Set up 2D/3D using Xwinnmr

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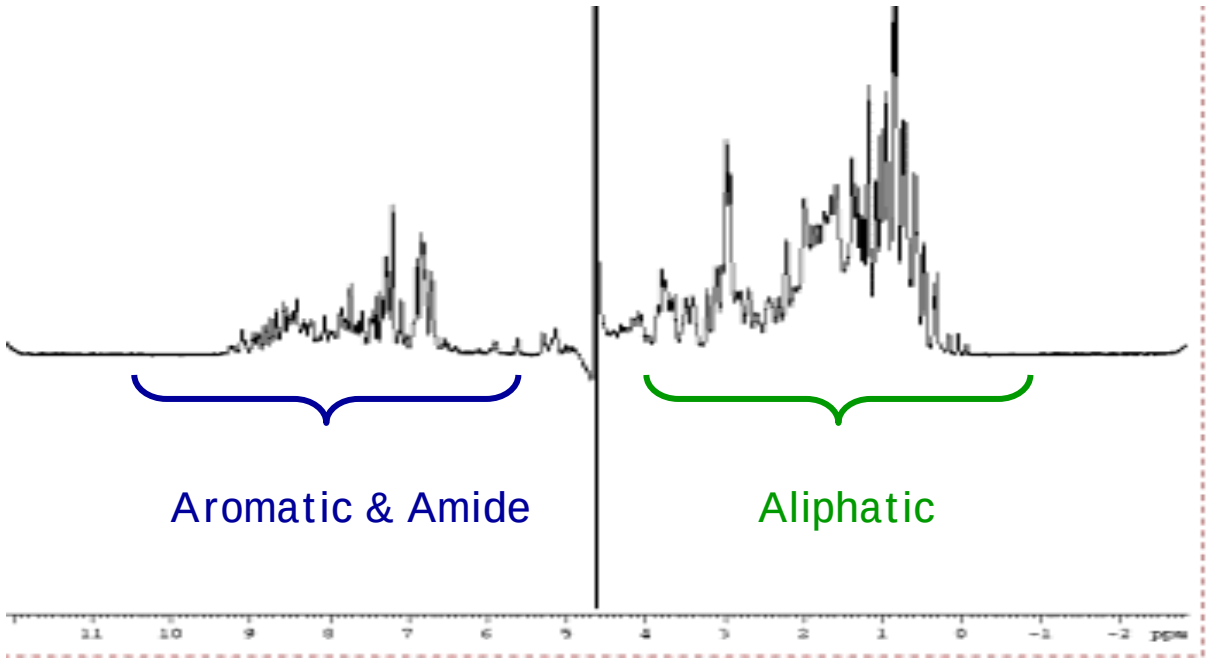
March 30, 2005

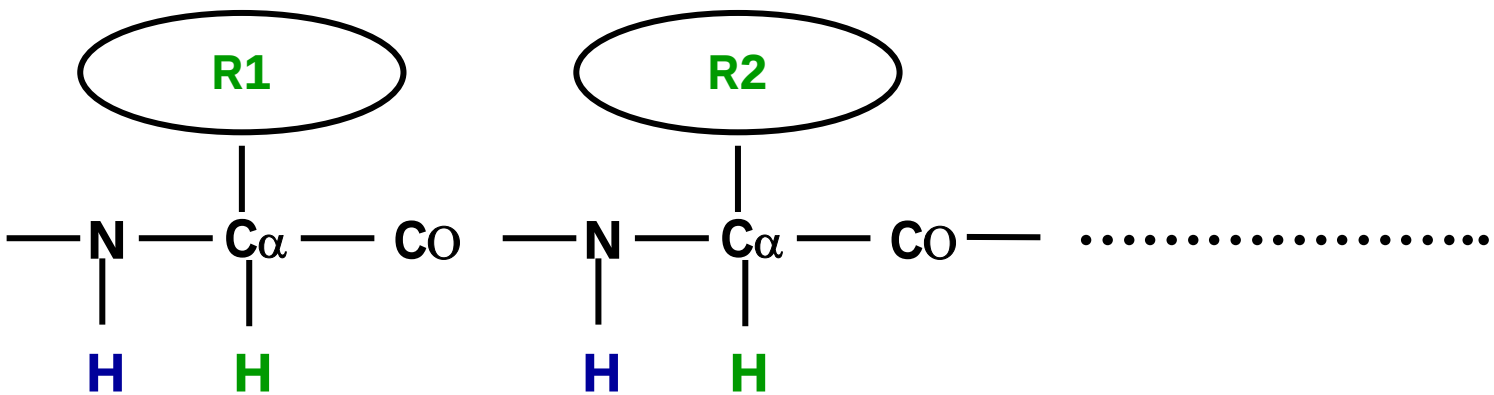
General Concept for 2D/3D experiment

(I) Why bother multi-dimension?

Homo Nuclear 1D NMR

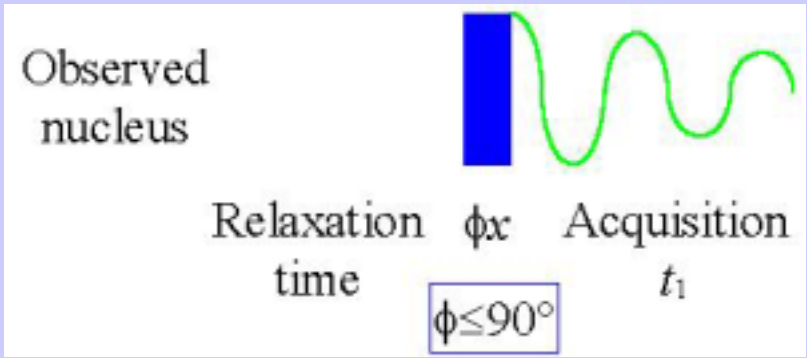
1D one pulse 1H

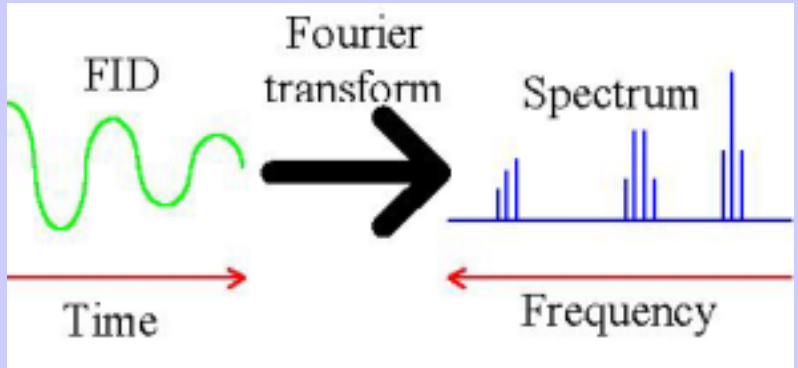




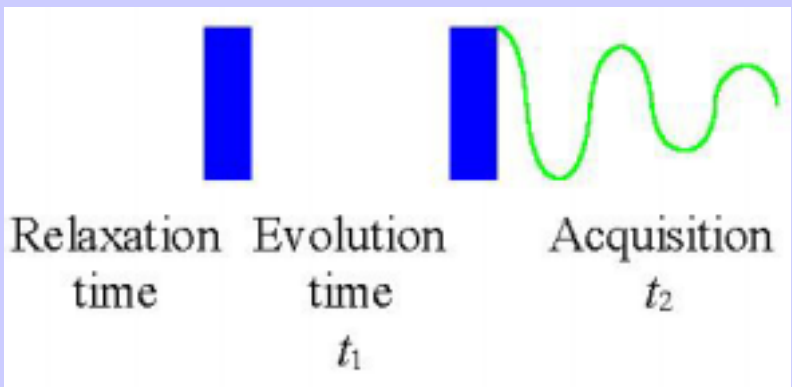
Homo/Hetro Nuclear 2D NMR

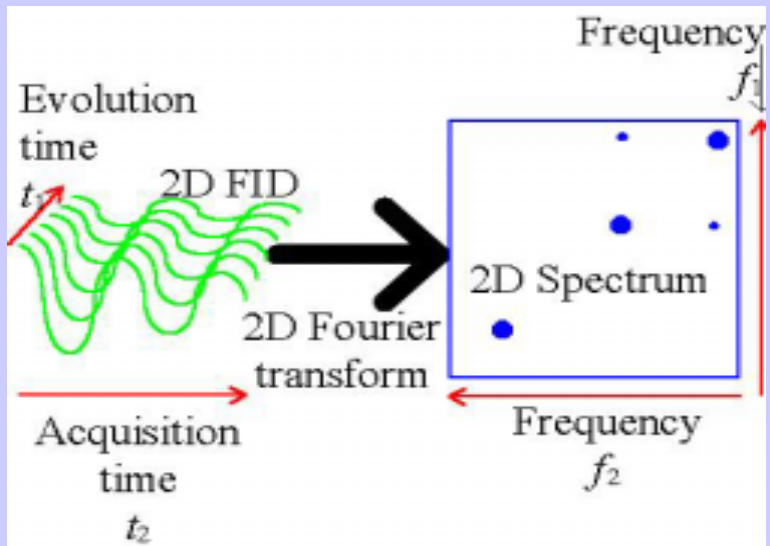
Basic 1D Experiment





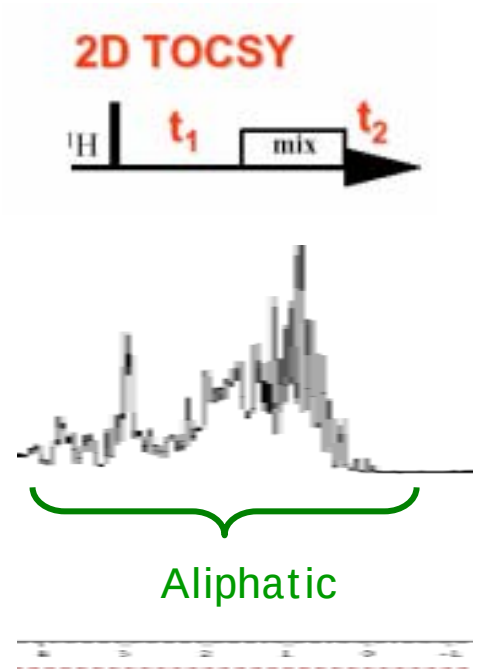
Basic 2D Experiment

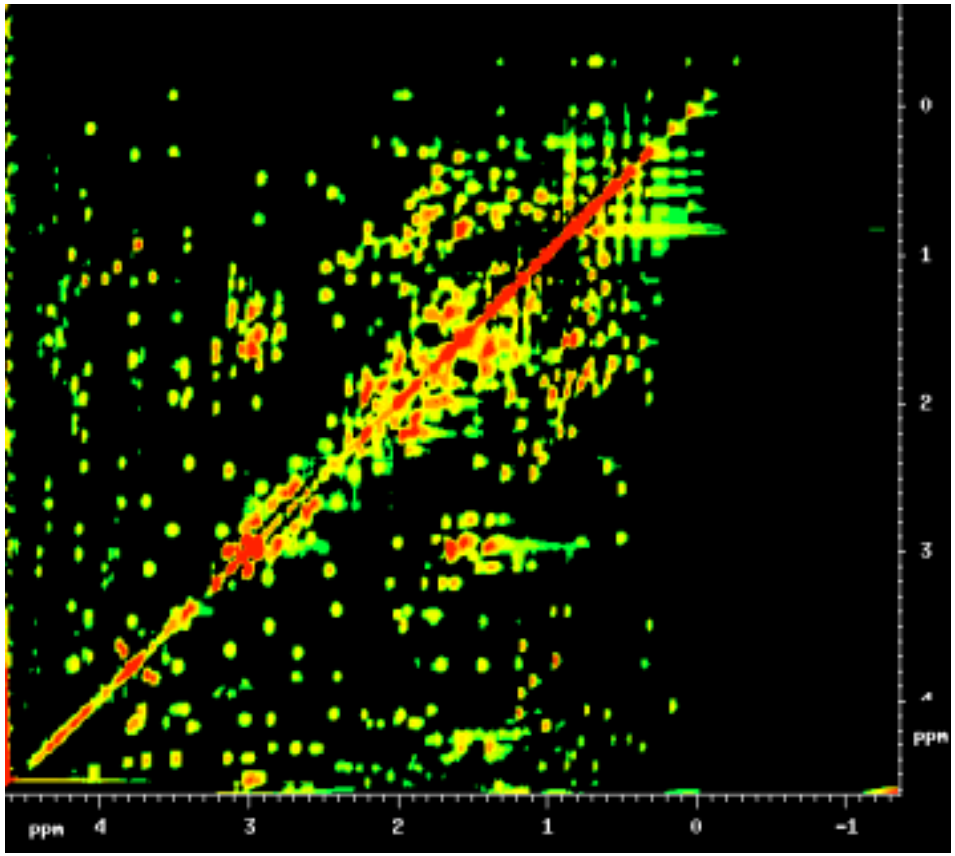


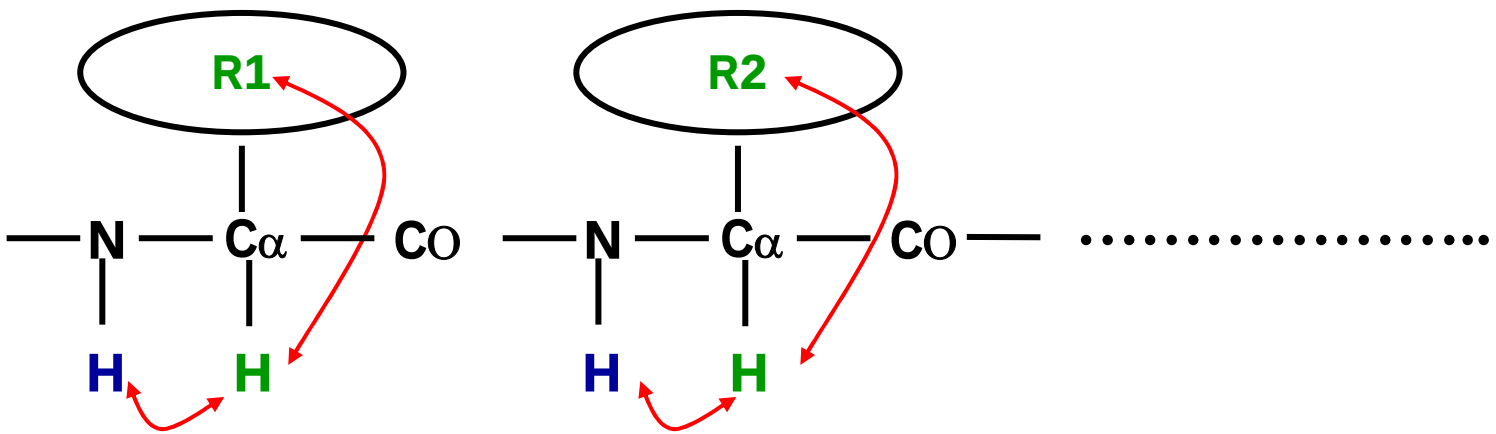


2D Homo Nuclear

1H-1H



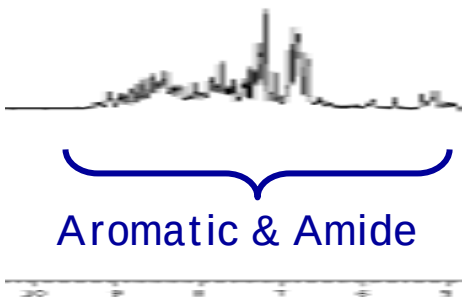


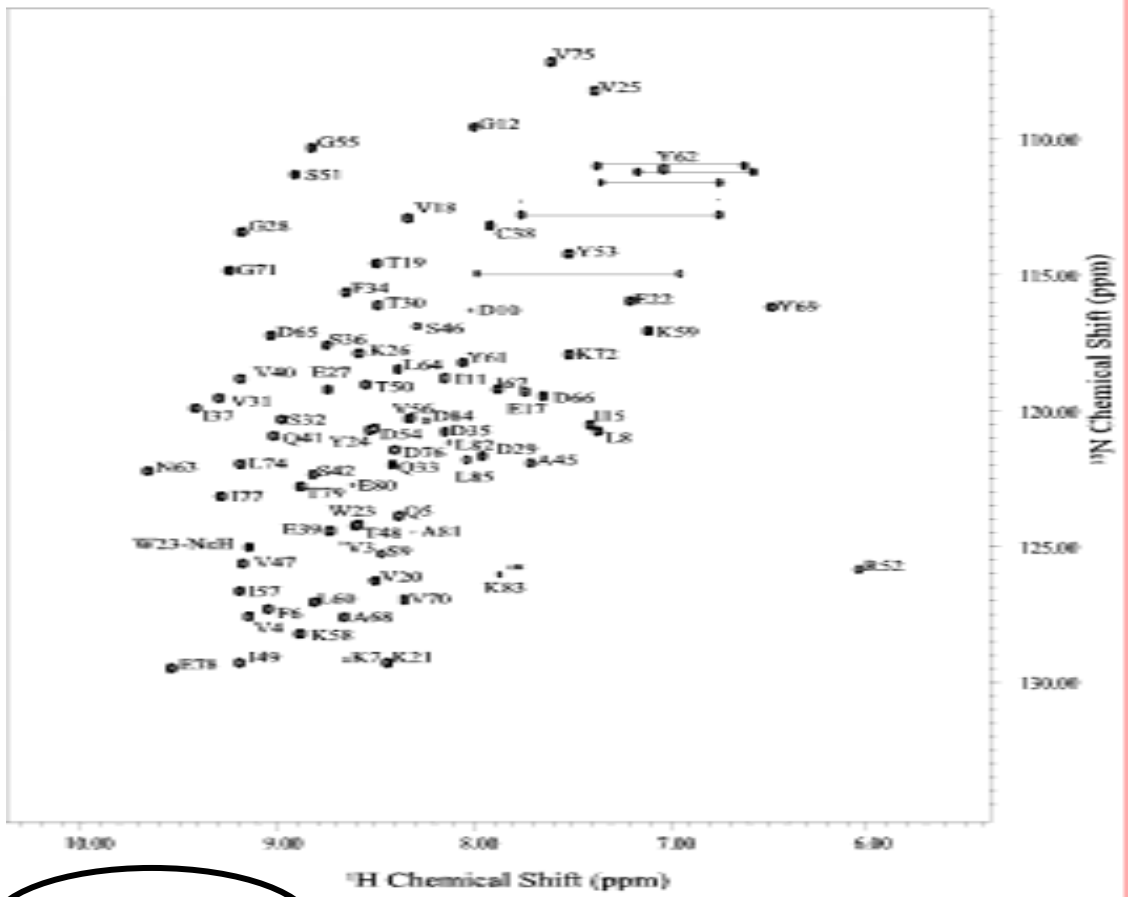


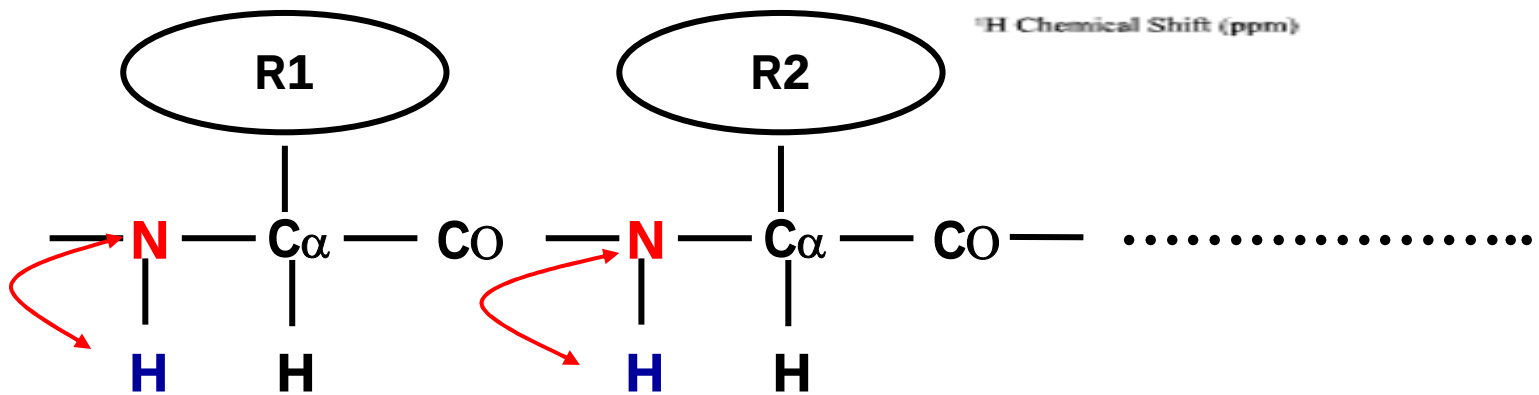
2D Hetero Nuclear

1H-15N

2D HSQC

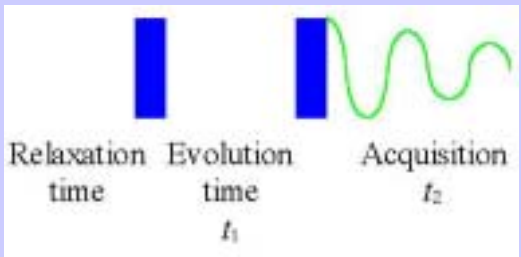




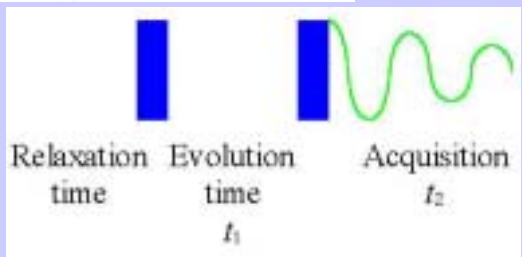


Multi-Dimensional NMR

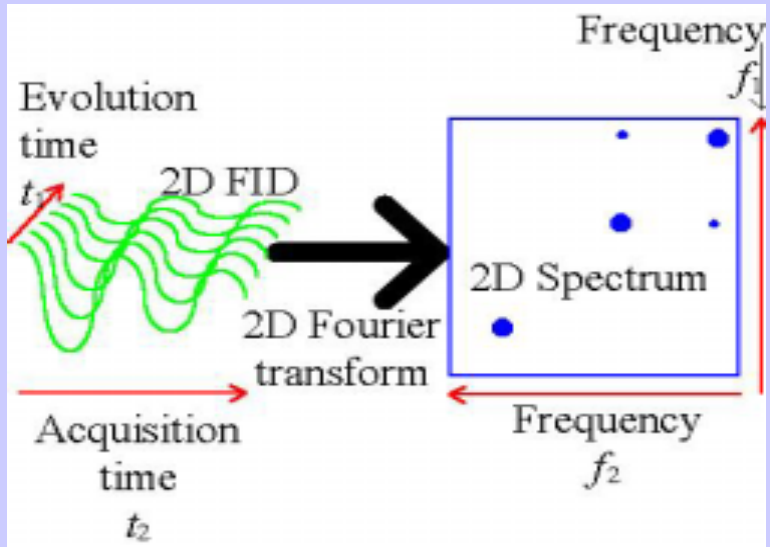
Basic 3D Experiment



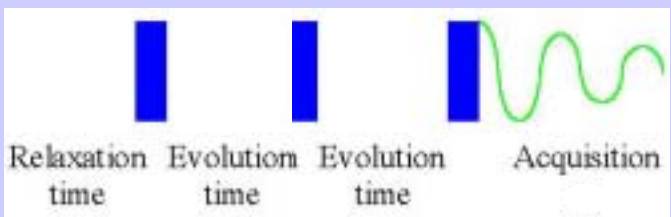
2D

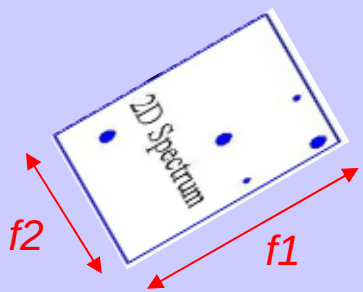


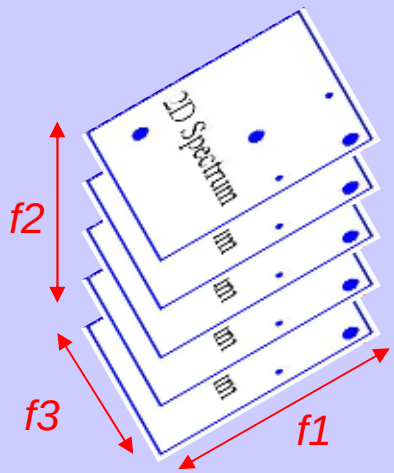
2D



3D



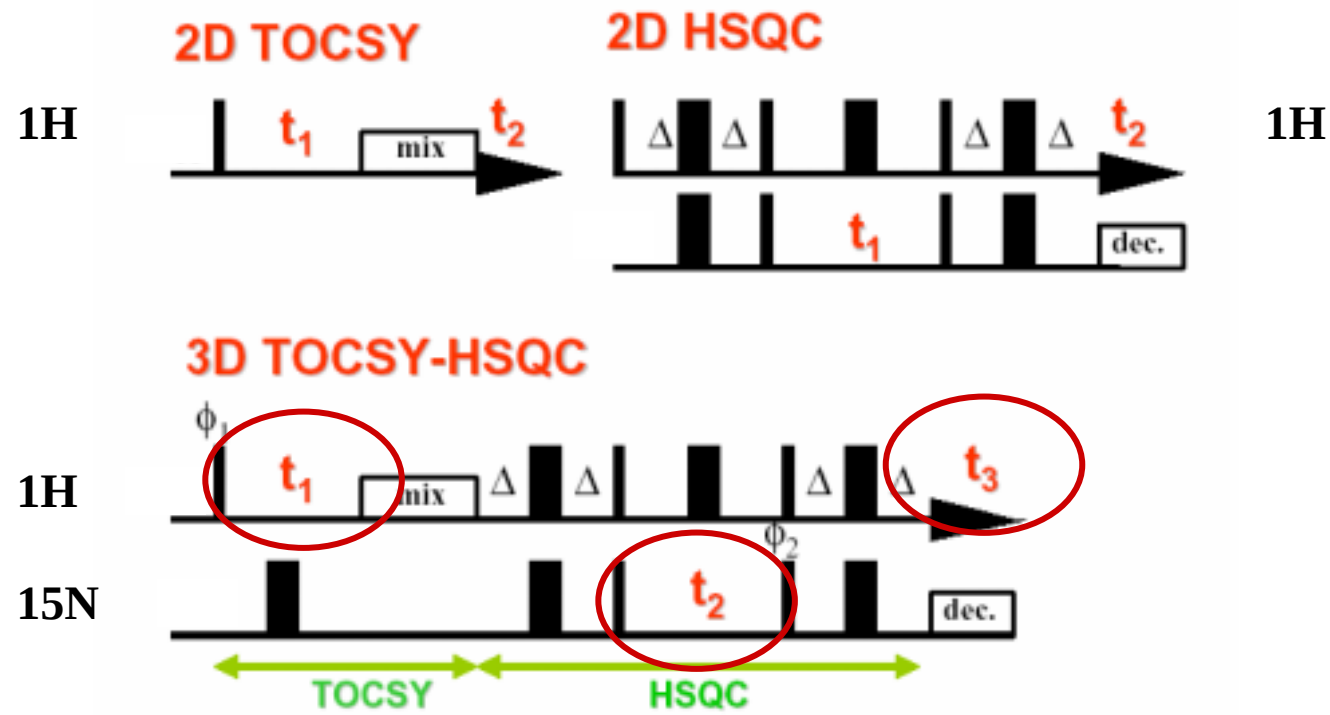


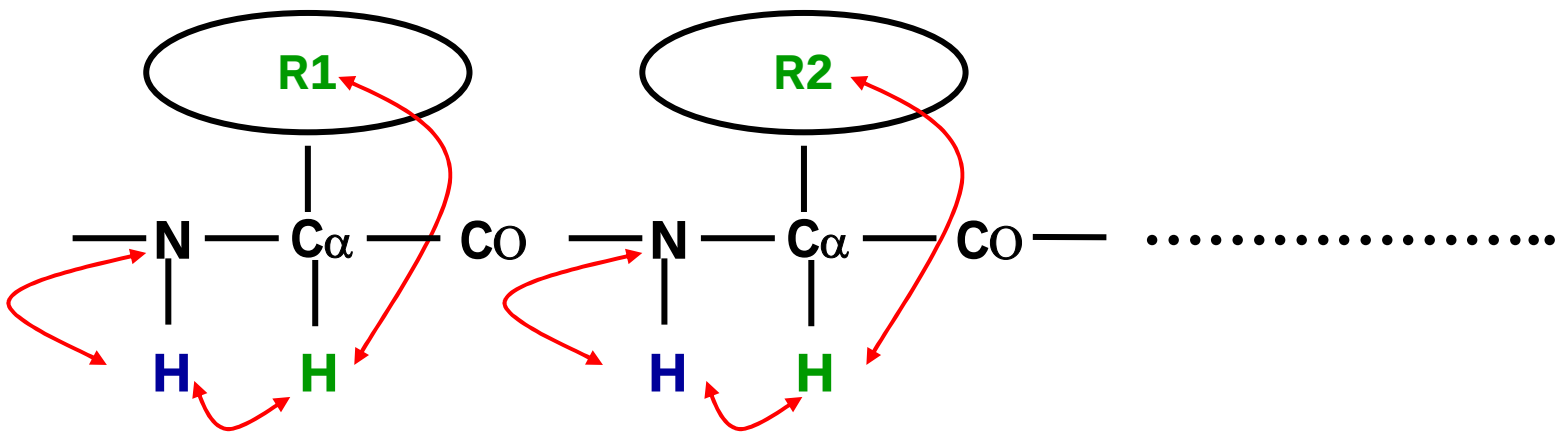


3D Hetero Nuclear

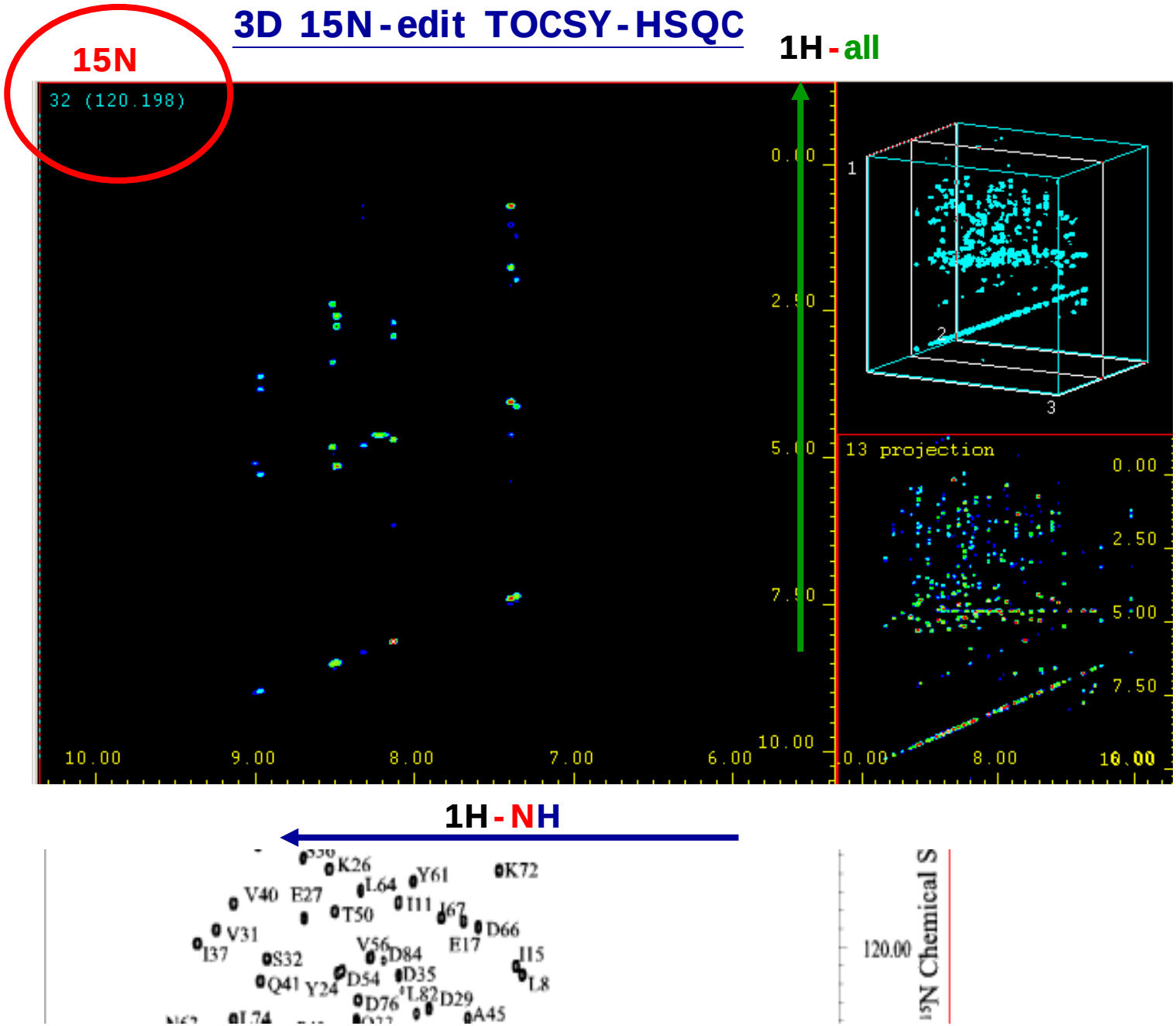
1H-15N-1H

Fig. 1. The 3D pulse sequence.

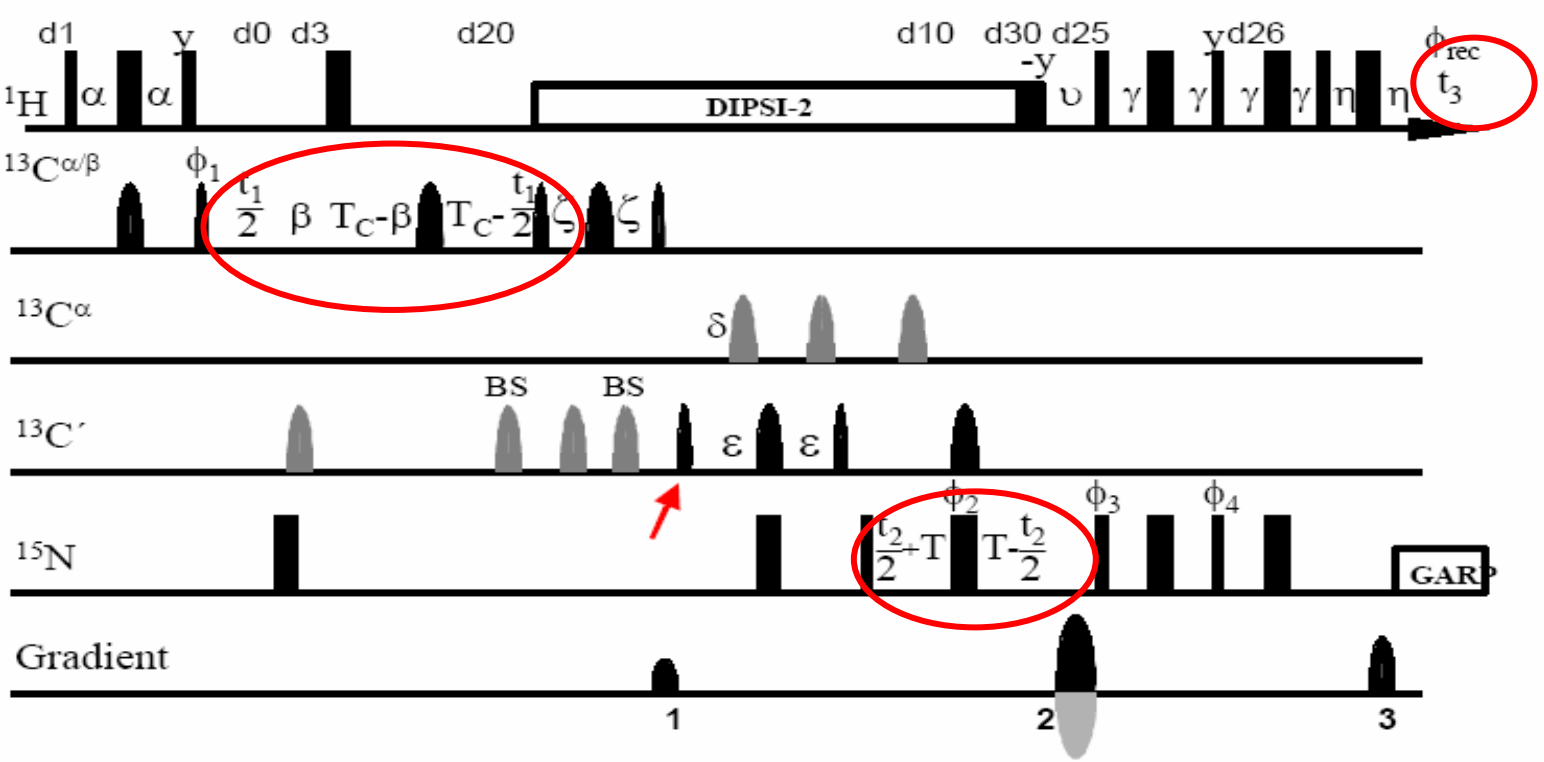




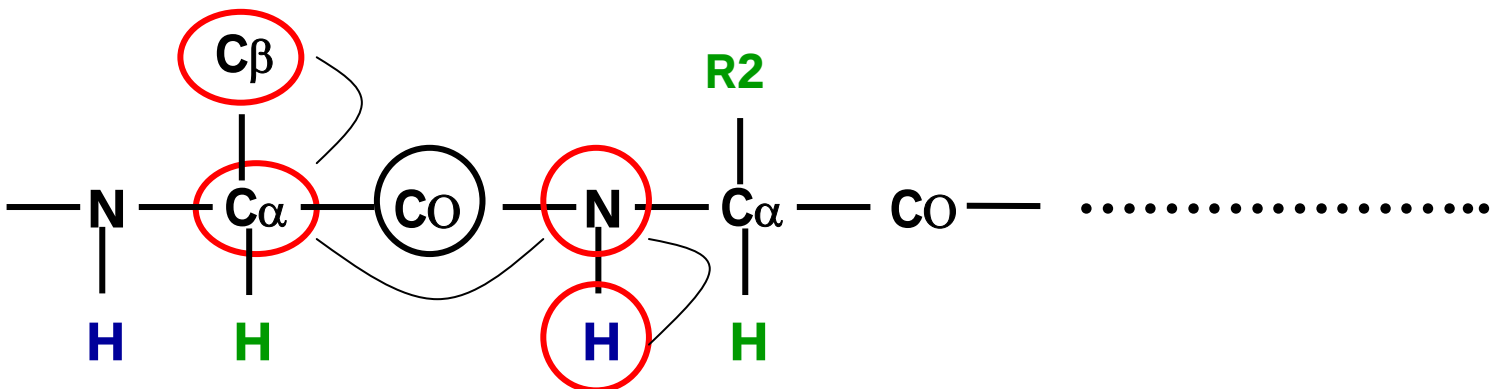
3D 15N-edit TOCSY-HSQC

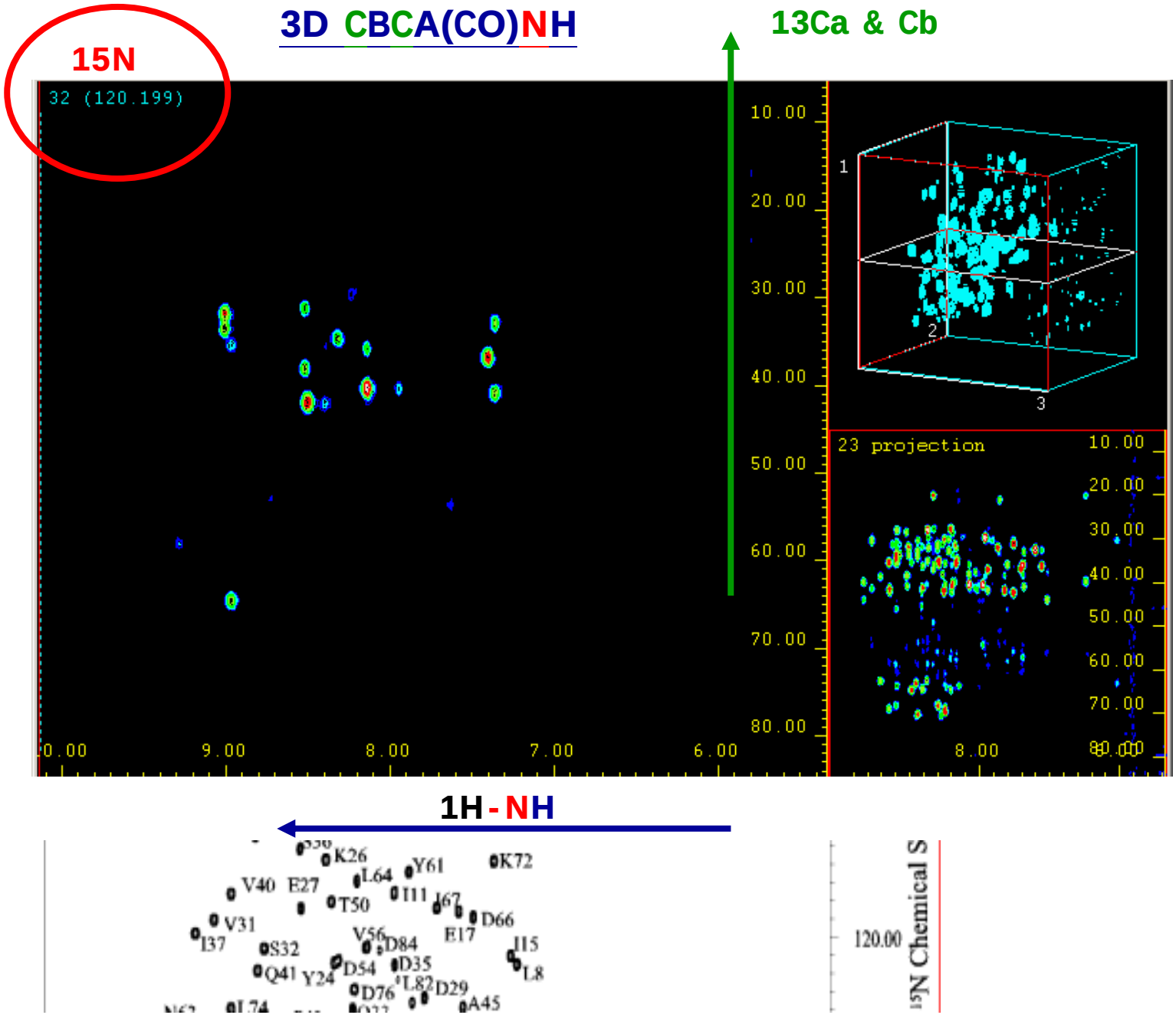


3D Hetero Nuclear 1H-15N-13C



$^1H^{\beta/\alpha}$ —(J_{CH})→ $^{13}C^{\beta/\alpha}(t_1)$ —($J_{C\alpha C\beta}$)→ $^{13}C^{\alpha}$ —($J_{C\alpha C'}$)→ $^{13}C'$ —($J_{C'N}$)→ $^{15}N(t_2)$ —(J_{NH})→ $^1H(t_3)$

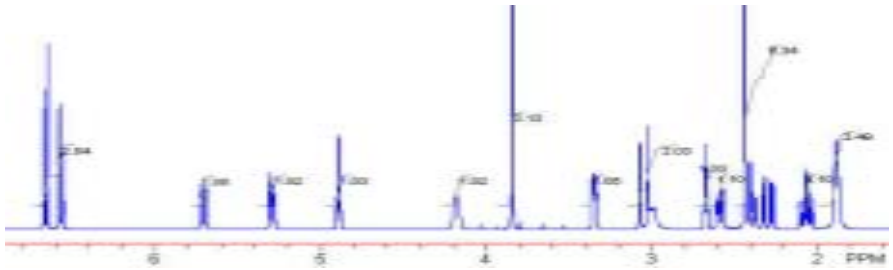



3D CBCA(CO)NH
13Ca & Cb
15N
32 (120.199)
10.00
20.00
30.00
40.00
50.00
60.00
70.00
80.00
9.00
8.00
7.00
6.00
1
2
3
23 projection
1H-NH
K26
L64
Y61
K72
V40
E27
T50
I11
I67
D66
V31
I37
S32
V56
D84
E17
I15
Q41
Y24
D54
D35
L8
D76
L82
D29
A45
120.00
Chemical S

General Concept for 2D/3D experiment

(II) Set up using Xwinnmr

1D NMR

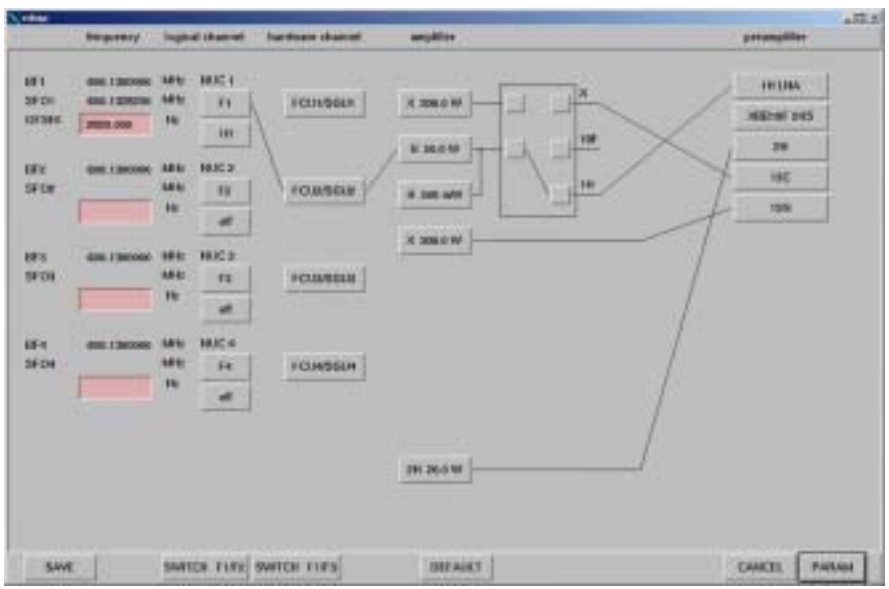


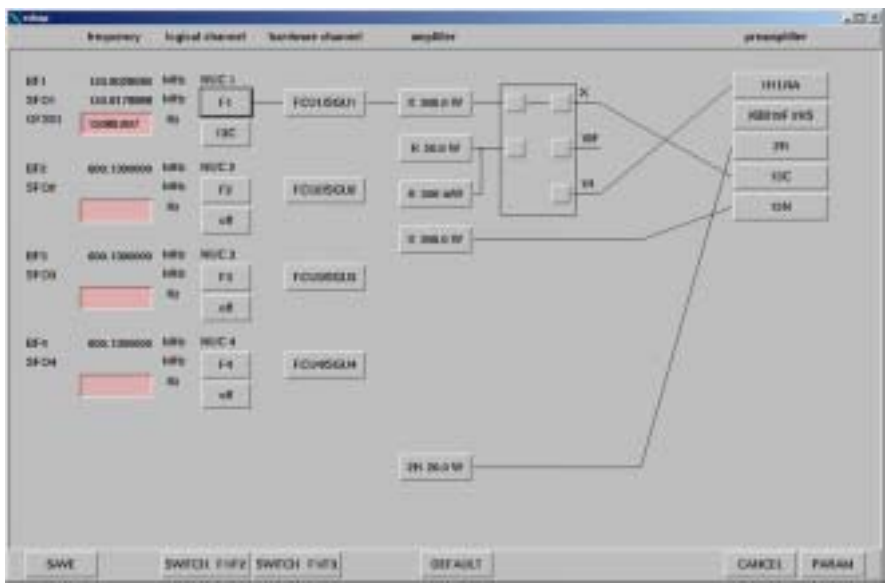
F1 dimension !!!

center (o1) at 4.5ppm

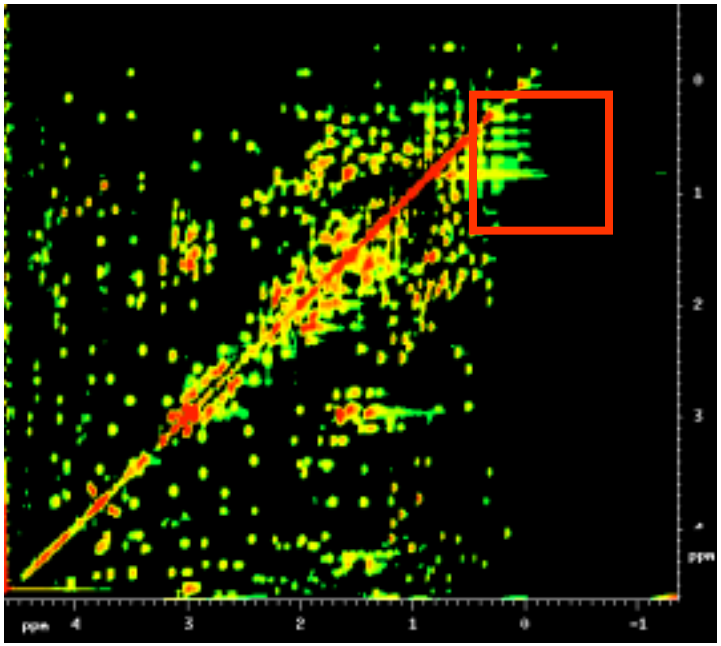
sw=6ppm

1.5ppm (=o1-sw/2) to 7.5ppm(=o1+sw/2)





2D NMR (homo)



F2 dimension !!!

F2: H (f1 channel)

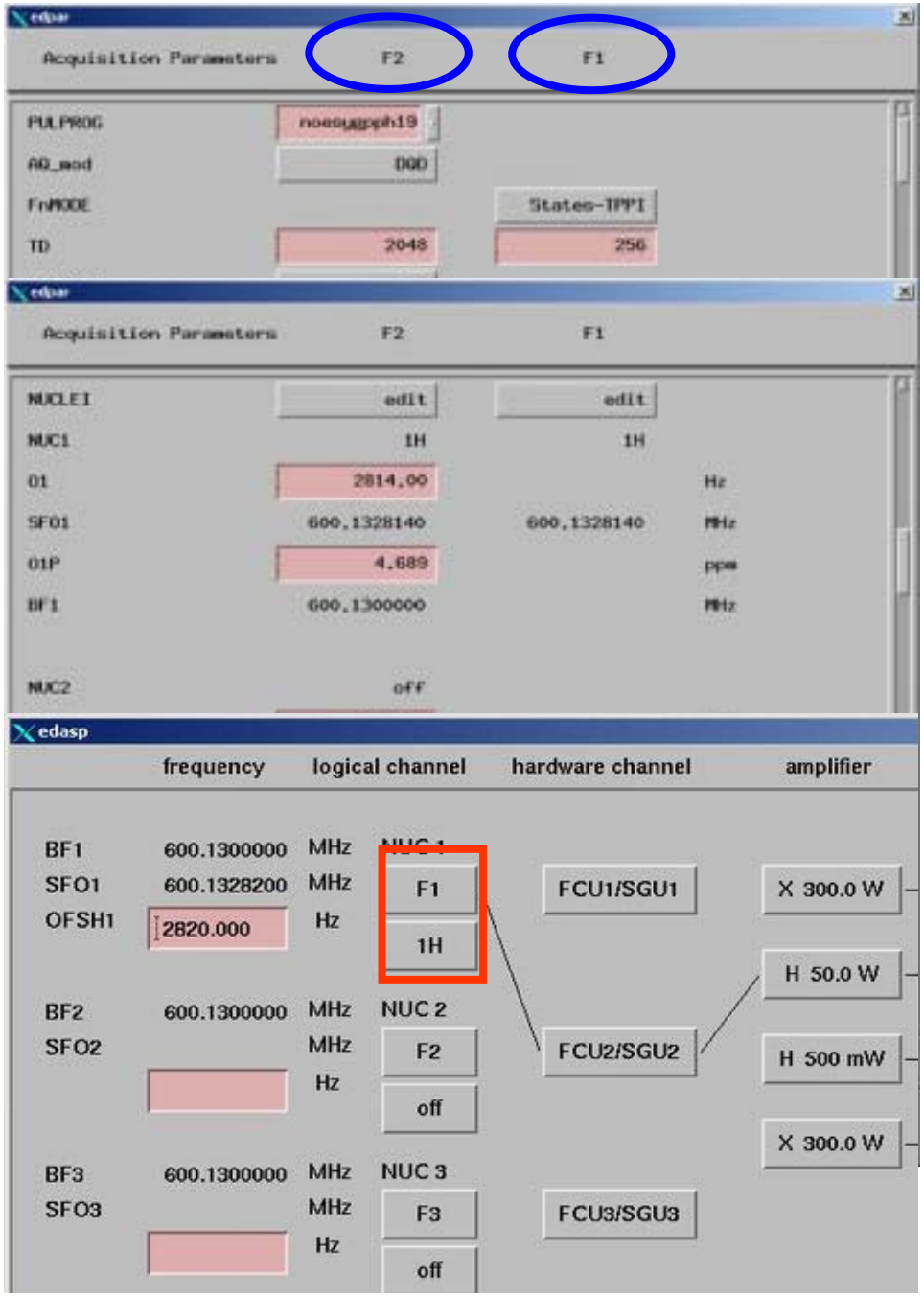
O1(f1 channel):1.8ppm (4.7)

2sw (F2 dimension): 6ppm (14)

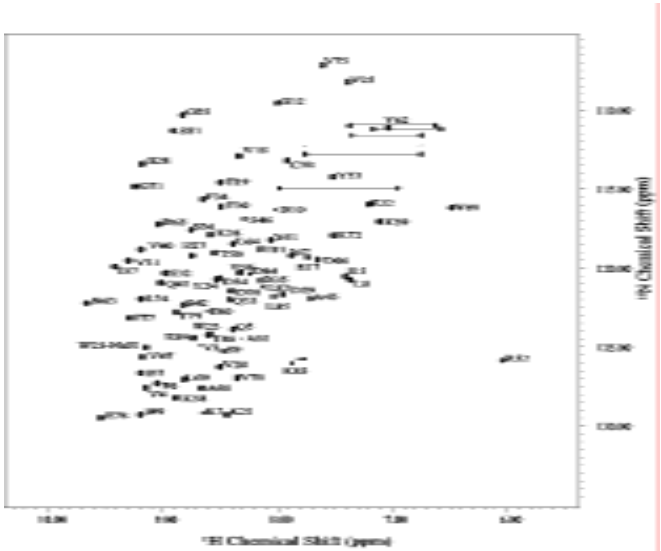
F1: H (f1 channel)

O1(f1 channel): 1.8ppm (4.7)

1sw (F1 diemnsion): 6ppm (14)

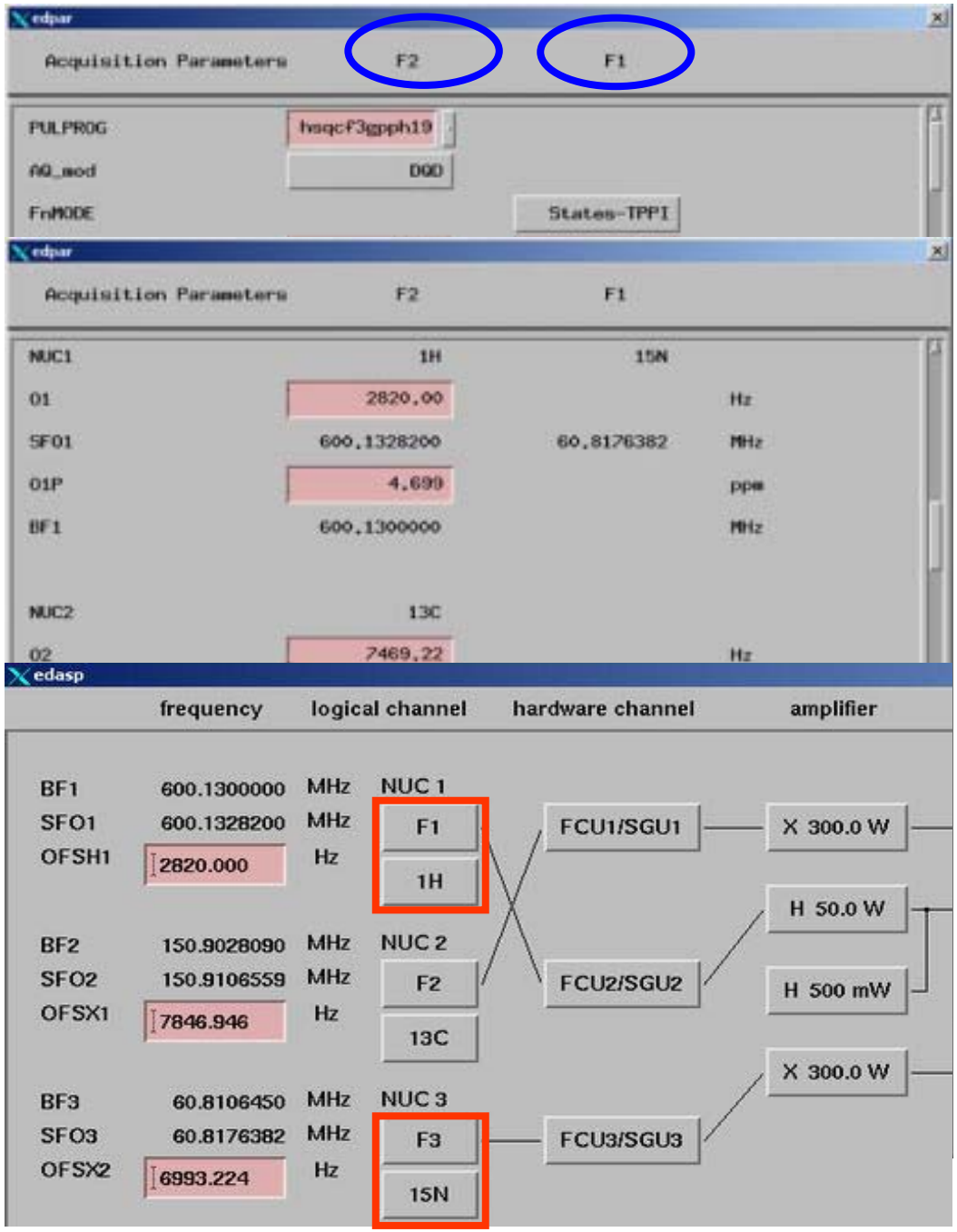


2D NMR (hetero)



F2 dimension !!!

F1 dimension !!!



F2: H (f1 channel)

O1(f1 channel):8ppm (4.7)

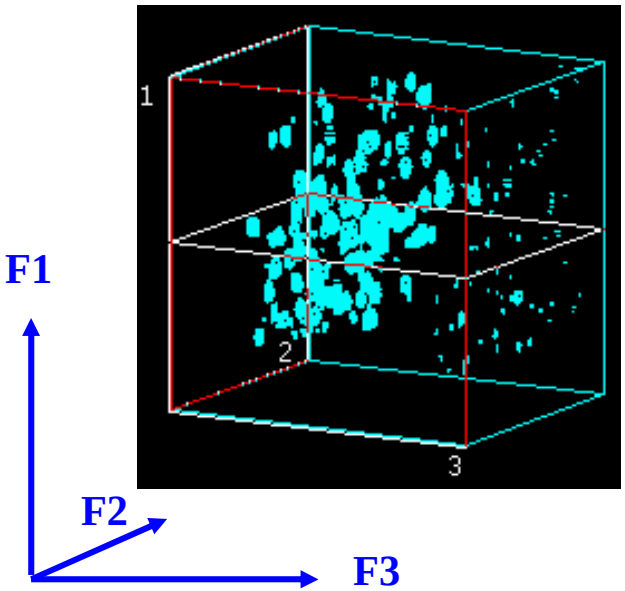
2sw (F2 dimension): 5ppm (13)

F1: N (f3 channel)

O3(f3 channel): 120ppm (115)

1sw (F1 diemnsion): 40ppm (40)

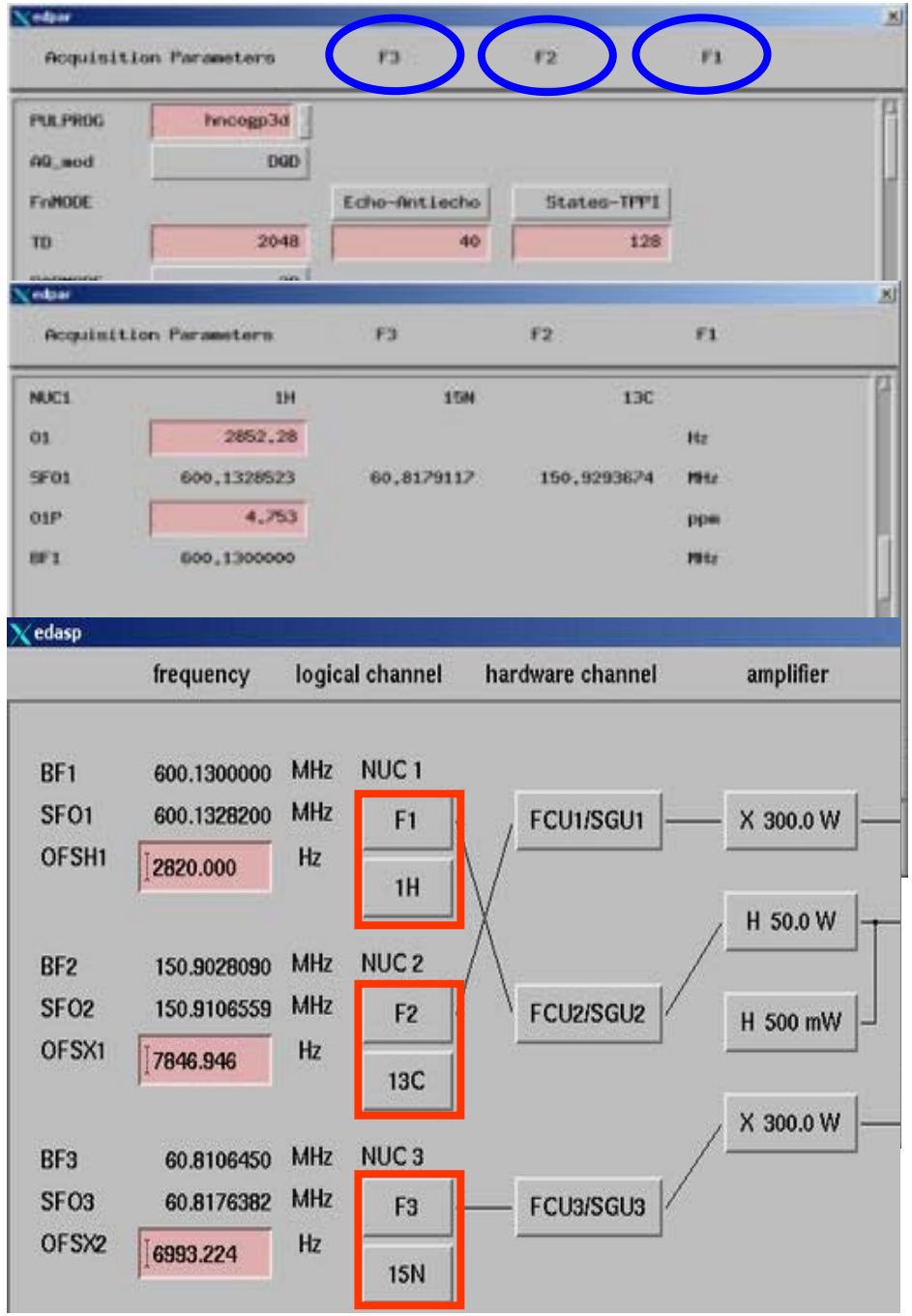
3D NMR / triple



F3: H (f1 channel)
O1(f1 channel):4.7ppm
2 sw (F3 dimension): 14ppm

F2: N (f3 channel)
O3(f1 channel):120ppm
2 sw (F2 dimension): 35ppm

F1: C (f2 channel)
O2(f2 channel): 173ppm
1 sw (F1 diemnsion): 22ppm



Parameters in pulse sequence

(Bruker version)

How to set up experiment? (follow the guide)

•**Experiment Name: 3D HNCO**

•**Experiment Type: Using Echo/antiecho , f1: H, f2:C, f3:N, F1(CO), F2(N), F3(H)**

•**Standard Parameter Set: std_3D_HNCO**

•**Pulse Program: hncogp3d**

•**AQ parameters to check**

1H pulses

pl1 (high power, ex: 0db), p1(90deg at pl1), p2(180deg at pl1)

pl19 (low power for dipsi2,pcpd1), p26(90deg at pl19), pcpd1(90deg ,ex: 40-50usec)

sp1 (shape pulse power for Sinc.1000) , p11(pulse length for sp1, ex: 2m)

Others

o1 (for 1H), o2 (for 13CO), o3 (for 15N)

1 sw, 1 td (for F1 dimension, ie: 13C)

2 sw, 2 td (for F2 dimension, ie:15N)

3 sw, 3 td (for F3 dimension, ie:1H)

d1, rg, ns(=8*n), ds (≧ 16)

Users need to adjust parameters in "red" (meaning of the parameter in "green")

What's going on? (inside the pulse program)

```
;hncogp3d
;avance-version (02/05/31)
;HNCO
;3D sequence with
;   inverse correlation for triple resonance using multiple
;      inept transfer steps
;
;      F1(H) -> F3(N) -> F2(C=O,t1) -> F3(N,t2) -> F1(H,t3)
;
;on/off resonance Ca and C=O pulses using shaped pulse
;phase sensitive (t1)
;phase sensitive using Echo/Antiecho gradient selection (t2)
;using constant time in t2
;(use parameterset HNCOGP3D)
;
;S. Grzesiek & A. Bax, J. Magn. Reson. 96, 432 - 440 (1992)
;J. Schleucher, M. Sattler & C. Griesinger, Angew. Chem. Int. Ed. 32,
;   1489-1491 (1993)
;L.E. Kay, G.Y. Xu & T. Yamazaki, J. Magn. Reson. A109, 129-133 (1994)


prosol relations=<triple>


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

Info & Ref.

Include files

;;"d0=3u"
;;"d11=30m"
;;"d13=4u"

"d21=5.5m"
"d23=12m"
"d26=2.3m"

"in29=in10"
"in30=in10"

"d10=d23/2-p14/2"
"d29=d23/2-p14/2-p26-d21-4u"
"d30=d23/2-p14/2"

"DELTA=d0*2+larger(p14,p22)-p14"
"DELTA1=p16+d16+d13+4u"
"DELTA2=d23-d21-p26"
"DELTA3=d21-p16-d16-4u"

"spoff2=0"
"spoff3=0"
"spoff5=bf2*(cnst22/1000000)-o2"
"spoff8=0"

aqseq 321

Const. & Equ.

1 d11 ze
 d11 pl16:f3
2 d11 do:f3
3 d1 **pl1:f1**
 p1 ph1
 d26 **pl3:f3**
 (center (p2 ph1) **(p22 ph1):f3**)
 d26 UNBLKGRAD
 (p1 ph2):f1

 4u pl0:f1
 (p11:sp1 ph1:r):f1
 4u
 p16:gp1
 d16

 (p21 ph3):f3
 d21 **pl19:f1**
 (p26 ph2):f1
 DELTA2 **cpds1:f1** ph1
 (center **(p14:sp3 ph1):f2** (p22 ph1):f3)
 d23
 (p21 ph1):f3

 d13 do:f1
 (p26 ph7):f1
 4u
 p16:gp2
 d16

Pulse sequence

(p13:sp2 ph4):f2
d0
(center **(p14:sp5 ph1):f2** (p22 ph1):f3)
d0
4u
(p14:sp3 ph1):f2
DELTA
(p14:sp5 ph1):f2
4u
(p13:sp8 ph1):f2

4u
p16:gp3
d16
(p26 ph2):f1
20u cpds1:f1 ph1

(p21 ph1):f3
d30
(p14:sp5 ph1):f2
d30
(center (p14:sp3 ph1):f2 (p22 ph8):f3)
d10
(p14:sp5 ph1):f2
d29
4u do:f1
(p26 ph7):f1
4u
p16:gp4*EA
d16
DELTA3 pl1:f1

Pulse sequence

```
(center (p1 ph1) (p21 ph5):f3 )
  d26
  (center (p2 ph1) (p22 ph1):f3 )
  d26
  (center (p1 ph2) (p21 ph6):f3 )
  d26
  (center (p2 ph1) (p22 ph1):f3 )
  d26
  (p1 ph1)
  DELTA1
  (p2 ph1)
  d13
  p16:gp5
  d16 pl16:f3
  4u BLKGRAD
  go=2 ph31 cpd3:f3
  d11 do:f3 mc #0 to 2
     F1PH(rd10 & rd29 & rd30 & ip4, id0)
     F2EA(igrad EA & ip6*2, id10 & id29 & dd30)
Exit

ph1=0
ph2=1
ph3=0 0 0 0 0 0 0 0 2 2 2 2 2 2 2 2
ph4=0 2
ph5=0 0 2 2
ph6=3 3 1 1
ph7=3
ph8=0 0 0 0 2 2 2 2
ph31=0 2 2 0 0 2 2 0 2 0 0 2 2 0 0 2
```

Pulse sequence

Phase

;pl0 : 120dB
;pl1 : f1 channel - power level for pulse (default)
;pl3 : f3 channel - power level for pulse (default)
;pl16: f3 channel - power level for CPD/BB decoupling
;pl19: f1 channel - power level for CPD/BB decoupling
;sp1: f1 channel - shaped pulse 90 degree (H2O on resonance)
;sp2: f2 channel - shaped pulse 90 degree (C=O on resonance)
;sp3: f2 channel - shaped pulse 180 degree (C=O on resonance)
;sp5: f2 channel - shaped pulse 180 degree (Ca off resonance)
;sp8: f2 channel - shaped pulse 90 degree (C=O on resonance)
; for time reversed pulse
;p1 : f1 channel - 90 degree high power pulse
;p2 : f1 channel - 180 degree high power pulse
;p11: f1 channel - 90 degree shaped pulse [2 msec]
;p13: f2 channel - 90 degree shaped pulse
;p14: f2 channel - 180 degree shaped pulse
;p16: homospoil/gradient pulse [1 msec]
;p21: f3 channel - 90 degree high power pulse
;p22: f3 channel - 180 degree high power pulse
;p26: f1 channel - 90 degree pulse at pl19

remarks

;d0 : incremented delay (F1 in 3D) [3 usec]
;d1 : relaxation delay; 1-5 * T1
;d10: incremented delay (F2 in 3D) = d23/2-p14/2
;d11: delay for disk I/O [30 msec]
;d13: short delay [4 usec]
;d16: delay for homospoil/gradient recovery
;d21: 1/(2J(NH) [5.5 msec]
;d23: 1/(4J(NCO) [12 msec]
;d26: 1/(4J'(NH) [2.3 msec]
;d29: incremented delay (F2 in 3D) = d23/2-p14/2-p26-d21-4u
;d30: decremented delay (F2 in 3D) = d23/2-p14/2
;cnst21: CO chemical shift (offset, in ppm)
;cnst22: Calpha chemical shift (offset, in ppm)
;o2p: CO chemical shift (cnst21)
;in0: 1/(2 * SW(CO)) = DW(CO)
;nd0: 2
;in10: 1/(4 * SW(N)) = (1/2) DW(N)
;nd10: 4
;in29: = in10
;in30: = in10
;NS: 8 * n
;DS: >= 16
;td1: number of experiments in F1
;td2: number of experiments in F2 td2 max = 2 * d30 / in30
;FnMODE: States-TPPI (or TPPI) in F1
;FnMODE: echo-antiecho in F2

remarks

;cpds1: decoupling according to sequence defined by cpdprg1
;cpd3: decoupling according to sequence defined by cpdprg3
;pcpd1: f1 channel - 90 degree pulse for decoupling sequence
;pcpd3: f3 channel - 90 degree pulse for decoupling sequence

;use gradient ratio: gp 1 : gp 2 : gp 3 : gp 4 : gp 5
; 60 : -40 : 10 : 80 : 8.1

;for z-only gradients:
;gpz1: 60%
;gpz2: -40%
;gpz3: 10%
;gpz4: 80%
;gpz5: 8.1%

;use gradient files:
;gpnam1: SINE.100
;gpnam2: SINE.100
;gpnam3: SINE.100
;gpnam4: SINE.100
;gpnam5: SINE.100

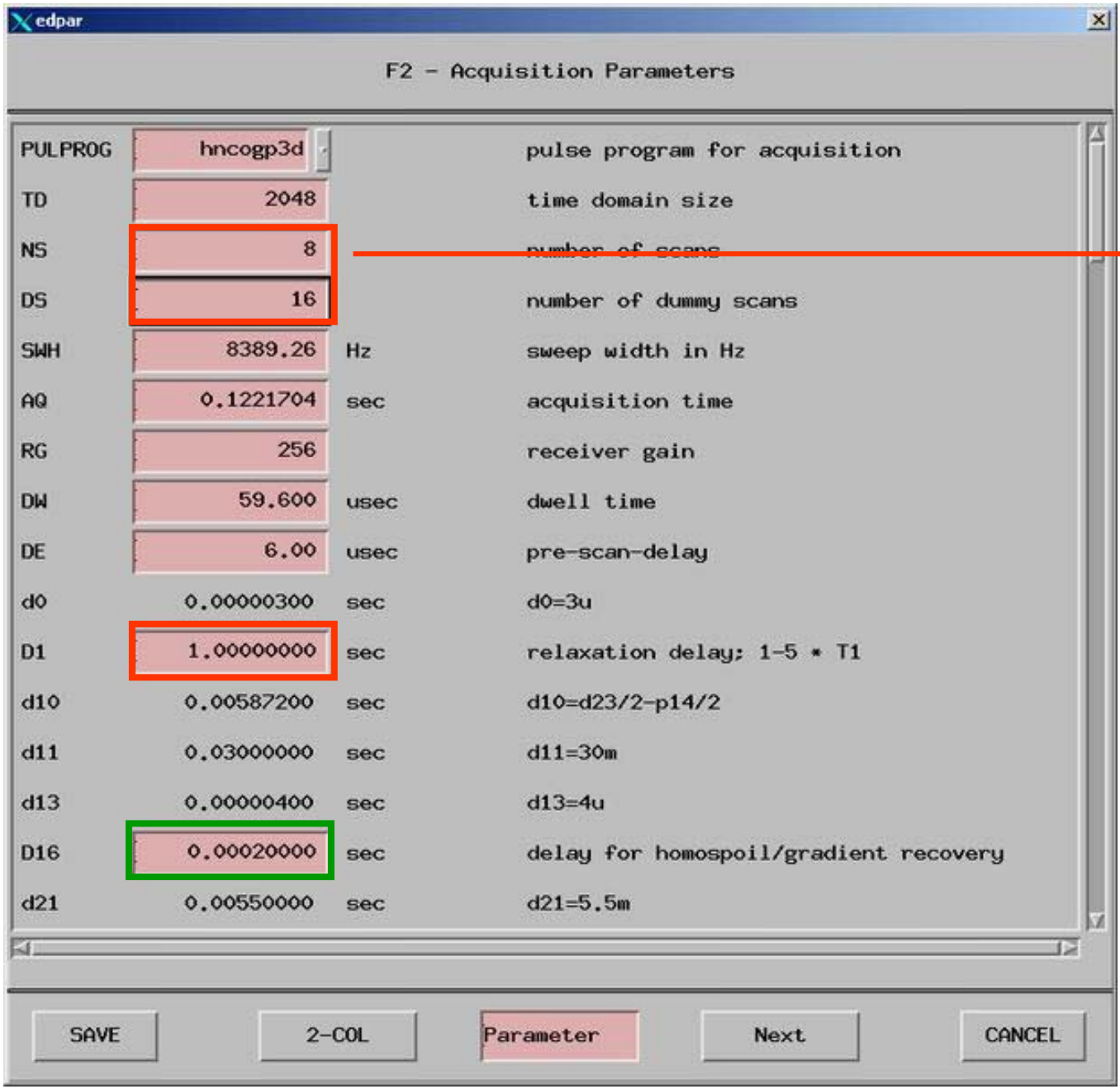
remarks

How to optimize condition? (only when there's no standard set available)

Steps: new → edasp → eda (select pulse program) → getprosol → ased

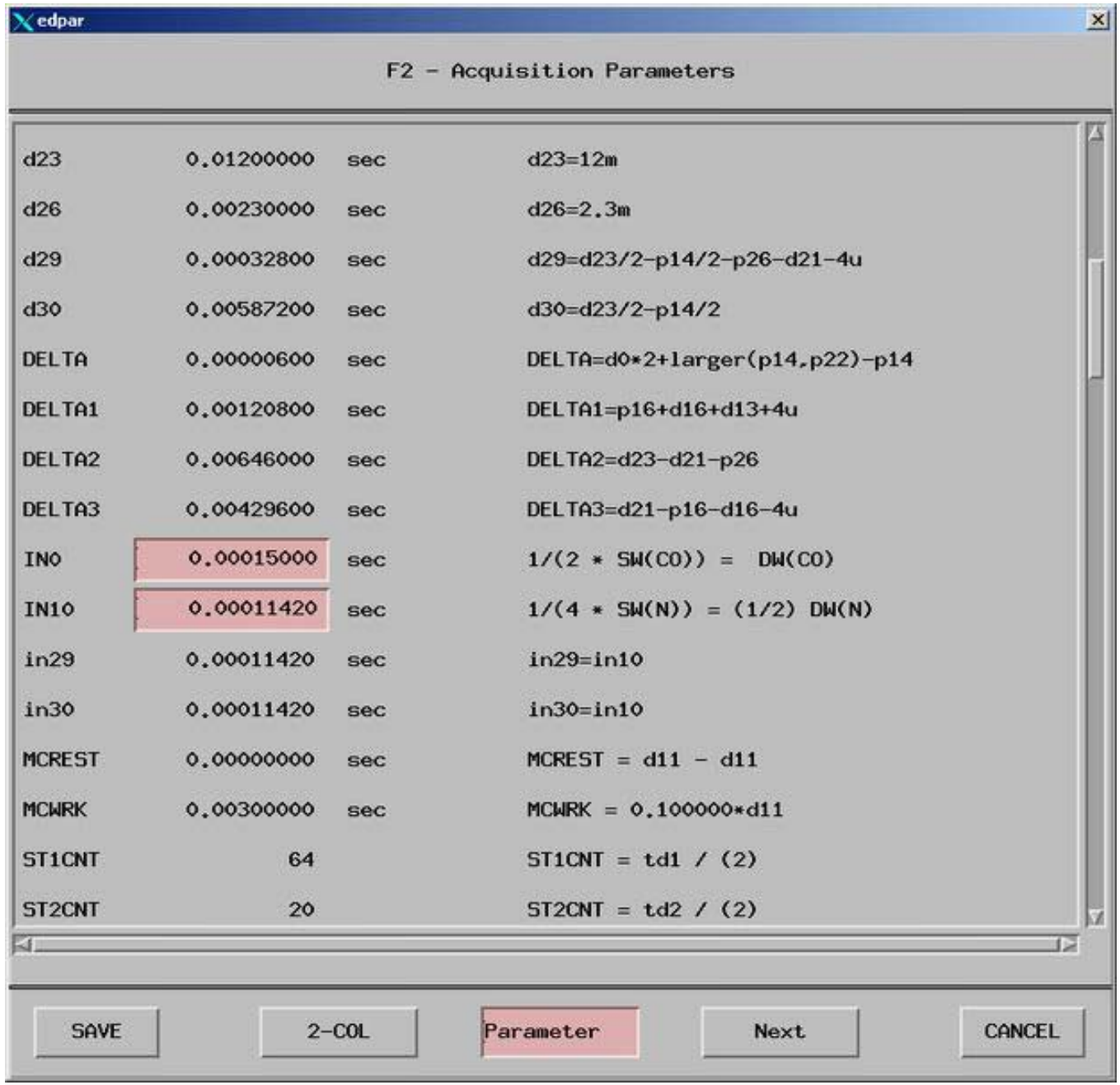
- follow pulse program
- maybe modify
- nd0 = number of d0 in the increment loops of 2D/3D
- in0=1/(nd0*sw)

Steps: new → edasp → eda (select pulse program) → getprosol → ased

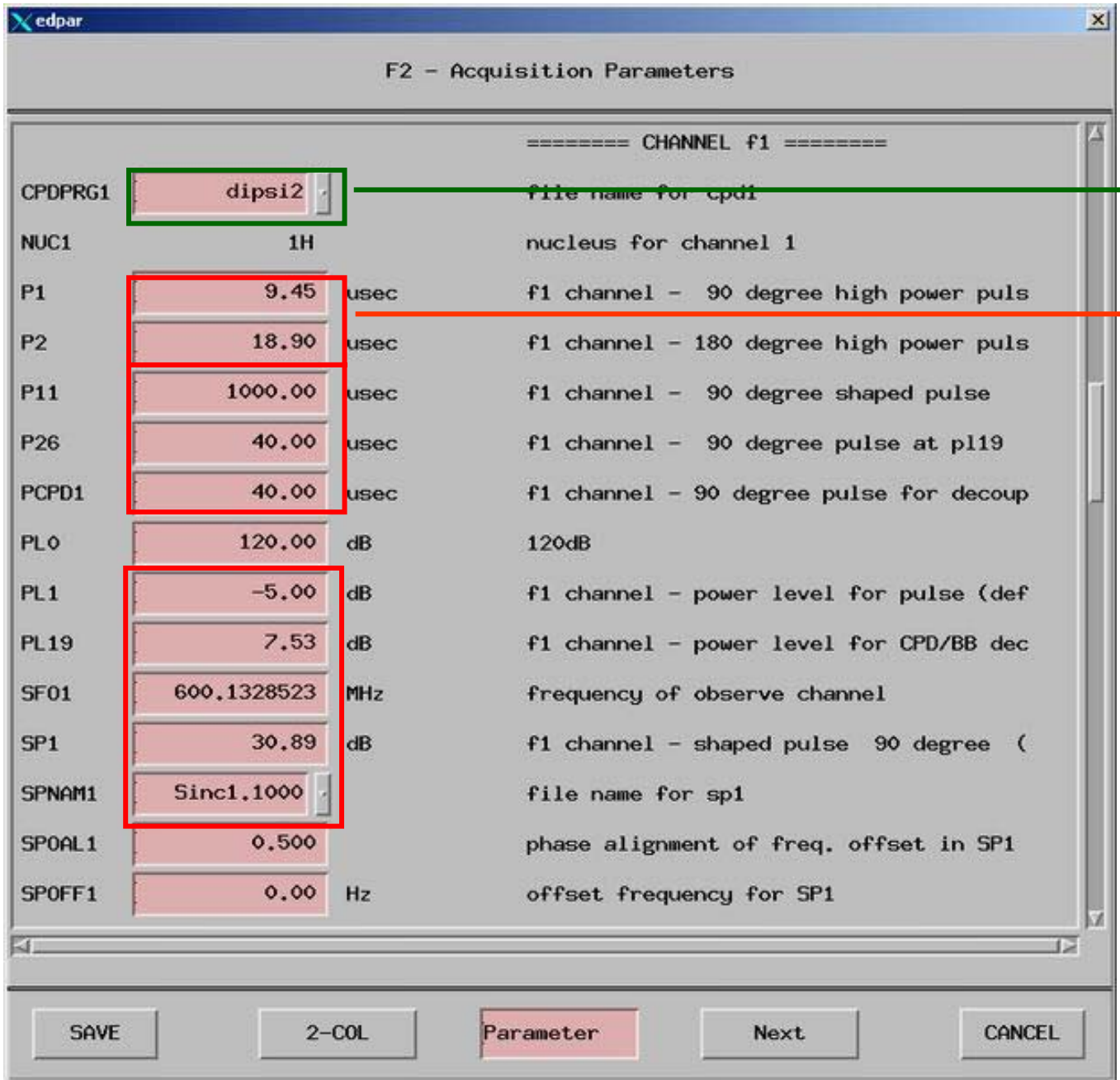


• sample dependent

Steps: new → edasp → eda (select pulse program) → getprosol → ased

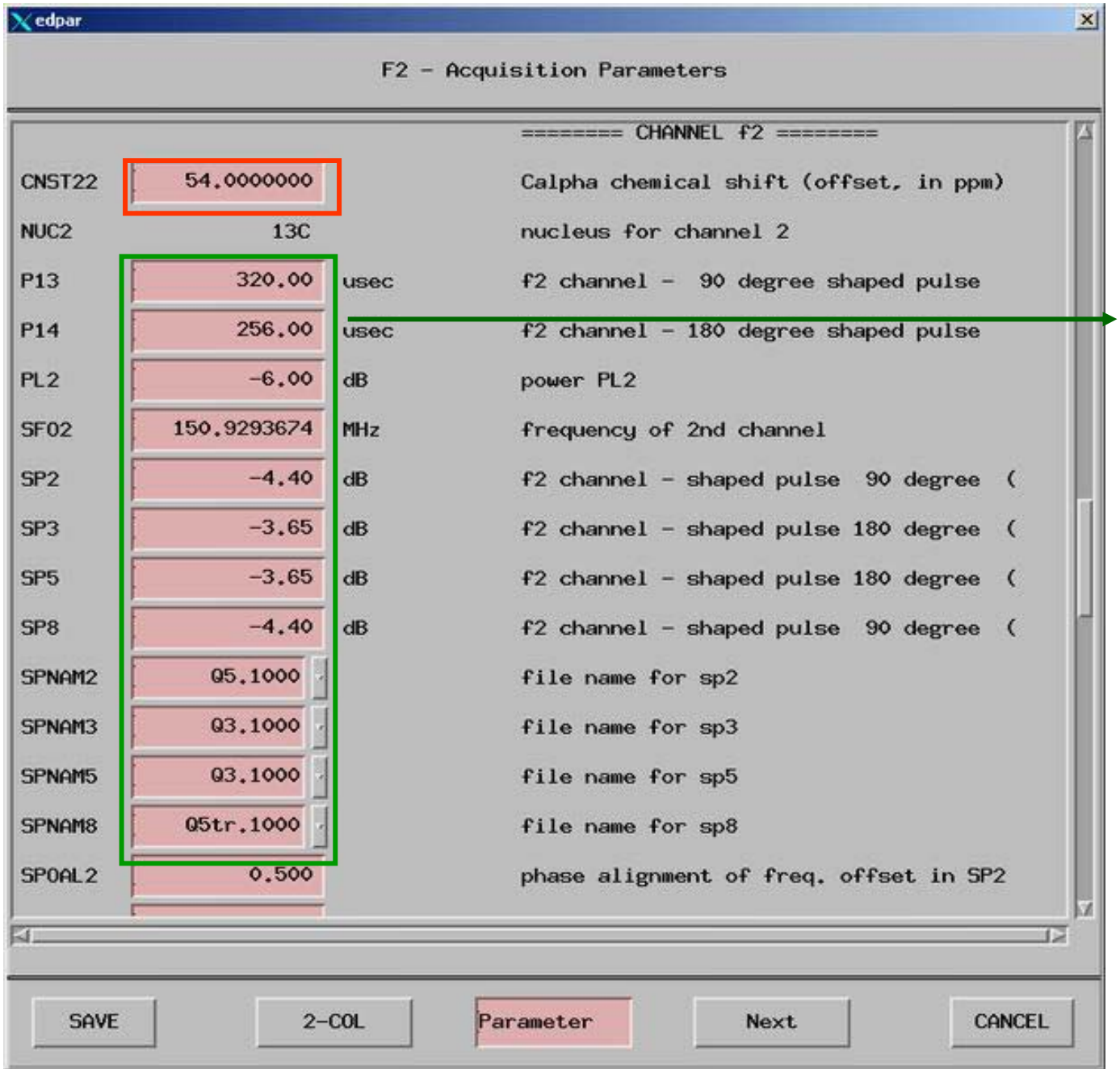


Steps: new → edasp → eda (select pulse program) → getprosol → ased



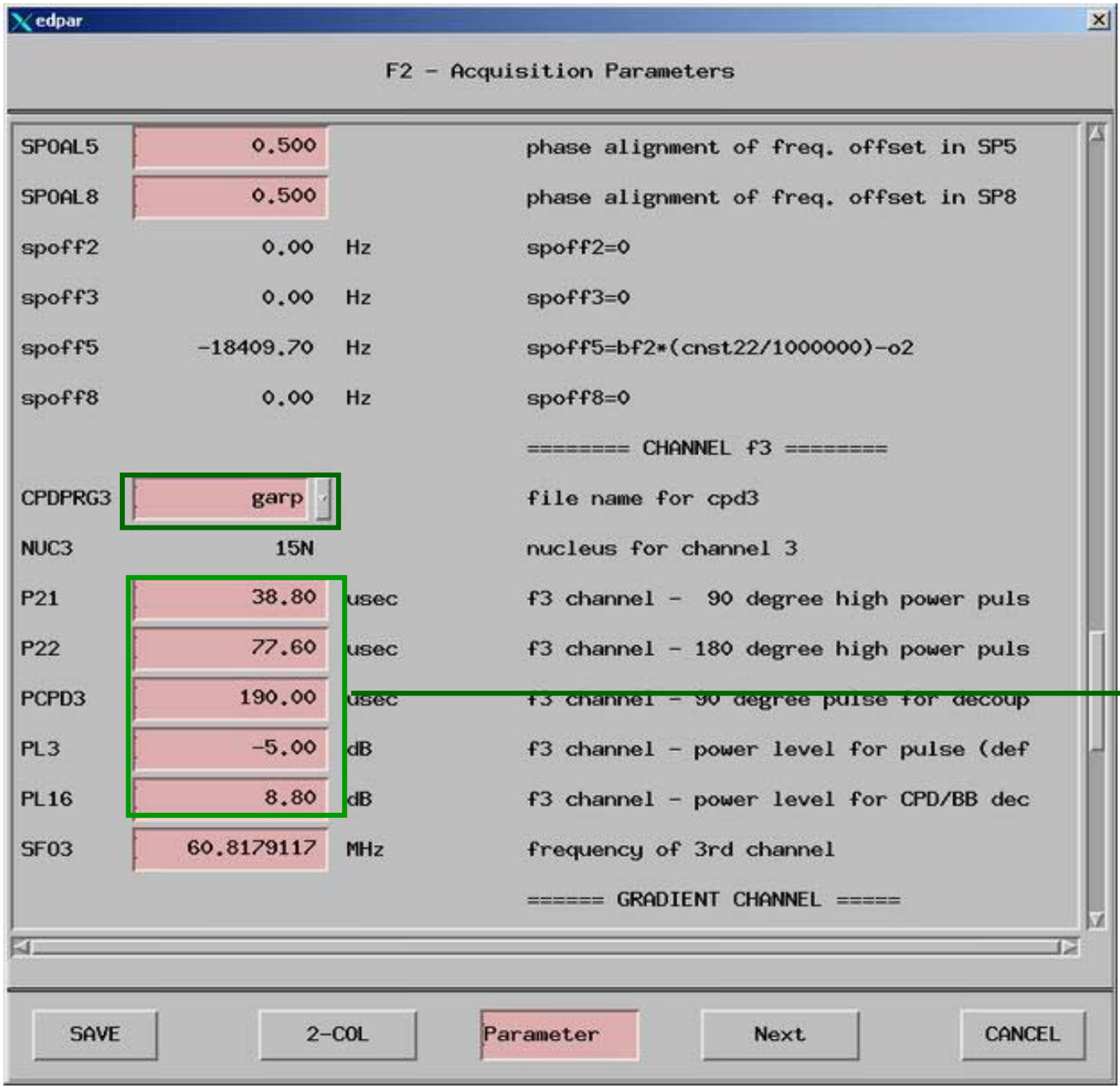
- use standard
- sample dependent

Steps: new → edasp → eda (select pulse program) → getprosol → ased



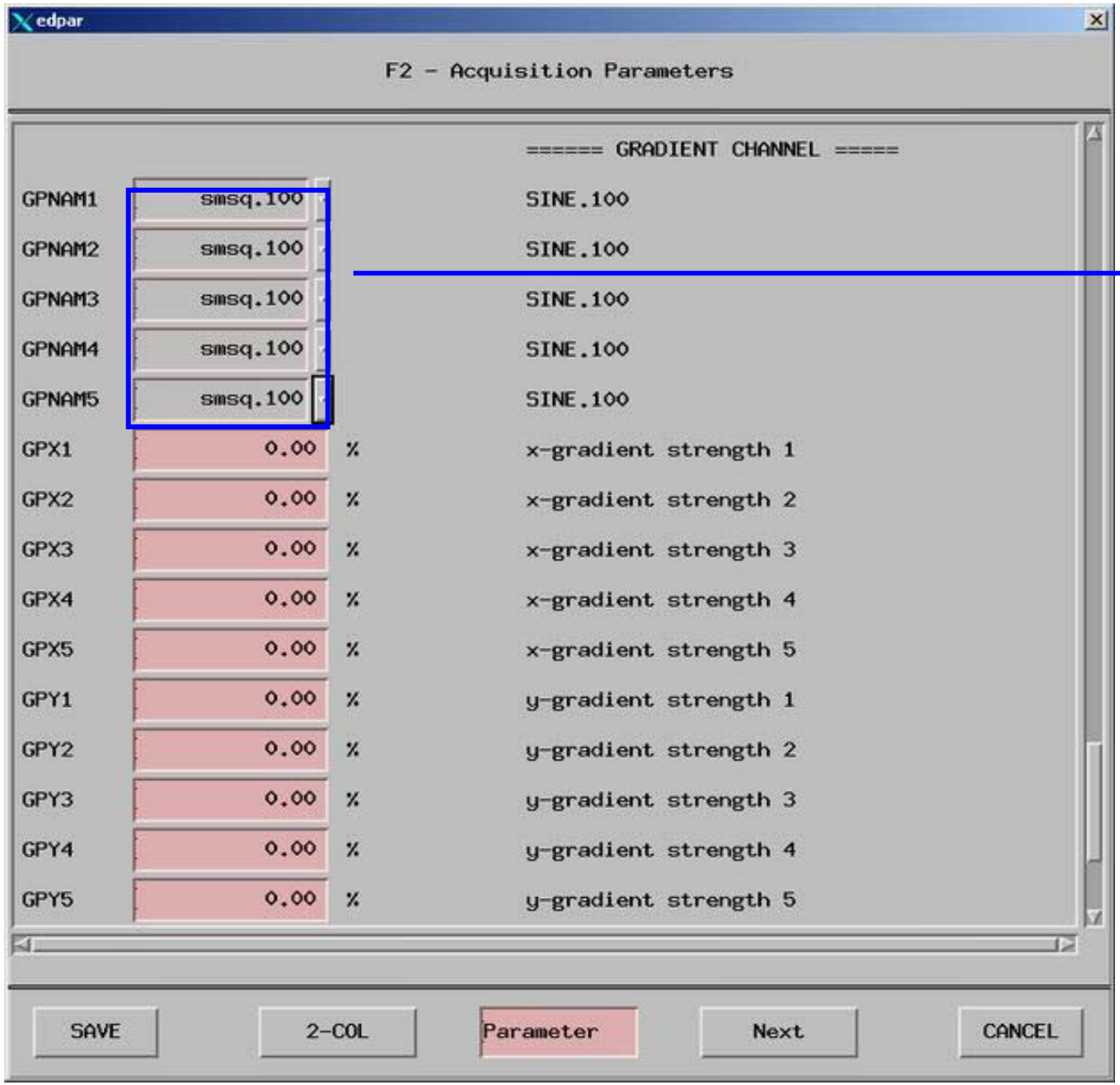
- use standard

Steps: new → edasp → eda (select pulse program) → getprosol → ased



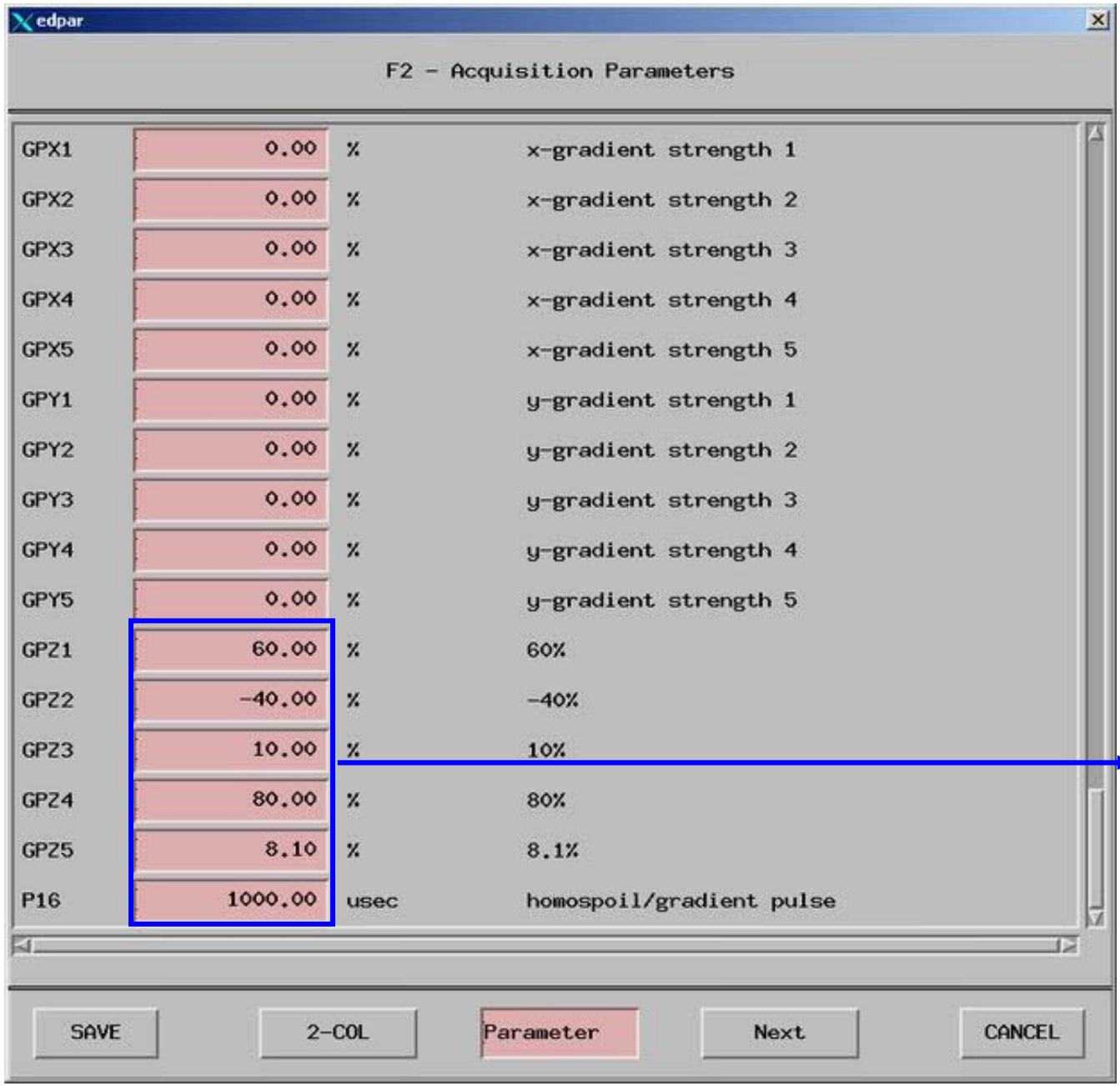
• use standard

Steps: new → edasp → eda (select pulse program) → getprosol → ased



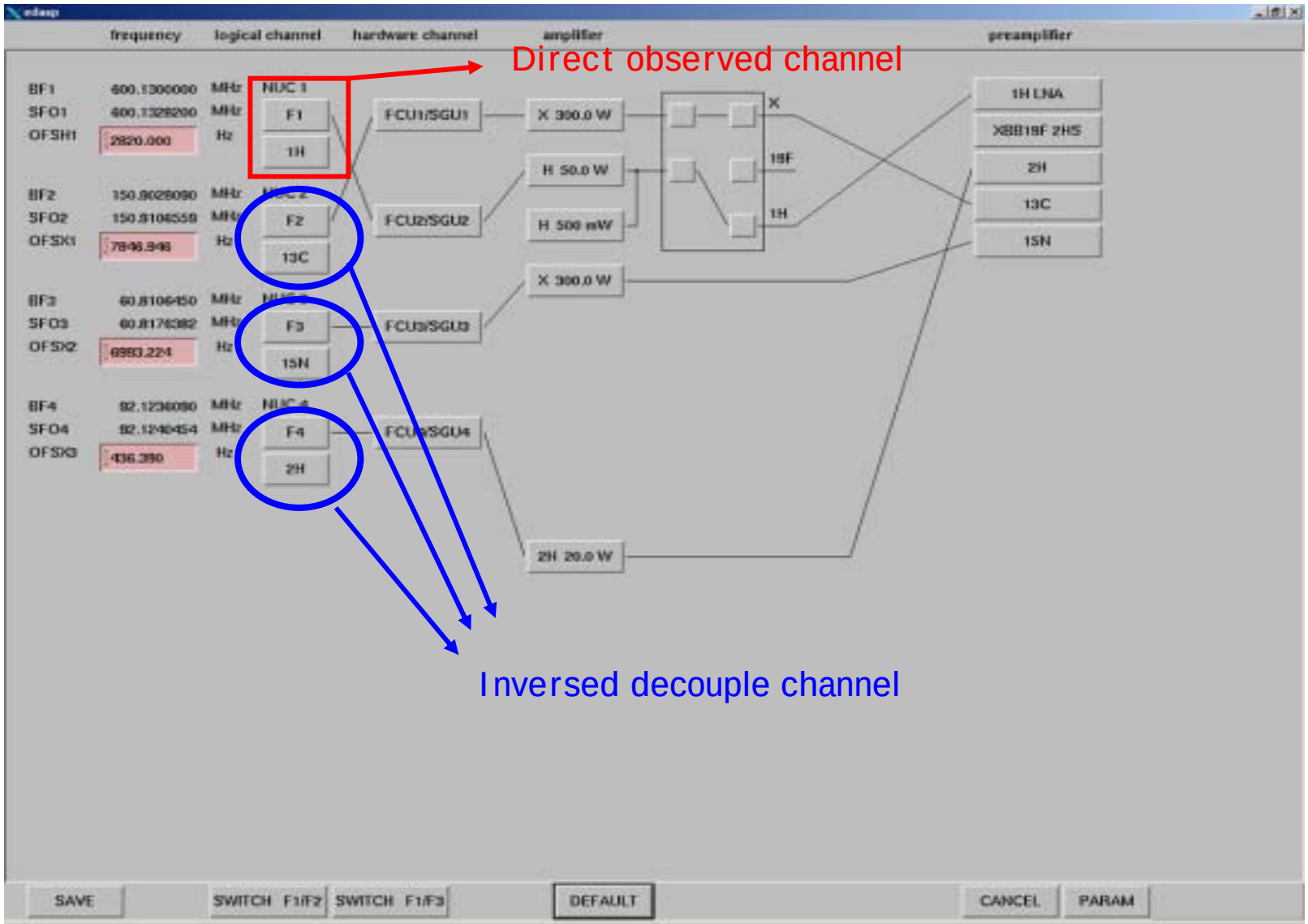
- follow pulse program
- maybe modify

Steps: new → edasp → eda (select pulse program) → getprosol → ased



• follow pulse program

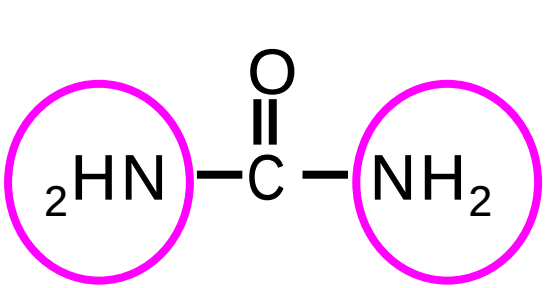
Pulse Calibration for decoupled channels (parameters in “prosol”)

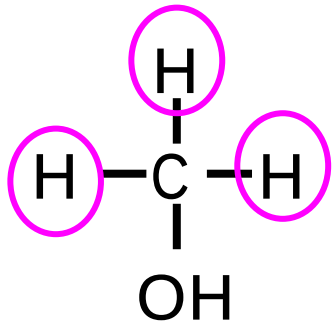


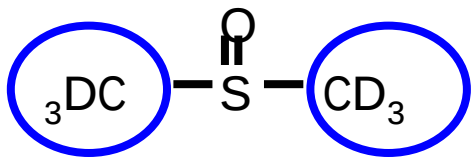
Pulse Calibration for decoupled channel

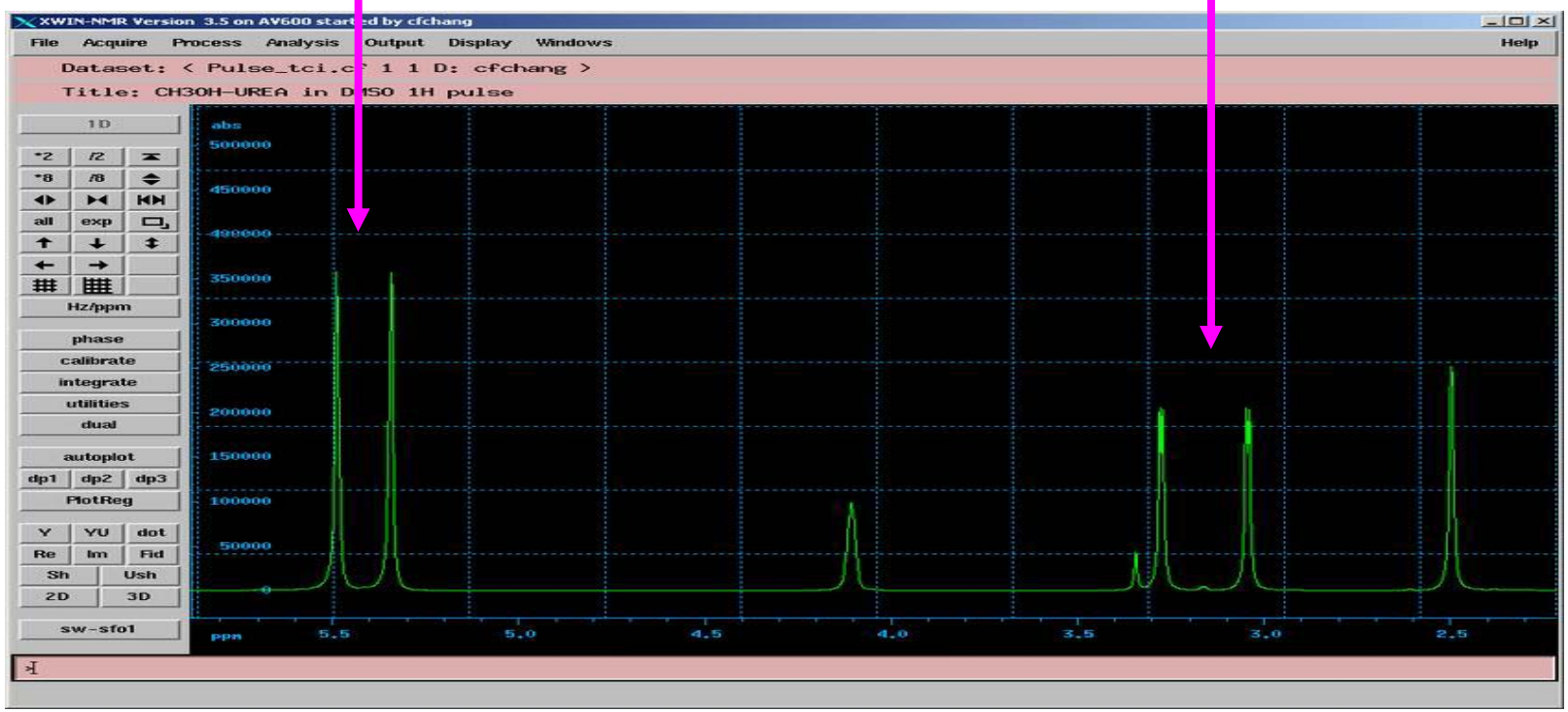
(I) Hard Pulses (13C/15N)

Sample: **0.1M 13CH3OH + 0.1M 15N-urea in DMSO-d6**



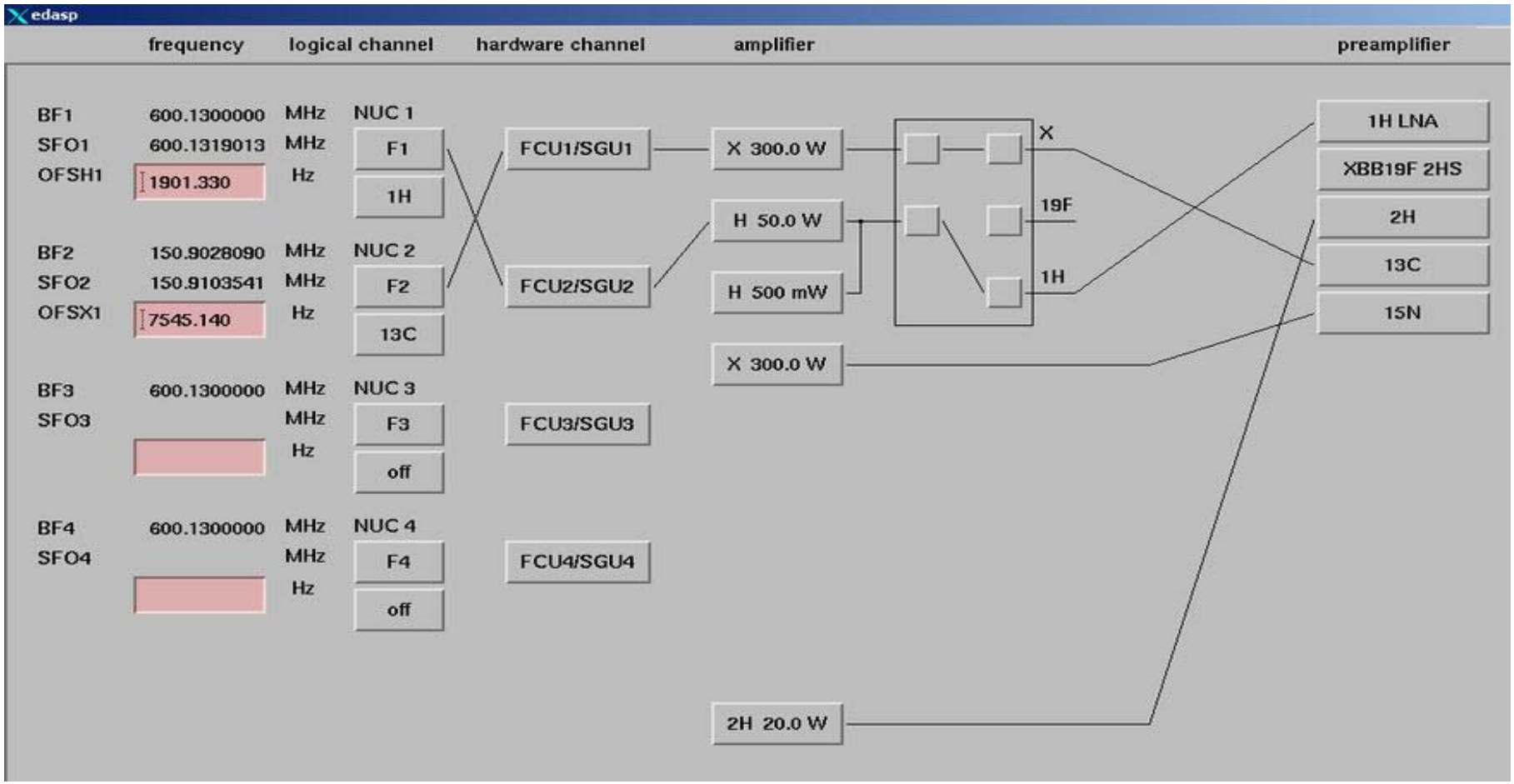






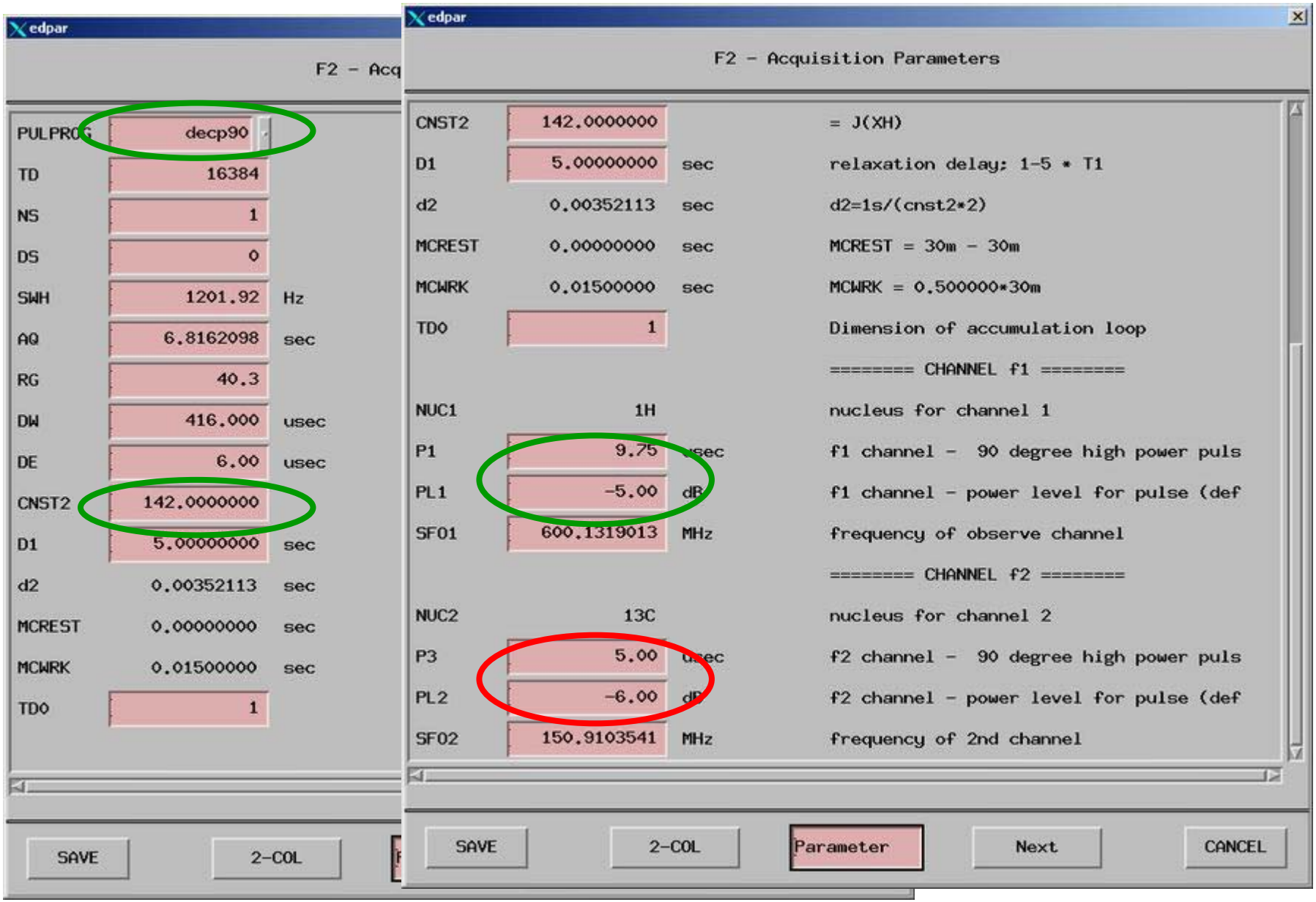
“Decouple channel” for 13C hard pulse

Step 1: edasp

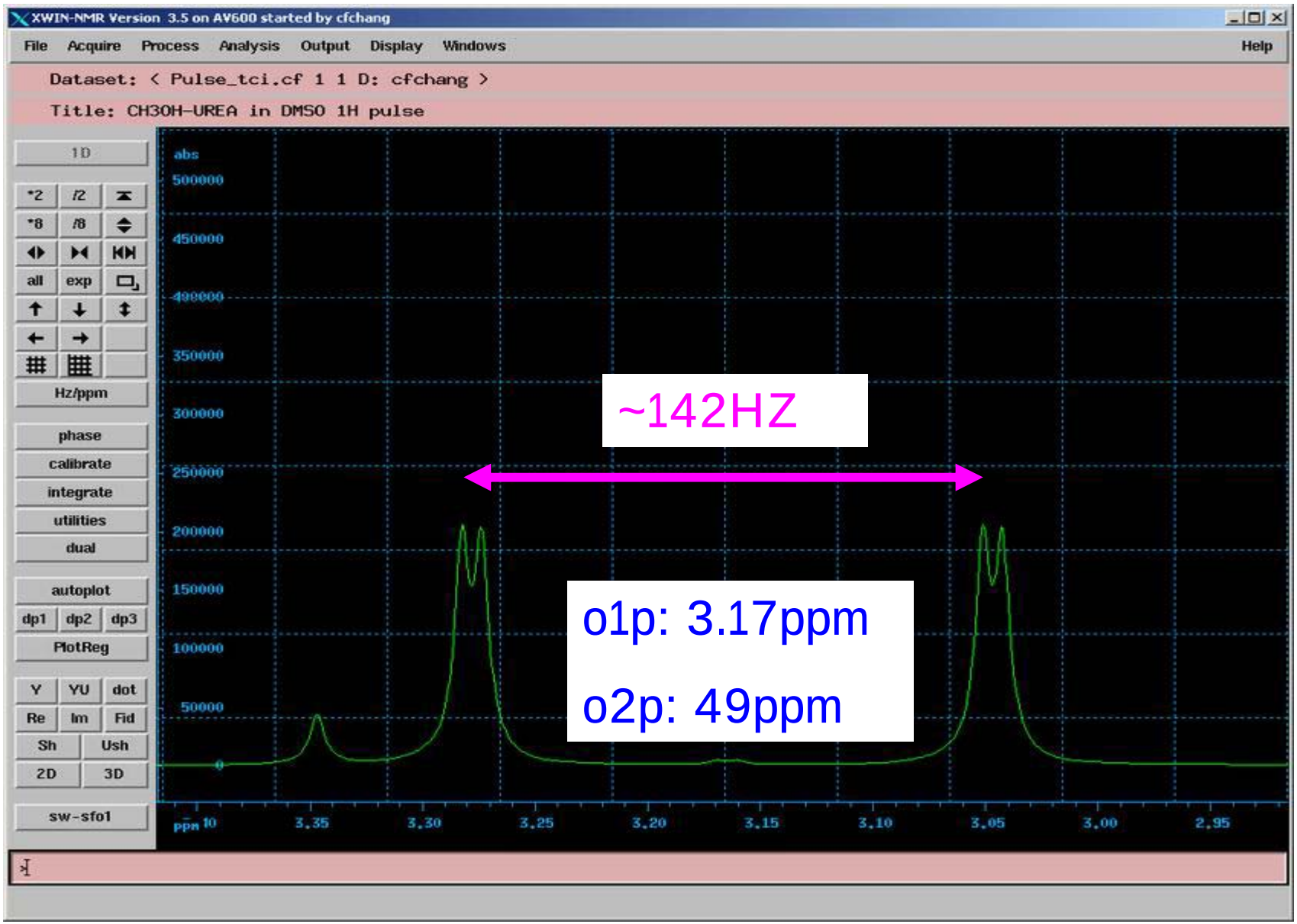


Decouple channel for 13C hard pulse (decp90)

Step 2: eda (parameter set up , PL2 vs. P3)



13CH3OH + 0.1M 15N-urea in DMSO-d6

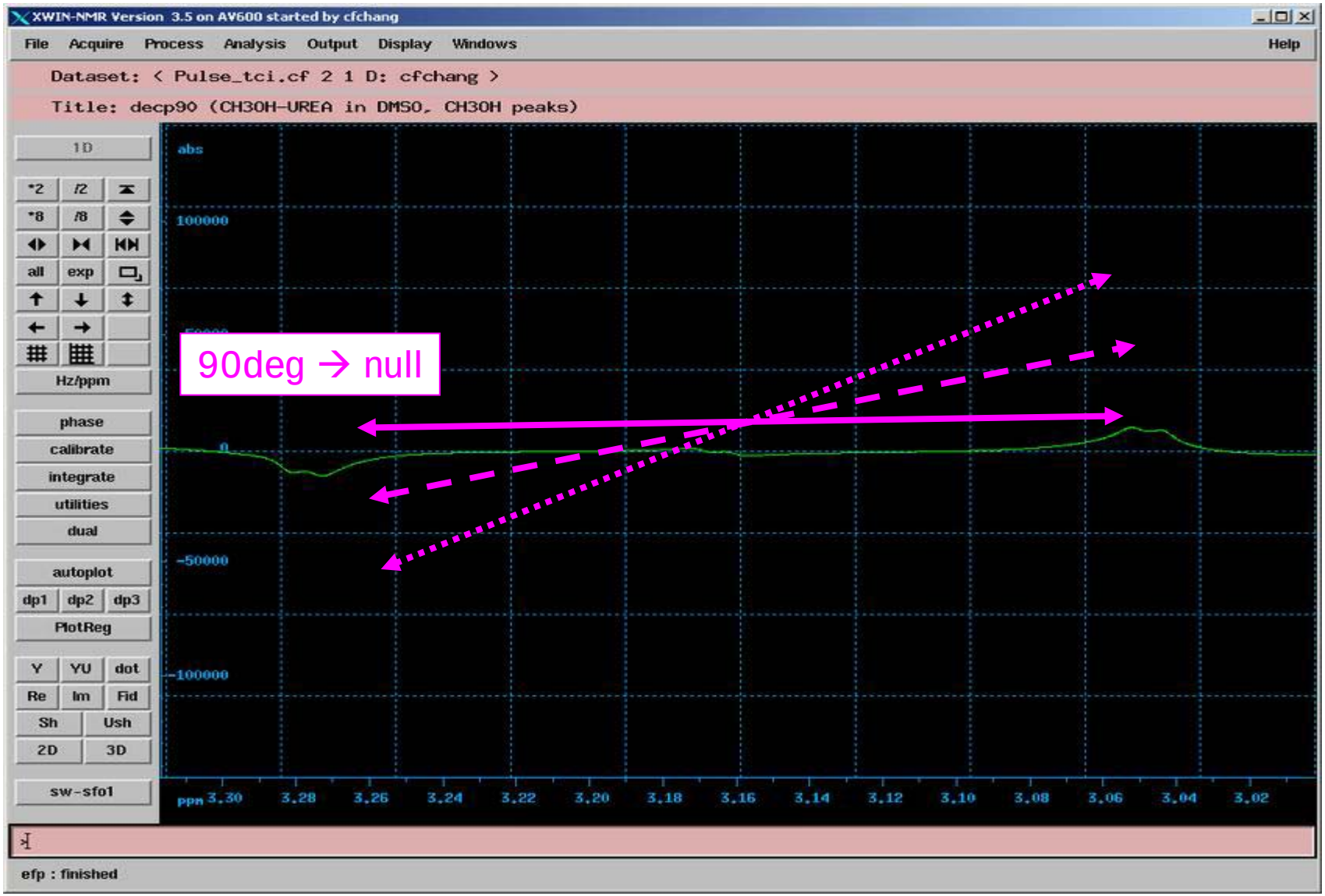


Decouple channel for 13C hard pulse (decp90)

Step 3: zg ,em, ft (pl2=-6dB, p3=5us)

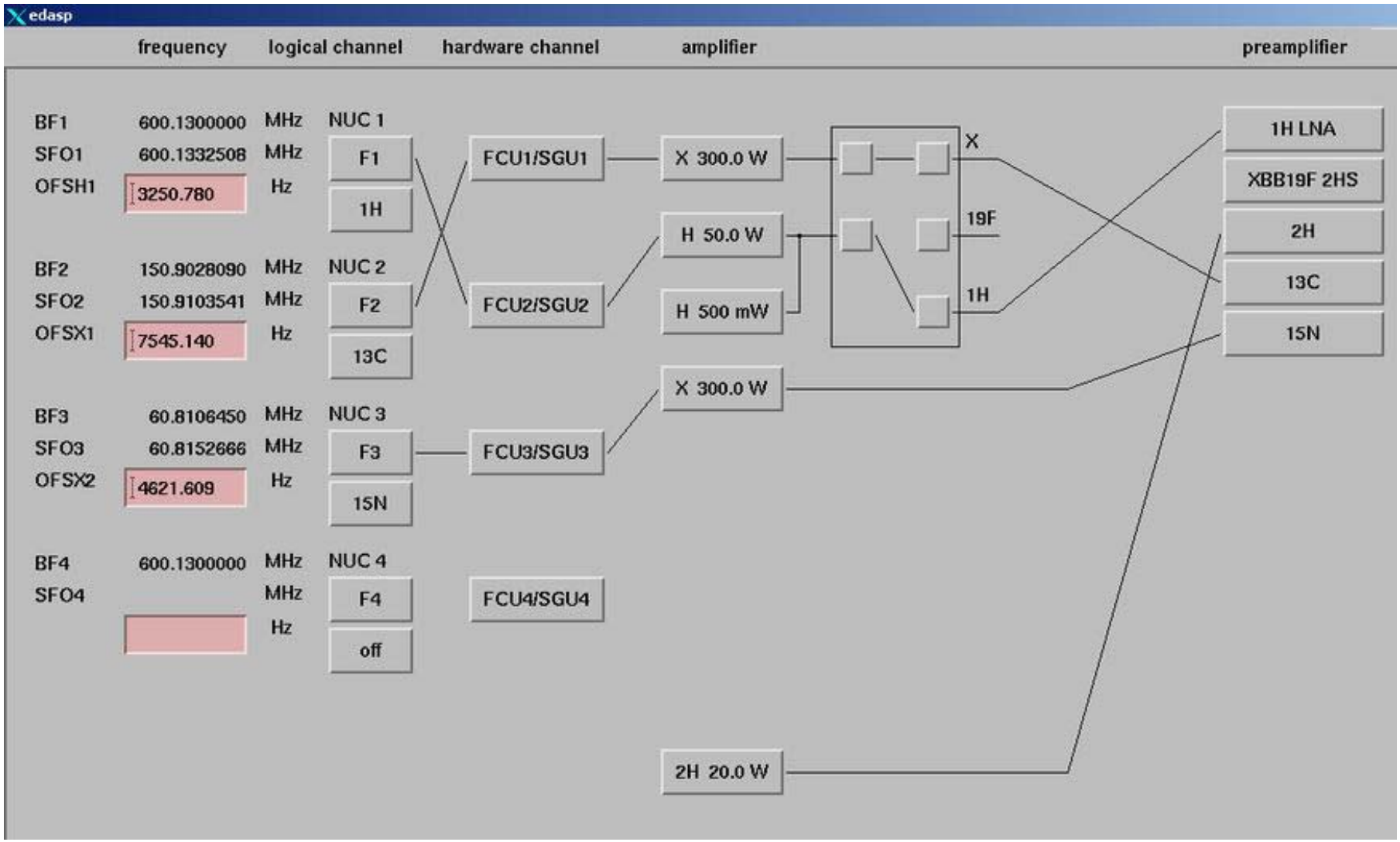
Decouple channel for 13C hard pulse (decp90)

Step 4: zg, em, fp (adjust p3 until reach null)



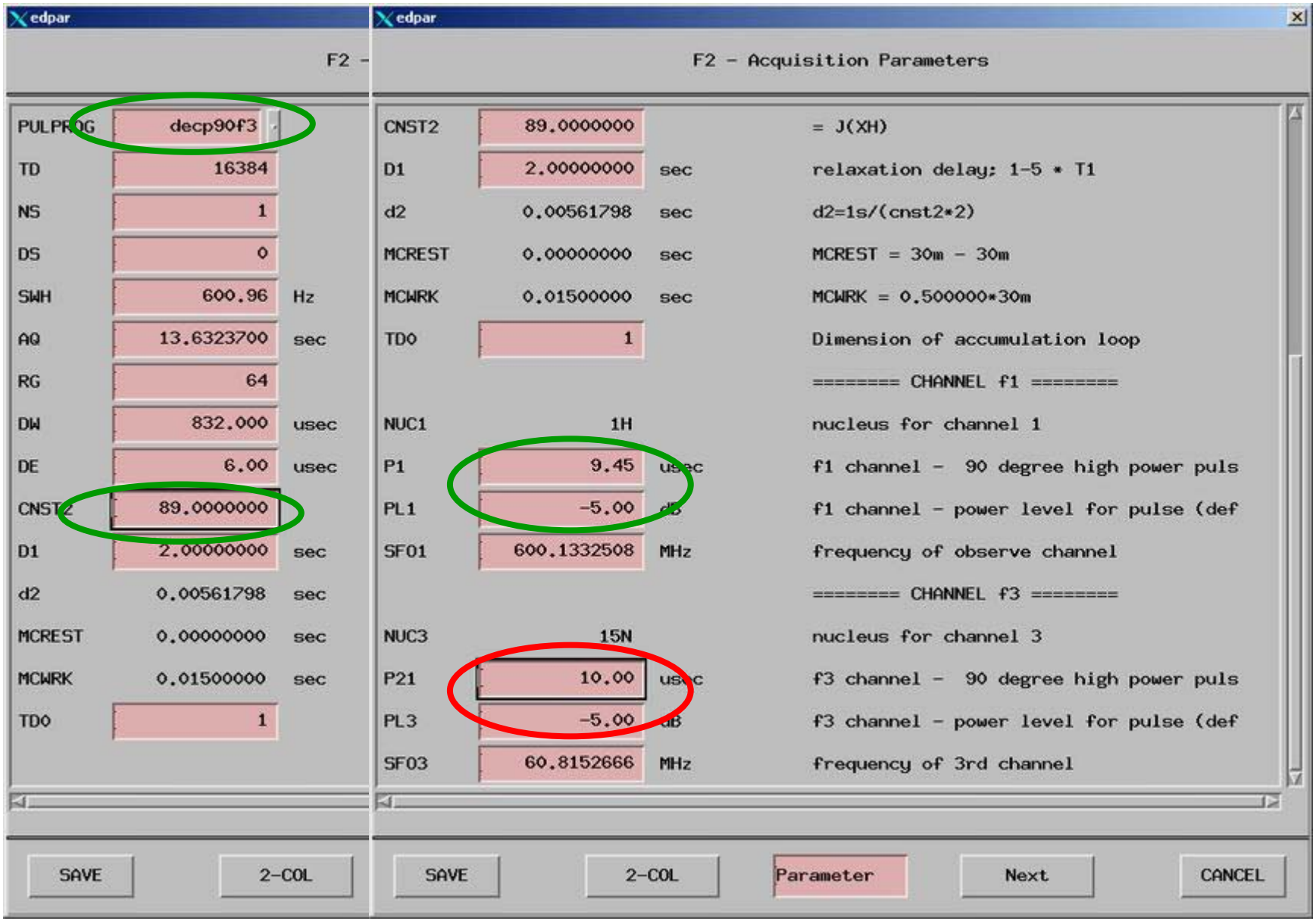
“Decouple channel” for 15N hard pulse

Step 1: edasp

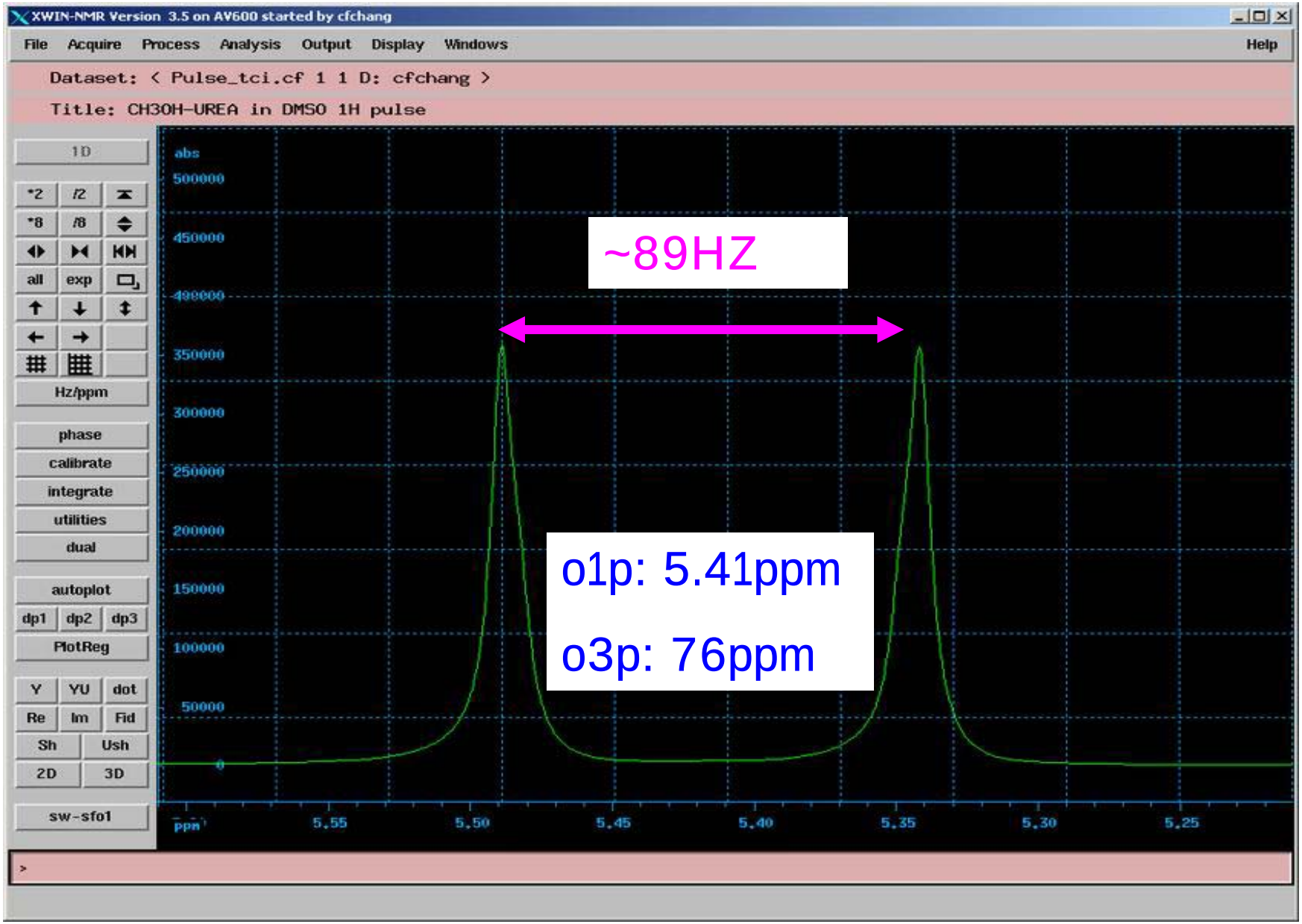


Decouple channel for 15N hard pulse (decp90f3)

Step 2: eda (parameter set up, PL3 vs. P21)

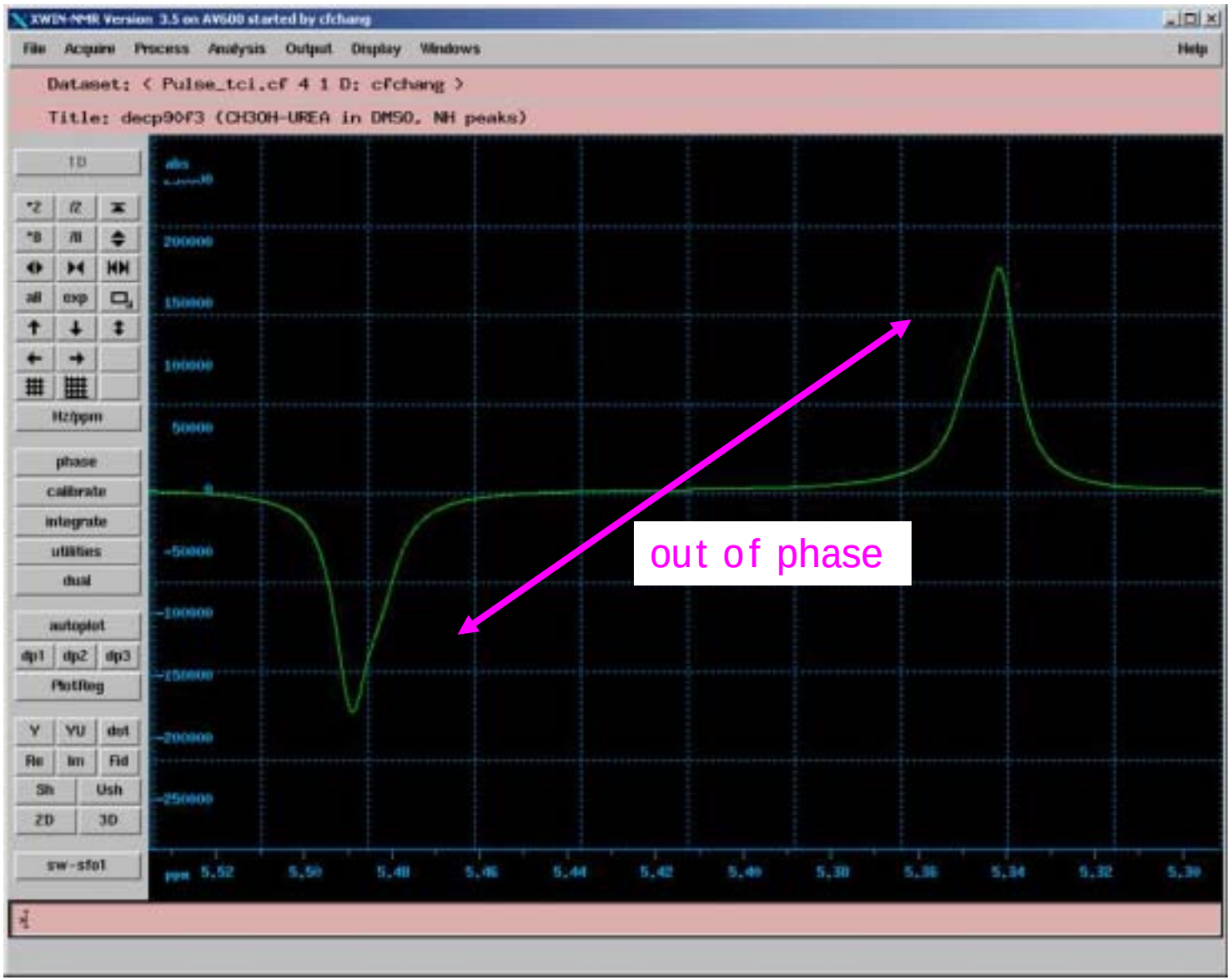


13CH3OH + **15N-urea** in DMSO-d6



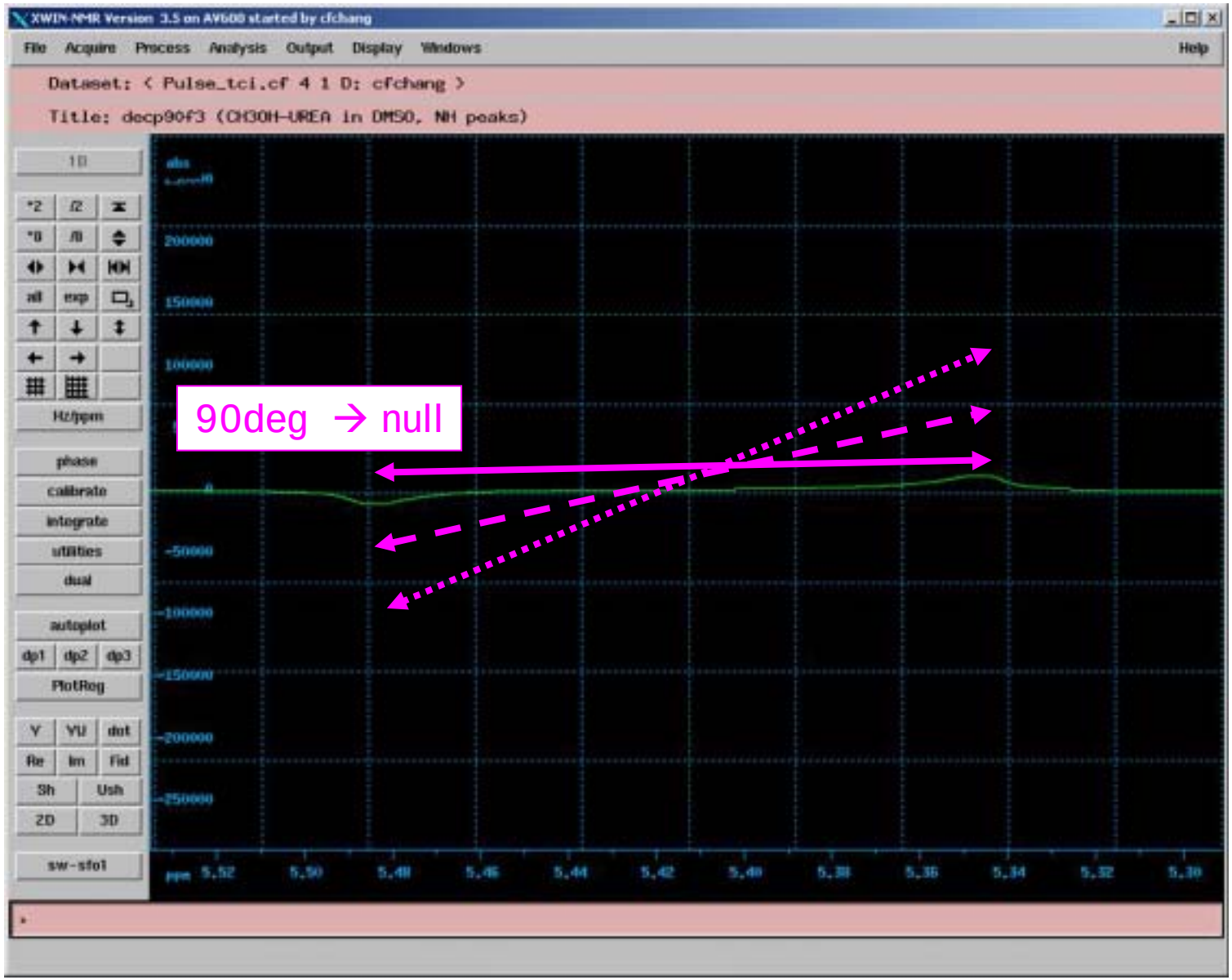
Decouple channel for 15N hard pulse (decp90f3)

Step 3: zg ,em, ft (pl3=-5dB, p21=5us)



Decouple channel for 15N hard pulse (decp90f3)

Step 4: zg, em, fp (adjust p21 until reach null)



Pulse Calibration for decoupled channel

(II) 13C Shape Pulses

How to optimize the pulse length for 13C shape pulse?

Excitation

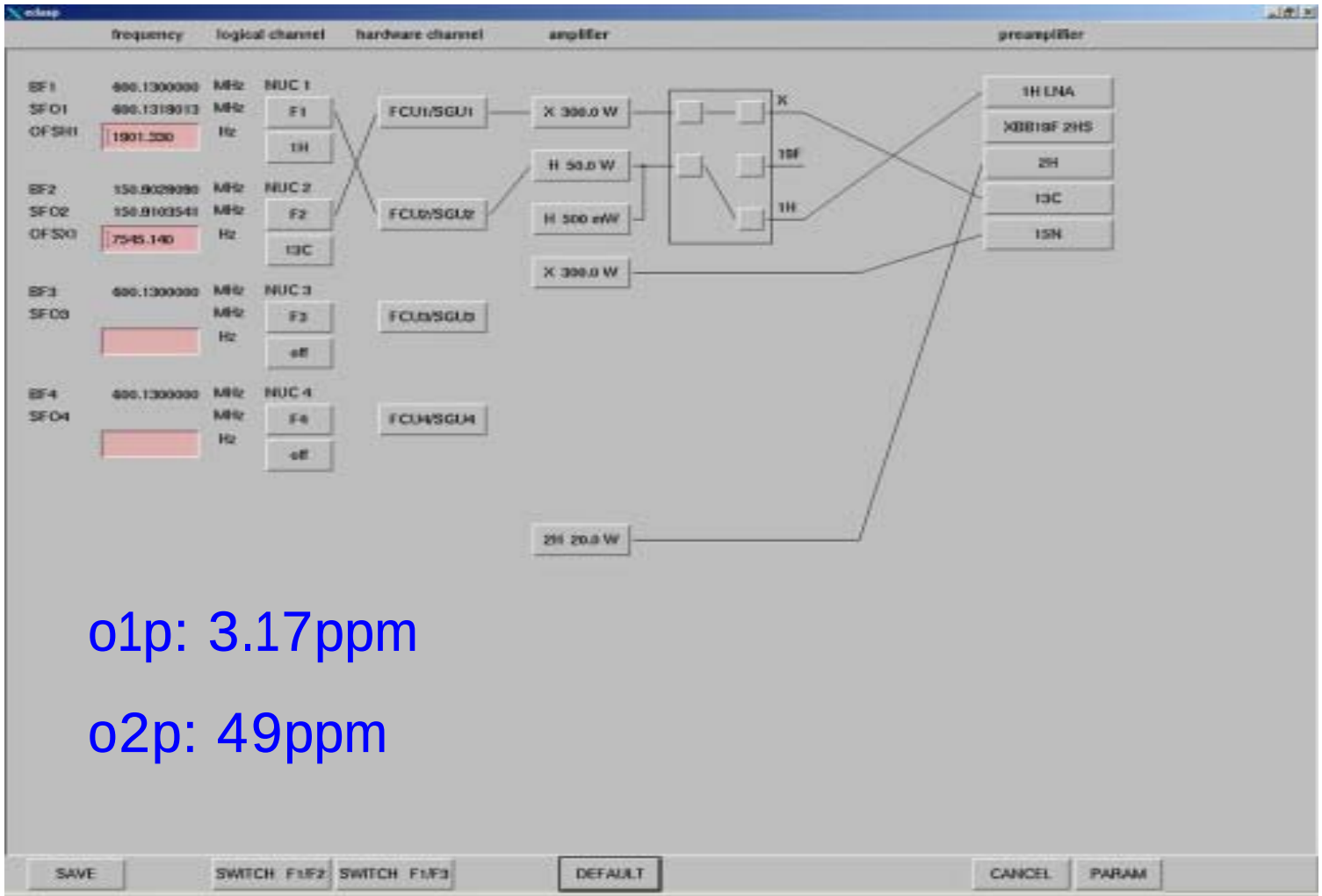
Shape	Pulse length	Cover frequency	500MHz (125Hz)	600MHz (150Hz)	800MHz (200Hz)
Q5.1000	410us	15073Hz	120ppm	100ppm	75ppm
Q5.1000	320u	19312Hz	154ppm	128ppm	96ppm

Inversion

Shape	Pulse length	Cover frequency	500MHz (125Hz)	600MHz (150Hz)	800MHz (200Hz)
Q3.1000	256us	13320Hz	106ppm	89ppm	66.6ppm
Q3.1000	768us	4440Hz	35ppm	29.6ppm	22.2ppm
Reburp.1000	1500u	3093Hz	24.75ppm		
Reburp.1000	1250u	3712Hz		24.75ppm	

Decouple channel for 13C 90 deg shape pulse

Step 1: edasp

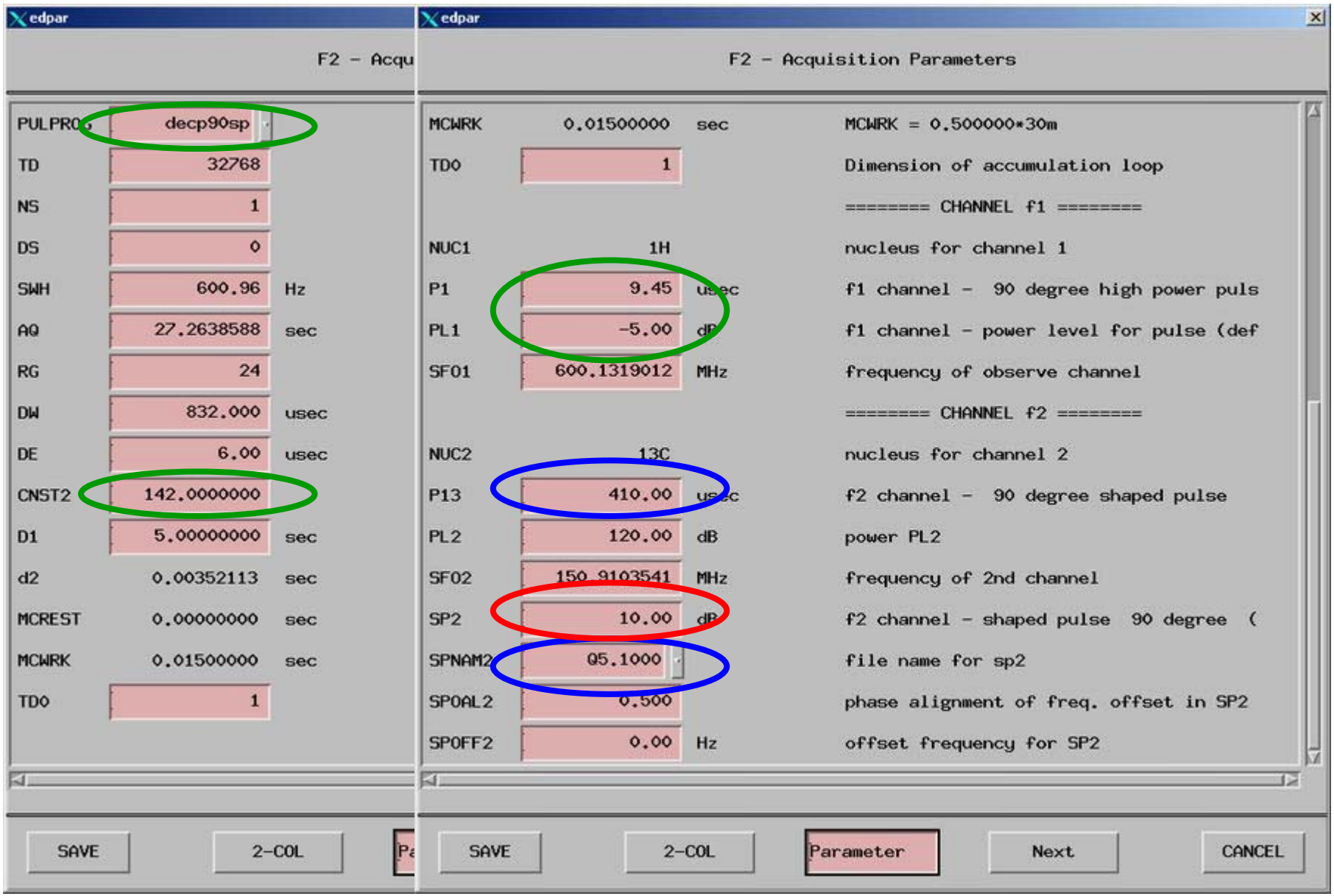


o1p: 3.17ppm

o2p: 49ppm

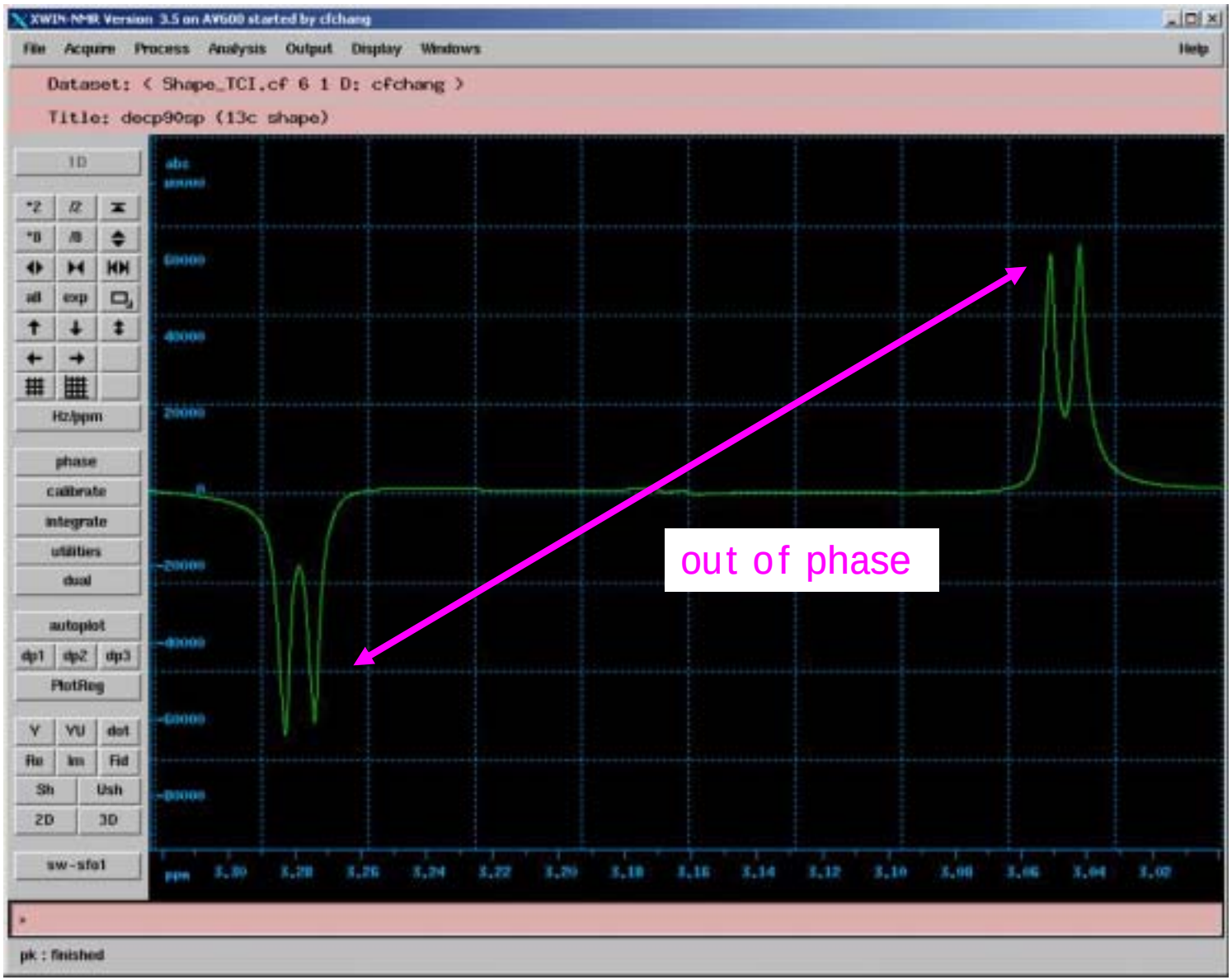
Decouple channel for 13C 90 deg shape pulse (decp90sp)

Step 2: eda (parameter set up , p13 vs. sp2 for SPNAM2)



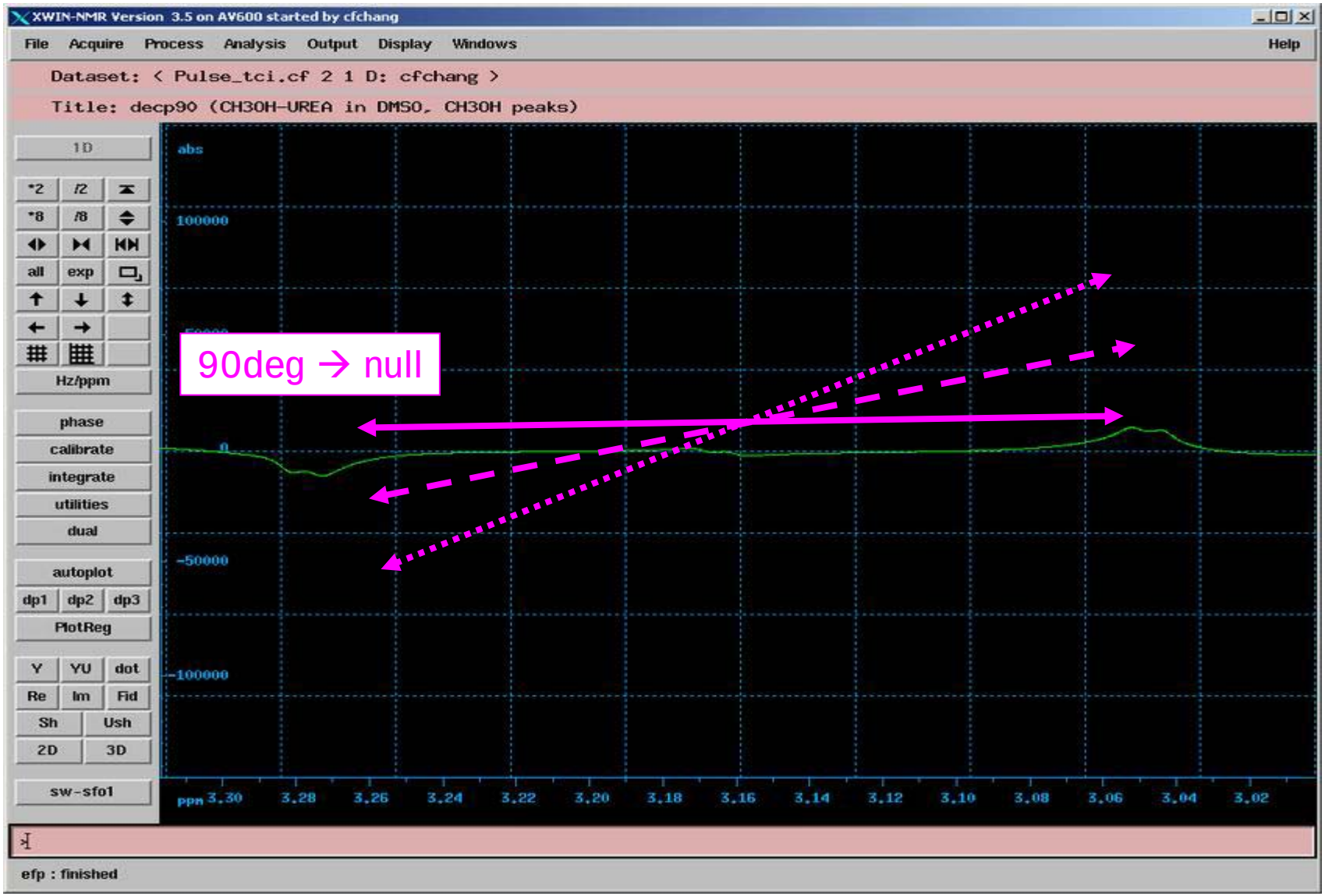
Decouple channel for 13C 90 deg shape pulse (decp90sp)

Step 3: zg ,em, ft (p13= 410us , sp2= 10dB , SPNAM2= Q5.1000)



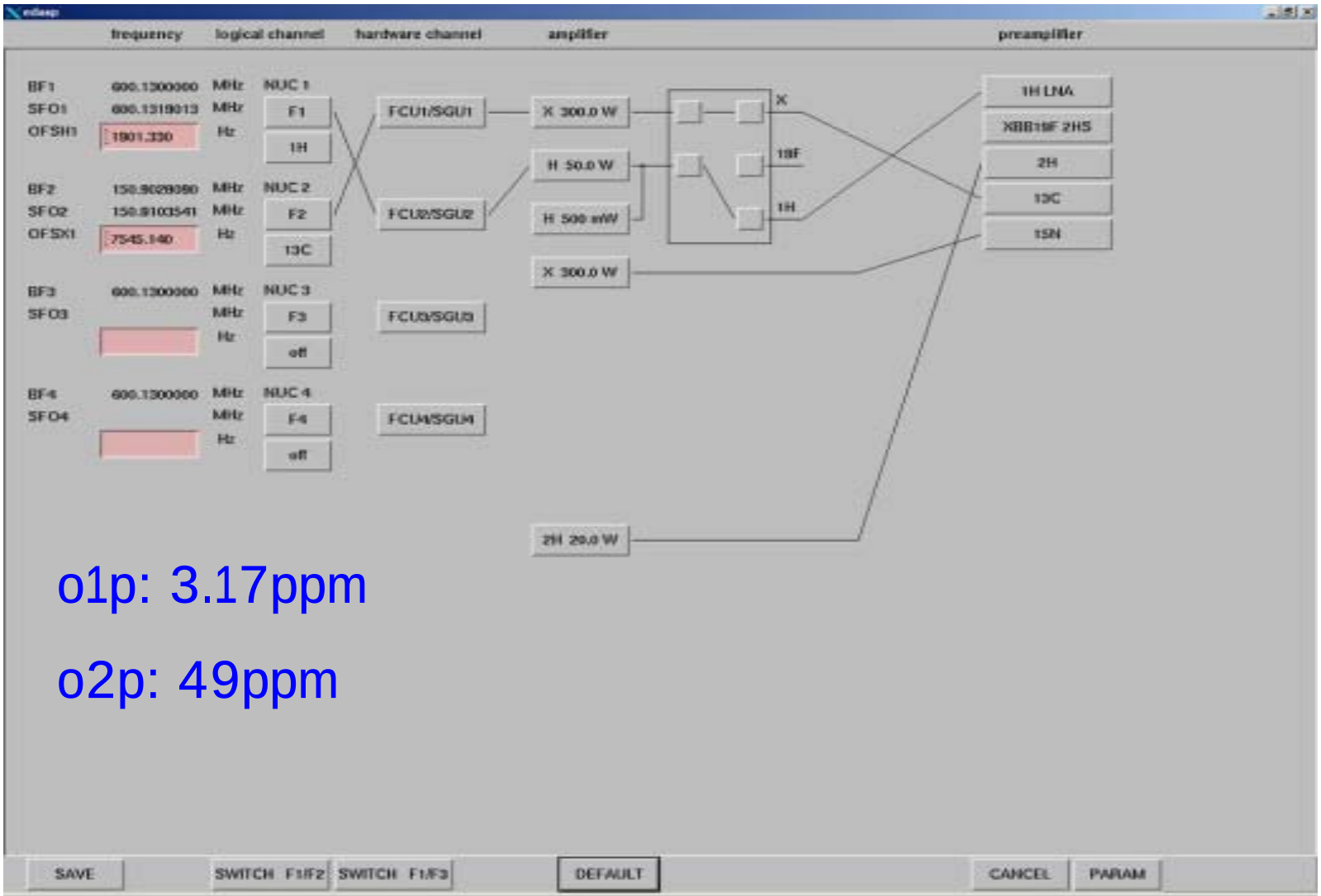
Decouple channel for 13C 90 deg shape pulse (decp90sp)

Step 4: zg, em, fp (adjust sp2 until reach null)



Decouple channel for 13C180 deg shape pulse

Step 1: edasp

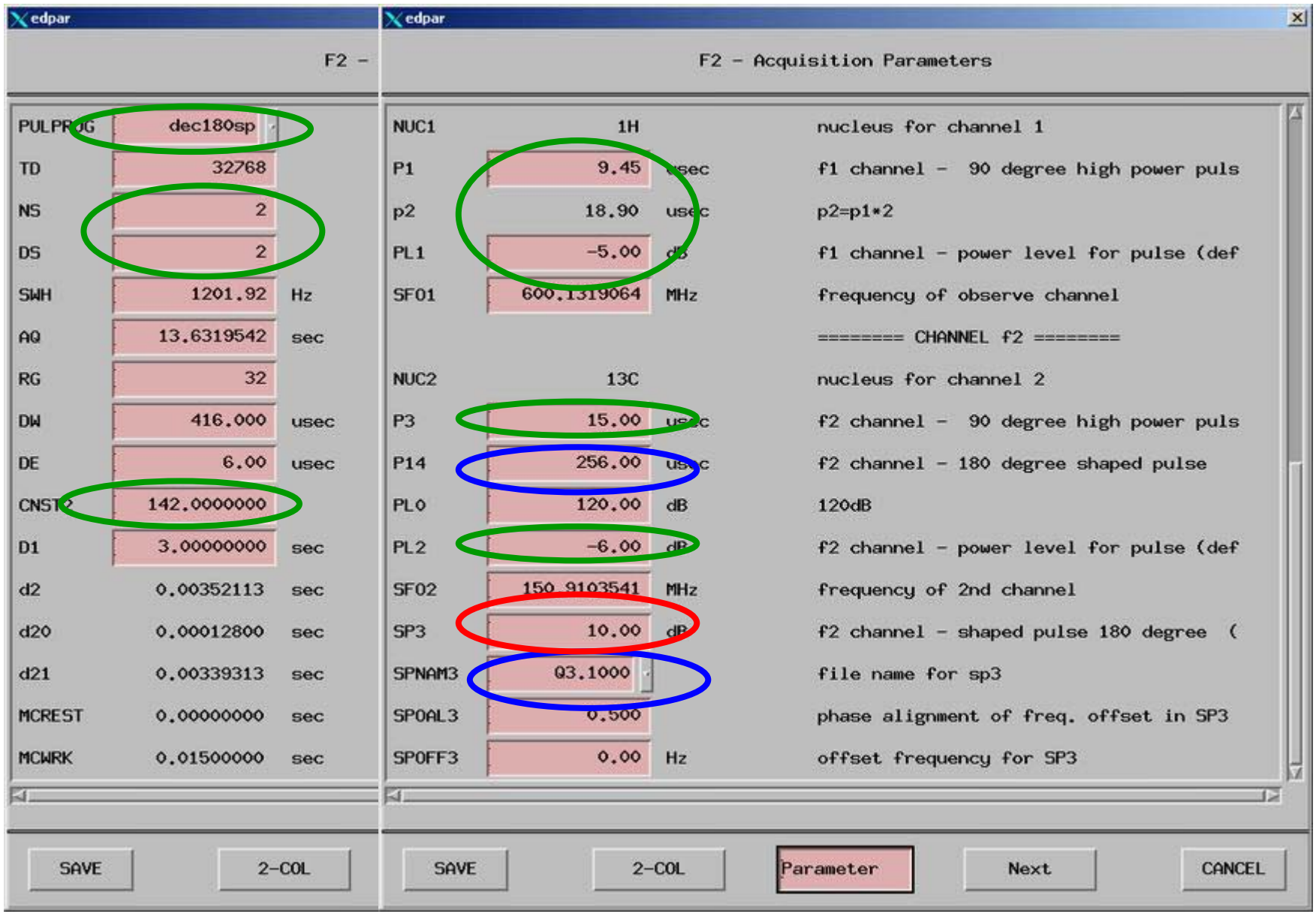


o1p: 3.17ppm

o2p: 49ppm

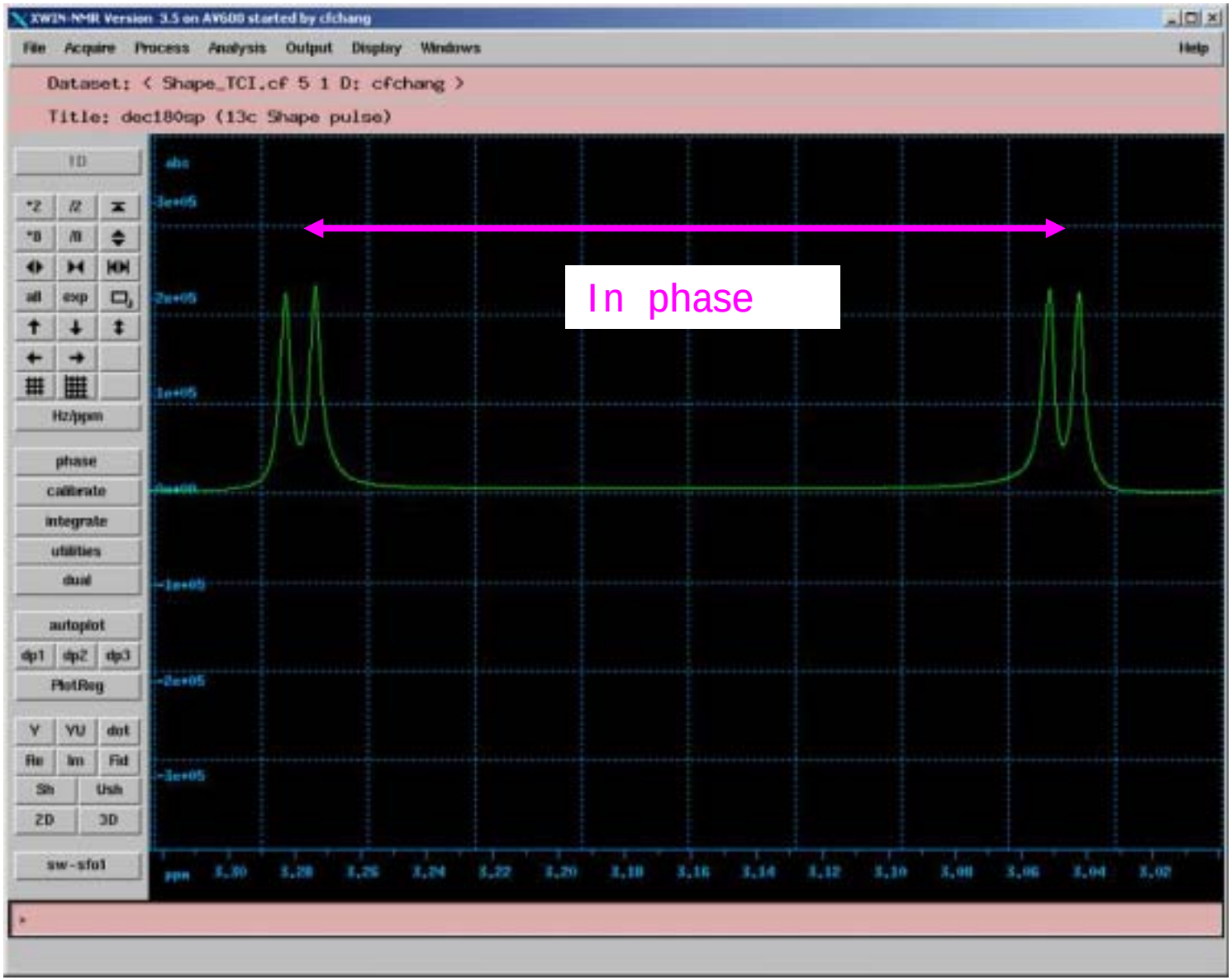
Decouple channel for 13C 180 deg shape pulse (decp180sp)

Step 2: eda (parameter set up , p14 vs. sp3 for SPNAM3)



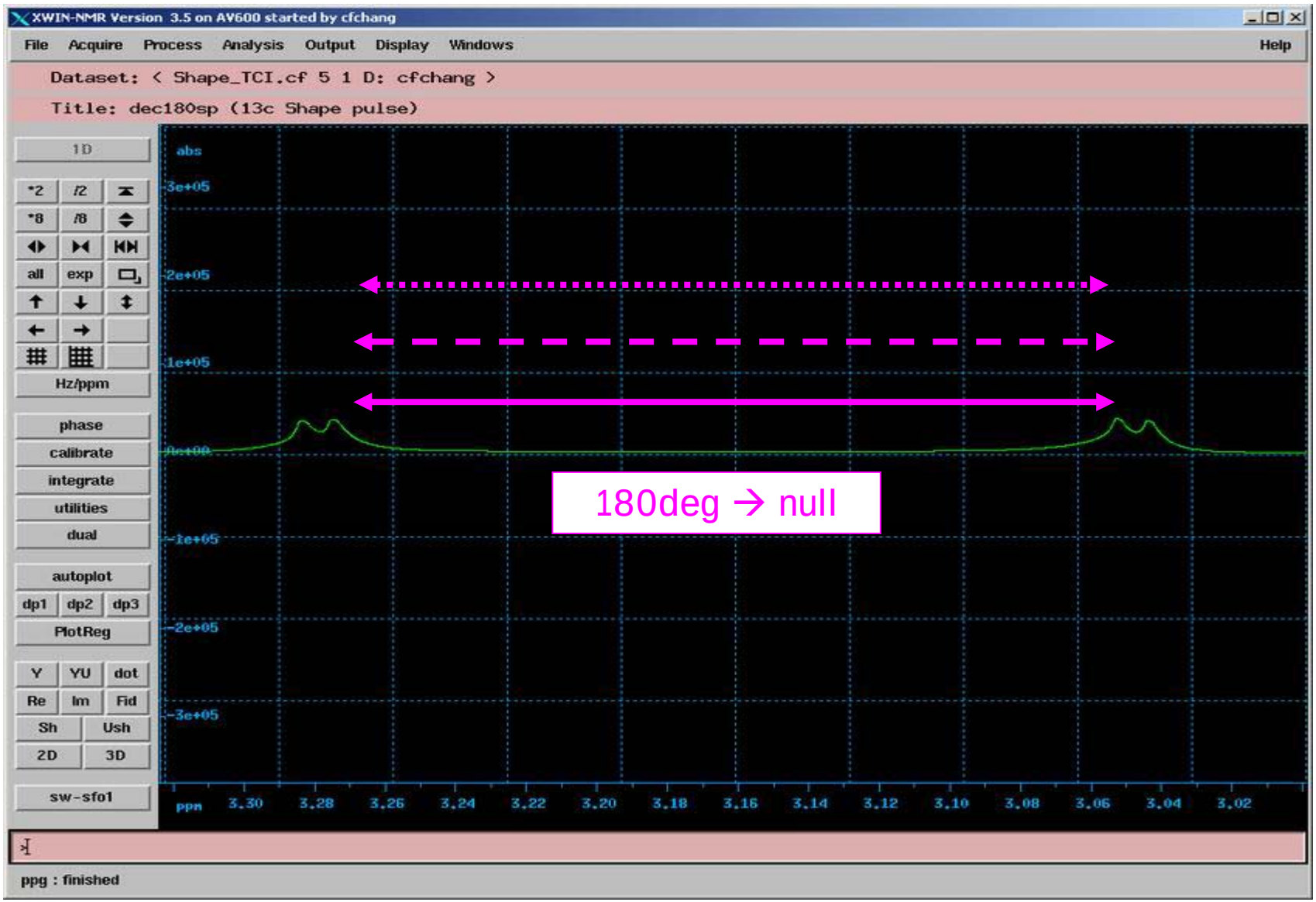
Decouple channel for 13C180 deg shape pulse (decp180sp)

Step 3: zg ,em, ft (p14= 256us , sp3= 10dB , SPNAM3= Q3.1000)



Decouple channel for 13C180 deg shape pulse (decp180sp)

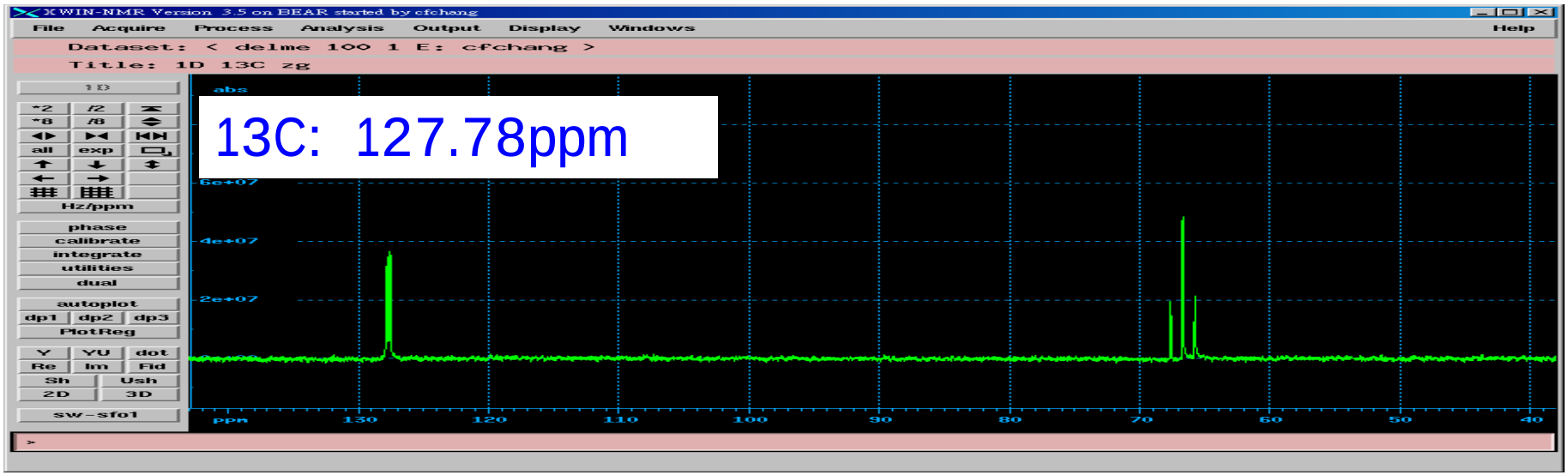
Step 4: zg, em, fp (adjust sp3 until reach null)

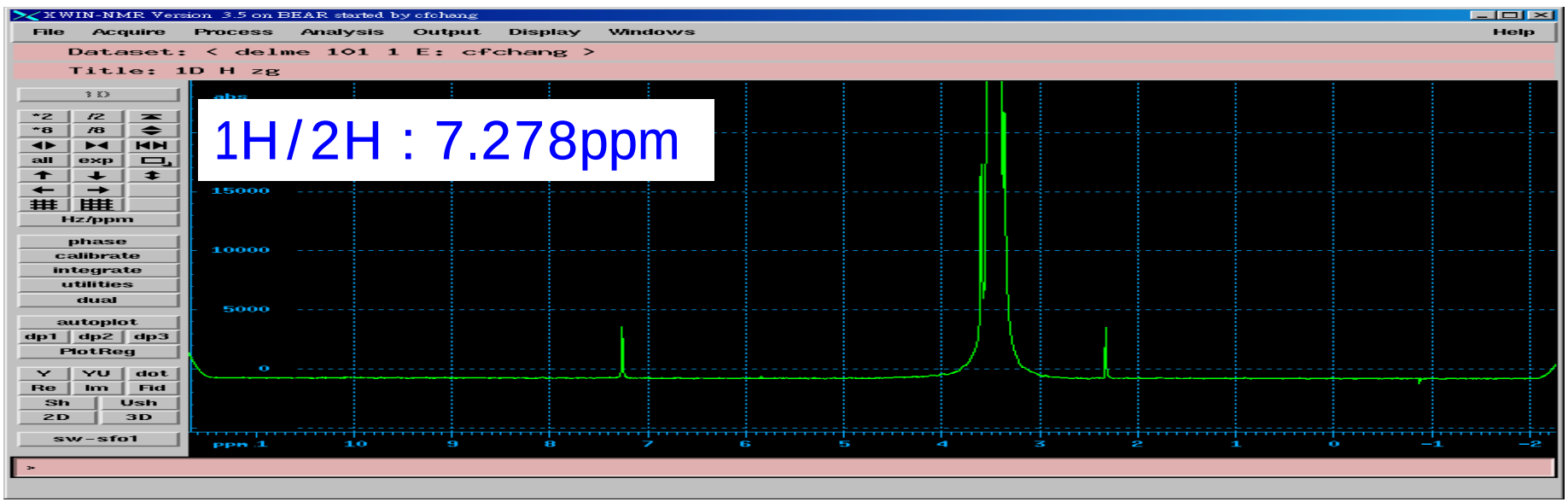


Pulse Calibration for decoupled channel

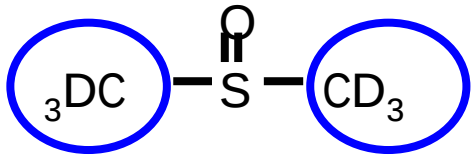
(III) 2H Hard Pulses

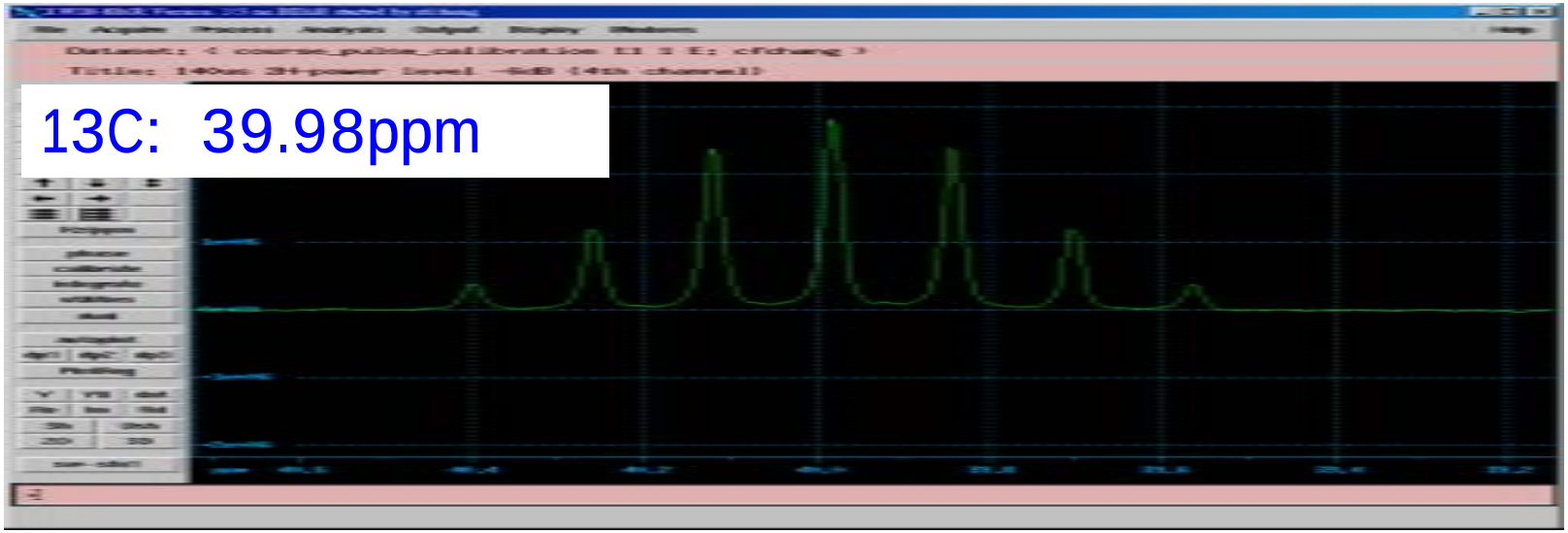
Sample: 60% Benzene-d6

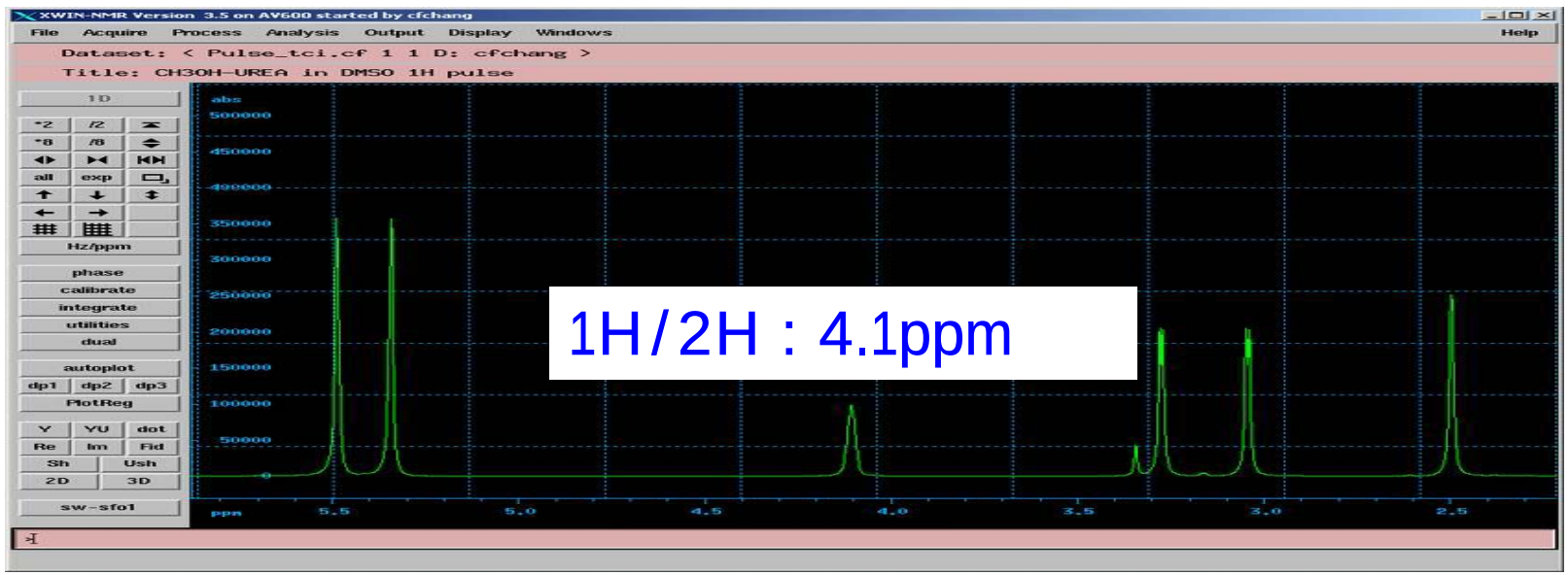




Sample: **0.1M 13CH3OH + 0.1M 15N-urea in DMSO-d6**

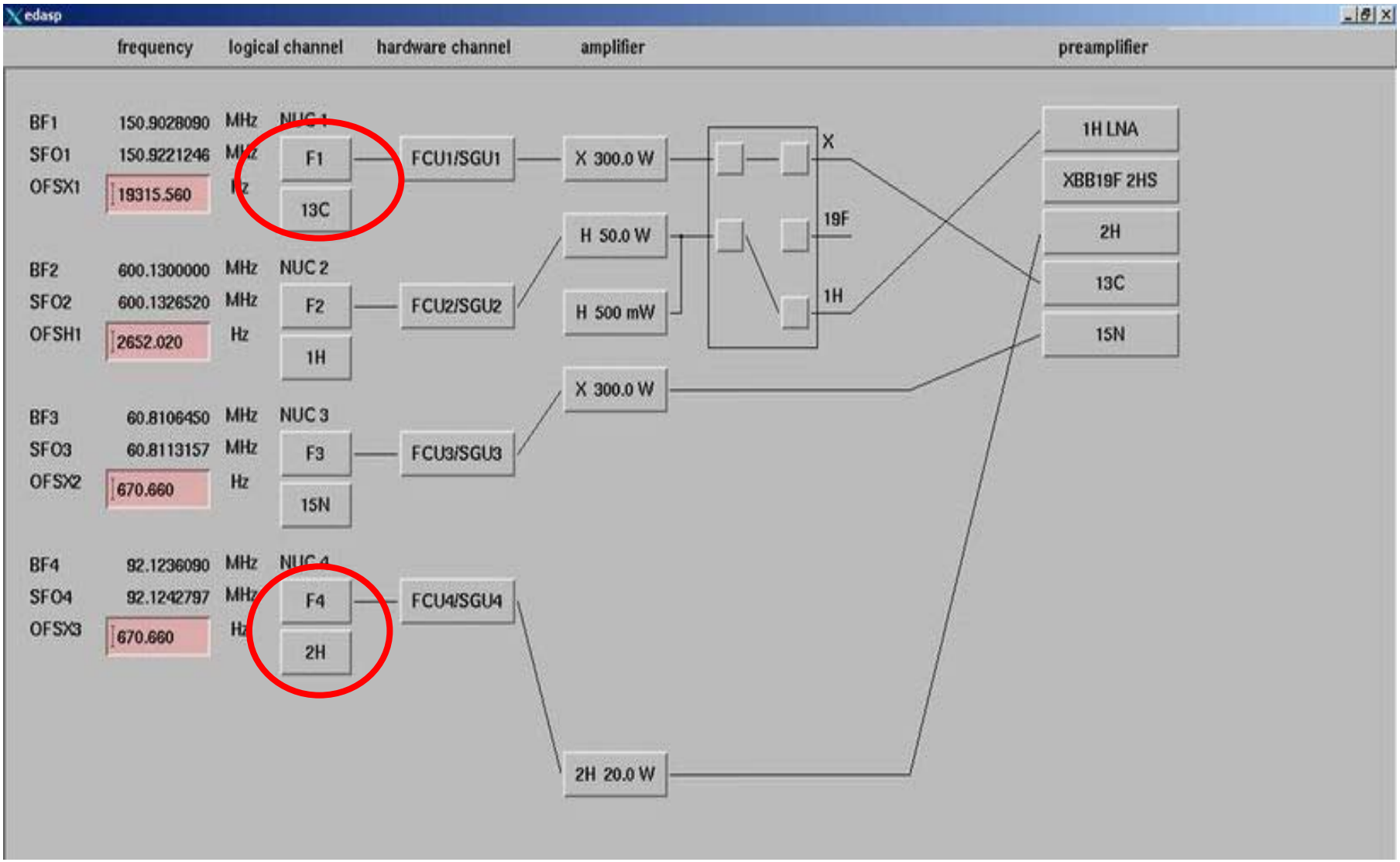






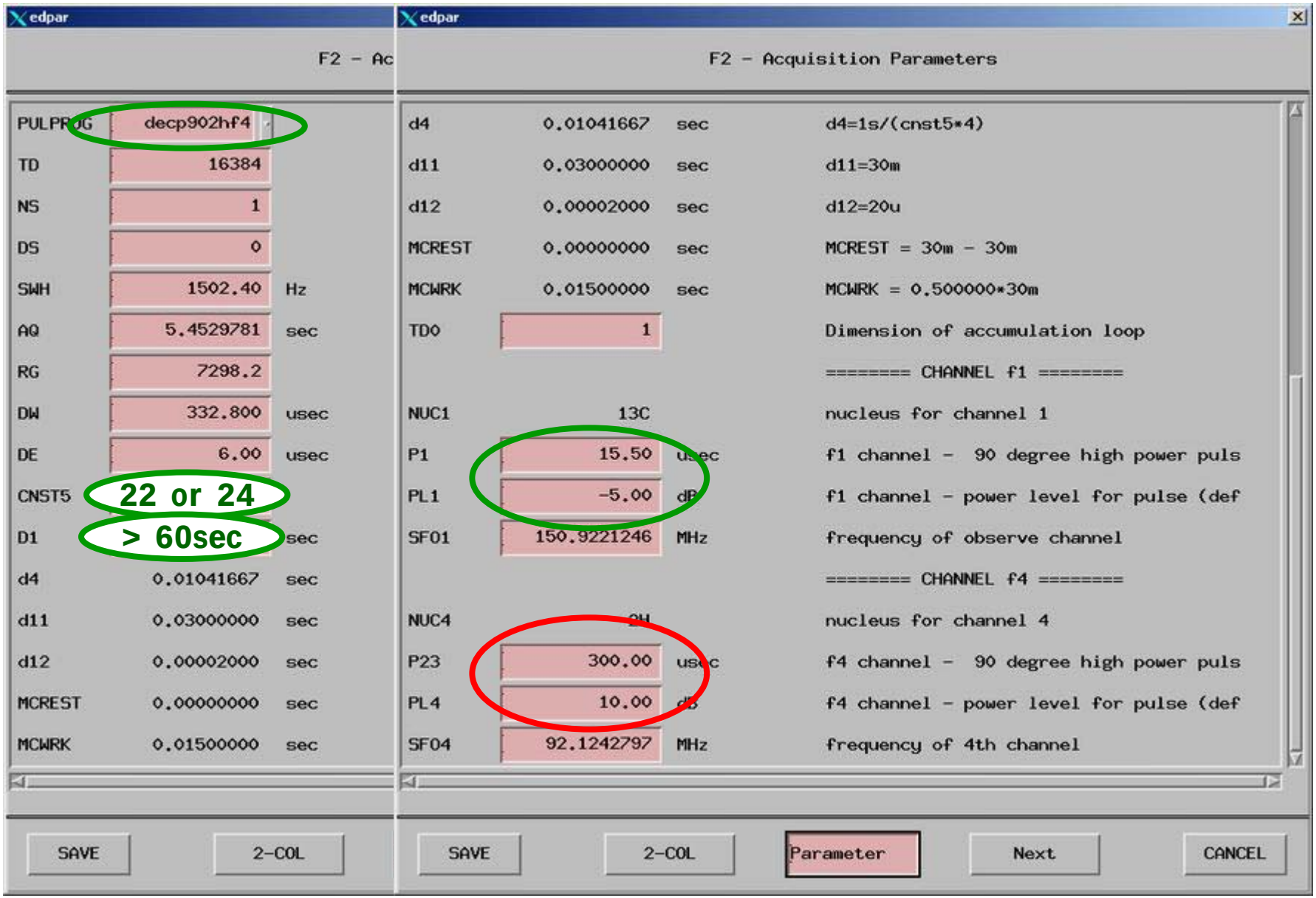
Decouple channel for 2H hard pulse

Step 1: edasp



Decouple channel for 2H hard pulse (decp902hf4)

Step 2: eda (parameter set up , PL4 vs. P23)

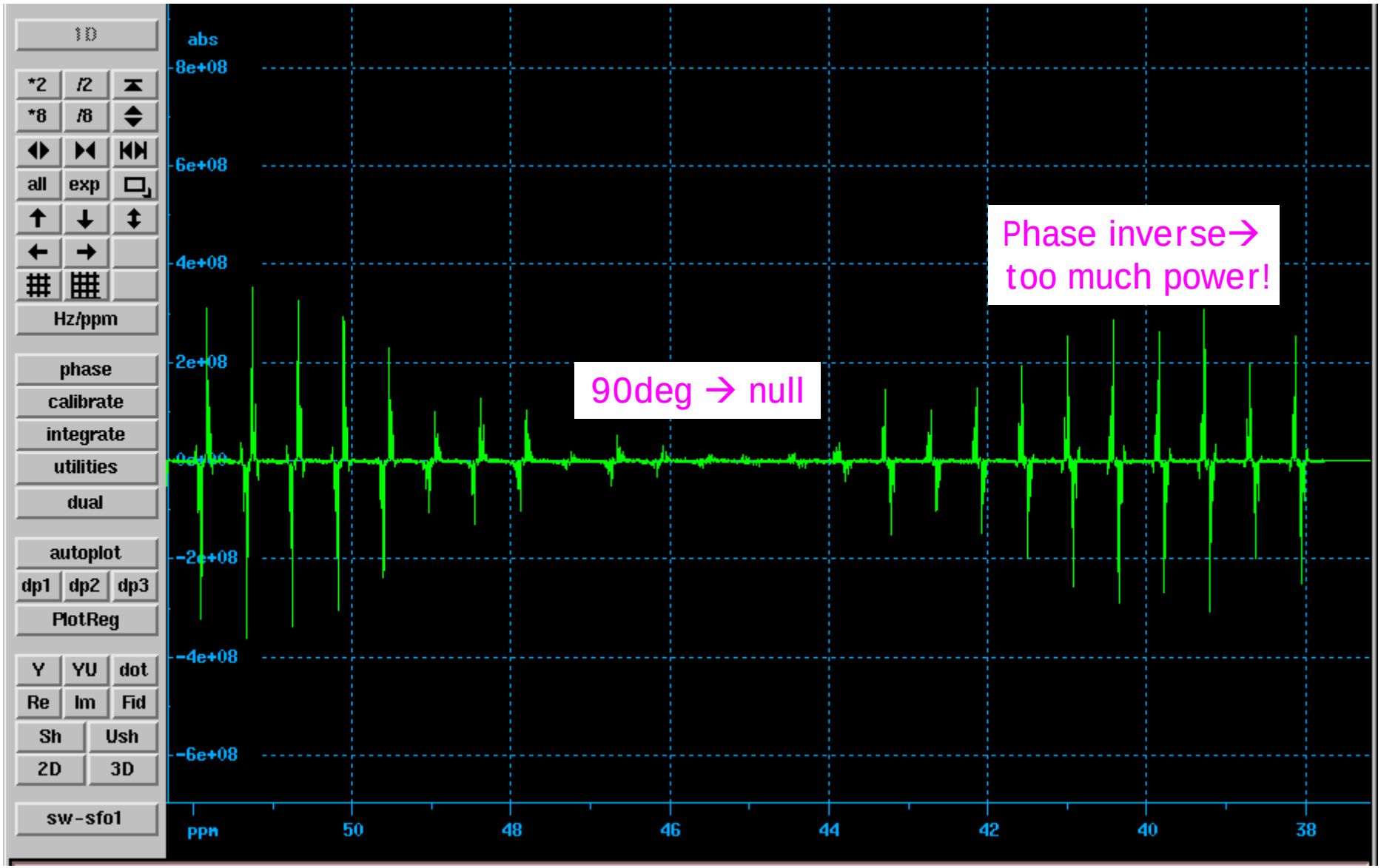


Decouple channel for 2H hard pulse (decp902hf4)

Step 3: zg ,em, ft (pl4=10dB, p23=200~300us)

Decouple channel for 2H hard pulse (decp902hf4)

Step 4: zg, em, fp (fix p23, adjust pl4 until reach null)



2005 NMR Training Course Lab Session

分組名單

示範人員	游春愛、方珮如	吳文晉	張七鳳
儀器名稱	AV600	AV500	AV600(in Chem)
2:00 ~	何孟儒(陳金榜) 黃愈志(陳金榜) 楊玉良(吳世雄) 蔡丞坤(黃太煌) 吳順家(磊葳)	駱啓仁(林達顯) 王誌慶(林達顯) 邱垂霖(程家維) 劉育男(呂平江) 吳英彥(磊葳)	盧弘捷(甘魯生) 饒淑娟(生化所) 林素卿(陳長謙) 詹雅玲(錢偉鈞) 羅彥中(王惠鈞)

P.S.由於此次Lab上機課程以有經驗者優先, 初學者請於下次基礎課程再上機.謝謝合作 !!

2005 NMR Training Course Lab Session

Outline (you should know how to shim, wobb, 1H pulse calibration…… already)

1. Hard pulse calibration for 13C/15N (decouple channel)

   a. rpar 1D 1H experiment

   b. edasp→ eda (select pulse program) → ased (set up parameters, cnst=142 or 89)

   c. Look for 90deg pulse

2. Shape pulse calibration for 13C (decouple channel)

   a. rpar 1D 1H experiment
   b. edasp→ eda (select pulse program) → ased (set up parameters, cnst=142)
   c. Look for 90deg or 180deg pulse

3. Standard HNCO type experiment (hncogpwg3d)

   a. rpar→ getprosol → look for “users” parameters

   b. double check “decouple pulses” which you obtain from (1) & (2)

4. New experiment from scratch

   new → eda (change to hncogp3d, and other parameters) → getprosol

   → ased (set up parameters)