Note

Conformational Change and Epimerization of Diketopiperazines Containing Proline Residue in Water

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In water, diketopiperazines cyclo(L-Pro-L-Xxx) and cyclo(L-Pro-D-Xxx) (Xxx=Phe, Tyr) formed an intramolecular hydrophobic interaction between the main skeleton part and their benzene ring, and both cyclo(L-Pro-L-Xxx) and cyclo(L-Pro-D-Xxx) took a folded conformation. The conformational changes from folded to extended conformation by addition of several deuterated organic solvents (acetone-d_6, metanol-d_4, dimethyl sulfoxide-d_6 (DMSO-d_6)) and the temperature rise were investigated using ^{1}H-NMR spectra. The results suggested that the intrarmolecular hydrophobic interaction of cyclo(L-Pro-D-Xxx) formed more strongtly than that of cyclo(L-Pro-L-Xxx). Under a basic condition of 1.0×10^{-1} mol/L potassium deuteroxide, enolization of O_1-C_1-C_9-H_9 moiety of cyclo(L-Pro-L-Xxx) occurred, while that of the O_4-C_4-C_3-H_3 moiety did not. Cyclo(L-Pro-L-Xxx) epimerized to cyclo(D-Pro-L-Xxx), while cyclo(L-Pro-D-Xxx) did not change.

Key words diketopiperazine; conformation; epimerization; intramolecular hydrophobic interaction; ^{1}H-NMR spectrum

The human immunodeficiency virus (HIV) protease selectively cleaves the peptide bonds L-Phe-L-Pro and L-Tyr-L-Pro. There is no mammalian protease that cleaves such peptide bonds, and it is a feature of retroviral protease. It is possible to design anti-HIV drugs with high selectivity and few side effects if this feature is utilized. Based on this concept, various HIV protease inhibitors incorporating substrate transition state analogues that mimicked the L-Phe-L-Pro of the HIV protease substrate peptide sequence have been developed.[1,2]

We have studied the basic properties of the L-Phe-L-Pro and L-Tyr-L-Pro moieties using a cyclic octapeptide cyclo (L-Pro-L-Phe)$_4$. As a result, it was found that cyclo (L-Pro-L-Phe)$_4$ took C2-symmetric comformation containing two cis peptide bonds in $CDCl_3$ and metanol-d_4.[3,4] However, since cyclo (L-Pro-L-Phe)$_4$ was insoluble in water, we decided to investigate the properties of water soluble diketopiperazines cyclo(L-Pro-L-Phe) and cyclo(L-Pro-L-Tyr) in water (Fig. 1). For comparison, the property of diketopiperazines cyclo(L-Pro-D-Phe) and cyclo(L-Pro-D-Tyr) was also examined (Fig. 1).

In this paper, the conformations, conformational changes, and epimerization of cyclo(L-Pro-Xxx) (Xxx=Phe, Tyr) in water are reported.

Experimental

NMR Experiments ^{1}H-NMR spectra were recorded at room temperature on a JEOL JMN-LA500 (Tokyo, Japan) operating at 500 MHz. D_2O was used as a solvent (99.9 atom% D; Wako Pure Chemical Industries, Ltd., Osaka, Japan). Chemical shift values are expressed in ppm downfield using sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) as an internal standard and all measurement temperature are 35°C. Also the NMR measurerment under basic condition using potassium deuteroxide (KOD) was performed at 35°C. The nuclear Overhauser effect (NOE) difference experiments were typically conducted with 32 K data points covering a spectral width of 10000 Hz and with *ca.* 5 s presaturation time. In measurement using H_2O, 3 mmϕNMR tube containing 180 μL D_2O was inserted into 5 mmϕNMR tube containing a diketopiperazine and 260 μL H_2O.

X-Ray Crystal Structure Analysis of Cyclo(L-Pro-L-Tyr) Cyclo(L-Pro-L-Tyr) was dissolved in ethyl acetate. Colorless needle crystals were obtained at room temperature after 1 d. A crystal of the cyclo(L-Pro-L-Tyr) was determined by X-ray crystallographic analysis at 213 K. The X-ray intensity data of 50502 reflections (of which 9783 were unique) were collected on a Rigaku RAXIS RAPID II imaging plate area detector with graphite monochromated Cu-K_α radiation (λ=1.54187Å). The data were corrected for Lorentz and polarization effects. The structure was solved by direct methods using SIR2011[5] and expanded using Fourier techniques.[6] The non-hydrogen atoms were refined anisotropically. Hydrogen atoms were refined using the riding model. The final cycle of full-matrix least-squares refinement on F^2 was based on 9741 observed reflections and 689 variable parameters and converged with unweighted and weighted agreement factors of $R=\Sigma||Fo|-|Fc||/\Sigma|Fo|=0.0673$ ($I>2.00\sigma(I)$), $Rw=[\Sigma(w(Fo^2-Fc^2)^2)/\Sigma\ w(Fo^2)^2]^{1/2}=0.2155$. The goodness of fit was 1.02. Unit weights were used. The maximum and minimum peaks on the final difference Fourier map corresponded to 0.54 and −0.22 e/Å^3, respectively. All calculations were performed using the CrystalStructure[7] crystallographic software package except for refinement, which was performed using SHELXL97.[8] Crystallographic data reported in this manuscript have been deposited with the Cambridge Crys-

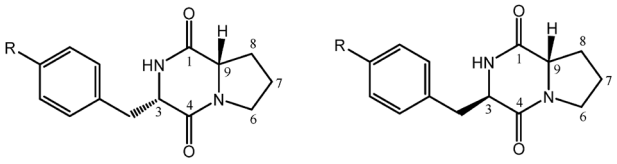

Fig. 1. Cyclo(Pro-Phe) and Cyclo(Pro-Tyr)

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tallographic Data Center as supplementary publication No. 1530756.

Results and Discussion

Conformation of Cyclo(L-Pro-Phe) and Cyclo(L-Pro-Tyr) in Water In the ^{1}H-NMR spectrum in D_2O, the proton signal for $H_{8\alpha}$ of cyclo(L-Pro-L-Xxx) (Xxx=Phe, Tyr) at 0.764, 0.768 ppm was observed in a higher magnetic field than that of cyclo(L-Pro-D-Xxx) (Xxx=Phe, Tyr) at 1.639, 1.646 ppm, and the proton signal for H_9 of cyclo(L-Pro-D-Xxx) at 2.471, 2.478 ppm was observed in a higher magnetic field than that of cyclo(L-Pro-L-Xxx) at 4.056, 4.034 ppm, respectively.

NOEs were observed between the proton signal for $H_{8\alpha}$ and the proton signals for the benzene ring in of cyclo(L-Pro-L-Xxx), and between the proton signal for H_9, $H_{8\beta}$ and the proton signals for the benzene ring of cyclo(L-Pro-D-Xxx), suggesting that the upfield shift in the proton signals for $H_{8\alpha}$ of cyclo(L-Pro-L-Xxx) and H_9 of cyclo(L-Pro-D-Xxx) resulted from the magnetic anisotropic shielding by the ring current from the benzene ring.

Judging from the above findings, intramolecular hydrophobic interactions were formed between the main skeleton of cyclo(L-Pro-L-Xxx) and cyclo(L-Pro-D-Xxx) and their benzene ring, and both cyclo(L-Pro-L-Xxx) and cyclo(L-Pro-D-Xxx) took a folded conformation in D_2O (Fig. 2). Sano *et al*. reported the intramolecular CH–π interaction of diketopiperazines bearing a benzyl group in dimethyl sulfoxide-d_6 (DMSO-d_6) and $CDCl_3$.[9–11]

The crystal structures of cyclo(L-Pro-L-Phe),[12] cyclo(L-Pro-

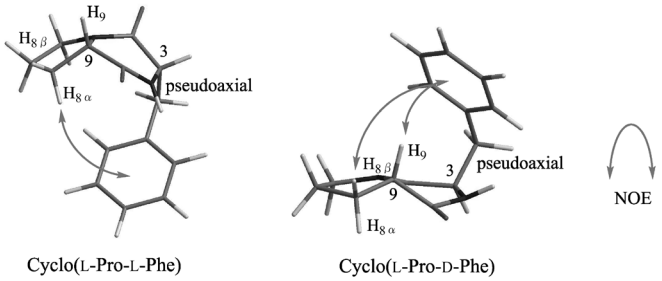

Fig. 2. Stereochemical Structures of Cyclo(L-Pro-L-Phe) and Cyclo(L-Pro-D-Phe) Black Both Arrows Indicate NOE

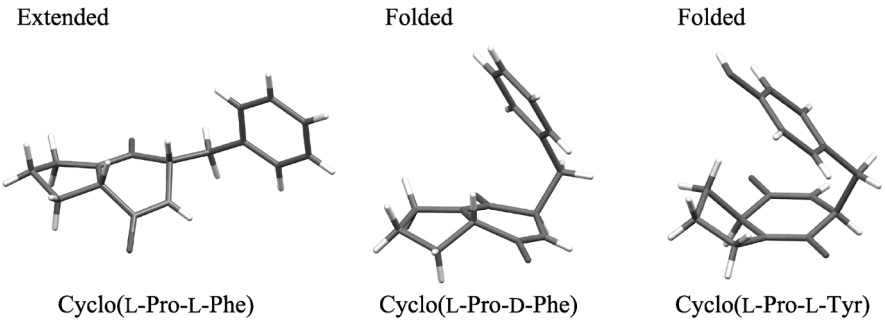

Fig. 3. Crystal Structures of Cyclo(Pro-Phe) and Cyclo(Pro-Tyr)

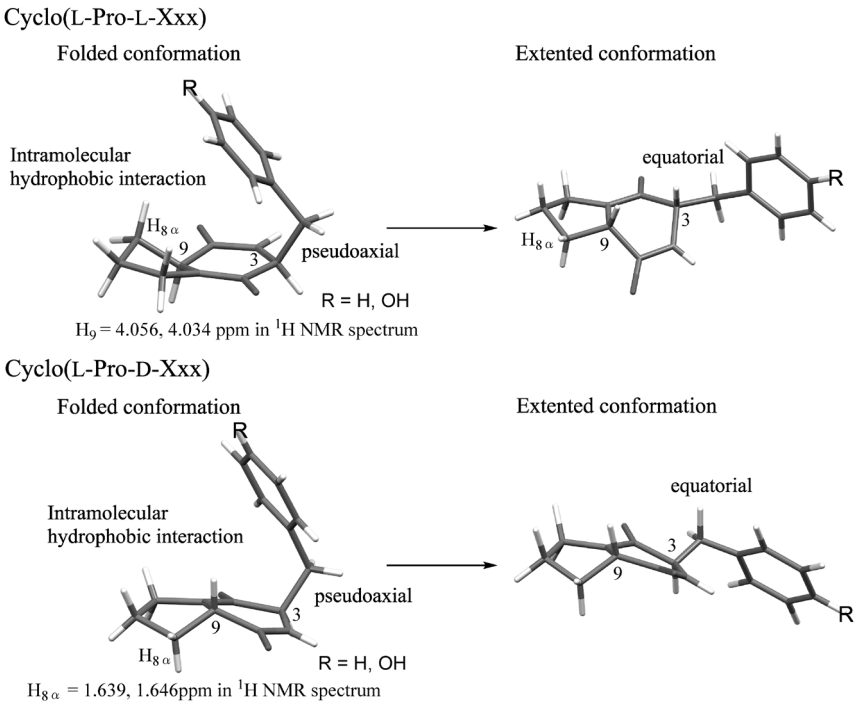

Fig. 4. Conformational Changes in Cyclo(L-Pro-L-Xxx) and Cyclo(L-Pro-D-Xxx) (Xxx=Phe, Tyr)

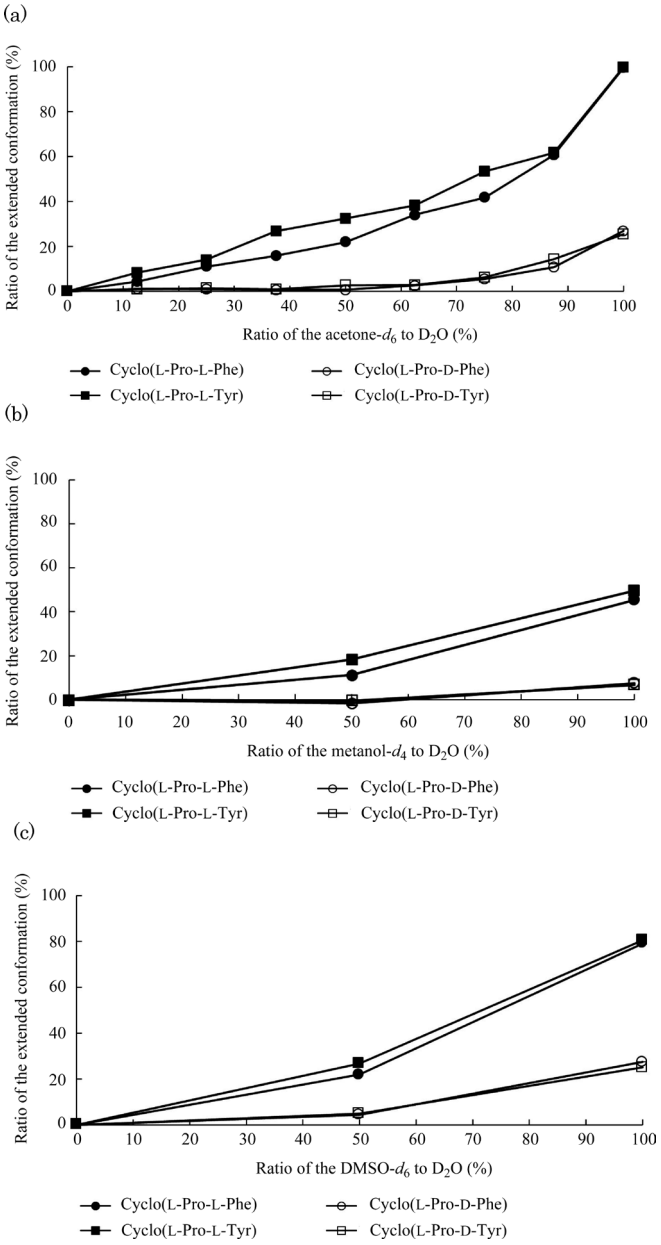

Fig. 5. Ratio of the Extended Conformation of Cyclo(L-Pro-L-Xxx) and Cyclo(L-Pro-D-Xxx) (Xxx=Phe, Tyr) with the Increase in (a) Acetone-d_6, (b) Metanol-d_4, and (c) DMSO-d_6

D-Phe),[13] and cyclo(L-Pro-L-Tyr) were investigated by X-ray crystallographic analysis. As shown in Fig. 3, cyclo(L-Pro-L-Phe) took extended conformation, on the other hand cyclo(L-Pro-D-Phe) and cyclo(L-Pro-L-Tyr) took folded conformation.

Conformational Change by Addition of an Organic Solvents and a Temperature Rise The conformational change of cyclo(L-Pro-L-Xxx) and cyclo(L-Pro-D-Xxx) (Xxx=Phe, Tyr) from folded to extended conformation was investigated by addition of deuterated organic solvent into D_2O (Fig. 4).

Changes in chemical shift values of the proton signal for $H_{8\alpha}$ of cyclo(L-Pro-L-Xxx) and the proton signal for H_9 of cyclo(L-Pro-D-Xxx) in the ^{1}H-NMR spectrum were measured while increasing the ratio of deuterated organic solvents (acetone-d_6, metanol-d_4, DMSO-d_6) against D_2O. Here, the chemical shift value (1.639, 1.646 ppm) of the proton signal for

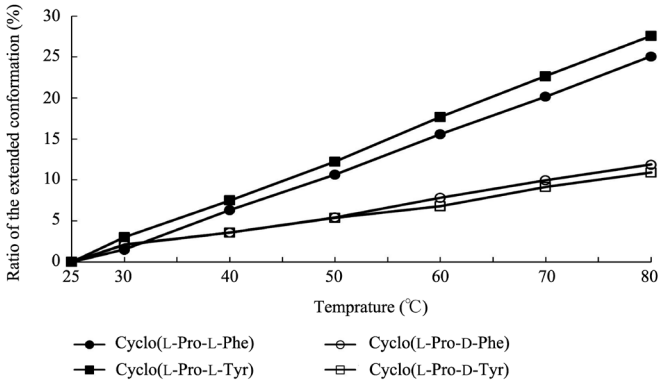

Fig. 6. Ratio of the Extended Conformation of Cyclo(L-Pro-L-Xxx) and Cyclo(L-Pro-D-Xxx) (Xxx=Phe, Tyr) with the Temperature Rise

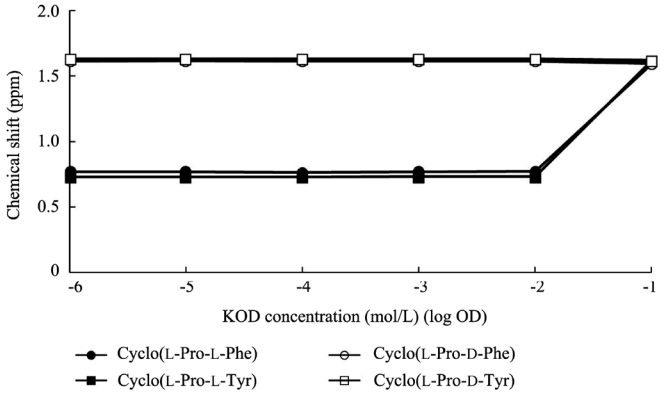

Fig. 7. Chemical Shift Changes in the ^{1}H-NMR Spectrum of Cyclo(L-Pro-L-Xxx) and Cyclo(L-Pro-D-Xxx) (Xxx=Phe, Tyr) with the Increase in the KOD Concentration

$H_{8\alpha}$ of cyclo(L-Pro-D-Xxx) was used as that of the cyclo(L-Pro-L-Xxx) (Xxx=Phe, Tyr) when they took the extented conformation in D_2O, and the chemical shift value (4.056, 4.034 ppm) of the proton signal for H_9 of cyclo(L-Pro-L-Xxx) was used as that of the cyclo(L-Pro-D-Xxx) when they takes extented conformation in D_2O, respectively.

Figure 5 shows the ratio of the extended conformation of cyclo(L-Pro-L-Xxx) and cyclo(L-Pro-D-Xxx) (Xxx=Phe, Tyr) *versus* the concentration of the deuterated organic solvent (acetone-d_6, metanol-d_4, DMSO-d_6). The ratio of the extended conformation of cyclo(L-Pro-L-Xxx) and cyclo(L-Pro-D-Xxx) was estimated by a shift value of the chemical shifts of the proton signal for $H_{8\alpha}$ of cyclo(L-Pro-L-Xxx) and H_9 of cyclo(L-Pro-D-Xxx), respectively.

The ratio of the extended conformation of cyclo(L-Pro-L-Xxx) increased more predominantly than that of cyclo(L-Pro-D-Xxx) with increasing concentration of deuterated organic solvents. In acetone-d_6, cyclo(L-Pro-L-Xxx) (Xxx=Phe, Tyr) reached extended conformation at a ratio of 99.6, 100%, on the other hand, cyclo(L-Pro-D-Xxx) (Xxx=Phe, Tyr) reached extended conformation at a ratio of only 26.9, 25.5%, as shown in Fig. 5a.

Nextly, the stability of the hydrophobic interaction of cyclo(L-Pro-L-Xxx) and cyclo(L-Pro-D-Xxx) to temperature rises was investigated. Figure 6 shows a ratio of the extended conformation of cyclo(L-Pro-L-Xxx) and cyclo(L-Pro-D-Xxx) (Xxx=Phe, Tyr) *versus* temperature change from 25°C to

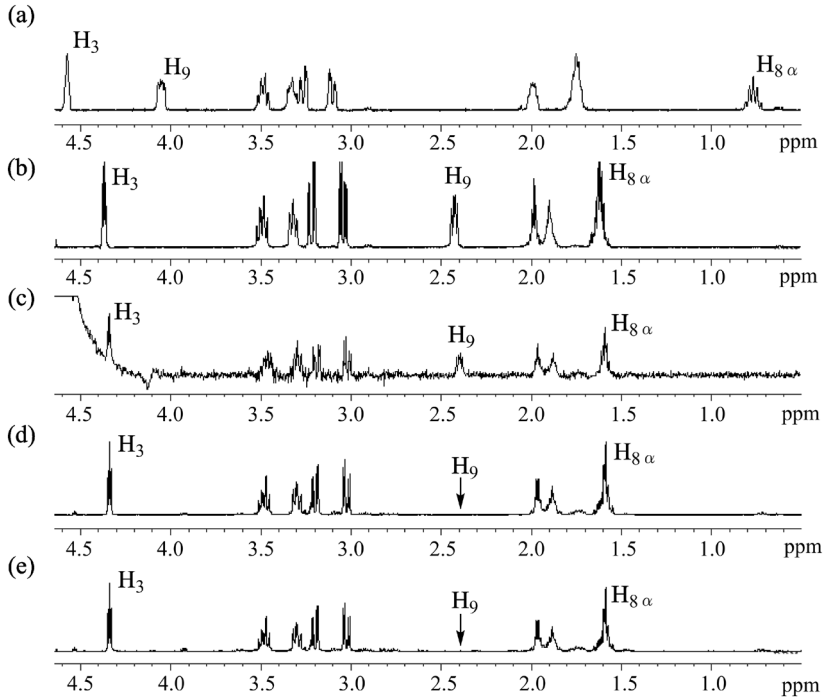

Fig. 8. ^{1}H-NMR Spectra

All ^{1}H-NMR measurement temperature are 35°C. (a) Cyclo(L-Pro-L-Phe) in D_2O. (b) Cyclo(L-Pro-D-Phe) in D_2O. (c) Cyclo(L-Pro-L-Phe) 1.0×10^{-1} mol/L KOH in H_2O. (d) Cyclo(L-Pro-L-Phe) 1.0×10^{-1} mol/L KOD in D_2O. (e) Cyclo(L-Pro-D-Phe) 1.0×10^{-1} mol/L KOD in D_2O.

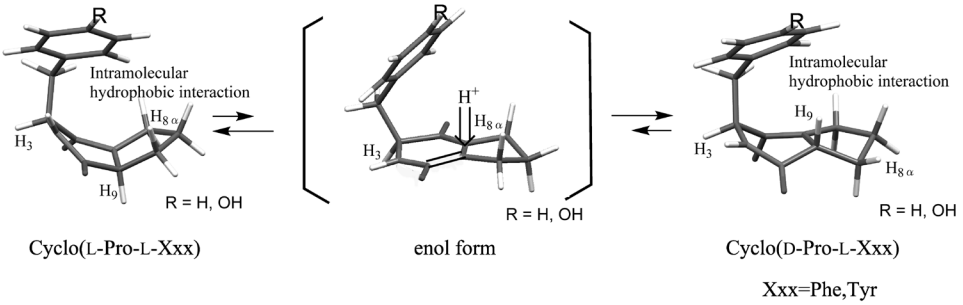

Fig. 9. Enolization and Epimerization of Cyclo(L-Pro-L-Xxx) (Xxx=Phe, Tyr)

80°C. The ratio of the extended conformation of cyclo(L-Pro-L-Xxx) increased more predominantly than that of cyclo(L-Pro-D-Xxx) with rising temperature. No significant chemical shift value was observed other than the shift values of the chemical shifts of proton signals for $H_{8\alpha}$ of cyclo(L-Pro-L-Xxx) and H_9 of cyclo(L-Pro-D-Xxx), suggesting that the conformational change from folded to extended conformation occurred due to the temperature rise.

Therefore, the results of the conformational change by addition of organic solvents and a temperature rise suggested that the intramolecular hydrophobic interaction of cyclo(L-Pro-D-Xxx) bound more strongly than that of cyclo(L-Pro-L-Xxx). It might be because in aqueous solution structure, the part that the main skeleton and benzene ring of cyclo(L-Pro-D-Xxx) (Xxx=Phe, Tyr) overlapped each other was larger than the part that those of cyclo(L-Pro-L-Xxx) (Xxx=Phe, Tyr) overlapped.

Epimerization of Cyclo(L-Pro-L-Phe) and Cyclo(L-Pro-L-Tyr) under Basic Conditions The isomerization of cyclo(L-Pro-L-Xxx) and cyclo(L-Pro-D-Xxx) (Xxx=Phe, Tyr) was investigated in the KOD concentration range from 1.0×10^{-6} to 1.0×10^{-1} mol/L using the proton signal for $H_{8\alpha}$ in ^{1}H-NMR spectrum.

No change in the ^{1}H-NMR spectrum of cyclo(L-Pro-D-Xxx) was observed in the range from 1.0×10^{-6} to 1.0×10^{-1} mol/L, and no change in the ^{1}H-NMR spectrum of cyclo(L-Pro-L-Xxx) was observed in the range from 1.0×10^{-6} to 1.0×10^{-2} mol/L. At 1.0×10^{-1} mol/L ^{1}H-NMR, the signal of cyclo(L-Pro-L-Xxx) changed to that of cyclo(L-Pro-D-Xxx) or cyclo(D-Pro-L-Xxx) (Figs. 7, 8).

In the ^{1}H-NMR spectrum of both cyclo(L-Pro-L-Xxx) and cyclo(L-Pro-D-Xxx) in D_2O the proton signal of H_9 was disappered at 1.0×10^{-1} mol/L KOD and the proton signal of H_3 remained, indicating that the enolization of the O_1-C_1-C_9-H_9 moiety of both cyclo(L-Pro-L-Xxx) and cyclo(L-Pro-D-Xxx) occurred, while that of the O_4-C_4-C_3-H_3 moiety of them did not occur (Fig. 8). Cyclo(L-Pro-L-Xxx) changed to cyclo(D-Pro-L-Xxx), while cyclo(L-Pro-D-Xxx) did not change.

Generally speaking, racemization occurs as a result of enolization. Actually, epimerization from cyclo(L-Pro-L-Xxx)

to cyclo(D-Pro-L-Xxx) occurred due to the enolization of the O_1-C_1-C_9-H_9 moiety (Fig. 9), while cyclo(L-Pro-D-Xxx) was not epimerized. Since cyclo(L-Pro-D-Xxx) and cyclo(D-Pro-L-Xxx) are enantiomer each other, it was thought that cyclo(D-Pro-L-Xxx) formed a strong intramolecular hydrophobic interaction between the main skeleton part and the benzene ring similar to cyclo(L-Pro-D-Xxx). Therefore, it is considered that cyclo(L-Pro-L-Xxx) may be epimerized to cyclo(D-Pro-L-Xxx) in order to obtain a stronger intramolecular hydrophobic interaction.

Conflict of Interest The authors declare no conflict of interest.

Supplementary Materials The online version of this article contains supplementary materials.

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