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Hybrid Diphenylalkyne–Dipeptide Oligomers Induce Multistrand β-Sheet Formation

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Dedicated to Professor Sir Alan R. Battersby FRS on the occasion of his 90th birthday

Abstract: Functionalized diphenylalkynes provide a template for the presentation of protein-like surfaces composed of multistrand β-sheets. The conformational properties of three-, four-, and seven-stranded systems have been investigated in the solid- and solution-state. This class of molecule may be suitable for the mediation of therapeutically relevant protein–protein interactions.

There is much current interest in the design of synthetic oligomers that mimic the recognition and folding properties of secondary structural domains on the surface of proteins.[1] These foldamers[2] have the potential to bind complementary protein targets and modulate their interactions with other proteins.[3] Considerable progress has been made in the design of various unnatural amino acid oligomers that are controlled by intramolecular hydrogen-bonding interactions. In particular, β-peptides,[4,5] γ-peptides,[6] and sequences containing aminoquinolines,[7] anthranilamides,[8,9] and dialkylamino acids[10–14] have been shown to adopt helical conformations of varying pitch and dimensions. The β-sheet is also an important secondary structural protein element, motivating researchers to develop a range of synthetic templation strategies for two-stranded systems.[12–18] Macrocyclic peptides have frequently been used to stabilize the formation of sheetlike structures and have provided insights into their supramolecular properties.[19] However, there are a limited number of templates that take advantage of intramolecular hydrogen bonding to stabilize structures formed of three or more strands.[20] These include turn mimics that incorporate D-Pro,[21,22] oligomers of α/β-amino acids,[23,24] and the self-assembly of two-stranded β-sheet mimics[25] and macrocycles[26,27] into larger complexes.

Nature provides many examples of extended and stable sheet structures, as both components of soluble globular proteins and insoluble aggregates such as amyloid fibrils. These provide two functionalized surfaces, formed from the side-chain residues projected above and below the folded frame. The β-meander protein is an example of a super-secondary protein element formed of several short peptide strands occupying extended conformations, and is itself a common component of larger structures such as the β-barrel (Figure 1 A).[28]

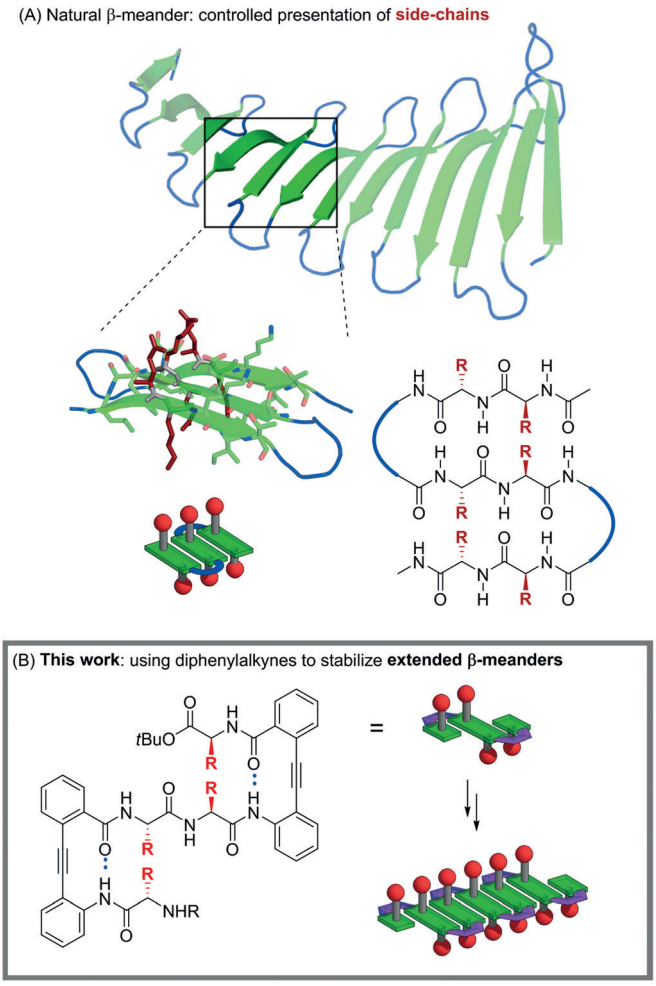

Figure 1. Multistranded β-sheets are key components of higher-order protein structures, and present large surface areas for interfacial protein–protein interactions: a) the β-repeat (also known as a β-meander), exemplified by Koide's protein self-assembly mimics (PDB 2HKD);[29] b) extended β-meander motifs based upon the diphenylalkyne linker as a turn motif.

Our interest lay in creating oligomeric scaffolds that stabilize extended β-sheet-like structures, with programmable surfaces projected above and below a common plane. Di-functionalized

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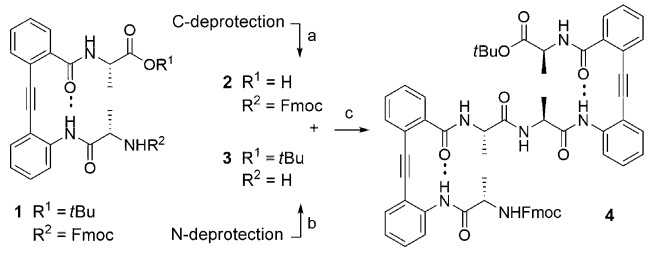
diphenylalkynes were introduced by Kemp as a non-peptidic anti-parallel β-turn inducer,[30,31] and have been extended by Spivey to display peptide loops,[32,33] and by our group to template bi-directional β-sheet formation.[34] The motif also has the potential to stabilize large surfaces as a modular unit of a foldamer, in which multiple peptide strands are linked in an anti-parallel fashion (Figure 1 B).

To explore the folding propensities of a series of anti-parallel two-peptide strands linked through diphenylalkynes, we synthesized **1** on a multigram scale from readily available starting materials (see the Supporting information). Alanine was selected as the amino acid for this proof-of-principle system due to its propensity to form β-sheets. Building block **1** can be orthogonally deprotected to reveal either the free *C*-terminus **2**, or *N*-terminus **3**. Amide coupling between **2** and **3** gave double turn motif **4** (Scheme 1). Further elaboration through iterative deprotection, amidation with a suitable two-amino acid unit (**2** or **3**), and capping of the resultant termini with protected amino acids gave three- (**5**) and four-stranded (**6**) mimics (Figure 2).



Scheme 1. Synthesis of a double turn motif with orthogonal *N*-/*C*-terminal protecting groups. Conditions: a) trifluoroacetic acid, CH_2Cl_2, 93%, b) piperidine:CH_2Cl_2 (0.1 M, 4:1 *v*/*v*), 95%; c) EDC, HOBt, iPr_2NEt, CH_2Cl_2, 62%. Dashed lines indicate hydrogen bonds. EDC = 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide, HOBt = hydroxybenzotriazole.

Extensive cross-strand correlations between the Cα-, Cβ-, and amide N-Hs of the ROESY (**5**) and NOESY (**6**) $CDCl_3$ spectra were consistent with the population of folded multistrand sheet conformations.[35,36] Support for the existence of well-conserved intramolecular hydrogen-bond networks is provided by N-H/D exchange between a solution of mimic in a mixture of $CDCl_3$ and CD_3OD, where hydrogen-bonded NHs that are protected from the solvent are expected to undergo exchange less rapidly than those exposed to solvent.[34,37] All NHs of single-stranded control molecules **8**–**10** underwent greater than 50% exchange after 9 min. Likewise, 50% exchange occurred for H_b/H_g (three-stranded **5**) and H_b/H_j (four-stranded **6**) in less than 5 min, suggesting that they occupy solvent exposed positions. H_c/H_f (of **5** and **6**), and H_i (of **6**), closest to the turn motifs, showed very slow rates with less than 12% exchange after 105 min. H_a (of **5**), H_d/H_e (of **5** and **6**), and H_g/H_h (of **6**), show intermediate rates of exchange, indicative of hydrogen bonds further from the turn motifs being of moderate strength. Consistent with the ROESY data, H_h (of **5**), and H_k (of **6**) reached 50% exchange within five minutes. It may be that the additional length of the *N*- compared to the *C*-terminal

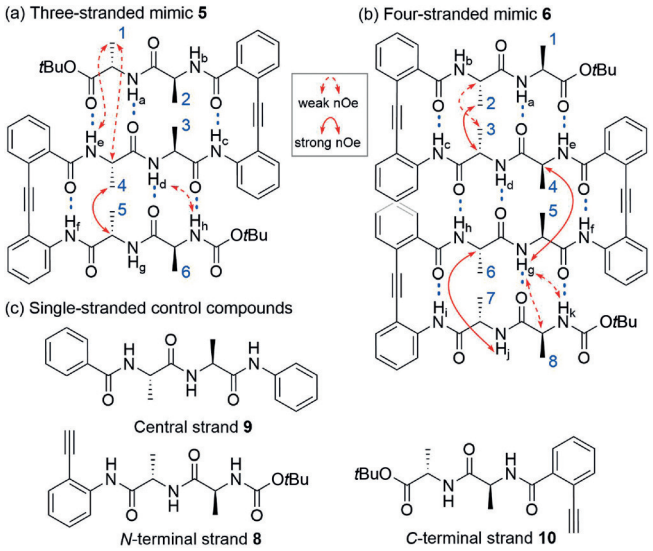


Figure 2. Solution-phase analysis of meander mimics: a) ROESY of three-stranded **5**; and b) NOESY of four-stranded **6**; c) single-stranded control compounds **8**, **9**, and **10**, which are incapable of intramolecular sheet formation. Solid and dashed red arrows indicate selected strong and weak nOe correlations respectively (13.33 mM in $CDCl_3$).

strand results in a steric clash between the *tert*-butyl group and the aromatic hydrogens, thus weakening the hydrogen bonding and causing fraying. Alternatively, weaker H-bonding at this position may be due to the reduced H-bond donor ability of carbamate, relative to amide, NHs (labeling of NHs is shown in Figure 2, full data in the Supporting Information).

The Cα-Hs of mimics **5** and **6** show a pronounced downfield shift in the ^{1}H NMR spectra when compared to single-stranded control molecules, consistent with increasing β-sheet character.[38,39] The non-terminal amino acids showed shifts of 0.78–1.04 (**5**) and 0.81–1.21 ppm (**6**) relative to the controls. Larger values in the latter case are likely due to the enforcement of greater sheet character by increased interstrand H-bond cooperativity. The corresponding range for terminal amino acids was 0.12–0.32 ppm, indicative of less-pronounced β-sheet structure (Figure 3 a). The assignment of a secondary structural propensity score using the Foreman–Kay method[40] allows contributions from different nuclei to be weighted according to their sensitivity to α- and β-structure. Mean β-sheet scores in excess of 94.0 and 99.8% for three- (**5**) and four-stranded mimics (**6**), respectively, were consistent with significant β-sheet character, whilst control molecules **8**–**10** showed minimal β-sheet propensity (between 0 and 31.7%).

Solution-phase conformational behavior in a more demanding medium for intramolecular H-bonding was explored by titration of $(CD_3)_2SO$ into a solution of sheet-mimic in $CDCl_3$. H_b/H_g (of **5**) and H_b/H_j (of **6**) shifted markedly downfield, suggesting greater exposure to solvent, whereas the other hydrogens shifted to an equal or greater degree upfield, consistent with adoption of a well-folded multi-strand sheet conformation (Figure 3 b).[41] For mimics **5** and **6** a quantitative comparison of ^{1}H NMR chemical shifts of the non-terminal amide NHs for spectra acquired in neat $CDCl_3$ and neat $(CD_3)_2SO$ gave a high

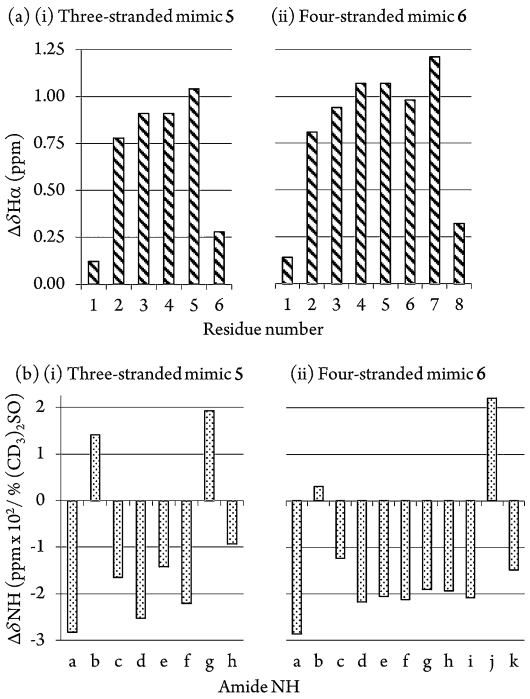


Figure 3. a) Secondary structural behavior: ^{1}H NMR chemical-shift values of the α-hydrogens in i) three-stranded **5**; and ii) four-stranded **6** mimics relative to single-stranded controls (**8**–**10**). Residue numbering indicated in Figure 2. b) Amide ^{1}H NMR behavior upon titration of $(CD_3)_2SO$ into a $CDCl_3$ solution of i) three-stranded **5** and ii) four-stranded **6** mimics. Labeling of NHs indicated in Figure 2. 10 mM in 500 μL of $CDCl_3$; $(CD_3)_2SO$ added in portions as follows: 10, 20, 20, 50, 50, 50 μL.

level of confidence for hydrogen-bonding networks consistent with well-folded multi-strand sheet conformation.[42] The hydrogens corresponding to the *C*- and *N*-terminal amino acids (H_e/H_h for **5**, H_e/H_k for **6**) gave intermediate values indicative of fraying, whereas the chemical shift change of the external hydrogens (H_b/H_g for **5**, H_b/H_j for **6**) suggested an absence of intramolecular H-bonding.[43]

X-ray crystallography of single crystals of **5** was consistent with a structure formed of three intramolecularly hydrogen-bonded strands. In agreement with the solution-phase work, a network of six intramolecular hydrogen bonds places three side-chains above the plane and three below (coloured orange and purple respectively, Figure 4a/b).[44] Average dihedral angles of $(\phi, \Psi) = (-135 \pm 32^\circ, 136 \pm 37^\circ)$ are in excellent agreement with those of a canonical β-sheet (−135, 135). See the Supporting Information for more details.[45]

With solid- and solution-phase data consistent with the three- and four-stranded mimics adopting multi-stranded β-sheet conformation, we sought to explore the preparation of extended systems with a greater number of strands. Further iteration of the synthetic route afforded seven-stranded meander mimic **7**, with protected termini allowing extension to larger surfaces (Figure 4c,d).[36]

Circular dichroism spectra of three- (**5**), four- (**6**), and seven-stranded mini-protein mimic (**7**) showed maxima at 253 nm and minima at 228 nm, consistent with β-sheet formation, whereas single-stranded control (**9**) gave a spectrum characteristic of random coil behavior. Maxima at 255 nm are due to chiral perturbations of the 2-amino-2′-diphenylalkyne chromophore (Figure 4e).[30,31]

In conclusion, we have shown that several short peptide strands may be linked in an anti-parallel arrangement by diphenylalkynes to give large surfaces presenting side-chains on two faces. The approach provides a template for the stabilization of multi-strand β-sheets and may find use in mediating protein–protein interactions. Work is underway to prepare extended systems containing longer sequences of amino acids per strand, and to incorporate hydrophilic side chains for functional studies in aqueous media.

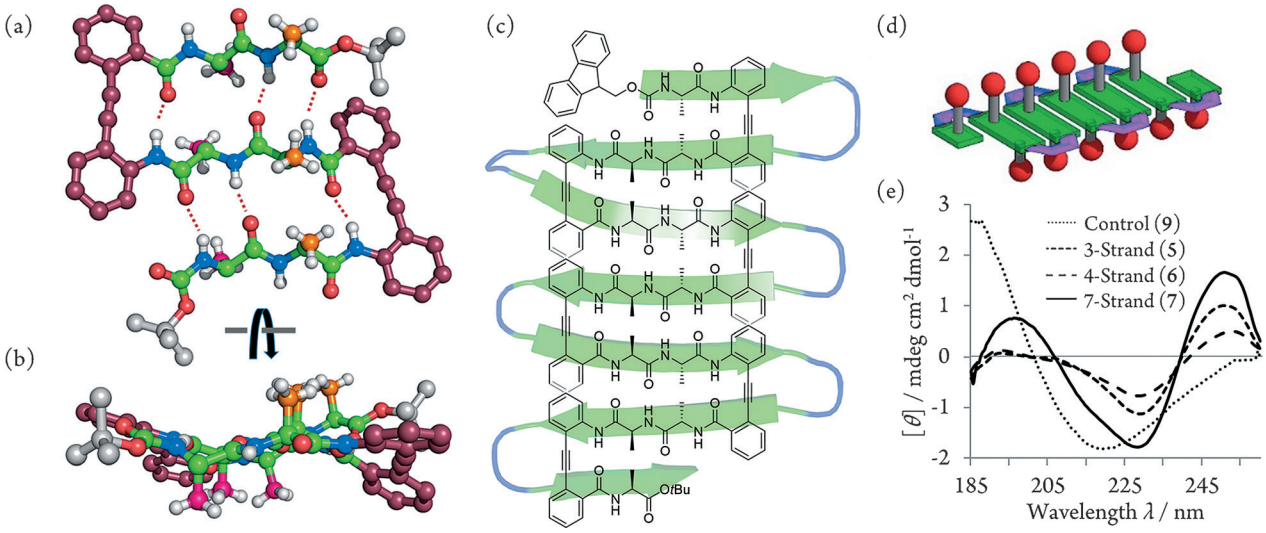


Figure 4. X-ray diffraction structure of three-stranded mimic **5**: a) top- and b) side-elevation. Side-chains highlighted in orange and purple (opposite face), diphenylalkyne turns in maroon, *tert*-butyl protecting groups in grey, dotted red lines represent hydrogen bonds, some hydrogens omitted for clarity. Mimicry of a seven-stranded β-meander **7**: c) structure; d) schematic representation; e) circular dichroism mean residue ellipticity ([θ]) of three- (**5**), four- (**6**), and seven-stranded (**7**) mimics relative to single-stranded control molecule (**9**); averages of ten acquisitions, 100 μM in trifluoroethanol.

Acknowledgements

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Keywords: foldamer · peptidomimetics · protein surface · protein–protein interaction · secondary structure · β-sheet

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[44] CCDC 1049256 (**5**) contains the supplementary crystallographic data for this paper. These data are provided free of charge by The Cambridge Crystallographic Data Centre.
[45] The relatively large errors in these values are due to fraying of the *C*- and *N*-terminal amino acids (see above).

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