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Conformational investigation of α,β-dehydropeptides. XVI. β-turn tendency in Ac-Pro-ΔXaa-NHMe: crystallographic and theoretical studies‡

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Abstract: The crystal structures of two diastereomeric α,β-dehydrobutyrine peptides Ac-Pro-(*Z*)-ΔAbu-NHMe (I) and Ac-Pro-(*E*)-ΔAbu-NHMe (II) have been determined. Both dehydropeptides adopt βI-turn conformation characterized by the pairs of (ϕ_{i+1}, ψ_{i+1}) and (ϕ_{i+2}, ψ_{i+2}) angles as −66, −19, −97, 11° for I and −59, −27, −119, 29° for II. In each peptide, the βI turn is stabilized by $(i+3) \rightarrow i$ intramolecular hydrogen bonds with N···O distance of 3.12 Å for I and 2.93 Å for II. These structures have been compared to the crystal structures of homologous peptides Ac-Pro-ΔVal-NHMe and Ac-Pro-ΔAla-NHMe. Theoretical analyses by DFT/B3LYP/6–31 + G** method of conformers formed by these four peptides and by the saturated peptide Ac-Pro-Ala-NHMe revealed that peptides with a (*Z*) substituent at the $C^{\beta}{}_{i+2}$ atom of dehydroamino acid, i.e. Ac-Pro-ΔVal-NHMe and Ac-Pro-(*Z*)-ΔAbu-NHMe, predominantly form β turns, both *in vacuo* and in polar environment. The tendency to adopt β-turn conformation is much weaker for the peptides lacking the (*Z*) substituent, Ac-Pro-(*E*)-ΔAbu-NHMe and Ac-Pro-ΔAla-NHMe. The latter adopts a semi-extended or an extended conformation in every polar environment, including a weakly polar solvent. The saturated peptide Ac-Pro-Ala-NHMe *in vacuo* prefers a β-turn conformation, but in polar environment the differences between various conformers are small. The role of π-electron correlation and intramolecular hydrogen bonds interaction in stabilizing the hairpin structures are discussed. Copyright © 2006 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: α,β-dehydropeptides; DFT calculation; X-ray crystallography; hydrogen bonds; solute/solvent interaction; β turn; extended form; peptide conformation

INTRODUCTION

α,β-Dehydroamino acids are common components of many microbial, plant and animal peptides. The presence of the sp^2-hybridized C^{α} and C^{β} atoms limits the conformation of the side chain to either (*Z*) or (*E*) orientation at the C^{β} atom in respect to the peptide chain. This limited conformational flexibility makes α,β-dehydroamino acids attractive in the search of novel functions and structural properties of peptide fragments containing these amino acids [2]. Most synthetic and conformational studies of α,β-dehydropeptides have been limited to those containing (*Z*)-dehydrophenylalanine [3–8]. Some works have described other dehydroamino acid residues, (*Z*)-dehydroleucine [3,4], dehydrovaline [9–12], dehydroalanine [10,13,14] and α,β-dehydrobutyrine [10,15–19].

α,β-Dehydrobutyrine is the simplest α,β-dehydroamino acid capable of forming both (*Z*) and (*E*) isomers. This residue has been found in several natural products. For example, the (*Z*) isomer of dehydrobutyrine is present in lantibiotics, a unique class of bacteriocins, which are synthesized ribosomally and modified posttranslationally [20–26]. In most of the known lantibiotics, the (*Z*)-dehydrobutyrine residue is further used for the biosynthesis of methyllanthionine [20]. (*Z*)-Dehydrobutyrine has also been found in cyclic depsipeptides, such as the antibiotic heptamycin [27] and antitumor peptide FR-901,228 [28]. (*E*)-Dehydrobutyrine has been found in other cyclic peptides and depsipeptides, such as *N*-methyl-tryptophan dehydrobutyrine diketopiperazine (TDD), an antitumor antibiotic [29], and in phomalide, a fungal toxin [30]. It was shown that the (*E*) configuration of α,β-dehydrobutyrine in these peptides is essential for their cytotoxicity and phytotoxicity, respectively, because the synthetic (*Z*)-Abu versions of the peptides are biologically inert [29,31]. Dehydrobutyrine is present in other toxins, hepatotoxines, such as microcystins and nodularins derived from cyanobacteria. Some of them are in (*Z*), and others are in (*E*) configuration [32–37]. It has been suggested that the (*E*) stereoisomer of

Abbreviations: As recommended in *J. Peptide Sci.* 2003; **9**: 1–8 with the following addition: Δ, α,β-dehydro.

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dehydrobutyrine residue may be associated with higher specific toxicity of some microcystins, as compared to those with the isomeric *N*-methyldehydroalanine [36].

Model Ac-Yaa-ΔXaa-NHMe triamide systems, where Yaa is any amino acid, and ΔXaa is (*Z*)-ΔPhe or (*Z*)-ΔLeu [4,5,9] typically adopt β-turn conformation [4,5,10] in crystal and in solution. Yet, α,β-dehydroamino acids that contain smaller unsaturated side chains, such as ΔVal [4,5,9], (*Z*)-ΔAbu [15,17,18], (*E*)-ΔAbu [15,18] and ΔAla [13,38], do not appear as profound in the induction of a β turn. Therefore, it seemed rational to further advance our understanding of the conformational flexibility of the dehydropeptides by focusing on the simplest diastereomeric α,β-dehydrobutyrine-containing model systems. Herein, we present the crystal structures of two peptides, Ac-Pro-(*Z*)-ΔAbu-NHMe (**I**) and Ac-Pro-(*E*)-ΔAbu-NHMe (**II**), and we compare them with the crystal structures of Ac-Pro-ΔVal-NHMe (**III**) and Ac-Pro-ΔAla-NHMe (**IV**) [9,13] which differ in the number of methyl substituents and their location at the C^{β} atom. Also, for comparison, theoretical calculations of conformational properties of these four peptides and the saturated homolog Ac-Pro-Ala-NHMe (**V**) have been performed by the DFT/B3LYP/6–31 + G** method. Through these studies we make an attempt to determine the steric requirements and limits for the formation of β turns.

MATERIALS AND METHODS

Materials

Ac-Pro-(*Z*)-ΔAbu-NHMe (**I**) and Ac-Pro-(*E*)-ΔAbu-NHMe (**II**) were obtained by condensation of Ac-Pro-NH_2 with α-oxo butyric acid in dry benzene in the presence of *p*-toluenosulfonic acid as a catalyst to furnish Ac-Pro-(*Z*)-ΔAbu (26%) and Ac-Pro-(*E*)-ΔAbu (13%) [39], and then reacting these acids

Table 1 Crystal data and structure refinement parameters for Ac-Pro-ΔAbu-NHMe

	I	**II**
CCDC Number[a]	275069	275068
Empirical formula	$C_{12}H_{19}N_3O_3$	$C_{12}H_{19}N_3O_3$
Formula weight	253.3	253.3
Crystal system	Orthorhombic	Monoclinic
Space group	$P2_12_12_1$	$P2_1$
Unit cell dimensions		
a (Å)	8.104(2)	8.494(2)
b (Å)	12.455(2)	8.306(2)
c (Å)	13.437(3)	9.602(3)
α (°)	90	90
β (°)	90	97.17(2)
γ (°)	90	90
Volume ($Å^3$); *Z*	1356.3(5); 4	672.1(3); 2
Density (calcd) (g cm^{-3})	1.241	1.252
Absorption coefficient (mm^{-1})	0.090	0.091
F(000)	544	272
Crystal size (mm)	0.2 × 0.27 × 0.4	0.3 × 0.35 × 0.4
Theta range for data collection (°)	2.23 to 27.57	2.14 to 25.05
Index ranges	$-6 \le h \le 10$, $-6 \le k \le 16$, $-6 \le l \le 17$	$0 \le h \le 10$, $0 \le k \le 9$, $-11 \le l \le 11$
Reflections collected	1858	1241
Independent reflections	1829 [$R_{int} = 0.024$]	1241
Data/parameters	1829/174	1241/174
Goodness of fit on F^2	1.077	1.073
Final *R* indices	$R_1 = 0.0482$	$R_1 = 0.0392$
[$I > 2\sigma(I)$]	$wR_2 = 0.1425$	$wR_2 = 0.0947$
R indices (all data)	$R_1 = 0.0964$	$R_1 = 0.0524$
	$wR_2 = 0.1546$	$wR_2 = 0.1002$
Extinction coefficient	—	0.069(9)
Diff. peak max and min (eA^{-3})	0.31 and −0.26	0.12 and −0.12

[a] Crystallographic data (excluding structure factors) for the crystal structures have been deposited with the Cambridge Crystallographic Data Centre. Copies of the data can be obtained on application to The Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (Fax: +44-1223-336-033; e-mail: deposit@ccdc.cam.ac.uk or www: http://www.ccdc.cam.ac.uk).

separately with isobutyl chlorocarbonate and methylamine to give **I** (77%) [39] and **II** (22%) [13].

X-ray Crystallography of I and II

Both peptides (**I** and **II**) were crystallized from a solution in a mixture of methanol and diethyl ether at −20 °C. X-ray diffraction measurements were made on a Syntex $P2_1$ diffractometer by the $\theta/2\theta$ scanning technique using a variable scan speed and the graphite-monochromated Mo Kα radiation ($\lambda = 0.71073$ Å). The background and integrated intensity for each reflection were evaluated from a profile analysis according to Lehmann and Larsen method [40,41]. Both structures were solved by direct methods and refined on F^2 by the full-matrix least-squares method using SHELXS93 [42] and SHELXL97 [43] programs, respectively. Non-H atoms were refined with anisotropic displacement parameters. The amide H atoms were located from the difference maps and their positional and U_{iso} parameters were refined. Positions of the remaining H atoms were calculated form the geometry, and the 'riding' model was used in the refinement. The carbon bonded H atoms were given isotropic factors of 1.2 U_{eq}(C atom). The crystallographic and experimental data and refinement statistics are given in Table 1.

Computational Procedures

Triamides Ac-Pro-Xaa-NHMe (where Xaa = (*Z*)-ΔAbu, (*E*)-ΔAbu, ΔVal, ΔAla and Ala) were the model compounds used to examine the conformational influence of dehydro amino acids on β-turn formation tendency. The starting structures for geometry optimization were the standard β-turn types, characterized by the torsion angles listed in Table 2 [44,45], with all *trans* peptide bonds. The starting conformations of the five-member pyrrolidine ring in proline were chosen to be puckered with the CH_2 group opposite to N–C^α bond both 'up', i.e. χ_1 (N–C^α–C^β–C^γ) > 0°, C^γ syn to proline amide group (+), and 'down', $\chi_1 < 0°$ (–) [46].

The geometries of all structures were fully optimized by the DFT/B3LYP/6–31 + G** method. To estimate the β-turn tendency, we calculated 'extended' conformations with the starting values of main-chain torsion angles equal 180°, except the proline torsion angle ϕ_1 (locked about −60°).

All calculations were performed using the Gaussian'03 program package [47]. The polarizable continuum model (PCM) developed by Tomasi [48] was applied to estimate the effect of

Table 2 Standard backbone angles for the four types of β turns with all trans amide bonds

Turn	ϕ_{i+1}	ψ_{i+1}	ϕ_{i+2}	ψ_{i+2}
βI	−60	−30	−90	0
βII	−60	120	80	0
βIII	−60	−30	−60	−30
βVIII	−60	−30	−120	120

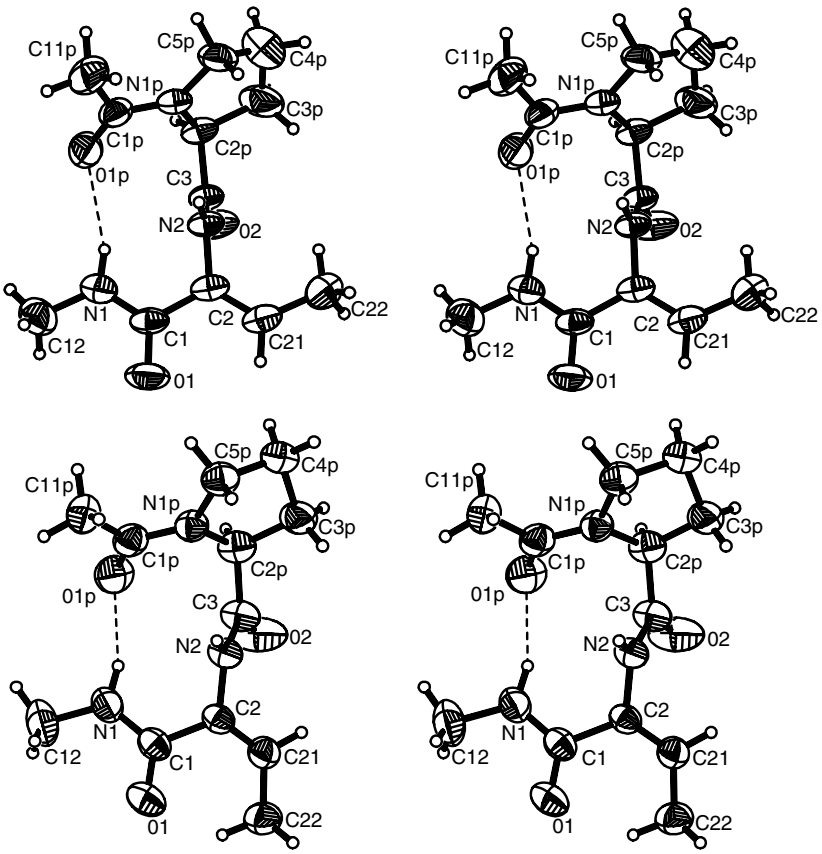

Figure 1 Stereoscopic drawing of molecules **I** (a) and **II** (b). The intramolecular N–H···O hydrogen bond is marked by a dashed line. Thermal ellipsoids are presented at 50% probability level.

solvation on β-turn stability. The energies in chloroform and water were calculated for all optimized *in vacuo* geometries.

RESULTS

Molecular and Crystal Structure

The crystals structures of **I** and **II** are shown in Figure 1, and their selected geometric parameters are listed in Table 3. Both isomers adopt the βI-turn conformation described by the pairs of (ϕ, ψ) torsion angles; their values are −66.0(3), −19.1(4), −96.5(3), 11.3(3)° for **I** and −58.5(4), −27.0(5), −119.0(4), 29.3(4)° for **II**, respectively. These conformers have the intramolecular $(i+3) \rightarrow i$ N–H···O hydrogen bond accompanied by two N–H···N interactions (Table 4). The N1 amide atom is involved in a three-center hydrogen-bond system: O 1p···H1n(N1)···N2, while the (N2)H2n

Table 3 Selected bond lengths (Å) and torsion angles (°) for Ac-Pro-ΔXaa-NHMe (ΔXaa = (*Z*)-ΔAbu (**I**), (*E*)-ΔAbu (**II**), ΔVal (**III**) and ΔAla (**IV**))

		I	**II**	**III** [9][a]	**IV** [13][b]
Bond lengths					
C(1)–O(1)		1.238(3)	1.231(4)	1.218	1.243
C(1)–N(1)		1.331(4)	1.336(4)	1.348	1.327
C(1)–C(2)		1.503(4)	1.493(4)	1.495	1.492
N(1)–C(12)		1.463(4)	1.455(5)	1.445	1.465
C(2)–C(21)		1.311(4)	1.318(4)	1.331	1.311
C(2)–N(2)		1.422(3)	1.429(4)	1.446	1.399
C(21)–C(22)		1.495(5)	1.494(5)	1.494; 1.526	
N(2)–C(3)		1.332(3)	1.351(4)	1.324	1.356
C(3)–O(2)		1.225(3)	1.216(4)	1.233	1.206
C(3)–C(2p)		1.521(4)	1.521(5)	1.519	1.522
N(1p)–C(1p)		1.342(4)	1.338(5)	1.324	1.333
C(1p)–O(1p)		1.229(4)	1.246(5)	1.242	1.236
C(1p)–C(11p)		1.509(4)	1.483(5)	1.520	1.509
N(1p)–C(2p)		1.461(3)	1.465(4)	1.468	1.456
N(1p)–C(5p)		1.471(4)	1.474(5)	1.464	1.485
C(2p)–C(3p)		1.544(5)	1.527(6)	1.512	1.531
C(3p)–C(4p)		1.464(7)	1.526(6)	1.527	1.443
C(4p)–C(5p)		1.493(6)	1.516(5)	1.493	1.461
Torsion angles					
O(1)–C(1)–N(1)–C(12)		−2.0(5)	1.0(6)	−3.0	−0.1
C(2)–C(1)–N(1)–C(12)	ω_{i+2}	177.5(3)	179.5(3)	176.9	−178.8
O(1)–C(1)–C(2)–C(21)		11.6(4)	33.3(5)	−17.0	171.8
N(1)–C(1)–C(2)–C(21)		−167.9(3)	−145.3(3)	163.1	−9.5
O(1)–C(1)–C(2)–N(2)		−169.2(3)	−152.1(3)	165.7	−6.4
N(1)–C(1)–C(2)–N(2)	ψ_{i+2}	11.3(3)	29.3(4)	−14.1	172.4
N(2)–C(2)–C(21)–C(22)		−0.9(5)	−172.4(3)	−1.6; 178.9	—
C(21)–C(2)–N(2)–C(3)		82.6(4)	55.7(5)	109.1	0.2
C(1)–C(2)–N(2)–C(3)	ϕ_{i+2}	−96.5(3)	−119.0(4)	−73.5	178.4
C(2)–N(2)–C(3)–O(2)		−5.1(5)	−11.5(6)	−4.4	−1.9
C(2)–N(2)–C(3)–C(2p)	ω_{i+1}	178.0(3)	171.8(3)	176.4	−179.3
C(2p)–N(1p)–C(1p)–O(1p)		4.9(4)	4.1(5)	6.1	1.8
C(2p)–N(1p)–C(1p)–C(11p)	ω_I	−174.7(3)	−176.7(3)	−175.2	179.5
O(2)–C(3)–C(2p)–N(1p)		164.0(3)	156.2(4)	160.7	165.8
N(2)–C(3)–C(2p)–N(1p)	ψ_{i+1}	−19.1(4)	−27.0(5)	−20.1	−16.8
C(1p)–N(1p)–C(2p)–C(3)	ϕ_{i+1}	−66.0(3)	−58.5(4)	−68.3	−71.4
Pro					
C(2p)–N(1p)–C(5p)–C(4p)		16.4(4)	17.7(4)	−16.7	−5.2
C(5p)–N(1p)–C(2p)–C(3p)		−2.3(3)	6.5(4)	−6.3	−7.8
N(1p)–C(2p)–C(3p)–C(4p)	χ_1	−13.1(4)	−28.1(3)	26.1	17.0
C(2p)–C(3p)–C(4p)–C(5p)		23.6(5)	39.0(4)	−36.9	−22.8
C(3p)–C(4p)–C(5p)–N(1p)		−24.2(5)	−34.3(4)	33.1	17.6

[a] Published estimated standard deviations (ESDs) are: 0.004–8 Å for C–C/N/O distances and 0.3–5° for torsion angles.
[b] Published ESDs are: 0.003–9 Å for C–C/N/O distances and 0.3–6° for torsion angles.

Table 4 Solid state intra- and intermolecular hydrogen bonds for Ac-Pro-ΔXaa-NHMe (ΔXaa = (*Z*)-ΔAbu (**I**), (*E*)-ΔAbu (**II**), ΔVal (**III**) and ΔAla (**IV**))

D–H	A	D–H (Å)	H···A (Å)	D···A (Å)	<(D–H···A) (°)
I					
N1–H1n	O 1p	0.89(3)	2.26(3)	3.117(4)	162(4)
N1–H1n	N2	0.89(3)	2.29(3)	2.747(4)	112(4)
N2–H2n	N 1p	0.73(3)	2.35(3)	2.768(4)	118(4)
N2–H2n	O1[a]	0.73(3)	2.15(3)	2.771(4)	142(4)
Symmetry code:[a] $x+0.5, 0.5-y, 1-z$.					
II					
N1–H1n	O 1p	0.92(4)	2.05(4)	2.934(5)	162(4)
N1–H1n	N2	0.92(4)	2.35(4)	2.760(5)	107(4)
N2–H2n	N 1p	0.75(4)	2.54(4)	2.812(5)	104(4)
C22–H22b	O1	0.96	2.31	2.978	126
N2–H2n	O1[a]	0.75(4)	2.14(4)	2.867(5)	164(4)
O 1p	C 11p–H 12p[b]	0.96	2.44	3.309(5)	150
O 1p	C 5p–H 52p[b]	0.97	2.50	3.331(5)	143
O2	C 3p–H 32p[c]	0.97	2.72	3.384(5)	126
O2	C 4p–H 42p[c]	0.97	2.69	3.388(5)	129
Symmetry codes:[a] $1-x, y-0.5, 1-z$: [b] $1-x, y+0.5, -z$: [c] $-x, y+0.5, -z$.					
III [9]					
N1–H1n	O 1p	0.95	2.18	3.06	153
N1–H1n	N2	0.95	2.20	2.71	113
N2–H2n	N 1p	0.95	2.31	2.78	109
C11p–H	O1[a]	0.95	2.72	3.42	123
C5p–H	O2[b]	0.95	2.71	3.38	120
Symmetry codes:[a] $-x, 0.5+y, 0.5-z$: [b] $-0.5-x, -y, z-0.5$.					
IV [13]					
N2–H2n	O1	1.02	2.07	2.56	107
C21–H	O2	1.08	2.21	2.90	119
C11p–H	O1[a]	1.08	2.31	3.39	173
C21–H	O 1p[b]	1.08	2.27	3.38	156
Symmetry codes:[a] $0.5+x, 1.5-y, 1-z$: [b] $0.5-x, 1-y, 0.5+z$.					

atom is directed to the proline N 1p. The D–H···A angles are equal 162° for the N1–H1n···O 1p bond in both crystals, whereas the N–H···N angles are in the range 104–118° indicating strains of these bonds. Comparison of the values of the N1···O 1p distance and (ϕ_{i+2}, ψ_{i+2}) torsion angles for **I** and **II** shows significant differences; shorter distance and larger angles are observed for **II** indicating a greater distortion of the standard βI turn (Table 2). The methyl C$^{\gamma}$ group in **II** is engaged in an intramolecular C–H···O bond with amide oxygen O1. The bond lengths and torsion angles remain similar to those reported for other short dehydropeptides [4]. The same O1 amide atom of **I** and **II** forms strong intermolecular hydrogen bond with the N2 atom of the neighboring molecule. Interestingly, there are no intermolecular C–H···O contacts shorter than 3.5 Å in the crystal of **I**, while in the crystal **II** (Figure 2 and Table 4) the O 1p and O2 atoms interact with the proline (C 3p, C 4p, C 5p) and acetyl (C 11p) carbons.

Theoretical Conformational Analysis

Table 5 lists the relative energies, torsion angles and selection parameters of β turns and extended conformers of **I**–**V** peptides together with the relative energies of solvated conformers for the studied molecules, obtained by the DFT/B3LYP/6–31 + G** method.

The optimization of geometry of **I**–**V** was carried out starting with five conformers corresponding to the standard βI, βII, βIII, βVIII (Table 2) and an extended conformation. For each peptide, the geometry optimization resulted in four minimum energy conformers because geometry optimization calculations starting from βIII turns were converging to the βI-turn result. Calculations initiated on βVIII-turn conformers of **IV** produced a semi-extended structure with planar dehydroalanine. Geometry optimization of **V** resulted in only three conformations; calculations starting from βIII turn produced βI turn, and those of βVIII turn produced an S-type conformer with two γ turns, similar to that reported in [49]. In addition, each of the conformers have been minimized in two variants of the proline ring

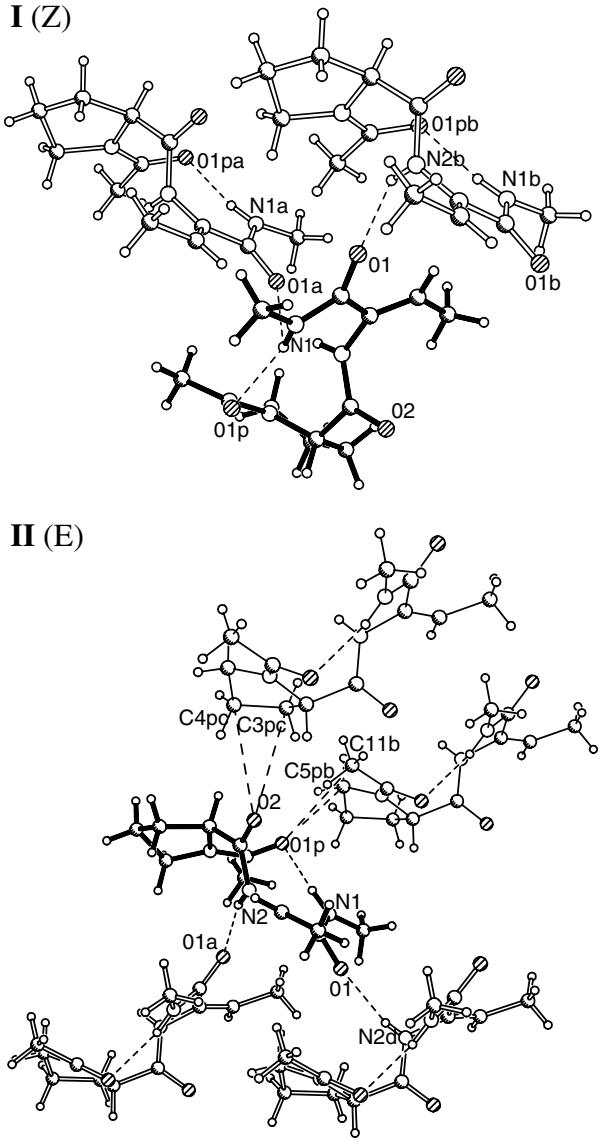

Figure 2 Association of molecules in the crystal structure of **I** and **II**. Hydrogen bonds are marked by dashed lines.

conformation, either with χ_1 in the range of 28 to 32° (C^{γ} 'up' (+)), or χ_γ between −15 and −23° (C^{γ} 'down' (−)). The calculated values of χ_1 agree with the values found experimentally in proline-containing peptides [46]. The structures with $|\tau| \leq 90°$, where τ is a 'virtual' dihedral angle $C^{\alpha}(i)$–$C^{\alpha}(i+1)$–$C^{\alpha}(i+2)$–$C^{\alpha}(i+3)$, which measures the degree of opening of a backbone fold [49], and with distance d ($C^{\alpha}(i)$–$C^{\alpha}(i+3)$) ≤ 7.00 Å [50] can be classified as β turns. Except of the extended conformers of all peptides, where $|\tau| \geq 90°$ and distance $d > 7.00$ Å, and except of the semi-extended conformers of Ac-Pro-ΔAla-NHMe which has $d > 7.00$ Å, all other conformers found by energy minimization satisfy the above-stated criteria for β turns. However, for some of the conformers of the peptides containing the residues ΔAla or (*E*)-ΔAbu, the torsion angles (ϕ_{i+1}, ψ_{i+1}) and (ϕ_{i+2}, ψ_{i+2}) are significantly distorted from the geometry of the standard β-turns listed in Table 2. The criterion of maximum deviation of any of ϕ_{i+1}, ψ_{i+1}, ϕ_{i+2} and ψ_{i+2} from the standard values by 40°, with one of the angles allowed to deviate by 50°, has been applied [45]. The conformers not satisfying this criterion were identified as distorted. The conformers βII+, βVIII+ and βVIII− of **II** are distorted at ϕ_{i+2} by 55–68°. The conformers βI+, βII+ and βII− of **IV** are even more distorted, at ϕ_{i+2} the distortion ranges 77–82° from the standard values, and moreover ψ_{i+1} of the conformer βI+ is by 94° greater that the standard, therefore making the structure a γ turn (C_7^{eq}) at Pro. In all the βI and βII conformers of all unsaturated peptides the angle ϕ_{i+2} is larger than the standard value for βI (−90°) and for βII (80°), respectively. This result illustrates the tendency of α,β-unsaturated amino acid residues, and particularly ΔAla and (*E*)-ΔAbu, to adopt an extended conformation, in which the π electrons of C^{α}=C^{β} conjugate with the π electrons of the preceding amide bond [51,52].

In vacuum, the lowest energy conformations for all unsaturated peptides are βII turns. As was expected [53], the lowest energy conformation of the reference peptide **V** is the turn βI+, and the next lowest is βII−. In chloroform and in water, βII turns are the conformations of the lowest energy for all the dehydropeptides except of **IV**. The conformation with minimum energy for the latter peptide in chloroform is semi-extended, and in water it is extended. Both in chloroform and in water **V** adopts extended conformation. Our results are in agreement with those in Ref. 10.

Table 6 lists the structural parameters for the internal N/C–H···O interactions in Ac-Pro-Xaa-NHMe, obtained by the DFT/B3LYP/6–31 + G** method. The O···H distance <2.4 Å and ∠N/C–H···O > 115° [54] were chosen as the selection criteria [55]. After the energy minimization the βI+ form of **IV** adopted a C_7^{eq} conformation (γ turn at Pro) with a strong N2–H···O 1p hydrogen bond. Except of this structure, all the βI and βII conformers of all peptides show a N1–H···O 1p hydrogen bond specific for β turn, with O···H 2.0–2.2 Å, N···O 3.0–3.1 Å and ∠N–H···O 152–169°. The βII+ and βII− conformers in **IV** and βII+ in **II** (Figure 3) reveal also additional N2–H···O 1p hydrogen bond (γ turn at Pro). The hydrogen bond N1–H···O 1p in all βI turns is longer and is less linear than the respective hydrogen bonds in βII turns, which explains partially the lower energies of the βII turns. The hydrogen bond N1–H···O 1p satisfying the selection criteria have neither been found in βVIII turns, nor in any of semi-extended or extended conformers. These conformers of dehydropeptides reveal only N2–H···O1 interactions (C_5 forms of the unsaturated residues), which are observed experimentally and predicted theoretically for diamide

Table 5 The relative energies, torsion angles and selection parameters of β turn and extended conformers together with relative energies of solvated conformers for the studied molecules, obtained by the B3LYP/6–31 + G** method

	ΔE_{vac}[a]	χ_1	ϕ_{i+1}	ψ_{i+1}	ϕ_{i+2}	ψ_{i+2}	τ	d	ΔE_{CHCl_3}[b]	ΔE_{aq}[c]
Ac-Pro-(*Z*)-ΔAbu-NHMe (**I**)										
βI+	1.78	29.1	−81.5	2.2	−113.3	9.7	47.2	5.47	1.87	1.84
βI−	2.47	−26.3	−69.5	−14.2	−111.8	12.3	39.7	5.29	1.95	1.54
βII+	0.00	28.6	−73.3	108.1	118.0	−8.9	23.5	5.51	0.60	1.08
βII−	0.08	−21.7	−63.6	117.7	115.2	−12.9	29.4	5.71	0.00	0.00
βVIII+	3.88	29.5	−85.0	−14.6	−133.7	155.0	30.2	6.82	3.19	2.69
βVIII−	4.62	−24.8	−72.5	−25.6	−132.2	155.2	22.1	6.63	3.81	3.18
Extended+	3.95	30.5	−69.3	145.5	−131.8	160.9	−176.4	10.32	2.88	1.74
Extended−	4.19	−22.2	−58.6	139.1	−132.9	160.1	179.5	10.22	2.90	1.78
Ac-Pro-(*E*)-ΔAbu-NHMe (**II**)										
βI+	1.76	30.1	−83.2	5.0	−127.6	19.3	38.3	5.22	1.77	1.97
βI−	2.48	−26.3	−69.7	−14.2	−120.4	20.1	33.6	5.11	1.79	1.54
βII+[d]	0.00	29.7	−78.4	93.5	148.3	−21.5	37.0	5.65	1.36	2.56
βII−	0.17	−20.9	−63.7	118.5	122.8	−20.7	36.5	5.80	0.00	0.00
βVIII+[d]	1.52	32.2	−90.6	0.5	−177.3	156.6	−0.5	6.88	1.68	2.18
βVIII−[d]	2.69	−22.5	−76.7	−15.2	−175.0	157.5	−12.2	6.87	2.38	2.70
Extended+	2.86	30.8	−72.5	142.6	176.0	163.6	130.0	10.01	2.83	2.27
Extended−	3.06	−22.5	−61.0	136.4	−175.7	161.5	128.6	9.84	2.67	1.81
Ac-Pro-ΔVal-NHMe (**III**)										
βI+	1.86	28.5	−80.9	−3.5	−113.2	16.5	44.5	5.29	2.07	2.02
βI−	2.40	−26.3	−69.9	−16.9	−112.2	18.7	38.4	5.16	2.11	1.90
βII+	0.04	28.2	−67.2	125.1	113.5	−18.5	34.4	5.72	0.32	0.55
βII−	0.00	−21.9	−60.4	124.8	114.0	−19.9	34.6	5.76	0.00	0.00
βVIII+	5.09	29.4	−84.9	−14.5	−122.8	133.3	44.4	6.51	4.23	3.74
βVIII−	5.81	−25.3	−71.1	−25.9	−120.5	132.3	36.9	6.17	4.81	4.18
Extended+	5.25	30.1	−69.3	141.7	−121.5	139.2	−164.5	10.09	3.90	2.72
Extended−	5.46	−21.5	−60.1	137.5	−121.4	138.7	−165.9	10.02	3.73	2.66
Ac-Pro-ΔAla-NHMe (**IV**)										
βI+[d]	1.41	31.8	−84.5	64.1	−167.9	18.7	62.2	6.56	2.05	3.52
βI−	3.96	−25.4	−70.8	−7.3	−134.1	20.6	26.0	5.06	3.28	4.53
βII+[d]	0.00	31.0	−80.8	77.3	161.9	−15.3	35.6	5.65	1.28	4.02
βII−[d]	0.81	−15.2	−77.6	84.8	156.8	−17.5	37.1	5.69	1.96	4.55
Semi-extended+	0.38	31.7	−89.9	−0.6	−179.4	164.2	−7.7	7.13	0.00	1.13
Semi-extended−	1.44	−23.9	−74.9	−17.2	−177.5	165.5	−21.1	7.15	0.55	1.38
Extended+	1.11	30.4	−72.4	140.0	178.5	−165.5	103.8	9.70	0.43	0.18
Extended−	1.39	−22.0	−61.2	134.3	178.3	−165.6	101.0	9.49	0.37	0.00
Ac-Pro-Ala-NHMe (**V**)										
βI+	0.00	28.1	−79.5	−1.5	−97.8	3.3	59.0	5.73	0.13	1.72
βI−	0.46	−27.0	−68.2	−16.4	−95.6	5.2	53.7	5.61	0.24	1.65
βII+	1.68	27.9	−63.7	130.2	72.1	11.2	6.1	5.27	0.92	1.92
βII−	1.51	−23.4	−57.3	129.3	73.4	8.8	5.8	5.36	0.43	1.22
Extended+	2.16	30.1	−71.1	137.4	−158.5	154.2	153.4	10.17	0.13	0.19
Extended−	2.39	−21.5	−60.8	133.6	−158.7	152.4	153.7	10.00	0.00	0.00

[a] Relative energies *in vacuo* (kcal mol^{-1}).
[b] Relative energies in $CHCl_3$ (kcal mol^{-1}).
[c] Relative energies in water (kcal mol^{-1}).
[d] Distorted conformations (see in the text).

derivatives of α,β-unsaturated amino acid residues [51,52].

All dehydropeptides except **I** show intramolecular C–H···O contacts where ∠C–H···O is 116–126° [54]. These interactions exist in the semi-extended and extended forms of the ΔAla containing peptide, where they connect C21 and O2, forming the C_6 structure which is similar to that found in the crystals of dehydroalanine homopeptides $(\Delta Ala)_n$ [14]. A C22–H···O1 interaction has been found in the turn conformers of **III**. In **II** either both the above-mentioned C–H···O contacts, or only one of them is present (Figure 3). The

Table 6 Structural parameters for the internal N/C–H···O interaction in the studied molecules, obtained by the B3LYP/6–31 + G** method

Conformation	N–H···O				C–H···O			
	H···O	N···O	∠N–H···O		H···O	C···O	∠C–H···O	
Ac-Pro-(*Z*)-ΔAbu-NHMe (**I**)								
βI+	2.154	3.119	158.2	N1–H···O 1p	—	—	—	—
βI–	2.106	3.082	160.6	N1–H···O 1p	—	—	—	—
βII+	2.027	3.029	168.8	N1–H···O 1p	—	—	—	—
βII–	2.009	3.013	169.4	N1–H···O 1p	—	—	—	—
Ac-Pro-(*E*)-ΔAbu-NHMe (**II**)								
βI+	2.180	3.119	153.1	N1–H···O 1p	2.289	2.985	119.9	C22-H···O1
βI–	2.128	3.088	157.1	N1–H···O 1p	2.291	2.994	120.5	C22-H···O1
βII+	1) 2.043	3.024	161.8	N1–H···O 1p	1) 2.320	2.963	116.2	C21-H···O2
	2) 2.322	2.965	120.3	N2–H···O 1p	2) 2.284	2.948	117.4	C22–H···O1
βII–	2.009	3.010	167.7	N1–H···O 1p	2.293	2.996	120.4	C22–H···O1
βVIII+	—	—	—	—	2.164	2.927	125.3	C21–H···O2
βVIII–	—	—	—	—	2.166	2.929	125.3	C21–H···O2
Extended+	—	—	—	—	2.148	2.915	125.6	C21–H···O2
Extended–	—	—	—	—	2.155	2.918	125.3	C21–H···O2
Ac-Pro-ΔVal-NHMe (**III**)								
βI+	2.170	3.100	151.7	N1–H···O 1p	2.225	2.889	117.5	C22–H···O1[a]
βI–	2.120	3.066	154.6	N1–H···O 1p	2.229	2.897	117.5	C22–H···O1[a]
βII+	2.030	3.019	163.9	N1–H···O 1p	2.236	2.902	117.4	C22–H···O1[a]
βII–	2.001	2.995	165.5	N1–H···O 1p	2.237	2.906	117.7	C22–H···O1[a]
Ac-Pro-ΔAla-NHMe (**IV**)								
βI+	1.881	2.840	154.7	N2–H···O 1p	—	—	—	—
βI–	2.159	3.133	160.1	N1–H···O 1p	—	—	—	—
βII+	2.113	3.096	163.0	N1–H···O 1p	—	—	—	—
	2.011	2.845	137.6	N2–H···O 1p				
βII–	2.082	3.068	163.4	N1–H···O 1p	—	—	—	—
	2.091	2.852	130.1	N2–H···O 1p				
Semi-extended+	—	—	—	—	2.263	2.915	116.8	C21–H···O2
Semi-extended–	—	—	—	—	2.263	2.915	116.9	C21–H···O2
Extended+	—	—	—	—	2.253	2.905	116.8	C21–H···O2
Extended–	—	—	—	—	2.257	2.907	116.7	C21–H···O2
Ac-Pro-Ala-NHMe (**V**)								
βI+	2.146	3.119	160.3	N1–H···O 1p	—	—	—	—
βI–	2.099	3.079	162.1	N1–H···O 1p	—	—	—	—
βII+	2.056	3.040	162.5	N1–H···O 1p	—	—	—	—
βII–	2.024	3.010	163.5	N1–H···O 1p	—	—	—	—

[a] C22 in ΔVal is in the (*E*) disposition, like in the (*E*)-ΔAbu residue.

turn conformers of **V** reveal the N1–H···O 1p hydrogen bonds specific for β turns, but the geometry of these bonds is less favorable than in dehydropeptides. The conformers of **V** also lack C–H···O contacts.

DISCUSSION

In crystals, the peptides Ac-Pro-(*Z*)-ΔAbu-NHMe (**I**) and Ac-Pro-(*E*)-ΔAbu-NHMe (**II**) adopt the βI– turn conformation. The peptide Ac-Pro-ΔVal-NHMe (**III**) adopts βI+ turn [9], and the peptide Ac-Pro-ΔAla-NHMe (**IV**) adopts a semi-extended+ conformation [13]. The related non-unsaturated peptide *i*PrCO-Pro-Ala-NH*i*Pr adopts a βII– turn [56]. The torsion angles (ϕ_{i+1}, ψ_{i+1}) and (ϕ_{i+2}, ψ_{i+2}) of all five peptides in the crystals (Table 3, Ref. 56) do not differ more than 40° from the angles calculated for the respective conformers (Table 5), and the best resemblance of the calculated and crystal conformers is observed for **I**, while the largest deviation is observed for **III**, which indicates that the effect of the crystal lattice is the weakest for (*Z*)-ΔAbu peptide and the strongest for that with ΔVal. Moreover, the number and the location of the

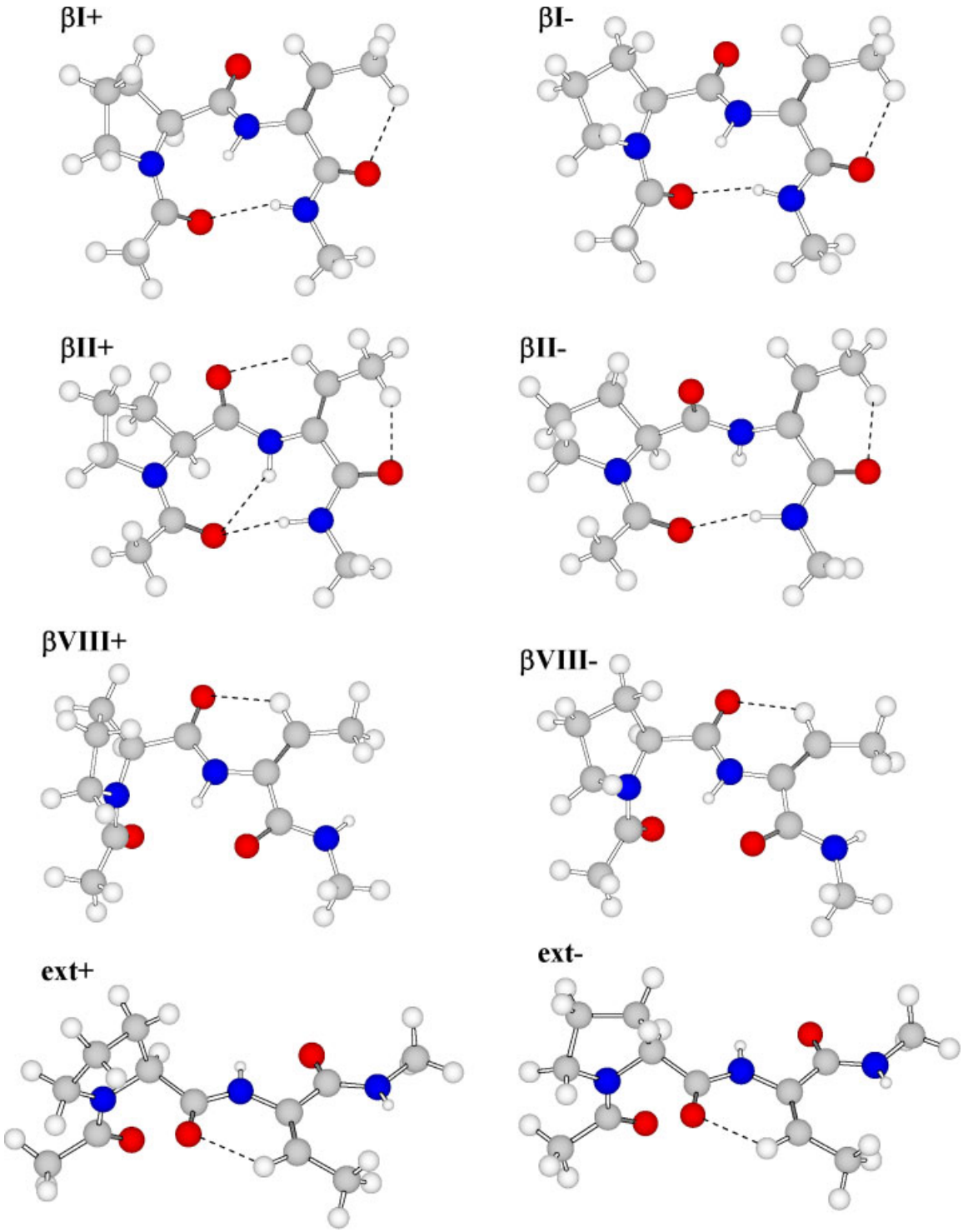

Figure 3 The Ac-Pro-(*E*)-ΔAbu-NHMe molecule in β turn and extended conformations obtained by B3LYP/6–31 + G** method.

intermolecular C–H···O contacts is also affected by the type and geometry of the unsaturated residue. In the crystal of **IV** the atoms C 11p and C21 are involved in such interactions, and the resulting intermolecular contacts are linear. In the crystal of **II** four atoms of the Ac-Pro fragment are chelating C–H donors, while in **III** only two of them are involved in C–H···O contacts. No C–H···O interactions are present in the crystal of **I**.

The calculations showed that for the peptides containing the residues ΔVal and (*Z*)-ΔAbu, the extended conformers have the highest relative energy, respectively, ~5 and ~4 kcal mol^{-1} higher than the energy of the βII+ turn, which shows the strong preference for adopting the turn conformations. This tendency is likely caused by the interaction of the *Z* substituent of the α,β-dehydro residue with the peptide backbone,

which prevents the conjugation of the π electrons within the residue. The tendency is more pronounced for dehydrovaline peptide than for the peptide with (*Z*)-ΔAbu, which is probably caused by C22–H···O1 contact within Pro-ΔVal in its βI and βII forms (Table 6), restricting the values of the torsion angle ψ_{i+2}. In **III** the difference in energy between the turn and extended forms is by approximately 1 kcal mol^{-1} larger than that for **I**, where C22–H···O1 contact does not exist.

In **II** and **IV** the tendency to form β–turns is lower than in **I** and **III**. The energy difference between βII+ and the extended form in vacuum is about 3 kcal mol^{-1} for (*E*)-ΔAbu peptide, and only 1.4 kcal mol^{-1} for the ΔAla peptide. This is a result of stabilization of the extended, βVIII and semi-extended forms by the π-electronic conjugation of the $C^{\alpha} = C^{\beta}$ and the adjacent amide bonds. The proof of the existence of such conjugations is provided by flat disposition of unsaturated residue (ϕ_{i+2} between 175.0 and 179.4°, ψ_{i+2} between 156.6° and 165.6°) (Table 5), and the shortening of C2–N2 distance and increase of N2–C3 distance in ΔAla (Table 3).

Moreover, the extended, βVIII and semi-extended forms of **II** and **IV** are stabilized by C_5 hydrogen bonds [51,52], supported by C21–H···O2 interactions [14]. The β-turn promoting interactions C22–H···O1 (Figure 3) can be found in the βI and βII forms of **II**. These interactions are similar, though longer than those in the ΔVal peptide, which causes the tendency to adopt β-turn conformation by the (*E*)-ΔAbu peptide to be higher than by the ΔAla peptide, but still lower than by the (*Z*)-ΔAbu and the ΔVal peptides.

The influence of two solvents, chloroform and water, on the conformation of studied peptides was estimated with the PCM model [48]. **I** and **III**, the two compounds showing the highest tendency to form β turns, adopt preferably the form βII in weakly polar $CHCl_3$, as well as in very polar environment of water. However, the difference of energy between solvated forms of turn βI, or βII, and those extended or semi-extended is decreased, as compared to the differences in vacuum. The energy of solvation of the extended conformers, which have no intramolecular hydrogen bonds, in $CHCl_3$ is larger by approximately 1 kcal mol^{-1} and in water is larger by approximately 2 kcal mol^{-1} than the solvation energy of the β-turn forms. This difference is predominantly a result of increased electrostatic interaction of solvents with the polar groups N–H and C=O in extended structures, where these groups are exposed to the environment. Thus, the polar environment decreases the tendency to form β turns by both **I** and **III**. NMR and IR experiments have proved that these two peptides adopt βII turns in $CHCl_3$ [16], whereas in water they prefer other forms [19]. In the crystals, Boc-Phe-(*Z*)-ΔAbu-NHMe is in βII-turns conformation; however, this compound and also two other peptides with (*Z*)-ΔAbu, Boc-Xaa-(*Z*)-ΔAbu-NHMe (Xaa = Ala, Val), did not form any detectable amount of β-turn conformers in $CHCl_3$ or in dimethylsulfoxide [18].

The energies of turn and extended conformers of **II** in different solvents are similar, and no definite solvent effect on the conformations of this compound is observed. This causes some conformational heterogeneity in polar environment. In chloroform a βII turns and another conformer with $\phi_{i+2} \sim 0°$ are observed [13]. In the same solvent the peptides Boc-Xaa-(*E*)-ΔAbu-NHMe (Xaa = Ala, Val, Phe), similar to their isomers with (*Z*)-ΔAbu, do not form any detectable amount of turn-containing conformers [18]. The semi-extended and the extended forms of **IV** in vacuum have only about 1.4 kcal mol^{-1} higher energy than the lowest βII+ turn, and the solvents apparently stabilize the extended forms. Therefore in $CHCl_3$ the form of lowest energy is semi-extended+, which is in accord with the experimental data [13]. The energies of various conformers of the reference peptide **V** are quite close, differing less than 1 kcal mol^{-1} for the forms in $CHCl_3$, and less than 1 kcal mol^{-1} for the forms in water. Accordingly, a mixture of β and γ turn is observed in $CHCl_3$ [16,57,58], and open forms are seen in water [58].

CONCLUSIONS

The present study compares the tendency to form β-turn conformers by the four homologous peptides Ac-Pro-ΔXaa-NHMe, where ΔXaa = (*Z*)-ΔAbu, (*E*)-ΔAbu, ΔVal and ΔAla, and by the reference peptide Ac-Pro-Ala-NHMe. The first three compounds adopt βI-turn forms in the solid state this work, [9], and βII turns in the solutions in $CHCl_3$ [13,16]. The fourth unsaturated peptide adopts a semi-extended conformation in the solid and in solution (chloroform). The reference peptide adopts respectively a βII turn in crystal [56], and a mixture of β and γ turn and open forms in chloroform [16,57,58]. All the peptides have the $(i + 3) \rightarrow i$ hydrogen bond in their β-turn forms.

The calculated lowest energy conformers of investigated dehydropeptides in vacuum are always βII turns. The second lowest energy conformer of Ac-Pro-ΔVal-NHMe and of Ac-Pro-(*Z*)-ΔAbu-NHMe is βI turn, Ac-Pro-(*E*)-ΔAbu-NHMe is βVIII+ turn, and that of Ac-Pro-ΔAla-NHMe is semi-extended+ conformation. The energy of βVIII+ turn of Ac-Pro-(*E*)-ΔAbu-NHMe is close to that of βI+ turn. The lowest energy conformation of the saturated peptide Ac-Pro-Ala-NHMe is βI turn. Thus, in the crystalline state the peptides adopt the respective conformations of the second lowest energy rather than those of the lowest energy found by theoretical analysis. These differences likely account for intermolecular interactions in the crystal lattice [57].

The tendency to form β turns is the most pronounced for the dehydropeptides having a (*Z*) substituent at the C^{β} atom of the unsaturated residue, specifically

for the peptides with ΔVal or with (*Z*)-ΔAbu residue. This tendency is related to the steric hindrance caused by the (*Z*) substituent, which forces the value of ϕ_{i+2} to be closer to ±90° rather than 0 or 180°. The β-turn conformation in the peptide containing ΔVal is additionally stabilized by the interaction C–H···O between $-CH_3$, its (*Z*)-β-substituent, and the carbonyl oxygen in $CONHCH_3$. Polar solvents decrease the tendency to form β turns by these peptides.

The two other studied dehydropeptides which lack the (*Z*) substituent at the C^{β} atom, (*E*)-ΔAbu and ΔAla, do not reveal any disposition to form particular turn or extended conformers. However, the polar solvents clearly stabilize extended forms of the peptide with ΔAla. No such effect was observed for the peptide containing (*E*)-ΔAbu residue. Various conformers of the saturated peptide Ac-Pro-Ala-NHMe have similar energies. The crystallographic and theoretical studies presented herein demonstrate that an intrinsic preference to form β turns by peptides containing the – Pro-Xaa – , where Xaa is an α,β-unsaturated amino acid residue, or Xaa = Ala, decreases in the following order ΔVal > (*Z*)-ΔAbu > (*E*)-ΔAbu > Ala > ΔAla. The peptide containing ΔAla in the condensed phase, even a weakly polar one, adopts always an extended conformation.

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