Summer 2008 Chem 637 – Lab # 6 Notes
VT, NOE-DIFF, HOMODEC, PRESAT, and NOESY/ROESY

Variable temperature (VT) operation is performed regularly on all the high-field spectrometers, so all high-field users must be aware of various issues with regard to setup and safe use of VT. An important component of the final checkout exam will ask all students to perform some basic gas-line hookup changes, and will have questions concerning VT operation. Otherwise, the lab for this week is optional; students should practice sections below that will be important for their research. Those that will be performing VT should run through setup and calibration procedures.

VARIABLE TEMPERATURE

See the VT notes in the VUG guide for step-by-step details, and for more about the safe use and operation of VT. Some of the more important points are:

a) INOVA-500 and 600: −80 to +80°C only
We are in the process of making the INOVA spectrometer switch from air to N2 gas for VT operation, similar to what is done on the UNITY-500. Pay attention to signs and notes in the labs; gaseous N2 must be used for all VT experiments (warm/hot and cold).

b) UNITY-500: −150 to +120°C

Always switch to N2 gas (and back to air when finished) for all VT experiments.

Do not assume other probes will behave similarly to* bbext*; only* bbext *gives sample temps close to thermocouple (setpoint) temps. Note e) below!

c) ALWAYS turn the heater off prior to disconnecting VT gas flow.

d) Always note the gas flow in your notebook (or text), as it affects temp calibrations.

e) Using VT usually requires accurate calibrations:
   − the actual temp is always outside the setpoint temp (**bbext** closer than other probes)
      → if setpoint = −80°C, actual temp will be ≤ −90°C
      → if setpoint = +80°C, actual temp will be ≥ +90°C
      → **equipment will freeze or boil samples of those not currently listening!**
         with significant probe damage possible (~ $5k in a recent sample explosion)!

1D NOE-DIFF

Unless there is an overriding reason to try NOE-DIFF, use NOESY1D instead. Although NOESY1D is a transient NOE experiment (and NOE-DIFF is steady-state), NOESY1D has been greatly superior in data quality in all cases so far experienced here to NOE-DIFF. Steady-state NOEs might, in some specific cases, provide some advantage to the transient experiment, but we've not yet seen an example in our labs.

HOMODEC

a)   Acquire a normal ^1H spectrum, then **homodec↵** to start the setup for a homonuclear decoupling experiment. These experiments have largely been replaced by COSY and its variants, but HOMODEC still has utility when observation of a "reduced" multiplet is needed.

b)   Set the cursor in the middle of the multiplet to be saturated (removing its coupling from other multiplets), and type **sd↵** , which sets **dof** correctly.

b)   Make sure the decoupler power **dpwr** is never > 25 (the software should prevent larger values from being set). Adjust the decoupler power to the lowest value possible, but still high enough to remove the coupling from the multiplet needing to be observed.

c)   Use **clradd spadd↵** (careful! this will delete exp5 without asking) with **addi↵** to see the normal ^1H spectrum on-screen with the homodec spectrum. Use **jexp1 s1 pl pscale(0) md(1,2) jexp2 r2 vp=vp+50 pl page↵** or similar to plot the two spectra; only **vp** and **vs** need be adjusted to give reasonable plots (or use NUTS).

PRESAT

Solvent suppression will be significantly better when using a ^1H direct (or inverse) probe, such as **hcx** on the INOVA-500, rather than the broadband probes **bbext** or **bbswgo**. The **presat** experiment will work fine on the broadband probes for removing relatively low concentration peaks (e.g., residual HOD from 100% D_2O, or solute methyls from t-butyl groups), but any sample in 90:10% $H_2O:D_2O$ should be run on **hcx**, or on the INOVA-600.

a)   Acquire a normal ^1H spectrum. It may be necessary to reduce **pw** (to **pw=1)** and **tpwr** (e.g., **tpwr=40** with **gain=0**) to acquire spectra without an ADC overflow for samples in 90:10% $H2O:D_2O$.

b)   Set the cursor in the middle of the solvent peak to be suppressed, and type

   nl movetof ga↵ , which sets the solvent peak on-resonance.

c)   **presat↵** sets up a presaturation experiment. PRESAT greatly reduces the intensity of solvent peaks (or other large singlet peaks).

d)   Start with a presaturation power of **satpwr =2** for the residual peak of D_2O (i.e., the HOD impurity peak), or other relatively small singlets (e.g., the methyls of t-butyl). Start with **satpwr=14** (up to **satpwr=24**) for $\geq$ 10% H_2O, or similar large solvent peaks.

e)   Set **satfrq=tof** ; i.e., always perform presat on-resonance to the solvent peak.

f)   **satdly** replaces **d1**. Typically then, **d1=0 satdly ~ T_1** is used. Leave **ss $\geq$ 2** .

g)   For high water content (e.g., 90:10% $H_2O:D_2O$), setting **tof** as prescribed above is not sufficient. Continue to improve the suppression as follows:

   − array **tof** and **satfrq** diagonally to find minimum solvent intensity as follows:

       array('tof',20,[tof-20]**,2)**   ;the 3rd element is (tof minus 20 Hz) as a number

       arraytof   ;diagonalizes the tof array with the satfrq array

       av   ;absolute value mode is best for seeing the solvent peak

       go dsa   ;choose the lowest solvent intensity from the array

       tof=[best one] **satfrq=tof**

       array('tof',20,[[best one]-4]**,0.5)**

       arraytof go dsa   ;run a fine array to get a more accurate tof

       tof=[best one] **satfrq=tof**

       ph   ;back to phased mode

   h)   If **pw** and/or **tpwr** were reduced to prevent ADC overflow, return them to their normal values. Now adjust **gain** properly. Acquire spectra with **nt≥4** and **ss≥4** .

   i)   The quality of the remaining solvent peak is critically dependent on the quality of the shims, in particular, the high order shims. See Charlie or Monika for assistance in learning how to gradient shim (Chap. 7 in VUG) if you will be doing a lot of work requiring solvent suppression.

NOESY and ROESY 2D Set-Up

- NOESY and ROESY are useful for samples in deuterated solvents; see notes elsewhere for setting up Watergate and wet (Biopack) versions of these sequences, wgnoesy and wgroesy

- Begin as normal with 1d acquisition, and optimize **sw** , typically by using **movesw** ; for **presat**, reduce **sw** directly (e.g., **sw=sw/1.5**) leaving **tof** unchanged (the best data quality is achieve when the solvent is on-resonance).

  Setup integral regions in the 1D spectrum, making sure the regions cover all areas of peak intensity. The integration setup is *strongly* recommended for its use with **bc** (baseline correction) in the 2D processing.

- Starting with this proton 1D spectrum, type **ROESY** or **NOESY**

  Note with **ROESY** setup that: $\gamma B_{SL} = 1/(4 \times slpw \times 1e\text{-}6) \sim 2\text{-}6$ kHz

   b) Critical parameters:

      np=1024 to 4096   ;if **np** or **ni** are changed, reapply the apodization (**gaussian**)
      nt=8   ;a minimum phase cycle
      ss=4   ;set to 4 for 1st row looks; set back to 32 or 64 for actual exp.
      d1 or **satdly≥2×T_1** ;pushing this parameter to smaller values can lead to serious problems/artifacts in the final data

       mix=0.2 to 1 s   ;mix $\leq$ 0.5 s for ROESY; acquiring multiple spectra with different mix times can provide data for a build-up curve, as well as confirm weak NOE/ROEs

c) acquire 1st row (can **aa** after), and phase carefully

    $\rightarrow$ do a **wft1 ff wti** and note if apodization is wrong

            gaussian wft1 wti will improve dramatically if so

    $\rightarrow$ if **lp $\neq$ 0** then run **calfa ga** to assist in flattening the baseline

    $\rightarrow$ note utility of **bc** for baseline improvement; note also that the defaulty spline fit used by **bc** will produce *big* errors if the integration regions do not cover *all* of the spectral intensity