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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 has 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.[2,3] 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,[4] while two 9-ribbons have been discovered for γ-peptides.[5] 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.

The β/γ-hybrid peptide manifold provides an interesting case which lends itself to further exploration. Confirming theoretical predictions,[8] the 11/13-helix[9] and the 13-helix[10] have been demonstrated as stable structures. The latter is of particular interest due to its analogy with Nature’s α-helix and successful isosteric replacement of short α-peptide segments by β/γ-peptide motifs in α-helical peptides has been carried out.[11] Conspicuously, however, no other folding manifolds based on shorter range interactions have been demonstrated nor anticipated for β/γ-peptides.

We speculated that it should be possible to rationally design a regular β/γ-peptide folding pattern based on short-range H-bonds. Applying a bottom-up design principle, we reasoned that the β-amino acid component should impart a dominant backbone constraint inducing strong (i–1→ i+1) 8-membered ring H-bonds (C8), while the γ-amino acid component should be essentially unconstrained in order to best adapt the backbone torsion angles to accommodate (i–1→ i+1) 9-membered ring (C9) folding patterns. Appropriate building blocks for such a study emerged as the highly constrained β-amino acid (1R,2R)-2-aminocyclobutane carboxylic acid (tACBC),[12] which has been shown to promote strong C8 structural features in peptides,[13,14] and γ-aminobutyric acid (GABA); although oligomers of GABA do not appear to adopt a regular folded structure and are adopted as flexible linkers in medicinal chemistry,[15] a GABA residue is able to support a C9 secondary features in γ-peptides.[16]

To test this hypothesis two series of peptides were proposed (Figure 1): Boc-(tACBC-GABA)$_n$-OBn (n = 1, 2 3; peptides **1**, **3**, **5**, respectively) and Boc-(GABA-tACBC)$_n$-OBn (n = 1, 2 3; peptides **2**, **4**, **6**, respectively). These peptides were synthesised using standard solution-state methods (see ESI†) and their conformational analysis was conducted using solution-state spectroscopic techniques and molecular modelling.§

^{1}H NMR spectra of all peptides in $CDCl_3$ 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 correlation experiments were then performed and the observed interactions are illustrated in Figure 1. Solution-state IR absorption spectra of all peptides were recorded in the same solvent and are illustrated in Figure 2.¶

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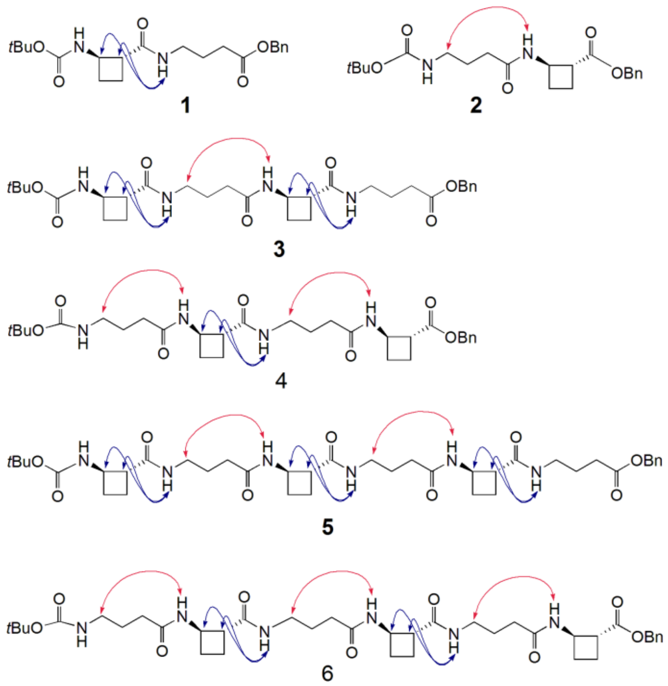

Figure 1 Molecular structures of β/γ-peptides **1-6** and ROESY correlations observed in $CDCl_3$ (10 mM).

As anticipated, in dipeptide **1** the *t*ACBC-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 *t*ACBC-induced C8 interactions were evident, gratifyingly accompanied by a correlation between H(γ) of GABA-2 and the NH of *t*ACBC-3 which indicating 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 two amide NHs but not for the carbamate NH of *t*ACBC-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 *t*ACBC-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 *t*ACBC-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 3330 cm^{-1}.

In dipeptide **2**, the *t*ACBC-2 NH showed a ROESY correlation with H(γ) 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 uninterrupt C9/C8/C9/C8/C9 H-bonded interaction series, substantiated b DMSO-d_6 titrations and complemented by the broad NH absorptio band in the range 3370-3240 cm^{-1} in the IR spectrum.

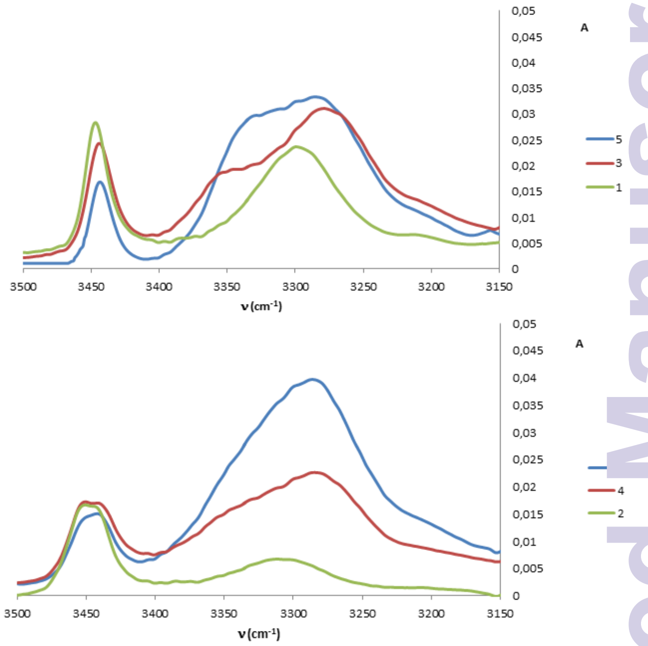

Figure 2 IR absorption specta of β/γ-peptides **1-6** in $CDCl_3$ (5 mM).

Molecular modelling of peptides **3-6** fully supported the stron propensity for the formation a continuous network of alternatin C9/C8 interactions in *t*ACBC/GABA peptides and revealed som engrossing facets of the GABA residues' behaviour and th peptides' topologies. A hybrid Monte Carlo Molecular Mechanic (MCMM) conformational search was carried out in chlorofor medium using Macromodel and the MMFF force field withou restraints. From 10 000 generated structures the lowest energy conformers (up to 10 kJ.mol^{-1}) were retained and sorted accor 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 no existent; occasionally C13 features were detected but were part conformers with significantly higher energies (see ESI†). The C9/C conformers of each peptide were subjected to *ab initio* geometric 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 (Figure 3). The rigid *t*ACBC units displayed highly uniform (*φ*, *θ*, *ψ*) torsion angle value (88°, –101°, 32°) very close to those which characterize this residue in an optimized 8-helix foldamer.[13a] GABA-2 adopted a favour ble g^+, g^+ local conformation for the (*θ*, *ζ*) torsion angles, facilita formation of the (*i*–1→ *i*+1) 9-membered ring H-bond an concomitant orientation of its NH towards an (*i*–2) carbonyl and o its C=O towards an (*i*+2) amide NH. Thus a fully structured C8/C9/C 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, bei flattened and resembling a ribbon. Although the ester C-terminal of GABA-4 was not involved in H-bonding, the (*θ*, *ζ*) torsion angles als adopted a g^+, g^+ local conformation.

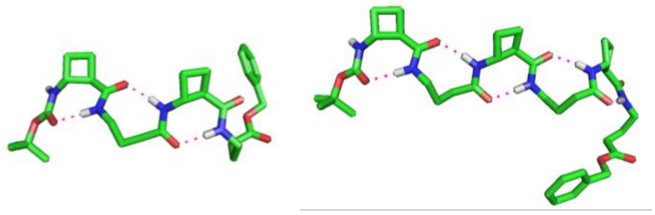

Figure 3 Top-view of the low energy conformers of β/γ-peptides **3** (left) and **5** (right), showing the alternating C9/C8 H-bonding network.

Peptide **5** also gave a single low-energy conformer (Figure 3). The *t*ACBC 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 *t*ACBC 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 (Figure 4), differing by no more than 0.4 kJ.mol^{-1} in energy. The *t*ACBC-2 residue imposed a C8 local structure as before while each GABA residue adopted a conformer which allowing formation of a C9 featured: 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.

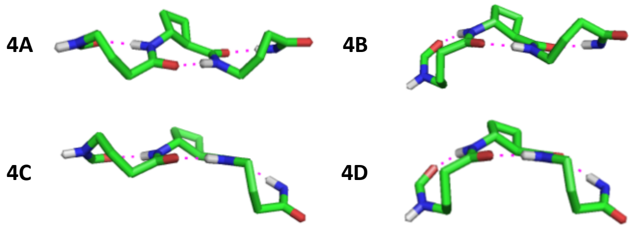

Figure 4 Side-view of the four low energy conformers of β/γ-peptide **4** showing the C9/C8/C9 H-bonding networks. Atoms not relevant to H-bonding have been removed for clarity.

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 (Figure 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" (∩);‡ 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 *t*ACBC 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–*t*ACBC-2 fragment followed by a "bent" *t*ACBC-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–*t*ACBC-2–GABA-3 segment while the second pair (G^+G^-) translates to a "straight-straight" GABA-3–*t*ACBC-4–GABA-5 segment.

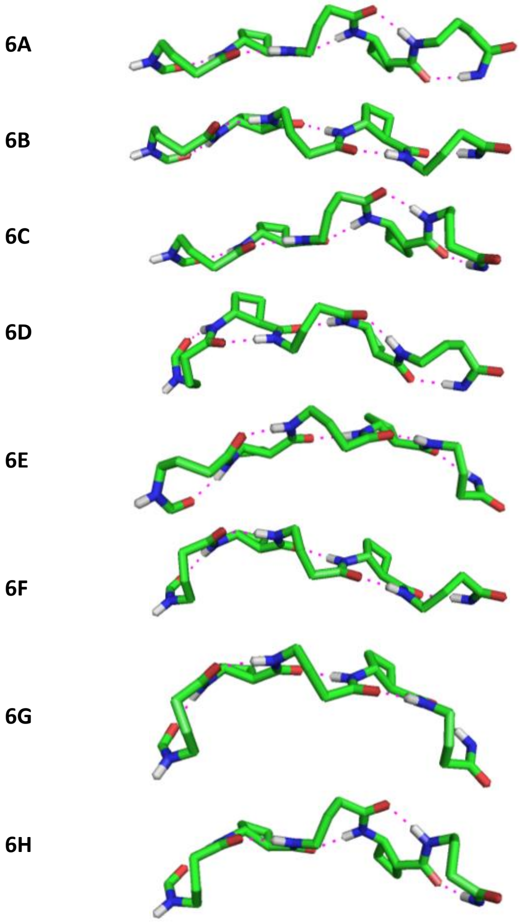

Figure 5 Side-view of the eight low energy conformers of β/γ-peptide **6** showing the C9/C8/C9/C8/C9 H-bonding network and the curved topology. Atoms not relevant to H-bonding have been removed for clarity.

Intriguingly, the mathematical nature of this code makes it impossible to generate a 9/8-ribbon conformer having three or more consecutive "straight" or "bent" fragments. This suggests that 9/8-ribbons cannot lie flat in a plane nor adopt highly-rounded structures; by benefiting from the continuous H-bonding network, they are intrinsically destined to adopt partly curved architectures. Retrospectively, the code allows the assignment of the central C9/C8/C9 fragment of the low energy conformer of peptide **5**: with a (G^+G^-) pair, a "straight-straight" topology is adopted.

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	**− − +**	∩ − ∩ ∩

In summary, these results illustrate the successful application of a bottom-up foldamer design leading to the unprecedented 9/8-ribbon. The unsymmetrical core structure disposes its β- and γ-residues on opposite sides of the ribbon propagation axis, which is partially curved in the perpendicular plane. The facile coexistence of several low energy 9/8-ribbon conformers is rendered possible due to the local flexibility of the GABA components, while the global topology portfolio remains within the limits of a coded curvature, which is governed by a combination of the stereochemical factors imparted by both the rigid *t*ACBC components (configuration) and the GABA components (conformation).[17] These observations underline the interest of ribbons in the foldamer field and further the understanding of the factors which control molecular shape.

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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 175° (–) and 126° to 128° (∩) for C9→C8, and in the ranges 164° to 167° (–) and –139° to –141° (∩) for C8→C9. See SI for more details.

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