

Fmoc-D-Leu-Val-Asp(OtBu)-D-Leu-OBzl (**21**)

To a solution of **19** (19.0 g, 26.5 mmol) in DMF (265 ml) was added NEt_2H (26.5 ml) and the solution was stirred at room temperature for 1 hour. After removal of the solvent, the residue was dissolved in AcOEt and rinsed with H_2O twice, and then dried (Na_2SO_4). After removal of the solvent, the crude product was purified by chromatography on silica gel (70 g), eluting with $CHCl_3$: MeOH = 200 : 0 ~ 10, to yield the product (**20**) (9.06 g, 69%).

To a solution of **20** (8.00 g, 16.3 mmol), Fmoc-D-Leu (6.33 g, 17.9 mmol, 1.1 eq) and HOBt (2.42 g, 17.9 mmol, 1.1 eq) in CH_2Cl_2 (65 ml) was added WSCI·HCl (3.43 g, 17.9 mmol, 1.1 eq) at an ice cooled temperature. This reaction mixture was stirred at this temperature for 2 hours and then at room temperature overnight. After removal of solvent, the residue was taken up to $CHCl_3$ and 10% aq. citric acid. The separated organic layer was rinsed with H_2O, 5% aq. $NaHCO_3$ and H_2O, and then dried (Na_2SO_4). After removal of the solvent, the crude product was purified by chromatography on silica gel (100 g), eluting with $CHCl_3$: MeOH = 200 : 0 ~ 5, to yield the product (11.7 g, 87%). MP 178 ~ 179°C. ESI-MS *m/z* 827 $(M+H)^+$. $[\alpha]_D^{26}$ +21.2° (*c* 1.05, $CHCl_3$).

Fmoc-Leu-D-Leu-Val-Asp(OtBu)-D-Leu-OBzl (**23**)

A solution of **21** (11.5 g, 13.9 mmol) in 20% piperidine in DMF (70 ml) was stirred at room temperature for 1 hour. After removal of the solvent, the crude product was purified by chromatography on silica gel (80 g), eluting with $CHCl_3$: MeOH = 200 : 0 ~ 10, to yield the product (**22**) (8.41 g, quant.).

To a solution of **22** (8.41 g, 13.9 mmol), Fmoc-Leu

(5.40 g, 15.3 mmol, 1.1 eq) and HOBt (2.07 g, 15.3 mmol, 1.1 eq) in CH_2Cl_2 (60 ml) was added WSCI·HCl (2.93 g, 15.3 mmol, 1.1 eq) at an ice cooled temperature. This reaction mixture was stirred at this temperature for 2 hours and then at room temperature overnight. After removal of solvent, the residue was taken up to AcOEt and 10% aq. citric acid. The separated organic layer was rinsed with H_2O, 5% aq. $NaHCO_3$ and H_2O, and then dried (Na_2SO_4). After removal of the solvent, the crude product was purified by chromatography on silica gel (100 g), eluting with $CHCl_3$: MeOH = 200 : 0 ~ 5, to yield the product which was solidified from $CHCl_3$ - Et_2O (10.5 g, 80%).

MP 181 ~ 182°C. ESI-MS *m/z* 940 $(M+H)^+$. $[\alpha]_D^{24}$ +48.9° (*c* 1.01, $CHCl_3$).

Fmoc-Gln(trt)-Leu-D-Leu-Val-Asp(OtBu)-D-Leu-OBzl (**25**)

A solution of **23** (4.00 g, 4.25 mmol) in 20% piperidine in DMF (20 ml) was stirred at room temperature for 20 minutes. After removal of the solvent, the crude product was purified by chromatography on silica gel (40 g), eluting with $CHCl_3$: MeOH = 200 : 0 ~ 5, to yield the product (2.94 g, 96%).

To a solution of this amine (2.94 g, 4.09 mmol), Fmoc-Gln(Trt) (2.75 g, 4.50 mmol, 1.1 eq) and HOBt (0.61 g, 4.5 mmol, 1.1 eq) in CH_2Cl_2 (20 ml) was added WSCI·HCl (0.86 g, 4.5 mmol, 1.1 eq) at an ice cooled temperature. This reaction mixture was stirred at this temperature for 1.5 hours and then at room temperature overnight. After removal of solvent, the residue was taken up to AcOEt and 10% aq. citric acid. The separated organic layer was rinsed with H_2O, 5% aq. $NaHCO_3$ and H_2O, and then dried (Na_2SO_4). After removal of the solvent, the crude product was purified by chromatography on silica gel (70 g), eluting with $CHCl_3$: MeOH = 200 : 0 ~ 5, to yield the product which was solidified from $CHCl_3$ - Et_2O (5.11 g, 95%).

MP 194 ~ 195°C. ESI-MS *m/z* 1310 $(M+H)^+$. $[\alpha]_D^{25}$ +42.4° (*c* 1.02, $CHCl_3$).

Fmoc-Gln(trt)-Leu-D-Leu-Val-Asp(OtBu)-D-Leu-OH (**26**)

A suspension of **25** (5.00 g, 3.81 mmol) and 5% Pd-C (0.80 g) in MeOH (120 ml) was reacted under a H_2 atmosphere (2 kg/cm^2) for 1.5 hours at room temperature. After filtration and evaporation, the crude product was purified by chromatography on silica gel (50 g), eluting with $CHCl_3$: MeOH = 200 : 0 ~ 15, to yield the product which was solidified from $CHCl_3$ - Et_2O (3.88 g, 83%).

(*R*)-Benzyl 3-Hydroxy-13-methyltetradecanoate (**27a**)

To a solution of **11** (5.00 g, 19.3 mmol) and Et_3N (2.7 ml, 19 mmol) in DMF (50 ml) was added benzyl bromide (2.3 ml, 19 mmol) at room temperature. This reaction mixture was stirred at room temperature overnight. After removal of the solvent, the residue was taken up to AcOEt and H_2O. The separated organic layer was rinsed with H_2O twice and then dried (Na_2SO_4). After removal of the solvent, the crude product was purified by chromatography on silica gel (30 g), eluting with $CHCl_3$: MeOH = 200 : 0 ~ 15, to yield the product which was recrystallized from *n*-hexane (3.33 g, 49%) and to recover the starting material 2.33 g (47%). MP 30°C. $[\alpha]_D^{25}$ −14.5° (*c* 1.00, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ 7.31 ~ 7.41 (5H, m, Ar-*H*), 5.15 (2H, s, CH_2Ph), 3.98 ~ 4.06 (1H, m, C*H*OH), 2.85 (1H, d, *J* = 3.9 Hz, CHO*H*), 2.56 (1H, dd, *J* = 3.2, 17 Hz, CH_2CO_2), 2.46 (1H, dd, *J* = 9.1, 16 Hz, CH_2CO_2), 1.47 ~ 1.57 (2H, m, CH_2), 1.37 ~ 1.47 (2H, m, CH_2), 1.19 ~ 1.37 (13H, m, CH_2), 1.11 ~ 1.19 (2H, m, CH_2), 0.86 (6H, d, *J* = 6.3 Hz, $CH(CH_3)_2$). *Anal* Calcd for $C_{22}H_{36}O_3$: C 75.8, H 10.4; Found C 75.9, H 10.5.

(*S*)-Benzyl 3-Hydroxy-13-methyltetradecanoate (**27b**)

MP 28 ~ 29°C. $[\alpha]_D^{25}$ +14.2° (*c* 1.00, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ 7.30 ~ 7.41 (5H, m, Ar-*H*), 5.15 (2H, s, CH_2Ph), 4.02 (1H, br s, C*H*OH), 2.86 (1H, d, *J* = 3.4 Hz, CHO*H*), 2.56 (1H, dd, *J* = 3.4, 17 Hz, CH_2CO_2), 2.46 (1H, dd, *J* = 8.8, 17 Hz, CH_2CO_2), 1.48 ~ 1.57 (2H, m, CH_2), 1.37 ~ 1.47 (2H, m, CH_2), 1.19 ~ 1.36 (13H, m, CH_2), 1.10 ~ 1.18 (2H, m, CH_2), 0.86 (6H, d, *J* = 6.3 Hz, $CH(CH_3)_2$). *Anal* Calcd for $C_{22}H_{36}O_3$: C 75.8, H 10.4; Found C 75.9, H 10.2.

Benzyl 13-Methyl-(*R*)-3-(Fmoc-Ile-O)-tetradecanoate (**28a**)

To a solution of **27a** (2.33 g, 6.69 mmol), Fmoc-Ile (2.60 g, 7.35 mmol, 1.1 eq) and DMAP (57 mg, 0.47 mmol, 0.07 eq) in CH_2Cl_2 (40 ml) was added DCC (2.07 g, 10.0 mmol, 1.5 eq) at an ice cooled temperature This was stirred at this temperature for 3 hours and the at room temperature overnight. After filtration and evaporation, the residue was taken up to AcOEt and 10% aq. citric acid. The separated organic layer was rinsed with H_2O, 5% aq. $NaHCO_3$ and H_2O, and then dried (Na_2SO_4). After removal of the solvent, the crude product was purified by chromatography on silica gel (70 g), eluting with Hex : AcOEt = 200 : 0 ~ 30, to yield the product

(4.57 g, quant.). $[\alpha]_D^{25}$ −0.35° (*c* 1.00, $CHCl_3$). ESI-MS *m/z* 684 $(M+H)^+$.

Benzyl 13-Methyl-(*S*)-3-(Fmoc-Ile-O)-tetradecanoate (**28b**)

$[\alpha]_D^{25}$ +2.82° (*c* 1.00, $CHCl_3$). ESI-MS *m/z* 684 $(M+H)^+$.

Benzyl 13-Methyl-(*R*)-3-(Fmoc-Gln(Trt)-Leu-D-Leu-Val-Asp(OtBu)-D-Leu-O)-tetradecanoate (**29a**)

A solution of benzyl **28a** (0.68 g, 0.99 mmol) and NEt_2H (1 ml) in DMF (10 ml) was stirred at room temperature for 1 hour. After removal of the solvent, the crude product was purified by chromatography on silica gel (25 g), eluting with $CHCl_3$: MeOH = 100 : 0 ~ 3, to yield the product (0.44 g, 90%).

To a solution of this amine (0.44 g, 0.90 mmol), Fmoc-Gln(trt)-Leu-D-Leu-Val-Asp(OtBu)-D-Leu-OH (1.10 g, 0.90 mmol) and HOBt (0.12 g, 0.90 mmol) in CH_2Cl_2 (20 ml) was added WSCI·HCl (0.17 g, 0.90 mmol) at an ice cooled temperature. This was stirred at this temperature for 2 hours and then at room temperature overnight. After removal of the solvent, the residue was taken up to AcOEt and 10% aq. citric acid. The separated organic layer was rinsed with H_2O, 5% aq. $NaHCO_3$ and H_2O, and then dried (Na_2SO_4). After removal of the solvent, the crude product was purified by chromatography on silica gel (30 g), eluting with $CHCl_3$: MeOH = 200 : 0 ~ 3, to yield the product which was solidified from Et_2O (1.48 g, 99%). $[\alpha]_D^{25}$ +38.2° (*c* 1.02, $CHCl_3$).

$[\alpha]_D^{24}$ +38.2° (*c* 1.02, $CHCl_3$). ESI-MS *m/z* 1663 $(M+H)^+$.

Benzyl 13-Methyl-(*S*)-3-(Fmoc-Gln(Trt)-Leu-D-Leu-Val-Asp(OtBu)-D-Leu-O)-tetradecanoate (**29b**)

$[\alpha]_D^{26}$ +41.6° (*c* 1.00, $CHCl_3$). ESI-MS *m/z* 1663 $(M+H)^+$.

Cyclo{13-methyl-(*R*)-3-[Gln(Trt)-Leu-D-Leu-Val-Asp(OtBu)-D-Leu-O]-tetradecanoate} (**30a**)

A suspension of **29a** (2.50 g, 1.50 mmol) and 5% Pd-C (2.50 g) in MeOH (100 ml) was reacted under a H_2 atmosphere (2 kg/cm^2) for 40 minutes at room temperature. After filtration and evaporation, the crude product was purified by chromatography on silica gel (60 g), eluting with $CHCl_3$: MeOH = 200 : 0 ~ 6, to yield the product (1.27 g, 54%).

A solution of this acid (0.60 g, 0.38 mmol) in 20% piperidine in DMF (20 ml) was stirred at room temperature for 1 hour. After removal of the solvent, the crude product was purified by chromatography on silica gel (60 g), eluting with $CHCl_3$: MeOH = 200 : 0 ~ 20, to yield the product (0.44 g, 85%).

To a solution of this depsipeptide (0.30 g, 0.22 mmol) in DMF (300 ml) was added DIPEA (0.15 ml, 0.89 mmol, 4 eq), HOAt (91 mg, 0.67 mmol, 3 eq) and HATU (0.25 g, 0.67 mmol, 3 eq) at room temperature. This resulting yellow solution was stirred at room temperature overnight. After removal of the solvent, the residue was taken up to AcOEt and 5% aq. $KHSO_4$. The separated organic layer was rinsed with H_2O and brine, and then dried (Na_2SO_4). After removal of the solvent, the crude product was purified by chromatography on silica gel (5 g), eluting with $CHCl_3$: AcOEt = 50 : 0 ~ 40, to yield the product (0.29 g, 95%).

$[\alpha]_D^{26}$ +30.0° (*c* 0.40, $CHCl_3$). ESI-MS *m/z* 1333 $(M+H)^+$.

Cyclo{13-methyl-(*S*)-3-[Gln(Trt)-Leu-D-Leu-Val-Asp(OtBu)-D-Leu-O]-tetradecanoate} (**30b**)

$[\alpha]_D^{26}$ +42.0° (*c* 1.00, $CHCl_3$). ESI-MS *m/z* 1333 $(M+H)^+$.

Cyclo{13-methyl-(*R*)-3-[Gln-Leu-D-Leu-Val-Asp-D-Leu-O]-tetradecanoate} (**1a**)

A solution of cyclo{13-methyl-(*R*)-3-[Gln(Trt)-Leu-D-Leu-Val-Asp(OtBu)-D-Leu-O]-tetradecanoate} (170 mg, 0.13 mmol) in TFA (5 ml) was stirred at room temperature for 30 min. After removal of the solvent, the residue was purified by chromatography on silica gel (5 g), eluting with $CHCl_3$: MeOH = 50 : 0 ~ 7, to yield the product (125 mg, 95%).

$[\alpha]_D^{26}$ −9.86° (*c* 0.40, CH_3OH).[lit.[1] $[\alpha]_D^{26}$ −11.2° (*c* 0.414, CH_3OH)] High-resolution FAB-MS (positive) *m/z* 1057.6923 [calcd for $C_{53}H_{94}N_8O_{12}Na$ $(M+Na)^+$; 1057.6889].

Cyclo{13-methyl-(*S*)-3-[Gln-Leu-D-Leu-Val-Asp-D-Leu-O]-tetradecanoate} (**1b**)

$[\alpha]_D^{26}$ −19.0° (*c* 0.50, CH_3OH). High-resolution FAB-MS (positive) *m/z* 1057.6960 [calcd for $C_{53}H_{94}N_8O_{12}Na$ $(M+Na)^+$; 1057.6889].

References

1) HIRAMOTO, S; N. KINOSHITA, S. HATANAKA & H. SETO: Stimulation of apolipoprotein E secretion in human hepatoma hep G2 cells by a cyclic acylpeptide, N-4909. J. Antibiotics 49: 949 ~ 952, 1996

2) YAMADA, N.; I. INOUE, M. KAWAMURA, K. HARADA, Y. WATANABE, H. SHIMANO, T. GOTOHDA, M. SHIMADA, K.

KOHZAKI, T. TSUKADA, M. SHIOMI, Y. WATANABE & Y. YAZAKI: Apolipoprotein E prevents the progression of atherosclerosis in Watanabe heritable hyperlipidemic rabbits. J. Clin. Invest. 89: 706~711, 1992

3) MAHLEY, R. W.; K. H. WEISGRAUBER, M. M. HUSSAIN, B. GREENMAN, M. FISHER, T. VOGAL & M. GORECKI: Intravenous infusion of apolipoprotein E accelerates clearance of plasma lipoprotein in rabbits. J. Clin. Invest. 83: 2125~2130, 1989

4) YAMADA, N.; H. SHIMANO, H. MOKUNO, S. ISHIBASHI, T. GOTOHDA, M. KAWAKAMI, Y. WATANABE, Y. AKANUMA, T. MURASE & F. TAKAKU: Increased clearance of plasma cholesterol after injection of apolipoprotein E into Watanabe heritable hyperlipidemic rabbits. Proc. Natl. Acad. Sci. U.S.A. 86: 665~669, 1989

5) HASUMI, K.; K. TAKIZAWA, F. TAKAHASHI, J. K. PARK & A. ENDO: Inhibition of acyl-CoA: Cholesterol acyltransferase by isohalobacillin, a complex of novel cyclic acylpeptides produced by Bacillus sp. A1238. J. Antibiotics 48: 1419~1424, 1995

6) SUGAI, T.; H. RITZEN & C.-H. WONG: Towards the chemoenzymatic synthesis of lipid A. Tetrahedron: Asymmetry 4: 1051~1508, 1993

7) HARUMI, K.; M. MIURA & K. ORITO: A short route to dihydrocapsaicinoids. Synthesis: 864~866, 1985

8) TATSUYOSHI, I; K. WADA: Total synthesis of (±)-diplodialide-A. J. C. S. Perkin I, 323~327, 1979