

**1D methods for <sup>13</sup>C**

The effect of the <sup>1</sup>H nOe vs. the effect of decoupling, calibration of decoupling using strychnine in CDCl<sub>3</sub> with CrAcac.

The maximum nOe (extreme narrowing condition for two isolated spins) is

$$\eta_{\{S\}} = \frac{\gamma_s}{\gamma_I} = \frac{I - I_0}{I_0}$$

This is the amount by which <sup>13</sup>C signal intensity is increased when <sup>1</sup>H is saturated (I), over the <sup>13</sup>C signal intensity when <sup>1</sup>H is not saturated (I<sub>0</sub>). Signals are also stronger-looking if they are not split by J coupling to their attached protons, thus, sensitivity gains are obtained by acquiring <sup>13</sup>C spectra with <sup>1</sup>H decoupling.

We will collect <sup>13</sup>C spectra without the nOe vs. with nOe by setting the <sup>1</sup>H decoupling mode to dm = 'n' vs. 'y' during d1. We will also collect <sup>13</sup>C spectra with and without <sup>1</sup>H decoupling during acquisition, again via dm. The d1 interval is the A status period in the s2pul pulse sequence, the at is the C status period and the B status period is essentially irrelevant because we have d2=0. vs. nOe (dm = 'yyy','yyn','nny','nnn'). Thus, for <sup>1</sup>H saturation only during d1 set dm = 'ynn', for decoupling only during at set dm = 'nny', for both set dm = 'yyy' (the usual) and for neither set dm = 'nnn'. <sup>1</sup>H decoupling also permits faster recycling between scans. To see the benefits of these different devices, compare spectra obtained with dm = 'yyy','yyn','nny','nnn'. Be sure that at is short (≈0.2 s) and d1 is long (≈3 s) to maintain good saturation with dm = 'yyn' and to not get an accidental nOe when dm = 'nny'. Maintain **dmm = 'w'** for waltz decoupling, which roughly doubles the bandwidth you achieve for a given decoupling field. For best results, the <sup>1</sup>H should be calibrated (see below).

Calibrating the <sup>1</sup>H decoupler to be used when observing <sup>13</sup>C

Collect a <sup>1</sup>H 1D as well as a <sup>13</sup>C 1D in advance. From the <sup>1</sup>H spectrum determine the frequency of the highest field line in the spectrum (lowest chemical shift) and the lowest field (highest chemical shift), using '**sd**' (write the frequencies down), use the same '**solvent**' as you will use in the decoupling calibration. From the <sup>13</sup>C spectrum determine the <sup>1</sup>J<sub>CH</sub> (C-H coupling constant) of the line whose decoupling you will assess (use the same line throughout), also calibrate the pw90 for <sup>13</sup>C, by direct observation of a strong resonance (such as solvent) while arraying pw.

**dof** is the parameter that sets the decoupler offset frequency, exactly as **tof** sets the transmitter offset (the center frequency). Thus, **dof** will determine the frequency at which <sup>1</sup>H decoupling is to be applied. In your <sup>13</sup>C experiment, set '**dof** = (lowest <sup>1</sup>H frequency), (highest <sup>1</sup>H frequency)', ie and array with two variables, for example **dof = -2200, 1500**. To be safe you could even use frequencies a bit beyond the spectra limits. Set **dmm = 'c'** and **dm = 'yyy'** or '**nny**'. set **dpwr** to the value for which you want a calibration, for example **dpwr=40**. You may need **nt = 16** or more, depending on your sample. **ga** to collect the two 1Ds. **ds(1)** to view the first, place the cursors on the two components of the <sup>13</sup>C line you are using to calibrate decoupling (use the same line throughout), and write down the splitting in Hz, **ds(2)** and do the same again. For the above case, and strychnine, we got residual couplings of 87 Hz and 94 Hz for a line with a (non-decoupled) splitting of 147 Hz. Type in **h2cal**. You will be prompted for the apparent (residual) coupling constants in each of **ds(1)** and **ds(2)**, the **dof** values for each (the low field value is requested first, give the one obtained using the high dof value, the positive one), and the true coupling constant for your <sup>13</sup>C line (obtained earlier from the <sup>13</sup>C spectrum without decoupling). The **h2cal** program will then return values for the strength of the decoupling field, the **pw90** for the decoupler at the **dpwr** employed and the third value will be the **dof** value that would be right on resonance for decoupling the <sup>1</sup>H attached to the <sup>13</sup>C peak you are observing. Gamma H2 = 2375 Hz, pw90 = 105.4 us, coalescence frequency = -360 Hz.

Note that **dmm='c'** is used for so-called CW (continuous wave or single frequency) decoupling, which at low **dpwr** (and low decoupling field or band width) can be very selective.

Now that you have calibrated the decoupler, use **dmm='www'** for waltz decoupling over a spectral window  $\approx 2*$  as wide as the decoupling field strength (half of **dmf**). **dmf** is defined as  $1/\text{pw90}$ , **dmf** is in Hz but **pw90** is in us, so you will enter  $\text{dmf}=1000000/\text{pw90}$  where **pw90** is the value you just got from **h2cal**. Because **dmf** =  $1/\text{pw90}$ , not  $1/\text{pw360}$ , **dmf** is actually  $4*$  the decoupler field strength.

If the above sounds too hairy, put the dioxane sample in the spectrometer (or ask Mr. Layton to do so), and load parameters for  $^1\text{H}$  decoupling calibration. Use the File, Set Directory, Parent, select 'vnmr\_6.1B2', change, select 'test' change, select 'gamah2.par' return, load. Correct **pw** and **tpwr** are not necessary but decent values for **dof** are. The values in the parameter set should be good for dioxane. One value of **dof** must be above the frequency of the proton to be decoupled and one must be below. Collect the two  $^{13}\text{C}$  1ds using these parameters, then invoke **h2cal** as above.

### DEPT, on strychnine

DEPT detection permits increased sensitivity detection of  $^{13}\text{C}$  coupled to  $^1\text{H}$ . By modifying the tip angle of the last pulse one can also use the sequence for spectral editing, i.e. to learn which Cs have 1 H bound, which have 2. The tip angle of the last pulse is set to  $90^\circ * \text{mult}$ , where **mult** is a parameter usually set to 0.5, 1.0 or 1.5. **mult** = 1.0 is ideal for CH groups ( $\text{CH}_2$  and  $\text{C}_3$  groups = 0), **mult** = 0.5 is best for  $\text{CH}_2$  (CH,  $\text{CH}_2$  and  $\text{CH}_3$  are all positive), **mult** = 1.5 gives positive C signals for CH and  $\text{CH}_3$  groups and negative signals for  $\text{CH}_2$  Cs. The selectivity of the **mult** = 1 spectrum reveals how close the  $\text{pp}^1\text{H}$  pulse is to a true **pw90**.

Set up a nice  $^{13}\text{C}$  1D spectrum and type '**dept**'.

You should retain the parameters you chose for a nice  $^{13}\text{C}$  spectrum, including **tof**, **sw**, **tpwr** and **pw**, based on direct observation of  $^{13}\text{C}$ . **tn='C13'** and **dn='H1'**. You can use **pw** and **tpwr** from a direct calibration of  $^{13}\text{C}$  pulse widths (collect a simple  $^{13}\text{C}$  1d, array **pw** and watch the solvent  $^{13}\text{C}$  resonance.) *The  $^1\text{H}$  pulses however will have to be appropriate for the decoupler channel.*

### $^1\text{H}$ pulse calibration on the decoupler channel using **d2pul**

Because your values for **pp** and **pplvl** (proton pulses and proton pulse level) will be used for the decoupler channel, they have to be based on a different calibration than the one in which **tn='H1'**. The **h2cal** routine above satisfies this criterion, however, since it involves decoupling over the duration of the at, it would be damaging to use this to calibrate high **dpwr** powers. Instead, either use **h2cal** for a low **dpwr** and calculate the equivalent **pw90** at a higher power (+6 db gives a factor of 2 shorter **pw**), or use the **d2pul** pulse sequence.

**d2pul** is the decoupler channel analog of **s2pul**. It is invoked by typing **d2pul**. Make sure that **homo='n'**, **dn='H1'** and **tn='H1'**. Set **dof** to the value you had for **tof** in a standard  $^1\text{H}$  1d. Array **pw** at a chosen **tpwr** (which now controls the decoupler). Check the **dps** at a high vs. a low **tpwr**, to confirm this latter point. Array **pw** to find the **pw360** and divide the value obtained by four to determine the  $90^\circ$  pulse width for the **dpwr** value used. These become **pp** and **pplvl** in the DEPT sequence, i.e. the length and power for a high power  $^1\text{H}$  pulse (as delivered by the decoupler channel). (Decent performance is obtained using the **pw90** and related **tpwr** you obtained by directly calibrating  $^1\text{H}$  in a  $^1\text{H}$  1D spectrum for **pp** and **pplvl**, but you will probably be  $\approx 0.5$  us off for a 12 us pulse, the decoupler channel usually gives slightly shorted **pw90**s).

### Other DEPT parameters:

**dpwr** is the  $^1\text{H}$  decoupling power, chosen based on calibrations using '**h2cal**', above such that the decoupler field obtained is  $\approx 1/2$  the width of the  $^1\text{H}$  spectrum you need to decouple (alternately, **dmf** is  $2*$  the  $^1\text{H}$  spectral

width), dof is the centre of the  $^1\text{H}$  spectrum (important) and dmf is  $1/(\text{the pw90 that applies at the stated dpwr})$ .

The  $^{13}\text{C}$  parameters (such as tof and sw) should be those used to collect a nice 1D, EXCEPT that you must use a **true  $90^\circ$  pulse for pw**. Also, do not forget to phase up your spectrum nicely. The easy one to do this on is with j set and mult = 0.5

You should use nt = a multiple of **4** and a multiple of **16** is suggested. **You must use dm='nny'**.

Set j1xh = an average of the C-H one-bond coupling constants in effect in your molecule (look at a coupled  $^{13}\text{C}$  spectrum for guidance). I recommend you use **satdly=0**.

**ga** to acquire data (using a good value of gain). **ds(1)** and set the **th** to be well above noise but still catch all *bona-fide* peaks. Type '**adept**' to analyze the **dept**, **pdept** to plot the results. The original spectra can be recovered by typing **wft**, and plotted as usual. **pdept** analyzes and plots the results.

### **INEPT $^{13}\text{C}$ spectrum, and indirect $^{13}\text{C}$ pulse calibration.**

Like DEPT, this is  $^{13}\text{C}$ -detected experiment, but magnetization is derived from excitation of  $^1\text{H}$ . Thus, tn='C13' and dn='H1'. Other aspects of the set up are as for DEPT.

#### INEPT transfer of $^1\text{H}$ magnetization to $^{13}\text{C}$ .

Collect a decent  $^{13}\text{C}$  spectrum (in practice this may be an old  $^{13}\text{C}$  of a related sample). It serves mainly to provide tof and sw.

Calibrate  $^{13}\text{C}$  pw at a high tpwr. You may do this by observing the strong signal of solvent.

In the  $^{13}\text{C}$  experiment, type **inept**.

You can provide your own one-bond J value and let the computer calculate delays for you. In that case, set **j=140** (or whatever is a better compromise). For the first half of the INEPT, the two delays are set to  $1/4J$ . This works well regardless of how many  $^1\text{H}$ s are coupled to a given  $^{13}\text{C}$ .

Set pp to the decoupler channel's proton pw90, for the decoupler power pplvl. These values can be calculated from the  $90^\circ$  pulse width given at a lower power by the **h2cal** macro (previous demo). Alternately, the decoupler  $^1\text{H}$  pulses can be calibrated directly using d2pul (above).

If focus='y' a refocussed INEPT will be performed, and there will be a second pair of delays. The best duration for these depends on the number of  $^1\text{H}$ s coupled to a  $^{13}\text{C}$ . With a value given for j, you don't get to set the delays yourself, however you can choose whether you would like an INEPT optimized for CH groups (doublets, set **mult=2**), for discrimination between  $\text{CH}_2$  and the other possibilities ( $\text{CH}_2$ s negative, CH and  $\text{CH}_3$  positive, **mult=3**) or a compromise enhancement of all Cs attached to Hs (**mult=4**).

You can override Varian's choices by setting j=0 and doing your own calculations of how long the delays should be. The first pair of delays together are d3 (each delay is  $d3/2$ ) and the second pair of delays is d2 ( $d2/2$  each). Thus you can set  $d3=1/2J$  for ideal performance or somewhat shorter for a sample with a very short  $T_2$  ( $T_2 \approx 1/2J$ ). Your choice for d2 will determine which type of C is best emphasized, and with what sign. For best observation of CH with no enhancement of  $\text{CH}_2$  or  $\text{CH}_3$ ,  $d2=1/2J$ . For negative  $\text{CH}_2$ s, positive CH and  $\text{CH}_3$   $d2=3/4J$ . For best observation of (positive)  $\text{CH}_2$   $d2=1/4J$ . For best observation of  $\text{CH}_3$   $d2=1/5J$  and for a good compromise spectrum  $d2=1/3.3J$ .