Advances in Cryogenic NMR Probes for Modern Labs

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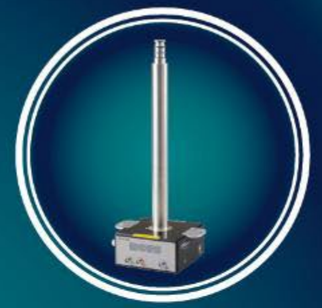
Overview

- Introduction to cryogenic probes
- JEOL's cryogenic NMR probes
- Choosing the right probe type
- SuperCOOL MARVEL probe
- SuperCOOL MARVEL example applications
- Q&A

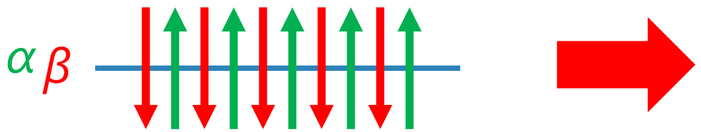
Introduction to Cryogenic NMR Probes



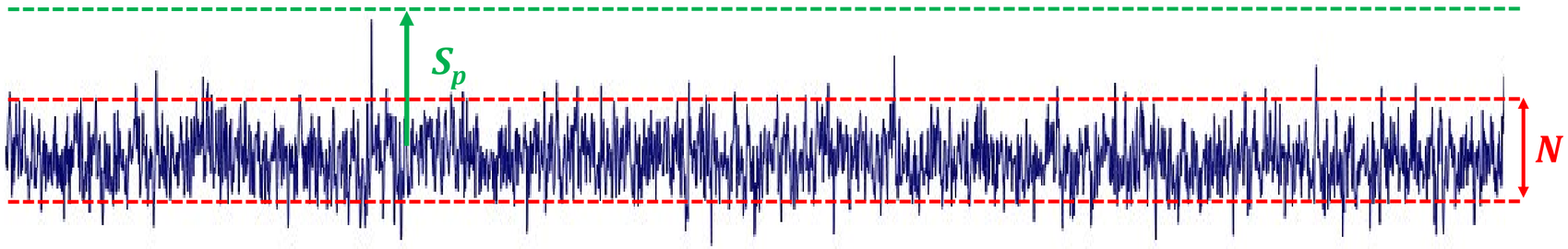
Sensitivity of NMR

- NMR is inherently insensitive compared to many other methods (e.g. MS)

$$B_0 = 0$$



- Low signal-to-noise ratio (SNR) in NMR spectrum



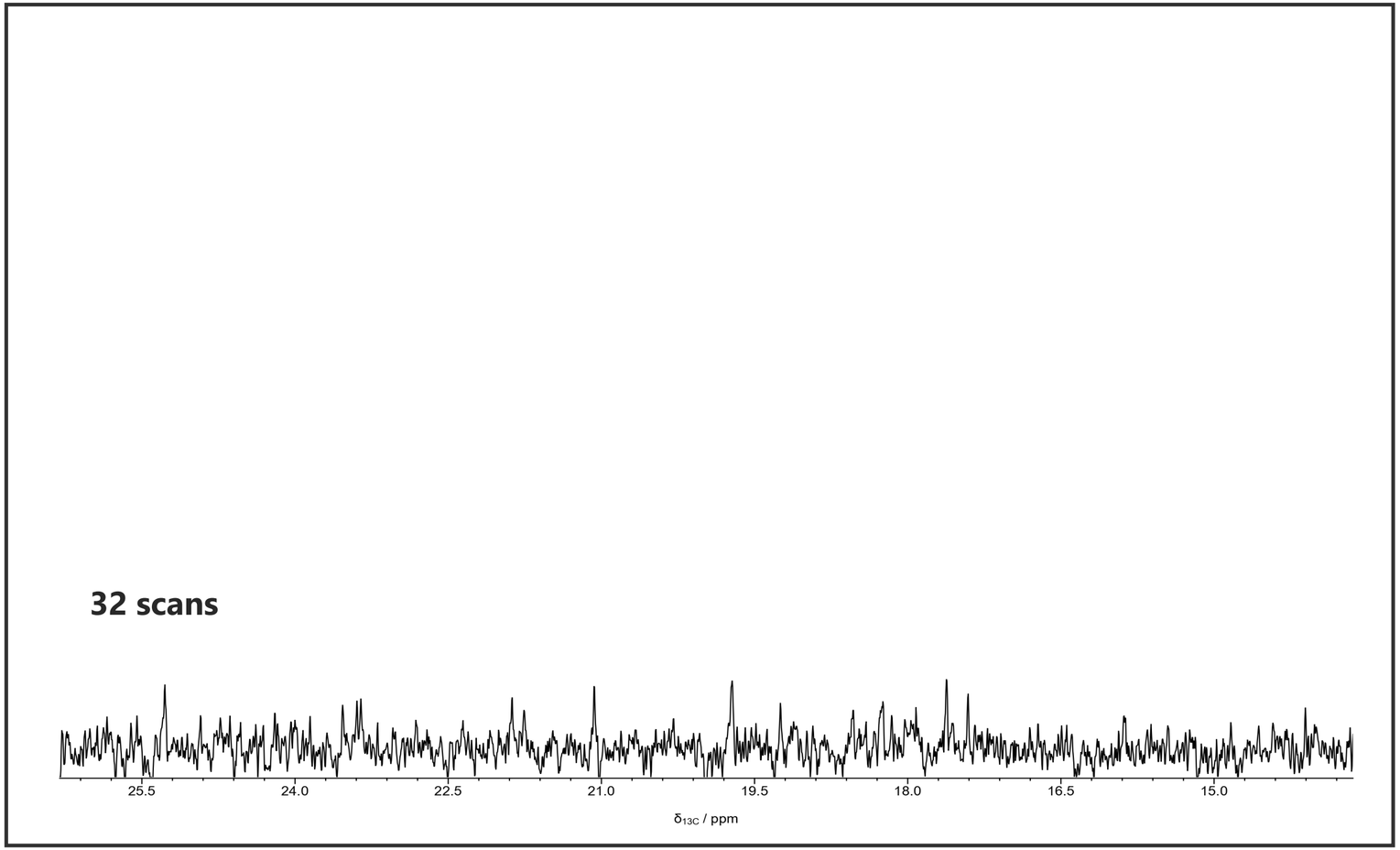
$$SNR = \frac{S_p}{2N_{RMS}}$$

Signal averaging

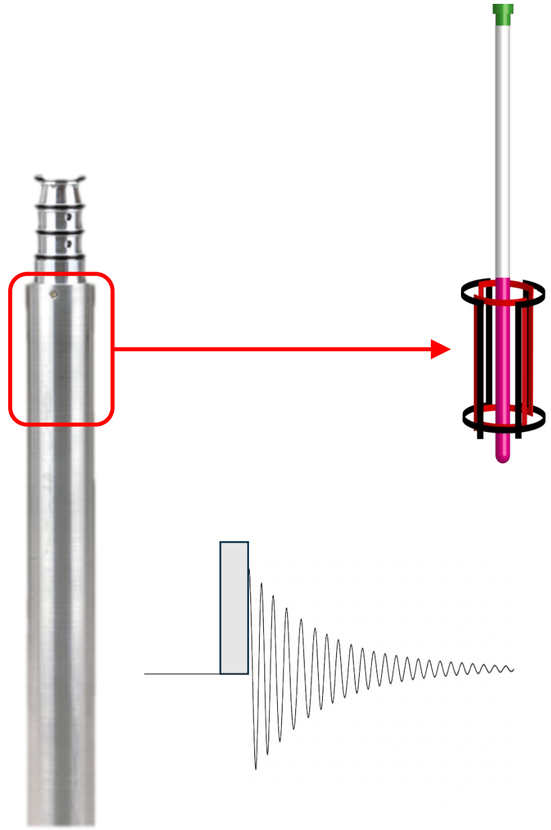
- Collecting more scans improves the signal-to-noise, but…. it takes time

$$SNR \propto \sqrt{scans}$$

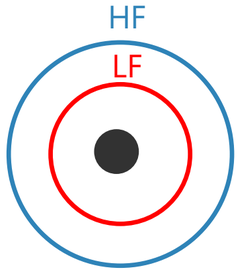
- Diminishing returns
- No substitute for higher intrinsic sensitivity



NMR probe design

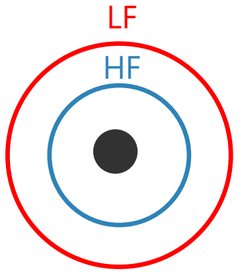


Direct geometry



- Optimized for low-band (e.g. ^{13}C) sensitivity
- Broadband probes (e.g. JEOL TH)

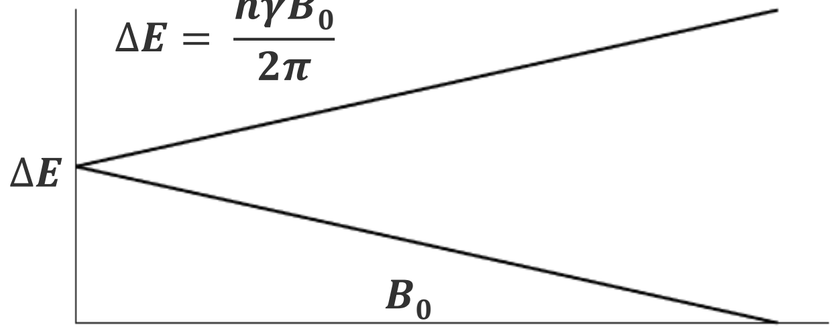
Inverse geometry



- Optimized for high-band (^{1}H and/or ^{19}F) sensitivity
- ID, HCN probes, etc.

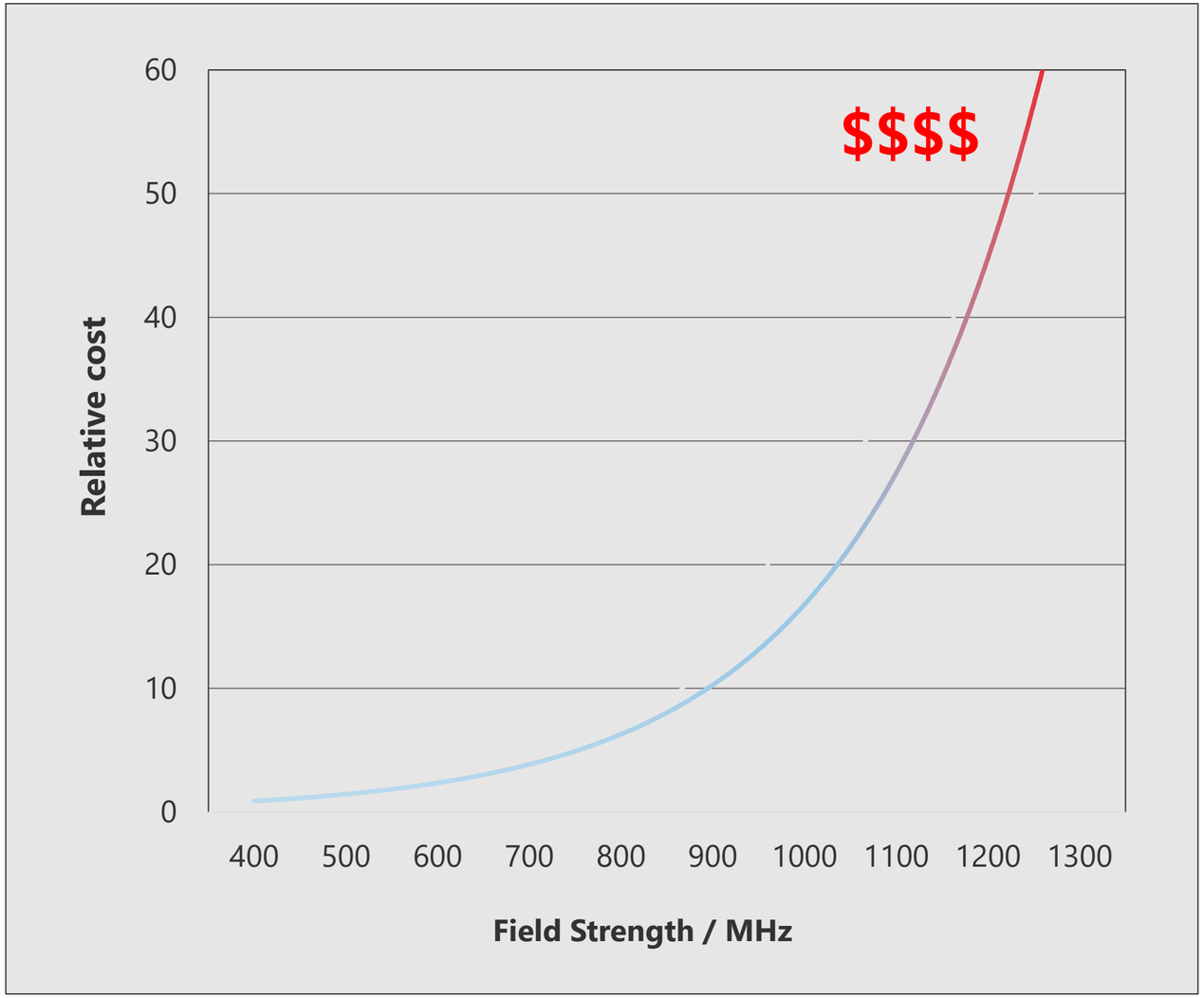
Improving NMR sensitivity: going to higher field strengths

$$\Delta E = \frac{h\gamma B_0}{2\pi}$$



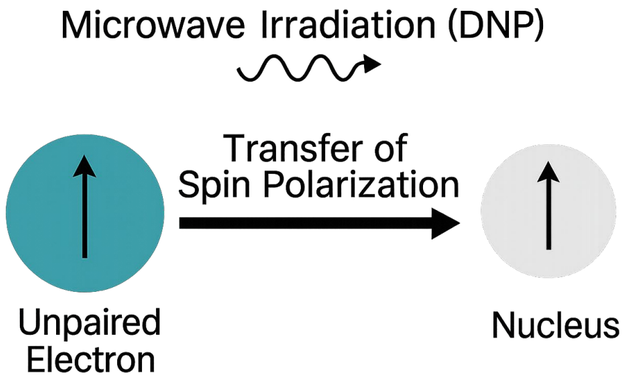
$$\frac{S}{N} \propto {B_0}^{\frac{3}{2}}$$

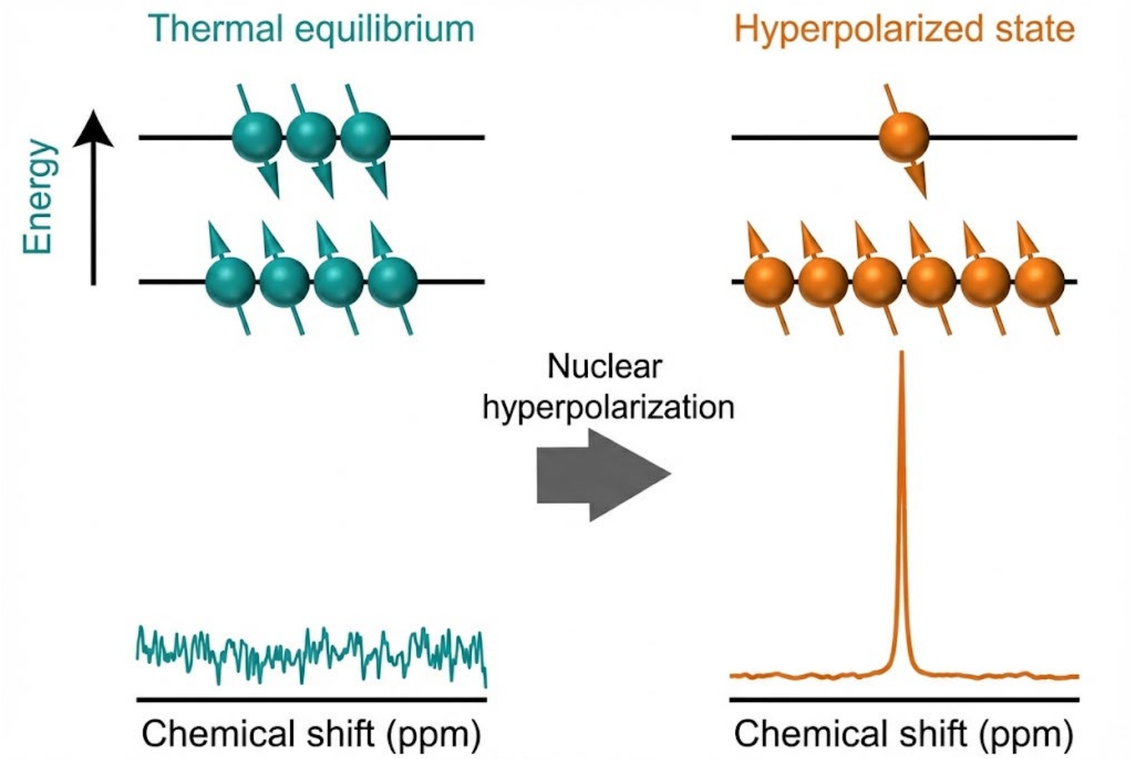
Field strength (T)	9.4	11.7	14.1	16.5	18.8
^{1}H Freq. (MHz)	400	500	600	700	800
Relative sensitivity	1	1.5	1.8	2.3	2.8



Improving NMR sensitivity: hyperpolarisation

- DNP, dissolution-DNP
- PHIP (PASADENA, SABRE, etc.)





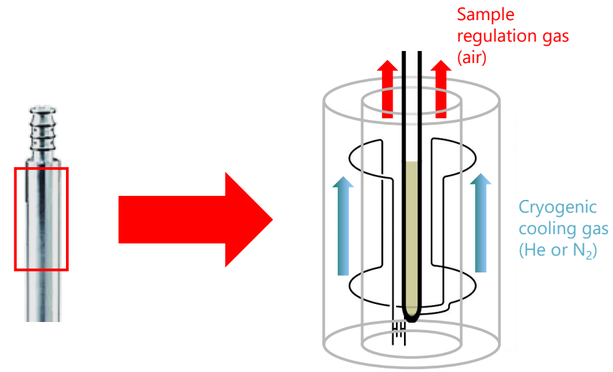
Improving NMR sensitivity: reducing the noise

$$S/N \propto \frac{1}{(T_C R_C + Ta(R_C + RS) + TSRS)^{\frac{1}{2}}}$$

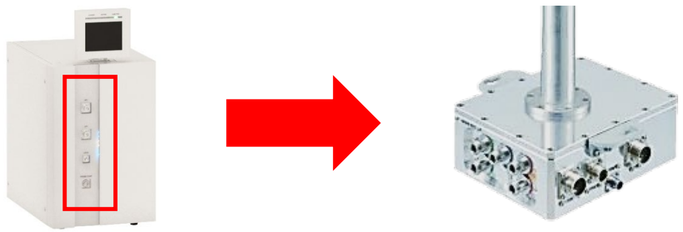
T_C	RF coil temperature
R_C	RF coil resistance
T_a	Noise temperature of preamp
R_S	Sample "resistance"
T_S	Sample temperature

Implications for NMR instrument design

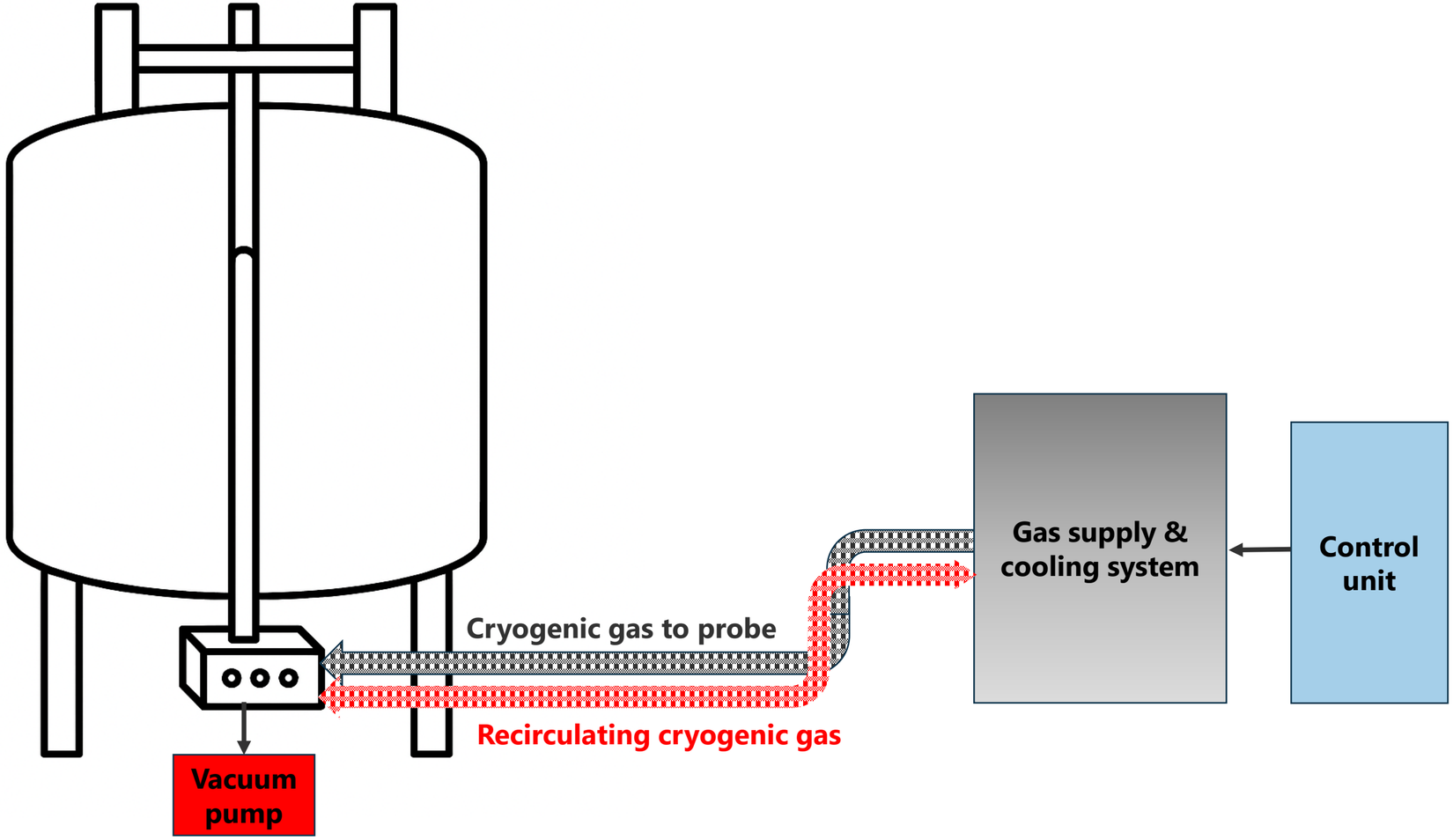
1. Cool down RF coils in probe to cryogenic temperature



2. Relocate preamps from head amp unit to inside probe and cool to cryogenic temperature



Cryogenic NMR probe systems



JEOL's family of cryogenic NMR probes

UltraCOOL (LHe temp.)	**SuperCOOL** (LN_2 temp.)	
Circulation (UC)	**Closed Type (SCC)**	**Open Type (SCO)**
		
➢5 mm inverse HCN ➢5 mm direct broadband ➢5 mm direct dual	➢5 mm direct ➢10 mm direct	➢5 mm direct ➢10 mm direct

UltraCOOL NMR probe cooling system (UC)

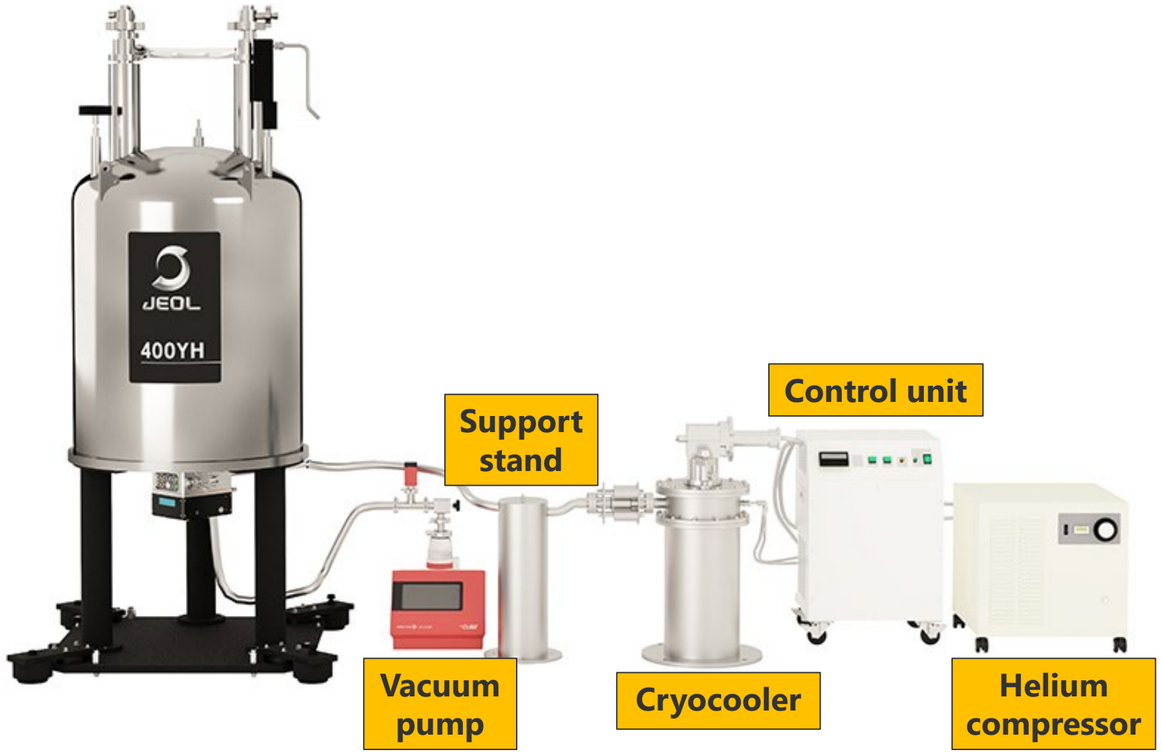
PROS

- Highest sensitivity – ~5 × RT probe
- Closed system, no need to refill weekly

CONS

- More complex, more infrastructure needed
- Requires helium
- More expensive to buy, run and maintain than SuperCOOL probes

Closed-type SuperCOOL NMR probe cooling system (SCC)



PROS

- 2-3 x higher sensitivity than RT probe; slightly higher than open-type
- Closed system, no need to refill weekly
- Simpler setup than UltraCOOL
- Significantly lower running costs (~1/4) than UltraCOOL

CONS

- Lower sensitivity than UltraCOOL probe
- Requires helium
- More expensive to buy, run and maintain than open-type SuperCOOL probes

Open-type SuperCOOL NMR probe cooling system (SCO)

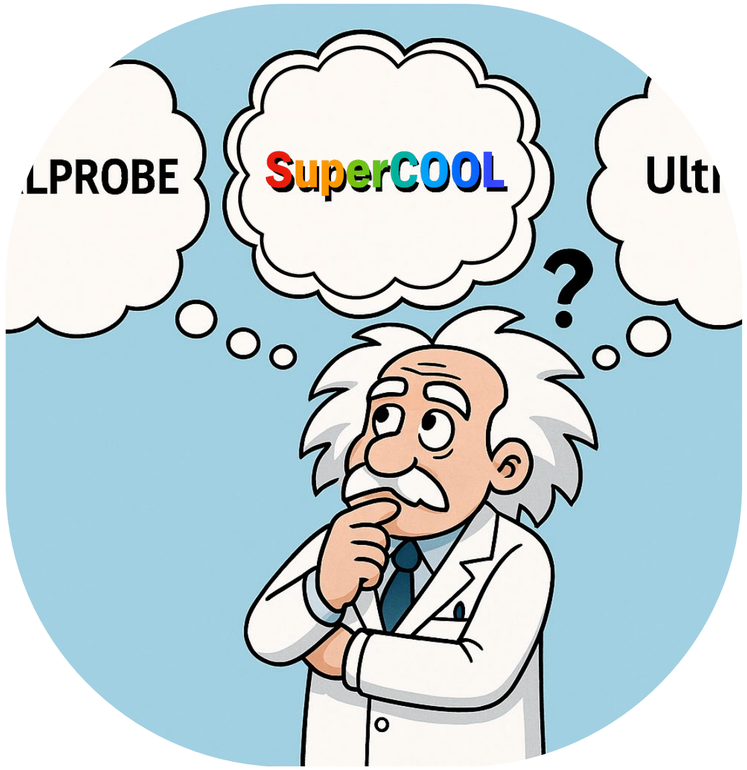
PROS

- No helium required
- Only slightly less sensitive than closed-type SuperCOOL (SCC)
- Simplest setup, fewer components (no chiller, no compressor)
- Cheaper to buy, run and maintain than UC and SCC

CONS

- Lower sensitivity than UC probe and slightly lower than SCC probe
- LN_2 dewar needs refilling every 7-10 days (but can be done during measurements)

Which type of NMR probe for your lab?

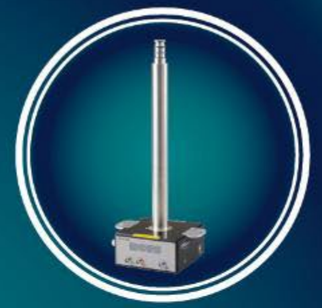


Considerations & trade-offs

- Performance
- Purchasing and maintenance costs
- Convenience
- Simplicity

SuperCOOL the ideal balance…?

SuperCOOL MARVEL Probe

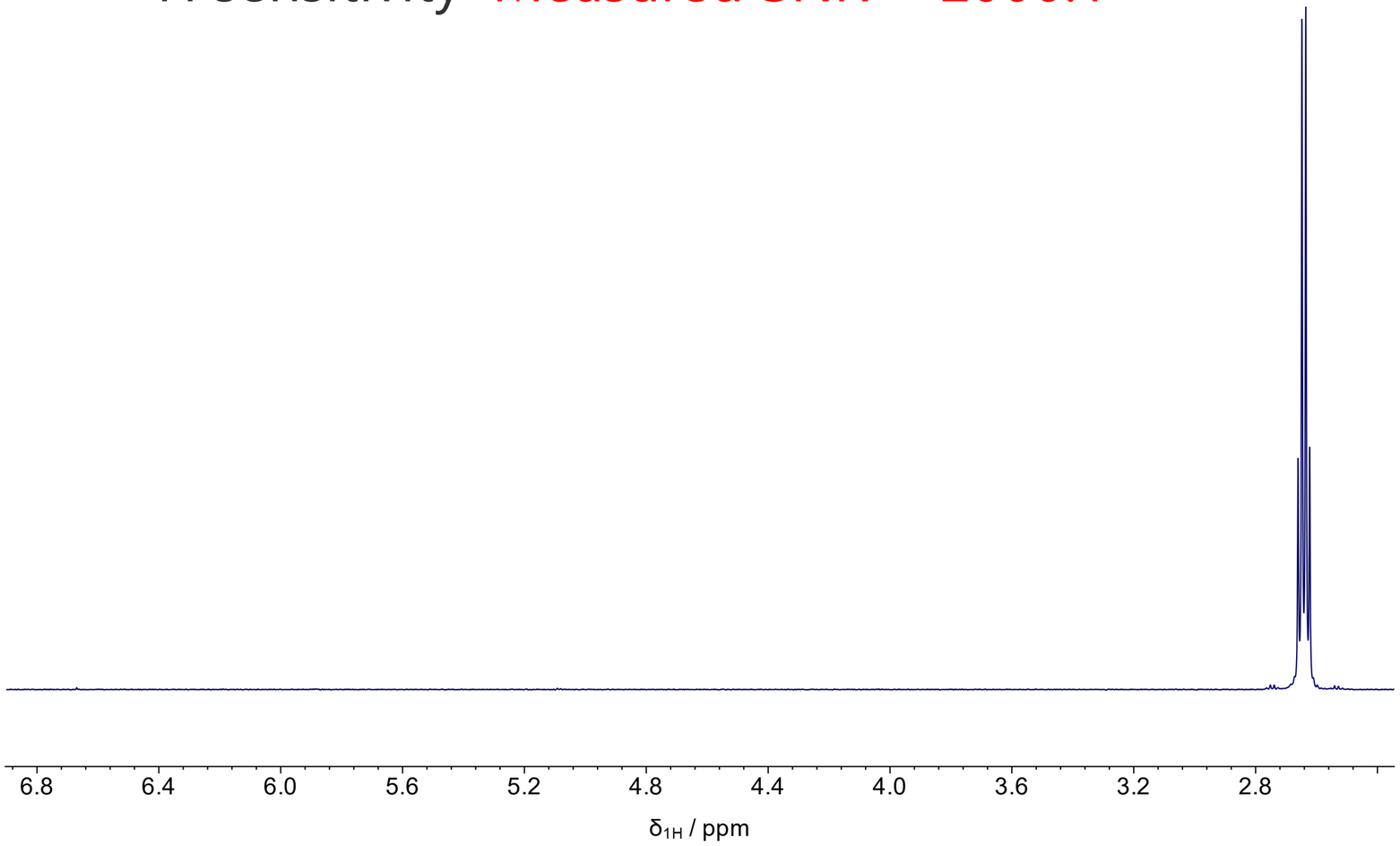


SuperCOOL MARVEL Probe

- Fourth-generation SuperCOOL model
- Completely redesigned and optimized electrical circuit
- Class-leading sensitivity
- 5 mm broadband design, balanced high- and low-frequency performance
- Offers outstanding price-performance ratio
- Excellent power efficiency for demanding applications

SuperCOOL MARVEL Probe : Balanced Performance

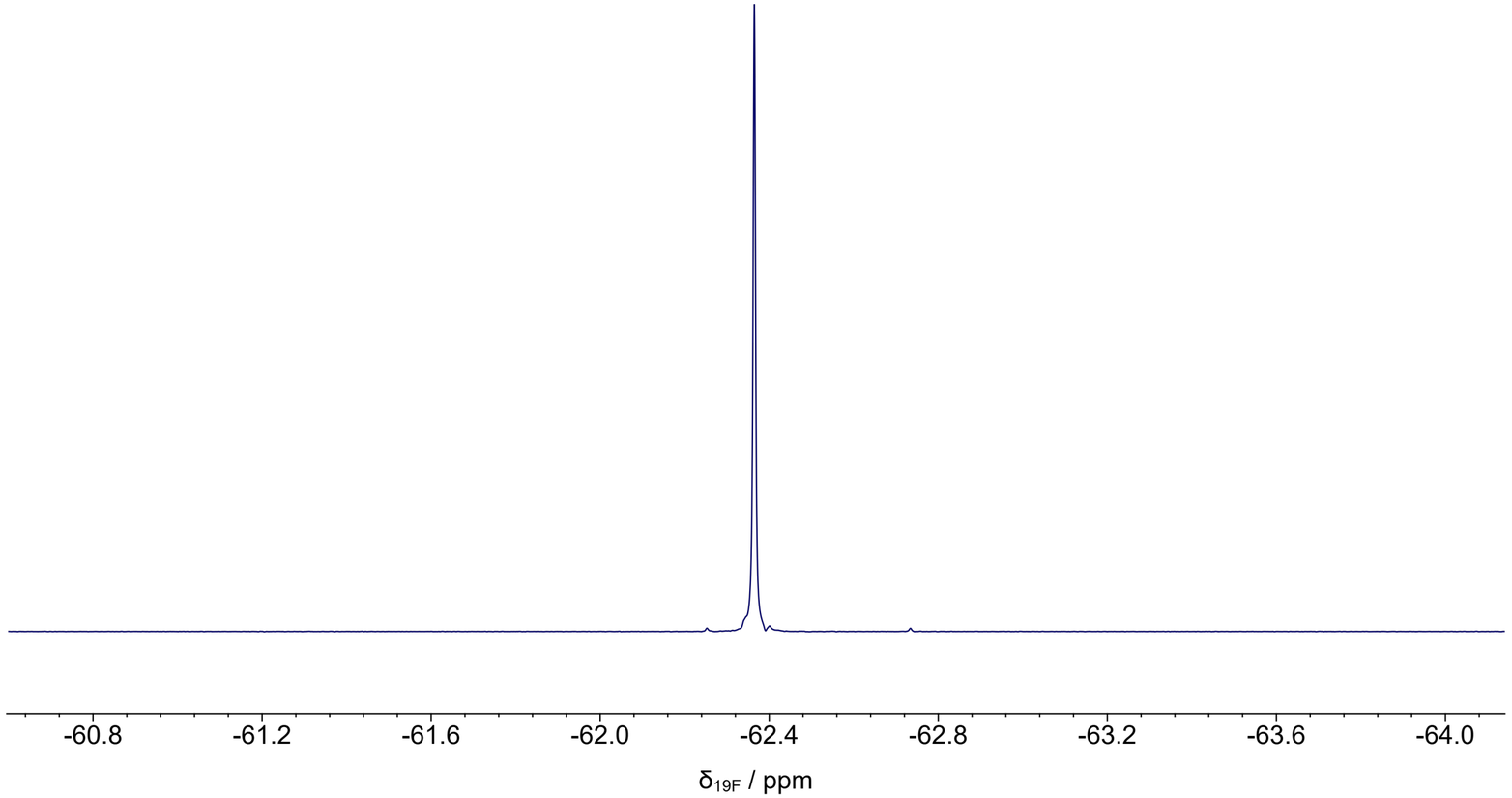
^{1}H sensitivity Measured SNR > 2000:1



Probe	**600 MHz SuperCOOL MARVEL**
Test	**^{1}H sensitivity**
Sample	0.1% ethyl benzene in $CDCl_3$
Acquisition parameters	1 scan, 90° pulse
Processing	1 Hz line broadening
SNR calculation	200 Hz-wide noise region (RMS)

SuperCOOL MARVEL Probe : Balanced Performance

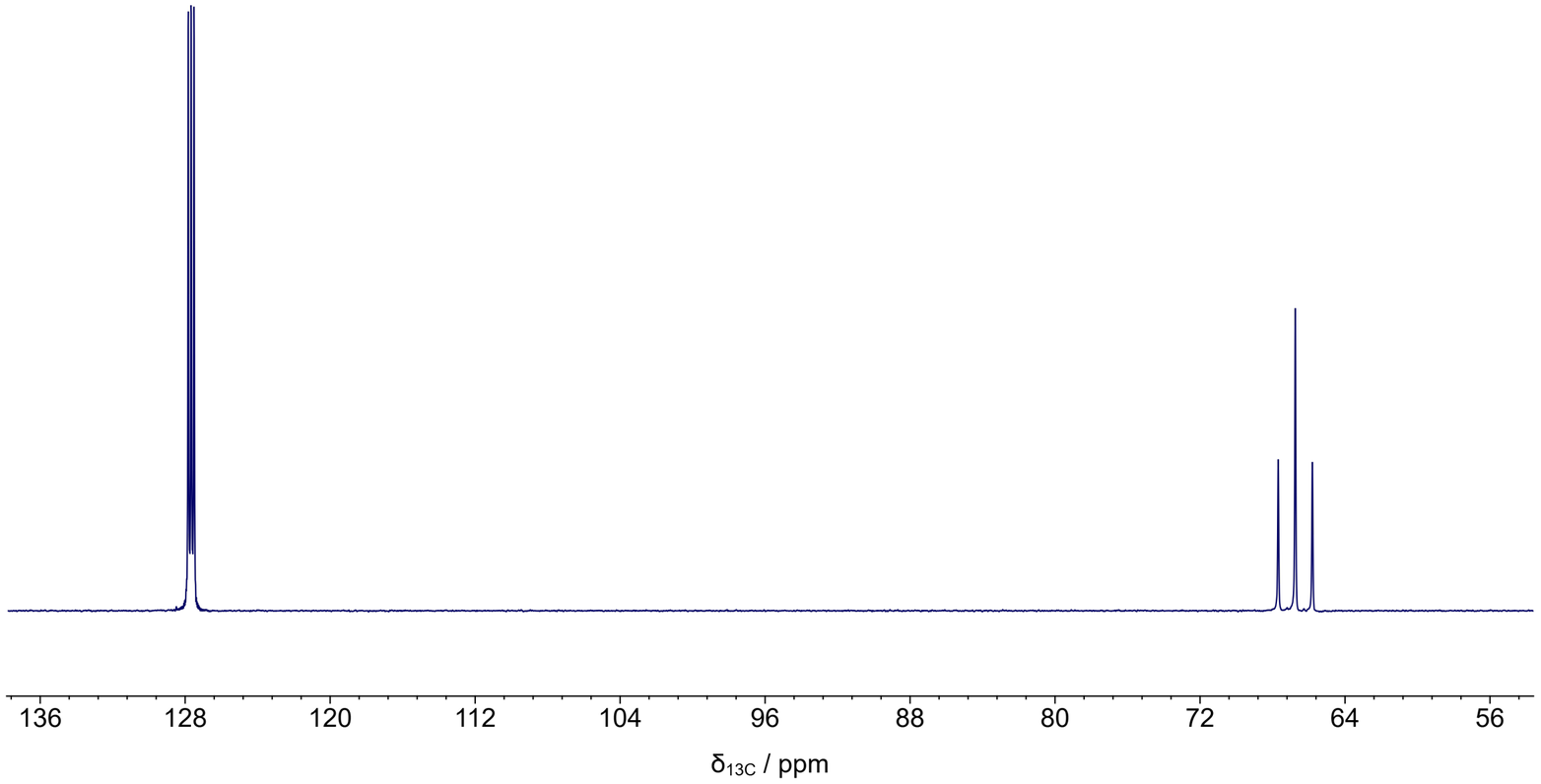
^{19}F sensitivity Measured SNR > 2200:1



Probe	**600 MHz SuperCOOL MARVEL**
Test	**^{19}F sensitivity (no dec.)**
Sample	0.05% trifluorotoluene in C_6D_6
Acquisition parameters	1 scan, 90° pulse
Processing	2 Hz line broadening
SNR calculation	1 ppm-wide noise region (RMS)

SuperCOOL MARVEL Probe : Balanced Performance

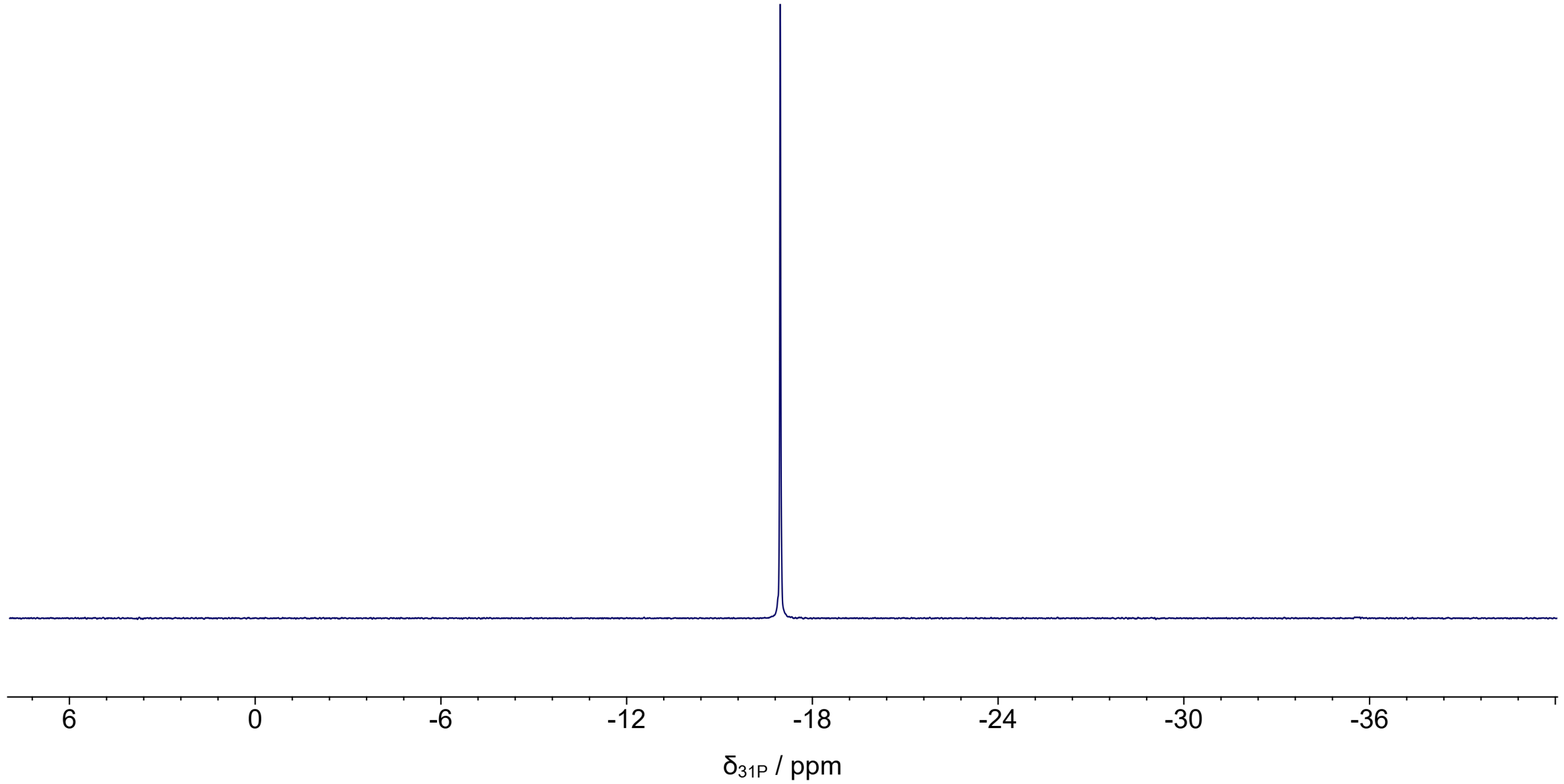
^{13}C sensitivity Measured SNR > 1000:1



Probe	**600 MHz SuperCOOL MARVEL**
Test	**^{13}C sensitivity**
Sample	40% p-dioxane in C_6D_6
Acquisition parameters	1 scan, 90° pulse
Processing	3.7 Hz line broadening
SNR calculation	1400 Hz (9.3 ppm)-wide noise region (RMS)

SuperCOOL MARVEL Probe : Balanced Performance

^{31}P sensitivity Measured SNR > 800:1

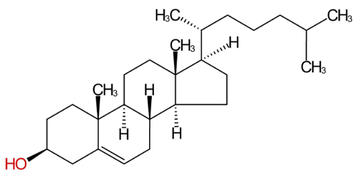


Probe	**600 MHz SuperCOOL MARVEL**
Test	**^{31}P sensitivity**
Sample	0.0485 M triphenyl phosphate
Acquisition parameters	1 scan, 90° pulse
Processing	5 Hz line broadening
SNR calculation	5 ppm-wide noise region (RMS)

SuperCOOL MARVEL Probe : Much Faster Measurements

	vs ROYALPROBE™	
Nuclide	**Ratio of sensitivity (est.)**	**Relative measurement time using MARVEL (est.)**
^{1}H	2.0	1/4
^{19}F	2.1	1/4
^{31}P	3.2	1/10
^{13}C	2.6	1/7

Cholesterol
1D carbon



SuperCOOL MARVEL Probe : Excellent Price-Performance Ratio

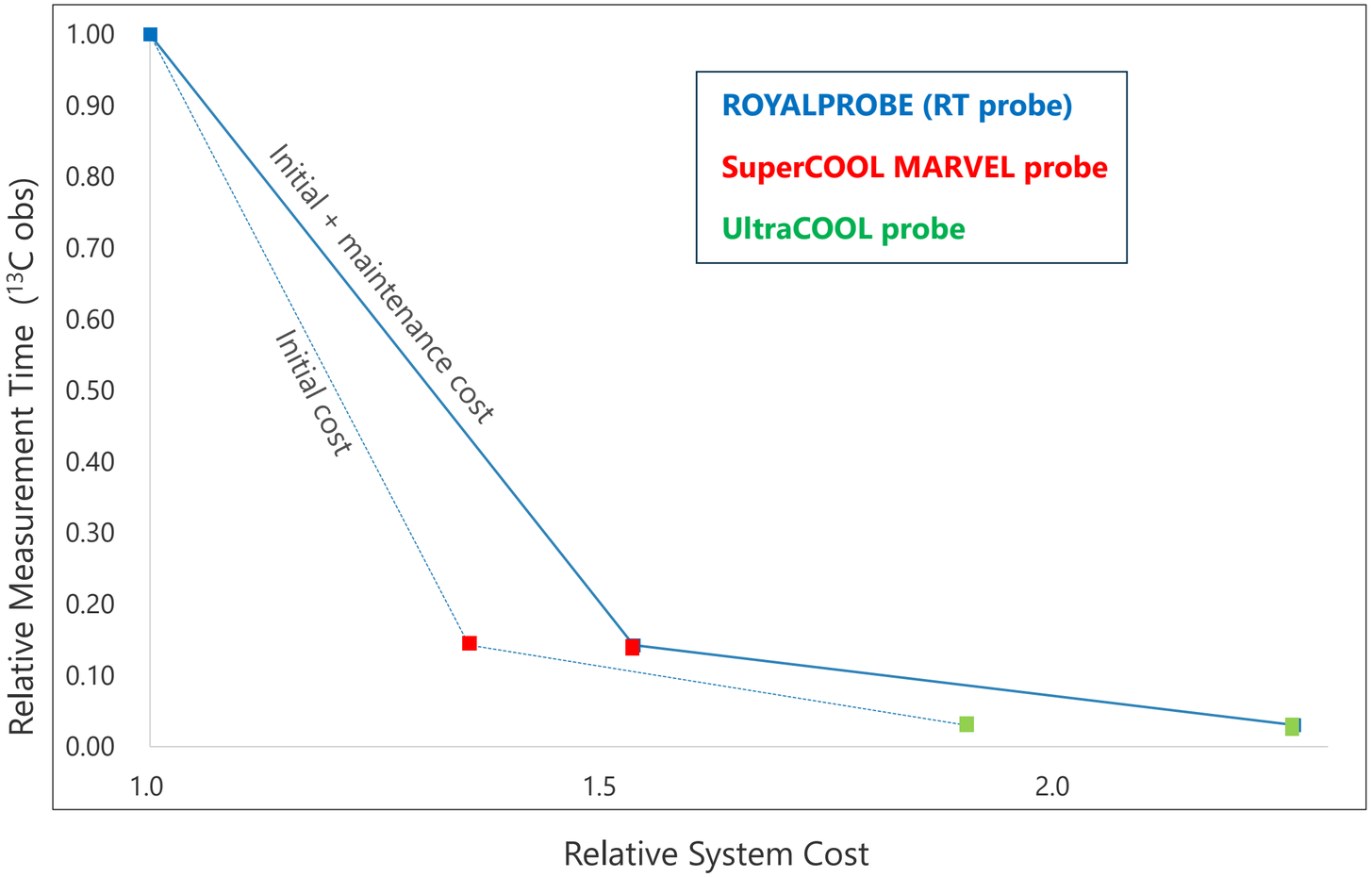
Performance

Example: ^{13}C measurement

- 10 hours on RT probe
- SuperCOOL MARVEL ~10 × faster → 1 hour
- UltraCOOL ~30 × faster → 20 mins

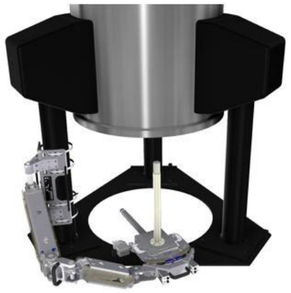
Price

- System with SuperCOOL costs ~40% more to buy
- System with UltraCOOL costs nearly double to buy
- Higher maintenance costs of UtlraCOOL increase ownership costs closer to 2.5 × higher



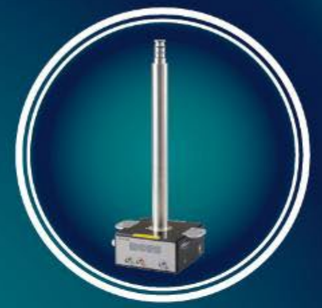
SuperCOOL MARVEL Probe : Affordable to Run, Easy to Use

- No helium
- No compressor or chiller to buy and maintain
- System can be refilled during use, during routine LN_2 refill
- Uninterrupted operation
- Optional Probe Arm/Probe Lifter makes probe swaps easy



SuperCOOL MARVEL Probe : One Probe. Many Applications

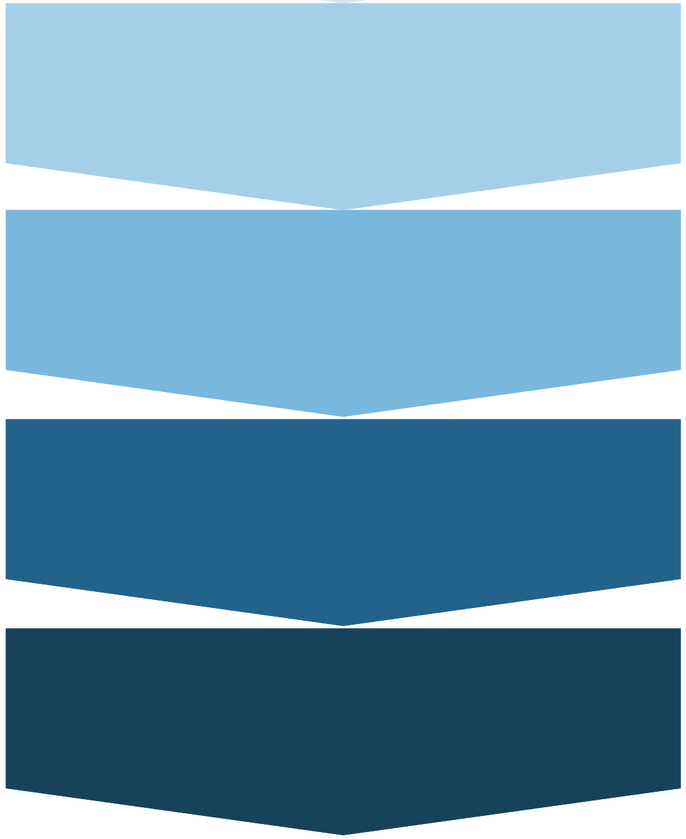
- Pharmaceutical and chemical analyses
- High-throughput measurements
- Low concentration samples
- Impurity and trace material analyses
- Fast degrading, unstable samples
- Advanced NMR methods
- Quantitative NMR (qNMR)



Example Applications

Overview

1. Rapid routine experiments



Examples and Expectations

Routine expectations with 1mg ~MW400

Overview

1. Rapid routine experiments
2. Robust {^{13}C} power handling

Examples and Expectations

Routine expectations with 1mg ~MW400

Unique ability to effectively handle ^{13}C decoupling

Overview

1. Rapid routine experiments
2. Robust {^{13}C} power handling
3. $^{N}J_{CH}$ by IPAP methods.

Examples and Expectations

Routine expectations with 1mg ~MW400

Unique ability to effectively handle ^{13}C decoupling

Use case where resolution and sensitivity matter

Overview

1. Rapid routine experiments
2. Robust {^{13}C} power handling
3. $^{N}J_{CH}$ by IPAP methods.
4. C-C by INADEQUATE
5. C-C by 1-1 ADEQUATE

Examples and Expectations

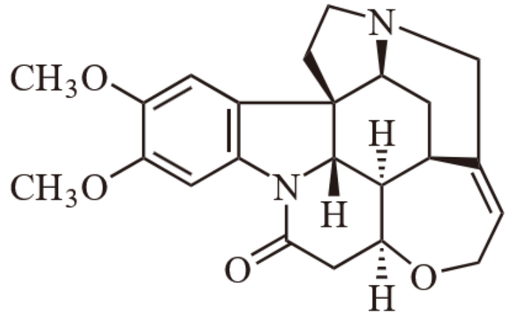
Routine expectations with 1mg ~MW400

Unique ability to effectively handle ^{13}C decoupling

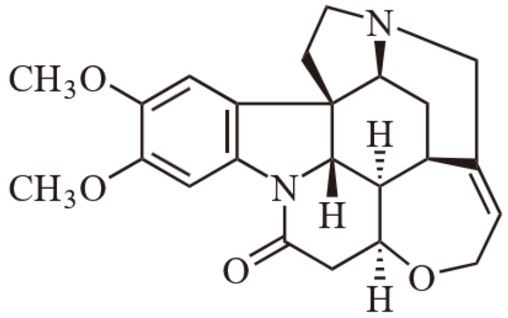
Use case where resolution and sensitivity matter

Examples of excellent balanced performance on both low and high-band channels.

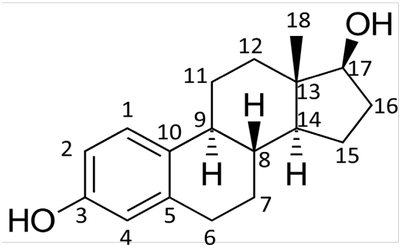
Brucine 1mg
Single Scan ^{1}H



Brucine 1mg
512 Scan ^{13}C
26 minutes



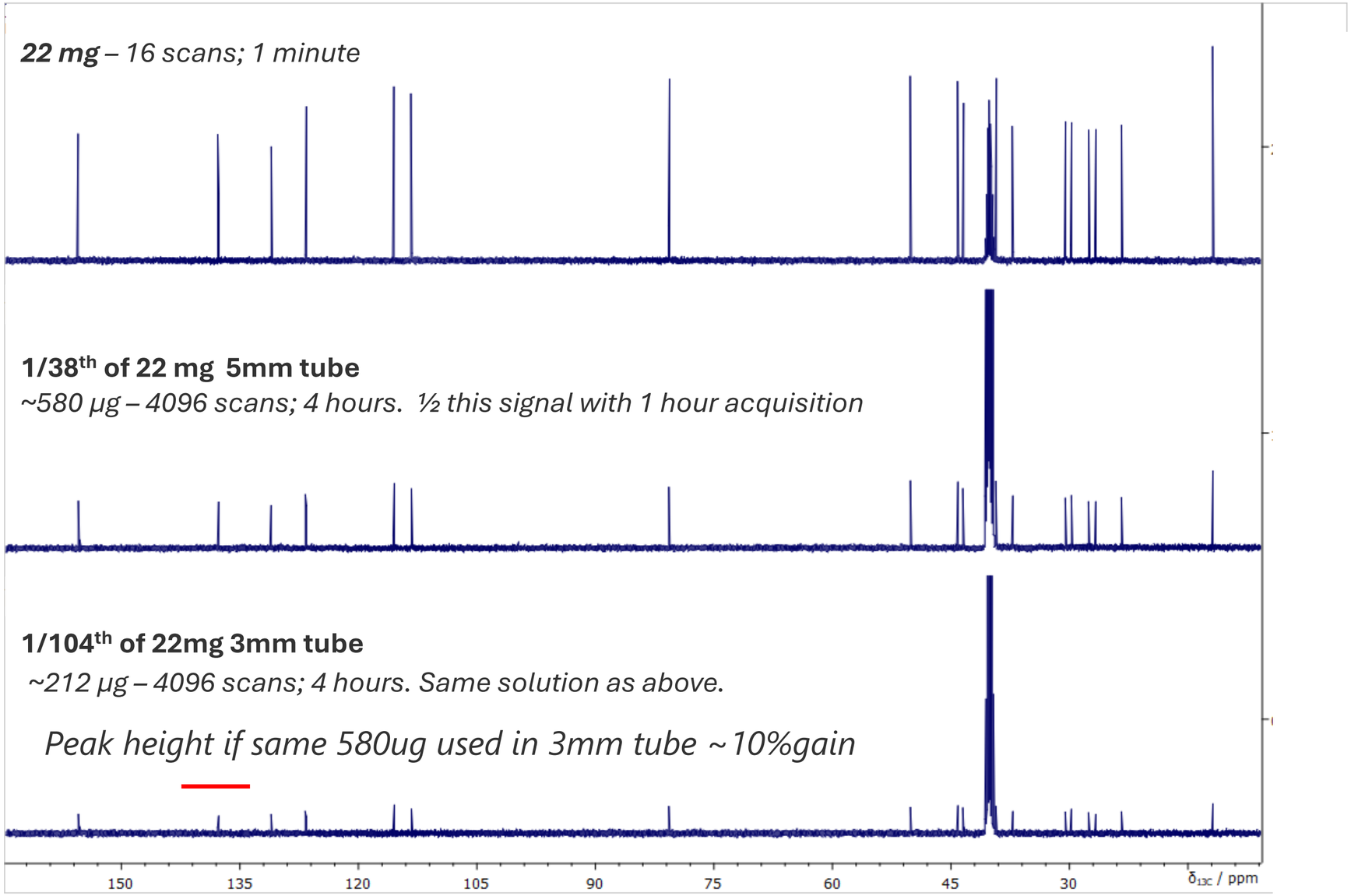
^{13}C sensitivity

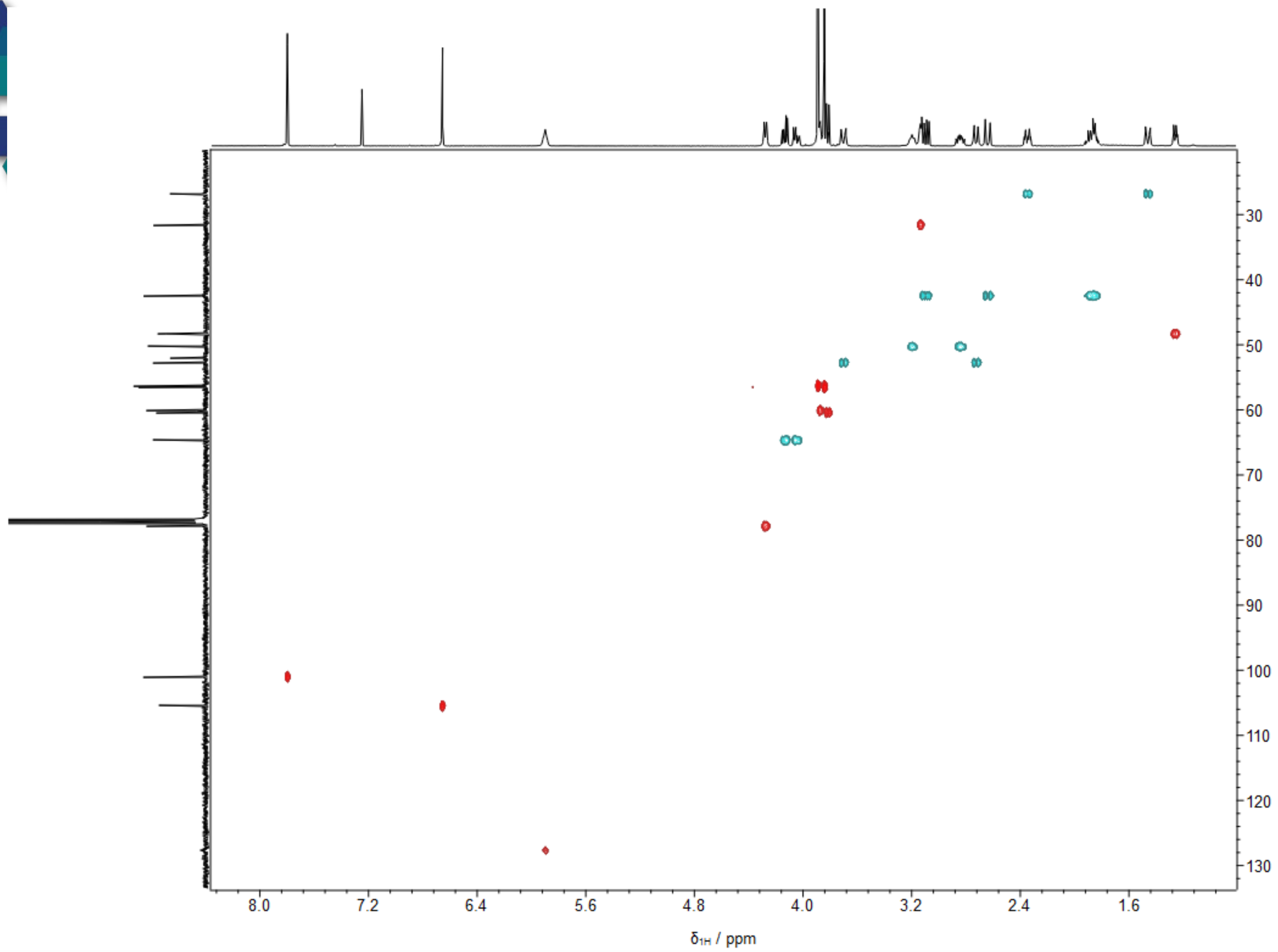


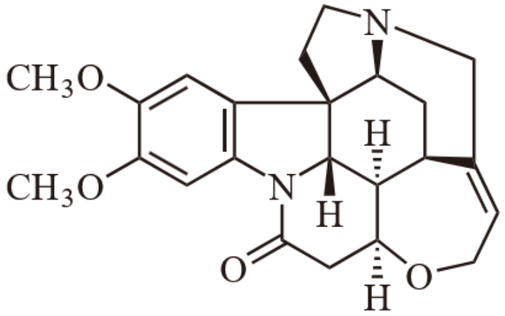
Estradiol

$^{13}C\{^{1}H\}$
90° tip angle
1.4 s acq. time
3.4 s rep. time

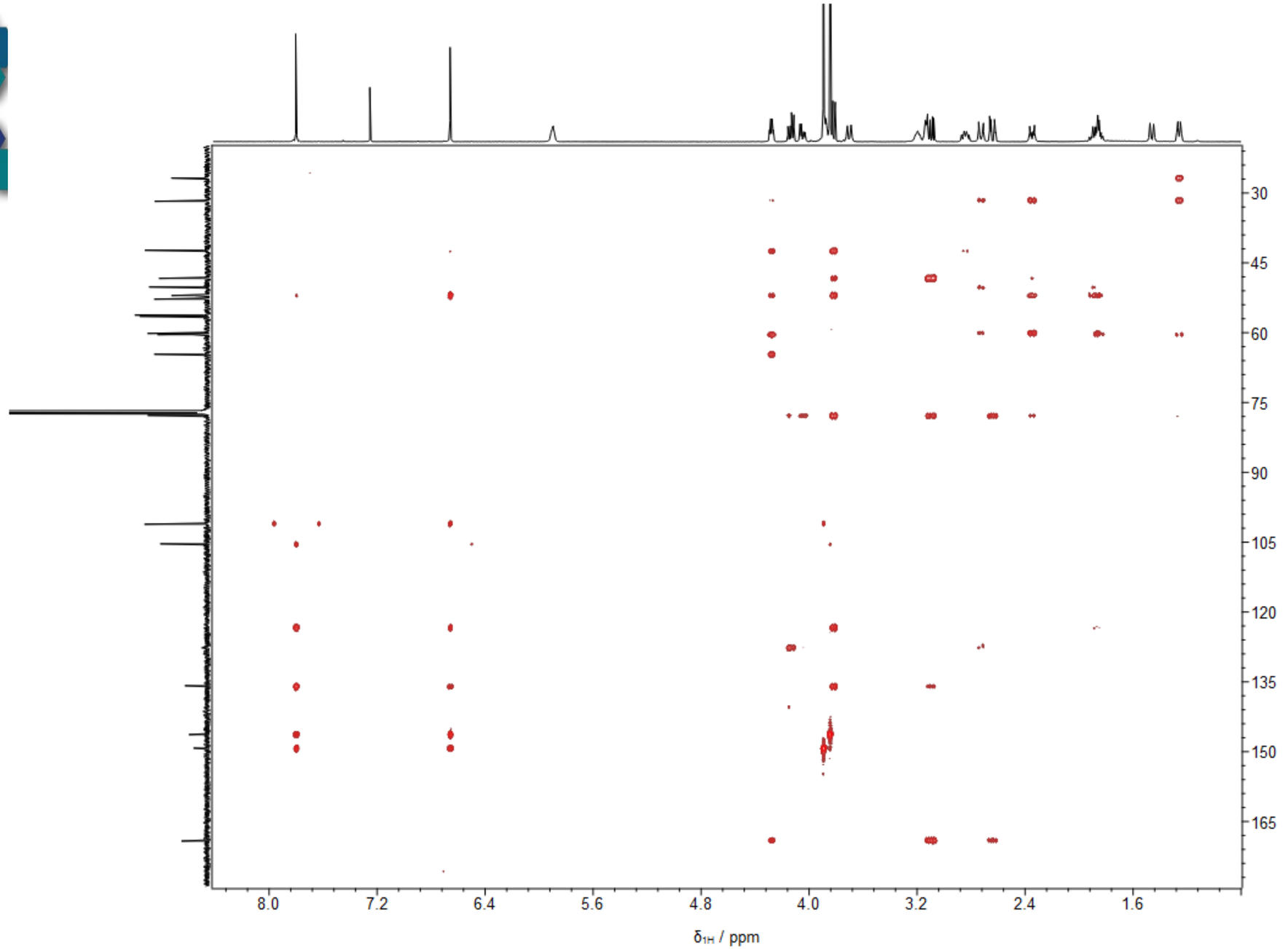
1 mg in ~15 - 30 min!

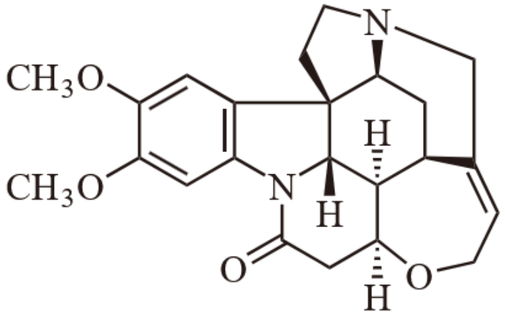






Brucine 1mg
gHSQCAD-2 scans
8 minutes
Primary Structure

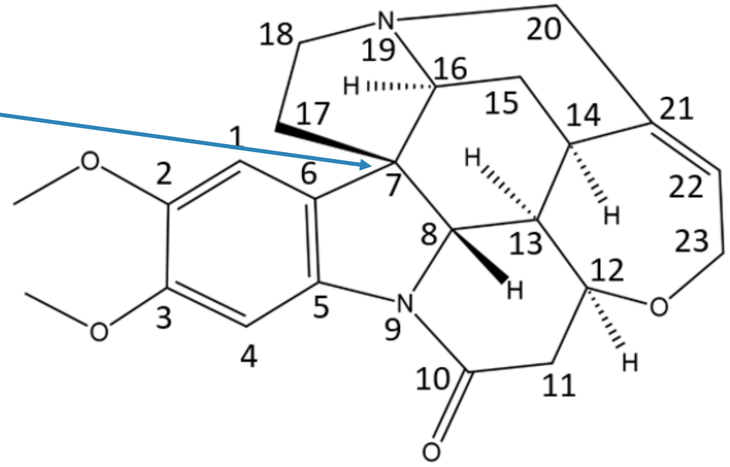




Brucine 1mg
gHMBC - 4 scans
38 minutes
Primary Structure

Each cross-peak arises From $^{N}J_{CH}$.

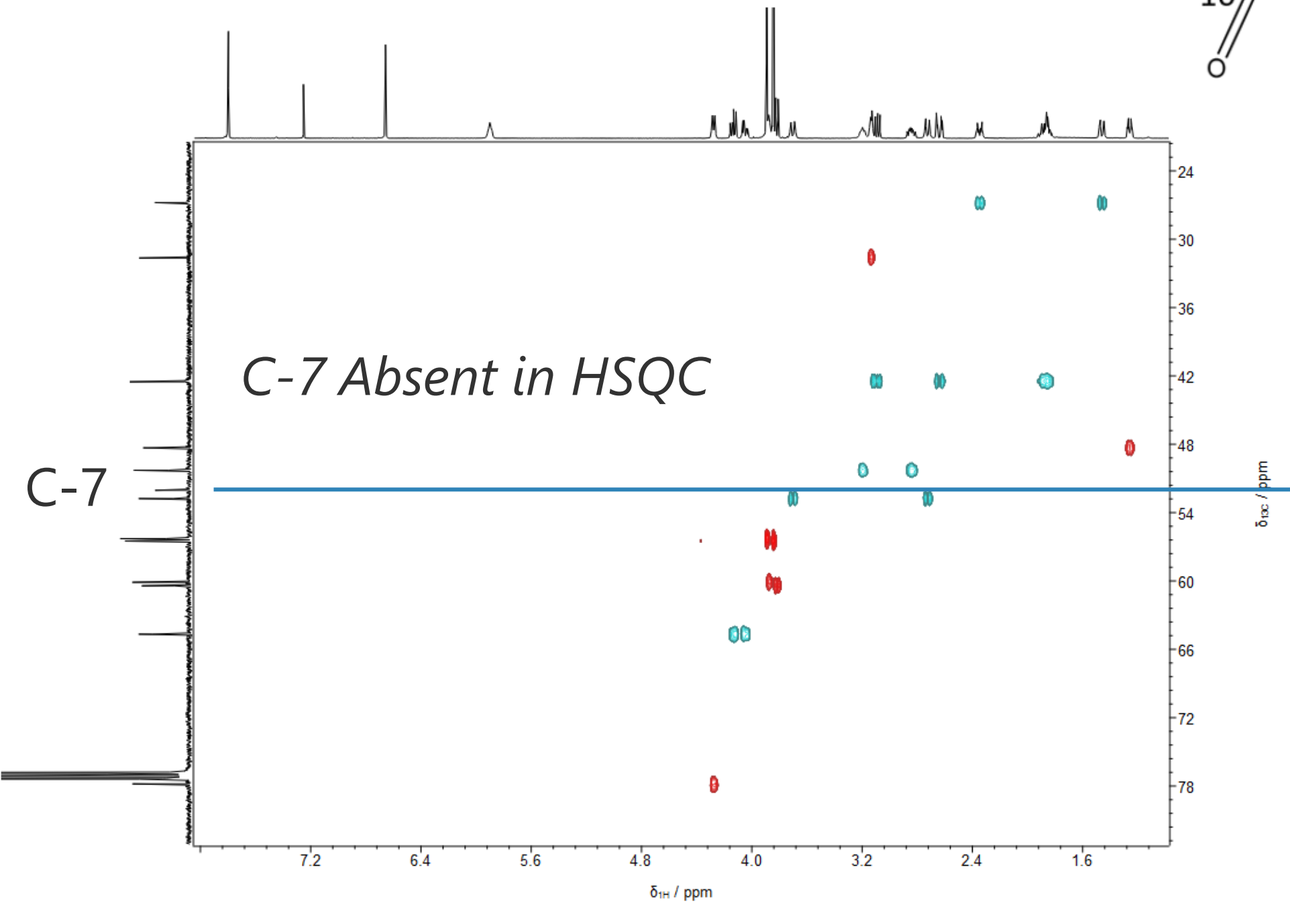
Quat C-7

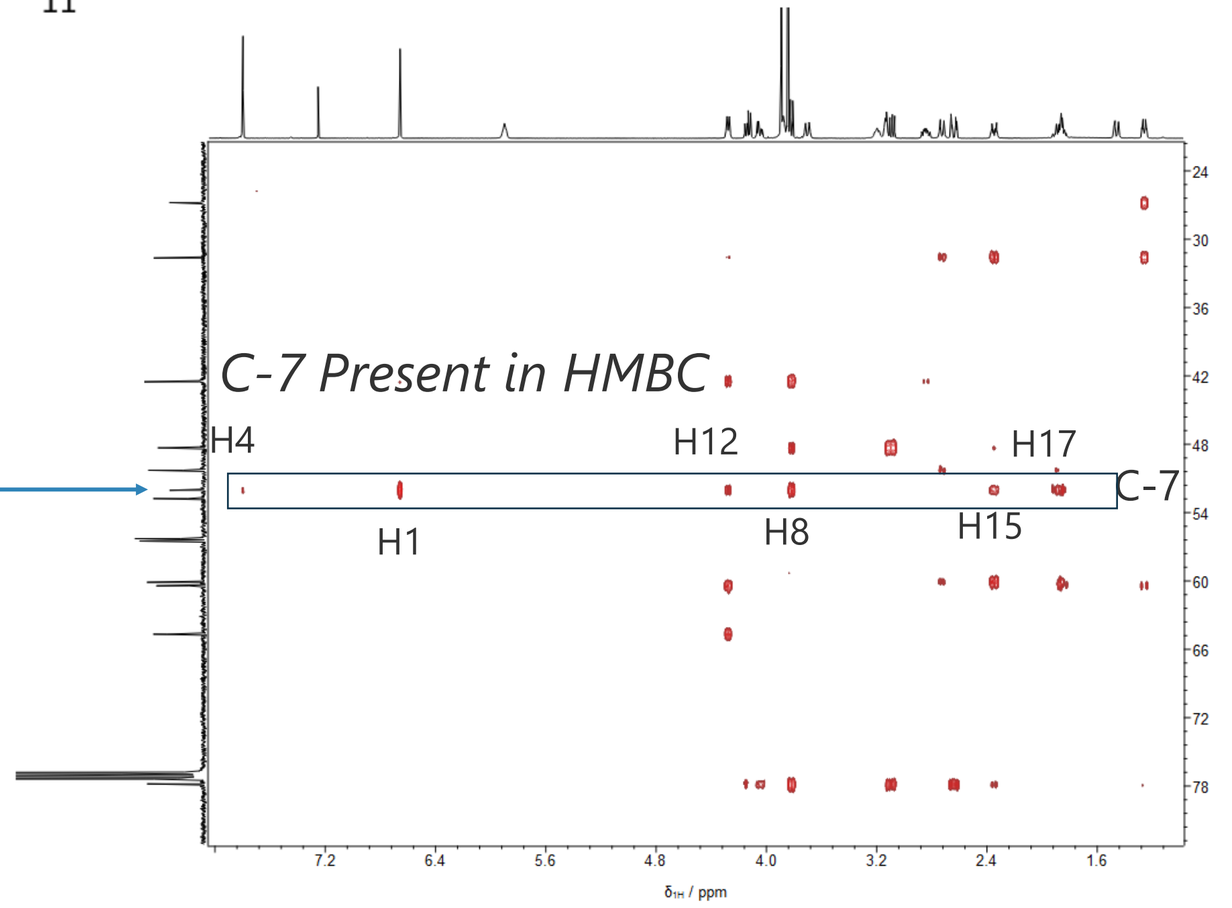


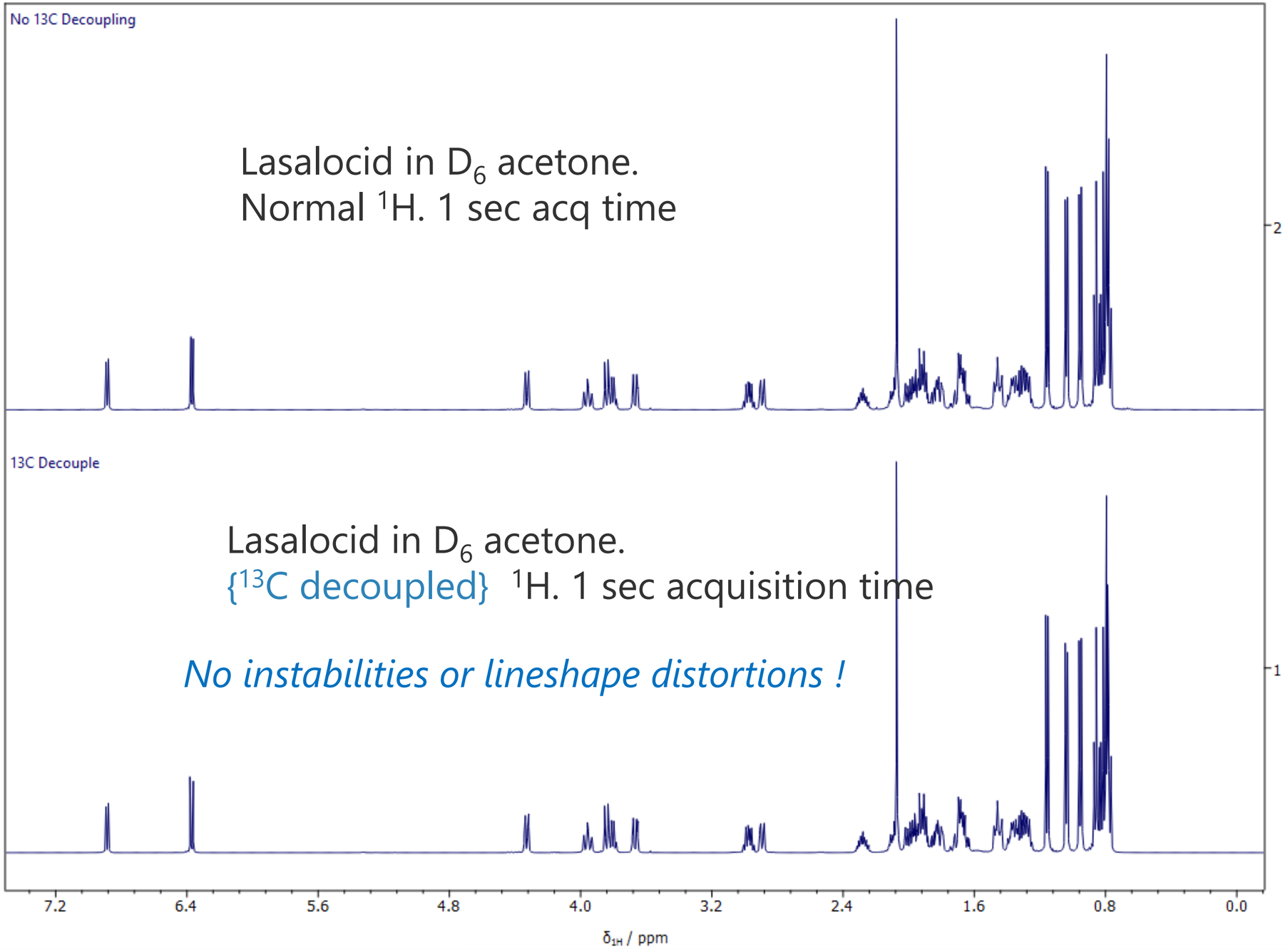
HMBC content review..

Each cross-peak arises From $^{N}J_{CH}$.

J magnitudes contain both Stereochemical and conformational information – *Secondary Structure*



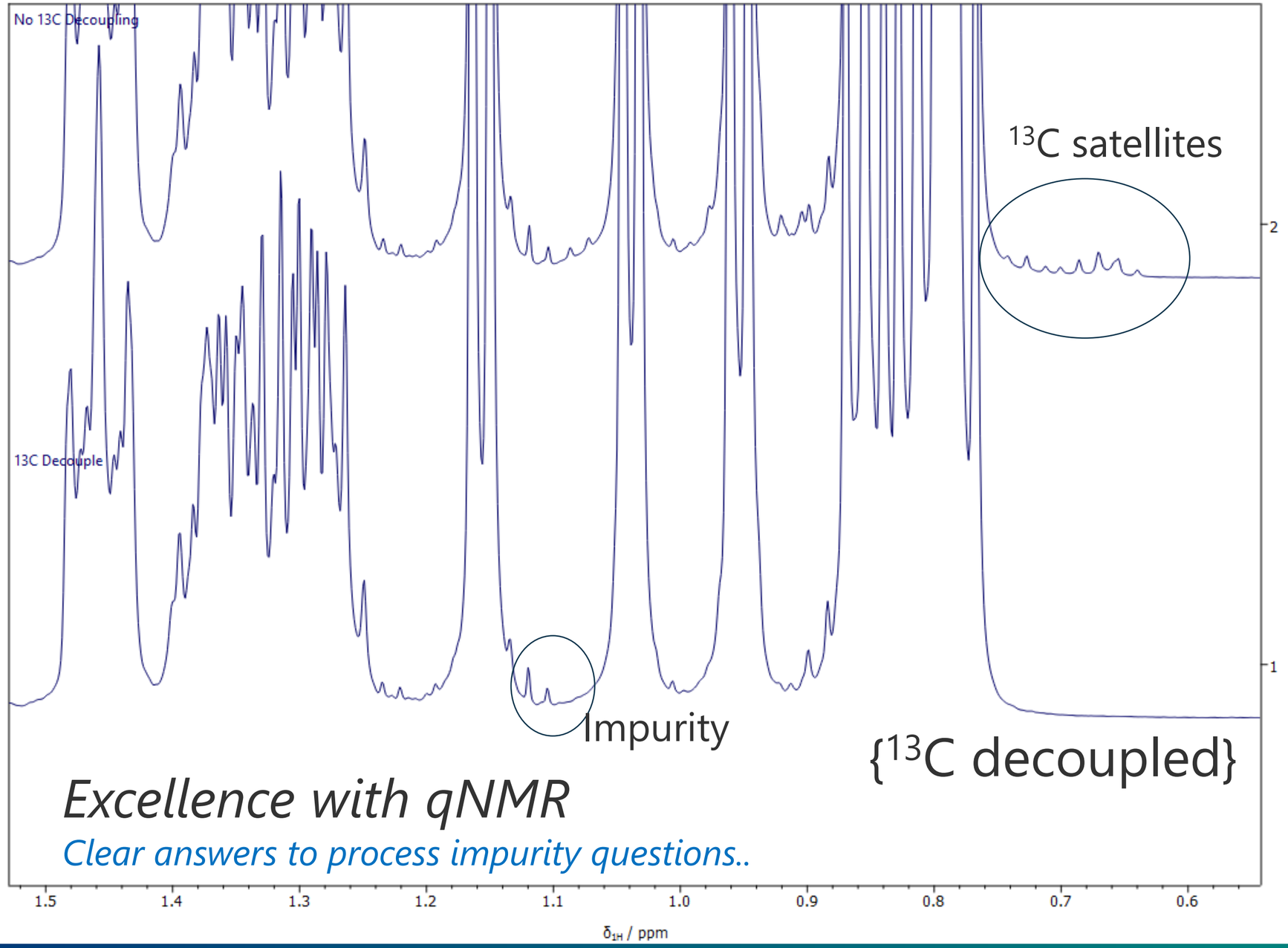




Stability!

500MHz MARVEL
SuperCool Probe

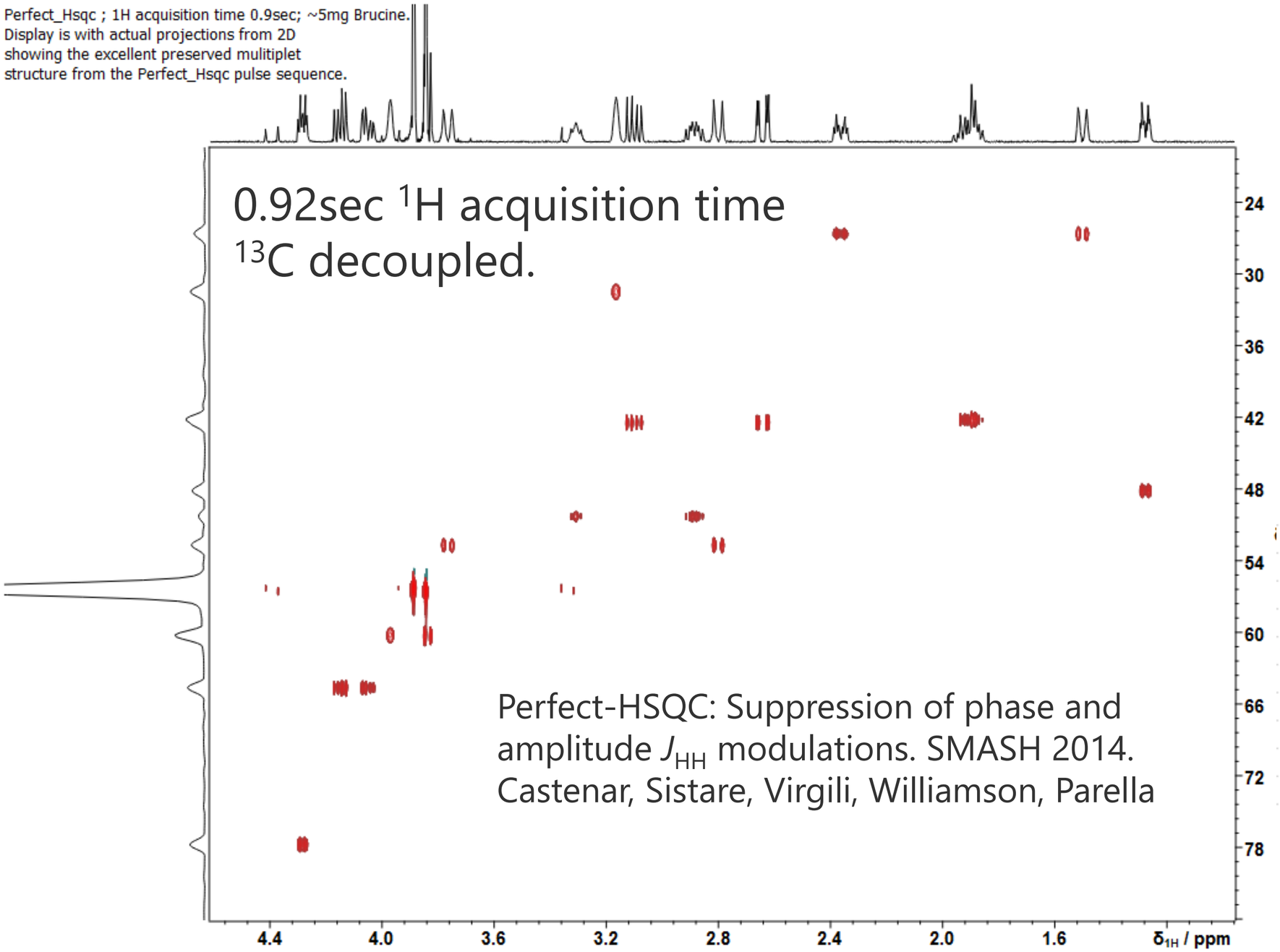
1 sec acq time



Excellence with qNMR

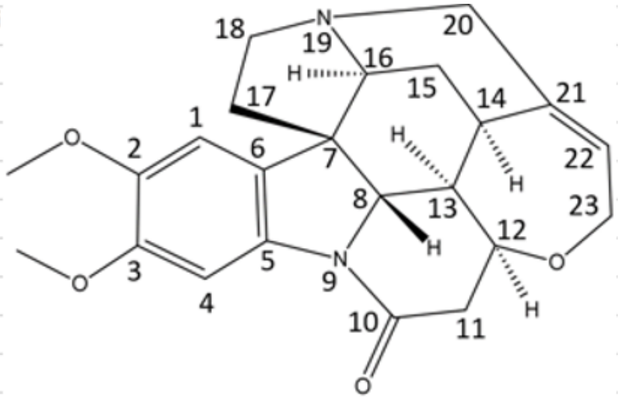
Clear answers to process impurity questions..

Perfect_Hsqc ; 1H acquisition time 0.9sec; ~5mg Brucine.
Display is with actual projections from 2D
showing the excellent preserved mulitiplet
structure from the Perfect_Hsqc pulse sequence.



As a precursor to IPAP hsqmbc we are looking at a hsqc acquired at moderately high (1.1Hz) F2 resolution & 8xZF.

Actual projections on top and sides of 2D plot. Note how perfect_hsqc preserves Multiplet structure.



From common tools… to less common ones

Having the ability to acquire higher resolution 2D experiments with ^{13}C decoupling affords improved confidence with measurements of long-range J_{CH} values.

Measuring $^{n}J_{CH}$ -> $^{N}J_{CH}$ are *J*umbled together with J_{HH} in ^{1}H spectrum at 1% intensity.

- Measure $^{N}J_{CH}$ by IPAP processed pip_hsqmbc.

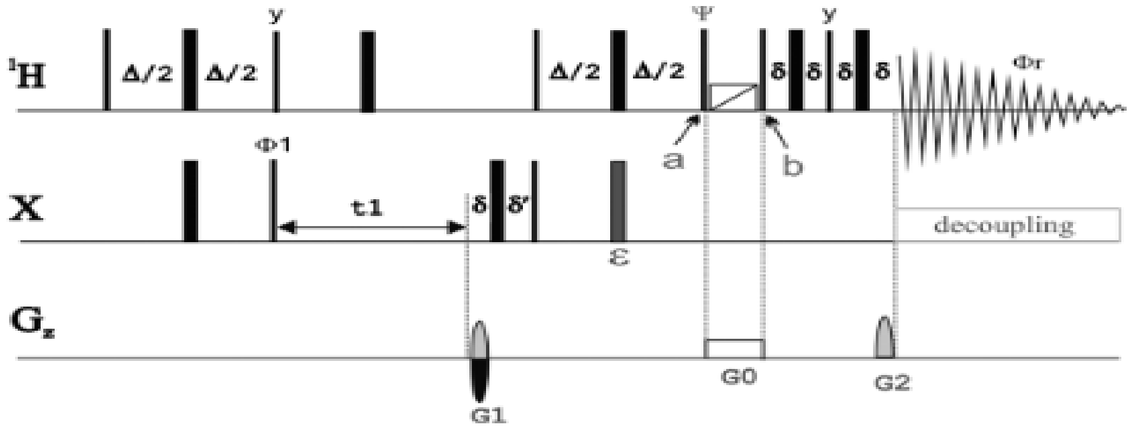
- Separates $^{N}J_{CH}$ from J_{HH} →by casting $^{N}J_{CH}$ as in-phase and anti-phase signals in two experiments with combination of ^{1}H and ^{13}C pulses. From there simple sum/difference arithmetic reveals the $^{N}J_{CH}$.

Pure In-Phase Heteronuclear Correlation NMR Experiments

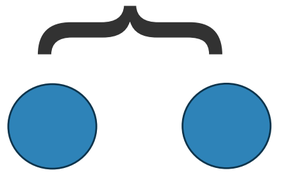
Angew. Chem. Int. Ed.
2014, 53, 1-5.
L. Castanar, J. Sauri, RT Williamson, A. Virgili, T. Parella.

(Closely related to the perfect-hsqc example show previously.)

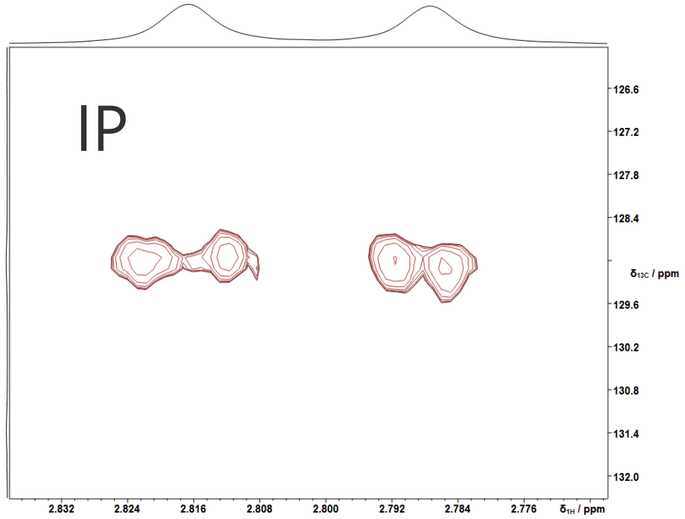
IPAP (In-Phase Anti-Phase) processing in NMR involves recording two separate HSQC experiments to generate in-phase and anti-phase cross-peaks. The sum and difference of these spectra allow for accurate measurement of scalar or dipolar coupling constants.

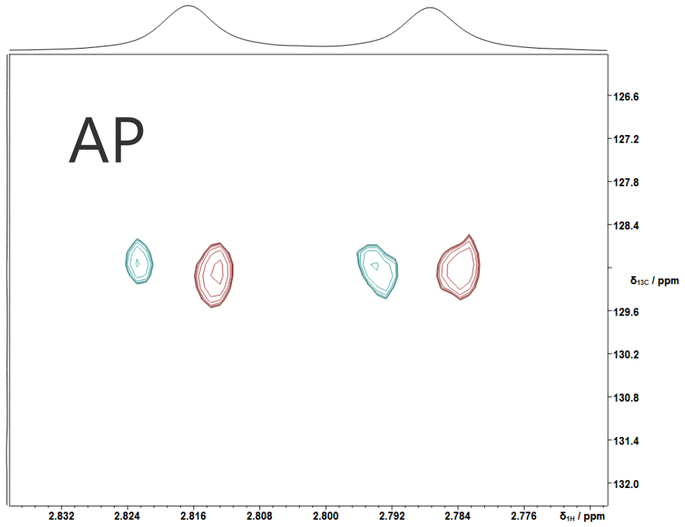


$^{N}J_{CH}$ IPAP 2D pair in F2

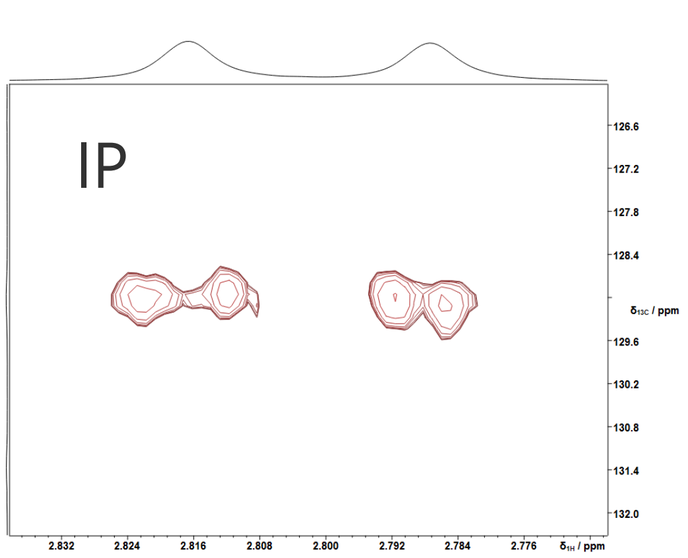


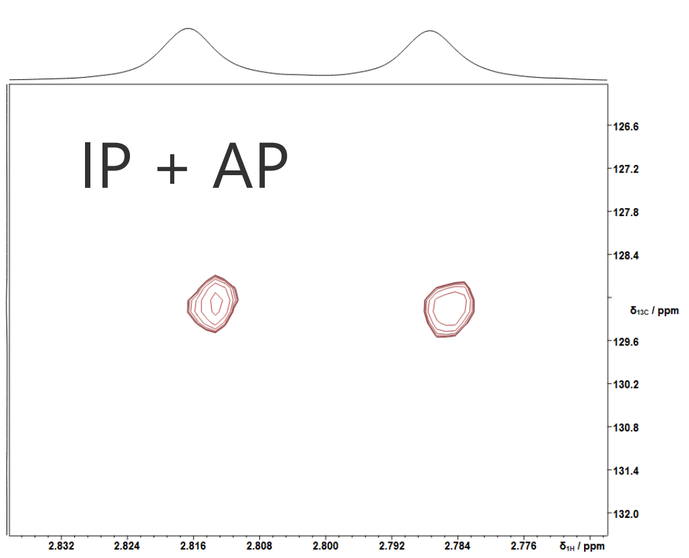
To a significant extent the accuracy of the measured $^{N}J_{CH}$ is dependent on ^{1}H acquisition time and IPAP technique.
Remember!! Zero-filling helps but Resolution = 1/acquisition time.

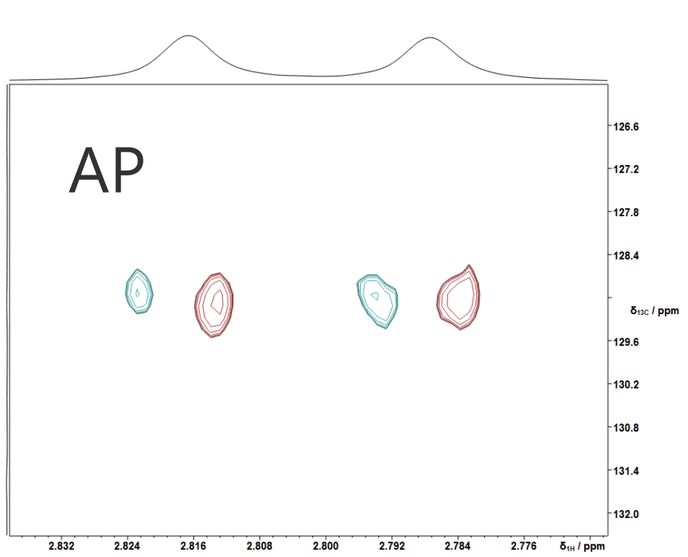


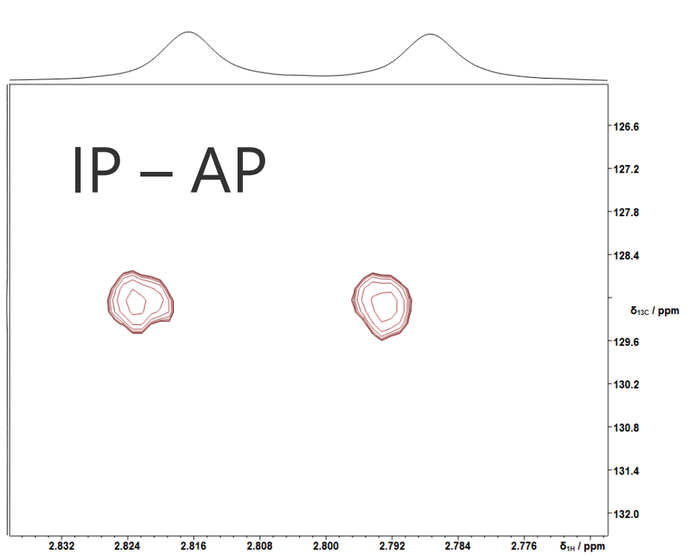


What is meant by IP/AP processing?

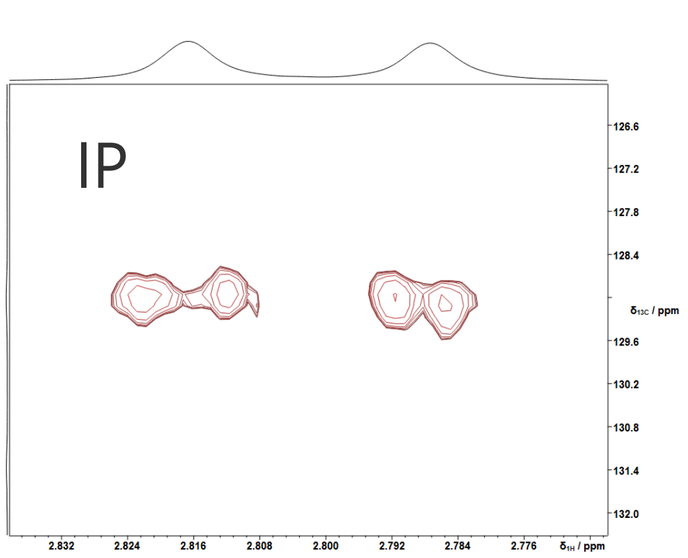


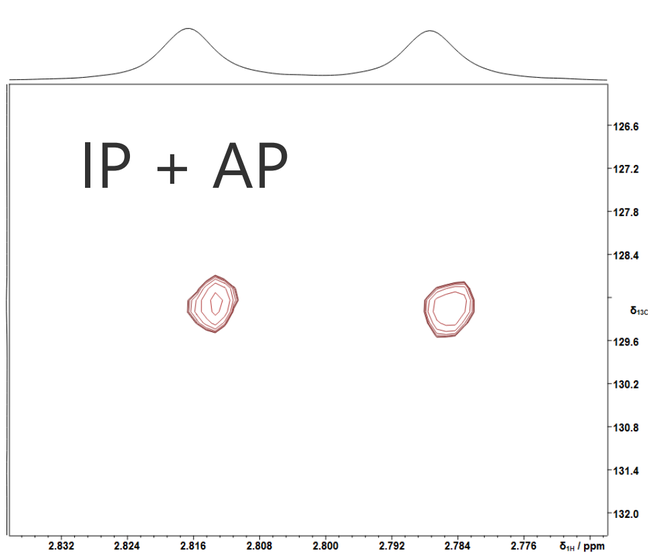


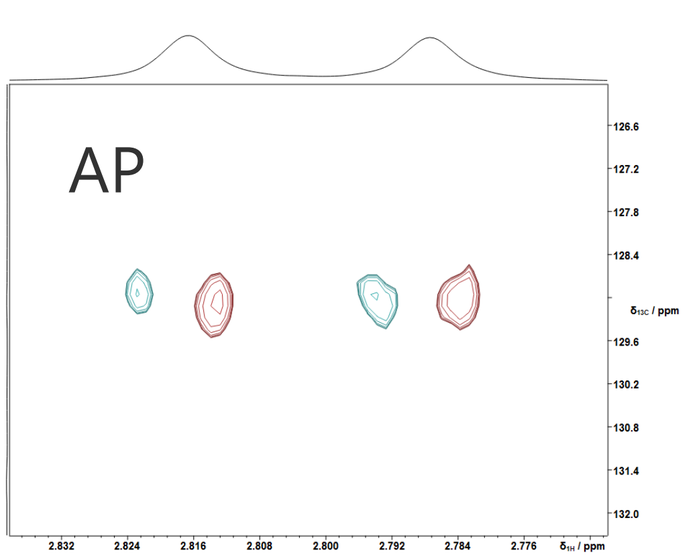


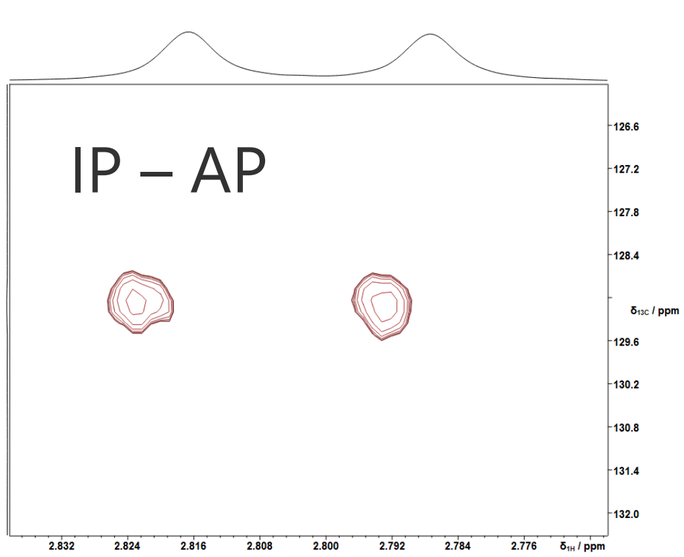


What is meant by IP/AP processing?

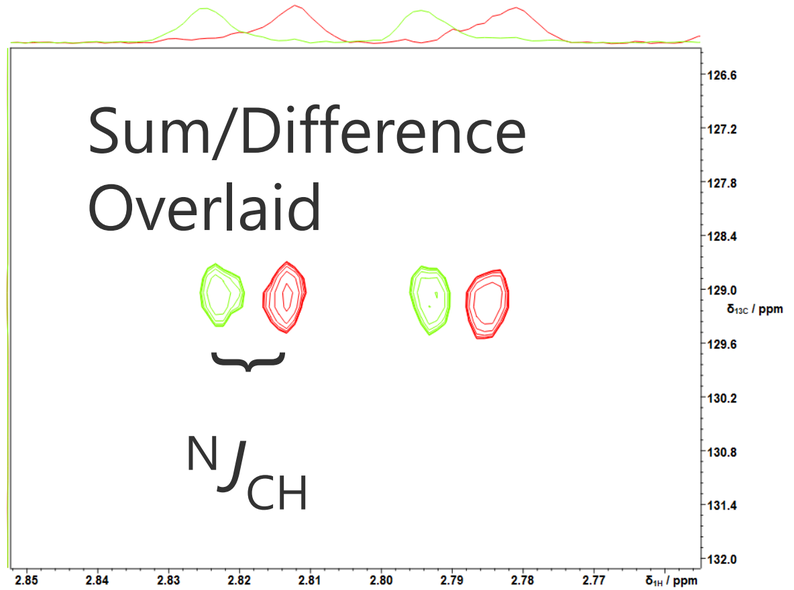


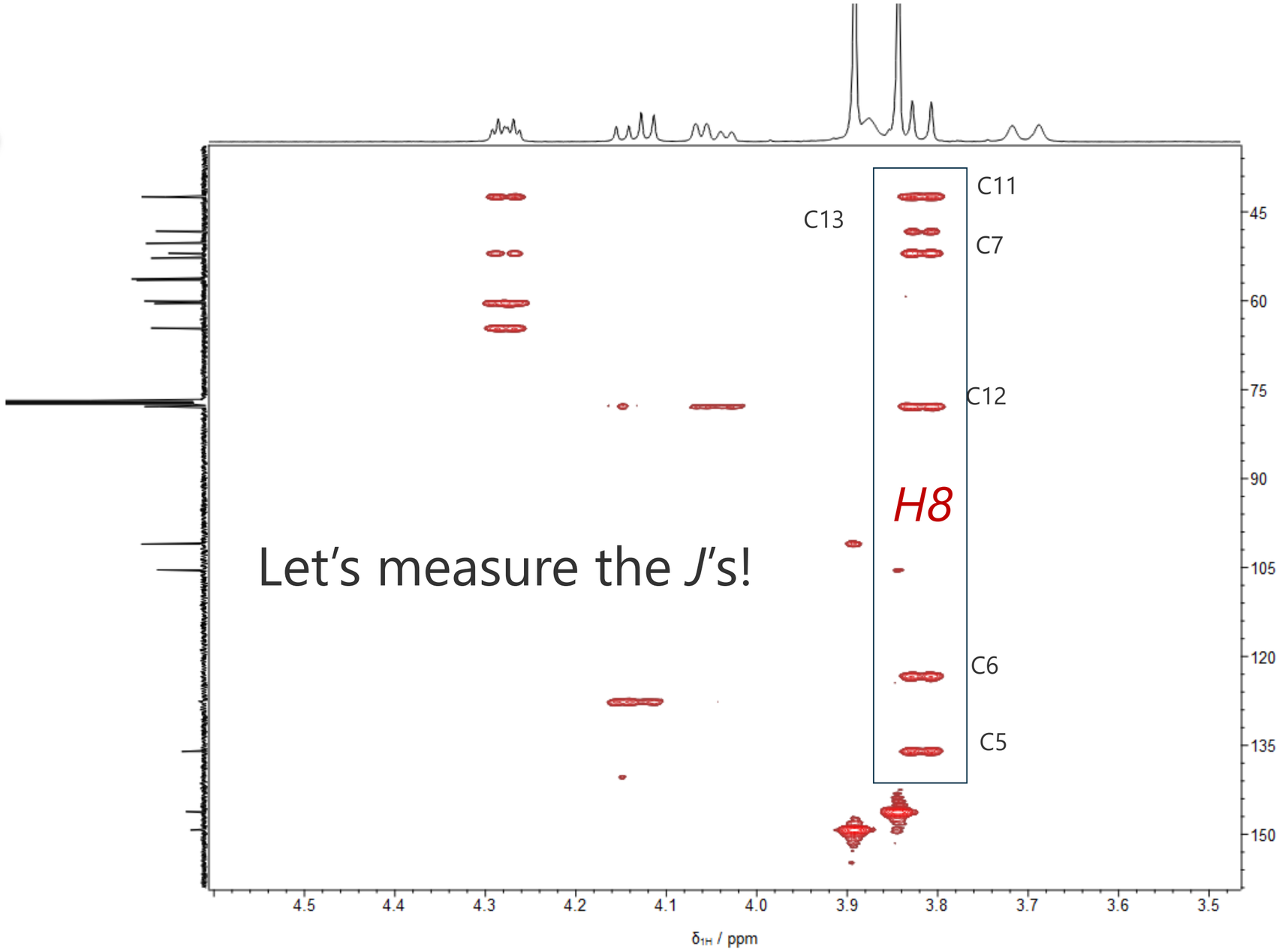


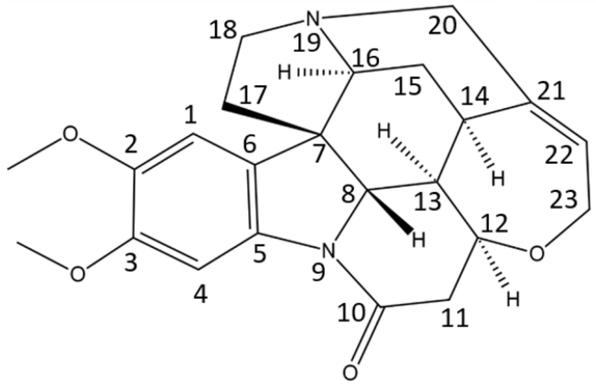




What is meant by IP/AP processing?

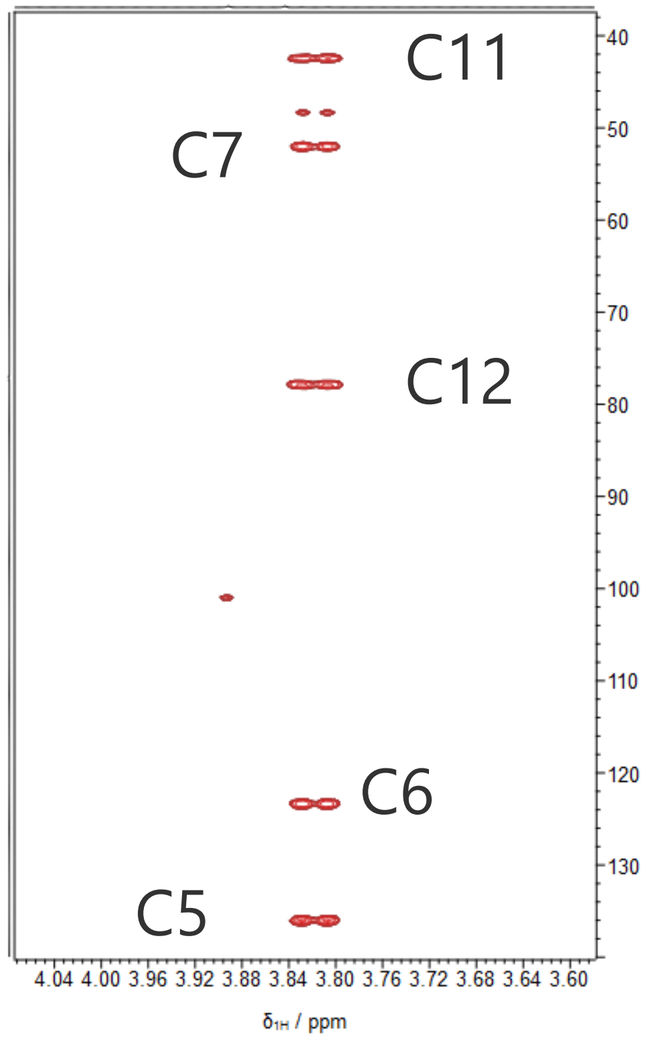




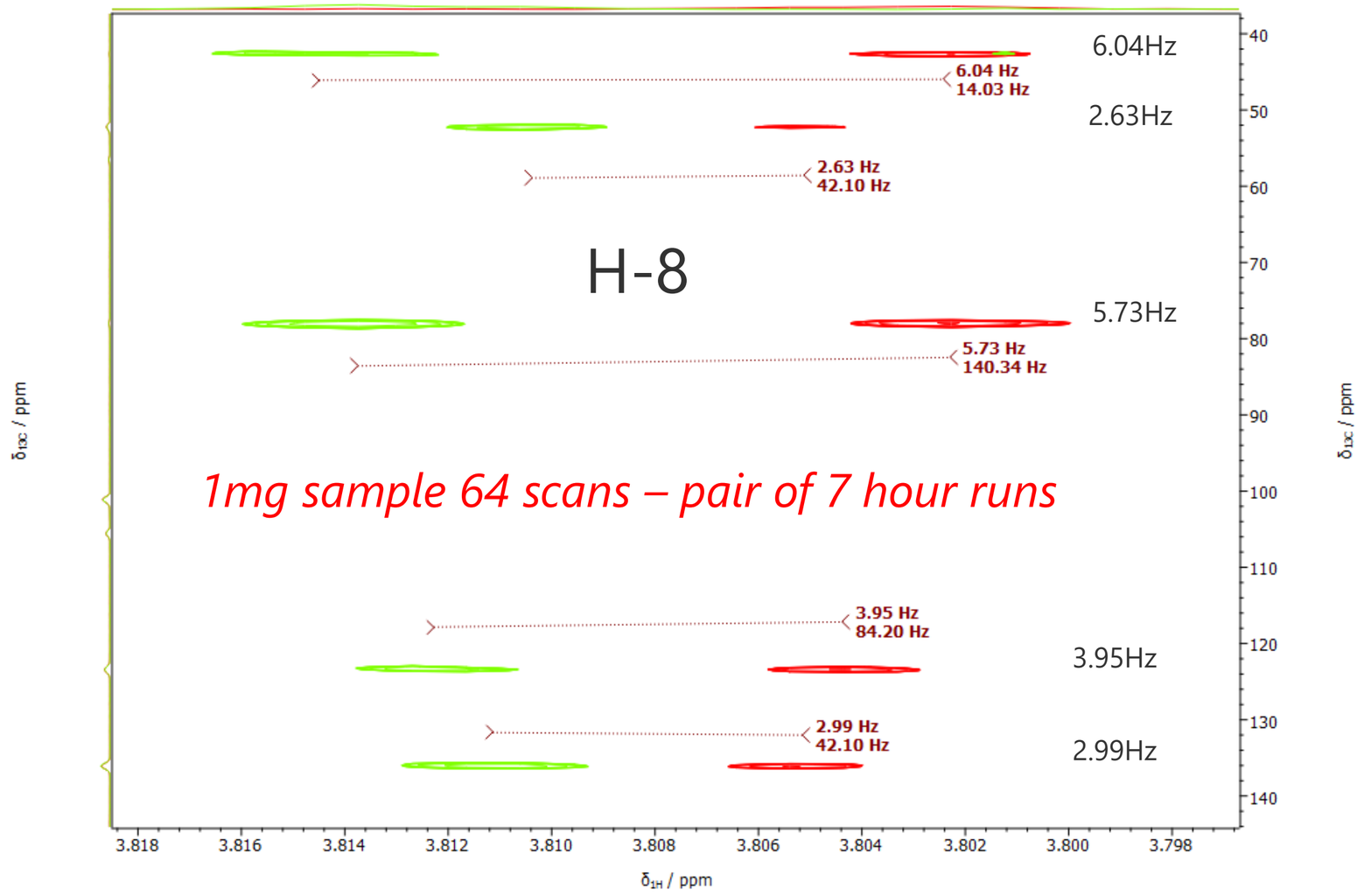


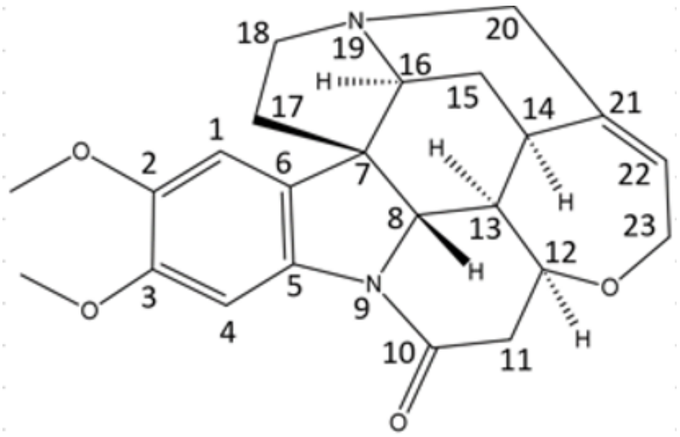
Brucine 1mg
gHMBC - 4 scans
38 minutes

HMBC



PIP-HSQMBC ipap processing





Expansion of IPAP processed
pip_hsqmbc 2D data pair; 8 scans/increment
1H (F2) acq time = 0.9sec
~5mg Brucine.

C-11
C-7
$^{n}J_{CH}$
C-12
H-16
H-8
6mg sample 8 scans – pair of 1.8 hour runs
C-6
C-5
C-3
C-2

δ_{13C} / ppm
δ_{1H} / ppm

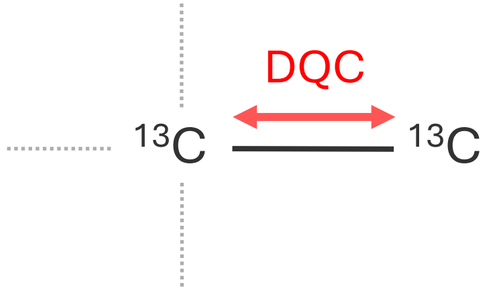
IPAP-processed
pip_hsqmbc pair
~5.8mg Brucine

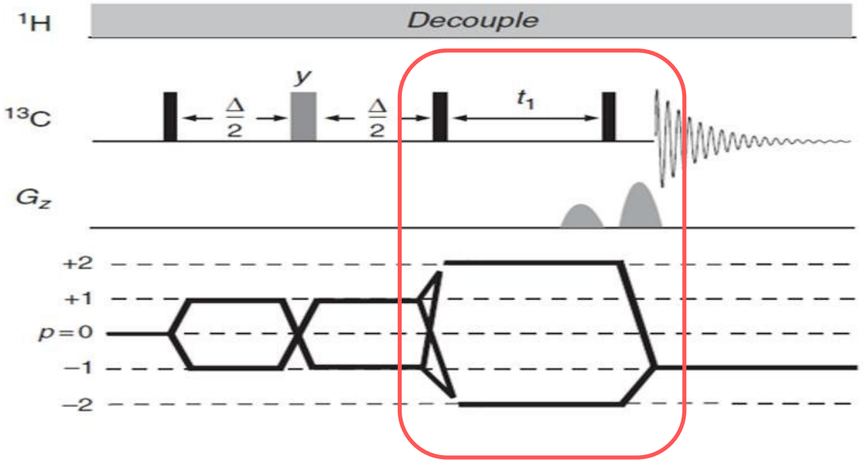
Couplings are in F2

A (much!) less common tool: 2D INADEQUATE
Incredible Natural Abundance DoublE QUantum Transfer Experiment

- 2D NMR technique used to identify **direct ^{13}C–^{13}C connectivities** in a molecule by detecting **one-bond couplings** between ^{13}C nuclei

- Exploits **double quantum coherence** to correlate pairs of **directly bonded ^{13}C nuclei**





Taken from *"High-Resolution NMR Techniques in Organic Chemistry"* by T.D.W. Claridge.

Used with permission from the author

- ***Potential*** to map out entire carbon skeleton in a molecule, but…

- Since natural ^{13}C abundance is only ~1.1%, both coupled carbons need to be ^{13}C → ~1 in ~10,000 molecules(!)

Original reference: Bax, Freeman, Frenkiel; J. Am. Chem. Soc. 1981, 103: 2102-2104

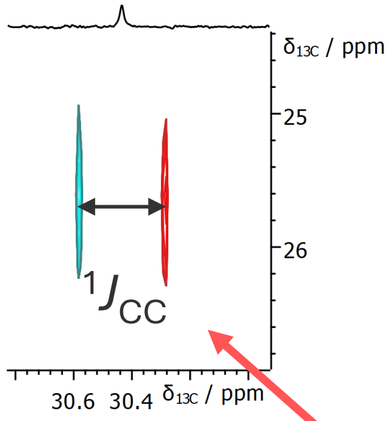
2D INADEQUATE

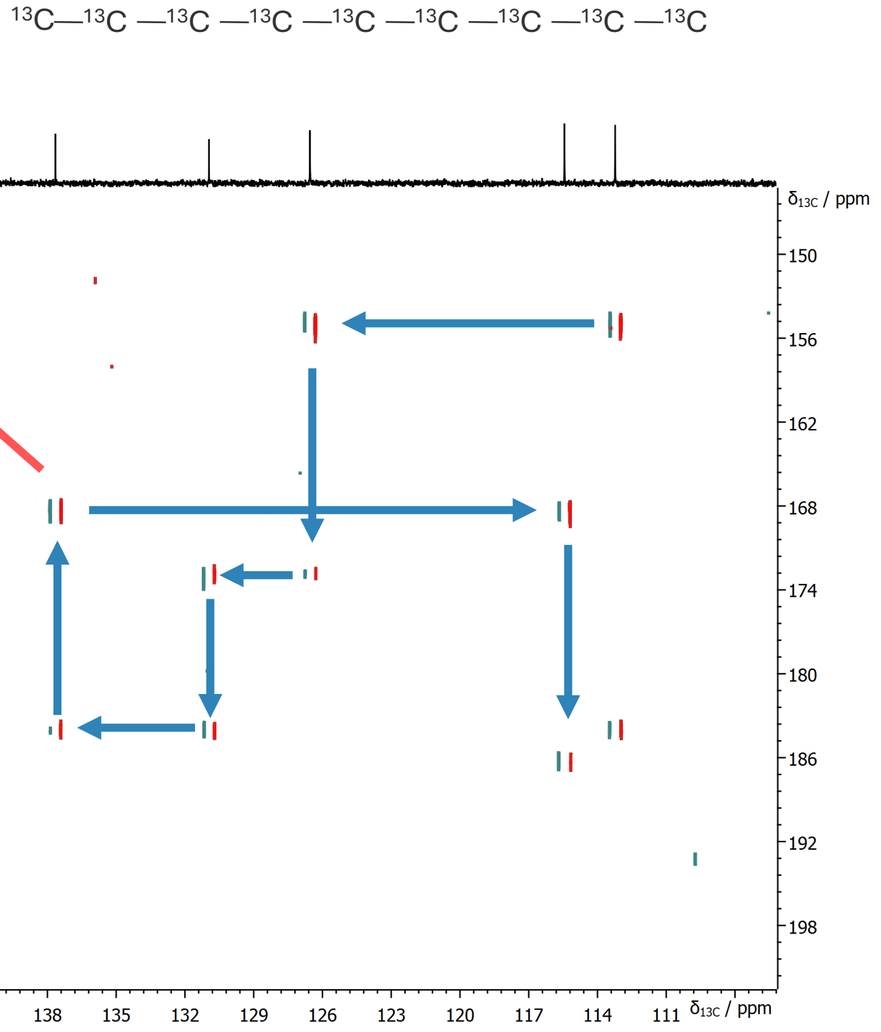
- F_2 axis shows ^{13}C chemical shift
- F_1 axis shows double-quantum (DQ) frequencies,

  or variants show single-quantum (SQ) frequencies
- Cross-peaks at the same F_1 frequency are from

  two adjacent carbon atoms in the molecule
- “Walk” stepwise through spectrum to sequentially

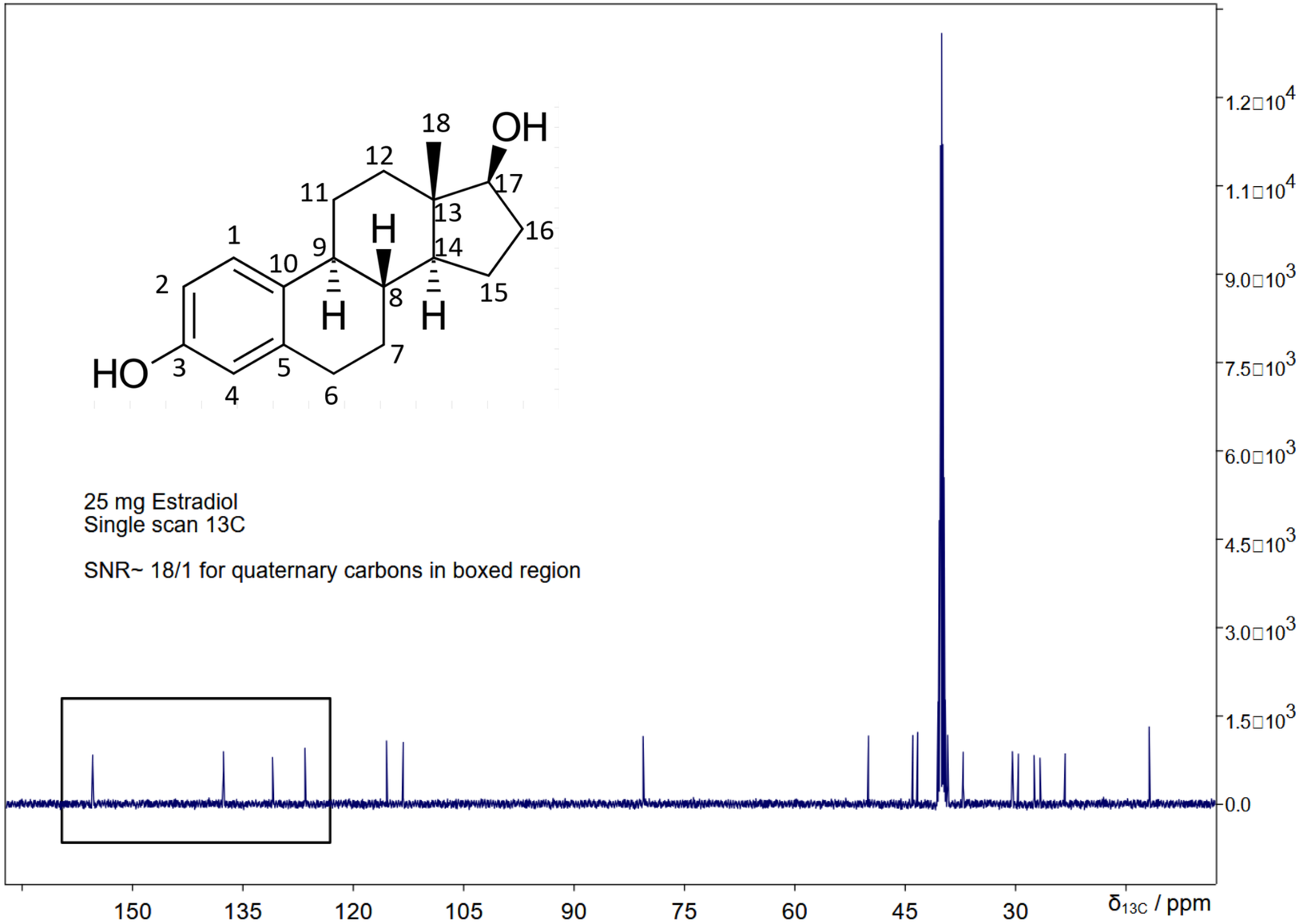
  assign carbon atoms



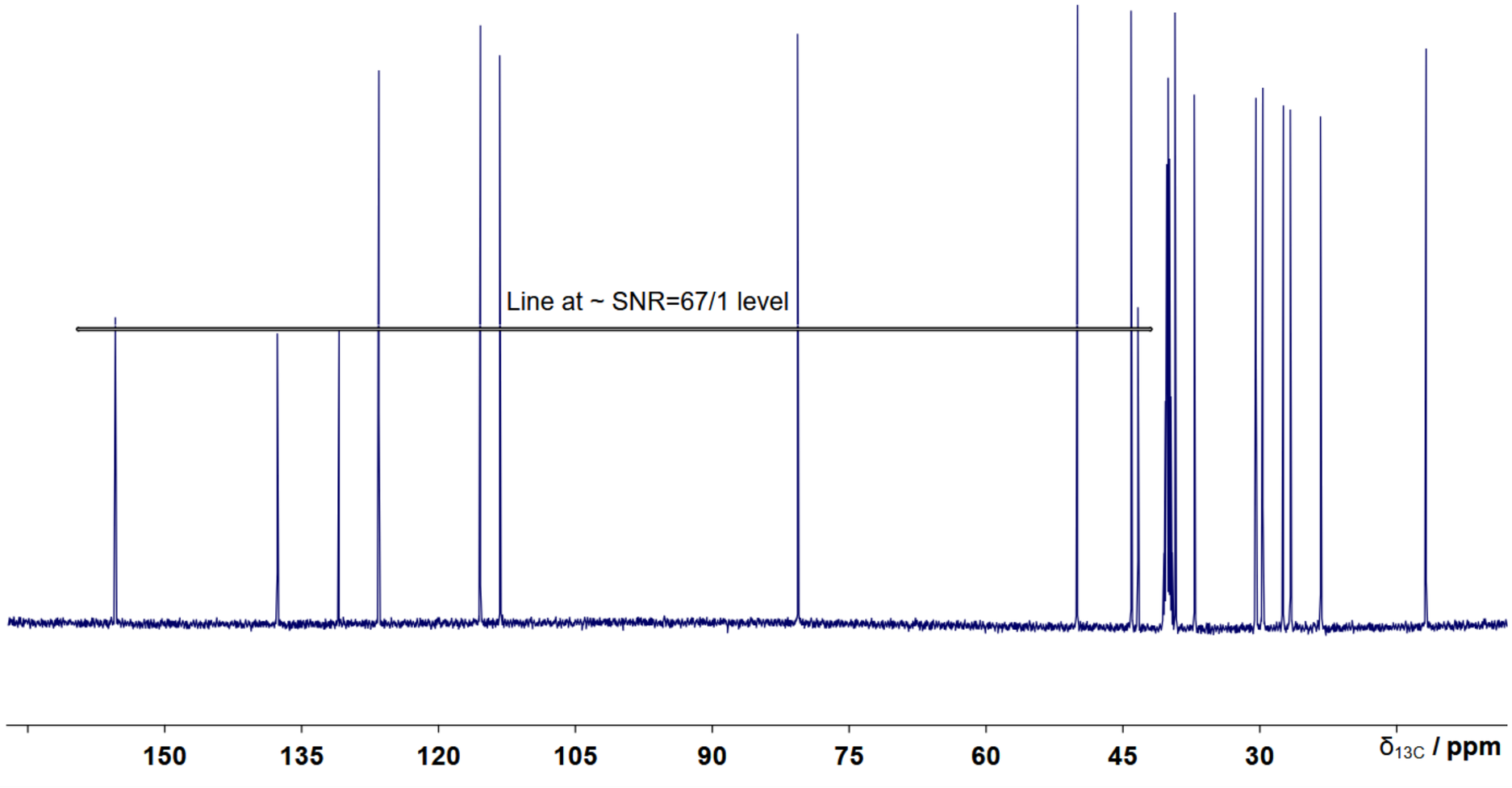


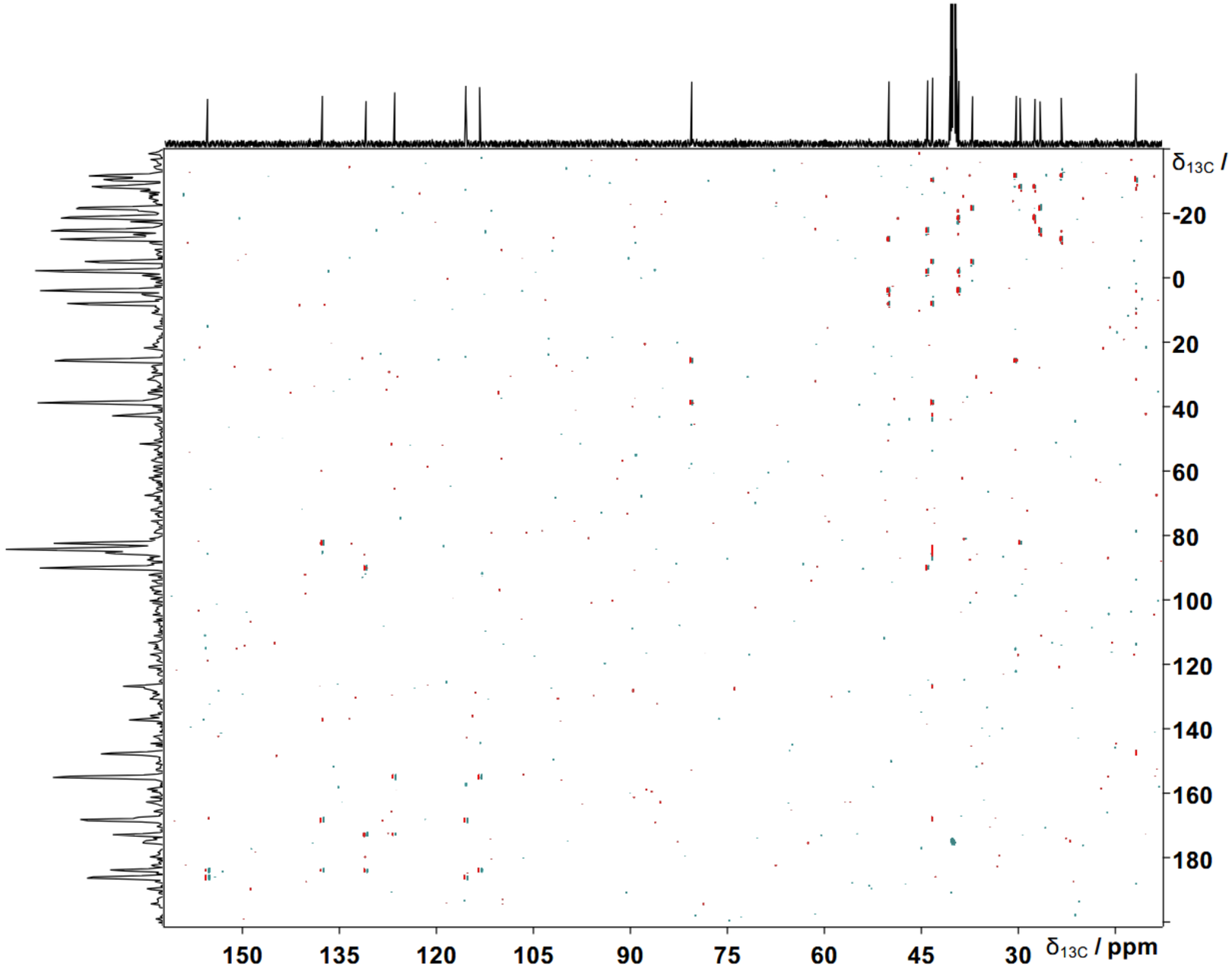
How might we ensure INADEQUATE will work?

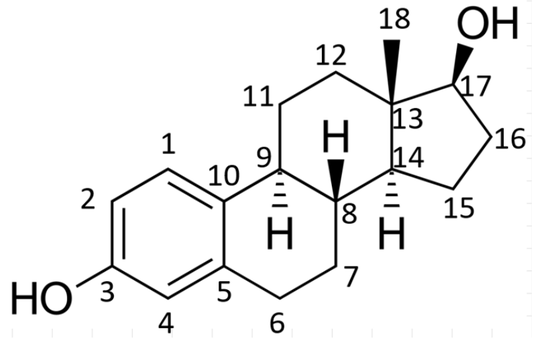
Run 2D with 1D ^{13}C parameters that achieve > 100/1 SNR



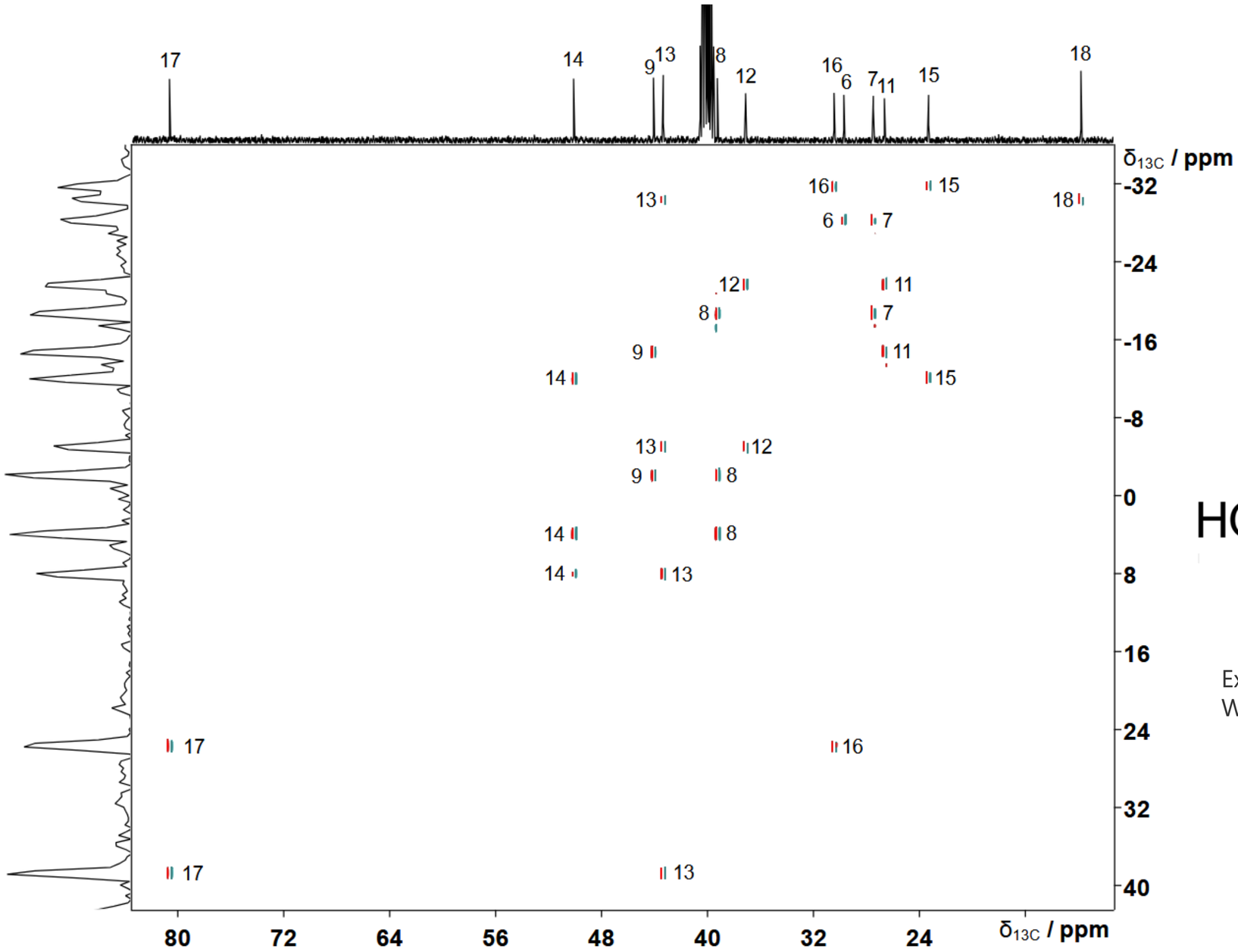
64 scan ^{13}C. 2sec rep rate with 0.42sec acquisition time. Using 128 scans would push SNR for long-relaxing carbons to ~100/1 ensuring good 2D INADEQUATE results.

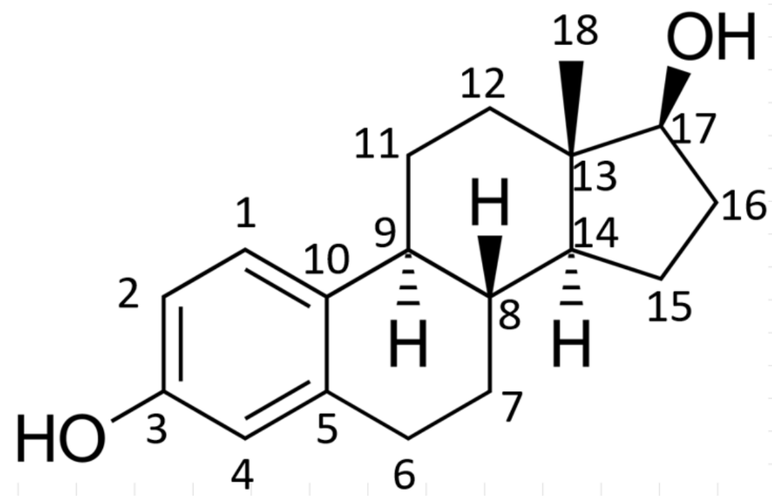






16.5hour 2D INADEQUATE
25mg Estradiol
128 scans/increment for 192 t1 points ; 50% NUS
Plotted at noise floor.
Phase-sensitive experiment yielding anti-phase correlations.



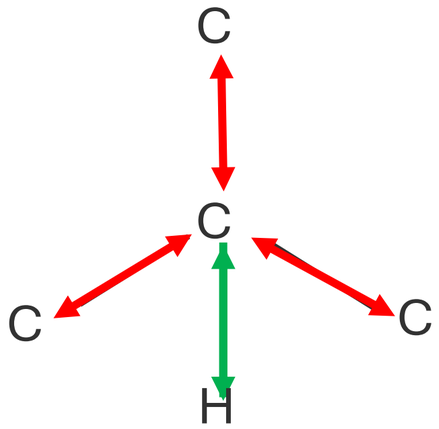


Expansion of high-field region
With DQ connectivities labelled.

ADEQUATE family of experiments

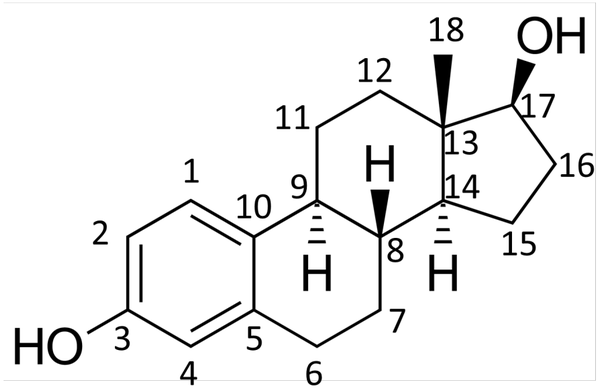
1,1 ADEQUATE

- ^{1}H-detected method (unlike INADEQUATE) → order of magnitude more sensitive
- Correlates two directly bonded carbons with a proton directly bonded to one of the carbons
- Only carbons and protons that are two bonds apart are correlated



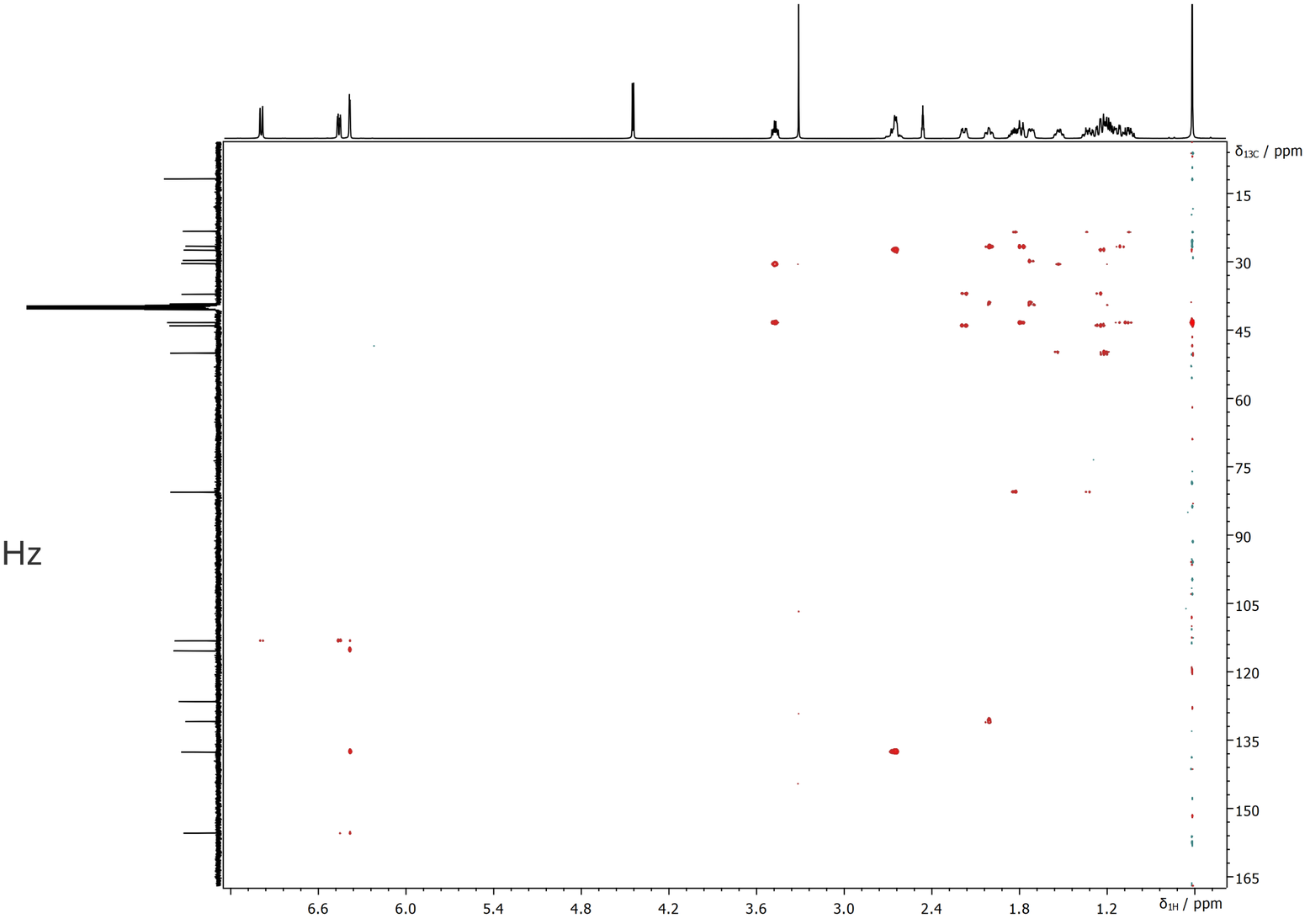
- INEPT transfer from H to directly attached C
- Generate DQC between adjacent carbon atoms (~1 in 10,000)
- t_1 evolution of ^{13}C DQC
- Conversion back to SQC
- Reverse INEPT from C back to H

1,1-ADEQUATE

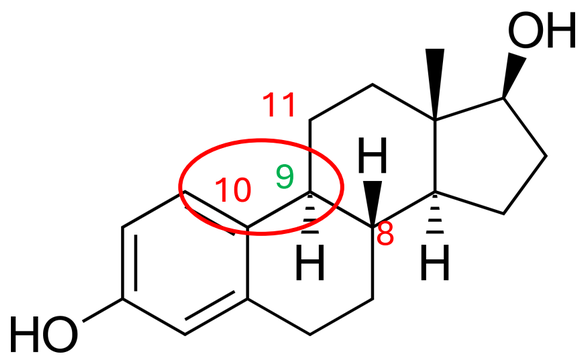


25 mg estradiol

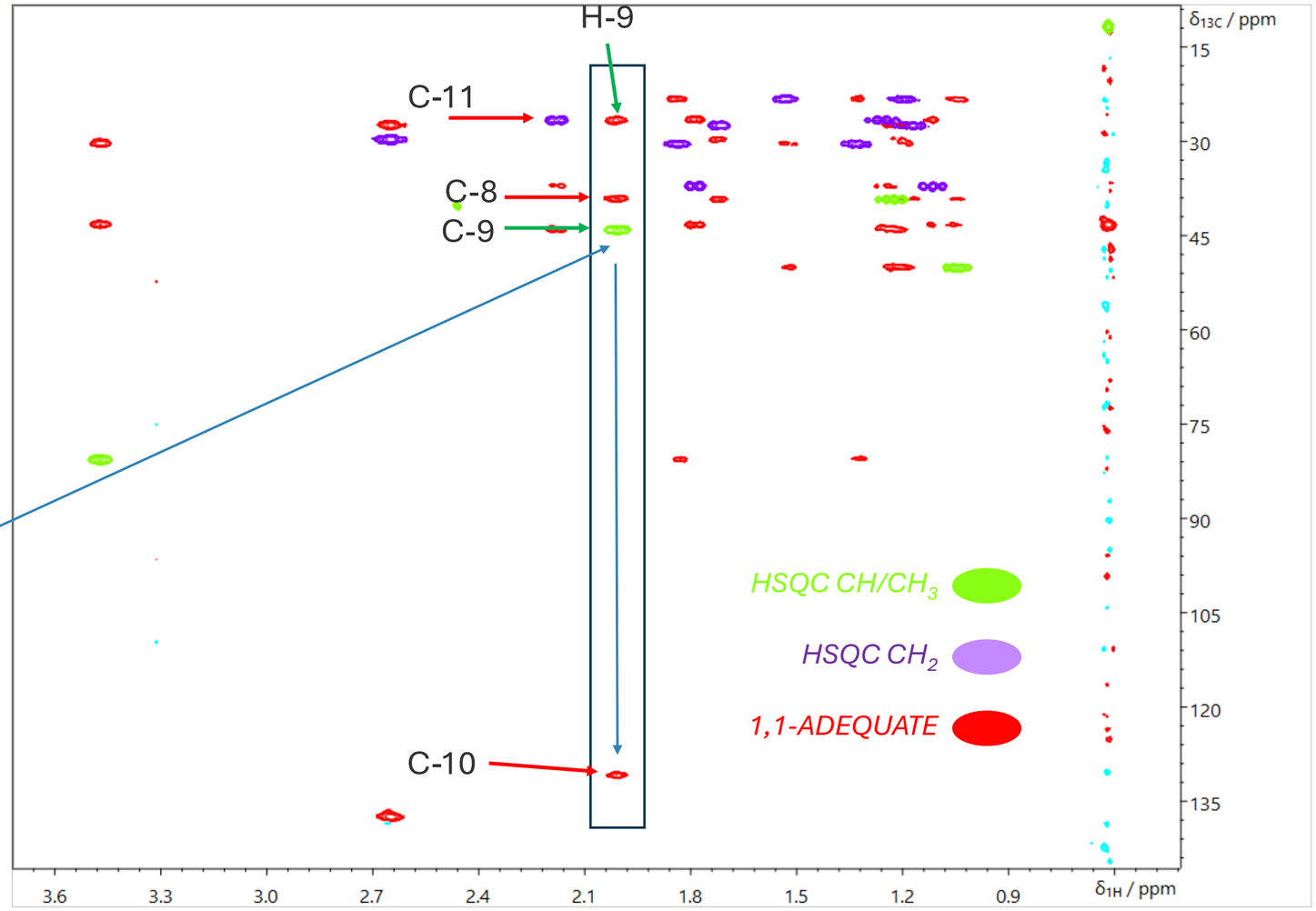
SuperCOOL MARVEL 500 MHz
7 h experiment



HSQC and 1,1-ADEQUATE overlaid

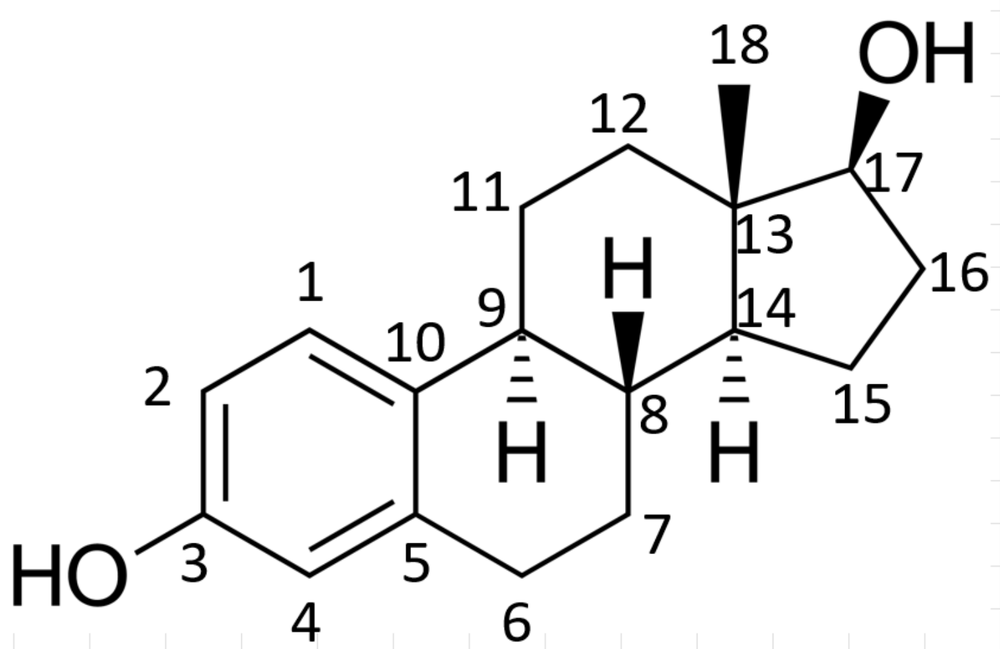


- Start from single HSQC correlation
- CH-9 is only aliphatic CH directly attached to an aromatic carbon (C-10)
- From there, possible to walk through all through overlaid spectra and make complete unambiguous assignments in a few minutes

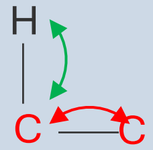
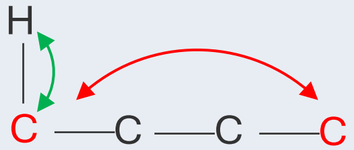
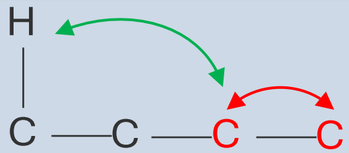
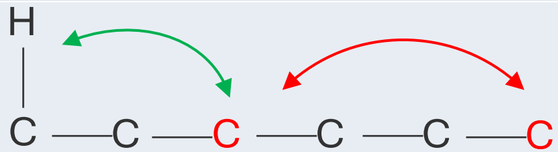


Assignment Table from C-C data 25mg Estradiol.

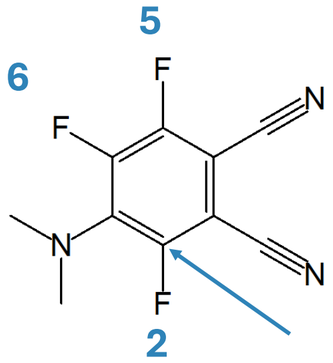
C-Pos (ppm)		Type		Label	H-Pos (ppm)		Assignment
155.39		quat			Q		3
137.65		quat			Q		5
130.98		quat			Q		10
126.53		C-H			6.983		1
115.45		C-H			6.386		4
113.23		C-H			6.457		2
80.613		C-H			3.469		17
50.05		C-H			1.039		14
44.049		C-H			1.997		9
43.333		quat			Q		13
39.226		C-H			1.21		8
37.113		CH2			1.788	1.106	12
30.417		CH2			1.839	1.324	16
29.686		CH2			2.656	2.634	6
27.478		CH2			1.713	1.169	7
26.619		CH2			2.17	1.255	11
23.307		CH2			1.525	1.184	15
11.78		CH3			0.613		18

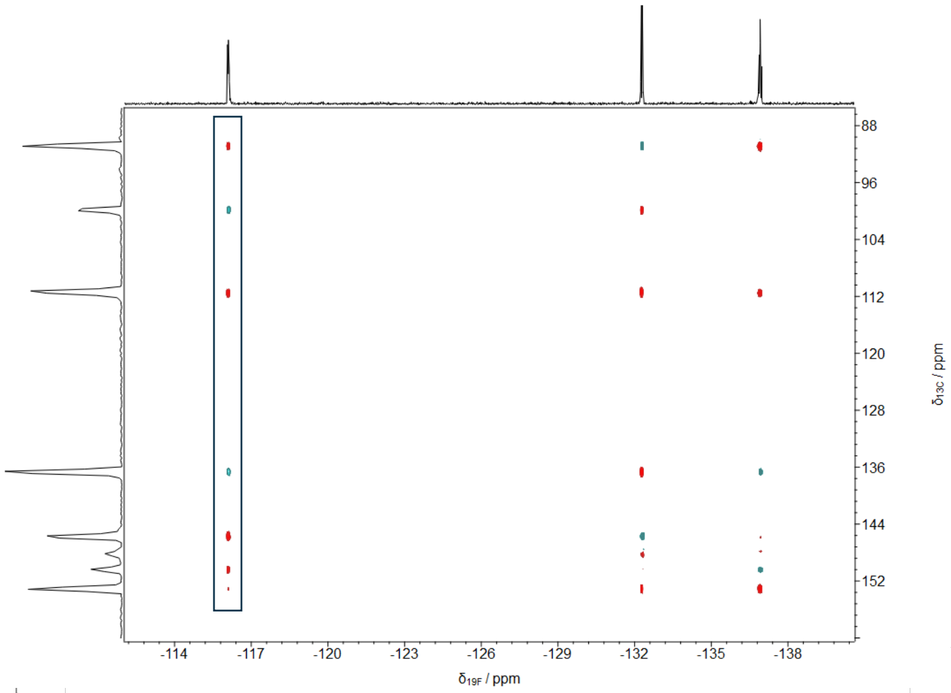


Other ADEQUATE variants

ADEQUATE variant	Couplings Exploited	Scheme	Max Bonds Correlated
1,1-	$^{1}J_{CH}$, $^{1}J_{CC}$		2
1,n	$^{1}J_{CH}$, $^{n}J_{CC}$		4
n,1	$^{n}J_{CH}$, $^{1}J_{CC}$		4
m,n	$^{m}J_{CH}$, $^{n}J_{CC}$		6

^{13}C Traces at 90.774ppm





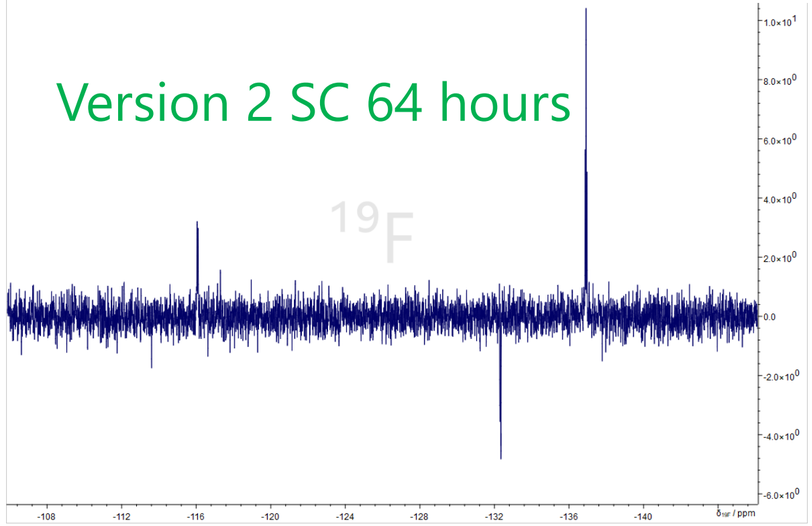
Progress!!

Greatly improved ^{19}F SNR

2.4^2 = 5.8 time factor

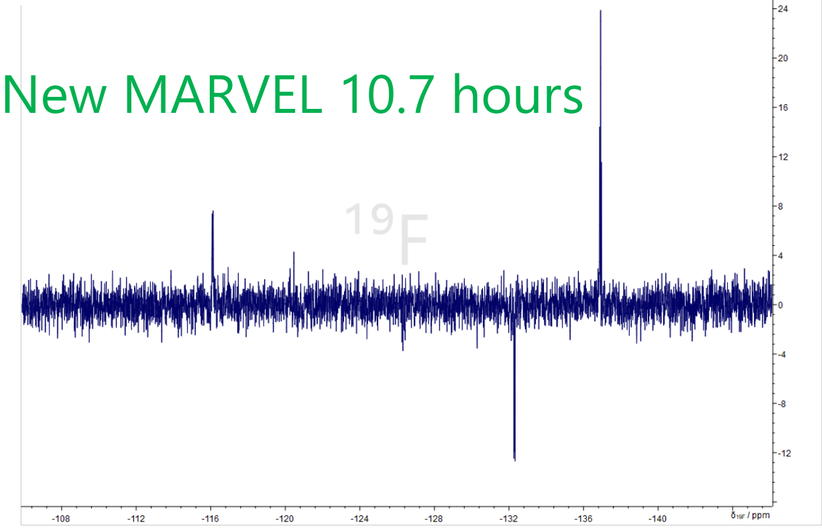
64hours/5.8 = 11hours

Version 2 SC 64 hours



MRC Volume 60, Issue 2
February 2022, Pages 210-220

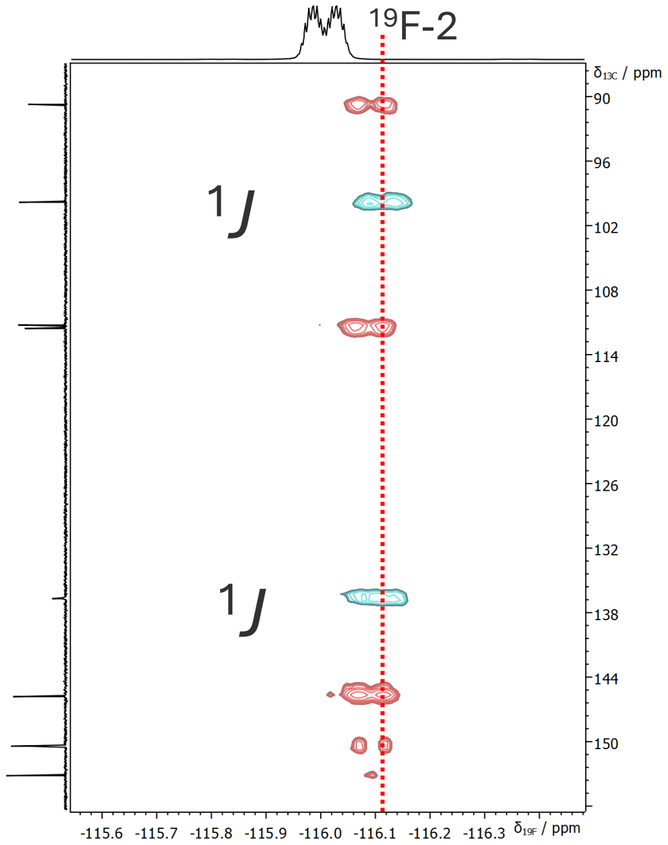
New MARVEL 10.7 hours

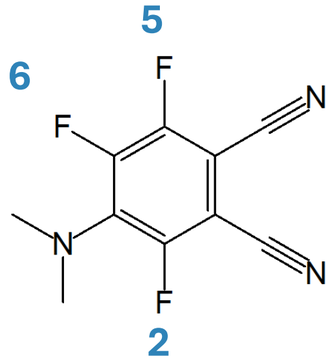


$^{n}J^{1}J$ edited F/C ADEQUATE Much more than a factor of 4 *less sensitive* that 1,1-ADEQUATE

^{19}F isotope shifts

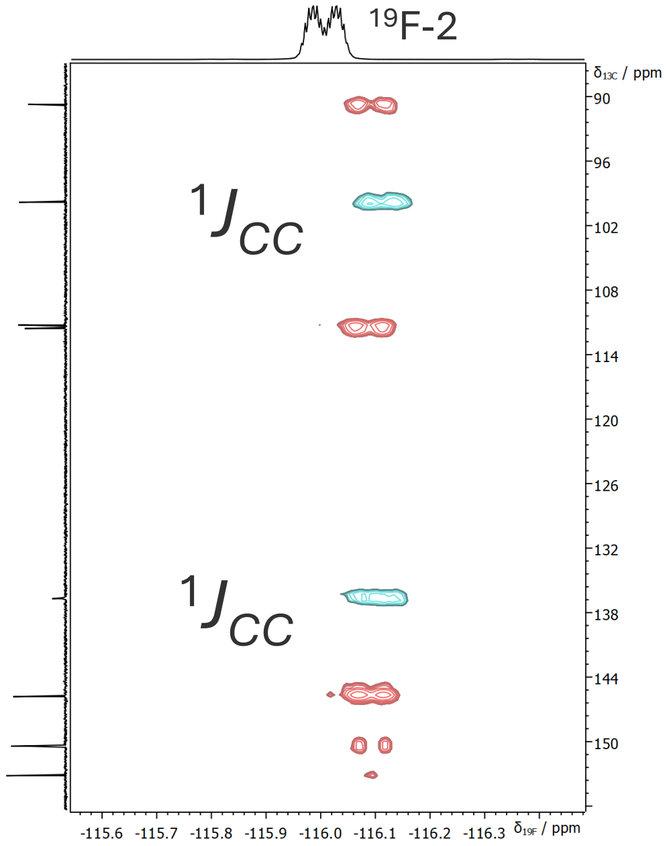
Large isotope shifts! Larger for ^{1}J



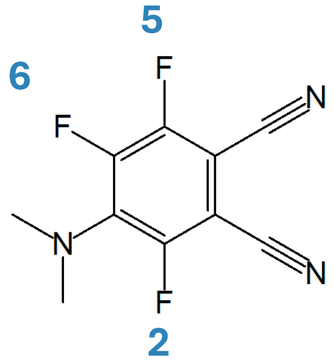


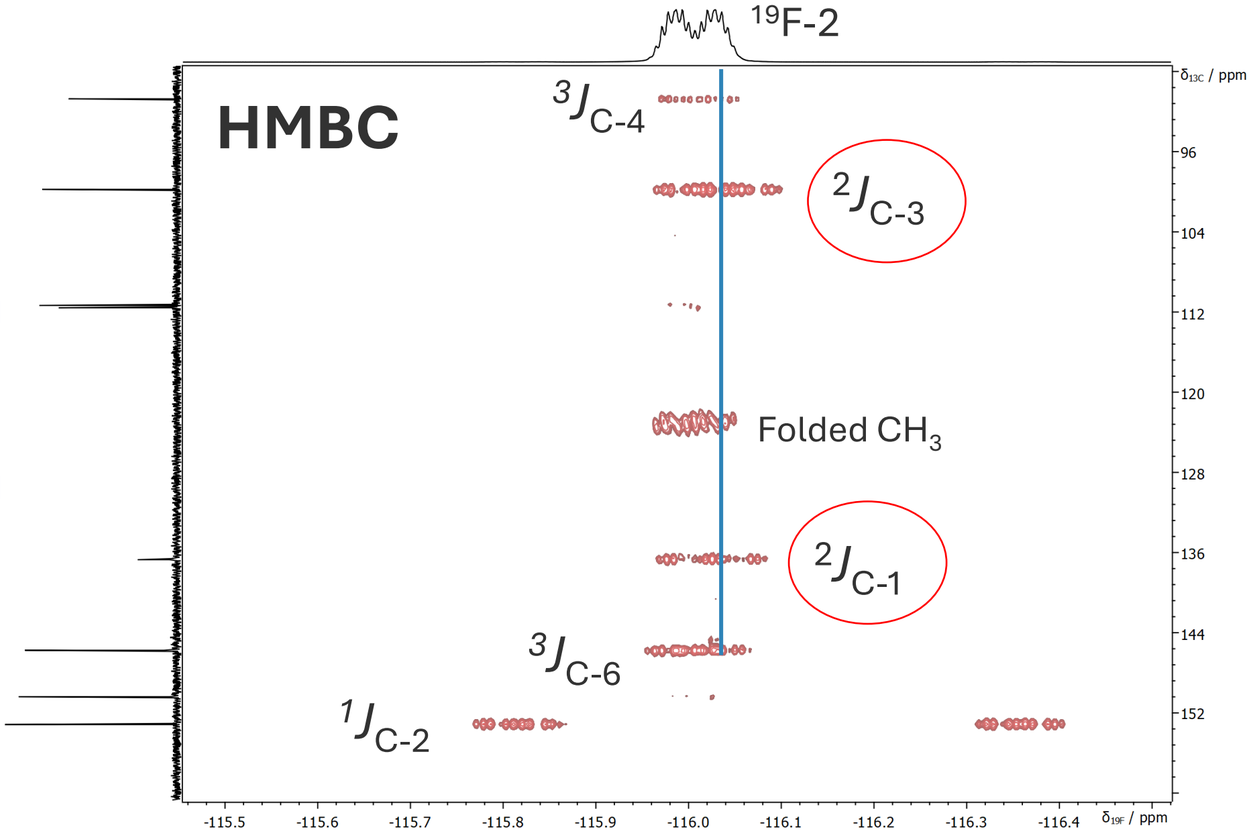
^{13}C-^{13}C connectivity relayed to ^{19}F

i-HMBC: exploiting the isotope shifts



^{13}C-^{13}C connectivity relayed to ^{19}F





^{19}F-^{13}C HMBC –> ^{19}F-^{13}C connectivity

i-HMBC: exploiting the isotope shifts

H-C isotope shifts ~ factor of 100 < F-C

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Unequivocal identification of two-bond heteronuclear correlations in natural products at nanomole scale by i-HMBC

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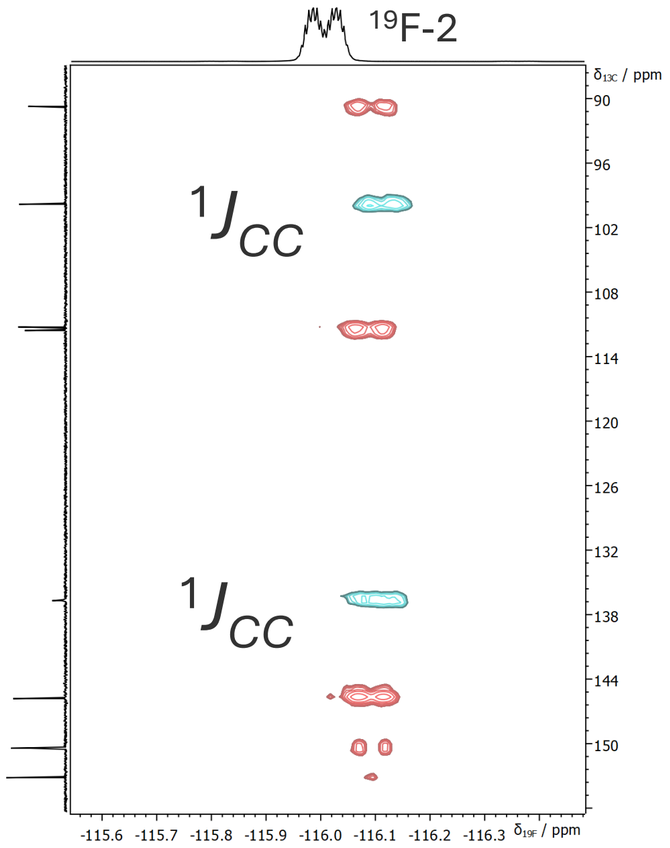
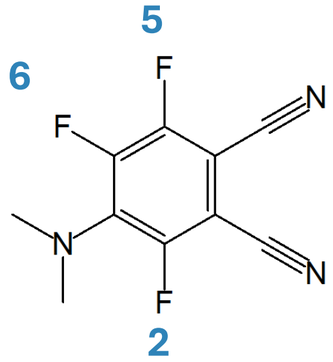
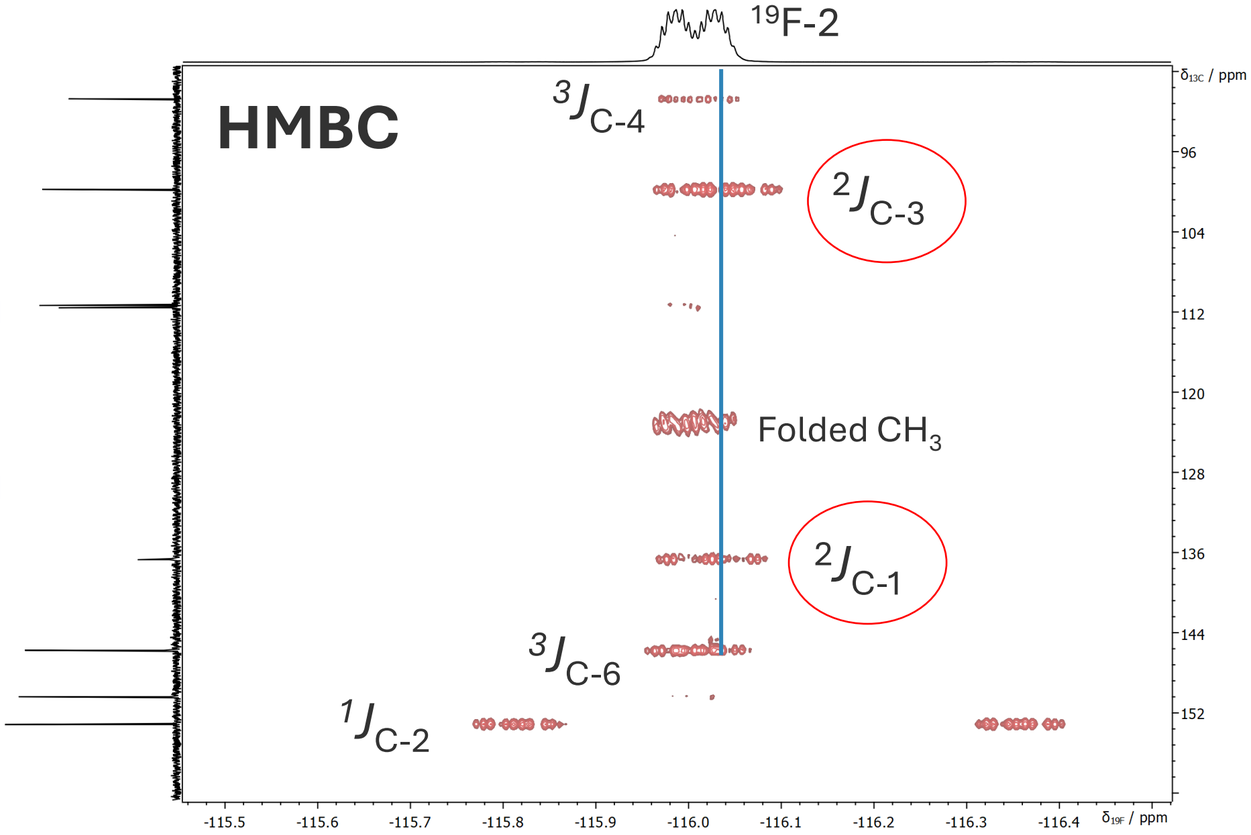


^{13}C-^{13}C connectivity relayed to ^{19}F

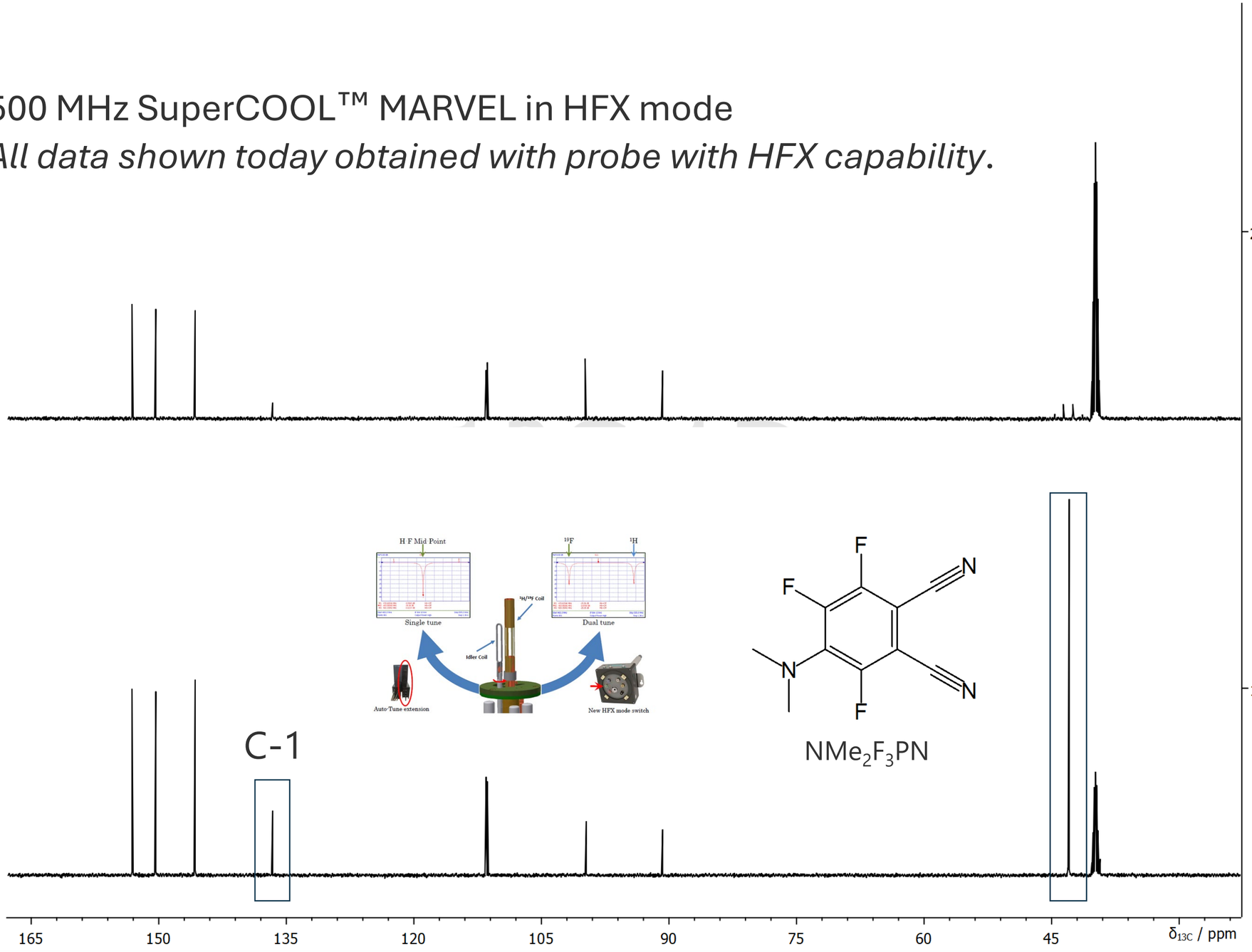




^{19}F-^{13}C HMBC –> ^{19}F-^{13}C connectivity

500 MHz SuperCOOL™ MARVEL in HFX mode

All data shown today obtained with probe with HFX capability.



Summary

- **Cryogenic NMR probes** are a **convenient** and **cost-effective** way to **significantly increase the sensitivity** of an NMR system.
- **Helium-cooled cryogenic probes** offer the **best performance**, but **purchase, infrastructure and maintenance costs** can be prohibitive for some labs.
- **SuperCOOL MARVEL** probe offers an **excellent price-performance ratio**, with **balanced high-band and low-band sensitivity** combined with **reasonable running costs.**
- **SuperCOOL MARVEL**'s superior performance makes it **ideal for many labs and applications**, from **routine measurements** to **more demanding** experiments.
- **SuperCOOL MARVEL** is also available as a **triple-resonance HFX probe** **…stay tuned for a future JEOL webinar on this probe**

JEOL USA, Peabody, MA

Q&A

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