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1D NMR Homodecoupled ^{1}H Spectra with Scalar Coupling Constants from 2D *NemoZS*-DIAG Experiments**

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In memory of Detlef Moskau

Abstract: *A two-dimensional liquid-state NMR experiment cleanly separating chemical shifts and scalar couplings information is introduced. This DIAG experiment takes advantage of a drastic reduction of the spectral window in the indirect dimension to be quickly recorded and of a new non-equidistant modulation of the selective pulse to improve the sensitivity of the broadband homodecoupling Zangger–Sterk sequence element by one order of magnitude. A simple automatic analysis results in 1D spectra displaying singlets and lists of the scalar couplings for first-order multiplets. This facilitates the analysis of 1D spectra by resolving multiplets based on their differences in chemical shifts and coupling structures.*

The complexity of 1D ^{1}H spectra is a long-standing problem in many liquid-state NMR applications. This is mostly because of the presence of scalar coupling making it difficult to identify the individual resonances because the multiplet structure causes serious signal overlap (see Figure 1 a). Ideally, these complex multiplets should be separated in an additional dimension. If the classic *J*-resolved experiment was close to this goal,[1] problems with the signal lineshape and the complexity of the multiplet structures in the presence of second-order effects found partial solutions[2] but not satisfactory enough to become generally applicable. One solution consists in taking advantage of the large chemical shift dispersion of ^{13}C and use decoupled HSQC[3] or more recent variants[4] of this experiment to resolve signals and facilitate the determination of coupling constants.

We developed an approach based on a 2D homonuclear sequence (see Figure 2) producing spectra where multiplets that are overlapping in 1D spectra (Figure 1 a) are spread along the indirect dimension (Figure 1 b). We called this experiment "DIAG" because it generates signals only along the diagonal (dashed line in Figure 1 b). Parallels could be drawn with earlier attempts to exploit the diagonal of COSY spectra.[5] But our approach, which is based on in-phase magnetization, avoids the difficulties of dealing with the numerous anti-phase magnetizations and the risk of signal cancellation because of the presence of positive and negative peaks. In order to simplify the structure of signals, the indirect F1 dimension is decoupled using the Zangger–Sterk sequence element (ZS)[6] because the constant-time incrementation used in many NMR pulse sequences cannot be applied to high-resolution homonuclear ^{1}H experiments.

The first part of the *nemoZS*-DIAG sequence (see Figure 2) is quite similar to the pure shift experiment.[7] But instead of using the ZS element to generate series of spin echos, it is used to decouple only the indirect dimension of a 2D spectrum. In this manner, the precious scalar coupling information is retained in the detected dimension. The δ-resolved[8] experiment developed by Giraud et al. was aiming at the same goal, but it is based on version of the ZS sequence using more than one selective pulse which is detrimental. Top-

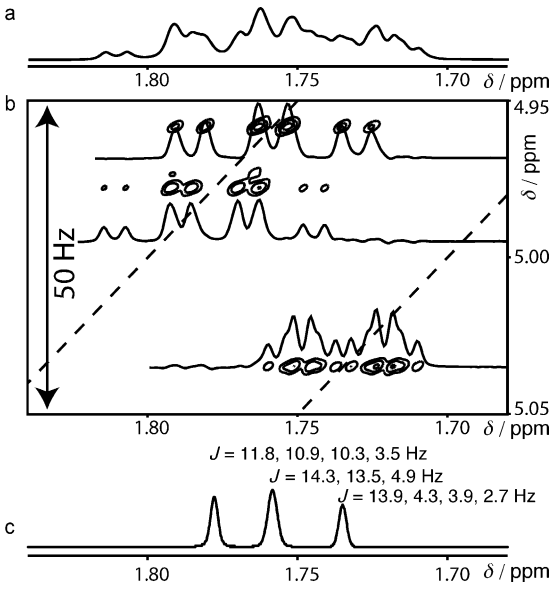

Figure 1. a) The overlap of the multiplets of three protons of androst-4-ene-3,17-dione makes this region of a normal 1D ^{1}H spectrum poorly characterized as "3H, *m*". b) In the 2D *nemoZS*-DIAG spectrum, the three multiplets of protons 8β, 1α, and 11α are clearly resolved along the zigzagging diagonal (dashed line). They can be easily analyzed to determine chemical shifts and coupling constants. c) The annotated reconstructed spectrum contains all the information of the multiplets: chemical shifts, scalar couplings and, after normalization with a 1D spectrum, semi-quantitative integrals.

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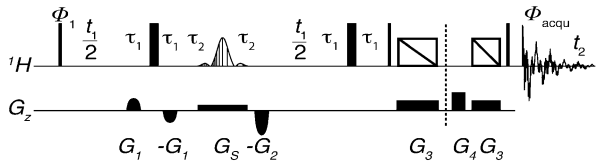


Figure 2. Pulse sequence of the *nemoZS*-DIAG experiment. Hard 90° and 180° pulses are shown with narrow and broad lines, respectively. The t_1 evolution time contains the Zangger–Sterk element using a non-equidistant modulated rsnob selective pulse. The DIAG mixing time includes a *z* filter using two chirp pulses represented with slashed rectangles combined with weak pulsed field gradients. All pulses have *x* phases. A two-step phase cycling alternates the signs of Φ_1 and Φ_{acqu}.

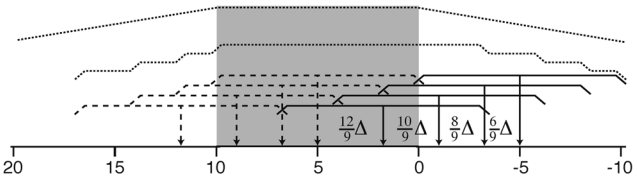


Figure 3. Distribution of the irradiation sites of the non-equidistant modulation (*nemo*) scheme. Two identical sets of irradiation sites (continuous and dashed vertical arrows) combined with an encoding gradient covering 10 ppm insure a homogeneous coverage (constant level of the dotted line) between 0 and 10 ppm (gray area). For clarity two sets of four irradiations are shown here (stepped dotted line) but typical applications use 2×40 to 2×60 tending to the coverage of the top dotted line.

quality spectra were obtained with ZQ-filters eliminating multiple-quantum artifacts.[9]

The new sequence can also be considered as a 2D TOCSY out of which the mixing element has been eliminated. In the absence of magnetization transfer during the mixing, the spectra contain no cross-peak and the diagonal signals are decoupled in the indirect F1 dimension[6] with the implementation of the ZS element using a selective pulse after the hard 180° pulse.[10]

An important drawback of ZS experiments is their low sensitivity. The presence of a pulsed field gradient during the selective radio-frequency pulse makes that only a small slice of the sample volume produces useful signal. For optimal sensitivity, one should therefore cover a small spectral domain and use short selective pulses to increase the size of the slices,[6] with the risk of causing signal artifacts because of a lack of selectivity (see the discussion and Figure S1 in the Supporting Information).

We recently addressed this problem using a modification of the ZS element where the selective pulse was applied simultaneously to *n* frequencies separated by a constant distance (see Figure S2).[11] This method increases the sensitivity by a factor *n* provided that pairs of coupling partners are not accidentally distant by the distance between the irradiation sites (Δ in Figure S2b). Applications using 10–50 irradiations worked quite well on the carbon spectra of ^{13}C-enriched compounds, but the probability of accidental selection of pairs of coupled spins is much higher in ^{1}H NMR spectroscopy and resulted to artifacts (see Figure S3). One solution would consists in avoiding this situation and generating lists of frequencies tailored to a specific sample,[12] but we preferred to face the problem in the perspective of a generally applicable method. We therefore introduce here an alternative scheme of modulation of the selective pulse where the modulation sites are non-equidistant. (Figure 3) It maintains the sensitivity of the modulated ZS but results in a drastic reduction of the artifacts (see Figure S3). Instead of adding up coherently for each spacial origin of the signal when the constant Δ is unfavorable, artifacts will differ for each irradiation site and average out when Δ is spread over a range of different values. This sequence element was called "*nemoZS*" for non-equidistantly modulated Zangger–Sterk. It can be systematically applied to the full 10 ppm domain of the ^{1}H spectrum.

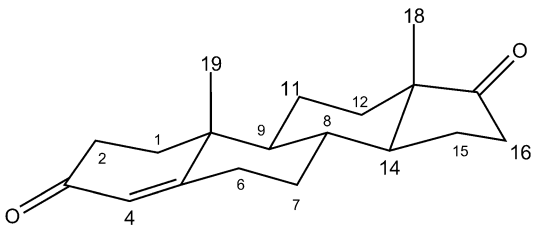
A typical application uses two sets of 50 modulations of 120 ms rsnob pulses[13] which increases the signal intensities by factors of 15–45. This is higher than any reported application of the ZS element and outperforms the recently introduced PSYCHE[14] decoupling scheme recorded with a 10° chirp pulses to avoid artifacts (see the discussion on the sensitivity in the Supporting Information).

High resolution in the decoupled dimension is essential to take advantage of the potential of the *nemoZS*-DIAG experiment. The indirect dimension was therefore recorded with a spectral window of 50 Hz, which corresponds to a 100-fold spectral aliasing[15a,b,c] at a 500 MHz ^{1}H Larmor frequency. The chemical shifts of the diagonal signals being measured in the direct dimension, the usual problem with chemical shift ambiguities caused by aliasing is simply avoided. The 2D experiment can therefore be recorded in a few minutes and results to signals with a typical width at half height close to 1 Hz. The quality of the spectra (Figure 1b and Figure 3a) is excellent making it easy for a computer program to automatically reconstruct a "decoupled" 1D spectrum and provide a list of the scalar couplings in first-order multiplets (see Figure 1c and Figure 3c).

We demonstrated the potential of "*nemoZS* DIAG" spectra for fruitful automated analysis using a simple processing program analyzing the multiplet structure to extract coupling constants and reconstruct a semi-quantitative decoupled 1D spectrum. It was applied to androst-4-ene-3,17-dione (see Scheme 1), a steroid displaying second-order effects, signal overlaps, and a complex coupling network including many protons coupling to five different partners.

The results of the analysis of the *nemoZS*-DIAG spectrum are shown in Figure 4 and coupling constants displayed in a graphical manner to facilitate their apprehension and comparison (see Figure S6 for a color-coded representation of the coupling constants). Homodecoupling was particularly useful to compress multiplets typically extending over more than 30 Hz to 1.2 Hz along the indirect dimension. The resolution of the two nearly equivalent protons at 2.03 ppm is particularly satisfactory (see the inserts at the top of Figure 4) because they were discriminated thanks to their different coupling patterns.

Signal assignment was clearly facilitated by the availability of the coupling constants. Knowing that all couplings above 9 Hz are geminal (12–20 Hz in the case of androsten) or *trans* diaxial (9–15 Hz), the visual inspection of the couplings



Scheme 1. Structure and carbon numbering of androst-4-ene-3,17-dione.

immediately identifies the axial protons. Pairing coupling partners based on equal values of the coupling constants is not very reliable except when the coupling is special as for $^{2}J_{16\alpha,16\beta} = 19.4$ Hz because of their α position relative to the carbonyl group. On the other hand, a comparison of the coupling constants with the results of DFT/GIOA calculations (or other method predicting chemical shifts and scalar coupling constants) is quite informative (see Figure S6). Usually, the assignments solely based on predicted chemical shifts are not conclusive, but including the scalar coupling constants makes the assignment of androsten possible without any additional spectrum such as 2D COSY or HSQC.

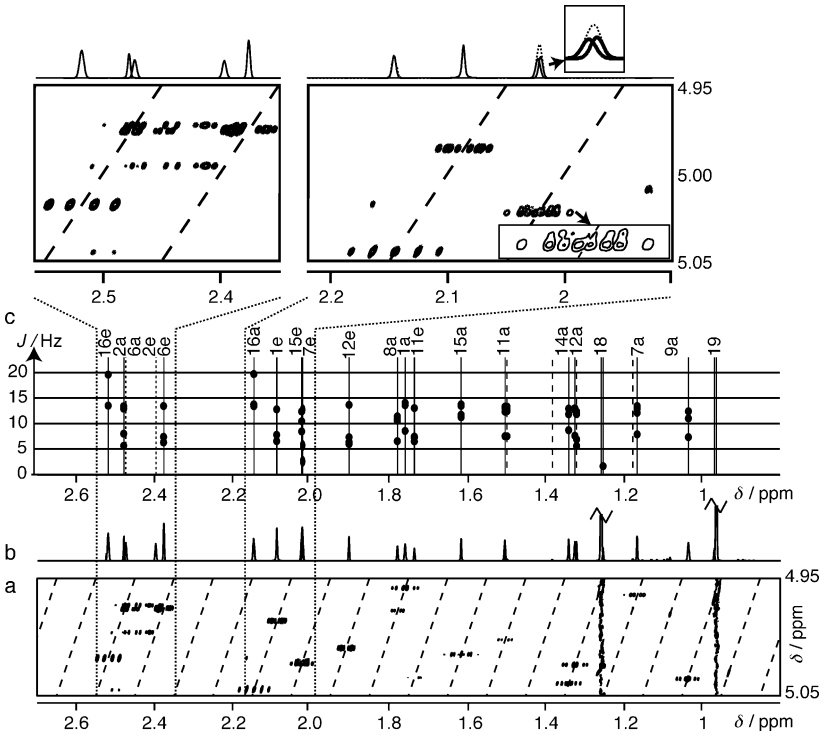


Figure 4. a) Spectrum of androsten obtained using the *nemoZS*-DIAG experiment with two sets of 50 non-equidistant modulations over 10 ppm. b) Singlets resulting from the analysis of the 2D spectrum. c) Graphical representation of the coupling constants. The dotted lines correspond to signals for which coupling constants could not be determined (only for the strongly coupling protons 2α and 2β). Dashed vertical lines indicate locations where small signals were identified and produced very small signals in (b).

Beside the production of homodecoupled 1D ^{1}H spectra, the ability of *nemoZS*-DIAG spectra to provide a reliable source of ^{1}H-^{1}H scalar coupling constants may be quite beneficial in the perspective of an automated analysis of complex NMR spectra. The *nemoZS* sequence element can be applied to 2D-TOCSY[16] and other NMR experiments and the DIAG approach used with other decoupling scheme.

Keywords: homonuclear decoupling · NMR spectroscopy · scalar coupling constants · structure elucidation

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