High-precision heteronuclear 2D NMR experiments using 10-ppm spectral window to resolve carbon overlap†

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The acquisition of a complementary heteronuclear 2D NMR experiment with 10-ppm carbon window allows chemists to improve by a factor 20–25 the spectral resolution and determine carbon chemical shifts with five figures from 2D spectra.

Heteronuclear ^{1}H–^{13}C NMR experiments usually have low signal resolution in the carbon-13 dimension. When a 250-ppm HSQC or HMBC spectrum is acquired using 512 time increments signals have a typical width as large as 1 (ppm) in the carbon dimension. The measured chemical shifts have four significant figures among which one is to the right of the decimal separator (see Fig. 1(a)) while 1D carbon spectra provide five figures. Close pairs of carbons are therefore unresolved making it difficult or impossible to assign signals. This is quite likely to occur in the study of mixtures of isomers, oligomers, polyketals, *etc*. but can also happen with the simplest molecules. Sophisticated NMR techniques[1] reduce the acquisition time of high-resolution experiments but most are not easily applicable for routine NMR use.

Increasing the resolution in the carbon dimension by a factor 20–25 with no significant increase in the acquisition time is possible by reducing the spectral width. This increases the duration of the t_1 increments and causes "spectral aliasing", the phenomenon making signals outside the spectral boundaries appearing as back-folded (or "aliased") into the smaller window (see Fig. 1(a)–(c)). This artifact is well known[2] and the power of computer optimization of spectral aliasing (COSA)[3] has been recently suggested to overcome ambiguities in chemical shifts. But two serious problems limit COSA to specialists or favorable situations. First, it requires a carbon (or DEPT) spectrum, a correct peak picking and running a program to optimize the spectral width. Berger and co-workers[4] proposed a solution to cope with this difficulty but it requires a special processing software to extract chemical shifts. The second problem is that the software used to visualize spectra has to be adapted so that the true chemical shifts of aliased signals can be determined.

Using 10-ppm spectra avoids all these problems, and requires no data processing. Determining the correct chemical shifts in aliased spectra is straightforward, thanks to a property of the modulo function. When looking at the spectra of 10-ppm scales, one can simply rely on one digit on the left of the period and two on the right (see Fig. 1(c) and (d)) and must ignore the first two digits of the observed chemical shift (in gray in Fig. 1(c)).

The combination of the values observed in the full-width and the 10-ppm spectra generates carbon chemical shifts with five digital figures. Pairing the signals is obvious even if signals accidentally overlap in the 10-ppm spectrum. The criteria are (1) equal proton chemical shifts and (2) equal values of the digits just before and after the period in the carbon dimension. When available, one can use 1D carbon or DEPT spectra in a verification process. The choice of 512 time increments (TD) for the full spectrum experiments is justified by the need to have sufficient redundancy in the carbon-13 chemical shift values measured in the two spectra. When applied to small molecules, the uncertainties for 512 increments are

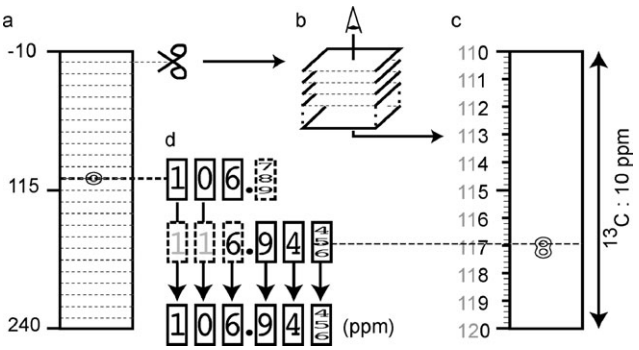

Fig. 1 A 10-ppm spectrum (c) corresponds to a normal spectrum (a) cut into 10-ppm broad stripes that overlap (b) except that signals are nearly 25 times narrower compared to a normal full 250-ppm spectrum. (d) The four digital figures of the chemical shift of the full spectrum (a) can be combined with the partially complementary four figures of the 10-ppm spectrum (c) to provide precise carbon chemical shifts (bottom). Signals overlapping in (a) may be resolved in (c). For the sake of clarity, the signals are only schematized above. When acquired with the recommended 512 increments, signals are narrower than drawn here.

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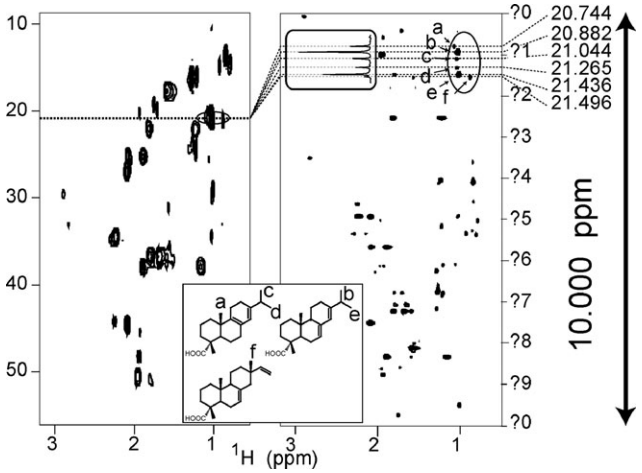

Fig. 2 Comparison of the full (left) and 10-ppm (right) HSQC spectra of a sample of pine resin in $CDCl_3$. The six signals in the ellipses overlap in the full spectrum but are clearly resolved in the 10-ppm spectrum. The top inset shows that the resolution in the 10-ppm spectrum with 512 increments is close to that of the 1D DEPT spectrum processed with 1 Hz line broadening. Only the diterpenes with signals in the ellipse are shown. The question marks indicate unknown figures.

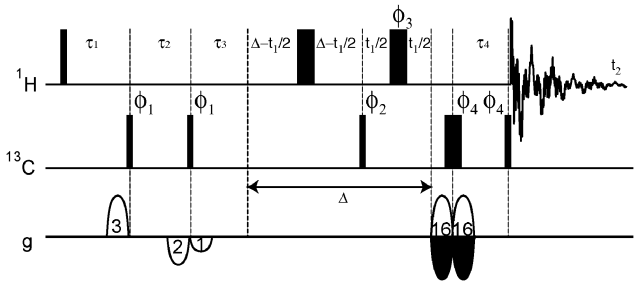

Fig. 3 The HMBC pulse sequence combines the phase-sensitive HMBC developed by Cicero *et al.* with the constant-time sequence of Furihata *et al.* Narrow and broad lines refer to 90 and 180° pulses respectively: Δ = TD × $T_{1inc}/2$; $\tau_1 = 1/2J_{CHmin}$, $\tau_2 = 1/2J_{CHmax}$, $\tau_3 = 1/2^nJ_{CH}$ = 65 ms, τ_4 insures equal times between the π pulse and the two π/2 pulses in the carbon channel. The phases of the pulses are $\phi_1 = 2 \times 0°, 2 \times 180°$; $\phi_2 = 0°, 180°$; $\phi_3 = 4 \times 0°, 4 \times 180°$; $\phi_4 = 8 \times 0°$, $8 \times 180°$; $\phi_{aq} = 4 \times (0°, 180°)$, $4 \times (180°, 0°)$ and 0° when not specified.

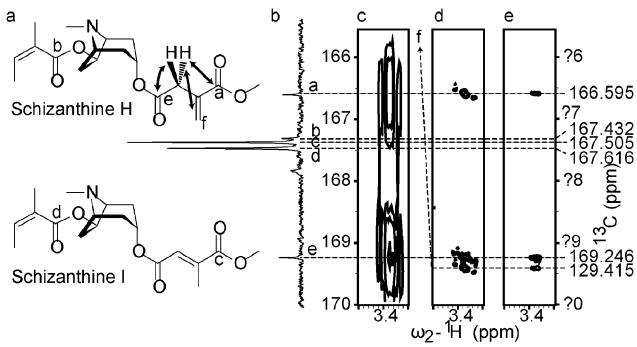

Fig. 4 (a) Two schizanthines of *Schizanthus grahamii*. The proton–carbon correlations (double arrows) correspond to the signal shown in the (c) full and (d, e) 10-ppm HMBC spectra of a sample dissolved in $CDCl_3$. Our new sequence (see Fig. 3) results in signals with less coupling structure (e) than the non-CT sequence (d). Note that carbonyls b–d have very similar chemical shifts and can only be resolved in the corresponding 10-ppm spectrum.

±0.05 and ±0.002 (ppm) in the full and 10-ppm spectra, respectively. When the spectra are expected to have sufficiently high signal-to-noise ratio for linear prediction[5] the number of time increments could be reduced to shorten the acquisition times. We illustrate herein the application of this methodology (without linear prediction) to HSQC and HMBC experiments separately.

The acquisition of 10-ppm HSQC spectra is straightforward. The standard experiments require only the carbon spectral width to be set to exactly 10 (ppm). When applied to a sample of pine resin containing eight diterpenes,[6] the 10-ppm spectrum resolves the overwhelming overlap of the full spectrum (see Fig. 2). When combined with the full and the 10-ppm HSQC-TOCSY and HMBC spectra (not shown) all signals of the two main components were assigned unequivocally and many fragments of the minor products could be characterized as well.

For long-range experiments we developed a new HMBC sequence (see Fig. 3) combining the phase-sensitive sequence of Cicero *et al.*[7] and the constant time HMBC developed by Furihata *et al.*[8] They were selected for the following reasons. Firstly, constant-time experiments have no coupling structures in the carbon dimension making it easy to identify the center of signals in crowded spectra. Secondly, the long t_1-evolution times of 10-ppm experiments are not gradient coded which avoids the signals of small molecules to be significantly damped due to molecular diffusion.[9] Thirdly, the quadrature detection in the carbon dimension results in higher signal-to-noise ratio because the constant time Δ has half the duration of single-channel sequences which gives relaxation less time to act. Finally, the small number of pulses results in a high signal-to-noise ratio even when experiments are acquired using a sample-changer, that is when the RF pulses are not calibrated for each sample.

We acquired the HMBC spectra of a sample containing two natural products extracted from the stem-bark of *Schizanthus grahamii*. The two alkaloids were difficult to separate by chromatography and could not be isolated in adequate quantity for NMR analysis. The 10-ppm HMBC spectra (see Fig. 4(d) and (e)) allowed unambiguous assignment of the critical carbonyl carbons (Fig. 4(b)) and permitted, for the first time, to prove the presence of schizanthine H and I[10] in *Schizanthus grahamii*.

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