# Circular Dichroism Spectrum of a Peptide $3_{10}$-Helix

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The $3_{10}$-helix, first predicted as a reasonably stable polypeptide secondary structure 55 years ago,[1] has only relatively recently attracted the attention of structural biochemists and protein crystallographers. Besides the classical α-helix and *β*-pleated sheet conformation, it represents the third principal structural element occurring in globular proteins and has been described at atomic resolution in model peptides and in peptaibol antibiotics.[2] The average conformational parameters of the α-helix are rather close to those of the $3_{10}$-helix, the latter being slightly tighter and more elongated.[2] However, the C═O···H—N intramolecular H-bonding schemes are quite different.

Electronic circular dichroism (CD) is the most extensively used spectroscopic technique to determine global secondary structural information in peptides and proteins in solution. The CD spectra for α-helix and *β*-pleated sheet are well characterized both theoretically and experimentally.[3] The right-handed α-helix gives rise to two negative bands at 222 and 208 nm of almost equal intensities and a strong positive band at about 192 nm. The spectrum for a *β*-pleated sheet shows a negative maximum near 217 nm and a positive maximum at 198 nm. In detailed studies on the theoretical aspects of $3_{10}$-helix CD, Manning and Woody[3c] proposed that one possible criterion for distinguishing the conformationally related $3_{10}$- and α-helices by CD is that they would have a different ratio $R = [\Theta]_{222}/[\Theta]_{208}$, much weaker for the $3_{10}$-helix (as mentioned above, largely α-helical polypeptides are known to have *R* values of ∼1). In addition, the $3_{10}$-helix is expected to display a positive band near 195 nm of much reduced intensity compared to that for the α-helix. The only published experimental CD investigation focusing specifically on the CD of α- *vs* $3_{10}$-helical peptides was carried out by Balaram and co-workers.[4] Using a set of seven peptides ranging in length from 10 to 21 amino acid residues, these authors concluded that, "$3_{10}$- and α-helical conformations cannot be distinguished by CD". In contrast to this pessimistic conclusion, in this communication we report the standard CD spectrum for a peptide $3_{10}$-helix, which turns out to be different, although not dramatically so, from that of an α-helix. In addition, it is in general agreement with the theoretically predicted $3_{10}$-helix CD features.

In this type of chirospectroscopic analysis, the main problem is the design of an appropriate optically active model compound. We started our reasoning from the consideration that monodispersed *homo*peptides from the prototype of $C^{\alpha}$-tetrasubstituted α-amino acids, the *achiral* Aib (α-aminoisobutyric acid), have been shown experimentally to fold *exclusively* into $3_{10}$-helical structures in solution as well as in the crystal state.[5] Formation of fully developed, stable $3_{10}$-helices in solution was observed only for the octamer and longer pleionomers.[6] Further, from a conformational analysis on short peptides, it was shown that, among the $C^{\alpha}$-tetrasubstituted *chiral* α-amino acids, the *β*-branched L-(α-Me)Val ($C^{\alpha}$-methyl valine) is the residue showing the most pronounced bias toward *right*-handed $3_{10}$-helices.[7] With this information in mind, we decided to synthesize three [L-(α-Me)Val]$_8$ homopeptide *tert*-butyl esters, whose different $N^{\alpha}$-blocking groups [acetyl (Ac), benzyloxycarbonyl (Z), and *p*-bromobenzoyl (*p*BrBz)] were expected to provide diverging solubility and spectroscopic properties. In particular, the $N^{\alpha}$-acetylated analog is *a priori* the most suitable for a far-UV CD analysis, lacking the aromatic chromophore at the N-terminus (potentially overlapping the $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ transitions of the peptide chromophores).

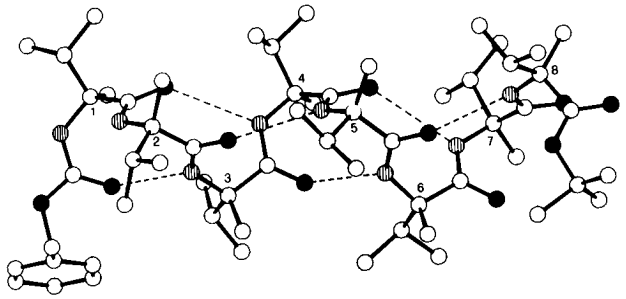


**Figure 1.** X-ray diffraction structure of *Z*-[L-(α-Me)Val]$_8$-O*t*Bu. Open circles represent carbon atoms, while vertically barred circles and filled circles represent nitrogen and oxygen atoms, respectively. The six intramolecular C═O···H—N hydrogen bonds are shown as dashed lines. Numbering of each amino acid residue is reported at the α-carbon.

*Z*-[L-(α-Me)Val]$_8$-O*t*Bu (O*t*Bu, *tert*-butyl ester) was synthesized step-by-step in solution using *Z*-L-(α-Me)Val-OH and H-L-(α-Me)Val-O*t*Bu as the starting materials.[8] Among the various activation methods tested to perform the coupling reaction between these sterically demanding residues, the acid fluoride method[9] gave the highest yields. The best experimental conditions were found to be an excess (1.5 equiv) of *Z*-L-(α-Me)Val-F and 0.75 equiv of base (*N*-methylmorpholine) in $CH_2Cl_2$ solution. From the *Z*-protected homooctapeptide ester, we also prepared the Ac and *p*BrBz $N^{\alpha}$-blocked analogs *via* catalytic hydrogenation, followed by the appropriate acylation. Complete characterization of the final products was achieved by determination of melting point, optical rotatory power, TLC $R_f$ values in three different solvent systems, and solid-state IR (KBr), $^1$H NMR, and mass spectra.[10]

The terminally blocked [L-(α-Me)Val]$_8$ sequence adopts a fully developed, right-handed $3_{10}$-helical conformation both in the crystal state and in solution. Figure 1 depicts the X-ray diffraction structure of the crystalline $N^{\alpha}$-benzyloxycarbonylated

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(1) Taylor, H. S. *Proc. Am. Phil. Soc.* **1941**, *85*, 1−7.
(2) Toniolo, C.; Benedetti, E. *Trends Biochem. Sci.* **1991**, *16*, 350−353.
(3) (a) Greenfield, N.; Fasman, G. D. *Biochemistry* **1969**, *8*, 4108−4116. (b) Woody, R. W. In *The Peptides*, *Vol. 7*; Hruby, V., Ed.; Academic Press: New York, 1985; pp 15−114. (c) Manning, M.; Woody, R. W. *Biopolymers* **1991**, *31*, 569−586.
(4) Sudha, T. S.; Vijayakumar, E. K. S.; Balaram, P. *Int. J. Pept. Protein Res.* **1983**, *22*, 464−468.
(5) (a) Prasad, B. V. V.; Balaram, P. *CRC Crit. Rev. Biochem.* **1984**, *16*, 307−348. (b) Toniolo, C.; Benedetti, E. *Macromolecules* **1991**, *24*, 4004−4009.
(6) (a) Toniolo, C.; Bonora, G. M.; Barone, V.; Bavoso, A.; Benedetti, E.; Di Blasio, B.; Grimaldi, P.; Lelj, F.; Pavone, V.; Pedone, C. *Macromolecules* **1985**, *18*, 895−902. (b) Kennedy, D. F.; Crisma, M.; Toniolo, C.; Chapman, D. *Biochemistry* **1991**, *30*, 6541−6548.
(7) Toniolo, C.; Crisma, M.; Formaggio, F.; Valle, G.; Cavicchioni, G.; Précigoux, G.; Aubry, A.; Kamphuis, J. *Biopolymers* **1993**, *33*, 1061−1072.
(8) Optically pure L-(α-Me)Val is a DSM product. For a pertinent reference, see: 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*, 1826−1827.
(9) Carpino, L. A.; Sadat-Aalaee, D.; Chao, H. G.; DeSelms, R. H. *J. Am. Chem. Soc.* **1990,** *112*, 9651−9652.
(10) Full experimental details on synthesis and characterization of the three $N^{\alpha}$-blocked L-(α-Me)Val octapeptide *tert*-butyl esters may be found in the supporting information.

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octamer.[11] The compound forms a perfect right-handed $3_{10}$-helix, stabilized by six consecutive intramolecular 1 ← 4 C═O···H−N H-bonds (type-III $\beta$-bends),[12] with mean $\varphi,\psi$ backbone torsion angle values of −54°,−32°, respectively, very close to the published average values (−57°,−30°).[2]

In previous detailed IR absorption studies in $CDCl_3$ solution, we have shown that the *Z*-$(Aib)_n$-O*t*Bu ($n = 8-11$) oligomers, which are completely folded in a $3_{10}$-helix, have the strongest bands at 3332−3323 (N−H stretching) and 1666−1661 $cm^{-1}$ (C═O stretching).[6] The corresponding values for the three L-(α-Me)Val octamers in the same solvent are found to be 3333−3331 and 1661 $cm^{-1}$. The solvent accessibilities of the NH protons, indicative of a possible participation in intramolecular H-bonds, have been examined for the most soluble $N^{\alpha}$-benzyloxycarbonylated analog as a function of added DMSO and free-radical TEMPO (2,2,6,6-tetramethylpiperidinyl-1-oxy) to the $CDCl_3$ solution using $^1H$ NMR.[13] As expected for a $3_{10}$-helix, only two NH protons are sensitive to the addition of the perturbing agents. We have identified them as the urethane N(1)H proton, which resonates at highest fields, and the N(2) proton, the latter by analogy with the results of the two-dimensional NMR study of the L-(α-Me)Val homotrimer.[14]

We have recently demonstrated that the *p*-bromobenzamido group at the N-terminus of a $3_{10}$-helical peptide chain is an excellent CD probe for the determination of helix screw sense.[15a] In TFE solution, *p*BrBz-[L-(α-Me)Val]$_8$-O*t*Bu exhibits a characteristically split CD curve, with a positive band at higher wavelengths (251 nm) and a negative band at lower wavelengths (228 nm).[16] The crossover point between the two intense, oppositely signed components is seen at 240 nm, *i.e.*, in the region of absorption of the *p*-bromobenzamido chromophore.[15b] The CD pattern supports our contention that the octameric sequence is right-handed $3_{10}$-helical also in TFE solution, as it is in the crystal state.

Having obtained evidence that the terminally blocked L-(α-Me)Val octameric sequence forms stable, right-handed $3_{10}$-helices in the crystal state and in structure-supporting solvents such as $CDCl_3$ and TFE, we recorded the CD spectrum of Ac-[L-(α-Me)Val]$_8$-O*t*Bu in TFE solution in the absorption region of peptide chromophores (Figure 2). *The right-handed $3_{10}$-helical peptide displays a negative CD band at 207 nm, accompanied by a shoulder centered near 222 nm. The ratio $R = [\Theta]_{222} / [\Theta]_{207}$ is 0.4.* These results are in good agreement with the theoretical calculations.[3c] In addition, the ellipticity at about 195 nm is positive, although only slightly. A further negative maximum is seen at 184 nm. The relatively low absolute ellipticity values are not surprising in view of the modest main-chain length of the peptide examined.[3c,4,17] Unfortunately, at present, there are no available synthetic methods to produce a high-molecular-weight homopolypeptide of limited polydispersity based on a $C^{\alpha}$-tetrasubstituted α-amino acid.[18]

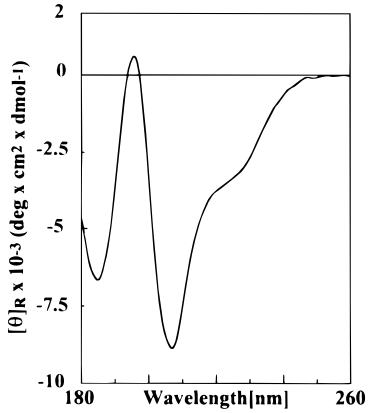


**Figure 2.** CD spectrum of Ac-[L-(α-Me)Val]$_8$-O*t*Bu in TFE solution (peptide concentration $0.6 \times 10^{-3}$ M). This spectrum represents the average of five measurements using five separate solutions. Residue molar ellipticity values, $[\Theta]_R$, are given.

In summary, we have reported the first unequivocal experimental CD spectrum for a right-handed $3_{10}$-helical peptide. It is gratifying to note that it is in general agreement with the theoretically predicted spectrum.[3c] On these bases, we tend to interpret the published CD spectra of peptaibol antibiotics[19] and model peptides containing Aib residues along with protein amino acids[4,17,19b,c,20] as arising from equilibrium mixtures of $3_{10}$- and α-helices and/or $3_{10}$- and α-helical segments coexisting in the same molecule.

**Supporting Information Available:** Experimental details for the preparation and characterization of the three L-(α-Me)Val homooctamers (31 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from ACS and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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(11) The details of the X-ray diffraction analysis for *Z*-[L-(α-Me)Val]$_8$-O*t*Bu may be found in the supporting information. The structure was solved on a Philips PW 1100 four-circle diffractometer (oriented graphite monochromator; Mo Kα $\lambda = 0.71073$ Å radiation). Full-matrix blocked least-squares anisotropic refinement for all non-H atoms yielded $R_1 = 0.0568$ [on $F \geq 4\sigma(F)$], $wR_2 = 0.1872$ (on $F^2$, all data), and $S = 0.715$. $w = 1/[\sigma^2 \cdot (F_o^2) + (0.0931 \times P)^2]$, where $P = (F_o^2 + 2F_c^2)/3$. Note: Some disorder, as reflected in the high thermal parameters of the corresponding atoms, was observed for the *Z*-phenyl ring as well as for some $C^{\gamma}$ atoms of the (α-Me)Val residues at positions 4, 5, 6, and 7. Restraints were applied to bond distances involving those atoms.

(12) Venkatachalam, C. M. *Biopolymers* **1986**, *6*, 1425−1436.

(13) (a) Kopple, K. D.; Ohnishi, M.; Go, A. *Biochemistry* **1969**, *8*, 4087−4095. (b) Pitner, T. P.; Urry, D. W. *J. Am. Chem. Soc.* **1972**, *94*, 1399−1400. (c) Kopple, K. D.; Schamper, T. J. *J. Am. Chem. Soc.* **1972**, *94*, 3644−3646.

(14) Formaggio, F.; Pantano, M.; Valle, G.; Crisma, M.; Bonora, G. M.; Mammi, S.; Peggion, E.; Toniolo, C.; Boesten, W. H. J.; Schoemaker, H. E.; Kamphuis, J. *Macromolecules* **1993**, *26*, 1848−1852.

(15) (a) Toniolo, C.; Formaggio, F.; Crisma, M.; Schoemaker, H. E.; Kamphuis, J. *Tetrahedron: Asymmetry* **1994**, *5*, 507−510. (b) Harada, N.; Nakanishi, K. *Circular Dichroic Spectroscopy: Exciton Coupling in Organic Stereochemistry*; University Science Books: Mill Valley, CA, 1983.

(16) In this study, the CD spectra were recorded with a Jasco Model J-710 spectropolarimeter. Cylindrical, fused quartz cells of 0.5 and 0.1 mm path lengths were used. The data are expressed in terms of $[\Theta]_R$, the residue molar ellipticity (deg $cm^2$ $dmol^{-1}$). 2,2,2-Trifluoroethanol (ACROS, Geel, Belgium) was employed as solvent.

(17) Vijayakumar, E. K. S.; Sudha, T. S.; Balaram, P. *Biopolymers* **1984**, *23*, 877−886.

(18) (a) Jones, D. J.; Kenner, G. W.; Preston, J.; Sheppard, R. C. *J. Chem. Soc.* **1965**, 6227−6239. (b) Froyer, G.; Sekiguchi, H. *Bull. Soc. Chim. Fr.* **1975**, 2321−2325. (c) Malcolm, B. R.; Walkinshaw, M. D. *Biopolymers* **1986**, *25*, 607−625.

(19) (a) Mc Mullen, A. I.; Marlborough, D. I.; Bayley, P. M. *FEBS Lett.* **1971**, *16*, 278−280. (b) Mayr, W.; Oekonomopulos, R.; Jung, G. *Biopolymers* **1979**, *18*, 425−250. (c) Oekonomopulos, R.; Jung, G. *Biopolymers* **1980**, *19*, 203−214. (d) Brückner, H.; Graf, H.; Bokel, M. *Experientia* **1984**, *40*, 1189−1197. (e) Matsuzaki, K.; Nakai, S.; Handa, T.; Takaishi, Y.; Fujita, T.; Miyajima, K. *Biochemistry* **1989**, *28*, 9392−9398. (f) Woolley, G. A.; Epand, R. M.; Kerr, I. D.; Sansom, M. S. P.; Wallace, B. A. *Biochemistry* **1994**, *33*, 6850−6858.

(20) Otoda, K.; Kimura, S.; Imanishi, Y. *J. Chem. Soc., Perkin Trans. 2* **1993**, 3011−3015.