Solvent-Dependent Conformations of Cyclic Tetrapeptide. II

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The solvent-dependent conformations of cyclo(−δ-Ava–L-Pro–)$_2$ were characterized by means of the ^{1}H and ^{13}H NMR, CD, and IR spectra. CD results in CH_2Cl_2, CH_3CN, CH_3OH, and H_2O indicated that the change in polarity of the solvent induces a reversible change in the conformation of cyclo(−δ-Ava–L-Pro–)$_2$, revealed by the decrease of the negative peak near to 230 nm and then by the gradual appearance of a negative trough near to 210 nm. The NMR data in CD_3Cl, CD_2Cl_2, CD_3CN, and CD_3OH confirmed that cyclo(−δ-Ava–L-Pro–)$_2$ has a C2 symmetric conformation consisting of all-*trans* peptide-bond backbones in all solvents, and that the change in polarity of the solvent induces a difference in the rotational states of the Pro $^\alpha$C–C=O single bond.

A Pro residue in naturally occurring bioactive peptides and proteins plays an important structural role in folding them.[1] So far, various peptides containing Pro residues have been synthesized in order to investigate the role of the Pro residues in peptides and proteins.[2]

Recently, we reported that cyclo(−γ-Abu–L-Pro–)$_2$,[3,4] in which two γ-Abu residues, –NH–$(CH_2)_3$–CO–, are used as a connector between two Pro residues, has a C2 symmetric conformation consisting of the *cis-trans-cis-trans* peptide bond backbone (with two *cis* γ–Abu–L-Pro bonds) in all solvents tested, and that the change in the polarity of the solvent induces an inversion of the *cis* and *trans* conformations around the Pro $^\alpha$C–C=O single bond.[5] (The *trans* and *cis* regions describe the rotational states of the Pro $^\alpha$C–C=O single bond in which the Pro $^\alpha$C–H is *trans* and *cis* to the Pro carbonyl oxygen atom, respectively)

In this paper, we report on the synthesis of cyclo(−δ-Ava–L-Pro–)$_2$, in which two δ-Ava residues, –NH–$(CH_2)_4$–CO–, are used as a connector between two Pro residues, and provide obvious experimental evidence of the interconversion among the three conformers consisting of all trans peptide bonds, which are induced by the difference in the rotational states of the Pro $^\alpha$C–C=O single bond.

Results and Discussion

This cyclic peptide was synthesized using a solution-phase methodology. Cyclization of H–δ-Ava–L-Pro–δ-Ava–L-Pro–ONSu (–ONSu, *N*-hydroxysuccinimide ester) was performed in pyridine (concentration of peptide in pyridine: 3×10^{-3}M, 1 M = 1 mol dm^{-3}) at 25 °C for 1 d. The cyclic peptide was purified by gel-filtration, followed by reprecipitation from methanol–ether. The cyclic tetrapeptide was obtained in a 56% yield. The homogeneity of cyclo(−δ-Ava–L-Pro–)$_2$ was confirmed by means of fast-atom bombardment (FAB) mass spectrometry, elemental analysis, amino acid analysis, high-performance liquid chromatography.

The evidence for the progressive conversion of the conformations of cyclo(−δ-Ava–L-Pro–)$_2$ in CD spectra is shown in Figs. 1 and 2. The spectrum in CH_2Cl_2 shows one negative

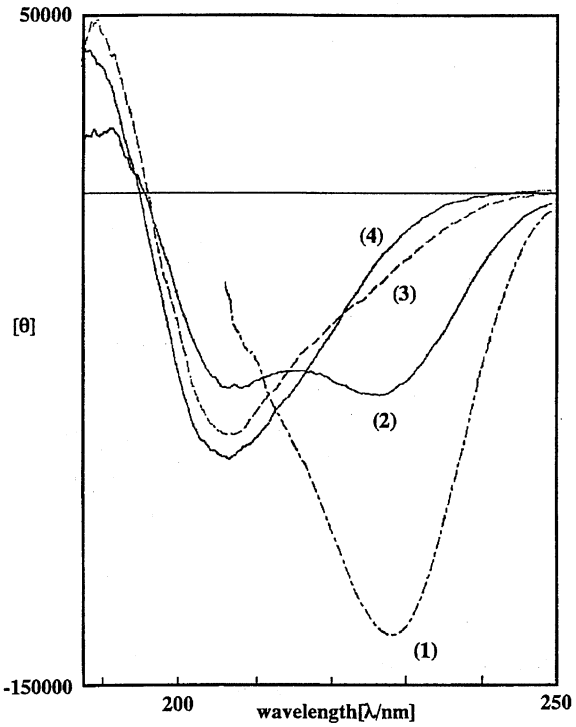

Fig. 1. CD spectra of cyclo(−δ-Ava–L-Pro–)$_2$ in various solvents. Data were obtained with a JASCO spectropolarimeter (model J-720w) using a 0.1 mm cell. The peptide concentration is 1.5 mM. (1) CH_2Cl_2; (2) acetonitrile; (3) MeOH; (4) H_2O.

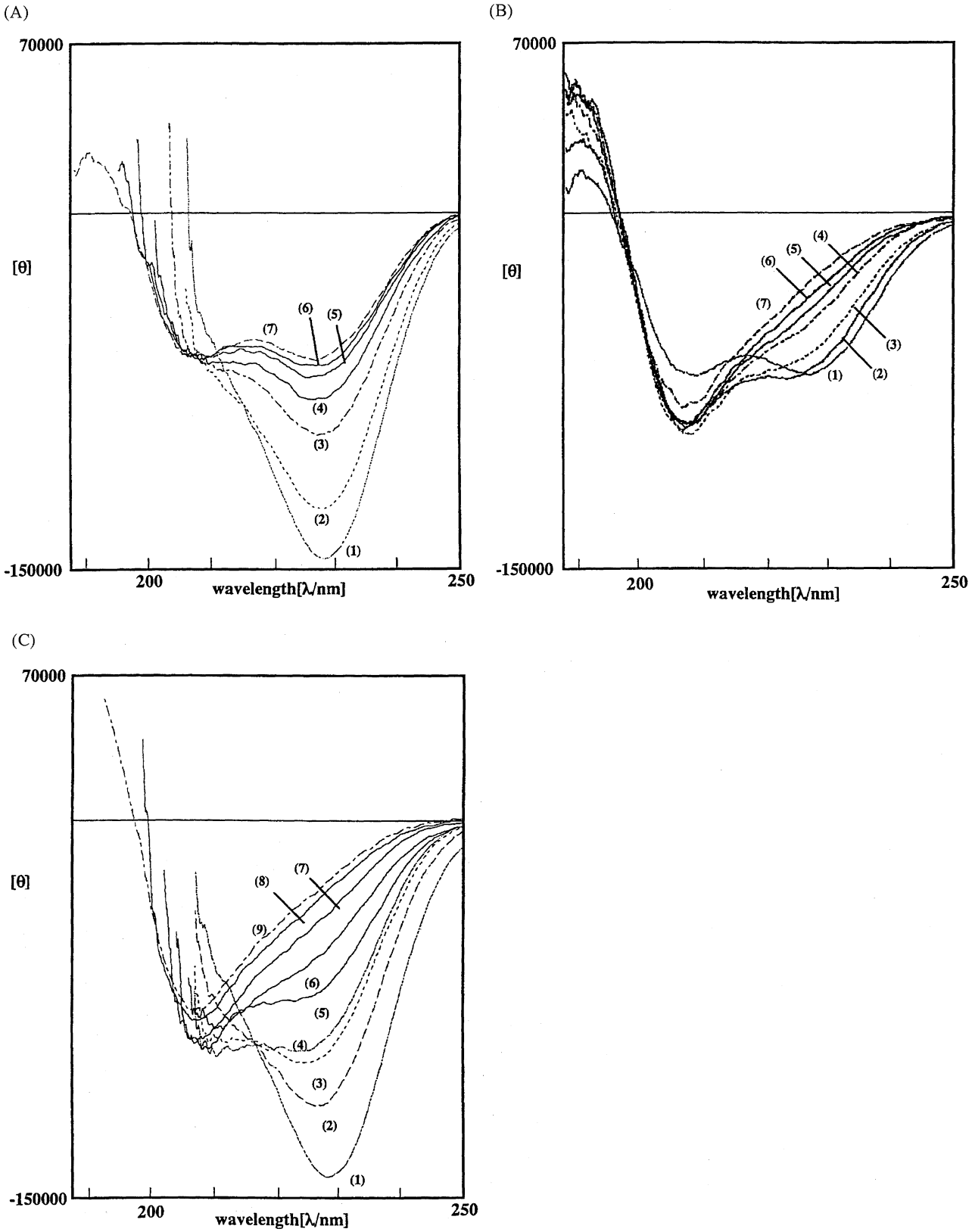

Fig. 2. CD spectra of cyclo(–δ-Ava–L-Pro–)$_2$ at room temperature in CH_2Cl_2/acetonitrile (**A**), acetonitrile/MeOH (**B**), and MeOH/CH_2Cl_2 (**C**) solvent mixture.
CH_2Cl_2/acetonitrile (**A**): (1) 100/0; (2) 80/20; (3) 60/40; (4) 40/60; (5) 20/80; (6) 10/90; (5) 0/100.
acetonitrile/MeOH (**B**): (1) 100/0; (2) 90/10; (3) 80/20; (4) 60/40; (5) 40/60; (6) 20/80; (7) 0/100.
MeOH/CH_2Cl_2 (**C**): (1) 100/0; (2) 95/5; (3) 90/10; (4) 85/15; (5) 80/20; (6) 60/40; (7) 40/60; (8) 20/80; (9) 0/100.

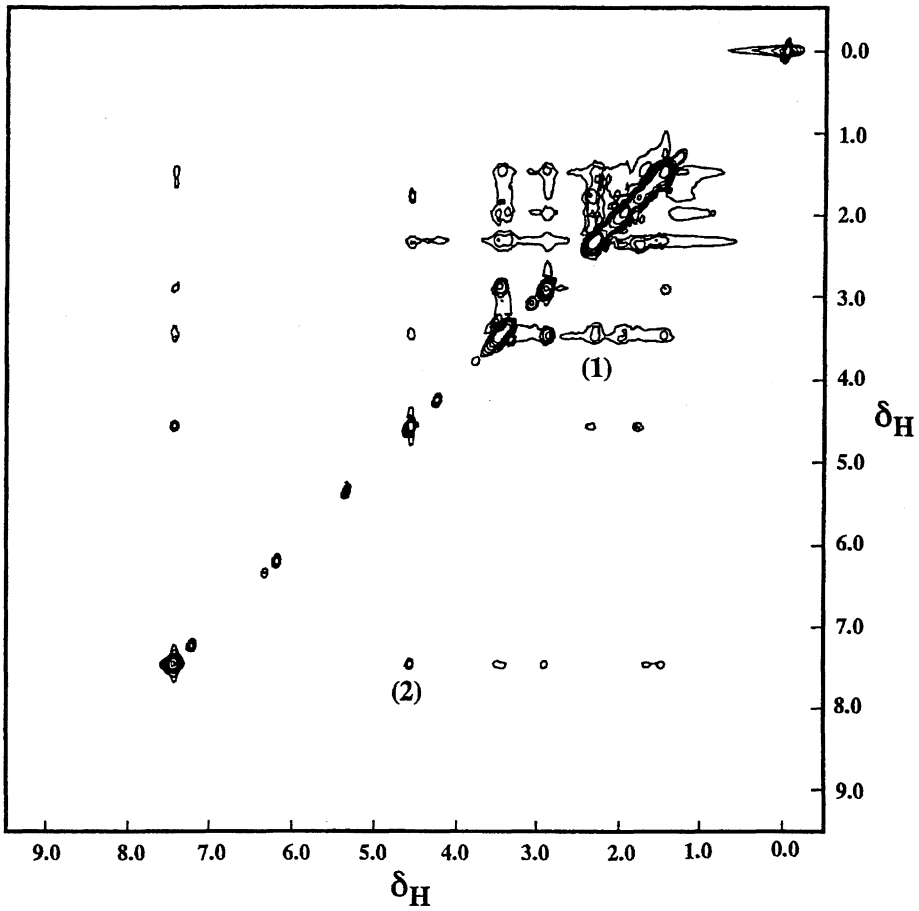

Fig. 3. NOESY spectrum of cyclo(–δ-AVa–L-Pro–)$_2$ in CD_2Cl_2 at 25 °C. (1) δ-Ava $^{\alpha}CH_2 \leftrightarrow$ Pro $^{\delta}CH_2$: (2) Ava $^{\delta}NH \leftrightarrow$ Pro $^{\alpha}CH$.

trough near to 230 nm. The CD pattern is characteristic for peptides having the γ-turn structure.[2b] On the other hand, the spectra in MeOH and H_2O show one negative band near to 207 nm. The patterns are similar to that of polyproline II.[6] In addition, the spectrum in acetonitrile shows a CD pattern of the α-helix type having two negative troughs near to 207 and 230 nm.[7] The addition of acetonitrile to the CH_2Cl_2 solution induces a progressive inversion of the conformation in CH_2Cl_2, revealed by a decrease in the ellipticity near to 230 nm, and also by the gradual appearance a trough of the negative band near to 207 nm (Fig. 2-A). With the addition of MeOH to a acetonitrile solution, the trough of the negative band near to 207 nm becomes deeper, and then the negative band near to 230 nm finally changes to a shoulder (Fig. 2-B). Further, upon the addition of MeOH to a CH_2Cl_2 solution, the CD spectrum in CH_2Cl_2 changes to the CD pattern in acetonitrile, and then finally the CD pattern in MeOH (Fig. 2-C). These results indicate that the change in the polarity of the

Table 1. NMR Parameters[a] and Ratios of Hydrogen Bonded NH to Total NH[b] of Cyclo-(–δ-AVa–Pro–)$_2$

Solvent	NH[c]	$\Delta\delta/\Delta T$[d]	C_β[e]	C_γ[e]	$\Delta\delta_{\beta-\gamma}$[f]	$NH_{hydrogen}/NH_{total}$[b]
$CDCl_3$	7.57	4	26.40	25.00	1.44	0.88
CD_2Cl_2	7.42	4	26.93	25.42	1.51	0.83
CD_3CN–CD_2Cl_2 (6 : 4 v/v)	7.19	3	28.20	25.41	2.79	—
CD_3OH	8.02	7	29.81	25.07	4.74	—

a) Data were obtained by ^1H (250 MHz) and ^{13}C (62.9 MHz) NMR spectra on a Bruker AM-250 instrument at 25 °C. Chemical shifts are downfield from internal tetramethylsilane. Peptide concentration is about 17 mg ml^{-1}. b) The ratios of signal area of H-bonded amide proton (3327 cm^{-1}) to that of total amide proton (3327 and 3423 cm^{-1}) in the NH stretch region. Data were obtained on JASCO FT/IR-230 for 1 mM sample in $CHCl_3$ and CH_2Cl_2 at 20 °C, after substation of the spectrum of pure $CHCl_3$ and CH_2Cl_2, respectively. c) Values of chemical shifts of $^{\delta}$NH δ-Ava (ppm). d) Temperature coefficients of chemical shifts of $^{\delta}$NH δ-Ava (ppb/°C). e) Values of chemical shifts of C_β and C_γ of Pro residue (ppm). f) Pro C_β–C_γ chemical shift differences (ppm).

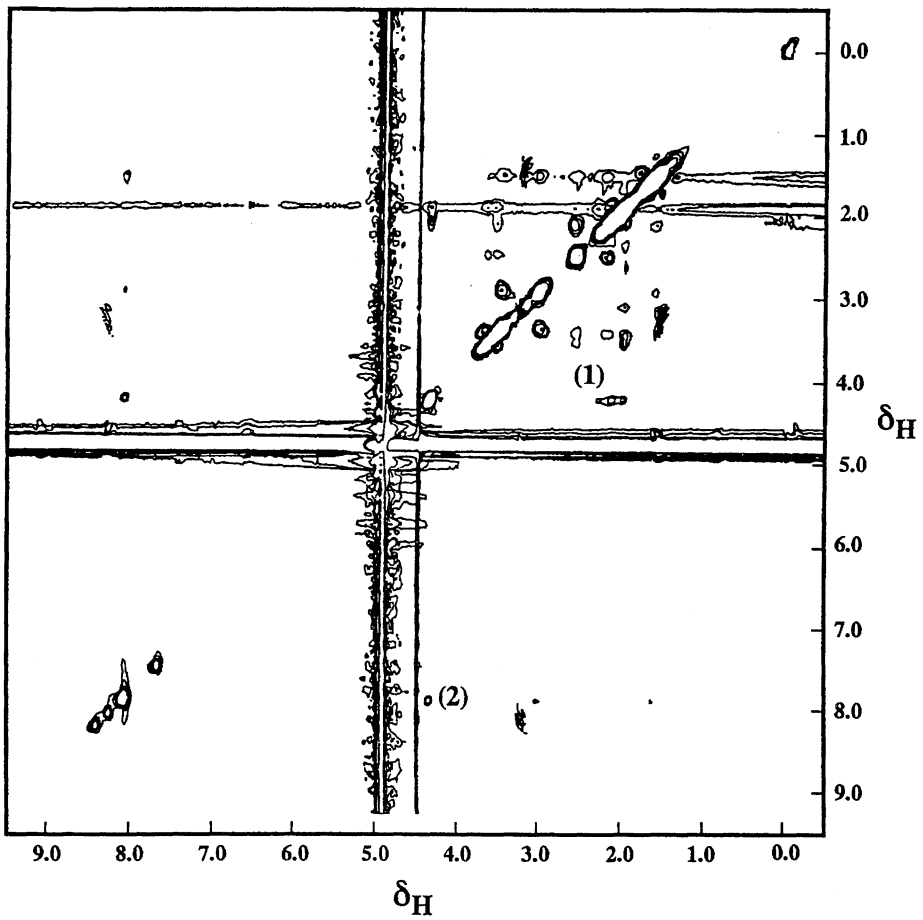

Fig. 4. NOESY spectrum of cyclo(–δ-Ava–L-Pro–)$_2$ in CD_3OH at 25 °C. (1) δ-Ava $^{\alpha}CH_2 \leftrightarrow$ Pro $^{\delta}CH_2$: (2) Ava $^{\delta}NH \leftrightarrow$ Pro $^{\alpha}CH$.

solvent induces a progressive inversion of the conformation, revealed by the disappearance of the negative peak near to 230 nm, and then by a gradual appearance of the negative trough near to 207 nm; also, the variations in CD spectra of cyclo(–δ-Ava–L-Pro–)$_2$ may be correlated to changes in the orientations of two amide bonds around the Pro residue.

Further conformational analyses of cyclo(–δ-Ava–L-Pro–)$_2$ were performed by means of ^1H (250 MHz) and ^{13}C (62.9 MHz) NMR spectra in $CDCl_3$, CD_2Cl_2, acetonitrile-d_3–CD_2Cl_2 (6 : 4), and CD_3OH, and FT-IR spectra in $CHCl_3$ and CH_2Cl_2; the data are summarized in Table 1. Acetonitrile -d_3–CD_2Cl_2 (6 : 4 v/v), in which the CD spectrum is similar to that in acetonitrile (Fig. 2-A), was used as a NMR solvent, since cyclo(–δ-Ava–L-Pro–)$_2$ is not sufficiently soluble in neat acetonitrile-d_3. In NMR studies, all protons and carbons were assigned by means of H-H COSY, C-H COSY, HOHAHA, and NOESY. (COSY = 2D chemical-shift correlation spectroscopy; HOHAHA = 2D homonuclear Hartmann–Hahn spectroscopy; NOESY = nuclear Overhauser effect spectroscopy.) The presence of a small amount of several conformers (< 20%) in these solvents is evident in the 1D NMR spectra, and the amount of the minor components show no significant concentration dependence in $CDCl_3$, CD_2Cl_2, acetonitrile-d_3–CD_2Cl_2 (6 : 4 v/v), and CD_3OH. The minor components have not been conformationally analyzed in detail. The main conformer of this cyclic peptide has C2 symmetry in the NMR time average, because only one amide proton resonance appears for the δ-Ava residue. The amide proton chemical shifts and their temperature dependences in $CDCl_3$, CD_2Cl_2, acetonitrile-d_3–CD_2Cl_2 (6 : 4 v/v), and CD_3OH were measured at a concentration of 17 mg ml^{-1}. The chemical shifts and temperature coefficients for δ-Ava $^{\delta}NH$ show no significant concentration dependence in $CDCl_3$, CD_2Cl_2, acetonitrile-d_3–CD_2Cl_2 (6 : 4 v/v), and CD_3OH, indicating that this cyclic peptide is monomeric over the entire range of concentration.

In $CDCl_3$ and CD_2Cl_2, the Pro $^{\alpha}H$ residue resonance shifted to a low field (4.65 and 4.54 ppm, respectively) and appeared as a double doublet due to the Pro $^{\beta}H$'s. The Pro $^{\beta}C$ in $CDCl_3$ and CD_2Cl_2 fairly shifted upfield from the usual position for a trans Pro $^{\beta}C$, and $\Delta\delta_{\beta-\gamma}$ in $CDCl_3$ and CD_2Cl_2 were 1.44 and 1.51 ppm, respectively (Table 1). In addition, the spatial NOE's in $CDCl_3$ and CD_2Cl_2 of cyclo(–δ-Ava–L-Pro–)$_2$ were observed between δ-Ava $^{\alpha}CH_A$ and Pro $^{\delta}CH$, and δ-Ava $^{\delta}NH$ and Pro $^{\alpha}CH$ (Fig. 3). These data indicate that cyclo(–δ-Ava–Pro–)$_2$ in $CDCl_3$ and CD_2Cl_2 adopts two γ-turn structures consisting of an all-*trans* peptide bond with a two-*trans* Pro $^{\alpha}C$–C=O bond.[2b,8]

In acetonitrile-d_3–CD_2Cl_2 (6 : 4 v/v), the Pro $^{\beta}C$ and $^{\gamma}C$ resonance in the field position usually found for the trans Pro $^{\beta}C$ resonance[8c] and its $\Delta\delta_{\beta-\gamma}$[8b] was 2.79 ppm (Table 1). The NOE cross peaks between δ-Ava $^{\alpha}CH_A$ and Pro $^{\delta}CH_A$,

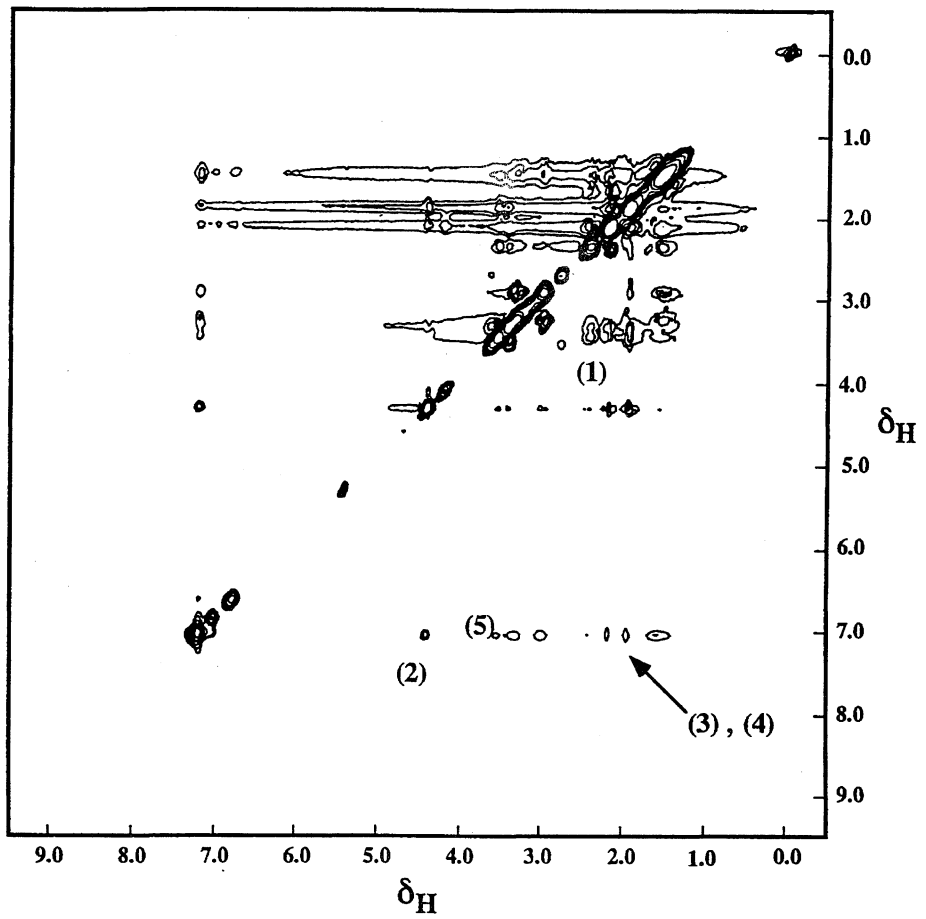

Fig. 5. NOESY spectrum of cyclo(–δ-Ava–L-Pro–)$_2$ in acetonitrile-d_3/CH_3Cl_2 (6 : 4 v/v) at 25 °C. (1) δ-Ava $^{\alpha}CH_2 \leftrightarrow$ Pro $^{\delta}CH_2$: (2) Ava $^{\delta}NH \leftrightarrow$ Pro $^{\alpha}CH$: (3) Ava $^{\delta}NH \leftrightarrow$ Pro $^{\beta}CH_A$: (4) Ava $^{\delta}NH \leftrightarrow$ Pro $^{\gamma}CH_A$: (5) Ava $^{\delta}NH \leftrightarrow$ Pro $^{\delta}CH_A$.

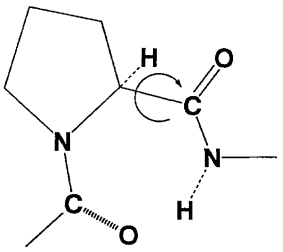

Fig. 6. Rotation about Pro $^{\alpha}$C–C=O single bonds.

and δ-Ava $^{\delta}$NH and Pro $^{\alpha}$CH. These results indicate that cyclo(–δ-Ava–Pro–)$_2$ in acetonitrile-d_3–CD_2Cl_2 (6 : 4 v/v) possesses a conformation with all-trans peptide bonds. In addition, spatial NOE cross peaks between δ-Ava $^{\delta}$NH and Pro $^{\beta}CH_A$, δ-Ava $^{\delta}$NH and Pro $^{\gamma}CH_A$, and δ-Ava $^{\delta}$NH and Pro $^{\delta}CH_A$ were observed (Fig. 5). In other solvents, additional NOE cross peaks were not. The observations of the additional NOE's indicate that the NH proton is close to the β, γ, and δ protons on one side of the pyrrolidine ring of the Pro residue; in other words, the rotation around the Pro$^{\alpha}$C–C=O single bond is within the cis region.[5c,8c] In CD and NMR studies of Ac–Pro–NHMe, Madison et al. reported that in the α-helix conformation of Ac–Pro–NHMe, the NH proton is close to the β, γ, and δ protons on one side of the pyrrolidine ring of the Pro residue, and that in the other conformations of Ac–Pro–NHMe (γ-turn and polyproline II conformation) it is not.[5c] The present NMR data in acetonitrile-d_3–CD_2Cl_2 (6 : 4 v/v) are in good agreement with the CD data in acetonitrile.

In CD_3OH, the Pro $^{\beta}$C resonance shifted to the field position usually found for the trans Pro $^{\beta}$C resonance; its $\Delta\delta_{\beta-\gamma}$ was 4.74 ppm (Table 1). The spatial NOE's in CD_3OH were observed between δ-Ava $^{\alpha}CH_A$ and Pro $^{\delta}$CH, and δ-Ava $^{\delta}$NH and Pro $^{\alpha}$CH (Fig. 4). These results indicated that cyclo(–δ-Ava–Pro–)$_2$ in CD_3OH possesses a conformation with all-*trans* peptide bonds with two-*trans* Pro $^{\alpha}$C–C=O bonds; the downfield movement of Pro $^{\beta}$C in CD_3OH can be correlated with conversion from one preferred rotamer in $CDCl_3$, CD_2Cl_2 to another within the trans region of the Pro $^{\alpha}$C–C=O bonds.[8b,8c]

The temperature coefficients[2d] of the chemical shifts of the δ-Ava $^{\delta}$NH resonances in $CDCl_3$, CD_2Cl_2, acetonitrile-d_3–CD_2Cl_2 (6 : 4 v/v) and CD_3OH (Table 1) suggest that the amide protons of the δ-Ava residues in $CDCl_3$, CD_2Cl_2 and acetonitrile-d_3–CD_2Cl_2 (6 : 4 v/v) are shielded from the solvents, while those in CD_3OH exposure to the solvents. In addition, an analysis of the amide N–H stretch region in theIR spectra[9] in $CHCl_3$ and CH_2Cl_2 indicates that the amide protons of the δ-Ava residues in $CDCl_3$, CD_2Cl_2 are involved in an intramolecular hydrogen bond.

In these studies, the $\Delta\delta_{\beta-\gamma}$ values of this cyclic peptide varied from 1.40 ppm in $CDCl_3$ to 4.74 ppm in CD_3OH according to the change in the polarity of the solvents used. The difference in the chemical shift between C^{β} and C^{γ} in the ^{13}C spectra ($\Delta\delta_{\beta-\gamma}$) was related to the C^{β}–C^{α}–C=O dihedral angle θ through the expression $\Delta\delta_{\beta-\gamma}$ (ppm) = $0.036|\theta|+0.73$ proposed for *trans* Pro peptides by Siemion et al.[8b] From an inspection of the Corey–Pauling–Koltun molecular models, using the $\Delta\delta_{\beta-\gamma}$ values of this cyclic peptide (Table 1), we assign values of θ = 19, 22, 57, and −111° to the cyclic peptide of $CDCl_3$, CD_2Cl_2, acetonitrile-d_3–CD_2Cl_2 (6 : 4 v/v), and CD_3OH, respectively. The increase in the $\Delta\delta_{\beta-\gamma}$ values in $CDCl_3$, CD_2Cl_2, acetonitrile-d_3–CD_2Cl_2 (6 : 4 v/v), and CD_3OH parallels the decrease in the negative CD band near to 230 nm for that series. Further, the contribution of the minor conformer to the CD spectra is nearly constant, because the fraction of the minor conformer is always between 15 and 20% in these solvents based on the 1D NMR spectra data. It is most likely that the most important change in the CD spectra upon transferring from CH_2Cl_2 to acetonitrile and MeOH (Fig. 2) can be correlated to changes in the orientations of two amide bonds around the Pro residue, induced by the difference in the rotational states of Pro $^{\alpha}$C–C=O single bonds of cyclo(−δ-Ava–L-Pro–)$_2$ having an all-*trans* peptide bond backbone (Fig. 6).

Similar solvent-dependent conformations of the Pro residue were obtained in CD and NMR studies of Ac–Pro–NHMe by Madison et al.[5c]

Recently, we reported that cyclo(−γ-Abu–L-Pro–)$_2$ has a C2 symmetric conformation consisting of the *cis-trans-cis-trans* peptide bond backbone (with two *cis* γ-Abu–L-Pro bonds) in all solvents tested, and that the change in polarity of the solvent induces a difference in the rotational states of the Pro $^{\alpha}$C–C=O single bond.[4] On the other hand, in the present studies, cyclo(−δ-Ava–L-Pro–)$_2$ has a C2 symmetric conformation consisting of all-*trans* peptide bond backbones in all solvents, and that the change in the polarity of the solvent induces a difference in the rotational states of the Pro $^{\alpha}$C–C=O single bond. Thus, the differences in the conformations between cyclo(−γ-Aba–L-Pro–)$_2$ and cyclo(−δ-Ava–L-Pro–)$_2$ were induced by the differences in the number of methylene groups (n = 3 and 4) of the γ-Abu and δ-Ava residues used as a connector between two Pro residues in each molecule. In addition, the present data concerning both cyclic peptides (cyclo(−γ-Abu–L-Pro–)$_2$ and cyclo(−δ-Ava–L-Pro–)$_2$) may be effectively used as a simple model for a conformational investigation of the Pro residue in proteins and peptides.

Experimental

The melting point of cyclo(−δ-Ava–L-Pro–)$_2$ was measured on a Ishii melting-point apparatus, and is uncorrected. An amino acid analysis of each hydrolysate of the peptides was carried out with a Hitachi 835 amino acid analyzer. The molecular weights of the cyclic products were determined using FAB mass spectrometry on a JEOL JMS-D-300 mass spectrometer (in Asahi Chemical Industry Company).

Syntheses of Cyclo(−δ-Ava–L-Pro–)$_2$. Boc–δ-Ava–L-Pro–δ-Ava–L-Pro–OBzl were prepared by stepwise elongation using 1-(dimethylamino propyl)-3-ethylcarbodiimide hydrochloride (WSCD·HCl) and 1-hydroxy benzotriazole (HOBt). The tetrapeptide was obtained in 62% yield from H–Pro–OBzl as a oil product. Boc–δ-Ava–L-Pro–δ-Ava–L-Pro–OBzl was converted into the corresponding acid by saponification, and was then converted into the corresponding succinimide esters using HONSu and WSCD·Cl. Boc–tetrapeptide–ONSu was treated with trifluoroacetic acid to remove the Boc group at the N-terminus. Tetrapeptide–ONSu trifluoroacetate was dissolved in small amounts of DMF, and the solutions were added dropwise into pyridine at 25 °C (concentration of the active esters was 3 mM). After the mixture was stirred for 1 d at 25 °C, the solvent was evaporated. The main product in the reaction mixtures was purified by semipreparative-HPLC using a Finepak SIL C18 column (10×250 mm, 10 μm particle size, JASCO, Japan) and by reprecipitation from methanol–ether–hexane. Cyclo(−δ-Ava–L-Pro–)$_2$ was obtained in 42% yield.

The analytical data for cyclo(−δ-Ava–L-Pro–)$_2$ were as follows: Mp 241—242 °C (decomp). Found: C, 58.41; H, 8.09; N, 13.45%. Calcd for $C_{20}H_{32}N_4O_4\cdot H_2O$: C, 58.52; H, 8.35; N, 13.65%. *m/z* 393 ($M+H^+$; 100%). The result of an amino acid analysis of cyclo(−δ-Ava–L-Pro–)$_2$ agreed closely with the theoretical values.

CD Spectroscopy. CD spectra were obtained with a JASCO spectropolarimeter (model J-720) using 0.1 mm cells at room temperature. CD spectra of cyclo(−δ-Ava–L-Pro–)$_2$ was measured in H_2O, MeOH, acetonitrile and CH_2Cl_2 solutions at a concentration of 1.5 mM.

IR Spectroscopy. IR spectra were obtained with a JASCO FT/IR-236 spectropolarimeter at 20 °C, after subtraction of the spectrum of pure $CHCl_3$ and CH_2Cl_2, respectively. The IR spectra of cyclo(−δ-Ava–L-Pro–)$_2$ were measured in $CHCl_3$ and CH_2Cl_2 solutions at a concentration of 1 mM.

NMR Spectroscopy. NMR [^{1}H NMR (250 MHz) and ^{13}C NMR (62.9 MHz)] spectra were measured in $CDCl_3$, CD_2Cl_2, acetonitrile-d_3–CD_2Cl_2 (6 : 4 v/v) and CD_3OH at 25 °C (peptide concentration: ca. 17 mg ml^{-1}) on a Bruker AC-250 using standard pulse sequences and software. H-HCOSY and HOHAHA spectra with 1 K points in F2 and 256 points in F1 were recorded with a sweep width of 2500 Hz in the phase-sensitive mode using the time-proportional phase incrementation. HOHAHA spectra were obtained with mixing times of 130 ms. NOESY spectra were obtained with a mixing time of 400 ms. The sizes of the time domain data were 1 K points in F2 and 256 points in F1. C-H COSY spectra with 4 K points in F2 and 256 points in F1 were recorded. The temperature coefficients of the chemical shifts of the amide protons were obtained from least-square fits to data of 20, 25, 30, 35, 40, 45, and 50 °C.

^{1}H NMR ($CDCl_3$) δ = 1.49 (2H, m, δ-Ava $^{\gamma}CH_2$), 1.50 (^{1}H, m, δ-Ava $^{\beta}CH_A$), 1.75 (^{1}H, m, δ-Ava $^{\beta}CH_B$), 1.81 (1H, m, Pro $^{\beta}CH_A$), 1.99 (^{1}H, m, Pro $^{\gamma}CH_A$), 2.14 (1H, m, Pro $^{\gamma}CH_B$), 2.29 (2H, m, δ-Ava $^{\alpha}CH_2$), 2.43 (1H, m, Pro $^{\beta}CH_B$), 2.92 (1H, m, δ-Ava $^{\delta}CH_A$), 3.38 (1H, m, Pro $^{\delta}CH_A$), 3.42 (1H, m, δ-Ava $^{\delta}CH_B$), 3.52 (1H, m, Pro $^{\delta}CH_B$), 4.62 (1H, dd, Pro $^{\alpha}$CH), 7.57 (1H, br s, δ-Ava $^{\delta}$NH).

^{1}H NMR (CD_2Cl_2) δ = 1.45 (2H, m, δ-Ava $^{\gamma}CH_2$), 1.61 (1H, m, δ-Ava $^{\beta}CH_2$), 1.73 (1H, m, δ-Ava $^{\beta}CH_B$), 1.78 (1H, m, Pro $^{\beta}CH_A$), 1.94 (1H, m, Pro $^{\gamma}CH_A$), 2.29 (2H, m, δ-Ava $^{\alpha}CH_2$), 2.35 (1H, m, Pro $^{\beta}CH_B$), 2.93 (1H, m, δ-Ava $^{\delta}CH_A$), 3.35 (1H, m, Pro $^{\delta}CH_A$), 3.42 (1H, m, δ-Ava $^{\delta}CH_B$), 3.53 (1H, m, Pro $^{\delta}CH_B$), 4.54 (1H, dd, Pro $^{\alpha}$CH), 7.42 (1H, br s, δ-Ava $^{\delta}$NH).

^{1}H NMR (CD_3CD-CD_2Cl_2 6 : 4 v/v) δ = 1.46 (^{1}H, m, δ-Ava $^{\gamma}CH_A$), 1.51 (1H, m, δ-Ava $^{\gamma}CH_B$), 1.56 (1H, m, δ-Ava $^{\beta}CH_A$),

1.60 (1H, m, δ-Ava $^{\beta}CH_B$), 1.92 (1H, m, Pro $^{\gamma}CH_A$), 1.93 (1H, m, Pro $^{\beta}CH_A$), 2.13 (1H, m, Pro $^{\gamma}CH_B$), 2.17 (1H, m, δ-Ava $^{\alpha}CH_A$), 2.20 (^{1}H, m, Pro $^{\beta}CH_B$), 2.41 (1H, m, δ-Ava $^{\alpha}CH_B$), 3.01 (1H, m, δ-Ava $^{\delta}CH_A$), 3.33 (1H, m, δ-Ava $^{\delta}CH_B$), 3.39 (1H, m, Pro $^{\delta}CH_A$), 3.52 (1H, m, Pro $^{\delta}CH_B$), 4.39 (1H, dd, Pro $^{\alpha}CH$), 7.19 (1H, br s, δ-Ava $^{\delta}NH$).

^{1}H NMR (CD_3OH) δ = 1.54 (^{1}H, m, δ-Ava $^{\gamma}CH_2$), 1.62 (1H, m, δ-Ava $^{\beta}CH_A$), 1.69 (1H, m, δ-Ava $^{\gamma}CH_B$), 1.76 (1H, m, δ-Ava $^{\beta}CH_B$), 1.97 (1H, m, Pro $^{\beta}CH_A$), 2.02 (2H, m, Pro $^{\gamma}CH_2$), 2.23 (1H, m, δ-Ava $^{\alpha}CH_A$), 2.58 (1H, m, δ-Ava $^{\alpha}CH_B$), 3.04 (1H, m, Pro $^{\delta}CH_B$), 3.49 (1H, m, δ-Ava $^{\delta}CH_B$), 3.55 (1H, m, Pro $^{\delta}CH_A$), 3.67 (1H, m, δ-Ava $^{\delta}CH_B$), 4.38 (1H, dd, Pro $^{\alpha}CH$), 8.02 (1H, br s, δ-Ava $^{\delta}NH$).

^{13}C NMR ($CDCl_3$) δ = 21.38 (δ-Ava $^{\beta}C$), 25.00 (Pro $^{\gamma}C$), 26.40 (Pro $^{\beta}C$), 28.46 (δ-Ava $^{\gamma}C$), 33.58 (δ-Ava $^{\alpha}C$), 38.53 (δ-Ava $^{\delta}C$), 47.27 (Pro $^{\delta}C$), 59.09 (Pro $^{\alpha}C$), 171.64 and 173.69 (δ-Ava and Pro C=O).

^{13}C NMR (CD_2Cl_2) δ = 21.97 (δ-Ava $^{\beta}C$), 25.42 (Pro $^{\gamma}C$), 26.93 (Pro $^{\beta}C$), 28.87 (δ-Ava $^{\gamma}C$), 34.11 (δ-Ava $^{\alpha}C$), 38.85 (δ-Ava $^{\delta}C$), 47.68 (Pro $^{\delta}C$), 59.58 (Pro $^{\alpha}C$), 171.19 and 173.32 (δ-Ava and Pro C=O).

^{13}C NMR (CD_3CD–CD_2Cl_2 6 : 4 v/v) δ = 21.38 (δ-Ava $^{\beta}C$), 25.41 (Pro $^{\gamma}C$), 28.20 (Pro $^{\beta}C$), 28.60 (δ-Ava $^{\gamma}C$), 33.98 (δ-Ava $^{\alpha}C$), 38.62 (δ-Ava $^{\delta}C$), 47.77 (Pro $^{\delta}C$), 60.23 (Pro $^{\alpha}C$), 171.87 and 173.37 (δ-Ava and Pro C=O).

^{13}C NMR (CD_3OH) δ = 20.84 (δ-Ava $^{\beta}C$), 25.07 (Pro $^{\gamma}C$), 27.74 (δ-Ava $^{\gamma}C$), 29.81 (Pro $^{\beta}C$), 33.11 (δ-Ava $^{\alpha}C$), 37.98 (δ-Ava $^{\delta}C$), 47.84 (Pro $^{\delta}C$), 60.96 (Pro $^{\alpha}C$), 174.93 and 175.00 (δ-Ava and Pro C=O).

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