Organic & Biomolecular Chemistry

RSC Publishing

PAPER

Original β,γ-diamino acid as an inducer of a γ-turn mimic in short peptides†

Cite this: *Org. Biomol. Chem.*, 2012, **10**, 9660

Sophie Thétiot-Laurent,[a] Francelin Bouillère,[a] Jean-Pierre Baltaze,[a] François Brisset,[a] Debby Feytens,[b,c] Cyrille Kouklovsky,[a] Emeric Miclet*[b] and Valérie Alezra*[a]

Received 18th September 2012,
Accepted 22nd October 2012

DOI: 10.1039/c2ob26828k

www.rsc.org/obc

Original αγα tripeptides containing one β,γ-diamino acid have been synthesized and their conformation determined by extensive NMR and molecular dynamic studies. These studies revealed the presence of a C_9 hydrogen bonded turn around the β,γ-diamino acid which was stabilized by bulky side chains of the preceding residue. This turn can be considered as a mimic of the well-known γ-turn.

Introduction

During the past two decades, the study of non-natural oligomers that fold into a specific regular structure, that closely resembles the secondary structures adopted by proteins, has gained unprecedented attention. This research field is now commonly referred to as foldamers.[1] A foldamer has been defined by S. Gellman in 1996 as "any polymer with strong tendency to adopt a specific compact conformation".[2] A predominant research area in this field concerns the peptidomimetic foldamers[3] that are considered as potential metabolically long-lived mimetics of bioactive alpha-polypeptides.[4] In this field, β-peptides have been extensively studied and have shown their ability to fold and to adopt a secondary structure such as helices, β-sheets, turns and β-hairpins.[5] In contrast to the intensive research on β-peptides, γ-peptides have received far less attention.[6] It has been discovered that γ-peptides form helical secondary structures in solution, detectable by NMR spectroscopy, with chain lengths as short as four residues.[7] Recently, hybrid peptides made of alternate α and γ-amino acids, or β and γ-amino acids have emerged and have proven to adopt also interesting conformations.[8] In this field, small peptides have been often neglected, as they are probably considered unable to adopt a defined secondary structure. Nevertheless, several crystallographic structures of di- or tripeptides, containing one γ-amino acid and two α-amino acids, have been described, showing the presence of C_7 and C_9 hydrogen bonded turns around the γ-amino acid.[9] A C_{12} hydrogen bond has also been observed in solution but not fully characterized.[10]

For a few years we have been interested in the asymmetric synthesis of β,γ-diamino acids starting from natural α-amino acids[11] and in their use as building blocks for γ-peptide synthesis, the second nitrogen being a source of molecular diversity or a hydrophilic substituent.[12] We have already shown that cyclic β,γ-diamino acids could be used to build α,γ-peptides that adopt a stable helical extended conformation devoid of any hydrogen bond.[13] We were then interested in the elaboration and the conformational studies of peptidic oligomers containing an acyclic β,γ-diamino acid. Here we describe αγα tripeptides containing an original β,γ-diamino acid that are able to form in solution a stable C_9 hydrogen bond.

Results and discussion

1. Synthesis of peptides

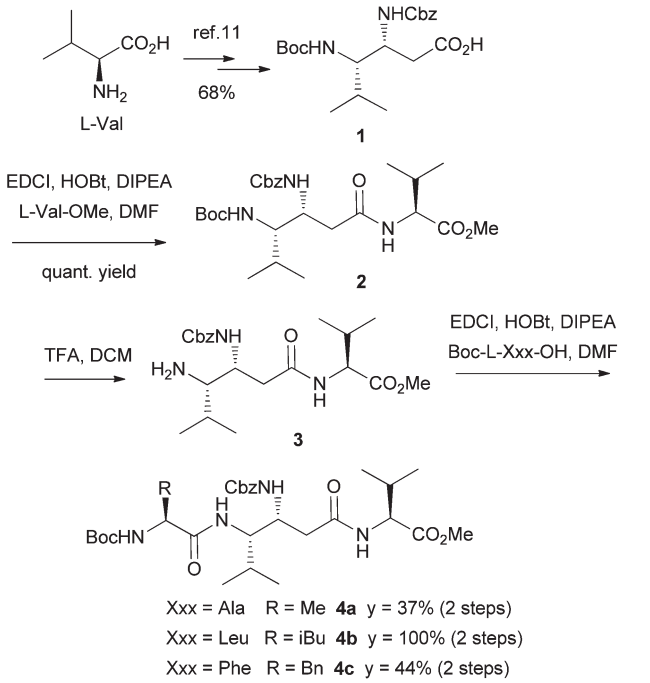
The αγα tripeptide sequences were based on compound **1**, which is readily obtained from L-valine.[11] Compound **1** was synthesized in 11 steps (starting from 10 g of L-valine) in 68% overall yield.[14] Peptide synthesis was then achieved under standard peptide coupling conditions with EDCI, HOBt, DIPEA in DMF to get three different tripeptides (Scheme 1).

2. Conformational studies of peptides

The conformations of the tripeptides were then explored. The IR spectra of these peptides in solution (5 mM in CH_2Cl_2) were recorded. In the NH-stretching region two absorption maxima were observed, corresponding to H-bonded and free amide (for **4a** at 3419 and 3340 cm^{-1}, for **4b** at 3415 and 3339 cm^{-1}, for **4c** at 3417 and 3339 cm^{-1}). Extensive NMR studies (TOCSY, NOESY, HSQC, HMBC) were then undertaken as a 5 mM

[a]*Laboratoire de Chimie des Procédés et Substances Naturelles, ICMMO, UMR 8182, Univ Paris-Sud, CNRS, Bât 410, Orsay F-91405, France. E-mail: valerie.alezra@u-psud.fr; Fax: (+33) 1 69 15 46 79; Tel: (+33) 1 69 15 76 50*
[b]*Laboratoire des BioMolécules, UMR 7203 CNRS-UPMC-ENS, UPMC Univ Paris 06, 4 Place Jussieu, Paris F-75005, France. E-mail: emeric.miclet@upmc.fr; Fax: (+33) 1 44 27 71 50; Tel: (+33) 1 44 27 31 15*
[c]*Organic Chemistry Department, Vrije Universiteit Brussel, Pleinlaan 2, B-1050 Brussels, Belgium*

†Electronic supplementary information (ESI) available. See DOI: 10.1039/c2ob26828k



Scheme 1 Synthesis of tripeptides.

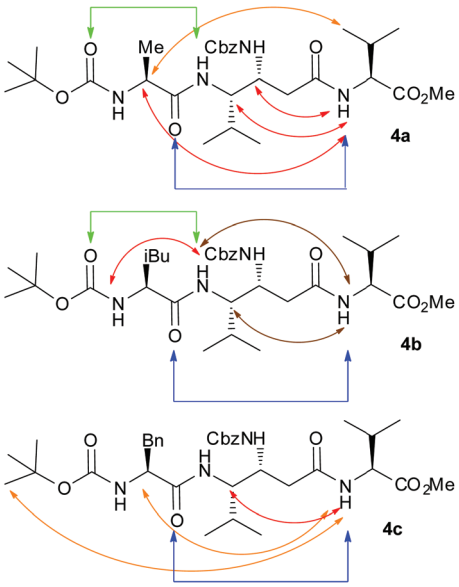


Fig. 1 Inter-residue NOE correlations (brown = strong, red = medium, orange = weak) and hydrogen bonds obtained from the simulated annealing protocol (green = C_7 and blue = C_9).

solution in $CDCl_3$ for all tripeptides and allowed complete proton and carbon resonance assignments. The ^1H NMR spectra showed downfield chemical shifts for the valine NH with $\delta > 7$ ppm (7.60 for **4a**, 7.78 for **4b** and 7.46 for **4c**), suggesting possible involvement in hydrogen bonds. Moreover solvent titration studies confirmed this hypothesis since low $\Delta\delta$ values (<0.20 ppm) were observed for the valine NH in the three tripeptides.[15] NOESY spectra were characterized by numerous cross peaks, which have been classified into weak, medium and strong correlations and accordingly converted into distance restraints. Among the inter-residues correlations, some are supporting a close proximity between the N- and the C-termini (Fig. 1). For instance, several NOE correlations imply the valine NH and either some protons of the first amino acid or the remote protons of the β,γ-diamino acid.

For each tripeptide, 200 structures have been calculated starting from extended folds. Vicinal coupling constants and NOE distance restraints were used in the simulated annealing protocol. Superimposition of the 10 lowest energy structures showed the presence of hydrogen bonds, forming a C_9 turn around the β,γ-diamino acid in the three peptides and also a standard C_7 turn around the first α-amino acid in **4a** and **4b** (Fig. 1 and 2). These results are in accordance with the observed NOE correlations. It is noteworthy that although they all present the same C_9 turn, these structures are slightly different regarding the backbone spatial arrangement. This means that the starting α-amino acid should play a significant role in the conformational space explored by the β,γ-diamino acid within the three peptides. Interestingly, the NH–Hα vicinal coupling constant of the α-amino acid residues increased with the steric hindrance of the first amino acid (from 6.2 Hz to 7.9 Hz).[16] This probably reflects a restricted flexibility for peptides **4b** and **4c** compared with peptide **4a**.

We therefore wanted to get further insights into the stability of the NMR structures at 300 K and undertook molecular dynamics calculations in $CHCl_3$ solvent boxes. Two crucial distance restraints observed in the NOESY spectra were kept during 50 ns simulations. The results obtained were highly dependent on the tripeptide.[16] For peptide **4a**, the C_9 turn was never observed at 300 K, the C_7 turn being present 40% of the total time.[17] In the corresponding conformation, the valine NH was close to the Cbz carbonyl group (2.20 Å), which could explain both its high δ_{NH} value and the small $\Delta\delta_{NH}$ measured during the titrations. For peptide **4b**, the C_9 turn was only rarely observed in contrast with the C_7 turn which largely dominated (88% of the total time). Actually, the careful examination of the trajectories predicted for both peptides **4a** and **4b** indicated that the β,γ residue rapidly exchanged between several conformational states, which are, on average, consistent with the NOE correlations.[18] In contrast, the C_9 turn was present 97% of the time for peptide **4c**, sometimes along with a C_7 turn (24% of total time), as observed in **4a** and **4b**. This stable C_9 turn was in very good agreement with the long-range NOE correlations observed for this peptide. As displayed on the dynamics trajectories (Fig. 3),[16] the conformational space appeared much more restricted for this peptide compared with peptide **4a** and, to a lesser extent, with peptide **4b**. Therefore in peptide **4c**, the first Phe residue provides sufficient steric hindrance to induce a stable C_9 hydrogen bonded turn.[19] As a γ-turn is defined by the existence of a hydrogen bond between the CO group of the *i*th residue and the NH group of the $(i + 2)$th residue, this C_9 hydrogen bonded turn can be viewed as a mimic of a γ-turn with a homologated residue.

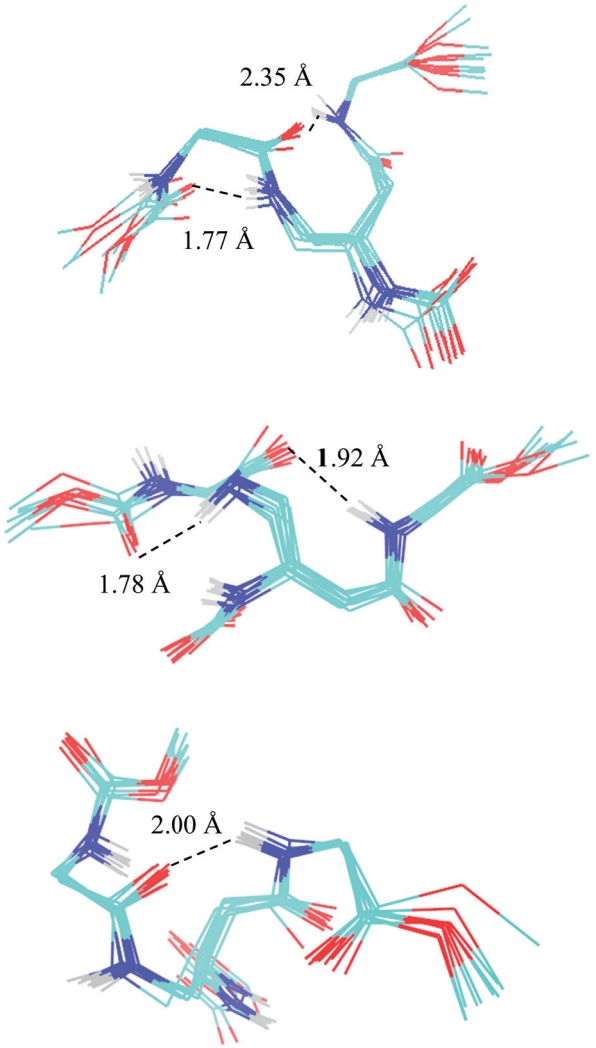


Fig. 2 Overlay of the 10 lowest energy structures of compounds **4a**, **4b** and **4c** (hydrogen-bonds are shown in dashed lines; for clarity, only the backbone is shown).

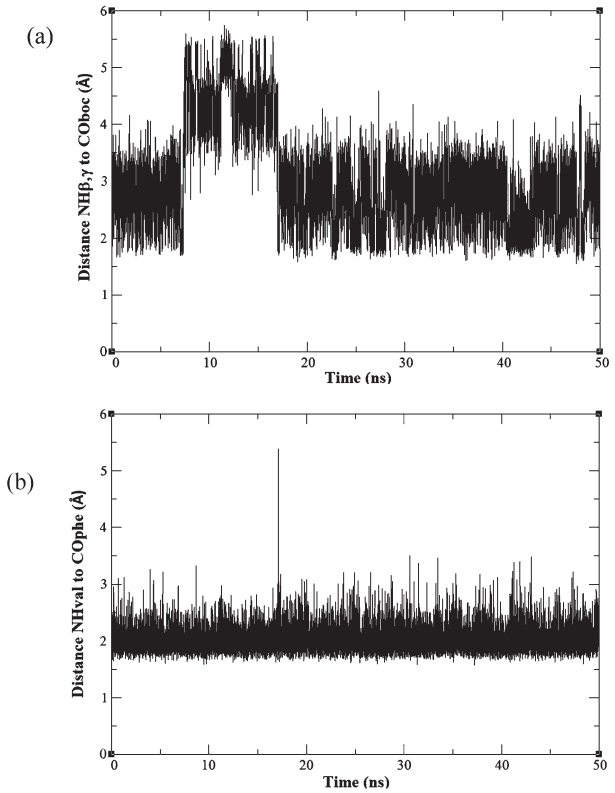


Fig. 3 Trajectories obtained for peptide **4c** in the time course of the MD simulation. NH···O=C distances revealing the formation of (a) a 7-membered ring hydrogen bond or (b) a 9-membered ring hydrogen bond.

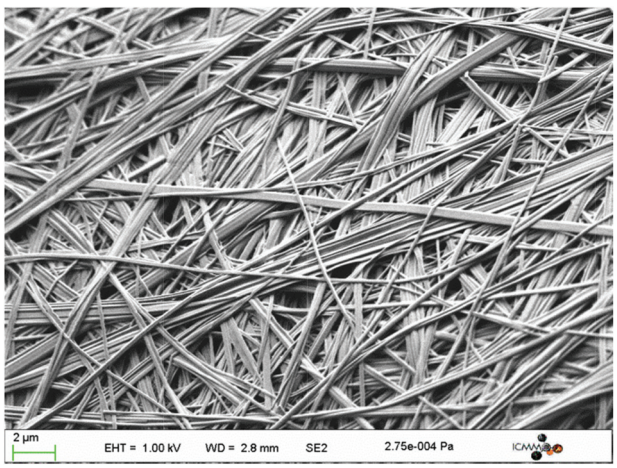


Fig. 4 SEM image of solid thin strands **4a**.

3. Macroscopic properties

Several attempts have been made in order to get crystals of the tripeptides. Although this was unsuccessful, slow evaporation of a solution of compound **4a** in a mixture AcOEt–petroleum ether (1 : 1) led to a white fibrous solid. Only one absorption maximum at 3321 cm^{-1} in the NH-stretching region was observed on the IR spectrum of this solid. This is consistent with H-bonded amides. In the carbonyl stretching region three absorption maxima were distinguished at 1739, 1690 and 1650 cm^{-1}, confirming therefore the presence of hydrogen bonds.[20]

A small piece of this solid was then observed by SEM (Fig. 4). Thin strands of 130 nm to 700 nm width and of several hundreds of microns length were visible. These strands are probably formed by self-assembly of peptide **4a**, stabilized by a network of hydrogen bonds as shown by the IR spectrum.

Moreover, compound **4c** showed also interesting supramolecular properties. A solution of compound **4c** (15 mM in $CHCl_3$ or CH_2Cl_2) forms rapidly a gel, that is probably significant of a self-assembly of peptide arising from specific interaction between molecules. These macroscopic properties have to be explored further to get real insight into the spatial arrangement of molecules.

Conclusions

We have shown that αγα peptide containing only three residues, including a β,γ-diamino acid, is able to adopt a defined stable conformation, as long as the first α-amino acid possesses the ideal steric hindrance. The propensity of this β,γ-diamino acid to form a C_9 hydrogen bond could be seen as an opportunity to have a superior homologue of the γ-turn of α-amino acids or as a good chance to get either a C_9 ribbon or a 9-helix by the repetition of this residue,[21] as this latter has been described as very stable by theoretical calculations.[22] Further studies in this field are currently under investigation in our laboratory.

Acknowledgements

This research was supported by the M.E.S.R. (a doctoral grant to F. B.) and by ANR (ANR grant no. ANR-08-JCJC0099). D. F. was a research assistant at the Fund for Scientific Research Flanders (FWO-Vlaanderen).

Notes and references

1 (*a*) I. Huc and S. Hecht, *Foldamers: Structure, Properties, and Application*, Wiley-VCH, Weinheim, 2007; (*b*) G. Guichard and I. Huc, *Chem. Commun.*, 2011, **47**, 5933.
2 (*a*) D. H. Appella, L. A. Christianson, I. L. Karle, D. R. Powell and S. H. Gellman, *J. Am. Chem. Soc.*, 1996, **118**, 13071; (*b*) S. H. Gellman, *Acc. Chem. Res.*, 1998, **31**, 173.
3 (*a*) P. G. Vasudev, S. Chatterjee, N. Shamala and P. Balaram, *Chem. Rev.*, 2011, **111**, 657; (*b*) T. A. Martinek and F. Fülöp, *Chem. Soc. Rev.*, 2012, **41**, 687.
4 C. Rosés, D. Carbajo, G. Sanclimens, J. Farrera-Sinfreu, A. Blancafort, G. Oliveras, A. D. Cirac, E. Bardají, T. Puig, M. Planas, L. Feliu, F. Albericio and M. Royo, *Tetrahedron*, 2012, **68**, 4406.
5 Reviews: (*a*) D. Seebach and J. Gardiner, *Acc. Chem. Res.*, 2008, **41**, 1366; (*b*) L. K. A. Pilsl and O. Reiser, *Amino Acids*, 2011, **41**, 709. Recent examples: (*c*) C. André, B. Legrand, C. Deng, C. Didierjean, G. Pickaert, J. Martinez, M.-C. Averlant-Petit, M. Amblard and M. Calmes, *Org. Lett.*, 2012, **14**, 960; (*d*) D. Balamurugan and K. M. Muraleedharan, *Chem.–Eur. J.*, 2012, **18**, 9516; (*e*) K. Basuroy, A. Rajagopal, S. Raghothama, N. Shamala and P. Balaram, *Chem.–Asian J.*, 2012, **7**, 1671.
6 F. Bouillère, S. Thétiot-Laurent, C. Kouklovsky and V. Alezra, *Amino Acids*, 2011, **41**, 687.
7 (*a*) S. Hanessian, X. Luo, R. Schaum and S. Michnick, *J. Am. Chem. Soc.*, 1998, **120**, 8569; (*b*) S. Hanessian, X. Luo and R. Schaum, *Tetrahedron Lett.*, 1999, **40**, 4925; (*c*) T. Hintermann, K. Gademann, B. Jaun and D. Seebach, *Helv. Chim. Acta*, 1998, **81**, 983; (*d*) D. Seebach, M. Brenner, M. Rueping, B. Schweizer and B. Jaun, *Chem. Commun.*, 2001, 207.
8 Reviews: (*a*) A. Roy, P. Prabhakaran, P. Kumar Baruah and G. J. Sanjayan, *Chem. Commun.*, 2011, **47**, 11593; (*b*) W. S. Horne and S. H. Gellman, *Acc. Chem. Res.*, 2008, **41**, 1399; Recent examples: (*c*) L. Guo, W. Zhang, I. A. Guzei, L. C. Spencer and S. H. Gellman, *Tetrahedron*, 2012, **68**, 4413; (*d*) L. Guo, W. Zhang, I. A. Guzei, L. C. Spencer and S. H. Gellman, *Org. Lett.*, 2012, **14**, 2582; (*e*) B. Dinesh, K. Basuroy, N. Shamal and P. Balaram, *Tetrahedron*, 2012, **68**, 4374; (*f*) A. Bandyopadhyay and H. N. Gopi, *Org. Lett.*, 2012, **14**, 2770; (*g*) L. Fischer, P. Claudon, N. Pendem, E. Miclet, C. Didierjean, E. Ennifar and G. Guichard, *Angew. Chem., Int. Ed.*, 2010, **49**, 1067.
9 (*a*) S. Aravinda, K. Ananda, N. Shamala and P. Balaram, *Chem.–Eur. J.*, 2003, **9**, 4789; (*b*) P. G. Vasudev, K. Ananda, J. Chatterjee, S. Aravinda, N. Shamala and P. Balaram, *J. Am. Chem. Soc.*, 2007, **129**, 4039; (*c*) J. Chatterjee, P. G. Vasudev, K. Ananda, S. Raghotama, N. Shamala and P. Balaram, *J. Org. Chem.*, 2008, **73**, 6595.
10 G. V. M. Sharma, V. B. Jadhav, K. V. S. Ramakrishna, P. Jayaprakash, K. Narsimulu, V. Subash and A. C. Kunwar, *J. Am. Chem. Soc.*, 2006, **128**, 14657.
11 (*a*) C. T. Hoang, V. Alezra, R. Guillot and C. Kouklovsky, *Org. Lett.*, 2007, **9**, 2521; (*b*) C. T. Hoang, F. Bouillère, S. Johannesen, A. Zulauf, C. Panel, D. Gori, A. Pouilhès, V. Alezra and C. Kouklovsky, *J. Org. Chem.*, 2009, **74**, 4177; (*c*) F. Bouillère, R. Guillot, C. Kouklovsky and V. Alezra, *Org. Biomol. Chem.*, 2011, **9**, 394.
12 E. A. Porter, X. Wang, M. A. Schmitt and S. H. Gellman, *Org. Lett.*, 2002, **4**, 3317.
13 F. Bouillère, D. Feytens, D. Gori, R. Guillot, C. Kouklovsky, E. Miclet and V. Alezra, *Chem. Commun.*, 2012, **48**, 1982.
14 The synthesis was performed as already described but almost no intermediate purification was actually necessary (see ESI† for more details).
15 Solvent titration studies were carried out by sequential additions of DMSO-d_6 (up to 140 μL) to a 5 mM solution of peptide in $CDCl_3$ (500 μL). In compound **4b**, both NH groups of the β,γ-diamino acid showed also $\Delta\delta < 0.20$ ppm.
16 See ESI† for further information.
17 Two other conformations were also populated in the MD simulation but were not in accordance with the observed NOE correlations.
18 D. Kruschela and B. Zagrovic, *Mol. BioSyst.*, 2009, **5**, 1606.
19 Some γ-amino acids, either unsubstituted or monosubstituted, have been shown to adopt a H-bonded conformation containing a C_9 ring, depending on their termination. See: (*a*) G. P. Dado and S. H. Gellman, *J. Am. Chem. Soc.*, 1994, **116**, 1054; (*b*) W. H. James III, E. G. Buchanan, L. Guo, S. H. Gellman and T. S. Zwier, *J. Phys. Chem. A.*, 2011, **115**, 11960.
20 G. Angelici, G. Falini, H.-J. Hofmann, D. Huster, M. Monari and C. Tomasini, *Chem.–Eur. J.*, 2009, **15**, 8037.
21 P. G. Vasudev, N. Shamala, K. Ananda and P. Balaram, *Angew. Chem., Int. Ed.*, 2005, **44**, 4972.
22 C. Baldauf, R. Günther and H.-J. Hofmann, *Angew. Chem., Int. Ed.*, 2004, **43**, 1594.