NMR Methods to Measure Residual Long-Range Proton-Carbon Dipolar Couplings in Aligned Anisotropic Media

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Residual Dipolar Couplings (RDCs) have a huge impact in the structure determination of biological macromolecules by NMR spectroscopy and their utility in the structure determination of small and medium-sized organic compounds is increasingly recognized. On the other hand, several new HSQMBC pulse schemes for measuring accurate heteronuclear long-range coupling constants ($^nJ_{CH}$; n >1) in solution conditions have recently been designed in our lab. In this work we evaluate some of these NMR methods to measure both the sign and the magnitude of long-range proton-carbon RDCs in a small molecule dissolved in a PMMA/$CDCl_3$ oriented media.

Methodology

Fig. 1 shows the pulse sequences used to measure $^nJ_{CH}$/$^nT_{CH}$: A) 2D ^{1}H-selective α/β-HSQMBC[1], B) 2D ^{1}H-selective α/β-HSQMBC-COSY[2] and C) 2D ^{1}H-selective α/β-HSQMBC-TOCSY[3] experiment. The most novel aspects of these experiments are: i) the application of frequency-selective 180º proton pulses that efficiently remove undesired J_{HH} coupling evolution during the INEPT transfer; ii) their application to measure $^nJ_{CH}$/$^nT_{CH}$ coupling constants on both protonated and non-protonated carbons, and iii) the availability to determine the sign and the magnitude of the coupling. In addition, our proposal uses the IPAP principle (**Fig.2**) where the extraction of $^nJ_{CH}$/$^nT_{CH}$ is realized by measuring of relative displacement of separate α- and β-cross-peak resulting of the addition/subtraction procedure of complementary pure-phase In-Phase (IP) and Anti-Phase (AP) HSQMBC data.

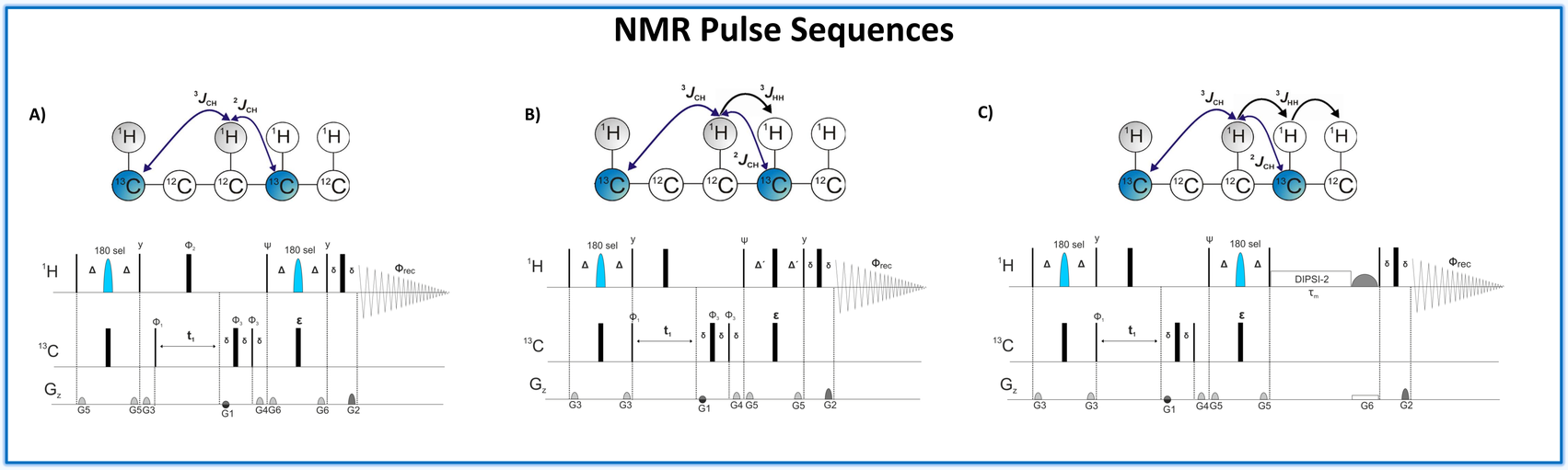


Figure 1. Pulse schemes and coupling pathways corresponding to the (A) 2D ^{1}H-sel α/β-HSQMBC, (B) 2D ^{1}H-sel α/β-HSQMBC-COSY and (C) 2D ^{1}H-sel α/β-HSQMBC-TOCSY experiments. Interpulse delays are optimized to Δ+p(180ºsel)/2=Δ´=1/(4* $^nJ_{CH}$). Two independent IP (Ψ=y, ε=on) and AP (Ψ=x, ε=off) data are collected as a function of ε and processed as described in Fig. 2.

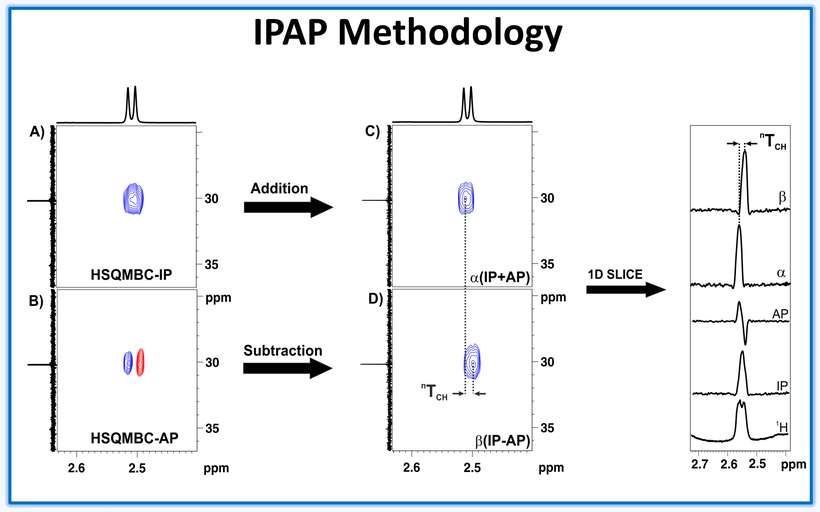
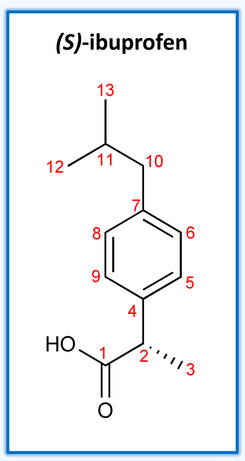


Figure 2. Basic protocol of the IPAP principle: (A) In-phase (IP) and (B) Anti-Phase (AP) data are acquired and further combined (AP±k*IP, where k is an optional scaling factor) to provide pure-phase (C) α–HSQMBC and (D) β–HSQMBC spectra.

Experimental Part

RDCs are observed as an additional contribution to the line splitting. The line splitting in anisotropic media is the sum of scalar coupling ($^nJ_{CH}$) and residual dipolar coupling ($^nD_{CH}$) and usually called total coupling constant ($^nT_{CH}$). Experimentally, two measurements are needed to obtain RDCs values: i) in isotropic solution to obtain $^nJ_{CH}$ and ii) in anisotropic media to obtain $^nT_{CH}$. Thus, $^nD_{CH}$ is obtained from the difference between the coupling values measured in both experiments.

$$^nT_{CH} = {}^nJ_{CH} + {}^nD_{CH}$$



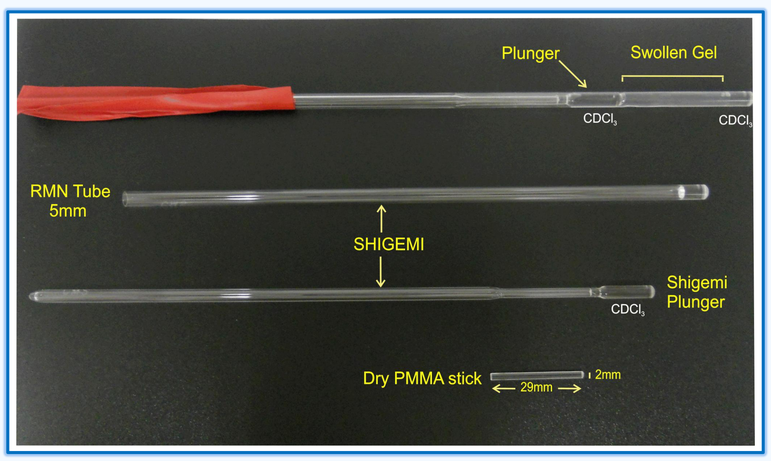


Figure 3. Materials needed for sample preparation.

The anisotropic alignment media used here is a strained aligning gel (SAG) of poly(methylmethacrylate) (PMMA).[4] The cross linked PMMA gel shows very good alignment properties when swollen with $CDCl_3$ whereas it presents small polymer background signal in HSQMBC spectra. The dry polymer stick was swollen inside of NMR tube with $CDCl_3$ only in the radial direction by blocking the vertical growth through a Shigemi tube plunger (**Fig. 3**). The solute used in this study was (*S*)-ibuprofen.

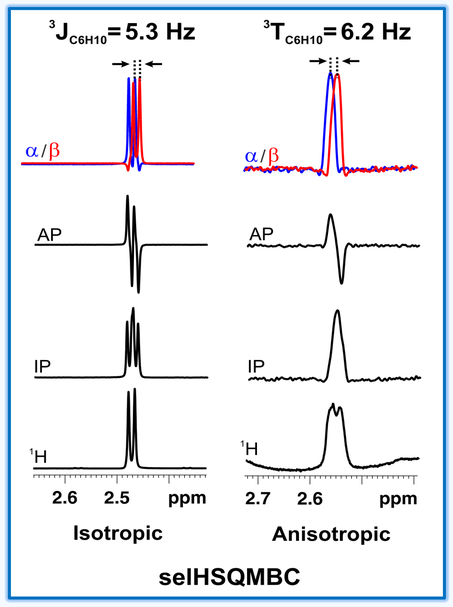


Figure 4. 1D slices extracted at the C_6 carbon frequency of the 2D H_{10}-selHSQMBC spectra in isotropic and anisotropic conditions.

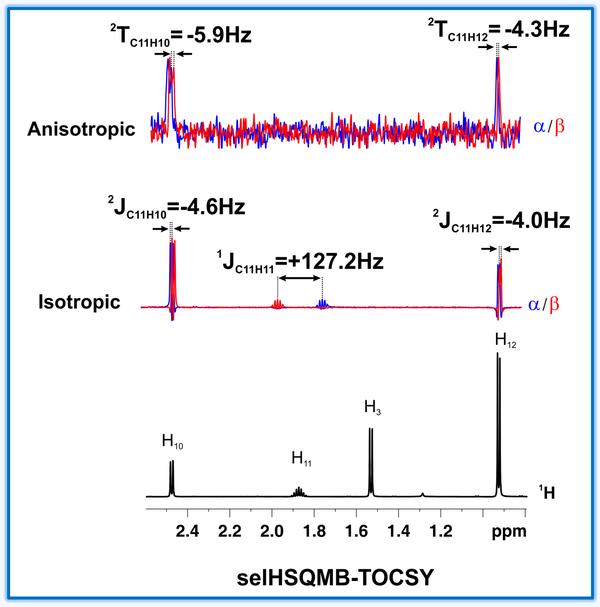


Figure 5. 1D slices extracted at the C_{11} carbon frequency of the 2D H_{10}-selHSQMBC-TOCSY spectra in isotropic and anisotropic conditions.

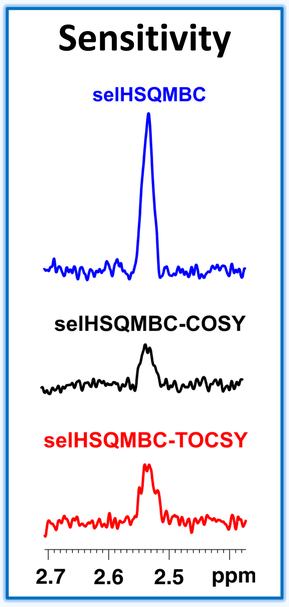


Figure 6. 1D slices showing the relative sensitivity of the pulse sequences displayed in Fig. 1. The signal corresponds to the H_{10}-C_7 cross-peaks in anisotropic media.

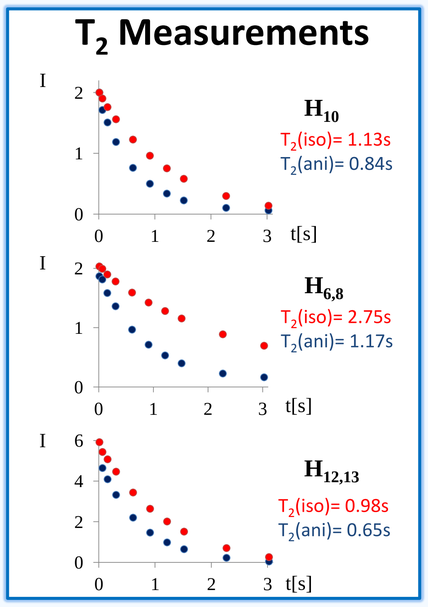


Figure 7. Curves of T_2 decays of some protons of ibuprofen in isotropic (red) and anisotropic (blue) media measured from the recently proposed CPMG-based PROJECT experiment.[5]

Conclusion

Although sensitivity is affected by the shorter T_2 values in anisotropic conditions, selHSQMBC experiments prove to be excellent NMR methods to obtain the sign and the magnitude of small heteronuclear scalar and residual dipolar coupling constants with high accuracy and simplicity. The combination of pure-phase multiplets and IPAP technique allows their application even in broad, non-resolved and complex multiplets.

References

[1] S. Gil, J.F. Espinosa, T. Parella, *J. Magn. Reson.* **2011**, 213, 145.

[2] J. Saurí, T. Parella, *Magn. Reson. Chem.* **2012,** In Press.

[3] J. Saurí, J.F. Espinosa, T. Parella, *Angew. Chem. Intl. Ed.* **2012,** 124, 3985.

[4] C. Gayathri, N. V. Tsarevsky, R. Gil. *Chem. Eur. J.* **2010,** 16, 3622.

[5] J.A. Aguilar, M. Nilsson, G. Bodenhausen, G.A. Morris, *Chem. Comm.* **2012,** 48, 811.