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Distinguishing two- and three-bond correlations for *all* ^{13}C multiplicities in heteronuclear NMR spectroscopy

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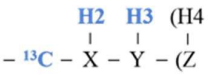
A novel two-dimensional method, SEA XLOC, for distinguishing two- and three-bond correlations in heteronuclear NMR spectroscopy is introduced and demonstrated on ibuprofen and by the complete set of correlations to a simple and a most complex quaternary ^{13}C multiplet in strychnine.

In heteronuclear NMR spectroscopy, exemplified by ^{1}H and ^{13}C, the size of one-bond J_{CH} coupling constants allows easy identification of one-bond correlations in two-dimensional (2D) spectra. On the other hand, the sizes of two- and three-bond J_{CH} are similar, so distinction between them based on the size of $^{n}J_{CH}$ is not possible. That is an inherent limitation of the original HMBC experiment for ^{1}H-^{13}C long-range correlation.[1]

To distinguish two- and three-bond correlations it is necessary to include interactions with further spins to obtain different responses for two- and three-bond correlations. For correlations to protonated ^{13}C the H2BC experiment[2, 3] works fine in identifying two-bond correlations and is quite complementary to HMBC. Alternatives include HAT HMBC[4] and ^{2}J,^{3}J-HMBC.[5]

These experiments all have their merits and there is no experiment fitting all variations in pertinent spin systems.[6, 7] And they have one shortcoming in common, namely that they do not work for correlation to quaternary ^{13}C that are present in most small molecules. Thus there is an analytical need for a simple, non-selective NMR method distinguishing two- and three-bond correlations to quaternary ^{13}C for all correlations present in an HMBC spectrum of the same excitation delay. That is the subject of this Communication with a method equally applicable to all ^{13}C multiplicities.

The origin of the idea can be illustrated by a three-spin system fragment, ^{13}C-^{1}H2-^{1}H3:



Three-bond J_{HH} and J_{CH} coupling constants are invariably positive and two-bond J_{CH} are in most cases negative. That can be used for distinction via states of heteronuclear zero- and double-quantum coherences (ZQC and 2QC, respectively) that are doublets in the above C-H2-H3 three-spin system. ZQC(C-H2), 2QC(C-H2), ZQC(C-H3), 2QC(C-H3) are split by $J_{H2H3} - J_{CH3}$, $J_{H2H3} + J_{CH3}$, $J_{H2H3} - J_{CH2}$, $J_{H2H3} + J_{CH2}$, respectively.[8] Given a negative sign for $^{2}J_{CH2}$ and positive signs for $^{3}J_{CH3}$ and $^{3}J_{H2H3}$, the narrower multiplet will for the two-bond correlation be the ZQC one whilst it will be the 2QC for the three-bond correlation. The difference in multiplet width (DMW) of two corresponding ZQC/2QC coherences is equal to twice the smaller of $^{3}J_{H2H3}$ and $^{2}J_{CH2}$ or $^{3}J_{CH3}$. Consequently, if one of these three *J*s vanishes there will be no DMW, and thus no two- and three-bond distinction in such cases.

Working with ZQC and 2QC spectra is tedious in routine applications, so while retaining the different ZQC/2QC multiplets above it is desirable to eliminate the ^{1}H chemical shifts in the two-spin coherence precession. That is accomplished by the XLOC approach[9] with pure ^{1}H and ^{13}C frequencies in the two dimensions of the 2D spectrum and where ZQC and 2QC correspond to echo and antiecho, respectively.

The new method requires echo and antiecho to be kept and inspected separately, thus the acronym SEA XLOC (Separate Echo and Antiecho XLOC). The pulse sequence is outlined in Fig. 1.

For quaternary ^{13}C where the multiplet width in F_1 usually is dominated by the digital resolution, the SEA XLOC sensitivity is down by about 30% compared to HMBC by virtue of echo and antiecho being kept separately. Passive ^{1}H spins coupling to both ^{1}H and ^{13}C of a ZQC/2QC pair will broaden one of the multiplets and narrow the other, whilst those coupling only to the ^{13}C spin will broaden both in comparison to HMBC. Passive spins coupling only to the ^{1}H spin contribute equally to the

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multiplet width in SEA XLOC and HMBC. For protonated ^{13}C the sensitivity is further divided over the one-bond splittings in F_1.

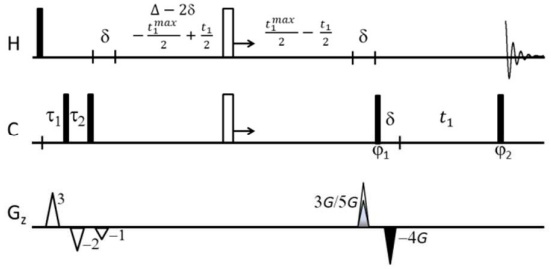


Figure 1. SEA XLOC pulse sequence employing a 2nd order low-pass *J* filter. Filled and open bars refer to π/2 and π pulses, respectively. $\tau_1 = 0.5[^1J_{min} + 0.146\ (^1J_{max} - {}^1J_{min})]^{-1}$, $\tau_2 = 0.5[^1J_{max} - 0.146\ (^1J_{max} - {}^1J_{min})]^{-1}$. ϕ_1 = {x, -x, -x, x}, ϕ_2 = {x, x, -x, -x}, and alternating receiver phase {x,-x}. Δ is the delay for evolution under heteronuclear long-range couplings, δ a gradient delay, and $t_1^{max} < 2\Delta - 4\delta$. The amplitude of the first three gradients can be set an order of magnitude lower than the amplitude of the two other ones selecting coherence transfer echo or antiecho.

If all pertinent passive *J*s are of the same sign, the 2QC multiplet is wider than the ZQC counterpart, which is the general picture for two-bond correlations. The opposite of the ZQC multiplet being wider than the 2QC counterpart means that a negative *J* is involved, which usually is $^2J_{CH}$, and thus the general picture for three-bond correlations. This clear two-/three-bond distinction hinges on negative $^2J_{CH}$, so one must be aware of the possibility of a positive $^2J_{CH}$ when the assignment puzzle does not resolve.

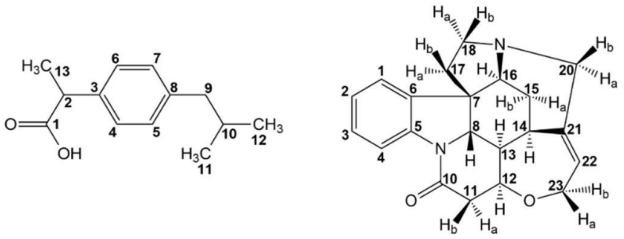
Geminal $^2J_{HH}$ coupling constants are invariably negative and contribute in the same direction as negative $^2J_{CH}$, i.e. according to the distinction above. The only exception to this is two-bond correlation with positive passive $^2J_{CH}$ to non-equivalent methylene ^1H.

Small DMWs require special attention because of the possible influence of four-bond couplings. In the model spin system above, DMW of the CH2 and CH3 peaks will be altered by twice the smaller of $\{^4J_{H2H4}, {}^4J_{CH4}\}$ and $\{^3J_{H3H4}, {}^4J_{CH4}\}$, respectively. In case e.g. a $^4J_{CH}$ is larger than a pertinent $^2J_{CH}$ both patterns can occur depending on the sign of $^4J_{CH}$. A way around this is at least in a first round of analysis to set a threshold or minimum DMW for two-/three-bond distinction of twice the expected largest ^4J.

Moreover, and just as in other long-range correlation spectra, four-bond J_{CH} can give rise to weak peaks. In other words, SEA XLOC spectra of molecules with unusually large four-bond coupling constants require extra attention and consistency check.

SEA XLOC assignment of protonated ^{13}C can be done in two ways. The 2Q/ZQ difference in the large "one-bond" splitting is for two- and three-bond peaks given by $2\cdot{}^3J_{HH}$ and $2\cdot{}^4J_{HH}$, respectively. Thus large differences are unequivocally associated with two-bond correlations. The two-/three-bond distinction can also be done from corresponding 2Q/ZQ ^1J multiplet components in analogy to quaternary assignment.

The merits of SEA XLOC shall be illustrated at hands of ibuprofen (left) and strychnine (right):



In Fig. 2 are shown all two- and three-bond correlations to the quaternary carbons and two protonated carbon correlations to non-aromatic protons in ibuprofen (aromatic $^2J_{CH}$ are vanishingly small).

H13-C2 is a two-bond correlation because of the large difference in one-bond splitting ($2\cdot{}^3J_{H2H13}$) whilst H11/12-C9 is a three-bond correlation because the wider individual triplet components in the ZQC (red) rather than in the 2QC (black) part and consistent with little difference in one-bond splitting due to the small $^4J_{H9H11/12}$.

C10 in strychnine shows three whilst C21 shows a total of nine correlations to protons within two or three bonds. Thus C21 contains a total of 512 ill-resolved resonances within an about 50 Hz wide multiplet. The complete correlation maps of both C10 and C21 are shown in Fig. 3. For the third aliphatic quaternary ^{13}C, C7, only three (see Supplementary Information) of the correlations exhibit clear patterns, mainly due to near methylene degeneracy leading to very strong coupling and because of overlap with C20 correlations.

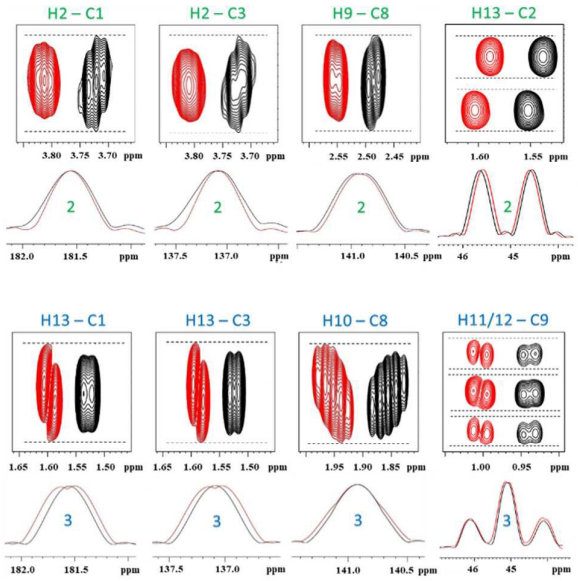


Figure 2. Excerpts from 500 MHz absolute-value SEA XLOC spectra of ibuprofen (0.5 M in $CDCl_3$) recorded on a Bruker Avance II spectrometer equipped with a TXI z-gradient probe showing two- and three-bond correlations of C1, C3 and C8 quaternaries along with a two-bond and a three-bond correlation of the protonated carbons C2 and C9, respectively. In the contour plots 2QC peaks (black) are displaced horizontally with respect to ZQC peaks (red) for better visualization. 1D sections were obtained by summing up columns across the peaks and scaled to the same intensity for comparison of multiplet widths. Numbers 2 and 3 indicate number of intervening bonds. The spectra were acquired using the pulse sequence in Fig. 1 with the parameters: Δ = 100 ms, $^1J_{min}$ = 125 Hz and $^1J_{max}$ = 165 Hz, spectral widths of 8.0 ppm (^1H) and 180.0 ppm (^{13}C), relaxation delay 1.6 s, 600 points in t_1 giving a digital resolution of 38 Hz/point

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with 8 scans per increment and 2048 data points in t_2. Coherence transfer echo or antiecho selection was achieved with gradient pulses 30% for ZQC or 50% for 2QC and -40.1% of maximum gradient strength (50 G/cm). A 30° shifted sine bell window function was applied in both dimensions, and the data were zero filled to 8K×8K prior to Fourier transformation. ^{1}H and ^{13}C 90° pulses were 11.0 and 15.7 μs, respectively and the sample temperature was 298 K. The smallest DMW for the correlations shown is 6 Hz. The full 2D echo spectrum can be found in Supplementary Information.

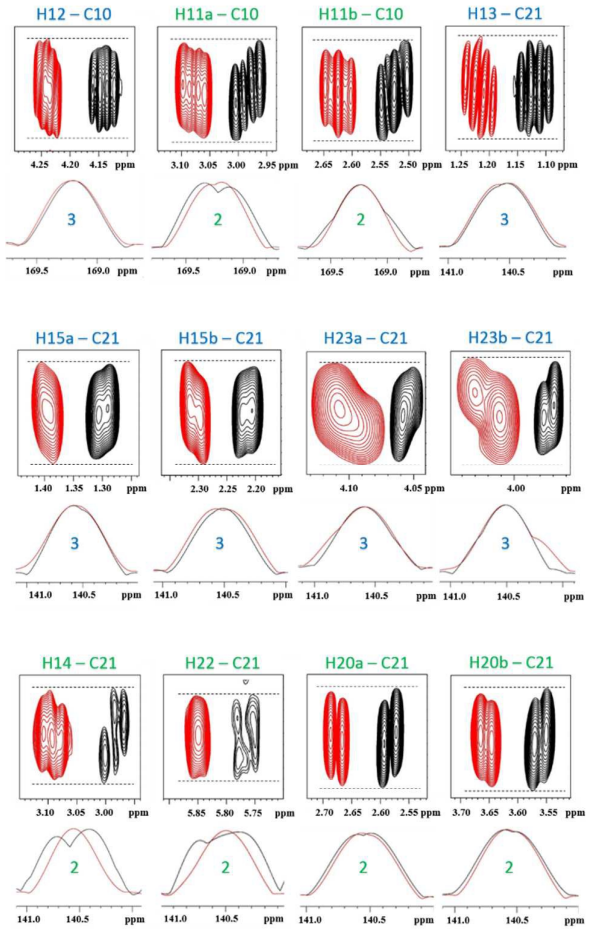

Figure 3. Excerpts from 700 MHz absolute-value SEA XLOC spectra of strychnine (0.4 M in $CDCl_3$) recorded on a Bruker Avance Neo spectrometer equipped with a TCI z-gradient prodigy probe showing two- and three-bond correlations of C10 and C21 quaternaries. In the contour plots 2QC cross peaks (black) are displaced horizontally with respect to ZQC peaks (red) for better visualization. 1D sections were obtained by summing up columns across the peaks and scaled to the same intensity for comparison of multiplet widths. Numbers 2 and 3 indicate number of intervening bonds. The spectra were acquired using the pulse sequence in Fig. 1 with the parameters: Δ =83 ms, $^{1}J_{min}$ = 125 Hz and $^{1}J_{max}$ = 165 Hz, spectral widths of 8.4 ppm (^{1}H) and 160.0 ppm (^{13}C), relaxation delay 1.3 s, 512 points in t_1 giving a digital resolution of 55 Hz/point with 16 scans per increment and 2048 data points in t_2. Coherence transfer echo or antiecho selection was achieved with gradient pulses 30% for ZQC or 50% for 2QC and -40.1% of maximum gradient strength (50 G/cm). A 30° shifted sine bell window function was applied in both dimensions, and the data were zero filled to 8K×8K prior to Fourier transformation. ^{1}H and ^{13}C 90° pulses were 7.3 and 12.0 μs, respectively and the sample temperature was 298 K. The smallest DMW for the correlations shown is 5 Hz. The full 2D echo spectrum can be found in Supplementary Information. Strychnine coupling constants can be found in Refs.[10-13]

Although the spectra presented were obtained with better digitization in t_1, tests have shown that even 70-80 Hz/point is adequate for comparison of multiplet widths for quaternary ^{13}C provided extensive zero-filling is applied to obtain well-defined F_1 profiles. Resolving one-bond splittings, however, normally requires better resolution than 70-80 Hz/point.

Apart from the techniques mentioned in the introduction, there are other approaches for tracing out the structure of molecules. Chief among them is INADEQUATE[14, 15] identifying the carbon backbone or its proton-detected versions.[7, 16-18] For these techniques sensitivity is an issue, because only molecules with two ^{13}C nuclei contribute.

Techniques requiring only one ^{13}C per molecule have everything else equal higher sensitivity and an HSQC element followed by a ^{1}H-^{1}H transfer can determine signs of $^{n}J_{CH}$ and this can also be used to distinguish two- and three-bond correlations, however, for protonated ^{13}C only.[19-23] The same principle applicable to quaternary carbons needs the HSQMBC approach[24, 25] requiring two long-range coherence transfers where the one amplitude-modulated by passive J_{HH} couplings particularly compromises sensitivity. The sensitivity of SEA XLOC benefits from the fact that such a coherence transfer is not part of the pulse sequence.

In conclusion, we have introduced a novel NMR method, SEA XLOC, for distinguishing two- and three-bond correlations applicable to all ^{13}C multiplicities and filling a significant analytical need for chemists in small-molecule NMR spectroscopy. The method is easy to implement and quite robust, as demonstrated by the application to a complex quaternary spin system of strychnine. It is mainly intended for quaternary ^{13}C, as H2BC usually will be superior for the protonated carbons, particularly for picking up the correlations with vanishing $^{2}J_{CH}$.

Conflicts of interest

There are no conflicts to declare.

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