Multiplicity editing in long-range heteronuclear correlation NMR experiments: Application to natural products

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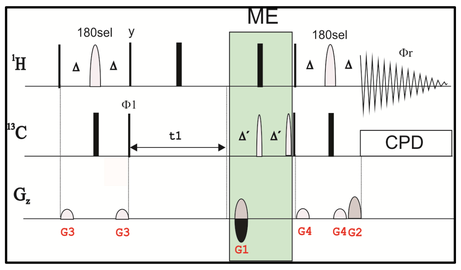
ABSTRACT

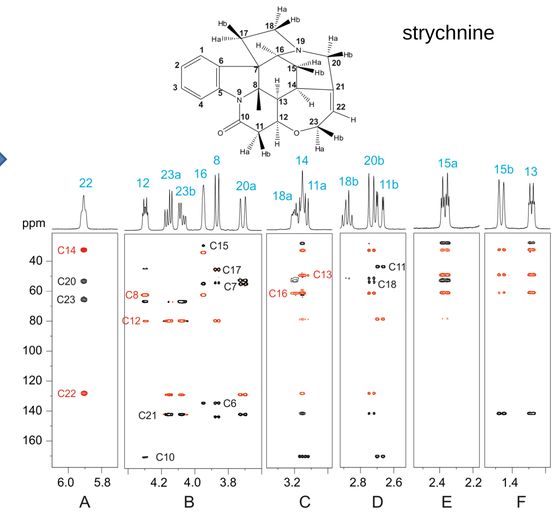
Even C/CH_2 and odd CH/CH_3 carbon-multiplicity information can be directly distinguished from the relative positive/negative phase of cross-peaks in a versatile Multiplicity-Edited ME-selHSQMBC experiment[1]. Optionally, the method can be extended by a TOCSY propagation step, and it is fully compatible with the precise and easy determination of long-range heteronuclear coupling constants ($^nJ_{CH}$). In addition, broadband homonuclear decoupling techniques can also be incorporated to enhance sensitivity and signal resolution by effective collapse of J_{HH} multiplets and to determine $^nJ_{CH}$ from simplified multiplets. The different features of the method are illustrated in the structure elucidation of several natural products.

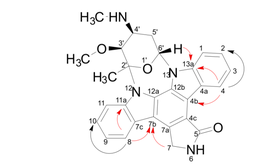
ME-selHSQMBC

Neglecting relaxation effects, the signal intensity of a given long-range correlation H-C_m in a ME-selHSQMBC experiment shows a dependence on $\sin^2(2\pi^nJ(CH)\Delta)*\cos^m(2\pi^1J(CH)\Delta')$, where *m* refers to the carbon multiplicity (m=0-3). Thus, cross-peaks belonging to a C/CH_2 carbon will show a positive intensity whereas those originating from CH/CH_3 carbons will present an opposite, negative intensity.

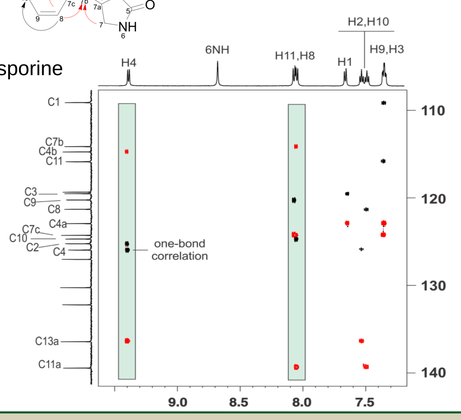


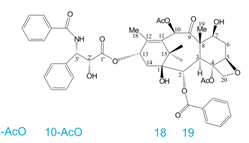
Pulse sequence scheme of the ME-selHSQMBC experiment. ^{1}H-Selective 180º pulses are applied in the middle of the INEPT blocks (Δ+p(180ºsel)/2=1/(4*$^nJ_{CH}$), where p180 is the duration of the selective 180º ^{1}H pulse) and ^{1}H data are acquired with broadband ^{13}C heteronuclear decoupling. The carbon-multiplicity editing block (Δ'=1/(2*^{1}J(CH)) includes a pair of shaped sweep synchronized adiabatic 180º ^{13}C pulses.



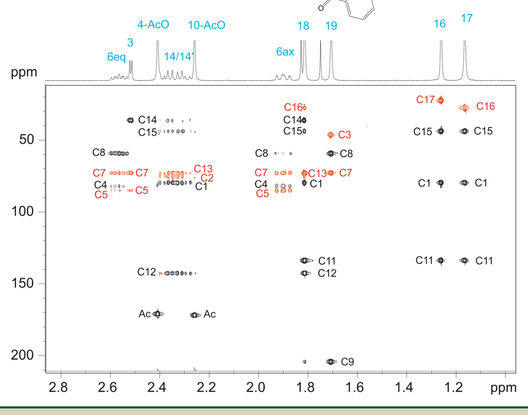


Staurosporine

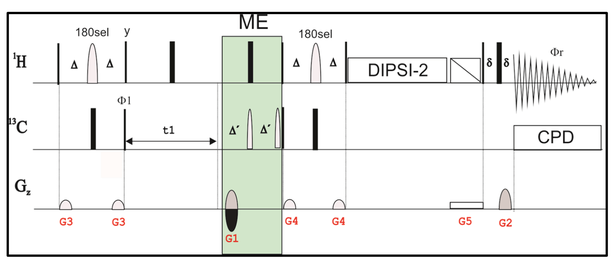




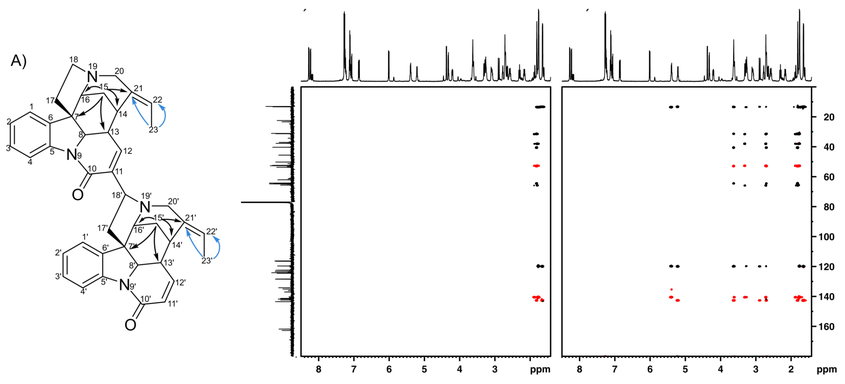
taxol

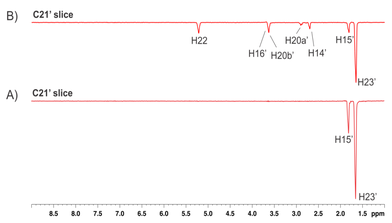


ME-selHSQMBC-TOCSY



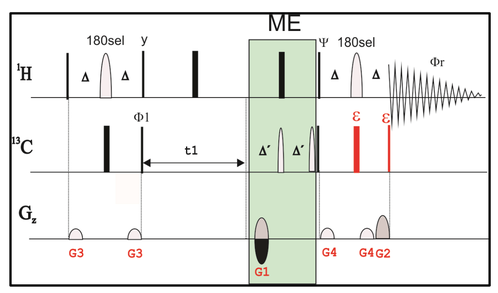
The proposed ME-selHSQMBC can also be used to obtain still broader structural information by appending a TOCSY mixing period to the pulse sequence in order to transfer the initial magnetization to more remote protons via J(HH).

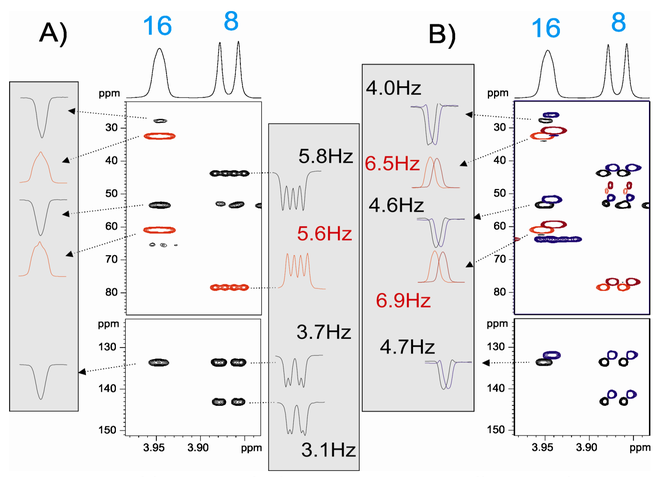




(left) ME-selHSQMBC experiment after selective inversion of the aliphatic protons H15/H15' and H23/H23' of sungucine; (right) ME-selHSQMBC-TOCSY experiment after inversion of the same protons as in B). Note that an increased number of cross-peaks are ready to be analyzed.

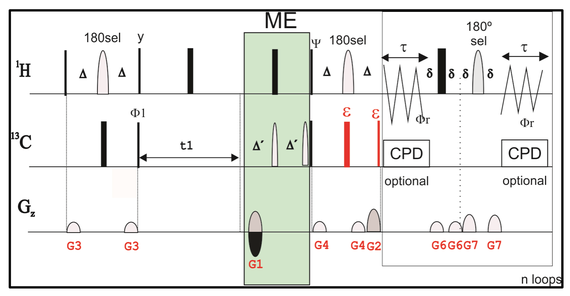
Measurement of $^nJ_{CH}$



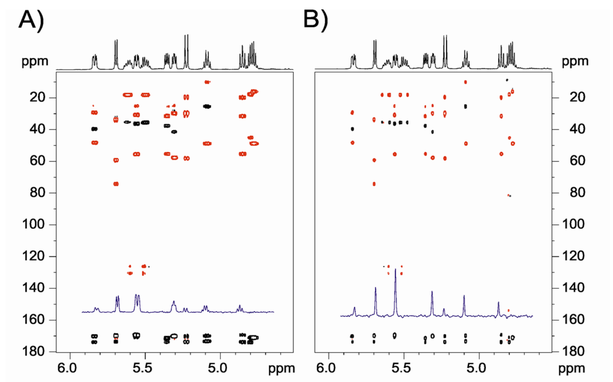


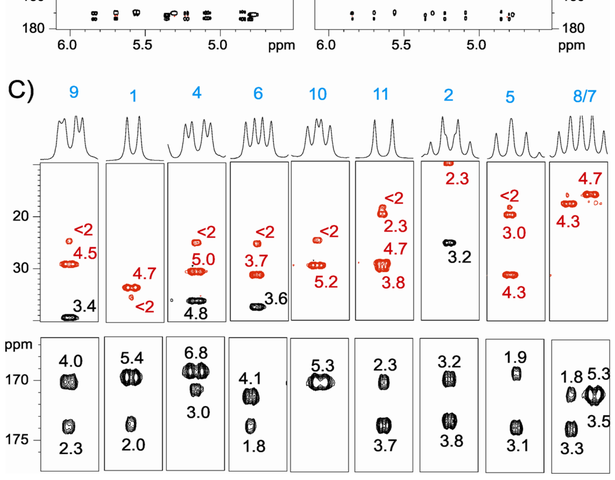
A) Expanded area corresponding to the CLIP-ME-HSQMBC; b) Expanded area corresponding to the ME-selHSQMBC-IPAP spectra of strychnine after band-selective inversion of the five proton frequencies centered at 4 ppm (α- and β- spectra are overlaid and relatively shifted in the vertical scale to visualize the J editing in spectrum. It is shown that $^nJ_{CH}$ can be directly extracted from the analysis of pure IP cross-peaks in resolved multiplets like the well defined H-8 proton in A) or from the relative α/β displacement in more complicated signals like the broad H-16 resonance in B).

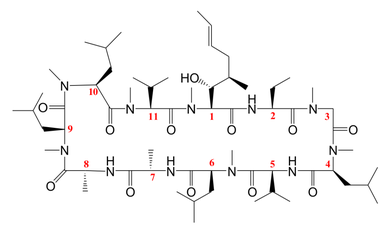
Incorporating Homonuclear Decoupling



ME-selHSQMBC methods can also benefit from the modern pure shift NMR concept, where multiplet patterns are collapsed by broadband homonuclear decoupling techniques. The objective is to obtain simplified doublet or singlet signals that are easier to analyze in both qualitative and quantitative terms







A) ME-selHSQMBC spectra of cyclosporine A using a REBURP 180 ^{1}H pulse of 5 ms centered at the Hα region.

B) HOBS-ME-selHSQMBC spectra acquired under the same experimental conditions as in A). Homo- and heteronuclear decoupling during acquisition has been applied. For comparison, 1D rows show the real sensitivity and signal resolution enhancements achieved by both approaches.

C) Expansions corresponding to the ^{13}C-coupled version of the HOBS-ME-selHSQMBC experiment, where each simplified cross-peak presents a clean IP doublet corresponding to the active $^nJ_{CH}$ value.

Conclusions

- Analysis of the up/down phase in ME-HSQMBC cross-peaks provide carbon multiplicity information.
- The ME-selHSQMBC sequence can be extended by a TOCSY transfer to other non-excited protons, where selective perturbation is not reliable.
- A pure shift ME-selHSQMBC experiment has been designed that incorporates broadband homo- and hetero-decoupling during acquisition, thereby enhancing sensitivity and signal resolution.
- The magnitude and/or the sign of $^nJ_{CH}$ couplings can be determined from ME-selHSQMBC and ME-selHSQMBC-TOCSY spectra. Alternatively, the ME-HOBS-selHSQMBC experiment affords simplified in-phase doublet cross-peaks, facilitating the analysis and automated peak-picking of $^nJ_{CH}$.

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1) J. Saurí, E. Sistaré, R. T. Williamson, G. E. Martin, T. Parella. *J. Magn. Reson*. 252 (**2015**) 170-175.