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ORIGINAL ARTICLE

β,γ-Diamino acid: an original building block for hybrid α/γ-peptide synthesis with extra hydrogen bond donating group

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Abstract Using a β,γ-diamino acid, several small hybrid α/γ peptides have been synthesized and their conformations investigated through extensive NMR studies and molecular dynamics. A tripeptide and a tetrapeptide have thus shown several hydrogen bonds in solution, including a 13-membered ring involving the β-nitrogen.

Keywords Peptide · γ-amino acid · Foldamer · NMR · Molecular dynamics · Structure

Introduction

For several years, the field of peptidic foldamers has produced a huge amount of interesting secondary structures, going from the α-helix mimetics to the β-turn analogues or more original new structures (Guichard and Huc 2011; Roy et al. 2011; Martinek and Fülöp 2012). In this area, the β-peptides have contributed to the major extent but the γ-peptides or hybrid peptides tend to increase their part (Vasudev et al. 2010; Bouillère et al. 2011b). Several secondary structures have thus been described such as 13-helix, with hybrid β/γ-peptide (Guo et al. 2010), β-turns (Chatterjee et al. 2009), or original structures like 9-helices (Sharma et al. 2006b), 12-helices (Dinesh et al. 2013; Jadhav et al. 2013), or 12/10-helices (Giuliano et al. 2013). The only limitation so far for peptides containing γ-amino acid residues lies in the synthesis of original and stereo-controlled γ-amino acids. Several diversely substituted γ^4-amino acids have been synthesized and incorporated in peptides but few of them contain heteroatom substituent (Machetti et al. 2000; Farrera-Sinfreu et al. 2004; Edwards et al. 2006; Sharma et al. 2006a, 2009; Mathieu et al. 2013) and only one of them is $\gamma^{3,4}$-disubstituted (without any substituent in 2-position) but no defined structure has been determined for it (Brenner and Seebach 2001).

For a few years we have developed a synthesis of orthogonally protected β,γ-diamino acids, ready to use in peptide synthesis (Hoang et al. 2007, 2009; Bouillère et al. 2011a, 2012). These types of compounds can be incorporated in peptides by the nitrogen in β-position or in γ-position, the second one being source of hydrosolubility (if deprotected), functionalization, or source of supplementary hydrogen bonding. We have already shown that tripeptides containing one β,γ-diamino acid issued from L-valine were able to present a C_9 hydrogen bonded turn around the β,γ-diamino acid (Thétiot-Laurent et al. 2012). In this paper, we describe the structure adopted by a tripeptide and a tetrapeptide containing a β,γ-diamino acid issued from L-leucine, with a different relative configuration (Fig. 1). The goal was to determine the influence of the relative configuration of the β-substituent.

Results and discussion

The synthesis of the β,γ-diamino acid was performed according to our developed strategy, starting from

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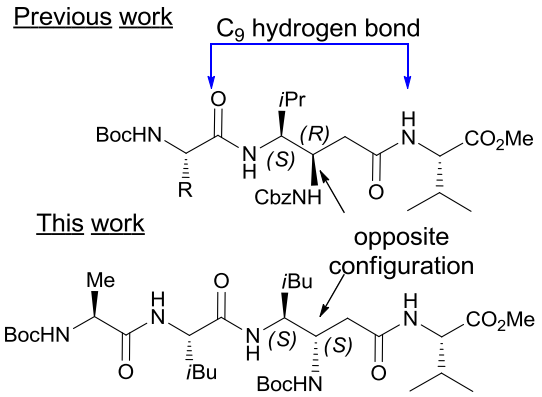
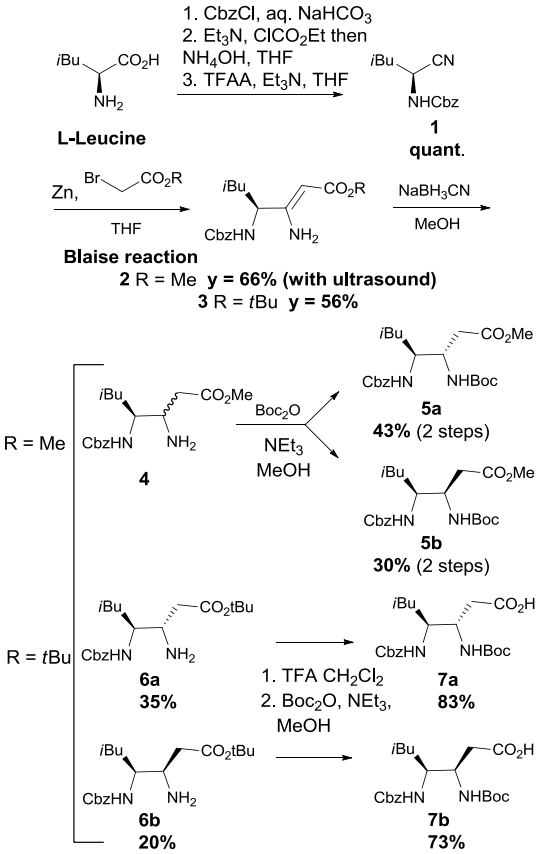
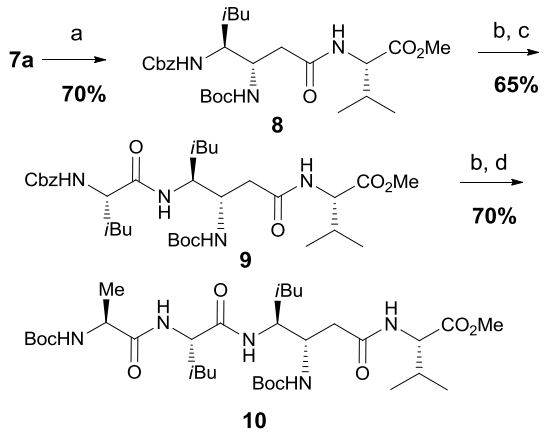


Fig. 1 Structures of former tripeptide (Thétiot-Laurent et al. 2012) and tetrapeptide of this work (color figure online)



Scheme 1 Synthesis of β,γ-diamino acid

L-leucine, the two key-steps being a Blaise reaction and the subsequent reduction of the double bond (Scheme 1). The synthesis was nevertheless slightly modified in few steps: the formation of the nitrile was achieved in quantitative yield using trifluoroacetic anhydride instead of phosphorus oxychloride and the Blaise reaction was conducted successfully directly on the monoprotected aminonitrile **1**.



Scheme 2 Synthesis of peptides. *a* L-Val, EDCI, HOBt, DIPEA, DMF; *b* H_2, Pd/C MeOH; *c* L-Leu, EDCI, HOBt, DIPEA, DMF; *d* L-Ala, EDCI, HOBt, DIPEA, DMF

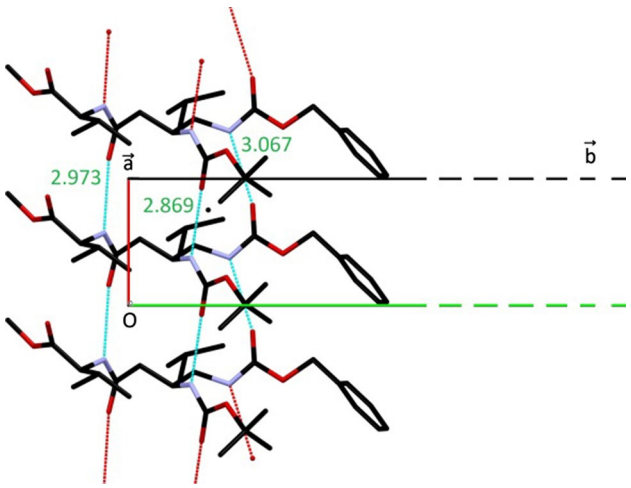


Fig. 2 Partial view of the packing of dipeptide **8** (color figure online)

It can be performed either using our previously developed conditions (Zn activated by 1,2-dibromoethane, tert-butyl-bromoacetate, yield = 56 %) or activated by ultrasound (Lee et al. 1997). This latter procedure led to a better yield (yield = 66 %) and also to a methyl ester which is orthogonal to the Boc protecting group. The resulting enaminoester **2** or **3** (present as a single stereoisomer) was then reduced into a mixture of diastereomers (dr close to 1:1 in both cases). In the case of the tert-butyl ester, the separation of the diastereomers was performed at this stage and the relative stereochemistry of compound **6a** was established thanks to its crystallographic structure. For the methyl ester, the diastereomer separation was efficient after protection. Orthogonally protected deoxyaminostatine **5a** (or **7a** via compound **6a**) was thus synthesized in 5 (6) steps in 28 % (16 %) yield instead of 11 steps in 5 % yield.

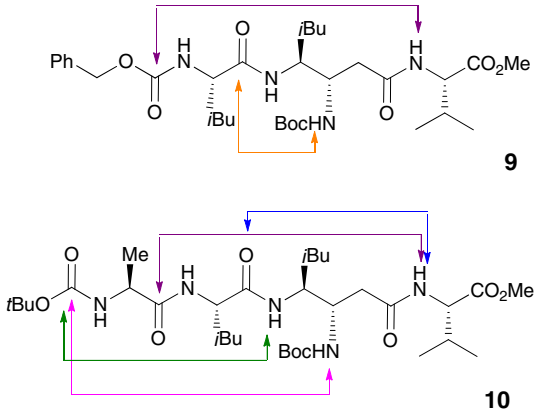


Fig. 3 Hydrogen bonds obtained from the simulated annealing protocol (*pink* C_{13}, *purple* C_{12}, *green* C_{10}, *blue* C_9, and *orange* C_7) of tripeptide **9** and tetrapeptide **10** (color figure online)

Compound **5a** could be quantitatively converted to compound **7a** by saponification. Compound **7a** was next engaged in peptide synthesis. We chose the same α-amino acids as before to have insights into the influence of the relative stereochemistry. The peptide synthesis was performed under classical conditions (Scheme 2). The dipeptide **8** was crystalline and the X-ray structure revealed the formation of a hydrogen-bonded ladder structure (Fig. 2). Every nitrogen atom and carbonyl group (except for the ester) are involved in a hydrogen bond. Successive dipeptides are parallel and the three N···O distances are around 2.97 Å and the N–H···O angles between 156° and 170°, which are significant of usual hydrogen bonding. The molecules are thus linked into infinite chain via hydrogen bond, the chains forming a parallel β-sheet like arrangement. The tripeptide **9** was then synthesized but no crystal structure could be obtained. We thus performed NMR studies, including solvent titration with DMSO. This latter showed that the NH-Val and the NHβ in the β,γ-diamino acid residue had very low $\Delta\delta$, which indicates that both could be involved in intramolecular hydrogen bond. This is supported by the NHβ chemical shift (δ = 6.43 ppm), significantly higher than those observed for free carbamate NH. NMR NOESY experiment showed among other a weak long-distance correlation between the NH-Val and the Cbz methylene. The NOESY correlations were converted into distance restraints thanks to the vicinal distance between Hα and Hβ of the valine residue (2.1 Å). Starting from extended folds, 200 structures have been calculated using a simulated annealing protocol and both vicinal coupling constants and NOE distance restraints. Superimposition of the 10 lowest energy structures showed the presence of two intramolecular hydrogen bonds. The first one forms a C_{12} turn and involves the NH-Val and the Cbz carbonyl group, and the second forms a C_7 turn and involves the NHβ in the β,γ-diamino acid residue and the L-leucine C=O (Fig. 3). We have never observed so far the implication of the NHβ in the β,γ-diamino acid residue and this conformation was very different from the one observed with the previous tripeptide [in which the β,γ-diamino acid is issued from L-Val (Thétiot-Laurent et al. 2012)]. We then decided to synthesize a tetrapeptide and added an L-alanine at the N-terminus.

The tetrapeptide **10** formed a gel in cyclohexane (c = 37 mmol L^{-1}) but was highly soluble in chloroform. At 5 mM, it showed downfield chemical shifts for some NH, in particular NH valine (δ = 7.8 ppm at 300 K) and also the NHβ in the β,γ-diamino acid residue (δ = 5.85 ppm), suggesting the presence of intramolecular hydrogen bonds. Extensive NMR experiments (COSY, TOCSY, NOESY, HMBC, HSQC) were recorded on a 5-mM solution in $CDCl_3$ and complete proton and carbon resonance assignment was performed. Solvent titration studies showed that very low $\Delta\delta$ were observed for the same NH (NH valine and NHβ in the β,γ-diamino acid residue). NOESY spectrum revealed the presence of numerous cross peaks, which were converted into distance restraints. Among the inter-residue correlations, some long-distance correlations were significant of hydrogen bonding and proximity of the Boc group and the β,γ-diamino acid. For instance, the tBu of Boc shows cross peaks with the NHγ and the NHβ in the β,γ-diamino acid.

Following the same protocol, vicinal coupling constants and NOE distance restraints were introduced in the simulated annealing protocol. Superimposition of the 10 lowest energy structures showed the presence of 2 three-centered hydrogen bonds (Figs. 3, 4). In the first one, the NH valine was located between the CO of leucine (forming a C_9 turn around the β,γ-diamino acid) and the CO of alanine (forming a C_{12} turn as found for the tripeptide **9**, d(NH···O) = 2.4 Å). The second three-centered hydrogen bond recalled the architecture observed in oligoureas foldamers where one hydrogen bond acceptor simultaneously interacts with two hydrogen bond donors (Guichard et al. 2008). In tetrapeptide **10**, the CO of the terminal Boc group was hydrogen bonded to both the NHγ and the NHβ of the β,γ-diamino acid, leading to C_{10} turn and C_{13} turns, respectively. Thus, the N-terminal elongation of peptide **9** had two consequences. First, the presence of the supplementary CO (of the terminal Boc group) made it possible to adopt the C_{10}/C_{13} double H-bonds fold. Secondly, since the C_{13} was not compatible with the C_7 pseudocycle, we observed the disruption of the latter and the formation of a hydrogen bond between the released CO (leucine) and the NH of the L-valine. This formed a C_9 turn around the β,γ-diamino acid, as reported before in tripeptide with a different relative configuration (Fig. 1) (Thétiot-Laurent et al. 2012). In this longer tetrapeptide, the presence of

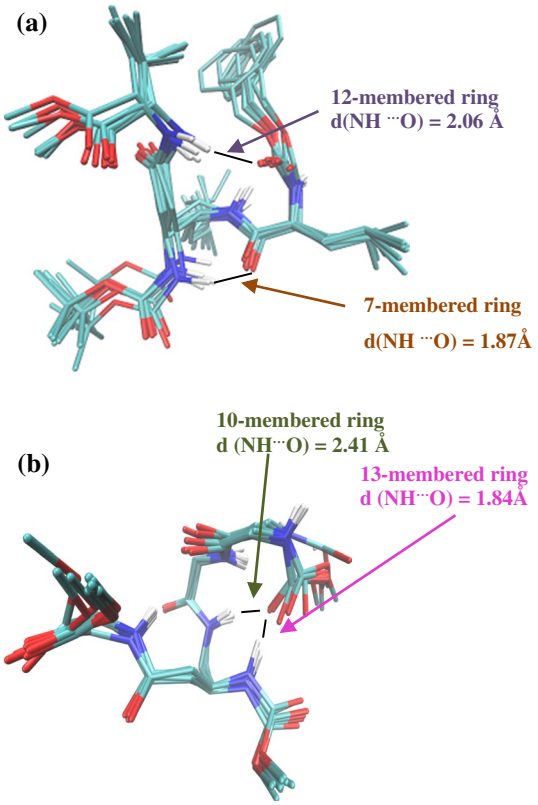


Fig. 4 Overlay of the 10 lowest energy structures of **a** tripeptide **9** and **b** tetrapeptide **10** (hydrogen-bonds are shown in *black lines*; for clarity, only the backbone is shown) (color figure online)

a supplementary CO (of the terminal Boc group) should favor longer hydrogen bonds, such as this C_{13} pseudo cycle, which reminds the hydrogen bonds present in the α helix. These structures, involving the β nitrogen, show the importance of having a supplementary nitrogen in the β-position.

We then wanted to assess the stability of the NMR structures at 300 K and performed molecular dynamics calculations in $CHCl_3$ solvent boxes. For peptide **9**, the 12- and 7-membered rings were conserved during the whole time course of the simulation, as attested by the short d(NH–O) distances (Fig. 5a, b). Similar calculations were carried out on peptide **10** which highlighted the stability of the C_{10} and C_{13} pseudo cycles involving the β,γ-diamino acid (Fig. 5c, d).

In contrast, it showed that the H-bond between the NH valine and CO Leucine was quickly disrupted, allowing the amide proton to specifically interact with the CO of the alanine residue (C_{12} turn, Fig. 5e). This hydrogen bond was stable in both peptides **9** and **10** and is probably favored by the (3*S*,4*S*) stereochemistry of the central β,γ-diamino acid, whereas the (3*R*,4*S*) configuration preferred the C_9 pseudocycle (Fig. 1) (Thétiot-Laurent et al. 2012).

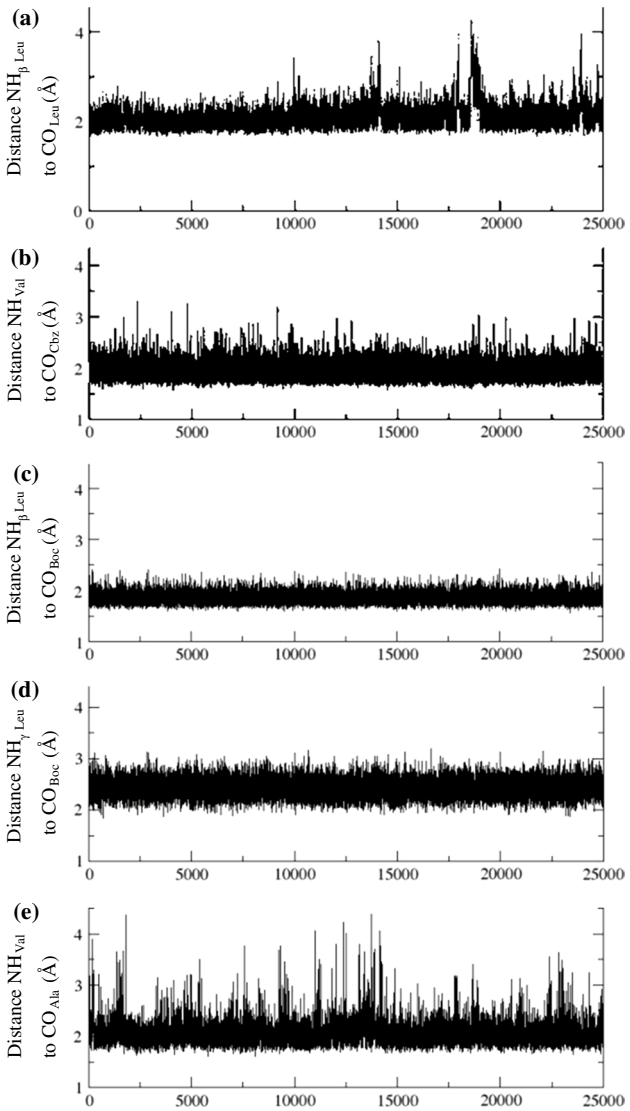


Fig. 5 Trajectories obtained for peptide **9** and **10** in the time course of the MD simulation (25 ns). NH···O=C distances revealing the formation for **9** of a 7-membered ring hydrogen bond (**a**) and a 12-membered ring hydrogen bond (**b**) and for **10** of a 13-membered ring hydrogen bond (**c**), a 10-membered ring hydrogen bond (**d**) and a 12-membered ring hydrogen bond (**e**)

Conclusion

To conclude, we have reported the synthesis of new hybrid α/γ-peptides incorporating a β,γ-diamino acid residue. NMR data, structure calculations and molecular dynamics have shown that this latter was involved in the formation of C_{10} and C_{13} turns leading to a stable three-centered hydrogen bonds arrangement. Such a backbone conformation required both a four-residues long peptide and a (3*S*,4*S*) configuration of the β,γ-diamino acid residue. In particular, a different backbone conformation was obtained for

tripeptide **9** which lacks an N-terminal CO group to establish a long C_{13} pseudocycle, as observed in the α-helices. The presence of a second amino group could then be seen as a tool for long-range peptide structuration. Further investigations with longer peptides are under study in our group.

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Conflict of interest The authors declare that they have no conflict of interest.

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