β-Strand mimetic foldamers rigidified through dipolar repulsion

Peptidomimetics

β-Strand Mimetic Foldamers Rigidified through Dipolar Repulsion**

Elizabeth A. German, Jonathan E. Ross, Peter C. Knipe, Michaela F. Don, Sam Thompson and Andrew D. Hamilton**

***Abstract**: Many therapeutically relevant protein-protein interactions have hot-spot regions, which contribute disproportionately to binding enthalpy, grouped on secondary structural elements. Mimicry of such α-helical regions has met with considerable success, however another key element – the β-strand, has received less attention. Here we present a foldamer for strand mimicry in which dipolar repulsion is a central determinant of conformation. Computation, solution- and solid-phase data are consistent with an ensemble weighted almost exclusively in favour of the desired conformation.*

The misregulation of protein-protein interactions (PPIs)[1] is implicated in many therapeutic areas, including HIV,[2] cancer,[3] diabetes[4] and neurodegeneration.[5] In order to target these interactions there is a need for non-peptidic mimics able to reproduce key distance and angular characteristics of protein surfaces.[6] Ideally, these scaffolds should be easily synthesised, stable under physiological conditions and allow the display of a broad range of functional groups.

Since secondary structural motifs are often found at the interfacial regions of protein partners there has been extensive work in the field of non-peptidic α-helix mimicry.[7]–[12] There are also many examples in which an isolated β-strand (Figure 1a) mediates an interaction between proteins,[13] yet β-strand mimicry remains relatively unexplored.[14]–[16] Possible peptidomimetic solutions should allow controlled positioning of side-chain functionality on specific vectors above and below a planar scaffold, as well as facile extension in a modular fashion to permit mimicry of larger surfaces.

Previous work in our group has focused on the mimicry of one recognition surface of the strand – that presenting the *i*, *i+2*, and *i+4* side-chain residues. Earlier generation scaffolds have included 2,2-disubstituted-indolin-3-ones[17] and aryl-linked hydantoins.[15] However, the development of these approaches was hindered by the need for asymmetric syntheses of all-carbon quaternary centres.

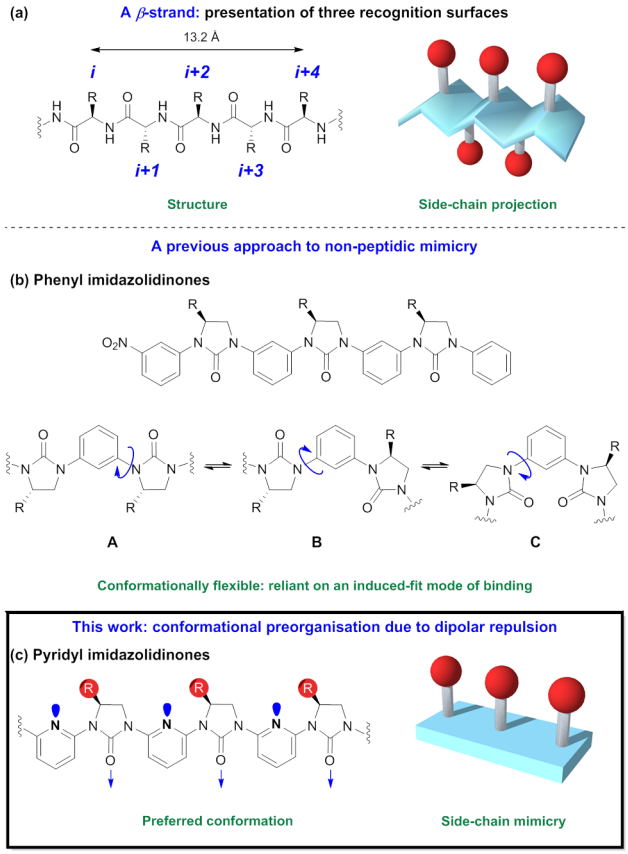

Figure 1. A non-peptidic approach to β-strand mimicry; a) schematic representation of a β-strand; and a pegboard representation showing side chain projection; b) previous work: aryl-linked imidazolidin-2-ones; c) this work: A 2,6-pyridyl-linked imidazolidin-2-one with dipole repulsion designed to impart conformational bias.

Recent work obviates this problem with the use of oligomers of alternating aryl-linked imidazolidin-2-ones, that are easily assembled from amino acid derivatives (Figure 1b).[16] In solution this scaffold undergoes relatively free rotation around the C-N single bonds and interconverts among at least three principal conformers – thus likely requiring an 'induced-fit' mode of protein binding (Figure 1b – structures A – C). Therefore we sought to impart conformational control on this system through replacement of the phenyl linkers with pyridines. This should retain the shape and planarity of the previous scaffold but would impart conformational bias through dipolar repulsion. A molecular mechanics calculation[18] of the extent of bias gave an energy profile from rotation about the C_{urea}-N_{pyr} bond relative to the same bond in the previous scaffold. For the pyridyl model this corresponds to 98% of the populated conformations being within +/- 15 degrees of the planar conformation in which the dipoles are perfectly opposed (Figure 2).

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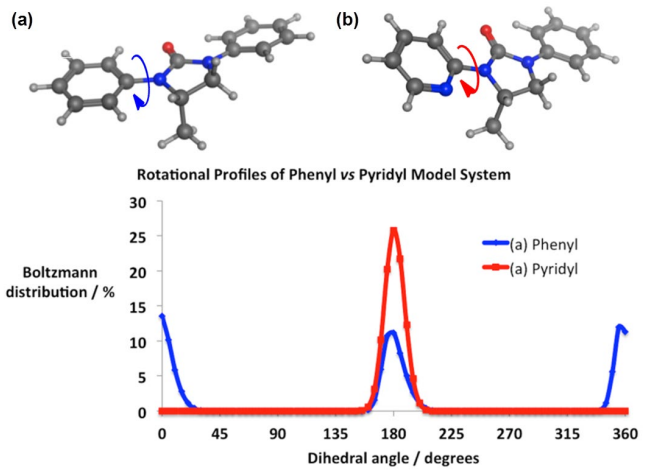
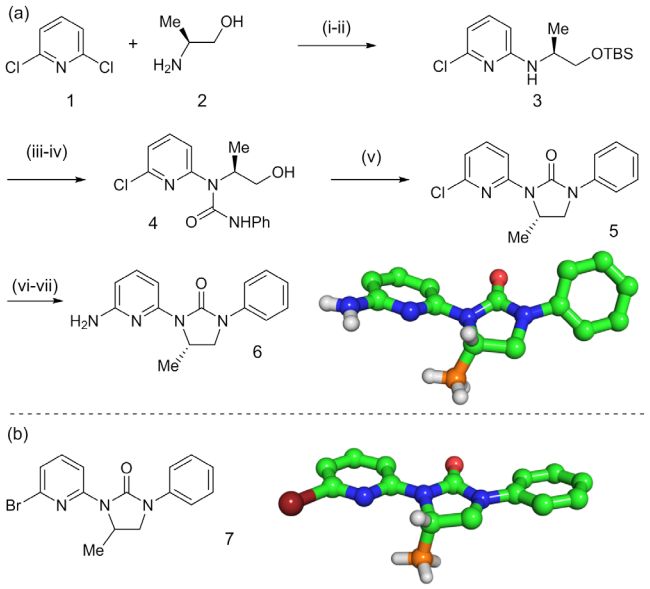


Figure 2. Computed room temperature Boltzmann-weighted populations of: (a) phenyl and (b) pyridyl systems as a function of dihedral angle.

With computational support in hand we sought proof of principle *via* the mimicry of three alanine side chains. Nucleophilic aromatic substitution of 2,6-dichloropyridine **1** with L-alaninol **2** gave an amino alcohol in 82 % yield,[19] followed by *O*-silylation with a TBS group to give **3**. Deprotonation of the amine and reaction of the resultant anion with phenyl isocyanate proceeded to give the urea in 68 % yield.

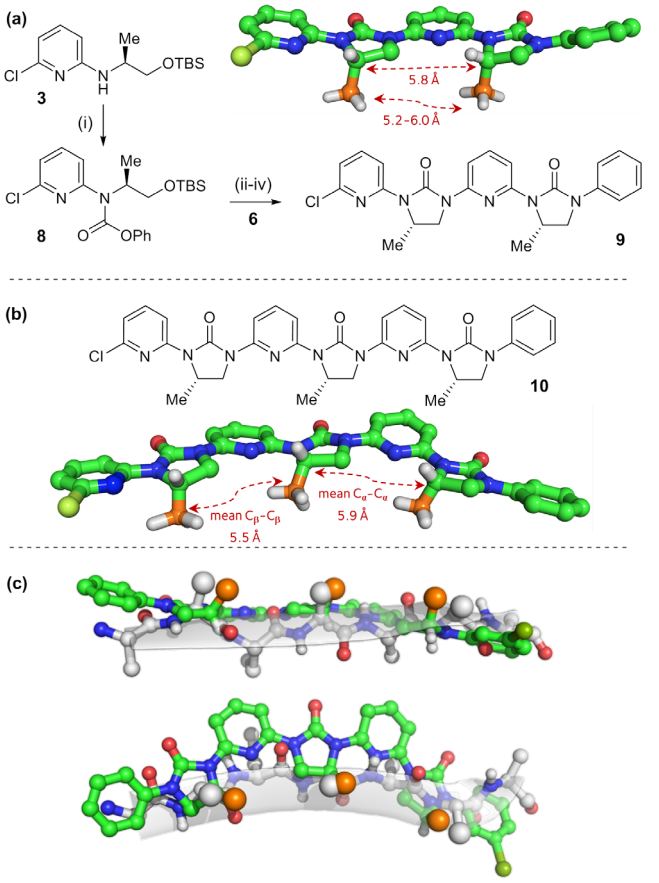


Scheme 1 (a) *Reagents and conditions:* (i) DIPEA, 180 ˚C, sealed tube, 82 %; (ii) TBSCl, imidazole, DMAP (cat.), DMF, 99 %; (iii) *n*-BuLi, THF *then* PhNCO, 68 %; (iv) TBAF, CH_3CO_2H, THF, 78 %; (v) PPh_3, DIAD, THF, 79 %; (vi) $Pd_2(dba)_3$, (±)-BINAP, NaO*t*-Bu, H_2NCH_2Ph, 1,4-dioxane, 95 %; (vii) $Pd(OH)_2/C$, H_2, CH_3CO_2H, CH_2Cl_2, 86 %. X-Ray diffraction structures of monomers (side-chains highlighted in orange, some hydrogens omitted for clarity) **6**; (b) (±)-**7** (with molecular structure, left. The (*S*)-enantiomer was chosen arbitrarily).

Desilylation to afford **4** followed by an intramolecular Mitsunobu reaction gave the imidazolidin-2-one monomer **5** in 62 % yield over two steps.[16] Buchwald-Hartwig coupling with benzylamine[20] followed by treatment with hydrogen and palladium on carbon unmasked aniline **6** in good yield (Scheme 1). The conformations of **6** and **7** (generated racemically by an analogous route, see the Supporting Information for full procedures) were examined by single crystal X-ray crystallography (Scheme 1).[21] Both were found to adopt the conformation expected on the basis of dipolar repulsion, with N_{pyr}-*C*-*N*-C_{urea} dihedral angles of 143° and 175° respectively. Amine **6** may be less planar due to the increased pyridine electron density reducing its π-conjugation with the urea, relative to the bromopyridine **7**. In both cases the side-chain methyl groups are projected into pseudo-axial positions by the puckered imidazolidinone rings, in a manner analogous to the projection of side-chains in natural β-strands.

Deprotonation of secondary amine **3** with *n*-BuLi and trapping with phenyl chloroformate gave carbamate **8** in 84 % yield. This was coupled with the lithium anilide of **6** to give the intermediate urea which following TBAF mediated deprotection and cyclisation afforded two-residue mimic **9**. Further iteration on **9** of this amination, coupling with **8** and cyclisation sequence afforded the three-residue mimic **10** (Scheme 2, see the Supporting Information).

Single-crystal X-ray structures were obtained of **9** and **10**.[21] For the two-residue mimic two different structures were present within the asymmetric unit. As a consequence of dipolar repulsion both present the side-chains on the same face of the molecule, with the α-carbons 5.8 Å, and the β-carbons 6.0 Å and 5.2 Å apart – distances within the range of those in natural β-strands (Scheme 2a).



Scheme 2 Two-residue mimic **9**: (a) *Reagents and conditions:* (i) *n*-BuLi, PhCOCl, THF, -78 ˚C, 84 %; (ii) *n*-BuLi, **6**, THF, -78 ˚C, 48 %; (iii) TBAF, CH_3CO_2H, THF, 81 %; (iv) PPh_3, DIAD, THF, 75 %; (b) Three-residue mimic **10**, see the Supporting Information for synthesis. X-Ray diffraction structures with side-chains highlighted in orange (some hydrogens omitted for clarity); (c) overlay of mimic (green) with natural beta strand (grey; extracted from PDB 3QXT, side-chains truncated for clarity), with six-point (α and β-carbons) RMSD of 1.1 Å.

The crystal structure of three-residue mimic **10** exhibited similar conformational preferences, with the methyl side-chains projected from the same face of an approximately planar scaffold. As with the dimer, two molecules of trimer **10** were present in the asymmetric unit, with mean α-carbon and β-carbon separations of 5.9 Å and

5.5 Å, respectively (Scheme 2b). As with dimer **9** and the monomeric species **6** and **7**, the conformation of all pyridyl-urea linkages exclusively placed the pyridine nitrogen *anti* to the urea carbonyl. This suggests that dipolar repulsion may be employed as a key determinant of global conformation. Comparison of the X-ray crystal structure of **10** with a natural β-strand revealed good overlap of strand C_α and C_β positions with the equivalent groups on the mimic, giving a 6-point RMSD of 1.1 Å (Scheme 2c). NMR experiments were conducted in order to provide insight into the solution phase conformational behaviour. NOESY analysis of **9** revealed an extremely weak correlation between H12 and H14, indicating that the illustrated conformation is the primary one (Figure 3).

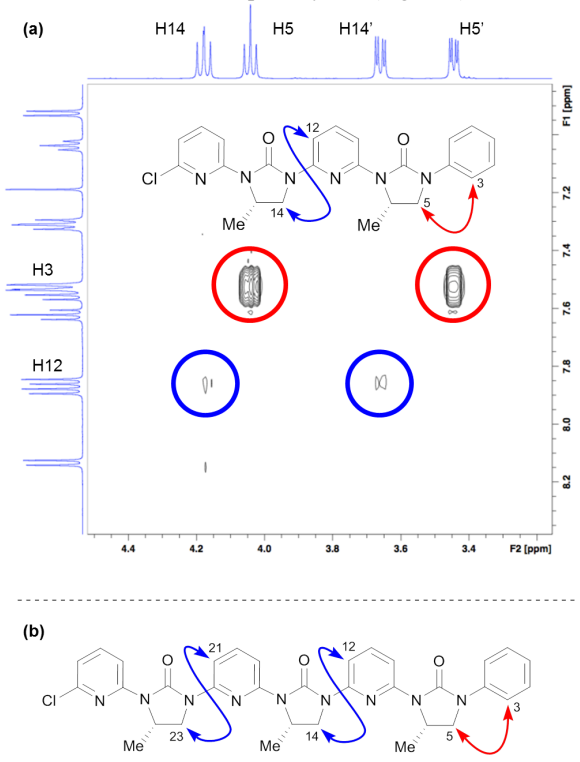


Figure 3. (a) Selected regions of NOESY spectrum for two-residue mimic **9** (500 MHz, $CDCl_3$). Arrows indicate relevant nOe correlations – with those in blue indicating weak interactions and those in red strong interactions ($CDCl_3$, 0.02 mM, see the Supporting Information for larger versions). (b) nOe correlations for three-residue mimic **10** (600 MHz, $CDCl_3$).

It is conceivable that the weak observed cross peak is a consequence of: (i) a small degree of conformational flexibility allowing access to a conformer resembling **B** (Figure 1b), with the low intensity reflecting its low population; or (b) complete conformational rigidity – the distance between H12 and H14 (see crystal structure) is 4.5 Å, which is on the edge of detection for the nOe effect, but may be sufficiently short to give some weak correlation. A semi-quantitative measure for the significance of these nOe results can be obtained by comparison with the H3-H5 cross-peak. In the absence of any conformational controlling effects, the expected nOe intensity of the H3-H5 cross peak would be approximately twice that of the H12-H14 peak (due to the C_2 symmetry of the phenyl group). The observed relative intensity of nOe signals (obtained by integration of the NOESY spectrum) is 39:1, suggesting an approximate 20:1 relative intensity of through-space interactions. The same behaviour is exhibited by the trimer **10**: H12-H14 and H21-H23 NOESY cross peaks have much lower intensity than the internal control H3-H5 correlation. Due to co-incidence between the cross-peaks these could not be integrated individually, but the ratio between the reference H3-H5 and the combined H12-H14 and H21-H23 cross-peaks was 18:1, indicating an average 18:1 relative intensity of through-space interactions. These solution state results are in good agreement with the initial premise of dipole-enforced conformational control, and with the X-ray crystallographic data. This indicates that in solution as well as the solid state the side-chains are indeed projected uniformly from a single face of the β-strand mimetic, in the manner of the side-chains of natural β-sheets and β-strands.

In conclusion we have designed and synthesised a non-peptidic scaffold capable of mimicking a recognition surface of a β-strand (in this case the side-chains of the *i*, *i+2* and *i+4* residues). Computation, solid- and solution-phase studies are consistent with the population of an ensemble in which the desired conformation is heavily biased by dipolar repulsion. The synthesis is modular, scalable and amenable to the incorporation of a wide variety of side-chains, including those with no proteinogenic congener. Work is underway in our laboratory to prepare mimics of therapeutically relevant β-strands using this scaffold, and to further exploit dipolar repulsion to exact conformational control over related systems.

Keywords: Mimic · peptidomimetic · secondary structure · protein-protein interaction · *β*-sheet · protein surface

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[21] CCDC 1030066 (**6**), 1030067 (**7**), 1030068 (**9**) & 1030069 (**10**) 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.

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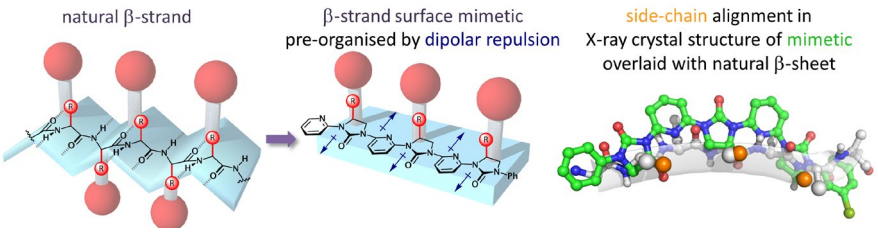

Secondary structural elements are commonly found at the interfacial regions of protein-protein interactions. A strategy to mediate these therapeutically crucial targets using peptidomimetics has been validated for the α–helix but there are few analogous examples for the β-strand. Here we present a foldamer for strand mimicry in which dipolar repulsion is a central determinant of conformation. Computation, solution- and solid-phase data are consistent with an ensemble weighted almost exclusively in favour of the desired conformation.