Selecting the Most Appropriate NMR Experiment to Access Weak and/or Very Long-Range Heteronuclear Correlations

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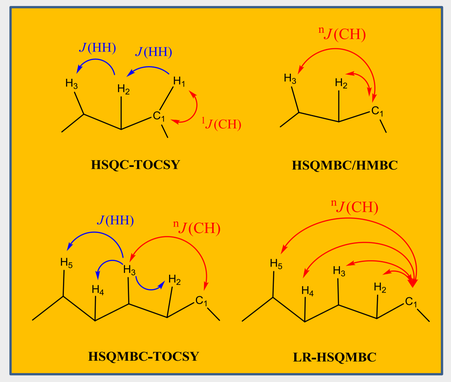
Introduction

Heteronuclear long-range NMR experiments are well established as essential NMR techniques for the structure elucidation of unknown natural products and small molecules. It is generally accepted that the absence of a given $^{n}J(XH)$ correlation in an HMBC or HSQMBC spectrum would automatically place the proton at least four bonds away from the carbon in question. This assumption can, however, be misleading in the case of a mismatch between the actual coupling constant and the delay used to optimize the experiment, which can lead to structural misassignments. Another scenario arises when an investigator, for whatever reason, needs to have access to very long-range correlations to confirm or refute a structure. In such cases, a conventional HMBC experiment will most likely fail to provide the requisite correlation, regardless of the delay optimization. Two recent methods for visualizing extremely weak or very long-range connectivities are the **LR-HSQMBC**[1] and the **HSQMBC-TOCSY**[2] experiments. Although they are intended to provide similar structural information, they utilize different transfer mechanisms, which differentiates the experiments, making each better suited for specific classes of compounds. In this work we have sought to examine the considerations implicit in choosing the best experiment to access weak or very long-range correlations for different types of molecules.[3]



Methodology

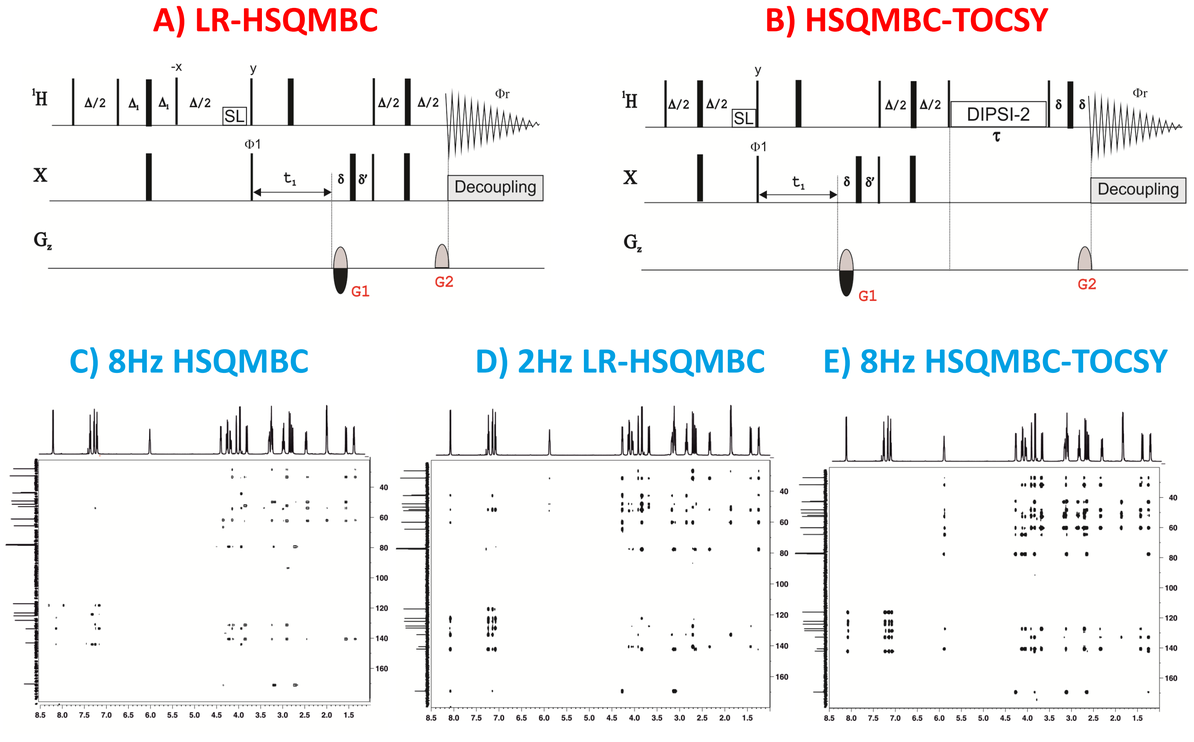
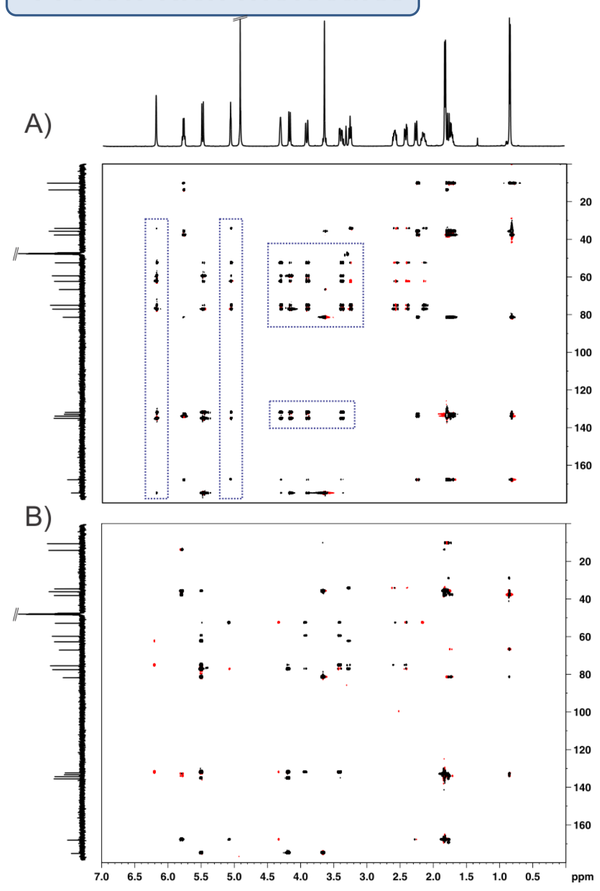


Figure 1: Pulse schemes for the A) LR-HSQMBC and B) HSQMBC-TOCSY experiments. The delay Δ is set to $1/[2*^{n}J(CH)]$, all ^{13}C 180º pulses were adiabatic CHIRP pulses for broadband inversion and refocusing, and broadband heteronuclear decoupling is applied during proton acquisition. In order to illustrate the different number of correlations observed in each experiment, the C) conventional 8-Hz optimized HSQC, D) 2-Hz optimized LR-HSQMBC and E) 8-Hz optimized HSQMBC-TOCSY (60ms) spectra of the alkaloid strychnine are shown.

Proton-Rich Molecules



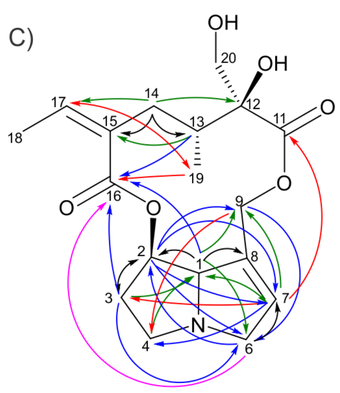
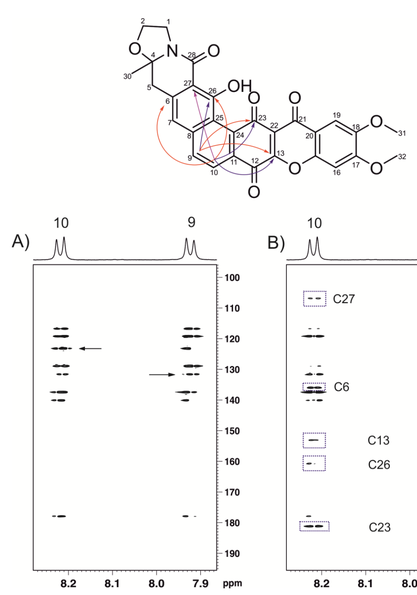


Figure 2. A) 8-Hz HSQMBC-TOCSY (mixing time was set to 60 ms) and B) 2-Hz LR-HSQMBC experiments of retrorsine. Both experiments were acquired under the same acquisition and processing conditions. Thirty-four additional correlations were obtained in A, most of them highlighted in the boxed regions. All additional correlations obtained in the HSQMBC-TOCSY that do not appear in the LR-HSQMBC are summarized on the structure shown in C. Color code: black arrows correspond to two-bond correlation, green arrows signify three-bond correlation, blue arrows denote four-bond correlation, red arrows indicate five-bond correlation, and the purple arrow identifies a six-bond correlation from H-6 to carbonyl C-16.

Proton-Deficient Molecules



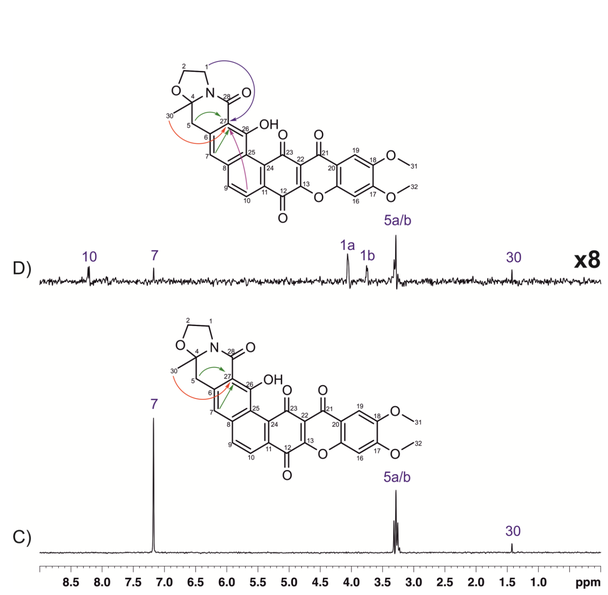


Figure 3. Expanded areas corresponding to the (A) 8-Hz HSQMBC-TOCSY and (B) 2-Hz LR-HSQMBC spectra, showing correlations for the aromatics protons H-10 and H-9 of cervinomycin A2. For these two protons, eight additional correlations were observed in B as marked in the chemical drawing. Color code: green arrows signify three-bond correlation, blue arrows denote four-bond correlation, red arrows indicate five-bond correlation, and the purple arrow identifies a six-bond correlation. C-D) 1D slices extracted at C27.

^{1}H-^{15}N HSQMBC-TOCSY vs LR-HSQMBC

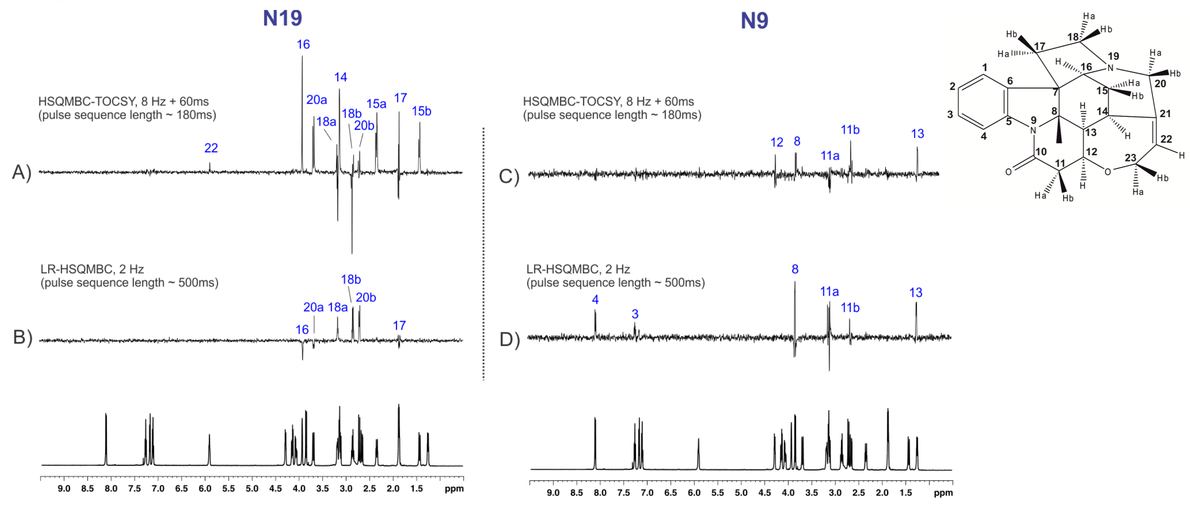
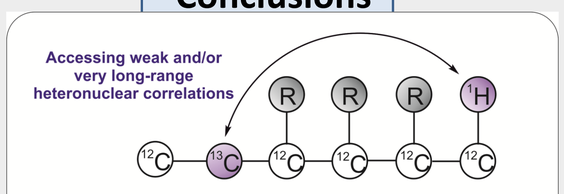


Figure 4. 1D slices extracted at both N-19 and N-9 nitrogens from the 2D ^{1}H–^{15}N HSQMBC-TOCSY and the ^{1}H–^{15}N LR-HSQMBC spectra of strychnine.

Conclusions



	HSQMBC-TOCSY	LR-HSQMBC
R = ^{1}H	GOOD s/n LARGE number of additional correlations	MEDIUM s/n MEDIUM number of additional correlations
R ≠ ^{1}H	GOOD s/n SMALL number of additional correlations	MEDIUM s/n LARGE number of additional correlations

References:

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3. J. Saurí, Y. Liu, T. Parella, R.T. Williamson, G.E. Martin, *J. Nat. Prod.* **2016**, 79, 1400-1406.

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