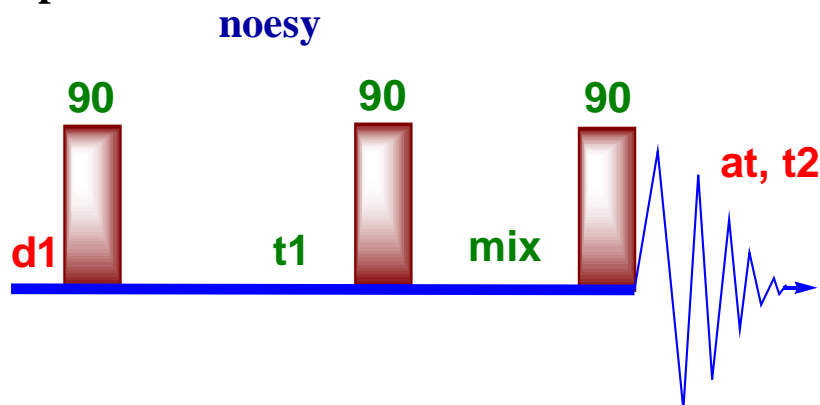


## NOESY experiment



Nuclear Overhauser Effect Spectroscopy is a 2D spectroscopy method whose aim is to identify spins undergoing cross-relaxation and to measure the cross-relaxation rates. Most commonly, NOESY is used as a homonuclear  $^1\text{H}$  technique. In NOESY, direct dipolar couplings provide the primary means of cross-relaxation, and so spins undergoing cross-relaxation are those which are close to one another in space. Thus, the cross peaks of a NOESY spectrum indicates which  $^1\text{H}$ 's are close to which other  $^1\text{H}$ 's in space. This can be distinguished from COSY, for example, which relies on J-coupling to provide spin-spin correlation, and whose cross peaks indicate which  $^1\text{H}$ 's are close to which other  $^1\text{H}$ 's through the bonds of the molecule.

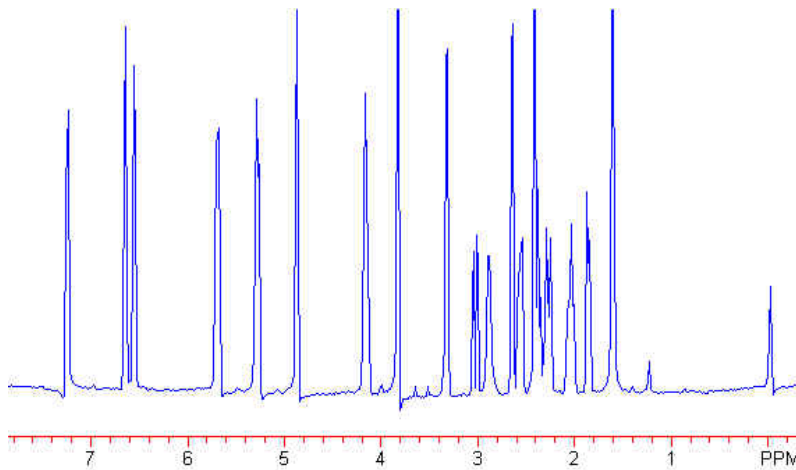
The basic NOESY sequence consists of three  $90^\circ$  pulses. The first pulse creates transverse spin magnetization. This precesses during the evolution time  $t_1$ , which is incremented during the course of the 2D experiment providing chemical shift information in the F1 domain. The second pulse transmits some of the magnetization to the Z axis and during the following mixing period, the non-equilibrium Z component will exchange magnetization through relaxation (dipole-dipole mechanism). This exchange of magnetization is known as NOE (Nuclear Overhauser Effect). Thus, the basic idea is to produce an initial situation for the mixing period  $\tau_m$  (the time during which cross relaxation occurs) where the longitudinal polarization of each spin is labelled by its resonance frequency. The longitudinal magnetization is allowed to relax during the mixing time  $\tau_m$ . Note that, for the basic NOESY experiment,  $\tau_m$  is kept constant throughout the 2D experiment. The third pulse creates transverse magnetization from the remaining longitudinal magnetization. Acquisition begins immediately following the third pulse, and the transverse magnetization is observed as a function of the time  $t_2$ . The NOESY spectrum is generated by a 2D Fourier transform with respect to  $t_1$  and  $t_2$ .

Axial peaks, which originate from magnetization that has relaxed during  $\tau_m$ , can be removed by the appropriate phase cycling.

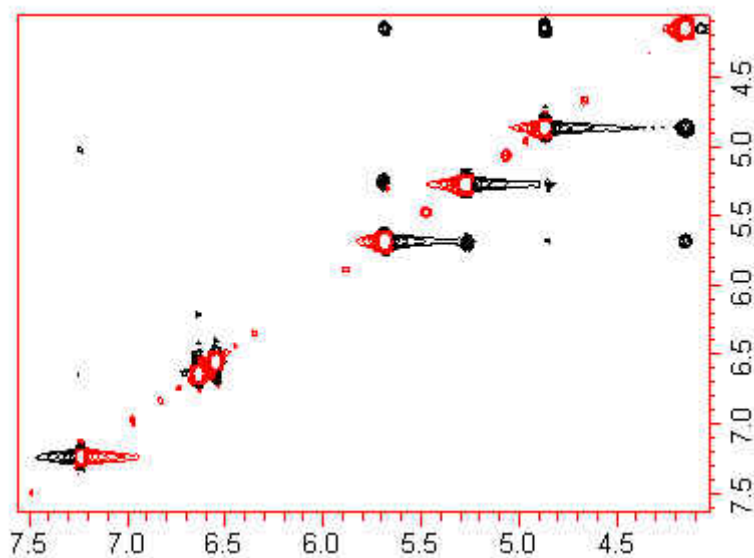
NOESY is one of the most useful techniques as it allows to correlate nuclei through space (distance smaller than  $5\text{\AA}$ ). By measuring cross peak intensity, distance information can be extracted.

## 2D NOESY Experiment

1. Run a 1D spectrum place two cursors at both sides  $\sim 1$  ppm away from the peak. . Move sweep width. Too short **sw** would cause phase distortion.



This would cause streaks in the contour plot as shown below.

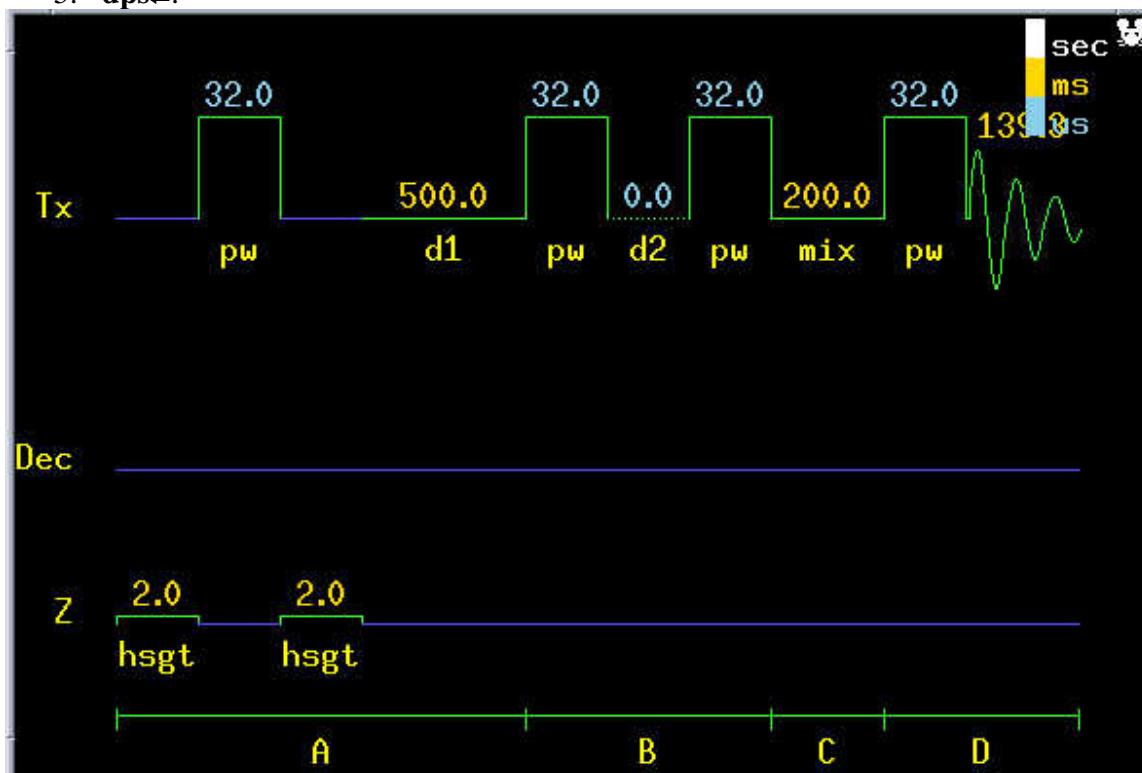


2. **mf(1,2) jexp2**.

3. **NOESY**↓. (for lower case **noesy**, **hsgt** is off).
4. **dg**↓.

ACQUISITION	TRANSMITTER	FLAGS	PROCESSING
seqfil	NOESY	tn H1	hs n gf 0.064
sw	3674.4	sfrq 399.729	sspul y gfs not used
at	0.139	tof 0	PFGflg y fn 2048
np	1024	tpwr 54	hsglvl 4854
ss	32	pw 32.000	hsgt 0.002000
d1	0.500	DECOUPLER	SAMPLE
nt	8	dn H1	date Mar 10 2004
ct	0	dm nnn	solvent CDC13
			sample undefined
2D ACQUISITION	NOESY		SPECIAL
sw1	3674.4	mix 0.200	temp not used
ni	200		spin not used
phase	arrayed		gain 30
PRESATURATION			
satmode	nnnn		
satfrq	0		
satdly	0		
satpwr	0		

5. **dps**↓.



6. **d1=0.5** ( $=2\sim 4T_1$ ,  $T_1=200$  ms), Too small d1 will cause very bad *t1* noise.
7. **np=2048**, this will set up at;  $at=np/(2sw)=0.139$ .
8. **ni=200**↓, this is the number of points in t1. The default number set by macro should be ok. Keep in mind that  $sw1/(2ni)$  should be **6~12 Hz/pt**, the digital resolution on F1.
9. **mix=0.2**↓, set it close to  $T_1(200$  ms) should provide maximized crosspeaks. For high MW, mix=0.3 should be ok. **mix** is the time period (in seconds) during which magnetization is transferred from one  $^1\text{H}$  to another  $^1\text{H}$ . **mix** must be long

enough to transfer magnetization between spins to "build up" the NOESY cross peak intensity between the two spins. If **mix** is too long though, the magnetization transferred to the second  $^1\text{H}$  will "diffuse" to additional spins, decreasing the NOESY cross-peak intensity between the two spins and creating new cross peaks between spins that are farther apart than 5Å. Typical values for mix range from 0.2 to 1.2 seconds depending upon the sample's molecular weight; 0.8 is generally a good starting point.

10. Optimize **gain**.

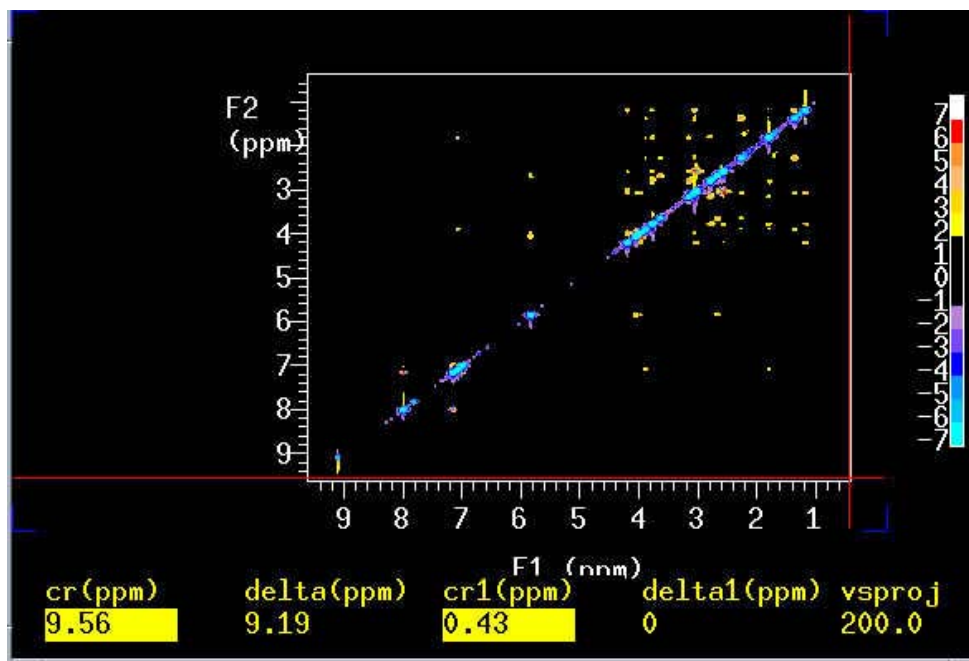
11. To optimize baseline flatness for the sw, sw1 you set up, please do the following to optimize **alfa** and **rof2**.

- acquire spectra with **ni = 2**, and phase carefully
- run **calfa** which uses the **lp** value from the phasing
- reacquire the **ni = 2** data and rephase; you should get a good phase with **lp=0**; if you do not, rerun **calfa** and require again
- if the baseline still has some curvature (either convex or concave), then you might want to:
  - note the values of **rof2** and **alfa**
  - keeping the sum **rof2+alfa** constant, change **rof2** and **alfa**. *Do array on both (see metal protein NMR)*
  - reacquire and note the effect of the change with **rof2** and **alfa**; one direction should move the baseline towards convex, the other towards concave; some combination should give optimal baseline flatness
  - write down the values of **sw rof2** and **alfa**; these should always work for this **sw**
- as a minimum, estimate *T1* using inversion-recovery null method to provide setting for **d1**

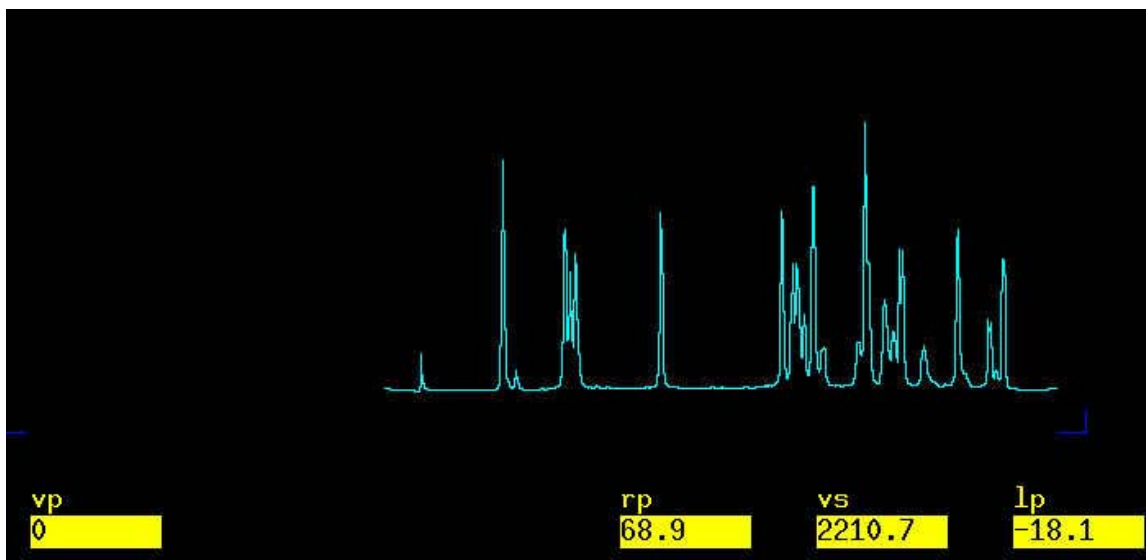
12. **go↵**.

13. save your original FID. **svf('cungen')** ↵.

14. Click at **MainMenu**, **AutoProcess**, you will see the following spectrum.

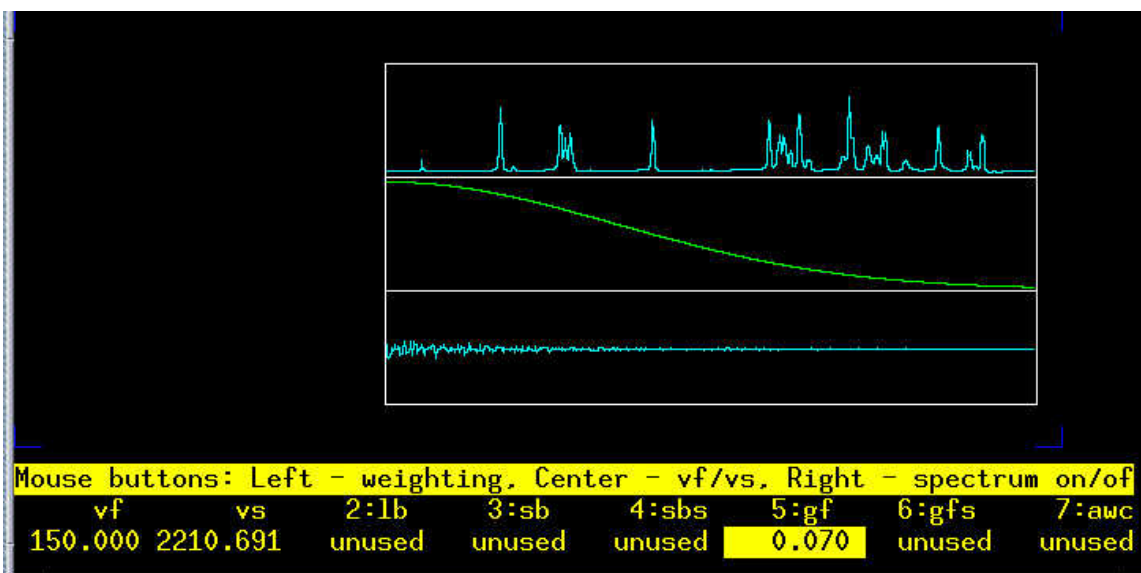


15. wft(1) ↵.



phase it as you did on a normal 1D.

16. wti ↵.



adjust *gf* to the end of FID.

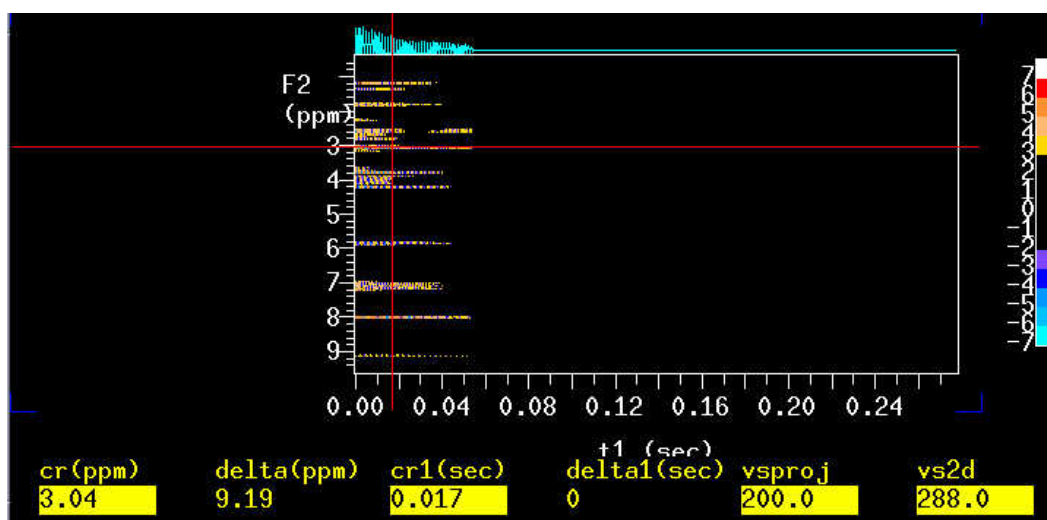
17. **wft1da**↵.

18. Click on *trace*.

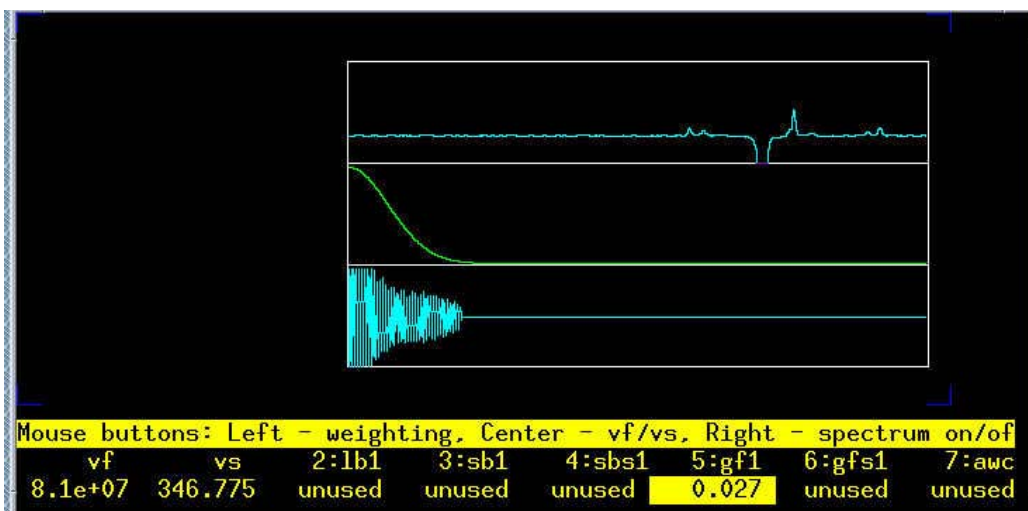
19. Choose one trace.

20. **ds**↵.

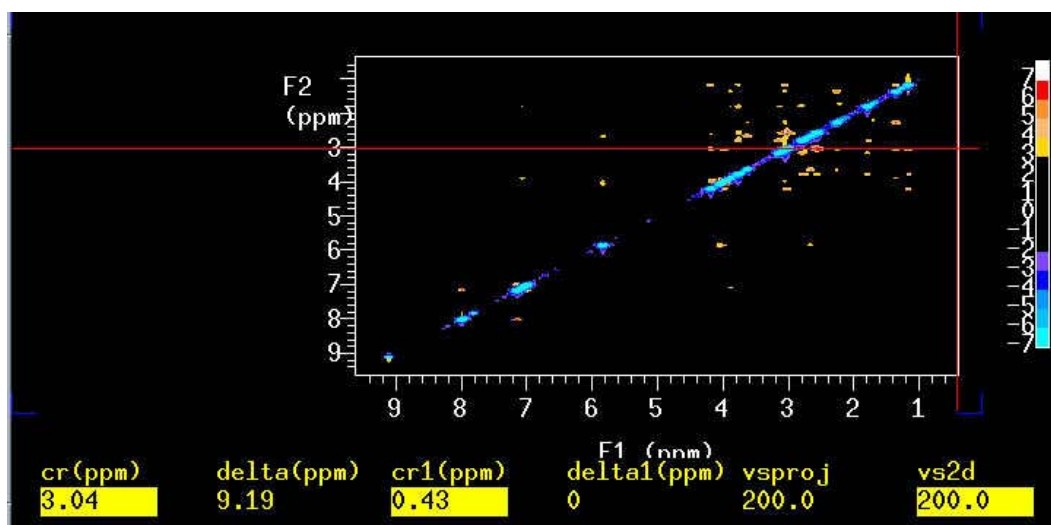
21. **wti**↵.



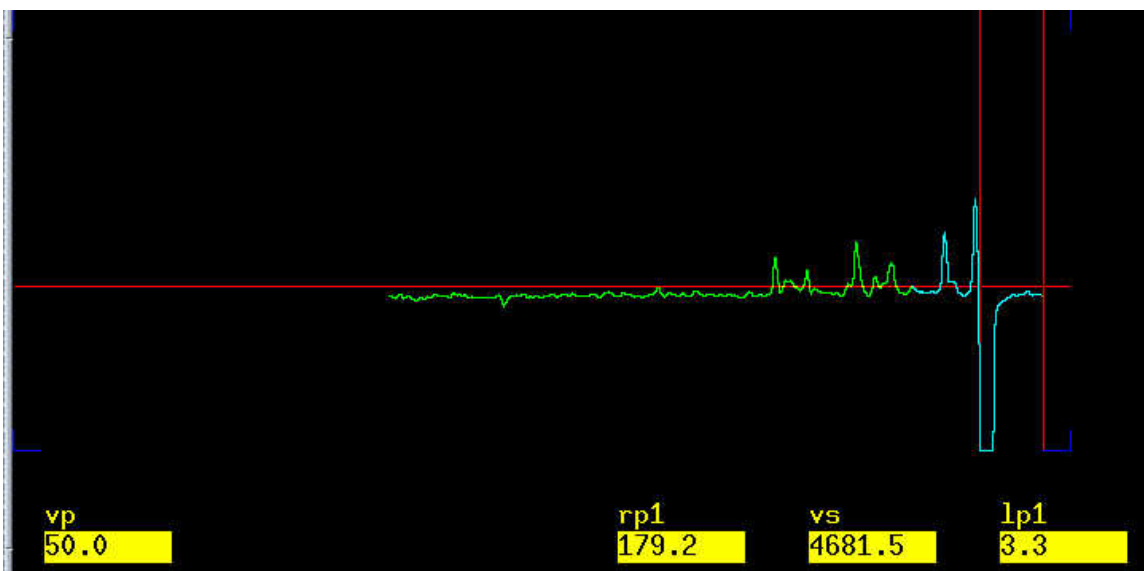
22.adjust *gf1* to the end of FID.



22. pmode='full' wft2da ↵.

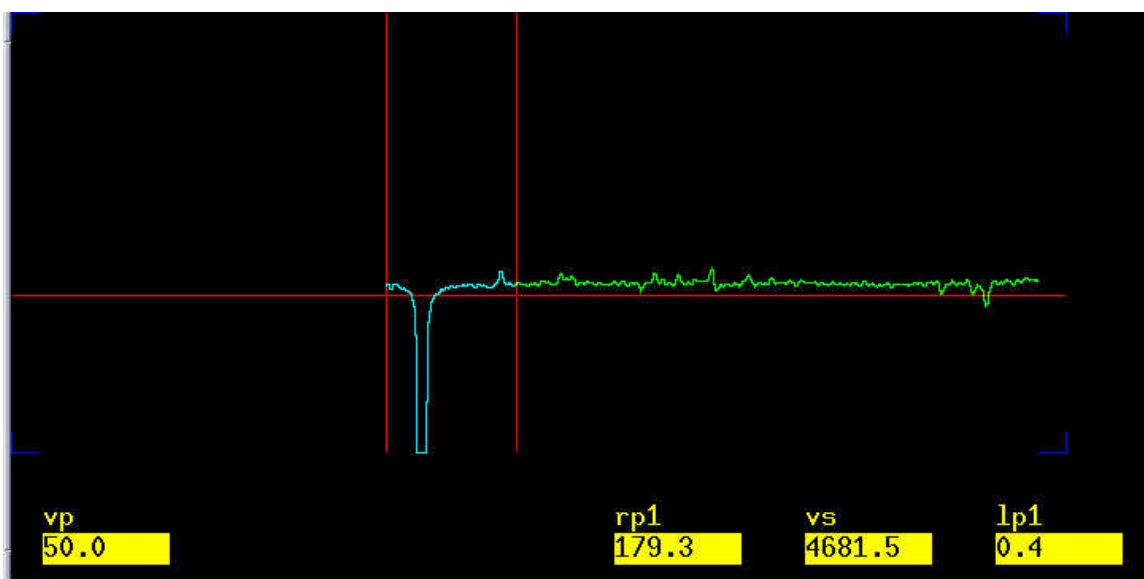


23. point the mouse at the right upper peak, ds ↵.



phase the the spectrum.

24. **dconi**. Point the mouse at the lower left peak, **ds**.



25. click at the right of the spectrum no adjustment please to finish the zero-order phase. This will keep the value you got from the first spectrum.

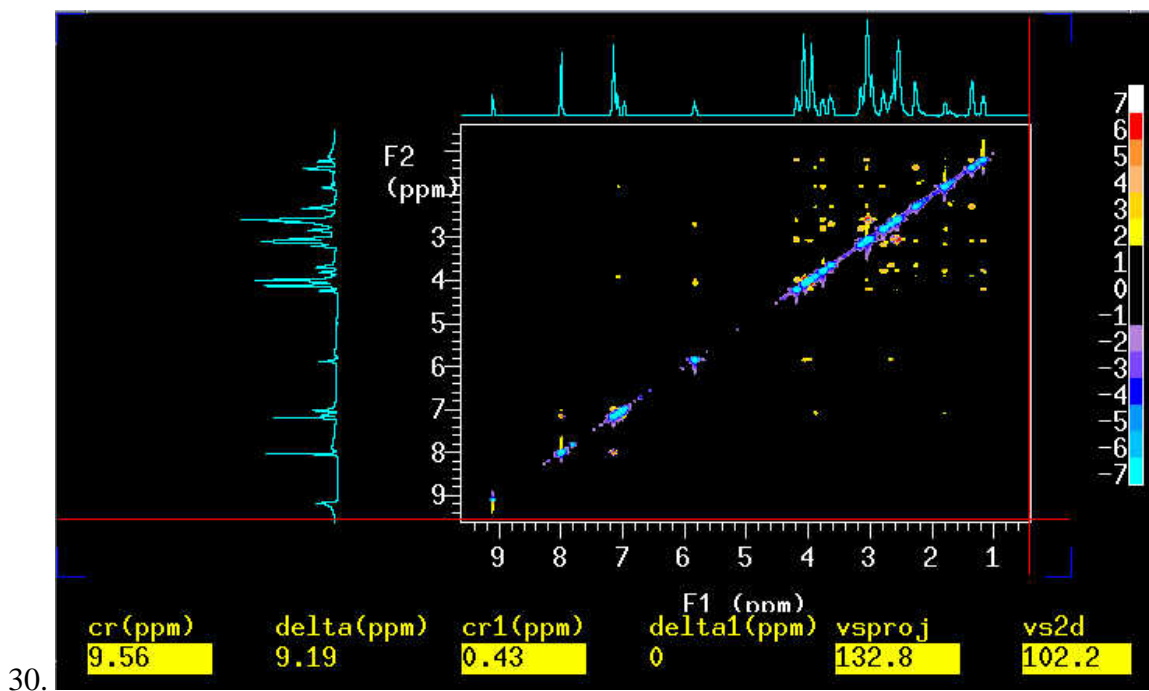
26. Phase the left peak. Adjust the baseline only. This is the first-order phase.

27. **dconi**.

28. **trace='f2'** **dconi**. Phase the 2D on F2 as you did on F1.

29. **dconi**.





31. To show 1D on both sides, dcon1 wc=130 wc2=130.↓ then click *ManMenu* → *Plot* → *Proj* → *Hproj(max){adjust vs}* → *Vproj(max)*.

32. Click *autoplot* to print out the 2D spectrum.

You have successfully done the 2D.