HSQMBC-TOCSY Experiment: A complementary tool for structure elucidation

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Introduction

Several versions of a HSQMBC-TOCSY experiment for the success application on structural elucidation of small molecules in solution are presented. We evaluate the possibility to obtain TOCSY information for a non-protonated carbon and we also extend this feature to other heteronuclei, such as ^{19}F or ^{77}Se. We compare experimental data obtained from the conventional HMBC and HSQC-TOCSY experiments with respect to this complementary HSQMBC-TOCSY experiment. Additionally, ^{1}H-selective versions will be also proposed for the accurate measurement of the magnitude of heteronuclear coupling constants[1], with special emphasis on the determination of the positive/negative sign of these couplings on non-protonated centers. We also evaluate the implementation of multiplicity-edited information, where CH/CH_3 cross peaks can be clearly distinguished from CH_2/C correlations thanks to their opposite up/down relative phase.

Methodology

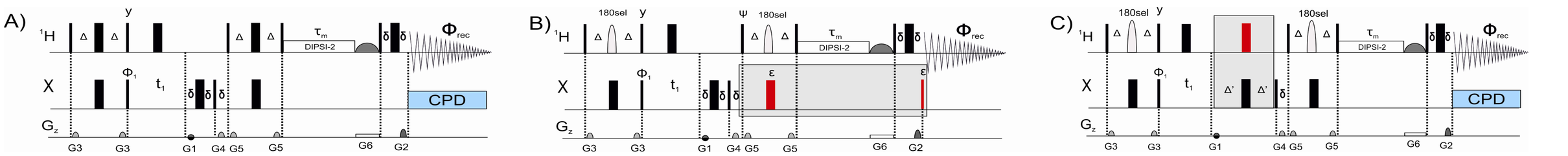

Fig. 1 Pulse sequences for the HSQMBC-TOCSY experiments: A) Broadband HSQMBC-TOCSY, B) ^{1}H-selHSQMBC-TOCSY IPAP (grey box stands to highlight the IPAP principle) and C) ^{1}H-ME-selHSQMBC-TOCSY (grey box stands to highlight the carbon multiplicity editing block). Rectangular 90° and 180° pulses are indicated by thin an thick black bars, respectively, and ^{1}H-selective 180° pulses as shaped bars. Phases are in all cases along x, unless indicated. The basic phase cycle was Φ_1=x,-x and Φ_{rec}=x,-x. In B) two independent IP (Ψ = y, ε = on) and AP (Ψ = x, ε = off) data are initially collected and further combined to provide complementary α/β data (IP±AP) in separate spectra. The inter-pulse delays (Δ+p180/2 = ¼*^{n}J(CH)) was optimized to 8 Hz. In C) the carbon multiplicity editing block was optimized to Δ'=1/(2*^{1}J(CH). The TOCSY transfer consisted of a z-filtered DIPSI-2 pulse train of duration t_m (40ms) with an additional G6 gradient simultaneously applied with a 30ms adiabatic smoothed *CHIRP* pulse to remove unwanted ZQ contributions. Gradients were optimized to G1:G2:G3:G4:G5:G6 = 80:20.1:17:50:11:3.

A) HSQMBC-TOCSY as a structural elucidation tool

HSQMBC-TOCSY vs. HMBC vs. HSQC-TOCSY

HSQC-TOCSY experiment provides remote heteronuclear correlations between protons and carbons belonging to the same spin system but its application is only limited to protonated carbons. In contrast, HMBC spectra provides connectivities between protons and heteronuclei separated by more than one bond, but their cross-peak intensities strongly depend of the magnitude of long-range proton-carbon coupling constants, and therefore some expected cross-peaks are missing or they present very low intensity.

The HSQMBC-TOCSY experiment allows us to obtain TOCSY information for a non-protonated carbon. In these spectra cross-peak intensities not only depend of the magnitude of long-range proton-carbon coupling constants but also depends of the TOCSY transfer between protons. In this way we can use HSQMBC-TOCSY experiment as a complementary structural elucidation tool in which we will be able to obtain some missing cross peaks that are absent in the HMBC spectra.

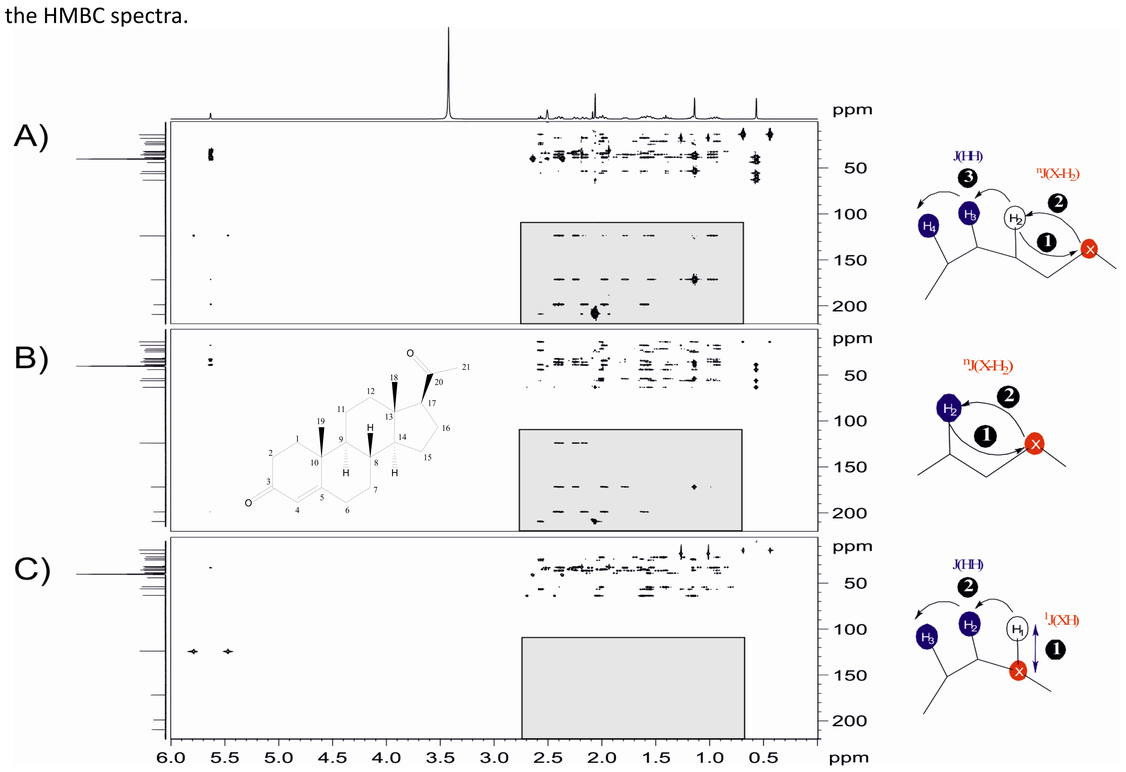

Fig. 2 A) HSQMBC-TOCSY, B) HMBC and C) HSQC-TOCSY spectra of progesterone. Grey box stands for a better visualization of the difference in the number of cross-peaks obtained from each experiment.

HSQMBC-TOCSY in other heteronuclei than ^{13}C

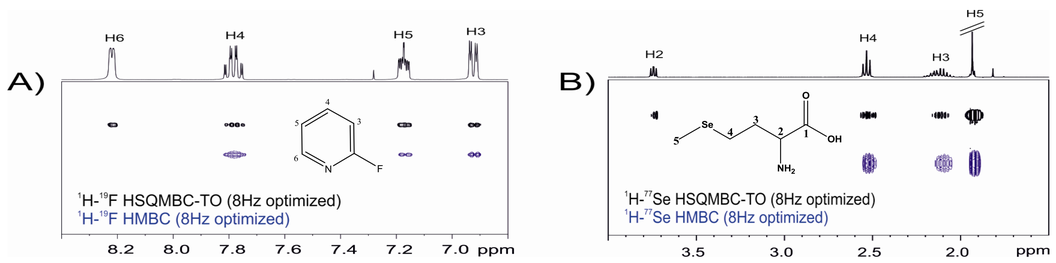

Fig. 3 shows how HSQMBC-TOCSY experiment is perfectly suitable for other heteronuclei than ^{13}C. A) Comparison of HMBC and ^{1}H-^{19}F-HSQMBC-TOCSY experiment in 2-fluoropyridine. B) Comparison of HMBC and ^{1}H-^{77}Se-HSQMBC-TOCSY experiment in Selenomethionine.

B) ^{1}H-selHSQMBC-TOCSY for the measurement of $^{n}J(CH)$

^{1}H- selective HSQMBC-TOCSY IPAP[1,2]

The selHSQMBC-TOCSY scheme (Fig. 1B) generates α/β multiplets (via IPAP methodology) by recording two separate and complementary in-phase (IP) and anti-phase (AP) data sets followed by a basic addition/subtraction data processing. The behavior of both J(HH) and ^{n}J(CH) evolution throughout the entire pulse sequence yields pure-phase multiplets and, in addition, the TOCSY period preserves the α/β ^{13}C spin-state information. The method allows the efficient measurement of the magnitude and the sign of ^{n}J(CH) by analyzing the relative displacement of α/β cross-peaks in the detected dimension with high resolution and accuracy even for very small values.

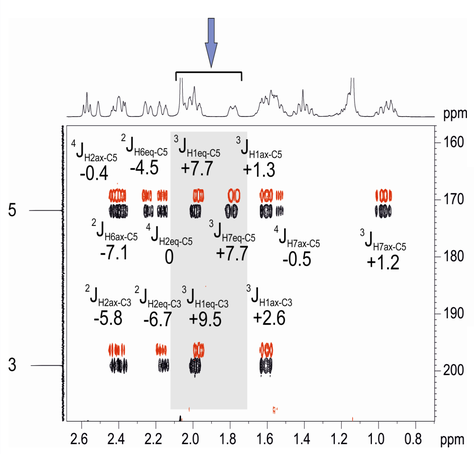

Fig. 4 Expanded area of the 2D ^{1}H-^{13}C α/β-selHSQMBC-TOCSY spectra of progesterone after selective inversion of all protons resonating in the region of 1.75–2.1 ppm.

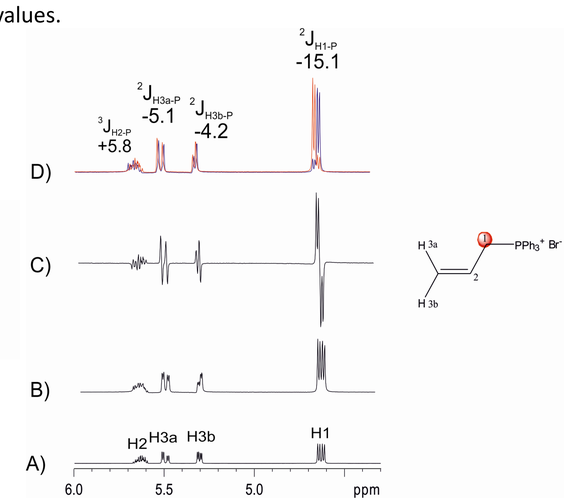

Fig. 5 1D ^{1}H-^{31}P α/β-selHSQMBC-TOCSY spectra of allyltriphenylphosphonium after selective inversion of H1 proton. A) ^{1}H NMR spectra, B) IP, C) AP, and D) α/β spectra.

C) Incorporating Multiplicity Editing in ^{1}H-selHSQMBC-TOCSY

A multiplicity-edited HSQMBC experiment (Fig. 1C) has been developed to distinguish directly long-range heteronuclear correlations belonging to even C/CH_2 carbons from those of odd CH/CH_3 carbons. The pure-phase spectrum offers a user-friendly representation where odd and even heteronuclear correlations can be directly ascertained from the different up/down signal pattern as traditionally performed in HSQC experiments.

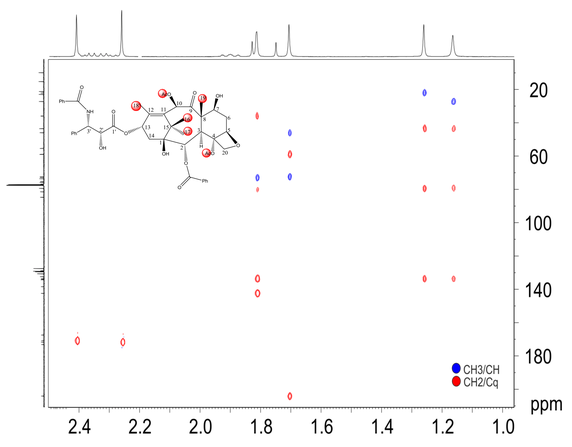

Fig. 6 2D ME-selHSQMBC spectrum after selective inversion of the methyl area in Taxol. CH_3/CH carbons show relative positive phase while CH_2/C show relative negative phase.

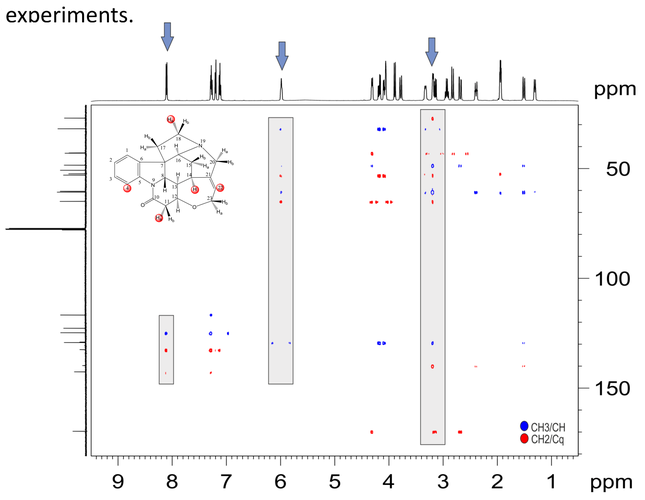

Fig. 7 2D ME-selHSQMBC-TOCSY spectrum after selective inversion of the protons H4, H22, and all the protons resonating at 3.15 ppm in strycnhine. CH_3/CH carbons show relative positive phase while CH_2/C show relative negative phase.

Conclusions

- Broadband version of HSQMBC-TOCSY works nicely as a complementary tool for structural elucidation when some cross-peaks are missing or present very low intensity in the HMBC experiment. In addition, TOCSY block is able to extend the information along the spin system including non-protonated carbon atoms. The experiment is also suitable for other heteronuclei than ^{13}C.
- ^{1}H-selHSQMBC-TOCSY IPAP experiment represents a definitive NMR solution for the measurement of the sign and the magnitude of small ^{n}J(CH) on protonated and non-protonated carbon atoms. The experiment is also suitable for other heteronuclei than ^{13}C.
- ^{1}H-ME-selHSQMBC-TOCSY experiment can become an important tool for chemical assignment proposes and structure elucidation of small molecules because provide long-range correlations and carbon multiplicity information in a single NMR experiment.

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1. J. Saurí, J. F. Espinosa, T. Parella. *Angew. Chem. Intl. Ed*. **2012**, 51, 3919-3922

2. J. Saurí, P. Nolis, T. Parella. *J. Magn. Reson*. 2013. DOI: http://dx.doi.org/10.1016/j.jmr.2013.08.013