

**NMR Spectroscopy**

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**Acquisition Regime for High-Resolution  
Heteronuclear 2D NMR Spectra**

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Two-dimensional NMR techniques are normally based on the detection of a set of one-dimensional spectra using a pulse sequence with an incremented delay within the evolution time.<sup>[1]</sup> These delays provide the second or indirect dimension and are incremented so that the set of time increments allows the sampling of all frequencies in the indirect dimension. The

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number of time increments and thus the resolution in the indirect dimension are always limited. The acquisition of heteronuclear 2D NMR spectra is time consuming, while their resolution is very poor when using the full spectral width in the indirect dimension. Compared with the resolution of a routine 1D  $^{13}\text{C}$  NMR spectrum, for example, this can be regarded as insufficient.

The  $^1\text{H}$ ,  $^{13}\text{C}$  HSQC and HMBC techniques are currently the most important routine methods that provide information about one- and multibond connectivities.<sup>[2]</sup> The problem of increasing the resolution in the indirect dimension without increasing the experiment time of a two-dimensional experiment is therefore of great importance. Significant solutions to this problem seem to belong to the realm of data handling using appropriate mathematical tools.<sup>[3]</sup> Covariance-based approaches<sup>[4,5]</sup> have been proposed for some homonuclear techniques which produce symmetric spectra. The methods suitable for high-resolution heteronuclear NMR spectroscopy,<sup>[3,6,7]</sup> such as Hadamard or filter diagonalization concepts, normally use different kinds of prior information about the chemical shifts in the indirect dimension and/or the number and shape of the peaks. An approach recently proposed by Jeannerat et al.<sup>[8,9]</sup> seems to be very promising within this context. It uses spectral aliasing as a means of reconstructing the full spectrum, when only a narrow band of the latter is physically acquired.

Unfortunately this method in its published form is also limited to the case when all frequencies of the  $^{13}\text{C}$  NMR spectrum are known beforehand. It then becomes possible to optimize the conditions for acquiring the aliased 2D spectrum. This is, however, not possible when working with an unknown substance, since there might be insufficient material available to acquire a  $^{13}\text{C}$  NMR spectrum with a significant signal-to-noise ratio in an acceptable time. Herein, we discuss a more general use of an aliasing technique to obtain HSQC- and HMBC-like spectra with high resolution in the  $^{13}\text{C}$  dimension but with no prior information on the  $^{13}\text{C}$  chemical shifts.

By aliasing one considers the appearance of artefact signals in the spectrum sampled with a too-narrow spectral width. Whereas this is suppressed on recent NMR spectrometers by filter techniques in the direct dimension, we will use this here in the indirect dimension. If a signal with the apparent chemical shift  $\delta_{\text{apparent}}$  is found within a too-narrow spectral width  $\text{SW}$  in the indirect dimension, the genuine value of the chemical shift  $\delta_{\text{genuine}}$  can be expressed as Equation (1), where  $k$  can be any arbitrary natural number.

$$\delta_{\text{genuine}} = \delta_{\text{apparent}} \pm k \times \text{SW} \quad (1)$$

Jeannerat et al. have proposed using the aliased spectra recorded in the indirect ( $^{13}\text{C}$ ) dimension under optimized conditions, thus allowing  $k$  to be determined unambiguously. The use of spectral widths of, for example, 1 ppm instead of the 150–250 ppm needed to span the whole carbon spectrum allows the resolution to be improved dramatically.

Instead of using a priori information about  $^{13}\text{C}$  chemical shifts, we propose here to acquire spectra with different SW values, for example, two— $\text{SW}_1$  and  $\text{SW}_2$ . At first, we assume a

single chemical shift in the  $^{13}\text{C}$  NMR spectrum corresponding to a given signal in the  $^1\text{H}$  direction as it is valid for HSQC. If the condition shown in Equation (2) is fulfilled for all possible  $k_1$  and  $k_2$  values within the full genuine spectral width  $\text{SW}_{\text{full}}$ , it becomes possible to unambiguously find the  $\delta_{\text{genuine}}$  value in Equation (1) from the two  $\delta_{\text{apparent}}$  values.

$$k_1 \times \text{SW}_1 \neq k_2 \times \text{SW}_2 \quad (2)$$

Such  $\text{SW}_i$  values that satisfy both Equation (2) and the intention to minimize the spectral width, and thus improve the resolution, can be chosen as follows. If one considers the  $\text{SW}_{\text{full}}$  value, given in ppm, and a desired resolution  $R$ , given in ppm per point, one can define the number of resolution points  $Z = \text{SW}_{\text{full}}/R$ , for example,  $Z = 150 \text{ ppm}/0.2 \text{ ppm per point} = 750$  points.

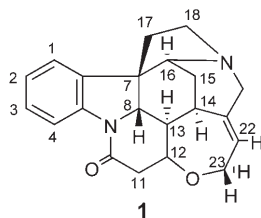
The numbers of the chosen points  $Z_1$  and  $Z_2$  that fulfil the condition  $k_1 Z_1 \neq k_2 Z_2$  must be larger than  $Z^{1/2}$ , since the product  $Z_i \times Z_j$  should be larger than  $Z$  to provide unequivocal aliasing. The easiest way to ensure the absence of common multiples less than  $Z$  is to choose  $Z_i$  and  $Z_j$  as prime numbers. In our example, these can be the numbers 29 and 31. For HSQC or HMQC type spectra, where each proton signal is only connected to one  $^{13}\text{C}$  frequency, two different  $Z_i$  values are sufficient and this provides an elegant option of obtaining HSQC spectra in very high resolution, as is required in the field of residual dipolar couplings.<sup>[10]</sup>

The situation is more complex for HMBC spectra, since each proton signal may be connected to several  $^{13}\text{C}$  frequencies. The number of HMBC spectra to be recorded in the indirect dimension with different spectral widths should be equal to  $N + 1$ , where  $N$  is the maximum number of expected correlations between one proton and the carbon atoms. The same principle should be used when an aliased HSQC spectrum exhibits additional peaks for a single proton frequency because of a strong overlap of protons. From our experience,  $N = 6$  is sufficient in most HMBC spectra. For the case  $\text{SW}_{\text{full}} = 200\text{--}230 \text{ ppm}$  and a desired resolution of 0.2 ppm, one can choose the prime numbers 37, 41, 43, 47, 53, 59, and 61. In our method these numbers directly serve as time domain (TD) points. Multiplication with the desired resolution  $R$  (0.2 ppm per point) yields the spectral widths  $\text{SW}_i$  of 7.4, 8.2, 8.6, 9.4, 10.6, 11.8, and 12.2 ppm. Thus, the seven HMBC spectra are recorded with a total number of 341 time increments.

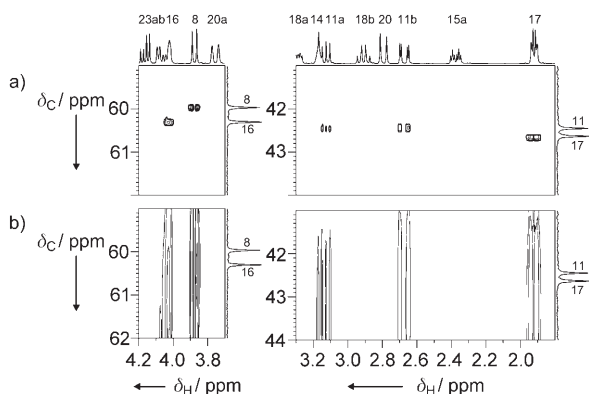
The spectra were reconstructed using a Matlab routine, which is available from the authors. It works as follows: Within each of the  $N + 1$  spectra, for each proton frequency, only the  $N$  most intensive maxima are considered as significant. The significant signal magnitudes are regarded as corresponding to all possible values of the hypothetical  $\delta_{\text{genuine}}$  value found by Equation (1) for all values of  $k$ . So each spectrum is “unfolded” from its  $\text{SW}_i$  to the  $\text{SW}_{\text{full}}$ . The geometric means of the magnitudes taken in the same points of the  $N + 1$  “unfolded” spectra produce the final reconstructed spectrum and remove artifacts. The final spectrum exhibits peaks in the points where the maxima in different individual “unfolded” spectra coincide. Zero-filling to 128 points in the  $^{13}\text{C}$  dimension resulted in different digital

resolution, which was subsequently corrected by the spline interpolation within our routine.

The reconstructed spectra were reimported into the Bruker Topspin software and further processing was performed there. Figure 1a shows some expansions of the reconstructed HSQC spectrum of strychnine (**1**) which



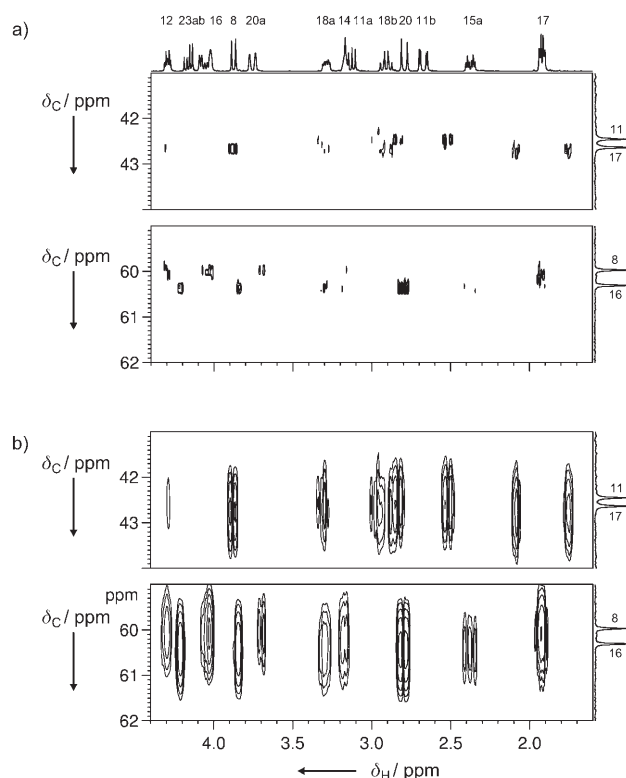
correspond to the two pairs of the most closely resonating  $^{13}\text{C}$  atoms, and Figure 1b shows the same expansions of the HSQC spectrum acquired within the same total experimental



**Figure 1.** Expansions of the HSQC spectrum of strychnine (**1**) for the regions  $\delta_{\text{C}} = 41\text{--}44$  ppm and  $\delta_{\text{C}} = 59\text{--}62$  ppm: a) Spectrum reconstructed from two recordings with SW = 5.8 ppm (TD = 29) and SW = 6.2 ppm (TD = 31) and interpolated to a digital resolution of 0.05 ppm per point; b) the conventional magnitude-mode HSQC recorded with TD = 60 within the full  $^{13}\text{C}$  spectral width of 150 ppm. The corresponding regions of the  $^1\text{H}$  and  $^{13}\text{C}$  spectra are shown along the axes.

time for the full spectrum without aliasing. The same regions of the reconstructed long-range HSQC (HMBC-like) spectrum versus the conventional long-range HSQC spectrum acquired within the same time (TD = 341) are shown in Figure 2a and b, respectively.

When any of the HMBC standard pulse sequences is used, the modulation by proton–proton spin coupling in the indirect dimension becomes clearly visible at this resolution. A variant of long-range HSQC<sup>[11–13]</sup> should be used instead. One developed from the approach of Ref. [11] (see the Supporting Information) has provided HMBC-like, but distortionless spectra. The magnitude-mode variants of the pulse sequences



**Figure 2.** Expansions of the long-range HSQC spectrum of strychnine (**1**) for the regions  $\delta_{\text{C}} = 41\text{--}44$  ppm and  $\delta_{\text{C}} = 59\text{--}62$  ppm: a) Spectrum reconstructed from seven long-range HSQC spectra recorded with different SW and TD values (see text) corresponding to a resolution of 0.2 ppm per point and interpolated to 0.1 ppm per point; b) The conventional magnitude-mode long-range HSQC spectrum recorded over the same time. The corresponding regions of the  $^1\text{H}$  and  $^{13}\text{C}$  spectra are shown along the axes.

were used throughout since our post-processing does not retain the phase information.

The reconstructed spectra exhibit all the peaks of the HSQC and HMBC spectra of strychnine. The digital resolution achieved through spline interpolation actually comprised 5 Hz per point for the HSQC spectrum and 10 Hz per point for the long-range HSQC spectrum, and allowed the closest  $^{13}\text{C}$  resonances to be distinguished. The sensitivity is reduced because of the very high splittings, according to the principle of “exclusive correlation spectroscopy” (E. COSY),<sup>[14]</sup> which is visible only in high resolution. For a comparison with the conventional spectrum and a discussion of the relative signal to noise ratios see the Supporting Information.

In conclusion, we have demonstrated here a new acquisition technique<sup>[15]</sup> for 2D NMR spectra that provides a significantly better resolution within the same given experimental time. Further developments will aim to fully reconstruct 1D  $^{13}\text{C}$  NMR spectra by using this approach.

## Experimental Section

The spectra of a 3% (w/v) solution of strychnine in  $\text{CDCl}_3$  were acquired on a Bruker Avance DRX400 spectrometer fitted with a

5 mm inverse probe head. For the pulse sequence used and spectrometer details see the Supporting Information.

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