Accurate measurement of J_{HH} in overlapped signals by a TOCSY-edited SERF Experiment

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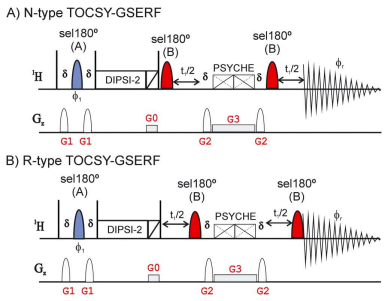
INTRODUCTION

Selective refocusing (GSERF[1] or the recent PSYCHEDELIC[2]) experiments were originally designed to determine all proton–proton coupling constants (J_{HH}) for a selected proton resonance. They work for isolated signals on which selective excitation can be successfully applied but, as it happens in other selective experiments, fail for overlapped signals. To circumvent this limitation, a doubly-selective TOCSY-GSERF scheme is presented for the measurement of J_{HH} in protons resonating in crowded regions[3]. This new experiment takes advantage of the editing features of an initial TOCSY transfer to uncover hidden resonances that become accessible to perform the subsequent frequency-selective refocusing.

NMR methodology

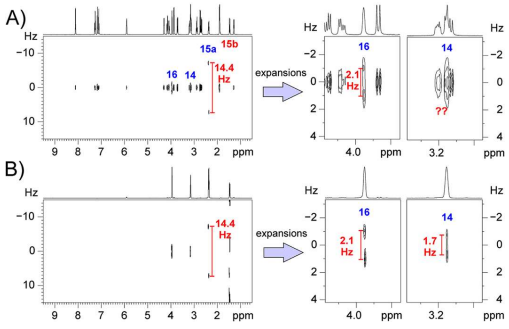
Pulse sequence description

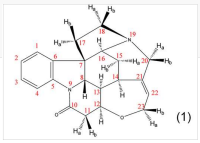


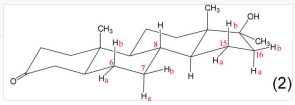
The pulse scheme of the two-dimensional TOCSY-GSERF experiment consists of two sequential steps. The first part is a conventional selTOCSY block applied on a well-isolated ^{1}H resonance (site A) in order to simplify the appearance of overlapped regions. The TOCSY mixing consists of a regular DIPSI-2 pulse train with zero-quantum (ZQ) filtration to afford pure in-phase multiplets. The second part is a GSERF block based on the PSYCHEDELIC scheme. The echo/anti-echo data combination approach is followed to provide pure absorption lineshapes and improved resolution that allows a much more accurate measurement of J_{HH}. The GSERF module is designed to refocus only protons selected by the TOCSY mixing and by the selective refocusing element to be applied on a relayed proton (site B). In practice, the two unique requirements for optimal experiment execution are the definition of the experimental conditions for selective excitation of the starting proton and for the selective refocusing of the relayed proton of interest. Therefore, TOCSY-GSERF affords clean 2D *J*-resolved spectra only displaying the signals filtered by the TOCSY element as a doublets along the indirect dimension. All irrelevant signals that would appear as singlets in the central F1 = 0 row in GSERF spectra will be missing in TOCSY-GSERF spectra, greatly facilitating the observation and measurement of small doublets in highly congested areas.

RESULTS

Example 1

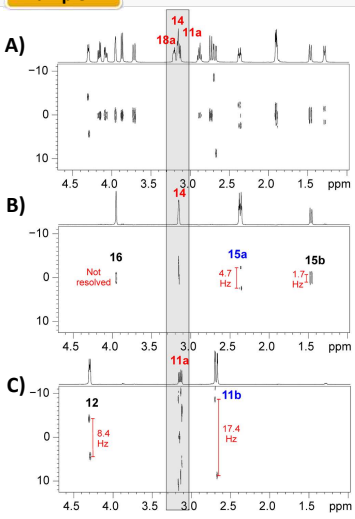






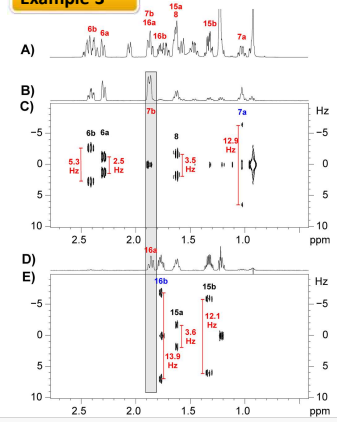
(A) 600.13 MHz 2D GSERF spectrum of strychnine (1) in $CDCl_3$ acquired with the PSYCHEDELIC pulse scheme. A reduced 2D version without the homodecoupling feature was used. The active H_{15b} proton was selectively refocused by a 20 ms Gaussian 180° ^{1}H pulse; (B) Equivalent TOCSY-GSERF spectrum acquired with the pulse sequence herein presented under the same experimental conditions as (A). The selective 180° pulses (20 ms) for TOCSY transfer (mixing of 60 ms) and for refocusing were both applied selectively on H_{15b}. On the right, expansions are shown to observe the excellent resolution achieved in F1 to resolve the small splittings corresponding to $^3J_{H15b,H16}$ and $^3J_{H15b,H14}$.

Example 2



(A) The GSERF spectrum resulting of the indiscriminate perturbation of H_{11a} and H_{14} sites protons at the same time by a 20 ms Gaussian-shaped pulse affords doublets that cannot be attributed to each individual signal. (B) and (C) show the corresponding TOCSY-GSERF spectra with double excitation on H_{15a}/H_{14} and H_{11b}/H_{11a}, respectively. Note that even in the case where H_{14} is a non-resolved broad resonance, vicinal J_{HH} values of 4.7 and 1.7 Hz can be accurately measured for H_{15a} and H_{15b}, respectively, whereas the small four-bond $J_{H14,H16}$ splitting is not resolved. In the second example, two clear doublets corresponding to the couplings of H_{11b} and H_{12} with the relayed H_{11a} proton are observed.

Example 3



Example 3 shows the potential of the TOCSY-GSERF experiment applied to a more challenging example, the steroid methyltestosterone (2). This molecule presents an overcrowded aliphatic region in the ^{1}H spectrum with a large number of signals between 0.9 and 2.5 ppm. Clearly, selTOCSY experiments applied on the isolated H_{7a} (B) and H_{16b} (D) protons facilitate the observation of cleaner 1D spectra that aids to the identification, analysis and assignment of signals. In particular, we are interested in the two fully overlapped signals resonating at 1.85–1.9 ppm that correspond to the H_{7b} and H_{16a} protons. The corresponding TOCSY-GSERF spectra affords 2D maps where accurate values of J_{HH} for both H_{7b} and H_{16a} can be determined (C and E, respectively). In the case of H_{7b}, its equatorial position can be established accordingly to the moderate vicinal J_{HH} values with H_{6a} (equatorial–equatorial interaction of 2.5 Hz), H_{6b} (equatorial–axial interaction of 5.3 Hz) and H8 (equatorial–axial interaction of 3.5 Hz) protons. On the other hand, the axial position of H_{16a} is established accordingly to the axial–axial interaction with H_{15b} (12.1 Hz) and the axial–equatorial interaction with H_{15a} (3.6 Hz) as well as to the large geminal coupling with H_{16b} ($^2J_{HH}$ = 13.9 Hz).

SUMMARY

The incorporation of a selTOCSY block for editing allows the obtention of GSERF spectra for overlapped signals. TOCSY-GSERF affords cleaner, excellent resolution in F1, great simplicity in the extraction of J_{HH} and unambiguous assignment. The use of the principles described for PSYCHE-type experiments ensures maximum sensitivity levels, improving by one order of magnitude those expected with the original spatially-encoded slice selection methods.

[1] Giraud, N., Béguin, L., Courtieu, J. and Merlet, D. (2010), *Nuclear Magnetic Resonance Using a Spatial Frequency Encoding: Application to J-Edited Spectroscopy along the Sample.* Angew. Chem. Int. Ed. **2010**, *49*, 3481.
[2] Sinnaeve, D., Foroozandeh, M., Nilsson, M., Morris, G.A., *A General Method for Extracting Individual Coupling Constants from Crowded 1H NMR Spectra.* Angew. Chem. Int. Ed. **2016**, *55*, 1090.
[3] Fredi, A., Nolis, P., and Parella, T. *Accurate measurement of J_{HH} in overlapped signals by a TOCSY-edited SERF Experiment.* Magn. Reson. Chem., **2017**, *55*,525

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