# Synthesis of Peptides with α,β-Dehydroamino Acids. XIII Photoisomerization of Ac-(*Z*)-ΔPhe-NHMe: Ac-(*E*)-ΔPhe-NHMe[1,2]

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**Easily accessible Ac-(*Z*)-ΔPhe-NHMe was photoisomerized to so far unknown Ac-(*E*)-ΔPhe-NHMe. Some parameters of the process leading to a diastereomeric mixture of ratio 90(*Z*) : 10(*E*) have been tested and the photoisomerization has been carried out on a preparative milligram scale. The isomers were separated *via* crystallization followed by preparative HPLC.**

**Key words** Ac-(*E*)-ΔPhe-NHMe; (*E*)-dehydrophenylalanine; α,β-dehydroamino acid; photoisomerization; (*Z*)/(*E*)-diastereoisomer separation; HPLC

One of the natural variants of common amino acids is α,β-dehydroamino acids, which have a double bond between the $C^{\alpha}$ and $C^{\beta}$ atoms. Thus, the chirality gets lost and (*Z*)/(*E*) isomerism appears. Both (*Z*)- and (*E*)-forms occur in nature.[3,4]

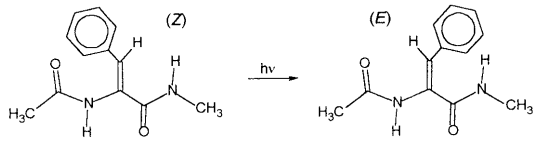
The introduction of the double bond into a peptide chain leads to a cross-conjugated system,[5,6] which may influence the conformation of the peptide backbone and the orientation of the amino acid side-chain. These special features have made α,β-dehydroamino acids valuable modifiers of peptides and consequently, α,β-dehydropeptides became attractive targets for conformational studies.[7,8] To these ends (*Z*)-dehydrophenylalanine is used most often, mainly perhaps because of its convenient chemical synthesis and the fact that all synthetic routes give exclusively the (*Z*)-isomer. On the other hand, receptor proteins discriminate quite precisely between the (*Z*) and (*E*)-disposition of the double bond in their α,β-dehydropeptide bioligands, as can be observed for the few available (*Z*)/(*E*)-ΔPhe couples of peptide analogs.[9—11] In contrast to the stability of dehydrophenylalanine in the (*Z*)-configuration, the (*E*)-configuration is quite unstable to the usual chemical conditions of peptide synthesis, converting always into the (*Z*)-configuration.[12,13] This synthetic limitation requires a procedure to invert the configuration of the dehydro unit from (*Z*) to (*E*) in the final stage of the synthesis. An appropriate method turned out to be photoisomerization.[9,10,14]

The significance of (*E*)-dehydrophenylalanine in the peptide modification generates a need for a deeper understanding of this amino acid conformational profile. Investigated was Ac-(*E*)-ΔPhe-NHMe, the simple model system, which mimics well the (*E*)-ΔPhe residue incorporated in a peptide chain. However, only theoretical structural study has been undertaken,[10] as the corresponding compound was unknown. Therefore, we provide here a synthesis of Ac-(*E*)-ΔPhe-NHMe. This was achieved *via* photoisomerization of easily accessible Ac-(*Z*)-ΔPhe-NHMe[15] (Table 1). With Ac-(*E*)-ΔPhe-NHMe in hand we were able to explore its conformational preferences experimentally.[16]

The photoisomerization of Ac-(*Z*)-ΔPhe-NHMe was performed in an ethanol solution. On an introductory testing by HPLC of some parameters of the process (Table 1), we selected for the final preparation of Ac-(*E*)-ΔPhe-NHMe the irradiation of $10^{-3}$ M solution of Ac-(*Z*)-ΔPhe-NHMe (Fig. 1a) with a 313-nm light over 16 h. As expected, the post-reaction mixture contained both isomers in a ratio of 90(*Z*) : 10(*E*) (Fig. 1b). Their separation and isolation require long-lasting operations, whereas (*E*)-2-phenyl-4-benzylidene-5(4*H*)-oxazolone was reported to isomerize to a 40(*Z*) : 60(*E*) equilibrium point, when left in an acetonitrile solution at room temperature in light for a few days.[17] Hence, the preparation of an (*E*)-dehydrophenylalanine peptide was carried out in the absence of direct light.[9] We therefore checked the stability of Ac-(*E*)-ΔPhe-NHMe under conditions of work-up at every stage of our procedure: concentration, crystallization and preparative HPLC separation.

A sample of the irradiated solution was left standing in direct light over 12 h and then condensed to about $5 \cdot 10^{-2}$ M concentration. The (*Z*) : (*E*)-isomer ratio did not change. The separation from this concentrated solution, of the majority of (*Z*)-isomer by crystallization affords a new mixture, enriched significantly in the (*E*)-isomer, in a ratio of 20(*Z*) : 80(*E*) (Fig. 1c). This is also stable to direct light (when irradiated with 313-nm light over 16 h, however, it passes to a ratio of 40(*Z*) : 60(*E*)). Preparative HPLC of this enriched mixture

Table 1. Photoisomerization of Ac-(*Z*)-ΔPhe-NHMe: the Content of Ac-(*E*)-ΔPhe-NHMe (by HPLC) as a Function of Wavelength, Irradiation Time and Solution Concentration




| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Time: 16 h; concentration: $10^{-5}$ M | | | | | | | |
| λ (nm) | 293[a] | 313[b] | 365[b] | | | | |
| *E* (%) | 8.1 | 10.8 | 5.2 | | | | |
| Wavelength: 313 nm; concentration: $10^{-5}$ M | | | | | | | |
| Time (h) | 0.7 | 5.5 | 6.5 | 10.5 | 12.0 | 16.0 | 22.0 |
| *E* (%) | 0.2 | 5.9 | 7.6 | 8.4 | 10.5 | 10.8 | 11.9 |
| Time: 16 h; wavelength: 313 nm | | | | | | | |
| Conc. (M) | $10^{-5}$ | $10^{-3}$ | $4 \cdot 10^{-3}$ [c] | | | | |
| *E* (%) | 10.8 | 10.1 | 3.5 | | | | |

*a*) Wavelength 293 nm resulted from the UV spectrum of Ac-(*Z*)-ΔPhe-NHMe. *b*) (*Z*)-Phenylalanine peptides[9,10] and (*Z*)-2-phenyl-4-benzylidene-5(4*H*)-oxazolone[14] were isomerized with 313-nm and 365-nm light, respectively. *c*) Presumably, this concentrated solution was poorly transmittable for 313-nm light and the isomerization proceeded only near the reactor wall. On dilution to $10^{-3}$ M, the process took a normal course.

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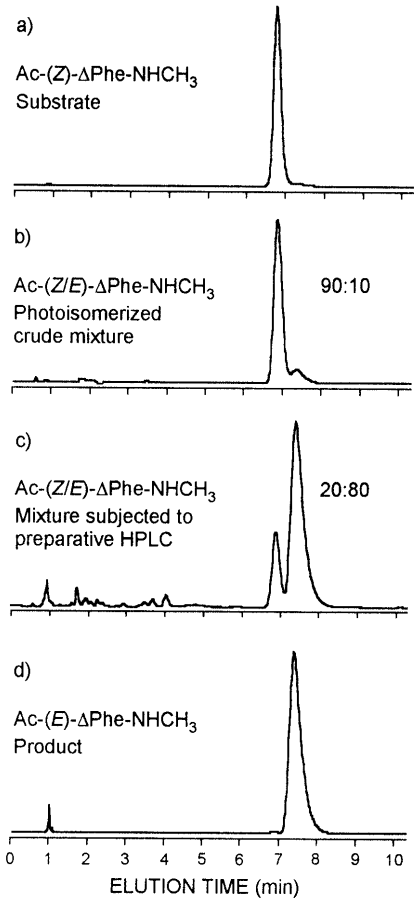


Fig. 1. HPLC Separation Profile of Ac-(*Z*/*E*)-ΔPhe-NHMe

Alltech Alltima, $C_{18}$, 5 $\mu$m, 150×4.6 mm column; acetonitrile : water (15 : 85); flow rate 1 ml/min. $t_R$ of Ac-(*Z*)-ΔPhe-NHMe 6.85 min; $t_R$ of Ac-(*E*)-ΔPhe-NHMe 7.35 min.

provides Ac-(*E*)-ΔPhe-NHMe of 99.8% purity (Fig. 1d). The compound was stored several months in a refrigerator with neither stereomutation nor other change. Its $^1$H-NMR spectrum is consistent with expectations as compared with the (*Z*)-isomer spectrum. For (*Z*)/(*E*) configuration assignment of an $\alpha,\beta$-dehydroamino acid residue, resonances $C^{\beta}$-H and NH-$C^{\alpha}$ can be of diagnostic value. We observed in the spectra of a few (*Z*)/(*E*) couples of constitutionally identical compounds that the vinyl and enamide protons of the (*Z*)-isomers resonate at lower and higher field, respectively, compared with the corresponding protons of the (*E*)-isomers.[1,13,18] In spectra of mixtures Ac-(*Z*/*E*)-ΔPhe-NHMe (taken in DMSO-$d_6$, due to the (*Z*)-compound solubility), the signal $C^{\beta}$-H(*E*) appears at 6.79 ppm while that of (*Z*)-isomer is at 7.04 ppm; in turn, the signal NH-$C^{\alpha}$(*E*) appears at 9.58 ppm and that of (*Z*)-isomer is at 9.36 ppm.

## Experimental

**General Experimental Procedures** Ac-(*Z*)-ΔPhe-NHMe, obtained according to the reported method,[15] was crystallized from ethanol to be of 100% purity by HPLC. Ethanol was distilled over NaOH and then through a Hempel column. The solvents from reaction mixtures and from column chromatographic separations were removed *in vacuo* on a rotary evaporator at a bath temperature not exceeding 30 °C. Analytical and preparative HPLC was performed on a Beckman "System Gold" chromatograph for Methods Development consisting of a Model 126 programmable module, a Model 168 diode array detector (working at 210 nm), a Model 210A injection valve and a PC386SX (Wearnes) with "System Gold" version 5.1 software for data collection and controller function. For analytical runs the following were used: an Alltech Alltima, $C_{18}$, 5 $\mu$m, 150×4.6 mm column, a 5 $\mu$l loop, acetonitrile : water (15 : 85) as a mobile phase and a flow rate of 1 ml/min; $t_R$ of Ac-(*Z*)-ΔPhe-NHMe 6.85 min, $t_R$ of Ac-(*E*)-ΔPhe-NHMe 7.35 min.

**Irradiations** Irradiations were performed at constant temperature of 22 °C, in 250 and 1500 ml bottles made of PET and equipped with a magnetic stirrer. Prior to the process, the solutions were bubbled with nitrogen for 5 min and thereafter space above the surface of the liquid was filled up with argon. The source of 293-nm and 313-nm light was a Photochemical Reactors, Ltd. 400-W medium-pressure mercury lamp fitted with appropriate filters. The source of 365-nm light was an 80-W lamp.

**Ac-(*E*)-ΔPhe-NHMe** Two solutions each Ac-(*Z*)-ΔPhe-NHMe (270 mg) in ethanol (1.2 l) were irradiated with 313-nm light over 16 h, then combined and concentrated to about 50 ml. A mixture of ethyl ether : hexane (1 : 1) (250 ml) was added and the whole left for crystallization. The resulting Ac-(*Z*)-ΔPhe-NHMe was filtered off (400 mg) and the filtrate concentrated. Precipitation and filtration were repeated once more to furnish the second crop of (*Z*)-izomer (60 mg), both of 99.8% purity by HPLC. The filtrate was evaporated to dryness to give a mixture of Ac-(*Z*/*E*)-ΔPhe–NHMe (20 : 80) (65 mg). This was dissolved in methanol (850 $\mu$l) and water (2550 $\mu$l) was added. The solution in 850 $\mu$l portions was applied with an 850 $\mu$l loop to an Alltech Alltima, $C_{18}$, 10 $\mu$m, 250×22 mm column. The column was eluted with water : methanol (80 : 20) at a flow rate of 20 ml/min and fractions were collected using a fraction collector Gilson 202. The fractions appropriate by analytical HPLC were combined and evaporated to dryness to afford Ac-(*E*)-ΔPhe-NHMe (15 mg) of 99.8% purity by HPLC.

$^1$HNMR (Tesla BS 567 100 MHz; a saturated solution in $CDCl_3$ with TMS as an internal standard) $\delta$ (ppm): 2.10 (s, 3H, $CH_3CO$), 2.67 (d, 3H, $NHC\underline{H}_3$), 6.62 (s, 1H $C^{\beta}$-H), 7.32 (m, 5H, Ph), 7.36 (q, 1H, $N\underline{H}CH_3$), 8.01 (s, 1H, NH-$C^{\alpha}$).

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## References and Notes

1) Part XII: Smełka L., Rzeszotarska B., Broda M. A., Kubica Z., *Org. Prep. Proced. Int.*, **29**, 696—701 (1997).
2) Abbreviations: Ac=acetyl, (*Z*)-ΔPhe=(*Z*)-dehydrophenylalanine, (*E*)-ΔPhe=(*E*)-dehydrophenylalanine.
3) Noda K., Shimohigashi Y., Izumiya N., "The Peptides. Analysis, Synthesis, Biology," Vol. 5, ed. by Gross E., Meienhofer J., Academic Press, New York-London, 1983, pp. 285—339.
4) Schmidt U., Lieberknecht A., Wild J., *Synthesis*, **1988**, 159—172.
5) Broda M. A., Rzeszotarska B., "Peptides. Proc. 25th Eur. Pept. Symp., Budapest 1998," ed. by Bajusz S., Hudecz F., Akadémiai Kiadó, Budapest, 1999, pp. 452—453.
6) Thormann M., Hofmann H.-J., *J. Mol. Struct. (Theochem)*, **431**, 79—96 (1998).
7) Pietrzyński G., Rzeszotarska B., *Polish J. Chem.*, **69**, 1595—1614 (1995).
8) Jain R., Chauhan V. S., *Biopolymers*, **40**, 105—119 (1996).
9) Nitz T. J., Shimohigashi Y., Costa T., Chen H.-Ch., Stammer Ch. H., *Int. J. Peptide Protein Res.*, **27**, 522—529 (1986).
10) Mosberg H. I., Dua R. K., Pogozheva I. D., Lomize A. L., *Biopolymers*, **39**, 287—296 (1996).
11) Edvards J. W., Fanger B. O., Cashman E. A., Eaton S. R., McLean L. R., "Peptides: Chemistry and Biology, Proc. 12th Am. Pept. Symp.," ed. by Smith J. A., Rivier J. E., Escom-Leiden, 1991, pp. 52—53; Edvards J. W., Fanger B. O., Patent No. 16105 (1993).
12) Nitz T. J., Holt E. M., Rubin R., Stammer Ch. H., *J. Org. Chem.*, **46**, 2667—2671 (1981).
13) Makowski M., Rzeszotarska B., Kubica Z., Pietrzyński G., Hetper J., *Liebigs Ann. Chem.*, **1986**, 980—991.
14) Ullman E. F., Baumann N., *J. Am. Chem. Soc.*, **92**, 5892—5899 (1970).
15) Aubry A., Allier F., Boussard G., Marraud M., *Biopolymers*, **24**, 639—646 (1985).
16) Broda M. A., Rzeszotarska B., Kubica Z., in preparation.
17) Brocklehurst K., Williamson K., *Tetrahedron*, **30**, 351—354 (1974).
18) Makowski M., Rzeszotarska B., Kubica Z., Wieczorek P., *Liebigs Ann. Chem.*, **1984**, 920—928; Makowski M., Rzeszotarska B., Kubica Z., Pietrzyński G., *ibid.*, **1985**, 893—900; Makowski M., Rzeszotarska B., Smełka L., Kubica Z., *ibid.*, **1985**, 1457—1464; Kubica Z., Rzeszotarska B., Makowski M., Główka M. L., Gałdecki Z., *Polish J. Chem.*, **62**, 107—113 (1988).