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Conformational investigation of α,β-dehydropeptides. *N*-acetyl-(*E*)-dehydrophenylalanine *N*′-methylamide: conformational properties from infrared and theoretical studies, part XIV‡

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Abstract: *N*-Acetyl-(*E*)-dehydrophenylalanine *N*′-methylamide [Ac-(*E*)-ΔPhe-NHMe], one of a few representative (*E*)-α,β-dehydroamino acids, was studied by FTIR in dichloromethane and acetonitrile. To support spectroscopic interpretations and to gain some deeper insight into the Ac-(*E*)-ΔPhe-NHMe molecule, the Ramachandran potential energy surface was calculated by the B3LYP/6-31G*//HF/3-21G method and the conformers localized were fully optimized at the B3LYP/6-31 + G** level. The spectra and calculations were compared with those of the related molecules Ac-ΔAla-NHMe and Ac-(*Z*)-ΔPhe-NHMe. The title compound assumes two conformational states in equilibrium in dichloromethane solution with a predominance of the extended conformer E. The Ac-(*E*)-ΔPhe-NHMe spectrum is like that of Ac-ΔAla-NHMe, particularly in the region of bands AI and AII, and unlike that of Ac-(*Z*)-ΔPhe-NHMe. The positions of bands AI and II together with the $\nu_s(N^1{-}H^1)$ band proves that the conformers E of both ΔAla and (*E*)-ΔPhe compounds are stabilized by the quite strong C_5 hydrogen bonds $N^1{-}H^1\cdots O^2$. The same conclusion is drawn from the Ramachandran diagrams. The conformers E of both compounds are placed in the global minima and the gaps in energy order between them and the second conformer are large. The conformers E of ΔAla and (*E*)-ΔPhe, apart from the $N^1{-}H^1\cdots O^2$ hydrogen bond, show the $C^\beta{-}H\cdots O^1$ interaction, and Ac-(*E*)-ΔPhe-NHMe displays the NH/π interaction with the $N^2{-}H^2$ projecting in the first carbon atom of the phenyl ring. The C_5 hydrogen bond is stronger in (*E*)-ΔPhe than that in the ΔAla compound. This is in agreement with interactions found in the calculated structures and can be explained by the influence of the phenyl ring in position (*E*). In acetonitrile, the molecule of Ac-(*E*)-ΔPhe-NHMe loses its C_5 hydrogen bond and becomes unfolded, whereas that of Ac-ΔAla-NHMe does not vary practically. Adopting conformation E in a non-polar solvent seems to be a general feature of the (*E*)-ΔXaa residues.

Keywords: *ab initio* calculation; FTIR spectroscopy; NH/π interaction; C_5 hydrogen bond; (*E*)-dehydrophenylalanine; π-electron conjugation; α,β-dehydroamino acids; amide II

INTRODUCTION

α,β-Dehydroamino acids have a double bond between the C^α and C^β atoms and thus chirality is lost and (*Z*)/(*E*) isomerism appears (Figure 1). Both (*Z*) and (*E*)-forms occur in nature and are used for conscious peptide modification. The prototypical molecule not having a β-substituent is dehydroalanine (ΔAla) and has been found in numerous members of the thiopeptide family of antibiotics [2,3]. Apart from this it serves in Michael addition with a range of amines and thiols, which provides an attractive route to the synthesis of natural and unnatural amino acid derivatives [4]. (*Z*)- and (*E*)-dehydrobutyrine [(*Z*)- and (*E*)-ΔAbu] are the simplest and most common (*Z*) and (*E*)-dehydroamino acids. They occur in ribosomal lantibiotics, peptides with unusual chemical diversity [5–8], microcystins and nodularins [9–13] as well as in other phytotoxins [14]. (*Z*)- and (*E*)-dehydrophenylalanine [(*Z*)- and (*E*)-ΔPhe)], chiefly the former, often serve as peptide modifiers [15–19]. Receptor proteins frequently discriminate quite precisely between the (*Z*) and (*E*)-disposition of the double bond $C^\alpha{=}C^\beta$ in their bioligands and compared with (*Z*)-ΔXaa counterparts, (*E*)-Δ-peptides are usually less active [15–20]. Much more is known about the conformational preferences of (*Z*)-α,β-dehydropeptides (for reviews see refs [19,21–24]) than those of their (*E*)-analogues, because most of the preparative procedures yield exclusively or predominantly the former isomers [22]. In recent years, there has been increasing interest in the (*E*)-dehydroamino acids evidenced by finding new efficient and stereoselective methods for the synthesis of (*E*) isomers [25,26] and by revitalizing the photoisomerization of (*Z*)-dehydrophenylalanine as a route to the (*E*)-dehydrophenylalanine [27,28].

The model dipeptides Ac-Xaa-NHMe with blocked amino and carboxyl groups are considered in order to mimic the incorporation of the Xaa amino acid residue into a peptide chain, and are the smallest structural core for building short-range interaction [29]. The conformational properties of such simple

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‡ For Part XIII in this series see Ref. [1].

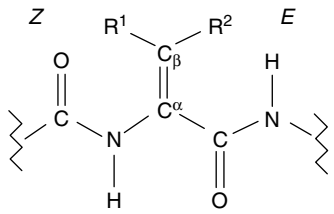

Figure 1 General scheme for α,β-dehydroamino acid residue with ascription of β-substituent.

α,β-dehydropeptide related molecules, Ac-ΔXaa-NHMe, where ΔXaa = ΔAla, ΔVal, (*Z*)-ΔLeu, (*Z*)-ΔPhe, (*Z*)- and (*E*)-ΔAbu were examined and compared with those of their saturated counterparts [30–32]. It has emerged that the α,β-dehydroamino acid derivatives in solution easily acquire a C_5 conformation that is not so readily accessible to their saturated analogues. The molecules of Ac-ΔAla-NHMe that are devoid of steric hindrance even have an unusual, strong C_5 hydrogen bond. The feature is also shared by the molecule of Ac-(*E*)-ΔAbu-NHMe without steric clash from the *N*-terminal end.

This paper studies the conformational properties of Ac-(*E*)-ΔPhe-NHMe. Phenylalanine seems to play an important role in recognition processes and is often located in a pharmacophoric region [33]. Therefore the constrained versions of ΔPhe with different degrees of control over the benzylic chain orientation are essential topographic tools for the rational design of peptide-based drugs [34]. The FTIR spectra of the sample compound in dichloromethane and acetonitrile were recorded and the modes of ν_s(N–H), amide I and amide II were analysed. To support the spectroscopic interpretations and to gain some deeper insight into the molecule, the Ramachandran potential energy surface was calculated on Ac-(*E*)-ΔPhe-NHMe with the B3LYP/6-31G*//HF/3-21G method and the conformers localized were fully optimized at the B3LYP/6-31 + G** level. The spectra and calculations were compared with those of the related molecules Ac-ΔAla-NHMe and Ac-(*Z*)-ΔPhe-NHMe.

MATERIALS AND METHODS

Material

Ac-(*E*)-ΔPhe-NHMe was obtained by irradiation of Ac-(*Z*)-ΔPhe-NHMe [28] and was of 99.8% purity as determined by HPLC. The analytical grade solvents, dichloromethane and acetonitrile, were dried further over P_2O_5, distilled and stored over freshly prepared molecular sieves.

FTIR Spectroscopy

The FTIR spectra were recorded at 20 °C on a Nicolet 540 Magna spectrometer, at 2 cm^{-1} nominal resolution, using a liquid cell (KBr, 0.1 and 0.2 mm). The spectra were analysed by the curve-fitting procedure with a mixed (Gauss-Lorentz) profile implemented in the GRAMS/386 program [35].

Computational Procedures

The theoretical conformational properties were examined on the free Ac-(*E*)-ΔPhe-NHMe, Ac-ΔAla-NHMe and Ac-(*Z*)-ΔPhe-NHMe molecules using the Gaussian 03 package [36]. Calculations were performed on the *trans*-acetyl group ($\omega_0 \sim 180°$). To generate the (ϕ, ψ) potential energy surfaces, 85 structures each calculated at the B3LYP/6-31G*//HF/3–21G level were used. In each of them, all geometrical parameters were fully relaxed, except the constrained torsion angles ϕ and ψ. Values of these angles were chosen by using a step size of 30°, within the range −180° to 180° for the angle ϕ, and from 0° to 180° for the angle ψ. Inversion through achiral α-carbon [i.e. $(\phi, \psi) \rightarrow (-\phi, -\psi)$] yielded equivalent structures; therefore full (ϕ, ψ) potential energy surface maps were obtained in this way [37]. The energy surface was created by way of the Surfer 8 program using the radial basis function as a gridding method [38]. The minima observed on the surface were then subjected to full geometry optimization at the B3LYP/6-31+G** level. A second derivative analysis (frequency) on the optimized structures established all of them to be minima. The geometrical parameters of the corresponding energy-minimized conformers were then further discussed. The calculated total energy of conformational minima was corrected for zero-point vibrational energy (without scaling factor). The accessible conformational space of the studied molecule was assumed on the basis of the close resemblance between the Ramachandran contact map and the energy contours map within the limit of 5 kcal mol^{-1} [39], as is also applied elsewhere [40,41]. The effect of electrostatic solute/solvent interaction on the solute energies was investigated within the SCRF method using the polarizable continuum model (PCM) [42].

As the overall conformational profiles of modified peptides can differ from those of common peptides, the energy-minimized conformers of the investigated molecules are described by the general short hand letter notation introduced by Zimmerman [40].

RESULTS

FTIR Spectra

The general formula, atom numbering and designation of selected torsion angles of Ac-(*E*)-ΔPhe-NHMe are given in Figure 2. Figure 3 and Table 1 present the amide mode and the $C^{\alpha}{=}C^{\beta}$ double bond stretching frequencies of this compound in dichloromethane along with the corresponding spectra of the cognate dehydroamino acid molecules, Ac-ΔAla-NHMe and Ac-(*Z*)-ΔPhe-NHMe. The characteristic feature of the spectrum of Ac-(*E*)-ΔPhe-NHMe is the spectral pattern

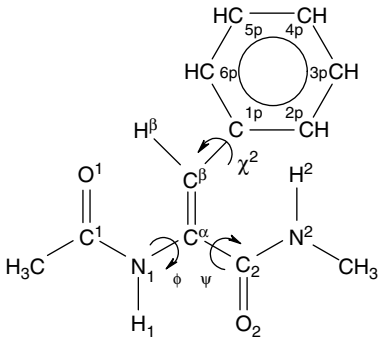

Figure 2 Atom numbering and selected torsion angles for Ac-(*E*)-ΔPhe-NHMe and other compounds.

Table 1 Amide and $C^{\alpha}=C^{\beta}$ Mode Frequencies (cm^{-1}) of Ac-(*E*)-ΔPhe-NHMe, Ac-ΔAla-NHMe, and Ac-(*Z*)-ΔPhe-NHMe in CH_2Cl_2

	(*E*)-ΔPhe	ΔAla [30]	(*Z*)-ΔPhe [30]
$\nu_s(N^1-H^1)$	3369 3407	3379	3401
$\nu_s(N^2-H^2)$	3440 3407	3466	3451
$AI(C^1=O^1)$	1692	1698	1698
$AI(C^2=O^2)$	1659 1670	1663 1674	1674
$\nu_s(C^{\alpha}=C^{\beta})$	1632	1631	1635
$AII(C^1-N^1H^1)$	1505 1490	1496	1477
$AII(C^2-N^2H^2)$	1543 1517	1536	1521

in the region of amide I and II like that of Ac-ΔAla-NHMe, which has a typical C_5 hydrogen-bonded conformation [30]. The band of $AI(C^2=O^2)$ at maximum 1659 cm^{-1} has a clearly elevated integral intensity. The band $AII(C^1-N^1H^1)$ has been found at 1505 cm^{-1} and is the most intensive one in the spectrum, as is the case for Ac-ΔAla-NHMe. The band $AI(C^2=O^2)$ lies at a frequency lower by 4 cm^{-1} and the band $AII(C^1-N^1H^1)$ at higher by 9 cm^{-1} than those respective bands of Ac-ΔAla-NHMe [30]. The positions of these bands suggests that the C_5 hydrogen bond in Ac-(*E*)-ΔPhe-NHMe is somewhat stronger than that in Ac-ΔAla-NHMe. Some small inflex can be recognized on the high-frequency edge of the $AI(C^2=O^2)$ band in the (*E*)-ΔPhe molecule like in the ΔAla molecule, which points to the presence of a small amount of another conformer. The curve-fitting procedure (Figure 4A) reveals that this band is at 1670 cm^{-1}, i.e. 11 cm^{-1} higher than that in the C_5 hydrogen-bonded conformation, exactly as in the ΔAla molecule (1663 cm^{-1} and 1674 cm^{-1}). The band $AI(C^1=O^1)$ has been discovered at 1692 cm^{-1}, i.e. 6 cm^{-1} lower than the corresponding band of Ac-ΔAla-NHMe and has also larger halfwidth (21 cm^{-1}) compared with the latter compound (14 cm^{-1}). This may be due to a stronger interaction of $C^{\beta}-H\cdots O^1$ than that within the dehydroalanine residue [43] (cf. Figure 8).

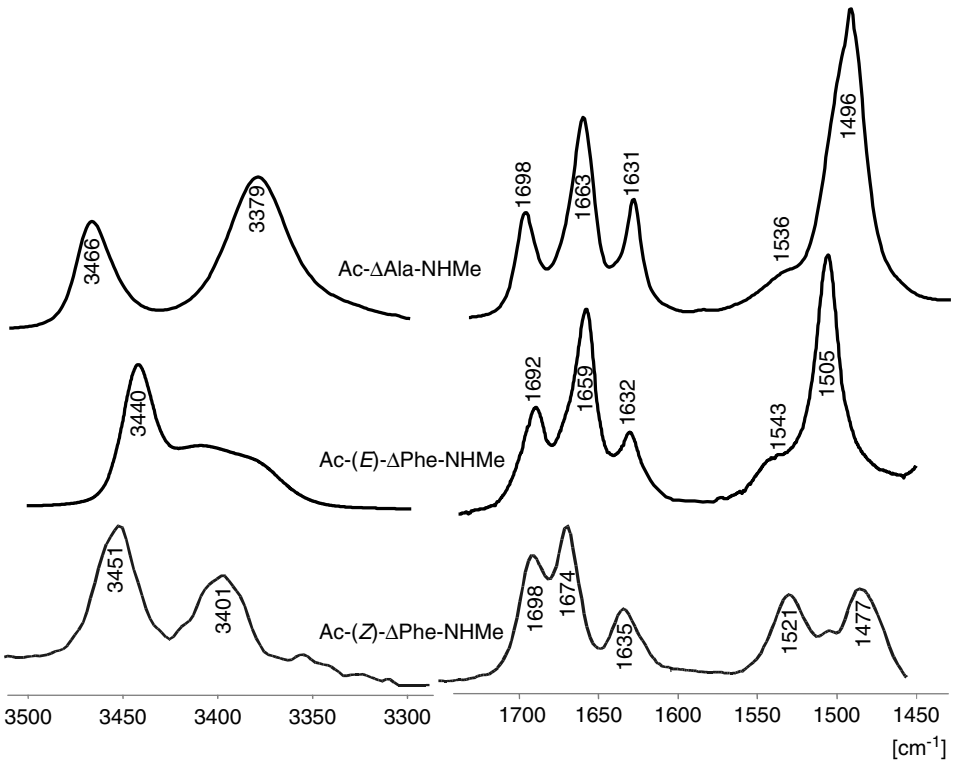

Figure 3 The FTIR spectra of Ac-(*E*)-ΔPhe-NHMe and the cognate molecules, Ac-ΔAla-NHMe and Ac-(*Z*)-ΔPhe-NHMe in the CH_2Cl_2 solution ($c = 5 \times 10^{-3}$); in the ν_s(N–H) region absorption scale extended three times.

In the N–H stretching region of Ac-(*E*)-ΔPhe-NHMe, three bands occur. The curve-fitting procedure yields the position of these bands: 3440, 3407 and 3369 cm^{-1} (Figure 4B). The bands at 3440 and 3369 cm^{-1} belong to the conformer involved in the C_5 hydrogen bond. The relatively narrow band at 3440 cm^{-1} originates from $\nu_s(N^2–H^2)$ and it should be noted that it lies 26 cm^{-1} lower than the respective band Ac-ΔAla-NHMe. This seems to be caused by the interaction of the $N^2–H^2$ with the phenyl ring π-electrons, and the value of the shift is quite large for such an interaction. The latter band, which is relevant to the $N^1–H^1$ group is 10 cm^{-1} lower than the corresponding frequency of Ac-ΔAla-NHMe. It also proves there is a stronger C_5 hydrogen bond in Ac-(*E*)-ΔPhe-NHMe than in Ac-ΔAla-NHMe. The band at 3407 cm^{-1} is the coincidental mode ν_s(N–H) of two N–H groups of the other conformer. Also in region AII, the curve-fitting procedure revealed two pairs of frequencies (Figure 4C), one pair, AII($C^1–N^1H^1$) 1505 cm^{-1} and AII($C^2–N^2H^2$) 1542 cm^{-1}, belongs to the C_5-hydrogen-bonded conformer and the other, AII($C^1–N^1H^1$) 1490 cm^{-1} and AII($C^2–N^2H^2$) 1517 cm^{-1}, belongs to the open conformer.

The spectrum Ac-(*Z*)-ΔPhe-NHMe (Figure 3) is quite different from that of Ac-(*E*)-ΔPhe-NHMe. Both amides I, AI($C^2=O^2$) and AI($C^1=O^1$) lie higher, at 1674 and 1698 cm^{-1}, respectively. Corresponding amides II, AII($C^1–N^1H^1$) and AII($C^2–N^2H^2$) occur lower, at 1477 and 1521 cm^{-1}. Two bands in the N–H stretching region are found at $\nu_s(N^2–H^2)$ 3451 cm^{-1} and $\nu_s(N^1–H^1)$ 3401 cm^{-1}.

The spectrum of Ac-(*E*)-ΔPhe-NHMe in acetonitrile in the amide I region (Figure 5) differs from that in CH_2Cl_2, which means a conformational change. Both AI bands become similar in intensity and move towards higher frequencies, the band AI($C^2=O^2$) shifts by as much as 9 cm^{-1} on 1668 cm^{-1} and the band AI($C^1=O^1$) shifts by 4 cm^{-1} on 1696 cm^{-1}. This proves a breaking of the C_5 hydrogen bond and a decrease in the strength of the $C^\beta–H\cdots O^1$ interaction. The bands of amide II are shifted toward the higher frequency, indicating that both N–H groups become bonded with acetonitrile. On the contrary, the spectrum of Ac-ΔAla-NHMe in acetonitrile is the same as that in dichloromethane. An exception is a small increase in the intensity of the inflex at 1674 cm^{-1}, which means that the concentration of the second conformer increases a little. This indicates an almost unchanged C_5 hydrogen-bonded conformation. In the spectrum of Ac-(*Z*)-ΔPhe-NHMe, the band AI($C^2=O^2$) does not change, AI($C^1=O^1$) shifts at the position of 1693 cm^{-1}

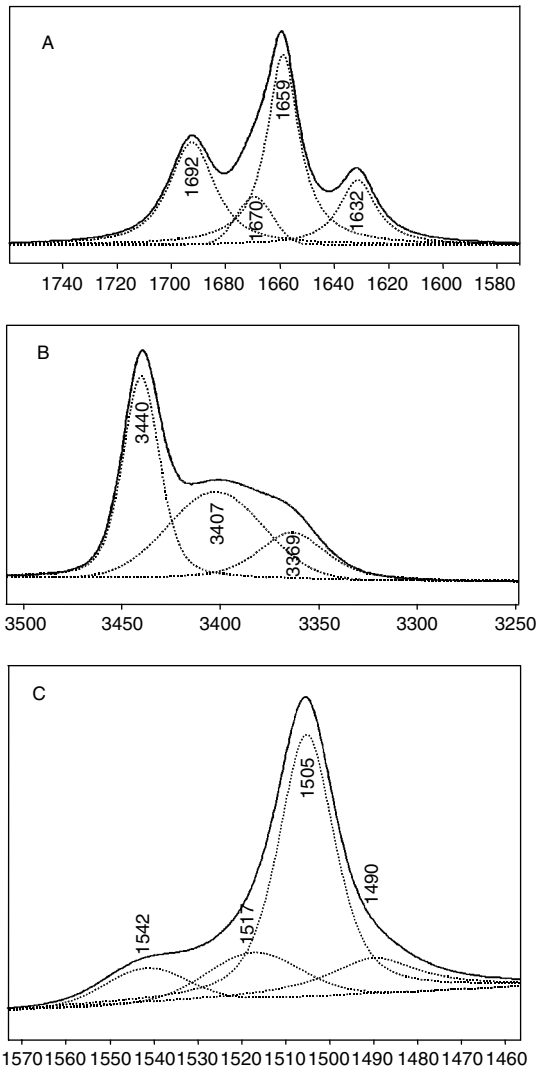

Figure 4 The curve-fitted spectra of Ac-(*E*)-ΔPhe-NHMe in the CH_2Cl_2 solution; (A) the AI and $\nu_s(C^\alpha=C^\beta)$ region; (B) the ν_s(N–H) region; (C) the AII region.

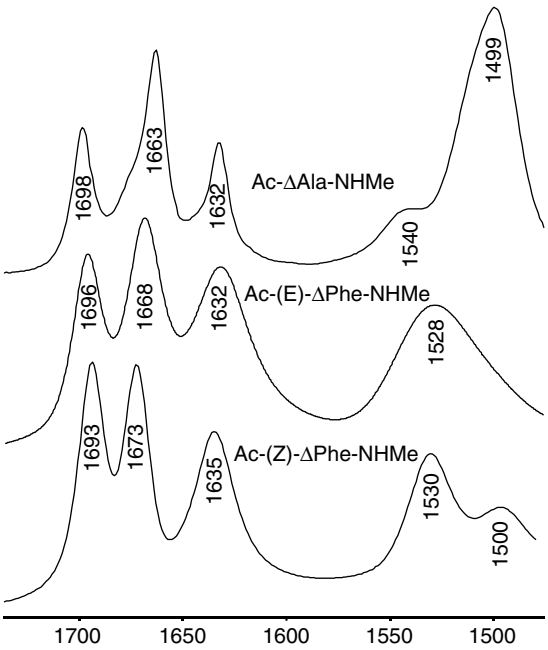

Figure 5 The FTIR spectra of Ac-(*E*)-ΔPhe-NHMe and the cognate molecules, Ac-ΔAla-NHMe and Ac-(*Z*)-ΔPhe-NHMe in acetonitrile solution in the AI, $\nu_s(C^\alpha=C^\beta)$ and AII region.

and both bands amide II shift toward a higher frequency showing both N–H groups bonded with acetonitrile.

Theoretical Conformation Analysis

Figure 6 shows the 2D-Ramachandran surface for the free Ac-(*E*)-ΔPhe-NHMe molecule along with those of Ac-ΔAla-NHMe and Ac-(*Z*)-ΔPhe-NHMe. Table 2 shows the calculated relative energies of all conformers obtained *in vacuo* and using PCM representation of dichloromethane and acetonitrile solutions also provides the selected conformational parameters in the

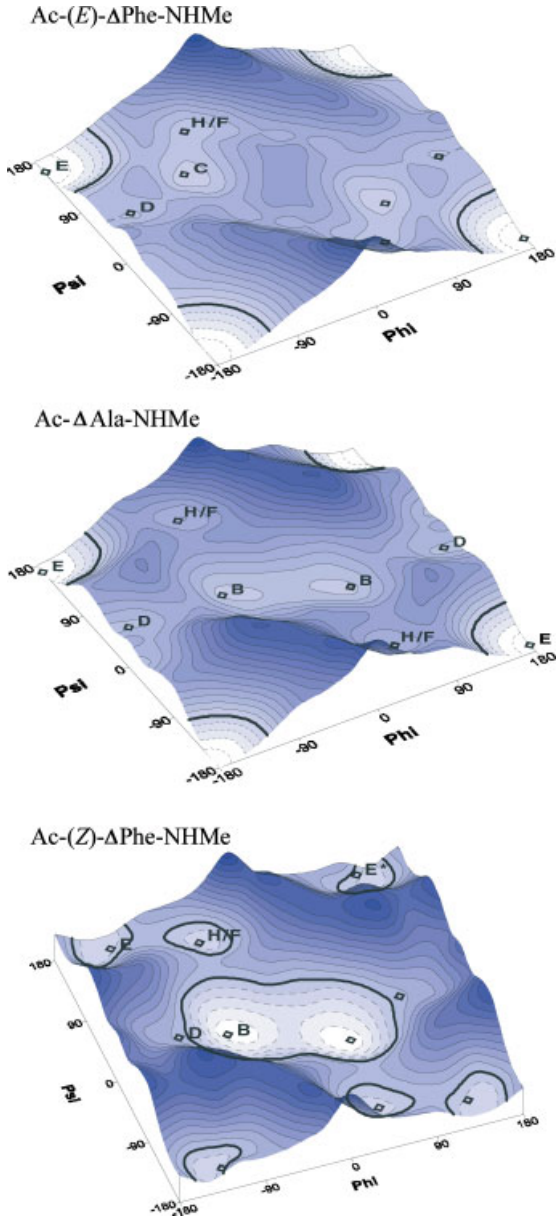

Figure 6 The landscape representation of the (ϕ, ψ) potential energy surface of the free Ac-(*E*)-ΔPhe-NHMe, Ac-ΔAla-NHMe and Ac-(*Z*)-ΔPhe-NHMe molecule calculated at the *ab initio* B3LYP/6-31G*//HF/3-21G level of theory along with the minima found on this surface with the B3LYP/6-31+G** method. The energy contours are drawn every 1 kcal mol^{-1}.

Table 2 Relative Energies and Selected Conformational Parameters of the Studied Molecules in all their B3LYP/6–31+G** Energy-minimized Conformers

Conformer	ΔE			ϕ	ψ	χ^2
	Vacuum	DCM	ACN			
			Ac-(*E*)-ΔPhe-NHMe			
E	0.00	0.96	2.34	−179	162	−56
C	4.23	2.17	2.80	−75	69	−24
H/F	4.37	0.00	0.00	−43	123	−31
D	6.66	3.63	3.75	−153	38	−25
			Ac-ΔAla-NHMe			
E[a]	—			−172.2	154.1	
E*[a]	—			162.6	−169.4	
E	0.00	0.00	0.00	180	169	—
B	5.12	3.86	3.60	−58	26	—
D	5.86	3.17	2.50	−165	25	—
H/F	5.98	2.08	1.21	−45	137	—
			Ac-(*Z*)-ΔPhe-NHMe			
F[b]	—			56.8	−148.7	39.8
B	0.00	1.60	2.47	−56	24	−33
H/F	1.28	0.00	0.00	−44	139	−29
E	1.32	1.82	2.17	−129	160	19
E*	1.54	2.45	2.79	133	170	−21
D	2.87	1.53	1.55	−112	10	21

Energy regions of the (ϕ, ψ) conformational map are denoted in terms of the short-hand letter notation introduced by Zimmerman *et al.* [40]. Relative energy (ΔE) in kcal mol^{-1}. Angles in degree (°). Energy of the lowest conformer (Hartree): Ac-(*E*)-ΔPhe-NHMe (−725.734447193), Ac-ΔAla-NHMe (−494.667583604), Ac-(*Z*)-ΔPhe-NHMe (−725.73304 6984).
[a] crystal structure [52].
[b] crystal structure [56].

solid state and in all energy-minimized free conformers for these molecules. Table 3 collects the structural parameters of the internal X–H···A interaction and C=O dipole attractions, based on Steiner's [44] and Allen [45] criteria, respectively.

The conformational map for Ac-(*E*)-ΔPhe-NHMe reveals four minima: E, C, H/F and D (Figure 7). The lowest-energy conformer, positioned in region E presents the fully extended structure with the ϕ, ψ torsion angles of −179°, 162° (Table 2) that ensure the most effective π-electron cross-conjugation. This conformer is also stabilized by the N^1–H···O^2 hydrogen bond and by the weaker C^β–H···O^1 and N–H/π interactions (Table 3). The second conformer is located in region C, with angles ϕ, ψ of −75°, 69° significantly deviating from planarity. It is stabilized by the N^2–H^2···O^1 hydrogen bond as well as by two other interactions, N^2–H^2···N^1 and C(Ph)–H···O^2. It has also one sheared parallel dipole C=O ►···◄ C=O attraction [45]. The conformer C, however, has a much higher energy than the conformer E (ΔE_{C-E} = 4.23 kcal mol^{-1}). The

Table 3 Structural Parameters for the Internal X–H···A Interactions and C=O ►···◄ C=O Dipole Attractions in the B3LYP/6–31+G** Geometries of the Studied Molecules

Parameter	Ac-(*E*)-ΔPhe-NHMe				Ac-ΔAla-NHMe				Ac-(*Z*)-ΔPhe-NHMe				
	E	C	H/F	D	E	B	D	H/F	B	H/F	E	E*	D
N–H···O													
H···O	2.02	1.98	—	—	2.07	1.84	—	—	1.83	—	2.14	2.11	—
N···O	2.59	2.87	—	—	2.62	2.79	—	—	2.78	—	2.65	2.63	—
∠N–H···O	113	144	—	—	111	154	—	—	154	—	109	109	—
∠C=O···H	87	109	—	—	86	104	—	—	103	—	86	65	—
N–H···N													
H···N	—	2.84	—	N) 2.60 C) 2.51	—	2.68	N) 2.48 C) 2.50	—	2.66	—	—	—	N) 2.92 C) 2.36
N···N	—	3.06	—	2.75	—	2.98	2.76	—	2.96	—	—	—	2.77
∠N–H···N	—	93	—	N) 87 C) 93	—	97	N) 95 C) 94	—	97	—	—	—	N) 71 C) 103
C–H···O													
H···O	2.13	2.19	2.81	N) 2.23 C) 2.08	2.24	2.45	N) 2.32 C) 2.55	—	2.30	—	2.84	—	2.36
C···O	2.90	3.12	3.68	N) 2.95 C) 2.98	2.90	2.78	N) 2.92 C) 2.87	—	2.76	—	3.06	—	2.81
∠C–H···O	125	143	137	N) 122 C) 140	117	96	N) 113 C) 96	—	103	—	91	—	103
∠C=O···H	102	96	68	N) 95 C) 111	104	84	N) 102 C) 79	—	84	—	70	—	82
N–H···C(Ph)													
H···C	2.34	—	—	—	—	—	—	—	2.74	2.84	—	—	—
N···C	3.07	—	—	—	—	—	—	—	3.09	3.10	—	—	—
∠N–H···C	129	—	—	—	—	—	—	—	100	96	—	—	—
C=O ►···◄ C=O													
$C^C \cdots O^N$	—	3.32	2.84	—	—	3.32	—	2.84	3.32	2.82	3.18	—	—
$C^N \cdots O^C$	—	—	3.37	—	—	—	—	3.24	—	3.18	—	—	—
type		III	II			III		II	III	II			

Data presented only for the X–H···A contacts (X = N, C; A = O, N, C(Ph)) in which H···A ≤3.2 Å and ∠X–H···A > 90° acc. to ref. [44] and for C=O ►···◄ C=O attractions in which C···O <3.6 Å acc. to ref. [45]. Distances are given in (Å). Angles are given in (°). N,C — ascribe the structural parameters to the *N*–terminal and *C*–terminal, respectively.

third conformer, situated in region H/F, has torsion angles ϕ, ψ of −43°, 123° also significantly deviating from planarity. This structure is not N–H···O-hydrogen-bonded. It is stabilized basically by the slightly sheared antiparallel motif involving a pair of dipole C=O ►···◄ C=O attractions [45], and to a smaller extent, by the C(Ph)–H···O^2 interaction. Interestingly, its energy is only slightly higher than that of the conformer C ($\Delta E_{H/F-C} = 0.14$ kcal mol^{-1}). Finally, the least stable is conformer D (ϕ, $\psi = -153°$, 38°; $\Delta E_{D-E} = 6.66$ kcal mol^{-1}). The main stabilizing forces are two antiparallel N–H···N hydrogen bonds and additionally the C^β–H···O and C(Ph)–H···O^2 interactions.

DISCUSSION

As can be seen, Ac-(*E*)-ΔPhe-NHMe in dichloromethane displays a mixture of two conformers (Figure 3), with a predominance of conformer E. The spectrum of Ac-(*E*)-ΔPhe-NHMe is like that of Ac-ΔAla-NHMe, particularly in the region of bands AI and AII. The positions of these bands together with the low-frequency shifted N^1–H^1 bands prove that the conformers E of both compounds are stabilized by the quite strong N^1–$H^1 \cdots O^2$ hydrogen bonds. The same conclusion can be drawn from the Ramachandran diagrams (Figure 6). The torsion angles ϕ and ψ of the extended conformers E of both the (*E*)-ΔPhe and ΔAla compounds reveal that a quite strong π-electron conjugation appears involving the C^α=C^β double bond and both *N*- and *C*-terminal amide moieties. Therefore, conformers E of both compounds are placed in the global minima and the gaps in energy between them and the second in energy order are relatively large (Table 2).

A comparison of the positions of the AI(C^2=O^2), AII(C^1–N^1H^1) and AI(C^1=O^1) bands (Table 1) shows

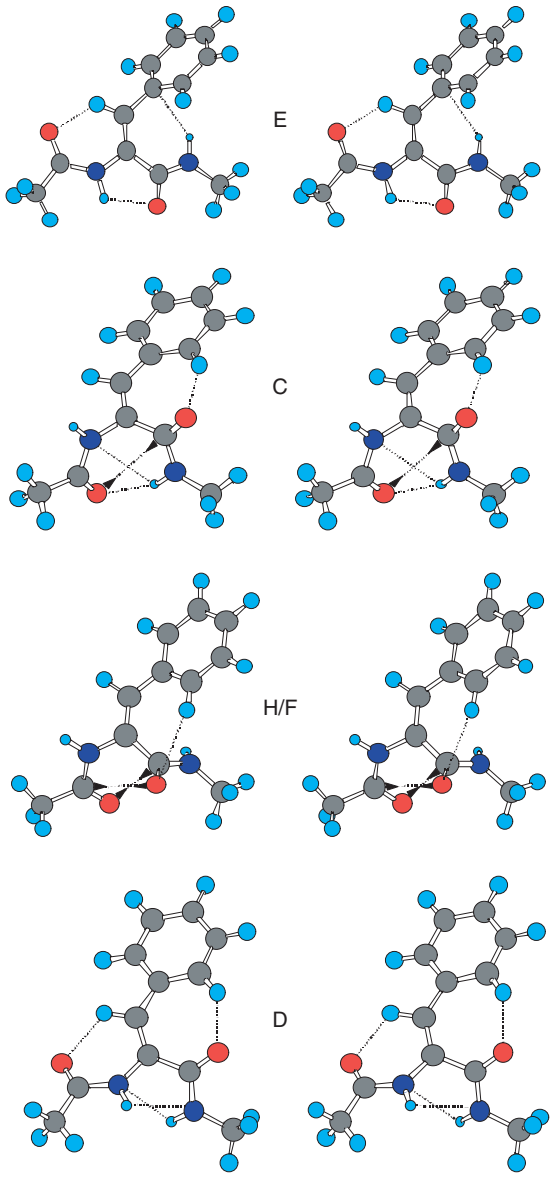

Figure 7 Stereo views of the conformers of Ac-(*E*)-ΔPhe-NHMe localized on the (ϕ, ψ) potential energy surface optimized at the B3LYP/6-311 + G** level of theory. Geometric parameters of hydrogen bonds and the C=O dipole attractions are collected in Table 3.

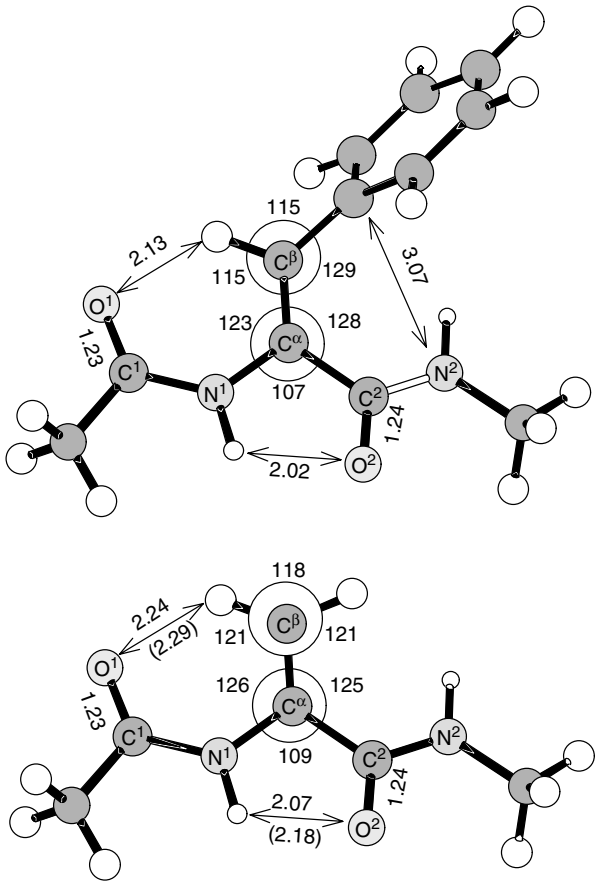

Figure 8 Selected structural parameters for the conformers E of the Ac-(*E*)-ΔPhe-NHMe and Ac-ΔAla-NHMe molecules. Crystal distances [43] are given in parenthesis.

that the C_5 hydrogen bond $N^1–H^1\cdots O^2$ and interaction $C^\beta–H\cdots O^1$ within Ac-(*E*)-ΔPhe-NHMe are somewhat stronger than those within Ac-ΔAla-NHMe. This is in agreement with interactions found in the calculated structures (Table 3), and can be explained by the influence of the phenyl ring in position *E*. The phenyl ring of Ac-(*E*)-ΔPhe-NHMe is not π-electron conjugated with the double bond $C^\alpha{=}C^\beta$ as the angle χ^2 amounts to -55°. There is a weak interaction between the phenyl ring π-system and the $N^2–H^2$ bond, instead. The distance (Figure 8) between the nearest C_{1p} carbon atom and the $N^2–H^2$ equals 3.07 Å, which can be relevant to the attraction forces ~1–2 kcal mol^{-1} [46,47]. This interaction is responsible for the shift of $\nu_s(N^2–H^2)$ band down to 3440 cm^{-1}, i.e. 26 cm^{-1} lower than the frequency of the corresponding band of the dehydroalanine analogue. Such interactions between the phenylalanine N–H and an aromatic ring occur often and shift the frequency of the ν_s (N–H) band by 11–50 cm^{-1} [19, 46–51]. As a result of the $N^2–H^2/\pi$ system interaction in Ac-(*E*)-ΔPhe-NHMe, an increase in the angle $C^\beta–C^\alpha–C^2$ and a decrease in the angles $N^1–C^\alpha–C^2$ and $N^1–C^\alpha–C^\beta$ ensue as the respective angles of Ac-ΔAla-NHMe are compared. The increase in the angle $C^\beta–C^\alpha–C^2$ effects a shortening of the distances $H^\beta\cdots O^1$ and $N^1–H^1\cdots O^2$ (cf. [43]), which causes the $N^1–H^1\cdots O^2$ and $C^\beta–H\cdots O^1$ interactions in Ac-(*E*)-ΔPhe-NHMe to be stronger than in the Ac-ΔAla-NHMe molecule.

In acetonitrile, the molecule of Ac-(*E*)-ΔPhe-NHMe loses its internal C_5 hydrogen bond and becomes unfolded, whereas that of Ac-ΔAla-NHMe does not vary. In conformer E of Ac-(*E*)-ΔPhe-NHMe, the $N^2–H^2$ is shielded by the phenyl ring and the molecule is weakly solvated. As a result, the conformational equilibrium shifts toward solvent-accessible open conformers and the conformer E disappears. On the contrary, the conformer E of Ac-ΔAla-NHMe is more stable

($\Delta E_{E-B} = 5.12$ kcal mol^{-1}), the uncovered N^2–H^2 moiety can be solvated with acetonitrile without a serious influence on the internal N^1–$H^1 \cdots O^2$ hydrogen bond and π-cross conjugation (the interaction between the model N-methyl acetamide and acetonitrile amounts to ~4.7 kcal mol^{-1}; Siodłak D; unpublished results). Thus, the conformer E of Ac-ΔAla-NHMe maintains its structure in acetonitrile and in the solid state as well [52]. To corroborate this reasoning and to answer the question of what sort of conformer of Ac-(*E*)-ΔPhe-NHMe in acetonitrile this is, the solvation energies of the ΔAla and ΔPhe compounds in this solvent were calculated. Data are given in Table 2 as ΔE_{ACN}. As seen, the interaction with the solvent can change the energetic orders of conformers. While for Ac-ΔAla-NHMe, the lowest-energy conformer continues to be conformer E, in the case of Ac-(*E*)-ΔPhe-NHMe, the conformer E is no longer the lowest-energy in acetonitrile. The lowest-energy one is the open conformer H/F.

Ac-(*Z*)-ΔPhe-NHMe in dichloromethane has a ν_s (N^1–H^1) band at 3401 cm^{-1}. This band is broad and seems to be composed of more than one component. Its position suggests the presence of a C_5 warped conformer [30] (Figure 3). In contrast to the deep conformational global minima of Ac-(*E*)-ΔPhe-NHMe and Ac-ΔAla-NHMe, driven by the main chain π-cross-conjugation, Ac-(*Z*)-ΔPhe-NHMe has a shallow potential energy surface resulting from a deficit in this conjugation (Table 2). The phenyl ring in Ac-(*Z*)-ΔPhe-NHMe divides the region of extended conformations in two, E and E*, and makes them warped and shifted toward the inside of the map (Figure 6). Therefore, the global minimum is occupied by conformer B [53,54]. Ac-(*Z*)-ΔPhe-NHMe, as calculated, in dichloromethane (Table 2, ΔE_{DCM}) and acetonitrile adopts conformation H/F. Similarly, as in vacuum, the intervals between the individual conformers are not large so that the gap between the highest and the lowest in energy conformer does not exceed 3 kcal mol^{-1}. Therefore, the conformation of this compound is not conclusive. As mentioned, in dichloromethane solution Ac-(*Z*)-ΔPhe-NHMe, a C_5 warped conformation is suggested. On the other hand, the low-frequency shifted ν_s(N^1–H^1) band at 3401 cm^{-1} may be also due to the interaction between the N^1–H^1 and phenyl ring π-system in the conformer H/F [55]. In solid state Ac-(*Z*)-ΔPhe-NHMe assumes conformation H/F [56].

As seen, only Ac-ΔAla-NHMe conserves conformer E in polar and H-bond forming solvent, i.e. acetonitrile, but the (*E*)-ΔPhe and probably (*Z*)-ΔPhe counterparts assume conformations H/F, whose H-bond donors are most exposed on the outside of the molecules. The solvation by H-bond forming solvent stabilized preferentially, sometimes by more than 3 kcal mol^{-1} [57,58], the open structures that are energetically unfavourable in the gas phase. This is expected in view of the greater potential for intermolecular hydrogen bonding possessed by these structures. So, it is not surprising that in acetonitrile, Ac-(*E*)-ΔPhe-NHMe loses its internally C_5 bonded conformation and adopts conformation H/F.

The FTIR and theoretical analyses show that Ac-(*E*)-ΔPhe-NHMe has a determined tendency to adopt the extended conformer E in a non-polar environment. The same tendency seems to be true in the case of the few existing examples of peptides with the (*E*)-ΔPhe residue. TFA-Gly-(*E*)-ΔPhe [59] and Pht-(*E*)-ΔPhe-NH*t*Bu [60] in the solid state are in extended conformation. In Boc-Ala-(*E*)-ΔPhe-Val-OMe, two NHs, (*E*)-ΔPhe NH and Val NH, participate in intramolecular hydrogen bonding, based on the NMR data. It has been concluded that the peptide takes in chloroform in two consecutive γ-turn conformers [27]. We suggest another alternative, plausible explanation. ΔPhe N^1–H^1 would be involved in the C_5 H-bonded conformation E and Val NH [i.e. (*E*)-ΔPhe N^2–H^2] would be engaged in the interaction with the phenyl ring as is the case for Ac-(*E*)-ΔPhe-NHMe. The remarkable NOE between Val NH and (*E*)-ΔPhe phenyl H in this molecule appears to support the supposition. The FTIR and theoretical analyses also indicate a similar tendency for Ac-(*E*)-ΔAbu-NHMe to adopt conformer E [31,53] in a non-polar environment. A more aggressive solvent such as acetonitrile, in the case of both the (*E*)-ΔPhe and (*E*)-ΔAbu compounds, disrupts the C_5 hydrogen bond and shifts the conformational equilibrium to populate the more open form. This conformational dimorphism seems to be a general feature of the (*E*)-ΔXaa residues and may be of biological interest as a function [61] or selectivity switch [62].

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