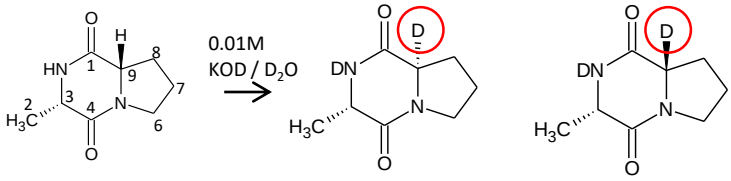
Analyze of stereoisomer by NMR

Product used : Nuclear Magnetic Resonance (NMR)

Certain organic compounds possess identical molecular structures but exhibit multiple stereoisomers due to differences in their stereochemistry. These stereoisomers often manifest distinct physical properties, reactivity, and physiological activities from one another. Consequently, it becomes essential to differentiate between them. While enantiomers cannot be discerned directly in solution NMR spectra, derivatization into diastereomers enables their discrimination within the NMR spectrum, facilitating the quantification of their respective ratios.
This discussion delves into the analysis of cyclic peptides using ^{1}H and ^{13}C NMR techniques. Specifically, a JNM-ECZL500G spectrometer, configured with a ^{2}H channel extension, and a ROYALPROBE™ were employed for these experiments, offering the capability of ^{13}C observation alongside simultaneous ^{1}H and ^{2}H decoupling.

D-substitution and diastereomerization reactions under basic conditions

Cyclo(L-Pro-L-Ala) is known to isomerize under basic conditions, leading to the formation of *DL* and *LL* forms, with deuterium incorporation at the H9 position.[1]



Cyclo(*L*-Pro-*L*-Ala) | Deuterated Cyclo(*D*-Pro-*L*-Ala) *DL* | Deuterated Cyclo(*L*-Pro-*L*-Ala) *LL*

Estimation of *DL* and *LL* ratio by ^{1}H NMR

Fig. 1 displays the ^{1}H spectra of the cyclic peptide cyclo(*L*-Pro-*L*-Ala) dissolved in D_2O solution (a) and 0.01M KOD/D_2O (b).
Fig. 1b illustrates the alteration in the ^{1}H spectral pattern and the absence of the H9 signal. Based on the integral ratio of H3 signals in the LL and DL stereoisomers, the *LL*:*DL* ratio is estimated to be 40:60.

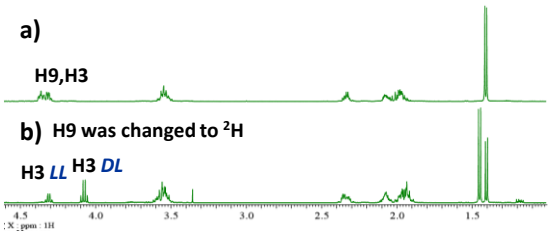


Fig. 1 : ^{1}H NMR spectra
a) D_2O solution, b) 0.01M KOD / D_2O solution

Effective observation of ^{13}C signals coupled to 2H

The diastereoisomerization reaction induces significant changes in the ^{13}C spectrum, as depicted in Fig. 2. Each ^{13}C signal shown in Fig. 2a undergoes splitting into two signals in Fig. 2b. Additionally, Fig. 3 presents an expanded region focused on C9. It is difficult to spot any signal in Fig. 3b due to ^{13}C-^{2}H splitting. Through simultaneous ^{1}H and ^{2}H decoupling, Fig. 3c reveals the presence of two signals corresponding to the *LL* and *DL* forms. This spectrum provides evidence of proper deuteration of the compound.

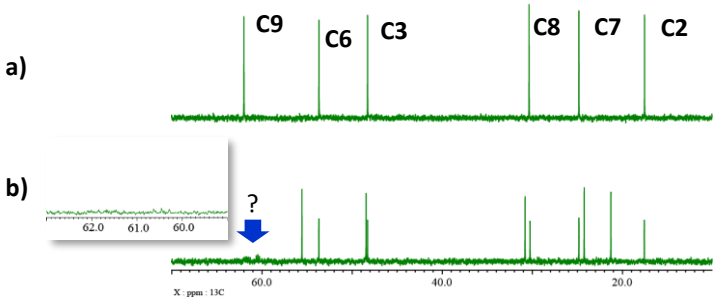


Fig. 2 : ^{13}C NMR spectra
a) D_2O solution, b) 0.01M KOD / D_2O solution

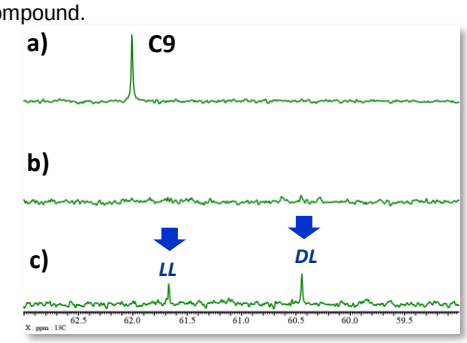


Fig. 3 : Expanded ^{13}C NMR spectra focused on C9
a)D_2O solution $^{13}C\{^{1}H\}$
b)0.01M KOD/D_2O solution $^{13}C\{^{1}H\}$
c)0.01M KOD/D_2O solution $^{13}C\{^{1}H\}\{^{2}H\}$

Sample courtesy of Prof. Takashi Ishizu
(Faculty of Pharmacy and Pharmaceutical Sciences, Fukuyama University)

Reference : [1] J. Am. Chem. Soc., 96(12), 3985-3989, 1974.

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