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Total synthesis and stereochemical reassignment of tasiamide B

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The first total synthesis of tasiamide B, an octapeptide bearing 4-amino-3-hydroxy-5-phenylpentanoic acid unit isolated from the marine cyanobacteria *Symploca* sp. is described. A simple and efficient way was found to avoid the pyroglutamylation of N^{α}-Me-Gln and led to a reassignment of the N^{α}-Me-L-Phe of tasiamide B to be N^{α}-Me-D-Phe, which was also supported by 1D and 2D NMR. Copyright © 2010 European Peptide Society and John Wiley & Sons, Ltd.

Supporting information may be found in the online version of this article

Keywords: tasiamide B; total synthesis; pyroglutamylation; reassignment

Introduction

Ocean is a unique resource that provides a diverse array of natural products. Since the mid-1980s, mounting evidence has suggested that marine microorganisms and cyanobacteria might be the real producers of many of the compounds isolated from the invertebrates such as sponges, tunicates, bryozoans, and mollusks [1]. From then on, scientists turned their attention to the cyanobacteria, and a series of new pharmacologically interesting natural products were found one after another.

Tasiamide B (**1**) (Figure 1), an 8-residue acyclic peptide, first isolated from the marine cyanobacteria *Symploca* sp. in 2003 by Moore *et al*., was found to be cytotoxic against KB cells with an IC_{50} value of 0.8 μM [2].

This natural product is structurally similar to tasiamide (**2**) isolated from cyanobacterium *Symploca* sp. in 2002, which is cytotoxic against KB and LoVo cells with IC_{50} values of 0.48 and 3.47 μg/ml, respectively [3]. Different from the latter, tasiamide B contains an Ahppa residue, which is a novel δ-amino-β-hydroxy acid and found in a metabolite of a marine cyanobacterium for the first time. Now, we have finished the total synthesis and structural reassignment of tasiamide [4], and its preliminary structure–activity relationship study is also ongoing. For a better understanding of the biological activity and the SAR of those compounds, we decided to explore an efficient synthesis of tasiamide B.

Results and Discussion

Our retrosynthetic analysis of **1** is outlined in Figure 2. Considering the minor steric hindrance, tetrapeptide **3** and **4** were selected as important synthetic intermediates. For fragment **4**, the more timesaving and economical [2 + 2] strategy rather than linear synthesis was employed.

The first effort was to synthesize the easily accessible fragment **3** (Scheme 1). After the preparation of L-proline methyl ester (**10**) and N^{α}-Boc-N^{α}-Me-phenylalanine (**9**) following established protocols [5,6], the two units were coupled using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) and 1-hydroxybenzotriazole (HOBt) as coupling reagents to afford dipeptide **11** (62.1%). The product was treated with 4 M HCl in ethyl acetate to remove the Boc group and then, coupled with Cbz-Ala-OH (**13**) to produce tripeptide **14** smoothly in 76.6% yield over two steps. Debenzyloxycarbonylation of **14** under catalytic hydrogenolysis gave primary amine **15**, which was coupled with Boc-Leu-OH (**16**) under the same conditions mentioned above to give the linear tetrapeptide **3** in 60% yield over two steps.

The next effort was to synthesize fragment **6** as shown in Scheme 2. L-Valine benzyl ester (**17**) which was prepared according to the literature [7] and commercially available L-lactic acid (**18**) were coupled using EDCI and 1-hydroxy-7-azabenzotriazole (HOAt) as coupling reagents to form **6**. However, NMR data indicated that significant racemization occurred during the condensation. The phenomenon could be explained by keto–enol tautomerism of the activated L-lactic acid, which was promoted by the unprotected hydroxy group (Scheme 2). In order to avoid the racemization, benzylate was employed to protect the hydroxy group first [8]. To our delight, no racemization was detected and dipeptide **6b** was obtained efficiently (95%).

In order to construct fragment **5**, N^{α}-Fmoc-N^{α}-Me-glutamine (**8**) must be first prepared. Though the majority of naturally occurring NMAs (*N*-methyl amino acids) are aliphatic type (MePhe, MeAla, MeGly, MeVal, MeIle, MeLeu), a number of others contain basic, acidic, and alcoholic side chains. The syntheses of these NMAs are often not straightforward. Many methods have been reported to synthesize NMAs, such as *N*-methylation by alkylation [9], reductive amination [10], 5-oxazolidinones [11], asymmetric syntheses [12],

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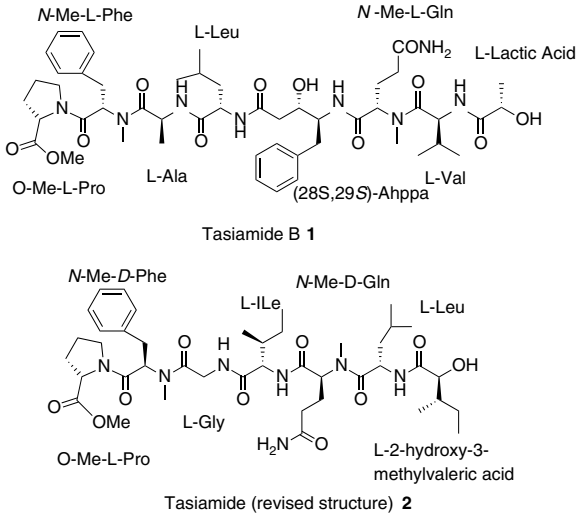


Figure 1. Structures of tasiamide B (**1**) and tasiamide (**2**).

and so on. Different strategies are applied for different amino acids. In this article, the widely used method 5-oxazolidinones was applied for the synthesis of (**8**) which has a reactive side chain amide.

Starting from commercially available N^{α}-Fmoc-N^{δ}-trityl-glutamine (**20**), (**8**) was obtained via oxazolidinone intermediate (**21**) and reductive ring-opening following Freidinger's procedure [13] as shown in Scheme 3.

The next effort was to construct (3*S*, 4*S*)-4-amino-3-hydroxy-5-phenylpentanoic acid (Ahppa), the most unique residue in tasiamide B. Several methods have been reported. Pedrosa *et al*. used α-aminoaldehyde as the starting material to obtain γ-amino-β-hydroxy acids via a Reformatsky reaction [14]. However, this method has a low stereoselectivity (*anti*:*syn* = 74: 26). Starting from phenyl acetaldehyde, Kumar *et al*. carried out the Wittig reaction and Sharpless aminohydroxylation to afford the amino alcohol. After several protection group manipulations, the target γ-amino β-hydroxy acids were furnished by the Wolff rearrangement [15]. Apparently, this method has a long route and a poor yield.

In this article, Ahppa was prepared according to the method reported by Hoffman in 1997 [16] (Scheme 4). First, **24** was obtained by methyl esterification and *N*,*N*-dibenzylation of commercially available phenylalanine **22**. Then, Claisen condensation of **24** with lithio *tert*-butyl acetate, followed by the reduction of β-ketoester **25** using sodium borohydride in absolute methanol, gave the major product *syn*-**26** (*dr* = 17: 1). It is worth mentioning that the alkaline hydrolysis of compound **24** was omitted because of the low yield and significant racemization during saponification. After dedibenzylation of *syn*-**26** under Palladium-catalyzed hydrogenolysis, *tert*-butyl (3*S*, 4*S*)-Ahppa (**7**) was obtained with satisfactory yields and high enantioselectivity.

With fragment **7** and **8** in hand, the stage was now set to carry out their coupling. First, amino acid **8** is to be activated by HOAt/EDCI before it was incorporated into the peptide chain. However, **8**-**H** rather than **5** was obtained as the major product (Scheme 5). What is worse, the minor but desired dipeptide **5** was found to be easily lactamizated when the protected group (Fmoc) was removed. Thus, **4b** could not be obtained employing the present strategy.

As it can be seen from Scheme 6, the undesired result could be attributed to the unprotected amide of glutamine. Although glutamine is usually used without side chain protection in peptide synthesis in solution, in our synthesis, as a flexible molecular, the presence of *N*-Me in glutamine not only enhanced the bend of the peptide chain but also the nucleophilicity of the secondary N atom. Further more, the amide of glutamine was also vulnerable

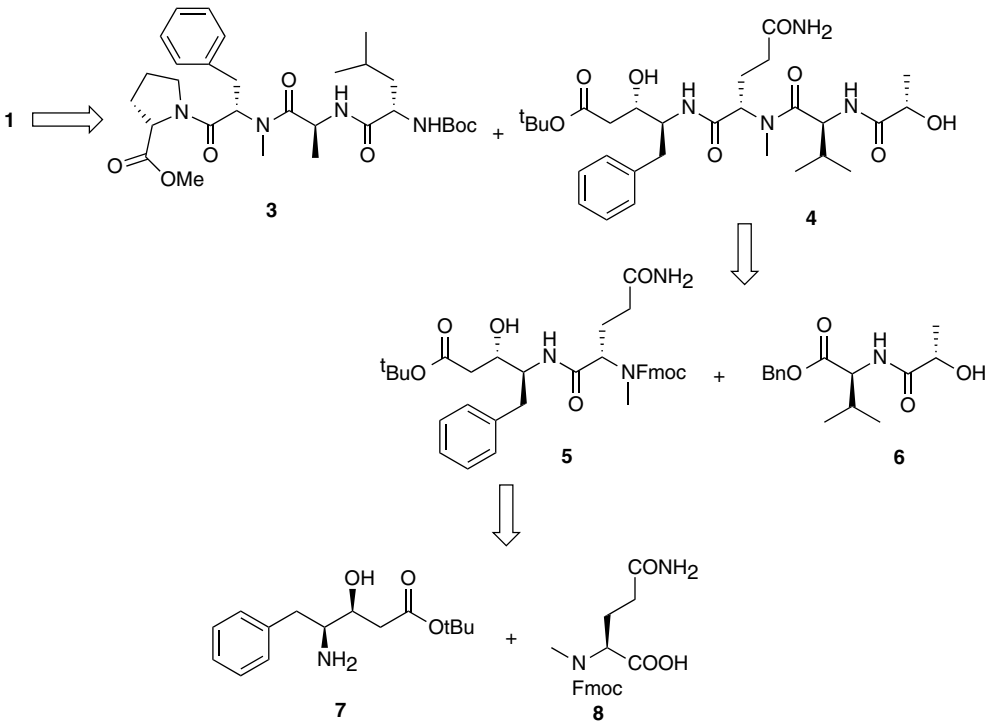
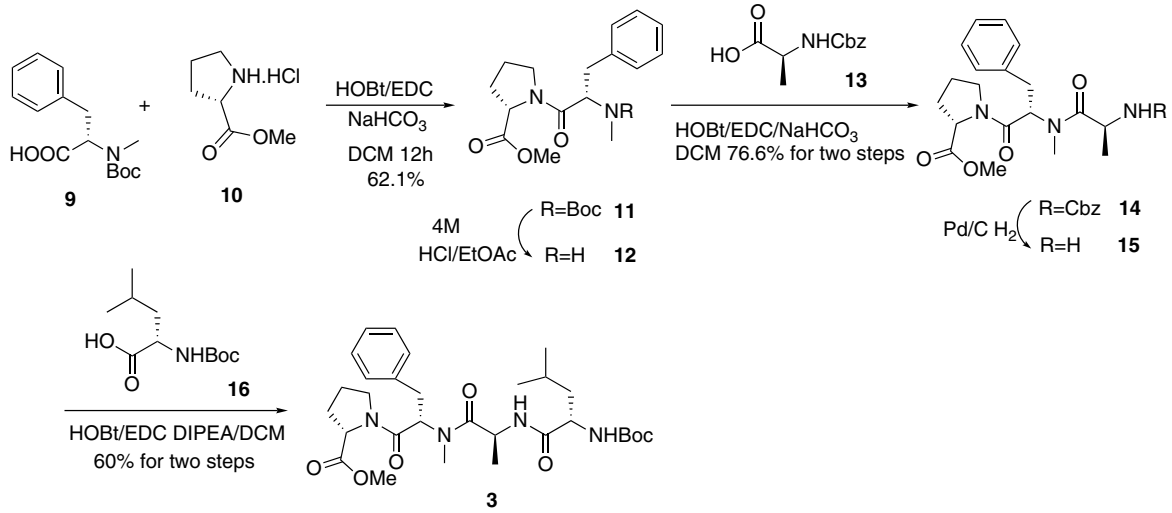
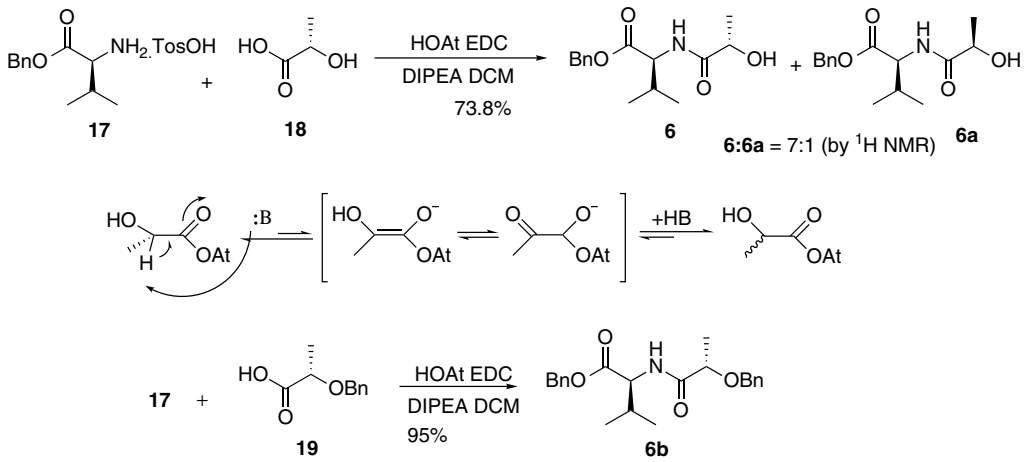


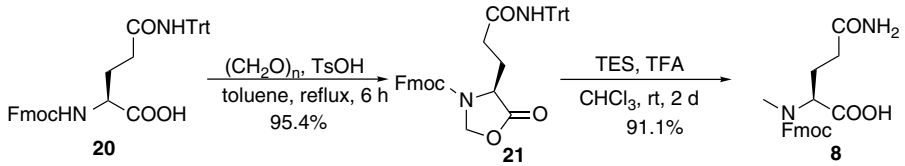
Figure 2. Retrosynthetic analysis of **1**.



Scheme 1. Synthesis of fragment **3**.



Scheme 2. Synthesis of fragment **6b**.



Scheme 3. Synthesis of N^{α}-Fmoc-N^{α}-Me-glutamine (**8**).

to nucleophilic attack. So N^{α}-Me-glutamine could be elongated neither from the *C*-terminus nor from the *N*-terminus, which was observed for the first time. Therefore, as for the liquid phase synthesis, the free amide group must be sealed for peptide elongation.

First, Trt was employed to protect the side chain amide of Fmoc-Gln-OH. However, desired product was not obtained because of the steric hindrance between Fmoc and Trt. Then, the commercially available reagent bearing smaller size protected group (**29**) was adopted. Trt group was employed to protect its amide and it did well [17] (Scheme 7). The following *N*-methylation was carried out to provide **28** using sodium hydride and methyl iodide [6]. Unavoidably, a small amount of **28b** was produced as a byproduct. The next step was to synthesize fragment **5b**. As expected, no pyroglutamylated byproduct was detected during this coupling step. Debenzyloxycarbonylation of **5b** produced **31** and alkaline hydrolysis of **6b** using $LiOH{\cdot}H_2O$ produced **27**, which were coupled to give linear tetrapeptide **4c** with a yield of 65% for three steps.

With tetrapeptides **4c** and **3** in hand, the stage was finally set to carry out their coupling (Scheme 8). First, the Trt group and *tert*-butyl ester of **4c** were removed using TFA–dichloromethane = 1 : 1, simultaneously under the same condition **33** was prepared by **3**. Then, the coupling of **32** and **33** provided octapeptide **34** (38% for three steps). Finally, the synthetic tasiamide B (**1**) was obtained smoothly after debenzylation of **34** under Palladium-catalyzed hydrogenolysis (91%).

To our surprise, the analytical data of **1** was inconsistent with those published for natural product. The optical rotation value of

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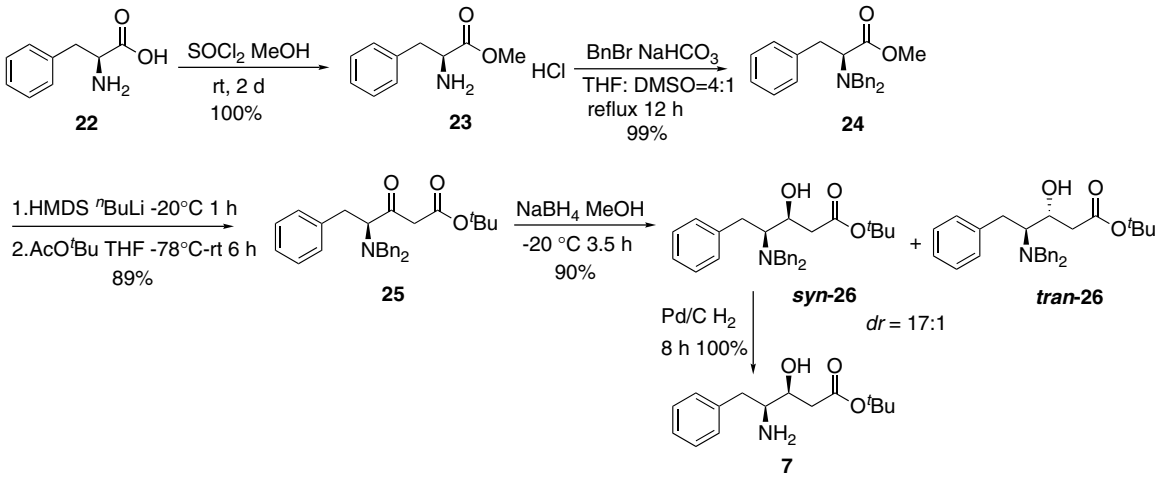
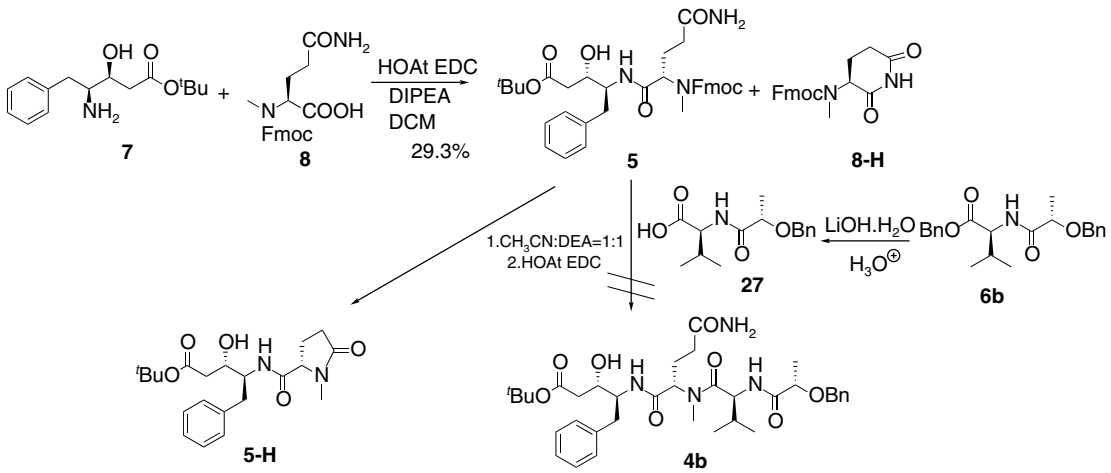
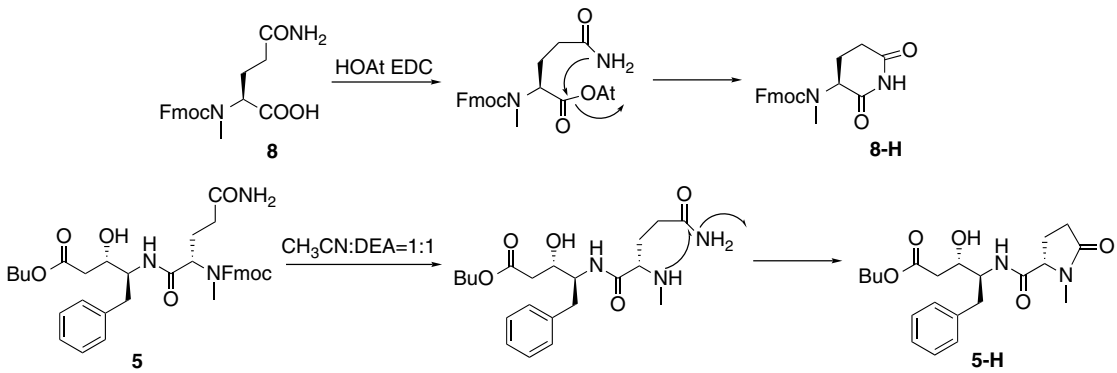


Scheme 4. Synthesis of protected (3*S*, 4*S*)-Ahppa (**7**).



Scheme 5. Unsuccessful synthesis of **4b**.

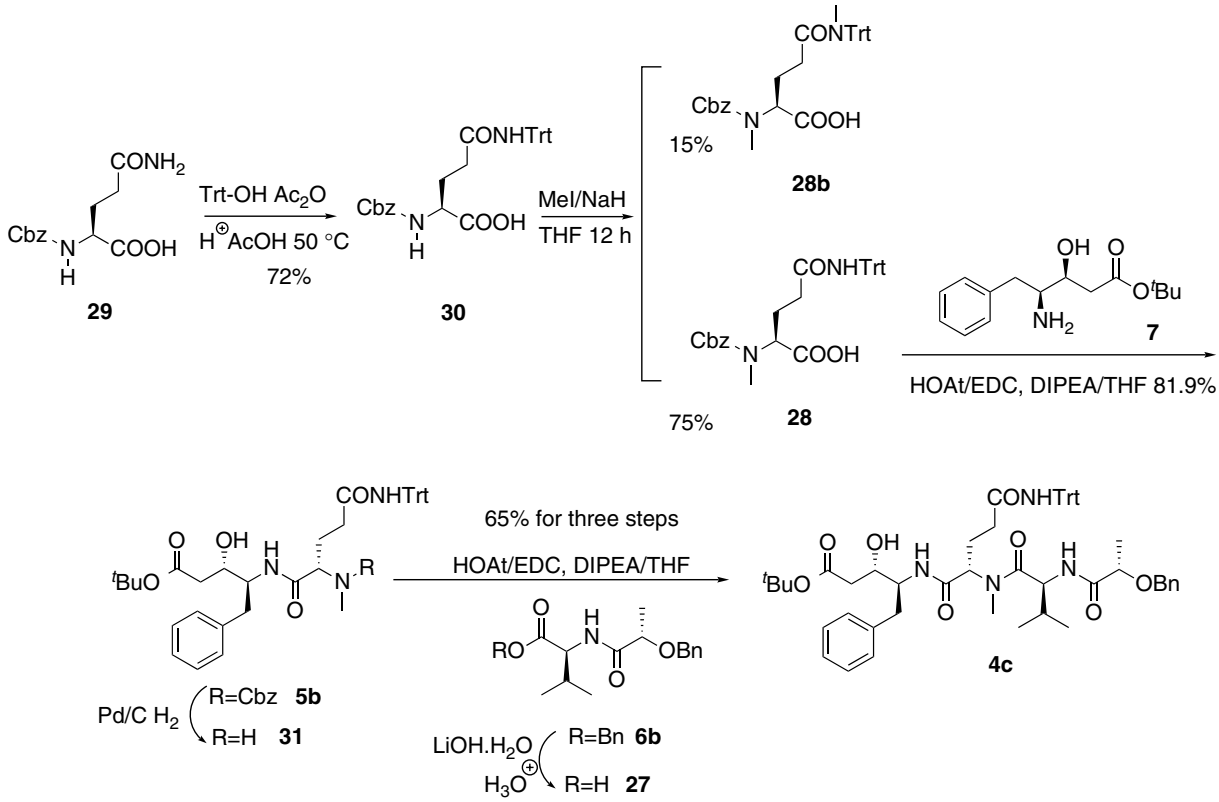


Scheme 6. Proposed mechanism for the formation of **8**-**H** and **5**-**H**.

the synthetic sample was much higher than that of the natural product {$[\alpha]^{20}_D = -89.6$ (*c* 0.4, MeOH) while lit. $[\alpha]^{21}_D = -28$ (*c* 0.4, MeOH)}. According to the HMQC/HMBC analysis, we found that the ^{13}C NMR signals assigned to N^{α}-Me-L-phenylalanine and L-alanine of **1** were quite different from those reported for the natural product (**Table 1**).

Meanwhile, we noticed that the structural analog tasiamide contained a N^{α}-Me-D-phenylalanine. Therefore, we inferred that the structure of the natural product might consist of N^{α}-Me-D-phenylalanine or D-alanine or both of them. To confirm our hypothesis, three diastereomers of **1** were designed and synthesized.

N^{α}-Me-L-phenylalanine of **1** was replaced by N^{α}-Me-D-phenylalanine to get diastereomer **1a**; L-alanine was replaced by D-alanine to give diastereomer **1b**; and both were replaced giving diastereomer **1c** (Figure 3).



Scheme 7. Synthesis of fragment **4c**.

The synthetic methods of the three diastereomers were mostly similar to the synthesis of **1**. Exception was the last condensation step, in which HATU instead of EDCI was employed as the coupling reagent to enhance the reaction efficiency (from 40% to 60% for three steps) and shorten the reaction time (from 48 to 15 h).

After complete NMR analysis (H–H COSY, HMQC, and HMBC; Supporting information), we found that all of the four synthetic diastereomers (**1, 1a, 1b**, and **1c**) showed no significant difference in their ^{1}H NMRs. However, their ^{13}C NMR data and optical rotations are quite different (Figure 4). Among them, **1a** provided a near-perfect match with the NMR data reported for tasiamide B, and the value of optical rotation of this synthetic sample was also the same as that of the natural product {$[\alpha]^{20}_{D} = -30.1$ (*c* 0.4, MeOH) while lit. $[\alpha]^{21}_{D} = -28$ (*c* 0.4, MeOH)}. All the details suggested that the N^{α}-Me-L-phenylalanine residue in tasiamide B has the D-configuration.

Lastly, all of the four synthesized octapeptides (**1, 1a, 1b**, and **1c**) were subjected to biological test on several cancer cell lines (A549, LoVo, KB, BEL-7402, P388, and HL-60). The results indicated that none of these synthetic peptides showed remarkable cytotoxicity. This was not fully consistent with Williams and co-workers' report, as compound **1a** (isolated from the marine cyanobacterium *Symploca* sp.) displayed an IC_{50} value of 0.8 μM against KB cells in their hands [2]. Further studies on tasiamide B structural modification are currently under way in our laboratory and will be reported in due course.

Conclusions

In summary, we have finished the first total synthesis of tasiamide B (**1**), a promising linear peptide with cytotoxic activity and configurational reassignment of the previously assigned N^{α}-Me-L-phenylalanine residue as N^{α}-Me-D-phenylalanine.

Experimental

General Information

Solvents were purified by standard methods. TLCs were carried out on Merck 60 F_{254} silica gel plates and visualized by UV irradiation or by staining with iodine absorbed on silica gel, ninhydrin solution or with aqueous acidic ammonium molybdate solution as appropriate. 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 spectrometers. Chemical shifts are reported in parts per million (ppm), relative to the signals due to the solvent. Data are described as followings: 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.

Chemistry

Synthesis of 3

*N^{α}-Boc-N^{α}-Me-Phe-L-Pro-OMe (**11**).* A solution of L-proline methyl ester (**10**) (1.19 g, 7.2 mmol) and N^{α}-Boc-N^{α}-Me-D-phenylalanine (**9**) (1.30 g, 4.6 mmol) in anhydrous CH_2Cl_2 (50 ml) was treated sequentially with HOBt (0.97 g, 7.2 mmol), EDCI (1.38 g, 7.2 mmol), and $NaHCO_3$ (0.76 g, 9.0 mmol) at 0 °C. The mixture was warmed to room temperature and stirred overnight. After diluted with

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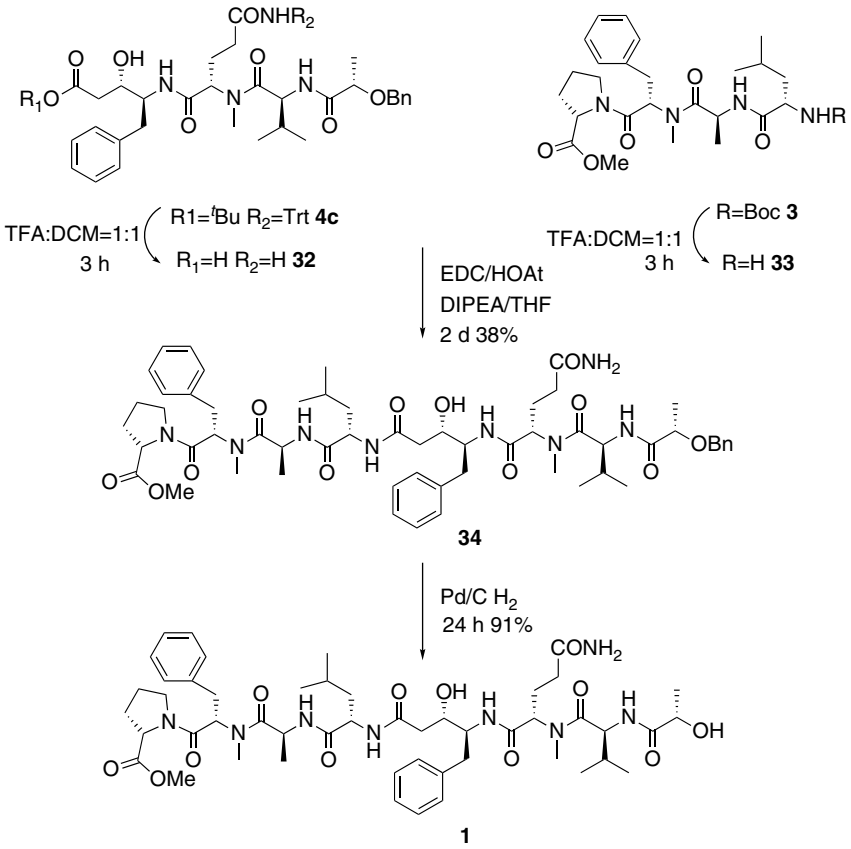
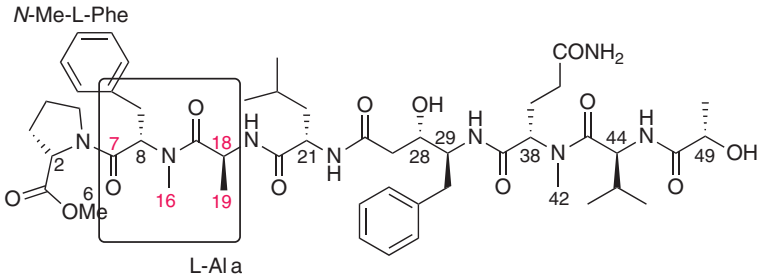


Scheme 8. Synthesis of **1**.

Table 1. Main differences of the ^{13}C NMR value between **1** and the reported tasiamide B

C no.	δ_C (lit.)	δ_C (compd. **1**)	$\Delta\delta_C$
7	168.1, s	169.2, s	+1.1
16	31.8, q	31.3, q	−0.5
18	45.2, d	45.8, d	+0.6
19	17.3, q	17.8, q	+0.5



EtOAc (200 ml), the whole mixture was washed with 10% citric acid (2 × 30 ml), 5% $NaHCO_3$ (2 × 30 ml), H_2O (2 × 30 ml), and brine (2 × 30 ml), dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by flash column chromatography with petroleum ether–EtOAc (20 : 1–5 : 1) to afford **11** as colorless oil (1.128 g, 62.1%). R_f 0.60 (1 : 1, petroleum ether–EtOAc); $[\alpha]^{21}_D = -130.18$ (c 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 600 MHz) four rotamers δ: 7.27–7.15 (m, 5 H, ArH), 5.27, 5.13 (dd, 0.5 H, 4 : 1, J = 6.1, 8.8 Hz, Phe α-H), 4.92, 4.80 (dd, 0.5 H, 4 : 1, J = 4.4, 10.4 Hz, Phe α-H), 4.48, 4.44 (dd, 1 H, 1 : 1, J = 2.8, 8.2 Hz, Pro α-H), 3.75, 3.70, 3.67 (s, 3 H, 5 : 4 : 1, Pro $COOCH_3$), 3.55 (m, 1 H, Pro δ-Ha), 3.36 (m, 1 H, Pro δ-Hb), 3.15, 3.05 (dd, 1 H, 5 : 4, J = 9.4, 18.2 Hz, Phe β-Ha), 2.97, 2.91 (dd, 1H, 4 : 1, J = 9.9, 14.3 Hz, Phe β-Hb), 2.82, 2.79, 2.69, 2.66 (s, 3H, 5 : 5 : 1 : 1, Phe *N*-CH_3), 2.16–1.92 (m, 4 H, Pro γ-H + β-H), 1.32, 1.16 (s, 9 H, 1 : 2, $C(CH_3)_3$); ^{13}C NMR ($CDCl_3$, 150 MHz) δ172.8, 172.7, 169.2, 138.1 (Ar C-1), 129.5 (Ar C-3 + C-5), 128.5 (Ar C-2 + C-6), 126.5 (Ar C-4), 59.3 (Pro α-C), 56.5 (Phe α-C), 52.3 (OCH_3), 46.4 (Pro δ-C), 35.0, 29.9, 29.2, 29.0, 28.1 ($C(CH_3)_3$), 24.9; ESI-MS (*m/z*): 391.2 $[M + H]^+$, 413.2 $[M + Na]^+$, 429.2 $[M + K]^+$ (calcd. 390.22).

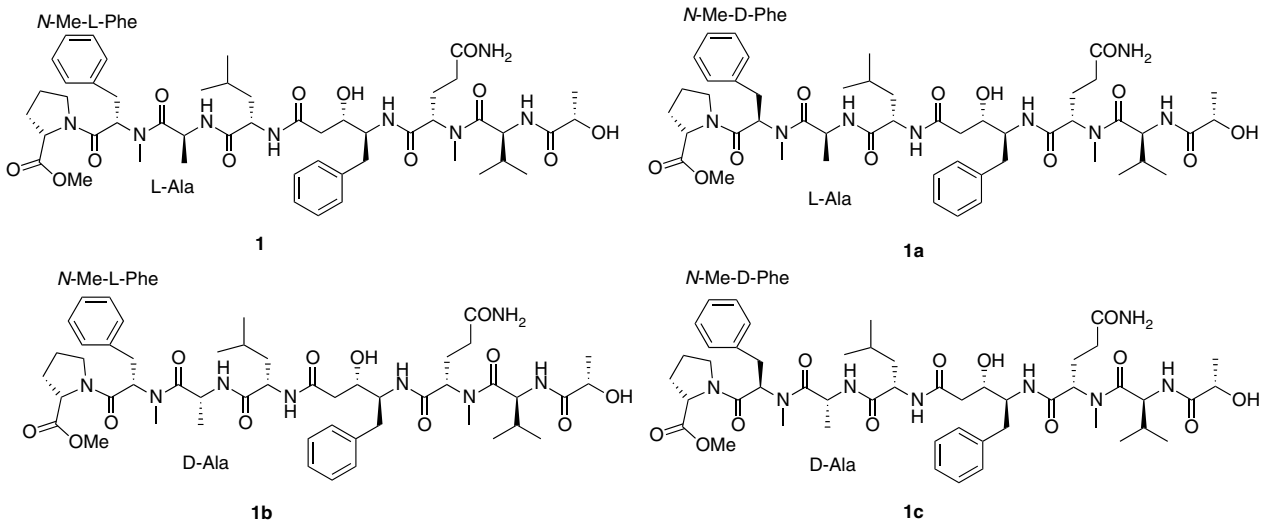


Figure 3. Structures of compound **1** and its three diastereomers (**1a, 1b**, and **1c**).

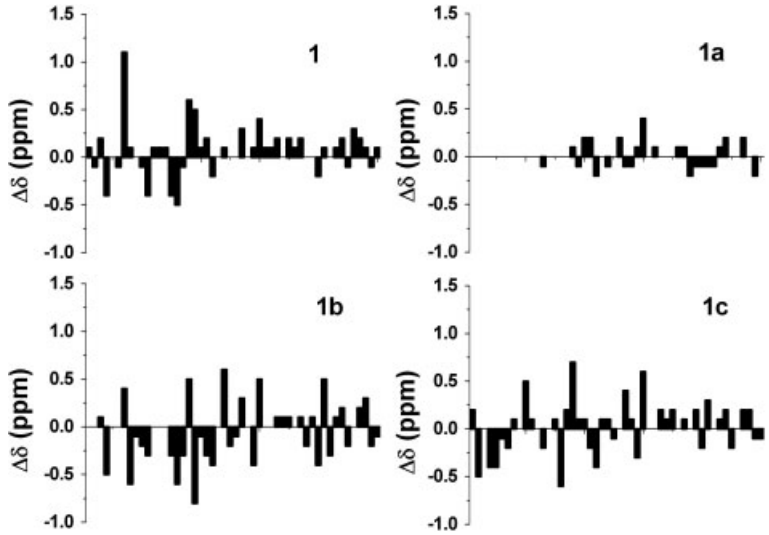


Figure 4. Differences in ^{13}C NMR shifts between the natural product, synthetic **1,1a, 1b**, and **1c**.

*Cbz-Ala-N$^{\alpha}$-Me-Phe-L-Pro-OMe (**14**).* To a solution of **11** (0.40 g, 1.02 mmol) in EtOAc (5 ml) at 0 °C, 4 M HCl/EtOAc (15 ml) was added. The mixture was stirred for 1 h and then evaporated, the residual oil was dissolved twice in CH_2Cl_2 (10 ml) with evaporation each time to give HCl salt **12**, which was used directly in the next step.

A solution of **12** and Cbz-Ala-OH (**13**) (0.27 g, 1.23 mmol) in anhydrous CH_2Cl_2 (25 ml) was treated sequentially with HOBt (0.21g, 1.54 mmol), EDCI (0.29 g, 1.54 mmol), and $NaHCO_3$ (0.17 g, 2.05 mmol) at 0 °C. The solution was stirred for 30 min, warmed to room temperature and stirred for another 12 h. And then, the mixture was treated as described for **11**. The residue was purified by flash column chromatography with petroleum ether–EtOAc (10 : 1–1 : 1) to afford **14** as a white solid (388.3 mg, 76.6%). R_f 0.45 (3 : 2, petroleum ether–EtOAc); $[\alpha]^{20}_D = -72.25$ (*c* 0.5, $CHCl_3$); ^1H NMR ($CDCl_3$, 600 MHz) two rotamers δ: 7.37–7.14 (m, 10 H, ArH), 5.59 (t, 1 H, $J = 7.7$ Hz, Phe α-H), 5.47 (d, 1H, $J = 8.3$ Hz, Ala α-H), 5.08 (s, 2H, PhCH_2O), 4.43 (dd, 1 H, $J = 4.4$, 8.8 Hz, Pro α-H), 3.70, 3.66 (s, 3 H, 9 : 2, Pro COOCH_3), 3.54 (m, 1 H, Pro δ-Ha), 3.35 (m, 1 H, Pro δ-Hb), 3.26 (dd, 1 H, $J = 7.1$, 14.3 Hz, Phe β-Ha), 3.11, 3.09 (s, 3 H, 1 : 5, Phe *N*-CH_3), 2.98 (dd, 1 H, $J = 8.3$, 14.3 Hz, Phe β-Hb) 2.15 (m, 1H, Pro β-Ha), 2.04 (s, 3H, Ala β-CH_3), 1.95–1.85 (m, 3H, Pro γ-H + β-Hb); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 173.1, 172.1, 169.4, 155.3 (NHCOOCH_2Ph), 136.6, 136.5, 129.2 (two), 128.6 (two), 128.5 (two), 128.2, 128.1 (two), 126.9, 66.8 (NHCOOCH_2Ph), 59.1 (α-C), 55.5 (α-C), 52.3 (OCH_3), 47.2, 46.9, 34.9 (Phe α-C), 31.1 (*N*-CH_3), 29.1 (Pro β-C), 24.9 (Pro γ-C), 18.7 (Ala β-C); ESI-MS (*m/z*): 518.2 $[M + Na]^+$, 534.2 $[M + K]^+$ (calcd. 495.24).

N$^{\alpha}$-Boc-Leu-Ala-N$^{\alpha}$-Me-Phe-L-Pro-OMe (3). Hydrogenation of **14** (373 mg, 0.75 mmol) was carried out in EtOAc–EtOH (1 : 4, 40 ml) in the presence of a catalytic amount of Pd-C (10%) under hydrogen at room temperature. Pd-C was removed by filtration and concentrated under reduced pressure to yield the amine **15**, which was used directly in the next step.

A solution of **15** and Boc-Leu-OH (**16**) (225 mg, 0.90 mmol) in anhydrous CH_2Cl_2 (40 ml) was treated sequentially with HOBt

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(152 mg, 1.13 mmol), EDCI (216 mg, 1.13 mmol), and $NaHCO_3$ (126 mg, 1.50 mmol) at 0 °C. The solution was stirred for 30 min, warmed to room temperature and stirred for another 12 h. And then, the reaction mixture was treated as described for **11**. The residue was purified by flash column chromatography with petroleum ether–EtOAc (5 : 1–1 : 2) to afford **3** as a white solid (257 mg, 60.0%). R_f 0.50 (EtOAc); $[\alpha]^{20}_D = -87.63$ (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.27–7.19 (m, 5 H, ArH), 6.74 (d, 1H, $J = 7.1$ Hz, Ala NH), 5.60 (t, 1 H, $J = 8.2$ Hz, Phe α-H), 4.73 (t, 1H, $J = 7.1$ Hz, Ala α-H), 4.43 (dd, 1 H, $J = 4.4$, 8.8 Hz, Pro α-H), 4.05 (d, 1H, $J = 3.8$ Hz, Leu α-H), 3.70, 3.69 (s, 3 H, 6 : 1, Pro COO*CH*$_3$), 3.55 (m, 1 H, Pro δ-Ha), 3.37 (m, 1 H, Pro δ-Hb), 3.26 (dd, 1 H, $J = 7.1$, 14.3 Hz, Phe β-Ha), 3.13, 3.07 (s, 3 H, 1 : 5, Phe *N*-CH_3), 2.97 (dd, 1 H, $J = 8.3$, 14.3 Hz, Phe β-Hb), 2.15 (m, 1H, Pro β-Ha), 1.97–1.88 (m, 3H, Pro γ-H × 2 + β-Hb), 1.61 (m, 1H, Leu γ-H), 1.51 (m, 1H, Leu β-Ha), 1.40–1.44 (m, 10H, Leu β-Hb + C(CH_3)$_3$), 1.26 (d, 3H, $J = 6.6$ Hz, Ala β-CH_3), 0.92 (d, 6H, $J = 6.6$ Hz, Leu δ-CH_3); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 172.7, 172.4, 171.7, 169.3, 155.6 (NHCOOC(CH_3)$_3$), 136.6 (Ar C-1), 129.1 (Ar C-3 + C-5), 128.6 (Ar C-2 + C-6), 126.9 (Ar C-4), 80.0 (NHCOO*C*(CH_3)$_3$), 59.1 (Pro α-C), 55.3 (Phe α-C), 53.2 (Leu α-C), 52.4 (OCH_3), 46.9 (Pro δ-C), 45.7 (Ala α-C), 41.8 (Leu β-C), 34.8 (Phe β-C), 31.0 (*N*-CH_3), 29.1 (Pro β-C), 28.4 (C(*C*H$_3$)$_3$), 25.0 (Leu γ-C), 24.8 (Pro γ-C), 23.1 (Leu δ-C × 2), 18.5 (Ala β-C); ESI-MS (*m/z*): 575.4 $[M + H]^+$, 597.3 $[M + Na]^+$, 613.3 $[M + K]^+$ (calcd. 574.34).

Synthesis of **4c**

H-Phe-OMe (***23***). To a solution of H-Phe-OH (**22**) (6.61 g, 40 mmol) in dry MeOH (40 ml) at −10 °C under argon, 12 ml of $SOCl_2$ was added dropwise. After stirring for 30 min at −10 °C, the reaction was allowed to warm to room temperature and stirred for another 2 days. Then, the reaction mixture was dissolved twice in MeOH (30 ml) with evaporation each time to yield the **23** as a white solid (8.6 g, 100%). R_f 0.70 (10 : 1, $CHCl_3$-MeOH); ^{1}H NMR (DMSO-d_6, 600 MHz) δ: 8.74 (s, 2H, NH), 7.34–7.23 (m, 5H, ArH), 4.23 (t, 1H, $J = 7.8$ Hz, α-H), 3.65 (s, 3H, OCH_3), 3.21 (dd, 1H, $J = 5.5$, 14.3 Hz, β-Ha), 3.10 (dd, 1H, $J = 7.7$, 14.3 Hz, β-Hb).

N,N-Bn$_2$*-Phe-OMe* (***24***). H-Phe-OMe (**23**) (4.31g, 20 mmol), BnBr (7.14 ml, 60 mmol), and $NaHCO_3$ (8.4 g, 100 mmol), were suspended in 50 ml THF and 12 ml DMSO and refluxed for 12 h. Then, the reaction was allowed to cool off to room temperature, diluted with water (50 ml), 5 ml of MeOH was added and stirred for 10 min. The solution was extracted with EtOAc (3 × 70 ml). The organic layer was then washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography with petroleum ether–EtOAc (80 : 1–60 : 1) to afford **24** as colorless transparent oil (7.1 g, 99%). R_f 0.70 (20 : 1, petroleum ether–EtOAc); ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.33–7.05 (m, 15H, ArH), 3.99 (d, 2H, $J = 13.7$ Hz, PhC*Ha*N), 3.77 (s, 3H, OCH_3), 3.71 (t, 1H, $J = 7.7$ Hz, α-H), 3.58 (d, 2H, $J = 13.7$ Hz, PhC*Hb*N), 3.16 (dd, 1H, $J = 7.1$, 13.7 Hz, β-Ha), 3.04 (dd, 1H, $J = 8.2$, 13.2 Hz, β-Hb).

tert-Butyl 4-(N,N-dibenzylamino)-3-oxo-5-phenylpentanoate (***25***). To a stirred solution of hexamethyldisiloxane (HMDS) (8 ml, 38.4 mmol) in 24 ml THF, ^{n}BuLi (14.4 ml, 36 mmol, 2.5 M in hexane) was added slowly at −20 °C under argon. The solution was stirred for 0.5 h, cooled down to −78 °C, then *tert*-butyl acetate (4.65 ml, 36 mmol) was added and stirred for 1 h. Then, the solution of **24** (4.32 g, 12 mmol) in 24 ml THF was added dropwise to the colorless solution of the lithium enloate at −78 °C under argon. The resulting mixture was stirred for 30 min, warmed to room temperature and stirred for another 6 h. The solution was then quenched with 1 M HCl (200 ml), and extracted with ethyl acetate (3 × 100 ml). The organic extracts were combined, washed with brine, dried (Na_2SO_4), and concentrated under reduced pressure. The residue was purified by flash column chromatography with petroleum ether-EtOAc (80 : 1–60 : 1) to afford **25** as colorless transparent oil (4.73 g, 89%). R_f 0.70 (20 : 1, petroleum ether-EtOAc); ^{1}H NMR ($CDCl_3$, 600 MHz) δ:7.34–7.15 (m, 15H, ArH), 3.81 (d, 2H, $J = 13.2$ Hz, 2PhC*Ha*N), 3.62 (dd, 1H, $J = 3.3$, 8.8 Hz, Phe α-H), 3.56 (d, 1H, $J = 18.2$ Hz, COC*Ha*CO), 3.54 (d, 2H, $J = 13.7$ Hz, 2PhC*Hb*N), 3.37 (d, 1H, $J = 15.4$ Hz, COC*Hb*CO), 3.20 (dd, 1H, $J = 8.8$, 13.2 Hz, Phe β-Ha), 2.92 (m, 1H, Phe β-Hb), 1.25 (s, 9H, C(CH_3)$_3$).

*(3S,4S)-tert-Butyl 4-(N, N-dibenzylamino)-3-hydroxy-5-phenylpentanoate (syn-****26****).* 3-Oxo ester **25** (463 mg, 1.25 mmol) was dissolved in anhydrous methanol (25 ml). The resulting solution was then cooled to −22 °C and treated with $NaBH_4$ (166 mg, 4.38 mmol). The reaction was monitored by TLC. After 3.5 h, the solution was quenched with H_2O (100 ml) at pH = 5–6 (adjusted by 1 M HCl), extracted with ether (3 × 100 ml), washed by brine (100 ml), dried (Na_2SO_4), and concentrated under reduced pressure. The residue was purified by flash column chromatography with petroleum ether–EtOAc (60 : 1–20 : 1) to afford *syn*-**26** as a colorless oil (397 mg, 85%); R_f 0.55 (10 : 1, petroleum ether–EtOAc); $[\alpha]^{21}_D = 14.3$ (c 1.35, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.19–7.36 (m, 15H, ArH), 4.01 (m, 2H, 2PhC*Ha*N), 3.95 (m, 1H, Ahppa H-3), 3.43 (d, 2H, $J = 13.1$ Hz, 2PhC*Hb*N), 3.11 (d, 1H, $J = 10.4$ Hz, Ahppa H-5a), 2.86 (m, 1H, Ahppa H-5b), 2.78 (m, 1H, Ahppa H-4), 2.40 (dd, 1H, $J = 9.9$, 16.0 Hz, Ahppa H-2a), 2.04 (m, 1H, Ahppa H-2b), 1.39 (s, 9H, C(CH_3)$_3$); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 192.5 (C-1), 134.6 (Ar C-1 × 3), 129.8, 129.4, 129.3, 129.2, 129.1, 128.8, 128.7, 81.9 (*C*(CH_3)$_3$), 63.0 (C-4), 54.9 (N(CH_2Ph)$_2$), 40.9 (C-2), 34.8 (C-5), 28.1 (C(*C*H$_3$)$_3$). ESI-MS (*m/z*): 446.2 $[M + H]^+$ (Calcd 445.3).

(3S,4S)-tert-Butyl 4-amino-3-hydroxy-5-phenylpentanoate (Ahppa) (***7***). Hydrogenation of *syn*-**26** (490 mg, 1.1 mmol) was carried out in EtOAc (40 ml) in the presence of a catalytic amount of Pd-C (10%) under hydrogen at room temperature. The reaction mixture was stirred for 8 h and the catalyst Pd-C was removed by filtration, then the resulting filtrate was reconcentrated under reduced pressure to yield the amine **7** as a white solid (292 mg, 100%); ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.20–7.31 (m, 5H, ArH), 3.90 (m, 1H, H-3), 2.99 (m, 1H, H-5a), 2.92 (m, 1H, H-5b), 2.64 (m, 1H, H-4), 2.56 (dd, 1H, $J = 8.8$, 13.8 Hz, H-2a), 2.45 (dd, 1H, $J = 8.8$, 16.0 Hz, H-2b).

N-Cbz-Gln(Trt)-OH (***30***). Cbz-Gln-OH (**29**) (4.2 g, 15 mmol), Trt-OH (7.8 g, 30 mmol), Ac_2O (3 ml, 30 mmol), and 75.3 µl conc. H_2SO_4 were suspended in 50 ml of glacial acetic acid and stirred for 3 h at 50 °C, The solution was then slowly added to 150 ml cold H_2O, the precipitate filtered off, dissolved in 150 ml ethyl acetate, washed with H_2O, dried, evaporated and crystallized from ethyl acetate-hexane to afford **30** as a white solid (5.6 g, 72%). R_f 0.25 (10 : 1, $CHCl_3$ – MeOH); ^{1}H NMR (DMSO-d_6, 600 MHz) δ: 8.62 (s, 1H, COOH), 7.54 (d, 1H, $J = 7.7$ Hz, NH), 7.15–7.36 (m, 20H, ArH), 5.04 (m, 2H, Ph*C*H$_2$O), 3.94 (m, 1H, α-H), 2.31–2.41 (m, 2H, γ-H × 2), 1.90 (m, 1H, β-Ha), 1.68 (m, 1H, β-Hb).

N-Cbz-N-Me-Gln(Trt)-OH (***28***). To a solution of *N*-Cbz-Gln (Trt)-OH (**30**) (1.04 g, 2 mmol) in dry THF (25 ml) at 0 °C under argon, NaH (60 wt % in mineral oil, 160 mg, 4 mmol) was added. After stirring for 30 min at 0 °C, MeI (186.8 µl, 3 mmol) was added. The reaction was allowed to warm to room temperature and stirred for another 16 h. The reaction mixture was quenched with water (2 ml) and then concentrated under reduced pressure. The residue was diluted with water (100 ml), washed twice with *n*-hexane (15 ml). The aqueous layer was acidified with HCl (0.1 M) until pH = 1–2 and extracted with EtOAc (3 × 50 ml). The organic layer was then washed with saturated $Na_2S_2O_3$ and then brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give **28** as a white solid (0.80 g, 75%). R_f 0.32 (10 : 1, $CHCl_3$–MeOH); $[\alpha]^{20}_D = -13.23$ (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 600 MHz) two coformers δ: 7.14–7.36 (m, 20H, ArH), 6.79, 6.59 (s, 1H, 2 : 1, CON*H*Trt), 5.01–5.20 (m, 2H, PhCH_2O), 4.77, 4.68 (d, 1H, J = 5.5, 9.9 Hz, 1 : 2, α-H), 2.88, 2.86 (s, 3H, *N*-Me), 2.72, 2.65 (m, 1H, γ-Ha), 2.20–2.35 (m, 3H, γ-Hb, β-H × 2). ESI-MS (*m/z*): 537.2976 $[M + H]^+$, 559.2601 $[M + Na]^+$ (Calcd 536.23).

N-Cbz-N-Me-Gln(Trt)-Ahppa-O^{t}Bu (***5b***). A solution of **28** (536.2 mg, 1.0 mmol) and **7** (292 mg, 1.1 mmol) in anhydrous THF (50 ml) was treated sequentially with HOAt (204.3 mg, 1.5 mmol), EDCI (287.6 mg, 1.5 mmol), and DIPEA (349.2 µl, 2.0 mmol) at 0 °C. The solution was stirred for 30 min, warmed to room temperature and stirred for another 12 h. And then, the mixture was treated as described for **11**. The residue was purified by flash column chromatography with petroleum ether–EtOAc (10 : 1–2 : 1) to afford **5b** as a white solid (642 mg, 81.9%). R_f 0.52 (1 : 1, petroleum ether–EtOAc); $[\alpha]^{20}_D = -64.63$ (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 600 MHz) two coformers δ: 716–7.39 (m, 25H, ArH), 6.72, 6.57 (s, 1H, CON*H*Trt), 6.36, 6.05 (d, 1H, J = 8.2 Hz, Ahppa N*H*), 5.15 (s, 2H, PhCH_2O), 4.61, 4.52 (m, 1H, Ahppa H-3), 4.07 (m, 1H, Ahppa H-4), 4.00 (m, 1H, Gln α-H), 2.74–2.89 (m, 2H, Ahppa H-5 × 2), 2.51 (s, 3H, *N*-CH_3), 2.14–2.34 (m, 5H, Ahppa H-2a + Gln γ-H × 2 + β-H × 2), 1.92 (m, 1H, Ahppa H-2b), 1.44 (s, 9H, C(CH_3)$_3$); ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 172.8, 170.8, 157.4 (PhCH_2OCO), 144.7, 137.8, 136.4, 129.3, 128.8, 128.6, 128.3, 128.1, 127.1, 126.5, 81.8 (*C*(CH$_3$)$_3$), 70.7 (Ahppa C-3), 67.8 (PhCH_2O), 58.3 (Glu α-C), 53.6 (Ahppa C-4), 39.6 (Ahppa C-2), 38.2 (Ahppa C-5), 33.8 (Glu γ-C), 29.6 (*N*-CH_3), 28.2 (C(CH_3)$_3$), 23.7 (Glu β-C); ESI-MS (*m/z*): 784.1 $[M + H]^+$, 806.1 $[M + Na]^+$ (Calcd 783.39).

(S)-Benzyl 2-((S)-2-(benzyloxy)propanamido)-3-methylbutanoate (BnO-Lac-Val-OBn) (***6b***). A solution of H-Val-OBn·TosOH (**17**) (242.0 mg, 0.666 mmol) and (*S*)-2-(benzyloxy)propanoic acid (**19**) (100.0 mg, 0.555 mmol) in anhydrous DCM (10 ml) was treated sequentially with HOAt (113.4 mg, 0.833 mmol), EDCI (159.7 mg, 0.833 mmol), and DIPEA (193.8 µl, 1.110 mmol) at 0 °C. The solution was stirred for 30 min, warmed to room temperature and stirred for another 12 h. And then, the mixture was treated as described for **11**. The residue was purified by flash column chromatography with petroleum ether–EtOAc (40 : 1–5 : 1) to afford **6b** as a pale-yellow viscous solid (194.8 mg, 95.0%). R_f 0.65 (2 : 1, petroleum ether–EtOAc); $[\alpha]^{20}_D = -51.99$ (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.29–7.37 (m, 10 H, Ar*H*), 7.12 (d, 1 H, J = 9.4 Hz, Val NH), 5.21 (d, 1 H, J = 12.1 Hz, PhC*Ha*OCO), 5.16 (d, 1H, J = 12.1 Hz, PhC*Hb*OCO), 4.63 (dd, 1 H, J = 5.0, 9.4 Hz, Val α-H), 4.62 (d, 1H, J = 11.6 Hz, PhC*Ha*OCH), 4.49 (d, 1H, J = 11.5 Hz, PhC*Hb*OCH), 3.97 (q, 1 H, J = 6.6, Hz, La α-H), 2.23 (m, 1 H, Val β-H), 1.43 (d, 3 H, J = 7.1 Hz, La β-CH_3), 0.93 (d, 3H, J = 6.6 Hz, Val γ-CH_3), 0.87 (d, 3H, J = 7.1 Hz, Val γ-CH_3); ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 173.4, 171.6, 137.2, 135.3, 128.6, 128.5, 128.4, 128.3, 128.0, 127.9, 76.3 (La α-C), 72.2 (PhCH_2OCH), 67.0 (PhCH_2OCO), 56.4 (Val α-C), 31.3 (Val β-C), 19.2 (Val γ-C), 19.0 (Val γ-C), 17.4 (La β-C); ESI-MS (*m/z*): 370.2 $[M + H]^+$, 392.2 $[M + Na]^+$, 408.1 $[M + K]^+$ (Calcd 369.19).

BnO-Lac-Val-N-Me-Gln(Trt)-Ahppa-O^{t}Bu (***4c***). Hydrogenation of **5b** (350 mg, 0.447 mmol) was carried out in EtOAc (30 ml) in the presence of a catalytic amount of Pd-C (10%) under hydrogen at room temperature for 5 h. Pd-C was removed by filtration and the resulting filtrate was reconcentrated under reduced pressure to yield the amine **31**. Meanwhile, to a solution of **6b** (190 mg, 0.514 mmol) in THF–MeOH–H_2O (4 : 1 : 2, 21 ml) at 0 °C, LiOH·H_2O (86.3 mg, 2.0 m mol) was added and stirred for 20 min, warmed to room temperature and stirred for another 1 h. The reaction mixture was then concentrated under reduced pressure. The residue was diluted with brine (100 ml), washed thrice with DCM (15 ml) to remove BnOH. The aqueous layer was acidified with HCl (0.1 M) until pH = 2–3 and extracted with EtOAc (3 × 50 ml), then concentrated under reduced pressure to yield **27**. A solution of residue **31** and **27** in THF (50 ml) was treated sequentially with HOAt (121.8 mg, 0.894 mmol), EDCI (171.4 mg, 0.894 mmol), and DIPEA (156.1 µl, 0.894 mmol). The solution was stirred at 0 °C for 30 min, warmed to room temperature and stirred for another 15 h. And then, the mixture was treated as described for **11**. The residue was purified by flash column chromatography with $CHCl_3$–MeOH (100 : 1–50 : 1) to afford the **4c** as a white solid (264 mg, 65%). R_f 0.63 (20 : 1, $CHCl_3$–MeOH); $[\alpha]^{20}_D = -61.47$ (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.15–7.41 (m, 26 H, ArH + Val α-H), 6.82 (d, 1H, J = 9.4 Hz, Ahppa N*H*), 6.76 (s, 1H, CON*H*Trt), 4.95 (m, 1H, Gln α-H), 4.50 (d, 1H, J = 11.6 Hz, PhC*Ha*O), 4.33 (d, 1H, J = 11.0 Hz, PhC*Hb*O), 4.25 (t, 1H, J = 7.7 Hz, Val α-H), 4.20 (dd, 1H, J = 7.7, 13.7 Hz, Ahppa H-4), 4.04 (m, 1H, La α-H), 3.96 (m, 1H, Ahppa H-3), 2.92 (m, 1H, Ahppa H-5a), 2.87 (s, 3H, *N*-CH_3), 2.78 (m, 1H, Ahppa H-5b), 2.26–2.35 (m, 4H, Ahppa H-2 × 2 + Gln γ-H × 2), 1.93–1.99 (m, 2H, Val β-H + Gln β-Ha), 1.81 (m, 1H, Gln β-Hb), 1.43 (d, 3H, J = 6.6 Hz, La β-CH_3), 1.41 (s, 9H, C(CH_3)$_3$), 0.99 (d, 3H, J = 7.1 Hz, Val γ-CH_3), 0.92 (d, 3H, J = 6.6 Hz, Val γ-CH_3); ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 175.1, 174.1, 172.8, 171.0, 169.9, 144.7, 144.6, 137.8, 137.2, 129.3, 128.7, 128.6, 128.4, 128.2, 128.0, 127.9, 127.7, 127.0, 126.8, 126.4, 81.6, 75.7, 72.1, 71.9, 70.4, 54.7, 54.2, 53.4, 40.9, 39.6, 38.0, 33.2, 31.0, 28.0 (C(*C*H$_3$)$_3$), 23.1, 18.5, 17.6; ESI-MS (*m/z*): 911.5 $[M + H]^+$, 933.4 $[M + Na]^+$, 949.5$[M + K]^+$ (Calcd 910.49).

Synthesis of **1**

BnO-Lac-Val-N-Me-Gln-Ahppa-Leu-Ala-N-Me-Phe-Pro-OMe (***34***). To a solution of **4c** (50.0 mg, 0.055 mmol) and **3** (37.8 mg, 0.066 mmol) in DCM (2 ml) at 0 °C, TFA (2 ml) was added and stirred for 20 min, warmed to room temperature and stirred for another 1.5 h. The solution was then dissolved twice in CH_2Cl_2 (2 ml) with evaporation each time to yield the acid **32** and the amine **33**. A solution of them in anhydrous THF (5 ml) was treated sequentially with HOAt (15.0 mg, 0.11 mmol l), EDCI (21.1 mg, 0.11 mmol), and DIPEA (50.0 µl, 0.14 mmol). The solution was stirred at 0 °C for 30 min, warmed to room temperature and stirred for another 16 h. And then, the mixture was treated as described for **11**. The residue was purified by flash column chromatography with $CHCl_3$–MeOH (80 : 1–20 : 1) to afford the **34** as a white solid (22.3 mg, 38%). R_f 0.60 (10 : 1, $CHCl_3$–MeOH).

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HO-Lac-Val-N-Me-Gln-Ahppa-Leu-Ala-N-Me-Phe-Pro-OMe (***1***). Hydrogenation of **34** (22.3 mg, 0.021 mmol) was carried out in EtOAc-EtOH (4 : 1, 5 ml) in the presence of a catalytic amount of Pd-C (10%) under hydrogen at room temperature for 17 h. The catalyst Pd-C was removed by filtration and the resulting filtrate was reconcentrated under reduced pressure to afford the **1** as a white solid (18.6 mg, 91%). R_f 0.28 (10 : 1, $CHCl_3$ – MeOH); $[\alpha]^{20}_D = -89.6$ (c = 0.45, MeOH); ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.49 (m, 1H, Leu NH), 7.32 (d, 1H, J = 7.3 Hz, Val NH), 7.16–7.25 (m, 10H, ArH), 7.10 (m, 1H, Ala NH), 7.07 (m, 1H, Ahppa N*H*), 5.51 (t, 1H, J = 7.3 Hz, Phe α-H), 5.04 (m, 1H, Gln α-H), 4.69 (t, 1H, J = 6.4 Hz, Ala α-H), 4.47 (t, 1H, J = 7.3 Hz, Val α-H), 4.41 (dd, 1H, J = 4.1, 9.1 Hz, Pro α-H), 4.34 (m, 1H, Leu α-H), 4.24 (m, 1H, Ahppa H-4), 4.19 (m, 1H, La α-H), 4.05 (m, 1H, Ahppa H-3), 3.70 (s, 3H, Pro OMe), 3.53 (m, 1H, Pro δ-Ha), 3.34 (m, 1H, Pro δ-Hb), 3.25 (dd, 1H, J = 6.9, 14.2 Hz, Phe β-Ha), 3.06 (s, 3H, Phe *N*-CH_3), 2.94–3.00 (m, 2H, Phe β-Hb + Ahppa H-5a), 2.85 (m, 1H, Ahppa H-5b), 2.81 (s, 3H, Gln *N*-CH_3), 2.49 (m, 1H, Ahppa H-2a), 2.36 (m, 1H, Ahppa H-2b), 2.11–2.24 (m, 4H, Pro β-Ha + Gln β-Ha + Gln γ-H × 2), 1.85–1.96 (m, 5H, Gln β-Hb + Pro β-Hb + Pro γ-H × 2 + Val β-H), 1.59 (m, 1H, Leu γ-H), 1.51 (m, 2H, Leu β-H × 2), 1.39 (d, 3H, J = 6.4 Hz, La β-CH_3), 1.25 (d, 3H, J = 6.4 Hz, Ala β-CH_3), 0.94 (d, 3H, J = 6.4 Hz, Val γ-CH_3), 0.91 (t, 6H, J = 5.5 Hz, Val γ-CH_3 + Leu δ-CH_3), 0.89 (d, 3H, J = 6.4 Hz, Leu δ-CH_3); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 176.3 (C-48), 175.6 (C-41), 173.5 (C-43), 172.7 (C-1), 172.3 (C-17), 171.8 (C-20), 171.6 (C-26), 170.1 (C-37), 169.2 (C-7), 138.0 (C-31), 136.6 (C-10), 129.1 (C-11, 15, 32, 36), 128.5 (C-12, 14), 128.4 (C-33, 35), 126.8 (C-13), 126.4 (C-34), 69.4 (C-28), 68.6 (C-49), 59.1 (C-2), 55.9 (C-38), 55.7 (C-8), 54.2 (C-44), 53.9 (C-29), 52.2 (C-6), 52.1 (C-21), 46.9 (C-5), 45.8 (C-18), 41.0 (C-27), 40.8 (C-22), 37.6 (C-30), 34.7 (C-9), 31.3 (C-16), 31.0 (C-40), 30.9 (C-42), 30.3 (C-45), 29.0 (C-3), 24.9 (C-4), 24.7 (C-23), 23.0 (C-24), 21.9 (C-25), 20.9 (C-50), 19.5 (C-47), 18.1 (C-46), 17.8 (C-19); HRESIMS calcd for $C_{50}H_{74}N_8O_{12}Na$ $[M + Na]^+$ 1001.5324, found 1001.5328.

Synthesis of **1a, 1b, 1c**

HO-Lac-Val-N-Me-Gln-Ahppa-Leu-Ala-N-Me-D-Phe-Pro-OMe (***1a***). Compound **1a** was obtained as a white solid as described for the synthesis of compound **1** but using the corresponding configuration of amino acids and HATU instead of DECI at the last condensation step. R_f 0.28 (10 : 1, $CHCl_3$ – MeOH); $[\alpha]^{20}_D = -30.1$ (c = 0.4, MeOH); ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.44 (d, 1H, J = 7.3 Hz, Leu NH), 7.32 (d, 1H, J = 7.8 Hz, Val NH), 7.15–7.23 (m, 10H, ArH), 7.11–7.14 (m, 2H, Ala NH + Ahppa NH), 7.01 (brs, 1H, CONHa), 6.85 (brs, 1H, CONHb), 5.63 (dd, 1H, J = 6.8, 9.2 Hz, Phe α-H), 5.03 (m, 1H, Gln α-H), 4.68 (q, 1H, J = 6.9 Hz, Ala α-H), 4.42–4.46 (m, 2H, Val α-H + Pro α-H), 4.32 (dd, 1H, J = 8.2, 14.2 Hz, Leu α-H), 4.20 (m, 1H, Ahppa H-4), 4.16 (q, 1H, J = 6.4 Hz, La α-H), 4.02 (m, 1H, Ahppa H-3), 3.72 (s, 3H, Pro OMe), 3.41 (m, 1H, Pro δ-Ha), 3.26 (m, 1H, Pro δ-Hb), 3.20 (dd, 1H, J = 6.4, 8.2 Hz, Phe β-Ha), 3.01 (s, 3H, Phe *N*-CH_3), 2.92–2.96 (m, 2H, Ahppa H-5a + Phe β-Hb), 2.78–2.84 (m, 4H, Ahppa H-5b + Gln *N*-CH_3), 2.43 (dd, 1H, J = 10.1, 13.7 Hz, Ahppa H-2a), 2.30 (dd, 1H, J = 3.7, 14.6 Hz, Ahppa H-2b), 2.17–2.21 (m, 4H, Pro β-Ha + Gln β-Ha + Gln γ-H × 2), 1.79–1.95 (m, 5H, Gln β-Hb + Pro β-Hb + Pro γ-H × 2 + Val β-H), 1.48–1.61 (m, 3H, Leu γ-H + Leu β-H × 2), 1.37 (d, 3H, J = 6.9 Hz, La β-CH_3), 0.94 (d, 3H, J = 6.4 Hz, Val γ-CH_3), 0.91 (d, 3H, J = 6.9 Hz, Val γ-CH_3), 0.87 (d, 3H, J = 6.4 Hz, Leu δ-CH_3), 0.84 (d, 3H, J = 6.4 Hz, Leu δ-CH_3), 0.82 (d, 3H, J = 7.3 Hz, Ala β-CH_3); ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 176.2 (C-48), 175.4 (C-41), 173.5 (C-43), 172.6 (C-1), 172.4 (C-17), 171.9 (C-20), 171.8 (C-26), 170.0 (C-37), 168.1 (C-7), 137.9 (C-31), 136.7 (C-10), 129.5 (C-11, 15), 129.1 (C-32, 36), 128.4 (C-12, 14), 128.2 (C-33, 35), 126.6 (C-13), 126.4 (C-34), 69.3 (C-28), 68.5 (C-49), 59.2 (C-2), 55.7 (C-38), 55.6 (C-8), 54.2 (C-44), 53.9 (C-29), 52.3 (C-6), 52.1 (C-21), 46.9 (C-5), 45.3 (C-18), 40.8 (C-22), 40.6 (C-27), 37.6 (C-30), 34.7 (C-9), 31.8 (C-16), 31.1 (C-40), 30.8 (C-42), 30.4 (C-45), 28.8 (C-3), 25.3 (C-4), 24.7 (C-23), 22.8 (C-24, 39), 21.9 (C-25), 20.8 (C-50), 19.5 (C-47), 17.8 (C-46), 17.2 (C-19); HRESIMS calcd for $C_{50}H_{75}N_8O_{12}$ $[M + H]^+$ 979.5504, found 979.5470.

HO-Lac-Val-N-Me-Gln-Ahppa-Leu-D-Ala-N-Me-Phe-Pro-OMe (***1b***). Compound **1b** was obtained as a white solid as described for the synthesis of compound **1** but using the corresponding configuration of amino acids and HATU instead of DECI at the last condensation step. R_f 0.28 (10 : 1, $CHCl_3$ – MeOH); $[\alpha]^{20}_D = -126.41$ (c = 0.4, MeOH); ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.40 (d, 1H, J = 5.9 Hz, Ala NH), 7.37 (d, 1H, J = 6.8 Hz, Leu NH), 7.30 (d, 1H, J = 7.8 Hz, Val NH), 7.16–7.24 (m, 10H, ArH), 6.99 (d, 1H, J = 8.7 Hz, Ahppa NH), 6.88 (brs, 1H, CONHa), 6.72 (brs, 1H, CONHb), 5.67 (dd, 1H, J = 5.5, 10.6 Hz, Phe α-H), 5.08 (m, 1H, Gln α-H), 4.59 (t, 1H, J = 6.9 Hz, Ala α-H), 4.41–4.45 (m, 2H, Val α-H + Pro α-H), 4.36 (m, 1H, Leu α-H), 4.23 (m, 1H, Ahppa H-4), 4.15 (m, 1H, La α-H), 4.05 (m, 1H, Ahppa H-3), 3.72 (s, 3H, Pro OMe), 3.48–3.51 (m, 2H, Pro δ-H × 2), 3.16 (dd, 1H, J = 5.5, 14.6 Hz, Phe β-Ha), 3.00–3.05 (m, 4H, Phe *N*-CH_3 + Phe β-Hb), 2.94 (dd, 1H, J = 7.3, 14.6 Hz, Ahppa H-5a), 2.81 (dd,1H, J = 9.7, 15.6 Hz, Ahppa H-5b), 2.73 (s, 3H, Gln *N*-CH_3), 2.42 (dd, 1H, J = 7.8, 13.7 Ahppa H-2a), 2.35 (m, 1H, Ahppa H-2b), 2.12–2.21 (m, 4H, Pro β-Ha + Gln β-Ha + Gln γ-H × 2), 1.85–1.95 (m, 5H, Gln β-Hb + Pro β-Hb + Pro γ-H × 2 + Val β-H), 1.44–1.57 (m, 3H, Leu γ-H + Leu β-H × 2), 1.38 (d, 3H, J = 6.9 Hz, La β-CH_3), 0.94 (d, 3H, J = 6.8 Hz, Val γ-CH_3), 0.91 (d, 3H, J = 6.4 Hz, Val γ-CH_3), 0.87 (d, 3H, J = 5.9 Hz, Leu δ-CH_3), 0.85 (d, 3H, J = 5.9 Hz, Leu δ-CH_3), 0.77 (d, 3H, J = 6.8 Hz, Ala β-CH_3); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 176.5 (C-48), 176.0 (C-41), 173.5 (C-43), 172.6 (C-1), 172.1 (C-17), 171.6 (C-20), 171.5 (C-26), 170.0 (C-37), 168.5 (C-7), 137.9 (C-31), 136.5 (C-10), 129.2 (C-11, 15), 129.0 (C-32, 36), 128.4 (C-12, 14), 128.3 (C-33, 35), 126.7 (C-13), 126.5 (C-34), 69.4 (C-28), 68.5 (C-49), 59.2 (C-2), 55.7 (C-38), 55.0 (C-8), 54.2 (C-44), 53.4 (C-29), 52.3 (C-6), 51.6 (C-21), 46.9 (C-5), 45.7 (C-18), 41.0 (C-27), 40.6 (C-22), 37.7 (C-30), 34.6 (C-9), 31.2 (C-16), 30.8 (C-40), 30.6 (C-42), 30.2 (C-45), 28.9 (C-3), 24.8 (C-4), 24.7 (C-23), 23.5 (C-24), 23.0 (C-39), 21.7 (C-25), 20.7 (C-50), 19.5 (C-47), 17.8 (C-46), 16.5 (C-19); HRESIMS calcd for $C_{50}H_{75}N_8O_{12}$ $[M + H]^+$ 979.5504, found 979.5478.

HO-Lac-Val-N-Me-Gln-Ahppa-Leu-D-Ala-N-Me-D-Phe-Pro-OMe (***1c***). Compound **1c** was obtained as a white solid as described for the synthesis of compound **1** but using the corresponding configuration of amino acids and HATU instead of DECI at the last condensation step. R_f 0.28 (10 : 1, $CHCl_3$ – MeOH); $[\alpha]^{20}_D = -50.01$ (c = 0.4, MeOH); ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 7.30–7.32 (m, 2H, Leu NH + Val NH), 7.27 (m, 1H, Ala NH), 7.10–7.23 (m, 10H, ArH), 7.03 (d, 1H, J = 8.2 Hz, Ahppa N*H*), 6.76 (brs, 1H, CONHa), 6.60 (brs, 1H, CONHb), 5.43 (dd, 1H, J = 5.9, 9.2 Hz, Phe α-H), 5.08 (m, 1H, Gln α-H), 4.82 (m, 1H, Ala α-H), 4.43–4.46 (m, 2H, Val α-H + Leu α-H), 4.39 (dd, 1H, J = 5.0, 8.8 Hz, Pro α-H), 4.29 (m, 1H, Ahppa H-4), 4.15 (d, 1H, J = 5.9 Hz, La α-H), 4.08 (m, 1H, Ahppa H-3), 3.70 (s, 3H, Pro OMe), 3.26–3.31 (m, 3H, Pro δ-H × 2 + Phe β-Ha), 3.12, 3.08 (s, 3H, 1 : 5, Phe *N*-CH_3), 2.95 (m, 1H, Ahppa H-5a), 2.83 (m, 1H, Ahppa H-5b), 2.73–2.76 (m, 4H, Phe β-Hb + Gln *N*-CH_3), 2.46 (dd, 1H, J = 7.7, 13.7 Hz, Ahppa H-2a), 2.40 (m, 1H, Ahppa H-2b), 2.08–2.18 (m, 4H, Pro β-Ha + Gln β-Ha + Gln γ-H × 2), 1.81–1.93

(m, 4H, Gln β-Hb + Pro β-Hb + Pro γ-Ha + Val β-H), 1.78 (m, 1H, Pro γ-Hb), 1.59–1.61 (m, 2H, Leu γ-H + Leu β-Ha), 1.51 (m, 1H, Leu β-Hb), 1.38 (d, 3H, J = 6.4 Hz, La β-CH_3), 1.32 (d, 3H, J = 6.9 Hz, Ala β-CH_3), 0.89–0.96 (m, 12H, Val γ-CH_3 × 2 + Leu δ-CH_3 × 2); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 176.4 (C-48), 175.8 (C-41), 173.5 (C-43), 172.8 (C-1), 172.6 (C-17), 171.8 (C-20), 170.0 (C-37), 167.9 (C-7), 137.9 (C-31), 137.2 (C-10), 129.6 (C-11, 15), 129.0 (C-32, 36), 128.4 (C-12, 14, 33, 35), 126.5 (C-13), 126.5 (C-34), 69.5 (C-28), 68.6 (C-49), 58.7 (C-2), 56.6 (C-8), 55.9 (C-38), 54.2 (C-44), 53.5 (C-29), 52.2 (C-6), 51.7 (C-21), 46.5 (C-5), 45.9 (C-18), 41.1 (C-27), 40.6 (C-22), 37.8 (C-30), 34.7 (C-9), 31.2 (C-16), 31.0 (C-40), 30.9 (C-42), 30.2 (C-45), 28.8 (C-3), 24.9 (C-4), 24.8 (C-23), 23.1 (C-39), 23.0 (C-24), 21.8 (C-25), 20.7 (C-50), 19.5 (C-47), 17.8 (C-46), 17.4 (C-19); HRESIMS calcd for $C_{50}H_{74}N_8O_{12}Na$ $[M + Na]^+$ 1001.5324, found 1001.5283.

Cytotoxic Evaluation *In Vitro*

The prepared compounds (**1a, 1b, 1c**, and **1**) were submitted to Shanghai Institute of Materia Medica Chinese Academy of Sciences to test their cytotoxicities. The cytotoxic effects were examined using sulforhodamine B (SRB) assay against A549, LoVo, KB, BEL-7402 and MTT tetrazolium dye assay against P388 and HL-60 cell lines.

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Supporting information

Supporting information may be found in the online version of this article.

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