



Di-oxanipecotic acids as more stable turn motifs than di-nipecotic acids

Bong-hyeon Baek, Myung-ryul Lee, Kwang-Yon Kim, Ung-In Cho, Doo Wan Boo* and Injae Shin*

Department of Chemistry, Yonsei University, Seoul 120-749, Republic of Korea

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Abstract—The folded structures of peptidomimetics containing dimers of oxanipecotic acid (Oxa) in loop segments were characterized and compared with those of the corresponding nipecotic acid (Nip)-based ones. According to structural studies using FT-IR and NMR spectroscopies, di-oxanipecotic acid adopted more stable turn conformations than di-nipecotic acid, and for tetramers, L,(S)-Oxa,(S)-Oxa,L and L,(S)-Oxa,(R)-Oxa,L formed hairpin-like structures but only L,(R)-Nip,(S)-Nip,L promoted the stable folded conformations. © 2003 Elsevier Science Ltd. All rights reserved.

Recently, α -aminoxy peptides have spurred considerable interests as novel pseudopeptides.^{1,2} Although monomers of α -aminoxy peptides are slightly different from those of β -peptides in that methylene groups in the β positions of the latter are replaced by oxygen atoms, their folded structures are remarkably altered. The α -aminoxy peptides composed of acyclic α -aminoxy acids adopt only γ -turnlike conformations (N–O turns) irrespective of the nature of monomers.^{1a} In contrast, β -peptides can form diverse secondary structures depending on monomeric residues.³

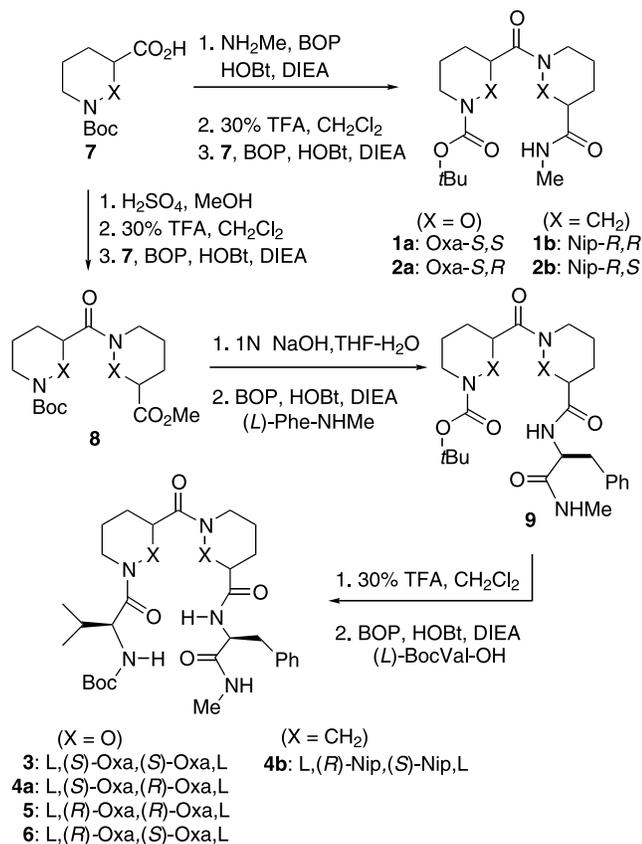
It was reported by Gellman and co-workers that di-nipecotic acids (Nip-dimers; dimers of six-membered β -amino acids) were able to form reverse turn structures.⁴ Previously we prepared oxanipecotic acid (six-membered α -aminoxy acid) as a nipecotic acid analogue for structural studies.^{2a,f,g} Although oxanipecotic acid (Oxa) has structural similarity to nipecotic acid (Nip), we postulated that peptidomimetics containing two types of cyclic monomers might adopt different folded structures because α -aminoxy peptides formed quite different conformations from β -peptides. Therefore, we characterized secondary structures of peptidomimetics containing di-oxanipecotic acids (Oxa-dimers) in loop segments and compared with those of the corresponding Nip-based ones.

For structural comparison, we synthesized Oxa-based dimers (**1a**, **2a**) and tetramers (**3–6**) as well as Nip-based dimers (**1b**, **2b**) and tetramer **4b**.^{5,6} Oxa-based peptidomimetics (**1–6**) were synthesized from BocOxa-OH (**7**) as shown in Scheme 1.^{2a} Monomer **7** was converted to dimers **1a** and **2a** by coupling of **7** to methylamine and subsequent condensation of Oxa-NHMe after Boc deprotection and **7** in the presence of BOP (benzotriazole-1-yl-oxy-tris(dimethylamino)phosphonium hexafluorophosphate)-HOBt (*N*-hydroxybenzotriazole)-DIEA. To prepare Oxa-based tetramers (**3–6**), monomer **7** was converted to **8** by esterification, Boc deprotection and condensation of the resulting Oxa-OME and **7**. After hydrolysis of methyl ester of **8**, the resulting acid was coupled to (*L*)-Phe-NHMe to produce trimers **9**. Finally, tetramers **3–6** were obtained by coupling of Boc-deprotected **9** to (*L*)-BocVal-OH. The Nip-based compounds (**1b**, **2b**, **4b**) were synthesized by modified known procedure.⁴

First, we characterized the folded structures of dimers **1** and **2** in chloroform by FT-IR and NMR spectroscopies at 1 mM concentrations, where aggregation was found to be negligible.⁷ According to FT-IR spectra of **1** and **2** in Figure 1, two of hydrogen-bonded and non-hydrogen-bonded NH stretching bands, whose ratio reflects the position of the conformational equilibrium, were observed.^{2f,4} The hydrogen-bonded and non-hydrogen-bonded amide NH bands for **1** and **2** appeared at 3350–3370 and 3450–3460 cm⁻¹, respectively. Since the IR spectra for homochiral Oxa-S,S (**1a**) exclusively displayed a hydrogen-bonded NH

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* Corresponding authors. Tel.: +82-2-2123-2631/2632; fax: +82-2-364-7050; e-mail: dwboo@yonsei.ac.kr; injae@yonsei.ac.kr



Scheme 1. Synthesis of dimers and tetramers.

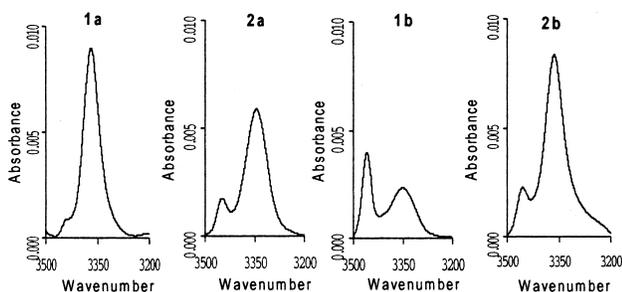


Figure 1. NH region of FT-IR spectra for dimers in CHCl_3 at room temperature (1 mM concentrations): (**1a**) maxima at 3370 cm^{-1} ; (**2a**) maxima at 3455 and 3354 cm^{-1} ; (**1b**) maxima at 3462 and 3354 cm^{-1} ; (**2b**) maxima at 3462 and 3369 cm^{-1} .

band, it was deduced that **1a** predominantly adopted the folded conformation. Given the higher ratio of hydrogen-bonded to non-hydrogen-bonded NH bands, the homochiral Oxa-*S,S* (**1a**) formed more stable β -turnlike structure than heterochiral Oxa-*S,R* (**2a**). Interestingly, these structural features are quite different from Nip-dimers whose preference is reversed as shown in Figure 1. For Nip-dimers, heterochiral dimer adopted the more stable folded conformation.^{4c}

The ¹H NMR chemical shift of amide NH has been used to ascertain which amide NH forms an internal hydrogen bond. We determined chemical shifts for major conformers of dimers **1** and **2** in CDCl_3 at 1 mM

concentrations. The amide NH signal for Oxa-*S,S* (**1a**) exhibited significantly downfield shift (7.79 ppm), suggesting that the NH group was exclusively involved in an internal hydrogen bond.⁴ Heterochiral dimers **2a** and **2b** showed downfield shifts of amide NHs (7.11 and 7.13 ppm, respectively). This indicated that both NH groups formed internal hydrogen bonds to a large extent. However, the NH resonance for Nip-*R,R* (**1b**) appeared relatively upfield (6.27 ppm), suggesting the formation of a moderate internal hydrogen bond. Taken together with IR and ¹H NMR analyses, Oxa-dimers favored the folded conformations, however, only heterochiral Nip-*R,S* (**2b**) promoted the stable turn structure.^{4c} Overall, Oxa-*S,S* formed the most stable folded structure among the four dimers tested.

Next, we carried out structural studies of Oxa-based tetramers (**3–6**) composed of di-oxanipeptotic acids in loop segments and α -peptide strands, and compared their structures with Nip-based tetramer **4b**. Figure 2 shows FT-IR data of **3–6** in CHCl_3 at 1 mM concentrations. For **3** and **4a**, two NH stretching bands were observed at 3448 and 3309 cm^{-1} ; the former band with weaker absorption is assigned to a non-hydrogen bonded NH and the latter with stronger absorption to an internal hydrogen-bonded NH. The tetramers **3** and **4a** exhibited high ratio of hydrogen-bonded to non-hydrogen-bonded NH bands, indicating that both tetramers adopted the folded conformations. However, the IR absorption bands of hydrogen-bonded NHs for **5** and **6** at 3307 and 3312 cm^{-1} , respectively, appeared to be very weak. In both cases, the shoulder peaks at 3424 and 3418 cm^{-1} for **5** and **6**, respectively, were observed

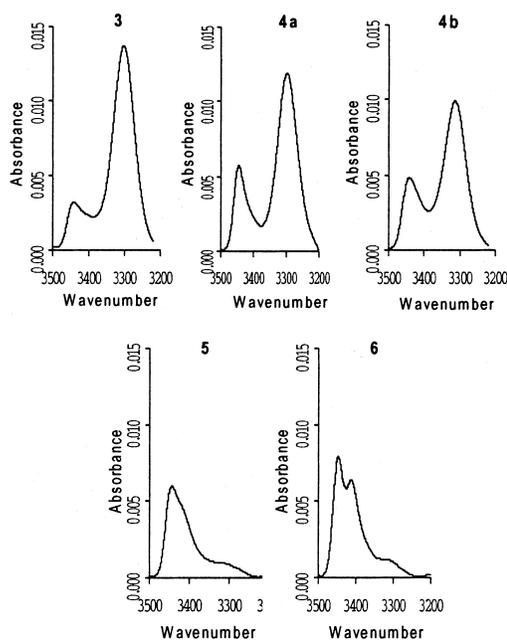


Figure 2. NH region of FT-IR spectra for tetramers in CHCl_3 at room temperature (1 mM concentrations): (**3**) maxima at 3448 and 3309 cm^{-1} ; (**4a**) maxima at 3448 and 3309 cm^{-1} ; (**5**) maxima at 3447, 3424 (shoulder) and 3307 cm^{-1} ; (**6**) maxima at 3450, 3418 (shoulder) and 3312 cm^{-1} ; (**4b**) maxima at 3449 and 3318 cm^{-1} .

and assigned to C_5 interactions.^{4,8} The IR data also revealed that NHs of **3** and **4a** formed the comparable hydrogen-bonding interactions to Nip-based tetramer **4b**.

We assigned the chemical shifts of amide NHs for tetramers **3–6** that participated in an internal hydrogen bond(s). Table 1 summarizes chemical shifts for major conformers of **3–6**. The downfield resonance of Phe NH (> 8.0 ppm) for **3**, **4a** and **4b** relative to AcNHPhNHMe (6.09 ppm) as a reference implied that Phe NH predominantly formed a strong internal hydrogen bond. However, the upfield signal of Phe NH (~ 6.6 ppm) for **5** and **6** suggested that both tetramers had large population of non-hydrogen-bonded Phe NH. The N-terminal Val NH for **3** and **4a** was involved in an internal hydrogen bond based on significant downfield shift (6.93 and 6.81 ppm, respectively) relative to 5.29 ppm of BocNHValNMe₂ as a reference. In contrast, Val NH resonance for **5** and **6** exhibited remarkably upfield shift (5.24 and 5.01 ppm, respectively), demonstrating that Val NH groups were not involved in internal hydrogen bonds. For Nip-based tetramer **4b**, Val NH signal appeared at 6.15 ppm, indicating that the amide NH group equilibrates between hydrogen-bonded and non-hydrogen-bonded states. The C-terminal amide NH signals for all the tetramers appeared upfield in the narrow range (5.6–6.1 ppm).⁹

The 2D ROESY NMR spectra of tetramers further supported the formation of hairpin-like conformations. As anticipated, tetramers **3**, **4a** and **4b** exhibited strong ROEs between *t*Bu and NH-CH₃ as well as Val C_β-H and Phe C_β-H on α -peptide strands (Fig. 3).⁷ However, tetramers **5** and **6**, which were found to lack the stable folded conformations, did not show the corresponding ROEs between α -strand residues. Taken together with IR and NMR analyses, it was concluded that **3** and **4a** adopted the stable hairpin-like conformations, whereas only L,(*R*)-Nip,(*S*)-Nip,L (**4b**) formed the folded structure.

Finally, we investigated the molecular basis of conformational differences between Oxa- and Nip-based peptidomimetics by comparing the ab initio energy-minimized structures of dimers **1** and **2** generated at RHF/6-31G(*d,p*) level.¹⁰ According to the energy-minimized structures, the Oxa-dimers (**1a**, **2a**) and Nip-*R,S* (**2b**) predominantly adopted the folded conformations, albeit the hydrogen bond distance of

Table 1. Chemical shifts of amide protons for the major conformers of **3–6** in CDCl₃ at 1 mM concentrations

	3	4a	4b	5	6
Phe NH	8.59	8.33	8.01	6.68	6.64
Val NH	6.93	6.81	6.15	5.24	5.01
Me NH	6.12	5.81	5.96	5.79	5.64

Percent major conformer of **3**, **4a**, **4b**, **5** and **6** is 87, 83, 82, 61 and 90%, respectively.

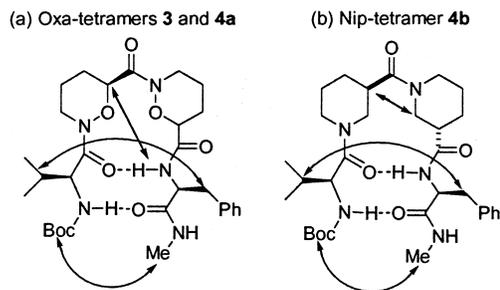


Figure 3. Summary of partial ROEs observed for tetramers **3** and **4** in CDCl₃ at room temperature (1 mM concentrations).

Nip-*R,S* was somewhat longer (Fig. 4). However, Nip-*R,R* (**1b**) exhibited hydrogen-bonded and non-hydrogen-bonded conformers with similar energies. As a consequence, the Nip-*R,R* equilibrated between hydrogen-bonded and non-hydrogen-bonded conformers and thus might adopt poorly folded structures. The distinct structural feature between two types of dimers is that the oxyamide group connecting the two rings of Oxa-dimers shows pyramidal conformation at the amide nitrogen atom, in contrast, the Nip-dimers exhibit nearly planar conformation at the corresponding amide group (Fig. 4). Another structural difference is the presence of steric hindrance between two β -CH₂ in the rings of Nip-dimers. Overall, these structural differences might dictate the tendency of the examined Oxa- and Nip-based peptidomimetics to form the folded structures.

In conclusion, we demonstrated that Oxa-dimers adopted more stable turn conformations than the corresponding Nip-dimers. In addition, we also revealed that for tetramers, L,(*S*)-Oxa,(*S*)-Oxa,L and L,(*S*)-Oxa,(*R*-

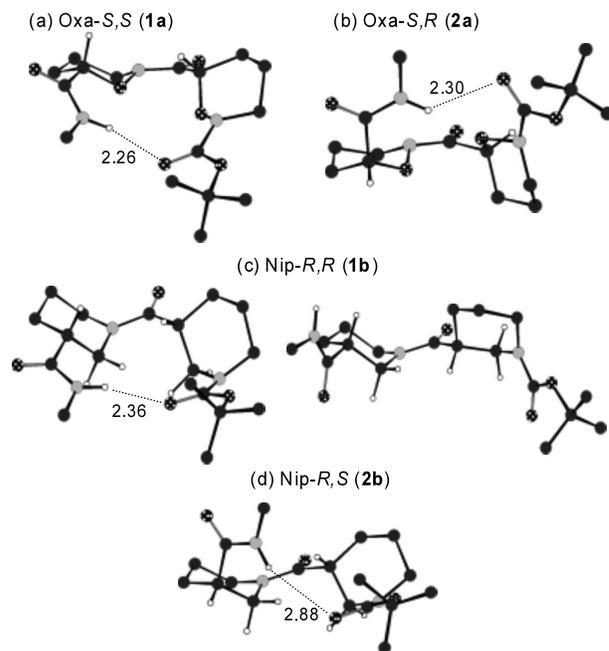


Figure 4. Energy-minimized structures of dimers generated at RHF/6-31G(*d,p*) level. Distances are shown in angstroms (black: C, gray: N, patterned: O, white: H).

Oxa,L formed folded structures, however, only L,(R)-Nip,(S)-Nip,L promoted the stable folded conformations.

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- Since *N*-acetylated peptidomimetics possessing di-oxanipeccotic acids were sparingly soluble in nonpolar solvents such as methylene chloride and chloroform, which are ideal for examining hydrogen bond formations by FT-IR and NMR, Boc-protected compounds were prepared for structural studies.
- According to FT-IR and NMR studies of four Boc-protected Nip-tetramers [L,(R)-Nip,(S)-Nip,L, L,(R)-Nip,(R)-Nip,L, L,(S)-Nip,(S)-Nip,L, L,(S)-Nip,(R)-Nip,L], only L,(R)-Nip,(S)-Nip,L (**4b**) exhibited folded conformations. Accordingly, we selected **4b** for these studies. In fact, this result is a little different from previous results that heterochiral acetylated Nip-tetramers (L,(R)-Nip,(S)-Nip,L and L,(S)-Nip,(R)-Nip,L) promote turn formations.^{4c} We assumed that carbonyl of Boc group is a worse hydrogen bond acceptor to C-terminal NH than that of acetyl group and thus only L,(R)-Nip,(S)-Nip,L forms the folded conformation.
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