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Transforming the C-terminal amide of a glycinylphenylalanine dipeptide into *N*-amidothiourea affords a β -turn structure in the formed dipeptide based *N*-amidothioureas, which can be readily identified by an induced CD signal from the achiral phenylthiourea chromophore.

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The β -turn is one of the important structural motifs for maintaining the secondary structures of proteins¹ and some of the peptides or proteins bearing β-turn structure have found significant pharmaceutical and biomedical applications.² Although it has been now shown that some of the amino acid residues, for example proline, would facilitate the formation of β-turn structure in peptides containing those residues,^{1b} it is not straightforward to predict which sequence would afford this structure and the use of such unmodified peptides as therapeutics is limited due to their poor pharmacokinetic properties. Azapeptides represent a family of modified peptides in which the β -turn structure is promoted, since the replacement by a nitrogen atom of the α-carbon of one or more amino acid residues in the peptide backbone leads to a more rigid N_{α} -C(O) motif and electronic repulsion between the lone pairs of the two adjacent nitrogen atoms, resulting in constrained φ and ψ dihedral angles (Scheme 1).³ The β -turn structure involves four amino acid residues, *i.e.* the *i* to i + 3 residues, and is stabilized by a ten-membered ring hydrogen bond. The four amino acid residues would therefore be included in a chiral environment if at least one of the amino acid residues, in particular the one within the "ring", i + 1or i + 2 amino acid residue, is chiral. This specific steric effect may allow the chirality to be transferred across a long distance and the induced chiral information such as the circular dichroism (CD)

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β-Turn structure in glycinylphenylalanine dipeptide

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based N-amidothioureas⁺

signal from the involved achiral chromophore may serve to identify the turn structure. We recently showed that *N*-amidothioureas exhibited much stronger hydrogen bonding ability towards oxoanions than the urea counterparts (the azaGly containing peptides,⁴ Scheme 1), partly because of the higher acidity of the thioureido –NH protons.⁵ Given the structural similarity of thioamide to oxoamide,⁶ we envisaged that within the peptide-based *N*-amidothioureas the β -turn structure might be created if the thioureido –NH is designed to act as a hydrogen bonding donor. Here we report our first attempt by capping the amino acid based *N*-amidothioureas (**1**, Scheme 2),⁷ with a simple achiral glycine residue at the N-terminal of the amino acid residue in **1**. Thus created glycinylphenylalanine dipeptide based *N*-amidothioureas (t-2 and D-2, Scheme 2) were shown to bear a β -turn structure in both solid state and solution phase. Despite the more stable β -turn structure in **2** than that in the urea counterpart



Scheme 2 Molecular structures of 1, 2, L-3, L-4a and L-4b. Chiral carbons in all molecules are indicated by " \star ".

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[†] Electronic supplementary information (ESI) available: Synthesis (Scheme S1) and characterization of **2**, *L*-**3**, *L*-**4a** and *L*-**4b** and spectral titration traces and data (Tables S1–S3, Schemes S1 and S2, Fig. S1–S15). CCDC 914552 and 926794. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c3cc44336a



it is nevertheless easier to be broken upon anion binding, again because of the higher hydrogen bonding ability of thiourea.

N-Amidothioureas L-2 and D-2 were readily prepared from (N,N-dimethylglycinyl)phenylalanine following a procedure outlined in Scheme S1 of ESI.[†] Fig. 1 presents the absorption and CD spectra of 2 in acetonitrile. Mirrored CD spectra of L-2 and D-2 are observed (Fig. 1b), confirming that the chirality originates from the phenylalanine residue. By comparison with the absorption spectrum, the CD signal at 235 nm is assigned to the chiral phenylalanine residue,8 while that at 270 nm arises from the achiral phenylthiourea chromophore. This observation is significant since no CD signal at 270 nm was observed from 1 suggesting that its phenylthiourea chromophore remains achiral (Fig. S1, ESI^{\dagger}).⁷ The thiourea moiety in 2 is thus turned chiral by simply capping 1 with an achiral glycine residue (Scheme 2), which demonstrates the key role that the glycine residue in 2 plays in facilitating the intramolecular chirality transfer, despite its achiral nature and its distance from the thiourea moiety.

The crystal of L-2 suitable for X-ray crystallography was successfully grown by slow evaporation of its solution in CH₂Cl₂. Crystal structure of L-2 (Fig. 2) shows two intramolecular five-membered ring hydrogen bonds (N2–H_h···N1, 2.298 Å; N5–H_k···N3, 2.260 Å) and one ten-membered ring hydrogen bond (N5–H_k···O1, 2.401 Å). The first five-membered ring hydrogen bond was also confirmed by the splitting of NMR signals of geminal CH₂ protons (H_a and H_b) in the achiral glycine residue since they are included in a fivemembered ring next to the chiral carbon center of the phenylalanine residue, which means that such a hydrogen bond exists at least in the tested three deuterated solvents of increasing polarity and hydrogen bonding capacity, CDCl₃, CD₃CN and DMSO-*d*₆ (Fig. S2, ESI[†]). The ten-membered ring hydrogen bond involves four amino acid residues and is stabilized by CO···HN hydrogen bond linking residues *i* and *i* + 3, termed β-turn in peptides and proteins.¹ This β-turn structure is of type II according to the Ramachandran φ and ψ dihedral angles (Table S1, ESI[†]). The β-turn structure causes the chiral phenylalanine residue and the achiral phenylthiourea moiety to come into close proximity and brings the achiral phenylthiourea moiety into a chiral environment, explaining the observed exciton-coupled CD signal of 2 (Fig. 1b). DFT calculations agree with the crystal structure of L-2 (Fig. S3, ESI[†]).

1D and 2D NMR data both show that the β -turn structure of 2 exists in solution phase as well. ¹H NMR spectra of 2 in DMSO-d₆-CD₃CN binary solvents of varying composition reveal that the signals of NH_h and NH_k protons are insensitive to variation in solvent composition, suggesting their involvement in the intramolecular hydrogen bonds, whereas those of NH_i and NH_i are sensitive which means that these two protons are solvent accessible (Fig. S4a, ESI⁺), as shown by the crystal structure of L-2 (Fig. 2). 2D NMR exhibits NOE signals for coupling of H_a/H_f and of H_b/H_f, providing direct evidence for the β -turn structure in the solution phase (Fig. S5, ESI⁺). It is therefore concluded that it is the β -turn structure in 2 that leads to the intramolecular chirality transfer from the phenylalanine residue to the phenylthiourea moiety. Because of the intramolecular hydrogen bonding nature, thus stabilized β-turn structure could exist in organic aqueous solutions with a limited extent of water. Our NMR titrations and CD spectral data in H_2O-CH_3CN solutions suggest that the β -turn structure may remain in up to 10-15% by volume of water (Fig. S4b and S6, ESI[†]). The observed temperature independent CD spectrum of L-2 in CH₃CN over 25–45 °C indicates that the β -turn structure is stable within the tested temperature range (Fig. S7, ESI⁺).

As one of the thioureido –NH protons is involved in the β -turn, it was expected that the β -turn structure may collapse upon addition of an anion since the anion will bind to the thiourea moiety, a well-known anion binding site.^{5,9} Fig. 3 shows absorption and



Fig. 2 X-ray crystal structure of L-2. Dashed pink lines highlight the intramolecular hydrogen bonds.



Fig. 3 Absorption (a, L-2) and CD (b, L-2; c, D-2) spectra of 2 in CH₃CN in the presence of the AcO⁻ anion. [2] = 40 μ M. AcO⁻ exists as the (*n*-Bu)₄N⁺ salt.



CD spectra of 2 in acetonitrile in the presence of the acetate anion. With increasing AcO⁻ concentration, the absorption of the phenylthiourea chromophore at 270 nm shifts to 266 nm, while a new band develops at 296 nm, with an isosbestic point at 244 nm (Fig. 3a). This means a clean interaction of AcO⁻ with 2. The absorption at 296 nm can be analogously assigned to the charge transfer transition of the anion binding complex.⁵ Interestingly, the CD signal at 270 nm of the phenylthiourea origin reverses gradually, along with the development of a new CD band at 304 nm. The resultant opposite and coupled Cotton effects at 304 nm and 270 nm suggest that the chirality of the phenylthiourea moiety in 2 has been overturned and extended to the anion-thiourea binding block.⁷ The mirror-imaged CD spectra of AcO⁻-L-2 and AcO⁻-D-2 solutions (Fig. 3) again confirm that the chirality originates from the phenylalanine residue. ¹H NMR titrations in CD₃CN on AcO⁻ binding to 2 showed enlarged splitting of the signals of magnetically nonequivalent geminal protons H_c and H_d at chiral carbon and of H_a and H_b protons at achiral carbon (Fig. S8 and S9, ESI⁺), suggesting a further rigidization of 2 upon binding to AcO^{-.10} Two structures for the AcO⁻-2 complex are possible (Scheme S2, ESI^{\dagger}), of which model I that contains a γ -turn is more stable than model II, in view of the steric effect (Scheme 3).

The urea counterpart of L-2, L-3 (Scheme 2) was prepared as a control to ascertain the contribution of the acidity of (thio)ureido -NH protons. Crystal structure of L-3 (Fig. S10, ESI⁺) is similar to that of L-2, showing a β -turn structure too. β -turn in L-3 however was found to be weaker than that in L-2, on the basis of the comparison of bond lengths and angles of the β -turns in the crystal structures (Table S2, ESI⁺) and the higher sensitivity of the NMR signal of the NH_k proton in L-3 towards the composition of the DMSO- d_6 -CD₃CN binary solvents (Fig. S11, ESI⁺). This agrees with the lower acidity of the ureido -NH protons than that of the thioureido -NH protons.5 In support of this assumption is the lower AcO⁻ binding affinity of L-3 than L-2 (Table S3, ESI⁺), deduced from the absorption titrations (Fig. S12, ESI⁺). It hence appears that, compared to L-3, the thiourea derivative L-2 takes a more stable β -turn structure itself and it is also more labile towards anion binding that breaks the β -turn and likely forms a γ -turn.

The substantial difference observed in the profiles of CD titrations of the amino acid based L-1 and the dipeptide based L-2 (Scheme 2) by AcO⁻ anion (Fig. S13, ESI[†]) again points to the vital function of the N-terminal achiral glycine residue in the transfer of chirality and the formation of the intramolecular hydrogen bonding network (Scheme 3). Control research into L-4a and L-4b of L-2 derivatives (Scheme 2) reveals that *N*-substitution on the glycine residue exerts negligible influence on the absorption

and CD spectral response toward AcO^- in CH₃CN, since they are similar to those of L-2 (Fig. S14 and S15, ESI[†]).

In summary, we report that, despite a minor difference by only an achiral glycine residue, the dipeptide based N-amidothiourea 2 differs dramatically from the amino acid based 1 in the conformation and chirality transfer, which affords a new entry to the β -turn structural motif by transforming the C-terminal amide of a dipeptide glycinylphenylalanine into an N-amidothiourea moiety. Thus created β -turn structure can be readily identified by a CD signal resulting from the achiral phenylthiourea chromophore. The β-turn structure in 2 is not only more stable than that in azapeptides but it is also more labile to anion binding. These observations, together with the interesting chirality transfer in both the dipeptide based N-amidothiourea and its anion binding complex, may help guide the design of peptidomimetic pharmaceuticals.¹¹ They also establish a new entry to chiral thioureas of intensive current interest in organocatalytic asymmetric syntheses.12 Work is now underway to validate the generality of the present strategy of creating a β -turn structure, despite being validated in our preliminary assays in a small library, and to evaluate chiral communication along the peptide backbone.

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