Adiabatic *z*-filtered *J*-spectroscopy for absorptive homonuclear decoupled spectra

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ABSTRACT

J-resolved NMR spectroscopy is a well-established method for measuring coupling constants or enhancing the resolution in homonuclear experiments. Classical *J*-spectra typically suffer from inevitable dispersive signal contributions and the occurrence of "ghost peaks" due to strong coupling effects.

In this article, a *J*-resolved-type experiment with an adiabatic *z*-filter element for the selection of inphase magnetization and quadrature detection in the indirect dimension is presented. The phase-sensitive recording scheme allows the distinction of strong coupling artefacts from symmetry relations and the full multiplet obtained by the experiment can be reduced by a simple pattern recognition algorithm. In addition to the *J*-resolved experiment, a second experiment is introduced that correlates strongly coupled spins. The possibility of homonuclear decoupling using the *J*-resolved experiment with the pattern recognition algorithm is demonstrated on a number of samples with a detailed description of potential strong coupling artefacts.

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1. Introduction

J-resolved spectroscopy or simply *J*-spectroscopy is a well-known NMR method that was introduced in the early seventies [1–3]. It allows precise measurement of coupling constants by refocussing field-inhomogeneities and can be used for homonuclear decoupling [4]. Recent applications can be found e.g. in the identification and characterization of metabolites [5,6], the measurement of one-bond heteronuclear couplings in large macromolecules [7,8], the increase in resolution in DOSY-type experiments [9], or the distinction of enantiomers in chiral alignment media [10,11]. One of the major drawbacks of conventional, homonuclear *J*-spectroscopy is the presence of dispersive antiphase contributions in the resulting spectra that prevent a phase correction into absorptive lineshapes. To circumvent this problem, data are usually presented by the absolute value of the complex spectra, retaining broadened signal feet due to dispersive contributions. Tilting the spectra with subsequent application of symmetrization algorithms helps to increase the spectral quality, but especially for samples with a high dynamic range signal overlap caused by broad magnitude lineshapes poses severe problems in the spectral read-out. Modified experiments that allow the generation of absorptive *J*-spectra are therefore of general interest.

A number of different approaches have been reported that in principle allow the construction of homonuclear decoupled spectra in absorption based on *J*-spectroscopy: Special processing techniques like the so-called "pseudo echo" [12] or the regularized resolvent transform [13] result in absorptive decoupled spectra, but relative intensities of the resulting signals are modified and ghost peaks originating from strong coupling artefacts cannot be distinguished; another, very elegant and highly sophisticated approach by Zangger and Sterk [14] uses simultaneous selective decoupling of all resonances by a frequency selective pulse while applying a pulsed field gradient. The resulting absorptive spectra retain the typical *J*-resolved cross peaks with multiplets tilted by 45° along the antidiagonal. The technique has been developed further recently by the groups of Keeler [15] and Morris [9]. The main disadvantage of the method is the significantly reduced signal intensity imposed by the decoupling scheme employed; a third type of effective homonuclear decoupling which was developed step by step by the Freeman group [16–19] is based on the acquisition of spectra that contain the full, pure phase 2D multiplets and the subsequent multiplet reduction into singlets using pattern recognition algorithms.

Based on this last approach it will be shown here that pure phase 2D spectra can be obtained by quadrature detection in the indirect dimension with a single scan per t_1-increment using a recently published adiabatic *z*-filter scheme [20,21]. Within the range of effective zero quantum suppression, resulting spectra can be decoupled with a simple pattern-recognition algorithm similar to previously published ones. Furthermore, we show that although strong coupling artefacts in the resulting spectra cannot be avoided at least the unambiguous identification of strongly coupled spins is straight-forward with the presented methods. Achievable spectral quality for the experiments are demonstrated using different test samples, including uniformly ^{13}C-labeled glucose.

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2. Theory

While pulsed field gradients effectively suppress most coherences, they do not affect homonuclear ZQ coherences. Phase cycling, on the other hand, can effectively remove ZQ coherence pathways, but all other z-order terms with coherence order 0 will be removed as well [22]. A z-filter with effective removal of ZQ coherences but maintenance of z-order terms was first introduced in [23] and is based on the sum of spectra with different effective ZQ-evolution periods. This original method has the disadvantage that a good removal of ZQ coherences involves the acquisition of a relatively large number of scans per increment.

The z-filter scheme introduced by Thrippleton and Keeler [20] overcomes this problem in a very elegant way: while a gradient is used for an offset distribution over the sample volume, the offset dependent trajectory of a simultaneously applied adiabatic inversion pulse creates different ZQ-evolution periods in a single scan, leading to an effective cancellation of ZQ coherences. The principle is nicely summarized in [24] and it has been shown that the application of cascaded ZQ suppression schemes leads to even improved spectral quality [21]. It has been applied in many experiments for spectral cleanup like in NOESY/TOCSY experiments [20,25], the z-COSY [26], and heteronuclear experiments [27].

Depending on the length of the adiabatic pulse τ_f applied during the element, the intensities of residual ZQ coherences I_{ZQ} will survive according to [20]

$$I_{ZQ} = \frac{\sin(\omega_{ZQ}\tau_f)}{\omega_{ZQ}\tau_f} \tag{1}$$

with the corresponding characteristic ZQ frequency $\omega_{ZQ} = 2\pi(\nu_1 - \nu_2)$ of two spins participating in the ZQ coherence.

The z-filter scheme not only suppresses ZQ coherences but also all other coherences present. Only z-order terms like I_{1z} and $2I_{1z}I_{2z}$ etc. survive the pulse sequence building block. In J-spectra only single quantum coherences evolve during the t_1-evolution period, resulting in transfers of the form $I_{1y} \rightarrow a_1 I_{1y} - a_2 2I_{1x}I_{2z} - a_3 4I_{1y}I_{2z}I_{3z} + \dots$ with the corresponding trigonometric prefactors a_i. If a 90_x° pulse is applied after the J-evolution period, the corresponding terms will be transferred to $a_1 I_{1z} + a_2 2I_{1x}I_{2y} - a_3 4I_{1z}I_{2y}I_{3y} - \dots$. The subsequent application of the z-filter will suppress all coherences and only terms with z-order 1, e.g. I_{1z} for spin 1, will survive the element. These are ideal conditions for spectral cleanup within the limitations of the adiabatic z-filter scheme. Since the prefactor a_1 for I_{1z} in a weakly coupled spin system corresponds to $\sum_{i=1}^{n}\cos(\pi J_{1i}t_1)$, and the detected signal after the final 90° pulse is as well cosine-modulated by $\sum_{i=1}^{n}\cos(\pi J_{1i}t_2)$, the resulting signal contains the full absorptive, inphase multiplet in both dimensions.

Depending on the phase of the 90° pulse applied directly before the z-filter element, either I_y or I_x magnetization present after the J-evolution period can be selected in an experiment as shown in Fig. 1A. In addition to conventional J-resolved techniques, this allows the application of recording schemes like States [28], TPPI [29], or States-TPPI [30] for the acquisition of complex data in the indirect dimension. Although only I_y terms are expected in weakly coupled spin systems, it turns out that strong coupling artefacts can be identified using a phase sensitive scheme (see below).

3. Absorptive J-resolved-type spectra

As mentioned above, conventional magnitude-mode J-spectra result in broad signal feet. An example is shown in Fig. 2B for a spectral region of menthol with signals from methyl groups close to signals with approximately 10 times lower intensities: Severe overlap distorts the lineshapes of the smaller signals so that they
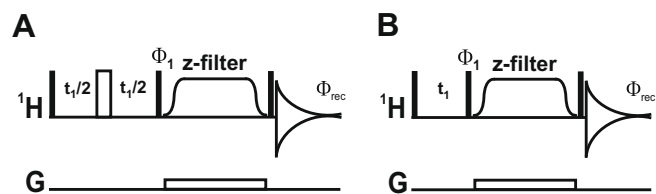

Fig. 1. Pulse sequences for adiabatic z-filtered J-spectroscopy (A) and correlation spectroscopy of strongly coupled spins (B). Filled, narrow bars and the open bar correspond to 90° pulses and a 180° pulse, respectively. The adiabatic z-filter consists of a CHIRP pulse of typically 30–50 ms duration with simultaneous application of a constant amplitude pulsed field gradient. For the setup of the inversion pulse and the corresponding gradient strength we refer the reader to the detailed instructions given in [20]. The phases Φ_1 and Φ_{rec} are incremented in States [28], TPPI[29], or States-TPPI [30] fashion to obtain quadrature detection in the indirect dimension.

can only be recognized at the edges of the multiplets with no intensive signals close-by.

An absorptive J-resolved-type experiment following the pulse sequence in Fig. 1A with incorporation of a z-filter is shown for comparison in Fig. 2C for the same spectral region of menthol dissolved in CDCl$_3$: Although the full multiplet patterns with increased overlap are present in the latter spectrum, signals in pure absorption mode show a significantly improved lineshape and allow the identification especially of low intensity multiplets. However, a reduction of complex multiplet patterns as with conventional J-spectra for simplified spectral interpretation is highly desirable. One way to achieve this is the application of a pattern recognition algorithm as derived in the following section.

4. Multiplet reduction

The multiplet reduction algorithm used previously for z-filtered J-spectra was based on the identification of the multiplet center and subsequent reduction of the expected peaks [19]. The aim of the algorithm introduced here, instead, tries to reduce the multiplets without a priori knowledge of the multiplet center. For this purpose two properties of the z-filtered J-resolved type experiment can be exploited: the center of any multiplet must always be the center in the J-dimension at frequency 0 Hz and the multiplet components are positioned symmetrically around the center of the signal in both dimensions. As long as we neglect strong coupling artefacts we further find a symmetry relation which implies that any eight peaks lying on a circle around the center of a multiplet should be of identical intensity (see Fig. 3A). It should be noted that these peaks also lie on a square around the same center.

Assuming equal resolution for both dimensions in the z-filtered J-resolved experiment (i.e. total acquisition times including zero-filling used for processing must be identical for the J-evolution period and the FIDs), the algorithm becomes quite simple, since the data describe an equidistant 2D grid where all eight peaks on the square (circle) can be directly addressed by the corresponding indices of the data matrix. In a defined sequence, the algorithm then constructs all possible eight positions with the desired symmetry conditions, subtracts the minimum signal height of these eight 2D data points from the original spectrum, and projects the signal intensities into a new data matrix at the chemical shift of the corresponding symmetry center of the square (circle) (Fig. 3B). The sum of all projections then leads to the "projected spectrum" and the sum of the projected spectrum along the J-dimension to the decoupled 1D-spectrum (Fig. 3C). The residual spectrum after application of the reduction algorithm contains all spectral features that do not fulfill the symmetry conditions of a weakly coupled multiplet.
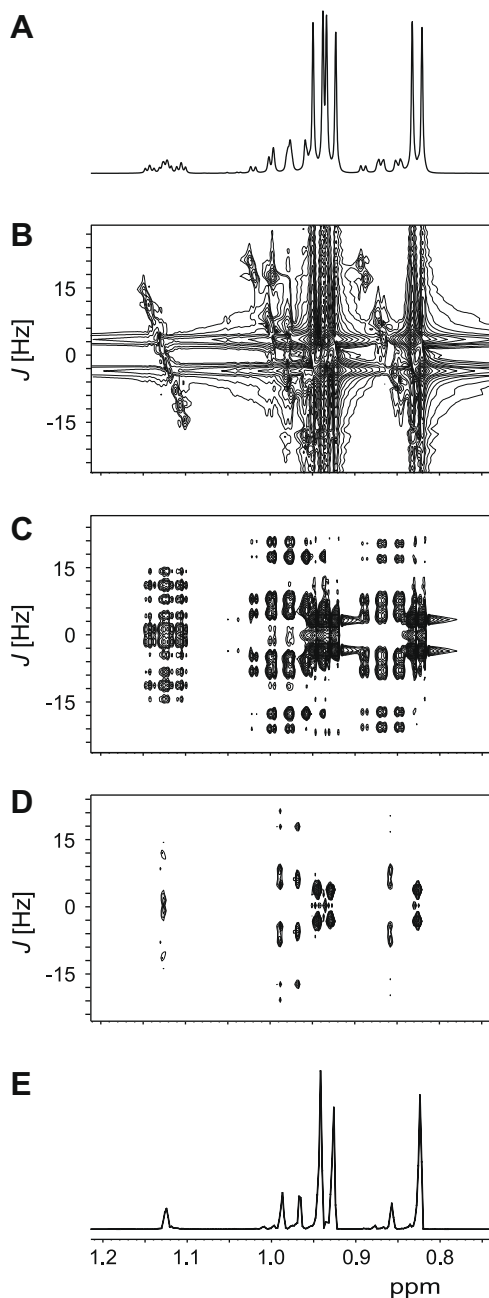
Fig. 2. ^{1}H 1D-spectrum (A), conventional J-spectrum (B), adiabatic z-filtered J-spectrum (C), the same spectrum after application of the multiplet reduction algorithm introduced in the main text (D), and the resulting effective homonuclear decoupled ^{1}H 1D-spectrum (E) for a spectral region of menthol dissolved in CDCl$_3$. Both J-spectra have been recorded with 2048 × 128 data points and a resolution of 1.17 Hz per point in both dimensions.

It should be noted that the sequence used for the construction of all possible symmetric data points is quite important. For a successful multiplet reduction the largest possible multiplet structures (corresponding to the largest indices of the data matrix in the J-dimension) must be tested first in order to avoid the mistaken subtraction of accidentally symmetric substructures (dashed arrows in Fig. 3A). For the same reason the symmetry centers have to be varied in a defined way, for example starting with the leftmost possible center and continuing to the right (dashed arrow with cross in Fig. 3A). Since all symmetric multiplet structures are then projected with the correct signal intensities, the resulting 2D projected spectrum and 1D decoupled spectrum have conserved integrals of the symmetric part of the original multiplets.

5. Strong coupling

In liquid state NMR spectroscopy two spins are usually coupled by a scalar coupling which *per se* has an isotropic secular coupling Hamiltonian

$$H_{iso}^J = 2\pi J \mathbf{I_1} \cdot \mathbf{I_2} = 2\pi J(I_{1x}I_{2x} + I_{1y}I_{2y} + I_{1z}I_{2z}). \qquad (2)$$

Since the zero quantum contribution to the Hamiltonian becomes non-secular in the presence of large chemical shift differences, the effective secular coupling contribution to the Hamiltonian can be approximated by $H_{eff}^J = 2\pi J\{I_{1z}I_{2z} + a(I_{1x}I_{2x} + I_{1y}I_{2y})\}$, with $0 < a \leqslant 1$. For weakly coupled spins with large chemical shift difference the zero quantum component of the coupling Hamiltonian is averaged out ($a \approx 0$) and the resulting multiplets are simply split by the coupling constant J. For small chemical shift difference (approximately $|\nu_1 - \nu_2| \leqslant 10J$), however, the secular zero quantum component cannot be neglected and the spins are in the so-called strong coupling limit of an AB-type spin system with $a > 0$.

The effective secular coupling Hamiltonian in this case resembles the one active during Hartmann–Hahn-type coherence transfer periods (ranging from coherence transfer under isotropic to cylindrical mixing conditions [31–36]), which, starting from I_{1x}, results in transfer to $I_{1y}I_{2z} - I_{1z}I_{2y}$ antiphase and I_{2x} inphase operators. A similar transfer can be seen in simulations using the full Hamiltonian including chemical shift terms: the Fourier-transformed spectrum of an FID with initial magnetization of spin A (spin B) in an AB spin system contains an antiphase and even a small inphase component at the chemical shift of spin B (spin A) and the combined spectrum shows the well-known roof effect as demonstrated by the simulations in Fig. 4A–C.

Correspondingly, in Fig. 4D, for example, a spectrum based on the strong coupling correlation experiment of Fig. 1B is simulated for an AB spin system: cross peaks with antiphase pattern along the multiplet antidiagonal are easily identified which connect the two individual spins. The result also explains strong coupling artefacts in J-spectra, which are simply the equivalent of the cross peaks observed in the chemical shift correlation experiment, just shifted in the indirect dimension due to the refocussed chemical shift in the J-spectrum (Fig. 4E). In this case, due to the 180° pulse in the center of the J-evolution period and the corresponding inversion of spin states, the 2D multiplet pattern of the cross peak shows all four components with antiphase in the J-dimension.

It is important to note that the strong coupling induced cross peak with the antiphase multiplet pattern does usually not fulfill the fourfold symmetry condition of the multiplet reduction algorithm and is easily identified in the residual spectrum after the algorithm is applied. Example cross peaks with moderate roof effect for demonstration are shown in Fig. 5 with the corresponding residual spectrum (the residual spectrum after subtraction of all fourfold symmetrical components by the multiplet reduction algorithm) in Fig. 5D, which proves the existence of strong coupling contributions to the multiplets. The signals of interest are still reduced to a singlet in the decoupled 1D spectrum without additional artefact (Fig. 5F), but since only the least intense signal of the multiplet is selected in the algorithm, a reduced signal intensity in the decoupled spectrum must be taken into account.

If more than two spins are strongly coupled or if the chemical shift differences of spins is on the order of their multiplet widths the situation becomes worse. In such a case the algorithm for multiplet reduction must fail for two reasons: firstly, the ZQ-suppression usually does not work properly in this case because of Eq. (1), and secondly, the symmetry relations of weakly coupled spins do not apply anymore and cannot be separated for spin systems with such significant strong coupling contributions.
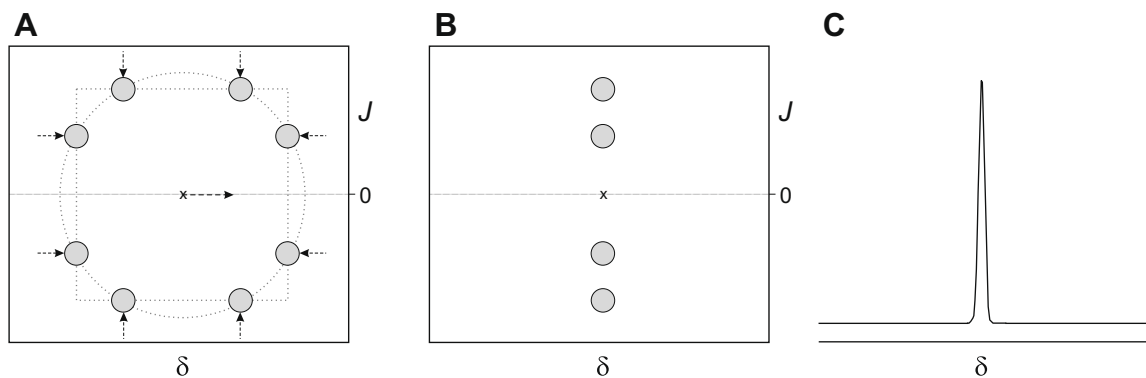
Fig. 3. Underlying principle of the basic symmetry-based multiplet reduction algorithm for z-filtered J-spectra used here: the center of every weakly coupled multiplet must be at 0 Hz in the J-dimension with a fourfold symmetry of its multiplet pattern. For each eight equivalent data points on a square (circle) (A) the minimum intensity of the points is subtracted from the spectrum and projected onto the central chemical shift value in a new data matrix (B). A decoupled 1D spectrum is obtained by the summation of the projected 2D spectrum along the J-dimension (C). Starting for example with the largest possible square at the left side of the spectrum, the whole 2D plane is scanned by this approach by first reducing the square step by step (A, dashed arrows) and then moving to the next chemical shift (A, dashed arrow with cross).

An example for insufficient ZQ-suppression is shown in Fig. 6: The chemical shift difference of the signals shown is only slightly larger than the multiplet width and the artefact signals marked by asterisks occur because of strong coupling contributions, as is simulated for a similar spin system in Fig. 4E. After application of the multiplet reduction algorithm the strong coupling contribution is nicely seen in the residual spectrum (Fig. 6B), but because of the fourfold symmetry of the artefact signals, an undesired ghost peak is created in the center between the two strongly coupled spins (Fig. 6C,D).

In Fig. 7, finally, a very extreme example of a complex spin system with largely overlapping multiplets is shown with norcamphor dissolved in DMSO. Altogether 8 spins contribute to the region shown with especially crowded areas around 1.38 ppm (two underlying spins) and 1.75 ppm (three underlying spins). Expectedly, the z-filtered J-spectrum (Fig. 7C) contains massive artefacts from strong coupling interactions which can again be identified in the residual spectrum (Fig. 7E). In addition, the z-filter must fail in the most crowded areas due to Eq. (1). As a result, the symmetry-driven multiplet reduction will lead to a number of ghost peaks between the overlapping and strongly coupled multiplets. Remarkably, only the signals in the most crowded regions are affected and although their resulting "decoupled" spectrum is not really decoupled, their multiplet widths are at least generally reduced (Fig. 7B and D).

In addition to the z-filtered J-spectrum, the experiment described in Fig. 1B can be of considerable interest for strongly coupled spins. The experiment correlates chemical shifts in both dimension without transfer of magnetization between the evolution periods. A spectrum of a weakly coupled spin system, therefore, would only contain the diagonal without any informational gain compared to a 1D-spectrum. As soon as strong coupling contributions are present, however, cross peaks between the coupled spins will occur as simulated in Fig. 4D. The corresponding strong coupling correlation spectrum for norcamphor is shown in Fig. 7F where the complete strongly coupled network can be easily identified.

6. Uniformly ^{13}C-labeled samples

The approach is of course not limited to proton spectra but can also be applied to hetero nuclei like ^{13}C. Especially in light of the renaissance of carbon-detected experiments in biomolecular NMR [38] with uniformly ^{13}C-labeled proteins and nucleic acids

an efficient method for homonuclear decoupling without significant loss in sensitivity is of potential interest.

As a small test system readily available in our laboratory, we chose uniformly ^{13}C-labeled glucose dissolved in D_2O for a demonstration of the z-filter approach. The molecule has a relatively narrow chemical shift range and all carbons are split by homonuclear one-bond couplings of 36–44 Hz. As can be seen in Fig. 8, second order artefacts are present in basically all signals as well as considerable overlap of multiplets of α- and β-glucose. We applied the z-filtered J-resolved spectra as shown in Fig. 1A with the addition of composite pulse decoupling on ^{1}H throughout the experiment. 8192 × 128 data points have been acquired in the indirect and direct dimension which were processed using a standard shifted quadratic sine-bell function. Subsequent application of the multiplet reduction algorithm led to the spectra shown in Fig. 8B–D. The resulting effectively decoupled ^{13}C-1D spectrum consists of the desired singlets.

7. Discussion

The J-resolved experiment demonstrated here is based on the z-filter approach originally described in [19] with the addition of a highly efficient z-filter [20] and sign-sensitive frequency evolution during t_1 using States-TPPI-type incrementation [28–30]. The experiment retains the full multiplet in both dimensions in pure absorption with the big advantage of narrow lineshapes, but for applications involving homonuclear decoupled spectra subsequent processing with a multiplet reduction algorithm is necessary.

Concerning technical details, the z-filter used in the experiments shown is based on a classical CHIRP-type adiabatic pulse [39] of 30–50 ms duration following the originally published procedure [20]. As pointed out previously, also non-adiabatic pulses with frequency sweep can be used in the z-filter approach [21]. With corresponding time-optimal inversion pulses like BIP [40] and BIBOP [41,42], shorter z-filters with equal performance will be applicable. While this is of little interest for small molecules, long periods with magnetization along z will lead to unwanted relaxation losses and NOE-type transfer for molecules with larger correlation times. However, following Eq. (1), a minimum z-filter duration of 5–10 ms should be kept to allow sufficient dephasing of zero quantum coherences.

Strong coupling between spins leads to antiphase contributions during the evolution of the FID as shown in Fig. 4. Using the phase-sensitive incrementation in the indirect dimension, the non-symmetric second order artefacts can easily be distinguished from
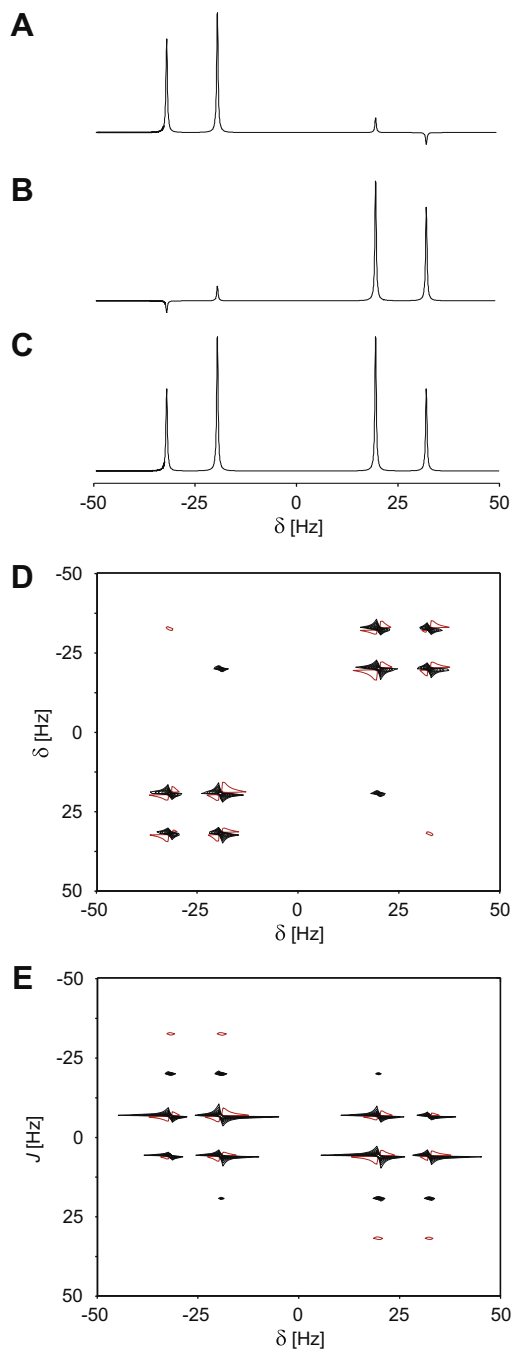
Fig. 4. Simulation of an AB spin system with $\nu_A = -25$ Hz, $\nu_B = 25$ Hz, and $J_{AB} = 12.5$ Hz. (A) Simulated spectrum with only spin A selectively excited. (B) The same spectrum simulated with selective excitation of spin B. (C) The resulting 1D-spectrum is the sum of the two components of the spin system with the typical roof effect caused by the individual antiphase contributions. (D) Simulated 2D strong coupling correlation spectrum for the same spin system. The simulation was performed using the pulse sequence of Fig. 1B assuming an ideal z-filter. (E) Corresponding simulated z-filtered J-spectrum. The resulting spectrum is similar to the experimental one shown in Fig. 6A without passive couplings.

Fig. 5. The z-filtered J-spectroscopy approach applied to strychnine dissolved in CDCl₃. (A) The fully coupled ¹H 1D-spectrum and (B) the resulting homonuclear decoupled spectrum. For a small region the z-filtered J-spectrum with overlapping signals (C) after symmetry-based multiplet reduction leads to the residual spectrum with signals indicative for antiphase contributions originating from strong coupling (D) and the projected spectrum with effective homonuclear decoupling in the chemical shift dimension (E). After summation along the J-dimension the corresponding decoupled 1D-spectrum is obtained (F). The J-spectrum was recorded with a single scan per increment and processed with standard shifted sine-bell functions and 4096 × 128 data points with 1.65 Hz resolution in both dimensions.

the symmetric coupling evolution of conventional signals in the weak coupling limit. With the chemical shift correlation experiment of Figs. 1B and 7F, the origin of the antiphase signals can be nicely identified as a kind of TOCSY transfer caused by the isotropic mixing contribution of the Hamiltonian.

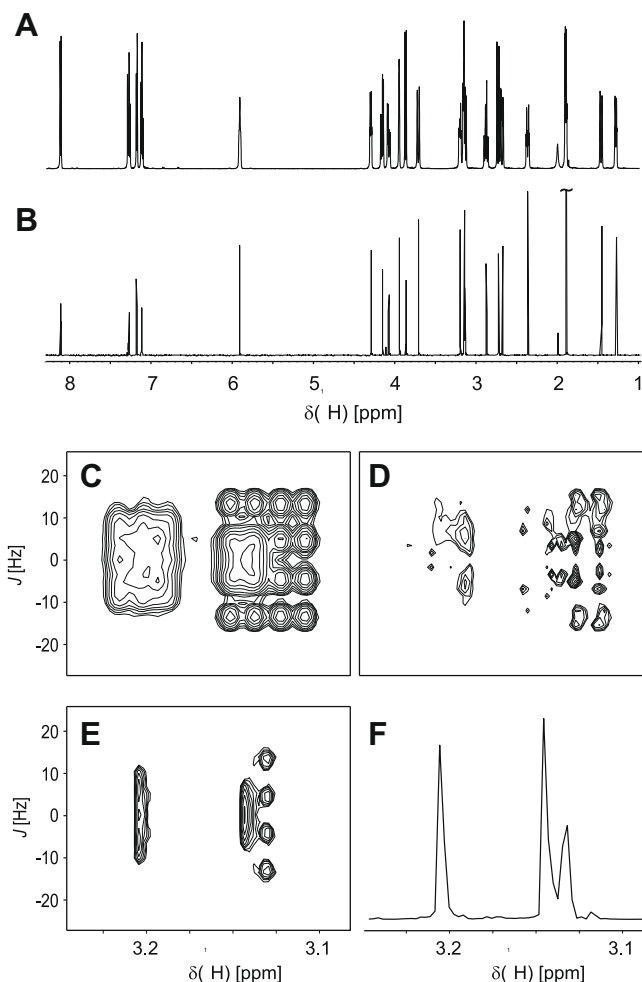The multiplet reduction algorithm used here is of the most simple form, only based on symmetry relations of weakly coupled spins and with the limitation that both dimensions of the z-filtered J-spectra have to be processed with the same digital resolution in Hz. More complex algorithms can be thought of, like introducing peak picking routines for the identification of multiplets, which are then independent of the digital resolution. Also the roof-effect can be taken into a fitting routine which then would even account to some extent for second order artefacts. This would, for example, improve the reliability of integrals measured in homonuclear decoupled spectra. The simple algorithm derived here has the advantage of not relying on any assumption concerning lineshapes or any beforehand specific knowledge of the multiplet patterns. As pointed out before, the integrals of weakly coupled spins are reliable and reduced signal intensities due to strong coupling artefacts can be deduced from the remaining spectrum after the algorithm is applied. Of course, for signals that do not fulfill the condition of Eq. (1) for zero quantum suppression, a general limit of the z-filter approach applies which results in multiplets of comparable complexity as in a conventional, non-decoupled 1D experiment. In this case, signals can only be deduced by a simulation taking into account all details of the experiment and the full spin system in question.
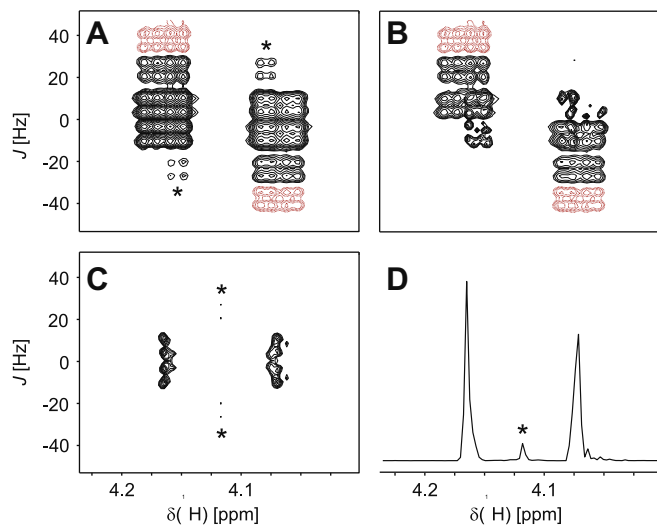
Fig. 6. Spectral region of the *z*-filtered *J*-spectrum of strychnine (see Fig. 5) with significant strong coupling contribution (A), the residual (B) and projected (C) spectrum after application of the multiplet reduction algorithm, and the resulting decoupled 1D-spectrum (D). Clearly the strong coupling contribution can be deduced from the antiphase signals at 4.17 ppm/30 Hz and 4.09 ppm/−30 Hz (A and B). In addition, these signals are partially mirrored in the *J*-dimension (A, marked with asterisks). Because of the fourfold symmetry of the artefact signal a small ghost peak appears in the decoupled spectrum (asterisks in C, D).

Fig. 8. Heteronuclear decoupled ^{13}C-spectra of uniformly ^{13}C-labeled glucose. A region of the conventional 1D-spectrum (A), the homonuclear decoupled spectrum (B), the *z*-filtered *J*-spectrum (C) with its projected spectrum after multiplet reduction (D) are shown. The *z*-filtered *J*-spectrum was recorded with a single scan per increment and processed with standard shifted sine-bell functions and 8192 × 128 data points with 1.09 Hz resolution in both dimensions.

The *z*-filtered *J*-spectrum is not the only approach for effective homonuclear decoupling of spins: The so-called pseudo-echo is able to provide absorptive conventional *J*-spectra by using symmetrization of the FID [12] and methods relying on the very elegant idea of Zangger and Sterk directly provide decoupled spectra [14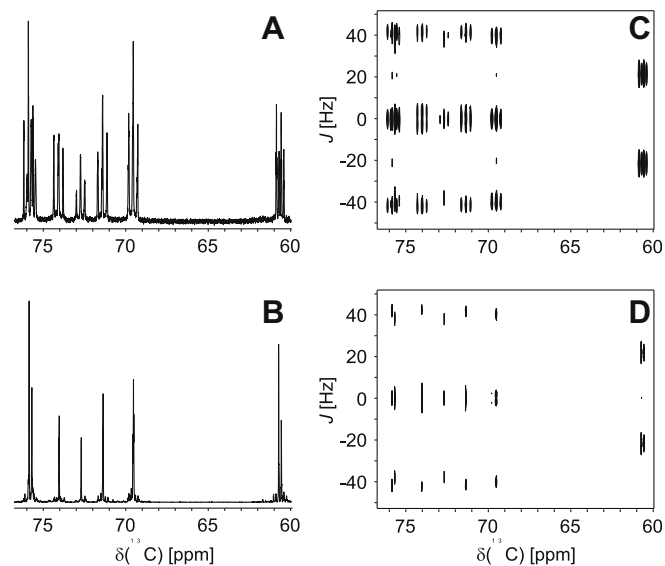,9,15]. However, all of these methods come at a considerable loss of signal intensity, typically significantly below 10% of the original signal-to-noise ratio. Another way of obtaining a homonuclear decoupled spectrum from conventional *J*-spectra is based on the impressive regularized resolvent transform [13] which results in a peak list with center frequencies of the corresponding multiplets. A disadvantage of this approach, however, is the limited
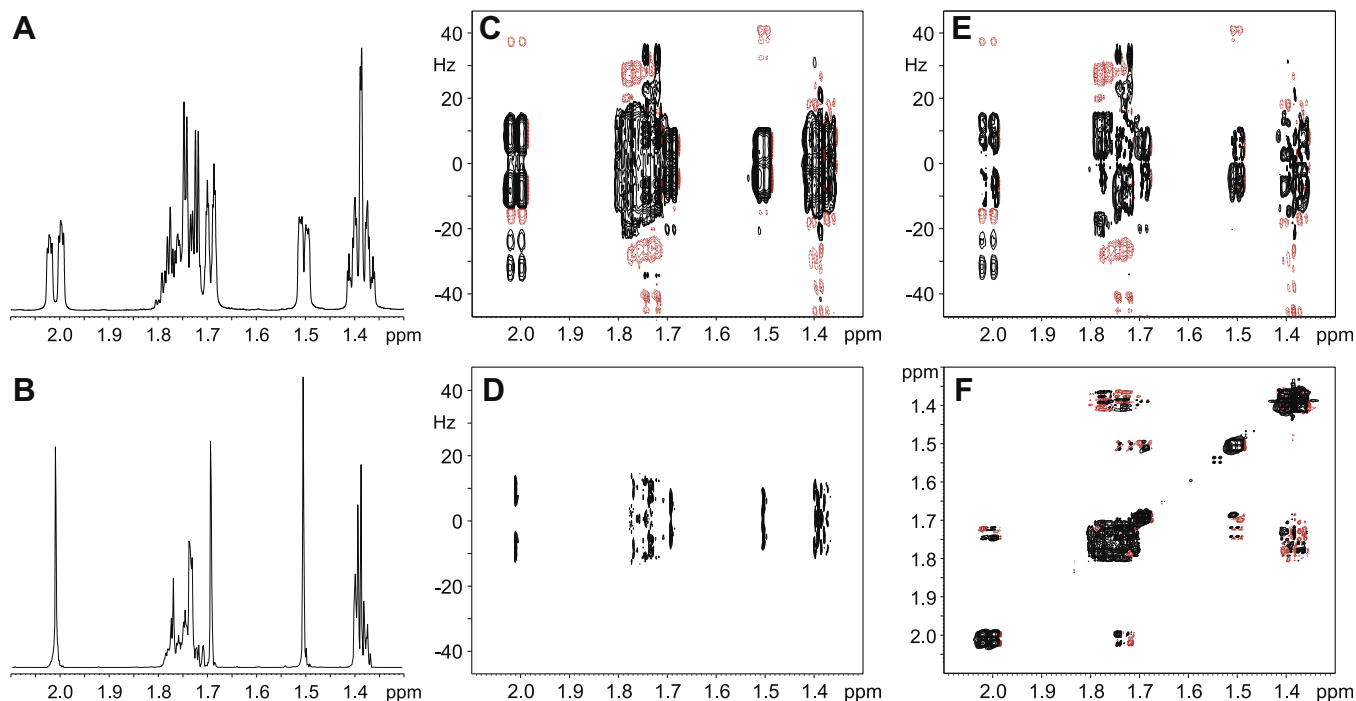

Fig. 7. Spectral region of norcamphor dissolved in DMSO-d_6. A conventional ^{1}H 1D-spectrum (A), the resulting homonuclear decoupled spectrum (B), the *z*-filtered *J*-spectrum (C) with the residual (D) and projected (E) spectra after multiplet reduction, and the strong coupling correlation experiment as described in Fig. 1B (F) are shown. Altogether 8 spins contribute to the spectral region. Overlap of coupled signals leads to a variety of ghost peaks and the signal regions at 1.38 and 1.75 ppm cannot effectively be decoupled but only reduced in their multiplet widths. The network of strong coupling contributions with individual coupling partners can nicely be identified in (F). The *z*-filtered *J*-spectrum was recorded with two scans per increment and processed with standard shifted sine-bell functions and 4096 × 128 data points with 1.0 Hz resolution in both dimensions. The strong coupling correlation experiment was recorded with 4096 × 2048 data points and otherwise identical treatment.

reliability of integrals and the occurrence of ghost peaks caused by strong coupling artefacts, which cannot be distinguished from real signals. The application of desymmetrized *J*-experiments [37] might be able to overcome the latter problem, but to our knowledge this has not been studied yet.

In contrast to these methods, the presented approach retains full sensitivity of the 2D-experiment with good integratability. Regarding potential applications, the inclusion of the described technique into DOSY-type experiments, for example, should be straight-forward. In this type of experiment it is most crucial to avoid overlap whenever possible [9,26] and homonuclear decoupled spectra are of special interest. In general, the *z*-filter *J*-resolved scheme might be incorporated in situations where conventional spectra do not allow for sufficient resolution. Therefore, another potential field of applications might be found in protein NMR spectroscopy of mainly α-helical or denatured molecules, where only directly carbon-detected experiments allows the unambiguous distinction of signals. So far, typical homonuclear decoupling schemes for uniformly $^{13}C,^{15}N$-labeled proteins like S^3E [43], IPAP [44] or DIPAP [45,38] decouple only the major one-bond couplings. The application of the *z*-filter approach in such experiments would also allow efficient decoupling of the multitude of long-range $^{13}C,^{13}C$-couplings without significant loss in sensitivity. The resulting linewidths would then be on the order of only a few Hz, providing the maximum possible resolution for these molecules with their notoriously poor spectral dispersion.

8. Conclusion

The experimental scheme introduced in this article allows the acquisition of absorptive *z*-filtered *J*-resolved spectra with only one scan per increment. Homonuclear decoupled spectra can be obtained by a multiplet reduction algorithm which is applied after conventional processing without significant loss in sensitivity. Limitations of the approach due to the occurrence of strong coupling have been studied in great detail. Pulse sequences for Bruker Avance spectrometers, example spectra, and the simple symmetry-based multiplet reduction algorithm written in Matlab will be made available on the website http://www.org.chemie.tu-muenchen.de/people/bulu.

References

[1] R.L. Vold, S.O. Chan, Modulated spin echo trains from liquid crystals, J. Chem. Phys. 53 (1970) 449–451.
[2] R. Freeman, H.D.W. Hill, High-resolution study of NMR spin echoes: *J*-spectra, J. Chem. Phys. 54 (1971) 301–313.
[3] G. Bodenhausen, R. Freeman, D.L. Turner, Two-dimensional *J*-spectroscopy: proton-coupled carbon-13 NMR, J. Chem. Phys. 65 (1976) 839–840.
[4] W.P. Aue, R.R. Ernst, Homonuclear broad band decoupling and two-dimensional *J*-resolved NMR spectroscopy, J. Chem. Phys. 64 (1976) 4226–4227.
[5] J.K. Nicholson, P.J.D. Foxall, M. Spraul, R.D. Farrant, J.C. Lindon, 750 MHz ^{1}H and ^{1}H–^{13}C NMR spectroscopy of human blood plasma, Anal. Chem. 67 (1995) 793–811.
[6] M.R. Viant, Improved methods for acquisition and interpretation of NMR metabolomic data, Biochem. Biophys. Res. Commun. 310 (2003) 943–948.
[7] B. Luy, J.P. Marino, JE-TROSY: comined *J*- and TROSY-spectroscopy for the measurement of one-bond couplings in macromolecules, J. Magn. Reson. 163 (2003) 92–98.
[8] J. Furrer, M. John, H. Kessler, B. Luy, *J*-spectroscopy in the presence of residual dipolar couplings: determination of one-bond coupling constants and separate dimension with scalable resolution, J. Biomol. NMR 37 (2007) 231–243.
[9] M. Nilsson, G.A. Morris, Pure shift proton DOSY: diffusion-ordered ^{1}H spectra without multiplet structure, Chem. Commun. (2007) 933–935.
[10] J. Farjon, D. Merlet, P. Lesot, J. Courtieu, Enantiomeric excess measurements in weakly oriented chiral liquid crystal solvents through 2D ^{1}H selective refocusing experiments, J. Magn. Reson. 158 (2002) 169–172.
[11] K. Kobzar, H. Kessler, B. Luy, Stretched gelatin gels as chiral alignment media capable to discriminate enantiomers, Angew. Chem. Int. Ed. 44 (2005) 3145–3147. Corrigendum: Angew. Chem. Int. Ed. 44 (2005) 3509.
[12] A. Bax, R. Freeman, G.A. Morris, A simple method for suppressing dispersion-mode contributions in NMR spectra: the pseudo echo, J. Magn. Reson. 43 (1981) 333–338.
[13] G.S. Armstrong, J.H. Chen, K.E. Cano, A. Shaka, V.A. Mandelshtam, Regularized resolvent transform for direct calculation of 45 degrees projections of 2D *J*-spectra, J. Magn. Reson. 164 (2003) 136–144.
[14] K. Zangger, H. Sterk, Homonuclear broadband-decoupled NMR spectra, J. Magn. Reson. 124 (1997) 486–489.
[15] A.J. Pell, J. Keeler, Two-dimensional *J*-spectra with absorption-mode lineshapes, J. Magn. Reson. 189 (2007) 293–299.
[16] P. Xu, X.-L. Wu, R. Freeman, Proton NMR spectra without spin–spin splittings, J. Am. Chem. Soc. 113 (1991) 3596–3597.
[17] P. Xu, X.-L. Wu, R. Freeman, Broadband-decoupled proton spectroscopy, J. Magn. Reson. 95 (1991) 132–148.
[18] M. Woodley, R. Freeman, Decoupled proton NMR spectra, J. Magn. Reson. Ser. A 109 (1994) 103–112.
[19] S. Simova, H. Sengstschmid, R. Freeman, Proton chemical-shift spectra, J. Magn. Reson. 124 (1997) 104–121.
[20] M.J. Thrippleton, J. Keeler, Elimination of zero-quantum interference in two-dimensional NMR spectra, Angew. Chem. Int. Ed. 42 (2003) 3938–3941.
[21] K.E. Cano, M.J. Thrippleton, J. Keeler, A.J. Shaka, Cascaded *z*-filters for efficient single-scan suppression of zero-quantum coherence, J. Magn. Reson. 167 (2004) 291–297.
[22] M.H. Levitt, Spin Dynamics, John Wiley & Sons, 2001.
[23] O.W. Sørensen, M. Rance, R.R. Ernst, Z-filters for purging phase-distorted or multiplet-distorted spectra, J. Magn. Reson. 56 (1984) 527–534.
[24] T. Schulte-Herbrüggen, Imaging techniques boost NMR spectroscopy: How to remove zero-quantum artifacts, Angew. Chem. Int. Ed. 42 (2003) 5270–5272.
[25] K. Kobzar, B. Luy, Analysis, extensions and comparison of three experimental schemes for measuring ($^nJ_{CH} + D_{CH}$)-couplings at natural abundance, J. Magn. Reson. 186 (2007) 131–141.
[26] A.J. Pell, R.A.E. Edden, J. Keeler, Broadband proton-decoupled proton spectra, Magn. Reson. Chem. 45 (2007) 296–316.
[27] P. Tzvetkova, S. Simova, B. Luy, P.E.HSQC: a simple experiment for simultaneous and sign-sensitive measurement of ($^1J_{CH} + ^1D_{CH}$) and ($^2J_{HH} + ^2J_{HH}$) couplings, J. Magn. Reson. 186 (2007) 193–200.
[28] D.J. States, R.A. Haberkorn, D.J. Ruben, A two-dimensional nuclear overhauser experiment with pure absorption phase in 4 quadrants, J. Magn. Reson. 48 (1982) 286–292.
[29] D. Marion, K. Wüthrich, Application of phase sensitive two-dimensional correlated spectroscopy (COSY) for measurement of ^{1}H–^{1}H spin–spin coupling constants in proteins, Biochem. Biophys. Res. Commun. 113 (1983) 967–974.
[30] R.R. Ernst, G. Bodenhausen, A. Wokaun, Principles of Nuclear Magnetic Resonance in One and Two Dimensions, Clarendon Press, Oxford, 1987.
[31] L. Braunschweiler, R.R. Ernst, Coherence transfer by isotropic mixing: application to proton correlation spectroscopy, J. Magn. Reson. 53 (1983) 521–528.
[32] S.J. Glaser, J.J. Quant, Homonuclear and heteronuclear Hartmann–Hahn transfer in isotropic liquids, in: W.S. Warren (Ed.), Advances in Magnetic and Optical Resonance, vol. 19, Academic Press, San Diego, 1996, pp. 59–252.
[33] O. Schedletzky, S.J. Glaser, Analytical coherence-transfer functions for the general AMX spin system under isotropic mixing, J. Magn. Reson. A 123 (1996) 174–180.
[34] B. Luy, S.J. Glaser, Analytical polarization transfer functions for four coupled spins $\frac{1}{2}$ under isotropic mixing conditions, J. Magn. Reson. 138 (1999) 19–27.
[35] B. Luy, S.J. Glaser, Analytical transfer functions for three dipolar coupled spins $\frac{1}{2}$, J. Magn. Reson. 142 (2000) 280–287.
[36] B. Luy, S.J. Glaser, Superposition of scalar and residual dipolar couplings: analytical transfer functions for three spins $\frac{1}{2}$ under cylindrical mixing conditions, J. Magn. Reson. 148 (2001) 169–181.
[37] M.J. Thrippleton, R.A.E. Edden, J. Keeler, Suppression of strong coupling artefacts in *J*-spectra, J. Magn. Reson. 174 (2005) 97–109.
[38] W. Bermel, I. Bertini, I.C. Felli, M. Piccioli, R. Pierattelli, ^{13}C-detected *protonless* NMR spectroscopy of proteins in solution, Prog. NMR Spectrosc. 48 (2006) 25–45.
[39] J.-M. Böhlen, G. Bodenhausen, Experimental aspects of CHIRP NMR spectroscopy, J. Magn. Reson. Ser. A 102 (1993) 293–301.
[40] M.A. Smith, H. Hu, A.J. Shaka, Improved broadband inversion performance for NMR in liquids, J. Magn. Reson. 151 (2001) 269–283.
[41] K. Kobzar, T.E. Skinner, N. Khaneja, S.J. Glaser, B. Luy, Exploring the limits of broadband excitation and inversion pulses, J. Magn. Reson. 170 (2004) 236–243.
[42] K. Kobzar, T.E. Skinner, N. Khaneja, S.J. Glaser, B. Luy, Exploring the limits of broadband excitation and inversion pulses II: RF-power optimized pulses, J. Magn. Reson. 194 (2008) 58–66.
[43] A. Meissner, J.O. Duus, O.W. Sørensen, Spin-state-selective excitation. Application for E.COSY-type measurement of J_{HH} coupling constants, J. Magn. Reson. 128 (1997) 92–97.
[44] M. Ottiger, F. Delaglio, A. Bax, Measurement of *J* and dipolar couplings from simplified two-dimensional NMR spectra, J. Magn. Reson. 131 (1998) 373–378.
[45] W. Bermel, I. Bertini, L. Duma, L. Emsley, I.C. Felli, R. Pierattelli, P. Vasos, Complete assignment of heteronuclear protein resonances by protonless NMR spectroscopy, Angew. Chem. Int. Ed. 44 (2005) 3089–3092.