

Nucleation of the β -hairpin structure in a linear hybrid peptide containing α -, β - and γ -amino acids

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Abstract

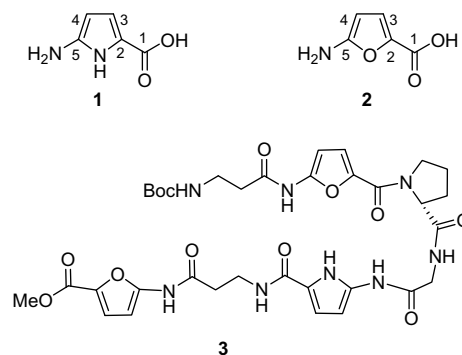
Synthesis and conformational studies of a short linear peptide containing a pyrrole amino acid (**1**, Paa) and a furan amino acid (**2**, Faa), namely Boc-hGly-Faa-D-Pro-Gly-Paa-hGly-Faa-OMe (**3**), were carried out in which it was established that peptide **3** adopted a well-defined β -hairpin structure in DMSO- d_6 .

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A large number of conformationally constrained designer scaffolds have been developed over the years and used successfully to induce β -turns in short peptides,¹ often a prerequisite to β -hairpin nucleation.² Studies have been carried out to show that hybrid sequences containing β -, γ - and δ -amino acids can be designed that adopt β -hairpin structures.³ A pyrrole-based δ -amino acid, 5-(amino-methyl)-pyrrole-2-carboxylic acid, was developed by us and served as a structurally restricted surrogate of the Gly- Δ Ala dipeptide isostere in the synthesis of peptides.⁴ In this Letter, we describe the use of pyrrole-based γ -amino acid **1** (Paa) as a peptide building block along with another constrained scaffold, a furan-based γ -amino acid **2** (Faa). The dipeptide hGly-Faa and the tripeptide Paa-hGly-Faa have been linked here through a centrally located type II' β -turn nucleating D-Pro-Gly motif⁵ giving the hybrid peptide **3** containing α -, β - and γ -amino acids. It was envisaged that the D-Pro unit with a φ value of $+60 \pm 20^\circ$ can induce a reverse turn that can be further stabilized by non-covalent interactions facilitated by the near planar disposition of the strategically placed Paa and Faa residues, especially

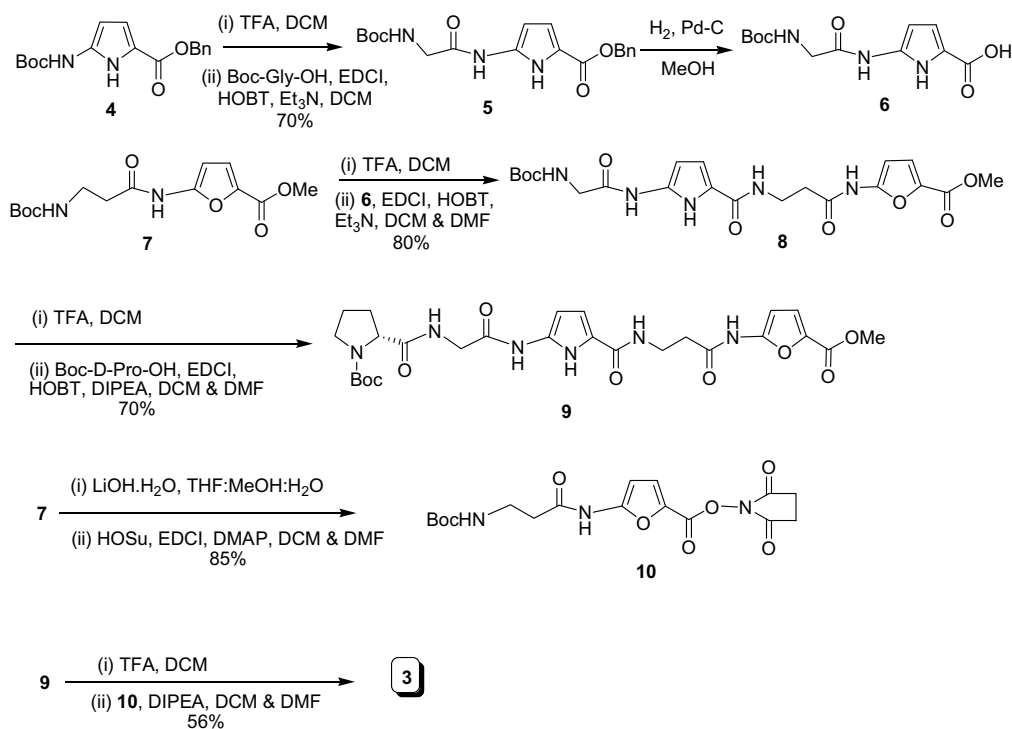
through the additional pyrrole-NH $\rightarrow$ furan-OH-bonds, leading to the formation of a stable hairpin architecture.



The synthesis of **3** is described in Scheme 1. The 5-amino-pyrrole-2-carboxylic acid **1** was first reported by Dervan and co-workers⁶ However, it was shown later by us⁷ that the compound prepared by them was actually the 4-amino congener. A practical synthesis of the required 5-amino version was then developed by us.⁷ The furan amino acid **2** was synthesized following the reported procedure.⁶ The peptides were synthesized by conventional

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Scheme 1. Synthesis of peptide 3.

solution phase methods⁸ using 1-ethyl-3-(3-(dimethyl-amino)-propyl)carbodiimide hydrochloride (EDCI) and 1-hydroxybenzotriazole (HOBt) as coupling agents and dry CH₂Cl₂ 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 (OMe) and benzyl esters (OBn). Deprotection of the Boc was achieved using TFA–CH₂Cl₂ (1:1), saponification of the methyl ester required LiOH in THF–MeOH–H₂O (3:1:1) and deprotection of the benzyl ester was carried out under catalytic hydrogenation.

Reaction of Boc-Gly-OH with H-Paa-OBn (**4**) under the conditions mentioned above gave the dimer, Boc-Gly-Paa-OBn (**5**). Hydrogenation of **5** gave **6**, which was coupled with the known dipeptide **7**,⁶ after Boc-deprotection, to furnish **8**. Boc-deprotection of **8** was followed by its coupling with Boc-D-Pro-OH to furnish **9**. Saponification of dipeptide **7** was followed by coupling with *N*-hydroxy-succinimide (HOSu) to give the active ester **10**. Reaction of **10** with **9**, after Boc-deprotection, furnished the desired peptide **3**. The product was purified by silica gel column chromatography⁹ and used for conformational studies.

NMR studies on compound **3** were carried out in DMSO-*d*₆ at 27 °C using a 600 MHz spectrometer. The poor solubility and existence of rotamers in CDCl₃ did not permit us to carry out the structural studies in detail in that solvent. Chemical shift assignments were made by gDQCOSY and TOCSY,¹⁰ and sequential assignment of the residues was made by ROESY¹¹ techniques. Temperature coefficients ($\Delta\delta/\Delta T$) of the NH chemical shifts mea-

Table 1

Temperature coefficients of the NHs present in compound **3**

NH	Temp coefficient ($\Delta\delta/\Delta T$) in ppb/K
hGly(1)NH	–5.4
Faa(2)NH	–4.1
Gly(4)NH	–5.4
Paa(5)NH	–2.0
Paa(5)PyrroleNH	–0.3
h-Gly(6)NH	–4.8
Faa(7)NH	–5.1

sured over a range of 33 K (from 300 K to 333 K) are listed in Table 1, which represent the strengths of the hydrogen bonds that they were involved in. Temperature coefficients of –2 ppb/K for Paa(5)-NH and –0.3 ppb/K for Paa(5)Pyrrole-NH suggest that they are strongly hydrogen bonded. A marginally higher value –4.1 ppb/K for Faa(2)NH is indicative of its involvement in a hydrogen bond of intermediate strength, while the remaining four NHs are solvent exposed and do not participate in hydrogen bonding.

Detailed analysis of the ROESY cross-peaks revealed a β -hairpin type structure for compound **3**. The presence of clear Paa(5)NH–Pro(3)C α H, Paa(5)NH–Pro(3)C δ H, Faa(2)C3H–Pro(3)C δ H, Faa(2)C3H–Pro(3)C α H, Paa(5)-C4H–Faa(2)C3H and Paa(5)PyrroleNH–Faa(2)C3H NOEs (Fig. 1) strongly support the formation of a β -turn by the D-Pro-Gly combination, which favours 10-membered hydrogen bonding between Paa(5)NH–Faa(2)CO. This D-Pro-Gly nucleated β -turn structure in DMSO has pro-

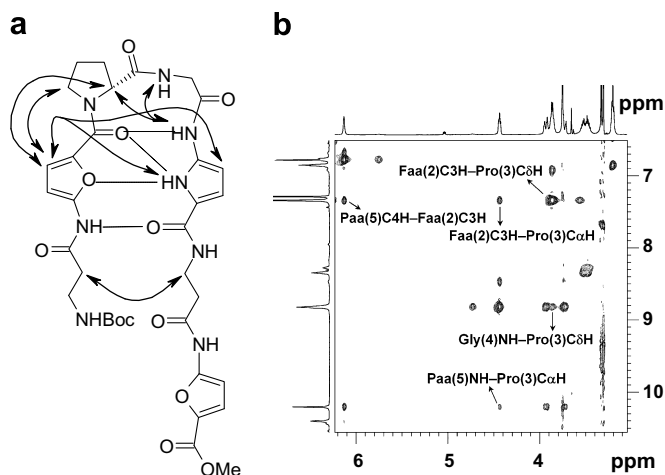


Fig. 1. (a) Schematic diagram showing the NOEs (in solid arrows) and the hydrogen bonds (in dashed lines) observed in the β -hairpin type structure of **3**; (b) ROESY expansion showing the key NOE cross-peaks.

moted a hairpin that is sustained over a new set of chains from the hGly(1) residue towards the NH-Boc end up to the hGly(6) towards the carboxyl end. The juxtaposition of these two residues is confirmed by the presence of an NOE between hGly(1)C α H–hGly(6)C β H (Fig. 1).

The intensities of the ROE cross-peaks were converted into distances and used in restrained molecular dynamics calculations.¹² 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 fraying out at the terminal residues is due to rapid molecular motions, as expected. The energy-minimized structure of one of these samples is shown in Figure 2. From the energy minimized structures, the β -turn hydrogen bond between Paa(5)NH–Faa(2)CO is estimated to be ~ 2.26 Å. The ϕ , ψ dihedral angles measured for D-Pro and Gly are -18 , -97 and -82 , 54° , respectively. The energy-minimized structures show the possibility that the β -hairpin is further stabilized by Paa(5)pyrroleNH–Faa(2)CO (~ 2.38 Å), Paa(5)pyrroleNH–Faa(2)furan 'O' (~ 2.41 Å) and Faa(2)NH–Paa(5)CO (~ 2.8 Å) hydrogen bonds across the chains, as shown in Figure 1.

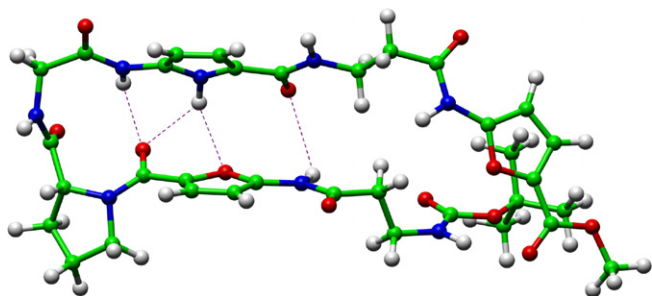


Fig. 2. One of the energy-minimized structures of **3** obtained from the MD simulations.

In summary, the furan and pyrrole rings of the hetero-aromatic γ -amino acids **1** and **2** nucleated additional hydrogen bonds stabilizing a well-defined β -hairpin structure in peptide **3**. Further work on these conformationally constrained peptidomimetic scaffolds is currently in progress.

Acknowledgements

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9. Selected physical data of **3**: ^1H NMR (DMSO- d_6 , 600 MHz, in δ scale): hGly(1): NH (6.85, t, 1H, $J_{\text{NH},\text{C}\alpha\text{H}} = 5.6$ Hz), C α H (2.55, m, 2H), C β H (3.20, m, 2H), Boc (1.34, s, 9H); Faa(2): FaaNH (11.34, s, 1H), C3H (7.33, d, 1H, $J_{\text{H}3,\text{H}4} = 3.5$ Hz), C4H (6.39, d, 1H, $J_{\text{H}3,\text{H}4} = 3.5$ Hz); Pro(3): C α H (4.43, dd, 1H, $J_{\text{C}\alpha\text{H},\text{C}\beta\text{H}} = 5.8$, 6.2 Hz), C β H (pro-*R*) (2.15, m, 1H), C β H (pro-*S*) (1.87, m, 1H), C γ H (pro-*S*) (2.08, m, 1H), C γ H (pro-*R*) (1.97, m, 1H), C δ H (3.85, m, 2H); Gly(4): NH (8.82, dd, 1H, $J_{\text{NH},\text{C}\alpha\text{H}} = 5.8$, $J_{\text{NH},\text{C}\alpha\text{H}'} = 4.6$ Hz), C α H (pro-*R*) (3.93, dd, 1H, $J_{\text{NH},\text{C}\alpha\text{H}} = 5.8$, $J_{\text{C}\alpha\text{H},\text{C}\alpha\text{H}'} = 17.0$ Hz), C α H' (pro-*S*) (3.73, dd, 1H, $J_{\text{NH},\text{C}\alpha\text{H}'} = 4.6$, $J_{\text{C}\alpha\text{H},\text{C}\alpha\text{H}'} = 17.0$ Hz); Paa(5): PaaNH (10.21, s, 1H), PyrroleNH (10.4, s, 1H), C3H (6.77, d, 1H, $J_{\text{H}3,\text{H}4} = 3.4$ Hz), C4H (6.12, d, 1H, $J_{\text{H}3,\text{H}4} = 3.4$ Hz); hGly(6): NH (8.34, t, 1H, $J_{\text{NH},\text{C}\alpha\text{H}} = 5.2$ Hz), C α H (2.62, m, 2H), C β H (3.50, m, 2H); Faa(7): FaaNH (11.59, s, 1H), C3H (7.27, d, 1H, $J_{\text{H}3,\text{H}4} = 3.6$ Hz), C4H (6.37, d, 1H, $J_{\text{H}3,\text{H}4} = 3.6$ Hz), OMe (3.75, s, 3H). MS (ESI): m/z 777 $[\text{M}+\text{Na}]^+$; HRMS (ESI): calcd for $\text{C}_{34}\text{H}_{42}\text{N}_8\text{O}_{12}\text{Na}$ $[\text{M}+\text{Na}]^+$, 777.2819; found, 777.2830.
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12. (a) Kessler, H.; Griesinger, C.; Lautz, J.; Muller, A.; van Gunsteren, W. F.; Berendsen, H. J. C. *J. Am. Chem. Soc.* **1988**, 110, 3393–3396; (b) The MD calculations were performed on the Insight-II-Discover platform. Simulated annealing for compound **3** was carried out by initial heating at 500 K for 500 ps and then cooling to 300 K in 1000 ps. Later dynamics were run at this equilibrated temperature for 5000 ps with a sampling time of 50 ps. Out of the 100 structures obtained, the minimum energy structure was subjected to restrained molecular dynamics for 1 ns with 1 fs step size and 100 ps history of sampling time.