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CONFORMATIONS OF CYCLO(L- OR D-Phe-L-Pro-Aca) AND CYCLO(L-Pro-L- OR D-Phe-Aca) CYCLIZED DIPEPTIDE MODELS FOR SPECIFIC TYPES OF β-BENDS

Hiroshige MIZUNO [a], Sannamu LEE [b], Hideaki NAKAMURA [c], Yasushi KODERA [d], Tetsuo KATO [b], Nobuhiro GŌ [a] and Nobuo IZUMIYA [b]

[a] *Department of Physics and* [b] *Laboratory of Biochemistry, Faculty of Science, Kyushu University, Fukuoka 812,* [c] *Department of Physics, Fukuoka Institute of Technology, Fukuoka 811-02 and* [d] *Department of Chemistry, Fukuoka University, Fukuoka 814-01, Japan*

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Conformational analyses on four cyclic model peptides of the β-bend, cyclo(L- or D-Phe-L-Pro-ε-aminocaproyl(Aca)) and cyclo(L-Pro-L- or D-Phe-Aca), were carried out both experimentally and theoretically. Cyclo(D-Phe-L-Pro-Aca) was shown to exist as a single conformer taking the type II′ β-bend. The comparison of its CD spectra with those of cyclo(L-Ala-L-Ala-Aca) revealed that type I and II′ β-bends, both with α-helix-like CD spectra, can be distinguished. Cyclo(L-Phe-L-Pro-Aca) was shown to exist as a single conformer with a *cis* L-Phe–L-Pro peptide bond, taking the type VI β-bend. Its CD spectrum has thus been observed for the first time for the bend containing a *cis* peptide bond. Cyclo(L-Pro-L-Phe-Aca) was shown to exist as a mixture of two conformers, the major one taking the type I β-bend with a *trans* Aca–L-Pro peptide bond and the minor one with a *cis* Aca–L-Pro peptide bond. Cyclo(L-Pro-D-Phe-Aca) was suggested to exist as a mixture of two conformers, the major one taking the type II β-bend with a *trans* Aca–L-Pro peptide bond and the minor one with a *cis* Aca–L-Pro peptide bond.

1. Introduction

The β-bends are one of the significant conformational features in peptides and proteins [1,2]. They are further classified into several types according to their conformational detail [2,3]. Those in oligopeptides in solution exist usually in a mixture of β-bends of different types. In many proteins of known structure, about 17–24% of residues are observed to be involved in β-bends [1]. Theoretical analyses have suggested that this local conformation plays an important role in folding into a compact globular structure, together with other local conformations such as α-helices or β-sheets [3,4]. The omnipresence of β-bends and their role have made characterization of this local conformation the subject of intensive investigation. Theoretical studies have elucidated various properties of β-bends; occurrence of various amino acids in them, and their conformational and energetic properties [5–15]. However, interpretation of various experimental results in terms of specific bend types is still difficult, because experimental parameters have not yet been attributed well to unique bend types [16–21].

In this paper, we synthesized four model peptides of β-bends, cyclo(L- or D-Phe-L-Pro-ε-aminocaproyl(Aca)) and their retro isomers, and carried out conformational analysis in an attempt to obtain experimental parameters on β-bends specific for these peptides. These peptides were selected because of the high frequency of occurrence of Pro in β-bends in proteins and in naturally occurring peptides. The dipeptide sequence,

D-Phe-L-Pro, which is one of the four model peptides to be studied here, is known to occur in gramicidin S as taking the type II′ β-bend. The short alkyl chain, Aca, is used to constrain the dipeptides to take β-bends. The chain length is 5.04 Å when fully extended [22]. This is still less than the limiting distance used for the definition of a bend by Lewis et al. [3]. Because Aca contains no functional groups and is optically inactive, most observed properties of the four peptides can be attributed to the terminally blocked dipeptide portions.

We carried out NMR and CD measurements on these molecules. However, spectroscopic methods alone cannot give sufficient information to elucidate detailed molecular conformations of the peptides in solution. The method of conformational energy calculation is powerful when used in conjunction with experimental methods. Therefore, we also carried out conformational energy calculation of these peptides. Stable conformations were obtained by calculating the conformational free energy. In this calculation, the conformational entropy was obtained by calculating the second derivative matrix numerically. The ring structure is exactly closed throughout the calculation. The possibility of energetically competitive *cis* and *trans* isomers of peptide bonds just before Pro was carefully examined. By comparison of the results from both experimental and theoretical analyses, we arrived at a clear interpretation of experimental results and obtained some useful optical characteristics for β-bends.

2. Method of free energy calculation

A stable conformation means low free energy. Therefore, we evaluated the conformational free energy, $G = H - TS$, by first minimizing the conformational energy, H, and then calculating the conformational entropy, S, at the minimum energy conformation.

2.1. Parameters used

The recommended nomenclature and conventions [23] are used for dipeptide conformations. The designation of dihedral angles in the Aca residue is shown in fig. 1. All dihedral angles except ϕ_{Pro}, which is fixed in the new version of the ECEPP computer program (ECEPP/2) [24], are treated as variable angles. The geometrical and energy parameters for Pro and Phe are those adopted in this program. The down puckering conformation of the pyrrolidine ring is assumed for Pro. The energy parameters for Ala′, which is used in the first step of the energy minimization (see below), are the same as in ECEPP/2, but the geometry is different. The geometry of Ala′ has the backbone of Phe and the side chain of a methyl radical. Both parameters for the Aca residue are the same as those used by Némethy et al. [22]. No solvent was included in the calculation.

2.2. Ring closure

In a cyclic structure with fixed bond lengths and bond angles, containing n bonds around which rotation is possible, $n - 6$ of the n dihedral angles are independent [25]. Dihedral angles (ω_0, ϕ_1, ψ_1, ω_1, ϕ_2, ψ_2, ω_2) in the dipeptide moiety are treated as independent variables. For a given set of independent dihedral angles, the ring closure program [25] finds one or more sets of values for the

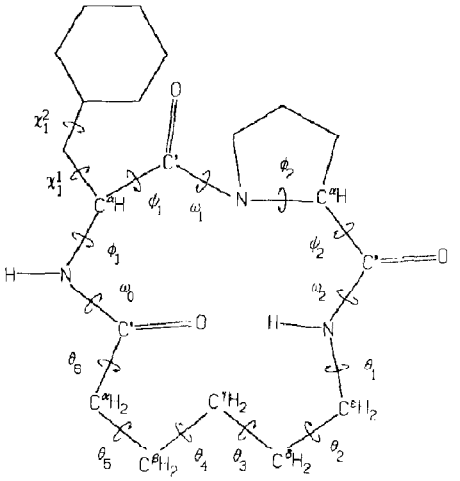

Fig. 1. Structural formula of cyclo(Phe-Pro-Aca), with the definition of the dihedral angles used to describe the conformation of the molecule.

remaining six dependent dihedral angles, θ_1–θ_6, in the Aca residue (fig. 1). When there are m sets, the conformational space of the cyclic molecule consists of m 'sheets' of $(n-6)$ dimensional space. Corresponding points on different sheets correspond to different conformations of the cyclic molecule. The difference is confined to the Aca chain. The conformations of the dipeptide part are the same. These conformations differing only in the Aca chain and therefore corresponding to different solutions from the ring closure program for the same set of independent variables are now defined to be conjugate to each other. Energy minimization has been carried out carefully to locate local minima on each separate sheet.

2.3. Energy minimization

Precise energy minimization becomes increasingly difficult when the degrees of freedom in a molecule become large. In order to keep the degrees of freedom minimum, we take advantage of the fact that low-energy backbone conformations of Phe and Ala residues are very similar [26], i.e., we replace a Phe residue by Ala residue in the first step (step A) of the minimization for each of the four cyclic molecules. However, the backbone geometry of the two residues, Phe and Ala, in the ECEPP treatment are slightly different. Therefore, we replace a Phe residue by an Ala′ residue, which has the backbone geometry of the Phe residue and the side chain geometry of the Ala residue. In the second step (step B) energy minimization is carried out by restoring the Ala′ residue to the Phe residue.

2.3.1. Step A

There are seven degrees of freedom in each of the four cyclic molecules in which the Phe residue is replaced by the Ala′ residue, namely, ψ_{Pro}, $\phi_{Ala'}$, $\psi_{Ala'}$, ω_0, ω_1, ω_2 and $\chi_{Ala'}$. Among them deviations of the ω angles from either 0° (*cis*) or 180° (*trans*) and deviation of $\chi_{Ala'}$ from 60° are expected to be small. For the purpose of making an initial rough search for the low-energy regions, we fix them at either 0° or 180° and 60°, respectively. Because the *cis-trans* choice is made for the three ω angles independently, there are eight combinations. For each combination the ring closure program is run at all lattice points in the three-dimensional 20° lattice space of ψ_{Pro}, $\phi_{Ala'}$ and $\psi_{Ala'}$. If a ring is closed, the conformational energy is calculated. The region within 10 kcal/mol of the global minimum for each of the four molecules is retained for further calculation. The global minimum energy for each combination is given in table 1. Then, the conformational energy is calculated in a finer 5° lattice space in regions containing the above low-energy regions. An energy contour map of ψ_{Pro} cross-sections is drawn for each of these low-energy regions (fig. 2). The two maps in this figure are localized on different sheets. Energy minimizations are then carried out in each sheet by varying all the seven independent variables by using the algorithm of Powell [27,28] and by starting from low-energy points on the contour maps. A convergence criterion of 0.001 kcal/mol is used, i.e., minimization is stopped when the energy decrease per step becomes less than 0.001 kcal/mol.

2.3.2. Step B

A methyl radical in the Ala′ residue is now replaced by a side chain of the Phe residue at the minimum energy conformations obtained above. Accordingly, we have now eight independent vari-

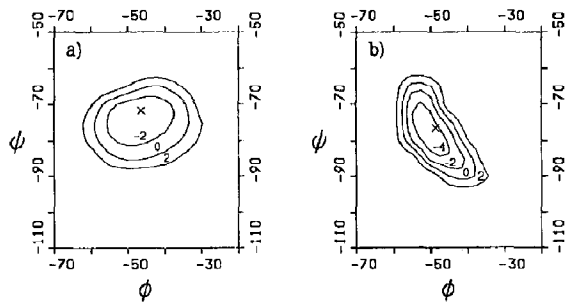

Fig. 2. Conformational energy contour maps of ψ_{Pro} cross-sections of low-energy regions in the three-dimensional 5° lattice space of (ψ_{Pro}, $\phi_{Ala'}$, $\psi_{Ala'}$) for cyclo(D-Phe-L-Pro-Aca). (a,b) Two maps are localized on different sheets. (×) Minimum energy points on each sheet from which subsequent energy minimizations of step A are started. The values on the contour lines denote the energy in kcal/mol above the minimum energy point; (a) $\psi_{Pro} = -20°$; (b) $\psi_{Pro} = -25°$.

Table 1

Lowest energies (kcal/mol) in 20° lattice space of (ψ_{Pro}, $\phi_{Ala'}$, $\psi_{Ala'}$)

Letters *t* and *c* designate that ω is assumed to be 180° (*trans*) and 0° (*cis*), respectively. (*) Larger than 10^2, (**) larger than 10^3.

Molecule	Three peptide configurations (ω_0, ω_1, ω_2)							
	ttt	*ttc*	*tct*	*tcc*	*ctt*	*cct*	*ctc*	*ccc*
Cyclo(D-Ala'-L-Pro-Aca)	−3.1	*	*	39.4	11.6	31.0	**	80.0
Cyclo(L-Ala'-L-Pro-Aca)	*	*	0.8	*	41.0	19.7	**	**
Cyclo(L-Pro-L-Ala'-Aca)	−7.3	16.5	**	**	−9.6	25.3	15.4	**
Cyclo(L-Pro-D-Ala'-Aca)	−6.7	18.2	**	**	−5.1	**	11.5	**

ables. To find low-energy conformations of the Phe side chain the energy contour maps are drawn by calculating energies at all 5° lattice points in the (χ_1, χ_2) space for the fixed values of the other independent variables (fig. 3). Usually two or three minimum energy points are located on such maps. Starting from these points, the energies of the molecule are further minimized in the full space of eight independent dihedral angles. In this step a convergence criterion of 0.00001 kcal/mol is used.

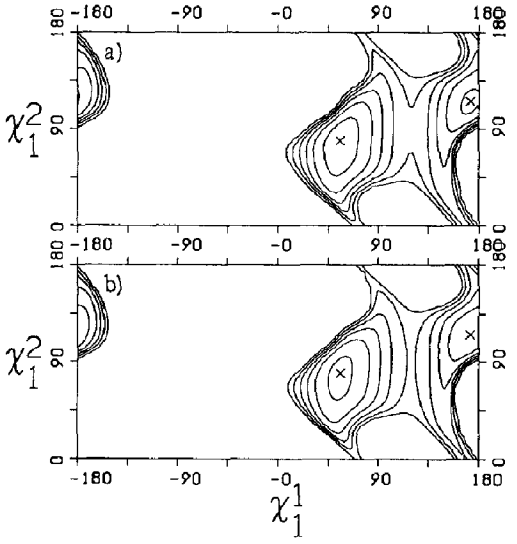

Fig. 3. Energy contour maps of cyclo(D-Phe-L-Pro-Aca) drawn by calculating energies at all 5° lattice points in the (χ_1^1, χ_1^2) space for the fixed values of the other independent variables. Maps a and b are those drawn for minimum energy conformations reached by energy minimizations of step A commencing from points shown by × in maps a and b, respectively, in fig. 2. Energy contour lines in the region of negative χ_1^2 are periodically the same as those shown in this figure. Local energy minimum is designated by ×.

In the resulting minimum energy conformations the values of the ω angles are often found to deviate by as much as 20°. This finding raises a question as to the validity of an implicit assumption made in the above steps of calculation, i.e., the assumption that low-energy regions can be roughly located by fixing ω at 0 or 180°. In order to check this point and possibly to find low-energy conformations missed in the above steps, additional energy minimizations are carried out by starting from all conformations conjugate to the minimum energy conformations obtained above.

2.4. Calculation of entropy

If the potential energy surface can be approximated for a linear molecule with fixed bond lengths and fixed bond angles by a multidimensional parabola around a minimized conformation, the second derivative matrix with respect to independent variables, *F*, at the minimized conformation determines the conformational entropy [29,30].

$$-TS = \frac{RT}{2}\ln[\det F]. \quad (1)$$

For a cyclic molecule, the conformational entropy term consists of a contribution not only from independent variables but also from dependent ones as given by [31]:

$$-TS = \frac{RT}{2}\ln[\det F] + RT \ln|J|. \quad (2)$$

The former term on the right-hand side of eq. 2 is the same as that in eq. 1, i.e., the second derivative matrix with respect to independent variables only. The latter term is determined from the conformation of the chain portion involving dependent variables. We calculated F numerically and J analytically.

3. Experimental procedure

3.1. Synthesis

Cyclic tripeptides are synthesized by the scheme shown in fig. 4. This procedure has been described in detail [20]. Thin-layer chromatography is employed to monitor workups and product purity.

Boc-tripeptide acids obtained by stepwise elongation from C-terminal aminocaproic acid methyl ester are converted to Boc-tripeptide *N*-hydroxysuccinimide esters. After the cleavage of Boc groups with trifluoroacetic acid, the tripeptide active esters are cyclized in pyridine (C, 3×10^{-3} M) overnight at room temperature. The crude cyclic peptides are purified using Sephadex LH-20 column chromatography. They are recrystallized from hot methanol or methanol water. The cyclic peptides are characterized by elemental analysis, 200 MHz proton and 50 MHz carbon NMR and EI mass spectroscopy. In the mass spectra the M^+ ion at 357 is prominent in all peptides. Their chemical and physical properties are summarized in table 2.

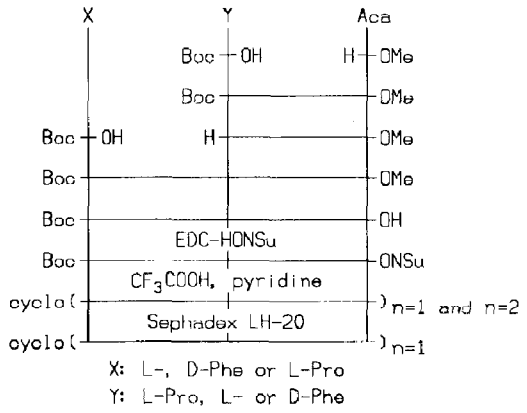

Fig. 4. Synthesis of the four cyclic molecules investigated.

3.2. NMR and CD measurements

NMR spectral data are obtained in DMSO-d_6 on a Jeol FX-200 spectrometer operating at 200 MHz for proton and 50 MHz for carbon. Two Pro δ-proton peaks are observed separately. But, they could not be identified uniquely as $H^{\delta 1}$ and $H^{\delta 2}$ defined by the conventions [23]. Therefore, these two peaks are designated as $H^{\delta A}$ and $H^{\delta B}$. Similar treatments are also done for Phe β-proton and Aca α- and ϵ-protons. CD spectra are taken on a Jasco J-40 spectropolarimeter in MeOH. Cell lengths are 0.1 cm.

4. Results

Computed minimum conformations with a relative free energy within 3 kcal/mol of the global

Table 2

Yields and physical constants of cyclic molecules

Satisfactory mass and TLC data were obtained for all the molecules. (f) Found; (c) calculated.

Molecule	Yield (%)	Melting point (°C)		Analysis (wt%) C	H	N
Cyclo(D-Phe-L-Pro-Aca)	32	228–229	(f)	66.71	7.59	11.77
			(c)	67.20	7.61	11.76
Cyclo(L-Phe-L-Pro-Aca)	5	210–212	(f)	66.86	7.57	11.34
Cyclo(L-Pro-L-Phe-Aca)	4	205–206	(f)	67.08	7.51	11.45
Cyclo(L-Pro-D-Phe-Aca)	35	280–281	(f)	67.13	7.69	11.69

minimum of each molecule are listed in table 3. Conformations with higher energy are not expected to contribute significantly to the equilibrium population. Classification of β-bend types in table 3 is done according to Lewis et al. [3]. Stereo views of representative conformations are shown in figs. 5 and 6. ^{1}H-NMR spectra are shown in fig. 7. Chemical shifts, coupling constants and temperature coefficients of Phe and Aca H^N groups and Pro H^α, $H^{\delta A}$ and $H^{\delta B}$ are summarized in table 4. CD spectra of the four molecules and gramicidin S are shown in fig. 8. Populations of three rotamers shown in fig. 9 of Phe side chains for cyclo(L- or D-Phe-L-Pro-Aca) are indicated in table 5.

4.1. *Cyclo(D-Phe-L-Pro-Aca)*

The ^{1}H-NMR spectrum indicates that this molecule exists as a (probably time-averaged) single conformer (fig. 7a). The difference ($\Delta^{\beta,\gamma}$ = 4.23 ppm) of ^{13}C-NMR chemical shifts of Pro C^β and C^γ indicates that this conformer has a *trans* D-Phe–L-Pro peptide bond. As indicated in table 4, the Aca H^N occurs at higher field (6.75 ppm) than the usual peptide H^N and has a low $\Delta\delta/\Delta T$ value (-1.4×10^{-3} ppm/deg). These results indicate a solvent-shielded or intramolecularly hydrogen-bonded NH group. The $\Delta\delta/\Delta T$ of Phe H^N (-4.7×10^{-3} ppm/deg) indicates a solvent-exposed proton. Possible values of ϕ_{Phe} deduced from the vicinal coupling constant ($J_{HN\alpha}$ = 7.2 Hz) by Ramachandran's equation [32] are 86 or 154° (table 4). The CD spectrum is characterized by double negative extrema in the 200–220 nm region as shown in fig. 8a. The experimental results described so far indicate that the backbone of the dipeptide portion of this molecule exists in a (probably time-averaged) single conformation with a *trans* D-Phe–L-Pro peptide bond, a possibly hydrogen-bonded Aca H^N and a solvent-exposed Phe H^N.

As for side chain configurations Pro $H^{\delta B}$ occurs at higher field (3.00 ppm) than usual. This suggests that this proton is located above the aromatic ring of the Phe residue. The population of the three rotamers of the Phe residue is estimated as $g_+ : t : g_-$ = 52 : 47 : 1 by Pachler's method [33] from the coupling constants (table 4) between α-

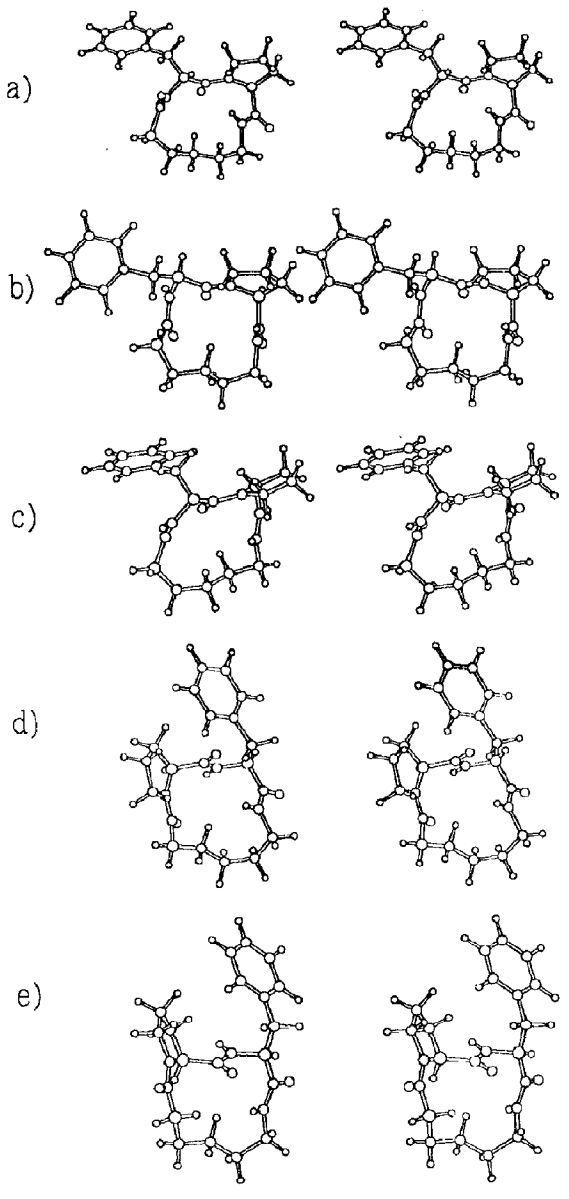

Fig. 5. Ball-and-stick representations of representative computed minimum energy conformations. (a,b) Conformations 1 and 5 of cyclo(D-Phe-L-Pro-Aca), which are the lowest energy conformations among those with bend types II′ and III, respectively; (c) cyclo(L-Phe-L-Pro-Aca) (conformation 1); (d,e) conformations 1 and 6 of cyclo(L-Pro-L-Phe-Aca) which are conformations with the lowest energy among those taking a *trans* and *cis* Aca–L-Pro peptide bond, respectively.

Table 3.1

Computed minimum energy conformations

Cyclo(D-Phe-L-Pro-Aca)

		Conformations											
		1 [e]	2	3 [e]	4	5	6	7	8 [e]	9 [e]	10 [e]	11 [e]	12 [e]
Free energy [a] (ΔG [b])		0.00	0.24	0.30	0.52	0.78	0.99	1.71	2.09	2.14	2.30	2.55	2.73
Enthalpy [a] (ΔH)		0.00	0.04	−0.22	−0.63	−0.97	−0.56	−0.20	−0.08	1.24	0.17	1.39	0.61
Entropy [a] ($-T\Delta S$)		0.00	0.20	0.51	1.15	1.74	1.56	1.91	2.16	0.90	2.13	1.16	2.11
Probability [c]		0.30	0.20	0.18	0.13	0.08	0.06	0.02	0.01	0.01	0.01	> 0.01	> 0.01
Bend type		II′	II′	II′	II′	III	III	III	III	II′	III	II′	III
Dihedral angles [d]	ω_0	−156	−163	−159	−166	−178	173	−179	−178	−150	172	−153	−179
	ϕ_1	126	100	123	91	−51	−49	−51	−50	126	−48	130	−49
	ψ_1	−132	−132	−126	−127	−70	−70	−72	−72	−137	−70	−136	−73
	ω_1	166	167	169	170	174	174	175	168	165	175	164	167
	χ_1^1	61	60	177	176	55	54	172	55	−61	173	171	−60
	χ_1^2	80	76	94	93	75	75	114	76	88	114	87	114
	ψ_2	−17	−12	−22	−14	−63	−59	−62	−62	−30	−58	−14	−65
	ω_2	174	177	174	179	174	−173	174	169	169	−174	173	169
	θ_1	−162	−163	−161	−166	−88	−150	−88	−158	−88	−151	−162	−155
	θ_2	67	75	66	75	−67	65	−67	56	−68	65	67	56
	θ_3	−165	−166	−163	−162	167	−112	167	78	160	−111	−166	78
	θ_4	146	120	149	120	−119	163	−120	−157	−170	163	147	−160
	θ_5	−68	−57	−68	−56	63	−74	63	69	55	−75	−69	70
	θ_6	103	128	103	133	−143	−92	−142	−118	54	−93	100	−117

[a] In kcal/mol.

[b] $\Delta G = G - G_0 = (H - TS) - (H_0 - TS_0)$, where H_0, $-TS_0$ and G_0, the absolute ECEPP values of conformation 1, are −12.24, 9.80 and −2.44 kcal/mol, respectively.

[c] Normalized among listed conformations with free energies less than 3.0 kcal/mol.

[d] In degrees.

[e] Found in the step of additional energy minimization (see section 2.3.2).

Table 3.2
Cyclo(L-Phe-L-Pro-Aca)

		Conformations		
		1	2	3
Free energy [a] (ΔG [f])		0.00	0.10	0.92
Enthalpy [a] (ΔH)		0.00	−0.18	0.59
Entropy [a] ($-T\Delta S$)		0.00	0.29	0.32
Probability [c]		0.49	0.41	0.10
Bend type		VI	VI	VI
Dihedral angles [d]	ω_0	155	160	155
	ϕ_1	−140	−145	−145
	ψ_1	141	136	142
	ω_1	−1	0	−1
	χ_1^1	−64	−177	59
	χ_1^2	101	91	93
	ψ_2	−13	−13	−11
	ω_2	178	179	176
	θ_1	−83	−81	−83
	θ_2	−79	−82	−82
	θ_3	173	171	174
	θ_4	−108	−108	−108
	θ_5	55	56	56
	θ_6	−114	−111	−110

[f] $\Delta G = G - G_0 = (H - TS) - (H_0 - TS_0)$, where H_0, $-TS_0$ and G_0 are −10.96, 11.63 and 0.67 kcal/mol, respectively.

and β-protons of the Phe residue (table 5). Simple geometric consideration indicates that among the two populated rotamers the aromatic ring of Phe comes close to Pro $H^{\delta 1}$ or $H^{\delta 2}$ only in the *t* rotamer, when the D-Phe–L-Pro peptide bond takes a *trans* configuration as indicated above (fig. 10a–c). Thus, the side chain of the Phe residue exists as a rapidly interconverting mixture of two rotamers, g_+ and *t*, with almost equal populations and in the latter this side chain is located above Pro $H^{\delta B}$.

Twelve minimum energy conformations are obtained from the calculation. All of them have all-*trans* peptide bonds. This is in agreement with the experimental results. From the very high calculated energies of conformations involving the *cis* configuration (table 1) we can safely conclude that this molecule in fact assumes the all-*trans* configuration. As judged from the values of the dihedral angles, they belong to bend types of either II′ or III (table 3.1). According to the calculation they are populated in these two bend types in the ratio 4:1. The experimentally ob-

Table 3.3
Cyclo(L-Pro-L-Phe-Aca)

		Conformations									
		1 [e]	2	3	4 [e]	5 [e]	6	7	8 [e]	9	10 [e]
Free energy [a] (ΔG [g])		0.00	0.61	0.77	1.43	1.45	2.08	2.09	2.32	2.49	2.69
Enthalpy [a] (ΔH)		0.00	0.49	0.51	1.19	0.85	1.67	1.15	2.01	1.91	2.36
Entropy [a] ($-T\Delta S$)		0.00	0.13	0.26	0.24	0.60	0.41	0.93	0.31	0.59	0.33
Probability [c]		0.52	0.19	0.14	0.05	0.05	0.02	0.02	0.01	0.01	0.01
Bend type		I	I	I	I	I	–	–	I	–	I
Dihedral angles [d]	ω_0	163	166	165	−177	163	−8	2	161	2	161
	ψ_1	−44	−39	−40	−11	−51	−26	−30	−52	−37	−33
	ω_1	179	−178	−177	173	177	178	178	177	177	166
	ϕ_2	−110	−114	−113	−127	−94	−97	−88	−103	−78	−135
	ψ_2	54	35	38	34	−30	56	−45	65	−47	99
	ω_2	−179	−178	−176	−171	179	−171	173	−179	176	−163
	χ_2^1	−54	−52	−52	−53	−55	−56	−57	−173	179	179
	χ_2^2	108	114	115	101	108	107	107	63	79	70
	θ_1	86	103	100	144	−154	135	−98	78	−98	74
	θ_2	61	61	60	−65	67	−71	−70	60	−71	58
	θ_3	−110	−105	−108	147	−150	141	141	−117	141	−170
	θ_4	168	165	166	−119	79	−85	−78	171	−78	81
	θ_5	−80	−83	−82	68	63	−77	−70	−75	−69	64
	θ_6	−81	−82	−81	−160	−155	167	172	−80	170	−159

[g] $\Delta G = G - G_0 = (H - TS) - (H_0 - TS_0)$, where H_0, $-TS_0$ and G_0 are −16.38, 10.32 and −6.06 kcal/mol, respectively.

Table 3.4
Cyclo(L-Pro-D-Phe-Aca) [h]

		Conformations									
		1	2	3	4	5	6	7 [e]	8 [e]	9 [e]	10
Free energy [a] (ΔG^i)		0.00	0.32	1.17	1.60	1.65	1.96	2.06	2.32	2.47	2.72
Enthalpy [a] (ΔH)		0.00	0.09	1.22	1.74	1.45	1.85	2.28	2.18	1.57	1.66
Entropy [a] ($-T\Delta S$)		0.00	0.23	−0.05	−0.14	0.20	0.11	−0.21	0.14	0.90	1.07
Probability [c]		0.51	0.30	0.07	0.03	0.03	0.02	0.02	0.01	0.01	0.01
Bend type		IV	IV	II	IV	II	IV	II	II	II	II
Dihedral angles [d]	ω_0	167	167	174	167	174	166	163	162	164	175
	ψ_1	69	69	99	72	101	73	85	92	81	97
	ω_1	−179	178	−174	−179	−173	179	178	178	−177	−173
	ϕ_2	133	131	99	129	93	127	96	86	92	100
	ψ_2	−61	−55	15	−60	22	−56	50	51	40	17
	ω_2	174	172	178	175	177	173	−174	−173	−176	178
	χ_2^1	58	57	57	172	177	171	61	−178	58	−68
	χ_2^2	82	82	76	117	102	117	78	102	75	96
	θ_1	−145	−147	168	−147	164	−147	90	89	81	167
	θ_2	58	58	−72	58	−73	58	64	65	39	−71
	θ_3	−117	−119	148	−117	149	−120	−137	−142	65	147
	θ_4	169	168	−122	168	−122	168	154	153	−170	−122
	θ_5	−81	−79	62	−82	62	−79	−78	−74	65	62
	θ_6	−83	−82	−156	−82	−155	−81	−83	−82	−109	−157

[h] Conformations having a *cis* X–Pro peptide bond with $\Delta G < 4.0$ kcal/mol are (ΔG; ω_0, ψ_1, ω_1, ϕ_2, ψ_2, ω_2, χ_2^1, χ_2^2, θ_1, θ_2, θ_3, θ_4, θ_5, θ_6) = (3.71; −7, 147, −172, 80, −70, 175, 61, 72, −83, −78, 144, −86, −79, 159) and (ΔG; ω_0, ψ_1, ω_1, ϕ_2, ψ_2, ω_2, χ_2^1, χ_2^2, θ_1, θ_2, θ_3, θ_4, θ_5, θ_6) = (3.89; 7, 132, −173, 86, 47, 179, −180, 100, 90, 78, −126, 77, −170, −170, 172).

[i] $\Delta G = G - G_0 = (H - TS) - (H_0 - TS_0)$, where H_0, $-TS_0$ and G_0 are −15.60, 10.30 and −5.30 kcal/mol, respectively.

served single conformer indicates that the molecule is in the state of bend type II′ (possibility 1) or III (possibility 2), or in a rapidly interconverting mixture of the two bend types II′ and III (possibility 3). We examine below these three possibilities by comparing calculated conformations with experimental results.

4.1.1. Possibility 1

According to table 3.1 we may consider only up to six conformations as those with type II′ bend. In conformations 3 and 4, a weak hydrogen bond is recognized between Aca NH and Aca CO, when Venkatachalam's criterion of the formation of a hydrogen bond [2] is applied. This agrees with the experimental results. Even though the hydrogen bond is not formed in other conformations, the Aca H^N is buried and the Phe H^N is exposed on the surface as exemplified in the case of conformation 1 in fig. 5a, which is still consistent with experimental results. Furthermore, conformations 3 and 4 have a *trans* rotamer of the Phe side chain and the Pro $H^{\delta 2}$ existing above (3.11 and 3.09 Å, respectively) the aromatic ring of the Phe residue. These results are in good agreement with experiments. The population of the three rotamers of the Phe residue calculated only among the six conformations with the type II′ bend is $g_+ : t : g_- = 61 : 38 : 1$. This is consistent with the experimentally deduced population. Values of ϕ_{Phe} distributing in a range of 90–130° agree with experimentally deduced values (86 or 154°). Thus, this case is in fact one of the possibilities.

4.1.2. Possibility 2

According to table 3.1, we may consider the remaining six conformations as those taking the type III bend. In all of them, the Aca H^N is buried and the Phe H^N is exposed on the surface as exemplified in the case of conformation 5 in fig.

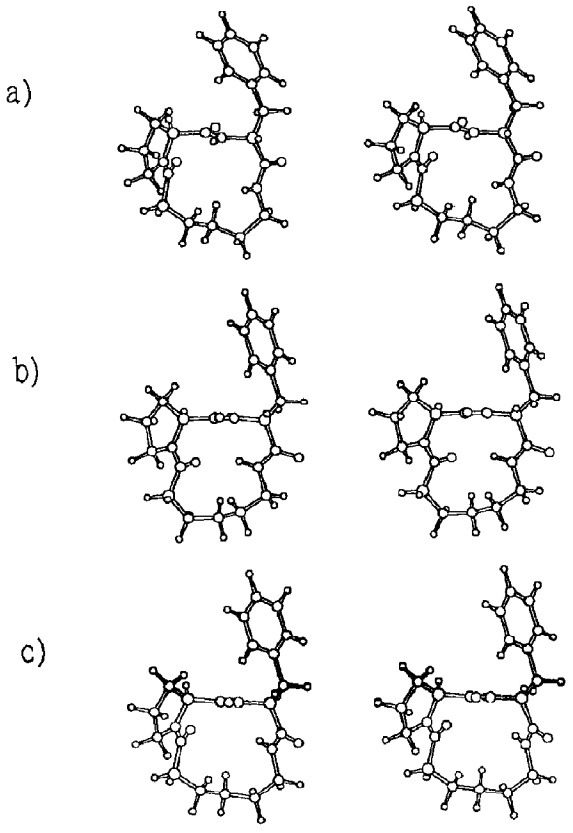

Fig. 6. Ball-and-stick representations of characteristic computed minimum energy conformations of cyclo(L-Pro-D-Phe-Aca). (a,b,c) Conformations 1, 3 and 7, respectively.

5b. However, the Aca H^N does not form a hydrogen bond. Values of ϕ_{Phe} (about $-50°$) which these conformations take do not agree with those deduced from the coupling constant. Both Pro $H^{\delta 1}$ and $H^{\delta 2}$ are far from the aromatic ring of the Phe residue (larger than 5.2 Å) in these conformations. In fact the aromatic ring of the Phe residue never comes close to either Pro $H^{\delta 1}$ or $H^{\delta 2}$ for a wide range of values of ψ_{Phe} around those of the calculated conformations considered here (fig. 10d). The population of the three rotamers of the Phe residue among the six conformations with the type III bend is calculated as $g_+ : t : g_- = 83 : 17 : 0$. This is in poor agreement with the experiments. From these considerations we conclude that this possibility is not the case.

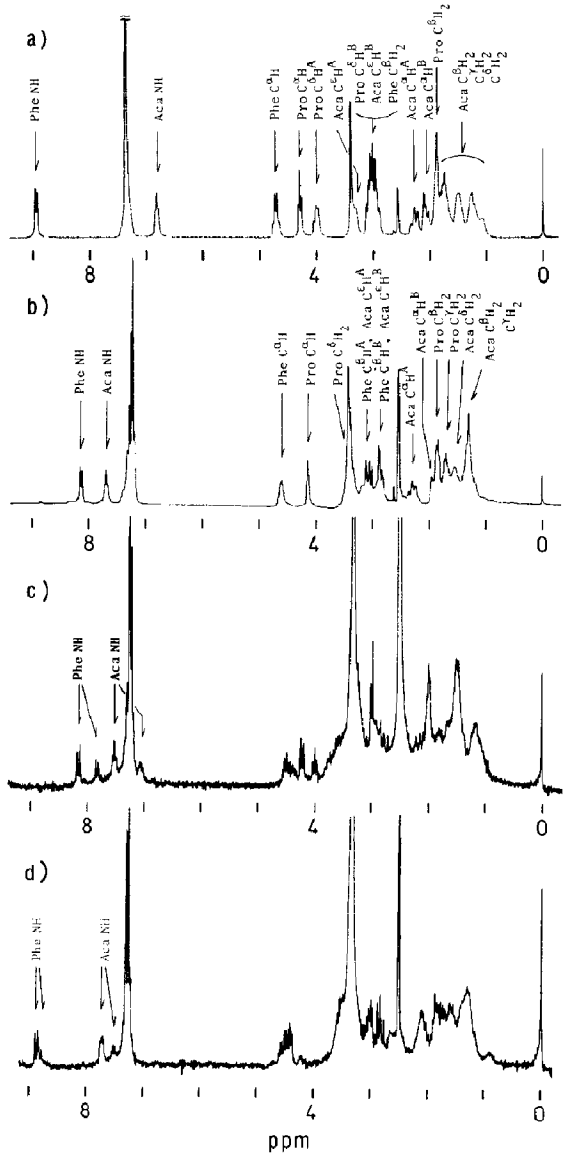

Fig. 7. ^{1}H-NMR spectra of (a) cyclo(D-Phe-L-Pro-Aca), (b) cyclo(L-Phe-L-Pro-Aca), (c) cyclo(L-Pro-L-Phe-Aca) and (d) cyclo(L-Pro-D-Phe-Aca) in DMSO-d_6 at 29 °C.

4.1.3. Possibility 3

We now consider all twelve calculated conformations. As a matter of course the degree of agreement between the calculations and the experiments becomes intermediate between those

Table 4

^{1}H-NMR data on peptide H^N resonance in DMSO-d_6 at 29°C

Molecule	Phe			Aca	
	δ [a]	$J_{HN\alpha}$ [b]	$\Delta\delta/\Delta T$ [c]	δ [a]	$\Delta\delta/\Delta T$ [c]
Cyclo(D-Phe-L-Pro-Aca) [d]	8.83	7.2 [e]	−4.7	6.75	−1.4
Cyclo(L-Phe-L-Pro-Aca) [f]	8.09	7.1 [g]	−4.8	7.65	−3.2
Cyclo(L-Pro-L-Phe-Aca)					
Major conformer	8.10	9.2 [h]	−4.0	7.47	−4.4
Minor conformer	7.78	5.0 [i]	−5.0	7.03	−2.3
Cyclo(L-Pro-D-Phe-Aca)					
Major conformer	8.80	9.1 [j]	−5.2	7.64	−5.0
Minor conformer	8.71	8.9 [k]	−2.5	7.46	−7.9

[a] Chemical shift in ppm downfield from TMS.
[b] Coupling constant in Hz.
[c] Temperature dependence of the chemical shift in 10^{-3} ppm/deg.
[d] Values of ^{1}H chemical shift δ of proline $H^{\delta A}$, $H^{\delta B}$ and H^{α} are 3.96, 3.00 and 4.31 ppm, respectively. The difference of ^{13}C chemical shifts in proline C^{β} and C^{γ}, $\Delta^{\beta,\gamma}$, is 4.23 ppm. The observed coupling constants between α- and β-protons of Phe are $J_{\alpha\beta_a}$ = 7.78 Hz, $J_{\alpha\beta_b}$ = 8.30 Hz and $J_{\beta_a\beta_b}$ = 13.29 Hz, respectively.
[e] Corresponds to ϕ_{Phe} = 86 or 154°.
[f] Values of ^{1}H chemical shift δ of proline $H^{\delta A}$, $H^{\delta B}$ are 3.2 ~ 3.6 and H^{α} is 4.13 ppm, respectively. The difference of ^{13}C chemical shifts in proline C^{β} and C^{γ}, $\Delta^{\beta,\gamma}$, is 9.11 ppm. The observed coupling constants between α- and β-protons of Phe are $J_{\alpha\beta_a}$ = 8.89 Hz, $J_{\alpha\beta_b}$ = 4.78 Hz and $J_{\beta_a\beta_b}$ = 12.70 Hz, respectively.
[g] Corresponds to ϕ_{Phe} = −154 or −86°.
[h] Corresponds to ϕ_{Phe} = −131 or −109°.
[i] Corresponds to ϕ_{Phe} = −170, −70, 31 or 89°.
[j] Corresponds to ϕ_{Phe} = 107 or 133°.
[k] Corresponds to ϕ_{Phe} = 104 or 136°.

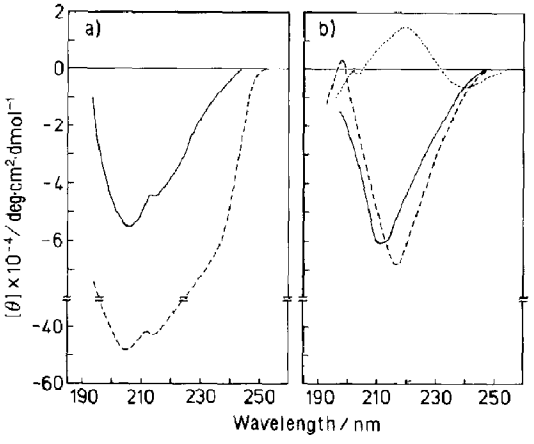

Fig. 8. Far-ultraviolet CD spectra in methanol at 29°C. (a) Cyclo(D-Phe-L-Pro-Aca) (———) and gramicidin S (- - - -); (b) cyclo(L-Phe-L-Pro-Aca) (———), cyclo(L-Pro-L-Phe-Aca) (- - - - - -) and cyclo(L-Pro-D-Phe-Aca) (· · · · · ·).

described in the above two possibilities. The population of three rotamers of the Phe residue is estimated here as $g_+ : t : g_- = 65 : 34 : 1$. Considering the accuracy of the calculation of relative populations this is still not inconsistent with the experiments.

Thus, we conclude that this molecule is in the state of bend type II′, or in a rapidly interconverting mixture of two bend types II′ and III. Even in the latter case the conformations with bend type II′ must be the major component because of the high-field shift of Pro $H^{\delta B}$.

4.2. *Cyclo(L-Phe-L-Pro-Aca)*

The ^{1}H-NMR spectrum indicates that this molecule exists as a (probably time-averaged) single conformer (fig. 7b). The difference ($\Delta^{\beta,\gamma}$ = 9.11 ppm) in the ^{13}C-NMR chemical shifts of Pro C^{β} and C^{γ} indicates that this conformer has a *cis* L-Phe–L-Pro peptide bond. As indicated in table

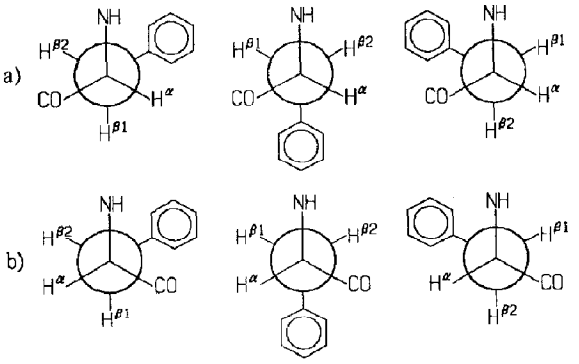

Fig. 9. Configurations of three typical rotamers of Phe side chains for (a) cyclo(D-Phe-L-Pro-Aca) and (b) cyclo(L-Phe-L-Pro-Aca). Calculated and experimentally deduced populations of the three rotamers shown are listed in table 5.

4, the Aca H^N has a somewhat low $\Delta\delta/\Delta T$ value (-3.2×10^{-3} ppm/deg). This indicates a solvent-shielded or weaker intramolecularly hydrogen-bonded NH group. The $\Delta\delta/\Delta T$ of Phe H^N (-4.8×10^{-3} ppm/deg) indicates a solvent-exposed proton. The coupling constant ($J_{HN\alpha} = 7.1$ Hz) suggests that ϕ_{Phe} takes a value around either -154 or $-86°$ (table 4). The CD spectrum is characterized by a strong negative extremum in the 210–213 nm region as shown in fig. 8b. The experimental results described above, in summary, indicate that this molecule exists in a (probably time-averaged) single backbone conformation with

Table 5
Experimentally deduced and calculated populations of Phe side chains

Molecule		Configuration		
		g_+	t	g_-
Cyclo(D-Phe-L-Pro-Aca)				
Calculation [a]	II′	61	38	1
	III	83	17	0
	II′ + III	65	34	1
Experiment		52	47	1
Cyclo(L-Phe-L-Pro-Aca)				
Calculation		10	40	50
Experiment		20	23	57

[a] Sets of values in rows designated by II′, III and II′ + III are populations calculated only among conformations as those with a type II′, III bend or both of them, respectively.

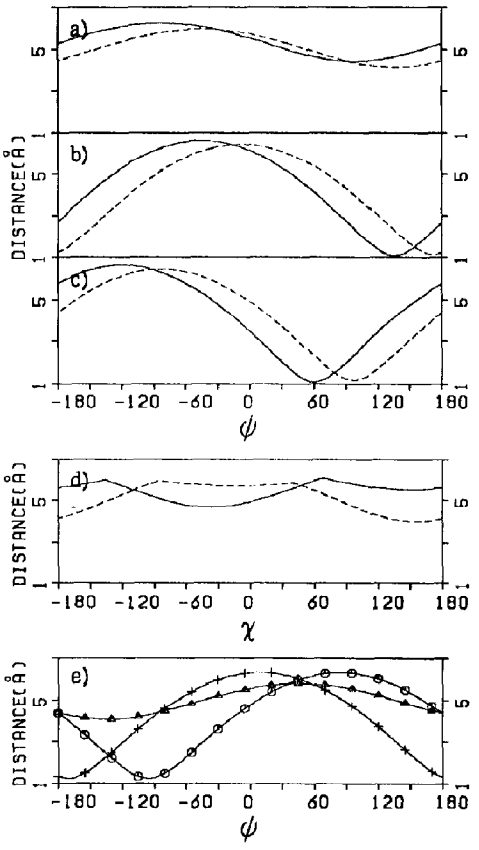

Fig. 10. Distances between the center of the aromatic ring of Phe and Pro $H^{\delta 1}$, $H^{\delta 2}$ or H^{α}. (a,b,c) Distances for $H^{\delta 1}$ (———) and $H^{\delta 2}$ (- - - - - -) when the Phe side chain configurations are in g_+, t and g_- rotamers, respectively, for a *trans* D-Phe–L-Pro peptide bond. (d) Minimum distances for $H^{\delta 1}$ (———) and $H^{\delta 2}$ (- - - - - -) at given values of χ_1^1 when ψ_{Phe} changes by 30° around $-70°$ which is the value of ψ_{Phe} of minimum energy conformations taking bend type III. (e) Distances for H^{α} when the Phe side chain configurations are in g_+ (○———○), t (+———+) and g_- (▲———▲), respectively, for a *cis* L-Phe–L-Pro peptide bond.

a *cis* L-Phe–L-Pro peptide bond, a buried or weaker hydrogen-bonded Aca H^N and an exposed Phe H^N.

As for side chain configurations the Pro H^{α} occurs at somewhat higher field (4.13 ppm) than usual. This result suggests that this proton exists above the aromatic ring of the Phe residue. The population of the three rotamers of the Phe re-

sidue (fig. 9b) is estimated as $g_+ : t : g_- = 20 : 23 : 57$ from the coupling constants (table 4) between α- and β-protons of the Phe residue (table 5). Simple geometric consideration indicates that among these three rotamers the aromatic ring of Phe cannot come close to Pro H^α in the g_- rotamer, when the L-Phe–L-Pro peptide bond takes a *cis* configuration as indicated above (fig. 10e). Therefore, we can conclude that the configuration where the aromatic ring of Phe is above Pro H^α is realized in either the g_+ or t rotamer, or in both. Thus, the side chain of the Phe residue exists as a rapidly interconverting mixture of three rotamers, g_+, t and g_- and in either or both of the g_+ and t rotamers the aromatic ring of the Phe residue is located above the Pro H^α.

Three minimum energy conformations are obtained from the calculation. All of them have a *cis* L-Phe–L-Pro peptide bond. This is in agreement with the experimental results. From the very high calculated energy of conformations involving *cis* configurations at the other two peptide bonds (table 1) we can safely conclude that this molecule in fact assumes a *trans-cis-trans* peptide configuration. This configuration of peptide bonds, i.e., the sequence having a *cis*-Pro in position $(i+2)$, indicates that the three calculated conformations belong to bend type VI. As indicated in table 3.2, the backbone conformations of the three calculated conformations are almost the same. This result indicates that a (probably time-averaged) single conformation shown by the ^{1}H-NMR spectrum really consists of a stable single backbone conformation. Values of ϕ_{Phe} of calculated conformations (−140 to −145°) fit with one of the experimentally deduced values (−154°). In all three conformations listed in table 3.2 the Aca H^N is buried and the Phe H^N is exposed on the surface as exemplified in the case of conformation 1 in fig. 5c. This is consistent with the experiment. As for side chain configurations, the aromatic ring of Phe is found to exist above the Pro H^α only in conformation 2. This conformer exists as a t rotamer of the Phe side chain (−177°). In the other two conformations in which the Phe side chains occur as g_- and g_+ rotamers, respectively, the aromatic ring of Phe is not close to the Pro H^α. Because the backbone is suggested to exist not in a mixture but really in a single conformation as resulting from the calculation, these results indicate that only in a t rotamer among experimentally analysed three rotamers does the aromatic ring of Phe exist above the Pro H^α. The population of the three calculated rotamers of the Phe residue is estimated as $g_+ : t : g_- = 10 : 40 : 50$ (table 5). This is roughly consistent with the experimentally deduced population. Thus, we conclude that this molecule is in a single stable backbone conformation taking the type VI bend with a side chain of Phe populating in a rapidly interconverting mixture of three rotamers and in the t rotamer the Phe side chain exists above the Pro H^α.

4.3. *Cyclo(L-Pro-L-Phe-Aca)*

The ^{1}H-NMR spectrum indicates that this molecule exists as a mixture of two conformers (fig. 7c). Their populations are estimated as 3 : 1. For the major conformer, the $\Delta\delta/\Delta T$ values of Aca H^N and Phe H^N (-4.4×10^{-3}, -4.0×10^{-3} ppm/deg) indicate that both protons are exposed to the solvent (table 4). The coupling constant ($J_{HN\alpha} = 9.2$ Hz) suggests that ϕ_{Phe} takes a value around −131 or −109° (table 4). For the minor conformer, the Aca H^N occurs at somewhat higher field (7.03 ppm) than the usual peptide H^N and has a low $\Delta\delta/\Delta T$ value (-2.3×10^{-3} ppm/deg) (table 4). These results indicate a solvent-shielded or intramolecularly hydrogen-bonded NH group. The $\Delta\delta/\Delta T$ of Phe H^N (-5.0×10^{-3} ppm/deg) indicates a solvent-exposed proton. Possible values of ϕ_{Phe} deduced from the coupling constant ($J_{HN\alpha} = 5.0$ Hz) are −170, −70, 31 or 89°. Determination of *cis* or *trans* configuration of the Aca–L-Pro peptide bond could not be made, because it was difficult to assign each peak in the ^{13}C-NMR spectrum. The CD spectrum of this molecule is characterized by a strong negative extremum ($[\theta] = -7\times10^4$ deg cm^2 dmol^{-1}) at 215 nm and a very weak positive extremum ($[\theta] = 3\times10^3$ deg cm^2 dmol^{-1}) at 197 nm (fig. 8b).

Ten minimum energy conformations are obtained from the calculation. Seven of these conformations have *trans* Aca–L-Pro peptide bonds. As judged from the values of the dihedral angles, they

belong to bend type I. The other three conformations have *cis* Aca–L-Pro peptide bonds. These two groups of conformations are populated in a ratio of 20 : 1 (table 3.3). Phe H^N is buried in all seven conformations of the major group as exemplified in the case of conformation 1 in fig. 5d. The Aca H^N is exposed in the lowest three conformations and conformation 10. In two other conformations, 4 and 8, Aca H^N is found to form a weak hydrogen bond. Aca H^N is buried in conformation 5. However, these three hydrogen-bonded Aca H^Ns are not the representative aspect reflecting this major group, because these three conformations have relatively high energies in the group. Even though the experimentally observed degree of exposure of Phe H^N is not precisely reproduced by the calculation, the experimentally observed tendency that Aca H^N is more exposed than Phe H^N is reproduced by the calculation. The values of ϕ_{Phe} in these conformations of the major group range between -135 and $-90°$. They are consistent with the experimentally expected value (-131 or $-109°$). In the minor group, the Phe H^N is buried in all three conformations as exemplified in the case of conformation 6 in fig. 5e. The Aca H^N forms a weak hydrogen bond in conformation 6 and is exposed in the other two conformations. Thus, Aca H^N is hydrogen-bonded in 40% of the population within the calculated minor group, which agrees rather well with experimental results on the minor conformer. Values of ϕ_{Phe} taken in these three conformations distributing in the range -100 to $-75°$ are consistent with the experimentally deduced value (-170, -70, 31 or 89°). From the clear dominance of the calculated major group having a *trans* Aca–L-Pro peptide bond and consistencies of values of ϕ_{Phe} between the calculations and experiments, we conclude that the major conformer of this molecule has a *trans* Aca–L-Pro peptide bond and corresponds to bend type I and the minor conformer has a *cis* Aca–L-Pro peptide bond.

4.4. Cyclo(L-Pro-D-Phe-Aca)

The ^{1}H-NMR spectrum indicates that this molecule exists as a mixture of two conformers (fig. 7d). They are populated in a ratio of 2 : 1. For the major conformer, the $\Delta\delta/\Delta T$ values of the Aca H^N and Phe H^N (-5.0×10^{-3}, -5.2×10^{-3} ppm/deg) indicate that both protons are exposed to the solvent. The coupling constant ($J_{HN\alpha} = 9.1$ Hz) suggests that ϕ_{Phe} takes a value around 107 or 133° (table 4). For the minor conformer, the Phe H^N has a low $\Delta\delta/\Delta T$ value (-2.5×10^{-3} ppm/deg). This indicates a solvent-shielded or intramolecularly hydrogen-bonded NH group. The $\Delta\delta/\Delta T$ value of Aca H^N (-7.9×10^{-3} ppm/deg) indicates a solvent-exposed proton. Possible values of ϕ_{Phe} deduced from the coupling constant ($J_{HN\alpha} = 8.9$ Hz) are 104 and 136° (table 4). The ^{13}C-NMR measurement to determine the *cis* or *trans* configuration of the Aca–L-Pro peptide bond cannot be carried out because of its poor solubility in DMSO-d_6. The CD spectrum of this molecule is characterized by a positive extremum ($[\theta] = 1.6 \times 10^3$ deg cm^2 dmol^{-1}) at 220 nm and a small shoulder at 205 nm (fig. 8b).

Ten minimum energy conformations are obtained from the calculation. All of them have all-*trans* peptide bonds. As judged from the values of the dihedral angles, they belong to bend types of either IV or II (table 3.4). According to the calculation they are populated in these two bend types in a ratio of 6 : 1. Calculations for the low-energy region of conformations with a *cis* Aca–L-Pro peptide bond found by the initial rough search (table 1) result in two conformations within 4.0 kcal/mol of the global minimum (3.6 and 3.9 kcal/mol). Considering the accuracy of the calculation without solvent, these conformations should not be neglected when we compare the calculated results with the experimental ones. Therefore, the experimentally observed mixture of two conformers should correspond either to those of bend types IV and II, or to those having *trans* and *cis* Aca–L-Pro peptide bonds. We examine below first the former possibility by comparing calculated and experimental results. All calculated conformations with the type IV bend have the inverse γ-turn hydrogen-bonded Phe H^N and the exposed Aca H^N. This is consistent with the experimental results on the minor conformer. All six conformations with the type II bend have the exposed Phe H^N. Three conformations have hydrogen-bonded Aca H^N (conformations 3, 5 and 10) and the

remaining three have the exposed Aca H^N (conformations 7–9). This is not inconsistent with the experimental results on the major conformer. We examine next whether these two bend conformations correspond to experimentally observed slowly interconverting conformations. The four conformations with the type IV bend have very similar backbone structures. The six conformations with the type II bend can be classified further into two groups. The first group (conformations 3, 5, 9 and 10) has the Phe peptide unit and (CH_2)-chain oriented differently from those of conformations with the type IV bend. The second group (conformations 7 and 8) also has the Phe peptide unit oriented differently but has the (CH_2)-chain oriented similarly to conformations with the type IV bend (fig. 6). An energy contour map for a single residue of D-Ala′ shows that backbones of D-Ala′ taken in these two bend conformations are connected by a low-energy valley. In fact, CPK model building shows that the D-Phe-Aca peptide unit in conformations 7 and 8 can be smoothly rotated to generate conformations with the type IV bend. Therefore, interconversion between these two conformations and those with the type IV bend is expected to be rapid. For conformations 3, 5, 9 and 10, CPK model building suggests that continuous adjustment of the conformation of the Aca chain to make it similar to those in the type IV bend conformations requires considerably large distortion of either the Aca-L-Pro or D-Phe-Aca peptide plane. However, such distortions could involve only a small conformational energy increase, if bond angles are allowed to vary as well. If this is the case, conformations classified into two bend types should not be regarded as corresponding to slowly interconverting conformers observed by ^{1}H-NMR measurement, but should correspond to the observed major conformer. In this case the observed minor conformation should correspond to those with a *cis* Aca–L-Pro peptide bond. In fact *cis-trans* isomerization of the peptide bond is known to be slow enough to show a spectrum as separate peaks in ^{1}H-NMR [34]. The two calculated conformations with a *cis* Aca–L-Pro peptide bond have the exposed Phe H^N. The Aca H^N of one conformation is hydrogen-bonded and that of the other is exposed. These results are inconsistent with experimental results on the minor conformer. The Aca H^N of most conformations with a *trans* Aca–L-Pro peptide bond is exposed as stated before, which is consistent with the experimental results. However, the configuration of the Phe H^N is not. Even though consistency in the configuration of Phe H^N and Aca H^N is poor between the calculations and experiments, we still believe from the kinetic consideration that the interpretation of the experimentally observed mixture as corresponding to those of *cis* and *trans* Aca–L-Pro peptide bonds is more plausible than the other interpretation as corresponding to those with bend types II and IV.

5. Discussion

Conformational energy calculation and such NMR parameters as coupling constants, temperature coefficients of amide protons and difference of Pro C^β and C^γ chemical shifts indicate that cyclo(D-Phe-L-Pro-Aca) is either in a state of bend type II′ or in a rapidly interconverting mixture of two bend types II′ and III with the former bend type being major. As shown in fig. 8a, the CD spectrum of this molecule is similar in its overall shape to that reported for gramicidin S in methanol in which the β-bend is characterized as type II′ [35]. Both spectra have double minima in the 200–230 nm region but with much smaller ellipticity in the former which may be explained by the large β-sheet in gramicidin S. Recently the dipeptide portion of cyclo(D-Phe-L-Pro-Aca) was shown by an X-ray diffraction study to take the type II′ β-bend [36]. This fact and the similarity of the CD spectra indicate that this molecule exists in the state of bend type II′, not in the state of a rapidly interconverting mixture of two bend types II′ and III.

These results suggest that the CD pattern of the D-aminoacyl-L-Pro sequence, which prefers the type II′ β-bend, has α-helix-like double minima in the 201–206 and 215–223 nm regions. A similar CD spectrum has also been observed for cyclo-(L-Ala-L-Ala-Aca) in methanol which takes the type I β-bend [20]. However, there is a clear difference between the two spectra. The first minimum at

201–206 nm is slightly shallower than the second in the 213–225 nm region in cyclo(L-Ala-L-Ala-Aca) (type I); on the other hand, the first minimum is deeper than the second in cyclo(D-Phe-L-Pro-Aca) (type II′). This means that type I and II′ β-bends, both with α-helix-like CD spectra, can be distinguished by their CD spectra. The dihedral angles and CD parameters of several related molecules showing the double minimum in the 200–230 nm region are listed in table 6. All of the ϕ and ψ values of cyclo(D-Phe-L-Pro-L-Val)$_2$, cyclo(D-Phe-L-Pro-Aca) and gramicidin S, all of which take the type II′ β-bend, are almost identical. However, the ϕ and ψ values of cyclo(L-Ala-L-Ala-Aca) which takes the type I β-bend differ from those in these three cyclic molecules. Comparison with the α-helical values of dihedral angles indicates that ϕ_1 and ψ_1 are close, and ϕ_2 and ψ_2 are different. Similar comparison of the values of dihedral angles in the α-helix and cyclo(D-Phe-L-Pro-Aca) indicates that ϕ_1 and ψ_1 are different, and ϕ_2 and ψ_2 are close. These small differences may explain the fact that the CD patterns in cyclo(L-Ala-L-Ala-Aca) and cyclo(D-Phe-L-Pro-Aca) are similar to that of the α-helix but the ellipticity of the double minima in these peptides has opposite relative values. Therefore, the CD spectrum of cyclo(D-Phe-L-Pro-Aca) will be of use in identifying and estimating the contribution of various bend types in acyclic and cyclic peptides.

Cyclo(L-Phe-L-Pro-Aca) is characterized as having the type VI bend on the basis of energy calculation and spectroscopic data. This bend structure contains a *cis* L-Phe–L-Pro peptide bond. This molecule takes the *cis* conformation as the preferred one because of steric hindrance between the L-Phe side chain and the Pro δ-methylene group brought out by the cyclization. The CD spectrum of this molecule shown in fig. 8b is the first example of a bend containing only a *cis* L-Phe–L-Pro peptide bond.

We also carried out NMR measurement of cyclo(L-Pro-L-Phe-Aca) in chloroform at room temperature. It was found that this molecule also exists as mixture of two conformations as in DMSO-d_6. This suggests that this molecule has two stable conformations rather generally in solution. The calculated major conformation takes the type I β-bend and it has been shown from conformational energy calculation that this bend type is one of the dominant conformations of Ac-L-Pro-L-Phe-NHMe [12]. It has also been reported that the sequence (Gly-L-Pro-L-Y)$_2$ (Y not Val) has a preference for the type I β-bend [18]. However, the CD spectrum is not the double-minimum type characteristic of the type I β-bend but is similar in shape to that of Piv-L-Pro-L-Leu-NHMe in methanol, exhibiting the type V β-bend [21]. We speculate that the contribution from the minor conformation erased the double-minimum character of the CD spectrum. The experimentally observed ratio, 3 : 1, of major and minor conformations is considerably different from that, i.e. 20 : 1, resulting from the calculation. However, this difference, corresponding to 1–2 kcal/mol in free energy, is within the accuracy of the calculation because interactions with solvent are not considered here. A small degree of lowering of the

Table 6

Dihedral angles and CD spectral parameters for related cyclic molecules

Molecule	Dihedral angles of cyclic molecules' CD spectral parameters									
	ϕ_1	ψ_1	ω_1	ϕ_2	ψ_2	ω_2	λ_{min} (nm)	θ [a] ($\times 10^{-3}$)	λ_{min} (nm)	θ [a] ($\times 10^{-3}$)
Cyclo(D-Phe-L-Pro-L-Val)$_2$ [b]	60	−135	−162	−86	−15	−176	223	−8.7	201	−16.3
Cyclo(D-Phe-L-Pro-Aca)	100	−132	165	−75	−12	177	215	−14.8	206	−19.0
Gramicidin S	55	−110	180	−60	−40	180	215	−38.2	205	−44.9
Cyclo(L-Ala-L-Ala-Aca)	−79	−47	176	−111	65	−179	220–2	−21	208	−20
α-Helix	−57	−47		−57	−47		222		209	

[a] These values are expressed as mean residue ellipticities, $m_\theta = M_\theta/n$, where n is the number of residues.

[b] Crystal structure was determined as in ref. 37. CD was measured in AcCN [18].

energy of the calculated minor conformation with a *cis* Aca–L-Pro peptide bond, due to solvation, can make it more populated.

Cyclo(L-Pro-D-Phe-Aca) has a CD spectrum which is similar to those that have been observed in methanol [21] for Piv-L-Pro-Aib-NHMe and Piv-L-Pro-D-Ala-NHMe whose central dipeptide portions with a *trans* Piv–L-Pro peptide bond are considered to take the type II β-bend from X-ray diffraction studies [38,39]. Also the CD spectrum of this molecule is similar in shape to those of poly(Ala_2-Gly_2) in ^2H_2O [16], cyclo(L-Orn-L-Pro-D-Phe)$_2$, cyclo(Gly-L-Pro-D-Phe)$_2$ in hexafluoro-2-propanol [17], cyclo(D-Ala-L-Pro-D-Ala)$_2$ in H_2O and TFE [18], cyclo(L-Ala-D-Ala-Aca) in methanol [20], Dnp-Gly-L-Pro-D-Ala-Gly-pNa in C^2H_3OH [40] and Boc-L-Pro-D-Ala-L-Leu in TFE [41], except for shifting about 10 nm to the red. The dipeptide portions, Ala-Gly, L-Pro-D-X (X = Phe or Ala) and L-Ala-L-Ala in these molecules are known to have a *trans* X–L-Ala or X–L-Pro peptide bond and take the type II β-bend. The similarities in CD spectra between cyclo(L-Pro-D-Phe-Aca) and many other molecules with the dipeptide portions being in the state of bend type II indicate that the state of this bend type is dominant in the major conformation of cyclo(L-Pro-D-Phe-Aca). Considering the accuracy of the calculation of relative populations, this fact is consistent with our conclusion stated in section 4.

At the final step of energy minimization, we carried out additional energy minimizations in all sheets conjugate to those containing minimum points which have already been reached. This calculation resulted in no additional stable conformations with a *cis* peptide bond just before Pro, but gave several minimum energy conformations with $\Delta G < 3.0$ kcal/mol for species with a *trans* configuration of this peptide bond. They have peptide bonds deviating considerably from planarity, suggesting strong steric restriction arising from Pro and ring cyclization. A few of them have such low free energy as to influence the population of bend types. These conformations are those that could not be found by the strategy starting from conformations with planar peptide bonds.

6. Conclusions

Four cyclic tripeptides including Pro and Phe were synthesized as models of β-bends and their conformations were examined both experimentally and theoretically.

In theoretical calculations, treatment of cyclization of the backbone is done carefully. For a given set of values of independent variable dihedral angles along a cyclic backbone, a few conjugate sets of values of dependent dihedral angles are determined and each set constitutes a distinct multi-dimensional sheet on which the ring is closed. The potential energy surface of the cyclic molecule exists on each sheet. These sheets and corresponding potential energy surfaces should be individually treated in calculations concerning backbone conformations.

Conformations of the four molecules are characterized as follows. The backbones of the minimum energy conformations of cyclo(D-Phe-L-Pro-Aca) and cyclo(L-Phe-L-Pro-Aca) are found to form β-bends of type II′ and VI, respectively. The CD spectrum of the former will be of use in identifying and estimating the contribution of various bend types in acyclic and cyclic peptides.

Type I and II′ β-bends, both with double-minimum α-helix-like CD spectra, were shown to be distinguishable by their relative values of the ellipticity of the double minima in the 200–230 nm region.

The CD curve of cyclo(L-Phe-L-Pro-Aca) is the first example observed of a bend containing a *cis* peptide bond.

Cyclo(L-Pro-L-Phe-Aca) was shown from both experimental observations and theoretical calculations to exist as a mixture of two conformations. It is indicated that major conformations have a *trans* Aca–L-Pro peptide bond taking the type I β-bend and minor conformations have a *cis* Aca–L-Pro peptide bond.

Cyclo(L-Pro-D-Phe-Aca) was also shown to exist as a mixture of two conformations in DMSO-d_6. Conformational calculations and the CD spectrum suggest that the major conformation has a *trans* Aca–L-Pro peptide bond taking the type II β-bend and the minor one has a *cis* Aca–L-Pro peptide bond.

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