KJM 9250

bfprf1bfprf2 Experiments on the AVIIIHD-800

Version 1.0

Topspin 3.5

Windows 7

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1.0 Introduction

Bruker pp's with CW presaturation on f1 require that it is applied at O1 = the spectral window midpoint. In cases where target compound signals are in the range (say) 0-6 ppm and the HOD or the other line to be presaturated occurs in the vicinity of 5 ppm, this requires that the presaturation experiment is set up with O1 = 5 ppm and SW = 10 ppm.

*aw coded bfprf1bfprf2 experiments can be used to apPLy dual CW presaturation at **CNST9 Hz on f1** <u>and</u> at **CNST21 Hz on f2**, <u>independent of one's choice of SW and O1.</u>*

All power levels, pulse types and pulse times used in aw coded bfprf1bfprf2 2D experiments are by default prosol Table linked and no FQLISTs are required.

Optimum outcomes are achieved in aw coded bfprf1bfprf2 experiments when the default prosol Table linked f1 and f2 presaturation powers (**PL9** and **PL21** respectively) are adjusted to be sufficient to suppress target signals while not suppressing correlations arising from other nearby signals.

Depending on the proximity of genuine signals this may require the PL9 and PL21 power levels to be reduced by 3-12 db from their default prosol Table values. This can be achieved by reducing (*ie* attenuating) the PL9 and PL21 power levels by <u>adding</u> 3-12 db to their default prosol Table power levels.

2.0 aw Coded bfprf1bfprf2 Parameter Sets

The following aw coded **bfprf1bfprf2** parameter sets are available on the **AVIIIHD-800:**

2.1	**awcosyqfbfprf1bfprf2**	(no gradients)
2.2	**awcosybfprf1bfprf2**	(with gradients)
2.3	**awtoscybfprf1bfprf2**	
2.4	**awclean-tocsybfprf1bfprf2**	
2.5	**awdipsi2bfprf1bfprf2**	
2.6	**awnoesybfprf1bfprf2**	
2.7	**awroesybfprf1bfprf2**	(with CW spin lock)
2.8	**awroesy2bfprf1bfprf2**	(with pulsed spin lock)

Prior to setting up a **bfprf1bfprf2** experiment determine the frequencies in **Hz** of the two signals to be presaturated in a standard ^{1}H spectrum and ascertain the desired 2D-spectral window (**SW**) in **ppm** and its intended **O1 value** in **Hz** (= spectral window midpoint).

2.1 Cosyqfbfprf1bfprf2 with a P0 excitation pulse, no gradients and CW presaturation on f1 at CNST9 Hz and f2 at CNST21 Hz

parameter set: **awcosyqfbfprf1bfprf2 (+ getprosol)**
pulse programme: **awcosyqfbfprf1bfprf2**

Type **eda** (enter) and enter **SW(F2) in ppm**, note the spectral window in **Hz** that appears in the **SWH(F2)** box and copy and paste this value into the **SWH(F1)** box.
Check **SWH (F2) = SWH(F1)** in Hz including all dp's.

Enter **O1 = O2 = CNST1 in Hz** *(in this order!)* where **O1** = the spectral window midpoint.
Enter **CNST9** and **CNST21 in Hz** of the signals to be presaturated on F1 and F2 respectively.

TD(F2) = 2K, **TD(F1)** = 128-256 (your choice).
NS = 2, 4, 8 (any number is OK), **DS** = 2, 4 or 8.
P0 = θ degree excitation pulse time, typically use a 45° or 90° pulse.
D1 = presaturation time = 1.5, 2.0 sec or other time of your choice.

Type **ased** (enter) and review parameters used in the job including **CNST1**, **CNST9** and **CNST21**. Check the **PL9** and **PL21** presaturation power levels are OK and if required adjust them appropriately.
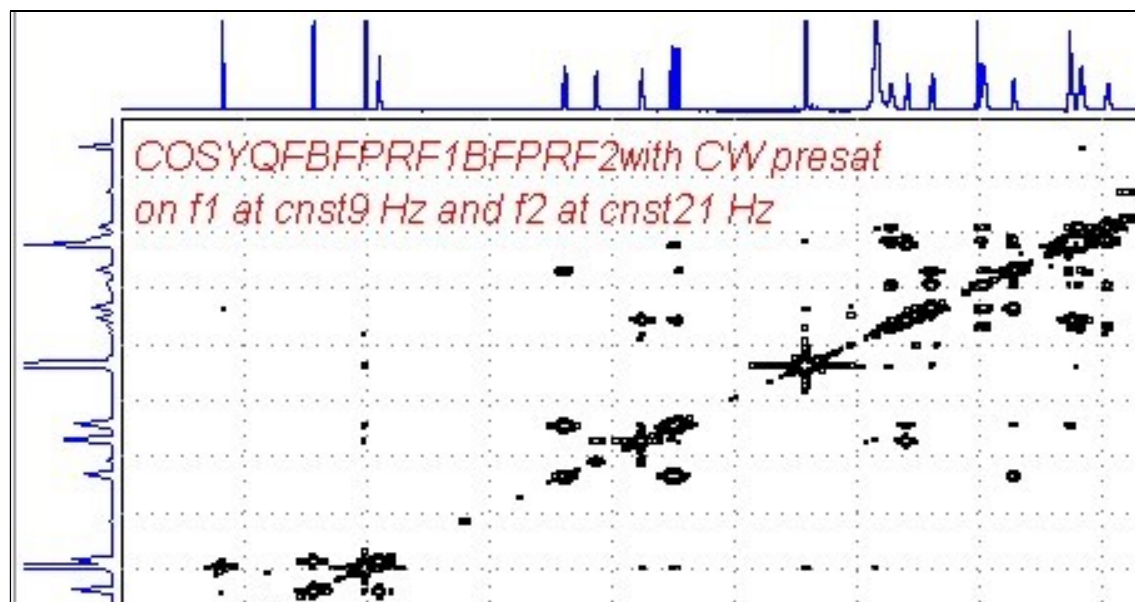
Set **receiver gain** using **RGA** *(important!).*

Process with: **SI(F2) = SI(F1) = 2K**
WDW(F1) = WDW(F2) = SINE
SSB(F2) = SSB(F1) = 0
xfb, abs1, abs2 and (optionally) **sym**

800 MHz Cosyqfbfprf1bfprf2 spectrum of quinine in D_6-DMSO with the HOD and DMSO lines at 3.38 ppm and 2.5 ppm suppressed. The spectrum is centered at 4.5 ppm (O1 = O2 = CNST1 = 3600.14 Hz).

2.2 Cosybfprf1bfprf2 with gradients and a P0 excitation pulse and CW presaturation on f1 at CNST9 Hz and f2 at CNST21 Hz

parameter set: **awcosybfprf1bfprf2 (+ getprosol)**
pulse programme: **awcosygpqfbfprf1bfprf2**

Type **eda** (enter) and enter **SW(F2) in ppm**, note the spectral window in **Hz** that appears in the **SWH(F2)** box and copy and paste this value into the **SWH(F1)** box. Check **SWH(F2) = SWH(F1)** in Hz including all dp's.

Enter **O1 = O2 = CNST1 in Hz** *(in this order!)* where **O1** = the spectral window midpoint. Enter **CNST9** and **CNST21 in Hz** of the signals to be presaturated on F1 and F2 respectively.

TD(F2) = 2K, **TD(F1)** = 128-256 (your choice).
NS = 2, 4, 8 (any number is OK), **DS** = 2, 4 or 8.
P0 = θ degree excitation pulse time, typically use a 45° or 90° pulse.
D1 = presaturation time = 1.5, 2.0 sec or other time of your choice.

Type **ased** (enter) and review parameters used in the job including **CNST1**, **CNST9** and **CNST21**. Check gradient and **PL9** and **PL21** presaturation power levels are OK and if required adjust them appropriately.
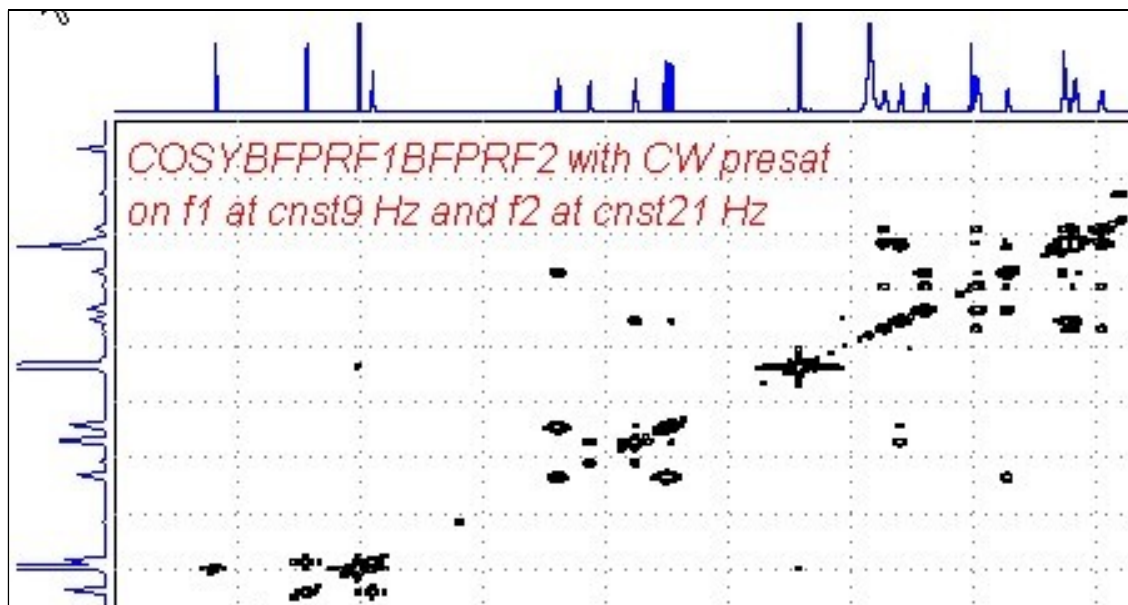
Set **receiver gain** using **RGA** *(important!)*.

Process with: **SI(F2) = SI(F1) = 2K**
               WDW(F1) = WDW(F2) = SINE
               SSB(F2) = SSB(F1) = 0
               xfb, abs1, abs2 and (optionally) **sym**

800 MHz Cosybfprf1bfprf2 spectrum of quinine in D_6-DMSO with the HOD and DMSO lines at 3.38 ppm and 2.5 ppm suppressed. The spectrum is centered at 4.5 ppm (O1 = O2 = CNST1 = 3600.14 Hz).

2.3 Tocsybfprf1bfprf2withCW presaturation onf1 at CNST9 Hz and f2 at CNST21 Hz

parameter set: **awtocsybfprf1bfprf2 (+ getprosol)**
pulse programme: **awtocsybfprf1bfprf2**

Type **eda** (enter) and enter **SW(F2) in ppm**, note the spectral window in **Hz** that appears
In the **SWH(F2)** box and copy and paste this value into the **SWH(F1)** box.
Check **SWH(F2)** = **SWH(F1)** in Hz including all dp's.

Enter **O1 = O2 = CNST1 in Hz** *(in this order!)* where **O1** = the spectral window midpoint.
Enter **CNST9** and **CNST21 in Hz** of the signals to be presaturated on F1 and F2 respectively.

TD(F2) = 2K, **TD(F1)** = 128-256 (your choice).
NS = 4, 8, 16 (multiPLe of 4 or 8 recommended), **DS** = 4 or 8.
D1 = presaturation time = 1.5, 2.0 sec or other time of your choice.
D9 = correlation time = 80 msec or other time of your choice (6-240 msec).

Type **ased** (enter) and review parameters used in the job including **CNST1**, **CNST9** and
CNST21. Check the **PL9** and **PL21** presaturation power levels are OK and if required adjust
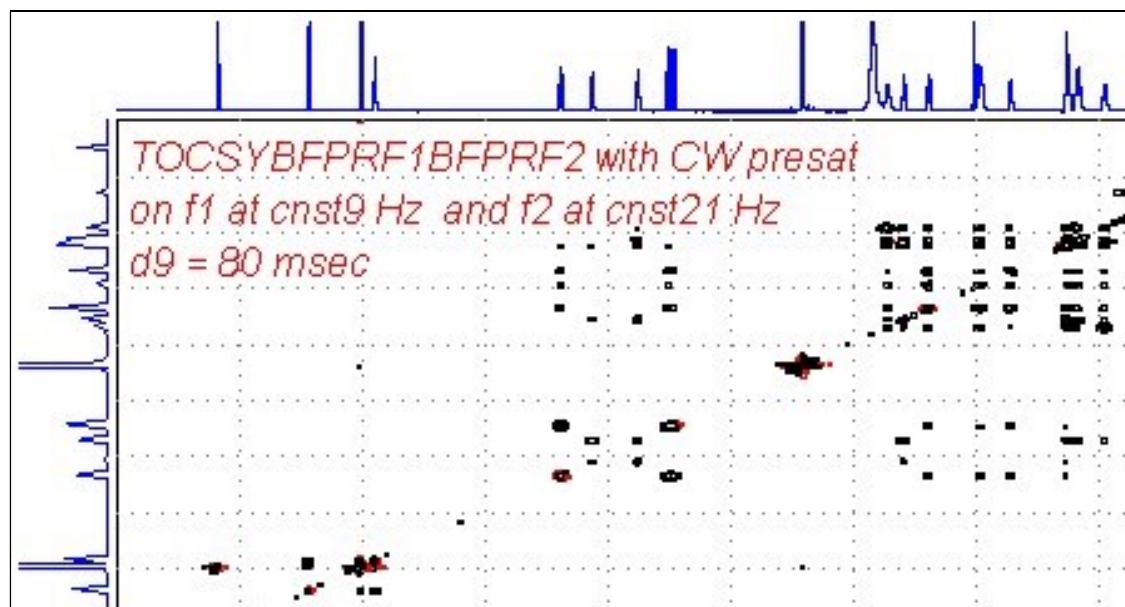them appropriately.

Set **receiver gain** using **RGA** *(important!)*.

Process with: **SI(F2) = SI(F1) = 2K**
     WDW(F1) = WDW(F2) = QSINE
     SSB(F2) = SSB(F1) = 2
     xfb, abs1, abs2 and optionally **syma**

800 MHz TOCSYbfprf1bfprf2 spectrum of quinine in D_6-DMSO with the HOD and DMSO
lines at 3.38 ppm and 2.5 ppm suppressed. The spectrum is centered at 4.5 ppm (O1 = O2 =
CNST1 = 3600.14 Hz).

2.4 Clean-tocsybfprf1bfprf2withCW presaturation on f1 at CNST9 Hz and f2 at CNST21 Hz

parameter set: **awcleantocsybfprf1bfprf2 (+ getprosol)**
pulse programme: **awclmlevbfprf1bfprf2**

Type **eda** (enter) and enter **SW(F2) in ppm**, note the spectral window in **Hz** that appears in the **SWH(F2)** box and copy and paste this value into the **SWH(F1)** box. Check **SWH(F2) = SWH(F1)** in Hz including all dp's.

Enter **O1 = O2 = CNST1 in Hz** *(in this order!)* where **O1** = the spectral window midpoint. Enter **CNST9** and **CNST21 in Hz** of the signals to be presaturated on F1 and F2 respectively.

TD(F2) = 2K, **TD(F1)** = 128-256 (your choice).
NS = 4, 8, 16 (multiPLes of 4 or 8 recommended), **DS** = 4 or 8
**D1** = presaturation time = 1.5, 2.0 sec or other time of your choice.
D9 = correlation time **= 80 msec** or other time of your choice (6-240 msec).

Type **ased** (enter) and review parameters used in the job including **CNST1**, **CNST9** and **CNST21**. Check the **PL9** and **PL21** presaturation power levels are OK and if required adjust them appropriately.

Set **receiver gain** using **RGA** *(important!).*

Process with: **SI(F2) = SI(F1) = 2K**
WDW(F1) = WDW(F2) = QSINE
SSB(F2) = SSB(F1) = 2
xfb, abs1, abs2 and optionally **syma**
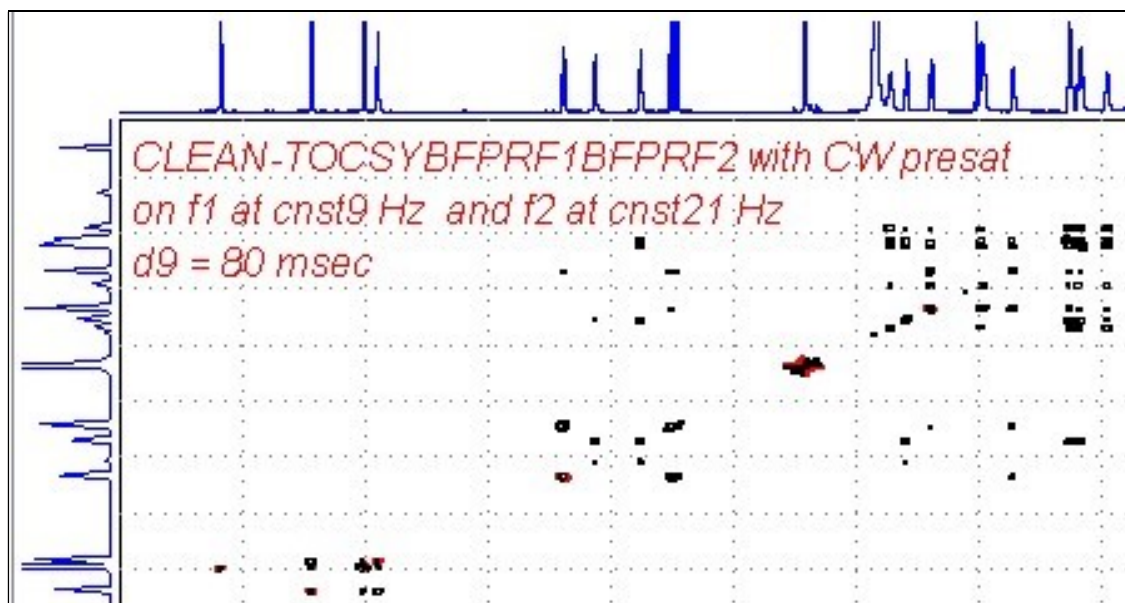
800 MHz Cleantocsybfprf1bfprf2 spectrum of quinine in D$_6$-DMSO with the HOD and DMSO lines at 3.38 ppm and 2.5 ppm suppressed. The spectrum is centered at 4.5 ppm (O1 = O2 = CNST 1 = 3600.14 Hz).

2.5 Dipsi2bfprf1bfprf2withCW presaturation on f1 at CNST9 Hz and f2 at CNST21 Hz

parameter set: **awdipsi2bfprf1bfprf2 (+ getprosol)**
pulse programme: **awdipsi2phbfprf1bfprf2**

Type **eda** (enter) and enter **SW(F2) in ppm**, note the spectral window in **Hz** that
appears in the **SWH(F2)** box and copy and paste this value into the **SWH(F1)** box.
Check **SWH(F2) = SWH(F1)** in Hz including all dp's.

Enter **O1 = O2 = CNST1 in Hz** *(in this order!)* where **O1** = the spectral window midpoint.
Enter **CNST9** and **CNST21 in Hz** of the signals to be presaturated on F1 and F2 respectively.

TD(F2) = 2K, **TD(F1)** = 128-256 (your choice).
NS = 2, 4, 8 (any number is OK), **DS** = 2, 4 or 8.
D1 = presaturation time = 1.5, 2.0 sec or other time of your choice.
D9 = correlation time = 80 msec or other time of your choice (6-240 msec).

Type **ased** (enter) and review parameters used in the job including **CNST1**, **CNST9** and
CNST21. Check the **PL9** and **PL21** presaturation power levels are OK and if required adjust
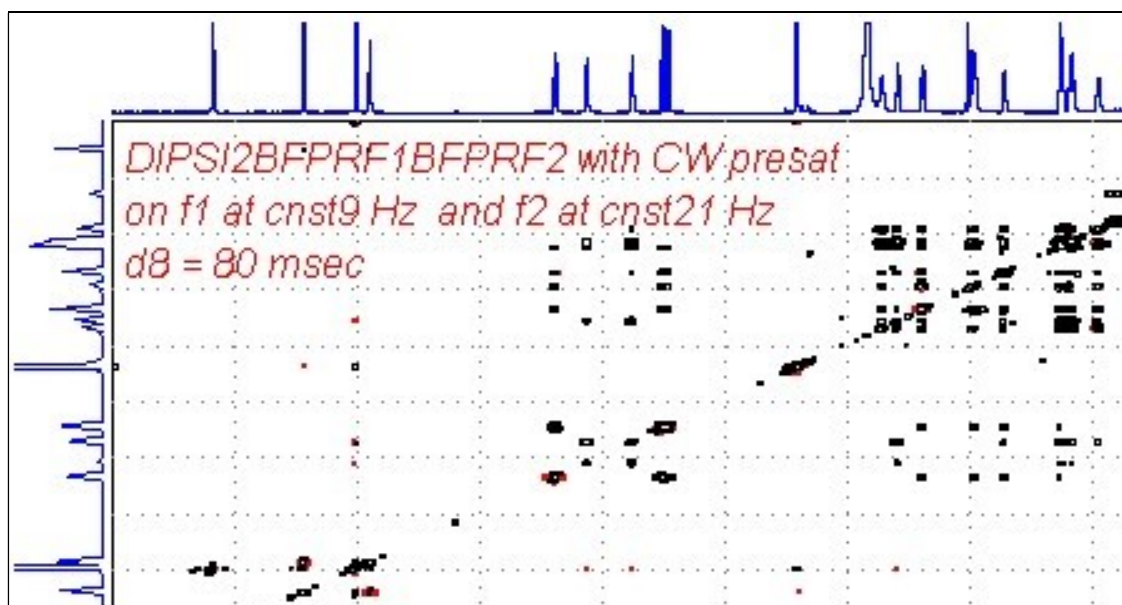them appropriately.

Set **receiver gain** using **RGA** *(important!)*.

Process with: **SI(F2) = SI(F1) = 2K**
WDW(F1) = WDW(F2) = QSINE
SSB(F2) = SSB(F1) = 2
xfb, abs1, abs2 and optionally **syma**

800 MHz Dipsi2bfprf1bfprf2 spectrum of quinine in D_6-DMSO with the HOD and DMSO lines
 at 3.38 ppm and 2.5 ppm suppressed. The spectrum is centered at 4.5 ppm (O1 = O2 = CNST 1 =
3600.14 Hz).

2.6 Noesybfprf1bfprf2 with CW presaturation on f1 at CNST9 Hz and f2 at CNST21 Hz

parameter set: **awnoesybfprf1bfprf2 (+ getprosol)**
pulse programme: **awnoesygpphbfprf1bfprf2**

Type **eda** (enter) and enter **SW(F2) in ppm**, note the spectral window in **Hz** that
appears in the **SWH(F2)** box and copy and paste this value into the **SWH(F1)** box.
Check **SWH(F2)** = **SWH(F1)** in Hz including all dp's.

Enter **O1 = O2 = CNST1 in Hz** *(in this order!)* where **O1** = the spectral window midpoint.
Enter **CNST9** and **CNST21 in Hz** of the signals to be presaturated on F1 and F2 respectively.

TD(F2) = 2K, **TD(F1)** = 128-256 (your choice).
NS = 4, 8 (multiPLes of 4 or 8 recommended), **DS** = 4 or 8.

D1 = presaturation time = 1.5, 2.0 sec or other time of your choice.
D8 = NOE mixing time = **0.5 sec** or other time of your choice.

Type **ased** (enter) and review parameters used in the job including **CNST1**, **CNST9** and
CNST21. Check the **PL9** and **PL21** presaturation power levels are OK and if required adjust
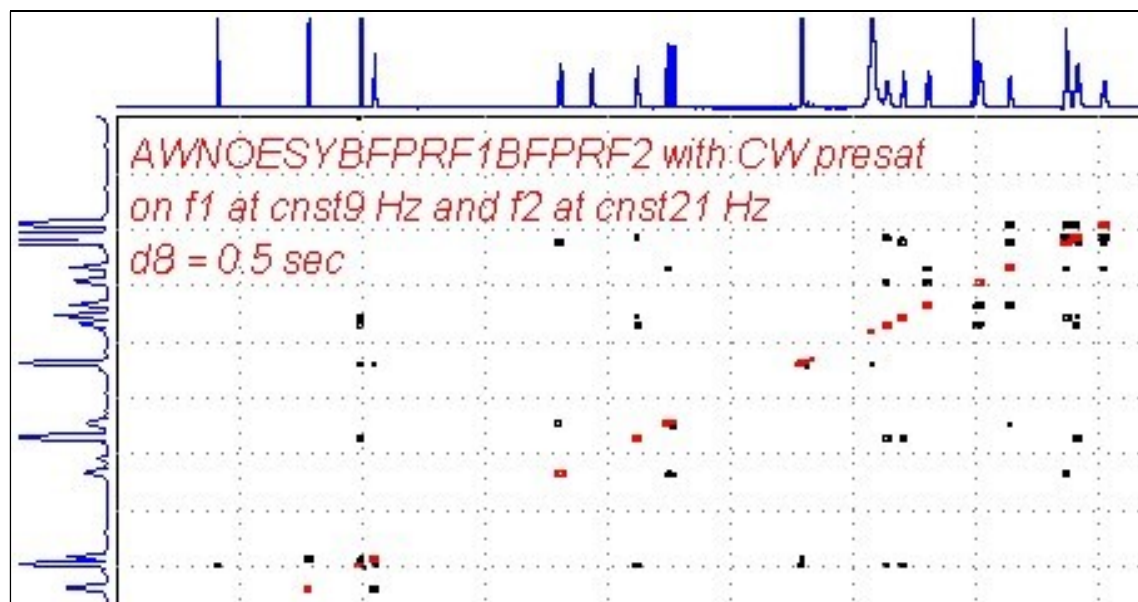them appropriately.

 Set **receiver gain** using **RGA** *(important!)*.

Process with: **SI(F2) = SI(F1) = 2K**
                WDW(F1) = WDW(F2) = QSINE
                SSB(F2) = SSB(F1) = 2
                xfb, abs1, abs2 and optionally **syma**

800 MHz Noesybfprf1bfprf2 spectrum of quinine in D_6-DMSO with the HOD and DMSO lines
at 3.38 ppm and 2.5 ppm suppressed. The spectrum is centered at 4.5 ppm (O1 = O2 = CNST 1 =
3600.14 Hz).

2.7 Roesybfprf1bfprf2 with CW presaturation on f1 at CNST9 Hz and f2 at CNST21 Hz

parameter set: **awroesybfprf1bfprf2 (+ getprosol)**
pulse programme: **awroesybfprfq1bfprf2** (with CW spinlock)

Type **eda** (enter) and enter **SW(F2) in ppm**, note the spectral window in **Hz** that
appears in the **SWH(F2)** box and copy and paste this value into the **SWH(F1)** box.
Check **SWH(F2)** = **SWH(F1)** in Hz including all dp's.

Enter **O1 = O2 = CNST1 in Hz** *(in this order!)* where **O1** = the spectral window midpoint.
Enter **CNST9** and **CNST21 in Hz** of the signals to be presaturated on F1 and F2 respectively.

TD(F2) = 2K, **TD(F1)** = 128-256 (your choice).
NS = 4, 8 (multiPLes of 4 or 8 recommended), **DS** = 4 or 8.
D1 = presaturation time = 1.5, 2.0 sec or other time of your choice.
P15 = spinlock time **= 250000 usec** (or 200000 usec) = 250 or 200 msec.

Type **ased** (enter) and review parameters used in the job including **CNST1**, **CNST9** and
CNST21. Check the **PL9** and **PL21** presaturation power levels are OK and if required adjust
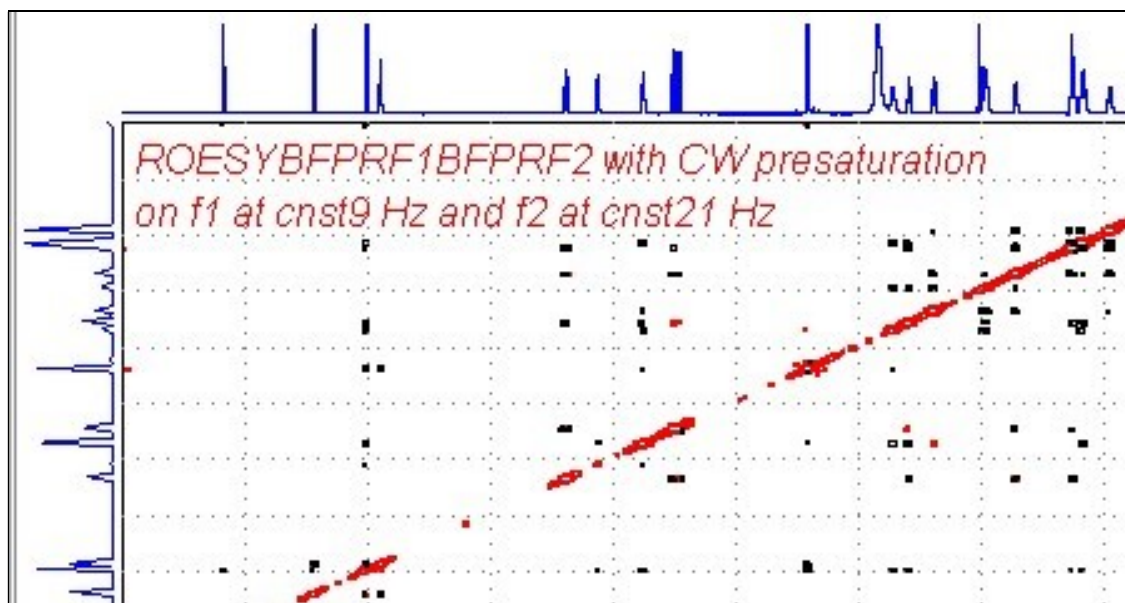them appropriately.

Set **receiver gain** using **RGA** *(important!)*.

Process with: **SI(F2) = SI(F1) = 2K**
 WDW(F1) = WDW(F2) = QSINE
 SSB(F2) = SSB(F1) = 2
 xfb, abs1, abs2 and optionally **syma**

800 MHz Roesybfprf1bfprf2 spectrum of quinine in D_6-DMSO with the HOD and DMSO lines
at 3.38 ppm and 2.5 ppm suppressed. The spectrum is centered at 4.5 ppm (O1 = O2 = CNST1 =
3600.14 Hz).

2.8 Roesy2bfprf1bfprf2 with CW presaturation on f1 at CNST9 Hz and f2 at CNST21 Hz

parameter set: **awroesy2bfprf1bfprf2 (+ getprosol)**
pulse programme: **awroesy2bfprf1bfprf2** (with pulsed spinlock)

Type **eda** (enter) and enter **SW(F2) in ppm**, note the spectral window in **Hz** that appears in the **SWH(F2)** box and copy and paste this value into the **SWH(F1)** box. Check **SWH(F2) = SWH(F1)** in Hz including all dp's.

Enter **O1 = O2 = CNST1 in Hz** *(in this order!)* where **O1** = the spectral window midpoint. Enter **CNST9** and **CNST21 in Hz** of the signals to be presaturated on F1 and F2 respectively.

TD(F2) = 2K, **TD(F1)** = 128-256 (your choice)
NS = 4, 8 (multiPLes of 4 or 8 recommended), **DS** = 4 or 8
D1 = presaturation time = 1.5, 2.0 sec or other time of your choice.
P15 = spinlock time = **250000 usec** (or **200000 usec**) = 250 or 200 msec.

Type **ased** (enter) and review parameters used in the job including **CNST1**, **CNST9** and **CNST21**. Check the **PL9** and **PL21** presaturation power levels are OK and if required adjust them appropriately.
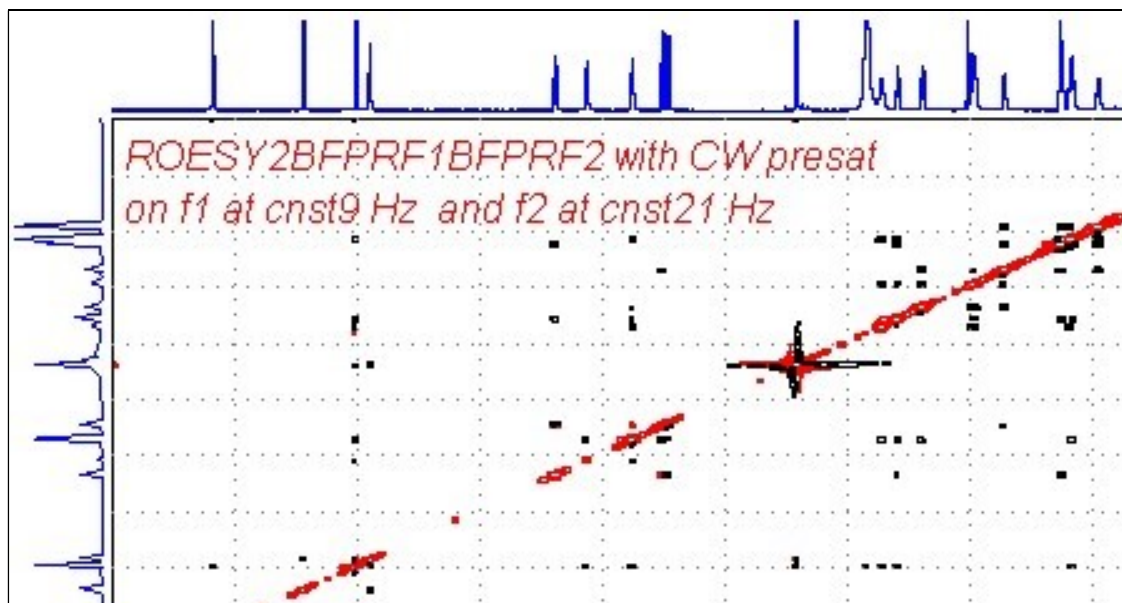
Set **receiver gain** using **RGA** *(important!).*

Process with: **SI(F2) = SI(F1) = 2K**
                WDW(F1) = WDW(F2) = QSINE
                SSB(F2) = SSB(F1) = 2
                xfb, abs1, abs2 and optionally **syma**

800 MHz Roesy2bfprf1bfprf2 spectrum of quinine in D_6-DMSO with the HOD and DMSO lines at 3.38 ppm and 2.5 ppm suppressed. The spectrum is centered at 4.5 ppm (O1 = O2 = CNST 1 = 3600.14 Hz).

Appendix

SamPLe aw coded bfprf1bfprf2 pulse programme with required "bf" lines and comments in red

```
;awcosyprfqf1prfqf2

;with fq lines in the pp for offset CW presaturation and
;  centered spectral window midpoint frequencies in Hz

;avance-version (12/01/11)
;2D homonuclear shift correlation
;with presaturation on f1 and f2 during relaxation delay
;using gradient pulses for selection
;
;$CLASS=HighRes
;$DIM=2D
;$TYPE=
;$SUBTYPE=
;$COMMENT=

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

"d11=30m"
"d12=20u"

"in0=inf1"
"d0=3u"

1 ze
2 d11

3 d12 PL9:f1 PL21:f2
  3m fq=CNST9 (bf hz):f1 fq=CNST21 (bf hz):f2
  d1 cw:f1 ph29 cw:f2 ph29
  4u do:f1 do:f2

  3m fq=CNST1 (bf hz):f1 fq=CNST1 (bf hz):f2

  d12 PL1:f1
  p1 ph1
  d0
  50u UNBLKGRAD
  p16:gp1
  d16
  p0 ph2
  4u
  p16:gp1
  d16
  4u BLKGRAD
  go=2 ph31
  d11 mc #0 to 2 F1QF(caldel(d0, +in0))
exit
```

```
ph1=0 2
ph2=0 0 2 2
ph29=0
ph31=0 2


;PL1 : f1 channel - power level for pulse (default)
;PL9 : f1 channel - power level for presaturation
;PL21 : f2 channel - power level for presaturation
;CNST9 : f1 presat freqency (Hz)
;CNST21 : f2 presat frequency (Hz)
;CNST1 : spectral window midpoint (Hz)
;p0 : f1 channel -  20 to 90 degree high power pulse
;p1 : f1 channel -  90 degree high power pulse
;p16: homospoil/gradient pulse
;d0 : incremented delay (2D)                     [3 usec]
;d1 : relaxation delay; 1-5 * T1
;d11: delay for disk I/O                         [30 msec]
;d12: delay for power switching                  [20 usec]
;d16: delay for homospoil/gradient recovery
;inf1: 1/SW = 2 * DW
;in0: 1/(1 * SW) = 2 * DW
;nd0: 1
;ns: 1 * n
;ds: 4, 8 or 16
;td1: number of experiments
;FnMODE: QF

;use gradient ratio:  gp 1
;                        10

;for z-only gradients:
;gpz1: 10%

;use gradient files:
;gpnam1: SMSQ10.100



;$Id: cosygpprqf,v 1.5 2012/01/31 17:49:22 ber Exp $
```