Tetrahedron Letters 50 (2009) 3926–3928

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

The imide-dipeptides that show strong and stable β-sheet-like interactions compared with natural sequence

Damei Ke [a,b], Chuanlang Zhan [a,*], Xiao Li [a,b], Yaobing Wang [a,b], Alexander D. Q. Li [c], Jiannian Yao [a,*]

[a] Beijing National Laboratory of Molecular Science (BNLMS), Laboratory of Photochemistry, Institute of Chemistry, Chinese Academy of Sciences, Beijing 100190, PR China
[b] Graduate School, Chinese Academy of Sciences, Beijing 100039, PR China
[c] Department of Chemistry, Washington State University, Pullman, WA 99164, USA

ARTICLE INFO

Article history:
Received 23 January 2009
Revised 14 April 2009
Accepted 17 April 2009
Available online 22 April 2009

ABSTRACT

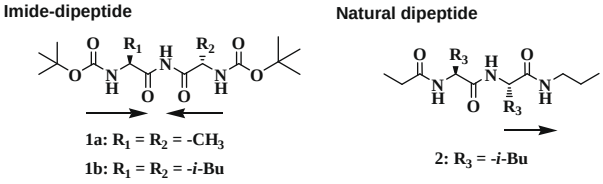
In this Letter, we report solution behavior of two imide-dipeptides containing L-alanine and L-leucine residues. In contrast to natural sequence, the imide-dipeptidyl backbone contains distinct features: self-pairing H-bonds, topochemical symmetry, a peptide polindron sequence, and different orientations of side chains. The solution behavior in chloroform reveals that both the imide-dipeptides adopt β-folding conformations and form β-sheet-like assembly. Most surprisingly, they form more stable and stronger H-bonds than the natural counterpart, and thus show different H-bonding patterns from the natural sequence.

Specific H-bonds between β-strand's edges play a key role in protein β-sheet interactions as well as protein–protein interactions. Intermolecularly, linear β-strand mimetics[1] behave and even functions like the important structural motifs or scaffolds formed by β-sheets. Current designs of linear β-strand mimetics usually incorporate rigid motifs into peptide backbone.[2–6] Since the discovery of natural urea-containing peptide backbones,[7] the urea unit has been used to develop a set of short peptidomimetics, which either adopt β-folding or form β-sheet-like assembly[8] or helix[9] in solutions through the self-pairing H-bonds between –C=O and –NHs of the urea unit. Similarly, the imide unit also provides a complementary hydrogen-bonding building block, and thus may be interesting to be incorporated into peptide backbone. We will focus on the imide unit and the characteristic behavior of peptidomimetics containing the imide moiety.

Natural peptide provides its backbone with a 'sense of direction'. In a cyclic peptide, reversing its residue sequence and inverting each residue's chirality produce its cyclo-retro-enantiomer.[10] Extending the retro-enantiomer concept to linear peptides generates the retro-inverso-peptide—a linear peptide isomer in which the backbone orientation is reversed in the middle of the chain and the side-chain orientation of each amino acid residue is also inverted.[11]

Accordingly, we integrate the 'retro-' concept and 'imide' unit to reverse the orientation of the dipeptide backbone, as nature selects,[7] to construct imide-dipeptides. Scheme 1 exemplifies that the imide-dipeptide backbone (**1**) introduces special chemical structural features unmatched in the natural sequence (**2**), including (i) self-pairing H-bonds; (ii) a peptide polindron sequence; (iii) topochemical symmetry (C_2 symmetry); (iv) different orientations of the two side chains. As preliminary investigations, we choose two imide-dipeptides (**1a** and **1b**[12]) containing L-alanine and L-leucine residues as models and report herein their solution behavior. Surprisingly, the imide-dipeptides show strong and stable intermolecular H-bonding interactions compared to those of the natural amino acid sequence.

Both **1a** and **1b** show excellent solubility in most of solvents such as cyclohexane, benzene, dichloromethane, chloroform, alcohol, except water. In chloroform, the α-protons of **1a** and **1b**, respectively, appear at 4.56 and 4.57 ppm, shifting 0.21 and 0.40 ppm downfield, respectively, from the random coil conformations of the L-alanine and L-leucine residues,[13] an evidence that the dipeptide chains adopt β-folding conformations. This is confirmed by the $^3J_{HN\alpha}$ value (8.0 Hz) of the α-protons of **1a**. The β-sheet-like assembly agrees with the ROE experiments of **1a** and **1b**. As shown in Figure 1, the interstrand ROE between the carbamate-protons and imide-protons, and the intrastrand ROEs between the α-protons and imide-protons and between the carbamate-/α-protons



Scheme 1. Chemical structures of the imide-dipeptides (**1**) and a selected natural sequence (**2**).

* Corresponding authors. Tel./fax: +86 10 82616517 (J.Y.).
E-mail addresses: clzhan@iccas.ac.cn (C. Zhan), jnyao@iccas.ac.cn (J. Yao).

0040-4039/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2009.04.067

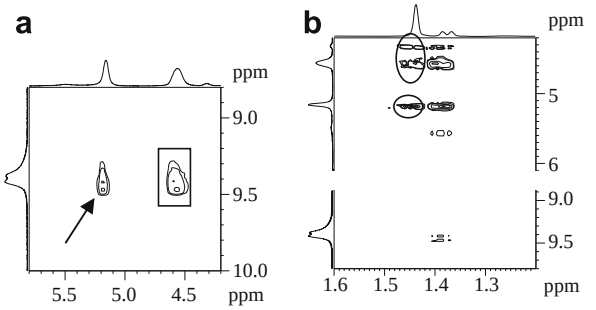


Figure 1. Both (a) and (b) show part of the ROESY spectra of **1a** in $CDCl_3$ solution carried out at 90 mM and 25 °C (mixing time 0.8 s). While the interstrand ROE signal between the imide- and carbamate-protons is indicated by an arrow, the intrastrand ROE signals between the α-protons and imide-protons and between the carbamate-/α-protons and Boc-protons are labeled by an unfilled square and unfilled circles, respectively.

and Boc-protons indicate the β-sheet-like assembly of **1a** and the β-folding conformations of the dipeptide chains as dominant species in chloroform. Similar interstrand and intrastrand ROE signals, for examples, those shown in Figure 2a, also indicate the β-sheet-like assembly of **1b** and the β-folding conformations of the dipeptide chains.

Surprisingly, the imide-dipeptides exhibit very different H-bonding behavior from that of the natural sequence. As shown in Figure 3a, the imide-protons and carbamate-protons of **1a** (down and up triangles) and **1b** (unfilled and filled circles) all reveal a weak dependence on their concentrations, hinting either very strong or weak intermolecular H-bonding interactions. This behavior is distinct to that of compound **2**[14] (Fig. 3b). The amide-protons in **2** are most likely free of H-bonds at low concentrations (typically, <7 mM). However, the chemical shifts continuously and nonlinearly become higher and higher when concentrations increase, suggesting the propensity to form H-bonds at high concentrations.

To clarify the ambiguity for H-bonding interactions of the imide-dipeptides, we added 6% (v/v) methanol into a 23 mM **1b**/$CDCl_3$ solution to disrupt the hydrogen-bonding network. First, the experimental results reveal a dramatically downfield shift for the imide-protons shifting from 9.03 to 9.90 ppm and for the carbamate-protons from 4.94 to 5.47 ppm (Fig. 3a and Table 1), respectively. This implies that the addition of methanol completely consumed the interpeptide H-bonds. Disrupting the interstrand H-bonds leads to the formation of new competitive H-bonds between methanol and –NHs which show a dramatically, nonlinearly upfield shift if the solution is diluted with pure $CDCl_3$ (Fig. 3a, filled and unfilled squares), as the chloroform molecules may replace some methanol molecules, effectively reducing H-bonding

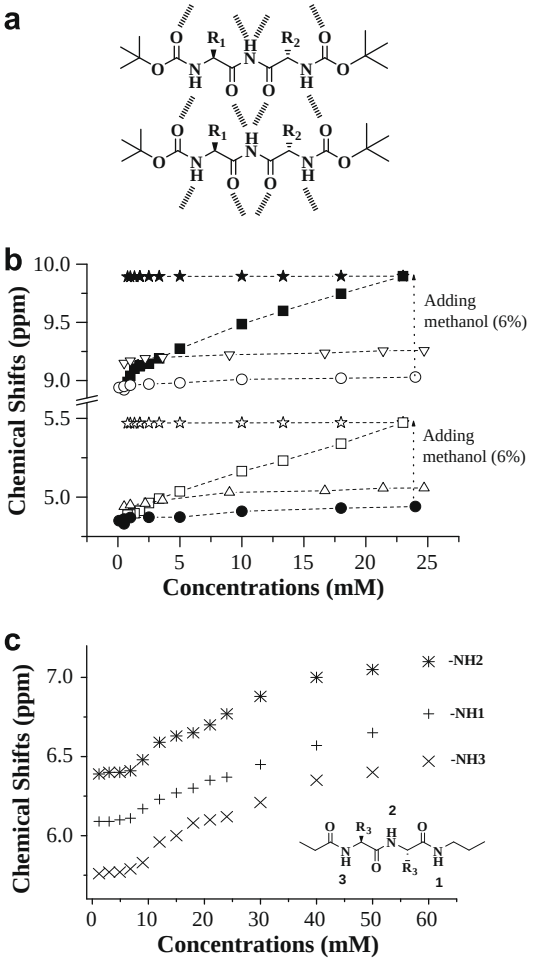


Figure 3. (a) Self-pairing and β-sheet-like assembly of **1**, (b) concentration-dependences of $\delta_{\text{-NH}}$s, respectively, for the imide- and carbamate-protons of **1a** in $CDCl_3$ (▽ and △), and **1b** in $CDCl_3$ (○ and ●) and in 6% $CH_3OH/CDCl_3$ (v/v) diluted, respectively, with pure $CDCl_3$ (■ and □) or with 6% $CH_3OH/CDCl_3$ (★ and ☆), (c) concentration-dependent $\delta_{\text{-NH}}$s of the amide-NHs of **2** in $CDCl_3$ (+, *, and ×).

opportunities between dipeptide molecules and methanol. When the solution of 23 mM **1b**/$CDCl_3$ + 6% methanol was further diluted using 6% methanol/$CDCl_3$ (v/v), however, the NHs exhibit a very weak dependence (Fig. 3a, filled and unfilled stars), since the further addition of methanol in 6% methanol/$CDCl_3$ keeps the concentration of methanol in the solution, and thus keeps H-bonding opportunities between dipeptide and methanol molecules. Second,

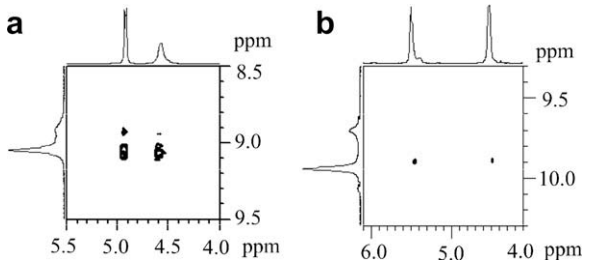


Figure 2. Part of the ROESY spectra of **1b**, respectively, in $CDCl_3$ (a) and in 6% $CH_3OH/CDCl_3$ (b) with a concentration of 23 mM (mixing time 0.8 s), showing that the ROE signals between the imide-protons and carbamate-/α-protons in 6% $CH_3OH/CDCl_3$ are much weaker than that in pure chloroform, which suggests disruption of the β-sheet-like assembly after addition of 6% methanol. Both the ROESY spectra are shown in the same zoom-in scales.

Table 1
Shows chemical shifts (δ, ppm) of **1a** (90 mM) and **1b** (23 mM) in different solvents

	Solvents	–NH1[a]	–NH2[b]	α-Hs	β-sheet
1a	$CDCl_3$	9.30	5.13	4.32	√[d]
	DMSO-d_6	10.69	7.13	4.32	×[d]
1b	Benzene-d_6	8.94	4.86	4.61	√
	$CDCl_3$	9.04	4.93	4.58	√
	6% MeOH/ $CDCl_3$ (v/v)	9.90	5.47	4.53	×
	DMSO-d_6	10.71	7.14	4.49	×
	CD_3OD	/[c]	/[c]	4.47	×

[a] Imide-NH.
[b] Carbamate-NHs.
[c] Protons at –NH units are replaced by deuterium atoms of CD_3OD.
[d] √ and × represent formation and disruption of the β-sheet-like assembly, respectively.

Table 2
Shows $\Delta\delta/\Delta T$ (ppb/K) of H-bonded-NHs of the imide-dipeptides **1a**, **1b**, and **2** in chloroform

	Carbamate-NH	Imide-NH	NH1	NH2	NH3
1a[a]	−3.1	−10.3			
1b[a]	−2.8	−7.9			
2[a]			−10.4	−11.6	−11.0

Note: (a) **1a** (25 mM), **1b** (23 mM), and **2** (30 mM).

ROE signals abated significantly when the β-sheet-like aggregates were disrupted (Fig. 2a and b); these ROE signals originated between the interstrand imide-protons and carbamate-protons and the intrastrand imide-protons and α-protons. The above two results suggest large scale disruption upon methanol addition, which suggests that both the imide-dipeptides form into highly strong intermolecular H-bonding interactions.

The β-sheet-like aggregates are supported again by X-ray structural analysis by depositing the **1b**/$CHCl_3$ solution, as an example, onto the Si substrate. The scattering pattern shows two strong reflections, respectively, positioning at $2\theta = 7.28^\circ$ and 19.70°. These two reflections give two *d*-spacings of 12.1 and 4.5 Å, respectively, corresponding to the stacking periodicity of the β-sheets and the spacing between peptide backbones running orthogonal to the β-sheet axis, typically characteristic of the β-sheet structures of **1b**.[15]

Other solvents that are capable of forming H-bonding interactions, such as DMSO-d_6, produce similar effects except for methanol. In non-H-bonding solvents such as benzene-d_6, however, the imide-dipeptides adopt β-folding conformations and form β-sheet-like assembly, similar to what is formed in $CDCl_3$, as listed in Table 1.

The temperature-variable NMR studies yielded much lower $\Delta\delta/\Delta T$ values for the carbamate-protons than for the imide-protons (Table 2), suggesting that the former forms stronger intermolecular hydrogen-bonds and contributes much more to stabilize the H-bonding network. Additionally, the lower $\Delta\delta/\Delta T$ values of the imide-dipeptides than that of the natural sequence confirm again that both of the imide-dipeptides possess much more stable H-bonding interactions.

In summary, the above solution behavior reveals that the imide-dipetides adopt β-folding conformations in non-H-bonding solvents, exhibit strong hydrogen-bonding propensity, and form much stronger and more stable β-sheet-like interactions than those of the natural sequence. The feature of the imide-dipeptides is notable. It opens an alternative way to create linear β-mimetic motifs that generate different backbone H-bonding patterns.

Acknowledgments

We thank NSFC (Nos. 20872145, 20733006), the Chinese Academy of Sciences, the National Research Fund for Fundamental Key Project 973 (2006CB806200, 2007CB936401), and the CAS/SAFEA International Partnership Program for Creative Research Teams.

References and notes

1. Loughlin, W. A.; Tyndall, J. D. A.; Glenn, M. P.; Fairlie, D. P. *Chem. Rev.* **2004**, *104*, 6085–6118.
2. Bailey, M. D.; Halmos, T.; Goudreau, N.; Lescop, E.; Llinas-Brunet, M. *J. Med. Chem.* **2004**, *47*, 3788–3799.
3. (a) Nowick, J. S.; Tsai, J. H.; Bui, Q.-C. D.; Maitra, S. *J. Am. Chem. Soc.* **1999**, *121*, 8409–8410; (b) Nowick, J. S.; Lam, K. S.; Khasanova, T. V.; Kemnitzer, W. E.; Maitra, S.; Mee, H. T.; Liu, R. *J. Am. Chem. Soc.* **2002**, *124*, 4972–4973; (c) Nowick, J. S.; Chung, D. M. *Angew. Chem., Int. Ed.* **2003**, *42*, 1765–1768; (d) Chung, D. M.; Dou, Y.; Baldi, P.; Nowick, J. S. *J. Am. Chem. Soc.* **2004**, *127*, 9998–9999.
4. (a) Phillips, S. T.; Rezac, M.; Abel, U.; Kossenjans, M.; Bartlett, P. A. *J. Am. Chem. Soc.* **2002**, *124*, 58–66; (b) Phillips, S. T.; Blasdel, L. K.; Bartlett, P. A. *J. Am. Chem. Soc.* **2005**, *127*, 4193–4198; (c) Hammond, M. C.; Bartlett, P. A. *J. Org. Chem.* **2007**, *72*, 3104–3107.
5. (a) Smith, A. B., III; Keenan, T. P.; Holcomb, R. C.; Sprengeler, P. A.; Guzman, M. C.; Wood, J. L.; Carroll, P. J.; Hirschmann, R. *J. Am. Chem. Soc.* **1992**, *114*, 10672–10674; (b) Smith, A. B., III; Guzman, M. C.; Sprengeler, P. A.; Keenan, T. P.; Holcomb, R. C.; Wood, J. L.; Carroll, P. J.; Hirschmann, R. *J. Am. Chem. Soc.* **1994**, *116*, 9947–9962; (c) Smith, A. B., III; Liu, H.; Hirschmann, R. *Org. Lett.* **2000**, *2*, 2037–2040.
6. Angelo, N. G.; Arora, P. S. *J. Am. Chem. Soc.* **2005**, *127*, 17134–17135.
7. Stefanelli, S.; Cavaletti, L.; Sarubbi, E.; Ragg, E.; Colombo, L.; Selva, E. *J. Antibiot.* **1995**, *48*, 332–334.
8. (a) Zhao, X.; Chang, Y.-L.; Fowler, F. W.; Lauher, J. W. *J. Am. Chem. Soc.* **1990**, *112*, 6627–6634; (b) Cbang, Y.-L.; West, M.-A.; Fowler, F. W.; Lauher, J. W. *J. Am. Chem. Soc.* **1993**, *115*, 5991–6000; (c) Zhang, X.; Rodrigues, J.; Evans, L.; Hinkle, B.; Ballantyne, L.; Pena, M. *J. Org. Chem.* **1997**, *62*, 6420–6423; (d) Page, P.; Bradley, M.; Walters, I.; Teague, S. *J. Org. Chem.* **1999**, *64*, 794–799; (e) Dales, N. A.; Bohacek, R. S.; Satyshur, K. A.; Rich, D. H. *Org. Lett.* **2001**, *3*, 2313–2316; (f) Moriuchi, T.; Tamura, T.; Hirao, T. *J. Am. Chem. Soc.* **2002**, *124*, 9356–9357; (g) Slater, M. J.; Amphlett, E. M.; Andrews, D. M.; Bamborough, P.; Carey, S. J.; Johnson, M. R.; Jones, P. S.; Mills, G.; Parry, N. R.; Somers, D. O'N.; Stewart, A. J.; Skarzynski, T. *Org. Lett.* **2003**, *5*, 4627–4630.
9. (a) Semetey, V.; Rognan, D.; Hemmerlin, C.; Graff, R.; Briand, J.-P.; Marraud, M.; Guichard, G. *Angew. Chem., Int. Ed.* **2002**, *41*, 1893–1895; (b) Violette, A.; Averlant-Petit, M. C.; Semetey, V.; Hemmerlin, C.; Casimir, R.; Graff, R.; Marraud, M.; Briand, J.-P.; Rognan, D.; Guichard, G. *J. Am. Chem. Soc.* **2005**, *127*, 2156–2164; (c) Sureshbabu, V. V.; Patil, B. S.; Venkataramanarao, R. *J. Org. Chem.* **2006**, *71*, 7697–7705.
10. (a) Shemyakin, M. M.; Ovchinnikov, A. Yu.; Ivanov, V. T. *Angew. Chem., Int. Ed. Engl.* **1969**, *8*, 492–499; (b) Wieland, T.; Penke, B.; Birr, C. *Liebigs Ann. Chem.* **1972**, *759*, 71–75.
11. (a) Goodman, M.; Chorev, M. *Acc. Chem. Res.* **1979**, *12*, 1–7; (b) Fletcher, M. D.; Campbell, M. M. *Chem. Rev.* **1998**, *98*, 763–795.
12. Ke, D.; C. Zhan; Li, X.; Li, A. D. Q.; Yao, J. *Synlett*, in press.
13. Wishart, D. S.; Sykes, B. D.; Richards, F. M. *Biochemistry* **1992**, *31*, 1647–1651.
14. *Characterizations of dipeptide* **2**: ^{1}H NMR δ (ppm, $CDCl_3$, 400 MHz, 24 mM): 6.77–6.75 (d, 1H, amide-NH, No. 2, ^3J = 8.1 Hz), 6.36 (t, 1H, amide-NH, No. 1, ^3J = 8.0 Hz), 6.11–6.09 (d, 1H, amide-NH, No. 3, ^3J = 7.9 Hz), 4.54–4.46 (m, 1H, α-proton, $^3J_{HN\alpha}$ = 8.4 Hz), 4.43–4.36 (m, 1H, α-proton, $^3J_{HN\alpha}$ = 8.4 Hz), 3.22–3.16 (q, 2H, *n*-Pr-αC-Hs, ^3J = 6.6 Hz), 2.26–2.20 (q, 2H, $-CH_2$, ^3J = 6.0 Hz), 1.80–1.45 (m, 8H, β-Hs of Leu, γ-Hs of Leu, and *n*-*Pr*-βC-Hs, ^3J = 3–8 Hz), 1.17–1.12 (t, 3H, $-CH_3$, ^3J = 7.5 Hz), 0.93–0.88 (q, 15H, δ-Hs of Leu and *n*-*Pr*-γC-Hs, ^3J = 6.0, ^3J = 6.8 Hz). ^{13}C NMR δ (ppm, $CDCl_3$, 100 MHz): 174.0, 172.3, 171.6, 51.9, 51.6, 41.3, 41.2, 40.8, 29.7, 29.5, 24.9, 24.8, 22.8, 22.7, 22.3, 22.2, 14.1, 11.3. ESI-Ms: 341, 364 (+Na^+).
15. Makin, O. S.; Serpell, L. C. *Fibre Diffr. Rev.* **2004**, *12*, 29–35.