An Unusual Turn Structure in Peptides Containing α-Aminoxy Acids

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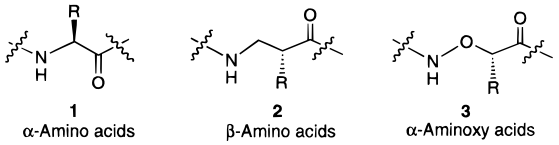
Important protein secondary structures such as α-helix, β-sheet, and turns are formed through intramolecular hydrogen bonding between α-amino acid residues (**1**).[1] There have been extensive studies on the conformation of peptides containing analogs of α-amino acids and their utility as peptidomimetics.[2] Since β-amino acids (**2**)[3] have excellent stability toward proteases, they also have been widely used as backbone-modified amino acids[4] in drug design. However, the extra carbon−carbon single bond ($C^{\alpha}-C^{\beta}$ bond) in a β-amino acid significantly increases the flexibility of peptide backbones.[5] Here, we report that a β-alanine analog, α-aminoxy acid residue (-NH-O-CH_2-CO-; **3** with R = H), has unusual conformational rigidity when incorporated into peptide backbones,[6] and it allows a strong eight-membered-ring hydrogen bond between adjacent amino acids.[7]



It is well-known that the N−O bond of hydroxylamine has unusual conformational properties due to the lone-pair electron repulsion.[8] We reasoned that replacing the β-carbon of a β-amino acid with an oxygen atom would result in an analog with more rigid conformations. *Ab initio* molecular orbital calculations were first carried out on amide **4** (Figure 1).[9] The most favorable conformation was found to be structure **5**. Although the N−O bond is about 20° out of the amide carbonyl plane, the *Z*-conformer in which the amide carbonyl is *cis* to the N−O bond is more stable than the *E*-conformer by about 2 kcal/mol, in agreement with the experimental data.[10,11] Unlike β-alanine, which prefers extended conformations, the O−CH_3 bond of *N*-oxy amide **4** strongly prefers out-of-plane orientations with the <CNOC angle close to 100° (or −100°), and the barrier for the N−O bond rotation through transition structure **6** is about 7 kcal/mol.[12] Interestingly, it was proposed that diamide **7** would adopt a rigid eight-membered-ring hydrogen-bonded structure **8**. The strong hydrogen bond in **8** is indicated by the short O···H distance and the nearly linear O···H−N angle. Despite about 4.4 eu less entropy, structure **8** is predicted to be significantly more stable than structure **9**, which benefits little from the six-membered-ring hydrogen-bonding interaction. Note that the hydrogen-bonding pattern of **8** is analogous to a γ-turn found in proteins except that it contains an extra oxygen atom in the backbone.[13]

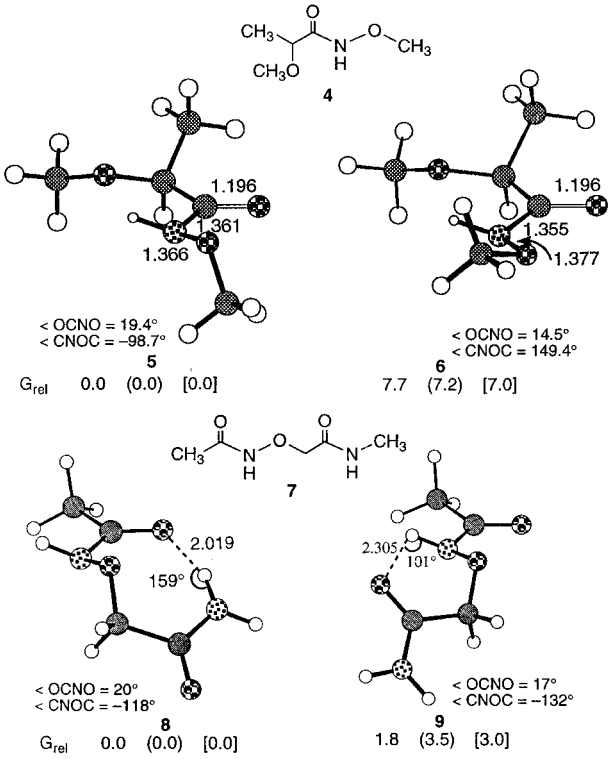


Figure 1. Structures of *N*-oxy amides. The calculated G_{rel} (kcal/mol) are in the order: HF/6-31G*, (MP2/6-31G*), and [HF/6-31G* $CHCl_3$ solvation].

Several diamides with the general formula *t*-Bu-CO-NH-O-CH_2-CO-NRR′ (compounds **10**−**12** with R, R′ = Et, Et; H, OMe; H, *i*-Bu) were synthesized and examined in dichloromethane by ^{1}H NMR and IR spectroscopies.[14,15]

(1) (a) Richardson, J. S. *Adv. Protein Chem.* **1981**, *34*, 167. (b) Creighton, T. E. *Proteins: Structures and Molecular Principles*, 2nd ed.; Freeman: New York, 1993.

(2) For a recent review, see: Goodman, M.; Ro, S. In *Burger's Medicinal Chemistry and Drug Discovery*, 5th ed.; Wolff, M., Ed.; Wiley: New York, 1995; Vol. I.

(3) (a) Drey, C. N. C. In *The Chemistry and Biochemistry of Amino Acids, Peptides and Proteins*; Weinstein, B., Ed.; Dekker: New York, 1976; Vol. 4, p 241. (b) Griffith, O. W. *Annu. Rev. Biochem.* **1986**, *55*, 855.

(4) Spatola, A. F. In *The Chemistry and Biochemistry of Amino Acids, Peptides and Proteins*; Weinstein, B., Ed.; Marcel Dekker: New York, 1983; Vol. 7, p 267.

(5) (a) Marraud, M.; Neel, J. *J. Polym. Sci.* **1975**, 271. (b) Dado, G.; Gellman, S. *J. Am. Chem. Soc.* **1994**, *116*, 1054. For a recent review on the structures of β-alanine containing peptides, see: (c) Benedetti, E. *Biopolymers (Peptide Science)* **1996**, *40*, 3.

(6) For literature reports on peptides containing α-aminoxy acids, see: (a) Schon, I.; Kisfaludy, L.; Nafradi, J.; Varga, L.; Varro, V. *Hoppe-Seyler's Z. Physiol. Chem.* **1978**, *Bd. 359*, 897. (b) Briggs, M.; Morley, J. S. *J. Chem. Soc., Perkin Trans. 1* **1979**, 2138.

(7) Hydrogen bonding between adjacent α-amino acids or β-amino acids has been shown to be unfavorable. See ref 5b and references cited therein.

(8) (a) Riddell, F. G. *Tetrahedron* **1981**, *37*, 849. (b) Raban, M.; Kost, D. *Tetrahedron* **1984**, *40*, 3345. (b) For an insightful discussion of the conformational preference of the N−O bond in relation to calicheamicin−DNA interactions, see: Walker, S.; Gange, D.; Gupta, V.; Kahne, D. *J. Am. Chem. Soc.* **1994**, *116*, 3197.

(9) All calculations were carried out with the GAUSSIAN 92/DFT program of Pople: Gaussian 92/DFT Revision F.2; Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Gill, P. M. W.; Johnson, B. G.; Wong, M. W.; Gomperts, R.; Andres, J. L.; Raghavachari, K.; Binkley, J. S.; Gonzalez, C.; Martin, R. L.; Fox, D. J.; Defrees, D. J.; Baker, J.; Stewart, J. J. P.; Pople; J. A. Gaussian, Inc.: Pittsburgh, PA, 1993. Geometry optimizations were first done with the HF/6-31G* method. For $CHCl_3$ solvation, the self-consistent reaction field method was used with the volume of the HF/6-31G* structure. Thermal energy and entropy were obtained by the HF/6-31G* frequency calculation.

(10) (a) Kolasa, T. *Tetrahedron* **1983**, *39*, 1753. (b) Brown, D. A.; Glass, W. K.; Mageswaran, R. *Magn. Reson. Chem.* **1988**, *26*, 970.

(11) For theoretical calculation on the favored conformations of *N*-oxy amides, see: (a) Fitzpatrick, N. J.; Mageswaran, R. *Polyhedron* **1989**, *8*, 2253. (b) Turi, L.; Dannenberg, J. J.; Rama, J. B.; Ventura, O. N. *J. Phys. Chem.* **1992**, *96*, 3709. In these studies, the *Z*-conformer was assumed to be planar and was found to be less stable than the *E*-conformer.

(12) This barrier is smaller than that for hydroxylamine because of the partial delocalization of the nitrogen lone pair to the carbonyl carbon.

(13) A similar extended γ-turn reported by Marraud involves an eight-membered-ring hydrogen bonding between an amide carbonyl group and the hydroxyl group of *N*-hydroxy amides. Dupont, V.; Lecoq, A.; Mangeot, J.-P.; Aubry, A.; Boussard, G.; Marraud, M. *J. Am. Chem. Soc.* **1993**, *115*, 8898.

S0002-7863(96)00515-X CCC: $12.00

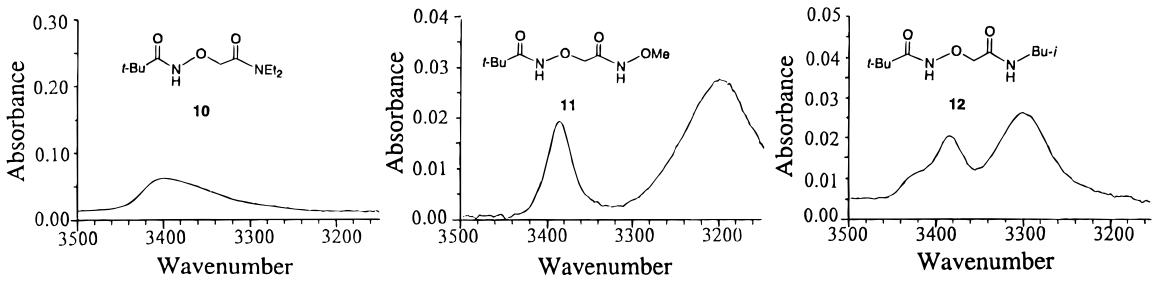


Figure 2. N−H stretch region FT-IR data for diamide solutions in CH_2Cl_2 at room temperature after subtraction of the spectrum of pure CH_2Cl_2. From left to right: **10** (5 mM), maximum at 3400 cm^{-1}; **11** (0.5 mM), maxima at 3386 and 3198 cm^{-1}; **12** (0.5 mM), maxima at 3427 (shoulder), 3384 and 3294 cm^{-1}.

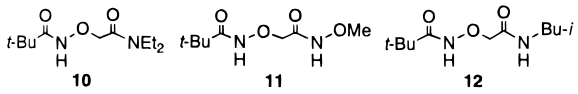


Figure 2 shows NH stretch region FT-IR data for compounds **10**−**12** at varying concentrations in CH_2Cl_2 at room temperature, after subtracting the spectrum of pure CH_2Cl_2. Throughout the concentration range of 1−40 mM, only a single peak was observed for compound **10** at 3400 cm^{-1}, which corresponds to the stretching frequency of the non-hydrogen-bonded NH of an *N*-oxy amide. This precludes the possibility of six-membered-ring hydrogen bonding between the NH and the C═O within one α-aminoxy acid residue (see structure **9**). Compared with **10**, diamide **11** has an extra amide proton. At low concentrations (0.5−20 mM), IR spectra of **11** showed two peaks: one at 3386 cm^{-1} and the other at 3198 cm^{-1}. Following the precedent of **10**, the former peak was assigned to a non-hydrogen-bonded amide NH (at the N-terminus) and the latter to an intramolecular hydrogen-bonded amide NH (at the C-terminus).

Compound **12** has one *N*-oxy amide unit and one *N*-isobutyl amide unit. Since these two types of amides have distinct stretching frequencies for both hydrogen-bonded NH and non-hydrogen-bonded NH, IR spectra of **12** may provide information on the extent of hydrogen bonding. Spectra of **12** at 0.5 mM showed two major peaks at 3384 and 3294 cm^{-1}. The former peak suggests that the *N*-oxy amide proton is solvent exposed (non-hydrogen-bonded) while the latter corresponds to the hydrogen-bonded *N*-isobutyl amide NH. The presence of a small shoulder at 3427 cm^{-1}, which was assigned to the non-hydrogen-bonded isobutyl amide NH, indicates that the intramolecular hydrogen-bonded structure of compound **12** (see structure **8**) is predominant in CH_2Cl_2.

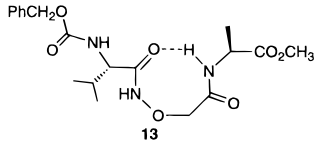
Table 1 summarizes the ^1H NMR chemical shift data for diamides **10**−**12** in CD_2Cl_2 at room temperature. The *N*-oxy amide NH of compound **10** at 5 mM appeared at 9.74 ppm and did not change upon further dilution. However, the chemical shifts of the two *N*-oxy amide protons of compound **11** at or below 1 mM are 11.41 and 8.71 ppm. The downfield signal corresponds to an intramolecular hydrogen-bonded NH, whereas the upfield signal corresponds to a non-hydrogen-bonded NH. For compound **12**, the singlet at 8.63 ppm was assigned to the *N*-oxy amide NH and the triplet at 8.28 ppm to the isobutyl amide NH. The unusually downfield chemical shift of the latter was attributed to the presence of an eight-membered-ring intramolecular hydrogen bond between the N-terminus C═O and the C-terminus amide NH.

It is interesting to probe whether the eight-membered-ring hydrogen bond is still favored in large peptides where there are other hydrogen-bonding possibilities between adjacent amino acid residues. Tripeptide **13** was thus analyzed.[14] In the ^1H NMR spectra of **13** at or below 1 mM, there are three amide NH signals at 9.25, 8.28, and 5.23 ppm, which were assigned to *N*-oxy amide NH, alanine NH, and valine NH, respectively, by 2D-COSY NMR experiment. Compared with that of dipeptide *N*-Cbz-Val-Ala-OMe in which δ(Ala-NH) and δ(Val-NH) were found to be 6.35 and 5.37 ppm (at 5 mM),[16] respectively, the eight-membered-ring intramolecular hydrogen bond Val-C═O···HN-Ala of tripeptide **13** was evident from its unusually downfield δ(Ala-NH) of 8.28 ppm.[17]

Table 1. Amide Proton NMR Chemical Shift for Diamides **10**−**12** in CD_2Cl_2 at 25 °C

compound	**10**[a]	**11**[b]	**12**[b]
δNH (ppm)	9.74 (s)	11.41 (s) 8.71 (s)	8.63 (s) 8.28 (t)

[a] 5 mM. [b] 0.25 mM.



The intramolecular eight-membered-ring hydrogen bonding induced by an α-aminoxy acid residue (-NH-O-CH_2-CO-) represents a novel type of backbone folding which we call the N−O turn. As *N*-oxy amides are readily available and have excellent biostability, the N−O turn should have potential in the molecular design of peptide analogs.[18]

Acknowledgment. We thank Prof. Samuel H. Gellman for advice on IR studies and Garnet Kin-Lic Chan and Crystal Yan Chan for synthesizing some intermediates. This work was supported by The University of Hong Kong, Leung Kau Kui/Run Run Shaw Research and Teaching Endowment Funds, Hong Kong University of Science and Technology, and Hong Kong Research Grants Council.

Supporting Information Available: Experimental details for preparation and characterization of compounds **10**−**13**, variable concentration ^1H NMR data and FT-IR data for **10**−**13**, and Cartesian coordinates for calculated structures **5**, **6**, **8**, and **9** (14 pages). See any current masthead page for ordering and Internet access instructions.

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(14) All new compounds were characterized by ^1H and ^{13}C NMR, IR, and HRMS. Syntheses and characterization data are provided in the supporting information.

(15) For excellent analysis of intramolecular hydrogen-bonding patterns using ^1H NMR and IR spectroscopy, see: (a) Gellman, S. H.; Dado, G. P.; Liang, G.-B.; Adams, B. R. *J. Am. Chem. Soc.* **1991**, *113*, 1164. (b) Reference 5b. (c) Nowick, J. S.; Abdi, M.; Bellamo, K. A.; Love, J. A.; Martinez, E. J.; Noronha, G.; Smith, E. M.; Ziller, J. W. *J. Am. Chem. Soc.* **1995**, *117*, 89.

(16) FTIR spectrum for 5 mM solution of dipeptide *N*-Cbz-Val-Ala-OMe in CH_2Cl_2 showed only one peak at 3424 cm^{-1}, which indicates that the two amide protons are non-hydrogen-bonded.

(17) FTIR data for tripeptide **13** also support the conclusion that the eight-membered-ring intramolecular hydrogen-bonded structure is predominant (see the supporting information).

(18) For recent examples of novel molecular scaffolds formed via intramolecular hydrogen bonding, see: (a) Nowick, J. S.; Holmes, D. L.; Mackin, G.; Noronha, G.; Shaka, A. J.; Smith, E. M. *J. Am. Chem. Soc.* **1996**, *118*, 2764. (b) Tsang, K. Y.; Diaz, H.; Graciani, N.; Kelly, J. W. *J. Am. Chem. Soc.* **1994**, *116*, 3988. (c) Winningham, M. J.; Sogah, D. Y. *J. Am. Chem. Soc.* **1994**, *116*, 11173. (d) Haque, T. S.; Little, J. C.; Gellman, S. H. *J. Am. Chem. Soc.* **1994**, *116*, 4105. (e) Gardner, R. R.; Liang, G.-B.; Gellman, S. H. *J. Am. Chem. Soc.* **1995**, *117*, 3280. (f) Burgess, K.; Ho, K.-K.; Pettitt, B. M. *J. Am. Chem. Soc.* **1994**, *116*, 799.