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Hydrogen bond surrogate stabilized water soluble 3_{10}-helix from a disordered pentapeptide containing coded α-amino acids

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ABSTRACT

Replacing a hypothetical i + 3 → i peptide H-bond in a disordered pentapeptide, that lacks any helicogenic C^{α}-tetrasubstituted residues, with a propyl linker and carbamylating the N-terminal nitrogen constrains it in the elusive 3_{10}-helical structure with high helicity and stability under varying conditions of temperature and pH, confirmed by NMR and CD analyses.

3_{10}-Helix is the second most abundant helical structure (10%)[1] in globular proteins next to the α-helix (90%) and contains the i + 3 → i intramolecular hydrogen bond between the backbone peptide N—H of the i + 3rd residue and C=O of the ith residue (Fig. 1). Although the (φ, ψ) backbone dihedral angles in 3_{10}-helices and α-helices fall in the same region of the Ramachandran plot,[1,2] the 3_{10}-helices are less stable and typically shorter (3–7 residues)[3] due to unfavourable van der Waals clashes and strict linear arrangement of the i + 3 → i hydrogen bonds.[3–5] 3_{10}-helices are often found at the termini of α-helices and play important roles as nucleation sites for helix formation during protein folding.[6]

Over the years, there has been great interest in developing methods to constrain short peptides in helical conformations. Synthetic models to constrain peptides into α-helix have primarily used two strategies: a) introducing a covalent surrogate for the main chain i + 4 → i H-bond (hydrogen bond surrogate, HBS, strategy)[7,8]; and b) introducing covalent[9–13], non-covalent[14–16] or metal coordination[17] interactions between i^{th} and i + 4th side chains. Non-peptidic models have also been successful in mimicking α-helical surfaces.[18] For mimicking the structurally more challenging 3_{10}-helices, the latter methods of introducing i···i + 3 side chain bridges, including lactam bridges[6], 1,2,3-triazole bridges[19], metathesis derived hydrocarbon bridges[20–22], photo- induced covalent bridges[23] and *p*-phenylenediacetic acid bridge[24], have been successful. The former strategy of introducing a covalent surrogate for the main chain i + 3 → i H-bond has however not yet been explored.

An important challenge about 3_{10}-helices is that, short peptides containing coded amino acids seldom form stable 3_{10}-helical structures outside their native protein context,[25] much lesser than those for α-helical structures.[26,27] As a result C^{α}-tetrasubstituted amino acids such as α-aminoisobutyric acid (Aib), whose allowed (φ, ψ) angles are extremely small and perfectly match with those of 3_{10}- and α-helices,[1,2,28,27] have been extensively incorporated (50% to 100%)[29,30] in peptide sequences to constrain them into 3_{10}-helical structures.[2,11,31] In fact, this is done in addition to introduction of other side chain constraints.[21,22] Such peptides hence suffer from poor solubility in water owing to their large apolar aliphatic surfaces and require specially designed C^{α}-tetrasubstituted α-amino acids functionalized with polar side chains to improve water solubility.[32] The steric bulk at C^{α} of the C^{α}-tetrasubstituted α-amino acids also encumber their backbone peptide bonds, which are crucial recognition elements, from being approached by molecules for interactions.

We envisioned the design of the first H-bond surrogate (HBS) (Fig. 1b) that can replace the main chain i + 3 → i H-bond, as a valuable method for stabilizing a short disordered peptide – that is devoid of C^{α}-tetrasubstituted amino acid residues – into 3_{10}-helix. As mentioned earlier, the HBS strategy,[7,33] (Fig. 1a) has been successful in constraining short, natural α-amino acid containing peptides into biologically relevant α-helical (n = 4)[34] and π-helical (n = 5)[35] conformations. But the 3_{10}-helical (n = 3) structures (Fig. 1b) have not been accessible by this method yet. Examination

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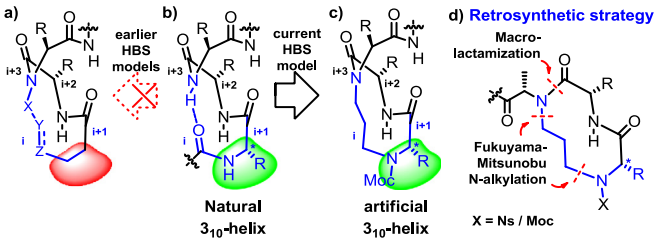
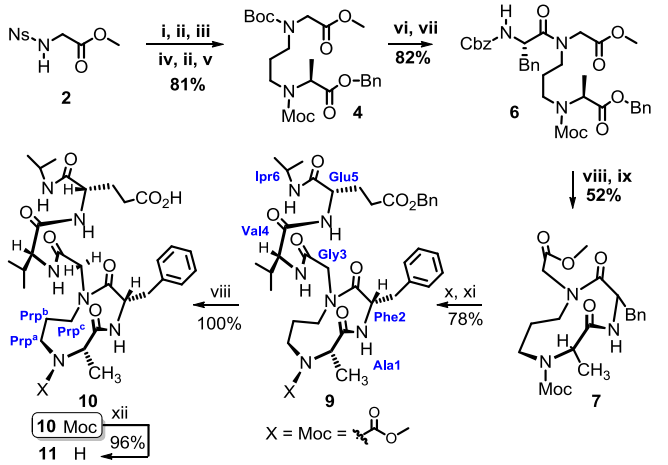


Fig. 1. ChemDraw rendition of: a) earlier hydrogen bond surrogate (HBS) models highlighting their lack of the i + 1st amino acid and N_{i+1}-CO group, hence deficient for constraining peptides in (b) the 3_{10}-helical conformation; c) current HBS model conserves the i + 1st residue and N_{i+1}-CO group, hence stabilizes even a completely disordered pentapeptide in the elusive 3_{10}-helical conformation. d) Retrosynthesis involves two initial Fukuyama-Mitsunobu N-alkylation reactions followed by macrolactamization.

of the two earlier HBS models[7,33] revealed that the i + 1st residue (Fig. 1a) is not conserved in them. The backbone groups (-NH-C^{α}R-) of the i + 1st residue get replaced by achiral groups (e.g. -CH_2-CH_2-), resulting in loss of crucial natural recognition elements and structural constraints.

Here we design a novel HBS model (Fig. 1c) where both these important structural elements (N and C^{α}R) of the i + 1st amino acid are conserved. The N-terminal i + 3 → i hydrogen bond (>N—H. . .O=CR—N<) is replaced with a propyl surrogate (>N—CH_2—CH_2—CH_2—N<) and a carbamyl group planarizes the i + 1st nitrogen. An efficient synthetic strategy is designed to incorporate this HBS model in a short disordered pentapeptide Boc-Ala^1-Phe^2-Gly^3-Val^4-Glu^5-Ipr^6 (**1**) (Ipr is isopropylamide), which is devoid of C^{α}-tetrasubstituted amino acids. Extensive 2D NMR and circular dichroism (CD) spectral analyses reveal that the current HBS-constrained peptide **10**, is uniquely restricted in a robust water-soluble 3_{10}-helix which is stable at different pHs and temperatures in spite of the absence of C^{α}-tetrasubstituted amino acids. Current method will provide access to 3_{10}-helical mimics with exclusively coded amino acid side chains in its sequence.



Legend: (i) PPh_3, DIAD, THF, 1,3-propanediol; (ii) PhSH, K_2CO_3, CH_3CN; (iii) $(Boc)_2O$, K_2CO_3, dioxane:H_2O; (iv) Ns-Ala-OBn (3), PPh_3, DIAD, THF; (v) Moc-Cl, K_2CO_3, CH_2Cl_2; (vi) TFA, CH_2Cl_2; (vii) Cbz-Phe-OH (5), ECF, NMM, THF;(viii) Pd/C/H_2, MeOH; (ix) EDC, HOBT, DIPEA, CH_3CN; (x) Li-OH,MeOH:H_2O; (xi)TFA-Val-Glu(OBn)-NHiPr (8), EDC, HOBT, DIPEA, CH_3CN; (xii) HBr/AcOH. Boc, tert-butyloxycarbonyl; Bn, benzyl; Ns, o-nitrobenzenesulfonyl; Moc, methyloxy carbonyl; Ipr, isopropylamine.

Scheme 1. Synthesis of the HBS-constrained 3_{10}-helix Moc-cyclo[Ala^1-Phe^2]-Gly^3-Val^4-Glu^5-Ipr^6 (**10**). cyclo[Ala^1-Phe^2]-Gly^3 denotes the propyl cross linker between N_{Ala1} and N_{Gly3}. The methylene groups of the propyl linker are labelled Prp^a, Prp^b, Prp^c from N- to C-terminus of **10**.conservation of which may prove crucial to mimicking and stabilizing the more stringent 3_{10}-helix.

1 has an order-breaking Gly residue and lacks any C^{α}-tetrasubstituted amino acids that promote 3_{10}-helix. AGADIR[36] calculations confirmed the lack of any helical structure in **1** (0.02% helicity) (S7.2). Any gain in 3_{10}-helicity in Moc-cyclo[Ala^1-Phe^2]-Gly^3-Val^4-Glu^5-Ipr^6 (**10**) (Scheme 1), the HBS constrained mimic of **1**, will hence be owing to the HBS-cross link in it.

In order to test the propensity of the Novel HBS to stabilize **1** in 3_{10}-helical conformation, we synthesized **10** (Scheme 1). Two Fukuyama-Mitsunobu reactions[37] using N-nosyl activated aminoesters **2** and **3** placed the propyl linker between the nitrogen atoms of Ala and Gly residues. The N-nosyl groups were replaced by Boc/Moc (**4**), which were better amenable to peptide coupling conditions. Boc-deprotection of **4** and coupling with **5** gave **6**. Reductive double deprotection of Cbz and Bn ester in **6** and macrolactamization yielded **7**. Ester deprotection of **7** and coupling with **8** gave C-terminal extended **9**. Reduction of side chain benzyl ester in **9** yielded **10**.

10 is water soluble. There are two segments in **10**: a) the HBS-constrained cyclic segment, Moc-cyclo[Ala^1-Phe^2], intended for 3_{10}-helix-nucleation; and b) the acyclic segment Gly^3-Val^4-Glu^5-Ipr^6 for propagation of one 3_{10}-helical cycle at its C-terminus. The solution structure of either segment in **10** was established by 2D NMR and circular dichroism (CD) spectral analyses (10% D_2O in H_2O) and molecular dynamic simulation analyses. First, 2D TOCSY and HSQC spectra were used to assign all the ^1H, ^{13}C NMR signals (S5.15, 16.) and ROE cross peaks (S5.17, 22.).

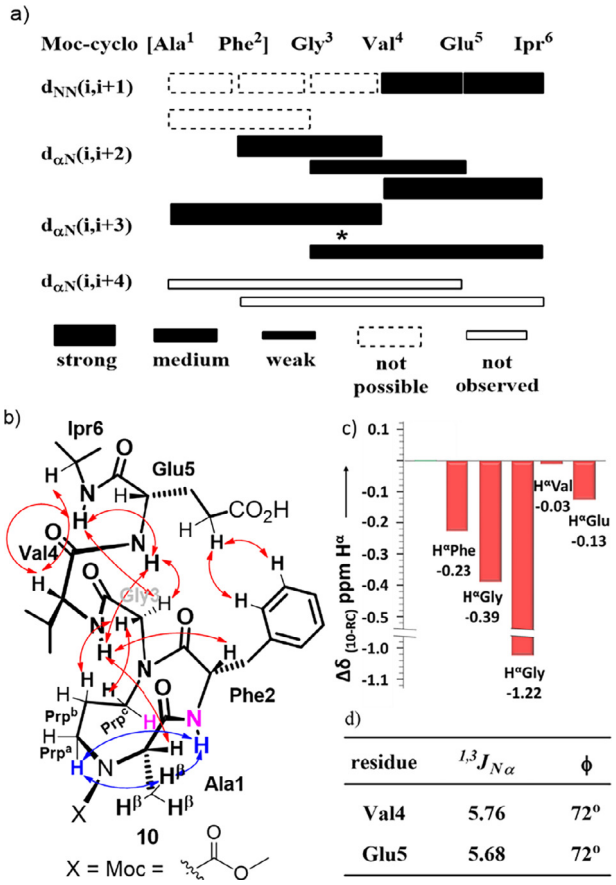


Fig. 2. a) ROE correlation chart for **10** showing sequential cross-peaks characteristic of 3_{10}-helical conformation in all its residues. Rectangular bars indicate ROE cross peak relative intensities. The Ala and Gly residues lack the backbone NH and hence the corresponding ROEs are not possible (S5.21.). b) The ChemDraw rendition of **10** representing all the key ROEs (10% D_2O/H_2O) that establish the 3_{10}-helical structure in it. c) Bar plot showing the negative secondary chemical shifts of H^{α} of residues in **10** from corresponding random coil (RC) values ($\Delta\delta_{\mathbf{10}\text{-RC}}H^{\alpha}$ ppm). d) Table of $^{3}J_{\alpha N}$ values and corresponding φ dihedral angles for Val4, Glu5 in **10** (S5.25).