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Development of 5-(aminomethyl)pyrrole-2-carboxylic acid as a constrained surrogate of Gly-ΔAla and its application in peptidomimetic studies

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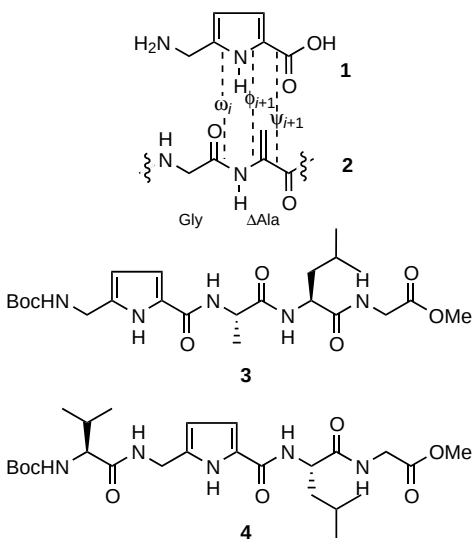
Abstract—A new peptidomimetic scaffold based on 5-(aminomethyl)pyrrole-2-carboxylic acid (**1**) is developed for the first time and used as a conformationally constrained surrogate of the Gly-ΔAla dipeptide isostere (**2**) to prepare peptides **3** and **4**. © 2002 Published by Elsevier Science Ltd.

Dehydro amino acids (ΔXaa) are found in many naturally occurring peptide antibiotics of bacterial origin as well as in some proteins.[1] They have also been used very extensively as rigid scaffolds in structural studies of peptides.[2] In this paper, we report the synthesis of a novel building block based on a pyrrole amino acid (Paa), 5-(aminomethyl)pyrrole-2-carboxylic acid (**1**), its application in peptidomimetic studies as a conformationally constrained surrogate of the Gly-ΔAla dipeptide residue (**2**) and the synthesis and conformational studies of peptides **3** and **4** prepared from this template.



One of the most widely used protocols to restrict conformational degrees of freedom in small peptides is by covalently stitching the "loose ends" together to fix various torsional angles at values characteristic of the target structure.[3] Bridging the terminal carbon of an olefinic analogue of a Gly unit and the Cβ atom of an adjacent ΔAla residue (**2**) leads to its constrained version **1** with fixed ω_i, φ_{i+1} and probably, ψ_{i+1} torsional angles; **1** was expected to nucleate interesting structures in peptides **3** and **4**.

The synthesis of compound **8**, i.e. the Boc-protected methyl ester of **1**, is outlined in Scheme 1. The starting material, 5-formylpyrrole-2-carboxylic acid methyl ester (**6**) was prepared from pyrrole (**5**) in three steps in 50% overall yield following known procedures: (a) a Vilsmeier–Haack reaction on pyrrole to furnish 2-formylpyrrole,[4] (b) Ag_2O oxidation of the resulting formyl group[5] followed by treatment with CH_2N_2 to give the 2-carbomethoxy group and finally (c) a second Vilsmeier–Haack reaction on pyrrole-2-carboxylic acid methyl ester to formylate its 5-position.[6] The formyl group of **6** was next reduced to an alcohol which was subsequently converted to an azido function[7] to furnish **7** in 52% yield in two steps. Reduction of the azido group of **7** was followed by in-situ Boc-protection leading to the protected intermediate **8** in 45% yield. While saponification of **8** provided the free carboxyl terminus, the N-terminus was deprotected using TFA/CH_2Cl_2 during the synthesis of compounds **3** and **4**. Peptide couplings were carried out under standard solution phase methods using 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide hydrochloride (EDCI) and 1-hydroxybenzotriazole (HOBt) as coupling agents and

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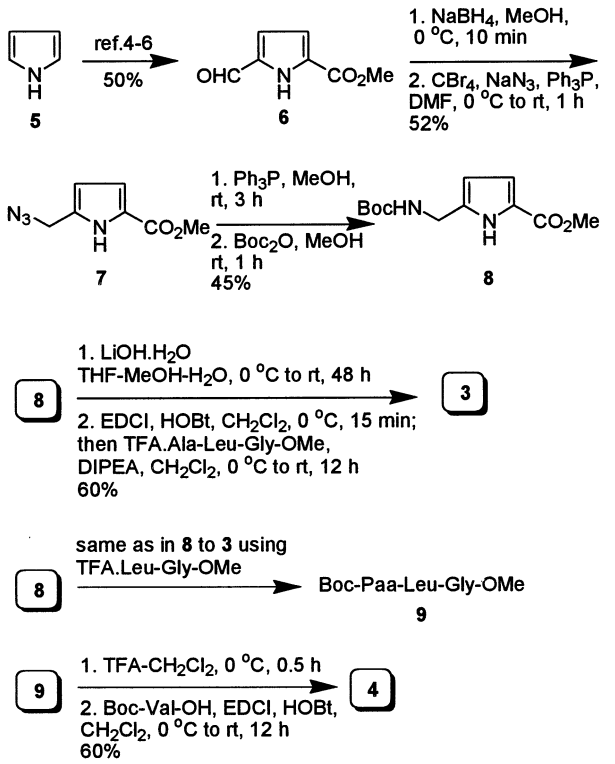

Scheme 1. Synthesis of Boc-Paa-OMe (**8**) and peptides **3** and **4**.

dry CH_2Cl_2 as solvent. The products were purified by silica gel column chromatography and used for conformational studies.[8]

Detailed conformational analysis of peptides **3** and **4** were performed using various NMR techniques. The assignments were carried out with the help of two-dimensional total correlation spectroscopy (TOCSY)[9] and were additionally confirmed by the rotating frame nuclear Overhauser effect spectroscopy (ROESY) experiments,[9] which provided the information on the proximity of the protons.

Although the NMR spectra of compounds **3** and **4** in DMSO-d_6 were very well resolved, they did not reveal significant turn structures. The temperature coefficients ($\Delta\delta/\Delta T$) of the amide protons of both **3** and **4**, obtained from variable temperature experiments (between 30 and 70 °C), had large magnitudes indicating the absence of any intermolecular H-bonding. However, the presence of strong to medium sequential NH–NH connectivity across the entire length of these molecules (Fig. 1), as shown clearly by the cross-peaks in their ROESY spectra, suggested that the φ,ψ torsional angles are in the α-region of the Ramachandran plot. The rOe cross peaks between AlaNH and pyrrole C3–H in **3** and LeuNH and pyrrole C3–H in **4** suggested near planar conformations with *trans* orientations for the φ and ψ torsions (both 180°) in the Paa segment of these molecules.

In a noncompetitive solvent such as $CDCl_3$, intramolecularly hydrogen bonded amide protons resonate downfield.[10] Compound **3** has only one amide proton, LeuNH with a chemical shift >7 ppm. In contrast, all the NH protons in compound **4** had the characteristic downfield chemical shifts of a hydrogen-bonded structure. To ascertain whether these shifts were due to hydrogen bond-mediated aggregation or not, NMR experiments were carried out with increasing amounts of solutes ranging from 1 to 33 mM concentrations. While all the NH signals in **3** were found to be highly concentration dependent, showing considerable downfield shifts ($\Delta\delta = 0.3$ to 0.9) with increase in concentration, there was less influence of aggregation in **4** ($\Delta\delta = 0.05$ to 0.1) within the concentration range studied. However, solvent titration studies revealed that the addition of increasing amounts of DMSO-d_6, a hydrogen bonding solvent, to a $CDCl_3$ solution (1 mM) of **4** (up to 25% v/v of DMSO-d_6 added) caused initial dips in all of its amide chemical shifts, except the pyrrole NH signal, indicating aggregation even at high dilution.[11] The pyrrole NH of **3** also showed minimum change during the solvent titration.

Due to the overlapping and broadening of spectral lines, NMR studies could not be carried out in detail in pure $CDCl_3$. However, a mixture of $CDCl_3$+12% DMSO-d_6 provided well-resolved spectra. Some of the important rOe cross peaks obtained in this solvent system are shown schematically in Fig. 1. As in DMSO-

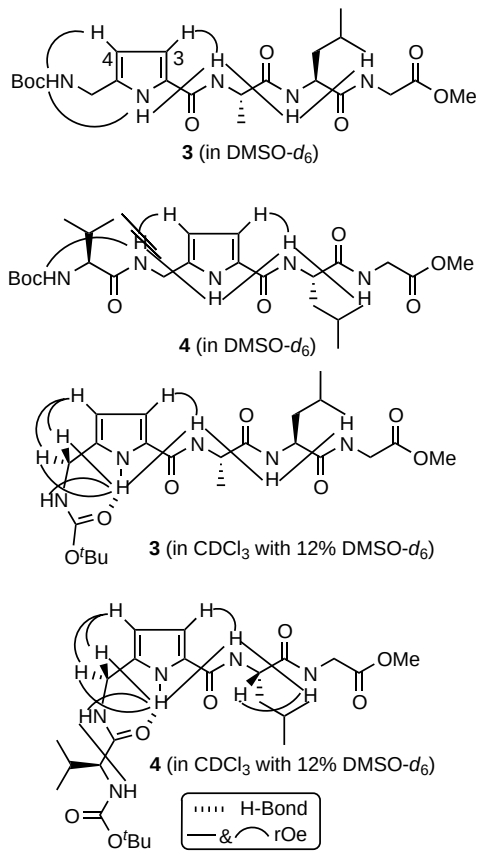

Figure 1. Schematic representation of the structures of **3** and **4** with the long-range rOes seen in their ROESY spectra.

d_6, here also strong sequential NH–NH connectivity can be seen across the entire length of these molecules. However, the absence of any cross peak between PaaNH and pyrrole C4–H, unlike those seen in both **3** and **4** in DMSO-d_6, can possibly be attributed to a γ-turn type structure as shown in Fig. 1 involving intramolecular hydrogen bonding between the pyrrole NH and the carbonyl of the previous residues, BocC=O (**3**) or ValC=O (**4**). This is in agreement with the known propensity of dehydroalanine (ΔAla) moieties to adopt near planar conformations in peptides with *trans* orientations for the φ and ψ torsions and induce an inverse γ-turn in the preceding residue.[2e] The characteristic rOe cross peaks and the small changes of the pyrroleNH chemical shifts during the solvent titration studies ($\Delta\delta = 0.3$ ppm for both **3** and **4** on addition of 25% v/v of DMSO-d_6) supported the proposed γ-turn structures for these molecules in the nonpolar solvent.

The rigid scaffold of the pyrrole amino acid described here can serve as a conformationally constrained template that may find useful application in developing novel peptidomimetics with interesting structures and useful properties. Further work is under progress.

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8. **3**: ^{1}H NMR (DMSO-d_6, 500 MHz): δ 11.08 (br s, 1H, Pyrrole N*H*), 8.27 (t, $J=5.9$ Hz, 1H, GlyN*H*), 7.94 (d, $J=8.5$ Hz, 1H, AlaN*H*), 7.86 (d, $J=8.5$ Hz, 1H, LeuN*H*), 7.09 (t, $J=5.3$ Hz, 1H, PaaN*H*), 6.74 (t, $J=2.8$ Hz, 1H, PaaC3*H*), 5.90 (t, $J=2.8$ Hz, 1H, PaaC4*H*), 4.41 (m, 1H, AlaαH), 4.33 (dt, $J=6.4$, 8.5, 1H, LeuαH), 4.07 (m, 2H, PaaC6H_2), 3.85 (dd, $J=5.9$, 17.3 Hz, 1H, GlyαH), 3.79 (dd, $J=5.9$, 17.3 Hz, 1H, GlyαH), 3.61 (s, 3H, OMe), 1.62 (m, 1H, LeuγH), 1.48 (m, 1H, LeuβH), 1.44 (m, 1H, Leu$\beta H'$), 1.37 (s, 9H, Boc), 1.28 (d, $J=7.1$ Hz, 3H, AlaβH), 0.86 (d, $J=6.6$ Hz, 3H, LeuδH), 0.82 (d, $J=6.6$ Hz, 3H, Leu$\delta H'$).
3: ^{1}H NMR ($CDCl_3$+12% DMSO-d_6, 500 MHz): δ 10.63 (br s, 1H, Pyrrole N*H*), 7.69 (t, $J=5.5$ Hz, 1H, GlyN*H*), 7.47 (d, $J=8.5$ Hz, 1H, LeuN*H*), 7.42 (d, $J=6.4$ Hz, 1H, AlaN*H*), 6.69 (t, $J=2.9$ Hz, 1H, PaaC3*H*), 6.08 (bt, 1H, PaaN*H*), 6.02 (t, $J=2.9$ Hz, 1H, PaaC4*H*), 4.53 (m, 1H, AlaαH), 4.51 (ddd, $J=4.9$, 8.5 and 9.8 Hz, 1H, LeuαH), 4.22 (m, 2H, PaaC6H_2), 4.04 (dd, $J=6.0$, 17.7 Hz, 1H, GlyαH), 3.92 (dd, $J=5.3$, 17.7 Hz, 1H, Gly$\alpha H'$), 3.71 (s, 3H, OMe), 1.72 (ddd, $J=4.9$, 8.7 and 13.4 Hz, 1H, LeuβH), 1.64 (m, 1H, LeuγH), 1.54 (ddd, $J=5.0$, 9.9 and 13.4 Hz, 1H, Leu$\beta H'$), 1.45 (d, $J=6.3$ Hz, 3H, AlaβH), 1.44 (s, 9H, Boc), 0.91 (d, $J=6.4$ Hz, 3H, LeuδH), 0.88 (d, $J=6.4$ Hz, 3H, Leu$\delta H'$).
4: ^{1}H NMR (DMSO-d_6, 500 MHz): δ 11.17 (br s, 1H, Pyrrole N*H*), 8.27 (t, $J=5.9$ Hz, 1H, GlyN*H*), 8.11 (t, $J=5.3$ Hz, 1H, PaaN*H*), 7.89 (d, $J=8.5$ Hz, 1H, LeuN*H*), 6.77 (t, $J=2.9$ Hz, 1H, PaaC3*H*), 6.63 (d, $J=9.0$ Hz, 1H, ValN*H*), 5.91 (t, $J=2.9$ Hz, 1H, PaaC4*H*), 4.51 (ddd, $J=4.9$, 8.5 and 10.1 Hz, 1H, LeuαH), 4.22 (dd, $J=5.6$, 15.5 Hz, 1H, PaaC6*H*), 4.21 (dd, $J=5.5$, 15.5 Hz, 1H, PaaC6*H'*), 3.84 (dd, $J=5.9$, 17.3 Hz, 1H, GlyαH), 3.80 (dd, $J=6.0$, 17.3 Hz, 1H, Gly$\alpha H'$), 3.76 (dd, $J=7.7$, 9.0 Hz, 1H, ValαH), 3.60 (s, 3H, OMe), 1.64 (m, 1H, LeuγH), 1.60 (ddd, $J=4.9$, 10.1 and 13.3 Hz, 1H, LeuβH), 1.54 (ddd, $J=4.9$, 9.5 and

13.3 Hz, 1H, Leu$\beta H'$), 1.37 (s, 9H, Boc), 0.89 (d, $J=6.5$ Hz, 3H, LeuδH), 0.85 (d, $J=6.5$ Hz, 3H, Leu$\delta H'$), 0.81 (d, $J=6.5$ Hz, 3H, ValγH), 0.80 (d, $J=6.5$ Hz, 3H, Val$\gamma H'$).

4: ^{1}H NMR ($CDCl_3$+12% DMSO-d_6, 500 MHz): δ 10.71 (br s, 1H, Pyrrole NH), 7.79 (t, $J=4.9$ Hz, 1H, PaaNH), 7.70 (t, $J=4.7$ Hz, 1H, GlyNH), 7.46 (d, $J=7.7$ Hz, 1H, LeuNH), 6.72 (t, $J=2.7$ Hz, 1H, PaaC3H), 6.03 (t, $J=2.7$ Hz, 1H, PaaC4H), 5.64 (d, $J=8.8$ Hz, 1H, ValNH), 4.68 (ddd, $J=5.0$, 7.7 and 9.8 Hz, 1H, LeuαH), 4.39 (dd, $J=5.9$, 15.3 Hz, 1H, PaaC6H), 4.31 (dd, $J=5.0$, 15.3 Hz, 1H, PaaC6H'), 4.00 (m, 1H, ValαH), 3.98 (dd, $J=5.7$, 17.8 Hz, 1H, GlyαH), 3.93 (dd, $J=5.5$, 17.8 Hz, 1H, Gly$\alpha H'$), 3.70 (s, 3H, OMe), 2.07 (m, 1H, ValβH), 1.72 (m, 1H, LeuγH), 1.72 (m, 1H, LeuβH), 1.70 (m, 1H, Leu$\beta H'$), 1.42 (s, 9H, Boc), 0.95 (d, $J=6.1$ Hz, 3H, LeuδH), 0.93 (d, $J=5.6$ Hz, 3H, Leu$\delta H'$), 0.92 (d, $J=6.6$ Hz, 3H, ValγH), 0.86 (d, $J=6.7$ Hz, 3H, Val$\gamma H'$).

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