On the interference of J(HH) modulation in HSQMBC-IPAP and HMBC-IPAP experiments

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Introduction

It is known that the evolution of homonuclear proton-proton coupling constants (J(HH)) during the long defocusing/refocusing periods in long-range heteronuclear correlation experiments, commonly referred as HMBC[1] or HSQMBC[2] experiments, causes important intensity and phase signal modulation effects. The resulting complex cross-peak shapes generally difficult a simple data analysis that often prevent an accurate and direct extraction of small proton-carbon coupling constant values ($^nJ(CH)$; n<1).

In this work, the effects of phase modulation due to J(HH) in HSQMBC-IPAP experiments are experimentally evaluated. We want to clarify if $^nJ(CH)$ can be efficiently measured from the resulting phase-distorted cross-peaks of a selHSQMBC experiment applied simultaneously on two mutually J-coupled protons. On the other hand, we want to extrapolate these rules in the evaluation of distorted cross-peaks obtained from broadband IPAP versions of equivalent HMBC and HSQMBC pulse trains. Finally, a discussion on the usefulness of complementary HMBC-COSY and HSQMBC-COSY experiments is made in order to detect and quantify $^nJ(CH)$ in a variety of conditions and especially when some expected cross-peaks are absent in original HMBC/HSQMBC experiments.

Methodology

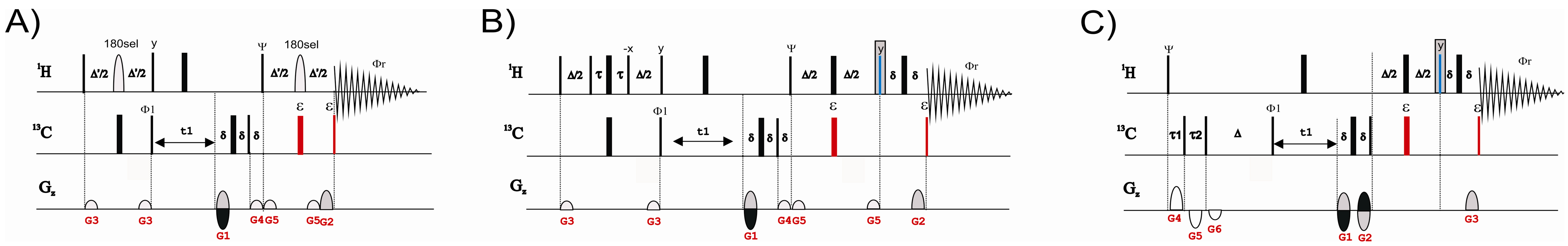


Figure 1: Pulse sequences for the IPAP versions of the 2D A) selHSQMBC, B) broadband HSQMBC and HSQMBC-COSY, and C) broadband HMBC and HMBC-COSY experiments. Rectangular 90° and 180° pulses are indicated by thin an thick black bars, respectively, and ^{1}H-selective 180° pulses as shaped bars. Phases are indicated above the pulses (where no phase is given, the pulse is applied along x). The basic phase cycle was ϕ_1=x,-x and ϕ_{rec}=x,-x. Two independent IP (Ψ = y, ε = on) and AP (Ψ = x, ε = off) data are initially collected and further combined to provide complementary α and β data (IP±AP) in separate spectra. In B), a BIRD element is inserted during the initial INEPT period to suppress direct $^1J(CH)$ correlations (τ=1/(2*$^1J(CH)$)). In C), direct correlations are attenuated by a two step low-pass J filter. The optional 90° ^{1}H pulse inserted into a box in B) and C) stands for COSY-like spectra. The inter-pulse delays were set to Δ= Δ'+p180=1/(2*$^nJ(CH)$, where p180 is the duration of selective 180° ^{1}H pulse. In selHSQMBC and broadband HSQMBC/HSQMBC-COSY experiments gradients were optimized to G1:G2:G3:G4:G5 = 80:20.1:11:50:17. In broadband HMBC/HMBC-COSY experiments the gradients were optimized to G1:G2:G3:G4:G5:G6 = 40:-40:20.1:15:-10:-5

Experimental

selHSQMBC experiment applied simultaneously on mutually J-coupled protons

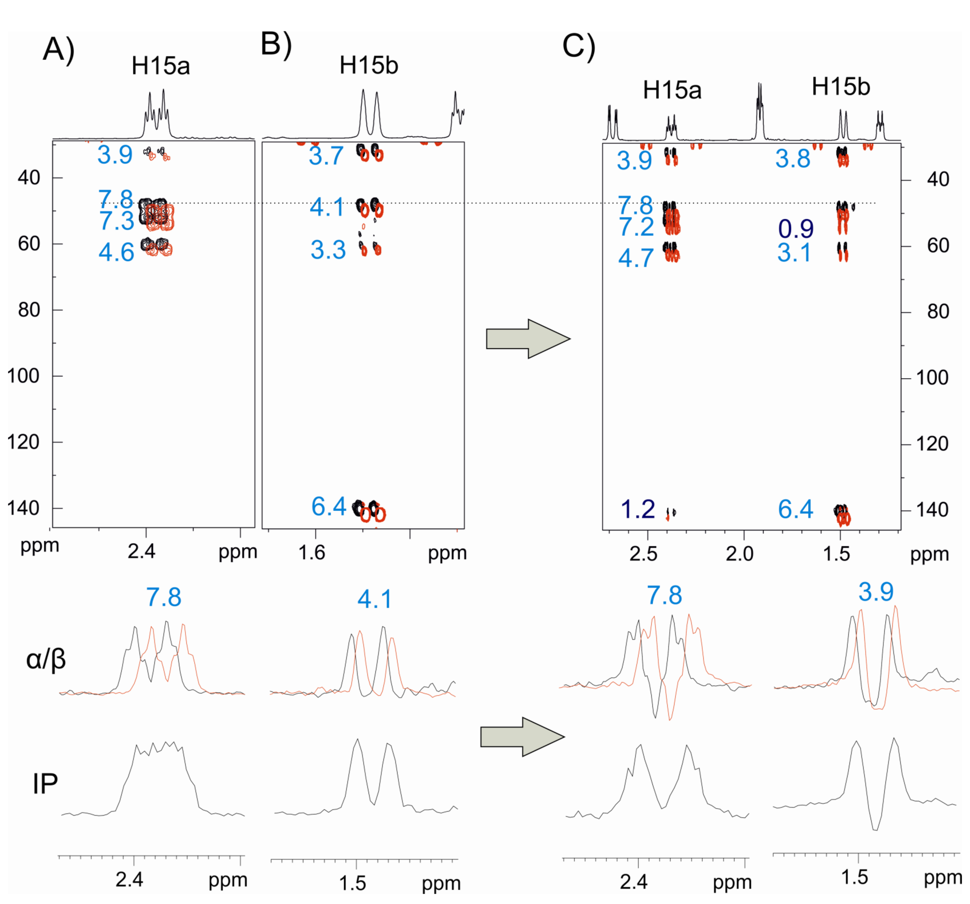


Figure 2: Effect of J(HH) modulation in selHSQMBC-IPAP experiments: A,B) single-frequency excitation and C) simultaneous dual-site excitation of H15b and H15a proton resonances. 1D traces taken at C13 carbon frequency are shown below. Whereas pure-phase IP and α/β components are obtained in A and B, distorted multiplets are obtained in C due to the evolution of its mutual J(HH). Even so, the extraction of $^nJ(CH)$ can be made even for α/β cross-peaks and with the same accuracy. The main explanation for such an observation is that IP and AP data only differ in the eventual application of ^{13}C pulses, and therefore, this does not produce a differential J(HH) modulation between them.

Evaluation of distorted cross-peaks obtained from broadband IPAP versions of HMBC & HSQMBC

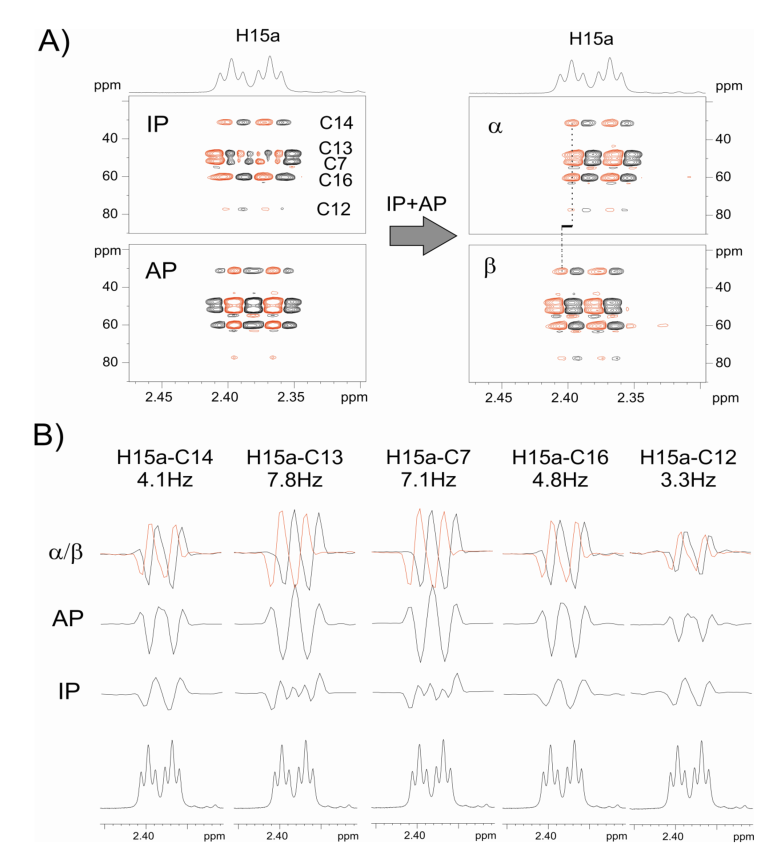


Figure 3: A) Expanded 2D areas corresponding to the H15a proton in IP/AP and α/β HMBC spectra, and B) 1D phase-twisted cross-peaks taken at different five carbon frequencies.

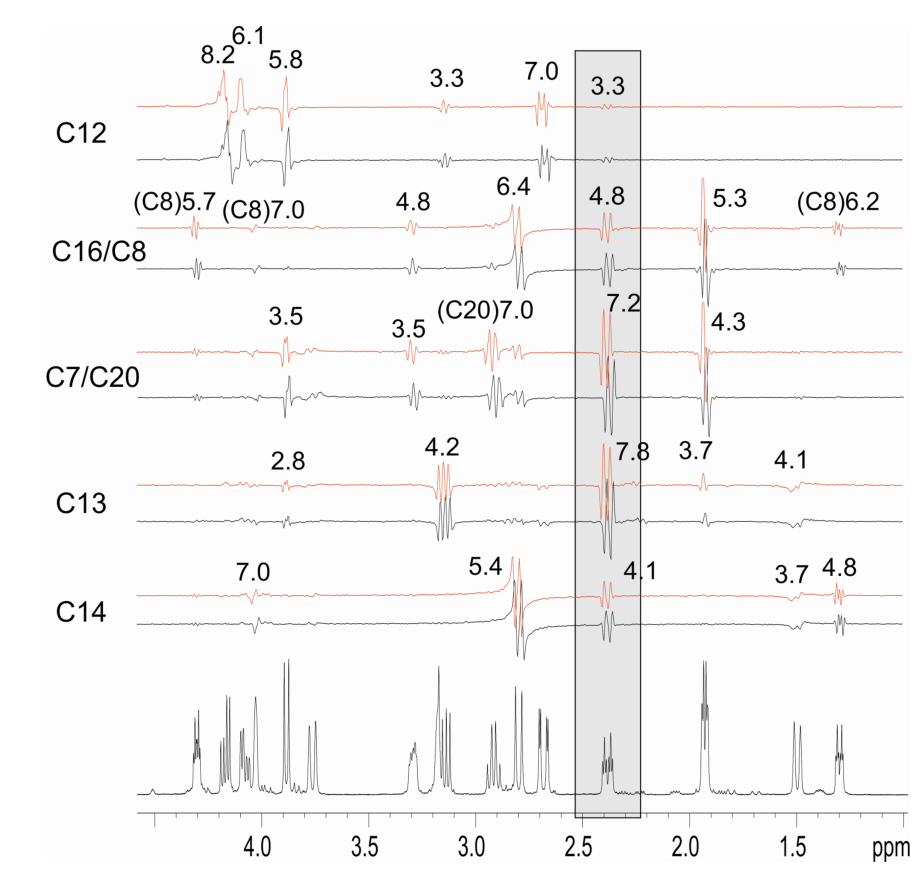
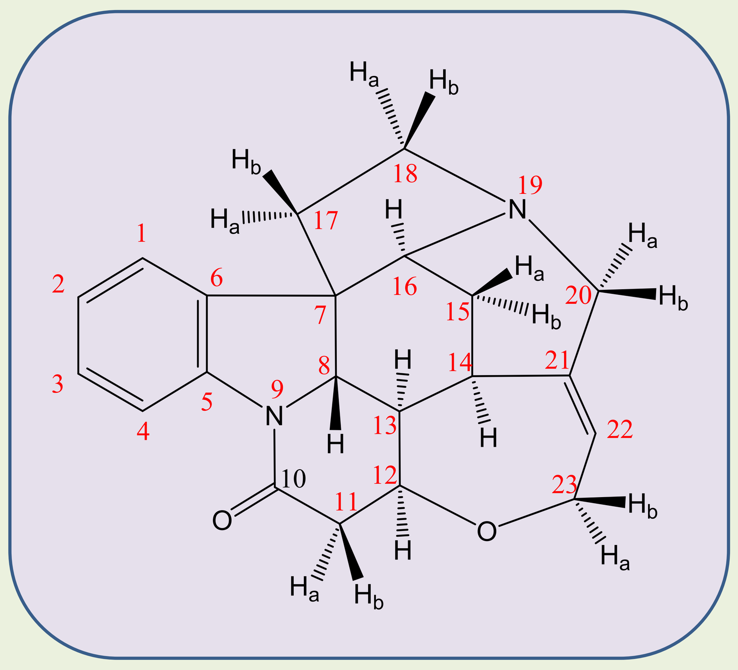


Figure 4: α/β 1D slices extracted from different carbon frequencies in the 2D HMBC-IPAP experiment optimized to 8 Hz using the pulse scheme of Fig. 1C.



HMBC-COSY and HSQMBC-COSY experiments as a complementary tool when some cross-peaks are missing

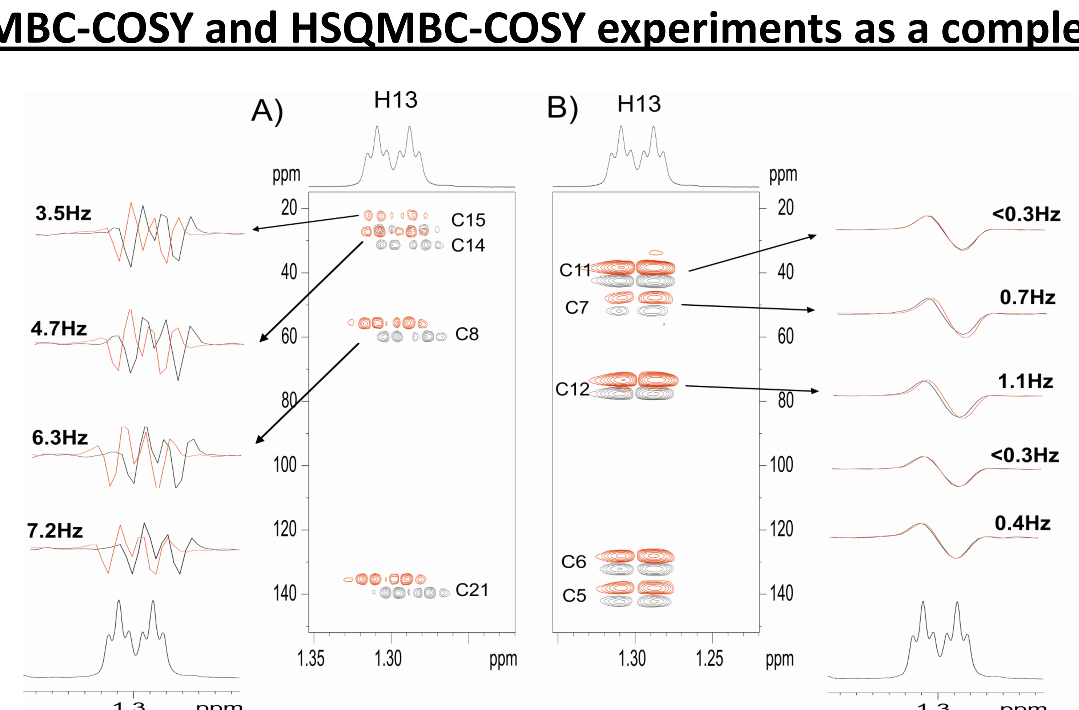


Figure 5: 2D expanded areas demonstrating the complementarity between A) HMBC-IPAP and B) HMBC-COSY-IPAP experiments. Whereas in HMBC, only the largest couplings from the H13 proton are observed and measured, very small $^nJ(CH)$ values can be extracted in the analog HMBC-COSY spectra.

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Conclusions

- Phase distortion caused by J(HH) modulation in selHSQMBC experiments is not a serious impediment for the successful performance of the IPAP technique on mutually coupled protons.
- IPAP technique can be successfully applied in both selective and non-selective versions, providing two equally distorted spin-state selective multiplets from which $^nJ(CH)$ can be measured along the F2 dimension irrespective of multiplet complexity and phase distortion.
- The concerted use of HMBC/HMBC-COSY experiments allows the detection and quantification of a complete set of $^nJ(CH)$, and particularly, HMBC-COSY can be a powerful method to measure very small $^nJ(CH)$ values in quaternary carbons.
- The proposed experiments can be applied to other heteronuclei X different to ^{13}C and therefore, they become general NMR methods to measure small heteronuclear $^nJ(XH)$ coupling constants.

1) R. A. E. Edden, J. Keeler. J. Magn. Reson. 2004, 166, 53–68. 2) R. T. Williamson, B. L. Márquez, W. H. Gerwick, K. E. Kövér. Magn. Reson. Chem. 2000, 38, 265–273.

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