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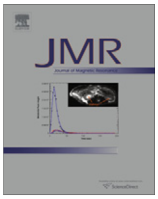

Accurate measurement of small heteronuclear coupling constants from pure-phase α/β HSQMBC cross-peaks

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ABSTRACT

A simple proton-selective α/β-HSQMBC experiment is proposed for the accurate measurement of long-range proton–carbon coupling constants ($^nJ_{CH}$) in small molecules without need for an individualized and time-consuming post-processing fitting procedure. The method acquires two pure-phase In-phase (IP) and Anti-phase (AP) multiplets completely free of any phase distortion due to the absence of J_{HH} evolution. Accurate $^nJ_{CH}$ values can be directly measured analyzing the relative displacement of the resulting IPAP cross-peaks. Discussion about signal intensity dependence and cross-talk is made for a range of experimental conditions. The robustness of the method is evaluated by comparing the $^nJ_{CH}$ value measured from the analysis of the three available IP, AP and IPAP multiplet patterns. Multiple-frequency and region-selective versions of the method can also be efficiently recorded provided that excited protons are not mutually coupled.

1. Introduction

For many years, it has been recognized that the use of long-range proton–carbon coupling constants ($^nJ_{CH}, n > 1$) is a very good complement to proton–proton coupling constants (J_{HH}) and/or NOE data for the structural and conformational analysis of natural-abundance molecules [1,2]. However, it is really surprising that the practical use of $^nJ_{CH}$ has been relatively scarce considering that most molecules are mainly composed by protons and carbons. It is known that these small $^nJ_{CH}$ coupling constants (ca. 0–10 Hz) present strong dependences with respect to coupling pathways, pattern substitutions and dihedral angles. However, the lack of extensive experimental data and trustworthy structural correlations often prevents its successful application to resolve routine problems.

The reason for this limited use of $^nJ_{CH}$ may be attributed to two main factors. First, there is no a single and general NMR method for their measurement [3,4] and, secondly, the accuracy of such measurements has always been a continuing source of discussion. Today is accepted that two general NMR strategies can be followed. On the one hand, HSQC-TOCSY pulse schemes [5–8] are the best approach to measure both the magnitude and the sign in protonated carbons but the method inevitably fails for non-protonated centers or when homonuclear TOCSY transfer is not efficient. Alternatively, long-range optimized correlation experiments (HMBC [9] and HSQMBC [10]) are highly suitable when quaternary carbons are involved. In these later experiments, the value of $^nJ_{CH}$ is usually extracted from an individualized and time-consuming post-processing fitting procedure of the resulting antiphase coupling pattern multiplets. Unfortunately, undesired mixed-phase multiplet distortions originated by the additional J_{HH}-coupling evolution during the long evolution INEPT-type periods introduce a common source of inaccuracy. The incorporation of G-BIRD and/or CPMG blocks into the INEPT periods has been proposed to minimize such modulations, but experimentally it is difficult to achieve this goal for all cross peaks into the same spectrum. In addition, the optimal setting of the inter-pulse delays [11] and pulse power levels [12] involved into the CPMG block is critical to minimize sample heating or heteroTOCSY/HEHAHA transfer whereas off-resonance effects and perfect suppression of J_{HH} modulation still remain a real challenge. Other related approaches have been also used, such as J-HMBC [13] or EXSIDE [14] experiments, which are based in the resolution and measurement of the $^nJ_{CH}$ value in the indirect carbon dimension.

In this work, a proton-selective version of the 2D IPAP-HSQMBC experiment [15] is proposed for the straightforward, direct and accurate measurement of $^nJ_{CH}$ in natural-abundance small molecules. The extraction of the $^nJ_{CH}$ value is realized without the need of the classical fitting procedure, simply by comparing the relative displacement of separate α- and β-cross-peaks that result of the time-domain addition/subtraction procedure of complementary In-Phase (IP) and Anti-phase (AP) HSQMBC data. In order to validate the accuracy of the measurement, the obtained $^nJ_{CH}$ value

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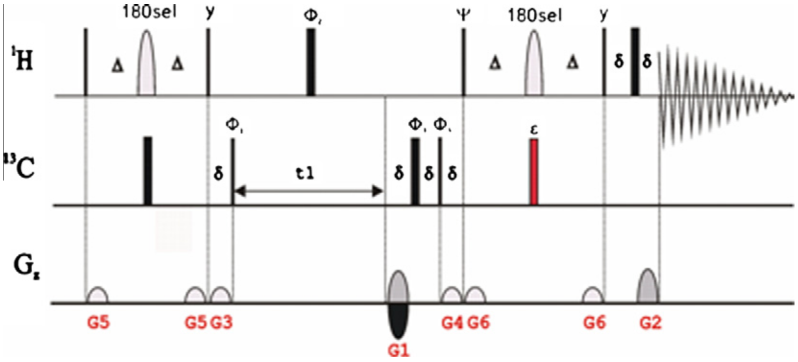

Fig. 1. Pulse scheme of the 2D proton-selective α/β-HSQMBC experiment. Proton 180° pulses applied in the middle of the evolution Δ (=1/(4$^{*n}J_{XH}$)) period can be frequency-selective, region-selection or a multiple-frequency. Two independent IP ($\Psi = y$, ε = on) and AP ($\Psi = x$, ε = off) data are initially collected and further combined to provide complementary α and β data (IP ± AP) in separate spectra.

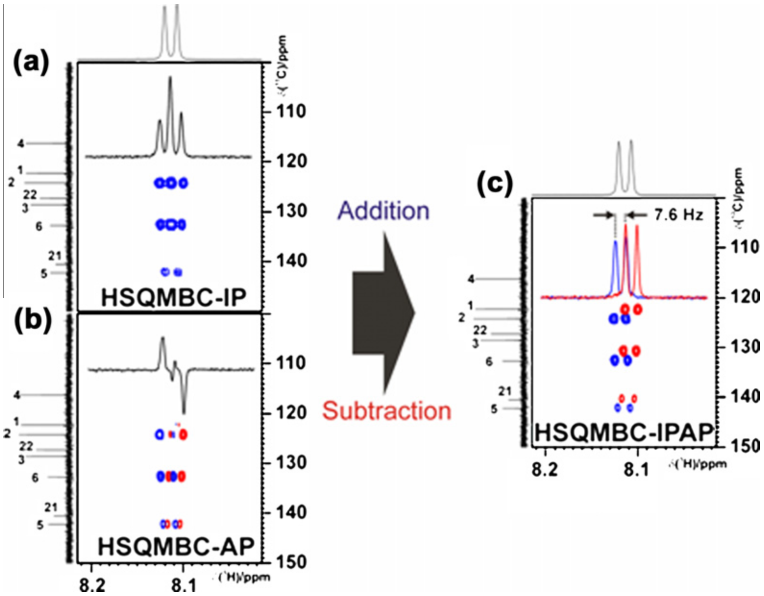

Fig. 2. Basic protocol to achieve α/β-HSQMBC spectra: 2D expanded areas corresponding to the H4 proton of strychnine in (a) HSQMBC-IP; (b) HSQMBC-AP and (c) spin-state-selective α/β-HSQMBC spectra are shown. Well separated α (blue) and β (red) cross-peaks permit an easy, direct and accurate measurement of $^nJ_{CH}$ by simple measurement of their relative displacements in the acquisition dimension.

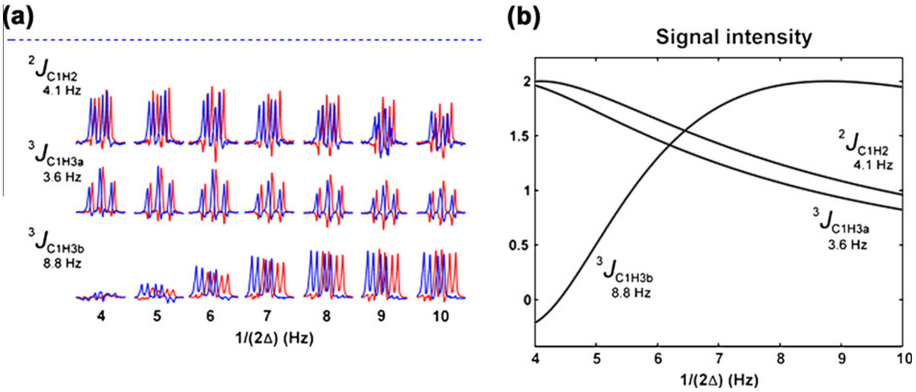

Fig. 3. (a) Experimental and (b) theoretical signal intensity dependences for several α/β-HSQMBC cross-peaks, involving the quaternary C1 carbon of DBPA, as a function of the optimized Δ period. Note clean IPAP editing for all range values that allow a general accurate measurement of $^nJ_{CH}$ irrespective of Δ optimization.

has been compared with those obtained from the acquired IP and AP multiplet patterns. A detailed discussion about the unwanted effects of cross-talk is also provided on a range of experimental conditions.