Stabilisation of β-hairpin conformations in a protein surface mimetic using a bicyclic template derived from (2*S*,3*R*,4*R*)-diaminoproline

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A trifunctional template, derived by formal coupling of (*S*)-aspartic acid and (2*S*,3*R*,4*R*)-diaminoproline (available from vitamin C) as a diketopiperazine, was incorporated by solid-phase peptide synthesis into a protein loop mimetic containing the sequence -Ala-Asn-Pro-Asn-Ala-Ala-; this was shown by NMR analysis to adopt a stable β-hairpin conformation in DMSO.

Many proteins exert their biological activity through interactions involving relatively small regions of their exposed surfaces. Small synthetic molecules that mimic surface features of proteins are therefore of potential interest in the design of novel drug candidates. Unlike folded proteins, however, short linear peptides are inherently flexible molecules. To overcome this problem, much attention has been focused recently on the design of templates to constrain peptide chains into biologically relevant secondary and tertiary structures.[1,2] We report here a novel bicyclic template, comprising a diketopiperazine derived from aspartic acid and (2*S*,3*R*,4*R*)-diaminoproline, which was designed to stabilize β-hairpin conformations, as typically found in protein loops connecting adjacent antiparallel β-strands.

In previous work,[3,4] we described the synthesis of template **1**, and its incorporation into the loop mimetic **2**, containing the NPNA (Asn.Pro.Asn.Ala) motif found in a tandemly repeated form in the circumsporozoite protein of *Plasmodium falciparum*.[5] Based on NMR and MD studies, we could show that **2** adopts a stable β-turn conformation within the NPNA motif, while the 4-amido N-atom in the 4-aminoproline moiety of the template prefers a pseudo-equatorial position,[3,6] as depicted in Fig. 1. Here, we set out to introduce an additional amino group in an axial position at the 3-position of the pyrrolidine ring, as in **3**, which could then be used as an anchoring group to more accurately position a peptide loop in a β-hairpin geometry, as in **4**.

A convenient gram-scale synthesis of (2*S*,3*R*,4*R*)-diaminoproline was established by exploiting a known route to the bicyclic β-lactam **5** from vitamin C, which has been implemented already on a multi-kilogram scale in a commercial synthesis of β-lactamase inhibitors.[7] As shown in Scheme 1, **5**

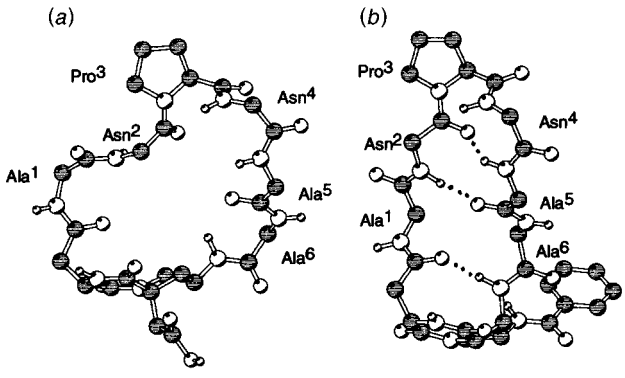
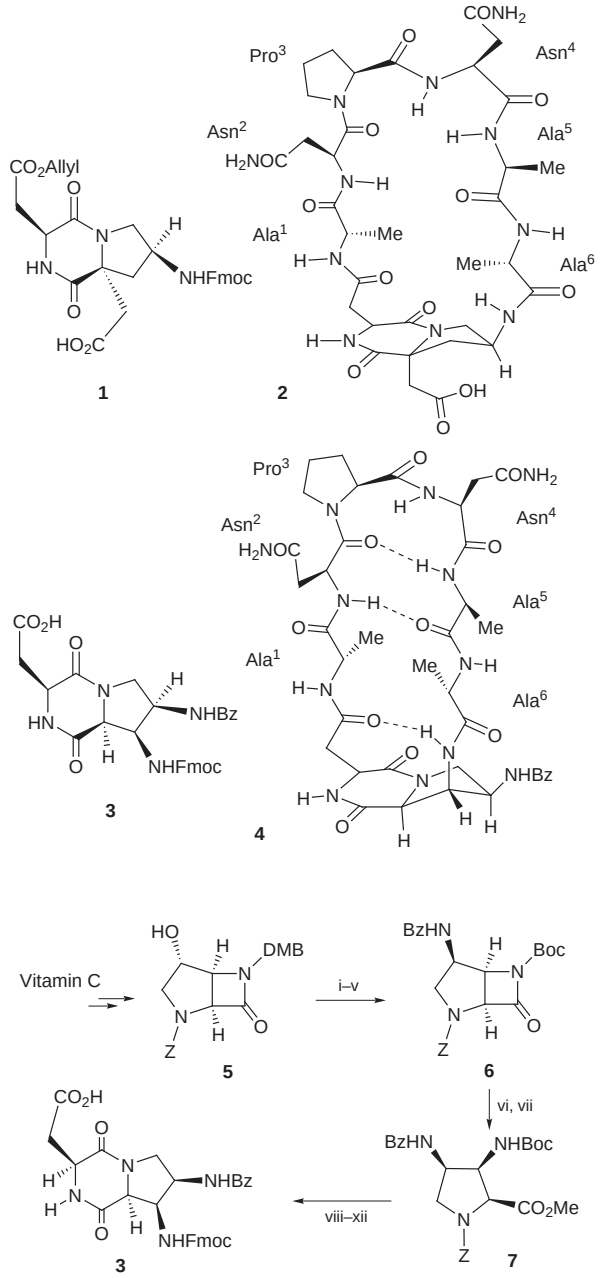


Fig. 1 Average solution structures of (*a*) **2** and (*b*) **4** deduced by SA (see text). The side-chains of Ala and Asn, and all hydrogen atoms apart from peptide NHs, are omitted for clarity. N, O and amide H atoms = white, C atoms = grey.



Scheme 1 *Reagents and conditions*: i, PhthH, Ph_3P, THF, DEAD (61%); ii, $MeNHNH_2$, DMF, 80 °C; iii, Bz_2O, Et_3N, CH_2Cl_2 (66% over 2 steps); iv, $K_2S_2O_8$, Na_2HPO_4, aq. MeCN, 78 °C (77%); v, Boc_2O, Et_3N, DMAP, CH_2Cl_2 (60%); vi, Na_2CO_3, aq. THF; vii, CH_2N_2, Et_2O (98% over 2 steps); viii, H_2, Pd/C, DMF (96%); ix, Z-Asp(OBu^t)-OH, HATU, HOAt, Pr^i_2EtN, CH_2Cl_2 (80%); x, H_2, Pd/C, DMF (100%); xi, TFA, CH_2Cl_2 (89%); xii, Fmoc-OSucc, Pr^i_2EtN, CH_2Cl_2 (60%)

Table 1 Temperature coefficients[a] ($-\Delta\delta/T$), H/D exchange rates[b] ($t_{1/2}$) for amide protons, and ^3J coupling constants[c] measured for **4**

			^3J/Hz			
Residue	$-(\Delta\delta/T)$/ppb K^{-1}	$t_{1/2}$/min	$^3J_{\alpha,NH}$	$^3J_{\alpha,\beta}$	$^3J_{\beta,\gamma}$	$^3J_{\gamma,\delta}$
Ala[1]	6.0	33	7.5	6.9	—	—
Asn[2]	3.2	280	7.6	5.0, 4.1	—	—
Asn[4]	1.4	350	9.4	10.3, 3.9	—	—
Ala[5]	0.0	260	7.0	6.8	—	—
Ala[6]	5.1	23	9.1	6.9	—	—
dApro[7]	1.9[d] 4.2[e]	$>10^4$ [d] 240[e]	8.9[d] 7.0[e]	3.5	4.3	10.1, 9.6
Asp[8]	5.1	4.5	<2.0	3.1, 4.1	—	—

[a] The temperature coefficients for the peptide amides are given; dApro[7] refers to the diaminoproline and Asp[8] to the aspartate moieties, respectively, of the template. Measurements were made in d_6-DMSO in the range 295–320 K. [b] The half-lifes ($t_{1/2}$) of amide resonances were determined by fitting residual peak intensities after dissolution in d_6-DMSO + 10% d_4-methanol to an exponential function. The exchange rates may be classified as: fast [Asp[8] NH and Asn[2]/Asn[4] side chain NHs (data not shown)]; medium (Ala[1], Ala[6]); slow [Asn[2], Asn[4], Ala[5], dApro[7] C(γ)-NH]; and very slow [dApro[7] C(β)-NH]. [c] Measured using 1D and/or E.COSY spectra. [d] For C(β)-NH. [e] For C(γ)-NH

was converted into **6** by a Mitsunobu reaction[8] and exchange of protecting groups, and the β-lactam ring was then opened to yield after esterification the orthogonally protected (2*S*,3*R*,4*R*)-diaminoproline derivative **7**. Thereafter, coupling to Z-Asp(OtBu)-OH, cyclisation to afford the diketopiperazine, and further manipulation of the protecting groups gave **3** in good overall yield.

The template **3** could be incorporated into the cyclic peptide **4** using standard solid-phase methods and Fmoc chemistry.[9] For example, **3** was coupled to Tentagel S-AC resin, and the peptide chain was then elaborated to afford H-Ala-Asn(Mtt)-Pro-Asn(Mtt)-Ala-Ala-Template-Resin. After cleavage from the resin with 1% TFA in CH_2Cl_2, the linear precursor was cyclized using HATU/HOAt‡ in DMF, and all side-chain protecting groups were then removed with TFA in CH_2Cl_2 (35:60) and TIPS (5% v/v). After purification by HPLC, the cyclic peptide **4** was obtained from **3** in 11% yield.

The preferred conformation of **4** was studied in d_6-DMSO (**4** has low solubility in water at pH 5) at 305 K, a temperature at which the amide NH protons are optimally resolved in 1D ^{1}H NMR spectra. A relatively stable β-hairpin conformation in the peptide backbone was indicated in NOESY spectra of **4** by NOEs connecting Ala[1] H(α) as well as Asn[2] NH with Ala[5] NH, which were not observed in earlier studies[3,6] of **2**. A β-turn in the NPNA motif was also indicated, in particular, by a relatively strong Asn[4] to Ala[5] d_{NN} NOE, as well as NOEs between Asn[2] H(β)s and Ala[5] NH, as observed in earlier studies[3,4,6] of **2**. Average solution structures were determined by dynamic simulated annealing§ (SA) using distance restraints derived from NOE build-up curves in a series of NOESY spectra with increasing mixing times. The SA structures showed no major distance restraint violations (*e.g.* >0.2 Å) and revealed a well defined β-hairpin backbone conformation, including a βI turn in the NPNA motif, as in the representative structure **4** shown in Fig. 1.

A critical test of the accuracy of the SA structures is to examine how well they also account for other experimental data, in particular, ^3J coupling constants, relative H/D exchange rates of amide protons, and amide proton chemical shift temperature coefficients. Structure **4** predicts intramolecular hydrogen-bonding across the hairpin, involving the C(β)-NH with O(δ) of the template, as well as Asn[2] NH with Ala[5] CO (indicated by dotted lines in **4** and in Fig. 1). We observe very low amide proton temperature coefficients for these two amide NH groups, as well as relatively slow amide NH exchange rates, measured in d_6-DMSO with 10% v/v d_4-methanol (Table 1), data which indicate the involvement of these NH groups in intramolecular hydrogen-bonding. In addition, the ^3J values for protons in the template, in particular within the diaminoproline moiety, show values consistent with the geometry found in the SA structures, with the C(β)-NH axial, and the C(γ)-NH equatorial. To a first approximation, therefore, the experimental data are interlocking and consistent with the derived SA structures, which indicate a significantly populated β-hairpin conformation in the backbone of **4**. The molecule should not be viewed as rigid, however, and MD simulations may provide a more detailed description of allowed conformational dynamics on the MD time-scale in this system.

Studies are now underway to determine how general this approach is to the construction of conformationally defined β-hairpin loop mimetics of diverse size and sequence. The amino functionality at C(γ) in the template may be useful in this context to allow its attachment to a solid-support for solid-phase syntheses, as well as for coupling to other carrier molecules.

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Notes and References

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‡ Abbreviations: HATU = *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate; HOAt = 1-hydroxy-7-azabenzotriazole; TIPS = triisopropylsilane.

§ The method used for SA calculations has been described in detail elsewhere (refs. 3 and 6).

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