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Switching the H-bonding network of a foldamer by modulating the backbone chirality and constitutional ratio of amino acids†

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This communication describes the folding propensity of a hetero-foldamer motif featuring proline (Pro) and anthranilic acid (Ant) residues in a 1 : 2 : 1 (α : β : α) constitutional ratio. Structural investigations unequivocally suggest that the hydrogen-bonding network of this foldamer motif can be switched between 9-membered and 6-membered by modulating the backbone chirality and constitutional ratio of the amino acid residues.

Proteins, referred to as polymers of amino acids, adopt innumerable and multifaceted secondary structures or shapes in their tertiary or quaternary levels, which is mainly due to the diversity in the dihedral angle preferences of each amino acid present.[1] The protein folding that involves the selection of an energetically favourable conformation to carry out specific biochemical reactions for particular biological responses turns out to be critical since any misfolding can lead to adverse effects.[2] The challenges in investigating and understanding the folding patterns and preferences arise when a protein lacks conformational ordering.[3] The study of conformationally ordered, discrete non-natural oligomers called foldamers with rigid backbone and dihedral angle preferences provides the platform to understand the intricate folding patterns in large biopolymers such as proteins.[4]

The concept of heterogeneous foldamers invokes unpredictability and diversity in folding behaviour, quite often depending on the torsion angle, backbone constraints, and steric and hydrogen-bonding preferences of individual amino acid residues.[5] The constrained aromatic β-amino acid – anthranilic acid (Ant), which induces a sheet-like secondary structure featuring intramolecular six-membered-ring hydrogen-bonding in its homo oligomers,[6] forms a right-handed helical structure with proline (Pro) in its hybrid oligomers (Fig. 1).[7]

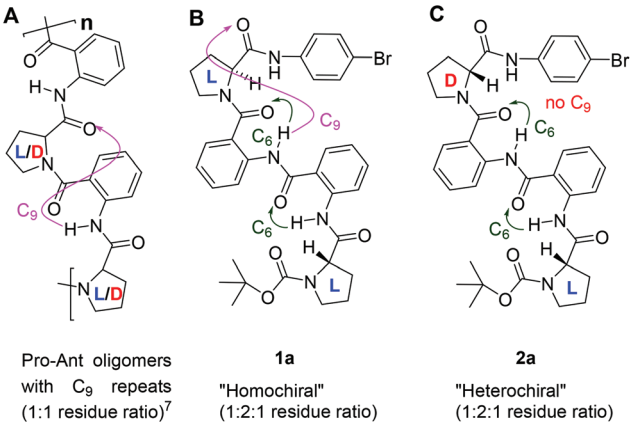

Fig. 1 Helical folding propensities of Pro–Ant oligomers featuring C_9 hydrogen-bonding patterns (A),[7a,b] homochiral Pro–Ant–Ant–Pro oligomers featuring C_6 and C_9 hydrogen-bonding patterns (B) and heterochiral Pr–Ant–Ant–Pro oligomers featuring only C_6 hydrogen-bonding patterns (C).

These α/β hybrid oligomers in a 1 : 1 ratio featured a repeating 9-membered hydrogen-bonding, mainly due to the steric and torsional constraints of Pro and Ant residues,[7a] and were found to be stable, displaying similar repeating hydrogen-bonding networks, but with opposite sense of chirality, upon altering the chirality of Pro residues.[7b] In this context, it was thought worthwhile to study the effect of chirality alteration[8] in Pro–Ant foldamers by investigating oligomers made of a 1 : 2 : 1 constitutional ratio of α/β/α amino acid residues with differing chiralities.

The results of conformational investigations obtained from crystal structure[9] and solution-state NMR show a three dimensional helical conformation for the homochiral oligomers featuring a combination of C_9 and C_6 hydrogen-bonding networks while the heterochiral oligomers show an exclusively C_6 hydrogen-bonding pattern – a three dimensional structure which is devoid of C_9 hydrogen-bonding. This contrasting

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result assumes significance, since a 9-membered robust hydrogen-bonded network is almost always observed when Pro succeeds Ant in synthetic peptide sequences.[7a–c] The formation of the C_9 turn had been shown to be caused by the intense steric clash between the aryl and the pyrolidine ring – forcing them to adopt antiperiplanar orientation bringing the Ant-NH and Pro-CO in close proximity, forming a tight 9-membered hydrogen-bonded network.[7a]

The homochiral oligomers **1a**, **1b** and heterochiral ones **2a**, **2b** (Fig. 2) were synthesised following straightforward synthetic steps, as detailed in the ESI (page S3†). The idea of introducing the 4-bromoanilide group at the C-terminus was to enhance crystal formation, as we observed earlier.[10] A closer look at the crystal structures of the homochiral enantiomeric pair **1a**, **1b** reveal a combination of C_6 and C_9 H-bonding networks. In contrast, the heterochiral enantiomeric pair **2a**, **2b** reveals an exclusively C_6 H-bonding pattern – a three dimensional structure which is devoid of C_9 H-bonding.

The differences in the folding behavior of **2a** and **2b** (heterochiral oligomers) with respect to **1a** and **1b** (homochiral oligomers) seem to be due to the significant torsional change (ψ values at Ant2 and Pro2, ~30° and ~15° respectively, Table S84, ESI†). Surprisingly, even the change of chirality at Pro2 [**2a** (D) and **2b** (L)] does not have any effect on the torsions, which exhibit similar values for ψ (although different signs). This significant conformational change for ψ at Ant2 and Pro2 of **2a** and **2b** could be due to the involvement of the anilide (4-Br) group in intramolecular C–H···π, intermolecular C–Br···O=C halogen-bonding interaction and dipolar C–Br···C=O[11] contact of the perpendicular motif (see ESI, pages S76–S79†).

It was noted that the dissimilar folding of homo- and heterochiral oligomers is accompanied by the observed dissimilar aromatic interactions[12] between Ant1 and 4-Br anilide rings. The two rings do not have any noticeable interactions in homochiral oligomers **1a** and **1b** featuring C_9 hydrogen-bonding between Ant2 NH and Pro CO, while in heterochiral oligomers **2a** and **2b**, these are engaged in a short and linear C–H···π interaction (see, ESI, pages S76–S79†), lacking C_9 hydrogen-bonding. This observation is also prevailing in the solution state, as evidenced by the characteristic NOEs: C9H *vs.* NH3 and C9H *vs.* C21H in a related heterochiral oligomer **2c** (see ESI, S73†) in which the NH3 and C21H of Pro2 are in close proximity to the Ant1 ring, thus revealing that both the rings [Ant1 and anilide (4-Br)] are in close proximity. Similar NOEs were observed for a related heterochiral oligomer (see, ESI, page S72†) which is devoid of a 4-Br anilide ring, thus nullifying the effect of an aromatic amide at the C-terminus and its involvement in edge-to-face interaction for modulating the conformation of peptides.[12] The corresponding NOE

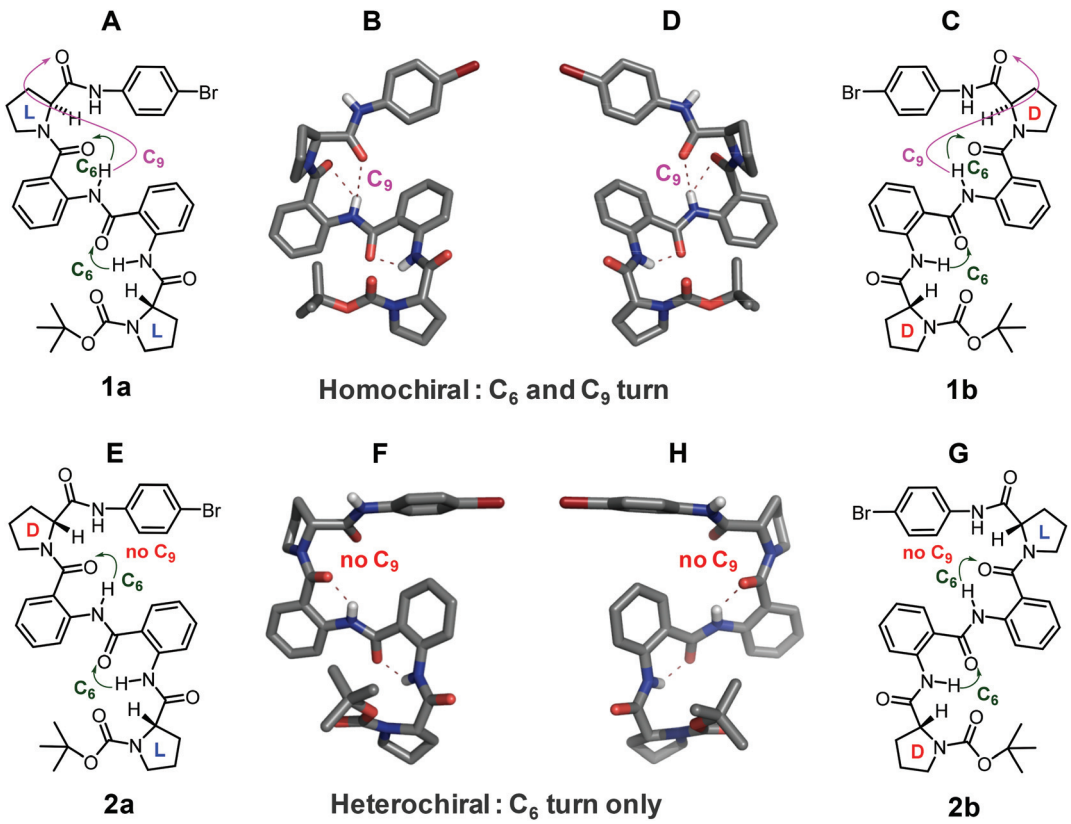

Fig. 2 Molecular and crystal structures, respectively, of homochiral tetramers **1a** (A, B) and **1b** (C, D) showing a combination of C_6 and C_9 hydrogen-bonding and the heterochiral tetramers **2a** (E, F) and **2b** (G, H) showing only C_6 hydrogen-bonding. *Note*: The peptides exist in two conformations in crystals: see ESI, pages S74–S84,† for extensive hydrogen-bonding and torsion angle investigations.

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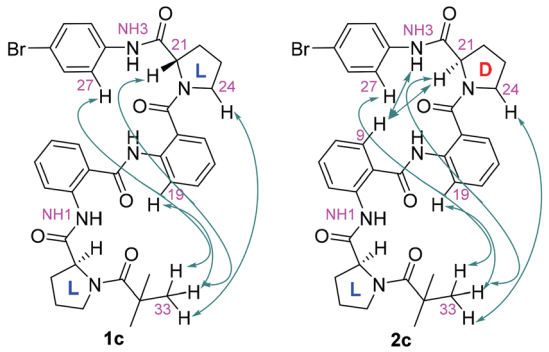

Fig. 3 Figure showing the selected NOE interactions observed for **1c** (homochiral) and **2c** (heterochiral) in the solution-state ($CDCl_3$, 400 MHz). Full NOESY spectra and their 2D extracts are available in the ESI, pages S71–S73.† *Note*: The pivaloyl group at the N-terminus was used to arrest the *cis–trans* isomerisation of the terminal Pro residue.[13]

interactions are absent in homochiral oligomer **1c** justifying the absence of edge-to-face interaction involving Ant2 and anilide (4-Br) rings (see, ESI, page S71†).

The solution-state NMR studies by 2D NOESY confirmed that the solid-state conformation is clearly reflected in the solution-state as well. Fig. 3 explains various NOE interactions supporting their 3-dimensional conformation (ESI, pages S71–S73† for 2D spectra). The strength of various H-bonding networks was examined by NMR DMSO-d_6 titration, dilution and variable temperature experiments in $CDCl_3$. All the NHs except NH3 show no shift, signifying their participation in intramolecular H-bonding (see, ESI, pages S50–S58†).

The difference in the chiral and conformational behaviour of the oligomers is indeed reflected in their CD spectra.[14] The homochiral oligomer **1a**, featuring C_6 and C_9 H-bonding, shows a maxima at 200 nm, zero-crossing at 216 nm, and a strong minimum at 245 nm which are mirrored for the homochiral oligomer **1b**. The heterochiral oligomer **2a** on the other hand shows entirely different spectral patterns exhibiting maxima at 200 nm and 222 nm, zero-crossing at 230 nm and a strong minimum at 244 nm. The heterochiral oligomer **2b** shows the exact mirror image of absorbance peaks. A weak second minimum is observed in **1a**, **1b**, **2a** and **2b** near 300 nm due to the presence of aromatic groups (Fig. 4).[14]

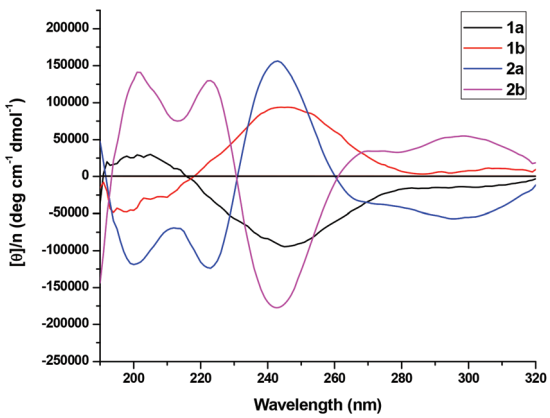

Fig. 4 Figure showing the CD absorption spectra (298 K) for oligomers **1a**, **1b**, **2a** and **2b** in trifluoroethanol (0.02 mM).

In summary, this work illustrates the effect of chirality alteration on Pro–Ant oligomers featuring a 1 : 2 : 1 constitutional ratio of α/β/α residues. Tetramers with homochiral proline residues show a combination of C_6 and C_9 hydrogen bonding networks while heterochiral residues feature only C_6 turns. The absence of C_9 turns in heterochiral residues is attributed to significant torsional changes (ψ) at Ant2 and Pro2, which could be due to the engagement of the anilide (4-Br) moiety in intra- and intermolecular interactions. This work provides an opportunity to understand the complexity in the folding behaviour of peptides using conformationally constrained synthetic oligomers called foldamers.[15,16]

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Notes and references

1 G. N. Ramachandran, C. Ramakrishnan and V. Sasisekharan, *J. Mol. Biol.*, 1963, **7**, 95.
2 R. L. Wiseman and J. W. Kelly, *Science*, 2011, **332**, 44.
3 V. N. Uversky, *Protein Sci.*, 2002, **11**, 739.
4 S. H. Gellman, *Acc. Chem. Res.*, 1998, **31**, 173.
5 (*a*) A. Roy, P. Prabhakaran, P. K. Baruah and G. J. Sanjayan, *Chem. Commun.*, 2011, **47**, 11593; (*b*) W. S. Horne and S. H. Gellman, *Acc. Chem. Res.*, 2008, **48**, 1399; (*c*) T. A. Martinek and F. Fulop, *Chem. Soc. Rev.*, 2011, **41**, 687.
6 Y. Hamuro, S. J. Geib and A. D. Hamilton, *J. Am. Chem. Soc.*, 1996, **118**, 7529.
7 (*a*) P. Prabhakaran, S. S. Kale, V. G. Puranik, P. R. Rajamohanan, O. Chetina, J. A. K. Howard, H.-J. Hofmann and G. J. Sanjayan, *J. Am. Chem. Soc.*, 2008, **130**, 17743; (*b*) S. S. Kale, A. S. Kotmale, A. K. Dutta, S. Pal, P. R. Rajamohanan and G. J. Sanjayan, *Org. Biomol. Chem.*, 2012, **10**, 8426; (*c*) V. H. Thorat, T. S. Ingole, K. N. Vijayadas, R. V. Nair, S. S. Kale, V. V. E. Ramesh, H. C. Davis, P. Prabhakaran, R. G. Gonnade, R. L. Gawade, V. G. Puranik, P. R. Rajamohanan and G. J. Sanjayan, *Eur. J. Org. Chem.*, 2013, 3529; (*d*) R. V. Nair, S. Kheria, S. Rayavarapu, A. S. Kotmale, B. Jagadeesh, R. G. Gonnade, V. G. Puranik, P. R. Rajamohanan and G. J. Sanjayan, *J. Am. Chem. Soc.*, 2013, **135**, 11477.
8 For the effect of chirality alteration on peptide backbones, see: (*a*) F. Freire, J. D. Fisk, A. J. Peoples, M. Ivancic, I. A. Guzei and S. H. Gellman, *J. Am. Chem. Soc.*, 2008, **130**, 7839; (*b*) M. G. Bomar, B. Song, P. Kibler, K. Kodukula and A. K. Galande, *Org. Lett.*, 2011, **13**, 5878; (*c*) Y. Demizu, Y. Yabuki, M. Doi, Y. Sato, M. Tanakac and M. Kurihara, *J. Pept. Sci.*, 2012, **18**, 466; (*d*) J. M. Aizpurua, C. Palomo, E. Balentová, A. Jimenez, E. Andreieff, M. Sagartzazu-Aizpurua, J. I. Miranda and A. Linden, *J. Org. Chem.*, 2013, **78**, 224.

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9 Crystallographic data of **1a**, **1b**, **2a** and **2b** have been deposited with the CCDC: 948398–948401.

10 (*a*) P. K. Baruah, N. K. Sreedevi, B. Majumdar, R. Pasricha, P. Poddar, R. Gonnade, S. Ravindranathan and G. J. Sanjayan, *Chem. Commun.*, 2008, 712; (*b*) K. N. Vijayadas, H. C. Davis, A. S. Kotmale, R. L. Gawade, V. G. Puranik, P. R. Rajamohanan and G. J. Sanjayan, *Chem. Commun.*, 2012, **48**, 9747.

11 R. J. Santos-Contreras, F. J. Martinez-Martinez, E. V. Garcia-Baez, I. I. Padilla-Martinez, A. L. Peraza and H. Hopfl, *Acta Crystallogr., Sect. C: Cryst. Struct. Commun.*, 2007, **63**, o239.

12 L. M. Salonen, M. Ellermann and F. Diederich, *Angew. Chem., Int. Ed.*, 2011, **50**, 4808.

13 B. Chatterjee, I. Saha, S. Raghothama, S. Aravinda, R. Rai, N. Shamala and P. Balaram, *Chem.–Eur. J.*, 2008, **14**, 6192.

14 S. M. Kelly and N. C. Price, *Curr. Protein Pept. Sci.*, 2000, **1**, 349.

15 For selected recent reviews, see: (*a*) G. Guichard and I. Huc, *Chem. Commun.*, 2011, **47**, 5933; (*b*) C. Tomasini, G. Angelici and N. Castellucci, *Eur. J. Org. Chem.*, 2011, 3648; (*c*) P. G. Vasudev, S. Chatterjee, N. Shamala and P. Balaram, *Chem. Rev.*, 2011, **111**, 657; (*d*) V. Azzarito, K. Long, N. S. Murphy and A. J. Wilson, *Nat. Chem.*, 2013, **5**, 161.

16 (*a*) V. V. E. Ramesh, G. Priya, A. S. Kotmale, R. G. Gonnade, P. R. Rajamohanan and G. J. Sanjayan, *Chem. Commun.*, 2012, **48**, 11205; (*b*) S. S. Kale, G. Priya, A. S. Kotmale, R. L. Gawade, V. G. Puranik, P. R. Rajamohanan and G. J. Sanjayan, *Chem. Commun.*, 2013, **49**, 2222; (*c*) P. Prabhakaran, G. Priya and G. J. Sanjayan, *Angew. Chem., Int. Ed.*, 2012, **51**, 4006; (*d*) G. Priya, A. S. Kotmale, R. L. Gawade, D. Mishra, S. Pal, V. G. Puranik, P. R. Rajamohanan and G. J. Sanjayan, *Chem. Commun.*, 2012, **48**, 8922; (*e*) V. V. E. Ramesh, S. S. Kale, A. S. Kotmale, R. L. Gawade, V. G. Puranik, P. R. Rajamohanan and G. J. Sanjayan, *Org. Lett.*, 2013, **15**, 1504; (*f*) C. Caumes, O. Roy, S. Faure and C. Taillefumier, *J. Am. Chem. Soc.*, 2012, **134**, 9553; (*g*) E. D. Santis, T. Hjelmgaard, C. Caumes, S. Faure, B. D. Alexander, S. J. Holder, G. Siligardi, C. Taillefumier and A. A. Edwards, *Org. Biomol. Chem.*, 2012, **10**, 1108; (*h*) M. I. Simone, A. A. Edwards, G. E. Tranter and G. W. J. Fleet, *Amino Acids*, 2011, **41**, 643.