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Synthesis of the cyclic nonapeptide of chlorofusin using a convergent [3+3+3]-fragment coupling strategy

Yan-Li Wang [a,b], Wen-Jian Qian [a], Wan-Guo Wei [a], Yue Zhang [b,*], Zhu-Jun Yao [a,*]

[a] State Key Laboratory of Bioorganic and Natural Products Chemistry, Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, 345 Lingling Road, Shanghai 200032, China
[b] Department of Chemistry and Pharmaceutical Engineering, Hebei University of Technology and Science, 70 East Yuhua Road, Shijiazhuang, Hebei 050018, China

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ABSTRACT

A [3+3+3]-fragment coupling strategy was successfully applied in the synthesis of the nonacyclopeptide of chlorofusin, a potent natural antagonist against *p*53–MDM2 interactions. The accomplished convergent synthesis includes parallel syntheses of three tripeptides and their sequential assembly, and macrocyclization of the linear precursor to the required 27-membered nonacyclopeptide.

1. Introduction

The tumour suppressor *p*53 induces cell death by apoptosis in response to various stress conditions, such as oncogene activation and DNA damage, and therefore plays a crucial role in the prevention of cancers.[1–4] The overexpression of MDM2 causes uncontrolled cell proliferation in a variety of human tumours,[5–8] and the binding of *p*53 with MDM2 has been found to result in the high risk of a number of human cancers.[9] As a new chemotherapy of cancers, discovery of potent small-molecule antagonists against *p*53-MDM2 interactions has attracted great attentions in medicinal chemistry in recent years. A number of small-molecular heterocyclic compounds have been successfully designed and developed with potent inhibitory activities against *p*53–MDM2 interactions, and thus showing great potentials for future drug development.[10,11]

Chlorofusin (**1**, Fig. 1), a unique nonacyclopeptide isolated from the fermentation broth of the fungal strain *Microdochium caespitosum*, has been found to inhibit the *p*53–MDM2 interaction with an IC_{50}=4.6 μM and a K_D=4.7 μM.[12,14] It thus represents a new lead structure of novel antagonists against the *p*53–MDM2 protein–protein interactions.[13] The structure of chlorofusin was initially proposed to contain a novel densely functionalized chromophore and a 27-membered nonacyclopeptide, through the linkage between its chromophore with a terminal amine of ornithine of the cyclopeptide.[12] Two total syntheses of chlorofusin and its enantiopure diastereomers have been accomplished in recent years, including revisions of relative and absolute configurations of its chromophore part.[15,16]

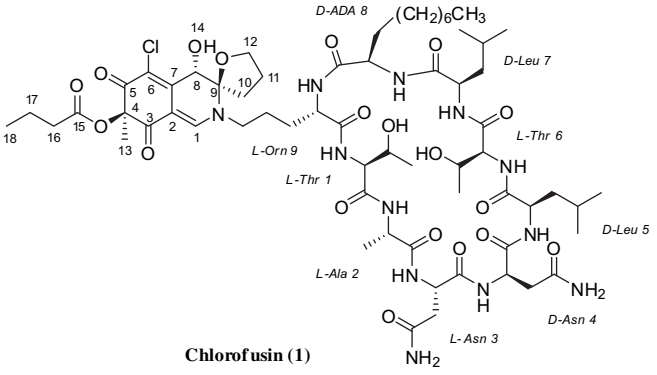

Figure 1. The revised structure of chlorofusin (**1**).

The cyclic peptide portion of chlorofusin (**1**, Fig. 1) was firstly determined by the corresponding spectroscopic studies and chemical degradation methods.[12] It includes two L-threonines, one L-alanine, one L- and one D-asparagine, two D-leucines, one D-2-aminodecanoic acid, and one L-ornithine. The two asparagine residues, Asn3 and Asn4, were suggested to have opposite stereochemistries (L- and D-form, respectively), but their order in the structure could not be determined at that moment. In 2003, Boger's group[17] and Searcey's group[18] synthesized the cyclic peptide portions of chlorofusin independently, and finally resulted in the assignment of L-Asn3 and D-Asn4. In 2007, Nakata's group successfully applied an [8+1]-fragment coupling strategy to the synthesis of the cyclic peptide.[19] Very recently, Boger's group reported their second synthesis of the cyclic peptide using a [2+2+2+3]-fragment assembly strategy.[20]

* Corresponding authors. Tel.: +86 21 5492 5133; fax: +86 21 6416 6128; e-mail address: yaoz@sioc.ac.cn.

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2. Results and discussion

2.1. Retrosynthesis

The first total synthesis of enantiopure (4*S*,8*R*,9*S*)-chlorofusin and other two diastereomers were successfully accomplished by our group in 2007.[15] To support our ongoing project on the second generation total synthesis of chlorofusin and its all seven chromophore diastereomers, a scalable synthesis of the cyclic peptide portion was studied. Herein, we report our synthesis of the cyclic peptide of chlorofusin, which is featured with a [3+3+3]-fragment coupling strategy (Fig. 2). Such an equal-cut retrosynthesis would result in a new convergent route to the cyclic peptide **2** and lead to high synthetic efficiency.

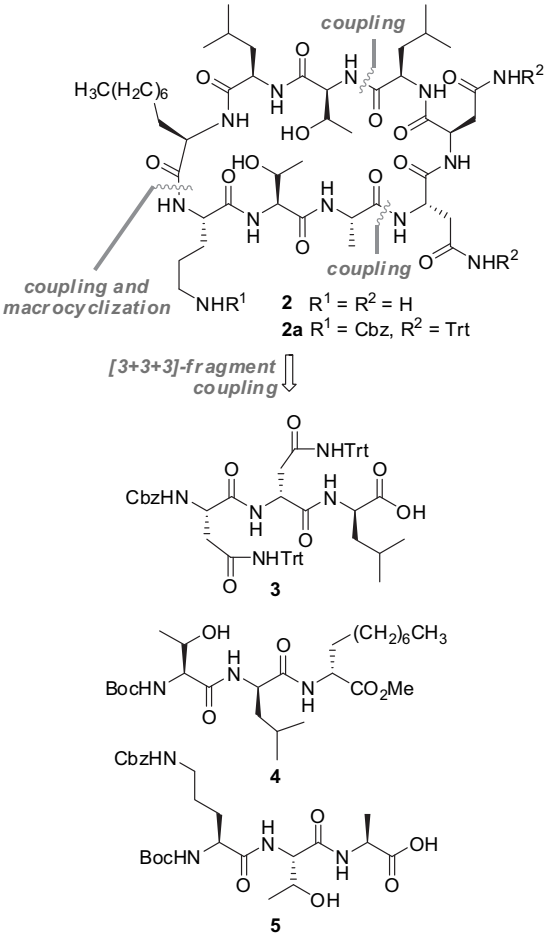
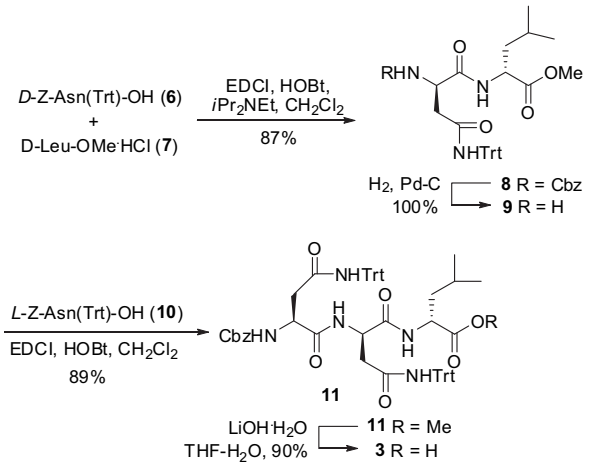


Figure 2. Retrosynthesis of the cyclic peptide **2**.

According to this design, the cyclopeptide **2** could be divided into three parallel tripeptides **3**, **4** and **5**. Compared to the dipeptide intermediates, tripeptides are usually more stable, and are able to avoid the production of undesired piperazine-2,5-dione derivatives via self-cyclization during their couplings. After sequential couplings of these three fragments, the final amide-bond formation between L-Orn9 and D-Ada8 was devised for the head-to-tail macrocyclization.

2.2. Synthesis of tripeptide 3

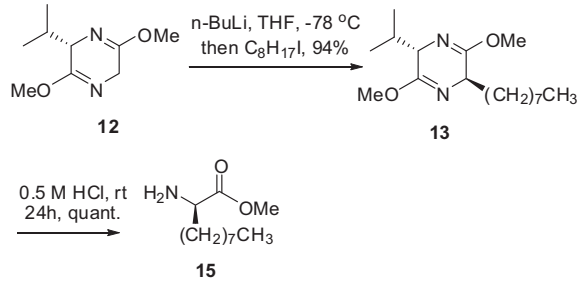
First of all, coupling of D-*Z*-Asn(Trt)–OH (**6**) with D-Leu–OMe·HCl (**7**) with EDCI and HOBt in the presence of *N*,*N*-diisopropylethylamine (DIEA) afforded dipeptide **8** in 87% yield (Scheme 1). Removal of the *N*-Cbz protecting group of **8** was carried out by hydrogenolysis in the presence of 10% Pd–C, affording quantitative yield of amine **9**. Further coupling of peptide amine **9** with L-*Z*-Asn(Trt)–OH (**10**) with EDCI and HOBt in DCM followed by ester hydrolysis with LiOH in THF–H_2O afforded the first tripeptide **3** in 81% yield (two steps).



Scheme 1.

2.3. Synthesis of tripeptide 4

Preparation of the unusual D-2-aminodecanic acid (D-Ada) started from the Schöllkopf reagent **12**[21] (Scheme 2). Deprotonation of **12** with *n*-BuLi followed by alkylation with 1-iodooctane in THF at −78 °C afforded **13** in 94% yield. Hydrolysis of **13** with 0.5 M aqueous HCl at 25 °C for 24 h afforded D-Ada–OMe (**15**) in a quantitative yield.

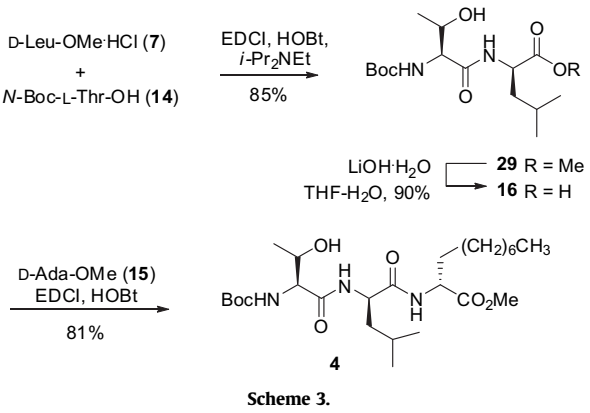


Scheme 2.

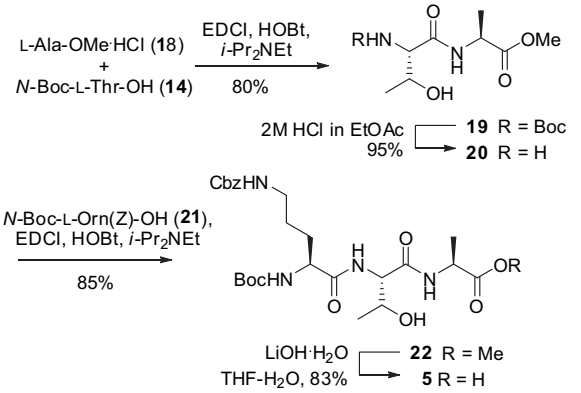
Coupling of *N*-Boc–L-Thr–OH (**14**) with D-Leu–OMe (**7**) in the presence of EDCI and HOBt in DCM followed by ester hydrolysis provided dipeptide **16** in 77% yield (Scheme 3). Further condensation of dipeptide-acid **16** with newly prepared amine **15** with EDCI and HOBt in DCM afforded the second tripeptide **4** in 81% yield.

2.4. Synthesis of tripeptide 5

Coupling of *N*-Boc–L-Thr–OH (**14**) with L-Ala–OMe (**18**) was carried out with EDCI and HOBt in DCM, affording dipeptide **19** in 80% yield (Scheme 4). Deprotection of *N*-Boc group of **19** with 2 M HCl in EtOAc followed by coupling with *N*-Boc–L-Orn(*Z*)–OH (**21**) with EDCI and HOBt in DCM afforded tripeptide **22** in 81% yield in two steps. Hydrolysis of **22** with LiOH·H_2O in THF–H_2O afforded the third tripeptide (acid) **5** in 83% yield.



Scheme 3.



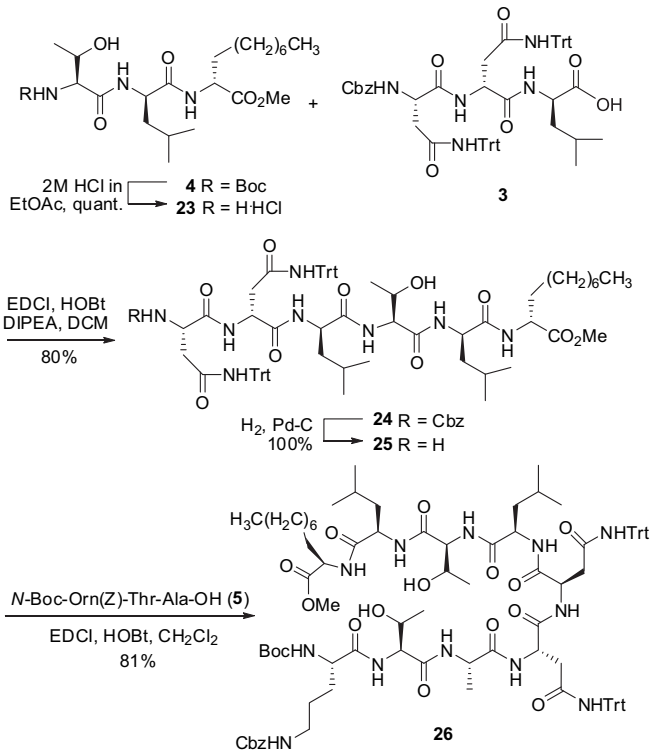
Scheme 4.

2.5. Synthesis of the linear nonapeptide

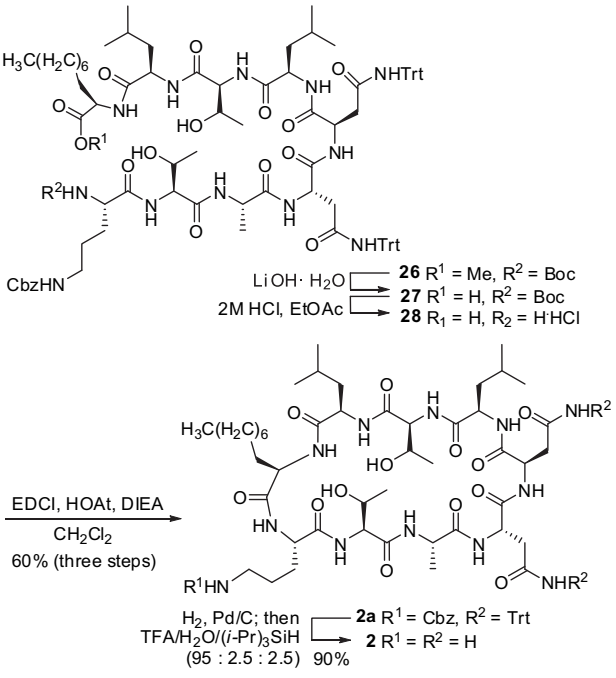
With all three tripeptides in hand, we turned our attention to their couplings and the final macrocyclization. The *N*-Boc protecting group of tripeptide **4** was removed with 2 M HCl in EtOAc, affording the corresponding peptide amine **23** as a hydrochloric acid salt (Scheme 5). Condensation of tripeptide acid **3** and tripeptide amine **23** was achieved by treatment with EDCI and HOBt in the presence of DIEA in DCM, providing hexapeptide **24** in 80% yield. The *N*-Cbz group of **24** was then removed by hydrogenolysis in MeOH in the presence of 10% Pd–C. The resulting crude amine **25** was immediately used in the subsequent coupling with tripeptide acid **5** in the presence of EDCI and HOBt in DCM, providing the needed nonapeptide **26** with protecting groups.

2.6. Macrocyclization

Hydrolysis of nonapeptide **26** with LiOH·H_2O in THF–H_2O followed by treatment with 2 M HCl in EtOAc afforded the linear head–tail free amino acid **28** (Scheme 6). Macrocyclization of the linear precursor **28** was carried out with EDCI and HOAt in the presence of DIEA in DCM, affording cyclopeptide **2a** (60% yields in three steps). Global deprotection of *N*-Cbz and *N*-Trt groups of cyclopeptide **2a** was accomplished by hydrogenolysis in the presence of 10% Pd–C in MeOH and subsequent treatment with TFA–H_2O–$^{i}Pr_3SiH$ (v/v/v=95:2.5:2.5), providing the target nonacyclopeptide **2** in 90% yield. NMR spectra and other physical characterizations of the synthetic sample of **2** obtained in this work are identical to those reported in the reference.[19]



Scheme 5.



Scheme 6.

3. Conclusion

In summary, a new synthesis of the cyclic nonapeptide of chlorofusin has been accomplished in 20 longest linear steps and 9% overall yield. Successful application of new convergent [3+3+3]-fragment assembly strategy and macrocylization at the

amide-bond position between Orn9 and Ada8 under mild conditions makes this synthesis very efficient and capable in material accumulation, and thus facilitates the further total synthesis of chlorofusin and other stereoisomers.

4. Experimental

4.1. General

All reactions were conducted using oven-dried glassware. All the temperatures were uncorrected. IR spectra were recorded on an FT-IR instrument. ^{1}H NMR spectra were recorded at 300 MHz or 400 MHz, and ^{13}C NMR spectra were recorded at 75 MHz or 100 MHz. Reference peaks for chloroform in ^{1}H NMR and ^{13}C NMR spectra were set at 7.27 ppm and 77.2 ppm, respectively. For DMSO-d_6, the reference peaks in ^{1}H NMR and ^{13}C NMR spectra were set at 2.50 ppm and 39.5 ppm. For acetone-d_6, the reference peaks in ^{1}H NMR and ^{13}C NMR spectra were set at 2.05 ppm, and 29.9 ppm and 206.7 ppm, respectively. Flash column chromatographies were performed on silica gel H (10–40 mesh). Petroleum ether and ethyl acetate were obtained from commercial suppliers and used without further distillations.

4.2. *N*-Cbz–D-Asn(Trt)-D-Leu–OMe (8)

A solution of D-*Z*-Asn(Trt)–OH (**6**, 3.1 g, 6.1 mmol) in dry DCM (60 mL) was treated with HOBt (0.9 mg, 6.6 mmol) and EDCI (1.3 g, 6.6 mmol) under stirring at 0 °C for 0.5 h. Then, D-Leu–OMe·HCl (**7**, 1.0 g, 5.5 mmol) and DIEA (0.9 g, 6.6 mmol) were added successively at 0 °C. The reaction mixture was allowed to warm to rt and stirred until completion. Saturated aqueous $NaHCO_3$ (50 mL) was added to quench the reaction. The whole mixture was extracted with EtOAc (100 mL×3). The combined EtOAc extracts were washed successively with water, aqueous 1 N HCl (40 mL×2), water and brine (40 mL), dried ($MgSO_4$) and concentrated in vacuo. The residue was chromatographied (CH_2Cl_2/MeOH=200:1 to 100:1) to afford **8** as a white foam (3.06 g, 87%). $[\alpha]_D^{25}$ 2.75 (*c* 1.90, $CHCl_3$); IR (KBr): ν_{max} 3307, 3058, 2955, 1659 cm^{-1}; ^{1}H NMR (acetone-d_6, 400 Hz, rt): δ 8.04 (1H, s), 7.56 (1H, d, *J*=7.6 Hz), 7.37 (5H, m), 7.26–7.22 (15H, m), 6.69 (1H, d, *J*=8.0 Hz), 5.08 (2H, s), 4.53 (1H, q, *J*=6.4 Hz), 4.45 (1H, q, *J*=7.2 Hz), 3.65 (3H, s), 2.95–2.89 (1H, m), 2.82–2.75 (1H, m), 1.70–1.66 (1H, m), 1.55 (2H, t, *J*=6.8 Hz), 0.89 (3H, d, *J*=4.0 Hz), 0.87 (3H, d, *J*=4.0 Hz); ^{13}C NMR (acetone-d_6, 100 Hz, rt): δ 173.5, 172.2, 170.3, 156.7, 145.7, 137.7, 129.6, 129.0, 128.5, 128.2, 127.2, 71.0, 66.8, 52.4, 52.2, 51.6, 41.0, 39.3, 25.2, 23.1, 21.9. HRMS (ESI, *m*/*z*) calcd for $C_{38}H_{41}N_3NaO_6$ [M+Na$^+$] 658.2893, found 658.2888.

4.3. *N*-Cbz–L-Asn(Trt)-D-Asn(Trt)-D-Leu–OMe (11)

A suspension of **8** (3.06 g, 4.8 mmol) and 10% Pd–C (300 mg) in MeOH (50 mL) was stirred under H_2 (1 atm) at rt for 4 h. The solid was removed by filtration through a pad of Celite and washed with EtOAc (20 mL). The filtrate was concentrated in vacuo to afford **9** (D-Asn(Trt)-D-Leu–OMe) as a white foam (2.75 g, 100%).

A solution of L-*Z*-Asn(Trt)–OH (**10**, 3.1 g, 6.0 mmol) in dry DCM (60 mL) was treated with HOBt (0.9 mg, 6.6 mmol) and EDCI (1.26 g, 6.6 mmol) under stirring at 0 °C for 0.5 h. Then, D-Asn(Trt)-D-Leu–OMe (**9**, 2.75 g, 5.5 mmol) was added and the mixture was stirred at 0 °C for 0.5 h. The reaction mixture was allowed to warm to rt, and stirred until completion. Saturated aqueous $NaHCO_3$ (40 mL) was added, and the whole mixture was extracted with EtOAc (80 mL×3). The combined EtOAc extracts were washed sequentially with water, aqueous 1 N HCl (30 mL×2), water and brine (40 mL), dried ($MgSO_4$) and concentrated in vacuo. The residue was chromatographied (CH_2Cl_2/MeOH=200:1 to 150:1) to afford **11** as a white foam (6.1 g, 89%). $[\alpha]_D^{26}$ −1.30 (*c* 1.80, $CHCl_3$); IR (KBr): ν_{max} 3059, 1734, 1668, 1493 cm^{-1}; ^{1}H NMR (acetone-d_6, 400 Hz, rt): δ 8.06 (2H, d, *J*=2.8 Hz), 7.87 (1H, d, *J*=8.0 Hz), 7.48 (1H, d, *J*=7.6 Hz), 7.33 (1H, d, *J*=2.4 Hz), 7.25–7.19 (30H, m), 6.63 (1H, d, *J*=7.6 Hz), 5.03 (2H, q, *J*=12.4 Hz), 4.75–4.70 (1H, m), 4.42–4.33 (2H, m), 3.00–2.79 (3H, m), 2.77–2.72 (2H, m), 1.65–1.58 (1H, m), 1.46–1.42 (2H, t, *J*=6.4 Hz), 0.81 (3H, d, *J*=5.1 Hz), 0.79 (3H, d, *J*=5.4 Hz); ^{13}C NMR (acetone-d_6, 75 Hz, rt): δ 173.4, 171.8, 171.7, 170.8, 170.2, 157.0, 145.8, 145.7, 137.6, 129.7, 129.6, 129.1, 128.7, 128.6, 128.5, 127.7, 71.1, 71.0, 67.1, 53.3, 52.1, 51.8, 50.6, 40.7, 39.0, 38.6, 25.3, 23.1, 22.0. HRMS (ESI, *m*/*z*) calcd for $C_{61}H_{61}N_5NaO_8$ [M+Na$^+$] 1014.4412, found 1014.4418.

4.4. *N*-Boc–L-Thr-D-Leu–OMe (29)

A mixture of *N*-Boc–L-Thr–OH (14, 5 g, 23 mmol), HOBt (3.4 g, 25 mmol) and EDCI (4.8 g, 25 mmol) in dry DCM (200 mL) was stirred at 0 °C for 0.5 h. Then, D-Leu–OMe·HCl (7, 3.73 g, 21 mmol) and DIEA (3.24 g, 25 mmol) were added successively at 0 °C. The reaction mixture was allowed to warm to rt and stirred until completion. Saturated aqueous $NaHCO_3$ (50 mL) was added to the reaction mixture. The whole mixture was extracted with EtOAc (100 mL×3). The combined EtOAc extracts were washed successively with water, aqueous 1 N HCl (40 mL×2), water and brine (40 mL), dried ($MgSO_4$) and concentrated in vacuo. The residue was chromatographied (CH_2Cl_2/MeOH=200:1 to 100:1) to afford **29** as a white foam (6.1 g, 85%). $[\alpha]_D^{27}$ 7.13 (*c* 1.50, $CHCl_3$); IR (KBr): ν_{max} 3308, 2966, 1748, 1662 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 Hz, rt): δ 8.12 (1H, d, *J*=8.0 Hz), 6.26 (1H, d, *J*=8.0 Hz), 4.69 (1H, d, *J*=5.6 Hz), 4.30–4.25 (1H, m), 3.89–3.84 (2H, m), 3.62 (2H, m), 1.62–1.58 (2H, m), 1.49–1.47 (1H, m), 1.39 (9H, s), 1.02 (3H, d, *J*=6.0 Hz), 0.88 (3H, d, *J*=6.4 Hz), 0.82 (3H, d, *J*=6.4 Hz); ^{13}C NMR (acetone-d_6, 100 Hz, rt): δ 172.9, 170.5, 155.2, 78.1, 67.0, 60.0, 51.7, 50.2, 28.1, 24.1, 22.8, 21.1, 19.9. HRMS (ESI, *m*/*z*) calcd for $C_{16}H_{30}N_2NaO_6$ [M+Na$^+$] 538.3463, found 538.3468.

4.5. *N*-Boc–L-Thr-D-Leu-D-Ada–OMe (4)

A solution of **29** (1.8 g, 5.2 mmol) in THF (40 mL) and H_2O (10 mL) was treated with LiOH·H_2O (440 mg, 10.4 mmol) at 0 °C for 1 h until 1 N aqueous HCl (10 mL) was added. The mixture was extracted with EtOAc (20 mL×5). The combined EtOAc extracts were dried ($MgSO_4$) and concentrated in vacuo to provide acid **16** as a white foam (1.55 g, 90%).

To a solution of **16** (0.55 g, 1.6 mmol) in dry DCM (20 mL) was added HOBt (0.24 g, 1.8 mmol) and EDCI (0.34 g, 1.8 mmol) at 0 °C. After stirring for 0.5 h, D-Ada–OMe (**15**, 0.3 g, 1.5 mmol) was added at 0 °C. The reaction mixture was allowed to warm to rt and stirred until completion. Saturated aqueous $NaHCO_3$ (10 mL) was added to quench the reaction. The mixture was extracted with EtOAc (20 mL×3). The combined EtOAc extracts were washed successively with water, aqueous 1 M HCl (10 mL×2), water and brine (20 mL), dried ($MgSO_4$) and concentrated in vacuo. The residue was chromatographied (CH_2Cl_2/MeOH=200:1 to 50:1) to afford **4** as a white foam (0.77 g, 81%). $[\alpha]_D^{27}$ 8.57 (*c* 1.50, $CHCl_3$); IR (KBr): ν_{max} 2959, 2930, 1747, 1647 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 Hz, rt): δ 8.13 (1H, d, *J*=7.2 Hz), 7.92 (1H, d, *J*=8.4 Hz), 6.41 (1H, d, *J*=7.2 Hz), 4.77 (1H, d, *J*=5.6 Hz), 4.33–4.32 (1H, m), 4.18–4.16 (1H, m), 3.87–3.81 (2H, m), 3.60 (3H, d, *J*=11.2 Hz), 1.66–1.60 (3H, m), 1.49–1.43 (2H, m), 1.38 (9H, s), 1.23 (12H, m), 1.02 (3H, d, *J*=6.0 Hz), 0.88–8.82 (9H, m); ^{13}C NMR (acetone-d_6, 100 Hz, rt): δ 173.2, 173.1, 171.9, 156.5, 79.5, 68.2, 61.2, 53.1, 52.5, 41.5, 32.5, 32.4, 29.9, 28.5, 26.3, 25.3, 23.2, 21.8, 19.8, 14.3. HRMS (ESI, *m*/*z*) calcd for $C_{26}H_{49}N_3NaO_7$ [M+Na$^+$] 538.3463, found 538.3468.

4.6. *N*-Boc–L-Thr-L-Ala–OMe (19)

A mixture of *N*-Boc–L-Thr–OH (**14**, 3.4 g, 15.5 mmol), HOBt (2.3 g, 17 mmol) and EDCI (3.3 g, 17 mmol) in dry DCM (160 mL) was stirred at 0 °C for 0.5 h. Then, L-Ala–OMe·HCl (**18**, 2.6 g, 14.1 mmol) and DIEA (3.0 mL, 17 mmol) were added successively at 0 °C. The reaction mixture was allowed to warm to rt and stirred until completion. Saturated aqueous $NaHCO_3$ (50 mL) was added to quench the reaction. The mixture was extracted with EtOAc (50 mL×3). The combined EtOAc extracts were washed sequentially with water, aqueous 1 N HCl (20 mL×2), water and brine (20 mL), dried ($MgSO_4$) and concentrated in vacuo. The residue was chromatographied (petroleum ether/EtOAc=20:1 to 10:1) to afford **19** as a white foam (3.8 g, 80%). $[\alpha]_D^{26}$ −49.2 (*c* 1.95, $CHCl_3$); IR (KBr): ν_{max} 3445, 2250, 2124, 1733 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz, rt): δ 8.18 (1H, d, *J*=7.2 Hz), 6.35 (1H, d, *J*=8.0 Hz), 4.73 (1H, d, *J*=5.6 Hz), 4.32–4.25 (1H, m), 3.85 (2H, s), 3.61 (3H, s), 1.39 (9H, s), 1.28 (3H, d, *J*=7.6 Hz), 1.05 (3H, d, *J*=6.0 Hz); ^{13}C NMR (acetone-d_6, 100 Hz, rt): δ 173.8, 171.1, 156.5, 79.4, 68.1, 59.9, 52.4, 48.8, 19.2, 17.9. HRMS (ESI, *m*/*z*) calcd for $C_{13}H_{24}N_2NaO_6$ [M+Na^+] 327.1532, found 327.1527.

4.7. *N*-Boc–L-Orn(Cbz)-L-Thr-L-Ala–OMe (22)

Dipeptide 1**9** (4.5 g, 14.8 mmol) was treated with 2 M HCl in EtOAc (50 mL) at rt for 1 h. The mixture was concentrated in vacuo to afford **20** as white foam, which was directly used in the next step without further purification.

To a solution of *N*-Boc–L-Orn(*Z*)–OH (**21**, 6.0 g, 16.3 mmol) in dry DCM (150 mL) was added HOBt (2.4 g, 17.8 mmol) and EDCI (3.4 g, 17.8 mmol) at 0 °C. After the mixture was stirred at 0 °C for 0.5 h, the above freshly prepared dipeptide amine **20** (4.3 g, 14.8 mmol) and DIEA (3.1 mL, 17.8 mmol) were added successively at 0 °C. The reaction mixture was stirred at 0 °C for 0.5 h, and then allowed to warm to rt and stirred until completion. Saturated aqueous $NaHCO_3$ (20 mL) was added to quench the reaction. The mixture was extracted with EtOAc (40 mL×3). The combined EtOAc extracts were washed sequentially with water, aqueous 1 N HCl (10 mL×2), water and brine (20 mL), dried ($MgSO_4$) and concentrated in vacuo. Purification by column chromatography (CH_2Cl_2/MeOH=200:1 to 100:1) afforded **22** as a white foam (7.0 g, 85%). $[\alpha]_D^{26}$ −30.5 (*c* 2.00, $CHCl_3$); IR (KBr): ν_{max} 3502, 2917, 1704, 1249 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz, rt): δ 8.11 (1H, d, *J*=6.4 Hz), 7.57 (1H, d, *J*=8.4 Hz), 7.38–7.30 (5H, m), 7.22 (1H, t, *J*=5.6 Hz), 7.03 (1H, d, *J*=7.6 Hz), 5.00 (2H, s), 4.83 (1H, d, *J*=4.4 Hz), 4.30–4.26 (1H, m), 4.20–4.17 (1H, m), 3.95–3.92 (2H, m), 3.60 (3H, s), 2.97 (2H, d, *J*=6.0 Hz), 1.63 (1H, m), 1.49–1.42 (9H, s), 1.28 (3H, d, *J*=7.6 Hz), 1.05 (3H, d, *J*=6.4 Hz); ^{13}C NMR (acetone-d_6, 100 Hz, rt): δ 172.8, 172.3, 172.0, 169.8, 156.1, 155.5, 137.2, 128.3, 127.7, 78.2, 66.6, 65.1, 60.5, 57.7, 54.2, 51.8, 47.6, 28.9, 28.2, 26.0, 19.6, 17.0, 14.0. HRMS (ESI, *m*/*z*) calcd for $C_{26}H_{40}N_4NaO_9$ [M+Na^+] 575.2688, found 575.2693.

4.8. *N*-Cbz-L-Asn(Trt)-D-Asn(Trt)-D-Leu-L-Thr-D-Leu-D-Ada–OMe (24)

Tripeptide *N*-Boc–L-Thr-D-Leu-D-Ada–OMe (**4**, 465 mg, 0.9 mmol) was treated with 2 M HCl in EtOAc (5 mL) at rt for 2 h. The mixture was concentrated in vacuo to afford the peptide amine **23** as white foam, which was directly used in the next step without further purification.

A solution of **11** (2.6 g, 2.6 mmol) in THF (40 mL) and H_2O (10 mL) was treated with LiOH·H_2O (110 mg, 2.6 mmol) and stirred at 0 °C for 2 h until 1 N aqueous HCl (10 mL) was added. The mixture was extracted with EtOAc (10 mL×5). The combined EtOAc extracts were dried ($MgSO_4$) and concentrated in vacuo. The crude tripeptide acid **3** was provided as white foam, and directly used in the next step without further purification.

To a solution of tripeptide acid **3** (802 mg, 0.82 mmol) in dry DCM (10 mL) was added HOBt (122 mg, 0.9 mmol) and EDCI (173 mg, 0.9 mmol) at 0 °C. The mixture was stirred at 0 °C for 0.5 h, and then the amine hydrochloride **23** (339 mg, 0.75 mmol) and DIEA (121 mg, 0.9 mmol) were added successively. After being stirred at 0 °C for 0.5 h, the reaction mixture was allowed to warm to rt and stirred until completion. Saturated aqueous $NaHCO_3$ (20 mL) was added to quench the reaction. The mixture was extracted with EtOAc (30 mL×3). The combined EtOAc extracts were washed successively with water, aqueous 1 N HCl (20 mL×2), water and brine (40 mL), dried ($MgSO_4$) and concentrated in vacuo. The residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH=200:1 to 100:1) to afford hexapeptide **22** as a white foam (825 mg, 80%). $[\alpha]_D^{26}$ 10.8 (*c* 3.15, $CHCl_3$); IR (KBr): ν_{max} 3300, 2955, 2926, 1650, 1529, 1448 cm^{-1}; ^1H NMR (DMSO-d_6, 400 Hz, rt): δ 8.62–8.47 (2H, m), 8.20–8.18 (1H, m), 7.88–7.65 (4H, m), 7.37–7.32 (5H, m), 7.20 (31H, m), 5.12–4.98 (2H, m),4.90–4.75 (1H, m), 4.58–4.49 (1H, m), 4.35–4.12 (5H, m), 3.92–3.91 (1H, m), 3.64 (3H, d, *J*=12.0 Hz), 2.76–2.60 (3H, m), 2.44–2.35 (1H, m), 1.64–1.46 (8H, m), 1.22 (12H, s), 0.98 (3H, d, *J*=6.0 Hz), 0.85–0.80 (15H, m); ^{13}C NMR (MeOH-d_4, 100 Hz, rt): δ 173.9, 173.4, 173.3, 173.1, 172.9, 172.2, 172.1, 171.8, 171.4, 170.5, 170.4, 157.4, 145.8, 145.5, 137.5, 129.7, 129.2, 128.7, 128.4, 127.4, 71.2, 68.0, 67.6, 61.3, 60.5, 54.9, 54.5, 53.5, 53.3, 52.9, 52.2, 52.1, 41.3, 41.1, 40.5, 39.9, 39.2, 38.7, 38.6, 38.3, 38.0, 32.5, 32.1, 26.3, 25.3, 25.1, 23.9, 23.3, 21.8, 21.3, 20.8, 20.2, 19.3, 14.4. HRMS (ESI, *m*/*z*) calcd for $C_{81}H_{98}N_8NaO_{12}$ [M+Na^+] 1397.7202, found 1397.7196.

4.9. *N*-Boc–L-Orn(Cbz)-L-Thr-L-Ala-L-Asn(Trt)-D-Asn(Trt)-D-Leu-L-Thr-D-Leu-D-Ada–OMe (26)

A suspension of **24** (0.99 g, 0.72 mmol) and 10% Pd–C (200 mg) in MeOH (10 mL) was stirred under H_2 (1 atm) at rt for 4 h. The solid was removed by filtration through a pad of Celite and washed with MeOH (10 mL). The filtrate and washings were concentrated in vacuo to afford peptide amine **25** as a white paste (893 mg, 100%).

A solution of **22** (1.7 g, 3.1 mmol) in THF (20 mL) and H_2O (5 mL) was treated with LiOH monohydrate (258 mg, 6.2 mmol) at 0 °C for 2 h until 1 M aqueous HCl (10 mL) was added. The mixture was extracted with EtOAc (5 mL×5). The combined EtOAc extracts were dried ($MgSO_4$) and concentrated in vacuo to provide tripeptide acid **5** as a white foam.

To a solution of tripeptide acid **5** (780 mg, 1.45 mmol) in dry DCM (20 mL) was added HOBt (213 mg, 1.58 mmol) and EDCI (304 mg, 1.58 mmol) at 0 °C. After being stirred for 0.5 h, the peptide amine **25** (1.6 g, 1.32 mmol) and DIEA (204 mg, 1.58 mmol) were added at the same temperature. The reaction mixture was allowed to warm to rt and stirred until completion. Saturated aqueous $NaHCO_3$ (20 mL) was added to quench the reaction. The mixture was extracted with EtOAc (50 mL×3). The combined EtOAc extracts were washed successively with water, aqueous 1 M HCl (20×2 mL), water and brine (20 mL), dried ($MgSO_4$) and concentrated in vacuo. The residue was purified by column chromatography (CH_2Cl_2/MeOH=200:1 to 100:1) on silica gel to afford the linear nonapeptide **26** as a white foam (1.88 g, 81%). $[\alpha]_D^{30}$ −11.8 (*c* 3.20, $CHCl_3$); IR (KBr): ν_{max} 3300, 2955, 2926, 1650, 1529, 1448 cm^{-1}; ^1H NMR (DMSO-d_6, 400 Hz, rt): δ 8.58 (2H, d, *J*=9.2 Hz), 8.22 (2H, m), 7.91 (1H, d, *J*=6.4 Hz), 7.80 (1H, d, *J*=8.4 Hz), 7.73 (1H, d, *J*=7.2 Hz), 7.66 (1H, d, *J*=8.0 Hz), 7.35–7.21 (5H, m), 7.20–7.14 (31H, m), 7.08 (1H, d, *J*=7.6 Hz), 4.99 (2H, s), 4.96 (1H, d, *J*=4.4 Hz), 4.73 (2H, d, *J*=5.6 Hz), 4.57–4.56 (1H, m), 4.50–4.49 (1H, m), 4.39–4.31 (3H, m), 4.26–4.24 (1H, m), 4.16–4.10 (2H, m), 4.02–4.00 (1H, m), 3.95–3.89 (2H, m), 3.56 (3H, s), 2.96 (2H, d, *J*=5.2 Hz), 2.72–2.59 (4H, m), 1.63–1.45 (12H, m), 1.38 (9H, s), 1.23 (15H, m), 1.01 (3H, d, *J*=6.0 Hz), 0.96 (3H, d, *J*=6.4 Hz), 0.85–0.79 (15H, m); ^{13}C NMR (MeOH-d_4, 100 Hz, rt): δ 174.8, 174.3, 173.9, 173.4, 173.2,

173.0, 172.4, 172.0, 171.6, 170.3, 170.2, 157.5, 157.3, 145.9, 145.7, 138.3, 129.8, 129.7, 129.1, 128.6, 128.5, 128.4, 128.3, 127.4, 127.3, 80 .3, 71.2, 71.1, 68.3, 68.2, 68.1, 67.2, 67.1, 66.4, 61.1, 60.1, 60.0, 56.3, 53.9, 53.2, 52.1, 50.7, 41.3, 41.1, 41.0, 40.1, 38.6, 32.5, 32.2, 29.9, 27.0, 26.2, 25.2, 25.1, 23.5, 23.3, 22.0, 21.3, 20.6, 20.2, 20.1, 16.7, 14.4. HRMS (ESI, *m*/*z*) calcd for $C_{98}H_{128}N_{12}NaO_{18}$ [M+Na$^+$] 1783.9362, found 1783.9367.

4.10. Cyclo(-L-Orn(Cbz)-L-Thr-L-Ala-L-Asn(Trt)-D-Asn(Trt)-D-Leu-L-Thr-D-Leu-D-Ada-) (2a)

To a solution of **26** (330 mg, 0.187 mmol) in THF (2 mL) and H_2O (0.5 mL) was added LiOH monohydrate (24 mg, 0.57 mmol) at 0 °C. The reaction mixture was stirred at 0 °C for 2 h, and then quenched with the addition of 1 M aqueous HCl (10 mL). The mixture was extracted with EtOAc (5 mL×5). The combined EtOAc extracts were dried ($MgSO_4$), and concentrated in vacuo to provide peptide acid **27** as a white foam, which was directly used in the next step without further purification.

The above freshly prepared peptide acid **27** was treated with 2 M HCl in EtOAc (1 mL). The resulting solution was stirred at rt for 2 h. The mixture was concentrated in vacuo to afford the crude linear amino acid **28** as a white foam, which was directly used in the next step.

To a stirred solution of HOBt (120 mg, 0.9 mmol), EDCI (172 mg, 0.9 mmol) and DIEA (116 mg, 0.9 mmol) in dry DCM (20 mL) was added **28** (309 mg, 0.187 mmol) in DMF (10 mL) via syringe pump at 0 °C. The reaction mixture was allowed to warm to rt and stirred until completion. Saturated aqueous $NaHCO_3$ (10 mL) was added to quench the reaction. The mixture was extracted with EtOAc (20 mL×3). The combined EtOAc extracts were washed successively with H_2O, aqueous 1 M HCl (10 mL×2), H_2O and brine (10 mL), dried ($MgSO_4$) and concentrated in vacuo. The residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH=200:1 to 50:1) to afford cyclopeptide **2a** as a white foam (186 mg, 61%). $[\alpha]_D^{30}$ 33.9 (*c* 1.80, $CHCl_3$); IR (KBr): ν_{max} 3436, 2918, 2249, 1333 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 Hz, rt): δ 9.36 (1H, s), 8.89 (1H, s), 8.65 (1H, s), 8.20 (1H, s), 7.84 (2H, t, *J*=1.2 Hz), 7.35–7.00 (37H, m), 6.88 (1H, m), 6.87 (1H, d, *J*=8.4 Hz), 6.32 (1H, m), 5.35 (1H, d, *J*=3.2 Hz), 4.99 (2H, s), 4.83–4.74 (1H, m), 4.52 (1H, d, *J*=9.2 Hz), 4.15 (3H, m), 3.94–3.79 (4H, m), 3.72 (1H, s), 3.30–3.22 (2H, m), 3.00–2.90 (3H, m), 2.38 (1H, d, *J*=15.6 Hz), 2.04–2.03 (1H, m), 1.87–1.79 (3H, m), 1.61–1.46 (5H, m), 1.33–1.24 (21H, m), 0.98 (3H, d, *J*=5.2 Hz), 0.86–0.74 (12H, m), 0.31 (3H, d, *J*=5.2 Hz); ^{13}C NMR (MeOH-d_4, 75 Hz, rt): δ 177.0, 175.6, 175.5, 172.6, 175.3, 175.0, 174.5, 174.4, 174.0, 173.4, 171.1, 170.0, 158.6, 146.0, 145.7, 138.3, 130.1, 129.9, 129.4, 128.9, 128.8, 127.8, 72 .1, 71.6, 71.5, 67.3, 67.1, 66.9, 66.6, 64.1, 55.4, 55.0, 53.7, 52.6, 51.0, 50.6, 41.0, 40.9, 40.5, 39.6, 33.0, 31.6, 30.7, 30.4, 30.3, 30.1, 27.8, 26.9, 26.0, 24.4, 23.8, 23.7, 21.3, 21.2, 20.9, 20.7, 16.9, 15.4, 14.5. HRMS (ESI, *m*/*z*) calcd for $C_{92}H_{116}N_{12}NaO_5$ [M+Na$^+$] 1651.8575, found 1651.8581.

4.11. Cyclo(-L-Orn-L-Thr-L-Ala-L-Asn-D-Asn-D-Leu-L-Thr-D-Leu-D-Ada-) (2)

A suspension of **2a** (124 mg, 0.076 mol) and 10% Pd–C (60 mg) in dry MeOH (2 mL) and AcOH (0.5 mL) was stirred under H_2 atmosphere (1 atm) at rt for 4 h. The solid was filtered and the filtrate was evaporated to afford the crude product (114 mg). A portion of this product (10 mg) was then treated with a mixture of TFA–H_2O–^{i}Pr$_3$SiH (0.26 mL, 95:2.5:2.5) at rt for 3 h. The solvents were removed under reduced pressure. The residue was washed with Et_2O (2 mL×5) to afford **2** (6.0 mg, 99%) as a colourless solid. $[\alpha]_D^{30}$ +5.13 (*c* 2.07, MeOH); IR (KBr): ν_{max} 3334, 2944, 2832, 1032 cm^{-1}; ^{1}H NMR (acetone-d_6, 400 Hz, rt): δ 9.09–9.04 (1H, m), 8.79 (1H, m), 8.67 (1H, s), 7.80–7.72 (5H, m), 7.50 (1H, d, *J*=8.8 Hz), 7.25 (1H, m), 7.09 (2H, m), 6.95 (3H, d, *J*=22 Hz), 6.70–6.66 (1H, m), 5.32–5.31 (1H, m), 4.99 (1H, m), 4.76 (1H, m), 4.58 (1H, m), 4.47–4.43 (1H, m), 4.37 (1H, m), 4.18–4.16 (1H, m), 4.00–3.98 (5H, m), 3.63 (1H, s), 2.91 (1H, d, *J*=12.4 Hz), 2.77–2.67 (3H, m), 2.57 (1H, m), 2.39–2.33 (1H, m), 1.77 (5H, m), 1.64–1.50 (6H, m), 1.37 (3H, m), 1.24 (13H, m), 1.14(3H, d, *J*=5.6 Hz), 1.08 (3H, s), 0.91–0.75 (15H, m); ^{13}C NMR (acetone-d_6, 75 Hz, rt): δ 174.1, 173.1, 173.0, 172.6, 172.4, 172.3, 172.0, 171.8, 171.5, 171.4, 170.4, 158.5, 158.1, 119.2, 115.2, 111.3, 65.0, 62.2, 54.0, 52.8, 52.1, 51.0, 50.8, 49.2, 40.3, 40.1, 39.8, 39.5, 39.2, 39.0, 38.7, 38.4, 37.4, 36.2, 31.3, 30.0, 28.6, 28.5, 27.8, 26.0, 24.2, 24.0, 23.3, 23.2, 22.9, 22.1, 20.6, 20.3, 20.2, 16.4, 13.9. HRMS (ESI, *m*/*z*) calcd for $C_{46}H_{82}N_{12}NaO_{13}$ [M+Na$^+$] 1033.6022, found 1033.6049.

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Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2010.03.032.

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