The discovery of 9/8-ribbons, β/γ-peptides with curved shapes governed by a combined configuration-conformation code†

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The de novo design of a β/γ-peptidic foldamer motif has led to the discovery of an unprecedented 9/8-ribbon featuring an uninterrupted alternating C9/C8 hydrogen-bonding network. The ribbons adopt partially curved topologies determined synchronistically by the β-residue configuration and the γ-residue conformation sets.

The emergence of peptide-based foldamers has had a major impact on the design of molecular architectures shaped by intramolecular non-covalent interactions, principally hydrogen bonds (H-bonds).1,2 The studies of different types of homo-, hetero- and hybrid oligopeptides containing custom-build β- or γ-amino acid building blocks have furnished a considerable collection of folded conformations. Most of these periodic structures are helical; indeed, helices have been prominent in non-peptide foldamers areas too.2,2 Other secondary structural patterns such as strands or turns have been established, but there are very few descriptions of peptide-based foldamer ribbons, which can be defined as flattened structures featuring a succession of regular, well-defined short-range H-bond patterns. Only one 8-ribbon β-peptide structure has been described,3 while two 9-ribbons have been discovered for γ-peptides.4 A bent γ-peptide 7-ribbon, featuring intra-residue H-bonds, was observed for a very short homooligomer of a highly constrained γ-amino acid.6 Very recently, a mixed 7/8-ribbon was described for a short α/β-hybrid peptide.7

The search for new types of foldamers remains a key objective in order to expand the array of organized secondary structures, particularly with unusual architectures. A bottom-up design of “foldable” oligomers should exploit predictable built-in conformational restraints which lead to local folding in a predictable manner.

† Electronic supplementary information (ESI) available: Synthetic procedures, IR and NMR analyses, molecular modelling data. See DOI: 10.1039/c5cc07136d

‡ H NMR spectra of all peptides in CDCl3 were sufficiently well defined and signals were conveniently dispersed, allowing confirmation of the structures and unambiguous attribution of all signals pertinent for conformational analysis, using standard 1D and 2D NMR sequences. ROESY experiments were then performed and the observed correlations are illustrated in Fig. 1.
Solution-state IR absorption spectra of all peptides were recorded in the same solvent and are illustrated in Fig. 2.§

As anticipated, in dipeptide 1 the tACBC-1 residue induced a strong 8-membered ring H-bonded (C8) feature, indicated by diagnostic ROESY interactions and corroborated by a low DMSO-$d_6$ amide NH titration coefficient in $^1$H NMR studies in CDCl$_3$ (see ESI† for details). The IR absorption spectrum of 1 showed a low frequency (H-bonded) amide NH band (3297 cm$^{-1}$) in addition to a free carbamate NH absorption (3447 cm$^{-1}$). In the ROESY analysis of tetrapeptide 3 two tACBC-induced C8 interactions were evident, gratifyingly accompanied by a correlation between H($\gamma$) of GABA-2 and the NH of tACBC-3 which indicated a 9-membered ring H-bond (C9) and thus a C8/C9/C8 conformer. This was corroborated by low $^1$H NMR DMSO-$d_6$ titration coefficients for the three amide NHs but not for the carbamate NH of tACBC-1. In the H-bonded region of the IR spectrum, the C8 absorption band at 3280 cm$^{-1}$ now had a shoulder at 3350 cm$^{-1}$, attributed to the NH of tACBC-3 implicated in the C9 interaction. ROESY analysis of hexapeptide 5 showed the appropriate sequence of correlations for an uninterrupted C8/C9/C8/C9/C8 network of H-bonded interactions while DMSO-$d_6$ titrations confirmed that only the tACBC-1 carbamate NH was not H-bonded. The IR absorption spectrum clearly showed the C8 band at 3286 cm$^{-1}$ with a pronounced C9 shoulder at 3340 cm$^{-1}$.

In dipeptide 2, the tACBC-2 NH showed a ROESY correlation with H($\gamma$) of GABA-1 and a lower $^1$H NMR DMSO-$d_6$ titration coefficient than that of the GABA NH, while an H-bonded amide NH absorption (3312 cm$^{-1}$) was observed in the IR spectrum. These data suggest a contribution from a C9 conformer implicating the GABA residue, which was again gratifying given its flexibility. Tetrapeptide 4 showed appropriate ROESY correlations to implicate a C9/C8/C9 conformer, in which only the N-terminal GABA-1 NH was entirely free on the basis of DMSO-$d_6$ titration coefficients. In the IR spectrum, the broad absorption in the range 3370–3240 cm$^{-1}$ comprises two C9 and one C8 H-bonded NH functions. ROESY analysis of hexapeptide 6 displayed an uninterrupted C9/C8/C9/C8/C9 H-bonded interaction series, substantiated by DMSO-$d_6$ titrations and complemented by the broad NH absorption band in the range 3370–3240 cm$^{-1}$ in the IR spectrum.

Molecular modelling of peptides 3–6 fully supported the strong propensity for the formation of a continuous network of alternating C9/C8 interactions in tACBC/GABA peptides and revealed some engrossing facets of the GABA residues’ behaviour and the peptides’ topologies. A hybrid Monte Carlo Multiple Minima (MCMM) molecular mechanics conformational search was carried out in chloroform medium using Macromodel and the MMFF force field without restraints. From 10 000 generated structures the lowest energy conformers (up to 10 kJ mol$^{-1}$) were retained and sorted according to their conformer family type; in all cases the conformational landscape was dominated by C9/C8-conformer families. It was notable that contributions from helical conformers were non-existent; occasionally C13 features were detected but were part of conformers with significantly higher energies (see ESI†). The C9/C8-conformers of each peptide were subjected to ab initio geometrical optimization by DFT using GAUSSIAN 09 and the B3LYP/6-311G(d,p) basis set in a chloroform medium.

Peptide 3 gave a single low-energy conformer (Fig. 3). The rigid tACBC units displayed highly uniform ($\phi$, $\theta$, $\psi$) torsion angle values ($88^\circ$, $-101^\circ$, $32^\circ$) very close to those which characterize this residue in an optimized 8-helix foldamer. GABA-2 adopted a favourable $\gamma$, $\gamma'$ local conformation for the ($\theta$, $\psi$) torsion angles, facilitating formation of the ($i - 1$ $\rightarrow$ $i + 1$) 9-membered ring H-bond and concomitant orientation of its NH towards an ($i - 2$) carbonyl and of its C==O towards an ($i + 2$) amide NH. Thus a fully

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**Fig. 1** Molecular structures of $\beta$/γ-peptides 1–6 and ROESY correlations observed in CDCl$_3$ (10 mM).

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**Fig. 2** IR absorption spectra of $\beta$/γ-peptides 1–6 in CDCl$_3$ (5 mM).

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structured C8/C9/C8 conformer was in evidence, with C–O · · · H–N distances in the range 1.87–1.91 Å. The structure does not have a helical topology, being flattened and resembling a ribbon. Although the ester C-terminal of GABA-4 was not involved in H-bonding, the (θ, ζ) torsion angles also adopted a g′, g′ local conformation.

Peptide 5 also gave a single low-energy conformer (Fig. 3). The tACBC units behaved essentially as above, dictating local C8 structures. GABA-2 behaved as its eponym in peptide 3, whereas GABA-4 adopted a g′, g′ local conformation for the (θ, ζ) torsion angles in order to accommodate its central C9 feature and both of the adjacent C8 structures. The uninterrupted C8/C9/C8/C9/C8 network, suggested by the spectroscopic studies, was clearly in evidence with all five C–O · · · H–N distances in the range 1.85–1.91 Å. The unsymmetrical 9/8-ribbon architecture neatly disposed the tACBC and GABA residues alternately on either side of the propagation axis.

The conformational analysis of peptides 4 and 6 was illuminating. Peptide 4 showed four C9/C8/C9 conformers (Fig. 4), differing by no more than 0.4 kJ mol⁻¹ in energy. The tACBC-2 residue imposed a C8 local structure as before while each GABA residue adopted a conformation which allowed formation of a C9 feature: this was achieved when the (θ, ζ) torsion angles corresponded to g′, g′ (hereafter G′) or g′, g′ (G′) local conformations, leading to all four possible combinations (G′G′, G′G′, G′G′, G′G′) on the low energy conformer landscape of 4. Each of the three H-bonds were near-planar in all four conformers, with C–O · · · H–N distances in the range 1.83–1.93 Å.

Peptide 6 showed eight C9/C8/C9/C8/C9 conformers, separated by less than 0.6 kJ mol⁻¹ in energy, a family within which all eight possible G′ and G′ combinations for the three GABA residues were present (Fig. 5). Once again, near-planar H-bonds were present in all conformers, with C–O · · · H–N distances in the narrow range 1.85–1.93 Å.

Inspection and comparison of the 9/8-ribbon conformer families of 4 and 6 revealed a fascinating feature: the ribbons showed a certain degree of curvature in the plane perpendicular to that of the propagation of the 9/8-ribbon axis. The extent of curvature for any given conformer could be characterized in terms of the relative orientations of consecutive H-bonded rings, qualified as “straight” (⊥) or “bent” (∩) [2], the latter relationship induces an incremental curvature of the conformer structure. This phenomenon does not correlate with any single GABA conformation (G′ or G′) as such, but instead with the combined local conformation types of the GABA pair on either side of the intervening tACBC residue in a given C9/C8/C9 segment. An unambiguous code exists between GABA-(i)GABA-(i + 2) conformation pairs and the relative orientations of the pairs of H-bonded rings around which they are folded (Table 1). Thus, for example, in conformer 4C the (G′G′) GABA conformer pair translates to a “straight” GABA-1-tACBC-2 fragment followed by a “bent” tACBC-2-GABA-3 fragment. In conformer 6B, with a (G′G′G′) GABA conformer set, the first pair (G′G′) correlates to a “straight-bent” GABA-1-tACBC-2-GABA-3 segment while the second pair (G′G′) translates to a “straight-straight” GABA-3-tACBC-4-GABA-5 segment.

Intriguingly, the mathematical nature of this code makes it impossible to generate a 9/8-ribbon conformer having three or
Table 1  Correlation between local GABA conformation sets and the global topology of peptides 4 and 6 (see text for symbol definitions)

| Peptide conformer | GABA conformer set (G' or G") | Relative orientations of successive H-ring |
|-------------------|-----------------------------|----------------------------------------|
| 4A                | + –                         | – –                                    |
| 4B                | – –                         | + –                                    |
| 4C                | + +                         | – –                                    |
| 4D                | – –                         | + –                                    |
| 6A                | + –                         | – –                                    |
| 6B                | – –                         | + –                                    |
| 6C                | + +                         | – –                                    |
| 6D                | – –                         | + –                                    |
| 6E                | ++                          | – –                                    |
| 6F                | + –                         | – –                                    |
| 6G                | + +                         | – –                                    |
| 6H                | – –                         | + –                                    |

Notes and references

† Amino acid residues are numbered in this paper using the conventional manner for peptides. All peptides were adequately soluble in chloroform but very poorly soluble in more polar solvents and not at all in water.

§ No change in the IR spectral profiles were observed over the range 1–10 mM, suggesting the absence of intermolecular interactions.

¶ For peptides 4 and 6, these qualitative appreciations correspond to dihedral angles between successive O–H–N planes in the ranges 164° to 178° (−) and 122° to 136° (γ) for C9 → C8, and in the ranges 160° to 168° (−) and −136° to −144° (γ) for C8 → C9. See ESI† for more details.

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