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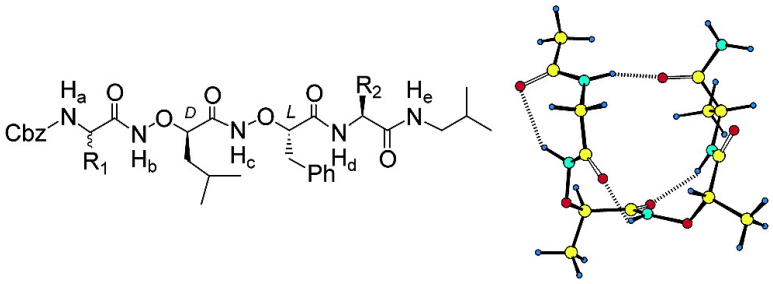
J. Am. Chem. Soc., **2003**, 125 (47), 14452-14457• DOI: 10.1021/ja029514j • Publication Date (Web): 29 October 2003



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A Reverse Turn Structure Induced by a D,L-α-Aminoxy Acid Dimer

Dan Yang,*,† Jin Qu,† Wei Li,† De-Ping Wang,‡ Yi Ren,‡ and Yun-Dong Wu*,‡

Contribution from the Department of Chemistry, The University of Hong Kong, Pokfulam Road, Hong Kong, and Department of Chemistry, The Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong, P. R. China

Received November 28, 2002; E-mail: yangdan@hku.hk; chydwu@ust.hk

Abstract: Our previous work revealed that two adjacent D-α-aminoxy acids could form two homochiral N−O turns, with the backbone folding into an extended helical structure (1.8_8-helix). Here, we report the conformational studies of linear peptides **3**−**6**, which contain a D,L-α-aminoxy acid dimer segment. The NMR and X-ray analysis of **3** showed that it folded into a loop conformation with two heterochiral N−O turns. This loop segment can be used to constrain tetrapeptides **4** and **6** to form a reverse turn structure. ^{1}H NMR dilution studies, DMSO-d_6 addition studies, and 2D-NOESY data indicated that tetrapeptides **4** and **6** folded into reverse turn conformations featured by a head-to-tail 16-membered-ring intramolecular hydrogen bond. In contrast, tetrapeptide **5** with L-Ala instead of Gly or D-Ala as the N-terminal amino acid could not form the desired reverse turn structure for steric reasons. Quantum mechanics calculations showed that model pentamide **7**, with the same substitution pattern of **4**, adopted a novel reverse turn conformation featuring two heterochiral N−O turns (each of an 8-membered ring hydrogen bond), a cross-strand 16-membered ring hydrogen bond, and a 7-membered ring γ-turn.

Introduction

Reverse turns, in which the peptide chain reverses its direction, are commonly observed secondary structures of proteins and peptides and play important roles in protein folding and receptor binding.[1] The most common type is the β-turn, defined by four residues at positions designated i to $i + 3$. Significant efforts have been made to search for mimics of various types of β-turns to modulate protein−protein and protein−peptide interactions.[2] Most of those β-turn mimics contain fused rings or macrocyclic structures to constrain conformations.[3] Recently, significant progress has been made in building reverse turns and hairpins using linear oligomers of unnatural units.[4−7] Seebach and co-workers reported an oligomer of acyclic β-amino acids adopted hairpin conformations in methanol.[4a] A reverse turn structure was observed by Gellman and co-workers in peptides containing a (*R*,*S*)-dinipecotic acid segment,[4c−f] which could promote the formation of hairpins in the adjacent residues. In addition, the configurations of the central two β-amino acids were critical to the stability of the reverse turn and hairpin structures. A D,L,D,L-tetrapyrrolinone designed by Smith and Hirschman was also shown to adopt a reverse turn conformation.[6] Here, we report that a D,L-α-aminoxy acid dimer segment can induce a novel reverse turn structure in the peptide backbone.

† The University of Hong Kong.

‡ The Hong Kong University of Science and Technology.

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10.1021/ja029514j CCC: $25.00

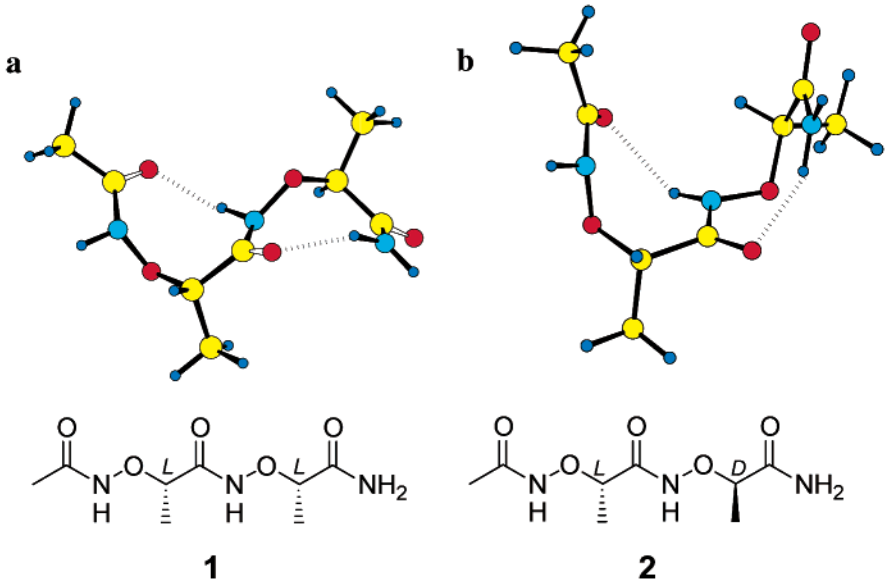

Figure 1. The calculated conformations of (a) two homochiral (L−L) N−O turns (**1**), and (b) two heterochiral (L−D) N−O turns (**2**).

Results and Discussion

Our early works on peptides of α-aminoxy acids revealed that they had a strong tendency to form an eight-membered-ring intramolecular hydrogen bond between adjacent residues (referred to as the N−O turn).[8] It was established that D-α-aminoxy acid induced a right-handed N−O turn while the L-enantiomer induced a left-handed N−O turn.[8] For dipeptide **1** with two consecutive α-aminoxy acids of the same configuration, the backbone folded into an extended helical structure (1.8_8-helix) with two homochiral N−O turns (Figure 1a).[9] Yet for dipeptide **2** with two heterochiral α-aminoxy acids, it is expected that the second N−O turn would bend backward, resulting in a loop conformation (Figure 1b). Theoretical calculations indeed revealed that the loop conformation of **2** was more stable than its helical counterpart (**2′** in the Supporting Information) by about 0.6 kcal/mol both in the gas phase and in CH_2Cl_2 solution.[10]

^{1}H NMR studies of triamide **3**, with a D-aminoxy leucine followed by an L-aminoxy phenylalanine (Figure 2), indicated that NH_b and NH_c were intramolecularly hydrogen-bonded because their chemical shifts changed little upon dilution with $CDCl_3$ or the addition of DMSO-d_6 to the $CDCl_3$ solution.[10] On the contrary, NH_a was solvent accessible because its chemical shift changed drastically. The NOESY spectrum of **3** displayed a NOE pattern similar to that observed in two consecutive homochiral N−O turns:[7a] strong NOEs between NH_i and αCH_i, but weak NOEs between NH_{i+1} and αCH_i (Figure 2a). Therefore, triamide **3** also preferred a conformation with two consecutive N−O turns in solution.

The X-ray analysis of triamide **3** confirmed the predicted loop conformation with two heterochiral N−O turns (Figure 2b).[10] The short NH- -O═C distances and the ideal N−H- -O angles of the two intramolecular hydrogen bonds in **3** (2.14 Å/156° for the first hydrogen bond from the N-terminus and 2.04 Å/157° for the second one) were comparable to those observed in two consecutive homochiral N−O turns.[8] The distance between $\alpha C_{(Piv)}$ and $\alpha C_{(i-Bu)}$ (6.23 Å) was below 7 Å. It was thus expected that the segment of two heterochiral N−O turns could be used to build a reverse turn structure.

Tetrapeptide **4** with one amino acid added to each end of the above loop segment was prepared and subjected to the ^{1}H NMR dilution and the DMSO-d_6 addition studies (Figure 3). In non-hydrogen-bonding solvents such as $CDCl_3$, a solvent accessible α-aminoxy amide NH usually appears in the range of 8.3−9.0 ppm, whereas a hydrogen-bonded NH falls in the range of 10.3−12.2 ppm.[8,9] As to normal α-peptide amide NH's, the non-hydrogen-bonded NH generally appears at about 5−6 ppm, while the hydrogen-bonded NH typically appears at about 7−8

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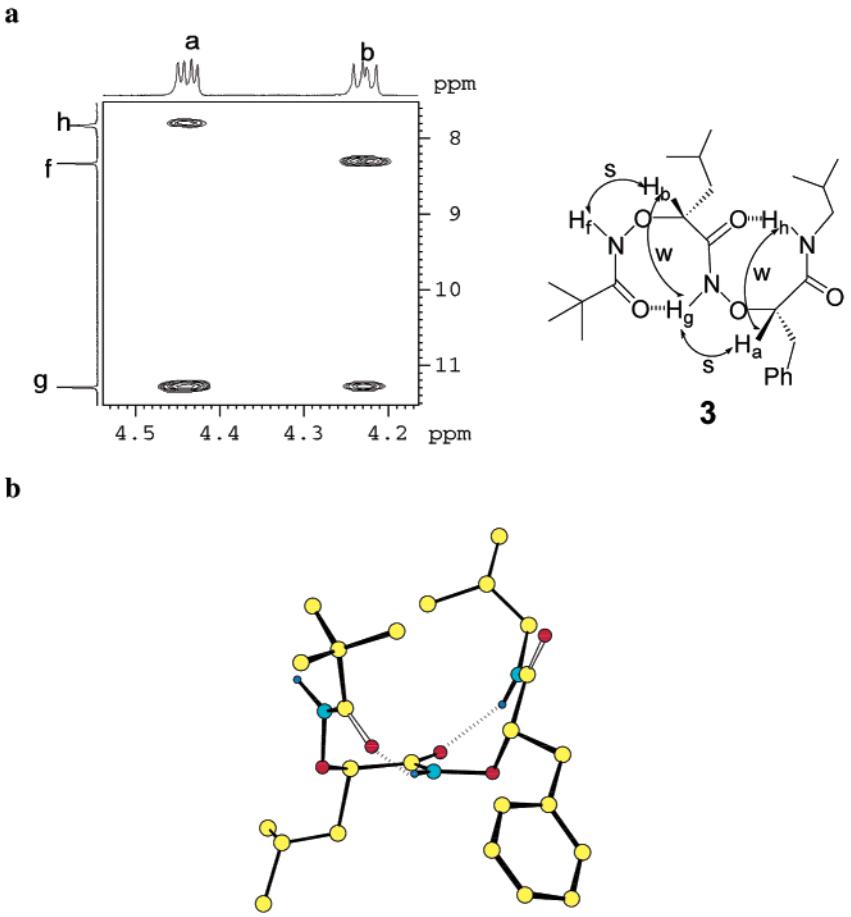

Figure 2. (a) Portion of the NOESY spectrum of **3** and the summary of NOEs observed (3 mM in $CDCl_3$ at 25 °C; s, strong NOE; w, weak NOE; all proton signals were assigned through the combined use of COSY and HMBC spectra). (b) Front view of the X-ray structure of **3**. (Except for three amide hydrogen atoms, all hydrogen atoms were omitted for clarity. The intramolecular hydrogen bonds were indicated as dashed lines.)

ppm.[11] One exception is the N-terminal carbamate NH such as Cbz protected NH. Because of the electron-donating feature of the Cbz group, solvent accessible carbamate NH's typically appear at about 5 ppm, while hydrogen-bonded NH's generally appear at about 6 ppm.[12] When the above criteria were applied to analyze the upfield limiting chemical shifts of amide NH's of tetrapeptide **4** at 1 mM concentration in $CDCl_3$ (Table 1),[13] the amides NH_c and NH_d, which appeared at about 11 and 8 ppm, respectively, were found to be intramolecularly hydrogen-bonded, whereas NH_e at about 6 ppm was a free amide NH. NH_b at about 9.5 ppm, between those hydrogen-bonded and non-hydrogen-bonded aminoxy amide NH's, was thus partially hydrogen-bonded. The N-terminal Cbz protected carbamate NH_a appeared at about 6 ppm, implying that it formed an intramolecular hydrogen bond.

It should be pointed out that aminoxy amide protons were more sensitive (with relatively larger $\Delta\delta_{NH}$ values) than normal amide protons in the ^1H NMR dilution and DMSO-d_6 addition studies because the aminoxy amide protons are much better hydrogen-bond donors. Therefore, different standards should be applied to analyze the $\Delta\delta_{NH}$ values for two kinds of amide NH's. The small $\Delta\delta_{NH}$ for NH_a, NH_c, and NH_d in **4** suggested that they are involved in intramolecular hydrogen bonds. The relatively large $\Delta\delta_{NH}$ for NH_e in **4** implied that it was solvent accessible. The $\Delta\delta_{NH}$ value observed for aminoxy NH_b was larger than that of aminoxy NH_c in the same molecule but was smaller than or comparable to that of the free aminoxy amide NH_a found in **3** ($\Delta\delta_{NH}$ = 0.83 ppm in the dilution study and $\Delta\delta_{NH}$ = 2.17 ppm in the DMSO-d_6 addition study), indicating that it was partially hydrogen-bonded. Accordingly, as compared with those assignments made directly by using the upfield limiting chemical shifts of amide NH's, the ^1H NMR dilution and DMSO-d_6 addition studies of **4** provided similar assignments of hydrogen-bonded and non-hydrogen-bonded amide NH's.

The observation on NH_c and NH_d of **4** could be readily explained because they were involved in two heterochiral N−O turns as in triamide **3**. The finding that NH_a instead of NH_e was intramolecularly hydrogen-bonded indicated that **4** adopted the expected reverse turn conformation, featuring a 16-membered-ring hydrogen bond between NH_a and the C-terminal L-Ala carbonyl group rather than an 18-membered-ring hydrogen bond between NH_e and the N-terminal Cbz carbonyl group.

More information on the conformation of tetrapeptide **4** was obtained from the NOESY experiment (Figure 4a). The NOE pattern for the D,L-aminoxy acid portion of **4** matched very well with that of triamide **3**, suggesting that this portion maintains the conformation of two heterochiral N−O turns. The observa-

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(13) The chemical shift changes for amide protons of **4** in the concentration range of 0.1−1 mM were below 0.02 ppm, indicating that no aggregation of **4** occurred at 1 mM (see Supporting Information).

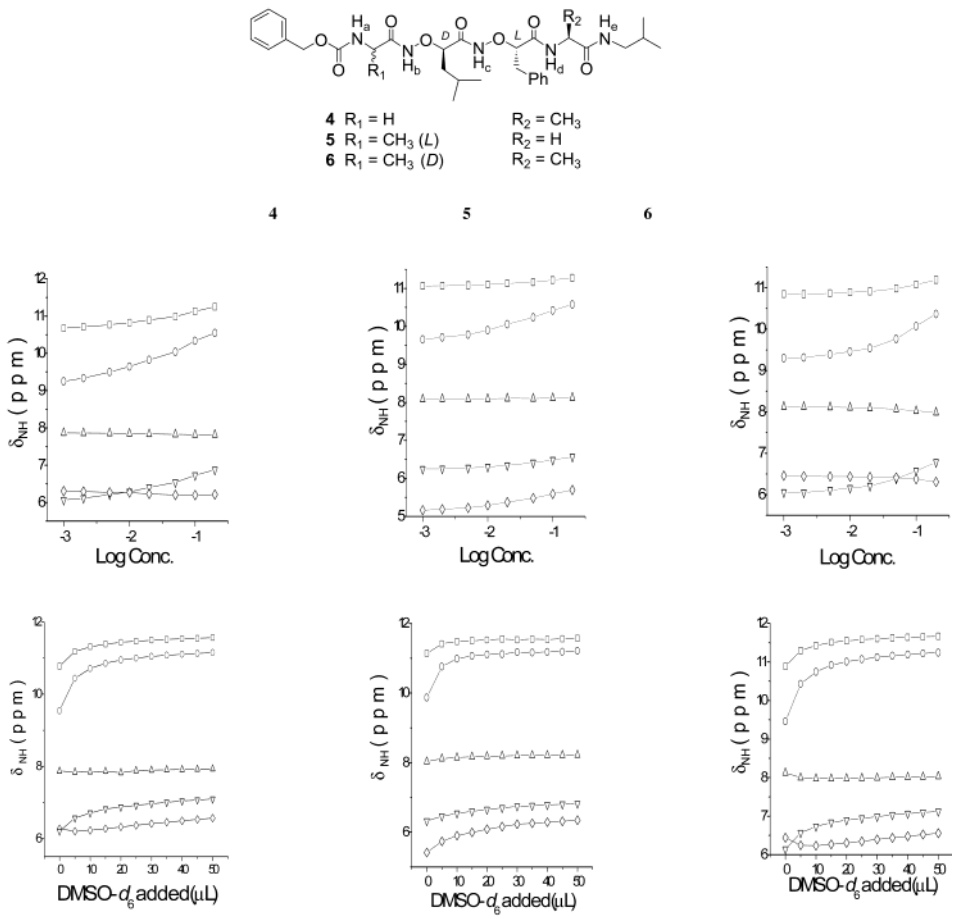

Figure 3. First row: amide proton chemical shifts as a function of the logarithm of the concentration in tetrapeptides **4**−**6** (in $CDCl_3$ at 25 °C). Second row: amide proton chemical shifts as a function of the amount of DMSO-d_6 added in a 5 mM solution of **4**−**6** (0.5 mL in $CDCl_3$ at 25 °C) (NH_a, ◇; NH_b, ○; NH_c, □; NH_d, △; NH_e, ▽).

Table 1. The Upfield Limiting Chemical Shifts and the Chemical Shift Changes ($\Delta\delta_{NH}$ Values) for ^{1}H NMR Dilution Studies (Dilu.) and the DMSO-d_6 Addition Studies (DMSO) of Tetrapeptides **4**−**6**

	NH_a (ppm)			NH_b (ppm)			NH_c (ppm)			NH_d (ppm)			NH_e (ppm)		
	δ^a	$\Delta\delta^b$ Dilu.	$\Delta\delta^c$ DMSO	δ^a	$\Delta\delta^b$ Dilu.	$\Delta\delta^c$ DMSO	δ^a	$\Delta\delta^b$ Dilu.	$\Delta\delta^c$ DMSO	δ^a	$\Delta\delta^b$ Dilu.	$\Delta\delta^c$ DMSO	δ^a	$\Delta\delta^b$ Dilu.	$\Delta\delta^c$ DMSO
4	6.31	0.09	0.29	9.25	1.29	1.60	10.68	0.57	0.79	7.87	0.05	0.06	6.07	0.81	0.89
5	5.16	0.53	0.93	9.66	0.92	1.42	11.06	0.22	0.44	8.08	0.04	0.19	6.24	0.32	0.50
6	6.46	0.15	0.12	9.30	1.07	1.79	10.84	0.34	0.78	8.13	0.14	0.09	6.05	0.73	0.98

[a] δ is the amide NH's chemical shift obtained from the ^{1}H NMR spectrum of the indicated compound at 1 mM concentration in $CDCl_3$ at 25 °C. [b] $\Delta\delta_{NH}$ in the dilution studies was calculated as $\Delta\delta_{NH} = \delta_{NH}$ (200 mM) − δ_{NH} (1 mM). [c] $\Delta\delta_{NH}$ in the DMSO-d_6 addition studies was calculated as $\Delta\delta_{NH} = \delta_{NH}$ (9% DMSO-d_6 in $CDCl_3$) − δ_{NH} ($CDCl_3$).

tion of a weak long-range NOE between the head (NH_a) and the tail (*i*-Bu group) residues, separated by the middle loop segment, further suggested that **4** is constrained to a reverse turn structure.

Quantum mechanics calculations of model pentamide **7**, which resembles the substitution pattern of **4**, were performed, and the geometry was fully optimized with the HF/6-31G* method (Figure 4b).[14] The lowest-energy conformation of **7** has the following features. (1) The distance between $\alpha C_{(i)}$ and $\alpha C_{(i+3)}$ was 5.80 Å (Figure 4c), well within the range for β-turns.[1] (2) With the presence of an additional oxygen atom in each α-aminoxy acid as compared with an α-amino acid, the α-carbon distance between i and $i + 1$ residues (4.64 Å) and that between $i + 1$ and $i + 2$ residues (4.68 Å) became longer accordingly than that between $i + 2$ and $i + 3$ residues (3.79 Å). (3) Structure **7** showed a reverse turn structure similar to that observed for **3** in the X-ray structure, and the four α-carbon atoms of **7** were superimposed very well with those of **3** except for the αC of the *i*-Bu group (Figure 4d). (4) The

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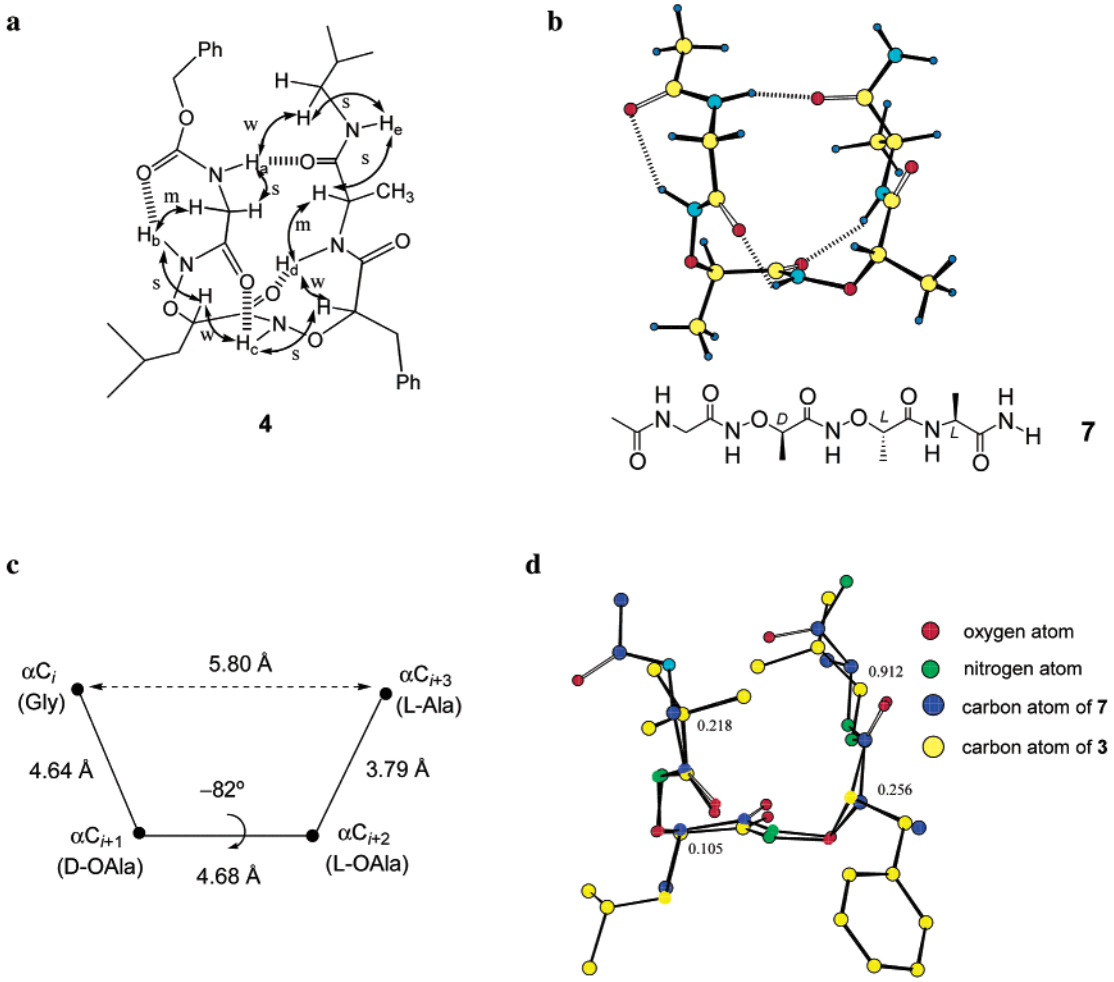

Figure 4. (a) The summary of NOEs observed in the NOESY spectrum of **4** in CD_2Cl_2 (3 mM at 278 K). (b) HF/6-31G* optimized lowest-energy conformation of the model tetrapeptide **7**. (c) Schematic diagram of α-carbon atoms of calculated structure **7**. (d) Superimposition of structure **7** with X-ray structure of **3**. The αC (**3**)- - -αC (**7**) distances are in angstroms.

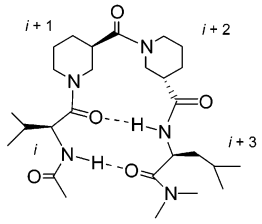

Figure 5. A reverse turn structure reported by Gellman et al.

pseudodihedral angle $\angle\alpha C_{(i)}-\alpha C_{(i+1)}-\alpha C_{(i+2)}-\alpha C_{(i+3)}$ of structure **7** was −82°, larger than those of typical type I′ β-turns (−40° to −60°) observed in proteins.[1] (5) A cross-strand $N-H_{(i)}$- -$O=C_{(i+3)}$ hydrogen bond (16-membered ring) was present with a favorable N- -O distance (3.01 Å), in agreement with the fact that H_a of **4** was involved in intramolecular hydrogen bonding. A similar 16-membered ring hydrogen bond has been reported by Gellman and co-workers to form between the NH of α-amino acid *i* and the C=O of α-amino acid *i* + 3 in a reverse turn induced by a (*R*,*S*)-nipecotic acid dimer (Figure 5).[4d] (6) The calculated structure **7** revealed a close distance between NH of the *i* + 1 residue and C=O of the *i* − 1 residue, suggesting the formation of a seven-membered-ring hydrogen bond (γ-turn). This was supported by the observation that NH_b of **4** was partially hydrogen-bonded.

Note that the N-terminus Gly residue was at the *i* position of the reverse turn structure found in **4**. It would be interesting to investigate whether the chirality of αC_i affects the reverse turn structure. Tetrapeptides **5** and **6** with L-Ala and D-Ala, respectively, at the *i* position were prepared accordingly. ^{1}H NMR dilution and DMSO-d_6 addition studies were performed for **5** and **6**, and the results are summarized in Figure 3 and Table 1. In both tetrapeptides, H_b was weakly hydrogen-bonded, indicating the presence of a γ-turn. However, **6** but not **5** displayed the same hydrogen bond pattern as **4**. As to tetrapeptide **5**, NH_a was solvent accessible because $\Delta\delta_{NHa}$ values were relatively large in both dilution and DMSO-d_6 addition experiments. The 2D NOESY experiments showed that the long-range cross-strand NOE signal observed in **4** and **6** was missing in **5**. This indicated that the incorporation of L-Ala as the N-terminal amino acid disfavored the reverse turn conformation. This can be explained by examining the calculated structure **7**. As shown in Figure 6a, the methyl group of D-Ala residue in **6** did not create any steric interaction for either the seven-membered-ring or the cross-strand hydrogen bond. However, the methyl group of the L-Ala residue at the *i* position in **5** would bump into the Cbz carbonyl group in the γ-turn region (Figure 6b). To maintain the γ-turn, NH_a of **5** would twist away from the appropriate orientation needed for the formation of the cross-strand hydrogen

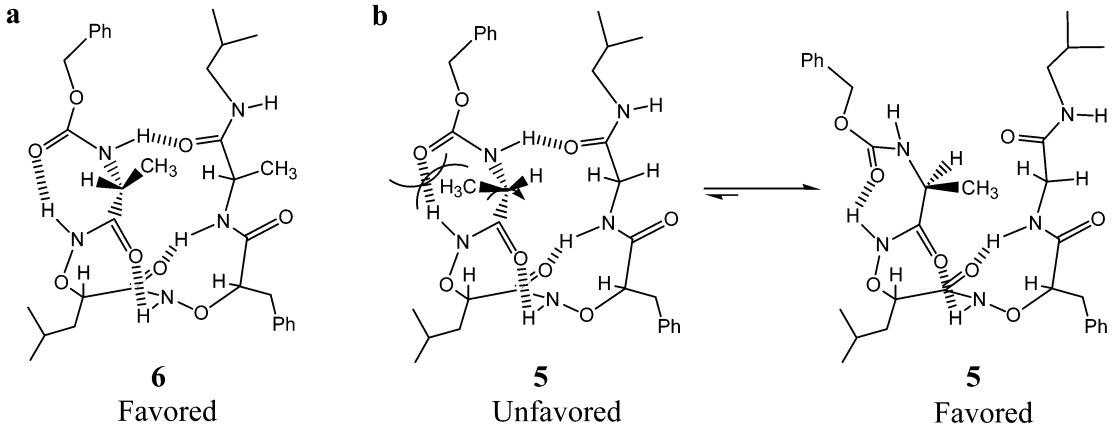

Figure 6. The conformational preferences of tetrapeptides **5** and **6**.

bond. The above results suggested the importance of cooperation between the γ-turn and the reverse turn in tetrapeptides **4**−**6**. The formation of the strong eight-membered-ring hydrogen bonds between C═O at the *i* position and NH at the $i + 2$ position of **4** may further polarize the N−H_b bond, favoring the formation of a γ-turn.

Conclusion

In summary, we have shown that, similar to the heterochiral dinipecotic acid segment, a D,L-α-aminoxy acid dimer can induce a novel reverse turn structure in peptides. This result, together with our previous reports on the formation of helix[9] and cyclic chloride ion receptor,[15] demonstrates that α-aminoxy acids are useful building blocks for the construction of a diverse range of new secondary structures.

Acknowledgment. This work was supported by The University of Hong Kong, The Hong Kong University of Science and Technology, Hong Kong Research Grants Council, and Bristol-Myers Squibb Foundation Unrestricted Grant in Synthetic Organic Chemistry (D.Y.). D.Y. and Y.-D.W. acknowledge the Croucher Foundation for the Senior Research Fellowship Award. We thank Professors M.-J. Zhang and G. D. Brown for advice on NMR studies, and Professor D. T.-W. Chan for obtaining the HRMS data.

Supporting Information Available: ^{1}H NMR dilution and DMSO-d_6 addition studies of **3**; ^{1}H NMR dilution studies of **4**; 2D ROESY data for **4**−**6**; preparation and characterization data of **3**−**6**; the Cartesian coordinates (in PDB format) of structures **1**, **2**, **2′**, and **7**; and X-ray structural analysis data of **3** containing tables of atomic coordinates, thermal parameters, bond lengths, and bond angles (PDF and CIF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA029514J

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