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The p-Bromobenzamido Chromophore as a Circular Dichroic Probe for the Assignment of the Screw Sense of Helical Peptides

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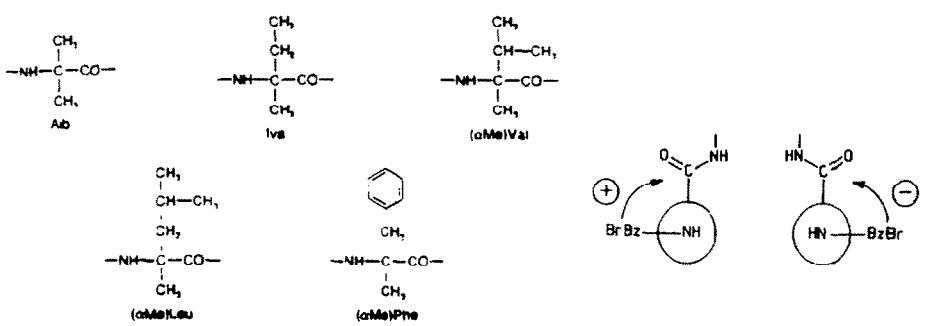
Abstract: The *para*-bromobenzoyl group linked to the N-terminus of a helical peptide chain turned out to be useful as a CD probe in the determination of the relationship between C^{α}-configuration of coded and non-coded α-amino acids and peptide helix screw sense in solution. The results of the present conformational investigation agree well with published crystal-state structural data on the same peptides obtained using X-ray diffraction where the *para*-bromobenzoyl group helped us to solve the phase problem by virtue of its heavy atom (bromine).

In our continuing X-ray diffraction investigation of the crystal-state preferred conformations of peptides rich in C^{α}-methylated α-aminoacids [1,2] (*Scheme 1*) we extensively took advantage of the *para*-bromobenzoyl (*p*BrBz) N^{α}-blocking group as a suitable tool to solve the phase problem by virtue of its heavy atom (bromine). As a general rule, this family of peptides tends to adopt the 3_{10}-helical structure [3] when the main-chain length is equal to or lower than eight residues. [4,5] A subtype of the 3_{10}-helix, the β-bend ribbon spiral, is adopted by specific sequences such as $(Aib\text{-}Pro)_n$. Remarkably, it was also found that the C^{α}-methylated aminoacids of L- (or S-) configuration with either a linear (isovaline, Iva) or a C^{β}-branched [(αMe)Val] side chain are strong inducers of the right-handed screw sense of the 3_{10}-helical structure (in analogy with all protein aminoacids), whereas the C^{α}-methylated L-aminoacids with a C^{γ}-branched [(αMe)Leu, (αMe)Phe] side chain tend to generate the opposite screw sense of helicity. Not surprisingly, FT-IR and ^{1}H NMR analyses have conclusively shown that the 3_{10}-helix of these conformationally constrained peptides is maintained in solution. [5]



Scheme 1. The α-amino acids methylated at the α-carbon and chiralities of the chromophores discussed in this work

In this communication we describe the application of the *para*-bromobenzamido chromophore linked to the N-terminus of the main chain to the CD assignment of the screw sense of 3_{10}-helical peptides. [6] Previous studies emanated from the Nakanishi's [7,8] and Nagai's [9] laboratories have provided clear evidence that interacting benzamido or *para*-bromobenzamido chromophores might be useful for determining the absolute configuration and the solution conformation of a variety of small organic molecules (diamines, diaminoacids, and aminosugars) by applying the CD exciton chirality method. [7]

Figure 1A illustrates the CD patterns in the 300-210 nm region in 2,2,2-trifluoroethanol (TFE) of three N^{α}-*para*-bromobenzoylated host-guest pentapeptides and one octapeptide where the host residue is the achiral C^{α}-methylated Aib and the single, chiral guest residues are the C^{α}-methylated L-(αMe)Val, D-(αMe)Leu, and L-(αMe)Phe and the protein aminoacid L-Leu (the latter in the octapeptide). In these peptides this spectral region is dominated by the contribution of the *para*-bromobenzamido chromophore, the absorption maximum of which is centred at 238-240 nm.

A characteristically split CD curve is observed for the L-(αMe)Val pentapeptide **1**, known to adopt a right-handed 3_{10}-helical structure. The Cotton effect at higher wavelengths (249.2 nm) is positive while that at lower wavelengths (227.0 nm) is negative. The cross-over point between the two intense, oppositely signed components is seen at 237.6 nm. A quite similar CD pattern is given by the right-handed 3_{10}-helical L-Leu octapeptide **4**.

Interestingly, the CD curve of the left-handed 3_{10}-helical L-(αMe)Phe pentapeptide **3** is also bisignated, but in this case the intense lobe at higher wavelengths is negative while the even more intense lobe at lower wavelengths is positive. The negative and positive CD maxima and the cross-over point are shifted by 3-5 nm towards the red compared with the corresponding wavelengths of the L-(αMe)Val pentapeptide and L-Leu octapeptide. This finding clearly indicates that the sign pattern of the split CD curve of the L-(αMe)Phe pentapeptide does reflect the screw sense of the helical peptide rather than the C^{α} configuration of the constituent chiral aminoacid.

Two oppositely signed CD bands (positive at higher wavelengths), although of much weaker strength, are visible in the CD spectrum of the right-handed 3_{10}-helical D-(αMe)Leu pentapeptide **2**. Here too, it is the screw sense of the helix rather than the configuration of the (αMe)Leu residue that is dominating the CD sign pattern. We are inclined to explain the low intensities of the CD Cotton effects of this pentapeptide on the basis of the experimental observation that D-(αMe)Leu-containing peptides exhibit only a moderate preference for the right-handed screw sense of the 3_{10}-helical conformation. This finding differs from the clear-cut tendency for a given screw sense shown by peptides characterized by the other C^{α}-methylated aminoacids. It is also worth noting that CD curves **1**, **3** and **4** of Figure 1A show only a modest variation by changing solvent polarity (from TFE to methanol, ethanol, and acetonitrile), whereas in the latter solvent the intensities of the two oppositely signed Cotton effects of curve **2** become comparable and somewhat higher. It seems reasonable to assume that the less polar acetonitrile would stabilize the right-handed 3_{10}-helix generated by the D-(αMe)Leu pentapeptide more than the polar TFE. No significant peptide concentration effect was noted in any of these CD curves.

We extended our study to two N^{α}-*para*-bromobenzoylated sequential (Aib/protein aminoacid) peptide series (typical examples are illustrated in Figure 1A). The CD spectrum of the right-handed 3_{10}-helical (Aib-L-Ala)$_3$ hexapeptide **5** is characterized by two strong Cotton effects centred at 250.0 nm (positive) and 226.0 nm (negative) and a cross-over point at 238.2 nm. Also the CD curve of the rigid, right-handed β-bend ribbon spiral forming Aib-(L-Pro-Aib)$_3$ heptapeptide **6** is split, with very intense Cotton effects at 249.0 nm (positive) and 226.2 nm (negative) and a cross-over point at 236.8 nm.

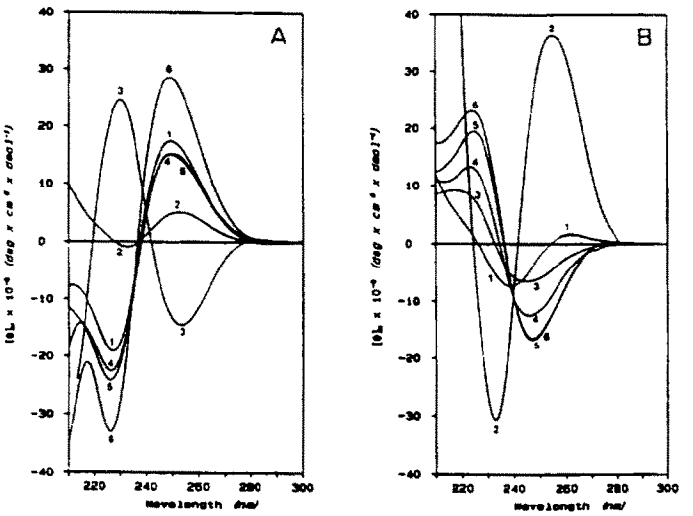


Figure 1. (A) CD spectra of *p*BrBz-$(Aib)_2$-Xxx-$(Aib)_2$-O*t*Bu [Xxx= L-(αMe)Val, **1**; D-(αMe)Leu, **2**; L-(αMe)Phe, **3**]; *p*BrBz-$(Aib)_5$-L-Leu-$(Aib)_2$-OMe, **4**; *p*BrBz-(Aib-L-Ala)$_3$-OMe, **5**; and *p*BrBz-Aib-(L-Pro-Aib)$_3$-OMe, **6**. (B) CD spectra of *p*BrBz-[D-(αMe)Leu]$_4$-O*t*Bu, **1**; *p*BrBz-[D-(αMe)Phe]$_4$-O*t*Bu, **2**; and *p*BrBz-$(D\text{-}Iva)_n$-O*t*Bu (n= 3-6, **3-6**). All spectra are recorded in TFE (peptide concentration: $1x10^{-3}$ M).

In going from the host/guest peptides of Figure 1A to the N$^{\alpha}$-*para*-bromobenzoylated homo-peptides (selected CD curves are shown in Figure 1B) a clearer picture seems to emerge. It is our contention that this result should be associated with the higher relative proportion of chiral residues in the homo-peptides and with the related increased preference of these peptides for a given screw sense of the 3_{10}-helical conformation. The series of the left-handed helical D-Iva oligomers (from trimer to hexamer **3-6**) shows a split CD pattern of increasing intensity with increasing main-chain length. This finding would probably reflect the higher helical content and the more defined screw sense preference of the higher oligomers. In the hexamer the CD bands are found at 247.0 (negative) and 224.0 nm (positive) and the cross-over point at 236.4 nm. The CD curve of the left-handed helical D-Iva homo-tetramer **4** is compared in Figure 1B with those of the corresponding right-handed helical oligomers from (αMe)Leu **1** and (αMe)Phe **2** of the same configuration. Again, as in the host/guest peptides, the sign pattern of the CD curves is related to the screw sense of the 3_{10}-helix rather than to the configuration of the C$^{\alpha}$-methylated aminoacid. The higher intensity of the Cotton effects of the (αMe)Phe homo-tetramer **2** might be attributed to the presence of four additional chromophores (the benzyl groups) also absorbing in this spectral region. [10] This phenomenon, although less significant, is also evident in curve **3** of Figure 1A.

In summary, we have shown that the *para*-bromobenzamido chromophore at the N-terminus of a peptide chain represents a useful CD probe for the assignment of the screw sense of 3_{10}-helical peptides, irrespective of the configuration of the constituent aminoacids. An important corollary of this investigation is the observation that exciton split CD patterns may be generated in peptides by a single *para*-bromobenzamido chromophore. A tentative explanation for this finding might invoke the interaction

between this chromophore and the amido chromophores [7] of the peptide molecules arranged in a right- or left-handed helical array (*Scheme I*). We do hope that these intriguing experimental results will stimulate a future theoretical investigation. In our laboratories we are currently investigating various types of *para*-substituted benzoyl groups linked to the N-terminus of the peptide main chain as potential tools for the CD determination of the screw sense of the classical α-helical conformation.

References and Notes

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