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Stabilization of β-hairpin structures *via* inter-strand π-π and hydrogen bond interactions in α-, β-, γ-hybrid peptides[¶]

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Abstract: Synthesis and conformational studies of α-, β-, γ-hybrid peptides containing a pyrrole amino acid (Paa, **1**) and a furan amino acid (Faa, **2**), namely Boc-β-Phe-Faa-D-Pro-Gly-Paa-β-HGly-Faa-OMe (**3**) and Boc-Paa-β-Phe-Faa-D-Pro-Gly-Paa-β-HGly-Faa-OMe (**4**), were carried out and they adopt β-hairpin structures stabilized *via* inter-strand π-π and hydrogen bonding interactions.

Keywords: pyrrole amino acid, furan amino acid, peptidomimetics, β-hairpin

While various weak interactions orchestrate the secondary and tertiary structures in proteins, conformational constraints of unnatural building blocks, like β-, γ- and δ-amino acids, have been extensively utilized to supplement these weak interactions in the rational design of many such secondary structures in small peptides.[1] A large number of conformationally constrained scaffolds have been developed over the years based on our understanding of the factors that nucleate the various folding patterns at local levels in proteins, like a two-residue turn with 10-membered hydrogen bonded structure bringing together two antiparallel strands in a β-hairpin which happens to be one of the most attractive targets for peptide chemists.[2] In this letter, we describe the use of two γ-amino acids, a pyrrole-based γ-amino acid (Paa, **1**) and a furan-based γ-amino acid (Faa, **2**) that were developed earlier,[3,4] in the synthesis of hybrid peptides **3** and **4** containing α-, β- and γ-amino acids. A dipeptide β-Phe-Faa and a tripeptide Paa-β-Phe-Faa have been linked here to a tripeptide Paa-β-HGly-Faa through a centrally located type II' β-turn-nucleating D-Pro-Gly motif giving rise to peptides **3** and **4**, respectively. Compound **3** showed the nucleation of β-turn with Paa(5)NH-Faa(2)CO and Paa(5)pyrroleNH-Faa(2)furan'O' hydrogen bonds leading to the formation of a 10- and 13-membered stable hairpin structures, respectively. In peptide **4**, hydrogen bonds between and Paa(6)NH-Faa(3)CO and Paa(6)pyrroleNH-Faa(3)furan'O' nucleated similar 10- and 13-membered stable hairpin structures. These hairpins continued further along the length of the peptide chains and stabilized via inter-strand π-π and hydrogen bonding interactions between β-Phe(1)NH-β-HGly(6)CO (in **3**) and Paa(1)pyrroleNH-Faa(8)furan'O' (in **4**).

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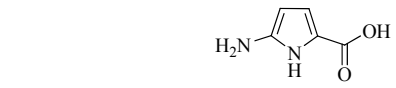

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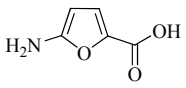

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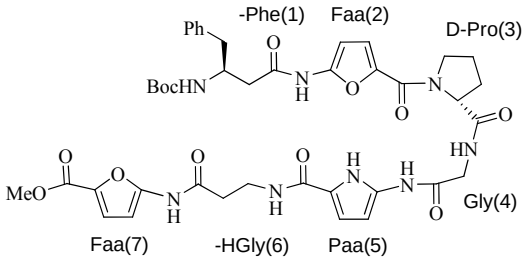

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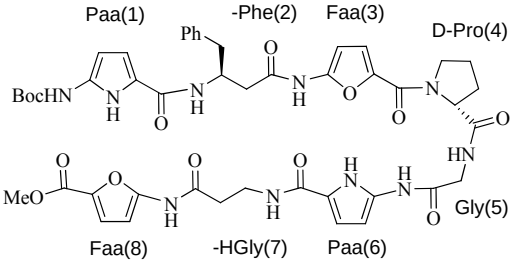

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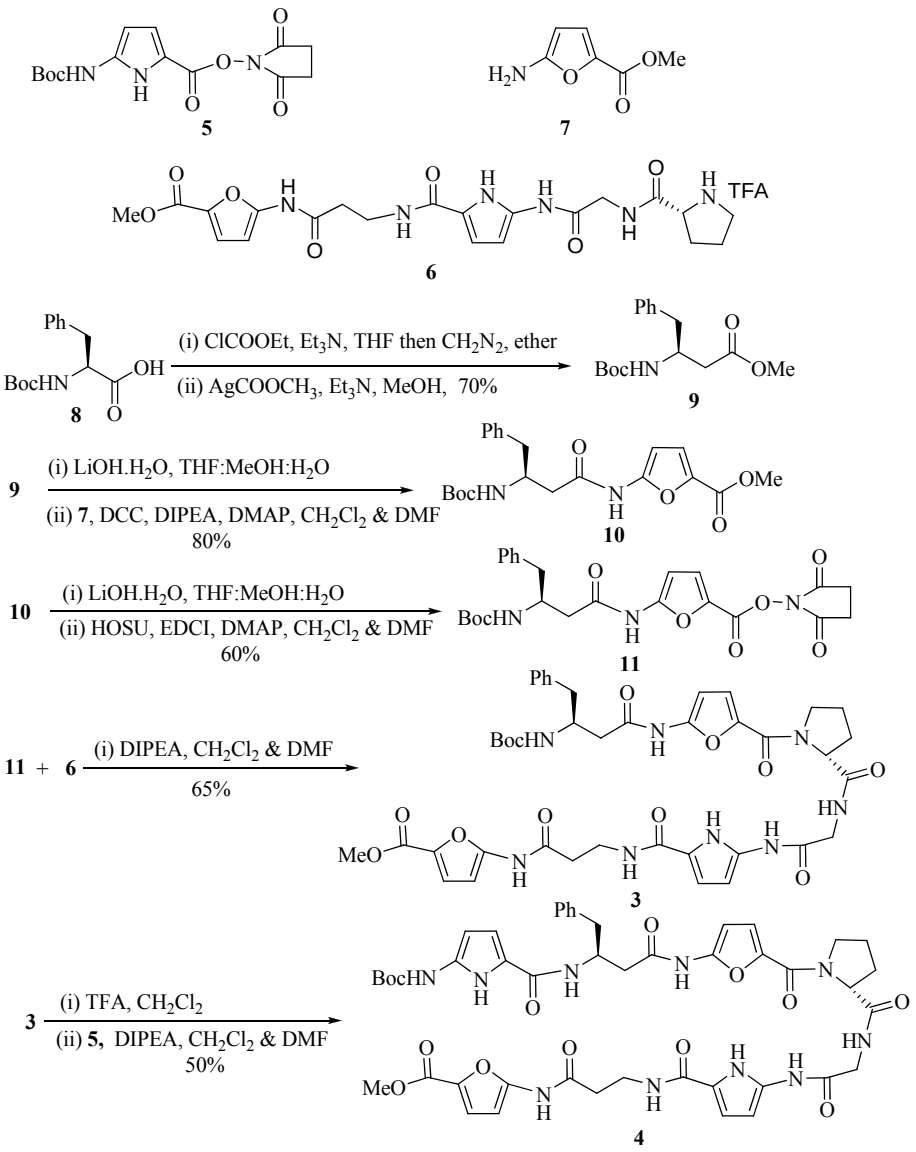
The syntheses of **3** and **4** are described in Scheme 1. The coupling of Boc-5-amino pyrrole amino acid succinimide ester **5** and TFA:D-Pro-β-Gly-Paa-β-HGly-Faa-OMe **6** was described in our earlier paper.[3] The synthesis of 5-amino-furan-2-methyl ester **7** was carried out following the reported procedure.[4] The peptides were synthesized by conventional solution phase methods[5] using dicyclohexylcarbodiimide (DCC) or 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide hydrochloride (EDCI) and 1-hydroxybenzotriazole (HOBt) as coupling agents and dry CH_2Cl_2 and/or amine-free dry DMF as solvents. While the *tert*-butoxycarbonyl (Boc) group was used for *N*-protection, the *C*-terminal was protected as methyl ester. Deprotection of the Boc was achieved using TFA–CH_2Cl_2 (1:1) and saponification of the methyl ester was carried out with LiOH in THF-MeOH-H_2O (3:1:1). Reaction of Boc-Phe-OH **8** with chloroethyl formate and diazomethane form diazoketone, which upon treatment with silver acetate[6] in MeOH gave monomer Boc-β-Phe-OMe **9**. Saponification of monomer **9** was followed by coupling with 5-amino-furan-2-methyl ester **7** under the conditions mentioned above to give the dimer, Boc-β-Phe-Faa-OMe **10**. Base hydrolysis of dimer **10** was followed by coupling with N-hydroxysuccinimide (HOSu) to give the active ester **11**. Reaction of **11** with **6** furnished the desired peptide heptamer **3**. Reaction of Boc-Paa-OSu **5** with **3**, after Boc-deprotection, afforded the desired peptide octamer **4**. The product was purified by silica gel column chromatography and used for conformational studies.



Scheme 1: Synthesis of peptides **3** and **4**.

Structural characterization of heptamer **3** and octamer **4** by NMR has been carried out in DMSO-d_6 at 300 K on 600 MHz spectrometer. Chemical shift assignments for the compound resonances have been made based on gDQCOSY, TOCSY and ROESY experiments. Temperature coefficients of NH chemical shifts calculated from variable temperature studies of peptides give preliminary information about their possible involvement in hydrogen bonding. Low $\Delta\delta/\Delta T$ values for β-Phe(1)NH, Paa(5)NH and Paa(5)pyrroleNH in **3** over a range of 300 – 338K confirm their involvement in hydrogen bondings.

Table 1. Temperature co-efficients of the NHs present in **3** and **4**.

Heptamer **3**		Octamer **4**	
NH	Temperature Co-efficient $\Delta\delta/\Delta T$ (ppb/K)	NH	Temperature Co-efficient $\Delta\delta/\Delta T$ (ppb/K)
β-Phe(1)NH	-1.0	Paa(1)NH	-6.4
Faa(2)NH	-5.5	Paa(1)PyrroleNH	-2.1
Gly(4)NH	-6.3	β-Phe(2)NH	-4.6
Paa(5)NH	-2.1	Faa(3)NH	-5.4
Paa(5)PyrroleNH	-0.5	Gly(5)NH	-5.7
β-HGly(6)NH	-6.3	Paa(6)NH	-2.1
Faa(7)NH	-6.5	Paa(6)PyrroleNH	-0.3
--	--	β-HGly(7)NH	-6.1
--	--	Faa(8)NH	-6.1

Unambiguous Paa(5)NH–Pro(3)CαH, Gly(4)NH–Pro(3)CδH, Faa(2)C3H–Pro(3)CαH, Faa(2)C3H–Pro(3)CδH and Paa(5)NH–Gly(4)NH ROEs observed in ROESY spectra of **3**, indicate the formation of a turn by D-Pro-Gly unit. Such a folding favors Paa(5)NH–Faa(2)CO 10-membered hydrogen bonding.[3] Paa(5)C4H–Faa(2)C3H ROE supports closer proximity of Paa(5) and Faa(2) residues which favors supplementary 13-membered Paa(5)pyrroleNH–Faa(2)furan'O' hydrogen bonding across these immediately following residues. Low $\Delta\delta/\Delta T$ (-2.1 ppb/K) for Paa(5)NH supports this possibility. Furthermore, these findings are confirmed by MD studies, which are discussed below.[3] In addition this ROE implies a face-to-face orientation of the aromatic planes in oppositely placed Faa(2) and Paa(5) residues, which favors aromatic π-π interactions[7-9] over the opposite strands of the hairpin and enhances the stability of the D-Pro-Gly induced β-turn extending further the turn to a hairpin fold. The extension of the hairpin fold along the chain is evident from the β-Phe(1)NH–β-HGly(6)CαH, β-Phe(1)CαH–β-HGly(6)CβH and β-Phe(1)CβH–β-HGly(6)CαH ROEs and participation of β-Phe(1)NH in hydrogen bonding. Placement of the β-Phe unit opposite to β-HGly has led to the resolved chemical shift resonances thereby helping in explicit and unambiguous ROEs.

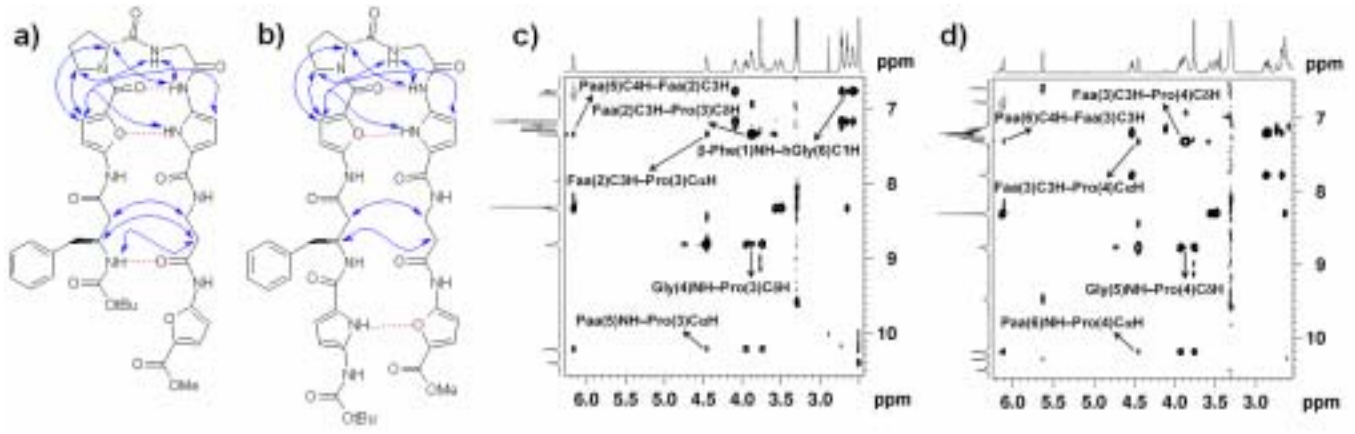


Figure 1. Schematic representation of specific NOEs (blue curves) and hydrogen bonding (red dotted lines) for **3** (**a**), and **4** (**b**). Expansions from ROESY spectra showing the characteristic NOEs for **3** (**c**), for **4** (**d**).

In order to examine the effect of aromatic interactions seen in **3** in a longer oligomer wherein two such aromatic residues are separated by more number of residues in between, we explored the synthesis and structural studies of the octamer **4**. Paa(1)pyrroleNH, Paa(6)NH and Paa(6)pyrroleNH in **4** exhibit low temperature coefficients as calculated over a temperature range 300 – 333K indicating their participation in hydrogen bonding. The observed Paa(6)NH–Pro(4)CαH, Gly(5)NH–Pro(4)CδH, Faa(3)C3H–Pro(4)CαH, Faa(3)C3H–Pro(4)CδH and Paa(6)NH–Gly(5)NH ROEs for **4** are consistent with those observed for **3**, designating the β-turn (D-Pro-Gly) unit involving in Paa(6)NH–Faa(3)CO 10-membered hydrogen bonding.[3] Manifestation of Paa(6)C4H–Faa(3)C3H ROE similar to the Paa(5)C4H–Faa(2)C3H ROE observed in **3**, and the homologous Phe(2)CαH–β-HGly(7)CβH and β-Phe(2)CβH–β-HGly(7)CαH ROEs further ascertain the secondary fold elongation and its stabilization through aromatic interactions. A significant change in the hydrogen bonding pattern is observed in the tail residues of **4**. The β-PheNH, which is originally involved in hydrogen bonding in **3** has now become idle whereas the Paa(1)pyrroleNH of Paa(1) residue in **4** has compensated its role. Such a difference can be explained based on the preferential aromatic π-π interactions between the Paa(1) and Faa(8) residues in **4** that are geometrically facing each other.

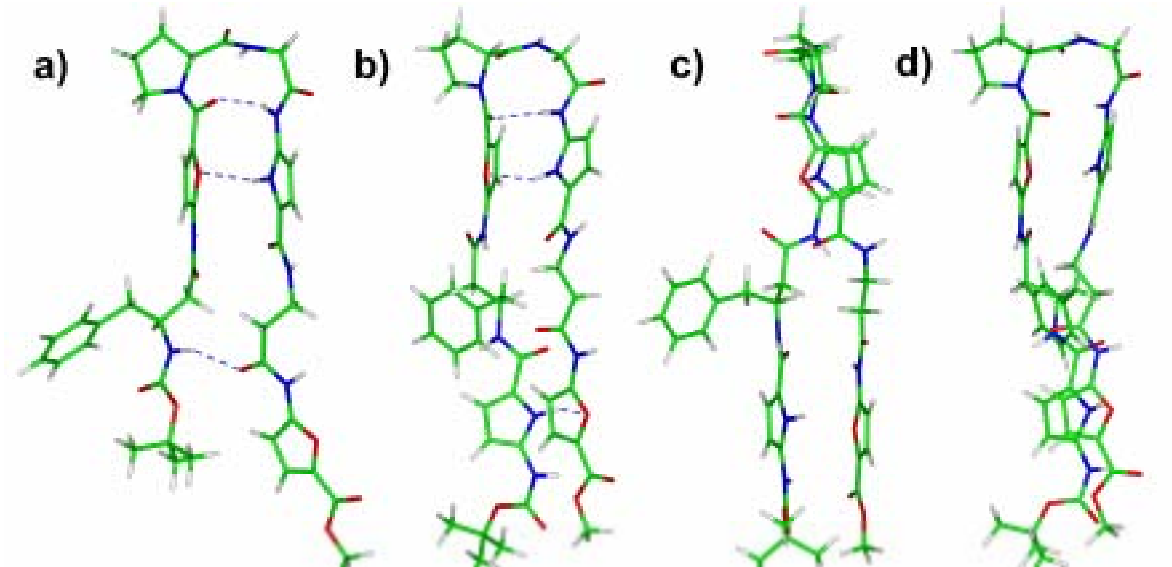


Figure 2. (**a**) Top view of one of the minimum energy structures obtained from MD studies for **3** and (**b**) for **4**; (**c**) and (**d**) are the side-views of the minimum energy structures for **4** showing the face-to-face orientation of aromatic γ-amino acid residues Paa and Faa.

The intensities of the ROE cross-peaks were converted into distances and used in restrained molecular dynamics calculations for both **3** and **4**. The 100 structures that were sampled during the MD simulations were energy-minimized and 30 low energy structures were aligned, which show a predominantly single conformation along the backbone. The energy-minimized structure of one of these samples for **3** and **4** are shown in Figure 2. Compounds **3** and **4** show the nucleation of β-turn with Paa(5)NH–Faa(2)CO and Paa(6)NH–Faa(3)CO 10-membered hydrogen bonds, respectively. This turn is further stabilized and transformed into a hairpin by a 13-membered Paa(5)pyrroleNH–Faa(2)furan'O' and Paa(6)pyrroleNH–Faa(3)furan'O' H-bonds, respectively, in **3** and **4**. This hairpin has continued further along the length of the peptide chains consisting inter-strand β-Phe(1)NH–β-HGly(6)CO and Paa(1)pyrroleNH–Faa(8)furan'O' H-bonds. The face-to-face orientation of the aromatic Paa and Faa residues across the strands is clearly evident in the MD structures. The aromatic plane centroids are separated by a distance of ≈ 4.4 Å. Paa(1) and Faa(8) aromatic interactions in **4** have now shown a case study of the role played by them in forming and stabilizing a long range secondary structural fold. We suspect that the twisted conformation of the β-hairpin in **4** is also a consequence of these aromatic interactions.

In order to facilitate the favored π-π interactions across the strands, the individual strands adopt variations in the local conformation. Accordingly as evident by $\Delta\delta/\Delta T$ values the resultant new conformations have H-bonding between Paa(1)pyrroleNH and Faa(8)furan'O' which also foregoes the β-Phe(1)NH–β-HGly(6)CO H-bonding that is observed in **3**. The observation of the preferential conformational changes to favor π-π interactions over a specific inter-strand H-bonding is remarkable in the present studies.

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