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Communication

Conformational properties of *N*-acetyl-*N*-methyl-α,β-dehydroalanine *N'*-methylamide⋆

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The conformational properties of Ac-Δ(Me)Ala-NHMe (*N*-acetyl-*N*-methyl-α,β-dehydroalanine *N'*-methylamide), as the simplest model of *N*-methyl-α,β-dehydroamino acids, was examined with theoretical methods and in comparison with Ac-ΔAla-NHMe and Ac-ΔAla-NMe_2. The N-terminal amide of the Δ(Me)Ala residue easily adopts the configuration *cis* and the torsion angles φ, ψ are highly flexible. The Δ(Me)Ala residue is a conformational flexibilizer as compared to the parent ΔAla, which is a conformational stiffener. This seems to be the reason why Δ(Me)Ala is found in small natural cyclic peptides, where it ensures the conformational flexibility necessary for biological action.

Keywords: α,β-dehydroamino acids, N-alkylpeptides, *ab initio*/DFT calculations, *cis-trans* isomerisation, peptide design, microcystins

Nature has continuously provided mankind with a broad and structurally diverse arsenal of bioactive compounds that have been utilised as new drugs or as lead structures for the development of novel synthetically derived analogues (Proksch *et al*., 2002). The search for potentially therapeutic compounds amongst natural products reveals a growing number of peptides with a wide range of biological activity (Ballard *et al*., 2002). However, the relationship between the structure and biological activity of these peptides remains an open question, as they very often contain non-coded amino acids of unknown conformational properties. One group of such amino acids are *N*-methyl-α,β-dehydroamino acids, Δ(Me)Xaa. *N*-Methyl-(*Z*)-dehydrophenylalanine was found in tentoxin, a naturally occurring cyclic tetrapeptide produced by several phytopathogenic fungi of the genus *Alternaria*. Tentoxin is a selective weed killer that causes chlorosis of many higher plants (Loiseau *et al*., 2002). *N*-Methyl-(*Z*)-dehydrobutyrine, Δ(Me)Abu, is a component of nodularins, cyclic pentapeptides from *Nodularia spumingena*. Similarly, *N*-methyldehydroalanine, Δ(Me)Ala, is a component of microcystins, cyclic heptapeptides produced primarily by *Microcystis aeruginosa*. Microcystins and nodularins constitute families of toxins produced by species of freshwater cyanobacteria. The primary target of these toxins is the liver where they cause cytoskeletal damage, necrosis and pooling of blood (Dawson, 1998; Łukomska *et al*., 2002). *N*-Methyldehydroalanine also occurs in a cyclic depsipeptide isolated from an evergreen plant, *Ardisia crenata sims* (Myrsinaceae). This depsipeptide, codenamed FR900359, inhibits platelet aggregation *in vitro* and *ex vivo* in rabbits, decreases blood pressure, and shows dose-related hypotensive action in anaesthetised normotensive rats (Miyamae *et al*., 1989).

This work describes the conformational properties of *N*-methyldehydroalanine (Fig. 1), a prototypical molecule of the whole family of *N*-methyldehydroamino acids, using theoretical methods, and compares these properties with those of non-methylated ΔAla and *N'N'*-dimethylamide ΔAla (Siodłak, 2004a).

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Abbreviations: ΔXaa, dehydroamino acids; Δ(Me)Ala, *N*-methyldehydroalanine; ΔAla, dehydroalanine; ΔAla-NMe, *N'N'*-dimethyldehydroalanine.

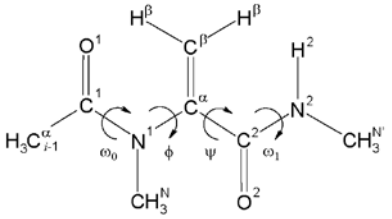

Figure 1. Atom numbering and torsion angles ϕ, ψ for the Ac-Δ(Me)Ala-NHMe molecule.
For the Ac-ΔAla-NHMe analogue, CH_3^N is replaced by H^1. For the Ac-ΔAla-NMe_2 analogue, CH_3^N is replaced by H^1, and H^2 is replaced by $CH_3^{N'}$.

METHODS

The theoretical conformational properties were examined for the free Ac-Δ(Me)Ala-NHMe molecule by the GAUSSIAN 03 package (Frisch *et al.*, 2003). Because of the known tendency of *N*-methylamino acids to adopt the *cis* configuration of the amide bond (Gilon *et al.*, 2002), calculations were performed with the *cis* (ω_0 ~ 0°) – *trans* (ω_1 ~ 180°) and *trans* (ω_0 ~ 180°) – *trans* (ω_1 ~ 180°) amide bond. Hereafter the former configuration is called the *cis* arrangement and the latter the *trans* arrangement. To generate the (ϕ, ψ) potential energy surfaces, 85 structures, each calculated at the B3LYP/6-31+G**// HF/3–21G level, were used. In each of them, the geometrical parameters were fully relaxed, except for the constrained torsion angles ϕ and ψ. Values for these angles were chosen by using a step size of 30°, within a range from –180° to 180° for the angle ϕ, and from 0° to 180° for the angle ψ. Inversion through achiral α-carbon (i.e. (ϕ, ψ) → (–ϕ, –ψ)) yields equivalent structures; therefore full (ϕ, ψ) potential energy surface maps were obtained in this way (Head-Gordon *et al.*, 1991). The energy surface was created by means of the Surfer 8 programme with the radial basis function as a gridding method (Surfer 8, 2002). The minima observed on the surface were then subjected to full geometry optimisation at the B3LYP/6-31+G** level. A second derivative analysis (frequency) on the optimised structures established all of them to be minima. The accessible conformational space of the studied molecule was based on the close resemblance between the Ramachandran contact map and the energy contours map within the limit of 5 kcal·mol^{-1} (Ramachandran & Sasisekharan, 1968), as has also been applied elsewhere (Zimmerman *et al.*, 1977; Herzberg & Moult, 1991). The effect of electrostatic solute/solvent interaction on the solute energies was investigated within the SCRF method using the polarizable continuum model (PCM) (Miertus *et al.*, 1977).

The conformers were designated by general short-hand letter notation (Zimmerman *et al.*, 1977) by analogy with the designation of the dehydroamino acids studied previously (Siodłak *et al.*, 2004a; 2004b; Broda *et al.*, 2005a; 2005b).

RESULTS

Figure 2 depicts the Ramachandran diagrams for both the *cis* (ω_0 ~ 0°) and *trans* (ω_0 ~ 180°) arrangements of the N-terminal amide bond for the free Ac-Δ(Me)Ala-NHMe molecule. It shows that the accessible ϕ,ψ conformational space of the Ac-Δ(Me)Ala-NHMe molecule within the 5.0 kcal·mol^{-1} limit equals 31% and 61% of the whole Ramachandran diagrams for the *cis* and the *trans* arrangements, respectively. These diagrams are accompanied by three conformers, B, E* and F, for both the *cis* and *trans* N-terminal amide bonds. The torsion angles ϕ,ψ of these conformers and the energy data for free molecules and those in water are listed in Table 1. Tables 2 and 3 collect the conformer geometric parameters of the hydrogen bonds and the dipole attractions.

Conformer *cis* B (ϕ, ψ = –111°, 8°) corresponds to the global minimum. The small value of the torsion angle ψ ensures the π-electron conjugation between the C^{α}=C^{β} double bond and the C-terminal amide bond. This conformer is also stabilised by the short N^2–H···N^1 and C^{β}–H···O^2 hydrogen bonds. The second *cis* conformer, E* (ϕ, ψ = 122°, 144°) is stabilised by the C^N–H···O^2 hydrogen bond. The highest energy conformer *cis*, F (ϕ, ψ = –49°, 146°) has the C^{α}_{i-1}–H···O^2 and the sheared parallel dipole C=O ▸···◂ C=O interactions between the carbonyl groups. Among the *trans* isomers, the lowest energy conformer B (ϕ, ψ = –76°, 38°) is stabilised primarily by the short C_7-type N–H···O hydrogen bond. In addition, it shows the C^{β}–H···O^2 and dipole C=O ▸···◂ C=O interactions. Conformer *trans* F (ϕ, ψ = –56°, 142°) has, instead of hydrogen bonds, two antiparallel dipole C=O ▸···◂ C=O interactions. The last *trans* conformer, E* (ϕ, ψ = 135°, 144°), is stabilised by the C^N–H···O^2 and C^{β}–H···O^1 interactions.

DISCUSSION

The introduction of the methyl group into the N-terminal amide bond of an amino-acid residue faciliate adopting the *cis* configuration of the N-methyl terminal amide bond (Gilon *et al.*, 2002). In the case of the investigated Δ(Me)Ala residue, the conformer *cis* B does correspond to the global minimum. This is due first of all to the planarity of the angle ψ, which ensures π-electronic conjugation between the C^{α}=C^{β} double bond and the C^2=O^2 group. This conjugation is the only π-electronic conjugation amongst the conformers of Ac-

Table 1. B3LYP/6-31+G conformers of the Ac-Δ(Me)Ala-NHMe molecule and their relative energies calculated for a free molecule and in water using the SCRF model**

Conformers	Torsion angles [°]		ΔE [kcal·mol^{-1}]	
	ϕ	ψ	vacuum	water
trans B	–76.2	38.1	2.01	3.16
trans F	–56.3	141.7	2.28	0.97
trans E*	134.7	144.2	3.92	2.92
cis B	–110.8	7.9	0.00	0.00
cis E*	122.3	143.9	2.56	0.56
cis F	–49.2	146.5	2.59	1.30
Energy of conformer *cis* B [Hartree]:			–533.9658967	–533.9843226

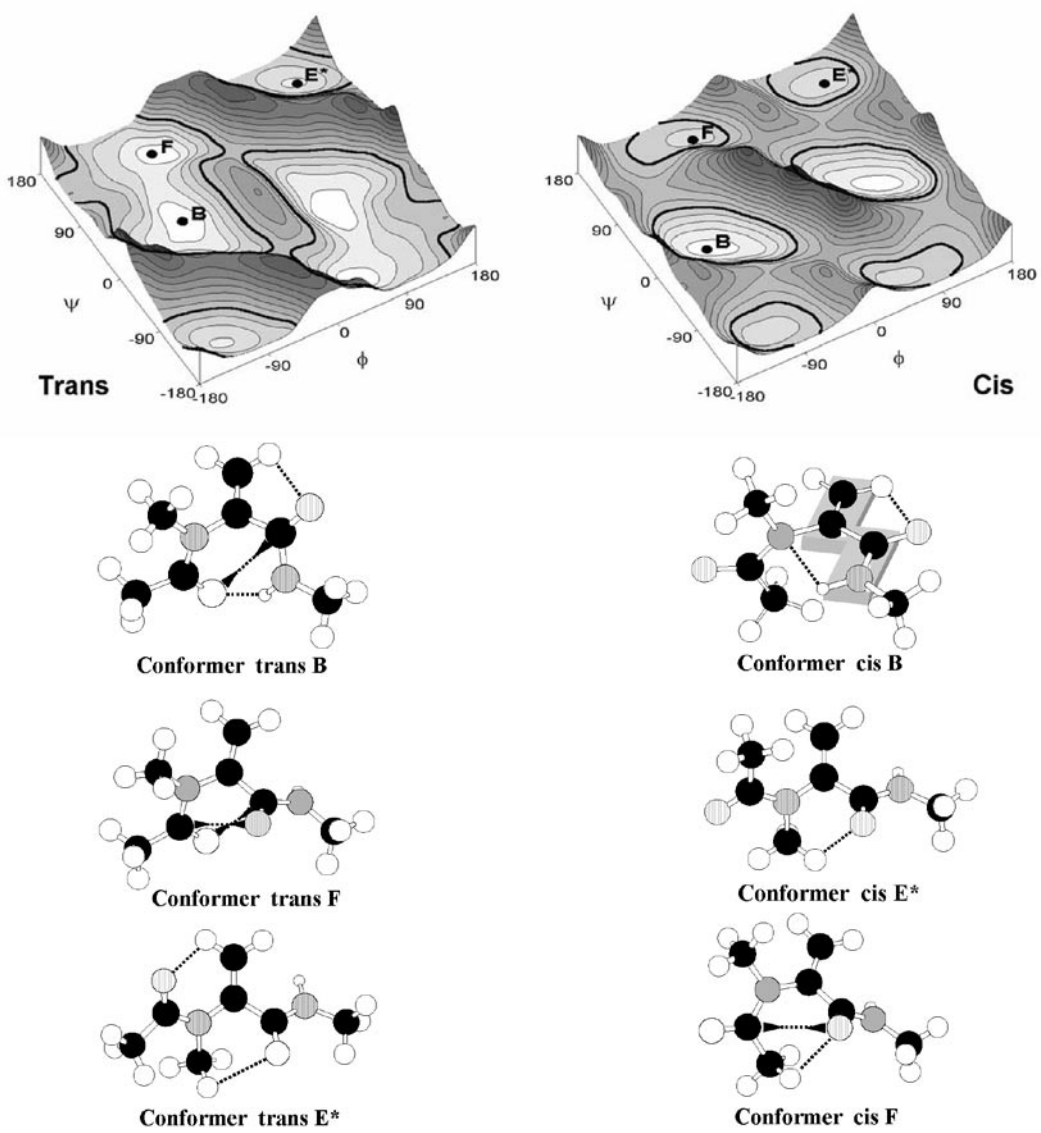

Figure 2. Landscape and contour representation of the *cis* (ω_0 = 0°) and *trans* (ω_0 = 180°) ϕ, ψ potential energy surfaces of the Ac-Δ(Me)Ala-NHMe molecule [a]together with its six conformers [b]after optimisation at the B3LYP/6–31+G level of theory.**

[a]The map was calculated at the B3LYP/6–31+G**//HF/3–21G level of theory *in vacuo*. The energy contours are drawn every 1 kcal·mol^{-1}. The 5.0 kcal·mol^{-1} contour is marked in bold. ● B3LYP/6–31+G** minima. [b](·····) hydrogen bond, (▸···◂) dipole attraction in the conformers. For geometric parameters of these interactions see Tables 2 and 3.

Table 2. Structural parameters for the internal X–H···A interactions in the B3LYP/6–31+G conformers of the Ac-Δ(Me)Ala-NHMe molecule[a]**

Interactions/ Parameters	Conformers					
	trans B	*trans* F	*trans* E*	*cis* B	*cis* E*	*cis* F
N–H···O						
H···O (Å)	1.930	–	–	–	–	–
N···O (Å)	2.840	–	–	–	–	–
∠N–H···O (°)	147.5	–	–	–	–	–
∠C=O···H (°)	101.7	–	–	–	–	–
N–H···N						
H···N (Å)	–	–	–	2.300	–	–
N···N (Å)	–	–	–	2.770	–	–
∠N–H···N (°)	–	–	–	106.9	–	–
C–H···O						
H···O (Å)	β) 2.580	–	β) 2.360 N) 2.660	β) 2.490	N) 2.680	α-1 2.560
C···O (Å)	β) 2.858	–	β) 2.810 N) 2.930	β) 2.820	N) 3.270	α-1 3.150
∠C–H···O (°)	β) 93.0	–	β) 103.5 N) 93.5	β) 96.0	N) 113.3	α-1 113.2
∠C=O···H (°)	β) 78.7	–	β) 99.7 N) 74.7	β) 83.0	N) 74.0	α-1 84.1

[a]Data presented only for the X–H···A contacts (X = N, C; A = O, N) in which H···A ≤ 3.2 Å and ∠X–H···A > 90° acc. to Steiner (2002). α, β, N denote C^{α}–H···O, C^{β}–H···O, and C^{N}–H···O hydrogen bonds, respectively.

(Me)ΔAla-NHMe, both *cis* and *trans*. Simultaneously, the π-electronic conjugation brings about the shortening of the N^2–H···N^1 and C^{β}–H···O^2 hydrogen bonds and, as a result, the bonds are strong. All the atoms involved in the hydrogen bonds are in such positions that they are not hindered sterically. In contrast, for the free molecule of the saturated analogue Ac-(Me)Ala-NHMe, the conformer with the lowest energy is *trans* C (ϕ,ψ = –90°, 81°) (Bágyi *et al.*, 2003). This means that the unsaturated compound adopts the *cis* configuration more easily. As can be seen, although the N–H···O hydrogen bond is the main stabilising force in peptide and protein structures, other interactions, each weaker than this bond, when acting together may have a considerable impact on the conformational preferences of Δ(Me)Ala. The energetic profit gained by the conformer *cis* B from the π-electron conjugation between the C^{α}=C^{β} double bond and the C-terminal amide bond strongly depends on the planarity of the torsion angle ψ. Thus, the area within the 5 kcal·mol^{-1} limit around the conformer *cis* B is smaller than that of the conformer *trans* B. Moreover, the gap in energy for the conformers *cis* B and F is greater (ΔE_{F-B} = 2.6 kcal·mol^{-1}) than that for the *trans* conformers (ΔE_{F-B} = 0.3 kcal·mol^{-1}). These two factors seem to influence the magnitude of the accessible ϕ,ψ *cis* area (ω_0 ~ 0°) that equals 31% and is two times less than the area for the *trans* (ω_0 ~ 180°) N-terminal amide bond.

Table 3. Structural parameters for the internal C=O ▸···◂ C=O dipole interactions in the B3LYP/6-31+G conformers of the Ac-Δ(Me)Ala-NHMe molecule [a]**

Conformers	Parameters								Type[b]
	C^{C}···O^{N}	C^{N}···O^{C}	C···C	O···O	∠(C=O)^{N}···C^{C}	∠O^{N} ···(C=O)^{C}	∠C^{N}···(O=C)^{C}	∠O^{C}···(C=O)^{N}	
trans B	3.265	–	3.303	4.480	80.9	–	–	81.3	I
trans F	2.836	3.220	2.930	3.320	82.4	102.6	65.6	83.9	II
cis F	3.243	–	3.086	4.230	–	–	71.7	138.5	I

[a]Data presented only for the C=O ▸···◂ C=O contacts, in which H···A ≤ 3.6 Å acc. to Allen *et al.* (1998). Distances are given in Å. Angles are given in degrees. ^{N,C} denote the *N*-terminal and *C*-terminal carbonyl group. [b]As given by Allen *et al.* (1998).

The second consequence of the introduction of the methyl group into the N-terminal amide bond of dehydroalanine reside is steric restriction on the torsion angle ϕ so that it is unable to adopt a value close to 0°. As a result, the extended conformer E and the semi-extended is not adopted by Δ(Me)Ala as compared to non-N-methylated dehydroalanine, ΔAla (E: ϕ, ψ = 180°, 169°; D: ϕ, ψ = 165°, 25°) and *N'N'*-dimethyldehydroalanine, ΔAla-NMe (E: ϕ, ψ = 180°, 153°; D: ϕ, ψ = 177°, 48°) (Siodłak *et al*., 2004a). Instead, Δ(Me)Ala adopts the conformation E* (ϕ, ψ = 135°, 144°) that is typical of (*Z*)-ΔXaa (Thormann & Hofmann, 1998; Siodłak *et al*., 2004a; 2004b) (but not for (*E*)-ΔXaa (Thormann & Hofmann, 1998; Broda *et al*., 1998, 2005a)). The N-methylation of the amide bond seems not to influence either the conformer B (ϕ, ψ = –76°, 38°) or the conformer F (ϕ, ψ = –56°, 142°). The former in ΔXaa has average torsion angles ϕ, ψ equal to –71°, and 41°, respectively. The latter was found in both ΔXaa and ΔXaa-NMe, with average torsion angles ϕ, ψ equal to –49°, 132° (Thormann & Hofmann, 1998; Siodłak *et al*., 2004a).

For ΔAla and ΔAla-NMe, the lowest energy conformer is E, stabilised mainly by the short C_5 hydrogen bond N^1–H···O^2 and the π-electron cross-conjugation extended between the double C^α=C^β bond and the N/C-terminal amide bonds. There is a considerable gap in energy between the conformer E and the second conformer B for ΔAla (ΔE_{B-E} = 5.1 kcal·mol^{-1}) or between the conformer E and the second conformer H/F for ΔAla-NMe ($\Delta E_{H/F-E}$ = 3.7 kcal·mol^{-1}). The conformational freedom of both residues is limited, but particularly that of ΔAla. The area within the 5 kcal·mol^{-1} limit equals 12% and 24% of the whole Ramachandran diagram for ΔAla and ΔAla-NMe, respectively (Siodłak *et al*., 2004a). The lack of conformer E in *trans* Δ(Me)Ala causes the conformer B to become the lowest in energy and the energetic difference between the conformer B and the second conformer F is very small (ΔE_{F-B} = 0.3 kcal·mol^{-1}). This, along with the small steric hindrance on the side chain of Δ(Me)Ala, affects the magnitude of the area within the 5 kcal·mol^{-1} limit of the *trans* Ramachandran diagram. It amounts to as much as 61% and is much more than for standard alanine and even glycine (Head-Gordon *et al*., 1991). Thus, the Δ(Me)Ala residue with the *trans* N-terminal amide bond experiences a great conformational freedom, in sharp contrast to the ΔAla and ΔAla-NMe analogues.

The influence of the polar environment on the conformational features of the Δ(Me)Ala residue was calculated using the SCRF method with water as the solvent (Table 1). The energetic order of the *cis* conformers remains unchanged and the lowest-energy conformer continues to be the conformer *cis* B. Amongst the *trans* conformers, the conformer F becomes the lowest in energy, as was found for ΔXaa-NMe (Broda *et al*., 2005b). The energetic gap between the highest and lowest *trans* conformers in water (ΔE_{B-F} = 2.2 kcal·mol^{-1}) is only slightly greater than that in vacuum (ΔE_{E^*-B} = 1.9 kcal·mol^{-1}). In contrast, the difference between the highest and lowest *cis* conformers is two times smaller in water than that in vacuum (ΔE_{F-B} = 1.3 and 2.6 kcal·mol^{-1}, respectively). Furthermore, the difference between the lowest *cis* and *trans* conformers i.e. the conformer *cis* B and *trans* F in water is also two times smaller (ΔE = 1 kcal·mol^{-1}) as compared to the conformers *cis* and *trans* B of the free molecule. Thus, the Δ(Me)Ala residue should in a polar environment conserve the *cis* amide bond arrangement as well as its ϕ,ψ conformational flexibility.

The conformational properties of Δ(Me)Ala help in understanding known facts. The FR900359 depsipeptide is so far the only known X-ray crystal structure which contains the Δ(Me)Ala residue (Miyamae *et al*., 1989). With the *trans* configuration of the amide bond, it adopts the conformation –F (ϕ, ψ = 45°, –136°), characteristic of the (*i* + 1)th position of the βII' turn. This amino-acid residue also displays large thermal parameters, and thus it is considered to be flexible. This agrees with the results of our calculations. Sivonen *et al*. (1992) showed that ΔAla substituted for Δ(Me)Ala decreases the biological activity of microcystin-LR. This can be explained by the opposing conformational preferences of both residues. Whereas Δ(Me)Ala experiences great flexibility, ΔAla strongly prefers an extended conformation and acts as a stiffener that restricts the conformation of the modified microcystin. The tendency to adopt the *cis* configuration by the N-terminal amide bond of *N*-methyldehydroamino acids can be inferred from the structural features of microcystin-LR and nodularin (Łukomska *et al*., 2002). NMR investigation reveals that both compounds have a similar conformation. The greatest difference concerns the configuration of the γ-peptide bond between the D-glutamic acid and *N*-methyldehydroamino acid residues. In the cyclic heptapeptide microcystin, the Δ(Me)Ala residue has the *trans* configuration of this bond, whereas in the cyclic pentapeptide nodularin, the Δ(Me)Abu residue has the *cis* configuration. It can be assumed, therefore, that the smaller nodularin reduces its conformational strain at relatively low energetic cost adopting the *cis* configuration at the N-terminal amide bond of the Δ(Me)Abu residue.

CONCLUSION

N-Methyl-α,β-dehydroamino acids, found in small natural cyclic peptides, belong to non-coded amino acids of unknown conformational features.

N-Methyldehydroalanine, prototypical of the whole family, is a component of microcystins that are toxins produced by species of freshwater cyanobacteria. This work presents a detailed theoretical study of the conformational properties of Ac-Δ(Me)Ala-NHMe (*N*-acetyl-*N*-methyl-α,β-dehydroalanine-*N'*-methylamide) as compared to those of Ac-ΔAla-NHMe and Ac-ΔAla-NMe_2.

The Δ(Me)Ala residue is characterised by the tendency for the *cis* N-terminal amide bond arrangement and shows great flexibility of the torsion angles φ, ψ. In addition, it easily adopts the conformation *trans* F, characteristic of the (*i* + 1)th position of the βII' turn, as proved by the X-ray crystal structure of the depsipeptide FR900359 (Miyamae *et al*., 1989) and confirmed with calculations. The Δ(Me)Ala residue has no extended conformers either *trans* or *cis*, whereas such extended *trans* conformers are typical for the ΔAla and ΔAla-NMe analogues. The area within the 5 kcal·mol^{-1} limit of the Ramachandran map amounts to 31% and 61% of the whole diagram for *cis* and *trans* N-terminal amide bonds, respectively. As can be seen, Δ(Me)Ala is a conformational flexibilizer, while ΔAla is a conformational stiffener. This seems to be the reason why Δ(Me)Ala is found in small natural cyclic peptides, where it ensures the conformational flexibility necessary for their biological action.

Acknowledgements

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