II. *X* Acquisition (e.g., ^{31}P or ^{13}C)

(22-Jun-98)

A. Discussion

- The spectrometer acquisition time of an *X* nucleus experiment should be estimated from similar experiments on the same equipment if possible using eqn (1) or (2) below. ^{13}C and ^{31}P experiment times on the 500's and 360 can be estimated from similar experiments on the AC-300 (Athena) using:

$$S/N \propto cB_o^{3/2}t^{1/2}x \qquad (1)$$

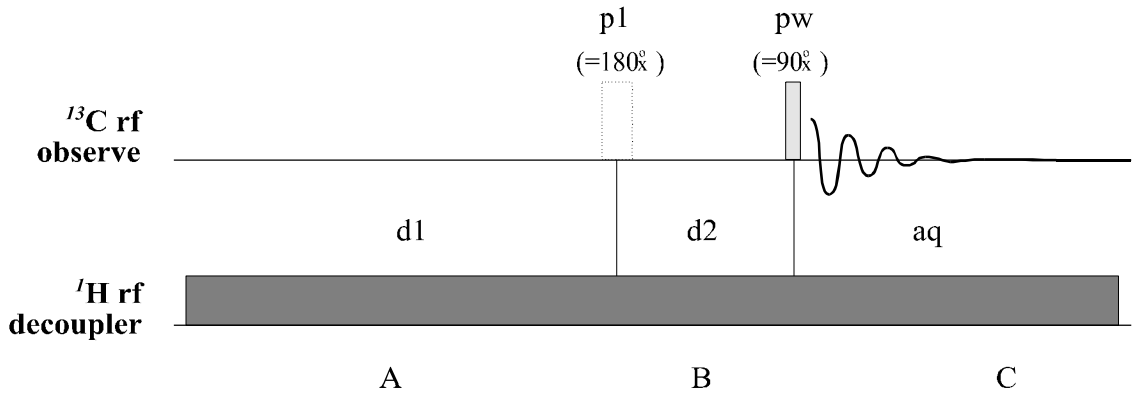
where c = concentration, B_o = magnetic field strength, t = experimental time, and x = probe filling factor. To estimate the time under different conditions that would give identical *S/N*, then:

$$t_{new} = t_{old} \cdot \left[\left(\frac{c_{old}}{c_{new}} \right)^2 \left(\frac{B_{o_{old}}}{B_{o_{new}}} \right)^3 \right] \quad . \qquad (2)$$

A reasonable estimate (although direct comparison to experiments on similar compounds is preferred) on the UW Chem MRF equipment can be made starting with the observation that a 0.1 M solution will typically give publication quality spectra in 20 min on the AC-300 (Athena).

- Standard decoupled (NOE-enhanced), quantitative decoupled (no-NOE; Bruker's INVGATE.AU) and NOE-enhanced coupled spectra (Bruker's GATEDEC.AU) can be obtained through use of the S2PUL sequence.
- Nuclei with negative γ values, such as ^{15}N and ^{29}Si, are best acquired using polarization transfer sequences; DEPT in general is the preferred sequence over INEPT if more than one J_{XH} value is involved; quaternary moieties of negative γ nuclei are best obtained through long-range coupling unless the $T_1(X)$ values are known to be reasonably short.

1d ^{13}C Acquisition (s2pul)



B. Critical Parameters

d1 – relaxation delay; **d1** > $T_1(^1H)$ to obtain optimum NOE
dof – usually =0 when **solvent** set correctly; should be within 5ppm of ^1H coupled to X nucleus of interest
dpwr – decoupler power (larger number is higher power); ***typically never > 46***
dmf – decoupling strength = [pw90(^1H at dpwr)]$^{-1}$
dmm – decoupler modulation mode; either **dmm='ccp' dseq='waltz16' dres=90** or **dmm='ccw'** [both are equivalent] is best for typical compounds
dm – decoupler on/off flag (see Table 6)

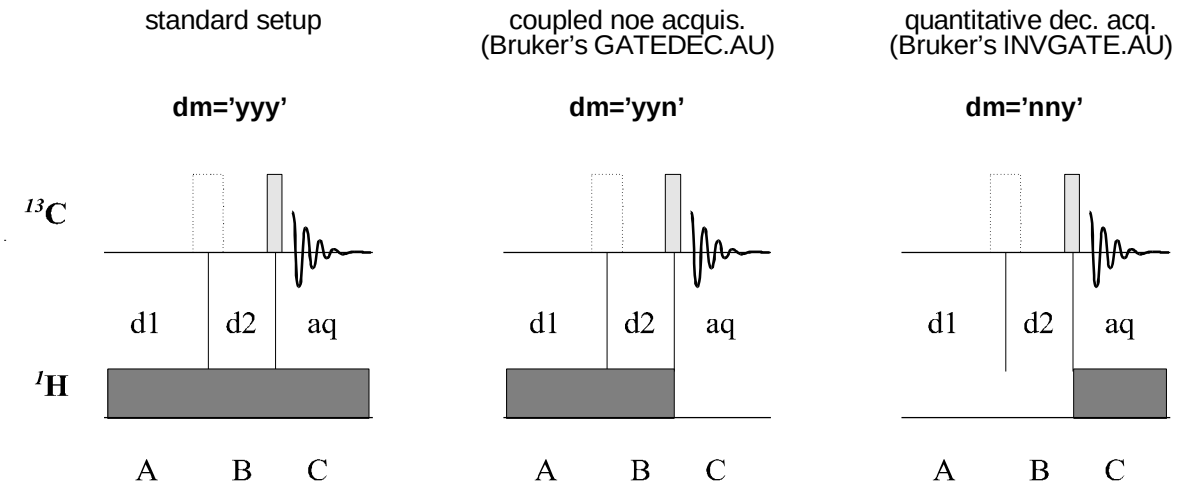
C. 1d X{^{1}H} *Acquisition*

- start by setting the probe parameter appropriately
- to setup parameters (method **b** is recommended)
  a) either read in a data file (rt) or parameter file (rtp) for standard $X\{^1H\}$ acquisition, or
☞ b) click on MAIN MENU SETUP and select nucleus and solvent
  c) alternatively, run s2pul macro (but normally will need to reset **spin='n'**, **gain=0**; check **temp** and **vttype**, and check **tpwr** (=58 typically) and **pw** (=5-8µs typically).
- use UWMACROS TUNE PROBE TUNE C13 [or **tunec** macro] and tune ^{13}C channel; reattach ^{13}C X cable to X Obs BNC
  – make sure ¼ wave cable is correct for ^{13}C
  – make sure low-pass (brown) filter is in-line
  – make sure correct probe cap is inserted (*none* in bbold probe)
  – make sure no external attenuator is in-line
- use UWMACROS TUNE PROBE TUNE H1 [or use **tuneh** macro (will have to reset gain after tunec if UWmacro is not use] and tune decoupler channel; reattach ^1H decoupler cable to decoupler BNC

Table 6. Common Decoupler Parameter Settings

Parameter Settings	Comments
dm='yyy' dpwr=46 su	typical for ^{13}C acquisition; full on decoupler; keep **dpwr £ 48**
dm='nnn' su	turns decoupler off; always finish with this command + **su**
dm='nny' su	inverse gated (Bruker's INVGATE.AU) mode; gives quantitative (assuming **d1**>5T_1 !) decoupled spectra
dm='yyn' su	gated spectra (Bruker's GATEDEC.AU); gives coupled spectra but with NOE buildup (DEPT is necessary for ^{29}Si, ^{15}N and other -γ nuclei)
dmm='p'	normal setup for ^{13}C acquisition (8/1/97; **dmm='w'** works fine)
dmm='ccp'	normal setup for DEPT and INEPT most 2d ^{13}C experiments, does hard pulses followed by pulsed decoupling during acquisition
dseq='waltz16' dres=90	normal setup for ^{13}C (and all X) acquisitions; ^1H waltz-16 decoupling
dseq='garp1' dres=1	normal setup for all inverse experiments; X garp-1 decoupling
dmf= [1/pw90]	sets decoupler pulsewidth for composite pulses, where pw90 is the ^1H decoupler 90° pulse width at the **dpwr** setting used for decoupling
dpwr= [typically ~42]	decoupler power in dB; typically want 90° pulsewidth = 100-150µs

1d ^{13}C Variations in VNMR



- use **dm='yyy' su** to turn on decoupler (or UWMACRO DECOUPLER ON; see Table 6 and figure below for more decoupler information)
- use **go** to start acquisition
  - **ga** will automatically apply a **wft** (weighted Fourier transform) following acquisition
  - use **wft** to manually transform
  - **dscale** will display the axis
  - **dsx** will apply wft dscale(-3)
- **gain = 30 to 40** should usually work for ^{13}C acquisition
  - if always getting ADC OVERLOAD beep, then receiver gain is too high
  - it is important the receiver gain be optimized for *X* nucleus experiments
  - see ^{1}H section above for how to set the gain accurately
- optimize **sw** by setting right (right mouse button) and left (left mouse button) cursors, and use **movesw**
- set reference by placing cursor close to peak, then type **nl rl** command, e.g., **rl(77p)**

D. ^{13}C *(X) pw90 and {*^{1}H*} Decoupler Calibrations*

- general rule of thumb for these calibrations is:
  - short runs use facility numbers
  - if probe problems are suspected, check pw90's of *X* and ^{1}H observe (not decouple)
    - if numbers are close to facility values, probe is likely OK
    - if pw90 is much less than facility value, you are doing something wrong (figure it out! :-)
    - if pw90 is much longer (>1μs) than facility value, find TA or facility staff
  - always perform calibrations for overnight or longer runs for PT-type experiments; for standard decoupled experiments, calibrations are rarely needed
- ^{13}C and other *X* nuclei pw90 calibrations require concentrated or labeled samples
  - for ^{13}C, use 50% benzene in acetone-d_6; do not degas these samples
  - addition of $GdCl_2$ or Cr-acac can improve T_1's dramatically

  - set **tpwr** to desired setting; see Table 2 (on lab wall for most current!!) for calibrations for probes in UW Chem MRF facility
  - make sure you are on resonance (set cursor on multiplet, then **movetof**)
  - set **d1=20s** or longer depending on sample T_1
  - use 360° pulse for final checks always!!
- once the observe pw90 is obtained, assuming in exp1 then **jexp2**
  - move ^1H cable to observe port, and find resonance for benzene doublet (take one scan, move cursor to middle of doublet and use **movetof**); write this value of tof down as ^1H dof
- assuming ^{13}C still in exp1 and ^1H in exp2, do **jexp1 mp(3) jexp3**
  - put ^1H on resonance by setting **dof** = ^1H dof from above
  - setup decoupler calibration experiment with UW macro **pwxdec90**
  - set **pwxlvl = 60** and find **pwx** where antiphase doublet nulls; this is decoupler hard 90
  - set **pplvl = pwxlvl** and **pp = pwx** for **hetcor** and **dept** experiments
  - set **pwxlvl** appropriately for decoupling (probe dependent; see Table 2 on wall) and find **pwx** where antiphase doublet nulls; set **dpwr = pwxlvl** and **dmf = 1/(pwx*1e-6)**

E. 1d X{^{1}H} Data Workup and Plotting

- typically will want **lb = 2** or **3** for ^{13}C experiments
- see 1d ^1H section E for plotting description