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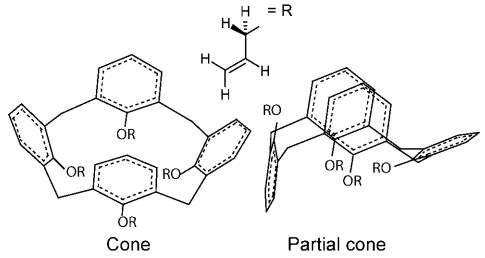
^{1}H NMR without Couplings

Pure Shift ^{1}H NMR: A Resolution of the Resolution Problem?**

*Juan A. Aguilar, Stephen Faulkner, Mathias Nilsson, and Gareth A. Morris**

NMR instrumentation has made enormous strides in sensitivity in recent years, notably through the development of cold-probe technology, but resolution has remained stubbornly tied to magnetic field strength: it has taken 28 years to double the highest field strength available, from 500 to 1000 MHz for ^{1}H. Methodological developments, especially multidimensional NMR, have helped, but even at the highest fields ^{1}H spectra are often extensively overlapped. The central problem is the multiplet structure caused by homonuclear scalar coupling; if this could be suppressed, the density of signals in a spectrum would typically decrease by almost an order of magnitude. A "pure shift" spectrum, without multiplets, would bring a resolution improvement to match that achieved in signal-to-noise ratio (SNR). Such spectra can be obtained,[1–13] but until recently have been very little used, for a variety of reasons including lack of generality, insensitivity, nonlinearity, and complexity. Here we show that second-generation pure shift methods are eminently practical for complex chemical systems, giving resolution equivalent to spectrometers of several GHz with mM sensitivity.

Figure 1 compares conventional and pure shift spectra, the latter acquired in 2.8 min, for a mixture (total concentration 9 mM) of the two tetraallyl calix[4]arenes of Scheme 1.



Scheme 1. Tetraallyl calix[4]arenes studied.

(The synthesis and study of these two calixarenes were part of a separate investigation and are described elsewhere.[14]) As the expansion of the allylic region around 6 ppm makes clear, suppressing multiplet structure gives a very large improvement in resolution, and in this mixture almost completely eliminates signal overlap. The region 5.7–6.2 ppm (Figure 1a) contains three of the four allylic methine multiplets, each

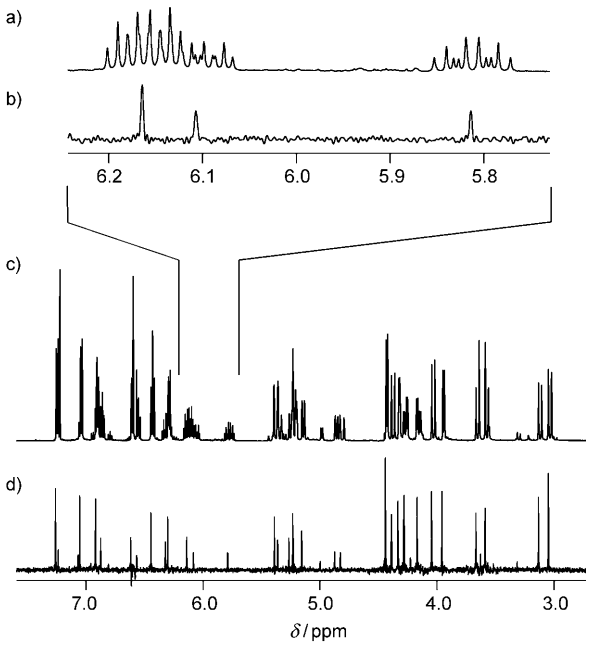


Figure 1. a,c) Normal ^{1}H NMR spectra for a mixture of the cone (3.8 mM) and partial cone (5.2 mM) calix[4]arenes of Scheme 1. b,d) Pure shift spectra measured in 2.8 min with the sequence of Figure 2a.

spanning over 40 Hz. The multiplets together cover over 120 Hz, but in the pure shift spectrum (Figure 1b) the three singlets occupy less than 5 Hz, over a 20-fold gain in resolution. The average resolution improvement over the whole spectrum is 10-fold; to obtain this by conventional means would require a 5 GHz spectrometer.

The pure shift spectrum of Figure 1b and d was acquired using the pulse sequence of Figure 2a. This is based on the experiment of Zangger and Sterk,[7] and is unusual in two respects: first in using a selective 180° pulse at its core that is selective both for chemical shift and for spatial position, and second in being used to build up a synthetic free induction decay or interferogram piece by piece. The weak field gradient under the selective 180° pulse is used to allow signals for all chemical shifts to be measured simultaneously, but each from a different horizontal slice of the sample; the 4 ppm width of Figure 1d corresponds to approximately a 1 cm range over the active volume of the sample used. Restricting measurements to a thin slice of the sample for a given chemical shift allows the combination of the selective and nonselective 180° pulses to invert all the spins in a given

[*] Dr. J. A. Aguilar, Dr. M. Nilsson, Prof. G. A. Morris
School of Chemistry, University of Manchester
Oxford Road, Manchester M13 9PL (UK)
Fax: (+44) 161-275-4598
E-mail: g.a.morris@manchester.ac.uk
Homepage: http://www.chemistry.manchester.ac.uk/aboutus/staff/showprofile.php?id=277
Prof. S. Faulkner
Inorganic Chemistry Laboratory
South Parks Road, Oxford, OX1 3QR (UK)

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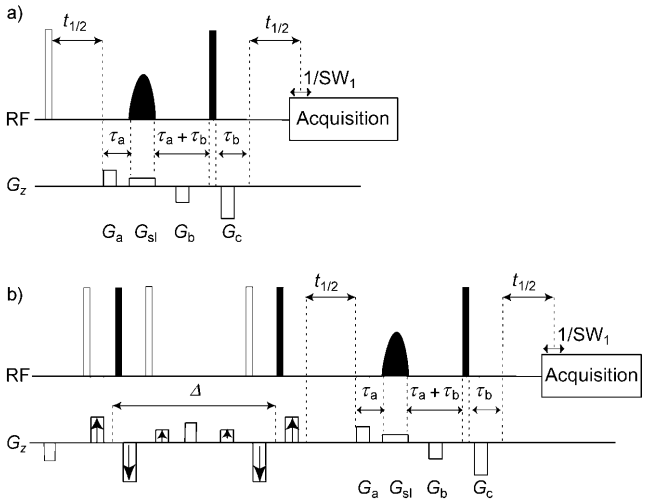

Figure 2. Pulse sequences used for pure shift ^{1}H NMR and pure shift DOSY. Open rectangles represent hard 90° pulses, filled rectangles 180° pulses, and solid semi-ellipses shaped soft 180° pulses. Vertical arrows indicate diffusion-encoding gradient pulses, which are incremented in sympathy. The delays τ accommodate gradient pulses and recovery intervals.

slice except those whose signals are being measured, thereby refocusing the effects of any scalar couplings to those spins. Over the whole evolution period, therefore, the average effect is to retain the chemical shift but to suppress the effects of scalar couplings—homonuclear broadband decoupling.

The decoupling effect is strictly achieved only at the exact time t_1. The obvious way to proceed is then to construct an interferogram one point at a time, but this would make the experiment impractically slow. Fortunately, scalar couplings evolve slowly, so it is possible to measure signals for a time $t_{acq} \ll J^{-1}$ before coupling effects intrude, acquiring a whole "chunk" of 10–20 ms of free induction decay containing n_{acq} complex data points for each value of t_1. An interferogram of n_{total} data points can then be constructed from only n_{total}/n_{acq} measurements, by concatenating successive chunks. The evolution time t_1 is incremented in steps of the chunk duration of $1/SW_1 = n_{acq}/SW$, where SW is the spectral width of the final spectrum. The timing of the sequence is adjusted to acquire only $n_{acq}/2$ data points for $t_1 = 0$, then for all remaining increments n_{acq} data points centered on the time t_1. In practice two extra data points are acquired, and discarded, before the start of the data required; this is because the digital signal processing used in modern spectrometers slightly distorts the first few data points measured. SW_1 is set so as to ensure that the small satellite signals introduced by the effect of J evolution during the chunk are negligible. Using chunks centered on the time t_1, rather than starting at time t_1, allows twice as long a chunk as in the original experiment of Zangger and Sterk, with a corresponding time saving.

In addition to doubling the width of the data chunks acquired, this second-generation sequence differs in two important ways from its parent.[7] First, the original slice-selective initial 270° pulse is replaced by a hard 90° pulse. This both simplifies the experiment and improves sensitivity, at the expense of a slight increase in experimental artefacts where the shift difference between two coupled protons accidentally matches the bandwidth of the selective 180° pulse. Second, pulsed field gradients are used to enforce a double spin echo coherence transfer pathway. This is important because of the need to suppress rigorously the strong signals of the passive spins. The net result is a quicker and cleaner experiment, up to 16 times faster than the original.

Two factors limit the homonuclear decoupling achieved. The first is the bandwidth of the selective pulse used, since decoupling requires that it affect only one coupling partner. The second, less tractable, factor is strong coupling, this leads to extra responses, mostly at frequencies between the two coupled chemical shifts; a typical example can be seen for an AB system at 3.6 ppm in Figure 1 d. There is a trade-off between sensitivity and the minimum chemical shift difference decoupled, since a narrower bandwidth corresponds to a thinner sample slice; the sensitivity is also inversely proportional to the slice select gradient G_{sl}. Since the latter determines the range of chemical shifts for which signals are measured, restricting measurements to the shift range of interest can significantly improve sensitivity, an element of control lacking in BIRD-based[3] pure shift methods.

Many existing NMR techniques can be adapted to use pure shift acquisition; Zangger and Sterk's original paper[7] described pure shift TOCSY, and very recently a pure shift HSQC based on Pines' BIRD method[3] has been published.[12] Early pure shift DOSY experiments[9] used a stimulated echo sequence; here we show the results of applying the Oneshot-based[15,16] sequence of Figure 2 b to a mixture containing the partial-cone and cone calix[4]arenes of Scheme 1 at 2.0 and 2.6 mM and the parent, unsubstituted, calixarene at 6.8 mM. Figure 3 shows that pure shift DOSY allows complete resolution of the substituted from the unsubstituted calixarenes, which differ in diffusion coefficient by 9 %; signal overlap prevents such a resolution in a normal Oneshot experiment.

Pure shift methods can, as these spectra illustrate, offer a major improvement in the resolution of proton NMR spectroscopy; it is straightforward to recover multiplet

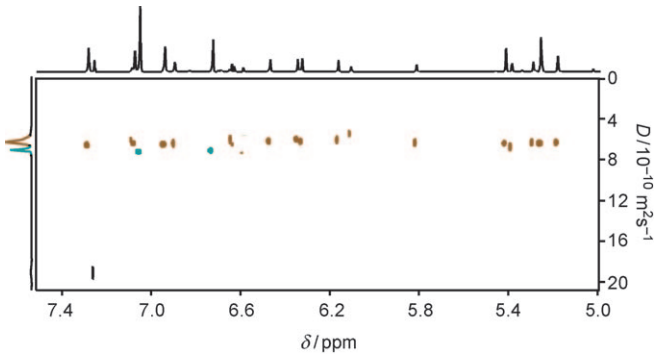

Figure 3. Pure shift DOSY spectrum of a mixture containing 2.0 mM and 2.6 mM, respectively, of the cone and partial-cone conformers of Scheme 1 (brown) and 6.8 mM of unsubstituted calix[4]arene (green) in $CDCl_3$ (residual $CHCl_3$ signal in blue), for the region 5–7.5 ppm, measured using the sequence of Figure 2 b. Apparent diffusion coefficients D were corrected for the experimentally determined spatial variation of gradient strength.[9,16]

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structure from an optional second dimension, so no information is lost. They allow individual chemical sites to be identified in complex species, and greatly extend the range of applicability of DOSY, in which high resolution in the diffusion dimension is predicated on fully resolved signals in the spectral dimension.

Experimental Section

Experiments were run at ambient temperature on a Varian VNMRS 500 spectrometer equipped with a triple resonance probe and a z gradient coil giving a maximum gradient of 66 G cm^{-1}. The sequence of Figure 2a used an rsnob selective 180° pulse[17] of duration 61.7 ms and bandwidth 30 Hz under a slice-selection gradient G_{sl} of 0.33 G cm^{-1}; 23 t_1 increments of 4 transients of 0.68 s each were acquired with $1/SW_1 = 21$ ms and a relaxation delay of 1 s, in a total time of 2.8 min. Coherence transfer selection gradient pulses were rectangular with width 0.5 ms and amplitudes G_a, G_b, and G_c of 5, −5, and −10 G cm^{-1}; the delays τ_a and τ_b were 2.5 and 2.0 ms, respectively.

DOSY data were acquired with the sequence of Figure 2b using a 37 ms rsnob pulse with 50 Hz bandwidth, with $G_{sl} = 0.18$ G cm^{-1}; 32 t_1 increments of 4 transients of 3.7 s each were acquired with $1/SW_1 = 21.7$ ms in a total time of 2.8 h. Coherence transfer selection gradient pulses were rectangular with width 1 ms and amplitudes G_a, G_b, and G_c of 20, −20, and −40 G cm^{-1}; the delays τ_a and τ_b were 3.0 and 2.0 ms, respectively. Diffusion-encoding gradient pulses were of 1 ms with levels quadratically spaced from 10 to 50 G cm^{-1}, with 0.5 ms stabilization delays and a 0.11 s diffusion time Δ.

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