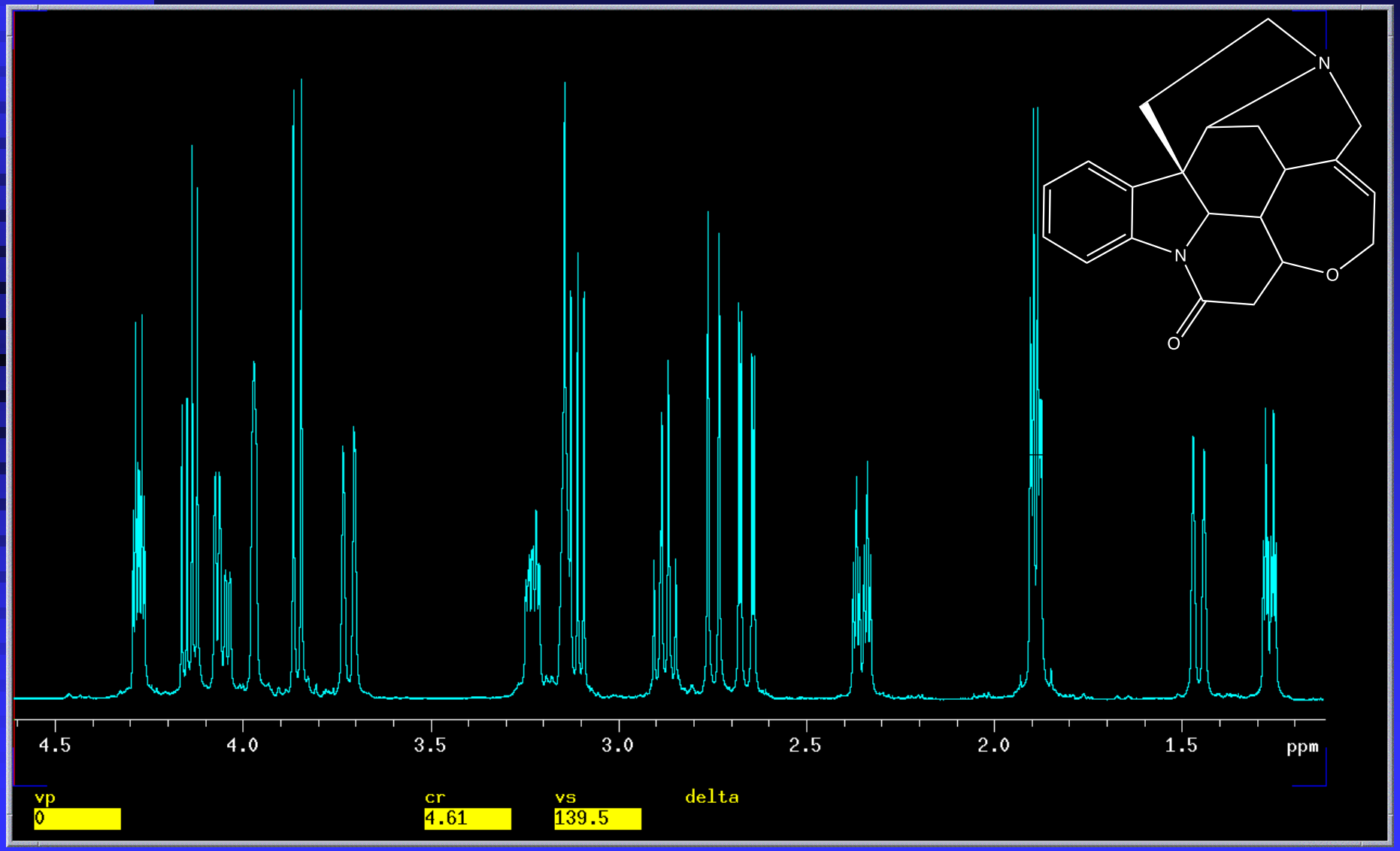
2D NMR Acquisition & Processing Workshop

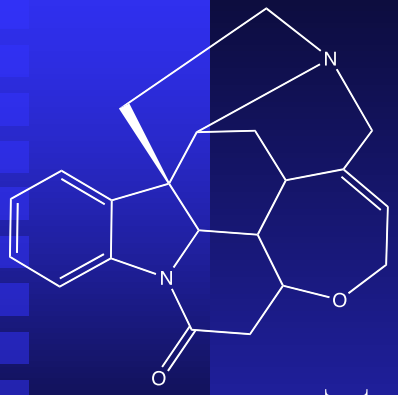
Michigan State University

September 23, 2020

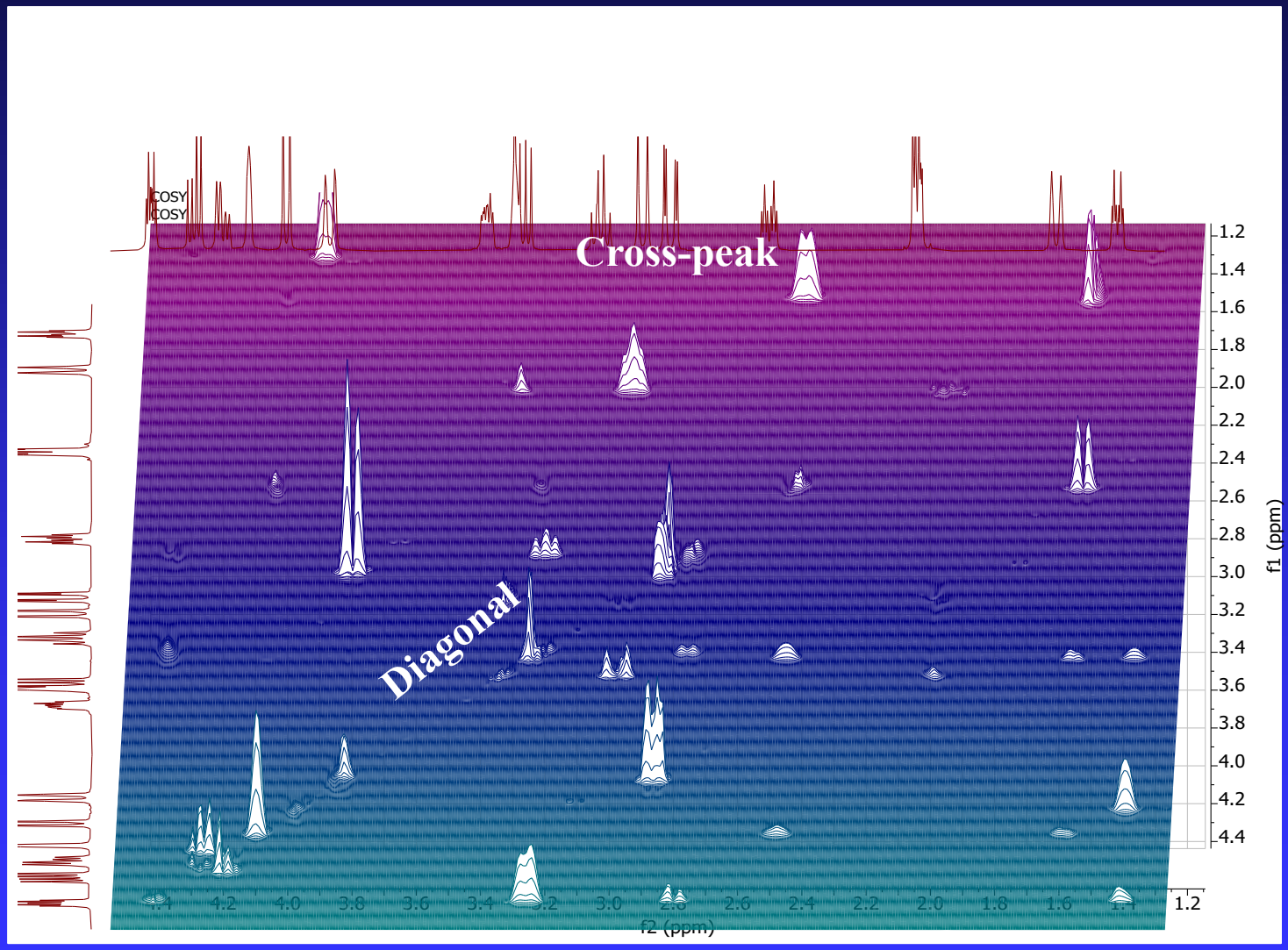
Can you Determine Coupling Partners from 1D Spectrum?



2D Homonuclear Correlation (COSY)

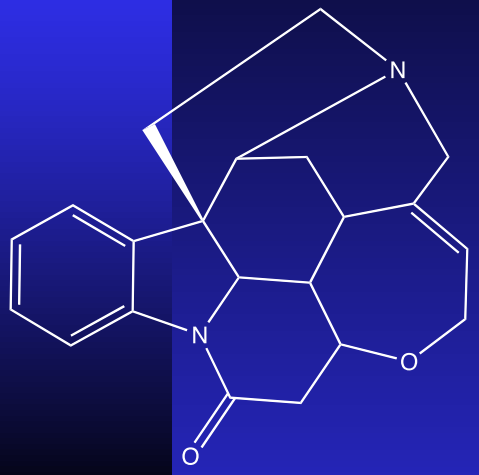


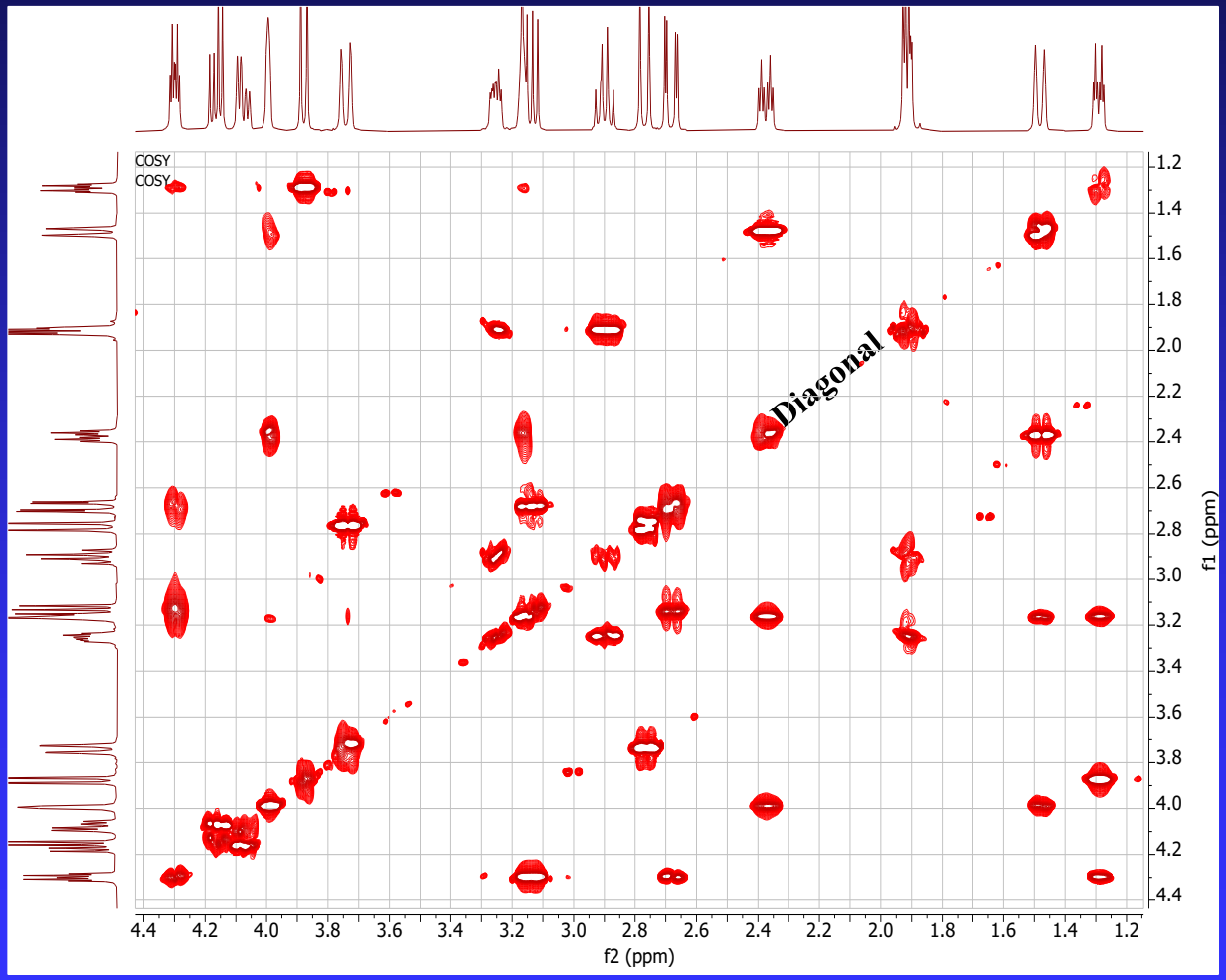
Indirect Dimension (F1)



Direct Dimension (F2)

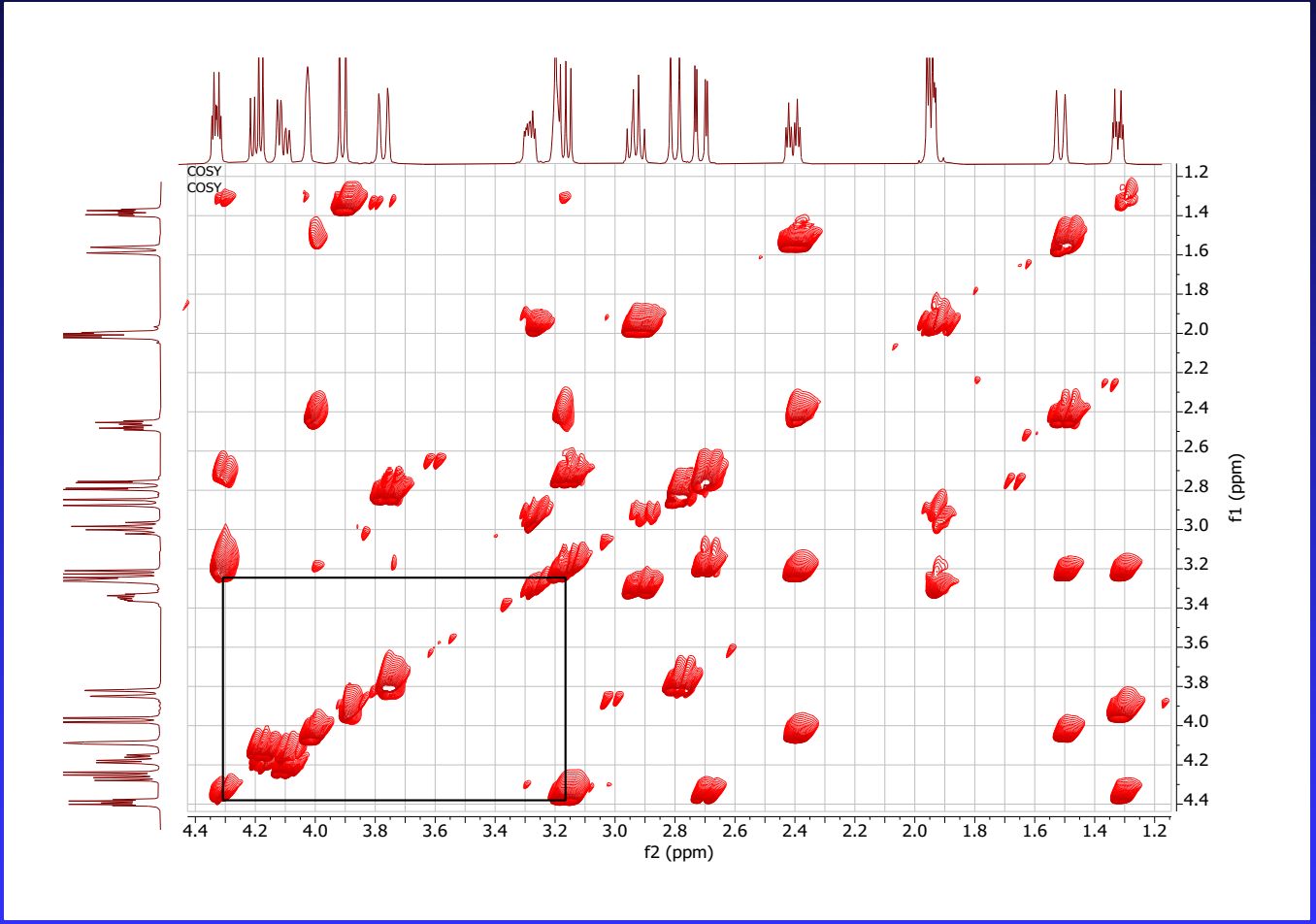
2D Homonuclear Correlation (COSY)

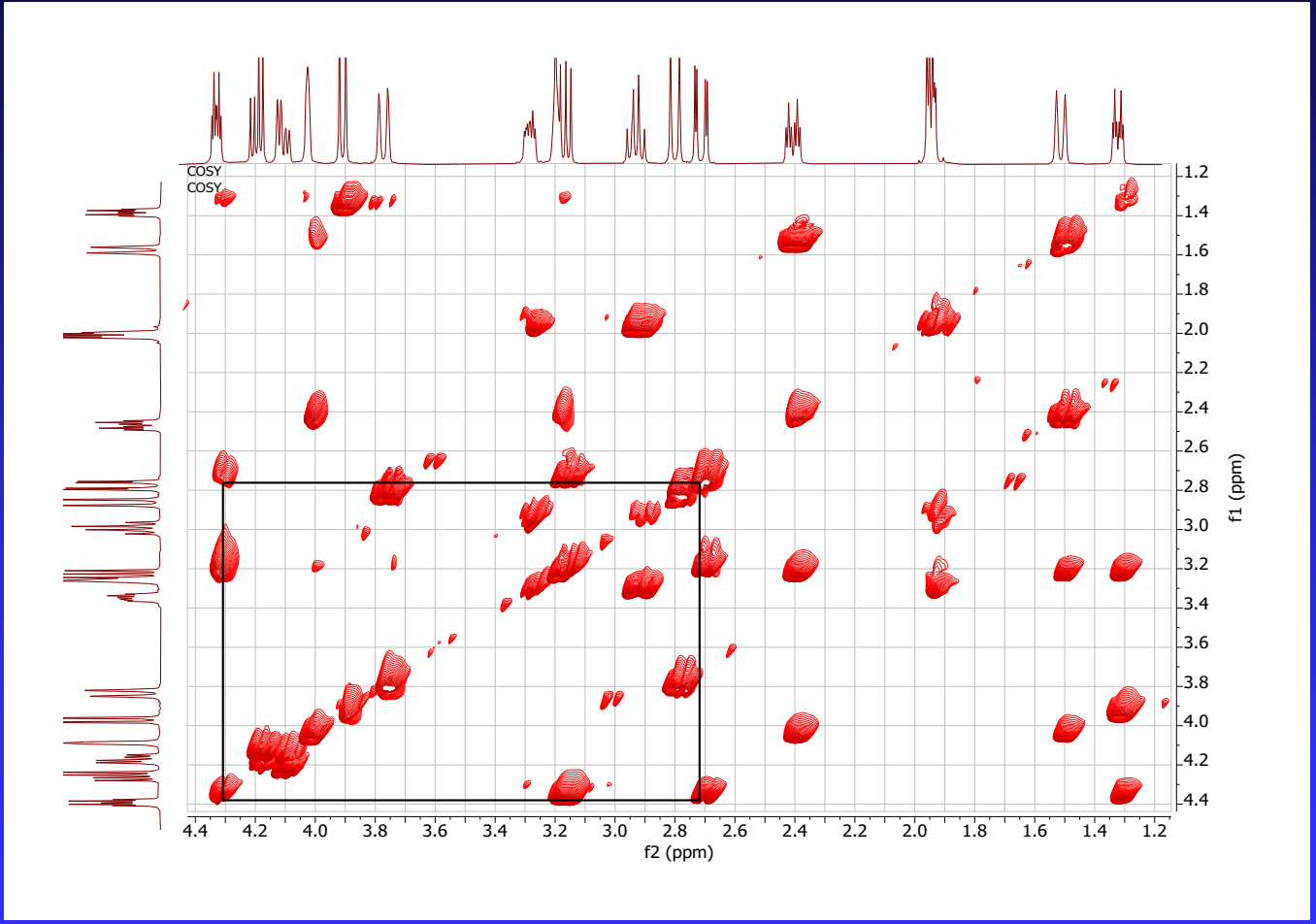


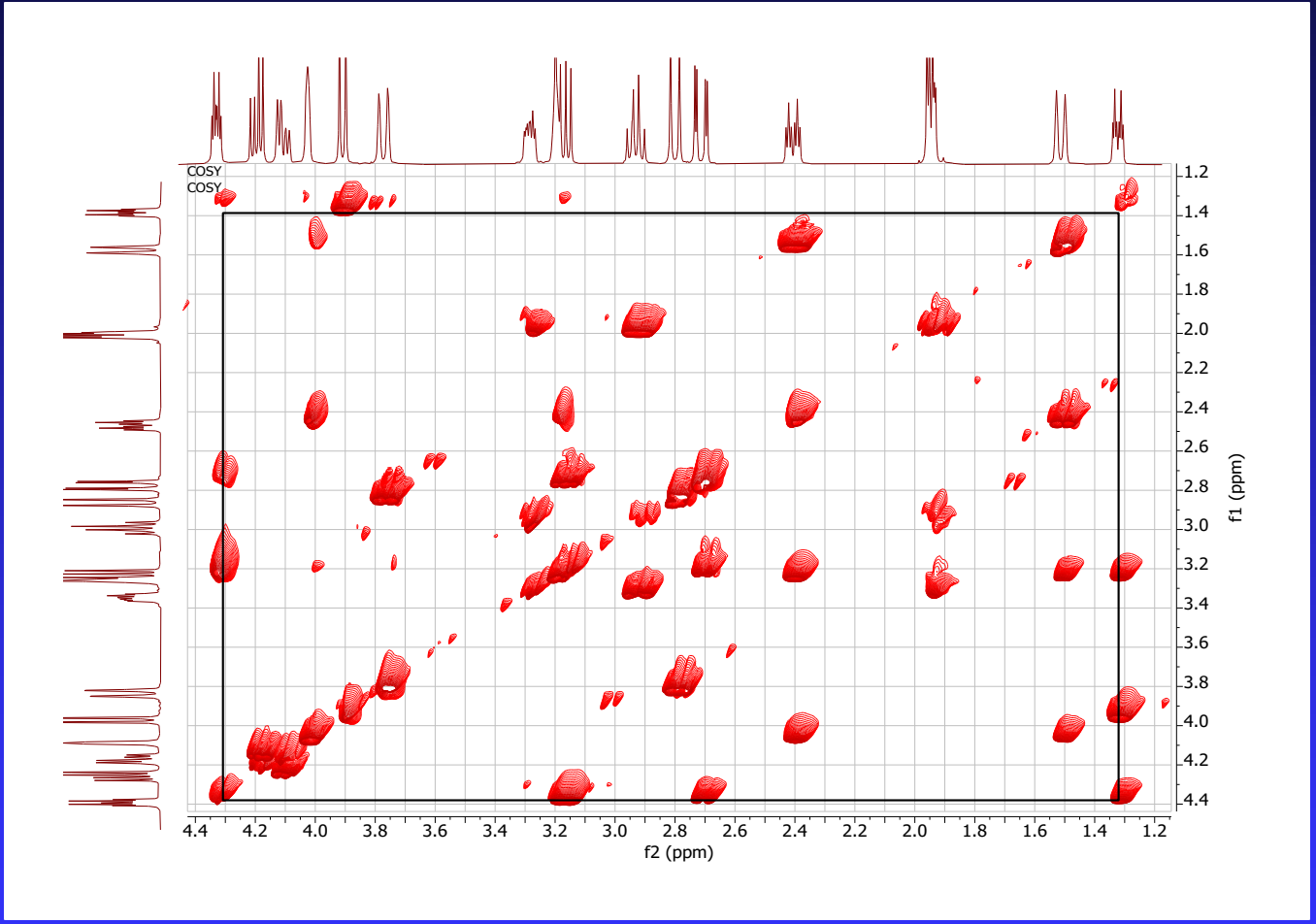


Indirect Dimension

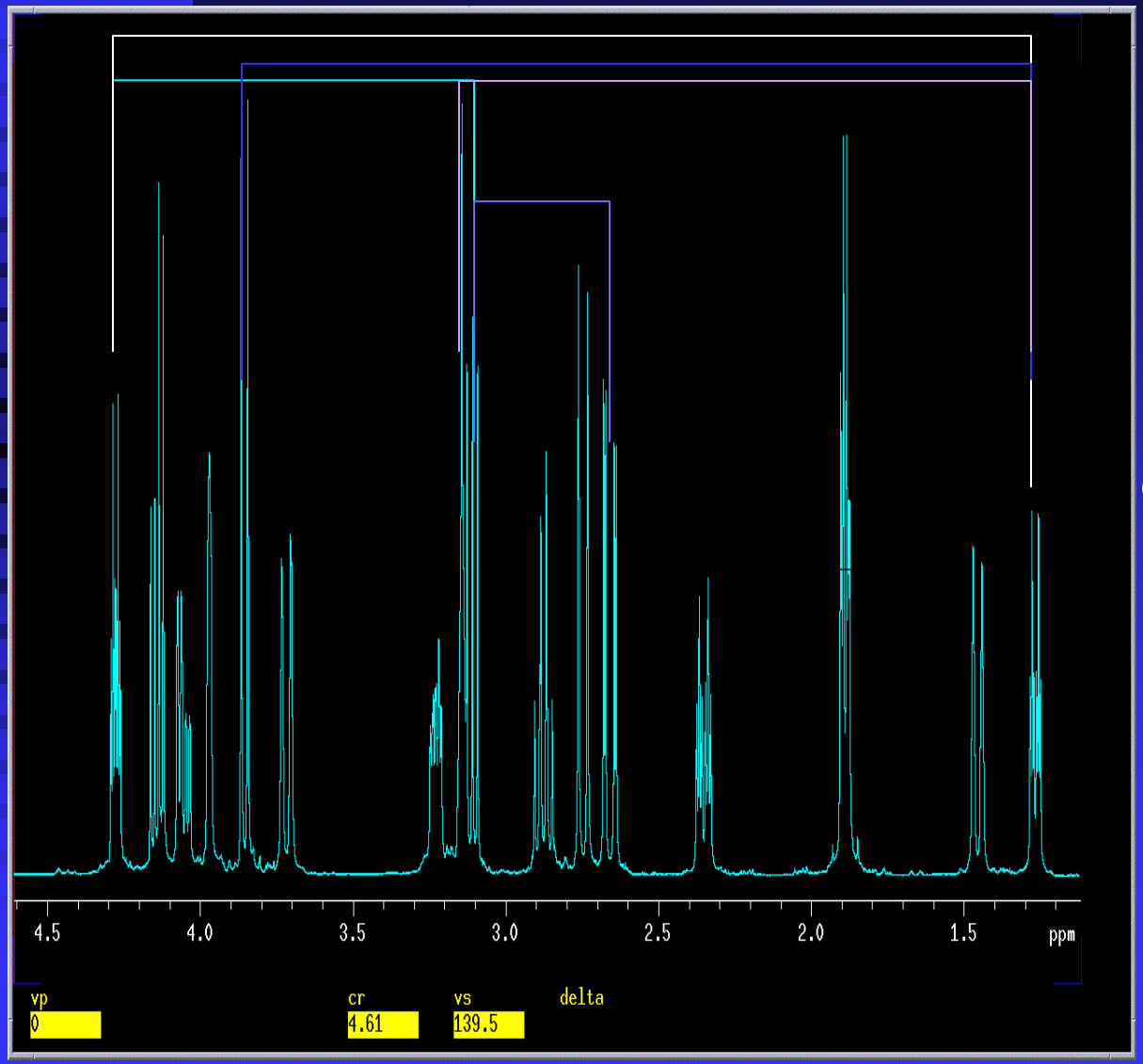
Direct Dimension

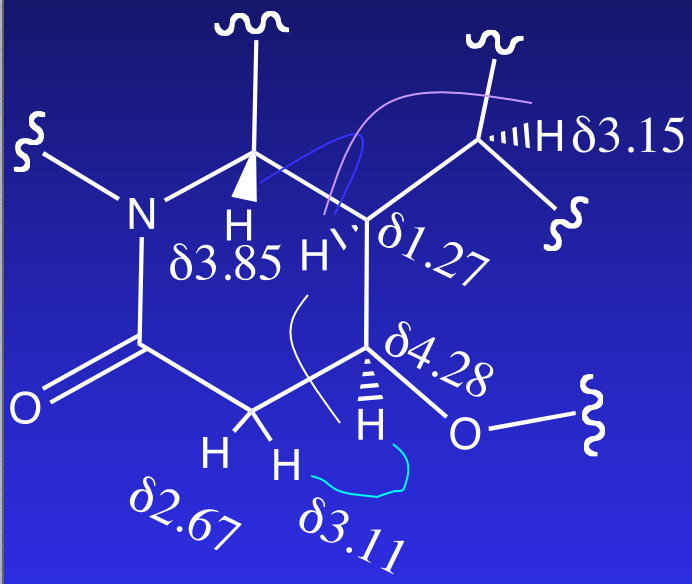

COSY
COSY
1.2
1.4
1.6
1.8
2.0
2.2
2.4
2.6
2.8
3.0
3.2
3.4
3.6
3.8
4.0
4.2
4.4
f1 (ppm)
4.4
4.2
4.0
3.8
3.6
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
f2 (ppm)


COSY
COSY
1.2
1.4
1.6
1.8
2.0
2.2
2.4
2.6
2.8
3.0
3.2
3.4
3.6
3.8
4.0
4.2
4.4
f1 (ppm)
4.4
4.2
4.0
3.8
3.6
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
f2 (ppm)


COSY
COSY
1.2
1.4
1.6
1.8
2.0
2.2
2.4
2.6
2.8
3.0
3.2
3.4
3.6
3.8
4.0
4.2
4.4
f1 (ppm)
4.4
4.2
4.0
3.8
3.6
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
f2 (ppm)

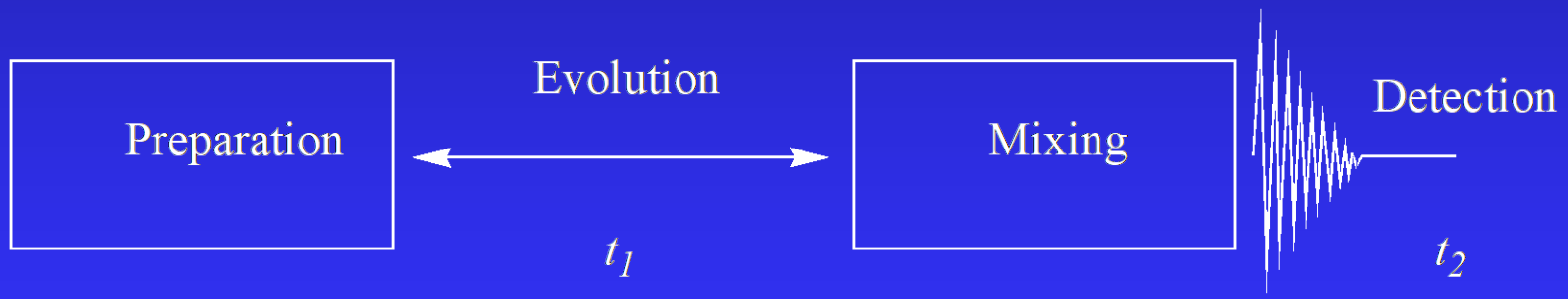
Can you Determine Coupling Partners from 1D Spectrum? Yes, w/ 2D Help!



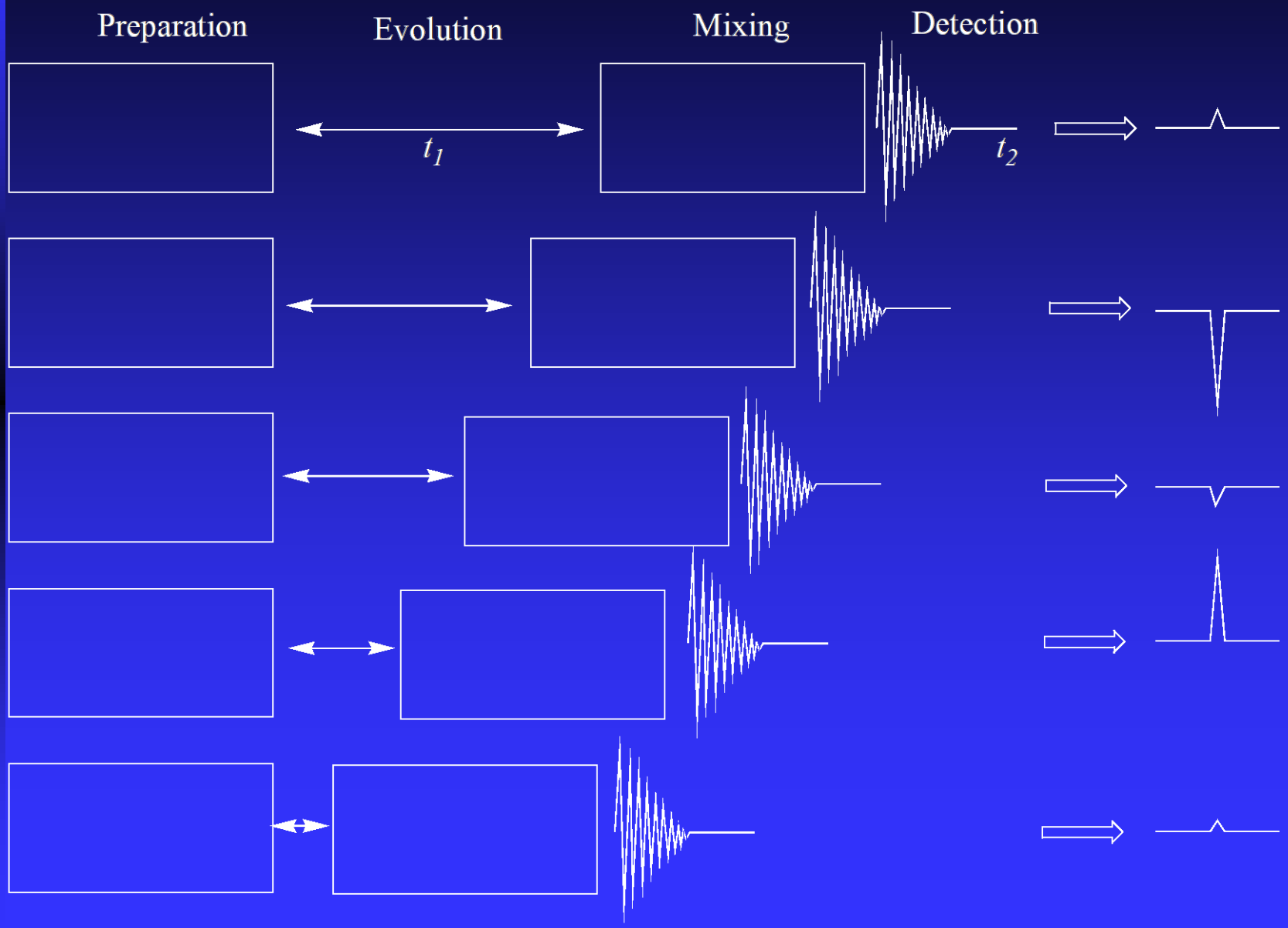


2-Dimensional Correlation Spectroscopy

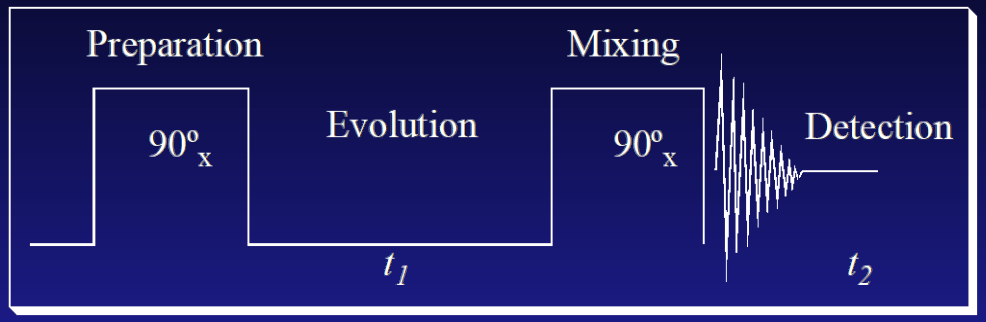
- 2D experiments employ an incremented delay (t_1) to generate the indirect dimension.

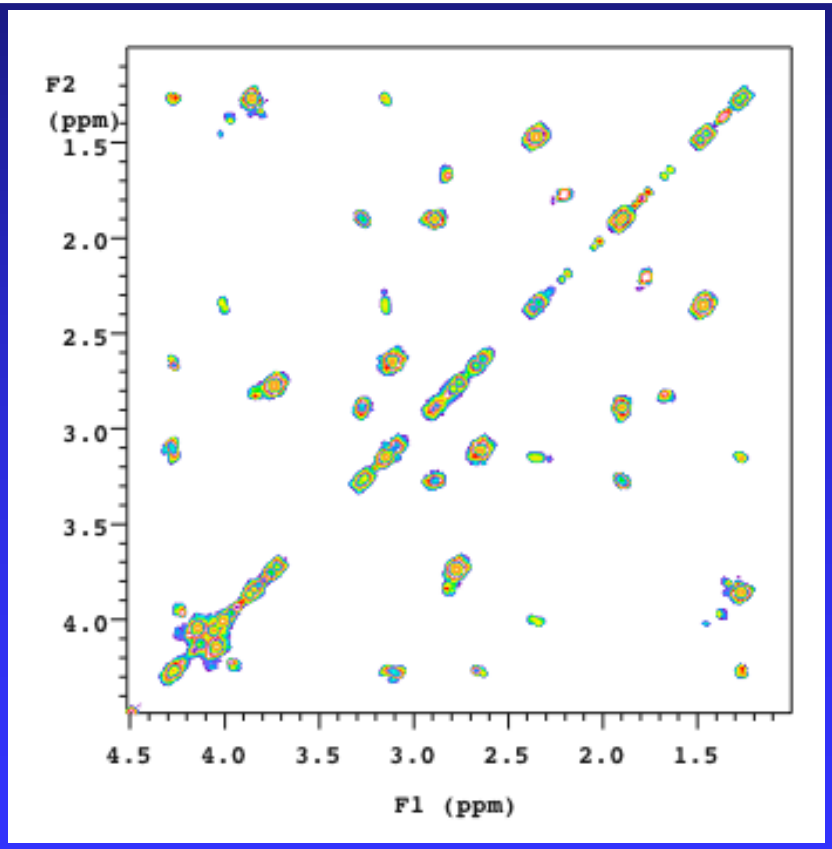


2-Dimensional Correlation Spectroscopy



COSY: Exemplary 2D experiment

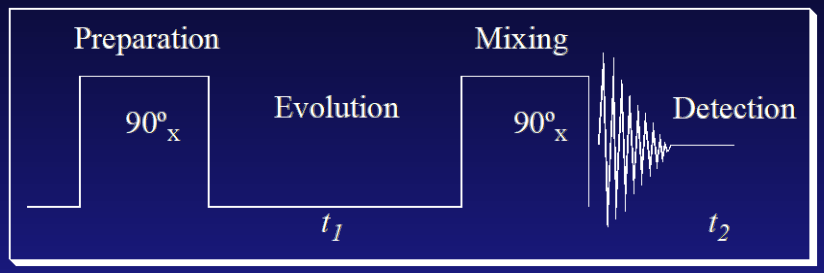




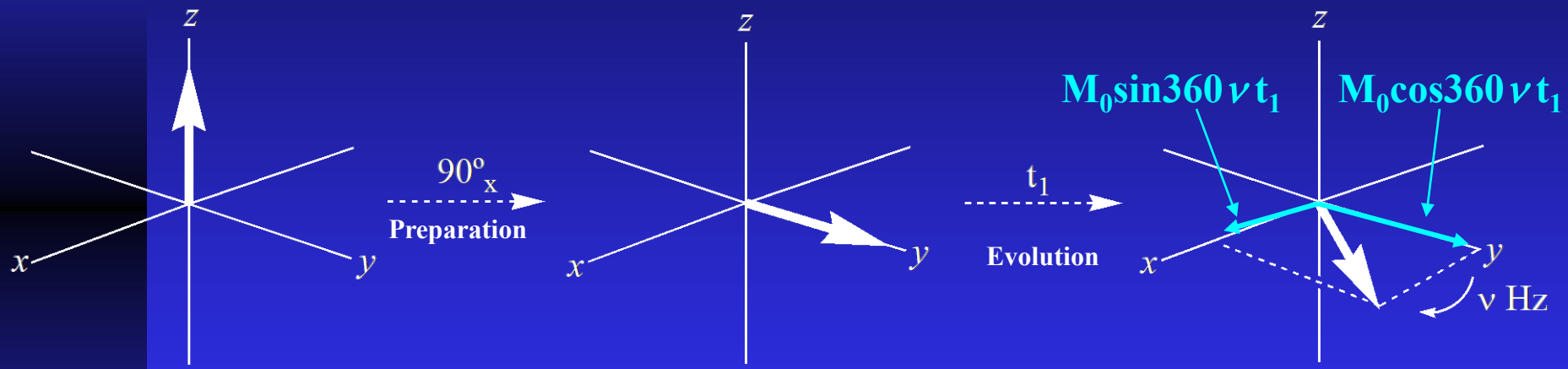
Direct Dimension (F2)

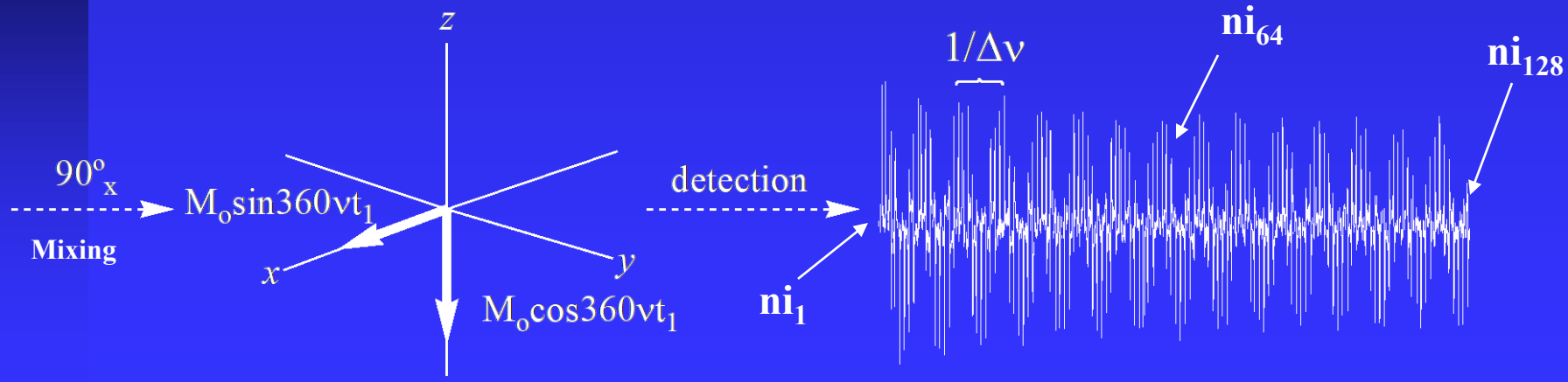
Indirect Dimension (F1)

COSY: Example of 2D expt.

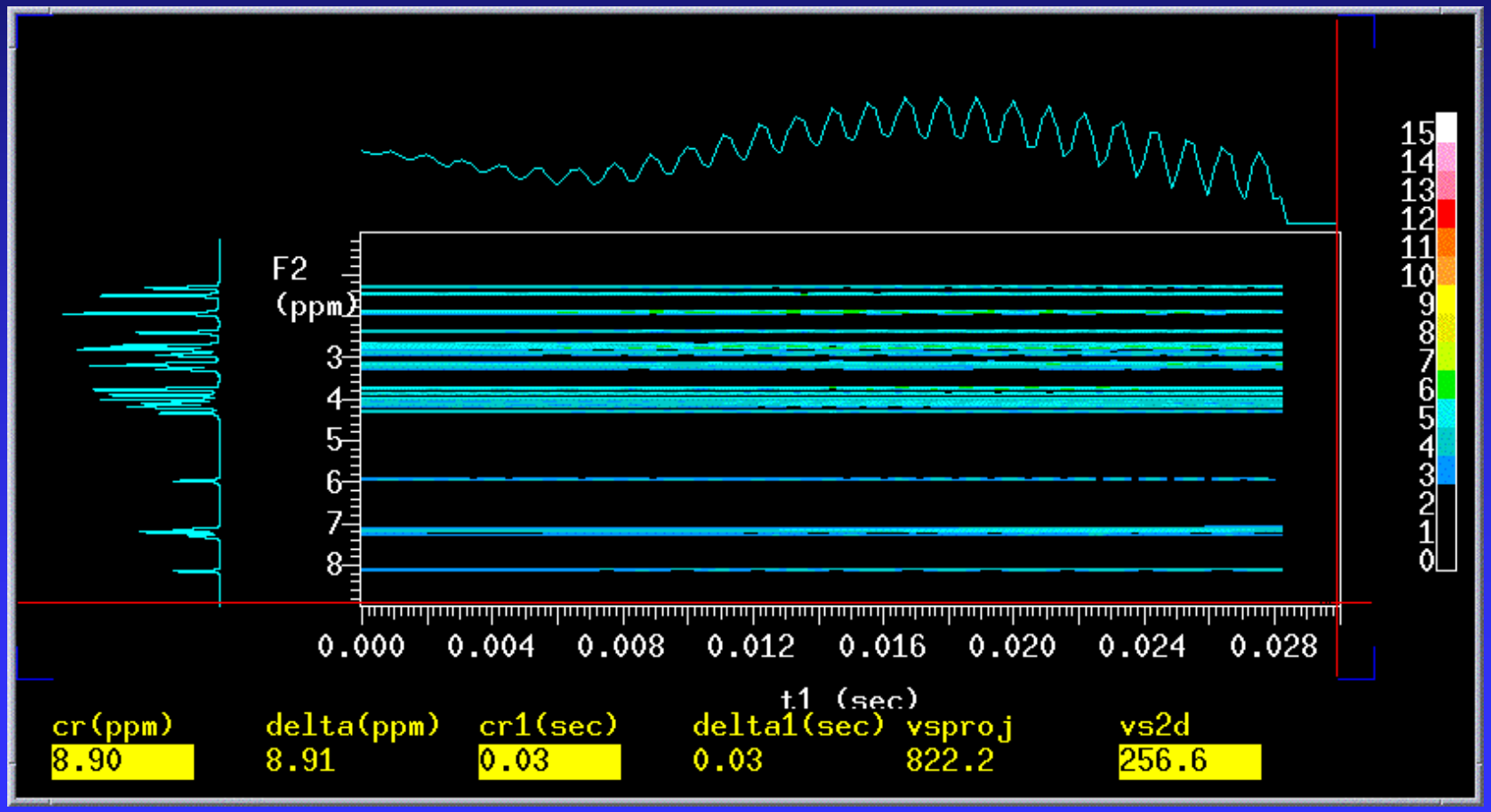


$$t_1 = \left\lfloor \frac{(ni-1)}{sw1} \right\rfloor_{ni=1,2,3,\ldots ni}$$

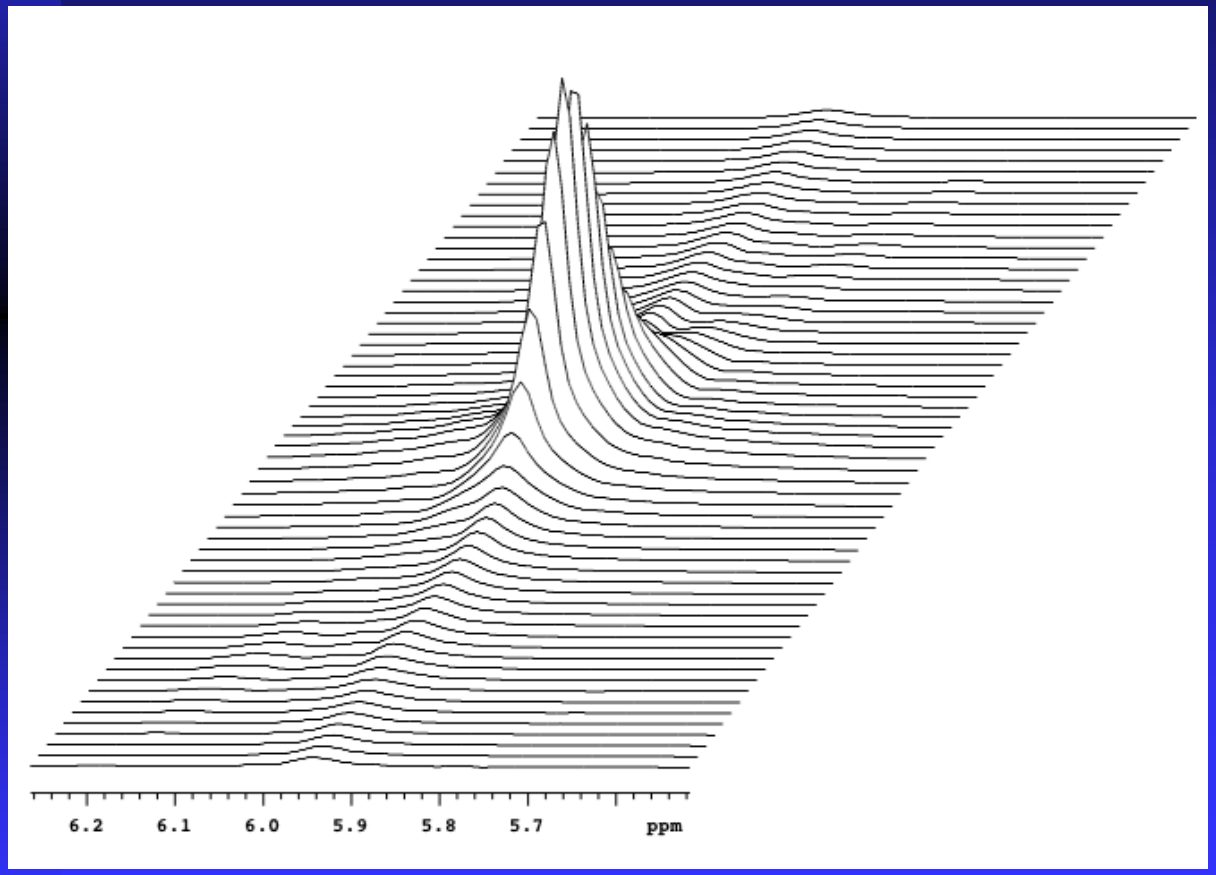




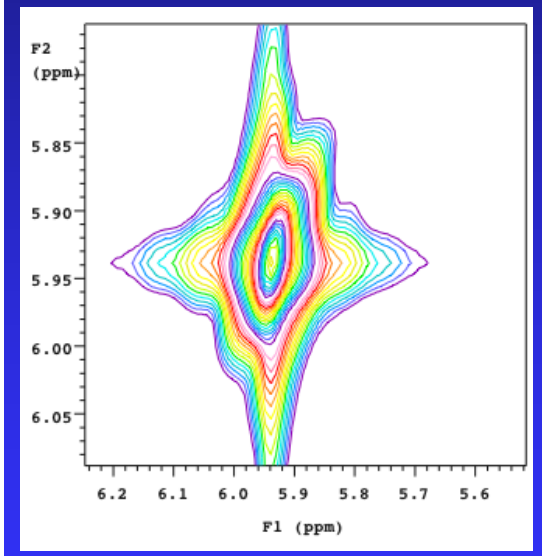
COSY: 1-dimensional transform



Transformed COSY Data: The Diagonal



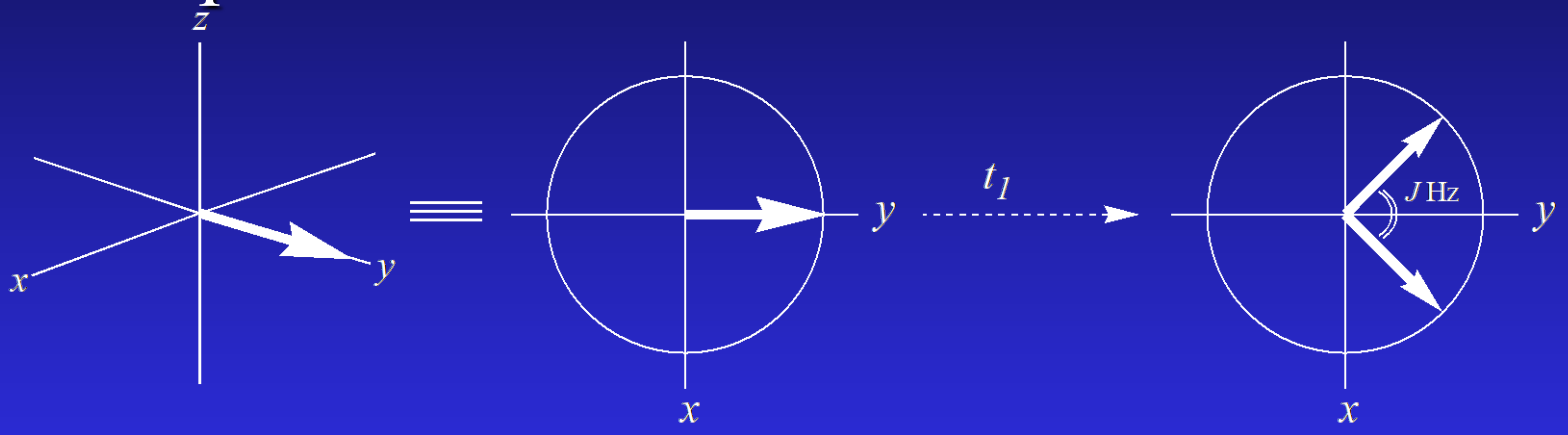
F2 (Direct Dimension)

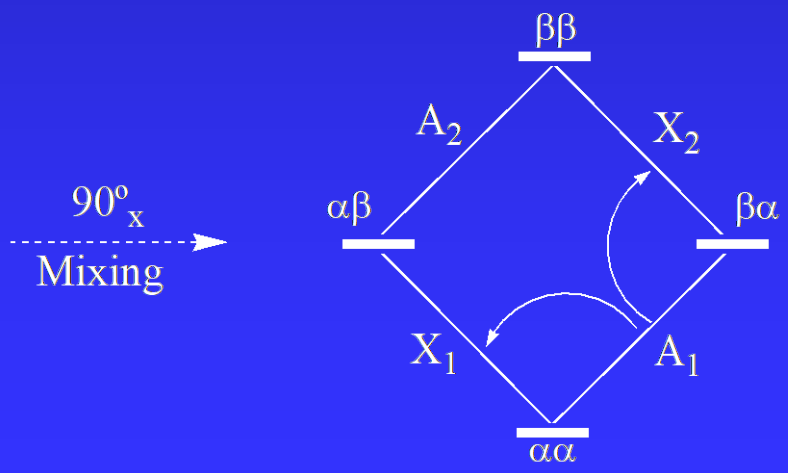


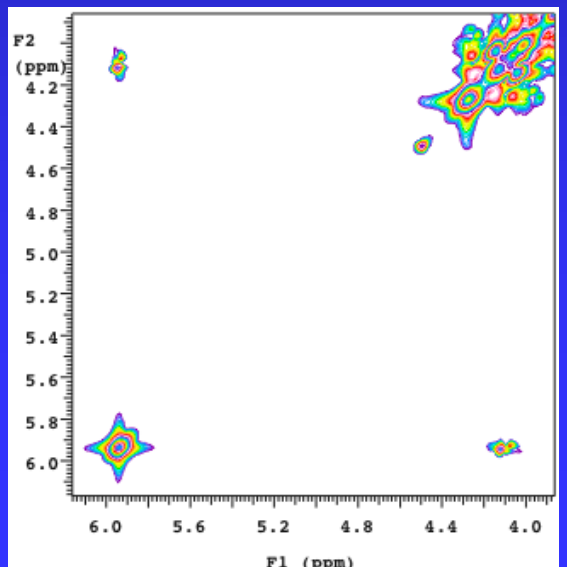
F1 (Indirect Dimension)

Transformed COSY Data: Cross-peaks

- Coherence transfer requires antiphase disposition of vectors.



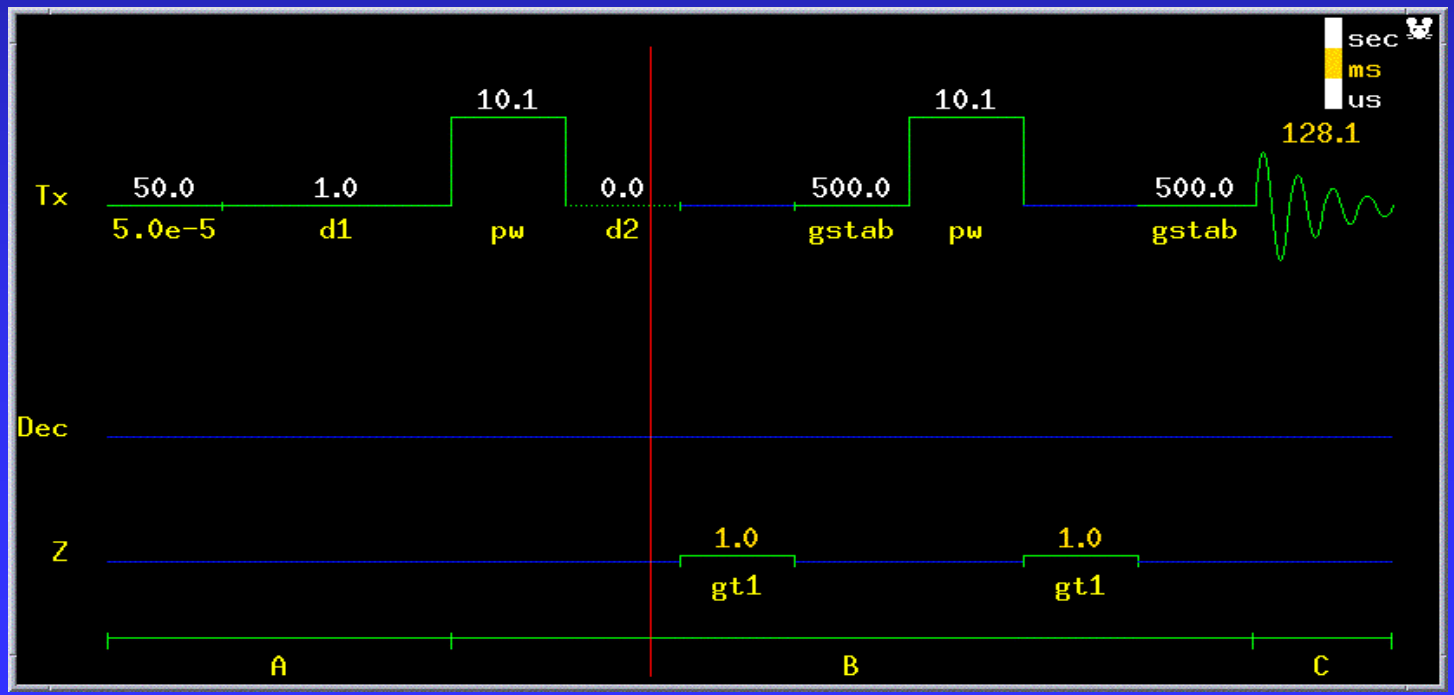




Transferred magnetization modulated by sin180Jt_1

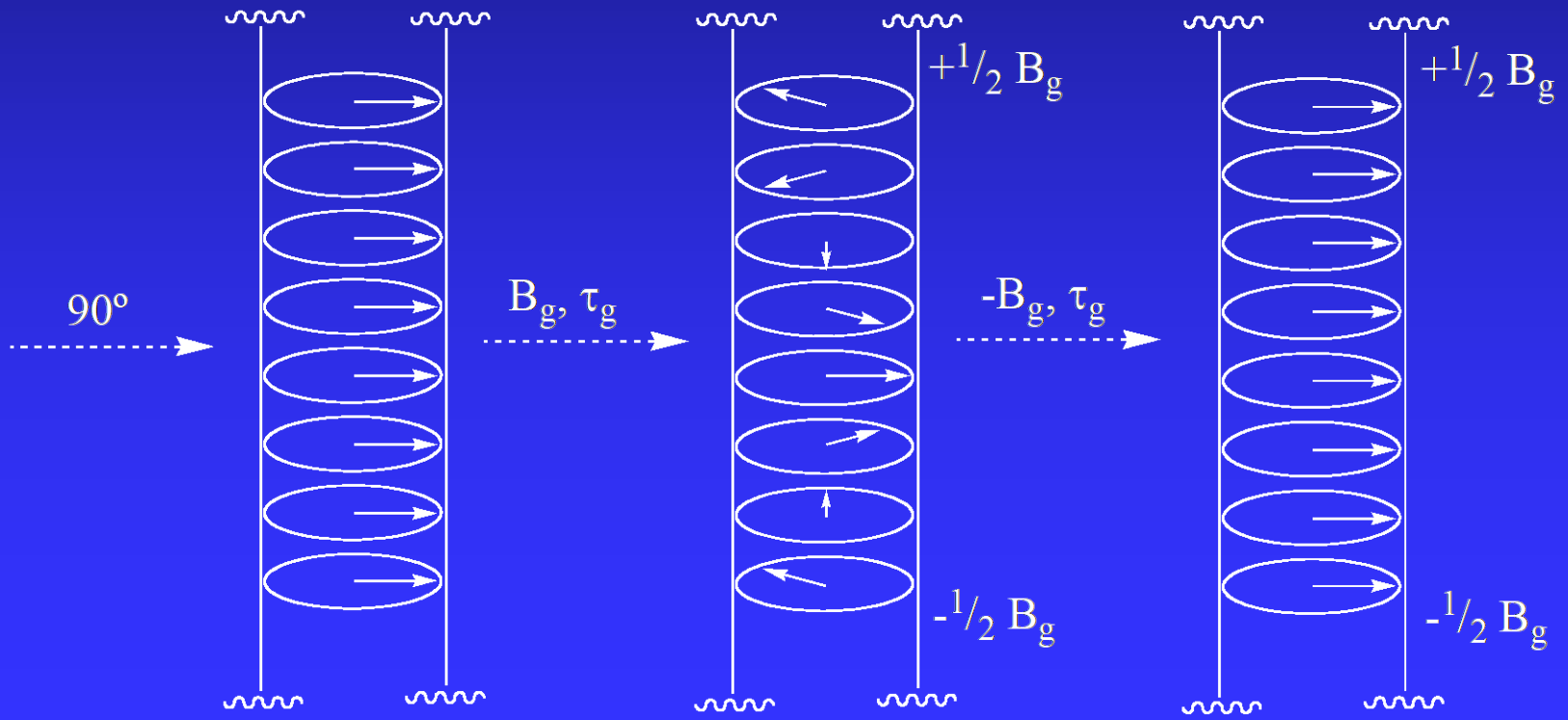
gCOSY: Pulse Sequence

- Pulse sequence implemented at MTR NMR.
- Used for routine acquisition.
  - Not phase sensitive, magnitude mode spectrum. No coupling information (i.e. No *J* coupling values) in cross-peak.
- Utilizes gradient selection.



Gradients

- Used either to destroy or select desired coherences (i.e. artifacts or peaks).
- Requires spatially defined nuclei: DO NOT SPIN SAMPLE!

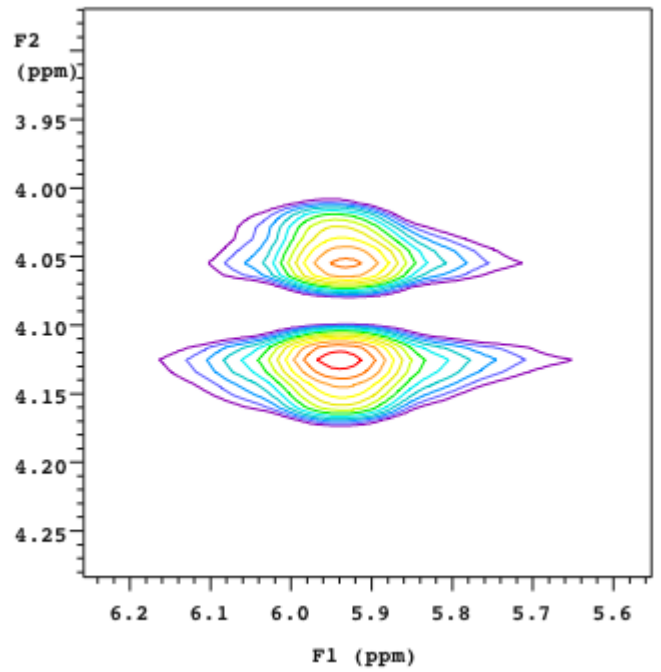


Acquiring gCOSY: ns vs. 1TD

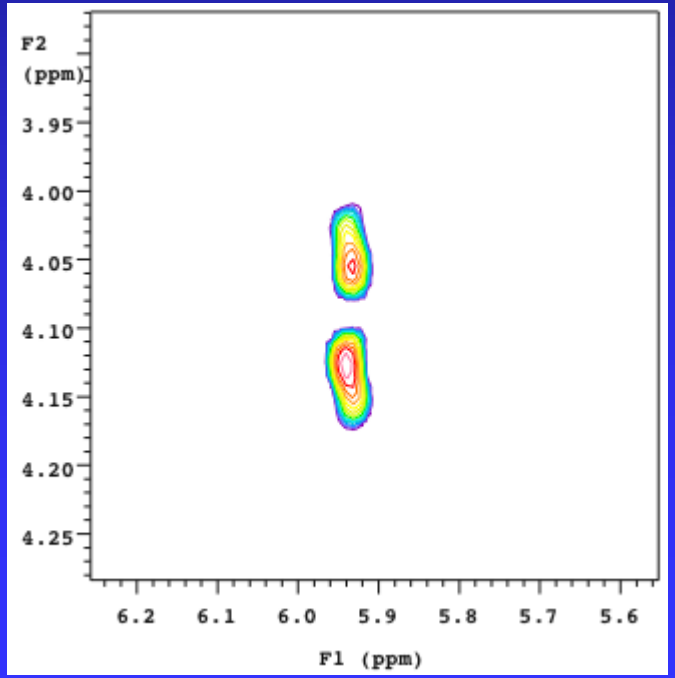
- nt determines the S/N for each FID used in constructing the indirect dimension (F1).
  - Recommended minimum: ns=4.
  - Increase for dilute samples or when looking for small coupling ($J < 3$ Hz): ns=8,16.
- 1TD sets resolution in indirect dimension (F1).
  - Default is 128, which gives ~15 Hz/point when used with Linear Prediction and zero-filling.
  - Increase for very crowded spectra. Consider using gDQCOSY for these samples.

Linear Prediction

- Apply up to 3 x linear prediction.
- If peaks are not resolved in the time domain, they cannot be resolved by linear prediction.



Cross peak without LP

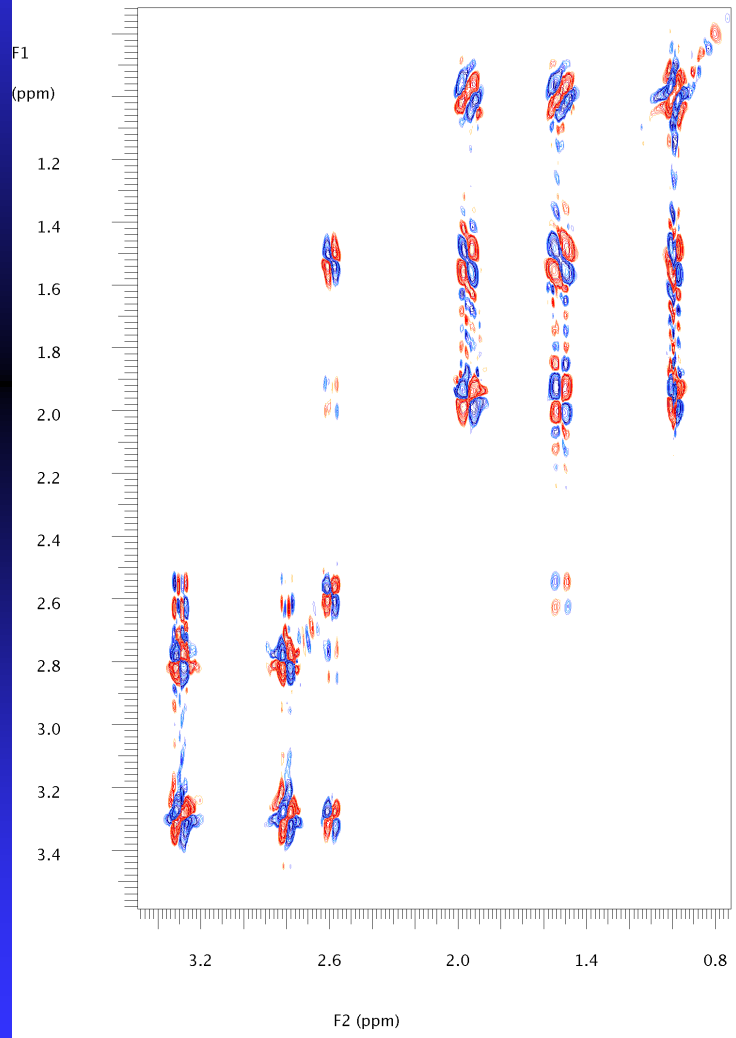


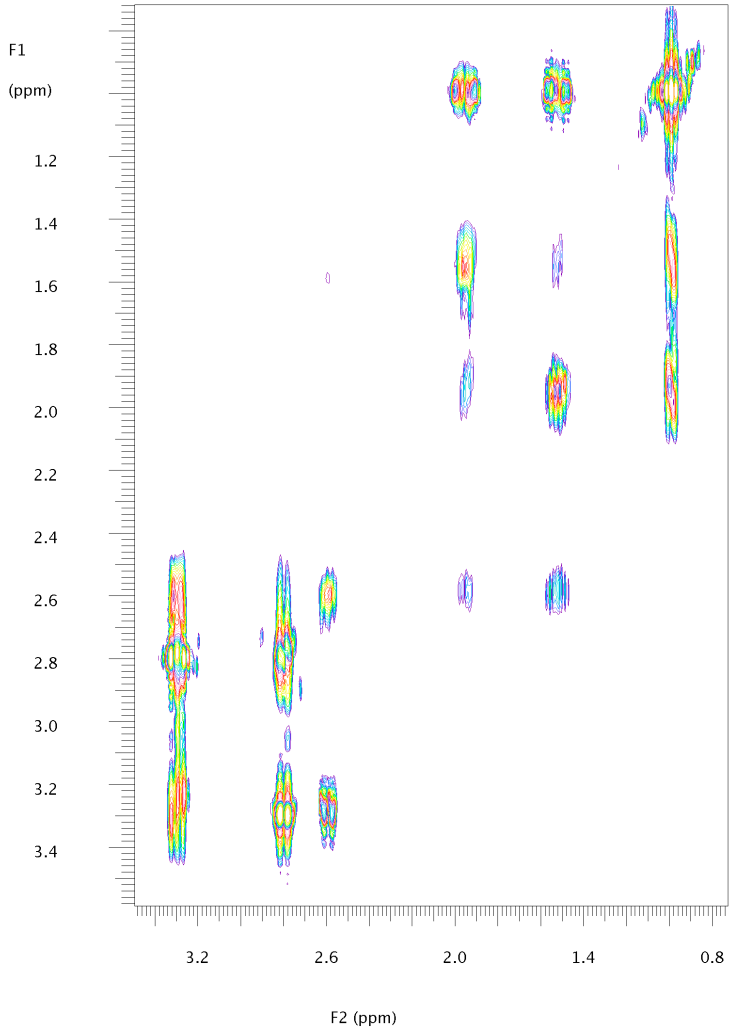
Cross peak with 3x LP

gDQFCOSY: An Alternative

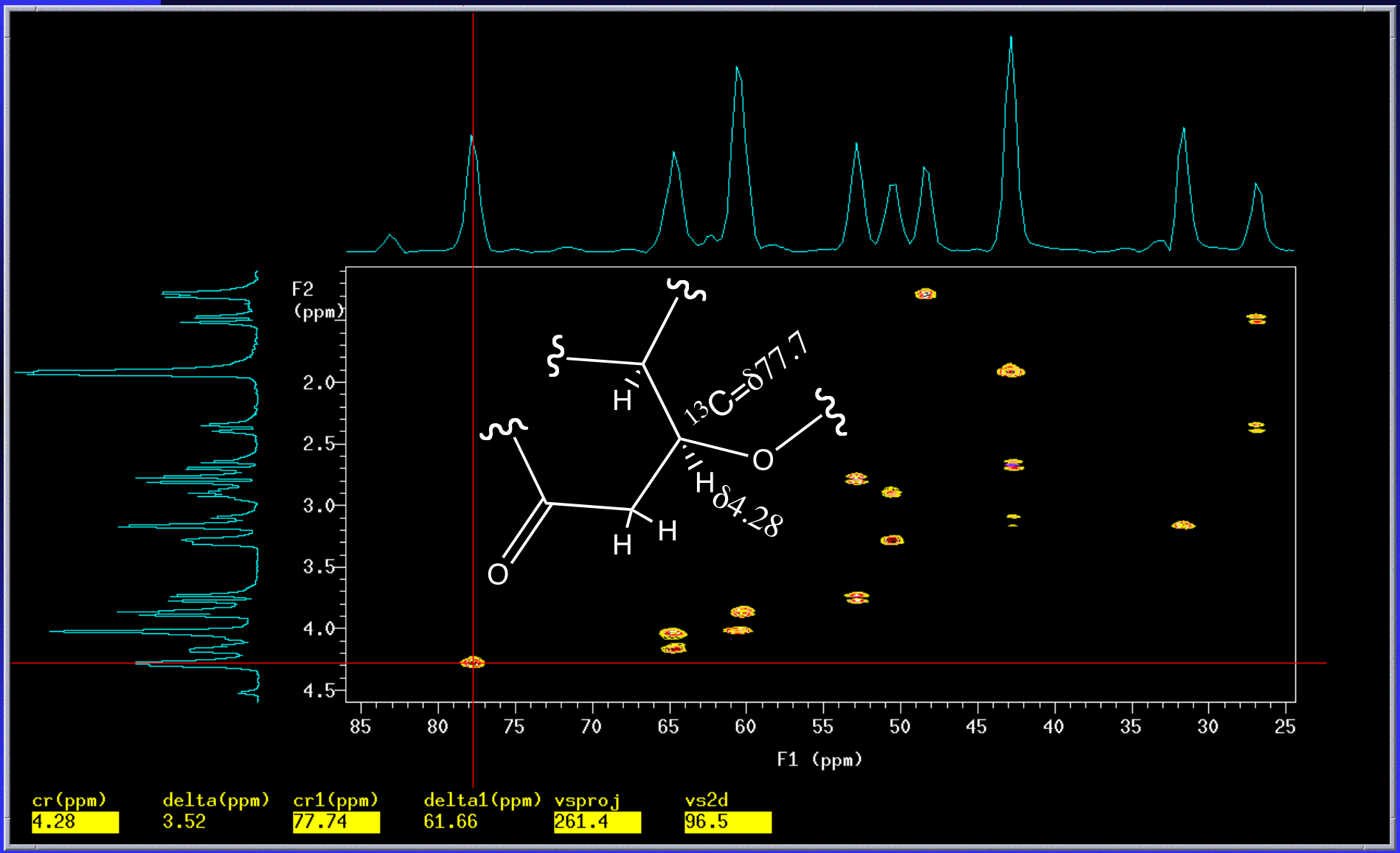
- Phase-sensitive: gives higher resolution.
- Can get magnitude of coupling constants.
- Suppresses singlets (good for spectra with large solvent peak or *t*-butyl group.
- May give better resolution around diagonal
- More sensitive to pulse calibration.

gDQFCOSY: An Alternative

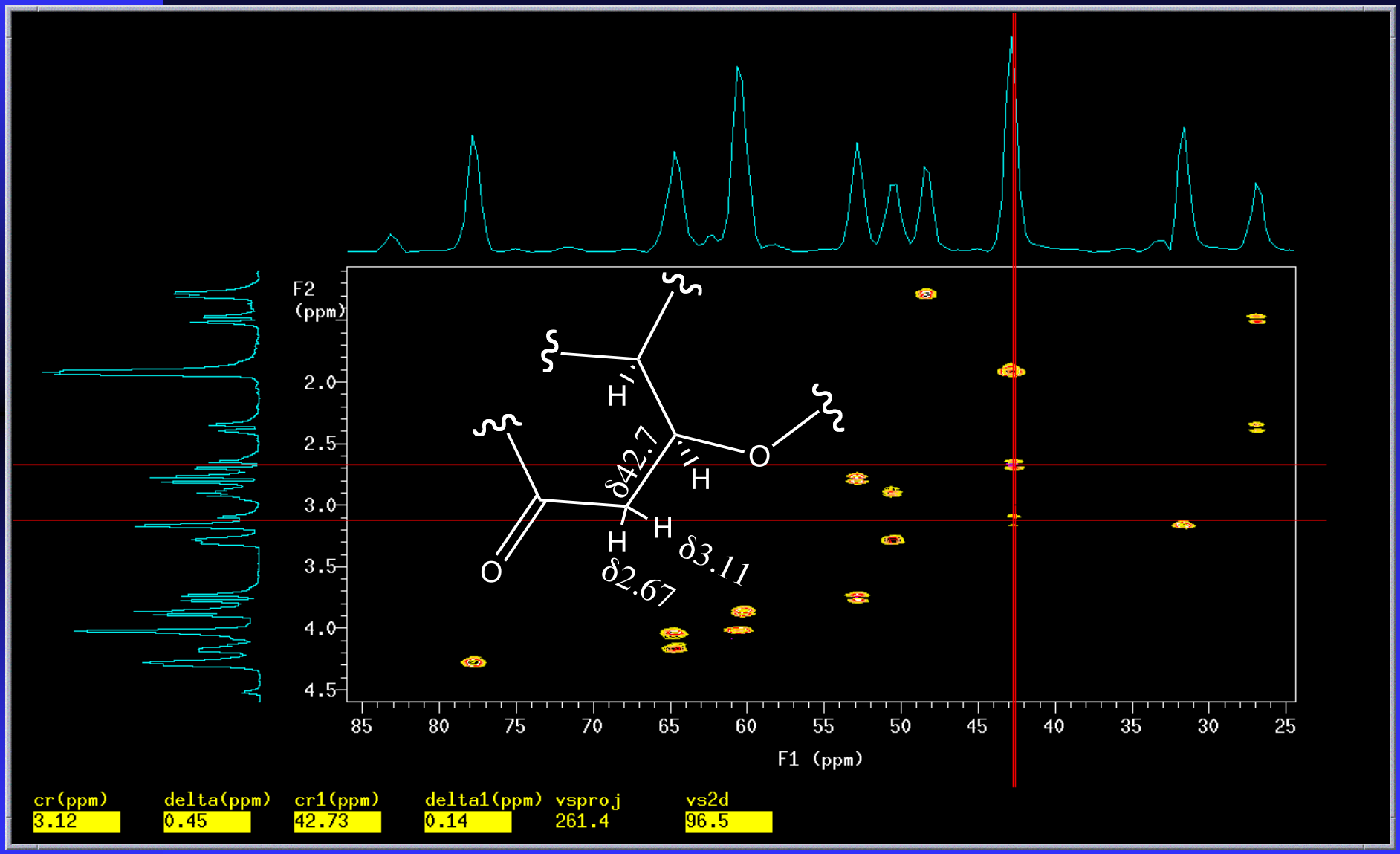




^{13}C-^{1}H Heteronuclear Correlation Spectrum

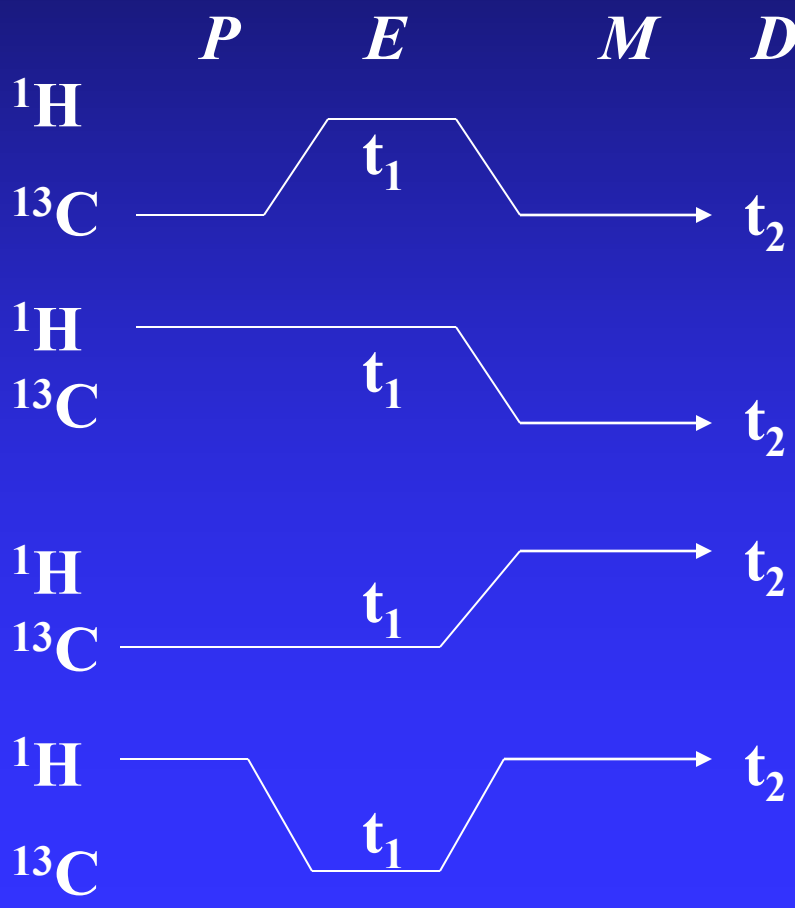


^{13}C-^{1}H Heteronuclear Correlation Spectrum



Heteronuclear Correlation: possible schemes

- $S/N \propto NAT^{-1}B_0^{3/2}\gamma_{exc}\gamma_{obs}^{3/2}T_2^{*}(NS)^{1/2}$



Relative Sensitivity

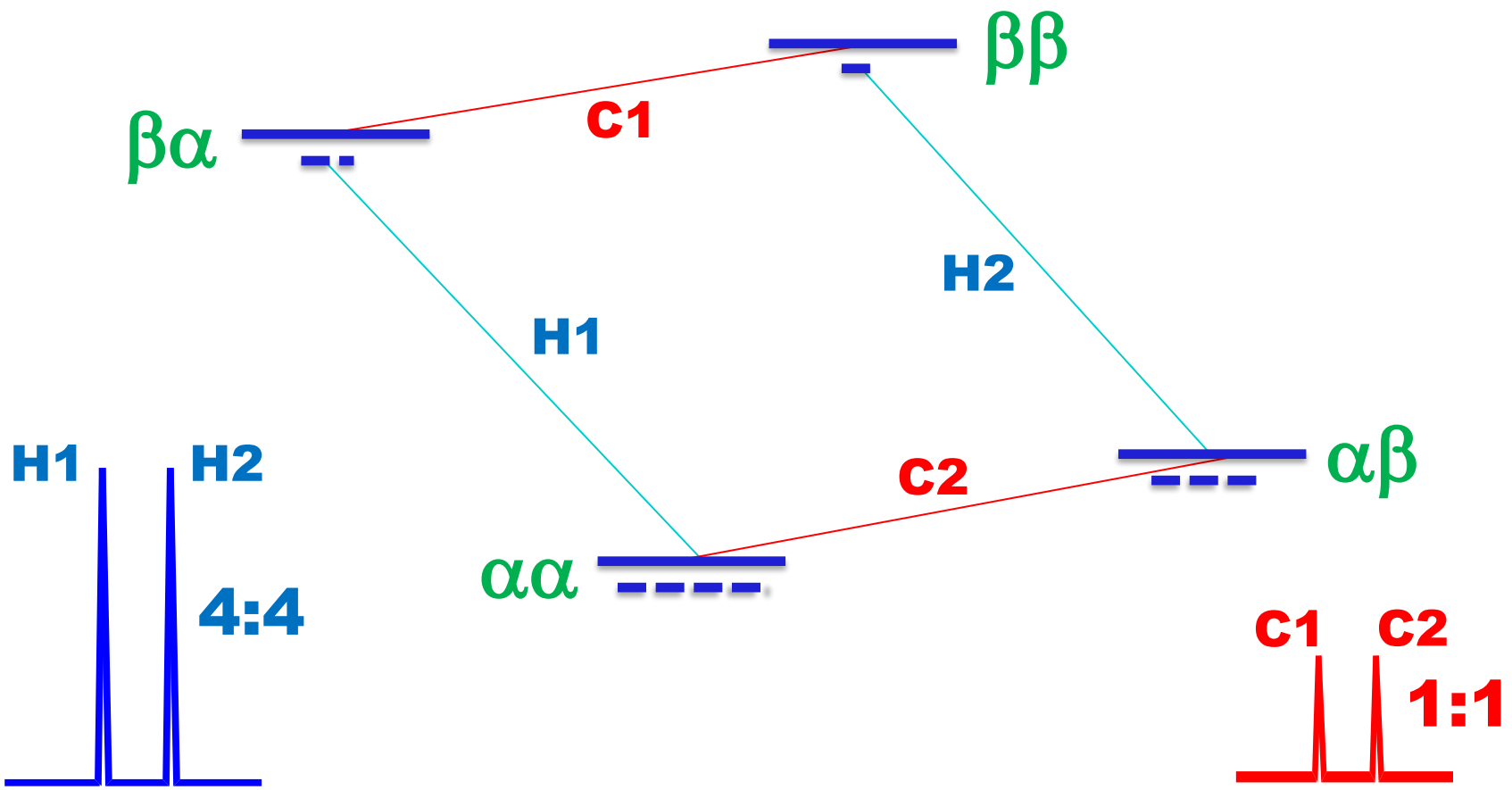
1

4 (HETCOR)

8

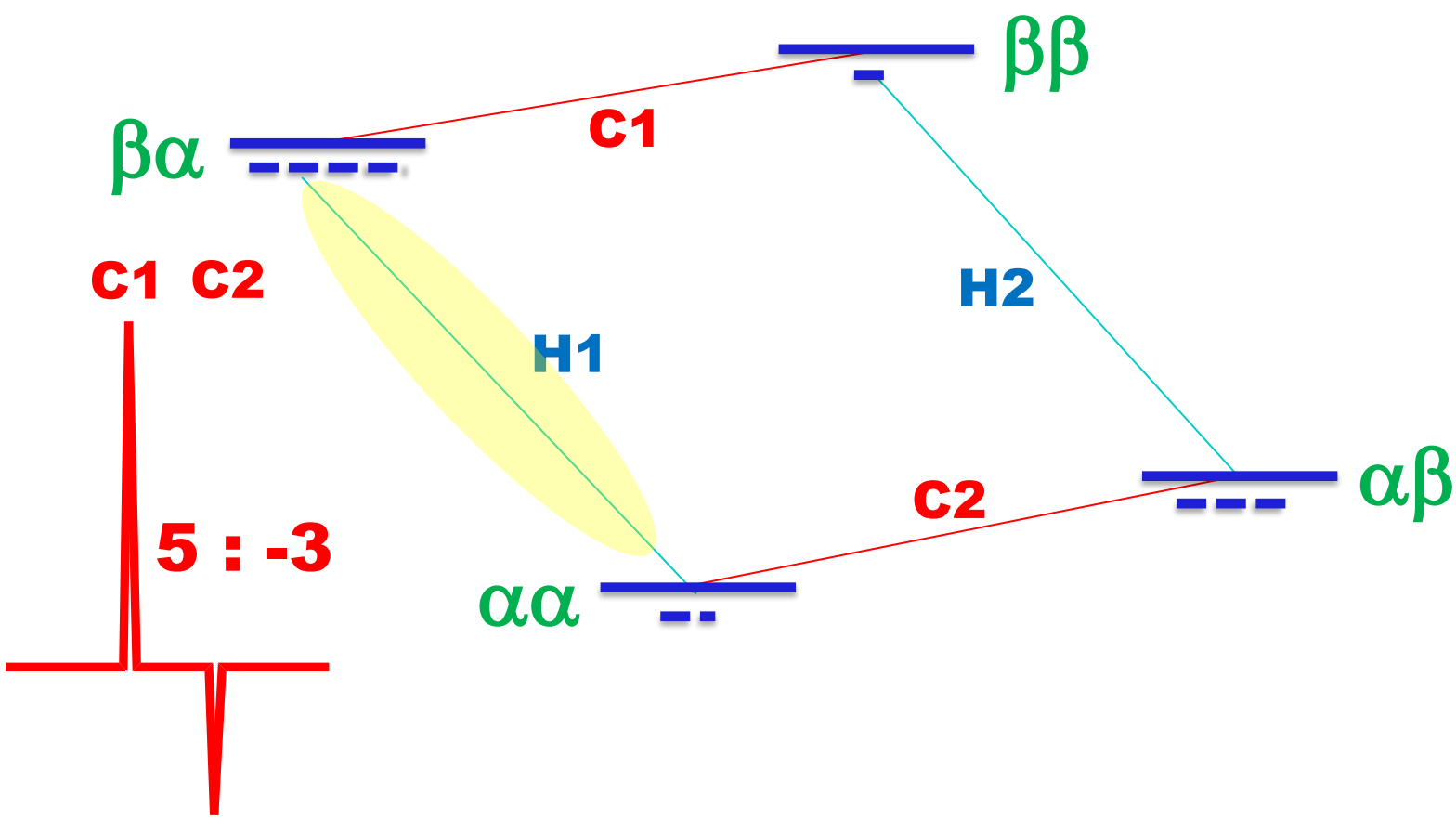
32 (gHMQC)

Equilibrium Energy Level Diagram



Adapted from http://u-of-o-nmr-facility.blogspot.com/

Polarization Transfer

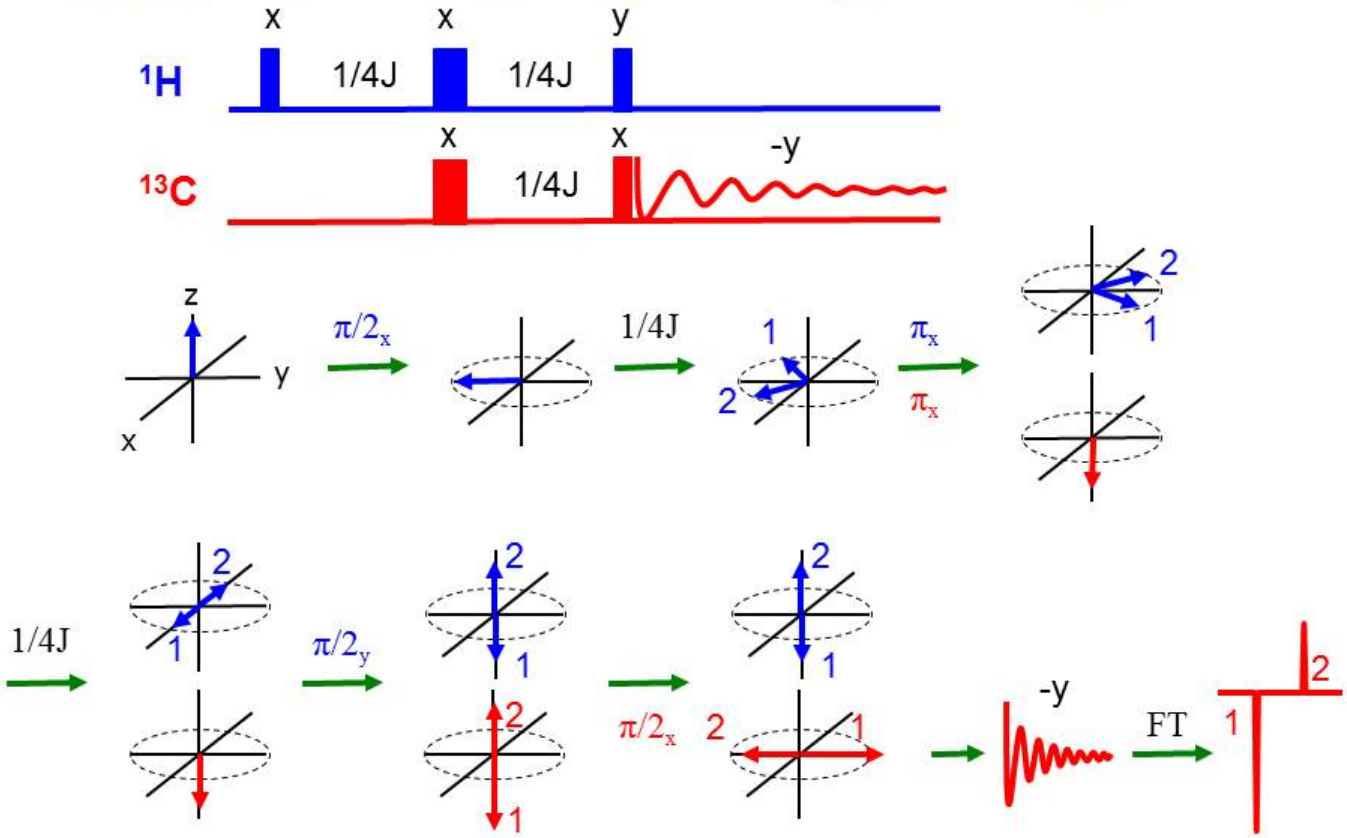


Adapted from http://u-of-o-nmr-facility.blogspot.com/

Polarization Transfer and Signal Enhancement

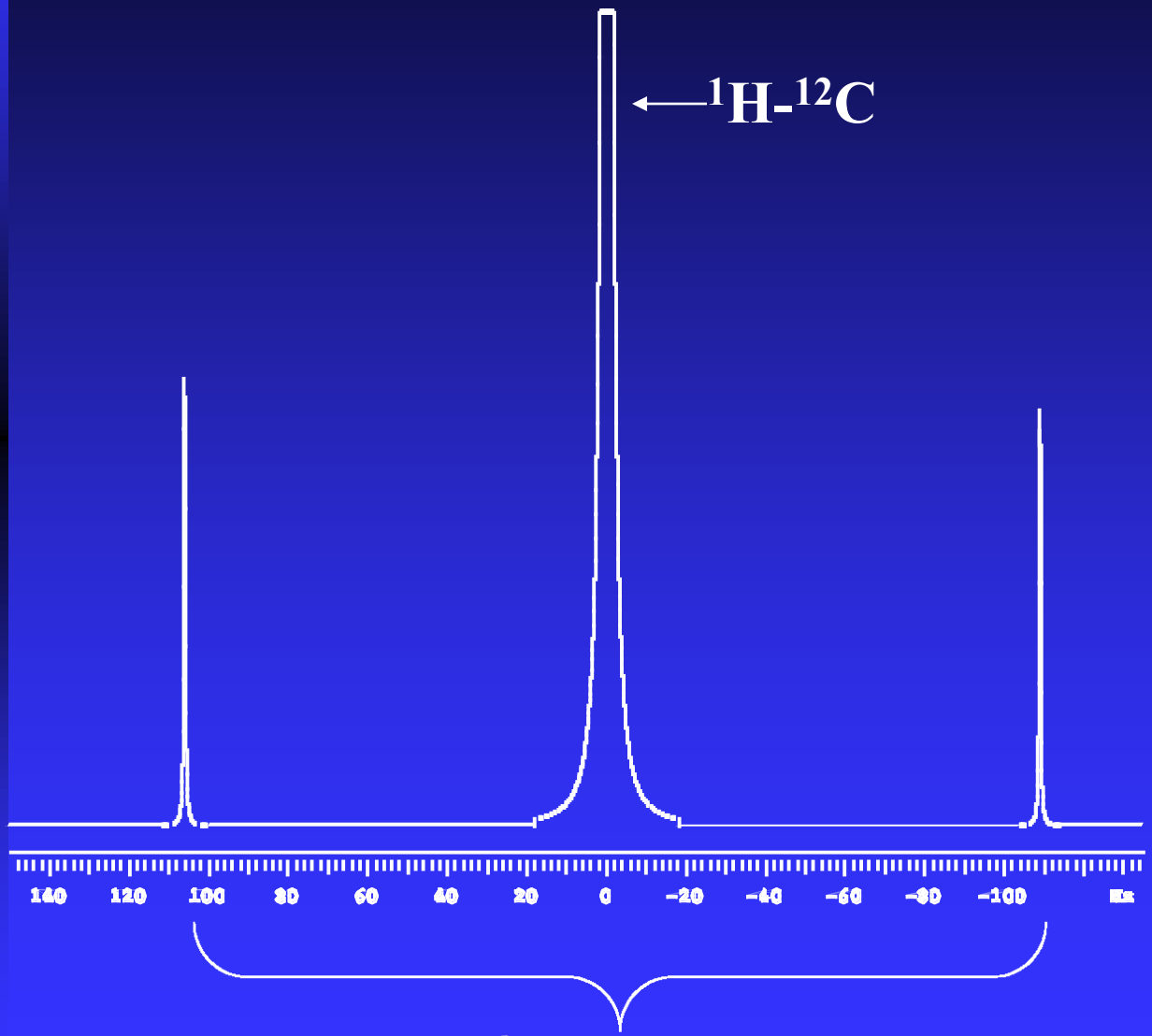
How Its Done – The "INEPT" Experiment

INEPT Insensitive Nuclei Enhanced by Polarization Transfer

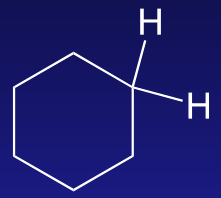


http://u-of-o-nmr-facility.blogspot.com/

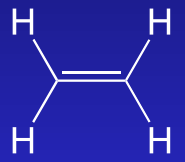
gHMQC/gHSQC:
Direct ^{13}C-^{1}H connectivity



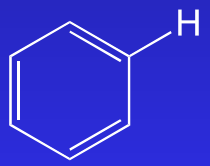
$^{1}J_{C\text{-}H}$ in Hz



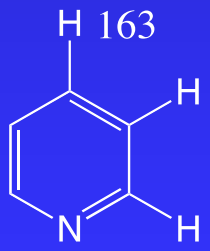
128



156



159

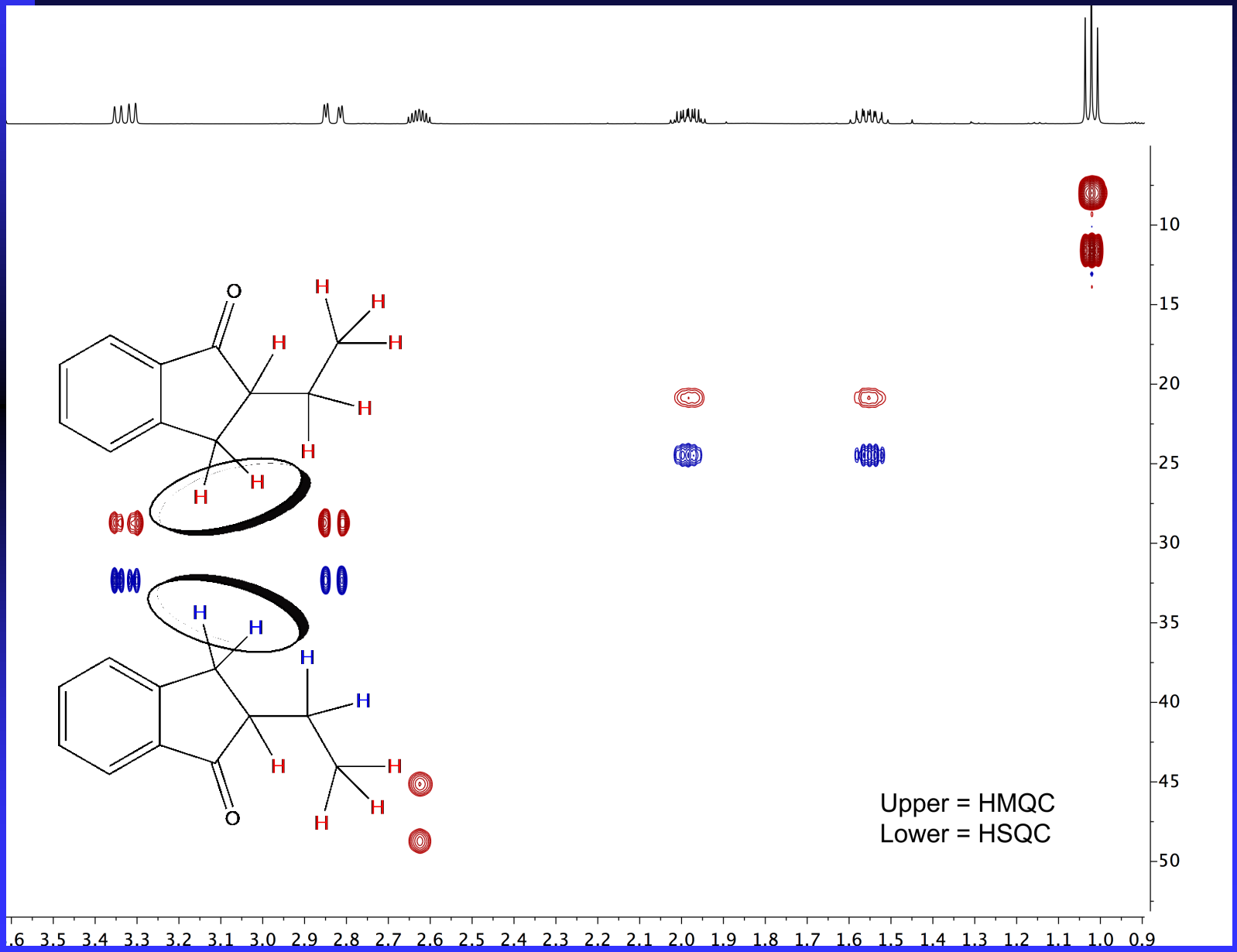


163

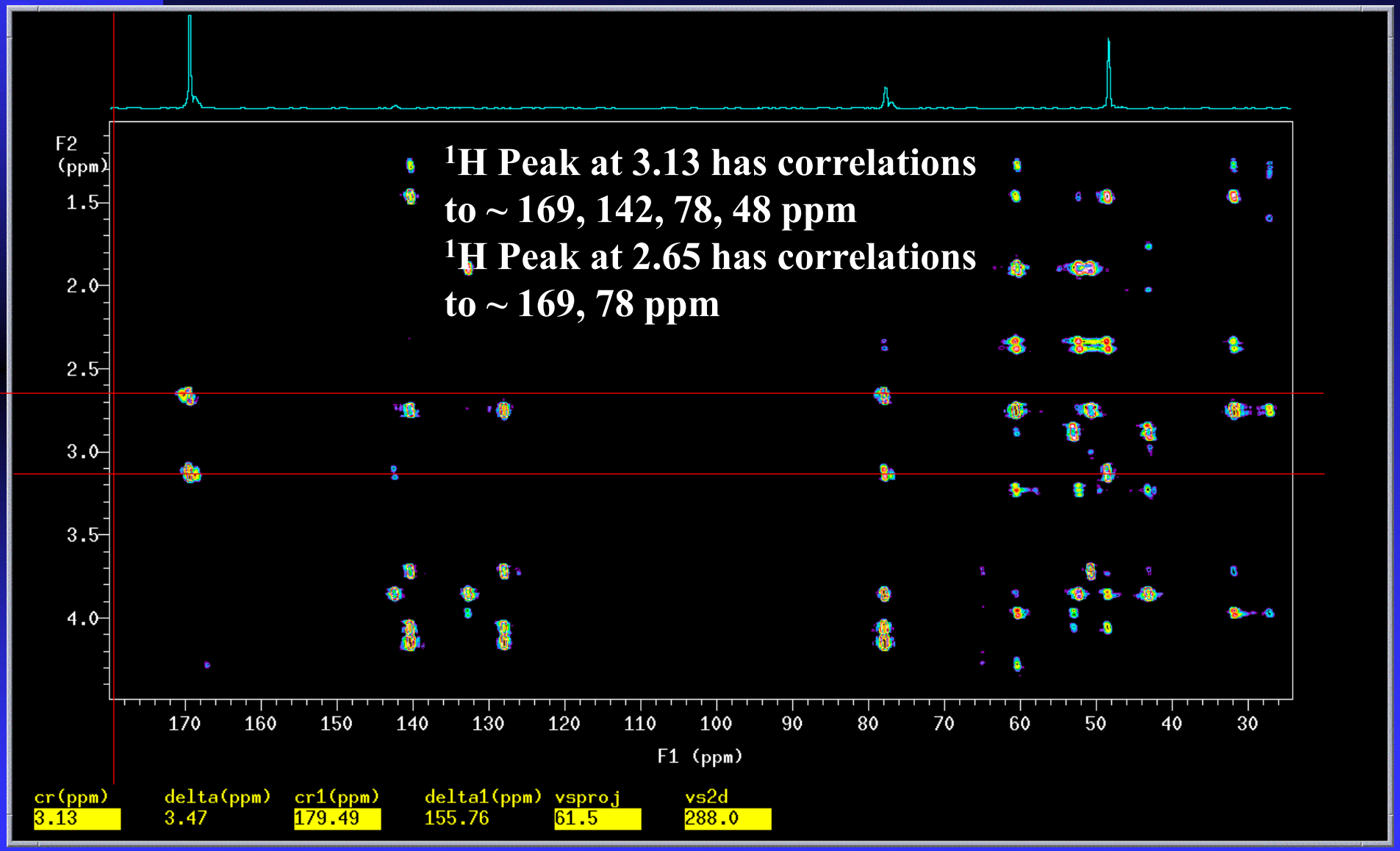
178

249

HSQC vs. HMQC



gHMBC: Long-range ^{13}C-^{1}H connectivity

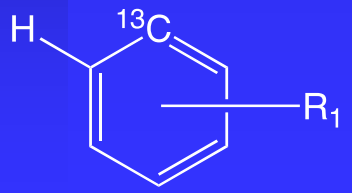


^{1}H Peak at 3.13 has correlations to ~ 169, 142, 78, 48 ppm

^{1}H Peak at 2.65 has correlations to ~ 169, 78 ppm

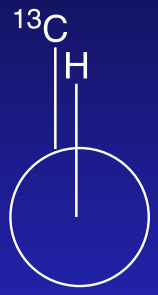
gHMBC: long-range coupling constants

	$\lvert {}^{2}J_{C\text{-}H}\rvert$ in Hz
H—C–^{13}C—	1 - 6
H—C=^{13}C—	0 - 16
H—C≡^{13}C—	40 - 60
H—C–^{13}C=O	5 - 8
H—C(=O)–^{13}C—	20 - 50

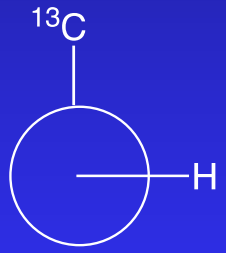


1 - 4

$\lvert {}^{3}J_{C\text{-}H}\rvert$ = 0 - 10 Hz

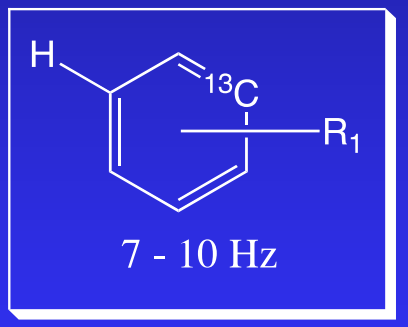


~ 6 Hz



~ 0 Hz

~ 9 Hz

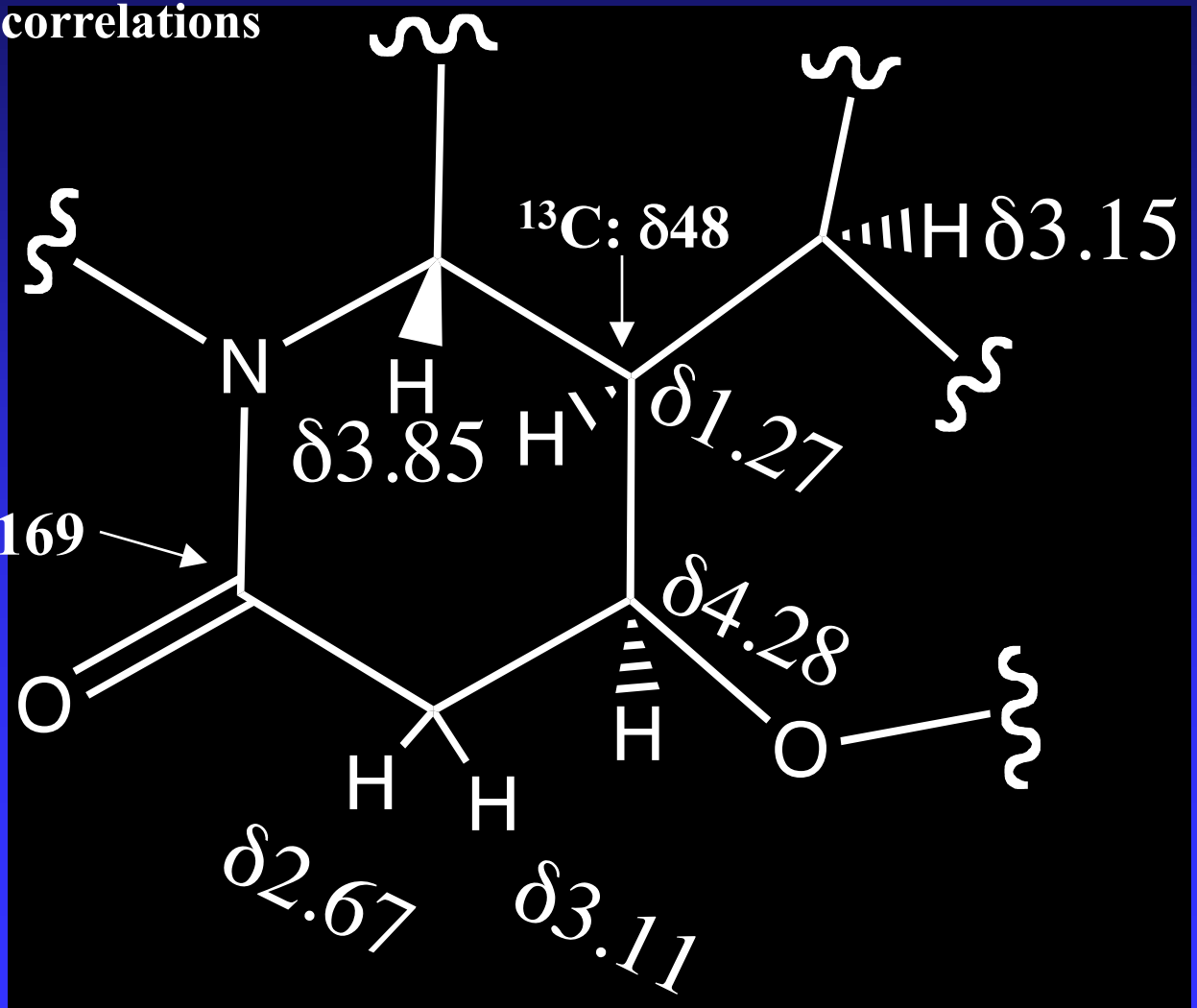


7 - 10 Hz

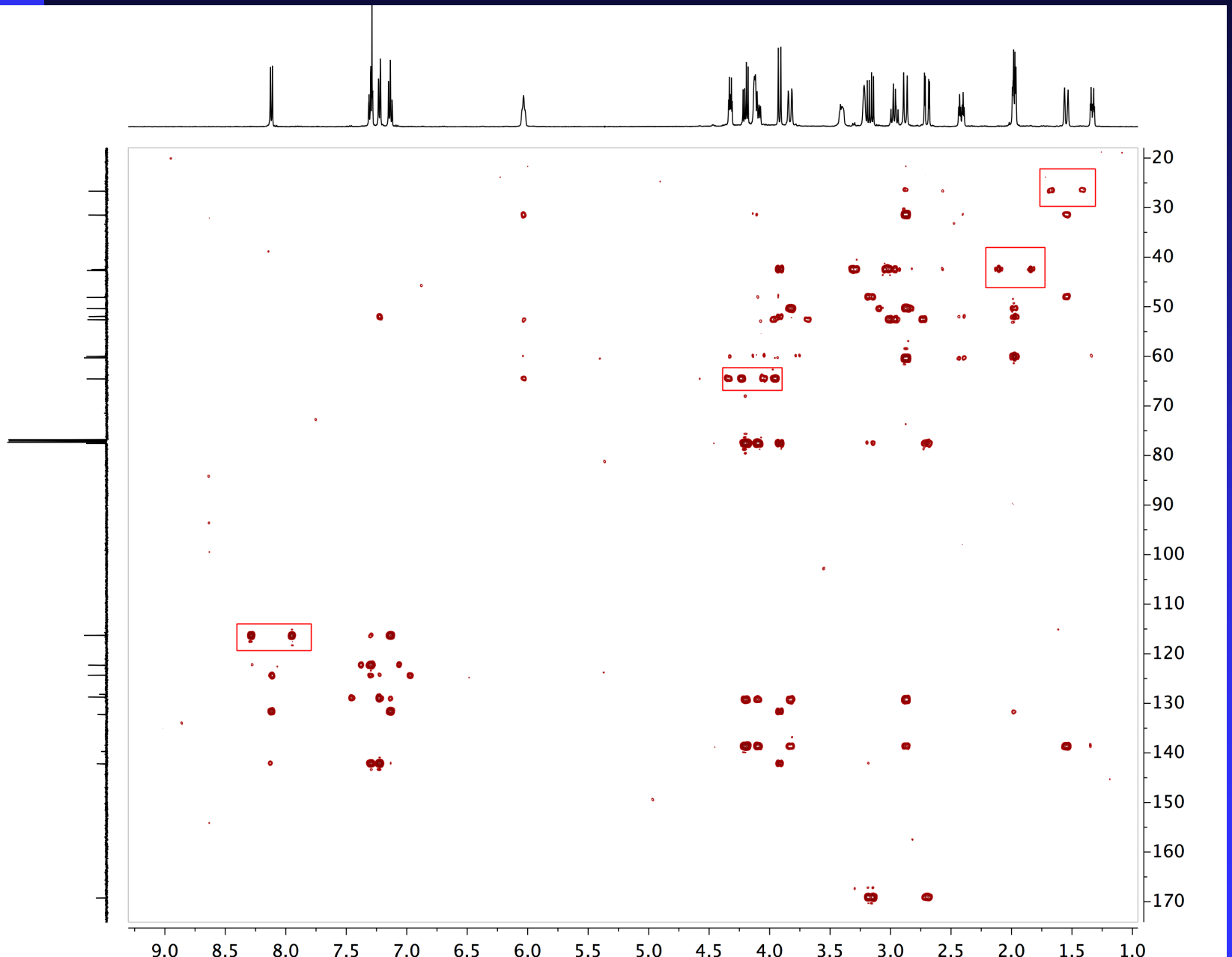
gHMBC: Long-range ^{13}C-^{1}H connectivity

^{1}H Peak at 3.13 has correlations to ~ 169, 142, 78, 48 ppm

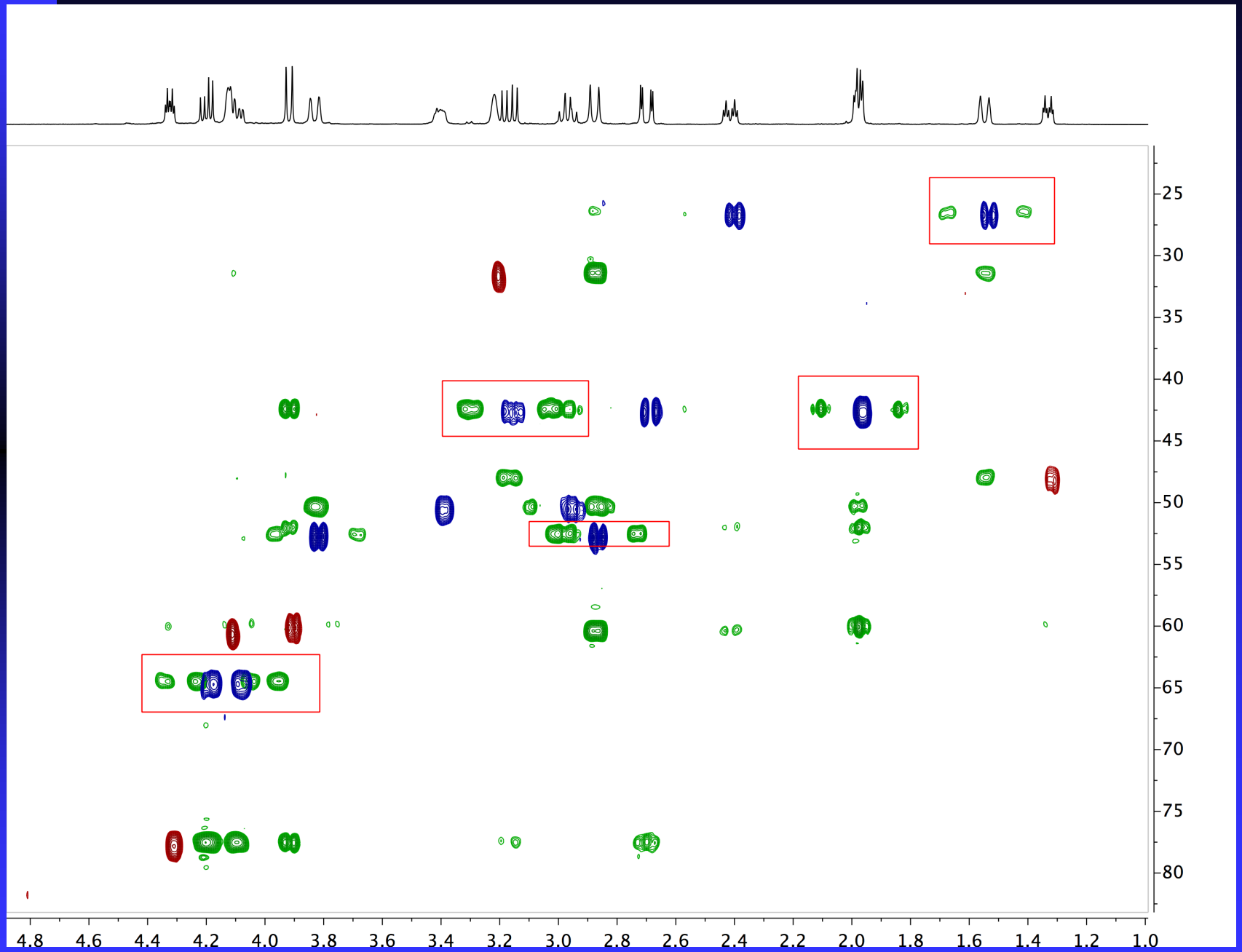
^{1}H Peak at 2.65 has correlations to ~ 169, 78 ppm



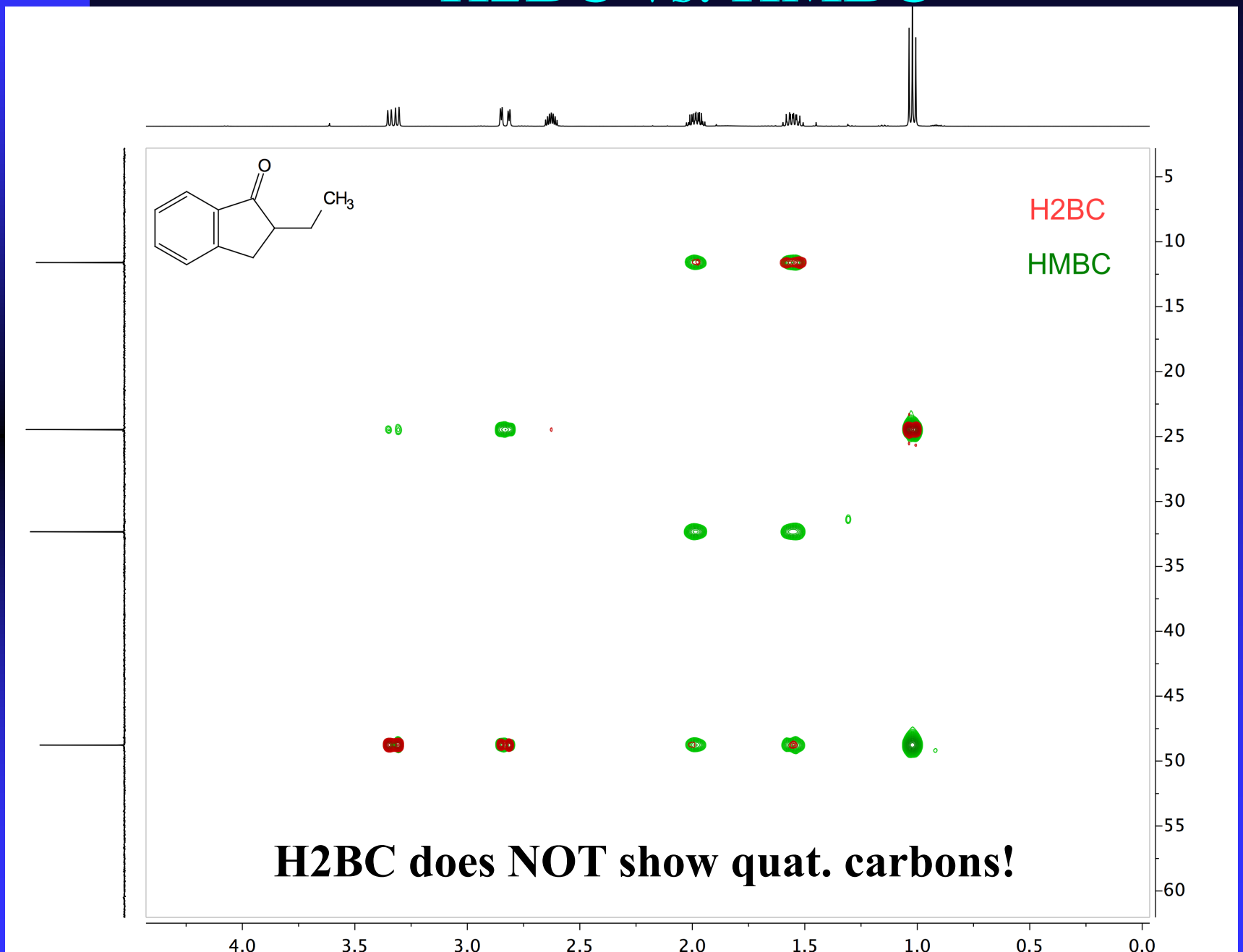
Artifacts in HMBC: one-bond breakthrough



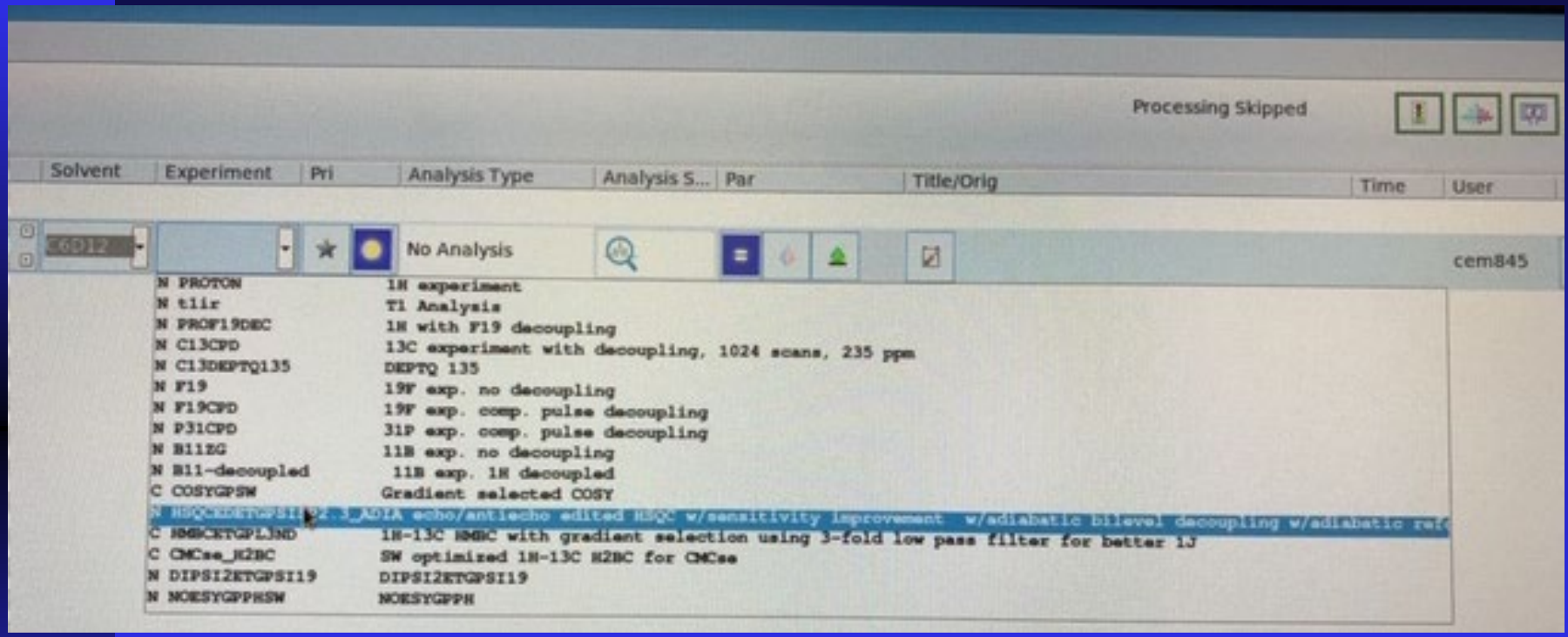
Artifacts in HMBC: HSQC Overlay

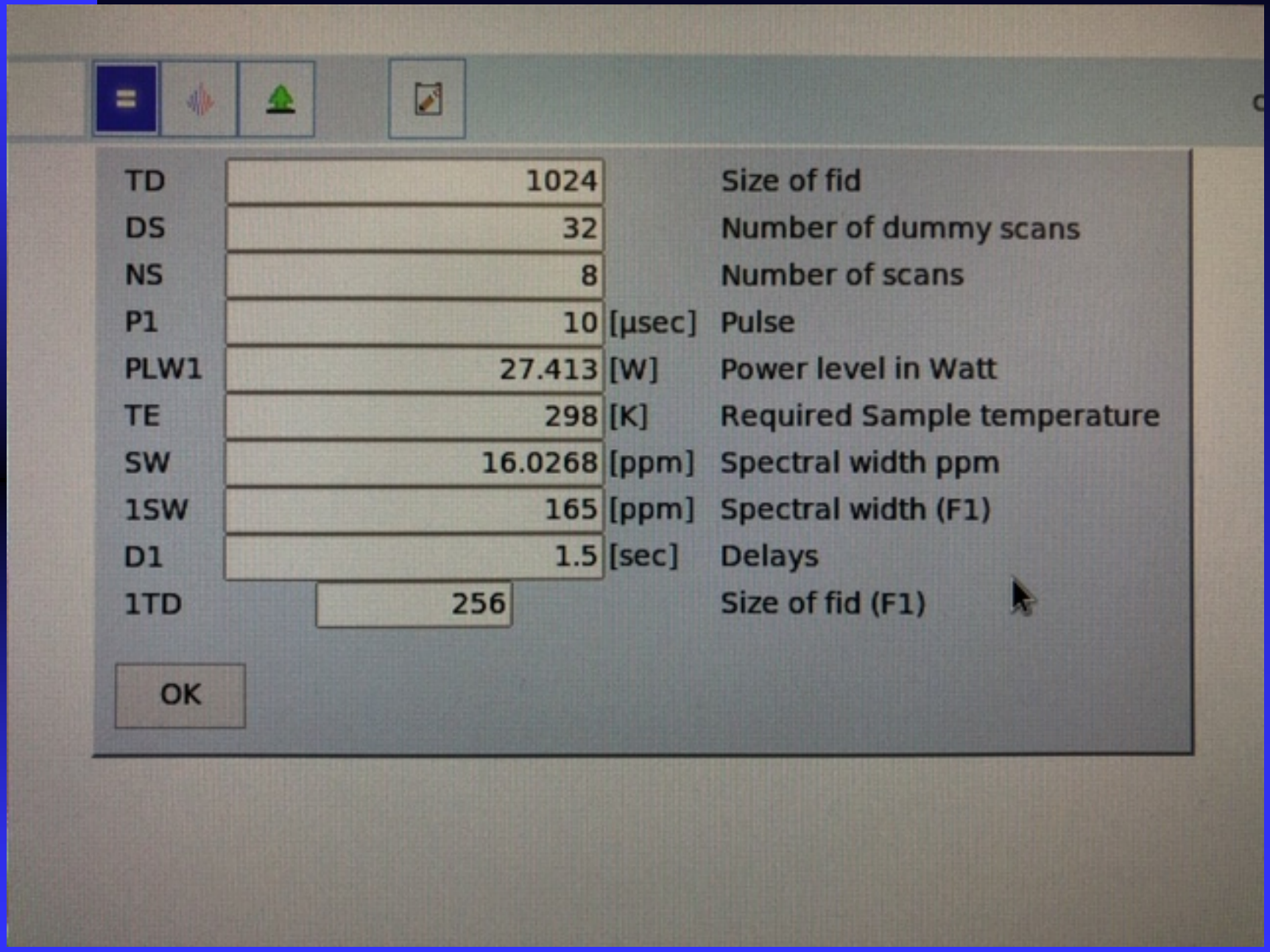


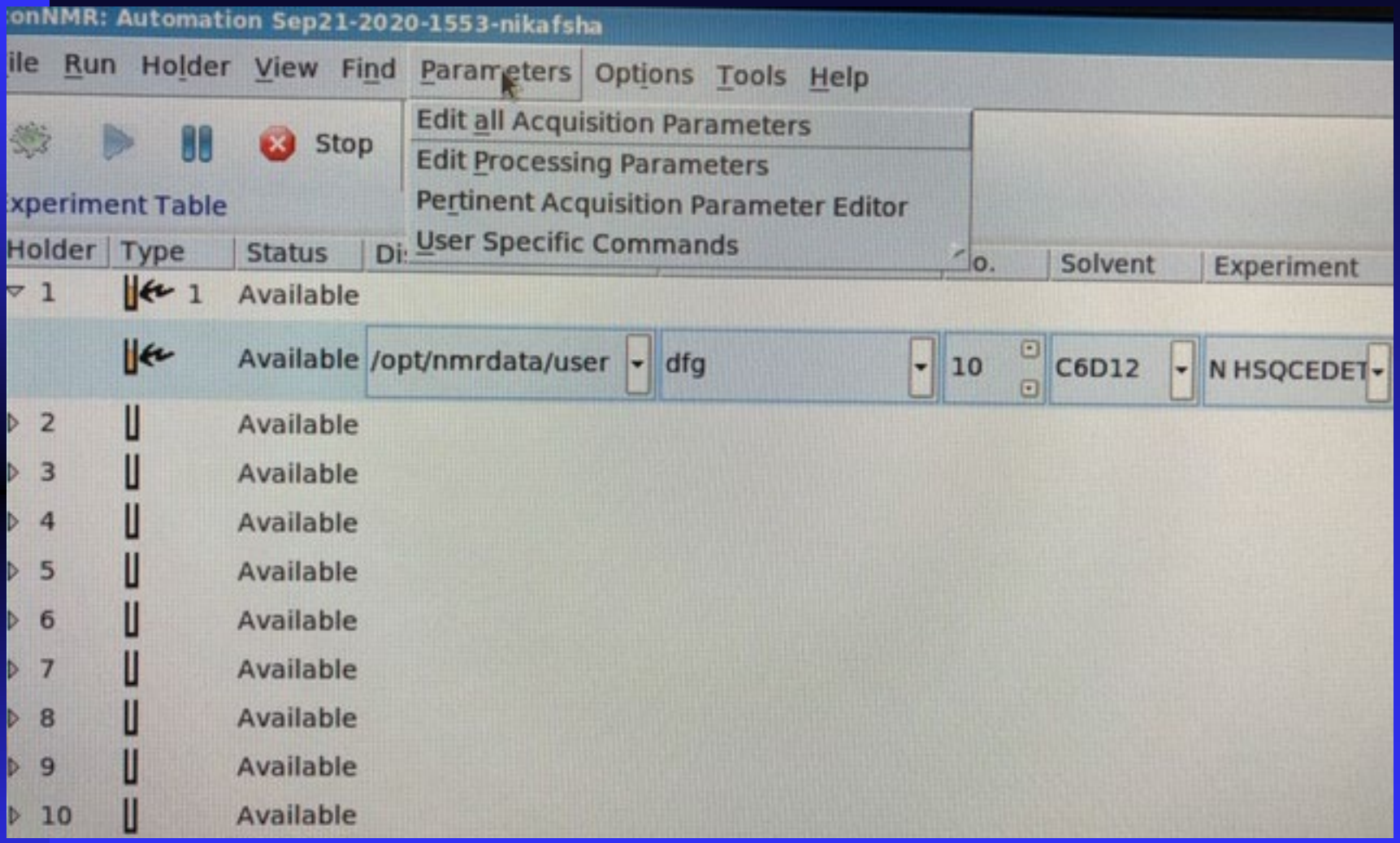
H2BC vs. HMBC



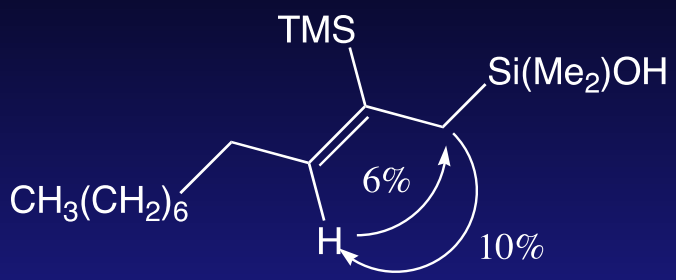
H2BC does NOT show quat. carbons!

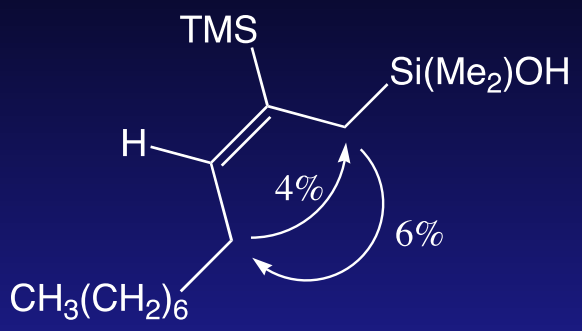

Processing Skipped
Solvent
Experiment
Pri
Analysis Type
Analysis S...
Par
Title/Orig
Time
User
No Analysis
cem845
N PROTON 1H experiment
N t1ir T1 Analysis
N PROF19DEC 1H with F19 decoupling
N C13CPD 13C experiment with decoupling, 1024 scans, 235 ppm
N C13DEPTQ135 DEPTQ 135
N F19 19F exp. no decoupling
N F19CPD 19F exp. comp. pulse decoupling
N P31CPD 31P exp. comp. pulse decoupling
N B11ZG 11B exp. no decoupling
N B11-decoupled 11B exp. 1H decoupled
C COSYGPSW Gradient selected COSY
C HMBCETGPLND 1H-13C HMBC with gradient selection using 3-fold low pass filter for better 1J
C CMCse_H2BC SW optimized 1H-13C H2BC for CMCse
N DIPSI2ETGPSI19 DIPSI2ETGPSI19
N NOESYGPPHSW NOESYGPPH

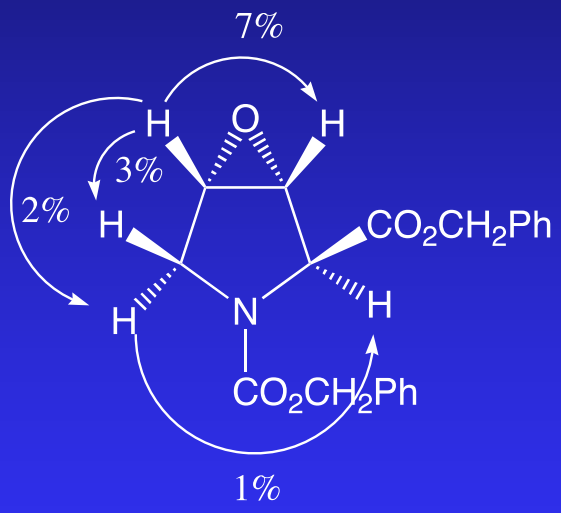

TD 1024 Size of fid
DS 32 Number of dummy scans
NS 8 Number of scans
P1 10 [µsec] Pulse
PLW1 27.413 [W] Power level in Watt
TE 298 [K] Required Sample temperature
SW 16.0268 [ppm] Spectral width ppm
1SW 165 [ppm] Spectral width (F1)
D1 1.5 [sec] Delays
1TD 256 Size of fid (F1)
OK

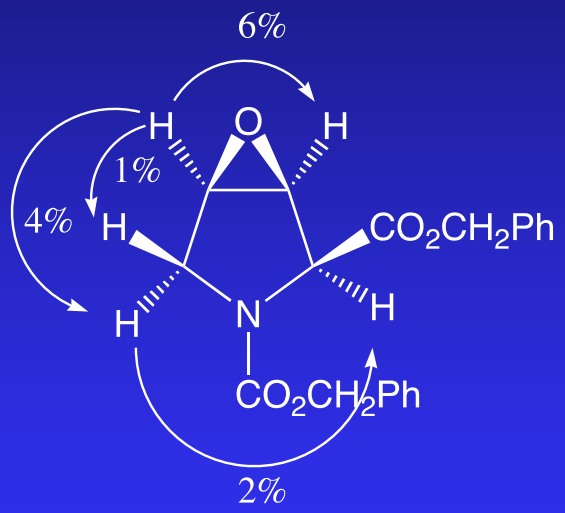

onNMR: Automation Sep21-2020-1553-nikafsha
ile Run Holder View Find Parameters Options Tools Help
Edit all Acquisition Parameters
Edit Processing Parameters
Pertinent Acquisition Parameter Editor
User Specific Commands
Stop
xperiment Table
Holder Type Status Di o. Solvent Experiment
1 1 Available
Available /opt/nmrdata/user dfg 10 C6D12 N HSQCEDET
2 Available
3 Available
4 Available
5 Available
6 Available
7 Available
8 Available
9 Available
10 Available

NOE's in Structural Assignments



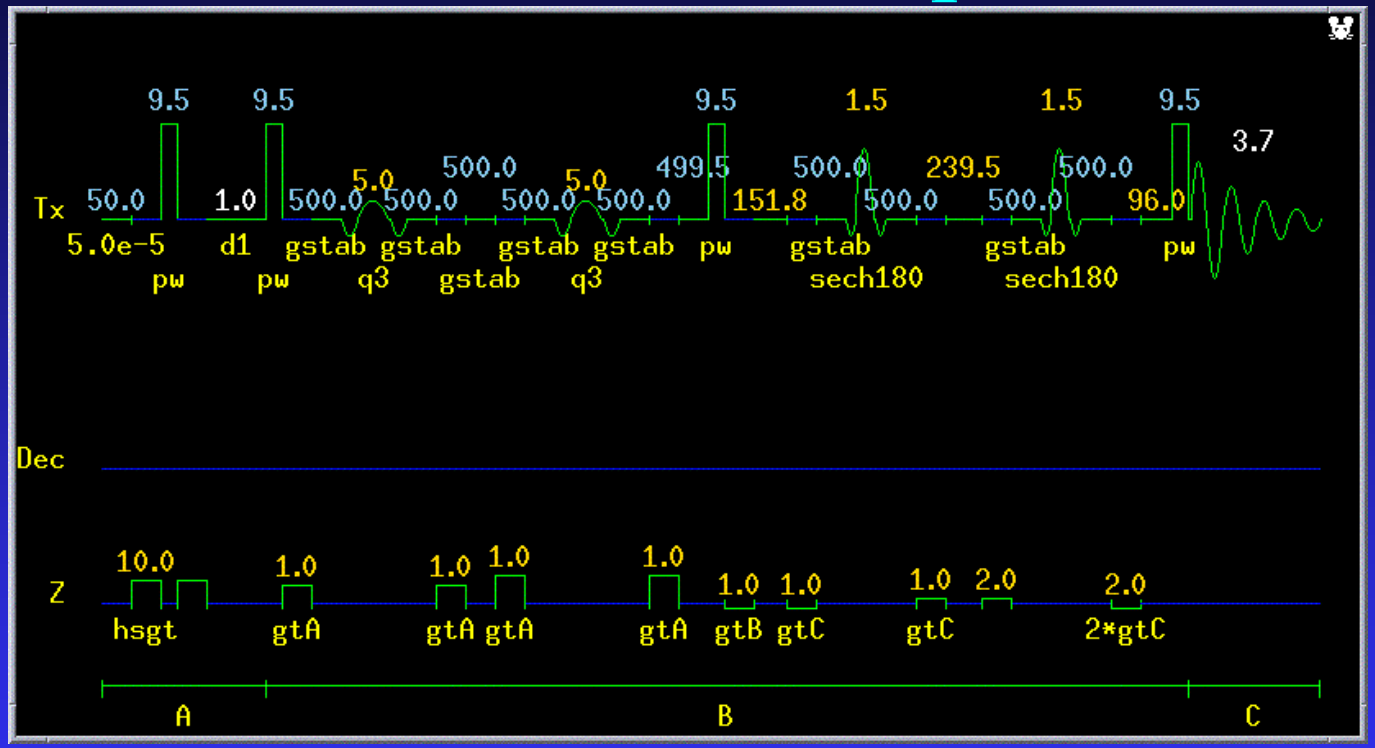






- NOE's are generated through-space.
- NOE's are relative values and can be ambiguous.

NOESY1D: One-Dimensional Nuclear Overhauser Experiment

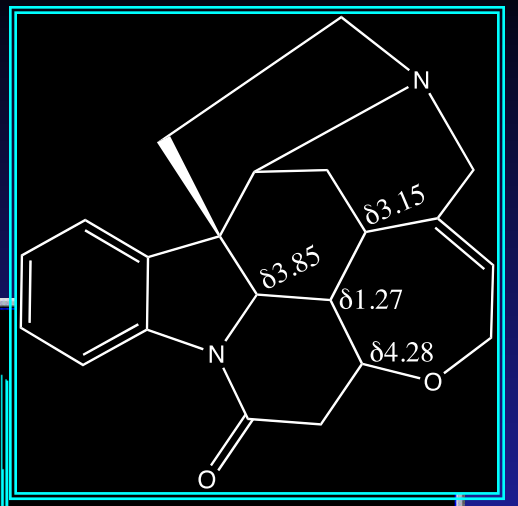


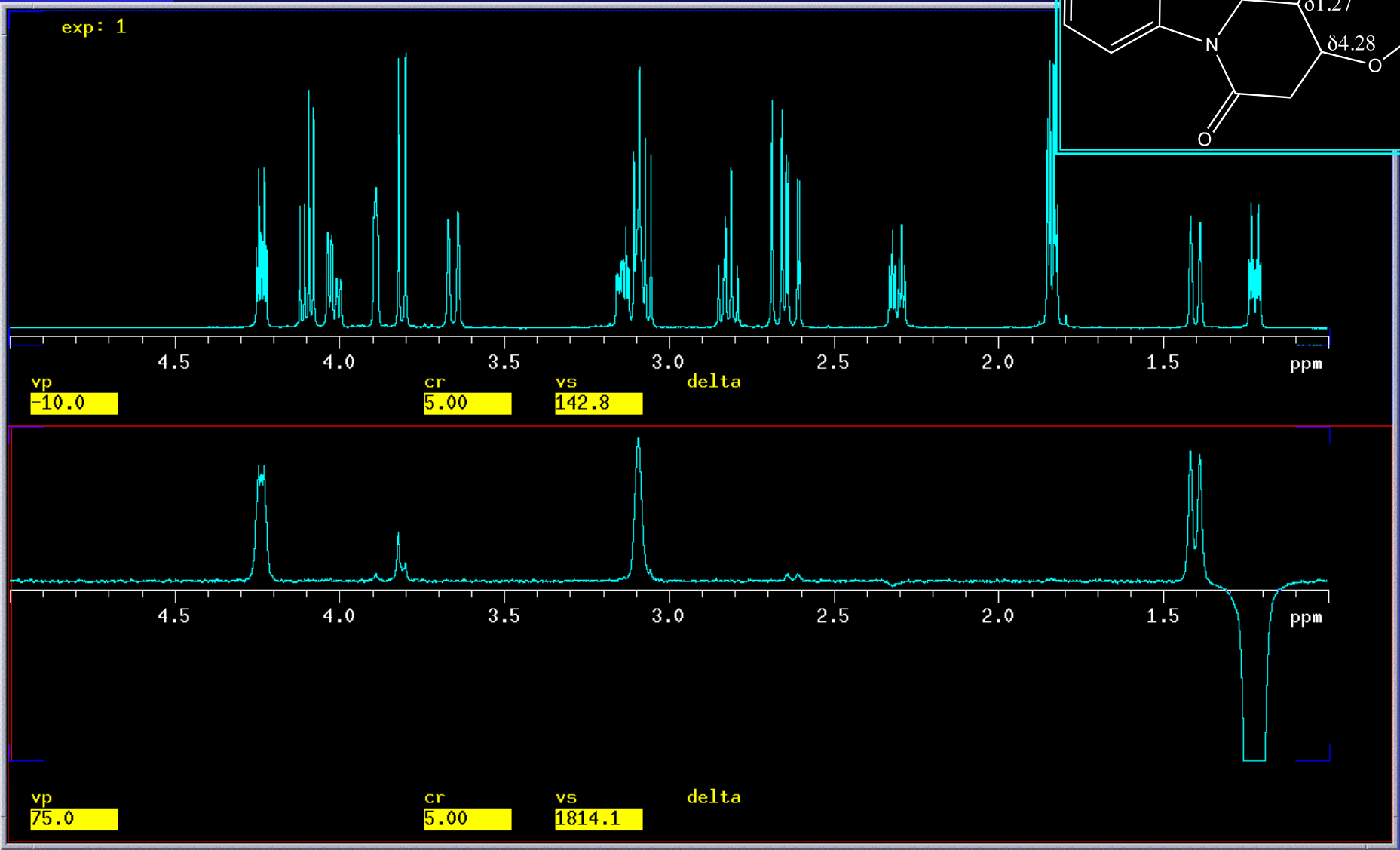
- Double Pulsed Field Gradient Spin-Echo (DPFGSE) transient NOE experiment.
- Z are the pulsed field gradients that allow for specific coherence selection.
- Spectral artifacts are low with this experiment.

NOESY1D: One-Dimensional Nuclear Overhauser Experiment

- Expect NOE's on the order of a few percent.
- Always degas your sample. Oxygen is a good source of relaxation and will decrease effective NOE's.
- DO NOT SPIN SAMPLE!
- Mix times of 0.5 seconds will typically show only short-range interactions. Long range NOE's can be seen when the mix time equals the T1's.
- Number of transients should be at least 64. More is better (e.g. 128,256).

NOESY1D: Strychnine





NOESY1D: Strychnine

δ1.40 on same side as 1.27, 3.85 on opposite side