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A family of macrocyclic antibiotics with a mixed peptide–peptoid ß-hairpin backbone conformation†

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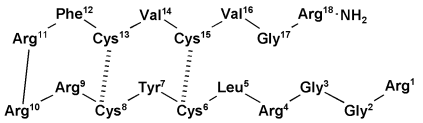
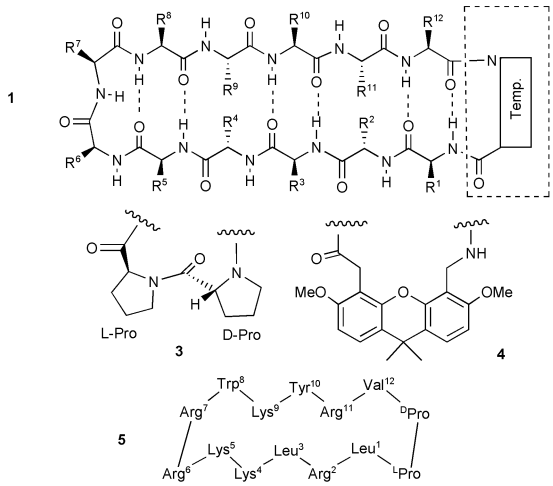
Macrocyclic peptidomimetics having a mixed peptide–peptoid backbone have been synthesized and shown to possess antibiotic activity against Gram-positive and -negative bacteria with a low hemolytic activity against human erythrocytes; one is shown to adopt a regular ß-hairpin conformation by NMR in aqueous solution.

The naturally occurring cationic antimicrobial peptides have stimulated enormous interest as a relatively new family of antibiotics with potential clinical value in the fight against multiple drug resistant microorganisms.[1] Their antimicrobial activity is related to their ability to adopt amphiphilic secondary structures, including α-helices or ß-hairpins, in a membrane-like environment.[2] Their principal mode of antibiotic action appears to involve depolarization or permeabilization of the bacterial cell membrane, although the detailed mechanisms are still not fully understood.[3]

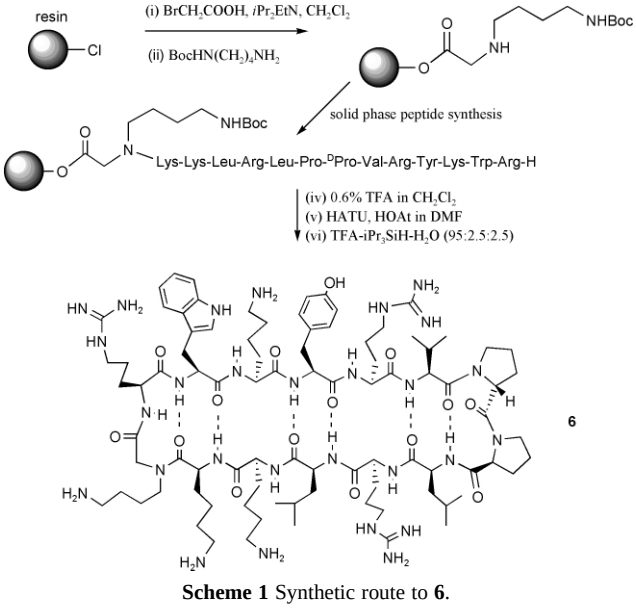
We recently described a new family of cyclic, template-bound antimicrobial peptidomimetics, represented by **1**, that are patterned on the naturally occurring cationic ß-hairpin antibiotic protegrin I (**2**).[4] Whereas the backbone of **2** is constrained into a ß-hairpin geometry by two disulfide bridges, the conformation of **1** is constrained by attachment of a 12-mer peptide loop to a hairpin-stabilizing template (as in **1**, where template = Temp = D-Pro-L-Pro (**3**) or xanthene **4**). The mimetic **5**, for example, shows antimicrobial activity against Gram-positive and -negative bacteria, and has a considerably lower hemolytic activity on red blood cells than the natural product **2**. NMR studies showed that **5** adopts an unordered structure in water, but undergoes a transition to a largely ß-hairpin conformation in a micellar environment.[4]

The scope for further structure–activity studies in this series would be extended if the peptide backbone of the hairpin loop could be modified to include other types of peptidomimetic scaffold, whilst retaining key side-chain functionality. Here we have examined the effects on structure and antimicrobial activity of introducing peptoid units[5] (*i.e.* with the amino acid side-chain moved from the C(α) position to the N-atom) into selected positions of the hairpin loop mimetic **5**, a change which may also improve proteolytic stability. For this, we focused first on residues 2, 4 and 6 whose peptide NHs would not be involved in potential cross-strand H-bonding in a regular ß-hairpin (see **1**). For the synthesis of **6**, the linear precursor was constructed on 2-chlorotrityl chloride–polystyrene resin using Fmoc chemistry[6] as shown in Scheme 1. After cleavage from the resin, macrocyclization was performed in DMF solution. Side-chain protecting groups (Boc, tBu and Pbf) were then removed in trifluoracetic acid, and the product was isolated in high yield (68%) after purification by reverse-phase HPLC (electrospray-MS: *m*/*z* 1850 [M+H]). The analogue **7** was prepared in a similar way. However, an attempted synthesis of **8** gave only very disappointing yields, and so **8** was not investigated further.





Since secondary structure can strongly influence antimicrobial activity, we investigated the structure of **6** in aqueous solution (H_2O–D_2O (9 : 1), pH 5.0, 300 K). The parent compound **5** appeared largely unstructured in aqueous solution,[4] so it is noteworthy that under these conditions **6** adopts a relatively stable ß-hairpin conformation, with the tertiary amide



Scheme 1 Synthetic route to **6**.

† Electronic supplementary information (ESI) available: ^{1}H-NMR (600 MHz) chemical shifts; ^3J coupling constants; upper distance restraints derived from integration of ROESY cross-peak volumes for the mimetic **6**, as well as average backbone torsion angles ϕ and ψ with standard deviation found in 15 NMR structures of the mimetic **6** as shown in Fig. 1; and CD spectra for **5**, **6** and **7**. See http://www.rsc.org/suppdata/cc/b3/b304310j/

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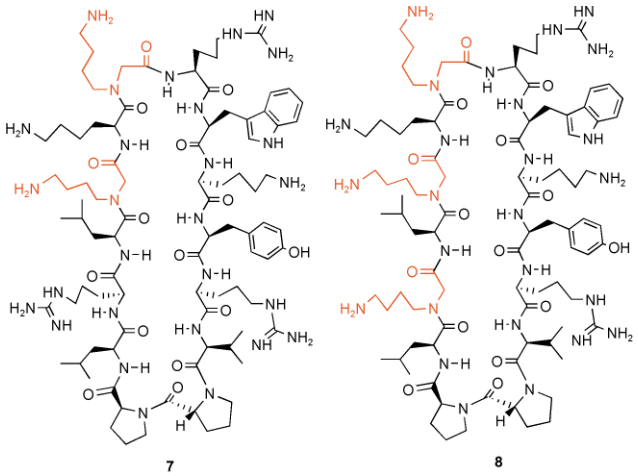

bond geometries as depicted in **6**. This was clearly evident from ROESY spectra of **6** in water which showed strong cross-strand NOEs between the C(α)-H protons of Arg[2] and Arg[11], as well as between those of Lys[4] and Lys[9]. Cross-strand NOEs were also seen between the peptide HNs of Leu[1] and Val[12], Leu[3] and Tyr[10], Lys[5] and Trp[8]. A relatively strong NOE was also seen between the NHs of Arg[7] and Trp[8] at the tip of the loop. Several ^3J (HN,H-C(α)) coupling constants characteristic of ß-structure (> 9.0 Hz; see supplementary information†) and relatively slow H–D exchange rates for those peptide NH groups expected to participate in cross-strand H-bonds (compare **1**), provide further support for a relatively stable ß-hairpin conformation.

A superimposition of 15 DYANA structures[7] calculated using NOE-derived distance restraints revealed a family of closely related ß-hairpin conformations having no significant restraint violations (Fig. 1). The RMSD to the mean structure

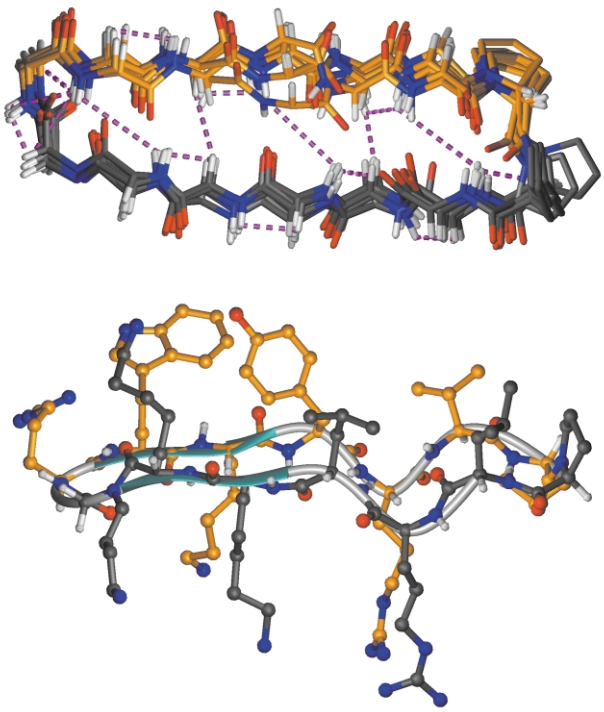

Fig. 1 *Upper*, 15 DYANA structures for **6**. The two sides of the ß-hairpin are colored grey and orange (N-atoms blue, O-atoms red), with the template on the right (compare with **6**) and side-chains omitted. The purple dots indicate key NOE distance restraints; *lower*, a typical NMR structure, again with the template right. The backbone is traced by the colored ribbon.

Table 1 Assays of antibiotic[a] activity and hemolytic activity[b]

	Peptide			
Test microorganism	**2**	**5**	**6**	**7**
E. coli ATCC25922	4	16	8	32
P. aeruginosa ATCC27853	4	8	4	8
S. aureus ATCC29213	4	32	24	32
S. aureus ATCC25923	4	64	64	64
% hemolysis at 100 μg ml^{-1}	37	1.4	0.5	1.4

[a] Antimicrobial activity is given as the minimal inhibitory concentration (MIC) in μg ml^{-1}, measured using the NCCLS broth microdilution method. [b] Hemolytic activity is the % lysis of fresh human red blood cells at a peptide concentration of 100 μg ml^{-1}.

after superimposition of backbone, and heavy atoms, of all residues was 0.75 and 1.52 Å, respectively. Cross-strand hydrogen bonds were present in many of the calculated structures for Leu[1] HN to Val[12] CO, and Val[12] HN to Leu[1] CO, from Leu[3] HN to Tyr[10] CO, as well as from Tyr[10] HN to Leu[3] CO (compare **1**). Many of the structures adopt a regular type-II′ ß-turn at the tip of the hairpin, with the *N*-aminobutylglycine (NBG) residue in the *i*+1 position.

The factors that stabilize ß-hairpins in peptides and proteins are currently attracting great interest. The importance of turn-forming residues at the tip of the hairpin,[8] as well as stabilizing cross-strand, side-chain to side-chain interactions[9] have been documented recently. In contrast to **5**, the regular hairpin structure deduced for **6** in water may reflect the enhanced stability of a II′-ß-turn at the hairpin tip in **6** due to the presence of the peptoid unit at the *i*+1 position,[10] a conclusion which may have general value in the design of other ß-hairpin peptidomimetics.

The antimicrobial and hemolytic activities (Table 1) of **6** and **7** were tested, as described in earlier work.[4] The assays revealed a slightly enhanced antimicrobial activity for **6** compared to **5**, whilst maintaining a much lower hemolytic activity against human erythrocytes compared to protegrin I (**2**). These results show that the presence of peptoid units in this family of mimetics is compatible with the selective targeting of bacterial cells over human erythrocytes. The chemical and biophysical properties of these and related peptoid-containing ß-hairpin mimetics will be the subject of future studies.

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