Journal of Peptide Science
J. Pept. Sci. 2008; **14**: 1139–1147
Published online 11 July 2008 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/psc.1051

JOURNAL OF PEPTIDE SCIENCE

Total synthesis and stereochemical reassignment of tasiamide

ZHEN-HUA MA, NI SONG, CHUN-XIA LI, WEI ZHANG, PENG WANG and YING-XIA LI*

Key laboratory of Marine Drugs, the Ministry of Education of China, School of Medicine and Pharmacy, Ocean University of China, Qingdao, 266003, China

Received 25 March 2008; Revised 6 May 2008; Accepted 7 May 2008

Abstract: The total synthesis of a marine acyclic peptide tasiamide and three diastereomers was reported for the first time. The synthesis has led to a reassignment of the N^{α}-Me-L-Gln of tasiamide to be N^{α}-Me-D-Gln, which was supported by ^{1}H NMR, ^{13}C NMR, COSY, HMQC, HMBC, and optical rotation. Copyright © 2008 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: tasiamide; synthesis; reassignment; diastereomer

INTRODUCTION

Marine cyanobacteria are well known to be a rich source of bioactive peptides and depsipeptides with pharmaceutical potential [1–3]. Among the cyanobateria, the vast majority of these extracts were isolated from the genus *Lyngbya*; however, for members of the genus *Symploca* (Oscillatoriaceae), very few reports of their chemistry or biological activity existed in the literature [4]. The potent antitumor agent dolastatin 10, originally isolated from the sea hare *Dolabella auricularia*, was again obtained from the marine cyanobacterium *Symploca* by Moore *et al.* [5]. From then on, the scientists turned their attention to the cyanobacterium *Symploca*, and a series of new pharmacologically interesting natural products were found one after another [5,6].

Tasiamide (**1**, Figure 1), an acyclic peptide isolated from the marine cyanobacterium *Symploca* sp. by Moore and co-workers in 2002, has been found to be cytotoxic against KB and LoVo cells with IC_{50} values of 0.48 and 3.47 μg/ml, respectively [7]. Shortly after this, another structural analog has also been isolated, namely, tasiamide B [6]. The structures of tasiamide and its congener were determined by a combination of NMR spectroscopy, MS, and chiral HPLC analysis of the chemical degradation. Structurally, both tasiamide and tasiamide B contain several structural features common among cyanobacterial peptides, including a hydroxy acid, two *N*-methylamides, and an ester [7]. Interestingly, they are both cytotoxic against KB cells with IC_{50} values of 0.48 and 0.8 μg/ml, respectively [6,7]. Attracted to the relationship between chemical structure and antitumor activity of the tasiamide family, herein, we detail the first total synthesis of tasiamide, together with a revision of its previously reported structure and assignment of the absolute stereochemistry.

*Correspondence to: Ying-Xia Li, Key laboratory of Marine Drugs, the Ministry of Education of China, School of Medicine and Pharmacy, Ocean University of China, Qingdao, 266003, China;
e-mail: liyx417@ouc.edu.cn

MATERIALS AND METHODS

General Information

Solvents were purified by standard methods. TLC was carried out on Merck 60 F_{254} silica gel plates and visualized by UV irradiation. Flash column chromatography was performed on silica gel (200–300 mesh, Qingdao, China). Optical rotations were obtained using a JASCO P-1020 digital polarimeter. NMR spectra were recorded on JEOL JNM-ECP 600 MHz spectrometer. Chemical shifts are reported in parts per million, relative to the signals due to the solvents. Data are described as the following: chemical shift, multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; br, broad; m, multiplet), coupling constants (Hz), integration, and assignment. Mass spectra were obtained on a Q-Tof Ultima Global mass spectrometer.

N^{α}-Boc-N^{α}-Me-D-Phe-L-Pro-OMe (5). A solution of L-Proline methyl ester (**6**) (644.3 mg, 3.89 mmol) and N^{α}-Boc-N^{α}-Me-D-phenylalanine (**7**) (1.09 g, 3.89 mmol) in anhydrous CH_2Cl_2 (20 ml) was treated sequentially with HOAt (634.2 mg, 4.66 mmol), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDCI) (894.4 mg, 4.66 mmol), and $NaHCO_3$ (391.4 mg, 4.66 mmol) at 0 °C. The reaction mixture was warmed to room temperature and stirred overnight. After diluted with EtOAc (100 ml), the whole mixture was washed with 10% citric acid (2 × 10 ml), 5% $NaHCO_3$ (2 × 10 ml), H_2O (2 × 10 ml), and brine (2 × 10 ml); dried over Na_2SO_4; and concentrated *in vacuo*. The residue was purified by flash column chromatography with petroleum ether–EtOAc (1 : 1) to afford **5** as a white solid (1.47 g, 96.7%). R_f 0.60 (1 : 1, petroleum ether–EtOAc); $[\alpha]^{21}_{D} = +10.1°$ (*c* 0.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ: 7.16–7.29 (m, 5H, Ar*H*), 5.17 (t, 1H, $J = 7.8$ Hz, α-C*H*-Phe), 4.87* (dd, 1H, $J = 9.4$, 5.3 Hz, α-C*H*-Phe), 4.47, 4.44* (dd, 1H, $J = 8.5$, 6.0 Hz, α-C*H*-Pro), 3.73, 3.70* (s, 3H, COOCH_3), 3.39–3.56 (m, 2H, δ-CH_2-Pro), 3.21, 3.12 (2 dd, 2H, $J = 13.7$, 7.3 Hz, β-CH_2-Phe), 2.90, 2.84* (s,

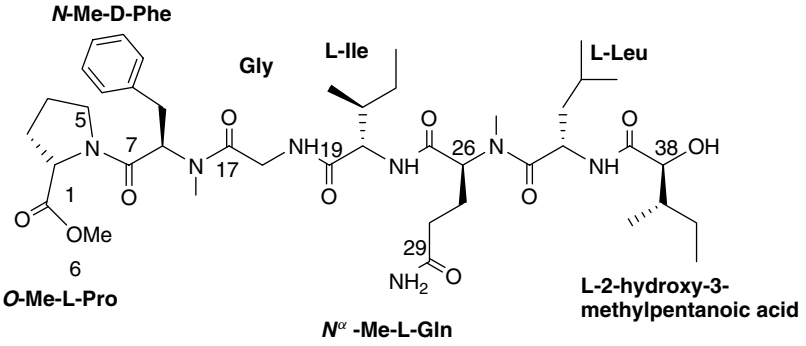


Figure 1 Chemical structure of tasiamide **1**.

1 : 1, 3H, *N*-CH_3), 2.93*, 2.87* (2 dd, 2H, $J = 14.0$, 5.8 Hz, β-CH_2-Phe), 1.84–2.22 (m, 4H, β-CH_2-Pro, γ-CH_2-Pro), 1.34, 1.26*, 1.20*, 1.17* (s, 9H, (CH_3)$_3$) (*as rotamer); ^{13}C NMR ($CDCl_3$) δ: 172.7, 172.3, 169.1, 168.8, 155.4, 154.7, 138.1, 137.7, 129.5, 128.3, 128.1, 126.3, 126.2, 80.0, 79.8, 59.5, 59.3, 59.0, 57.6, 52.1, 46.5, 46.2, 34.8, 29.6, 28.9, 28.2, 28.0, 25.2; ESIMS: calcd for $C_{21}H_{30}N_2O_5$ $[M + H]^+$ 391.2; found 391.3.

Fmoc-Gly-N$^{\alpha}$-Me-D-Phe-L-Pro-OMe (3). To a solution of **5** (1.16 g, 2.97 mmol) in EtOAc (10 ml) at 0 °C was added 4 M HCl/EtOAc (20 ml). The mixture was stirred for 1.5 h and then reconcentrated from CH_2Cl_2 two times to remove excess HCl. A solution of the HCl salt **5*** and Fmoc–glycine (1.06 g, 3.57 mmol) in anhydrous CH_2Cl_2 (40 ml) was treated sequentially with HOAt (485.9 mg, 3.57 mmol), EDCI (684.4 mg, 3.57 mmol), and $NaHCO_3$ (300 mg, 3.57 mmol). The reaction mixture was stirred at 0 °C for 30 min and for another 12 h at room temperature. The reaction mixture was treated as described for **5.** The residue was purified by flash column chromatography with petroleum ether–EtOAc (1 : 1) to afford **3** as a white solid (1.30 g, 76.5%). R_f 0.37 (1 : 1, petroleum ether–EtOAc); $[\alpha]^{21}{}_D = +16.6°$ (*c* 0.3, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ: 7.76 (d, 2H, $J = 7.3$ Hz, 4,5-C*H*-Fluorenyl), 7.60 (d, 2H, $J = 7.7$ Hz, 1,8-C*H*-Fluorenyl), 7.40 (t, 2H, $J = 7.5$ Hz, 3,6-C*H*-Fluorenyl), 7.31 (t, 2H, $J = 7.5$ Hz, 2,7-C*H*-Fluorenyl), 7.18–7.28 (m, 5H, Ar*H*), 5.68 (t, 1H, $J = 4.8$ Hz, N*H*-Gly), 5.60 (t, 1H $J = 7.5$ Hz, α-C*H*-Phe), 4.42 (dd, 1H, $J = 8.4$, 5.5 Hz, α-C*H*-Pro), 4.38 (t, 2H, $J = 6.8$ Hz, CH_2-Fluorenyl), 4.21 (t, 1H, $J = 7.3$ Hz, 9-C*H*-Fluorenyl), 4.09, 3.87 (2 dd, 2H, $J = 17.2$, 4.4 Hz, α-CH_2-Gly), 3.73 (s, 3H, COOCH_3), 3.34–3.43 (m, 2H, δ-CH_2-Pro), 3.29, 2.85 (2 dd, 2H, $J = 13.5$, 7.3 Hz, β-CH_2-Phe), 3.00 (s, 3H, *N*-CH_3), 1.80–2.19 (m, 4H, β-CH_2-Pro, γ-CH_2-Pro); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 172.4, 168.1, 167.9, 156.1, 143.8, 141.2, 136.9, 129.4, 128.3, 127.7, 127.0, 126.7, 125.1, 119.9, 67.0, 58.9, 56.3, 52.2, 47.1, 46.8, 42.6, 34.9, 29.6, 28.8, 25.0; ESIMS: calcd for $C_{33}H_{35}N_3O_6$ $[M + H]^+$ 570.3; found 570.3.

N$^{\alpha}$-Fmoc-N$^{\alpha}$-Me-L-Gln-L-Ile-OBn (8). A solution of L-isoleucine benzyl ester (**10**) (307.3 mg, 0.78 mmol) and *N*$^{\alpha}$-Fmoc-*N*$^{\alpha}$-Me-L-glutamine (**9**) (291.0 mg, 0.76 mmol) in anhydrous CH_2Cl_2 (20 ml) was treated sequentially with HOAt (127.0 mg, 0.94 mmol), EDCI (180.0 mg, 0.94 mmol), and NMM (173.1 µl, 1.56 mmol) at 0 °C. The reaction mixture was warmed to room temperature and stirred overnight. After diluting with EtOAc (100 ml), the whole mixture was washed with 10% citric acid (2 × 10 ml), 5% $NaHCO_3$ (2 × 10 ml), H_2O (2 × 10 ml), and brine (2 × 10 ml); dried over Na_2SO_4; and concentrated *in vacuo*. The residue was purified by flash column chromatography with EtOAc–petroleum ether (1 : 3) to afford **8** as a foam solid (445.0 mg, 96.8%). R_f 0.42 (EtOAc); $[\alpha]^{25}{}_D = -41.1°$ (*c* 0.4, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ: 7.77 (d, 2H, $J = 7.3$ Hz, 4,5-C*H*-Fluorenyl), 7.57 (d, 2H, $J = 7.3$ Hz, 1,8-C*H*-Fluorenyl), 7.40 (t, 2H, $J = 7.3$ Hz, 3,6-C*H*-Fluorenyl), 7.29–7.38 (m, 7H, 2,7-C*H*-Fluorenyl), 6.67 (d, 1H, $J = 7.8$ Hz, N*H*-Ile), 5.83, 5.44 (2 brs, 2H, NH_2-Gln), 5.19, 5.10 (2 d, 2H, $J = 12.4$ Hz, CH_2Ph), 4.67 (t, 1H, $J = 6.9$ Hz, α-C*H*-Gln), 4.55 (dd, 1H, $J = 6.9$, 4.1 Hz, α-C*H*-Ile), 4.40–4.48 (m, 2H, CH_2-Fluorenyl), 4.24 (t, 1H, $J = 6.4$ Hz, 9-C*H*-Fluorenyl), 2.85, 2.88*, 2.95* (s, 3H, *N*-CH_3), 1.99–2.26 (m, 4H, β-CH_2-Gln, γ-CH_2-Gln), 1.89 (brs, 1H, β-C*H*-Ile), 1.07–1.31 (m, 2H, γ-CH_2-Ile), 0.80–0.84 (m, 6H, γ-CH_3-Ile, δ-CH_3-Ile); ^{13}C NMR ($CDCl_3$) δ: 174.3, 171.6, 170.2, 157.3, 143.7, 141.3, 135.3, 128.6, 128.5, 128.4, 127.8, 124.9, 120.1, 68.0, 67.1, 58.0, 56.6, 47.2, 37.4, 32.0, 30.0, 25.0, 24.0, 15.6, 11.5; ESIMS: calcd for $C_{34}H_{39}N_3O_6$ $[M + H]^+$ 586.3; found 586.3.

Fmoc-L-Leu-N$^{\alpha}$-Me-L-Gln-L-Ile-OBn (4). To a solution of compound **8** (359.0 mg, 0.61 mmol) in CH_3CN (10 ml) at room temperature was added diethylamine (DEA) (10 ml). The reaction mixture was stirred for 6 h and then reconcentrated from CH_2Cl_2 two times to remove excess DEA. A solution of residue **8*** and Fmoc–leucine (260.1 mg, 0.74 mmol) in anhydrous CH_2Cl_2 (12 ml) was treated sequentially with EDCI (141.1 mg, 0.74 mmol) and HOAt (100.2 mg, 0.74 mmol). The reaction mixture was stirred at 0 °C for 30 min and for another 12 h at room temperature. The reaction mixture was treated as described for **8.** The residue was purified by flash column chromatography with EtOAc–petroleum ether (1 : 3) to afford **4** as a white foam solid (207.0 mg, 48.3%). R_f 0.20 (1 : 4, petroleum ether–EtOAc); $[\alpha]^{25}{}_D = -72.9°$ (*c* 0.4, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ: 7.76 (d, 2H, $J = 7.3$ Hz, 4,5-C*H*-Fluorenyl), 7.60 (d, 2H, $J = 6.2$ Hz, 1,8-C*H*-Fluorenyl), 7.40 (t, 2H, $J = 7.3$ Hz, 3,6-C*H*-Fluorenyl), 7.31–7.37 (m, 7H, 2,7-C*H*-Fluorenyl, Ar*H*), 6.77 (d, 1H, $J = 8.7$ Hz, N*H*-Ile), 5.91, 5.38 (2 brs, 2H, NH_2-Gln), 5.61 (d, 1H, $J = 8.7$ Hz, N*H*-Leu), 5.21, 5.11 (2 d, 2H, $J = 12.4$ Hz, CH_2Ph), 4.63 (td, 1H, $J = 9.4$, 3.2 Hz, α-C*H*-Leu), 4.56 (dd, 1H, $J = 8.7$, 5.0 Hz, α-C*H*-Ile), 4.31–4.42 (m, 2H, CH_2-Fluorenyl), 4.21 (t, 1H, $J = 7.3$ Hz, 9-C*H*-Fluorenyl), 3.02, 2.84* (s, 3H, *N*-CH_3), 2.00–2.24 (m, 4H, β-CH_2-Gln, γ-CH_2-Gln), 1.90 (brs, 1H, β-C*H*-Ile), 1.72–1.78 (m, 1H, γ-C*H*-Leu), 1.35–1.52 (m, 2H, β-CH_2-Leu), 1.07–1.34 (m, 2H, γ-CH_2-Ile), 0.98, 0.94 (2 d, 6H, $J = 6.4$ Hz, 2 δ-CH_3-Leu), 0.84 (t, 6H, $J = 6.9$ Hz, γ-CH_3-ILe, δ-CH_3-ILe)

DOI: 10.1002/psc

(*as rotamer); ^{13}C NMR ($CDCl_3$) δ: 176.0, 174.3, 174.1*, 171.6, 171.5*, 171.3*, 169.9, 156.5, 143.8, 143.7, 141.3, 135.3, 135.0, 128.7, 128.6, 128.5, 128.4, 128.3, 127.8, 127.2, 125.2, 125.0*, 120.0, 67.3, 67.0, 56.5, 55.6, 49.9, 47.1, 37.5, 31.6, 30.5, 29.0, 25.0, 24.9, 24.7, 23.5, 23.4, 21.4, 15.7, 15.6*, 11.5, 11.4* (* as rotamer); ESIMS: calcd for $C_{40}H_{50}N_4O_7$ $[M+H]^+$ 699.4; found 699.5.

Synthesis of Compound 1

Fmoc-L-Leu-N$^{\alpha}$-Me-L-Gln-L-Ile-Gly-N$^{\alpha}$-Me-D-Phe-L-Pro-OMe (2). Hydrogenation of **4** (90.8 mg, 0.13 mmol) was carried out in EtOAc–EtOH (1 : 4, 10 ml) in the presence of a catalytic amount of Pd–C (10%) under hydrogen at room temperature. Pd–C was removed by filtration and the resulting filtrate was reconcentrated from CH_2Cl_2 two times to yield the acid **4***. The Fmoc group of **3** (90.6 mg, 0.16 mmol) was removed by DEA as described above to give the amine **3***. A solution of residue **3*** and **4*** in CH_2Cl_2–DMF (3 : 1, 6 ml) was treated sequentially with HOAt (27.0 mg, 0.20 mmol), EDCI (38.0 mg, 0.20 mmol), and DIPEA (35.0 mg, 0.27 mmol). The reaction mixture was stirred at 0 °C for 30 min and for another 12 h at room temperature. After diluting with $CHCl_3$ (50 ml), the whole mixture was washed with 10% citric acid (2 × 5 ml), 5% $NaHCO_3$ (2 × 5 ml), H_2O (2 × 5 ml), and brine (2 × 5 ml); dried over Na_2SO_4; and concentrated *in vacuo*. The residue was purified by flash column chromatography with $CHCl_3$–MeOH (20 : 1) to afford the **2** as colorless oil (30.1 mg, 24.7%). R_f 0.23 (10 : 1, $CHCl_3$–MeOH); $[\alpha]^{25}_D = -34.8°$ (*c* 0.4, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ: 7.76 (d, 2H, J = 7.3 Hz, 4,5-C*H*-Fluorenyl), 7.60 (d, 2H, J = 7.3 Hz, 1,8-C*H*-Fluorenyl), 7.41 (t, 2H, J = 7.3 Hz, 3,6-C*H*-Fluorenyl), 7.32 (t, 2H, J = 7.3 Hz, 2,7-C*H*-Fluorenyl), 7.18–7.27 (m, 5H, Ar*H*), 6.95 (brs, 1H, N*H*-Gly), 6.85 (brs, 1H, N*H*-Ile), 6.42, 5.46 (2 brs, 2H, NH_2-Gln), 5.66 (d, 1H, J = 8.7 Hz, N*H*-Leu), 5.48 (t, 1H, J = 7.3 Hz, α-C*H*-Phe), 5.03 (t, 1H, J = 7.3 Hz, α-C*H*-Gln), 4.66 (td, 1H, J = 11.0, 2.6 Hz, α-C*H*-Leu), 4.32–4.42 (m, 4H, CH_2-Fluorenyl, α-C*H*-Pro, α-C*H*-Ile), 4.21 (t, 1H, J = 7.3 Hz, 9-C*H*-Fluorenyl), 4.15, 3.87 (2 dd, 2H, J = 17.9, 4.8 Hz, α-CH_2-Gly), 3.72 (s, 3H, COOCH_3), 3.32 (td, 2H, J = 11.5, 5.9 Hz, δ-CH_2-Pro), 3.26 (dd, 1H, J = 13.7, 8.3 Hz, β-CH_2-Phe), 3.04 (s, 3H, *N*-CH_3-Gln), 3.00 (s, 3H, *N*-CH_3-Phe), 2.81 (dd, 1H, J = 13.7, 8.3 Hz, β-CH_2-b-Phe), 1.81–2.32 (m, 9H, β-CH_2-Gln, γ-CH_2-Gln, β-CH_2-Pro, β-C*H*-Ile, γ-CH_2-Pro), 1.07–1.75 (m, 5H, γ-C*H*-Leu, β-CH_2-Leu, γ-CH_2-Ile), 0.99, 0.93 (2 d, 6H, J = 6.4 Hz, 2 δ-CH_3-Leu), 0.87 (m, 6H, γ-CH_3-Ile, δ-CH_3-Ile); ^{13}C NMR ($CDCl_3$) δ: 174.5, 173.9, 172.5, 170.8, 170.1, 168.3, 167.8, 156.4, 143.8, 143.7, 141.3, 137.0, 129.3, 128.5, 127.8, 127.1, 126.8, 125.2, 125.1, 120.0, 67.0, 59.0, 57.9, 56.5, 55.6, 52.3, 49.8, 47.1, 46.8, 41.8, 41.2, 36.4, 34.9, 31.6, 30.5, 29.9, 28.8, 25.0, 24.6, 24.4, 23.4, 23.3, 21.4, 15.8, 11.5; ESIMS: calcd for $C_{51}H_{67}N_7O_{10}$ $[M+Na]^+$ 960.5; found 959.9.

Hmp-L-Leu-N$^{\alpha}$-Me-L-Gln-L-Ile-Gly-N$^{\alpha}$-Me-D-Phe-L-Pro-OMe (1). The Fmoc group of **2** (30.1 mg, 0.03 mmol) was removed by DEA as described above to give amine **2***. A solution of amine **2*** and Hmp (5.1 mg, 0.04 mmol) in THF (2 ml) was treated sequentially with EDCI (7.5 mg, 0.04 mmol) and HOAt (5.4 mg, 0.04 mmol). The reaction mixture was stirred at 0 °C for 30 min and for another 12 h at room temperature. The reaction mixture was treated as described for **2**. The residue was purified by flash column chromatography with $CHCl_3$–MeOH (20 : 1) to afford **1** as a white solid (7.5 mg, 28.2%). R_f 0.18 (20 : 1, $CHCl_3$–MeOH); $[\alpha]^{21}_D = -12.6°$ (*c* 0.4, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.19–7.35 (m, 5H, Ar*H*), 6.99 (brs, 1H, N*H*-Gly), 6.80, 6.29 (2 brs, 2H, NH_2-Gln), 5.52 (t-like, 1H, J = 7.3, 7.8 Hz, α-C*H*-Phe), 5.11 (t, 1H, J = 7.1 Hz, α-C*H*-Gln), 4.94 (m, 1H, O*H*-Hmp), 4.81 (brs, 1H, α-C*H*-Leu), 4.41 (dd, 1H, J = 8.3, 5.5 Hz, α-C*H*-Pro), 4.28 (t, 1H, J = 7.3 Hz, α-C*H*-Ile), 4.13, 3.89 (2 dd, 2H, J = 17.4, 4.6 Hz, α-CH_2-Gly), 3.72 (s, 3H, COOCH_3), 3.34 (m, 2H, δ-CH_2-Pro), 3.28, 2.81 (2 dd, 2H, J = 13.7, 8.3 Hz, β-CH_2-Phe), 3.13 (s, 3H, *N*-CH_3-Gln), 3.00 (s, 3H, *N*-CH_3-Phe), 1.80–2.99 (m, 10H, γ-CH_2-Gln, β-CH_2-Gln, β-CH_2-Pro, γ-CH_2-Pro, β-C*H*-Ile, β-C*H*-Hmp), 1.10–1.75 (m, 7H, γ-C*H*-Leu, β-CH_2-Leu, γ-CH_2-Ile, γ-CH_2-Hmp), 0.98 (d, 3H, J = 6.9 Hz, γ-CH_3-Hmp), 0.96, 0.95 (2 d, 6H, J = 7.0 Hz, 2 δ-CH_3-Leu), 0.89 (t, 3H, J = 7.3 Hz, δ-CH_3-Hmp), 0.87 (d, 3H, J = 6.9 Hz, γ-CH_3-Ile), 0.85 (t, 3H, J = 7.3 Hz, δ-CH_3-Ile); ^{13}C NMR ($CDCl_3$) δ: 175.5 (C-37), 174.1 (C-29), 174.0 (C-31), 172.5 (C-1), 171.2 (C-19), 170.4 (C-25), 167.9 (C-7), 167.9 (C-17), 137.0 (C-10), 129.4 (C-11,15), 128.5 (C-12,14), 126.8 (C-13), 76.2 (C-38), 59.0 (C-2), 58.0 (C-20), 56.5 (C-8), 55.4 (C-26), 52.3 (C-6), 47.8 (C-32), 46.8 (C-5), 41.2 (C-18), 41.0 (C-33), 38.6 (C-39), 36.5 (C-21), 35.0 (C-9), 31.5 (C-28), 31.0 (C-30), 29.9 (C-16), 28.8 (C-3), 25.0 (C-22), 24.9 (C-34), 24.4 (C-4), 23.9 (C-27), 23.4 (C-40), 23.2 (C-35), 21.4 (C-36), 15.7 (C-23), 15.5 (C-41), 11.8 (C-42), 11.3 (C-24); ESIHRMS: calcd for $C_{42}H_{68}N_7O_{10}$ $[M+H]^+$ 830.5028; found 830.5035.

Synthesis of Compounds 1a, 1b, and 1c

N$^{\alpha}$-Boc-L-Ile-Gly-N$^{\alpha}$-Me-D-Phe-L-Pro-OMe (11). The Fmoc group of **3** (926.9 mg, 1.63 mmol) was removed by DEA as described above. A solution of amine **3*** and Boc–isoleucine (471.0 mg, 1.96 mmol) in anhydrous CH_2Cl_2 (30 ml) was treated sequentially with HOAt (266.8 mg, 1.96 mmol), EDCI (375.8 mg, 1.96 mmol), and $NaHCO_3$ (164.6 mg, 1.96 mmol). The reaction mixture was stirred at 0 °C for 30 min and for another 12 h at room temperature. The reaction mixture was treated as described for **3**. The residue was purified by flash column chromatography with $CHCl_3$–MeOH (20 : 1) to afford **11** as a white foam solid (730.0 mg, 80.0%). R_f 0.26 (1 : 1, petroleum ether–EtOAc); $[\alpha]^{21}_D = +19.3°$ (*c* 0.4, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ: 7.18–7.29 (m, 5H, Ar*H*), 6.81 (brs, 1H, N*H*-Ile), 5.58 (t, 1H, J = 7.5 Hz, α-C*H*-Phe), 4.42 (dd, 1H, J = 8.2, 6.0 Hz, α-C*H*-Pro), 3.91–4.13 (m, 3H, α-C*H*-Ile, α-CH_2-Gly), 3.73 (s, 3H, COOCH_3), 3.31–3.42 (m, 2H, δ-CH_2-Pro), 3.28, 2.84 (2 dd, 2H, J = 13.7, 7.3 Hz, β-CH_2-Phe), 2.99 (s, 3H, *N*-CH_3), 1.81–2.20 (m, 5H, β-CH_2-Pro, β-C*H*-Ile, γ-CH_2-Pro), 1.09–1.44 (m, 11H, γ-CH_2-Ile, (CH_3)$_3$), 0.89–0.93 (m, 6H, γ-CH_3-Ile, δ-CH_3-Ile); ^{13}C NMR ($CDCl_3$) δ: 172.5, 171.5, 167.9, 137.0, 129.4, 128.6, 128.4, 126.8, 126.7, 79.9, 59.3, 59.0, 56.2, 52.3, 46.8, 41.2, 37.5, 35.0, 29.7, 28.8, 28.3, 25.0, 24.6, 15.7, 11.6; ESIMS: calc for $C_{29}H_{44}N_4O_7$ $[M+H]^+$ 561.3; found 561.4.

N$^{\alpha}$-Fmoc-N$^{\alpha}$-Me-L-Gln-L-Ile-Gly-N$^{\alpha}$-Me-D-Phe-L-Pro-OMe (12). To a solution of compound **11** (100.0 mg, 0.18 mmol) in CH_2Cl_2 (4 ml) at room temperature was added TFA (4 ml). The reaction mixture was stirred for 2 h and then reconcentrated from CH_2Cl_2 two times to remove excess TFA. A solution of the TFA salt **11*** and **9** (98.8 mg, 0.26 mmol) in THF (10 ml) was treated sequentially with HOAt (58.0 mg, 0.43 mmol), EDCI (82.0 mg, 0.43 mmol), and NMM (48 µl, 0.43 mmol). The reaction mixture was stirred at 0 °C

DOI: 10.1002/psc

for 30 min and then stirred at room temperature for another 20 h. The reaction mixture was treated as described for **8**. The residue was purified by flash column chromatography with CH_2Cl_2–MeOH (20 : 1) to afford **12** as a white foam solid (116.1 mg, 79.0%). R_f 0.61 (5 : 1, $CHCl_3$–MeOH); $[\alpha]^{21}{}_D = -1.3^\circ$ (*c* 0.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ: 7.77 (d, 2H, $J = 7.3$ Hz, 4,5-C*H*-Fluorenyl), 7.56 (t, 2H, $J = 7.5$ Hz, 1,8-C*H*-Fluorenyl), 7.41 (t, 2H, $J = 7.3$ Hz, 3,6-C*H*-Fluorenyl), 7.32 (t, 2H, $J = 7.3$ Hz, 2,7-C*H*-Fluorenyl), 7.18–7.28 (m, 5H, Ar*H*), 6.97 (brs, 1H, N*H*-Gly), 6.79 (d, 1H, $J = 7.7$ Hz, N*H*-Ile), 6.56, 5.55 (2 brs, 2H, NH_2-Gln), 5.46 (t, 1H, $J = 7.5$ Hz, α-C*H*-Phe), 4.61–4.63 (m, 1H, α-C*H*-Gln), 4.38–4.47 (m, 3H, CH_2-Fluorenyl, α-C*H*-Pro), 4.31–4.34 (m, 1H, α-C*H*-Ile), 4.25 (t, 1H, $J = 6.6$ Hz, 9-C*H*-Fluorenyl), 4.17, 3.86 (2 dd, 2H, $J = 17.6$, 4.4 Hz, α-CH_2-Gly), 3.68*, 3.73, 3.74* (s, 3H, $COOCH_3$), 3.31–3.34 (m, 2H, δ-CH_2-Pro), 3.26, 2.84 (2 dd, 2H, $J = 13.6$, 7.3 Hz, β-CH_2-Phe), 3.01, 3.03* (s, 3H, *N*-CH_3-Gln), 2.87 (s, 3H, *N*-CH_3-Phe), 1.80–2.33 (m, 9H, β-CH_2-Gln, γ-CH_2-Gln, β-CH_2-Pro, β-C*H*-Ile, γ-CH_2-Pro), 1.03–1.37 (m, 2H, γ-CH_2-Ile), 0.82 (t, 6H, $J = 7.6$ Hz, γ-CH_3-Ile, δ-CH_3-Ile) (* as rotamer); ^{13}C NMR ($CDCl_3$) δ: 174.7, 172.5, 170.9, 170.5, 168.4, 167.8, 157.3, 149.8, 143.7, 141.3, 137.0, 134.7, 129.3, 128.5, 127.8, 127.1, 126.9, 124.9, 120.1, 68.2, 59.0, 58.1, 57.6, 56.6, 52.3, 47.1, 46.8, 41.1, 36.1, 34.9, 31.9, 29.9, 29.7, 28.8, 25.1, 24.4, 23.9, 15.8, 11.5; ESIMS: calcd for $C_{45}H_{56}N_6O_9$ $[M + H]^+$ 824.5; found 825.5.

***N$^\alpha$*-Fmoc-*N$^\alpha$*-Me-D-Gln-L-Ile-Gly-*N$^\alpha$*-Me-D-Phe-L-Pro-OMe (12d).** Compound **12d** (78.2 mg, 53.3%) was obtained as a white solid as described for the synthesis of compound **12** but using **9d** instead of **9**. R_f 0.61 (5 : 1, $CHCl_3$–MeOH); $[\alpha]^{21}{}_D = +13.0^\circ$ (*c* 0.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ: 7.77 (d, 2H, $J = 7.3$ Hz, 4,5-C*H*-Fluorenyl), 7.60 (d, 2H, $J = 6.6$ Hz, 1,8-C*H*-Fluorenyl), 7.40 (t, 2H, $J = 7.5$ Hz, 3,6-C*H*-Fluorenyl), 7.32 (m, 2H, 2,7-C*H*-Fluorenyl), 7.17–7.23 (m, 5H, Ar*H*), 6.97 (brs, 1H, N*H*-Ile), 6.83 (brs, 1H, N*H*-Gly), 5.92, 5.61 (2 brs, 2H, NH_2-Gln), 5.50 (t, 1H, $J = 7.3$ Hz, α-C*H*-Phe), 4.67 (brt, 1H, α-C*H*-Gln), 4.45–4.52 (m, 2H, CH_2-Fluorenyl), 4.40 (dd, 1H, $J = 8.8$, 5.8 Hz, α-C*H*-Pro), 4.32 (brt, 1H, α-C*H*-Ile), 4.28 (m, 1H, 9-C*H*-Fluorenyl), 4.06, 3.83 (2 dd, 2H, $J = 17.9$, 4.8 Hz, α-CH_2-Gly), 3.71, 3.73* (s, 3H, $COOCH_3$), 3.31 (t, 2H, $J = 6.2$ Hz, δ-CH_2-Pro), 3.25, 2.80 (2 dd, 2H, $J = 13.6$, 8.0 Hz, β-CH_2-Phe), 2.95, 2.96* (s, 3H, *N*-CH_3-Gln), 2.84 (s, 3H, *N*-CH_3-Phe), 1.76–2.33 (m, 9H, β-CH_2-Gln, γ-CH_2-Gln, β-CH_2-Pro, β-C*H*-Ile, γ-CH_2-Pro), 1.11–1.43 (m, 2H, γ-CH_2-Ile), 0.87 (t, 6H, $J = 7.3$ Hz, γ-CH_3-Ile, δ-CH_3-Ile) (* as rotamer); ^{13}C NMR ($CDCl_3$) δ: 172.5, 167.9, 143.9, 143.8, 141.3, 136.9, 129.4, 128.4, 127.8, 127.1, 126.7, 125.0, 120.1, 68.0, 59.1, 58.9, 58.0, 56.3, 52.3, 47.2, 46.8, 41.1, 37.0, 35.0, 32.2, 29.8, 29.7, 28.8, 25.0, 24.8, 24.1, 15.6, 11.3; ESIMS: calcd for $C_{45}H_{56}N_6O_9$ $[M + H]^+$ 824.5; found 825.5.

Hmp-L-Leu-OBn (13). A solution of compound **14** (1.57 g, 4.00 mmol) and Hmp (528.4 mg, 4.00 mmol) in anhydrous CH_2Cl_2 (10 ml) was treated sequentially with HOAt (653.3 mg, 4.80 mmol), EDCI (920.2. mg, 4.80 mmol), and NMM (532 µl, 4.80 mmol). The reaction mixture was stirred at 0 °C for 30 min and for another 12 h at room temperature. After diluting with EtOAc (200 ml), the whole mixture was washed with 10% citric acid (2 × 20 ml), 5% $NaHCO_3$ (2 × 20 ml), H_2O (2 × 20 ml), and brine (2 × 20 ml); dried over Na_2SO_4; and concentrated *in vacuo*. The residue was purified by flash column chromatography with EtOAc–petroleum ether (1 : 3) to afford **13** as a colorless oil (1.20 g, 89.3%). R_f 0.56 (2 : 1, petroleum ether–EtOAc); $[\alpha]^{21}{}_D = -28.2^\circ$ (*c* 0.8, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ: 7.33–7.39 (m, 5H, Ar*H*), 6.83 (d, 1H, $J = 8.3$ Hz, N*H*-Leu), 5.17, 5.15 (2 d, 2H, $J = 15.0$ Hz, CH_2Ph), 4.69 (td, 1H, $J = 9.2$, 5.0 Hz, α-C*H*-Leu), 4.04 (t, 1H, $J = 4.2$ Hz, α-C*H*-Hmp), 2.84 (t, 1H, $J = 5.5$ Hz, O*H*-Hmp), 1.85–1.92 (m, 1H, β-C*H*-Hmp), 1.57–1.70 (m, 3H, β-CH_2-Leu, γ-C*H*-Leu), 1.17–1.45 (m, 2H, γ-CH_2-Hmp), 0.99 (d, 3H, $J = 6.8$ Hz, γ-CH_3-Hmp), 0.93, 0.92 (2 d, 6H, $J = 3.6$ Hz, 2 δ-CH_3-Leu), 0.89 (t, 3H, $J = 7.3$ Hz, δ-CH_3-Hmp); ^{13}C NMR ($CDCl_3$) δ: 173.4, 173.1, 135.3, 128.6, 128.4, 128.2, 76.3, 67.2, 50.4, 41.2, 38.7, 24.9, 23.3, 22.9, 21.6, 15.5, 11.8; ESIMS: calcd for $C_{19}H_{29}NO_4$ $[M + H]^+$ 336.2; found 336.1.

Hmp-D-Leu-OBn (13d). Compound **13d** (305 mg, 90.9%) was obtained as a white solid as described for the synthesis of compound **13** but using **14d** instead of **14**. R_f 0.56 (2 : 1, petroleum ether–EtOAc); $[\alpha]^{21}{}_D = +1.5^\circ$ (*c* 0.8, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ: 7.33–7.39 (m, 5H, Ar*H*), 6.70 (d, 1H, $J = 8.7$ Hz, N*H*-Leu), 5.17, 5.16 (2 d, 2H, $J = 18.8$ Hz, CH_2Ph), 4.71 (td, 1H, $J = 8.7$, 5.4 Hz, α-C*H*-Leu), 4.05 (dd, 1H, $J = 5.5$, 3.6 Hz, α-C*H*-Hmp), 2.35 (t, 1H, $J = 5.5$ Hz, O*H*-Hmp), 1.84–1.92 (m, 1H, β-C*H*-Hmp), 1.57–1.72 (m, 3H, β-CH_2-Leu, γ-C*H*-Leu), 1.15–1.45 (m, 2H, γ-CH_2-Hmp), 1.00 (d, 3H, $J = 7.3$ Hz, γ-CH_3-Hmp), 0.93, 0.92 (2 d, 6H, $J = 1.3$ Hz, 2 δ-CH_3-Leu), 0.87 (t, 3H, $J = 7.3$ Hz, δ-CH_3-Hmp); ^{13}C NMR ($CDCl_3$) δ: 172.9, 172.6, 135.3, 128.6, 128.4, 128.2, 76.3, 67.1, 50.4, 41.4, 39.0, 24.9, 23.0, 22.8, 21.8, 15.5, 11.8; ESIMS: calcd for $C_{19}H_{29}NO_4$ $[M + H]^+$ 336.2; found 336.2.

***Hmp-L-Leu-N$^\alpha$*-Me-D-Gln-L-Ile-Gly-N$^\alpha$*-Me-D-Phe-L-Pro-OMe (1a).** The benzyl group of **13** (100.0 mg, 0.30 mmol) was removed by 10% Pd/C and the Fmoc group of **12d** (20.0 mg, 0.02 mmol) was removed by DEA as described above. A solution of the two crude products in CH_2Cl_2–DMF (4 : 1, 5 ml) was treated sequentially with HOAt (49.4 mg, 0.36 mol), EDCI (69.6 mg, 0.36 mmol), and NMM (40.2 µl, 0.36 mmol). The reaction mixture was stirred at 0 °C for 30 min and then at room temperature for another 12 h. After diluting with $CHCl_3$ (50 ml), the whole mixture was washed with 10% citric acid (2 × 5 ml), 5% $NaHCO_3$ (2 × 5 ml), H_2O (2 × 5 ml), and brine (2 × 5 ml); dried over Na_2SO_4; and concentrated *in vacuo*. The residue was purified by flash column chromatography with CH_2Cl_2–MeOH (20 : 1) to afford **1a** as a white solid (10 mg, 49.7%). R_f 0.18 (20 : 1, $CHCl_3$–MeOH); $[\alpha]^{21}{}_D = +15^\circ$ (*c* 0.4, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.18–7.27 (m, 5H, Ar*H*), 7.16 (d, 1H, $J = 8.7$ Hz, N*H*-Leu), 7.09 (d, 1H, $J = 9.2$ Hz, N*H*-Ile), 7.02 (t, 1H, $J = 4.1$ Hz, N*H*-Gly), 5.98, 5.63 (2 brs, 2H, NH_2-Gln), 5.53 (t, 1H, $J = 7.6$ Hz, α-C*H*-Phe), 5.05 (t, 1H, $J = 7.5$ Hz, α-C*H*-Gln), 4.94 (dt, 1H, $J = 10.6$, 6.4 Hz, α-C*H*-Leu), 4.73 (d, 1H, $J = 4.1$ Hz, O*H*-Hmp), 4.38 (dd, 1H, $J = 8.7$, 5.5 Hz, α-C*H*-Pro), 4.30 (dd, 1H, $J = 9.1$, 6.8 Hz, α-C*H*-Ile), 4.08, 3.83 (2 dd, 2H, $J = 17.4$, 4.6 Hz, α-CH_2-Gly), 3.73*, 3.71, 3.66* (s, 3H, $COOCH_3$), 3.29–3.36 (m, 2H, δ-CH_2-Pro), 3.29, 2.82 (2 m, 2H, β-CH_2-Phe), 3.01 (s, 3H, *N*-CH_3-Gln), 2.99 (s, 3H, *N*-CH_3-Phe), 2.82 (m, 1H, β-CH_2-Phe), 2.00–2.31 (m, 6H, β-CH_2-Gln, γ-CH_2-Gln, β-CH_2-Pro), 1.77–1.93 (m, 6H, γ-CH_2-Pro, β-CH_2-Pro, β-C*H*-Ile, β-C*H*-Hmp), 1.55–1.66 (m, 3H, γ-C*H*-Leu, β-CH_2-Leu), 1.11–1.47 (m, 4H, γ-CH_2-Hmp, γ-CH_2-Ile), 0.96 (t, 9H, $J = 5.5$ Hz, γ-CH_3-Hmp, 2 δ-CH_3-Leu), 0.85–0.90 (m, 9H, δ-CH_3-Hmp, γ-CH_3-Ile, δ-CH_3-Ile) (* as rotamer); ^{13}C NMR ($CDCl_3$) δ: 174.5 (C-31), 174.2 (C-29), 174.1 (C-37), 172.5 (C-1), 171.4 (C-19), 169.7 (C-25), 167.9

DOI: 10.1002/psc

(C-7), 167.7 (C-17), 136.9 (C-10), 129.4 (C-11,15), 128.4 (C-12,14), 126.7 (C-13), 76.5 (C-38), 58.9 (C-2), 57.8 (C-20), 56.4 (C-26), 56.2 (C-8), 52.3 (C-6), 47.2 (C-32), 46.8 (C-5), 41.1 (C-18), 41.0 (C-33), 38.3 (C-39), 37.0 (C-21), 35.1 (C-9), 32.2 (C-28), 31.1 (C-30), 29.7 (C-16), 28.8 (C-3), 24.9 (C-4,34), 24.7 (C-22), 23.8 (C-40), 23.1 (C-27), 23.0 (C-35), 22.0 (C-36), 15.6 (C-23), 15.5 (C-41), 11.8 (C-42), 11.2 (C-24); ESIHRMS calcd for $C_{42}H_{68}N_7O_{10}$ $[M+H]^+$ 830.5028; found 830.5045.

Hmp-D-Leu-N$^{\alpha}$-Me-L-Gln-L-Ile-Gly-N$^{\alpha}$-Me-D-Phe-L-Pro-OMe (1b). Compound **1b** (34.4 mg, 30.9%) was obtained as a white solid as described for the synthesis of compound **1a** but using **12** and **13d** instead of **12d** and **13**, respectively. R_f 0.18 (20 : 1, $CHCl_3$–MeOH); $[\alpha]^{22}_D = -15.6^\circ$ (*c* 0.4, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.37 (dd, 2H, J = 12.5, 8.1 Hz, N*H*-Leu, N*H*-Ile), 7.15–7.28 (m, 5H, Ar*H*), 7.08* (t, 2H, J = 7.9 Hz, N*H*-Leu, N*H*-Ile), 7.00 (dd, 1H, J = 8.4, 4.4 Hz, N*H*-Gly), 6.74, 6.67*, 6.44, 5.74* (2 brs, 2H, NH_2-Gln), 5.51, 5.47* (t, 1H, J = 7.5 Hz, α-C*H*-Phe), 5.12, 4.93* (dd, 1H, J = 9.7, 5.7 Hz, α-C*H*-Gln), 4.89–4.93, 4.75–4.79* (m, 1H, α-C*H*-Leu), 4.40, 4.39* (dd, 1H, J = 5.7, 1.8 Hz, α-C*H*-Pro), 4.28 (dd, 1H, J = 8.0, 6.6 Hz, α-C*H*-Ile), 3.87–4.15 (m, 4H, O*H*-Hmp, α-CH_2-Gly, α-C*H*-Hmp), 3.72*, 3.71, 3.66*, 3.65* (s, 3H, COOCH_3), 3.23–3.36 (m, 2H, δ-CH_2-Pro), 3.26, 2.82 (2 dd, 2H, J = 6.8, 2.0 Hz, β-CH_2-Phe), 3.11*, 3.08 (s, 3H, *N*-CH_3-Gln), 3.00, 2.99* (s, 3H, *N*-CH_3-Phe), 1.99–2.39 (m, 7H, β-CH_2-Gln, γ-CH_2-Gln, β-C*H*-Ile, β-CH_2-Pro), 1.78–1.97 (m, 5H, γ-CH_2-Pro, β-CH_2-Pro, β-C*H*-Hmp), 1.04–1.74 (m, 7H, γ-C*H*-Leu, β-CH_2-Leu, γ-CH_2-Hmp, γ-CH_2-Ile), 0.97 (d, 3H, J = 6.6 Hz, γ-CH_3-Hmp), 0.92–0.95 (m, 6H, 2 δ-CH_3-Leu), 0.81–0.90 (m, 9H, δ-CH_3-Hmp, γ-CH_3-Ile, δ-CH_3-Ile) (* as rotamer); ^{13}C NMR ($CDCl_3$) δ: 174.7*, 174.6 (C-31), 174.2 (C-29), 174.1 (C-37), 174.0*, 173.6*, 172.5 (C-1), 171.1 (C-19), 171.0*, 170.3 (C-25), 168.3*, 168.2*, 167.9 (C-7), 167.8 (C-17), 136.9 (C-10), 129.4 (C-11,15), 129.3*, 128.4 (C-12,14), 126.8*, 126.7 (C-13), 76.3 (C-38), 75.9*, 59.0 (C-2), 58.2*, 57.9 (C-20), 56.5*, 56.4 (C-26), 54.8 (C-8), 52.3 (C-6), 48.0 (C-32), 46.8 (C-5), 41.5 (C-18), 41.1 (C-33), 39.1*, 38.4 (C-39), 36.5 (C-21), 36.0*, 35.0 (C-9), 34.9*, 32.0 (C-28), 31.9*, 31.2 (C-30), 30.5*, 29.9*, 29.8*, 29.7 (C-16), 28.8 (C-3), 25.0 (C-4), 24.8 (C-34), 24.6 (C-22), 23.9 (C-40), 23.6 (C-27), 23.3 (C-35), 21.6 (C-36), 21.2*, 15.7*, 15.6 (C-23), 15.5 (C-41), 15.4*, 11.8 (C-42), 11.3 (C-24) (* as rotamer); ESIHRMS calcd for $C_{42}H_{68}N_7O_{10}$ $[M+H]^+$ 830.5028; found 830.5008.

Hmp-D-Leu-N$^{\alpha}$-Me-D-Gln-L-Ile-Gly-N$^{\alpha}$-Me-D-Phe-L-Pro-OMe (1c). Compound **1c** (24.1 mg, 58.1%) was obtained as a white solid as described for the synthesis of compound **1a** but using **13d** instead of **13**. R_f 0.18 (20 : 1, $CHCl_3$–MeOH); $[\alpha]^{21}_D = +18.5^\circ$ (*c* 0.4, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.21–7.25 (m, 5H, Ar*H*), 7.19 (m, 1H, N*H*-Leu), 7.11(brs, 1H, N*H*-Ile), 7.02 (brs, 1H, N*H*-Gly), 6.06, 5.76 (2 brs, 2H, NH_2-Gln), 5.52 (dd, 1H, J = 8.4, 7.0 Hz, α-C*H*-Phe), 5.05 (t, 1H, J = 7.3 Hz, α-C*H*-Gln), 4.93 (m, 1H, α-C*H*-Leu), 4.77 (brs, 1H, O*H*-Hmp), 4.38 (dd, 1H, J = 8.8, 5.5 Hz, α-C*H*-Pro), 4.30 (dd, 1H, J = 8.8, 6.6 Hz, α-C*H*-Ile), 4.08, 3.82 (2 dd, 2H, J = 17.6, 5.2 Hz, α-CH_2-Gly), 3.73*, 3.71, 3.66* (s, 3H, COOCH_3), 3.29–3.35 (m, 2H, δ-CH_2-Pro), 3.28, 2.81 (2 m, 2H, β-CH_2-Phe), 3.04*, 3.00 (s, 3H, *N*-CH_3-Gln), 2.99 (s, 3H, *N*-CH_3-Phe), 1.99–2.30 (m, 4H, β-CH_2-Gln, γ-CH_2-Gln), 1.76–1.92 (m, 6H, β-CH_2-Pro, β-C*H*-Ile, γ-CH_2-Pro, β-C*H*-Hmp), 1.51–1.66 (m, 3H, γ-C*H*-Leu, β-CH_2-Leu), 1.10–1.45 (m, 4H, γ-CH_2-Hmp, γ-CH_2-Ile), 0.93–0.97 (m, 9H, γ-CH_3-Hmp, 2 δ-CH_3-Leu), 0.84–0.90 (m, 9H, δ-CH_3-Hmp, γ-CH_3-Ile, δ-CH_3-Ile) (* as rotamer); ^{13}C NMR ($CDCl_3$) δ: 174.5 (C-29), 174.3 (C-31), 174.1 (C-37), 172.5 (C-1), 171.5 (C-19), 169.8 (C-25), 167.9 (C-7), 167.7 (C-17), 136.9 (C-10), 129.4 (C-11,15), 128.4 (C-12,14), 126.7 (C-13), 76.4 (C-38), 58.9 (C-2), 57.8 (C-20), 56.4 (C-26), 56.2 (C-8), 52.2 (C-6), 47.3 (C-32), 46.7 (C-5), 41.1 (C-18), 40.9 (C-33), 38.3 (C-39), 37.0 (C-21), 35.0 (C-9), 32.2 (C-28), 31.1 (C-30), 29.7 (C-16), 28.8 (C-3), 24.9 (C-4,34), 24.7 (C-22), 23.7 (C-40), 23.2 (C-27), 23.0 (C-35), 22.0 (C-36), 15.6 (C-23), 15.5 (C-41), 11.8 (C-42), 11.2 (C-24); ESIHRMS calcd for $C_{42}H_{68}N_7O_{10}$ $[M+H]^+$ 830.5028; found 830.5035.

RESULTS AND DISCUSSION

Our retrosynthetic analysis of tasiamide **1** is outlined in Figure 2. As tasiamide contains two *N*-methylamides, the most critical problem in the assembly is the connection of sterically hindered units with other amino acid residues. Due to small steric hindrance of glycine residue and absence of racemization during fragment

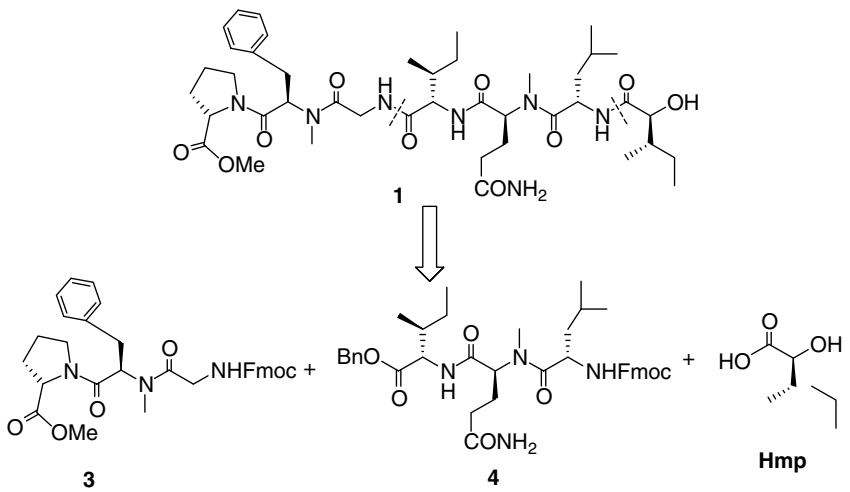


Figure 2 Retrosynthetic analysis of tasiamide **1**.

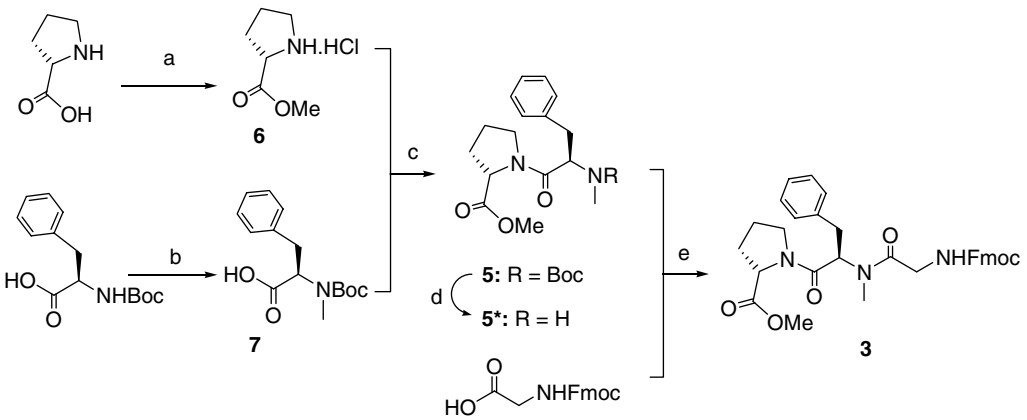
DOI: 10.1002/psc

coupling, we envisaged that the amide bond between Gly and L-Ile could be formed in the late stage of connection. With a consideration toward the use of protecting groups and to minimize the opportunities for racemization, the amide bond linking N^{α}-Me-L-Gln and L-Leu should be conducted at the early stage of the synthesis. Our plan therefore consisted of tripeptide fragment **3**, tripeptide fragment **4,** and unprotected hydroxy acid Hmp. Thus, a convergent [3 + 3 + 1] strategy was applied to the total synthesis of tasiamide.

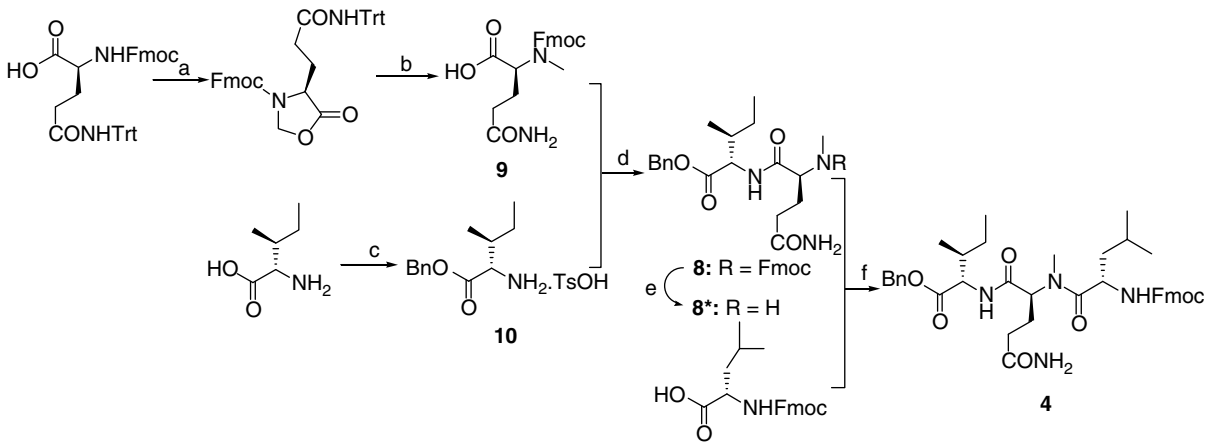
The efficient formation of amide bonds with *N*-methyl amino acids is a challenging job because racemization and diketopiperazine formation are common side reactions. Many reagents and methods have been developed to facilitate the acylation of *N*-methyl amino acid derivatives. In recent years, EDCI and the effective additive HOAt were widely used as coupling reagents for hindered peptide synthesis [8] because they show a good activity, facile incorporate, and are reasonably cheap. Boger applied EDCI/HOAt to the synthesis of the vancomycin aglycon AB ring system [9] and they were also enlisted by Rinehart *et al.* to form Pro-MeTyr linkage through route to didemnin A [10]. Encouraged by these studies, herein we selected EDCI/HOAt as coupling reagents for our target. All the couplings were performed overnight to guarantee the completion.

The first effort was to elaborate fragment **3** as shown in Scheme 1. L-Proline methyl ester (**6**) and N^{α}-Boc-N^{α}-Me-D-phenylalanine (**7**) were first prepared following established protocols [11,12]. Then, their condensation was carried out by using EDCI/HOAt as coupling reagents to provide dipeptide **5** in 97% excellent yield; meanwhile, we found $NaHCO_3$ was better than NMM as acid scavenger during this process. Removal of the Boc group of **5** with 4 M HCl in ethyl acetate and incorporation with Fmoc–glycine (EDCI, HOAt, $NaHCO_3$) produced the target tripeptide **3** smoothly in 77% yield over two steps.

In order to construct fragment **4** (Scheme 2), N^{α}-Fmoc-N^{α}-Me-glutamine (**9**) was first prepared from commercially available N^{α}-Fmoc-N^{δ}-trityl-glutamine via the oxazolidinone intermediate and its reductive open ring following Freidinger's procedure [13]. L-Isoleucine benzyl ester (**10**) was obtained from L-isoleucine

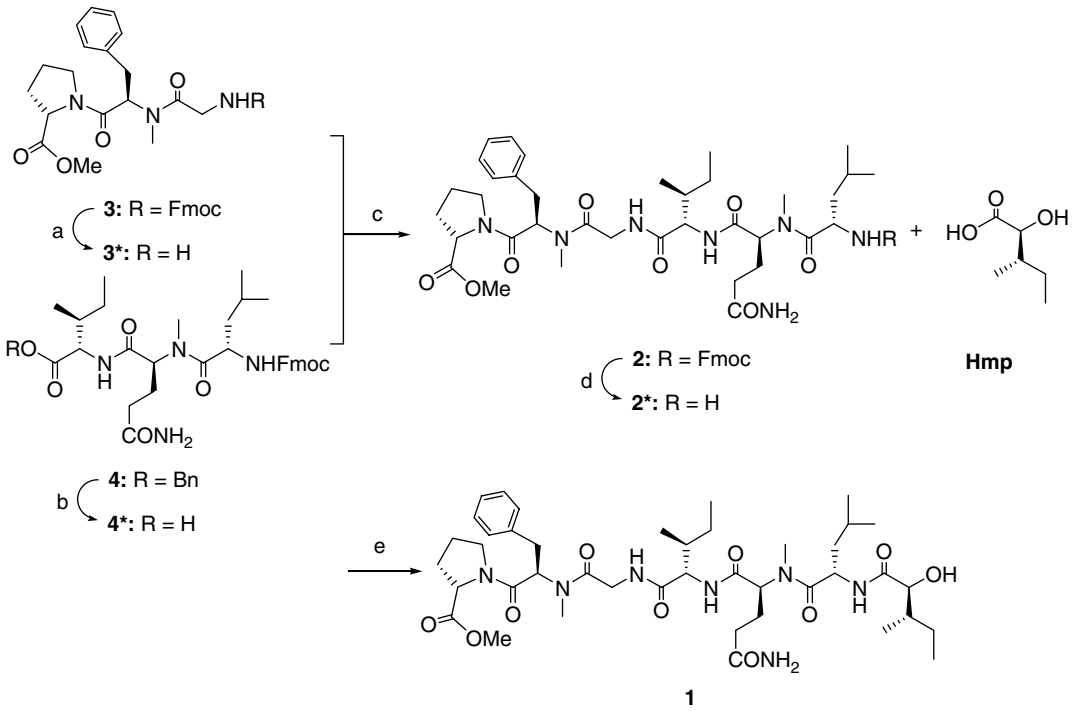


Scheme 1 Reagents and conditions: (a) $SOCl_2$, MeOH, 65%; (b) NaH, THF, CH_3I, quantitative; (c) EDCI, HOAt, $NaHCO_3$, 97%; (d) 4 M HCl/EtOAc; (e) EDCI, HOAt, $NaHCO_3$, 77% in two steps.

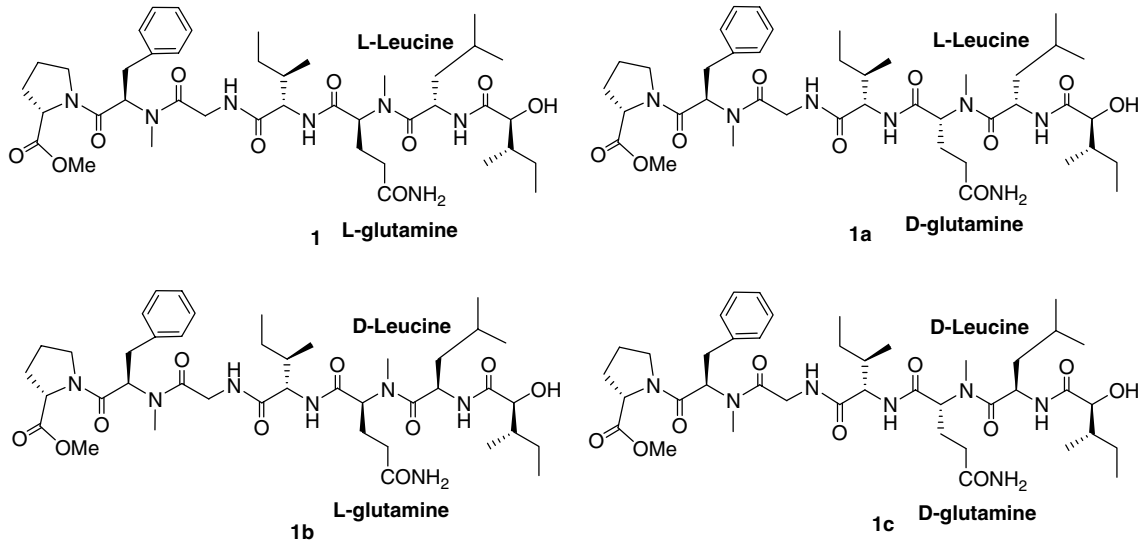


Scheme 2 Reagents and conditions: (a) $(CH_2O)_n$, cat. $TsOH \cdot H_2O$, toluene, reflux, 6 h, 83%; (b) TES, TFA, $CHCl_3$, rt, 2d, 83%; (c) BnOH, *p*-TSA, toluene, reflux, 56%; (d) EDCI, HOAt, NMM, 97%; (e) DEA, CH_3CN; (f) EDCI, HOAt, NMM, 48% in two steps.

DOI: 10.1002/psc



Scheme 3 Reagents and conditions: (a) DEA, CH_3CN; (b) 10% Pd/C, H_2; (c) EDCI, HOAt, NMM, 25% in three steps; (d) DEA, CH_3CN; (e) EDCI, HOAt, NMM, 28% in two steps.



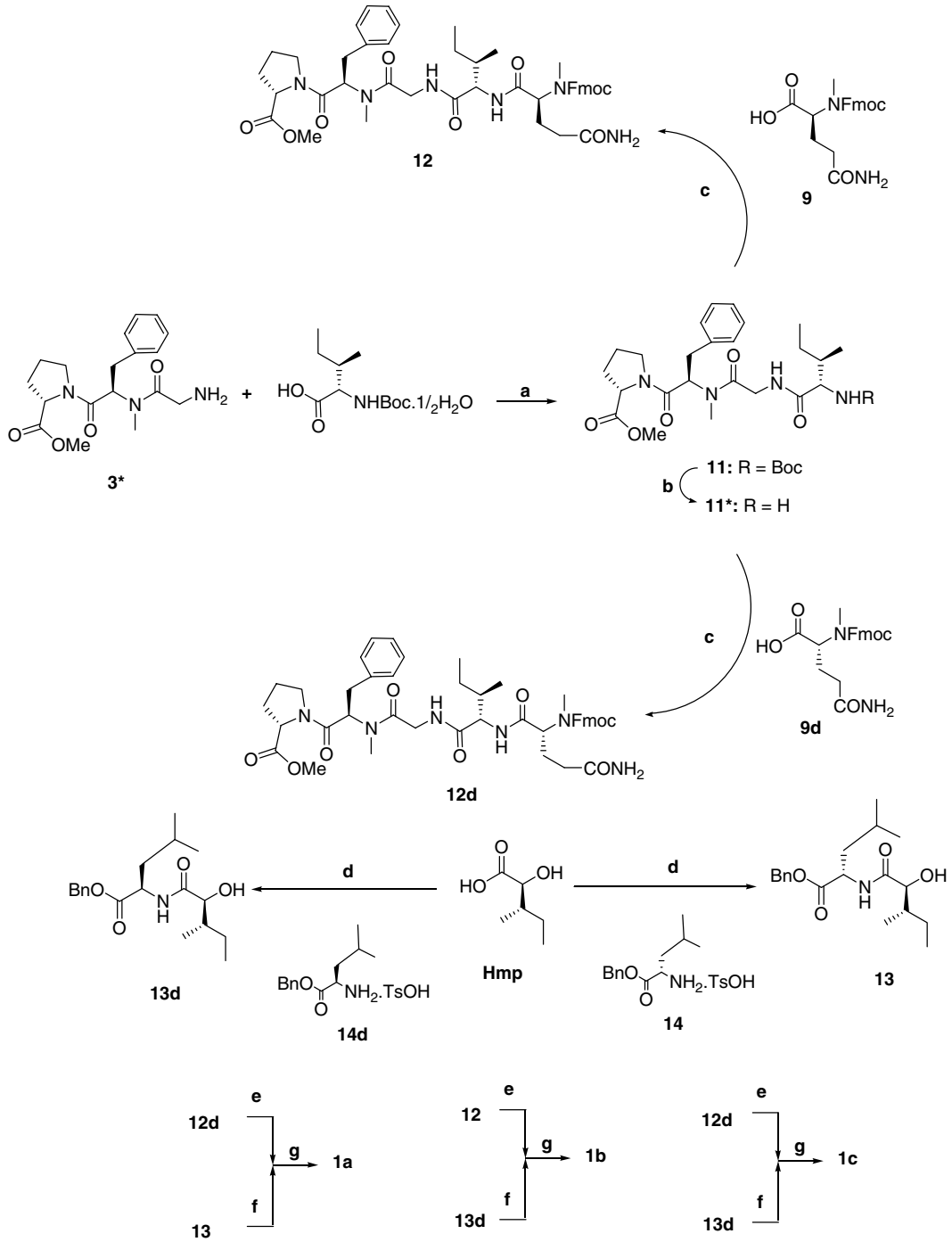
Scheme 4 Tasiamide (**1**) and its three other diastereomers (**1a,1b** and **1c**).

according to the literature [14]. Then incorporation of **9** to amine **10** using EDCI/HOAt as coupling reagents gave **8** at a high yield of 97%, while NMM worked better than $NaHCO_3$ as acid scavenger in this condition. After deprotection of the Fmoc group of **8** in the presence of DEA, the *N*-methyl amide linkage of L-Leu-N^{α}-Me-L-Gln was formed by coupling with Fmoc–leucine under the same coupling conditions to give the target fragment **4** at 48% yield in two steps.

With fragment **3** and **4** in hand, their coupling was then carried out. Treatment of **3** with DEA in CH_3CN, removal of the benzyl group of **4** with 10% Pd/C, and finally coupling gave the linear hexapeptide **2** at a 25% yield in three steps. Finally, Fmoc deprotection of **2** and incorporation with Hmp, prepared from L-Ile according to the literature procedure [15], gave the target product **1** as a white solid (Scheme 3).

However, the NMR data of **1** showed considerable differences from those reported for the natural product, and the value and sign of optical rotation of the synthetic sample were quite different from those of natural product {$[\alpha]^{21}_{D} = -12.6^{\circ}$ (*c* 0.4, $CHCl_3$) while lit. $[\alpha]^{21}_{D} = +15^{\circ}$ (*c* 0.4, $CHCl_3$)}. By carefully comparison of NMR data, it was found that the ^1H NMR and

DOI: 10.1002/psc



Scheme 5 Route of synthesis compounds **1a, 1b**, and **1c**. (a) EDCI, HOAt, NMM; (b) 4 M HCl/EtOAc; (c) EDCI, HOAt, NMM; (d) EDCI, HOAt, NMM; (e) DEA, CH_3CN; (f) 10% Pd/C, H_2; (g) EDCI, HOAt, NMM.

^{13}C NMR signals assigned to N^{α}-Me-L-glutamine and L-leucine of **1** were most different from the reported data for the natural product. We inferred that the structure of the natural product consisted of N^{α}-Me-D-glutamine or D-leucine or both of them. To confirm our hypothesis, three diastereomers of tasiamide **1** were designed and synthesized. N^{α}-Me-L-glutamine of **1** was replaced by N^{α}-Me-D-glutamine to get the diastereomer **1a**; L-leucine was replaced by D-leucine to give the diastereomer **1b**; and both were replaced to obtain the diastereomer **1c**. The stereochemical structures of the three diastereomers are shown as Scheme 4.

To obtain the three diastereomers in a convenient manner, a different route of synthesis was carried out as shown in Scheme 5. All the three diastereomers were divided into two parts from the linkage between N^{α}-Me-glutamine and leucine.

The target compound **1a** could be afforded from the intermediate **12d** and **13**. Fmoc removal of **3** followed by coupling with Boc–isoleucine (EDCI, HOAt)

DOI: 10.1002/psc

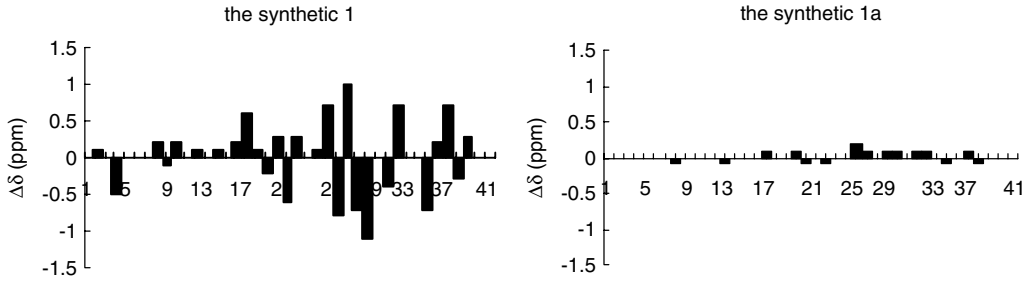


Figure 3 Differences in ^{13}C NMR shifts between the natural product **1a** and synthetic **1**.

gave tetrapeptide **11** at 80% yield. N^{α}-Fmoc-N^{α}-Me-D-glutamine (**9d**) was prepared from N^{α}-Fmoc-N^{δ}-trityl-D-glutamine in the same way as **9**. Removal of the Boc protection of **11** (4 M HCl/EtOAc) and coupling with **9d** under the same coupling conditions gave the key intermediate **12d** at a 53% yield in two steps.

The L-leucine benzyl ester (**14**) was prepared from L-leucine by method similar to that of compound **10**. The intermediate **13** was then readily synthesized by coupling Hmp with **14**. Deprotection of the intermediate **12d** and **13** under standard conditions followed by coupling with each other directly gave **1a** as a white solid at 50% yield over three steps.

The same method was applied to prepare **1b** and **1c**. Compound **1b** was synthesized by using **9** and D-leucine benzyl ester (**14d**) instead of **9d** and **14,** while **1c** was obtained by using **9d** and **14d** as starting materials. To our delight, **1a** provided a near-perfect match with the NMR data reported for tasiamide, and the value and sign of optical rotation of **1a** are also the same as those of the natural product {$[\alpha]^{21}_{D} = +15^{\circ}$ (*c* 0.4, $CHCl_3$), lit. $[\alpha]^{21}_{D} = +15^{\circ}$ (*c* 0.4, $CHCl_3$)}. All the details established that the N^{α}-Me-L-glutamine residue in tasiamide has the D-configuration. The differences in ^{13}C NMR shifts between the reported natural product **1a** and the synthetic **1** were shown in Figure 3.

CONCLUSION

In summary, we have described the first total synthesis of tasiamide, a promising linear peptide with cytotoxic activity and configurational reassignment of the previously assigned N^{α}-Me-L-glutamine residue as N^{α}-Me-D-glutamine. Further studies of the structure–activity relationship will be reported in due course.

Acknowledgements

This work was financially supported by the National Natural Science Foundation of China (30572245) and Hi-Tech Research and Development Program of China (2006AA09Z409).

REFERENCES

1. Jorge I. Jime'nez and Paul J. Scheuer, new lipopeptides from the caribbean cyanobacterium *Lyngbya majuscule*. *J. Nat. Prod.* 2001; **64**: 200–203.
2. Hendrik L, Yoshida WY, Moore RE, Paul VJ, Mooberry SL, Corbett TH. Symplostatin 3, a new dolastatin 10 analogue from the marine cyanobacterium *Symploca* sp. VP452. *J. Nat. Prod.* 2002; **65**: 16–20.
3. Lik Tong T, Sitachitta N, Gerwick WH. The guineamides, novel cyclic depsipeptides from a papua new guinea collection of the marine cyanobacterium *Lyngbya majuscule*. *J. Nat. Prod.* 2003; **66**: 764–771.
4. Williams PG, Yoshida WY, Moore RE, Paul VJ. Tasipeptins A and B: new cytotoxic depsipeptides from the marine cyanobacterium *Symploca* sp. *J. Nat. Prod.* 2003; **66**: 620–624.
5. Hendrik L, Moore RE, Paul VJ, Mooberry SL, Corbett TH. Isolation of dolastatin 10 from the marine cyanobacterium *Symploca* species VP642 and total stereochemistry and biological evaluation of its analogue symplostatin 1. *J. Nat. Prod.* 2001; **64**: 907–910.
6. Williams PG, Yoshida WY, Moore RE, Paul VJ. The isolation and structure elucidation of tasiamide B, a 4-Amino-3-hydroxy-5-phenylpentanoic acid containing peptide from the marine cyanobacterium *Symploca* sp. *J. Nat. Prod.* 2003; **66**: 1006–1009.
7. Williams PG, Yoshida WY, Moore RE, Paul VJ. Tasiamide, a cytotoxic peptide from the marine cyanobacterium *Symploca* sp. *J. Nat. Prod.* 2002; **65**: 1336–1339.
8. Zhang W, Ma Z-H, Mei D, Li C-X, Zhang X-L, Li Y-X. Total synthesis and reassignment of stereochemistry of obyanamide. *Tetrahedron* 2006; **62**: 9966–9972.
9. Boger DL, Miyazaki S, Heon Kim S, Wu JH, Castle SL. Olivier Loiseleur, and Oing Jin. Total Synthesis of the vancomycin aglycon. *J. Am. Chem. Soc.* 1999; **121**: 10004–10011.
10. Rinehart KL, Kishore V, Nagarajan S, Lake RJ, Gloer JB, Bozich FA, Li K-M, Maleczka RE Jr, Todsen WL, Munro MHG, Sullins DW, Sakai R. Total synthesis of Didemnins A, B, and C. *J. Am. Chem. Soc.* 1987; **109**: 6846–6848.
11. Erlanger BF, Sachs H, Brand E. The synthesis of peptides related to gramicidin S. *J. Am. Chem. Soc.* 1954; **76**: 1806–1810.
12. McDermott JR, Benoiton NL. *N*-Methylamino acids in peptide synthesis. I. A new synthesis of *N*-benzyloxycarbonyl, *N*-methylamino acids. *Can. J. Chem.* 1973; **51**: 1915.
13. Selçuk Ç, Morales Ramos AI, Lipton MA. Solid-phase synthesis and configurational reassignment of callipeltin E. Implications for the structures of callipeltins A and B. *J. Org. Chem.* 2006; **71**: 6351–6356.
14. Heinz G, Schröder E. Peptidsynthesen, III. Synthesen von arginin-haltigen peptiden. *Justus Liebigs Ann. Chem.* 1961; **642**: 145–162.
15. Bates RB, Brusoe KG, Burns JJ, Caldera S, Cui W, Gangwar S, Gramme MR, McClure KJ, Rouen GP, Schadow H, Stessman CC, Taylor SR, Vu VH, Yarick GV, Zhang J, Pettit GR, Bontems R. Dolastatins 26. Synthesis and stereochemistry of dolastatin 11[1a]. *J. Am. Chem. Soc.* 1997; **119**: 2111–2113.