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# N- versus C-Terminal Control over the Screw-Sense Preference of the Configurationally Achiral, Conformationally Helical Peptide Motif $Aib_8GlyAib_8$

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Helical conformations are commonly adopted by both biological and synthetic polymers, and the chirality of the monomers from which they are built generally determines the screw sense of the helix.[1] The amino acid aminoisobutyric acid (Aib), while strongly promoting the adoption of helical peptide conformations,[2] is achiral, and otherwise achiral oligomers containing Aib can be induced to prefer one of the two screw senses by ligation with a single terminal amino acid.[3,4] In this work, we have used a simple NMR method to show that screw-sense control is more effective when applied from the N-terminus rather than from the C-terminus. We also report the crystal structure of nearly six full turns of a peptide $3_{10}$ helix, the longest $3_{10}$ helix ever observed in the crystalline state.

The degree of screw-sense bias at any site in a configurationally achiral but conformationally fluxional helix (a class which includes oligomers of Aib[5]) can be probed using NMR spectroscopy by incorporating into the helix a pair of potentially diastereotopic reporter groups, such as a $CH_2$ unit.[4−6] At slow exchange, the local chiral environment would render the $CH_AH_B$ signals anisochronous regardless of the relative populations of the two helical screw-sense conformers. In a rapidly inverting, configurationally achiral helix (i.e., one built entirely of achiral monomers) the two "reporter" nuclei $H_A$ and $H_B$ can become isochronous (Figure 1a), but only if the population of the two helical screw-sense conformers is equal. When a remote chiral influence X* or Y* succeeds in preferentially inducing one screw sense in the helical oligomer (i.e., $K_{eq} \neq 1$), the symmetry of the local environment of the reporter is broken. Provided that the chiral influence is located sufficiently far away to avoid direct interaction with the reporter, the anisochronicity of the $H_A$ and $H_B$ signals results from a weighted average of two pseudoenantiomeric environments and is directly proportional to the induced level of screw-sense control (Figure 1b).[4,5] Measuring the anisochronicity as a chemical shift difference $\Delta\delta$ (in ppb) allows the relative effectiveness of the screw-sense control supplied by a series of controllers X* or Y* to be quantified.

We previously used such a method to show that the helicity of hybrid Aib−Gly peptides containing up to 20 residues can be controlled from a single N-terminal residue.[4] Here we show that placing a reporter group in the *middle* of a peptide chain allows a direct comparison to be made between the screw-sense control achievable from the N- and C-termini of a configurationally achiral helical peptide motif. We chose as a reporter the methylene group of a single Gly residue flanked on either side by an $Aib_8$ motif. A series of $XaaAib_8GlyAib_8Yaa$ 17−19-mer peptides were constructed, as shown in Scheme 1: the azido-Aib octamer **1**[4,7] was reduced to give **3** and coupled with either CbzPhe (giving **4**) or CbzGly (giving **6**). C-terminal deprotection of the latter gave **7**, which was coupled with a choice of capping C-terminal amino ester or amide Yaa to provide C-terminal-moiety decamers **8**. Ligation of the peptide 9- and 10-mers **6** and **8** with **2** or **5** via their azlactones[7] gave the $Aib_8GlyAib_8$-containing 17−19-mers **9a**−**e**.

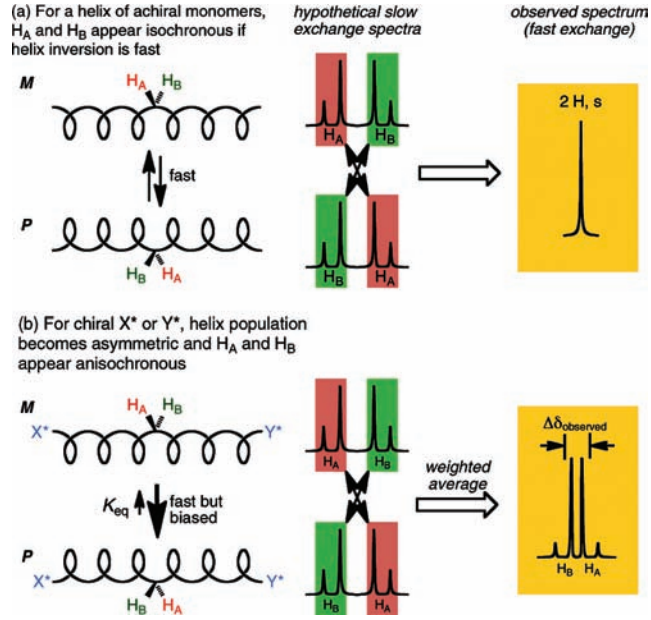
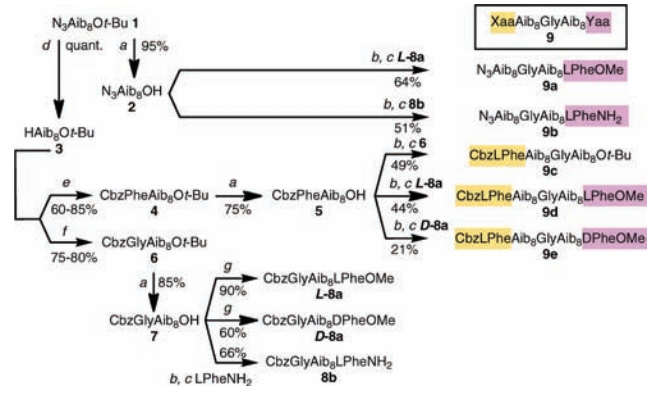



***Figure 1.*** Detection of a helical preference by NMR spectroscopy in the fast-exchange regime.

***Scheme 1.*** Synthesis of the 17−19-mers[a]




[a] Conditions: (*a*) $CF_3CO_2H$; (*b*) $Ac_2O$, 120 °C; (*c*) deprotection of coupling partner (conditions *d*) then MeCN, Δ; (*d*) $H_2Pd/C$, MeOH; (*e*) CbzLPheOH, PyBOP, $iPr_2NEt$, $CH_2Cl_2$, DMF; (*f*) CbzGlyOH, EDC, HOBt, $CH_2Cl_2$, DMF; (*g*) coupling partner given, PyBOP, *i*-$Pr_2NEt$, $CH_2Cl_2$, DMF.

In each case, the chemical shift difference within the diastereotopic Gly $CH_2$ group was determined by $^1H$ NMR spectroscopy at 500 MHz in $CD_3OD$ at 23 °C, and the results are shown in Table 1. In the 9-mers **6** and **7**, the N-terminal Gly residue displays a 2H singlet, as expected, since the *M* and *P* conformers of helical Aib oligomers interconvert rapidly on the NMR time scale at ambient temperature.[6] Incorporation of an N-terminal Cbz-L-Phe residue (in **9c**), however, causes the Gly $CH_2$ to appear as a clear AB system,[4]



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with a peak separation $\Delta\delta = 74$ ppb. We interpret this collapse of local symmetry at the Gly residue as evidence of a thermodynamic bias toward one helical sense, in this case *P* (right-handed).[8] This bias must arise from a local organizational effect of the N-terminal Cbz-L-Phe residue, which, because of the powerful helicogenic properties of the Aib residues, is relayed through the 27 bonds separating it from the Gly residue.

***Table 1.*** Anisochronicity in the Central Gly $CH_2$ of **9a**−**e** and in the N-Terminal Gly $CH_2$ of **8a** and **8b**

| entry | compound | N-terminal residue Xaa | C-terminal residue Yaa | $\Delta\delta$ (ppb) |
|---|---|---|---|---|
| 1 | **9c** | Cbz-L-Phe | −[b] | 74 |
| 2 | **9a** | −[a] | L-PheOMe | 0 |
| 3 | **9b** | − | L-PheNH$_2$ | 47 |
| 4 | **9d** | Cbz-L-Phe | L-PheOMe | 74 |
| 4 | **9e** | Cbz-L-Phe | D-PheOMe | 67 |
| 5 | **8a** | −[c] | L-PheOMe | 35 |
| 6 | **8b** | −[c] | L-PheNH$_2$ | 100 |

[a] N-terminal $N_3$Aib unchanged from **1**. [b] C-terminal AibO*t*-Bu unchanged from **6**. [c] N-terminus is reporter CbzGly.

Incorporating L-PheOMe at the C terminus (in **9a**) led to no detectable anisochronicity in the signals arising from the Gly $CH_2$ group. Since the local environments at the Gly reporters in **9a** and **9c** are essentially identical, this result indicates that an N-terminal chiral amino acid exhibits a much greater propensity to control the screw sense of the helix than the same residue placed at the C-terminus. The similarity of the $\Delta\delta$ values for **9d** and **9e**, which contain potentially matched and mismatched pairs of Phe residues, confirms the relative weakness of screw-sense control from the C terminus: in both cases, the anisochronicity of the central Gly $CH_2$ group was hardly changed from that observed in **9c**. However, replacement of the C-terminal ester of **9a** with the C-terminal amide of **9b** (which can in contrast participate in hydrogen bonding through its NH bond) improved the conformational control. The $\Delta\delta$ value within the Gly $CH_2$ group of **9b** increased to 47 ppm, though still falling short of that seen with the N-terminal controller. Comparison of the anisochronicities $\Delta\delta$ in the diastereotopic N-terminal Gly $CH_2$ groups of **8a** and **8b** also indicates that an amide-capped C-terminal residue provides a greater screw-sense bias than an ester-capped C-terminal residue.

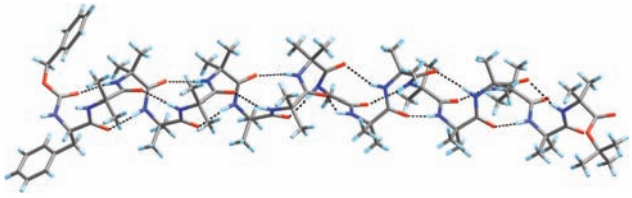

***Figure 2.*** X-ray crystal structure of **9c**: five turns of a $3_{10}$ helix.

Crystals of **9c** were obtained by slow precipitation from acetonitrile.[9] The conformation of **9c** in the crystalline state is shown in Figure 2. It consists of nearly six turns of a continuous $3_{10}$ helix (the longest ever observed crystallographically[7c]) stretching over 17 of the 18 residues of the peptide. Only the C-terminal AibO*t*-Bu residue is excluded from the helix, forming a characteristic "Schellmann motif".[10] The lack of control exerted by a C-terminal ester residue is most likely due to the tendency of C-terminal esters to form such conformationally flexible features.

Tabulation of hydrogen-bond distances and bond angles (see the Supporting Information) indicated that the $3_{10}$ helix (with H bonds between residues *i* and $i + 3$) is distorted toward an α helix (with H bonds between residues *i* and $i + 4$) in the vicinity of the central Gly residue. Values for $\psi$ remain close to the 30° characteristic of a $3_{10}$ helix[11] over most of the peptide but drift up to 40° at residues 7−10, leading to the slight kink evident in Figure 2. However, close H-bonded contacts exist for every C═O and the $i + 3$ N−H except for the three C-terminal carbonyl groups. One unexpected feature of the crystal structure is the fact that the helix is left- and not right-handed;[8] this is presumably a crystal packing effect.[3a,b]

Long-range conformational control is a promising biomimetic strategy for the communication of information on the nanometer scale,[12,13] and screw-sense switching is a plausible method of communication over such distances.[13] We conclude that the potential to use the screw sense of an oligo-Aib helix as a means of communcation is greater if the "input" of information is incorporated at the N-terminus rather than the C-terminus of the helix.

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**Supporting Information Available:** Experimental details and characterization of new compounds, CD spectra, and crystallographic data (CIF). This material is available free of charge via the Internet at http://pubs.acs.org.

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