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The N-Terminal Nonapeptide of Cephaibols A and C: A Naturally Occurring Example of Mismatched Helical Screw-Sense Control

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Abstract: The N-terminal nonapeptide domain of the fungal nonribosomal peptide antibiotics cephaibol A and cephaibol C (AcPheAib$_4$LeuIvaGly-Aib) is reported to adopt a right-handed helical conformation in the crystalline state. However, this conformation is at odds with the left-handed helicity observed in solution in related synthetic oligomers capped with Ac-L-PheAib$_4$ fragments. We report the synthesis of four diastereoisomers of the cephaibol N-terminal nonapeptide, and show by NMR and CD spectroscopy that the peptide containing the chiral amino acids Phe and Leu in the naturally occurring relative configuration exists in solution as an interconverting mixture of helical screw-sense conformers. In contrast, the nonapeptide containing the unnatural relative configuration at Phe and Leu adopts a single, stable helical screw-sense, which is left handed when the N-terminal Phe residue is L and right-handed when the N-terminal Phe residue is D.

Keywords: conformation · foldamers · NMR spectroscopy · peptaibiotics · peptides

Introduction

Peptide chains of achiral amino acids, even though they contain no asymmetric centres, may be induced into either a left- or a right-handed helical conformation by an external chiral influence. For example, homo-oligomers of the helicogenic[1–3] quaternary amino acid Aib[4,5] (2-aminoisobutyric acid), or hetero-oligomers of Aib and other achiral residues (such as Gly,[6–8] Ac6c,[8–10] or dehydroamino acids[9,11]), may be induced to adopt a preferred screw sense by chiral residues bonded either covalently[6,8–13] or non-covalently[13,14] at their N or C terminus. A remarkable naturally occurring achiral peptide motif of this type occurs towards the N-terminus of the antibiotic fungal metabolites cephaibol A, C, D and E (Figure 1).[15] These non-ribosomal peptides contain a string of no less than five achiral amino acids (Aib-Aib-Aib-Aib-Gly) which adopt, in the solid state, a right-handed helical conformation.[16] The achiral pentapeptide segment is capped at the N- and C-terminus by the chiral residues Ac-L-Phe and L-Leu, respectively, with one or two further achiral residues linking this AcPheAib$_4$GlyLeu oligomer to the proline/hydroxyproline-rich C-terminal domain of the 16-residue chain.

At first sight, it seems reasonable to assume that the right-handed screw-sense preference in the solid state structure of this achiral pentapeptide segment arises from the absolute configuration of the flanking L-Phe and L-Leu residues. Peptide helices containing L-amino acids typically adopt a right-handed screw sense, and the two chiral residues may induce the unbiased remainder of the N-terminal domain to adopt the same conformation. However, recent studies have revealed that protected chiral amino acids such as Ac-L-Phe, when located at the N-terminus of a peptide, typically induce a left-handed screw sense in nearby achiral residues.[17] This observation calls into question the origin of the screw-sense preference at the N-terminus of the cephaibols, a preference that may play a crucial role in the antibacterial and anthelmintic activity of these and related antibiotics.

The cephaibols belong to a broader class, known as the peptaibols, of fungal non-ribosomal peptides containing between five and twenty amino acid residues.[18] Peptaibols are characterized by a high content of non-proteinogenic, α,α-dialkylated amino acids such as Aib or Iva (isovaline, or 2-amino-2-methylbutyric acid), along with a C-terminal 1,2-amino alcohol and (usually) acylation at the N-terminus.[19,20] The broad spectrum of antibacterial activities of peptaibols derives from their interaction with biological phospholipid membranes.[21] Because these antimicrobial peptides permeabilize membranes without specificity towards a protein target, the likelihood that bacterial resistance may develop is low. Understanding their mechanism of action is thus crucial for the development of peptaibols or their analogues as useful anti-microbial agents, which could address the increasingly serious, worldwide health problem of multidrug antibiotic resistance.

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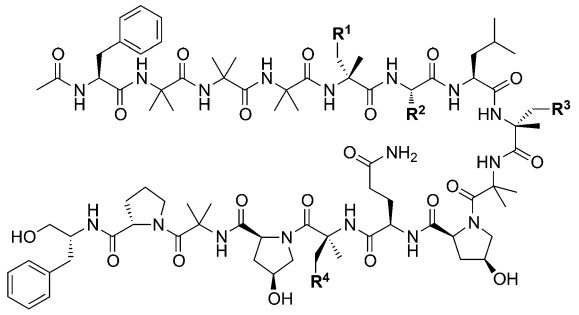
Peptaibols fold into helical structures both in solution and when membrane-bound.[19] The most studied member of this family is alamethicin, a 20-residue pore-forming peptide from the fungus *Trichoderma viridans*.[22,23] Depending on peptide concentration and/or transmembrane potential, alamethicin forms pores by the so-called "barrel-stave" mechanism, in which peptide helices insert in a transmembrane orientation and aggregate in a cylindrical superstructure.[23,24] This pore-forming mechanism has been unambiguously demonstrated only for alamethicin and melittin, the lytic toxin from honeybee venom.[25] However, in view of both the closely related amino acid composition and physico-chemical properties of other long-chain (i.e. ca. 20-residue) peptaibols, it is conceivable that they too could form pores in a similar way. Support for the "barrel-stave" mode of action is also provided by the fact that the length of alamethicin means it can almost exactly span the thickness of bacterial membranes. By contrast, another important subfamily of membrane-active peptaibols has less than 15 residues:[26] representative members include antiamoebins,[27–29] cephaibols[15] and zervamicins.[27,30] Their membrane-perturbing mechanism of action is still under debate.

The cephaibols were isolated from the soil fungus *Acremonium tubakii*, DSM 127741.[15] In addition to their considerable antibacterial potency, they exhibit pronounced anthelmintic activity against ectoparasites.[15] The amino acid sequence of cephaibols is highly conserved among the members of the group (Figure 1) and the principal points of variation are residues 5, 8 and 12, where either Aib or its one-carbon homologue D-Iva[31] may be found. A 10-fold variation in the antibacterial potency is observed in this peptaibol



	R^1	R^2	R^3	R^4
cephaibol A	H	H	Me	Me
cephaibol A2	H	Me	Me	Me
cephaibol B	Me	H	Me	Me
cephaibol C	H	H	Me	H
cephaibol D	H	H	H	H
cephaibol E	H	H	H	Me
antiamoebin	Me	H	H	Me

Figure 1. Chemical structure of cephaibols and comparison with antiamoebin.

sub-class, so the insertion of these additional methylene groups seems to play a major role in the antimicrobial action of these peptides.[15]

The three-dimensional structures of various peptaibols have been determined both in the solid state and in solution.[29,30,32] These studies indicate that many of these molecules adopt a helical conformation consisting of an N-terminal domain followed by a bend at a proline or a hydroxyproline residue located close to the middle of the helix. While the crystal structures of cephaibols A, B and C have already been reported,[16] no structural information in solution is available for this group of peptaibols.[33]

Our earlier studies on the screw-sense preference induced by chiral amino acids in achiral oligomers made use of compounds whose structure was inspired by the N-terminal sequence of the cephaibols, and we showed that a series of 'cephaibologues' Cbz-L-Phe$(Aib_4Gly)_nAib_4AibCH_2OH$ ($n=$ 0–3) displayed features in their ^{1}H NMR spectra indicating that the entire achiral segment of the oligomers adopts to some extent a preferred screw sense.[6] We developed NMR-based methods to quantify screw-sense preference in similar achiral helices,[34] and used related Aib oligomers to relay information from a switchable centre or binding site to a remote isotopically labelled enantiomerically enriched NMR reporter.[4,6–8,34,35] This work revealed that, contrary to expectations, Cbz-L-Phe$(Aib_4Gly)_nAib_4AibCH_2OH$ and related $(Aib)_n$ chains carrying N-terminal L-amino acids adopt left-handed helicity in solution, unless the N-terminal L-amino acid is quaternary, in which case right-handed helicity is preferred.[35] We further showed that the preference for left- or right-handed helicity arises from the detailed structure of the β-turn at the N terminus: tertiary L-amino acids induce a left-handed screw sense by favouring a Type II β-turn, while quaternary L amino acids induce a right-handed screw sense by favouring a Type III β-turn.[17]

Despite the similarity of the N-terminal Ac-L-PheAib_4Gly sequence of cephaibols A and C to the sequences of the left-handed helical oligomers Cbz-L-Phe-$(Aib_4Gly)_nAib_4AibCH_2OH$, the N-terminal helices of both cephaibols A and C are right-handed in the solid state (Figure 2).[16] Moreover, the usual strong propensity of Aib-rich peptides to populate 3_{10} helical conformations[1] is not evident in the X-ray crystal structure of the cephaibols' N-terminal domain, which is folded into an α-helix until the ninth residue.

We therefore set out to synthesise the four configurational diastereoisomers **1** of the N-terminal domain of cephaibols A and C (Figure 3) and to explore the dependence of their conformation in solution on the absolute and relative configurations of the three chiral amino acids that they contain. We hoped to achieve deeper insights into the origin of screw-sense preference in these bioactive peptides and to clarify whether their solid state conformational preferences are representative of their solution structures.

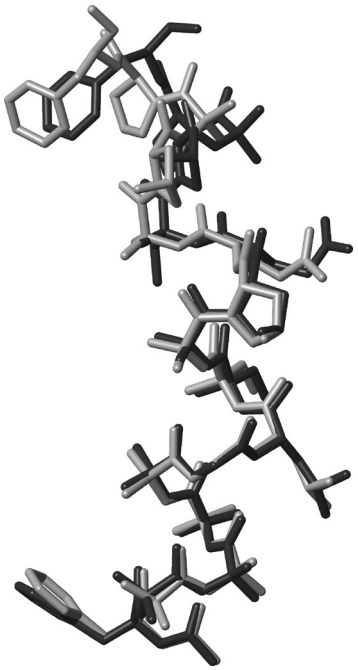


Figure 2. Superimposition of the solid state structures of cephaibols A (black; PDB entry: 1OB4) and C (grey; PDB entry: 1OB7) (see ref. [16]).

a) R-L-**Phe**-Aib-Aib-Aib-Aib-Gly-L-**Leu**-L-**Iva**-Aib-O*t*Bu (**L**, **L**, **L**)

b) R-L-**Phe**-Aib-Aib-Aib-Aib-Gly-D-**Leu**-L-**Iva**-Aib-O*t*Bu (**L**, **D**, **L**)

c) R-D-**Phe**-Aib-Aib-Aib-Aib-Gly-L-**Leu**-L-**Iva**-Aib-O*t*Bu (**D**, **L**, **L**)

d) R-D-**Phe**-Aib-Aib-Aib-Aib-Gly-D-**Leu**-L-**Iva**-Aib-O*t*Bu (**D**, **D**, **L**)

Figure 3. The diastereoisomeric nonapeptide targets **1a–d** (R = Ac) and **2a–d** (R = Cbz).

Results and Discussion

Synthesis of the nonapeptides: The primary sequences of the four peptides **1** include several quaternary (C^{α}-dialkylated) residues, which precluded the possibility of using solid-phase peptide-synthesis methods. We therefore used a fragment-based solution-phase approach, shown in Scheme 1, starting from L-Iva.

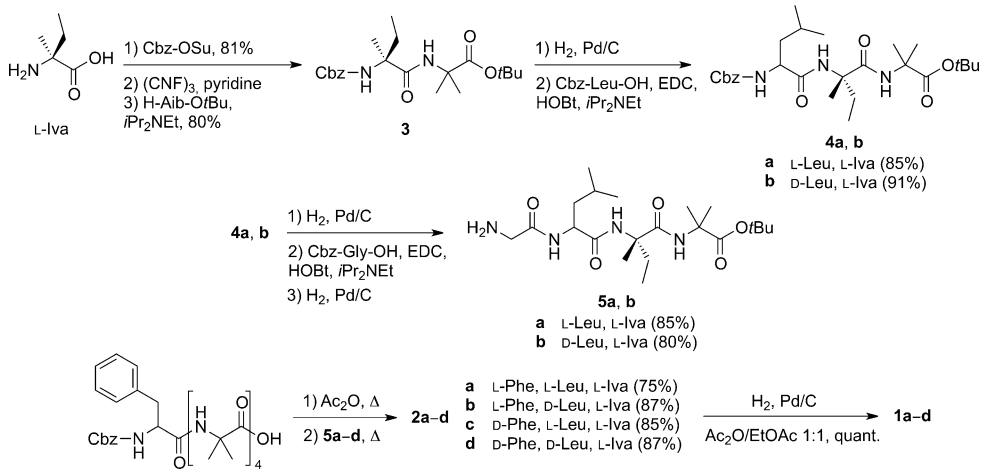
We reasoned that the presence of a Gly residue in the middle of the target peptides **1a–d** would facilitate the synthesis by allowing the azlactone of Cbz-Phe-Aib_4-OH[36] to be ring opened by the reactive Gly amino group of tetrapeptide **5**. The synthesis began with the sterically demanding coupling between Aib *tert*-butyl ester and the acyl fluoride derivative[37] of Cbz-L-Iva-OH, giving dipeptide **3**[38] in 80% yield. Standard peptide coupling and deprotection using EDC/HOBt with Cbz-L- or Cbz-D-Leu-OH, then with Cbz-Gly-OH, each followed by deprotection, provided the two diastereoisomers **5a** and **5b**, each in 47% overall yield from Cbz-L-Iva-OH. Conversion of each enantiomer of Cbz-Phe-Aib_4-OH[36] to its azlactone with acetic anhydride and ring opening with each of the amines **5** afforded each of the diastereoisomers of **2**. Final Cbz deprotection and N-acetylation to yield **1a–d** was achieved by hydrogenation in a solution of EtOAc/Ac_2O. The four peptides were purified by preparative HPLC.

Conformational studies

CD spectroscopy: CD spectra[39] of the four diastereoisomers of **2** were acquired in MeOH (a solvent that prevents aggregation of related peptides) and are shown in Figure 4.

The CD spectra of the two pairs **2a**,**d** and **2b**,**c** are near mirror images, despite all four compounds containing the same L-Iva residue. This observation indicates that the configuration of the Iva residue relative to the rest of the peptide exerts little effect on the peptide's overall conformation. Iva's C^{α} Me and Et groups are similar in size, and Iva is typically seen to be screw-sense indifferent.[16,35,40] Thus **2a**,**d** and **2b**,**c** appear to be essentially pseudoenantiomeric pairs.

Importantly, however, the shape of the spectra of the **2a**,**d** pair is very different from that of the **2b**,**c** pair. **2b** and **2c**,



Scheme 1. Synthesis of the diastereoisomers of **1**, the N-terminal domain of cephaibols A and C.

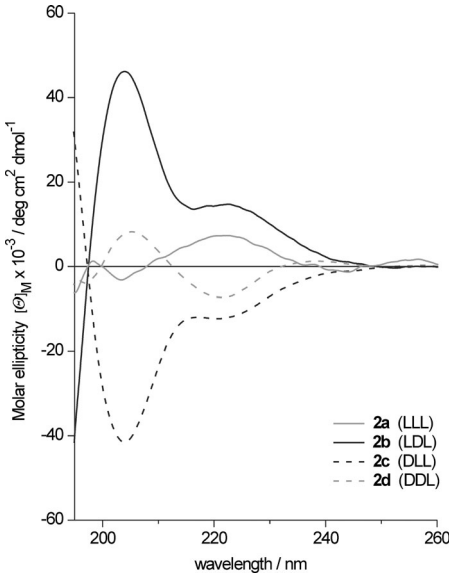


Figure 4. CD spectra of the four configurational isomers of Cbz-protected **2** in MeOH. Peptide concentration: 1 mM.

in which the absolute configurations of Phe and Leu are opposite, display a profile typical of a highly populated 3_{10}-helix,[41] with **2b** and **2c** being left- and right-handed, respectively. 3_{10}-Helical conformations are characteristic of peptides rich in quaternary amino acids such as Aib and Iva. On the other hand, no clear conclusions can be drawn from the CD on the secondary structure adopted by peptides **2a**,**d**, where both Phe and Leu have the same configuration, despite the same high proportion of helix-promoting Aib and Iva residues.

When the Cbz group of **2** is replaced with the acetyl group of **1**, as found at the N-terminus of the cephaibols, no significant change is evident in the CD curves of the conformation of **1b**,**c** in MeOH (Figure 5a): again, they appear to adopt well-structured 3_{10}-helices. Indeed, the increased intensity of the CD signal is consistent with an even higher population of folded structures.[42] The same 3_{10}-helical character is likewise evident in CH_3CN (Figure 5b) and 2,2,2-trifluoroethanol (TFE) (Figure 5c).

However, the CD curves of the N-acetylated peptide pair **1a**,**d** reveal a few differences from those of their Cbz-protected counterparts **2a**,**d**. As illustrated in Figure 5, the conformation of the less well-structured peptides **1a**,**d** appears to be more affected by solvent than that **1b**,**c**. Remarkably, an inversion in the Cotton effect seems to take place for **1a**,**d** when moving from CH_3CN to TFE.[43]

These CD results indicate that the relative configuration of the Phe and Leu residues of peptides **1b** and **1c** work together in solution to maintain a well-defined, solvent-independent, mostly 3_{10}-helical conformation. In other words, they are stereochemically 'matched', despite having opposite absolute configurations. This conclusion is fully consistent with our previous observations that an N-terminal L-amino acid induces a left-handed screw sense, even though L-amino acids incorporated within a helical motif typically favour right-handed screw sense. The left-handed screw sense evident in the CD spectrum of **1b** is induced by the N-terminal L-Phe and reinforced by the left-handed screw sense favoured by the D-Leu[7] of the helix. Likewise the right-handed screw-sense evident in the CD spectrum of **1c** is induced by the N-terminal D-Phe and reinforced by the right-handed screw-sense favoured by the L-Leu[7]. The relative configuration of the L-Iva[8] has no effect on the overall screw sense.

In contrast, the corresponding CD spectra of **1a** and **1d** show that these peptides may either fold into a mixture of

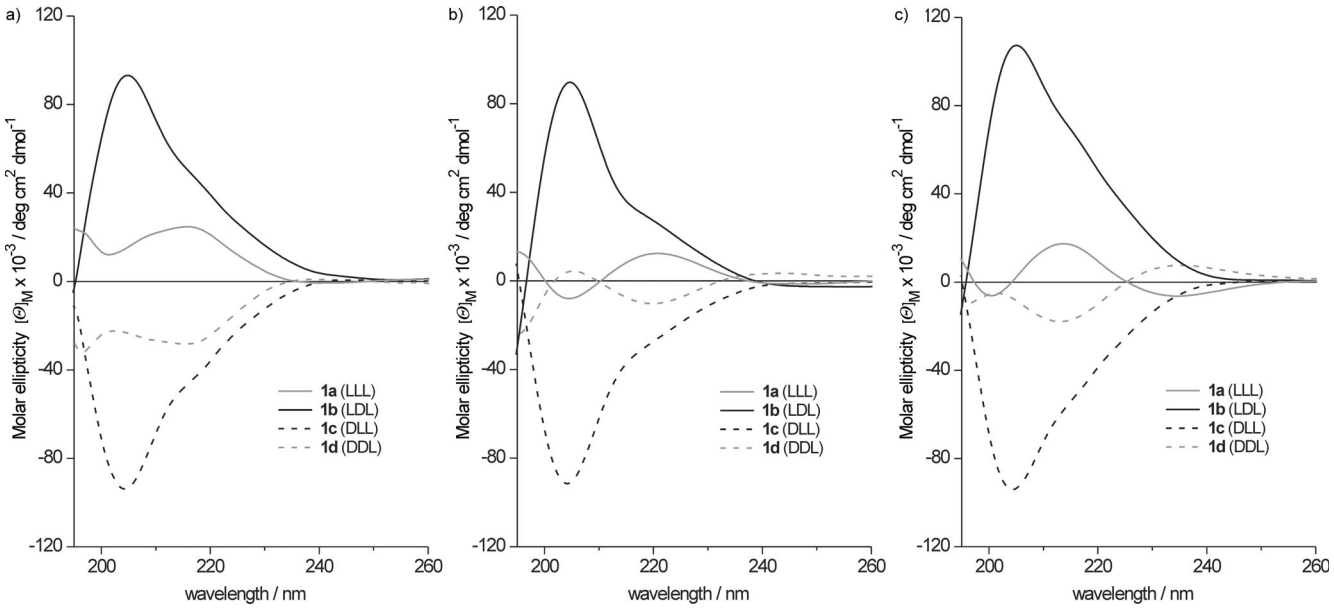


Figure 5. CD spectra of the four configurational isomers of **1** in MeOH (a), CH_3CN (b) and TFE (c). Peptide concentration: 1 mM.

interconverting left- and right-handed helices or may not adopt any organized conformation at all. The configurations of the Phe and Leu residues in **1a** and **1d** are mismatched, and work against one another to frustrate the adoption of a well-defined structure. Remarkably, it is one of these two peptides, **1d**, that has the relative stereochemistry (though the opposite absolute stereochemistry) of natural cephaibols A and C.

NMR spectroscopy: CD spectroscopy provides evidence of helicity in peptides **1b** and **1c**, but fails to give detailed information on the preferred conformation of peptides **1a** and **1d**. Detailed structural characterization of the peptides was therefore carried out using 2D NMR spectroscopy. We focused our analysis on peptides **1a** (with the two principal chiral residues conformationally 'mismatched') and its epimer **1b** (whose two principal chiral residues are 'matched'). Experiments were performed in CD_3OH solution in order to preserve amide NH proton signals, to avoid complications arising from aggregation in chlorinated solvents, and to allows us to correlate results with those obtained by CD spectroscopy.

Overall assignment of proton resonances of both peptides **1a** and **1b** was made by means of homo- and heteronuclear 2D NMR spectra (see the Experimental Section). The amide proton region of the ROESY spectrum of 'matched' peptide **1b** is shown in Figure 6. All NH···NH sequential interactions are visible (apart from those obscured by diagonal peaks), consistent with the presence of a helical conformation.

Further relevant 3D structural information is obtained from the $C^{\alpha(\beta)}H$–NH regions of the ROESY spectrum

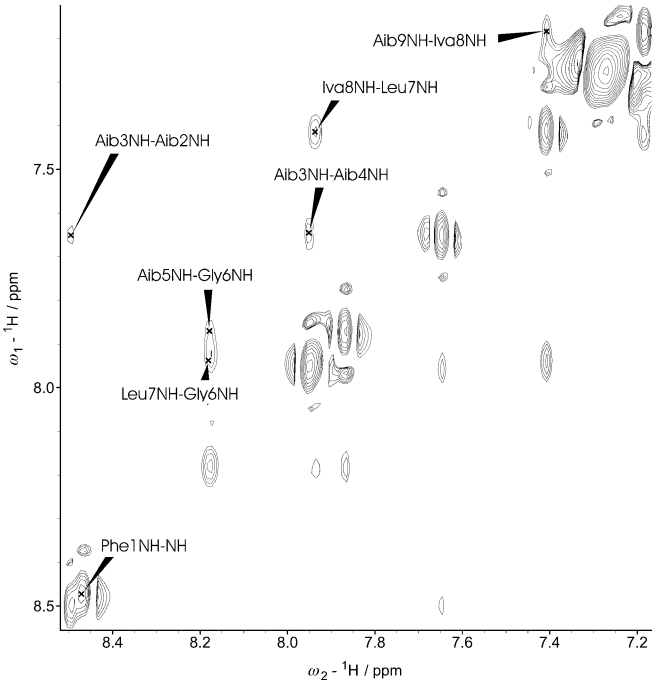


Figure 6. Amide proton region of the ROESY spectrum (τ_m 200 ms, 298 K, 500 MHz) of peptide **1b** in CD_3OH (5 mM).

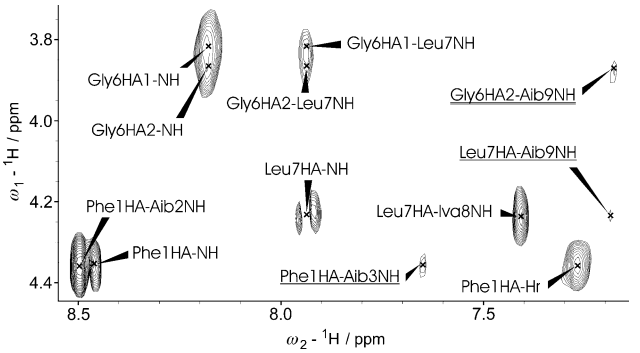


Figure 7. $C^{\alpha}H$–NH fingerprint region of the ROESY spectrum (τ_m 200 ms, 298 K, 500 MHz) of **1b** in CD_3OH (5 mM). Medium range $C^{\alpha}H_i \rightarrow NH_{i+2}$ (typical for a 3_{10}-helix) and $C^{\alpha}H_i \rightarrow NH_{i+3}$ (3_{10}- or α-helix) cross-peaks are underlined.

(Figure 7 and 8). Many diagnostic $C^{\alpha(\beta)}H_i$–NH_{i+3} and $C^{\alpha(\beta)}H_i$–NH_{i+2} cross peaks are visible, confirming the presence of a mainly 3_{10} helix throughout the sequence of peptide **1b**. In particular, the two $C^{\alpha(\beta)}H_i$–NH_{i+3} [2→5 (Figure 8) and 6→9 (Figure 7)] connectivities support the hypothesis that peptide **1b** is folded into a helical conformation. Moreover, the two $C^{\alpha}H_i$–NH_{i+2} [1→3 and 7→9 (Figure 7)] cross-peaks are diagnostic of a mainly 3_{10}-helical secondary structure throughout the peptide sequence.[44] The α-helical contribution, if present, is not significant, since no $C^{\alpha(\beta)}H_i$–NH_{i+4} are detectable. The 2D NMR study supports the conclusions drawn from the CD spectra that this nonapeptide displays a strong preference to fold into a 3_{10}-helix in methanol.

In the case of the conformationally 'mismatched' peptide **1a**, 3D structural NMR analysis is less straightforward.

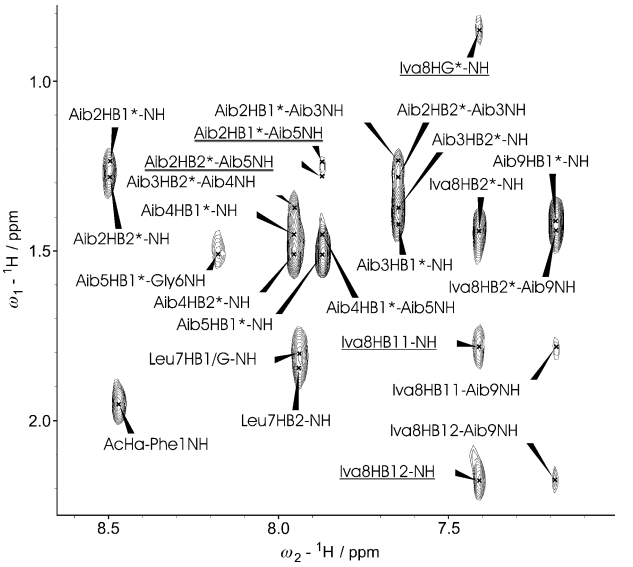


Figure 8. $C^{\beta}H$–NH correlation region of the ROESY spectrum (τ_m 200 ms, 298 K, 500 MHz) of peptide **1b** in CD_3OH (5 mM). Medium-range interactions $C^{\beta}H_i \rightarrow NH_{i+3}$ diagnostic for helical structures, and intraresidue Iva cross-peaks, are underlined. (HB1*: Aib pro-R methyl group; HB2*: Aib pro-*S* methyl group).

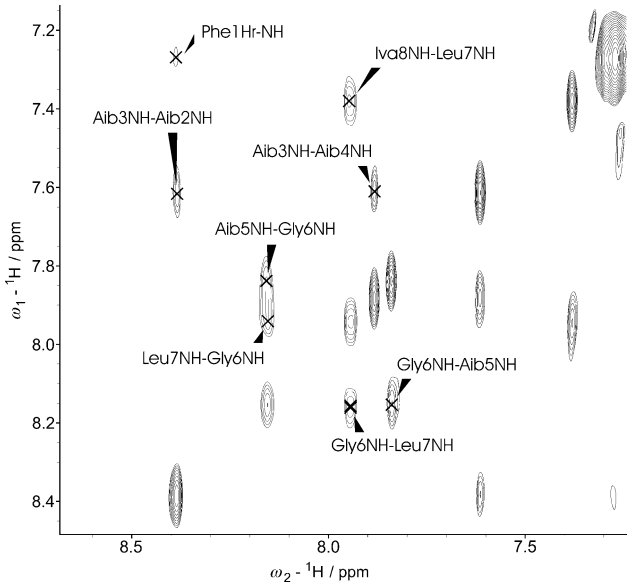


Figure 9. Amide proton region of the ROESY spectrum (τ_m 200 ms, 298 K, 500 MHz) of peptide **1a** in CD_3OH (5 mM).

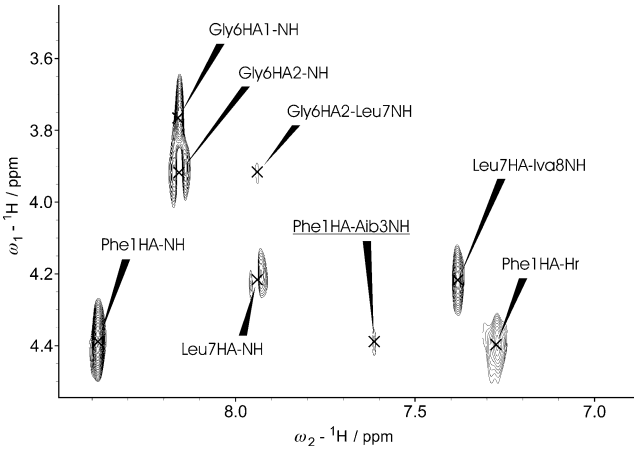


Figure 10. $C^{\alpha}H$–NH region of the ROESY spectrum (τ_m 200 ms, 298 K, 500 MHz) of **1a** in CD_3OH (5 mM). The medium range cross-peak $C^{\alpha}H_i \rightarrow NH_{i+2}$ (3_{10}-helix) is underlined.

Figure 9 shows the amide proton region of the ROESY spectrum of peptide **1a**.

As with peptide **1b**, all the sequential, inter-residue NH-NH interactions are also detectable in the case of peptide **1a**. Furthermore, inspection of the fingerprint region of its ROESY spectrum (Figure 10 and 11) reveals a few medium-range cross-peaks accounting for the presence of a helical structure ($C^{\beta}H_i$–NH_{i+3}, 2→5 and 4→7, Figure 11) of the 3_{10}-type ($C^{\alpha}H_i$–NH_{i+2}, 1→3, Figure 10). This seems to suggest that peptide **1a** is helical, despite its inconclusive CD spectrum.

In order to identify the presence of α- or 3_{10} helical conformations in **1a** and **1b**, the variation in chemical shift with temperature of each NH signal was measured. In the Supporting Information, Figure S1 shows the different NMR spectra for peptides **1a** and **1b** between the range 278–308 K.

Figure 12 reports the temperature coefficients of the chemical shifts of the amide NH signals ($\Delta\delta$(HN)/ΔT) measured for peptides **1a** and **1b**, indicating that both peptides behave similarly with variation of temperature. The highest values are exhibited by amide NHs located at the N-terminus, which are exposed to the solvent and therefore are more affected than the rest of the intramolecular H-bond network by a change in temperature. This VT-NMR analysis confirms the helical nature of both peptides **1a** and **1b**, though the magnitude of the coefficient of Aib^3 NH also suggests that they exist as a mixture of 3_{10}/α-helical structures in MeOH solution.

Another valuable indicator of helical character in Aib-rich peptides is the anisochronicity of the ^{13}C NMR signals of a pair of diastereotopic methyl groups in each Aib residue.[46] The ^{13}C NMR signals of such *gem*-dimethyl pairs in unfolded structures, even when directly adjacent to a stereogenic centre, are usually separated by less than 0.5 ppm. However, within a stable helical conformation, this anisochronicity value (also called "chemical nonequivalence" or CNE[46]) can rise to more than 2 ppm. Table 1 reports the anisochronicities in the ^{13}C NMR spectrum of the methyl groups of the five Aib residues of peptides **1a** and **1b**.

Despite their difference being just one chiral centre, only peptide **1b** (the 'matched' structure) shows high (>2 ppm) anisochronicity values in the main body of the Aib_4 se-

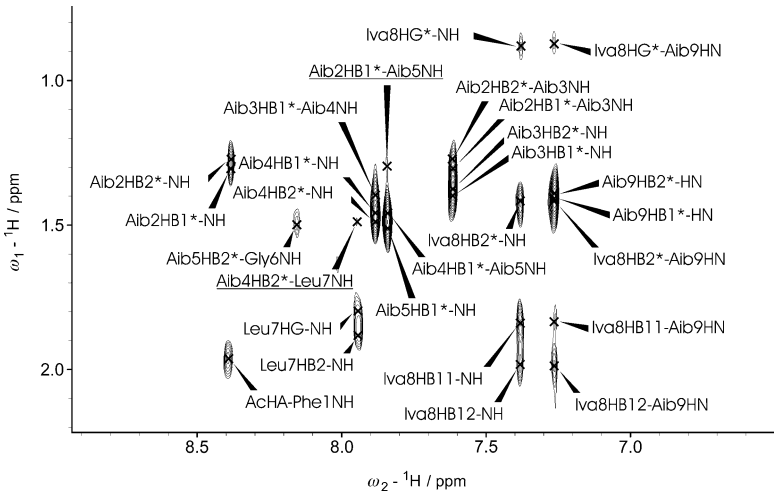


Figure 11. $C^{\beta}H$–NH correlation region of the ROESY spectrum (τ_m 200 ms, 298 K, 500 MHz) of peptide **1a** in CD_3OH (5 mM). Medium-range interactions $C^{\beta}H_i \rightarrow NH_{i+3}$ diagnostic for helical structures are underlined.

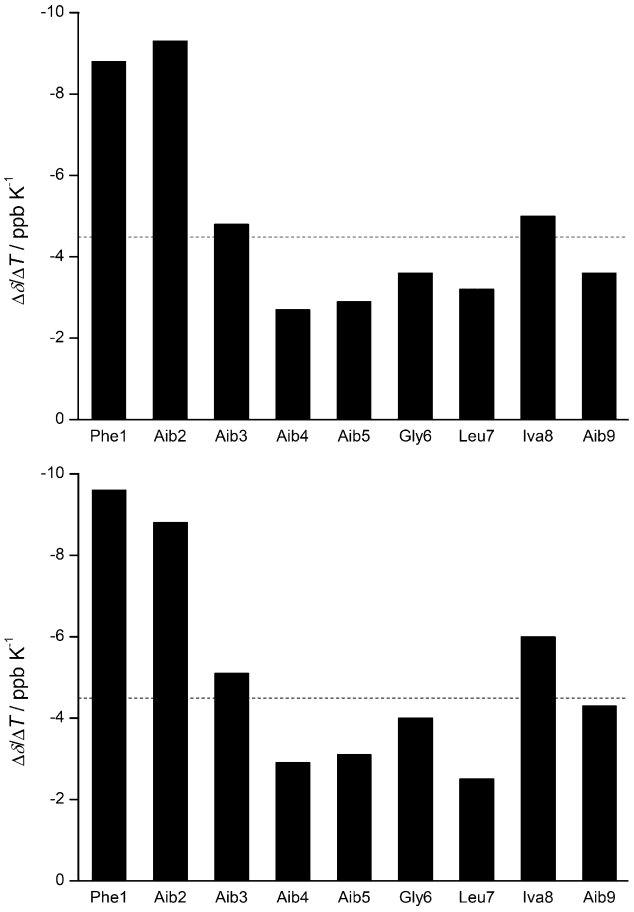


Figure 12. Temperature coefficients for amide H-atom chemical shifts (temperature range: 278–308 K) of peptide **1a** (top) and **1b** (bottom). The dashed line at $-4.5\,\mathrm{ppb\,K^{-1}}$ illustrates the proposed level (see refs. [3] and [45]) that divides intramolecularly hydrogen-bonded from free-amide NHs.

Table 1. Anisochronicity or CNE values (ppm) of $^{13}C_\beta$ of methyl groups of the Aib residues of peptides **1a** and **1b**.

residue	**1a** [ppm]	**1b** [ppm]
Aib[2]	1.61	2.85
Aib[3]	1.08	3.16
Aib[4]	0.75	2.95
Aib[5]	<1	2.91
Aib[9]	<1	0.54

quence, lending further support to the hypothesis that this molecule adopts in solution a well-developed 3_{10} helix. On the other hand, peptide **1a** (the 'mismatched' structure) shows smaller values for the anisochronicities of the methyl groups in each of its Aib residues. These values indicate that this peptide is not well structured as a helix with a single screw sense, and may instead be undergoing rapid (on the NMR time scale) conformational exchange, possibly between right- and left-handed helical conformations.

It has been reported that the chemical-shift difference between the diastereotopic β-CH_2 protons of an Iva residue embedded in a peptide chain may be correlated to the screw sense adopted by the helix, provided that the configuration of the Iva residue is known (Table 2).[47]

Indeed, when embedded into the diastereoisomeric peptides **1a** and **1b**, the L-Iva[8] residues behave differently, as shown in Table 3:

Table 3. NMR parameters relative to the L-Iva[8] residues in the two peptides **1a** and **1b**.

NMR parameter	**1a** [ppm]	**1b** [ppm]
$\delta\ ^{\gamma}CH_3$ (^{1}H NMR)	0.870	0.851
$\Delta\delta\ ^{\beta}CH_2$	0.148	0.393
$\delta\ ^{\beta}CH_2$ (^{13}C NMR)	30.98	28.30

By comparing the two sets of values with those reported in Table 2, it is fair to conclude that while the parameters obtained from L-Iva[8] of peptide **1b** are consistent with a left-handed (*M*) helix, those obtained from peptide **1a** do not show a clear propensity for only one helical screw sense over the other, but rather supports the view of a system undergoing fast conformational interconversion.

2D NMR analysis of peptide **1a** indicates that in solution it adopts principally a helical structure. Conversely, the CD data and the data in Tables 1 and 3 are consistent with the interpretation either that peptide **1a** is unfolded, or that if it is helical it does not adopt a single favoured screw sense. We therefore deduce that while peptide **1b** is a stable left-handed helix in solution, peptide **1a** exists as a rapidly interconverting mixture of left- and right-handed helices. The difference results from either a match or a mismatch between the effects of the Phe and Leu residues. Both peptides are rich in quaternary amino acids, and so favour the population of helical conformations. In both **1a** and **1b**, the N-terminal L-Phe induces a left-handed screw sense, and in **1b** this conformational preference is reinforced by D-Leu[7], giving a thermodynamically stable left-handed helical structure. In **1a**, however, the left-handed screw-sense favoured by the N-terminal L-Phe is opposed by the right-handed screw sense preference of L-Leu[7]. Both screw senses are therefore populated, and rapid conformational exchange results. The same

Table 2. Summary of the NMR parameters used to assess the absolute configuration of Iva residues in a right-handed peptide (see ref. [47]).[a]

NMR parameter	Value for D-Iva [ppm]	Value for L-Iva [ppm]
^{1}H NMR chemical shift of the γ-methyl protons	$\delta < 0.89$	$\delta > 0.91$
Difference between the chemical shifts ($\Delta\delta$) of the two β-methylene protons	$\Delta\delta > 0.28$	$\Delta\delta < 0.20$
^{13}C NMR chemical shift of the β-methylene carbon atom	$\delta < 29$	$\delta > 33$

[a] For a left-handed helical peptide, the parameters must be reversed.

conclusions can be drawn for peptides **1c** and **1d**, whose CD spectra suggest almost identical behaviour to **1b** and **1a**, respectively, given the weakness of the role played by inverted relative stereochemistry at the Iva reside.

Conclusion

The conformational analysis carried out for the four diastereoisomers of the N-terminal domain of cephaibols A and C indicates that while all four adopt helical conformations, only in those (**1b**, **1c**) with the unnatural relative configuration between the residues Phe[1] and Leu[7] is this conformation a stable, single screw sense (left handed for **1b**; right handed for **1c**). In peptides **1a** and **1d**, the helical conformation is fluxional in its screw sense, and the population of different conformers varies in a solvent-dependent manner.

Intriguingly, the relative configuration displayed by the natural products cephaibols A and cephaibols C corresponds to (though is enantiomeric with) that of **1a**. This suggests that the N-terminal domain of the natural compound may also be characterised by conformational fluxionality. The apparently well-folded right-handed helix observed for this domain in the X-ray crystal structure of these cephaibols may therefore be a crystallographic artefact, or may possibly be stabilised by interactions with the C-terminal domain missing from **1a**. Detailed studies of the conformation of the intact cephaibols in solution have never been carried out: 2D NMR work was used to confirm their primary sequence but not their secondary structure.[15,16]

Whether this conformational mobility plays a role in the bioactivity of the cephaibols remains unclear, but it is an intriguing thought that the incorporation of the N-terminal L-Phe residue by the fungus is the result of evolutionary selection for organisms producing antibiotics with a disordered N-terminal helix. Similar proposals have been made for other peptaibols whose conformational mobility contributes to their bioactivity.[29,48]

Experimental Section

Synthesis and characterization of peptides: The synthetic strategy used in this work is shown in Scheme 1. Characterization details are reported in the Supporting Information.

Circular dichroism: CD spectra were obtained on a Jasco (Tokyo, Japan) J-815 spectropolarimeter. A quartz cell (Hellma) of 0.1 mm path length was used. The values are expressed in terms of $[\theta]_M$, molar ellipticity ($deg\,cm^2\,dmol^{-1}$). Spectrograde MeOH, CH_3CN and TFE (Sigma Aldrich) were used as solvents.

NMR spectroscopy: NMR experiments were carried out on a Bruker 500 MHz spectrometer. The peptide concentration was 5 mM in CD_3OH. The alcohol OH signal was suppressed by presaturation during the relaxation delay or applying a WATERGATE gradient program. All homonuclear spectra were acquired by collecting 256 experiments, each one consisting of 32 scans and 2 K data points. The spin systems of protein amino acid residues were identified by using standard DQF-COSY and TOCSY spectra (spin-lock pulse: 70 ms).[49] The stereospecific assignment of the Aib residues methyl groups was achieved through the HMQC spectra (256 experiments of 200 scans each, spectral width in F1 is 200 ppm, centred at 95 ppm).[50] HMBC experiments[51] were performed by using a long-range coupling constant of 7.5 Hz, a spectral width in F1 of 220 ppm centred at 100 ppm, 128 t_1 experiments of 500 scans, and 1 K points in F2. ROESY experiments were used for sequence-specific assignment. After analysis of the build-up curves, a mixing time of 240 ms was employed to acquire the ROESY experiments. The splitting patterns (see the Supporting Information) are abbreviated as follows: (s) singlet, (d) doublet, (t) triplet, (m) multiplet.

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