Supporting Information

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

Tamás Gyöngyösi, Tamás Milán Nagy, Katalin E. Kövér, Ole W. Sørensen
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1. Figure S1. 500 MHz full SEA XLOC echo spectrum of ibuprofen
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3. Figure S3. Selected strychnine correlations from the same SEA XLOC spectra as in Fig. 3.
F2 cross sections taken from the SEA XLOC echo spectrum of strychnine shown in Fig. S2. Multiple-bond correlations are color coded as before. Four-bond correlations are indicated by red arrows. One-bond residuals having survived the low-pass $J$ filter are indicated by * in the C15 row.
H20a-C15 is the only four-bond peak of appreciable intensity in the strychnine SEA XLOC spectrum. This arises from the fact that the H20a homonuclear coupling network essentially only consists of its geminal partner, H20b, which is highly beneficial for the sensitivity compared to many of the more complicated and broader strychnine proton multiplets and compensates the small $^{4}J_{\text{C15-H20a}}$ of about 1 Hz. However, a H20a-C15 correlation will not show up as a two-bond correlation in an H2BC spectrum if that spectrum is recorded, because such an H2BC peak would require a non-vanishing $^{5}J_{\text{H15-H20a}}$. Otherwise, the identification of the H20a-C15 peak as a four-bond correlation is to emerge from the complete analysis of the full SEA XLOC spectra and consistency checks. Note that the H20a-C15 peak though a bit distorted has the characteristic of a three-bond correlation because the relative sign of the pertinent passive $^{2}J$, $^{2}J_{\text{H20a-H20b}}$ and $^{4}J_{\text{C15-H20b}}$, is negative.

The strychnine peaks H15a-C7 and H15b-C7 appear with clear three-bond characteristic, but do represent a bit of “luck”, because the pertinent passive $^{2}J_{\text{H16C7}} = -0.33$ Hz is small though negative and there is a passive $^{4}J_{\text{H14C7}} = -0.39$ Hz. Both of them pull in the direction of narrowing the 2Q relative to the ZQ multiplet, but had $^{4}J_{\text{H14C7}}$ been positive and a bit larger the pattern would have been opposite. Misassignments of such types are avoided working with a threshold of a minimum acceptable DMW.

In summary, 24 two-bond and 28 three-bond correlations in the non-aromatic part of strychnine could be unambiguously assigned based on the characteristics of the pertinent ZQ/DQ multiplets and a threshold of DMW = 3.5 Hz. In addition to the problems with C7 mentioned in the main text further correlations (H16-C8, H8-C16 and H18b-C20) could not be analyzed due to spectral overlap.

Aromatic regions in SEA XLOC spectra are in general not reliable because aromatic heteronuclear two-bond coupling constants are notoriously small and can have both signs. In fact, only 4 aromatic two-bond peaks in strychnine could be assigned with full certainty and correctly within the DMW threshold of 3.5 Hz. They are shown below. Obviously, three-bond correlations associated with small positive $^{3}J_{\text{CH}}$ of aromatic spin systems have the patterns of two-bond correlations. However, they are easily identified as such by an H2BC spectrum where they typically are absent.
4. SEA XLOC pulse sequence code for Bruker spectrometers (Avance II, III and NEO systems)

; sea-xloc_ek_ow
; advance-version - tested on Avance II and NEO systems, TopSpin 2.1 and TopSpin 4.0.2

; 2D H-1/X correlation via heteronuclear zero (ZQ) or double quantum (DQ) coherence
; recorded in two separate (SEA = separate echo-antiecho) experiments
; optimized on long range couplings
; with second order low-pass J-filter to suppress one-bond correlations
; no decoupling during acquisition
; using gradient pulses for coherence selection
; using hard or shaped pulses for inversion on f2 - channel

; February 21, 2018 KEK, TGY, TMN

; This pulse sequence is part of
; Tamás Gyöngyösi, Tamás Milán Nagy, Katalin E. Kövér, Ole W. Sørensen; "Distinguishing two-
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; manuscript in preparation

; The pulse sequence has been coded for test purposes only and
; may contain errors.
; The functionality of the pulse sequence itself may differ depending on
; the hardware as well as the software used to execute it. Functionality
; on differing systems cannot be granted.
; Any use of this pulse sequence on a spectrometer is at your own risk.
;
; By using this pulse sequence, or any modification of it in any published material
; you agree to acknowledge the above-mentioned publication.
```c
#include <Avance.incl>
#include <Grad.incl>
#include <Delay.incl>

"p2=p1*2"
"p4=p3*2"

d11=30m"
"in0=inf1/2"
"in20=in0"

d23=1s/(cnst14*4)"
"d20=d23-p16-d16"

"d0=3u"

"DELTA1=1s/(2 * (cnst6 + 0.146 * (cnst7-cnst6)))-p16-d16"
"DELTA2=1s/(2 * (cnst7 - 0.146 * (cnst7-cnst6)))-p16-d16"
"DELTA3=d23-p16-d16-d0"

1 ze
2 d11 pl1:f1
3 d1
{p1 ph1)
DELTA1 UNBLKGRAD ;second order low-pass J-filter to suppress one-bond correlations
p16:gp4
dl6 pl2:f2
{p3 ph1):f2
DELTA2
p16:gp5
dl6
{p3 ph1):f2
;heteronuclear long-range coupling evolution starts
p16:gp6
dl6
DELTA3
d0 ;incremented
{center (p2 ph1) (p4 ph1):f2)
 ;(center (p2 ph1) (p14:sp3 ph1):f2) ;using shaped inversion pulse - optional
d20 ;decremented
p16:gp1 ;use gpz1 = 50 % for DQ-selection, gpz1 = 30 % for ZQ-selection
dl6 pl2:f2
{p3 ph4):f2
p16:gp2
dl6 BLKGRAD

d0
d0
{p3 ph5):f2
go=2 ph31

;;; TopSpin 2.1
;;; d11 mc #0 to 2 F1QF(id0 & dd20)

;;; TopSpin4.0.2
d11 mc #0 to 2 F1QF(caldel(d0, +in0) & caldel(d20, -in20))

exit
```
ph1=0
ph4=0 2 2 0
ph5=0 0 2 2
ph6=2
ph31=0 2

;pl1 : f1 channel - power level for pulse (default)
;pl2 : f2 channel - power level for pulse (default)
;sp3: f2 channel - shaped pulse (180 degree inversion)
;spnam3: Crp60,0.5,20.1
;pl1 : f1 channel - 90 degree high power pulse
;pl2 : f1 channel - 180 degree high power pulse
;pl3 : f2 channel - 90 degree high power pulse
;pl4 : f2 channel - 180 degree high power pulse
;pl14: f2 channel - 180 degree shaped pulse for inversion
;     = 500 usec for Crp60,0.5,20.1
;pl6: homospoil/gradient pulse
;d0 : incremented delay (2D) [3 usec]
;d1 : relaxation delay; 1-5 * T1
;d2 : 1/(2J)XH
;d23 : delay for evolution of long range couplings
;d11: delay for disk I/O [30 msec]
;d16: delay for homospoil/gradient recovery

;d20: decremented delay
;cnst2: = J(XH) = 145 Hz
;cnst6: = J(XH)min = 125 Hz
;cnst7: = J(XH)max = 165 Hz
;cnst14: = J(XH) long range = ca. 6 Hz

;inf1: 1/SW(X) = 2 * DW(X)
in0: 1/(2 * SW(X)) = DW(X)
nd0: 2
in20: = in0
NS: 4 * n
DS: >= 16
;td1: number of experiments
;FnMODE: QF

;use gradient ratio:
;for z-only gradients:

;gpz1: 50% for DQ-selection or 30% for ZQ-selection for C-13
;gpz2: -40.1% for C-13

;gpz4: 6%
;gpz5: -4%
;gpz6: -2%

;use gradient files:
;gpnam1: SINE.100
;gpnam2: SINE.100
;gpnam4: SINE.100
;gpnam5: SINE.100
;gpnam6: SINE.100