Extending long-range heteronuclear connectivities by modified HSQMBC Experiments

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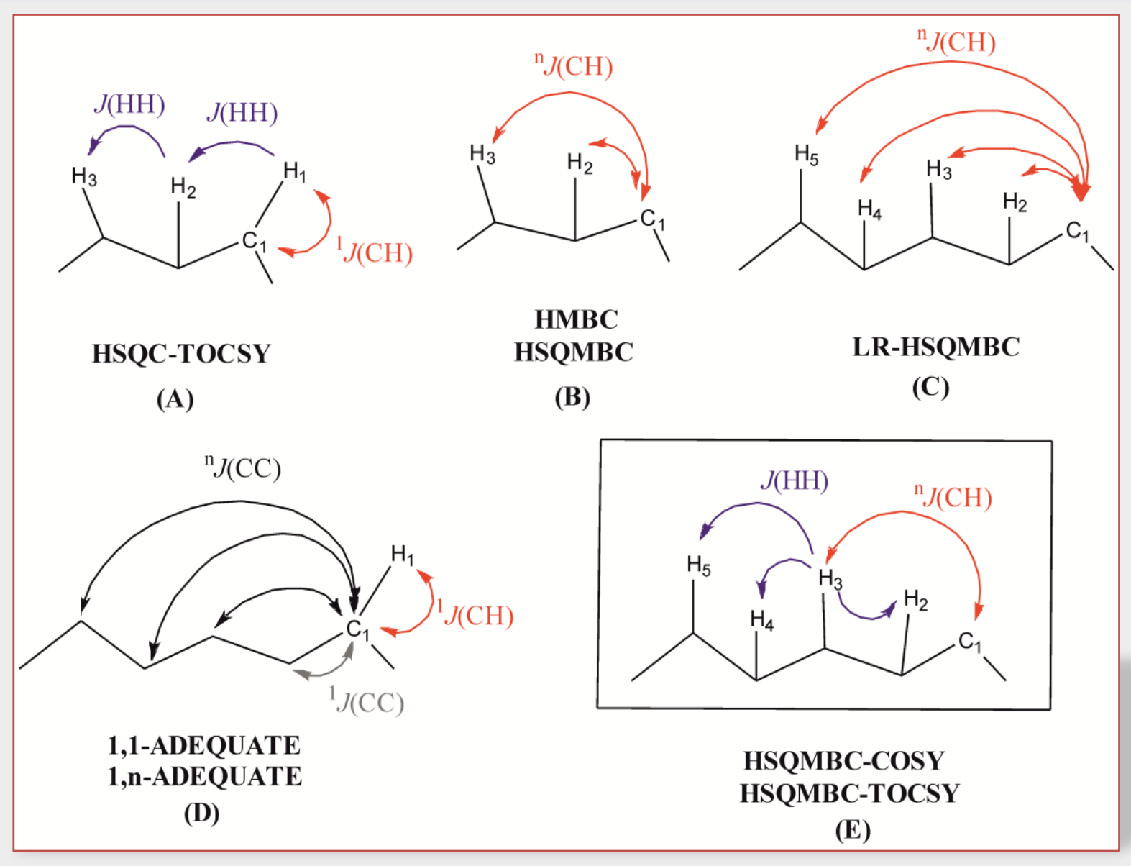
Introduction

The detection of long-range heteronuclear correlations presenting *J*(CH) coupling values smaller than 1-2 Hz is a challenge in the structural analysis of small molecules and natural products. HSQMBC-COSY and HSQMBC-TOCSY pulse schemes are evaluated as complementary NMR methods to standard HMBC/HSQMBC experiments. Incorporation of an additional *J*(HH) transfer step in the basic HSQMBC pulse scheme can favor the sensitive observation of traditionally missing or very weak correlations and, in addition, facilitates the detection of a significant number of still longer-range connectivities to both protonated and non-protonated carbons under optimum sensitivity conditions. A comparative ^{1}H-^{13}C study is performed using strychnine as a model compound and several examples are also provided including ^{1}H-^{15}N applications.



Methodology

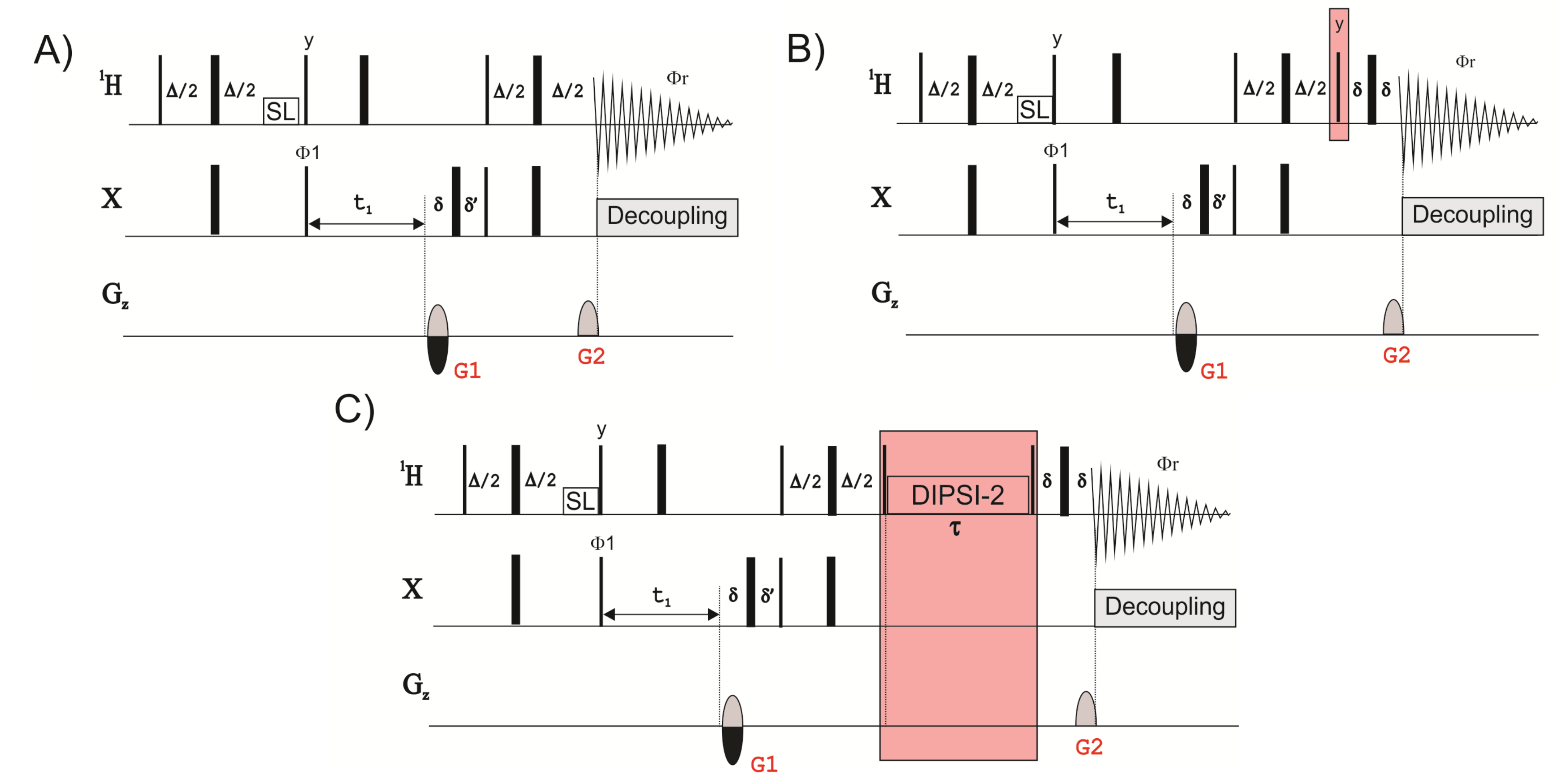


Figure 1: Pulse schemes for the A) HSQMBC, B) HSQMBC-COSY, and C) HSQMBC-TOCSY heteronuclear correlation experiments. The delay Δ is set to $1/[2*{}^{n}J(CH)]$ and all ^{13}C 180º pulses were adiabatic CHIRP pulses for broadband inversion and refocusing and broadband heteronuclear decoupling is applied during proton acquisition. The added COSY and TOCSY blocks in B and C are marked with a box.

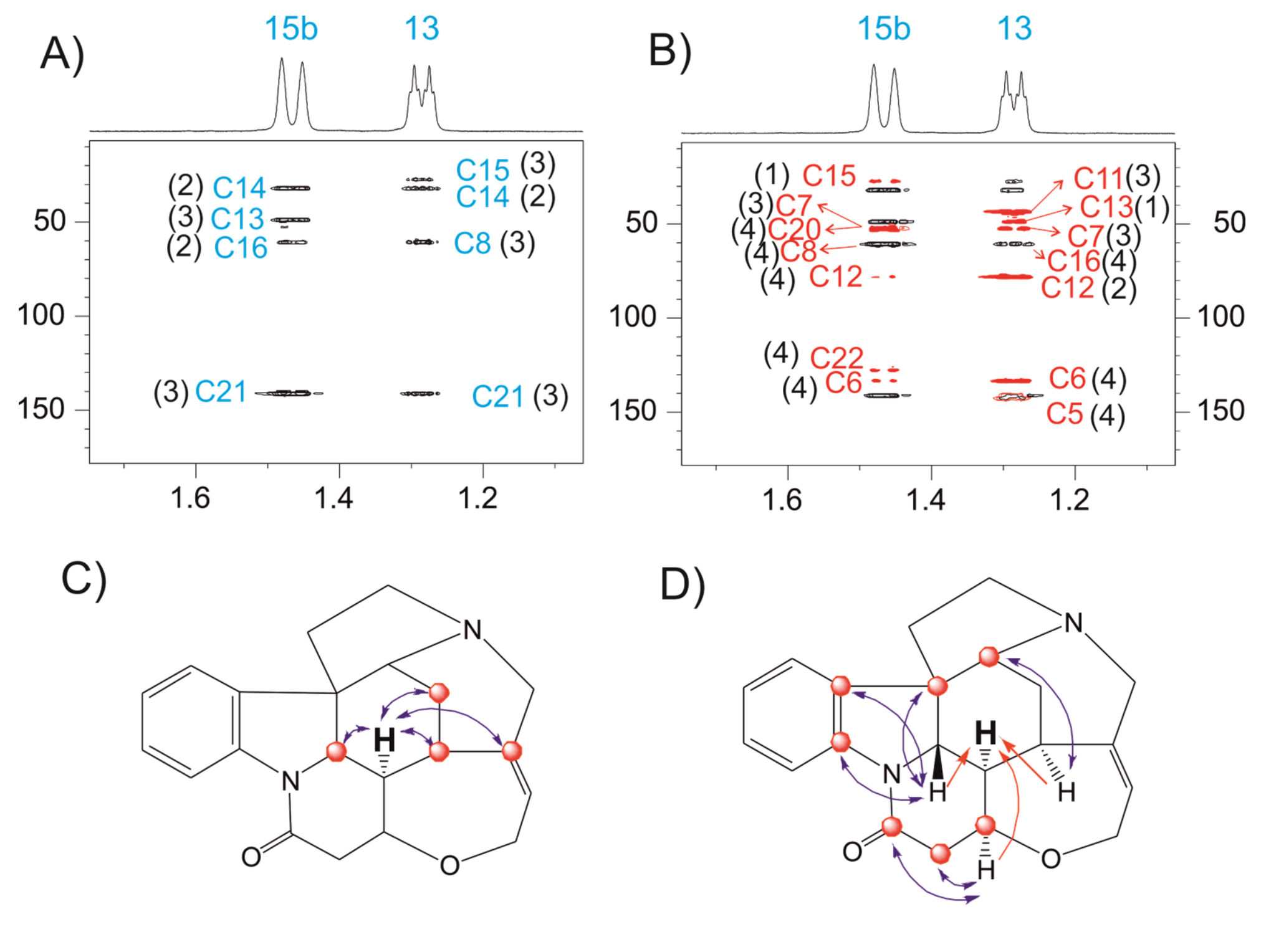


Figure 3: Expanded areas corresponding to the A) 8 Hz HSQMBC (see Figure 2A) and B) 8 Hz HSQMBC-TOCSY spectra with a 40 ms TOCSY mixing time of **1**. The number of bonds across which the correlation is observed is shown parenthetically. Schematic illustration showing the C) direct ^{1}H-^{13}C HSQMBC and D) relayed HSQMBC-TOCSY correlations observed for the H13 proton.

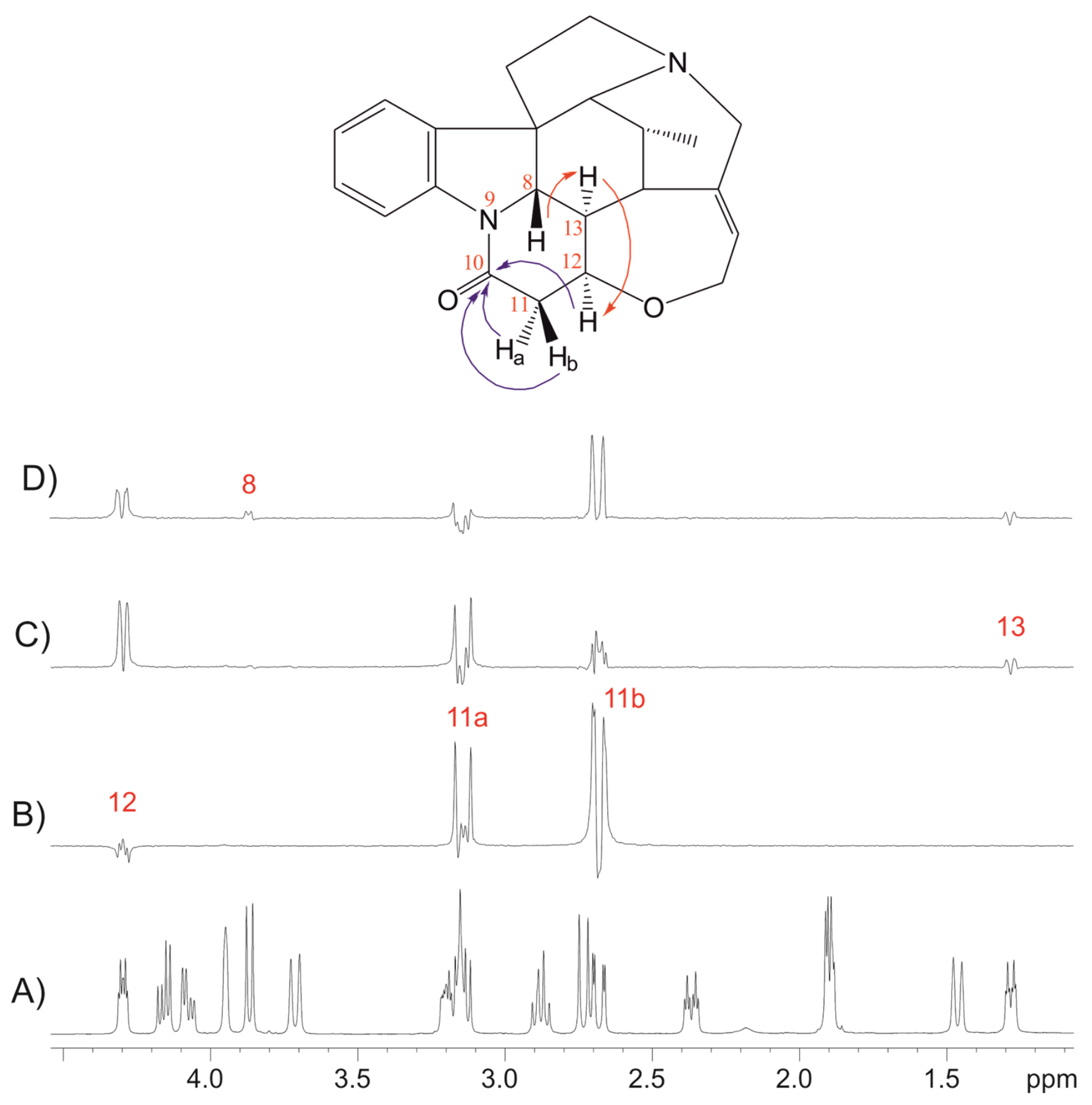


Figure 4: 1D slices corresponding to the C10 carbon frequencies extracted from the B) 8 Hz HSQMBC and C-D) 8 Hz HSQMBC-TOCSY (40 and 60 ms, respectively) spectra of **1**. Purple arrows stand for correlations from B whereas the red arrows stand for correlations from C-D).

NMR Spectra

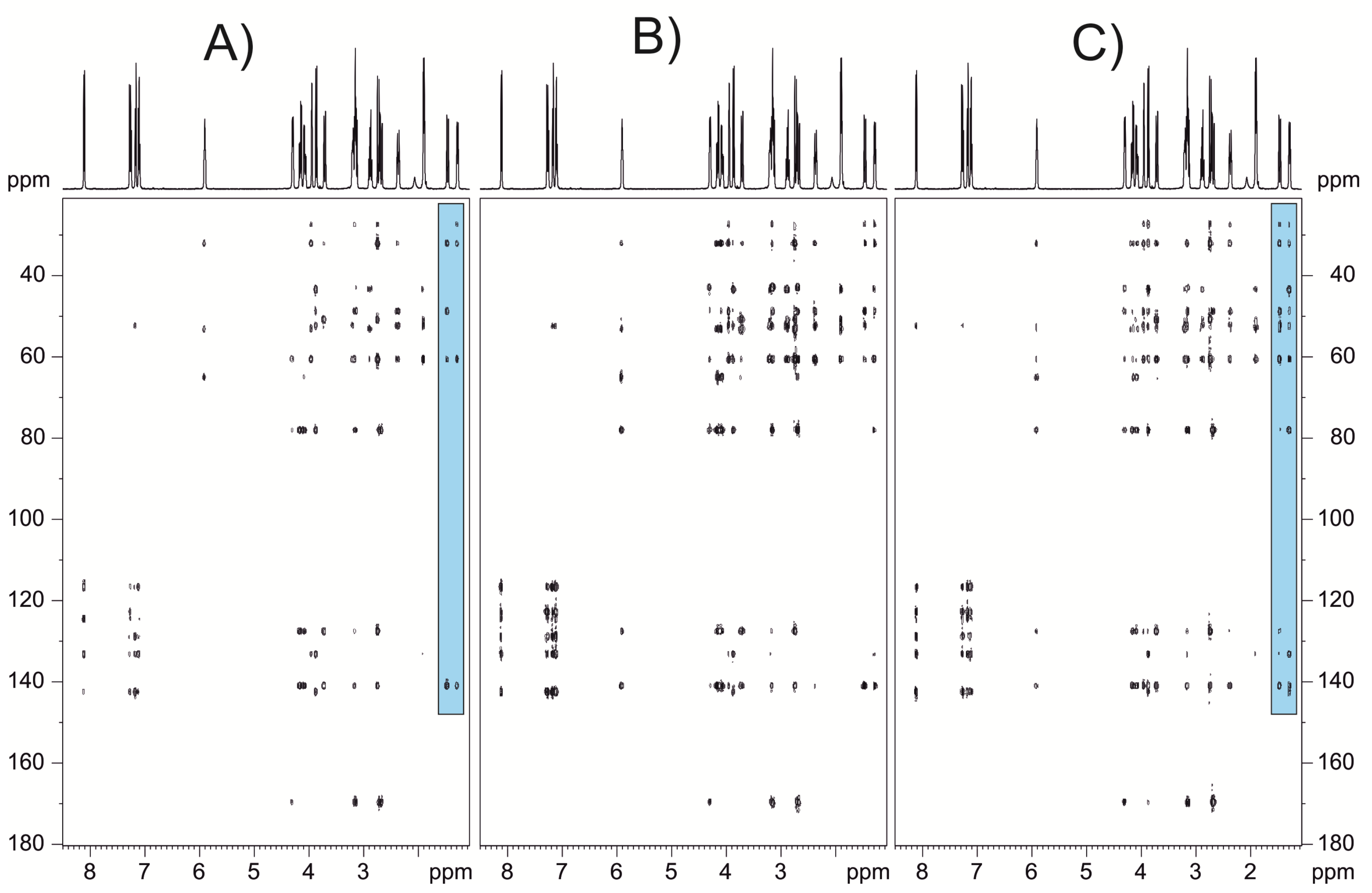
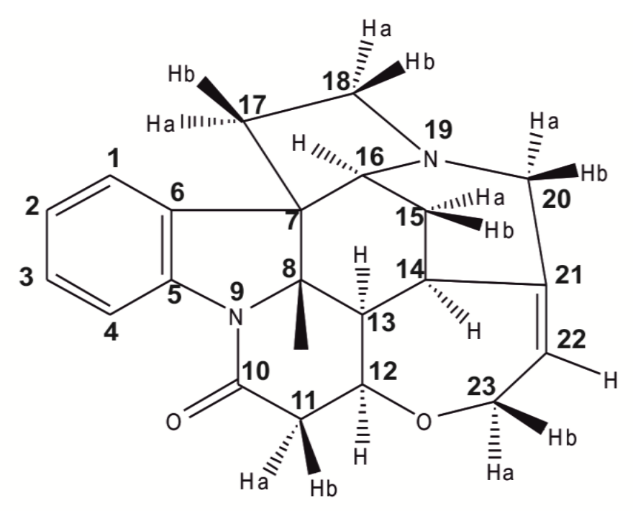


Figure 2: 8 Hz optimized A) HSQMBC, B) HSQMBC-COSY and C) HSQMBC-TOCSY spectra of the alkaloid strychnine (**1**). For a detailed comparison, the marked areas are expanded in Fig. 3.



	^{2}J	^{3}J	^{4}J	^{5}J	^{6}J	Total $>^{3}J$	Total
2 Hz LR-HSQMBC [a]	33	59	55	11	2	68	160
2 Hz D-HMBC [a]	29	43	34	8	2	44	116
2 Hz HMBC [a]	34	54	43	10	1	54	142
8 Hz HMBC [a]	34	53	36	4	1	41	129
HSQC_TOCSY [b]	23	15	11	2	0	13	51
8 Hz HSQMBC-Refoc [c]	36	58	40	1	0	41	135
8 Hz HSQMBC-COSY [d]	38	58	58	14	2	74	170
8 Hz HSQMBC-TOCSY [e]	38	58	65	18	3	86	182

Table 1: Comparison of the number and the nature of long-range heteronuclear responses observed in different NMR experiments performed on **1**.

[a] Taken from: R.T: Williamson, A.V. Buevich , G.E. Martin, T. Parella , *J Org Chem.* 2014;79:3887-3894.
[b] Measured in this work using the pulse sequence of Fig. 1C optimized to 140 Hz.
[c] Measured in this work using pulse sequence of Fig. 1A optimized to 8 Hz.
[d] Measured in this work using the pulse sequence of Fig. 1B optimized to 8 Hz.
[e] Measured in this work using the pulse sequence of Fig. 1C optimized to 8 Hz and using a mixing time of 40 ms.

PIP-HSQMBC-TOCSY

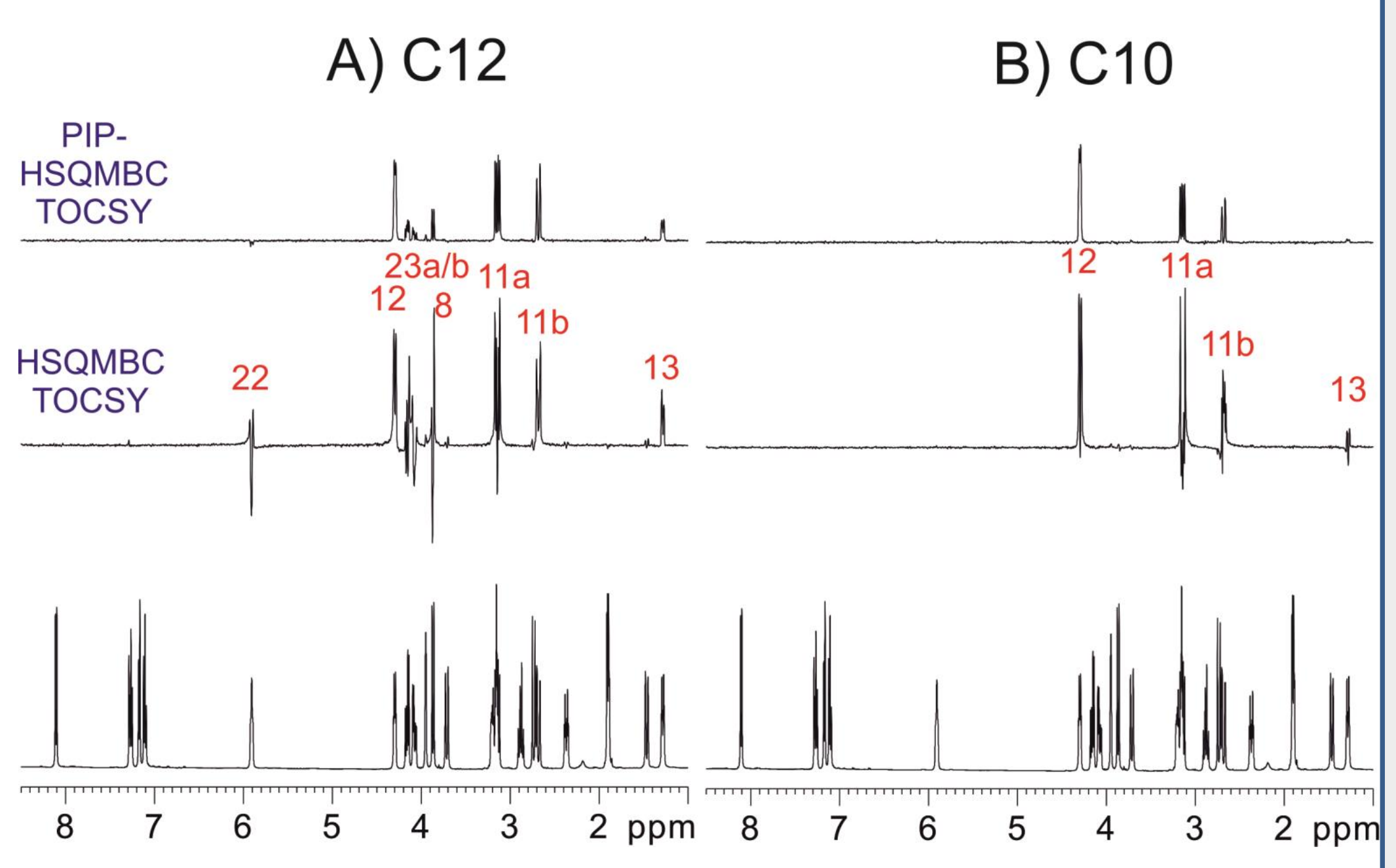


Figure 5: 1D slices extracted from the A) C12 and B) C10 carbon frequencies of the 8 Hz optimized HSQMBC-TOCSY spectrum of **1** acquired (middle) without and (top) with a ZQF element inserted after the z-filtered DIPSI-2 element (40 ms) in Fig. 1C.

Non-Uniform Sampling

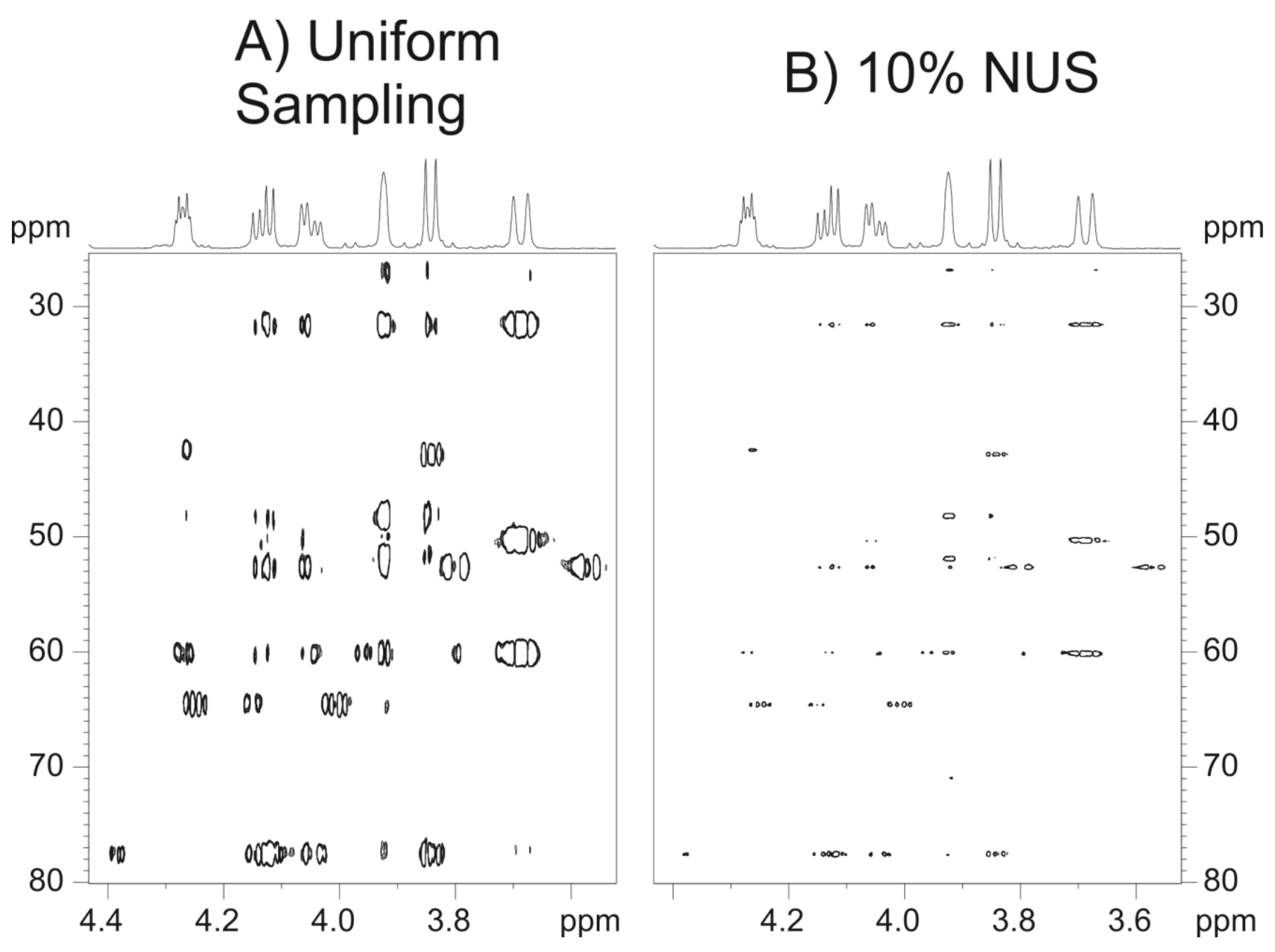


Figure 6: Comparison of 8 Hz optimized HSQMBC-TOCSY spectra of **1** acquired with A) uniform sampling (128 t_1 increments) and B) 10% NUS. Both datasets have been acquired with the same experimental time (14 min).

Conclusions

- HSQMBC-COSY and HSQMBC-TOCSY experiments can be valuable and complementary tools to the conventional HSQMBC experiment.
- These experiments can provide additional ^{1}H-^{13}C correlations even in the extreme case that the corresponding $^{n}J(CH) \sim 0$ Hz.
- They offer better sensitivity than some complementary experiments like the recently proposed LR-HSQMBC that use longer evolution delays or the less sensitive ADEQUATE or HCNMBC experiments, which are based on ^{13}C-^{13}C and ^{13}C-^{15}N transfers at natural abundance, respectively.
- The HSQMBC-TOCSY experiment can be easily tuned for quantitative measurements using the PIP-HSQMBC-TOCSY version which affords pure in-phase multiplets amenable for a direct determination of $^{n}J(CH)$ or using a coupled/decoupled approach.
- NUS can be incorporated providing important gains in resolution along the F1 dimension or reducing experimental times
- Application to any type of heteronucleus is perfectly suitable, for instance ^{1}H-^{15}N correlation experiments.

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Reference: J. Saurí, N. Marcó, R.T. Williamson, G.E. Martin and T. Parella, J. Magn. Reson., in press (2015)

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