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A His-Pro-Aib Peptide That Exhibits an Asx-Pro-Turn-Like Structure

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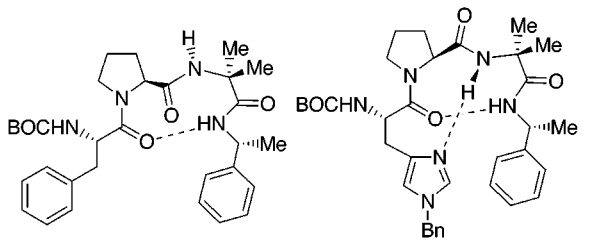
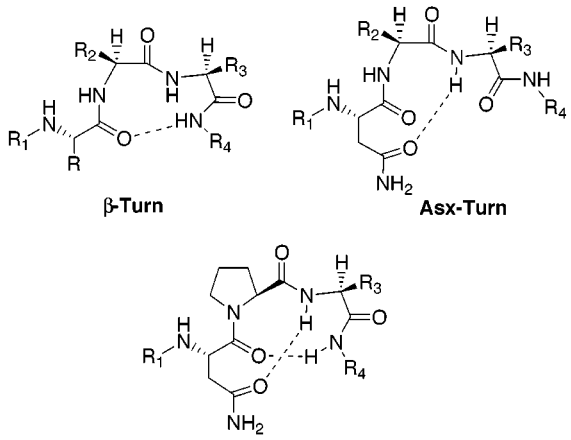
ABSTRACT



1, β–Turn **2**, Asx-Pro-like Turn

Small peptides 1 and 2, which differ only in that 1 possesses a BOC-Phe residue at the N-terminus, where 2 bears a BOC-(τ-benzyl)His, were found to exhibit very different structures. In both the solid state (X-ray) and in solution (NMR and IR), peptide 1 exists as a β-turn, whereas peptide 2 exists in a conformation that resembles the Asx-Pro motif.

Low molecular weight peptides that adopt well-defined conformations continue to be of interest in the design of bioactive molecules and more recently as synthetic catalysts for organic reactions. In these contexts, the various β-turns have achieved a privileged status, in part because so many biological receptors recognize peptides in this conformation.[1] Within our studies in the field of enantioselective catalysis, we have found that this motif often provides a suitably asymmetric environment such that enantioselective reactions can be carried out by appropriately functionalized β-turns.[2] An additional motif that has received significant attention recently is the so-called Asx-turn.[3] In addition to occurring naturally with a high degree of regularity, this motif has been proposed to be a structural element recognized by oligosaccharyl transferase as a prelude to asparagine-linked protein glycosylation.[4] Indeed, considerable effort is now focused on the discovery of small molecule mimics of the Asx-turn as inhibitors of this enzyme.[5] A hybrid structure, known as the Asx-Pro-turn, has also been characterized with some



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frequency in protein structures.[6] The Asx-Pro-turn exhibits a merged hydrogen bonding network featuring both the main chain-to-main chain hydrogen bond of the β-turn, as well as the side chain-to-main chain hydrogen bond of the Asx-turn.

Analysis of the Brookhaven PDB by Wilson and Finlay revealed that, whereas Asn-Pro-X sequences result in Asx-Pro-turns with a high degree of fidelity, His-Pro-X sequences do not result in analogous turns with nearly the same regularity.[6] We were therefore somewhat surprised to find that peptide **2** crystallizes in a form that strongly resembles the Asx-Pro-turn conformation. This is of particular interest in comparison to peptide **1**, a control peptide that differs from **2** only in that the phenyl ring of **1** is replaced with the benzyl imidazole of **2**. In Figure 1, the X-ray crystal structures of **1** and **2** are juxtaposed.[7]

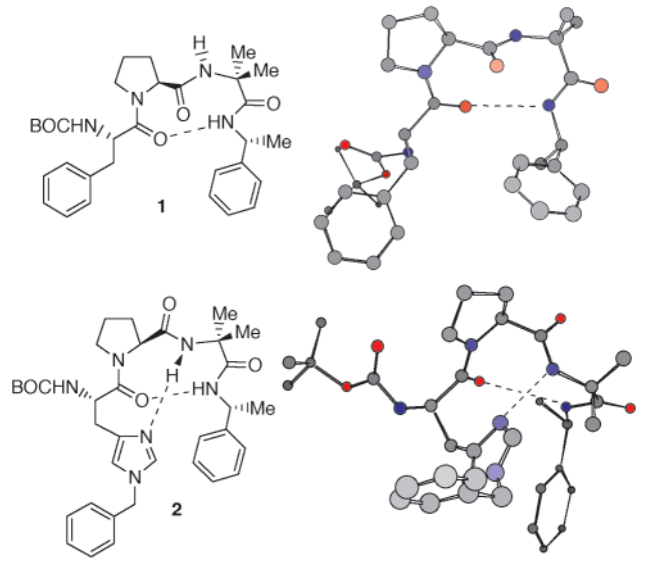

Figure 1. X-ray crystal structures for β-turn peptide **1** and "Asx-Pro-turn-like" **2**.

Several key features of the structures in the solid state dictate the assignments. In particular, for structure **1**, the relevant dihedrals are as follows: $\phi_{i+1} = -51°$, $\psi_{i+1} = +128°$; $\phi_{i+2} = +62°$, $\psi_{i+1} = +25°$. In addition, the O−N distance for the Phe C═O oxygen to the C-terminal nitrogen is 3.039 Å, consistent with the type II β-turn structure. The structure of peptide **2** is considerably different, with the following dihedrals: $\phi_{i+1} = -48°$, $\psi_{i+1} = -37°$; $\phi_{i+2} = -62°$, $\psi_{i+1} = -22°$. These values most closely approximate those defined for the type III turn.[8] Strikingly, the His side chain protrudes into the center of the turn, and the N−N distance between the π N of the His and the Aib N is short (3.074 Å). Other data of interest include the O−N distance between the His C═O and the C-terminal N, which is 3.129 Å. This distance indicates that a degree of β-turn character is retained in the solid-state structure of **2**. These data show that the solid-state structure of **2** exhibits significant Asx-Pro-turn-like character (Figure 2). Clearly, the inherent β-turn nature of this small peptide is not completely disrupted as a dual H-bonding network is observed.

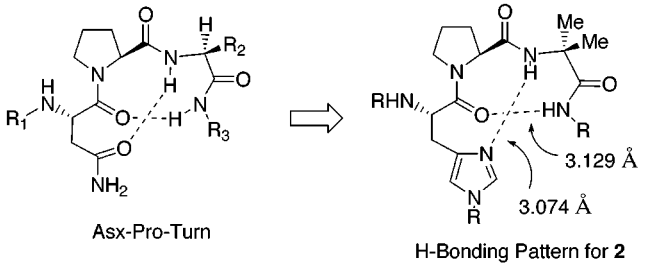

Figure 2. Structural similarity between the Asx-Pro-turn and the solid-state structure of **2**.

When peptide **2** is dissolved in C_6D_6, the structural features observed are similar to those present in the crystalline form. At ambient temperature, the spectrum exists as a sharp set of unique resonances. Examination of the chemical shift dependence of the amide NH protons on solvent composition reveals the following (Figure 3a): for peptide **2**, both the C-terminal NH (H_a) and the Aib NH (H_b) resonances are found at chemical shifts that are nearly insensitive to increasing DMSO concentration ($\Delta\delta < 0.25$ ppm).[9] These data point to a solution structure of **2** in a nonpolar medium where both H_a and H_b are involved in intramolecular hydrogen bonds. In contrast, the NMR spectrum exhibited by peptide **1** contains two sets of resonances at 25 °C, which coalesce to a unique set when the peptide sample is heated to 70 °C. Complicating a direct comparison of the spectra of peptide **1** to peptide **2** are the overlapping resonances of the various aromatic protons of peptide **1** and the amide NH signals. However, examination of the solution IR spectra (0.005 M/CH_2Cl_2) exhibited by the two peptides provides some evidence that the conformational differences observed in the solid state are mirrored in solution (Figure 3b). Peptide **2** exhibits a comparatively more intense absorbance at 3350 cm^{-1} and a weaker absorbance at 3440 cm^{-1}. This is consistent with a preference for a conformation where there are two intramolecular hydrogen bonds and one NH that is

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(7) As per journal policy, the crystallographic data for peptide **2** will be deposited in the Cambridge Crystallographic Data Center. The X-ray structural data for **1** has been published previously: see ref 2c.

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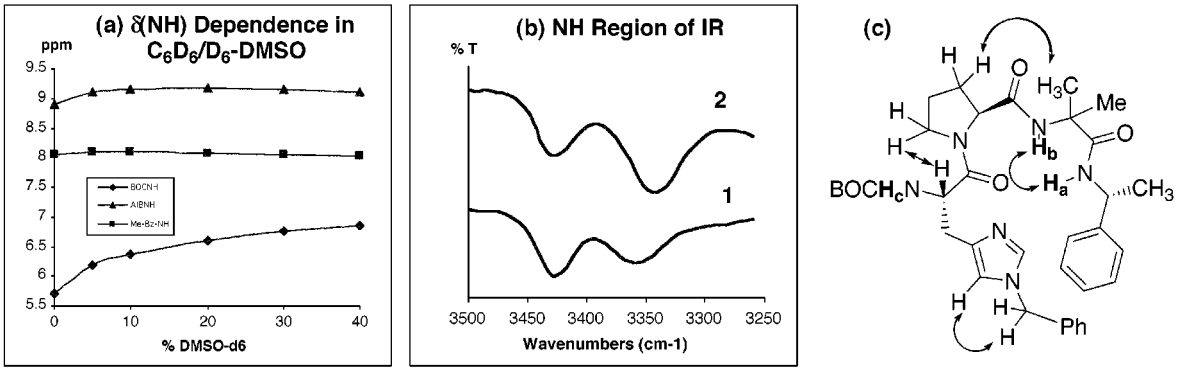

Figure 3. (a) Chemical shift data for peptide **2** as a function of solvent composition (%DMSO in C_6D_6). (b) NH region of the infrared spectra of peptides **1** and **2** (0.005 M/CH_2Cl_2). (c) Significant NOE enhancements.

not hydrogen bonded.[10] In contrast, peptide **1** exhibits less well-defined absorbances, a fact that supports the existence of conformational heterogeneity.

An evaluation of the NOESY (C_6D_6 solvent) spectrum of **2** also revealed intramolecular features that resemble the solid-state structure as well. As shown in Figure 3c, a number of significant NOEs were observed. Most significant is that between the Aib(NH_b) and the C-terminal NH_a. The proximity of these two amide protons is indicative of a compact, rather than extended, structure.

In conclusion, we have found that a simple His-Pro-X peptide may adopt a well-defined, compact structure that resembles the Asx-Pro-turn in both its crystalline form and in organic solvent. The stability of this secondary structure in a more biologically relevant aqueous medium could of course be jeopardized by competing hydrogen bonds to solvent.[11] Nevertheless, peptidomimetics[12] based on the incorporation of a His-like residue in the place of Asn could prove valuable as mimics of the Asx-Pro-turn motif.

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Supporting Information Available: Characterization data, including complete crystallographic data for peptide **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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