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A novel azetidinyl γ-lactam based peptide with a preference for β-turn conformation†

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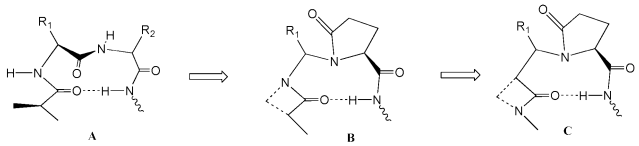
Novel azetidinyl γ-lactam based peptides 1–3 have been synthesized with only compound 1 showing a preference for the β-turn conformation.

The physiological role displayed by a large number of peptides has stimulated researchers all over the world to design and synthesize similar molecules, which are now collectively called peptidomimetics.[1] Natural peptides seldom can be used therapeutically as drugs because of the problems associated with low absorption, rapid metabolism and poor oral bioavailability.[2] Peptidomimetics, designed on the basis of modification of the natural sequence of amino acids in bioactive peptides, have the advantage of providing new functionalities that can circumvent these problems.[3] However, since small molecules are usually conformationally flexible and hence have to cross a considerable entropy barrier to adopt the bioactive conformation, it would be favourable to design molecules with rigid conformation. Rigidity in conformation can be achieved by introducing features in the structure that will induce the molecule to adopt a particular conformation.[4] An intriguing challenge in the design of peptidomimetics is the development of templates that stabilize structures resembling secondary structure motifs of peptides. Reverse turn mimetics[5] have so far been the prime target in this area. The localization of turns on the surface of proteins has led to the belief that these must play an important role in receptor–peptide recognition events.[6] The β-turn which is the most common in peptides, is a tetrapeptide sequence in a non-helical region, in which the distance between Cα(*i*) to the Cα ($i + 3$) is less than or equal to 7 Å and the donor/acceptor distance ($i + 3$)NH $\cdots$ OC(*i*) of the turn-stabilizing hydrogen bond (typically, 1.8–2.5 Å for H $\cdots$ O distance and 2.6–3.2 Å for N $\cdots$ O distance).[7] The induction of β-turns in non-peptide molecules is driven by the formation of such an intramolecular H-bond. Herein we report our investigation of the design, synthesis and conformational characterization of a novel azetidinyl γ-lactam based peptide that illustrates this concept. In addition, the identification of the structural parameters along with the stereochemistry involved in the stabilization of lactam-based peptidomimetics is also reported.

The natural β-turn is represented by structure **A** in which a 10-membered H-bond network is formed. Our idea to design the lactam-based peptidomimetic represented by **B** is based upon the consideration of the following: a cyclic moiety replaces a C(α)–N bond of the $i + 2$ amino acid, (ii) carbonyl of ($i + 1$) amino acid is excluded from the backbone and placed in the side chain and (iii) most importantly, connecting the N of ($i + 1$) amino acid and C(α) of amino acid (*i*) by a methylene bridge. An intramolecular H-bond as shown can then be visualized which will ensure the retention of the β-turn. An alternative structural design **C** can also be thought of in which the N of ($i + 1$) and C(α) amino acid interchange their positions. This should not disturb the H-bond and should retain the turn structure as conceived. Since structure **C** is more amenable towards synthesis, we had to work on its synthesis and to study its conformational characteristics.[8]



Before embarking on the actual synthesis, a conformational analysis was performed. Low-energy conformers were generated by molecular mechanics force field calculations using Spartan'04 VI 0.0 (Fig. 1).[9] Only the *trans* peptide was shown to adopt a conformation in which the glycine NH and the β-lactam carbonyl are within a distance of 1.813 Å, indicating strong intramolecular H-bonding possibility. No such preferences could be seen for the *cis* peptides.

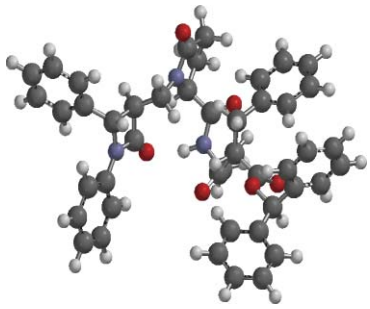


Fig. 1 Energy minimized conformation of **1**.

The synthesis of the peptides relied upon the availability of the 3-pyroglutamylmethyl β-lactam in *cis* and *trans* forms, preferably enanatiomerically pure. To prepare these compounds, the Kinugasa reaction[10] was our method of choice because of its mild conditions coupled with the easy access to various propargyl acetylenes used as the 2-electron component for these reactions. An asymmetric version of the Kinugasa reaction is also quite well known.[11] Our synthetic strategy is shown in Scheme 1. The propargyl ethyl pyroglutamate **6** was prepared and the Kinugasa reaction between **6** and the diphenyl nitrone **7** was carried out in CH_3CN solution in presence of triethylamine and cuprous iodide. Interestingly, the reaction produced three diastereomers: one *trans* isomer **8** and a pair of *cis* isomers **9** and **10**. It appears that only one of the *cis* isomer **10** has epimerized to the *trans* compound. The other *cis* isomer **9** is configurationally more stable and is resistant to epimerization. The two *cis* isomers had similar polarity on Si-gel and we decided to carry out the synthesis of the peptides with the mixture. However, elaboration

† Electronic supplementary information (ESI) available: Experimental procedures, ^{1}H NMR, ^{13}C NMR, VT NMR and NOESY spectra of representative compounds, energy minimized conformation of **1** and ORTEP diagram and X-ray crystallographic data for compound **11**. See DOI: 10.1039/b511029g

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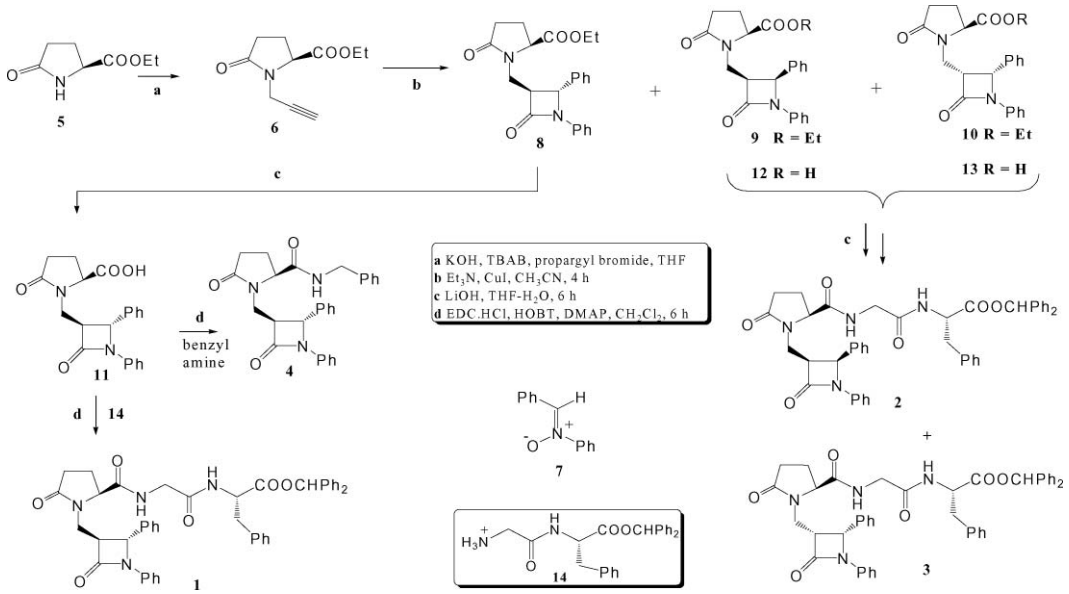

Scheme 1 Synthesis of the tripeptides **1–3**.

to the peptide was first carried out with the *trans* isomer as this was one pure diastereomer and for that, the ester in the pyroglutamic acid moiety needed to be hydrolysed. Initially, considering the sensitivity of β-lactams towards nucleophiles, enzymatic hydrolysis was considered; both porcine pancreatic lipase (PPL) and porcine liver esterase (PLE) gave poor yield of free acid **11**. Finally, LiOH in THF–H_2O[12] was found to provide the free acid **11** in acceptable yield (75%). Since the configuration at C-2 was prefixed as we have started from *S*-pyroglutamic acid and since the configuration at this centre remains unaltered as can be seen from the production of only one diastereomer upon coupling, the absolute configuration of the acid **11** could be ascertained from the single crystal X-ray structure (ORTEP diagram shown in Fig. 2).[13] The acid was then coupled with the C-protected dipeptide **14** in presence of EDCI–HOBT to afford the protected tripeptide **1** as a white solid. Similarly, the *cis* isomers were deprotected to the free acids **12/13** and were coupled to the free amine to prepare the protected tripeptides **2/3** which could only be separated by HPLC.

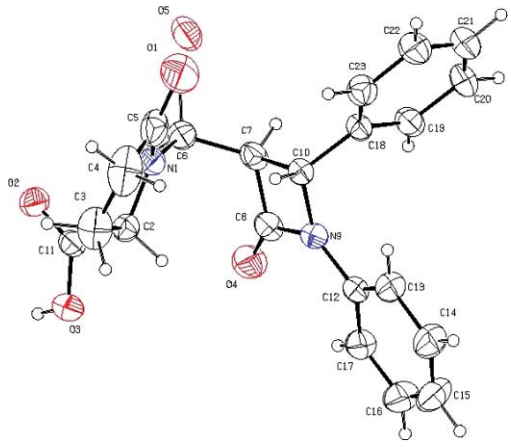

Fig. 2 ORTEP diagram of **11**.

The peptides were fully characterized by NMR and mass spectroscopy. In the ^{1}H NMR of the *trans* peptide **1** in $CDCl_3$, the two NH's were overshadowed by the aromatic signals, which were, however, clearly visible when the spectrum was recorded in d_6-DMSO. The glycine NH appeared as a triplet at δ 8.21 while the phenyl alanine NH appeared as a doublet at δ 8.27. The C-4 hydrogen in the azetidinone moiety appeared as a doublet at δ 4.88 with a coupling constant of 2.1 Hz characteristic of *trans* configuration. The *cis*-isomers behaved similarly with the NH's appearing as triplet and doublet in the most downfield region. C-4 hydrogen expectedly appeared as a doublet with a characteristic coupling constant of 6 Hz.

The tripeptide model compounds, namely the *trans* and the corresponding *cis* isomers, were studied by ^{1}H NMR spectroscopy to measure the temperature coefficients of the two NH protons. This method has become a useful tool to determine the presence of intramolecular H-bonding. The temperature coefficients of the various NH's are listed in Table 1. In the *trans* peptide **1** the temperature coefficient NH of glycine is the lowest and is close to the Kessler limit[14] of 3 ppb suggesting strong intramolecular H-bonding. That the phenyl alanine-NH has a higher temperature coefficient than the glycine-NH can be clearly seen. With a rise in temperature the phenyl alanine-NH signal shifted upfield at a higher rate and ultimately crossed the signal for the glycine NH (Fig. 3). For both the *cis* tripeptides **2** and **3**, the temperature coefficients of chemical shifts for all the NHs are above 5.0, which indicate the absence of a definite β-turn motif in these systems. Thus, it seems that while a β-turn like-conformation is preferred in *trans* peptide **1**, no such preferences could be seen in the *cis* peptides **2** or **3**. Hence the stereochemistry of the β-lactam ring plays a key role in controlling the conformation of the peptides. For the *trans* peptide **1**, in order to know which carbonyl is involved in intramolecular H-bonding, the truncated amide **4** was synthesized. In this case, intramolecular H-bonding is present (temperature coefficient is 4.8 ppb) although to a lesser extent as compared to the tripeptide **1**. Presence of some degree of intramolecular H-bonding indicates that the β-lactam carbonyl participates in intramolecular H-bond with the glycine amide NH.

Table 1 NMR Temperature coefficients of NH chemical shifts in DMSO-d_6[a]

Compound no.	NH Glycine	NH Phenylalanine	NH Benzylamine
1	3.4	5.6	
2	6.0	5.7	
3	6.2	6.0	
4			4.8

[a] $\Delta\delta/\Delta T$ (ppb K^{-1}).

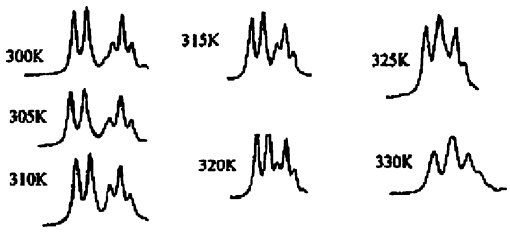

Fig. 3 ^{1}H-NMR signals of phenylalanine and glycine NHs of peptide **1** at various temperatures.

We have thus successfully designed and synthesized a new class of azetidinyl γ-lactam-based peptide. From VT-NMR experiments it has been shown that the peptide with *trans* stereochemistry in the azetidinone moiety adopts a β-turn like conformation unlike the corresponding *cis* isomers for which the NHs are either mostly intermolecularly H-bonded or exposed to the solvent. Current attempts are aimed towards making the water-soluble analogs of **1** suitable for study under biological conditions.

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