VOL. 52 NO. 9, SEPT. 1999 THE JOURNAL OF ANTIBIOTICS pp. 835 — 838

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

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(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 group[3].

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. LEE[4] developed a method to prepare cyclooctadepsipeptide PF1022A analogs using the oxime resin which was developed by KAISER[5] and used to make cyclic peptides[6,7]. LEE prepared a tetradepsipeptide in advance to avoid an intramolecular displacement.

We used his method to examine the posibility 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 (CH_2Cl_2) 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/CH_2Cl_2, this was cleaved along with cyclization using triethylamine and AcOH[8] 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 test[9], all reactions proceeded almost quantitatively. The cyclized depsipeptide (**7**) was treated with 5% Pd-C under H_2 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. ($[\alpha]_D^{26}$ −13.1° (*c* 1.26, CH_3OH). [lit.[1] $[\alpha]_D^{26}$ −11.2° (*c* 0.414, CH_3OH)]) The ^1H NMR and other physical data were identical to those of natural product.

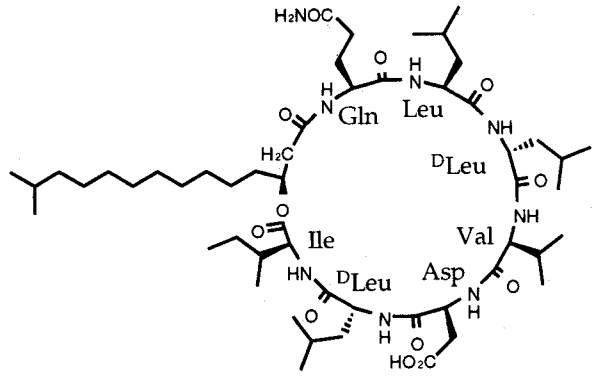
In conclusion, we have found that oxime resin was very useful for preparing N-4909 (**1**) concisely and this method would be taken to develop a combinatorial library of N-4909 analogs.

Experimental

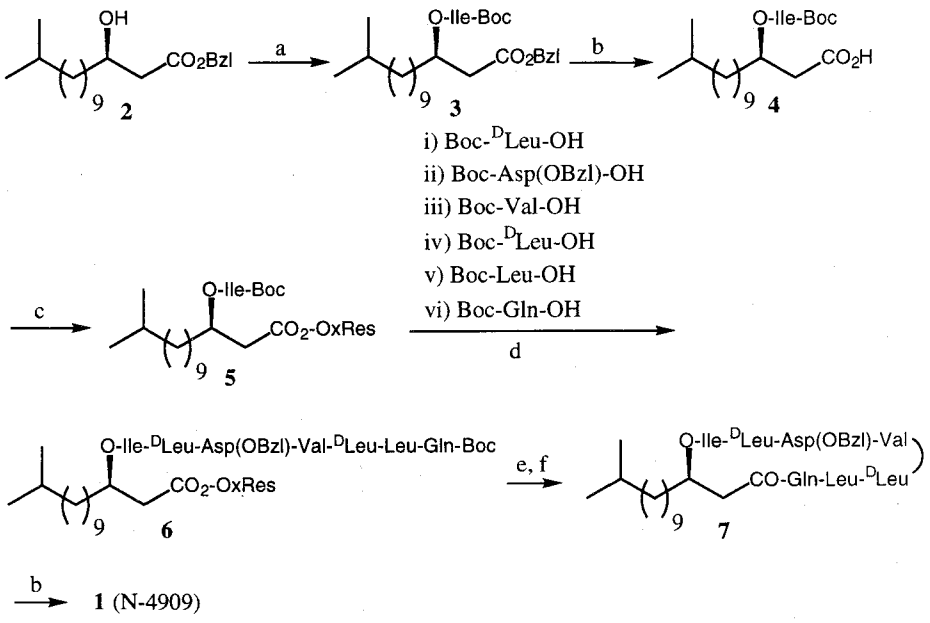
General

Optical rotations were obtained on a JASCO DIP-370 digital polarimeter. ^1H NMR spectra were recorded at

Fig. 1. Structure of N-4909 **1**.



Scheme 1. Synthesis of N-4909 using oxime resin.



a) Boc-Ile-OH, DCC, DMAP, CH_2Cl_2, 84 %; b) H_2, 5% Pd-C, MeOH, 99 %; c) Oxime Resin, HATU, DIEA; d) 1) 25 % TFA, CH_2Cl_2, 2) amino acid, HATU, DMF; e) 25 % TFA, CH_2Cl_2; TEA, AcOH:

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 · 1/2H_2O (1.20 g, 5.02 mmol, 1.1 eq) and 4-(dimethylamino)pyridine (39 mg, 0.32 mmol, 0.07 eq) in CH_2Cl_2 (30 ml) was added 1,3-dicyclohexylcarbodiimide (1.41 g, 6.84 mmol, 1.5 eq) at an ice cooled temperatre. 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 H_2O, 5% aq. $NaHCO_3$ and H_2O, and then dried over anhydrous Na_2SO_4. 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° (*c* 1.03, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ 7.29~7.40 (5H, m, Ar-*H*), 5.27 (1H, quint., *J*=6.3 Hz, C*H*$(CH_2)_2O$), 5.12 (2H, s, CH_2Ph), 4.99 (1H, d, *J*=8.8 Hz, N*H*), 4.22 (1H, dd, *J*=4.4, 8.8 Hz, C*H*NHCO), 2.69 (1H, dd, *J*=6.8, 16 Hz, CH_2CO_2), 2.59 (1H, dd, *J*=5.9, 16 Hz, CH_2CO_2), 1.84 (1H, br s, C*H*$(CH_3)_2$), 1.62 (2H, br s, CH_2), 1.44 (9H, s), 1.04~1.51 (19H, m, CH_2), 0.92 (3H, d, *J*=6.3 Hz, $CH(CH_3)_2$), 0.89 (3H, t, *J*=7.3 Hz, $CH(CH_3)_2$), 0.86 (6H, d, *J*=6.3 Hz, $CH(CH_3)_2$). 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 H_2 atomosphere (~2 kg/cm^2) at room temperature for 1 hour. After filtration and evaporation, the product was obtained 1.75 g (99 %).

^1H NMR (400 MHz, $CDCl_3$) δ 5.25 (1H, quint., *J*=6.2 Hz, C*H*$(CH_2)_2O$), 5.02 (1H, d, *J*=8.3 Hz, N*H*), 4.20 (1H, dd, *J*=4.9, 8.8 Hz, C*H*NHCO), 3.16 (1H, br s, CO_2H), 2.67 (1H, dd, *J*=6.8, 16 Hz, CH_2CO_2), 2.61 (1H, dd, *J*=5.7,

16 Hz, CH_2CO_2), 1.85 (1H, br s, $CH(CH_3)_2$), 1.57~1.75 (2H, m, CH_2), 1.44 (9H, s), 1.06~1.55 (19H, m, CH_2), 0.94 (3H, d, J=7.3 Hz, $CH(CH_3)_2$), 0.91 (3H, t, J=7.8 Hz, $CH(CH_3)_2$), 0.86 (6H, d, J=6.3 Hz, $CH(CH_3)_2$). ESI-MS m/z 472 $(M+H)^+$.

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_2Cl_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, 1.5 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_2Cl_2 (20 ml), twice with 20 ml of CH_2Cl_2 : ethyl alcohol (1 : 1) solution and twice with CH_2Cl_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_2Cl_2 solution (20 ml). The mixture was stirred for 30 minutes at room temperature and filtered off. The resin was washed twice with CH_2Cl_2 (20 ml), isopropyl alcohol (20 ml), 4 times with CH_2Cl_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_2O (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_2Cl_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**).

Cyclo{13-methyl-(*R*)-3-[Gln-Leu-D-Leu-Val-Asp(OtBu)-D-Leu-O]-tetradecanoate} (**7**)

To a stirred (by Argon gas) suspension of 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_2O. The separated organic layer was rinsed with H_2O 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 atomosphere (~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_2O=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_2O : TFA=93 : 7 : 0.05; flow rate, 30 ml/min; detection, UV 222 nm) to yield the product (126 mg, 58.1%).

$[\alpha]_D^{26}$ −13.1° (*c* 1.26, CH_3OH). [lit.[1]) $[\alpha]_D^{26}$ −11.2° (*c* 0.414, CH_3OH)]. ^1H NMR (400 MHz, $CDCl_3$) 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 $C_{53}H_{94}N_8O_{12}$ $(M+H)^+$; 1035.7069]

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