Communications to the Editor

Reduction of Inhomogeneous Line Broadening in Two-Dimensional High-Resolution MAS NMR Spectra of Molecules Attached to Swelled Resins in Solid-Phase Synthesis

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The great advances and widespread application of solid-phase synthesis[1] of peptides,[2] oligonucleotides,[3] oligosaccharides,[4] and small organic molecules in combinatorial chemistry[5] call for effective analytical techniques for analysis of the products. In combinatorial chemistry the solid support is typically polystyrene beads equipped with a spacer and a linker to which the reacting molecules are attached. Clearly, if the product of the synthesis were cleaved off from the support and purified, the usual arsenal of analytical techniques would be available just as in classical organic synthesis. However, that process is destructive and time-consuming, in particular if each stage of a multistep synthesis needs to be investigated.

On this background, magic-angle-spinning (MAS) NMR of intact systems of molecules attached to resins swelled in organic solvents rapidly established itself as a prime analytical method in combinatorial chemistry.[6] By swelling the resins, sufficient mobility is introduced that the resonances are significantly narrower than for dry resins, and by adding MAS, liquid-state quality NMR spectra are approached. Nevertheless, it would be highly desirable to get below typical line widths of several hertz. That would improve the sensitivity and make possible accurate measurement of *J* coupling constants. This paper introduces a general scheme to that end and shows an application to two-dimensional (2D) ^{1}H homonuclear correlation spectroscopy.

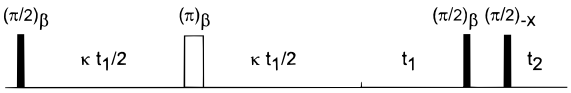

Figure 1. Pulse sequence for multiple-quantum-filtered COSY[9] or E.COSY[8] with scaling in the evolution period. In the case of dominating and disturbing signals from the resin or the spacer, it is advantageous to combine the pulse sequence with presaturation, CPMG, or a spin-lock multipulse sequence.

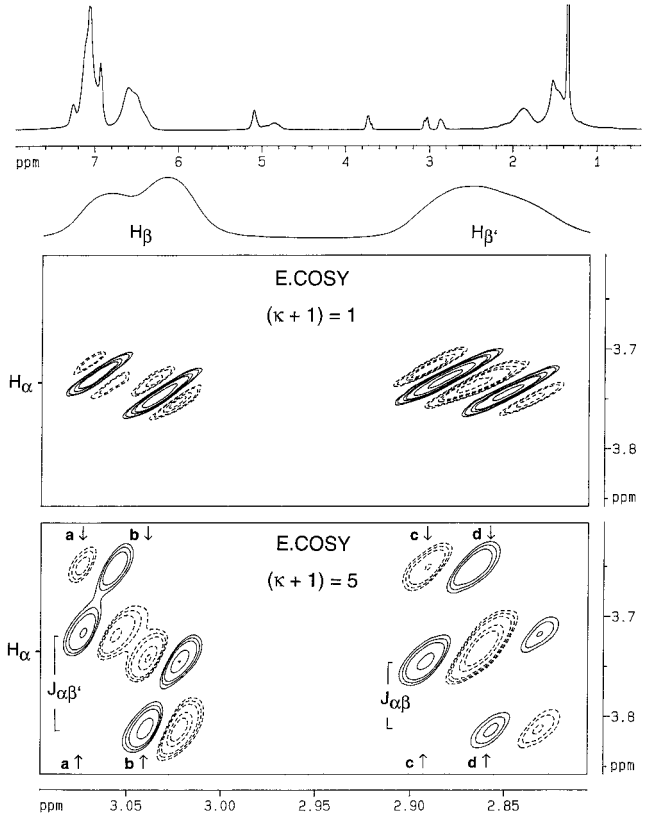

Figure 2. 1D spectrum and excerpts from $\kappa = 0$ and $\kappa = 4$ E.COSY spectra of *O-tert*-butyltyrosine attached to a Wang resin. All spectra shown were recorded at 300 K; in the FIDs, 2764 time domain data points covered a spectral width of 3788 Hz and a sinebell window function shifted $2\pi/5$ was employed in all dimensions. TPPI was applied in the 2D spectra that employed both $(1 + \kappa)t_1^{max} = 370$ ms and a prescan delay of 5 s. For the $\kappa = 0$ ($\kappa = 4$) spectrum the parameters were the following: number of t_1 experiments, 370 (74); number of scans per t_1 experiment, 12 (60); data matrix 370 × 2764 (74 × 2764) zero-filled to 4096 × 16384 (512 × 16384) prior to 2D Fourier transformation. The standard E.COSY $N = K = 3$ phase cycle[8c] was applied.

The clue to the problem is the fact that, in spite of swelling and MAS, inhomogeneity is still expected across the sample. (The sizes of the beads and also the surroundings of the individual molecules bound can vary.) This hypothesis is supported by the typical appearance of homonuclear 2D *J*-resolved spectra[7] of resin-bound molecules where narrower peaks are observed. But that method is not generally satisfactory because overlap of peaks is not uncommon and because the inherent phase-twisted peak shapes call for an absolute value display, reducing resolution. In fact, the method has been largely abandoned in liquid-state NMR for measurement of *J*

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coupling constants. Instead E.COSY[8] type methods are used for this purpose, and that would also be desirable in MAS NMR of combinatorial chemistry samples. In order to make that possible, the inhomogeneous contribution to the line widths must be reduced.

Since the inhomogeneity is associated with the chemical shift terms of the Hamiltonian, a solution is to scale down these terms relative to the *J* coupling terms. That is done by inserting the element $\kappa t_1/2-\pi-\kappa t_1/2$ into the evolution period of any existing 2D correlation sequence as indicated for multiple-quantum-filtered COSY[9] or E.COSY in Figure 1. In this way the chemical shifts are scaled by a factor of $(\kappa + 1)^{-1}$ while the *J* couplings are unaffected. However, since in practice it is more convenient to retain a COSY-type display format, the spectra show an apparent upscaling of the *J* coupling constants by a factor of $\kappa + 1$.

We should note that the scaling scheme presented is not new but has been used by other authors on homogeneous liquid samples for a different purpose.[10] Presuming a well-shimmed magnet and a homogeneous sample, the benefit of scaling is only one of being able to do with a coarser digital resolution in the ω_1 dimension of 2D spectra while still resolving multiplet structures. In contrast, the application to entire resin complexes in combinatorial chemistry yields true line narrowing in ω_1 but has of course also the benefit related to the digital resolution.

Experimental tests have been carried out on a Bruker AMX-2 400 MHz NMR spectrometer equipped with a 4 mm HR-MAS probehead using a sample of *O-tert*-butyltyrosine attached to a Wang resin (0.92 mmol/g loading; purchased from Bachem, Switzerland) and swelled in $CDCl_3$. An insert in the rotor was used to create a spherical sample compartment of 25 μL volume.

On top of Figure 2 is shown the full ^{1}H NMR spectrum while an expansion of the region of the β protons is displayed above the excerpt from the standard E.COSY spectrum (i.e. $\kappa = 0$). At the bottom, the same excerpt from the $\kappa = 4$ E.COSY spectrum is shown. The tilts and elongated shapes of the cross peak multiplet components reflect the inhomogeneity across the sample[11] and the value of κ. In order to have a reliable comparison of sensitivity and resolution, $(1 + \kappa)t_1^{max}$ and the total number of FIDs acquired were identical for both 2D experiments.

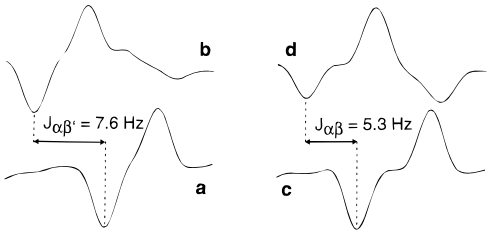

Figure 3. Traces indicated by the arrows in Figure 2 and suitable for E.COSY-type extraction of the vicinal coupling constants between the α and β protons.

Because of overlap of multiplet components caused by inhomogeneous line broadening in the normal E.COSY spectrum, no *J* coupling constants can be extracted from the $H_\alpha-H_{\beta'}$ multiplet, and it is questionable whether displacements can be measured with reliability in the $H_\alpha-H_\beta$ multiplet because the geminal *J* coupling constant is incompletely resolved. In contrast, the position of pertinent multiplet components in the $\kappa = 4$ E.COSY spectrum are very well defined, making it possible to extract the vicinal coupling constants with the usual high accuracy of E.COSY. In Figure 3 are shown the traces indicated by the arrows in the $\kappa = 4$ E.COSY spectrum.

In conclusion, we have presented a general scheme for reduction of inhomogeneous line broadening in high-resolution MAS NMR for nondestructive on-bead analysis of products from solid-phase synthesis. Incorporation of this scheme into, for example, E.COSY makes it possible to extract crucial *J* coupling constants otherwise buried in the line width. The scheme applies equally well to other applications of high-resolution MAS probeheads, for example, foodstuff, fruits, tissue, plants, and algae. Also other applications of NMR to inhomogeneous systems, for example, in-vivo spectroscopy, could benefit from the proposed scheme for reduction of inhomogeneous line broadening.

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