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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 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.^{2,3} 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-helix⁹ and the 13-helix¹⁰ 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 ($i - 1 \rightarrow i + 1$) 8-membered ring H-bonds (C8), while the γ -amino acid component should be essentially unconstrained in order to best adapt its backbone torsion angles to accommodate ($i - 1 \rightarrow i + 1$) 9-membered ring (C9) folding pattern. Appropriate building blocks for such a study emerged as the highly constrained β -amino acid (1*R*,2*R*)-2-aminocyclobutane carboxylic acid (*t*ACBC),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 (Fig. 1): Boc-(*t*ACBC-GABA)_{*n*}-OBn (*n* = 1, 2, 3; peptides 1, 3, 5, respectively) and Boc-(GABA-*t*ACBC)_{*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.‡

¹H 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.

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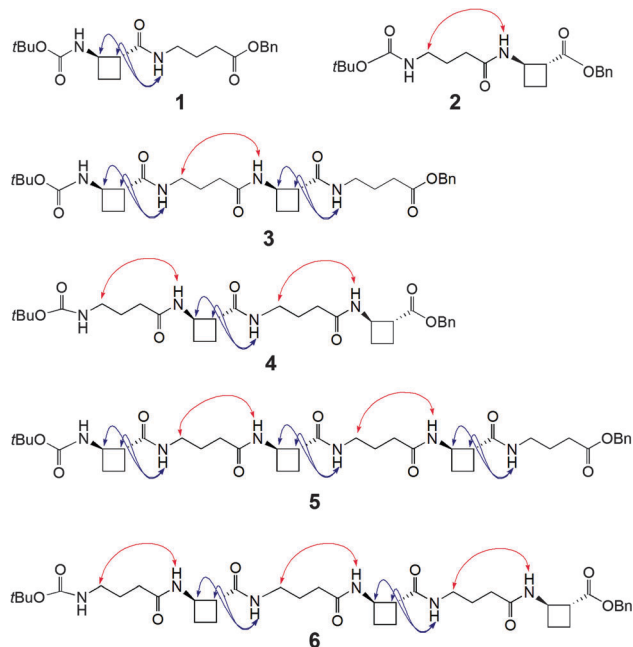


Fig. 1 Molecular structures of β/γ -peptides **1–6** and ROESY correlations observed in CDCl_3 (10 mM).

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 $\text{DMSO-}d_6$ amide NH titration coefficient in ^1H 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 $\text{H}(\gamma)$ 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 ^1H NMR $\text{DMSO-}d_6$ 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 $\text{DMSO-}d_6$ 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 $\text{H}(\gamma)$ of GABA-1 and a lower ^1H NMR $\text{DMSO-}d_6$ 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 $\text{DMSO-}d_6$ titration coefficients. In the IR spectrum, the broad absorption in the range $3370\text{--}3240\text{ cm}^{-1}$ 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 $\text{DMSO-}d_6$ titrations and complemented by the broad NH absorption band in the range $3370\text{--}3240\text{ cm}^{-1}$ 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 (MCM) 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 (φ , θ , ψ) torsion angle values (88° , -101° , 32°) very close to those which characterize this residue in an optimized 8-helix foldamer.^{13a} GABA-2 adopted a favourable g^+ , g^+ local conformation for the (θ , ζ) 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

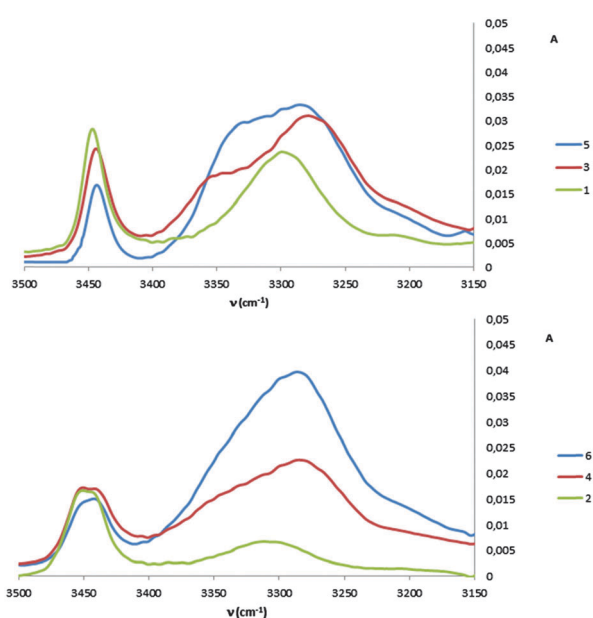


Fig. 2 IR absorption spectra of β/γ -peptides **1–6** in CDCl_3 (5 mM).



