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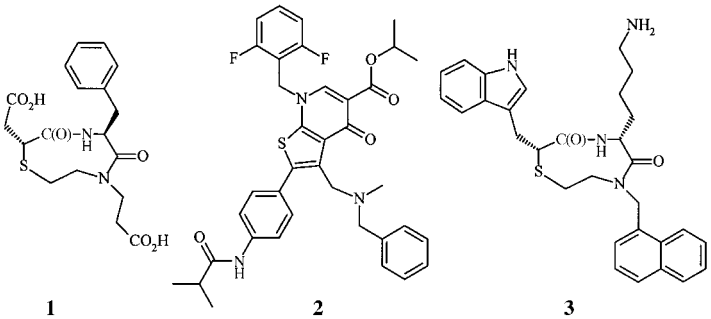
Are β-Turn Mimetics Mimics of β-Turns?

Gerhard Müller,* Gerhard Hessler, and Helene Y. Decornez

Dedicated to Dr. Pol Bamelis on the occasion of his 60th birthday

At the molecular level almost all important physiological and pathophysiological processes in the human organism are modulated by peptide – protein and protein – protein interactions. Consequently for pharmaceutical research these recognition phenomena represent attractive target systems for therapeutic intervention in a broad spectrum of disease states.[1] From the viewpoint of structural chemistry β-turns have been attributed a special importance as carriers of molecular recognition since within the context of pharmacophore arrangement they allow a sterically controlled presentation of two to four interaction-mediating amino acid side chains.[2] In medicinal chemistry the retention of the orientation of the amino acid side chains with simultaneous replacement of the peptide backbone by nonpeptide druglike structures, so-called "β-turn mimetics", has progressed to become an established topic in the structural design of peptide mimetics.[3]

The relevance of the design element "β-turn" with variable decoration in the molecular periphery has recently been convincingly confirmed by combinatorial chemistry and classical medical chemistry. This work resulted in, for example, high-affinity and selective peptidomimetic $\alpha_4\beta_1$-integrin antagonists such as **1**,[4] LHRH antagonists such as **2**,[5] and somatostatin antagonists such as **3**.[6]



1 **2** **3**

Here we take up the problem of the conformational compatibility of β-turn mimetics with the secondary structural elements that are to be imitated, and we introduce a simulation procedure with which is possible to evaluate not

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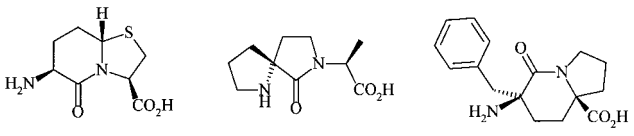
only the compatibility, but also the turn-inducing potential of a designed β-turn mimetic. This evaluation can be made in advance of a possibly cumbersome chemical synthesis.

We have investigated a series of β-turn mimetics with respect to the preferred conformation by deterministic (molecular dynamics, MD) and stochastic (Monte Carlo, MC) molecular mechanics simulation procedures. All dipeptide mimetics were built into the $i+1-i+2$ turn position of a cyclic hexapeptide analogue (Figure 1) that already exhibited a tendency to form and stabilize a β,β-turn pattern because of its remaining tetrapeptide sequence (-Ala-D-Ala-Ala-Ala-). It is known from cyclic hexapeptides of this structural type that amino acids in the D-configuration preferably occupy the $i+1$ position of βII′ turns and thus induce the corresponding turn pattern.[7]

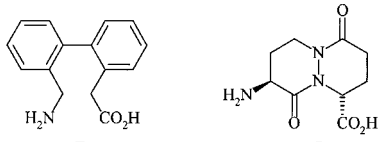
Figure 1. Construction principle of the cyclic hexapeptide analogues cyclo(-βTM-Ala-D-Ala-Ala-Ala) with which the β-turn mimetics were investigated by MD simulation for structural compatibility. βTM (β-turn mimetic) indicates the position of the mimetic; all starting conformations were generated so that the illustrated β,β-turn pattern was preinduced.

By MD simulation over 100 ps the stability of the β,β-turn pattern was investigated under explicit treatment of an aqueous solvent environment for the turn mimetics under discussion.

The unexpected conformational heterogeneity of dipeptide modules described as β-turn mimetics, as identified by the methods introduced here, is demonstrated on the basis of compounds **4**–**8**. Whereas the β,β-turn pattern remains stable over the trajectory of the MD simulation for mimetics **4**–**6** and **8**, analysis of the MD simulation of the *ortho,ortho'*-disubstituted biphenyl derivative **7** reveals a significant incompatibility with the turn geometry that should actually be stabilized (Figure 2).



4 **5** **6**



7 **8**

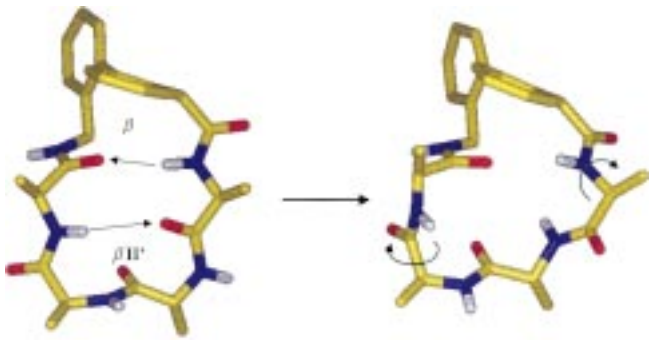


Figure 2. Starting conformation (left) of the cyclic hexapeptide analogue cyclo(7-Ala-D-Ala-Ala-Ala with a β,β-turn structure (transannular hydrogen bonds are indicated as arrows) that was relinquished during the MD simulation. The mimetic **7** clearly destabilized the regular turn conformation in favor of the conformation illustrated on the right. The rotations around bonds indicated by arrows (right) lead to the loss of the βII′-turn in the lower part of the molecule.

To obtain a differentiated image of the conformational characteristics of the turn mimetics investigated here the potential for turn induction (adequate requirement) of each mimetic was investigated by computer methods in addition to the compatibility (necessary requirement for a mimetic). The preferred conformations of all dipeptide analogues that were inserted as a central building block into the model linear peptide sequence Ac-Ala-Ala-βTM-Ala-Ala-NHMe was investigated by MC-based simulated annealing protocol. Thus, the direct consideration of the influence of a minimal flexible peptidic environment is guaranteed. The stochastic scanning of the conformational space spanned by all rotatable bonds gave for each turn mimetic a conformer ensemble consisting of 1000 structures, which was analyzed on the basis of geometric turn diagnostic parameters (Figure 3).

The distribution profile determined from the conformer ensemble, illustrated in Figure 3, clearly shows that the potential for turn induction decreases in the order **6** > **5** > **4** > **8** > **7**. In terms of turn induction, the β-turn mimetic BTD **4** (bicyclic turned dipeptide), the "classic" of all mimetics, was actually surpassed by the spiro compound **5** and the βVI turn mimetic **6** ($i+1-i+2$ *cis*-amide), which may be seen from the sharp maxima of the distribution profile of the geometric descriptors analyzed (Figure 3). The structural convergence in the turn region of mimetics **5** and **6** derived from the MC calculations is illustrated by the conformation families shown in Figure 4.

The conformational analysis of the hydrazide derivative **8** based on MC gives an extremely interesting and unexpected finding: Apart from the only moderately populated conformation family corresponding to the design in which the mimetic occupies the central positions ($i+1-i+2$) of the β-turn being mimicked, a second, clearly energetically more favored, preferred conformation was identified. In this conformation **8** stabilizes a regular β-turn, which in the basic turn pattern is displaced by a backbone position in the direction of the N terminus (Figure 5).

A comparison of the distribution profile for the topology that should actually be stabilized (**8** in Figure 3) with the distribution profile of the arrangement displaced by one

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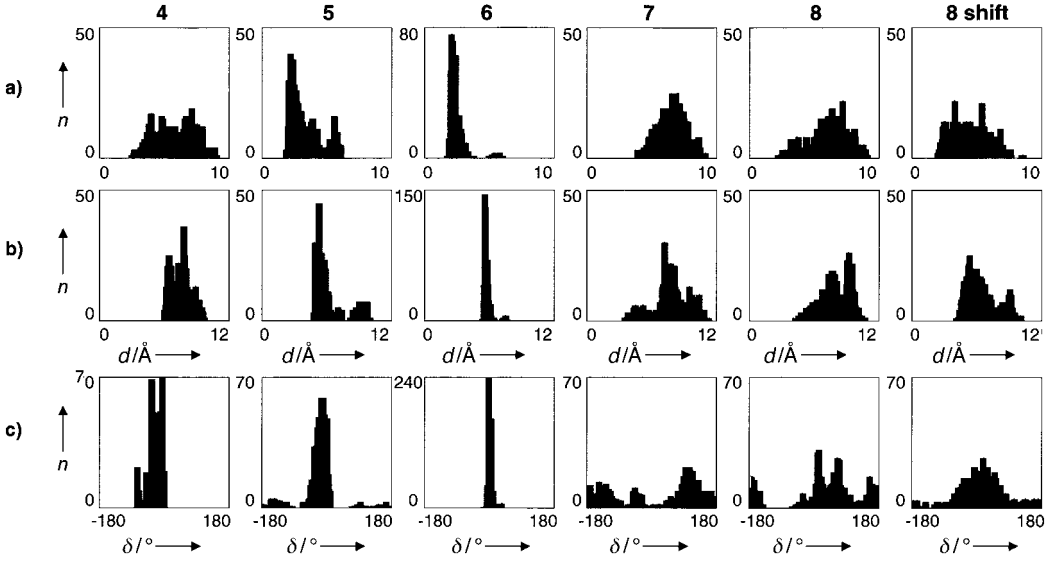

Figure 3. Statistical evaluation of the conformation ensemble produced by MC simulation. The following distribution profiles are illustrated: a) donor–acceptor distance ((HN_{i+3})–$(C{=}O_i)$) of the turn-stabilizing hydrogen bond;[9] b) the $C\alpha_i$–$C\alpha_{i+3}$ distance characteristic of β-turns (ideal value: 4.1–4.8 Å), according to Lewis et al.;[10] c) pseudodihedral angle encompassing four consecutive C_α atoms of the turn-forming amino acids or dipeptide analogues (ideal value: $50^\circ > \Theta > -50^\circ$). The ideal values are taken from the geometries of known β-turns[2a] (βI, βI′, βII, βII′, βVia, βVib).

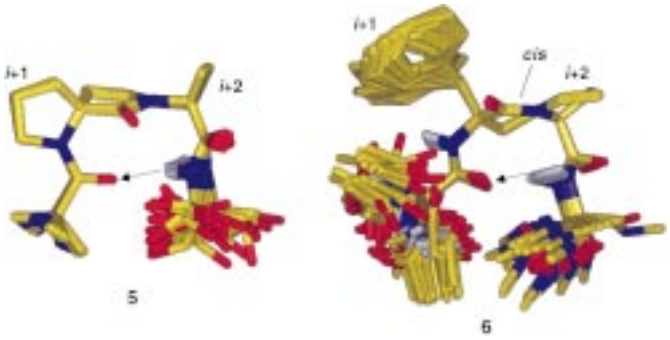

Figure 4. Superimposition of representative examples from the most highly populated conformation families derived from MC calculations for the β-turn mimetics **5** (left) and **6** (right) built into the model peptide. The reference atoms for the superimposition are the heavy atoms of the peptide-analogous backbone of the mimetic, including the atoms of the inwardly and outwardly directed amide bond. For reasons of clarity only the heavy atoms (C: yellow, N: blue, O: red) and amide protons (white) are shown.

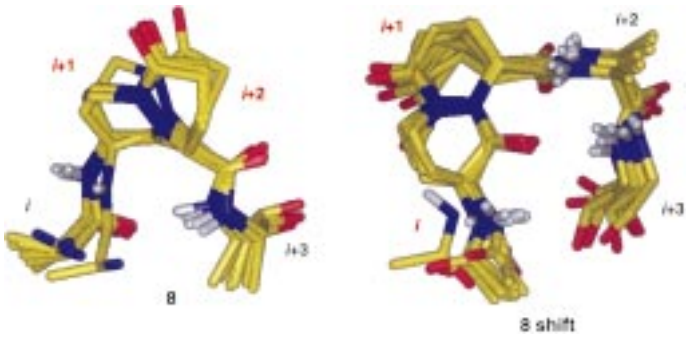

Figure 5. Structural comparison of the turn topologies identified by MC and stabilized by the mimetic **8**. In the less populated conformation family (left) the mimetic adopts the central position ($i+1-i+2$) of the β-turn, whilst in the most highly populated family (right) the turn positions $i-i+1$ are imitated by the mimetic.

position (**8shift** in Figure 3) suggests that the latter geometry is the more plausible turn arrangement (Figure 5).

In view of the results obtained from the simulation procedures for the predictive assumption of the turn-inducing potential, we suggest that the design of potential turn mimetics should be supported by the computer methods described here, for clearly not all of the compounds catagorized as β-turn mimetics are to be described as such. The value of the simulation procedure lies in the early identification and elimination of "false-positive" β-turn mimetics, that is, those compounds which were designed as turn mimetics but exhibit no corresponding compatibility in a three-dimensional structural context. Moreover, the molecular modeling method described here is suitable for the ranking of structure mimetics that in recent years have become established as attractive peptide mimetic templates for the rational design of combinatorial libraries but are to some extent cumbersome to synthesize.

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