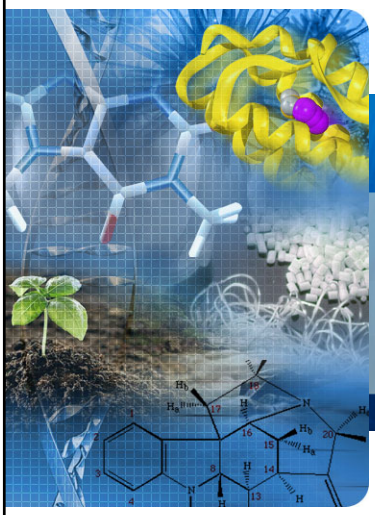
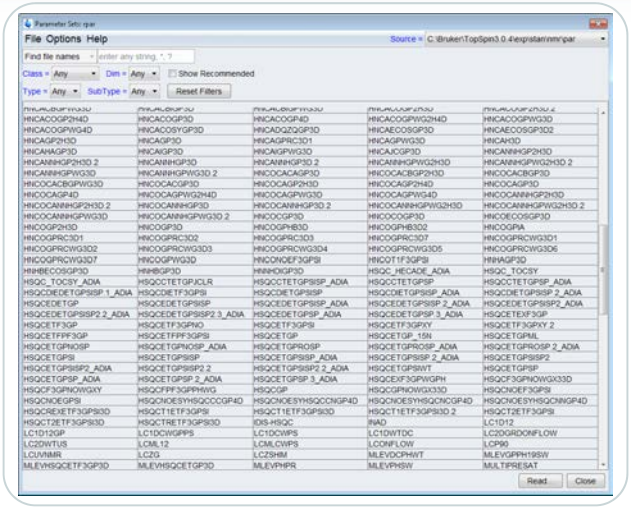


Which Experiment Should I Choose?

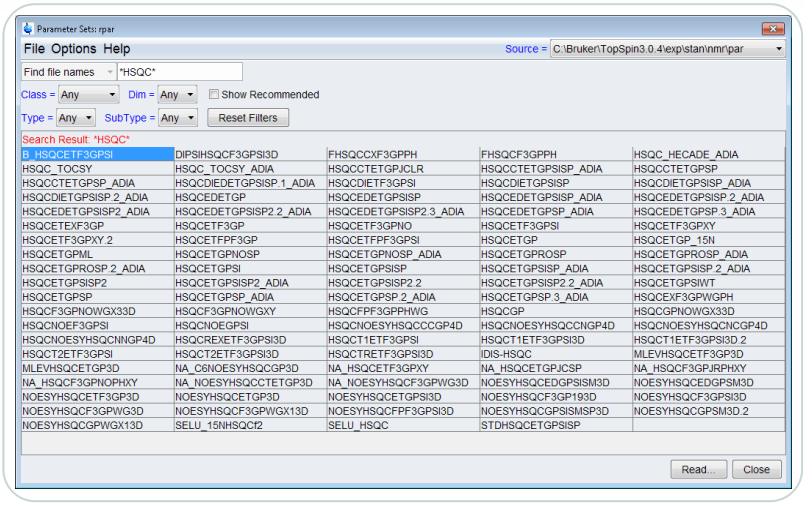
Bruker BioSpin

So Many to Choose From….



So Many to Choose From…

99 parameter sets, and 144 pulse sequences with "HSQC"



B_HSQCETF3GPSI	DIPSIHSQCF3GPSI3D	FHSQCCXF3GPPH	FHSQCF3GPPH	HSQC_HECADE_ADIA
HSQC_TOCSY	HSQC_TOCSY_ADIA	HSQCCTETGPJCLR	HSQCCTETGPSISP_ADIA	HSQCCTETGPSP
HSQCCTETGPSP_ADIA	HSQCDIEDETGPSISP.1_ADIA	HSQCDIETF3GPSI	HSQCDIETGPSISP	HSQCDIETGPSISP_ADIA
HSQCDIETGPSISP.2_ADIA	HSQCEDETGP	HSQCEDETGPSISP	HSQCEDETGPSISP_ADIA	HSQCEDETGPSISP.2_ADIA
HSQCEDETGPSISP2_ADIA	HSQCEDETGPSISP2.2_ADIA	HSQCEDETGPSISP2.3_ADIA	HSQCEDETGPSP_ADIA	HSQCEDETGPSP.3_ADIA
HSQCETEXF3GP	HSQCETF3GP	HSQCETF3GPNO	HSQCETF3GPSI	HSQCETF3GPXY
HSQCETF3GPXY.2	HSQCETFPF3GP	HSQCETFPF3GPSI	HSQCETGP	HSQCETGP_15N
HSQCETGPML	HSQCETGPNOSP	HSQCETGPNOSP_ADIA	HSQCETGPROSP	HSQCETGPROSP_ADIA
HSQCETGPROSP.2_ADIA	HSQCETGPSI	HSQCETGPSISP	HSQCETGPSISP_ADIA	HSQCETGPSISP.2_ADIA
HSQCETGPSISP2	HSQCETGPSISP2_ADIA	HSQCETGPSISP2.2	HSQCETGPSISP2.2_ADIA	HSQCETGPSIWT
HSQCETGPSP	HSQCETGPSP_ADIA	HSQCETGPSP.2_ADIA	HSQCETGPSP.3_ADIA	HSQCEXF3GPWGPH
HSQCF3GPNOWGX33D	HSQCF3GPNOWGXY	HSQCFPF3GPPHWG	HSQCGP	HSQCGPNOWGX33D
HSQCNOEF3GPSI	HSQCNOEGPSI	HSQCNOESYHSQCCCGP4D	HSQCNOESYHSQCCNGP4D	HSQCNOESYHSQCNCGP4D
HSQCNOESYHSQCNNGP4D	HSQCREXETF3GPSI3D	HSQCT1ETF3GPSI	HSQCT1ETF3GPSI3D	HSQCT1ETF3GPSI3D.2
HSQCT2ETF3GPSI	HSQCT2ETF3GPSI3D	HSQCTRETF3GPSI3D	IDIS-HSQC	MLEVHSQCETF3GP3D
MLEVHSQCETGP3D	NA_C6NOESYHSQCGP3D	NA_HSQCETF3GPXY	NA_HSQCETGPJCSP	NA_HSQCF3GPJRPHXY
NA_HSQCF3GPNOPHXY	NA_NOESYHSQCCTETGP3D	NA_NOESYHSQCF3GPWG3D	NOESYHSQCEDGPSISM3D	NOESYHSQCEDGPSM3D
NOESYHSQCETF3GP3D	NOESYHSQCETGP3D	NOESYHSQCETGPSI3D	NOESYHSQCF3GP193D	NOESYHSQCF3GPSI3D
NOESYHSQCF3GPWG3D	NOESYHSQCF3GPWGX13D	NOESYHSQCFPF3GPSI3D	NOESYHSQCGPSISMSP3D	NOESYHSQCGPSM3D.2
NOESYHSQCGPWGX13D	SELU_15NHSQCf2	SELU_HSQC	STDHSQCETGPSISP	

The "Secret Decoder Ring"

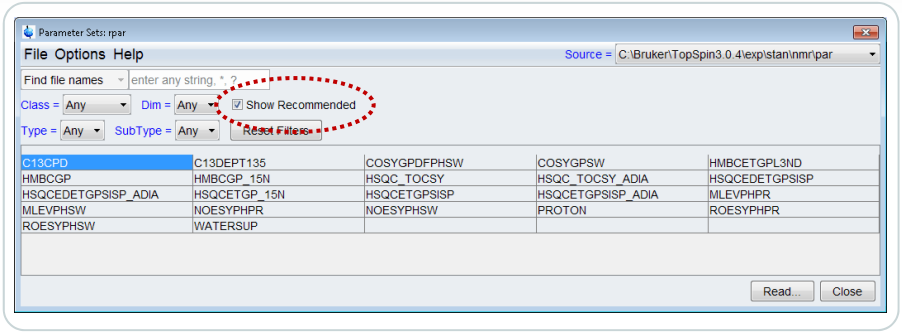
<TopSpinHome>\exp\stan\nmr\lists\pp

"Pulprog.info"

- **ea** phase sensitive using Echo/Antiecho method
- **ed** with multiplicity editing
- **et** phase sensitive using Echo/Antiecho-TPPI method
- **f3** using f3 - instead of f2 – channel
- **gp** using gradients with ":gp" syntax
- **ph** phase sensitive using States-TPPI, TPPI, States or QSEQ
- **pr** with presaturation
- **si** sensitivity improved
- **sp** using a shaped pulse
- **HSQCEDETGP = hsqc + ed + et + gp**
  - multiplicity edited HSQC using echo/ antiecho detection and gradient pulses

New in TopSpin 3.0

"Show Recommended"



- **"Recommended" parameter sets for some of the most commonly used Small Molecule Experiments**

Not Rules Written in Stone ……
Just Things to Think About

^{1}H Observe

- **PROTON**
  - zg30
  - ^{1}H acquire with 30° pulse
    - $\cos(\Theta) = e^{-(d1+aq)/T1}$
      - 30 ° pulse is a nice compromise of signal and time for most T1 values
        - The zg pulse sequence uses a 90° pulse
    - Not many options outside of D1, NS and SW/O1P
      - Keep in mind that DW=1/sw
        - » Number of points stay constant, so changing sw affects the acquisition time.
- **WATERSUPP**
  - noesygppr1d
    - Presaturation applied during D1, and d8
      - Narrower residual water peak

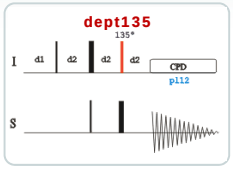
^{1}H Observe
Additional Parameter Sets for Automation

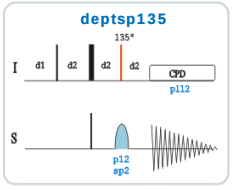
- **CMCQ_PROTON**
  - zg30
    - For quantitation purposes, so longer D1
    - AU program (cmcq_acquQuant) that does a pulse calibration on each sample
- **WATER**
  - zgcppr
    - Presaturation using composite 90 ° pulse
    - AU program (au_watersc) that does a scout scan to find the most intense signal and sets O1 there
- **LC1DWTDC**
  - wetdc
    - WET with ^{13}C decoupling during WET and AQ
    - AU program to automatically find solvent peaks and create the wet shape
      - Number of peaks to suppress defined by L30

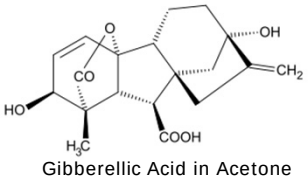
^{13}C Observe

- **C13CPD**
  - zgpg30
    - ^{13}C acquire with 30° pulse, and power gated decoupling during D1, and AQ
    - Not many options outside of D1, NS and SW/O1P
      - Keep in mind that DW=1/sw
        - » Number of points stay constant, so changing sw affects the acquisition time.
- **C13DEPT135**
  - deptsp135
    - Most common DEPT experiment showing all protonated carbons
      - Uses an adiabatic 180° pulse

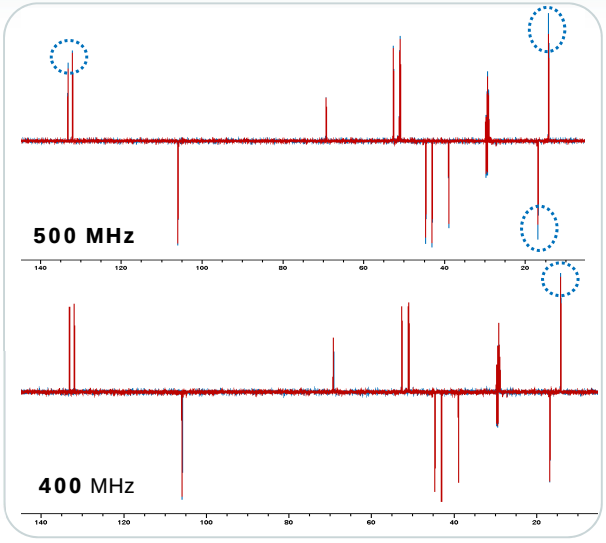
^{13}C Observe
Adiabatic Pulses





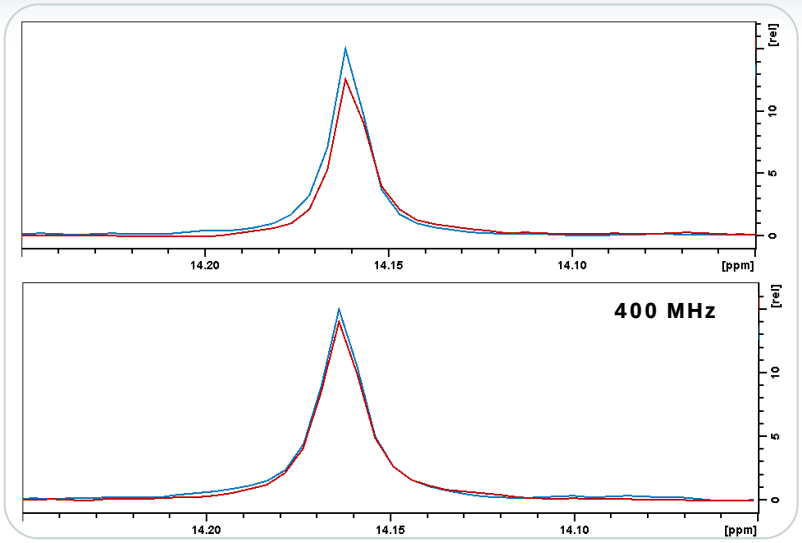


Gibberellic Acid in Acetone



^{13}C Observe
Adiabatic Pulses

dept135
deptsp135

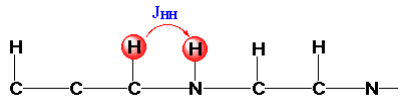


^{13}C Observe

- **C13CPD**
  - zgpg30
    - ^{13}C acquire with 30° pulse, and power gated decoupling during D1, and AQ
    - Not many options outside of D1, NS and SW/O1P
      - Keep in mind that DW=1/sw
        - » Number of points stay constant, so changing sw affects the acquisition time.
- **C13DEPT135**
  - deptsp135
    - Most common DEPT experiment showing all protonated carbons
      - Uses an adiabatic 180° pulse
- **Other Sequences**
  - zgig30
    - Sequence with inverse gated decoupling, so only during acquisition
  - dept45sp
  - dept90sp

^{1}H-^{1}H Homonuclear 2D Experiments

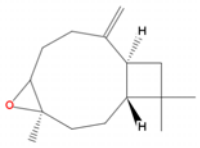
Through Bond



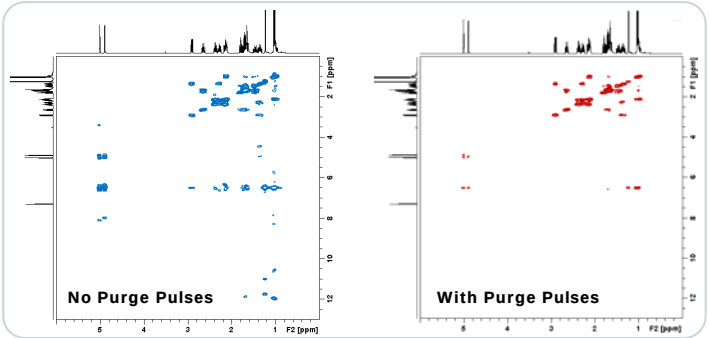
COSY

^{1}H-^{1}H Homonuclear 2D Experiments
COSY

- **COSYGPSW**
  - cosygpppqf -- Magnitude mode COSY (qf) with gradients (gp) and purge pulses (pp)
    - + Gradient selected, so ns ≥ 1
    - + Purge pulse to reduce artifacts from not waiting long enough for D1
      - » D1=0.1sec, AQ=0.8

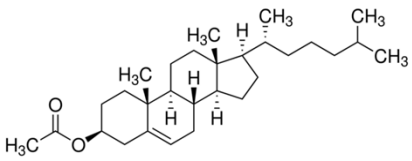


Caryophyllene Oxide in DMSO

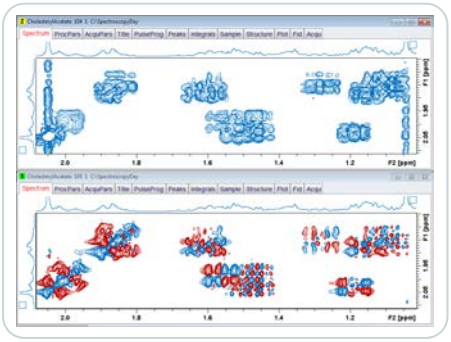


^{1}H-^{1}H Homonuclear 2D Experiments
COSY

- **COSYGPDFPHSW**
  - cosygpmfphpp -- COSY with gradient pulses (gp), multiple quantum filter (mf), phase sensitive (ph), and purge pulses (pp)
    - \+ Double quantum filter simplifies the diagonal
    - \+ Phase sensitive information (active/passive coupling)



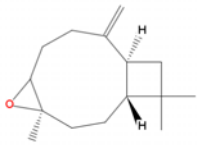
Cholesterol Acetate in $CDCl_3$



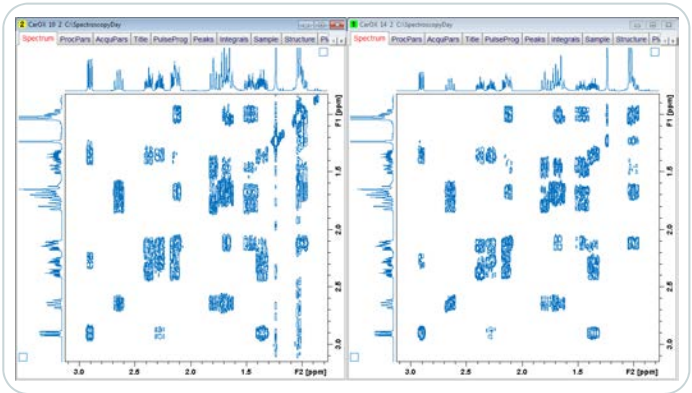
- − Difficult for a beginner to phase

^{1}H-^{1}H Homonuclear 2D Experiments
Another COSY Option

- cosygpmfppqf -- Magnitude mode (qf) COSY, with gradients (gp), multiple quantum filter (mf), and purge pulses (pp)
  - \+ Double quantum filter to simplify the diagonal
  - \+ Still magnitude mode so no phase necessary

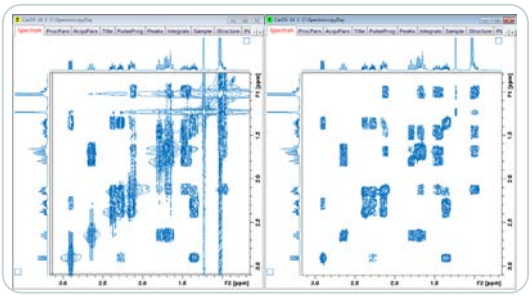


Caryophyllene Oxide in DMSO



^{1}H-^{1}H Homonuclear 2D Experiments
Another COSY Option

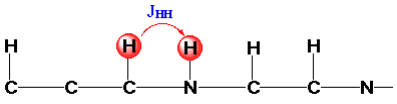
+ Double quantum filter to simplify the diagonal – Especially if the window function is adjusted to bring out more signal (ssb = 4)



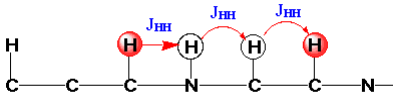
- **CMCse_COSY**
  - cosygpmfppqf
    - » Because the parameter set was designed for CMCse, there is more resolution (512 increments) than other parameter sets
      - – Longer experiment
      - – Brings out peaks that are weakly coupled

^{1}H-^{1}H Homonuclear 2D Experiments

Through Bond



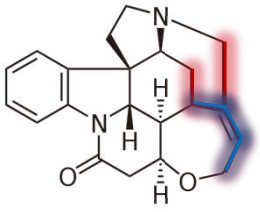
COSY



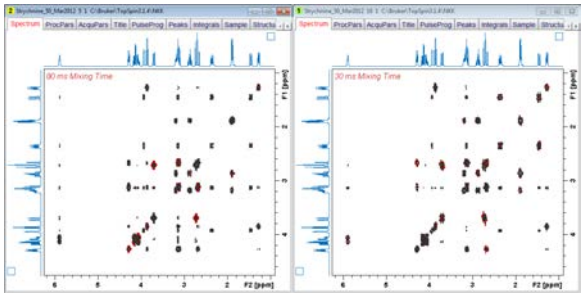
TOCSY

^{1}H-^{1}H Homonuclear 2D Experiments
TOCSY

- **MLEVPHSW**
  - mlevphpp -- Homonuclear Hartman-Hahn using MLEV17 sequence, phase sensitive (ph), and purge pulses (pp)
- **MLEVPHPR**
  - mlevphpr.2 -- Homonuclear Hartman-Hahn using MLEV17 sequence, phase sensitive (ph), and presat (pr),
    - » TOCSY Mixing Time is defined by d9
      - – Default is 0.08 seconds

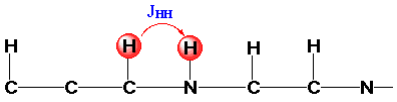


Strychnine in $CDCl_3$

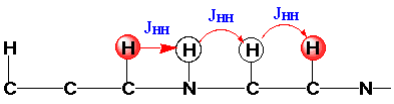


^{1}H-^{1}H Homonuclear 2D Experiments

Through Bond

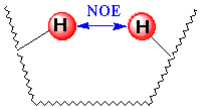


COSY



TOCSY

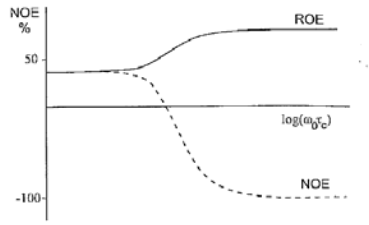
Through Space



NOESY
ROESY

^{1}H-^{1}H Homonuclear 2D Experiments
NOESY / ROESY

- **NOESYPHSW**
  - noesygpphpp -- NOESY with gradient pulses during mixing time, phase sensitive (ph), and purge pulses (pp)
    - » Mixing time is defined by d8
      - – Default is 0.3 seconds
- **ROESYPHSW**
  - roesyphpp.2 -- ROESY sequence, phase sensitive (ph), and purge pulses (pp), using 180x-180x pulses for spin lock to suppress TOCSY artifacts (.2)
    - » Mixing time is defined by p15
      - – Default is 200 milliseconds



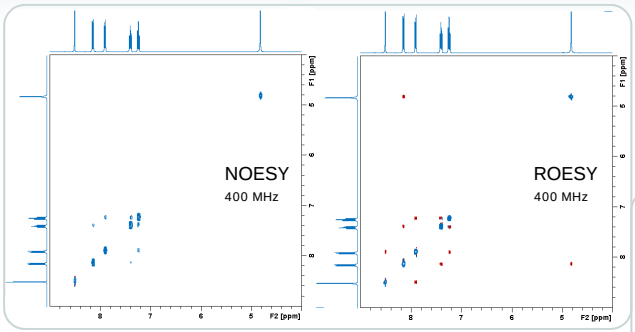
High-Resolution NMR Techniques in Organic Chemistry
Timothy D.W. Claridge 1999

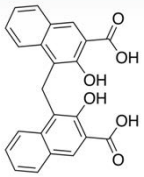
Zero Crossing Depends on:

- Magnetic Field
- Size of Molecule
- Temperature
- Viscosity

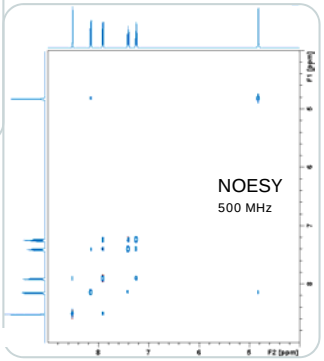
Around 1,000 – 2,000 Daltons

^{1}H-^{1}H Homonuclear 2D Experiments
NOESY / ROESY



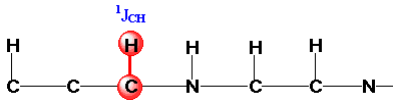


Pamoic Acid
MW=388
DMSO at 292 K



Small Molecule	Large Molecule	Exchange Peak
– NOESY – ROESY	+ NOESY – ROESY	+ NOESY + ROESY

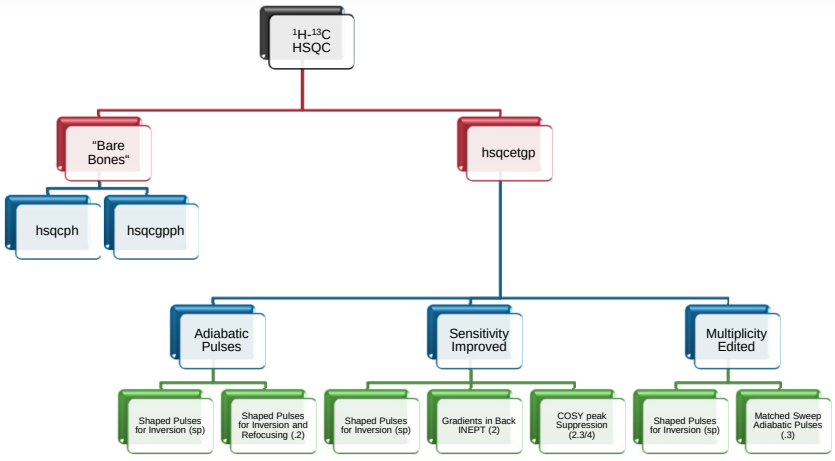
^{1}H-^{13}C Heteronuclear 2D Experiments



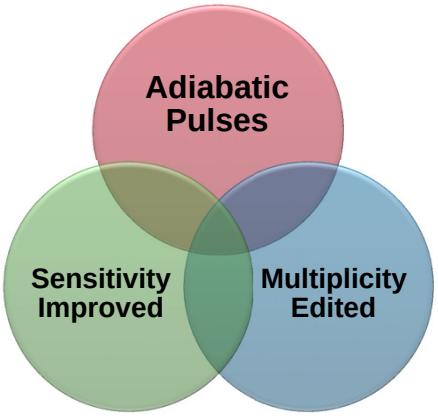
Single Bond

HSQC

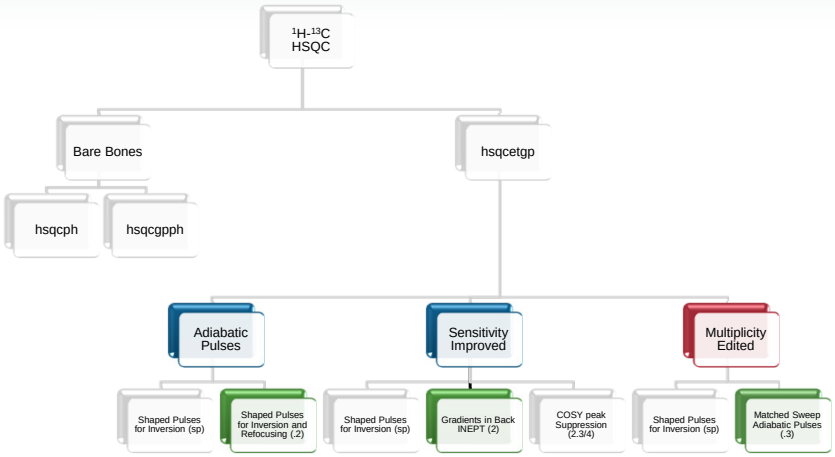
^{1}H-^{13}C Heteronuclear 2D Experiments
HSQC



^{1}H-^{13}C Heteronuclear 2D Experiments
HSQC

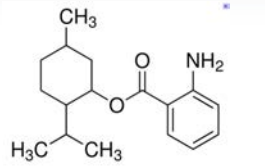


^{1}H-^{13}C HSQC – Things to Consider
HSQCEDETGPSISP_ADIA and HSQCETGPSISP_ADIA

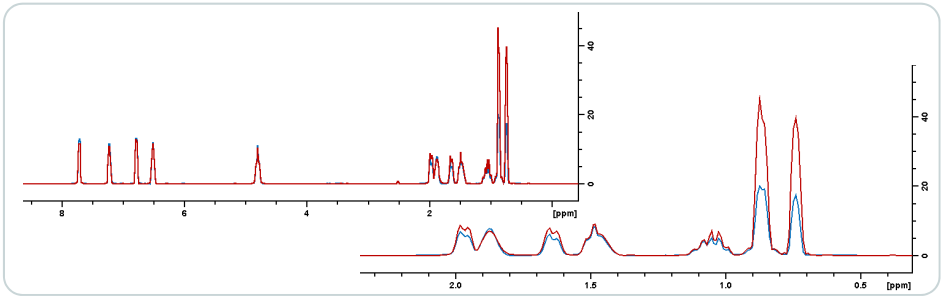


^{1}H-^{13}C HSQC – Things to Consider
Multiplicity Edited or Not?

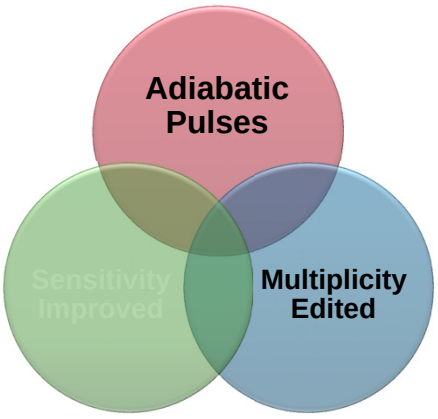
- **HSQCETGP**
  - hsqcetgp
    - Simple Gradient HSQC – non Edited
- **HSQCEDETGP**
  - hsqcedetgp
    - Simple Multiplicity Edited Gradient HSQC



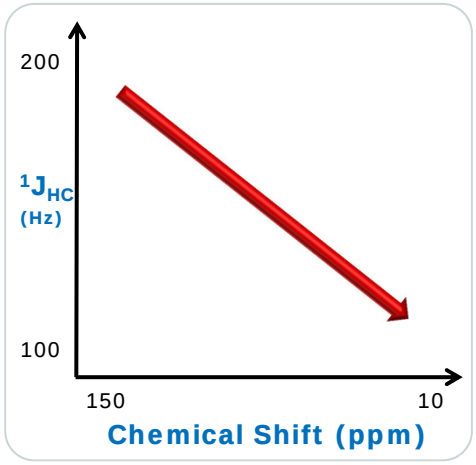
Menthyl Anthranilate in DMSO



^{1}H-^{13}C Heteronuclear 2D Experiments
HSQC



"Matched Sweep" Adiabatic Pulses
Removing the J Dependence



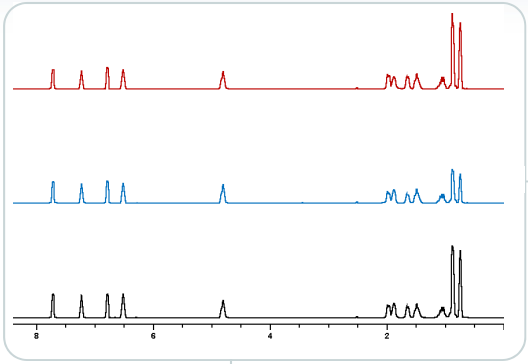
d21 = 1 / 2J_{xh}

If J= 180 hz → 2.7ms
If J=100 hz → 5 ms

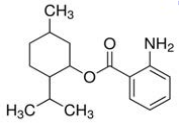
The Matched Sweep Adiabatic Pulse

Sweeps through the ^{13}C frequency range so that it inverts signals closer to when the time matches the 1/2J condition

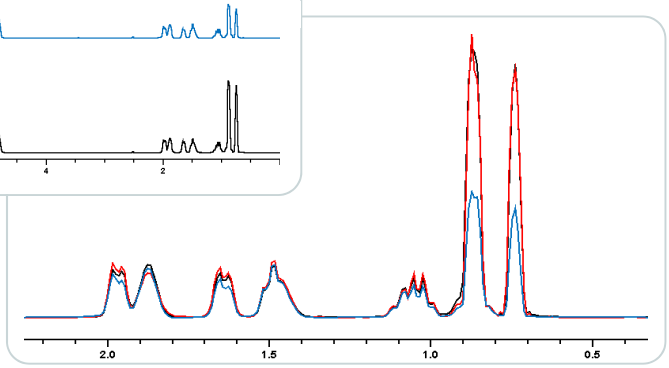
^1H-^{13}C HSQC – Things to Consider
Multiplicity Edited or Not?



- **hsqcetgp**
- **hsqcedetgp**
- **hsqcedetgpsp.3**



Menthyl Anthranilate in DMSO

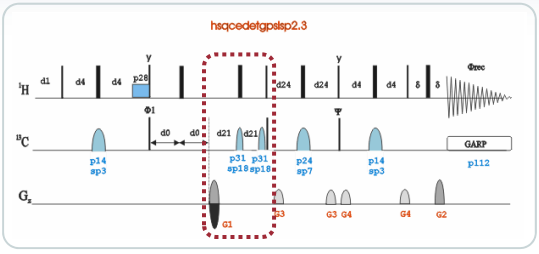


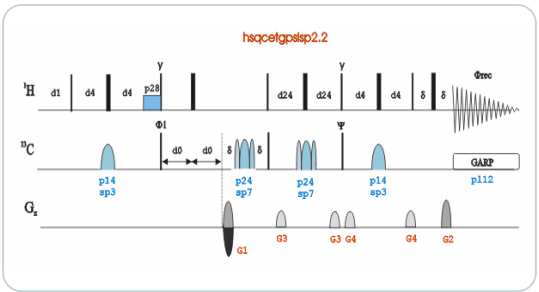
^{1}H-^{13}C HSQC – Things to Consider
Multiplicity Edited or Not?

- **HSQCEDETGPSISP_ADIA**
  - hsqcedetgpsisp2.3 w/ bi_p5m4sp_4sp.2 decoupling
    - Multiplicity Edited (ed)
      - + You get the DEPT type information in addition to the ^{1}H-^{13}C connectivity
    - Adiabatic Pulses (sp) – Including a Matched Sweep Adiabatic (.3)
      - + No significant loss in sensitivity
    - Sensitivity Improved (si)

- **HSQCETGPSISP_ADIA**
  - hsqcetgpsisp2.2 w/ bi_p5m4sp_4sp.2 decoupling
    - **Not** Multiplicity Edited
      - + Simple, all peaks are Positive
    - Adiabatic Pulses (sp) – for both Inversion and Recovery (.2)
    - Sensitivity Improved (si)

^{1}H-^{13}C HSQC – Things to Consider
Multiplicity Edited or Not?





d21= $1/2J_{xh}$ = 3.6 ms
vs.
δ = gradient recovery delay = .2ms

~ 7 ms longer of a sequence

Depending on the T_2 relaxation rates of the molecule the non-edited version might be more sensitive:

But is it worth sacrificing the multiplicity information?

^{1}H-^{13}C HSQC – Things to Consider

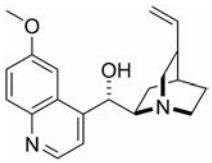
Multiplicity Edited or Not?

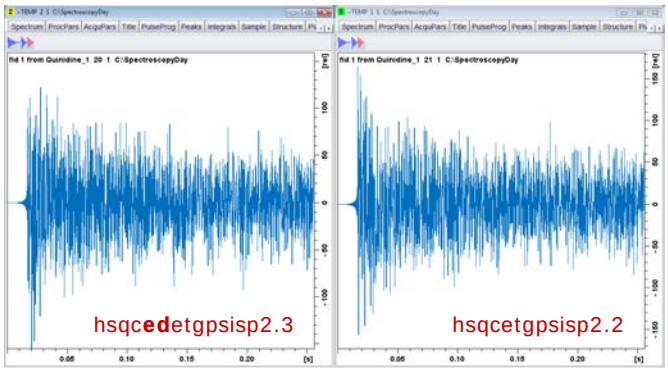
Multiplicity Editing:

~ **7 ms** longer of a sequence

1 mg/ml Quinidine

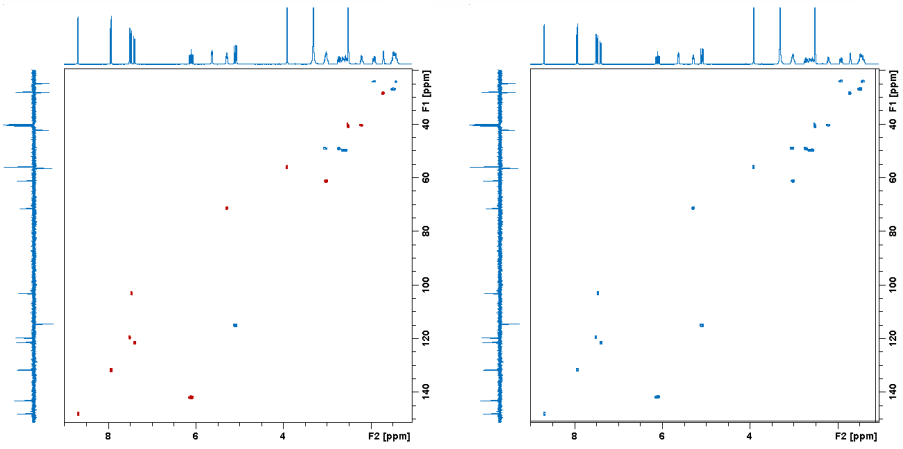
1st fid from an HSQC





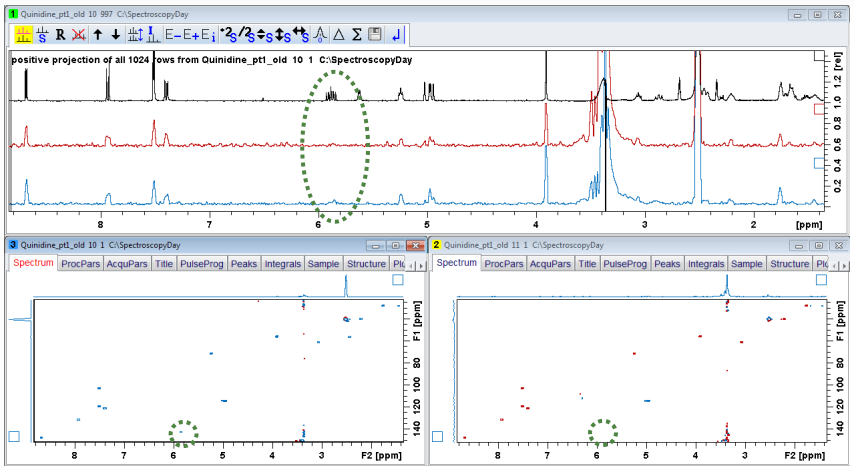
^{1}H-^{13}C HSQC – Things to Consider

Multiplicity Edited or Not?



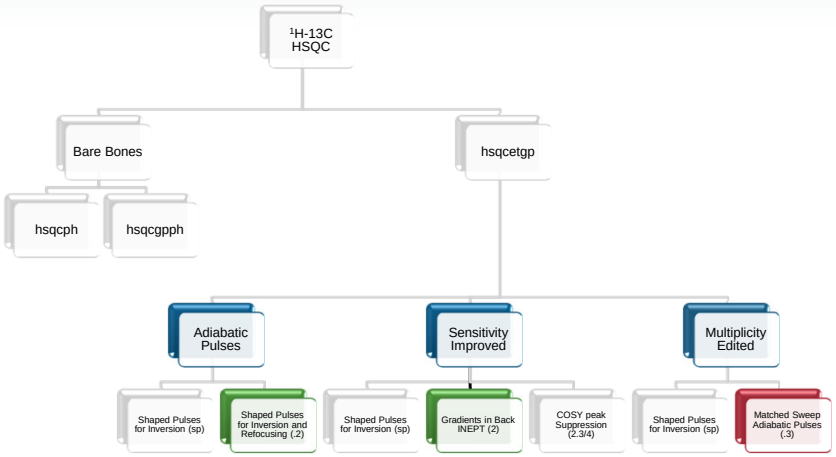
1 mg/ml Quinidine, 1 hour 20 Min each HSQC w/ 9 hour DEPT as projection

^{1}H-^{13}C HSQC – Things to Consider
Multiplicity Edited or Not?



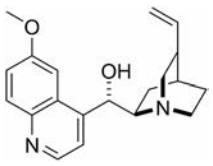
0.1 mg/ml Quinidine, 10 hour each HSQC spectra w/ no DEPT

^{1}H-^{13}C HSQC – Things to Consider
Matched Sweep Adiabatic Pulse?

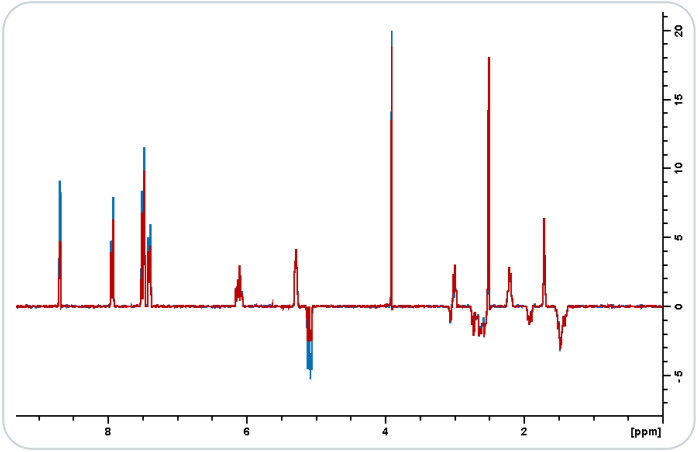


^{1}H-^{13}C HSQC – Things to Consider
Benefit of Matched Sweep

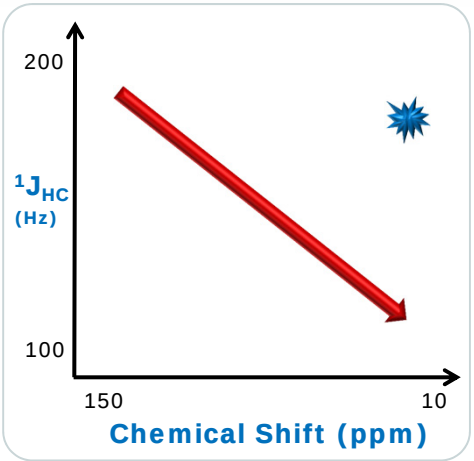
hsqcedetgpsisp2.2 **hsqcedetgpsisp2.3**



Quinidine in DMSO



^{1}H-^{13}C HSQC – Things to Consider
Benefit of Matched Sweep



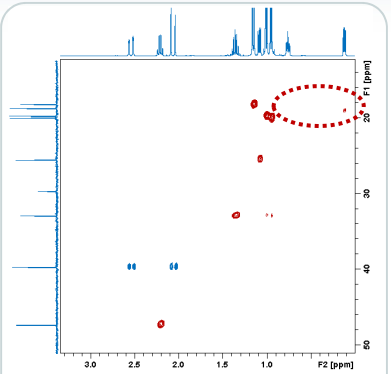
d21= $1/2J_{xh}$

If J= 180 hz → 2.7ms
If J=100 hz → 5 ms

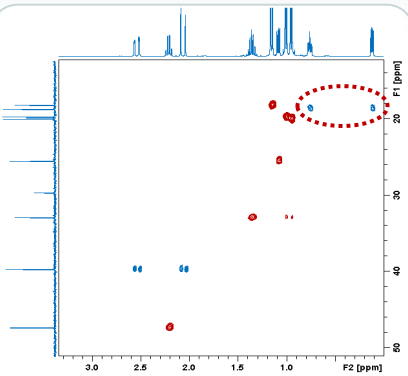
The Matched Sweep Adiabatic Pulse

Sweeps through the 13C frequency range so that it inverts signals closer to when the time matches the 1/2J condition

^{1}H-^{13}C HSQC – Things to Consider
Matched Sweep Adiabatic Pulse?

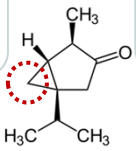


hsqcedetgpsisp2.3
Matched Sweep Pulse



hsqcedetgpsisp2.2
Non Matched Sweep Pulse

J_{hc} = 158 Hz



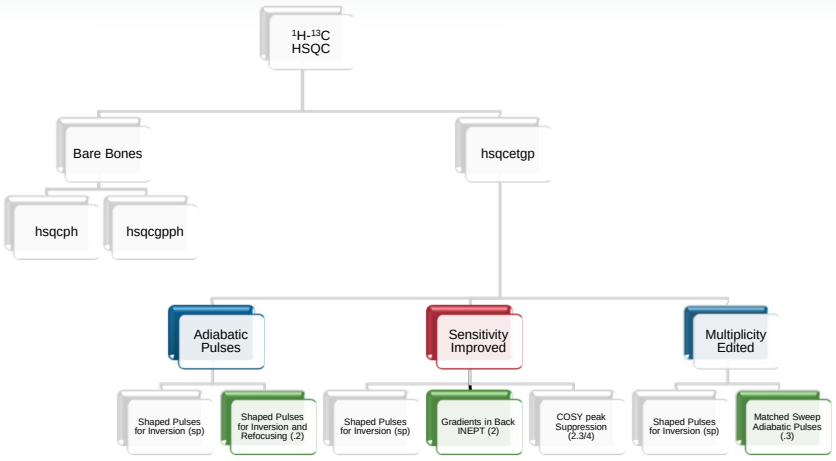
α-Thujone in DMSO

^{1}H-^{13}C HSQC – Things to Consider
Matched Sweep Adiabatic Pulse?

- **hsqcedetgpsisp2.3**
  - Multiplicity Edited
  - Matched Sweep Adiabatic Pulse
    - \+ Works well when J scales with Chemical Shift
    - − Problematic when J differs
- **hsqcedetgpsisp2.2**
  - Multiplicity Edited
  - Regular Adiabatic Pulses
    - \+ Less Sensitive to deviations in J
    - − No benefit from the matched sweep for “normal” resonances

^{1}H-^{13}C HSQC – Things to Consider

Sensitivity Improved or Not?



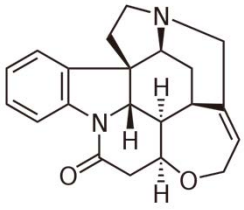
^{1}H-^{13}C HSQC – Things to Consider

Sensitivity Improved or Not?

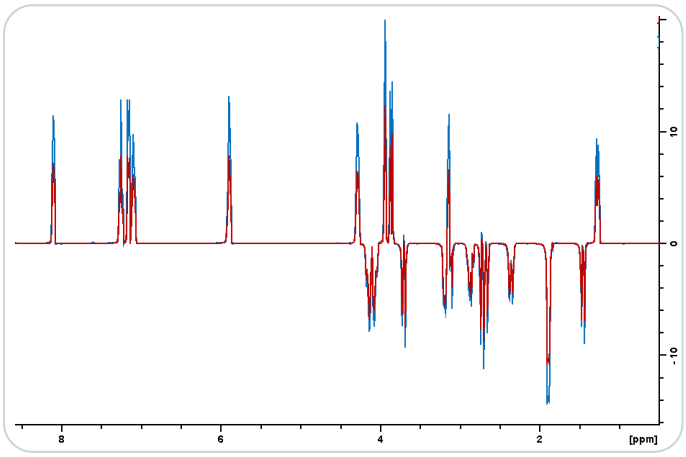
- **HSQCEDETGPSISP_ADIA**
  - hsqcedetgpsisp2.3
- **HSQCETGPSISP_ADIA**
  - hsqcetgpsisp2.2
    - **S**ensitivity **I**mproved Element
      - \+ Possible sensitivity improvement of ~ √2
- **HSQCEDETGPSP.3_ADIA**
  - hsqcedetgpsp.3
    - Multiplicity edited with Matched Sweep Adiabatic
- **HSQCETGPSP.2_ADIA**
  - hsqcetgpsp.2
    - Non Multiplicity Edited
    - **No** Sensitivity Improved Element
      - − In general, less sensitive than the SI version

^{1}H-^{13}C HSQC – Things to Consider
Sensitivity Improved or Not?

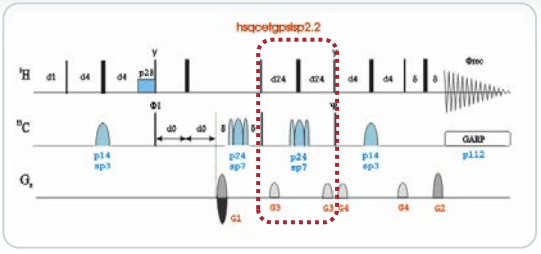
hsqcedetgpsp.3 hsqcedetgp**si**sp2.3



Strychnine in $CDCl_3$

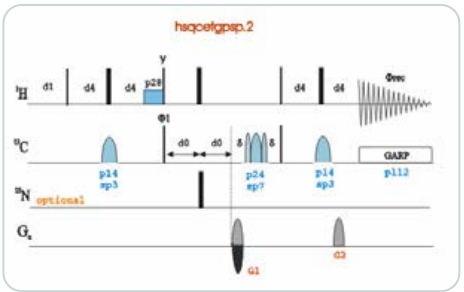


^{1}H-^{13}C HSQC – Things to Consider
Sensitivity Improved or Not?



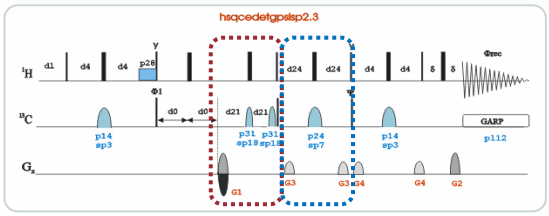
d24= $1/8J_{xh}$ = 0.89m

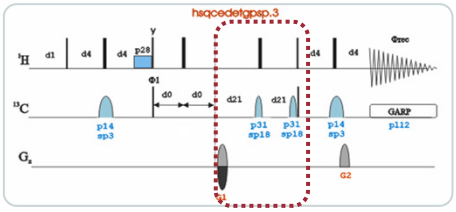
~ 2ms longer of a sequence



Depending on the T_2 relaxation rates of the molecule of interest, the non-si version might be actually be more sensitive

^{1}H-^{13}C HSQC – Things to Consider
Sensitivity Improved or not?

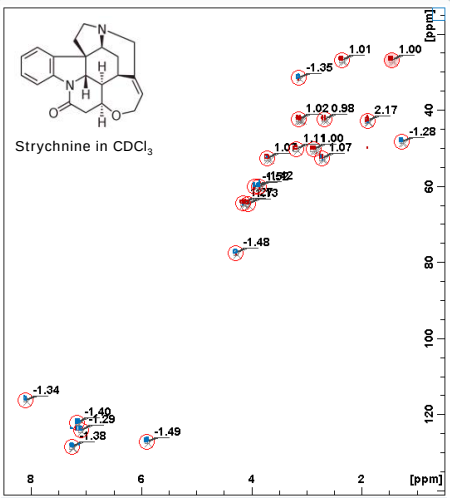




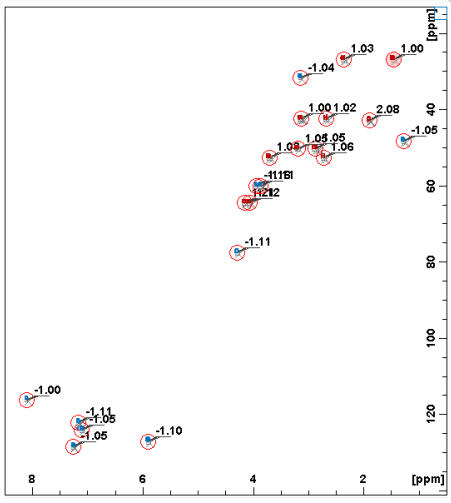
Matched Sweep Adiabatic Pulses can be use (p31,sp10) to compensate for J_{xh} in d21.

But no compensation available for J_{xh} in d24

^{1}H-^{13}C HSQC – Things to Consider
Sensitivity Improved or not?



hsqcedetgpsisp2.3

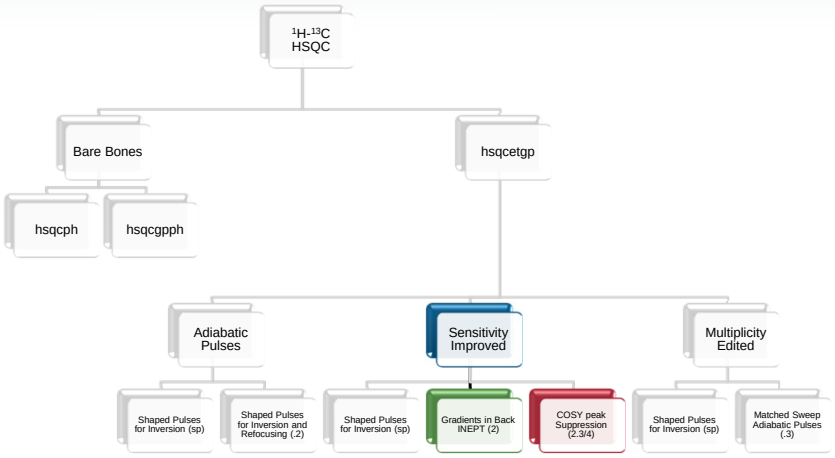


hsqcedetgpsp.3

^{1}H-^{13}C HSQC – Things to Consider
Sensitivity Improved or Not?

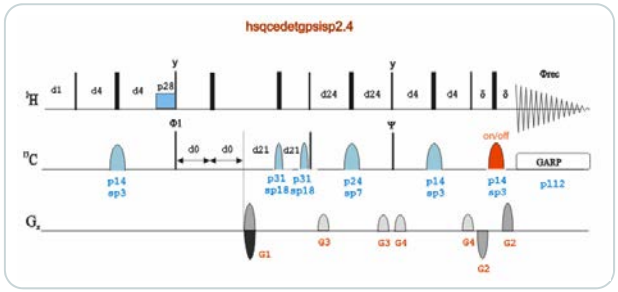
- **HSQCEDETGPSISP_ADIA**
  - hsqcedetgpsisp2.3
    - Multiplicity Edited
    - **"Sensitivity Improved" INEPT element**
    - Matched Sweep Adiabatic Pulses
      - \+ More Sensitive
      - − Non quantitative
- **HSQCEDETGPSP.3_ADIA or CMCse_HSQC**
  - hsqcedetgpsp.3
    - Multiplicity Edited
    - **Without "Sensitivity Improved" INEPT element**
    - Matched Sweep Adiabatic Pulses
      - − Less Sensitive
      - \+ Quantitative integrals
        - Used in CMCse

^{1}H-^{13}C HSQC – Things to Consider
COSY Peak Suppression



^{1}H-^{13}C HSQC – Things to Consider
COSY Peak Suppression

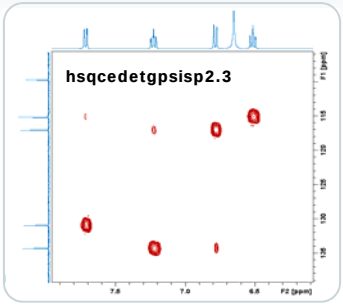
- **hsqcedetgpsisp2.4**
  - Sensitivity Improved
  - Multiplicity Edited
    - Matched Sweep
  - COSY Suppression
- **hsqcetgpsisp2.3**
  - Sensitivity Improved
  - Non Multiplicity Edited
  - COSY Suppression

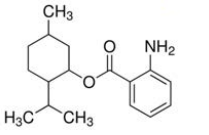


\+ Removes the COSY artifacts that arise when using the “si” versions

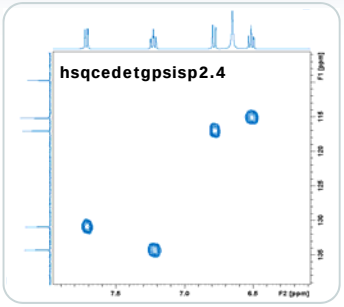
− Less sensitive than regular “si” versions

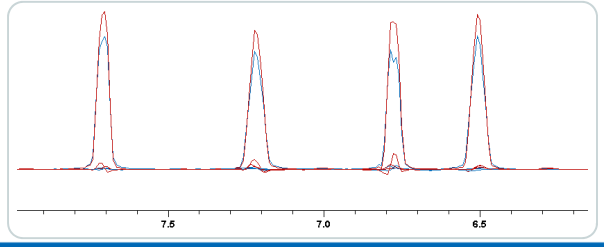
^{1}H-^{13}C HSQC – Things to Consider
COSY Peak Suppression



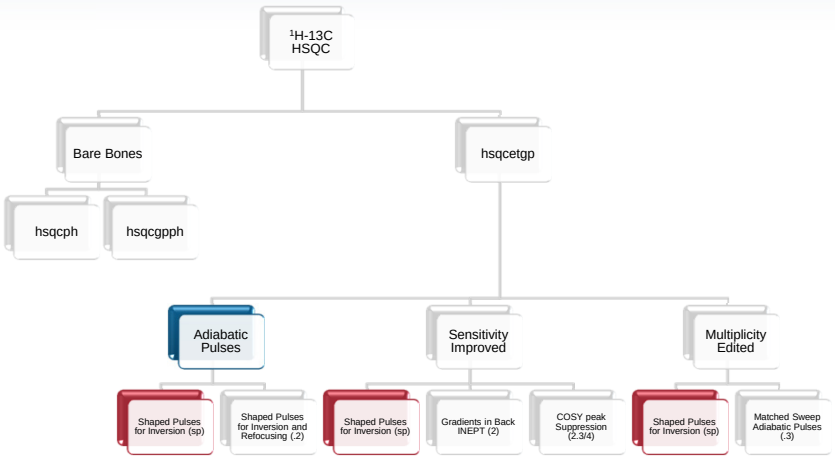


Menthyl Anthranilate in DMSO

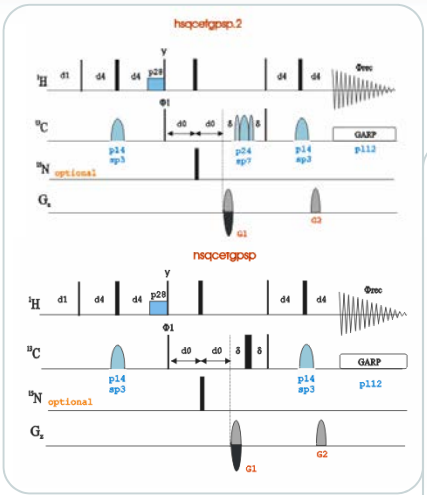




^{1}H-^{13}C HSQC – Things to Consider
Long Refocusing Pulse

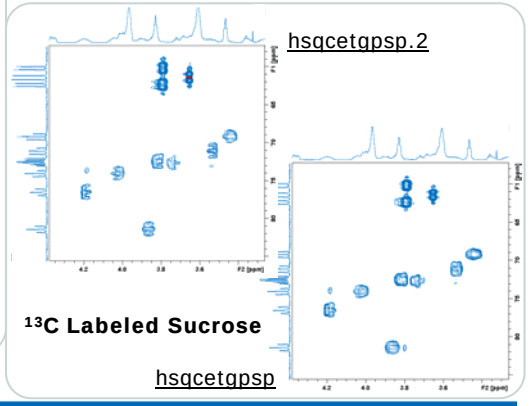


^{1}H-^{13}C HSQC – Things to Consider
Long Refocusing Pulse



Adiabatic pulses:
Inversion (p14) = .5 ms
Refocusing (p24) = 2 ms

Hard 180 Pulse:
16 us

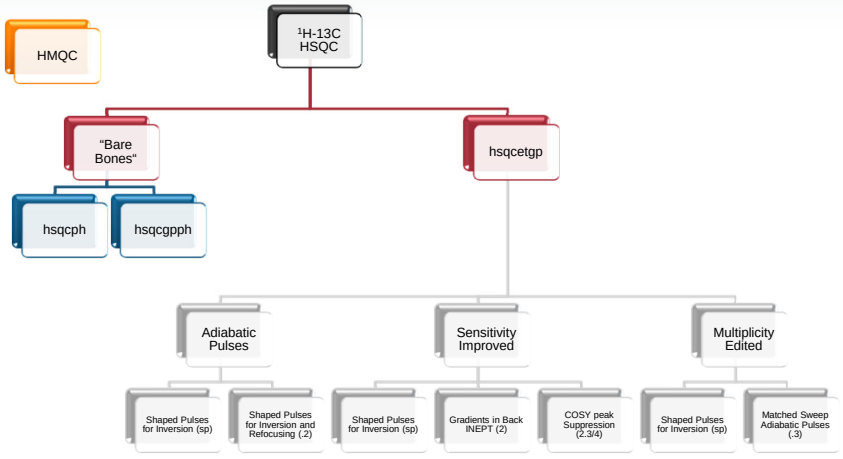


hsqcetgpsp.2

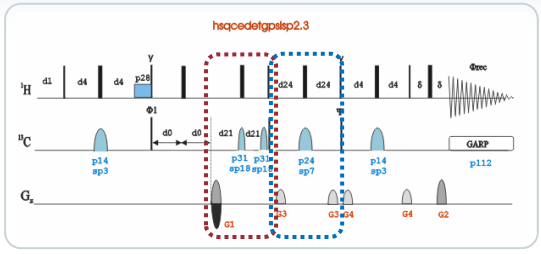
^{13}C Labeled Sucrose

hsqcetgpsp

^{1}H-^{13}C HSQC – Things to Consider
When is Simple Better?



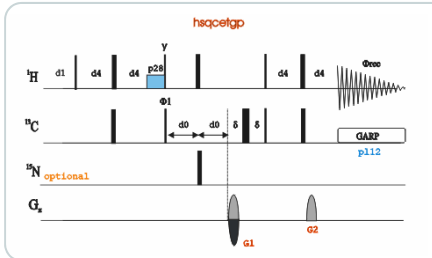
HSQC – Things to Consider
When Is Simple Better?

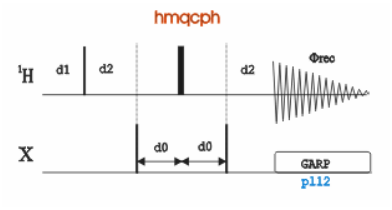


$d21 = 1/2J_{xh} = 3.6$ ms
$d24 = 1/8J_{xh} = 0.89$ ms

Adiabatic pulses:
Inversion = .5 ms
Refocusing = 2 ms

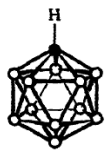
Hard 180 Pulse:
16 us





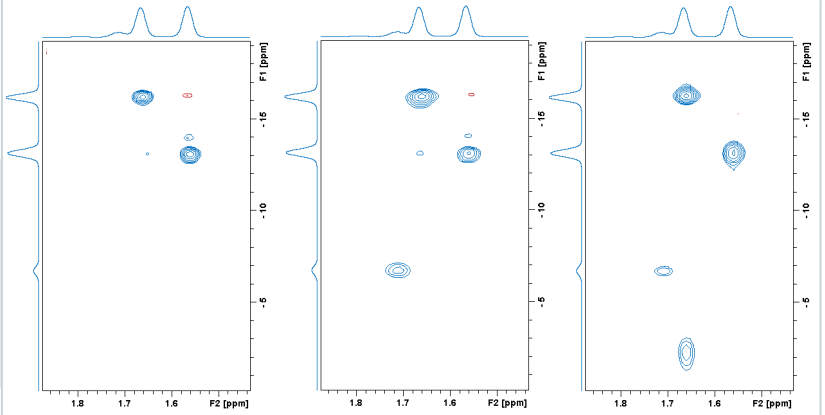
HSQC – Things to Consider
When Is Simple Better?

^{1}H - ^{11}B Spectra



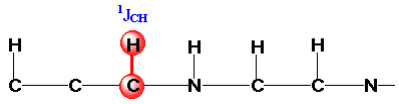
$CB_{11}H_{12}$

● CH
O BH



hsqcetgpsi2 hsqcetgp hmqcph

^{1}H-^{13}C Heteronuclear 2D Experiments

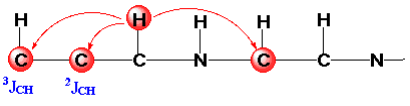


Single Bond

HSQC / HMQC

Multiple Bond

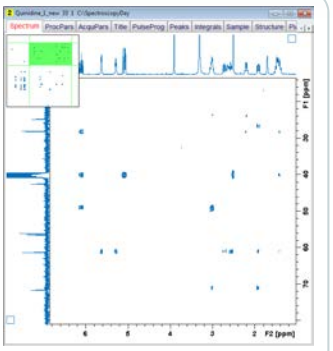
HMBC



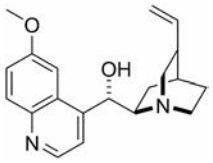
^{1}H-^{13}C Heteronuclear 2D Experiments
HMBC

- **HMBCGP**
  - hmbcgplpndqf
    - Gradients for coherence selection (gp)
    - Low pass filter (lp)
    - No decoupling during acquisition (nd)
    - Magnitude Mode (qf)
      - \+ Simple
      - \+ No 180° pulses
- **HMBCETGPL3ND**
  - hmbcetgpl3nd
    - Echo Anti Echo (et)
    - Gradients for coherence selection (gp)
    - 3rd order Low Pass filter (l3)
      - \+ Better suppression of ^{1}J correlation peaks
      - \+ More sensitive because of Echo Anti Echo Detection
      - − More difficult to process (xfb + xf2m)

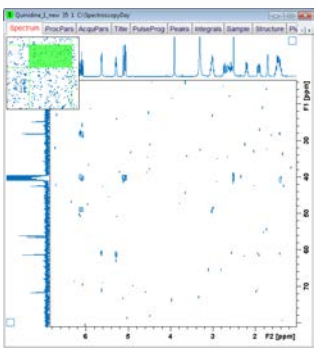
^{1}H-^{13}C Heteronuclear 2D Experiments
HMBC - Sensitivity



HMBCETGPL3ND



Quinidine in DMSO
1 mg/ ml

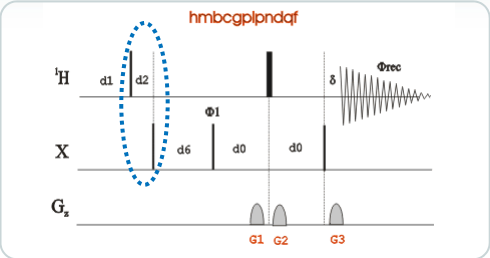


HMBCGP

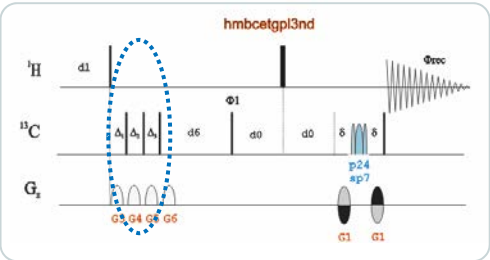
32 Scans, 256 Increments = 6 hours each

^{1}H-^{13}C Heteronuclear 2D Experiments
HMBC – Low Pass Filter

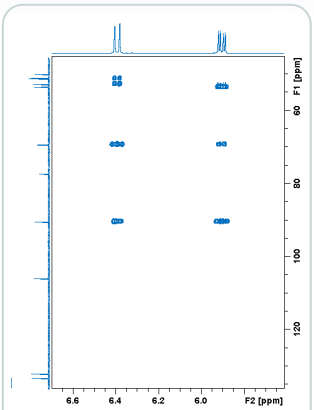
- hmbcgplpndqf
  - d2=1/2J_{xh}



- hmbcetgpl3nd
  - Δ1=1/(2($J_{xh\text{-}min}$ + .07($J_{xh\text{-}max}$- $J_{xh\text{-}min}$))
  - Δ2=1/($J_{xh\text{-}min}$ + $J_{xh\text{-}max}$)
  - Δ3=1/(2($J_{xh\text{-}max}$ - .07($J_{xh\text{-}max}$- $J_{xh\text{-}min}$))

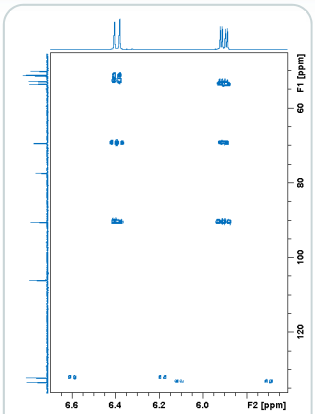


^{1}H-^{13}C Heteronuclear 2D Experiments
HMBC – Suppression of ^{1}J correlations



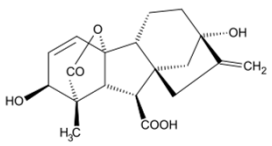
HMBCETGPL3ND

$^{1}J_{xh\ (max)}$ = 170 Hz
$^{1}J_{xh\ (min)}$ = 120 Hz



HMBCGP

$^{1}J_{xh}$ = 145 Hz

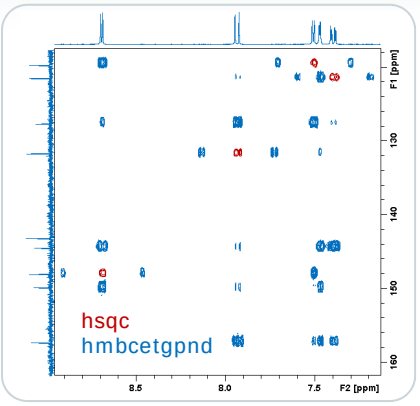


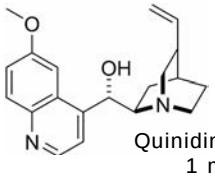
Gibberellic Acid
in Acetone

Long Range J_{xh}
8 Hz

^{1}H-^{13}C Heteronuclear 2D Experiments
Another HMBC option

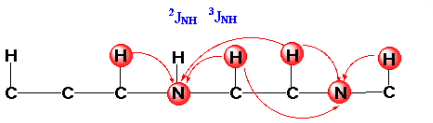
- **hmbcetgpnd**
  - Gradients for coherence selection
  - Echo Anti Echo
    - \+ Similar sensitivity to hmbcetgpl3nd
  - No Low Pass filter
    - \+ ^{1}J correlations are often useful when interpreting the data instead of the HSQC





Quinidine in DMSO
1 mg/ ml

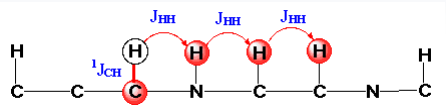
Heteronuclear 2D Experiments
Not Just ^{13}C – ^{1}H / ^{15}N also



- **HMBCGP_15N**
  - hmbcgpndqf
    - ^{15}N is routed through f2
    - Gradients for coherence selection
      - \+ Ratio set to select for ^{1}H/^{15}N instead of ^{1}H/^{13}C
      - » Other nuclei are possible with the AU program “gradratio”

- **HSQCETGP_15N**
  - hsqcetgpsi2
    - ^{15}N is routed through f2
    - Echo-anti echo
    - Sensitivity improved
      - Gradients in the back inept
    - Gradients for coherence selection
      - \+ Ratio set to select for ^{1}H/^{15}N

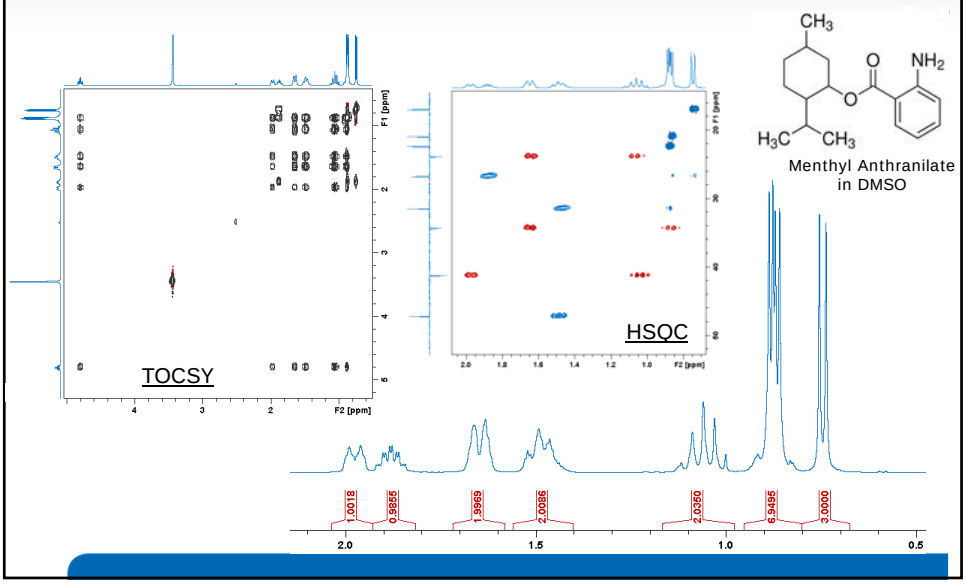
^{1}H-^{13}C Heteronuclear 2D Experiments
HSQC_TOCSY_ADIA



- **HSQC_TOCSY_ADIA**
  - hsqcdietgpsisp.2
    - DIPSI2 for Hartman-Hahn Mixing
    - Using adiabatic pulses
    - Sensitivity Improved
    - All Peaks Positive

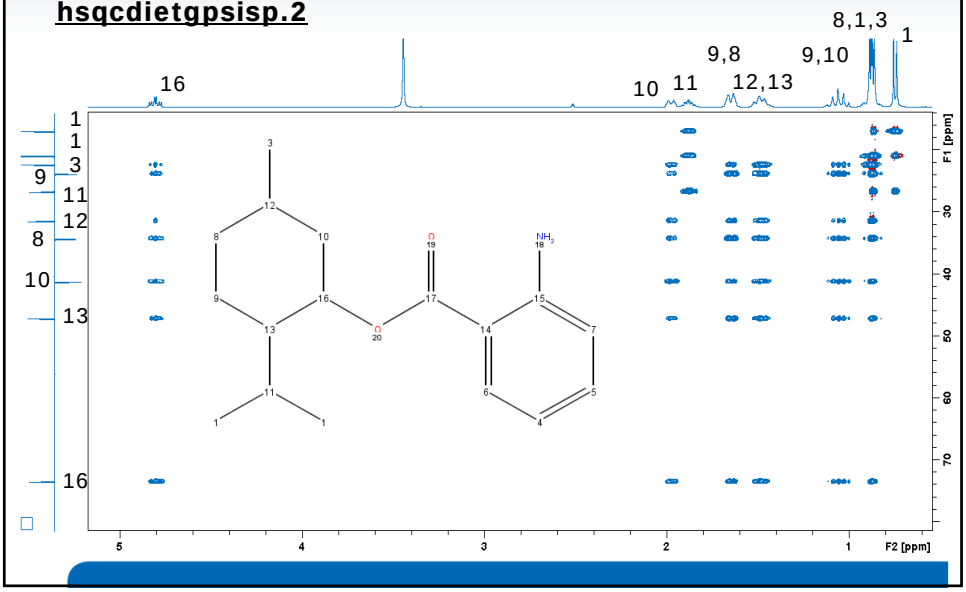
- **Other Options**
  - hsqcdiedetgpsisp.2
    - Inversion of directly coupled protons
      - "HSQC" are **+**
      - "TOCSY" are **-**
  - hsqcdiedetgpsisp.3
    - Fully Edited
      - "HSQC" → CH/CH_3 + & CH_2 –
      - "TOCSY" → CH/CH_3 – & CH_2 +

^{1}H-^{13}C Heteronuclear 2D Experiments
HSQC_TOCSY_ADIA



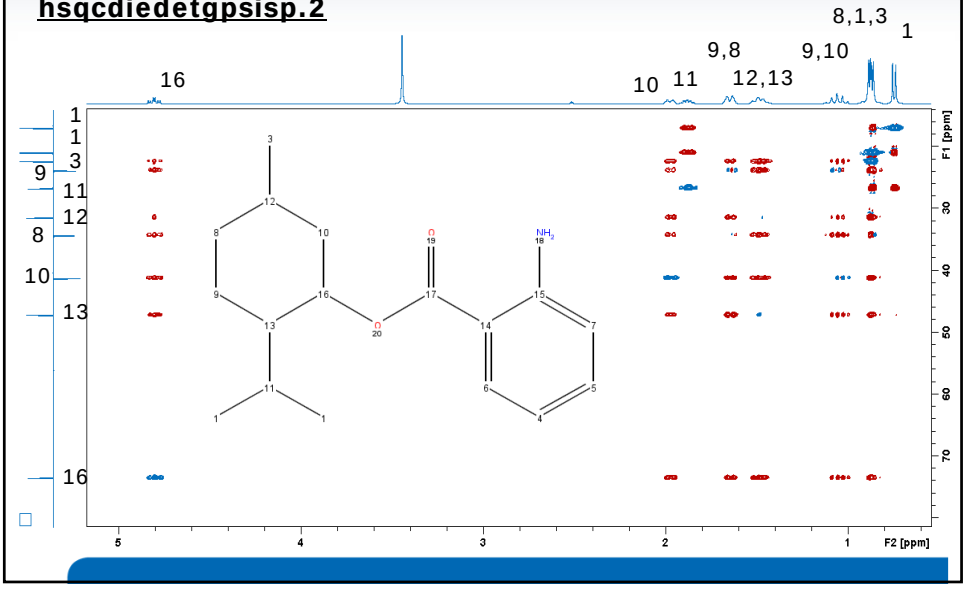
^{1}H-^{13}C Heteronuclear 2D Experiments
HSQC_TOCSY_ADIA

hsqcdietgpsisp.2

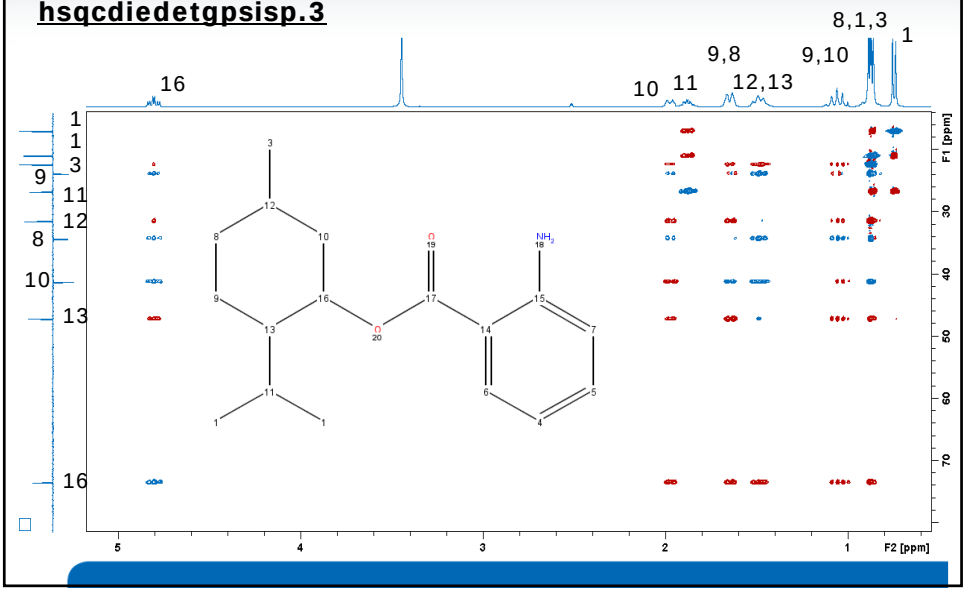


^{1}H-^{13}C Heteronuclear 2D Experiments
HSQC_TOCSY_ADIA

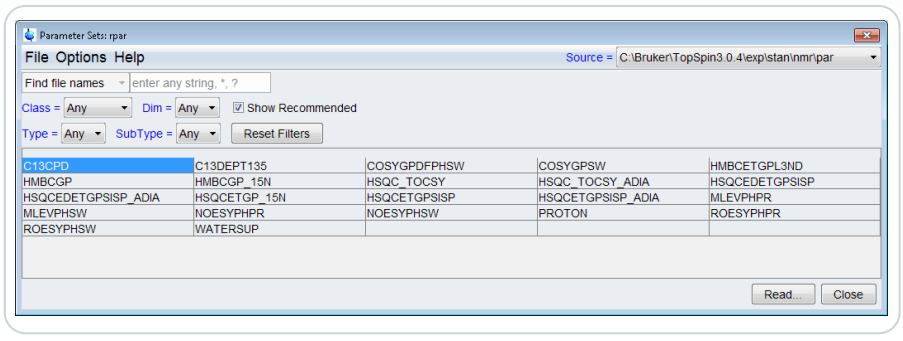
hsqcdiedetgpsisp.2



^{1}H-^{13}C Heteronuclear 2D Experiments
HSQC_TOCSY_ADIA

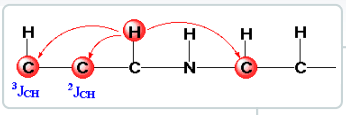


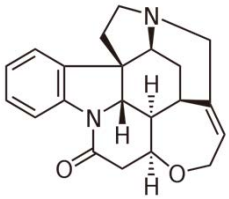
New in TopSpin 3.0
"Show Recommended"



But There's More If These Don't Answer Your Question

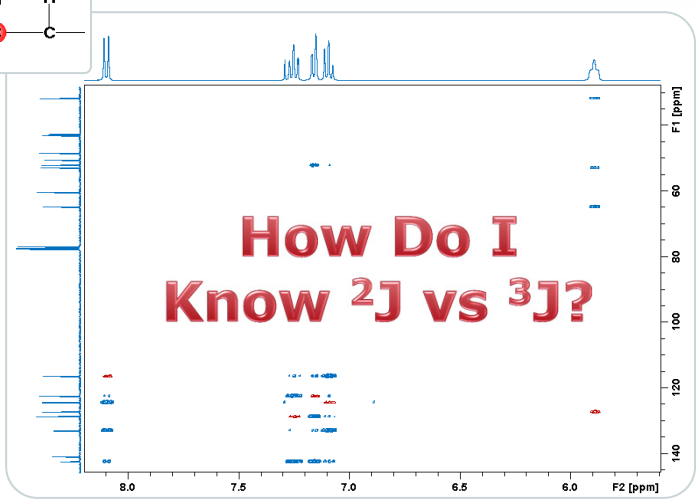
^{1}H-^{13}C Heteronuclear 2D Experiments
HMBC



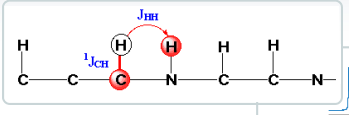


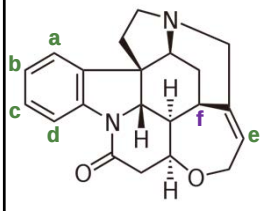
Strychnine in $CDCl_3$

HSQC
HMBC



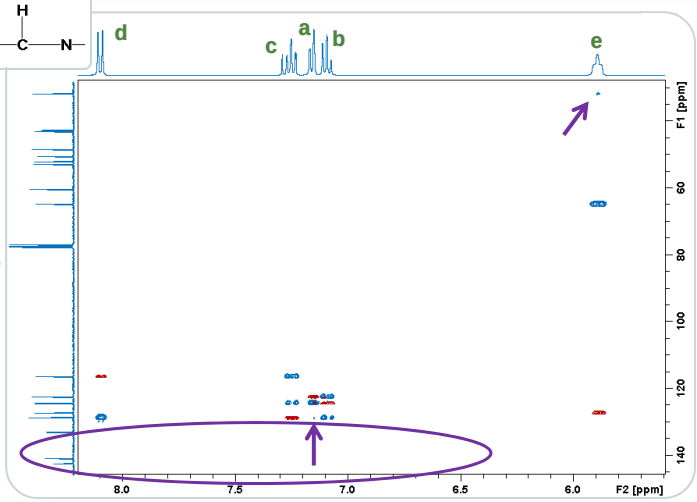
H2BC (AKA HMQC-COSY)
Heteronuclear 2 Bond Correlation





Strychnine in $CDCl_3$

HSQC
h2bcetgpl3



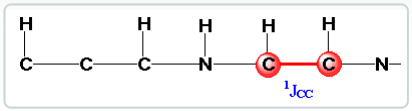
H2BC
Experimental Details

+ **Advantages of the H2BC**:
  - It helps solve the problem of distinguishing two- and three-bond correlations in HMBC or HSQC-TOCSY
  - Is independent of occasionally vanishing $^{2}J_{CH}$ coupling constants, which alleviates the problem of missing two-bond correlations in HMBC spectra

− **Disadvantages of the H2BC**:
  - Only protonated carbons are observed (no 4*)
  - Relies on $^{3}J_{HH}$ to get "2 Bond" correlations
    - $^{4}J_{HH}$ Couplings are not uncommon, and if large enough (>1Hz) will also be observed

- No Parameter Set in TopSpin
  - Contact the Applab, we do have one
- Pulse Sequence → h2bcetgpl3
- Processing → xfb + xf2m

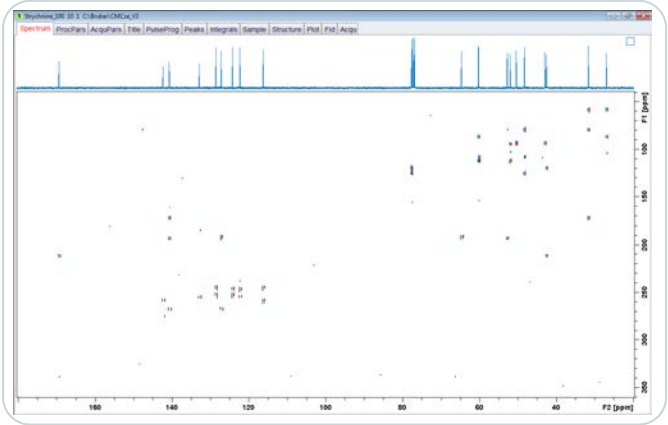
INADEQUATE
Experimental Details



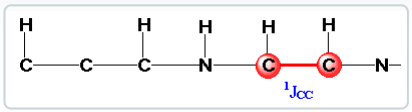
+ **Advantages:**
  - Information rich!

− **Disadvantages:**
  - Insensitive
    - Relies on ^{13}C next to another ^{13}C

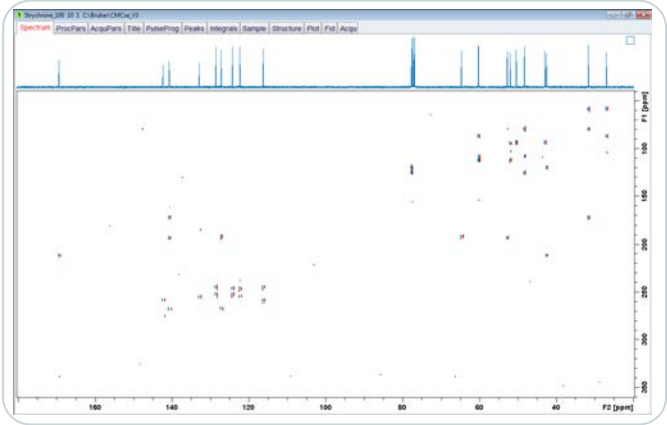
- 100 mg/ml Strychnine on a RT 400 MHz BBFO Smart Probe → **2.5 DAYS**
  - Single Scan 1D-^{13}C S:N of 100:1



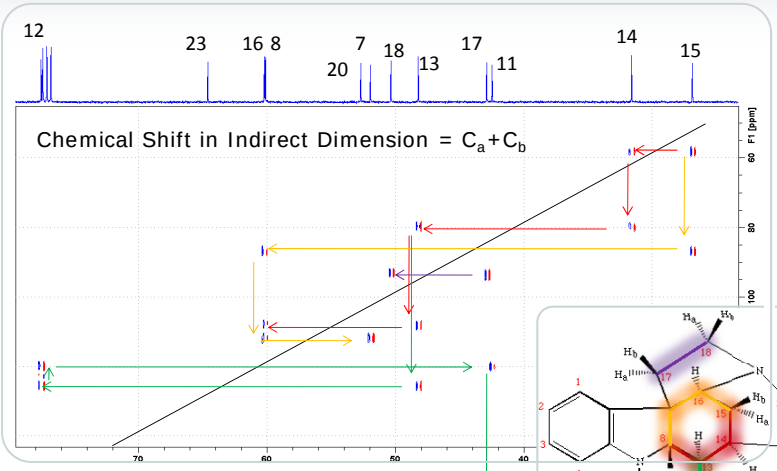
INADEQUATE
Experimental Details



- **Pulse Sequence:**
  - inadphsp
- **Experimental Details**:
  - SW in F2 = ^{13}C Spectrum
  - SW in F1 = 2 x ^{13}C SW in F2
- **Referencing:**
  - Center of spectrum in F1 = 2x O1p

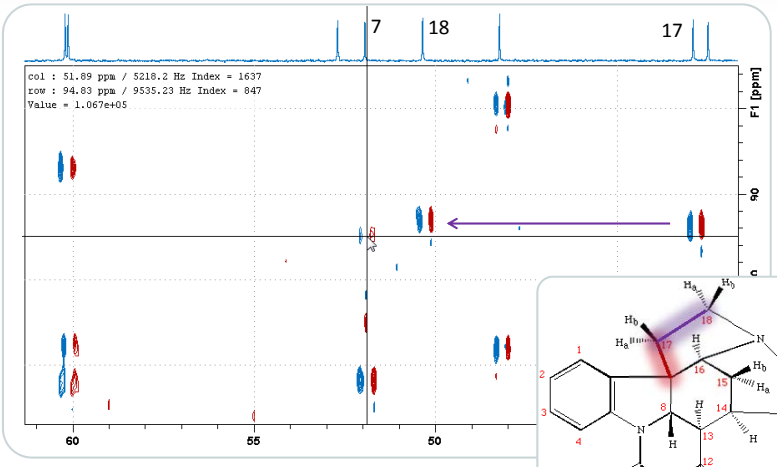


INADEQUATE
How to Interpret



INADEQUATE
Benefit of Phase Sensitive

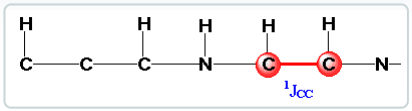
Chemical Shift in Indirect Dimension = C_a+C_b



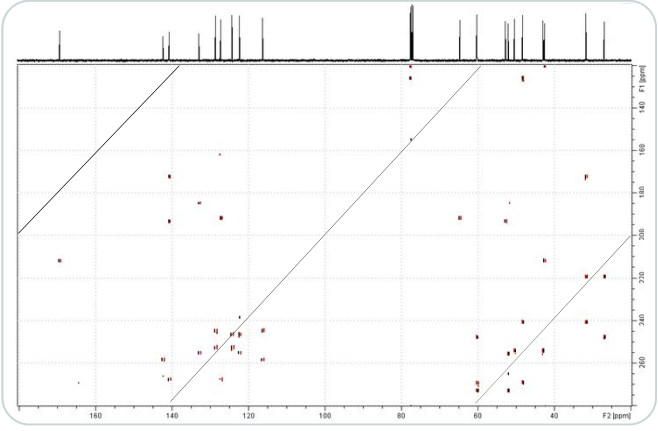
Correlation at 51.8 ppm and 95 ppm

C7 = 51.9 ppm + C17 = 42.9 ppm ➔ 94.8

INADEQUATE
Experimental Details - Folding

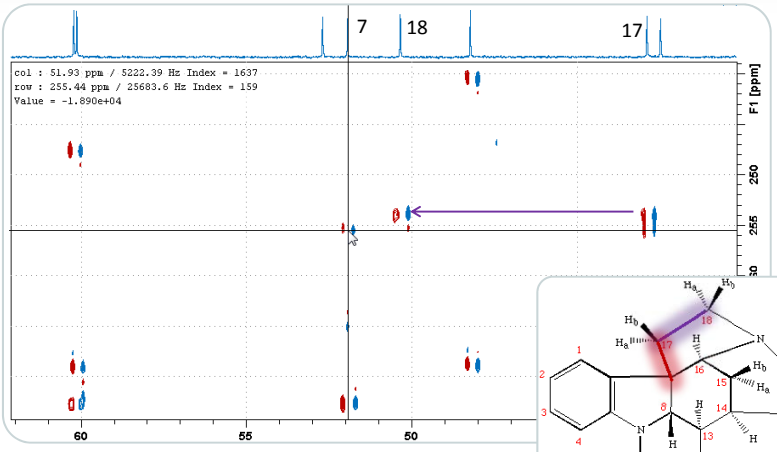


- **Pulse Sequence:**
  - inadphsp
- **Experimental Details**:
  - SW in F2 & F1 = ^{13}C Spectrum
- **Referencing:**
  - Center of spectrum in F1 = 2x O1p
- **Position of Folded Peaks** = SW +C_a +C_b



INADEQUATE
Benefit of Folding

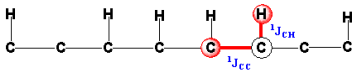
Chemical Shift in Indirect Dimension = SW + C_a+C_b



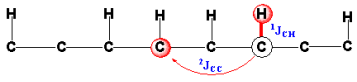
Correlation at 51.9 ppm and 255 ppm

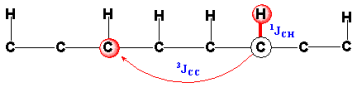
C7 = 51.9 ppm + C17 = 42.9 ppm + SW = 160 ppm ➔ 254.8

ADEQUATE
Proton Detected ^{13}C-^{13}C Correlations

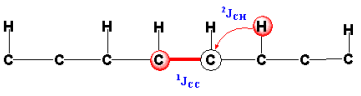


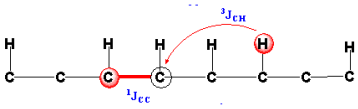
1,1-ADQUATE



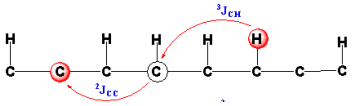


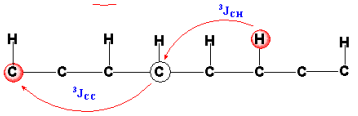
1,n-ADQUATE





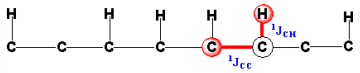
n,1-ADQUATE





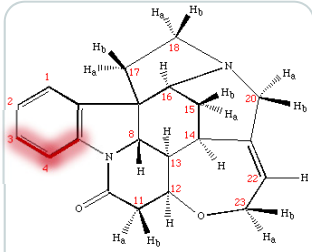
n,n-ADQUATE

ADEQUATE
Proton Detected ^{13}C-^{13}C Correlations

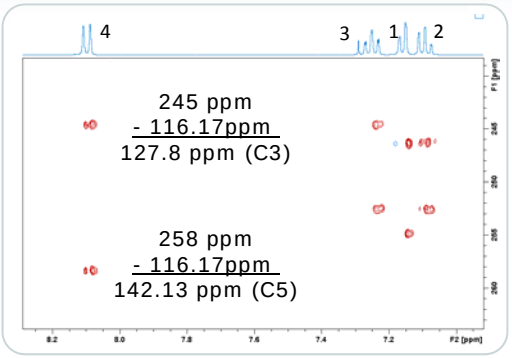


1,1-ADQUATE

adeq11etgpsp



50 mg/ml Strychnine in CDCl3
Room Temp 400 MHz BBFO
Smart Probe → 16 hours

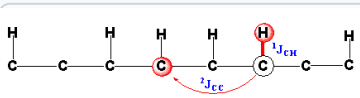


Correlation at H_a / C_a + C_b

From HSQC → H_4 = 8.1ppm C_4 = 116.17 ppm

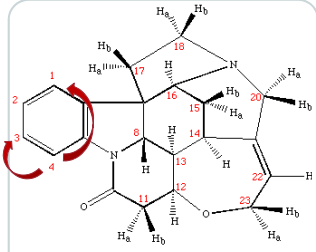
ADEQUATE Peaks at 244.5 ppm and 258.3
C4 Next to Carbons at 127.8 (C3) and 142.13 (C5)

ADEQUATE
Proton Detected ^{13}C-^{13}C Correlations

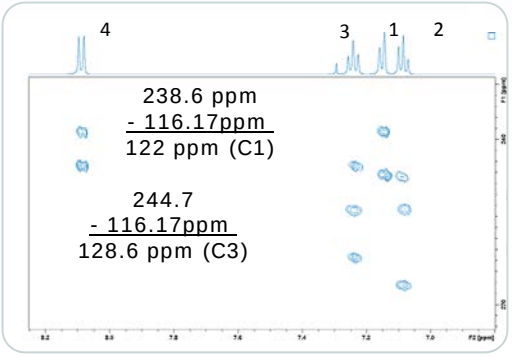


1,n-ADQUATE

adeq1netgpsp



50 mg/ml Strychnine in CDCl3
500 MHz Prodigy → 4 days 4 Hours



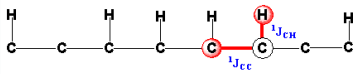
Correlation at H_a / C_a + C_b

From HSQC → H_4 = 8.1ppm C_4 = 116.17 ppm

ADEQUATE Peaks at 238.4 ppm and 244.7
C4 Next to Carbons at 124.0 (C1) and 132.67 (C3)

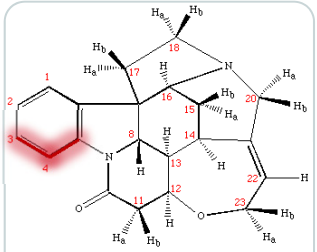
ADEQUATE

Proton Detected ^{13}C-^{13}C Correlations

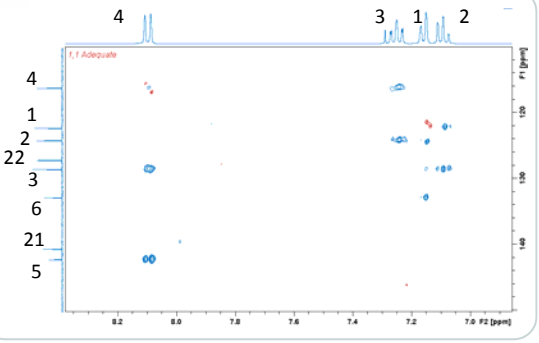


Refocused 1,1-ADQUATE

adeq11etgp**rd**sp.2



50 mg/ml Strychnine in CDCl3
Room Temp 400 MHz BBFO
Smart Probe → 16 hours



Correlation at H_a / C_b

Can Interpret like an HMBC/H2BC
Know it is Neighboring ^{13}C (J_{CC})
Unlike H2BC – correlations to 4° Carbons are possible

BRUKER

Innovation with Integrity