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Bispidine as a helix inducing scaffold: examples of helically folded linear peptides†

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We designed and synthesized bispidine-anchored peptides and showed that these peptides as small as B3 (containing four chiral α-amino acid residues) adopt a right handed helical conformation. Bispidine anchored linear peptide B3 adopts a helical conformation in solution and in the solid state.

Controlling the 3D structure of proteins is the underlying theme of many recent research activities. The alpha helical shape is one of the topologies employed by nature to give structural and functional form to biological molecules. The prevalence of a helical conformation in many protein–protein interactions prompted chemists to mimic this conformation *via* a variety of methods.[1] For example, different peptidomimetic approaches for mimicking helical conformations have been investigated. Fairlie *et al.*[2] and Arora *et al.*[3] used cyclization approaches for restricting the conformation of the peptide to a helix. Their approaches involved the stapling of side chains using amide bonds or click reactions. These covalent cross-links stabilized the helical conformation if the targeted linking residues were installed at appropriate positions. Hamilton *et al.*[4] used non-peptide systems, such as biphenyl, to generate a helical topology and to effectively inhibit protein–protein interactions.

A variety of folding paradigms were investigated by Gellman and coworkers for specific types of folded molecules (namely, foldamers), thereby opening a new area of study. Gellman *et al.*[5] and Horne *et al.*[6] used different combinations of α and β amino acids to synthesize a variety of folded molecules. The continuing saga of protein folding and the engineering of peptides with predictable 3D structures still requires a more fundamental understanding before perfect designs can be obtained.[7]

Template-facilitated peptide folding is an attractive option for engineering secondary structures.[8] Recently, turns have been hypothesized to act as helix-nucleating elements in peptides.[9] Approaches based on proline-like structures to induce secondary structures in peptides were successfully demonstrated by various groups. β-Aminocyclopentanecarboxylic acid (ACPC),[10] β-amino cyclohexanecarboxylic acid (ACHC),[6a] aminopiperidine carboxylic acid,[11] 1-(aminomethyl)cyclohexaneacetic acid (gabapentin),[12] bicyclic systems,[13] and diproline[14] systems are effective in inducing secondary structures in peptides (Fig. 1). We conceived the incorporation of a cyclic structure into peptide backbones as a strategy to induce a turn that, if further reinforced by appropriate hydrogen bonds, might facilitate nucleation of a specific secondary structure.

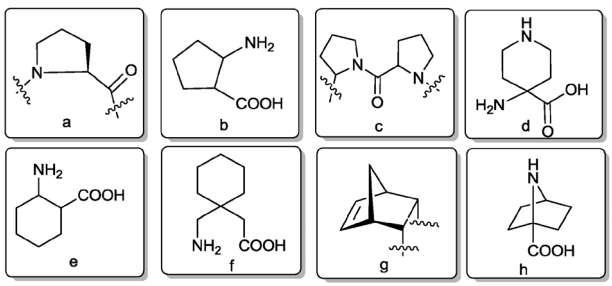

Fig. 1 Proline-inspired design of a template for secondary structure nucleation.

We envisaged that bispidine – with its two fused piperidine rings – would provide rigidity and kink in the backbone due to its bicyclic architecture with co-facially arranged nitrogen atoms. The two closely spaced nitrogens of bispidine keep the appended peptide chains in close proximity, which is favorable for intramolecular interactions leading to secondary structure formation.[15] We envisaged the closely appended nitrogen, decorated with peptides in an antiparallel way, as an attractive option for secondary structure nucleation.

In order to accomplish this, we converted bispidine into an imino acid-like structure, by linking one of the nitrogens to bromoacetic acid. In other words, incorporation of bispidine into the peptide backbone could be visualized as incorporation of an unnatural amino acid surrogate, where one side of the bispidine was functionalized with bromoacetic acid to obtain the imino acid (highlighted region in Fig. 2). The antiparallel arrangement of anchored peptide chains on the bispidine provides ample opportunities for intramolecular hydrogen bonding.

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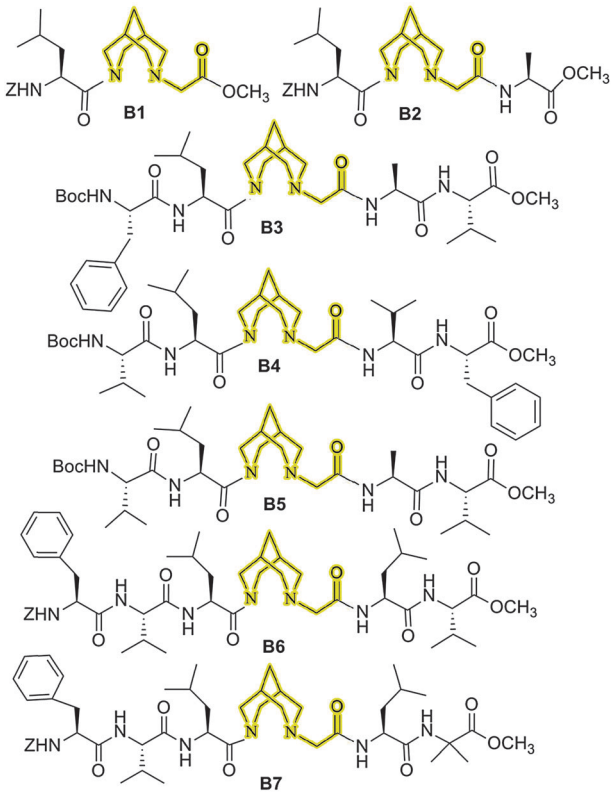

Fig. 2 Structures of the bispidine anchored peptides **B1–B7**.

We designed and synthesized bispidine-conjugated peptides **B1–B7** (Fig. 2). Selective functionalization[16] of the two nitrogen atoms yielded asymmetrically-appended bispidine with peptides (Scheme S1, ESI†). One of the two bispidine nitrogen atoms was functionalized with peptides *via* amide linkage, and the other nitrogen atom was functionalized by reacting with peptides functionalized with bromoacetic acid at the terminus. The two different linkages on bispidine-conjugated peptides offer possibilities for intramolecular hydrogen bonding because of the proximity of the complementary groups (*i.e.*, amide NHs and COs). Tetrapeptides **B3–B5** and pentapeptides **B6** and **B7** incorporating a bispidine moiety were synthesized by solution phase peptide synthesis under DCC/*N*-hydroxysuccinimide-based coupling conditions (ESI†).

The solution conformations of bispidine-anchored peptides were studied by NMR, IR and circular dichroism (CD) studies. The ^{1}H NMR spectra of tetrapeptides **B3**, **B4** and **B5** in $CDCl_3$ showed highly dispersed NHs. Careful analysis of the NMR spectra of **B1** and **B2** showed exchange peaks of amide NH as a result of the rotation of the imide carbonyls. The rotamer population decreases with increased peptide length (Table S1, ESI†). Detailed 2D NMR spectroscopic analyses of these compounds were performed using TOCSY, ROESY, and HSQC (Fig. S1–S4, ESI†). The ROESY spectrum of compound **B3** in $CDCl_3$ showed NOE peaks (Fig. S1 and S2, ESI†) between PheNH/LeuNH, ValNH/AlaNH, LeuNH/AlaNH, AlaαCH/ValNH and Leu NH/PheαCH; these are typical for helical conformations (Fig. S5b, ESI†). To test for the presence of intramolecular H-bonding, we performed ^{1}H NMR titration of the peptides in $CDCl_3$ with DMSO-d_6.[17] The Val NH showed a shift of ($\Delta\delta$) 0.332 at a DMSO concentration of 22% (v/v), indicating non-hydrogen-bonded solvent-exposed NH. Conversely, Leu NH ($\Delta\delta$ = 0.12), Ala NH ($\Delta\delta$ = 0.07), and Phe NH ($\Delta\delta$ = 0.03) showed almost no change in the amide protons, indicative of intramolecular H-bonding (Fig. S5, ESI†). The solution FT-IR studies of **B3** in chloroform showed bands at 3328 cm^{-1} and 3426 cm^{-1}, typical for hydrogen-bonded and non-hydrogen-bonded amide NHs (Fig. S6, ESI†). The amide I band at 1666 cm^{-1} supported a helical conformation.[18]

In order to investigate the intramolecularly H-bonded pattern in **B3**, we studied the simpler peptides **B1** and **B2**. The solution FT-IR spectrum of **B1** showed a band at 3424 cm^{-1}, indicating that the predominant form in solution was non-bonded NHs (Fig. S6, ESI†). NMR titration studies of **B1** in $CDCl_3$ with DMSO-d_6 showed exposed NH, as expected (Fig. S7, ESI†). FT-IR and DMSO-d_6 titration studies of **B2** showed that both the NHs were intramolecularly hydrogen-bonded (Fig. S6–S8, ESI†). Comparing **B1** and **B2**, the possible interactions could be ascribed to Leu NH with Ala CO and Ala NH with Leu CO. This was further supported by molecular dynamics studies of **B2** in chloroform. All possible H-bonded conformations of **B2** were modelled and analyzed to find the most favoured hydrogen bonding. It was found that the involvement of Leu NH/Ala CO and Ala NH/Leu CO is the most favoured compared to other possible hydrogen bonds (Table S2, ESI†). The three-dimensional structures of **B3** in chloroform and methanol were calculated using molecular dynamics simulation (SANDER, Amber 10). The ten lowest-energy structures showed a right handed helical structure (Fig. S9, ESI†) and a backbone RMSD of 0.21–0.59 Å as compared to the crystal structure.

CD spectra of the peptides **B3–B7** showed minima at 214 nm and 228 nm, which are characteristic of a right handed helical conformation (Fig. 3 and Fig. S10b–e, ESI†). The small red shift in the wavelength was attributed to the presence of an unnatural moiety in the backbone, or to deviations from the typical α-helical protein structure. Such a shift in the CD spectra is known in many synthetic systems.[19] **B1** and **B2** showed no helical characteristics in the CD spectrum (Fig. S10a, ESI†). The longer peptides **B6** and **B7** showed enhanced helicity (Fig. S10d–e, ESI†) compared to the shorter peptides **B3**, **B4** and **B5**. A minimum of four residues were required for helical conformation, as observed from the CD studies. **B3** and **B4** remained fully helical during CD melting experiments at temperatures from 10–55 °C, indicating that they are structurally robust (Fig. S11, ESI†). The addition of trifluoroethanol (TFE) showed only a slight increase in the CD signal (Fig. 3 and Fig. S12, ESI†).

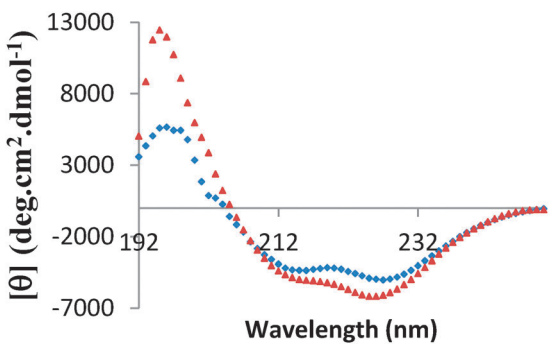

Fig. 3 CD spectrum of **B4** ◇ in methanol and Δ after addition of 50% trifluoroethanol.

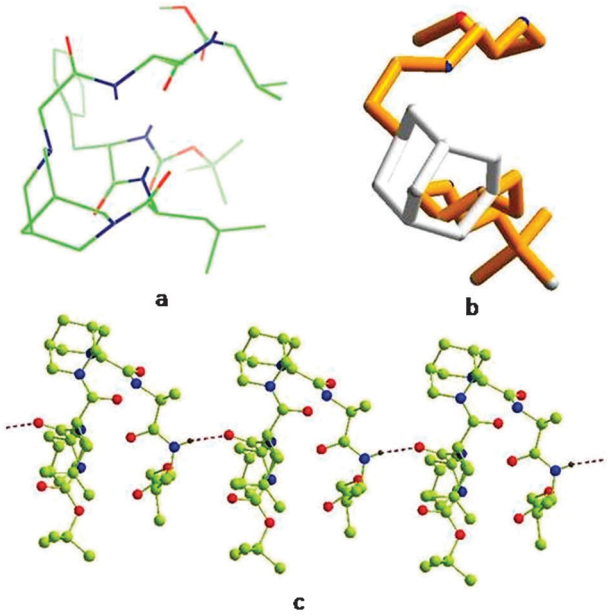

Fig. 4 X-ray crystal structure of **B3**: (a) capped stick representation, (b) backbone of **B3** showing a right handed helical conformation. The peptide backbone is coloured yellow. (c) Intermolecularly H-bonded assembly in the solid state showing H-bonds between Val NHs and Phe COs.

The protonation of the tertiary nitrogen in bispidine was expected to result in a conformational change (Fig. S13, ESI†). The CD spectrum of **B3** changed from a helical conformation to the one similar to a polyproline type I (PPI) conformation with the addition of trifluoromethane sulfonic acid or methane sulfonic acid (Fig. S13, ESI†). The CD spectrum displayed a maximum at 225 nm, indicative of a PPI conformation.[20] The CD, NMR and FT-IR studies on **B6** and **B7** also showed helical conformation (Fig. S14–19, ESI†).

Compound **B3** was crystallized from acetonitrile and dimethylformamide (Table S3, ESI†). The X-ray crystal structure of **B3** showed topological similarity to a right-handed helical conformation (Fig. 4) with torsional angles (Table S5, ESI†) different from a typical α-helix. The two piperidine rings of bispidine adopted chair conformations, resulting in a chair–chair (CC) conformation.[21] The distance between the two nitrogens in the bispidine unit is 2.93 Å. The intramolecular hydrogen bonds were observed between Leu-CO with Ala NH (2.338 Å), Ala CO with Leu NH (2.072 Å) and Phe NH with Val CO (2.398 Å) (Fig. S20, ESI†). These hydrogen bonds resulted in 10, 11, and 12-membered rings (Fig. S20, ESI†). The crystal structure further showed that free Val NH present in **B3** is involved in an intermolecular H-bond with the Phe CO of another molecule to form a linear chain of helical molecules (Fig. 4c).

In conclusion, we have identified bispidine as a moiety that can induce a helical conformation. The NMR, IR, CD and X-ray structure supported the right handed helical conformation in solution and in the solid state. The conformational transition from a helix to PPI-type conformation is an additional observation. The effectiveness of bispidine in facilitating the folding of peptides with no significant preference for a helical conformation is a noteworthy finding.

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