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 (I), 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 (I) 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 stereochernistry 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 injection4,5 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).
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.\textsuperscript{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\textsubscript{3}N) 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).

\[
\begin{align*}
\text{CHO} & \quad \text{a) (EtO)}_2\text{POCH}_2\text{CO}_2\text{Et, NaOMe, 82 \%; b) LAH, 53 \%; c) PCC, Zeolite, 67 \%; d) BrCH}_2\text{CO}_2\text{Et, Zn, PhH-Et}_2\text{O, 55 \%; e) 1) DHP p-TsOH, quant., 2) KOH, 80 \%; f) Bzl-Br, NEt}_3, 44 \%; g) p-TsOH, 61 \%}
\end{align*}
\]

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 %


\[
\begin{align*}
45 \quad R = \text{CH}_2\text{CH}_2\text{CH}(\text{CH}_3)_2 & \quad 53 \% \\
46 \quad R = \text{(CH}_2)_2\text{CH}_3 & \quad 50 \% \\
47 \quad R = \text{(CH}_2)_2\text{CH}_3 & \quad 58 \%
\end{align*}
\]

45 R = CH_2CH_2CH(CH_3)_2 53 % 46 R = (CH_2)_2CH_3 50 % 47 R = (CH_2)_2CH_3 58 %

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

(DMAP) in 94% yield. The N-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 (2E)-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). After deprotection, cyclization
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 Na-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.

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 D-Ala 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).

<table>
<thead>
<tr>
<th>No</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>apo E (%) 1.0 μM</th>
<th>apo E (%) 5.0 μM</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>Leu</td>
<td>0-Leu</td>
<td>Val</td>
<td>0-Leu</td>
<td>Ile</td>
<td>457</td>
<td>1046</td>
</tr>
<tr>
<td>4</td>
<td>Ala</td>
<td>0-Leu</td>
<td>Val</td>
<td>0-Leu</td>
<td>Ile</td>
<td>156</td>
<td>315</td>
</tr>
<tr>
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<td>Val</td>
<td>0-Leu</td>
<td>Ile</td>
<td>127</td>
<td>319</td>
</tr>
<tr>
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<td>Ala</td>
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<td>Ile</td>
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<td>531</td>
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<tr>
<td>7</td>
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<td>0-Leu</td>
<td>Val</td>
<td>0-Ala</td>
<td>Ile</td>
<td>188</td>
<td>339</td>
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<td>0-Leu</td>
<td>Val</td>
<td>0-Leu</td>
<td>Ala</td>
<td>134</td>
<td>311</td>
</tr>
<tr>
<td>9</td>
<td>Leu</td>
<td>Leu</td>
<td>Val</td>
<td>0-Leu</td>
<td>Ile</td>
<td>210</td>
<td>280</td>
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<tr>
<td>10</td>
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<td>0-Leu</td>
<td>Val</td>
<td>Leu</td>
<td>Ile</td>
<td>223</td>
<td>416</td>
</tr>
<tr>
<td>11</td>
<td>Leu</td>
<td>Leu</td>
<td>Val</td>
<td>Leu</td>
<td>Ile</td>
<td>134</td>
<td>268</td>
</tr>
<tr>
<td>12</td>
<td>0-Leu</td>
<td>Val</td>
<td>0-Leu</td>
<td>Ile</td>
<td></td>
<td>133</td>
<td>197</td>
</tr>
<tr>
<td>13</td>
<td>0-Leu</td>
<td></td>
<td>Val</td>
<td>0-Leu</td>
<td>Ile</td>
<td>117</td>
<td>238</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>R</th>
<th>(14)</th>
<th>(15)</th>
<th>(16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH₃CH₂CH(CH₃)₂</td>
<td>172</td>
<td>119</td>
<td>423</td>
</tr>
<tr>
<td>CH₃CH₂CH₃</td>
<td>132</td>
<td>130</td>
<td>483</td>
</tr>
</tbody>
</table>

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).

<table>
<thead>
<tr>
<th>(μM)</th>
<th>(14)</th>
<th>(15)</th>
<th>(16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>370</td>
<td>273</td>
<td></td>
</tr>
<tr>
<td>5.0</td>
<td>255</td>
<td>101</td>
<td></td>
</tr>
</tbody>
</table>

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 $\alpha$-amino function of amino acids was protected by the Boc or Fmoc group. The $\beta$-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-DLeu-Val-Asp(OMe)-D-Leu-Ile-O]-tetradecanoyl] (2)
To a solution of N-4909 (1) (50mg, 0.048mmol) in MeOH (1 ml) was added a diethyl ether solution of CH$_2$N$_2$ 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$_{8}$O$_{12}$ (M+H)$^+$; 1049.7231].

Benzyl 3-Hydroxytetradecanoate (20)
To a solution of 3-hydroxymyristic acid (19) (15.0 g, 61.4 mmol) and Et$_3$N (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$_2$O, H$_2$O twice and then dried (Na$_2$SO$_4$). 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$_3$) $\delta$ 7.33~7.40 (5H, m, Ar-H), 5.16 (2H, s, CH$_2$Ph), 3.95~4.05 (1H, m, CHO$_2$), 2.85 (1H, d, $J$=4.4 Hz, COH), 2.56 (1H, dd, $J$=2.9, 17 Hz, CH$_2$CO$_2$), 2.46 (1H, dd, $J$=9.0, 17 Hz, CH$_2$CO$_2$), 1.20~1.60 (20H, m, CH$_2$), 0.88 (3H, d, $J$=6.8 Hz, CH$_3$). 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$_2$Cl$_2$ (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$_2$SO$_4$). 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 (8.8 mes, 94%). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.30~7.39 (5H, m, Ar-H), 5.24~5.33 (1H, m, N#), 5.12 (1H, brs, CH$_2$), 5.08~5.12 (1H, d, $J$=2.5 Hz, CH$_2$Ph), 4.98~5.05 (1H, m, CHO$_2$), 4.15~4.25 (1H, m, COOC$_2$), 2.56~2.72 (2H, m, CH$_2$CO$_2$), 1.75~1.90 (1H, m, CH$_2$(CH$_3$)$_2$), 1.50~1.70 (2H, m, CH(CH$_3$)CH$_2$CH$_3$), 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 rinsed with Na$_2$SO$_4$. Removal of the solvent gave the product 22 (3.78 g, quant.). ESI-MS m/z 448 (M+H)$^+$.
eq) in CH₂Cl₂ (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 H₂O, 5% NaHCO₃ and H₂O, and then dried (Na₂SO₄). After removal of the solvent, the crude product was purified by chromatography on silica gel (80 g), eluting with n-Hexane:AcOEt=200:10 to yield the product (5.58 g, quant.). ¹H NMR (400 MHz, CDCl₃) δ 7.30-7.39 (5H, m, Ar-H), 6.60-6.70 (1H, m, N#), 5.07-5.14 (2H, m, C//2Ph), 4.75-4.95 (1H, m, CO), 4.45-4.55 (1H, m, NHC//), 4.10-4.20 (1H, m, CH₂), 2.55-2.71 (2H, m, CH₂), 1.80-1.95 (1H, m, C//(CH₃)CH₂), 1.50-1.70 (3H, m, CH(CH₃)C//2CH₃+CH₂C//(CH₃)₂), 1.35-1.50 (2H, m, CH₂CH₂CH₂), 1.45 (9H, s, C(C₃)₃), 1.00-1.35 (20H, m, CH₂), 0.85-0.95 (15H, m, CH₃). ESI-MS m/z 661 (M+H)+.

Benzyl 3-[Fmoc-Val-Asp(OtBu)-DLeu-Ile-O]-tetradecanoate (27)

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

Benzyl 3-[Fmoc-DLeu-Val-Asp(OtBu)-DLeu-Ile-O]-tetradecanoate (29)

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

Benzyl 3-[Fmoc-Leu-DLeu-Val-Asp(OtBu)-DLeu-Ile-O]-tetradecanoate (31)

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

Benzyl 3-[Fmoc-Gln(Mbh)-Leu-DLeu-Val-Asp(OtBu)-DLeu-Ile-O]-tetradecanoate (33)

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

Cyclo[3-(Gln-Leu-DLeu-Val-Asp-DLeu-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% NaHCO₃ and extracted with 10% MeOH in CHCl₃. The combined organic layers were dried (Na₂SO₄). After removal of the solvent, the crude product was purified by chromatography on silica gel (20 g), eluting with CHCl₃:MeOH=200:0 to yield the product (0.23 g, 85%). ¹H NMR (400 MHz, CDOD) 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 C₅₂H₉₃N₈O₁₂ (M+H)+; 1021.6936].

Cyclo[3-(Gln-Ala-DLeu-Val-Asp-DLeu-Ile-O)-tetradecanoyl] (4)

High-resolution FAB-MS (positive) m/z 979.6436 [calcd for C₄₉H₇₉N₈O₁₂ (M+H)+; 979.6448].

Cyclo[3-(Gln-Leu-DAla-Val-Asp-DLeu-Ile-O)-tetradecanoyl] (5)

High-resolution FAB-MS (positive) m/z 979.6441 [calcd for C₄₉H₇₉N₈O₁₂ (M+H)+; 979.6448].
Cyclo[3-(Gln-Leu-DLeu-Ala-Asp-DLeu-Ile-O)-tetradecanoyl] (6)  
High-resolution FAB-MS (positive) m/z 993.6578 [calcd for C_{50}H_{89}N_{8}O_{12} (M+H)+; 993.6605]  
Cyclo[3-(Gln-Leu-DLeu-Val-Asp-DLeu-Ile-O)-tetradecanoyl] (7)  
High-resolution FAB-MS (positive) m/z 979.6436 [calcd for C_{49}H_{87}N_{8}O_{12} (M+H)+; 979.6448]  
High-resolution FAB-MS (positive) m/z 979.6436 [calcd for C_{49}H_{87}N_{8}O_{12} (M+H)+; 979.6448]  
Cyclo[3-(Gin-Leu-Leu-Val-Asp-DLeu-Ile-O)-tetradecanoyl] (9)  
High-resolution FAB-MS (positive) m/z 1021.6942 [calcd for C_{52}H_{93}N_{8}O_{12} (M+H)+; 1021.6918]  
Cyclo[3-(Gln-Leu-DLeu-Val-Asp-Leu-Ile-O)-tetradecanoyl] (10)  
High-resolution FAB-MS (positive) m/z 1021.6945 [calcd for C_{52}H_{93}N_{8}O_{12} (M+H)+; 1021.6918]  
High-resolution FAB-MS (positive) m/z 1021.6945 [calcd for C_{52}H_{93}N_{8}O_{12} (M+H)+; 1021.6918]  
Cyclo[3-(Gln-DLeu-Val-Asp-DLeu-Ile-O)-tetradecanoyl] (12)  
ESI-MS m/z 908 (M+H)+.  
Cyclo[3-(Gln-Val-Asp-DLeu-Ile-O)-tetradecanoyl] (13)  
High-resolution FAB-MS (positive) m/z 795.5262 [calcd for C_{46}H_{71}N_{6}O_{10} (M+H)+; 795.5234]  
Ethyl (2E)-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-methylpropanal (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 H2O was added. The separated aqueous layer was extracted with n-hexane. The combined organic layers were rinsed with H2O and brine, and then dried (MgSO4). After removal of the solvent, the residue was distilled to yield the product (34.3 g, 82%). bp 65–67°C (20 mmHg).  
1H NMR (400 MHz, CDCl3) δ 6.95 (1H, dd, J = 6.8, 15.6 Hz, C-3-H), 5.77 (1H, dd, J = 1.5, 15.6 Hz, C-2-H). 4.18 (2H, q, J = 6.8 Hz, OCH2CH3), 2.45 (1H, dsep., J = 6.8 Hz, CH(CH3)2), 1.29 (3H, t, J = 6.8 Hz, OCH2CH2), 1.06 (6H, d, J = 6.8 Hz, CH(CH3)2).  

4-Methylpentan-1-ol (39)  
To a suspension of lithium aluminum hydride (7.6 g, 0.20 mol) in diethyl ether (Et2O) (200 ml) was added dropwise a solution of 38 (17.3 g, 0.135 mol) in Et2O. 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 Et2O twice. The combined organic layers were rinsed with H2O, sat. NaHCO3 and brine, and then dried (MgSO4). 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).  
1H NMR (400 MHz, CDCl3) δ 3.62 (2H, m, C2OH), 1.56 (2H, m, C2CH2OH), 1.24 (3H, m, C2C(CH3)2), 0.89 (6H, d, J = 6.4 Hz, CH(CH3)2).  

4-Methylpentanal (40)  
To a suspension of pyridium chlorochromate (23.7 g, 0.29 mmol) and Zeolite A3 (12.0 g) in CH2Cl2 (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 florasil. After removal of the solvent, the residue was treated with IPE and H2O. The separated organic layer was dried (MgSO4). 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, v): 2958 (s), 2933 (s), 2872 (s), 2819 (m), 2719 (m), 1727 (s), 1469 (m), 1414 (w), 1387 (m), 1368 (m), 1321 (w), 1263 (w), 1160 (m), 1126 (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 Et2O (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. H2SO4 (100 ml) was added slowly with an ice bath. The separated organic layer was rinsed with sat. aq. NaHCO3 and brine, and then dried (MgSO4). 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%). \(^\text{1}H\) NMR (400 MHz, CDCl\(_3\)) \(\delta\) 4.17 (2H, q, \(J=6.8\) Hz, \(\text{OCH}_2\text{CH}_3\)), 3.97 (1H, m, \(\text{CHOH}\)), 2.97 (1H, d, \(J=3.9\) Hz, \(\text{OCH}_2\)), 2.53 (1H, dd, \(J=2.9, 17\) Hz, \(\text{CH}_2\text{CO}_2\)), 2.40 (1H, dd, \(J=9.3, -16\) Hz, \(\text{CH}_2\text{CO}_2\)), 1.53 (3H, br s, \(\text{C}_4\text{-H} + \text{C}_6\text{-H}\)), 1.30 (2H, brs, \(\text{C}_5\text{-H}\)), 1.28 (3H, t, \(J=7.0\) Hz, \(\text{OCH}_2\text{CH}_3\)), 0.893 (3H, d, \(J=6.4\) Hz), 0.897 (3H, d, \(J=6.8\) Hz). IR (Film, v): 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\(_2\)O (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, v): 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\(_2\)O (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\(_2\)O and IPE. The separated aqueous layer was neutralized (pH 3) with dil HC\(_1\) and extracted with IPE three times. The combined organic layers were rinsed with H\(_2\)O and brine, and then dried (MgSO\(_4\)). Removal of the solvent gave the product 42 (5.20 g, 80%). IR (Film, v): 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\(_2\)O (100 ml). The separated aqueous layer was extracted with IPE. The combined organic layers were rinsed with H\(_2\)O 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, v): 3064 (w), 3033 (w), 2952 (s), 2869 (m), 1793 (s), 1737 (s), 1700 (s), 1498 (w), 1456 (m), 1393 (m), 1384 (m), 1286 (m), 1260 (m), 1167 (m), 1132 (m), 1117 (m), 1077 (m), 1026 (s), 988 (m), 870 (w), 750 (m), 698 (m) cm\(^{-1}\).

Benzyl 6-Methyl-3-[Fmoc-Gln(Mbh)-Leu-DLeu-Val-Asp(OtBu)-DLeu-Ile-O]-heptanoate (45)

\(^{1}H\) NMR (400 MHz, CD\(_3\)OD) \(\delta\) 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 6-Methyl-3-[Fmoc-Gln(Mbh)-Leu-DLeu-Val-Asp(OtBu)-DLeu-Ile-O]-octanoate (46)

\(^{1}H\) NMR (400 MHz, CD\(_3\)OD) \(\delta\) 7.68–7.80 (2H, m), 7.53–7.62 (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 6-Methyl-3-[Fmoc-Gln(Mbh)-Leu-DLeu-Val-Asp(OtBu)-DLeu-Ile-O]-hexadecanoate (47)

\(^{1}H\) NMR (400 MHz, CD\(_3\)OD) \(\delta\) 7.69–7.79 (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).
8.8 Hz), 6.84 (2H, d, J = 8.8 Hz), 6.83 (2H, d, J = 8.3 Hz), 6.10 (1H, s), 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-[Glenn(Mbh)-Leu-D-Leu-Val-Asp(OtBu)-D-Leu-Ile-O]-hexadecanoyl] (50)

To a solution of the depsipeptide (47) (1.28g, 0.77mmol) was added NEt2H (1.5ml) and this was stirred at room temperature for 3 hours. After removal of the solvent, the residue was purified by chromatography on silica gel (20g), eluting with CHCl3 : 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 (0.08g) in MeOH (25ml) was shaken at H2 atmosphere for 4 hours. After filtration, the solvent was removed. The residue was dissolved with 4-methylmorpholine (0.13ml, 1.14mmol, 2 eq) and HOBt-H2O (0.35g, 2.28mmol, 4 eq) in THF (130ml). This was added dropwise to a suspension of CsCl (0.96 g, 5.69 mmol, 10 eq), KCl (0.38g, 5.13mmol, 9 eq) and WSCI-HCl (0.98g, 5.13mmol, 9 eq) in a mixture of THF (260ml) and DMF (130ml). This reaction mixture was stirred at room temperature for 11 days. After dilution with EtOAc (150ml), this was rinsed with H2O, 5% NaHCO3, H2O, 10% citric acid and H2O, and then dried (Na2SO4). After removal of the solvent, the residue was purified by chromatography on silica gel (30 g), eluting with ^-hexane : EtOAc=200:0~6, to yield the product which was solidified with Et2O-rc-hexane (0.60g, 79%). *H NMR (400MHz, CD3OD) 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), 4.04~4.11 (1H, m), 3.77, 3.76 (6H, s), 2.64~2.91 (2H, m), 2.51~2.59 (1H, m), 2.15~2.43 (3H, m), 1.81~2.04 (4H, m), 1.51~1.78 (11H, 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]-hexanoyl] (14)

To a solution of Z-Gln(Mbh)-Leu-OtBu (52) (1.56g, 3.07mmol) and Leu-OtBu HCl (0.82g, 3.67mmol, 1.2 eq) in CH2C12 (20ml) were added TEA (0.47ml, 3.38mmol, 1.1 eq), WSCI-HCl (0.65g, 3.38mmol, 1.1 eq) and HOBt-H2O (0.46g, 3.38mmol, 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 CHCl3 and H2O. The separated organic layer was rinsed with sat. NaHCO3, H2O, 10% citric acid and H2O, and then dried (Na2SO4). Removal of the solvent gave the product (2.06g, 98%). *H NMR (400 MHz, CDCl3) δ 7.31~7.34 (5H, m), 7.13~7.22 (5H, m), 6.83~6.86 (4H,
Fmoc-Leu\textsuperscript{-}DLeu\textsuperscript{-}OBzl (55)

\begin{align*}
\text{H NMR (400 MHz, CDCl}_3 \delta & 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).
\end{align*}

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

A suspension of 52 (0.68g, 1.00mmol) and 5% Pd-C (0.15g) in a mixture of MeOH (35ml) and CHC\textsubscript{13} (15ml) was shaken at H\textsubscript{2} atmosphere for 6 hours. After filtration, removal of the solvent gave the product. H NMR (400MHz, CDCl\textsubscript{3}) \( \delta \) 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-DLeu-OBzl (56)

To a solution of 55 (6.29g, 11.3mmol) in DMF (120ml) was added NEt\textsubscript{2}H (12 mL). This reaction mixture was stirred at room temperature for 3 hours. After removal of the solvent, Fmoc-Gln(Mbh) (6.72g, 11.3mmol) and HOBt-H\textsubscript{2}O (1.73g, 11.3mmol) were added and dissolved in a mixture of CH\textsubscript{2}C\textsubscript{12} (80ml) and DMF (30ml). To this was addedWSCl2-HCl (2.17g, 11.3mmol) 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 CH\textsubscript{2}C\textsubscript{12} and 10% citric acid. The separated organic layer was rinsed with H\textsubscript{2}O, 5% NaHCO\textsubscript{3} and H\textsubscript{2}O, and then dried (Na\textsubscript{2}SO\textsubscript{4}). After removal of the solvent, the residue was crystallized from Et\textsubscript{2}O-CHCl\textsubscript{3} to yield the product (8.09g, 79%). H NMR (400MHz, CDCl\textsubscript{3}) \( \delta \) 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 (11H, 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)+.

Fmoc-Val-Asp(OtBu)-DLeu-Ile-O-tetradecanoic Acid (58)

\begin{align*}
\text{H NMR (400 MHz, CDCl}_3 \delta & 7.76 (2H, d, J=7.3 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 (11H, m), \\
& 5.13-5.26 (1H, m), 4.86-4.99 (11H, 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).
\end{align*}
**N-α-[3-(Fmoc-Val-Asp-DLeu-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 CHCl₃ (5 ml) and H₂O (5 ml). To this was added 5% NaHCO₃ to pH 8 and dilute HCl was added to pH 1−2. To this was added CHCl₃ and MeOH to make this solution. This solution was rinsed with H₂O and then dried (MgSO₄). After removal of the solvent, the residue was solidified from CHCl₃-Et₂O-^-hexane. The resulting solids were collected and dried to yield the product (0.61 g, 91%). ¹H NMR (400 MHz, CDCl₃+CD₃OD) 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)-DLeu-Ile-O]-tetradecanoyl]-Gln(Mbh)-Leu-DLeu-OBzl (60)**

To a solution of 57 (1.10 mmol, 1.1 eq), 58 (0.96 g, 1.00 mmol) and HOBt-H₂O (0.15 g, 1.00 mmol) in CH₂Cl₂ (10 ml) was added WSCI-\(\text{HCl}\) (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 CHCl₃ and H₂O. The separated organic layer was rinsed with 5% NaHCO₃, H₂O, 10% citric acid and brine, and then dried (Na₂SO₄). After removal of the solvent, the residue was purified by chromatography on silica gel (60 g), eluting with CHCl₃ : MeOH=100:0−3.5, to yield the product which was purified by rechromatography on silica gel (45 g), eluting with CHCl₃:EtOAc=100:0−40, to yield the product (0.75 g, 46%). ¹H NMR (400 MHz, CDCl₃) 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-\(\text{DLeu-Ile-O}\))−tetradecanoyl]-Gln-Leu-\(\text{OBzl}\) (60)**

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 H₂ atmosphere (2.0−2.5 kg/cm²) 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 CHCl₃ (10 ml) and H₂O (10 ml). To this was added 5% NaHCO₃ to pH 8 and dil HCl was added to pH 1−2. To this was added Et₂O and n-hexane. The formed solid was collected and dissolved in CHCl₃ and MeOH. To this was added Et₂O and n-hexane and the formed solid was collected. This was purified by chromatography on silica gel (20 g), eluting with CHCl₃:EtOAc=100:0−20, to yield the product which was purified by rechromatography on silica gel (16 g), eluting with CHCl₃:MeOH=100:5−10, to yield the product (0.18 g, 31%). ¹H NMR (400 MHz, DMSO-d₆) 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 1284.7505 [calcd for C₆₇H₁₀₄N₈O₁₅ (M+Na)+; 1283.7524].

**References**


