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Turn Conformation of β-Amino Acid-Based Short Peptides Promoted by an Amidothiourea Moiety at C-Terminus

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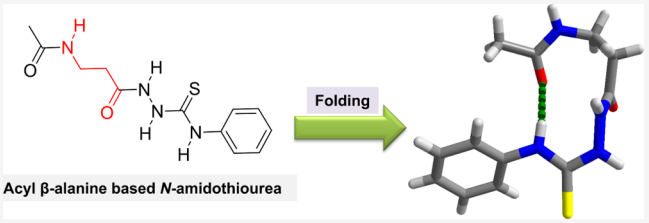
ABSTRACT: A C-terminal amidothiourea motif is shown to promote a β-turn-like folded conformation in a series of β-amino acid-based short peptides in both the solid state and solution phase by an intramolecular 11-membered ring hydrogen bond.



INTRODUCTION

Turn is a fundamental secondary structural element that stabilizes the global conformation of proteins/peptides and takes part in a variety of biological molecular recognition processes.[1] Synthetic short peptides with a turn structure have also found applications in molecular recognition, organocatalysis, and drug design.[2] Despite being facilitated by an intramolecular hydrogen bond, the turn structure is determined by the dihedral angles of the peptide backbones, which depends on the sequence and stereochemistry of the peptide chain.[3] Structurally rigid amino acids, for example, proline,[4] or modified amino acids, for example, azaamino acids,[5] have therefore been preferably considered to facilitate the turn structures in peptides.

β-Amino acids, homologues of α-amino acids (Scheme 1), have become attractive structural entities into designing synthetic peptides with improved biomedical utility, for example, as enzyme inhibitors and antimicrobial peptides, because of the enhanced proteolytic stability and improved biomedical utility.[6] Despite with only one more carbon than the α-amino acid residue, the β-amino acid residue in the peptide backbone is structurally much more flexible, which thereby imposes an extremely high challenge to form a turn structure. In this context, specific rigid β-amino acids, for example, 2-aminocyclopentanecarboxylic acid[7] and orthanilic acid,[8] and β-amino acid analogues, for example, α-aminoxy acids[9] and hydrazino peptides,[10] have been employed to promote the folded turn conformation of the short peptides or oligopeptides. These complicated, unnatural rigid β-amino acids and analogues,[11] however, are not easily accessible while for simple but structurally flexible β-amino acids, for example, the naturally occurring β-alanine, it remains hard to form such turn structures.

Inspired by the success of the otherwise much less accessible γ-turn-like structure in tripeptides containing a β-amino acid analogue, the α-aminoxy acid, by the N−O turn,[9] we decided to create the folded structure in short peptides that contain a flexible β-amino acid residue by using our previously discovered amidothiourea motif.[12−14] α-Amino acid-based short peptides equipped with an amidothiourea motif at the C-terminus, for example, *S*-**6** in Scheme 1 that is also referred to as an azapeptide,[12b] exhibit a folded β-turn structure maintained in an intramolecular 10-membered hydrogen bond (10-HB, Scheme 1).[12−14] This folded turn structure is driven by the twisted conformation of the N−N bond in the amidothiourea moiety while the enhanced acidity of the thioureido −NH proton leads to a stronger intramolecular hydrogen bond to stabilize the turn structure.[12] It has also been noted that the substitution of amide with a thioamide in peptides could lead to increased carbonyl−carbonyl n → π* interaction[15] and additional aromatic−thioamide interaction through the C_{sp^2}−H···S_{amide} hydrogen bond,[16] together with a shorter C−N bond and a higher C−N rotational barrier,[17] likely stabilizing the turn structure too. We thus envisaged that if a folded turn structure would exist in the β-amino acid-based short peptides containing an amidothiourea moiety at C-terminus, similar to the α-amino acid-based analogues, an 11-membered intramolecular hydrogen bond would form (11-HB, Scheme 1). This, by referring to Kuhn's topologies,[18] could be much less possible to an exponential extent. To our delight, experiments showed that such β-turn-like structure does exist in the acyl β-amino acid-based short peptides **1**−**5** (Scheme 1).

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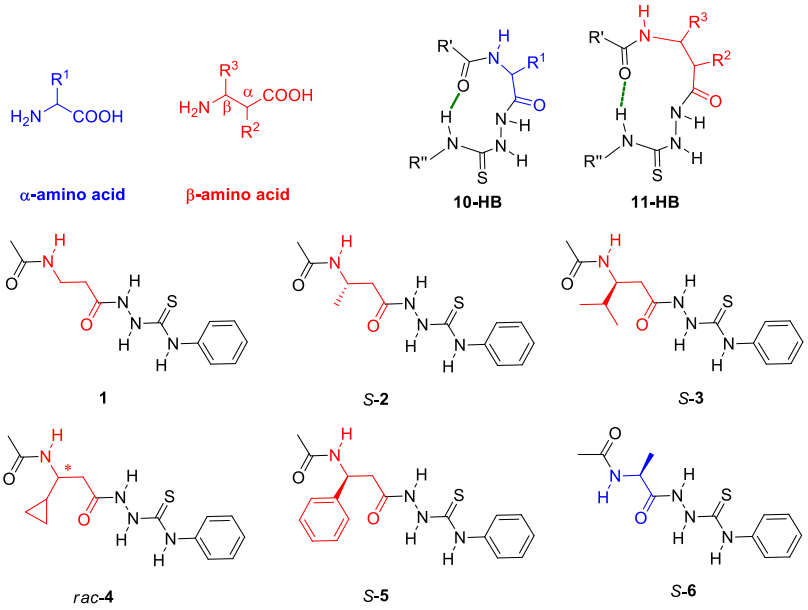
Scheme 1. Structures of α-Amino Acid and β-Amino Acid and Intramolecular 10- and 11-Membered Ring Hydrogen Bonds (10-HB and 11-HB) in α-Amino Acid- and β-Amino Acid-Based *N*-Amidothioureas; Chemical Structure of Designed Acyl β-Amino Acid-Based *N*-Amidothioureas (1, *S*-2, *S*-3, *rac*-4, and *S*-5) and α-Alanine-Based *S*-6



RESULTS AND DISCUSSION

Achiral β-alanine is the only naturally existing β-amino acid and it is the simplest and structurally most flexible one. We were fortunate to grow the crystals of β-alanine-based *N*-amidothiourea, **1** (Scheme 1), by slow evaporation of its solution in CH_3CN/CH_3OH (Figure S1 and Table S1, Supporting Information). A β-turn-like folded conformation was identified in the structure of **1** (Figure 1a), which is maintained by an 11-membered ring intramolecular hydrogen bond between thioureido $-NH^d$ proton and acetyl $C{=}O^i$, with a H···O distance of 2.414 Å, N···O distance of 3.103 Å, and N−H···O angle of 137.60° (Table S2). The torsion of the N−N bond (φ_{i+2}) was shown to be −101.75° (Table S3), confirming the twisted conformation of the amidothiourea moiety, the structural motif that is assumed to promote the turn structure.[12]

The amide/thioamide bonds in **1** are of trans-form, so there may be carbonyl−carbonyl n → π* interactions to help stabilize the turn structure (Figure S2).[15,19] In view of the crystal structure of **1** (Figure S2), only one n → π* interaction is possible, that is, that between $C^i{=}O^i$ and $C^{i+1}{=}O^{i+1}$, with a $O^i{\cdots}C^{i+1}$ distance of 2.923 Å (Figure 1a). However, the angle of $O^i{\cdots}C^{i+1}{=}O^{i+1}$ is 86.35°, out of the optimal range from 99 and 119°,[19] suggesting that the contribution of this n → π* interaction in stabilizing the turn structure is negligible. The distance between $H^{h'}$ on the C-terminal phenyl group and the S atom is 2.705 Å, with a $C{-}H^{h'}{\cdots}S$ angle of 109.30° (Figure 1a), suggesting that the overlap of their orbitals, and thereby the presence of an aromatic $C_{sp^2}{-}H{\cdots}S_{amide}$ interaction,[16] is possible. This could afford a certain extent of stabilization on the turn structure, but it is not dominant because this interaction is outside of the turn structure. In crystals, one molecule of **1** is involved in six intermolecular hydrogen bonds with adjacent three molecules, that is, four N−H···O=C and two N−H···S=C hydrogen bonds (Figure S3 and Table S4).

1D and 2D NMR spectra in DMSO-d_6/CD_3CN or CD_3CN support the folded conformation of **1** in the solution phase as well. The assignment of the protons in **1**, for example, four −NH protons, was based on 2D COSY and NOESY spectra in CD_3CN and DMSO-d_6 (Figures S4−S7). Changing the solvent from CD_3CN to hydrogen bonding DMSO-d_6, signals of the −NH protons in **1** shift to downfield (Figure S8). Solvent accessibility calculated from the difference of the chemical shifts of the −NH protons in DMSO-d_6 and in

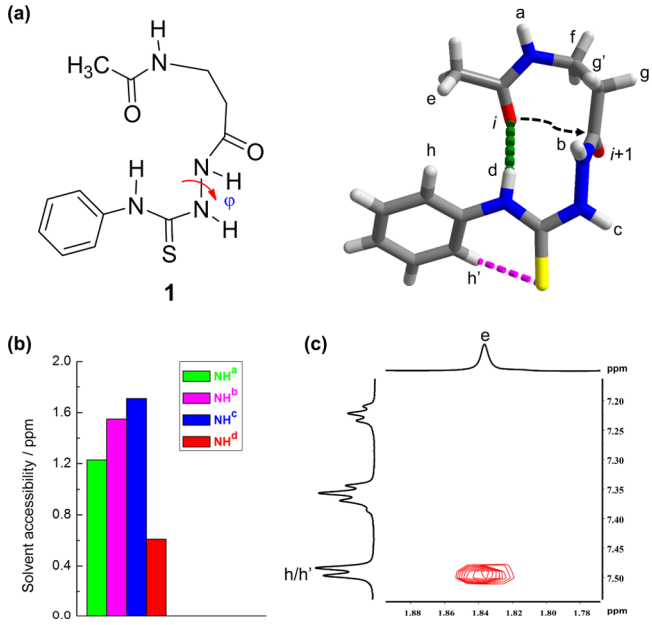


Figure 1. (a) Molecular and crystal structure of **1**. Dashed green line highlights the $N{-}H^d{\cdots}{}^iO{=}C$ hydrogen bond, dashed pink line highlights the aromatic $C_{sp^2}{-}H{\cdots}S_{amide}$ interaction while the dashed black arrow indicates the carbonyl−carbonyl n → π* interaction. (b) Solvent accessibility of −NH protons in **1** at 25 °C. Solvent accessibility is given in the difference of δ_{NH} in DMSO-d_6 from δ_{NH} in CD_3CN. (c) Partial 2D NOESY spectrum of **1** in CD_3CN at 25 °C. [**1**] = 2 mM.

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CD_3CN shows that the thioureido $-NH^d$ exhibits the lowest solvent accessibility in all of the four −NH protons (Figure 1b and Table S5), suggesting that it takes part in the intramolecular hydrogen bonding,[20] as shown in the crystal structure of **1** (Figure 1a). 2D NOESY supports the folded conformation of **1** in CD_3CN (Figure 1c) by the coupling between $-CH^e$ and $-CH^{h/h'}$ protons that are located at two termini of molecule **1** and separated by 12 atoms or 13 bonds. The NOE couplings observed between $NH^d-CH^{g/g'}$ and NH^d-CH^e, as well as NH^d-NH^b, are also in line with the folded conformation (Figure S9).

The α-amino acid counterpart of **1**, *S*-**6** (Scheme 1), shows a β-turn structure with an intramolecular 10-membered hydrogen bond (H···O length 2.312 Å, N···O length 3.153 Å, and N−H···O angle 162.60°) in its crystal structure,[12b] stronger than the 11-membered ring hydrogen bond in **1**, by referring to the bond length and angle parameters (Figure S10 and Table S2). The lower solvent accessibility of proton $-NH^d$ in *S*-**6** (0.21 ppm) than that in **1** (0.61 ppm) again demonstrates a stronger intramolecular hydrogen bond in *S*-**6** (Figure S11). The capability of bringing the highly flexible $-CH_2-CH_2-$ fragment in β-alanine residue into a folded turn conformation of **1** supports the robust role of the C-terminal amidothiourea motif in promoting the turn structure.

More rigid β-amino acids, 3-aminobutanoic acid, 3-amino-4-methylpentanoic acid, 3-amino-3-cyclopropylpropanoic acid, and β-phenylalanine, were next introduced to create *S*-**2**, *S*-**3**, *rac*-**4**, and *S*-**5** as analogues of **1**, respectively, but with a β-substituent of varying size (Scheme 1). The "foldedness" or the stability of the folded turn structures in **1**−**5** is described by the strength of the intramolecular 11-membered ring hydrogen bond. Solvent accessibility of the −NH protons indicates the intramolecular hydrogen bonding of $-NH^d$ in *S*-**2**, *S*-**3**, *rac*-**4**, and *S*-**5** (Fig. S12), as that in **1** (Figure 1b).[20] Comparison of the solvent accessibilities of $-NH^d$ reveals that *S*-**2**, *S*-**3**, and *rac*-**4** afford more stable intramolecular hydrogen bond than **1**, of which *S*-**3** is the strongest, whereas the hydrogen bond in *S*-**5** that contains a bulky phenyl substitute at β-carbon is weaker than that in **1** (Figure 2a). The α-methylene $-CH^gH^{g'}$ protons

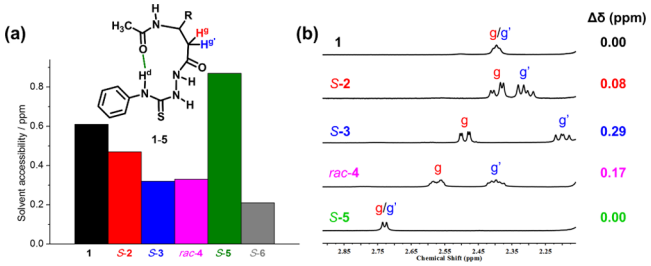

Figure 2. (a) Solvent accessibility of $-NH^d$ in **1**, *S*-**2**, *S*-**3**, *rac*-**4**, *S*-**5**, and *S*-**6**. (b) ^{1}H NMR signals of α-$CH^gH^{g'}$ protons in **1**, *S*-**2**, *S*-**3**, *rac*-**4**, and *S*-**5** and the splitting $\Delta\delta$ in CD_3CN. [**1**] = [*S*-**2**] = [*S*-**3**] = [*rac*-**4**] = [*S*-**5**] = [*S*-**6**] = 2 mM.

in achiral **1** exhibit one set of NMR signal while those in *S*-**2**, *S*-**3**, and *rac*-**4** that are locate adjacent to the chiral β-carbon center are magnetically inequivalent, showing split signals (Figure 2b).[21] However, *S*-**5**, despite chiral too, does not show split signals from the $-CH^gH^{g'}$ protons. The splitting $\Delta\delta$ is therefore not only determined by the adjacent chiral center but also by the stabilization of the turn structure that increases the rigidity of the structure around this methylene group. Comparison of the splitting $\Delta\delta$ of $-CH^gH^{g'}$ protons in **1**−**5** in CD_3CN suggests that the most favorite folded conformation occurs in *S*-**3** (Figure 2b) while the worst one in *S*-**5**. This trend agrees with that shown by the solvent accessibility (Figure 2a). It thus appears that a rigid β-substituent, for example, isopropyl in *S*-**3**, could strengthen the intramolecular hydrogen bond so to enhance the stability of the folded conformation, yet too large a bulky substituent, for example, phenyl in *S*-**5**, may do harm.

Chiral peptides *S*-**2**, *S*-**3**, and *S*-**5** in CH_3CN show the CD signal at *ca.* 270 nm, assigned to the achiral phenylthiourea chromophore by referring to their absorption spectra (Figure S13).[12] This can be expected from the turn structure of these compounds because the 11-membered ring hydrogen bond brings the achiral chromophore into a chiral environment, allowing the chirality of the β-amino acid residue to be transferred to the distant achiral chromophore. The *g* values of *S*-**2**, *S*-**3**, and *S*-**5** at 270 nm, -0.92×10^{-4}, -1.48×10^{-4}, and -0.71×10^{-4}, are comparable (*S*-**2** and *S*-**5**) to or even higher (*S*-**3**) than that (-0.90×10^{-4}) of *S*-**6** (Figure S14), suggesting a considerable efficiency of chirality transfer in the β-amino acid-based short peptides, despite *via* a large, 11-membered ring hydrogen-bonding network.

The β-turn-like folded conformation was found to exist in aqueous solutions too. Variable-temperature ^{1}H NMR experiments show that for **1**, *S*-**2**, *S*-**3**, and *rac*-**4**, the temperature coefficient of the chemical shift of $-NH^d$ is more positive than −4.5 ppb/°C in 90:10 (v/v) H_2O/CD_3CN (Figure S15), suggesting that it remains intramolecularly hydrogen bonded in aqueous solutions while other −NH protons that are intermolecularly hydrogen bonded to water exhibit more negative temperature coefficients (Figure 3a).[22] *S*-**3** shows the

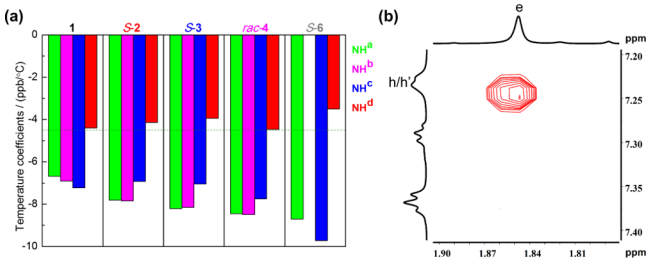

Figure 3. (a) Temperature coefficients of chemical shifts of −NH protons of **1**, *S*-**2**, *S*-**3**, *rac*-**4**, and *S*-**6** in 90:10 (v/v) H_2O/CD_3CN. (b) Partial 2D NOESY spectrum of **1** in the same solvent at 25 °C. [**1**] = [*S*-**2**] = [*S*-**3**] = [*rac*-**4**] = [*S*-**6**] = 2 mM.

most positive temperature coefficient of $-NH^d$ among **1**−**4** (−3.94 ppb/°C, Figure 3a), suggesting that the intramolecular hydrogen bond remains the most stable in aqueous solutions. However, it is weaker than that of *S*-**6** (−3.32 ppb/°C for $-NH^d$, Figure 3a) with a β-turn structure in aqueous solutions. Note that couplings of $CH^e-CH^{h/h'}$ (Figure 3b) and $NH^d-CH^{g/g'}$ (Figures S16 and S17) in 2D NOESY of **1** in 90:10 (v/v) H_2O/CD_3CN were observed that further supports the folded conformation of **1** in aqueous solutions. The 2D NOESY spectrum of *S*-**3** is also indicative of its folded structure in aqueous solutions (Figure S18). Similar experiments with *S*-**5** were not carried out because of limited solubility.

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J. Org. Chem. 2020, 85, 9844−9849

CONCLUSIONS

In conclusion, we demonstrate that acyl β-amino acid is made into folded short peptides by equipping a C-terminal amidothiourea motif. An 11-membered intramolecular ring hydrogen bond is shown to maintain the turn structure in the solid state, in CH_3CN, and in aqueous solution as well. The success with a series of β-amino acids bearing varying β-substituents unveils that an increase in the rigidity of this substituent to some extent leads to enhanced strength of the intramolecular 11-membered ring hydrogen bond, but too large a substituent reduces the stability of the folded conformation. These results establish that the C-terminal amidothiourea motif represents a robust scaffold to promote the folded conformation in short peptides containing the much more flexible β-amino acid residue, even with the most flexible β-alanine residue. Given the potential bioapplications of β-amino acid residues and the feasibility of the structural modification, the current protocol is expected to be of significance in developing functional short peptides bearing a β-amino acid residue.

EXPERIMENTAL SECTION

General Experimental Information. All reagents were purchased from commercial sources and were used without further purification. The solvent for spectroscopy was acetonitrile.

Details of the synthesis of **1** to **5** are given together with full characterization by ^{1}H NMR, ^{13}C NMR, and HRMS. ^{1}H NMR, ^{13}C NMR, COSY, and NOESY spectra were recorded on a Bruker AV500MHz, AV600MHz, or AV850MHz spectrometer; high-resolution mass spectra (HRMS) were acquired on a Bruker En Apex Ultra 7.0 TFT-MS spectrometer. Circular dichroism (CD) spectra were obtained on Jasco J-1500. Absorption spectra were obtained on a Thermo Evolution 300 spectrometer with a 1 cm standard quartz cell. Crystallographic details of **1** are shown in the Supporting Information.

Absorption and CD spectral measurements were carried out using a stock solution of *S*-**2**, *S*-**3**, *S*-**5**, and *S*-**6** in CH_3CN. [*S*-**2**] = [*S*-**3**] = [*S*-**5**] = [*S*-**6**] = 40 μM. 1D and 2D NMR spectra were recorded using a solution of **1**−**6** of 2 mM in CD_3CN or DMSO-d_6 or CD_3CN/DMSO-d_6 mixtures or H_2O/CD_3CN mixtures.

General procedures for preparation of **1**−**5** are given in Schemes S1 and S2.

General Procedure for 1B. **1A** (0.89 g, 10 mmol) was added into 35 mL of EtOH, followed by the slow addition of 2 mL of $SOCl_2$ (28 mmol) under an ice bath and then refluxed in an oil bath overnight. The solvent was removed *via* evaporation *in vacuo* to give **1B**.

General Procedure for 1C. **1B** (1.53 g, 10 mmol) and 35 mL of CH_2Cl_2 were added to a 100 mL round-bottom flask equipped with magnetic stirring, and Et_3N (2.8 mL, 20 mmol) and acetyl chloride (1.6 mL, 20 mmol) were slowly added dropwise. The reaction mixture was stirred at room temperature overnight. The solvent was removed by evaporating *in vacuo* to give a white solid. The solid residue was dissolved in AcOEt, and then the solution was washed successively with 1% $NH_3{\cdot}H_2O$, 1% HCl, and saturated NaCl solutions. After the solution was dried over anhydrous Na_2SO_4 and evaporated *in vacuo*, 1C was obtained. (1.35 g, 85% yield).

General Procedure for 1D. Excess $N_2H_4{\cdot}H_2O$ (85%, 4.0 mL) was added to **1C** in EtOH (40 mL), and the mixture was refluxed in an oil bath for 24 h. The solvent was removed by evaporating *in vacuo* to give a white solid. The solid was washed with CH_3CN and Et_2O several times to afford white solid product **1D** (0.87 g, 60% yield).

General Procedure for 1. Phenyl isothiocyanate (0.50 mL) was added to **1D** (0.19 g, 1 mmol) in CH_3CN (25 mL), and the mixture was refluxed in an oil bath overnight. The solvent was removed by filtration, and the crude product was washed with Et_2O several times to afford pure white solid product **1** (0.224 g, 80% yield), mp 150.0−150.2 °C. ^{1}H NMR (850 MHz, DMSO-d_6): δ (ppm) 9.91 (s, 1H), 9.50 (s, 2H), 7.93 (s, 1H), 7.44 (s, 2H), 7.33 (s, 2H), 7.16 (s, 1H), 3.28 (s, 2H), 2.34 (s, 2H), 1.78 (s, 3H); ^{13}C{^{1}H} NMR (214 MHz, DMSO-d_6): δ (ppm) 180.7, 170.5, 169.3, 139.0, 127.9, 125.8, 125.0, 34.8, 33.7, 22.5; HRMS (ESI) *m*/*z*: [M + Na]$^+$ calcd for $C_{12}H_{16}N_4O_2SNa$, 303.0892; found, 303.0893.

The procedures for the syntheses of *S*-**2**, *rac*-**4**, and *S*-**5** are similar to those of **1**.

General Procedure for *S*-3C. *S*-Boc-**3A** (2.31 g, 10 mmol) was added to 35 mL of EtOH and slowly 2 mL of $SOCl_2$ (28 mmol) was added under an ice bath and then refluxed in an oil bath overnight. The solvent was removed by evaporating *in vacuo* to give *S*-**3C**.

General Procedure for *S*-Boc-3B. CH_2Cl_2 (20 mL) and CF_3COOH (20 mL) were added to the above liquid, and the mixture was reacted at room temperature for 4 h and then dried to give an oily liquid of *S*-3B. CH_2Cl_2 (30 mL) and Et_3N (3 mL) were added to the above oily liquid, and 1 mL of acetyl chloride (12.8 mmol) was slowly added dropwise and stirred overnight, and the solvent was evaporated *in vacuo* to give an oily liquid. The oily liquid was dissolved in AcOEt, and then, the solution was washed successively with 1% $NH_3{\cdot}H_2O$, 1% HCl, and saturated NaCl solutions. After the solution was dried over anhydrous Na_2SO_4 and evaporated *in vacuo*, *S*-**3C** was obtained. (1.51 g, 75% yield).

General Procedure for *S*-3D. Excess $N_2H_4{\cdot}H_2O$ (85%, 4.0 mL) was added to *S*-**3C** in EtOH (40 mL), and the mixture was refluxed in an oil bath for 24 h. The solvent was removed by evaporating *in vacuo* to give a white solid. The solid was washed with CH_3CN and Et_2O several times to afford white solid product *S*-**3D** (1.22 g, 65% yield).

General Procedure for *S*-3. Phenyl isothiocyanate(0.50 mL) was added to *S*-**3D** (0.19 g, 1 mmol) in CH_3CN (25 mL), and the mixture was refluxed in an oil bath overnight. The solvent was removed by filtration, and the crude product was washed with CH_3CN and Et_2O several times to afford pure white solid product *S*-**3** (0.255 g, 79% yield), mp 170.0−170.3 °C. ^{1}H NMR (600 MHz, DMSO-d_6): δ (ppm) 9.92 (s, 1H), 9.58 (s, 1H), 9.32 (s, 1H), 7.73 (s, 1H), 7.47 (s, 2H), 7.33 (s, 2H), 7.15 (s, 1H), 4.04 (s, 1H), 2.39 (d, *J* = 10.4 Hz, 1H), 2.15 (s, 1H), 1.77 (s, 3H), 1.74−1.67 (m, 1H), 0.85 (s, 7H); ^{13}C{^{1}H} NMR (214 MHz, DMSO-d_6): δ (ppm) 180.7, 170.3, 169.3, 139.0, 127.9, 125.6, 124.9, 50.9, 36.9, 31.2, 22.5, 18.9, 18.0; HRMS (ESI) *m*/*z*: [M + Na]$^+$ calcd for $C_{15}H_{22}N_4O_2SNa$, 345.1361; found, 345.1359.

Compound S-2. It is obtained as a white solid; 0.241 g, 82% yield; mp 166.6−166.8 °C. ^{1}H NMR (600 MHz, DMSO-d_6): δ (ppm) 9.92 (s, 1H), 9.54 (s, 1H), 9.47 (s, 1H), 7.86 (s, 1H), 7.46 (d, *J* = 6.9 Hz, 2H), 7.33 (t, *J* = 7.6 Hz, 2H), 7.15 (t, *J* = 6.9 Hz, 1H), 4.14 (dt, *J* = 13.1, 6.5 Hz, 1H), 2.33 (dd, *J* = 14.1, 6.6 Hz, 1H), 2.25 (dd, *J* = 14.1, 7.1 Hz, 1H), 1.76 (s, 3H), 1.09 (d, *J* = 6.5 Hz, 3H); ^{13}C{^{1}H} NMR (214 MHz, DMSO-d_6): δ (ppm) 180.8, 170.1, 168.6, 139.2, 128.0, 125.8, 125.1, 42.1, 41.0, 22.7, 20.5; HRMS (ESI) *m*/*z*: [M + Na]$^+$ calcd for $C_{13}H_{18}N_4O_2SNa$, 317.1048; found, 317.1049.

Compound rac-4. It is obtained as a white solid; 0.253 g, 79% yield; mp 197.6−198.1 °C. ^{1}H NMR (600 MHz, DMSO-d_6): δ (ppm) 9.94 (s, 1H), 9.57 (s, 1H), 9.36 (s, 1H), 7.93 (s, 1H), 7.46 (d, *J* = 7.5 Hz, 2H), 7.32 (t, *J* = 7.8 Hz, 2H), 7.15 (t, *J* = 7.2 Hz, 1H), 3.61 (dt, *J* = 15.6, 8.0 Hz, 1H), 2.48−2.43 (m, 1H), 2.33 (dd, *J* = 13.9, 8.3 Hz, 1H), 1.76 (s, 3H), 0.92 (dd, *J* = 12.4, 4.5 Hz, 1H), 0.42 (d, *J* = 8.3 Hz, 1H), 0.36 (d, *J* = 7.1 Hz, 1H), 0.23 (dd, *J* = 10.7, 5.8 Hz, 2H); ^{13}C{^{1}H} NMR (214 MHz, DMSO-d_6): δ (ppm) 180.8, 170.0, 168.9, 139.2, 128.0, 125.7, 125.1, 49.9, 22.7, 15.8, 3.2, 2.9; HRMS (ESI) *m*/*z*: [M + Na]$^+$ calcd for $C_{15}H_{20}N_4O_2SNa$, 343.1205; found, 343.1205.

Compound S-5. It is obtained as a white solid; 0.289 g, 81% yield; mp 209.0−209.3 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ (ppm) 10.01 (s, 1H), 9.63 (s, 1H), 9.27 (s, 1H), 8.38 (d, *J* = 7.4 Hz, 1H), 7.43 (d, *J* = 7.4 Hz, 2H), 7.37−7.28 (m, 7H), 7.24−7.14 (m, 3H), 5.29 (dd, *J* = 15.1, 8.5 Hz, 1H), 2.64 (dd, *J* = 18.3, 7.4 Hz, 2H), 1.79 (s, 3H); ^{13}C{^{1}H} NMR (214 MHz, DMSO-d_6): δ (ppm) 180.7, 169.1, 168.5, 142.6, 138.9, 128.2, 127.9, 126.8, 126.3, 125.7, 125.1, 49.2, 40.5, 22.5; HRMS (ESI) *m*/*z*: [M + Na]$^+$ calcd for $C_{18}H_{20}N_4O_2SNa$, 379.1205; found, 379.1198.

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J. Org. Chem. 2020, 85, 9844−9849

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.joc.0c01139.

Detailed synthetic procedures, characterization, crystal data, spectral analysis, and others (PDF)

Crystallographic information for **1** (CCDC 1964197) (CIF)

General procedures for the syntheses of **1**, *S*-**2**, *rac*-**4** and *S*-**5**, general procedures for the syntheses of *S*-**3**, ORTEP diagram for crystal structure of compound **1**, crystallographic data and structure refinement for **1**, bond lengths and bond angles of intramolecular hydrogen bonds revealed by X-ray crystal structures of **1** and *S*-**6**, backbone torsional angles observed in the X-ray crystal structure of **1**, intermolecular hydrogen bonds of one molecule of **1** with adjacent three molecules, 2D COSY spectrum of **1**, 2D NOESY spectrum of **1**, influence of DMSO-d_6 volume fraction, chemical shifts, partial 2D NOESY spectra of **1**, crystal structures of *S*-**6** and **1**, solvent accessibility of −NH protons, absorption (a) and CD (b) spectra, anisotropy *g* factors of *S*-**2**, *S*-**3**, *S*-**5**, and *S*-**6** in CH_3CN, and influence of temperature on −NH proton resonances (PDF)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) (a) Rose, G. D.; Glerasch, L. M.; Smith, J. A. Turns in Peptides and Proteins. *Adv. Protein Chem.* **1985**, *37*, 1−109. (b) Hruby, V.; Balse, P. Conformational and Topographical Considerations in Designing Agonist Peptidomimetics from Peptide Leads. *Curr. Med. Chem.* **2000**, *7*, 945−970. (c) Tyndall, J. D. A.; Pfeiffer, B.; Abbenante, G.; Fairlie, D. P. Over One Hundred Peptide-Activated G Protein-Coupled Receptors Recognize Ligands with Turn Structure. *Chem. Rev.* **2005**, *105*, 793−826.

(2) (a) Blank, J. T.; Miller, S. J. Studies of folded peptide-based catalysts for asymmetric organic synthesis. *Biopolymers* **2006**, *84*, 38−47. (b) Shults, M. D.; Imperiali, B. Versatile Fluorescence Probes of Protein Kinase Activity. *J. Am. Chem. Soc.* **2003**, *125*, 14248−14249. (c) Proulx, C.; Sabatino, D.; Hopewell, R.; Spiegel, J.; García Ramos, Y.; Lubell, W. D. Azapeptides and their therapeutic potential. *Future Med. Chem.* **2011**, *3*, 1139−1164. (d) Metrano, A. J.; Abascal, N. C.; Mercado, B. Q.; Paulson, E. K.; Hurtley, A. E.; Miller, S. J. Diversity of Secondary Structure in Catalytic Peptides with β-Turn-Biased Sequences. *J. Am. Chem. Soc.* **2017**, *139*, 492−516.

(3) Lewis, P. N.; Momany, F. A.; Scheraga, H. A. Folding of Polypeptide Chains in Proteins: A Proposed Mechanism for Folding. *Proc. Natl. Acad. Sci. U.S.A.* **1971**, *68*, 2293−2297.

(4) (a) Hutchinson, E. G.; Thornton, J. M. A revised set of potentials for β-turn formation in proteins. *Prot. Sci.* **1994**, *3*, 2207−2216. (b) Fu, H.; Grimsley, G. R.; Razvi, A.; Scholtz, J. M.; Pace, C. N. Increasing protein stability by improving beta-turns. *Proteins* **2009**, *77*, 491−498.

(5) (a) Reynolds, C. H.; Hormann, R. E. Theoretical Study of the Structure and Rotational Flexibility of Diacylhydrazines: Implications for the Structure of Nonsteroidal Ecdysone Agonists and Azapeptides. *J. Am. Chem. Soc.* **1996**, *118*, 9395−9401. (b) Lee, H.-J.; Song, J.-W.; Choi, Y.-S.; Park, H.-M.; Lee, K.-B. A theoretical study of conformational properties of N-methyl azapeptide derivatives. *J. Am. Chem. Soc.* **2002**, *124*, 11881−11893.

(6) (a) Cheng, R. P.; Gellman, S. H.; DeGrado, W. F. β-Peptides: From Structure to Function. *Chem. Rev.* **2001**, *101*, 3219−3232. (b) Seebach, D.; Gardiner, J. β-Peptidic Peptidomimetics. *Acc. Chem. Res.* **2008**, *41*, 1366−1375. (c) Laursen, J. S.; Engel-Andreasen, J.; Olsen, C. A. β-Peptoid Foldamers at Last. *Acc. Chem. Res.* **2015**, *48*, 2696−2704.

(7) (a) Appella, D. H.; Christianson, L. A.; Klein, D. A.; Powell, D. R.; Huang, X.; Barchi, J. J.; Gellman, S. H. Residue-based control of helix shape in β-peptide oligomers. *Nature* **1997**, *387*, 381−384. (b) Berlicki, Ł.; Pilsl, L.; Wéber, E.; Mándity, I. M.; Cabrele, C.; Martinek, T. A.; Fülöp, F.; Reiser, O. Unique α,β- and α,α,β,β-Peptide Foldamers Based on cis-β-Aminocyclopentanecarboxylic Acid. *Angew. Chem., Int. Ed.* **2012**, *51*, 2208−2212.

(8) Kale, S. S.; Priya, G.; Kotmale, A. S.; Gawade, R. L.; Puranik, V. G.; Rajamohanan, P. R.; Sanjayan, G. J. Orthanilic acid-promoted reverse turn formation in peptides. *Chem. Commun.* **2013**, *49*, 2222−2224.

(9) (a) Yang, D.; Ng, F.-F.; Li, Z.-J.; Wu, Y.-D.; Chan, K. W. K.; Wang, D.-P. An Unusual Turn Structure in Peptides Containing α-Aminoxy Acids. *J. Am. Chem. Soc.* **1996**, *118*, 9794−9795. (b) Li, X.;

Wu, Y.-D.; Yang, D. α-Aminoxy acids: New possibilities from foldamers to anion receptors and channels. *Acc. Chem. Res.* **2008**, *41*, 1428−1438.
(10) Günther, R.; Hofmann, H.-J. Hydrazino Peptides as Foldamers: An Extension of the β-Peptide Concept. *J. Am. Chem. Soc.* **2001**, *123*, 247−255.
(11) (a) Seebach, D.; Beck, A.; Capone, S.; Deniau, G.; Grošelj, U.; Zass, E. Enantioselective Preparation of β^2-Amino Acid Derivatives for β-Peptide Synthesis. *Synthesis* **2009**, 1−32. (b) Kiss, L.; Fülöp, F. Synthesis of Carbocyclic and Heterocyclic β-Aminocarboxylic Acids. *Chem. Rev.* **2014**, *114*, 1116−1169.
(12) (a) Yan, X.-S.; Wu, K.; Yuan, Y.; Zhan, Y.; Wang, J.-H.; Li, Z.; Jiang, Y.-B. β-Turn structure in glycinylphenylalanine dipeptide based *N*-amidothioureas. *Chem. Commun.* **2013**, *49*, 8943−8945. (b) Yan, X.-S.; Luo, H.; Zou, K.-S.; Cao, J.-L.; Li, Z.; Jiang, Y.-B. Short Azapeptides of Folded Structures in Aqueous Solutions. *ACS Omega* **2018**, *3*, 4786−4790.
(13) Yuan, Y.; Yan, X.-S.; Li, X.-R.; Cao, J.-L.; Li, Z.; Jiang, Y.-B. Folded short azapeptide for conformation switching-based fluorescence sensing. *Chem. Commun.* **2017**, *53*, 13137−13140.
(14) (a) Cao, J.; Yan, X.; He, W.; Li, X.; Li, Z.; Mo, Y.; Liu, M.; Jiang, Y.-B. C−I···π Halogen Bonding Driven Supramolecular Helix of Bilateral *N*-Amidothioureas Bearing β-Turns. *J. Am. Chem. Soc.* **2017**, *139*, 6605−6610. (b) Yan, X.; Zou, K.; Cao, J.; Li, X.; Zhao, Z.; Li, Z.; Wu, A.; Liang, W.; Mo, Y.; Jiang, Y. Single-handed supramolecular double helix of homochiral bis(*N*-amidothiourea) supported by double crossed C-I···S halogen bonds. *Nat. Commun.* **2019**, *10*, 3610.
(15) (a) Choudhary, A.; Gandla, D.; Krow, G. R.; Raines, R. T. Nature of Amide Carbonyl−Carbonyl Interactions in Proteins. *J. Am. Chem. Soc.* **2009**, *131*, 7244−7246. (b) Newberry, R. W.; VanVeller, B.; Guzei, I. A.; Raines, R. T. n→π* Interactions of Amides and Thioamides: Implications for Protein Stability. *J. Am. Chem. Soc.* **2013**, *135*, 7843−7846.
(16) Laursen, J. S.; Engel-Andreasen, J.; Fristrup, P.; Harris, P.; Olsen, C. A. *Cis*−*Trans* Amide Bond Rotamers in β-Peptoids and Peptoids: Evaluation of Stereoelectronic Effects in Backbone and Side Chains. *J. Am. Chem. Soc.* **2013**, *135*, 2835−2844.
(17) (a) Huang, Y.; Jahreis, G.; Fischer, G.; Lücke, C. Atomic Polarizability Dominates the Electronic Properties of Peptide Bonds upon Thioxo or Selenoxo Substitution. *Chem.—Eur. J.* **2012**, *18*, 9841−9848. (b) Nelli, Y. R.; Antunes, S.; Salaün, A.; Thinon, E.; Massip, S.; Kauffmann, B.; Douat, C.; Guichard, G. Isosteric Substitutions of Urea to Thiourea and Selenourea in Aliphatic Oligourea Foldamers: Site-Specific Perturbation of the Helix Geometry. *Chem.—Eur. J.* **2015**, *21*, 2870−2880.
(18) (a) Kuhn, B.; Mohr, P.; Stahl, M. Intramolecular Hydrogen Bonding in Medicinal Chemistry. *J. Med. Chem.* **2010**, *53*, 2601−2611. (b) Caron, G.; Kihlberg, J.; Ermondi, G. Intramolecular hydrogen bonding: An opportunity for improved design in medicinal chemistry. *Med. Res. Rev.* **2019**, *39*, 1707−1729.
(19) (a) Hinderaker, M. P.; Raines, R. T. An electronic effect on protein structure. *Protein Sci.* **2003**, *12*, 1188−1194. (b) Bartlett, G. J.; Choudhary, A.; Raines, R. T.; Woolfson, D. N. n→π* interactions in proteins. *Nat. Chem. Biol.* **2010**, *6*, 615−620.
(20) (a) André, F.; Vicherat, A.; Boussard, G.; Aubry, A.; Marraud, M. Aza-peptides. III. Experimental structural analysis of aza-alanme and aza-asparagine-containing peptides. *J. Pept. Res.* **1997**, *50*, 372−381. (b) Copeland, G. T.; Jarvo, E. R.; Miller, S. J. Minimal Acylase-Like Peptides. Conformational Control of Absolute Stereospecificity. *J. Org. Chem.* **1998**, *63*, 6784−6785.
(21) (a) Solà, J.; Helliwell, M.; Clayden, J. N- versus C-Terminal Control over the Screw-Sense Preference of the Configurationally Achiral, Conformationally Helical Peptide Motif Aib8GlyAib8. *J. Am. Chem. Soc.* **2010**, *132*, 4548−4549. (b) De Poli, M.; Zawodny, W.; Quinonero, O.; Lorch, M.; Webb, S. J.; Clayden, J. Conformational photoswitching of a synthetic peptide foldamer bound within a phospholipid bilayer. *Science* **2016**, *352*, 575−580.
(22) (a) Baxter, N. J.; Williamson, M. P. Temperature dependence of 1H chemical shifts in proteins. *J. Biomol. NMR* **1997**, *9*, 359−369. (b) Cierpicki, T.; Otlewski, J. Amide proton temperature coefficients as hydrogen bond indicators in proteins. *J. Biomol. NMR* **2001**, *21*, 249−261. (c) Bomar, M. G.; Song, B.; Kibler, P.; Kodukula, K.; Galande, A. K. An Enhanced β Turn in Water. *Org. Lett.* **2011**, *13*, 5878−5881.

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