Association of model peptides and dehydropeptides: *N*-acetyl-ʟ-butyrine and (*Z*)-dehydrobutyrine *N′*,*N′*-dimethylamides⋆

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These comparative studies on the aggregation behaviour of Ac-(*Z*)-ΔAbu-NMe$_2$ and Ac-ʟ-Abu-NMe$_2$ in carbon tetrachloride were performed by the analysis of their FTIR spectra and by theoretical calculations. The percentage of the monomeric form (α) decreased as concentration increased and this occurred to a higher degree for the (*Z*)-ΔAbu derivative than for its saturated analogue. The dimerization constant K_D, calculated on the basis of the intensity of the monomer and associate bands in the v_s(N-H) vibration region, is by three orders of magnitude larger for Ac-(*Z*)-ΔAbu-NMe$_2$ than for Ac-ʟ-Abu-NMe$_2$. The obtained dimer geometries of the dehydrocompound were calculated by the B3LYP/6-31+G** method.

The introduction of conformationally constrained amino-acid residues into bioactive peptides is a useful and frequently applied modification method for studying the resulting active conformations and to design new potent analogues (Hruby *et al.*, 1997; Hruby & Balse, 2000; Hruby, 2001). Residues found in naturally occurring peptides comprise, among others, α,β-dehydroamino acids. The results of the presence of a double bond between C$^\alpha$ and C$^\beta$ are their unique features such as a fixed value of the χ^1 torsion angle and an enlarged C′–C$^\alpha$–N valence angle. Any β-substituent in the α,β-dehydroamino acid also provides steric conformational constraints on the peptide backbone torsion ϕ, ψ angles and restricts the orientation of the amino-acid side chain. The introduction of an α,β-dehydroamino acid into a peptide chain changes not only the conformational properties of the backbone and side chain but in some cases may also affect the strength of the H-bonds formed by these residues and alter the association tendency of the peptide. In a previous paper we investigated the associative properties of Ac-ΔAla-NMe$_2$ and its saturated analogue, Ac-ʟ-Ala-NMe$_2$, and found a significantly greater tendency to self-association for the former compound, which was due to stronger hydrogen bonds (Broda *et al.*, 2005a).

In this communication we report our comparative studies on the association of another homologous pair of peptides, Ac-(*Z*)-ΔAbu-NMe$_2$ and Ac-ʟ-Abu-NMe$_2$ (Fig. 1). The conformational preference of the title compounds were investigated by means of the IR method and theoretical calculations (Siodłak *et al.*, 2004a; Broda *et al.*, 2005a). In a weakly polar environment, Ac-(*Z*)-ΔAbu-NMe$_2$ occurs mainly in the extended conformation E, stabilized by the hydrogen bond C$_5$, and is accompanied by the conformers D and F in small amounts. 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 (Rzeszotarska, *et al.*, 2002; Siodłak *et al.*, 2003; 2004a; 2004b; 2004c; Broda *et al.*, 2005a; 2005b; 2005c). To study the association, as was the case for conformation, we also first used the FTIR measurements. To support spectroscopic interpretation and gain some deeper insight into the nature of associates, the geometries of the Ac-(*Z*)-ΔAbu-NMe$_2$ dimers were optimised by the B3LYP/6-31+G** method. The relative energies, the energies of dimer interactions and selected geometrical parameters of dimers are given and the dependence of association on conformation is discussed.

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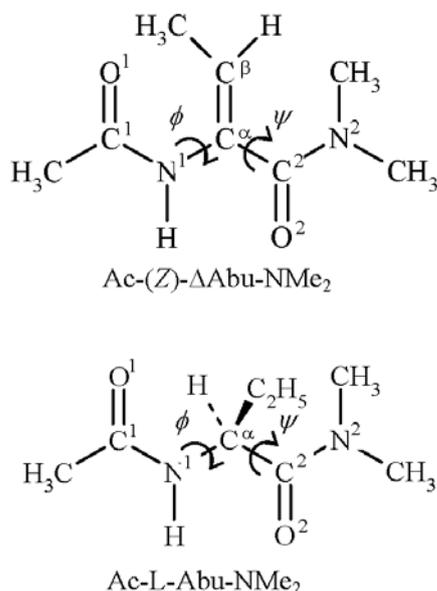
Figure 1. General formula, atom numbering and torsion angles ϕ, ψ for the Ac-(Z)-ΔAbu-NMe₂ and Ac-L-Abu-NMe₂ molecules.

METHODS

The IR spectra in the ν_s(N–H) region were recorded at 20°C using a Nicolet Nexus spectrometer equipped with a DTGS detector and flushed with dry nitrogen during the measurements. All spectra represent an average of 100 scans at 1 cm⁻¹ resolution. KBr liquid cells (0.2 and 2.86 mm) were used. Two series of CCl₄ solutions of Ac-(Z)-ΔAbu-NMe₂ and Ac-L-Abu-NMe₂ were prepared covering the concentration ranges 5.60×10^{-4}–1.35×10^{-3} mol dcm⁻³ and 1.25×10^{-3}–5.70×10^{-2} mol dcm⁻³, respectively. All spectra were analysed by the GRAMS/386 program by means of the second derivative and the curve fitting procedure (the mixed Gaussian-Lorentzian functions) (GRAMS/386, 1994).

The properties of the Ac-(Z)-ΔAbu-NMe₂ dimers were investigated *in vacuo* using the GAUSSIAN'03 program package (Frisch *et al.*, 2003). Three kinds of dimer species for the studied molecules were taken into account: a cyclic dimer with two intermolecular $C^2=O^2\cdots H^1–N^1$ hydrogen bonds, and two linear dimers with only one intermolecular H-bond; either $C^2=O^2\cdots H^1–N^1$ or $C^1=O^1\cdots H^1–N^1$. The starting structures for all the dimers were constructed from all conformers. Initially, optimizations HF/3-21G were carried out on all the dimers to provide good starting geometries for the post-Hartree-Fock calculations. Then, the geometries of the lowest-energy dimers were re-optimized by the DFT calculations using the B3LYP/6-31+G** method. The vibrational frequencies were calculated to verify the nature of the stationary points found on the potential energy surface. The hydrogen-bonding energies

of the studied dimers were corrected both with basis set superposition error (BSSE) and zero-point vibrational energies (ZPVE). The BSSE was estimated using the counterpoise (CP) method (Boys *et al.*, 1970).

RESULTS

Figure 2 shows the ν_s(N–H) region of the FTIR spectrum together with the second derivatives of Ac-(Z)-ΔAbu-NMe₂ (A) and Ac-L-Abu-NMe₂ (B) in CCl₄ solution. In the spectrum of Ac-L-Abu-NMe₂, two strong bands of the N–H stretching vibration are present. They originate from the free N–H group of the monomer at 3416 cm⁻¹ with a shoulder at 3435 cm⁻¹ and from the N–H bonded group of the associates at about 3300 cm⁻¹ with a small sub band at about 3200 cm⁻¹. The band at 3416 cm⁻¹ is ascribable to the extended conformer E with the internal H-bond. The band shoulder indicates an open form (Broda *et al.*, 2005b). In the range of the NH bonded group at about 3300 cm⁻¹, the second derivative results in peaks at the frequencies 3374, 3318 and 3286 cm⁻¹. The first is probably the stretching frequency of the free-end N–H group, present in linear associated species, and the two remaining
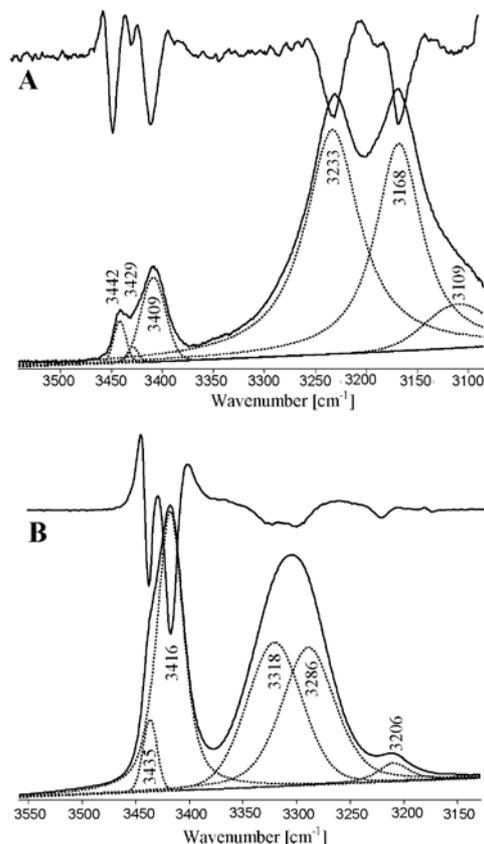
Figure 2. The ν_s(N–H) region of the FTIR spectrum in CCl₄ together with the second derivative (A) Ac-(Z)-ΔAbu-NMe₂ ($c_0 = 1.9 \times 10^{-2}$ mol dcm⁻³) and (B) Ac-L-Abu-NMe₂ ($c_0 = 1.9 \times 10^{-2}$ mol dcm⁻³).
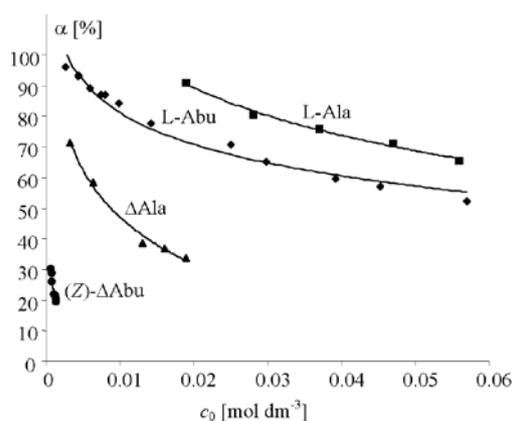
Figure 3. Percentage of the monomeric form of Ac-ΔXaa-NMe$_2$ and Ac-L-Xaa-NMe$_2$ as a function of the concentration c_0 in CCl$_4$.

peaks correspond to the N–H bonded groups. The spectral pattern of Ac-(Z)-ΔAbu-NMe$_2$ is quite different. The spectrum in the ν_s(N–H) region has at least four distinct bands. The band at 3409 cm^{-1} is assigned to the monomer in the extended conformation E and the band at 3442 cm^{-1} corresponds to the open conformer F (Broda *et al.*, 2005b). Bands at 3233 and 3168 cm^{-1} are indicative of association. The second derivative additionally yields two other bands at 3429 and 3189 cm^{-1}. They can, respectively, be assigned to conformer D of the monomer (Broda *et al.*, 2005b) and another associate form.

At low concentrations (below 2×10^{-3} mol dm^{-3}), the absorption-concentration plots give straight lines, which means that the compounds exist completely in a monomer form at such concentrations. The molar absorption coefficient (ε_M) at peak height can be determined, from the slope of the lines, to be 95 and 97 dm^3 mol^{-1} cm^{-1} for Ac-(Z)-ΔAbu-NMe$_2$ and Ac-L-Abu-NMe$_2$, respectively. These values are similar to those reported for Ac-ΔAla-NMe$_2$ and Ac-L-Ala-NMe$_2$ (Broda *et al.*, 2005a). In more concentrated solutions, the concentration of the monomer c_M can be calculated using Beer's law $A_M = \varepsilon_M \times b \times c_M$, where A_M, ε_M, b are, respectively, the absorbance of the monomer band, its molar absorption coefficient, and the optical pathlength of the cell. We initially assumed such a model of association that takes into account the dimers only. Dimerisation constants K_D for both studied compounds were determined according to the formula $K_D = (c_0 - c_M) / 2c_M^2$ (c_0, total concentration). We obtained values of K_D from 6467 to 7966 and from 8.0 to 15.4 mol^{-1} dm^3 for Ac-(Z)-ΔAbu-NMe$_2$ and Ac-L-Abu-NMe$_2$, respectively. As the concentration increases, so does the value of K_D. This means that in the investigated range of concentration, the association proceeds beyond dimers. Generally, however, the K_D values for Ac-(Z)-ΔAbu-NMe$_2$ are by three orders of magnitude larger than for Ac-L-Abu-NMe$_2$. The percentage of the mono-

mer form in CCl$_4$ solutions is $\alpha = c_M/c_0 \times 100\%$. As expected, with decreasing concentration c_0, α increases gradually. In agreement with the results of the dimerisation constants K_D, the percentage of the monomer for Ac-L-Abu-NMe$_2$ is visibly higher than for the unsaturated compound, which means that smaller amounts of associated species are present in solution (Fig. 3).

Table 1 lists relative energies and selected geometrical parameters of the Ac-(Z)-ΔAbu-NMe$_2$ molecules for the four energetically lowest cyclic dimers obtained by the B3LYP/6-31+G** method, and Fig. 4 illustrates these dimers. The lowest energy dimer **1**

Table 1. Energies and selected geometrical parameters of Ac-(Z)-ΔAbu-NMe$_2$ cyclic dimers obtained by the B3LYP/6–31+G method**

Parameters	Dimers			
	1	**2**	**3**	**4**
ΔE^a	0.00^c	0.35	5.36	6.13
E_{int}^b	17.48	17.13	13.30	7.61
ϕ_1	–49.1	48.5	121.3	–126.
ψ_1	127.4	–126.1	142.3	117.7
ϕ_2	–48.9	–48.5	54.9	121.6
ψ_2	127.1	126.1	–131.8	143.3
N–H···O				
H···O	1.861	1.879	1.886	1.916
	1.861	1.879	1.950	1.938
N···O	2.882	2.897	2.875	2.935
	2.883	2.897	2.965	2.845
∠N–H···O	172.2	170.6	161.8	173.5
	172.2	170.6	171.4	167.9
N–H···N				
H···N	3.002	3.069	3.167	
	3.002	3.069		
N···N	3.392	3.460	3.780	
	3.392	3.460		
∠N–H···N	103.5	103.7	119.7	
	103.5	103.7		
C–H···O				
H···O	2.667	2.734	2.497	2.486
	2.667	2.734		
C···O	3.559	3.604	3.431	3.419
	3.559	3.604		
∠C–H···O	138.4	136.3	142.9	142.8
	138.4	136.3		

Data presented only for the X–H···A contacts, in which H···A ≤ 3.2 Å and ∠X–H···A > 90° acc. to (Steiner, 2002). $^a\Delta E$ (kcal mol^{-1}) = relative energy (with ZPE correction). $^b E_{int}$ (kcal mol^{-1}) = intermolecular H-bond energy (corrected by BSSE and ZPE). $^c E$ = –1146. 597891 a.u.
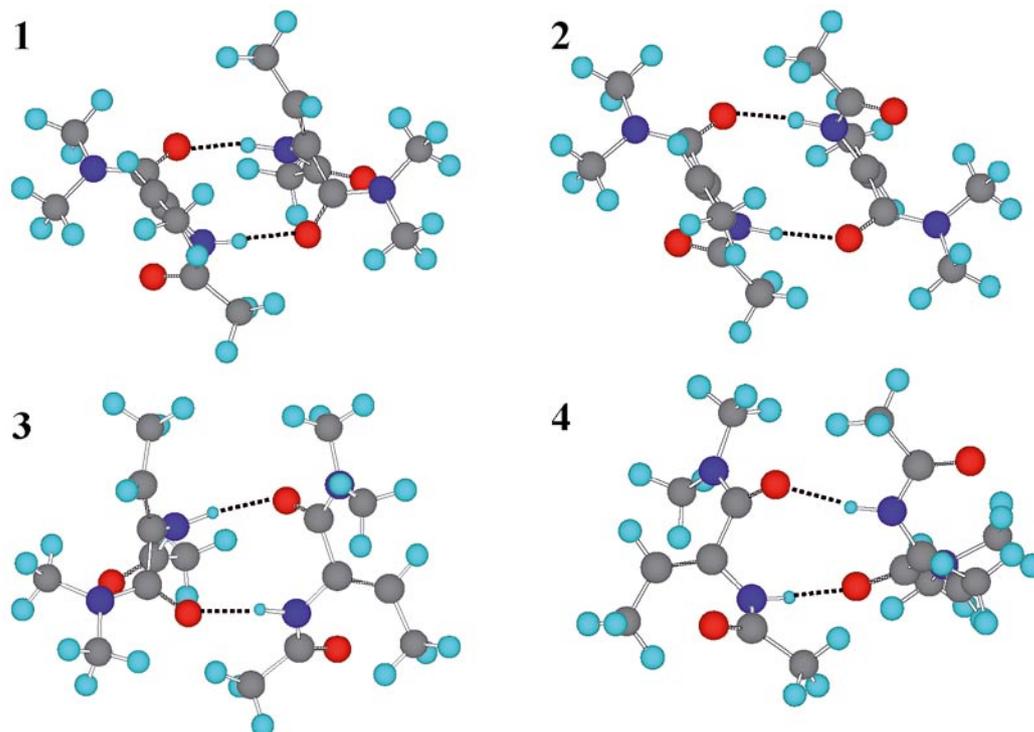
Figure 4. Cyclic dimers of Ac-(Z)-ΔAbu-NMe$_2$ calculated by B3LYP/6-31+G** method.

is built from two conformers H/F and exhibits two short intermolecular N–H⋯O hydrogen bonds with a small, about 10º, deviation from linearity. Additionally, the dimer is stabilised with a few weaker internal interactions, *viz.* two N–H⋯N and two C–H⋯O interactions. The formation of the dimer requires only a slight distortion (about 4º for both angles φ, ψ) in the isolated molecule (φ, ψ = –45°, 131°) (Siodłak *et al.*, 2004a). This complex has the highest energy of the intermolecular interaction. A similar geometry of the N–H⋯O bonds and interaction energies can be observed in dimer **2**. The dimer is constructed from conformer H/F and its enantiomer H/F*. Among the Ac-(Z)-ΔAbu-NMe$_2$ dimers, **2** is second in the energy order, and is only about 0.35 kcal mol^{-1} higher in energy than the former one. Complex **3** also exhibits two N–H⋯O hydrogen bonds, but is considerably higher in energy. It is built of the extended molecule E* and a molecule in conformation H/F*. The molecules have H⋯O distances and one N⋯O distance longer than those in dimers **1** and **2**. Moreover, they have a N–H⋯N and one C–H⋯O interaction. The formation of the hydrogen bonds induces a distinct distortion of the monomer geometry. The φ angle in molecule E* departs by about 12º and the angle ψ in molecule H/F* departs by about 10° from the values in the isolated molecule E* (φ, ψ = 121°, 154°) and F* (φ, ψ = 45°, –131°) (Siodłak *et al.*, 2004a). The underlying cause of the warping of the ψ angle and the altered geometry of the hydrogen bonds is the steric interaction of two methyl groups at the termini of both molecule. The dimer highest in ener-

gy, dimer **4**, consists of monomers E and E*. It has two N–H⋯O hydrogen bonds and only one C–H⋯O interaction and is considerably distorted at two ψ angles, by 30° and 12°, respectively, compared to the values in the isolated molecules. The remaining dimers are linear. The calculations at the B3LYP/6-31+G*//HF/3-21G level show the linear dimers to be higher in energy than the cyclic ones. For instance, the linear dimer lowest in the energy order had the energy of ≈ 6.5 kcal mol^{-1} (not shown in Table 1). This suggests that, in the case of Ac-(Z)-ΔAbu-NMe$_2$, *in vacuo* and probably in a non-polar environment as well, the formation of cyclic dimers, especially those of dimers **1** and **2**, is favoured.

DISCUSSION

The dehydrobutyrine peptide Ac-(Z)-ΔAbu-NMe$_2$ shows a greater tendency to self-associate than its saturated counterpart, Ac-L-Abu-NMe$_2$. This is proved by the smaller percentage of the monomeric form α (Fig. 3) than in the case of Ac-L-Abu-NMe$_2$. The separation between the dimer and monomer IR bands for the dehydro peptide appears visibly larger than the separation for the saturated one (Fig. 2). This means stronger H-bonds in the Ac-(Z)-ΔAbu-NMe$_2$ dimers. Such strong hydrogen bonds are formed for two reasons. Firstly, the Ac-(Z)-ΔAbu-NMe$_2$ molecule is capable of adopting the H/F conformation. This open, low-energy conformer with φ, ψ = –45°, 131° has its N–H and C=O groups

exposed and shows a great steric potential for the formation of intermolecular H-bonds. Secondly, the α,β-dehydroamino acid N–H and C=O groups take part in resonance with the $C^\alpha = C^\beta$ double bond and have an increased polarizability. Similar relations between the properties of hydrogen bonds were observed in the pair of Ac-ΔAla-NMe₂/Ac-L-Ala-NMe₂ compounds. So, it is supposed that the capability of forming strong intermolecular hydrogen bonds is a characteristic feature of the structural fragment –ΔXaa-NMe–.

The positions of the association IR bands for the saturated peptides Ac-L-Abu-NMe₂ and Ac-L-Ala-NMe₂, and their dimerization constants K_D, estimated basing on IR spectra, are similar. By analogy with the L-Ala derivative (Broda *et al.*, 2005a), it can also be assumed for the L-Abu derivative that the bands in the H-bonded ν_s(N–H) region (about 3300 cm⁻¹) originate from two dimers: one of them cyclic and the second one linear. The relation $\alpha = f(c_0)$ for both these compounds shows a somewhat smaller fraction of the monomeric form for the L-Abu derivative as a result of a more warped conformation, which is more conducive to the formation of associates. This agrees with an earlier work on the association of the similar Ac-L-Xaa-NMe₂ (Mizuno *et al.*, 1979).

The geometry of the calculated low energy cyclic dimers for Ac-ΔAla-NMe₂ $\phi, \psi = -43°, 126°$ and for Ac-(Z)-ΔAbu-NMe₂ $\phi, \psi = -49°, 127°$ agree perfectly with that of the crystal structures of these and other Ac-ΔXaa-NMe₂ molecules (average $\phi, \psi = -48°$, 130°), where ΔXaa = ΔAla, (Z)-ΔAbu, (Z)-ΔPhe, (Z)-ΔLeu, ΔVal (El-Masdouri *et al.*, 1992; Rzeszotarska *et al.*, 2002; Siodłak *et al.*, 2003; 2004b; 2004c). This confirms the given description of the associate species in solutions of the so far known Ac-ΔXaa-NMe₂. The spectral patterns in the ν_s(N–H) regions of the associates of Ac-(Z)-ΔAbu-NMe₂ and Ac-ΔAbu-NMe₂ are different. In the spectrum of Ac-ΔAla-NMe₂, a wider range of dimeric forms was identified than in the spectrum of Ac-(Z)-ΔAbu-NMe₂, which is in accordance with the results of theoretical calculations (Broda *et al.*, 2005a). But more amazing is the disparity in the tendency for self-association of compounds Ac-(Z)-ΔAbu-NMe₂ and Ac-ΔAla-NMe₂. The estimated dimerization constants K_D differ substantially. The constant for compound (Z)-ΔAbu is two orders of magnitude larger. Such a distinct difference is surprising, since the strength of the hydrogen bonds in the dimers of both compounds is similar, as indicated by the positions of their IR bands as well as the energy of their interaction, as calculated by the DFT method. Because the energy of the H-bonds appears to be similar, the difference in the tendency for self-association follows most probably from the different molecular conformational preferences of both derivatives. In the case of both compounds, the lowest-energy molecular conformation is the extended conformation E, stabilised by the internal hydrogen bond C_5, and the second in the energy order is the open conformer H/F. The gap in energy between conformers E and H/F, as calculated by B3LYP/6-31+G** (Siodłak *et al.*, 2004a) amounts to 3.7 kcal mol⁻¹ for Ac-ΔAla-NMe₂, and for Ac-(Z)-ΔAbu-NMe₂ it is only 1.9 kcal mol⁻¹. This means that the latter molecule can readily adopt the open conformation pertinent to the formation of the lowest-energy cyclic dimers and so associates easily.

CONCLUSION

Conformational analysis of free Ac-ΔXaa-NMe₂ molecules shows that the lowest energy conformer, either the first or second in energy order, is the open conformer H/F characterized by the angles $\phi, \psi = -41 \pm 4°, 128 \pm 3°$ (Siodłak *et al.*, 2004a). This conformer appears in the Ramachandran map region not easily accessible for common amino-acid residues and is particularly favorable to forming intermolecular H-bonds, because of the N-H and C=O groups exposed outside. Such a monomer conformation of Ac-ΔXaa-NMe₂ occurs in the dimers both in the solid state as indicated by X-ray analysis (El-Masdouri *et al.*, 1992, Rzeszotarska *et al.*, 2002; Siodłak *et al.*, 2003; 2004b; 2004c) and in solution (Broda *et al.*, 2005a; this work) as calculated by the DFT method. From the experimental and theoretical results, it can be concluded that the molecular arrangement in the associated dimers of Ac-ΔXaa-NMe₂ can be regarded as a conformational model of a unit structure of collagen chains of two types (ϕ, ψ –51°, 153°; ϕ, ψ –45°, 148°) and the polyproline-II helix (–78°, 149°). The secondary structures given by the –ΔXaa-NMe– motif should be especially stable because of the stronger H-bonds formed by α,β-dehydroamino acid N–H and C=O groups.

Acknowledgements

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