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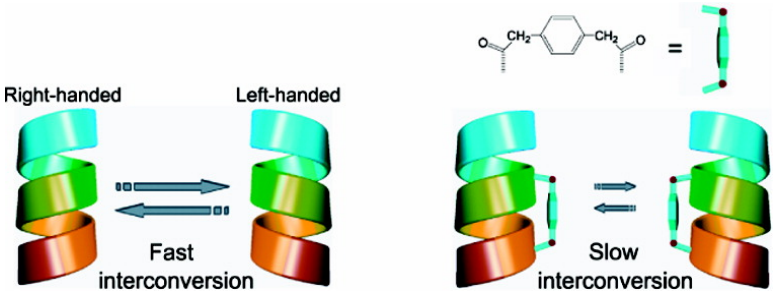
J. Am. Chem. Soc., **2008**, 130 (2), 463-465 • DOI: 10.1021/ja077857h



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Intramolecular Crosslinking of an Optically Inactive 3_{10}-Helical Peptide: Stabilization of Structure and Helix Sense

Naoki Ousaka,[†] Tomohiro Sato,[†] and Reiko Kuroda*[,†,‡]

Japan Science and Technology Agency, ERATO-SORST Kuroda Chiromorphology Team, 4-7-6, Komaba, Meguro-ku, Tokyo, Japan 153-0041 and Department of Life Sciences, Graduate School of Arts and Sciences, The University of Tokyo, 3-8-1 Komaba, Meguro-ku, Tokyo, Japan 153-8902.

Received October 13, 2007; E-mail: ckuroda@mail.ecc.u-tokyo.ac.jp

A helical structure is one of the most significant structural motifs in living system. In the last two decades, chemists have synthesized various kinds of well-defined artificial helical oligomers (so-called "foldamers"[1a−c]) and polymers[1d−f] that mimic biological structures. The biological helices predominantly adopt a particular helical screw sense (right- or left-handed) in response to the chiral monomer units such as L-α-amino acids and D-sugars. If a helical molecule is composed of only achiral units, it adopts left- and right-handed helices equally in symmetry-conserving media with interconversion between the enantiomers. The rate of interconversion usually depends on chain-lengths,[2] solvents,[2b] and guest molecules which may be present in the solution.[3a] These artificial helical molecules sometime exhibit slow interconversion of enantiomeric conformers[2] and even retain the helix sense for a long period of time.[3] In contrast, peptide backbones based on α-amino acid residues which exhibit such slow inversion have never been developed despite their importance in biological systems, because such peptide design is more restricted than that of the artificial backbone.

Herein, we report a novel finding that a single side-chain crosslinking stabilizes the entire structure and decelerates helix-inversion of a dynamically optically inactive 3_{10}-helical peptide[4] which is composed of only achiral C^{α}-tetrasubsituted α-amino acids.[5] The 3_{10}-helix[5b] is characterized by three amino acids per turn and the intramolecular hydrogen bonds between residue *i* (C═O) and *i* + 3 (NH). The side-chain crosslinking of residue *i* and *i* + 3 in 3_{10}-helix has been investigated for the case of ring-closing metathesis[6a] and salt-[6b] and lactam-bridge formations.[6c,d] We have presumed that such a crosslinking stabilizes the structure and interrupts the helix reversal, if an appropriate bridging moiety is chosen.[7]

An octapeptide with the sequence Cbz-Aib_2-Api(Boc)-Aib_2-Api-(Boc)-Aib_2-OMe (Cbz = benzyloxycarbonyl; Aib = α-aminoisobutyric acid; Api(Boc) = 1-Boc-piperidine-4-amino-4-carboxyric acid; Boc = *tert*-butoxycarbonyl; OMe = methoxy) (**P1**) was designed. Short oligopeptides containing Aib residues are known to promote the 3_{10}-helices strongly,[4−6] and achiral Api residue possessing the functional group at the side-chain is also known to stabilize the helical structure.[6b,8] Starting from Cbz-Api(Boc)-OH,[8a] we prepared the achiral octapeptide **P1** by activating the amino acid carboxyl group with HOAt (7-aza-1-hydroxy-1,2,3-benzotriazole)/HATU (HOAt uronium salt derivative)[9] in dry methylene chloride in the presence of *N*,*N*-diisopropylethylamine (for details of the peptide synthesis and characterization, see Supporting Information). Our modeling study indicates that *p*-phenylenediacetic acid is one of the most suitable and relatively rigid bridging moieties for the crosslinking between the side-chains of Api residues at the residue

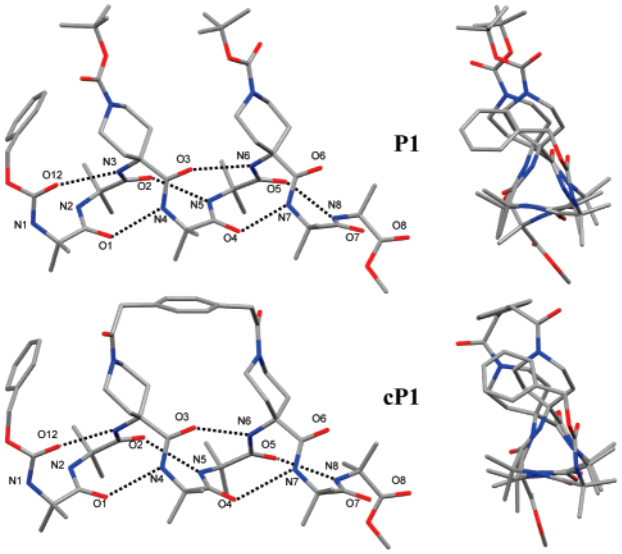
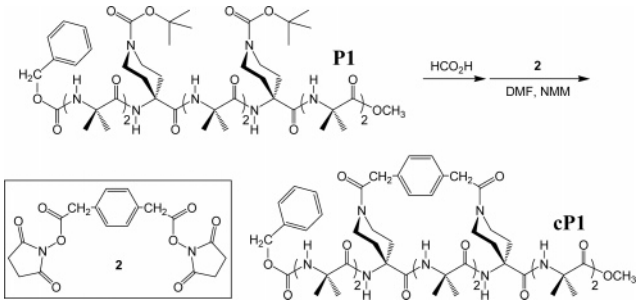


Figure 1. Side (left) and top (right) views of the structures of **P1** and **cP1** in the crystals. (For **cP1**, one of the two crystallographically independent molecules, molecule A, is shown) Only the right-handed helices are shown. Hydrogen atoms and cocrystallized solvent molecules are omitted for clarify. Dotted lines represent intramolecular hydrogen bonds.

Scheme 1. Synthesis of Side-Chain Crosslinked Peptide **cP1** from **P1**



i and *i* + 3 positions. The side-chain crosslinked peptide, **cP1**, was prepared by the activated ester method in a dilute solution (Scheme 1).[10]

Figure 1 shows X-ray crystal structures of peptides **P1** and **cP1**. In the crystal of **cP1**, two crystallographically independent molecules (molecules A and B) are contained in the asymmetric unit, each with similar molecular conformation (Figures S1, S2). Peptides **P1** and **cP1** take a regular 3_{10}-helix for residues 1−7 with a consecutive-type III *β*-turn but an opposite chirality for the last residue. The partial reversal is usually observed in helical peptides which contain Aib residue at the C-terminus.[11] All the piperidine rings have the amino group in an axial orientation, and the urethane

[†] Japan Science and Technology Agency.
[‡] The University of Tokyo.

10.1021/ja077857h CCC: $40.75

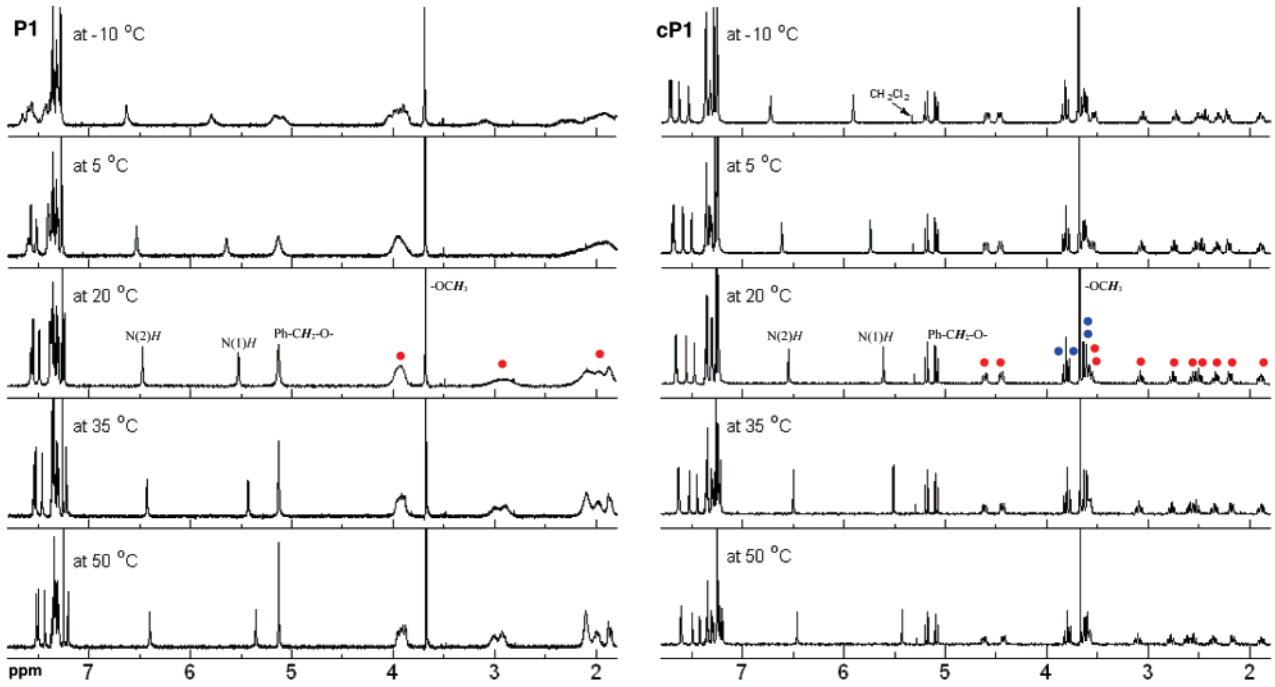


Figure 2. Variable temperature ^{1}H NMR (500 MHz) spectra (7.8−1.8 ppm) of **P1** (left) and **cP1** (right) in $CDCl_3$; [peptide] = 3.5 mM. Red and blue circles indicate protons of piperidine rings and $-CH_2-Ph-CH_2-$, respectively. The full region spectra are provided in Figure S6.

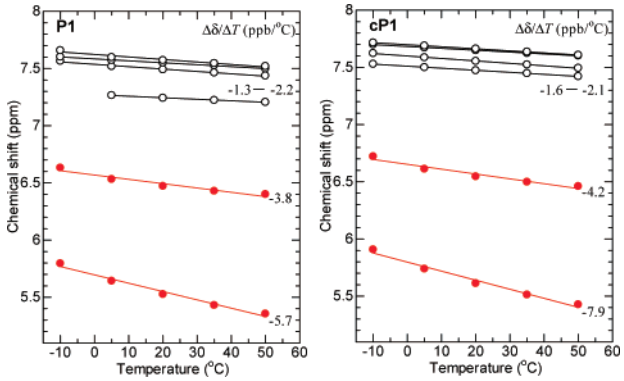


Figure 3. Plots of the NH chemical shifts in the ^{1}H NMR spectra (Figure 2) as a function of temperature in $CDCl_3$ for **P1** (left) and **cP1** (right).

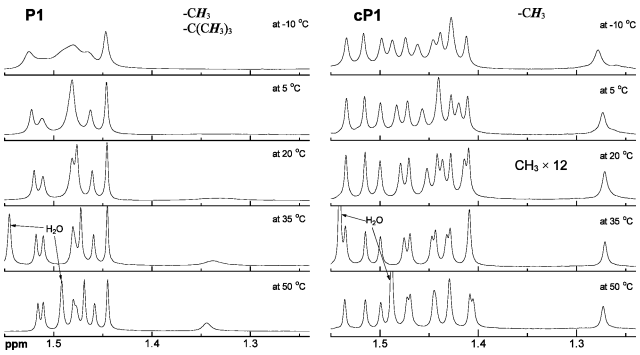


Figure 4. Methyl regions of variable temperature ^{1}H NMR spectra of **P1** (left) and **cP1** (right), which are shown in Figure S6.

or amide carbonyl orientations of the two side-chains are opposite direction to each other. A recent report shows that side-chain crosslinking gives rise to slightly disturbing regularity to the helix.[6a] In contrast, **cP1** exhibits high regularity of 3_{10}-helix except for the last residue, and the structure is very similar to that of **P1**, with the root-mean-square deviations for the backbone atoms of **P1** and **cP1**

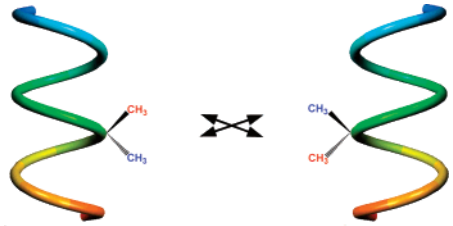


Figure 5. Schematic representation of left-right helical interconversion. The interconversion exchanges the chemical environments of the β-methyl protons on each residue in the helical peptides.[4a,b] Only one of the six sets of β-methyl protons is shown for clarity.

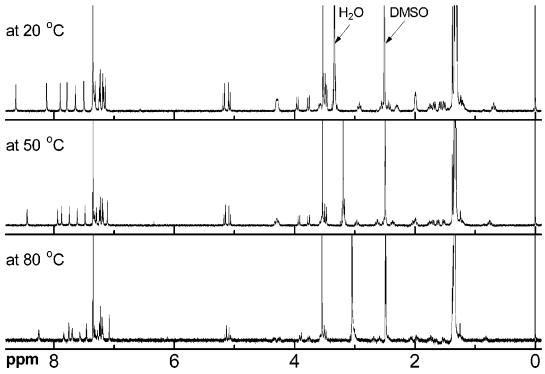


Figure 6. Variable temperature ^{1}H NMR spectra of **cP1** in DMSO-d_6; [peptide] = 3.5 mM.

of 0.371 and 0.333 Å, for molecule A and B, respectively (averaged $|\phi|/|\psi|$ torsion angles (degree) of **P1** and **cP1** are 53/31 and 55/30, respectively). The results of least-square superpositioning of the molecular structures are shown in Figures S2−S4, and the backbone torsion angles and hydrogen-bonding parameters are summarized in Tables S1−S6.

Solution conformations of **P1** and **cP1** were determined by ^{1}H NMR technique, and additionally by computational analysis. ^{1}H NMR experiments were performed to confirm a helix type (3_{10}- or α-helix) in $CDCl_3$ solutions. The signals of urethane NH and another

signal are located at relatively higher field (below 6.6 ppm) than other NH proton signals (above 7.2 ppm) for both peptides (Figure 2). These two protons are assigned to N(1)H and N(2)H.[12] Indeed, N(1)H and N(2)H signals of both peptides show slightly bigger temperature coefficients (−5.7, −3.8 for **P1**, and −7.9, −4.2 ppb/°C for **cP1**, as shown in red in Figure 3) than others (−1.3 to −2.2 ppb/°C)(shown in black, Figure 3). These results suggest lack of intramolecular H-bonding for the two protons.[12,13] The energy calculations also support the 3_{10}-helical structures in both peptides.[10] It is also known that side-chain crosslinking of residues *i* and *i* + 3 in the peptide helix promotes the 3_{10}-helical structure and destabilizes an α-helix.[6c,d]

The proton signals of the piperidine rings of **P1** are broadened, suggesting that a chair−chair interconversion or (and) urethane CO−N(CH_2−)$_2$ bond rotation is relatively slow on the NMR time scale at 20 °C (Figure 2 left). These resonances are slightly sharper at higher temperatures and much broader at lower temperatures (Figure 2). Interestingly, all the protons except for the aromatic and amide protons of **cP1** are quite different from those of **P1** (Figure 2). All the proton signals of piperidine rings of **cP1** are very sharp and distributed over a wide chemical-shift range (assigned by 2D TOCSY spectrum at 20 °C, see Figure S8). Positions and half bandwidths of all protons on piperidine rings are almost independent of temperatures ranging from −10 to 50 °C (Figure 2 right). Thus, the motion of the two piperidine rings is constrained. Remarkably, both methylene protons of −C*H*$_2$−C$_6$H$_4$−C*H*$_2$− (around 3.6−3.8 ppm) in the bridging moiety show double doublets. Moreover, N-terminal methylene protons of the Cbz group (around 5.13 ppm) also show diastereotopic motif, although they are placed distant from the bridging region (Figure 2 right).[14] These splitting resonances are almost independent of temperature. These results indicate that **cP1** as a whole exhibits slow helix-inversion.

Further evidence of slower inversion of **cP1** is seen in the region of β-methyl protons of Aib residues, which are found in the region of 1.54−1.27 ppm (Figure 4). All the β-methyl protons of **cP1** are individually distinct. The left-right helicity interconversion exchanges the chemical environment of the β-methyl protons (*pro*-S and *pro*-R) on each residue in the helical peptides (Figure 5).[4a,b] If the interconversion is sufficiently slow on the NMR time scale, these *pro*-S and *pro*-R β-methyl protons must be distinguishable with different chemical shifts. Indeed, 12 signals of β-methyl protons in the spectrum at 20 °C are observed (Figure 4).[15] Even at higher temperatures of up to 50 °C, 10 signals of β-methyl protons are clearly observed (Figure 4).

In DMSO-d_6, coalescence temperature of all the diastereotopic splitting of **cP1** is above 80 °C (Figure 6). The *J* value of methylene protons on the Cbz group is more susceptible to temperature in DMSO-d_6 than in $CDCl_3$ where it is almost constant. These data indicate that the rate of interconversion depends on the solvents (DMSO is a strong hydrogen-bond acceptor and thus destabilizes peptide helices).

As shown in Figure 1 (**cP1**, bottom right), the two amide bonds of Api side-chains are twisted anticlockwise along the right-handed helical axis, and the amide carbonyl orientations are opposite direction to each other as described above. On the basis of these structural features, the slower interconversion may originate from cooperative restriction on the amide bonds, as simultaneous 180° rotations of the two amide bonds of Api side chains are necessary for the helix reversal. In contrast to **cP1**, the β-methyl proton peaks of **P1** did not show diastereotopic splitting, even at lower temperature (−10 °C, Figure 4). These data obviously indicate that the single side-chain crosslinking increased the stability of the entire peptide structure and slowed the helix inversion.

In summary, we have demonstrated the first example that side-chain crosslinking of *i* and *i* + 3 residues not only stabilizes the structure without disruption of the helix regularity but also decelerates the enantiomerization in the dynamically optically inactive 3_{10}-helical peptide. This is one of the stiffest helical peptides,[12,16] which is composed of only the strongly helix-promoting C$^{\alpha}$-tetrasubstituted α-amino acids with a side-chain crosslinking. The sequence -Api-Aib$_2$-Api- bridged with *p*-phenylenediacetic acid may serve as a key structural unit for the design of optically active peptides composed of only achiral building blocks and/or a variety of peptides with enhanced structural rigidity.

Supporting Information Available: Synthetic procedure, characterization, spectroscopic data, and crystallographic data in CIF format. This material is available free of charge via the Internet at http://pubs.acs.org.

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