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DESIGN OF A NATURAL *cis* PEPTIDE BOND MOTIF TO FORM TYPE VI β-TURN MIMETIC

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Abstract: Starting from a pyro-glutamic acid residue, which has a natural *cis* amide bond motif, and extending the chain appropriately from its C^{γ} atom, a type VI β-turn mimetic can be designed. The stability of the model can be enhanced by making a spiro compound linking the C^{γ} atom to a previous N in the extended chain. Three out of a possible four values defining the β-turn are constrained by local cyclisations giving a well defined and rigid model for a type VI β-turn.

The occurrence of *cis* peptide bonds in peptides[1] and proteins[2] has been receiving increasing attention recently. Methods have been proposed and tested to synthesize peptides with this characteristic[3]. Here we present the design of a peptide with a mandatory *cis* peptide unit and with specific secondary structural features. This follows directly from earlier studies[4] in this laboratory which showed that peptides with bridged γ-lactams and N-terminal γ-lactams have unusual properties in terms of the constraints imposed on them by the lactam rings on the two degrees of conformational freedom ϕ and ψ. These studies indicated that (a) in a bridged γ-lactam system, the conformational parameter ψ is constrained to a value near -120° for an L -residue and +120° for a D-residue and (b) in a pyro-glutamic acid residue (Glp), which is an N-terminal γ-lactam the conformational parameter ϕ is constrained to around +120° for a L-residue and -120° for a D-residue.

Here the results of these two studies are combined and extended to propose a type VI β-turn model. As a Glp residue (figure 1) is characterized by a *cis* amide bond in the ring, the problem is to ascertain whether starting from such a motif the peptide chain can be extended appropriately in both directions to produce the desired secondary structural characteristics. This can be done by visualizing the $C^{\gamma}C^{\delta}O^{\epsilon}NHC^{\alpha}$ of a Glp residue as a peptide unit $C^{\alpha}CONHC^{\alpha}$ with the sidechains at both the C^{α}'s being fused at C^{β}. Now, depending on the way the chain is extended at the C^{γ} of Glp (C^{α}_{2} say) and C^{α} of Glp (C^{α}_{3} say), viz. depending on the chirality at these C^{α}'s, different conformational characteristics are obtained.

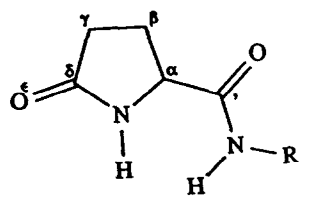

Figure 1 : A pyroglutamic acid (Glp) residue. The $C^{\gamma}C^{\delta}O^{\epsilon}NHC^{\alpha}$ unit can be thought of as a dipeptide system $C^{\alpha}_{2}CONHC^{\alpha}_{3}$ with the side chains of C^{α}_{2} and C^{α}_{3} being fused at C^{β}.

These can be easily derived [4] and figure 2 shows all possible combinations of this central motif along with their torsion values about the C_2^α-C bond (ψ_2) and N-C_3^α bond (ϕ_3). The logical extension is to see which of these units with obligatory *cis* peptide bonds forms interesting secondary structures.

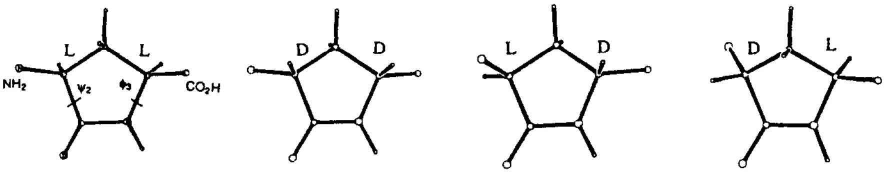

Figure 2 : The peptide chain can be extended at C_2^α to NH_2 and at C_3^α to CO_2H and can have four possible combinations of torsions about C_2^α-C′ (ψ_2) and N-C_3^α (ϕ_3) bonds depending on the chirality at C_2^α and C_3^α This follows from the fact these torsions (ψ_2 and ϕ_3) are also defined within the ring and are constrained to be close to 0° Therefore when defined as part of the peptide backbone, ψ_2 and ϕ_3 will have values close to ±120° depending on the chirality at C_2^α and C_3^α. For combinations of chirality at C_2^α and C_3^α given by (L,L), (D,D),(L,D) and (D,L); the (ψ_2,ϕ_3) values are (a) (-120°,120°), (b)(120°,-120°), (c) (-120°,-120°) and (d) (120°,120°) respectively

β-turns are stable secondary structural features found in proteins and peptides defined over a length of four amino-acid residues, encompassing a tripeptide unit, which cause a reversal in the direction of the peptide chain[5]. Conformationally, these turns are defined by the (ϕ,ψ) values of the two central amino-acid residues. In the case of small peptides (like naturally occurring hormones)β-turns have been implicated in the bio-active structure[6]. Consequently there has been a great deal of interest in making β-turn mimetics [7] especially of the well known β-turns (called types I,II,III and their inverses) which are characterized by all *trans* peptide bonds. However with the recent increase in reports of β-turn conformations in small peptides with a *cis* peptide bond between the middle two residues, interest has also focused on such *cis* structures Earlier analysis[8] of amino-acid sequences in proteins containing a *cis* peptide bond has identified two β-turn types called VIa and VIb for which approximate conformational parameters (ϕ_2,ψ_2) and (ϕ_3,ψ_3) were defined. More recently[9], crystal structures of small linear and cyclic peptides have revealed that conformational parameters (ϕ_2,ψ_2),(ϕ_3,ψ_3) are closer to (-60,120), (-120,60) for type VIa and (-100,120), (-120,80) for type VIb. We have used this definition in the paper.

Clearly, from figure 2 the cases represented by (a) and (b) may be able to form β-turns as their constrained dihedral angles ψ_2 and ϕ_3 match approximately the required value for a type VI β-turn or its inverse. In particular case (D,D) given in figure 2(b) has conformational parameters close to ψ_2 , ϕ_3 of a type VIa β-turn.

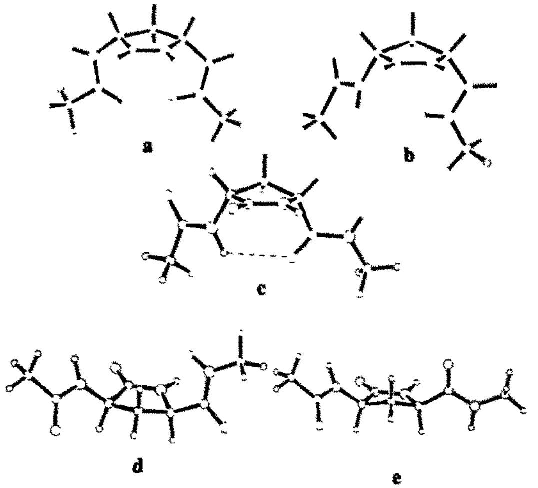

Figure 3 : Conformational searching using molecular dynamics simulations of a tripeptide system which included case (D,D) of figure 2 revealed five minima within 2.5 kcal/mol of each other. The simulations were performed from three different starting points for a total time of 150 picoseconds. The minimised conformations (a)-(e) are given in decreasing order of energy. Conformation (a) has (φ,ψ) values typical of type VIa β-turn. From the relative dispositions of the hydrogen substituents on C^{α}_{2} and the N preceding it it is clear from the figure that only for conformation (a) can a spiro compound at C^{α}_{2} be made.

Indeed conformational searching using molecular dynamics simulations (with a valence force field[10]) on a tripeptide which included this case (i.e. D,D) with its obligatory *cis* peptide motif indicates that one of the five minima located is in a type VIa β-turn with its attendant 4->1 hydrogen bond as shown in figure 3.

An interesting extension to this is that only in the type VIa β-turn conformation (figure 3a) can a spiro compound be made which connects the C^{α}_{2} and the previous N by a five-membered ring. Such a conformation in its minimised form is shown in figure 4. This molecule is expected to confer additional conformational stability as the torsion angle about N-C^{α}_{2} (i.e. ϕ_2) is also constrained and is close to the required value of a type VIa β turn or its inverse as the case may be. In this system 3 out of the 4 (φ,ψ) angles needed to define the β-turn i.e. ϕ_2 , ψ_2 and ϕ_3 have restricted values owing to local cyclisations. These conformational parameters in the minimised conformation (figure 4) are ϕ_2= -65.3°, ψ_2= 132°; ϕ_3= -121° and ψ_3= 71°, which is in excellent agreement with crystal structures of small peptides[9].

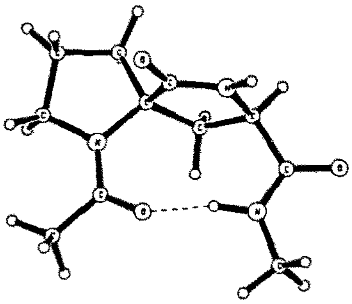

Figure 4 : Minimised conformation showing a spiro compound from figure 3a as a type VIa β-turn mimetic.

Thus, an effective type VI β-turn mimetic can be made starting from a Glp residue which is a natural motif for a *cis* peptide bond and extending it appropriately from its C^{γ} atom. Such a system will have torsion angles conducive to form type VI β-turns. The stability of the system can be enhanced by making a spiro compound.

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