Conformational Communication

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Nanometer-Range Communication of Stereochemical Information by Reversible Switching of Molecular Helicity**

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Stable helical molecular structures are ubiquitous in both biological and synthetic polymers,[1] and in general the handedness (screw-sense) of a helical polymer is dictated by the chirality of the monomers from which it is built (for example, DNA built from D-deoxyribose is typically right-handed, as are α helices built from L-amino acids).[2] It occurred to us that the switching of a molecular helix between a left- and a right-handed form could constitute a very simple means of communicating information along the axis of the helix over distances commensurate with the helical cooperativity length.[3] Changes in conformation are commonly used by biological systems to communicate information, for example, in receptors or in allosteric proteins.[4] However, simple switching between two helical forms of a typical peptide is not suitable for such a purpose because of the substantial difference in stability between diastereoisomeric helices built from chiral monomers.[2] There are, however, many synthetic oligomers which favor helical structures even though they are constructed from nonchiral subunits.[1,5] We exploited one of these classes of oligomers in this study.

Pfeiffer and Quehl showed in 1931 that a configurational preference could be induced in a stereolabile cation by a chiral anion.[6] A screw-sense preference in a stereolabile helical oligomer or polymer[7] can likewise be induced by coordination to a chiral counterion,[8] or by the incorporation of (even a very weak) chiral influence into the oligomer itself.[3,9] At the limit, the screw sense of a helical oligomer may be induced solely by thermodynamic control from a chiral influence located at the terminus of the helix.[8c,10,11] With a switchable chiral influence, helix inversion has been achieved under the influence of factors such as temperature,[12a] solvent polarity,[12b] irradiation,[12c] and electrochemistry.[12d]

Signal transduction can result from conformational changes in artificial molecular structures as a result of a chemical stimulus;[13] however, the switching of a helix has not previously been coupled with the local detection of helical screw sense at a remote site for the communication of information over multi-nanometer distances. We envisaged a molecule (Figure 1) in which such a helix was attached at one end to a switchable controller—a chiral "input" which would induce a local left- or right-handed preference. The propensity of the molecule to adopt a consistent helical structure

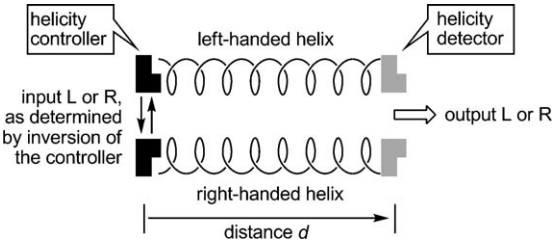
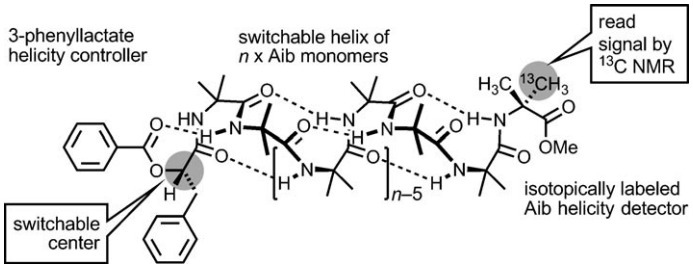


Figure 1. A mechanism for communication on the basis of molecular helicity. L and R correspond to left- and right-handed helicity, respectively.

would cause this local influence to propagate through the helix (provided the rate of inversion of helicity were sufficiently fast); signal transduction in this way would enable the information encoded in the handedness of the controller to be read remotely by a detector of helix chirality located at the helix terminus. Interactions within the helicity detector would result in measurably different outputs from left- and right-handed helices, so that information could be communicated over the distance *d* (Figure 1).

Peptide-like oligomers of the achiral amino acid aminoisobutyric acid (Aib) adopt stable hydrogen-bonded helical structures (usually 3_{10} helices)[14] with a low barrier to inversion between enantiomeric left- and right-handed forms.[15] Since we also knew that Aib mediates the propagation of a given helical preference with a high degree of fidelity,[11] we chose to use Aib oligomers of the general structure shown in Scheme 1 to mediate the information



Scheme 1. A switchable helix of Aib monomers, capped by a 3-phenyllactate controller and an isotopically labeled Aib detector.

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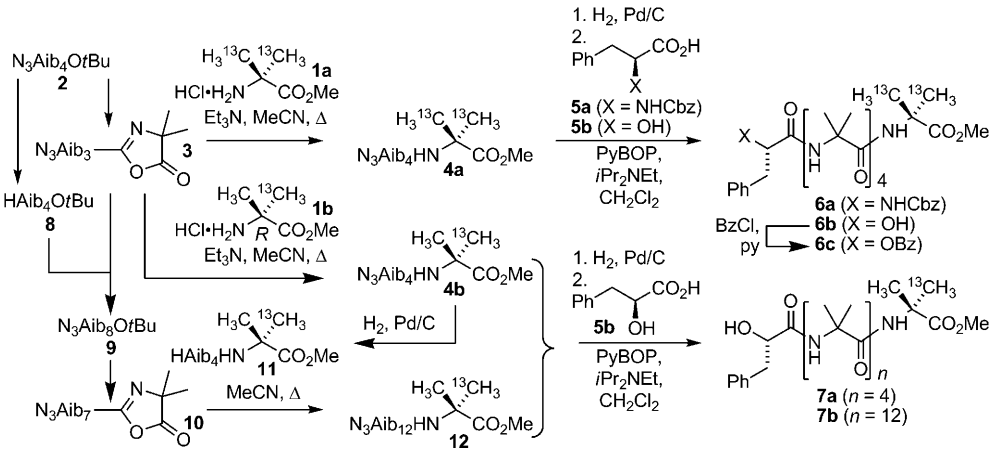
transfer. For the stereochemical input, we selected the benzoate ester of 3-phenyllactic acid (2-hydroxy-3-phenylpropionic acid), first because of its structural similarity to protected phenylalanine (which is known to be able to control helicity in oligo-Aib systems[11]), and second because the benzoate esters can be conveniently switched between their two mirror-image forms by means of the Mitsunobu reaction.[16]



Scheme 2. Synthesis of the capped helices. Bz = benzoyl, Cbz = carbobenzyloxy, py = pyridine, PyBOP = benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate.

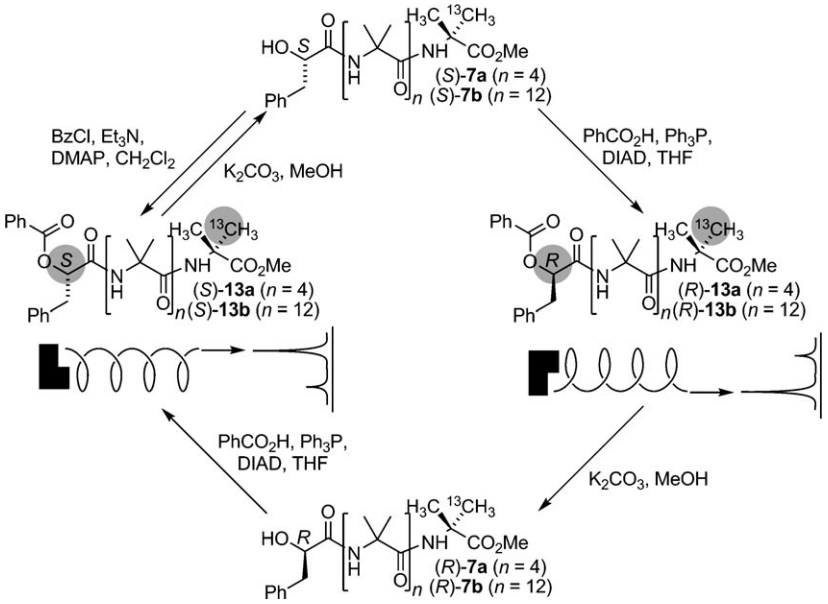
Information about the stereochemical input would be encoded in the screw sense of the helix; a remote asymmetric probe was required to decode this information. For this purpose, we chose to incorporate an isotopic label into the terminal Aib monomer: the stereochemical environment of the label could then be conveniently read by using the noninvasive technique of NMR spectroscopy. The use of a chemically silent isotopic label minimizes the influence of the probe on the configuration of the helix.[17]

To identify the location of the two diastereotopic ^{13}C NMR signals for the C-terminal Aib residue of the helix, and to establish that the two signals exhibited a detectable chemical shift difference, we used doubly ^{13}C labeled Aib, **1a**,[18] to open the azlactone[19] **3** derived from the Aib tetramer **2**[11] with its N terminus protected as an azide (Scheme 2). The doubly ^{13}C labeled helically racemic pentamer **4a** displayed a single strong peak at $\delta = 25.37$ ppm in its ^{13}C NMR spectrum in CD_3OD. This result indicated that the two methyl groups of the C-terminal Aib residue are in enantiomeric environments (i.e. they are not diastereotopic). Oligomer **4a** must therefore, as expected,[15] undergo rapid helix inversion at ambient temperature on the NMR timescale.

Ligation with either Cbz-L-Phe (**5a**) or L-3-phenyllactate (**5b**) gave peptides **6a**,**b**; peptide **6b** was benzoylated to yield **6c**. Compounds **6a** and **6c** showed two strong signals centered at 25.35 ppm owing to the now diastereoisomeric environments of the methyl groups of the C-terminal Aib residue (Figure 2a). Fourfold serial dilution of **6a** did not change the peak separation. We could therefore conclude that the anisochronicity of the two ^{13}C NMR signals was due to the intramolecular influence of the N-terminal controller rather than any supramolecular association.[20]

A singly ^{13}C labeled Aib probe **1b** was synthesized as an unequal mixture of enantiomeric isotopomers (*R*/*S* 77:23) by a modification of the method of Kouklovsky and co-workers.[21] Ligation of the tetra-Aib azlactone **3** with **1b** (to give **4b**) and then with L-3-phenyllactic acid (**5b**) gave the helical hexapeptide **7a** with a switchable stereocenter at the N terminus and a spectroscopic probe at the C terminus.

Sequential Mitsunobu esterification and hydrolysis enabled stereochemical switching, as shown structurally and schematically in Scheme 3. Thus, invertive esterification with benzoic acid in the presence of triphenylphosphane and diisopropylazodicarboxylate (DIAD) gave the ester (*R*)-**13a**, which displayed in its ^{13}C NMR spectrum two strong diastereotopic peaks at $\delta = 26.01$ and 24.69 ppm, precisely coincident with those of **6c**, but in a 78:22 ratio (Figure 2b). The ester (*R*)-**13a** was transformed into the alcohol (*R*)-**7a** by methanolysis, and the N-terminal controller was converted back into the ester (*S*)-**13a** by a second inversion under



Scheme 3. Helix switching. DMAP = 4-dimethylaminopyridine.

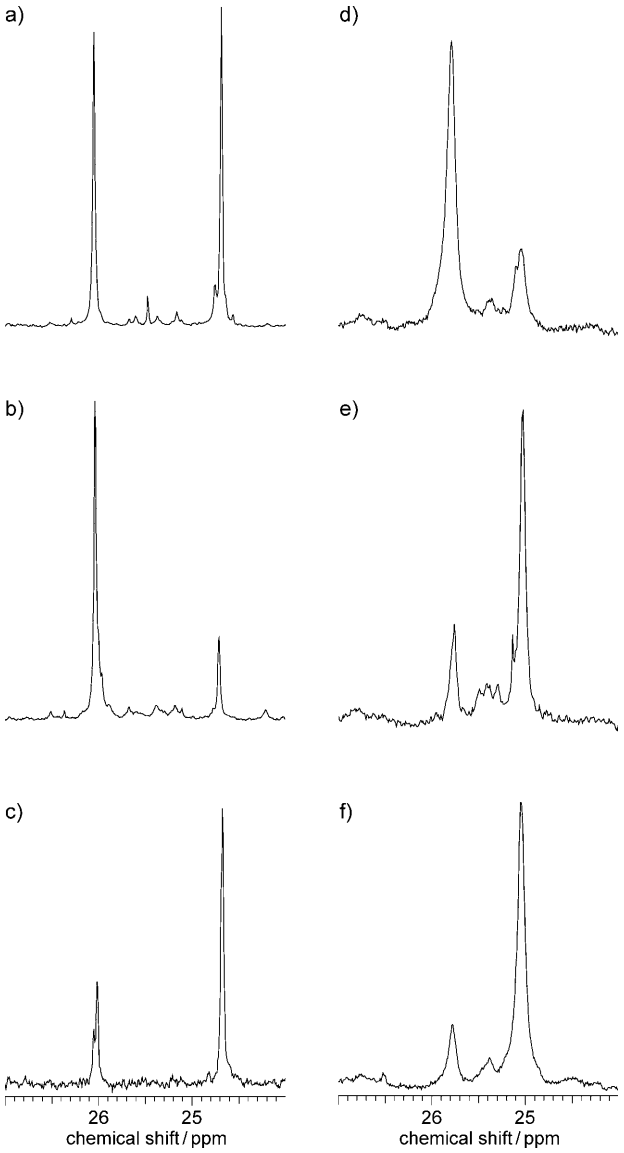


Figure 2. Portion of the ^{13}C NMR spectrum of a) **6c** (50:50 ratio); b) (*R*)-**13a** (after one inversion of (*S*)-**7a**; 78:22 ratio); c) (*S*)-**13a** (after two inversions of (*S*)-**7a**; 28:72 ratio implying coincidence of the minor peak and some "unlabeled" Me signals); d) (*R*)-**13b** (after one inversion of (*S*)-**7b**; 75:25 ratio); e) (*S*)-**13b** (after two inversions of (*S*)-**7b**; 20:80 ratio); f) (*S*)-**13b** (after benzoylation of (*S*)-**7b**; 23:77 ratio).

Mitsunobu conditions. Figure 2c shows the response of the remote reporter: a switch in the location of the major signal from 26.05 to 24.65 ppm. Although we were unable to deduce the magnitude of the helical excess observed, N-terminal L-amino acids typically induce P helicity.[22]

Given the low incidence of helix reversal in Aib oligomers,[11] extension of the Aib-oligomer segment of the peptides should enable communication over longer distances. To prove this principle, we synthesized longer peptides by dimerizing **2** via **3** and **8** to yield **9**, which was converted into its azlactone **10** and then coupled with **11**, the reduction product of **4b**. Reduction of the azide and ligation with **5b** gave the tetradecapeptide (*S*)-**7b**, which was converted into the benzoate ester (*R*)-**13b** by invertive Mitsunobu esterification as before. Use of an unequal mixture of enantiomers enabled us not only to decode the sense of helicity of the oligomer, but also to quantify the extent to which helicity is controlled as the chain is lengthened.[23] Peak separation in the ^{13}C NMR spectrum (Figure 2d) was less than for **13a** as a result of erosion of the signal with the lengthening chain,[11] but induction of helicity through the 12 Aib residues between the N-terminal controller and C-terminal reporter resulted in a clear 75:25 ratio of ^{13}C NMR signals at $\delta = 25.76$ and 25.02 ppm (Figure 2d). Upon the inversion of (*R*)-**13b** to (*S*)-**13b** by methanolysis and Mitsunobu esterification, the information about the switch was transmitted through all 40 bonds lying between the N- and C-terminal residues of the peptides, and the population of the ^{13}C reporter was inverted (Figure 2e). The identity of (*S*)-**13b** formed in this way, and hence the completion of the switching cycle, was confirmed by comparing its spectrum with that of (*S*)-**13b** formed by simple acylation of (*S*)-**7b** with benzoyl chloride (Figure 2f). The essentially identical spectra furthermore confirm the fully invertive nature of the Mitsunobu steps (> 97 % inversion for each of the two steps).

The transmission of stereochemical information from the N-terminal residue of 14-mer **13b** to the C-terminal probe of helicity is, as far as we are aware, the first example of the use of the handedness of a helical structure to convey quantified information between spatially separated points, whether on a molecular scale or otherwise. Typically, 3_{10} helices have a 1.94 Å rise per residue,[24] and the 2.5 nm length of **13b** is commensurate with the thickness of a typical phospholipid bilayer.[25] Aib oligomers **13** have considerable homology with a family of membrane-spanning oligomers known as the peptaibols.[26] We expect future modification and generalization of the encoding and decoding steps, with control of helicity retained as the means of propagating the information, to advance the development of artificial mimics of biological receptors capable of transmitting information across chemically impermeable membrane barriers.

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