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Quantitative analysis of cyclic β-turn models

ANDRÁS PERCZEL AND GERALD D. FASMAN

Graduate Department of Biochemistry, Brandeis University, Waltham, Massachusetts 02254-9110

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Abstract

The β-turn is a frequently found structural unit in the conformation of globular proteins. Although the circular dichroism (CD) spectra of the α-helix and β-pleated sheet are well defined, there remains some ambiguity concerning the pure component CD spectra of the different types of β-turns. Recently, it has been reported (Hollósi, M., Kövér, K.E., Holly, S., Radics, L., & Fasman, G.D., 1987, *Biopolymers 26*, 1527-1572; Perczel, A., Hollósi, M., Foxman, B.M., & Fasman, G.D., 1991a, *J. Am. Chem. Soc. 113*, 9772-9784) that some pseudohexapeptides (e.g., the *cyclo*[(δ)Ava-Gly-Pro-Aaa-Gly] where Aaa = Ser, Ser(O^{t}Bu), or Gly) in many solvents adopt a conformational mixture of type I and the type II β-turns, although the X-ray-determined conformation was an ideal type I β-turn. In addition to these pseudohexapeptides, conformational analysis was also carried out on three pseudotetrapeptides and three pseudooctapeptides. The target of the conformation analysis reported herein was to determine whether the ring stress of the above β-turn models has an influence on their conformational properties.

Quantitative nuclear Overhauser effect (NOE) measurements yielded interproton distances. The conformational average distances so obtained were interpreted utilizing molecular dynamics (MD) simulations to yield the conformational percentages. These conformational ratios were correlated with the conformational weights obtained by quantitative CD analysis of the same compounds. The pure component CD curves of type I and type II β-turns were also obtained, using a recently developed algorithm (Perczel, A., Tusnády, G., Hollósi, M., & Fasman, G.D., 1991b, *Protein Eng. 4*(6), 669-679). For the first time the results of a CD deconvolution, based on the CD spectra of 14 β-turn models, were assigned by quantitative NOE results. The NOE experiments confirmed the ratios of the component curves found for the two major β-turns by CD analysis. These results can now be used to enhance the conformational determination of globular proteins on the basis of their CD spectra.

Keywords: circular dichroism; cyclic β-turn models; molecular dynamics; NMR

Circular dichroism spectroscopy is a widely used conformational probe (Greenfield & Fasman, 1969; Brahms & Brahms, 1980; Yang et al., 1986), as is the NMR technique (Gierasch et al., 1981; Bruch et al., 1985; Dyson et al., 1988a,b; Wright et al., 1988; Kessler et al., 1990) for monitoring conformational changes of polypeptides and proteins (Smith & Pease, 1980). Because there is no direct way to assign molecular conformations using CD spectra, the power of this method is often underestimated or neglected. In the present work both quantitative NOE and MD simulations have been used to analyze the whole conformational set adopted by the models and to assign the CD spectra of different β-turns.

Only a few cyclopeptide derivatives have been designed especially for conformational investigations, although their cyclic structures incorporate β- and/or γ-turns. The CD spectrum of a polypeptide chain is mainly determined by the spatial arrangement of all the amide chromophores around the chiral centers (C^α atoms of the amino acids). Therefore only a carefully designed model can be expected to properly reproduce the CD properties of the investigated substructure (e.g., the β-turn substructure is only a fragment of the whole molecule). Such a model design must include the correct number of chromophores as well as the correct degree of conformational rigidity. All the herein-analyzed models have only three amide groups with a maximum of two chiral centers embedded in differently rigid molecules.

With the object of obtaining the perfect β-turn model, by synthesizing linear and bridged cyclopeptide models (e.g., Bandekar et al., 1982; Aubry et al., 1984; Marraud

Reprint requests to: Gerald D. Fasman, Rosenfield Professor of Biochemistry, Graduate Department of Biochemistry, Brandeis University, Waltham, Massachusetts 02254-9110.

Abbreviations: Aaa, alpha amino acid; (δ)Ava, 5-amino valeric acid; (ε)Aca, 6-amino capric acid; CD, circular dichroism; CI, chemical ionization; COSY, correlated spectroscopy; ϵ^b, dielectric constant; EI, electric ionization; NOE, nuclear Overhauser effect; NOESY, nuclear Overhauser and exchange spectroscopy; CCA, convex constraint analysis; DMSO, dimethyl sulfoxide; MD, molecular dynamics; OBzl, O-benzyl; O^{t}Bu, O-^{t}butyl.

& Aubry, 1984; Hollósi et al., 1985, 1987; Perczel et al., 1990), a systematic research effort was started several years ago. The interesting CD spectrum of *cyclo*[(ϵ)Aca-Ala-Ala] (Bandekar et al., 1982) led to the initiation of studies on the effect of the backbone length and the amino acid sequence on the β-turn models. The basic idea was to introduce a single β-turn moiety in a rigid cyclic structure. However, it was not clear what the optimal ring size was for such a model (see Fig. 1). The ring stress, induced by the ring size, on the conformation has been investigated by Karle (1981), and it was found that in models containing less than four α-amino acid residues (less than 12 backbone atoms) the *cis*-amide bond was required. Karle (1989) also demonstrated that when an odd number of amino acids was incorporated into the cyclic peptide, the structure cannot have internal symmetry and therefore the CD spectra must originate from more than a simple β-turn moiety. For example, in a cyclic pentapeptide (Karle, 1989; Stradley et al., 1990) a β- and a γ-turn are simultaneously present, and consequently the CD spectra was the sum of the two substructures.

On the basis of a statistical analysis of β-turn fragments, taken from X-ray diffraction studies on proteins, several -Xxx-Yyy- dipeptides were found to be potential candidates for β-turn models. Using the frequency values first determined by Chou and Fasman (1977) and recently by Wilmot and Thornton (1988), one can estimate a low probability of β-turn formation for -Ala-Ala- or -Pro-Ala- dipeptides at $(i + 1)$ and $(i + 2)$ positions of a β-turn. By contrast a -Pro-Ser- amino acid pair is predicted to have a high potential for β-turn formation. This dipeptide is not only frequently found in β-turns of globular proteins (elastase [residues 28, 29], subtilisin BPN′ [residues 52, 53; 172, 173], hemoglobin [residues 32, 33], etc.), but it has also been observed to be the target of several posttranslational modifications (*O*-glycosylation and/or *O*-phosphorylation), which may also be related to β-turn conformations (Hollósi et al., 1990). The design of some cyclic models, having a different ring size but always incorporating only the -Pro-Ser- chiral moiety, was made in the form of pseudotetrapeptides (*cyclo*[(ϵ)Aca-Pro-Ser(OBzl)] [*1*], *cyclo*[(ϵ)Aca-Pro-Ser(OH)] [*2*], *cyclo*[(ϵ)Aca-Ala-Ala] [*3*]), pseudohexapeptides (*cyclo*[(δ)Ava-Gly-Pro-Aaa-Gly], where Aaa = Ser, Ser(O^{t}Bu), or Gly), and pseudooctapeptides (*cyclo*[Pro-Ser(OBzl)-(ϵ)Aca-Pro-Ser(OBzl)-(ϵ)Aca] [*4*], *cyclo*[Pro-Ser-(ϵ)Aca-Pro-Ser-(ϵ)Aca] [*5*], *cyclo*[Pro-Ala-(ϵ)Aca-Pro-Ala-(ϵ)Aca] [*6*]) (Fig. 1; Kinemages 1-6). During the selection of these models, in addition to the already described factors, other parameters were also considered; e.g., chiral amino acids were only introduced in the central part of the β-turn (positions $i + 1$ and $i + 2$), whereas glycines were used elsewhere (therefore positions i and $i + 3$ had no CD contribution), and the whole β-turn hairpin subunit was bridged by a suitable nonchiral ϵ or δ amino acid. The conformational mobility of these models was decreased by fixing the ϕ_{i+1} torsion angle at a favorable β-turn value ($\phi_{i+1} \approx -60°$) by using the proline residue and by the protection of the serine side chain with a bulky ^{t}butyl or benzyl ether.

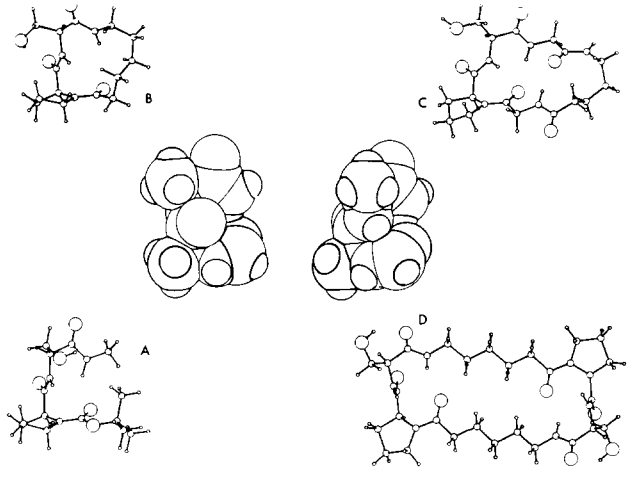


Fig. 1. Four different β-turn models, encompassing the -Pro-Ser- chiral subunit. Boc-Pro-Ser-NHMe (**A**); *cyclo*[(ϵ)Aca-Pro-Ser(OH)] (**B**); *cyclo*[(δ)Ava-Gly-Pro-Ser(OH)-Gly] (**C**); *cyclo*[Pro-Ser-(ϵ)Aca-Pro-Ser-(ϵ)Aca] (**D**). In the center are the Van der Waals models for the type I and the type II β-turns. See Kinemages 1-6 for three-dimensional figures.

On the basis of different theoretical calculations (Appelquist, 1982; Woody, 1985; Manning & Woody, 1987; Manning et al., 1988) performed on a triamide chiral unit in a β-turn conformation (Aca-Ala-Ala-$NHCH_3$), it is known that the backbone dihedral angles (ϕ_1, ψ_1, ϕ_2, ψ_2) have a basic influence on the $n\pi^*$ and $\pi\pi^*$ rotational strengths. Manning et al. (1988) recently demonstrated that even small backbone conformational distortions can cause strong changes in the CD signal. (This is the reason why CD can monitor conformational changes with high sensitivity.) Woody (1974) distinguished four classes (A, B, C, and D) of CD spectral patterns for β-turns. Despite the fact that in some linear as well as cyclic peptides such patterns have already been observed, there is not an unambiguous correlation between the β-turn types and the above spectral classes.

The rationale of this study is twofold: first, to investigate different β-turn geometries as thermodynamically stable local structures, which may play an important role in the initial stages of polypeptide and protein folding (Montelione & Scheraga, 1989; Sundaralingam & Sekharudu, 1989); and second, to describe the pure component CD spectra of different β-turns. The latter is required for the estimation of the secondary structural elements of proteins using their CD spectra (<240 nm) (Yang et al., 1986), which will only be possible when the pure component spectra of all major secondary structures (including β-turns) are known. Using the linear combina-

tion of the weighted pure CD component curves, one can calculate the measured CD spectra of polypeptides or proteins. In contrast to the pure CD spectra of the α-helix and the β-pleated sheet, the pure CD spectra of β-turns have only been tentatively assigned, even though several theoretical and empirical approaches have been made. The CD spectra of β-turns can be used as an independent spectroscopic probe in the same manner as NMR was used for structural assignment (Dyson et al., 1988a).

Although NOEs yield interproton distances, CD can provide information on the relative orientation of the amide bonds (ϕ, ψ) in their chiral environment (see Fig. 2). However, the correlation of quantitative CD and NMR data is lacking. The two methods can yield different types of conformational data on the same structure. Therefore the strategy of the present conformational analysis was, first, to determine the complete conformational sets of all cyclic models from MD trajectory analysis; and second, to use appropriate interproton distances obtained from MD conformations to quantify the NOE distances. Finally, conformational data obtained by NMR were compared to the quantitative CD results independently obtained.

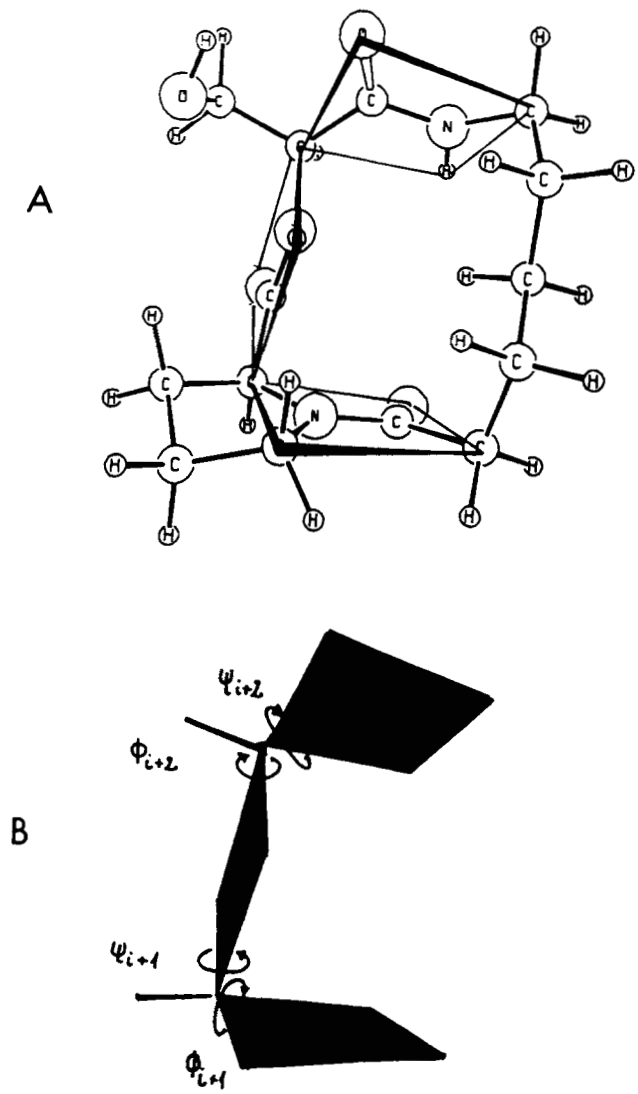


Fig. 2. **A**: *Cyclo*[(ϵ)Aca-Pro-Ser(OH)] with the amide planes highlighted. **B**: The three amide planes of a triamide system with the torsional angles determining their conformations.

Results and discussion

Molecular dynamics simulations

Molecular dynamics attempts to simulate the motion of a molecule using a set of constraints, resulting in coordinates and velocities describing the atomic motions of the system over time. Conformational comparisons performed on the β-turn part of the different cyclic models, based on the trajectory analyses obtained by MD, yielded similar geometries (see Table 1). The fully relaxed backbone conformations (obtained in acetonitrile [dielectric constant 38.8]) are also close to the so-called ideal β-turn conformations. The ring size has, however, a basic influence on the number and on the conformation of the adopted backbone geometries. For the cyclic pseudotetrapeptides (*1–3*), regardless of the amino acid side chains, only two drastically different backbone conformations were found. The first conformational type, $\phi_{i+1} \approx -60°$, $\psi_{i+1} \approx -30°$, $\phi_{i+2} \approx -130°$, $\psi_{i+2} \approx \pm 40°$, is close to a type I β-turn conformation, whereas the second type shows a resemblance to a type II β-turn, $\phi_{i+1} \approx -60°$, $\psi_{i+1} \approx +100°$, $\phi_{i+2} \approx +70°$, $\psi_{i+2} \approx \pm 40°$ (see Table 1). However, the torsional angles of the $(i + 2)$th residue (ϕ_{i+2} and ψ_{i+2}) deviated significantly from values predicted by Venkatachalam (1968): $\phi_{i+1} \approx -60°$, $\psi_{i+1} \approx -30°$, $\phi_{i+2} \approx -90°$, $\psi_{i+2} \approx 0°$ for type I and $\phi_{i+1} \approx -60°$, $\psi_{i+1} \approx +120°$, $\phi_{i+2} \approx +80°$, $\psi_{i+2} \approx 0°$ for type II β-turn conformations. A similar ring-size-induced conformational distortion for both β-turn conformations was previously observed for *cyclo*[(ϵ)Aca-Ala-Gly] (Némethy et al., 1981) and *cyclo*[(ϵ)Aca-Ala-Ala] (Bandekar et al., 1982) using molecular mechanics (MM)-type calculations. It is therefore evident that in *cyclo*-pseudotetrapeptide models the distortion of the ideal β-turn conformation is induced by the ring stress and not by the composition of the amino acid side chain. The enlargement of the ring size reduced the distorting forces but simultaneously resulted in a larger number of conformations (see Table 1) for cyclic pseudohexapeptides. Beside the already discussed torsional angle combinations for type I and type II β-turns, a new type of backbone conformation was also found during trajectory analyses. This conformation ($\phi_{i+1} \approx -60°$, $\psi_{i+1} \approx -45°$, $\phi_{i+2} \approx -75°$, $\psi_{i+2} \approx -30°$) is close to an ideal type III β-turn structure and is partially adapted by cyclic hexapeptides and cyclic octapeptides, as well as some linear β-turn models (see Table 1). Even if type I and type III β-turn

Table 1. *Molecular dynamics conformations in acetonitrile based on trajectory analysis*

Compound[a]	ϕ_{Pro}	ψ_{Pro}	ϕ_{Ser}	ψ_{Ser}	M1	M2	M3	Turn type
A	−60°	−27.5°	−135°	−45°	3.5 ± 0.15	3.0	[b]	I
				+45°				
	−60°	+81°	+65° ± 20°	−45°	2.2 ± 0.15	2.2	[b]	II
				+45°				
B	−80°	−30°	−120°	−30°	3.5	3.0	1.8	I
				+45°			2.6	
	−60°	+120°	+75°	0°	2.2	2.3	2.7	II
C	−60°	−45°	−135°	+45°	3.5	2.9	2.33 ± 0.2	I
				−30°			1.75 ± 0.2	
	−60°	−45°	−60°	−45°	3.5	2.95	2.35 ± 0.2	III (or I)
	−60°	+30°	+60°	+30°	2.85	2.2	2.9 ± 0.15	
	−60°	+70°	+60°	+30°	2.15	2.2	2.2 ± 0.2	II
				−75°				
	−60°	+115°	+60°	+30°	2.15	2.2	2.2 ± 0.2	
D	−60°	−45°	−75°	−25°	3.5	2.9	2.55 ± 0.25	III (or I)
	−60°	+52°	−155°	−25°	2.6 ± 0.25	2.2	1.8 ± 0.15	
				+25°				
	−60°	+135°	+125°	+25°	2.25	2.25	2.8 ± 0.2	II

[a] A, *cyclo*[(ε)Aca-Pro-Ser]; B, *cyclo*[(ε)Aca-Ala-Ala]; C, *cyclo*[(δ)Ava-Gly-Pro-Ser(OH)-Gly]; D, *cyclo*[(ε)Aca-Pro-Ala-(ε)Aca-Pro-Ala] or *cyclo*[(ε)Aca-Pro-Ser-(ε)Aca-Pro-Ser].
[b] The calculated interproton distance (M3) between NH_{i+2} and NH_{i+3} is not representative due to large alternations.

backbone geometries differ significantly, the calculated marker-distance values (M1, M2, and M3) are very similar in both of the above two conformers (3.5 Å, 2.95 ± 0.05 Å, and 2.30 ± 0.2 Å, respectively), and therefore an NOE experiment is not expected to distinguish (based on M1 and M2) between these two structures. However, these two β-turn forms differ from each other significantly in the orientation of the third amide plane ($\phi_{i+2} \approx -130°$ or $\phi_{i+2} \approx -70°$ and $\psi_{i+2} \approx +40°$ or $\psi_{i+2} \approx -45°$). Because CD spectroscopy is expected to reflect the relative orientation of the amide bonds, it is reasonable to presume that type I- and type III-related CD spectra will be different. (The CD spectra of the two conformers will be discussed later.)

The conversion of the measured NOE percentages to interproton distances requires a reference distance (d_{ref}, see Equation 1, below) that must be *insensitive* toward conformational changes (e.g., geminal interproton distance [d_{HCH}] of a suitable methylene group). By contrast, the different backbone conformations are assigned through conformation-*sensitive* marker distances (e.g., M1, M2, etc.) (see Table 2). For example, M1 and M2 are expected to effectively monitor an interconversion between the type I and type II forms of a β-turn. The calculated conformational percentages, from Equation 3, on the basis of quantitative NOEs, depend strongly on the actual values of the marker distances (see for example M1, M2, and M3 in Table 2) found in the appropriate pure conformations. However, the calculated pure conformers may differ from the ideal conformers, therefore resulting in different marker distance values (see Table 1) than previously determined for the ideal conformers.

Table 2. *Selected marker distances in the ideal type I and type II β-turns*

Conformer type	Suitable interproton distance	Labeled as	Interproton distance in an ideal conformer[a] (Å)
Type I β-turn	NH_{Ser}-H^{α}_{Ser}	Marker 2 (M2)	2.9
	NH_{Ser}-H^{α}_{Pro}	Marker 1 (M1)	3.5
	NH_{Ser}-NH_{i+3}	Marker 3 (M3)	[b]
Type II β-turn	NH_{Ser}-H^{α}_{Ser}	Marker 2 (M2)	2.3
	NH_{Ser}-H^{α}_{Pro}	Marker 1 (M1)	2.1
	NH_{Ser}-NH_{i+3}	Marker 3 (M3)	[b]

[a] Venkatachalam (1968).
[b] Due to the large range of ψ_{Ser} ($-45° < \psi_{Ser} < +45°$) the interproton distance of NH_{Ser}-NH_{i+3} is ambiguous. Therefore M3 is rarely used for conformational assignment.

However, the numerical resemblance of the herein-calculated M1 and M2 values for the investigated compounds with the M1 and M2 values associated with the ideal conformers is significant (compare M1 and M2 values in Table 1 with appropriate values in Table 2). These

similarities of the appropriate marker distance values suggest that -Pro-Ser- dipeptides may adopt β-turn conformations with a high resemblance to the ideal hairpin geometries. However, even small alterations of the M1 and M2 distances may induce significant differences in the estimation of conformational weights based on Equation 3. (The reference distance dependence on the appropriate torsional angle[s] is discussed later.)

Conformational interconversion between the two major β-turn backbone types (type I and type II) was observed only for pseudooctapeptides on the time scale of our dynamics simulation at 300 °K. For smaller rings the two forms of β-turns (type I and type II) seem to be separated by a high-energy barrier. The global implication of the present dynamic simulation is that these cyclic structures, depending on the size of the backbone ring, always share several conformers (more than two), but a large percentage of these conformations is similar to an ideal type I or to type II β-turn conformations (see Table 1).

The cyclo[(ϵ)Aca-Ala-Ala] conformations in d_6-DMSO

During the last decade *cyclo*[(ϵ)Aca-Ala-Ala] (*3*) (Bandekar et al., 1982) has been the most promising model for an ideal type I β-turn. The detailed conformational analysis, not based on ^{1}H-{^{1}H}-NOE or X-ray data, suggested that *3* exhibits a single conformation in different solvents whose geometry is close to type I β-turn structure. The fact that *3* contains alanine residues at both positions $i + 1$ and $i + 2$ of the β-turn makes the assignment of some observed enhancements rather complicated (cf. Fig. 3). The NOE data presented herein (measured in d_6-DMSO 100.0 atom%) contradict the unique conformational hypothesis as shown above.

Followed by the irradiation of the NH_{Aca} (see Fig. 3C), the strong enhancement ($\sigma = 15.5\%$) of the $H^{\alpha}_{Ala_2}$ suggests a *cis*oid orientation of these two protons, presuming a $\psi_{Ala_2} \simeq +60°$ (Fig. 4). (According to MD calculations, $d_{NH(Aca)\text{-}H^{\alpha}(Ala_1)}$ is larger than 3.5 Å, therefore $\sigma_{NH(Aca)\text{-}H^{\alpha}(Ala_1)} \simeq 0$.) Such a conformation is in good agreement with the MD-calculated value of $\psi_{Ala_2} = +45°$, and with the lack of $\sigma_{NH(Aca)\text{-}H^{\alpha}(Ala_1)}$ cross-relaxation rate ($d \simeq 0$ as shown on Fig. 4). In such a type I β-turn backbone conformation ($\phi^{\text{type I}}_{Ala_1} = -60° \pm 30°$, $\psi^{\text{type I}}_{Ala_1} = -30° \pm 30°$, $\phi^{\text{type I}}_{Ala_2} = -90° \pm 30°$, $\psi^{\text{type I}}_{Ala_2} = +60° \pm 30°$), the second alanine residue adopts an inverse γ-turn conformation (γ-turn, γ_L, or C_7^{eq} with torsional angles $\phi^{\gamma} = -75° \pm 30°$, $\psi^{\gamma} = +75° \pm 30°$), which in itself is a favorable geometry stabilized by an intramolecular H bond. Based on ab initio calculations (Scarsdale et al., 1983; Balázs, 1990) as well as infrared experiments in the solid state (Koyama & Shimanouchi, 1971) in inert solvents (Avignon et al., 1973), the minimal energy conformation of the *N*-acetyl-alanine-*N'*-methylamide was always found around the C_7^{eq}. Therefore, it is not surprising that a similar backbone conformation can also be assigned in the Ala_2 moiety of *cyclo*[(ϵ)Aca-Ala_1-Ala_2]. The NOE-based $d_{NH(Ala_1)\text{-}H^{\beta}(Ala_1)} = 2.5$ Å ($\sigma = 8\%$) (see Figs. 3A, 4) and the $d_{NH(Ala_1)\text{-}H^{\alpha}(Aca)} = 2.22$ Å($\sigma = 16.6\%$) distances imply $\phi_1 \approx -75°$ (see Fig. 4), which torsional angle is in perfect agreement with the results of the MD-obtained conformation ($\phi_1 \approx -60°$).

The measured 2.7 Å for $d_{NH(Ala_1)\text{-}NH(Ala_2)}$ based on $\sigma = 5\%$, which is larger than the expected value for an ideal type I β-turn ($d^{\text{type I ideal}}_{NH(Ala_1)\text{-}NH(Ala_2)} = 2.25$ Å), gives rise to the question of whether *3* may adopt more than a single conformation. Moreover, the size of the observed NOEs on the two $H^{\alpha}_{Ala_1}$ protons, followed by the selective irradiation of NH_{Ala_2} (see Fig. 3B), definitely contradicts the idea that *3* exclusively adopts a type I β-turn conformation. The quantitative conformational analysis is feasible, even if the two alanine alpha protons overlap, by solving a five-variable algebraic equation system. Assuming the presence of only two conformations, where the probability of a type I β-turn is p_1 and therefore the probability of a type II β-turn is $(1 - p_1)$, the equation system has the following form:

$$a + b = 0.121$$

$$M1^{6} = 0.7 * 1.75^{6}/a$$

$$M2^{6} = 0.7 * 1.75^{6}/b$$

$$M1^{-6} = p_1 * 3.5^{-6} + (1 - p_1) * 2.2^{-6}$$

$$M2^{-6} = p_1 * 3.0^{-6} + (1 - p_1) * 2.3^{-6},$$

using Equations 1 and 2 (see below), where M1 and M2 are the actual values of the marker distance 1 and the marker distance 2 in the two adopted conformations. In the calculated type I and type II β-turn conformations (see Table 1) these values are 3.5 Å, 2.2 Å and 3.0 Å, 2.3 Å, respectively, because the conformation insensitive interproton distance is 1.75 Å between H^{ϵ}_a and H^{ϵ}_b protons in both conformations. The selective irradiation of the H^{ϵ}_a proton in Aca resulted in a cross-relaxation rate of ≈ 0.7 on H^{ϵ}_b, and this value was used for quantification of the NOEs obtained for *3*. Therefore, the measured σ_{ref} is 0.7 (related to interproton distance = 1.75 Å) and σ_{obs} is 0.121, which is the sum of $\sigma_{Ala_2NH\text{-}Ala_1H^{\alpha}}$ and $\sigma_{Ala_2NH\text{-}Ala_2H^{\alpha}}$ (see Fig. 4). Solving the above equation system, the population for a type I β-turn was found to be 69.7%. This suggests a conformational mixture of 70% type I and 30% type II β-turn instead of the previously suggested 100% type I conformation (Bandekar et al., 1982). The interpretation of the measured NOEs clearly demonstrates that *3* simultaneously adopts at least two conformations. The fact that MD simulations carried out in acetonitrile ($\epsilon^b = 38.8$), in DMSO ($\epsilon^b = 46.7$),

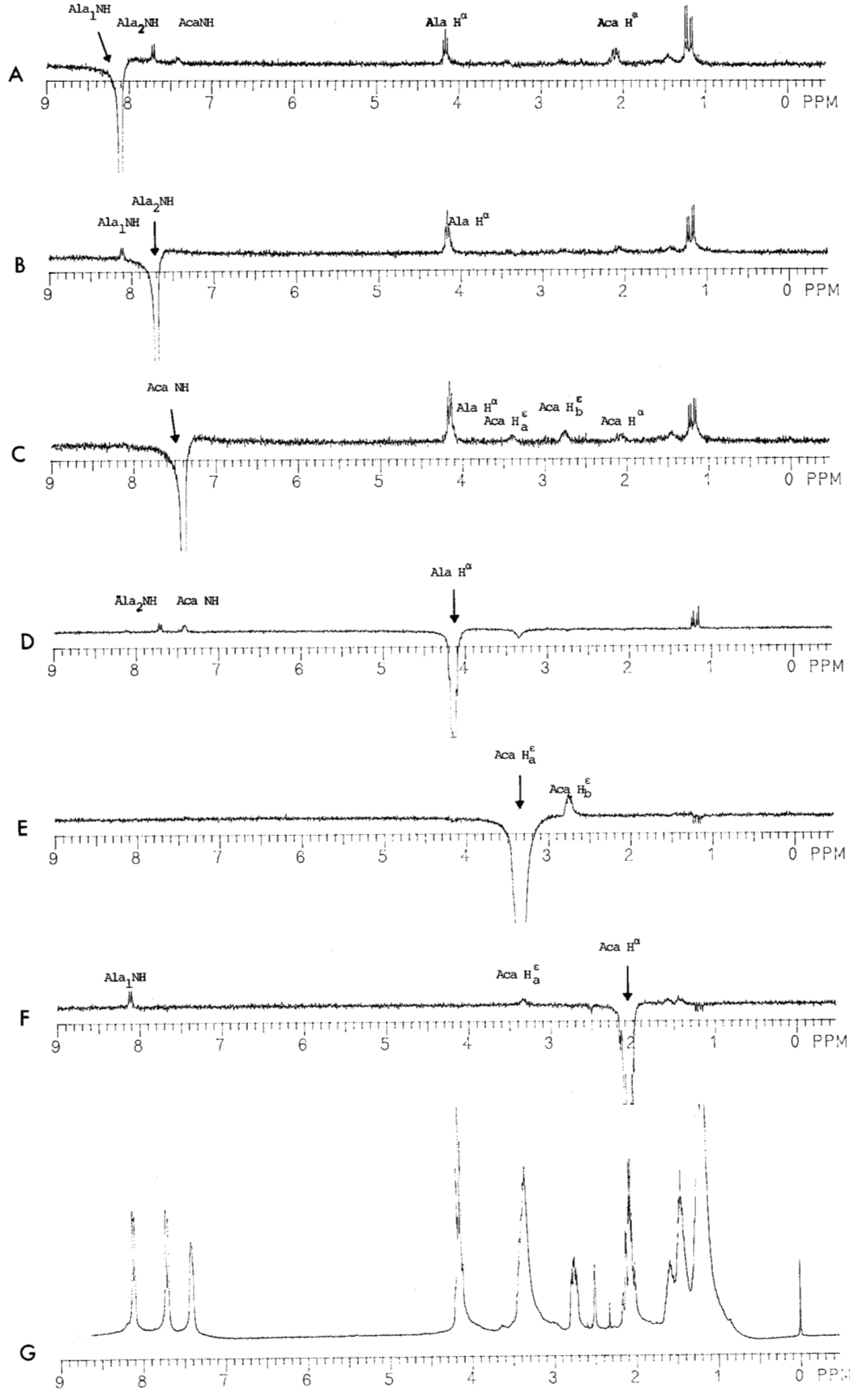


Fig. 3. One-dimensional NOE difference spectra of *cyclo*[(ϵ)Aca–Ala–Ala] in d_6-DMSO (100 atom%). **A**: Ala_1 NH saturated. **B**: Ala_2 NH saturated. **C**: Aca NH saturated. **D**: $AlaH^{\alpha}$ saturated. **E**: Aca H^{ϵ}_{a} saturated. **F**: Aca H^{ϵ}_{b} saturated. **G**: Aca H^{α} saturated. **H**: The whole proton spectra recorded at 301 °K.

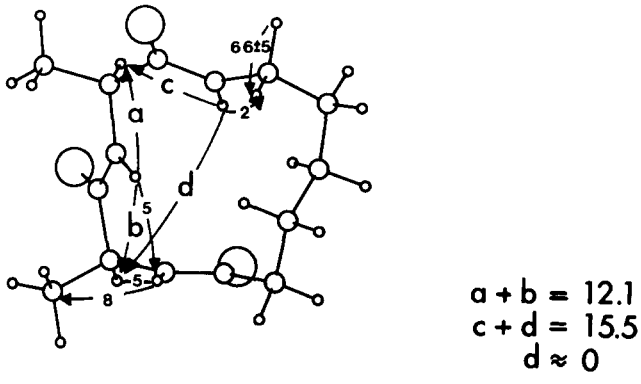


Fig. 4. The observed cross-relaxation rates multiplied by 100 between selected protons in *cyclo*[(ϵ)Aca-Ala-Ala] measured in d_6-DMSO 100.0 atom%.

and in water ($\epsilon^b = 78.5$) resulted in identical conformations probably implies that the previously published CD spectra in different solvents (Bandekar et al., 1982) also reflect the simultaneous presence of two or more conformers (see Table 1).

As shown here for *3* and suggested previously using quantitative NOE methods for some linear (Boc-Pro-Ser-$NHCH_3$, Boc-Pro-D-Ser-$NHCH_3$, Boc-Val-Ser-$NHCH_3$, Boc-Val-D-Ser-$NHCH_3$ [Perczel et al., 1991b]) and cyclic β-turn models (*cyclo*[(δ)Ava-Gly-Pro-Ser-Gly] [Perczel et al., 1991a], *cyclo*[Gly-Pro-Ala-D-Phe-Pro] [Stradley et al., 1990], *cyclo*[Gly-Pro-Asn-D-Phe-Pro] [Stradley et al., 1990]), it is very improbable that the conformational properties of the ideal type I and/or type II β-turn can be determined simply by synthesizing the perfect β-turn model. The decrease of the ring size seems to eliminate some nontypical backbone conformations (the whole conformational hypersurface may have fewer minima) but may also induce the distortion of the backbone ring. As a final trial to obtain the perfect model the introduction of the rigid proline into the $(i + 1)$ position of a β-turn was made as shown in Scheme 1. Surprisingly, the cyclization of the -(ϵ)Aca-Pro-Ala- linear fragment did not result in any monomer according to mass spectroscopy, and only the *cyclo*[(ϵ)Aca-Pro-Ala-(ϵ)Aca-Pro-Ala] was isolated. By contrast, the -(ϵ)Aca-Pro-Ser(OBzl)- peptide was successfully closed into a pseudotetra as well as a pseudoocta ring.

cyclo[(ϵ)Aca-Ala-Ala]
↓ ***first, change Ala_1 → Pro***
cyclo[(ϵ)Aca-Pro-Ala]
↓ ***second, change Ala_2 → Ser***
cyclo[(ϵ)Aca-Pro-Ser]

Scheme 1.

Quantitative cross-relaxation rate analysis of -Pro-Ser- cyclic models

Gierasch and coworkers (Stradley et al., 1990) pointed out that interproton distances can be determined on the basis of quantitative NOEs, even for multiple-spin systems (Noggle & Schirmer, 1971; Bruch et al., 1985), to an accuracy of ±0.1 Å. When the cross correlations between spins are negligible and a high irradiation power is used (Stradley et al., 1990), all dipolar interactions may be characterized by a single correlation time, and consequently the ratio between two interproton distances (d_i and d_{ref}) is

$$\left(\frac{d_i}{d_{ref}}\right)^6 = \frac{\sigma_{ref}}{\sigma_i}, \tag{1}$$

where d_i is the H_i to H_j distance, d_{ref} is the suitable interproton distance, and σ_i and σ_{ref} are the individual cross-relaxation rates via dipole-dipole interaction. The σ_{ref} value is determined as a reference, using an appropriate methylene interproton distance, $d_{ref} = 1.75$ Å, e.g., $-C^{\beta}H_2-$ of a serine side chain or a favorable $-CH_2-$ of the proline ring. If the preirradiation time is large compared to T_{1i}, then the individual cross-relaxation rates via dipole-dipole interaction (σ_i) (in the absence of solvent-solute NOE) is

$$\sigma_i = \eta_i(j)/T_{1i}, \tag{2}$$

where $\eta_i(j)$ is the enhancement of H_i when H_j is saturated and T_{1i} is the relaxation time of H_i. As mentioned above, the variation of marker distance 1 (M1) such that $d_{NH(Ser)-H^{\alpha}(Pro)}$, estimated from $\sigma_{NH(Ser)-H^{\alpha}(Pro)}$, is related to ψ_{Pro}. The larger the observed σ is, the closer the ψ_{Pro} is to the value of 120° (cf. Fig. 5A). On the other hand the $d_{NH(Ser)-H^{\alpha}(Ser)}$ marker distance 2 (M2) obtained from $\sigma_{NH(Ser)-H^{\alpha}(Ser)}$ is related to ϕ_{Ser} (cf. Fig. 5B). In both cases, the function [$f = f$(torsion)] has single minima and maxima (cf. Fig. 5A,B), but in the first case [$f = f(\psi_{i+1})$] the ^1H-{^1H}-NOE cannot be measured in the $-150° < \psi_{i+1} < +20°$ region (cf. Fig. 5A). The less frequently used marker distance 3 (cf. Fig. 5C) depends on both ϕ and ψ torsions (see Fig. 5C). The equidistant area where the M3 is shorter than 3 Å is a relatively broad conformational valley; therefore, due to the ambiguous ψ and ϕ assignment, the M3 is less frequently used for conformational identification.

The measured interproton distances in a conformational mixture are the weighted sum of those marker distances, which may be determined from the pure conformers:

$$\frac{1}{r_i^6} = \sum_{j=1}^{\text{all conformers}} p_j \frac{1}{r_{ij}^6}, \tag{3}$$

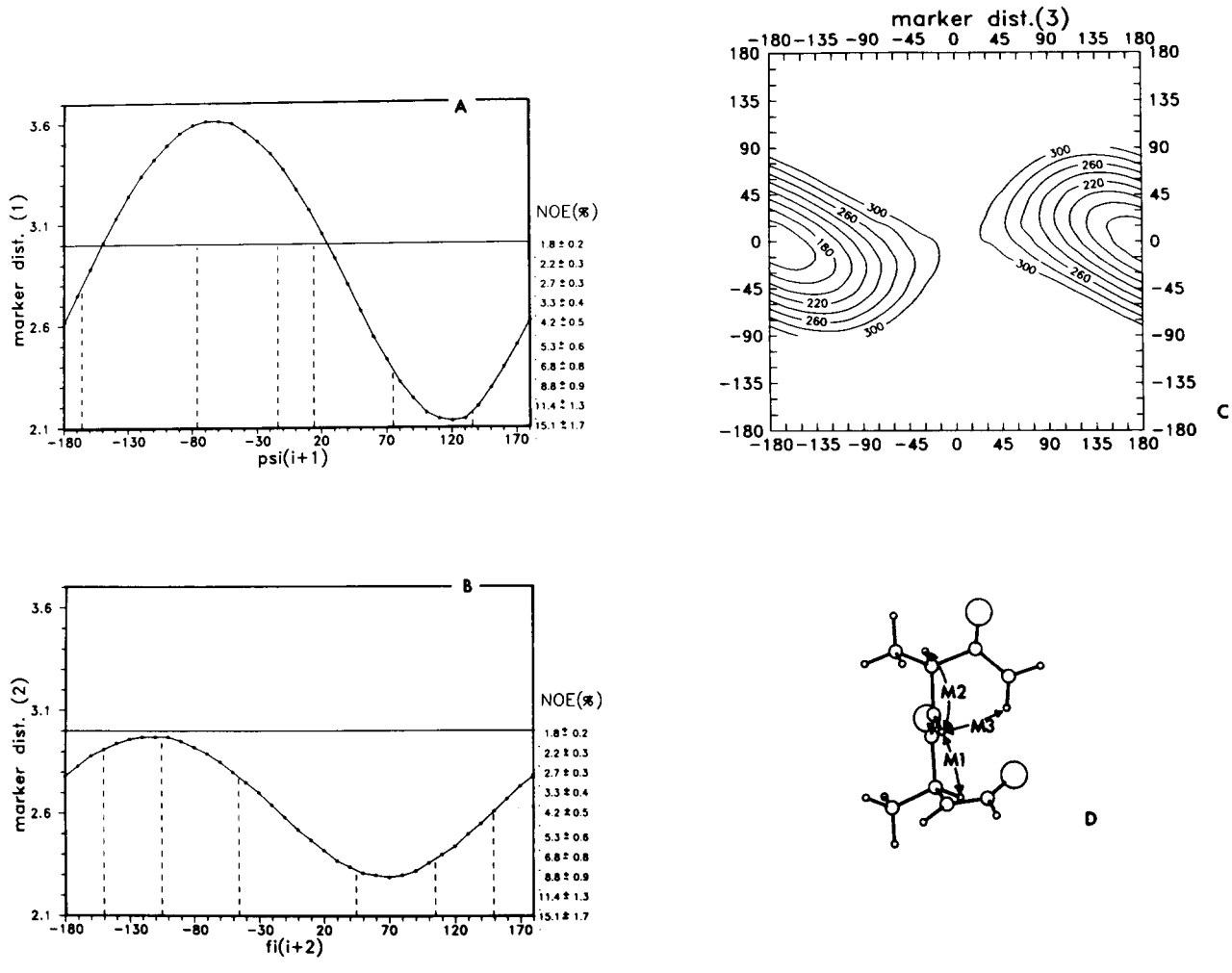


Fig. 5. The backbone torsional angle dependence of the marker distances. **A**: Calculated NH_{Ser}-H^{α}_{Pro} distance (Marker 1) ψ_{i+1} dependence. The distance equivalent NOE%s were calculated, with $T_1 = 1$ s according to Equation 2. **B**: Calculated NH_{Ser}-H^{α}_{Ser} distance (Marker 2) ϕ_{i+2} dependence. The distance equivalent NOE%s were calculated with $T_1 = 1$ s according to Equation 2. **C**: Calculated NH_{Ser}-NH_{i+3} distance (Marker 3) ϕ_{i+2}, ψ_{i+2} dependence. The equidistant areas are marked below 3.00 Å. **D**: The schematic representation of M1, M2, and M3.

where p_j is the probability of the jth conformation where the analyzed interproton distance is r_{ij} (Noggle & Schirmer, 1971; Stradley et al., 1990). Based on the MD calculations, the resulting marker distances found for *cyclo*-pseudotetrapeptides are reported in Table 1 for type I (M1(Ser NH – Pro H^{α}) ≃ 3.5 Å, M2(Ser NH – Ser H^{α}) ≃ 3.0 Å) and for type II β-turns (M1(Ser NH – Pro H^{α}) ≃ 2.3 Å, M2(Ser NH – Ser H^{α}) ≃ 2.3 Å). Using these marker distances and assuming the maximum number of conformers, the population of the two β-turns can be estimated. As shown in Figure 6A–D for two conformers, the actual value of a marker distance (M1 or M2) has a basic influence on p_j. By changing the range (maximum and minimum values) of M1 and/or M2 the calculated conformational weights are different (see Fig. 6A–D). If the models were sharing only the two β-turn conformations (type I and type II), then the conformational percentages obtained from any of the two marker distances (M1 and M2) would be similar. Any significant alteration of the calculated weights (using M1 and M2) could originate from an inconsistent experimental result or from the presence of more than two conformations. As demonstrated previously for *3*, an algebraic equation system can always be used if $(n - 1)$ independent marker distances can be measured for the conformational mixture having n components.

As suggested by MD simulations, models having a pseudotetrapeptide size (*1–3*), due to the internal ring stress, adopt only two combinations of ψ_{Pro} and ϕ_{Ser} torsional angle values close to type I and type II β-turn conformations (cf. Table 1). The identity of the NOE-determined conformational weights in acetonitrile, using M1 or M2 for *1* or *2* (see Table 3), suggests that only two conformers are present: one close to a type I and

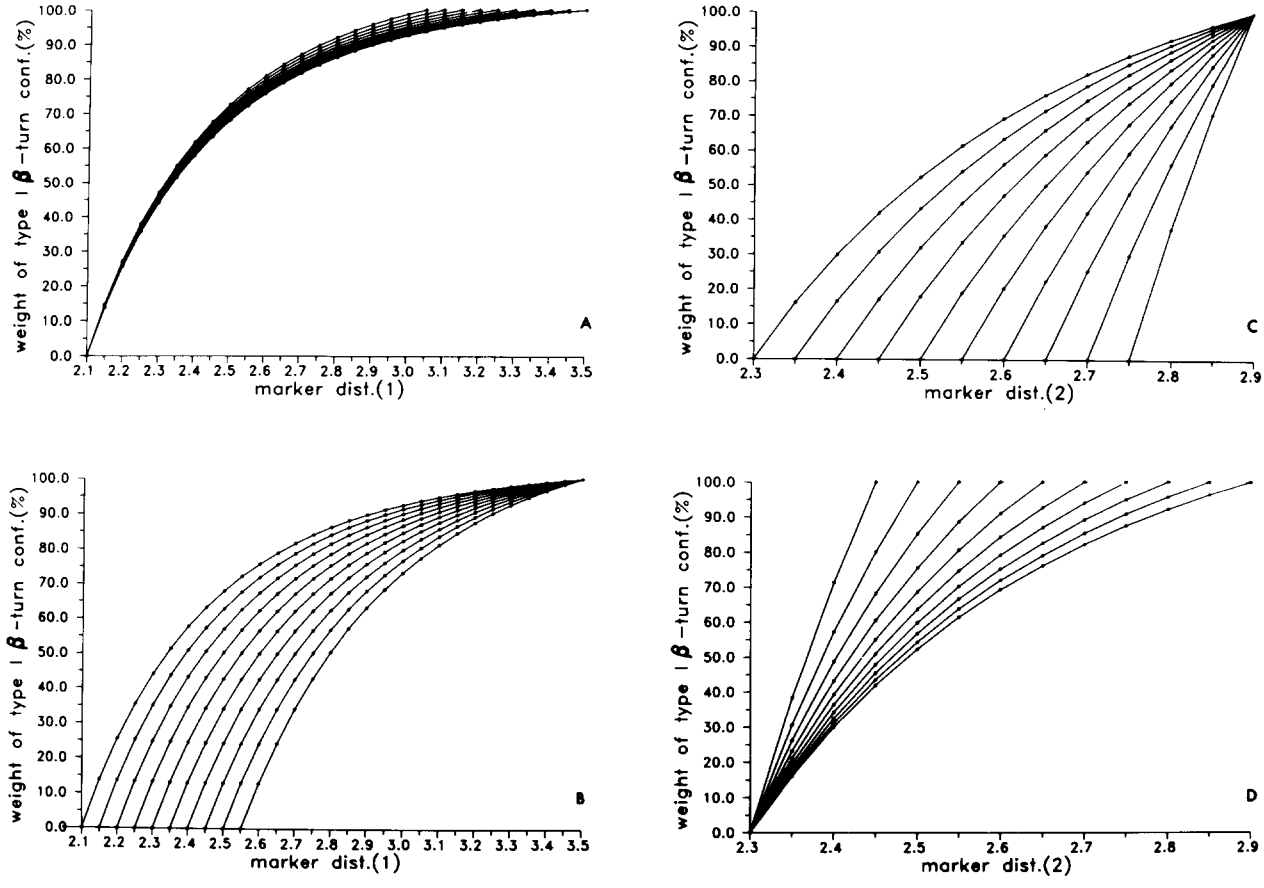


Fig. 6. Calibration curves for type I β-turn percentage determination from the NOE distance, using $(r_{NOE})^{-6} = p_{type\ I}(r^{mark}_{type\ I})^{-6} + (1 - p_{type\ I})(r^{mark}_{type\ II})^{-6}$ equation. **A**: Marker distance 1 was used with 3.1 Å $\leq r_{type\ I} \leq$ 3.50 Å, $r_{type\ II}$ = 2.1 Å. **B**: Marker distance 1 was used with 2.1 Å $\leq r_{type\ II} \leq$ 2.55 Å, $r_{type\ I}$ = 3.5 Å. **C**: Marker distance 2 was used with 2.3 Å $\leq r_{type\ II} \leq$ 2.75 Å, $r_{type\ I}$ = 2.9 Å. **D**: Marker distance 2 was used with 2.45 Å $\leq r_{type\ II} \leq$ 2.90 Å, $r_{type\ II}$ = 2.3 Å.

the other similar to a type II β-turn. For the two previously reported pseudohexapeptides *cyclo*[(δ)Ava-Gly-Pro-Ser(OX, X = H or ^{t}Bu)-Gly] (Perczel et al., 1991a) the small deviations ($\pm$7%) of the M1- and M2-determined conformational weights can be an indication that more than two conformers may be present. As suggested by MD simulations when $\psi_{Pro} = -45°$, the ϕ_{Ser} has the two values, $-60°$ and/or $-135°$, respectively, yielding the overall type I β-turn conformation for *cyclo*[(δ)Ava-Gly-Pro-Ser-Gly]. These observations harmonize perfectly with results obtained for *4*, which is the dimer of *1* with a C_2 internal symmetry (see Figs. 7, 8). The quantitative analysis of the one-dimensional NOE difference spectra of *4* shows that when the Ser NH was selectively saturated (Fig. 9A) enhancement was observed on Ser H^{α} and on Pro H^{α}, respectively. The M1 and M2 distances found for *4* yield slightly different conformational weights, which may again indicate that more than two conformers are present. Using the M3 distance value based on the value of $\sigma_{NH(Ser)/NH(Aca)}$ (10.4%), the calculated appropriate distance is 2.25 Å. The equidistant level 2.2-2.3 Å, as shown on Figure 5C, may be related with several ϕ, ψ torsional angle pairs, therefore M3 can only ambiguously assign conformations when more than one conformer is present. Due to the conformational assignment based on M1 and M2, *4* predominantly adopts a type I β-turn (see Table 3), presuming a $\phi_{Ser} \approx -80°$, $\psi_{Ser} \approx -25°$, which is also possible on the basis of M3. No ^{1}H-{^{1}H}-NOEs were obtained for *5* in d_6-DMSO (100% atom). Cross-relaxation rates obtained for *6* in d_6-DMSO are reported in Figure 10.

Solution state conformations on the basis of CD spectra

Circular dichroic spectra of the herein-reported peptides originate from the relative orientation of the amide planes around chiral centers (cf. Fig. 2B). By assuming the additivity of the CD contribution of those conformations, the measured CD spectra are the weighted sum of the pure conformer's CD $g_i(\lambda)$:

$$f(\lambda) = \sum_{i=1}^{\text{all conformers}} p_i * g_i(\lambda) + \text{noise}, \qquad (4)$$

Table 3. *Comparison of quantitative NOE-determined conformational weights (using MD conformations) with circular dichroism spectra deconvolution-determined conformational weights in acetonitrile for various compounds*

Compound[a]	Selected interprotons	NOE: Measured distance (Å)	NOE: Marker distance values[b] (Å)		NOE: Type I (%)	NOE: Type II (%)	CD: $p_1 + p_4$[c] (%)	CD: $p_2 + p_3$[d] (%)
A	NH_{Ser}-H^{α}_{Ser}	2.78	2.9	2.3	89 ± 3	11 ± 3	100	0
	NH_{Ser}-H^{α}_{Pro}	3.17	3.5	2.3	88 ± 2	12 ± 2		
B	NH_{Ser}-H^{α}_{Ser}	2.64	2.9	2.3	74 ± 1	26 ± 1	70	30
	NH_{Ser}-H^{α}_{Pro}	3.17	3.5	2.3	72 ± 2	28 ± 1		
C	NH_{Ser}-H^{α}_{Ser}	2.68	2.9	2.2	83 ± 2	17 ± 2	92	8
	NH_{Ser}-H^{α}_{Pro}	3.03	3.5	2.25	87 ± 4	13 ± 4		
E	NH_{Ser}-H^{α}_{Ser}	2.70	2.9	2.3	82	18	77	23
	NH_{Ser}-H^{α}_{Pro}	2.70	3.5	2.15	78	22		
F	NH_{Ser}-H^{α}_{Ser}	2.50	2.9	2.3	40 + 5	60 + 5	65	35
	NH_{Ser}-H^{α}_{Pro}	2.30	3.5	2.15	53	47		
G	NH_{Ser}-H^{α}_{Ser}	3.00	3.0	2.3	100	0	43	57
	NH_{Ser}-H^{α}_{Pro}	2.70	3.45	2.2	72	28		
H	NH_{Ser}-H^{α}_{Ser}	2.85	2.25	2.9	7	93	16	84
	NH_{Ser}-H^{α}_{Pro}	2.30	3.45	2.25	3	97		

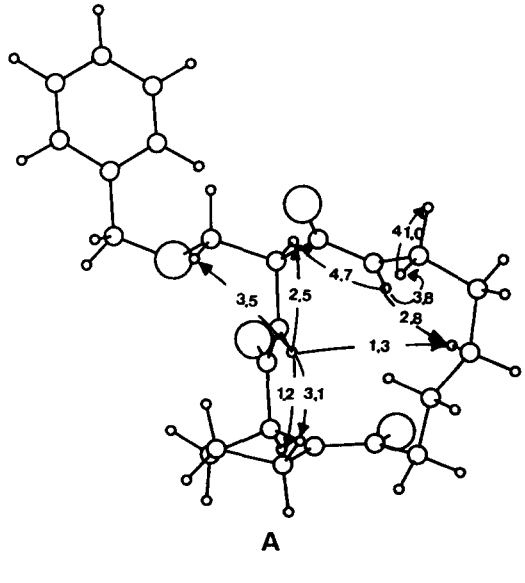
[a] A, *cyclo*[(ϵ)Aca-Pro-Ser(OBzl)]; B, *cyclo*[(ϵ)Aca-Pro-Ser]; C, *cyclo*[(ϵ)Aca-Pro-Ser(OBzl)-(ϵ)Aca-Pro-Ser(OBzl); D, *cyclo*[(ϵ)Aca-Pro-Ser-(ϵ)Aca-Pro-Ser]; E, *cyclo*[(δ)Ava-Gly-Pro-Ser(O^{t}Bu)-Gly]; F, *cyclo*[(δ)Ava-Gly-Pro-Ser(OH)-Gly]; G, Boc-L-Pro-L-Ser-$NHCH_3$; H, Boc-L-Pro-D-Ser-$NHCH_3$. D was not soluble in CD_3CN.

[b] The values are from fully optimized structures, where input geometry was taken from Table 1.

[c] Summary weights of pure component CD curves 1 and 4.

[d] Summary weights of pure component CD curves 2 and 3.

where $f(\lambda)$ is the measured CD curve and p_i is the weight of $g_i(\lambda)$. Because CD reflects the overall conformation, one cannot simply correlate the measured CD spectra with any structure directly. As mentioned previously, up to the present, all synthesized β-turn models exhibited more than a single conformation in all solvents. Therefore, all reported spectra were obtained by measuring conformational mixtures. Acknowledging that type I



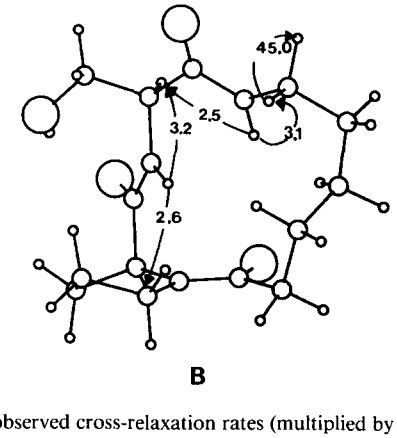


Fig. 7. The observed cross-relaxation rates (multiplied by 100) between selected protons in *cyclo*[(ϵ)Aca-Pro-Ser(OBzl)] (**A**) and *cyclo*[(ϵ)Aca-Pro-Ser] (**B**) in CD_3CN. The arrow points from the saturated proton to the proton with enhanced intensity. The marked $\sigma * 100$ is calculated according to Equation 2.

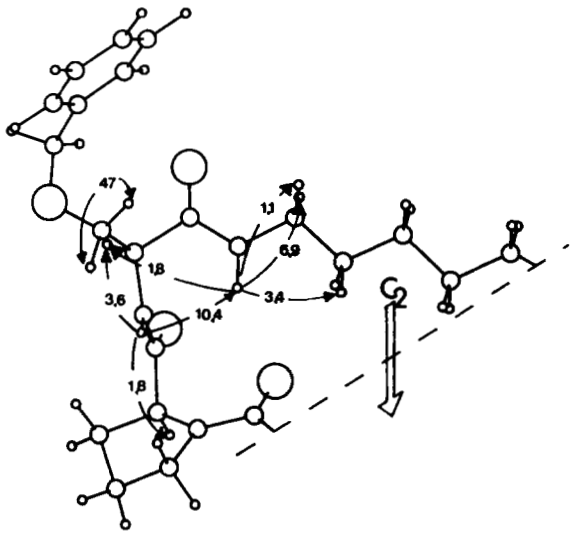


Fig. 8. The observed cross-relaxation rate (multiplied by 100) between selected protons in *cyclo*[(ε)Aca-Pro-Ser(OBzl)-(ε)Aca-Pro-Ser(OBzl)] in CD_3CN. The arrows are between NOE interrelated protons. The interaction is expressed in $\sigma * 100$ according to Equation 2. Due to the C_2 type internal symmetry of the model compound only half of the molecule is shown.

and type II β-turns are always found simultaneously, it was decided herein to solve the problem utilizing a CD spectra deconvolution method (Perczel et al., 1989, 1991b). This procedure operates conversely to a linear combination, aiming to determine simultaneously the weights p_i and the pure component curves $g_i(\lambda)$. Such a minimization is feasible when a set of measured CD spectra ($f[\lambda]$) is simultaneously analyzed:

$$\left\{\left[\sum_{j=1}^{N} f_j^m(\lambda) - \sum_{j=1}^{N} f_j^c(\lambda)\right]^2 = \left[\sum_{j=1}^{N} f_j^m(\lambda) - \sum_{j=1}^{N}\sum_{i=1}^{P} p_{ij} * g_i(\lambda)\right]\right\}^2 \rightarrow \text{minimized.} \tag{5}$$

Assuming that all other determining factors of CD, such as temperature, solvent shift, concentration, number of chromophores, etc., are constant, the resulting pure CD conformational curve and weight matrix are related to the amide conformations because the conformation is the unique variable.

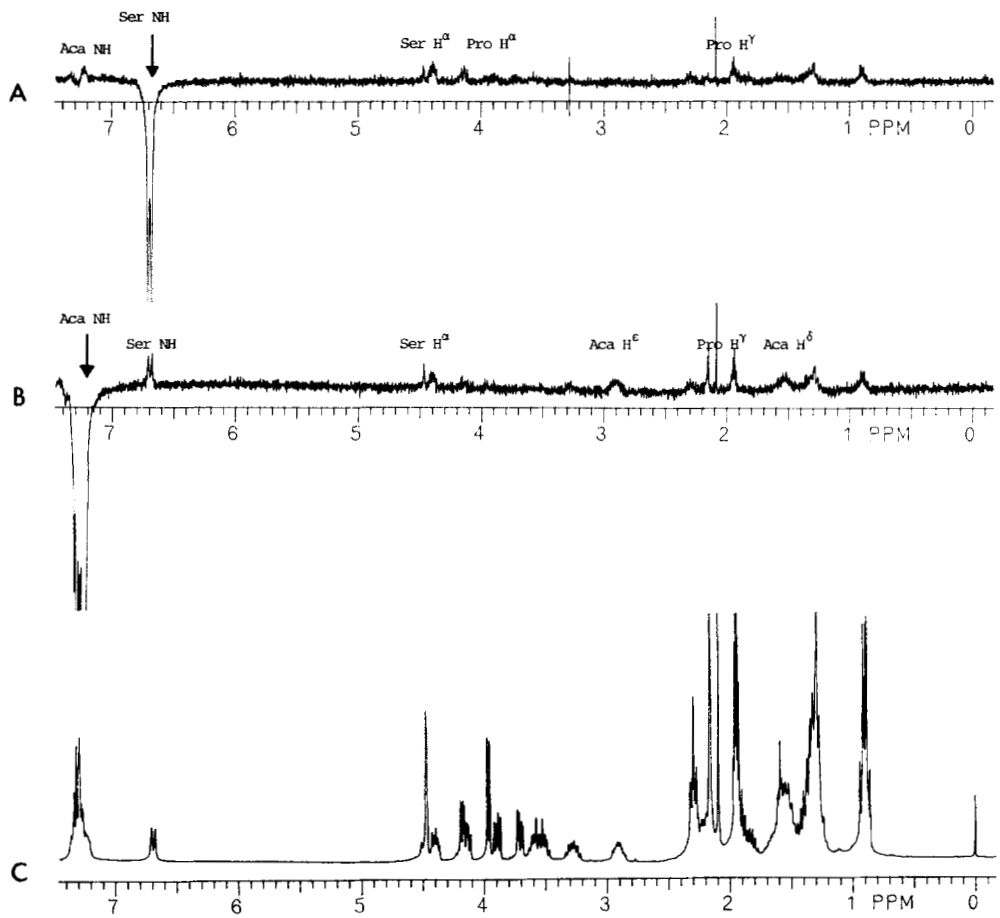


Fig. 9. The one-dimensional NOE difference spectra of *cyclo*[(ε)Aca-Pro-Ser(OBzl)-(ε)Aca-Pro-Ser(OBzl)] in CD_3CN. **A**: Ser NH is saturated. **B**: Aca NH (and the aromatic protons) are saturated. **C**: The whole proton spectra at 300 °K.

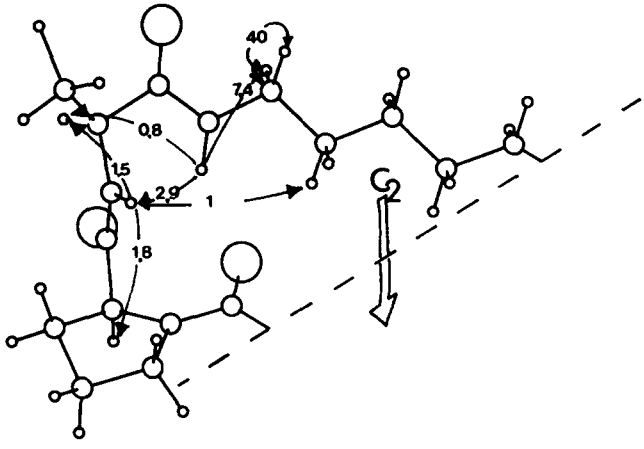


Fig. 10. The observed cross-relaxation rate (multiplied by 100) between selected protons in *cyclo*[(ϵ)Aca-Pro-Ala]$_2$ in d_6-DMSO. Due to the internal C_2 symmetry of the molecule only half of the model is reported. The arrows are between NOE interconnected protons.

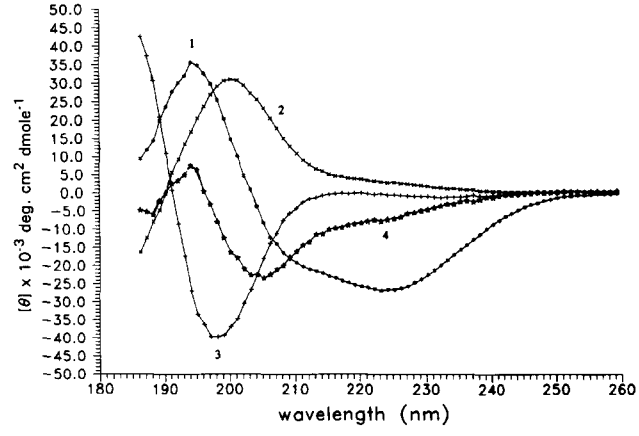


Fig. 11. The four component CD spectra resulting from deconvolution. Components 1 and 4 are the two forms of type I β-turns, component 2 is related to the type II β-turn, and component 3 is generally interpreted as the CD spectra of open conformation(s).

The target of the herein-reported deconvolution was the CD spectra of 14 β-turn models recorded on the same instrument at the same solvent at the same temperature. Using the resulting conformational weights (cf. Table 4) and the appropriate pure component curves (cf. Fig. 11), any measured $f_j^m(\lambda)$ spectra can be reconstructed:

$$f(\lambda) = p_1 * g_1(\lambda) + p_2 * g_2(\lambda) + P_3 * g_3(\lambda) + p_4 * g_4(\lambda), \quad (6)$$

with a given tolerance (for the accuracy of the fitted CD spectra of a -Pro-Ser- model, see Fig. 12).

The assignment of the four pure component curves was made by using NOE data and by comparing the shape of the pure component curves to literature data. The computer-assisted conformational deconvolution-obtained weights were compared with NOE-determined conformational percentages (cf. Table 3). Circular dichroic spectra indicate the presence of two type I β-turns, a type II β-turn-like conformation, and a nontypical conformation.

Table 4. *Conformational weights of 14 β-turn models recorded on the same instrument in the same solvent (CH_3CN) at the same temperature (22 °C)*[a]

	Model[b]													
	1	2	3	4	5	6	7	8	9	10	11	13	14	15
p_1	22	14	20	16	24	19	0	17	0	10	0	94	18	70
p_2	4	17	22	0	8	9	22	84	79	0	18	0	8	8
p_3	23	40	55	50	68	64	50	0	5	23	16	0	0	22
p_4	50	28	3	34	0	8	28	0	16	67	67	6	74	0

[a] For the shape of the 4 pure conformational curves having p_1, p_2, p_3, and p_4 contributions see Figure 11. For models see Table 13.

[b] Models 12, 16, 17, and 18 (see Table 13) were not soluble in 100% acetonitrile.

The sum of $p_1 + p_4$, which is thought to be the global type I folded conformation, can be correlated accurately with the NOE-determined conformational weights for the same conformational type. The dominance of the type I β-turn was suggested by NOEs (89 ± 3%), which was confirmed by CD (94 ± 6%) for *1* (see Table 3). The unprotected side-chain-containing cyclic structures (*cyclo*[(ϵ)Aca-Pro-Ser] [*2*]) have a significantly higher type II β-turn content (≈30%) than *1*, which was also confirmed by the NOE measurement (≈27%). The *cyclo*[(δ)Ava-Gly-Pro-Ser(O^{t}Bu)-Gly] yielded a class C CD spectrum, which is composed of 76.5% of the global type I conformation in accordance with the NOE-determined value of ≈80%. *Cyclo*[(δ)Ava-Gly-Pro-Ser-Gly] is made up of 65% of type I conformation on the basis

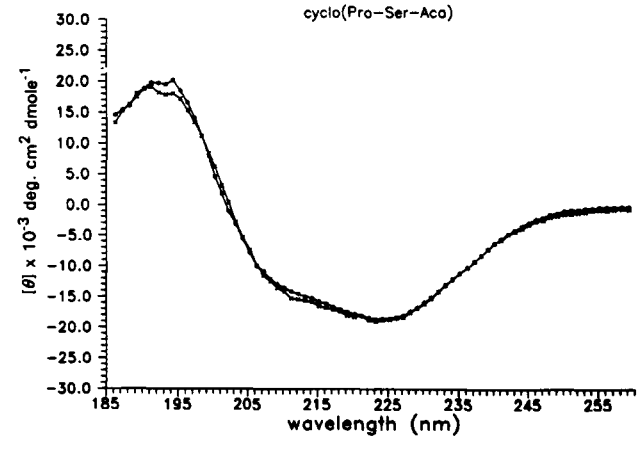


Fig. 12. The measured and the calculated CD spectra of *cyclo*[(ϵ)Aca-Pro-Ser]. Calculated curves are obtained from a linear combination, where the weights and the curves are from the deconvolution. (For weights see Table 4 and for the pure component curves see Fig. 11.) For the calculation of [θ] the reduced molecular weights were used. (For values see Table 13.) Other model CD spectra are on deposit.

of CD deconvolution, which is slightly underestimated by the NMR method (M1 results yield 53%, whereas M2 yields 40 + 5%). As suggested previously, the strong marker distance dependence of the conformational weights indicates more complex conformational problems.

When these β-turn models were designed, the main attention was focused on the type I conformation. Therefore, not surprisingly, the resulting CD deconvolution data concerning this conformational type are more detailed. In fact, two class C pure component spectra were found during analysis: component curves 1 and 4 (see Fig. 11). Both have an exiton-couplet near 200 nm (positive band below 196 nm; negative band near 206 nm) and a negative $n\pi^*$ transition near 222 nm, fitting into the class C category (Woody, 1974, 1985). As mentioned above, the sum of the two pure components $p_1 + p_4$ correlates with NOE-determined type I conformations. The significant difference between these two curves of related structures must be due to the orientation of the third amide plane. As found by MD calculations, the average value of ψ_{Ser} is around 0°, previously suggested by Venkatachalam (1968). However, this average is the sum of ψ_{Ser} torsional values around −45° and +45°, which observation was confirmed by the recent analysis of Wilmot and Thornton (1988). As found for *cyclo*[(ϵ)Aca-Ala-Ala], the $d_{NH(Aca)-NH(Ala_2)} = 2.25$ Å is theoretically related with $\psi_{Ser} \approx 80°$ (or $\psi_{Ser} \approx 160°$) values (cf. Fig. 5A). (However, no cyclic pseudotetrapeptide conformation can have $\psi_{Ser} \approx 160°$.) In *cyclo*[(ϵ)Aca-Pro-Ser(OX, X = H or Bzl)] the appropriate $d_{NH(Aca)-NH(Ser)}$ was around 2.8 Å, which implies a $\psi_{Ser} \approx +45°$. The fact that a p_1 conformation is dominant only in *cyclo*-pseudotetrapeptides, where the ring stress distorts the third amide plane to a value around $\psi_{Ser} \approx +60°$, explains this observation. By contrast, when the backbone ring stress is negligible (all other β-turn models reported in Table 4), this distorted type I β-turn ($\phi_{Pro} = -60°$, $\psi_{Pro} = -30°$, $\phi_{Ser} = -90°$, $\psi_{Ser} = +60°$) has a minimum contribution. This was observed, for example, in the CD spectra deconvolution of *cyclo*[(δ)Ava-Gly-Pro-Ser(O^{t}Bu)-Gly] ($p_1 = 67\%$, $p_4 = 10\%$), which also agrees with the results of the solid-state conformation analysis (Perczel et al., 1991a), yielding $\psi_{Ser} = 4.5°$. (One must note the extreme sensitivity of CD toward a small conformational distortion.)

Pure component CD spectrum 2 (see Fig. 11) resembles those correlated with the type II β-turn conformation and labeled as class C′ according to Woody (1974, 1985). This component has a significant contribution to the overall CD spectra only for the two -L-D- models (see Table 4). The dominant conformation (type II) on the basis of CD (≈80%) was also predominantly found on the basis of NOE experiments (≈90%) (cf. Table 4). Pure component 3, having a single minimum below 200 nm, is usually assigned to the open or "Z" conformation. The fact that in cyclopeptides the contribution of this conformer is weak or negligible agrees with such an explanation. For linear models, the analyzed weight (p_3) of this conformer is higher, demonstrating that in noncyclic models, the open conformation may always be present as the largest amount of the various conformations.

Conclusions

It has been demonstrated that the two major forms of β-turns (type I and type II) are interrelated. In solution both forms are simultaneously present (a conformational mixture) and observed. The mobility demonstrated herein may provide a key role for the hairpin conformation in proteins (Sundaralingham & Sekharudu, 1989). By conformational deconvolution the pure CD spectra of these two conformers were obtained. Using these pure component curves and their conformational weights, it is now possible to reconstruct (using Equation 6), with high accuracy, the originally measured CD spectra of the models. The conformational weights, calculated from the CD spectra, were found to be similar to those calculated from NMR. Due to the fact that the CD-obtained weights and the four pure component CD curves are determined from the same procedure, the CD-established component spectra have been verified by NOE experiments. The two independent quantitative methods (NMR and CD) yielded a very similar conformer distribution for the investigated models. Therefore, the resulting CD conclusions can be accepted with a high degree of confidence.

Materials and methods

Synthesis

Classical solution techniques were used to prepare the cyclic compounds (Bodanszky & Bodanszky, 1984) with chemicals purchased from Calbiochem, Sigma Chemical, and Schweizerhall. *Cyclo*[(ϵ)Aca-Ala-Ala] (*3*) was synthesized as described earlier (Bandekar et al., 1982). Other bridged cyclopeptides (*1, 2, 4-6*) were synthesized according to Scheme 2, by coupling with the mixed anhydride method (Anderson et al., 1967) and deprotecting using standard conditions (1.8 mol/L HCl/CH_3OH) (Bodanszky & Bodanszky, 1984).

Boc-Pro-Ser(OBzl)-(ϵ)Aca-ONSuc was prepared from 0.4 mmol of Boc-Pro-Ser(OBzl)-(ϵ)Aca-OH and 0.5 mmol of HONSuc in anhydrous acetonitrile (8–10 mL) in the presence of 212 mg of morpho CDI, as suggested for similar compounds by Bandekar et al. (1982). After 6 h, the solution mixture was evaporated in vacuo, the remaining residue was dissolved in EtOAc, and washed with water and 5% of $NaHCO_3$. The solution was dried over $MgSO_4$, filtered, and the organic solvent was evaporated, resulting in a crude oil (approximate yield 80%) R_f(EtOAc:Puff/9:1) = 0.55. The product was analyzed by ^{13}C spectroscopy recorded in $CDCl_3$ at ambient temperature: C'_1 (171.64), C'_2 (169.57), C'_3 (169.14), C'_4 (168.45), C'_5 (155.73), C^t_{Bu} (80.93), C_{Bzl} (73.16), C^{β}_{Ser} (69.36), C^{α}_{Pro} (61.09), C^{α}_{Ser} (52.98), C^{δ}_{Pro} (47.35), C^{ϵ}_{Aca}

Boc-Ser(OBzl)-OH $HCl \cdot H$-(ϵ)Aca-OCH_3

↘ *coupling* ↙

Boc-Ser(OBzl)-(ϵ)Aca-OCH_3

deprotection ↓

Boc-Pro-OH $HCl \cdot H$-Ser(OBzl)-(ϵ)Aca-OCH_3

↘ *coupling* ↙

Boc-Pro-Ser(OBzl)-(ϵ)Aca-OCH_3

deprotection ↓

Boc-Pro-Ser(OBzl)-(ϵ)Aca-OH

activation ↓

Boc-Pro-Ser(OBzl)-(ϵ)Aca-ONSuc

deprotection ↓

$F_3CCOOH \cdot H$-Pro-Ser(OBzl)-(ϵ)Aca-ONSuc

cyclization ↓

cyclo[(ϵ)Aca-Pro-Ser(OBzl)] *cyclo*[(ϵ)Aca-Pro-Ser(OBzl)-(ϵ)Aca-Pro-Ser(OBzl)]

↓ ***deprotection*** ↓

cyclo[(ϵ)Aca-Pro-Ser] *cyclo*[(ϵ)Aca-Pro-Ser-(ϵ)Aca-Pro-Ser]

Scheme 2.

(39.23), C^{α}_{Aca} (30.72), C^{β}_{Pro} (29.76), C^{δ}_{Aca} (28.57), C_{Bu} (28.17), C^{γ}_{Pro} (25.79), C_{Suc} (25.51), C^{β}_{Aca} (24.49), C^{γ}_{Aca} (24.16).

To prepare H-Pro-Ser(OBzl)-(ϵ)Aca-ONSuc·CF_3COOH, Boc-Pro-Ser(OBzl)-(ϵ)Aca-ONSuc (2.5 mmol) was dissolved in 6.6 mL of CF_3COOH at 0 °C. After 20 min the solution was evaporated in vacuo, and the remaining oil was solidified under absolute ether. R_f(EtOAc: Puff/4:1) = 0.72. The product was analyzed by ^{13}C spectroscopy recorded in $CDCl_3$ at ambient temperature: C'_1 (169.62), C'_2 (169.47), C'_3 (168.67), C'_4 (168.46), C_{Bzl} (73.37), C^{β}_{Ser} (69.95), C^{α}_{Pro} (59.49), C^{α}_{Ser} (53.47), C^{δ}_{Pro} (46.68), C^{ϵ}_{Aca} (39.29), C^{α}_{Aca} (30.77), C^{β}_{Pro} (30.12), C^{δ}_{Aca} (29.65), C^{γ}_{Pro} (28.39), C_{Suc} (25.51), C^{β}_{Aca} (24.28), C^{γ}_{Aca} (24.03).

To produce *cyclo*[(ϵ)Aca-Pro-Ser(OBzl)] (*1*) and *cyclo*[(ϵ)Aca-Pro-Ser(OBzl)-(ϵ)Aca-Pro-Ser(OBzl)] (*4*), H-Pro-Ser(OBzl)-(ϵ)Aca-ONSuc·CF_3COOH (1 g) was dissolved in absolute DMF (5 mL) and added drop-wise to constantly stirred absolute pyridine (400 mL). After stirring for 4 h, 1 g of DCCI was added to the solution. After 240 h, the solution was evaporated and the residual crude powder was dissolved in CH_3OH:H_2O (1:1) and DCU was partially filtered off. The product was crystallized from absolute ethanol and the dimer (*4*) (M^+ = 774.6) was obtained (yield 7%). For NMR data on *4* see Tables 5 and 6. The solution (ethanol) was evaporated in vacuo, and the remaining residue was flash chromatographed on a Kieselgel 60 column using EtOAc:hexane (7:3). The monomer (M^+ = 387.3) was recrystallized from absolute ethanol (yield 9%). The cyclic peptides were finally purified by high performance liquid chromatography (HPLC) and analyzed by mass spectroscopy (EI and CI methods) as well as NMR methods (cf. Tables 5, 7). The HPLC was run on an Ultrasphere-ODS 5 μ column (4.6 × 250 mm ALTEX) using the gradient method (A = 1,000 mL H_2O plus 1 mL TFA, B = 1,000 mL CH_3CN plus 1 mL TFA) at a flow rate of 1 mL/min

Table 5. *^{13}C NMR data on various compounds*

		Compound[a]				
		1	*2*	*4*	*5*	*6*
Pro	α	62.88	62.26	62.84	60.51	61.37
	β	29.26	30.06	29.33	29.41	29.73
	γ	25.29	26.85	25.37	26.36	26.53
	δ	47.49	47.75	47.64	46.85	47.01
Ser	α	52.16	53.36	53.78	55.86	48.05[b]
	β	68.98	69.63	68.15	61.37	17.11[c]
CH_2^{Bzl}	δ	72.58	72.77	–	–	–
Ava	α	34.22	34.46	34.23	33.75	33.89
	β	21.60	25.11	21.73	24.46	24.55
	γ	23.65	25.03	23.08	24.42	24.49
	δ	26.60	29.85	26.49	29.61	29.54
	ϵ	38.66	39.76	37.46	[d]	37.2[d]
C′	1	174.58	174.36	174.74	172.91	172.90
	2	172.33	172.08	172.80	172.06	171.67
	3	169.63	169.48	171.34	169.48	171.55

[a] *1*, *cyclo*[(ϵ)Aca-Pro-Ser(OBzl)] in CD_3CN; *2*, *cyclo*[(ϵ)Aca-Pro-Ser(OBzl)-(ϵ)Aca-Pro-Ser(OBzl)] in CD_3CN; *4*, *cyclo*[(ϵ)Aca-Pro-Ser] in CD_3CN; *5*, *cyclo*[(ϵ)Aca-Pro-Ser-(ϵ)Aca-Pro-Ser] in d_6-DMSO; *6*, *cyclo*[(ϵ)Aca-Pro-Ala-(ϵ)Aca-Pro-Ala] in d_6-DMSO.

[b] C^{α}_{Ala}.

[c] C^{β}_{Ala}.

[d] Chemical shift assignment is tentatively due to the overlap with d_6-DMSO.

Table 6. *300-MHz ^1H NMR data on cyclo[(ϵ)Aca-Pro-Ser(OBzl)-(ϵ)Aca-Pro-Ser(OBzl)] in CD_3CN*

	Pro[a]	Ser[b]	Aca	
δ_{NH} (ppm)	–	6.69 (0.52)[c]	7.23 (0.54)	δ_{NH} (ppm)
δH^{α} (ppm)	4.13 (1.10)	4.34 (1.10)	3.23 (0.94) 2.85 (0.52)	δH^{ϵ} (ppm)
δH^{β} (ppm)	2.18 (0.83) 1.79 (0.54)	3.86 (0.44) 3.66 (0.44)	1.48 (0.44)	δH^{δ} (ppm)
$^3J_{HN-\alpha H}$ (Hz)	–	8.7	1.22 (0.46)	δH^{γ} (ppm)
$^3J\alpha_{H-\beta H}$ (Hz)	6.3 8.8	4.0 5.5	1.46 (0.43) 1.55 (0.44)	δH^{β} (ppm)
$^2J\beta_{H-\beta H}$ (Hz)	–	9.7	2.26 (0.44)	δH^{α} (ppm)

[a] Pro 1.87 (0.60); δH^{δ} (ppm) of Pro 3.55 (0.44), 3.47 (0.43).
[b] δCH_2 (ppm) of Bzl 4.42 (0.95); δCH (ppm) of Bzl ≃ 7.34; δH^{γ} (ppm).
[c] T_1 relaxation time, seconds (in parentheses).

Table 8. *Cyclo[(ϵ)Aca-Pro-Ser(OH)] 300-MHz ^1H NMR data in CD_3CN*

	Pro[a]	Ser	Aca	
δ_{NH} (ppm)	–	6.69 (2.09)[b]	6.84 (1.85)	δ_{NH} (ppm)
δH^{α} (ppm)	4.16 (1.54)	4.37 (2.79)	3.44 (1.47) 2.87 (1.18)	δH^{ϵ} (ppm)
δH^{β} (ppm)	2.19 (3.50) 1.86 (1.42)	3.79 (1.65) 3.63 (2.10)	1.65 (1.09) 1.53 (1.07)	δH^{δ} (ppm)
$^3J_{HN-\alpha H}$ (Hz)	–	9.2	1.20 (2.00) 0.86 (2.53)	δH^{γ} (ppm)
$^3J\alpha_{H-\beta H}$ (Hz)	7.6	6.3 5.2	1.88 (1.51) 1.50 (1.08)	δH^{β} (ppm)
$^2J\alpha_{H-\alpha H}$ (Hz)	–	–	2.44 (1.35) 2.18 (3.50)	δH^{α} (ppm)

[a] δH^{γ} (ppm) of Pro 1.88; δH^{δ} (ppm) of Pro 3.54 (1.50), 3.60 (1.78).
[b] T_1 relaxation time, seconds (in parentheses).

yielding a k′ (for *1*) = 8.7 and k′ (for *4*) = 12.87. (k′ was calculated usually as $k' = [t - t_0]/t_0$; detection was performed at 214 nm.)

To synthesize *cyclo*[(ϵ)Aca-Pro-Ser] (*2*), *cyclo*[(ϵ)Aca-Pro-Ser(OBzl)] (*1*) was dissolved in CH_3COOH and hydrogenated (Pd/C) for 8 h yielding quantitatively a white solid powder. The purity of *4* was checked using HPLC and mass spectroscopic analysis (EI and CI methods) as well as NMR methods (cf. Tables 5, 8).

Cyclo[Pro-Ser-(ϵ)Aca-Pro-Ser-(ϵ)Aca] (*5*) was produced by dissolving *cyclo*[(ϵ)Aca-Pro-Ser(OBzl)-(ϵ)Aca-Pro-Ser(OBzl)] (*4*) in CH_3COOH and hydrogenated (Pd/C) for 8 h yielding quantitatively a white solid powder. The purity of *5* was checked using HPLC and mass spectroscopic analysis (EI and CI methods) as well as NMR methods (cf. Tables 5, 9).

Table 7. *300-MHz ^1H NMR data on cyclo[(ϵ)Aca-Pro-Ser(OBzl)] in CD_3CN*

	Pro[a]	Ser[b]	Aca	
δ_{NH} (ppm)	–	6.67 (1.38)[c]	6.87 (1.31)	δ_{NH} (ppm)
δH^{α} (ppm)	4.17 (3.73)	4.59 (2.75)	3.41 (1.13) 2.88 (1.29)	δH^{ϵ} (ppm)
δH^{β} (ppm)	2.22 (2.5) 1.80	3.84 (1.24) 3.75	1.62 (0.8) 1.55 (0.8)	δH^{δ} (ppm)
$^3J_{HN-\alpha H}$ (Hz)	–	9.2	1.22 (1.2) 0.97 (0.6)	δH^{γ} (ppm)
$^3J\alpha_{H-\beta H}$ (Hz)	7.3	4.4 7.6	1.80 (1.22) 1.55 (0.8)	δH^{β} (ppm)
$^2J\beta_{H-\beta H}$ (Hz)	Not defined	10.3	2.45 (1.34) 2.23 (2.71)	δH^{α} (ppm)

[a] δH^{γ} (ppm) of Pro 1.91 (2.05); δH^{δ} (ppm) of Pro 3.56, 3.67 (1.08).
[b] δCH_2 (ppm) of Bzl 4.52, 4.47 (2.44); δCH (ppm) of Bzl 7.31, 7.33, 7.34 (9.2).
[c] T_1 relaxation time, seconds (in parentheses).

Cyclo[Pro-Ala-(ϵ)Aca-Pro-Ala-(ϵ)Aca] (*6*) was cyclized under argon from 880 mg (2.6 mmol) of H-Pro-Ala-(ϵ)Aca-OH as described (Perczel et al., 1991a) earlier by dissolving 4.6 g (10.5 mmol) benzotriazol-1-yloxytris(dimethylamino) phosphonium hexafluorophosphate (Castro et al., 1975, 1977) (Aldrich) and 1.42 g (10.5 mmol) HOBt in 900 mL of absolute DMF. Then, 1.15 mL of *N*-methyl-morpholine was added dropwise and stirred for 20 h. DMF was removed in vacuo, and the remaining powder was dissolved in $EtOH/Et_2O$. The collected crystal fractions (yield 180 mg) were combined and recrystallized from absolute ethanol. The purity of *6* was checked using HPLC and mass spectroscopic analysis (EI and CI methods) as well as NMR methods (cf. Tables 5, 10). (No monomer has been found on the basis of mass spectroscopy and HPLC.)

Cyclo[(ϵ)Aca-Ala-Ala] (*3*) was synthesized as described previously (Bandekar et al., 1982). Mass spectros-

Table 9. *300-MHz ^1H NMR data on cyclo[(ϵ)Aca-Pro-Ser-(ϵ)Aca-Pro-Ser] in d_6-DMSO 100.0 atom%*

	Pro[a]	Ser	Aca	
δ_{NH} (ppm)	–	8.13 (0.32)[b]	7.27 (0.35)	δ_{NH} (ppm)
δH^{α} (ppm)	4.03 (0.93)	4.08 (0.85)	3.13 (0.40) 2.76 (0.29)	δH^{ϵ} (ppm)
δH^{β} (ppm)	2.11 (0.30) 1.74 (0.35)	3.63 (0.25) 3.43 (0.35)	1.43 (0.25)	δH^{δ} (ppm)
$^3J_{HN-\alpha H}$ (Hz)	–		1.22 (0.33)	δH^{γ} (ppm)
$^3J_{HN-\alpha H}$ (Hz)			1.49 (0.28)	δH^{β} (ppm)
$^2J_{HN-\alpha H}$ (Hz)	–	–	2.28 (0.28) 2.43 (0.20)	δH^{α} (ppm)

[a] δH^{γ} (ppm) of Pro 1.82 (0.36); δH^{δ} (ppm) of Pro 3.43 (0.33), 3.70 (0.25).
[b] T_1 relaxation time, seconds (in parentheses).

Table 10. *300-MHz ^1H NMR data on cyclo[(ϵ)Aca-Pro-Ala-(ϵ)Aca-Pro-Ala] in d_6-DMSO*

	Pro[a]	Ala	Aca	
δ_{NH} (ppm)	–	7.84 (0.35)[b]	7.21 (0.34)	δ_{NH} (ppm)
δH^{α} (ppm)	4.01 (1.10)	4.13 (1.20)	3.02 (0.45) 2.84 (0.35)	δH^{ϵ} (ppm)
δH^{β} (ppm)	2.13 1.69	1.17	1.48 (0.29)	δH^{δ} (ppm)
$^3J_{HN\text{-}\alpha H}$ (Hz)	–	8.9	1.26 (0.38)	δH^{γ} (ppm)
$^3J\alpha_{H\text{-}\beta H}$ (Hz)	6.8 8.7	6.8	1.47 (0.29)	δH^{β} (ppm)
$^2J\alpha_{H\text{-}\alpha H}$ (Hz)	–	–	2.24 (0.30) 2.40 (0.23)	δH^{α} (ppm)

[a] δH^{γ} (ppm) of Pro 1.81 (0.38); δH^{δ} (ppm) of Pro 3.57 (0.36), 3.49 (0.70).
[b] T_1 relaxation time, seconds (in parentheses).

Table 11. *300-MHz ^1H NMR data on cyclo[Ala-Ala-(ϵ)Aca] in d_6-DMSO*

	Ala_1	Ala_2	Aca	
δ_{NH} (ppm)	8.06 (0.41)[a]	7.65 (0.46)	7.38 (0.45)	δ_{NH} (ppm)
δH^{α} (ppm)	4.16 (1.10)	4.16 (1.20)	3.44 (no) 2.75 (0.22)	δH^{ϵ} (ppm)
δH^{α} (ppm)	1.23 (0.37)	1.17 (0.45)	1.47 (0.23)	δH^{δ} (ppm)
$^3J_{HN\text{-}\alpha H}$ (Hz)	7.2	8.3	1.20 (0.37)	δH^{γ} (ppm)
$^3J_{HN\text{-}\beta H}$ (Hz)	7.2	6.7	1.59 (0.23)	δH^{β} (ppm)
$^2J\alpha_{H\text{-}\alpha H}$ (Hz)	–	–	2.08 (0.28)	δH^{α} (ppm)

[a] T_1 relaxation time, seconds (in parentheses).

copy-confirmed material was investigated (for NMR data see Table 11).

NMR measurement

^1H- and ^{13}C-NMR measurements were performed on a Varian XL-300 spectrometer at ambient temperature (25 °C), using TMS as external standard. *1*, *2*, and *4* were dissolved in CD_3CN, and *3*, *5*, and *6* were dissolved in d_6-DMSO (100% atom) and degassed using at least 10 freeze-pump-thaw cycles. Assignments of ^1H signals were made with two-dimensional decoupling experiments. COSY data consisted of 256 t_1 increments with a relaxation delay of 1 s. The two-dimensional J-resolved spectra were recorded with a total of 16 or 64 transients per t_1 value, where t_1 was always equal to 64. The recorded two-dimensional ^1H-^{13}C correlated spectra were obtained using 64 t_1 values with 512 transients at each

Table 12. *Circular dichroism characteristics of the analyzed cyclic β-turn models*

	Acetonitrile			Water			TFE		
Compound[a]	c (mg/mL)	γ_{nm}	$[\theta]_M$[b]	c (mg/mL)	γ_{nm}	$[\theta]_M$[b]	c (mg/mL)	γ_{nm}	$[\theta]_M$[b]
1	0.60	224.0	−43,800	0.91	220.5	−48,750	0.6	219.5	−36,200
		210.0	−33,300		206.8	−25,000		207.5	−25,000
		194.0	+57,700		191.5	+49,375		192.5	+64,450
2	0.95	223.5	−31,370	1.05[c]	218.5	−36,950	0.45	218.0	−36,885
		205.0	−67,650		204.5	−53,500		203.5	−72,130
		193.5	+70,600		192.5	+47,500		191.5	+60,500
3	0.69[d]	222.5	−44,400	0.90	217.0	−58,500		[e]	
		204.0	−50,000		205.0	−50,000			
		187.0	+62,900		187.0	+62,900			
4	0.60	225.0	−24,500	0.82	221.0	−38,000	1.05	220.0	−28,040
		211.0	−20,000		210.0	−23,200		210.0	−21,400
		192.0	+24,400		190.5	+32,700		190.0	+34,160
5	1.50[f]	221.0	−21,200	1.13	220.0	−24,050	1.54	220.0	−16,970
		204.0	−43,650		205.5	−38,700		207.0	−24,000
		186.0	+14,000		186.5	+17,600		188.0	+14,110
6	0.64[d]	220.0	−19,000	0.43	225.0	−16,500[g]	2.05	218.5	−9,800
		203.0	−55,000		205.0	−53,000		205.5	−15,500

[a] *1*, *Cyclo*[(ϵ)Aca-Pro-Ser(OBzl)]; *2*, *cyclo*[(ϵ)Aca-Pro-Ser(OBzl)-(ϵ)Aca-Pro-Ser(OBzl)]; *3*, *cyclo*[(ϵ)Aca-Ala-Ala]; *4*, *cyclo*[(ϵ)Aca-Pro-Ser(OBzl)-(ϵ)Aca-Pro-Ser(OBzl)]; *5*, *cyclo*[(ϵ)Aca-Pro-Ser-(ϵ)Aca-Pro-Ser]; *6*, *cyclo*[(ϵ)Aca-Pro-Ala-(ϵ)Aca-Pro-Ala].
[b] For mean residue ellipticity the molecular weights used are in Table 13.
[c] Acetonitrile (16%) was added.
[d] Water (9%) was added.
[e] See Bandekar et al. (1982).
[f] Water (20%) was added.
[g] Shoulder.

Table 13. *The synthesized 18 β-turn models*

	MW[a]	MW_{core}[b]	MW/MW_{core}
1 Aca-Pro-Ser-NHMe	257	229	1.12
2 Boc-Pro-Ser-NHMe	315	229	1.38
3 Boc-Val-Ser-NHMe	317	230	1.38
4 Boc-Pro-Thr-NHMe	329	242	1.36
5 Boc-Leu-Thr-NHMe	345	258	1.34
6 Boc-Val-Thr-NHMe	331	244	1.36
7 Boc-Gly-Ser-NHMe	275	188	1.46
8 Boc-Pro-D-Ser-NHMe	315	229	1.38
9 Boc-Val-D-Ser-NHMe	317	230	1.38
10 *Cyclo*[(δ)Ava-Gly-Pro-Ser(O^{t}Bu)-Gly]	453	229	1.99
11 *Cyclo*[(δ)Ava-Gly-Pro-Ser(OH)-Gly]	397	229	1.74
12 *Cyclo*[(δ)Ava-Gly-Pro-Gly-Gly]	367	199	1.84
13 *Cyclo*[(ε)Aca-Pro-Ser(OBzl)]	387	229	1.69
14 *Cyclo*[(ε)Aca-Pro-Ser(OBzl)-(ε)Aca-Pro-Ser(OBzl)]	774	229	3.38
15 *Cyclo*[(ε)Aca-Pro-Ser(OH)]	297	229	1.30
16 *Cyclo*[Pro-Ser-(ε)Aca-Pro-Ser-(ε)Aca]	594	229	2.59
17 *Cyclo*[Pro-Ala-(ε)Aca-Pro-Ala-(ε)Aca]	562	213	2.64
18 *Cyclo*[(ε)Aca-Ala-Ala]	255	183	1.39

[a] MW is the molecular weight of the peptide.
[b] MW_{core} is the reduced molecular weight of the peptides. The CD properties of the common For-Aaa-Aaa-NH_2 substructures were compared directly.

cycle. The relaxation delay was 2.5 s with a mixing time of 0.5 s for NOESY spectra measured for *1*. One-dimensional NOE experiments were performed in the difference mode where at least 256 transients were accumulated. The relaxation delay varied between 8 and 10 s, with a minimum required decoupler power to achieve a total saturation of the selected resonance line. The observed pulse was set to 90° with a spectral window of 4 kHz and 32,000 data points were collected. The concentrations were in the range of 1-3 mg/mL.

Molecular dynamics simulations

Energy minimizations and dynamics trajectory calculations for the models were performed using CHARMm (Brooks et al., 1983) with QUANTA (POLYGEN) on a Silicon Graphics computer. The initial estimate of the different conformers for all compounds were obtained from trajectory analysis of simulations carried out at 500 °K (100 ps). The different conformers were minimized first with backbone torsional constraints, then fully relaxed. Final molecular simulation was obtained by heating (from 0 °K to 300 °K in 10 ps), equilibration (300 °K, 50 ps), and simulation steps (200-800 ps at 300 °K) starting from all previously found initial conformers (see Tables 1, 2).

CD measurement

All the CD measurements were performed on a Jobin-Yvon Mark V autodichrograph using circular quartz cells with the path lengths of 0.01, 0.02, or 0.05 cm (Opticell and Helma), with a sensitivity setting at 1×10^{-6} to 1×10^{-5}. The response time setting for the spectrometer was 2 or 3 s, with data acquisition time of 5 s for the Apple IIe computer connected to the spectrometer, which ensured noise reduction as well as full-magnitude signal conversion from analog to digital. Each measurement was the average result of five repeated scans in steps of 0.2 nm at 22 °C. The CD spectra of peptides were usually measured at 0.5 mg/mL, which is lower than that used for ^{1}H-NMR acquisition (1-3 mg/mL). In order to achieve a direct comparison of the two types of conformational data, CD spectra were recorded in a wide concentration range, with extremes of 0.5 mg/mL and 2.3 mg/mL. No significant concentration dependence has been observed, but the band intensities below 200 nm were slightly smaller at higher concentrations. For NMR comparison, the curves reported in Table 12 were used. For mean residue ellipticity calculation the molecular weights used were normalized to For-Aaa-Aaa-NH_2 as shown in Table 13.

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