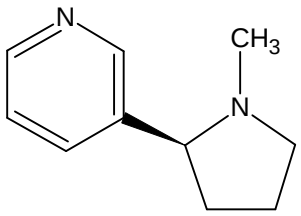
Chem 636, Fall 2008 HW #7

Due at the beginning of lab, the week of Oct 28

☞ Use ***Athena*** (not Homer) and the **150mg nicotine in $CDCl_3$** sample for this lab.



13C NMR Spectroscopy: Introduction

There are a number of new techniques and concepts introduced with this lab:

i) Observing *13C* involves double-resonance:

observe at 62.9 MHz for *13C* (on Phoenix):

SW, **O1**, **SF**, etc. → now will now be *13C*–specific observe parameters

+ high-power ***decoupling*** at 250.13 MHz for *1H*:

O2 ≡ *offset frequency* for the 2nd (decoupler, *1H*) channel;
defines the center frequency of the decoupler;
should equal (or be close to) **O1** of the corresponding proton jobfile;

ii) High-power decoupling can be accomplished in many different ways, and is therefore a complex subject. We will use only WALTZ-16, or **CPD**, decoupling in this lab. Three commands control the decoupler on AC spectrometers:

PO ≡ *power off* turns the decoupler off
DO ≡ *decoupler on* turns the decoupler unit on, but rf power is gated off to the probe
this command takes ~30 sec to stabilize
CPD ≡ *composite pulse decoupling*, which has the more common name, WALTZ-16.

The proper sequence to turn on and off the decoupler is:

PO → DO → CPD → DO → PO

Skipping the **DO** steps can cause problems with the decoupler unit, and might lead to acquisition of data with significant artifacts.

iii) ***Improper setting of the decoupler power can cause extensive damage to the spectrometer.***

Decoupler power is set by the parameters:

DP ≡ *decoupler power*: this name is a misnomer!!

DP is actually the ***DECOUPLER ATTENUATION***. Higher numbers are lower power. Use of 0H in **CPD** mode ***will*** damage the probe, and possibly also the high-power transmitters.

H and **L** denote high-power and low-power respectively.

Use **18H** on Athena (should setup correctly with **RJ**). **22H** is correct for Homer.

S1 or **S2** ≡ parameter used for decoupler power(attenuation!) in some automation routines (such as INVGATE.AU and GATEDEC.AU)

iv) Automation routines provide additional capability, allowing pulse sequences to go beyond the simple **GO** (the last part of **ZG**: **ZE** plus **GO**) command for more complex experiments on AC spectrometers:

AS ≡ *automation setup* lists the automation sequence, then runs through each acquisition parameter in the order they appear in the sequence.

AU ≡ *automation acquisition* runs the current sequence; it is a good idea to type the name of the sequence to insure the correct sequence is being run.

v) ^{13}C spectra are typically processed using **LB** = 0.5 to 2 Hz (1 to 2 Hz being most common). **LB** trades off resolution for signal-to-noise (S/N); smaller values provide better resolution, larger values reduce more noise in the latter parts of the FID.

Standard (CPD) ^{13}C Spectroscopy: ^{1}H–^{13}C NOE and Decoupling

^{13}C spectra have significant utility in showing the number of carbons (spin counting), and in assisting with assignments. Since the carbon-13 nucleus is 1.1% abundant—the rest being carbon-12 with I=0—signal-to-noise is a significant issue to obtaining usable data. In this lab and the next, we will experiment with different methods for obtaining and working with ^{13}C spectra.

☞ 1. Perform a normal ^{1}H acquisition on the nicotine in $CDCl_3$ sample in the lab.

PLOT1 → Make a research-quality plot of the ^{1}H spectrum and turn in.

The large range of ^{13}C chemical shifts typically provides sufficient dispersion in ^{13}C spectra, but do not become relaxed with respect to shimming. Poor shims will degrade sensitivity, and sensitivity is the crucial issue with ^{13}C NMR work. Checking that shims are reasonably good by acquiring a ^{1}H spectrum is always recommended prior to running ^{13}C NMR experiments.

In very concentrated samples (such as used in the lab demo), ^{1}H spectra can be broadened by a relaxation mechanism called "radiation dampening"*; do not expect a great ^{1}H spectrum from such samples. The ^{1}H spectrum is still useful in making sure the shims are reasonable (i.e., the peaks are symmetric; if not, Z2 is likely misset) and that the sample is reasonably clean (e.g., not containing a lot of impurities). If a research sample is so concentrated that radiation dampening prevents important multiplets from being resolved, remove 5-10% of the sample to another tube and dilute with more solvent. From this new sample you can obtain a good quality ^{1}H spectrum.*

☞ 2. Obtain a standard ^{13}C FID using **NS=32**. "Standard" ^{13}C spectra, often denoted $^{13}C\{^{1}H\}$—the curly brackets specifying the nucleus being decoupled—will always be acquired with **CPD** decoupling.

The typical sequence for setting up and running a standard ^{13}C spectrum is:

i) read in the proper jobfile with **RJ**
ii) perform an **II** [This important command switches the hardware correctly for a ^{13}C experiment. It will also power up the decoupler unit to mode **DO** .]
iii) wait ~30s, then turn on the decoupler with **CPD** (the jobfile + **II** sets the decoupler to **DO)**
iv) acquire with **ZG**

NS = –1 is often used in ^{13}C experiments: the spectrometer will acquire forever, until *halted* with a **CTRL–H**. In this case, you would use **TR** to transfer the spectrum as it is acquiring to another job; transform (***always*** *set LB ≥ 0.5 and then use EF)* and inspect the spectrum in EP-mode. Allow the FID to continue to acquire until the S/N is adequate for your needs.

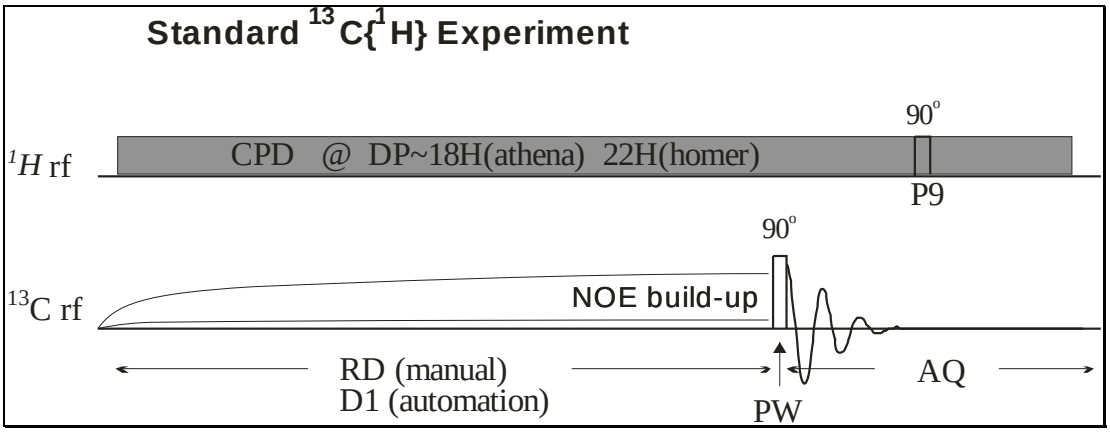
☞ Acquire a second FID using the same **NS** (=32), but now with the decoupler gated off with **DO**.

The DO spectrum demonstrates the effect of forgetting to turn the decoupler on (using CPD). It also shows what would happen if one attempts to obtain a coupled spectrum by simply turning the decoupler off. Coupled spectra should be acquired using either GATEDEC.AU, as described below, or DEPT-45, as will be done next in next week's lab).

PLOT2 → Plot both spectra, processed with **LB**=2, on the same page using NUTS's buffer (**BU**) routine; make sure they are properly annotated.

*Note that the **DO** spectrum has very poor relative signal-to-noise compared to the **CPD** spectrum. Nuclear Overhauser Enchancement (NOE) from the protons to the carbon-13 nuclei—from the ^{1}H decoupling during **RD**—leads to an enhancement in the ^{13}C signal of typically a factor of 2.5 to 3. With the decoupler gated off, we do not obtain this enhancement.*

Q1 → Give a brief explanation as to why **RG** does not have to be changed for ^{13}C spectra.



NOEs are crucial in routine ^{13}C spectroscopy: they enable sufficient signal-to-noise, S/N, to be obtained with moderate concentrations (roughly 0.2M on a 300 MHz instrument) in reasonable periods of time (<10 min). The size of the NOE is dependent on the number and proximity of protons to each ^{13}C nucleus: quaternary carbons, for example, will be significantly reduced in intensity relative to the other carbons in standard (CPD) ^{13}C spectra because there are no nearby (1-bond) protons. Protonated carbons will also show variations in intensity with respect to each other due to differences in ^{1}H distances, number of close-by ^{1}H, and relaxation times. Normal $^{13}C\{^{1}H\}$ spectra are therefore non-quantitative.

*A significant aspect in normal ^{13}C spectra is the repetition delay, **RD**. The proton-initiated NOE to distant (quaternary) carbons takes more time; quaternary carbons also typically have longer spin-lattice relaxation times. It can be useful to increase **RD** in the standard CPD acquisition; by doing so, quaternary carbons, in particular, will become larger relative to protonated carbons.*

☞ 3. Take a standard (CPD) $^{13}C\{^{1}H\}$ 8-scan spectrum of nicotine with **RD** increased to 20S.

PLOT3 → Plot the 8-scan RD=20 and the 32-scan RD=2 spectra on the same page using NUTS' buffer (**BU**) routine. Annotate with **NS=** and **RD=** correct for each spectrum. Note the resulting changes in *relative* intensities of the peaks.

Q2 →Which carbons are most affected? Why? [**RD** is the critical parameter here, not **NS**.]

Quantitative ^{13}C Spectroscopy: Decoupling Without ^{1}H–^{13}C NOEs

To obtain quantitative ^{13}C spectra, ^{1}H–^{13}C NOEs must be avoided. Quantitative data are sometimes required for kinetic studies where the useful region of the ^{1}H spectrum is too overlapped to provide accurate integrals or deconvolution. [Note: Always use ^{1}H spectroscopy for quantitative studies if possible. Integrals, although simple to setup and perform, are significantly less accurate than line shape fitting procedures (deconvolution).]

*The most troublesome aspects of quantitative ^{13}C spectroscopy, other than the amount of sample required, are the ^{13}C spin-lattice relaxation times. In general, the repetition delay, **D1**, should be set > $5\times T_1(^{13}C)$ of the slowest relaxing ^{13}C nuclei of interest. Because of the spread of $T_1(^{13}C)$ values in common compounds (2 to >20s), there is no simple recipe for how to set **D1**.*

4. The Bruker sequence INVGATE.AU is designed to avoid ^{1}H–^{13}C NOEs, but at the same time acquire a decoupled spectrum. INVGATE.AU is therefore used when quantitative ^{13}C spectra are required. *Always read the proper ^{13}C jobfile prior to setting up INVGATE.AU.*

   Use **AS INVGATE.AU** to setup the parameters for this experiment; a ^{13}C jobfile must be read in prior to running the **AS** command.

   → **D1** replaces **RD** as the recycle delay in *all* .AU experiments.

→ **RD=0** is now required!! Leaving **RD ≠ 0** will insert an unplanned delay into the **GO** portion of the sequence, which will usually ruin the data.

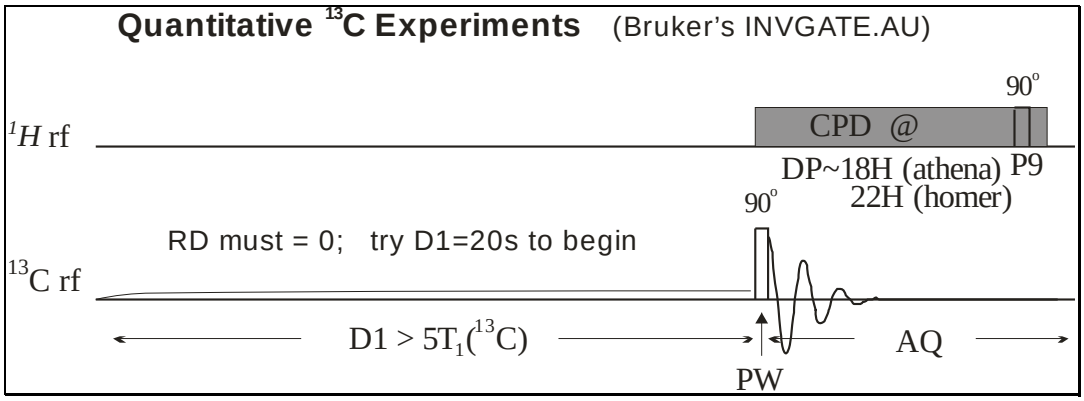
Set **D1=20 RD=0** and **NS=8** , and check that **S2=18H** (which replaces **DP**).

☞ To acquire, use the command **AU INVGATE.AU** (do not use **ZG**). Save this spectrum.

PLOT4 → Do something unusual (for ^{13}C NMR), and integrate this INVGATE ^{13}C spectrum. Plot the spectrum showing those integrals. Is this spectrum quantitative or not?

Q3 → Show the calculation to obtain the concentration of this solution: 150 mg nicotine (MW=162) and 0.6 ml solvent.

Q4 → The signal-to-noise (S/N) of NMR spectra improves as the square-root of the number of scans, and increases in direct proportion to the concentration. Provide a calculation showing how many scans would be needed for the same INVGATE experiment run on a 50 mM nicotine sample to obtain identical signal-to-noise. How long would this experiment take? How long would a standard CPD $^{13}C\{^{1}H\}$ acquisition (AQ=1.5 RD=2) take using the same number of scans?



Coupled ^{13}C Spectroscopy: ^{1}H–X NOEs without decoupling

We saw earlier that obtaining a coupled spectrum using ***DO*** *and* ***ZG*** *gives very poor S/N. The primary problem is the loss of the NOE, reducing the S/N by ~3. Carbon-proton couplings also reduce peak intensities. An apparent doublet of a methine (≡CH) is in general reduced by more than 1/2 compared to a decoupled singlet; long-range ^{1}H–^{13}C couplings will split, or broaden, each part of the doublet, often significantly. Thus, coupled spectra are usually not obtained in ^{13}C spectroscopy. There are better ways to determine the* multiplicity *of the ^{13}C nuclei—quaternary, methine, methylene, methyl—as we saw with the automation DEPT experiments run earlier (and further investigated in next week's lab).*

Even so, coupled spectra are occasionally needed, e.g., to measure couplings in a novel compound. In these cases, one possible experiment is to re-obtain the NOE to improve S/N, while doing away with the decoupler during the acquisition: Bruker's GATEDEC.AU sequence does just that.

The most optimal S/N is provided by a DEPT-45 experiment (next week's lab). Properly setting up DEPT spectra, however, requires knowledge of the heteronuclear J-couplings, J_{CH}. GATEDEC.AU has utility for acquiring coupled spectra of novel compounds where J_{XH} is unknown, or possibly for measuring long-range coupling constants of quaternary carbons.

5. Similar to above, set up and run the "gated" coupling experiment **GATEDEC.AU**. This time set **D1=2** (**RD=0** still required!) and **NS=–1**. Use **AS GATEDEC.AU↵** to check/run through all the parameters. **S1** is used now, as it can be a few dB (the unit of attenuation[power]) higher than **DP** or **S2**; i.e., **S1=21H** or **22H** is fine here.

   Setting the decoupler to higher attenuation/lower power can help prevent sample heating from the decoupler, OK here because the decoupler is not being used to decouple protons, but rather to provide an NOE signal enhancement (and yes, you can easily boil a sample with an NMR spectrometer—keep in mind that 0H will not only boil the sample, but damage the probe and high-power amplifier).

PLOT5 → Plot and annotate this spectrum with the standard 32-scan ^{13}C spectrum from part 2 on the same page using NUTS' buffer (**BU**) routine.

Turn in 5 plots, and answers to 4 questions.