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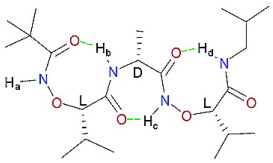

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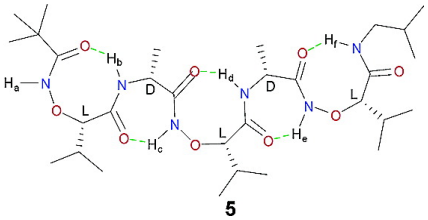

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A New Strategy to Induce γ-Turns: Peptides Composed of Alternating α-Aminoxy Acids and α-Amino Acids

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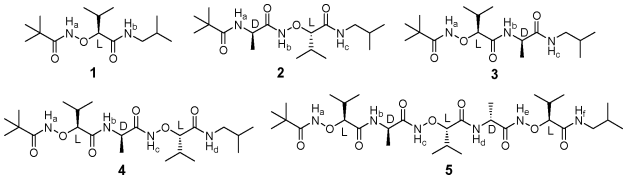
As one type of reversed turn secondary structures of proteins, γ-turns are formed by a 3→1 hydrogen bond between the CO group of amino acid residue *i* and the NH group of amino acid residue *i* + 2.[1] While γ-turns are less frequently observed in proteins than β-turns,[1c] they have been shown to play important roles in biological recognition.[2] For example, the γ-turn present in RGD (Arg-Gly-Asp) sequence of vitronectin was found to contribute to the specific recognition by integrin receptor $\alpha_V\beta_3$.[3] However, it has been challenging to investigate the roles of γ-turns in protein−peptide recognition because they seldom exist in short, linear peptides. Most of the γ-turn mimics constrain the peptide conformation through ring formation.[4,5] Here we report that the γ-turns can be initiated by the following N−O turns, an eight-membered-ring intramolecular hydrogen bond induced by an α-aminoxy acid.[6]



Peptides **1**−**5** composed of alternating L-aminoxy valine and D-alanine were prepared according to a convergent synthetic scheme. In **2** and **3**, an α-amino acid was placed before or after an α-aminoxy acid, whereas in **4** and **5**, each α-amino acid was sandwiched by two adjacent α-aminoxy acids.

Chemical shifts of amide NHs of **1**−**5** taken in CD_2Cl_2 at very low concentration (1−2 mM) are summarized in Table 1. For the regular amides, only the NH_a of dipeptide **2** and NH_c of dipeptide **3** fell in the range of non-hydrogen-bonded amide protons. In contrast, those regular amide NHs immediately after an α-aminoxy acid residue in **1**−**5** were downfield shifted by about 2 ppm, suggesting that they form strong intramolecular hydrogen bonds, most likely the N−O turns. For the *N*-terminal aminoxy amide NHs of peptides **1** and **3**−**5**, they appeared in the range of 8.36−8.48 ppm, assigned to be non-hydrogen-bonded NHs. The unusually downfield chemical shifts of the other aminoxy amide protons (δ 9.63−9.83 ppm) suggested the formation of seven-membered-ring intramolecular hydrogen bonds (the γ-turn) between aminoxy amide NH_i and $C{=}O_{i-2}$. The higher acidity of aminoxy amide protons may contribute to the formation of the observed γ-turns.

^{1}H NMR studies of peptides **1**−**5** were also carried out at 10 mM in CD_3OH at −30 °C (Table 1). For each peptide, the hydrogen-bonding patterns in CD_3OH were found to be similar to those in nonpolar solvent CD_2Cl_2. The chemical shifts of NH_a of **1** and **3**−**5** appeared at about 11.45 ppm, more downfield than in CD_2Cl_2 due to the formation of intermolecular hydrogen bond to CD_3OH, whereas the peaks of other aminoxy amide protons were downfield shifted to 11.9 ppm. Furthermore, after solvent suppression, the peaks of non-hydrogen-bonded aminoxy amide NH_a disappeared due to the fast exchange with solvent, while the signals of hydrogen-bonded aminoxy amides still remained but became weaker. As to the regular amide NHs, the chemical shifts of NH_a of **2** and NH_c of **3** were rather upfield, assigned to be free amides. Other regular amide NHs were all downfield shifted by more than 0.3 ppm compared to the free amide NH_c of **3**. These data suggested that the alternating N−O turns and γ-turns still exist in methanol.

Table 1. Chemical Shifts of Amide NHs of **1**−**5** in CD_2Cl_2 at 25 °C at Low Concentration (1−2 mM), and in CD_3OH (10 mM) at −30 °C[a]

	δH_a		δH_b		δH_c		δH_d		δH_e		δH_f	
	CD_2Cl_2	CD_3OH	CD_2Cl_2	CD_3OH	CD_2Cl_2	CD_3OH	CD_2Cl_2	CD_3OH	CD_2Cl_2	CD_3OH	CD_2Cl_2	CD_3OH
1	8.36	11.46	8.15	8.94								
2	5.91	8.01	9.63	11.89	7.94	8.90						
3	8.46	11.46	8.60	9.01	6.37	8.55						
4	8.45	11.42	8.82	9.11	9.66	11.93	8.07	8.86				
5	8.48	11.44	8.85	9.31	9.83	11.92	8.45	8.93	9.74	11.89	8.02	8.85

[a] Aminoxy amide NHs are underlined.

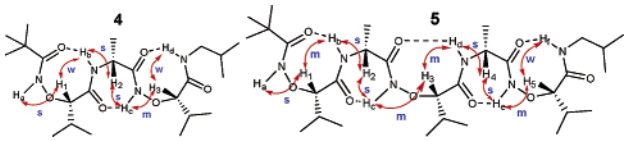


Figure 1. Summary of NOEs observed of compounds **4** and **5** at 25 °C (5 mM in CD_2Cl_2; s, strong NOE; m, medium NOE; w, weak NOE).

NOESY results of oligomers **4** and **5** in CD_2Cl_2 are summarized in Figure 1. For each α-D-alanine residue, similar intensities of NOE between $C_\alpha H_i$ and NH_i and those between $C_\alpha H_i$ and NH_{i+1} suggested that the α-proton takes an axial position in the γ-turn, resulting in an inverse γ-turn conformation.[1c,7] As to the aminoxy acid residues, according to our previous theoretical calculation, $C_\alpha-C_\beta$ bond is anti to the N−O bond in the most stable N−O turn conformation; thus, stronger NOE between NH_i and $C_\alpha H_i$ than that between NH_{i+1} and $C_\alpha H_i$ is expected.[6c,d] This was indeed observed for the first and the last α-L-aminoxy acid residues of **4** and **5**. Interestingly, another conformer of the left-handed N−O turn was observed in the middle α-L-aminoxy acid residue of pentapeptide **5**, in which the NOE between H_c and H_3 was of similar intensity to that of the NOE between H_3 and H_d. This NOE pattern agreed with the second lowest-energy N−O turn conformation revealed by theoretical calculations.[6d] In this conformer, the $O-C_\alpha$ bond was rotated slightly with the $C_\alpha-C_\beta$ bond gauche to the N−O bond. This conformational change probably rendered the two α-methyl

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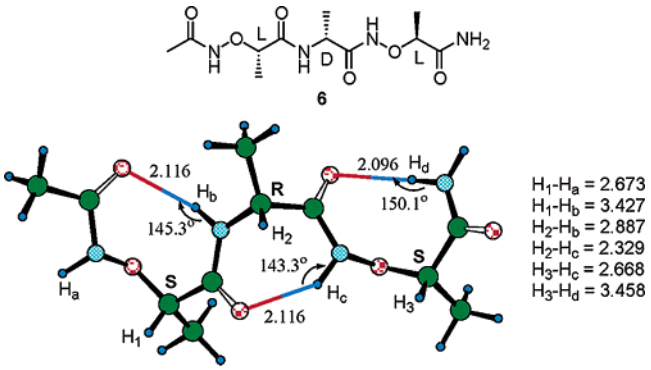

Figure 2. Calculated, most stable conformation of compound **6** in CH_2Cl_2. Interatomic distances are in Å.

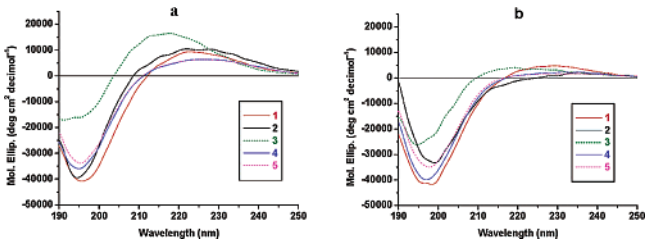

Figure 3. CD spectra of compounds **1**−**5** at 25 °C: (a) 0.4 mM in 2,2,2-trifluoroethanol; (b) 4.0 mM in methanol.

groups of the α-D-alanine residues parallel to each other so as to alleviate their steric repulsions.

Theoretical calculations have been carried out on several di-, tri-, and tetra-peptide models (see SI for detailes). These peptides have a stong tendency to form alternating N−O turn and γ-turn. Figure 2 shows the most stable conformation of model compound **6** in CH_2Cl_2. In the alanine residue, the α-methyl group took an equatorial position, and the dihedral angles (φ,ψ) for the γ-turn are (83.8, −88.1), characteristic of an inverse γ-turn.[1c,7] For the aminoxy acid residues, the $C_\alpha-C_\beta$ bond is anti to the N−O bond. The calculated short O- - -H distances and large O- - -H−N angles indicate the formation of strong hydrogen bonds. The calculated interatomic H/H distances agree well with the observed NOE pattern for tripeptide **4**. Overall, the three turns form an extended helix structure.

CD spectra of compounds **1**−**5** taken at room temperature in 2,2,2-trifluoroethanol are shown in Figure 3a. Because α-aminoxy acid backbone is predisposed to the N−O turn formation and the energy cost to initiate the secondary structure is lower than that of an α-amino acid, α-aminoxy acid residues are expected to make a major contribution to the CD absorption. Thus, the CD signals were normalized for the concentration and the number of backbone N−O turns of each compound. Peptide **3** showed a different CD curve compared with others, possibly because the γ-turn between NH_c and C═O of aminoxy acid of **3** was not formed as revealed by the above ^{1}H NMR studies. The CD curves of oligomers **4** and **5** were almost superimposable to each other with a maximum at 227 nm, a minimum at 195 nm, and a zero crossing at 211 nm, suggesting that oligomers **4** and **5** adopt the same type of secondary structure, a novel mixed 7−8 helix. CD curves of peptides **1**−**5** in methanol (Figure 3b) showed patterns similar to those in 2,2,2-trifluoroethanol, suggesting the secondary structures remain unchanged in methanol.

In summary, the conformational studies suggested that oligomers **4** and **5** form N−O turns and γ-turns simultaneously in solution, even in protic solvent methanol. ^{1}H NMR studies of peptides **2** and **3** implied that the γ-turn could only be initiated by the following N−O turn,[8] which means that this hydrogen bond must involve an acidic aminoxy amide NH. Therefore, we have developed a new strategy to induce a γ-turn at specific sites of short peptides by putting an α-aminoxy acid immediately after the particular α-amino acid of interest.

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Supporting Information Available: Synthetic scheme and characterization data of compounds **1**−**5**; ^{1}H NMR studies of **4** and **5**; ^{1}H NMR spectra of **1**−**5**; 2D NOESY spectra of **4** and **5**; theoretical calculations (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(7) The inverse γ-turn is identified by an equatorial $i+1$ side chain orientation while the classical γ-turn contains an axial $i+1$ side chain. For D-amino acids, the inverse γ-turn with (φ,ψ) values generally appear in the range (70 to 95, −75 to −45).

(8) For the corresponding L,L-isomer of **2**, a γ-turn was also found. Unpublished results.

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