Solid-Phase Synthesis of Cyclooctadepsipeptide N-4909 Using a Cyclization-Cleavage Method with Oxime Resin

Toshio Suguro1 and Makoto Yanai1

1st Pharmaceutical Laboratory, Pharmaceutical Research Laboratories, Nisshin Fleur Milling Co., Ltd., 5-3-1 Tsurugaoka, Ohi-machi, Iruma-gun, Saitama 356-8511, Japan
2Research Laboratories, Nisshin Kyorin Pharmaceutical Co., Ltd., 5-3-1 Tsurugaoka, Ohi-machi, Iruma-gun, Saitama 356-8511, Japan

(Received for publication May 25, 1999)

N-4909 (1), which has a stimulating activity on apolipoprotein E secretion in human hepatoma Hep G2 cells, was isolated from the culture broth of Bacillus sp. No. 4691 by Hiramoto et al.1) This compound (1) was also isolated from Bacillus sp. A1238 as an inhibitor of acyl-CoA; cholesterol acyltransferase by Hasumi et al.2) This cyclooctadepsipeptide (1) and its diastereomer were synthesized for the first time using solution phase chemistry by our group3). To develop a structure-activity relationship, we needed a library of analogs of this compound and decided to use a combinatorial technology. To prepare a combinatorial library of its analogs, we had to develop a more concise method than the previous solution phase chemistry because the latter required multiple steps and extended time. Lee4) developed a method to prepare cyclooctadepsipeptide PF1022A analogs using the oxime resin which was developed by Kaiser5) and used to make cyclic peptides6)7). Lee prepared a tetradepsipeptide in advance to avoid an intramolecular displacement.

We used his method to examine the possibility of creating the desired cyclic depsipeptide libraries. We chose optically active (R)-3-(N-Boc-isoleucinyl)oxy-13-methyl-tetradecanoic acid (4) as a starting material which was prepared by reacting Boc-Ile-OH with (R)-benzyl 3-hydroxy-13-methyltetradecanoate (2)3) and then deprotecting the benzyl group by hydrogenation. First, the compound (4) was coupled with the oxime resin using O-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU) to give oxime resin (R)-3-(N-Boc-isoleucinyl)oxy-13-methyl-tetradecanoate (5). This was deprotected with TFA/methylene chloride (CH2Cl2) and coupled with Boc-D-Leu-OH using HATU. This procedure was repeated until preparing oxime resin (R)-3-(N-Boc-Gln-Leu-D-Leu-Val-Asp(OBzl)-D-Leu-Ile)oxy-13-methyl-tetradecanoate (6). After deprotection of the Boc with TFA/CH2Cl2, this was cleaved along with cyclization using triethylamine and AcOH8) to yield cyclo{13-methyl-(R)-3-[Gln-Leu-D-Leu-Val-Asp(OtBu)-D-Leu-O]-tetradecanoate} (7) in 43% yield. According to the confirmation at each step by Kaiser test9), all reactions proceeded almost quantitatively. The cyclized depsipeptide (7) was treated with 5% Pd-C under H2 atmosphere to remove the benzyl protection group of Asp. There were no purification process until the last stage. The desired product (1) was 63% pure by HPLC analysis. The crude product was purified by HPLC to afford N-4909 (1) in 45% yield. ([α]D28o = −13.1° (c 1.26, CH3OH). [lit.1] [α]D28o = −11.2° (c 0.414, CH3OH)). The 1H NMR and other physical data were identical to those of natural product.

In conclusion, we have found that oxime resin was very useful for preparing N-4909 (1) concisely and this method would be taken to develop a combinatorial library of N-4909 analogs.

Experimental

General

Optical rotations were obtained on a JASCO DIP-370 digital polarimeter. 1H NMR spectra were recorded at
400 MHz on a JEOL JNM-EX400 spectrometer. ESI-MS spectra were obtained on a Micromass Quattro II instrument.

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

(R)-Benzyl 3-(N-Boc-isoleucinyl)oxy-13-methyl-tetradecanoate (3)

To a stirred and cooled (0°C) solution of (R)-benzyl 3-hydroxy-13-methyltetradecanoate (2) (1.59 g, 4.56 mmol), Boc-Ile-OH·H2O (1.20 g, 5.02 mmol, 1.1 eq) and 4-(dimethylamino)pyridine (39 mg, 0.32 mmol, 0.07 eq) in CH2Cl2 (30 ml) was added 1,3-dicyclohexylcarbodiimide (1.41 g, 6.84 mmol, 1.5 eq) at an ice cooled temperature. The mixture was stirred at 0°C for 2 hours and then at room temperature for 3 days. After filtration and evaporation, the residue was taken up to AcOEt and 10% aq. citric acid. The separated organic layer was rinsed with H2O, 5% NaHCO3 and H2O, and then dried over anhydrous Na2SO4. After removal of the solvent, the crude product was purified by chromatog. on silica gel (50 g), eluting with hex:AcOEt=200:0~25, to yield the product 2.14 g (84%).

\[[\alpha]_{D}^{25}+4.47^\circ\] (c 1.03, CHCl3).

1H NMR (400 MHz, CDCl3) δ 7.29~7.40 (5H, m, Ar-H), 5.27 (1H, quint., J=6.3 Hz, CH(CH2)2O), 5.12 (2H, s, CH2Ph), 4.99 (1H, d, J=8.8 Hz, CH(CH2)2O), 2.69 (1H, dd, J=6.8, 16 Hz, CH2CO2), 2.59 (1H, dd, J=5.9, 16 Hz, CH2CO2), 1.84 (1H, br s, CH2), 1.62 (2H, br s, CH2), 1.44 (9H, s), 1.04~1.15 (19H, m, CH2), 0.92 (3H, d, J=6.3 Hz, CH(CH3)2), 0.89 (3H, t, J=7.3 Hz, CH(CH3)2), 0.86 (6H, br s, CH2), 0.67 (6H, J=6.3 Hz, CH(CH3)2). ESI-MS m/z 562 (M+H)+.

(R)-3-(N-Boc-isoleucinyl)oxy-13-methyl-tetradecanoic acid (4)

A suspension of the benzyl ester (3) (2.10 g, 3.74 mmol) and 5% Pd-C (0.42 g) in MeOH (80 ml) was reacted under H2 atmosphere (~2 kg/cm2) at room temperature for 1 hour. After filtration and evaporation, the product was obtained 1.75 g (99%).

1H NMR (400 MHz, CDCl3) δ 5.25 (1H, quint., J=6.2 Hz, CH(CH3)2O), 5.02 (1H, d, J=8.3 Hz, CH2), 4.20 (1H, dd, J=4.9, 8.8 Hz, CH(NHCO)), 3.16 (1H, br s, CO2H), 2.67 (1H, dd, J=6.8, 16 Hz, CH2CO2), 2.61 (1H, dd, J=5.7, 16 Hz, CH2CO2).
Oxime Resin (R)-3-(N-Boc-isoleucinyl)oxy-13-methyl-tetradecanoate (5)

To a stirred (by Argon gas) suspension of 1.00 g (2.0 mequiv.) of oxime resin in CH$_2$Cl$_2$ (20 ml) was added (R)-3-(N-Boc-isoleucinyl)oxy-13-methyl-tetradecanoic acid (4) (472 mg, 1.00 mmol) and HATU (380 mg, 1.00 mmol) followed by addition of diisopropylethylamine (190 mg, 1.50 mmol) dropwise at room temperature. After stirring for 18 hours at room temperature, the suspension was filtered off and the resin was washed twice with CH$_2$Cl$_2$ (20 ml), twice with 20 ml of CH$_2$Cl$_2$:ethyl alcohol (1:1) solution and twice with CH$_2$Cl$_2$ (20 ml), and dried under reduced pressure. The weight of the resin was increased about 450 mg which showed the reaction was proceeded quantitatively.

Oxime Resin (R)-3-(N-Boc-Gln-Leu-D-Leu-Val-Asp(OBzl)-D-Leu-Ile)oxy-13-methyl-tetradecanoate (6)

The resin (0.5 mmol) was suspended in 25% TFA in CH$_2$Cl$_2$ solution (20 ml). The mixture was stirred for 30 minutes at room temperature and filtered off. The resin was washed twice with CH$_2$Cl$_2$ (20 ml), isopropyl alcohol (20 ml), 4 times with CH$_2$Cl$_2$ (20 ml) and then dried under reduced pressure.

To a stirred (by Argon gas) suspension of the obtained resin in DMF (20 ml) was added Boc-D-Leu-OH·H$_2$O (374 mg, 1.50 mmol) and HATU (380 mg, 1.00 mmol) followed by addition of diisopropylethylamine (420 mg, 3.25 mmol) dropwise at room temperature. After stirring for 40 minutes at room temperature, the suspension was filtered off and the resin was washed 4 times with DMF (20 ml) and twice with CH$_2$Cl$_2$ (20 ml), and dried under reduced pressure. The small portion of resin was tested by Kaiser reagent to see the reaction was completed.

This procedure was repeated using Boc-Asp(OBzl)-OH, Boc-Val-OH, Boc-D-Leu-OH, Boc-Leu-OH, Boc-Gln-OH instead of Boc-D-Leu-OH to yield oxime resin (R)-3-(N-Boc-Gln-Leu-D-Leu-Val-Asp(OBzl)-D-Leu-Ile)oxy-13-methyl-tetradecanoate (6) (0.5 mmol) in DMF (20 ml) was added AcOH (60 μl, 1.00 mmol) and triethylamine (139 μl, 1.00 mmol). After stirring for 24 hours at room temperature, the suspension was filtered off and the resin was washed with DMF (20 ml). The combined organic solution was evaporated. The residue was treated with AcOEt and H$_2$O. The separated organic layer was rinsed with H$_2$O and dried over anhydrous MgSO$_4$. Removal of the solvent gave the product (7) (240 mg, 42.6% from 3). ESI-MS m/z 1126 (M+H)$^+$. N-4909 (1)

A suspension of the benzyl ester (7) (240 mg, 0.21 mmol) and 10% Pd-C (60 mg) in EtOH (30 ml) was reacted under H$_2$ atmosphere (~1 kg/cm$^2$) at room temperature for 3 hours. After filtration and evaporation, the crude product was obtained. This had 63% purity by HPLC analysis (column, PEGASIL ODS (4.6×250 mm); mobile phase, MeOH:H$_2$O=95:5; flow rate, 1.0 ml/min; detection, UV 222 nm). This was purified by HPLC under the following conditions (column, Inertsil PREP-ODS (30×250 mm); mobile phase, MeOH:H$_2$O:TFA=93:7:0.05; flow rate, 30 ml/min; detection, UV 222 nm) to yield the product (126 mg, 58.1%).

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[a]_D^{26} = -13.1° ~ (c ~ 1.26, CH$_3$OH), \text{[lit.]} [a]_D^{26} = -11.2° ~ (c ~ 0.414, CH$_3$OH). \]

1H NMR (400 MHz, CDC$_3$) δ 12.30 (1H, br s), 8.37 (1H, d, J = 6.8 Hz), 8.30 (1H, d, J = 7.8 Hz), 8.11 (1H, d, J = 7.3 Hz), 7.79~7.96 (4H, m), 7.30 (1H, s), 6.86 (1H, s), 4.90~5.02 (1H, m), 4.43~4.67 (2H, m), 4.03~4.29 (5H, m), 2.73 (1H, d, J = 11.2 Hz), 2.65 (1H, d, J = 7.3 Hz), 2.25~2.44 (2H, m), 1.95~2.15 (3H, m), 1.67~1.93 (3H, m), 1.07~1.63 (29H, m), 0.61~0.93 (36H, m). High-resolution FAB-MS (positive) m/z 1035.7100 [calcd for C$_{53}$H$_{94}$N$_8$O$_{12}$ (M+H)$^+$; 1035.7069]

References

4) LEE, BYUNG H.: Solid-phase synthesis of cyclooctadecapeptide PF1022A analogs using a cyclization-cleavage method with oxime resin. Tetrahedron Letters 38:


