

## Fall 2014 Chem 636 – Lab #4

Assignment due at beginning of lab, week 5 (Sept 30 – Oct 2).

**Use Athena (AC-300) for section I, and Persephone (AVANCE-500) for Sections II and III. Always login to your own account when working. Although you may work in groups, each of you should setup and acquire your own data, as well as process and hand in that *individual* set of data.**

**Reading** – A primary reference for this lab is the guide “Acquisition Step-by-Step” located on the facility website in the User Guides → Avance section.

- Data sampling: filtering and folding: ZJ 11.2.7,11.3.5; Clar 3.2.3
- $^{13}\text{C}$  NMR: ZJ is better with general description; Clar is much better with INEPT&DEPT ZJ 4.3, decoupling 12.4, PT 12.9,12.10,13.3.4; Clar PT 4.3,4.4 decoupling 4.2.3
- Tuning the probe: Zerbe+Jurt (ZJ) 3.4; Claridge (Clar) 3.4.2,3.4.3;

**Goals** – Experiment with spectral windows, filters, and folding.  
Learn  $^{13}\text{C}$  NMR on Avance 500 (Persephone) running Topspin.

The SampleCase robot will accept samples in tubes 7” to 9” long, and any cap style (including J-Young, Shigemi, sealed samples, etc.).

You can decorate your thinking cap as you wish, but please keep it on in the lab!

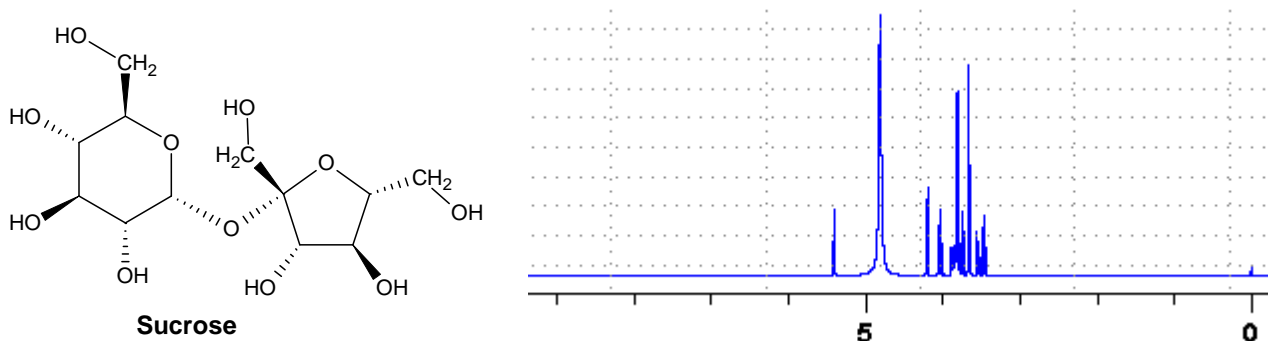
### Digital and Analog Filters, and Folding

New spectrometers, such as the Bruker AVANCE III console, are equipped with digital filtering technology. The data points are acquired at high speed using a fast digitizer: on the AVANCE III, the effective digitization is fixed at one complex point acquired every 50 ns, or a rate of 20 MHz. The data are not all saved! A common  $^1\text{H}$  spectrum will have  $\text{AQ} = 4$  s, so a single 1D spectrum has 80 million complex points digitized. The spectrometer performs a *digital filter* on the data to exclude undesired frequencies — above and below the requested spectral window, defined by **SW** and **O1p** — and then decimates the number of points to some reasonable value as requested by that parameter **TD**. The digital filter can be made quite sharp: i.e., it reduces the size of peaks outside the spectral window by a lot very quickly. Older “analog” spectrometers could not achieve such sharp filtering, and spectral *folding* can then occur. We’ll observe this effect in the next section, using the AC-300 spectrometer.

There are two major reasons for looking at spectral windows at this time:


- a) The spectral window is a key concept for acquiring NMR data. Experimentation is the best way to get comfortable with it.  $^1\text{H}$  and  $^{13}\text{C}$  1D spectra are usually taken with fixed spectral windows, but all other nuclei are not so simple.  $^{31}\text{P}$  and  $^{19}\text{F}$  usually require optimization of the spectral window, in particular reduction of **SW**, to obtain high-quality data. Metal nuclei often have very large chemical shift ranges that require considerable thought and experimentation with **SW** and **O1p** to obtain spectra.
- b) Spectral window optimization is a critical task for all 2D NMR data. Moreover, filtering cannot be performed on the indirect detection dimension. Folding is therefore an important issue in 2D NMR.

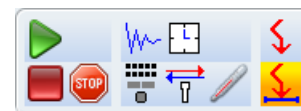
There are many ways to perform spectral window optimizations, the best involving graphical tools as implemented in TopSpin. [Unfortunately, the tools in IconNMR are not so good, so we'll return later to this topic when using Icon.] On Athena, the Bruker AC-300, the tools are quite cryptic and take a long time to get comfortable with. This HW will therefore simply provide, then, **SW** and **O1p** values to enter so you can see the effect of the changes. [There is no **O1p**, center of spectrum stated in ppm, on the AC-300. The center of the spectrum, set by **O1**, must therefore be entered in Hz.]



In the spectrum of sucrose, the spectral window (as always) is set by the parameters **SW** and **O1p**. **SW** is the width of the spectrum, approx 9.8 ppm. **O1p** is the center of the spectrum, which can be eyeballed, or calculated to be more precise:

$$\begin{aligned} \mathbf{O1p} &= [(\text{left edge}) + (\text{right edge})] / 2 \\ &= [9.3\text{ppm} + (-0.5\text{ppm})] / 2 = 4.4\text{ppm} \end{aligned}$$

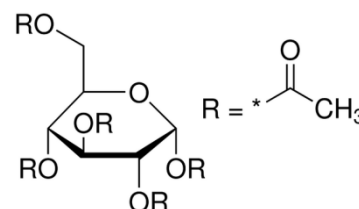
Fortunately, we can graphically set the spectral window in TopSpin by simply expanding the spectrum to the desired region. Clicking then on the  icon (or typing .setsw) will automatically set **SW** and **O1p** to acquire just that window. [Remember that when SW is changed, one should always check AQ to set it to a reasonable value.]



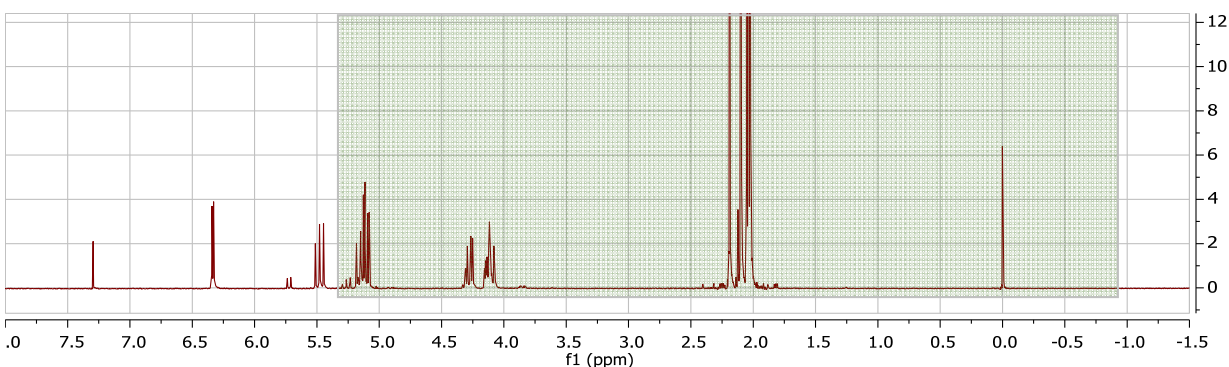
### I. Spectral Window and Folding on Analog Spectrometer, the Bruker AC-300:

Use  $\alpha$ -glucose pentaacetate in  $\text{CDCl}_3$  for sections I and II. Use Athena in similar manner as done for HW 2.

- a) Acquire a “standard”  $^1\text{H}$  spectrum of  $\alpha$ -glucose pentaacetate in  $\text{CDCl}_3$ . Use the standard spectral window (RJ CDCL3.1DJ) for this experiment and work up the spectrum as before.



- b) Now use “optimized” parameters. We want you to see the difference using analog filters, rather the digital filters used in the next section on the 500. So for this part, we'll provide the parameters to you. The following parameters set the spectral window to the shaded area in the figure below: change the sweep width **SW** to 1888 and **O1** to 3446 (both in Hz; 6.3 ppm and 2.2 ppm, respectively). Acquire the  $^1\text{H}$  spectrum, and work up as before.



1☞ Stack the two spectra from a) and b) [just EFP, phase, and annotate], and turn in .mnova and .pdf files using a normal upload.

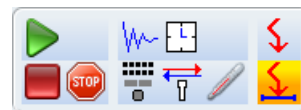
Q1 Notice the set of new peaks appearing between 4.3 and 5.3 ppm in the second spectrum. How do you explain this? Can you phase them with the other peaks?

Q2 What is the small peak at 3.42ppm?

## II. Experimenting with the Spectral Window in TopSpin on the AVANCE-500P (Persephone)

a) Acquire a spectrum of  $\alpha$ -glucose pentaacetate in  $\text{CDCl}_3$  with the full standard spectral window (SW=20ppm, O1p=6ppm).

b) Acquire another spectrum with an optimized spectral window narrowing the SW to give ~10% baseline at each edge of the spectrum [a typical optimization done to setup a 2D spectrum]. Use the graphical tools to accomplish this. Note again the change in AQ when you make the change in SW. Correct AQ back to a “standard” value.



2☞ Plot this new spectrum showing the full spectral width. You don't need to do more than FT, phase and annotate the plot.

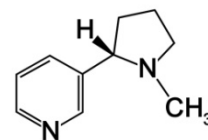
c) Now acquire a spectrum excluding part of the spectrum, similar to that done in section I b).

3☞ Turn-in another plot stacking the spectra from a) to c). You don't need to do more than FT, phase and annotate the plot.

Q3 What happened to the downfield and upfield peaks in the spectrum from part c)? Expand vertically; can you see any evidence of them in the spectra?

## III. $^{13}\text{C}$ NMR (NOE-involved) Using TopSpin on Persephone (the Bruker AVANCE-500P):

A. Acquire a standard  $^{13}\text{C}$  spectrum of nicotine in  $\text{CDCl}_3$ . The parameters set / experiment is C13CPD\* in the Bruker standard library. Acquire the spectrum using NS = jx8 [the commands **tr** and something like **halt 32** are important]. 32 scans is likely sufficient.



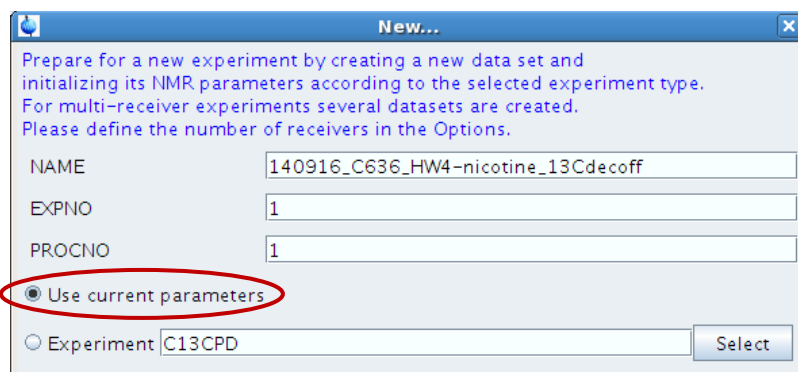
4☞ Plot this spectrum with standard processing for  $^{13}\text{C}\{^1\text{H}\}$  spectra†:

\* CPD is a Bruker acronym for *composite pulse decoupling*, i.e., the normal form of  $^1\text{H}$  decoupling.

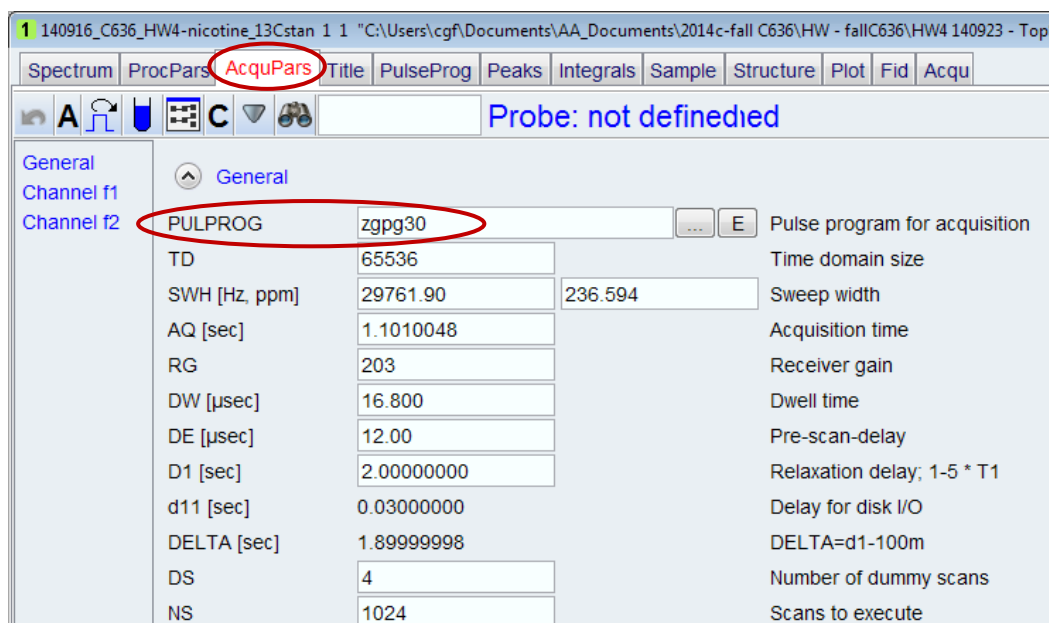
† The experiment where  $^{13}\text{C}$  is detected and  $^1\text{H}$  is decoupled is often written as  $^{13}\text{C}\{^1\text{H}\}$ .

- Exponential apodization, usually with  $\mathbf{lb} = 1$ .
  - One zerofill, then standard fast fourier transformation.
  - Phase correction, similarly done as with  $^1\text{H}$  spectra: 0<sup>th</sup>-order phase correct with toggle point at right or left peak (excluding solvent peaks), then 1<sup>st</sup>-order phase correct on opposite side of spectrum.
  - Baseline correction as normal.
  - It is common to do a peak pick on a carbon spectrum, and leave the peak picks in ppm. Unless a mass spec has been done, perform a check that the # carbons in the spectrum matches the number of carbons in the proposed compound structure.
- Q4:** When acquiring any type of spectrum, one should always take a  $^1\text{H}$  1D spectrum (if you haven't already, do so now!). There are two important reasons for doing this: state both and turn in with this HW.
- Q5:** Think about why one would proton decouple a  $^{13}\text{C}$  spectrum. Describe briefly two reasons why this is a good thing to do, and give one reason why one might not want to do this.
- Q6:** State a couple reasons why the carbon count might not match that in the proposed structure, even when the structure is correct and the signal-to-noise of the  $^{13}\text{C}$  spectrum is good.
- B.** Acquire a  $^1\text{H}$ -coupled  $^{13}\text{C}$  spectrum by simply turning the  $^1\text{H}$  decoupler off in the previous experiment. Use the same **NS** as used in part A.

There are numerous ways to acquire this spectrum, but be careful! Remember that Bruker saves the data automatically upon completion of the acquisition. There is no File → Save As.... The data or parameters must be copied to a new experiment area, or you will overwrite the spectrum obtained in part A. The most versatile method is to use the Create Dataset button or **new** command, rename the file (or increment the EXPNO , and check the “Use current parameters” button:



To turn the decoupler off, change the pulse sequence **PULPROG** = zgpg30 to zg30 (by typing in the new sequence name; see fig next page). The decoupler section will disappear upon an **ased**. **zg** will now acquire a  $^{13}\text{C}$  spectrum, but without  $^1\text{H}$  decoupling during any portion of the experiment.




- C. Acquire another  $^1\text{H}$ -coupled  $^{13}\text{C}$  spectrum, but now use “gated decoupling”. We’ll follow the same procedure here: “Create Dataset” or **new**, make a new name or increment EXPNO, check “Use current parameters”. Then change **PULPROG** = zgpg30, and acquire with **zg**. Once again use the same **NS** as used in part A and B.

5. Plot the A, B and C spectra as a stack.

- Q7:** While on the spectrometer, view the three pulse sequences as shown in the demo lab session. Briefly describe the differences in intensities you observe for the three spectra.

**Upload 5 plots as .mnova and .pdf files, and hand in answers to 7 questions.**

**Common commands in TopSpin:**

- Hover the mouse over an icon and it will provide a description with the command. Hovering is especially important with the Flow Toolbars.
- Right-click in TopSpin to obtain useful context-dependent menus.
- Use  TopSpin's Help → Commands for a complete (brief) listing of all commands.

<b>new</b> ≡ <b>edc</b>	create a new dataset
<b>iexpno</b>	increment and copy to next exp #; e.g., if in exp 4 copies params to exp 5
<b>re 4 1</b>	switch to specified exp # and proc #, here experiment 4 and process 1
<b>wra 40</b>	copies dataset to exp 40; 40 should not exist previously
<b>*2 *8 /2 /8</b>	multiply or divide vertical scale by 2 or 8
<b>.all .hr</b>	show complete spectrum rescaled (.all) or not rescaled (.hr)
<b>ased</b>	show brief list of acquisition parameters
<b>getprosol</b>	read in probe & solvent dependent params (e.g., pulse widths and powers)
<b>atma</b>	automatically tune the probe as specified by parameter set
<b>topshim gui</b>	open auto-shimming tool panel
<b>ns 32</b>	sets ns = 32 (just use a space between ns and 32)
<b>tr tr 16</b>	tr transfers data after current scan; tr 16 transfers after 16 <sup>th</sup> scan complete
<b>halt #</b>	halts after specified number of scans (usually multiple of 4 or 8)
<b>efp</b>	<b>em</b> then <b>ft</b> then <b>pk</b> ; em uses lb, pk applies previous phase correction
<b>apk</b>	automatic phase correction
<b>abs n</b>	automatic baseline correction without integrals

**Common parameter sets in TopSpin:**

Parameter set	Comments	Pulse Sequence
<b>PROTON</b>	<b>standard 1H 1d</b> ; d1=1 aq=4 lb=.3 p1=30°	zg30
<b>C13CPD</b>	<b>13C 1d with proton decoupling</b> ; d1=2 aq=1.3 lb=1 p1=30°	zpgg30
<b>C13DEPT135</b>	no quats, CH and CH3 positive, CH2 negative cnst2=145 sw=160p o1p=80p d1=2 aq=2 lb=1	deptsp135
<b>C13DEPT90</b>	no quats, CH positive, CH2 and CH3 nulled; cnst2=145	dept90
<b>C13DEPT45</b>	no quats, CH CH2 CH3 positive; correct sequence for 29Si, etc	dept45
<b>C13APT</b>	<b>attached proton test</b> : cnst11=1 quat and CH2 pos, CH CH2 neg cnst11=2 quats only (CH CH2 CH3 nulled)	jmod
<b>C13IG</b>	inverse gate → <b>quantitative carbon</b> ; d1 must be ≥ 3×T1	zgif30
<b>C13GD</b>	gated decoupling → <b>proton-coupled 13C 1d</b>	zggd30
<b>P31</b>	<b>31P 1d without decoupling</b> or noe (i.e., with proton coupling)	zg30
<b>P31CPD</b>	<b>31P 1d with proton decoupling</b> ; d1=2 aq=0.5 lb=1 p1=30°	zpgg30
<b>PROP31DEC</b>	<b>1H 1d with 31P decoupling</b> ; set O2 close to 31P of interest	zgif
<b>F19</b>	<b>19F 1d without decoupling</b> or noe (i.e., with proton coupling)	zgf1qn
<b>F19CPD</b>	<b>19F 1d with proton decoupling</b> ; d1=2 aq=0.5 lb=1 p1=30°	zghfigqn.2
<b>PROF19DEC</b>	<b>1H 1d with 19F decoupling</b> ; set O2 close to 19F of interest	zghfigqn
<b>COSYGPSW</b>	<b>standard gradient COSY</b> ; use setlimits button for sw, ns≥1	cosygpppqf
<b>COSYGPDPHPSW</b>	<b>DQF COSY</b> ; remove strong singlets & solvent peaks, measure J(HH)	cosygpmpfphpp
<b>HMBCETGPL3ND</b>	<b>HMBC J(HC) n-bond</b> ; cnst13=8 Hz; 3-pass J1(HC) removal	hmbcetgpl3nd
<b>HMBCGP</b>	<b>HMBC J(HC) n-bond</b> ; cnst13=10 Hz; 1-pass J1(HC) removal	hmbcgp1pndqf
<b>HMBCGP 15N</b>	<b>HMBC J(HN) n-bond</b> ; cnst13=5 Hz; no J1(HN) removal	hmbcgpndqf
<b>HSQCETGPSISP</b>	<b>multiplicity-edited HSQC 1-bond J1(HC)</b> ; CH CH3 pos, CH2 neg	hsqcedetgpsisp2.3
<b>HSQCETGP 15N</b>	<b>HSQC 1-bond J1(HN)</b> ; cnst2=90	hsqcetgps2
<b>HSQCETGPSISP</b>	<b>HSQC 1-bond J1(HC)</b> ; cnst2=145, all peaks positive	hsqcetgpsisp2.2
<b>MLEVPHPR</b>	<b>TOCSY 2d w solvent preset</b> ; d9[mix]=80ms; no gradients, NS=n*8	mlevphpr.2
<b>MLEVPHSW</b>	<b>TOCSY 2d</b> ; d9[mix]=80ms; no gradients, NS=n*8	mlevphpp
<b>NOESYPHPR</b>	<b>NOESY 2d w solvent preset</b> ; d8[mix]=0.3s; no gradients, NS=n*8	noesyphpr
<b>NOESYPHSW</b>	<b>NOESY 2d</b> ; d8[mix]=0.3s; with gradients, NS=n*2	noesygpphpp
<b>ROESYPHPR</b>	<b>ROESY 2d w solvent preset</b> ; p15[mix]=0.2s; no gradients, NS=n*8	roesyphpr.2
<b>ROESYPHSW</b>	<b>ROESY 2d</b> ; p15[mix]=0.2s; flip-flop splk; no gradients, NS=n*8	roesyphpp.2

**Example for C13APT:**

The sequence listing is given below, along with the comments at the end of the pulse sequence. Look at the comments:

- pl1, pl12: power levels which we should very rarely change (getprosol sets these!)
- p1, p2: pulse widths: we may update these on occasion, but getprosol again usually does the job
- d1: hopefully you know what to do here [default is 2 sec — typical for an organic compound; 5 sec wouldn't be unusual if "remote" carbons are present (problematic quaternaries), and/or sample is sealed O<sub>2</sub>-free]
- d20: here's where the comments are useful, but only in combination with the sequence itself; note that d20 is set within the sequence listing, and is dependent on cnst2 and cnst11 (i.e., you don't change d20 directly, only by changing cnst2 or cnst11)
- cnst2: typically set = 145 [Hz]
- cnst11: =1 is dept135 analog, =2 is dept90 analog
- NS: end comments are often useful for minimum NS; use multiples of 4 for NS!

```
#include <Avance.incl>
#include <Delay.incl>

"p2=p1*2"
"d20=1s/(cnst2*cnst11)"

"DELTA=p1*4/3.1416"
;pl1 : f1 channel - power level for pulse (default)
;pl12: f2 channel - power level for CPD/BB decoupling
;p1 : f1 channel - 90 degree high power pulse
;p2 : f1 channel - 180 degree high power pulse
;d1 : relaxation delay; 1-5 * T1
;d20: 1/(J(XH)) X, XH2 positive, XH, XH3 negative
; 1/(2J(XH)) X only
;cnst2: = J(XH)
;cnst11: = 1 X, XH2 positive, XH, XH3 negative
; 2 X only
;NS: 4 * n, total number of scans: NS * TD0
;DS: 4
;cpd2: decoupling according to sequence defined by cpdprg2
;pcpd2: f2 channel - 90 degree pulse for decoupling sequence

1 ze
  30m pl12:f2
  30m cpd2:f2
2 30m
  d1
  4u do:f2
  p1 ph1
  d20
  (p2 ph2):f1
  d20 cpd2:f2
  DELTA
  go=2 ph31
  30m mc #0 to 2 F0(zd)
  d20 do:f2
exit

ph1=0 0 0 0 1 1 1 1 2 2 2 2 3 3 3 3
ph2=0 2 1 3 1 3 2 0 1 3 2 0 2 0 1 3
ph31=0 0 2 2 1 1 3 3
```