

Indirect observation of ^{13}C and its correlations with ^1H , by gHSQC. Strychnine in CDCl_3 with CrAcAc

From Eq. 4.2 of your text, signal-to-noise, S/N increases with the static field strength, H_0 , the gyromagnetic ratio of the nucleus from which magnetization derives, γ_{exc} , and the gyromagnetic ratio of the nucleus detected, γ_{obs} .

$$S/N \propto \frac{NA}{T} H_0^{3/2} \gamma_{\text{exc}} \gamma_{\text{obs}}^{3/2} T_2^* \sqrt{nt} \quad ,$$

where N is the number of molecules in the sample, A is the abundance of the isotope being observed, T is the absolute temperature, T_2^* is the effective transverse relaxation time and nt is the number of transients averaged together.

The enhancement available from the heteronuclear nOe is $\eta_{I\{S\}} = \frac{\gamma_S}{2\gamma_I}$ so there is an nOe of 2 and the ^{13}C signal is three times larger in the presence of the ^1H nOe. This, is however not as good as a whole factor of $\frac{\gamma_S}{\gamma_I}$ obtained by switching the roles of the I and S nuclei.

INEPT transfer enables you to exploit the high γ_{exc} of ^1H but still relies on the lower γ_{obs} of ^{13}C for detection. Thus the next step up in sensitivity is to use ^1H for both γ_{exc} and γ_{obs} . This is the strategy implemented by HSQC, HMQC and HMBC. The cost involved in the first two is that only C directly bonded to ^1H is observed (these are single bond methods). The HMBC (MB = multiple bonds) allows observation of Cs two bonds from a ^1H , and thus most quaternary compounds.

We will obtain gHSQC and gHMQC spectra of menthol (concentrated, with short T_1 and T_2). Whenever possible, gradient versions of experiments are used, because the results they give are cleaner.

preamble to HSQC

As usual, first obtain a nice ^1H 1d with good choices of sw, tof, gain and a calibrated pw90. (We had tpwr=59, pw90 = 11.8 on thing 2)

You will also need to know about the spectral range covered by your ^{13}C resonances. In the real world when we often don't have strong enough samples to obtain a ^{13}C spectrum directly, we usually start with standard parameters that contain a built-in overestimate of the sw.

You will need calibrated high power (short) and low power (long) pulses for ^{13}C . The former will be used for broad-band excitation and inversion pulses. The latter will be used for decoupling ^{13}C during ^1H detection. Since at is many ms long, the decoupling power must be kept low, this is especially important for decoupling of heteronuclei such as ^{13}C and ^{15}N . You could do these calibrations directly observing ^{13}C , which would get you the right values for our 400 MHz spectrometers. However, for other machines, and for conservative practise, you should calibrate in accordance with the mode in which a channel will be used. Since tn will be ^1H and ^{13}C will be dn, for the HSQC, we will use the same assignments in calibrating. This is accomplished using **pwxc**al ('X nucleus pw calibration').

pwxcal for calibration of ^{13}C pulses

In an experiment that has your good ^1H 1d parameters, type **pwxc**. You can override the defaults of calibrating ^{13}C with channel 2, but we won't.

Set the ^1H pw and tpwr to result in a 90° pulse and set the J value correctly ($j\text{C13} = 151$ for methyl iodide). To determine a good value, look at either a ^1H coupled ^{13}C spectrum, or the ^{13}C satellites in your ^1H spectrum. Make your decision based on either the J value for important resonances, or a compromise between the different Js represented by typical resonances. J is the separation in Hz between lines in a multiplet, or between the two ^{13}C satellites. Set dof to be near the ^{13}C resonances in your spectrum. This is especially important for low power calibrations, where dof needs to be within 500 Hz of the ^{13}C coupled to the ^1H under observation. In practice, you could use the TMS satellite signals, or resort to a separate calibration sample, such as ^{13}C enriched methyl iodide.

Set dpwr to 57 for high power pulses. Array pw_x1 from 1 to 15. **ga**. The satellite signals ONLY should decrease in amplitude and then invert as the ^{13}C spins to which they are attached are tipped off the Z axis through the XY plane and then through to the negative Z axis. The spectrum in which the satellites are at a null represents a ^{13}C 90° pulse. Write down that pulse length and the associated dpwr (7 μs at dpwr=57 on thing 2). **MAKE SURE THAT dm = 'nnn' or 'n' !**

Set dpwr to a low value (40) and repeat the above, with pw_x1 arrayed between 40 and 70. Write down the low power pw_x1 which gives a null for the satellites, this is the low power pw₉₀ (pw_x90 = 50 μs for 40 = dpwr). (At this lower power, a poor choice of dof may result in failure of the satellites to invert. If you encounter this problem, take the pw_x1 value that has the strongest effect on the satellites and array dof over 20,000Hz in 1,000 Hz steps. The value at which the satellites are best inverted should be used as dof in another array of pw_x1, where the value that gives a null for the satellites is the pw_x90).

Return to HSQC

In a third experiment, set up with the good ^1H 1d parameters, type **gHSQC**. This will install parameters for a decent gHSQC. Confirm that you have correct ^1H pw₉₀ and tpwr etc. in place. Enter the high ^{13}C power and associated pw_x1 for pw_xlv1 and pw_x. You will also have to enter a good value for the H-C J coupling, j_{1xh}. This is used to calculate the INEPT transfer delays. Finally, note that sw₁ is not the same as sw. This is because you are now observing different nuclei on the different axes. Your old ^1H sw will appear as the new sw, because you are still directly detecting ^1H . However you are indirectly detecting ^{13}C in the t1 (d2) interval. Thus, you will need a decent estimate of the sw of your ^{13}C spectrum (only those ^{13}C coupled to at least one ^1H), for sw₁. When in doubt, overestimate, you can always trim back. Similarly, you will need a decent estimate for dof, the ^{13}C centre frequency, from the tof of a ^{13}C 1d of a related sample. For now, set **dm='nnn'** and **dmm='w'**.

Confirm that the experiment works using **phase=1, ni=1, nt=1, pw_x=0, <your value for pw_x> **ga**. The second spectrum should show only the ^{13}C coupled peaks right way up. The first spectrum should show nothing (gHSQC) (but if you don't have gradients and you are running HSQC, the first spectrum will include all peaks, inverted. Check for cancellation of ^1H coupled to ^{12}C with nt=2 vs. nt=1, using your value for pw_x). The use of gradients suppresses protons attached to ^{12}C even better than the phase cycle, and a 'TANGO' presequence also suppresses them, and can be activated with **nullflg='y'**.**

Decoupling of ^{13}C must be done with extreme care for the hardware. Ideally you would like to decouple over the full sw₁. When using Waltz decoupling this would imply you should set dmf to $2*\text{sw}_1$. In practice, we can rarely afford this. Your choice of dmf implies a choice of dpwr because $1/\text{dmf}$ is the 90° pulse and dpwr must be the corresponding power. In the relatively easy case of menthol/benzene, sw₁ = 8000, so the ideal dmf=16000 which implies a 61.25 μs decoupling 90° and therefore dpwr \approx 39. Even this is not tolerable for at > .15 s. We therefore compromise at dmf \approx sw₁, and also keep at short, \approx .15 s. (for menthol in benzene, dof=5424, j_{1xh}=131).

Parameters drawn **from the ^1H spectrum** and their use in the gHSQC

tof -> tof
sw -> sw

Parameters drawn **from the ^{13}C spectrum** and their use in the gHSQC

tof -> dof
sw -> sw1
tpwr -> pwxlvl
pw -> pwx

For dpwr and dmf, see above.

Others: the proton power/pulse calibration must be done in such a way that the high-band channel performance is being calibrated, NOT the full band channel that normally functions in s2pul or std1h. To trick the machine into using the high band channel you can run one of those two experiments and calibrate pw90 as usual as long as **dm = 'C13'** (then **su**). Alternatively, calibrate ^1H using **d2pul** or **h2cal**, as described in previous exercises.

From the h2cal method

dpwr / pw90 -> tpwr / pw calculate a high power tpwr for ^1H (h2cal is performed at low dpwr, such as 40, to calculate the pw90 that would result for dpwr = 58 take the obtained pw90 and divide by 8 (you should be able to figure out why it is a factor of 8).

from the d2pul method

tpwr / pw -> tpwr / pw calibrate as usual by arraying pw, tpwr controls the decoupler in this pulse sequence.

From the s2pul method

tpwr / pw -> tpwr / pw Set **dm = 'C13'** then **su**, then calibrate as usual by arraying pw, tpwr now controls the high band because whenever C13 is invoked it is given the full band channel *on our 400 MHz spectrometers*. The same is not necessarily true on other machines, only in cases where there is a single full band. If in doubt either use one of the methods above or ask someone who is familiar with the hardware.

PFGflg sets up gradient-90-gradient suppression of left-over magnetization from the previous scan, set = 'y'.

nullflg sets TANGO suppression of resonances of ^1H coupled to ^{12}C .

null \neq 0 implements BIRD suppression of resonances of ^1H coupled to ^{12}C . TANGO is recommended on the 400s, BIRD will work well without gradients (i.e. on the gemini).

Processing is similar to that for TOCSY. All cross peaks should be absorptive and positive. Use **rl(3p)** for the direct dimension and **rl1(70d)** for the ^{13}C dimension, with 3 and 70 chosen simply as examples.

gctHSQCafm2

AFM's gradient constant-time HSQC, which distinguishes between CH₂ groups and Cs with odd numbers of attached Hs (CH and CH₃).

For this one, load a parameter set or data set used previously for this pulse sequence, such as HSQC_CT_afm7nov01.fid. Also make sure that the pulse sequence itself is present in the psglib directory of your account (copy gctHSQCafm2.c over if the pulse sequence is not present, then, in vnmr, type **seqgen(seqfil)** to compile the pulse sequence in your own account). You will now have to change relevant parameters to your choice of values: sw, tof, tpwr, pw for ¹H; sw1, dof, pwxlvl, pwX for ¹³C. Keep at short, <<0.15 s, unless you turn off ¹³C decoupling during acquisition (**dm='nnn'**). Set j1xh to the one-bond HC J coupling constant that typifies your Cs of interest. Set **nullflg='y'** for suppression of H coupled to ¹²C (recommended). If you want your CH₂ signals to have the opposite phase to the CH and CH₃s, set **mltflg='y'** (multiplicity flag). If you are using this for an H-C 2d, you don't need to touch the gradients. However if you are doing HN, you will (not covered). Use **sspul='y'** and d1 = 1 - 1.3* the T₁ of your Hs (note that this is much shorter than the T₁ of your Cs, another advantage of getting the Cs via the Hs). As for the HSQC, the maximum value at1 of the indirect detection delay, t₁, will be ni/sw1. Calculate this value and make sure that BigT is longer by at least 1 ms (**BigT = .001+(ni/sw1)**). As always, the cost of a large ni (and BigT) is loss of signal to T₂ relaxation.