Effect of the ΔPhe Residue Configuration on a Didehydropeptides Conformation: A Combined CD and NMR Study

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ABSTRACT:

*Conformations of two pairs of dehydropeptides with the opposite configuration of the ΔPhe residue, Boc-Gly-Δ^ZPhe-Gly-Phe-OMe (**Z-OMe**), Boc-Gly-Δ^EPhe-Gly-Phe-OMe (**E-OMe**), Boc-Gly-Δ^ZPhe-Gly-Phe-p-NA (**Z-p-NA**), and Boc-Gly-Δ^EPhe-Gly-Phe-p-NA (**E-p-NA**) were compared on the basis of CD and NMR studies in MeOH, trifluoroethanol (TFE), MeCN, chloroform, and dimethylsulfoxide (DMSO). The CD results were used as the additional input data for the NMR-based determination of the detailed solution conformations of the peptides. It was found that **E-OMe** is unordered and **Z-OMe**, **Z-p-NA**, and **E-p-NA** adopt the β-turn conformation. There are two overlapping β-turns in each of those peptides: type II and type III′ in **Z-OMe** and **Z-p-NA**, and two type III in **E-p-NA**. The ordered structure-inducing properties of Δ^ZPhe and Δ^EPhe in the peptides studied depend on the C-terminal blocking group. In methyl esters, the Δ^ZPhe residue is a strong inducer of ordered conformations whereas the Δ^EPhe one has no such properties. In p-nitroanilides, both isomers of ΔPhe cause the peptides to adopt ordered structures to a similar extent.* © *2010 Wiley Periodicals, Inc. Biopolymers 93: 1055–1064, 2010.*

Keywords: dehydropeptides; dehydrophenylalanine configuration; circular dichroism; nuclear magnetic resonance; dehydropeptide conformation

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INTRODUCTION

α,β-Dehydroamino acid residues (α,β-unsaturated or Δ-amino acid residues) contain a double bond between the C^α and C^β atoms. This structural feature endows them with the ability to limit the conformational freedom of peptides. These Δ-amino acids, which have different substituents on the C^β carbon atom, can exist in two forms — as isomers Z, with the older substituent in position *cis* to the nitrogen atom, and isomers E, with this substituent *trans* to nitrogen. Isomers Z are more thermodynamically stable than their E counterparts and hence easier to

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obtain. It has been shown recently, though, that poly-ΔAbu exists in alternate Δ^{Z}Abu and Δ^{E}Abu form rather than the all Z-form.[1] A great majority of articles published so far on dehydropeptides deal with Δ-amino acid residues of the Z configuration. The most studied dehydroamino acid residue has been dehydrophenylalanine. It has been found that in the solid state a ΔPhe residue of the Z configuration induces β-turns in short sequences[2–6] and a 3_{10}-helix in longer ones or peptides with more than one dehydro residue.[4,7–19] Similar conformational properties of Δ^{Z}Phe have been observed in solution by NMR[8,11,19–34] and CD,[18,19,27–44] though other, unusual structures have also been reported.[45] The Δ^{Z}Phe residues have also a potential to introduce long-range interactions in peptides[46,47] or to be a β structure breaker.[48]

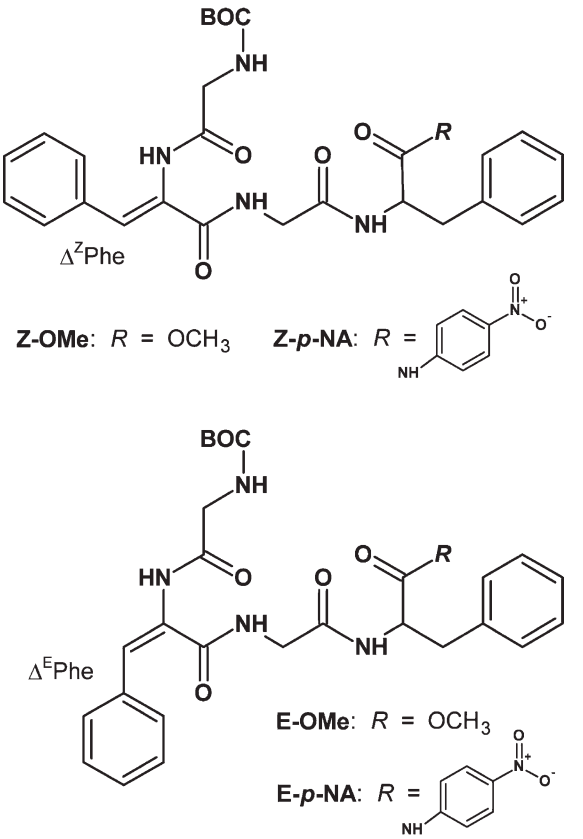
Much less is known about the conformational preferences of a ΔPhe residue of the E configuration. Few crystal structures of Δ^{E}Phe-containing peptides show that this residue occupies the $i + 1$ position in type II or II′ β-turn.[17,49,50] NMR and CD studies on Δ^{E}Phe-containing peptides[51–54] revealed that this residue favors formation of the folded conformations of the β-turn type.

So far, in the literature there has been no article with a direct comparison of the conformational properties of dehydropeptides containing a single ΔPhe residue and differing only by its configuration. Therefore, we wanted to check how the dehydroamino acid residue configuration influences a dehydropeptide conformation. In this connection, we investigated the conformations of two following peptides (Scheme 1):

Boc-Gly-Δ^{Z}Phe-Gly-Phe-OMe (**Z-OMe**)
Boc-Gly-Δ^{E}Phe-Gly-Phe-OMe (**E-OMe**)

The absorption and CD spectra of **Z-OMe** in MeOH have been presented in Ref. 17, but the peptide has been used there as a model compound and its conformation has not been discussed at all. We investigated the conformations of the two methyl esters by CD in MeOH, TFE, MeCN, and chloroform, and NMR in $CDCl_3$ and DMSO-d_6.

So far, the detailed solution conformations of dehydropeptides have been derived from the NMR alone. This approach has some disadvantages because a peptide in solution is a dynamic system. In the cases of small and dynamic systems when there is a small number of ROE contacts per residue and often ambiguous $^{3}J_{HH}$ couplings information, the NMR is not capable of sufficient solution structure determination. In such a case the CD can give additional information on the peptide conformation, for example, whether a peptide is folded or unordered or what type of a β-turn dominates in its conformational equilibrium. In this article, we applied an integrated approach that consists in combining the NMR and CD results



SCHEME 1 Structures of **Z-OMe**, **E-OMe**, **Z-*p*-NA**, and **E-*p*-NA**.

to obtain the detailed solution conformations of the peptides studied. We used successfully such an approach in our conformational studies on the decapeptide fragment of ubiquitin.[55]

Basing on CD and NMR studies we present the detailed solution conformations of **Z-OMe** and **E-OMe**. The conformational properties of the methyl esters were compared with those of two very similar peptides (Scheme 1):

Boc-Gly-Δ^{Z}Phe-Gly-Phe-*p*-NA (**Z-*p*-NA**)
Boc-Gly-Δ^{E}Phe-Gly-Phe-*p*-NA (**E-*p*-NA**)

differing from the first pair only by the C-terminal blocking group. The X-ray[1] and CD studies[17] have shown that **Z-*p*-NA** adopts a type II β-turn conformation at the Δ^{Z}Phe[2] and Gly[3] residues, in the solid state and solution. Both *p*-nitroanilides have also been studied by NMR,[18,51] but these investigations have not allowed to present the detailed solution conformations of the peptides. In this connection, we carried out anew the NMR studies on **Z-*p*-NA** and **E-*p*-NA** in dimethylsulfoxide (DMSO). The latter peptide was also studied by CD in MeOH, MeCN, TFE, and DMSO. The CD spectrum of **Z-*p*-NA** in DMSO was measured as well. The CD and NMR studies on **Z-*p*-NA** and **E-*p*-NA** yielded the detailed solution

structures of both peptides. The results presented in this article may have implications for the de novo design of peptides with specific conformations in solution.

EXPERIMENTAL PROCEDURES

Synthesis of Peptides

The syntheses of **Z-OMe**, **Z-*p*-NA**, and **E-*p*-NA** have been described in Refs. 18,56, and 51, respectively.

Boc-Gly-Δ^{E}Phe-Gly-Phe-OMe (E-OMe). Boc-Gly-Δ^{E}Phe-Gly-OH was obtained from Boc-Gly-Δ^{E}Phe-Gly-OMe[51] by a standard basic hydrolysis with a 30% excess of 1*M* NaOH in MeOH. Yield 98%; mp 127–130°C; elemental analysis calcd (%) for $C_{18}H_{23}N_3O_6$ (377.39): C 57.29, H 6.14, N 11.40; found: C 57.59, H 6.00, N 11.11.

TEA (0.143 mL, 1.05 mmol) was added to the solution of Boc-Gly-Δ^{E}Phe-Gly-OH (0.189 g, 0.5 mmol) and HCl · Phe-OMe (0.108 g, 0.5 mmol) in MeCN (3 mL). After 5 min, TBTU (0.168 g, 0.55 mmol) was added and the solution was stirred for 10 h at room temperature. Then MeCN was evaporated and the resulting oil was dissolved in ethyl acetate (50 mL). The solution was washed with 1*M* HCl (3 × 3 mL), saturated solution of $KHCO_3$ (3 × 3 mL), and brine (3 mL). Then it was dried with $MgSO_4$ and ethyl acetate was evaporated in vacuo. Crude **E-OMe** was crystallized from $CHCl_3$-ethyl acetate (9:1, v/v)/hexane. Yield 0.247 g (92%); mp 100–102°C; elemental analysis calcd (%) for $C_{28}H_{34}N_4O_7$ (538.58): C 62.44, H 6.36, N 10.40; found: C 62.56, H 6.20, N 10.21. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 1.43 (9H, s, 3 × CH_3 Boc); 3.09 (2H, d-d, $C^{\beta}H_2$ Phe^4); 3.66 (3H, s, $COOCH_3$); 3.71 (1H, d-d, $C^{\alpha}H^1$ Gly^3); 3.85 (1H, d-d, $C^{\alpha}H^2$ Gly^3); 3.90 (2H, broad s, $C^{\alpha}H_2$ Gly^1); 4.75 (1H, q, $C^{\alpha}H$ Phe^4); 5.37 (1H, t, NH Gly^1); 6.39 (1H, broad t, NH Gly^3); 7,11 (1H, d, NH Phe^4); 7.19–7.31 (11H, m, aromatic of Phe^4 and Δ^{E}Phe^2; $C^{\beta}H$ Δ^{E}Phe^2); 8.69 (1H, broad s, NH Δ^{E}Phe^2).

Absorption Spectra

Absorption spectra were measured with a Cary 500 Scan UV–vis–NIR spectrophotometer in MeOH, at room temperature. Pathlength of 1 mm was used. Concentration of the solutions was 5×10^{-4}*M*.

Circular Dichroism Spectroscopy

CD spectra were recorded on a Jasco J-600 spectropolarimeter, at room temperature. Spectra were measured in MeOH, TFE, MeCN, chloroform, and DMSO. Pathlength of 1 mm was used. Concentrations of the solutions were in the range of 0.17–0.21 mg/mL ($2.6–3.9 \times 10^{-4}$*M*). Each spectrum represents the average of at least four scans. The data are presented as molar ellipticity [θ].

Table I Absorption Features of Z-OMe, E-OMe, Z-*p*-NA, and E-*p*-NA in MeOH, in the Near-UV Region

Peptide	λ_{max} (nm)	ε_{max}
Z-OMe[a]	280	19,500
E-OMe	280	22,900
Z-*p*-NA[a]	286	30,500
E-*p*-NA	289	30,200

[a] Ref. 17.

NMR Spectroscopy

NMR spectra of **Z-OMe** and **E-OMe** were recorded at 11.7 T on a Varian Unity +500 spectrometer at 25°C, in $CDCl_3$ and DMSO-d_6. NMR spectra of **Z-*p*-NA** and **E-*p*-NA** were measured at 11.7 T with a Bruker Avance 500 spectrometer at 25°C, in DMSO-d_6. Two-dimensional (2D) homonuclear NMR spectra were processed with NMRPipe[57] and analyzed with SPARKY[57] programs. Complete assignments of the ^{1}H and ^{13}C resonances for all the peptides were done by application of a standard procedure[58] based on inspection of the 2D homonuclear TOCSY (with mixing times 10 and 80 ms) and ROESY (with mixing times 300 and 500 ms) experiments. Accuracies of the estimated proton chemical shifts and $^3J_{HH}$ coupling constants are 0.02 ppm and 0.4 Hz, respectively. The temperature coefficients of amide protons of methyl esters and *p*-nitroanilides were measured in $CDCl_3$ and DMSO-d_6, respectively, at the temperature range from 291 to 318 K, with a temperature interval of 3 K. Chemical shifts are given as δ in relation to $SiMe_4$ as internal standard. In all cases 15 m*M* peptide solutions were used. Calculations of lowest-energy structures, carried out for peptides that exhibited ordered conformations in solution according to CD, were performed with an X-PLOR NIH 2.21 program package.[59] Figures presenting the calculated structures were prepared with a MolMol software.[60] Supplementary data are available from the authors upon request.

RESULTS

Absorption Spectra

The absorption features of **Z-OMe**, **E-OMe**, **Z-*p*-NA**, and **E-*p*-NA** in the near-UV region are presented in Table I. The spectra of **Z-OMe** and **E-OMe** show a large, broad band at 280 nm, similar to those observed for other mono-unsaturated Δ^{Z}Phe peptides.[35,36,42] It is an intramolecular charge-

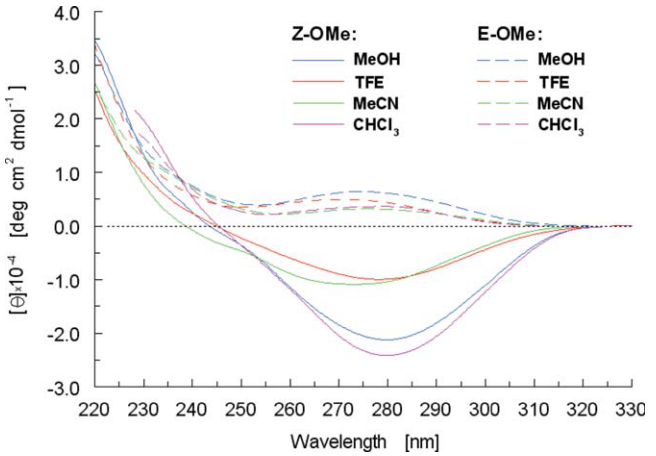


FIGURE 1 CD spectra of **Z-OMe** and **E-OMe**.

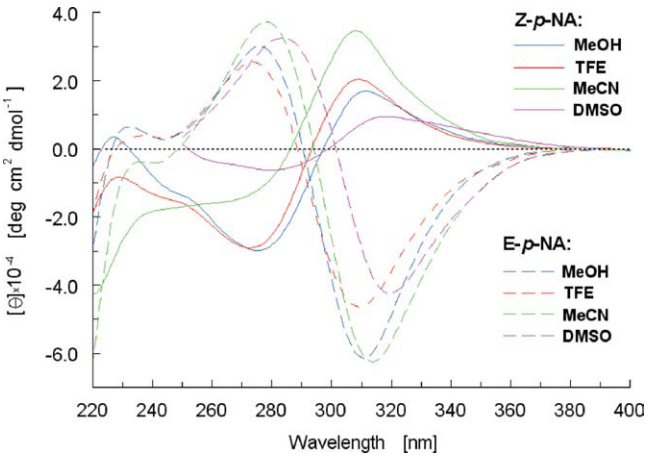


FIGURE 2 CD spectra of **Z-*p*-NA** and **E-*p*-NA**.

transfer band originating from the cinnamic chromophore $C_6H_5C{=}C{-}C{=}O$ of the ΔPhe residue, from the highest occupied orbital of the electron-donating styryl group to the vacant orbital of the electron-accepting carbonyl group.[35,61] In the case of **Z-*p*-NA** and **E-*p*-NA**, the ΔPhe residue band at 280 nm is overlapped by a charge-transfer band of the *p*-NA group at 315 nm.[18] The spectra of **Z-*p*-NA** and **E-*p*-NA** are very similar. They show a band at 286 and 289 nm, respectively, with a shoulder at about 325 nm that is quite distinct for **Z-*p*-NA** and poorly visible for **E-*p*-NA**. The spectra of both pairs of peptides show that the ΔPhe residue configuration has only a slight influence on their shape.

CD Studies

The CD spectra of **Z-OMe** and **E-OMe** in MeOH, TFE, MeCN, and chloroform are presented in Figure 1. The spectra of **Z-OMe** in MeOH and chloroform are very similar. They show a large negative band at 280 nm, a little more intensive in the latter solvent. In trifluoroethanol (TFE) and MeCN this band, at 278 and 273 nm, respectively, is about twice smaller than in chloroform and MeOH. The CD spectra of **E-OMe** are much less differentiated. They show a very small, broad, positive band at 270–279 nm, the most intensive in MeOH and the smallest in MeCN.

The CD spectra of **Z-*p*-NA** and **E-*p*-NA** in MeOH, TFE, MeCN, and DMSO are shown in Figure 2. They look to a some extent like mirror images. The spectra of **Z-*p*-NA** in MeOH and TFE are very similar. There is a large, positive *p*-NA band at 311 and 308 nm, and a large, negative Δ^ZPhe band at 275 and 273 nm, in those spectra. The spectrum of **Z-*p*-NA** in MeCN differs from those in MeOH and TFE by the bands intensity. In MeCN, the *p*-NA band at 308 nm is much stronger, and the Δ^ZPhe residue band at 273 nm much weaker, than in MeOH and TFE. There is a large difference between the CD spectra of **Z-*p*-NA** in DMSO and other solvents. In DMSO, the positive *p*-NA band at 318 nm and the negative Δ^ZPhe band at 280 nm are distinctly smaller than in other solvents, especially the latter. The spectra of **E-*p*-NA** are less differentiated than those of **Z-*p*-NA**. In MeOH, TFE, and MeCN, there is a large negative *p*-NA band at 309–314 nm and a large positive Δ^EPhe band at 274–278 nm. The spectrum in DMSO is similar in its shape to the spectra in other solvents but it is shifted somewhat to longer wavelengths, with a negative and a positive band at 319 and 284 nm, respectively.

NMR Studies

To detect intramolecular hydrogen bonds in the peptides investigated, the temperature dependence of their amide protons was measured. It is well known that amide proton chemical shifts are sensitive to temperature changes. If a proton forms a hydrogen bond, then its chemical shift shows a very small dependence on the temperature. The parameter that indicates the presence or absence of hydrogen bonds is a $d\delta/dT$ (ppm K^{-1}) coefficient. It has been established that amide protons in protein systems, which are characterized by the temperature coefficient value lower than 0.0046 ppm K^{-1}, are involved in hydrogen bonds.[62] This value is often used for checking if any amide protons are involved in hydrogen bonds. The temperature coefficients of amide protons in the studied peptides are presented in Table II. They show that there are two hydrogen bonds in **Z-OMe**, **Z-*p*-NA**, and **E-*p*-NA**. They are formed by amide protons of Gly^3 and Phe^4 in **Z-OMe** and **Z-*p*-NA**, and of Phe^4 and *p*-NA in **E-*p*-NA**. The values of temperature coefficients of Gly^3 and Phe^4 amide protons in **Z-OMe** are larger than the 0.0046 limit value, but other results presented below confirm that the two protons form hydrogen bonds. A low value of the

Table II Temperature Coefficients for Amide Protons of Z-OMe, E-OMe, Z-*p*-NA, and E-*p*-NA

	$d\delta/dT$ (ppm $K^{-1} \times 1000$)			
Residue	**Z-OMe**[a]	**E-OMe**[a]	**Z-*p*-NA**[b]	**E-*p*-NA**[b]
Gly^1	7.2	6.9	5.6	6.4
ΔPhe^2	7.4	7.2	4.8	5.5
Gly^3	5.2[c]	8.1	3.0[c]	6.6
Phe^4	5.4[c]	4.7	0.7[c]	3.5[c]
p-NA	—	—	5.6	2.6[c]

[a] In $CDCl_3$.
[b] In DMSO-d_6.
[c] Protons forming hydrogen bonds.

Phe^4 amide proton temperature coefficient in **E-OMe**, lower than for Gly^3 and Phe^4 amide protons in **Z-OMe**, suggests that this proton also forms an intramolecular hydrogen bond. Still, other results obtained for that peptide, described below, are in contradiction with such a possibility. Another way of checking for the presence of intramolecular hydrogen bonds in peptides is determination of the dependence of the amide protons chemical shifts on the solvent polarity. In the case of protons involved in hydrogen bonds that dependence is distinctly smaller than for nonhydrogen bonded ones. The results obtained for the methyl esters, measured in $CDCl_3$ and DMSO-d_6, are shown in Table III. They confirm the presence of two hydrogen bonds in **Z-OMe**, formed by amide protons of Gly^3 and Phe^4, and indicate the absence of a hydrogen bond in **E-OMe**. Such studies were not performed for the *p*-nitroanilides due to their poor solubility in chloroform.

Important information on the conformational properties of **Z-OMe**, **E-OMe**, **Z-*p*-NA**, and **E-*p*-NA** were obtained from the ROESY spectra. On their basis, interatomic contacts in the peptides studied could be detected. Trivial, intraresidue contacts are present in each investigated peptide. Nontrivial contacts were observed only for **Z-OMe**, **Z-*p*-NA**, and **E-*p*-NA**. They are presented in Table IV. Such contacts are absent in **E-OMe**, confirming the lack of intramolecular hydrogen bonds in that peptide. These contacts were used as the input data for calculations of the lowest-energy conformers of **Z-OMe**, **Z-*p*-NA**, and **E-*p*-NA**. Besides them, the calculations were based only on the unambiguous results obtained at the same time by the NMR and CD. For example, if the NMR spectrum of a peptide indicated that two amide protons are involved in intramolecular hydrogen bonds, suggesting the presence of two β-turns, and the CD spectrum showed that there are two β-turns, one of a given type, at specific residues and stabilized by a hydrogen bond formed by one of those amide protons detected by NMR as hydrogen-bonded, and the other whose type and location could not be determined exactly by CD, then only this first β-turn with its hydrogen bond were used in the calculations. Such an approach was applied for **Z-OMe** and **Z-*p*-NA**. In the case of **E-*p*-NA**, the CD results are not unambiguous. The CD spectra show the presence of two β-turns whose position could be determined only by the hydrogen bonds detected by the NMR (Table II). These turns are located at the $\Delta^E Phe^2$-Gly^3 and Gly^3-Phe^4 residues. They can be either of type II′ or III. It gives four possibilities: II′/II′, II′/III, III/II′, and III/III. It was found that independently of which of those combinations was chosen, the calculations yielded very similar results. The results presented for **E-*p*-NA** (Table V) were obtained with the two hydrogen bonds, Phe^4 NH · · · Gly^1 CO and *p*-NA NH · · · $\Delta^E Phe^2$ CO, and the β-turns at the $\Delta^E Phe^2$-Gly^3 and Gly^3-Phe^4 residues, assumed to be of type II′ and III, respectively, as the input data. In the case of calculations based on combined NMR and CD results, it was important to have the results from the both methods that could be directly compared. Such a situation took place in the case of **Z-OMe** and **E-OMe** whose NMR spectra were measured in chloroform that was also one of the solvents used for CD studies. Because of a very poor solubility of **Z-*p*-NA** and **E-*p*-NA** in chloroform, the NMR spectra of those peptides were measured in DMSO. Therefore, the CD spectra of **Z-*p*-NA** and **E-*p*-NA** were measured in DMSO as well. For **E-*p*-NA**, there is a large similarity between the CD spectra measured in DMSO and other solvents. However, the CD spectra of **Z-*p*-NA** in DMSO and other solvents differ very much from each other (see Figure 2). The CD spectrum of **Z-*p*-NA** in DMSO indicates that its conformation, contrary to that in MeOH, TFE, and MeCN and to the NMR results, is unordered to a large extent. Still, it is rather not the case here. So for that peptide the NMR results combined with the CD ones obtained not for DMSO but for MeOH, TFE, and MeCN, were used for the conformational calculations. The conformation of **Z-*p*-NA** in DMSO and the way the calculations were performed for that peptide will be discussed in the

Table III Dependence of Amide Protons Chemical Shifts on the Solvent Polarity for Z-OMe and E-OMe

	$\Delta\delta = \delta_{NH}(DMSO\text{-}d_6) - \delta_{NH}(CDCl_3)$	
Residue	**Z-OMe**	**E-OMe**
Gly^1	1.47	1.61
ΔPhe^2	1.53	1.30
Gly^3	0.72[a]	2.24
Phe^4	0.80[a]	1.22

[a] Protons forming hydrogen bonds.

Table IV Interatomic Nontrivial Contacts in Z-OMe, Z-*p*-NA, and E-*p*-NA Detected from the ROESY Spectra

Interatomic Contacts in:		
Z-OMe[a]	**Z-*p*-NA[b]**	**E-*p*-NA[b]**
$C^{\alpha}H_2$ Gly^1 ↔ NH $\Delta^Z Phe^2$	NH $\Delta^Z Phe^2$ ↔ $C^{\alpha}H_2$ Gly^1	NH $\Delta^E Phe^2$ ↔ $C^{\alpha}H^{\alpha 1}$ Gly^1
$C^{\alpha}H_2$ Gly^1 ↔ NH Gly^3	NH $\Delta^Z Phe^2$ ↔ NH Gly^1	NH $\Delta^E Phe^2$ ↔ $C^{\alpha}H^{\alpha 2}$ Gly^1
NH $\Delta^Z Phe^2$ ↔ NH Gly^3	NH $\Delta^Z Phe^2$ ↔ NH Gly^3	NH $\Delta^E Phe^2$ ↔ NH Gly^3
NH Gly^3 ↔ NH Phe^4	NH $\Delta^Z Phe^2$ ↔ aromatic H's Phe^4	NH Gly^3 ↔ NH Phe^4
$C^{\alpha}H_2$ Gly^3 ↔ NH Phe^4	NH Gly^3 ↔ $C^{\alpha}H_2$ Gly^1	Aromatic H's Phe^4 ↔ $C^{\beta}H$ $\Delta^E Phe^2$
	NH Gly^3 ↔ $C^{\beta}H$ $\Delta^Z Phe^2$	NH *p*-NA ↔ $C^{\beta}H$ $\Delta^E Phe^2$
	NH Gly^3 ↔ NH Phe^4	NH *p*-NA ↔ $C^{\alpha}H$ Phe^4
	$C^{\alpha}H^{\alpha 1}$ Gly^3 ↔ NH Gly^1	NH *p*-NA ↔ NH Phe^4
	NH Phe^4 ↔ $C^{\alpha}H_2$ Gly^1	
	NH Phe^4 ↔ $C^{\alpha}H^{\alpha 2}$ Gly^3	
	NH *p*-NA ↔ $C^{\alpha}H$ Phe^4	
	NH *p*-NA ↔ NH Phe^4	

[a] In $CDCl_3$.
[b] In DMSO-d_6.

next section. The calculated lowest-energy conformers of **Z-OMe**, **Z-*p*-NA**, and **E-*p*-NA** are presented in Figures 3a–3c and the values of their dihedral angles are given in Table V. Figures 3d–3f also show the ensembles of 50 lowest-energy structures of those peptides, chosen from the 500 calculated peptide conformers.

DISCUSSION

The CD spectra of dehydropeptides in the far-UV region are difficult to analyze since there are overlapping contributions of the peptide, aromatic, and unsaturated chromophores. In this connection, we limited our analysis of the spectra of four peptides studied to the near-UV region only. The peptides contain a ΔPhe residue that gives a large CD band in that region, at about 280 nm, which is very sensitive to a dehydropeptide conformation.[18,19,27–44] Thus, this residue is a very good conformational probe in the studies on dehydropeptides. Moreover, **Z-*p*-NA** and **E-*p*-NA** contain the *p*-NA group that has also been found to be a useful tool in the conformational studies on peptides.[63–66]

A large intensity of the negative Δ^ZPhe CD bands of **Z-OMe** at 280 nm in MeOH, TFE, and MeCN (see Figure 1) shows that the peptide adopts a chiral, rigid structure of the same type in each solvent. This structure must involve the Phe^4 residue because otherwise two enantiomeric forms of that conformation would be present in the conformational equilibrium of **Z-OMe** resulting in a very small intensity of the Δ^ZPhe band. It has been found that a negative Δ^ZPhe band at 280 nm is typical of dehydropeptides adopting a type II β-turn, with a Δ^ZPhe residue at the $i + 2$ position.[42] The presence of such a band in the CD spectra of **Z-OMe** suggests that there is a type II β-turn in that peptide at the Gly^1 and $\Delta^Z Phe^2$ residues, stabilized by the intramolecular hydrogen bond between the amide proton of Gly^3 and the carbonyl oxygen of the Boc group. But this conformation does not involve the Phe^4 residue whereas the CD spectra show that this residue must also be a part of the ordered structure of **Z-OMe**. It suggests the presence of another β-turn in that peptide, at the $\Delta^Z Phe^2$ and Gly^3 or Gly^3 and Phe^4 residues. Since the β-turn at Gly^3 and Phe^4 could not be stabilized by the

Table V Torsion Angles Values of Z-OMe, Z-*p*-NA, and E-*p*-NA Calculated on the Basis of NMR and CD Parameters with an X-PLOR Program

	Z-OMe		Z-*p*-NA		E-*p*-NA	
Residue	φ	Ψ	φ	Ψ	φ	Ψ
Gly^1	−48° (±6°)	114° (±25°)	−56° (±2°)	114° (±5°)	143° (±89°)	−116° (±101°)
ΔPhe^2	49° (±11°)	18° (±21°)	30° (±2°)	30° (±2°)	−26° (±3°)	−34° (±2°)
Gly^3	77° (±36°)	10° (±34°)	39° (±4°)	53° (±11°)	−54° (±3°)	−7° (±3°)
Phe^4	−41° (±87°)	—	−61° (±12°)	27° (±90°)	−65° (±4°)	−40° (±3°)

Standard deviations for 50 lowest-energy structures are given in brackets.

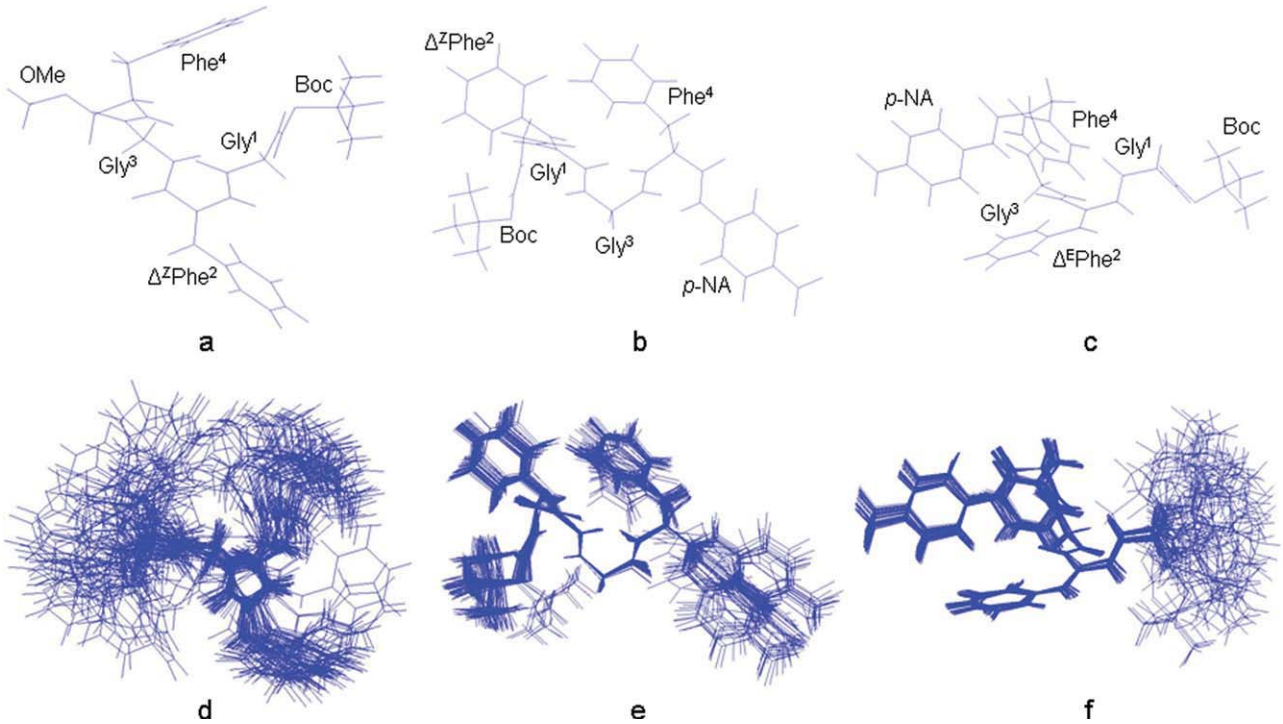


FIGURE 3 The lowest-energy conformers of **Z-OMe** (a), **Z-*p*-NA** (b), and **E-*p*-NA** (c), and ensembles of the 50 lowest-energy structures of **Z-OMe** (d), **Z-*p*-NA** (e), and **E-*p*-NA** (f).

intramolecular 4→1 hydrogen bond due to the lack of a proton donor, the β-turn at ΔPhe^2 and Gly^3 stabilized by the hydrogen bond between the amide proton of Phe^4 and the carbonyl group of Gly^1 is much more probable. These conclusions were confirmed by the NMR studies on **Z-OMe**. They show the presence of two intramolecular hydrogen bonds, formed by the amide protons of Gly^3 and Phe^4 (Tables II and III). The dihedral angles of successive residues of **Z-OMe** (Table V) indicate that these hydrogen bonds stabilize two overlapping β-turns, of type II at Gly^1 and $\Delta^Z Phe^2$ and type III′ at ΔPhe^2 and Gly^3. Such a sequence of β-turns occurs very often in the case of Aib-containing peptides[67,68] in which the N-terminal β-turn is followed by a left-handed 3_{10} helical conformation, that is, type III′ β-turns. It has also been observed for Boc-L-Val-Δ^ZPhe-Δ^ZPhe-Δ^ZPhe-L-Val-OMe.[28] The type III′ β-turn in **Z-OMe** corresponds very well with the CD spectra of that peptide. It has been found that Δ^ZPhe-containing dehydropeptides in the type III β-turn conformation give a positive CD band at about 280 nm and a negative band at about 235 nm.[42] It shows that dehydropeptides in the type III′ β-turn conformation should give a negative CD band at 280 nm and a positive one at 235 nm. This spectral pattern agrees with the CD spectra of **Z-OMe** in each solvent. Indeed no positive band at about 235 nm in those spectra is observed, which may be due to its overlap with a stronger positive band at shorter wavelengths, but there are positive ellipticities in that region, except the spectrum in MeCN. The intensities of the negative Δ^ZPhe band at 280 nm suggest that the ordered conformation of **Z-OMe** is the most highly populated in chloroform and MeOH, whereas in MeCN and TFE its population is decreased. The lowest-energy conformation of **Z-OMe** and a comparison of its 50 lowest-energy conformers are shown in Figures 3a and 3d, respectively. The ensemble of 50 lowest-energy structures indicates that **Z-OMe** is quite flexible in solution and it has a substantial conformational freedom.

A small intensity of the Δ^EPhe CD bands of **E-OMe** (see Figure 1) at 270–280 nm in each solvent suggests that the peptide is unordered. The same result was obtained from the NMR studies, which do not show any interatomic contacts that would suggest the ordered conformation of that peptide. Positive CD bands of very small intensity at 280 nm have been observed in water and MeOH for trifluoroacetate (TFA) of H-Tyr-D-Ala-Δ^ZPhe-Gly-NH_2 for which the unordered structure was postulated.[37] The intensities of the positive Δ^EPhe band of **E-OMe** in MeOH and TFE are about twice as large as those observed for TFA · H-Tyr-D-Ala-Δ^ZPhe-Gly-NH_2. It does not allow to exclude the presence of some amounts of ordered structures in the conformational equilibria of **E-OMe**. These structures could possibly account for the surprisingly small temperature coefficient of the Phe^4 amide proton (Table II) that suggests the presence of an

intramolecular hydrogen bond. Still, the NMR and CD studies show that an unordered structure is by far the dominating conformation of **E-OMe** in each solvent.

At the beginning of the discussion of the results obtained for **Z-*p*-NA**, the matter of whether the NMR results obtained for the peptide in DMSO may be directly compared with those obtained by CD in other solvents should be addressed. The NMR studies on **Z-*p*-NA** in DMSO show that there are two intramolecular hydrogen bonds in that peptide, formed by the Gly^3 and Phe^4 amide protons (Table II). It indicates that **Z-*p*-NA** adopts the folded conformation of the β-turn type. This conclusion is confirmed by a large number of nontrivial interatomic contacts detected for **Z-*p*-NA** from the ROESY spectra (Table IV), especially by contacts NH $\Delta^Z Phe^2 \leftrightarrow$ aromatic H's Phe^4, NH $Gly^3 \leftrightarrow C^\alpha H_2$ Gly^1, $C^\alpha H^{\alpha 1}$ $Gly^3 \leftrightarrow$ NH Gly^1, and NH $Phe^4 \leftrightarrow C^\alpha H_2$ Gly^1, which suggest the presence of a β-turn conformation. Table IV shows that the largest number of contacts was detected for that very peptide. At the same time the CD spectrum of **Z-*p*-NA** in DMSO shows a very small, negative band of the Δ^ZPhe residue at 282 nm and a positive band of the *p*-NA group that is distinctly smaller than those observed in other solvents. It suggests that the population of the ordered structures of **Z-*p*-NA** in DMSO is markedly decreased as compared with MeOH, TFE, and MeCN. Still, these ordered structures seem to be of the same type as in the other solvents. Their decreased population in DMSO is in some contradiction to the NMR results that indicate an evidently ordered conformation of **Z-*p*-NA** in DMSO. Since the NMR and CD must reflect the same conformation of **Z-*p*-NA** in DMSO, then it should be assumed that the CD spectrum of the peptide corresponds to its ordered structure seen by the NMR. It is difficult, however, to explain in such a case this substantial decrease of the CD bands of **Z-*p*-NA** in DMSO. We can not give at the present any reliable explanation for that. One can speculate that the Δ^ZPhe side chain of **Z-*p*-NA** in DMSO adopts such a conformation that its optical activity is strongly diminished or that the peptide is present in two conformations with the opposite dispositions of the Δ^ZPhe side chain whose CD contributions largely cancel each other. Since the CD spectra of **Z-*p*-NA** suggest the presence of the same ordered structures in each solvent, then it seems reasonable to assume that conclusions drawn from the CD spectra of **Z-*p*-NA** in MeOH, TFE, and MeCN correspond to the NMR results for **Z-*p*-NA** in DMSO. It was confirmed by the conformational calculations performed for **Z-*p*-NA** on the basis of NMR results for DMSO and the CD results for MeOH, TFE, and MeCN. As can be seen in Figure 3b, all interatomic contacts detected for **Z-*p*-NA**, among them those of significant importance for the conformation of the peptide (NH $\Delta^Z Phe^2 \leftrightarrow$ aromatic H's Phe^4, NH $Gly^3 \leftrightarrow C^\alpha H_2$ Gly^1, $C^\alpha H^{\alpha 1}$ $Gly^3 \leftrightarrow$ NH Gly^1, and NH $Phe^4 \leftrightarrow C^\alpha H_2$ Gly^1), are preserved in the final, lowest-energy structure. It proves the correctness of our approach.

The CD spectra of **Z-*p*-NA** (see Figure 2) in the near-UV in MeOH, TFE, and MeCN show that the peptide adopts the type II β-turn conformation at the Gly^1 and $\Delta^Z Phe^2$ residues, stabilized by the intramolecular hydrogen bond between the amide proton of Gly^3 and the carbonyl oxygen of the Boc group.[18] The presence of such a conformation is consistent with the NMR studies, which show that the Gly^3 amide proton is involved in a hydrogen bond (Table II) and by the conformational calculations (Table V). The analogous reasoning as in the case of **Z-OMe** indicates that there is another β-turn, either at the $\Delta^Z Phe^2$ and Gly^3 or Gly^3 and Phe^4 residues, stabilized by the corresponding $4 \rightarrow 1$ hydrogen bond. A large intensity of the positive band of the *p*-NA group suggests that this group should also be a part of the second β-turn of **Z-*p*-NA**, hence the β-turn at the Gly^3 and Phe^4 residues, with a hydrogen bond between *p*-NA NH and $\Delta^Z Phe^2$ CO, seems more probable than the one at $\Delta^Z Phe^2$ and Gly^3. It is consistent with the solid state structure of **Z-*p*-NA** in which two type II β-turns, at Gly^1-$\Delta^Z Phe^2$ and Gly^3-Phe^4, are present.[2] Meanwhile the NMR studies showed that **Z-*p*-NA** adopts the same conformation as **Z-OMe**. The second intramolecular hydrogen bond in **Z-*p*-NA** is formed not by the *p*-NA group, a good hydrogen bond donor, but by the Phe^4 amide proton (Table II). This result was unexpected since in such a case the *p*-NA group is not a part of any ordered structure. In this connection, it is difficult to explain a large intensity of its CD band. A distinctly low value of the temperature coefficient of the Phe^4 amide proton indicates that a hydrogen bond formed by that proton is very strong. The calculations (Table V) showed that the second β-turn at the $\Delta^Z Phe^2$ and Gly^3 residues, stabilized by the Phe^4 NH $\cdots$ Gly^1 CO hydrogen bond, is of type III′. The privileged solution conformation of **Z-*p*-NA** is presented in Figure 3b. The calculated lowest-energy conformers of that peptide (Figure 3e) show that its conformational flexibility is distinctly reduced as compared with **Z-OMe**.

In the case of **E-*p*-NA**, its CD spectra (see Figure 2) in each solvent used are quite similar to each other, indicating small solvent-dependence of its conformation. A large intensity of the Δ^EPhe and especially *p*-NA CD bands at about 280 and 310 nm, respectively, suggests that, like in the case of **Z-OMe** and **Z-*p*-NA**, **E-*p*-NA** adopts the conformation composed of two β-turns, stabilized by intramolecular hydrogen bonds. One of those β-turns should be located at the Gly^3 and Phe^4 residues since otherwise the *p*-NA group would not be a part of the ordered structure. The positive band of the $\Delta^E Phe^2$ residue indicates that those β-turns may be both of

type III or II′, or type III/II′.[42] The temperature coefficients of the amide protons of **E-*p*-NA** (Table II) show the presence of two hydrogen bonds formed by NH's of Phe^4 and *p*-NA. It indicates that there are two overlapping β-turns in **E-*p*-NA**, located at the $\Delta^{E}Phe^2$-Gly^3 and Gly^3-Phe^4 residues. They are stabilized by 4→1 hydrogen bonds between Phe^4 NH and Gly^1 CO and between *p*-NA NH and $\Delta^{E}Phe^2$ CO. The calculated lowest-energy conformation of **E-*p*-NA** is presented in Figure 3c. The values of dihedral angles of the peptide (Table V) show that its both β-turns are of type III. A very large intensity of the negative *p*-NA band in the CD spectrum of **E-*p*-NA** shows that this group is much more fixed in that peptide than in **Z-*p*-NA**. It is connected to the intramolecular hydrogen bond formed by the *p*-NA group in **E-*p*-NA** that is absent in the case of **Z-*p*-NA**. A comparable intensity of the ΔPhe^2 CD bands in the spectra of **Z-*p*-NA** and **E-*p*-NA** suggests that both peptides are ordered to a similar extent. The ensemble of 50 lowest-energy structures of **E-*p*-NA** indicates that this peptide is the most rigid of all the peptides studied. Its only part that possesses quite a substantial conformational freedom is the Boc group. It agrees very well with this group not being a part of the ordered conformation of **E-*p*-NA**.

CONCLUSIONS

For the first time the conformations of two pairs of dehydropeptides, differing only by the configuration of a dehydroamino acid residue, are directly compared. A combined use of the CD and NMR methods let determine the detailed solution structures of **Z-OMe**, **E-OMe**, **Z-*p*-NA**, and **E-*p*-NA**. It was found that the conformation of **Z-OMe** is ordered, with two overlapping β-turns, whereas the conformation of **E-OMe** is unordered. It shows that the configuration of the ΔPhe residue plays an essential role in the conformational properties of the methyl esters. In the case of *p*-nitroanilides, both peptides are ordered to a similar extent but in a different way. The conformation of **Z-*p*-NA** is the same as that of **Z-OMe**. A comparison of that conformation with the crystal structure of **Z-*p*-NA** shows that the peptide preserves only partly its solid state structure in solution. In **E-*p*-NA**, there are two overlapping β-turns but their position is different than in **Z-*p*-NA**. The types of β-turns present in **Z-OMe**, **Z-*p*-NA**, and **E-*p*-NA** show that the peptides adopt the incipient 3_{10} helical conformation, left-handed in **Z-OMe** and **Z-*p*-NA**, and right-handed in **E-*p*-NA**. It follows from the results obtained for **Z-*p*-NA** and **E-*p*-NA** that in their case the ΔPhe residue configuration is important for the kind of ordered structures they adopt but it does not affect the extent to which these peptides are ordered. Since the only difference within the two pairs of peptides studied is the configuration of a ΔPhe residue, it seems that the conformational differences between isomeric peptides result mainly from the different steric interactions between the ΔPhe side chain and other parts of the peptides. The results presented also show the influence of the C-terminal blocking group on a dehydropeptide conformation in relation to the configuration of a dehydropeptide. In the case of peptides containing the $\Delta^{Z}Phe$ residue, the substitution of the methyl group with the *p*-NA one does not bring about any conformational changes whereas such a substitution causes very large conformational changes in dehydropeptides with the $\Delta^{E}Phe$ residue. It shows that in the latter case a ΔPhe residue of the E configuration alone is not sufficient to induce the ordered conformation in a peptide.

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