Left-handed helical preference in an achiral peptide chain is induced by an L-amino acid in an N-terminal Type II β-turn

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ABSTRACT: Oligomers of the achiral amino acid Aib adopt helical conformations in which the screw-sense may be controlled by a single N-terminal residue. Using crystallographic and NMR techniques, we show that the left- or right-handed sense of helical induction arises from the nature of the β-turn at the N terminus: the tertiary amino acid L-Val induces a left handed Type II β-turn in both the solid state and in solution, while the corresponding quaternary amino acid L-α-methylvaline induces a right handed Type III β-turn.

Introduction

Oligomers of the achiral quaternary α-amino acid Aib (2-aminoisobutyric acid) adopt helical conformations in which each monomer is hydrogen bonded to the monomer three positions further along the chain (Figure 1). The stability of this conformation, known as a 310-helix, over the more typical peptide α-helix is a result of the ‘conformational Thorpe-Ingold effect’: the geminally dimethylated quaternary carbon atom of the monomer favours more tightly twisted chain conformations.
Figure 1: The right handed 3₁₀ helical conformation of an Aib₉ oligomer capped by an N-terminal Cbz-L-α-methylvaline [L-(αMe)Val] residue.¹⁰

Because Aib is achiral, 3₁₀-helices containing only Aib display no preference for left- or right-handed screw-sense, and indeed Aib oligomers undergo conformational inversion on a time scale of milliseconds or less.⁷⁻⁹ However, we,¹⁰ and others,¹¹ have shown that this dynamic equilibrium may be biased in favour of either the left or right handed screw-sense by incorporating a chiral residue at the chain terminus,¹⁰ or by non-covalent interaction with chiral ligands.¹¹ We developed a simple NMR method to report on the degree of control obtained:¹² for example, an N-terminal Cbz-L-valine induces a 65:35 screw-sense preference while an N-terminal Cbz-L-α-methylvaline [L-(αMe)Val] induces a 76:24 screw-sense preference in the oligomer shown in Figure 1.

During this work it was evident that the preferred screw-sense induced in the helix depended not only on the configuration of the chiral N-terminal residue, but also on whether it was tertiary (as in the ‘normal’ proteinogenic amino acids L-Phe and L-Val) or quaternary (for example, L-α-methylvaline or L-isovaline [L-Iva]).¹³ L-Val and L-Phe induce left handed helicity in an oligo-Aib helix, while L-(αMe)Val or L-Iva induce right-handed helicity. This seemed all the more remarkable, given that peptide helices made of Cα-trisubstituted L-amino acids are typically right handed.¹⁴,¹⁵

The hydrogen-bonding pattern in a 3₁₀-helix matches that in a peptide β-turn. According to the definition of Venkatachalam,¹⁶ these are regions involving four consecutive amino acid residues where the polypeptide chain folds back on itself by nearly 180°, classified as type I, II, and III (Figure 2). Specifically, they differ in the conformation of the chain held by a hydrogen bond between C=O of residue i and NH of residue i + 3, which is de-
scribed by $\phi$, $\psi$ torsion angles of $(i + 1)$ and $(i + 2)$ residues of the turn (Table 1). An extended $3_{10}$-helix is in essence a stack of type III $\beta$-turns (Figure 2).

Table 1. Torsion angle values for three major types of $\beta$-turn

<table>
<thead>
<tr>
<th>$\beta$-turn</th>
<th>$\phi$ $(i + 1)$</th>
<th>$\psi$ $(i + 1)$</th>
<th>$\phi$ $(i + 2)$</th>
<th>$\psi$ $(i + 2)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type I</td>
<td>-60°</td>
<td>-30°</td>
<td>-90°</td>
<td>0°</td>
</tr>
<tr>
<td>Type II</td>
<td>-60°</td>
<td>+120°</td>
<td>+80°</td>
<td>0°</td>
</tr>
<tr>
<td>Type III</td>
<td>-60°</td>
<td>-30°</td>
<td>-60°</td>
<td>-30°</td>
</tr>
</tbody>
</table>

Of these three conformations, it is worth noting that the sense of twist in type II and type III $\beta$-turns is opposite because two torsion angles are of opposite sign. It is reasonable to infer that the opposite screw sense induced by tertiary and quaternary amino acids is due to their differing preferences for type II vs. type III $\beta$-turn conformations,\textsuperscript{17} and that this difference leads to opposite senses of twist that are propagated into the remainder of the oligo-Aib helix through the hydrogen-bonding network.\textsuperscript{2,17,18}

Figure 2. The three ideal types of $\beta$-turns.
In this paper we present the experimental evidence that this is indeed the case. Studies were carried out on four helical peptides 1-4.

Peptide 1: Ac-L-Val-Aib₄-Gly-NH₂

Peptide 2: Ac-L-(αMe)Val-Aib₄-Gly-NH₂

Peptide 3: Ac-L-Val-Aib₄-Ot-Bu

Peptide 4: Ac-L-(αMe)Val-Aib₄-OH

**Results**

1. *Conformational analysis in the crystalline state*

The first experimental evidence for the correlation between peptide helix screw sense and different types of turns at their N-termini was obtained by means of X-ray diffraction analysis. Peptides 1 and 2 did not crystallize under all conditions we tried, but the closely related peptides Ac-L-Val-Aib₄-Ot-Bu 3 and Ac-L-(αMe)Val-Aib₄-OH 4, which differ from 1 and 2 only in the C-terminal residue, have crystals suitable for single crystal X-ray diffraction studies. Their X-ray crystal structures are shown in Figures 3 and 4. Crystal data and structure refinements are reported in Table S1. Backbone (φ, ψ) torsion angles and intramolecular H-bond distances are reported in Table 2.
Figure 3. X-ray crystal structure of Ac-L-Val-Aib$_4$-Ot-Bu 3.

Figure 4. X-ray crystal structure of Ac-L-(αMe)Val-Aib$_4$-OH 4.
Table 2. Structural parameters for Ac-L-ValAib₄Or-Bu (3) and Ac-L-(αMe)ValAib₄OH (4).

<table>
<thead>
<tr>
<th>residue</th>
<th>3</th>
<th>4</th>
<th>i – 1 C=O to i + 2 H-N H-bonding distance (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>φ (°)</td>
<td>ψ (°)</td>
<td>φ (°)</td>
</tr>
<tr>
<td>Val¹ or (αMe)Val¹</td>
<td>-69.0</td>
<td>166.4</td>
<td>-50.0</td>
</tr>
<tr>
<td>Aib²</td>
<td>57.3</td>
<td>27.6</td>
<td>-51.6</td>
</tr>
<tr>
<td>Aib³</td>
<td>46.3</td>
<td>43.8</td>
<td>-54.8</td>
</tr>
<tr>
<td>Aib⁴</td>
<td>65.1</td>
<td>19.9</td>
<td>-50.7</td>
</tr>
<tr>
<td>Aib⁵</td>
<td>-33.9</td>
<td>-179.6</td>
<td>-48.5</td>
</tr>
</tbody>
</table>

\(^{a}\)hydrogen bond detected between residue Aib³(C=O) and Aib⁷(COOH).

Inspection of (φ, ψ) backbone torsion angles in Table 2 reveals that they belong to the 3₁₀/α-region of the Ramachandran plot\(^{19,20}\) and confirms the strong folding propensities of Aib-rich short peptides. Both compounds form mainly 3₁₀-helical structures in the body of their (Aib)\(_n\) chains, stabilized by consecutive intramolecular 1←4 C=O...H-N bonds (type III β-turns).\(^{16}\) The mean backbone (φ, ψ) values of peptide 3 and 4 are (56°, 30°) and (−51°,−37°), respectively, very close to the published average values (−57°,−30°).

Interestingly, the (φ, ψ) values in the two peptides are of similar magnitude but of opposite sign, reflecting the reversed screw sense observed in the two structures (apart from φ of Val¹ in peptide 3 which retains a negative value as expected for L amino acids\(^{31}\)). In particular, sign inversion takes place at ψ of Val¹ of peptide 3 (see Table 2) which corresponds to position i + 2 of the first helix β-turn.\(^{16}\) This fact accounts for the presence of a dis-
torted β-turn of type II at the peptide N-terminus, which propagates a left-handed screw sense through the rest of the Aib chain.

When L-(αMe)Val is at peptide N-terminus (peptide 4) the structure folds into a well-developed series of type III β-turns, maintained until the last, C-terminal residue where a hydrogen bond is detected between COOH of Aib1 and C=O of Aib3. The C-terminal Aib4 residue of peptide 3, on the other hand, shows a reversal of the helix screw sense (negative φ, ψ values) resulting from the presence of the C-terminal ester (and unrelated to the N-terminal structure). This conformational feature relieves the unfavourable O····O interaction taking place between the C=O of Aib3 and either oxygen atom of the ester functionality of C-terminal Aib5 residue, by rotating its φ torsion by 180°.22

2. Conformational analysis in solution

We have previously used circular dichroism (CD) to show that the principal conformations of peptides 1 and 2 in methanol are helices with opposite screw-senses, and time-dependent DFT calculations allowed us to correlate the CD spectrum of 1 with a left-handed helix and the CD spectrum of 2 with a right-handed helix. However, CD did not reveal sufficiently detailed information for us to draw conclusions about the structure of the N-termini of peptides 1 and 2 in solution. We therefore carried out a detailed investigation using NMR spectroscopy. Experiments were carried out in CD3OH in order to preserve amide NH proton signals, allowing their identification, and to allow us to compare our findings with those previously obtained by CD under the same experimental conditions.

To detect the proposed N-terminal type-II β-turn in peptide 1, two independent NMR methods were used: firstly, inter-proton distances estimated using nOe spectroscopy were compared with those found both in calculated structures and in the N-terminal segment of the X-ray crystal structure of the related peptide 3, and secondly experimental coupling constants were used to calculate dihedral angles using the Karplus equation.
Overall assignment of proton and carbon signals for both peptides 1 and 2 was achieved using the standard procedure described by Wüthrich25 (Tables S2 and S3, Supporting Information). The spin systems of the Val and Gly residues were identified using DQF-COSY and TOCSY spectra,26,27 while HMQC28 and HMBC29 experiments were used to assign the Aib and (αMe)Val residues.

The $^{13}$C atoms of the two methyl groups (indicated in Table S1 and S2 as $\beta$ and $\beta'$) of each Aib residue were found to resonate at different chemical shifts, above and below 25 ppm. Jung et al.30 have shown that the anisochronicity of the $^{13}$C NMR signals of a pair of diastereotopic methyl groups may be used as a marker of helix stability in solution. Typically, in the absence of higher order structures, the $^{13}$C NMR signals of a gem-dimethyl group, even directly adjacent to a stereogenic centre, are separated by less than 0.5 ppm. However, within a stable helical conformation, the anisochronicity (also known as ‘chemical non-equivalence’ or CNE30) of the $^{13}$C NMR signals of a gem-dimethyl group may exceed 2 ppm. Tables S4 and S5 (see Supporting Information) report the anisochroncities in the $^{13}$C NMR spectrum of the methyl groups of the four Aib residues of peptides 1 and 2. Despite the short length of both peptides, all anisochroncities were found to be around 2 ppm, confirming the adoption of a stable helical conformation in MeOH solution. Stereospecific assignment of the two diastereotopic methyl groups in each Aib residue was achieved by means of HMQC spectra. As reported in the literature, each Aib residue of a peptide chain shows two distinct $^{13}$C chemical shifts, one above and one below 25ppm. This general behaviour of Aib residues in polypeptides is a consequence of the different shielding tensors experienced by the pro-R (located in the position of the $^6$H in a trisubstituted residue) and pro-S methyl groups.31,32

The sequential assignments for peptides 1 and 2 were obtained from HMBC and NOESY spectra.27,33 To avoid spin diffusion, the build-up curve of NOE cross-peak intensities was first determined as a function of mixing time ($\tau_m = 50$ to 500 ms). The optimal mixing time $\tau_m$, balancing cross-peak intensity against relayed magnetization transfer, was found to be 280 ms for both the peptides. Relevant regions of the NOESY spectrum of peptide
are shown in Figures 5, 6 and 7, whilst the amide region of the NOESY spectrum of peptide 2 is reported in Figure 8.

Figure 5. Amide region of the NOESY spectrum (500 MHz, $\tau_m = 280$ ms) of peptide 1 (15 mM in MeOH-$d_3$, 298 K). *: sequential cross-peak overlapped by diagonal peaks. Gly6 HN21 and HN22: C-terminal amide protons.
Figure 6. Fingerprint region of the NOESY spectrum (500 MHz, $\tau_m = 280$ ms) of peptide 1 (15 mM in MeOH-$d_3$; 298 K). Medium-range ($i \rightarrow i + 2$) interaction is marked in colour. Gly6 HN21: C-terminal amide proton.
Figure 7. Region of the NOESY spectrum (500 MHz, $\tau_m = 280$ ms) of peptide 1 (15 mM in MeOH-$d_3$; 298 K). Medium-range ($i \rightarrow i + 2$), diagnostic of the presence of a $3_{10}$-helical structure, and ($i \rightarrow i + 3$) interactions are marked in blue and red, respectively. Gly6 HN21 and HN22: C-terminal amide protons.
Figure 8. Amide region of the NOESY spectrum (500 MHz, $\tau_m = 280$ ms) of peptide 2 (15 mM in MeOH-$d_3$; 308 K). Gly6 HN21: C-terminal amide proton.

In the portions of the NOESY spectrum of peptide 1 shown in Figures 5 and 6, a strong cross-peak can be detected between Val$^1$C$\alpha$H and Aib$^2$NH, while the intra-residue Val$^1$C$\alpha$H–NH correlation peak has a much lower intensity and the Val$^1$NH–Aib$^2$NH cross-peak is hardly visible. This fact alone immediately suggests the presence of a type II $\beta$-turn at the peptide N-terminus, this being the only possible conformation where the Val$^1$C$\alpha$H and Aib$^2$NH protons are in close spatial proximity.$^{17}$ All other expected diagnostic C$^{\alpha(\beta)}$H($i$)–NH($i+3$), C$^{\alpha(\beta)}$H($i$)–NH($i+2$) and sequential NH–NH cross peaks are visible (apart from those obscured by diagonal
peaks) and confirm the presence of a mainly $3_{10}$-helical structure throughout the rest of the sequence. Thus the presence of a different type of turn at the peptide N-terminus does not appear to affect the conformation of the rest of chain.

All the non-overlapping cross-peaks detected in the NOESY spectrum of peptide 1 were integrated, and the values used to estimate inter-proton distances as described in the Experimental section. The most relevant NMR-derived distances are reported (with their standard deviations) in Table 3, along with the corresponding values obtained from the calculated model and the N-terminal portion of the X-ray crystal structure of peptide 3. There is a good fit between the three sets of distances, justifying our interpretation of the NOE cross peaks associated with the Val$^1$ residue. In particular, all the distances involving the Ac-Val$^1$-Aib$^2$ sequence are consistent with the presence of a type II β-turn and exclude the possibility of a type III β-turn.
Table 3. Distances (Å) obtained by 2D-NMR, X-ray and calculated model structures for peptide 1

<table>
<thead>
<tr>
<th>Distances</th>
<th>2D-NMR</th>
<th>X-ray</th>
<th>Calculation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Val$^1$ H$^a$ – HN</td>
<td>2.9 ± 0.3</td>
<td>2.80</td>
<td>2.87</td>
</tr>
<tr>
<td>Val$^1$ H$^a$ – Aib$^2$ HN</td>
<td>2.2 ± 0.2</td>
<td>2.39</td>
<td>2.13</td>
</tr>
<tr>
<td>Val$^1$ H$^a$ – Aib$^3$ HN$^b$</td>
<td>3.6 ± 0.4</td>
<td>3.59</td>
<td>3.77</td>
</tr>
<tr>
<td>Val$^1$ H$^a$ – Aib$^4$ HN$^b$</td>
<td>-$^a$</td>
<td>4.74</td>
<td>5.07</td>
</tr>
<tr>
<td>Val$^1$ H$^a$ – Aib$^5$ HN$^b$</td>
<td>-$^a$</td>
<td>-</td>
<td>7.24</td>
</tr>
<tr>
<td>Val$^1$ H$^a$ – Aib$^6$ βH$^b$</td>
<td>-$^a$</td>
<td>6.12</td>
<td>6.64</td>
</tr>
<tr>
<td>Val$^1$ Ac – Aib$^2$ HN</td>
<td>-$^a$</td>
<td>6.01</td>
<td>5.14</td>
</tr>
<tr>
<td>Val$^1$ Ac – Aib$^3$ HN$^b$</td>
<td>-$^a$</td>
<td>5.45</td>
<td>4.08</td>
</tr>
<tr>
<td>Val$^1$ Ac – Aib$^4$ HN$^b$</td>
<td>-$^a$</td>
<td>4.58</td>
<td>4.96</td>
</tr>
<tr>
<td>Val$^1$ HN - Aib$^2$ HN</td>
<td>4.4 ± 0.4</td>
<td>4.64</td>
<td>4.46</td>
</tr>
<tr>
<td>Aib$^2$ HN – Aib$^3$ HN</td>
<td>2.9 ± 0.3</td>
<td>2.91</td>
<td>2.72</td>
</tr>
<tr>
<td>Aib$^3$ HN – Aib$^4$ HN</td>
<td>2.8 ± 0.3</td>
<td>2.83</td>
<td>2.79</td>
</tr>
<tr>
<td>Aib$^5$ HN – Gly$^6$ HN</td>
<td>2.8 ± 0.3</td>
<td>-</td>
<td>2.76</td>
</tr>
<tr>
<td>Gly$^6$ HN – CONH$_2$</td>
<td>2.8 ± 0.3</td>
<td>-</td>
<td>2.62</td>
</tr>
</tbody>
</table>

$^a$not detected in the NOESY spectrum; $^b$correlations that, if present, account for the presence of a helical conformation.
The existence of a type II β-turn at the N-terminus of peptide 1 was further supported by analysis of the dihedral angles obtained from coupling constants (Table 4). $^1J_{\text{C}	ext{a}	ext{H}a}$ and $^3J_{\text{N}	ext{H}a}$ of Val$^1$ were measured using non-decoupled HMQC$^{29}$ and phase-sensitive DQF-COSY.$^{34}$ Values of 141.1 Hz for $^1J_{\text{C}	ext{a}	ext{H}a}$ and 5.4 Hz for $^3J_{\text{N}	ext{H}a}$ were found. Both values deviate from those in residues embedded into a helical structure and are closer to those expected for either a random coil or a polyproline-type II structure.$^{35,36}$

Table 4. Comparison between observed and characteristic coupling constants in different protein secondary structures$^{36}$

<table>
<thead>
<tr>
<th>constants</th>
<th>found</th>
<th>α-helical</th>
<th>random coil</th>
<th>poly-(Pro) II</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^3J_{\text{HN}a}$</td>
<td>5.4</td>
<td>4.8</td>
<td>7.5</td>
<td>6.5</td>
</tr>
<tr>
<td>$^1J_{\text{C}	ext{a}	ext{H}a}$</td>
<td>141.1</td>
<td>146.3</td>
<td>141.5</td>
<td>142.6</td>
</tr>
</tbody>
</table>

The $^3J_{\text{N}	ext{H}a}$ coupling constant is related to the torsion angle $\phi$ by the Karplus equation (1):$^{37}$

$$^3J_{\text{N}H\alpha} = 6.4 \cos^2 \theta - 1.4 \cos \theta + 1.9 \text{ (where } \theta = |\phi - 60^\circ|)$$ (1)

For $J = 5.4$ Hz, and taking into account the allowed ($\phi, \psi$) torsion angle regions$^{38}$ of the Ramachandran plot for L-Val, the dihedral angle $\phi$ was calculated to be $-70^\circ$. Consequently, this value was used to determine $\psi$ dihedral angles by means of equation (2):$^{35}$

$$^1J_{\text{C}	ext{a}	ext{H}a} = 140.3 + 1.4 \sin (\psi + 138^\circ) - 4.1 \cos 2 (\psi + 138^\circ) + 2.0 \cos 2 (\phi + 30^\circ)$$ (2)
The calculated $\psi$ torsion angles falling into the allowed regions\textsuperscript{38} of the Ramachandran plot for L-Val are $\psi = 106^\circ$ and $\psi = 160^\circ$, both positive values. This observation explains why the N-terminal $\beta$-turn gives rise to a left-handed screw-sense in peptide 1. Furthermore, as shown in Figure 9, the angles $(\phi, \psi = -69.0^\circ, +166.4^\circ)$ observed at Val\textsuperscript{1} in the X-ray structure of peptide 3 fit nicely with one of the two possible pairs of $(\phi, \psi)$ values obtained by combining equations (1) and (2), specifically $(\phi, \psi) = (-70^\circ, +160^\circ)$. This finding further proves that the N-terminal segment of peptide 1 is folded into a type II $\beta$-turn.

Figure 9. First turn $(\phi, \psi)$ torsion angles as observed in the x-ray crystal structure of peptide 3 (the rest of the molecule is omitted for clarity).

As regards peptide 2, the analysis was complicated by extensive overlapping in both the NH–NH and in the $^6$CH$_3$–NH proton correlation regions (Figure 8). To overcome this problem a range of temperatures was screened. The variation of the NMR spectrum with temperature is consistent with the presence of highly populated $3_{10}$-helical structures. Indeed, as shown in Figure S1 and Table S6 (Supporting Information), the temperature dependence of NH chemical shifts in CD$_3$OH solution groups the NH protons into two distinct types: the two N-terminal NH protons exhibit significantly larger temperature coefficients (9 ppb/$^\circ$C) than all other NH
protons (between 4 and 3 ppb/°C).\textsuperscript{39,40} Finally, the behaviour of the two C-terminal amide protons suggests that one is intramolecularly hydrogen-bonded while the other is not.

Some of the diagnostic peaks indicating a 3\textsubscript{10}-helical conformation were visible in the NOESY spectrum of peptide 2, although signal overlapping prevented us performing an in-depth analysis of the 2D NMR data, as done for peptide 1. Furthermore, the strong helicogenic propensities of L-(\(\alpha\)Me)Val are well known.\textsuperscript{41,42} When embedded into a peptide sequence, this residue folds preferentially into right-handed 3\textsubscript{10}-helices, as seen in the X-ray crystal structure of peptide 4 (Figure 4). It is safe to conclude therefore that peptide 2 adopts a well developed, right-handed 3\textsubscript{10}-helical conformation in solution similar to the one observed in the solid state.

**Discussion**

This work indicates that the role of proteinogenic, tertiary amino acids (such as valine) in controlling the handedness of Aib helices is dependent on their position in the sequence. L-Val in a non-terminal position induces right-handed helicity in an Aib tetramer,\textsuperscript{23} and L-amino acids in general participate in right-handed helical structures.\textsuperscript{14} Related studies have been carried out on achiral peptides containing dehydroamino acids.\textsuperscript{17,43,44} Furthermore, amino acids possessing a \(\beta\)-branched, hydrophobic side chain (such as Val or Ile) are known to destabilize helical structures,\textsuperscript{45,46} while favouring \(\beta\)-sheet formation.\textsuperscript{47,48}

The effect on the N-terminal turn of the relatively small difference between the L-Val and L-(\(\alpha\)Me)Val residue can be analysed by viewing the first turn of each helix in the form of a Newman projection (Figure 10). A type II turn with a tertiary L-amino acid at position \(i + 1\) allows the amino acid side chain (an isopropyl group in the case of L-Val) to adopt an unhindered position gauche to the carbonyl group of the same residue (Figure 10a). The acylated amino group is poised to hydrogen bond with the NH group of residue \(i + 3\) without incurring unfavourable eclipsing interactions, since only the \(C^\alpha\)–H bond need eclipse the chain (specifically, the C–N bond of the amide linkage between \(i + 1\) and \(i + 2\)). This hydrogen bond leads to a left-handed (\(M\)) helix.
On exchanging the L-tertiary amino acid for an L-quaternary amino acid, the C$^\alpha$–H bond must be replaced by a C–C bond, resulting in an eclipsing interaction. The result is a destabilisation of the type II $\beta$-turn, and the adoption instead of a type III $\beta$-turn (Figure 10b). In this structure, one of the two C$^\alpha$ substituents (preferably the more hindered group—isopropyl in the case of L-(\(\alpha\)Me)Val) is perpendicular to the C=O group, while the other finds itself gauche to the C=O group. Hydrogen bonding between the C=O of the acylated amino group and the NH of residue $i + 3$ again continues a helix, but this time a right handed ($P$) one.

These conformations represent only the most stable of those possible: we know that the left or right handed screw-sense preference induced by both L-Val and L-(\(\alpha\)Me)Val is only of the order of 3:1, so other conformations must also be adopted to a lesser extent, populating the less favourable screw-sense. For L-(\(\alpha\)Me)Val, these might conceivably include a Type III' $\beta$-turn, i.e. a type III structure with a left-handed twist in which the Me and i-Pr groups have changed places. However it seems unlikely that an N-terminal L-Val will adopt a Type II' $\beta$-turn, in which there would be a severe eclipsing interaction. Therefore it may be that the type III $\beta$-turn is also populated by the L-Val-capped peptide 1 to a lesser extent.$^{59}$
Figure 10: Newman projections of the N-terminal turns of (a) L-Val and (b) L-(aMe)Val capped helices. For acetyl, X= Me

Analogous behaviour by other N-terminal tertiary amino acids in the solution phase is supported by both our conformational analysis of 1 and 3 and CD spectra of similar Aib-rich peptides. Furthermore, this conformational feature arises regardless of the identity of the N-terminal amino acid side chain or protecting group, as shown by the common screw-sense also observed in phenylalanine and alanine capped peptides. It seems reasonable therefore to conclude that the left-handed screw sense (M helicity) exhibited by these Aib-rich peptides is the result of an N-terminal type II β-turn conformation.
Conclusion

Previous work\textsuperscript{13} had shown, by using circular dichroism spectroscopy and time-dependent DFT calculations, that L-valine induces a left-handed screw-sense when attached to the N-terminus of an Aib oligomer, while its quaternary analogue L-\(\alpha\)-methylvaline induces a right handed screw sense. X-ray crystallography of two representative Aib oligomers suggests that the origin of the difference lies in the contrasting \(\beta\)-turn conformations adopted at the N-terminus of the helix. The detailed NMR investigations presented here support this view, and we conclude that in solution tertiary L-amino acids (of which L-valine is representative) at the N-terminus of an Aib oligomer induce left handed helicity by promotion of a Type II \(\beta\)-turn, while quaternary L-amino acids (of which L-\(\alpha\)-methylvaline is representative) conversely induce right handed helicity by promotion of a Type III \(\beta\)-turn.

Experimental section

Synthesis. The synthesis of peptides 1 and 2 is published.\textsuperscript{13}

\textit{Ac-Val-Aib\textsubscript{4}-Ot-Bu}. Cbz-L-Val-Aib\textsubscript{4}-Ot-Bu\textsuperscript{13,23} (687 mg, 1.06 mmol) was dissolved in 5 mL of acetic anhydride under a N\textsubscript{2} atmosphere. Pd/C (10\%, 70 mg) was carefully added and the reaction stirred at r.t. under a H\textsubscript{2} atmosphere until TLC indicated complete consumption of the starting material (overnight). The mixture was filtered through a pad of Celite\textsuperscript{®} and the filtrate concentrated under reduced pressure. Toluene (3mL) was added and the mixture concentrated again to remove residual Ac\textsubscript{2}O azeotropically. This process was repeated four times. No further purification was required. White solid. Yield: 573 mg, 97\%. \textit{Rf}: 0.45 (CH\textsubscript{2}Cl\textsubscript{2}/MeOH, 95:5); \textit{mp} = 235-237\textdegree C; \textit{[\(\alpha\)]\textsubscript{D}}\textsuperscript{25} = -26.8 (c = 1, MeOH); \textit{IR} (ATR, cm\textsuperscript{-1}) 3311, 1652, 1532, 1147; \textit{\textsuperscript{1}H-NMR} (300 MHz, CDCl\textsubscript{3}) \textit{\delta}H 7.30 (s, 1H), 7.13 (s, 1H), 6.92 (s, 1H), 6.43 (s, 1H), 6.22 (d, \(J = 5.4\) Hz, 1H), 3.69 (dd, \(J = 7.0, 5.6\) Hz, 1H), 2.07 (m, \(J = 7.0\) Hz, 1H), 2.05 (s, 3H), 1.47-1.38 (m, 33H), 1.03 (d, \(J = 7.0\) Hz, 3H), 1.00 (d, \(J = 7.0\) Hz, 1H).
Hz, 3H); 13C-NMR (75 MHz, CDCl3) δc 174.3, 173.9, 173.5, 172.1, 171.8, 80, 61, 57.3, 57.2, 56.9, 56.3, 28.1, 26.2, 25.2, 25.0-24.4 (overlapping signals), 23.2, 19.4, 19.3; HRMS (TOF ES+, MeOH) calcd. for C27H50N5O7 [M+1]+ 556.3702, found 556.3705.

Ac-(aMe)Val-Aib4OH 4. The t-buty] ester of 4 was prepared as described for peptide 3 starting from Cbz-L-(aMe)Val-Aib4-OtBu.13 Ac-(aMe)Val-Aib4-OtBu was obtained as a white solid. Yield: 460 mg, 98%. Rf: 0.55 (CH2Cl2/MeOH, 95:5); mp = 212-214°C; [α]D 25 = +16.0 (c = 1, MeOH); IR (ATR, cm⁻¹) 3298, 1667, 1643, 1538, 1148; 1H-NMR (400 MHz, CDCl3) δh 7.51 (s, 1H), 7.35 (s, 1H), 7.31 (s, 1H), 6.32 (s, 1H), 6.12 (s, 1H), 2.04 (s, 3H), 2.03 (m, J = 5.5 Hz, 1H), 1.47-1.39 (m, 36H), 0.97 (d, J = 5.0 Hz, 3H), 0.91 (d, J = 5.0 Hz, 3H); 13C-NMR (100 MHz, CDCl3) δc 174.1, 174.0, 173.9, 173.8, 172.4, 171.2, 79.8, 62.9, 57.1, 56.9, 56.7, 56.1, 35.1, 27.9, 26.5, 26.3, 26.1, 25.2, 24.5-23.9, 17.2, 17.0; MS (ES+, MeOH) m/z = ([M+Na]+, 100%); HRMS (TOF ES+, MeOH) calcd. for C28H52N5O7 [M+1]⁺ = 570.3870, found 570.3862.

To a stirred solution of Ac-(aMe)Val-Aib4-OtBu (400 mg, 0.70 mmol) in distilled CH2Cl2 (3 mL) was added trifluoroacetic acid (3 mL) dropwise. The course of the reaction was followed by TLC. After reaction completion (4 hs) the mixture was concentrated in vacuo. Et2O was added to the residue (10 mL) and the solvent evaporated. This process was repeated 5 times. No further purification was required. Compound 4 was obtained as a white solid. Yield: 342 mg, 95%. Rf: 0.35 (CH2Cl2/MeOH, 95:5); mp = 273-275°C; [α]D 25 = +19.2; IR (ATR, cm⁻¹) 3273, 3353, 1651, 1644, 1527, 1170; 1H-NMR (500 MHz, CD3OD) δh 7.94 (brs, 1H), 7.93 (brs, 1H), 7.85 (brs, 1H), 2.04 (s, 3H), 2.02 (m, J = 7.0 Hz, 1H), 1.51-1.38 (m, 27H), 0.97 (d, J = 7.0 Hz, 3H), 0.97 (d, J = 7.0 Hz, 3H); 13C-NMR (100 MHz, CD3OD) δc 178.4, 176.9, 176.8, 174.6, 174.5, 174.3, 62.0, 61.9, 58.1, 58.0, 57.9, 57.2, 30.1, 27.0, 26.9, 26.8, 26.5, 25.9, 24.7-24.13 (overlapping signals), 22.5, 19.7; MS (ES+, MeOH) m/z = ([M+Na]+, 100%); HRMS (TOF ES+, MeOH) calcd. for C24H44N5O7 [M+1]⁺ = 514.3236, found 514.3232.
**X-ray diffraction.** Crystals suitable for X-ray analysis were obtained by slow evaporation from methanol for both peptides 3 and 4. Crystals of peptide 3 are monoclinic, space group P2₁, with unit-cell dimensions a = 9.2540 (14), b = 17.036 (3), c = 10.3534 (16) Å, and β = 94.202 (3) and those of peptide 4 are orthorhombic, with space group P2₁ and unit cell dimensions a = 11.926 (2), b = 8.7132 (17), and c = 13.814 (3) Å, and β = 94.826 (4). The crystal data, refinement details and structural parameters are summarized in Tables 1 and 2. Data collection for both peptide 3 and 4 was carried out on a CCD diffractometer with graphite monochromated Mo Ka radiation, cryocooling to 100 K. The structures were solved by direct methods and refined by full-matrix least squares against F².

In 3, there is a water molecule in the asymmetric unit. All non-H atoms were refined anisotropically. H atoms bonded to C were included in calculated positions; those hydrogen atoms bonded to nitrogen and oxygen were found by difference Fourier methods and refined with Ueq values 1.2 (nitrogen) or 1.5 (oxygen) times that of the parent atom. The crystal data for 3 and 4 have been deposited with the Cambridge Crystallographic Data Centre, deposition numbers 907153 and 907154, respectively.

**Nuclear Magnetic Resonance.** Peptides 1 and 2 were dissolved in CD₃OH (peptide concentrations: 15 mM). All NMR experiments were performed on a Bruker Avance II+ 500 MHz spectrometer using the TOPSPIN 2.1 software package. Suppression of the alcohol OH signal was achieved using the WATERGATE pulse sequence. Phase sensitive DQF-COSY, NOESY, and TOCSY were acquired by collecting 512, 480 and 400 increments, respectively, each one consisting of 16-40 scans and 16K data points. The spin-lock pulse for the TOCSY experiments was 70 ms long. To optimize the digital resolution in the carbon dimension, HMQC experiments were acquired using a spectral width of 30 ppm centered at 22 ppm in F₁. The HMQC experiments were recorded with 200 t₁ increments, of 92 scans and 1K points each. The non-decoupled HMQC experiment used
to measure $^1J_{\text{Cafl}}$ coupling constants was performed using a spectral width in $F_1$ of 150 ppm centered at 80 ppm, 512 $t_1$ experiments of 72 scans, and 16K points in $F_2$. All suitable, non-overlapping cross-peaks of the NOESY spectrum of peptide 1 were integrated using the SPARKY 3.111 software package. Each interproton distance ($d_i$) was obtained from the corresponding cross-peak volume $V_i$ applying the equation: $d_i = [(d_0 \cdot V_0)/V_i]^{1/6}$, where $d_0$ and $V_0$ are the calibration distance and volume. Distance calibration is usually based on the average of the integration values of the cross peaks due to interactions between $\beta$-geminal protons (e.g. $\text{CH}_2$ of Glycines and/or of other amino acid residues) set to a distance of 1.8 Å. However, in peptide 1 the $\beta$-geminal protons of Gly$^6$ are very strongly coupled, and an important scalar contribution to their nOe cross-peaks could not be avoided at any of the mixing times tested. Another established way to calibrate distances when $\beta$-geminal protons are not available is the use of sequential distances [for instance $d(H_i^a, H_{i+1}N)$ or $d(H_iN, H_{i+1}N)$] in regular secondary structural elements.$^{52,53}$ This being the case for the amino acid sequence Aib$^3$-Gly$^7$ of peptide 1, calibration was performed by calculating the average of cross-peak integration values due to interactions between amide protons of the above-mentioned peptide sequence (with the clear exclusion of Aib$^5$N – Aib$^4$HN, completely overlapped by the diagonal peaks, see Figure 5) set to a distance of 2.80 Å. For all of the distances obtained, a standard deviation of ± 10% was estimated.

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Supporting Information

NMR spectra and X-ray data for 3 and 4. Detailed data from NMR experiments on 1 and 2. This material is available free of charge via the Internet at http://pubs.acs.org/.

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L-Val or L-α-methyl-Val induces left-handed helicity via a Type II β-turn.

L-α-methyl Val induces right-handed helicity via a Type III β-turn.