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E/*Z*-CONFIGURATIONAL ASSIGNMENT OF DEHYDROPEPTIDES: DIFFERENTIAL NOE ENHANCEMENT BETWEEN THE VINYL AND AMIDE PROTONS OF AN α,β-DEHYDRO AMINO ACID DERIVATIVE

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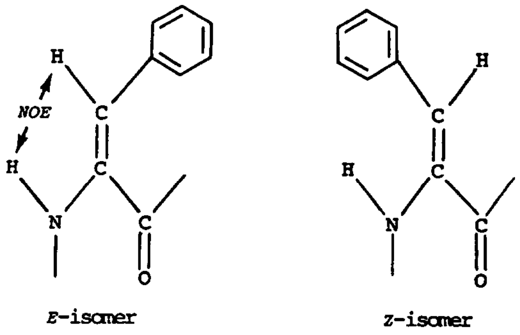
Summary: The nuclear Overhauser enhancement difference technique applied to vinyl and amide protons of an α,β-dehydroamino acid derivative allows the assignment of the *E* or *Z* configuration; *E*-form, NOE = 26-37% and *Z*-form, NOE = 0%.

The incorporation of α,β-dehydroamino acids into peptide hormones has been recognized as a useful structural variation for the elucidation of dynamic structure-activity relationships.[2] The restricted orientation of the β-substituent on the double bond is a probe for the specific interaction of this group with the receptors as demonstrated for D-Ala^2, Δ^ZPhe^4-enkephalins.[3,4] Thus, it is important to the synthesis of dehydropeptides to know the configuration of the dehydro unit. Some empirical rules for determining *E*/*Z*-configurations by NMR spectroscopy have been reported in recent years: (i) a comparison of chemical shifts of the vinyl proton when the spectrum is measured in $CDCl_3$ and trifluoroacetic acid;[5] (ii) a comparison of the vinyl proton chemical shift before and after *N*-methylation;[5,6] (iii) a difference in the vicinal coupling between the carbonyl carbon and the vinyl proton.[7]

Using a Nicolet NMC-500 NMR spectrometer operated at 500.09 MHz for ^{1}H-NMR spectroscopy, we have measured the nuclear Overhauser effect (NOE)[8,9] on the vinyl and amide protons of Cbz-ΔPhe-OEt, in order to discriminate the *E*- and *Z*-isomers (Fig. 1). The syntheses and conformational assignments by X-ray and ^{13}C-NMR have been reported previously for each pure isomer.[6]



E-isomer *Z*-isomer

Fig. 1. The structures of dehydro unit of the ΔPhe residues with the *E*- and *Z*-configurations.

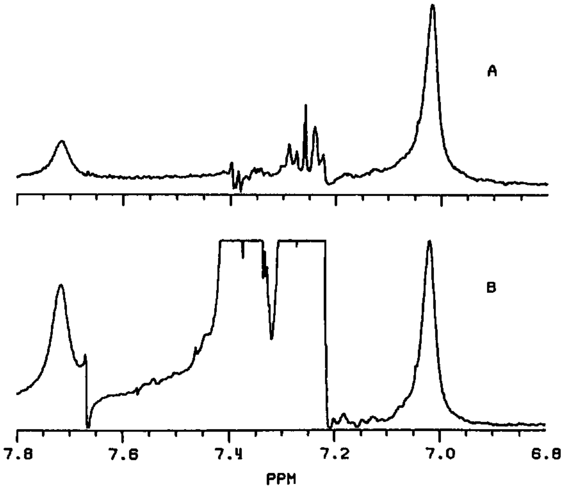


Fig. 2. 500 MHz ^{1}H-NMR spectra of Cbz-Δ^EPhe-OEt: (A) NOE difference spectrum with irradiation of the amide proton (7.017 ppm) and (B) spectrum without irradiations.

The H-{H} NOE difference spectrum in $CDCl_3$ was obtained by irradiation of either the vinyl or amide proton signal and was gated during a waiting time of 5 sec prior to each scan. Fig. 2 shows the NOE difference spectrum of *E*-isomer, Cbz-Δ^EPhe-OEt, after irradiation of the amide proton (7.017 ppm) (Fig. 2A) together with a spectrum without NOE (Fig. 2B). The amide proton was assigned by H-D exchange using CD_3OD, and observed enhancements as negative NOEs were 37% for the vinyl proton signal (7.712 ppm) and 5.5% for the phenyl proton signals of the Cbz group (7.255 ppm). By irradiation of the vinyl proton, the enhancements were 26% for the amide proton signal and 4.6% for the signals of Δ^EPhe-phenyl protons (7.393 ppm). Thus, the observation of large negative NOEs indicates that the vinyl proton is very close in space to the amide proton in the Δ^EPhe residue, but in the *Z*-isomer, no enhancement of the vinyl proton signal (7.514 ppm in $CDCl_3$) was observed by irradiation of the amide proton (6.304 ppm). The large effect on the aromatic envelope, which loses 28% of signal intensity, suggests a very close spacing between the amide proton and the phenyl ring (Fig. 1).

The present study clearly shows that a NOE difference spectroscopy is an efficient and direct method for the *E*/*Z*-configurational assignment of α,β-dehydroamino acid residues.

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