Solvent-dependent conformations of a cyclic tetrapeptide

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The conformations of cyclo(-γ-Abu-L-Pro-)$_2$ in several solvents have been characterized by means of ^{1}H and ^{13}C NMR, CD and IR spectra. CD results indicate that the change in polarity of the solvent induces an inversion of the conformation, revealed by the disappearance of the positive peak near 210 nm and then by the gradual appearance of a negative trough near 210 nm having opposite chirality. The data of NMR spectroscopy in $CDCl_3$, CD_2Cl_2, CD_3OH, $(CD_3)_2SO$ and D_2O indicate that cyclo(-γ-Abu-L-Pro-)$_2$ has a C_2 symmetric conformation consisting of the *cis–trans–cis–trans* peptide bond backbone (with two *cis* γ-Abu-L-Pro bonds) in all solvents, and that the change of polarity of solvent induces the inversion of *cis* and *trans* conformations around the Pro $^{\alpha}$C–C=O single bond. (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 direction of the Pro carbonyl oxygen atom, respectively). The *cis–trans–cis–trans* conformation containing the two *cis* Pro $^{\alpha}$C–C=O bonds in $CDCl_3$ and CD_2Cl_2 is stabilized by the presence of seven-membered rings consisting of intramolecular hydrogen bonds between $^{\gamma}$NH and $^{\alpha}$C=O in the γ-Abu residue.

cis–trans Isomerism has been observed about X-Pro peptide bonds where the rotation between two isomers of similar energy is slow enough on the NMR timescale to permit observation of separated resonances for each form.[1] On the other hand, the principal degree of torsional freedom is in the rotation about Pro $^{\alpha}$C–C=O single bonds.[2] Various peptides containing Pro residues have been synthesized, in order to investigate the roles of the Pro residue in peptides and proteins, but the role of the rotation about Pro $^{\alpha}$C–C=O single bonds has been little studied.[2]

In this study, we report the synthesis of cyclo(-γ-Abu-L-Pro-)$_2$,[3] in which two γ-Abu residues are each used as a connector between two Pro residues (γ-Abu is here used to represent γ-amino butyric acid, although the more usual term is GABA.) and provide obvious experimental evidence of an interconversion between the two conformers, 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 solution-phase methodology. Cyclization of H-γ-Abu-L-Pro-γ-Abu-L-Pro-ONSu (-ONSu = *N*-hydroxysuccinimide ester) was performed in pyridine (concentration of peptide in pyridine: 3×10^{-3} M) at 25 °C for 1 day. The cyclic peptide was purified by gel filtration, followed by reprecipitation from methanol–diethyl ether. The cyclic tetrapeptide was obtained in 56% yield. The homogeneity of cyclo(-γ-Abu-L-Pro-)$_2$ was confirmed by means of fast-atom bombardment (FAB) mass spectrometry, elemental analysis, amino acid analysis, and high-performance liquid chromatography (HPLC). The values of $[\alpha]_D^{20}$ (*c* 1.0) in $CHCl_3$, CH_2Cl_2, MeOH, dimethylformamide (DMF) and water are −111.7, −109.3, +6.38, +7.38 and $+30.4 \times 10^{-1}$ deg cm^2 g^{-1}, respectively, suggesting that the change in the polarity of the solvents induces the changes in the conformations of cyclo(-γ-Abu-L-Pro-)$_2$.

Evidence for the progressive conversion of the conformations of cyclo(-γ-Abu-L-Pro-)$_2$ in CD spectra are shown in Figs. 1 and 2. The spectrum in water showed one positive band near 210 nm and one shoulder near 230 nm. On the other hand, the spectrum in CH_2Cl_2 showed one negative trough near 210 nm and one shoulder near 220 nm. Its features are the mirror image of those in water. The CD spectrum in MeOH was similar to that in water, but the height of the positive band near 210 nm is half that in water. Furthermore, the positive band near 210 nm in water and MeOH was smaller in acetonitrile. Addition of MeOH to the aqueous solution induced the progressive inversion of the conformation in water, revealed by the decrease in ellipticity (Fig. 2, 1–5). With addition of CH_2Cl_2 to a MeOH solution, the trough of the negative band near 200–220 nm becomes deeper (Fig. 2, 6–11). On the other hand, the positive band near 210–230 nm gradually shifted toward longer wavelength and finally changed to a shoulder. These results indicate that the change in polarity of the solvent induces the progressive inversion of the conformation, revealed by the disappearance of the positive peak and then by the gradual appearance of that having opposite chirality. Thus, the variations in CD spectra of cyclo(-γ-Abu-L-Pro-)$_2$ can be correlated to changes in the orientations of two amide bonds around the Pro residues.

Further conformational analyses of cyclo(-γ-Abu-L-Pro-)$_2$ were performed by means of ^{1}H (250 MHz) and ^{13}C (62.9 MHz) NMR spectra in $CDCl_3$, CD_2Cl_2, CD_3OD, $(CD_3)_2SO$ and D_2O, and Fourier transform (FT)-IR spectra in $CHCl_3$ and CH_2Cl_2, and the data are summarized in Table 1. In NMR studies, all protons and carbons were assigned by means of H–H COSY, C–H COSY, HOHAHA and NOESY.† The presence of a small amount of several conformers (<20%) in these solvents was evident in the 1D NMR spectra, but the minor components were not conformationally analyzed in detail. The main conformer of this cyclic peptide has C_2 symmetry in the NMR time-average, because only one amide proton resonance

† COSY = 2D chemical-shift correlation spectroscopy; HOHAHA = 2D homonuclear Hartmann–Hahn spectroscopy; NOESY = nuclear Overhauser effect spectroscopy.

Table 1 NMR parameters[a] and ratios of hydrogen-bonded NH to total NH[b] of cyclo (-γ-Abu-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]
cyclo(-γ-Abu-L-Pro-)$_2$	$CDCl_3$	7.35	3	31.53	22.71	8.82	0.97
	CD_2Cl_2	7.15	3	32.00	23.17	8.83	0.90
	CD_3OH	8.15	6	33.42	23.87	9.55	—
	$(CD_3)_2SO$	8.00	6	31.46	22.15	9.31	—
	D_2O–water (1 : 9 v/v)	8.26	7	34.47	25.06	9.41	—

[a] Data were obtained by ^1H (250 MHz) and ^{13}C (62.9 MHz) NMR spectroscopy on a Bruker AM-250 instrument at 25 °C. Chemical shifts are downfield from internal tetramethylsilane. Peptide concentration ~ 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 IR stretch region. Data were obtained on a JASCO FT/IR-230 for a 1 mM sample in $CHCl_3$ and CH_2Cl_2 at 20 °C, after subtration of the spectrum of pure $CHCl_3$ and CH_2Cl_2, respectively. [e] Values of chemical shifts of $^{\gamma}$NH γ-Abu (ppm). [d] Temperature coefficients of chemical shifts of $^{\gamma}$NH γ-Abu (ppb/°C). [e] Values of chemical shifts for C^{β} and C^{γ} of Pro residue (ppm). [f] Pro C^{β}–C^{γ} chemical-shift differences (ppm).

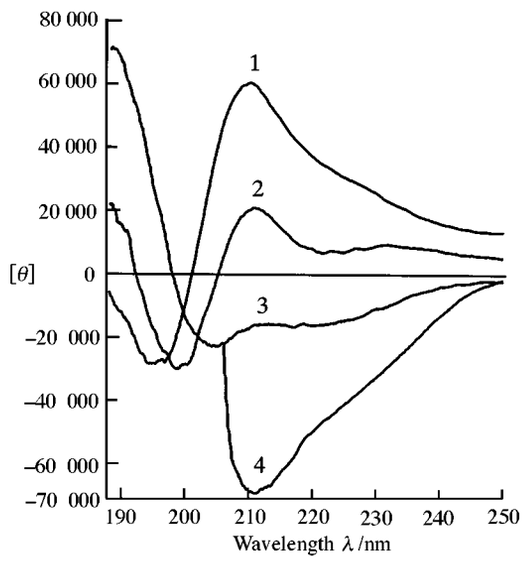

Fig. 1 CD spectra of cyclo(-γ-Abu-L-Pro-)$_2$ in various solvents. Data were obtained with a JASCO spectoropolarimeter (model J-720w) using a 0.1 mm cell. The peptide concentration was 1.5 mM. (1) water; (2) MeOH; (3) CH_3CN; (4) CH_2Cl_2.

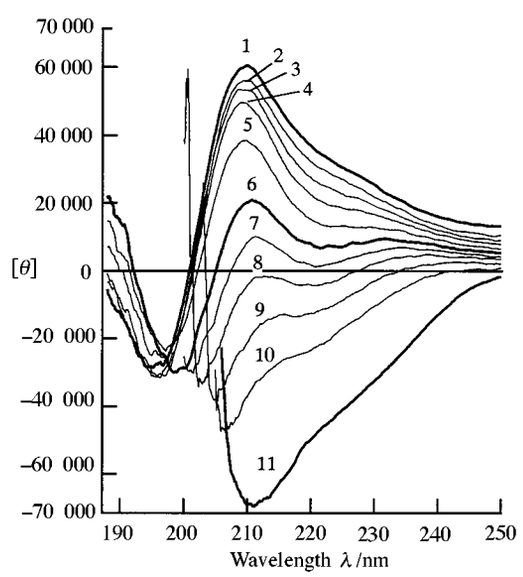

Fig. 2 CD spectra of cyclo (-γ-Abu-L-Pro-)$_2$ at room temp. in water/MeOH and MeOH/CH_2Cl_2 solvent mixture. Water/MeOH: (1) 100/0; (2) 80/20; (3) 60/40; (4) 40/60; (5) 20/80. MeOH/CH_2Cl_2: (6) 100/0; (7) 80/20; (8) 60/40; (9) 40/60; (10) 20/80; (11) 0/100.

appears for the γ-Abu residue. The chemical shifts of the Pro $^{\beta}$C and $^{\gamma}$C, and the Pro $^{\beta}$C–$^{\gamma}$C chemical-shift difference $\Delta\delta\beta$–γ (Table 1) in $CDCl_3$, CD_2Cl_2, CD_3OH, $(CD_3)_2SO$ and D_2O indicate that cyclo(-γ-Abu-L-Pro-)$_2$ has a C_2 symmetric conformation consistent with a *cis–trans–cis–trans* peptide bond backbone (with two *cis* γ-Abu-L-Pro bonds)[4] in these solvents.

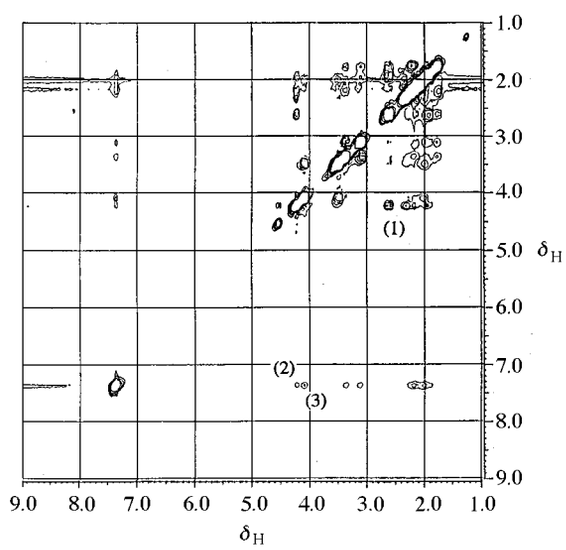

Fig. 3 NOESY spectrum of cyclo(-γ-Abu-L-Pro-)$_2$ in $CDCl_3$ at 25 °C. (1)–(3) indicate the protons giving NOEs by NOESY. (1) γ-Abu $^{\alpha}CH_A$ ⟷ Pro$^{\alpha}$CH; (2) γ-Abu $^{\gamma}$NH ⟷ Pro$^{\alpha}$CH; (3) γ-Abu $^{\gamma}$NH ⟷ Pro$^{\delta}CH_A$

To obtain further information on the rotation about Pro $^{\alpha}$C–C=O single bonds, we measured the NOESY spectrum at 25 °C (Figs. 3 and 4). The strong NOE between γ-Abu $^{\alpha}CH_A$ and Pro $^{\alpha}$CH observed in all solvents is related to the *cis* X-Pro bond. In addition, in $CDCl_3$ and CD_2Cl_2, strong NOE cross-peaks between γ-Abu $^{\gamma}$NH and Pro $^{\delta}CH_A$ and weak NOE cross-peaks between γ-Abu $^{\gamma}$NH and Pro $^{\alpha}$CH were observed (Fig. 3). On the other hand, in CD_3OH, $(CD_3)_2SO$ and D_2O–water (1 : 9 v/v), strong NOE cross-peaks between γ-Abu $^{\gamma}$NH and Pro $^{\alpha}$CH were observed, but NOE cross-peaks between γ-Abu $^{\gamma}$NH and Pro $^{\delta}CH_A$ were not (Fig. 4). The NMR data suggest that cyclo(-γ-Abu-L-Pro-)$_2$ has one preferred rotamer within the Pro *cis* region in $CDCl_3$ and CD_2Cl_2, but in the Pro *trans* region in CD_3OH, $(CD_3)_2SO$ and D_2O. (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 direction of the Pro carbonyl oxygen atom, respectively) (Fig. 5).

Owing to the fact that the fraction of minor conformer is always between 15 and 20% in these solvents (from the 1D NMR spectra data) the contribution of the minor conformer to the CD spectra is nearly constant. It is most likely that the most important change in the CD spectrum on transferring from CH_2Cl_2 to water (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(-γ-Abu-L-Pro-)$_2$ having a *cis–trans–cis–trans* peptide bond backbone (with two *cis* γ-Abu-L-Pro bonds).

The temperature coefficients[1d] of the chemical shifts of the γ-Abu $^{\gamma}$NH resonances in $CDCl_3$, CD_2Cl_2, CD_3OH, $(CD_3)_2SO$

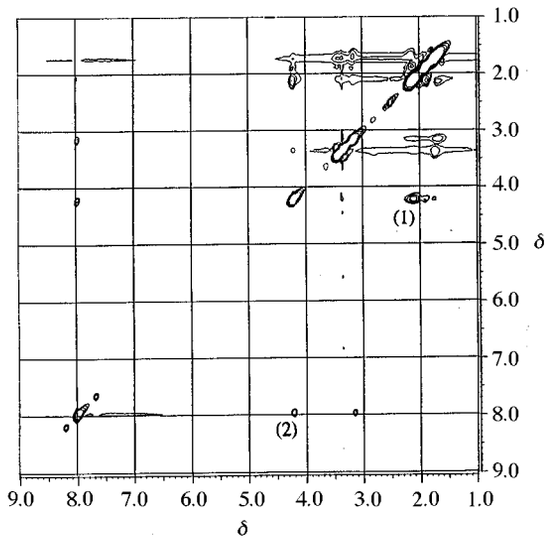

Fig. 4 NOESY spectrum of cyclo(-γ-Abu-L-Pro-)$_2$ in $(CD_3)_2SO$ at 25 °C. (1) and (2) indicate the protons giving NOEs by NOESY. (1) γ-Abu $^{\alpha}CH_A$ ⟷ Pro$^{\alpha}$CH; (2) γ-Abu $^{\gamma}$NH ⟷ Pro$^{\alpha}$CH

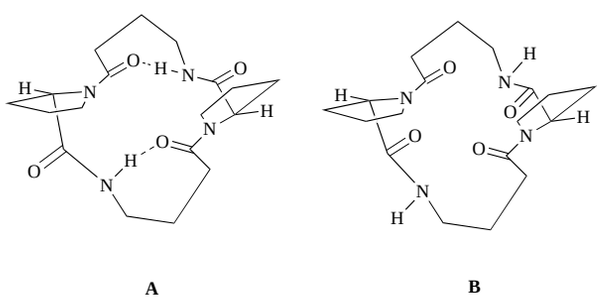

Fig. 5 Schematic representations of conformations proposed for cyclo(-γ-Abu-L-Pro-)$_2$ in solution: (A) the *cis–trans–cis–trans* conformation containing the two *cis* Pro $^{\alpha}$C–C=O bonds and two intramolecular hydrogen bonds between $^{\gamma}$NH and $^{\alpha}$C=O in γ-Abu residue. (B) the *cis–trans–cis–trans* conformation containing the two *trans* Pro $^{\alpha}$C–C=O bonds.

and D_2O–water (1 : 9 v/v), and an analysis of the amide N–H stretch region in IR spectra[5] of this cyclic peptide in $CHCl_3$ and CH_2Cl_2 (Fig. 6 and Table 1) indicate that the amide protons of γ-Abu residues in $CDCl_3$ and CD_2Cl_2 are shielded from the solvents and are involved in a rigid intramolecular hydrogen bond, while those in CD_3OH, $(CD_3)_2SO$ and D_2O are exposed to the solvents. From an inspection of Corey–Pauling–Koltun molecular models, it should be possible for cyclo(-γ-Abu-L-Pro-)$_2$ in $CDCl_3$ and CD_2Cl_2 to adopt a conformation with seven-membered rings stabilised by a hydrogen bond between $^{\gamma}$NH and $^{\alpha}$C=O in the γ-Abu residues (Fig. 5A). These results indicate that the presence of intramolecular hydrogen bonds in cyclo(-γ-Abu-L-Pro-)$_2$ contribute to the stabilization of the *cis–trans–cis–trans* conformation containing the two *cis* Pro $^{\alpha}$C–C=O bonds in $CDCl_3$ and CD_2Cl_2.

The present data demonstrate the unique conformational interconversion of a cyclic tetrapeptide containing two Pro residues, which may also be of interest as a model for conformational investigation of Pro residues in peptides.

Experimental

The mp of cyclo(-γ-Abu-Pro-)$_2$ was measured on an Ishii mp apparatus and is uncorrected. Amino acid analysis of each hydrolysate of the peptides was carried out with an Hitachi 835 amino acid analyzer. Relative molecular masses of the cyclic products were determined by using FAB mass spectrometry on a JEOL JMS-D-300 mass spectrometer (at the Asahi Chemical Industry Company).

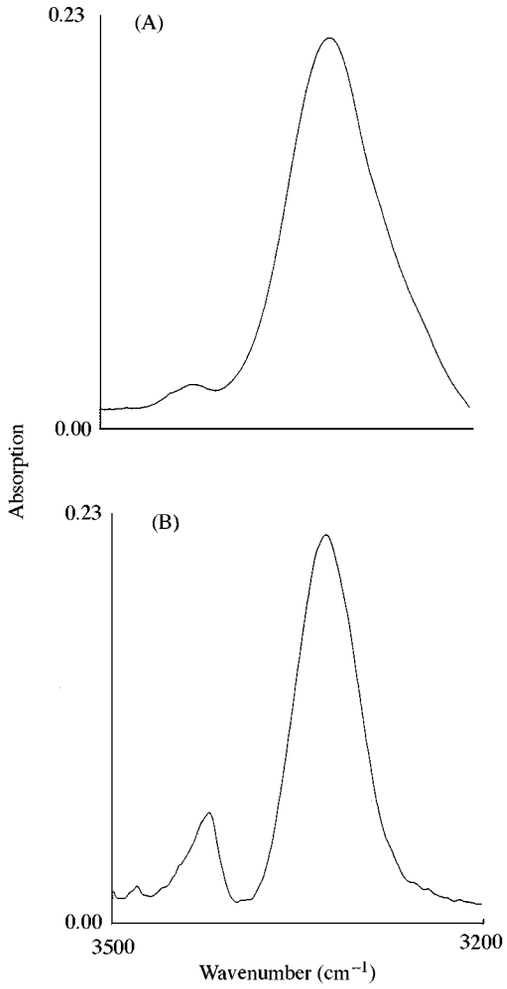

Fig. 6 FT-IR spectra of N–H stretch region of cyclo(-γ-Abu-L-Pro-)$_2$ for a 1 mM sample in $CHCl_3$ (A) and CH_2Cl_2 (B) at 20 °C, after subtraction of the spectrum of pure $CHCl_3$ and CH_2Cl_2, respectively

Syntheses of cyclo(-γ-Abu-Pro-)$_2$

Boc-γ-Abu-Pro-γ-Abu-Pro-OBzl was prepared by stepwise elongation using 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (WSCD·HCl) and 1-hydroxybenzotriazole (HOBt). The tetrapeptide was obtained in 60% yield from H-Pro-OBzl as a oily product. Boc-γ-Abu-Pro-γ-Abu-Pro-OBzl was converted into the corresponding acid by saponification, and this was then converted into the corresponding succinimide esters using HONSu and WSCD·HCl. Boc-pentapeptide-ONSus were treated with trifluoroacetic acid (TFA) 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 to pyridine at 25 °C (concentration of the active esters was 3 mM). After the mixture had been stirred for 1 day at 25 °C, the solvent was evaporated off. 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–diethyl ether–hexane. *Cyclo*(-γ-*Abu*-L-*Pro*-)$_2$ was obtained in 56% yield. Analytical data for cyclo(-γ-Abu-L-Pro-)$_2$ are as follows: mp 315–316 °C (decomp.) (Found: C, 59.40; H, 7.70; N, 15.09. $C_{18}H_{28}N_4O_4$ requires C, 59.32; H, 7.74; N, 15.37%); *m/z* 365 ($M + H^+$, 75%).

The results of amino acid analysis of cyclo(-γ-Abu-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 temp. CD spectra of

cyclo(-γ-Abu-L-Pro-)$_2$ were measured in water, 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 spectrophotometer at 20 °C, after subtraction of the spectrum of pure $CHCl_3$ and CH_2Cl_2, respectively. IR spectra of cyclo(-γ-Abu-L-Pro-)$_2$ were measured in $CHCl_3$ and CH_2Cl_2 solutions at a concentration of 1 mM.

NMR Spectroscopy

NMR [^{1}H (250 MHz) and ^{13}C (62.9 MHz)] spectra were measured in $CDCl_3$, CD_2Cl_2, CD_3OH, $(CD_3)_2SO$ and D_2O at 25 °C (peptide concentration ~17mg ml^{-1}) on a Bruker AC-250 spectrometer using standard pulse sequences and software. H–H COSY 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 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 1K points in F2 and 256 points in F1. C–H COSY spectra with 4K points in F2 and 256 points in F1 were recorded. Temperature coefficients of the chemical shifts of amide protons were obtained from least-square fits to data recorded at 20, 25, 30, 35, 40, 45 and 50 °C.

^{1}H NMR ($CDCl_3$). δ 1.79 (1 H, m, γ-Abu $^{\beta}CH_A$), 1.95 (1 H, m, γ-Abu $^{\alpha}CH_A$), 1.98 (1 H, m, Pro $^{\gamma}CH_A$), 2.09 (1 H, m, Pro $^{\gamma}CH_B$), 2.19 (1 H, m, Pro $^{\beta}CH_A$), 2.20 (1 H, m, γ-Abu $^{\beta}CH_B$), 2.32 (1 H, m, Pro $^{\beta}CH_B$), 2.64 (1 H, m, γ-Abu $^{\alpha}CH_B$), 3.09 (1 H, m, γ-Abu $^{\gamma}CH_A$), 3.36 (1 H, m, γ-Abu $^{\gamma}CH_B$), 3.50 (1 H, m, Pro $^{\delta}CH_A$), 4.10 (1 H, m, Pro $^{\delta}CH_B$), 4.20 (1 H, dd, Pro $^{\alpha}CH$) and 7.35 (1 H, br s, γ-Abu $^{\gamma}NH$).

^{1}H NMR (CD_2Cl_2). δ 1.70 (1 H, m, γ-Abu $^{\beta}CH_A$), 1.90 (1 H, m, γ-Abu $^{\alpha}CH_A$), 1.97 (2 H, m, Pro $^{\gamma}CH_2$), 2.10 (1 H, m, Pro $^{\beta}CH_A$), 2.12 (1 H, m, γ-Abu $^{\beta}CH_B$), 2.30 (1 H, m, Pro $^{\beta}CH_B$), 2.57 (1 H, m, γ-Abu $^{\alpha}CH_B$), 3.08 (1 H, m, γ-Abu $^{\gamma}CH_A$), 3.23 (1 H, m, γ-Abu $^{\gamma}CH_B$), 3.46 (1 H, m, Pro $^{\delta}CH_A$), 4.02 (1 H, m, Pro $^{\delta}CH_B$), 4.15 (1 H, dd, Pro $^{\alpha}CH$) and 7.15 (1 H, br s, γ-Abu $^{\gamma}NH$).

^{1}H NMR (CD_3OH). δ 1.85 (2 H, m, γ-Abu $^{\beta}CH_2$), 1.88 (2 H, m, Pro $^{\gamma}CH_2$), 2.08 (1 H, m, Pro $^{\beta}CH_A$), 2.18 (1 H, m, γ-Abu $^{\alpha}CH_A$), 2.27 (1 H, m, Pro $^{\beta}CH_B$), 2.28 (1 H, m, γ-Abu $^{\alpha}CH_B$), 3.35 (1 H, m, γ-Abu $^{\gamma}CH_A$), 3.42 (1 H, m, γ-Abu $^{\gamma}CH_B$), 3.50 (1 H, m, Pro $^{\delta}CH_A$), 3.62 (1 H, m, Pro $^{\delta}CH_B$), 4.33 (1 H, dd, Pro $^{\alpha}CH$) and 8.15 (1 H, br s, γ-Abu $^{\gamma}NH$).

^{1}H NMR [$(CD_3)_2SO$]. δ 1.65 (1 H, m, γ-Abu $^{\beta}CH_A$), 1.69 (2 H, m, Pro $^{\gamma}CH_2$), 1.77 (1 H, m, γ-Abu $^{\beta}CH_B$), 1.91 (1 H, m, Pro $^{\beta}CH_A$), 2.07 (1 H, m, Pro $^{\beta}CH_B$), 2.11 (2 H, m, γ-Abu $^{\alpha}CH_2$), 3.14 (1 H, m, γ-Abu $^{\gamma}CH_A$), 3.17 (1 H, m, γ-Abu $^{\gamma}CH_B$), 3.41 (2 H, m, Pro $^{\delta}CH_2$), 4.22 (1 H, dd, Pro $^{\alpha}CH$) and 8.00 (1 H, t, γ-Abu $^{\gamma}NH$).

^{1}H NMR (D_2O–water 1:9 v/v). δ 1.77 (1 H, m, γ-Abu $^{\beta}CH_A$), 1.85 (1 H, m, γ-Abu $^{\beta}CH_B$), 1.92 (1 H, m, Pro $^{\gamma}CH_A$), 1.99 (1 H, m, Pro $^{\gamma}CH_B$), 2.08 (1 H, m, Pro $^{\beta}CH_A$), 2.10 (1 H, m, γ-Abu $^{\alpha}CH_A$), 2.30 (1 H, m, γ-Abu $^{\alpha}CH_B$), 2.35 (1 H, m, pro $^{\beta}CH_B$), 3.38 (2 H, m, γ-Abu $^{\gamma}CH_2$), 3.48 (1 H, m, Pro $^{\delta}CH_A$), 3.55 (1 H, m, Pro $^{\delta}CH_B$), 4.47 (1 H, dd, Pro $^{\alpha}CH$) and 8.26 (1 H, br s, γ-Abu $^{\gamma}NH$).

^{13}C NMR ($CDCl_3$). δ_C 20.05 (γ-Abu $^{\beta}C$), 22.71 (Pro $^{\gamma}C$), 31.53 (Pro $^{\beta}C$), 32.73 (γ-Abu $^{\gamma}C$), 41.14 (γ-Abu $^{\alpha}C$), 47.14 (Pro $^{\delta}C$), 62.00 (Pro $^{\alpha}C$), 172.95 and 173.13 (γ-Abu and Pro C=O).

^{13}C NMR (CD_2Cl_2). δ_C 20.08 (γ-Abu $^{\beta}C$), 23.17 (Pro $^{\gamma}C$), 32.00 (Pro $^{\beta}C$), 33.06 (γ-Abu $^{\gamma}C$), 41.35 (γ-Abu $^{\alpha}C$), 47.48 (Pro $^{\delta}C$), 62.49 (Pro $^{\alpha}C$), 173.19 and 173.45 (γ-Abu and Pro C=O).

^{13}C NMR (CD_3OH). δ_C 23.87 (Pro $^{\gamma}C$), 25.59 (γ-Abu $^{\beta}C$), 32.86 (γ-Abu $^{\alpha}C$), 33.42 (Pro $^{\beta}C$), 40.13 (γ-Abu $^{\gamma}C$), 48.28 (Pro $^{\delta}C$), 62.62 (Pro $^{\alpha}C$), 174.22 and 175.21 (γ-Abu and Pro C=O).

^{13}C NMR [$(CD_3)_2SO$]. δ_C 22.15 (Pro $^{\gamma}C$), 23.32 (γ-Abu $^{\beta}C$), 30.59 (γ-Abu $^{\alpha}C$), 31.46 (Pro $^{\beta}C$), 37.96 (γ-Abu $^{\gamma}C$), 46.19 (Pro $^{\delta}C$), 59.97 (Pro $^{\alpha}C$), 170.68 and 171.71 (γ-Abu and Pro C=O).

^{13}C NMR (D_2O–water 1:9 v/v). δ_C 25.06 (Pro $^{\gamma}C$), 26.95 (γ-Abu $^{\beta}C$), 34.47 (Pro $^{\beta}C$), 34.57 (γ-Abu $^{\alpha}C$), 41.66 (γ-Abu $^{\gamma}C$), 50.04 (Pro $^{\delta}C$), 64.07 (Pro $^{\alpha}C$), 177.27 and 177.43 (γ-Abu and Pro C=O).

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References

1 (*a*) D. E. Dorman and F. A. Bovey, *J. Org. Chem.*, 1973, **38**, 2379; (*b*) V. Madison, M. Atreyi, C. M. Deber and E. R. Blout, *J. Am. Chem. Soc.*, 1974, **96**, 6725; (*c*) H. Kessler, *Angew. Chem., Int. Ed. Engl.*, 1982, **21**, 512; (*d*) D. E. Stewart, A. Sarkar and J. E. Wampler, *J. Mol. Biol.*, 1990, **214**, 253; (*e*) C. Francart, J.-M. Wieruszeski, A. Tartan and G. Lippens, *J. Am. Chem. Soc.*, 1996, **118**, 7019.

2 A. E. Tonelli, *J. Am. Chem. Soc.*, 1973, **95**, 5946; C. M. Deber, E. C. Fossel and E. R. Blout, *J. Am. Chem. Soc.*, 1974, **96**, 4015; V Madison and K. D. Kopple, *J. Am. Chem. Soc.*, 1980, **102**, 4855.

3 The abbreviations for amino acids and peptides are in accordance with the rules of the IUPAC-IUB Commission on Biological Nomenclature. *Eur. J. Biochem.*, 1984, **138**, 9.

4 A. F. Spatola, L. M. Gierasch and A. L. Rockwell, *Biopolymers*, 1983, **22**, 147; I. Z. Siemion, T. Wieland and K.-H. Pook, *Angew. Chem., Int. Ed. Engl.*, 1975, **14**, 702; C. M. Deber, V. Madison and E. R. Blout, *Acc. Chem. Res.*, 1976, **9**, 106.

5 R. R. Gardner, G. Liang and S. H. Gellman, *J. Am. Chem. Soc.*, 1995, **117**, 3280.

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