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EASY ROESY: Reliable Cross-Peak Integration in Adiabatic Symmetrized ROESY

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Determination of the 3D structure of small- to medium-sized organic and biomolecular compounds relies on the use of several NMR parameters. Information about dihedral angles from homo- and heteronuclear ^3J scalar couplings[1–3] is usually combined with information on distances from the nuclear Overhauser effect (NOE).[4] Even when using the recently (re)introduced (residual) dipolar couplings[5] to study flexible compounds,[6–8] distance information from the NOE is still essential for structure determination. The build-up rate of the NOE, however, depends on the correlation time τ_c of the compound and the observation frequency ω. The NOE changes sign at $\omega\tau_c \approx 1.12$, which leads to the well-known phenomenon that little or no NOE is observed for medium-sized compounds (MW $\approx 1000\,g\,mol^{-1}$). This impedes the extraction of distance information from NOESY spectra of these compounds.

This problem can be solved by using rotating-frame nuclear Overhauser effect spectroscopy (ROESY),[9,10] which yields negative cross-peaks (corresponding to positive Overhauser enhancements) for all values of $\omega\tau_c$. In the interpretation of ROESY, however, several experimental problems—namely direct cross-peaks due to J coupling, Hartmann–Hahn matching (leading to TOCSY, total correlation spectroscopy, cross-peaks) and offset dependence[11]—have to be avoided. The offset dependence influences the cross-peak integrals of all spins depending on their offset, whereas the other two phenomena can degrade the line shapes and integrals of ROESY cross-peaks. TOCSY artefacts also impede the use of ROESY for detecting chemical exchange.[12] These problems lead to serious complications, when distances or exchange rates are to be extracted from ROESY spectra.

Several ROESY pulse sequences have been proposed for removing these artefacts,[13–15] but they are cumbersome to set up, reduce sensitivity too much, or show pronounced offset dependence. Thus a robust, convenient procedure is needed that yields high-quality, high-sensitivity spectra that can be reliably integrated even in the presence of J coupling.

We have previously shown that jump-symmetrized ROESY (JS-ROESY) combines negligible offset dependence with close-to-optimal suppression of TOCSY,[14] but the experimental setup is quite laborious as pulses with fine-tuned power levels (gray in Figure 1 A) are needed. Here we show that by bracketing two off-resonance spin-lock pulses with adiabatic pulses[16], we obtain high-quality, high-sensitivity spectra with no need for a sample-specific setup (Figure 1 B). The resulting spectra can be reliably quantified and TOCSY transfer is as well suppressed as in conventional JS-ROESY. The superior performance of this convenient

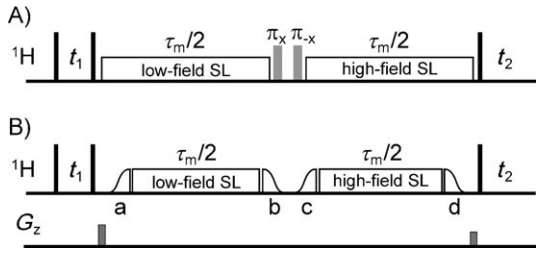

Figure 1. Pulse sequences of the previously described JS-ROESY (A) and EASY ROESY (B). A) A pair of specially calibrated 180° pulses[14] (gray) is needed to transfer magnetization from the low- to the high-field spinlock (SL). B) Adiabatic ramps at times a–d transfer magnetization to the spinlocks. After EASY ROESY has been set up once, parameters are calculated automatically. Gradient pulses (G_z) suppress unwanted signals. Solvent suppression can easily be added to the sequence.

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ROESY approach, which we name EASY-ROESY (**E**fficient **A**diabatic **SY**mmetrized ROESY), is demonstrated below by comparing ROESY and NOESY-derived distance estimates for a small protein (BPTI), and by measuring exchange rates in a Grubbs II-type complex, an olefin metathesis pre-catalyst.

In JS-ROESY (Figure 1 A), the mixing time (τ_m) is divided into two equal parts, in which a continuous-wave spin lock is applied to the high- and low-field regions of the spectrum. This averages out the offset dependence of the cross-peak integrals to a good approximation. At the same time, each spin lock is applied outside of the spectrum so that TOCSY transfer is minimized almost to the theoretical limit.[14] In EASY ROESY (Figure 1 B), adiabatic ramps rotate the magnetization from the z axis to the spin-lock axis (time points a, c) or back (time points b, d), removing the requirement for specially calibrated pulses, which are key elements of JS-ROESY (Figure 1 A). Thus, the advantages of JS-ROESY are retained with no need for sample-specific set-ups.

We demonstrate the excellent spectral qualities of EASY ROESY here by presenting spectra of a small protein (Figure 2 A,B) and an organometallic complex (Figure 2 C). While the spectrum acquired with an alternative (phase-alternating) ROESY sequence[13] (Figure 2 B) contains strong J coupling artefacts (COSY-type antiphase cross-peaks), which hamper interpretation and integration, the spectra recorded with EASY ROESY are free of artefacts due to J couplings, a precondition for reliable integration in crowded spectral
regions.

We used the small protein BPTI to test how well EASY ROESY spectra can be integrated, by comparing with a NOESY spectrum. A small protein is suitable for this purpose because the ratio of ROE/NOE rates depends only on the correlation time and the spectrometer frequency, if internal motions can be neglected.[17] Figure 3 shows that ROESY and NOESY integrals correlate very well, demonstrating that EASY ROESY spectra can be integrated to derive distance constraints. Furthermore, the slope of the correlation line agrees with a predicted value, indicating that EASY ROESY shows the expected sensitivity. For medium-sized molecules, EASY ROESY has 75% of the achievable sensitivity, (see Supporting Information), a small trade-off for the excellent suppression of TOCSY artefacts.

A common problem for quantification of ROESY is offset dependence, that is, that the integrals of cross-peaks depend on their location in the spectrum. Because NOESY has no offset dependence, the ROE/NOE integral ratio can also be used as a sensitive indicator of offset dependence in ROESY experiments. As shown in supplementary Figure 1, EASY ROESY displays no detectable offset dependence, in agreement with JS-ROESY simulations.[14] Furthermore, ROESY- and NOESY-derived distances correlate with an R^2 of 0.953 (Figure 2 in the Supporting Information), indicating that distance restraints can be derived from EASY ROESY without a need for offset correction.

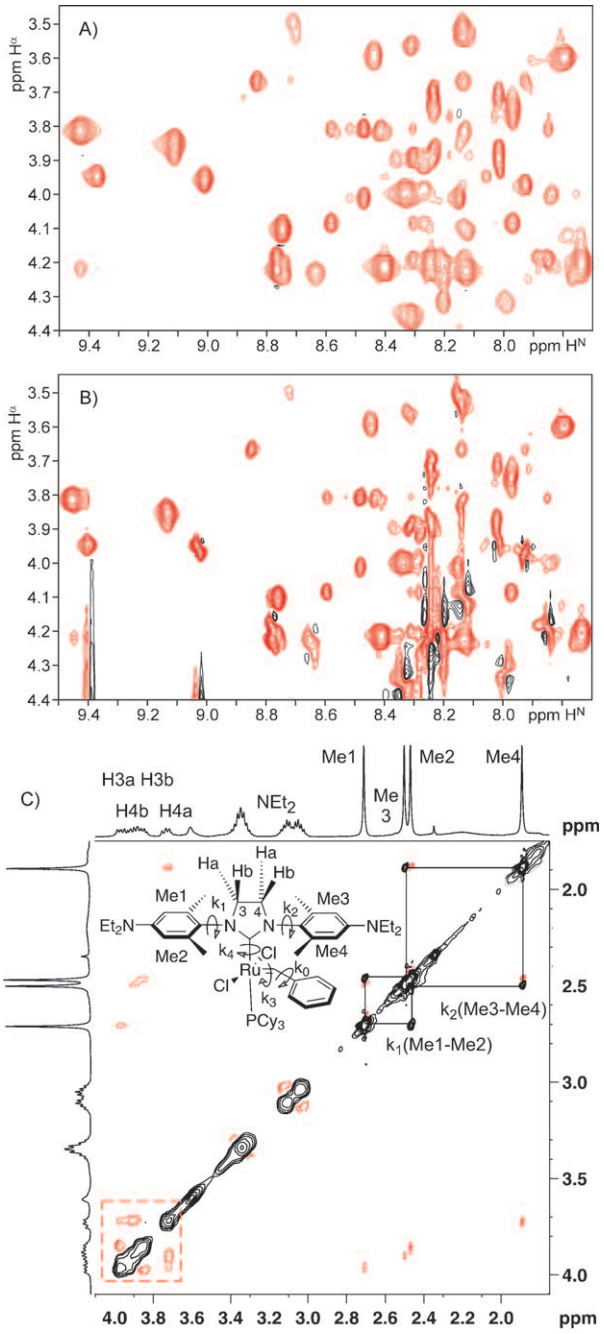

Figure 2. Expansions of ROESY spectra of a small protein and an organometallic complex. Experimental details are provided in the Supporting Information. A) and B) H^N,H^α region of the small protein BPTI recorded with A) EASY ROESY and B) a phase-alternating ROESY sequence.[13] C) The aliphatic spectral region of a Grubbs II-type complex[12] at 238 K is free of TOCSY cross-peaks for all resolved peaks. The rotations of the two mesityl flaps (k_1, Me1–Me2; k_2, Me3–Me4) are indicated by black squares. The rotation of the benzylidene unit (k_3) can be excluded at that temperature, since only ROE signals are observed in the region of the NHC backbone protons, as indicated by the red dashed box.

Further examples of the excellent properties of EASY ROESY are provided by spectra obtained in analyses of the exchange pathways in a Grubbs II-type complex. An objective of our investigations of the π-face donor properties of

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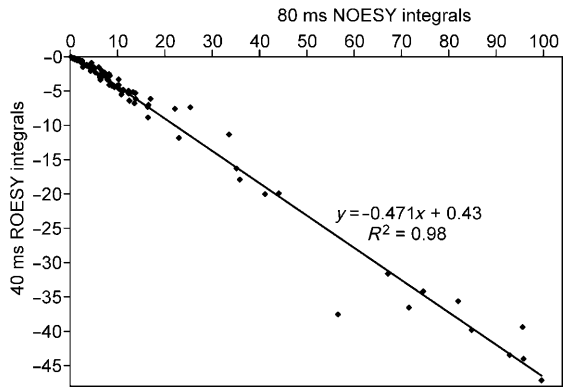

Figure 3. Correlation of cross-peak integrals obtained from NOESY and EASY ROESY spectra. A ROESY/NOESY integral ratio of −0.45 is predicted from experimental parameters, as described in the Supporting Information. Integration error, estimated from the noise of the spectra, is around 0.1% for the strongest signals and proportionally larger for weaker ones.

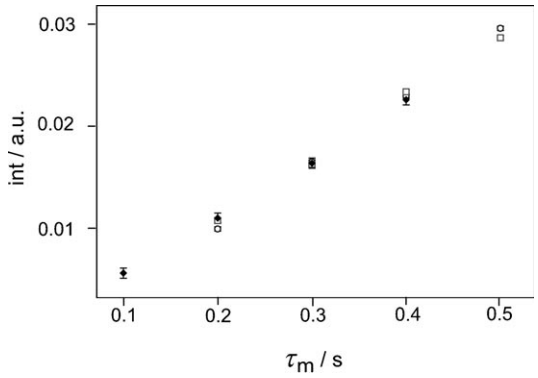

Figure 4. Comparison of integrals (extracted using the PANIC approach)[18,19] from EASY ROESY experiments with those from 1D PFGSE NOE at several mixing times and 238 K. The slope yields the rate constant k_2, determined by using the H3′-H4′ cross-peaks in the same aromatic moiety as Me3–Me4. Open circles indicate the values extracted from the H3′–H4′ cross-peak, open squares are extracted from the symmetry-related H4′–H3′ cross-peak, and filled diamonds the values obtained from 1D PFGSE NOE[12]. Error bars indicate the absolute error of integrals, obtained by integrating the noise in an empty spectral region. A color version of this figure can be found in the Supporting Information.

N-heterocyclic carbenes[12] (Figure 2C) was to distinguish and quantify several dynamic processes in these complexes. It was especially important to distinguish the rotation of the benzylidene unit (k_3) from the rotation of mesityl flaps (k_1 and k_2). This is only possible by monitoring the NHC backbone protons (H3a+H3b and H4a+H4b). Cross-peaks among these protons with the same sign as the diagonal could indicate exchange. However, since these protons are scalar coupled, they could also be due to TOCSY transfer. Thus, it is especially important to be able to completely suppress Hartmann–Hahn transfer between scalar-coupled spins in this case. As can be nicely seen in Figure 2C, the rotation of the benzylidene unit (k_3) can be excluded at that temperature, since only ROE signals are observed in the region of the NHC backbone protons, as indicated by the red dashed box. However, rotation of the mesityl flaps (k_1 and k_2) does occur (black boxes).

Integration of EASY ROESY cross-peaks was used to extract exchange rate constants for the rotation of the mesityl flaps under these conditions. As can be seen in Figure 4 and Table 1, the rate constants extracted from EASY ROESY experiments are in excellent agreement with rate constants that we previously determined in 1D PFGSE NOE measurements,[12] again highlighting the reliable integration properties of the new technique.

Table 1. Comparison of exchange rates k in s^{-1} for the rotation of the mesityl flaps at 238 K obtained from 1D PFGSE[12] and EASY ROESY experiments.

	k_1 (Me1–Me2) [s^{-1}]	k_2 (Me3–Me4) [s^{-1}]	k_2 (H3′–H4′)[a] [s^{-1}]
k from 1D PFGSE[12]	0.050 (2)	0.055 (3)	0.055 (1)
k from EASY ROESY	0.051 (10)	0.057 (10)	0.056 (1)

[a] H3′ and H4′ are the protons next to Me3 and Me4 on the mesityl flaps above the benzylidene unit. Values in brackets are the errors of the last digit.

Conclusion

EASY ROESY yields high-quality spectra with high sensitivity, which can be integrated and used for distance calculations without offset correction, and no need for sample-specific set-up. These features make EASY ROESY the technique of choice for structure determination and studies of exchange phenomena.

Experimental Section

The EASY ROESY pulse sequence, descriptions of the adiabatic pulses, details of the data analysis, and the experimental parameters of BPTI and the Grubbs II complex are provided in Supporting Information. In general, parameters that provide a good compromise between sensitivity and suppression of TOCSY can be determined once for a hardware configuration. Because exact calibration is not needed for adiabatic pulses, no sample-specific set-up is needed for routine use of EASY ROESY.

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Keywords: distance constraints · EASY ROESY · NMR spectroscopy · structure elucidation

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