Conformational Studies of Cyclo(L-Phe–L-Pro–Gly–L-Pro)$_2$ by ^{1}H- and ^{13}C-Nuclear Magnetic Resonance Spectroscopy, and Its Enantioface-Differentiating Ability

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Analyses of the ^{1}H- and ^{13}C-nuclear magnetic resonance (^{1}H- and ^{13}C-NMR) spectra of the cyclooctapeptide cyclo (L-Phe–L-Pro–Gly–L-Pro)$_2$ (3) in $CDCl_3$ with the aid of the C–H correlated spectroscopy (C–H COSY) two-dimensional NMR spectrum (Fig. 2) suggested that two kinds of C_2-symmetric conformation with all *trans* and *cis–trans–trans–trans* forms coexist. When 0.5 eq of CsSCN or 1 eq of D- and L-PheOMe · HCl (D/L ratio = 1/2) was added to a solution of the cyclooctapeptide (3) in $CDCl_3$, the ^{1}H- and ^{13}C-NMR spectra (Fig. 3) suggested the presence of only one C_2-symmetric conformation (all *trans*), resulting from the formation of complexes with CsSCN or D- and L-PheOMe · HCl. The ^{13}C-NMR spectra of the complexes of the cyclooctapeptide (3 or 4) with D- and L-PheOMe · HCl displayed separate resonances for each carbon atom of D-PheOMe · HCl and L-PheOMe · HCl. Furthermore, the ability of 3 to distinguish the D from the L enantiomer, is superior to that of 4 (Table II).

Keywords cyclooctapeptide; C_2-symmetric conformation; ^{1}H-NMR spectrum; ^{13}C-NMR spectrum

Some cyclopeptides possess potent biological activities as antibiotics, toxins, hormones, and ion-transport agents. Recent progress in relating the activity of these peptides to their conformational states has been remarkable. The role of intermolecular and intramolecular forces in producing a functional conformation can be explored through conformational studies by using many kinds of synthetic cyclopeptide as model compounds. For this purpose we tried to synthesize the various cyclooctapeptides which consist of L-Phe, Gly, and L-Pro residues (Chart 1), in order to study their conformations. The Pro residue enhances the lipophilicity of the peptide and allows a *cis–trans* isomerization of the peptide bond. The latter feature increases the number of available conformations of the peptide, which has been considered to be favorable for complex formation.

The conformations of three cyclooctapeptides have been determined by using ^{13}C-NMR spectroscopy. Madison[1] reported that cyclo(Gly–L-Pro)$_4$ (**1**) existed completely as a C_4-symmetric conformation in all *trans* form in $CDCl_3$, and has a wide range of complexing power in the binding of metal ions. Further, compound **1** demonstrated an ability to distinguish D from L enantiomers of amino acid salts.[2] Kimura[3] reported that cyclo(L-Phe–L-Pro)$_4$ (**5**) took a C_2-symmetric conformation containing two *cis* peptide bonds in $CDCl_3$, Me_2SO-d_6, and CD_3OD. Cyclo(D-Phe–L-Pro–Gly-L-Pro)$_2$[1] was reported to exhibit multiple conformations in $CDCl_3$, and to yield only one C_2-symmetric conformation with all *trans* form upon addition of 0.5 eq of CsSCN to the solution.

This paper focuses on the conformational states of cyclo(L-Phe–L-Pro–Gly–L-Pro)$_2$ (**3**) (Chart 2) based on the results of ^{1}H- and ^{13}C-NMR spectral examinaton with the aid of the C–H COSY two-dimensional NMR spectrum, and the enantioface-differentiating abilities of **3** and cyclo[(L-Phe–L-Pro)$_2$–(Gly–L-Pro)$_2$] (**4**).

Results and Discussion

The cyclooctapeptide **4** was synthesized by the liquid phase method shown in Fig. 1. A similar synthesis of the cyclooctapeptide **3** has been reported in a preliminary communication.[4] Cyclo[L-Phe–L-Pro–(Gly–L-Pro)$_3$] (**2**) could not be synthesized, since the yield of the reaction to prepare the active esters Boc[(Gly–L-Pro)$_3$–L-Phe–L-Pro]-OSu and Boc[(Gly–L-Pro)$_2$–L-Phe–L-Pro–Gly–L-Pro]OSu as its precursors was too low.

The conformation of the cyclooctapeptide **3** in $CDCl_3$ was determined by ^{1}H- and ^{13}C-NMR spectroscopy (Table I). The signals were assigned on the basis of the C–H COSY two-dimensional NMR spectrum (Fig. 2). In the region of carbonyl group signals in the ^{13}C-NMR spectrum, four intense and three minor signals appeared at 168.30, 169.82, 170.70, 171.23 ppm and at 169.16, 171.91, 173.59 ppm, respectively (two signals were overlapping in one of these seven signals). Two signals for each carbon arom of the L-Phe and L-Pro residues appeared except for the L-Phe C_m[5]

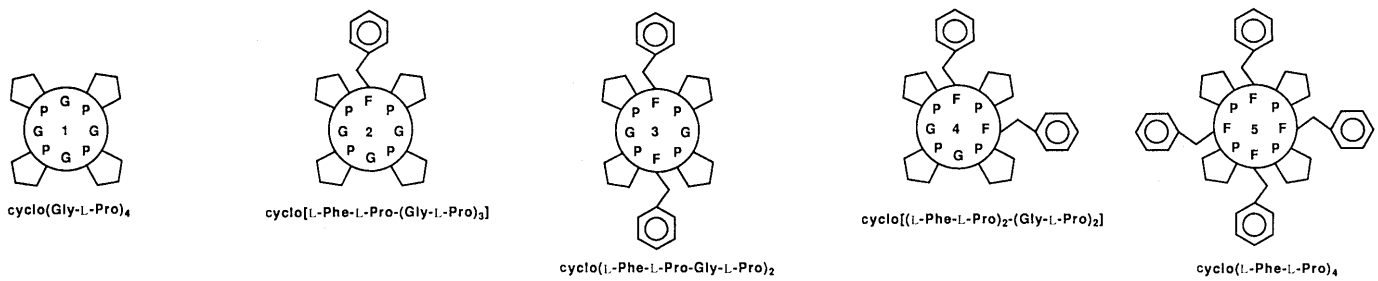

Chart 1

and L-Pro C_α atoms. On the other hand, only one signal for Gly C_α was observed at 42.59 ppm. Furthermore, in the ^{1}H-NMR spectrum, two signals for each proton of $C_\alpha H$ and $C_\beta H_2$ of the L-Phe residue appeared at 4.50 and 4.95 ppm, and at 2.90, 2.94, 3.05, and 3.17 ppm, respectively, while one signal for each proton of Gly $C_\alpha H_2$ was observed at 4.01 and 4.17 ppm. In the ^{13}C-NMR spectrum, the chemical shifts of the L-Pro C_β atoms at 28.00, 28.39, 29.28 ppm and C_γ atoms at 24.25, 25.43, 25.61 ppm indicated three *trans* Xxx-L-Pro peptide bonds (Xxx = L-Phe or Gly), and those of L-Pro C_β at 31.76 ppm and C_γ at 21.97 ppm indicated one *cis* bond, because the difference is about 10 ppm. A correlation between the chemical shifts of the C_β and C_γ atoms of the Pro residues and *cis* and *trans* forms of the Xxx-Pro bond has been reported.[6-8] This suggested that the L-Phe–L-Pro peptide bonds coexist one *cis* and one *trans*, while the Gly–L-Pro peptide bonds are both *trans*. This led to the conclusion that the cyclooctapeptide **3** coexists in two kinds of C_2-symmetric conformation with all *trans* and *cis–trans–trans–trans* forms in $CDCl_3$ (on the NMR time scale). The signals appearing at 51.92 and 38.62 ppm were assigned to L-Phe C_α and C_β of the all *trans* form, respectively, and the signals appearing at 55.00 and 35.21 ppm were assigned to those of the *cis–trans–trans–trans* form, respectively, judging from the chemical shifts in the ^{13}C-NMR spectrum of the complex of the cyclooctapeptide **3** with CsSCN (Table I).

Adding 0.5 eq[9] of CsSCN to a solution of the cyclooctapeptide **3** in $CDCl_3$ decreased the total number of signals in the ^{1}H- and ^{13}C-NMR spectra to almost half (Fig. 3 and Table I). Single signals appeared for each carbon and proton of the L-Phe, L-Pro, and Gly residues of **3**. The L-Phe–L-Pro and Gly–L-Pro peptide bonds were assigned as all *trans*, because in the ^{13}C-NMR spectrum, the signals of L-Pro C_β and C_γ atoms appeared at 27.83 and 29.40 ppm

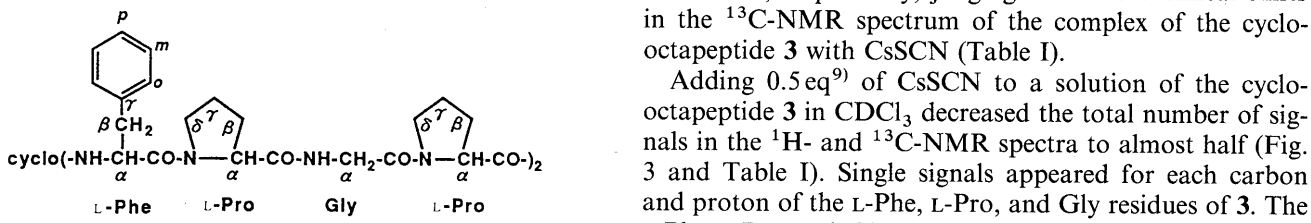

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Chart 2

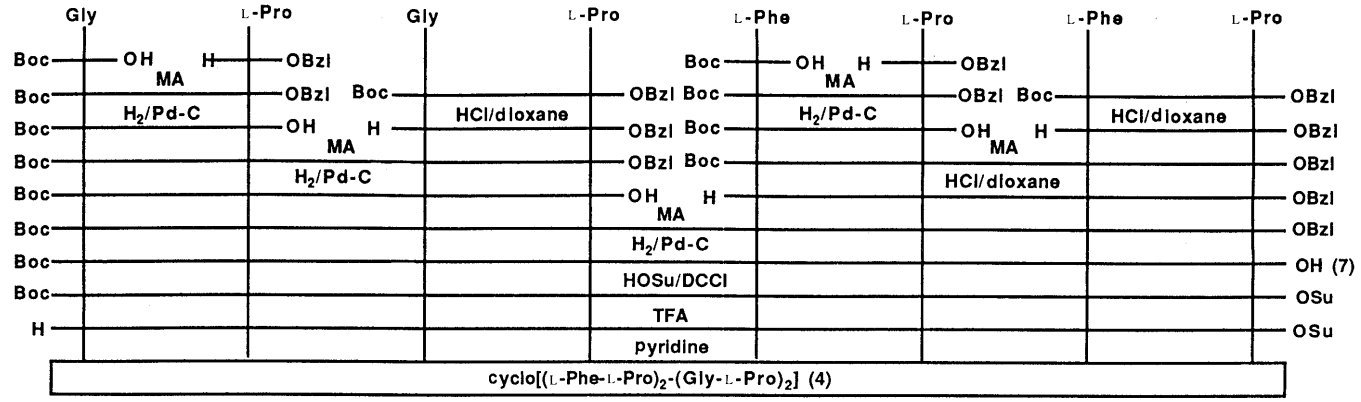

Fig. 1. Synthetic Route to Cyclo[(L-Phe–L-Pro)$_2$–(Gly–L-Pro)$_2$] (**4**)

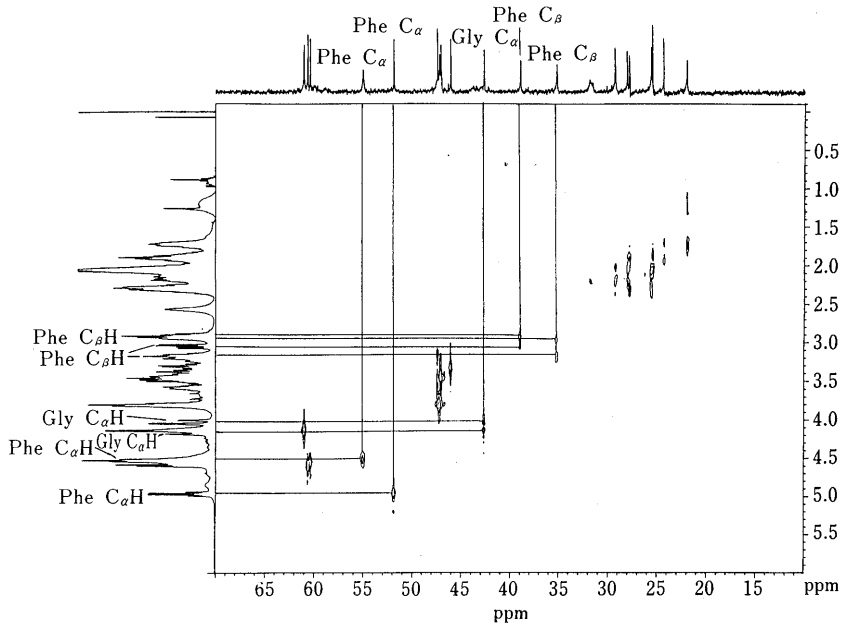

Fig. 2. C–H COSY Two Dimensional NMR Spectrum of Cyclo(L-Phe–L-Pro–Gly–L-Pro)$_2$ (**3**) in $CDCl_3$

TABLE I. ^{13}C-NMR Spectral Data (ppm, in $CDCl_3$) of the Cyclooctapeptide (**3**), **3**+CsSCN, and **4**+CsSCN

Carbon	**3**	**3**+CsSCN[a]	**4**+CsSCN[a]	Carbon	**3**	**3**+CsSCN[a]	**4**+CsSCN[a]
C=O	173.59 (s)[b]	171.65	172.44	PheC_α	55.00	51.75	52.62
	171.91 (s)	170.58	172.27		51.92		52.50
	171.23 (l)[b]	170.23	170.99	ProC_δ	47.51	47.57	47.54
	170.70 (l)	169.93	170.78		47.23	46.70	47.23
	169.82 (l)		169.51		47.06		46.47
	169.16 (s)		169.38		46.14		46.21
	168.30 (l)			GlyC_α	42.59	42.37	42.58
PheC_γ	138.05	136.44	136.69				42.35
	136.11		136.16	PheC_β	38.62	37.85	38.78
PheC_o	129.70	129.53	129.74		35.21		36.70
	128.95		129.17	ProC_β	31.76 (c)[c]	29.40 (t)	29.22 (t)
PheC_m	128.30	128.28	128.39		29.28 (t)[c]	27.83 (t)	28.13 (t)
			128.29		28.39 (t)		27.95 (t)
PheC_p	126.88	126.87	126.86		28.00 (t)		
	126.61			ProC_γ	25.61 (t)	25.33 (t)	25.52 (t)
ProC_α	61.00	61.06	60.72		25.43 (t)	24.10 (t)	25.15 (t)
	60.68	60.07	60.40		24.25 (t)		24.54 (t)
	60.42		60.16		21.97 (c)		23.69 (t)

a) The cyclooctapeptide (**3** or **4**) (23.880 mg, 3.0×10^{-5} mol) and CsSCN (2.865 mg, 1.5×10^{-5} mol) were dissolved in $CDCl_3$ (700 μl). *b*) s and l are small and large signals, respectively. *c*) c and t are *cis* and *trans* signals, respectively.

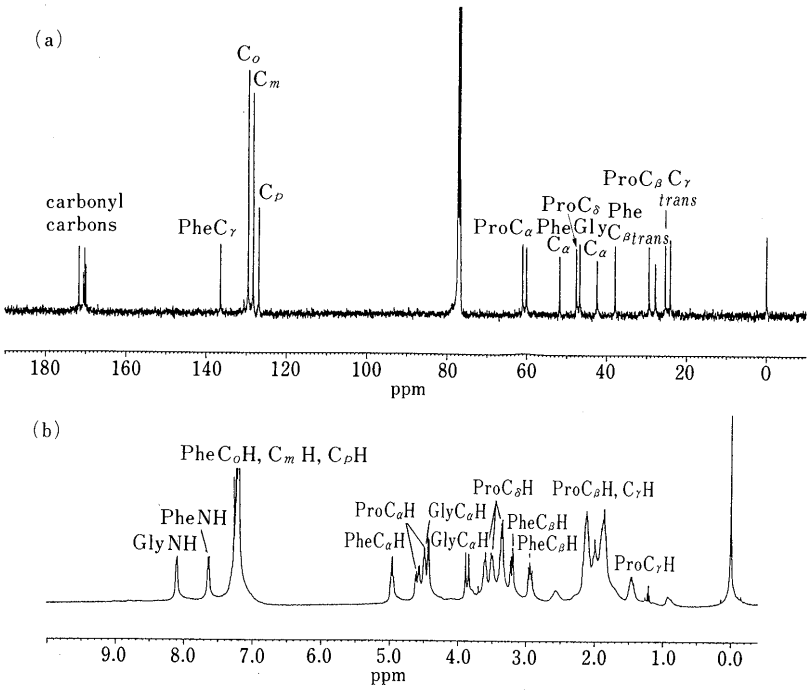

Fig. 3. ^{13}C-NMR (a) and ^{1}H-NMR (b) Spectra of Cyclo(L-Phe–L-Pro–Gly–L-Pro)$_2$ (**3**) with CsSCN[a] in $CDCl_3$

a) Compound **3** (23.880 mg, 3.0×10^{-5} mol) and CsSCN (2.865 mg, 1.5×10^{-5} mol) were dissolved in $CDCl_3$ (700 μl).

and at 24.10 and 25.33 ppm, respectively.[6-8] It is therefore concluded that only one C_2-symmetric conformation of all *trans* form results from the complex formation with CsSCN.

Similarly, by adding 0.5 eq[9] of CsSCN to a $CDCl_3$ solution of the cyclooctapeptide **4**, of which the ^{13}C-NMR spectrum suggested the presence of multiple (probably two or three) asymmetric conformations, the total number of signals decreased to less than half (Table I). Two signals appeared for most of the carbon atoms of L-Phe, L-Pro, and Gly residues of **4** (Table I). As the signals of L-Pro C_β and C_γ appeared at 27.95, 28.13, 29.22 ppm (two signals were overlapping in one of these three signals) and at 23.69, 24.54, 25.15, 25.52 ppm, respectively, the L-Phe–L-Pro and Gly–L-Pro peptide bonds were assigned as all *trans*.[6-8] This led to the conclusion that only one asymmetric conformation (all *trans* form) results from the complex formation with CsSCN.

Adding 1 eq of D- and L-PheOMe·HCl (D/L ratio = 1/2) to a solution of the cyclooctapeptide **3** or **4** in $CDCl_3$ induced a conformational change similar to that caused by the addition of CsSCN. The conformations of the cyclooctapeptides **3** and **4** changed to a single C_2-symmetric conformation of all *trans* form, and a single asymmetric conformation of all *trans* form, respectively.

The ^{13}C-NMR spectra of the complexes of the cyclooctapeptide **3** or **4** with D- and L-PheOMe·HCl displayed

TABLE II. ^{13}C-NMR Data for HCl·PheOMe (D, L) in $CDCl_3$

Cyclooctapeptide	Chemical shifts δ (ppm) of HCl·PheOMe (D, L)[a]							
	Form	CO	C_γ	C_o	C_m	C_p	C_α	CH_3	C_β
Cyclo(L-Phe–L-Pro–Gly–L-Pro)$_2$ (**3**)	D	[b]	135.43	130.02	128.54	127.07	55.09	52.77	36.76
	L	[b]	134.83	129.97	128.40	[c]	54.84	[c]	36.56
Cyclo[(L-Phe–L-Pro)$_2$–(Gly–L-Pro)$_2$] (**4**)	D	170.69	134.66	129.35	128.73	127.42	54.98	52.73	36.66
	L	[c]	134.54	[c]	128.67	127.36	54.93	52.78	[c]

a) Cyclooctapeptide : L-form : D-form = 1.5 (11.940 mg, 1.5×10^{-5} mol) : 1.0 (2.157 mg, 1.0×10^{-5} mol) : 0.5 (1.079 mg, 0.5×10^{-5} mol) in $CDCl_3$ (400 μl). *b*) The signal of HCl·PheOMe (D, L) could not be assigned because of the overlapping of the cyclooctapeptide signals. *c*) No splitting of the signal.

separate resonances[2] for each carbon atom of D-PheOMe·HCl and L-PheOMe·HCl (Table II). These spectra resulted from the formation of diastereomeric pairs of the complexes. It was found from these results that the two cyclooctapeptides **3** and **4** could distinguish D-PheOMe·HCl and L-PheOMe·HCl. Furthermore, larger splits of the signals of C_α, C_m, and C_γ of D- and L-PheOMe·HCl were observed in the ^{13}C-NMR spectrum of the complexes of **3** than in those of **4**. It was shown that the enantioface-differentiating ability of **3** is superior to that of **4**.

Experimental

^{13}C-NMR spectra were determined with a Brucker AM-400 (400 MHz) in $CDCl_3$ at 25 °C using tetramethylsilane (TMS) as an internal standard. Fast-atom-bombardment mass spectra (FAB-MS) were recorded with a JEOL JMS DX-300 data system. Thin-layer chromatography (TLC) was run with Kieselgel 60 F_{254} (Merck). Spot detection was carried out by spraying with 47% hydrobromic acid and then ninhydrin, by UV absorbance measurement at 254 nm, or by exposure to I_2 vapor.

Synthesis of Cyclo(L-Phe–L-Pro–Gly–L-Pro)$_2$ (3) and Cyclo[(L-Phe–L-Pro)$_2$–(Gly–L-Pro)$_2$](4) The synthetic route to the cyclooctapeptide (**4**) is shown in Fig. 1. The linear octapeptides Boc(L-Phe–L-Pro–Gly–L-Pro)$_2$–OH (**6**) and Boc[(Gly–L-Pro)$_2$–(L-Phe–L-Pro)$_2$]–OH (**7**) were obtained by fragment condensation, which was carried out using the standard mixed anhydride (MA) method [isobutylchloroformate (IBCF) and *N*-methylmorpholine (NMM)]. The *tert*-butoxycarbonyl (Boc) group was removed by treatment with 4 N HCl/dioxane, and the benzyl ester group by hydrogenation with 10% Pd–C.

Compound **6** *Rf* in AcOEt–pyridine–AcOH–H_2O (120 : 20 : 6 : 11): 0.23. FAB-MS *m/z*: 915 ($M+H^+$), and 937 ($M+Na^+$). Compound **7** *Rf* in the same solvent system; 0.25. FAB-MS *m/z*: 915 ($M+H^+$), and 937 ($M+Na^+$).

Dicyclohexylcarbodiimide (DCCI, 744 mg) was added to a solution of the linear octapeptide Boc(L-Phe–L-Pro–Gly–L-Pro)$_2$–OH (**6**) (3.0 g) and *N*-hydroxysuccinimide (HOSu, 491 mg) in dimethylformamide (DMF) (25 ml) at 0 °C. After stirring overnight, the solution was concentrated *in vacuo*. The residue was dissolved in AcOEt, a few drops of AcOH were added, and the resulting DCC urea was removed by filtration. The filtrate was washed with 5% $NaHCO_3$ aqueous solution and water, and dried over Na_2SO_4. The solvent was evaporated off *in vacuo*. The residue was triturated repeatedly with petroleum ether and each time the supernatant was decanted to leave a white solid. Trifluoroacetic acid (TFA, 7 ml) was added to it at 0 °C. The mixture was stirred for 30 min, then the volatile matter was evaporated *in vacuo*. The residue was solidified by the addition of ether to yield a white solid, which was washed several times with ether by decantation and dried over NaOH for 1 d to give a white powder. It was dissolved in dry DMF (15 ml) containing a few drops of AcOH, and the solution was added dropwise to a large amount of pyridine (1 l), with stirring at 35 °C, over a period of 6 h. After stirring overnight at room temperature, the solvents were evaporated off completely *in vacuo* at below 45 °C. The residue was dissolved in a CH_3OH–H_2O (4 : 1) (50 ml) mixture, and the slution was treated successively with Dowex 1 (OH^- form, 20 g) and Dowex 50 (H^+ form, 20 g) with stirring for 1 h each at room temperature. The resins were removed by filtration, and the solvents were evaporated *in vacuo* to obtain a white solid, which was dissolved in a small amount of CH_3OH (*ca.* 2 ml) and applied to a Sephadex LH-20 column, which was developed with the same solvent. Fractions containing the desired peptide were determined by TLC (spot detection by I_2 vapor), and concentrated *in vacuo* to give the cyclooctapeptide cyclo(L-Phe–L-Pro–Gly–L-Pro)$_2$ (**3**) (525 mg, 20.1% yield from **6**) as a white solid. *Anal.* Calcd for $C_{42}H_{52}N_8O_8 \cdot 2H_2O$: C, 60.56; H, 6.78; N, 13.45. Found: C, 60.23; H, 6.57; N, 13.17. FAB-MS *m/z*: 797 ($M+H^+$). ^{1}H-NMR of **3** ($CDCl_3$) δ: 1.71 (m, Pro $C_\gamma H_2$), 1.82—2.33 (m, Pro $C_\beta H_2$ and $C_\gamma H_2$), 2.90 (dd, J=7.1, 13.7 Hz, Phe $C_\beta H_2$), 2.94 (m, Phe $C_\beta H_2$), 3.05 (dd, J=6.6 Hz, Phe $C_\beta H_2$), 3.15—3.49 (m, Pro $C_\delta H_2$), 3.17 (dd, J=6.9, 16.5 Hz, Phe $C_\beta H_2$), 3.57 (m, Pro $C_\delta H_2$), 3.79 (m, Pro $C_\delta H_2$), 4.01 (dd, J=4.4, 18.0 Hz, Gly $C_\alpha H_2$), 4.12 (m, Pro $C_\alpha H$), 4.17 (dd, J=3.4 Hz, Gly $C_\alpha H_2$), 4.50 (m, Phe C_α), 4.51—4.59 (m, Pro $C_\alpha H$), 4.95 (m, Phe $C_\alpha H$), 7.15—7.32 (m, Phe C_oH, C_mH, C_pH), 7.47 (d, J=8.0 Hz, Phe NH), 7.65 (br s, Gly NH), ^{1}H-NMR of **3** with 0.5 eq of CsSCN ($CDCl_3$) δ: 1.46 (1H, m, Pro $C_\gamma H_2$), 1.86—2.17 (7H, m, Pro $C_\beta H_2$, $C_\gamma H_2$), 2.94 (1H, dd, J=7.7, 14.0 Hz, Phe $C_\beta H_2$), 3.22 (1H, dd, J=6.0 Hz, Phe $C_\beta H_2$), 3.35 (2H, m, Pro $C_\gamma H_2$), 3.51 (1H, m, Pro $C_\delta H_2$), 3.61 (1H, m, Pro $C_\delta H_2$), 3.87 (1H, d, J=16.9 Hz, Gly $C_\alpha H_2$), 4.46 (1H, d, Gly $C_\alpha H_2$), 4.47 (1H, m, Pro $C_\alpha H$), 4.59 (1H, dd, J=6.9, 9.1 Hz, Pro $C_\alpha H$), 4.97 (1H, m, Phe $C_\alpha H$), 7.21—7.27 (5H, m, Phe C_oH, C_mH, C_pH), 7.64 (1H, d, J=7.9 Hz, Phe NH), 8.10 (1H, m, Gly NH).

Upon work-up as described above, the linear octapeptide Boc[(Gly–L-Pro)$_2$–(L-Phe–L-Pro)$_2$]–OH (**7**) (1.83 g) gave the cyclooctapeptide cyclo[(L-Phe–L-Pro)$_2$-(Gly–L-Pro)$_2$] (**4**) (253 mg, 15.9% yield from **7**) as a white solid. *Anal.* Calcd for $C_{42}H_{52}N_8O_8 \cdot 2H_2O$: C, 60.56; H, 6.78; N, 13.45. Found: C, 60.85; H, 6.60; N, 13.75. FAB-MS *m/z*: 797 ($M+H^+$).

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References and Notes

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5) C_o, C_m, and C_p mean carbon atoms at the *ortho*, *meta*, and *para* positions of phenyl groups, respectively.
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9) No change in the ^{13}C-NMR spectrum (Fig. 3 and Table I) occurred after the addition of more than 0.5 eq of CsSCN to a solution of the cyclooctapeptide (**3** or **4**) in $CDCl_3$.