Spectroscopic Methods

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Combined NMR and UV/Vis Spectroscopy in the Solution State: Study of the Geometries of Strong OHO Hydrogen Bonds of Phenols with Carboxylic Acids**

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It has become a routine approach in the structure determinations of organic compounds to employ a set of different methods, such as NMR, IR, Raman, UV/Vis spectroscopy and mass spectrometry, exploiting their complementary benefits.[1] These experiments are usually performed using different samples prepared according to the specific requirements of the particular method. However, different samples of a given system may exhibit a different composition which can lead to a different degree of molecular aggregation and hence molecular conformations. The molecular conformations are temperature and solvent dependent and difficult to analyze. Thus, to ensure the compatibility of spectra obtained by different techniques, measurements performed on the same sample and under the same conditions may be crucial. For this reason, combined methods such as Raman spectroscopy/UV/Vis spectroscopy/fluorescence spectroscopy,[2] X-ray photoemission spectroscopy/ultraviolet photoemission spectroscopy/flame emission spectroscopy (XPS/UPS/FES),[3] and EPR spectroscopy/UV/Vis spectroscopy/gas chromatography (GC)[4] methods have been proposed. Recently, Hunger and co-workers have described a way to perform combined UV/Vis absorption and magic-angle spinning (MAS) NMR measurements.[5] This has incited us to combined low-temperature UV/Vis and solution-state NMR spectroscopy (UVNMR) which provides new insights into the acid–base chemistry of strongly hydrogen-bonded complexes dissolved in aprotic solvents. These systems are very sensitive to sample concentration,[6] solvent,[7,9] and temperature.[8,9] The use of low-temperature NMR spectroscopy has the advantage that the regime of slow proton exchange between hydrogen-bonded complexes can be reached, which allows their NMR parameters and hence information about their structure to be obtained.[10,11] Moreover, it allows the influence of the solvent polarity which is strongly temperature dependent to be studied.[12]

The benefits of UVNMR will be demonstrated using the example of a phenol carboxylate complex dissolved in CD_2Cl_2. The reason to choose this system is two-fold. Firstly, previous UV/Vis studies indicated that the position of the absorption bands of phenol groups is sensitive to their protonation state, both in protic[13] and aprotic[14,15] media; however, no information about hydrogen-bond geometries could be derived from UV/Vis measurements alone. Secondly, the UV/Vis spectra of solutions of a phenol with bases generally exhibit broad overlapping absorption bands indicating the presence of several species in different hydrogen bond and protonation states.[16–18] Herein, we demonstrate that UVNMR allows the electronic excitation frequencies to be correlated with NMR chemical shifts which in turn provide information about hydrogen-bond geometries.

To build a combined UVNMR probe an existing Bruker 5 mm low-temperature ^1H–^{13}C probe was equipped with a guiding channel for the insertion of a fiber optic reflection probe. The optical probe with six illumination fibers and one read fiber was a custom variation of the regular probe with 200 μm fibers and 2.5 mm tip by Avantes (Eerbeek, Netherlands). In Figure 1, a schematic representation of the measurement region of the modified NMR probe is given. The tip of the optical probe is located centrally underneath the bottom of the NMR sample tube. The illumination fibers are connected to a halogen/deuterium light source (Avantes) and the light reflected to the read fiber is analyzed by a AvaSpec 2048 spectrometer (operating range 240–800 nm). Reflection of sufficient amounts of light is achieved by placing a polytetrafluoroethylene (PTFE) insert inside the sample tube, leaving only a thin layer of solution between the inner glass surface and the bottom of the insert (0.02–0.5 mm, depending on the shape of the insert; the "effective" optical path length can be estimated using solutions of a substance with known extinction coefficient). In this work, the PTFE

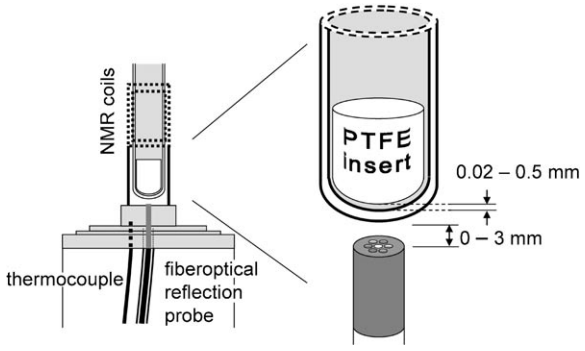

Figure 1. Schematic view of the top area of the NMR probe (bottom area of the NMR sample tube) modified for the combined NMR and UV/Vis spectroscopic measurements.

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inserts were principally cylindrical but flattened vertically at four sides to allow the sample solution to pass; they were round bottomed, in approximation of the shape of the inner bottom surface of the sample tubes. We note, however, that to ensure a constant layer thickness it will be necessary to use flat-bottom sample tubes and PTFE inserts. The radio-frequency (RF) coils for NMR experiments are located above the top of the PTFE insert and thus the usual sample volume is available for NMR detection. In the current setup, the glassware of the light guide and the NMR sample tubes allows experiments to be performed in the wavelength range between 200 nm and 2000 nm. However, the measurements in the NIR region would require changes in the material of the reflecting insert and the optical spectrometer used. Unfortunately, it is difficult to reach the IR spectral range at present.

To demonstrate the advantages of UVNMR we have studied mixtures of 2-chloro-4-nitrophenol (**1**), tetraethylammonium-3-phenylpropionate (**2**), 3-phenylpropionic acid (**3**) in CD_2Cl_2 at 175 K. Typical UVNMR results are depicted in Figure 2.

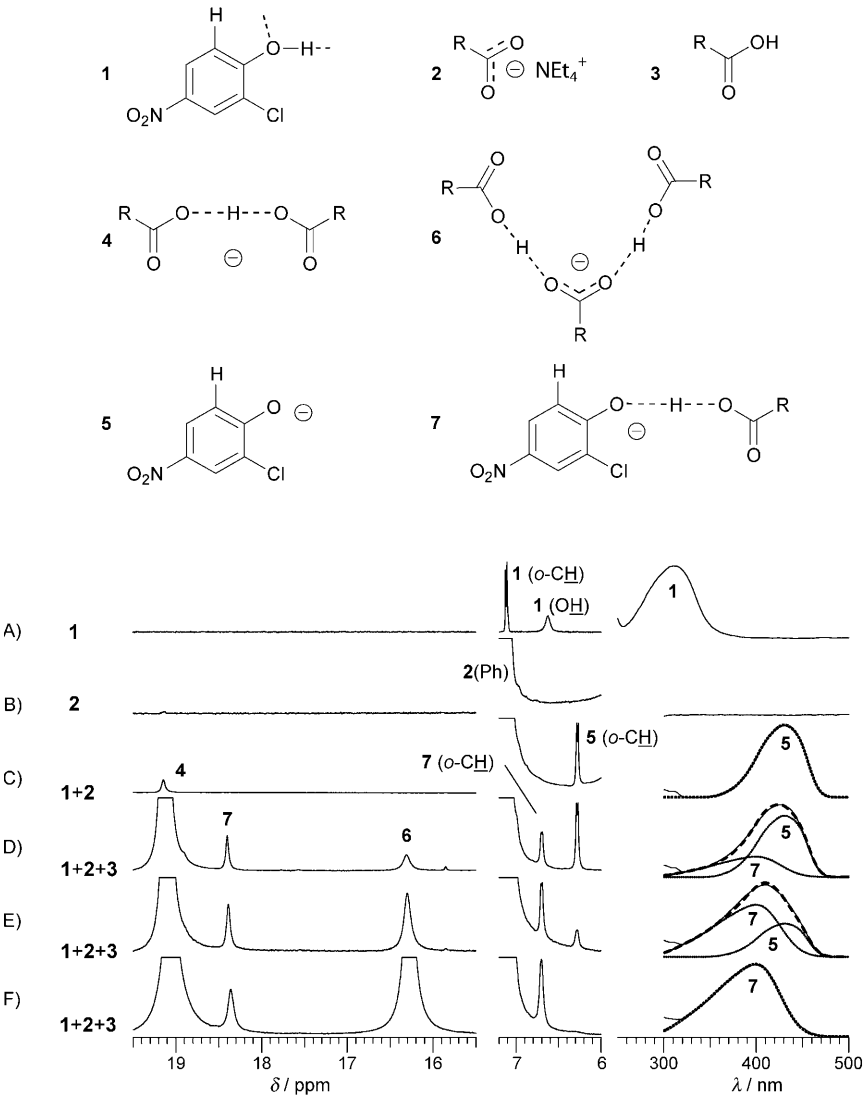

Figure 2. Combined ^{1}H NMR (left) and UV/Vis (right) spectra of **1**–**3** dissolved in CD_2Cl_2 recorded at 175 K; R = $PhCH_2CH_2$; dotted lines in UV/Vis spectra indicate the deconvolution of the bands. Concentrations of **1**/**2**/**3** in mM: A) 1:0:0, B) 0:29:0, C) 1:28:0, D) 1:23:17, E) 1:22:19, F) 1:21:23.

Sample A, which contains approximately 1 mM of **1** does not exhibit any signal in the downfield region of the ^{1}H spectrum. However, a sharp doublet appears around $\delta = 7.1$ ppm (*o*-C<u>H</u> coupled to *m*-C<u>H</u>) and a broader singlet around $\delta = 6.6$ ppm (O<u>H</u>). The signal s of the *m*-C<u>H</u> atoms resonating at $\delta = 7.8$ ppm and 8.1 ppm are not displayed. The UV/Vis absorption band maximum is located at 311 nm.

Sample B contains 29 mM **2**. Its phenyl residue gives rise to a large NMR multiplet between $\delta = 7.1$ and 7.3 ppm. This multiplet appears in all subsequent samples and is not discussed further. No signal is observed in the downfield ^{1}H NMR region and no absorption is detected in the UV/Vis region covered. This demonstrates 1) the absence of strong hydrogen bonds and 2) the transparency of the carboxylate and the counterion between 300 nm and 500 nm.

Sample C was obtained from Sample B by adding a small amount of phenol **1**. Compound **1** is almost entirely converted into the phenolate **5**, as indicated by the appearance of the *o*-C<u>H</u> signal at $\delta = 6.2$ ppm. Such upfield shifts of signals of phenolic *o*-C<u>H</u> and *p*-C<u>H</u> atoms upon deprotonation of the hydroxy group have been observed in a number of systems forming intramolecular hydrogen bonds.[19] The deprotonation is confirmed by the observation of an absorption maximum at 430 nm, in view of the observation that **1** dissolved in water at low pH values contributes a band at 315 nm and at high pH values a band at 400 nm to the UV/Vis spectra.[20] Interestingly, a small signal appears at $\delta =$ 19.1 ppm which is typical for the homoconjugate anion **4**, in which the downfield shift arises from the formation of a very strong hydrogen bond.[10]

Samples D to F were obtained by successive addition of **3**. The main result is a strong increase of the signal assigned to **4**. However, 1) a new signal at $\delta = 16.3$ ppm appears and becomes stronger, and 2) two signals in a 1:1 ratio grow together at $\delta =$ 18.4 ppm and 6.7 ppm. The assignment of the $\delta = 16.3$ ppm signal is straightforward: it is characteristic for doubly proton-bridged tricarboxylate **6** as has been shown for acetic acid.[10b] We assign the $\delta = 18.4$ ppm signal to the novel complex **7** which also exhibits a strong hydrogen bond. This assignment is confirmed by the finding that the *o*-C<u>H</u> proton of **7** appears halfway between the corresponding signals of **1** and **5**. This NMR result is supported by the UV/Vis spectra: because **4** and **6** do not contribute to these, the spectra consist only of a superposition of two bands arising from **5** and **7**. In the case of Sample F, only **7** contributes to the spectrum. The relative contributions of both species to the spectra of Samples D and E

were obtained by line-shape analysis as depicted in Figure 2. The two spectral components were taken from the spectra of Samples C and F. However, note that the intensities of the two UV/Vis spectral components do not necessarily reflect the molar ratio of the two species because of a possible difference in their extinction coefficients. By contrast, NMR spectroscopy provides the correct mole fractions.

The set of spectra of Figure 2 shows one advantage of combined UVNMR, that is, to provide an improved way to monitor the composition of samples defined by complex equilibria. A second advantage is to determine the integrated relative extinction coefficients taking into account the NMR signal intensities. Moreover, in the present case, UVNMR provides information about the hydrogen-bond geometries of **7**. NMR spectroscopy demonstrates the formation of a very strong hydrogen bond. However, in the case of OHO hydrogen bonds it is difficult to determine by NMR spectroscopy whether the H is located on the left or on the right side of the hydrogen-bond center, or whether there is a fast proton transfer between two tautomeric states.[10a] In this case, UV/Vis spectroscopy is helpful: the electronic absorption band of **7** is located closer to the band of the anion **5** rather than to the band of the neutral form **1**. This result suggests that **7** has a structure in which H is located on average closer to the carboxylate. This situation is in contrast to the finding that in aqueous solution the carboxylic acid **3** is more acidic than the phenol **1**. That the UV/Vis absorption band of **7** lies in between the absorption bands of **1** and **5** indicates a correlation between the electronic absorption frequencies of phenol carboxylate complexes and their hydrogen-bond geometries. Moreover, as the nature of the different species cab be determined by NMR spectroscopy, we are going to analyze the electronic band of **7** in more detail in the future to look for an intrinsic barrier or a solvent barrier for the proton motion.[21,22]

In conclusion, the setup described herein may be useful whenever sample compositions are very sensitive to the concentrations of the interacting components and to temperature, particularly in systems undergoing slow chemical reactions or photoreactions.[23] Combined UVNMR as proposed herein might also have an impact on the understanding of the connection between the hydrogen-bond structures and the optical properties of the chromophores in the active site of signaling proteins, such as PYP.[24]

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