Structure Elucidation of Amide Bonds with Dipolar Chemical Shift NMR Spectroscopy

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The structure of the amide bonds of gluconamide has been elucidated and compared to acetanilide by the combined application of ^{13}C and ^{15}N double- and triple-resonance solid-state NMR spectroscopy. The length of the amide bond has been determined from the dipolar spectrum using a SEDOR type experiment, and the orientation of the principal axis systems of both the ^{13}C and ^{15}N chemical shift tensors have been determined by employing dipolar chemical shift NMR spectroscopy in conjunction with CSA spectroscopy. The groups exhibit for amide bonds typical approximately 120° bond angles between −CO, −CN, −CR, and −NC, −NH, −NR. Comparing the structure of the gluconamide with the corresponding structure of the acetanilide, two major differences are visible: the orientations of the CSA tensors in the amide plane with respect to the CN-bond direction are different (12° for the ^{13}C tensor and 10° for the ^{15}N tensor), and the directions of least shielding and intermediate shielding are interchanged in the gluconamide as compared to the acetanilide. Since the chemical shielding tensors of the ^{15}N are strongly influenced by hydrogen bonding, these different orientations are an indication of the different hydrogen bond structure of the gluconamide as compared to the acetanilide.

1. Introduction

In recent years ^{13}C and ^{15}N solid-state NMR spectroscopies have become important tools for the structure elucidation of small peptides and proteins.[1] On one hand, the chemical shift interaction is a sensitive probe of structural features such as the primary and secondary structure of the molecule; however, for the interpretation of the corresponding CSA tensors one usually has to employ ab initio calculations of the molecular groups under investigation. Due to the importance of the amide bond as the structure-determining part of the peptide and thus the proteins, these tensors have been investigated in numerous studies of amide and peptide bonds. Magnetic dipolar interactions give direct geometrical information about intra- and intermolecular distances; therefore, this type of interaction has been used in various studies of bond length or intramolecular distances. Due to the axial symmetry of the interaction most information about the relative orientation of the coupled nuclei is not accessible. It is advantageous to apply a combined analysis of chemical shielding anisotropy and dipolar interaction. This approach allows for an orientation of the dipolar vector in the frame of the chemical shielding interaction. Recent studies of ^{13}C-CSA and ^{15}N-CSA tensors and $^{13}C-^{15}N$ dipolar interaction using various solid-state NMR techniques[1] demonstrated the usefulness of this approach. The prime result of all these studies showed the principal components of the CSA tensors of the amide carbon and nitrogen in the various polypeptides investigated to be very similar.[2] The chemical shielding interaction is a local interaction that depends mostly on the molecular and electronic structure close to the nuclei of interest. It was found that for the ^{13}C-CSA tensor the most shielded direction σ_{33} is perpendicular to the NCO plane and the intermediate component σ_{22} is approximately along the −CO bond. For the ^{15}N-CSA the most shielded component points approximately into the direction of the $^{13}C-^{15}N$ bond and the intermediate component is perpendicular to the amide plane.

In the present study ^{13}C and ^{15}N solid state NMR spectroscopies are used for comparing the structure of two different amides, namely, acetanilide (which has been recently studied[1a]) as a reference substance for the NMR structure of amide bonds and *N*-1-octyl-D-gluconamide. The latter compound assembles in bulk water to form micellar fibers in the form of extended quadruple helixes. Such H-bonded fibers have been isolated in the solid state. The molecular conformation of the gluconamide headgroup region has been elucidated by solid-state ^{13}C CP-MAS − NMR spectroscopy[3] and comparisons made with crystal structures of the second, crystalline modification of the *N*-1-octyl-D-gluconamide. It has been found that the linear arrangement of the *N*-1-octyl-D-gluconamide in the crystal (all-trans) is disturbed at C-2. The amide bond conformation was assumed to be trans in all cases, and chemical shift differences of the carboxamide carbon were related to different hydrogen bond lengths. Supporting evidence came from crystal structure and ^{13}C NMR solid-state spectra of the crystals.

In a first step the ^{15}N and ^{13}C dipolar interaction was determined by a variant of the SEDOR[4] experiment. Then the static powder spectra of the ^{15}N and ^{13}C nuclei were measured, and their CSA tensors and the orientation of the dipolar tensors in these CSA frames were determined. The ^{15}N and ^{13}C CSA tensors of the two amides were correlated by dipolar-chemical shift NMR spectroscopy,[1a,h,i] allowing one to determine the relative orientation of the two CSA tensors. Due to the axial symmetry of the dipolar interaction, the two tensors can be rotated along the dipolar axis and there exists a cylindrical ambiguity for the relative orientation.[1a] The usual approach to resolve this ambiguity is the application of symmetry arguments, comparing the system to related systems or ab initio calculations. However one has to be careful in the application of these second-hand arguments.

The rest of the article is organized as follows. After a brief introduction into the theoretical background necessary for understanding how the CSA tensors are correlated with respect to each other, a short description of our experimental setup, sample synthesis, and preparation is given. Then the experimental results are presented and discussed and finally summarized.

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