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Synthesis of Fellutamide C and its Diastereomer

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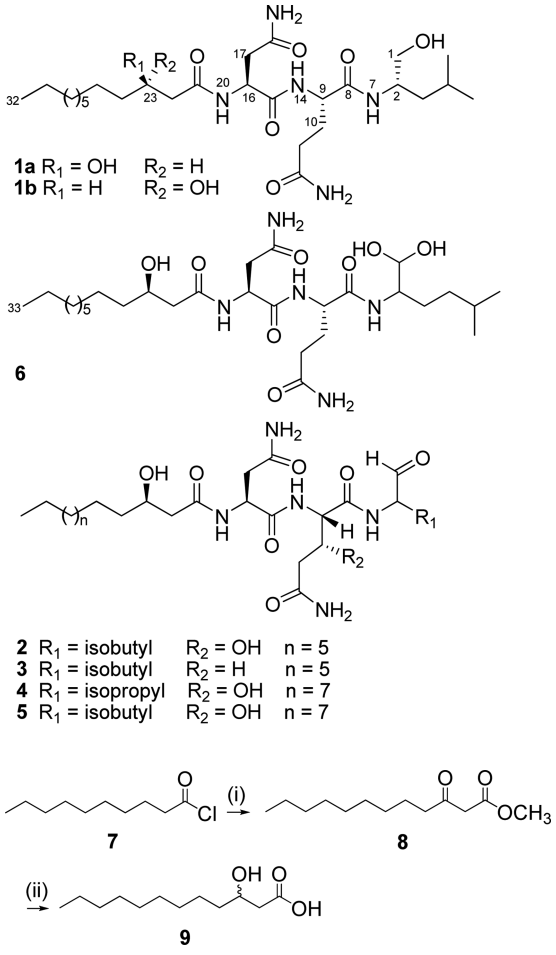
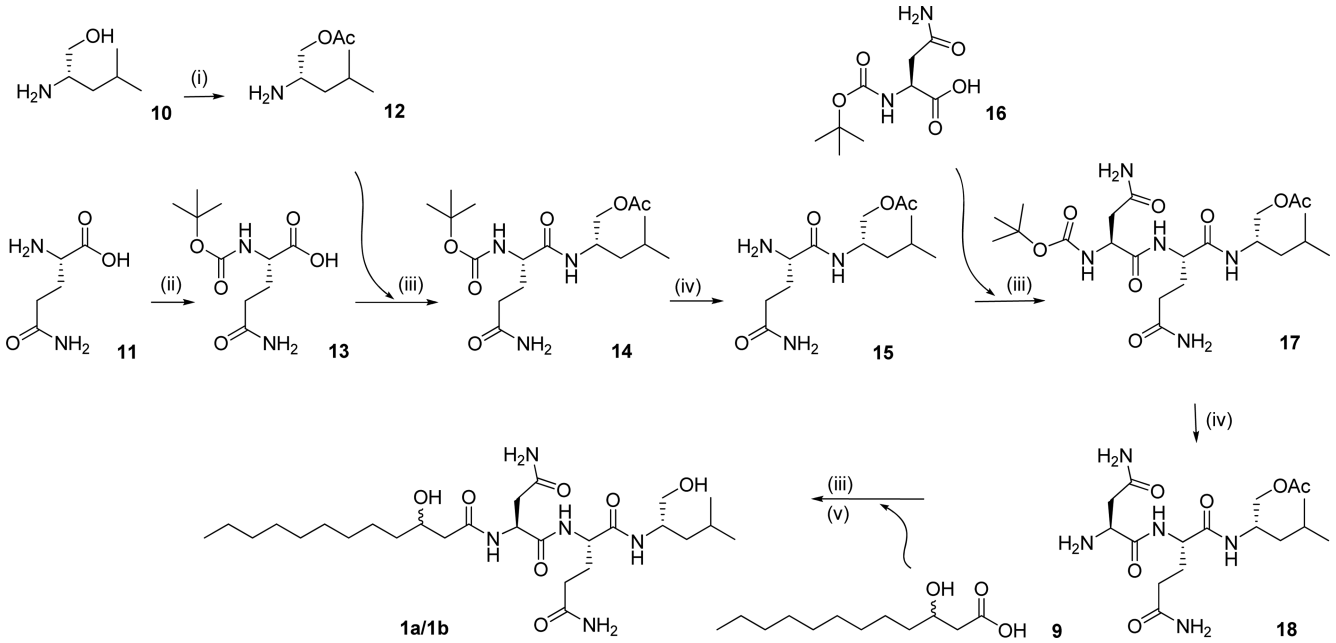
In our previous study on cytotoxic constituents from the sponge-derived fungus *Aspergillus versicolor*, a cytotoxic lipopeptide named fellutamide C (**1a**) was isolated and defined.[1] This lipopeptide exhibited interesting cytotoxic effects against the human solid cancer cell lines SK-MEL-2, XF498, and HCT15. Fellutamide C is structurally related to fellutamide A (**2**) and B (**3**), which were first isolated from the fish-derived fungus *Penicillium fellutanum*,[2] and subsequently, this class of lipopeptides were found to have several interesting biological activities.[2-6] Recently, fellutamides **4** and **5** with different β-hydroxy aliphatic tail (C_{14}) and terminal unit (valinal in **4**) were isolated from a fungus referred to as *Metulocladosporiella* (not otherwise described).[7] Another congener fellutamide F (**6**) was isolated from the fungus *A. versicolor*, which also produces fellutamide C (**1a**). Furthermore, fellutamide F was found to be cytotoxic to XF498 cells (a human CNS cancer cell line) and HCT15 cells (a human colon cancer cell line) and to have potencies comparable to those of doxorubicin.[8]

Our interest in bioactive sponge lipopeptides prompted us to establish a simple synthetic pathway for fellutamide C and for its analogs in order to explore their biological activities and structure-activity relationships. To establish a simple, synthetic route, we used a classical solution phase method based on the efficient and well-established Boc-chemistry[9] rather than the more expensive Fmoc-chemistry[10] to synthesize fellutamide B (compound **3**).[4]

Our synthetic strategy for compound **1** is summarized in Schemes 1 and 2. Scheme 1 describes the preparation of 3-hydroxy dodecanoic acid (3-HDA, **9**) in a racemic form. Previous studies on fellutamides have shown that 3*R*-hydroxy fatty acid is important for the biologic activities of fellutamides.[4,7] This type of fatty acid is also the most common fatty acid constituent found in the Lipid A, which an important lipophilic component of lipopolysaccharides in the cell surfaces of Gram-negative bacteria.[11] 3-Hydroxy fatty acids isolated from various natural sources have been found to display significant antimicrobial, antiviral and antitumor activities.[12-14] Therefore, the synthesis of pure enantiomers of this type of fatty acid has been the subject of several synthetic studies.[15-17] To synthesize fellutamide B, a pure enantiomer of 3-hydroxy dodecanoic acid was first prepared and coupled with a tripeptide moiety.[4] However, the synthesis of an enantiopure 3-hydroxy fatty acid required at least 6 steps. In the present study, to reduce the number of steps, a racemic 3-hydroxy fatty acid was employed, because we considered that the coupling of a racemic 3-hydroxy fatty acid with the tripeptide moiety of fellutamide C would produce two diastereomers that could be separated using a routine chromatographic method. Accordingly, the β-keto ester (**8**) was synthesized by condensing Meldrum's acid



Scheme 1. Synthesis of 3-hydroxydodecanoic acid (3-HDA)[a]. [a]Reagents and conditions: (i) Meldrum's acid, pyridine, CH_2Cl_2, 0 °C to rt, 2 h, then MeOH, reflux, 5 h, yield 60%; (ii) $NaBH_4$, MeOH, 0 °C to rt, 3 h, then NaOH, H_2O, 12 h, yield 70%.



Scheme 2. Synthesis of fellutamide C and its diastereomer[a]. [a]Reagents and conditions: (i) Acetyl chloride, 6 N HCl: acetic acid, yield 90%; (ii) NaOH/$(BOC)_2O$ (di-*tert*-butyl dicarbonate), 0 °C to rt, 4 h, yield 85%; (iii) HOBt, DIPEA, EDCI, DMF, 0 °C, 12 h, yield 50%; (iv) CF_3COOH: CH_2Cl_2, 30 min, yield 95%; (v) LiOH, H_2O/THF, rt, 12 h, yield 70%.

(2,2-dimethyl-1,3-dioxane-4,6-dione) with decanoyl chloride (**7**) and subsequent methanolysis, as previously described.[18] 3-Hydroxydodecanoic acid (3-HDA, **9**) was obtained by reducing the β-keto ester (**8**) with $NaBH_4$ in methanol and then hydrolyzing with aq. NaOH.

As shown in Scheme 2, the core peptide sequence was constructed by solution phase synthesis. First, the hydroxyl group of L-leucinol (Lol, **10**) was selectively protected by acetylation of leucinol with acetyl chloride in acetic acid.[19] The amine groups of the two amino acid moieties (L-glutamine and L-asparagine) were then protected with di-*tert*-butyl dicarbonate/$(Boc)_2O$, and the carbodiimide coupling of H-Lol-Ac (**12**) with Boc-Gln-OH (**13**) in DMF was then accomplished using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDCI) and 1-hydroxybenzotriazole (HOBt) in the presence of *N,N*-diisopropylethylamine (DIPEA). The *N*-*tert*-butyloxycarbonyl (Boc)-protecting group was then cleaved from the dipeptide Boc-Gln-Lol-Ac (**14**) with TFA in CH_2Cl_2. Subsequent coupling with Boc-Asn-OH and deprotection yielded the key tripeptide moiety H-Asn-Gln-Lol-Ac (**18**), which was coupled with 3-HDA (**9**), and then hydrolyzed with LiOH in THF/H_2O to give the target lipopeptide **1** as a two component diastereomeric mixture (**1a** and **1b**). These two diastereomers were readily separated by routine RP-HPLC (ODS eluted with a 50% CH_3CN and 0.01% TFA). The first diastereomer (**1a**) eluted was identified as (*R*)-(-)-3-HDA-Asn-Gln-Leu-OH, since its spectroscopic data were identical to those of natural fellutamide C. In addition, compound **1a** showed the same sign of optical rotation ($[\alpha]_D$ –112.0) as natural fellutamide C ($[\alpha]_D$ –128).[1] The second diastereomer eluted (**1b**) was unambiguously identified defined as (*S*)-(+)-3-HDA-Asn-Gln-Leu-OH, because the chemical shifts of protons around the chiral C-23 of the β-hydroxy aliphatic tail and protons of the asparagine moiety were distinct from those of natural fellutamide C. Furthermore, compound **1b** showed an opposite sign of optical rotation ($[\alpha]_D$ +105.0) compared to those of natural and synthetic fellutamide C ($[\alpha]_D$ –128 and –112.0, respectively).

Summarizing, we describe a simple and efficient means for synthesizing the natural fellutamide C. The two diastereomers **1a** and **1b** bearing (*R*)-(–)-3-HDA and (*S*)-(+)-3-HDA, respectively, were obtained using the described procedure, which is considerably less complicated and faster than those previously described.[4,15-17]

Experimental Section

General Procedures. Optical rotations were measured using a JASCO P-1020 digital polarimeter, and ^1H and ^{13}C NMR spectra were recorded on Varian Unity 400 MHz spectrometers. Chemical shifts are reported with reference to the respective residual solvent or deuterated solvent peak (δ_H 3.30 and δ_C 49.0 for CD_3OD, δ_H 2.50 and δ_C 39.5 for DMSO). FABMS data was obtained on a JEOL JMS SX-102A spectrometer. HPLC was performed on a YMC ODS-H80 column (250 × 10 mm, 4 μm, 80 Å) or a C18-5E Shodex packed column (250 × 10 mm, 5 μm, 100 Å) using a Shodex RI-71 detector. All chemical reagents were purchased from Sigma-Aldrich and used as received.

Methyl 3-Oxododecanoate (8): Lauroyl chloride (**7**, 1 equiv) was added to a solution of Meldrum's acid (2,2-dimethyl-1,3-dioxane-4,6-dione, 1 equiv) and pyridine (2 equiv) in CH_2Cl_2 at 0 °C over 10 min. The reaction mixture

was then allowed to warm to rt over 2 h whereupon the solution turned red and a white precipitate was formed. The solution was then washed with 1.0 M aq HCl and H_2O, filtered, concentrated, and dried in vacuo. The residue was re-dissolved in MeOH and the resultant solution was heated at reflux for 5 h. After being allowed to cool to rt, the solution was concentrated under vacuo to give a white semisolid. This was then recrystallized from MeOH at 20 °C to give the β-ketoester (**8**), a white semisolid; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.71 (3H, s, -OCH_3), 3.42 (2H, s, H-2), 2.50 (2H, t, J = 7.6 Hz, H-4), 1.57 (2H, m, H-5), 1.23 (12H, brs, -CH_2), 0.85 (3H, t, J = 6.8 Hz, H-12).

3-Hydroxydodecanoic Acid (9): To a solution of methyl 3-oxododecanoate (**8**) in methanol at 0 °C, $NaBH_4$ (1 equiv) was added and stirred at room temperature for 3 h. Subsequently, a solution of NaOH (1.2 equiv) in water was added and the mixture was stirred for 12 h at room temperature. The reaction was neutralized with 1 N HCl, extracted with EtOAc, washed with H_2O, dried over $MgSO_4$, and evaporated to give a crude product, which was purified by RP HPLC 80% to afford 3-hydroxydodecanoic acid (**9**). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.01 (1H, m, H-3), 2.56 (1H, dd, J = 16.8, 3.2 Hz, H-2b), 2.43 (1H, dd, J = 16.8, 9.2 Hz, H-2a), 1.54-1.24 (14H, m, -CH_2), 0.85 (3H, t, J = 6.8 Hz, H-12).

H-Lol-Ac (12): Leucinol (**10**) was dissolved in a solution of 6 N HCl and acetic acid (1:1), and cooled to 0 °C in an ice bath. Excess acetyl chloride was then added slowly to the mixture and stirred for 30 minute. After evaporation under vacuo the product was obtained as a white semisolid, which was used for the next reaction without purification. White semisolid; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.35 (1H, dd, J = 16.4, 3.2 Hz, H-1b), 4.15 (1H, dd, J = 16.4, 7.2 Hz, H-1a), 3.55 (1H, m, H-2), 2.10 (3H, s, -CO$\underline{CH_3}$), 1.75 (1H, m, H-4), 1.44 (1H, m, H-3b), 1.28 (1H, m, H-3a), 0.95 (3H, d, J = 6.8 Hz, -CH_3), 0.92 (3H, d, J = 6.8 Hz, -CH_3).

Boc-Gln-OH (13): A solution of di-*tert*-butyl dicarbonate ($(Boc)_2O$, 1.5 equiv) in dioxane was added to an ice-cold solution of L-glutamine (**11**, 1 equiv) in 1 N NaOH. The mixture was stirred at 0 °C for 30 min, allowed to warm to room temperature over 4 h, concentrated to a half of its original volume by rotary evaporation, cooled in an ice-water bath, acidified to pH 2-3 by the slow addition of 1 N $KHSO_4$, and then extracted with ethyl acetate. Combined extracts were dried over $MgSO_4$ and concentrated under reduce pressure to give Boc-Gln-OH (**13**) as a colorless semisolid in 85% yield. ^{1}H NMR (DMSO, 400 MHz) δ 7.25 (1H, brs, $CONH_2$), 7.05 (1H, d, J = 3.4 Hz, Boc-$\underline{NH}$), 6.70 (1H, brs, $CONH_2$), 3.80 (1H, m, H-2), 2.10 (2H, t, J = 7.2 Hz, H-4), 1.90 (1H, m, H-3b), 1.65 (1H, m, H-3a), 1.31 (9H, s, -CH_3).

Boc-Gln-Lol-Ac (14): To a mixture of H-Lol-Ac (**12**, 1 equiv), Boc-Gln-OH (**13**, 1 equiv), DIPEA (*N,N*-diisopropylethylamine, 3 equiv), and HOBt (*N*-hydroxybenzotriazole, 1.5 equiv) in CH_2Cl_2 at 0 °C was added EDCI (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide, 1.5 equiv), and stirred at the same temperature for 12 h. After working up according to the standard method, the mixture was purified by RP-HPLC (YMC ODS-H80) using 80% aqueous CH_3CN as eluant to give the dipeptide **14**. Colorless oil; ^{1}H NMR (CD_3OD, 400 MHz) δ 4.23-3.88 (5H, m, H-1, H-2, and H-9), 2.28 (2H, t, J = 7.2 Hz, H-11), 2.02 (3H, s, -$COCH_3$), 1.97 (2H, m, H-10), 1.83 (1H, m, H-4), 1.67 (1H, m, H-3b), 1.43 (9H, s, -OC($\underline{CH_3}$)$_3$), 1.27 (1H, m, H-3a), 0.92 (3H, t, J = 6.8 Hz, H-5), 0.89 (3H, t, J = 6.8 Hz, H-6).

H-Gln-Lol-Ac (15): Boc-Gln-Lol-Ac (**14**) was dissolved in a mixture of CH_2Cl_2 and trifluoroacetic acid (1:1). The reaction mix was maintained at room temperature for 1 h and then evaporated under vacuo to give dipeptide **15**, which was used for the next reaction without purification. Colorless oil; ^{1}H NMR (CD_3OD, 400 MHz) δ 4.24 (1H, m, H-9), 3.88 (3H, m, H1 and H-2), 2.45 (2H, t, J = 7.2 Hz, H-11), 2.03 (3H, s, -$COCH_3$), 2.08 (2H, m, H-10), 1.67 (1H, m, H-4), 1.45 (1H, m, H-3b), 1.31 (1H, m, H-3a), 0.95 (3H, t, J = 6.8 Hz, H-5), 0.93 (3H, t, J = 6.8 Hz, H-6).

Boc-Asn-OH (16): The Boc-protected asparagine **16** was prepared from L-asparagine using the same procedure used to synthesize Boc-Gln-OH (**13**). ^{1}H NMR (DMSO, 400 MHz) δ 7.31 (1H, brs, $CONH_2$), 6.92 (1H, brs, $CONH_2$), 6.82 (1H, d, J = 3.2 Hz, Boc-$\underline{NH}$), 4.20 (1H, m, H-2), 2.42 (2H, m, H-3), 1.32 (9H, s, -CH_3).

Boc-Asn-Gln-Lol-Ac (17): The tripeptide **17** was prepared by coupling H-Gln-Lol-Ac (**15**) and Boc-Asn-OH (**16**) using the procedure used to prepare the dipeptide Boc-Gln-Lol-Ac (**14**). Colorless oil; ^{1}H NMR (CD_3OD, 400 MHz) δ 4.37-3.91 (6H, m, H-1, H-2, H-9 and H-16), 2.73 (1H, dd, J = 15.6, 6.0 Hz, H-17b), 2.62 (1H, dd, J = 15.6, 6.4 Hz H-17a), 2.31 (2H, t, J = 7.2 Hz, H-11), 2.15 (1H, m, H-10b), 2.02 (3H, s, -$COCH_3$), 1.92 (1H, m, H-10a), 1.64 (1H, m, H-4), 1.44 (9H, s, -OC($\underline{CH_3}$)$_3$), 1.37 (1H, m, H-3b), 1.25 (1H, m, H-3a), 0.93 (3H, t, J = 6.8 Hz, H-5), 0.88 (3H, t, J = 6.8 Hz, H-6).

H-Asn-Gln-Lol-Ac (18): The Boc-deprotected tripeptide **18** was obtained using the procedure used to prepare H-Gln-Lol-Ac (**15**) Colorless oil; ^{1}H NMR (CD_3OD, 400 MHz) δ 4.36-3.90 (6H, m, H-1, H-2, H-9 and H-16), 2.93 (1H, dd, J = 17.2, 5.2 Hz, H-17b), 2.62 (1H, dd, J = 17.2, 8.0 Hz H-17a), 2.34 (2H, t, J = 7.6 Hz, H-11), 2.10 (1H, m, H-10b), 2.02 (3H, s, -$COCH_3$), 1.93 (1H, m, H-10a), 1.67 (1H, m, H-4), 1.46 (1H, m, H-3b), 1.29 (1H, m, H-3a), 0.93 (3H, t, J = 8.0 Hz, H-5), 0.88 (3H, t, J = 6.8 Hz, H-6).

3-HDA-Asn-Gln-Lol-OH (1): The coupling of 3-HDA (**9**) with the Boc-deprotected tripeptide H-Asn-Gln-Lol-Ac (**18**) was performed using the procedure used to prepare the dipeptide Boc-Gln-Lol-Ac (**14**) and the tripeptide Boc-Asn-Gln-Lol-Ac (**17**). Subsequent hydrolysis of 3-HDA-Asn-Gln-Lol-Ac by LiOH (1.2 equiv) in THF/H_2O yielded compound **1** as a diastereomeric mixture, which was readily separated by RP HPLC using a 50% CH_3CN and 0.01% TFA mobile phase to give the pure diastereomers **1a** and **1b**.

Compound 1a – Colorless oil; $[\alpha]_D$ –112 (*c* 0.15, MeOH); ^{1}H NMR (400 MHz, CD_3OD) 0.88 (t, 3H, J = 7.0 Hz), 0.89 (d, 3H, J = 5.4 Hz), 0.91 (d, 3H, J = 5.2 Hz), 1.29 (br, 14H), 1.33 (m, 1H), 1.46 (m, 3H), 1.64 (m, 1H), 1.92

(m, 1H), 2.18 (m, 1H), 2.30 (m, 2H), 2.31 (m, 1H), 2.41 (dd, 1H, J = 11.2, 3.2 Hz), 2.73 (dd, 1H, J = 12.4, 4.8 Hz), 2.77 (dd, 1H, J = 12.4, 5.2 Hz), 3.45 (d, 2H, J = 4.4 Hz), 3.95 (m, 1H), 3.97 (m, 1H), 4.27 (dd, 1H, J = 11.2, 3.2 Hz), 4.63 (t, 1H, J = 7.2 Hz). FABMS (*m/z*) 580 $[M + Na]^+$.

Compound 1b – Colorless oil; $[\alpha]_D$ +105 (*c* 0.16, MeOH); ^{1}H NMR (400 MHz, CD_3OD) 0.89 (t, 3H, J = 7.2 Hz), 0.89 (d, 3H, J = 5.4 Hz), 0.92 (d, 3H, J = 5.6 Hz), 1.29 (br s, 14H), 1.34 (m, 1H), 1.47 (m, 3H), 1.65 (m, 1H), 1.91 (m, 1H), 2.19 (m, 1H), 2.30 (m, 2H), 2.32 (m, 1H), 2.47 (dd, 1H, J = 11.2, 3.2 Hz), 2.73 (dd, 1H, J = 12.8, 4.8 Hz), 2.81 (dd, 1H, J = 12.8, 5.2 Hz), 3.44 (dd, 2H, J = 4.4, 0.8 Hz), 3.96 (m, 1H), 3.98 (m, 1H), 4.25 (dd, 1H, J = 8.0, 3.2 Hz), 4.55 (t, 1H, J = 7.0 Hz). FABMS (*m/z*) 580 $[M + Na]^+$.

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