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

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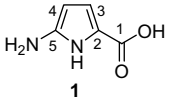
Abstract

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.

Keywords: Peptide mimetics; Pyrrole amino acid; Furan amino acid; Hydrogen bonding; Conformation; NMR

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-ΔAla 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 motif[5] 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 $+60 \pm 20°$ 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→furan-OH-bonds, leading to the formation of a stable hairpin architecture.



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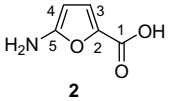

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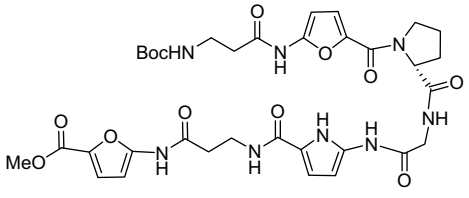

3

The synthesis of **3** is described in Scheme 1. The 5-amino-pyrrole-2-carboxylic acid **1** was first reported by Dervan and co-workers[6] However, it was shown later by us[7] 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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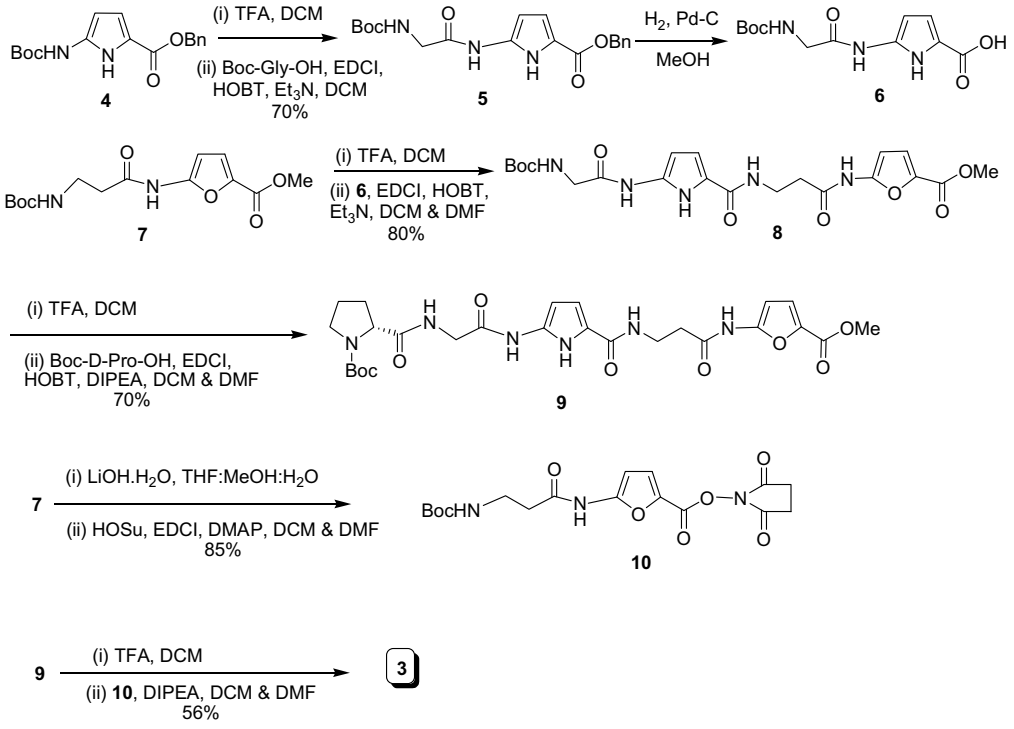

Scheme 1. Synthesis of peptide **3**.

solution phase methods[8] using 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 (OMe) and benzyl esters (OBn). Deprotection of the Boc was achieved using TFA–CH_2Cl_2 (1:1), saponification of the methyl ester required LiOH in THF–MeOH–H_2O (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*-hydroxysuccinimide (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 chromatography[9] 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 $CDCl_3$ 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 ROESY[11] techniques. Temperature coefficients ($\Delta\delta/\Delta T$) of the NH chemical shifts measured 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.

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

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)CαH, Paa(5)NH–Pro(3)CδH, Faa(2)C3H–Pro(3)CδH, Faa(2)C3H–Pro(3)CαH, 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-

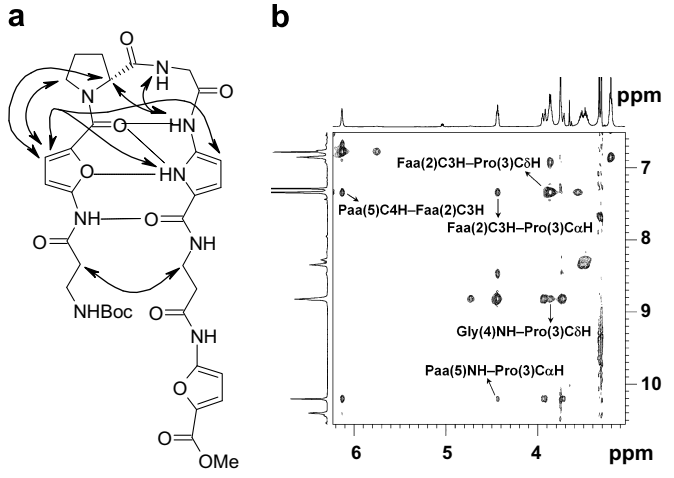

Fig. 1. (a) Schematic diagram showing the NOEs (in solid arrows) and the hydrogen bonds (in dashed lines) observed in the β-hairpin type structure of **3**; (b) ROESY expansion showing the key NOE cross-peaks.

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)CαH–hGly(6)CβH (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.

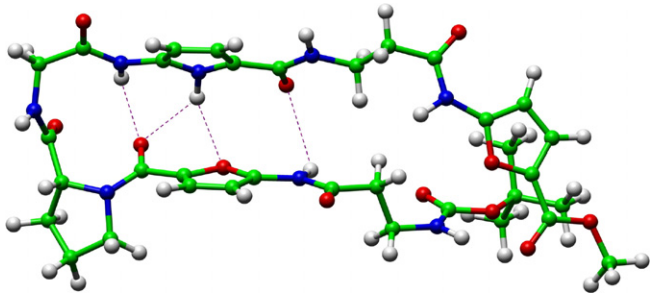

Fig. 2. One of the energy-minimized structures of **3** obtained from the MD simulations.

In summary, the furan and pyrrole rings of the heteroaromatic 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**: ^{1}H NMR (DMSO-d_6, 600 MHz, in δ scale): hGly(1): NH (6.85, t, 1H, $J_{NH,C\alpha H} = 5.6$ Hz), CαH (2.55, m, 2H), CβH (3.20, m, 2H), Boc (1.34, s, 9H); Faa(2): FaaNH (11.34, s, 1H), C3H (7.33, d, 1H, $J_{H3,H4} = 3.5$ Hz), C4H (6.39, d, 1H, $J_{H3,H4} = 3.5$ Hz); Pro(3): CαH (4.43, dd, 1H, $J_{C\alpha H,C\beta H} = 5.8$, 6.2 Hz), C$\beta$H (pro-*R*) (2.15, m, 1H), CβH (pro-*S*) (1.87, m, 1H), CγH (pro-*S*) (2.08, m, 1H), CγH (pro-*R*) (1.97, m, 1H), CδH (3.85, m, 2H); Gly(4): NH (8.82, dd, 1H, $J_{NH,C\alpha H} = 5.8$, $J_{NH,C\alpha H'} = 4.6$Hz), CαH (pro-*R*) (3.93, dd, 1H, $J_{NH,C\alpha H} = 5.8$, $J_{C\alpha H,C\alpha H'} = 17.0$Hz), C$\alpha$H′ (pro-*S*) (3.73, dd, 1H, $J_{NH,C\alpha H'} = 4.6, J_{C\alpha H,C\alpha H'} = 17.0$Hz); Paa(5): PaaNH (10.21, s, 1H), PyrroleNH (10.4, s, 1H), C3H (6.77, d, 1H, $J_{H3,H4} = 3.4$ Hz), C4H (6.12, d, 1H, $J_{H3,H4} = 3.4$ Hz); hGly(6): NH (8.34, t, 1H, $J_{NH,C\alpha H} = 5.2$ Hz), CαH (2.62, m, 2H), CβH (3.50, m, 2H); Faa(7): FaaNH (11.59, s, 1H), C3H (7.27, d, 1H, $J_{H3,H4} = 3.6$ Hz), C4H (6.37, d, 1H, $J_{H3,H4} = 3.6$ Hz), OMe (3.75, s, 3H). MS (ESI): *m*/*z* 777 $[M+Na]^+$; HRMS (ESI): calcd for $C_{34}H_{42}N_8O_{12}Na$ $[M+Na]^+$, 777.2819; found, 777.2830.
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