Which Experiment Should I Choose?

So Many to Choose From....
So Many to Choose From...
99 parameter sets, and 144 pulse sequences with “HSQC”

- 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
**1H Observe**

**PROTON**
- zg30
- 1H 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

**1H 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**
- zgcpr
  - Presaturation using composite 90° pulse
  - AU program (au_watersc) that does a scout scan to find the most intense signal and sets O1 there

**LC1DWTDCC**
- wetdc
  - WET with 13C 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]

- **dept135**
  - 500 MHz

- **deptsp135**
  - 400 MHz
• **C13CPD**
  - `zpg30`
    - $^{13}$C acquire with $30^\circ$ 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 $180^\circ$ pulse

• **Other Sequences**
  - `zgig30`
    - Sequence with inverse gated decoupling, so only during acquisition
  - `dept45sp`
  - `dept90sp`
**1H–1H Homonuclear 2D Experiments**

**COSY**

**Through Bond**

![Through Bond Diagram]

- COSY GPSW
  -.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**

![Caryophyllene Oxide in DMSO]

- No Purge Pulses
- With Purge Pulses
**1H-1H Homonuclear 2D Experiments**

**COSY**

- **COSYGPDPHPSW**
  - 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 CDCl₃

- Difficult for a beginner to phase

**1H-1H Homonuclear 2D Experiments**

**Another COSY Option**

- cosygmfppqf -- 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
**1H-1H 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
  - cosvqpmfppqf

  » 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

---

**1H–1H Homonuclear 2D Experiments**

**Through Bond**

- COSY
- TOCSY
$^1$H-$^1$H Homonuclear 2D Experiments

**TOCSY**

- **MLEVPWSW**
  - mlevphpp -- Homonuclear Hartman-Hahn using MLEV17 sequence, phase sensitive (ph), and purge pulses (pp)

- **MLEVPHPHR**
  - mlevphpr.2 -- Homonuclear Hartman-Hahn using MLEV17 sequence, phase sensitive (ph), and presat (pr),
  - TOCSY Mixing Time is defined by $d_9$
    - Default is 0.08 seconds

Strychnine in CDCl$_3$

$^1$H-$^1$H Homonuclear 2D Experiments

**Through Bond**

COSY

**Through Space**

NOESY

ROESY
**1H-1H Homonuclear 2D Experiments**

**NOESY/ROESY**

- **NOESYPHWSW**
  - *noesypphpp* -- 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

- **ROESYPHWSW**
  - *roesypphpp.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

**Zero Crossing Depends on:**
- Magnetic Field
- Size of Molecule
- Temperature
- Viscosity

Around 1,000 – 2,000 Daltons

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**1H-1H Homonuclear 2D Experiments**

**NOESY/ROESY**

- **NOESY**
  - 400 MHz

- **ROESY**
  - 400 MHz

Small Molecule
- NOESY
- ROESY

Large Molecule
+ NOESY
+ ROESY

Exchange Peak
+ NOESY
+ ROESY

Pamoic Acid
MW = 388
DMSO at 292 K

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High-Resolution NMR Techniques in Organic Chemistry
Timothy D.W. Claridge 1999
**1H-13C Heteronuclear 2D Experiments**

### Single Bond

![Diagram](image1.png)

**HSQC**

- Bare Bones
- Enhanced Sensitivity
- Adiabatic Pulses
- Multiplicity Edited

- Shaped Pulses for Inversion
- Shaped Pulses for Inversion and Refocusing
- COSY peak Suppression
- Shaped Pulses for Inversion and Adiabatic Pulses
**1H–13C Heteronuclear 2D Experiments**

**HSQC**

- **Adiabatic Pulses**
- **Sensitivity Improved**
- **Multiplicity Edited**

---

**1H–13C HSQC – Things to Consider**

**HSQCEDETGPSISP_ADIA and HSQCETGPSISP_ADIA**

- Bare Bones
  - *hsqqph*
  - *hsqqpph*

  - **Adiabatic Pulses**
  - **Sensitivity Improved**
  - **Multiplicity Edited**

  - Shaped Pulses for Inversion
  - Shaped Pulses for Inversion and Refocusing
  - Shaped Pulses for Inversion
  - Gradient in Black
  - COSY peak Suppression
  - Shaped Pulses for Inversion
  - Matched Sweep
  - Shaped Pulses for Inversion
$^{1}H^{13}C$ HSQC – Things to Consider

Multiplicity Edited or Not?

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

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

HSQC

Adiabatic Pulses

Sensitivity Improved

Multiplicity Edited
**“Matched Sweep” Adiabatic Pulses**

**Removing the J Dependence**

\[ d_{21} = \frac{1}{2J_{zh}} \]

If \( J = 180 \text{ hertz} \) → 2.7 ms
If \( J = 100 \text{ hertz} \) → 5 ms

**The Matched Sweep Adiabatic Pulse**

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

---

**\(^1\text{H}-^{13}\text{C}\) HSQC – Things to Consider**

**Multiplicity Edited or Not?**

- \text{hsqcetgp}
- \text{hsqcedetgp}
- \text{hsqcedetgpsp.3}

Menthyl Anthranilate in DMSO
**1H-13C 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 1H-13C 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)

\[ d_{21} = \frac{1}{2}J_{xy} = 3.6 \text{ ms} \]

\[ \delta = \text{gradient recovery delay} = .2\text{ms} \]

\[ \sim 7 \text{ ms longer of a sequence} \]

Depending on the T2 relaxation rates of the molecule the non-edited version might be more sensitive:

**But is it worth sacrificing the multiplicity information?**
1H-13C 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 mg/ml Quinidine, 1 hour 20 Min each HSQC w/ 9 hour DEPT as projection
$^1$H-$^{13}$C HSQC – Things to Consider

Multiplicities Edited or Not?

Matched Sweep Adiabatic Pulse?

0.1 mg/ml Quinidine, 10 hour each HSQC spectra w/ no DEPT
**1H-13C HSQC – Things to Consider**

**Benefit of Matched Sweep**

*hsqcedetgpsisp2.2  hsqcedetgpsisp2.3*

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**Quinidine in DMSO**

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**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

\[ d_{21} = \frac{1}{2J_{xh}} \]

If \( J = 180 \text{ hz} \rightarrow 2.7 \text{ms} \)

If \( J = 100 \text{ hz} \rightarrow 5 \text{ ms} \)
**1H-13C 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

J_{hc} = 158 Hz

α-Thujone in DMSO
1H-13C HSQC – Things to Consider

Sensitivity Improved or Not?

- **HSQCEDETGPSISP_ADIA**
  - hsqcedetgpsisp2.3

- **HSQCETGPSISP_ADIA**
  - hsqcetgpsisp2.2
  - Sensitivity Improved Element
    - Possible sensitivity improvement of ~ \(\sqrt{2}\)

- **HSQCEDETGPS.P3_ADIA**
  - hsqcedetgpsp.3
  - Multiplicity edited with Matched Sweep Adiabatic

- **HSQCETGPS.P2_ADIA**
  - hsqcetgpsp.2
  - Non Multiplicity Edited
  - No Sensitivity Improved Element
    - In general, less sensitive than the SI version
1H-13C HSQC – Things to Consider
Sensitivity Improved or Not?

Sensitivity Improved or Not?

\[ d_{24} = \frac{1}{8}J_{\omega H} = 0.89 \text{m} \]

\[ \sim 2\text{ms longer of a sequence} \]

Depending on the T2 relaxation rates of the molecule of interest, the non-si version might be actually be more sensitive.
$^1\text{H} - ^{13}\text{C}$ HSQC – Things to Consider
Sensitivity Improved or not?

$d_{24} = \frac{1}{8}J_{xh}$
$d_{21} = \frac{1}{2}J_{xh}$

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

Strychnine in CDCl$_3$
**1H-13C HSQC – Things to Consider**

**Sensitivity Improved or Not?**

- **HSQCEDETGPSISP_ADIA**
  - `hsgcedetgpsisp2.3`
    - Multiplicity Edited
    - “Sensitivity Improved” INEPT element
    - Matched Sweep Adiabatic Pulses
      - More Sensitive
      - Non quantitative

- **HSQCEDETGPSP.3_ADIA or CMCse_HSQC**
  - `hsgcedetgpsp.3`
    - Multiplicity Edited
    - Without “Sensitivity Improved” INEPT element
    - Matched Sweep Adiabatic Pulses
      - Less Sensitive
      - Quantitative integrals
        - Used in CMCse

**1H-13C HSQC – Things to Consider**

**COSY Peak Suppression**

- Bare Bones
- `hsgph`
- `hsggph`
- Adiabatic Pulses
  - Shaped Pulses for Inversion (sp)
  - Shaped Pulses for Inversion and Refocusing (sp)
- Sensitivity Improved
  - Shaped Pulses for Inversion and Refocusing (sp)
  - INEPT (2)
- Multiplicity Edited
  - COSY peak Suppression (2.3/4)
  - Shaped Pulses for Inversion (sp)
  - Matched Sweep Adiabatic Pulses (sp)
1H-13C 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

Menthyl Anthranilate in DMSO
$^1$H-13C HSQC – Things to Consider

Long Refocusing Pulse

Adiabatic Pulses:
- Inversion ($p_{14}$) = 0.5 ms
- Refocusing ($p_{24}$) = 2 ms

Hard 180 Pulse:
- 16 us

13C Labeled Sucrose

hsqcetgpsp,2
$^{1}H-^{13}C$ HSQC – Things to Consider

When is Simple Better?

- Adiabatic Pulses
- Sensitivity Improved
- Multiplicity Edited

- Shaped Pulses for Inversion
- Shaped Pulses for Inversion and Refocusing
- Adiabatic Pulses for Inversion and Refocusing
- Shaped Pulses for Inversion and Refocusing
- COSY peak Suppression
- INEPT

- Matched Gradient Adiabatic Pulses

$d_{21} = 1/2J_{x_h} = 3.6$ ms
$d_{24} = 1/8J_{x_h} = 0.89$ ms

Adiabatic pulses:
- Inversion = 0.5 ms
- Refocusing = 2 ms

Hard 180 Pulse:
- 16 us
HSQC – Things to Consider
When Is Simple Better?

$^1$H – $^{11}$B Spectra

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

Single Bond
HSQC/HMQC

Multiple Bond
HMBC
1H-13C 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**
  - hmbcetapl3nd
    - 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)

1H-13C Heteronuclear 2D Experiments
HMBC - Sensitivity

- Quinidine in DMSO
  - 1 mg/ml

HMBCETGPL3ND
HMBCGP

32 Scans, 256 Increments = 6 hours each
**1H-13C Heteronuclear 2D Experiments**

**HMBC – Low Pass Filter**

- hmbcgpplndqf
  
  \[ d_2 = \frac{1}{2J_{xh}} \]

- hmbcetgp3nd
  
  \[ \Delta_1 = \frac{1}{2(J_{xh\text{min}} + 0.07J_{xh\text{max}})} \]
  \[ \Delta_2 = \frac{1}{J_{xh\text{min}} + J_{xh\text{max}}} \]
  \[ \Delta_3 = \frac{1}{2(J_{xh\text{max}} - 0.07J_{xh\text{max}} - J_{xh\text{min}})} \]

**1H-13C Heteronuclear 2D Experiments**

**HMBC – Suppression of 1J correlations**

Gibberellic Acid in Acetone

- $J_{xh\text{(max)}} = 170$ Hz
- $J_{xh\text{(min)}} = 120$ Hz
- $J_{xh} = 145$ Hz

Long Range $J_{xh}$ 8 Hz
**1H-13C Heteronuclear 2D Experiments**  
*Another HMBC option*

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

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

- **HMBCGP_15N**
  - **hmbcgpndaf**
    - $^{15}$N is routed through $f_2$
    - Gradients for coherence selection
      - Ratio set to select for $^1H/^{15}$N instead of $^1H/^{13}$C
      - Other nuclei are possible with the AU program "gradratio"

- **HSQCETGP_15N**
  - **hsqcetpsij2**
    - $^{15}$N is routed through $f_2$
    - Echo-anti echo
    - Sensitivity improved
      - Gradients in the back inept
      - Gradients for coherence selection
    - Ratio set to select for $^1H/^{15}$N
**1H-13C Heteronuclear 2D Experiments**

**HSQC_TOCSY_ADIA**

- **Other Options**
  - **hsqcdiedetgpsisp.2**
    - Inversion of directly coupled protons
      - “HSQC” are +
      - “TOCSY” are -
  - **hsqcdiedetgpsisp.3**
    - Fully Edited
      - “HSQC” → CH/CH₃ + & CH₂ -
      - “TOCSY” → CH/CH₃ - & CH₂ +

---

**Menthyl Anthranilate in DMSO**

**HSQC**

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

**HSQC_TOCSY_ADIA**

**hsqcdiedetgpsisp.3**

New in TopSpin 3.0

“Show Recommended”

But There’s More If These Don’t Answer Your Question
1H-13C Heteronuclear 2D Experiments

HMBC

How Do I Know $^2$J vs $^3$J?

H2BC (AKA HMQC-COSY)
Heteronuclear 2 Bond Correlation

Strychnine in CDCl₃

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.
**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

---

**How to Interpret**

Chemical Shift in Indirect Dimension = $C_a + C_b$
**INADEQUATE**

**Benefit of Phase Sensitive**

Chemical Shift in Indirect Dimension = $C_a + C_b$

Correlation at 51.8 ppm and 95 ppm

$C_7 = 51.9$ ppm + $C_{17} = 42.9$ ppm $\Rightarrow 94.8$

**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 $\rightarrow$ 16 hours

Correlation at $H_a / C_a + C_b$
From HSQC $\rightarrow$ $H_a = 8.1$ ppm, $C_a = 116.17$ 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 $\rightarrow$ 4 days 4 Hours

Correlation at $H_a / C_a + C_b$
From HSQC $\rightarrow$ $H_a = 8.1$ ppm, $C_a = 116.17$ ppm
ADEQUATE Peaks at 238.4 ppm and 244.7
C4 Next to Carbons at 124.0 (C1) and 132.67 (C3)
ADEXUATE
Proton Detected $^{13}$C-$^{13}$C Correlations

Refocused 1,1-ADQUATE
adeq11etgprds.2

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

Correlation at H$_2$ / C$_6$
Can Interpret like an HMBC/H2BC
Know it is Neighboring $^{13}$C (J$_{CC}$)
Unlike H2BC – correlations to 4 Carbons are possible