❚ Protein–Protein Interactions |*Hot Paper*|

A Modular Synthesis of Conformationally Preorganised Extended β-Strand Peptidomimetics

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Abstract: A promising strategy for mediating protein–protein interactions is the use of non-peptidic mimics of secondary structural protein elements, such as the α-helix. Recent work has expanded the scope of this approach by providing proof-of-principle scaffolds that are conformationally biased to mimic the projection of side-chains from one face of another common secondary structural element—the β-strand. Herein, we present a synthetic route that has key advantages over previous work: monomers bearing an amino acid side-chain were pre-formed before rapid assembly to peptidomimetics through a modular, iterative strategy. The resultant oligomers of alternating pyridyl and six-membered cyclic ureas accurately reproduce a recognition domain of several amino acid residues of a β-strand, demonstrated herein by mimicry of the i, $i+2$, $i+4$ and $i+6$ residues.

The inhibition or stabilization of protein–protein interactions (PPIs) represents a potential goldmine for medicinal chemistry,[1] yet the rational design of synthetic agents able to achieve this goal has been seen by many as intractable until recently.[2] There is growing interest in the synthesis of non-peptidic mimics of secondary[3] and supersecondary[4] protein elements and the side-chain residues they present for the recognition of cognate partners. There has been considerable success in mimicking the α-helical structures that are ubiquitous at interfacial peptide regions, prompting researchers to develop scaffolds for the projection of groups that reproduce side-chain vectors from one[5] or two[6] faces of the helix.

PPIs mediated by extended regions of proteins, such as β-strands (Figure 1 A),[7] are also of great interest for our group[8] and others,[9] inspiring strategies for the synthesis of minimalist frameworks that replicate their key domains (Figure 1 B).

Recently, we described a homochiral foldamer of alternating 2,6-disubstituted pyridyl groups linked by imidazolidin-2-ones
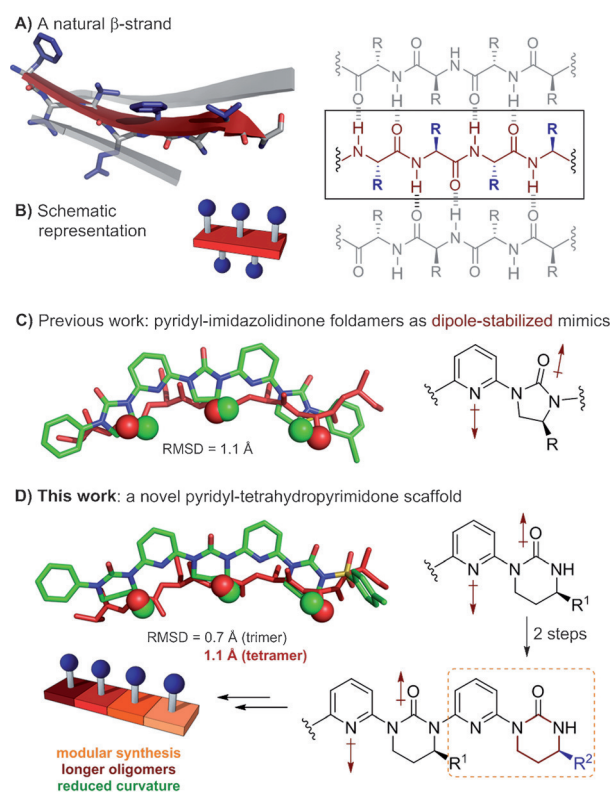

Figure 1. β-Strand peptidomimicry. A) Canonical β-strand; B) schematic of side-chain projection; C) previous work: overlay of a three-residue mimic rigidified through dipolar repulsion (green) with a strand (red); D) this work: a modular synthesis from preformed monomers provides rapid access to foldamers of alternating six-membered rings. Increased linearity over extended distances allows mimicry of longer peptide sequences. For example the six-point (trimer) RMSD is reduced to 0.7 Å, and the eight-point value for the tetramer is 1.1 Å. β-Strand from PDB: 3QXT, side-chains represented as spheres for clarity, α- and β-carbons used for calculation.[12]

bearing methyl groups, resulting in the mimicry of side-chains from the i, $i+2$, and $i+4$ positions of one face of a β-strand.[10] An important conformational feature of this motif is preorganization of the scaffold by dipolar repulsion between the pyridyl nitrogen lone pair and the carbonyl groups of adjacent imidazolidinones. The resultant projection of three groups for side-chain mimicry is in good agreement with those of one face of a canonical strand (Figure 1 C).

Extending the scope of this design for the mimicry of amino acid sequences over longer stretches of peptide requires two challenges to be overcome: 1) the alternation of five- and six-

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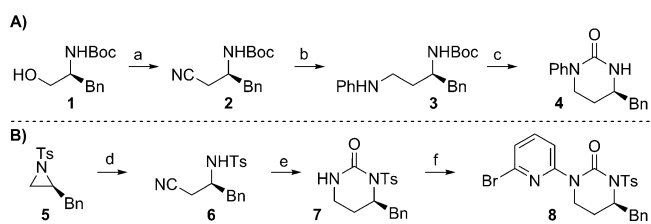   andrew.hamilton@chem.ox.ac.uk
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membered rings introduces a backbone curvature that is increasingly divergent from that of a canonical strand; and 2) a linear synthesis, in which each side-chain mimic is formed on the growing oligomeric strand, that is inherently inefficient. A potential solution is to pre-form pyridyl–urea monomers that mimic a given amino acid side-chain prior to oligomerization. As one-carbon homologues of the imidazolidin-2-one, the resultant foldamer is much more linear—thus, more closely reproducing the side-chain vectors of a β-strand.[11] Calculation suggests a reduction in six-point root-mean-square deviation (RMSD) value from 1.1 to 0.7 Å for the five- and six-membered urea mimics, respectively.[12a,b] Pre-forming each monomer has the potential to drastically reduce the longest linear synthetic sequence and more readily allows a peptidomimic of several residues to be varied at a particular position and generated in an iterative fashion (Figure 1 D). There are numerous commercially available derivatives of enantiomerically pure α-amino alcohols bearing a wide variety of side-chains, making them an ideal starting material for monomer construction.

As a representative amino acid found commonly in β-strands we chose to explore a proof-of-principle strategy based on phenylalanine. One-carbon homologation of N-Boc-L-phenylalaninol (1; Boc = $tert$-butyloxycarbonyl) through O-mesylation and displacement with sodium cyanide gave nitrile 2, which was transformed to the aniline derivative 3 by diisobutylaluminium hydride (DIBAL-H) mediated reduction followed by reductive amination with sodium cyanoborohydride and aniline. Trifluoroacetic acid mediated removal of the Boc group and cyclization with triphosgene gave terminally capped monomer 4 (Scheme 1 A).

The iterative monomer 8 was synthesized from commercially available aziridine 5. Opening of the three-membered ring with sodium cyanide gave 6, followed by nickel boride mediated reduction and cyclization with triphosgene to produce 7 in 40 % overall yield. Subsequent Buchwald–Hartwig amination of 2,6-dibromopyridine with 7 gave tosyl-protected monomer 8 in 54 % yield (Scheme 1 B).

Monomers 4 and 8 were coupled under Buchwald–Hartwig conditions to give two-residue mimic 9 in an excellent yield of 96 %. The oligomer can be rapidly extended to a mimic of three- 11 and four-residues 13 by an iterative two-step sequence of N-tosyl deprotection with magnesium in methanol followed by Buchwald–Hartwig cross-coupling with bromide 8 (Scheme 2).
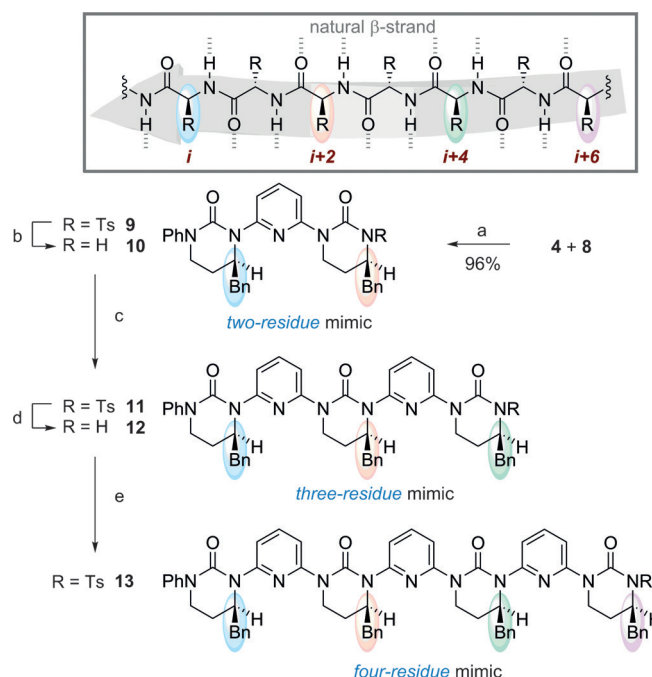
Scheme 2. Oligomer homologation to form conformationally rigidified peptidomimetics of two (9, 10), three (11, 12) and four (13) side-chain residues from one face of a natural β-strand. Reagents and conditions: a) conditions X, 96 %; b) conditions Y, 56 %; c) conditions X, 72 %; d) conditions Y, 10 %; e) conditions X, 52 %. Conditions X: XantPhos (30 mol %), [Pd₂dba₃] (10 mol %), CsCO₃ (2 equiv), dioxane, 90 °C, 16 h; conditions Y: Mg (25 equiv), MeOH, sonication, RT, 10 min.

Single crystals suitable for X-ray diffraction were obtained for monomer 8.[13] The solid-state conformation is consistent with dipolar repulsion between the urea carbonyl group and the pyridine nitrogen atom favouring an $anti$-relationship (Figure 2 A). The largely planar cyclic urea adopts a puckered conformation placing the benzyl group in a pseudo-axial position (Figure 2 B and the Supporting Information).[14] To probe the preferences of the higher order homologues 9–13, we conducted a computational search of their lowest-energy conformations.[12b,c] The validity of this method was confirmed by the close agreement between the solid-state structure of monomer 8 and the lowest-energy conformer by calculation (RMSD 0.5 Å; see the Supporting Information). Calculations for mimics 8–13 gave lowest-energy structures adopting fully extended conformations with urea carbonyl groups $anti$ to pyridine nitrogens, and with R groups in pseudo-axial positions (see the Supporting Information). Consequently, the phenylalanine side-chain mimics are projected from a common face, and are thus in good agreement with those of a canonical β-strand. Trimer
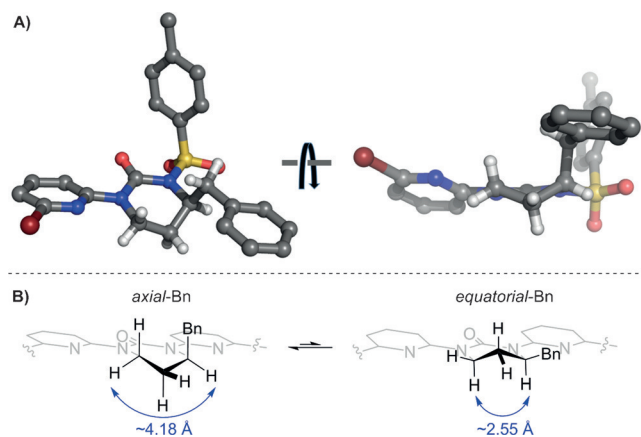

Scheme 1. Synthesis of monomeric cyclic ureas bearing an amino acid side-chain mimic for: A) uni-directional functionalization; and B) bi-directional functionalization. Reagents and conditions: (a, i) MsCl (1.2 equiv), Et₃N (1.5 equiv), CH₂Cl₂, 0 °C→rt, 10 min; (ii) NaCN (2.5 equiv), DMF, 60 °C, 3 h, 66 % over two steps, (b, i) DIBAL-H (2.5 equiv), CH₂Cl₂, −78 °C→0 °C, 1 h; (ii) aniline (2 equiv), NaBH₃CN (3 equiv), AcOH, MeOH, rt, 16 h, 19 % over two steps, (c, i) trifluoroacetic acid, CH₂Cl₂, rt, 0.5 h; (ii) triphosgene (0.5 equiv), iPr₂NEt (4 equiv), rt, 1 h, 47 % over two steps, (d) NaCN (1.35 equiv), MeCN, H₂O, reflux, 1.5 h, 84 %, (e, i) NiCl₂ (2 equiv), NaBH₄ (10 equiv, portion-wise), MeOH, 0 °C→rt, 10 min; (ii) triphosgene (0.5 equiv), iPr₂NEt (2 equiv), rt, 2 h, 47 % over two steps, (f) 2,6-dibromopyridine (2 equiv), 4,5-bis(diphenylphosphino)-9,9-dimethylxanthene (XantPhos; 30 mol %), [Pd₂dba₃] (10 mol %; dba = dibenzylideneacetone), CsCO₃ (2 equiv), dioxane, 80 °C, 1.5 h, 54 %.

Figure 2. Conformational behaviour of β-strand mimics **8–13**. A) X-ray crystal structure of monomer **8** showing the benzyl substituent in a pseudo-axial position; B) equilibrium between ring conformers placing the Bn side-chain mimic in pseudo-axial and pseudo-equatorial positions. Selected inter-hydrogen distances given for each conformer based on MM calculations.[12b,c, 14, 15] The difference in inter-hydrogen distance allows the conformers to be distinguished by NOE observations.

11 mimics the *i*, *i*+2, *i*+4 residues with a six-point RMSD value of 0.7 Å, whereas tetramer **13** adds the *i*+6 residue and has an eight-point RMSD value of 1.1 Å (Scheme 2 and the Supporting Information).[12a]

Solution-phase conformational behaviour was probed with NOESY and ROESY NMR experiments in CDCl₃ (Figure 3). NOE signals between the pyridine and cyclic urea hydrogens, for example, H8↔H15 and H20↔H17 for dimer **9**, were not observed for compounds **8–13** (Figure 3B, dashed red arrows). However, strong correlations were observed between urea hydrogens and the terminal phenyl/tosyl hydrogens by way of comparison, for example, H6$_{eq}$↔H3 and H22$_{eq}$↔H29 (Figure 3B and D, green arrows and the Supporting Information).[15c, 16] These data are consistent with the previously reported five-membered imidazolidin-2-one analogues,[10] indicating that the predominant species in solution adopts the desired extended conformation, in which the urea carbonyl groups are *anti*- to the pyridine nitrogen atoms as depicted in Figures 3B and D. This result highlights the utility of dipolar repulsion as a conformational determinant for pre-organisation of peptidomimetics.

Further inspection of the NOESY NMR spectra of **8–13** revealed interesting features regarding the position of the phenylalanine mimic. For dimer **9**, the absence of trans-annular NOE correlations between H6 and H8, and between H20 and H22, was consistent with their occupying pseudo-equatorial positions,[15a] with the side-chain mimic in a pseudo-axial site (Figure 3C and D). Interestingly, the only NOE signal in this region, corresponds to two hydrogens (H8 and H20) located on adjacent cyclic ureas.[15b] Additional evidence for this ring conformation was given by cross-peaks between the cyclic urea and benzylic hydrogens. For example, the NOE signal between benzylic H9 and one of the diastereotopic H6 hydrogens (Figure 3D, green arrows) is much stronger than it is with the H7 hydrogen (49:1 by direct integration of cross-peaks). Moreover, one of the diastereotopic H7 hydrogens has no
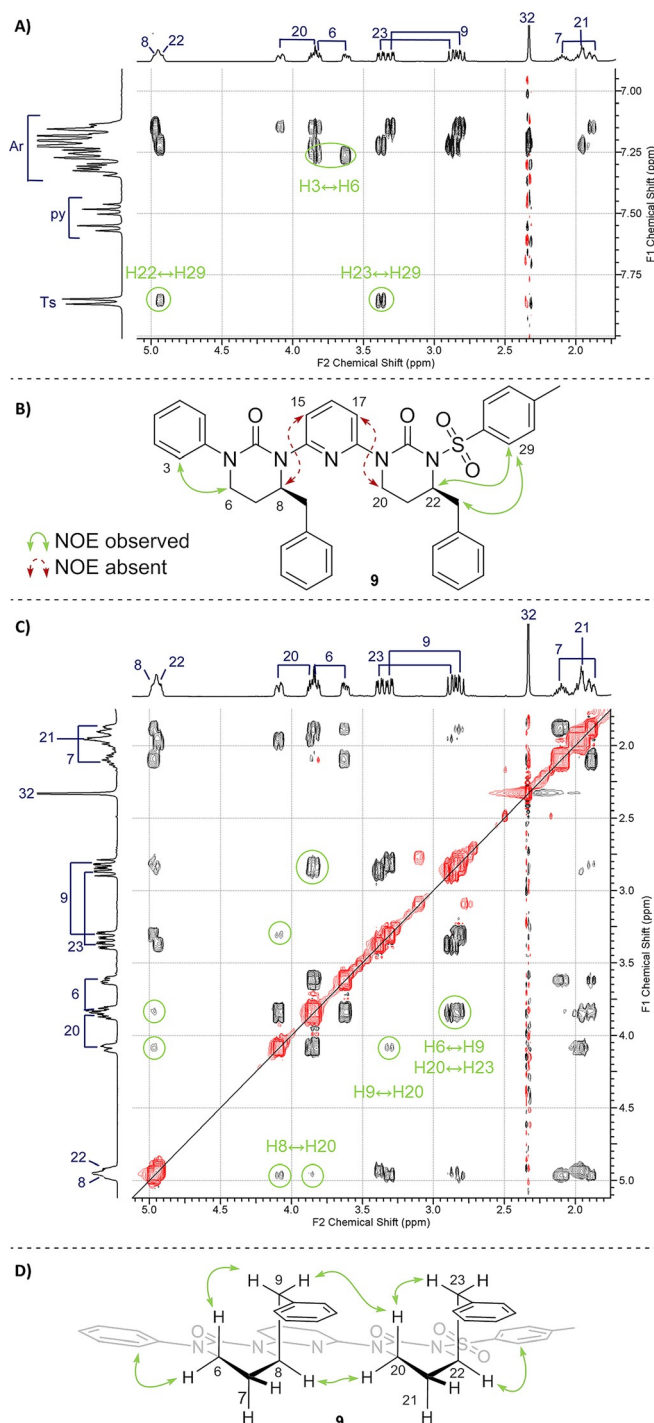

Figure 3. Solution-phase conformational analysis of dimer **9**. A) Selected region of the NOESY NMR spectrum focusing on cross-peaks with the pyridine signals (py); B) conformation based on NOE data; C) selected region of the NOESY NMR spectrum focusing on cross-peaks between the cyclic urea hydrogens; and D) perspective view of conformation based on NOE data. Key observed (green) and absent (red, dashed) NOE correlations are indicated (CDCl₃, 400 MHz, 298 K).[16]

cross-peak with the aromatic region, indicative of occupation of a pseudo-axial position.[16]

In conclusion, we have designed a synthetic route to an oligomer consisting of alternating pyridyl and six-membered cyclic urea groups. The resultant foldamer is conformationally

pre-organized by dipolar repulsion and ring puckering to project groups along the same vectors as the side-chains of amino acid residues from one face of a β-strand, as was shown by X-ray crystallography, NMR and MM calculations. As proof-of-principle, we demonstrated this for mimics of two, three and four phenylalanine side-chains. A particularly attractive aspect of the approach is that an iterative two-step process allows the foldamer to be extended by incorporation of pre-formed monomers. Work is underway to evaluate the conformational behaviour of mimics of specific peptide sequences containing a range of proteinogenic amino acids, and to assess their efficacy in mediating protein–protein interactions in aqueous media.

Acknowledgements

[1] S. Jones, J. M. Thornton, *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 13–20.

[2] a) M. R. Arkin, J. A. Wells, *Nat. Rev. Drug Discovery* **2004**, *3*, 301–317; b) M. R. Arkin, Y. Tang, J. A. Wells, *Chem. Biol.* **2014**, *21*, 1102–1114.

[3] a) M. J. Adler, A. G. Jamieson, A. D. Hamilton in *Small-Molecule Inhibitors of Protein–Protein Interactions, Vol. 348* (Eds.: L. Vassilev, D. Fry), Springer, Berlin Heidelberg, **2011**, pp. 1–23; b) M. K. P. Jayatunga, S. Thompson, A. D. Hamilton, *Bioorg. Med. Chem. Lett.* **2014**, *24*, 717–724; c) H. Moon, H.-S. Lim, *Curr. Opin. Chem. Biol.* **2015**, *24*, 38–47.

[4] a) O. V. Kulikov, S. Thompson, H. Xu, C. D. Incarvito, R. T. W. Scott, I. Saraogi, L. Nevola, A. D. Hamilton, *Eur. J. Org. Chem.* **2013**, *17*, 3433–3445; b) L. Nevola, J. M. Rodriguez, S. Thompson, A. D. Hamilton, *Supramol. Chem.* **2013**, *25*, 586–590.

[5] a) V. Azzarito, K. Long, N. S. Murphy, A. J. Wilson, *Nat. Chem.* **2013**, *5*, 161–173; b) B. B. Lao, I. Grishagin, H. Mesallati, T. F. Brewer, B. Z. Olenyuk, P. S. Arora, *Proc. Natl. Acad. Sci. USA* **2014**, *111*, 7531–7536; c) G. M. Burslem, H. F. Kyle, A. L. Breeze, T. A. Edwards, A. Nelson, S. L. Warriner, A. J. Wilson, *ChemBioChem* **2014**, *15*, 1083–1087; d) V. Azzarito, J. A. Miles, J. Fisher, T. A. Edwards, S. L. Warriner, A. J. Wilson, *Chem. Sci.* **2015**, *6*, 2434–2443; e) A. Barnard, K. Long, H. L. Martin, J. A. Miles, T. A. Edwards, D. C. Tomlinson, A. Macdonald, A. J. Wilson, *Angew. Chem. Int. Ed.* **2015**, *54*, 2960–2965; *Angew. Chem.* **2015**, *127*, 3003–3008; f) O. V. Kulikov, S. Kumar, M. Magzoub, P. C. Knipe, I. Saraogi, S. Thompson, A. D. Miranker, A. D. Hamilton, *Tetrahedron Lett.* **2015**, *56*, 3670–3673.

[6] a) S. Marimganti, M. N. Cheemala, J.-M. Ahn, *Org. Lett.* **2009**, *11*, 4418–4421; b) S. Thompson, R. Vallinayagam, M. J. Adler, R. T. W. Scott, A. D. Hamilton, *Tetrahedron* **2012**, *68*, 4501–4505; c) S. Thompson, A. D. Hamilton, *Org. Biomol. Chem.* **2012**, *10*, 5780–5782; d) K.-W. Jung, K. Vanommeslaeghe, M. E. Lanning, J. L. Yap, C. Gordon, P. T. Wilder, A. D. MacKerell, S. Fletcher, *Org. Lett.* **2013**, *15*, 3234–3237.

[7] a) H. Remaut, G. Waksman, *Trends Biochem. Sci.* **2006**, *31*, 436–444; b) A. M. Watkins, P. S. Arora, *ACS Chem. Biol.* **2014**, *9*, 1747–1754.

[8] 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.

[9] W. A. Loughlin, J. D. A. Tyndall, M. P. Glenn, T. A. Hill, D. P. Fairlie, *Chem. Rev.* **2010**, *110*, PR32–PR69.

[10] E. A. German, J. E. Ross, P. C. Knipe, M. F. Don, S. Thompson, A. D. Hamilton, *Angew. Chem. Int. Ed.* **2015**, *54*, 2649–2652; *Angew. Chem.* **2015**, *127*, 2687–2690.

[11] S. W. Fanning, J. R. Horn, *Protein Sci.* **2011**, *20*, 1196–1207.

[12] a) For the Cα and Cβ atoms of a canonical strand, MMFF94x with Born solvation for chloroform; b) *Molecular Operating Environment (MOE)*, version 2014.09, Chemical Computing Group, Inc., Montreal, QC, Canada, **2014**; c) LowModeMD; (see Supporting Information).

[13] 1056842 (**8**) contains the supplementary crystallographic data. These are provided free of charge by The Cambridge Crystallographic Data Centre.

[14] a) Cremer–Pople puckering coordinates (D. Cremer, J. A. Pople, *J. Am. Chem. Soc.* **1975**, *97*, 1354–1358) calculated using PLATON: b) A. L. Spek, *Utrecht University, Utrecht, The Netherlands* **2001**, *20*; c) A. L. Spek, *J. Appl. Cryst.* **2003**, *36*, 7–13.

[15] Calculations[12] give inter-nuclear hydrogen distances of: a) ≈4.18 Å between 1,3-di-pseudo-equatorial positions; b) ≈2.8 Å between pseudo-equatorial positions on neighbouring ureas; c) >4 Å between the pyridine and urea hydrogens (see the Supporting Information).

[16] ¹H NMR and NOESY spectra for dimer **9** were chosen for Figure 3 due to superior resonance dispersion. Observations for trimer **11** and tetramer **13** were consistent with the population of analogous conformational ensembles, see the Supporting Information.