ORIGINAL RESEARCH

Impact of the ΔPhe configuration on the Boc-Gly-ΔPhe-NHMe conformation: experiment and theory

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Abstract

Conformational propensities of *N-t*-butoxycarbonyl-glycine-(*E/Z*)-dehydrophenylalanine *N′*-methylamides (Boc-Gly-(*E/Z*)-ΔPhe-NHMe) in chloroform were investigated by NMR and IR techniques. The low-temperature crystal structure of the *E* isomer was determined by single crystal X-ray diffraction and the experimental data were elaborated by theoretical calculations using DFT (B3LYP, M06-2X) and MP2 approaches. The β-turn tendencies for both isomers were determined in the gas phase and in the presence of solvent. The obtained results reveal that the configuration of ΔPhe residue significantly affects the conformations of the studied dehydropeptides. The tendency to adopt β-turn conformations is significantly lower for the *E* isomer (Boc-Gly-(*E*)-ΔPhe-NHMe), both in gas phase and in chloroform solution.

Keywords ^{1}H NMR · ^{13}C NMR · IR spectroscopy · X-ray crystal structure analysis · Peptide conformational analysis · β-turn tendency · DFT-GIAO calculations · Dehydrophenylalanine · *Z* isomer · *E* isomer

Introduction

Biological activity of numerous small size molecules is directly related to their conformational properties. It is possible to control pharmaco-kinetic properties of naturally occurring peptides by introduction of nonstandard amino acid residues into their backbone chain which could produce derivatives showing more desired pharmacological properties, for example, resistance to enzymatic degradation, receptor selectivity, enhanced potency, or bioavailability [1–5]. For example, it is possible to introduce a dehydroamino acid residue and forcing a specific conformation of the chain fragment [6].

α,β-Dehydroamino acids are non-coded amino acids [7, 8] in which the $C^\alpha = C^\beta$ bond freezes the χ^1 torsion angle and sets the β-substituents in *Z* or *E* position.

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Both isomers of the dehydroresidues occur in nature and they often exhibit different biological properties [7, 9]. α,β-Dehydropeptides are more stable and resistant toward proteolytic degradation and thus could be used to design synthetic analogs of biologically active peptides [10–12]. According to the literature on α,β-dehydropeptides, the (*Z*)-dehydrophenylalanine [13–15] is the most often studied residues. It is known that (*Z*)-ΔPhe residue stabilizes β-turns in short peptides and 3_{10} helix in longer ones [13, 16]. The conformational profile of the isomer *E* is much less recognized. According to literature data, (*E*)-ΔPhe adopts in its crystal structure the β (φ, ψ ~ − 42°, 124°) conformation or the helical α_L (φ, ψ ~51°, 49°) one [17–20]. In non-polar solvents, the extended conformer C_5 (φ, ψ ~ − 179°, 162°) can be also found [21]. Spectroscopic and theoretical investigations of dehydropeptides analogs with *E* or *Z* isomers of dehydroamino acid residues in peptide chain suggest their different conformational preference in solution [22–26] has different conformational preferences in solution.

Our DFT study on Ac-Gly-(*E/Z*)-ΔPhe-NHMe [26] pointed out that (*E*)-ΔPhe has strong tendency to adopt the extended conformation while the *Z* isomer of Ac-Gly-ΔPhe-NHMe has the disposition to occur in the gas
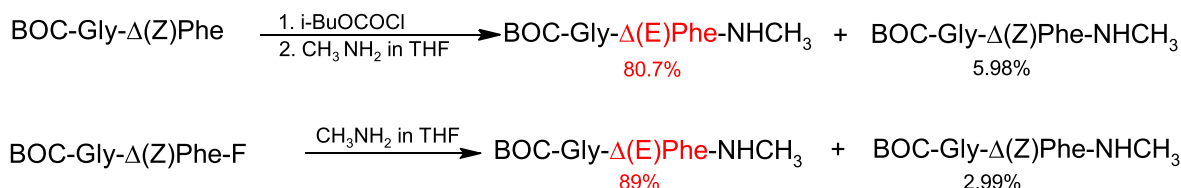
phase as type II and in solvents as type I β-turn conformation. However, the dihedral angles ϕ_1, ψ_1, and ϕ_2, ψ_2 are uncommon in standard amino acids.

The aim of the current study is to support the above theoretical results by experimental data. We were interested in determination of the impact of dehydrophenylalanine configuration on the formation of β-turn by the peptide backbone. Thus, we report on the single crystal X-ray study of E isomer of N- and C-protected dipeptide Boc-Gly-ΔPhe-NHMe. In addition, we conducted spectroscopic (FTIR and NMR) conformational research for both E and Z isomers and detailed theoretical analysis.

Experimental procedures

Synthesis of peptides

BOC-Gly-Δ(Z)Phe   →(1. i-BuOCOCl / 2. CH_3NH_2 in THF)→   BOC-Gly-Δ(E)Phe-$NHCH_3$ + BOC-Gly-Δ(Z)Phe-$NHCH_3$
                                                     80.7%   5.98%

BOC-Gly-Δ(Z)Phe-F   →(CH_3NH_2 in THF)→   BOC-Gly-Δ(E)Phe-$NHCH_3$ + BOC-Gly-Δ(Z)Phe-$NHCH_3$
                                                       89%   2.99%

Boc-Gly-ΔPhe-$NHCH_3$

Z and E isomers of Boc-Gly-ΔPhe-NHMe, abbreviated as compounds (**1**) and (**2**), were derived from Boc-Gly-(Z)-ΔPhe, synthesized using a previously reported methodology [27, 28]. In particular, *solution 1* was obtained following a simple procedure using THF (2.5 mL) cooled to −18 °C and next saturated with methylamine (30 min). Subsequently, the temperature of Boc-Gly-(Z)-ΔPhe (solution of 0.161 g, 0.5 mmol) and TEA (0.07 mL, 0.5 mmol in 2.5 mL of THF was decreased to −15 °C and subsequently, 0.065 mL, (0.5 mmol) of isobutyl chloroformate was added and next (after about 90 s) *solution 1* was poured in. The obtained liquid mixture was saturated with methylamine for 30 min and mixed (22 h) at room temperature. The excess of THF was evaporated and the oily residue dissolved in 2.5 mL of ethyl acetate. As result, a mixture of (Z) and (E) isomers was obtained. The two isomers were separated with the help of column chromatography filled with silica gel H60 (Merck). As eluent methanol in ethyl acetate was selected (from 0.05 to 0.45% of methanol). The individual components were obtained in a crystalline form from a solution $CHCl_3$ - diethyl ether (1:20, v/v)/hexane. The following yield of products was obtained: isomer E 0.135 g (80.7%); mp 180–182 °C, isomer Z 0.01 g (5.98%); mp 193–195 °C.

In parallel, a second procedure was used 0.094 mL of diisopropylethylamine (DiPEA, 0.55 mM) in 1.25 mL of THF cooled to −20 °C, saturated 30 min with methylamine. In the next step Boc-Gly-(Z)-ΔPhe-F solution (0.177 g, 0.55 mM as fluorine source) in 1.25 mL THF was added to *solution 1*, saturated with methylamine for 20 min and next mixed 22 h at 298 K. The remaining synthesis stages were identical as for the first procedure. The total yield of this synthesis was 0.149 g (89%) for isomer E (mp. 180–182.5 °C) and 0.005 g (2.99%) for isomer Z (mp. 194–196 °C). The results of elemental analysis are as follows: calcd. (%) for $C_{17}H_{23}N_3O_4$ (334.395), C—61.25%, H—6.95%, N—12.60%; found (Z): C—61.17%, H—6.89%, N—12.66%; (E): C—61.15%, H—6.83%, N—12.67%.

X-ray crystal structure analysis

The low-temperature (85 K) intensity data for Boc-Gly-(E)-ΔPhe-NHMe were measured on an Xcalibur diffractometer, using the graphite monochromated MoKα ($\lambda = 0.71073$ Å) radiation, and a cooler made by Oxford Cryosystems. The *CrysAlis CCD* and *CrysAlis Pro* programs enable both data collection and cell refinement, as well as entire processing of data reduction [29]. The crystal structure was obtained from direct methods and additionally refined on F^2, applying a full-matrix least-squares method with the help of SHELX program [30]. The positions of all non-H atoms were subsequently refined using anisotropic displacement parameters. On the contrary, the hydrogen atoms were located using Fourier difference maps. Hydrogen atoms directly attached to nitrogen atoms were refined using geometrical restrains (SADI command of SHELXL) [30]. The riding model, incorporating idealized structural parameters, including bond lengths and angles, was applied to the remaining hydrogen atoms. The isotropic displacement parameters of hydrogen atoms were taken with coefficients 1.5 and 1.2 times higher than the corresponding ones methyl carbon and the remaining carbon/nitrogen atoms, respectively. Structural drawings were obtained with *Mercury* program [31].

Crystal data for Boc-Gly-(E)-ΔPhe-NHMe

$C_{17}H_{23}N_3O_4$, $M = 333.38$, crystal size $0.48 \times 0.48 \times 0.25$ mm, monoclinic, space group $P2_1/c$, $a = 17.0537$ (6), $b = 5.9680$ (2), $c = 17.6177$ (5) Å, $\beta = 103.647$ (3)°, $V = 1742.45$ (10) Å^3, $\rho_{calcd} = 1.271$ g/cm^3, $Z = 4$, $\mu = 0.092$ mm^{-1}, reflections collected 12,547, $R_{int} = 0.0364$, data/parameters 4451/230, GOF on F^2 1.021, R_1 (all data) = 0.0550, wR_2 (all data) = 0.1100.

The crystallographic data for Boc-Gly-(E)-ΔPhe-NHMe have been deposited at the Cambridge Crystallographic Data Centre as supplementary publication no CCDC 1814248. The data can be obtained free of charge via ww.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223 336,033; e-mail: deposit@ccdc.cam.uk).

Theoretical calculations

Gaussian 09 program package was used in all theoretical studies in this work [32].

Geometry optimization

Starting geometries of the E and Z forms of the dipeptide Boc-Gly-ΔPhe-NHMe (see Fig. 1) were obtained from previously published conformers of Gly and ΔPhe moieties [26]. The dihedral angles φ and ψ, characteristic for minima of Ac-Gly-NHMe and Ac-(E/Z)-ΔPhe-NHMe, are collected in Table S1 (see the supplementary material). In addition, the standard β-turn conformations (characterized by main-chain torsion angles listed in Table S2 in the supplementary material) were also used as the starting structures in geometry optimization. We also took advantage of the achiral character of the studied molecules. Thus, only one conformer, defined by $\phi_1, \psi_1, \phi_2, \psi_2$ dihedral angles were calculated. The other one with $-\phi_1, -\psi_1, -\phi_2, \psi_2$, being a mirror reflection was not considered since the conformational enantiomers have the same energy and spectral properties.

The initial structures were calculated using B3LYP/6-311++G(d,p) level of theory [33, 34]. As result, 28 local minima were

obtained (15 and 13 for **1** and **2**, respectively). The polarized continuum model PCM [35, 36] was used to include the effect of solvent. The presence of true minimum energy structures was confirmed by lack of imaginary harmonic frequencies. In addition, single point energy calculations were performed for all found conformers in the gas phase and solution using different level of theory, which better accounts dispersion interactions (MP2/6-311++G(d,p) and M06-2X/6–311++G(d,p) [37]).

NMR calculations Fully optimized B3LYP/6-311++G(d,p) structures of **1** and **2** in chloroform within the PCM model were applied as input for all nuclear magnetic shielding calculations using a standard gauge including atomic orbitals (GIAO) approach [38] and B3LYP functional. The calculated NMR parameters are sensitive to the electron correlation presence, method, and basis set. As result of previous tests, we decided to apply aug-cc-pVTZ-J basis set [39–41] in our NMR calculations. This basis set was downloaded from EMSL [42]. Obviously, it is a tailor-made basis set for spin-spin coupling constants in case of small molecules and as a good choice in the "locally dense basis set approach" [43, 44] for larger molecules. Fortunately, this "compact" basis set produces also good shieldings [45]. Chemical shifts were obtained using benzene and TMS as a reference. In addition, for efficient calculation of nuclear shieldings, we also used a newly modified STO-3G$_{mag}$ basis set [46]. Thus, we decided to test a performance of this basis set in case of NMR parameters of model peptides.

IR spectra

The analytical grade $CHCl_3$ was dried and distilled freshly before analysis. The infrared spectra were measured at room temperature (20 °C) with a Nicolet Nexus spectrometer flushed with dry N_2. The spectral resolution was set to 2 cm^{-1}. Each spectrum was an average of 256 scans. The corresponding solvent spectra were used as a background removed during spectral processing. The solution of $8.6 \cdot 10^{-3}$–$1.7 \cdot 10^{-2}$ mol l^{-1} concentration were measured in the 2.86 mm KBr liquid cell. All data processing was performed with the
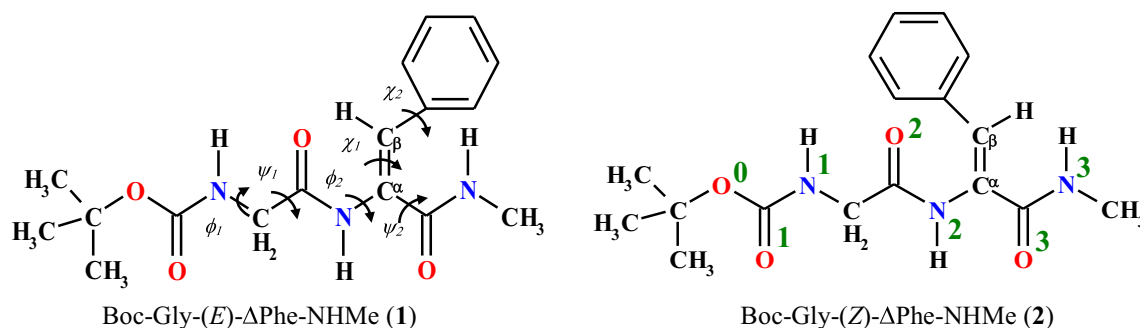
Fig. 1 General formula, atom numbering and selected torsion angles for the studied compounds. Boc-Gly-(E)-ΔPhe-NHMe (**1**) Boc-Gly-(Z)-ΔPhe-NHMe (**2**).

GRAMS AI software [47]. The second derivatives and Fourier self-deconvolution techniques were applied to obtain the initial positions and numbers of bands in the N-H region of spectra. The accurate bands positions and intensities were estimated from mixed Gauss-Lorentz curve-fitting.

NMR spectroscopy

Standard 1D ^{1}H and ^{13}C NMR spectra were recorded in CDCl$_3$ with TMS as internal standard using Bruker Ultrashield 400 MHz NMR Spectrometer at room temperature. Data acquisition and processing were performed with standard Bruker TopSpin version 2.1 program. Unequivocal assignments of the ^{1}H and ^{13}C peaks for the studied peptides were accomplished according to a standard procedure [48] with the help of 2D homonuclear NOESY and ROESY experiments adjusting the values of mixing times and spinlock at 300 ms.

Results and discussion

X-ray crystal structure characterizes conformation and association pattern

The molecular structure of Boc-Gly-(*E*)-ΔPhe-NHMe (**1**) is depicted in Fig. 2. The majority of dimensions of the studied compound are, in principal, in agreement with related compounds [49–51]. There are, however, some differences, due to different intra- and intermolecular interactions. The torsion
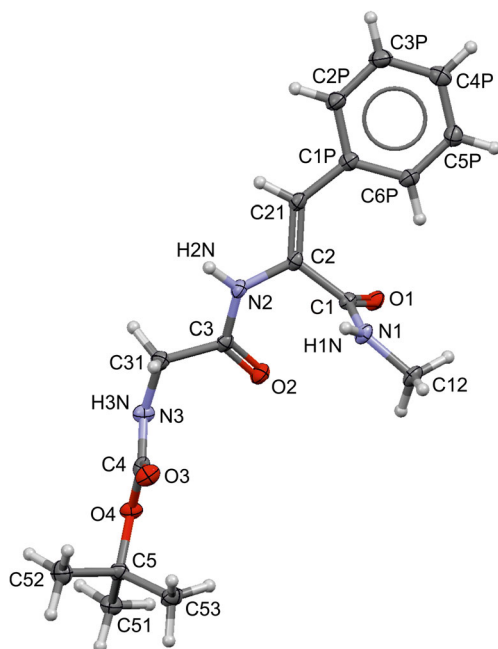
Fig. 2 The molecular structure of Boc-Gly-(*E*)-ΔPhe-NHMe (**1**) in the crystalline state, showing the molecular conformation and the atom numbering scheme

ϕ_1, ψ_1, ϕ_2, ψ_2 angles for Boc-Gly-(*E*)-ΔPhe-NHMe are 100.33(12)°, 173.32(9)° (Gly), − 23.38(15)° and − 73.51(12)° (*E*-ΔPhe). The opposite −φ and −ψ angles were also found for the corresponding symmetry-related molecules. Apart from ϕ_2, torsion C3-N2-C2-C21 (158.70(10)°), N2-C2-C21-C1P (173.53(11)°), and C2-C21-C1P-C2P (179.21(11)°) show that phenyl ring, C2 = C21 double bond, and N-terminal amide are basically coplanar indicating possible extended π-electron conjugation, whereas the C-terminal amide group is in perpendicular position resulting from a steric hindrance imposed by the phenyl ring at *E* position.

Figure 3 presents the association pattern of Boc-Gly-(*E*)-ΔPhe-NHMe in the crystal structure. Perpendicular position of the amide groups enables formation of the intermolecular N–H⋯O hydrogen bonds, which are responsible for the molecular association (Table S3). Each Boc-Gly-(*E*)-ΔPhe-NHMe molecule (serving as both hydrogen-bond donor and acceptor) is involved in six N–H⋯O hydrogen bonds, in which four are created by the (*E*)-ΔPhe flanking amide groups. The C-terminal amide group forms three N–H⋯O hydrogen bonds, involving C=O as bifurcated acceptor, whereas the N-terminal amide is involved as single N-H donor. As a result, the molecules bend in a way, which makes access to the N-terminal C=O group difficult. The weaker C–H⋯O intramolecular bonds play a supporting role and further stabilize the adopted molecular conformation (Table S3).

For *Z* analogue Boc-Gly-(*Z*)-ΔPhe-NHMe [52], the torsion ϕ_1, ψ_1, ϕ_2, ψ_2 angles are 57.2(6)°, − 141.2(4)° (Gly), − 71.5(6)°, and − 7.2(6)° (*Z*-ΔPhe), respectively. The values of torsion ϕ_2, ψ_2 indicate that C-terminal amide bond is coplanar with double bond and phenyl ring, and due to steric crowding imposed by the phenyl ring at the position *Z*, the N-terminal amide group is perpendicular to this molecular fragment. As a result, the perpendicular position of the N-terminal amide group enables formation of intermolecular N–H⋯O hydrogen bonds (Fig. 4). As can be seen, position of the phenyl ring in space, *Z* or *E*, changes the conformation of the ΔPhe residue and influences the intermolecular pattern of hydrogen bonds interactions. It also has a profound effect on the torsion angles of neighboring Gly residue, and in consequence, on the whole molecular conformation.

Theoretical calculations

α,β-Dehydrophenylalanine contains a sp^2 hybridized carbon alpha resulting in conformations different from those shown by standard amino acids. In order to simplify the nomenclature and correctly characterize the non-typical conformation, we used the more versatile and convenient Zimmerman [53] notation. The E, E*, H (or F), C, D, and G* conformations correspond to the extended (C$_5$), α$_D$, polyproline-like (β or P$_{II}$), C$_7$, β$_2$, and α' structures, reported in the literature. The direct comparison of the above-mentioned nomenclature
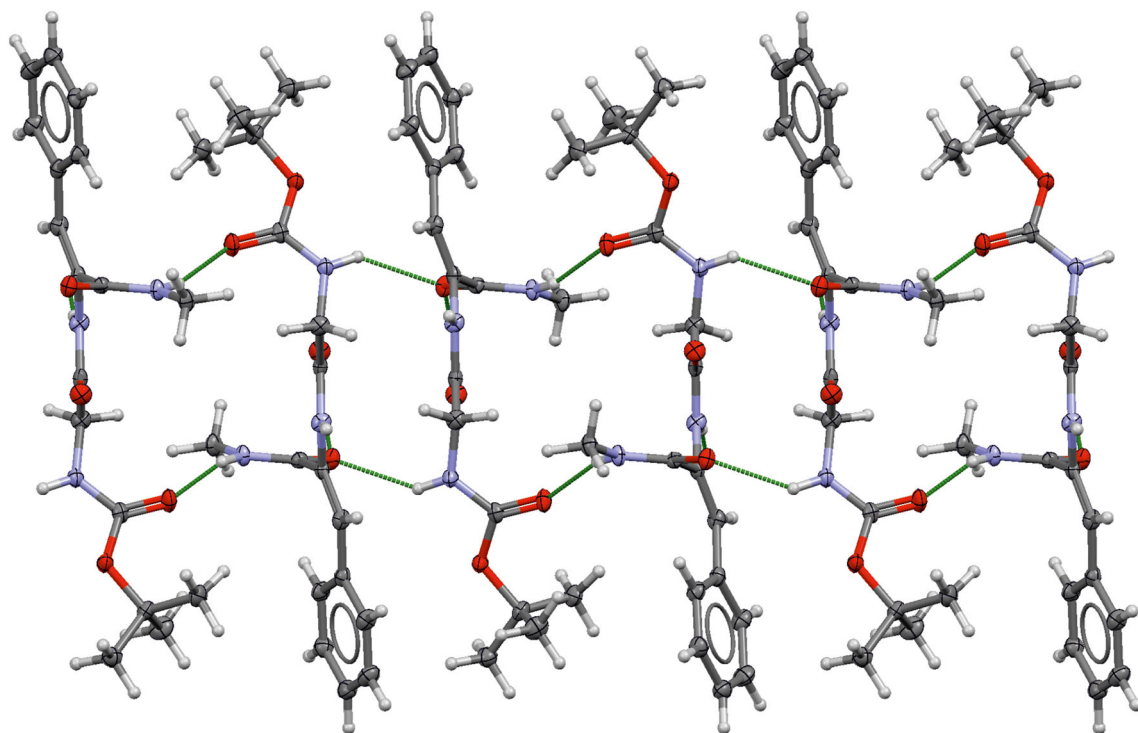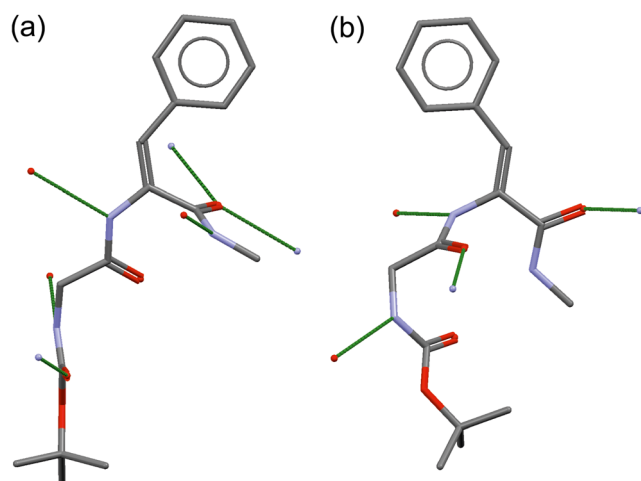
Fig. 3 The association pattern of Boc-Gly-(*E*)-ΔPhe-NHMe (**1**) molecules in the crystal structure (only one single chain is shown for clarity). The green, dotted lines indicate the N-H···O hydrogen bonds

methods related to the characterization of backbone conformation and their position on the Ramachandran map is shown in Fig. S1 in the supplementary material. In the course of calculations, we decided to classify some geometries as β-turns by using the τ and d parameters as selection criteria [54, 55]. The dihedral angle τ (O^0-C^2-C^α-C^4) (Fig. 1) indicates the openness of a backbone chain, and d measures the separation between the O^0···C^4 atoms. The τ and d parameters are gathered in Tables 1 and 3. For β-turn structures, marked

Fig. 4 The different conformations adopted by Boc-Gly-(*E*)-ΔPhe-NHMe (**a**) and Boc-Gly-(*Z*)-ΔPhe-NHMe (**b**) isomers in their crystal structures. The dotted green lines represent intermolecular hydrogen bonds interactions (hydrogen atoms have been omitted for clarity)

in bold in the tables, the following relations hold: $|\tau| \leq 90°$ and $d \leq 7.0$ Å.

Boc-Gly-(*E*)-ΔPhe-NHMe (1)

The selected parameters, including relative B3LYP/6-311++ G(d,p) calculated energies of Boc-Gly-(*E*)-ΔPhe-NHMe in the gas phase and immersed in a polarizable continuum, whose dielectric constant matches that of chloroform or water, are gathered in Table 1. Only the conformers of (**1**) with relative energies lower than 1 kcal/mol in each environment are shown. An extended version of this table, showing all found conformers with relative energy less than 5 kcal/mol, is included in the supplementary material as Table S5. Additionally, both M06-2X and MP2 single point calculations, using the same basis set, were performed (see two last columns in Tables 1 and S5). According to the results of all used methods, in the gas phase the three most stable conformers of (**1**), with nearly identical energy are B*E*, EE*, and B*E. In all of them, the (*E*)-ΔPhe residue adopts an extended conformation. The remaining conformers have much higher relative energies. Also in the presence of chloroform and water, two or three lowest energy conformers have the (*E*)-ΔPhe residue in the extended conformation. Recently, we reported that the model Ac-(*E*)-ΔPhe-NHMe diamide [49], both in the gas phase and in solution, has a strong tendency to adopt extended conformation, although polar solvent significantly reduces energy difference between this

Table 1 The Boc-Gly-(E)-ΔPhe-NHMe conformers in the gas phase and in chloroform and water (within PCM model) characterized by dihedral angles and relative energies obtained by B3LYP, M06-2X, MP2/6–311++G(d,p) methods

Conformer code [ref. 53]	$\phi_1[°]$	$\psi_1[°]$	$\phi_2[\ ]$	$\psi_2[°]$	$\chi_2[°]$	$\tau[°]$	d [Å]	ΔE [kcal/mol]	ΔE	ΔE
Gas phase								**B3LYP**	**M06-2X**	**MP2**
B*E*	114.5	− 12.2	178.0	− 155.1	52.5	− 12.5	7.3	**0.00**	**0.00**	**0.00**
EE*	− 177.1	− 179.2	179.7	163.1	− 58.7	− 174.1	10.6	0.05	0.28	1.74
B*E	112.5	− 9.3	− 177.6	161.2	− 57.3	17.8	7.3	0.18	0.15	0.15
chloroform								**B3LYP**	**M06-2X**	**MP2**
B*E*	111.3	− 7.2	178.7	− 148.0	47.2	− 10.4	7.3	**0.00**	**0.00**	**0.00**
B*E	111.9	− 5.2	− 175.2	148.4	− 45.8	28.8	7.4	0.22	0.20	0.13
E*E	177.3	− 178.5	− 179.2	153.7	− 50.5	− 163.7	10.6	0.87	1.08	2.58
water								**B3LYP**	**M06-2X**	**MP2**
B*E*	105.2	− 1.2	179.3	− 143.3	44.3	− 6.0	7.2	**0.00**	**0.00**	0.10
B*E	106.1	0.0	− 177.4	144.5	− 44.2	32.7	7.3	0.02	0.04	0.16
B*C	107.3	− 0.6	− 40.8	127.1	− 33.3	173.9	9.3	0.86	0.51	0.06
BC	− 104.6	1.5	− 41.2	127.4	− 33.5	149.0	9.1	0.96	0.48	**0.00**
X-ray structure										
E*H	100.3	173.3	− 23.4	− 73.51	− 1.9	− 31.2	7.3	–	–	–

conformation and a second conformation H. Similarly, in larger system Ac-Gly-(E)-ΔPhe-NHMe, the (E)-ΔPhe residue in (E)-ΔPhe-NHMe fragment exists in an extended conformation in three of the lowest energy minima [26]. In the currently studied dipeptide **1**, we observe a similar conformational behavior of the (E)-ΔPhe residue.

Furthermore, the Gly residue adopts the B (ϕ_1, ψ_1 ≈ 113°, − 10°) conformation in the majority of found structures of dipeptide **1**. It is rather surprising because the results of the DFT and DFT-D3 calculations [56] indicate that the energy of this conformer for Ac-Gly-NHMe diamide model in the gas phase is rather high (its relative energy is about 2 kcal/mol). For this reason, we decided to check how the exchange of the Ac- by Boc- group affects the conformational preferences of Gly derivative.

The results of B3LYP/6–311++G(d,p) calculations for Ac-Gly-NHMe (**3**) and Boc-Gly-NHMe (**4**) in the gas phase and in water are shown in Table 2.

As can be concluded from the results of the calculations presented in Table 2, exchange of Ac- group on Boc- has a negligible effect on the geometric parameters and the relative energies of E and C conformers. In contrast, there is a clear reduction of the relative energy of B conformer. The B structures of **3** and **4** are stabilized by N-H⋯N intramolecular hydrogen bonds and this interaction is stronger and shorter in the case of Boc-Gly-NHMe molecule. The N⋯H distances are 2.37 Å and 2.31 Å for **3** and **4**, respectively. For both of the studied molecules, **3** and **4** water most strongly stabilizes conformer B which in aqueous environment becomes a global

Table 2 Selected conformational parameters and relative energies for Ac-Gly-NHMe (**3**) and Boc-Gly-NHMe (**4**) conformers in the gas phase and water calculated by B3LYP/6–311++G(d,p) method

Conformer	$\phi[°]$	$\psi[°]$	ΔE [kcal/mol]	$\phi[°]$	$\psi[°]$	ΔE [kcal/mol]
Ac-Gly-NHMe (**3**)	Gas phase			Water		
E (C$_5$)	− 179.9	179.9	0.42	180.0	180.0	0.45
C (C$_7$)	82.5	− 71.0	0.00	83.1	− 61.7	0.61
B	112.7	− 17.1	2.03	99.9	0.0	0.00
F	–	–	–	− 77.9	158.2	0.92
Boc-Gly-NHMe (**4**)	Gas phase			Water		
E (C$_5$)	177.3	178.6	0.85	180.0	180.0	1.00
C (C$_7$)	82.6	− 69.0	0.00	83.7	− 58.8	1.24
B	117.9	− 13.0	0.75	105.1	− 0.7	0.00
F	–	–	–	− 77.0	167.0	1.17

Fig. 5 The global minimum (B*E*) and the β-turn conformation (C*D) for Boc-Gly-(E)-ΔPhe-NHMe (**1**) optimized at the B3LYP/6–311++G** level of theory in the gas phase. The hydrogen bonds are marked by dashed lines
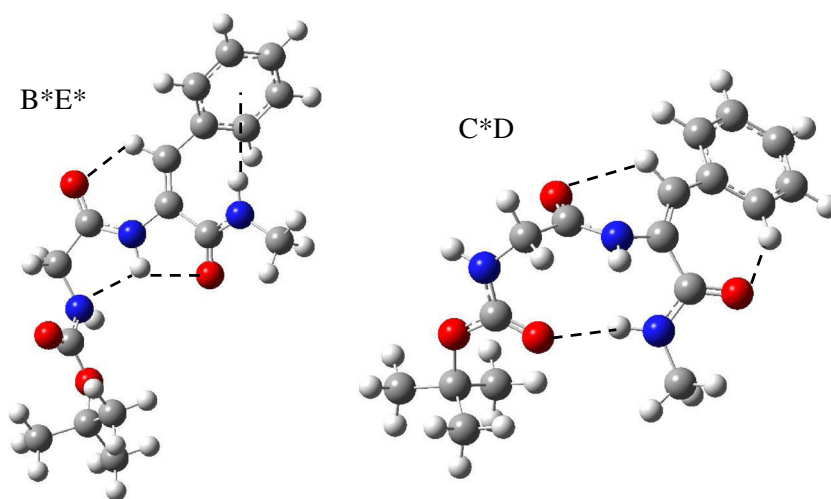
minimum. In the water environment for both molecules **3** and **4,** we found additional local minimum F, stabilized mainly by attractive interaction of the two carbonyl groups. This structure is similar to the conformation of the Gly residue in the Boc-Gly-(E)-ΔPhe-NHMe crystal (φ,ψ = 100°, 173°).

The presence of a number of subtle and simultaneous noncovalent interactions determines the conformation of a polypeptide chain. We assume in this study that both H-bond (N-H···O, N-H···N, N-H···π, C-H···O) [57] and dipole-dipole forces [58] are the main factors, which determine the peptide conformation. On the other hand, we omit hyperconjugative effects [59, 60] in our considerations. The characteristic structural parameters for all intramolecular hydrogen bonds stabilizing conformers of the studied dipeptides are shown in Table S4 in the supplementary material.

It is important to notice that E conformation of (E)-ΔPhe residue is stable due to an extremely short N^2-H···O^3 C_5 hydrogen bond (H···O^3 distance of 2.00 Å) and by C^β-H···O^2 resonance assisted intramolecular H-bond (RAHB) [61]. This interaction leads to a formation of a six-member ring. In addition, the stabilization of this conformation causes the N^3-H···π hydrogen bonding with phenyl ring (Fig. 5).

According to B3LYP results in the gas phase, chloroform, and in water, the structure B*E* in Fig. 5 is the lowest energy conformer of **1**. This structure is stabilized by above-described interactions within the conformer E and B of (E)-ΔPhe and Gly residues, respectively. Second in energy order is the extended conformer EE*, stabilized mainly by two N-H···O C_5 H-bonds. It is worth noting that increasing polarity of the environment causes a marked destabilization of this conformer. Moreover, the single point energies calculated with MP2 are much higher for this conformer in all studied environments (by about 1.7 kcal/mol and 1.5 kcal/mol in comparison to the B3LYP and M06-2X results, respectively).

Conformer C*D of **1** is the lowest in energy β-turn structure and is characterized by a fairly high relative energy of 4.35 kcal/mol (Table S5 in the Supporting Information). This β-turn conformer contains 1 ← 4 H-bond with the N^3-H···O^1 distance equal to 2.11 Å. In addition, there are present two C-H···O interactions (see Fig. 5, as well as Table S4 in the Supporting Information). The torsion angle χ2 is only − 21° and this suggests a partial π-electron conjugation of the phenyl ring of (E)-ΔPhe residue with the double bond $C^\alpha = C^\beta$. A comparison of backbone angles with the values for typical β-turns (see Table S2) indicates the C*D conformation is a distorted Type II β-turn.

For the model diamide Ac-(E)-ΔPhe-NHMe, we observed a decrease of relative energy of helical conformer A* as a result of increased solvent polarity [20]. A similar phenomenon was observed for a larger model derivative of (E)-ΔPhe [26, 49]. Also the investigation in this work molecule (**1**) shows an increased tendency to adopt a helical conformation with increasing polarity of the environment. Among the low energy conformers of (**1**) (ΔE < 5 kcal/mol) the (E)-ΔPhe residue adopts conformer A for 0, 2, and 4 structures in vacuo, chloroform, and water, respectively (see Table S5 in the Supporting Information).

Boc-Gly-(Z)-ΔPhe-NHMe (2)

In the four lowest-energy structures (ΔE ≤ 1 kcal/mol) of (**2**) in the gas phase (Table 3 and Table S6 in Supporting Information) obtained by B3LYP density functional the (Z)-ΔPhe residue adopts B conformation ($\phi_2, \psi_2 \approx −60°, 20°$) which is stabilized by C_7 N^3-H···O^2 H-bond. According to this method, the lowest energy structure of the **2** in vacuum is the conformer B*B with the previously described conformation B* on Gly residue stabilized by N^2-H···N^1 hydrogen bond, and B conformation on the (Z)-ΔPhe residue (Fig. 6, Table S4). The BB conformer which is the second in energy order (ΔE = 0.43 kcal/mol), is stabilized by an almost identical system of hydrogen bonds as the B*B conformer.

Table 3 The Boc-Gly-(**Z**)-ΔPhe-NHMe conformers in the gas phase and in chloroform and water (within PCM model) characterized by dihedral angles and energy differences obtained by B3LYP, M06-2X, MP2/6–311++G(d.p) methods

Conformer code [Ref. 53]	φ₁[°]	ψ₁[°]	φ₂[°]	ψ₂[°]	χ₂[°]	τ[°]	**d** [Å]	ΔE [kcal/mol]	ΔE	ΔE
Gas phase								**B3LYP**	**M06-2X**	**MP2**
B*B	119.0	−11.1	−56.8	25.1	−27.5	139.9	8.60	**0.00**	1.04	0.46
BB	−114.8	9.5	−54.4	23.9	−39.0	133.7	8.43	0.43	2.02	1.67
AB	**−76.5**	**−13.3**	**−80.3**	**−3.6**	**−39.3**	**65.3**	**5.88**	0.88	**0.00**	**0.00**
EB	−171.0	177.5	−56.9	23.6	−35.2	−54.9	8.16	1.04	2.79	4.56
CB*	**−62.6**	**127.2**	**109.3**	**−11.8**	**−18.8**	**37.7**	**5.76**	1.11	0.84	0.48
in chloroform								**B3LYP**	**M06-2X**	**MP2**
AA	**−72.0**	**−19.5**	**−64.1**	**−15.2**	**−22.7**	**71.4**	**5.94**	**0.00**	**0.00**	**0.00**
BF*	−115.9	7.7	46.0	−141.2	28.6	−154.7	9.25	0.80	2.51	1.85
CB*	**−62.3**	**127.1**	**105.4**	**−12.3**	**−20.1**	**31.9**	**5.71**	1.23	1.51	1.55
B*B	119.6	−8.9	−60.2	26.9	−25.7	136.8	8.49	1.28	3.30	3.00
in water								**B3LYP**	**M06-2X**	**MP2**
AA	**−71.0**	**−20.9**	**−64.2**	**−16.2**	**−15.2**	**69.9**	**5.90**	**0.00**	**0.00**	**0.00**
BF*	−115.4	5.7	47.0	−142.9	28.0	−155.4	9.30	1.40	3.12	2.20
B*F*	106.9	−3.9	46.3	−142.4	27.6	−140.5	9.00	1.34	3.30	2.62
FB*	**−61.4**	**131.6**	**94.4**	**−8.4**	**−14.0**	**24.5**	**5.70**	1.73	2.06	2.68
X-ray structure [52]										
FB*	**−57.2**	**141.2**	**71.5**	**7.2**	**0.5**	**19.4**	**5.28**	–	–	–

We also found two conformations of **2,** which fulfill the β-turn criteria: AB and CB*. The corresponding relative energies of these structures are 0.9 and 1.1 kcal/mol. Both conformations are stabilized by 1 ← 4 hydrogen bonds and their relative energy confirms repeatedly described in the literature tendency of (**Z**)-ΔPhe residue to stabilize β-turn conformation. It should be noted that the A conformation of the Gly residue was not found for the model molecules either **3** or **4**. It should rather be regarded as modified B conformation, because it is also stabilized by N²-H···N¹ hydrogen bond. Moreover, according to the results of MP2 and M06-2X methods conformer AB is the global minimum of **2,** and the second β-turn structure (CB*) has a much lower relative energy than is provided by B3LYP method. The latter structure is the global minimum of previously studied model dehydropeptide Ac-Gly-(**Z**)-ΔPhe-NHMe according the B3LYP and MP2 methods [26].

The results of B3LYP, M06-2X, and MP2 calculations indicate a significant stabilization of the β-turn structure of **2** by solvent. In chloroform and water environment, the global minimum is AA, which evolved from the AB conformation after small changes in φ₂, ψ₂ torsion angles within the (**Z**)-ΔPhe residue. Both residues in this conformer exhibit a helical conformation, with φ₂, ψ₂ angles of − 64° and − 15°. Similarly, the

Fig. 6 The global minimum (B*B) and the β-turn conformation (AB) for Boc-Gly-(**Z**)-ΔPhe-NHMe (**2**) obtained by B3LYP/6–311++G(d,p) method in the gas phase. The H-bonds are marked as dotted lines
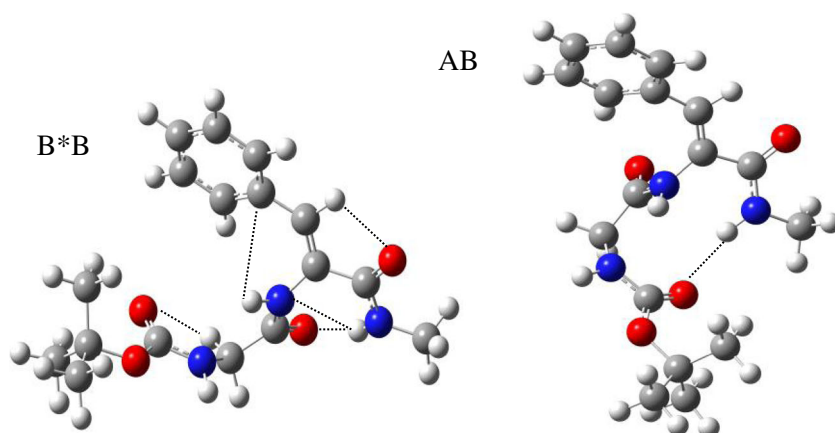
**Fig. 7** The ν_s(N-H) region of IR spectra of (*E*) and (*Z*) isomer of Boc-Gly-ΔPhe-NHMe in CHCl$_3$ solution
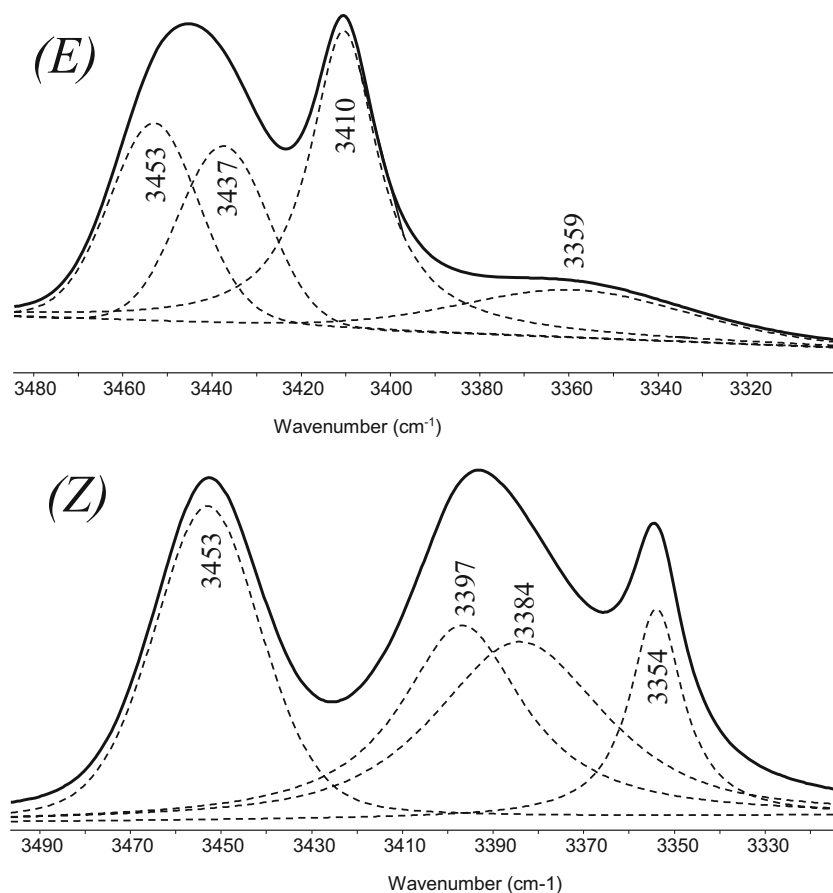
3_{10}-type helix induced by solvent was recently observed for Ac-(*Z*)-ΔPhe-NHMe [49] and also in case of longer peptide chain containing (*Z*)-ΔPhe residue [26]. Boc-Gly-(*Z*)-ΔPhe-NHMe exists in crystalline state as the β-turn Type II. This

Table 4 B3LYP calculated and experimental ^{13}C and ^{1}H NMR chemical shifts for **E** and **Z** isomers of Boc-Gly-ΔPhe-NHMe in chloroform

Atom	Izomer **E**			Izomer **Z**		
	aug-ccpVTZ-J	STO-3G$_{mag}$	Exp.	aug-ccpVTZ-J	STO-3G$_{mag}$	Exp.
CH$_3$ [Boc]	29.30	27.22	26.55	29.34	29.28	28.24
CH$_3$ [NHMe]	29.12	28.26	28.28	29.59	29.30	29.71
C$^\alpha$ [Gly]	42.00	48.26	45.09	50.88	43.01	45.16
C$^\gamma$ [Boc]	83.07	81.74	80.55	92.64	82.66	81.32
C$^\alpha$ [Δ$^{E/Z}$Phe]	132.51	134.03	134.79	131.66	128.54	128.76
C$^\beta$ [Δ$^{E/Z}$Phe]	118.41	117.62	119.82	132.71	135.62	133.53
C$^\gamma$ [Δ$^{E/Z}$Phe]	139.49	140.19	129.29	137.80	138.17	
C$^\zeta$ [Δ$^{E/Z}$Phe]	127.15	127.39	128.12	130.57	129.29	129.08
C$^{\delta1,2}$; C$^{\epsilon1,2}$ [Δ$^{E/Z}$Phe]	128.72	129.94	128.87	128.62	131.52	130.91
C=O [Boc]	155.89	153.20	156.03	158.37	155.50	156.84
C=O [Gly]	169.02	166.76	168.28	167.32	165.62	169.07
C=O [Δ$^{E/Z}$Phe]	166.16	164.37	164.76	164.32	165.07	165.32
CH$_3$ [Boc]	1.46	1.31	1.48	1.44	1.31	1.45
CH$_3$ [NHMe]	2.57	2.40	2.66	2.80	2.67	2.91
H$^\alpha$ [Gly]	3.96	3.65	3.93	3.67	3.21	3.86
HN [Gly]	4.58	4.18	5.60	4.83	5.00	5.28
H$^\beta$ [Δ$^{E/Z}$Phe]	8.88	8.86	8.02	7.91	7.66	7.49
H$^{\delta1,2}$; H$^{\epsilon1,2}$ [Δ$^{E/Z}$Phe]	7.20	7.13	7.34	7.51,	7.63,	7.58, 7.36
				7.20	7.10	
HN [Δ$^{E/Z}$Phe]	8.91	8.70	8.42	7.14	6.34	6.81
HN [NHMe]	5.26	5.03	5.18	7.28	7.82	7.86
RMS	**1.74**	**1.73**		**2.18**	**1.68**	
cpu	33d 18 h 26 min	16 h 51 min		26d 6 h 17 min	17 h 52 min	

Fig. 8 Calculated vs. experimental ^{1}H and ^{13}C chemical shifts for E and Z isomers of Boc-Gly-ΔPhe-NHMe in deuterochloroform
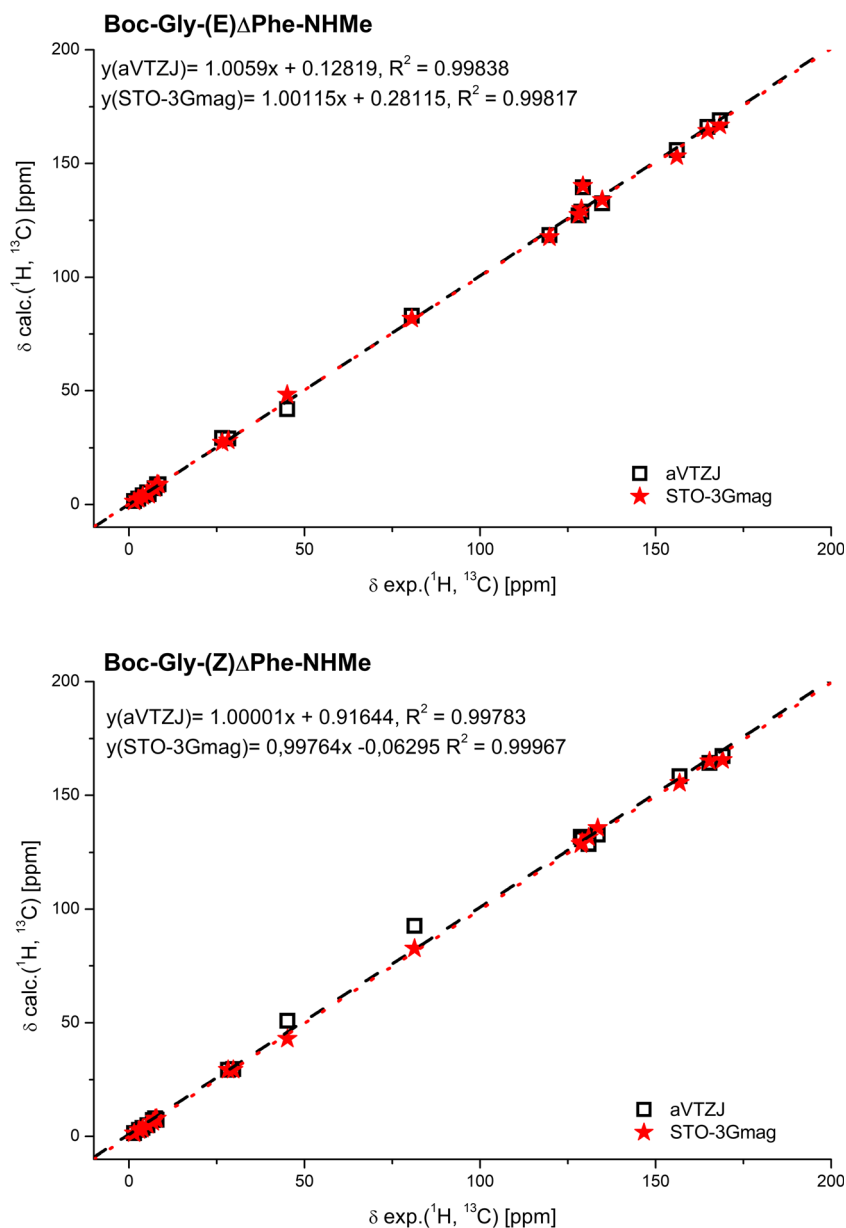
conformation has the backbone angles (ϕ_1, ψ_1, ϕ_2, ψ_2 = − 57°, 141°, 72, 7°) [52], analogues to those theoretically predicted for conformer FB* in water.

From the relative energies, it was possible to determine the ratio of β-turn structures for **2** as 15, 62, and 79% in the gas phase, chloroform, and water, respectively. Such results indicate the high preference of dipeptide with (Z)-ΔPhe residue in the ($i + 2$) position to exist in polar media in bended form.

FT-IR spectra

Three ν_s(N-H) bands, at 3445, 3410, and 3359 cm^{-1}, are visible in the FT-IR spectrum of Boc-Gly-(E)-ΔPhe-NHMe (**1**) in CHCl$_3$ solution (Fig. 7). On the basis of comparison between of Ac-(E)-ΔPhe-NHMe spectra in CH$_2$Cl$_2$ solution

[21], we can assign the 3410 band to ν_s(N^2-H) vibration in C$_5$ conformation of (E)-ΔPhe residue. The 3359 cm^{-1} band is related to NH groups involved in intermolecular hydrogen bonds because the intensity of this band decreases with decreasing concentration of the peptide solution. The band at 3445 has a large half-width and is the result of a superposition of two bands: 3453 and 3437 cm^{-1} due to N^1−H and N^3−H stretch modes, respectively. This assignment of band positions is consistent with the calculated IR spectrum of conformer B*E*. Thus, comparing IR spectrum of Boc-Gly-(E)-ΔPhe-NHMe (**1**) in CHCl$_3$ solution with experimental spectra of similar molecular systems and with theoretical spectrum of B*E* conformer, one could assume that in chloroform solution peptide **1** exist in extended conformation within (E)-ΔPhe residue. In Fig. S3 in the Supplementary Material

are presented scaled theoretical infrared bands of N-H stretch modes superimposed on experimental spectra of (E) and (Z) isomers of Boc-Gly-ΔPhe-NHMe showing a reasonable agreement between predicted and observed spectra.

In the ν_s(N-H) region of the Z isomer spectrum three overlapping broad bands are observed. The accurate positions of four peaks were determined from a curve fitting: 3453, 3397, 3384, and 3354 cm^{-1}. The 3354 cm^{-1} band is concentration independent and, therefore, may be assigned to an intramolecularly hydrogen-bonded N-H group. Comparison with the calculated IR spectra for different Boc-Gly-(Z)-ΔPhe-NHMe conformers allows us to assign this band to N^3-H group in β-turn structures CB* or AA. The 3453 cm^{-1} originates from N^1-H group, and the bands 3397 and 3384 cm^{-1} come from N^2-H group in two different β-turn conformation. This conclusion is consistent with the results of previous spectroscopic studies [62] on Boc-Ala-(Z)-ΔPhe-NHMe conformation in chloroform, where the authors also showed the occurrence of a significant population of type II β-turn structures with some evidence for the population of type I β-turn.

^{1}H and ^{13}C NMR spectra

Our earlier experiences [63] with prediction of proton and carbon NMR parameters in CDCl$_3$ and DMSO-d$_6$ solutions, calculated using B3LYP/aug-cc-pVTZ-J and PCM approach, pointed out the possibility of distinguishing between E and Z isomers of Ac-ΔPhe-NMe$_2$.

In the current study, the experimental H-1 and C-13 chemical shifts of E and Z isomers of Boc-Gly-ΔPhe-NHMe in CDCl$_3$ are accurately predicted (see Table 4). For E and Z isomers the calculated values are obtained for B*E* and AA conformers, respectively (they are the lowest energy structures in chloroform according to our calculations). Their presence in solution was also supported by IR data.

In case of N^{2}H signal, one can notice the biggest discrepancies between both isomers (shifted by about 1.6 ppm to lower magnetic field in E isomer) due to formation of intramolecular H-bond. Besides, H$^\beta$ signal in E isomer is about 0.6 ppm shifted to the lower magnetic field. Very similar effects were observed in our earlier studies on E and Z isomers of Ac-ΔPhe-NMe$_2$.[63]. Such large proton chemical shift differences could be explained by resonance-assisted H-bond in C$_5$ conformation of (E)-ΔPhe residue. In addition, a very large difference is observed for chemical shift of N^{3}H signal in E and Z isomers (5.18 and 7.49 ppm). This is due to formation of 1 ← 4 intramolecular H-bond by N^{3}H which stabilizes the β-turn structure. As expected, the chemical shift of tert-butyl group at about 1.5 ppm is very similar for both Z and E isomers.

In general, a nice theoretical reproduction of experimental positions of carbon signals is observed (Table 4). However, due to problems with solubility of the studied compounds in chloroform, the presence (and assignment) of C$^\gamma$ [Δ$^{E/Z}$Phe]

signal in the spectra is controversial (the difference between a theoretical chemical shift and a spectral feature buried in the noise is about 10 ppm).

The obtained results indicate a good performance of small STO-3G$_{mag}$ basis set with respect to a significantly larger aug-cc-pVTZ-J one (RMS of 2.74 vs. 2.76 ppm for isomer E, and 2.61 vs. 1.34 ppm for isomer Z in Table 4). The former basis set, being about half the size of the second one, yielded the magnetic shieldings significantly faster (CPU time of 17 h vs. 34 days, see Table 4).

The signal assignment of ^{1}H and ^{13}C spectra of the studied compounds was also supported by a linear correlation between theory and experiment (Fig. 8). The obtained linear equations indicate very similar performance of ^{1}H and ^{13}C chemical shifts of Boc-Gly-(E)-ΔPhe-NHMe isomer in CDCl$_3$ ($y = 1.006\ x + 0.128$, $r^2 = 0.9984$ for aug-cc-pVTZ-J and $y = 1.001\ x + 0.281$, $r^2 = 0.9982$ for STO-3G$_{mag}$). The correlations are similar for the Z isomer: $y = 1.000\ x - 0.916$ ppm, $r^2 = 0.9978$ and $y = 0.9976\ x - 0.0630$, $r^2 = 0.9997$, for aug-cc-pVTZ-J and STO-3G$_{mag}$ basis respectively. In addition, we notice an overlap of points, calculated with both kinds of basis sets.

On the basis of the presented results, it is evident that molecular modeling is capable of selecting the dominant conformer in solution using the direct comparison between predicted and measured proton and carbon NMR signals [64].

Conclusions

Among the promising ways of obtaining potentially active peptidomimetics is the inclusion of a dehydrophenylalanine residue into the peptide chain. Such structural modification leads to compounds with better proteolytic stability and increased hydrophobicity, resulting in improved blood-to-brain penetration, and rigidity of the molecular fragment.

Extensive theoretical calculations, as well as experimental X-ray, NMR, and IR methods were used to determine the conformational properties of E and Z isomers of Boc-Gly-ΔPhe-NHMe in chloroform. The presence of particular conformers of the studied isomers in solution, visible in IR and NMR spectra, was supported by theoretical calculations. Our studies revealed that the conformational preference of peptide backbone is strongly influenced by the position of the side chain in the ΔPhe residue. The E isomer of dehydrophenylalanine shows a tendency to form an extended conformation both in vacuum and in polar media. However, increased polarity of the environment promotes the helical conformations of peptide with (E)-ΔPhe moiety.

The Z isomer of ΔPhe promotes the formation of β-turn structure. According to our theoretical modeling and infrared spectra, the Boc-Gly-(Z)-ΔPhe-NHMe in chloroform solution exists as a mixture of βII and βIII turns. Our results in solution are in good agreement with earlier X-ray studies. Methods

which account for dispersion (MP2 and M06-2X) work better than B3LYP in predicting the order of peptide conformers with respect to their relative energy. In this study, we also demonstrated a good agreement observed between chemical shifts calculated using small STO-3G$_{mag}$ basis sets and the dedicated ones.

Compliance with ethical standards

Conflict of interest   The authors declare that they have no conflict of interest.

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