

## Fall 2014 Chem 636 – Lab #5 Advanced $^{13}\text{C}$ 1D NMR: PT and Multiplicity Editing

Assignment due at beginning of lab, week 6 (Oct 7 – Oct 10).

**Use Callisto for this week's HW #5.**

**Although you may work in groups, each of you should setup and acquire your own data, and process and hand in that individual set of data.**

**Reading** – Spin Physics: Claridge section 4.3 (for those interested)  
 – Polarization Transfer experiments: Claridge section 4.4 (primary reference)  
 – edited  $^{13}\text{C}$  experiments: Claridge section 4.3, 4.4 (both are advanced discussions)

**Goals** – Learn the major polarization transfer (PT)  $^{13}\text{C}$  1D experiments, and their correct setup. Gain some impression of sensitivity issues with  $^{13}\text{C}$  NMR. Introduction to 2D NMR via the 1-bond  $^1\text{H}$ - $^{13}\text{C}$  heterocorrelation experiment, HSQC.

This write-up is rather long. Some descriptive sections will be *italicized*, to separate them from the practical experimental details and requests.

In this lab, you will acquire a variety of spectra using pulse sequences that utilize *polarization transfer* (PT). This technique is applicable to many nuclei: for  $^{13}\text{C}$ , it enhances sensitivity and enables “multiplicity analysis”. The most popular variant is the DEPT experiment. You will also look at APT and INEPT experiments, and will be given suggestions as why those alternatives exist. Heterocorrelation 2D experiments provide similar information, but add to that significantly by correlating each  $^{13}\text{C}$  nucleus to its coupled proton(s). You will obtain data using the 1-bond multiplicity-edited version, specifically  $^1\text{H}$ - $^{13}\text{C}$  HSQC.

1. *Keep your thinking hat on, please.*
2. Ask questions! And document and report — email is usually best — any problems you observe with the instrument. Don't assume staff will already be aware of the problem.

*Considerations for PT experiments: DEPT is usually superior to INEPT*

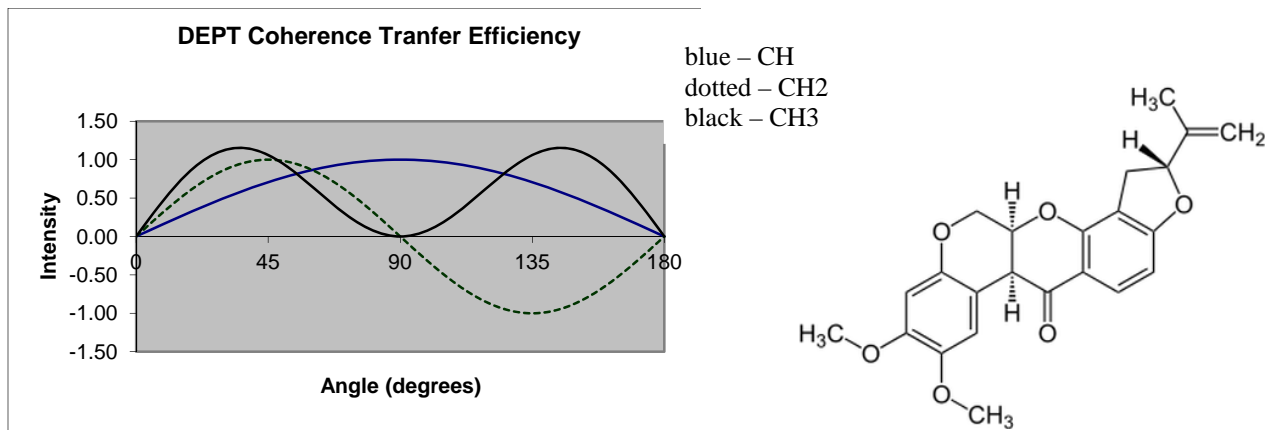
- DEPT generates less distortion — thus the acronym “*distortionless enhancement by polarization transfer*” — compared to INEPT when a number of different  $J$ -couplings exist in the sample. This situation is true for  $^1\text{J}_{\text{CH}}$  in organic compounds, where e.g.,

$$^1\text{J}_{\text{CH}} \sim 110\text{-}130\text{Hz for aliphatic carbons, and}$$

$$^1\text{J}_{\text{CH}} \sim 145\text{-}160\text{Hz for aromatic carbons.}$$

DEPT is therefore usually the preferred experiment for  $^{13}\text{C}$  NMR.

- Both experiments (but most commonly DEPT) can be used for “spectrum editing,” or “multiplicity analysis.” The editing is based on the phase of the  $^{13}\text{C}$  nucleus resulting from the *number* of attached protons, as shown in the figure below. Angle refers to the final *nutaton* of the magnetization, and is set with a pulse length (here **P0**) being 1/2, 1 or 3/2 times the  $90^\circ$  pulse (**P1**) in the experiment, leading to DEPT-45, -90 or -135 spectra respectively. INEPT spectra can be run similarly, but rely on a change in delay (**D3**) rather than nutation.



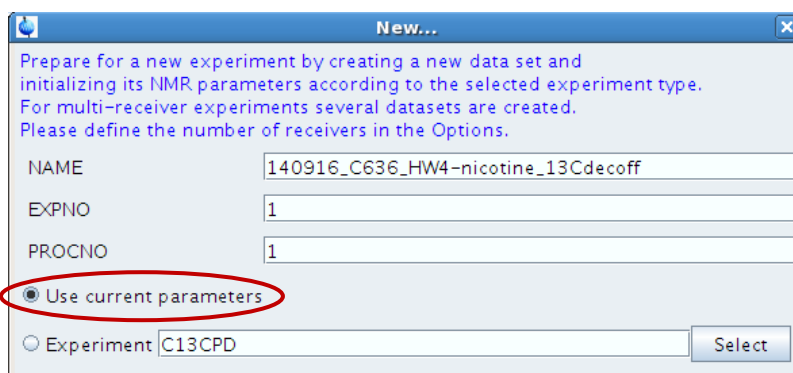
### I. Multiplicity-editing using PT


- Acquire a “standard”  $^{13}\text{C}$  spectrum of the 50mM sample of rotenone in  $\text{CDCl}_3$  using  $\text{NS}=8$ .
- Acquire DEPT-45, DEPT-90 and DEPT-135 spectra (use PULPROG changes for setups). Use  $2\times$  the minimum  $\text{NS}$  required for these experiments. Use the pulse sequence comments — at the bottom of the pulse sequence listing — to obtain this information.

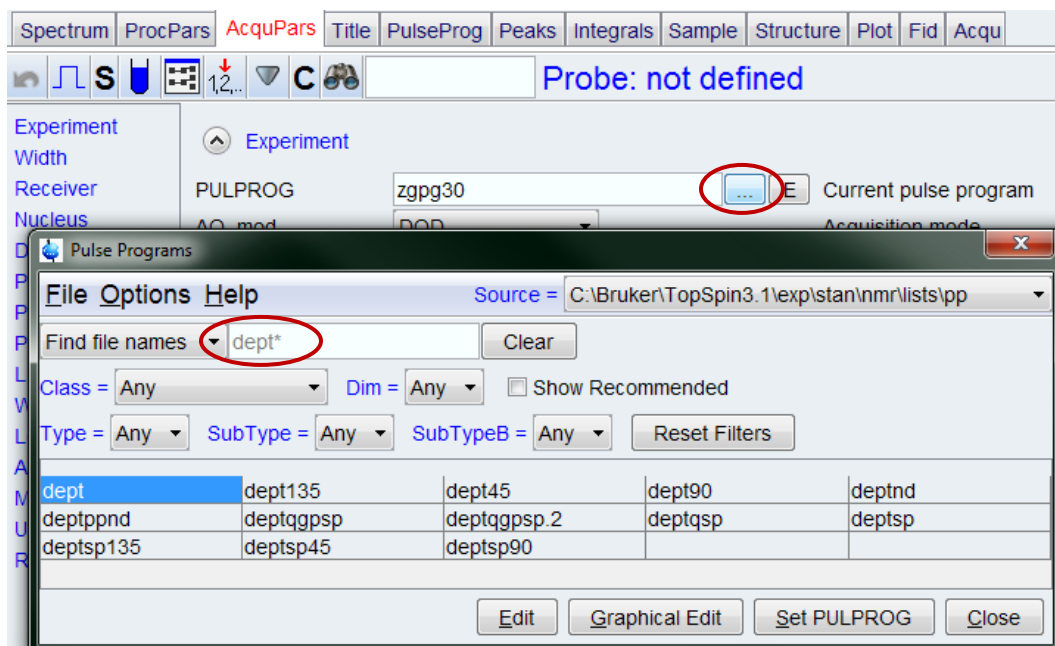
*This brings us back to having many ways to setup and run these experiments. Changing the pulse sequence is definitely one way, similar to what we did in the previous HW, and we’ll proceed with that method for the DEPT experiments. But later we’ll run into a serious issue with Bruker pulse sequence libraries: the naming of the pulse sequences often works against discovering which one to choose for a particular experiment. A particular strong example is the APT experiment, as we’ll see soon.*

*The advantage of changing pulse sequences is that other parameters such as **SW** and **O1p** (i.e., the spectral window) are kept the same. A disadvantage, in addition to the naming issue, is that other parameters important to the new pulse sequence may not be set correctly. Proper use of parameter sets can avoid most of these issues. We’ll use that method in the next section.*

- 👉 To acquire a DEPT-135 experiment, start with the acquired  $^{13}\text{C}\{^1\text{H}\}$  spectrum from part a). Use **new** with the “Use current parameters” box checked, as in the previous HW#4:



Change the filename, or increment the EXPNO. The  $^{13}\text{C}\{^1\text{H}\}$  parameters will be copied to the new experiment. Now change the **PULPROG = deptsp135**.<sup>1</sup> One way to find pulse sequences is to click on the  button, and then select the desired sequence:



Now we see some of the problems with the pulse sequence library: there are often many variations, with little direct help to assist in deciding which to choose. Here the help is written into this document: **deptsp45**, **deptsp90** and **deptsp135** are, we believe, the better sequences.<sup>2</sup> But what if you don't have notes around?

Set **NS** = 2×(minimum phase cycle) as described at the bottom of the pulse sequence listing. Keep in mind that you (should!) have already locked, tuned the probe (atma), and shimmed the sample. The change in pulse sequence has not affected these functions, so you can now simply do **rga** and then **zg**.

Repeat the procedure above to obtain DEPT-45 and DEPT-90 spectra, each having **NS** = 2× the minimum phase cycle recommended in the pulse sequence.

1 Plot the three DEPT and the  $^{13}\text{C}\{^1\text{H}\}$  spectra as a stack plot. Do a full multiplicity analysis: determine the number of CH, CH<sub>2</sub>, CH<sub>3</sub>, and quat carbons in the sample. Make at least two assignments, i.e., the two that should be obvious from simple chemical shift considerations. See if you can assign the three CH<sub>2</sub> moieties (if you've had Chem 605, this will be simple).

Q1 Did the DEPT-45 have any value for the multiplicity analysis? In what way did the DEPT-90 help? Could you have managed with just the  $^{13}\text{C}$  spectrum and the DEPT-135?

<sup>1</sup> PULPROG = dept or dept135 (or other variants) would also work, but the "shaped pulse" in the deptsp135 is the best version.

<sup>2</sup> Pulse sequence variations are created as new versions are published. Newer sequences are usually (but not always!) better. Researchers, even so, tend to stick to what they know, so Bruker keeps the older sequences around. Some variations arise from competing research groups, and in such cases there may be no consensus as to which is best.

- c) Acquire an INEPT-135<sup>3</sup> spectrum, again with 2× minimum NS (using the facility Experiment / Parameter file for the setup).

*We could setup this experiment as done in the previous section using **PULPROG = ineptd**. **cnst2** and **cnst11** might not be set correctly, but checking the pulse sequence comments would assist in getting those right.*

✎ Instead, we can look for the appropriate parameter set by selecting the Experiment in the **new** window. If you look in the Bruker **standard** area, using C13\* or \*INEPT\*, you won't find anything (rather odd...). In the **uwchem/par** area, on the other hand, there is **C13inept135**. Choose this experiment to setup the parameters: you will find that **cnst2=145** and **cnst11=3** (the setting for the dept135 analog), as examples. Set **NS=2×minimum**.

2✎ Plot the DEPT-135 and INEPT-135 spectra as a stack.

- Q2** Is the INEPT-135 spectrum substantively different than the DEPT-135? What parameter can be changed in the INEPT-135 to change to an INEPT-90 (or better said, a DEPT-90 analog)?

*As mentioned above, for many compounds INEPT will produce significant distortions due to variations in  $^1J_{CH}$ . These distortions may make the spectrum difficult to phase, and DEPT is superior. [Note: Modern spectrometers and pulse sequences — **ineptd** is an improved version of **inept** — reduce such issues, so differences may be subtle in your spectra.] So why bother with INEPT at all? It turns out that INEPT is nearly 1/3 the length of a DEPT experiment, e.g., 4 ms vs 10 ms in total length. This savings can be critical in some situations:*

- *INEPT can provide better sensitivity for experiments where a single J-coupling value is involved, e.g.,  $^{29}\text{Si}$  spectra of a compound with one silicon nucleus.*
- *INEPT can be significantly better than DEPT when J-couplings are small (making some delays in the experiment long), or when the MW or viscosity is large (e.g., when the temperature is close to the freezing point of the solvent). The INEPT-45 (all peaks positive) analog is shortest, and is the preferred experiment in such situations.*

✎ **The standard recommendation for 1D  $^{13}\text{C}$  NMR is to acquire a standard  $^{13}\text{C}\{^1\text{H}\}$  spectrum and a DEPT-135 spectrum.** *This combination is considered to be optimal in spectrometer time needed, and often provides unambiguous multiplicity analysis. If some confusion exists in the analysis, adding a DEPT-90 spectrum will help. The above recommendation is common, but not completely accepted. Some researchers, it seems especially in Europe, prefer obtaining a single spectrum (rather than two) that has multiplicity editing similar to DEPT-135, but also contains all quaternary carbons. The Attached Proton Test (APT) and DEPTQ-135 both provide data of this type (see Claridge sections 4.3.2 and 4.4.4 for more information).*

- c) Acquire an APT (Attached Proton Test) spectrum [with 2× minimum NS].

For setting up this experiment, use **new** and select C13APT from the Bruker *standard* parameter set library. Set **NS=2×minimum** but looking at the comments in the pulse sequence listing.

<sup>3</sup> INEPT = insensitive nuclei enhanced by polarization transfer

**Q3** From the pulse sequence listing, what are two parameters that are important for PT and multiplicity editing?

Note that **PULPROG = jmod**. Here we see full force the problems with attempting to use pulse sequence changes: what the heck is **jmod** ?? The name, arising J-modulated, dates back to the initial publication of this type of experiment. So it means something to older spectroscopists, and those with detailed literature knowledge. But it is essentially meaningless to the vast majority of chemists and biochemists. The facility staff could rename the pulse sequence, but it is simpler to use the parameter set / experiment method, as that also get other parameters set to typical values: reading in C13APT makes sense, and we'll pretty much standardize on this method for future experiments (not always, but most of the time).

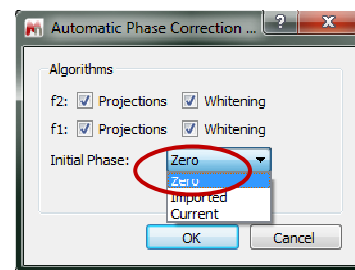
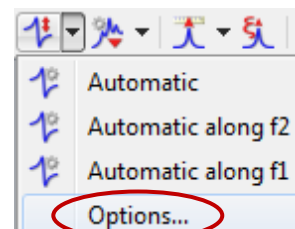
d) Acquire a DEPTQ-135 spectrum [with 2× minimum NS].

The parameter set is in the **uwchem/par** facility folder.

3 Plot the APT, DEPTQ-135, and  $^{13}\text{C}\{^1\text{H}\}$  spectra as a stack.

**UhOh!** MNova often has problems with experiments it does not recognize.

And unhappily, in those situations its behavior is not always consistent. DEPTQ-135 is one of those experiments. On my (cgf) laptop, MNova decides to perform a magnitude calculation on the spectrum: all the peaks are made positive, removing the multiplicity information. To fix this, click Options in the Phase dropdown, and select Zero for Initial Phase. You may need to do the same procedure with your HSQC spectrum (see below).



**Q4** What seems to be the primary issue with these data? If you wanted to perform a full multiplicity analysis, what limitations might you run into? What part of the spectrum dominates how long the experiment would have to be run?

Considerations for PT experiments: it's not completely clear, so choose your favorite [again, the majority opinion is that the combination of  $^{13}\text{C}$  plus DEPT-135 is best.]

You may have a postdoc in your group that likes APT. It has a fan-base (especially in European labs). Perhaps an improved version is used over there? We have an old version provided by Bruker, and it is definitely problematic with distortions, similar to (the original) INEPT. If you like the simplicity of a single  $^{13}\text{C}$  spectrum with multiplicity-editing and quat carbons, DEPTQ is a newer and (likely) superior sequence.

- The Attached Proton Test, APT, is an experiment that shows quaternary carbons and multiplicity edited-protonated carbons in a single spectrum. The version implemented on our Bruker spectrometers is old, and distortions due to variations in  $^1J_{\text{CH}}$  will be common.
- A new form of DEPT called DEPTQ+ is similar to APT but eliminates its problems: quaternary and edited protonated carbons are observed in a single “distortionless” spectrum (see Claridge Fig. 4.36).
- Both APT and DEPTQ+ are difficult to optimize for observation of quats, and will almost always give poorer signal-to-noise than a standard  $^{13}\text{C}\{^1\text{H}\}$ . Keeping things simple with a combination of standard  $^{13}\text{C}\{^1\text{H}\}$  plus DEPT-135 is recommended (but edited-HSQC...!).

## II. Multiplicity-editing $^1\text{H}$ - $^{13}\text{C}$ HSQC

For many situations, a  $^1\text{H}$ - $^{13}\text{C}$  1-bond heterocorrelation 2D experiment (HSQC) is far superior to any combination of DEPT, INEPT or APT experiments. The chemical and pharmaceutical industries have standardized their protocol for acquiring “routine” NMR data: the minimum protocol is to always acquire  $^1\text{H}$  1D and  $^1\text{H}$ - $^{13}\text{C}$  edited-HSQC 2D spectra for every sample submitted. This inclusion of  $^1\text{H}$ - $^{13}\text{C}$  HSQC arises from two major advantages of this experiment:

A. HSQC is significantly more efficient than  $^{13}\text{C}$ -detected experiments: for dilute samples, HSQC is  $> 100\times$  faster than a  $^{13}\text{C}$  1D experiment. For samples where direct  $^{13}\text{C}$  data cannot be obtained, HSQC can provide complete  $^{13}\text{C}$  chemical shift data for all protonated carbons.

B. In addition to providing accurate  $^{13}\text{C}$  chemical shifts for all protonated carbons, HSQC also connects each carbon to its attached proton(s). This information is very useful for structure verification and elucidation.

a) Acquire a (multiplicity-)edited-HSQC spectrum. The Experiment / parameter set to choose in the **new** panel is **HSQCEDETGPSISP**.<sup>4</sup>

**Ugh!** You might be wondering what the heck is up with the acronyms for the parameter sets?! Here’s a breakdown for HSQCEDETGPSISP, just for kicks:<sup>5</sup>

HSQC	→	heteronuclear single-quantum correlation (are you happier?)
ED	→	multiplicity- <b>ed</b> ited
ET	→	echo/anti-echo- <b>TPPI</b> method (feeling better?)
GP	→	using gradients with “: <b>gp</b> ” syntax (yee-haa!)
SI	→	sensitivity improved
SP	→	using a shaped pulse


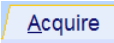
*Initially this doesn’t help much, but after a while, it starts to make sense (to NMR jocks). The facility staff will soon update the experiment setups (they’ll all go into the uwchem/par folder), and we’ll rename them to be more plain-chemist English. But in general, users of Bruker spectrometers learn to keep a list of the most common experiments they use close by.*


b) Use  $2\times$  the minimum number of **NS**, and use the defaults for (almost) everything else. Other important parameters for HSQC are: **TD[2]**, **TD[1]**, **DS**, **SW[2]**, **SW[1]**, **O1p[2]**, **O1p[1]**, **RG**, **CNST2**, and **AQ[2]**.<sup>6</sup>


<sup>4</sup> The very similar HSQCEDETGPSISP\_ADIA might be slightly better on a 500. The \_ADIA (**adiabatic**) gains significant utility on very high-field spectrometers by providing better bandwidth coverage for the  $^{13}\text{C}$  180° pulses.

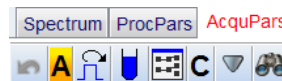
<sup>5</sup> Bruker provides a table of the primary acronyms used in the pulse sequence folder. These can be accessed by going to /opt/topspin3.2.5/exp/stan/nmr/lists/pp (or type the alias **cdpp** in a Unix window) and view the \*.info files.

<sup>6</sup> 2D spectra always have an F2 directly-detected axis (in TopSpin and MNova, always horizontal or abscissa), and an F1 indirectly detected axis (always vertical, ordinate). For HSQC,  $^1\text{H}$  is directly detected and is therefore on the F2 axis. Parameters associated with the  $^1\text{H}$  detection are indicated above by [2].  $^{13}\text{C}$  is indirectly detected, and is on the F1 axis using parameters [1]. You don’t type in the [2] or [1]: to change either value of **SW** in TopSpin, just enter **sw** at the command line. Or use the ACQUPARS tab to find the **SW** row: F2 and F1 columns are labeled at the top. More discussion of 2D NMR will start in lecture and labs in a few weeks.


c) The only other change for the HSQC that we'll make in this HW is to optimize the  $^1\text{H}$  spectral window. TopSpin provides very good tools for making this adjustment, with the new  button on the  flowbar [it shows up only for 2D data]. Click the SetLimits button and bring in the  $^1\text{H}$  1D spectrum (e.g., by left-dragging it in from TopSpin's Browser). Set the spectral region, and make sure ~10% baseline is included on both edges. Click OK, and TopSpin will set **SW[2]** and **O1p[2]**.

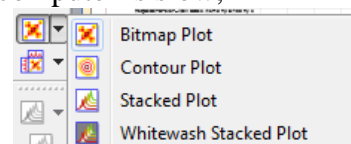
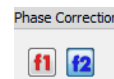
 It is very important to check **AQ[2]** to insure that it remains  $\leq 0.2\text{s}$ . **Failure to do so may result in damage to the probe** [as stated on the taped-on notes on the spect. monitors].

Look at the parameter listing specific to the pulse sequence: click the  button in ACQPARS, or type **ased** in the TopSpin command line. Yup, this is a much more complex pulse sequence with a lot more parameters. The normal parameters are still important: e.g., D1, NS, SW, and O1p.  $^1\text{H}$  parameters are above  $^{13}\text{C}$  — in the carbon experiments they were the other way around — because here we are *detecting* protons, and *indirectly* obtaining the correlated  $^{13}\text{C}$  chemical shifts. Click **A** in ACQPARS and you'll see a bigger listing, now separated by F2 and F1 columns. For 2D spectra, you'll need to switch back and between these displays.



**Q5** In the **ased** display, what is the new parameter that is important, and has been present for all the polarization transfer experiments we've been running? [Be a chemist; which one has information that is simple and you recognize?]

 Bring the HSQC into MNOVA, and properly phase it. Phasing works essentially the same as for 1Ds, but now can be applied in both F1 and F2 (you'll see the buttons to switch between them in the Phase Correct box of MNOVA). If your computer is slow, turn off Contours and use Bitmap Plot. If all the peaks are positive, perform the same Initial Phase  $\rightarrow$  Zero trick as shown above for the DEPTQ.



Open the  $^1\text{H}$  and  $^{13}\text{C}$  spectra in the same MNOVA document, then drag them onto the HSQC. They will automatically be placed as Traces on the spectrum. Rolling the mouse over the trace and using the middle wheel will adjust the intensity of the 1D traces. Typically, the 2D plots looked best displayed in contour mode.

Plot the 2D spectrum with  $^1\text{H}$  and  $^{13}\text{C}$  1D traces, and upload as normal.

**Q6** Do you get the same carbon count in the HSQC 2D spectrum as in the  $^{13}\text{C}\{^1\text{H}\}$  experiment? How about the DEPT-135? If not, why not?

**Upload 4 plots as .mnova and .pdf files, and answer the 6 questions.**