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# *N*-Methyldehydroamino acids promote a configuration *cis* of *N*-methylamide bond

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**Abstract**

Dehydroamino acids with a methylated N-terminal tertiary amide bond occur in natural small cyclic peptide toxins. To investigate their conformational preferences a systematic theoretical analysis was performed on *N*′-methylamides of *N*-acetyl-*N*-methyldehydroamino acids (Ac-Δ(Me)Xaa-NHMe, where Xaa = (*Z*)-Abu, (*E*)-Abu, Val, (*Z*)-Phe, and (*E*)-Phe) considering the configuration *trans* and *cis* of the tertiary amide bond. The $\phi$, $\psi$ potential energy surfaces were calculated at the B3LYP/6-31+G**//HF/3-21G level with inclusion of the solvent (water) effect (SCRF method). The conformers localised were fully optimised at the B3LYP/6-31+G** in vacuo. The accessible areas of the potential energy surfaces; the number of conformers and the stabilising internal forces were compared for all the studied molecules. The main feature of the studied *N*-methyldehydroamino acids is their considerable tendency to adopt the configuration *cis* for the N-terminal tertiary amide bond. It results from the specific ability of these dehydroamino acids to be able to gain stability from the π-electron conjugation between the $C^{\alpha}{=}C^{\beta}$ double bond and the neighbouring C-terminal amide group. This stabilising force concomitant with the short N—H···N hydrogen bond makes the conformer *cis* $C7_{eq}$ ($\varphi$, $\psi \sim -105°$, 8°) the lowest in energy. The data presented hereinto indicates that *N*-methyldehydroamino acids can potentially be new promoters of *trans–cis* isomerisation of the amide bond.


*Keywords:* N-Methylation; Dehydroamino acids; Tentoxin; Microcystin; Nodularin; *trans–cis* isomerisation; Potential energy surface

## 1. Introduction

Dehydroamino acids are frequent structural modifications of both main and side peptide chains [1,2]. Amongst naturally occurring dehydroamino acids there is a subgroup which have a methylated N-terminal amide bond. They can be defined, in short, as *N*-methyldehydroamino acids. *N*-Methyldehydroalanine, Δ(Me)Ala, and *N*-methyl-(*Z*/*E*)-dehydrobutyrine, (*Z*/*E*)-Δ(Me)Abu, are primarily components of microcystins and nodularins; families of toxins produced by cyanobacteria (blue-green algae). These hepatoxins constantly attract a great interest as blooms of many cyanobacterial species commonly occur in reservoirs of drinking water and present a hazard for public health safety [3–10]. *N*-Methyl-(*Z*)-dehydrophenylalanine, (*Z*)-Δ(Me)Phe, is a component of tentoxin produced by several phytopathogenic fungi of the genus *Alternaria* that can be found for example, in naturally contaminated tomatoes, both home grown and from supermarkets [11,12]. It seems to be a rule that *N*-methyldehydroamino acids occur in natural small cyclic peptide toxins.

*N*-Methyldehydroamino acids play an important role for the biological action of these toxins. Microcystin-LR with *N*-methyldehydroalanine reveals considerably greater toxicity than that contained in a non-methylated dehydroalanine [13]. *N*-Methyl-(*Z*)-dehydrophenylalanine is indispensable when adopting a biologically active conformation of tentox-

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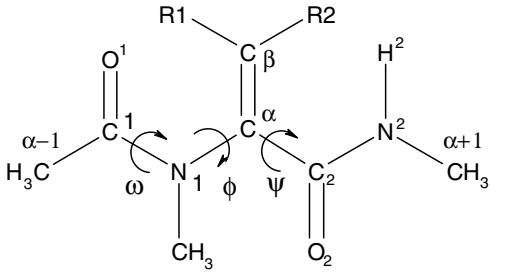


| | | |
|---|---|---|
| R1 = Me, R2 = H | ω ~ 0° | *cis*-Ac-(*Z*)-Δ(Me)Abu-NHMe |
| | ω ~ 180° | *trans*-Ac-(*Z*)-Δ(Me)Abu-NHMe |
| R1 = H, R2 = Me | ω ~ 0° | *cis*-Ac-(*E*)-Δ(Me)Abu-NHMe |
| | ω ~ 180° | *trans*-Ac-(*E*)-Δ(Me)Abu-NHMe |
| R1 = R2 = Me | ω ~ 0° | *cis*-Ac-Δ(Me)Val-NHMe |
| | ω ~ 180° | *trans*-Ac-Δ(Me)Val-NHMe |
| R1 = Ph, R2 = H | ω ~ 0° | *cis*-Ac-(*Z*)-Δ(Me)Phe-NHMe |
| | ω ~ 180° | *trans*-Ac-(*Z*)-Δ(Me)Phe-NHMe |
| R1 = H, R2 = Ph | ω ~ 0° | *cis*-Ac-(*E*)-Δ(Me)Phe-NHMe |
| | ω ~ 180° | *trans*-Ac-(*E*)-Δ(Me)Phe-NHMe |

Fig. 1. General formula, atom numbering, and selected torsion angles of the dipeptides studied in this work.

ine [14,15]. Interestingly, naturally occurring isotentoxine, which contains *N*-methyl-(*E*)-dehydrophenylalanine, (*E*)-Δ(Me)Phe, has no or only a very small bioactivity [16]. It should be noted, that *N*-methyl-(*Z*)-dehydrobutyrine in nodularin and *N*-methyl-(*Z*)-dehydrophenylalanine in tentoxin adopt configuration *cis* of the methylated amide bond [17,18]. This seems to be an interesting feature of the *N*-methyldehydroamino acids, important for adopting biological conformations of the peptides they constitute.

Our previous theoretical [19] and crystallographic [20] studies were focused on *N*-methyldehydroalanine, a prototypical molecule of the *N*-methyldehydroamino acids. The studies also revealed a considerable tendency of the methylated amide bond to adopt configuration *cis*.

In this paper, we report the results of theoretical conformational studies of *N*-methyl-(*Z*/*E*)-dehydroamino acids on selected diamide models (Fig. 1). The single amino acid diamides have been successfully applied in determining peptide conformational properties of the saturated analogues [21]. Similarly, we have tried to analyse how the stabilising forces created within the given amino acid residue influence conformational preferences, i.e. the number of conformers and their relative energy. The analysis was performed on the assumption that the hydrogen bonds/contacts (X—H···A) [22] and, amongst the dipole–dipole attractions, those between the carbonyl groups (C=O ►···◄ C=O) [23] are the main stabilising internal forces.

## 2. Methods

Calculations were performed using the Gaussian03 package [24]. The theoretical conformational properties of the free molecules having a N-terminal (methylated) amide group both in the *trans* ($\omega_0 \sim 180°$) and *cis* ($\omega_0 \sim 0°$) configuration were examined. Hereafter the former configuration is called the *trans* and the latter the *cis*. The $(\phi,\psi)$ potential energy surface of each molecule was generated on the basis of 84 structures calculated at the HF/3-21G level. In each of these structures, the geometrical parameters were fully relaxed, except for the constrained torsion angles $\phi$ and $\psi$. The values of these angles were chosen using a step size 30°, within the range −180° to 150° for the torsion angles $\phi$ and within the range 0–180° for the torsion angles $\psi$. Inversion through achiral α-carbon (i.e. $(\phi,\psi) \rightarrow (-\phi,-\psi)$) yields equivalent structures; therefore full $(\phi,\psi)$ potential energy surface maps were obtained in this way. Next, for the HF/3-21G geometries obtained in vacuo, the effect of electrostatic solute/solvent (water) interaction on the solute energies was investigated with the SCRF method using the polarisable continuum model (PCM) [25] and calculated at the B3LYP/6-31+G** level. The potential energy surface maps were created using the Surfer 8 programme with the radial basis function as a gridding method [26]. Based on the maps, the minima were localised and their geometries fully optimised at the B3LYP/6-31+G** level in vacuo. A second derivative analysis (frequency) of the optimised structures established all of them to be minima. The SCRF/PCM method was used to calculate the effect of electrostatic solute/solvent (chloroform, water) interaction on the energies of these minima. The conformational features of the studied molecules were then analysed in terms of the number of conformers, their internal stabilising forces, and the accessible conformational $\phi$, $\psi$ space.

The conformers of amino acid residues, defined in terms of torsion angles $\phi$ and $\psi$, are labelled variously by different research groups [27]. For the convenience of readers in Table 1 and in Fig. 4 three of the most commonly used notations have been given.

## 3. Results

Fig. 2 presents the Ramachandran maps of the potential energy surfaces for the molecules of Ac-(*Z*)-Δ(Me)Abu-NHMe, Ac-(*E*)-Δ(Me)Abu-NHMe, and Ac-Δ(Me)-Val-NHMe. Similarly, Fig. 3 presents the maps for Ac-(*Z*)-Δ(Me)Phe-NHMe and Ac-(*E*)-Δ(Me)Phe-NHMe. For each molecule two maps have been generated, respectively, for the configuration *trans* and *cis* of the N-terminal tertiary amide group. On each map the energy minimised conformers are depicted and their notation, torsion angles $\phi$, $\psi$, and energies are collected in Table 1. Due to the achiral α-carbon of the studied molecules, their maps are symmetrical in relation to the point $(\phi, \psi = 0°, 0°)$. Therefore, to discuss the results obtained, the minima found on the upper halves of the maps have been considered.

For the studied *N*-methyldehydroamino acids, except (*E*)-dehydrophenylalanine, three conformers can generally be found, $C7_{eq}$, $\alpha_D$ and β for both configuration *trans* and *cis* of the N-terminal amide bond (Fig. 4 and Table 1). All