NMR Spectroscopic Methods (2)

A DMSO-Compatible Orienting Medium: Towards the Investigation of the Stereochemistry of Natural Products**

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The elucidation of the relative stereochemistry of asymmetric centers of organic molecules is an important challenge in chemistry since it requires the simultaneous determination of conformation and configuration. Whereas conventional NMR spectroscopy parameters such as NOE and ^3J coupling constants, which provide internuclear distances and dihedral angles, yield the configuration of stereocenters in rigid compounds, this approach is difficult or impossible in cases in which the molecule is flexible or the stereocenters are remote in the bonding network. Residual dipolar couplings (RDCs) provide additional structural restraints, as has been abundantly shown in structural biology,[1] and might enhance the power of NMR spectroscopy in the determination of the conformation and configuration of small organic molecules at the same time. They rely on the weak alignment of molecules in solution and provide angular as well as distance information that are not contained in the NOE or *J* coupling values. RDCs have proven to be very efficient in the stereochemical assignment of moieties and hold promise as parameters for the determination of the stereochemistry, even in nonrigid molecules.[2]

Although a large variety of alignment media exist for aqueous solutions, such as filamentous phages,[3] phospholipid bicelles,[4] Otting phase,[5] and gels,[6] only few are currently available for organic molecules that cannot be dissolved in water. So far, only chiral liquid-crystalline media such as poly-γ-benzyl-L-glutamate (PBLG) or poly-γ-ethyl-L-glutamate (PELG),[2] which are compatible with $CHCl_3$, CH_2Cl_2, *N*,*N*-dimethylformamide (DMF), THF, and 1,4-dioxane and cross-linked polystyrene (PS),[7] which is compatible with $CHCl_3$ and THF, have been used. These media are limited to the mentioned solvents. Furthermore, PBLG aligns more-complex organic molecules so strongly that the resolution and sensitivity of ^{1}H spectra are dramatically lowered by line broadening. The PS orients weakly enough; however, it is incompatible with polar organic solvents. Therefore, additional alignment media are required.

Herein we introduce a copolymeric crosslinked polyacrylamide (PH gel), the first alignment medium compatible with dimethyl sulfoxide (DMSO), which has excellent solvent properties and is widely used in organic chemistry. The amount of alignment is much lower than that of PBLG, as can already be appreciated from the narrow ^{1}H-resonance lines observed for (+)-menthol in DMSO in PH-gel (Figure 1). Moreover, our results show that PH-gel is also compatible with DMF and D_2O.

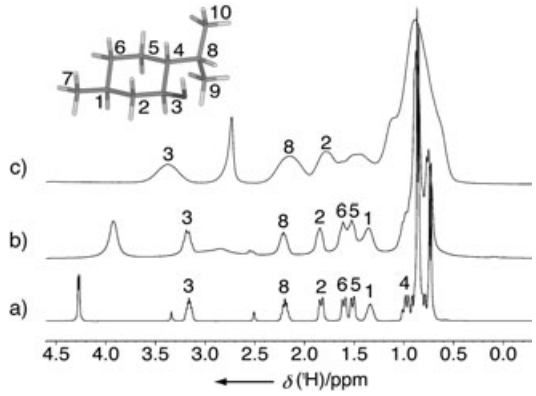

Figure 1. ^{1}H NMR spectra (400 MHz, 298 K) of (+)-menthol in a) DMSO, b) PH gel/DMSO, and c) PBLG/$CDCl_3$.

The PH gel can easily be obtained by the copolymerization of 2-(acrylamido)-2-methylpropanesulfonic acid (AMPS; ionizable monomer) with *N*,*N*-dimethylacrylamide (DMAA; comonomer) in the presence of *N*,*N*′-methylenebisacrylamide (BIS) as linker and ammonium persulphate (APS) as radical initiator.[8] The washed PH gel is dried on a glass capillary. It is then introduced into a 5-mm NMR tube, and the desired solvent is added (Figure 2). Within 1–2 days, the gel reswells and is compressed owing to the constraints of the glass wall.[7] The capillary allows the gel to be centered between the bottom of the tube and the surface of the solvent level within the NMR tube, which facilitates the reswelling of the gel. In contrast to the observations of Grzesiek et al.[6b] no significant stretching on the capillary could be determined. After 5 days, the deuterium spectra of the solvent show a quadrupolar splitting up to 4 Hz, indicating the alignment in the sample. The gel concentration of the NMR samples mentioned below needs to be adopted to obtain the described alignment.

The versatility of the PH gel is demonstrated on four different molecule/solvent-systems: (+)-menthol in DMSO and in DMF, the cyclic depsiheptapeptide hormaomycin[9] in DMSO, and a decasaccharide[10] in water (chemical formulas are shown in the Supporting Information). To measure the RDCs, t_2-coupled ^{1}H,^{13}C-HSQC[11] spectra were recorded in the presence (anisotropic solution) and in the absence (isotropic solution) of the orienting PH gel. The scalar $^1J_{CH}$

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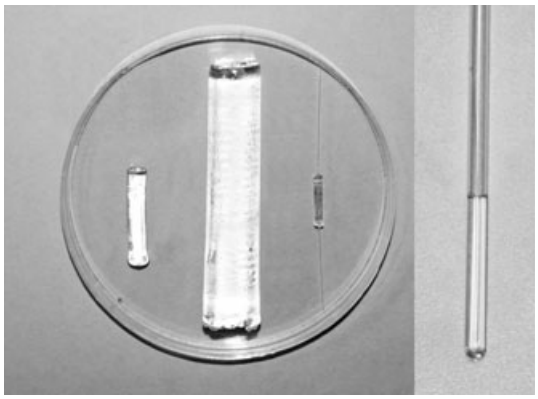

Figure 2. Photograph of the PH gel in the different states during preparation. From left to right : PH gel after polymerization, after washing in water, dried on a glass capillary, reswollen in the NMR tube with the desired solvent.

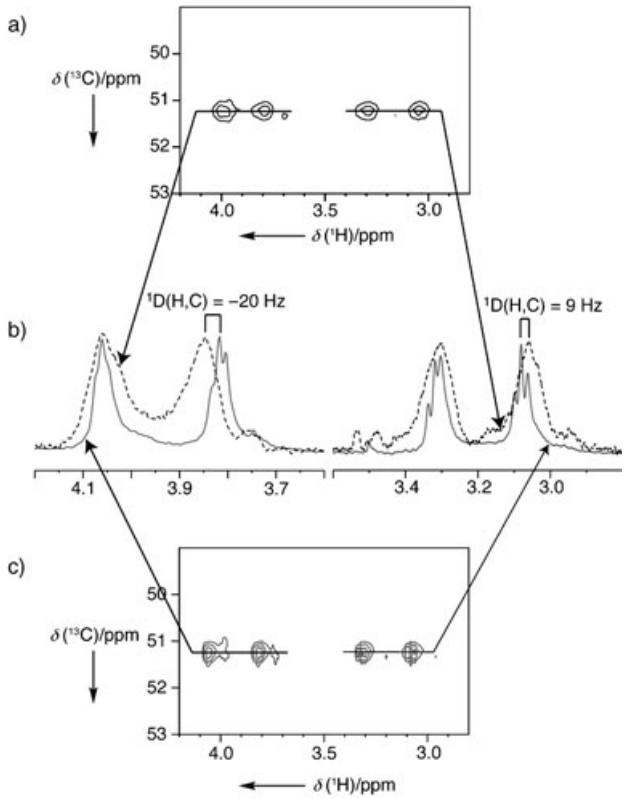

Figure 3. $^{1}H,^{13}C$-HSQC spectra (t_2-coupled) of a sample of hormaomycin in DMSO (50 mM) recorded on a 600-MHz spectrometer equipped with a TXI cryoprobe at 298 K. a) Section of the anisotropic HSQC spectrum, b) superimposed traces through the anisotropic HSQC section (dotted line) and the isotropic HSQC section (solid line), c) section of the isotropic HSQC spectrum.

and total couplings $^{1}J_{CH}+{}^{1}D_{CH}$ can then be measured individually after extraction of the HSQC traces in the F2 dimension for all ^{13}C-bound protons of the studied molecule. The difference between the two coupling constants then results in the RDCs $^{1}D_{CH}$.[12] However, this method proved unreliable in cases in which the size of the RDC is small. To improve the precision of the RDCs, the corresponding traces from the isotropic and anisotropic HSQC spectra were superimposed and then fitted, based on the peak shapes (Figure 3). For the more difficult cases in which the spectral pattern of the isotropic and anisotropic traces were different, an exponential apodization function was applied to the spectra obtained from isotropic solution before the superimposition. This method determines the dipolar couplings directly and results in a significantly improved accuracy. The direct comparison of the upfield components of the anisotropic and isotropic spectra and similarly for the downfield components of the anisotropic and isotropic spectra as opposed to the extraction of the J value from the isotropic and the $J+D$ value from the anisotropic spectra is a small change in the extraction procedure, but is essential and beneficial owing to the strong coupling effects.

As a first test molecule, (+)-menthol was dissolved in [D_6]DMSO and the solution was introduced with a polyacrylamide gel stick (PH gel concentration 8 % *w*/*v*) into an NMR tube. The resulting quadrupolar splitting on the deuterium spectrum of the solvent was 4 Hz. The sign of the $^{1}J_{CH}$ couplings is always positive.[13] In weakly orienting media, $^{1}D_{CH}$ couplings are generally smaller than $^{1}J_{CH}$ couplings, and therefore the total coupling $^{1}J_{CH}+{}^{1}D_{CH}$ has a positive sign. All dipolar couplings are in the range 1.8–4.8 Hz. The single-value-decomposition module (SVD)[14] of the program PALES[15] was used to fit ten experimental dipolar couplings (excluding the $^{1}D_{CH}$ couplings of the isopropyl group because of its flexibility) to a reference structure. As there is no available crystal structure for (+)-menthol, we used the modified crystal structure of (−)-menthol, for which the chirality was inverted and the proton positions were optimized by conjugate-gradient energy minimization. The correlation factor R is 0.97. A similar fit ($R=0.96$) was obtained with a (+)-menthol/PH gel sample (PH gel concentration 12 % *w*/*v*) in DMF (see Supporting Information). The range of the dipolar couplings for this sample is 0.6–2.6 Hz. Menthol is a rather spherical molecule and it therefore orients more weakly than nonspherical molecules of similar size.

Finally, the following two experiments demonstrate the versatility of the PH gel. First, the cyclic depsiheptapeptide hormaomycin, which has a molecular weight of 1130 g mol^{-1}, was oriented in a PH gel/DMSO alignment medium resulting in a quadrupolar deuterium splitting for [D_6]DMSO of 2 Hz. Sections of the isotropic and anisotropic HSQC spectra show RDCs that range from −20 to 29 Hz, a range in which they can be evaluated easily and accurately. Experimental traces for two geminal methylene protons of the 4-[(*Z*)-propenyl]-proline residue are depicted in Figure 3. In the second application, we investigated a decasaccharide with a molecular weight of 1804 g mol^{-1}. It was dissolved in D_2O and aligned with the PH gel, causing a quadrupolar deuterium splitting for D_2O of 3 Hz. The measured $^{1}D_{CH}$ ranged from −22 to 28 Hz. The amount of alignment is scalable (see Experimental Section).

In summary, we have shown that a reliable alignment can be obtained by using PH gel with DMSO and other solvents for complex organic molecules of various sizes. The magnitude of the dipolar couplings is in a range in which they can be

evaluated accurately since they are large enough to be measured with an acceptable spectral resolution since $^1H,^1H$ dipolar couplings do not cause extensive line broadening. Further research is underway in which this gel is being used to solve stereochemical problems in organic molecules that can neither be crystallized nor dissolved in water or chloroform.[16]

Experimental Section

PH-gel: AMPS, DMAA, and BIS (1:1:0.026) were dissolved in purified water to a total monomer concentration of 0.75 mol L^{-1}. The pre-gel solution was inserted into a gel cylinder with an inner diameter of 5.4 mm and polymerized for 2 h at 70 °C initiated by ammonium persulphate ($\approx$0.0015 g mL^{-1}). The gels were washed once with NaOH solution (0.02 M) and at least four times with water, each time for several hours. The swollen gels were dried on a glass capillary (diameter ~0.3–0.4 mm) under vacuum at 25–30 °C. The alignment is scalable by varying the diameter of the gel cylinder.

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