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Synthesis of N-4909 Analogs

Part I. A Stimulant of Apolipoprotein E Secretion in Human Hepatoma G2 Cells

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Analogs of N-4909 (**1**), which had a stimulating activity for apolipoprotein E (apo E) secretion in Human hepatoma Hep G2 cells, were prepared and their activities examined. Cyclic analogs which had different kinds of amino acids or different number of amino acids from N-4909 (**1**) showed less effect on apo E secretion from Hep G2 cells. The length of acyl chain was found to be an important factor for the activity. Shorter chain reduced the activity. Linear analogs were also prepared. One of their analogs, N-5849 (**17**), which had six amino acids was found to have strong activity.

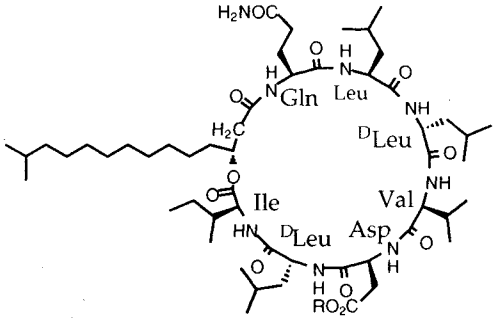
N-4909 (**1**) was isolated from the culture broth of *Bacillus* sp. No. 4691 as a stimulator of apolipoprotein E (apo E) secretion from human hepatoma Hep G2 cells. This was identified as the subcomponent of isohalobacillin. The stereochemistry of its β-oxyacyl residue was determined by HIRAMOTO *et al.*,[1] and this was synthesized by YANAI *et al.*[2,3]

Apo E was shown to cause a marked decrease of plasma cholesterol levels in hyperlipidemic rabbits by intravenous injection[4,5] and to prevent the progression of atherosclerosis in Watanabe heritable hyperlipidemic rabbits.[6] Therefore, we estimated that stimulators of apo E secretion from the liver will increase the apo E levels in plasma and may show hyperlipidemic and antiatherogenic activities.

N-4909 (**1**) is a cyclic depsipeptide which consists of seven amino acids and (*R*)-3-hydroxy-13-methyltetradecanoic acid as shown in Figure 1. Unfortunately, N-4909 (**1**) was not orally active. Therefore, we decided to modify this cyclic depsipeptide to find a more active compound than N-4909 (**1**).

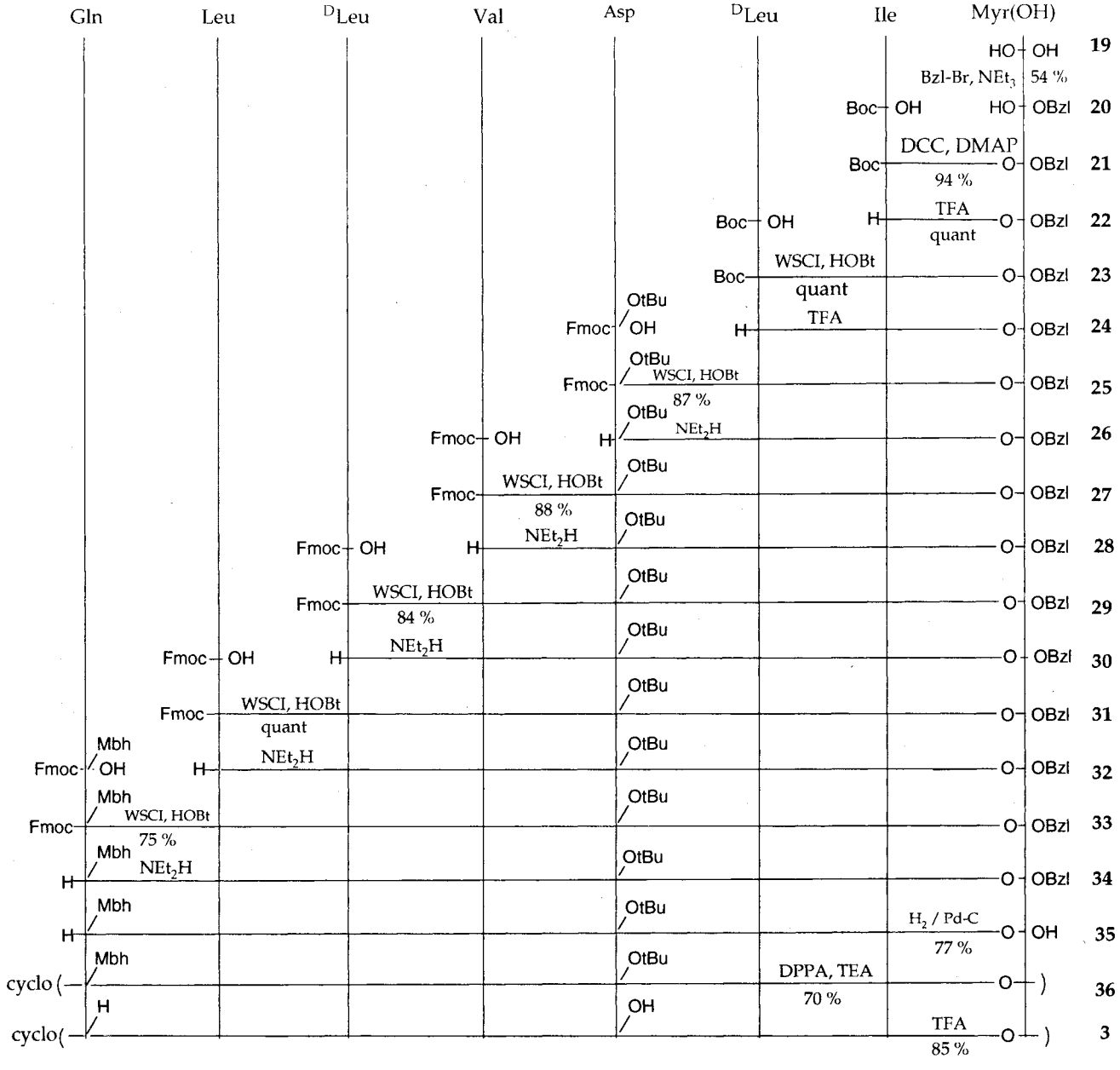
In this paper, we report the synthesis of depsipeptide analogs of N-4909 (**1**) and show their effects on apo E secretion in Hep G2 cells.

Fig. 1. Structure of N-4909 (**1**) and its methyl ester (**2**).



1 R = H
2 R = Me

Scheme 1. Synthesis of cyclic depsipeptide (**3**).



Chemistry

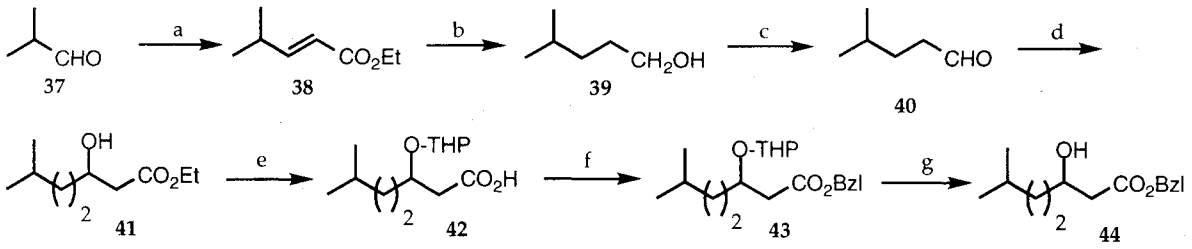
First, we modified and changed amino acids of N-4909 (**1**) to prepare cyclic depsipeptide analogs (**2**~**13**).

A methyl ester (**2**) of β-carboxylic acid in Asp was synthesized in 46% yield by treating N-4909 (**1**) with diazomethane in methanol.

When we synthesized N-4909 (**1**), the cyclization precursor was constructed from the hexapeptide and the amino ester.[2] For preparing cyclic depsipeptide analogs which contain myristic acid and various kinds of amino acids, we decided to construct the cyclization precursors stepwise as shown in Scheme 1. The other analogs (**4**~**13**) were also prepared by the same stepwise method.

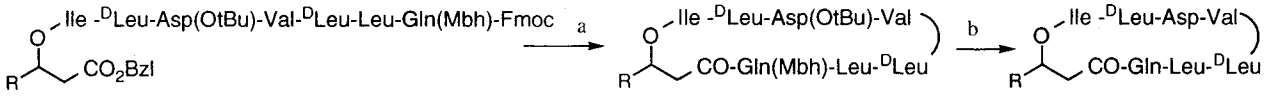
Myristic acid (**19**) was converted to its benzyl ester (**20**) using benzyl bromide and triethylamine (Et_3N) in 53% yield. Benzyl 3-(Boc-Ile-O)-tetradecanoate (**21**) was obtained by coupling Boc-Ile-OH with benzyl 3-hydroxytetradecanoate (**20**) with dicyclohexylcarbodiimide (DCC) and a catalytic amount of 4-dimethylaminopyridine

Scheme 2. Synthesis of benzyl 3-hydroxy-6-methylheptanoate (**44**).



a) $(EtO)_2POCH_2CO_2Et$, NaOMe, 82 %; b) LAH, 53 %; c) PCC, Zeolite, 67 %; d) $BrCH_2CO_2Et$, Zn, PhH-Et_2O, 55 %; e) 1) DHP p-TsOH, quant., 2) KOH, 80 %; f) Bzl-Br, NEt_3, 44 %; g) p-TsOH, 61 %

Scheme 3. Synthesis of cyclic depsipeptide analogs (**14**~**16**).



45 R = $CH_2CH_2CH(CH_3)_2$	53 %	**48** R = $CH_2CH_2CH(CH_3)_2$	78 %	**14** R = $CH_2CH_2CH(CH_3)_2$
46 R = $(CH_2)_4CH_3$	50 %	**49** R = $(CH_2)_4CH_3$	73 %	**15** R = $(CH_2)_4CH_3$
47 R = $(CH_2)_{12}CH_3$	58 %	**50** R = $(CH_2)_{12}CH_3$	70 %	**16** R = $(CH_2)_{12}CH_3$

a)1) NEt_2H, 2) 5% Pd-C, H_2, 3) WSCI, HOBt, NMM, KCl, CsCl; b) TFA

(DMAP) in 94% yield. The N^{α}-deprotected product (**22**) of the ester (**21**) was coupled with Boc-^{D}Leu-OH by the WSCI-HOBt (1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide-1-hydroxybenzotriazole) method to yield benzyl 3-(Boc-^{D}Leu-Ile-O)-tetradecanoate (**23**) quantitatively. The same procedure was repeated to prepare the protected cyclization precursor, benzyl 3-[Fmoc-Gln(Mbh)-Leu-^{D}Leu-Val-Asp(OtBu)-^{D}Leu-Ile-O]-tetradecanoate (**33**). Then, Fmoc and benzyl groups were removed in the usual manner. Cyclization was achieved by a high dilution method in DMF with diphenylphosphoryl azide (DPPA) at room temperature. The protected cyclization product, cyclo[3-[Gln(Mbh)-Leu-^{D}Leu-Val-Asp(OtBu)-^{D}Leu-Ile-O]-tetradecanoyl] (**36**), was obtained in 70% yield. The removal of the protecting groups of Asp and Gln by TFA gave the product, cyclo[3-(Gln-Leu-^{D}Leu-Val-Asp-^{D}Leu-Ile-O)-tetradecanoyl] (**3**), in 85% yield.

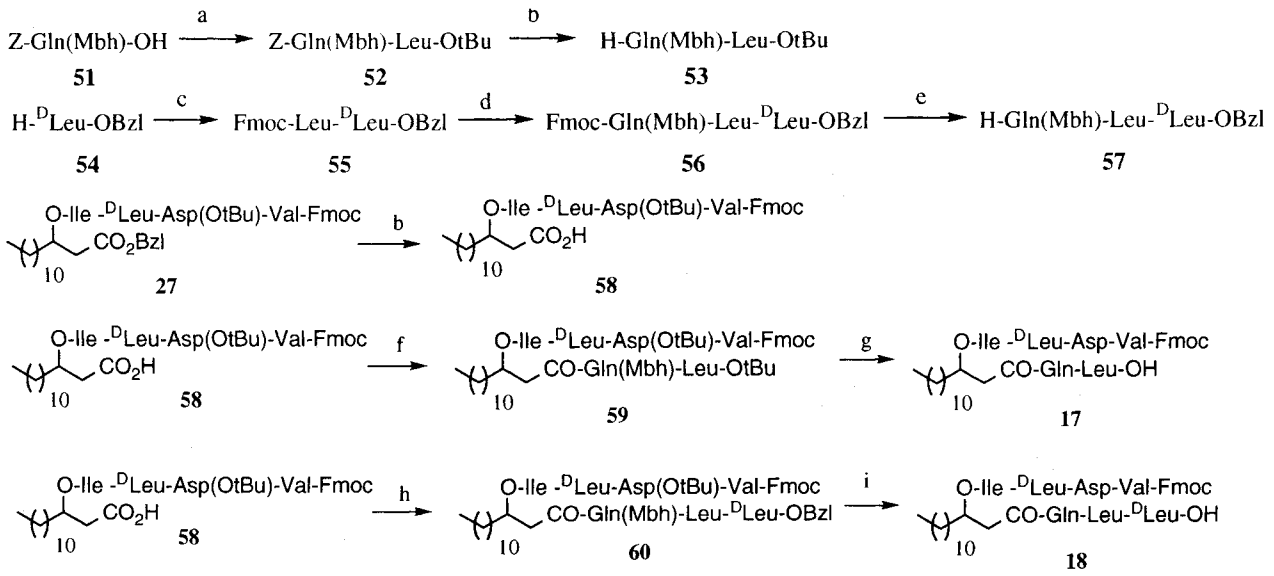
Secondly, we wanted to prepare cyclic depsipeptide analogs (**14**~**16**) which have different β-hydroxy carboxylic acids from myristic acid.

Benzyl 3-hydroxy-6-methylheptanoate (**44**) was synthesized as shown in Scheme 2.

Ethyl (2*E*)-4-methylpent-2-enoate (**38**) was prepared from 2-methylpropanal (**37**) by Horner-Emmons Reaction with ethyl diethylphosphonoacetate in 82%. After reduction of (**38**) by lithium aluminum hydride in 53% yield, the resulting 4-methylpentanol (**39**) was oxidized to 4-methylpentanal (**40**) by pyridium chlorochromate in 67% yield. Ethyl 3-hydroxy-6-methylheptanoate (**41**) was synthesized from (**40**) by Reformatsky Reaction with ethyl bromoacetate and zinc in 55% yield. This β-hydroxy ester (**41**) was reacted with dihydropyran to give a tetrahydropyranyl (THP) ether and then hydrolyzed with KOH to give 6-methyl-3-(tetrahydropyranyloxy)heptanoic acid (**42**) in 80% yield. This was converted to benzyl 6-methyl-3-(tetrahydropyranyloxy)heptanoate (**43**) using benzyl bromide and Et_3N in 44% yield, and then THP was deprotected by a catalytic amount of *p*-TsOH in MeOH to give benzyl 3-hydroxy-6-methylheptanoate (**44**) in 61% yield.

We prepared the cyclization precursors (**45**~**47**) from the amino esters and the hexapeptide, Fmoc-Gln(Mbh)-Leu-^{D}Leu-Val-Asp(OtBu)-^{D}Leu-OH, by the same manner as we synthesized N-4909 (**1**)[2]. After deprotection, cyclization

Scheme 4. Synthesis of the linear depsipeptide analogs (**17**, **18**).



a) Leu-OtBu, HOBt, WSCI, 98 %; b) 5% Pd-C, H_2; c) Fmoc-Leu, HOBt, WSCI; d) 1) NEt_2H, 2) Fmoc-Gln(Mbh), HOBt, WSCI, 79 % for 2 steps; e) NEt_2H; f) **53**, HOBt, WSCI, 59 %; g) TFA, 91 %; h) **57**, HOBt, WSCI, 46 %; i) 1) 5% Pd-C, H_2, 2) TFA, 31 % for 2 steps.

was performed by the WSCI-HOBt method as shown in Scheme 3 to yield the protected cyclization products (**48**~**50**) which were deprotected with TFA to give the target analogs (**14**~**16**).

Finally, the linear depsipeptide analogs (**17**, **18**) were synthesized as shown in Scheme 4.

Dipeptide (**52**) and tripeptide (**56**) were synthesized by the WSCI-HOBt method. The N^{α}-deprotected peptides (**53**, **57**) were coupled with the carboxylic acid (**58**) prepared from the previously synthesized intermediate (**27**) by hydrogenolysis. Deprotection of these coupling compounds (**59**, **60**) gave the linear analogs (**17**, **18**) of N-4909 (**1**).

Results and Discussion

At first, to see the active site of N-4909 (**1**), β-carboxylic acid of Asp was modified to methyl ester (**2**). This methyl ester analog (**2**) showed a significant drop of the activity of apo E secretion (Table 1). This indicates that a free β-carboxylic acid of Asp is important for the activity of N-4909 (**1**).

Secondly, to see the importance of the consisting amino acids of N-4909 (**1**), we changed the amino acids and examined the effects on the activity.

For preparing the analogs, we used a racemic myristic acid (**19**) as β-hydroxy carboxylic acid instead of (*R*)-3-hydroxy-13-methyltetradecanoic acid in N-4909 (**1**) because the analog (**3**) which had the same amino acids as N-4909 (**1**) and a racemic myristic acid (**19**) showed almost the same effects as N-4909 (**1**) on the secretion of apo E by Hep G2 cells.

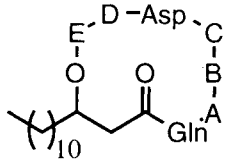
Table 1. Effects of N-4909 (**1**) and its methyl ester (**2**) on the secretion of apo E by Hep G2 cells (% of each control value).

(μM)	N-4909 (**1**)	its methyl ester (**2**)
1.0	428	118
5.0	937	163

To find the most important amino acid for the activity, each one of the amino acids except for Asp and Gln of N-4909 (**1**) were changed to Ala or DAla which had the same stereochemistry of the corresponding amino acid (**4**~**8**). Table 2 showed that all analogs (**4**~**8**) decreased their activity and suggested that every amino acid has almost the same importance for the activity.

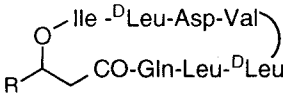
To see the importance of the stereochemistry of D-Leu, these were changed to L-Leu (**9**~**11**). These analogs also dropped their activity and these results suggested that their

Table 2. Effects of cyclic depsipeptide analogs of N-4909 (**1**) on the secretion of apo E by Hep G2 cells (% of each control value).



No	A	B	C	D	E	apo E (%) 1.0 μM	apo E (%) 5.0 μM
3	Leu	^{D}Leu	Val	^{D}Leu	Ile	457	1046
4	Ala	^{D}Leu	Val	^{D}Leu	Ile	156	315
5	Leu	^{D}Ala	Val	^{D}Leu	Ile	127	319
6	Leu	^{D}Leu	Ala	^{D}Leu	Ile	160	531
7	Leu	^{D}Leu	Val	^{D}Ala	Ile	188	339
8	Leu	^{D}Leu	Val	^{D}Leu	Ala	134	311
9	Leu	Leu	Val	^{D}Leu	Ile	210	280
10	Leu	^{D}Leu	Val	Leu	Ile	223	416
11	Leu	Leu	Val	Leu	Ile	134	268
12	-	^{D}Leu	Val	^{D}Leu	Ile	133	197
13	-	-	Val	^{D}Leu	Ile	117	238

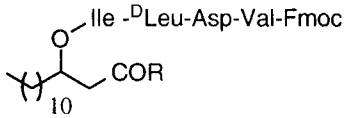
Table 3. Effects of various β-hydroxy carboxylic acids on the secretion of apo E by Hep G2 cells (% of each control value).



14 : R = $CH_2CH_2CH(CH_3)_2$
15 : R = $(CH_2)_4CH_3$
16 : R = $(CH_2)_{12}CH_3$

(μM)	(**14**)	(**15**)	(**16**)
1.0	172	119	423
5.0	132	130	483

Table 4. Effects of the number of amino acids in the linear depsipeptide analogs on the secretion of apo E by Hep G2 cells (% of each control value).



17 : R = Gln-Leu-OH
18 : R = Gln-Leu-^{D}Leu-OH

(μM)	(**17**)	(**18**)
1.0	370	273
5.0	255	101

unnatural D forms are important for their activities (Table 2).

To see the importance of the number of the composing amino acids, we synthesized cyclic depsipeptides (**12**, **13**) consisting of five or six amino acids. Both compounds dropped their activity significantly (Table 2). We assumed that more than seven amino acids are necessary for their activities.

Third, we changed β-hydroxy carboxylic acid to see its importance for the activity.

Table 3 suggests that the length of the acyl chains are important. The shorter acyl chains (**14**, **15**) dropped their activities even it had the isopropyl group at the end (**14**). Longer acyl chain (**16**) has remained the activity but myristic acid analog (**3**) which has the same length as N-4909 (**1**) showed the best result (Table 2).

Finally, we checked the importance of the ring.

Table 4 showed that six amino acids (**17**) has the better effects than seven amino acid (**18**). At 1.0 μM, the analog

(**17**) has a significant activity among all analogs in the present study. For preparation, the linear analogs are easier than the cyclic analogs. From these reasons, we decided to modify this linear analog (**17**), N-5849, to improve the activity. These studies are now under investigation.

Experimental

General

Melting points were determined on a micro melting point apparatus and were uncollected. ^{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. IR spectra were recorded on a Nicolet FT-IR spectrometer Impact 420.

Biological Activity

Effects of N-4909 (**1**) and its analogs (**2**~**18**) 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 Boc or Fmoc group. The β-carboxyl group of Asp was protected by the *tert*-Bu group. The carbamoyl group of Gln was protected by 4,4′-dimethoxy benzhydryl (Mbh) group. The protecting group for fatty acids was Bzl.

Cyclo[(*R*)-13-methyl-3-[Gln-Leu-^{D}Leu-Val-Asp(OMe)-^{D}Leu-Ile-O]-tetradecanoyl] (**2**)

To a solution of N-4909 (**1**) (50 mg, 0.048 mmol) in MeOH (1 ml) was added a diethyl ether solution of CH_2N_2 until the solution became slightly yellow. After stirring for 10 minutes at room temperature, an excess amount of AcOH was added. After removal of the solvent, diethyl ether was added to solidify the product which was filtered off and dried *in vacuo* to yield the product (23 mg, 46%). High-resolution FAB-MS (positive) *m/z* 1049.7224 [calcd for $C_{54}H_{97}N_8O_{12}$ $(M+H)^+$; 1049.7231].

Benzyl 3-Hydroxytetradecanoate (**20**)

To a solution of 3-hydroxymyristic acid (**19**) (15.0 g, 61.4 mmol) and Et_3N (8.56 ml, 61.4 mmol) in DMF (150 ml) was added benzyl bromide (7.30 ml, 61.4 mmol) at room temperature. This reaction mixture was stirred at room temperature for 2 days. After removal of the solvent, the residue was taken up to EtOAc 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 (150 g), eluting with $CHCl_3$: MeOH=200 : 0~20, to yield the product which was recrystallized from *n*-hexane (11.0 g, 54%) and to recover the starting material (6.49 g, 43%). MP 30°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33~7.40 (5H, m, Ar-*H*), 5.16 (2H, s, CH_2Ph), 3.95~4.05 (1H, m, C*H*OH), 2.85 (1H, d, *J*=4.4 Hz, CHO*H*), 2.56 (1H, dd, *J*=2.9, 17 Hz, CH_2CO_2), 2.46 (1H, dd, *J*=9.0, 17 Hz, CH_2CO_2), 1.20~1.60 (20H, m, CH_2), 0.88 (3H, d, *J*=6.8 Hz, CH_2CH_3). *Anal* Calcd for $C_{21}H_{34}O_3$: C 75.4, H 10.3; Found C 75.4, H 10.6.

Benzyl 3-(Boc-Ile-O)-tetradecanoate (**21**)

To a solution of **20** (3.00 g, 8.97 mmol), Boc-Ile (2.22 g, 9.10 mmol, 1.1 eq) and DMAP (77 mg, 0.63 mmol, 0.07 eq) in CH_2Cl_2 (50 ml) was added DCC (2.78 g, 13.5 mmol, 1.5 eq) at an ice cooled temperature. This was stirred at this temperature for 1 hour and then at room temperature for 2 hours. After filtration and evaporation, the residue was taken up to EtOAc and 0.5 N HCl. The separated organic layer was rinsed with sat. $NaHCO_3$ and brine, and then dried (Na_2SO_4). After removal of the solvent, the crude product was purified by chromatography on silica gel (100 g), eluting with *n*-Hexane : EtOAc=200 : 0~15, to yield the product (4.62 g, 94%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30~7.39 (5H, m, Ar-*H*), 5.24~5.33 (1H, m, N*H*), 5.12 (1H, br s, CH_2Ph), 5.10 (1H, d, *J*=2.5 Hz, CH_2Ph), 4.98~5.05 (1H, m, C*H*OCO), 4.15~4.25 (1H, m, COC*H*NH), 2.56~2.72 (2H, m, CH_2CO_2), 1.75~1.90 (1H, m, C*H*$(CH_3)CH_2$), 1.50~1.70 (2H, m, CH$(CH_3)CH_2CH_3$), 1.44 (9H, s, $C(CH_3)_3$), 1.10~1.45 (20H, m, CH_2), 0.86~0.93 (9H, m, CH_3). ESI-MS *m/z* 565 $(M+NH_4)^+$.

Benzyl 3-(H-Ile-O)-tetradecanoate (**22**)

A solution of **21** (4.62 g, 8.43 mmol) in TFA (9 ml) was stirred at room temperature for 15 minutes. After removal of TFA, the residue was taken up to EtOAc and sat. $NaHCO_3$. The separated organic layer was dried (Na_2SO_4). Removal of the solvent gave the product **22** (3.78 g, quant.). ESI-MS *m/z* 448 $(M+H)^+$.

Benzyl 3-(Boc-^{D}Leu-Ile-O)-tetradecanoate (**23**)

To a solution of **22** (3.78 g, 8.43 mmol), Boc-^{D}Leu·H_2O (2.10 g, 8.43 mmol), HOBt·H_2O (1.25 g, 9.28 mmol, 1.1

eq) in CH_2Cl_2 (50 ml) was added WSCI·HCl (1.78 g, 9.28 mmol, 1.1 eq) at an ice cooled temperature. This was stirred at this temperature for 1 hour and then at room temperature overnight. After removal of the solvent, the residue was taken up to AcOEt and 10% citric acid. The separated organic layer was rinsed with H_2O, 5% $NaHCO_3$ and H_2O, and then dried (Na_2SO_4). After removal of the solvent, the crude product was purified by chromatography on silica gel (80 g), eluting with *n*-Hexane : AcOEt=200 : 10~25, to yield the product (5.58 g, quant.). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30~7.39 (5H, m, Ar-*H*), 6.60~6.70 (1H, m, N*H*), 5.25~5.30 (1H, m, N*H*), 5.07~5.14 (2H, m, CH_2Ph), 4.75~4.95 (1H, m, C*H*OCO), 4.45~4.55 (1H, m, NHC*H*CO), 4.10~4.20 (1H, m, NHC*H*CO), 2.55~2.71 (2H, m, CH_2CO), 1.80~1.95 (1H, m, C*H*(CH_3)CH_2), 1.50~1.70 (3H, m, CH(CH_3)CH_2CH_3+CH_2C*H*(CH_3)$_2$), 1.35~1.50 (2H, m, CH_2CH(CH_3)$_2$), 1.45 (9H, s, C(CH_3)$_3$), 1.00~1.35 (20H, m, CH_2), 0.85~0.95 (15H, m, CH_3). ESI-MS *m/z* 661 $(M+H)^+$.

Benzyl 3-[Fmoc-Val-Asp(OtBu)-^{D}Leu-Ile-O]-tetradecanoate (**27**)

ESI-MS *m/z* 1053 $(M+H)^+$.

Benzyl 3-[Fmoc-^{D}Leu-Val-Asp(OtBu)-^{D}Leu-Ile-O]-tetradecanoate (**29**)

ESI-MS *m/z* 1166 $(M+H)^+$.

Benzyl 3-[Fmoc-Leu-^{D}Leu-Val-Asp(OtBu)-^{D}Leu-Ile-O]-tetradecanoate (**31**)

ESI-MS *m/z* 1279 $(M+H)^+$.

Benzyl 3-[Fmoc-Gln(Mbh)-Leu-^{D}Leu-Val-Asp(OtBu)-^{D}Leu-Ile-O]-tetradecanoate (**33**)

ESI-MS *m/z* 1633 $(M+H)^+$.

Cyclo[3-[Gln(Mbh)-Leu-^{D}Leu-Val-Asp(OtBu)-^{D}Leu-Ile-O]-tetradecanoyl] (**36**)

To a solution of **33** (1.85 g, 1.13 mmol) in DMF (20 ml) was added NEt_2H (2 ml) at room temperature. This was stirred at this temperature for 1 hour and then evaporated. To the residue were added MeOH (60 ml) and 5% Pd-C (0.20 g). This was reacted under H_2 atmosphere for 3 hours. After filtration and evaporation, the residue was purified by chromatography on silica gel (30 g), eluting with $CHCl_3$: MeOH=200 : 0~30, to yield the product (**35**) (1.16 g, 77%).

To a solution of **35** (0.50 g, 0.38 mmol) in DMF (200ml) were added DPPA (0.09 ml, 0.42 mmol, 1.1 eq) and Et_3N (0.06 ml, 0.42 mmol, 1.1 eq) at an ice cooled temperature. This was stirred at this temperature for 3 hours and then at room temperature overnight. After cooled with an ice bath, DPPA (0.99 ml, 0.42 mmol, 1.1 eq) and Et_3N (0.06 ml, 0.42 mmol, 1.1 eq) were added. This was stirred at this temperature for 4 hours and then at room temperature for four days. After removal of the solvent, the residue was treated with AcOEt and 10% citric acid. The separated organic layer was rinsed with H_2O, 5% $NaHCO_3$ and H_2O, and then dried (Na_2SO_4). After removal of the solvent, the crude product was purified by chromatography on silica gel (25 g), eluting with $CHCl_3$: MeOH=200 : 0~4, to yield the product (**36**) (0.35 g, 70%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.07~7.20 (4H, m), 6.80~6.92 (4H, m), 6.01~6.10 (1H, m), 5.14~5.29 (1H, m), 4.67~4.78 (1H, m), 4.21~4.45 (5H, m), 4.06~4.16 (1H, m), 3.77 (3H, s), 3.76 (3H, s), 2.62~2.85 (2H, m), 2.47~2.58 (1H, m), 2.21~2.44 (3H, m), 1.81~1.94 (4H, m), 1.53~1.81 (10H, m), 1.44 (9H, s), 1.08~1.53 (21H, m), 0.74~1.08 (33H, m). ESI-MS *m/z* 1303 $(M+H)^+$.

Cyclo[3-(Gln-Leu-^{D}Leu-Val-Asp-^{D}Leu-Ile-O)-tetradecanoyl] (**3**)

A solution of **36** (0.35 g, 0.27 mmol) in TFA (5 ml) was stirred at room temperature for 1.5 hours. After removal of the solvent, the residue was neutralized with 5% $NaHCO_3$ and extracted with 10% MeOH in $CHCl_3$. The combined organic layers were dried (Na_2SO_4). After removal of the solvent, the crude product was purified by chromatography on silica gel (20 g), eluting with $CHCl_3$: MeOH=100 : 0~50, to yield the product (0.23 g, 85%). ^{1}H NMR (400 MHz, CD_3OD) δ 5.20~5.30 (1H, m), 4.80~4.90 (1H, m), 4.20~4.50 (5H, m), 4.10~4.20 (1H, m), 2.80~2.95 (2H, m), 2.40~2.55 (2H, m), 2.10~2.25 (2H, m), 1.80~2.00 (4H, m), 1.45~1.80 (10H, m), 1.10~1.45 (21H, m), 0.70~1.10 (33H, m). High-resolution FAB-MS (positive) *m/z* 1021.6942 [calcd for $C_{52}H_{93}N_8O_{12}$ $(M+H)^+$; 1021.6918]

Cyclo[3-(Gln-Ala-^{D}Leu-Val-Asp-^{D}Leu-Ile-O)-tetradecanoyl] (**4**)

High-resolution FAB-MS (positive) *m/z* 979.6436 [calcd for $C_{49}H_{87}N_8O_{12}$ $(M+H)^+$; 979.6448]

Cyclo[3-(Gln-Leu-^{D}Ala-Val-Asp-^{D}Leu-Ile-O)-tetradecanoyl] (**5**)

High-resolution FAB-MS (positive) *m/z* 979.6441 [calcd for $C_{49}H_{87}N_8O_{12}$ $(M+H)^+$; 979.6448]

Cyclo[3-(Gln-Leu-^{D}Leu-Ala-Asp-^{D}Leu-Ile-O)-tetradecanoyl] (**6**)

High-resolution FAB-MS (positive) *m/z* 993.6578 [calcd for $C_{50}H_{89}N_8O_{12}$ $(M+H)^+$; 993.6605]

Cyclo[3-(Gln-Leu-^{D}Leu-Val-Asp-^{D}Ala-Ile-O)-tetradecanoyl] (**7**)

High-resolution FAB-MS (positive) *m/z* 979.6436 [calcd for $C_{49}H_{87}N_8O_{12}$ $(M+H)^+$; 979.6448]

Cyclo[3-(Gln-Leu-^{D}Leu-Val-Asp-^{D}Leu-Ala-O)-tetradecanoyl] (**8**)

High-resolution FAB-MS (positive) *m/z* 979.6436 [calcd for $C_{49}H_{87}N_8O_{12}$ $(M+H)^+$; 979.6448]

Cyclo[3-(Gln-Leu-Leu-Val-Asp-^{D}Leu-Ile-O)-tetradecanoyl] (**9**)

High-resolution FAB-MS (positive) *m/z* 1021.6942 [calcd for $C_{52}H_{93}N_8O_{12}$ $(M+H)^+$; 1021.6918]

Cyclo[3-(Gln-Leu-^{D}Leu-Val-Asp-Leu-Ile-O)-tetradecanoyl] (**10**)

High-resolution FAB-MS (positive) *m/z* 1021.6945 [calcd for $C_{52}H_{93}N_8O_{12}$ $(M+H)^+$; 1021.6918]

Cyclo[3-(Gln-Leu-Leu-Val-Asp-Leu-Ile-O)-tetradecanoyl] (**11**)

High-resolution FAB-MS (positive) *m/z* 1021.6945 [calcd for $C_{52}H_{93}N_8O_{12}$ $(M+H)^+$; 1021.6918]

Cyclo[3-(Gln-^{D}Leu-Val-Asp-^{D}Leu-Ile-O)-tetradecanoyl] (**12**)

ESI-MS *m/z* 908 $(M+H)^+$.

Cyclo[3-(Gln-Val-Asp-^{D}Leu-Ile-O)-tetradecanoyl] (**13**)

High-resolution FAB-MS (positive) *m/z* 795.5262 [calcd for $C_{40}H_{71}N_6O_{10}$ $(M+H)^+$; 795.5234]

Ethyl (2*E*)-4-methylpent-2-enoate (**38**)

To NaOMe (28% in MeOH, 66.0 g, 0.342 mol) was added a solution of ethyl diethylphosphonoacetate (73.0 g, 0.327 mol) at an ice cooled temperature. After stirred at 0°C for 30 minutes, a solution of 2-methyl propanal (**37**) (23.5 g, 0.327 mol) in THF (50 ml) was added at this solution. The resulting reaction mixture was stirred at this temperature for 1 hour. Then, this was diluted with *n*-hexane and H_2O was added. The separated aqueous layer was extracted with *n*-hexane. The combined organic layers were rinsed with H_2O and brine, and then dried ($MgSO_4$). After removal of the solvent, the residue was distilled to yield the product (34.3 g, 82%). bp 65~67°C (20 mmHg). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.95 (1H, dd, J=6.8, 15.6 Hz, C3-*H*), 5.77 (1H, dd, J=1.5, 15.6 Hz, C2-*H*). 4.18 (2H, q, J=6.8 Hz, OCH_2CH_3), 2.45 (1H, dsep., J=6.8 Hz, $CH(CH_3)_2$), 1.29 (3H, t, J=6.8 Hz, OCH_2CH_3), 1.06 (6H, d, J=6.8 Hz, $CH(CH_3)_2$).

4-Methylpentan-1-ol (**39**)

To a suspension of lithium aluminum hydride (7.6 g, 0.20 mol) in diethyl ether (Et_2O) (200 ml) was added dropwise a solution of **38** (17.3 g, 0.135 mol) in Et_2O. After stirred at reflux for 2 hours, EtOAc (15 ml) and dil HCl were added to this at an ice cooled temperature. The separated aqueous layer was extracted with Et_2O twice. The combined organic layers were rinsed with H_2O, sat. $NaHCO_3$ and brine, and then dried ($MgSO_4$). After removal of the solvent, the residue was purified by distillation twice to yield the product (7.33 g, 53%). bp 62~65°C (18 mmHg). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.63 (2H, m, CH_2OH), 1.56 (2H, m, CH_2CH_2OH), 1.24 (3H, m, $CH_2CH(CH_3)_2$). 0.89 (6H, d, J=6.4 Hz, $CH(CH_3)_2$).

4-Methylpentanal (**40**)

To a suspension of pyridium chlorochromate (23.7 g, 110 mmol) and Zeolite A3 (12.0 g) in CH_2Cl_2 (100 ml) was added **39** (7.33 g, 73.2 mmol) at room temperature. After stirred at room temperature for 2 hours, this was diluted with IPE (200 ml) and filtered through florisil. After removal of the solvent, the residue was treated with IPE and H_2O. The separated organic layer was dried ($MgSO_4$). After removal of the solvent, the residue was purified by distillation to yield the product (4.88 g, 67%). bp 55°C (18 mmHg). IR (Film, ν): 2958 (s), 2933 (s), 2872 (s), 2819 (m), 2719 (m), 1727 (s), 1469 (m), 1414 (w), 1387 (m), 1368 (m), 1328 (w), 1263 (w), 1180 (m), 1126 (w), 1106 (w), 1025 (w) cm^{-1}.

Ethyl 3-Hydroxy-6-methylheptanoate (**41**)

A small amount of a solution of 4-methylpentanal (**40**) (4.80 g, 48.7 mmol) and ethyl bromoacetate (10.0 g, 60.0 mmol) in benzene (100 ml) and Et_2O (20 ml) was added to Zinc powder (3.80 g, 58.5 mmol). This was heated to start the reaction. To this reaction mixture was added a rest of the solution slowly to keep the reflux. After the addition, this was refluxed for 30 minutes. After cooled to room temperature, 10% aq. H_2SO_4 (100 ml) was added slowly with an ice bath. The separated organic layer was rinsed with sat. aq. $NaHCO_3$ and brine, and then dried ($MgSO_4$). After removal of the solvent, the crude product was purified by chromatography on silica gel (100 g),

eluting with *n*-hexane : EtOAc=100 : 20, to yield the product (5.01 g, 55%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.17 (2H, q, J=6.8 Hz, OCH_2CH_3), 3.97 (1H, m, C*H*OH), 2.97 (1H, d, J=3.9 Hz, O*H*), 2.53 (1H, dd, J=2.9, 17 Hz, CH_2CO_2), 2.40 (1H, dd, J=9.3, 16 Hz, CH_2CO_2), 1.53 (3H, br s, C4-*H*+C6-*H*), 1.30 (2H, br s, C5-*H*), 1.28 (3H, t, J=7.0 Hz, OCH_2CH_3), 0.893 (3H, d, J=6.4 Hz), 0.889 (3H, d, J=6.8 Hz). IR (Film, ν): 3454 (m), 2956 (s), 2935 (s), 2870 (m), 1736 (s), 1468 (m), 1407 (w), 1371 (m), 1300 (m), 1251 (m), 1176 (s), 1030 (m) cm^{-1}.

6-Methyl-3-(tetrahydropyranyloxy)heptanoic Acid (**42**)

To a solution of **41** (5.00 g, 26.6 mmol) in Et_2O (100 ml) were added dihydropyran (8.00 g, 95.1 mmol) and p-TsOH (0.20 g) at room temperature. After stirred at room temperature for 2 hours, this was rinsed with sat. $NaHCO_3$ twice and brine, and then dried ($MgSO_4$). Removal of the solvent gave the THP ether (7.33 g, quant.). IR (Film, ν): 2653 (m), 2870 (m), 2852 (m), 1737 (s), 1467 (m), 1367 (m), 1176 (m), 1132 (m), 1116 (m), 1077 (m), 1026 (s), 988 (m), 903 (w), 871 (m), 814 (w) cm^{-1}.

To a solution of this THP ether (7.33 g, 26.7 mmol) in MeOH (50 ml) was added a solution of KOH (85%, 1.75 g, 26.6 mmol) in H_2O (20 ml) at an ice cooled temperature. After stirred for 30 minutes at this temperature, KOH (2.00 g, 30.3 mmol) was added. After 30 minutes, another KOH (2.00 g, 30.3 mmol) was added and stirred for 30 minutes. After removal of the solvent, this was treated with H_2O and IPE. The separated aqueous layer was neutralized (pH 3) with dil HCl and extracted with IPE three times. The combined organic layers were rinsed with H_2O and brine, and then dried over anhydrous $MgSO_4$. Removal of the solvent gave the product **42** (5.20 g, 80%). IR (Film, ν): 3000 (br), 2935 (s), 2870 (m), 2700 (br w), 1736 (s), 1711 (s), 1467 (m), 1384 (m), 1286 (m), 1132 (m), 1116 (m), 1077 (m), 1026 (s), 988 (m), 904 (w), 870 (m) cm^{-1}.

Benzyl 6-Methyl-3-(tetrahydropyranyloxy)heptanoate (**43**)

A solution of **42** (5.20 g, 21.3 mmol), benzyl bromide (7.28 g, 42.6 mmol) and NEt_3 (4.30 g, 42.6 mmol) in DMF (80 ml) was stirred at room temperature for 3 days. This was treated with IPE (100 ml) and H_2O (100 ml). The separated aqueous layer was extracted with IPE. The combined organic layers were rinsed with H_2O and brine, and then dried ($MgSO_4$). After removal of the solvent, the residue was purified by chromatography on silica gel, eluting with *n*-hexane : EtOAc=100 : 10, to yield the product (3.11 g, 44%) and to recover the starting material (2.00 g). IR (Film, ν): 3064 (w), 3033 (w), 2952 (s), 2869 (m), 1737 (s), 1498 (w), 1456 (m), 1384 (m), 1285 (m), 1260 (m), 1167 (m), 1132 (m), 1117 (m), 1077 (m), 1026 (s), 988 (m), 870 (w), 750 (m), 698 (m) cm^{-1}.

Benzyl 3-Hydroxy-6-methylheptanoate (**44**)

To a solution of **43** (3.11 g, 9.30 mmol) in MeOH (70 ml) was added p-TsOH (0.35 g). This was refluxed for 1 hour and cooled to room temperature. This was treated with H_2O and IPE. The separated aqueous layer was extracted with IPE. The combined organic layer was rinsed with H_2O, sat. $NaHCO_3$ and brine, and then dried ($MgSO_4$). After removal of the solvent, the residue was purified by chromatography on silica gel (70 g), eluting with *n*-hexane : AcOEt=100 : 15, to yield the product (1.38 g, 61%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30~7.40 (5H, m, Ar-*H*), 5.16 (2H, s, CH_2Ph), 3.96~4.04 (1H, m, C*H*OH), 2.57 (1H, dd, J=2.9, 17 Hz, CH_2CO_2), 2.46 (1H, dd, J=8.8, 17 Hz, CH_2CO_2), 1.40~1.60 (3H, m, C4-*H*+C6-*H*), 1.25~1.39 (1H, m, C5-*H*), 1.10~1.24 (1H, m, C5-*H*), 0.88 (6H, 2d, J=6.5 Hz). IR (Film, ν): 3453 (m), 3066 (w), 3034 (w), 2954 (s), 2869 (m), 1734 (s), 1498 (w), 1456 (m), 1384 (m), 1290 (m), 1256 (m), 1166 (s), 1119 (w), 1078 (w), 1054 (w), 1030 (w), 749 (m), 697 (m) cm^{-1}.

Benzyl 6-Methyl-3-[Fmoc-Gln(Mbh)-Leu-^{D}Leu-Val-Asp(OtBu)-^{D}Leu-Ile-O]-heptanoate (**45**)

^{1}H NMR (400 MHz, CD_3OD) δ 7.75 (2H, d, J=7.3 Hz), 7.54~7.62 (2H, m), 7.37 (2H, t, J=7.6 Hz), 7.24~7.34 (7H, m), 7.15 (4H, d, J=8.3 Hz), 6.84 (4H, d, J=8.3 Hz), 6.12 (1H, s), 5.16~5.27 (1H, m), 5.02~5.14 (2H, m), 4.84 (1H, br s), 4.40~4.50 (2H, m), 4.12~4.50 (6H, m), 4.03 (1H, d, J=6.3 Hz), 3.76 (6H, s), 2.91~2.98 (1H, m), 2.54~2.78 (3H, m), 2.39 (2H, t, J=7.6 Hz), 1.95~2.17 (3H, m), 1.81~1.95 (1H, m), 1.33~1.81 (13H, m), 1.42 (9H, s), 1.08~1.25 (3H, m), 0.76~1.03 (36H, m).

Benzyl 3-[Fmoc-Gln(Mbh)-Leu-^{D}Leu-Val-Asp(OtBu)-^{D}Leu-Ile-O]-octanoate (**46**)

^{1}H NMR (400 MHz, CD_3OD) δ 7.68~7.80 (2H, m), 7.53~7.63 (2H, m), 7.18~7.43 (9H, m), 7.13 (4H, d, J=8.3 Hz), 6.82 (4H, d, J=8.3 Hz), 6.09 (1H, s), 5.13~5.29 (1H, m), 4.98~5.13 (2H, m), 4.10~4.60 (9H, m), 3.98~4.06 (1H, m), 3.74 (6H, s), 2.52~3.00 (4H, m), 2.31~2.50 (2H, m), 1.96~2.20 (4H, m), 1.47~1.75 (10H, m), 1.41 (9H, s), 1.08~1.45 (9H, m), 0.70~1.00 (33H, m).

Benzyl 3-[Fmoc-Gln(Mbh)-Leu-^{D}Leu-Val-Asp(OtBu)-^{D}Leu-Ile-O]-hexadecanoate (**47**)

^{1}H NMR (400 MHz, CD_3OD) δ 7.69~7.79 (2H, m), 7.56~7.63 (2H, m), 7.23~7.40 (9H, m), 7.14 (4H, d, J=

8.8 Hz), 6.84 (2H, d, J=8.8 Hz), 6.83 (2H, d, J=8.3 Hz), 6.10 (1H, s), 5.18~5.27 (1H, m), 5.03~5.10 (2H, m), 4.21~4.61 (8H, m), 4.13~4.20 (1H, m), 4.00~4.05 (1H, m), 3.75 (6H, s), 2.53~2.91 (4H, m), 2.40 (2H, t, J= 7.6 Hz), 2.10~2.19 (1H, m), 1.95~2.06 (2H, m), 1.83~ 1.93 (1H, m), 1.48~1.81 (10H, m), 1.42 (9H, s), 1.13~ 1.46 (25H, m), 0.79~1.00 (33H, m).

Cyclo[3-[Gln(Mbh)-Leu-^{D}Leu-Val-Asp(OtBu)-^{D}Leu-Ile-O]-hexadecanoyl] (**50**)

To a solution of the depsipeptide (**47**) (1.28 g, 0.77 mmol) was added NEt_2H (1.5 ml) and this was stirred at room temperature for 3 hours. After removal of the solvent, the residue was purified by chromatography on silica gel (20 g), eluting with $CHCl_3$: MeOH=100 : 0~3.5, to yield the amine (0.82 g, 74%).

A suspension of the resulting amine (0.82 g, 0.57 mmol) and 5% Pd-C (0.08 g) in MeOH (25 ml) was shaken at H_2 atmosphere for 4 hours. After filtration, the solvent was removed. The residue was dissolved with 4-methylmorpholine (0.13 ml, 1.14 mmol, 2 eq) and HOBt·H_2O (0.35 g, 2.28 mmol, 4 eq) in THF (130 ml). This was added dropwise to a suspension of CsCl (0.96 g, 5.69 mmol, 10 eq), KCl (0.38 g, 5.13 mmol, 9 eq) and WSCI·HCl (0.98 g, 5.13 mmol, 9 eq) in a mixture of THF (260 ml) and DMF (130 ml). This reaction mixture was stirred at room temperature for 11 days. After dilution with EtOAc (150 ml), this was rinsed with H_2O, 5% $NaHCO_3$, H_2O, 10% citric acid and H_2O, and then dried (Na_2SO_4). After removal of the solvent, the residue was purified by chromatography on silica gel (30 g), eluting with n-hexane : EtOAc=200 : 0~6, to yield the product which was solidified with Et_2O-n-hexane (0.60 g, 79%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.10~7.16 (4H, m), 6.82~6.89 (4H, m), 6.04~6.09 (1H, m), 5.11~5.25 (1H, m), 4.69~4.76 (1H, m), 4.22~4.51 (5H, m), 4.03~4.13 (1H, m), 3.77 (3H, s), 3.76 (3H, s), 2.64~2.92 (2H, m), 2.51~2.58 (1H, m), 2.15~2.43 (3H, m), 1.81~2.04 (4H, m), 1.51~1.78 (10H, m), 1.44 (9H, s), 1.11~1.48 (25H, m), 0.77~1.05 (33H, m).

Cyclo[6-methyl-3-[Gln(Mbh)-Leu-^{D}Leu-Val-Asp(OtBu)-^{D}Leu-Ile-O]-heptanoyl] (**48**)

^{1}H NMR (400 MHz, CD_3OD) δ 7.10~7.17 (4H, m), 6.82~6.88 (4H, m), 6.03~6.09 (1H, m), 5.15~5.23 (1H, m), 4.69~4.83 (1H, m), 4.22~4.51 (5H, m), 4.04~4.11 (1H, m), 3.77, 3.76 (6H, 2s), 2.64~2.91 (2H, m), 2.51~ 2.59 (1H, m), 2.15~2.45 (3H, m), 1.82~2.02 (3H, m), 1.36~1.78 (13H, m), 1.44 (9H, s), 1.13~1.33 (4H, m), 0.76~1.02 (36H, m).

Cyclo[3-[Gln(Mbh)-Leu-^{D}Leu-Val-Asp(OtBu)-^{D}Leu-Ile-O]-octanoyl] (**49**)

^{1}H NMR (400 MHz, CD_3OD) δ 7.10~7.16 (4H, m), 6.82~6.88 (4H, m), 6.04~6.09 (1H, m), 5.09~5.25 (1H, m), 4.70~4.77 (1H, m), 4.23~4.51 (5H, m), 4.03~4.13 (1H, m), 3.77 (3H, s), 3.76 (3H, s), 2.64~2.91 (2H, m), 2.51~2.58 (1H, m), 2.15~2.43 (3H, m), 1.82~2.03 (3H, m), 1.51~1.78 (11H, m), 1.44 (9H, s), 1.13~1.49 (9H, m), 0.77~1.04 (33H, m).

Cyclo[3-(Gln-Leu-^{D}Leu-Val-Asp-^{D}Leu-Ile-O)-hexadecanoyl] (**16**)

A solution of **50** (0.60 g, 0.45 mmol) in TFA (7 ml) was stirred at room temperature for 3 hours. After removal of the solvent, the residue was neutralized with 5% $NaHCO_3$ and extracted with 10% MeOH in $CHCl_3$. The combined organic layers were dried (Na_2SO_4). After removal of the solvent, the residue was purified by chromatography on silica gel (20 g), eluting with n-hexane : EtOAc=100 : 0~ 40, to yield the product which was solidified with Et_2O-n-hexane (0.33 g, 70%). ^{1}H NMR (400 MHz, CD_3OD) δ 5.12~5.33 (1H, m), 4.67~4.80 (1H, m), 4.26~4.63 (5H, m), 4.04~4.20 (1H, m), 2.38~2.93 (4H, m), 1.50~2.34 (16H, m), 1.17~1.50 (25H, m), 0.81~1.09 (33H, m). High-resolution FAB-MS (positive) m/z 1049.7224 [calcd for $C_{54}H_{97}N_8O_{12}$ $(M+H)^+$; 1049.7231]

Cyclo[6-methyl-3-(Gln-Leu-^{D}Leu-Val-Asp-^{D}Leu-Ile-O)-hexanoyl] (**14**)

High-resolution FAB-MS (positive) m/z 937.6008 [calcd for $C_{46}H_{81}N_8O_{12}$ $(M+H)^+$; 937.5978]

Cyclo[3-(Gln-Leu-^{D}Leu-Val-Asp-^{D}Leu-Ile-O)-octanoyl] (**15**)

High-resolution FAB-MS (positive) m/z 937.6003 [calcd for $C_{46}H_{81}N_8O_{12}$ $(M+H)^+$; 937.5978]

Z-Gln(Mbh)-Leu-OtBu (**52**)

To a solution of Z-Gln(Mbh)-OH (**51**) (1.56 g, 3.07 mmol) and Leu-OtBu·HCl (0.82 g, 3.67 mmol, 1.2 eq) in CH_2Cl_2 (20 ml) were added TEA (0.47 ml, 3.38 mmol, 1.1 eq), WSCI·HCl (0.65 g, 3.38 mmol, 1.1 eq) and HOBt·H_2O (0.46 g, 3.38 mmol, 1.1 eq) at an ice cooled temperature. This was stirred at room temperature overnight. After removal of the solvent, the residue was treated with $CHCl_3$ and H_2O. The separated organic layer was rinsed with sat. $NaHCO_3$, H_2O, 10% citric acid and H_2O, and then dried (Na_2SO_4). Removal of the solvent gave the product (2.06 g, 98%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31~7.34 (5H, m), 7.13~7.22 (5H, m), 6.83~6.86 (4H,

m), 6.53 (1H, d, J=7.6 Hz), 6.21 (1H, d, J=8.4 Hz), 5.76 (1H, d, J=7.6 Hz), 5.10 (1H, d, J=12 Hz), 5.05 (1H, d, J=12 Hz), 4.37 (1H, ddd, J=4.8, 8.4, 13 Hz), 4.16~4.19 (1H, m), 3.77 (3H, s), 3.76 (3H, s), 2.37~2.40 (2H, m), 2.07 (2H, q, J=6.8 Hz), 1.43 (9H, s), 1.25~1.72 (3H, m), 0.84~0.90 (6H, m).

Fmoc-Leu-^{D}Leu-OBzl (**55**)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (2H, d, J=7.3 Hz), 7.57 (2H, d, J=7.3 Hz), 7.39 (2H, t, J=7.6 Hz), 7.27~7.36 (7H, m), 6.52 (1H, d, J=7.8 Hz), 5.19 (1H, d, J=7.8 Hz), 5.14 (1H, d, J=12 Hz), 5.09 (1H, d, J=12 Hz), 4.60~4.68 (1H, m), 4.34~4.46 (2H, m), 4.21 (1H, t, J=7.1 Hz), 4.17~4.29 (1H, m), 1.43~1.76 (6H, m), 0.93 (6H, d, J=5.4 Hz), 0.89 (6H, d, J=5.9 Hz).

H-Gln(Mbh)-Leu-OtBu (**53**)

A suspension of **52** (0.68 g, 1.00 mmol) and 5% Pd-C (0.15 g) in a mixture of MeOH (35 ml) and $CHCl_3$ (15 ml) was shaken at H_2 atmosphere for 6 hours. After filtration, removal of the solvent gave the product. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 (1H, d, J=7.2 Hz), 7.72 (1H, d, J=7.6 Hz), 7.15~7.19 (4H, m), 6.79~6.84 (4H, m), 6.11 (1H, d, J=7.6 Hz), 5.22 (2H, br s), 4.35 (1H, dt, J=6.0, 8.4 Hz), 3.90 (1H, t, J=6.8 Hz), 3.75 (3H, s), 3.74 (3H, s), 2.48~2.55 (2H, m), 2.08~2.19 (2H, m), 1.44~1.70 (3H, m), 1.41 (9H, s), 0.90 (3H, d, J=6.8 Hz), 0.87 (3H, d, J=6.8 Hz). ESI-MS m/z 542 $(M+H)^+$.

Fmoc-Gln(Mbh)-Leu-^{D}Leu-OBzl (**56**)

To a solution of **55** (6.29 g, 11.3 mmol) in DMF (120 ml) was added NEt_2H (12 ml). This reaction mixture was stirred at room temperature for 3 hours. After removal of the solvent, Fmoc-Gln(Mbh) (6.72 g, 11.3 mmol) and HOBt·H_2O (1.73 g, 11.3 mmol) were added and dissolved in a mixture of CH_2Cl_2 (80 ml) and DMF (30 ml). To this was added WSCI·HCl (2.17 g, 11.3 mmol) at an ice cooled temperature. This was stirred at this temperature for 2 hours and at room temperature overnight. After removal of the solvent, the residue was treated with 10% MeOH in $CHCl_3$ and 10% citric acid. The separated organic layer was rinsed with H_2O, 5% $NaHCO_3$ and H_2O, and then dried (Na_2SO_4). After removal of the solvent, the residue was crystallized from Et_2O-$CHCl_3$ to yield the product (8.09 g, 79%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.51 (1H, d, J=8.3 Hz), 8.23 (1H, d, J=7.8 Hz), 7.86 (2H, d, J=7.8 Hz), 7.78 (1H, d, J=7.8 Hz), 7.70 (2H, t, J=5.9 Hz), 7.47 (1H, d, J=7.8 Hz), 7.39 (2H, t, J=7.3 Hz), 7.26~7.36 (7H, m), 7.14 (4H, dd, J=1.5, 8.8 Hz), 6.83 (4H, dd, J=2.4, 8.8 Hz), 6.02 (1H, d, J=8.3 Hz), 5.06 (2H, s), 4.35~4.46 (1H, m), 4.17~4.34 (4H, m), 4.00~4.08 (1H, m), 3.71 (3H, s), 3.70 (3H, s), 2.21~2.34 (2H, m), 1.90~2.01 (1H, m), 1.74~1.87 (1H, m), 1.47~1.64 (4H, m), 1.44 (2H, t, J=7.1 Hz), 0.75~0.91 (12H, m). ESI-MS m/z 911 $(M+H)^+$.

H-Gln(Mbh)-Leu-^{D}Leu-OBzl (**57**)

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.61 (1H, d, J=8.8 Hz), 8.47 (1H, d, J=7.8 Hz), 7.96 (1H, d, J=8.3 Hz), 7.28~7.40 (5H, m), 7.14 (4H, d, J=8.8 Hz), 6.86 (4H, d, J=7.8 Hz), 6.00 (1H, d, J=8.3 Hz), 5.09 (2H, s), 4.38~4.47 (1H, m), 4.25~4.34 (1H, m), 3.71 (6H, s), 3.14 (1H, dd, J=4.4, 8.3 Hz), 2.15~2.34 (3H, m), 1.80~1.92 (1H, m), 1.46~1.66 (6H, m), 1.42 (2H, t, J=7.1 Hz), 0.83~0.87 (9H, m), 0.80 (3H, d, J=5.9 Hz).

3-(Fmoc-Val-Asp(OtBu)-^{D}Leu-Ile-O)-tetradecanoic Acid (**58**)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (2H, d, J=7.8 Hz), 7.56~7.66 (3H, m), 7.38~7.42 (2H, m), 7.29~7.33 (2H, m), 6.75~7.04 (1H, m), 6.61~6.71 (1H, m), 5.51~5.68 (1H, m), 5.13~5.26 (1H, m), 4.86~4.99 (1H, m), 4.33~4.55 (4H, m), 4.20~4.24 (1H, m), 3.98~4.01 (1H, m), 2.75~2.93 (2H, m), 2.37~2.60 (2H, m), 2.07~2.20 (1H, m), 1.39 (9H, s), 1.09~1.94 (26H, m), 0.83~1.00 (2H, m).

N-α-[3-[Fmoc-Val-Asp(OtBu)-^{D}Leu-Ile-O]-tetradecanoyl]-Gln(Mbh)-Leu-OtBu (**59**)

To a solution of **53** (1.00 mmol), **58** (0.96 g, 1.00 mmol) and HOBt·H_2O (0.15 g, 1.00 mmol) in CH_2Cl_2 (10 ml) was added WSCI·HCl (0.21 g, 1.00 mmol) at an ice cooled temperature. This was stirred at this temperature for 2 hours and at room temperature overnight. After removal of the solvent, the residue was treated with $CHCl_3$ and H_2O. The separated organic layer was rinsed with 5% $NaHCO_3$, H_2O, 10% citric acid and brine, and then dried (Na_2SO_4). After removal of the solvent, the residue was purified by chromatography on silica gel (30 g), eluting with $CHCl_3$: MeOH=200 : 5, to yield the product which was solidified with Et_2O-*n*-hexane. This was purified by rechromatography on silica gel (40 g), eluting with $CHCl_3$: EtOAc=100 : 30, to yield the product (0.87 g, 59%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74~7.76 (2H, m), 7.57~7.62 (2H, m), 6.97~7.48 (14H, m), 6.80~6.85 (4H, m), 6.17~6.20 (1H, m), 5.60~5.77 (1H, m), 5.08~5.19 (1H, m), 4.81~4.94 (1H, m), 4.28~4.50 (6H, m), 4.18~4.22 (1H, m), 3.92~4.00 (1H, m), 3.75 (6H, s), 2.72~2.80 (2H, m), 2.28~2.45 (4H, m), 2.00~2.14 (3H, m), 1.40, 1.43 (18H, 2s), 1.19~1.98 (29H, m), 0.81~0.96 (27H, m).

N-α-[3-(Fmoc-Val-Asp-^{D}Leu-Ile-O)-tetradecanoyl]-Gln-Leu-OH (**17**)

A solution of **59** (0.87 g, 0.59 mmol) in TFA (5 ml) was stirred at room temperature for 2 hours. After removal of TFA, the residue was taken up to $CHCl_3$ (5 ml) and H_2O (5 ml). To this was added 5% $NaHCO_3$ to pH 8 and dilute HCl was added to pH 1~2. To this was added $CHCl_3$ and MeOH to make this solution. This solution was rinsed with H_2O and then dried ($MgSO_4$). After removal of the solvent, the residue was solidified from $CHCl_3$-Et_2O-*n*-hexane. The resulting solids were collected and dried to yield the product (0.61 g, 91%). ^{1}H NMR (400 MHz, $CDCl_3$+CD_3OD) δ 7.77~7.79 (2H, m), 7.65~7.72 (2H, m), 7.28~7.40 (4H, m), 5.11~5.20 (1H, m), 4.65~4.71 (1H, m), 4.36~4.48 (5H, m), 4.22~4.31 (2H, m), 3.81~3.84 (1H, m), 2.94~3.00 (2H, m), 2.79~2.87 (2H, m), 2.44~2.58 (2H, m), 2.28~2.34 (2H, m), 2.03~2.12 (2H, m), 1.15~1.99 (28H, m), 0.84~1.00 (27H, m). ESI-MS *m/z* 1148 $(M+H)^+$.

N-α-[3-[Fmoc-Val-Asp(OtBu)-^{D}Leu-Ile-O]-tetradecanoyl]-Gln(Mbh)-Leu-^{D}Leu-OBzl (**60**)

To a solution of **57** (1.10 mmol, 1.1 eq), **58** (0.96 g, 1.00 mmol) and HOBt·H_2O (0.15 g, 1.00 mmol) in CH_2Cl_2 (10 ml) was added WSCI·HCl (0.21 g, 1.00 mmol) at an ice cooled temperature. This was stirred at this temperature for 2 hours and at room temperature overnight. After removal of the solvent, the residue was treated with $CHCl_3$ and H_2O. The separated organic layer was rinsed with 5% $NaHCO_3$, H_2O, 10% citric acid and brine, and then dried (Na_2SO_4). After removal of the solvent, the residue was purified by chromatography on silica gel (60 g), eluting with $CHCl_3$: MeOH=100:0~3.5, to yield the product which was purified by rechromatography on silica gel (45 g), eluting with $CHCl_3$: EtOAc=100:0~40, to yield the product (0.75 g, 46%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 (2H, d, *J*=7.8 Hz), 6.69~7.61 (22H, m), 6.78~6.82 (4H, m), 6.14, 6.19 (1H, 2d, *J*=7.8 Hz), 5.62~5.94 (1H, m), 4.77~5.19 (4H, m), 4.17~4.61 (8H, m), 3.96~4.06 (1H, m), 3.74 (3H, s), 3.72 (3H, s), 2.64~2.81 (2H, m), 2.24~2.44 (4H, m), 1.92~2.18 (3H, m), 1.68 (9H, s), 1.19~1.89 (32H, m), 0.81~0.93 (33H, m).

N-α-[3-(Fmoc-Val-Asp-^{D}Leu-Ile-O)-tetradecanoyl]-Gln-Leu-^{D}Leu-OH (**18**)

A suspension of **60** (0.75 g, 0.46 mmol) and 5% Pd-C (0.13 g) in a mixture of MeOH (20 ml) and DMF (20 ml) was shaken at 40°C under H_2 atmosphere (2.0~2.5 kg/cm^2) for 3 hours. After filtration and evaporation, the residue was dissolved in TFA (5 ml). This was stirred at room temperature for 1 hour. After removal of the solvent, the residue was taken up to $CHCl_3$ (10 ml) and H_2O (10 ml). To this was added 5% $NaHCO_3$ to pH 8 and dil HCl was added to pH 1~2. To this was added Et_2O and *n*-hexane. The formed solid was collected and dissolved in $CHCl_3$ and MeOH. To this was added Et_2O and *n*-hexane and the formed solid was collected. This was purified by chromatography on silica gel (20 g), eluting with $CHCl_3$: MeOH=100:10~20, to yield the product which was purified by rechromatography on silica gel (16 g), eluting with $CHCl_3$: MeOH=100:5~10, to yield the product (0.18 g, 31%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.83~7.90 (2H, m), 7.70~7.77 (2H, m), 7.26~7.43 (4H, m), 5.00~5.17 (1H, m), 3.86~4.60 (10H, m), 2.31~2.56 (2H, m), 0.94~2.16 (39H, m), 0.75~0.93 (33H, m). High-resolution FAB-MS (positive) *m/z* 1283.7505 [calcd for $C_{67}H_{104}N_8O_{15}$ $(M+Na)^+$; 1283.7524]

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