Preparation and Complex Formation of Cyclic Octapeptides Containing L-Proline Residues with Phenylalanine Methylester Hydrochloride

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Cyclic octapeptides, cyclo[L-Tyr(Bzl)-L-Pro]$_4$ (2) and cyclo(L-Tyr-L-Pro)$_4$ (3), were prepared and their conformations investigated. A C_2-symmetric conformation containing two *cis* peptide bonds was found in 2 in $CDCl_3$ and 3 in CD_3OD. Cyclo(L-Phe-L-Pro)$_4$ (1) formed a 1:1 complex with L-phenylalanine methylester hydrochloride (L-PheOMe·HCl). The ^{13}C-NMR spectra of the complexes of 1 and 2 with 1 eq of D- and L-PheOMe·HCl (D/L ratio = 1/2) in $CDCl_3$ displayed separate resonances for several carbon atoms of D-PheOMe·HCl and L-PheOMe·HCl owing to the formation of diastereomeric pairs of the complexes.

Keywords cyclic octapeptide; C_2-symmetric conformation; ^{13}C-NMR spectrum; stoichiometry

Cyclic peptides having various residues and sizes of ring skeleton have been synthesized, for study of the relationship between their conformational properties and metal-ion complexation. They were synthesized as artificial ionophores.[1-3] However, little work has been done on host–guest complex formation between cyclic peptides and organic compounds.[4] The main reasons for this are that some hydrogen bonds block the cavity of the cyclic peptides, and the degree of freedom of the cavity is too large for convenient design of a host molecule.

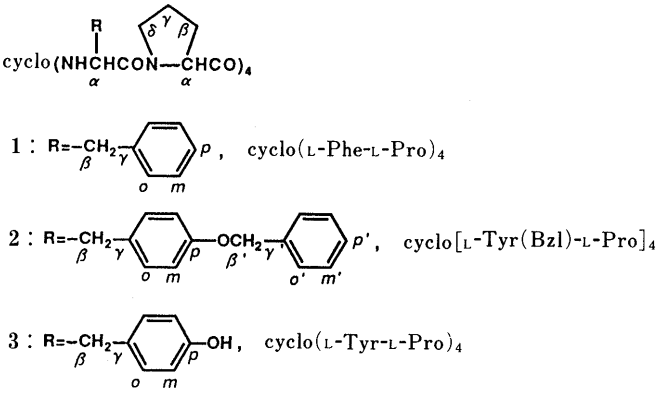
The final goal of our investigation is to design and synthesize artificial receptor using cyclic peptides. This paper focuses on the interaction of cyclic octapeptides (**1** and **2**) with PheOMe·HCl.

Results and Discussion

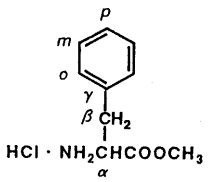
Cyclo[L-Tyr(Bzl)-L-Pro]$_4$ (**2**) and cyclo(L-Tyr-L-Pro)$_4$ (**3**) were prepared by liquid phase method [mixed anhydride (MA) method and activated ester method]. ^{13}C-NMR spectra were measured in $CDCl_3$ or CD_3OD to obtain information about the conformations in solution. These two cyclic octapeptides contain Pro residues, so that *cis* and *trans* peptide bonds can be formed; the isomerization is slow on the NMR time scale, so that signals arising from each conformational state can be distinguished. Thus, some of the conformations detected by NMR spectra represent the peptide bond isomers.

The ^{13}C-NMR spectrum of **2** in $CDCl_3$, except for signals of the aromatic carbons, is similar to that of cyclo(L-Phe-L-Pro)$_4$ (**1**), which was prepared by Kimura and Imanishi[5] (Table I). This similarity suggested that the conformation of **2** is the same as that of **1**, so that **2** was considered to take a C_2-symmetric conformation containing two *cis* peptide bonds in $CDCl_3$.

The assignment of signals in the ^{13}C-NMR spectrum of **2** is based on the chemical shifts of **1**.[5] The appearance of two signals for each carbon atom of the L-Pro and L-Tyr (Bzl) residues except for the $C_{m'}$ and $C_{p'}$ carbons of the L-Tyr(Bzl) residue indicates that **1** takes a C_2-symmetric conformation in this solvent. The signals of the C_β and C_γ carbons of the L-Pro residues were seen at 30.74 and 29.49 ppm, and 25.65 and 21.55 ppm, respectively. A correlation between the chemical shifts of the C_β and C_γ carbons of the Pro residues and the *cis* and *trans* forms of the Xxx-Pro bond has been reported.[6] Accordingly, the



phenylalanine methylester hydrochloride (**PheOMe · HCl**)



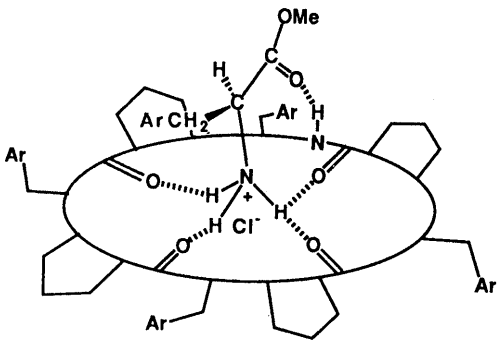


Fig. 1. Schematic Representation of the Complex between Cyclic Octapeptide (**1** or **2**) and L-PheOMe·HCl

singals at 29.49 and 25.65 ppm and those at 30.74 and 21.55 ppm were ascribed to the alkyl carbon atoms adjacent to *trans* and *cis* peptide bonds, respectively.

The ^{13}C-NMR spectrum of **3** in CD_3OD is similar to that of **1** in $CDCl_3$ except for the aromatic carbons (Table I). Therefore, the conformation of **3** in CD_3OD is also C_2-symmetric containing two *cis* peptide bonds (**3** was insoluble in $CDCl_3$).

The stoichiometry of the complex of **1** with L-PheOMe·HCl was considered by means of ^{13}C-NMR measurement in $CDCl_3$ at 25 °C. Figure 2 shows a plot of the changes of the chemical shifts of the four carbonyl carbons of **1** at 172.29, 171.30, 170.33 and 168.85 ppm *vs.* the molar ratio of L-PheOMe·HCl to **1**. The upfield and downfield shifts of two carbonyl carbons are marked. This behavior indicates that **1** forms a complex with L-PheOMe·HCl. The reaction ratio between **1** and L-PheOMe·HCl was determined by the molar ratio method to be 1 : 1 (Fig. 2). From the data in Fig. 2, the formation constant of the complex was calculated by a non-linear least-squares method as $1160 \pm 10\,M^{-1}$. The theoretical curve calculated on the basis of the assumption that L-PheOMe·HCl binds to **1** at a 1 : 1 ratio is also shown in Fig. 2, and coincides well with the data obtained. This fact indicated that the stoichiometry of the complex between **1** and L-PheOMe·HCl is indeed 1 : 1.

A schematic representation of the 1 : 1 complex between **1** or **2** and L-PheOMe·HCl is shown in Fig. 1, as indicated by Madison *et al.*[3] By the addition of 1 eq of **1** to a solution of L-PheOMe·HCl in $CDCl_3$, the ^{13}C-NMR signal of the carbonyl carbon atom of L-PheOMe·HCl at 169.30 ppm was shifted to 170.55 ppm. This suggested that a hydrogen bond is formed between the carbonyl group of L-PheOMe·HCl and the amino group of **1**.

When 1 eq of D- and L-PheOMe·HCl (D/L ratio = 1/2) was added to a solution of the cyclic octapeptide (**1** or **2**) in $CDCl_3$, the ^{13}C-NMR spectra of the complexes of **1**

TABLE I. ^{13}C-NMR Spectral Data (ppm) for the Cyclic Octapeptides (**1** and **2** in $CDCl_3$, and **3** in CD_3OD)

Carbon	**1** (Xxx = L-Phe)	**2** [Xxx = L-Tyr(Bzl)]	**3** (Xxx = L-Tyr)
C=O	172.29	172.31	173.98
	171.30	171.23	173.52
	170.33	170.45	173.21
	168.85	168.71	170.03
XxxC_γ	138.20	127.95 ($C_{\gamma'}$ 137.09)	130.38
	135.63	127.86 ($C_{\gamma'}$ 136.89)	127.57
XxxC_o	129.78	130.85 ($C_{o'}$ 128.60)	131.83
	128.95	129.80 ($C_{o'}$ 128.55)	130.90
XxxC_m	128.79	115.34 ($C_{m'}$ 127.30)	116.81
	128.29	114.70	116.14
XxxC_p	127.57	158.05 ($C_{p'}$ 127.30)	158.18
	126.52	157.40	157.17
L-ProC_α	60.48	60.53	61.94
	59.69	59.65	60.75
XxxC_α	54.86	54.87	56.52
	54.41	54.60	56.25
L-ProC_δ	47.56	47.44	[a]
	46.72	46.71	47.85
XxxC_β	38.25	37.38 ($C_{\beta'}$ 69.95)	38.16
	35.68	34.84 ($C_{\beta'}$ 69.90)	35.85
L-ProC_β	30.70 (*c*)[b]	30.74 (*c*)	31.56 (*c*)
	29.51 (*t*)[b]	29.49 (*t*)	30.53 (*t*)
L-ProC_γ	25.65 (*t*)	25.65 (*t*)	26.61 (*t*)
	21.47 (*c*)	21.55 (*c*)	22.36 (*c*)

a) One of the two signals of L-ProC_δ overlaps with the solvent signals. *b*) *c* and *t* are *cis* and *trans*, respectively.

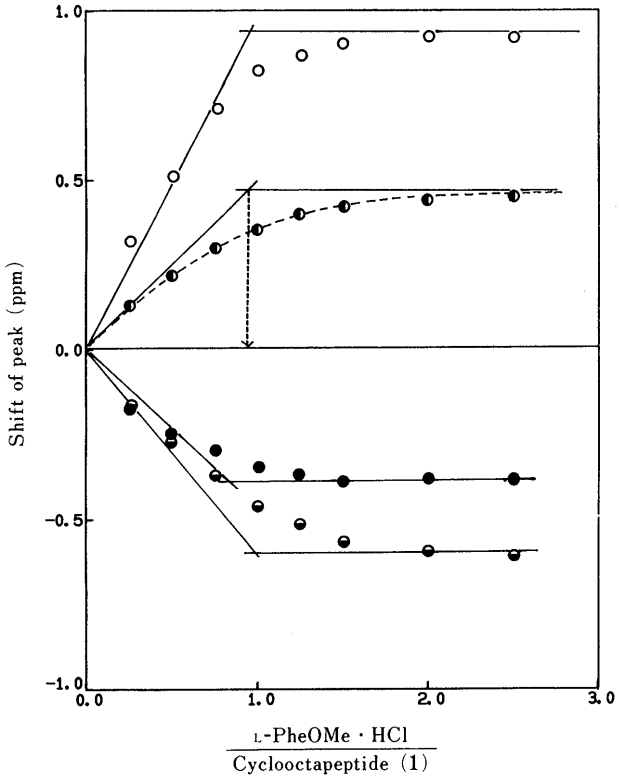


Fig. 2. The Shifts of the ^{13}C-NMR Signals of Carbonyl Carbons of the Cyclic Octapeptide (**1**) upon Addition of L-PheOMe·HCl

The ^{13}C-NMR signals at 172.29 (○), 171.30 (◐), 170.33 (●), and 168.85 (◒) ppm shift upfield or downfield upon addition of L-PhOMe·HCl. The theoretical curve (------) was calculated on the basis of the reaction ratio [cyclic octapeptide (**1**): L-PheOMe·HCl = 1 : 1] and the formation constant ($1160 \pm 10\,M^{-1}$).

TABLE II. ^{13}C-NMR Spectral Data for D- and L-PheOMe·HCl in the Complex with Cyclic Octapeptide (**1** or **2**) in $CDCl_3$[a,b]

Cyclic octapeptide	Form	Chemical shifts δ (ppm) of D- and L-PheOMe·HCl							
		CO	C_γ	C_o	C_m	C_p	C_α	CH_3	C_β
No cyclic octapeptide		169.30	134.05	129.62	128.95	127.70	54.45	53.00	36.32
1	D	170.58	134.63	129.68	128.55	127.68	55.00	52.80	36.65
	L	[c]	134.40	[c]	[c]	127.62	54.79	[c]	36.40
2	D	170.13	134.81	129.65	128.91	127.60	55.11	52.77	36.86
	L	170.71	134.53	[c]	[c]	[c]	54.82	[c]	36.48

a) Cyclic octapeptide (**1** or **2**): L-form: D-form = 1.5 [14.640 mg (**1**) or 21.000 mg (**2**), 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). *b*) The chemical shifts of **1** or **2** upon complex formation are given in Experimental. *c*) No splitting of the signal was observed.

or **2** with D- and L-PheOMe·HCl displayed separate resonances[4] for several carbon atoms of the D-PheOMe·HCl and L-PheOMe·HCl. Compounds **1** and **2** split the signals of the C_γ, C_p, C_α, C_β and CO, C_γ, C_α, C_β carbons of D- and L-PheOMe·HCl, respectively. Table II shows the values (in ppm) of the splitting, which reflect the formation of diastereomeric pairs of the complexes between **1** or **2** and D- and L-PheOMe·HCl.

Experimental

^{13}C-NMR spectra were determined with a Bruker AM-400 (400 MHz) in $CDCl_3$ or CD_3OD 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 Octapeptides (2—4) The linear octapeptide Boc[L-Tyr(Bzl)-L-Pro]$_4$OH (4) was obtained by fragment condensation, which was carried out using the 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 methylester group by hydrolysis with 1 N NaOH aqueous solution.

Boc[L-Tyr(Bzl)-L-Pro]$_4$OH (**4**) *Rf* in CH_3Cl–CH_3OH (10:1) 0.40. FAB-MS *m/z*: 1541 ($M+Na^+$).

Cyclization of **4** by the activated ester method using dicyclohexylcarbodiimide (DCCI) and *N*-hydroxysuccinimide (HOSu) gave cyclo[L-Tyr(Bzl)-L-Pro]$_4$ (**2**) (155 mg, 29% from **4**) as a white solid. *Anal.* Calcd for $C_{84}H_{88}N_8O_{12}\cdot 2H_2O$: C, 70.18; H, 6.45; N, 7.79. Found: C, 69.81; H, 6.37; N, 7.64. *Rf* in $CHCl_3$–CH_3OH (10:1) 0.44. FAB-MS *m/z*: 1401 ($M+H^+$).

The cyclic octapeptide (**2**) was treated with anhydrous HF containing *p*-cresole to give cyclo(L-Tyr-L-Pro)$_4$ (**3**) (131 mg, 65% from **2**) as a white solid. *Anal.* Calcd for $C_{56}H_{64}N_8O_{12}\cdot 7H_2O$: C, 57.62; H, 6.74; N, 9.60. Found: C, 57.34; H, 6.21; N, 9.57. *Rf* in $CHCl_3$–CH_3OH (5:2) 0.52. FAB-MS *m/z*: 1041 ($M+H^+$), 1063 ($M+Na^+$).

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