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The Bip Method, Based on the Induced Circular Dichroism of a Flexible Biphenyl Probe in Terminally Protected -Bip-Xaa*-Dipeptides, for Assignment of the Absolute Configuration of β-Amino Acids

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Abstract: An induced axial chirality of the biphenyl core of the Bip (2′,1′:1,2;1″,2″:3,4-dibenzcyclohepta-1,3-diene-6-amino-6-carboxylic acid) residue in the terminally protected dipeptides Boc-Bip-β-Xaa*-OMe (β-Xaa* = L-β^3-HAla, L-β^3-HVal, L-β^3-HLeu, L-β^3-HPro, *trans*-(1*S*,2*S*)-ACHC, *trans*-(1*R*,2*R*)-ACHC, *trans*-(1*S*,2*S*)-ACPC, *trans*-(1*R*,2*R*)-ACPC) resulted in an induced circular dichroism, revealing the usefulness of the Bip method for a reliable and fast assignment of the absolute configuration of chiral β-amino acids. Remarkably, the Bip method was also applied to the unique spin-labeled, cyclic, β-amino acids *cis/trans*-β-TOAC and *trans*-POAC. In particular, this study allowed the assignment of the unknown absolute configurations of the enantiomers of the latter compound.

Introduction

Within the past decade and stimulated by the early findings of Seebach[1a] and Gellman,[1b] short oligomers of β-amino acids (β-peptides) have attracted considerable attention because of their propensities to adopt a variety of stable helical secondary structures (mainly 2.6_{12}, $2.7_{10/12}$, and 3_{14} helices),[2] different from those typically adopted by oligomers of protein α-amino acids and C^{α}-tetrasubstituted α-amino acids (3.6_{13}- or α-helix and 3_{10}-helix, respectively).[3] Furthermore, β-peptides offer the considerable advantage of combined folding tendency and resistance to proteolytic degradation in vitro and in vivo, a useful starting point which has led to the successful design, synthesis, and evaluation of functional mimics of biologically active natural peptides and proteins.[2,4] Accordingly, robust synthetic methods for the production of a variety of β-amino acids as well as for the determination of their enantiomeric purity have been investigated.[2,5]

However, development of methods allowing a reliable and fast assignment of the absolute configuration of β-amino acids still remains a challenge. An interesting approach takes advantage of the configurationally flexible biphenyl chromophore for which induction of axial chirality by interaction with chiral auxiliaries is well documented and has been exploited in molecular recognition studies as well as in asymmetric catalysis. Central-to-axial transfer of chirality results in an induced circular dichroism (ICD) of the biphenyl chromophore, which has been used as a tool for the determination of the absolute configuration of chiral alcohols and carboxylic acids as well as α-amino acids.[6] We have previously reported that in peptides as short as dimers an axial chirality can be induced in the biphenyl moiety of 2′,1′:1,2;1″,2″:3,4-dibenzcyclohepta-1,3-diene-6-amino-6-carboxylic acid (Bip), a conformationally labile, atropoisomeric, C^{α}-tetrasubstituted α-amino acid.[7] Unequal populations of -Bip-Xaa*- dipeptide diastereoisomeric conformers [Xaa* = Ala, Val, Leu, (αMe)Val, and (αMe)Leu] were detected by CD and ^{1}H NMR techniques. The magnitude of this effect is particularly remarkable when Xaa* is positioned at the C-terminus of Bip. Moreover, the signs of the ICD bands correlate with the α-amino acid absolute configuration. More specifically, in the N^{α}-protected α-amino esters Boc-Bip-Xaa*-OMe (Boc, *tert*-butyloxycarbonyl; OMe, methoxy), the C-terminal L-Xaa* and D-Xaa* residues preferentially induce a negative and a positive Cotton effect at 250 nm, and a **P** and a **M** torsion in the biphenyl chromophore, respectively. This ICD phenomenon represents

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(1) (a) Seebach, D.; Matthews, J. L. *J. Chem. Soc., Chem. Commun.* **1997**, 2015–2022. (b) Gellman, S. H. *Acc. Chem. Res.* **1998**, *31*, 173–180.
(2) (a) For leading review articles, see : Cheng, R. P.; Gellman, S. H.; DeGrado, W. F. *Chem. Rev.* **2001**, *101*, 3219–3232. (b) Seebach, D.; Beck, A. K.; Bierbaum, B. J. *Chem. Biodiv.* **2004**, *1*, 1111–1239.
(3) (a) Karle, I. L.; Balaram, P. *Biochemistry* **1990**, *29*, 6747–6756. (b) Toniolo, C.; Benedetti, E. *Trends Biochem. Sci.* **1991**, *16*, 350–353.
(4) Gademann, K.; Hintermann, T.; Schreiber, J. V. *Current Med. Chem.* **1999**, *6*, 905–925.
(5) Juaristi, E. *Enantioselective Synthesis of β-Amino Acids*; Wiley-VCH: New York, 1997, and references therein.
(6) (a) Superchi, S.; Casarini, D.; Laurita, A.; Bavoso, A.; Rosini, C. *Angew. Chem.* **2001**, *113*, 465–468. *Angew. Chem. Int. Engl. Ed.* **2001**, *40*, 451–454. (b) Superchi, S.; Bisaccia, R.; Casarini, D.; Laurita, A.; Rosini, C. *J. Am. Chem. Soc.* **2006**, *128*, 6893–6902.
(7) (a) Mazaleyrat, J.-P.; Gaucher, A.; Šavrda, J.; Wakselman, M. *Tetrahedron: Asymmetry* **1997**, *8*, 619–631. (b) Formaggio, F.; Crisma, M.; Toniolo, C.; Tchertanov, L.; Guilhem, J.; Mazaleyrat, J.-P.; Gaucher, A.; Wakselman, M. *Tetrahedron* **2000**, *56*, 8721–8734.

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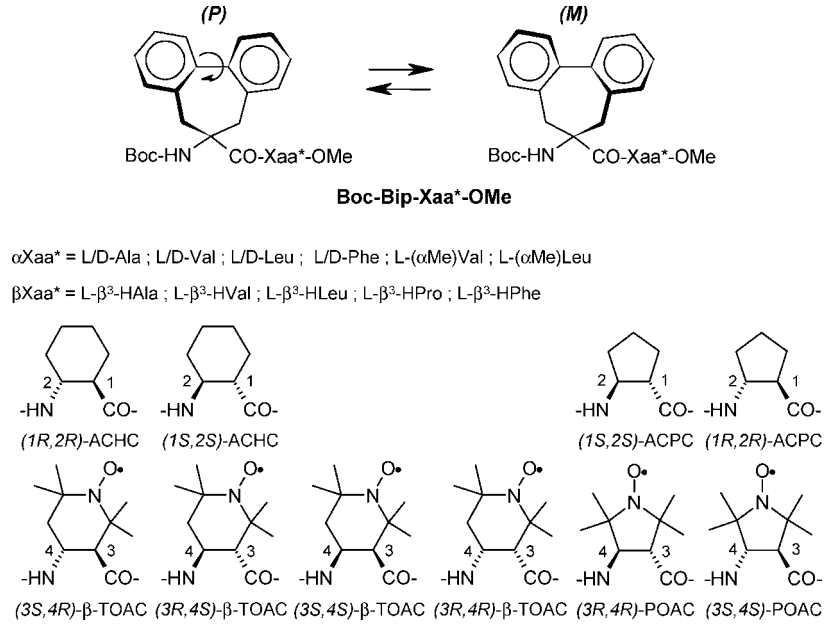

Figure 1. Conformational equilibrium of the Boc-Bip-Xaa*-OMe dipeptides. The abbreviations ACHC, ACPC, β-TOAC, and POAC stand for 2-amino-cyclohexanecarboxylic acid, 2-amino-cyclopentanecarboxylic acid, 4-amino-1-oxyl-2,2,6,6,-tetramethylpiperidine-3-carboxylic acid, and 4-amino-1-oxyl-2,2,5,5-tetramethylpyrrolidine-3-carboxylic acid, respectively.

the basis for the Bip method, an easy and fast configurational assignment of chiral α-amino acids.[8] In the present paper, we wish to report the full set of our results relative to the extension of the Bip method to β-amino acids (β-Xaa*): L-β^3-HAla, L-β^3-HVal, L-β^3-HLeu, L-β^3-HPro, L-β^3-HPhe, *trans* (1*R*,2*R*)/(1*S*,2*S*)-ACHC, *trans* (*1R*,2*R*)/(*1S*,2*S*)-ACPC (Figure 1),[9] as well as the *unique spin-labeled, cyclic, chiral* $\beta^{2,3}$*-amino acids* synthesized and/or resolved in our groups, *cis/trans*-β-TOAC[10] and *trans*-POAC.[11,12]

Experimental Section

Synthesis of Dipeptides. A new synthesis of Boc-Bip-OH,[7a] as well as syntheses of all new dipeptides discussed in this work, are reported in the Supporting Information.

Infrared Absorption. The FT-IR absorption spectra were recorded using a Perkin-Elmer model 1720 X FT-IR spectrophotometer, nitrogen-flushed, equipped with a sample-shuttle device, at 2 cm^{-1} nominal resolution, averaging 100 scans. Cells with path lengths of 0.1, 1.0, and 10 mm (with CaF_2 windows) were used. Spectrograde deuterochloroform (99.8% *d*) was purchased from Aldrich (St Louis, MO). Solvent (baseline) spectra were recorded under the same conditions.

Nuclear Magnetic Resonance. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker WM300 spectrometer operating at 300 and 77 MHz, respectively, the solvent $CDCl_3$ (^{1}H: $\delta = 7.27$ ppm; ^{13}C: $\delta = 77.00$ ppm) or CD_3OD (^{1}H: $\delta = 3.31$ ppm) being used as internal standard. Spectrograde deuterochloroform (99.5% *d*) and methyl alcohol d_4 (99.8% *d*) were purchased from Eurisotop (Saclay, France).

Circular Dichroism. The CD spectra were obtained on a Jasco (Tokyo, Japan) J-715 spectropolarimeter. Cylindrical fused quartz cells (Hellma) of 0.1 mm path length were used. The values are expressed in terms of $[\theta]_T$, total molar ellipticity ($deg \cdot cm^2 \cdot dmol^{-1}$). Spectrograde methanol (MeOH) (Fluka, Buchs, Switzerland) was used as solvent.

Results and Discussion

Synthesis. The N^{α}-protected amino acid derivative Boc-Bip-OH was readily prepared in a different way than previously reported,[7a] by bisalkylation of ethyl isocyanoacetate with 2,2′-bis(bromomethyl)-1,1′-biphenyl under the solid–liquid phase transfer conditions developed by Kotha and Brahmachary,[13] with potassium carbonate as a base and tetra-*n*-butylammonium hydrogen sulfate (TBAHS) as the catalyst, in acetonitrile at

(8) (a) Mazaleyrat, J.-P.; Wright, K.; Gaucher, A.; Toulemonde, N.; Wakselman, M.; Oancea, S.; Peggion, C.; Formaggio, F.; Setnička, V.; Keiderling, T. A.; Toniolo, C. *J. Am. Chem. Soc.* **2004**, *126*, 12874–12879. (b) Mazaleyrat, J.-P.; Wright, K.; Gaucher, A.; Toulemonde, N.; Dutot, L.; Wakselman, M.; Broxterman, Q. B.; Kaptein, B.; Oancea, S.; Peggion, C.; Crisma, M.; Formaggio, F.; Toniolo, C. *Chem. Eur. J.* **2005**, *11*, 6921–6929.

(9) Preliminary reports of this work have been published: (a) in which evolution of the ^{1}H NMR spectra of Boc-Bip-L-β^3-HAla-OMe in CD_3OD as a function of temperature is presented: Dutot, L.; Gaucher, A.; Wright, K.; Wakselman, M.; Mazaleyrat, J.-P.; Oancea, S.; Peggion, C.; Formaggio, F.; Toniolo, C. *Tetrahedron: Asymmetry* **2006**, *17*, 363–371. (b) Dutot, L.; Gaucher, A.; Wright, K.; Wakselman, M.; Mazaleyrat, J.-P.; Peggion, C.; Formaggio, F.; Toniolo, C. In *Peptides 2006, Proceedings of the 4th International and 29th European Peptide Symposium*, Rolka, K., Rekowski, P., Silberring, J., Eds.; Kenes International: Geneva, Switzerland, 2007; pp 76–77. (c) Wright, K.; Dutot, L.; Gaucher, A.; Wakselman, M.; Mazaleyrat, J.-P.; Peggion, C.; Formaggio, F.; Toniolo, C. In *Peptides for Youth, Proceedings of the 20th American Peptide Symposium*, in press [abstract in Biopolymers (Pept. Sci.) 2007, *88*, 535].

(10) (a) Wright, K.; Crisma, M.; Toniolo, C.; Török, R.; Péter, A.; Wakselman, M.; Mazaleyrat, J.-P. *Tetrahedron Lett.* **2003**, *44*, 3381–3384. (b) Wright, K.; de Castries, A.; Sarciaux, M.; Formaggio, F.; Toniolo, C.; Toffoletti, A.; Wakselman, M.; Mazaleyrat, J.-P. *Tetrahedron Lett.* **2005**, *46*, 5573–5576. (c) Wright, K.; Sarciaux, M.; de Castries, A.; Wakselman, M.; Mazaleyrat, J.-P.; Toffoletti, A.; Crisma, M.; Formaggio, F.; Toniolo, C. *Eur. J. Org. Chem.* **2007**, 3133–3144.

(11) Tominaga, M.; Barbosa, S. R.; Poletti, E. F.; Zukerman-Schpector, J.; Marchetto, R.; Schreier, S.; Paiva, A. C. M.; Nakaie, C. R. *Chem. Pharm. Bull.* **2001**, *49*, 1027–1029.

(12) (a) Wright, K.; Formaggio, F.; Toniolo, C.; Török, R.; Péter, A.; Wakselman, M.; Mazaleyrat, J.-P. *Tetrahedron Lett.* **2003**, *44*, 4183–4186. (b) Péter, A.; Török, R.; Wright, K.; Wakselman, M.; Mazaleyrat, J.-P. *J. Chromatogr. A* **2003**, *1021*, 1–10. (c) Wright, K.; Dutot, L.; Wakselman, M.; Mazaleyrat, J.-P. Crisma, M.; Formaggio, F.; Toniolo, C. *Tetrahedron* **2008**, in press.

(13) Kotha, S.; Brahmachary, E. *J. Org. Chem.* **2000**, *65*, 1359–1365.

Table 1. Chemical Shifts (δ) of the -$COOCH_3$ Singlet at 293 and 233 K (Two Diastereoisomers) and Calculated Diastereoisomeric Ratios (dr) for the Dipeptides Boc-Bip-β-Xaa*-OMe in CD_3OD Solution

	δ-$COOCH_3$ (ppm)		
dipeptides	293 K	233 K	dr
Boc-Bip-L-β^3-HAla-OMe[9b]	3.69	3.72 and 3.65	68:32
Boc-Bip-L-β^3-HVal-OMe	3.69	3.75 and 3.63	72:28
Boc-Bip-L-β^3-HLeu-OMe	3.68	3.79 and 3.63	61:39
Boc-Bip-L-β^3-HPhe-OMe	3.68	3.69 and 3.68[a]	~60:40[a]
Boc-Bip-L-β^3-HPro-OMe	3.67	3.68 and 3.64	64:36
Boc-Bip-*(1S,2S)*-ACPC-OMe[b]	3.69	3.74 and 3.64	62:38
Boc-Bip-*(1S,2S)*-ACHC-OMe[c]	3.69	3.74 and 3.62	74:26

[a] $\Delta\delta$ (-$COOCH_3$) between the two diastereoisomers was too small (shoulder) to allow calculation of the corresponding dr with accuracy. [b] Or Boc-Bip-(1*R*,2*R*)-ACPC-OMe. [c] Or Boc-Bip-(1*R*,2*R*)-ACHC-OMe.

80 °C. The resulting 2′,1′:1,2;1″,2″:3,4-dibenzcyclohepta-1,3-diene-6-isocyano-6-carboxylic acid ethyl ester was not isolated, but rather the crude reaction mixture was submitted to acid hydrolysis to afford directly the α-amino ester H-Bip-OEt (OEt, ethoxy)[7a] in 61% yield, from which Boc-Bip-OEt[7a] (72%) and then the desired compound Boc-Bip-OH[7a] (85%) were obtained.

The syntheses and characterizations of the terminally protected dipeptides Boc-Bip-α-Xaa*-OMe [α-Xaa* = L/D-Ala, L/D-Val, L/D-Leu, L/D-Phe, L-(αMe)Val, L-(αMe)Leu] have been previously reported.[7,8] The Boc-Bip-β-Xaa*-OMe[9b] dipeptides (β-Xaa* shown in Figure 1) were prepared in solution by coupling of Boc-Bip-OH with the amino ester hydrochlorides HCl·H-β-Xaa*-OMe or with H-β-Xaa*-NHC_6H_{11}. Full details are given in the Supporting Information.

^{1}H NMR Analysis. The chirality transfer phenomenon in the Boc-Bip-Xaa*-OMe dipeptides could be observed by ^{1}H NMR in CD_3OD for both β^3-Xaa* and $\beta^{2,3}$-Xaa* amino esters, as well as previously reported[8] for α-Xaa*-containing dipeptide esters. Indeed, two sets of signals, each corresponding to the presence of one diastereoisomeric conformer, exchanging slowly on the NMR time scale, are seen at low temperature (233 K) with unequal populations, while a single set of sharp signals is observed at 333 K under fast-interconverting conditions with coalescence occurring at about 293 K, as expected from the rotational energy barrier of 14 kcal mol^{-1} along the 1–1′ bond of the biphenyl moiety.[7] The diastereoisomeric ratio (dr) was determined at 233 K by integration of the singlet corresponding to the carboxylic ester methyl group, which generally appears at different chemical shifts for the two stereoisomers (Table 1).

Significantly high dr values (ranging from 60:40 to 74:26) are observed at 233 K in CD_3OD for all combinations of Bip with both β^3- and cyclic $\beta^{2,3}$-amino acids. However, it is interesting to note that the dr values for β^3-HAla, β^3-HVal, and β^3-HLeu are always lower than those for the corresponding α-amino acids Ala (81:19), Val (77:23), and Leu (89:11), respectively,[8b] except for β^3-HPhe versus Phe (58:42)[8b] (in this last case both dipeptides show relatively low dr values). As expected, the dipeptides Boc-Bip-(1*R*,2*R*)-ACPC-OMe and Boc-Bip-(1*R*,2*R*)-ACHC-OMe exhibit identical ^{1}H NMR spectra and dr values as Boc-Bip-(1*S*,2*S*)-ACPC-OMe and Boc-Bip-(1*S*,2*S*)-ACHC-OMe, respectively, clearly indicating that enantiomeric β-amino acids induce an opposite chirality of the Bip pro-atropoisomeric residue.

CD Analysis. The biphenyl chromophore is known to exhibit an intense electronic absorption near 240–250 nm, assigned to the A band,[14] followed by an even more intense absorption at ca. 210–215 nm (C band). In the CD spectra of biphenyl-based chiral molecules a negative maximum corresponding to the A band is associated with a **P** torsion of the $C_{Ar}-C_{Ar}$ bond.[15] We performed a CD analysis in MeOH solution of all of the Boc/OMe terminally protected -Bip-β-Xaa*-dipeptides listed in Figure 1 and compared them with the corresponding -Bip-α-Xaa*-dipeptides. The spectra show that the ICD resulting from the induced axial chirality in the biphenyl core of the Bip residue gives a clear information on the β-Xaa* configuration for both β^3- and cyclic $\beta^{2,3}$-amino acids. Taking all data together, the most relevant points are as follows:

(i) A comparison between the CD spectra of Boc-Bip-L-β^3-HVal-OMe and Boc-Bip-L-Val-OMe, as well as Boc-Bip-L-β^3-HLeu-OMe and Boc-Bip-L-Leu-OMe, shows that in these dipeptides, as previously observed for Boc-Bip-L-β^3-HAla-OMe and Boc-Bip-L-Ala-OMe,[8b,9b] a negative sign of the Bip Cotton effect at 250 nm is preferentially biased by both the α-Xaa* and β-Xaa* amino acids of the L configuration (Figure 2 and Table 2). In the case of β-Xaa*-containing dipeptides, the CD signal is weaker but still quite informative. Only Boc-Bip-L-β^3-HPhe-OMe (spectrum not shown) exhibits a positive Cotton effect for the A band (Table 2), probably resulting from a coupling of the aromatic chromophore present in the amino acid

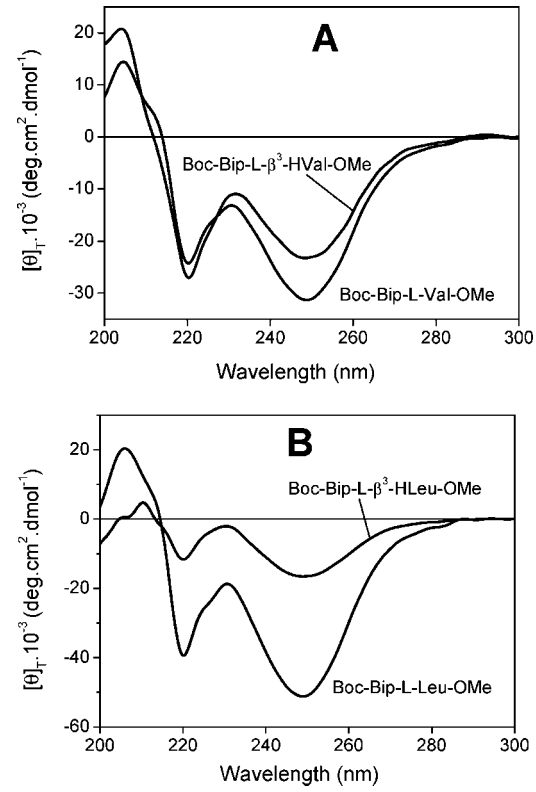

Figure 2. Far-UV CD spectra in MeOH solution of (A) Boc-Bip-L-β^3-HVal-OMe and Boc-Bip-L-Val-OMe, and (B) Boc-Bip-L-β^3-HLeu-OMe and Boc-Bip-L-Leu-OMe. Peptide concentration: 1 mM.

(14) Suzuki, H. *Electronic Absorption Spectra and Geometry of Organic Molecules*, Academic Press: New York, 1967.
(15) (a) Mislow, K.; Glass, M. A. W.; O'Brien, R. E.; Rutkin, P.; Steinberg, D. H.; Weiss, J.; Djerassi, C. *J. Am. Chem. Soc.* **1962**, *84*, 1455–1478. (b) Mislow, K.; Bunnenberg, E.; Records, R.; Wellman, K.; Djerassi, C. *J. Am. Chem. Soc.* **1963**, *85*, 1342–1349. (c) Loncar-Tomascovic, L.; Sarac-Arneri, R.; Hergold-Brundic, A.; Nagl, A.; Mintas, M.; Sandström, J. *Helv. Chim. Acta* **2000**, *83*, 479–494.

Table 2. Intensity and Sign of the A Band, and Absolute Configuration of the Asymmetric Center(s) Correlated with the **P** or **M** Torsion of the Biphenyl Core, for the Dipeptides Boc-Bip-Xaa*-OMe in MeOH Solution (Peptide Concentration: 1mM)

dipeptide derivative	Cotton effect (A band) intensity[a]	Cotton effect (A band) sign	central asymmetry	biphenyl torsion
Boc-Bip-L-β^3-HAla-OMe[17a]	15	(−)	(*S*)	**P**
Boc-Bip-L-Ala-OMe[16b]	30	(−)	(*S*)	**P**
Boc-Bip-L-β^3-HVal-OMe	22	(−)	(*R*)[b]	**P**
Boc-Bip-L-Val-OMe[16]	30	(−)	(*S*)	**P**
Boc-Bip-L-β^3-HLeu-OMe	18	(−)	(*S*)	**P**
Boc-Bip-L-Leu-OMe[16b]	50	(−)	(*S*)	**P**
Boc-Bip-L-β^3-HPro-OMe	21	(−)	(*S*)	**P**
Boc-Bip-L-β^3-HPhe-OMe	28	(+)	(*S*)	**M**
Boc-Bip-(1*S*,2*S*)-ACPC-OMe	15	(−)	(1*S*,2*S*)	**P**
Boc-Bip-(1*R*,2*R*)-ACPC-OMe	15	(+)	(1*R*,2*R*)	**M**
Boc-Bip-(1*S*,2*S*)-ACHC-OMe	28	(−)	(1*S*,2*S*)	**P**
Boc-Bip-(1*R*,2*R*)-ACHC-OMe	29	(+)	(1*R*,2*R*)	**M**

[a] Expressed by the total molar ellipticity $[\Theta]_T \times 10^{-3}$ (deg·cm^2·dmol^{-1}). [b] Absolute configuration (*R*) according to the accepted nomenclature, but corresponding to the same spatial arrangement as (*S*)-β^3-HAla, (*S*)-β^3-HLeu, and (*S*)-L-β^3-HPro.

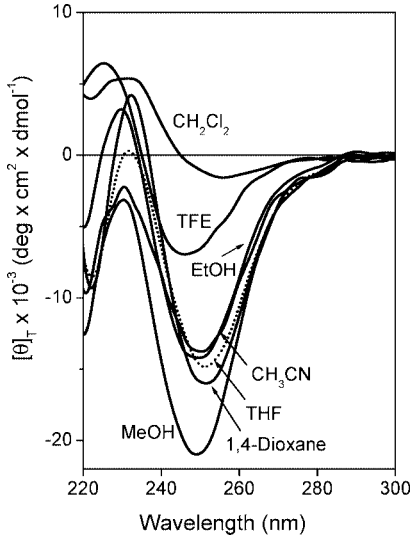

Figure 3. Far-UV CD spectra of Boc-Bip-L-β^3-HLeu-OMe in MeOH, 1,4-dioxane, THF, CH_3CN, EtOH, TFE (2,2,2-trifluoroethanol), and CH_2Cl_2 solutions. Peptide concentration: 1 mM.

side chain with that of Bip,[16] as reported for its Boc-Bip-L-Phe-OMe analogue.[8b]

(ii) We also checked the role of the solvent on the ICD of the Boc-Bip-β-Xaa*-OMe dipeptides. Figure 3 illustrates the CD spectra of Boc-Bip-L-β^3-HLeu-OMe in a variety of solvents. A strong negative maximum at about 250 nm is seen in all solvents tested with the following rank order for the ellipticity: MeOH > 1,4-dioxane > THF > $CH_3CN \approx$ EtOH > TFE > CH_2Cl_2. A similar solvent rank order was previously observed in the CD spectra of several Boc-Bip-α-Xaa*-OMe dipeptides, the dr values of which, extracted from an NMR analysis, were found to be higher in CD_3OD than in CD_3CN and $CDCl_3$.[8b] Therefore, the nature of solvent does play a remarkable role in

(16) (a) Beychok, S. In *Poly-α-Amino Acids: Protein Models for Conformational Studies*, Fasman, G. D., Ed.; Dekker: New York, 1967; pp 293–337. (b) Goodman, M.; Toniolo, C. *Biopolymers* **1968**, *6*, 1673–1689. (c) Woody, R. W. *J. Polymer Sci., Macromol. Rev.* **1977**, *12*, 181–320.

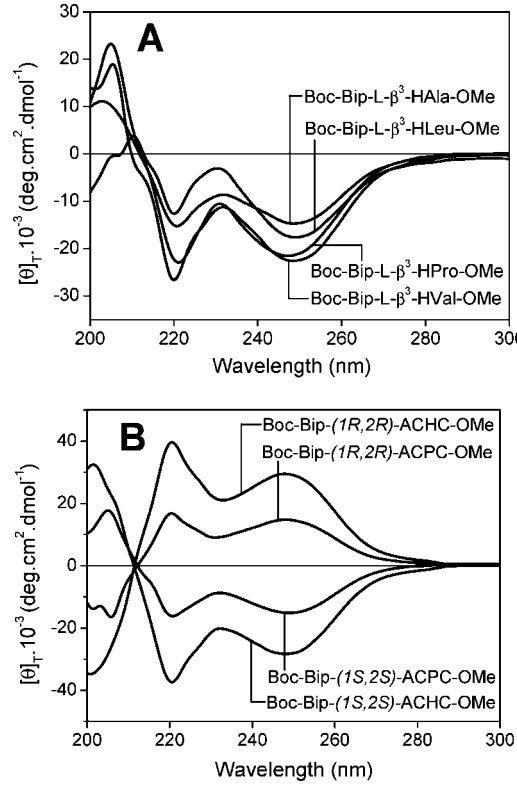

Figure 4. Far-UV CD spectra in MeOH solution of Boc-Bip-L-β^3-HAla-OMe, Boc-Bip-L-β^3-HVal-OMe, Boc-Bip-L-β^3-HLeu-OMe, and Boc-Bip-L-β^3-HPro-OMe (A), and Boc-Bip-(1*R*,2*R*)-ACHC-OMe, Boc-Bip-(1*S*,2*S*)-ACHC-OMe, Boc-Bip-*(1R,2R)*-ACPC-OMe, and Boc-Bip-(1*S*,2*S*)-ACPC-OMe (B). Peptide concentration: 1 mM.

this phenomenon, but no inversion of the sign of the 250 nm band is observed.

(iii) A negative sign for the strong Cotton effect of the A band is seen for both series of terminally protected Bip dipeptides containing L-β^3-Xaa* amino acids (except for that based on L-β^3-HPhe, as mentioned above) or (1*S*,2*S*)-$\beta^{2,3}$-cyclic amino acids (ACHC, ACPC) (Figure 4 and Table 2), characterized by the same absolute configuration at their β-carbon atom. Obviously, the CD spectra of the enantiomeric Boc-Bip-(1*S*,2*S*)-ACHC-OMe and Boc-Bip-(1*R*,2*R*)-ACHC-OMe, as well as Boc-Bip-(1*S*,2*S*)-ACPC-OMe and Boc-Bip-(1*R*,2*R*)-ACPC-OMe, are mirror images (Figure 4B).

(iv) The enantiomeric dipeptides containing both O-acetyl protected side-chain nitroxide β-amino acid derivatives *cis*- and *trans*-β-TOAC(Ac) exhibit intense Cotton effects (Figure 5A). Remarkably, the sign of the A band in the CD spectra of both Boc-Bip-(3*S*,4*R*)-β-TOAC(Ac)-OMe and Boc-Bip-(3*R*,4*R*)-β-TOAC(Ac)-OMe is positive, as that of Boc-Bip-(1*R*,2*R*)-ACHC-OMe. Therefore, the sign of the Cotton effect at 250 nm is determined by the absolute configuration of the carbon bearing the nitrogen atom (4*R*, 4*R*, and 2*R*, respectively), not by that of the carbon bearing the carboxyl group (*3S*, 3*R*, and 1*R*, respectively). Again, it is worth noting that the CD spectra of the two enantiomeric pairs Boc-Bip-(3*R*,4*S*)/(3*S*,4*R*)-β-TOAC(Ac)-OMe and Boc-Bip-(3*S*,4*S*)/(3*R*,4*R*)-β-TOAC(Ac)-OMe are mirror images. In the same manner, as reported elsewhere,[12c] the O-acetyl protected side-chain nitroxide-based dipeptides Boc-Bip-(−)-POAC(Ac)-OMe and Boc-Bip-(+)-POAC(Ac)-OMe show a strong Cotton effect at 250 nm, the sign of which is the same as that of Boc-Bip-(1*R*,2*R*)-ACPC-OMe and Boc-Bip-(1*S*,2*S*)-ACPC-OMe, respectively (Figure 5B). This finding

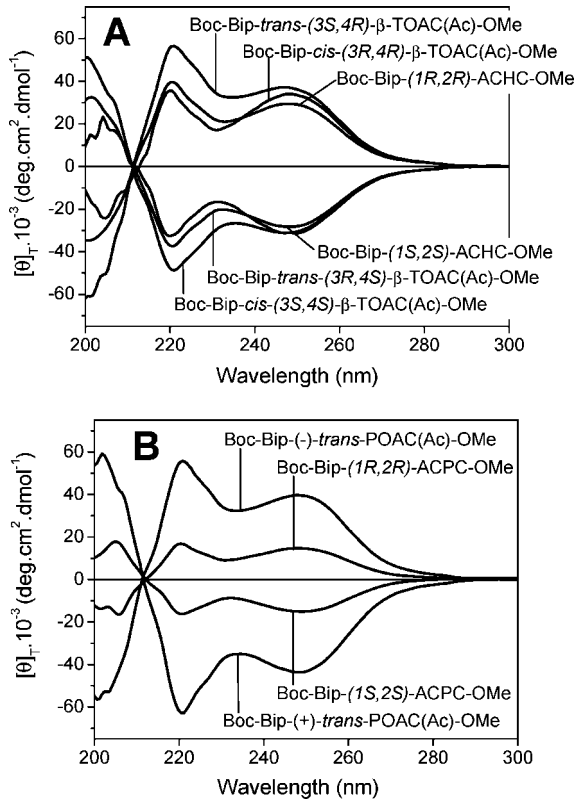

Figure 5. Far-UV CD spectra in MeOH solution of Boc-Bip-(3*S*,4*R*)-*β*-TOAC(Ac)-OMe, Boc-Bip-(3*R*,4*S*)-*β*-TOAC(Ac)-OMe, Boc-Bip-(3*R*,4*R*)-*β*-TOAC(Ac)-OMe, Boc-Bip-(3*S*,4*S*)-*β*-TOAC(Ac)-OMe, Boc-Bip-(1*R*,2*R*)-ACHC-OMe, and Boc-Bip-(1*S*,2*S*)-ACHC-OMe (A), and Boc-Bip-(−)-POAC(Ac)-OMe, Boc-Bip-(+)-POAC(Ac)-OMe, Boc-Bip-(1*R*,2*R*)-ACPC-OMe, and Boc-Bip-(1*S*,2*S*)-ACPC-OMe (B).[12c] Peptide concentration: 1 mM.

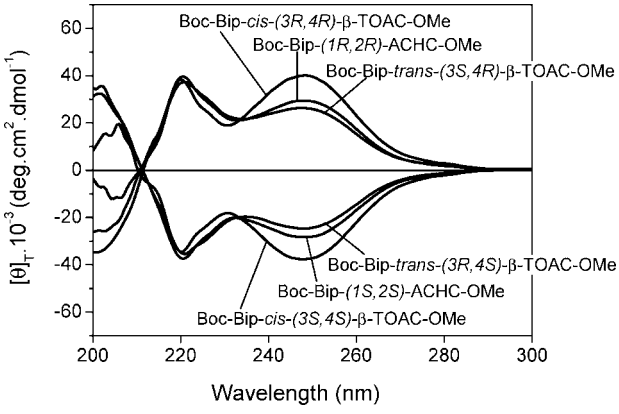

Figure 6. Far-UV CD spectra in MeOH solution of Boc-Bip-*(3S,4R)*-*β*-TOAC-OMe, Boc-Bip-*(3R,4S)*-*β*-TOAC-OMe, Boc-Bip-*(3R,4R)*-*β*-TOAC-OMe, and Boc-Bip-*(3S,4S)*-*β*-TOAC-OMe, Boc-Bip-*(1R,2R)*-ACHC-OMe, and Boc-Bip-*(1S,2S)*-ACHC-OMe. Peptide concentration: 1 mM.

has allowed us to assign the previously unknown (−)-(3*S*,4*S*) and (+)-(3*R*,4*R*) absolute configurations to the enantiomers of *trans*-POAC(Ac).

(v) Analogous conclusions were extracted from the CD spectra of both enantiomers of the two dipeptides Boc-Bip-*trans*-*β*-TOAC-OMe and Boc-Bip-*cis*-*β*-TOAC-OMe, containing side-chain unprotected nitroxide moieties, as compared to the corresponding enantiomers of Boc-Bip-ACHC-OMe (Figure 6). Again, an extension of this study to the enantiomers of *trans*-POAC allowed the assignment of their absolute configurations,

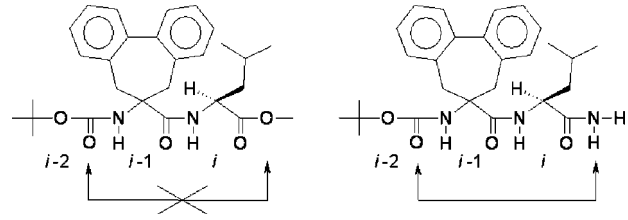

Figure 7. Interaction C═O (*i*-2)···O (*i*) (unfavorable) for Boc-Bip-L-Leu-OMe and C═O (*i*-2)···H−N (*i*) (favorable) for Boc-Bip-L-Leu-NH_2 in the postulated folded (nascent 3_{10}-helical) conformation (not depicted here).

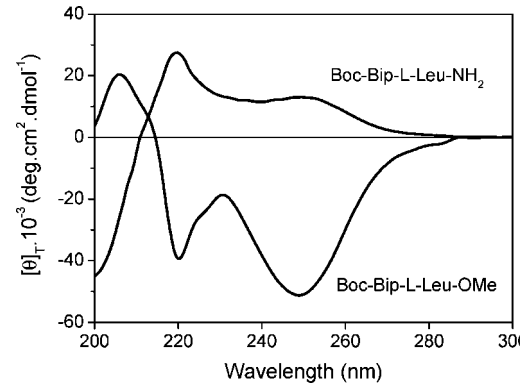

Figure 8. CD spectra in MeOH solution of Boc-Bip-L-Leu-OMe and Boc-Bip-L-Leu-NH_2. Peptide concentration: 1 mM.

(-)-(3S,4S) and *(+)-(3R,4R)*. This result was achieved by comparing the CD spectra of Boc-Bip-*(-)*-POAC-OMe and Boc-Bip-*(+)*-POAC-OMe (not shown) with those of Boc-Bip-*(1R,2R)*-ACPC-OMe and Boc-Bip-*(1S,2S)*-ACPC-OMe, respectively (Figure 5B). Altogether, the "Bip method" appears to allow the determination of the absolute configuration of spin-labeled *β*-amino acids, even without the preliminary protection of their side-chain nitroxide function. Application of this methodology to POAC is of special interest since incorporation of this labeled residue into peptides for structural investigations has been reported.[11]

Relationship between ICD and Conformation of the Bip Dipeptides. In the present study a few observations are pertinent to the central-to-axial chirality transfer process in the -Bip-Xaa*- dipeptides. In particular, we have noted that the magnitude of the ICD is generally lower for the β^3-Xaa* residues than for their α-Xaa* counterparts for each pair Boc-Bip-L-Ala-OMe/Boc-Bip-L-β^3-HAla-OMe, Boc-Bip-L-Val-OMe/Boc-Bip-L-β^3-HVal-OMe, and Boc-Bip-L-Leu-OMe/Boc-Bip-L-β^3-HLeu-OMe (Table 2). However, in these three pairs, the number of bonds separating the asymmetric center of Xaa* and the chiral axis of Bip is just the same. Therefore, the factor playing the major role in the induced axial chirality is apparently not a through-bond coupling between the side chain of the asymmetric C(NH) carbon atom of the Xaa* residue and the biphenyl group of the Bip residue, but rather one has to consider a specific through-space (conformational) coupling which could be responsible for the chirality transfer.

The most significant results of our previous conformational studies in this area[3b,7b,8] suggest that *the central-to-axial chirality transfer process in the Boc-Bip-*α*-Xaa*-OMe dipeptides is likely to be related to their nascent 3_{10}-helical secondary structure*. This hypothesis is supported by a comparison of the CD spectra for the dipeptide ester Boc-Bip-L-Leu-OMe and the corresponding dipeptide amide Boc-

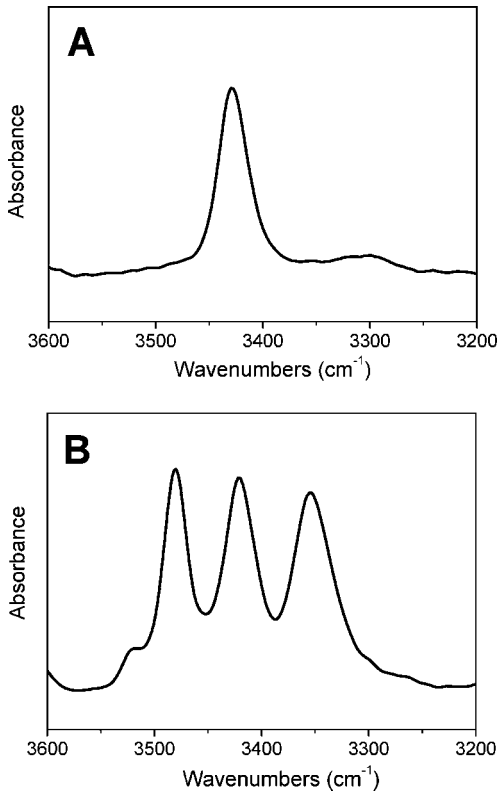

Figure 9. FT-IR absorption spectra (N−H stretching region) of Boc-Bip-L-Leu-OMe (A) and Boc-Bip-L-Leu-NH_2 (B) in $CDCl_3$ solution. Peptide concentration: 1 mM.

Bip-L-Leu-NH_2 in which, in the case of a folded (nascent 3_{10}-helical) conformation, the interaction C═O (*i*-2)···O (*i*) and C═O (*i*-2)···H−N (*i*), respectively, should change from unfavorable to favorable (Figure 7). Indeed, the CD spectra of the two dipeptides present a dramatic change of the Cotton effect at 250 nm (A band), the sign of which inverts from negative for the ester to positive for the amide (Figure 8).

The FT-IR absorption spectra for these two compounds in $CDCl_3$ solution, a solvent of relatively low polarity and known to support the ordered secondary structure of peptides, also show a dramatic change in the conformationally sensitive N−H stretching (amide A) region (3470–3300 cm^{-1}).[17] For Boc-Bip-L-Leu-OMe a single band is seen, centered at 3428 cm^{-1} and assigned to the free urethane/amide NH groups (Figure 9A), while for Boc-Bip-L-Leu-NH_2 three intense bands are present (Figure 9B). The bands at 3420 and 3481 cm^{-1} may be assigned to the free urethane/secondary amide NH groups and the C-terminal primary amide -NH_2 group, respectively, while the band at 3353 cm^{-1} is typical of intramolecularly H-bonded amide NH groups. These results strongly support the view that in Boc-Bip-L-Leu-NH_2 a folded conformation, stabilized by an intramolecular H-bond, is highly populated in $CDCl_3$ solution. On the basis of the length of the peptide and the known 3D-structural properties of the Bip residue,[7b,8] we believe that the

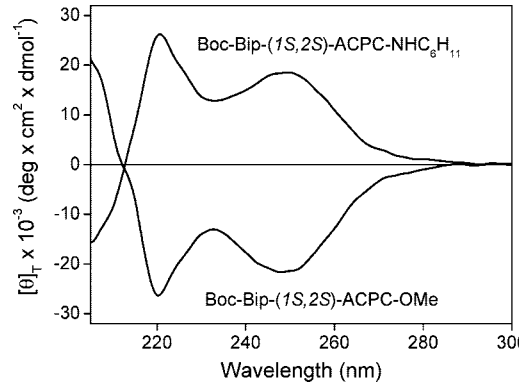

Figure 10. CD spectra in MeOH solution of Boc-Bip-(1*S*,2*S*)-ACPC-OMe and Boc-Bip-(1*S*,2*S*)-ACPC-NHC_6H_{11}. Peptide concentration: 1 mM.

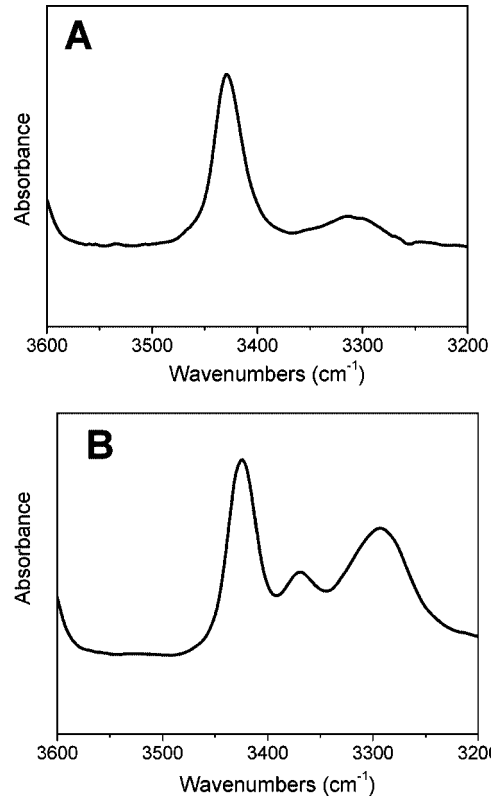

Figure 11. FT-IR absorption spectra (N−H stretching region) of Boc-Bip-(1*S*,2*S*)-ACPC-OMe (A) and Boc-Bip-(1*S*,2*S*)-ACPC-NHC_6H_{11} (B) in $CDCl_3$ solution. Peptide concentration: 1 mM.

folded conformation observed is a C_{10} (β-turn),[18] the basic unit of the 3_{10}-helix.

Altogether, the evidence for a β-turn conformation in Boc-Bip-L-Leu-NH_2, combined with the observed sign inversion of the Cotton effect for this amide versus the corresponding ester Boc-Bip-L-Leu-OMe, confirms the paramount importance of the (nascent) 3_{10}-helical structure, induced by the conformational properties of the Bip residue, in the central-to-axial chirality transfer process and in the resulting ICD observed for the Boc-Bip-α-Xaa*-OMe dipeptides.

As for the Boc-Bip-β-Xaa*-OMe dipeptide series, an analogous process of chirality transfer related to the onset of a turn

(17) (a) Cung, M. T.; Marraud, M.; Néel, J. *Ann. Chim. (Paris)* **1972**, 183–209. (b) Palumbo, M.; Da Rin, S.; Bonora, G. M.; Toniolo, C. *Makromol. Chem.* **1976**, *177*, 1477–1492. (c) Bonora, G. M.; Mapelli, C.; Toniolo, C.; Wilkening, R. R.; Stevens, E. S. *Int. J. Biol. Macromol.* **1984**, *6*, 179–188. (d) 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.

(18) (a) Venkatachalam, C. M. *Biopolymers* **1968**, *6*, 1425–1436. (b) Toniolo, C. *CRC Crit. Rev. Biochem.* **1980**, *9*, 1–44. (c) Rose, G. D.; Gierasch, L. M.; Smith, P. J. *Adv. Protein Chem.* **1985**, *37*, 1–109.

(nascent helical) conformation may be expected, since Gellman and co-workers have shown that for mixed α/β-peptides the 11-helical structure is favored by the presence of cyclic, conformationally constrained, β-residues (typically ACPC) and C^{α}-tetrasubstituted α-residues (typically Aib).[19] Figure 10 shows clearly that, even for an N^{α}-protected Boc-Bip-β-Xaa*-dipeptide sequence with a rigidified β-amino acid (ACPC), the signs of the biphenyl CD bands change on going from the ester to the alkyl (cyclohexyl) amide functionality.

The corresponding FT-IR absorption spectra in the N−H stretching region are reported in Figure 11. It is evident that a band indicative of intramolecularly H-bonded (C_{11}) species (at 3370 cm^{-1}),[17c] although of modest intensity, appears only in the spectrum of the alkyl amide dipeptide. We conclude that the onset of an incipient helix in a Bip dipeptide alkylamide based on a structurally restricted β-amino acid may have profound consequences in the process of chirality transfer, as observed for -Bip-α-Xaa*-dipeptides. Apparently however, when in a α/β-dipeptide the rigid ACPC is replaced by a more flexible β-amino acid, such as L-β^3-HLeu, the variations of the biphenyl CD bands from the ester to the alkylamide are less clear-cut and more difficult to interpret (spectra not shown).

Conclusions

A remarkable central-to-axial induction of chirality from the C-terminal β^3-Xaa*-OMe (β^3-HAla, β^3-HVal, β^3-HLeu, β^3-HPro) or cyclic $\beta^{2,3}$-Xaa*-OMe (ACHC, ACPC) amino ester residues to the N^{α}-Boc protected, pro-atropoisomeric, C^{α}-tetrasubstituted α-amino acid Bip has been revealed in simple linear dipeptides, thus demonstrating that the Bip residue may be used as a convenient CD probe for assignment of the absolute configuration of not only α-amino acids as previously reported,[8] but β-amino acids as well. Interestingly, we have also applied the Bip method to the spin-labeled, cyclic, chiral $\beta^{2,3}$-amino acids *cis* and *trans* β-TOAC and even to the assignment of the unknown absolute configurations of the *trans*-POAC enantiomers.

Taking our results together, we suggest that (i) MeOH is the best solvent for the application of the Bip method to α- or β-amino acid configurational issues for its excellent solubilizing properties and high CD response and (ii) Boc-Bip-Xaa*-dipeptide ester is the sequence of choice, as the N^{α}-protecting Boc moiety lacks any aromatic group potentially interfering with the biphenyl CD band near 250 nm, and the C-terminal ester functionality is devoid of any H-bonding donor and therefore unable to contribute to the stability of dipeptide folded forms which, in turn, may not be beneficial for an unambiguous interpretation of the chirospectroscopic data.

Supporting Information Available: Details of the synthesis of the dipeptides. This material is available free of charge via the Internet at http://pubs.acs.org.

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(19) Schmitt, M. A.; Choi, S. H.; Guzei, I. A.; Gellman, S. H. *J. Am. Chem. Soc.* **2005**, *127*, 13130–13131.