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The First Experimental Evidence of Differential Hydration of *E/Z* Isomers of Sterically Hindered Amides: One Dimensional Steady-State Selective Intermolecular ^{13}C, ^{1}H Overhauser Effect Study of *Tert*-butylformamide

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Abstract: One dimensional steady-state selective intermolecular ^{13}C, ^{1}H Overhauser effect study of *tert*-butylformamide provides the first experimental evidence of differentical hydration of the *Z* isomer, compared to the *E* isomer, due to the presence of the bulky *tert*-butyl group; the significant decrease in the population of the *Z* isomer should be attributed to steric effects and deformation of the amide bond with hydration differences playing a significant role.

Amide bond isomerization is important in many processes that require alternation of peptide and protein structure, among them transport of peptides through membranes, oligomerization, folding and catalytic activity of peptides and proteins[1-3]. Hydration might play a crucial role in the difference in stability between *E* and *Z* peptide bonds. NMR spectroscopy is probably the most widely used experimental method for determining the presence of *E/Z* equilibria in amides and peptides and for investigating the effects of solvents[4-8]. However, to the best of our knowledge, no report has so far been published in investigating the interaction of water with *E/Z* isomers of amides at a molecular level. There have been several developments in the past few years which promise to extend NMR methodology as a powerful method for investigating the interaction of water with molecules of biological interest via 2D and 3D NOE and ROE type dipolar cross relaxation processes between water protons and polypeptide protons[9-16]. Heteronuclear ^{13}C, ^{1}H Overhauser effect spectroscopy (HOESY)[17,18] can provide a valuable probe for investigating hydration of the amide and peptide group because of the strategic role of the peptide carbonyl carbon. Complementary information can also be obtained for the solvation state of sidechain carbons. However, this method has not yet been explored with only one report so far reported on intermolecular hydrogen bonding between water and the amide group of 2-pyridones[19]. The present communication describes the first application of a steady-state selective intermolecular carbon-13, proton-H HOESY 1D experiment to the study of *E* and *Z* isomerism of amides (*tert*-butylformamide) and the first demonstration of differentical hydration of *E/Z* amide carbons due to the presence of the bulky *N-tert*-butyl group.

The amount of the *E* form of *N-tert*-butylformamide (TBF) was found to be strongly solvent dependent. At 298 K it is about 25% in CD_3CN, 45% in CD_2Cl_2, 48% in $CDCl_3$ and 46% in H_2O solutions[20]. Unequivocal assignment of the *Z* isomer was achieved by 2D ^{1}H-^{1}H NOESY experiments which demonstrate the through space proximity of the C(O)H and NH protons. It is important to minimize magnetization transfer between the two isomers by the use of low temperature (278 K) and to choose the appropriate combination of selective, low-power decoupling followed by a high-power broadband decoupling during the observing pulse and the acquisition of free induction decays. It is not trivial to define the optimum

time of selective low-power decoupling of the water resonance in a multispin system to obtain HOESY peaks of significant intensity[19,21]. The ^{13}C longitudinal relaxation times for *E/ Z*- N-*tert*-butylformamide were measured at 278 K by the inversion-recovery method and were found to be: H$\underline{C}$(O), 2.07 s (*E*) and 1.52 s (*Z*); $\underline{C}(CH_3)_3$, 15.33 s (*E*) and 12.01 s (*Z*); C($\underline{C}H_3)_3$, 1.15 s (*E*) and 1.16 s (*Z*). Since carbon T_1 relaxation times cover a wide range, different optimum irradiation times should be used to map out all the dipole-dipole solute-solvent interactions. In practice, optimization of the irradiation time was achieved by following the build up of heteronuclear NOE.

Figure 1 shows a comparison of the ^{13}C, ^{1}H HOESY experiment of *tert*-butylformamide in H_2O/ D_2O (85%/ 15%) with the conventional 1D ^{13}C NMR spectrum. Strong hydration of both isomers is clearly demonstrated by the significant NOE peaks between water protons and the carbon atoms of *tert*-butylformamide. The integrated peak intensities of the resonances indicate that the NOE cross peaks from the water proton resonance to the CO carbons of the two isomers (Figure 1 (A)) are significantly different compared to the conventional 1D ^{13}C spectrum (Figure 1 (B)). Thus the *Z* amide CO NOE peak ($\delta \sim 162.00$ ppm) is smaller compared to the *E* isomer ($\delta \sim 164.20$ ppm) while the respective integrals are reversed in the conventional 1D ^{13}C NMR spectrum. This indicates that the water clustering of the water molecules around the *Z* amide CO group is significantly reduced compared to the *E* CO group. Similar results were obtained for selective low-power irradiation times of the water resonance of 0.9 and 2.8 s. On the contrary, the integrated NOE peak intensities of the *E/ Z* C($\underline{C}H_3)_3$ carbons appear to be the same compared with the conventional 1D ^{13}C spectrum. This indicates that the water clustering around the *tert*-butyl group is nearly the same for both isomers.

Our results demonstrate that the *Z* amide CO group of *tert*-butylformamide is sterically hindered from interactions with the water solvent due to the presence of the bulky *tert*-butyl group[22]. Interestingly, ^{17}O NMR studies of N-substituted amides indicate a deshielding of the ^{17}O NMR resonance by ~20 ppm of *Z*-*tert*-butylformamide compared to the *E* isomer[20,23]. This was attributed to a significant out-of-plane (torsion-angle) deformation of the amide bond which leads to greater double bond (aldehyde) character of the amide oxygen and/ or reduced hydration of the Z isomer[23]. Thus the significant decrease in the population of the *Z* isomer, compared to the *E* isomer, should be attributed to the combined effect of reduced hydration of the *Z* amide CO group and out-of-plane ω torsion- angle deformation of the amide plane.

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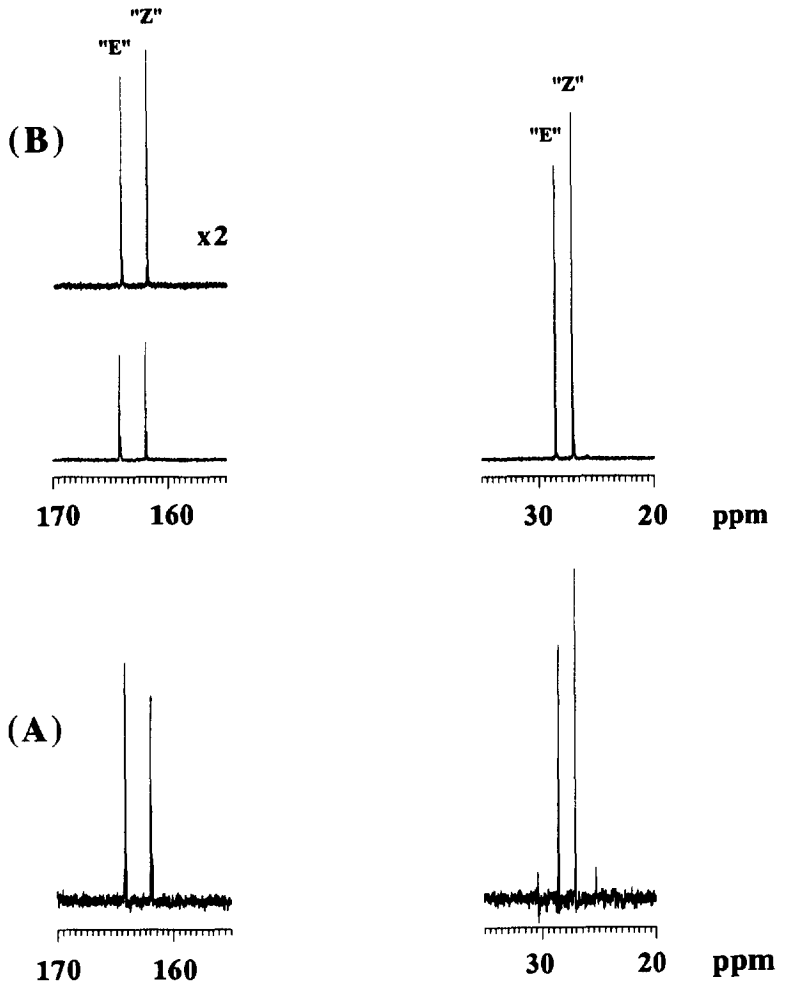

Figure 1. (A) 1D ^{13}C-^{1}H HOESY experiment of 2M *tert*-butylformamide in 85% H_2O / 15% D_2O, at 278 K, 5 mm sample tube, on a Brüker AMX-400 MHz instrument. Spectral acquisition parameters: 0.5 s acquisition time, 16 KHz spectral width, 320 number of scans and 1.2 s selective low-power decoupling of the water resonance. A 10 s waiting time was allowed between each pulse sequence. (B) Conventional ^{13}C NMR spectrum, 10 s recycling delay time, 16 number of scans. The *E/Z* resonances (marked by "E" and "Z" respectively) are shown above each peak.

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