

76. Synthesis, Conformation, and Interactions with Small Molecules of Bis(Cyclic Dipeptides)

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Summary

Bis(cyclic dipeptides), $\text{cyclo}(\overline{\text{Lys-Pro}})\text{cyclo}(\overline{\text{Glu-Pro}})$ and S, S' -bis($\text{cyclo}(\text{hemiCys-Pro})$), were synthesized. These bis(cyclic dipeptides) very efficiently formed complexes with Ba^{2+} and Na^+ owing to intramolecular cooperation of two cyclic-dipeptide moieties, the *bis-effect*. $\text{Cyclo}(\overline{\text{Lys-Pro}})\text{cyclo}(\overline{\text{Glu-Pro}})$ stacked sodium 8-anilino-1-naphthalensulfonate in aqueous solution. These properties of complexation were controlled by the nature of the bridge connecting two cyclic dipeptide moieties. The geometry of the complex between S, S' -bis($\text{cyclo}(\text{hemiCys-Pro})$) and metal ion was investigated with the help of circular dichroism, nuclear magnetic resonance, and *Raman* spectroscopy.

Introduction. – A number of studies on the interactions between metal ions and synthetic cyclic peptides as ionophore models have been reported [1]. Cyclic tetra-[1], penta-[2], hexa-[1], octa-[3], and decapeptides [3] form complexes with metal ions as a result of intramolecular cooperation of carbonyl groups. In cyclic dipeptides, the carbonyl groups cannot cooperate to form intramolecular complexes. However, $\text{cyclo}(\text{Sar})_2$ forms insoluble complexes with various metal ions in ethyl acetate [4]. The crystalline complex between $\text{cyclo}(\text{Sar})_2$ and LiClO_4 has a network structure, in which four carbonyl groups of four cyclic dipeptides coordinate to Li^+ [5]. Therefore a corresponding bis(cyclic dipeptide) might be an efficient ligand for metal ions because its complexation should be favored over that of simple-cyclic dipeptides by the entropy term. We shall call the enhanced complexation caused by intramolecular cooperation of two covalently connected cyclic dipeptide moieties the *bis-effect*. Such *bis-effects* have already been postulated and studied in the case of S, S' -bis($\text{cyclo}(\text{hemiCys-peptides})$) with regard to the complexation of metal ions and their conformations ([6–8] and ref. therein).

The *bis-effect* is expected to depend strongly on the length and the rigidity of the bridge connecting the two cyclic peptide moieties; so by making a proper choice of the nature of the bridge, one might be able to realize a certain ion selectivity. Peptide complexes of transition-metal ions are particularly interesting, because they might provide models for electron-transporting proteins acting in biological membranes.

In this paper, we describe the synthesis and complexation with small molecules of cyclo(Lys-Pro) cyclo(Glu-Pro) and *S,S'*-bis(cyclo(hemiCys-Pro)), bis(cyclic dipeptides) in which two cyclic dipeptide moieties are linked respectively by an amide bond and a disulfide bond through their side chains. In the complexation with metal ions or dyestuff the *bis-effect* was manifested, and the mode of complexation was strongly dependent on the character of bridge connecting two cyclic dipeptide moieties.

To elucidate the *bis-effect*, the conformation of bis(cyclic dipeptide) was analyzed by spectroscopy. Circular dichroism (CD.) and *Raman* spectra yielded information about the internal rotation around the *S,S*-bond, and use of a lanthanide probe, Yb(fod)₃, enabled the structure of the complex between *S,S'*-bis(cyclo(hemiCys-Pro)) and metal ions to be determined.

Experimental Part

Synthesis. – The synthetic routes of two bis(cyclic dipeptides) are shown in Figure 1 and 2.

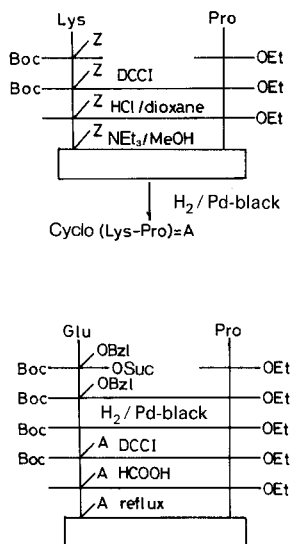


Fig. 1. Synthetic route of cyclo(Lys-Pro) cyclo(Glu-Pro) (**bis(I)**)

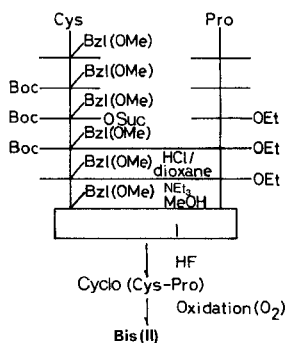


Fig. 2. Synthetic route of bis-*S,S'*-cyclo(hemiCys-Pro) (**bis(II)**)

Cyclo(Lys-Pro) Boc-Glu-Pro-OEt. Boc-Glu-Pro-OEt and cyclo(Lys-Pro) were synthesized by a conventional liquid-phase method. To a solution of Boc-Glu-Pro-OEt (0.25 g) and cyclo[Lys(HCl)-Pro] (0.175 g) in CH_2Cl_2 , triethylamine (93 μl) and dicyclohexylcarbodiimide (DCCI, 0.138 g) were added at 0° , and stirred overnight at RT. The solution was evaporated in vacuum and the residue was dissolved in ethyl acetate. Precipitated dicyclohexylurea was filtered off and the filtrate was extracted with water. The product was extracted from the aqueous solution by excess butanol. The butanol phase was dried (Na_2SO_4), evaporated in vacuum, and the residue was recrystallized from ethyl acetate; yield 40%. Thin layer chromatography (TLC.) on *Kieselgel G* was performed using the eluents: I, CHCl_3 /methanol/acetic acid 95:5:3; II, butanol/acetic acid/water/pyridine 30:6:24:20. Rf(I) 0.18; Rf(II) 0.70.

$\text{C}_{28}\text{H}_{45}\text{N}_5\text{O}_8$ (597.712) Calc. C 56.28 H 7.87 N 11.72% Found C 56.63 H 7.92 N 10.93%

Cyclo(Lys-Pro) cyclo(Glu-Pro) [bis(I)]. A solution of cyclo(Lys-Pro) Boc-Glu-Pro-OEt (0.3 g) in 30 ml of formic acid was stirred for 2 h at RT. and concentrated under reduced pressure. The oily residue was dissolved in a mixture of 60 ml of 2-butanol and 15 ml of toluene. The solution was refluxed for 2 h, then concentrated under reduced pressure. The solid residue was recrystallized from ethyl acetate; yield 80%. TLC.: Rf(I) 0; Rf(II) 0.63.

$\text{C}_{21}\text{H}_{31}\text{N}_5\text{O}_5 \cdot \text{H}_2\text{O}$ (451.525) Calc. C 55.88 H 7.32 N 15.52% Found C 55.68 H 7.11 N 16.09%

Cyclo(Cys[Bzl(OMe)]-Pro). Boc-Cys[Bzl(OMe)]-Pro-OEt, synthesized by a conventional liquid-phase method, was treated with 4N HCl/dioxane. The white solid obtained after evaporation was dissolved in methanol containing triethylamine equimolar to the peptide. After standing for 24 h, the solution was concentrated under reduced pressure leaving a white solid, which was dissolved in ethyl acetate, washed with a small amount of water, and dried (Na_2SO_4). After evaporation, the residue was recrystallized from ethyl acetate/2-propanol; yield 61%. TLC.: Rf(I) 0.35; Rf(II) 0.75; m.p. $135\text{--}136^\circ$.

$\text{C}_{16}\text{H}_{20}\text{N}_2\text{O}_3\text{S}$ Calc. C 60.00 H 6.25 N 8.75 S 10.00%
(320.406) Found „ 59.91 „ 6.38 „ 8.69 „ 10.13%

Cyclo(Cys-Pro). Anisole (1 ml) was added to cyclo(Cys[Bzl(OMe)]-Pro) and HF was led into the apparatus cooled by dry ice/acetone. After stirring for 30 min at 0° , HF was removed under a reduced pressure at 0° , and the residue was kept under a reduced pressure for 5 h. The solid residue was triturated with hexane and dried (NaOH); yield 63%. TLC.: Rf(I) 0.45.

S,S'-Bis(cyclo(hemiCys-Pro)) [bis(II)]. CO_2 -free air was bubbled for 24 h through the solution of cyclo(Cys-Pro) in water (pH 6.5). The solution was concentrated to dryness under reduced pressure, and the residual solid was purified by recrystallization. TLC.: Rf(I) 0.12.

$\text{C}_{16}\text{H}_{22}\text{N}_4\text{O}_4\text{S}_2 \cdot \frac{3}{2} \text{H}_2\text{O}$ Calc. C 45.16 H 6.13 N 13.11 S 14.99%
(452.52) Found „ 45.62 „ 5.83 „ 12.75 „ 15.05%

Spectroscopic measurements. – Circular dichroism (CD.), fluorescent, and UV. spectra were measured at RT. using JASCO J-20 spectropolarimeter, Hitachi MPF-4 fluorescence spectrophotometer, and Shimadzu UV-210 spectrophotometer, respectively. NMR. spectra were measured using a Bruker WH 270-type Fourier transform spectrometer at 23° by the courtesy of Prof. T. Miyazawa of University of Tokyo. Raman spectra were measured by the courtesy of Prof. Y. Kyogoku of Protein Research Institute of Osaka University.

Conformation of bis(cyclic dipeptides). The conformations available to cyclic dipeptides containing Pro-residue are in general limited because of their bicyclic structure, and the structure of the diketopiperazine backbone is restricted to a bowsprit-boat conformation [9]. Figure 3 shows the CD. spectra of **bis(I)** in various solvents. In aqueous solution a positive $n\text{--}\pi^*$ transition and a positive $\pi\text{--}\pi^*$ transition (long-wavelength lobe) are observed, which indicates that each cyclic dipeptide in **bis(I)** takes a boat conformation [9]. The CD. spectra of **bis(I)** depended on the solvent, the sign of the $n\text{--}\pi^*$ transition being apparently reversed in CHCl_3 and CH_2Cl_2 . The change of the CD. spectra may be caused either by the change of

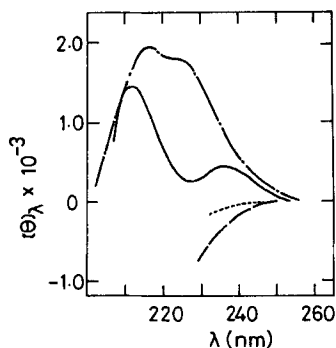


Fig. 3. CD. spectra of $\text{cyclo}(\overline{\text{Lys-Pro}})\text{cyclo}(\overline{\text{Glu-Pro}})$ [**bis(I)**] in ---- H_2O , — EtOH , CH_2Cl_2 , and -.- CHCl_3

orientation of two cyclic dipeptides or by the change of the state of electronic transition with solvent, allowing for the rigid skeleton of $\text{cyclo}(\text{Pro-cyz})$. The former seems to be the origin of the spectral change in the present case for the following reasons. The temperature dependence of NMR.-chemical shifts of three amide protons of **bis(I)** in D_6 -dimethylsulfoxide (D_6 -DMSO) were measured; these were 3.3×10^{-3} , 4.8×10^{-3} and 6.0×10^{-3} ppm/°C. The rate of exchange with deuterium in D_4 -methanol was slow for the first of three amide protons. These facts suggest that a certain amide proton is involved in the intramolecular H-bonding. Therefore, the change of CD. spectra with solvent should reflect the variation of intramolecular interaction of two cyclic dipeptide moieties of **bis(I)** as a result of the solvent effect on the H-bonding. Consequently, in nonpolar solvents intramolecular H-bonding and amide-amide interaction between two cyclic dipeptide moieties favours a folded structure of **bis(I)**, while in aqueous solution **bis(I)** adopts an extended conformation because the intramolecular hydrogen bond is broken.

In the NMR. spectra of **bis(II)** in CDCl_3 and D_6 -DMSO, only one signal appeared for each proton, so this molecule must adopt a C_2 -symmetric conformation on the NMR. time scale. The CD. spectrum of **bis(II)** in ethanol showed a positive $n\text{-}\pi^*$ transition and a negative $\pi\text{-}\pi^*$ transition, which differs from the CD. spectra of **bis(I)**. In the case of **bis(II)** the shortness of the bridge connecting the two cyclic dipeptide moieties results in their proximity allowing sufficient amide-amide interaction. This state is similar to the folded conformation of **bis(I)** in non polar solvents. The pattern of CD. spectra of **bis(II)** must have arisen from such a state.

The chemical shift of the amide proton of **bis(II)** in CDCl_3 is 6.47 ppm which is located at a higher magnetic field compared with 7.37 ppm of $\text{cyclo}(\text{Cys-Pro})$. This could be accounted for by the magnetic shielding effect of the other diketopiperazine unit, an explanation which is consistent with the previous conclusion about the closeness of the two diketopiperazines of **bis(II)**, although an alternative possibility that the ring skeleton takes a different conformation from the bowsprit-boat conformation is not precluded.

Interaction with metal ions. The variation of CD. spectrum of **bis(I)** in ethanol with the addition of AgClO_4 is shown in Figure 4. Similar changes of the $\pi\text{-}\pi^*$

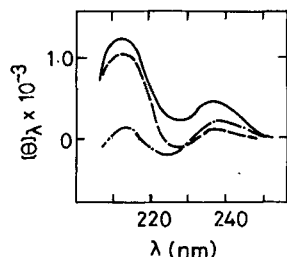


Fig. 4. CD. spectra of $\text{cyclo}(\overline{\text{Lys-Pro}}) \text{cyclo}(\overline{\text{Glu-Pro}})$ [**bis(I)**] in ethanol with or without AgClO_4 . Molar ratio of $\text{AgClO}_4/\text{bis(I)}$: — 0, ---- 1, - · - 4.

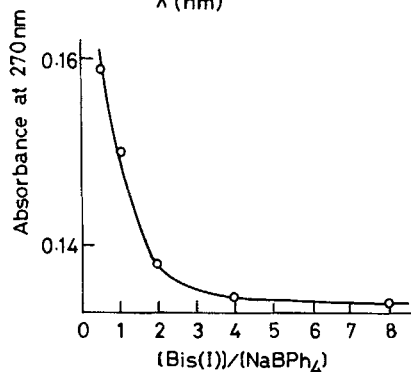


Fig. 5. Extraction of NaBPh_4 from water to CH_2Cl_2 with $\text{cyclo}(\overline{\text{Lys-Pro}}) \text{cyclo}(\overline{\text{Glu-Pro}})$

transition are observed with the addition of Na^+ or Ba^{2+} ion, indicating the formation of complex between **bis(I)** and metal ions.

Figure 5 shows the result of an extraction experiment of sodium tetraphenylborate from water to dichloromethane with **bis(I)**, carried out with varying concentrations of **bis(I)** in CH_2Cl_2 , and the absorption of residual BPh_4^- at 270 nm in aqueous solution was determined. Since the intersection with the abscissa by extrapolation of the initial linear part is two, the stoichiometry of the complex should be peptide/metal ion 2:1. Adopting this stoichiometry, the binding constants were determined for Ba^{2+} and Na^+ complexes from the change of ellipticity in the CD. spectra [10]. Variation of CD. spectra was similarly observed with the addition of metal ions to **bis(II)**. Assuming the same stoichiometry of this complex, the binding constants were also obtained with **bis(II)**. The binding constants are given in Table 1. For a simple cyclic dipeptide a value of $26\text{--}31 \text{ l} \cdot \text{mol}^{-1}$ was reported for the $\text{cyclo}(\text{Sar-Gly})/\text{Eu}^{3+}$ complex in CHCl_3 [1], but the complexation in polar solvents has not been reported. The large binding constants observed with **bis(I)** in polar solvents may thus be ascribed to the *bis-effect*.

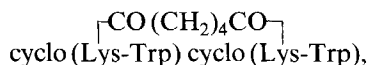
The complexation of **bis(I)** is ion-selective; that is, **bis(I)** is more apt to form a complex with Ba^{2+} than Na^+ . On the other hand, **bis(II)** did not discriminate

Table 1. Binding constants ($\text{l}^2 \cdot \text{mol}^{-2}$) of $\text{cyclo}(\overline{\text{Lys-Pro}}) \text{cyclo}(\overline{\text{Glu-Pro}})$ (**bis(I)**) and S,S' -*bis*($\text{cyclo}(\text{hemiCys-Pro})$) (**bis(II)**) with NaClO_4 or $\text{Ba}(\text{ClO}_4)_2$ in EtOH

	bis(I)	bis(II)
Na^+	4.9×10^4	3.2×10^4
Ba^{2+}	2.9×10^5	3.7×10^4

between Ba^{2+} and Na^+ . However, we cannot directly relate the binding constant with the nature of peptide ligand without taking into account other factors such as a different charge number between Ba^{2+} and Na^+ . This consideration could lead to a different plausible explanation, that the structure of **bis** (II) is so rigid that the cooperative action of the carbonyl groups is more suited for capturing Na^+ than Ba^{2+} and the binding constants appear to be comparable.

The binding constants observed with **bis** (I) are in general larger than those with **bis** (II). This implies that in the case of **bis** (II) with a short linkage, conformational adaptation for two cyclic dipeptides to coordinate to metal ion is more difficult than in the case of **bis** (I) with a long, flexible linkage. For comparison,



which possesses a very long bridge composed of twelve methylene groups and two amide groups, was synthesized and the binding constants for Ba^{2+} and Na^+ ions were lower than those of **bis** (I) and **bis** (II) [11]. The very long bridge connecting two cyclic dipeptide moieties made their intramolecular cooperation to coordinate to a metal ion unfavorable. The *bis-effect* thus depends on the length of bridge, and selective binding of metal ions can be achieved by a proper choice of bridge.

Interactions with dyestuff and iodine. With the addition of **bis** (I) to an aqueous solution of 8-anilino-1-naphthalenesulfonate (ANS), the quantum yield of fluorescence of ANS increased and the maximum wavelength of emission shifted to a shorter wavelength (Fig. 6). These phenomena were not observed with the addition of $\text{cyclo}(\text{Leu})_2$ or **bis** (II). Therefore, **bis** (I) stacked an ANS molecule with two cyclic-dipeptide moieties [12]. If the same stacking had occurred with two molecules of $\text{cyclo}(\text{Leu})_2$, it would have been accompanied by a large entropy loss. In the case of **bis** (II) the space between the two cyclic dipeptide moieties is too narrow to accommodate a bulky ANS molecule. Dipole-dipole interactions and hydrophobic interactions involving the alkyl side chains of Pro- and Lys-residues are supposed to be the attractive force between **bis** (I) and ANS. The binding constant was estimated to be $1.1 \times 10^2 \text{ l} \cdot \text{mol}^{-1}$ at 20° , and increased to $4 \times 10^2 \text{ l} \cdot \text{mol}^{-1}$ on

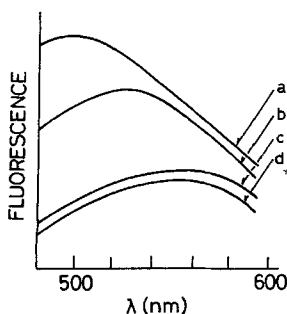


Fig. 6. Fluorescence spectra of 8-anilino-1-naphthalenesulfonate (ANS) in aqueous solution. a: $2 \times 10^{-5} \text{ mol} \cdot \text{l}^{-1}$ ANS + $4 \times 10^{-4} \text{ mol} \cdot \text{l}^{-1}$ **bis** (I); b: $2 \times 10^{-5} \text{ mol} \cdot \text{l}^{-1}$ ANS + $1 \times 10^{-4} \text{ mol} \cdot \text{l}^{-1}$ **bis** (I); c: $2 \times 10^{-5} \text{ mol} \cdot \text{l}^{-1}$ ANS + $4 \times 10^{-4} \text{ mol} \cdot \text{l}^{-1}$ $\text{cyclo}(\text{Leu})_2$; d: $2 \times 10^{-5} \text{ mol} \cdot \text{l}^{-1}$ ANS.

raising the temperature to 50°, supporting the explanation that the hydrophobic interaction stabilizes ANS stacked by **bis**(I).

Complex formation between **bis**(I) and iodine was illustrated by the UV. spectrum. The binding constant was estimated to be $2.1 \times 10^2 \text{ l} \cdot \text{mol}^{-1}$ in CHCl_3 , which is larger than $8.01 \cdot \text{mol}^{-1}$ and $1.01 \cdot \text{mol}^{-1}$ reported for iodine complexes of acetylsarcosine dimethylamide and cyclo(Sar)₂, respectively [1]. The complex formation between peptides and iodine should be based on the dipole-induced dipole interaction, and the strong complexation of **bis**(I) with iodine may have resulted from a strong dipolar field produced by the *bis*-effect.

Structure of complex between bis(II) and metal ion CD. CD. spectra due to S, S'-bond of **bis**(II) in ethanol are shown in Figure 7. The maximum wavelength in this region can be correlated with the dihedral angle θ_s around the S, S'-bond. A positive helical sense, that is, $90^\circ < \theta_s < 180^\circ$, was deduced for **bis**(II) [13]. Since no shift of the maximum wavelength was observed with the addition of Ag^+ , θ_s does not seem to change upon complexation.

Raman spectrum. In the Raman spectrum of **bis**(II) in methanol with the addition of Ba^{2+} ion, absorption was observed at 510 cm^{-1} (Fig. 8). Miyazawa *et al.* [14] showed the correlation between the molecular structure around C(β), S-bond of Cys-residue and Raman spectrum to correspond to the complex of **bis**(II) adopting a *gauche* form around C(β), S-bond.

Lanthanide ion probe method. Upon adding Yb^{3+} [15], four kinds of molecular species of **bis**(II), P_f , P_b , $\text{P}_f \cdot \text{L}_n$, and $\text{P}_b \cdot \text{L}_n$, are present, but their interconversion is so rapid that the signals are averaged.

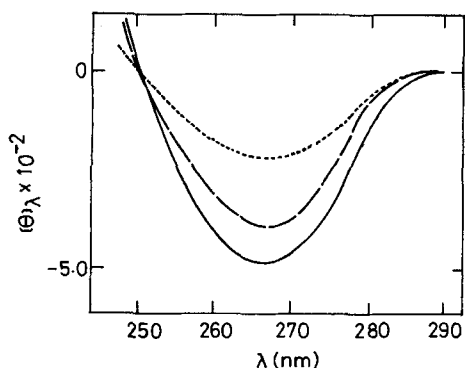


Fig. 7. CD. spectra of S,S'-bis(cyclo(hemiCys-Pro)) (**bis**(II)) with or without AgClO_4 in EtOH. Molar ratio of $\text{AgClO}_4/\text{bis(II)}$: — 0, --- 50, 100.

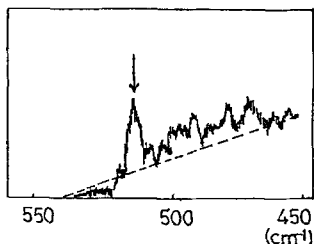
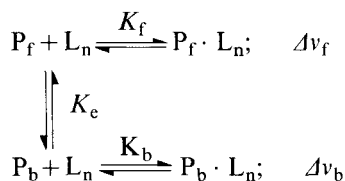


Fig. 8. Raman spectra of S,S'-bis(cyclo(hemiCys-Pro)) (**bis**(II)) with $\text{Ba}(\text{SCN})_2$ in MeOH



P_f = Peptide taking a conformation free from *bis-effect*; P_b = Peptide taking a conformation manifesting *bis-effect*; L_n = Lanthanide probe; $\Delta\nu_f$ = Shift of proton signal in P_f upon binding the probe; $\Delta\nu_b$ = Shift of proton signal in P_b upon binding the probe.

Since bis(cyclic dipeptides) far more easily form complexes with metal ions than simple cyclic dipeptides, $K_c \cdot K_b \gg K_f$. Therefore, in the presence of a small amount of Yb^{3+} , $\Delta\nu_b$ should be much larger than $\Delta\nu_f$, and the observed relative chemical shifts can be approximated by the ratio of $\Delta\nu_b$, leading to information about the structure of the complex in which two cyclic dipeptide moieties cooperate intramolecularly.

The relative chemical shifts of **bis(II)** caused by the addition of $Yb(fod)_3$ in $CDCl_3$ are summarized in Table 2. Assuming the coordination of Yb^{3+} to a carbonyl group of either Pro- or Cys-residue and varying the coordination (ϕ , θ , d) of Yb^{3+} , the relative chemical shifts of protons in **bis(II)** were calculated, then compared with the observed values (Table 2), and the appropriateness of the calculation was judged by the agreement factor (R) [16]. The coordination of each atom of cyclo-(Cys-Pro) was taken from the X-ray diffraction analysis of cyclo(Leu-Pro) [17]. Figure 9 shows a contour line map which was calculated assuming the coordination of Yb^{3+} to the carbonyl group of Pro-residue. If Yb^{3+} was assumed to coordinate to the carbonyl group of Cys-residue, R exceeded unity, which is improbable. We concluded that Yb^{3+} coordinates to the carbonyl group of Pro-residue and the position is determined by $\phi = 100^\circ$ and $\theta = 282^\circ$.

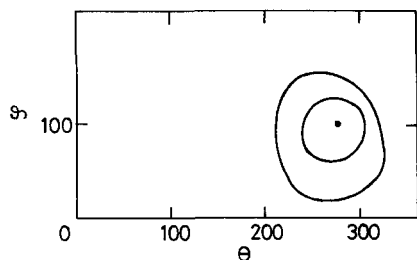


Fig. 9. Map of agreement factor (R). $C(\alpha)$, $C(\beta)$ and $C(\gamma)$ of Pro-residue lie on the x, y -plane, and the direction from $C(\alpha)$ to $C(\beta)$ is positive along the x -axis. θ and ϕ are the angles of Yb^{3+} from x -axis and z -axis, respectively, on the spherical coordinates with the origin at the oxygen of carbonyl group. The contour lines represent 0.55 and 0.70. The distance between Yb^{3+} and carbonyl oxygen (d) is taken as 3.0 Å.

Table 2. Ratio of $Yb(fod)_3$ -induced shift (QB(I)) of proton signals of S,S'-bis(cyclo(hemiCys-Pro)) (**bis(II)**)

Proton	QB(I)	Proton	QB(I)
NH of Cys	1.0	H-C(δ) of Pro	0.792
H-C(α) of Pro	0.631		0.792
H-C(β) of Pro	0.408	H-C(α) of Cys	0.541
	0.567	H-C(β) of Cys	0.312
H-C(γ) of Pro	0.286		0.435
	0.255		

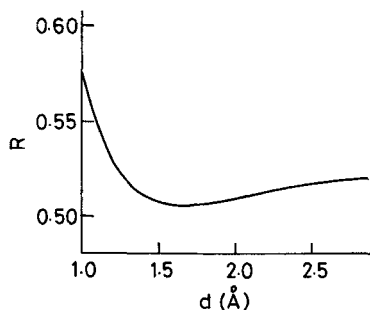


Fig. 10. The dependence of agreement factor (R) on the distance between Yb^{3+} and carbonyl O -atom (d)

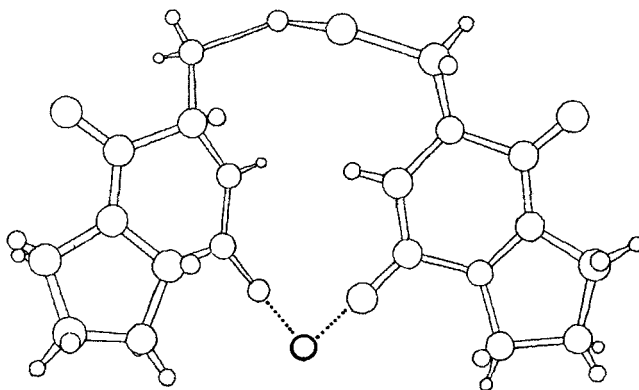


Fig. 11. Proposed conformation of $\text{S,S}'$ -bis(cyclo(hemiCys-Pro))/metal ion complex

Figure 10 shows the dependence of R on the distance between the carbonyl oxygen and Yb^{3+} (d). No drastic change of R is seen when $d > 1.5 \text{ \AA}$, thus confirming the above calculation.

The rotation angle χ_1 around the $\text{C}(\alpha)\text{C}(\beta)$ -bond of Cys-residue was determined to be *ca.* -60° , when the calculated shift ratio of $\text{H}-\text{C}(\beta)$ agreed well with the observed values. The probable conformation of **bis(II)**/ Yb^{3+} complex is shown in Figure 11, drawn with $\chi_1 = -50^\circ$, $\chi_2 = -50^\circ$, and the dihedral angle around S,S -bond $= 140^\circ$.

Conclusion. – Cyclic dipeptides are too small to form a metal ion complex of inclusion type, but with bis(cyclic dipeptides) **bis(I)** and **bis(II)** complexes were formed with Ba^{2+} and Na^+ . A complex of inclusion type surrounded by hydrophobic exterior is believed to work effectively as an ion carrier through a lipid membrane. Bis(cyclic peptides) are very useful for the design of ionophore models.

The *bis-effect* depends strongly on the nature of the bridge connecting two cyclic moieties and the ability of complex formation decreased in the following order: $-(\text{CH}_2)_2-\text{CONH}-(\text{CH}_2)_4->-\text{CH}_2-\text{S}-\text{S}-\text{CH}_2->-(\text{CH}_2)_4-\text{NHCO}-(\text{CH}_2)_4\text{CONH}-(\text{CH}_2)_4-$. High selectivity in complex formation may be obtained by using and choosing a suitable bis(cyclic peptide).

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