Peptidomimetics Hot Paper

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# Diphenylacetylene-Linked Peptide Strands Induce Bidirectional β-Sheet Formation**

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***Abstract:*** *In the search for synthetic mimics of protein secondary structures relevant to the mediation of protein–protein interactions, we have synthesized a series of tetrasubstituted diphenylacetylenes that display β-sheet structures in two directions. Extensive X-ray crystallographic and NMR solution phase studies are consistent with these proteomimetics adopting sheet structures, displaying both hydrophobic and hydrophilic amino acid side chains.*

A key element of the structure and function of β-sheet domains in proteins is the almost perpendicular presentation of side-chain residues in alternating periodicity above and below the plane of the hydrogen-bonded network (Figure 1 a). These residues are critical in forming intramolecular contacts with other domains in many folded proteins, or in determining the intermolecular associations that stabilize certain protein–protein interactions (PPIs). β-Strand/β-strand interactions[1] between different protein chains to form interchain β-sheets account for around 16% of all PPIs and have been implicated in a range of diseases, including cancer.[2] Furthermore, human pathologies, such as Alzheimer's and Parkinson's diseases, and dialysis-related amyloidosis are associated with amyloid fiber formation involving primarily the intermolecular stacking interaction of β-strands and β-sheets.[3]

There is much interest in the development of molecules capable of mimicking the structure and recognition mechanisms of β-sheets as potential disruptors of PPIs.[4] In addition, the ability to construct easily variable functionalized surfaces, in the manner of an extended β-sheet, has many potential applications in supramolecular chemistry for the formation of new materials or catalysts. Whereas much progress has been made in the development of synthetic mimics of α-helices,[5] the development of analogous β-sheet constructs has been slower. In a seminal study, Kemp and co-workers described a β-turn mimic derived from a substituted diphenylacetylene that templates intramolecular hydrogen-bonded β-sheet formation.[6] This broad approach of covalently linking one end of a β-sheet has been extended by others using tolan derivatives,[7] substituted biphenyl,[8] dibenzofuran,[9] oligoanthranilamide,[10] and urea scaffolds,[11] as well as more unusual motifs such as ferrocene[12] or other metal-chelating groups[13] to template the two strands. Others have exploited the simple strategy of cyclizing peptide or peptidomimetic strands[14] to form a length of β-sheet flanked by two β-turn units (Figure 1 b). Unnatural amino acids have been used to induce β-sheet formation (Figure 1 c)[15] and cross-linking strands using disulfide bonds has also been employed (Figure 1 d).[16]

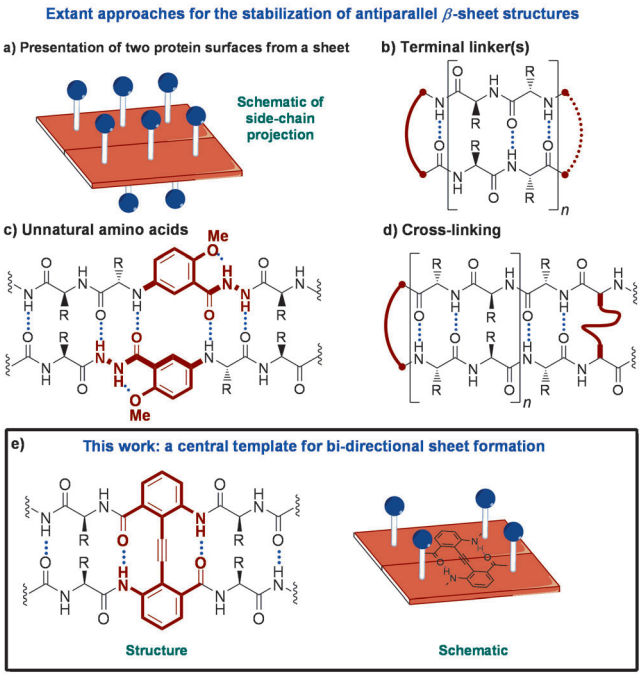


***Figure 1.*** Strategies to template β-sheet formation.

In the course of our investigations of synthetic analogues of protein secondary structure, we have reported the use of completely unnatural backbone oligomers as β-strand mimetics.[17] Herein, we report a different strategy, using a tetrasubstituted diphenylacetylene as both a rigidifying and a linking spacer between two peptide strands. This approach has the potential to promote sheet formation in two directions, and differs structurally from many existing templates that use terminal linkers. Incorporating amino acids into each quadrant results in a scaffold from which four substituents project from a single face in conceptual analogy to the positioning of residues on a β-sheet (Figure 1 e).

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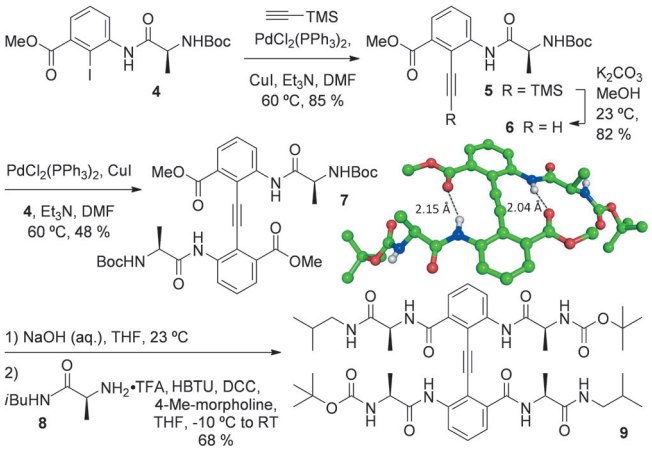
As alanine is a good example of an amino acid preferentially adopting the antiparallel β-sheet structure,[18] we used it as a model residue in a convergent synthesis that readily allows the incorporation of other amino acids. Reduction of nitro benzoate **1**[19] provided aniline **2**, which was coupled with *N*-Boc-alanine **3** (Boc = *tert*-butoxycarbonyl) to give iodide **4** (not shown, see the Supporting Information). Iodide **4** is a common precursor for both fragments of the target molecule. A Sonogashira reaction with TMS-acetylene followed by basic removal of the silyl group gave the second coupling fragment, alkyne **6**. A second Sonogashira reaction is used to link the fragments in reasonable yield. The structure of two-residue mimic **7** was solved from single crystal X-ray diffraction data,[20] and presented a broadly planar arrangement with hydrogen bonds between the amide NH and the ester carbonyl on each side of the diphenylacetylene. The C=O···N distances are between 3.10 and 2.84 Å, with N–H···O distances of 2.15 and 2.04 Å (dashed black lines in Scheme 1; some hydrogens omitted for clarity). Installation of two further amino acids began with basic hydrolysis of the methyl esters of **7** to give a dicarboxylic acid followed by coupling with amino amide **8**, derived from L-alanine, to give fully elaborated β-sheet analogue **9** (Scheme 1).




***Scheme 1.*** Synthesis of a four-alanine residue sheet mimic, with an X-ray diffraction structure of precursor **7**.

A ROESY spectrum of **9** in chloroform (Figure 2a) showed correlations between the Cα hydrogens of the two alanine groups on the linked strands of the β-sheet, as well as between the *tert*-butyl group hydrogens of the Boc group and the *iso*-butyl hydrogens of the amide. These indicate that the ends of the two strands are in close proximity, which is consistent with the *iso*-butyl amide NH forming a hydrogen bond to the Boc carbonyl on the adjacent strand. A comparison of the Cα–H shifts of iodide **10** with those of sheet mimic **9** shows that both have moved downfield, from 4.6 to 5.2 ppm and 4.4 to 5.5 ppm, respectively, which is consistent with the two strands of **9** having formed a β-sheet (Figure 2).[21]

Further support for this structure comes from deuterium exchange experiments where the hydrogen-bonded amide-NH groups would be expected to exchange less rapidly than

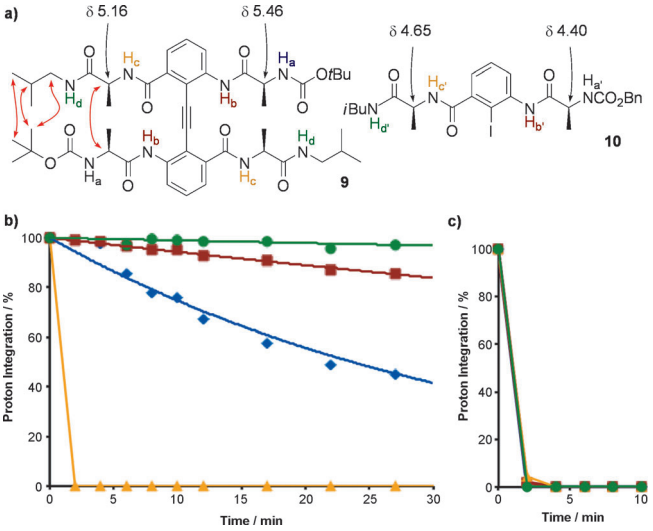



***Figure 2.*** Solution-phase analysis in $CDCl_3$. a) $^1H$ shifts of Cα-hydrogens in sheet and open structures. ROESY experiment of **9** with correlations shown in red (symmetry-related correlations ommitted). Rate of [$D_4$]methanol exchange for NH groups: b) sheet mimic **9**, c) control **10**; $H_a/H_{a'}$ (blue), $H_b/H_{b'}$ (red), $H_c/H_{c'}$ (yellow), $H_d/H_{d'}$ (green).

those exposed to solvent.[22] Figure 2b shows the rates at which amide hydrogens are exchanged for deuterons after the addition of a small amount of [$D_4$]methanol to a solution of **9** in $CDCl_3$. $H_d$ (terminal amide, green) and $H_b$ (anilide, red) exchange more slowly with the solvent than $H_a$ (carbamate, blue) and $H_c$ (amide, yellow), which is consistent with the hydrogen-bonding network proposed. Figure 2c shows the rates of exchange of the four equivalent hydrogens in model system **10**, which is incapable of intramolecular hydrogen bonding. In this case, hydrogens $H_{d'}$ and $H_{b'}$ (green and red) exchange much faster than the equivalent hydrogens in sheet analogue **9**, thus supporting the fact that these hydrogens are shielded from the solvent in the latter.

Although it was not possible to obtain satisfactory single crystals of **9**, substitution of the Boc groups with acetyl groups gave crystals of **11** suitable for X-ray diffraction experiments (Figure 3).[20] Consistent with the solution-phase studies, four intramolecular hydrogen bonds stabilize the desired β-sheet conformation, with the alanine side-chains displayed on the same face of the molecule. The C=O···N distances range between 3.03 and 2.75 Å, with N–H···O distances between 2.21 and 1.90 Å. The interstrand Cα/Cα distances of 4.0 and 4.5 Å are within the range of those found in natural β-sheets.[23] Additional intermolecular NH···O=C hydrogen bonds were formed with adjacent molecules in the crystal, resulting in supramolecular structures featuring unidirectional side-chain projection.

To demonstrate the generality of this approach for the incorporation of natural or unnatural amino acids, we followed an analogous synthetic route to prepare the four-residue mimic **12**, incorporating two lysine and two glutamic acid residues (Figure 4; see the Supporting Information for the synthesis of these compounds).

Hydrophilic mimetic **12** was insoluble in chloroform, and so we first probed its solution-phase conformation by

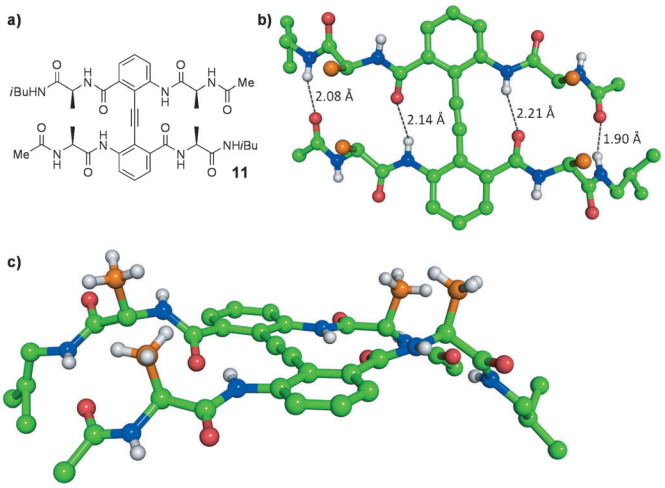



***Figure 3.*** a) Sheet mimic **11**; b) top and, c) side-projections of X-ray structure; some hydrogens omitted for clarity, alanine side chains highlighted in orange.

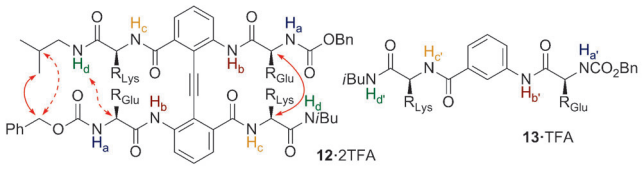



***Figure 4.*** Solution-phase analysis of mimic **12** and control compound **13**. [$D_6$]DMSO ROESY correlations shown as red arrows (dashed arrows represent weaker interactions). For color coding of amide hydrogens for variable temperature and titration studies, see text.

conducting a series of NMR experiments in [$D_6$]DMSO and compared the results to those of control compound **13**, which is incapable of intramolecular H-bonding (Figure 4; full data and analysis are given in the Supporting Information). A ROESY spectrum of **12** showed cross-strand correlations consistent with the population of a sheet conformation analogous to those observed for **9**. Further evidence for the existence of a β-sheet structure in solution was provided by a variable temperature NMR study. Temperature-dependent coefficients ($\Delta\delta/\Delta K$) for $H_b$ (anilide, red) and $H_d$ (terminal amide, green) of −0.89 and −3.85 ppb $K^{-1}$, respectively, are consistent with intramolecular H-bonding, whereas coefficients of −7.45 and −6.88 ppb $K^{-1}$ for $H_a$ (carbamate, blue) and $H_c$ (amide, yellow), respectively, suggest interaction with solvent only.[24]

The presence of a H-bonding network was also probed by the addition of both $CDCl_3$ and 2,2,2-trifluoroethanol to [$D_6$]DMSO solutions. Upon increasing titration with $CDCl_3$, $H_b$ is essentially unperturbed, whereas the upfield shift of $H_d$ is markedly smaller than those of $H_a$ and $H_c$.[25] This behavior is consistent with previous studies in which a lowering of the proportion of a powerfully H-bond-accepting solvent leads to greater perturbations for solvent-exposed amide NHs relative to those in intramolecular H-bonds.[26] Under these conditions it is expected that solvent-exposed NH groups will be more susceptible to a change in solvent composition than those "protected" within intramolecular H-bonds. The addition of 2,2,2-trifluoroethanol to **12** in [$D_6$]DMSO caused $H_b$ and $H_d$ to move slightly downfield, whereas $H_a$ and $H_c$ moved upfield to a greater degree. These observations can be explained by 2,2,2-trifluoroethanol H-bonding to the solvent-exposed carbonyl groups of amides bearing $H_b$ and $H_d$, thus leading to electron withdrawal and a downfield shift. The upfield shifts of $H_a$ and $H_c$ likely come from a reduction in their H-bonding to [$D_6$]DMSO as a result of competition with added 2,2,2-trifluoroethanol.[27]

To explore the conformational behavior of sheet mimic **12** in aqueous media, we added $H_2O$ incrementally to a [$D_6$]DMSO solution and recorded ROESY spectra, and temperature-dependent coefficients of the backbone amide NHs. We observed the same interstrand correlations as those in [$D_6$]DMSO, at up to 40% $H_2O$. Increasing the ratio of $H_2O$ to 70% required a high level of solvent suppression that led to silencing of the region in which the key resonances of the Cα-, and benzylic-hydrogens reside, making it difficult to gather further data. However, over this range (0–70% $H_2O$), the temperature-dependent coefficients of $H_b$ and $H_d$ did not change significantly,[28] which suggests the maintenance of interstrand hydrogen bonding[29] and thus the persistence of a sheet conformation (see the Supporting Information, Chapter 4 "Conformational analysis").

In conclusion, we have developed a general strategy to protein secondary structure mimics capable of displaying β-sheet-like structures in two directions, and have demonstrated its efficiency for the incorporation of both hydrophobic and hydrophilic amino acid side chains. The use of a central rigidifying and templating group covalently attached to four peptide strands is distinct from extant approaches to β-sheet mimicry, and thus offers a new method for the display of protein surfaces. Solid- and solution-phase conformational studies in a range of solvent systems are consistent with the interstrand hydrogen bonds between peptide chains, and the projection of amino acid side chains, such as those found in natural β-sheets. Development of this class of β-sheet mimic for use in mediating PPIs and as a basis for forming analogues of higher order protein structure is underway.

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[1] H. Yin, G. Lee, H. S. Park, G. A. Payne, J. M. Rodriguez, S. M. Sebti, A. D. Hamilton, *Angew. Chem.* **2005**, *117*, 2764–2767; *Angew. Chem. Int. Ed.* **2005**, *44*, 2704–2707.
[2] J. S. Nowick, D. M. Chung, K. Maitra, S. Maitra, K. D. Stigers, Y. Sun, *J. Am. Chem. Soc.* **2000**, *122*, 7654–7661.
[3] F. Chiti, C. M. Dobson, *Nat. Chem. Biol.* **2009**, *5*, 15–22.
[4] W. A. Loughlin, J. D. A. Tyndall, M. P. Glenn, T. A. Hill, D. P. Fairlie, *Chem. Rev.* **2010**, *110*, PR32–PR69.
[5] a) M. K. P. Jayatunga, S. Thompson, A. D. Hamilton, *Bioorg. Med. Chem. Lett.* **2014**, *24*, 717–724; b) V. Azzarito, K. Long, N. S. Murphy, A. J. Wilson, *Nat. Chem.* **2013**, *5*, 161–173; c) S. Thompson, R. Vallinayagam, M. J. Adler, R. T. W. Scott, A. D.

Hamilton, *Tetrahedron* **2012**, *68*, 4501 – 4505; d) S. Thompson, A. D. Hamilton, *Org. Biomol. Chem.* **2012**, *10*, 5780 – 5782.
[6] a) D. S. Kemp, Z. Q. Li, *Tetrahedron Lett.* **1995**, *36*, 4175 – 4178; b) D. S. Kemp, Z. Q. Li, *Tetrahedron Lett.* **1995**, *36*, 4179 – 4180.
[7] a) A. C. Spivey, J. McKendrick, R. Srikaran, B. A. Helm, *J. Org. Chem.* **2003**, *68*, 1843 – 1851; b) D. A. Offermann, J. E. McKendrick, J. J. P. Sejberg, B. Mo, M. D. Holdom, B. A. Helm, R. J. Leatherbarrow, A. J. Beavil, B. J. Sutton, A. C. Spivey, *J. Org. Chem.* **2012**, *77*, 3197 – 3214.
[8] C. L. Nesloney, J. W. Kelly, *J. Am. Chem. Soc.* **1996**, *118*, 5836 – 5845.
[9] H. A. Lashuel, S. R. LaBrenz, L. Woo, L. C. Serpell, J. W. Kelly, *J. Am. Chem. Soc.* **2000**, *122*, 5262 – 5277.
[10] Y. Hamuro, A. D. Hamilton, *Bioorg. Med. Chem.* **2001**, *9*, 2355 – 2363.
[11] J. S. Nowick, E. M. Smith, G. Noronha, *J. Org. Chem.* **1995**, *60*, 7386 – 7387.
[12] S. Chowdhury, G. Schatte, H.-B. Kraatz, *Angew. Chem.* **2008**, *120*, 7164 – 7167; *Angew. Chem. Int. Ed.* **2008**, *47*, 7056 – 7059.
[13] A. C. Laungani, J. M. Slattery, I. Krossing, B. Breit, *Chem. Eur. J.* **2008**, *14*, 4488 – 4502.
[14] a) J. A. Robinson, *Acc. Chem. Res.* **2008**, *41*, 1278; b) J. A. Robinson, S. DeMarco, F. Gombert, K. Moehle, D. Obrecht, *Drug Discovery Today* **2008**, *13*, 944 – 951; c) F. Freire, S. H. Gellman, *J. Am. Chem. Soc.* **2009**, *131*, 7970 – 7972; d) F. Freire, S. H. Gellman, *J. Am. Chem. Soc.* **2011**, *133*, 12318.
[15] J. S. Nowick, *Acc. Chem. Res.* **2008**, *41*, 1319 – 1330.
[16] A. M. Almeida, R. Li, S. H. Gellman, *J. Am. Chem. Soc.* **2012**, *134*, 75 – 78.
[17] a) P. N. Wyrembak, A. D. Hamilton, *J. Am. Chem. Soc.* **2009**, *131*, 4566 – 4567; b) A. G. Jamieson, D. Russell, A. D. Hamilton, *Chem. Commun.* **2012**, *48*, 3709 – 3711; c) C. L. Sutherell, S. Thompson, R. T. W. Scott, A. D. Hamilton, *Chem. Commun.* **2012**, *48*, 9834 – 9836.
[18] B. Rost, C. Sander, *J. Mol. Biol.* **1993**, *232*, 584 – 599.
[19] E. C. Y. Woon, A. Dhami, M. F. Mahon, M. D. Threadgill, *Tetrahedron* **2006**, *62*, 4829 – 4837.
[20] CCDC 915930 (**7**) & 915931 (**11**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.
[21] D. S. Wishart, B. D. Sykes in *Methods in Enzymology* (Ed.: T. L. James), Academic Press, New York, **1994**, 363.
[22] L. R. Steffel, T. J. Cashman, M. H. Reutershan, B. R. Linton, *J. Am. Chem. Soc.* **2007**, *129*, 12956 – 12957.
[23] C.-I. Brändén, J. Tooze, *Introduction to Protein Structure*, Garland Publishing, New York, **1999**.
[24] a) E. S. Stevens, N. Sugawara, G. M. Bonora, C. Toniolo, *J. Am. Chem. Soc.* **1980**, *102*, 7048 – 7050; b) A. Ballardin, A. J. Fischman, W. A. Gibbons, J. Roy, I. L. Schwartz, C. W. Smith, R. Walter, H. R. Wyssbrod, *Biochemistry* **1978**, *17*, 4443 – 4454; c) Coefficients for $H_{a'}$-$H_{d'}$ in control molecule **13** were between $-4.92$ and $-7.33$ ppb $K^{-1}$, suggesting interactions with solvent only.
[25] Increasing $CDCl_3$ content in DMSO from 44 to 90 % gave the following perturbations: $H_a$ $-24.3$, $H_b$ $+0.5$, $H_c$ $-16.7$, $H_d$ $-3.9$ ppb $K^{-1}$.
[26] H. Kessler, *Angew. Chem.* **1982**, *94*, 509 – 520; *Angew. Chem. Int. Ed. Engl.* **1982**, *21*, 512 – 523.
[27] a) M. Llinas, M. P. Klein, *J. Am. Chem. Soc.* **1975**, *97*, 4731 – 4737; b) D. W. Urry, M. M. Long, *CRC Crit. Rev. Biochem.* **1976**, *4*, 1 – 45.
[28] On going from 0 to 70 % $H_2O$, $H_b$ and $H_d$ moved upfield 1.3 and 0.95 ppb $K^{-1}$, respectively (to $-1.5$ and $-4.8$ ppb $K^{-1}$).
[29] T. Cierpicki, J. Otlewski, *J. Biomol. NMR* **2001**, *21*, 249 – 261.

  