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Cyclic dipeptides exhibit potency for scavenging radicals

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ABSTRACT

Twenty kinds of cyclic dipeptides containing L-leucine were synthesized, and their antioxidant activity against ·OH and O_2^- was investigated. Compounds possessing polar amino acid residues, such as Asp, Cys, Glu, Lys, Pro, Ser, and Trp, exhibited higher antioxidant activity against ·OH than vitamin E. However, only cyclo(L-Cys–L-Leu) scavenged O_2^-.

1. Introduction

Cyclicpeptides play important roles in biology. Many drugs, such as cyclosporine and colistin, contain a cyclicpeptide structure. Cyclosporin is an immunosuppressant and is used for treatment of autoimmune disease and rejection of internal organ transplants. Colistin treats multiple-drug-resistant *Pseudomonas aeruginosa*. RAs[1] and trapoxin,[2] which are cyclic hexapeptide and cyclic tetrapeptide, respectively, were reported to exhibit antitumor activity. Thus, cyclicpeptides indicate various biological activities. Among them, cyclicdipeptides (CDPs) belong to a group of compounds with the simplest structure.

CDPs are generated by cyclization of two amino acids, and they characteristically contain a 2,5-diketopiperazine skeleton. CDPs are found in various foods and beverages such as cocoa,[3] roasted coffee,[4] beer[5] and Japanese sake.[6] Their bitter taste is unique. CDPs were also isolated from marine fungus[7–11] and were reported to have biological activity, antimicrobacterial activity,[12] antitumor activity,[13] platelet-activating factor inhibitory activity,[14] etc.

We previously reported that several CDPs were contained in the distillation residue of awamori spirits,[15] and they exhibited antioxidant activity by the bleomycin-Fe method. Most CDPs contained of L-Leu and other amino acids, and exhibited nearly identical activities. Moreover, we reported the protective effects of cyclo(L-Leu-L-Tyr) against postischemic myocardial dysfunction.[16] In this way, cyclic dipeptides can exhibit interesting biological activities. In this study, we synthesized CDPs containing L-Leu and examined their antioxidant activity by using ESR to investigate the amino acid contribution to the activity.

2. Chemistry

A general synthetic scheme for most cyclic dipeptides is shown in Figure 1. A *N*-protected-L-amino acid and a L-amino acid methyl ester were used as starting materials.[17] They were treated with *N*,*N*́-dicyclohexylcarbodiimide (DCC) in the presence of triethylamine (TEA) to give a *N*-protected-dipeptide methyl ester (**1**). The dipeptides except for compounds **1P** and **1Q** were treated with 4 M HCl–1,4-dioxane followed by refluxing in 0.1 M acetic acid–2-butanol with *N*-methylmorphorine (NMM) for 3 h to give CDPs (**3**). *N*-Boc-L-Leu-L-Pro methyl ester (**1P**) was converted into L-Leu-L-Pro methyl ester by treatment with 4 M HCl–1,4-dioxane, then the ester was refluxed in benzene for 3 days to afford cyclo(L-Leu-L-Pro) (**3P**). On the other hand, N^{α}-Z-N^{ω}-Trt-L-Gln-L-Leu methyl ester (**1Q**) was treated with Pd-C in methanol to remove the Z-group. The resulting dipeptide methyl ester in 0.1 M acetic acid–2-butanol was refluxed 1 h with NMM to afford cyclo(N^{ω}-Trt-L-Gln-L-Leu) (**2Q**). Compound **2Q** was further treated with 50% trifluoroacetic acid-dichloromethane to give cyclo(L-Gln-L-Leu) (**3Q**).[18] Cyclo (L-Cys-L-Leu) (**3C**),[19] cyclo(L-His-L-Leu) (**3H**),[20] cyclo(L-Glu-L-Leu) (**3E**), cyclo(L-Leu-L-Lys) (**3K**) and cyclo(L-Arg-L-Leu) (**3R**)[21] were

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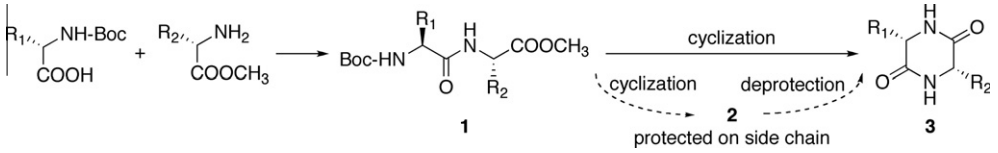

Figure 1. General scheme for preparation of cyclic dipeptides.

prepared by deprotection on the side chains, respectively, after generation of diketopiperazines (**2**).

3. Antioxidant activity

We investigated antioxidant activities of CDPs against ·OH and $O_2^{\cdot-}$ using ESR spectra.[22] Among the CDPs tested, some (**3S**, **3M**, **3W**, **3P**, **3C**, **3D**, **3E** and **3K**) exhibited higher antioxidant activity against ·OH at a concentration of 2.5×10^{-5} M than vitamin E used as a positive control (37.7% inhibition). In particular, **3D** showed the highest antioxidant activity (95.0%, IC_{50} 4.0×10^{-6} M). On the other hand, only **3C** exhibited high activity against $O_2^{\cdot-}$ (83.3%, 8.8×10^{-4} M) at the same concentration, but other CDPs did not exhibit antioxidant activity as shown in Table 1.

4. Discussion

We synthesized 20 kinds of CDPs, which all contain L-Leu, and investigated antioxidant activities by using ESR. Most of the CDPs scavenged ·OH. Radicals on the α-position of the peptide carbonyl group can be conveniently produced by H-atom abstraction from CDPs by ·OH.[23] According to this theory, the antioxidant activity of CDPs is demonstrated by this mechanism, and CDP radicals would become hydroxyL-CDPs or CDP dimers. Moreover the potency of antioxidant activity against ·OH should be almost identical. However, among the CDPs tested, some (**3S**, **3M**, **3W**, **3P**, **3C**, **3D**, **3E** and **3K**) exhibited higher antioxidant activity against ·OH than vitamin E, and some did not. On the basis of the evidence that most of the CDPs that possessed high antioxidant activity tend to have the highly polar functional group on their side chains, such as carboxylic group and amino group, the radical scavenging effect of these functional groups would contribute more to antioxidant activity than the generation of radical on the α carbon as mentioned above. We tried to identify products of the oxidation reaction by hydroxyl radical. However, the amount of the products were too small to discuss their structure. So the precise mechanism for scavenging radicals on the side chain of the amino acid residue is not clear yet, and its investigation is in progress. On the other hand, only **3C** exhibited antioxidant activity against $O_2^{\cdot-}$ (83.3%, IC_{50} 8.8×10^{-4} M) at a concentration of 2.5×10^{-5} M, but other CDPs did not exhibit antioxidant activity. The potency of compound **3C** should due to the thiol group on the side chain to form S–S linkage to exhibit the radical scavenging effect.

As mentioned above, the contribution of an amino acid residue with highly polar functional groups in the side chains to the antioxidant activity is critical. In addition to investigating the mechanism of antioxidant activity, we are now examining the activity of CDPs that consist of an amino acid with a highly polar side chain.

5. Experimental

5.1. General

Infrared spectra were recorded on the FT-IR-410 spectrophotometer. ^1H and ^{13}C NMR spectra were recorded on the JEOL ECA-500 (^1H: 500 MHz and ^{13}C: 125 MHz) and JEOL JNM A-400 (^1H: 400 MHz and ^{13}C: 100 MHz) spectrometers. Chemical shifts for ^1H and ^{13}C NMR are given in parts per million (δ) relative to solvent signal (methanol-d_4; δ_H 3.30 and δ_C 49.0, and chloroform-*d*; δ_H 7.24 and δ_C 77.7) as the internal standard. FAB-MS were obtained with a JEOL JMS HX-110 spectrometer, and *m*-nitrobenzyl alcohol was used as a matrix. Analytical TLC was performed on silica gel

Table 1
Radical scavenging activity of cyclic dipeptides

	·OH		$O_2^{\cdot-}$	
	Inhibition rate[a] (%)	IC_{50} (M)	Inhibition rate[a] (%)	IC_{50} (M)
Cyclo(Gly-L-Leu) (**3G**)	0.0 ± 0.10	—	0.0 ± 0.13	—
Cyclo(L-Ala-L-Leu) (**3A**)		—	0.0 ± 0.15	—
Cyclo(L-Leu-L-Val) (**3V**)	12.3 ± 0.12	—	0.0 ± 0.23	—
Cyclo(L-Ile-L-Leu) (**3I**)	9.7 ± 0.09	—		
Cyclo(L-Leu-L-Leu) (**3L**)	26.0 ± 0.14	—	0.0 ± 0.05	—
Cyclo(L-Leu-L-Ser) (**3S**)	95.2 ± 0.04	1.0×10^{-3}	0.0 ± 0.25	—
Cyclo(L-Leu-L-Thr) (**3T**)	0.0 ± 0.11	—	0.0 ± 0.17	—
Cyclo(L-Leu-L-Met) (**3M**)	79.0 ± 0.11	8.0×10^{-4}	0.0 ± 0.08	—
Cyclo(L-Cys-L-Leu) (**3C**)	88.9 ± 0.02	1.0×10^{-4}	83.3 ± 0.01	8.8×10^{-4}
Cyclo(L-Leu-L-Phe) (**3F**)	23.5 ± 0.12	—	0.0 ± 0.24	—
Cyclo(L-Leu-L-Tyr) (**3Y**)				—
Cyclo(L-Leu-L-Trp) (**3W**)	66.0 ± 0.33	1.8×10^{-3}	0.0 ± 0.21	—
Cyclo(L-His-L-Leu) (**3H**)	83.9 ± 0.05	8.5×10^{-4}	0.0 ± 0.17	—
Cyclo(L-Asp-L-Leu) (**3D**)	95.0 ± 0.02	4.0×10^{-6}	0.0	—
Cyclo(L-Glu-L-Leu) (**3E**)	97.8 ± 0.21	8.0×10^{-5}	0.0 ± 0.26	—
Cyclo(L-Asn-L-Leu) (**3N**)	96.0 ± 0.15	1.7×10^{-4}	0.0 ± 0.30	—
Cyclo(L-Gln-L-Leu) (**3Q**)	19.0 ± 0.14	—	0.0 ± 0.19	—
Cyclo(L-Arg-L-Leu) (**3R**)	—			—
Cyclo(L-Leu-L-Lys) (**3K**)	96.0 ± 0.05	1.6×10^{-4}	0.0 ± 0.25	—
Cyclo(L-Leu-L-Pro) (**3P**)	86.2 ± 0.03	1.1×10^{-3}	0.0 ± 0.02	—
Vitamin E	37.7		32.3	

[a] At 2.5×10^{-3} M.

60 F254 (Merck). Column chromatography was carried out on silica gel BW-820MH (Fuji Silysia Chemicals, Co. Ltd, Seto, Japan). Develosil ODS MG-5 (φ20 × 250 mm, Nomura Chemical, Seto, Japan) was used for preparative HPLC. ESR spectra were recorded on the JEOL JES FR-30 spectrometer. The operated conditions for the ESR to estimate the concentration of superoxide anion radicals were as follows: magnetic field, 335.7 ± 5 mT; power, 4 mW 9.414 GHz; modulation, 100 kHz 1 × 0.079 mT; response, 0.1 s; temperature, 25 °C; amplitude, 160; and sweep time, 2 min, respectively. For the determination of hydroxy radicals, the magnetic field, power, modulation, response, temperature, amplitude, and sweep time were 335.7 ± 5 mT, 4 mW 9.414 GHz, 100 kHz 1 × 0.079 mT, 0.1 s, 25 °C, 160 and 2 min, respectively. Hypoxantine, diethylenetriaminepentaacetic acid (DETAPAC) and xantine oxidase from bovine milk were purchased from Sigma–Aldrich Co. 5,5-Dimethyl-1-pyrroline *N*-oxide (DMPO) was purchased from Labotec (Tokyo). Other chemicals used were of analytical grade.

5.2. General procedure for the synthesis of *N*-Boc-dipeptide me thyl ester (1)

To a solution of L-amino acid methyl ester hydrochloride in dichloromethane, triethylamine (1 equiv) and *N*-Boc-L-amino acid were added at −15 °C, and DCC was added as a dichloromethane solution at 0 °C. The reaction mixture was stirred at 0 °C for 1 h. Then the solution was stirred at room temperature for 3 h. The dicyclohexylurea was removed by filtration from the reaction mixture. The solution was washed with 5% $NaHCO_3$, 1 M HCl, and brine. The organic layer was dried over anhydrous Na_2SO_4 and evaporated. The product was purified by silica gel column chromatography.

5.2.1. *N*-Boc-L-Ala-L-Leu methyl ester (1A)

Yield 97%; white powder; $[\alpha]_D^{25}$: −47.0 (*c* 0.10, MeOH); IR ν_{max} (Film): 3019, 2961, 1739, 1683 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$, δ_H): 0.83 (3H, d, *J* = 6.6 Hz), 0.85 (3H, d, *J* = 6.6 Hz), 1.19 (3H, d, *J* = 7.3 Hz), 1.34 (9H, s), 1.51 (2H, m), 1.61 (m), 3.60 (3H, s), 3.99 (1H, br d, *J* = 6.4 Hz), 4.36 (1H, dd, *J* = 5.9, 9.2 Hz); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C); 17.9, 21.7, 22.7, 24.7, 28.2, 41.3, 49.3, 49.8, 50.6, 52.2, 80.0, 155.5, 172.4, 173.2: HRMS–FAB: *m*/*z* 317.2083 $[M+H]^+$ calcd for $C_{15}H_{29}N_2O_5$ 317.2076; FAB-MS (positive): *m*/*z* 317 $[M+H]^+$.

5.2.2. *N*-Boc-S-4-methoxybenzyL-L-Cys-L-Leu methyl ester (1C)

Yield 82%; white powder; $[\alpha]_D^{25}$: −25.9 (*c* 0.10, MeOH); IR ν_{max} (Film): 3314, 2956, 2932, 1744, 1682, 1657 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, δ_H): 0.90 (3H, d, *J* = 6.6 Hz), 0.92 (3H, d, *J* = 6.6 Hz), 1.30 (1H, m), 1.40 (9H, s), 1.55 (2H, m), 2.70 (1H, dd, *J* = 7.0, 14.3 Hz), 2.83 (1H, dd, *J* = 5.5, 14.3 Hz), 3.69 (3H, s), 3.77 (3H, s), 4.20 (1H, br s), 4.57 (1H, dt, *J* = 4.8, 8.4 Hz), 5.24 (1H, br s), 6.67 (1H, br s), 6.82 (2H, d, *J* = 9.6 Hz), 7.23 (2H, d, *J* = 9.6 Hz); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C): 21.8, 22.8, 24.7, 28.2, 33.4, 33.9, 35.9, 41.4, 50.9, 52.2, 55.2, 80.3, 114.0, 129.8, 130.1, 155.3, 158.7, 170.4, 172.8; HRMS–FAB: *m*/*z* 469.2369 $[M+H]^+$ calcd for $C_{23}H_{37}N_2O_6S$ 469.2372; FAB-MS (positive): *m*/*z* 469 $[M+H]^+$.

5.2.3. *N*-Boc-4-benzyL-L-Asp-L-Leu methyl ester (1D)

Yield 89%; white powder; $[\alpha]_D^{25}$: −23.5 (*c* 0.10, MeOH); IR ν_{max} (KBr): 3416, 3332, 2959, 1739, 1684 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, δ_H): 0.91 (3H, d, *J* = 6.0 Hz), 0.92 (3H, d, *J* = 6.0 Hz), 1.45 (9H, s), 1.54 (1H, m), 1.64 (2H, m), 2.72 (1H, dd, *J* = 6.4, 17.0 Hz), 3.02 (1H, dd, *J* = 4.5. 17.0 Hz), 3.70 (3H, s), 4.55 (1H, m), 5.14 (1H, dd, *J* = 12.0, 17.0 Hz), 6.88 (1H, br d, *J* = 7.2 Hz), 7.34 (5H, m); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C): 21.8, 22.8, 24.7, 28.2, 36.0, 41.3, 50.5, 50.9, 52.0, 66.8, 80.5, 128.2, 128.3, 128.6, 135.4, 155.5, 170.5, 171.8, 172.9; HRMS–FAB: *m*/*z* 451.2407 $[M+H]^+$ calcd for $C_{23}H_{35}N_2O_7$ 451.2444; FAB-MS (positive): *m*/*z* 451 $[M+H]^+$.

5.2.4. *N*-Boc-5-benzyL-L-Glu-L-Leu methyl ester (1E)

Yield 94%; white powder; $[\alpha]_D^{25}$: −31.7 (*c* 0.20, MeOH); IR ν_{max} (KBr): 3338, 2954, 1745, 1726, 1685, 1656 cm^{-1}; ^1H NMR (500 MHz, CD_3OD, δ_H): 0.89 (3H, d, *J* = 6.8 Hz), 0.93 (3H, d, *J* = 6.8 Hz), 1.42 (9H, s), 1.59 (3H, m), 1.95 (1H, m), 2.06 (1H, m), 2.47 (2H, t, *J* = 7.4 Hz), 3.66 (3H, s), 4.12 (1H, t, *J* = 6.2 Hz), 4.45 (1H, dd, *J* = 5.7, 9.2 Hz), 5.12 (2H, s), 7.33 (5H, m); ^{13}C NMR (125 MHz, CD_3OD, δ_C): 21.8, 23.3, 25.8, 28.5, 28.5, 28.7, 31.3, 34.8, 41.3, 52.1, 57.7, 64.9, 67.4, 80.6, 129.2, 129.5, 137.6, 157.7, 174.3, 174.4, 174.5; HRMS–FAB: *m*/*z* 465.2578 $[M+H]^+$ calcd for $C_{25}H_{39}N_2O_7$ 465.2601; FAB-MS (positive): *m*/*z* 465 $[M+H]^+$.

5.2.5. *N*-Boc-L-Leu-L-Phe methyl ester (1F)

Yield 77%; white powder; $[\alpha]_D^{25}$: −17.3 (*c* 0.10, MeOH); IR ν_{max} (KBr): 3329, 3285, 2954, 1748, 1686, 1657 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, δ_H): 0.86 (3H, d, *J* = 6.6 Hz), 0.89 (3H, d, *J* = 6.6 Hz), 1.42 (9H, s), 1.61 (2H, m), 1.94 (1H, m), 3.06 (1H, dd, *J* = 7.7, 13.6 Hz), 3.15 (1H, dd, *J* = 7.7, 13.6 Hz), 3.69 (3H, s), 4.05 (1H, br s), 4.74 (1H, br s), 4.82 (1H, dd, *J* = 5.8, 13.6 Hz), 6.43 (1H, d, *J* = 9.7 Hz), 7.25 (5H, m); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C): 21.9, 22.8, 24.7, 28.3, 33.9, 37.9, 41.2, 49.1, 52.3, 53.1, 80.0, 127.1, 128.5, 129.3, 135.7, 156.7, 171.6, 172.1; HRMS–FAB: *m*/*z* 393.2386 $[M+H]^+$ calcd for $C_{21}H_{33}N_2O_5$ 393.2389; FAB-MS (positive): *m*/*z* 393 $[M+H]^+$.

5.2.6. *N*-Boc-Gly-L-Leu methyl ester (1G)

Yield: 83%; white powder; $[\alpha]_D^{25}$: −22.4 (*c* 0.06, MeOH); IR ν_{max} (KBr): 3261, 3237, 3067, 1690, 1625 cm^{-1}; ^1H NMR (500 MHz, CD_3OD, δ_H): 0.90 (3H, d, *J* = 5.6 Hz), 0.93 (3H, d, *J* = 5.6 Hz), 1.31 (1H, m), 1.44 (9H, s), 1.61 (2H, m), 3.69 (3H, s), 3.71 (2H, s), 4.48 (1H, m); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C): 21.8, 22.7, 24.7, 28.2, 41.5, 44.3, 50.6, 52.3, 80.3, 156.0, 169.2, 173.2; HRMS–FAB: *m*/*z* 325.1746 $[M+Na]^+$ calcd for $C_{14}H_{26}N_2O_5Na$ 325.1739; FAB-MS (positive): *m*/*z* 325 $[M+Na]^+$.

5.2.7. N^{α}-Boc-N^{π}-BOM-L-His-L-Leu methyl ester (1H)

Yield 70%; white powder; $[\alpha]_D^{25}$: −20.2 (*c* 0.20, MeOH); IR ν_{max} (Film): 3422, 3330, 3214, 3015, 2959, 1742, 1678 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, δ_H): 0.85 (3H, d, *J* = 6.6 Hz), 0.87 (3H, d, *J* = 6.6 Hz), 1.39 (9H, s), 1.45 (3H, m), 3.04 (1H, dd, *J* = 7.7, 15.4 Hz), 3.10 (1H, dd, *J* = 7.6, 15.4 Hz), 3.68 (3H, s), 4.48 (1H, m), 4.50 (2H, s), 4.50 (2H, m), 6.92 (1H, br s), 7.30 (5H, m), 7.61 (br s); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C): 21.7, 22.7, 24.6, 26.6, 28.2, 41.2, 50.6, 52.2, 53.7, 69.9, 73.0, 80.2, 127.2, 128.1, 128.3, 128.7, 129.4, 135.9, 155.4, 170.6, 172.7; HRMS–EI: *m*/*z* 502.2773 $[M]^+$ calcd for $C_{26}H_{38}N_4O_6$ 502.2791; EI-MS: *m*/*z* 502 $[M]^+$.

5.2.8. *N*-Boc-L-Ile-L-Leu methyl ester (1I)

Yield 44%; white powder; $[\alpha]_D^{25}$: −46.4 (*c* 0.10, MeOH); IR ν_{max} (KBr): 3330, 3276, 3078, 2963, 1756, 1685, 1648 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, δ_H): 0.92 (12H, m), 1.42 (9H, s), 1.57 (5H, m), 1.84 (1H, br s), 3.70 (3H, s), 3.89 (1H, dd, *J* = 6.6, 8.8 Hz), 4.60 (1H, dt, *J* = 4.8, 8.6 Hz), 5.00 (1H, br d, *J* = 7.0 Hz), 6.16 (1H, br d, *J* = 6.6 Hz); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C): 11.3, 15.4, 21.8, 22.7, 24.7, 24.8, 37.1, 41.4, 50.6, 52.2, 59.2, 79.8, 155.7, 171.4, 173.1; HRMS–FAB: *m*/*z* 359.2549 $[M+H]^+$ calcd for $C_{18}H_{35}N_2O_5$ 359.2546; FAB-MS (positive): *m*/*z* 359 $[M+H]^+$.

5.2.9. N^{α}-Boc-N^{ε}-Z-L-Lys-L-Leu methyl ester (1K)

Yield 44%; white powder; $[\alpha]_D^{25}$: −23.8 (*c* 0.10, MeOH); IR ν_{max} (Film): 3431, 3329, 3017, 2957, 1707, 1675 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$, δ_H): 0.85 (6H, d, *J* = 5.5 Hz), 1.36 (9H, s), 1.47 (3H, m), 1.61 (5H, m), 1.78 (m), 3.12 (2H, m), 3.61 (3H, s), 4.02 (m), 4.52 (m), 5.02 (2H, s), 7.37 (5H, m); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C): 21.7, 22.3, 22.9, 24.7, 24.9, 28.2, 29.3, 31.7, 40.3, 41.2, 50.7, 52.3, 54.1, 66.6, 80.0, 128.1, 128.4, 136.6, 155.7, 156.6, 171.9,

173.3; HRMS–FAB: m/z 508.3012 $[M+H]^+$ calcd for $C_{26}H_{42}N_3O_7$ 508.3023; FAB-MS (positive): m/z 508 $[M+H]^+$.

5.2.10. *N*-Boc-L-Leu-L-Leu methyl ester (1L)

Yield 57%; white powder; $[\alpha]_D^{25}$: −44.1 (c 0.10, MeOH); IR ν_{max} (Film): 3434, 3346, 3019, 2961, 1740, 1697, 1682 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, δ_H): 0.92 (12H, m), 1.24 (9H, s), 1.60 (6H, m), 3.71 (3H, s), 4.06 (1H, br s), 4.59 (1H, dt, J = 5.1, 9.2 Hz), 4.81 (1H, br s), 6.37 (1H, br d, J = 7.0 Hz); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C): 21.8, 22.1, 22.8, 24.7, 28.2, 33.9, 40.8, 41.5, 50.6, 52.2, 52.9, 80.0, 155.7, 172.2, 173.1; HRMS–FAB: m/z 359.2512 $[M+H]^+$ calcd for $C_{18}H_{35}N_2O_5$ 359.2546; FAB-MS (positive): m/z 359 $[M+H]^+$.

5.2.11. *N*-Boc-L-Met-L-Leu methyl ester (1M)

Yield 71%; white powder; $[\alpha]_D^{25}$: −27.0 (c 0.10, MeOH); IR ν_{max} (Film): 3422, 3333, 3017, 2960, 1743, 1680 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$, δ_H): 0.89 (6H, d, J = 5.5 Hz), 1.41 (9H, s), 1.52 (1H, m), 1.63 (2H, m), 1.90 (1H, m), 2.03 (1H, m), 2.03 (3H, s), 2.56 (2H, t, J = 7.3 Hz), 3.69 (3H, s), 4.27 (1H, m), 4.56 (1H, ddd, J = 4.1, 8.7, 12.9 Hz), 5.19 (1H, d, J = 7.8 Hz), 6.58 (1H, d, J = 6.9 Hz); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C): 12.5, 21.7, 22.8, 24.7, 28.2, 30.0, 31.3, 50.7, 52.2, 53.1, 80.1, 156.5, 171.3, 173.0; HRMS–FAB: m/z 377.2094 $[M+H]^+$ calcd for $C_{17}H_{33}N_2O_5S$ 377.2110; FAB-MS (positive): m/z 377 $[M+H]^+$.

5.2.12. *N*-Boc-L-Asn-L-Leu methyl ester (1N)

Yield 46%; white powder; $[\alpha]_D^{25}$: −35.3 (c 0.10, MeOH); IR ν_{max} (KBr): 3412, 3317, 3206, 2958, 1741, 1685, 1667 cm^{--1}; ^1H NMR (500 MHz, CD_3OD, δ_H): 0.92 (3H, d, J = 6.4 Hz), 0.93 (3H, d, J = 6.4 Hz), 1.43 (9H, s), 1.62 (2H, m), 1.71 (1H, m), 2.56 (1H, dd, J = 7.8, 15.6 Hz), 2.65 (1H, dd, J = 5.0, 15.6 Hz), 3.69 (3H, s), 4.45 (2H, m); ^{13}C NMR (125 MHz, CD_3OD, δ_C): 21.8, 23.3, 25.8, 27.0, 28.5, 28.6, 38.1, 41.5, 52.2, 52.7, 52.7, 157.5, 174.0, 174.4, 175.0; HRMS–FAB: m/z 360.2147 $[M+H]^+$ calcd for $C_{16}H_{30}N_3O_6$ 360.2135; FAB-MS (positive): m/z 360 $[M+H]^+$.

5.2.13. *N*-Boc-L-Leu-L-Pro methyl ester (1P)

Yield 87%; white powder; $[\alpha]_D^{25}$: −85.1 (c 0.10, MeOH); IR ν_{max} (KBr): 3328, 2958, 1750, 1709, 1648 cm^{-1}; ^1H NMR (500 MHz, CD_3OD, δ_H): 0.92 (6H, d, J = 6.2 Hz), 1.38 (9H, s), 1.40 (m), 1.90 (5H, m), 2.22 (m), 3.57 (m), 3.65 (3H, s), 3.80 (1H, br dd, J = 5.8, 14.7 Hz), 4.33 (1H, br s), 4.42 (1H, m); ^{13}C NMR (125 MHz, CD_3OD, δ_C): 21.9, 23.6, 25.8, 25.9, 29.9, 34.8, 41.3, 48.2, 50.9, 52.6, 60.4, 80.5, 158.0, 174.0, 174.1; HRMS–FAB: m/z 343.2222 $[M+H]^+$ calcd for $C_{17}H_{31}N_2O_5$ 343.2233; FAB-MS (positive): m/z 343 $[M+H]^+$.

5.2.14. N^{α}-Z-N^{ω}-Trt-L-Gln-L-Leu methyl ester (1Q)

Yield 45%; white powder; $[\alpha]_D^{25}$: −1.9 (c 0.14, MeOH); IR ν_{max} (KBr): 3310, 3030, 2956, 1670 cm^{-1}; ^1H NMR (500 MHz, CD_3OD, δ_H): 0.88 (3H, d, J = 8.7 Hz), 0.90 (3H, d, J = 8.7 Hz), 1.57 (2H, t, J = 6.8 Hz), 1.68 (1H, m), 1.85 (1H, m), 2.01 (1H, m), 2.44 (2H, m), 3.64 (3H, s), 4.14 (1H, dd, J = 5.7, 8.5 Hz), 4.42 (1H, t, J = 7.4 Hz), 5.07 (2H, d, J = 2.3 Hz), 7.22 (20H, m); ^{13}C NMR (125 MHz, CD_3OD, δ_C): 21.8, 23.3, 25.9, 29.2, 33.6, 41.2, 49.9, 52.2, 52.7, 55.5, 67.7, 71.6, 127.8, 128.7, 128.9, 129.0, 130.0, 138.2, 145.9, 158.3, 174.3, 174.6; HRMS–FAB: m/z 650.7981 $[M+H]^+$ calcd for $C_{39}H_{45}N_3O_6$ 650.7974; FAB-MS (positive): m/z 650 $[M+H]^+$.

5.2.15. N^{α}-Boc-N^{G}-nitro-L-Arg-L-Leu methyl ester (1R)

Yield 86%; white powder; $[\alpha]_D^{25}$: −6.3 (c 0.10, MeOH); IR ν_{max} (Film): 2959, 2871, 1741, 1651 cm^{-1}; ^1H NMR (500 MHz, CD_3OD, δ_H): 0.91 (3H, d, J = 6.0 Hz), 0.92 (3H, d, J = 6.0 Hz), 1.43 (9H, s), 1.67 (7H, m), 3.26 (2H, br s), 3.69 (3H, s), 4.09 (1H, br.t, J = 7.3 Hz), 4.46 (1H, dd, J = 5.5, 9.6 Hz); ^{13}C NMR (125 MHz, CD_3OD, δ_C): 21.8, 23.3, 25.8, 26.5, 27.0, 30.5, 41.4, 41.8, 52.1, 52.7, 80.7, 157.6, 161.0, 174.5, 174.8; HRMS–FAB: m/z 447.2565 $[M+H]^+$ calcd for $C_{18}H_{35}N_6O_7$ 447.2567; FAB-MS (positive): m/z 447 $[M+H]^+$.

5.2.16. *N*-Boc-L-Leu-L-Ser methyl ester (1S)

Yield 57%; white powder; $[\alpha]_D^{25}$: −14.0 (c 0.10, MeOH); IR ν_{max} (Film): 3432, 3019, 1743, 1677 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, δ_H): 0.92 (3H, d, J = 6.6 Hz), 0.93 (3H, d, J = 6.6 Hz), 1.43 (9H, s), 1.48 (1H, m), 1.64 (2H, m), 3.77 (3H, s), 3.92 (2H, m), 4.07 (1H, m), 4.62 (1H, dt, J = 3.7, 7.3 Hz), 4.99 (1H, d, J = 7.0 Hz), 6.93 (1H, br d, J = 7.3 Hz); ^{13}C NMR (125 MHz, CD_3OD, δ_C): 21.9, 23.5, 25.9, 28.7, 42.0, 52.8, 54.5, 56.1, 62.8, 80.7, 157.9, 172.000, 175.8; HRMS–FAB: m/z $[M+H]^+$ 333.2014 calcd for $C_{15}H_{29}N_2O_6$ 333.2026; FAB-MS (positive): m/z 333 $[M+H]^+$.

5.2.17. *N*-Boc-L-Thr-L-Leu methyl ester (1T)

Yield 97%; white powder; $[\alpha]_D^{25}$: −37.7 (c 0.10, MeOH); IR ν_{max} (Film): 3329, 2960, 2872, 1744, 1700, 1654 cm^{-1}; ^1H NMR (400 MHz, CD_3OD, δ_H): 0.91 (3H, d, J = 6.8 Hz), 0.93 (3H, d, J = 6.8 Hz), 1.19 (3H, d, J = 5.5 Hz), 1.45 (9H, s), 1.60 (2H, m), 1.70 (1H, m), 3.69 (3H, s), 4.02 (2H, m), 4.48 (1H, dd, J = 5.9, 9.2 Hz); ^{13}C NMR (125 MHz, CD_3OD, δ_C): 17.9, 21.6, 22.8, 24.7, 28.2, 41.0, 50.7, 52.3, 57.6, 66.7, 80.3, 156.4, 171.5, 173.1; HRMS–FAB: m/z 347.2197 $[M+H]^+$ calcd for $C_{16}H_{31}N_2O_6$ 347.2182; FAB-MS (positive): m/z 347 $[M+H]^+$.

5.2.18. *N*-Boc-L-Leu-L-Val methyl ester (1V)

Yield 67%; white powder; $[\alpha]_D^{25}$: −33.4 (c 0.1, MeOH); IR ν_{max} (Film): 3436, 3328, 3070, 2964, 1739, 1686 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, δ_H): 0.92 (12H, m), 1.42 (9H, s), 1.43 (1H, m), 1.65 (2H, m), 2.15 (1H, m), 3.71 (3H, s), 4.09 (1H, br s), 4.51 (1H, dd, J = 4.9, 8.5 Hz), 4.82 (1H, br s), 6.52 (1H, br d, J = 4.8 Hz); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C): 17.5, 18.9, 22.1, 22.8, 24.7, 28.2, 31.2, 40.6, 52.1, 56.9, 80.0, 155.7, 172.1, 172.4; HRMS–FAB: m/z 345.2383 $[M+H]^+$ calcd for $C_{17}H_{33}N_2O_5$ 345.2389; FAB-MS (positive): m/z 345 $[M+H]^+$.

5.2.19. *N*-Boc-L-Trp-L-Leu methyl ester (1W)

Yield 94%; white powder; $[\alpha]_D^{25}$: −14.7 (c 0.10, MeOH); IR ν_{max} (Film): 3329, 2960, 2872, 1744, 1700, 1654 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, δ_H): 0.82 (3H, d, J = 6.7 Hz), 0.84 (3H, d, J = 6.7 Hz), 1.41 (9H, s), 1.48 (3H, m), 3.16 (1H, dd, J = 6.3, 14.7 Hz), 3.26 (1H, dd, J = 5.1, 14.7 Hz), 3.61 (3H, s), 4.42 (1H, br s), 4.48 (1H, m), 5.57 (1H, br s), 6.22 (1H, d, J = 8.1 Hz), 7.02 (1H, br s), 7.09 (1H, t, J = 8.7 Hz), 7.16 (1H, t, J = 8.7 Hz), 7.32 (1H, d, J = 8.7 Hz), 7.63 (1H, d, J = 8.7 Hz), 8.32 (s); ^{13}C NMR (125 MHz, CD_3OD, δ_C): 21.9, 23.3, 25.7, 28.6, 34.8, 41.6, 52.0, 52.6, 56.7, 80.6, 110.9, 112.2, 119.4, 122.3, 124.6, 128.9, 138.0, 157.6, 174.3, 174.8; HRMS–FAB: m/z 432.2485 $[M+H]^+$ calcd for $C_{23}H_{34}N_3O_5$ 432.2498; FAB-MS (positive): m/z $[M+H]^+$ 432.

5.2.20. *N*-Boc-L-Leu-L-Tyr methyl ester (1Y)

Yield 65%; white powder; $[\alpha]_D^{25}$: −16.5 (c 0.10, MeOH); IR ν_{max} (Film): 3323, 2960, 1746, 1697, 1657 cm^{-1}; ^1H NMR (400 MHz, CD_3OD, δ_H): 0.88 (3H, d, J = 6.6 Hz), 0.92 (3H, d, J = 6.6 Hz), 1.15 (1H, m), 1.42 (9H, s), 1.48 (3H, m), 2.91 (1H, dd, J = 7.7, 14.0 Hz), 3.02 (1H, dd, J = 5.5, 14.0 Hz), 3.66 (3H, s), 4.05 (1H, dd, J = 7.3, 16.1 Hz), 4.60 (1H, t, J = 7.3 Hz), 6.68 (2H, d, J = 8.4 Hz), 6.98 (2H, d, J = 8.4 Hz); ^{13}C NMR (125 MHz, CD_3OD, δ_C): 22.5, 23.8, 29.2, 38.1, 42.6, 53.1, 55.9, 55.6, 81.1116.8, 128.9, 131.8, 157.9, 158.2, 173.8, 175.9; HRMS–FAB: m/z 409.2339 $[M+H]^+$ calcd for $C_{21}H_{33}N_2O_6$ 409.2339; FAB-MS (positive): m/z 409 $[M+H]^+$.

5.3. General procedure for the synthesis of cyclic dipeptide (1 to 2 or 3)

N-Boc-dipeptide methyl ester was treated with 4 M HCl–1,4-dioxane at room temperature for 30 min, and the reaction mixture

was evaporated. The residue was dissolved in 0.1 M acetic acid–2-butanol, and one equivalent *N*-methylmorpholine (NMM) was added to the solution. The reaction mixture was refluxed for 3 h under N_2. The reaction mixture was concentrated in vacuo to a small volume. Cyclic dipeptide was collected by filtration, and was washed with small amounts of 2-butanol. The cyclic dipeptide can be further obtained from the filtrate by silica gel column chromatography.

5.3.1. Cyclo(L-Ala-L-Leu) (3A)

Yield 36%; white powder; $[\alpha]_D^{25}$: −25.1 (*c* 0.15, MeOH); IR ν_{max} (KBr): 3197, 3085, 2958, 1687 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6, δ_H): 0.86 (3H, d, *J* = 6.6 Hz), 0.88 (3H, d, *J* = 6.6 Hz), 1.26 (3H, m), 1.45 (1H, m), 1.60 (1H, m), 1.81 (1H, m), 3.75 (1H, br s), 3.85 (1H, dd, *J* = 5.0, 11.9 Hz), 8.08 (1H, s), 8.10 (1H, s); ^{13}C NMR (125 MHz, DMSO-d_6, δ_C): 19.5, 21.8, 22.9, 23.6, 4.6, 49.9, 52.6, 168.3, 168.8; HRMS–FAB: *m*/*z* 185.1290 $[M+H]^+$ calcd for $C_9H_{17}N_2O_2$ 185.1264; FAB-MS (positive): *m*/*z* 185 $[M+H]^+$.

5.3.2. Cyclo(L-Leu-L-Phe) (3F)

Yield 34%; white powder; $[\alpha]_D^{25}$: −98.2 (*c* 0.2, MeOH); IR ν_{max} (KBr): 3247, 3209, 2957, 1667, 1459 cm^{-1}; ^1H NMR (400 MHz, CD_3OD, δ_H) 0.06 (1H, ddd, *J* = 4.8, 9.9, 13.9 Hz), 0.67 (3H, d, *J* = 6.6 Hz), 0.72 (3H, d, *J* = 6.6 Hz), 0.85 (1H, ddd, *J* = 4.4, 9.5, 13.9 Hz), 1.43 (1H, m), 2.93 (1H, dd, *J* = 4.8, 13.6 Hz), 3.27 (1H, m), 3.65 (1H, ddd, *J* = 1.1, 4.4, 9.9 Hz), 4.23 (1H, ddd, *J* = 1.1, 4.0, 8.5 Hz):; ^{13}C NMR (125 MHz, DMSO-d_6, δ_C): 21.4, 22.9, 22.9, 38.5, 43.7, 52.3, 55.5, 126.8, 128.2, 130.5, 136.1, 166.2, 167.5; HRMS–FAB: *m*/*z* 261.1607 $[M+H]^+$ calcd for $C_{15}H_{21}N_2O_2$ 261.1603; FAB-MS (positive): *m*/*z* 261 $[M+H]^+$.

5.3.3. Cyclo(Gly-L-Leu) (3G)

Yield 50%; white powder; $[\alpha]_D^{25}$: −11.1 (*c* 0.07, MeOH); IR ν_{max} (KBr): 3237, 3067, 2956, 1688, 1625, 1558 cm^{-1}; ^1H NMR (500 MHz, D_2O, δ_H): 0.63 (3H, d, *J* = 6.2 Hz), 0.67 (3H, d, *J* = 6.2 Hz), 1.35 (3H, m), 3.58 (2H, d, *J* = 2.3 Hz), 3.95 (1H, t, *J* = 6.8 Hz); ^{13}C NMR (125 MHz, D_2O, δ_C): 10.2, 11.9, 14.2, 30.0, 43.7, 156.0, 169.4; HRMS–FAB: *m*/*z* 171.1140 $[M+H]^+$ calcd for $C_8H_{15}N_2O_2$ 171.1134; FAB-MS (positive): *m*/*z* 171 $[M+H]^+$.

5.3.4. Cyclo(L-Ile-L-Leu) (3I)

Yield quant.; white powder; $[\alpha]_D^{25}$: −56.5 (*c* 0.1, MeOH); IR ν_{max} (KBr): 3191, 3054, 2960, 1661 cm^{-1}; ^1H NMR (400 MHz, CD_3OD, δ_H): 0.96 (12H, m), 1.25 (m), 1.58 (2H, m), 1.74 (ddd, *J* = 4.4, 8.8, 13.6 Hz), 1.88 (m), 3.84 (dd, *J* = 1.1, 4.1 Hz), 3.94 (ddd, *J* = 1.1, 4.4, 9.2 Hz); ^{13}C NMR (125 MHz, CD_3OD, δ_C): 11.8, 15.1, 21.8, 23.1, 23.5, 24.3, 38.3, 43.7, 52.3, 58.8, 166.8, 168.4; HRMS–FAB: *m*/*z* 227.1759 $[M+H]^+$ calcd for $C_{12}H_{23}N_2O_2$ 227.1760; FAB-MS (positive): *m*/*z* 227 $[M+H]^+$.

5.3.5. Cyclo(L-Leu-L-Leu) (3L)

Yield 63%; white powder; $[\alpha]_D^{25}$: −46.4 (*c* 0.10, MeOH); IR ν_{max} (KBr): 3189, 3054, 2958, 1670 cm^{-1}; ^1H NMR (400 MHz, CD_3OD, δ_H): 0.95 (6H, d, *J* = 6.6 Hz), 0.96 (6H, d, *J* = 6.6 Hz), 1.62 (2H, ddd, *J* = 5.1, 8.8, 18.0 Hz), 1.71 (1H, ddd, *J* = 4.8, 8.8, 13.6 Hz), 1.83 (2H, m), 3.89 (2H, dd, *J* = 4.8, 9.2 Hz); ^{13}C NMR (100 MHz, CD_3OD, δ_C): 21.9, 23.5, 25.3, 45.9, 65.1, 171.2; HRMS–FAB: *m*/*z* 227.1795 $[M+H]^+$ calcd for $C_{12}H_{23}N_2O_2$ 227.1760; FAB-MS (positive): *m*/*z* 227 $[M+H]^+$.

5.3.6. Cyclo(L-Leu-L-Met) (3M)

Yield 23%; white powder; $[\alpha]_D^{25}$: −47.1 (*c* 0.07, MeOH); IR ν_{max} (KBr): 3321, 3199, 3095, 2958, 1681 cm^{-1}; ^1H NMR (500 MHz, CD_3OD, δ_H): 0.96 (3H, d, *J* = 6.4 Hz), 0.98 (3H, d, *J* = 6.4 Hz), 1.61 (1H, m), 1.72 (1H, m), 1.85 (1H, m), 2.12 (3H, m), 2.61 (2H, m), 3.95 (1H, ddd, *J* = 0.9, 4.6, 8.7 Hz), 4.08 (1H, dd, *J* = 4.6, 8.7 Hz); ^{13}C NMR (125 MHz, DMSO-d_6, δ_C): 14.3, 21.8, 22.9, 23.5, 28.9, 33.0, 42.9, 52.0, 53.0, 167.7, 168.4; HRMS–FAB: *m*/*z* 245.1324 $[M+H]^+$ calcd for $C_{11}H_{21}N_2O_2S$ 245.1324; FAB-MS (positive): *m*/*z* 245 $[M+H]^+$.

5.3.7. Cyclo(L-Asn-L-Leu) (3N)

Yield 63%; white powder; $[\alpha]_D^{25}$: −23.2 (*c* 0.50, MeOH); IR ν_{max} (KBr): 3403, 3343, 3205, 3091, 2957, 1681 cm^{-1}; ^1H NMR (400 MHz, CD_3OD, δ_H): 0.94 (3H, d, *J* = 6.4 Hz), 0.96 (3H, d, *J* = 6.4 Hz), 1.72 (2H, m), 1.86 (1H, m), 2.66 (1H, dd, *J* = 8.1, 15.8 Hz), 2.83 (1H, dd, *J* = 4.4, 15.8 Hz), 3.96 (1H, ddd, *J* = 1.1, 5.1, 8.1 Hz), 4.32 (1H, ddd, *J* = 1.1, 4.0, 8.1 Hz); ^{13}C NMR (125 MHz, DMSO-d_6, δ_C): 21.7, 23.0, 23.5, 38.1, 42.1, 51.2, 52.4, 167.8, 168.4, 171.1; HRMS–FAB: *m*/*z* 228.1347 $[M+H]^+$ calcd for $C_{10}H_{18}N_3O_2$ 228.1348; FAB-MS (positive): *m*/*z* 228 $[M+H]^+$.

5.3.8. Cyclo(L-Leu-L-Ser) (3S)

Yield 46%; white powder; $[\alpha]_D^{25}$: −286.5 (*c* 0.20, MeOH); IR ν_{max} (KBr): 3464, 3363, 3190, 3051, 2960, 1670 cm^{-1}; ^1H NMR (400 MHz, CD_3OD, δ_H): 0.93 (3H, d, *J* = 6.9 Hz), 0.95 (3H, d, *J* = 6.9 Hz), 1.80 (3H, m), 3.67 (1H, dd, *J* = 4.0, 12.4 Hz), 3.90 (3H, m); ^{13}C NMR (125 MHz, CD_3OD, δ_C): 21.8, 23.6, 25.1, 54.6, 59.0, 63.9, 65.3, 168.8, 171.4; HRMS–FAB: *m*/*z* 201.1257 $[M+H]^+$ calcd for $C_9H_{17}N_2O_3$ 201.1239; FAB-MS (positive): *m*/*z* 201 $[M+H]^+$.

5.3.9. Cyclo(L-Leu-L-Thr) (3T)

Yield 38%; white powder; $[\alpha]_D^{25}$: −56.5 (*c* 0.07, MeOH); IR ν_{max} (KBr): 3323, 3201, 3058, 2963, 1677 cm^{-1}; ^1H NMR (400 MHz, CD_3OD, δ_H): 0.91 (3H, d, *J* = 6.8 Hz), 0.93 (3H, d, *J* = 6.8 Hz), 1.23 (3H, d, *J* = 6.8 Hz), 1.38 (m), 1.85 (2H, m), 3.71 (1H, m), 3.85 (1H, dd, *J* = 3.4, 9.5 Hz), 4.17 (1H, ddd, *J* = 2.4, 6.6, 13.4 Hz); ^{13}C NMR (125 MHz, DMSO-d_6, δ_C): 20.1, 21.5, 23.1, 23.3, 45.0, 52.7, 60.5, 66.6, 166.7, 168.6; HRMS–FAB: *m*/*z* 215.1383 $[M+H]^+$ calcd for $C_{10}H_{19}N_2O_3$ 215.1396; FAB-MS (positive): *m*/*z* 215 $[M+H]^+$.

5.3.10. Cyclo(L-Leu-L-Val) (3V)

Yield 29%; white powder; $[\alpha]_D^{25}$: −71.2 (*c* 0.10, MeOH); IR ν_{max} (KBr): 3100, 3054, 2959, 1662 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6, δ_H): 0.85 (9H, m), 0.93 (3H, d, *J* = 6.9 Hz), 1.43 (1H, ddd, *J* = 4.6, 8.6, 13.3 Hz), 1.82 (1H, m), 2.09 (1H, m), 3.60 (1H, t, *J* = 2.8 Hz), 3.74 (1H, t, *J* = 4.1 Hz), 8.03 (1H, s), 8.16 (1H, s); ^{13}C NMR (125 MHz, DMSO-d_6, δ_C): 17.3, 18.7, 21.7, 23.1, 23.5, 31.5, 43.9, 52.4, 59.5, 166.8, 168.4; HRMS–FAB: *m*/*z* 213.1603 $[M+H]^+$ calcd for $C_{11}H_{21}N_2O_2$ 213.1603; FAB-MS (positive): *m*/*z* $[M+H]^+$.

5.3.11. Cyclo(L-Leu-L-Trp) (3W)

Yield 73%; yellow powder; $[\alpha]_D^{25}$: −2.6 (*c* 0.14, MeOH); IR ν_{max} (KBr): 3212, 3057, 2957, 1670 cm^{-1}; ^1H NMR (400 MHz, CD_3OD, δ_H): 0.18 (1H, m), 0.45 (3H, d, *J* = 6.6 Hz), 0.58 (3H, d, *J* = 6.6 Hz), 0.65 (1H, m), 1.14 (1H, m), 3.47 (1H, dd, *J* = 3.7, 14.7 Hz), 3.57 (1H, dd, *J* = 1.1, 4.8 Hz), 4.48 (1H, s), 6.98 (1H, dt, *J* = 1.1, 8.8 Hz), 7.06 (1H, s), 7.06 (1H, dt, *J* = 1.1, 8.8 Hz), 7.31 (1H, d, *J* = 8.8 Hz), 7.58 (1H, d, *J* = 8.8 Hz); ^{13}C NMR (125 MHz, DMSO-d_6, δ_C): 21.3, 22.6, 22.8, 29.1, 43.6, 52.3, 55.5, 108.1, 111.1, 118.3, 118.9, 120.7, 124.6, 127.7, 135.9, 167.0, 167.4; HRMS–FAB: *m*/*z* 300.1708 $[M+H]^+$ calcd for $C_{17}H_{22}N_3O_2$ 300.1712; EI-MS: *m*/*z* 299 $[M+H]^+$.

5.3.12. Cyclo(L-Leu-L-Tyr) (3Y)

Yield 57%; white powder; $[\alpha]_D^{25}$: +222.3 (*c* 0.10, MeOH); IR ν_{max} (KBr): 3312, 3204, 3084, 2953, 1676, 1661 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6, δ_H): 0.14 (1H, ddd, *J* = 5.0, 9.2, 13.8 Hz), 0.63 (3H, d, *J* = 6.6 Hz), 0.65 (3H, d, *J* = 6.6 Hz), 0.76 (1H, ddd, *J* = 5.0, 11.9, 13.8 Hz), 1.42 (1H, m), 2.68 (1H, dd, *J* = 4.6, 13.3 Hz), 3.01 (1H, dd, *J* = 3.7, 13.8 Hz), 3.45 (1H, br dd, *J* = 4.1, 8.3 Hz), 4.06 (1H, br s), 6.63 (2H, d, *J* = 8.3 Hz), 6.89 (2H, d, *J* = 8.3 Hz), 8.03 (1H, s), 8.04 (1H, s), 9.20 (1H, s); ^{13}C NMR (125 MHz, DMSO-d_6, δ_C): 21.3,

22.8, 23.0, 37.8, 43.7, 52.3, 55.7, 114.9, 125.9, 131.3, 156.4, 166.3, 167.5; HRMS–FAB: *m/z* 277.1516 $[M+H]^+$ calcd for $C_{15}H_{21}N_2O_3$ 277.1552; FAB-MS (positive): *m/z* 277 $[M+H]^+$.

5.3.13. Cyclo(*S*-4-MeO-benzyL-L-Cys-L-Leu) (2C)

Yield 55%; white powder; $[\alpha]_D^{25}$: −16.9 (*c* 0.10, MeOH); IR ν_{max} (Film): 3188, 3049, 2958, 2898, 1666 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, δ_H): 0.92 (3H, d, *J* = 6.6 Hz), 0.94 (3H, d, *J* = 6.6 Hz), 1.62 (1H, m), 1.72 (1H, m), 1.85 (1H, m), 2.67 (1H, dd, *J* = 8.8, 13.9 Hz), 3.06 (1H, dd, *J* = 3.3, 13.9 Hz), 3.58 (2H, m), 3.78 (3H, s), 3.94 (2H, m), 6.46 (1H, br s), 6.50 (1H, br s), 6.83 (2H, d, *J* = 9.8 Hz), 7.20 (2H, d, *J* = 9.8 Hz); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C): 21.1, 23.3, 24.2, 35.7, 36.1, 43.3, 53.3, 54.0, 55.3, 114.2, 129.3, 130.0, 158.9, 166.8, 168.2; HRMS–FAB: *m/z* 337.1591 $[M+H]^+$ calcd for $C_{17}H_{25}N_2O_3S$ 337.1586; FAB-MS (positive): *m/z* 337 $[M+H]^+$.

5.3.14. Cyclo(4-benzyL-L-Asp-L-Leu) (2D)

Yield 36%; white powder; $[\alpha]_D^{25}$: −55.6 (*c* 0.10, MeOH); IR ν_{max} (KBr): 3209, 3091, 3066, 2958, 1723, 1689 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6, δ_H): 0.84 (3H, d, *J* = 6.6 Hz), 0.85 (3H, d, *J* = 6.6 Hz), 1.46 (1H, m), 1.63 (1H, m), 1.85 (1H, m), 2.76 (2H, d, *J* = 5.5 Hz), 3.86 (1H, br dd, *J* = 4.8, 7.6 Hz), 4.25 (1H, br.t, *J* = 5.4 Hz), 5.09 (2H, dd, *J* = 2.4, 15.3 Hz), 7.36 (5H, m), 8.11 (1H, s), 8.16 (1H, s); ^{13}C NMR (100 MHz, DMSO-d_6, δ_C): 21.8, 22.9, 23.6, 36.6, 41.4, 50.9, 52.4, 65.6, 127.9, 128.0, 128.4, 136.0, 167.3, 166.7, 169.7; HRMS–FAB: *m/z* 319.1648 $[M+H]^+$ calcd for $C_{17}H_{23}N_2O_4$ 319.1658; FAB-MS (positive): *m/z* 319 $[M+H]^+$.

5.3.15. Cyclo(5-benzyL-L-Glu-L-Leu) (2E)

Yield 20%; white powder; $[\alpha]_D^{25}$: −28.1 (*c* 0.10, MeOH); IR ν_{max} (KBr): 3198, 3094, 3063, 2957, 1721, 1679 cm^{-1}; ^1H NMR (400 MHz, CD_3OD, δ_H): 0.94 (3H, d, *J* = 6.6 Hz), 0.95 (3H, d, *J* = 6.6 Hz), 1.66 (2H, m), 1.83 (1H, m), 2.12 (2H, m), 2.57 (2H, m), 3.92 (1H, ddd, *J* = 3.7, 4.8, 8.8 Hz), 3.97 (1H, t, *J* = 5.9 Hz), 5.11 (2H, s), 7.32 (5H, m); ^{13}C NMR (125 MHz, CD_3OD, δ_C): 21.0, 23.1, 24.2, 28.9, 29.9, 43.0, 53.2, 54.1, 66.7, 128.3, 128.4, 128.6, 135.5, 167.9, 168.9, 172.6; HRMS–FAB: *m/z* 333.1815 $[M+H]^+$ calcd for $C_{18}H_{25}N_2O_4$ 333.1814; FAB-MS (positive): *m/z* 333 $[M+H]^+$.

5.3.16. Cyclo(N^{π}-BOM-L-His-L-Leu) (2H)

Yield 32%; white powder; $[\alpha]_D^{25}$: −24.0 (*c* 0.14, MeOH); IR ν_{max} (KBr): 3193, 3057, 2959, 1668 cm^{-1}; ^1H NMR (500 MHz, CD_3OD, δ_H): 0.76 (1H, m), 0.82 (6H, d, *J* = 6.8 Hz), 1.29 (1H, m), 1.61 (1H, m), 3.14 (1H, dd, *J* = 4.1, 15.6 Hz), 3.35 (1H, dd, *J* = 5.1, 15.6 Hz), 3.80 (1H, m), 4.26 (1H, m), 4.45 (2H, d, *J* = 1.4 Hz), 4.60 (1H, br s), 5.43 (1H, d, *J* = 11.0 Hz), 5.51 (1H, d, *J* = 11.0 Hz), 6.87 (1H, br s), 7.30 (5H, m), 7.78 (1H, br s); ^{13}C NMR (125 MHz, CD_3OD, δ_C): 22.2, 24.0, 25.5, 29.2, 46.0, 54.8, 56.7, 67.4, 71.9, 75.8, 128.5, 129.4, 129.6, 130.0, 130.8, 138.7, 140.4, 169.4, 171.2; HRMS–EI: *m/z* 370.2019 $[M]^+$ calcd for $C_{20}H_{26}N_4O_3$ 370.2005; EI-MS: *m/z* 370 $[M]^+$.

5.3.17. Cyclo(L-Leu-N^{ε}-Z-L-Lys) (2K)

Yield 61%; white powder; $[\alpha]_D^{25}$: −25.3 (*c* 0.14, MeOH); IR ν_{max} (KBr): 3339, 3209, 3089, 2958, 1733, 1716, 1684, 1653 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, δ_H): 0.94 (3H, d, *J* = 7.3 Hz), 0.95 (3H, d, *J* = 7.3 Hz), 1.65 (9H, m), 3.13 (2H, t, *J* = 6.6 Hz), 3.93 (2H, m), 5.05 (2H, s), 7.30 (5H, m); ^{13}C NMR (125 MHz, DMSO-d_6, δ_C): 21.7, 21.8, 22.9, 23.5, 29.0, 33.1, 43.2, 52.5, 54.0, 65.0, 127.6, 128.3, 137.2, 156.0, 167.8, 168.3; HRMS–FAB: *m/z* 376.2213 $[M+H]^+$ calcd for $C_{20}H_{30}N_3O_4$ 376.2236; FAB-MS (positive): *m/z* 376 $[M+H]^+$.

5.3.18. Cyclo(N^G-nitro-L-Arg-L-Leu) (2R)

Yield 21%; white powder; $[\alpha]_D^{25}$: –43.7 (*c* 0.05, MeOH); IR ν_{max} (KBr): 3201, 3103, 2960, 2928, 1687, 1659 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6, δ_H): 0.83 (3H, d, *J* = 6.8 Hz), 0.84 (3H, d, *J* = 6.8 Hz), 1.57 (7H, m), 3.15 (2H, m), 3.75 (2H, m), 8.15 (1H, d, *J* = 2.2 Hz), 8.19 (1H, d, *J* = 2.2 Hz); ^{13}C NMR (125 MHz, DMSO-d_6, δ_C): 21.8, 23.0, 23.6, 24.2, 30.9, 40.2, 43.4, 52.6, 53.9, 159.3, 167.9, 168.4; HRMS–FAB: *m/z* 315.1789 $[M+H]^+$ calcd for $C_{12}H_{23}N_6O_4$ 315.1781; FAB-MS (positive): *m/z* 315 $[M+H]^+$.

5.4. Preparation of cyclo(N^{ω}-Trt-L-Gln-L-Leu) (2Q)

1Q (112 mg, 0.17 mmol) was dissolved in methanol (25 mL), and Pd-C (10%) was added to the solution. The reaction mixture was stirred at room temperature for 30 min under H_2. Pd-C was removed by filtration, and the solution was evaporated. The residue was dissolved in 0.1 M acetic acid–2-butanol (5 mL), and NMM (17 mg, 0.17 mmol) was added. The reaction mixture was refluxed for 1 h under N_2. The reaction mixture was concentrated in vacuo to a small volume. Product was collected on a filter and washed with small amounts of 2-butanol. Cyclo(N^{ω}-Trt-L-Gln-L-Leu) **(2Q)** was obtained (72 mg, 88%) as a white powder.

Compound **2Q**: $[\alpha]_D^{25}$: −17.7 (*c* 0.30, MeOH); IR ν_{max} (KBr): 3019, 2962, 1680 cm^{-1}; ^1H NMR (500 MHz, CD_3OD, δ_H): 0.93 (3H, d, *J* = 6.8 Hz), 0.95 (3H, d, *J* = 6.8 Hz), 1.62 (1H, m), 1.75 (1H, m), 1.82 (1H, m), 2.02 (2H, m), 2.47 (2H, m), 3.83 (1H, t, *J* = 5.7 Hz), 3.89 (1H, dd, *J* = 4.5, 8.5 Hz), 7.24 (15H, m); ^{13}C NMR (125 MHz, CD_3OD, δ_C): 18.4, 22.0, 23.5, 25.3, 31.7, 33.4, 33.4, 45.3, 54.5, 55.4, 58.3, 71.6, 127.8, 128.7, 130.0, 146.0, 170.1, 171.2, 174.0; HRMS–FAB: *m/z* 484.6211 $[M+H]^+$ calcd for $C_{30}H_{34}N_3O_3$ 484.6204; FAB-MS (positive): *m/z* 484 $[M+H]^+$.

5.5. Preparation of cyclo(L-Leu-L-Pro) (3P)

Compound **1P** (362 mg, 1.06 mmol) was treated with 4 M HCl–1,4-dioxane (20 mL) at room temperature for 30 min. The reaction mixture was evaporated, and the residue was dissolved in benzene (50 mL). The reaction mixture was refluxed with Molecular Sieves 4A for 3 days under N_2. Molecular Sieve 4A was removed and the reaction mixture was evaporated. The product was purified by silica gel column chromatography eluting with chloroform–acetone (5:1). Cyclo(L-Leu-L-Pro) (**3P**) was obtained as a white powder (85 mg, 38%).

Compound **3P**: $[\alpha]_D^{25}$: −101.0 (*c* 0.10, MeOH); IR ν_{max} (KBr): 3413, 1653, 1606, 1503 cm^{-1}; ^1H NMR (400 MHz, CD_3OD, δ_H): 0.95 (3H, d, *J* = 6.7 Hz), 0.96 (3H, d, *J* = 6.7 Hz), 1.51 (1H, m), 2.29 (1H, m), 3.50 (2H, m), 4.13 (1H, m), 4.25 (1H, m); ^{13}C NMR (125 MHz, CD_3OD, δ_C): 22.2, 23.3, 23.6, 25.8, 29.0, 39.4, 46.4, 54.6, 60.3, 168.9, 172.8; HRMS–FAB: *m/z* 211.1453 $[M+H]^+$ calcd for $C_{11}H_{19}N_2O_2$ 211.1447; FAB-MS (positive): *m/z* 211 $[M+H]^+$.

5.6. Preparation of cyclo(L-Cis-L-Leu) (3C)

2C (143 mg, 0.43 mmol) was dissolved in trifluoroacetic acid (30 mL). The reaction mixture was refluxed for 2 h under N_2 and the reaction mixture was evaporated. The product was purified by silica gel column chromatography eluting with chloroform–acetone. Cyclo(L-Cys-L-Leu) (**3C**) was obtained (75 mg, 81%) as a white powder.

Compound **3C**: $[\alpha]_D^{25}$: −44.9 (*c* 0.05, MeOH); IR ν_{max} (KBr): 3192, 3054, 2959, 2894, 2561, 1669 cm^{-1}; ^1H NMR (400 MHz, CD_3OD, δ_H): 0.91 (3H, d, *J* = 6.7 Hz), 0.93 (3H, d, *J* = 6.7 Hz), 1.80 (2H, m), 1.88 (1H, m), 2.83 (1H, dd, *J* = 4.0, 14.3 Hz), 3.04 (1H, dd, *J* = 4.0, 14.3 Hz), 3.98 (1H, m), 4.26 (1H, m); ^{13}C NMR (125 MHz, CD_3OD, δ_C): 22.0, 23.6, 25.1, 28.6, 45.6, 54.4, 57.6, 168.3, 171.1; HRMS–FAB: *m/z* 217.1024 $[M+H]^+$ calcd for $C_9H_{17}N_2O_2S$ 217.1011; FAB-MS (positive): *m/z* 217 $[M+H]^+$.

5.7. Preparation of cyclo(L-His-L-Leu) (3H)

Compound **2H** (33 mg, 0.09 mmol) was dissolved in 80% acetic acid aqueous solution (10 mL). The reaction mixture was stirred

with 10% Pd-C at room temperature for 30 min under H_2. Pd-C was removed, and the reaction mixture was evaporated. The product was purified by silica gel column chromatography eluting with chloroform–acetone (4:1). Cyclo(L-His-L-Leu) (**3H**) was obtained as a white powder (9 mg, 40%).

Compound **3H**: $[\alpha]_D^{25}$: −67.7 (*c* 0.10, MeOH); IR ν_{max} (KBr): 3195, 2958, 1670 cm^{-1}; ^1H NMR (500 MHz, CD_3OD, δ_H): 0.73 (1H, m), 0.82 (3H, d, *J* = 6.7 Hz), 0.84 (3H, d, *J* = 6.7 Hz), 1.26 (1H, m), 1.61 (1H, m), 3.00 (1H, dd, *J* = 4.6, 14.7 Hz), 3.19 (1H, dd, *J* = 5.0, 14.7 Hz), 3.79 (1H, m), 4.23 (1H, m), 6.89 (1H, br s), 7.62 (1H, br s); ^{13}C NMR (125 MHz, CD_3OD, δ_C): 21.7, 23.5, 24.9, 32.2, 45.3, 54.3, 56.7, 120.0, 132.8, 136.3, 169.3, 170.7; HRMS–FAB: *m*/*z* 251.1509 $[M+H]^+$ calcd for $C_{12}H_{19}N_4O_2$ 251.1508; FAB-MS (positive): *m*/*z* 251 $[M+H]^+$.

5.8. Preparation of cyclo(L-Asp-L-Leu) (3D)

Compound **2D** (40 mg, 0.12 mmol) was dissolved in methanol (10 mL). The reaction mixture was stirred for 30 min at room temperature under H_2 in the presence of 5% Pd-C. Pd-C was removed by filtration, and the reaction mixture was evaporated. Cyclo(L-Asp-L-Leu) (**3D**) was obtained as a white powder (28 mg, quant).

Compound **3D:** $[\alpha]_D^{25}$: −61.0 (*c* 0.10, MeOH); IR ν_{max} (KBr): 3441, 3197, 3097, 3059, 2959, 1678 cm^{-1}; ^1H NMR (500 MHz, pyridine-d_5, δ_H): 0.90 (3H, d, *J* = 6.0 Hz), 0.91 (3H, d, *J* = 6.0 Hz), 2.13 (1H, m), 2.15 (2H, m), 3.25 (dd, *J* = 8.2, 16.5 Hz), 3.57 (1H, dd, *J* = 3.7, 16.5 Hz), 4.31 (1H, m), 4.94 (1H, m), 9.30 (1H, s), 9.39 (1H, s); ^{13}C NMR (125 MHz, pyridine-d_5, δ_C): 21.8, 24.7, 39.5, 43.8, 53.1, 65.2, 168.6, 169.5, 173.5; HRMS–FAB: *m*/*z* 229.1181 $[M+H]^+$ calcd for $C_{10}H_{17}N_2O_4$ 229.1188; FAB-MS (positive): *m*/*z* 229 $[M+H]^+$.

5.9. Preparation of cyclo(L-Glu-L-Leu) (3E)

Compound **2E** (60 mg, 0.18 mmol) was prepared in the same manner as that of **3D**. The product was purified by HPLC equipped with an ODS column by using methanol–water (50:50) as a mobile phase. Cyclo(L-Glu-L-Leu) (**3E**) was obtained as a white powder (21 mg, 58%).

Compound **3E**: $[\alpha]_D^{25}$: −27.5 (*c* 0.14, MeOH); IR ν_{max} (KBr): 3326, 3196, 3055, 2956, 2929, 1719, 1673 cm^{-1}; ^1H NMR (400 MHz, CD_3OD, δ_H): 0.96 (3H, d, *J* = 6.6 Hz), 0.97 (3H, d, *J* = 6.6 Hz), 1.69 (2H, m), 1.85 (1H, ddd, *J* = 6.5, 8.4, 15.0 Hz), 2.02 (1H, m), 2.16 (1H, m), 2.36 (1H, m), 3.91 (1H, m), 3.95 (1H, m); ^{13}C NMR (125 MHz, DMSO-d_6, δ_C): 21.8, 22.9, 23.6, 28.8, 29.5, 43.0, 52.5, 51.4, 167.7, 168.5, 173.9; HRMS–FAB: *m*/*z* 243.1371 $[M+H]^+$ calcd for $C_{11}H_{19}N_2O_4$ 243.1345; FAB-MS (positive): *m*/*z* 243 $[M+H]^+$.

5.10. Preparation of cyclo(L-Gln-L-Leu) (3Q)

Compound **2Q** (72 mg, 0.15 mmol) was dissolved in 50% trifluoroacetic acid–dichloromethane (15 mL). The reaction mixture was refluxed for 5 min under N_2. The reaction mixture was evaporated. Cyclo(L-Gln-L-Leu) (**3Q**) was obtained as a white powder (43 mg, 99%).

Compound **3Q**: $[\alpha]_D^{25}$: −38.9 (*c* 0.14, MeOH); IR ν_{max} (KBr): 3395, 3199, 1683, 1653 cm^{-1}; ^1H NMR (600 MHz, CD_3OD, δ_H): 0.97 (3H, d, *J* = 6.5 Hz), 0.98 (3H, d, *J* = 6.5 Hz), 1.63 (2H, m), 1.85 (1H, m), 2.14 (2H, m), 2.36 (2H, t, *J* = 7.6 Hz), 3.93 (1H, dd, *J* = 4.0, 14.8 Hz), 3.96 (1H, t, *J* = 5.8 Hz); ^{13}C NMR (125 MHz, CD_3OD, δ_C): 22.0, 23.5, 25.3, 31.9, 48.9, 55.1, 55.6, 71.5, 170.1, 171.2, 177.4; HRMS–FAB: *m*/*z* 242.1505 $[M+H]^+$ calcd for $C_{11}H_{20}N_3O_3$ 242.1541; FAB-MS (positive): *m*/*z* 242 $[M+H]^+$.

5.11. Preparation of cyclo(L-Arg-L-Leu) (3R)

Compound **2R** (17 mg, 0.06 mmol) was dissolved in 5% formic acid–methanol (25 mL). The reaction mixture was stirred for 4 h at room temperature under H_2 with 10% Pd-C. Pd-C was removed by filtration, and the reaction mixture was evaporated. Cyclo(L-Arg-L-Leu) (**3R**) was obtained as a white powder (15 mg, quant).

Compound **3R**: $[\alpha]_D^{25}$: −43.7 (*c* 0.05, MeOH); IR ν_{max} (KBr): 3201, 3103, 2960, 2928, 1687, 1659 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6, δ_H): 0.83 (3H, d, *J* = 6.8 Hz), 0.84 (3H, d, *J* = 6.8 Hz), 1.57 (7H, m), 3.15 (2H, m), 3.75 (2H, m), 8.15 (1H, d, *J* = 2.2 Hz), 8.19 (1H, d, *J* = 2.2 Hz); ^{13}C NMR (125 MHz, DMSO-d_6, δ_C): 21.8, 23.0, 23.6, 24.2, 30.9, 40.2, 43.4, 52.6, 53.9, 159.3, 167.9, 168.4; HRMS–FAB: *m*/*z* 315.1789 $[M+H]^+$ calcd for $C_{12}H_{23}N_6O_4$ 315.1781; FAB-MS (positive): *m*/*z* 315 $[M+H]^+$.

5.12. Preparation of cyclo(L-Leu-L-Lys) (3K)

Compound **2K** (151 mg, 0.41 mmol) was dissolved in methanol (50 mL). The reaction mixture was stirred for 4 h at room temperature under H_2 in the presence of 10% Pd-C. Pd-C was removed by filtration, and the reaction mixture was evaporated. The product was purified by silica gel column chromatography using chloroform–methanol (3:1) to afford cyclo (L-Leu-L-Lys) (**3K**) as a white powder (36 mg, 41%).

Compound **3K**: $[\alpha]_D^{25}$: −46.7 (*c* 0.14, MeOH); IR ν_{max} (KBr): 3495, 3316, 3201, 3094, 2958, 2870, 1772, 1682 cm^{-1}; ^1H NMR (500 MHz, CD_3OD, δ_H): 0.95 (3H, d, *J* = 6.4 Hz), 0.96 (3H, d, *J* = 6.4 Hz), 1.53 (5H, m), 1.79 (4H, m), 2.71 (2H, t, *J* = 6.9 Hz), 3.92 (2H, m); ^{13}C NMR (125 MHz, DMSO-d_6, δ_C): 21.7, 21.8, 23.0, 23.6, 32.4, 33.4, 41.1, 43.3, 52.5, 54.1, 167.9, 168.3; HRMS–FAB: *m*/*z* 242.1861 $[M+H]^+$ calcd for $C_{12}H_{24}N_3O_2$ 242.1869; FAB-MS (positive): *m*/*z* $[M+H]^+$.

5.13. Antioxidant assay for scavenging ·OH and O_2^-

Antioxidant assay against ·OH and O_2^- by ESR was carried out as reported previously.[15]

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2012.01.050. These data include MOL files and InChiKeys of the most important compounds described in this article.

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