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Conformational investigation of α,β-dehydropeptides. XV: *N*-acetyl-α,β-dehydroamino acid *N′N′*-dimethylamides: conformational properties from infrared and theoretical studies‡

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Abstract: The FTIR spectra were analysed in the region of the ν_s(N–H), AI(C=O) and $\nu_s(C^{\alpha}=C^{\beta})$ bands for a series of Ac-ΔXaa-NMe_2, where ΔXaa = ΔAla, (*Z*)-ΔAbu, (*Z*)-ΔLeu, (*Z*)-ΔPhe and ΔVal, to determine a predominant solution conformation of these α,β-dehydropeptide-related molecules. Measurements were taken in CCl_4, DCM and MeCN solutions. In the same way, spectra of saturated analogues Ac-Xaa-NMe_2, where Xaa = Ala, Abu, Leu, Phe and Val, were investigated. To help interpret the spectroscopic results, conformational maps were calculated by the B3LYP/6–31+G** method. Also, the relative energies of all conformers of the dehydro compounds *in vacuo* as well as in the studied solvents in addition to the theoretical IR frequencies of these conformers were calculated. For comparison, molecules of two saturated analogues, Ac-L-Ala-NMe_2 and Ac-L-Phe-NMe_2, were calculated in a similar way. Both unsaturated and saturated compounds, which have an aliphatic side chain, occur in CCl_4 and DCM mainly as a mixture of extended conformers with the C_5 H-bond and open conformers. As solvent polarity increases, participation of the open conformers also increases, and in MeCN, the model amides are almost exclusively in the open form, except Ac-ΔAla-NMe_2, which shows a small amount of the H-bonded conformer. Ac-ΔAla-NMe_2 and Ac-ΔAbu-NMe_2 have stronger C_5 hydrogen bonds than those of their saturated counterparts. As the calculations indicate, the open conformation of the unsaturated amides is conformer H/F with ϕ, ψ $-44 \pm 5°$, $127 \pm 4°$. This is the second lowest in energy conformer *in vacuo* and in CCl_4 and the lowest one in more polar solvents. The open conformation of Ac-L-Ala-NMe_2 constitutes conformer C with ϕ, ψ $-101.5°$, $112.7°$. For Ac-ΔAla-NMe_2 and Ac-ΔAbu-NMe_2, FTIR also reveals the presence of a third conformer. Calculations indicate that is the semiextended conformer D with the N^1-$H^1\cdots N^2$ hydrogen bond/contact. In all solvents, Ac-L-Phe-NMe_2 and Ac-(*Z*)-ΔPhe-NMe_2 show only the extended E and the open H/F, respectively. In both there is an amide/π(Ph) interaction.

Keywords: α,β-dehydroamino acids; density functional theory calculations; FTIR spectroscopy; theoretical IR frequencies; conformation; C_5 hydrogen bond; solute/solvent interaction; amide/π(Ph) interaction

INTRODUCTION

Conformational constraints, introduced into a peptide structure by a peptide modifier, could influence the selectivity of a biological action, since the biological effects mediated by peptides greatly depend on their conformational properties [2–4]. One group of the popular peptide modifiers is α,β-dehydroamino acids. They can confer local constraints on both the backbone of the main chain (the angle ϕ, ψ) and the side chain of the amino acid residue (the configuration *Z* as usual or *E* as exceptional) [5,6]. On the other hand, a lot of peptide modifications are based on a tertiary amide bond, which can provide, among others hydrophobicity and metabolic stability. Some peptides are synthetically processed to the *C*-terminal *N′*,*N′*-dimethylamides [7]. *N*-methyl peptides have been recognized for a long time [8–14] while peptoid peptidomimetics (*N*-substituted glycines) are a relatively new class of potential pharmacological tools and drugs [15, 16].

The simplest compounds, which combine structural features of α,β-dehydroamino acids with the tertiary amide group, are *N*-acetyl-α,β-dehydroamino acid *N′*,*N′*-dimethylamides Ac-ΔXaa-NMe_2 (Figure 1). They (where ΔXaa = ΔAla [17], (*Z*)-ΔAbu [18], (*Z*)-ΔLeu [19], ΔVal [20] and (*Z*)-Δ Phe [21]) have been proven to have extremely conserved structure. In the solid state, all of them assume the open conformation which features the angles ϕ, ψ = ∼−45°, ∼130° (for Val, $\phi = -60°$). The conformation is independent of the side chain [H, Me, $CH(Me)_2$, Ph, 2 × Me] and intermolecular contacts. It has not been observed for the known crystal structures of the saturated *N*-acetyl *N′*,*N′*-dimethylamides Ac-L/DL-Xaa-NMe_2, which have various conformations in the solid state [17–21,22–24]. The conformational maps for the unsaturated dimethylamides *in vacuo* also indicate in all cases the presence of this conserved

Abbreviations: As recommended in *J. Peptide Sci.* 2003; **9**: 1–8 with the following addition: acetonitrile, MeCN.

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‡ For Part XIV in this series see Reference [1]. The long delay between receipt and revision was caused by a communications failure.

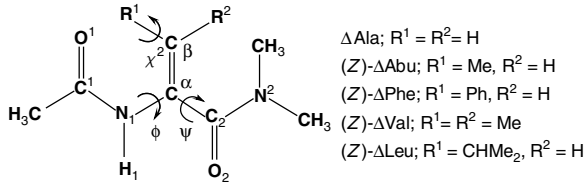

Figure 1 General formula, atom numbering and selected torsion angles for the studied α,β-dehydroamino acid residues with ascription of the β-substituent.

structure [21,25]. The lowest in energy order is the extended conformer E with the $N^1{-}H^1\cdots O^2{=}C^2$ hydrogen bond, but the second is this open conformer. For Ac-(*Z*)-ΔPhe-NMe_2, the latter conformer is in fact the lowest in energy. This conserved conformer is called H/F, because it is on the borderline between two conformers, H and F, according to the general shorthand letter notation introduced by Zimmerman [26,27]. The conformational map is quite different for Ac-L-Ala-NMe_2. Again the lowest in energy is the extended conformer E, but the second is the open conformer C, with ϕ, ψ −101.5°, 112.7°. The remaining conformers are open, high-energetic ones [28]. It would be interesting to know which conformer(s) would be adopted by Ac-ΔXaa-NMe_2 in solutions of various polarity. To this end, we recorded the FTIR spectra of Ac-ΔXaa-NMe_2 in CCl_4, DCM and MeCN and for comparison, those of Ac-L-Xaa-NMe_2. The relative energies of all conformers of Ac-ΔXaa-NMe_2, Ac-L-Ala-NMe_2 and Ac-L-Phe-NMe_2 *in vacuo* and in the studied solvents as well as the theoretical IR frequencies of these conformers were calculated to help interpret the spectroscopic results.

MATERIALS AND METHODS

Materials

Ac-ΔAla-NMe_2 and Ac-(*Z*)-ΔPhe-NMe_2 were obtained according to references [17] and [21], respectively. Ac-(*Z*)-ΔAbu-NMe_2, Ac-(*Z*)-ΔLeu-NMe_2 and Ac-ΔVal-NMe_2 were synthesized according to reference [29]. Ac-Xaa-NMe_2, where X = Ala, DL-Abu, DL-Leu, Phe and Val, were yielded according to references [17], [18], [19], [30], [21], respectively. All of them were above 98.6% purity by HPLC. The analytical grade CCl_4, DCM and MeCN were dried over P_2O_5, distilled and stored over freshly prepared molecular sieves.

FTIR Spectra

The FTIR spectra were recorded at 20 °C on a Nicolet Magna 860 spectrometer equipped with a DTGS detector and flushed with dry nitrogen during the measurements. All represent an average of 512 scans at 2 cm^{-1} resolution. Quartz cells (15 and 100 mm) and KBr liquid cells (0.2 and 2.86 mm) were used. If necessary, the spectra were analysed with the GRAMS/386 program and the curve fitting procedure with the mixed Gaussian-Lorentzian sum functions was applied [31].

Computational Procedures

The theoretical conformational properties were examined on the free Ac-ΔAla-NMe_2, Ac-(*Z*)-ΔAbu-NMe_2, Ac-ΔVal-NMe_2, Ac-(*Z*)-ΔPhe-NMe_2 and Ac-L-Ala-NMe_2 molecules using the Gaussian 03 package [32]. Calculations were performed on the *trans*-acetyl group ($\omega_0 \sim 180°$). To generate the (ϕ, ψ) potential energy surfaces of Ac-ΔXaa-NMe_2, 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 for the constrained torsion angles ϕ and ψ. The values of these angles were chosen using a step size 30°, within the range −180° to 180° for the angle ϕ, and 0° to 180° for the angle ψ. Inversion through achiral α-carbon [i.e. (ϕ, ψ) → ($-\phi$, $-\psi$)] yields equivalent structures; thus the full (ϕ, ψ) potential energy surface maps were obtained in this way [33]. To generate the (ϕ, ψ) potential energy surfaces of Ac-L-Ala-NMe_2, 144 structures were used, and the values of the angles ϕ, ψ were chosen using a step size 30°, within the range −180° to 180°. The potential energy surfaces were created using the Surfer 8 program with the radial basis function as a gridding method [34]. The minima observed on the surfaces have been fully optimized at the B3LYP/6–31 + G** level [21,25,28]. To obtain the theoretical IR frequencies, a second derivative analysis on the optimized structures was performed. A variable scaling procedure was used with different scaling factors depending on the different types of vibrational modes, i.e. 0.95 for ν_s(N–H) and 0.97 for $\nu_s(C^\alpha{=}C^\beta)$ and amide I modes [35]. The effect of electrostatic solute/solvent interaction on the solute energies was investigated on the geometries of solutes *in vacuo* within the SCRF method using the polarizable continuum model (PCM) [36].

As the overall conformational profiles of the modified peptides can differ from those of common peptides, the energy-minimized conformers of the investigated molecules are described by the general shorthand letter notation introduced by Zimmerman [26].

RESULTS

FTIR Spectra

Figure 2 shows the N–H stretching bands of the saturated dimethylamides Ac-Xaa-NMe_2. The amides with the aliphatic side chain have two ν_s(N–H) bands in CCl_4 solutions. One derives from an open conformer having the frequencies 3435–3437 cm^{-1} and the other from the extended conformer with the C_5 hydrogen bond, having the frequencies 3416–3420 cm^{-1} [37,38]. The intensity of the former band increases with the bulkiness of the side chain and the greatest amount of open form is for Ac-Val-NMe_2. In DCM, both bands shift toward lower frequencies by several cm^{-1}. The open conformer is higher than that in CCl_4 and also increases with the bulkiness of the side chain, so the compounds with Leu [38] and Val predominantly adopt the open form. In MeCN, there is only one band, which lies in the range of 3389–3393 cm^{-1} and can be ascribed to the open form. The spectrum of Ac-Phe-NMe_2 in the ν_s(N–H) region in all studied solvents

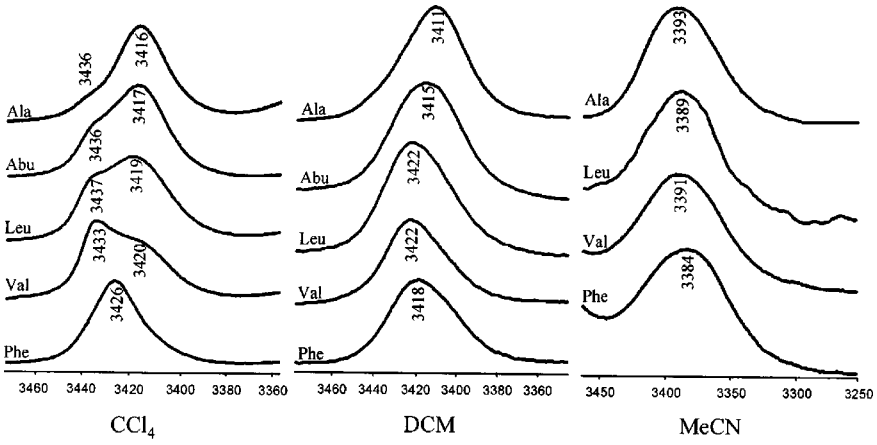

Figure 2 The FTIR spectra of saturated Ac-Xaa-NMe_2 in the ν_s(N–H) region.

shows only one band [38], the frequency of which is up to ~10 cm^{-1} lower than those of the open conformers of these peptides with the aliphatic side chain. The saturated dimethylamides show two bands in the region of AI(C=O), (Table 1). The band higher in frequency, 1679–1684 cm^{-1} (CCl_4), belongs to AI(C^1=O^1) and that of lower frequency, 1645–1652 cm^{-1}, to AI(C^2=O^2) mode [37]. The frequency of AI(C^1=O^1) and AI(C^2=O^2) in DCM decreases compared with the spectra in CCl_4, and in MeCN. The former's frequency remains almost constant, but the latter's increases and becomes narrower.

Figure 3 presents the N–H stretching bands originated from monomers of the dimethylamides Ac-ΔXaa-NMe_2 in CCl_4, DCM and MeCN solutions. The spectrum of Ac-ΔAla-NMe_2 in CCl_4 has one broad band at 3407 cm^{-1} with an inflexion on the high frequency edge at 3444 cm^{-1}. It indicates conformer E with the C_5 H-bond as preferential and the open conformer with the free N–H group as marginal. Also for Ac-(*Z*)-ΔAbu-NMe_2, two bands are visible at 3442 cm^{-1} and 3406 cm^{-1}, the latter with the inflexion on the high-frequency edge. The curve-fitting procedure (Figure 4A) gives a third band at 3429 cm^{-1}. Three bands in the monomer region prove the presence of three conformers. The band at 3442 cm^{-1} belongs to the open conformer and that at 3406 cm^{-1} to conformer E. The band at 3429 cm^{-1} comes from the third conformer, in which the N–H is engaged in a hydrogen bond weaker than that in conformer E. The spectrum of Ac-ΔVal-NMe_2 shows two bands, at 3438 and 3418 cm^{-1}, derived from the open conformer and conformer E, respectively. The position of the latter proves that the hydrogen bond is weaker than that in the ΔAla and ΔAbu derivatives. The spectrum Ac-(*Z*)-ΔPhe-NMe_2 has exclusively one narrow band with $\Delta\nu_{1/2} = 12$ cm^{-1}, which means that the compound occurs only in one conformation. This band has an untypical, decreased position with the maximum at 3424 cm^{-1}, which is ~20 cm^{-1} lower compared with the maxima of the open conformers of the analogues with the aliphatic side chains and represents a conformer with an amide/π(Ph) interaction.

Table 1 The FTIR Frequencies of Ac-ΔXaa-NMe_2 and Ac-Xaa-NMe_2 in Various Solvents in the AI(C=O) and $\nu_s(C^\alpha{=}C^\beta)$ Modes Region

Xaa	AI(C^1=O^1)	AI(C^2=O^2)	$\nu_s(C^\alpha{=}C^\beta)$
CCl_4			
ΔAla	1703	1628	1644
(Z)-ΔAbu	1701	1631	1666
(Z)-ΔLeu	1700	1632	1662
ΔVal	1694	1637	1665
(Z)-ΔPhe	1706	1652	1644
Ala	1679	1652	—
Abu	1681	1650	—
Leu	1683	1651	—
Val	1684	1648	—
Phe	1679	1645	—
DCM			
ΔAla	1695	1624 1633	1647
(Z)-ΔAbu	1693	1632	1667
(Z)-ΔLeu	1692	1633	1662
ΔVal	1688	1627	1665
(Z)-ΔPhe	1695	1651	1638
Ala	1670	1644	—
Abu	1672	1642	—
Leu	1673	1644	—
Val	1675	1641	—
Phe	1672	1644	—
MeCN			
ΔAla	1697	1635	1649
(Z)-ΔAbu	1690	1636	1667
(Z)-ΔLeu	1690	1637	1663
ΔVal	1688	1633	1665
(Z)-ΔPhe	1694	1649	1638
Ala	1674	1650	—
Abu	1674	1650	—
Leu	1674	1650	—
Val	1674	1646	—
Phe	1675	1650	—

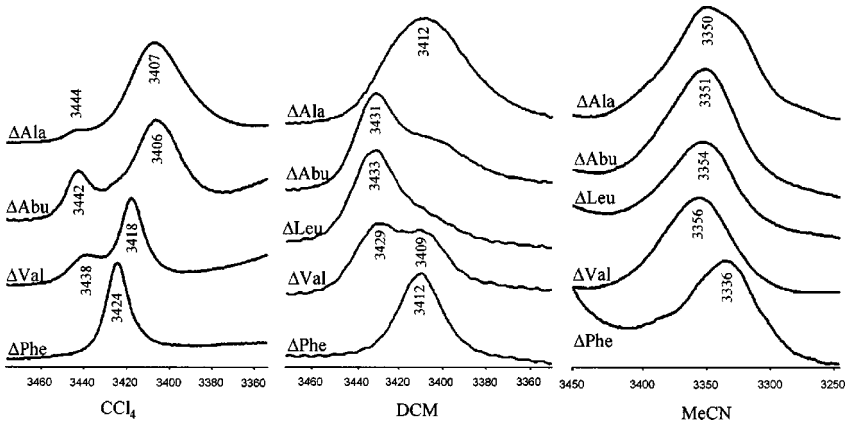

Figure 3 The FTIR spectra of unsaturated Ac-ΔXaa-NMe_2 in the ν_s(N–H) region.

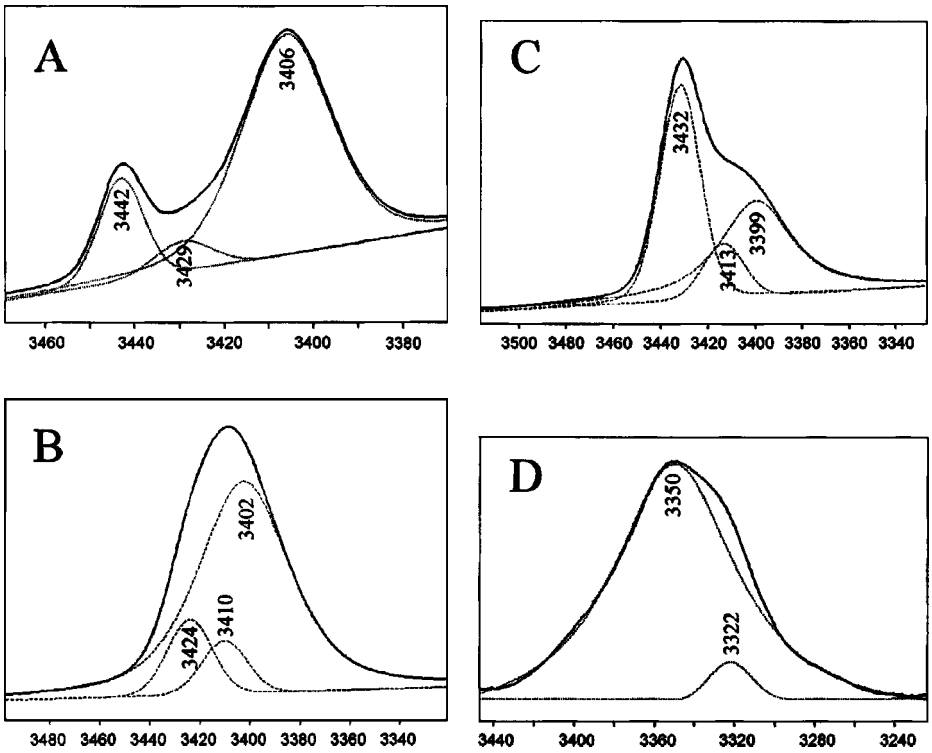

Figure 4 The curve-fitted spectra of unsaturated Ac-ΔXaa-NMe_2 in the ν_s(N–H) region. (A) ΔAbu in CCl_4; (B) ΔAla in DCM; (C) ΔAbu in DCM; (D) ΔAla in ACN.

When going from CCl_4 to DCM, the ν_s(N–H) bands of the unsaturated compounds become substantially broadened and lowered in position by ~5–20 cm^{-1} and those, which derive from the open conformers become more intensive. In the case of Ac-ΔAla-NMe_2 and Ac-(*Z*)-ΔAbu-NMe_2, the curve-fitting procedure resolves the recorded bands each into three components, the former at 3424, 3410 and 3402 cm^{-1} and the latter at 3432, 3413 and 3399 cm^{-1} with the assignments like those for Ac-(*Z*)-ΔAbu-NMe_2 in CCl_4 solution and with a preference to the open form for the ΔAbu peptide (Figure 4B and C). For Ac-(*Z*)-ΔLeu NMe_2 and Ac-ΔVal-NMe_2, the bands at 3432 (3431) and 3410 (3408) cm^{-1} have been ascribed, respectively, to the open conformer, which was major, and conformer E, which was minor. Ac-(*Z*)-ΔPhe-NMe_2 shows only one band, relatively narrow, with the maximum at 3412 cm^{-1}. The band, as previously in CCl_4, has a lowered frequency of the N–H group in relation to the aliphatic analogues with the free N–H group. In MeCN solution spectra, the unsaturated peptides have, as a rule, only one band in the range 3350–3356 cm^{-1}. The frequency of Ac-(*Z*)-ΔPhe-NMe_2 is, as usually lower, i.e. at maximum 3336 cm^{-1}. Ac-ΔAla-NMe_2, shows an inflexion at the low-frequency edge, which originates from a small amount of conformer E with the internal

H-bond. After the curve fitting procedure, the position of this band was found to be at 3322 cm^{-1} (Figure 4D).

As can be seen, in CCl_4 the spectra of the unsaturated compounds with the aliphatic side chains are dominated by extended H-bonded forms. In DCM the open forms are preferred. An exception is Ac-ΔAla-NMe_2 where conformer E prevails. Also in MeCN, only open forms are observed for Ac-ΔXaa-NMe_2 with the N–H groups H-bonded with the solvent, $N^1–H^1\cdots N{\equiv}CCH_3$. In addition to this bond, a small portion of Ac-ΔAla-NMe_2 exists in a form which has the bifurcated hydrogen bond of the $N^1–H^1$ group with the $C^2{=}O^2$ and acetonitrile N≡C group. The conformations of the Phe and ΔPhe peptides will be discussed separately.

The spectra of Ac-ΔXaa-NMe_2 in the region of AI(C=O) and $\nu_s(C^\alpha{=}C^\beta)$ have three bands (Table 1). The exception is the spectrum of Ac-ΔAla-NMe_2 in DCM. It shows four bands including two AI($C^2{=}O^2$), where one comes from the H-bonded C=O group and another from the free group. The highest in frequency bands of Ac-ΔXaa-NMe_2 in CCl_4, 1694–1706 cm^{-1}, belong to AI($C^1{=}O^1$) in agreement with the frequencies of AI($C^1{=}O^1$) of Ac-ΔXaa-NHMe [40]. The remaining two bands are ascribed either to the isolated AI($C^2{=}O^2$) and $\nu_s(C^\alpha{=}C^\beta)$ or to the coupled vibrators of the $C^\alpha{=}C^\beta$ and $C^2{=}O^2$ groups (Table 3) [41]. When going from CCl_4 to DCM, the frequencies of AI($C^1{=}O^1$) and AI($C^2{=}O^2$) lower by several cm^{-1}. In MeCN, these for the former bands remain constant and for the latter slightly increase.

Theoretical Conformation Analysis

Figure 5 presents the 2D-Ramachandran surfaces for Ac-ΔXaa-NMe_2 and Ac-L-Ala-NMe_2 *in vacuo* and Table 2 lists the energy-minimized conformers of these compounds and Ac-L-Phe-NMe_2 *in vacuo* in CCl_4, DCM and MeCN. The maps have the same number of minima and the same energy order as the maps obtained by the HF/6–31G*//HF/3–21G method [21,25,28]. They are very similar to those previously described but the conformational profiles of the maps, obtained by the B3LYP/6–31G*//HF/3–21G method better fit, however, to the minima optimized by the B3LYP/6–31 + G** method. As can be seen, for the dehydro compounds with the aliphatic side chain, conformers E are the lowest-energy ones *in vacuo* and in CCl_4. Conformers H/F constitute the second lowest. In the latter, the N–H groups are exposed outside the molecules and are capable of forming a stronger interaction with the solvent than those in conformer E. The energy gap between conformers H/F and E diminishes with an increase in solvent polarity. Finally, in MeCN, conformers H/F are the lowest in energy. For Ac-(*Z*)-ΔPhe-NMe_2 *in vacuo*, the stability order is reversed; the most stable is conformer H/F and the second is conformer E. This reversed order results from an amide/π(Ph) interaction within conformer H/F. The interaction with the solvent stabilizes this conformer more, so the energy gap between conformers H/F and E increases with the increase in solvent polarity. In the case of Ac-L-Ala-NMe_2, in all solvents, the lowest in energy is conformer E and the second is conformer C. Along with the increase in the solvent polarity, the energetic gap between the C conformer and E decreases because the open conformer C is stabilized by the solvents more. For Ac-L-Phe-NMe_2,

Table 2 Selected Conformational Parameters and Relatives Energies *In Vacuo* and in Solvents of the Studied Molecules in their B3LYP/6–31 + G** Energy-Minimized Conformers

Conformer	ϕ	ψ	ΔE			
			Vac.	CCl_4	DCM	MeCN
		Ac-ΔAla-NMe_2				
E	−179.7	152.9	0.00	0.00	0.00	0.62
H/F	−39.5	125.2	3.67	2.30	0.31	0.00
D	−176.7	47.5	4.29	3.52	2.42	2.55
		Ac-(*Z*)-ΔAbu-NMe_2				
E	−133.2	148.6	0.00	0.00	0.43	1.00
H/F	−45.0	131.4	1.90	0.94	0.00	0.00
E*	120.5	154.2	3.00	2.62	2.34	2.58
D	−127.3	46.9	3.08	1.45	0.33	0.25
H*	24.3	61.5	4.84	3.62	2.50	2.44
G*	124.2	50.2	6.24	5.55	5.07	5.29
		Ac-ΔVal-NMe_2				
E	−120.1	122.6	0.00	0.00	0.31	0.65
H/F	−48.6	124.2	0.68	0.31	0.00	0.00
E*	111.8	136.1	3.37	2.89	2.25	2.06
		Ac-(*Z*)-ΔPhe-NMe_2				
H/F	−38.6	127.6	0.00	0.00	0.00	0.00
E	−127.7	146.3	1.13	1.85	2.58	2.82
E*	124.4	155.6	3.36	3.97	4.34	4.36
D	−114.7	49.1	4.79	4.23	3.98	3.85
G*	126.0	50.6	6.92	7.05	6.97	6.86
		Ac-L-Ala-NMe_2				
E	−153.7	159.6	0.00	0.00	0.00	0.00
C	−101.5	112.7	1.30	1.11	0.57	0.44
G	−158.3	−47.9	7.76	7.33	6.62	6.40
A	−67.1	−35.8	7.84	6.29	4.33	2.81
A*	50.1	53.4	8.33	6.97	4.38	2.90
D*	114.1	−59.7	8.46	7.57	6.40	6.00
F*	75.7	165.1	8.90	7.97	6.32	5.57
		Ac-L-Phe-NMe_2				
E(t)	−152.7	141.1	0.00	0.00	0.00	0.23
E(g)	−153.0	162.6	1.35	1.24	0.87	0.92
E(-g)	−124.2	152.6	1.69	1.39	0.80	0.68
C(-g)	−102.5	111.4	1.72	1.15	0.18	0.00
A(-g)	−59.8	−39.3	7.19	6.14	4.73	4.39
A*(-g)	48.4	54.2	7.21	6.14	4.10	3.01

Energy regions of the (ϕ, ψ) conformational map are denoted in terms of the short-hand letter notation introduced by Zimmerman *et al.* [26]. Relative energy (ΔE) in kcal mol^{-1}. Angles in degree (°).

Table 3 The Scaled[a] Theoretical IR Frequencies of the Studied Molecules Obtained by the B3LYP/6–31 + G** Method (in the parenthesis experimental value in CCl_4 solutions)

Conformer	$\nu_s(N^1-H^1)$	AI($C^1=O^1$)	AI($C^2=O^2$)	$\nu_s(C^\alpha=C^\beta)$
	Ac-ΔAla-NMe_2			
E	3398 *(3407)*	1698 *(1703)*	1631asym *(1628)*	1642sym *(1644)*
H/F	3452 *(3444)*	1707	1667	1643
D	3447	1708	1675asym	1641sym
	Ac-(*Z*)-ΔAbu-NMe_2			
E	3401 *(3406)*	1699 *(1701)*	1632 *(1631)*	1665 *(1666)*
H/F	3448 *(3442)*	1704	1659	1670
D	3431 *(3429)*	1702	1671asym	1646sym
E*	3415	1725	1634	1665
H*	3452	1716	1658	1675
G*	3443	1720	1681asym	1664sym
	Ac-ΔVal-NMe_2			
E	3415 *(3418)*	1695 *(1694)*	1634 *(1637)*	1670 *(1665)*
H/F	3440 *(3438)*	1699	1651	1663
E*	3424	1723	1639	1653
	Ac-(*Z*)-ΔPhe-NMe_2			
H/F	3425 *(3424)*	1705 *(1706)*	1663 *(1652)*	1638 *(1638)*
E	3389	1710	1630	1647
E*	3395	1733	1630	1641
D	3416	1696	1656asym	1628sym
G*	3433	1728	1674asym	1646sym
	Ac-L-Ala-NMe_2			
E	3415 *(3416)*	1679 *(1679)*	1654 *(1652)*	—
C	3446 *(3436)*	1679	1658	—
	Ac-L-Phe-NMe_2			
E(t)	3430 *(3426)*	1677 *(1679)*	1645 *(1645)*	—
E(g)	3417	1678	1658	—
E(-g)	3422	1691	1653	—
C(-g)	3451	1678	1656	—
A(-g)	3424	1702	1662	—
A*(-g)	3449	1705	1675	—

[a] Scaling factors [35]:
for $\nu_s(N^1-H^1)$ mode −0.95,
for AI(C=O) and $\nu_s(C^\alpha=C^\beta)$ modes −0.97.

the orientation of the aromatic ring influences the backbone conformation [42]. In order to monitor this effect, Ac-L-Phe-NMe_2 was computed for the two lowest energy conformers E and C on three staggered χ^1 rotamers, *trans* ($\chi^1 \sim 180°$), *gauche* (−) ($\chi^1 \sim -60°$) and *gauche* (+) ($\chi^1 \sim +60°$) (the angles ϕ, ψ in the starting structures were the same as the angle ϕ, ψ in the Ac-L-Ala-NMe_2 minima). Only the six lowest energy conformers are presented in Table 2. Conformer E with χ^1 in the *trans* region proves to correspond to the global minimum.

Table 3 shows the scaled theoretical ν_s(N–H), AI(C=O) and $\nu_s(C^\alpha=C^\beta)$ IR frequencies of the conformers of the studied molecules, yielded by the B3LYP/6–31 + G** method and these are compared with the experimental frequencies in CCl_4 solutions. The mean frequency deviation between the experimental data and those calculated in CCl_4 amounts to 4.5 cm^{-1} for ν_s(N–H) and 2.6 cm^{-1} for the others, which denotes an excellent consistency in both sets of findings [35].

DISCUSSION

Comparing the ν_s(N–H) frequencies of the unsaturated and saturated compounds, which have the aliphatic side chains, it can be seen that both series in CCl_4 and DCM occur mainly as a mixture of conformers E and the open ones (Figure 6). As the solvent polarity increases, the participation of the open conformers also increases, and in MeCN, all sample model compounds adopt almost exclusively the latter form. The ν_s(N–H) bands of conformers E of the ΔAla and ΔAbu derivatives lie ~10 cm^{-1} lower than those of the saturated compounds. This indicates stronger hydrogen bonds in the case of these dehydro analogues. However, within the ΔVal derivative, two β-methyl groups are

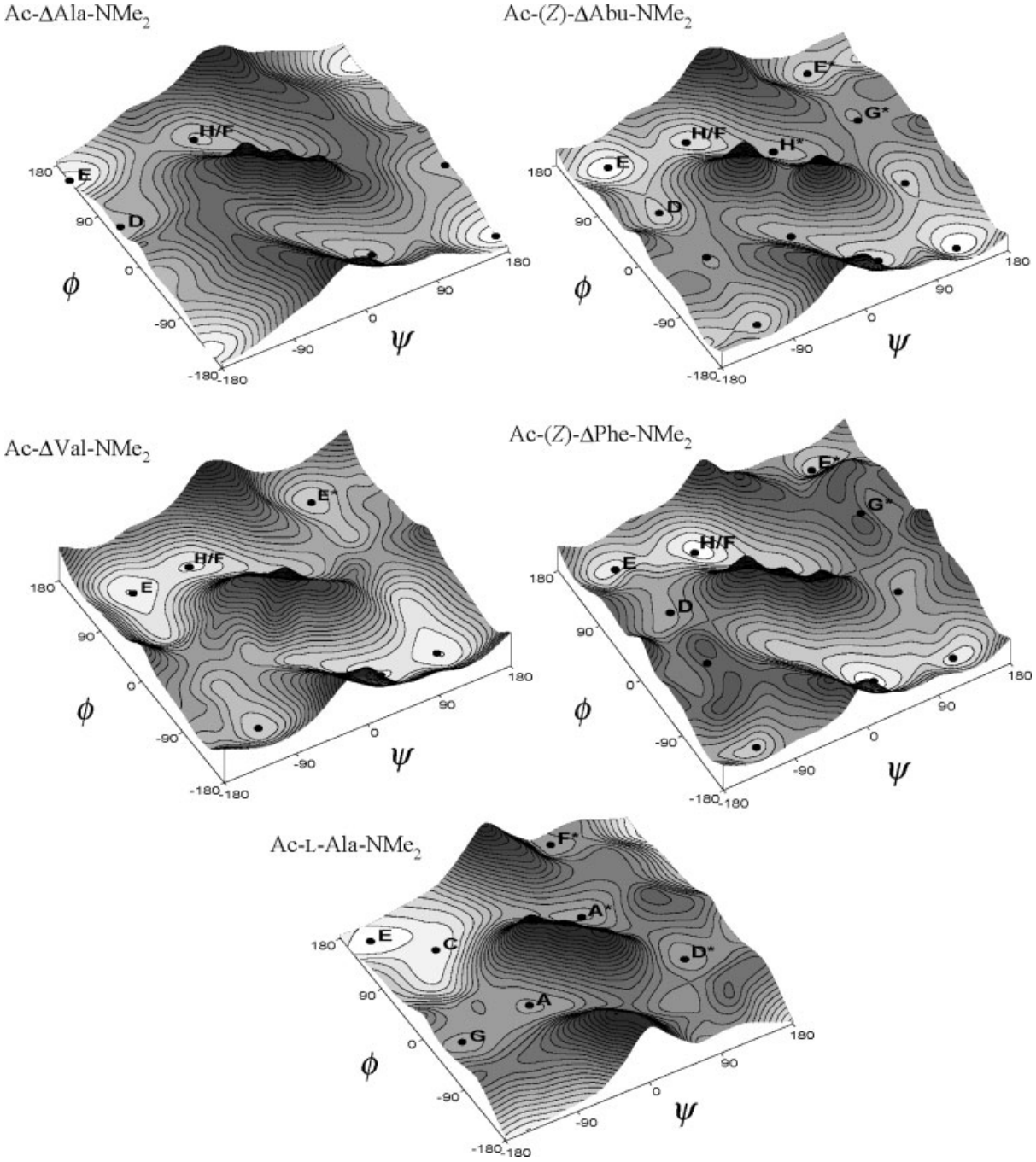

Figure 5 The landscape representations of the (ϕ, ψ) potential energy surface of the studied free molecules calculated at the B3LYP/6–31G*//HF/3–21G level of theory along with the minima found on these surfaces with the B3LYP/6–31 + G** method. The energy counters are drawn every 1 kcal mol^{-1}.

prevented the molecule from adopting such an extended conformation as that accommodated by the two former compounds. The hydrogen bond is weaker and its geometry departs from the optimal parameters. The ν_s(N–H) band is higher in position and equals that of the Val derivative. Ac-L-Phe-NMe$_2$ in CCl_4 and DCM is in conformation E with the side chain in the form of the *trans* χ^1 rotamer, but not in an open form as suggested earlier [38]. In MeCN, the N–H group of the discussed dimethylamides and the C≡N group of the solvent are hydrogen bonded. The bands ν_s(N–H) of the dehydro compounds have frequencies lower by ~40 cm^{-1} than those of the saturated ones, which denotes the formation of stronger hydrogen bonds by the former. Similar differences (~60 cm^{-1}) in the position of the ν_s(N–H) bands in MeCN were observed in the case of a series of Ac-ΔXaa-NHMe compared with Ac-ΔXaa-NHMe [40]. They seem to result from a greater polarizability of the N–H group of the dehydro compounds. Also, the nature of the

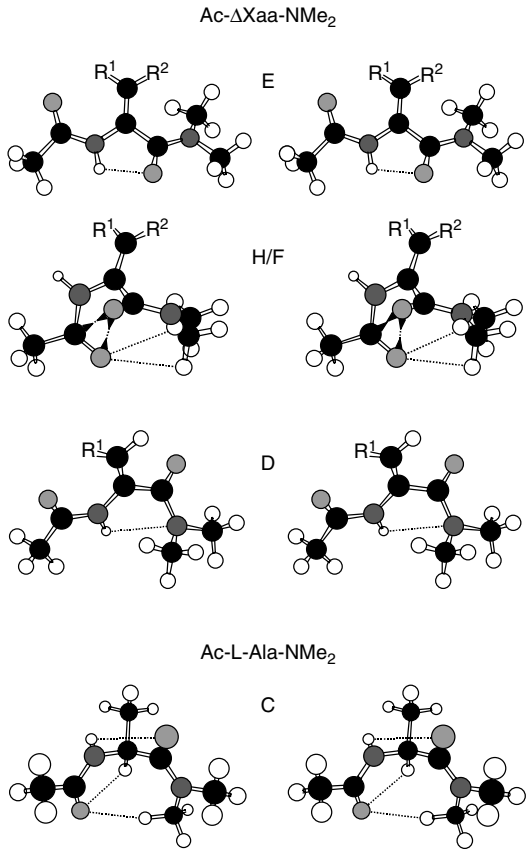

Figure 6 The stereo views of the E, H/F and D conformers found in solutions of the studied Ac-ΔXaa-NMe_2 molecules and the C conformer for Ac-L-Ala-NMe_2 along the stabilizing internal hydrogen/contact (···) and the C=O dipole–dipole attractions (►···◄).

$C^1{=}O^1$ group is altered by the influence of the $C^\alpha{=}C^\beta$ bond. The frequencies of AI($C^1{=}O^1$) of the unsaturated dimethylamides are higher by 10–27 cm^{-1} (Table 1) than those of the saturated counterparts [40]. The greatest difference is for the pair ΔAla–Ala and the smallest is for the pair ΔVal–Val. These differences in position result from a diminution in amidic resonance in relation to the saturated amides by reason of the π-electron conjugation of the $C^\alpha{=}C^\beta$ double bond with the *N*-terminal amide group. The greater the angle ϕ, the greater the participation of the resonance structure B and smaller the participation of the structure C (Figure 7). Hence the highest frequency AI($C^1{=}O^1$) has the ΔAla derivative (lack of steric hindrance enables the extended conformation) and the lowest has the ΔVal derivative. The frequencies of AI($C^2{=}O^2$) of the unsaturated analogues are lower by 11–24 cm^{-1} than those of the saturated compounds [40]. The underlying cause is the conjugation of the $C^\alpha{=}C^\beta$ double bond with the *C*-terminal carbonyl (the canonical form D) as well as formation of the $N^1{-}H^1{\cdots}O^2{=}C^2$ hydrogen bond.

In MeCN solutions, the compounds of both series, unsaturated and saturated, occur mainly in the open conformations. As is indicated by the calculations (Table 2), the open conformer of the unsaturated compounds is conformer H/F with $\phi,\ \psi \sim -44 \pm 5^\circ$, $\sim 127 \pm 4^\circ$. This is the conserved structure, $\phi,\ \psi \sim -45^\circ,\ 130^\circ$ (for ΔVal, $\phi = -60^\circ$), adopted by all of the unsaturated amides in the solid state. This conformation neither occurs on the conformational maps of the saturated amino acids Ac-Xaa-NHMe [43,44] nor on the crystals of proteins [26,45], but occurs in the maps of Ac-ΔXaa-NHMe, although it presents a much higher position in energetic order [46]. The structure H/F is, then, the inherent conformational feature of the α,β-dehydro amino acid molecules. Conformer H/F is stabilized by a pair of slightly sheared non-covalent antiparallel dipole C=O ►···◄ O=C attractions [21,25,47], not mediated by hydrogen atoms, and two chelating intramolecular $N^2{-}CH_3 \vdots\vdots O^1$ hydrogen bonds [21,25,48] (Figure 6). The latter is exceptionally favourable for Ac-ΔVal-NMe_2 with the shortest distance observed of the studied Ac-ΔXaa-NMe_2 [21]. The open conformer of the saturated compounds is most probably conformer C, with $\phi,\ \psi \sim -100,\ \sim 115^\circ$ [27]. In conformers H/F of the unsaturated compounds and conformer C of the saturated ones, the N–H and C=O groups are directed outside the molecule, toward the molecules of the solvents and are eager for interactions with them.

Our calculations indicate that conformer H/F of Ac-(*Z*)-ΔPhe-NMe_2 is the lowest in energy order *in vacuo* and in all studied solvents. This distinguished conformational feature, compared with the dehydro compounds with the aliphatic side chain, is caused by the additional stabilization gained by the interaction of the *N*-terminal amide with phenyl ring. The distance between the ring centre and the nitrogen atom N–H group is 4.13 Å. The angle between N–H and ring midpoint amounts to 109°, so this contact is rather a stacked interaction than a hydrogen bond [49,50]. The results of the theoretical studies on the aromatic–amide complexes [50,51] show that such an interaction can achieve significant stabilization energy (1.0–4.0 kcal mol^{-1}) over a wide configurational space. The vicinity of the phenyl ring reduces the ν_s(N–H) frequency. The scaled theoretical ν_s(N–H) frequency of conformer H/F of the ΔPhe derivative is 20 cm^{-1} lower than that of other dehydro amides, which is in a perfect agreement with the experimental results.

The positions of ν_s(N–H) bands of the ΔPhe and Phe derivatives in CCl_4 are very similar (3424 and 3426 cm^{-1}, respectively) although the compounds have different conformations. Ac-ΔPhe-NMe_2 has conformation H/F with amide–phenyl interaction and Ac-Phe-NMe_2 has extended conformation E with conventional N–H···O=C hydrogen bond. The ν_s(N–H) bands of both

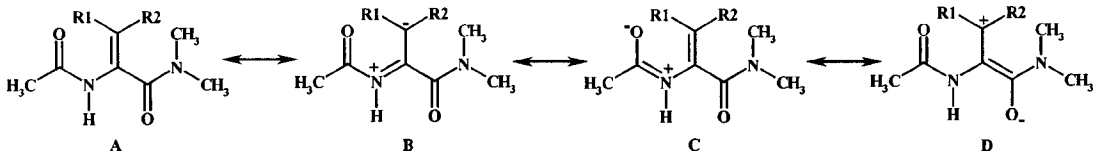

Figure 7 The resonance structures of Ac-ΔXaa-NMe_2.

compounds, despite having the same positions, distinctly differ in $\Delta\nu_{1/2}$ values; that of the saturated analogue is twice as broad as that of the unsaturated one. The narrowing of the ΔPhe band comes from the limited conformational freedom of the phenyl ring resulting from the presence of the $C^{\alpha}=C^{\beta}$ double bond. Hence the pertinent geometry of the N–H/π(Ph) contact is possible only in a narrow range of conformational parameters.

Experimentally for Ac-ΔAla-NMe_2 in DCM and Ac-(*Z*)-ΔAbu-NMe_2 in CCl_4 and DCM (Figure 4A, B and C), we have small amounts of a third conformer. Theoretical conformational analysis of Ac-ΔAla-NMe_2 (Table 2) points to the possible presence of three conformers, E, H/F and D. In DCM, conformer D is stabilized by the solvent and it is this third conformer which we have observed in solution. It has the N^1–$H^1\cdots N^2$ hydrogen bond [25] (Figure 6), which is weaker than the N^1–$H^1\cdots O^2=C^2$ hydrogen bond in conformer E. In the case of Ac-(*Z*)-ΔAbu-NMe_2, the calculations of the energy in the solvents (Table 2) and theoretical the IR frequencies (Table 3) have also hinted at conformer D as being this third conformer. However, within this peptide, the N^1–$H^1\cdots N^2$ geometry is less favourable. The small separation N^1–$H^1\cdots N^2 = 2.97$ Å is accompanied by the angle $\angle N^1$–$H^1\cdots N^2 = 74^\circ$ [25]. So, we can only speak about the presence of some N^1–$H^1\cdots N^2$ contact in conformer D of Ac-(*Z*)-ΔAbu-NMe_2, but not about a hydrogen bond.

CONCLUSION

The Ac-ΔXaa-NMe_2 compounds, which combine the α,β-dehydroamino acid units and the tertiary amides, have specific conformational properties. In a polar environment, i.e. in the solid state and polar solvents, they accommodate the conserved conformation H/F, ϕ, $\psi \sim -45^\circ$, $\sim 130^\circ$. Their N–H and C=O groups protrude outside molecules towards other molecules, with which they readily form the intermolecular hydrogen bonds. However, in non-polar solvent, conformation E is adopted with the internal hydrogen bond, as is the case for saturated dimethylamides. The unsaturated dimethylamides also have specific spectral features. They have the AI($C^1=O^1$) mode higher by up to 27 cm^{-1}, the AI($C^2=O^2$) mode lower by up to 24 cm^{-1} and in acetonitrile the ν_s(N–H) mode lower $\sim$40 cm^{-1} compared with the respective saturated counterparts, which proves the presence of the amide-$C^{\alpha}=C^{\beta}$ resonances within the molecules of Ac-ΔXaa-NMe_2.

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