# Theoretical conformational analysis of acetyl-alanine–methylamide double bond and methyl-substituted double-bond isosteres of the peptide bond

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## 1. INTRODUCTION

In [1–3] the replacement of the peptide bond by an E-carbon–carbon double bond has been described. This isosteric replacement was introduced to minimize the enzymic degradation of the peptide bonds involved and to increase lipophilicity of the peptide, which should facilitate the passage through the blood–brain barrier [1]. The replacement was proposed on the basis of the similar geometry of the *trans*-amide and E-alkene bonds [1].

Here, we report a comparative theoretical analysis of the conformational properties of E-alkene bonds and amide bonds in dipeptide units. We show that dipeptides with *trans*-amide and with E-alkene bonds, occupy the same conformational space. The double bond–dipeptide unit conformations however, are spread more evenly over the allowed space, thereby showing a greater conformational mobility in comparison to the amide bond–dipeptide unit.

Furthermore, we investigated the effect of substitution of the alkene bond by a methyl group, thus introducing a bulky substituent at the site of the amide oxygen atom. This analogue shows an increased preference for the α-helix region as compared to the amide dipeptide unit, and its incorporation into peptide molecules opens interesting perspectives in the design of conformationally constrained analogues.

## 2. EXPERIMENTAL

Conformational energy maps were calculated in 20° steps of $\Phi$ and $\Psi$ torsion angles (fig.1, fig.4). The $\omega_1$ and $\omega_2$ angles were fixed at 180°. Angles are defined according to IUPAC-rules [4].

The geometrical parameters adopted are those proposed [5] for polypeptides. Geometrical data for the double bond moiety are from [6]. The semi-empirical conformational calculation method used is extensively described in [7].

Values for the fractional charges on the atoms, used in the conformational energy calculations, were obtained by ab initio methods. The ab initio calculations were performed by the Gaussian 70 program [8] using Pople's minimal STO-3G basis set [9,10].

## 3. RESULTS AND DISCUSSION

As a model for the incorporation of double bonds in peptides, we chose the dipeptide unit *N*-acetyl-Ala[$\Psi$(CH=CH,*trans*)]NHMe (fig.1).

The conformational energy for each $\Phi,\psi$ pair was calculated. With these energies the population of each $\Phi,\psi$ conformation at room temperature was calculated according to a Boltzmann distribution. A population diagram was thus established as shown in fig.2. Fig.3 gives the same diagram for *N*-acetyl-Ala–NHMe for comparison.

The same regions of the diagram are occupied for the *N*-acetyl-Ala–NHMe and for its double-bond isostere. For the latter, the energy differences between the different allowed conformations are

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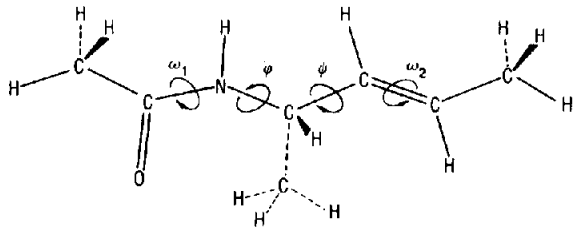

Fig.1. Structure of *N*-acetyl-Ala[Ψ(CH=CH,*trans*)]-NHMe.

levelled, and therefore the conformations in the allowed regions of the Ramachandran map are more evenly populated in the case of the double-bond isostere than in the case of the amide. This can be explained in terms of steric interactions. In the double-bond derivative, the oxygen is replaced by a proton, which has a much smaller steric volume and so allows a greater conformational freedom. Since the atomic charges on the carbonyl and the NH entities which produced an electronic constraint in the Ac-Ala—NHMe derivative, have practically disappeared in the double-bond isostere, this also results in a loss of conformational rigidity.

These calculations show that wherever the electronic properties of carbonyl and NH groups are not necessary for interaction with the receptor or another target molecule (e.g., by hydrogen bonding), one can replace the amide bond by an E-alkene bond without loosing the conformational properties of the system.

To reduce the conformational flexibility of the double-bond analogue, one can introduce a substituent to mimic or accentuate the steric role of the oxygen atom in amide bonds. Analogous calculations were therefore performed on the methyl-substituted double-bond analogue (fig.4).

The conformational population diagram for the *N*-acetyl-Ala[Ψ(C(CH$_3$)=CH,*trans*)]NHMe isosteric analogue is shown in fig.5.

The difference with Ac-Ala—NHMe is rather striking, as could be expected from the replacement of an oxygen atom by the sterically more bulky methyl group. We see that the A-region of the map ($\Phi$, $-100° \rightarrow -40°$; $\Psi$, $-80° \rightarrow -20°$) is much more populated than for Ac-Ala—NHMe, with a population of $>50\%$ ($\sim 1.4\%$ population for this region in Ac-Ala—NHMe. This dipeptide unit thus has a distinct preference for the $\alpha$-helix conformation. This important feature of this derivative is of great importance for the construction of constrained peptide molecules, especially for peptides where $\beta$-turn-type conformations are important for activity.

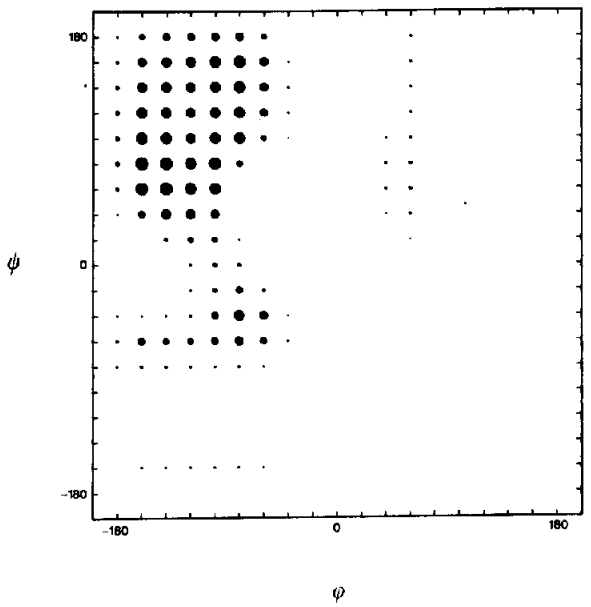


Fig.2. Population diagram for the $\Phi,\Psi$ conformers of *N*-acetyl-Ala[Ψ(CH=CH,*trans*)]NHMe at room temperature (298 K). The diameter of the black circles is proportional to the population for each conformer. Notice the similarities with the diagram of fig.3 for *N*-acetyl-Ala—NHMe.

Since a $\beta$I-turn is characterised by the stereochemical code '*ae*' for the second and third residues in the turn, one can expect that a peptide where the dipeptide unit with methylated double bond is incorporated as the $i+1$st and $i+2$nd residue, will have strong $\beta$I turn forming tendencies. One can also form a type III $\beta$-turn where the methylated double bond is between the 2nd and 3rd residue, or between the 3rd and 4th residue of the turn, since this turn is characterised by the conformation '*aa*' for the central residues.

Incorporation of such 'forced $\beta$-turns' into biologically active peptides is of much interest. Such reverse turns have been suggested [11] to provide recognition sites for proteins and peptides in their interaction with receptors. The systematic incor-

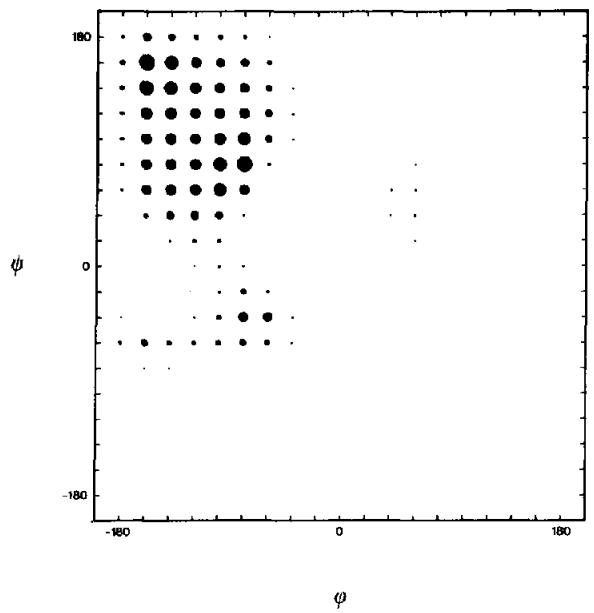


Fig.3. Population diagram for the Φ,Ψ conformers of *N*-acetyl-Ala–NHMe at room temperature.

poration of isosteric modifications of peptide bonds is also a valuable tool in receptor mapping, especially if these modifications bring along conformational constraints [12]. In this respect the methylated double-bond analogues are complementary to α-methylated amino acids [12].

For example, the incorporation of methylated double-bond analogues in enkephalin, would allow us to test various proposed hypotheses.

Since the $Gly^2CO$ in enkephalin is important, but not essential for biological activity, its role being to guide the $Phe^4$ and $Met^5$ sidechains to their binding sites [13], one can replace that peptide bond by a methylated alkene bond (not shown).

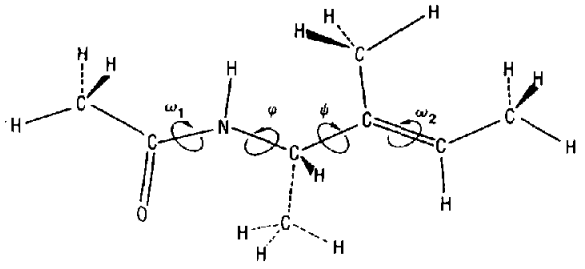


Fig.4. Structure of *N*-acetyl-Ala[Ψ(C($CH_3$)=CH,*trans*)]NHMe.

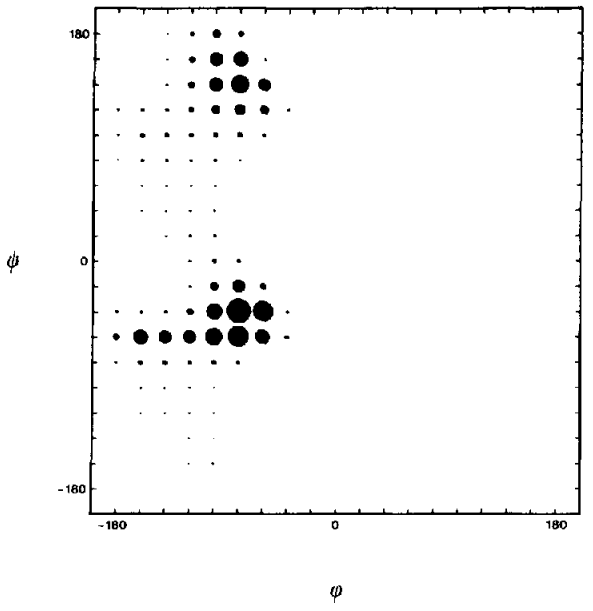


Fig.5. Population diagram for the Φ,Ψ conformers of *N*-acetyl-Ala[Ψ(C($CH_3$)=CH,*trans*)]NHMe at room temperature. The α-helix region (lower left) is much more populated than in the case of *N*-acetyl-Ala–NHMe. Conformations are much more constrained to this region of the diagram.

Hereby, the Tyr–Gly–Gly–Phe segment is forced into a βI-turn. A βI-type turn has been proposed by different authors as one of the possible conformational features of enkephalins [14–16], and is the only proposed reverse-turn structure that is in agreement with findings from cyclic enkephalin derivatives [17,18]. The proposed enkephalin derivative should make it possible to assess whether or not a βI-type turn on Tyr–Gly–Gly–Phe is involved in the receptor conformation of enkephalin.

Work in this direction is underway in our laboratory.

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