

UI500NB Spectrometer: Advanced 1D and 2D NMR Experiments

With Application to Structure Elucidation of Small Organic Molecules

You should have finished basic NMR lab training
with NMR staff before proceeding to train on
UI500NB for advanced 1D and 2D NMR experiments.

The following knowledge and skills are presumed:

- Tuning and Matching the UI500NB Probe
- pw90 Calibration
- T1 Determination
- Solvent Pre-saturation
- Decoupling

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A. Introduction to UI500NB Spectrometer

- 1) **Spectrometer:** A Varian (Agilent) Inova 500 MHz spectrometer: a three channel, multinuclear solution FT-NMR instrument with Z Pulse Field Gradient (PFG) capability. All channels have waveform generators with pulse shaping capability.
- 2) **Probe:** The default probe on this instrument is the indirect-detection triple-resonance 5mm $^1\text{H}\{^{13}\text{C}/^{15}\text{N}\}$ Z-gradient probe (hcn). It is optimized for the observation of ^1H ONLY and ^1H detected 2D NMR experiments, which means that it is best used for 1D ^1H and NOE difference, 1D/2D ^1H - ^1H COSY, TOCSY, NOESY, ROESY, and 2D ^1H - ^{13}C or ^1H - ^{15}N HMQC and HMBC, and any other ^1H -observed experiments. ^{13}C sensitivity is so low (80:1) that running ^{13}C -observed experiments on this instrument is strongly discouraged. In fact, the APT and HETCOR experiments have been disabled on this instrument. The standard ^{13}C 1D experiment should be used solely for the purpose of setting the ^{13}C parameters in experiments such as HMQC and HMBC.
A 5mm $^1\text{H}\{^{31}\text{P}/\text{X}\}$ Z-gradient probe (hpx) is available as a backup and for experiments requiring different combinations of nuclei than ^1H - ^{13}C - ^{15}N .
A 10mm broadband probe (^{15}N - ^{31}P) is also available for this instrument. However, most experiments requiring this probe are normally done on the U500, VXR500 and UI600. Contact Dean Olson (4-0564) if you need to use this probe or have any other special requests.
- 3) **VT Setup:** The temperature limits for the hcn probe (and for most gradient probes) are -20°C to $+80^\circ\text{C}$. Use FTS unit to cool the system below room temperature (see VT user manual in a separate document).
As always, you will need to be checked out for VT before you can use the FTS unit. If you have not been checked out on VT, please do the VT training/checkout, which will allow you to use VT on this instrument.
For long 2D experiments, it is recommended that the experiments are collected at a fixed temperature. For example, set temp = 25°C for room temperature experiments.
- 4) **Probe Tuning:** You must be trained and checked by lab staff before you are allowed to tune the probe. Instruction for tuning the probe is given in this document and is also given in a separate handout.
- 5) **Shimming:** You must be trained again on shimming (even if you've done it in the basic NMR training section). Due to the design of the experiments running on UI500NB, you will collect your spectra mostly without sample spinning; therefore you should shim the system both in spinning shimming and none spinning shims following the procedure given in this document (more information can be found in the SCS NMR website).
- 6) **Scheduling:** Check the SCS NMR website and the following page for the current rules and regulations.

TABLE 1
Comparison of Sensitivity (Sens) for NMR Spectrometers

NMR Instrument, Probe	¹ H Sens (1)	¹⁹ F Sens (2)	¹³ C Sens (3)	³¹ P Sens(4)	Variable Temperature Range (°C)
U400, QUAD (¹ H, ¹⁹ F, ¹³ C, ³¹ P) 5mm switchable	~160:1 ~120:1	~150:1	~150:1 ~150:1	~110:1 ~150:1	-100 °C to +100 °C -100 °C to +100 °C
UI400, QUAD (¹ H, ¹⁹ F, ¹¹ B, ³¹ P)	~120:1	~120:1	~130:1 (disabled)	~90:1	-100 °C to +100 °C
U500, QUAD (¹ H, ¹⁹ F, ¹³ C, ³¹ P)	~260:1	~270:1	~210:1	~300:1	-100 °C to +100 °C
VXR500, QUAD PFG Z (¹ H, ¹⁹ F, ¹³ C, ³¹ P) ¹ H{X} switchable	~260:1 ~300:1	~280:1	~230:1 ~210:1	~200:1	-80 °C to +100 °C -100 °C to +100 °C
UI500NB ¹ H{ ¹³ C/ ¹⁵ N} PFG Z ¹ H{ ³¹ P/X} PFG Z ¹⁵ N- ³¹ P BB (10mm)	~750:1 ~700:1 NA		~80:1 NA ~550:1		-20 °C to +80 °C -30 °C to +50 °C -100 °C to +100 °C
UI600 ¹ H{ ¹³ C/ ¹⁵ N} PFG X,Y,Z AutoX (¹⁵ N- ³¹ P and ¹ H or ¹⁹ F) ¹⁵ N- ³¹ P BB (10mm)	~1000:1 ~370:1 NA	~475:1 NA	~125:1 ~320:1 ~1100:1	~206:1 ~550:1 (~80:1 for 5mm tube)	-20 °C to +80 °C -80 °C to +130 °C -150 C to +150 C
VNS750NB ¹ H{ ¹³ C/ ¹⁵ N} PFG X,Y,Z ¹⁵ N- ³¹ P BB (10mm) ¹³ C{ ¹ H} 5 mm ¹³ C{ ¹ H} 3 mm	~1350:1		~180:1 ~1200:1 ~530:1 ~220:1	~780:1	-20 °C to +80 °C -150 °C to +150 °C -100 °C to +100 °C -100 °C to +100 °C

Numbers in () indicate sample used, see corresponding list at the bottom.

- (1) ¹H Sensitivity Standard: 0.1% ethylbenzene/CDCl₃ (5mm NMR tube)
- (2) ¹⁹F Sensitivity Standard: 0.05% CF₃C₆H₅/C₆D₆ (5mm NMR tube)
- (3) ¹³C Sensitivity Standard: ASTM, 40% dioxane/C₆D₆ (5mm NMR tube)
- (4) ³¹P Sensitivity Standard: 0.0485M Triphenylphosphate/CDCl₃ (5mm NMR tube)
- (5) For 10mm BB probe, ¹³C and ³¹P Sensitivity Standards are in 10mm NMR tube

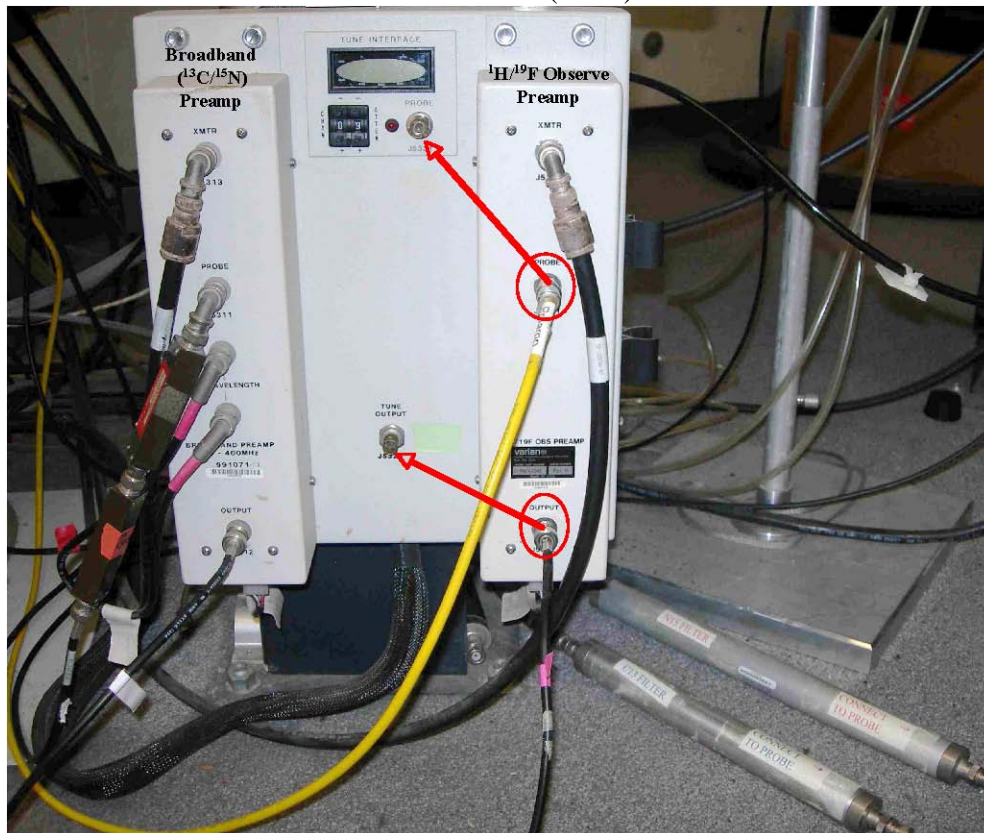
B. Probe tuning for the hcn probe on UI500NB

Insert your sample into the magnet bore,

1) In the VNMR command line, type: **tune('H1','C13')** or **tune('H1','C13','N15')**
This sets the tuning CHAN 1 to ^1H and CHAN 2 to ^{13}C (and CHAN 3 to ^{15}N)

2) Tune the ^1H channel:

2-1. Disconnect the J5301 cable and connect it to J5321 on the Probe Tune Interface and disconnect the J5302 cable and connect it to J5323 (Tune) on the Tune Interface.



2-2. Change CHAN to 1, by pushing the bottom button on the tune interface labeled +, leave the setting on the right at 9. The TUNING INTERFACE readout should turn green and give a reading.



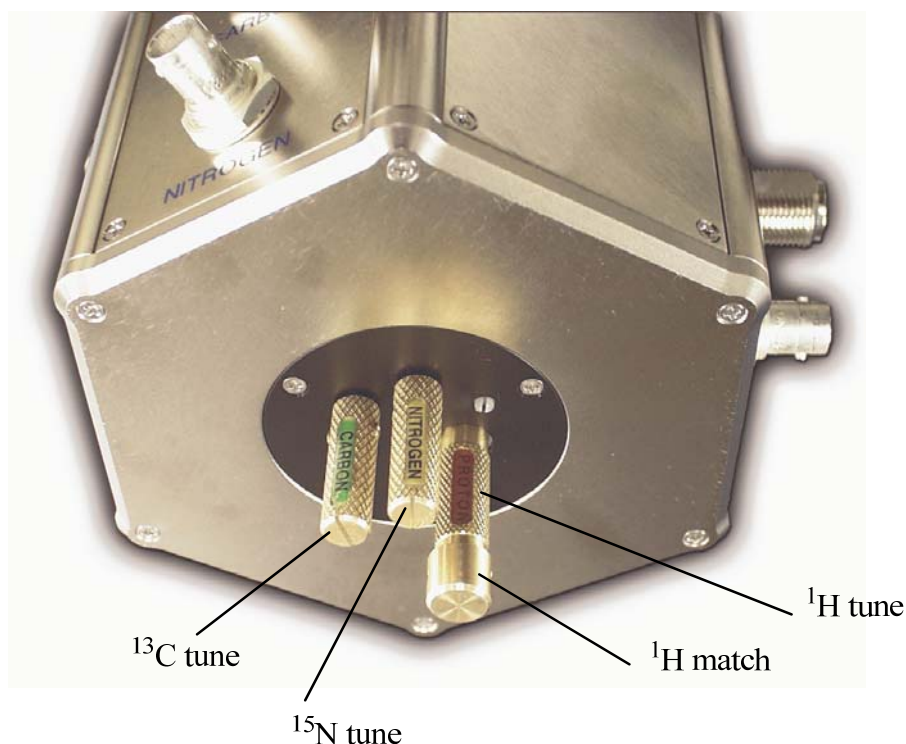
2-3. Locate the large, shiny brass knob underneath the probe. It is labeled "Proton" and some red

color is visible on the knob. This proton adjustment knob has a large, lower, shiny portion and a smaller, upper, knurled portion. The upper portion is for Tuning, while the lower portion is for Matching. Turn these knobs, *carefully* in one direction and watch the output change on the tune interface readout panel. If the readout increases, turn the Tune knob the other direction, until a *minimum* value is reached. Adjust the Match knob to decrease the value further. Continue to turn the tune and match knobs until you reach the smallest number possible (ideally under 5, however, this is sample-and solvent-dependent).

2-4. Change CHAN back to 0

2-5. Return the cables back to their original positions (J5321 @J5301 and J5323 @J5302).

NOTE: DO NOT FORCE THE KNOB TO TURN. IF YOU FEEL RESISTANCE, STOP! Otherwise, you could break the rod.



Placement of tuning knobs for Carbon, Nitrogen, and Proton under the probe. The largest, brassy red knob is for proton and is the only one with tune and match.

3) Tune the ^{13}C channel:

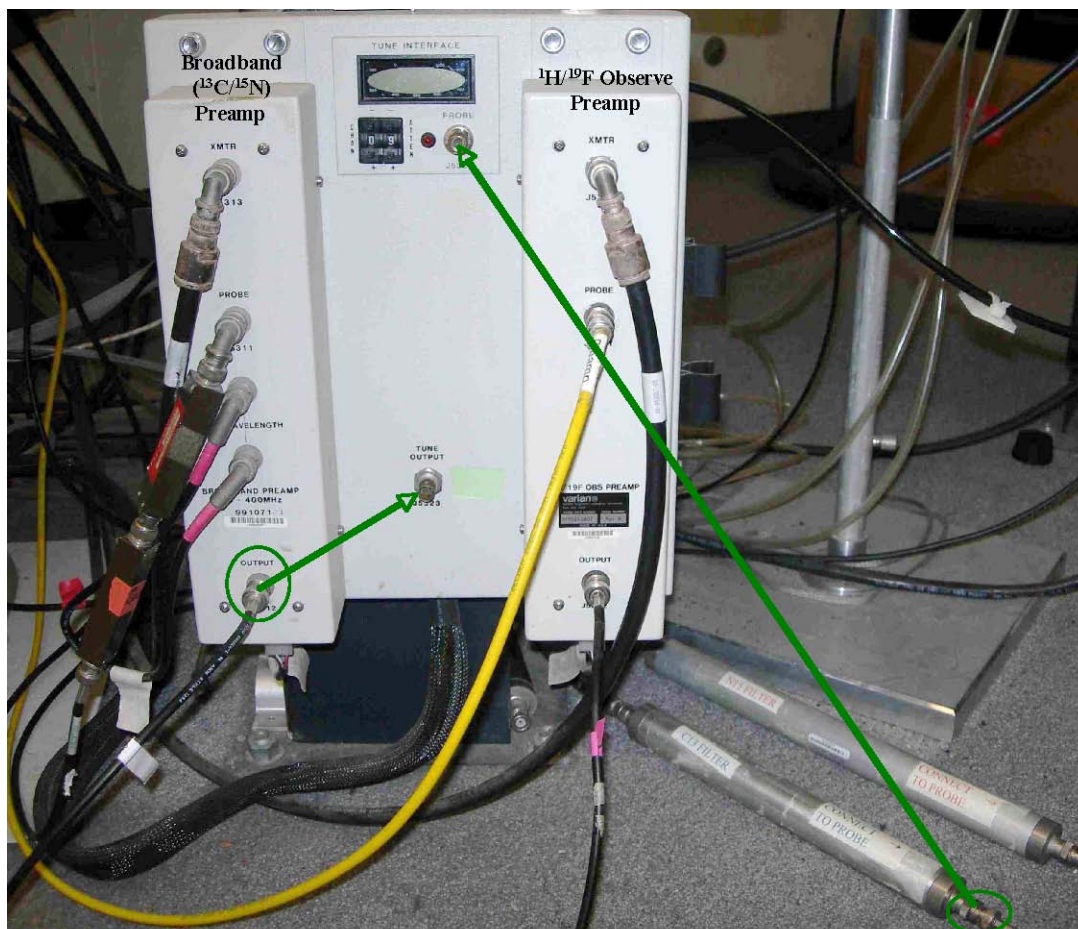
3-1. Disconnect the cable from the carbon filter on the floor (see the photo in the next page) and connect it to J5321 on the Probe Tune Interface. Disconnect the J5312 cable and connect it to J5323 (Tune) on the Tune Interface.

3-2. Change CHAN to 2, by pushing the button on the tune interface labeled +. Again, leave the setting on the right at 9.

3-3. Turn the Green knob underneath the probe, labeled “carbon” to tune the channel. Note, there is only 1 section to the carbon tuning knob. Turn this knob *carefully* in one direction and watch the value on the tune interface readout. If this number increases, turn the Tune knob in

the other direction until a *minimum* value is reached. Continue to adjust the Tune knob until the smallest readout value is achieved (ideally under 5, however, this is sample-and solvent-dependent).

- 3-4. Change CHAN back to 0 and return the cables back to their original positions (J5321 @carbon filter and J5323 @J5312).



Please note the carbon cable connected to the filter circled in green at the lower right-hand corner.

NOTE: DO NOT FORCE THE KNOB TO TURN. IF YOU FEEL RESISTANCE, STOP! Otherwise, you could break the rod.

- 4) Tune the ^{15}N channel (if needed):
 - 4-1. Disconnect the cable from the nitrogen filter on the floor and connect it to J5321 on the Probe Tune Interface. Disconnect the J5312 cable and connect it to J5323 (Tune) on the Tune Interface.
 - 4-2. Change CHAN to 3, by pushing the button on the tune interface labeled +. Again, leave the setting on the right at 9.
 - 4-3. Turn the knob underneath the probe, labeled “nitrogen” to tune the channel in a similar way as tuning ^{13}C channel. Note, there is also only 1 section to the nitrogen tuning knob.
 - 4-4. Change CHAN back to 0 and return the cables back to their original positions (J5321 @nitrogen filter and J5323 @J5312).

NOTES

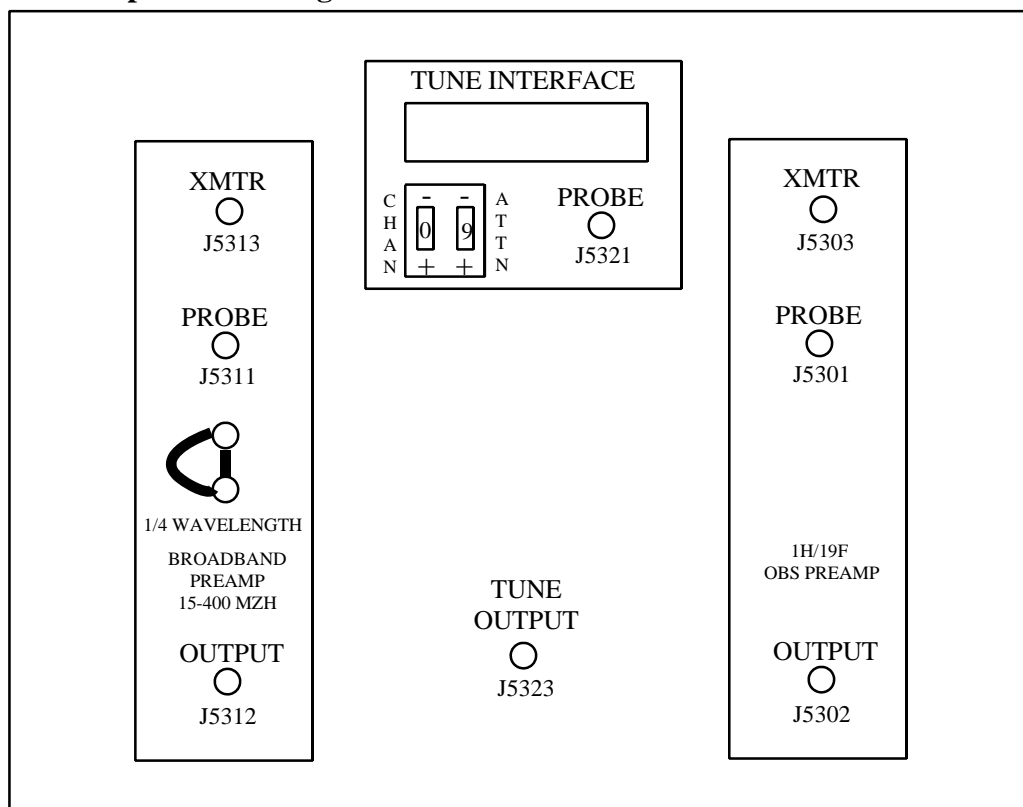
Tuning and matching is performed to optimize sensitivity. Typically, 2D experiments take a long time to acquire, and optimization of sensitivity can make the most effective use of the data acquisition time. Greater sensitivity means larger signals.

The instrument is designed to measure ^{13}C and ^{15}N indirectly and these channels normally calibrated by NMR lab staff. Consequently, the pw90 for the proton is the only pw that requires careful calibration.

Typical Values on the Tune Interface Meter

Solvent	Proton	Carbon	Nitrogen
CDCl_3	007	001	001
CD_2Cl_2	024	002	001
DMSO-D_6	010	002	001
D_2O	045	001	001

Scheme of the Preamplifier Housing



C. Shimming

For 1D/2D gradient NMR experiments, you will be collecting your data without sample spinning. Therefore you should shim your sample both in spinning and none spinning shims. The following is the basic shim procedure, more can be found on the SCS NMR website. Again, this procedure is written on the bases that you've trained on shimming previously when you first come to the NMR lab.

- 1) Load the system shim map by typing `rts('hcn')` on the command line
- 2) Lock your sample by adjusting `z0` value (check `lockphase` value also)
- 3) Shim spinning shims first (Z1 to Z5) while turning on the spin (spin rate at 20 Hz) by clicking spin "ON" button
 - 3-1. Adjust Z1 then, Z2, then Z1 again, iteratively. After the maximum lock signal (level) reaches,
 - 3-2. Adjust Z3 (clockwise) to decrease the signal intensity about 20%, then adjust Z1 and Z2 (iteratively) to maximize the signal. Continue changing Z3 in the same direction if the lock signal is higher than it was initially, and then adjust Z1 and Z2 until reach to maximum. If the lock signal is worse, adjust Z3 (count-clockwise) to decrease the signal intensity about 20%, then adjust Z1 and Z2 again.
 - 3-3. Adjust Z4 as the same fashion as Z3 (step 3-2), that is, every change in Z4 must be followed by the optimization of Z1 and Z2 until the highest possible lock level is obtained.
 - 3-4. Adjust Z5. The best way is the same as above, or you can adjust the Z5 value to maximize the lock signal, then check Z1 and Z2 again.
- 4) Repeat steps in step 3 iteratively until the highest possible lock level is obtained.
- 5) If you are satisfied with Z shims, turn off the spin by click spin "OFF" button, watch the lock level. If the lock level drops more than 5 units, you need to shim the none-spinning shims (X1, Y1, XZ, YZ, and XY and X2Y2).
 - 4-1. Adjust X1 and Y1 iteratively to maximize the lock signal.
 - 4-2. Adjust XZ in one direction to decrease the signal intensity about 20%. Adjust X1 to maximize the signal. If it's better, adjust XZ more in the same direction. If it becomes worse, adjust XZ in the other direction until signal maximized.
 - 4-3. Adjust YZ in one direction to decrease the signal intensity about 20%. Adjust Y1 to maximize the signal as above.
 - 4-4. Repeat step 4-1.(Note: the following is non-routine)
 - 4-5. Maximize lock level by shimming XY, then repeat step 4-1.
 - 4-6. Maximize lock level by shimming X2Y2 then repeat step 4-5.
 - 4-7. Repeat steps 4-1 to 4-3.
 - 4-8. Repeat steps in step 3 (shim on Z again) with spin "ON".

Finally if you have a good shimmed system, there should not be a big difference (< 10%) in lock levels between sample spinning (spin=20 Hz) and none-spinning (spin=0). You may want to collect a quick 1D proton spectrum to inspect the quality of the shims.

D. Setting up 1D/2D NMR experiments for structure elucidation of small organic molecules

NOTE: These instructions assume that you will be collecting a full data set for structure elucidation of small organic molecules, including both HMQC (inverse HETCOR) or HSQC and HMBC (inverse long-range HETCOR) on the same sample. Instructions are also given for a 1D/2D TOCSY, NOESY or ROESY data collection. The experiment library will be as follows (and you will have much less trouble if you always follow a routine such as this, in terms of your data collection):

exp1: ^1H spectrum
exp2: ^1H pw90 calibration or (optional) ^{13}C spectrum with parameters for gHMQC and/or gHMBC
exp3: 2D ^1H - ^1H gCOSY
exp4: 2D ^1H - ^{13}C gHMQC or 2D ^1H - ^{13}C gHSQC
exp5: 2D ^1H - ^{13}C gHMBC

The following experiments are optional, but sometimes necessary

exp6: 2D ^1H - ^1H gDQCOSY
exp7: 2D ^1H - ^1H TOCSY
exp8: 1D ^1H - ^1H TOCSY
exp9: 2D ^1H - ^1H NOESY
exp10: 1D ^1H - ^1H NOESY1D
exp11: 2D ^1H - ^1H ROESY
exp12: 1D ^1H - ^1H ROESY1D

1. Setting up the ¹H Experiment

- 1) Insert your sample in the spectrometer. Lock and shim the sample. NOTE: You can lock and shim while the sample is equilibrating if the temperature change is <5°. However, you should touch up the shims once the sample has equilibrated.
- 2) In exp1, set up parameters for a standard ¹H experiment, set the **temp** parameter if you are going to use VT, then enter su.
- 3) If using VT, wait until the temperature has reached the set point and your sample has equilibrated. Then tune the probe according to the instructions. NOTE: You should have your parameters for a standard ¹H experiment loaded before tuning the probe, or alternatively type command **tune('H1')** or **tune('1H', 'C13')** or **tune('1H', 'C13', 'N15')** as described in probe tuning section on page 4.
- 5) Optimize the ¹H parameters; mainly **sw** by setting the two cursors ~0.5 ppm beyond last proton peak on both sides of spectrum, then type the command
movesw
- 2) Re-acquire 1D ¹H experiment; make sure the parameters are correct. Then phase and reference the ¹H spectrum.
- 5) Determine the ¹H pw90 of the sample (more details of this operation can be found at the SCS NMR website named “90 Degree Pulse Width Determination”):

mf(1,2) jexp2 wft

gain?

A number will show up in the command line

gain=the number

Array experiment won't run if you have gain='n'

nt=1

type: **array** <rtn>

NOTE: The next four items are the answers to the questions posed by the array macro

Parameter to be arrayed: **pw** <rtn>

Enter number of steps in array: **10** <rtn>

Starting value: **20** <rtn> (an example. This number should be set to approximately
[(4*pw90 from the default setup) - 2])

Array increment: **1** <rtn>

Then type:

pw[1]=5

Replaces first array element, 20, with the value 5 (for setting up the correct phase)

da

Displays current arrayed values for pw

d1=10

ai vsadj vp=70

Sets absolute intensity mode and adjust the peak height; places spectrum about half-way up on the display.

ga

Make sure gain = “a number”, do not use auto-gain

As the spectra accumulate, use **dssh dssl** to view them. You can terminate the experiment with **aa** when you have determined the pw360 (where the signal is near zero).

NOTE: if the second spectrum is already positive, reset the array with a smaller starting value.

After you determine the pw360 of your sample,

jexp1

pw90=(the numeric value for the 360 found above!)/4; e.g., pw90=pw360/4

pw=pw90

ga

- 6) Quick determination of the T_1 for the sample (more details of this operation can be found in the SCS website named "T1 Measurement"):

NOTE: this step is optional. For most small molecules, 1.5 seconds of delay (d1) is normal. You could use 2.0-2.5 seconds for optimal signal-to-noise)

mp(1,2) jexp2

gain=the number found previously

dot1 <rtn>

NOTE: The next three items are the answers to the questions posed by the dot1 macro)

Minimum T1 expected: **0.5 <rtn>**

Maximum T1 expected: **5 <rtn>**

Number of scans: **1 <rtn>**

ga

As the spectra accumulate, use **dssh** to view them. You can terminate the experiment with **aa** when you have determined the T_1 of interest.

$$T_1 = 1.443 \times \tau_{(\text{null})}$$

(Derived from $M = M_o(1 - 2e^{-\tau/T_1})$)

M: magnetization at time τ after a 180 degree inversion pulse,

M_o : magnetization at equilibrium

$\tau_{(\text{null})}$: the time when the magnetization is zero)

- 7) To obtain the ^1H spectrum used for the following experiments

jexp1

optimize **d1** as necessary (based on the T1 if attempting a "good" integration (d1 ~ 5s))

optimize **nt** (nt ~ 8)

ga

Then phase, reference, and save the ^1H spectrum.

2. Setting up the 2D ¹H-¹H gCOSY Experiment

The following setup assumes you have done steps 1-7 in **Setting up the ¹H Experiment**. If you have not, do them now before continuing.

- 1) To load a gCOSY experiment

```
jexp3  
mf(1,3)  
gcosy
```

- 2) check **ni** - it should be at least 256 for a typical spectral width of 10 ppm, but may be smaller for a smaller sw.

Check:

```
d1=1-2 s or the value optimized in exp1 (~ 1.5T1)
```

```
pw=pw90
```

```
np=2048
```

```
sw1=sw
```

```
phase=1
```

```
nt=2 or multiple of 2 for better S/N
```

```
ni=128 or 256
```

if you change np or ni, readjust sb = -at/2 and sb1 = -ni/(2*sw1)

if you change np or ni, you may need to reset fn and fn1

- 3) Check **time** by typing time<rtm>, this experiment normally runs less than 10 mins for nt=2. Adjust nt if necessary; there is no minimum.

Make sure the **sample is not spinning!!** To turn off, go to the VNMR Acquisition window, LC Lock, LC spin off. While this window is open, make sure that the lock level is > 60% - you should adjust only the gain, if possible. When you start the experiment with go (below), make sure that the lock level stays above 20%.

enter **go** to start

After the acquisition is complete, save your spectrum.

WORKUP: use **wft2d** to process.

Note:

The gradient gCOSY experiment provides homonuclear chemical shift correlation information via the J-coupling interaction, revealing 2-bond (germinal) and 3-bond (vicinal) spin-spin coupled pairs. Sensitivity of this experiment is not normally an issue; it is acquired and processed in absolute value (magnitude) mode and usually requires no more than a few minutes to acquire. Other COSY type experiments are given later in the document; the gradient double-quantum filtered phase-sensitive COSY experiment, gDQCOSY, provides information about the adjacent two spin systems. TOCSY experiment has a very good S/N ratio, revealing the spin-spin correlation throughout the spin systems in a molecule. TOCSY1D: 1D version of 2D TOCSY; giving a clean one spin-spin system at a time.

3. Setting up the 2D ^1H - ^{13}C gHMQC (or gHSQC) Experiment

The following setup assumes you have done steps 1-7 in **Setting up the ^1H Experiment**. If you have not, do them now before continuing. You should have an optimized ^1H spectrum setup in exp1 before proceeding with the setup below.

- 1) (Optional) **jexp2** and set up parameters for a standard ^{13}C experiment (nt=1 or 4 should be sufficient to see the solvent peak). Acquire a spectrum with **ga**, then phase and reference. Check **sw** and change with **movesw** if desired. Remember, if you are only acquiring an HMQC spectrum, you can narrow the ^{13}C **sw** to include only protonated carbons. If you are also acquiring an HMBC spectrum, the **sw** should be set for both protonated and quaternary carbons. Please also remember that the pw90 of ^{13}C needs to be calibrated but this has already been done on a standard organic sample by NMR staff, you can use the default ^{13}C values. Please talk to the NMR staff if you want to calibrate the ^{13}C data on your particular sample.

- 2) To load a HMQC (or HSQC) experiment

jexp4

mf(1,4)

ghmqc (or gHSQC)

(enter the answers accordingly)

- 3) Check parameters

at should be around 0.2 seconds (np ~ 2048)

d1 should be set so that:

a) **$at/(at+d1) < 0.15$**

THIS CONDITION MUST BE MET! If necessary, reduce **np** (i.e., $np=np/2$) and/or increase **d1**.

b) **$d1+at = 1 \text{ to } 1.5 \times T1$**

This is a recommendation. If your T1's are long, you can try the default setting of $d1=2$. If you set **d1** for less than the recommended time, you will sacrifice sensitivity. However, if your sample concentration is adequate, the savings in time will more than balance this loss.

nt should be set according to sample concentration, no minimum required (2 or 4 is adequate).

ni should be set between 256 and 512.

phase=1,2

- 4) By default, **j1xh=140**, which is the average one-bond ^1H - ^{13}C coupling constant for your sample. If you do not see a correlation you believe should be visible, it may be because the coupling constant for this particular C-H bond differs too much from this average value. If you re-run the experiment optimized for the new coupling constant, the correlation should appear.
- 5) If you chose to set ^{13}C parameters manually, set the following:
sw1 = (sw of ^{13}C spectrum collected previously in step 1, otherwise use the default value)
dof = (tof of ^{13}C spectrum collected previously in step 1, otherwise use the default value)
- 6) check **time**, adjust nt if necessary
(It normally takes 20-40 min depending on sample concentration)

Make sure the sample is not spinning!! To turn off, go to the VNMR Acquisition window, LC Lock, LC spin off. While this window is open, make sure that the lock level is > 60% - you should adjust only the gain, if possible. When you start the experiment with go (below), make sure that the lock level stays above 20%.

enter **dps** to check the pulse sequence
enter **go** to start

After the acquisition is complete, save your spectrum.

WORKUP: use **wft2da** to process; use **twod** to setup proper display window.

The difference between HMQC and HSQC

Both ^1H - ^{13}C HMQC (Heteronuclear Multiple Quantum Coherence, eg. $2I_xS_y$) and HSQC (Heteronuclear Single-Quantum Coherence, eg. $2I_zS_x$) detect the correlation between directly bonded ^1H and ^{13}C nuclei via the one-bond ^1H and ^{13}C J coupling interaction. The cross peaks in the 2D spectrum contain both chemical shifts information of the carbon and its directly attached protons, providing a correlation map between the coupled spins.

HMQC has shorter relaxation pathway and is more robust to poorly calibrated pulses. However the cross peaks are modulated by homonuclear proton J coupling which broadens the carbon dimension. All cross peaks in HMQC spectrum have the same sign.

HSQC cross peaks are not affected by homonuclear proton J coupling and has a superior resolution in the carbon dimension. The edited gradient-enhanced HSQC (gHSQC) inverts the CH_2 signals, leaving these cross peaks negative. The CH and CH_3 cross peaks are positive in the 2D spectrum. If you work with a well calibrated system, gHSQC is preferred.

4. Setting up the 2D ^1H - ^{13}C gHMBC Experiment

The following setup assumes you have done steps 1-7 in **Setting up the ^1H Experiment**.

- 1) To load a gHMBC experiment

jexp5

mf(1,5)

ghmbc

(enter the answers accordingly)

- 2) Check parameters

nt should be 4 to 8 times of that necessary for the gHMQC experiment,

ni should be set between 256 and 512.

pw=pw90

phase=0

jnxh=8 is the default value, the average long range ^1H - ^{13}C coupling constant. Typical values are between 5 and 10. If you are missing some correlations, you may need to run the experiment again with a different value of **jnxh**.

By default, **j1xh=140**, the average one-bond ^1H - ^{13}C coupling constant for aliphatic proton/carbon pairs, aromatic 1-bond ^1H - ^{13}C coupling constants are 170-250 Hz, so **j1xh=180** if you want to filter out one-bond ^1H - ^{13}C aromatic peaks.

- 3) check **time**, adjust **nt** if necessary (It normally takes 1-12 hrs depending on sample concentration)

Make sure the sample is not spinning!!

enter **dps** to check the pulse sequence

enter **go** to start

After the acquisition is complete, save your spectrum.

WORKUP: use **wft2da** to process; use **twod** to setup proper display window.

5. Setting up the 2D ^1H - ^1H gDQCOSY Experiment

The following setup assumes you have done steps 1-7 in **Setting up the ^1H Experiment**. If you have not, do them now before continuing.

- 1) To load a gDQCOSY experiment

```
jexp6  
mp(1,6)  
gDQCOSY
```

- 2) check **ni** - it should be at least 256 for a typical spectral width of 10 ppm, but may be smaller for a smaller sw.

Check:

d1=1-2 s or the value optimized in exp1

pw=pw90

np=4096

nt=4 or multiples of 4 for greater S/N

sw1=sw

phase=1,2

ni=200 or 256 or more

if you change np or ni, readjust sb = -at/2 and sb1 = -ni/sw1, and sbs = -at/2 and sbs1=-ni/sw1 (squared sine bell with 90 degree shift)

if you change np or ni, you may need to reset fn and fn1

- 3) Check **time**, this experiment normally runs less than 20 mins for **nt=4**. Adjust **nt** if necessary.

Make sure the **sample is not spinning!!** To turn off, go to the VNMR Acquisition window, LC Lock, LC spin off. While this window is open, make sure that the lock level is > 60% - you should adjust only the gain, if possible. When you start the experiment with go (below), make sure that the lock level stays above 20%.

enter **go** to start

After the acquisition is complete, save your spectrum.

WORKUP: use **wft2da** to process.

Note:

The gradient double-quantum filtered phase-sensitive COSY (gDQCOSY) removes the intense singlets and observes only the magnetization associated with double-quantum coherence multiplets. The diagonal peaks are in absorption mode, giving much narrower diagonal peaks compared with gCOSY which has dispersive diagonal peaks. Therefore gDQCOSY presents less interference to the cross peaks near the diagonal. This experiment reveals germinal and vicinal spin-spin coupled pairs and measures the proton-proton J coupling constants. However its S/N is half of that of gCOSY therefore twice as many scans are needed to achieve the same S/N as in gCOSY.

Example: Measurement of proton-proton J coupling constants*

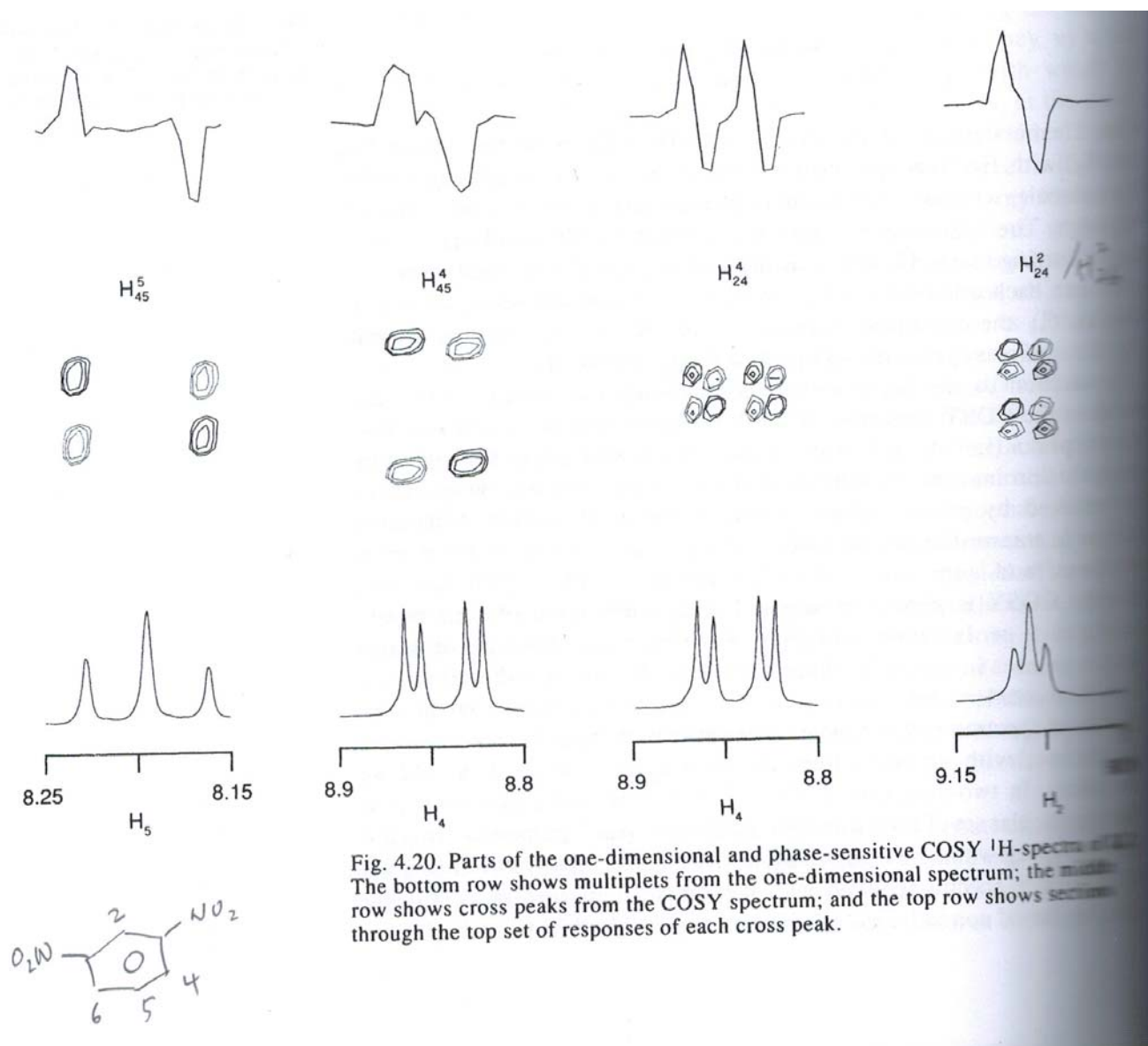


Fig. 4.20. Parts of the one-dimensional and phase-sensitive COSY ^1H -spectra of 2,4-dinitrophenol. The bottom row shows multiplets from the one-dimensional spectrum; the middle row shows cross peaks from the COSY spectrum; and the top row shows second-order derivative spectra through the top set of responses of each cross peak.

*: from "Modern NMR Spectroscopy, a guide for chemists" by J. Sanders and B. Hunter, 2nd Edition (Oxford University Press), Page 116.

6. Setting up the 2D ^1H - ^1H TOCSY Experiment

The TOCSY mixing time, τ_m , is related to how far the spin-spin relay carries throughout the spin system, in the range of 60-100ms, is typically useful to observe 4- and 5-bond coupling correlations. Its default value is 80 ms for a small molecule of size less than 1000 da. The larger the molecule is, the shorter of the mixing time.

The following setup assumes you have done steps 1-7 in **Setting up the ^1H Experiment**. If you have not, do them now before continuing.

- 1) To load a TOCSY experiment

```
jexp7  
mp(1,7)  
TOCSY
```

- 2) Check **ni** - it should be at least 256 for a typical spectral width of 10 ppm, but may be smaller for a smaller sw.

Check:

d1=1-2 s or the value optimized in exp1

pw=pw90

np=4096 (or smaller, e.g. 2048)

nt=2 or multiples of 2 for greater S/N

sw1=sw

phase=1,2

ni=200 or 256 or more

mix=0.08 (80 ms) or other value as described above

if you change np or ni, readjust $sb = at/2$ and $sb1 = ni/(2*sw1)$

if you change np or ni, you may need to reset fn and fn1

- 3) Check **time**, this experiment normally runs less than 20 mins for nt=2. Adjust nt if necessary.

Make sure the **sample is not spinning!!** To turn off, go to the VNMR Acquisition window, LC Lock, LC spin off. While this window is open, make sure that the lock level is > 60% - you should adjust only the gain, if possible. When you start the experiment with go (below), make sure that the lock level stays above 20%.

enter **go** to start

After the acquisition is complete, save your spectrum.

WORKUP: use **wft2da** to process.

7. Setting up the 1D ¹H-¹H TOCSY Experiment

1D TOCSY1D is a one-dimensional version of 2D TOCSY. By selectively irradiating one proton with soft shaped pulse, only its spin-spin correlation to other protons can be detected. This 1D experiment has advantages over 2D experiment by eliminating potential overlaps to provide an effective and clean TOCSY spectrum.

The following setup assumes you have done steps 1-7 in **Setting up the ¹H Experiment**. If you have not, do them now before continuing.

- 1) To load a 1D TOCSY experiment

```
jexp8  
mf(1,8)  
wft full  
pw=pw90  
TOCSY1D('ds')
```

- 2) Expand around first peak to be irradiated (inverted). Center cursors around desired peak ensuring that the full peak is between the two cursors, then,

LC Select Selects current region between cursors for selective inversion

Repeat Select for all desired peaks. When completed,

LC Proceed Calculates shaped pluses and completes TOCSY1D setup

You will need to answer the following questions:

Enter reference 90 degrees pulse width (μsec):

Enter your determined pw90 from pw90 calibration you've done early

Enter reference power level:

Enter the tpwr value used in pw90 calibration

da Displays array. Make sure all your desired peaks have been selected. They will appear with name similar to TOCSY1D_5_67p.RF, where the numbers refer to the chemical shift of the peak.

- 3) Check

nt=16 (or 32 for a better S/N)

d1; normally ~ 2 seconds (or longer)

mix=0.08 (80ms) or other value as describe in 2D TOCSY section

- 4) Check **time**, adjust **nt** if necessary.

Make sure the sample is not spinning!!

enter **go** to start

After the acquisition is complete, save your spectrum.

WORKUP: use **wft** to process.

Do not auto-phase the spectrum (aph). First type **phase(180)** (if necessary) to have the irradiated peak in the positive direction, then phase manually, the TOCSY peaks will be in the same positive direction.

dssh or **dssa** to view all spectrum in array

To view individual spectrum, type **ds(#)**, where # is the number of the desired spectrum.

To print all spectra in the array:

pl('all') pscale page

To print individual spectrum:

ds(#)

pl pscale page

or

pl pir ppf pscale page (add ppf or pir for peak picking and integration)

8. Setting up the 2D ^1H - ^1H NOESY Experiment

The 2D ^1H - ^1H NOESY experiment measures NOE (Nuclear Overhauser Enhancement) between protons within a molecule, providing information about distance between two protons in space. The stronger the NOE is, the closer of the two nuclei locate.

Sign of NOE cross peaks:

- (1) For small organic molecule, all NOE cross peaks have the opposite phase to the diagonal peaks, in contrast the cross peaks from chemical or conformational exchange are in phase with the diagonal.
- (2) For large molecules, all NOE cross peaks and the exchange cross peaks are in phase with the diagonal. In such case, ROESY experiment can be perform to distinguish between them if needed.

The following setup assumes you have done steps 1-7 in **Setting up the ^1H Experiment**. If you have not, do them now before continuing.

- 1) To load a NOESY experiment

```
jexp9  
mf (1,9) wft full  
pw=pw90  
NOESY
```

- 2) Check parameters

```
nt=32 (or more for better signal-to-noise ratio)  
ni=200 or more for a typical spectral width of 10 ppm, but may be smaller for a smaller sw  
(e.g. ni=200).
```

```
sw1=sw
```

```
d1=~ 2 seconds (or longer)
```

```
phase=1,2
```

```
mix=0.25 – 0.5 (250 – 500 ms), the mixing time for the NOE build-up. The mixing time is correlated to the molecular weight of the molecule. Generally 300 ms is adequate for MW > 1000, and ~500 ms for MW < 1000. If a mixing time is too short, it will not allow the NOE to buildup; if a mixing time is too long, it results in spin diffusion and you will get feak NOE peaks. Sometimes, a couple of NOESY experiments are collected for different mixing time. mix=0.5 (500ms) is the default value.
```

- 3) Check **time**, adjust **nt** if necessary;

Make sure the sample is not spinning!!

```
enter go to start
```

After the acquisition is complete, save your spectrum.

WORKUP: use **wft2da** to process.

9. Setting up the 1D ¹H-¹H NOESY Experiment

1D ¹H-¹H NOESY is a one-dimensional version of 2D NOESY. By selectively irradiating one proton with soft shaped pulse, only its NOE to other protons can be detected. This 1D experiment has advantages over 2D NOESY experiment by eliminating potential overlaps to provide an effective and clean NOE spectrum.

The following setup assumes you have done steps 1-7 in **Setting up the ¹H Experiment**. If you have not, do them now before continuing.

- 1) To load a 1D NOESY experiment

```
jexp10  
mf(1,10) wft full  
pw=pw90  
NOESY1D('ds')
```

- 2) Expand around first peak to be irradiated (inverted). Center cursors around desired peak ensuring that the full peak is between the two cursors, then,

LC Select Selects current region between cursors for selective inversion

Repeat Select for all desired peaks. When completed,

LC Proceed Calculates shaped pluses and completes NOESY1D setup

You will need to answer the following questions:

Enter reference 90 degrees pulse width (μsec):

Enter your determined pw90 from pw90 calibration you've done early

Enter reference power level:

Enter the tpwr value used in pw90 calibration

da Displays array. Make sure all your desired peaks have been selected. They will appear with name similar to NOESY1D_5_67p.RF, where the numbers refer to the chemical shift of the peak.

- 3) Check

nt=64 (or 128, or 256.. for a better S/N)

d1; normally ~ 2 seconds (or longer)

mix=0.5 (500ms) or other value as describe in 2D NOESY section

- 4) Check **time**, adjust **nt** if necessary;

- 5) Set **bs=16** (or 32), **il='y'** so that the data will be collected for the first FID (first arrayed peak) in the block size of 16 (or 32), then go to the second FID..... instead of finishing the first FID (first arrayed peak) then go to the second arrayed peak. Such if the S/N is good enough, you can always stop the experiment.

Make sure the sample is not spinning!!

enter **go** to start

After the acquisition is complete, save your spectrum.

WORKUP: use **wft** to process.

Do not auto-phase the spectrum (aph). First type **phase(180)** (if necessary) to have the irradiated peak in the negative direction, then phase manually, the NOESY peaks will be in the positive direction, and exchange peaks will in the negative direction for small molecules.

dssh or **dssa** to view all spectrum in array

To view individual spectrum, type **ds(#)**, where # is the number of the desired spectrum.

To print all spectra in the array:

pl('all') pscale page

To print individual spectrum:

ds(#)

pl pscale page

or

pl pir ppf pscale page (add ppf or pir for peak picking and integration)

10. Setting up the 2D ^1H - ^1H ROESY Experiment

The ROESY (Rotating frame Overhauser Effect Spectroscopy) experiment measures NOE in the rotating frame, providing information about distance between two protons in space. It is generally used for compounds with a molecular weight of 1000-3000 for which NOESY is approximately close to zero. ROESY cross peaks are always having opposite sign to the diagonal peaks, contrary to any TOCSY or exchange peaks if exists in the ROESY spectrum.

The following setup assumes you have done steps 1-7 in **Setting up the ^1H Experiment**. If you have not, do them now before continuing.

- 1) To load a ROESY experiment

```
jexp11  
mf(1,11) wft full  
pw=pw90  
ROESY
```

- 2) Check parameters

nt=32 (or more for better signal-to-noise ratio)

ni=200 or more for a typical spectral width of 10 ppm, but may be smaller for a smaller sw
(e.g. ni=200).

sw1=sw

d1=~ 2 seconds (or longer)

phase=1,2

mix=0.1 – 0.3 (100 – 300 ms), the mixing time for the ROE build-up. The mixing time is correlated to the molecular weight of the molecule. Long mixing time is needed for a small molecule. Generally 100-150ms is adequate for MW ~400-2000, and 200-300 ms if you want to observe longer mixing time ROE; for MW < ~ 400, 300-500 ms for long mixing time is generally used. All ROE cross peaks are in the opposite sign of the diagonal peaks, while TOCSY or exchange peaks are in phase with the diagonal. mix=0.2 (200ms) is the default value.

- 3) Check **time**, adjust **nt** if necessary

Make sure the sample is not spinning!!

enter **go** to start

After the acquisition is complete, save your spectrum.

WORKUP: use **wft2da** to process.

11. Setting up the 1D ¹H-¹H ROESY Experiment

1D ROESY1D is a one-dimensional version of 2D ROESY (Rotating frame Overhauser Effect Spectroscopy) experiment. By selectively irradiating one proton with soft shaped pulse, only its ROE to other protons can be detected. This 1D experiment has advantages over 2D ROESY experiment by eliminating potential overlaps to provide an effective and clean ROE spectrum.

The following setup assumes you have done steps 1-7 in **Setting up the ¹H Experiment**. If you have not, do them now before continuing.

- 1) To load a 1D ROESY experiment

```
jexp12  
mf(1,12) wft full  
pw=pw90  
ROESY1D('ds')
```

- 2) Expand around first peak to be irradiated (inverted). Center cursors around desired peak ensuring that the full peak is between the two cursors, then,

LC Select Selects current region between cursors for selective inversion

Repeat Select for all desired peaks. When completed,

LC Proceed Calculates shaped pluses and completes ROESY1D setup

You will need to answer the following questions:

Enter reference 90 degrees pulse width (μsec):

Enter your determined pw90 from pw90 calibration you've done early

Enter reference power level:

Enter the tpwr value used in pw90 calibration

da Displays array. Make sure all your desired peaks have been selected. They will appear with name similar to ROESY1D_5_67p.RF, where the numbers refer to the chemical shift of the peak.

- 3) Check

nt=64 (or 128, or 256.. for a better S/N)

d1; normally ~ 2 seconds (or longer)

mix=0.2 (200ms) or other value as describe in 2D ROESY section

- 4) Check **time**, adjust **nt** if necessary.

- 5) Set **bs=16** (or 32), **il='y'** so that the data will be collected for the first FID (first arrayed peak) in the block size of 16 (or 32), then go to the second FID..... instead of finishing the first FID (first

arrayed peak) then go to the second arrayed peak. Such if the S/N is good enough, you can always stop the experiment.

Make sure the sample is not spinning!!

enter **go** to start

After the acquisition is complete, save your spectrum.

WORKUP: use **wft** to process.

Do not auto-phase the spectrum (aph). First type **phase(180)** (if necessary) to have the irradiated peak in the negative direction, then phase manually, the ROESY peaks will be in the positive direction, and exchange peaks will in the negative direction for small molecules.

dssh or **dssa** to view all spectrum in array

To view individual spectrum, type **ds(#)**, where # is the number of the desired spectrum.

To print all spectra in the array:

pl('all') pscale page

To print individual spectrum:

ds(#)

pl pscale page

or

pl pir ppf pscale page (add ppf or pir for peak picking and integration)

E. Guideline for 2D Data Acquisition

You can make a good approximation of the number of scans needed for your 2D data acquisition by looking at your 1D ^1H spectrum. The ^1H 1D spectrum needs to be acquired under the following conditions:

1. $\text{pw}=\text{pw90}$
2. $\text{nt}=1$
3. Check the S/N to the smallest peak of interest in your spectrum.
4. Check that number versus the suggested numbers below.

gcosy: if the S/N > 50:1, use $\text{nt}=1$; the experiment time is ~ 5 minutes

ghmqc: if the S/N > 250:1, use $\text{nt}=1$; the experiment time is ~ 15 minutes
if the S/N > 100:1, use $\text{nt}=4$; the experiment time is ~ 1 hour

ghmbc: if the S/N > 250:1, use $\text{nt}=8$; the experiment time is ~ 45 minutes. It should be 4 to 8 times of that necessary for the gHMQC experiment.

Note:

In a 1D experiment, if you want to double the S/N, you need to increase 4 times nt used.

F. 2D Data Processing (This section will be expanded later)

Unless you have been informed of a macro that will do the proper data processing of your spectrum for you, you will need to process the data manually. Below is a table showing the optimum processing apodizations (window functions), and the proper processing commands in Vnmr software.

Experiments	Apodization 1	Apodization 2	Processing	Process Shortcut
gCOSY ** (phase=1)	sb=-at/2	sb1=-ni/(2*sw1)	wft2d('t2dc','1,0,0,1') foldt (optional)	wft2d foldt
TOCSY (phase=1,2)	gf=at/2	gf1=ni/(2*sw1)	wft2da	
gHMQC (phase=1,2)	gf=at/2	gf1=ni/(2*sw1)	wft2da	
gHMBC (phase=0)	gf=at/2	gf1=ni/(2*sw1)	av av1 wft2da	

**For gCOSY, gf=at/2 and gf1=ni/(2*sw1) can be used to enhance S/N

For plotting, you can use **cosyplot** and **hetcorplot**. Check the appropriate handouts for more information.

cosyplot can be used on the instrument or the SUNDS.

If you want to use **hetcorplot**, you need to have acquired a “good” 1D ¹³C spectrum on an instrument other than the ui500nb. You can then plot on the SUNDS.

To plot using **hetcorplot** on the SUNDS:

1. logon to the ui500nb
2. jexp1
3. load your 1D ¹H fid and process
4. jexp4
5. load your ghmqc data and process
6. logon to the instrument with your 1D ¹³C fid
7. jexp2
8. load your 1D ¹³C fid and process
9. jexp4
10. dcon1 to display 2D data
11. set the window as you want, then type **hetcorplot** and answer the questions as appropriate.

Some useful commands:

pcon page used to plot 2D spectrum as displayed on the screen
dcon1 used to redisplay 2D after changes, e.g., vs2d
fullt sizes the 2D display so that 1D spectra can be plotted on the side and top of the 2D plot
vs2d used to rescale the 2D plot; used dcon1 after using this command

Supplemental material on processing 2D spectrum:

1) At end of experiment, set appropriate weighting functions and linear prediction parameters, type commands:

```
setLP1  
sqisinebell
```

2) If f_n parameter now equals 4096, process will be slow, then type:

```
fn = np  
fn1 = fn
```

3) Switch f_1 and f_2 axes (make f_2 the x-axis), type

```
trace = 'f2' or trace = 'f1'
```

4) Display full across screen (removes error message)

```
full
```

5) Display full ppm scale

```
f
```

6) Display as contour plot, type command:

```
dp10
```

7) Spectrum should be appropriately referenced already, but you should confirm this; if necessary re-reference by putting cursor on appropriate diagonal peak and type (assuming CDCl_3):

```
rl(7.26p)  
r11(77d)  
dp10
```

8) Adjust vertical scale with vs +20% and vs -20% menu buttons or with middle mouse button or manually changing the parameter, vs2d, so vs2d = 100 (the lower the number, the less noise displayed), then redraw 2D with dp10 command

9) Print with full rectangle, type:

```
full  
dp10  
pcon(10,1.2) page
```

10) Save data, type:

```
svf('filename')
```


G. Chemical Shift Referencing:

¹H Spectrum:

You can use either an internal chemical shifts standard (e.g. TMS: 0 ppm) or a residual ¹H resonance from the deuterated solvent (e.g. 7.26 ppm for CDCl₃). Less commonly you can use a capillary containing a standard to avoid any chemical shifts changes due to solvent conditions such as pH and concentration etc.

¹¹B Spectrum:

The commonly used ¹¹B chemical shift standard is 15% (v/v) BF₃.OEt₂ in CDCl₃, and is referenced to 0.0 ppm. When you use the standard parameter loaded from the manual bar, the BF₃.OEt₂ in CDCl₃ will appeared at 0.0 ppm.

¹³C Spectrum:

Commonly a residual ¹³C resonance from the deuterated solvent is used as the reference.

¹⁹F Spectrum:

The chemical shift standard for ¹⁹F is neat CFCl₃, which is referenced to 0.0 ppm; which giving TFA at -73.6 ppm, or 0.05% C₆H₅CF₃ in C₆D₆ at -62.9 ppm.

³¹P Spectrum:

85% phosphoric acid (H₃PO₄) in D₂O is used, giving a reference peak at 0.0 ppm, or 0.0485M triphenyl phosphate in CDCl₃ is reference at -17.9 ppm with respect to the phosphoric acid.

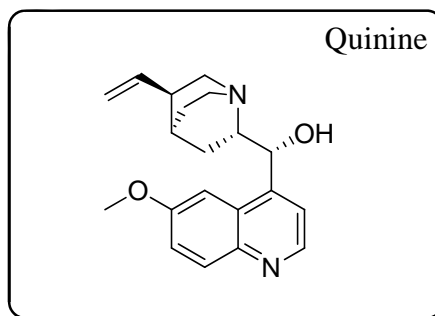
¹⁵N Spectrum:

Different referencing schemes have been in common use as reported in literature. Setting the chemical shift of nitromethane at 0.0 ppm results in most compounds have negative values of ¹⁵N resonances. The chemical shift of liquid ammonia (used for bio-samples) is 380 ppm away from nitromethane (used for organic compounds). Here is the table listing the ¹⁵N Chemical shifts expressed with respect to both reference compounds.

Chemical Shift Referencing Schemes for ¹⁵N

Compound	δ(NH ₃)	δ(CH ₃ NO ₂)
Amines	-49	-429
NH₃	0	-380
NH ₄ NO ₃	21	-359
NH ₄ Cl	39	-341
Amides	119	-261
CH ₃ CN	243	-137
Nitriles	258	-122
Pyridine	319	-61
Imines	343	-37
NH ₄ NO ₃	376	-4
CH₃NO₂	380	0
Nitrates	388	8

H. Structure Elucidation: Quinine as an Example



Chemical Formula: C₂₀H₂₄N₂O₂

Exact Mass: 324.18

Molecular Weight: 324.42

H-1: ¹H NMR proton data (CDCl₃): The results of ¹H NMR spectroscopic analysis (Figure H-1) are consistent with the structure of Quinine. A total of 23 protons were observed examined by integration with a long T₁ recovery delay (The OH proton was missing in the spectrum due to its broadness) including 5 aromatic protons, 3 vinyl protons, and 15 aliphatic protons. The aliphatic protons was further separated to 4 methine, 4 methylene groups and 1 methyl group as drawn from logic of further work shown below.

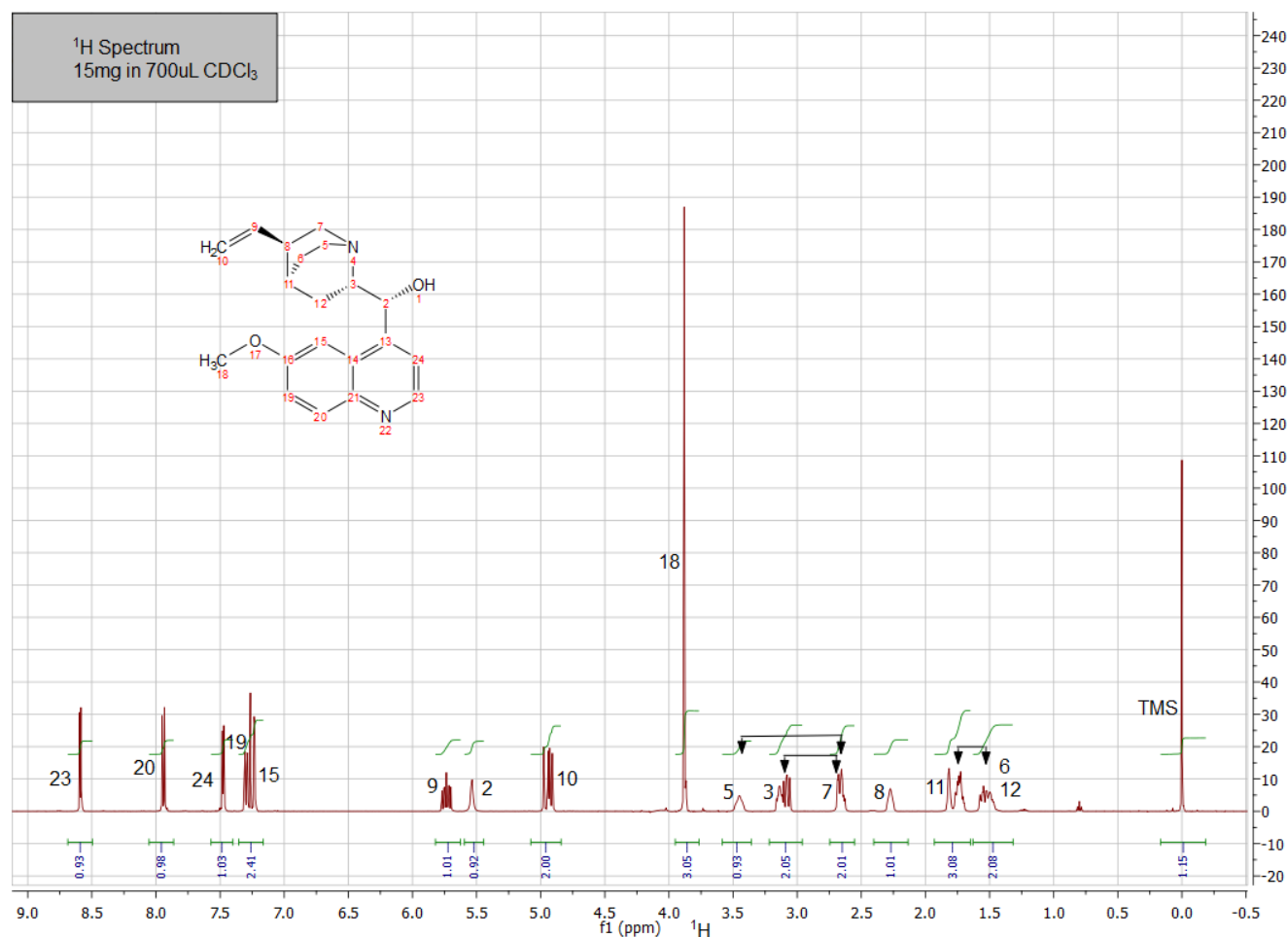


Figure H-1: Proton Spectrum of Quinine.

H-2: ^{13}C NMR carbon data (CDCl_3): The Attached Proton Test (APT) ^{13}C data collected with a fully decoupled proton domain is present in Figure H-2. The J-modulated ^{13}C data exhibit up and down peaks depending upon the numbers of its directly attached protons giving CH and CH_3 up and CH_2 and quaternary carbons down or vice versa. A total of 20 carbon peaks were observed, consistent with the structure of Quinine.

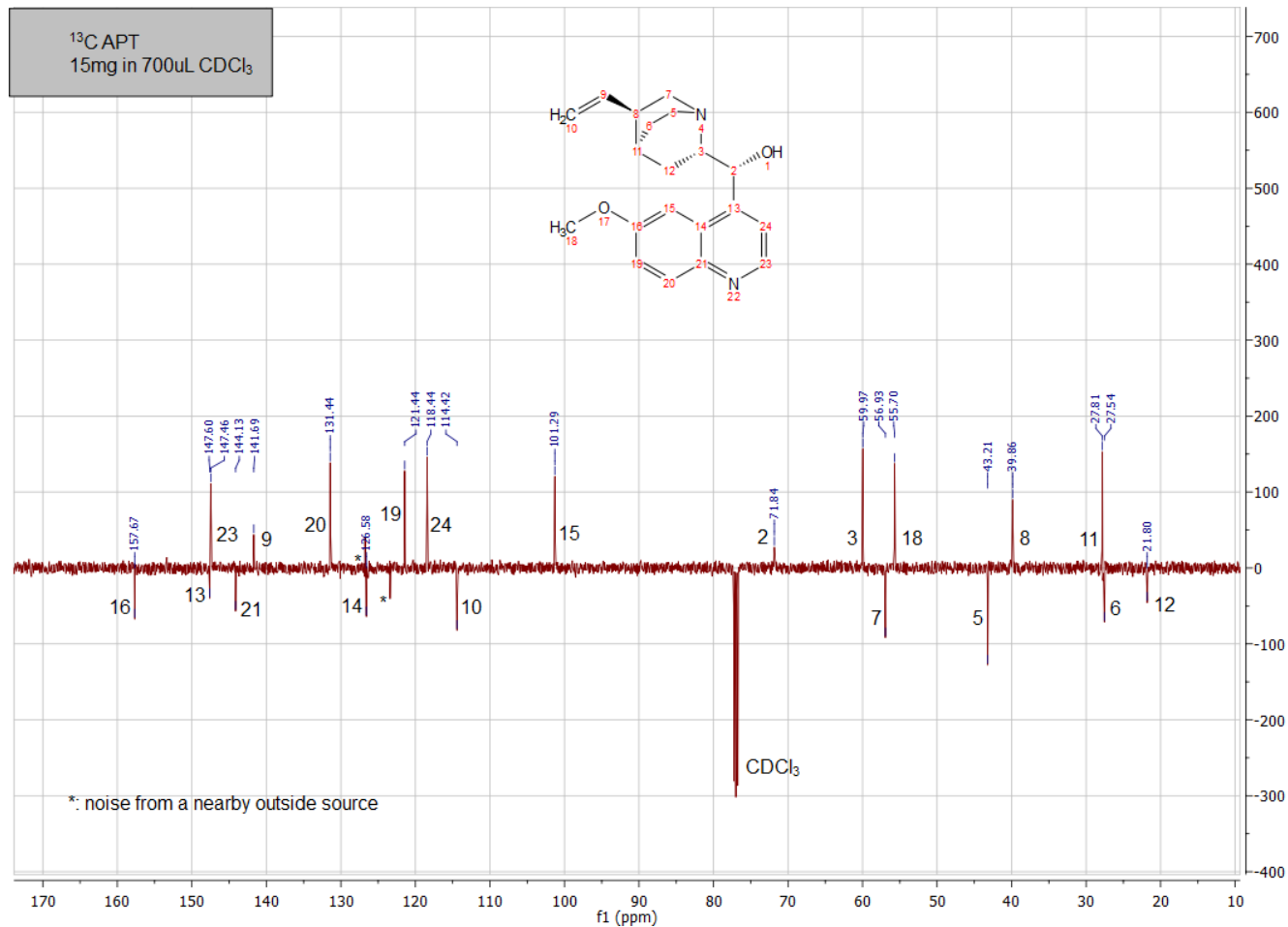


Figure H-2: Carbon-13 spectrum of Quinine

H-3: ^1H - ^1H NMR 2D gCOSY data (CDCl_3): The data developed from the 2D gCOSY experiment are illustrated in Figure H-3. These data reveal isolated contiguously coupled spin systems. Two aromatic spin systems and one continuously coupled spin system (including aliphatic and vinyl protons) are observed.

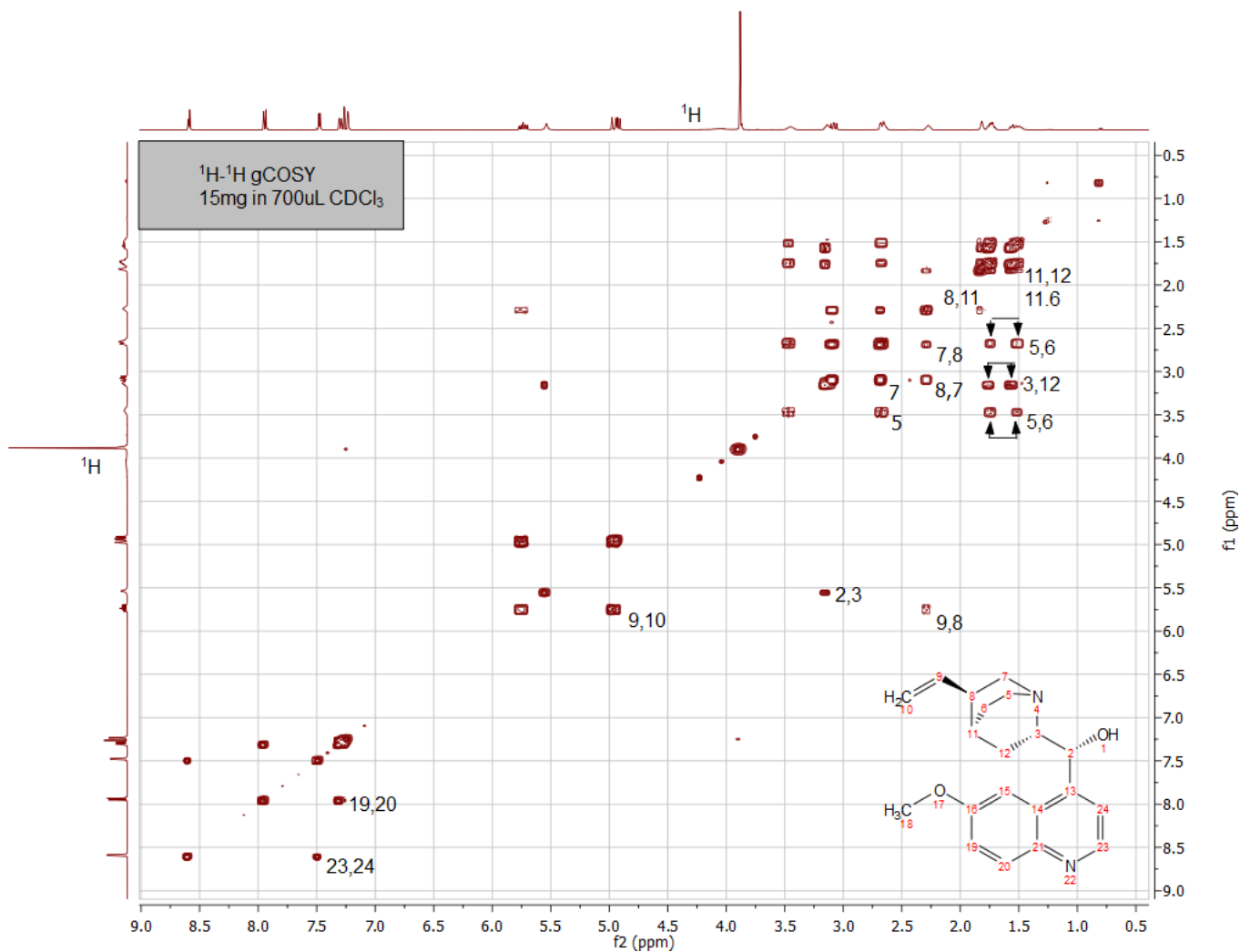


Figure H-3: ^1H - ^1H 2D gCOSY spectrum of Quinine

H-4: ^1H - ^{13}C NMR 2D HSQC data (CDCl_3): The data developed from the 2D ^1H - ^{13}C HSQC experiment are illustrated in Figure H-4. These data reveal one bond cross correlations between protons and carbons in a two dimensional format. Assignments in either domain allow direct assignment of the opposite domain from their cross contours. The assignments illustrated in Figure H-4 are based upon data and conclusions obtained from other experiments, i.e. 2D gCOSY and 1D APT carbon data, chemical shifts and coupling constant considerations. These data are consistent with the structure of Quinine.

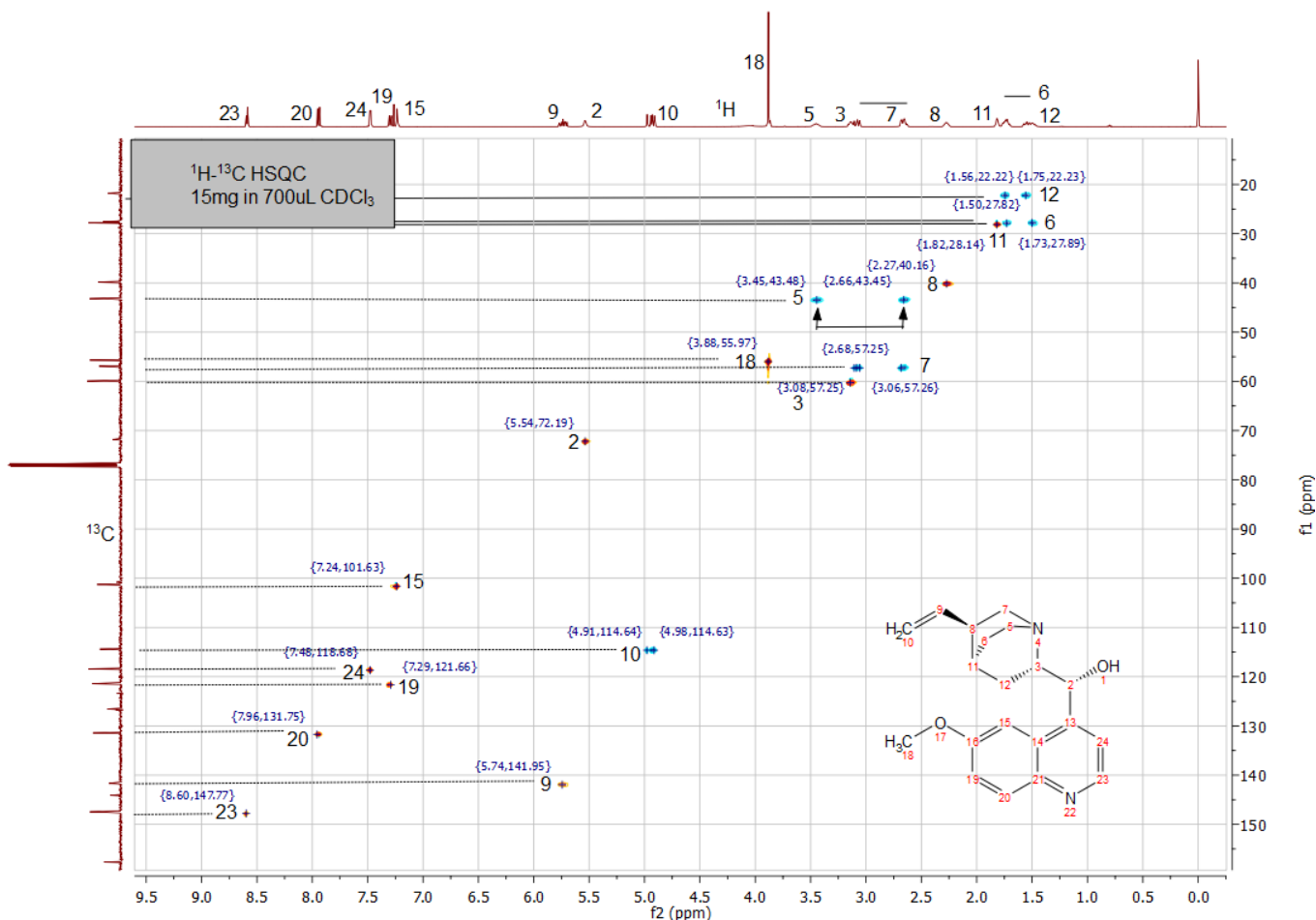


Figure H-4: ^1H - ^{13}C 2D HSQC Spectrum of Quinine

H-5: ^1H - ^{13}C 2D HMBC data (CDCl_3): The data developed from the 2D HMBC experiment are illustrated in Figure H-5. These data reveal multiple-bond cross correlations between protons and carbons in a two dimensional format. The assignments illustrated in Figures H-5 are primarily from 3-bond proton-to-carbon cross correlations. The experiment is optimized with a time delay for 8 Hz coupling ($^3J_{\text{HC}}$ coupling). Occasionally 2-bond and 4-bond correlations will appear. Furthermore, the suppression on one-bond couplings also appears in the spectra due to non-optimal bird-pulse suppression. Some of the important 3-bond correlations are summarized in the structural illustration below:

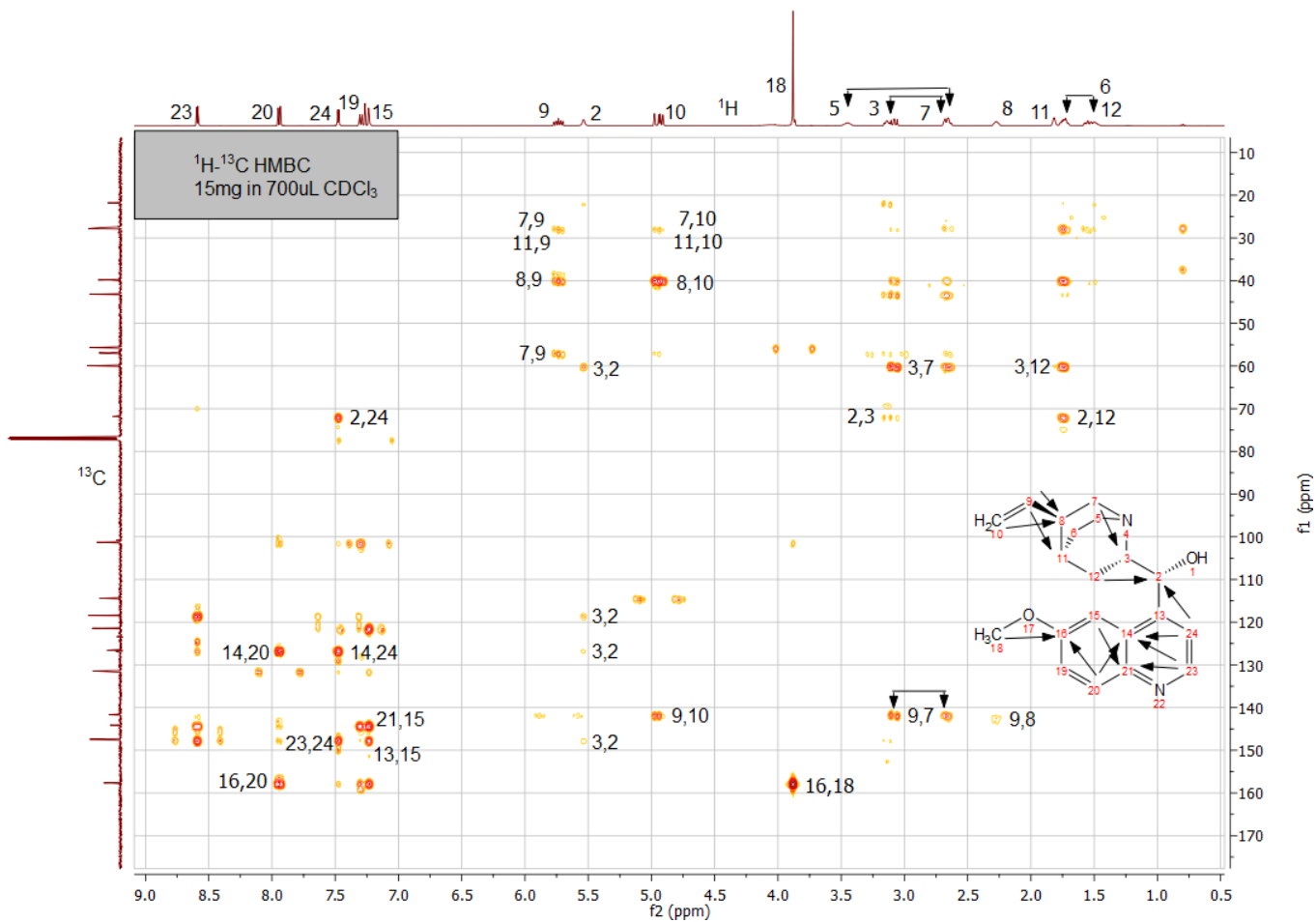


Figure H-5: ^1H - ^{13}C 2D HMBC Spectrum of Quinine

H-6: ^1H - ^1H NMR 2D NOE data (CDCl_3): The results of ^1H - ^1H NMR 2D NOE spectroscopic analysis (Figures H-6) are consistent with the structure of Quinine. These data reveal through space or proximal located spins and are based upon relaxation phenomenon. Thus, strong NOE observed between protons 2 and 5, and strong NOE observed between protons 9 and 3 suggest that the relative stereochemistry of this compound is as shown.

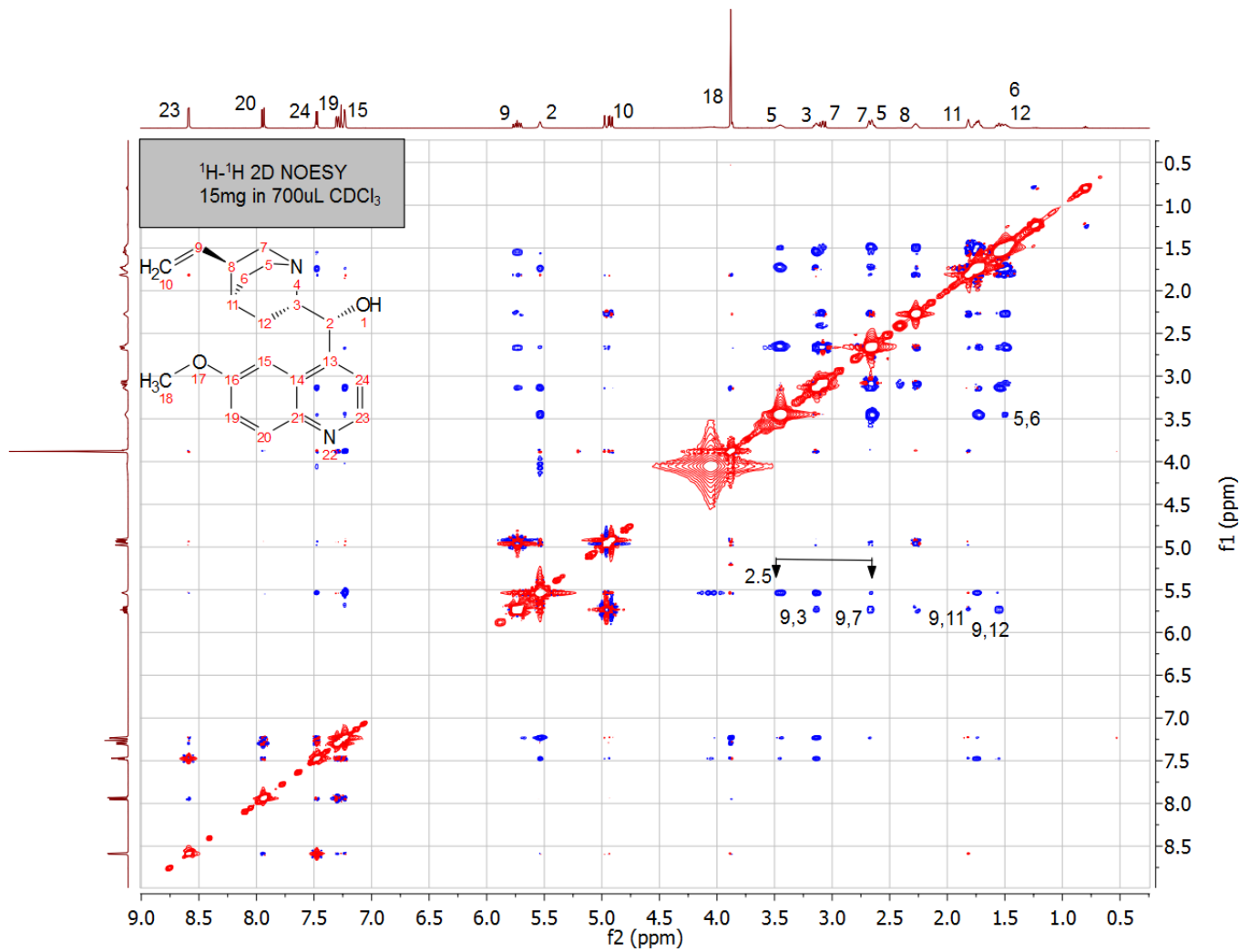


Figure H-6: ^1H - ^1H 2D NOESY Spectrum of Quinine