

Vibrational Circular Dichroism of β -Hairpin Peptides

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Abstract: Analysis of vibrational absorption and vibrational circular dichroism (VCD) for synthetic peptides designed to adopt β -hairpin conformations reveals characteristic well-resolved amide I absorption and VCD bands. All β -hairpins with a type II' β -turn segment yield an intense negative VCD band in the ~ 1643 – 1659 cm^{-1} region, and a weak positive VCD band at ~ 1693 cm^{-1} . These spectral features are diagnostic of β -hairpins and distinct from those observed for other secondary structures. Comparison of the electronic CD spectra of the β -hairpin peptides Boc-Leu-Val-Val-^DPro-Gly-Leu-Val-Val-OMe (**1**) and Boc-Leu-Phe-Val-^DPro-Gly-Leu-Phe-Val-OMe (**2**) reveals that cross-strand aromatic interactions result in anomalous CD spectra in the region 200–240 nm for peptide **2**. Similar anomalous electronic CD are observed in the three-stranded β -sheet peptide Boc-Leu-Phe-Val-^DPro-Gly-Leu-Val-Leu-Ala-^DPro-Gly-Phe-Val-Leu-OMe (**3**), while the VCD spectrum is characteristic of β -hairpin conformations. The identical VCD spectra obtained for the peptides **1** and **2** emphasize the utility of VCD, as compared to electronic CD, in the conformational analysis of peptides containing aromatic residues.

Introduction

Vibrational circular dichroism (VCD)¹ is a technique that has been finding increasing application in conformational analysis of peptides.² It can provide important conformational information in cases where electronic circular dichroism (CD) is complicated by overlap of the backbone amide and side chain aromatic chromophores. This feature is particularly relevant for studies of peptides containing Phe, Tyr, and Trp residues which can contribute significantly to electronic absorption in the far-UV region (190–230 nm), which is generally considered to arise exclusively from backbone amide contribution. Characteristic VCD spectra have been reported for peptide helices,³ β -sheet,^{3c–e} random coil,^{3c–e} β -turns,⁴ double helices,^{2b,h} etc. In calibrating

VCD methods for the conformational analysis of peptides and proteins, it is necessary to unambiguously determine characteristic spectroscopic signatures for various secondary structural motifs. The availability of structurally well-defined synthetic peptides permits the assignment of specific spectroscopic bands to unique conformational features.

The use of centrally positioned ^DPro-Xxx segments to nucleate β -hairpin conformation in synthetic oligopeptides is well established.⁵ However, VCD studies of β -hairpin structure were not reported. In this paper we describe the observation of diagnostic VCD bands in a series of synthetic peptides with centrally positioned ^DPro-Gly or ^DPro-Ala segments, whose β -hairpin structure was confirmed by NMR or X-ray. And these peptides are restricted to the type II' β -turn at the ^DPro-Xxx segment. The absorption and VCD spectra show a distinct pattern characteristic of the β -hairpin conformation in all these peptides. We also demonstrate the utility of VCD as a sensitive probe for conformational analysis of peptides containing aromatic residues, where the interpretation of electronic circular dichroism becomes ambiguous.

Experimental Section

Peptide Synthesis. Peptides were synthesized by conventional solution-phase methods by using a fragment condensation strategy.^{5b} The *tert*-butyloxycarbonyl (Boc) group was used for N-terminal protection, and the C-terminus was protected as a methyl ester. Deprotections were performed using 98% formic acid and by saponification for N- and C-terminus, respectively. Couplings were mediated by dicyclohexylcarbodiimide/1-hydroxybenzotriazole (DCC/HOBt). All the intermediates were characterized by ¹H NMR (80 MHz) and thin-layer chromatography (TLC) on silica gel and used without further

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purification. The final peptides were purified by reverse-phase, medium-pressure liquid chromatography (C_{18} , 40–60 μ m) and by high-performance liquid chromatography (HPLC) on a reverse-phase C_{18} column (5–10 μ m, 7.8 mm \times 250 mm) using methanol/water gradients. The chemical structures of the peptides studied are as follows: Boc-Leu-Val-Val- D Pro-Gly-Leu-Val-Val-OMe (1); Boc-Leu-Phe-Val- D Pro-Gly-Leu-Phe-Val-OMe (2); Boc-Leu-Phe-Val- D Pro-Gly-Leu-Val-Leu-Ala- D Pro-Gly-Phe-Val-Leu-OMe (3); Boc-Leu-Val-Val- D Pro-Ala-Leu-Val-Val-OMe (4); Boc-Leu-Phe-Val- D Pro-Ala-Leu-Phe-Val-OMe (5); Boc-Met-Leu-Val-Val- D Pro-Gly-Leu-Val-Val-Phe-OMe (6); Boc-Met-Leu-Phe-Val- D Pro-Ala-Leu-Val-Val-Phe-OMe (7); and Boc-Leu-Val-Val- D Pro- P Ala-Leu-Val-Val-OMe (8). The purified peptides were analyzed by mass spectrometry on a Kratos PC-Kompact MALDI-TOF mass spectrometer: MNa^+_{obs} peptide 1, 931.7 (M_{calc} 908.6); MNa^+_{obs} peptide 2, 1029.3 (M_{calc} 1005.3); MNa^+_{obs} peptide 3, 1578.3 (M_{calc} 1555.6); MNa^+_{obs} peptide 4, 946.1 (M_{calc} 922.6); MNa^+_{obs} peptide 5, 1041.7 (M_{calc} 1019.3); MNa^+_{obs} peptide 6, 1210.9 (M_{calc} 1187.6); MNa^+_{obs} peptide 7, 1273.0 (M_{calc} 1249.6); MNa^+_{obs} peptide 8, 945.8 (M_{calc} 922.6). All peptides were fully characterized by 500 MHz 1H NMR.

NMR Spectroscopy. NMR studies were carried out on a Bruker DRX-500 spectrometer. All 2D experiments were done in the phase-sensitive mode using time proportional phase incrementation. DQF-COSY,⁶ TOCSY,⁷ and ROESY⁸ experiments were performed collecting 1 K data points in f_2 and 512 data points in f_1 using a spectral width of 5500 Hz. Solvent suppression was achieved using presaturation (using a 55 dB pulse in recycle delay of 1.5 s). Data were processed on a Silicon Graphics Indy work station using Bruker XWIN NMR software. Typically, a sine-squared window function, phase shifted by $\pi/2$ rad, was applied in both the dimensions. Data in f_1 were zero-filled to 1K points. 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 ~ 5 mM and the probe temperature was maintained at 300 K. Coupling constants were measured from resolution-enhanced one-dimensional spectra.

Electronic Circular Dichroism. 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, using 1 nm bandwidth with a step size of 0.2 nm. Typically, 8 scans were acquired from 260 to 195 nm using a 50 nm/min scan speed. The resulting data were baseline corrected and smoothed. Peptide concentrations were ~ 0.5 mg/mL.

Vibrational Circular Dichroism. All VCD spectra were recorded on a commercial Chiralir spectrometer (BomemBiotools, Canada). The concentrations were ~ 5 mg/mL in chloroform and the temperature was 20 $^\circ$ C. All spectra were collected for 1 h at a resolution of 8 cm^{-1} , and the contribution of solvents has been subtracted.

Results and Discussion

Vibrational Circular Dichroism Vibrational absorption and VCD spectra of six peptides in the amide I (C=O stretch) region are shown in Figure 1. Similar spectral features are observed in these spectra. Consider the absorption and VCD spectra of peptide 1 as an example. The absorption bands (Figure 1a, bottom trace) are observed at 1740, 1697, 1659, and 1636 cm^{-1} . The VCD spectrum (Figure 1a, top trace) shows a strong negative band at 1655 cm^{-1} and a weak positive band at 1666 cm^{-1} , and a weak negative couplet is observed at higher frequency with the negative component at 1678 cm^{-1} and the positive component at 1697 cm^{-1} . The absorption and VCD spectra in Figure 1a are clearly distinguishable from those observed for

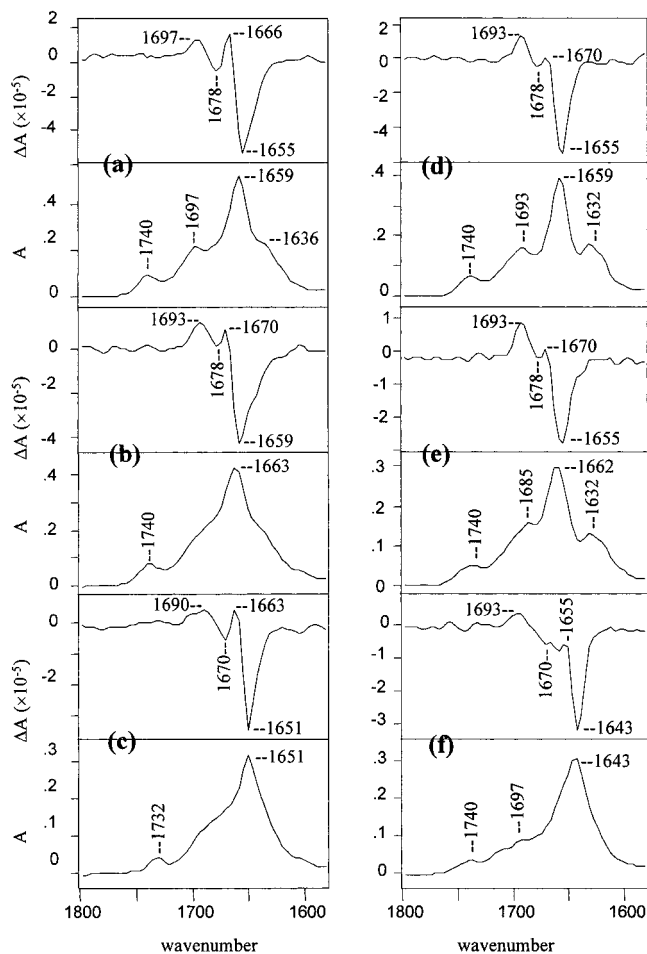


Figure 1. Vibrational absorption (bottom) and VCD (top) spectra of β -hairpin peptides in chloroform solution: (a) Boc-Leu-Val-Val- D Pro-Gly-Leu-Val-Val-OMe; (b) Boc-Leu-Phe-Val- D Pro-Gly-Leu-Phe-Val-OMe; (c) Boc-Leu-Phe-Val- D Pro-Gly-Leu-Val-Leu-Ala- D Pro-Gly-Phe-Val-Leu-OMe; (d) Boc-Leu-Val-Val- D Pro-Ala-Leu-Val-Val-OMe; (e) Boc-Leu-Phe-Val- D Pro-Ala-Leu-Phe-Val-OMe; and (f) Boc-Met-Leu-Val-Val- D Pro-Gly-Leu-Val-Val-Phe-OMe.

helix and β -sheet peptides. In the latter two cases, usually one unresolved absorption band was obtained in the amide I region. The VCD spectra of right-handed helical peptides show a bisignate couplet for the amide I band, with a negative component at higher frequency.^{2d,3} The VCD spectra of β -sheet peptides show one⁹ or two^{3c,d} negative amide I bands, but do not show VCD at 1697 cm^{-1} .

We believe that the absorption and VCD spectra in Figure 1a show the characteristic feature of a type II' β -turn incorporated in a β -sheet structure, leading to a β -hairpin structure. The strong absorption band at 1659 cm^{-1} and the negative VCD band at 1655 cm^{-1} are characteristic of a β -sheet structure. The weak positive VCD band at 1666 cm^{-1} was also observed in some cases for β -sheet structure.^{3e,f} The absorption bands at 1636 and 1697 cm^{-1} and the VCD bands at 1697 and 1678 cm^{-1} are assigned to β -turn structure. It was reported⁴ that when proline is contained in the peptide linkage, distinctive absorption bands were observed in amide I region. In a X-Pro peptide linkage where a tertiary amine is involved, the $C=O_{(X)}$ stretching frequency was observed around 1640–1655 cm^{-1} , whereas in the Pro-X peptide linkage where a secondary amine is involved, the $C=O_{(Pro)}$ is observed at 1670–1685 cm^{-1} . So in Figure 1a, the absorption band at 1636 cm^{-1} is due to the carbonyl group in the Val- D Pro linkage, whereas the band at 1697 cm^{-1} is due

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to the carbonyl group in the D Pro-Gly linkage. The weak VCD couplet observed in Figure 1a in the ~ 1695 – 1670 cm^{-1} region is assigned to that of a type II' β -turn conformation.

To confirm the type II' β -turn conformation, β -turn models of peptide **1** were built for different types (type I, type II, type III, type I', type II', and type III') and VCD spectral predictions were made using coupled oscillator theory.¹⁰ Only type II' shows the high-frequency negative VCD couplet, corresponding to the couplet in the ~ 1695 – 1670 cm^{-1} region in Figure 1a. The β -hairpin structure model with a type II' turn was also built for peptide **1**. The calculated VCD spectrum resembled the experimental VCD spectrum in Figure 1a, except that the positive VCD in the experimental spectrum at 1666 cm^{-1} did not have a counterpart in the calculated spectrum.

The absorption spectra of the other five peptides shown in Figure 1 are similar to that of peptide **1**, except that, in some cases, the bands are not as well resolved as they are for peptide **1**. The VCD spectra for the six peptides shown in Figure 1 are also similar, except that weak negative VCD at 1678 and positive VCD at 1666 cm^{-1} , seen clearly for some peptides, are not as clear, possibly due to their overlap, for other peptides.

The absorption and VCD spectra of peptide **7** and **8** are not shown here. The signals of peptide **7** are weak due to the lack of adequate amount of sample (1.9 mg). The VCD spectrum of peptide **8**, which was determined by NMR to be β -hairpin with a type I' β -turn, is somewhat different from those in Figure 1.

Peptide Conformations. The conformational analysis of peptide **1** has been previously reported in solution and in the solid state. NMR studies in chloroform solutions have clearly established a β -hairpin conformation stabilized by four cross-strand hydrogen bonds.^{5a,b} This conformation has also been observed by X-ray diffraction studies of single crystals grown from methanol solution.^{5c} NMR studies also establish that peptide **2** adopts a predominantly β -hairpin conformation in solution. In addition, the peptide module Leu-Phe-Val- D Pro-Gly-Leu-Phe-Val has been shown to have a β -hairpin conformation in the crystal structure of a 17-residue peptide, Boc-Val-Ala-Leu-Aib-Val-Ala-Leu-Gly-Gly-Leu-Phe-Val- D Pro-Gly-Leu-Phe-Val-OMe (Aib = α -aminoisobutyric acid), containing this segment.¹¹ The NH proton chemical shifts in peptide **2** are very similar to that observed in peptide **1**. Figure 2 shows the nuclear Overhauser effects (NOE) between backbone protons. The observation of strong interresidue $C_i^{\alpha}\text{H}-N_{i+1}\text{H}$ NOEs and weaker intraresidue $C_i^{\alpha}\text{H}-N_i\text{H}$ NOEs for residues 1–3 and 6–8 along with the absence of sequential $N_i\text{H}-N_{i+1}\text{H}$ confirms the strand conformation for these segments. The observation of large $^3J_{\text{NH}-\text{C}^{\alpha}\text{H}}$ [J (Hz): Leu(1) (8.5), Phe(2) (8.8), Val(3) (9.2), Leu(6) (8.9), Phe(7) (7.5), Val(8) (9.4)] for residues 1–3 and 6–8 further supports the extended backbone conformation of these segments. The interstrand NH–NH NOEs between residues Leu(1)–Val(8) and Val(3)–Leu(6) confirms that a β -hairpin conformation is predominantly populated for peptide **2** in solution. The octapeptides Boc-Leu-Val-Val- D Pro-Ala-Leu-Val-Val-OMe (**4**) and Boc-Leu-Val-Val- D Pro- D Ala-Leu-Val-Val-OMe (**8**) have also been shown to adopt β -hairpin conformation in methanol solution differing only in the nature of the β -turn, type II' in **4** and type I' in **8**. The β -hairpin conformation in peptide **4** has also been established by X-ray diffraction studies (submitted for publication). The 14-residue three-stranded

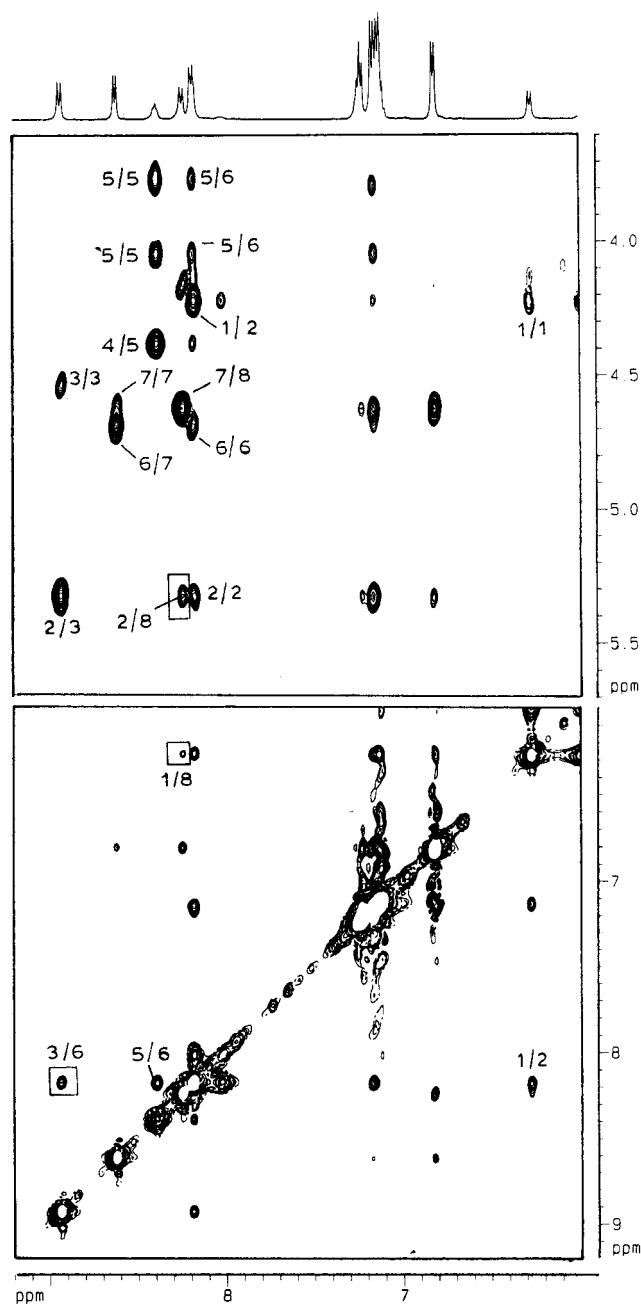


Figure 2. Partial 500 MHz ROESY spectra of peptide **2** in CD_3OH at 300 K: (top panel) $\text{C}^{\alpha}\text{H} \leftrightarrow \text{NH}$ NOEs and (bottom panel) $\text{NH} \leftrightarrow \text{NH}$ NOEs. The residue numbers are used to label the cross-peaks. Long-range NOEs diagnostic of β -hairpin conformations are boxed.

β -sheet peptide **3** has been earlier characterized in chloroform and methanol solutions by ^1H NMR spectroscopy.¹² Peptides **5**, **6**, and **7** have also been characterized by 500 MHz ^1H NMR spectroscopy and the data are fully consistent with a predominant population of β -hairpins in these peptides (data not shown).

Comparison of Electronic CD and VCD in Peptides Containing Aromatic Residues. Figure 3 shows the electronic CD spectra of peptides **1** and **2** in methanol and 2,2,2-trifluoroethanol (TFE). Peptide **1** shows a broad negative band centered at 218 nm, a feature characteristic of β -hairpin peptides.^{5a,13} In TFE a small blue shift of ~ 2 – 3 nm is observed

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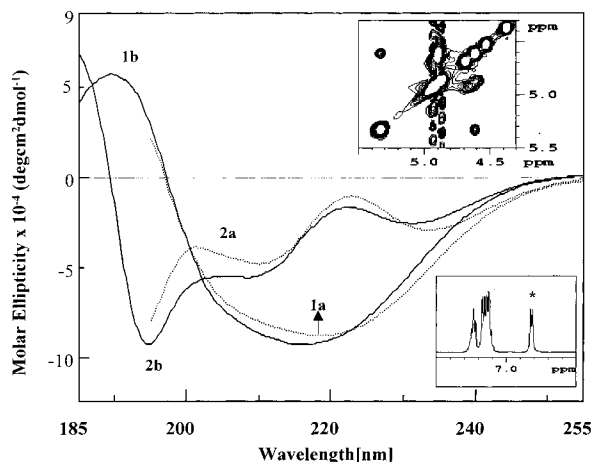


Figure 3. Electronic CD spectra of the peptides **1** and **2** in the far-UV region in methanol (a) and trifluoroethanol (b) (300 K). The inset (bottom, right) highlights the ring current shifted H2 and H6 protons (*) of Phe 7 in peptide **2** in CD₃OH (300 K). The inset (top, right) illustrates a portion of the ROESY spectrum of **2** in CD₃OH at 300 K, highlighting the C^αH connectivity between Phe 2 and Phe 7.

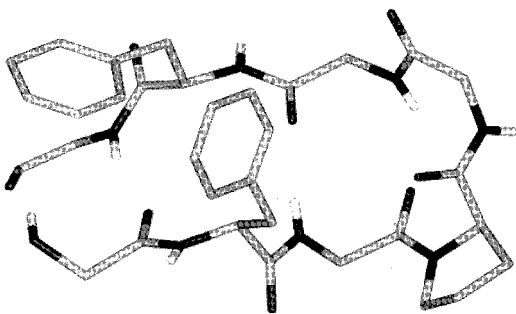


Figure 4. Schematic illustration of the β -hairpin conformation in peptide **2** indicating the close proximity of the two aromatic rings of Phe 2 and Phe 7. The structure has been generated using idealized geometries and backbone dihedral angles determined for the segment Leu-Phe-Val-D₂Pro-Gly-Leu-Phe-Val in the X-ray structure of a 17-residue peptide (ref 11). The figure was generated using MolMol.¹⁴

without any significant change in band shape or intensity. In contrast, peptide **2** shows a distinctly anomalous spectrum in both solvents. Peptide **2** which contains two Phe residues exhibits two negative bands at 232 and 210 nm in methanol. Clearly, the anomalous electronic CD spectra of this peptide hairpin must arise due to the presence of aromatic residues. The inset to Figure 3 shows the anomalous chemical shift observed for the H2 and H6 protons of Phe(7). The high-field position of 6.8 ppm arises due to the shielding effect of Phe(2), with two aromatic rings participating in a strong cross-strand interaction (Figure 4). The population of β -hairpin structures is established by the observation of critical cross-strand NOEs. The NOE between the C^αH protons of Phe(2) and Phe(7)

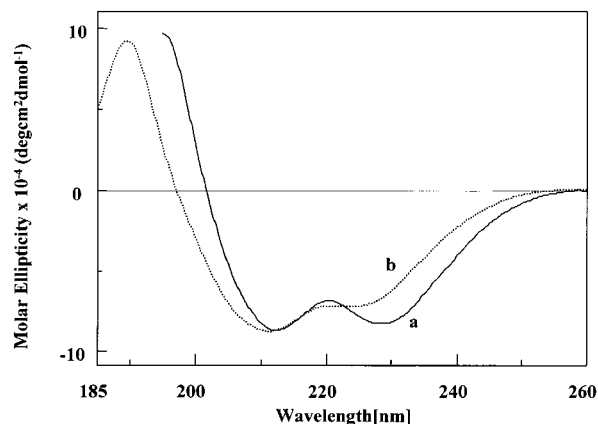


Figure 5. Electronic CD spectra of the three-stranded β -sheet peptide **3** in the far-UV region in methanol (a) and trifluoroethanol (b) (300 K).

observed in the ROESY spectrum of **2** is illustrated in the inset to Figure 3. Figure 5 shows the electronic CD spectra of the three-stranded β -sheet peptide **3** in methanol and TFE. Distinct negative bands can be seen at 228 and 212 nm in methanol, and at 225 and 211 nm in TFE. A low-wavelength positive band is observed in TFE at 189 nm while in methanol the exact position of the positive band could not be determined due to solvent absorption. Clearly, the CD spectra are anomalous and do not reflect the three-stranded β -sheet structure determined for the peptide by NMR. In contrast, the VCD spectrum shown in Figure 1c shows the pattern observed for β -hairpin peptides **1** and **2** (Figure 1a,b).

Conclusion

Our results clearly establish a characteristic VCD pattern for the amide I band in β -hairpin peptides. The utility of VCD as a conformational probe is also illustrated by the application to a system containing aromatic residues where anomalous electronic CD is observed. Application of VCD methods will be of particular importance in view of the growing realization that aromatic contribution to far-UV electronic CD cannot be ignored.¹⁵

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