Bend-ribbon forming γ-peptides†

Abhishek Kothari, M. Khurram N. Qureshi, Elizabeth M. Beck and Martin D. Smith*

Received (in Cambridge, UK) 30th April 2007, Accepted 6th June 2007
First published as an Advance Article on the web 15th June 2007
DOI: 10.1039/b706528k

Homo- and heterochiral tetrameric γ-peptide derivatives in which the backbone is constrained by a five-membered ring populate a bend-ribbon conformation in solution stabilized by intramolecular hydrogen bonds.

Amongst abiotic folding backbones, a subset comprising ring-constrained β-peptides has been intensively studied.[1] This has demonstrated that a change in either the size or the stereochemistry of the side-chain ring constraint has a profound influence on the solution (and solid state) conformation. Thus, peptides composed of 2,3-*trans*-cyclohexane β-amino acids adopt a 14-helical conformation[2] whilst those possessing a 2,3-*trans*-cyclopentane[3] or 2,3-*cis*-oxetane[4] constraint adopt 12- and 10-helical conformations respectively. In contrast, an 8-helical conformation is populated by 2,3-*trans*-β-peptides based upon oxanorbornene.[5] It has also been shown that strand[6] and alternating 12/10 helical conformations may be accessed through homo- or heterochiral combinations of either 2,3-*cis*- or 2,3-*trans*-cyclopentane β-amino acids,[7] and that α/β-peptides containing *cis*-cyclopropane β-amino acids adopt stable helical folds.[8] In comparison, little is known about the effects of variation of the ring size in γ-peptides.[9] An interesting study of 2,3-*trans*-β-aminoxy acid oligomers demonstrated that conformation is independent of ring size for five- or six-membered *trans*-configured constraints; a repeating turn structure stabilized by 9-membered ring hydrogen bonds was observed in both cases.[10] This is consistent with insightful theoretical studies that have predicted that 9- and 14-membered ring hydrogen bonded helices are the most stable conformations for γ-peptides.[11,12] As part of a programme aimed at investigating the factors that affect the preference for intermolecular rather than intramolecular hydrogen bonding in short γ-peptides, we have shown that simple cyclopropane γ-peptide derivatives such as **1** self-assemble into parallel sheet structures in the solid state (Fig. 1).[13] These sheets are stabilized by intermolecular bifurcated hydrogen bonds in which the amide N–H protons and the C–H bond on the cyclopropane adjacent to the amide group function as hydrogen bond donors. In this paper we describe how the introduction of a five-membered ring constraint on the backbone of γ-amino acid units permits exploration of the intramolecular rather than intermolecular hydrogen bonding manifolds, and investigate the conformational effects of generating homo- and heterochiral systems.

Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge, UK CB2 1EW. E-mail: mds44@cam.ac.uk; Fax: 44 1223 336362; Tel: 44 1223 336511

† Electronic supplementary information (ESI) available: Full experimental and spectroscopic details for all compounds; ^{1}H and ^{13}C NMR spectra for oligomers. Conformational data for tetramers **4** and **6**: TOCSY, COSY, NOESY, HMQC and HMBC spectra and DMSO titration. See DOI: 10.1039/b706528k

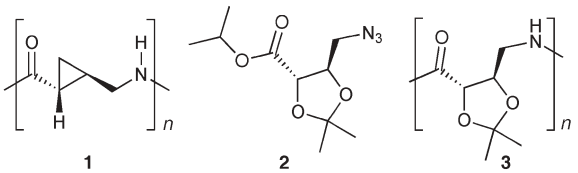

Fig. 1 Ring-constrained γ-peptide derivatives.

For this study we have utilized a 2,3-*trans*-dioxolane-constrained γ-amino acid derivative **2**. The use of a dioxolane ring system rather than a cyclopentane as a five-ring constraint is envisaged to have little impact on the overall conformation whilst simplifying the synthetic approach and easing analysis of NMR spectra. The synthesis of both enantiomers of this γ-amino acid derivative **2** is easily achieved from dimethyl tartrate, and oligomeric derivatives of this building block **3** are efficiently assembled by an iterative coupling strategy.† Tetrameric and hexameric homooligomers **4** and **5** respectively (composed of L-tartrate derived units) were prepared, and a series of heterooligomeric molecules including **6** and **7** consisting of alternating D- and L-tartrate derived monomers were synthesized (Fig. 2) and their conformational preferences investigated.[14]

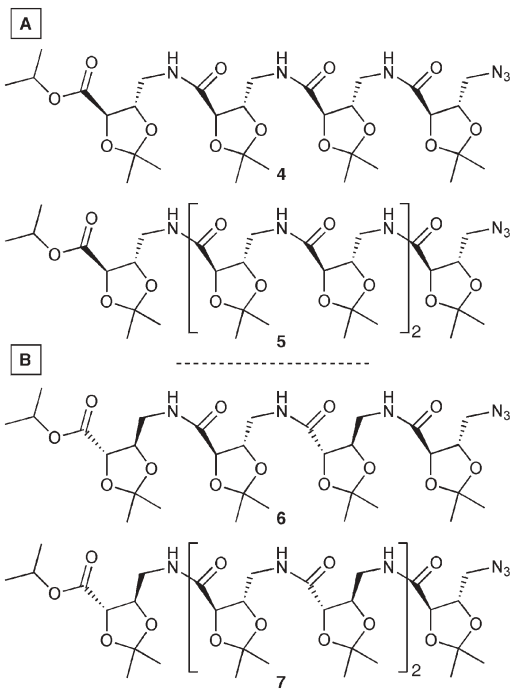

Fig. 2 [A] Homochiral oligomeric γ-peptide derivatives. [B] Alternating heterochiral oligomeric γ-peptide derivatives.

The solution conformations of the tetrameric derivatives **4** and **6** were studied by NMR in organic solvents, and it was found that benzene-d_6 gave the best dispersion of resonances, particularly in the amide region (Fig. 3A). For both of these derivatives, resonance dispersion is excellent considering the repeating unit and was found to be independent of concentration below 10 mM, which is consistent with a highly populated solution conformation. For **4** and **6**, all resonances could be unambiguously assigned through a combination of 2D experiments. COSY and TOCSY spectra allowed assignment within each residue, and semi-selective long-range heteronuclear correlations[15] were used to establish unambiguous through-bond connectivity. Correlations between $H2^i$ and $C{=}O^i$ and also from NH^{i+1} and $H4^{i+1}$ to $C{=}O^i$ were observed, allowing all neighbouring residues to be identified. NOEs between the backbone protons (H^2, H^3, H^4, $H^{4'}$) are only seen *within* each monomer unit, and all inter-residue NOEs involve the amide protons (Fig. 4).[16]

Analysis of the amide regions of the ^{1}H NMR spectra for both series of tetrameric and hexameric oligomers indicated two distinct groups of amide proton resonances (Fig. 3A). The chemical shifts of amide protons are sensitive to the presence of hydrogen bonding; a decrease in diamagnetic shielding due to involvement in a hydrogen bond should result in a downfield chemical shift. For the tetramer **4**, such a shift is observed for two of the three protons (NH^B, δ_H 7.50 and NH^C, δ_H 7.42), suggesting they are involved in hydrogen-bond formation. The remaining amide (NH^D) resonates at lower frequency (δ_H 6.78) characteristic of an amide proton that experiences no hydrogen-bonding. This shift is similar to that observed for a dimeric derivative (δ_{NH} 6.84)† that is unable to form a hydrogen bond analogous to those proposed. An equivalent pattern is observed for the hexamer **5**, which exhibits four high frequency amide protons (NH^B, NH^C, NH^D, NH^E) and one again at lower frequency (NH^F).† The ^{1}H NMR spectrum of heterochiral tetramer **6** (Fig. 3A) demonstrates a similar pattern in which two amide protons are shifted downfield (NH^B, δ_H 7.55 and NH^C, δ_H 7.45), and an analogous arrangement is observed for

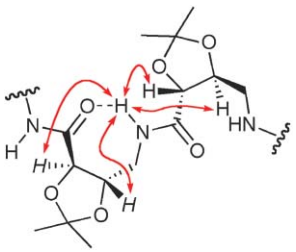

Fig. 4 Representative NOEs for tetramers **4** and **6** involving amide protons (NOEs to the methylene adjacent to the amide proton are omitted for clarity).

higher oligomers.[17]† Titrations in which aliquots of DMSO-d_6 were sequentially added to a solution of the oligomers **4** and **6** in benzene-d_6 were performed to probe these hydrogen bonding interactions (Fig. 3B). In both cases, those protons that are shielded from the strongly H-bonding solvent are less sensitive to perturbation on DMSO addition (NH^B, NH^C) whilst the solvent-exposed amide proton (NH^D) shifts progressively to higher frequency, consistent with its lower chemical shift.

This pattern of shielded and deshielded amide protons is consistent with a repeating structural unit and suggests that both tetrameric derivatives **4** and **6** populate bend-ribbon (or strand-type) conformations in benzene solution stabilized by intramolecular 7-membered-ring nearest-neighbour hydrogen bonds (Fig. 5). Compounds **5** and **7** populate similar conformations. This conformation leaves the *C*-terminal amide (NH^D) solvent-exposed, which is consistent with an ester being a poorer hydrogen-bond acceptor than an amide.[18,19]

In this conformation the isopropylidene groups project away from the peptidic backbone, which favours an intra-residue hydrogen bond despite the *trans*-configured ring constraint. These results are consistent with elegant model studies on the folding of γ-amino butyric acid derivatives which demonstrated that both 7- and 9-membered nearest neighbour hydrogen bonds

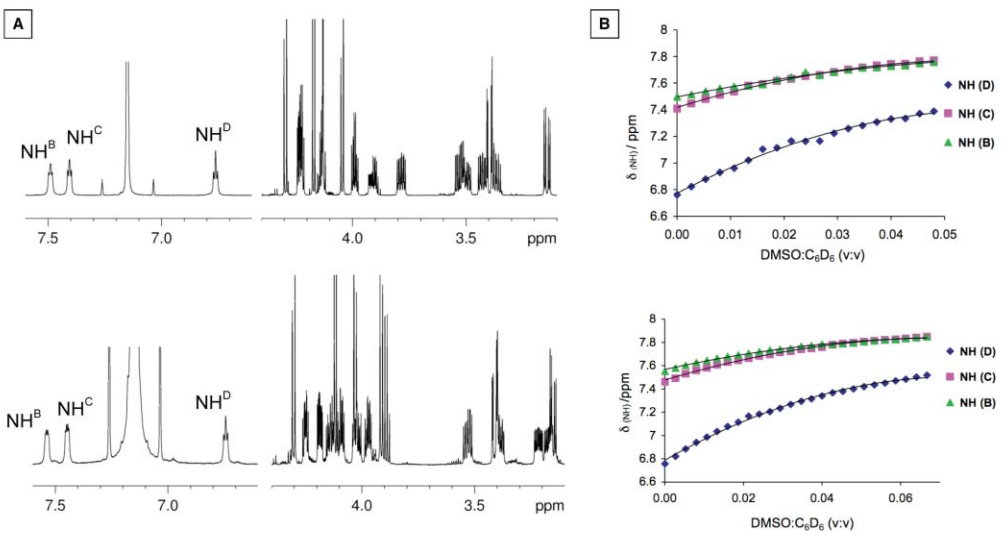

Fig. 3 [A] Partial ^{1}H NMR spectra for tetramers **4** (upper plot) and **6** (lower plot) (700 MHz, 5.6 mM, benzene-d_6 @ 7.15 ppm, 298 K). [B] Plots showing behaviour of amide NH chemical shifts of **4** (upper plot) and **6** (lower plot) on titration of DMSO-d_6 to a 5.6 mM benzene-d_6 solution (2 μl aliquots, 400 MHz, 298 K, referenced to TMS @ 0 ppm).

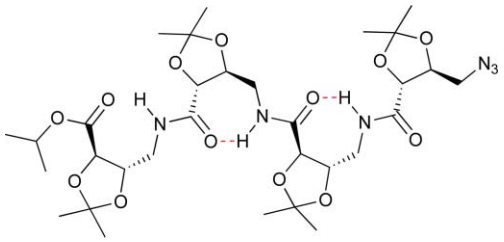

Fig. 5 Observed bend-ribbon solution conformation of **4** (hydrogen bonds in red).

are favoured.[20] It is interesting to speculate why this *trans*-dioxolane constrained amino acid prefers to adopt an intra-residue hydrogen bond when the corresponding 2,3-*trans*-cyclopentane β-aminoxy acid oligomers, which effectively differ only by the substitution of the methylene group in monomers such as **2** for an oxygen, form 9-membered inter-residue hydrogen bonds. The larger, nine-membered ring could be anticipated to be favoured as the geometry for the hydrogen bond can approach linearity. However, it is likely that this is more a reflection of a specific conformational preference of the N–O bond, and the ability of the oxygen of the N–O bond to function as a bifurcated hydrogen bond acceptor in concert with a carbonyl group.

This paper has confirmed that the conformations of ring constrained γ-peptides may be modulated by changing the size of the ring constraint[13] and demonstrates that intra-residue nearest-neighbour hydrogen bonds may be favoured when the flexibility of a ring constraint can permit their formation. In this scenario, the propensity for the formation of such hydrogen bonds appears to override the influence of the absolute configuration of individual residues, as seen in the heterochiral derivatives **6** and **7**. The generation of other ring constrained γ-peptides should elucidate in detail the factors that govern the conformations populated by these systems.

The authors would like to thank the Royal Society for a URF (to MDS), Dr Tim Claridge (University of Oxford) for the band-selective constant-time HMBC sequence, Mr Duncan Howe and Mr Andrew Mason for assistance with NMR spectroscopy, the Pakistani HEC (to MKNQ), the Hinduja Cambridge Trust (to AK), AstraZeneca (for a Research Support Grant) and the EPSRC National Mass Spectrometry Centre (Swansea).

Notes and references

1 S. H. Gellman, *Acc. Chem. Res.*, 1998, **31**, 173; R. P. Cheng, S. H. Gellman and W. F. DeGrado, *Chem. Rev.*, 2001, **101**, 3219; D. Seebach, D. F. Hook and A. Glättli, *Biopolymers*, 2005, **84**, 23; D. J. Hill, M. J. Mio, R. B. Prince, T. S. Hughes and J. S. Moore, *Chem. Rev.*, 2001, **101**, 3893; F. Fulop, *Chem. Rev.*, 2001, **101**, 2181; N. Rathore, S. H. Gellman and J. J. de Pablo, *Biophys. J.*, 2006, **91**, 3425.

2 D. H. Appella, L. A. Christianson, I. L. Karle, D. R. Powell and S. H. Gellman, *J. Am. Chem. Soc.*, 1996, **118**, 13071; D. H. Appella, L. A. Christianson, I. L. Karle, D. R. Powell and S. H. Gellman, *J. Am. Chem. Soc.*, 1999, **121**, 6206.

3 D. H. Appella, L. A. Christianson, D. A. Klein, D. R. Powell, X. L. Huang, J. J. Barchi and S. H. Gellman, *Nature*, 1997, **387**, 381; D. H. Appella, L. A. Christianson, D. A. Klein, M. R. Richards, D. R. Powell and S. H. Gellman, *J. Am. Chem. Soc.*, 1999, **121**, 7574.

4 T. D. W. Claridge, J. M. Goodman, A. Moreno, D. Angus, S. F. Barker, C. Taillefumier, M. P. Watterson and G. W. J. Fleet, *Tetrahedron Lett.*, 2001, **42**, 4251.

5 R. J. Doerksen, B. Chen, J. Yuan, J. D. Winkler and M. L. Klein, *Chem. Commun.*, 2003, 2534.

6 T. A. Martinek, G. K. Tóth, E. Vass, M. Hollósi and F. Fülop, *Angew. Chem., Int. Ed.*, 2002, **41**, 1718; S. Chandrasekhar, B. N. Babu, A. Prabhakar, A. Sudhakar, M. S. Reddy, M. U. Kiran and B. Jagadeesh, *Chem. Commun.*, 2006, 1548.

7 T. A. Martinek, I. M. Mándity, L. Fülop, G. K. Tóth, E. Vass, M. Hollósi, E. Forró and F. Fülop, *J. Am. Chem. Soc.*, 2006, **41**, 13539.

8 S. De Pol, C. Zorn, C. D. Klein, O. Zerbe and O. Reiser, *Angew. Chem., Int. Ed.*, 2004, **43**, 511.

9 For examples of ring-constrained γ-peptide foldamers see: M. G. Woll, J. R. Lai, I. A. Guzei, S. J. C. Taylor, M. E. B. Smith and S. H. Gellman, *J. Am. Chem. Soc.*, 2001, **123**, 11077; J. Farrera-Sinfreu, L. Zaccaro, D. Vidal, X. Salvatella, E. Giralt, M. Pons, F. Alberico and M. Royo, *J. Am. Chem. Soc.*, 2004, **126**, 6048; P. K. Baruah, N. K. Sreedevi, R. Gonnade, S. Ravindranathan, K. Damodaran, H. J. Hofmann and G. J. Sanjayan, *J. Org. Chem.*, 2007, **72**, 636.

10 D. Yang, D. W. Zhang, Y. Hao, Y. D. Wu, S. W. Luo and N. Y. Zhu, *Angew. Chem., Int. Ed.*, 2004, **43**, 6719.

11 C. Baldauf, R. Günther and H. J. Hofmann, *Helv. Chim. Acta*, 2003, **86**, 2573.

12 C. Baldauf, R. Günther and H. J. Hofmann, *J. Org. Chem.*, 2005, **70**, 5351.

13 M. K. N. Qureshi and M. D. Smith, *Chem. Commun.*, 2006, 5006.

14 A series of oligomers (dimer, trimer, tetramer, pentamer) up to the hexamer level was prepared for the heterochiral series.

15 T. D. W. Claridge and I. Perez-Victoria, *Org. Biomol. Chem.*, 2003, **1**, 3632; W. Bermel, K. Wagner and C. Griesinger, *J. Magn. Reson.*, 1989, **83**, 223; H. Kessler, P. Schmeider, M. Köck and M. Kurz, *J. Magn. Reson.*, 1990, **88**, 615; R. C. Crouch, T. D. Spitzer and G. E. Martin, *Magn. Reson. Chem.*, 1992, **30**, 595; J.-M. Bernassau and J.-M. Nuzillard, *J. Magn. Reson., Ser. B*, 1994, **103**, 77.

16 Residues are labelled alphabetically from the N- to the C-terminus, with protons within each residue being numbered from the highest ranking carbon according to IUPAC recommendations.

17 The ^{1}H NMR spectrum of hexamer **7** is complicated by resonance overlap and unambiguous assignment of every resonance was not possible. However, a pentameric derivative was completely assigned and demonstrated an analogous pattern to tetramer **4**.

18 E. A. Gallo and S. H. Gellman, *J. Am. Chem. Soc.*, 1993, **115**, 9774.

19 A 12-helical conformation stabilized by $NH^{i} \rightarrow C{=}O^{i+1}$ hydrogen bonds would produce the same pattern of shielded and deshielded amide protons but would require a hydrogen bond to the C-terminal ester (and is inconsistent with the patterns of NOE enhancements observed).

20 G. P. Dado and S. H. Gellman, *J. Am. Chem. Soc.*, 1994, **116**, 1054.

