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The effect of β-methylation on the conformation of α, β-dehydrophenylalanine: a DFT study

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Dehydroamino acids are non-coded amino acids that offer unique conformational properties. Dehydrophenylalanine (ΔPhe) is most commonly used to modify bioactive peptides to constrain the topography of the phenyl ring in the side chain, which commonly serves as a pharmacophore. The Ramachandran maps (in the gas phase and in $CHCl_3$ mimicking environments) of ΔPhe analogues with methyl groups at the β position of the side chain as well as at the *C*-terminal amide were calculated using the B3LYP/6-31+G method. Unexpectedly, β-methylation alone results in an increase of conformational freedom of the affected ΔPhe residue. However, further modification by introducing an additional methyl group at *C*-terminal methyl amide results in a steric crowding that fixes the torsion angle ψ of all conformers to the value 123°, regardless of the *Z* or *E* position of the phenyl ring. The number of conformers is reduced and the accessible conformational space of the residues is very limited. In particular, (*Z*)-Δ(βMe)Phe with the tertiary *C*-terminal amide can be classified as the amino acid derivative that has a single conformational state as it seems to adopt only the β conformation. Copyright © 2009 European Peptide Society and John Wiley & Sons, Ltd.**

Keywords: dehydroamino acids; methylation; conformational analysis; dehydrophenylalanine; DFT calculations

Introduction

Dehydroamino acids belong to the nonstandard amino acids found in nature. They constitute, for example, nisins [1,2], thiopeptide antibiotiotics [3], dolastatins [4], microcystins and nodularins [5–8], AM-toxin [9] and tunichromes [10].

The presence of a $C^{\alpha}{=}C^{\beta}$ double bond generates unique conformational properties. There is no asymmetry on the C^{α} carbon atom and each conformer of the dehydroamino acid has a counterpart which has opposite values of torsion angles φ and ψ. Such pairs of conformers belong both to the L series of standard amino acids as well as to the D series of their enantiomers and are equally accessible. Potentially, this should make dehydroamino acid residues more flexible. On the other hand, the shorter bonds of the C^{α} carbon atom, having sp^2 hybridisation, and the possible π-electron cross conjugation involving the $C^{\alpha}{=}C^{\beta}$ double bond and neighbouring amide groups impose conformational restrictions.

Dehydroalanine (ΔAla) is a perfect example of this phenomenon. The short side chain does not impose any steric constraints and this fact enables π-electron cross conjugation to be extended over the double bond and both *N*- and *C*-terminal amide groups. All data gathered so far indicate that ΔAla prefers an extended conformation [11–13]. As a consequence, it can serve as a single conformational building block for the protein and peptide design.

The $C^{\alpha}{=}C^{\beta}$ double bond of dehydroamino acids makes the rotation of the side chain impossible and only the positions *Z* or *E* can be adopted. Thus, dehydrophenylalanine (ΔPhe) is often used to constrain the topography of the phenyl ring, which commonly serves as a pharmacophore [14–20]. Each geometrical (*Z*/*E*) isomer of ΔPhe possesses different conformational features. In its crystal state, (*Z*)-ΔPhe prefers primarily the conformation α_L with the torsion angles φ, ψ ∼ 60°, 20° and the counterpart conformation with φ, ψ ∼ −60°, −20° [21] [Searching The Cambridge Crystallographic Data Centre (November 2007) gave 121 Z-dehydrophenylalanine residues. Fifty-two residues were found to have conformation in the range of values of torsion angles φ from 46° up to 106° and ψ from −5° up to 45° with the average φ,ψ (64°, 21°). Sixty-nine residues were found to have counterpart conformation in the range of values of torsion angles φ from −39° up to −99° and ψ φ from −43° up to 3° with the average φ,ψ (−61°, −21°]. The conformer β (φ, ψ ∼ −62°, 148°) should also be taken into consideration [22–24] and this conformation is probably preferred in solution [25]. According to the scarce literature data, (*E*)-ΔPhe adopts in its crystal state the conformation β (φ, ψ ∼ −42°, 124°) and α_L (φ, ψ ∼ 51°, 49°) [26,27]. In solution, the extended conformer C_5 (φ, ψ ∼ −179°, 162°) can also be found [25].

The chosen examples reveal that dehydroamino acids are units of specific conformational properties, useful in peptide design and structure–activity research, and that they are worthy of further investigation. Particularly, ΔPhe derivatives are constantly attracting attention [28–30].

Recently, some interest was focused on dehydroamino acid having two β-substituents. β-Methyldehydrophenylalanine (Δ(βMe)Phe) was synthesised as intermediate for catalytic hydrogenation to obtain threo- and erythro-(βMe)Phe [31]. Among

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β,β'-disubstituted dehydroamino acids β-(pyren-1-yl)dehydroaminobutyric acid and β-(pyren-1-yl)dehydrophenylalanine derivatives were prepared to evaluate their electrochemical and photophysical properties as these may be potentially useful for conformational studies of peptides and proteins [32]. Also, β,β'-diaryldehydroalanines were studied and some preliminary antimicrobial properties reported [33].

Two bulky groups on the C^{β} atom can impose a conformational restriction. Dehydrovaline with a methylated *C*-terminal amide adopts only the conformation with a torsion angle $\psi \sim \pm 125^{\circ}$ [34]. Methylation of the *C*-terminal amide limits the conformational freedom of the dehydroamino acid residue regardless of the β-substituent in the side chain [35]. Our aim was to combine the conformational limitations of dehydroamino acid derivatives containing a methylated *C*-terminal amide (ϕ,ψ torsion angles of the main chain) with the topographical restriction of the phenyl ring in the position *Z* or *E* and an additional methyl group on the Cβ atom (χ torsion angles of the side chain). Therefore, we have investigated model derivatives of the β-methyldehydrophenylalanine ($\Delta(\beta$Me)Phe) as a potential building block of well-defined conformational properties.

Experimental Methods

The theoretical conformational properties of the *E* and *Z* isomers of the Bz-$\Delta(\beta$Me)Phe-NHMe (**1,2**) and Bz-$\Delta(\beta$Me)Phe-NMe_2 (**3,4**) (Figure 1) molecules were examined using the Gaussian 03 package [36]. Theoretical calculations are most often performed on diamide models protected by the *N*-acetyl group and as *C*-terminal amides. However, the *N*-benzoyl group is synthetically more convenient [31] which opens the way to further investigations of such diamide models experimentally and to explicit comparison with the theoretical results. Calculations were performed on the *trans*-amide groups (ω_0, $\omega_1 \sim 180^{\circ}$). The ϕ,ψ potential surfaces of the studied molecules were calculated at the B3LYP/6-31+G**//HF/3-21G level of theory with resolutions of 30° for the main-chain dihedral angles (ϕ and ψ). Due to the achiral nature of the molecules, only half of the maps were computed since $E(\phi,\psi)$ equals to $E(-\phi,-\psi)$. To obtain an estimate of the solvation effects on the shape of the Ramachandran map, single-point calculations were also conducted in each grid point using a self-consistent reaction field (SCRF) model. Specifically, we chose the polarisable continuum model (PCM) developed by Tomasi and co-workers to describe a $CHCl_3$ mimicking environment [37,38]. The energy surface was created using the Surfer 8 program (Golden Software, Inc. 2002). Possible energy minima on the surface were investigated on every low-energy region of the map by full geometry optimisation at the B3LYP/6-31+G** level *in vacuo* and in the $CHCl_3$ mimicking environment using the PCM model. Frequency analyses were carried out to verify the nature of the minimum state of all the stationary points obtained and to calculate the zero-point vibrational energies (ZPVEs) and both thermal and entropic corrections.

Results

Figures 2 and 3 show the $E = f(\phi,\psi)$ potential energy surfaces and the energy-minimised conformers for the molecules **1**, **2**, **3** and **4**. The maps were calculated for the isolated molecules (*in vacuo*) and in the polar environment of chloroform where the dielectric constant mimicks an environment when the amino acid residue is buried inside a folded protein. Tables 1 and 2 present the calculated relative energies of all conformers obtained *in vacuo* and in $CHCl_3$-mimicking environment, respectively.

Dehydroamino acids differ significantly in their structure from standard amino acids. The definition of some conformers is sometimes ambiguous because the values of the torsion angles ϕ, ψ and/or the kind of the stabilising forces involved do not fit to those of the conformers of saturated analogues [39,40]. Therefore, a detailed analysis of the conformers of the studied molecules has been given to explain the influence of the structural modifications introduced. The analysis was performed on the assumption that the internal X–H···A hydrogen bonds and, amongst the dipole–dipole attractions, those between the carbonyl groups C=O ▶ ··· ◀ C=O are the main stabilising internal forces [41,42]. Tables 3 and 4 collect, respectively, the geometric parameters of the hydrogen bonds and the dipole interactions between the carbonyl groups, based on the relative conformer stability of the isolated molecules being discussed. Because of the achiral α-carbon of the compounds, their maps are symmetrical in relation to the point (ϕ, $\psi = 0^{\circ}$, 0°). Therefore, to discuss the results obtained, the minima found on the upper halves of the maps have been considered.

Bz-(*E*)-$\Delta(\beta$Me)Phe-NHMe (1)

The conformational maps of compound **1** reveal four minima, both in the gas phase and in the theoretical chloroform solvent. For the molecule *in vacuo* the conformers are in the following order of energy: C_5, C_7, $\beta 2$ and α_L. The energy rises gradually and the energy gap between the highest and lowest energy conformers is 5.8 kcal/mol. The two lowest energy conformers, C_5 ($\phi,\psi = -129^{\circ}, 171^{\circ}$) and C_7 ($\phi,\psi = -80^{\circ}, 86^{\circ}$), are stabilised primarily by the N–H···O hydrogen bonds N^1–H^1···O^2 and N^2–H^2···O^1, respectively. There is also N^2–H^2···π interaction in the extended conformer C_5. The conformers $\beta 2$ ($\phi,\psi = -126^{\circ}$,

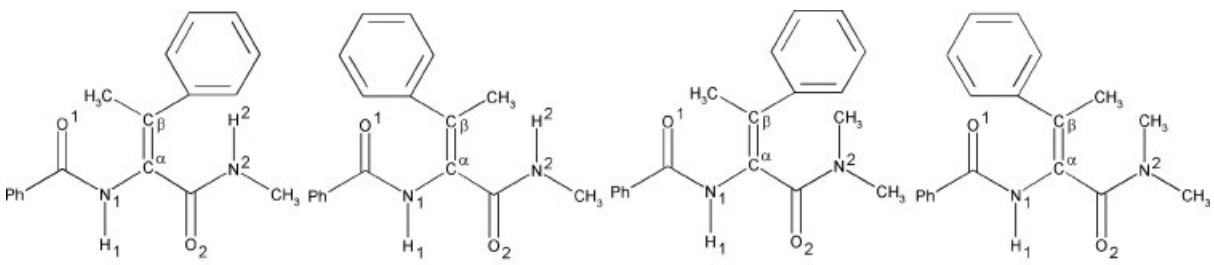

Figure 1. General formula and atom numbering for the diamides studied in this work.

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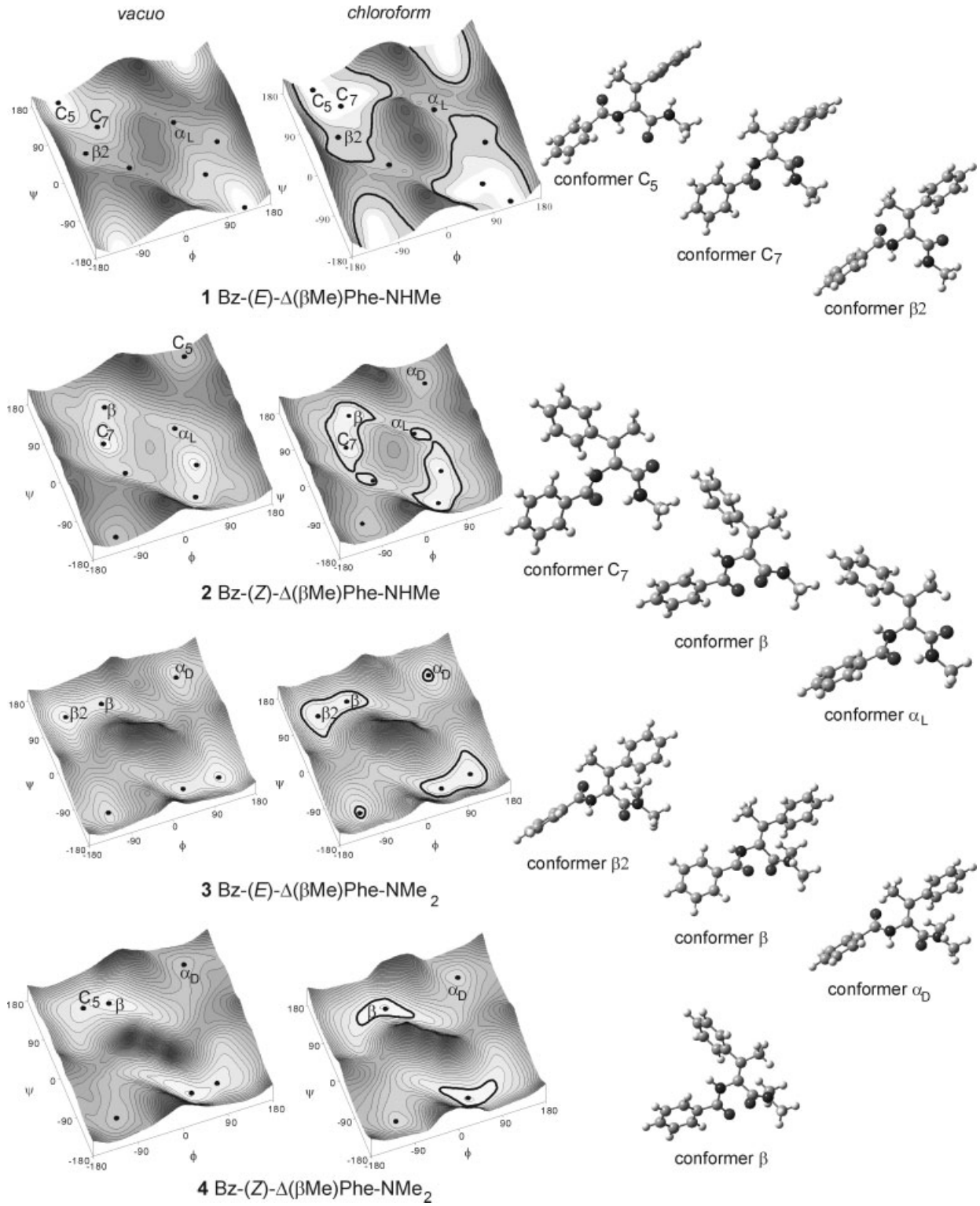

Figure 2. The ϕ, ψ potential energy surfaces, *in vacuo* and in chloroform mimicking environment, of the diamides 1–4 calculated at the B3LYP/6-31+G**//HF/3–21G level of theory together with the most stable conformers in the solvent environment after optimisation at the B3LYP/6–31+G** level of theory. Energy contours are drawn every 1 kcal/mol. The accessible conformational space of the residues in solvent environment within 2.0 kcal/mol equals 22, 15, 11 and 5% of the whole ϕ,ψ space for the compounds **1**, **2**, **3** and **4**, respectively.

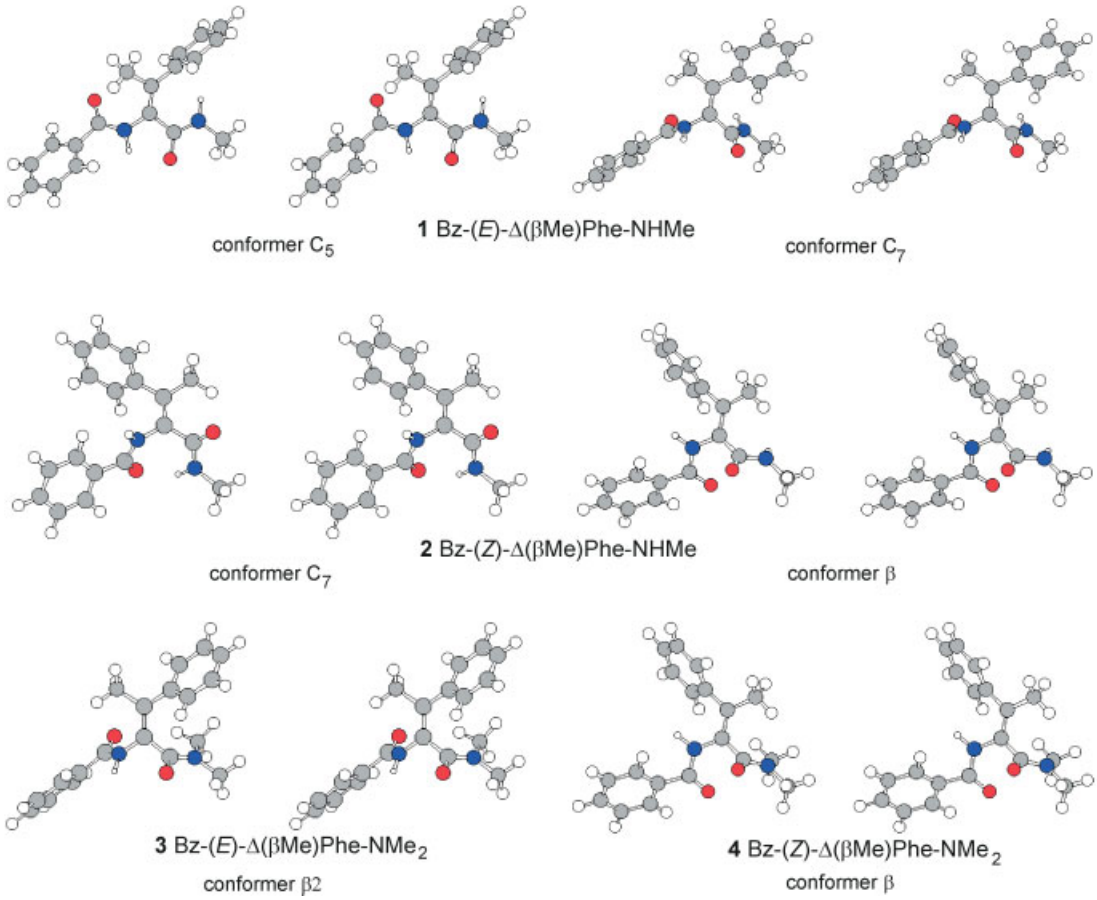

Figure 3. Stereo drawings of selected conformers of the compounds **1**–**4**.

31°) and α_L (ϕ, ψ = 57°, 33°) are stabilised by weaker N–H···N hydrogen bonds.

The solvent environment influences considerably the geometry of the conformer C_7 (ϕ, ψ = −93°, 119°) but not of the conformer C_5 (ϕ, ψ = −127°, 169°). The solvent stabilisation energy is higher for the first conformer than for the later (ΔG_{sol} = 14.4 and 10.5 kcal/mol, respectively) that is why in the solvent system they have almost the same energy. The conformers $\beta 2$ (ϕ, ψ = −122°, 54°) and α_L (ϕ, ψ = 57°, 40°) also undergo some changes, although to a lesser extent. The difference in energy between the lowest and highest energy conformers decreases to 2.4 kcal/mol.

Bz-(*Z*)-Δ(*β*Me)Phe-NHMe (2)

The conformational maps of the isomer *Z* **2** also reveal four minima in both the gas phase and in the chloroform system. There are some changes in comparison to the isomer *E*. For the isolated molecule the conformers are in the following order C_7, β, α_L and C_5. The lowest energy conformer C_7 (ϕ, ψ = −75°, 52°) is stabilised mainly by the N^2–H^2···O^1 hydrogen bond. The second is the conformer β (ϕ, ψ = −47°, 121°). Its presence on the map can be explained by internal stabilisation via the N–H···π hydrogen bond [43] as well as by the relatively short and strong dipole C═O ▶ ··· ◀ C═O attraction [42] between the carbonyls of the amide bonds (Tables 3 and 4). The third conformer, α_L (ϕ, ψ = 61°, 30°), is stabilised by the N–H···N and N–H···π hydrogen bonds. The phenyl ring in the position *Z* disturbs considerably the geometry of the extended conformer C_5 (ϕ, ψ = 128°, 162°) so that it becomes the highest energy conformer, even if it is stabilised by the N^1–H^1···O^2 hydrogen bond. The energy gap between the highest and lowest energy conformer equals 4.3 kcal/mol. It does not exceed, however, 1.2 kcal/mol between the lowest energy conformers C_7, β and α_L.

The solvent environment diminishes the difference between the conformers' energies to a value of 2.9 kcal/mol. It influences significantly the extended conformer, which shifts into the area of the conformer α_D (ϕ, ψ = 115°, 132°). The geometry of the remaining conformers does not change much and the differences in energy between the lowest energy conformers C_7, β and α_L are generally maintained (0.9 kcal/mol). The conformer β becomes the lowest in energy.

Bz-(*E*)-Δ(*β*Me)Phe-NMe_2 (3)

Conversion of the methylamide into dimethylamide changes the conformational profile of compound **3** in comparison to **1** and **2**. Only three conformers can be observed on the Ramachandran maps of **3**, both *in vacuo* and in the solvent environment, i.e. $\beta 2$, β and α_D. The lowest energy conformer is placed in the region defined as $\beta 2$ (ϕ, ψ = −122°, 119°). It is stabilised by the N^1–H^1···O^2 hydrogen bond, characteristic for extended conformers as well as by the dipole C═O ▶ ··· ◀ C═O interaction. For the second conformer β (ϕ, ψ = −44°, 121°), no internal hydrogen bond/contact can be found. Its presence on the map can be explained by the short and strong dipole C═O ▶ ··· ◀

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Table 1. Conformers of the molecules **1**–**4** and their related non-methylated derivatives *in vacuo*

Conformer	ϕ (°)	ψ (°)	Energy (Hartrees)	ΔE (kcal/mol)
Bz-(*E*)-Δ(βMe)Phe-NHMe **(1)**				
C_5	−129.2	171.0	−956.7962928	0.00
C_7	−80.0	85.5		2.87
$\beta 2$	−123.6	30.9		4.34
α_L	57.3	33.0		5.80
Bz-(*Z*)-Δ(βMe)Phe-NHMe **(2)**				
C_7	−74.5	51.7	−956.7946144	0.00
β	−47.3	121.1		1.04
α_L	60.5	29.8		1.22
C_5	128.0	162.3		4.33
Bz-(*E*)-Δ(βMe)Phe-NMe_2 **(3)**				
$\beta 2$	−121.7	119.4	−996.1005773	0.00
β	−44.2	121.3		1.73
α_D	118.5	125.2		3.31
Bz-(*Z*)-Δ(βMe)Phe-NMe_2 **(4)**				
β	−39.8	118.3	−996.0999244	0.00
C_5	−90.3	119.8		1.69
α_D	116.8	135.1		5.89
Ac-(*E*)-ΔPhe-NHMe [25]				
C_5	−179	162	−725.7344472	0.00
C_7	−75	69		4.23
β	−43	123		4.37
$\beta 2$	−153	38		6.66
Ac-(*Z*)-ΔPhe-NHMe [25]				
C_7	−56	24	−725.7330470	0.00
β	−44	139		1.28
C_5	−129	160		1.32
C_5'	133	170		1.54
$\beta 2$	−112	10		2.87
Ac-(*Z*)-ΔPhe-NMe_2 [35]				
β	−38.6	127.6	−765.0360394	0.00
C_5	−127.7	146.3		1.13
C_5'	124.4	155.6		3.36
$\beta 2$	−114.7	49.1		4.79
$\beta 2'$	126.0	50.6		6.92

Table 2. Conformers of the molecules **1**–**4** in chloroform mimicking environment

Conformer	ϕ (°)	ψ (°)	Energy (Hartrees)	ΔE (kcal/mol)
Bz-(*E*)-Δ(βMe)Phe-NHMe **(1)**				
C_7	−92.6	119.2	−956.8108577	0.00
C_5	−126.9	168.5		0.05
$\beta 2$	−121.7	53.9		0.92
α_L	57.4	40.0		2.42
Bz-(*Z*)-Δ(βMe)Phe-NHMe **(2)**				
β	−49.9	126.2	−956.8121075	0.00
C_7	−80.5	58.1		0.38
α_L	57.8	37.3		0.85
α_D	115.0	132.0		2.87
Bz-(*E*)-Δ(βMe)Phe-NMe_2 **(3)**				
$\beta 2$	−120.4	114.2	−996.1149504	0.00
β	−54.2	126.2		0.14
α_D	118.2	125.7		1.02
Bz-(*Z*)-Δ(βMe)Phe-NMe_2 **(4)**				
β	−41.1	121.1	−996.1158654	0.00
α_D	111.5	130.7		3.82

C═O attraction between the amide carbonyls. The highest energy conformer α_D (ϕ, ψ = 119°, 125°) is stabilised by a N^1–$H^1\cdots O^2$ hydrogen bond of bad geometry and a weak dipole C═O ▶ ⋯ ◀ C═O interaction.

The chloroform mimicking system does not affect either the energy order or the geometry of the conformers, which are as follows: $\beta 2$ (ϕ, ψ = −120°, 114°), β (ϕ, ψ = −54°, 126°), α_D (ϕ, ψ = 118°, 126°). It decreases, however, the energy gap between the conformers from 3.3 kcal/mol for the molecules *in vacuo* to 1.0 kcal/mol in the solvent environment.

Bz-(*Z*)-Δ(βMe)Phe-NMe_2 (4)

The conformational maps of the isomer *Z* **4** also reveal three minima in the gas phase. The conformer β (ϕ, ψ = −40°, 118°) is the lowest in energy, stabilised by the N^1–$H^1\cdots\pi$ hydrogen bond and dipole C═O ▶ ⋯ ◀ C═O interaction. The second conformer C_5 (ϕ, ψ = −90°, 120°) is stabilised by the N^1–$H^1\cdots O^2$ hydrogen bond. The two weaker hydrogen bonds C^{N2}–$H\cdots O^1$ and C^{γ} (phenyl)–$H\cdots O$ should be also considered. The third and highest energy conformer is present in the area of the conformer α_D (ϕ, ψ = 117°, 135°). It is stabilised by the N^1–$H^1\cdots O^2$ hydrogen bond and a weak dipole C═O ▶ ⋯ ◀ C═O interaction. There is a considerable energy gap of 5.9 kcal/mol between the highest and lowest energy conformers in the gas phase.

The solvent environment reduces the number of conformers, and the conformer C_5 is absent. Only the conformers β (ϕ, ψ = −41°, 121°) and α_D (ϕ, ψ = 112°, 131°) are present with almost unchanged geometry in comparison to the molecules *in vacuo*. The energy difference equals 3.8 kcal/mol.

Discussion

In the case of coded amino acids the side chain conformation can be controlled by introducing an alkyl group at the position β [44]. Such a modification allows to restrict the orientation of the aromatic ring of an aromatic amino acid residue, and enhances the lipophilicity of a peptide molecule, which helps a peptide analogue to overcome the blood–brain barrier. Moreover, this modification does not disturb much the backbone conformation.

The side chain topology of the α,β-dehydroamino acids is completely different from that of their saturated counterparts. Correspondingly the effect of β-methylation is expected to be different, but had not been studied yet. In order to evaluate the conformational consequences of introducing a methyl group at the C^{β} atom of α,β-dehydrophenylalanine, we compared the calculated Ramachandran maps of the the compounds **1**, **2**, **3** and **4** to the maps calculated for their non-methylated analogues.

Effect of β-Methylation on the Conformation of *N*-Benzoyl-Dehydrophenylalanine Methylamide

Comparison of the conformational map and minima calculated for **1** to that of the analogue without the β-methyl group (Ac-(*E*)-

Table 3. Structural parameters for the internal X–H···A interactions in the B3LYP/6-31+G** geometries of the compounds **1**–**4**[a]

	N–H···O				N–H···N or N–H···π[c]				C–H···O[b]			
Conformer code	H···O	N···O	∠N–H···O	∠C=O···H	H···N	N···N	∠N–H···N	H···O	C···O	∠C–H···O	∠C=O···H	
Bz-(*E*)-Δ(βMe)Phe-NHMe **(1)**												
C_5	2.037	2.585	111.1	88.1	2.247[c]	2.980[c]	128.4[c]	2.464	2.914	103.0	109.6	β
C_7	2.212	3.015	134.9	109.1	–	–	–	2.503	3.188	120.1	91.5	Ph
$\beta 2$	–	–	–	–	2.480	2.768	95.6	2.609	3.053	103.2	102.8	β
					2.755	2.768	80.1					
α_L	–	–	–	–	2.401	2.820	104.0	–	–	–	–	
Bz-(*Z*)-Δ(βMe)Phe-NHMe **(2)**												
C_7	1.899	2.827	149.9	105.1	2.716	2.967	94.0	2.327	3.014	119.4	87.1	β
β	–	–	–	–	2.613[c]	2.874[c]	94.5[c]	–	–	–	–	
α_L	–	–	–	–	2.364	2.793	104.5	2.257	2.943	119.1	92.8	β
					2.545[c]	2.839[c]	96.2[c]					
C_5	2.149	2.625	106.5	84.0	–	–	–	–	–	–	–	
Bz-(*E*)-Δ(βMe)Phe-NMe_2 **(3)**												
$\beta 2$	2.461	2.832	100.8	77.0	–	–	–	2.586	3.041	103.8	105.2	γ
β	–	–	–	–	–	–	–	–	–	–	–	
α_D	2.770	2.835	83.2	65.2	–	–	–	2.476	2.930	103.4	110.0	γ
Bz-(*Z*)-Δ(βMe)Phe-NMe_2 **(4)**												
β	–	–	–	–	2.590[c]	2.870[c]	95.6[c]	–	–	–	–	
C_5	2.709	2.890	89.8	77.4	–	–	–	2.550	3.436	137.8	114.7	N
								2.498	3.389	138.6	91.2	Ph
α_D	2.646	2.778	86.7	69.7	–	–	–	–	–	–	–	

Distances are given in Å. Angles are given in degrees.
[a] Data presented only for X–H···A (X = N, C; A = O, N) in which H···A ≤ 3.2 Å and ∠X–H···A > 90° acc. to [41].
[b] α, β, N, Ph denote C^{α}–H···O, C^{β}–H···O, C^{N}–H···O, and C^{γ} (phenyl)–H···O.
[c] N–H···π interaction [43] where distances are H···C^{γ} (phenyl), N···C^{γ} (phenyl) and bond angle ∠X–H···C^{γ} (phenyl).

ΔPhe-NHMe [25]) (Table 1) shows that both residues have a tendency to adopt the extended conformer C_5, which is stabilised mainly by the N^1–H^1···O^2 and N^2–H^2···π hydrogen bonds. In the case of the (*E*)-Δ(βMe)Phe derivative, however, the β-methyl group imposes a steric effect on the torsion angle ϕ (127°), and the conformation C_5 is considerably distorted. It diminishes the stabilisation gained from π-electron conjugation involving the $C^{\alpha} = C^{\beta}$ double bond and the neighbouring amide groups. The energy difference between the lowest energy conformers C_5 and C_7 is smaller for the β-methylated residue than for the non-methylated compound (2.9 and 4.2 kcal/mol, respectively). These different conformational properties are also observed in the solvent environment. This leads to a much greater accessible conformational space for the (*E*)-Δ(βMe)Phe derivative than for the related (*E*)-ΔPhe compound; it is the greatest among the studied molecules **1**–**4**. These results indicate that the introduction of the β-methyl group influences torsion angles in the main chain and increases the conformational freedom of the Bz-(*E*)-Δ(βMe)Phe-NHMe.

The Ramachandran diagram of Ac-(*E*)-ΔPhe-NHMe shows the conformer β ($\phi, \psi = -43°, 123°$). In polar environment, it becomes the global conformer of α,β-dehydroamino acids, often adopted in the solid state [25–27]. The compound **1** does not have the conformer β. Nevertheless, the energy contour of the map for **1** (Figure 2) indicates that this area is accessible, and possibly, such a conformer can be adopted. In contrast, β-methylation makes the helical conformation α_L accessible, which is not present for (*E*)-ΔPhe.

A comparison of the (*Z*)-Δ(βMe)Phe derivative **2** to its non-methylated (*Z*)-ΔPhe analogue shows that both residues assume the low-energy conformation β and α_L. For both residues the calculation *in vacuo* places the conformer C_7 as the lowest in energy. The solvent environment disfavours the conformers C_7 of both residues but to a much greater extent for non-methylated (*Z*)-ΔPhe [25] than for (*Z*)-Δ(βMe)Phe. For the latter, the conformers β, C_7 and α_L differ in energy by less than 0.9 kcal/mol. It seems that the introduction of the β-methyl group increases the conformational freedom of the *Z* isomer, although the accessible area of the map (15% of the whole ϕ,ψ space in the chloroform environment) is smaller than in the case of the isomer *E*.

Effect of both β- and *C*-terminal Methyl Groups on the Conformation of *N*-Benzoyl-Dehydrophenylalanine

Upon conversion of the *C*-terminal methylamide into dimethylamide a considerable conformational change can be observed. The conformer C_7 is lacking as the tertiary amide prevents the N^2–H^2···O^1 hydrogen bond. Interestingly, regardless of the position *Z* or *E* of the phenyl ring, the torsion angle ψ of all conformers has a strictly defined value of about 123°. This conformational feature results from the steric constraint imposed by the presence of methyl substituent at both the β carbon atom in the side chain and at *C*-terminal tertiary amide, similar to the case of dehydrovaline *N*,*N*′-dimethylamide [34]. For compound **3**, the three conformers $\beta 2$, β and α_D are accessible and the energy difference between them is very small in the solvent environment (1 kcal/mol). Nevertheless, the intrinsic steric crowding of the molecule diminishes

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Table 4. Structural parameters for the internal C=O▶ ··· ◀C=O dipole interactions in the B3LYP/6–31+G** geometries of the compounds **1**–**4**[a]

Conformer code	Parameters								Type[b]
	C^C-O^N	C^N-O^C	C–C	O–O	$\angle(C{=}O)^N-C^C$	$\angle O^N-(C{=}O)^C$	$\angle C^N-(O{=}C)^C$	$\angle O^C-(C{=}O)^N$	
Bz-(*E*)-Δ(βMe)Phe-NHMe **(1)**									
C_7	3.324	–	3.211	4.452	74.0	152.1	34.5	96.0	I
α_L	3.010	–	3.113	3.703	83.2	115.2	32.2	63.6	I
Bz-(*Z*)-Δ(βMe)Phe-NHMe **(2)**									
C_7	3.347	–	3.302	4.580	79.2	175.8	17.5	87.7	I
β	2.853	3.415	2.970	3.438	83.2	108.1	58.7	80.7	II
α_D	3.018	–	3.117	3.750	83.1	117.6	30.4	64.3	I
Bz-(*E*)-Δ(βMe)Phe-Nme_2 **(3)**									
β	2.827	3.355	2.954	3.388	83.6	106.3	60.7	81.0	II
Bz-(*Z*)-Δ(βMe)Phe-NMe_2 **(4)**									
β	2.751	3.386	2.946	3.277	86.6	104.2	58.9	74.4	II
α_D	–	3.563	3.505	4.029	60.1	84.8	77.3	103.6	III

Distances are given in Å. Angles are given in degrees. ^{N,C} denote the *N*-terminal and *C*-terminal carbonyl group.
[a] Data presented only for the C=O▶ ··· ◀C=O contacts, in which C···O ≤ 3.6 Å acc. to [42].
[b] As given by [42].

significantly in the areas around the conformers (11% of the whole φ,ψ space in chloroform). As a consequence, the (*E*)-Δ(βMe)Phe residue with a tertiary *C*-terminal amide shows one of the smallest conformational spaces among dehydroamino acids.

In the case of the (*Z*)-Δ(βMe)Phe derivative **4** steric crowding primarily reduces the number of conformers. In the solvent environment there are two conformers β and α_D with the latter one showing a relatively high energy in comparison to the conformer β. It seems that the conformer β is the primary choice for the (*Z*)-Δ(βMe)Phe residue with the tertiary *C*-terminal amide. The accessible conformational space within 2.0 kcal/mol corresponds only to 5% of the whole φ,ψ space in the solvent environment.

Conclusion

Dehydroamino acids offer conformational properties which could well be exploited for peptide design. Among the known dehydroamino acids, dehydrophenylalanine is the most commonly used to modify bioactive peptides because it is the most stable and relatively easy to obtain. On the other hand, among topographically constrained amino acids, β-methylphenylalanine is very often used as the phenyl ring commonly serves as a pharmacophore [45–51]. This is an important feature as the advances in structure–activity relationships have shown that restrictions not only of the conformations (φ,ψ torsion angles) but also of topographies (χ torsion angles) of a single amino acid residues can affect the bioactivities of peptides [52].

As it was shown in the present study, the topography of the side chain can affect the conformation of the main chain of dehydrophenylalanine residues and related derivatives. These results seem to contradict those observed with the saturated analogue β-methylphenylalanine, which is applied to control the topography of the side chain [44] based on the assumption that it does not change considerably the conformation of the main chain. It means, however, that such dependence can be used to obtain a residue featuring the desired properties.

Conformational analysis of the Bz-(*Z*/*E*)-Δ(βMe)Phe-NHMe compounds **1** and **2** revealed that β-methylation unexpectedly results in an increase of conformational freedom. Conformational analysis of the dimethylamide derivatives Bz-(*Z*/*E*)-Δ(βMe)Phe-NMe_2 (**3,4**) shows that the introduction of the methyl groups at the β carbon and at the *C*-terminal amide results in steric crowding that changes the conformational preferences of such modified residues. The torsion angle ψ of all the conformers has a value of about 123°, regardless of the position *Z* or *E* of the phenyl ring. The flexibility of the amino acid residue is considerably limited. This indicates that the Δ(βMe)Phe residue with the *C*-terminal tertiary amide exhibits significantly restricted conformational compared to dehydrophenylalanine. Particularly, (*Z*)-Δ(βMe)Phe can be classified as an amino acid that adopts a single conformational state, and thus it can potentially be applied for peptide design. Indeed the model compounds were synthesised and the conformational preferences confirmed as will be reported elsewhere.

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