Studies on Synthesis of a Cycloheptapeptide and Effects of Different Metal Ions on the Cyclization†

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A cyclic heptapeptide, c(Gly-Ile-Pro-Tyr-Ile-Ala-Ala), which was isolated and identified from *Stellaria yunnanensis* Franch (M), was synthesized by solution method for the first time. Protected heptapeptide Z-Gly-Ile-Pro-Tyr-Ile-Ala-Ala-OBzl was synthesized with 3-(diethoxy-phosphoryloxy)-1,2,3-benzotriazin-4(3*H*)-one (DEPBT) as a coupling reagent. After deprotection of benzyl and benzyloxycarbonyl groups, a free linear heptapeptide H-Gly-Ile-Pro-Tyr-Ile-Ala-Ala-OH was cyclized in DMF (2×10^{-3} mol/L) in the presence of nine different metal ions, *i.e.*, Li^+, Na^+, K^+, Cs^+, Mg^{2+}, Ca^{2+}, Ba^{2+}, Ni^{2+} and Cr^{3+} respectively, using DEPBT as a coupling reagent. Univalent metal ion Cs^+ enhanced both the cyclization yield and cyclization rate of H-Gly-Ile-Pro-Tyr-Ile-Ala-Ala-OH, while some bivalent metal ions, such as Mg^{2+}, Ca^{2+} and Ni^{2+} decreased the yields of c(Gly-Ile-Pro-Tyr-Ile-Ala-Ala) drastically. Trivalent metal ion Cr^{3+} even inhibited the cyclization reaction completely.

Keywords linear heptapeptide, cyclic heptapeptide, DEPBT, metal ion, cyclization

Introduction

Cyclic peptides, which are constrained conformationally and more resistant to protease digestions than their linear precursors, have been of great interest as synthetic targets both as potential drug leads and as models for conformational analysis.[1-4] Current methods for syntheses of cyclic peptides generally involve a partially or fully protected linear precursor which is then cyclized in organic solvents by solution method or solid phase method.[5-8] Recently, thioester method has been successfully applied to the peptide cyclization in aqueous buffered solution.[9,10] Cyclic peptides can also be obtained by enzymatic cyclization.[11] Our efforts were focused on starting with partially protected linear peptides as precursors to synthesize cyclic products by using 3-(diethoxy-phosphoryloxy)-1,2,3-benzotriazin-4(3*H*)-one (DEPBT) (Fig. 1), an organophosphorus coupling reagent.[12-14]

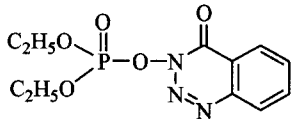

Fig. 1 3-(Diethoxy-phosphoryloxy)-1,2,3-benzotriazin-4(3*H*)-one (DEPBT).

As to our previous work,[15] univalent metal ions can enhance both cyclization yields and cyclization rates of two linear pentapeptides, H-Tyr-Leu-Ala-Gly-Pro-OH and H-Ala-Gly-Pro-Tyr-Leu-OH, and a linear heptapeptide, H-Gly-Tyr-Gly-Gly-Pro-Phe-Pro-OH. In order to prove the influence of metal ions on the cyclization yields, we chose the other different cyclic heptapeptide, c(Gly-Ile-Pro-Tyr-Ile-Ala-Ala), which was isolated and identified from *Stellaria yunnanensis* Franch (M),[16] as a model peptide. Herewith the synthesis of this cycloheptapeptide by solution method is reported for the first time. The influence of different metal ions on the cyclization yields was studied systematically.

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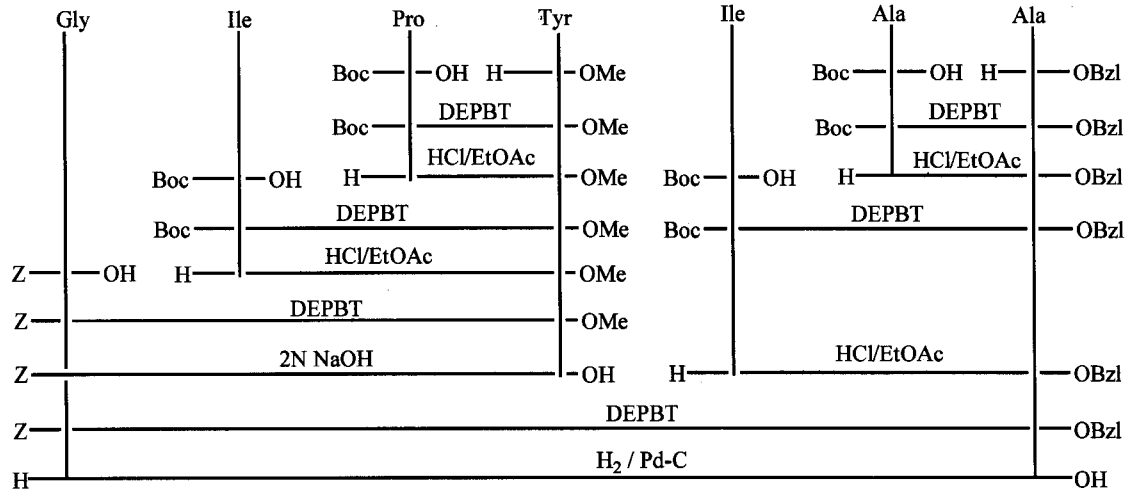
Scheme 1



Results and discussion

The protected linear heptapeptide Z-Gly-Ile-Pro-Tyr-Ile-Ala-Ala-OBzl (**1**) was synthesized by a 4 + 3 segment condensation by solution method (Scheme 1).

Firstly, protected tripeptide Boc-Ile-Ala-Ala-OBzl (**2**) and tetrapeptide Z-Gly-Ile-Pro-Tyr-OMe (**3**) were synthesized respectively by using DEPBT as a coupling reagent. H-Ile-Ala-Ala-OBzl (**2a**) was obtained after deprotection of **2** and Z-Gly-Ile-Pro-Tyr-OH (**3a**) was obtained after saponification of **3**. Compound **1** was then obtained after coupling of **2a** and **3a** with DEPBT. Compound **1** was purified by silica gel column before catalytic hydrogenation for deprotecting Z- and -Bzl group.

The by-products of ester exchange were found during catalytic hydrogenation. H-Gly-Ile-Pro-Tyr-Ile-Ala-Ala-OMe (**4a**) and an unknown compound **4b** were obtained in methanol solution. About half amount of the product was converted to its ethyl ester, H-Gly-Ile-Pro-Tyr-Ile-Ala-Ala-OEt (**4c**) in ethanol solution. Catalytic hydrogenation of compound **1** was also carried out in isopropanol solution, but the reaction was very slow and side-reaction was serious. After many trials, the free heptapeptide, H-Gly-Ile-Pro-Tyr-Ile-Ala-Ala-OH (**4**) was obtained with using H_2/Pd-C and acetic acid as a solvent, but it must be purified by RP-HPLC for the next cyclization.

Cyclization of **4** was carried out in DMF (2×10^{-3} mol/L) using DEPBT as a coupling reagent and DIEA as a base. Nine salts of chloride, corresponding to nine different metal ions, Li^+, Na^+, K^+, Cs^+, Mg^{2+}, Ca^{2+}, Ba^{2+}, Ni^{2+} and Cr^{3+} were dissolved in water and added to each reaction respectively. After 1 h, 3 h, 5 h, 24 h of reaction time, 20 μL of reaction solution was injected into HPLC by using minimal injector. Molar concentration of **4** was about 2 mmol/L and each metal ion was 10 mmol/L. Content of water in the system of reaction was about 1.3%. The results of cyclization were shown in Table 1.

Table 1 Cyclization yields (%) of H-Gly-Ile-Pro-Tyr-Ile-Ala-Ala-OH detected by RP-HPLC

Metal ions[a]	Reaction time (h)			
	1	3	5	24
None	17	32	39	55
Li^+	24	37	47	60
Na^+	18	35	44	59
K^+	19	34	41	57
Cs^+	22	38	51	66
Mg^{2+}	< 5	9	16	22
Ca^{2+}	< 5	< 5	< 5	5
Ba^{2+}	19	36	42	59
Ni^{2+}	0	0	0	Trace
Cr^{3+}	0	0	0	0

[a] Concentration of each metal ion was 5 equiv.. Water content (φ) in reaction system was 1.3%.

Among the univalent metal ions, Cs^+ could improve the yield of c(Gly-Ile-Pro-Tyr-Ile-Ala-Ala) (**5**) from 55% to 66% as compared with the cyclization of **4** without any metal ions. While the cyclization yields of **5** were decreased drastically when bivalent metal ion was added

to reaction except for Ba^{2+}. No cyclic heptapeptide **5** could be detected by HPLC when trivalent metal ion Cr^{3+} was added to the cyclization reaction. Comparing the results of cyclization of **4** with our previous work, there was some similar regularities between them, of which Na^+ was favorable for the cyclization of linear pentapeptides (yield was increased from 22% to 39%, 67% to 80% respectively) and Cs^+ was favorable for the cyclization of linear heptapeptides[15] (yield was increased from 60% to 78%); some bivalent metal ions and trivalent metal ion inhibited the reaction of cyclization.

When the content of water was switched from 1.3% to 50%, it seemed that Cs^+ could still improve the yield of cyclization (Table 2), but the coupling reagent DEPBT decomposed seriously. So the minimum water that dissolved the salt of chloride was enough for univalent metal ion Cs^+ to promote the cyclization of **4**.

Table 2 Effect of different water contents on the yields of **4**

Water content (φ)	Adding Cs^+ [a]		Without metal ions	
	1 h	3 h	1 h	2 h
50	26	29	18	22
1.3[b]	22	38	17	32

[a] Concentration of Cs^+ was 5 equiv.. [b] Yields from Table 1.

If the radius of metal ion is greater than 0.076 nm (including 0.076 nm), such as Cs^+, they could improve the yields of the cyclization except for Ca^{2+}, the radius of which is 0.100 nm. If the ratio of radius to charge of metal ion is greater than 0.050, such as Cs^+, they could improve the yields of cyclization, *vice versa*. So both radius and charge of metal ion are important factors for the metal ion to affect the cyclization of linear heptapeptide **4** (Table 3).

We suppose that the C-terminal carboxyl group together with the nitrogen of the N-terminal amino group in the linear peptide coordinates the metal ion to draw them close to each other. Therefore, it is favorable for the ring closure. On the other hand, bivalent or trivalent metal ion, such as Ni^{2+} or Cr^{3+}, which can coordinate strongly to the carboxyl and the amino groups in the linear peptide, prevents the amino group and carboxyl group from forming a cyclic structure. Therefore, the yields of cyclic heptapeptide **5** decreased or no reaction occurred when these metal ions were introduced. The results were similar to those of c(Gly-Tyr-Gly-Gly-Pro-Phe-Pro), which have been reported previously.[15] Further study is focused on the interaction between metal ions and linear peptides using CD, UV, ^1H NMR and fluorescence spectra. The result will be reported elsewhere.

The yield of cyclic heptapeptide **5** was about 55% with semi-preparation RP-HLPC in the absence of metal ions, which was in accordance with the HPLC yield in Table 1. The sequence of **5** (containing six *L*-amino acid residues and one glycine) was the same as that of natural sample, but the specific rotation (-88.8, *c* 0.124, MeOH) was different from that of natural sample (-116.8, *c* 0.125, MeOH).[16] No configuration of the amino acid residues in natural sample was reported by Ref. 16. Thus it needs for further study of the natural sample if it contains *D*-amino acid residue.

Experimental

^1H NMR spectra were taken on Bruker ARX200,

Table 3 Radius and ratio of radius to charge of metal ions

Metal ions	Radius of ions[a] (nm)	Effect on cyclization[b]	Metal ions	r/n[c]	Effect on cyclization[b]
Cs^+	0.174	+	Cs^+	0.174	+
K^+	0.151	+	K^+	0.151	+
Ba^{2+}	0.142	+	Na^+	0.102	+
Na^+	0.102	+	Li^+	0.076	+
Ca^{2+}	0.100	−	Ba^{2+}	0.071	+
Li^+	0.076	+	Ca^{2+}	0.050	−
Mg^{2+}	0.072	−	Mg^{2+}	0.036	−
Ni^{2+}	0.069	−	Ni^{2+}	0.035	−
Cr^{3+}	0.062	−	Cr^{3+}	0.021	−

[a] Data from Common Chemistry Handbook, Ed.: Fu, B., Beijing, 1997, pp. 9—15. [b] "+" Promoting cyclization, "−" inhibiting cyclization. [c] "*n*" Charge of metal ion.

ARX400 spectrometers. Tetramethylsilane (TMS) was used as an internal standard. Elemental analyses were carried out on an Elementar Vario EL (Germany). Melting points were determined with a Yanaco apparatus and uncorrected. Mass spectra were recorded on VG-ZAB-HS, Bruker APEX II and Bruker BIFLE XIII spectrometers. Optical rotation was determined with a Perkin Elmer 341LC. Freeze-drying was carried out on Flexi-Dry™ μP, FTS SYSTEMS, INC. (USA). Amino acid analysis was recorded on a Beckman 121 MB. Samples were detected by HPLC on HP1100 System. Semi-preparation of cycloheptapeptide was carried out on Waters 600E LC System, Water 486 monitor. Amino acids used in this paper are all of *L*-configuration.

Abbreviations standard abbreviations for amino acids and peptides derivatives are according to the suggestions of the IUPAC-IUB Commission on Biochemical Nomenclature (1984) *Eur. J. Biochem.* 138, 9—37. Other abbreviations: Boc, *tert*-butyloxycarbonyl; Z, Benzyloxycarbonyl; Et, ethyl; Bzl, benzyl; Me, methyl; Tos, 4-toluenesulphonyl; THF, tetrahydrofuran; DMF, *N*,*N*-dimethylformamide; DIEA, diisopropyl ethyl amine; CD, circular dichroism. MALDI, Matrix-assisted laser desorption ionization; MS, mass spectrometry; NMR, nuclear magnetic resonance; TOF, time of flight. The solvents were dried according to standard methods. Acetonitrile was of chromatographic purity and used to HPLC after ultra filtration.

AlaOBzl·TosOH (**6**)

AlaOBzl·TosOH was synthesized according to the reported mothod.[17] Yield 56%, m. p. 112—113 ℃, $[\alpha]_D^{20}$ −5.2 (*c* 4, MeOH) [Lit.[18] m.p. 116—118 ℃, $[\alpha]_D^{26}$ −6.0 (*c* 4, MeOH)]; ^{1}H NMR ($CDCl_3$, 200 MHz) δ: 1.43 (d, J = 7.2 Hz, 3H), 2.29 (s, 3H), 4.05 (q, J = 7.2 Hz, 1H), 5.04 (q, J = 9.2 Hz, 2H), 7.06 (d, J = 8.2 Hz, 2H), 7.72 (d, J = 8.2 Hz, 2H), 7.20—7.40 (m, 5H), 8.02—8.48 (br, 2H).

Boc-Ala-Ala-OBzl (**7**)

Boc-Ala-Ala-OBzl was synthesized according to the reported mothod.[19] Yield 86%. m.p. 69—70 ℃, $[\alpha]_D^{20}$ −15.4 (*c* 1, EtOAc) (lit.[19] Yield 86%, m.p. 73—74 ℃); ^{1}H NMR ($CDCl_3$, 200 MHz) δ: 1.18—1.65 (m, 15H), 4.10—4.30 (m, 1H), 4.45—4.65 (m, 1H), 4.82—5.05 (br, 1H), 5.17 (s, 2H), 6.60 (d, J = 7.2 Hz, 1H), 7.20—7.55 (m, 5H); MS (FAB) *m/z* (%): 351 [$(M+H)^+$, 80]. Anal. calcd for $C_{18}H_{26}N_2O_5$: C 61.68, H 7.48, N 8.00; found C 61.67, H 7.29, N 7.90.

Boc-Ile-Ala-Ala-OBzl (**2**)

Boc-Ala-Ala-OBzl (1.025 g, 2.93 mmol) was dissolved in solution of saturated HCl in 5 mL of anhydrous EtOAc and stirred at room temperature for fifteen minutes. The solution was evaporated to dryness *in vacuo*. The excess HCl was removed by 10 mL of anhydrous methanol twice and anhydrous benzene twice by evaporation *in vacuo* and the residue was suspended in 20 mL of THF. Boc-Ile-OH (1.016 g, 4.39 mmol) and DEPBT (1.313 g, 4.39 mmol) were added. The pH of solution was adjusted to 9—10 by DIEA. The reaction mixture was stirred at room temperature overnight. A drop of acetic acid was added to adjust pH < 7 and the solvent was evaporated *in vacuo* (55—60 ℃). The residue was dissolved in a mixture of ethyl acetate (80 mL) and water (20 mL). The organic phase was extracted with a 5% solution of citric acid in water (20 mL), water (20 mL), 5% Na_2CO_3 (20 mL), water (20 mL) and saturated brine (20 mL), dried over anhydrous Na_2SO_4 and evaporated to dryness under reduced pressure. The crude product obtained was purified by column chromatography on silica gel with petroleum ether/ethyl acetate (from 10 : 1 to 1:1) as eluent. Recrystallization from petroleum ether/ethyl acetate afforded the protected tripeptide 1.015 g. Yield 75%, m.p. 111—114 ℃, $[\alpha]_D^{20}$ −19.8 (*c* 1, EtOAc); ^{1}H NMR ($CDCl_3$, 200 MHz) δ: 0.92 (d, J = 4.0 Hz, 6H), 1.02—1.25 (m, 2H), 1.28—1.64 (m, 6H), 1.44 (s, 9H), 1.75—2.02 (m, 1H), 3.93 (t, J = 5.8 Hz, 1H), 4.42—4.68 (m, 2H), 4.85—5.18 (br, 1H), 5.18—5.30 (m, 2H), 6.45—6.68 (br, 1H), 6.72—6.88 (br, 1H), 7.35 (s, 5H); MS (FAB) *m/z* (%): 351 [$(M+H)^+$, 6]. Anal. calcd for $C_{24}H_{37}N_3O_6$: C 62.17, H 8.05, N 9.07; found C 62.14, H 7.97, N 9.05.

Boc-Pro-Tyr-OMe (**9**)

Protected dipeptide **9** was prepared from Boc-Pro-OH and Tyr-OMe employing the same procedure described for

compound **2**. Yield 93%, m.p. 60—61 ℃, $[\alpha]_D^{20}$ −36.0 (*c* 1, MeOH) [lit.[20] m.p. 63—64 ℃, $[\alpha]_D^{20}$ −36.0 (*c* 1, MeOH)].

Boc-Ile-Pro-Tyr-OMe (**8**)

Protected tripeptide derivative **8** was prepared employing the same procedure described for compound **7**. Yield 83%, m.p. 72—75 ℃, $[\alpha]_D^{20}$ −57.1 (*c* 1.25, MeOH) [lit.[21] m.p. 68 ℃, $[\alpha]_D^{20}$ −55.9 (*c* 1, MeOH)]; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.74—1.00 (m, 6H), 1.00—1.22 (m, 1H), 1.40 (s, 9H), 1.48—1.62 (m, 1H), 1.62—1.81 (m, 2H), 1.85—2.12 (m, 3H), 2.12—2.30 (m, 1H), 3.00 (d, J = 5.8 Hz, 2H), 3.52—3.64 (m, 1H), 3.67 (s, 3H), 3.71—3.86 (m, 1H), 4.27 (t, J = 9.1 Hz, 1H), 4.44—4.59 (m, 1H), 4.62—4.81 (m, 1H), 5.20 (d, J = 9.4 Hz, 1H), 6.71 (d, J = 8.4 Hz, 2H), 6.90 (d, J = 7.4 Hz, 1H), 6.98 (d, J = 8.4 Hz, 2H); MS (FAB) *m/z* (%): 506 [$(M+H)^+$, 65]; 528 [$(M+Na)^+$, 52].

Z-Gly-Ile-Pro-Tyr-OMe (**3**)

Protected tetrapeptide **3** was prepared employing the same procedure described for compound **7**. Yield 89%, m.p. 97—99 ℃, $[\alpha]_D^{20}$ −45.4 (*c* 1, MeOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.73 (t, J = 7.3 Hz, 3H), 0.80—0.88 (m, 1H), 0.93 (d, J = 6.6 Hz, 3H), 1.46—1.58 (m, 1H), 1.62—1.90 (m, 3H), 1.90—2.16 (m, 2H), 2.93 (q, J = 6.0 Hz, 1H), 3.06 (q, J = 4.4 Hz, 1H), 3.50—3.62 (m, 1H), 3.65 (s, 3H), 3.74—4.02 (m, 3H), 4.21 (s, 1H), 4.44 (t, J = 8.2 Hz, 1H), 4.70 (q, J = 5.6 Hz, 1H), 4.92—5.21 (m, 2H), 6.54 (br, 1H), 6.64 (d, J = 8.2 Hz, 2H), 6.91 (d, J = 5.6 Hz, 1H), 6.97 (d, J = 8.3 Hz, 2H), 7.18—7.38 (m, 6H), 7.84 (br, 1H); HRMS calcd for $C_{31}H_{40}N_4O_8$ 597.2924 $(M+H)^+$, found 597.2912.

Z-Gly-Ile-Pro-Tyr-OH (**3a**)

2 N NaOH (0.5 mL) was added to a solution of **3** (0.28 g, 0.47 mmol) in methanol (1 mL) with stirring at room temperature. The mixture was stirred for 2 h. Dilute hydrochloric acid (1 N, about 1 mL) was added and the pH was adjusted to 7. The methanol was removed *in vacuo*. The aqueous solution was cooled in an ice-water bath and stirred during acidification (about 2 mL of 1 N HCl). The final pH was adjusted to 1—2. After storage in the cold for 1 h the precipitate was collected on a filter, thoroughly washed with water and dried in air. Yield 81%, m.p. 119—123 ℃, $[\alpha]_D^{20}$ −31.9 (*c* 1, MeOH); ^{1}H NMR (*d*-DMSO, 400 MHz) δ: 0.80 (t, J = 7.5 Hz, 3H), 0.86 (d, J = 6.7 Hz, 3H), 0.92—1.12 (m, 1H), 1.36—1.58 (m, 1H), 1.58—1.76 (m, 1H), 1.77—1.94 (m, 3H), 1.99 (s, 1H), 2.74—2.96 (m, 2H), 3.49—3.84 (m, 4H), 4.24—4.46 (m, 3H), 5.03 (s, 2H), 6.64 (q, J = 8.4 Hz, 2H), 7.04 (q, J = 8.4 Hz, 2H), 7.21—7.52 (m, 6H), 8.01 (q, J = 8.5 Hz, 2H), 9.19 (s, 1H), 12.58 (s, 1H); MS (MALDI-TOF) *m/z* (%): 605.5 $(M+Na)^+$, 621.5 $(M+K)^+$; HRMS calcd for $C_{30}H_{38}N_4O_8$ 583.2768 $(M+H)^+$, found 583.2766.

Z-Gly-Ile-Pro-Tyr-Ile-Ala-Ala-OBzl (**1**)

Protected heptapeptide **1** was prepared employing the same procedure described for compound **8**. Yield 88%, m.p. 118—122 ℃, $[\alpha]_D^{20}$ −20.6 (*c* 0.5, DMF); ^{1}H NMR (*d*-DMSO, 400 MHz) δ: 0.66—0.92 (m, 12H), 0.94—1.14 (m, 2H), 1.19 (d, J = 7.1 Hz, 3H), 1.30 (d, J = 7.3 Hz, 3H), 1.33—1.56 (m, 2H), 1.58—1.76 (m, 3H), 1.76—1.89 (m, 3H), 1.90—2.05 (m, 1H), 2.73 (q, J = 8.2 Hz, 1H), 2.88 (q, J = 4.5 Hz, 1H), 3.46—3.66 (m, 3H), 3.66—3.81 (m, 1H), 4.16 (t, J = 8.2 Hz, 1H), 4.22—4.46 (m, 5H), 5.02 (s, 2H), 5.10 (d, J = 4.5 Hz, 2H), 6.61 (d, J = 8.4 Hz, 2H), 7.00 (d, J = 8.5 Hz, 2H), 7.20—7.50 (m, 10H), 7.79 (q, J = 9.0 Hz, 2H), 8.06 (t, J = 6.9 Hz, 2H), 8.29 (d, J = 6.9 Hz, 1H), 9.15 (s, 1H); MS (MALDI-TOF) *m/z* (%): 950.8 $(M+Na)^+$, 966.8 $(M+K)^+$; HRMS calcd for $C_{49}H_{65}N_7O_{11}$ 928.4820 $(M+H)^+$, found 928.4811.

H-Gly-Ile-Pro-Tyr-Ile-Ala-Ala-OH (**4**)

A solution of **4** (150 mg, 0.16 mmol) in acetic acid (3 mL) was prepared in a 25 mL round bottom flask provided with a magnetic stirrer and gas inlet-outlet tube. The air was displaced by a slow steam of nitrogen and a 10% palladium-on-charcoal catalyst (30 mg) was added. Once again a slow steam of nitrogen was led through the

flask for a few minutes, then the introduction of a slow steam of hydrogen was started. The catalyst was kept in suspension by vigorous stirring at room temperature. The reaction was terminated after 8 h monitored by TLC. The catalyst was removed by filtration. The filtrate was evaporated under reduced pressure (60—65 ℃). The residual acetic acid was evaporated with benzene twice *in vacuo*. The residue was dissolved in 10 mL of water for freeze-drying affording 94.5 mg of crude **4**. Yield 84%. Crude **4** (70 mg, 0.1 mmol) was purified by HPLC affording pure **4** (39 mg, 57 μmmol). $[\alpha]_D^{20}$ − 23.2 (*c* 0.25, MeOH).

Amino acid analysis of **4** Tyr 0.9 (1), Ile 0.85 ×2 (2), Gly 1.0 (1), Pro 1.0 (1), Ala 2.0 (2). HRMS calcd for $C_{34}H_{53}N_7O_9$ 704.3977 $(M+H)^+$, found 704.3968. **4a**: MS (FAB) *m/z* (%): 718 [$(M+H)^+$, 15], **4b**: MS (FAB) *m/z* (%): 732 [$(M+H)^+$, 90], **4c**: MS (FAB) *m/z* (%): 732 [$(M+H)^+$, 10]. HPLC semi-preparation condition: Waters 600E LC System, Vydac™ column, C_{18} (250 × 10 mm) with a 30 min linear gradient of 5%—50% CH_3CN in H_2O containing 0.1% TFA at a flow rate of 2 mL/min at 220 nm.

Cyclization of H-Gly-Ile-Pro-Tyr-Ile-Ala-Ala-OH (**4**)

Linear heptapeptide **4** (2.3 mg, 3.27 μmol) and DEPBT (1.0 mg, 3.34 μmol) were dissolved in anhydrous DMF (1.5 mL) with stirring at room temperature. DIEA (1.8 μL, 10.3 μmol) was added to the mixture. At the same time, adding **A**: water (20 μL); **B**: $LiCl\cdot H_2O$ (1.06 mg, 15 μmol, dissolved in 20 μL of H_2O); **C**: NaCl (0.88 mg, 15 μmol, dissolved in 20 μL of H_2O); **D**: KCl (1.12 mg, 15 μmol, dissolved in 20 μL of H_2O); **E**: CsCl (2.53 mg, 15 μmol, dissolved in 20 μL of H_2O); **F**: $MgCl_2\cdot 6H_2O$ (3.05 mg, 15 μmol, dissolved in 20 μL of H_2O); **G**: $CaCl_2$ (1.67 mg, 15 μmol, dissolved in 20 μL of H_2O); **H**: $BaCl_2\cdot 2H_2O$ (3.67 mg, 15 μmol, dissolved in 20 μL of H_2O); **I**: $NiCl_2\cdot 6H_2O$ (3.57 mg, 15 μmol, dissolved in 20 μL of H_2O); **J**: $CrCl_3\cdot 6H_2O$ (4.00 mg, 15 μmol, dissolved in 20 μL of H_2O), to the mixture respectively at room temperature. HPLC monitored the cyclization reaction at 1 h, 3 h, 5 h and 24 h of reaction time. HPLC analysis condition: Microsorb column, C_{18} (250 × 4.6 mm) with a 30 min linear gradient of 5%—50% CH_3CN and a 10 min linear gradient of 50%—5% CH_3CN in H_2O containing 0.1% TFA at a flow rate of 1 mL/min at 275 nm.

Semi-preparation of c(Gly-Ile-Pro-Tyr-Ile-Ala-Ala) (**5**)

Cycloheptapeptide **5** was prepared from **4** (24.9 mg, 35.4 μmol) by employing the same procedure described for the cyclization of **4** without adding any metal ions. After 24 h of reaction, the solution was condensed to 1 mL. Semi-preparation by HPLC and the following freeze-drying afforded the cycloheptapeptide **5** (9.6 mg). Yield 55%. $[\alpha]_D^{20}$ − 88.8 (*c* 0.124, MeOH) [Lit.[16] $[\alpha]_D^{19}$ − 116.8 (*c* 0.125, MeOH)].

Amino acid analysis of **5** Tyr 0.9 (1), Ile 0.85 ×2 (2), Gly 1.1 (1), Pro 0.9 (1), Ala 2.0 (2). HRMS calcd for $C_{34}H_{51}N_7O_8$ 686.3872 $(M+H)^+$, found 686.3865. HPLC semi-preparation condition was the same as that of **4**.

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