Molecular Carpentry: Piecing Together Helices and Hairpins in Designed Peptides

Chittaranjan Das, S. Channaveerappa Shankaramma, and Padmanabhan Balaram*[a]

Abstract: The design of a peptide that contains two distinct elements of secondary structure, helix and β-hairpin, is described. Two designed 17-residue peptides: Boc-Val-Ala-Leu-Aib-Val-Ala-Leu-Gly-Gly-Leu-Phe-Val-D-Pro-Gly-Leu-Phe-Val-OMe (**I**) and Boc-Leu-Aib-Val-Ala-Leu-Aib-Val-Gly-Gly-Leu-Val-Val-D-Pro-Gly-Leu-Val-Val-OMe (**II**) have been conformationally characterized by NMR spectroscopy. Peptides **I** and **II** contain a seven-residue helical module at the N terminus and a eight-residue β-hairpin module at the C terminus, which are connected by a conformationally flexible Gly-Gly segment. The choice of the secondary-structure modules is based upon prior crystallographic and spectroscopic analysis of the individual modules. Analysis of 500 MHz ^{1}H NMR data, recorded as solutions in methanol, suggests that the observed pattern of chemical shifts, $^3J_{HN-C^{\alpha}H}$ values, temperature coefficients of the NH chemical shifts, and backbone inter-residue nuclear Overhauser effects favor helical structures for residues 1–7 and β-hairpin structures for residues 10–17. The spectroscopic data are compatible with termination of the helical segment by formation of a Schellman motif; this restricts Gly(8) to a left-handed α-helical conformation. Gly(9) is the only residue with multiple conformational possibilities in ϕ,ψ space. Possible orientations of the two secondary-structure modules are considered. This study validates the use of stereochemically rigid peptide modules as prefabricated elements in the construction of synthetic protein mimics.

Keywords: β-hairpin · conformational analysis · helical structures · peptides · synthesis design · tertiary structures

Introduction

In protein structures, distinct secondary structural elements, such as helices and strands, are assembled into a compact fold that is stabilized by a complex network of tertiary interactions. The driving force for the compaction of protein sequences is solvophobic in nature, with nonpolar side chains tending to cluster in the interior of globular structures and the consequent exclusion of the solvent, water, from the protein core.[1] In attempts to design synthetic polypeptide mimics for protein structures, a specifically designed patterning of polar and apolar residues along the synthetic sequence is generally employed in order to direct the orientation of secondary-structural elements.[2] These approaches have led to significant success in the design of synthetic helical bundles that possess specific binding and catalytic properties.[3] Recent work has also emphasized the importance of specific β-turn segments in nucleating β-hairpins and multistranded, β-sheet structures.[4] Unlike native proteins, the designed structures possess poorly packed interiors and generally resemble molten globule states in their physical properties.[5]

We have been pursuing an alternate approach to the construction of complex polypeptide structures, in which a primary goal has been to design molecules of defined shape that are soluble in poorly interacting organic solvents. In this approach, the driving forces that determine polypeptide-chain folding are enthalpic in nature and consist primarily of hydrogen bonding and van der Waals interactions. The key to this strategy is the ability to construct well-defined units of secondary structure, such as helices, reverse turns, and hairpins. Subsequent assembly of these relatively rigid, prefabricated modules of secondary structures can, in principle, be achieved by careful choice of linking segments. The construction of complex polypeptides by using well-defined conformational modules may be termed a "Meccano- or Lego-set approach" to peptide design.[6] The construction of individual elements such as helices and β-hairpins may be achieved by using conformationally constrained amino acids to nucleate specific backbone conformations.[7] Peptide helices ($3_{10}/\alpha$) are readily obtained by incorporation of α,α-dialkylated amino acid residues,[7–9] most notably α-amino-isobutyric acid (Aib). Aib-containing peptides have been

[a] Prof. P. Balaram, C. Das, S. C. Shankaramma
Molecular Biophysics Unit, Indian Institute of Science
Bangalore 560 012 (India)
Fax: (+91) 80-3600683, (+91) 80-3600535
E-mail: pb@mbu.iisc.ernet.in

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extensively investigated, and cylindrical helical conformations have been well characterized by X-ray diffraction in peptides ranging in length from 7–16 residues.[7, 8] β-Hairpins are generated by positioning a central D-Pro-Gly segment in a synthetic sequence.[10] The tendency of D-Pro-Xxx sequences to adopt type II′ β-turn conformations is important for nucleation of a tight turn of appropriate stereochemistry;[11] this then facilitates hairpin formation, stabilized by cross-strand hydrogen bonding. Indeed, β-hairpins have been observed in crystals of a synthetic octapeptide with a central D-Pro-Gly sequence.[10d] The use of multiple D-Pro-Xxx segments for the nucleation of three- and four-stranded β-sheets has also been reported.[4c,e,f]

In this report, we examine the conformations of characterized helical and hairpin segments that are connected by a glycylglycine (Gly-Gly) linking segment. This study has been undertaken to establish the conformational integrity of the individual modules when they are inserted into larger structures. Two 17-residue sequences: Boc-Val-Ala-Leu-Aib-Val-Ala-Leu-Gly-Gly-Leu-Phe-Val-D-Pro-Gly-Leu-Phe-Val-OMe (**I**) and Boc-Leu-Aib-Val-Ala-Leu-Aib-Val-Gly-Gly-Leu-Val-Val-D-Pro-Gly-Leu-Val-Val-OMe (**II**) have been chosen for spectroscopic analysis. The helical module used in peptide **I**, Val-Ala-Leu-Aib-Val-Ala-Leu, has been extensively characterized in crystals of peptides of varying lengths.[12] This module contains only a single, centrally positioned Aib residue. As a consequence, it possesses some degree of conformational fragility. The helical module used in peptide **II**, Leu-Aib-Val-Ala-Leu-Aib-Val, which contains two helix-stabilizing Aib residues, has been conformationally characterized by X-ray diffraction in crystals of two decapeptide sequences: Boc-Leu-Aib-Val-Ala-Leu-Aib-Val-Ala-Leu-Aib-OMe and Boc-Leu-Aib-Val-Ala-Leu-Aib-Val-D-Ala-D-Leu-Aib-OMe.[13] A superposition of the observed helical fold for the module is shown in Figure 1a. The hairpin module Leu-Val-Val-D-Pro-Gly-Leu-Val-Val used in peptide **II** has also been independently characterized in crystals. (See Figure 1b for a superposition of the two crystal-state conformations.)[10d] The octapeptide Leu-Phe-Val-D-Pro-Gly-Leu-Phe-Val, which is analogous to the hairpin module in peptide **II**, has been shown by NMR spectroscopy to adopt a β-hairpin structure (unpublished results). The choice of Gly-Gly as the linking segment was dictated by the possibility that the achiral Gly residue at position eight would act as a helix terminator by facilitating formation of the Schellman motif;[14] this requires positive ϕ,ψ values in the left-handed helical (α_L) region of conformational space.[15] Gly(9) would then, in principle, be the sole conformationally flexible residue in the two peptide sequences. The NMR results described in this paper establish that both helical and hairpin modules retain their conformational integrity and that a relatively small number of conformational possibilities occur at Gly(9); this determines the spatial orientation of the two rigid modules of secondary structure.

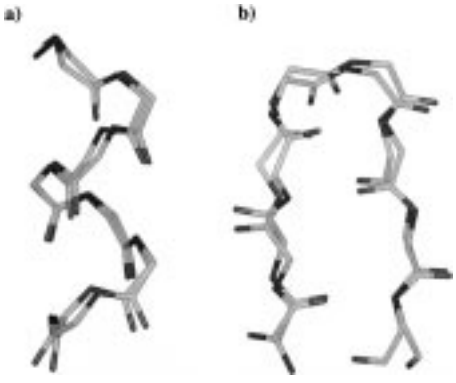

Figure 1. a) Superposition of two backbone conformations of the fragment Leu-Aib-Val-Ala-Leu-Aib-Val obtained in the crystal structures of Boc-Leu-Aib-Val-Ala-Leu-Aib-Val-Ala-Leu-Aib-OMe and Boc-Leu-Aib-Val-Ala-Leu-Aib-Val-D-Ala-D-Leu-Aib-OMe.[13] b) Superposition of two backbone conformations obtained in the crystal structure of Boc-Leu-Val-Val-D-Pro-Gly-Leu-Val-Val-OMe. The peptide crystallized in the triclinic system with two independent molecules per asymmetric unit.[10d]

Results and Discussion

Peptides **I** and **II** were freely soluble in a variety of organic solvents. In chloroform, both peptides had NMR spectra that revealed significant overlap of NH resonances and some evidence for aggregation-induced broadening of resonances. In dimethyl sulfoxide, peptide **II** had a well-resolved ^{1}H NMR spectrum. However, preliminary studies revealed that several expected inter-residue NOEs, characteristic of hairpin segments, were absent. In methanol, both peptides had well-resolved ^{1}H NMR spectra with excellent dispersion of NH and C$^\alpha$H resonances. The observed line widths suggest the absence of aggregation effects. The detailed conformational analysis of peptides **I** and **II** is therefore restricted to methanolic solutions. Sequence-specific assignments of backbone- and side-chain-proton resonances were readily achieved by using a combination of TOCSY and ROESY/NOESY experiments. The relevant chemical shifts and coupling constants are summarized in Table 1. Figures 2 and 3 illustrate nuclear Overhauser effects observed between backbone protons of **I** and **II**, respectively. In helical segments,

Table 1. Characteristic ^{1}H NMR parameters for peptides **I** and **II**.[a]

	δ [ppm]				dδ/dT [ppb K^{-1}]		$^3J_{HN-C^\alpha H}$ [Hz]	
	NH		C$^\alpha$H					
Residues[b]	**I**	**II**	**I**	**II**	**I**	**II**	**I**	**II**
Val/Leu(1)	7.05	7.13	3.76	3.84	10.0	10.3	3.9	5.0
Ala/Aib(2)	8.54	8.57	4.13	–	8.3	9.0	3.7	–
Leu/Val(3)	7.72	7.38	4.15	3.74	4.3	1.0	6.0	6.4
Aib/Ala(4)	7.61	7.58	–	4.06	1.3	−0.9	–	5.1
Val/Leu(5)	7.78	7.88	3.80	4.11	5.6	6.9	4.9	6.0
Ala/Aib(6)	7.91	7.70	4.16	–	4.0	2.9	3.9	–
Leu/Val(7)	7.89	7.66	4.44	4.09	3.0	4.8	7.3	7.2
Gly(8)	8.07	8.04	4.01	3.91	4.0	3.0	–	–
Gly(9)	8.10	8.14	3.85, 4.00	3.80, 4.03	4.3	5.5	–	–
Leu(10)	7.61	7.79	4.44	4.51	2.3	2.8	7.5	8.1
Phe/Val(11)	8.13	7.93	5.20	4.66	9.7	9.8	9.0	9.0
Val(12)	8.80	8.73	4.48	4.48	8.7	6.9	9.3	9.0
D-Pro(13)	–	–	4.40	4.34	–	–	–	–
Gly(14)	8.35	8.35	3.79, 4.01	3.67, 4.01	10.7	9.2	–	–
Leu(15)	8.11	8.16	4.62	4.62	4.0	2.6	8.7	8.8
Phe/Val(16)	8.55	8.24	4.59	4.48	12.3	10.2	7.8	9.0
Val(17)	8.24	8.65	4.23	4.42	4.3	7.4	9.2	8.8

[a] Solvent: CD_3OH, $T = 300$ K. [b] Residues for peptide **I** and **II**, respectively.

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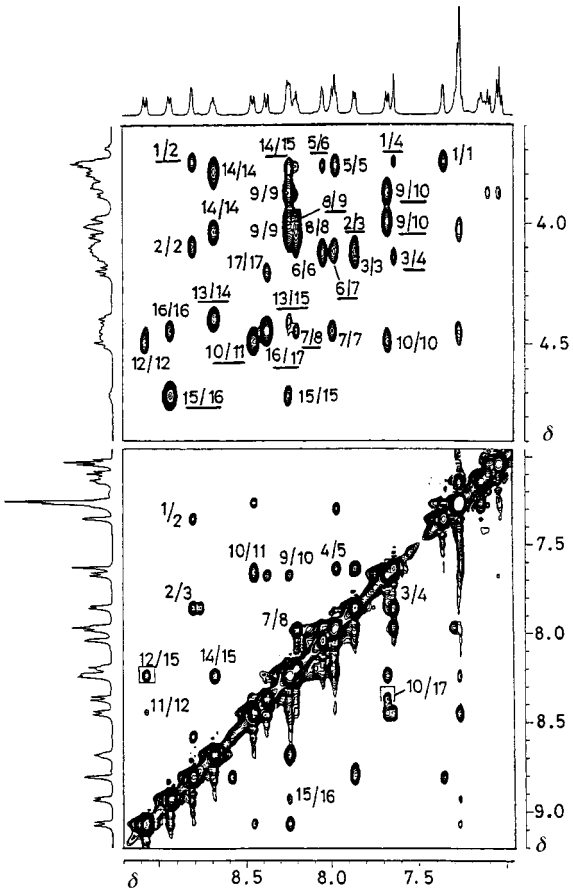

Figure 2. Partial 500 MHz NOESY spectra of peptide **I** in CD_3OH at 270 K. The top panel shows $C^{\alpha}H \rightleftharpoons NH$ NOEs, and the lower panel shows $NH \rightleftharpoons NH$ NOEs. Residue numbers are used to label the cross-peaks. Underlined cross-peaks refer to inter-residue connectivities. Long-range $NH \rightleftharpoons NH$ NOEs diagnostic of β-hairpin conformation in the segment 10–17 are boxed.

intense sequential $N_iH \rightleftharpoons N_{i+1}H$ (d_{NN}) connectivities are expected, while the inter-residue $C_i^{\alpha}H \rightleftharpoons N_{i+1}H$ ($d_{\alpha N}$) connectivity is generally weaker than the corresponding intra-residue $C_i^{\alpha} \rightleftharpoons N_iH$ ($d_{N\alpha}$) NOE. β-Hairpin segments are characterized by long-range, interstrand $NH \rightleftharpoons NH$ and $C^{\alpha}H \rightleftharpoons C^{\alpha}H$ connectivities, while the β-turn segment can be recognized by the inter-residue d_{NN} connectivity for the residue "$i+2$" [Gly(14)] in peptides **I** and **II**. In addition, the extended strand segments are expected to exhibit strong inter-residue $d_{\alpha N}$ connectivities. Inspection of the spectra in Figures 2 and 3 reveal that all the crucial backbone connectivities expected for the helical and hairpin segments in both peptides are, indeed, observed. The NOE data that relate intra-residue and sequential backbone protons for both peptides are schematically summarized in Figure 4. Clearly, the characteristic NOEs expected for helices are present across the segment that contains residues 1–7, while the diagnostic NOEs for β-hairpins are observed for the segment 10–17. Figure 5 illustrates the cross-strand $C^{\alpha}H \rightleftharpoons C^{\alpha}H$ NOE between residues 11 and 16 in the C-terminal hairpin of both peptides.

The backbone $^3J_{HN-C^{\alpha}H}$ coupling constants listed in Table 1 establish that low values of J (≤ 6.4 Hz) are observed for residues 1–6 in both the peptides. Significantly higher vicinal-coupling constants ($^3J = 7.5$ Hz) are observed for the C-terminal residues; this suggests that the N-terminal-helical and C-terminal-strand segments are clearly delineated.[16] The NMR evidence presented thus far supports the original premise behind our design strategy: that the chosen modules of secondary structure would retain their conformations in a larger assembly. A critical question which arises at this point is the nature of the conformation of the Gly-Gly segment, which will ultimately define the orientation of the two modules.

In order to probe the possible involvement of the Gly NH groups in intramolecular hydrogen bonding, the temperature dependence of NH chemical shifts in methanol were determined.[16a, 17] The observed temperature coefficients are summarized in Table 1. As anticipated for the helix, the resonances of residues 1 and 2 in both peptides have high $d\delta/dT$ values (> 8 ppb K^{-1}), characteristic of solvent-exposed groups. Residues 3, 4, 6, and 7 have low $d\delta/dT$ values (≤ 5 ppb K^{-1}); this suggests that they are involved in intra-

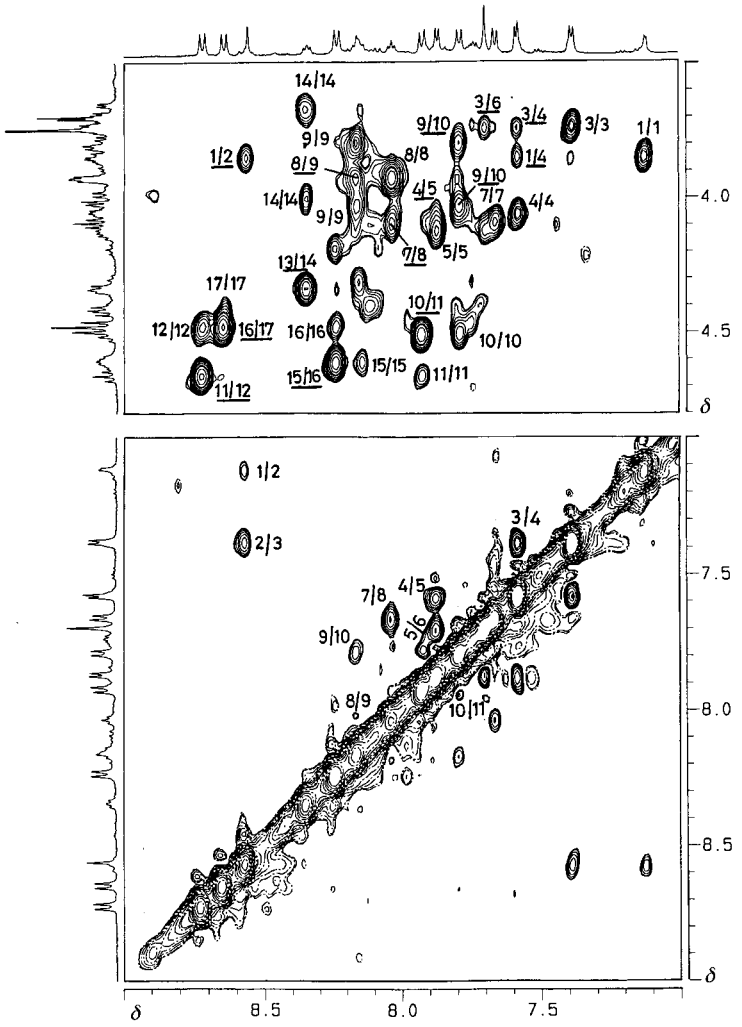

Figure 3. Partial 500 MHz ROESY spectra of peptide **II** in CD_3OH at 300 K. The top panel shows $C^{\alpha}H \rightleftharpoons NH$ NOEs, and the lower panel shows $NH \rightleftharpoons NH$ NOEs. The cross-peaks are labeled with residue numbers. Underlined cross-peaks highlight inter-residue connectivities.

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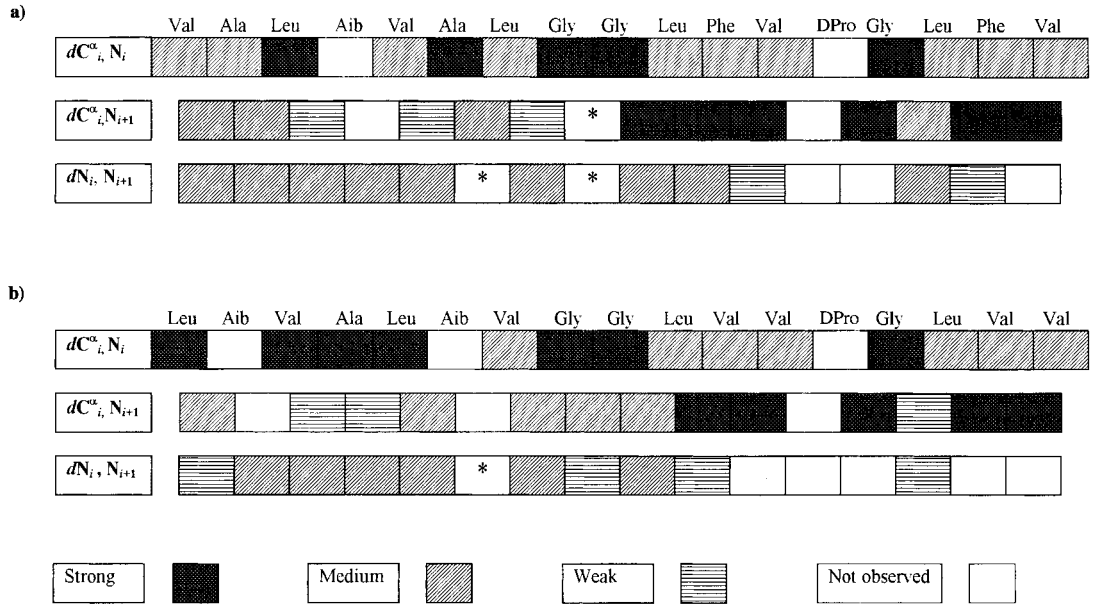

Figure 4. Summary of intra-residue and sequential NOEs observed between backbone protons for peptides **I** (a) and **II** (b) in CD_3OH. NOEs which could not be observed due to resonance overlap are indicated by an asterisk.

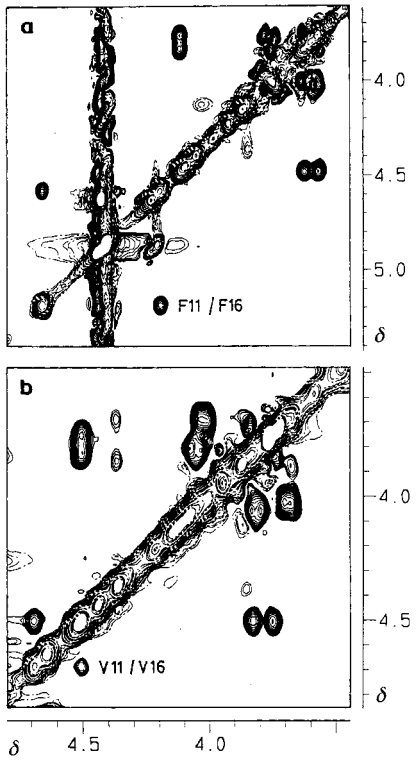

Figure 5. $C^{\alpha}H \rightleftharpoons C^{\alpha}H$ cross-strand NOEs observed for peptides **I** and **II** in CD_3OH at 300 K: a) Portion of the ROESY spectra of peptide **I**, b) portion of the ROESY spectra of peptides **II**.

molecular hydrogen bonding. Residue 5 has a moderately high $d\delta/dT$ value; this indicates greater solvation of the helix in this region. In the hairpin segment (residues 10–17), the NH groups of residues 10, 15, and 17 have relatively low coefficients, suggestive of their participation in cross-strand interaction. The NH groups of residues 11, 14 ("$i+2$" residue of the β-turn), and 16 are expected to be exposed to solvent and, indeed, exhibit extremely high $d\delta/dT$ values. The NH groups of Val(12), to some extent, and Val (17) in peptide **II** exhibit moderately high $d\delta/dT$ values; this may indicate some solvent invasion of the β-hairpin. A hydroxylic solvent, such as methanol, may be expected to compete for hydrogen bonding.

A qualitative idea of the involvement of peptide NH groups in intramolecular hydrogen bonds was also obtained from an H/D exchange experiment[16a, 17] carried out by dissolving the peptides in CD_3OD. Figure 6b shows the course of the disappearance of the NH resonances in peptide **II**. The NH groups involved in helical hydrogen bonding: Val(3), Ala(4), Aib(6), Val(7), and, to a lesser extent, Leu(5) exchange at a slower rate than the remaining NH groups. In the hairpin segment, Val(12) and Val(17), which are expected to be internally hydrogen bonded, have lower rates of exchange. The behavior of the Val(11) and Val(16) NH groups is anomalous. It should be noted that the anomalous exchange rates and solvent dependence of NH chemical shifts in extended strands may be a consequence of the proximity of the CO group of the same residue in "stretched out" conformations. Indeed, the C_5 interaction has been considered to be a hydrogen bond in fully extended peptides.[18] Stereoelectronic considerations would dictate the approach of solvent to NH groups in such a situation, with the approach of a solvent molecule being impeded by the proximal carbonyl group.[10f, 19] A similar experiment carried out with peptide **I** (Figure 6a) reveals that two of the NH groups that are expected to be involved in cross-strand hydrogen bonding, those on Val(12) and Val(17), exchange slowly. In this peptide as well, the Phe(11) NH exchanges anomalously slowly. Interestingly, in peptide **I**, the helical segment that contains only a single Aib residue at the 4-position is appreciably more fragile, with the NH groups of Leu(3) and Aib(4) exchanging rapidly. Indeed, the Val(5) NH is the only group in this segment to exchange very slowly. Studies of the isolated

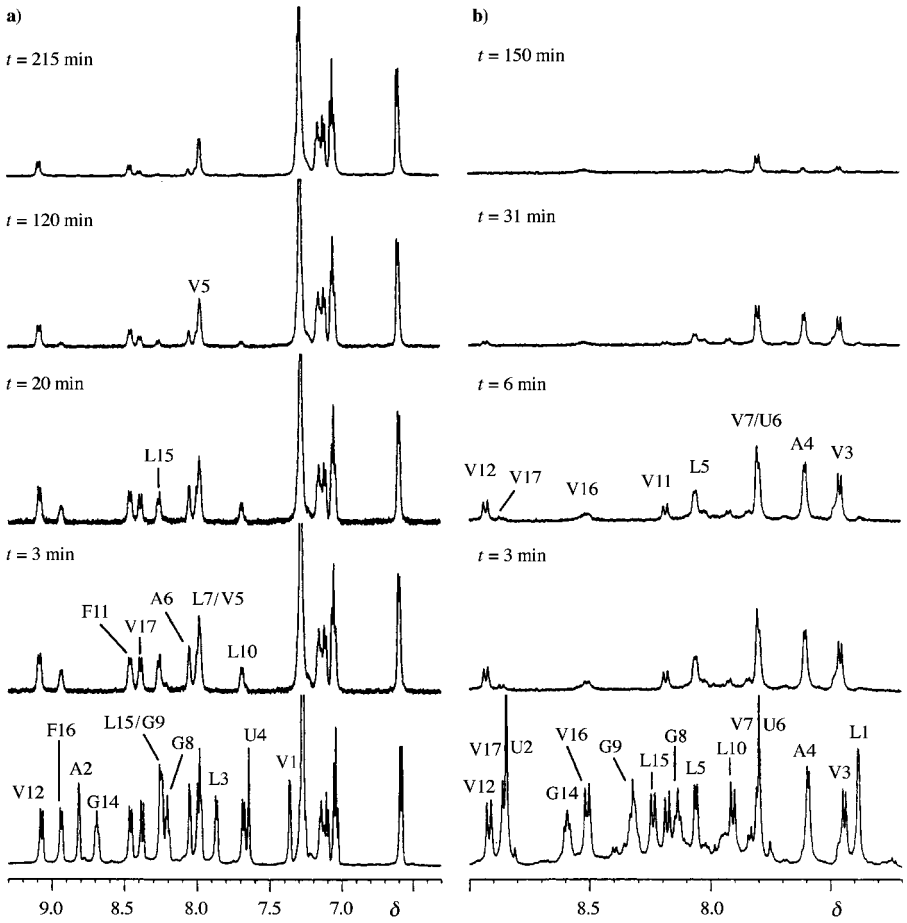

Figure 6. Partial 500 MHz ^{1}H spectra of peptides **I** (a) and **II** (b) in CD_3OD, which illustrate the rate of exchange of NH groups. Peptides **I** and **II** ($\sim$1 mg) were dissolved in 500 μL of precooled CD_3OD (at 270 K for peptide **I**; 277 K for peptide **II**), and the one-dimensional spectra at different time intervals were acquired as indicated. The lowest spectra in a) and b) correspond to the spectra of peptides **I** and **II** recorded in CD_3OH at 270 and 277 K, respectively. The letters correspond to the standard abbreviations for amino acids with U for Aib.

heptapeptide module Boc-Val-Ala-Leu-Aib-Val-Ala-Leu-OMe have indeed demonstrated significant fragility of the helix in crystals and in solution.[20] Most importantly, a crystal structure determination of peptide **I** reveals that the two N terminus residues, Val(1) and Ala(2), adopt unfolded, extended conformations and are involved in intermolecular hydrogen-bond formation in the solid state.[21]

The integrity of the β-hairpin in peptide **I** is also supported by the observation of a resonance anomalously shifted to high field that corresponds to the H2/H6 protons of Phe(16) (inset, Figure 7). Such a shift of the Phe ring protons is possible only if there is a strong cross-strand interaction that allows the ring current of one of the Phe residues to influence the chemical shifts of the proximal aromatic protons. A schematic conformation for the hairpin segment, based on the crystal structure of peptide **I**,[21] is shown in Figure 7. The significant upfield shift suggests that the majority of the residues 10–17 are in a β-hairpin conformation in peptide **I**.

The Gly(8) NH has very little temperature dependence of chemical shifts. The observed $d\delta/dT$ values for this residue in both peptides **I** and **II** strongly support structures in which the NH group is solvent shielded; this suggests that the helical hydrogen-bonding pattern extends to the NH group of Gly (8), a feature which implies a helical conformation at the preceding residue. Interestingly, the Gly(9) NH also has a low $d\delta/dT$ value, consistent with its involvement in intramolecular hydrogen bonding. In the anticipated structure, Gly(8) is expected to adopt a left-handed helical (α_L) conformation, this results in helix termination by formation of a Schellman motif.[14, 15, 22] In such a structure, the Gly(9) NH should be involved in a $6 \rightarrow 1$ hydrogen bond with the CO group of residue at the 4-position. The NMR data thus support helix termination by Gly(8) forming the site of chiral reversal. In the crystal structures of peptides that adopt the Schellman motif, the dihedral angle, $\phi(N-C^{\alpha})$, of the Thr(1) residue is considerably larger in magnitude ($\sim -100^{\circ}$). Interestingly, in both peptides, the $J_{HN-C^{\alpha}H}$ for residue seven is significantly greater ($\sim$7 Hz) than the values observed for the preceding residues in the helix.

Possible conformations: The NMR results clearly establish that residues 1–7 in both peptides **I** and **II** adopt a largely helical folding pattern, while

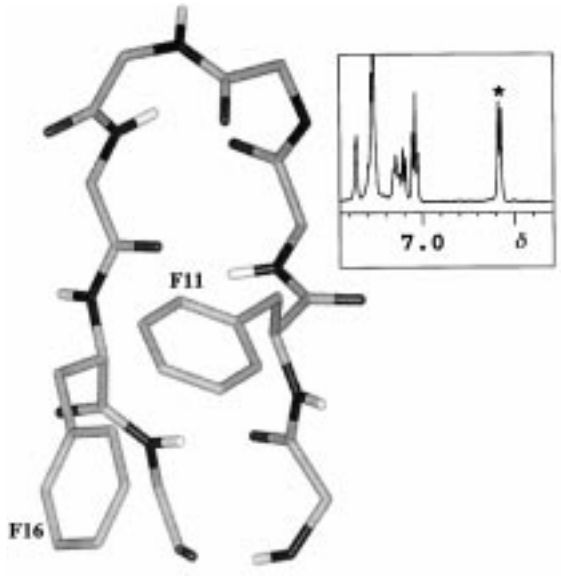

Figure 7. Model for the β-hairpin component of peptide **I** built with ϕ and ψ values obtained from the crystal structure,[21] which illustrates the orientation of aromatic residues in the strands. Inset: the upfield shift of the Phe(16) ring protons, H2 and H6, marked with an asterisk.

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residues 10–17 favor a β-hairpin conformation. The helical segment in peptide **I** is considerably more fragile; this suggests solvent invasion of the backbone. The solvent-shielded nature of the Gly(9) NH group constrains Gly(8) to an α_L conformation ($\phi \sim +60^\circ$, $\psi \pm 30^\circ$). The orientation of the helical and hairpin segments are then determined solely by the conformation at Gly(9). Four major conformational possibilities may be envisaged at this residue: α_R ($\phi \sim -65^\circ$, $\psi \sim -35^\circ$), α_L ($\phi \sim +65^\circ$, $\psi \sim +35^\circ$), β ($\phi \sim -150^\circ$, $\psi \sim +150^\circ$) or β' ($\phi \sim +150^\circ$, $\psi \sim -150^\circ$), which lie in the four quadrants of the Ramachandran map.[23] The β and β' conformations become closely related as the residue approaches a fully extended conformation ($\phi \sim 180^\circ$, $\psi \sim 180^\circ$). Figure 8 illustrates ribbon

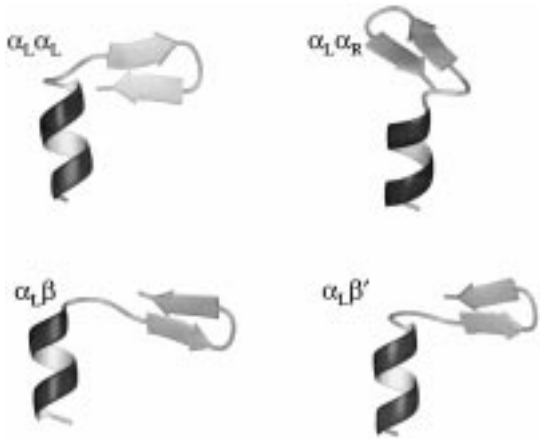

Figure 8. Four distinct stereochemical alternatives for the helix-linker hairpin peptides, **I** and **II**. Ribbon representations of the backbone conformations are highlighted. This figure was generated by using the program MolMol.[32] Idealized backbone ϕ and ψ values at Gly 9 ($\alpha_L = +65^\circ, +35^\circ$; $\alpha_R = -65^\circ, -35^\circ$; $\beta = -150^\circ, +150^\circ$; $\beta' = +150^\circ, -150^\circ$) were used to arrive at these models. In all these cases, Gly (8) was fixed in the ideal α_L conformation. Residues 1–7 were placed in the α_R conformations, while for residues 10–12 and 15–17, ideal extended strand values of $\phi = -130^\circ$ and $\psi = +130^\circ$ were used. The β-turn segment at residues 13 and 14 was fixed in ideal type II′ geometry ($\phi_{i+1} = +60^\circ$, $\psi_{i+1} = -120^\circ$; $\phi_{i+2} = -80^\circ$, $\psi_{i+2} = 0^\circ$).

representations of the molecule; these were obtained by using idealized values for Gly(9) in the four broad conformational regions. The Gly-Gly linker conformations $\alpha_L\beta$ and $\alpha_L\beta'$ lead to very similar overall folds. The $\alpha_L\alpha_L$ conformation, however, leads to a different orientation of the strands with respect to the helix, while the $\alpha_L\alpha_R$ linker results in a distinct orientation. In three of the conformational families, the β-hairpin axis and the helix axis are orthogonal, while the $\alpha_L\alpha_R$ linker results in a more elongated conformation in a direction parallel to the helix axis. Significantly, a crystal structure determination of peptide **I** reveals the $\alpha_L\beta'$ conformation at the linking Gly-Gly segment, with the helical and hairpin segments lying perpendicular to one another.[21] The possibility that the peptide can assume a more compact structure than the conformations illustrated in Figure 8 is excluded, because there is only a single linking residue, Gly(9), between the two secondary structure segments. The NMR data show that the conformations of Gly(8) are constrained. At the present level of analysis, the possibility of dynamic interconversion between the conformational families cannot be excluded.

Circular Dichroism: CD spectra for peptides **I** and **II** in methanol are shown in Figure 9. Both peptides exhibit intense CD bands in the region below 230 nm; this is indicative of the presence of the substantial secondary structure. The difference in the spectra of peptides **I** and **II** may arise from the

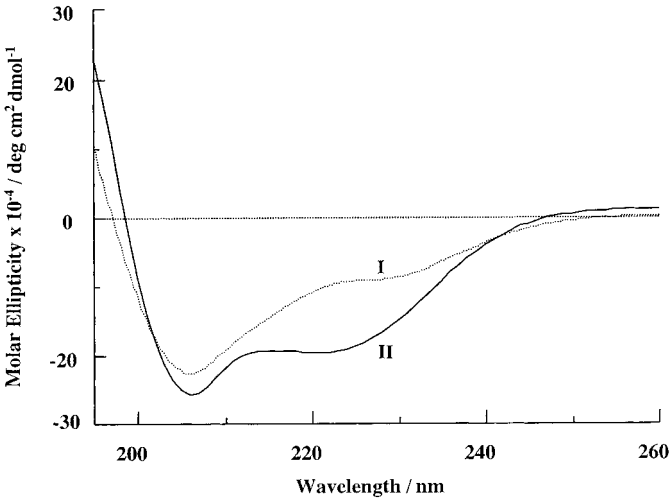

Figure 9. CD spectra of peptides **I** and **II** in methanol (300 K).

contribution of the two Phe residues to the far-UV CD spectrum of peptide **I**. Aromatic contributions to the far-UV CD spectra of peptides cannot be ignored[24] and, in the present case, the proximity of Phe(11) and Phe(16) in a β-hairpin may be expected to significantly distort the spectrum.[25] Inspection of the spectra in Figure 9 suggests that both helical and hairpin components are indeed contributing. While short helical segments are expected to have a weak band at 218–220 nm, which accompanies a relatively strong band at 205–208 nm,[26] β-hairpins exhibit a broad negative band at 214–220 nm.[4a,b, 10b]

Conclusion

The results presented in this report establish that conformationally well-defined modules of peptide secondary structure can be covalently assembled into larger units, without significantly altering the local conformation of the module. The structural analyses described in the present work have been carried out in methanol and generally support both isolated helical and hairpin conformations. In less polar media, such as chloroform, aggregation, presumably by association of the outer faces of the β-strands, becomes important. In a more strongly solvating medium such as DMSO, solvent invasion of the peptide backbone limits the extent of secondary-structure formation. Strategies for achieving globularity may be important in protecting intramolecular hydrogen bonds from disruption by strongly interacting solvents. The ability to construct short peptide segments that adopt relatively robust helical and β-hairpin conformations may be used to advantage in developing a "Lego" approach to the elaboration of synthetic polypeptides that adopt complex folding patterns. The use of backbone

conformational constraints as a means of nucleating specific conformations allows design strategies to be dictated by the appropriate choice of amino acid building blocks. This approach places fewer demands on solvent forces in maintaining peptide-folding patterns. In its present state of development, this approach may be termed "molecular carpentry" as it results in polypeptide objects of reasonably well-defined shapes. Extension by the positioning of functionally active residues on these scaffolds may allow evolution of this strategy to the domain of molecular engineering. Indeed, small peptides that possess pre-organized β-turn and β-hairpin scaffolds have been used to generate moderately enantioselective peptide catalysts for acyl-transfer reactions.[27]

Experimental Section

Peptide synthesis: Peptides **I** and **II** were synthesized by conventional solution-phase methods[10f] by using a fragment condensation strategy. The *tert*-butyloxycarbonyl (Boc) group was used for N-terminal protection, while the C terminus was protected as a methyl ester. Deprotections were performed with 98% formic acid and saponification for the N and C termini, respectively. Couplings were mediated by dicyclohexylcarbodiimide/1-hydroxybenzotriazole (DCC/HOBt). The final couplings of both of the 17-residue peptides were achieved by the fragment condensation of Boc-Val-Ala-Leu-Aib-Val-Ala-Leu-OH and H-Gly-Gly-Leu-Phe-Val-D-Pro-Gly-Leu-Phe-Val-OMe for peptide **I** and Boc-Leu-Aib-Val-Ala-Leu-Aib-Val-OH with H-Gly-Gly-Leu-Val-Val-D-Pro-Gly-Leu-Val-Val-OMe for peptide **II** with DCC/HOBt as coupling agents. All the intermediates were characterized by ^{1}H NMR (80 MHz) and thin-layer chromatography (TLC) on silica gel and used without further purification. The final peptides were purified by reverse-phase, medium-pressure liquid chromatography (C_{18}, 40–60μ) and by HPLC on a reverse-phase C_{18} column (5–10 μ, 7.8 mm × 250 mm) with methanol/water gradients. The purified peptides were analyzed by mass spectrometry on a Kratos PC-Kompact MALDI-TOF mass spectrometer: MNa^+_{obs} peptide **I**, 1794.8 (M_{calcd} 1771.2); MNa^+_{obs} peptide **II**, 1712.1 (M_{calcd} 1689.3). Both peptides were fully characterized by 500 MHz ^{1}H NMR.

NMR spectroscopy: NMR studies were carried out on a Bruker DRX-500 spectrometer. All two-dimensional experiments were done in the phase-sensitive mode with time-proportional phase incrementation. DQF-COSY,[28] TOCSY,[29] NOESY,[30] and ROESY[31] experiments were performed by collecting 1000 data points in f2 and 512 data points in f1 with a spectral width of 5500 Hz. Solvent suppression was achieved by using presaturation (with a 55 dB pulse in recycle delay of 1.5 s). Data were processed on a Silicon Graphics Indy work station with Bruker XWIN NMR software. Typically, a sine-squared window function, phase shifted by $\pi/2$ radians, was applied in both dimensions. Data in f1 were zero-filled to 1000 points. Mixing times of 300 ms were used in NOESY experiments. A spin-lock mixing time of 300 ms was used in ROESY experiments and a 70 ms mixing time was used for TOCSY experiments. The sample concentration was ~3 mM and the probe temperature was maintained at 300 K or 270 K as desired. Coupling constants were measured from resolution-enhanced one-dimensional spectra.

Circular dichroism (CD): CD spectra were recorded on a JASCO J-715 spectropolarimeter. The instrument was calibrated with D(+)-10-camphor sulfonic acid. The path length used was 1 mm. The data were acquired in the wavelength scan mode, with a 1 nm band width and a step size of 0.2 nm. Spectra were acquired at 300 K with peptide concentrations of 0.1 mg mL^{-1}. Typically, 8 scans were acquired from 260–195 nm by using a scan speed of 50 nm min^{-1}. The resulting data were baseline-corrected and smoothed.

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[1] T. E. Creighton, *Proteins: Structures and Molecular Properties*, 2nd ed., Freeman, New York, **1993**; b) K. A. Dill, S. Bromberg, K. Yue, K. M. Fiebig, D. P. Yee, P. D. Thomas, H. S. Chan, *Protein Sci.* **1995**, *4*, 561–602.

[2] a) S. Kamtekar, J. M. Schiffer, H. Xiong, J. M. Babik, M. H. Hecht, *Science* **1993**, *262*, 1680–1685; b) W. F. DeGrado, C. M. Summa, V. Pavone, F. Nastri, A. Lombardi, *Annu. Rev. Biochem.* **1999**, *68*, 779–819; c) S. T. R. Walsh, H. Cheng, J. W. Bryson, H. Roder, W. F. DeGrado, *Proc. Natl. Acad. Sci. USA*. **1999**, *96*, 5486–5490; d) B. I. Dahiyat, S. L. Mayo, *Science* **1997**, *278*, 82–87; e) E. Ilyina, V. Roongta, K. H. Mayo, *Biochemistry* **1997**, *36*, 1301–1315; f) M. D. Struthers, R. P. Cheng, B. Imperiali, *Science* **1996**, *271*, 342–345; g) P. B. Harbury, J. J. Plees, B. Tidor, T. Alber, P. S. Kim, *Science* **1998**, *282*, 1462–1467; h) T. Kortemme, M. Ramirez-Alvarado, L. Serrano, *Science* **1998**, *281*, 253–256; i) C. E. Schafmeister, S. L. LaPorte, J. W. Miercke, R. M. Stroud, *Nat. Struct. Biol.* **1997**, *4*, 1039–1046.

[3] a) T. Sasaki, E. T. Kaiser, *J. Am. Chem. Soc.* **1989**, *111*, 380–381; b) S. Sakamoto, I. Obataya, A. Ueno, H. Mihara, *Chem. Commun.* **1999**, 1111–1112; c) L. Regan, W. F. DeGrado, *Science.* **1988**, *241*, 976–978; d) J. S. Johansson, B. R. Gibney, J. J. Skalicky, A. J. Wand, P. L. Dutton, *J. Am. Chem. Soc.* **1998**, *120*, 3881–3886; e) B. R. Gibney, J. S. Johansson, F. Rabanal, J. J. Skalicky, A. J. Wand, P. L. Dutton, *Biochemistry*, **1997**, *36*, 2798–2806; f) B. Gutte, S. Klauser in *Peptides: Synthesis, Structures and Applications* (Ed.: B. Gutte), Academic Press, New York, **1995**, pp. 363–390; g) L. Baltzer, *Curr. Opin. Struct. Biol.* **1998**, *8*, 466–470; h) K. S. Broo, H. Nilsson, J. Nilsson, A. Flodberg, L. Baltzer, *J. Am. Chem. Soc.* **1998**, *120*, 4063–4066; i) S. Nastri, A. Lombardi, G. Morelli, O. Maglio, G. D'Auria, C. Pedone, V. Pavone, *Chem. Eur. J.* **1997**, *3*, 340–349. j) A. Lombardi, C. M. Summa, S. Geremia, L. Randaccio, V. Pavone, W. F. DeGrado, *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 6298–6305; k) J. Nilsson, L. Baltzer, *Chem. Eur. J.* **2000**, *6*, 2214–2220.

[4] a) M. R. Alvarado, F. J. Blanco, L. Serrano, *Nature Struct. Biol.* **1996**, *3*, 604–612; b) A. J. Maynard, G. J. Sharman, M. S. Searle, *J. Am. Chem. Soc.* **1998**, *120*, 1996–2007; c) H. L. Schenck, S. H. Gellman, *J. Am. Chem. Soc.* **1998**, *120*, 4869–4870; d) G. J. Sharman, M. S. Searle, *J. Am. Chem. Soc.* **1998**, *120*, 5291–5300; e) C. Das, S. Raghothama, P. Balaram, *Chem. Commun.* **1999**, 967–968; f) C. Das, S. Raghothama, P. Balaram, *J. Am. Chem. Soc.* **1998**, *120*, 5812–5813; g) M. D. Struthers, R. P. Cheng, B. Imperiali, *J. Am. Chem. Soc.* **1996**, *118*, 3073–3081.

[5] a) S. F. Betz, D. P. Raleigh, W. F. DeGrado, *Curr. Opin. Struct. Biol.* **1993**, *3*, 601–610; b) J. W. Bryson, S. F. Betz, H. S. Lu, D. J. Suich, H. X. Zhou, W. F. DeGrado, *Science* **1995**, *270*, 935–941; c) D. P. Raleigh, S. F. Betz, W. F. DeGrado, *J. Am. Chem. Soc.* **1995**, *117*, 7558–7559.

[6] a) P. Balaram, *Pure Appl. Chem.* **1992**, *64*, 1061–1066; b) P. Balaram, *J. Peptide Res.* **1999**, *54*, 195–199; c) I. L. Karle, J. L. Flippen-Anderson, M. Sukumar, K. Uma, P. Balaram, *J. Am. Chem. Soc.* **1991**, *113*, 3952–3956.

[7] R. K. Kaul, P. Balaram, *Bioorg. Med. Chem.* **1999**, *7*, 105–117.

[8] a) B. V. V. Prasad, P. Balaram, *CRC Crit. Rev. Biochem.* **1984**, *16*, 307–348; b) P. Balaram, *Curr. Opin. Struct. Biol.* **1992**, *2*, 845–851; c) I. L. Karle, *Acc. Chem. Res.* **1999**, *32*, 693–701; d) I. L. Karle, P. Balaram, *Biochemistry* **1990**, *29*, 6747–6756; e) C. Toniolo, E. Benedetti, *ISI Atlas of Science: Biochemistry* **1988**, pp 225–230; f) C. Toniolo, E. Benedetti, *Trends Biochem. Sci.* **1991**, *16*, 350–353.

[9] a) I. L. Karle, R. B. Rao, S. Prasad, R. Kaul, P. Balaram, *J. Am. Chem. Soc.* **1994**, *116*, 10355–10361; b) I. L. Karle, R. Gurunath, S. Prasad, R. Kaul, R. B. Rao, P. Balaram, *Int. J. Peptide Protein Res.* **1996**, *47*, 376–382; c) S. Vijayalakshmi, R. B. Rao, I. L. Karle, P. Balaram, *Biopolymers* **2000**, *53*, 84–98, and references therein.

[10] a) T. S. Haque, J. C. Little, S. H. Gellman, *J. Am. Chem. Soc.* **1994**, *116*, 4105–4106; b) S. K. Awasthi, S. Raghothama, P. Balaram, *Biochem. Biophys. Res. Commun.* **1995**, *216*, 375–381; c) T. S. Haque, J. C.

  0947-6539/01/0704-0846 $ 17.50+.50/0

Little, S. H. Gellman, *J. Am. Chem. Soc.* **1996**, *118*, 6975–6985; d) I. L. Karle, S. K. Awasthi, P. Balaram, *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 8189–8193; e) T. S. Haque, S. H. Gellman, *J. Am. Chem. Soc.* **1997**, *119*, 2303–2304; f) S. R. Raghothama, S. K. Awasthi, P. Balaram, *J. Chem. Soc. Perkin Trans. 2* **1998**, 137–143.

[11] a) B. L. Sibanda, J. M. Thornton, *Nature* **1985**, *316*, 170–174; b) B. L. Sibanda, T. L. Blundell, J. M. Thornton, *J. Mol. Biol.* **1989**, *206*, 759–777; c) K. Gunasekaran, C. Ramakrishnan, P. Balaram, *Protein Eng.* **1997**, *10*, 1131–1141.

[12] L. Karle, R. Gurunath, S. Prasad, R. Kaul, R. B. Rao, P. Balaram, *J. Am. Chem. Soc.* **1995**, *117*, 9632–9637.

[13] S. Aravinda, S. Datta, A. Pramanik, N. Shamala, P. Balaram, unpublished results.

[14] C. Schellman in *Protein Folding* (Ed.: R. Jaenicke) Elsevier, Amsterdam, **1980**, pp. 53–61.

[15] a) H. A. Nagarajaram, R. Sowdhamini, C. Ramakrishnan, P. Balaram, *FEBS Lett.* **1993**, *321*, 79–83; b) R. Aurora, R. Srinivasan, G. D. Rose, *Science* **1994**, *264*, 1126–1130; c) K. Gunasekaran, H. A. Nagarajaram, C. Ramakrishnan, P. Balaram, *J. Mol. Biol.* **1998**, *275*, 917–932.

[16] In peptides and proteins, low J values (2–6 Hz) are characteristic of helical conformations that correspond to $\phi \sim -60^\circ \pm 30^\circ$ for L-residues , while β-strands have high J values ($> >8$ Hz) with $\phi \sim -120^\circ \pm 60^\circ$. a) K. Wüthrich, *NMR of Proteins and Nucleic Acids*, Wiley, New York, **1986**; b) A. Pardi, M. Billeter, K. Wüthrich, *J. Mol. Biol.* **1984**, *180*, 741–751.

[17] A. Stern, W. A. Gibbons, L. C. Craig, *Proc. Natl. Acad. Sci. USA* **1968**, *61*, 734–741.

[18] E. Benedetti, C. Toniolo in *Polymeric Materials Encyclopedia*, *Vol. 8* (Ed.: J. C. Salamone), CRC, Boca Raton, Florida, **1996**, pp. 6472–6481.

[19] S. Raghothama, M. Chadda, P. Balaram, *J. Phys. Chem.* **1996**, *100*, 19666–19671.

[20] I. L. Karle, J. L. Flippen-Anderson, K. Uma, P. Balaram, *Biopolymers* **1993**, *33*, 827–837.

[21] I. L. Karle, C. Das, P. Balaram, *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 3034–3037.

[22] a) I. L. Karle, J. L. Flippen-Anderson, K. Uma, P. Balaram, *Int. J. Peptide Protein Res.* **1993**, *42*, 401–410; b) S. Datta, N. Shamala, A. Banerjee, A. Pramanik, S. Bhattacharjya, P. Balaram, *J. Am. Chem. Soc.* **1997**, *119*, 9246–9251; c) A. Banerjee, S. Datta, A. Pramanik, N. Shamala, P. Balaram, *J. Am. Chem. Soc.* **1996**, *118*, 9477–9483; d) S. Datta, M. V. Uma, N. Shamala, P. Balaram, *Biopolymers* **1999**, *50*, 13–22.

[23] a) G. N. Ramachandran, C. Ramakrishnan, V. Sasisekharan, *J. Mol. Biol.* **1963**, *7*, 95–99; b) C. Ramakrishnan, N. Srinivasan, *Curr. Sci.* **1990**, *59*, 851–861; c) C. Ramakrishnan, N. Srinivasan, D. Prasanth, *Int. J. Peptide Protein Res.* **1987**, *29*, 629–637.

[24] a) N. Sreerama, M. C. Manning, M. E. Powers, J. Zhang, D. P. Goldenberg, R. W. Woody, *Biochemistry* **1999**, *38*, 10814–10822; b) R. W. Woody, *Eur. J. Biophys.* **1994**, *23*, 253–262; b) A. Chakrabartty, T. Kortemme, S. Padmanabhan, R. L. Baldwin, *Biochemistry* **1993**, *32*, 5560–5565.

[25] A comparison of the electronic CD spectra of model β-hairpin peptides: Boc-Leu-Val-Val-D-Pro-Gly-Leu-Val-Val-OMe and Boc-Leu-Phe-Val-D-Pro-Gly-Leu-Phe-Val-OMe, clearly reveals anomalous electronic CD bands in the latter, while the vibrational CD (amide I region) spectra are almost identical. C. Zhao, P. L. Polavarapu, C. Das, P. Balaram, unpublished results.

[26] a) T. S. Sudha, E. K. S. Vijaykumar, P. Balaram, *Int. J. Peptide Protein Res.* **1983**, *22*, 464–468; b) G. Yoder, A. Polese, R. A. G. D. Silva, F. Formaggio, M. Crisma, Q. B. Broxterman, J. Kamphuis, C. Toniolo, T. A. Keiderling, *J. Am. Chem. Soc.* **1997**, *119*, 10278–10285; c) N. H. Andersen, Z. Liu, K. S. Prickett, *FEBS Lett.* **1996**, *399*, 47–52.

[27] E. R. Jarvo, G. T. Copeland, N. Papaioannou, P. J. Bonitatebus, Jr., S. J. Miller, *J. Am. Chem. Soc.* **1999**, *121*, 11638–11643.

[28] U. Piantini, O. W. Sorensen, R. R. Ernst, *J. Am. Chem. Soc.* **1982**, *104*, 6800–6801.

[29] L. Braunschweiler, R. R. Ernst, *J. Magn. Reson.* **1983**, *53*, 521–528.

[30] A. Kumar, R. R. Ernst, K. Wüthrich, *Biochem. Biophys. Res. Commun.* **1980**, *95*, 1–6.

[31] a) A. Bax, D. G. Davis, *J. Magn. Reson* **1985**, *63*, 207–213; b) A. A. Bothner-By, R. L. Stephens, J. Lee, C. D. Warren, R. W. Jeanloz, *J. Am. Chem. Soc.* **1984**, *106*, 811–812.

[32] R. Koradi, M. Billeter, K. Wüthrich, *J. Mol. Graphics* **1996**, *14*, 51–55.

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