Helical Peptides

DOI: 10.1002/anie.201107583

Induction of Unexpected Left-Handed Helicity by an N-Terminal L-Amino Acid in an Otherwise Achiral Peptide Chain**

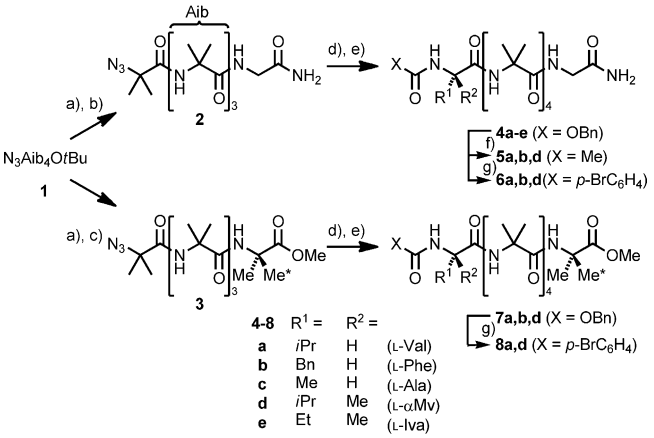
*Robert A. Brown, Tommaso Marcelli, Matteo De Poli, Jordi Solà, and Jonathan Clayden**

Helices composed of L-α-amino acids are typically more stable when they adopt right-handed (rather than left-handed) helicity, due to steric interactions between the L-amino acid side chains and the main-chain carbonyl groups.[1] Left-handed helices are rare motifs in protein structures (about 0.4% in a nonredundant subset of the Protein Data Bank), and the vast majority of these helices are short (four residues at most), stabilized by interhelical hydrogen bonds and other weak interactions. Many left-handed helices are important for the stability of the protein, for ligand binding, or as part of the active site.[2] Collagen is an exception: its quaternary structure adopts right-handed helicity, but its individual peptide chains are long single-stranded left-handed helices, stabilized through intermolecular hydrogen bonds.[3] Necessarily, all-D proteins,[4] or those synthesized as racemates,[5] contain left-handed helical domains.[6]

Peptides made entirely of achiral amino acids, even though they may form helical structures, can have no preference for left- or for right-handed helicity. For example, octamers of the achiral quaternary amino acid Aib (2-aminoisobutyric acid) form 3_{10} helices that interconvert rapidly in solution between the left- and right-handed helical conformations.[7] A single N-terminal L-amino acid is nonetheless sufficient to perturb measurably[8] the conformational ratio and can induce an absolute helical preference[9] in an entire chain of up to 20 achiral amino acids.[10] Induction of helical preference[11] from the terminus of an otherwise achiral chain, mediated by stabilization of one of the two helical conformations, has been reported in several other classes of synthetic foldamers,[12] including dehydropeptides,[13] polyisocyanates,[14] polyureas,[15] and aromatic oligoamides.[16] Switching the configuration of the chiral terminal residue switches the helical preference of the entire oligomer,[17] and the literature suggests[9] that in solution an L-amino acid induces right-handed helicity in an achiral Aib_n peptide chain.[18]

Herein we report a combined NMR spectroscopy, circular dichroism (CD) spectroscopy, and computational study that shows that an oligo(Aib) 3_{10} helix carrying an N-terminal L-amino acid residue (specifically L-valine (L-Val), L-alanine (L-Ala) or L-phenylalanine (L-Phe)) adopts left-handed (*M*) helicity. Corresponding 3_{10} helices carrying an N-terminal α-methylated (quaternary) amino acid such as L-α-methylvaline (L-αMv) or L-isovaline (L-Iva) conversely adopt the expected right-handed (*P*) helicity.

Achiral tetramers of Aib were made as their N-terminal azide and C-terminal *tert*-butyl ester, N_3Aib_4OtBu (**1**),[10] and coupled at their C terminus with either $GlyNH_2$ to give **2** or with enantiomerically enriched mono-^{13}C-labeled (*R*)-Aib*[19] to give **3** (Scheme 1). After reduction of the N-terminal azide, ligation of **2** or **3** with a selection of Cbz-protected L-amino acids gave the sets of compounds **4** and **7** shown in Scheme 1. The L-α-amino acids employed were the tertiary α-amino acids L-Val (**a**), L-Phe (**b**), and L-Ala (**c**) as well as the quaternary α-amino acids L-αMv (**d**) and L-Iva (**e**).



Scheme 1. Peptides used in the study. Reagents and conditions: a) CF_3CO_2H, CH_2Cl_2, 24 h; b) Ac_2O, 120°C, 3 h then $HGlyNH_2{\cdot}HCl$, Et_3N, MeCN, Δ, 3 days; c) as for (b) but with HAib*OMe·HCl; d) H_2, 10% Pd/C, MeOH, 24 h; e) CbzXxxOH, PyBOP, iPr_2NEt, CH_2Cl_2, 24 h for **a**,**b** or CbzXxxF, iPr_2NEt, MeCN, 72 h for **c**–**e**; f) H_2, 10% Pd/C, EtOH, AcOH, 24 h then AcCl, py, CH_2Cl_2/DMF (1:1), 24 h; g) H_2, 10% Pd/C, EtOH, AcOH, 48 h then *p*BrBzCl, iPr_2NEt, CH_2Cl_2, 48 h. Me* = $^{13}CH_3$. Gly = glycine, Cbz = benzyloxycarbonyl, PyBOP = 1-benzotriazolyloxytris(pyrrolidino)phosphonium hexafluorophosphate, py = pyridine.

[*] R. A. Brown, Dr. T. Marcelli, Dr. M. De Poli, Dr. J. Solà, Prof. J. Clayden
School of Chemistry, University of Manchester
Oxford Road, Manchester M13 9PL (United Kingdom)
E-mail: clayden@man.ac.uk
Dr. T. Marcelli
Dipartimento di Chimica, Materiali e Ingegneria Chimica "Giulio Natta", Politecnico di Milano
via Mancinelli 7, 20131 Milano (Italy)

[**] This work was supported by the BBSRC, EPSRC, and ERC. We are grateful to Prof. Fernando Formaggio, Prof. Claudio Toniolo, Prof. Tadashi Mori, and Dr. Alison Edwards for helpful discussions, and to Dean Holt and Romina Wechsel for synthetic assistance. J.C. is the recipient of a Royal Society Wolfson Research Merit award. T.M. acknowledges the MIUR (Rientro dei Cervelli 2008) and the Politecnico di Milano for financial support. We are indebted to CILEA for the generous allocation of computer time.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201107583.

CD spectroscopy gives reliable information on the screw sense of helical structures,[20] and in helical peptides the sign and magnitude of absorption bands associated with the n–π* and π–π* transitions of the amide carbonyl groups report on the nature and absolute orientation of the helicity.[21] To minimize interference from transitions owing to aromatic chromophores associated directly with the chiral amino acid, peptides **4a**, **b**, and **d** were deprotected and converted into their *N*-acetyl derivatives **5**. For comparison with reported spectra,[9,22] they were also converted into their *N*-*p*-bromobenzamide derivatives **6**. CD spectra of **5** were acquired at 20 °C in methanol (Figure 1) and in 2,2,2-trifluoroethanol.

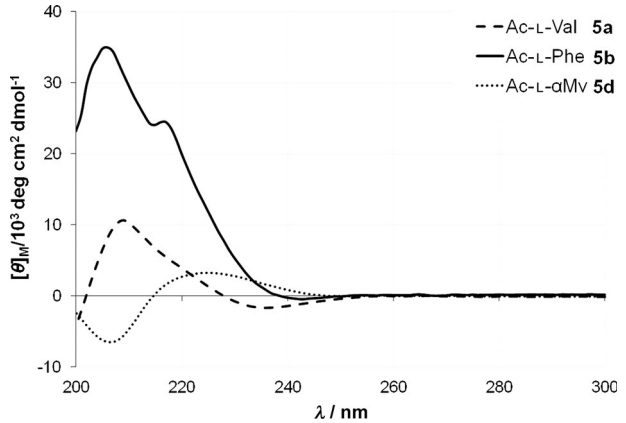


Figure 1. CD spectra of Ac-L-XxxAib$_4$GlyNH$_2$ **5a**, **5b**, and **5d** in MeOH.

The three compounds **5a**, **b**, and **d** showed bands with characteristics of 3_{10} helices with maxima at 208 nm accompanied by shoulders at 220 nm,[9] and comparison of the form of the CD spectra of the peptides **5a**,**b** bearing tertiary L-amino acid residues with that of peptide **5d** bearing a quaternary L-amino acid residue suggested that the sense of helicity is opposite for the two classes.[23]

Final confirmation that L-Val-capped Aib oligomers and L-αMv-capped Aib oligomers adopt opposite helical conformations was provided by ^{13}C NMR spectroscopy. The two methyl groups of each Aib residue in an Aib$_n$ helix, if they are distant from any chiral residue, are in magnetically inequivalent environments owing to the chirality of the helix,[7] and the environments of the diastereotopic methyl groups switch when the absolute sense of helicity switches. By incorporating in a position remote from the chiral N terminus an Aib residue differentially and enantioselectively labeled with ^{13}C in each of its enantiotopic methyl groups, it is therefore possible to compare the absolute sense of helicity of different helical peptides by observing whether the label appears mainly in the upfield or downfield member of the pair of diastereotopic signals in the ^{13}C NMR spectrum. We have previously used such a method to report the controlled inversion of screw sense in an Aib oligomer.[17] Figure 2 shows portions of the ^{13}C NMR spectra at 23 °C in methanol of such labeled peptides **7a**,**b**,**d** and **8a**,**d**, each carrying a C-terminal Aib*OMe residue with a ^{13}C label located approximately 70 % in the pro-*R* methyl group and 30 % in the pro-*S* methyl

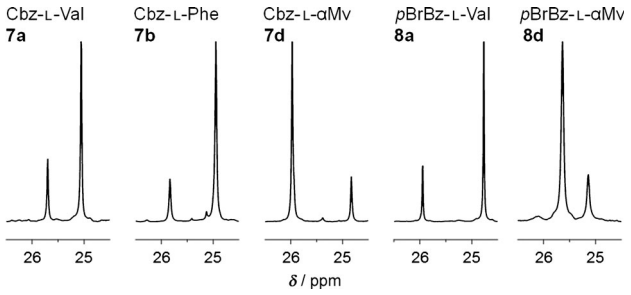


Figure 2. Sections of ^{13}C NMR spectra (CD$_3$OD, 23 °C) of XxxAib$_4$-Aib*OMe **7a**,**b**,**d** and **8a**,**d**.

group (i.e. made from (*R*)-Aib* of ca. 40 % *ee*).[19] With a tertiary L-amino acid at the N terminus (**7a,b,** or **8a**) the label appears predominantly in the more upfield member of the pair of diastereotopic signals; with a quaternary L-amino acid at the N terminus (**7d**, **8d**) the label appears predominantly in the more downfield member of the pair of diastereotopic signals.[24]

Aib is the most powerful known stabilizer of β turns[25] and specifically stabilizes a type I or type III β turn at positions $i+1$ and $i+2$ and a type II β turn at position $i+2$.[26] The N terminus of peptides such as **5** is therefore expected to adopt either a type II or a type III β turn conformation[27] with the chiral L-amino acid and Aib located at the $i+1$ and $i+2$ positions, respectively (Figure 3). The

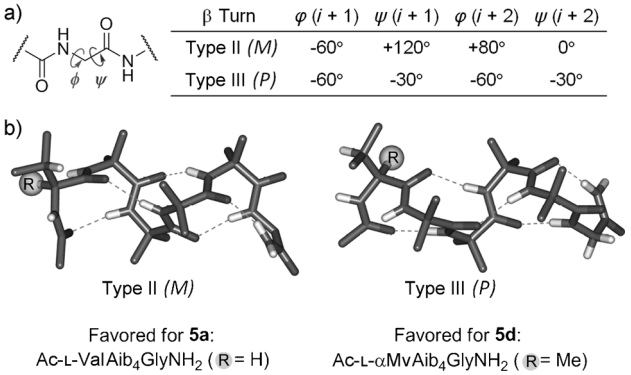


Figure 3. a) Ideal torsions for type II and III peptide β turns. b) Optimized 3_{10} helical geometries for peptide **5** in a left-(*M*) and right-handed (*P*) helical conformation. Methyl hydrogen atoms are omitted for clarity.

direction of twist in each of these β turns is opposite (Figure 3a), so it seemed possible that the opposite helicities evident in peptides capped by tertiary or quaternary L-amino acids could arise from a difference in the relative stability of the two types of turn containing the two classes of amino acids.

To explore this hypothesis, we used density functional theory (DFT) calculations to study the conformational preferences of the peptides.[28] For compounds **5a** and **5d**, a left-handed (*M*) helix with a type II turn at the N terminus and a right-handed (*P*) helix with a type III turn at the N terminus were found to have the lowest energy, differing by less than 2.4 kJ mol^{-1} (Figure 3b). *M* helices containing N-

terminal type III turns and *P* helices containing N-terminal type II turns were found to be less stable by 6.8–15.9 kJ mol^{-1}. These calculated energy differences are, however, too small to allow a reliable prediction of helical preferences. To this end, we used time-dependent DFT (TD-DFT) calculations to construct CD profiles for the four optimized conformations of **5a** and **5d**. Overlaid calculated and experimental CD spectra are shown in Figure 4. TD-DFT calculations yield very similar

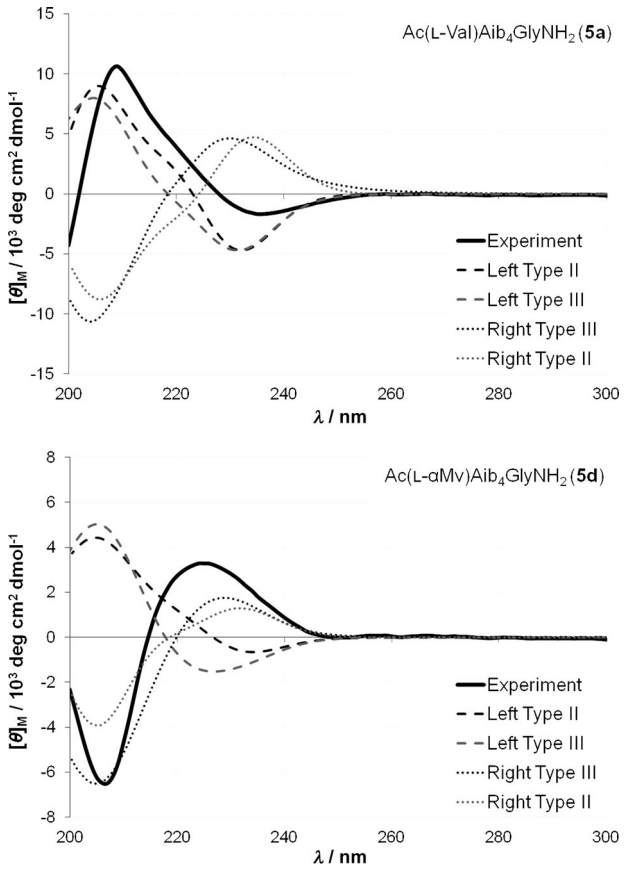


Figure 4. Experimental and calculated CD spectra for peptides **5a** and **5d** as left-handed (– – –) and right-handed (•••••) helices with type II or type III turns at their N termini.

results for both compounds, indicating that replacement of the α-hydrogen atom of L-Val with the α-methyl group of L-αMv has little effect on the chiroptical properties of the peptide. Likewise the geometry of the first turn of the helix has a minor impact on the overall shape of the predicted spectra, with CD curves for type II and type III turns being similar. On the other hand, the calculations predict that *P* and *M* helices give rise to opposite Cotton effects, thus pinpointing the overall sense of helicity as the main factor determining the CD profile of compounds **5a** and **5d**. The comparison between the calculated and measured CD spectra shows an excellent level of agreement between theory and experiment, which strongly suggests that the L-Val residue of peptide **5a** induces it to adopt left-handed (*M*) helicity, while the L-αMv induces peptide **5d** to adopt right-handed (*P*) helicity. Correlation with the CD and ^{13}C NMR spectra (Figure 1 and Figure 2) of Aib oligomers bearing other amino acids leads us to the general conclusion that peptides W-Xxx-Aib$_n$-Y (where W is a carbamate or amide protecting group and Y is a C-terminal ester or amide) adopt left-handed helicity if Xxx is a tertiary L-amino acid and right-handed helicity if Xxx is a quaternary L-amino acid (see Figure 3b, with R = H and R = Me, respectively).[29]

A reported study of absolute helicity in Aib oligomers bearing an N-terminal chiral amino acid[9] had also employed CD spectroscopy, but had come to the conclusion that both L-Val and L-αMv induce the same, right-handed helicity. Toniolo et al. had proposed that N-*p*-bromobenzamide (*p*BrBz) protection allows the sense of helicity of an Aib$_n$ chain to be correlated with the sign of the Cotton effect arising from the *p*BrBz chromophore in the 210–300 nm region of the spectrum.[22] However, we find that the CD spectra of the *p*BrBz-protected peptides **6a**,**b**,**d** and **8a**,**d** all show the same Cotton effect (Figure 5 and the Supporting

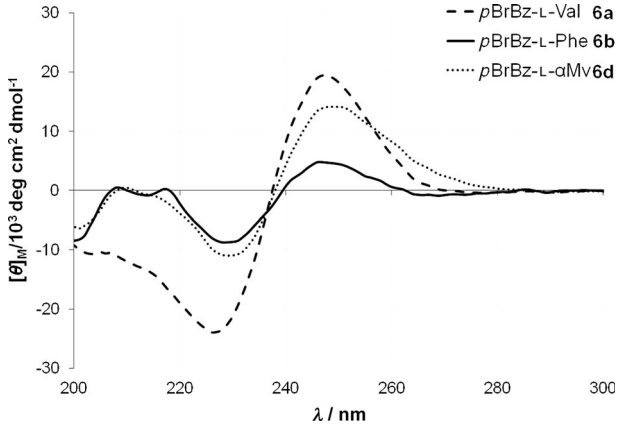


Figure 5. CD spectra of peptides *p*BrBz-L-XxxAib$_4$GlyNH$_2$ **6a**, **b**, **d**.

Information) whether they carry N-terminal tertiary or quaternary L-amino acids, despite the clear switch in helicity between **5a** and **5d** shown by CD spectroscopy (Figure 4) and between **8a** and **8d** shown by ^{13}C NMR spectroscopy (Figure 2).[30] This mismatch indicates that the CD spectrum of *p*BrBz derivatives is not an indicator of helicity when the adjacent amino acid is chiral, presumably because its chromophore is influenced more strongly by the configuration of the adjacent chiral amino acid (which is here L in all cases) than by the overall helicity of the peptide.[31]

Support for this explanation of the unreliability of an N-terminal *p*BrBz chromophore as a reporter of helicity came from TD-DFT calculations on peptides **6a** and **6d**. In this case, we explored only the two most stable arrangements (left-handed helix with type II turn and right-handed helix with type III turn). We found the small torsional angle between the phenyl ring and the carbonyl group of the *p*-bromobenzamide chromophore (χ) to be the key factor governing the appearance of the CD spectrum in the 230–280 nm region (illustrated for **6a** in Figure 6).[32] For peptides containing an N-terminal amino acid, the preference for a certain value of χ originates directly from the local stereogenic center in the surroundings of the *p*BrBz chromophore and not from the overall screw sense of the helix. For this

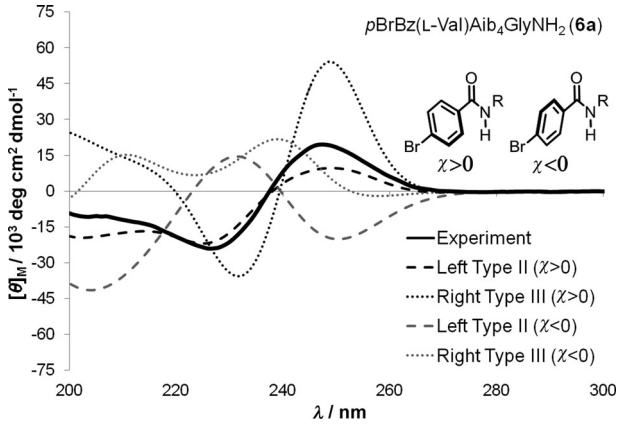


Figure 6. Experimental and calculated CD spectra for peptide **6a**.[32]

reason, the sense of helicity cannot be assigned based on CD bands associated with this chromophore.[33]

We conclude that C-α-methylation of an N-terminal L-amino acid is sufficient to cause a switch in the sense of helicity of an oligomer of achiral amino acids from left to right, probably by altering the conformational preference of the N-terminal β turn from type II to type III. We find that previous reports that an N-terminal tertiary L-amino acid induces right-handed helicity in an Aib oligomer to be erroneous, owing to the unreliability of an N-terminal *p*BrBz chromophore as an indicator of screw-sense preference when it lies adjacent to a chiral amino acid. In the light of recent reports of the ongoing use of the *N*-*p*BrBz chromophore as a marker of helix screw sense,[34] it is worth pointing out that there is no reason to doubt its effectiveness as a reporter when it lies remote from the chiral amino acid.

Absolute helicity induced in an achiral peptide chain by a single N-terminal L-amino acid may evidently be opposite to that induced in a peptide in which L-amino acids are abundant, and we are currently exploring the role of variations of the nature, number, and position of chiral residues located close to the N terminus of peptides built largely from achiral residues in determining the sense and magnitude of the overall helical preference of the chain.

Received: October 27, 2011
Published online: January 3, 2012

Keywords: circular dichroism · conformation analysis · foldamers · helical structures · peptides

[1] C.-I. Brändén, J. Tooze, *Introduction to Protein Structure*, Garland Publishing, New York, **1999**.
[2] M. Novotny, G. J. Kleywegt, *J. Mol. Biol.* **2005**, *347*, 231 – 241.
[3] M. D. Shoulders, R. T. Raines, *Annu. Rev. Biochem.* **2009**, *78*, 929 – 958.
[4] L. E. Zawadzke, J. M. Berg, *Proteins Struct. Funct. Genet.* **1993**, *16*, 301 – 305; L. W. Hung, M. Kohmura, Y. Ariyoshi, S. H. Kim, *Acta Crystallog. Sect. D* **1998**, *54*, 494 – 500.
[5] B. L. Pentelute, Z. B. Gates, M. R. Sawaya, T. O. Yeates, S. B. H. Kent, *Chem. Commun.* **2010**, *46*, 8174 – 8176; J. R. Banigan, K. Mandal, M. R. Sawaya, V. Thammavongsa, A. P. Hendrickx, O. Schneewind, T. O. Yeates, S. B. H. Kent, *Protein Sci.* **2010**, *19*, 1840 – 1849.
[6] Pauling's first diagram of an α helix (L. Pauling, R. B. Corey, H. R. Branson, *Proc. Natl. Acad. Sci. USA* **1951**, *37*, 205 – 211) famously shows a left-handed helix with D-amino acids; see: D. Dunitz, *Angew. Chem.* **2001**, *113*, 4295 – 4301; *Angew. Chem. Int. Ed.* **2001**, *40*, 4167 – 4173.
[7] a) R.-P. Hummel, C. Toniolo, G. Jung, *Angew. Chem.* **1987**, *99*, 1180 – 1182; *Angew. Chem. Int. Ed. Engl.* **1987**, *26*, 1150 – 1152; b) M. Kubasik, A. Blom, *ChemBioChem* **2005**, *6*, 1187 – 1190; c) M. Kubasik, J. Kotz, C. Szabo, T. Furlong, J. Stace, *Biopolymers* **2005**, *78*, 87 – 95.
[8] J. Solà, G. A. Morris, J. Clayden, *J. Am. Chem. Soc.* **2011**, *133*, 3712 – 3715.
[9] B. Pengo, F. Formaggio, M. Crisma, C. Toniolo, G. M. Bonora, Q. B. Broxterman, J. Kamphius, M. Saviano, R. Iacovino, F. Rossi, E. Benedetti, *J. Chem. Soc. Perkin Trans. 2* **1998**, 1651 – 1658.
[10] J. Clayden, A. Castellanos, J. Solà, G. A. Morris, *Angew. Chem.* **2009**, *121*, 6076 – 6079; *Angew. Chem. Int. Ed.* **2009**, *48*, 5962 – 5965.
[11] a) E. Yashima, K. Maeda, H. Iida, Y. Furusho, K. Nagai, *Chem. Rev.* **2009**, *109*, 6102 – 6211; b) K. Maeda, E. Yashima, *Top. Curr. Chem.* **2006**, *265*, 47 – 88; c) T. Nakano, Y. Okamoto, *Chem. Rev.* **2001**, *101*, 4013 – 4038.
[12] a) S. Hecht, I. Huc, *Foldamers*, Wiley-VCH, Weinheim, **2007**; b) D. J. Hill, M. J. Mio, R. B. Prince, T. S. Hughes, J. S. Moore, *Chem. Rev.* **2001**, *101*, 3893 – 4012; c) S. H. Gellman, *Acc. Chem. Res.* **1998**, *31*, 173 – 180.
[13] a) Y. Inai, Y. Kurokawa, T. Hirabayashi, *Biopolymers* **1999**, *49*, 551 – 564; b) Y. Inai, Y. Kurokawa, A. Ida, T. Hirabayashi, *Bull. Chem. Soc. Jap.* **1999**, *72*, 55 – 61; c) Y. Inai, Y. Kurokawa, N. Kojima, *J. Chem. Soc. Perkin Trans. 2* **2002**, 1850 – 1857; d) Y. Inai, Y. Ishida, K. Tagawa, A. Takaso, T. Hirabayashi, *J. Am. Chem. Soc.* **2002**, *124*, 2466 – 2473. For reports of induction of helicity by noncovalent binding to the N terminus of a dehydropeptide, see: N. Ousaka, Y. Inai, *J. Org. Chem.* **2009**, *74*, 1429 – 1439, and references therein.
[14] D. Pijper, B. L. Feringa, *Angew. Chem.* **2007**, *119*, 3767 – 3770; *Angew. Chem. Int. Ed.* **2007**, *46*, 3693 – 3696.
[15] a) J. Clayden, L. Lemiègre, G. A. Morris, M. Pickworth, T. J. Snape, L. H. Jones, *J. Am. Chem. Soc.* **2008**, *130*, 15193 – 15202; b) J. Clayden, M. Pickworth, L. H. Jones, *Chem. Commun.* **2009**, 547 – 549.
[16] a) H. Jiang, C. Dolain, J.-M. Léger, H. Gornitzka, I. Huc, *J. Am. Chem. Soc.* **2004**, *126*, 1034 – 1035; b) C. Dolain, H. Jiang, J.-M. Léger, P. Guionneau, I. Huc, *J. Am. Chem. Soc.* **2005**, *127*, 12943 – 12951; c) A. M. Kendhale, L. Poniman, Z. Dong, K. Laxmi-Reddy, B. Kauffmann, Y. Ferrand, I. Huc, *J. Org. Chem.* **2011**, *76*, 195 – 200.
[17] J. Solà, S. P. Fletcher, A. Castellanos, J. Clayden, *Angew. Chem.* **2010**, *122*, 6988 – 6991; *Angew. Chem. Int. Ed.* **2010**, *49*, 6836 – 6839.
[18] A consistent exception is when an L-amino acid finds itself at the C-terminal residue of a peptide ester (see Ref. [9] for example)—an effect noted by Schellman (C. Schellman in *Protein Folding* (Ed.: R. Jaenicke), Elsevier, Amsterdam **1980**). The alamethicin fragment Boc-Pro-Aib-Ala-Aib-Ala-OH (R. Bosch, G. Jung, H. Schmitt, G. M. Sheldrick, W. Winter, *Angew. Chem.* **1984**, *96*, 440 – 443; *Angew. Chem.* **1984**, *23*, 450 – 453) may also be a left-handed helix in solution. In achiral dehydropeptides, a single terminal chiral amino acid induces left-handed helicity from both N and C termini,[13] and the screw-sense preference of dehydropeptides with chiral amino acids at other positions in the chain is residue- and solvent-dependent.[13d] Left-handed helicity has been observed in related structures in the crystalline state

(see, for example: I. L. Karle, D. Ranganathan, C. Lakshmi, *Biopolymers* **2001**, *59*, 301 – 304 and Ref. [24]).
[19] S. P. Fletcher, J. Solà, D. Holt, R. A. Brown, J. Clayden, *Beilstein J. Org. Chem.* **2011**, *7*, 1304 – 1309.
[20] a) N. Berova, L. Di Bari, G. Pescitelli, *Chem. Soc. Rev.* **2007**, *36*, 914 – 931; b) D. A. Lightner, J. E. Gurst, *Organic Conformational Analysis and Stereochemistry from Circular Dichroism Spectroscopy*, John Wiley & Sons, New York, **2000**.
[21] a) M. C. Manning, R. W. Woody, *Biopolymers* **1991**, *31*, 569 – 586; b) C. Toniolo, A. Polese, F. Formaggio, M. Crisma, J. Kamphuis, *J. Am. Chem. Soc.* **1996**, *118*, 2744 – 2745.
[22] C. Toniolo, F. Formaggio, M. Crisma, H. Schoemaker, J. Kamphuis, *Tetrahedron: Asymmetry* **1994**, *5*, 507 – 510.
[23] CD Spectra in 2,2,2-trifluoroethanol were almost identical. See the Supporting Information.
[24] The magnitude of the anisochronicity also provides a measure of relative helical conformational preference: see Ref. [8], [10] and J. Solà, M. Helliwell, J. Clayden, *J. Am. Chem. Soc.* **2010**, *132*, 4548 – 4549.
[25] a) C. M. Venkatachalam, *Biopolymers* **1968**, *6*, 1425 – 1436; b) P. Y. Chou, G. D. Fasman, *J. Mol. Biol.* **1977**, *115*, 135 – 175; c) C. Tonlolo, E. Benedetti, *CRC Crit. Review Biochem.* **1980**, *9*, 1 – 44; d) G. D. Rose, L. M. Gierasch, J. A. Smith, *Adv. Protein Chem.* **1985**, *37*, 1 – 109; e) C. Toniolo, E. Benedetti, *Trends Biochem. Sci.* **1991**, *16*, 350 – 353; f) G. R. Marshall in *Intra-Science Chemistry Report*, *Vol. 5* (Ed.: N. Kharasch), Gordon & Breach, New York, **1971**, pp. 305 – 316; g) I. L. Karle, P. Balaram, *Biochemistry* **1990**, *29*, 6747 – 6756; h) C. Toniolo, M. Crisma, F. Formaggio, C. Peggion, *Biopolymers* **2001**, *60*, 396.
[26] C. Toniolo, M. Crisma, F. Formaggio, G. Valle, G. Cavicchioni, G. Précigoux, A. Aubry, J. Kamphuis, *Biopolymers* **1993**, *33*, 1061 – 1072.
[27] M. Crisma, E. Andreetto, M. De Zotti, A. Moretto, C. Peggion, F. Formaggio, C. Toniolo, *J. Pept. Sci.* **2007**, *13*, 190 – 205, and references cited therein.
[28] See the Supporting Information for calculated energies (Table S1 in the Supporting Information).
[29] This conclusion suggests that Aib oligomers and the dehydropeptides behave more similarly than previously thought: see Ref. [13].
[30] These NMR spectra rule out an alternative possibility, that some amino acids when N-protected with *p*BrBz induce opposite helicity from the same amino acids N-protected with Ac or Cbz.
[31] The conclusions of Toniolo et al. in Refs. [9] and [22] with regard to *p*BrBz-protected peptides which do not have a chiral N-terminal amino acid are not affected by this discovery.
[32] The calculated spectra of **6a** and **6d** (which are very similar to those of **6a**: see the Supporting Information) were shifted by −22 nm, which is the difference between calculated (264 nm) and experimental (242 nm) maximum UV absorption of this class of compounds. See Ref. [9].
[33] A similarly strong dependence of chiroptical properties from the same torsional angle in benzamides was recently observed by Meijer, Palmans, and co-workers in a combined CD/TD-DFT study on supramolecular helical polymers: Y. Nakano, T. Hirose, P. J. M. Stals, E. W. Meijer, A. R. A. Palmans, *Chem. Sci.* **2012**, *3*, 148 – 155.
[34] N. Ousaka, Y. Takeyama, H. Iida, E. Yashima, *Nat. Chem.* **2011**, *3*, 856 – 861.

