Chirality Transfer

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Quantifying End-to-End Conformational Communication of Chirality through an Achiral Peptide Chain**

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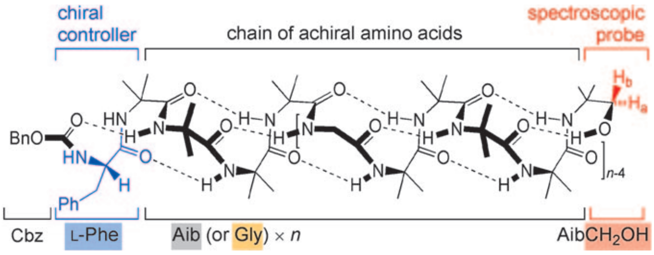
Helicity is a widespread characteristic of the secondary structure of peptides: those built of L-amino acids typically adopt right-handed helical structures, even when most of the chain is achiral.[1] Helical peptide motifs may be stabilized by the incorporation of quaternary amino acids such as Aib (aminoisobutyric acid),[2] and oligomers of Aib adopt racemic helical conformations.[3,4] Favored adoption of a left- or right-handed helix may be controlled by judicious distribution of chiral monomers along any polymer chain.[5] A bias towards one absolute sense of helicity may also arise from a terminal chiral residue bound covalently[6,7] or noncovalently[8] to an otherwise achiral peptide chain. At the limit, absolute helicity (that is, a left- or right-handed helical preference) may be induced in an otherwise configurationally achiral oligomer by a single terminal amino acid. The work of Toniolo et al. (for short Aib$_n$ oligomers)[6] and of Inai et al. (for oligomers of Aib-Δ^ZPhe (Δ^ZPhe = (Z)-didehydrophenylalanine)[7,8] has shown that covalent or noncovalent attachment of a terminal chiral residue leads to a helicity preference[9] in at least part of the peptide structure, detectable by circular dichroism.

What we now establish is how far the asymmetric influence of a terminal chiral residue can persist through a single isolated helical structure—in other words the fidelity with which a helical peptide built of achiral monomers can carry information about a terminal residue over ever increasing distances. Previous studies have used CD to detect helicity in the oligomer as a whole, but no information on asymmetry localized at the helix terminus can be gathered by this method.[10] As a helix of achiral monomers is lengthened, every achiral residue must carry a finite chance of helix inversion, leading to erosion of the asymmetric environment at the terminus of the growing oligomer. Using a simple spectroscopic technique, we have now evaluated the local asymmetry of a pair of geminal "reporter" ^{1}H nuclei at the C terminus of a peptide containing a single N-terminal chiral residue, and hence have quantified both the distance over which oligomers retain a helical preference in solution and the fidelity with which each achiral amino acid transmits a helical preference along the chain.

The method relies on the observation by NMR spectroscopy of anisochronicity between two ^{1}H nuclei which are indistinguishable unless they find themselves in a chiral environment. In a helix built entirely of achiral monomers, and inverting rapidly on the NMR timescale, the two "reporter" nuclei are in fast exchange and must be isochronous.[11] If a remote chiral influence succeeds in inducing preferentially one absolute helicity in the oligomer, the symmetry of the local environment of the nuclei will be broken, rendering the diastereotopic nuclei anisochronous. Provided the chiral controller is located sufficiently far away to avoid direct interaction with the reporter nuclei, the degree of anisochronicity reflects a weighted average of two pseudoenantiomeric environments and therefore reflects the local excess of one absolute helicity over the other. Quantifying the anisochronicity as a chemical-shift difference between these nuclei, and observing its decay with increasing helix length, may thus provide an empirical measure of the distance over which a chiral influence can persist in a peptide.

We chose 1-aminoisobutyric acid (Aib (**1**)) as the achiral monomeric amino acid from which to build the helix since oligomers incorporating Aib are well-known to form 3_{10}-helical structures[2–4] which undergo helix inversion rapidly on the NMR timescale at room temperature.[4] The generalized structure of our targets is shown in Scheme 1: an N-terminal chiral amino acid (in most cases, Cbz-L-Phe[12]) provides a stereochemical influence which we hope will propagate through a helix of achiral amino acids (Aib or glycine). Remote from the chiral controller, a pair of diastereotopic protons provide a spectroscopic probe,[13] reporting on the local asymmetry at the C terminus.



Scheme 1. General structure of the target molecules studied.

The achiral Aib oligopeptides were built up from a C-terminal protected ester **3a** or alcohol **3b** by the iterative method[14] illustrated in Scheme 2: acylation with 1-azidoisobutyryl chloride (AzibCl (**2**)) to give **4a** or **4b** followed by

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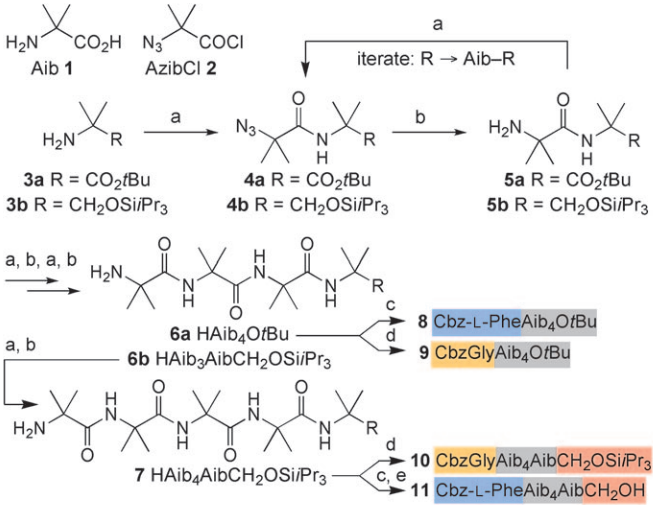

Scheme 2. Oligopeptide synthesis: a) AzibCl (**2**), Et_3N, CH_2Cl_2; b) H_2, Pd/C, MeOH; c) Cbz-L-Phe, PyBOP, *i*Pr_2NEt, CH_2Cl_2; d) CbzGly, EDC, HOBt, CH_2Cl_2; e) HCl, MeOH. PyBOP: benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate; EDC: 3-(3-dimethylaminopropyl)-1-ethylcarbodiimide; HOBt: 1-hydroxybenzotriazole.

hydrogenation of the azide gave the chain-extended dipeptides **5a** or **5b**. Repetition of the procedure two and three times gave achiral tetrapeptides **6a** or **6b** and pentapeptide **7**, respectively. These oligopeptides were capped at the N terminus with Cbz-L-Phe to yield **8** and (after desilylation) **11**, or with CbzGly to yield **9** and **10**.

From these pentapeptide building blocks we constructed polypeptides of the general structure shown at the top of Figure 1. Direct coupling between Aib oligomers was difficult to achieve using standard coupling methods,[14] but the Gly linkers allowed us to build decapeptide **12** (by deprotection and coupling of pentapeptides **8** and **10**), pentadadecapeptide **13** (similarly from **8**+**9**+**10**), and icosapeptide **14** (similarly from **8**+**9**+**9**+**10**) as shown in Figure 1. ^{1}H NMR spectra of these four peptides Cbz-L-PheAib$_4$[GlyAib$_4$]$_n$AibCH$_2$OH ($n=0$–3) were acquired in deuterated methanol, and the form of the CH_2OH AB signal for each is shown in Figure 1.

Each of the peptides **11**–**14** contains a single chiral residue, the terminal L-Phe, with a stereogenic center located respectively 16, 31, 46, and 61 bonds from the C-terminal reporter nuclei. Nonetheless, all displayed ^{1}H NMR spectra which indicated that the diastereotopic reporter group finds itself in a locally chiral environment which is independent of concentration.[15] Lengthening the peptide by five residues decreased the anisochronicity $\Delta\delta$ of the signals by approximately a factor of two, but the AB system persisted even with nineteen achiral amino acids—61 bonds—intervening between the single stereogenic center and the C-terminal diastereotopic pair. Anisochronicity was also evident in the methylene groups of the glycine linkers of **12**, **13**, and **14**.

We interpret the remote effect of the N-terminal Phe residue as being the result of perturbation of the equilibrium between left- and right-handed helices. The observed local asymmetry at the C terminus is the consequence of this thermodynamically controlled imbalance. Several further

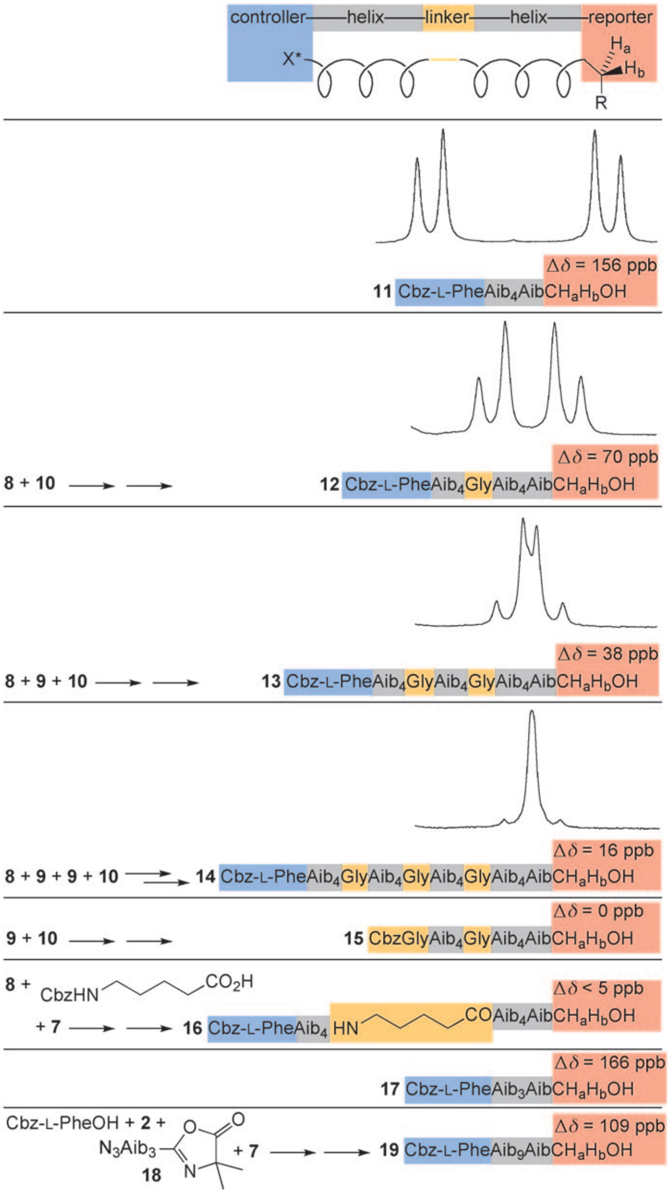

Figure 1. Construction of peptides Cbz-L-PheAib$_4$[GlyAib$_4$]$_n$AibCH$_2$OH ($n=0$–3; **11**–**14**) and controls **15**–**17** (syntheses involve sequential deprotections and couplings: see the Supporting Information), and form of their CH_2OH signals and the chemical-shift separation $\Delta\delta$ of the AB system (^{1}H NMR in CD_3OD, 23 °C).

experiments were carried out to confirm that the anisochronicity arises from induction of chirality through the helix under thermodynamic control rather than by any form of direct inter- or intramolecular interaction between the terminal chiral residue and the reporter group: a) The achiral Gly-terminated decapeptide **15** displayed no anisochronicity in its terminal reporter group (nor its glycine methylene groups) at temperatures between −20 and +20 °C, confirming that helix inversion in peptides constructed of Aib oligomers is fast on the NMR timescale,[16] and that racemic helicity alone cannot give rise to anisochronicity in these systems; b) decapeptide **16**, in which the two Aib$_4$ segments are insulated from one another[17] by a conformationally flexible

5-aminopentanoyl residue, displayed almost (though interestingly not quite) zero anisochronicity ($\Delta\delta < 5$ ppb) in its CH_2OH group; c) the NMR spectrum of (±)-**12** is identical in all respects—including the anisochronicity of the CH_2OH group—with that of (−)-**12**; d) an equimolar mixture of **12** and **15** shows a superimposed singlet and AB system, each identical with that of the pure components. These last three results, along with the concentration independence of $\Delta\delta$ in CD_3OD,[15] indicate that anisochronicity is the result of intramolecular communication only, and not intermolecular interactions.

The observed decay of anisochronicity with increasing chain length presumably results from equalization of the populations of local *M* and *P* helicity at the C terminus as random helix “errors” accumulate in longer chains. The decay is in fact consistent with a simple model in which there is a constant probability that a helix fault (inversion) will occur at any given residue, leading to an exponential erosion of helix preference with chain length. On this model (see the Supporting Information), the average fault rate in the $(GlyAib_4)_n$ sections of **11**–**14** (Figure 2, blue squares) is of the order of 6% per residue (blue line). However, given the difference in helix stability of Gly- and Aib-containing peptides,[2a,b,e] we suspected that the helix faults would be located principally at the Gly residues. We therefore made “all-Aib” tetrapeptide **17** (Cbz-L-PheAib$_3$AibCH$_2$OH; $\Delta\delta$ = 166 ppb) and, by an alternative route employing an azlactone derivative **18** of the azidotetrapeptide precursor to **7**, “all-Aib” decapeptide **19** (Cbz-L-PheAib$_9$AibCH$_2$OH). Anisochronicity $\Delta\delta$ in the reporter CH_2OH group of **19** was quantified as 109 ppb (see Figure 1), significantly greater than that observed in the Gly-containing homologue **12**. From the $\Delta\delta$ values for **17**, **11**, and **19** (Figure 2, red squares) we deduce that the apparent fault rate (probability of helix inversion) at an Aib residue is only 3.5% (Figure 2, orange line). To give correct values for the Gly-containing oligomers, the fault rate at a Gly residue must consequently be of the order of 17.5% (Figure 2, green line).

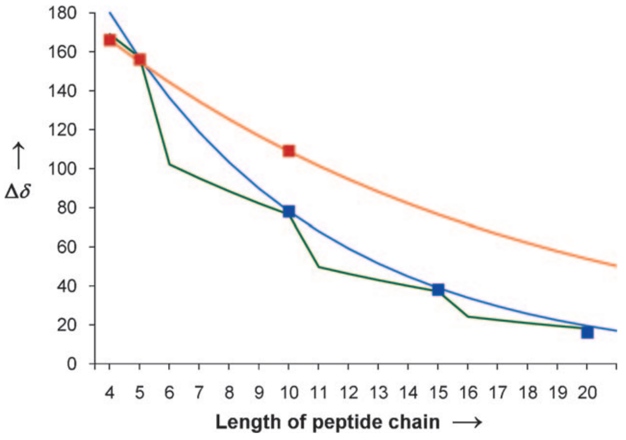

Figure 2. Experimental and modeled anisochronicity in ■ Cbz-L-PheAib$_4$[GlyAib$_4$]$_n$AibCH$_2$OH oligomers (length of peptide chain = 5x + 5) and ■ Cbz-L-PheAib$_n$AibCH$_2$OH oligomers (length of peptide chain = y + 1); modeled signal decay —— (blue line) fault rate of 6.0%; —— (orange line) fault rate of 3.5%; —— (green line) fault rates of 3.5% for Aib monomers and 17.5% for Gly monomers.

Peptides rich in Aib typically form 3_{10} helices, and NMR experiments (see the Supporting Information) allowed us to ascertain that only two of the NH groups of **12** were not involved in intramolecular hydrogen bonds in $CDCl_3$, a result consistent with the formation of a 3_{10} helix (rather than an α helix, in which three NH groups are excluded from intramolecular hydrogen-bonding). CD experiments[18] (see the Supporting Information) indicated a right-handed (*P*) helix. The adoption of a *P*-3_{10}-helical conformation with a helix rise per residue of 1.94 Å[19] by icosapeptide **14** generates a structure of 3.9 nm length, and future work will seek to exploit the nanoscale organizational properties of these, and related, synthetic peptidic structures. Biological systems make extensive use of conformational changes to mediate signal transduction, and we propose to employ helical peptides related to **14** (which is longer than the typical thickness of a cell membrane) to mediate communication on a similar scale in artificial systems.[20]

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[13] A CH_2OH group was chosen as the probe (as opposed, for example, to the diastereotopic methyl groups of the C-terminal Aib (see ref. [4])) since it displayed a well-separated AB system in an uncluttered region of the spectrum. Protected CH_2OR analogues showed poor anisochronicity, perhaps due to freer bond rotation in the absence of a C-terminal hydrogen bond.

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