Pergamon

Tetrahedron: Asymmetry Vol. 6, No. 3, pp. 687-690, 1995

0957-4166/95 $9.50+0.00

0957-4166(95)00062-3

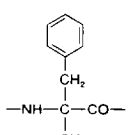
Helical Screw Sense of Homo-Oligopeptides of Cα-Methylated α-Amino Acids as Determined with Vibrational Circular Dichroism

Gorm Yoder,[1] Timothy A. Keiderling,[1]* Fernando Formaggio,[2] Marco Crisma,[2] Claudio Toniolo,[2]* and Johan Kamphuis[3]

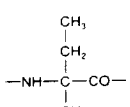
[1]Department of Chemistry, University of Illinois at Chicago, Chicago, IL 60607-7061, USA; [2]Biopolymer Research Center, CNR, Department of Organic Chemistry, University of Padova, 35131 Padova, Italy; [3]DSM Research, Bio-organic Chemistry Section, 6160 MD Geleen, The Netherlands

Abstract: Vibrational circular dichroism spectra for three homo-oligopeptides, *p*-BrBz-[D-(αMe)Phe]$_{4,5}$-O*t*Bu and *p*-BrBz-(D-Iva)$_5$-O*t*Bu, are presented and interpreted to show that the C$^\alpha$-methylated α-amino acids (αMe)Phe and Iva of the same optical configuration favor helical conformations of the opposite screw sense. The D-(αMe)Phe tetra- and pentapeptides are folded in a right-handed 3_{10}-helix, which is consistent with previous findings regarding homo-oligomers of C$^\alpha$-methylated amino acids with γ-branched side chains. On the other hand, the pentapeptide of D-Iva, with a linear side chain, is folded in a left-handed helical sense, in agreement with the behavior of peptides based on the D-enantiomers of protein amino acids.

Many homo-oligomers of C$^\alpha$-methylated amino acids [1] (*Scheme I*) are known to form stable 3_{10}-helical structures. More specifically, recent X-ray studies have indicated that, in the crystal state, C$^\alpha$-methylated L-amino acids with linear side chains predominently lead to the common right-handed helical form, whereas in homo-oligomers of C$^\alpha$-methylated L-amino acids with γ-branched side chains the unusual left-handed helical form largely prevails. [2] As we have demonstrated in previous reports,[3] vibrational circular dichroism (VCD), the measurement of CD for fundamental infrared-active vibrations, can provide a unique tool for identification of the 3_{10}-helix in peptides and discrimination from the classical α-helical form. In a conformational study of peptides a major advantage of VCD over electronic CD is the inherent resolution capability of the infrared region which allows discrimination against interfering transitions, in particular those associated with the aromatic moieties of blocking groups and amino acid side chains, thereby focusing on the peptide backbone. [4] Therefore, it seemed natural to us to exploit VCD to investigate the helical



(αMe)Phe



Iva

Scheme 1. The C$^\alpha$-methylated α-amino acids discussed in this work.

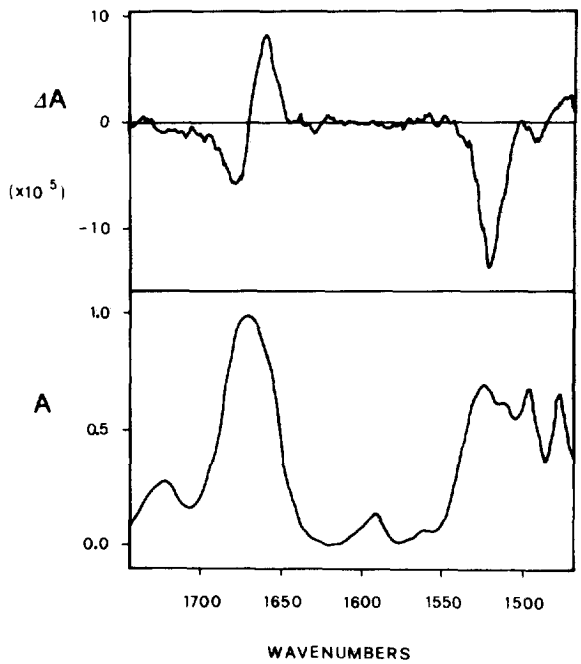


Figure 1. IR absorption and VCD spectra in the 1750-1475 cm^{-1} region of *p*-BrBz-[D-(αMe)Phe]$_4$-O*t*Bu in $CDCl_3$ solution (cell pathlength 100μ, peptide concentration 1.25 mg/100μL).

structure of selected C^{α}-methylated homo-oligopeptides in solution both in terms of its nature and screw sense.

The synthesis and characterization of *p*-BrBz-[D-(αMe)Phe]$_{4,5}$-O*t*Bu [*p*-BrBz, *para*-bromobenzoyl; (αMe)Phe, C^{α}-methyl phenylalanine; O*t*Bu, *tert*-butoxy] and *p*-BrBz-(D-Iva)$_5$-O*t*Bu (Iva, isovaline) have already been reported. [5] VCD spectra were recorded in $CDCl_3$ solution in the amide I and amide II regions using a UIC dispersive VCD instrument previously described [6] and a 10-sec time constant as the average of eight scans. Identical scans of the solvent were subtracted for baseline correction. All spectra were collected with a resolution of 9 cm^{-1} and calibrated for instrument response using our standard techniques.[6]

The IR absorption and VCD spectra of *p*-BrBz-[D-(αMe)Phe]$_4$-O*t*Bu and *p*-BrBz-(D-Iva)$_5$-O*t*Bu in the 1750-1475 cm^{-1} region in $CDCl_3$ solution are shown in Figures 1 and 2, respectively. For the purpose of comparison, all spectra were normalized to have a peak absorbance of 1.0 for the amide I component. Thus, the plots read ΔA/A directly on the amide I absorbance peak and offer an approximate normalization of the VCD to peptide concentration.

The D-(αMe)Phe tetrapeptide VCD in the amide I region shows a positive couplet (*i.e.*, negative to higher energy) with a positive bias. The zero crossing point lies close to the maximum of the large absorption band centered at about 1670 cm^{-1}. The amide II VCD consists of a strong negative band with its maximum at 1520 cm^{-1}, only slightly shifted down in frequency from the absorption maximum. These VCD data are comparable in shape, frequency and intensity to those found experimentally for right-handed 3_{10}-helical structures [3] and are quite different from the right-handed α-helical pattern [7] in terms of the

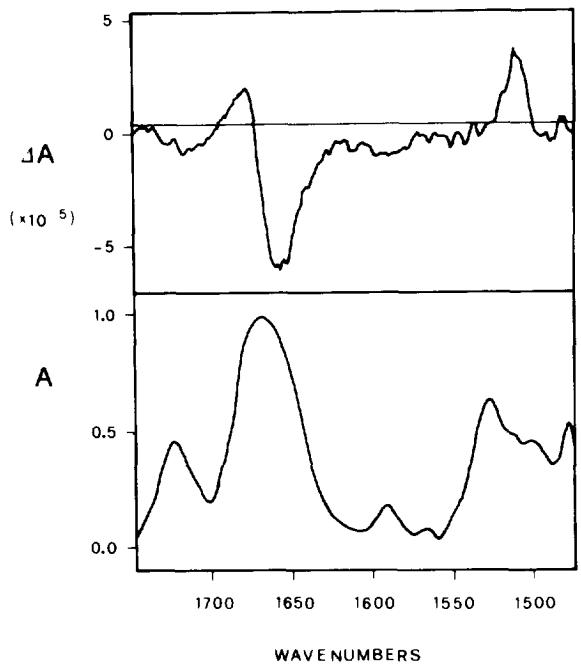


Figure 2. IR absorption and VCD spectra in the 1750-1475 cm^{-1} region of *p*-BrBz-(D-Iva)$_5$-O*t*Bu in $CDCl_3$ solution (cell pathlength 100 μ, peptide concentration 1.25 mg/100 μL).

relative intensity of the amide II band and the bandshape of the amide I. Here the important features indicating right-handed 3_{10}-helix formation are the intense, negative amide II band, seen more weakly in right-handed α-helices, and the positive bias of the positive amide I VCD couplet, where the α-helix positive VCD couplet is negatively biased. The D-(αMe)Phe pentapeptide gave qualitatively similar results, but had a somewhat more intense amide I and less intense amide II VCD (not shown). These observed VCD spectra are those expected for right-handed 3_{10}-helices formed by protein L-amino acids. However, the two (αMe)Phe oligomers were synthesized using an amino acid of the opposite (D) configuration. Therefore, the favored conformation for the oligomers of L-(αMe)Phe is indicated to be left-handed helical. Furthermore, due to the C^{α}-methyl substitution, the preferred helix appears to be 3_{10}-helical rather than α-helical.

For the D-Iva pentapeptide the amide I region VCD consists of a negatively biased couplet with a negative lobe to lower energy. The absorption band, which has its maximum at 1667 cm^{-1}, lies close to the zero crossing point of the VCD spectrum. The amide II component exhibits a positive bandshape whose maximum is centered at 1510 cm^{-1}, which is significantly lower in energy than the absorbance maximum (1525 cm^{-1}) and is much weaker in intensity than the amide I VCD. Unlike the D-(αMe)Phe homopeptides, the VCD spectrum for the D-Iva pentapeptide does not uniquely suggest the helical form to be 3_{10} in nature. However, all other available experimental data for this oligomer (crystal structure, infrared absorption and nuclear magnetic resonance) [5b] indicate formation of a 3_{10}-helix. In addition, the VCD spectral features are in favor of the left-handed nature of the all-D oligomer of Iva, making its preferred handedness the same as that adopted by peptides based on the D-enantiomers of protein amino acids.

In summary, the present VCD findings are in agreement with previous crystal-state conformational results, [2] in particular that a C^{α}-methylated, L-amino acid with a linear side chain (Iva) favors a right-handed helix, while a C^{α}-methylated L-amino acid with a γ-branched side chain [(αMe)Phe] tends to form a left-handed helix. We also confirmed that the dominant helix formed by the (αMe)Phe homo-peptides is of the 3_{10} type. Finally, we showed that the screw sense of a helix formed by a peptide of (αMe)Phe is easily determinable by VCD in the amide I and amide II regions, whereas the interpretation of its electronic CD spectrum may be much less straightforward due to interference by the transitions of the aromatic group.

References and Notes

1. For the large-scale production of the optically pure (αMe)Phe and Iva enantiomers we have exploited an economically attractive, chemo-enzymatic synthesis described in: Kruizinga, W.H.; Bolster, J.; Kellogg, R.M.; Kamphuis, J.; Boesten, W.H.J.; Meijer, E.M.; Schoemaker, H.E. *J. Org. Chem.* **1988**, 53, 1826.
2. For a review-article see: Toniolo, C.; Crisma, M.; Formaggio, F.; Valle, G.; Cavicchioni, G.; Précigoux, G.; Aubry, A.; Kamphuis, J. *Biopolymers* **1993**, 33, 1061.
3. a) Yasui, S.C.; Keiderling, T.A.; Bonora, G.M.; Toniolo, C. *Biopolymers* **1986**, 25, 79. b) Yasui, S.C.; Keiderling, T.A.; Formaggio, F.; Bonora, G.M.; Toniolo, C. *J. Am. Chem. Soc.* **1986**, 108, 4988.
4. a) Yasui, S.C.; Keiderling, T.A. *Biopolymers* **1986**, 25, 5. b) Yasui, S.C.; Keiderling, T.A.; Sisido, M. *Macromolecules* **1987**, 20, 2403.
5. a) Pantano, M.; Formaggio, F.; Crisma, M.; Bonora, G.M.; Mammi, S.; Peggion, E.; Toniolo, C.; Boesten, W.H.J.; Broxterman, Q.B.; Schoemaker, H.E.; Kamphuis, J. *Macromolecules* **1993**, 26, 1980. b) Formaggio, F.; Crisma, M.; Bonora, G.M.; Pantano, M.; Valle, G.; Toniolo, C.; Aubry, A.; Bayeul, D.; Kamphuis, J. *Pept. Res.* **1995**, in press.
6. a) Keiderling, T.A. *Appl. Spectroscop. Rev.* **1981**, 17, 189. b) Keiderling, T.A. In *Practical Fourier Transform Spectroscopy*, Ferraro, J.R.; Krishnan, K., Eds., Academic Press, New York, 1990, pp. 203-283.
7. a) Singh, R.D.; Keiderling, T.A. *Biopolymers* **1981**, 20, 237. b) Lal, B.; Nafie, L.A. *Biopolymers* **1982**, 21, 2161. c) Sen, A.C.; Keiderling, T.A. *Biopolymers* **1984**, 23, 1519. d) Yasui, S.C.; Keiderling, T.A.; Katakai, R. *Biopolymers* **1987**, 26, 1407. e) Birke, S.S.; Agbaje, I.; Diem, M. *Biochemistry* **1992**, 31, 450.

(*Received in UK* 23 *January* 1995)