KJM 9250

AVII-600 MHz HSQC, HMBC and H2BC Experiments

Version 7.3

Topspin 3.2 Windows 7 AVII600

AVII-600 MHz HSQC, HMBC and H2BC Experiments

1.0 Introduction

^{1}H detected aw coded **HSQC, HMB**C and **H2BC** parameter sets are set up with 1K or 2K acquired ^{1}H points and 128 to 256 ^{13}C increments.

[1]H and ^{13}C spectral windows and their mid points should be determined before setting up **HSQC, HMBC** or **H2BC** experiments.

1.1 Processing

HSQC experiments are phase sensitive experiments. These spectra should be phased **before** using the **abs1** and **abs2** commands. Low level ^2J correlations may occasionally be observed in **HSQC** spectra.

HMBC experiments are absolute value experiments. Phasing is not required

H2BC experiments are acquired in phase sensitive mode and transformed to afford an absolute value spectrum using the **xfb** and **xf2m** commands.

2.0 Experiments and Parameter Sets

The following ^{1}H detected HSQC, HMBC and H2BC experiments and parameter sets have been set up on the 600 MHz MHz spectrometer.

2.1	**hsqcetgp**	not multiplicity edited, DEPT45 like
2.2	**hsqcedetgp**	multiplicity edited, DEPT135 like
2.3	**hsqcetdgpsisp2.3-135**	multiplicity edited, DEPT135 like
2.4	**hsqcedetgpsisp2.3-135pr**	with CW presaturation
2.5	**hsqcedefgpsisp2.3-135adia**	with adiabatic ^{13}C decoupling
2.6	**hsqc-tocsy**	not multiplicity edited, DEPT45 like
2.7	**hsqc-dipsi2.45**	DEPT45 like correlations
2.8	**hsqc-dipsi2.135**	DEPT135 like correlations
2.9	**hsqc-noesy**	
2.10	**hsqc-roesy**	CW spin locked
2.11	**hsqc-roesy2**	pulsed spin locked
2.12	**hmbc**	with ^nJ selection
2.13	**hmbcpr**	with ^nJ selection and CW presaturation
2.14	**hmbclp2**	with $^1J_{min/max}$ filter and ^nJ selection
2.15	**hmbc-cigar**	with ^{13}C decoupling
2.16	**h2bc**	for ^2J correlations

2.1 HSQCETGP

Parameter set: **awhsqcetgp (+ getprosol)**
Pulse programme: **hsqcetgp**

Type **eda** (enter) and enter **SW (^{1}H)** and **SW (^{13}C)** in ppm.
Enter **O1P = ^{1}H** spectral window midpoint in ppm.
Enter **O2P = ^{13}C** spectral window midpoint in ppm.
TD(F2) = 1K or 2K, **TD(F1)** = 128-256 (your choice).

NS = 4, 8, 16 (multiple of 4 or 8 recommended), **DS** = 8 or 16.
D1 = repetition delay = **1.5 sec** or other time of your choice.
CNST2 = ^1J coupling constant = **145 Hz** or other value of your choice (eg: 125-160 Hz).

Type **ased** (enter) and review parameters used in the job.
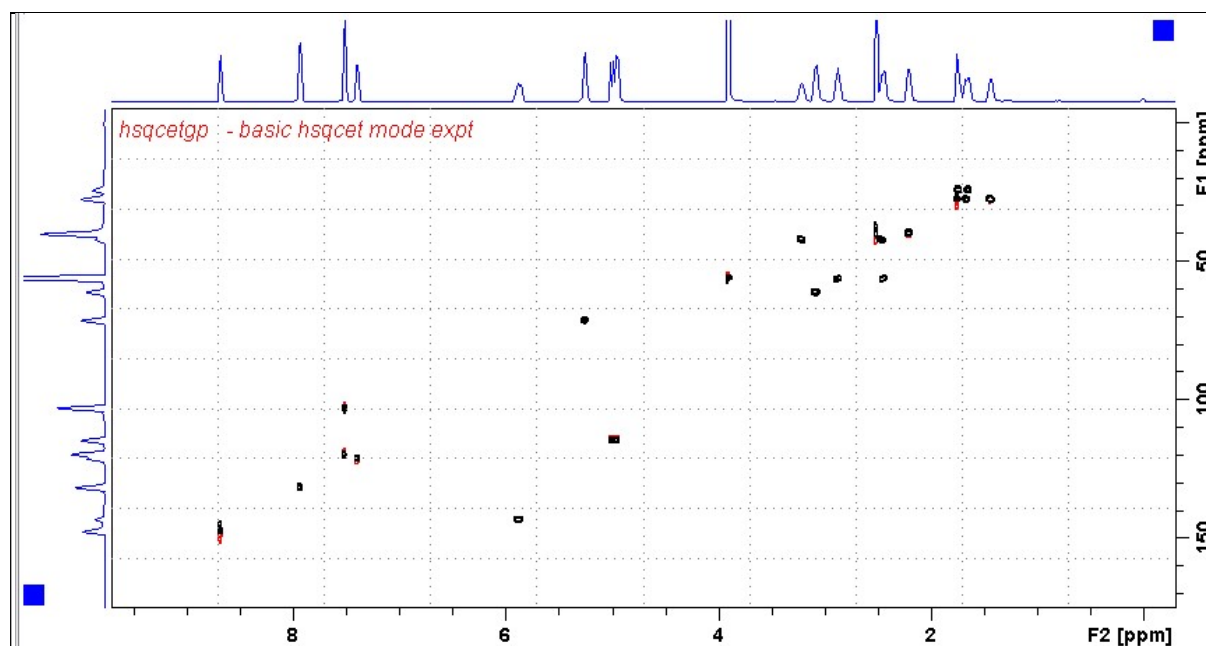Check gradients are OK.

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

Process with: **SI(F2) = SI(F1) = 1K or 2K**
WDW(F1) = WDW(F2) = QSINE
SSB(F2) = SSB(F1) = 2
xfb, abs1 and abs2

AVII-600 **HSQCETGP** spectrum (not edited) of quinine in D$_6$-DMSO.

2.2 HSQCEDETGP

Parameter set: **awhsqcedetgp-135 (+ getprosol)**
Pulse programme:**awhsqcedetgp-135**
        d21 is automatically calculated from **cnst2**

Type **eda** (enter) and enter **SW (^{1}H)** and **SW (^{13}C)** in ppm.
Enter **O1P = ^{1}H** spectral window midpoint in ppm.
Enter **O2P = ^{13}C** spectral window midpoint in ppm.
TD(F2) = 1K or 2K,v**TD(F1)** = 128-256 (your choice).

NS = 4, 8, 16 (multiple of 4 or 8 recommended), **DS** = 8 or 16.
D1 = repetition delay = **1.5 sec** or other time of your choice.
CNST2 = ^1J coupling constant = **145 Hz** or other value of your choice (eg: 125-160 Hz).

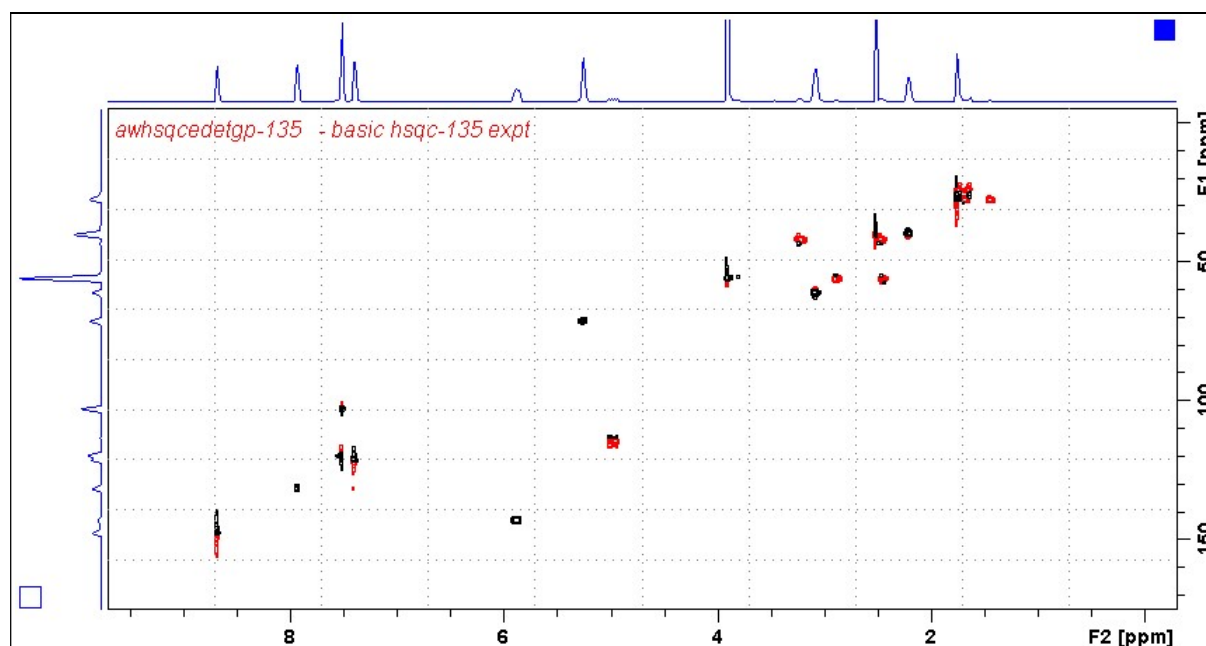Type **ased** (enter) and review parameters used in the job.
Check gradients are OK.

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

Process with: **SI(F2) = SI(F1) = 1K or 2K**
            WDW(F1) = WDW(F2) = QSINE
            SSB(F2) = SSB(F1) = 2
            xfb, abs1 and abs2

AVII-600 **HSQCEDETGP** spectrum (DEPT135-like) of quinine in D_6-DMSO plotted
with CH and CH$_3$ positive (black) and CH$_2$ negative (red).
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2.3 HSQCEDETGPSISP2.3-135

parameter set: **awhsqcedetgpsisp2.3-135 (+ getprosol)**
        fast load: **awhsqc135 (+ getprosol)**
pulse programme: **awhsqcedetgpsisp2.3-135**
        d21 and **d24** are automatically calculated from **cnst2**

Type **eda** (enter) and enter **SW (^{1}H)** and **SW (^{13}C)** in ppm.
Enter **O1P = ^{1}H** spectral window midpoint in ppm.
Enter **O2P = ^{13}C** spectral window midpoint in ppm.
TD(F2) = 1K or 2K, **TD(F1)** = 128-256 (your choice).

NS = 4, 8, 16 (multiple of 4 or 8 recommended), **DS** = 8 or 16.
D1 = repetition delay = **1.5 sec** or other time of your choice.
CNST2 = ^1J coupling constant = **145 Hz** or other value of your choice (eg: 125-160 Hz).

Type **ased** (enter) and review parameters used in the job.
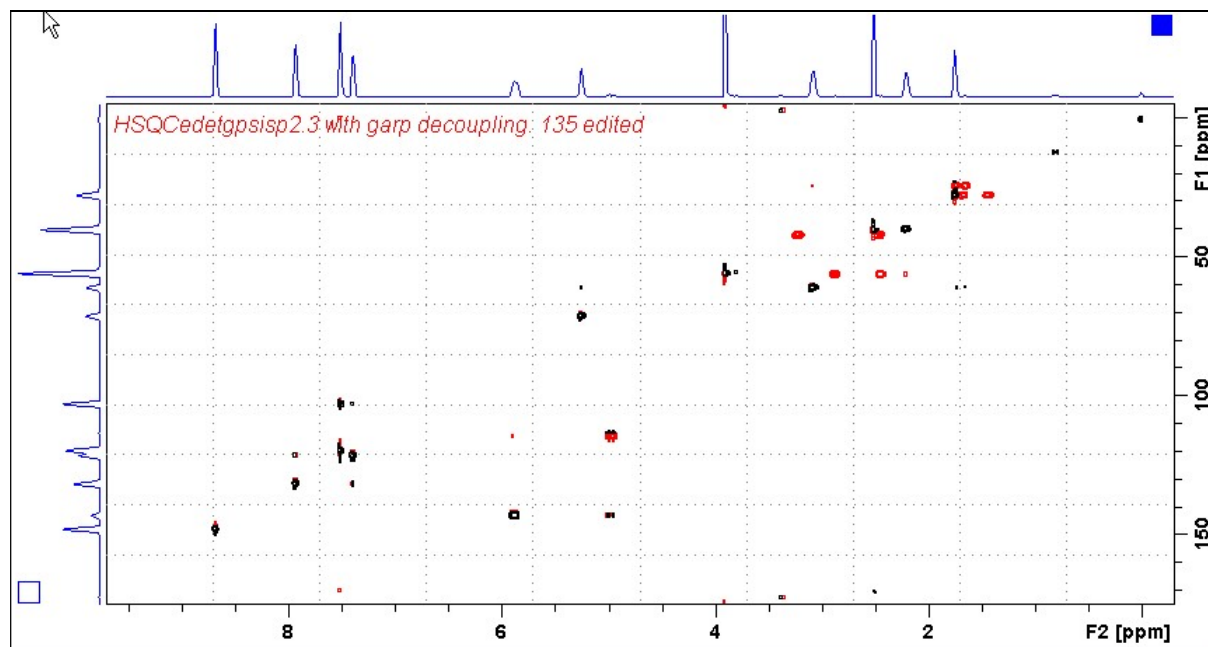Check gradients and shaped pulses are OK.

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

Process with: **SI(F2) = SI(F1) = 1K or 2K**
            WDW(F1) = WDW(F2) = QSINE
            SSB(F2) = SSB(F1) = 2
            xfb, abs1 and abs2

AVII-600 **HSQCEDETGPSISP2.3-135** spectrum of quinine in D6-DMSO plotted with CH and CH$_3$ correlations positive (black) and CH$_2$ correlations negative (red).

2.4 HSQCEDETGPSISP2.3-135PR

Parameter set: **awhsqcedetgpsisp2.3-135pr (+ getprosol)**
      Fast load: **awhsqc135pr (+ getprosol)**
Pulse programme: **awhsqcedetgpsisp2.3-135pr**
      d21 and **d24** are automatically calculated from **cnst2**

Type **eda** (enter) and enter **SW (^{1}H)** and **SW (^{13}C)** in ppm.
Enter **O1 = ^{1}H** frequency to be presaturated during **D1.**
      Check **SW** = spectral window is wide enough.
Enter **O2P = ^{13}C** spectral window midpoint in ppm.
TD(F2) = 1K or 2K, **TD(F1)** = 128-256 (your choice).

NS = 4, 8, 16 (multiple of 4 or 8 recommended), **DS** = 8 or 16.
D1 = repetition delay = **1.5 sec** or other time of your choice.
PL9 = CW presaturation power level.
CNST2 = ^1J coupling constant = **145 Hz** or other value of your choice (eg: 125-160 Hz).

Type **ased** (enter) and review parameters used in the job.
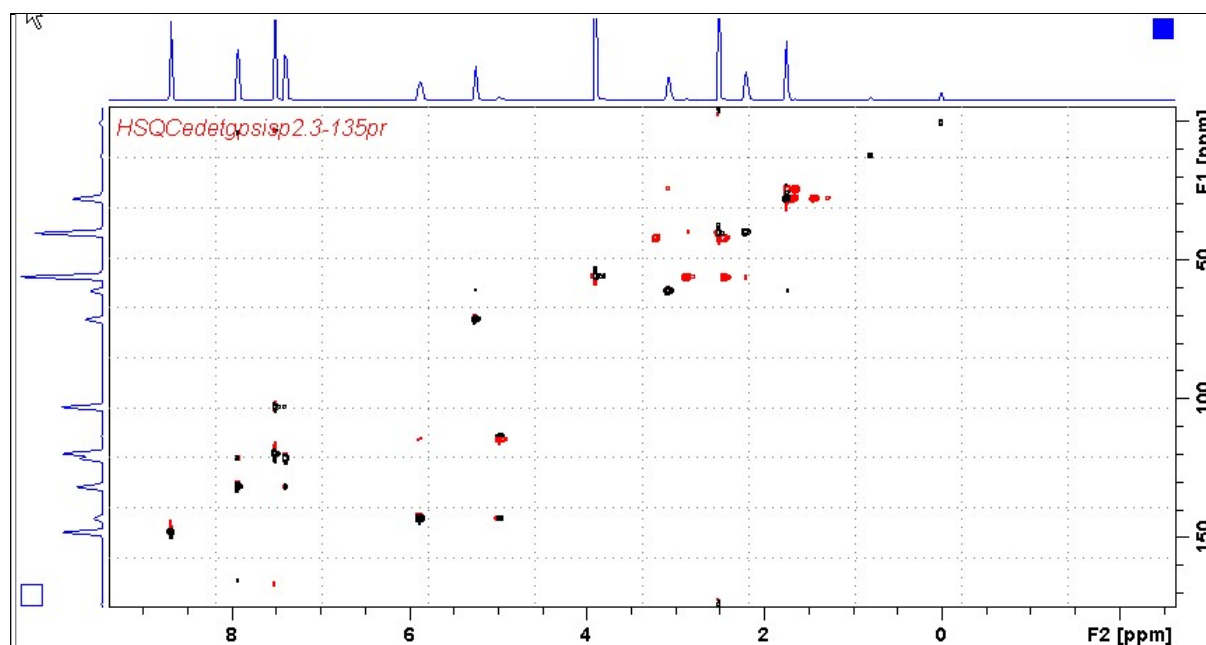Check gradients and shaped pulses are OK.

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

Process with: **SI(F2) = SI(F1) = 1K** or **2K**
                 WDW(F1) = WDW(F2) = QSINE
                 SSB(F2) = SSB(F1) = 2
                 xfb, abs1 and abs2

AVII-600 **HSQCEDETGPSISP2.3-135PR** spectrum of quinine in D$_6$-DMSO with CW presaturation of the HOD line at 3.37 ppm. The spectrum is plotted with positive CH and CH$_3$ correlations (black) and negative CH$_2$ correlations (red).

2.5 HSQCEDETGPSISP2.3-135ADIA

Parameter set: **awhsqcedetgpsisp2.3-135adia**
Pulse programme**: awhsqcedetgpsisp2.3-135**
   d21 and **d24** are automatically calculated from **cnst2**

Type **eda** (enter) and enter **SW (^{1}H)** and **SW(^{13}C)** in ppm
Enter**O1P = ^{1}H** spectral window midpoint in ppm.
Enter **O2P = ^{13}C** spectral window midpoint in ppm.
TD(F2) = 1K or 2K, **TD(F1)** = 128 - 256 (your choice).

NS = 4, 8, 16 (multiple of 4 or 8 recommended), **DS** = 8 or 16.
D1 = repetition delay = **1.5 sec** or other time of your choice.
CNST2 = ^1J coupling constant = **145 Hz** or other value of your choice.

Type **ased** (enter) and review parameters used in the job.
Check **CPDPRG2 = bi_p5m4sp_4sp.2** for adiabatic ^{13}C decoupling.
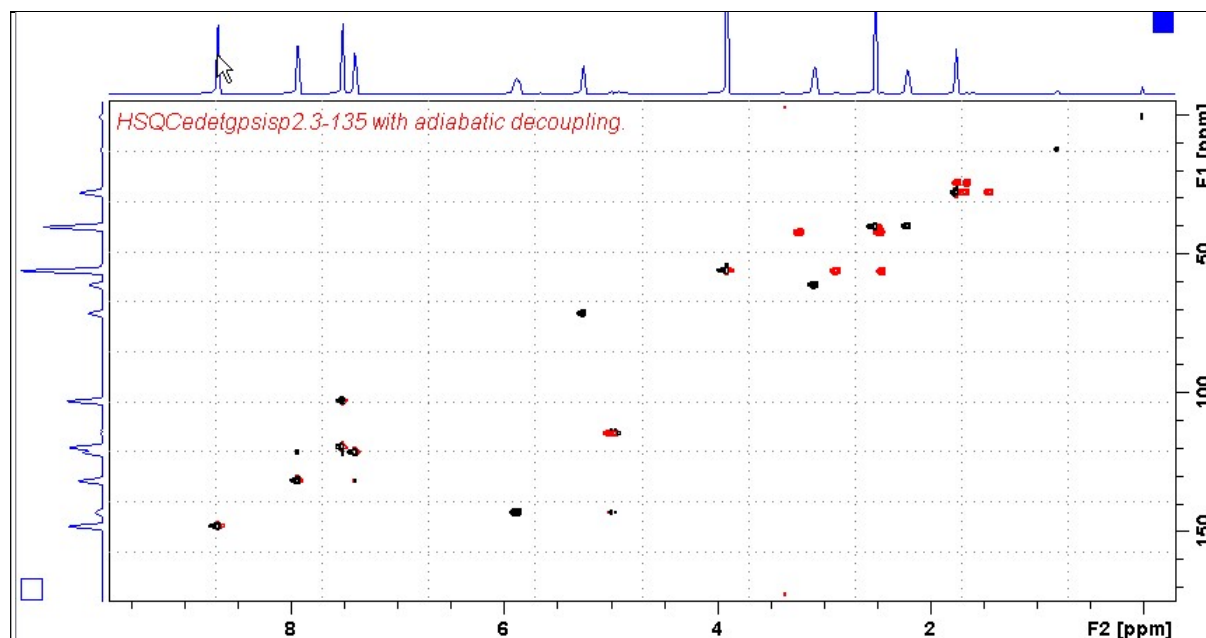Check gradients and shaped pulses are OK.

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

Process with: **SI(F2) = SI(F1) = 1K or 2K**
               WDW(F1) = WDW(F2) = QSINE
               SSB(F2) = SSB(F1) = 2
               xfb, abs1 and abs2

AVII-600 **HSQCEDETGPSISP2.3-135ADIA** spectrum of quinine in D$_6$-DMSO with adiabatic^{13}C decoupling. The spectrum is plotted with positive CH and CH$_3$ correlations (black) and negative CH$_2$ correlations (red).

2.6 HSQC-TOCSY

Parameter set: **awhsqc-tocsy (+ getprosol)**
Pulse programme: **hsqcetgpml**

Type **eda** (enter) and enter **SW (^{1}H)** and **SW (^{13}C)** in ppm.
Enter **O1P = ^{1}H** spectral window midpoint in ppm.
Enter **O2P = ^{13}C** spectral window midpoint in ppm.
TD(F2) = 1K or 2K, **TD(F1)** = 128-256 (your choice).

NS = 4, 8, 16 (multiple of 4 or 8 recommended), **DS** = 8 or 16.
D1 = repetition delay = **1.5 sec** or other time of your choice.
D9 = correlation time = **80 msec** or other value of your choice (6-240 msec).
CNST2 = ^1J coupling constant = **145 Hz** or other value of your choice (eg: 125-160 Hz).

Type **ased** (enter) and review parameters used in the job.
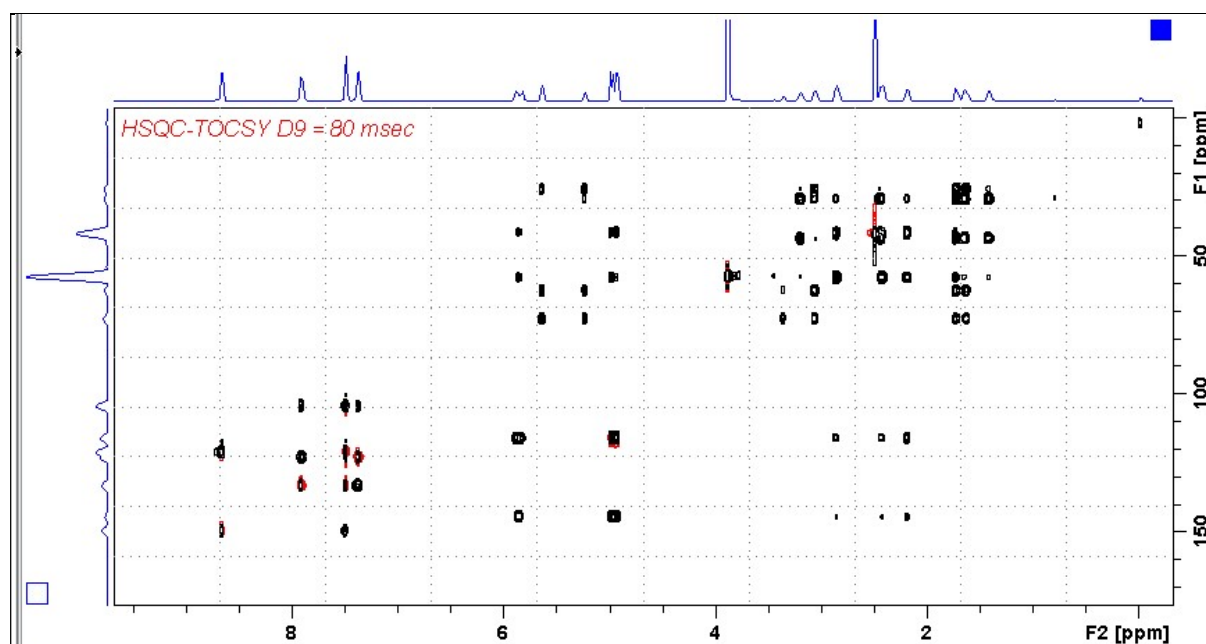Check gradients are OK.

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

Process with: **SI(F2) = SI(F1) = 1K or 2K**
    WDW(F1) = WDW(F2) = QSINE
    SSB(F2) = SSB(F1) = 2
    xfb, abs1 and abs2

AVII-600 **HSQC-TOCSY** spectrum of quinine in D6-DMSO. HSQC and correlated
TOCSY peaks are positively phased.

2.7 HSQC-DIPSI2.45

Parameter set: **awhsqc-dipsi2.45 (+ getprosol)**
Pulse programme: **awhsqcdipsi2.45**
        With auto calculation of **d24** from **cnst2**

Type **eda** (enter) and enter **SW (^{1}H)** and **SW (^{13}C)** in ppm.
Enter **O1P** = ^{1}H spectral window midpoint in ppm.
Enter **O2P** = ^{13}C spectral window midpoint in ppm.
TD(F2) = 1K or 2K, **TD(F1)** = 128-256 (your choice).

NS = 4, 8, 16 (multiple of 4 or 8 recommended), **DS** = 8 or 16.
D1 = repetition delay = **1.5 sec** or other time of your choice.
D9 = correlation time = **80 msec** or other value of your choice (6-240 msec).
CNST2 = ^1J coupling constant = **145 Hz** or other value of your choice (eg: 125-160 Hz).

Type **ased** (enter) and review parameters used in the job.
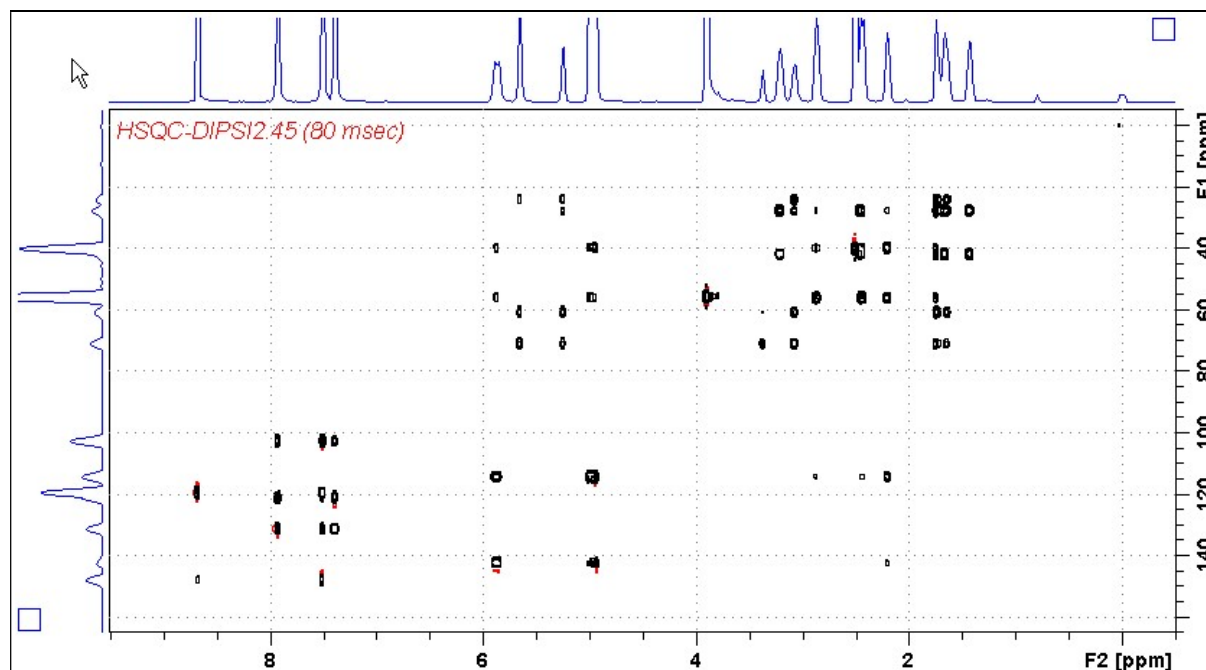Check gradients and shaped pulses are OK.

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

Process with: **SI(F2) = SI(F1) = 1K or 2K**
                WDW(F1) = WDW(F2) = QSINE
                SSB(F2) = SSB(F1) = 2
                xfb, abs1 and abs2

AVII-600 **HSQC-DIPSI2.45** spectrum of quinine in D$_6$-DMSO.

2.8 HSQC-DIPSI2.135

Parameter set: **awhsqc-dipsi2.135 (+ getprosol)**
Pulse programme**: awhsqcdipsi2.135**
      With auto calculation of **d24** from **cnst2**

Type **eda** (enter) and enter **SW (^{1}H)** and **SW (^{13}C)** in ppm.
Enter **O1P** = ^{1}H spectral window midpoint in ppm.
Enter **O2P** = ^{13}C spectral window midpoint in ppm.
TD(F2) = 1K or 2K, **TD(F1)** = 128-256 (your choice).

NS = 4, 8, 16 (multiple of 4 or 8 recommended), **DS** = 8 or 16.
D1 = repetition delay = **1.5 sec** or other time of your choice.
D9 = correlation time = **80 msec** or other value of your choice (6-240 msec).
CNST2 = ^1J coupling constant = **145 Hz** or other value of your choice (eg: 122-160 Hz).

Type **ased** (enter) and review parameters used in the job.
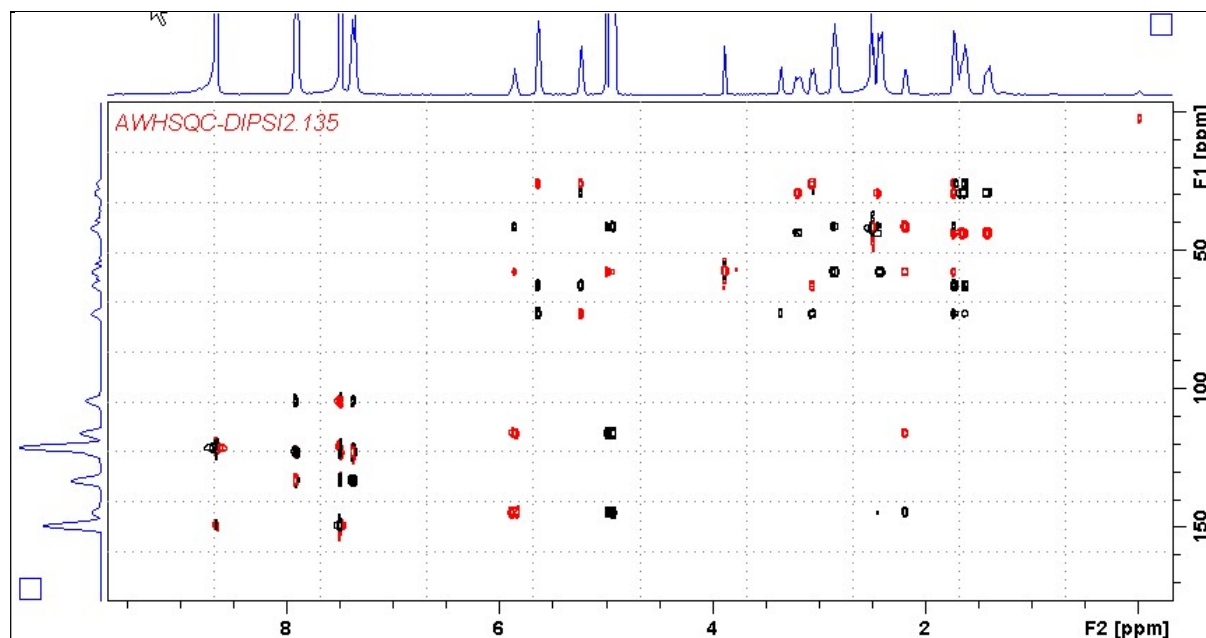Check gradients and shaped pulses are OK.

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

Process with: **SI(F2) = SI(F1) = 1K or 2K**
              WDW(F1) = WDW(F2) = QSINE
              SSB(F2) = SSB(F1) = 2
              xfb, abs1 and abs2

AVII-600 **HSQC-DIPSI2.135** spectrum of quinine in D$_6$-DMSO.

2.9 HSQC-NOESY

Parameter set: **awhsqc-noesy (+ getprosol)**
Pulse programme: **hsqcetgpnosp**

Type **eda** (enter) and enter **SW (^{1}H)** and **SW (^{13}C)** in ppm.
Enter **O1P = ^{1}H** spectral window midpoint in ppm.
Enter **O2P = ^{13}C** spectral window midpoint in ppm.
TD(F2) = 1K or 2K, **TD(F1)** = 128-256 (your choice).

NS = 4, 8, 16 (multiple of 4 or 8 recommended), **DS** = 8 or 16,
D1 = repetition delay = **1.5 sec** or other time of your choice.
D8 = NOESY correlation time = **0.5 sec** or other value of your choice (0.3-0.8 sec),
CNST2 = ^1J coupling constant = **145 Hz** or other value of your choice (eg: 125-160 Hz).

Type **ased** (enter) and review parameters used in the job.
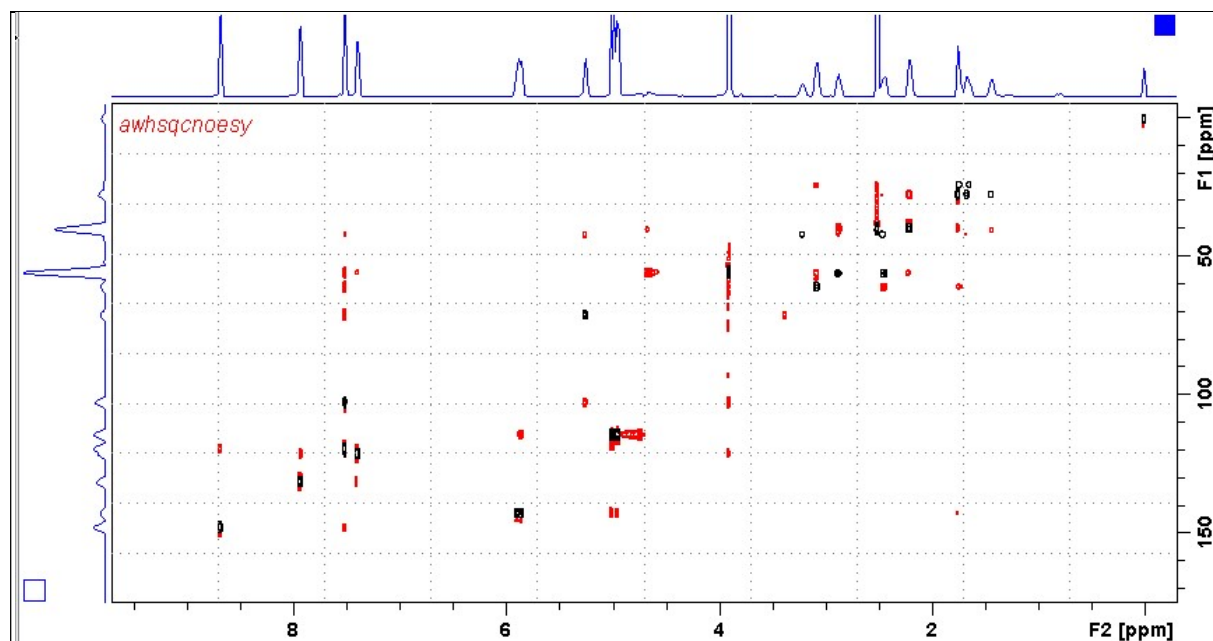Check gradients and the shaped pulse are OK.

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

Process with: **SI(F2) = SI(F1) = 1K or 2K**
WDW(F1) = WDW(F2) = QSINE
SSB(F2) = SSB(F1) = 2
xfb, abs1 and abs2

AVII-600 **NOESY** spectrum of quinine in D6-DMSO. HSQC signals (black) are positivelyy phased. Correlated NOESY signals (red) are negatively phased. HSQC signal levels were reduced relative to NOESY signals levels using the **edlev** command.

2.10 HSQC-ROESY

Parameter set: **awhsqc-roesy (+ getprosol)**
Pulse programme: **hsqcetgprosp** (with CW spin lock)

Type **eda** (enter) and enter **SW (^{1}H)** and **SW (^{13}C)** in ppm.
Enter **O1P** = ^{1}H spectral window midpoint in ppm.
Enter **O2P** = ^{13}C spectral window midpoint in ppm.
TD(F2) = 1K or 2K, **TD(F1)** = 128-256 (your choice).

NS = 4, 8, 16 (multiple of 4 or 8 recommended), **DS** = 8 or 16.
D1 = repetition delay = **1 .5 sec** or other time of your choice.
CNST2 = ^1J coupling constant = **145 Hz** or other value of your choice.
P15 = ROESY correlation time **200000** or **250000** usec (= 200 or 250 msec).

Type **ased** (enter) and review parameters used in the job.
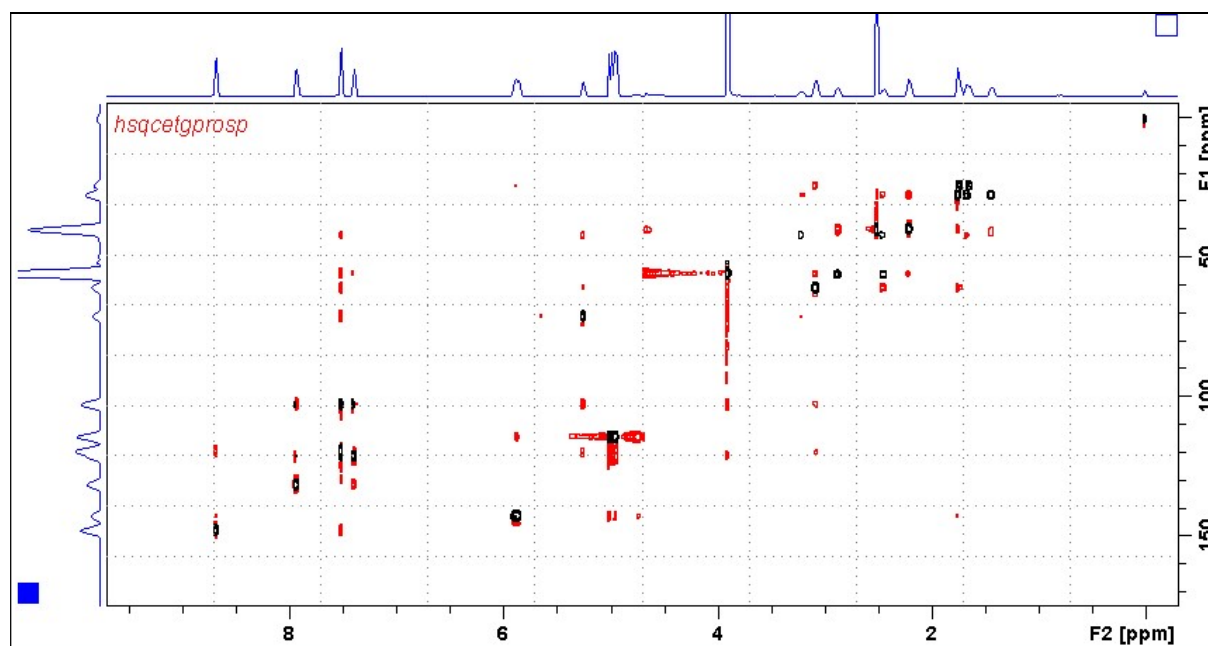Check gradients and shaped pulses are OK.

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

Process with: **SI(F2) = SI(F1) = 1K or 2K**
                  WDW(F1) = WDW(F2) = QSINE
                  SSB(F2) = SSB(F1) = 2
                  xfb, abs1 and abs2

AVII-600 **HSQC-ROESY** spectrum of quinine in D$_6$-DMSO. HSQC signals (black) are positively phased. Correlated ROESY signals (red) are negativeely phased. HSQC signal levels were reduced relative to ROESY signal levels using the **edlev** command.

2.11 HSQC-ROESY2

Parameter set: **awhsqc-roesy2 (+ getprosol)**
Pulse programme: **hsqcetgprosp.2** (with pulsed spin lock)

Type **eda** (enter) and enter **SW (^{1}H)** and **SW (^{13}C)** in ppm.
Enter **O1P** = ^{1}H spectral window midpoint in ppm.
Enter **O2P** = ^{13}C spectral window midpoint in ppm.
TD(F2) = 1K or 2K, **TD(F1)** = 128-256 (your choice).

NS = 4, 8, 16 (multiple of 4 or 8 recommended), **DS** = 8 or 16.
D1 = repetition delay = **1.5 sec** or other time of your choice.
CNST2 = ^1J coupling constant = **145 Hz** or other value of your choice
P15 = ROESY correlation time = **200000** or **250000** usec (= 200 or 250 msec).

Type **ased** (enter) and review parameters used in the job.
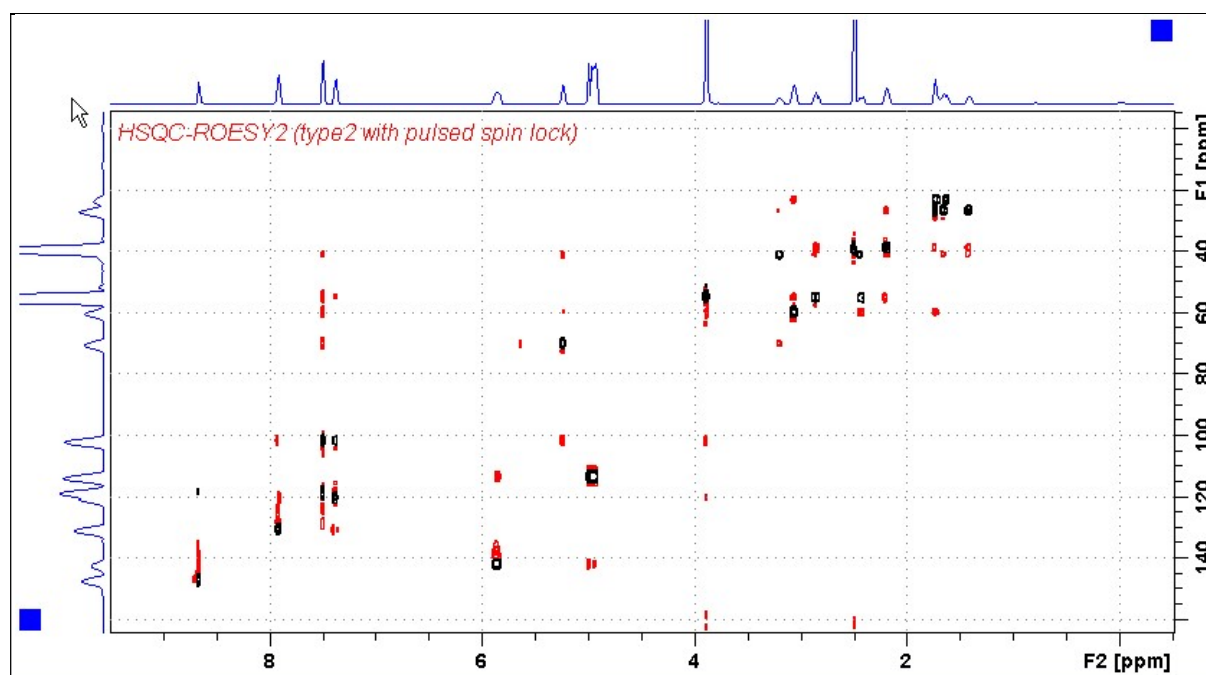Check gradients and shaped pulses are OK.

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

Process with: **SI(F2) = SI(F1) = 1K or 2K**
                WDW(F1) = WDW(F2) = QSINE
                SSB(F2) = SSB(F1) = 2
                xfb, abs1 and abs2

AVII-600 **HSQC-ROESY2** spectrum of quinine in D$_6$-DMSO. HSQC signals (black) are positively phased. Correlated ROESY2 signals (red) are negatively phased. HSQC signal levels were reduced relative to ROESY2 signal levels using the **edlev** command.

2.12 HMBC

Parameter set: **awhmbc (+ getprosol)**
Pulse programme: **hmbcgplpndqf**

Type **eda** (enter) and enter **SW (^{1}H)** and **SW (^{13}C)** in ppm.
Enter **O1P** = ^{1}H spectral window midpoint in ppm.
Enter **O2P** = ^{13}C spectral window midpoint in ppm.
TD(F2) = 1K or 2K, **TD(F1)** = 128-256 (your choice).

NS = 4, 8, 16 (multiple of 4 or 8 recommended), **DS** = 8 or 16.
D1 = repetition delay = **1.5 sec** or other time of your choice.
CNST2 = ^1J coupling constant = **145 Hz** or other value of your choice.
CNST13 = ^nJ selection filter = **8 Hz** or other value of your choice.

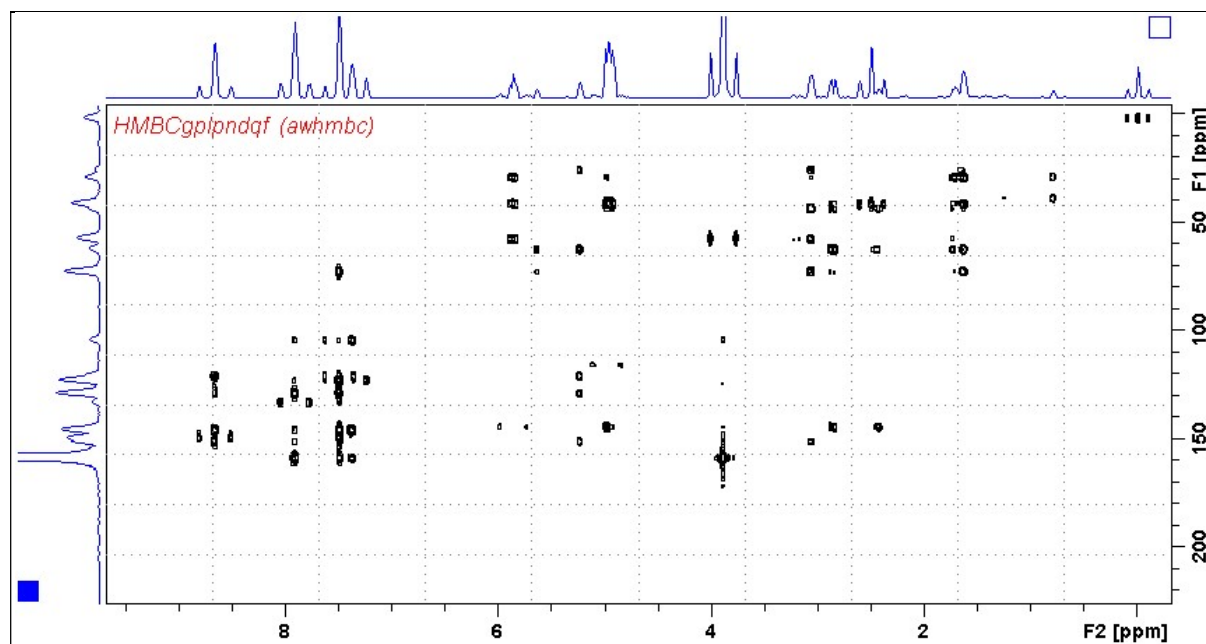Type **ased** (enter) and review parameters used in the job.
Check gradients are OK.

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

Process with: **SI(F2) = SI(F1) = 1K** or **2K**
                WDW(F1) = WDW(F2) = SINE
                SSB(F2) = SSB(F1) = 0
                xfb, abs1 and abs2

AVII-600 **HMBC** spectrum of quinine in D$_6$-DMSO.

2.13 HMBCPR

Parameter set: **awhmbcpr (+ getprosol)**
Pulse programme: **awhmbcgplpndprqf**

Type **eda** (enter) and enter **SW (^{1}H)** and **SW (^{13}C)** in ppm.
Enter **O1 = ^{1}H** frequency to be presaturated during **D1.**
        Check **SW =** spectral window is wide enough.
Enter **O2P = ^{13}C** spectral window midpoint in ppm.
TD(F2) = 1K or 2K, **TD(F1)** = 128-256 (your choice).

NS = 4, 8, 16 (multiple of 4 or 8 recommended), **DS** = 8 or 16.
D1 = repetition delay = **1.5 sec** or other time of your choice.
PL9 = CW presaturation power applied during **D1**.
CNST2 = ^1J coupling constant = **145 Hz** or other value of your choice.
CNST13 = ^nJ selection filter = **8 Hz** or other value of your choice.

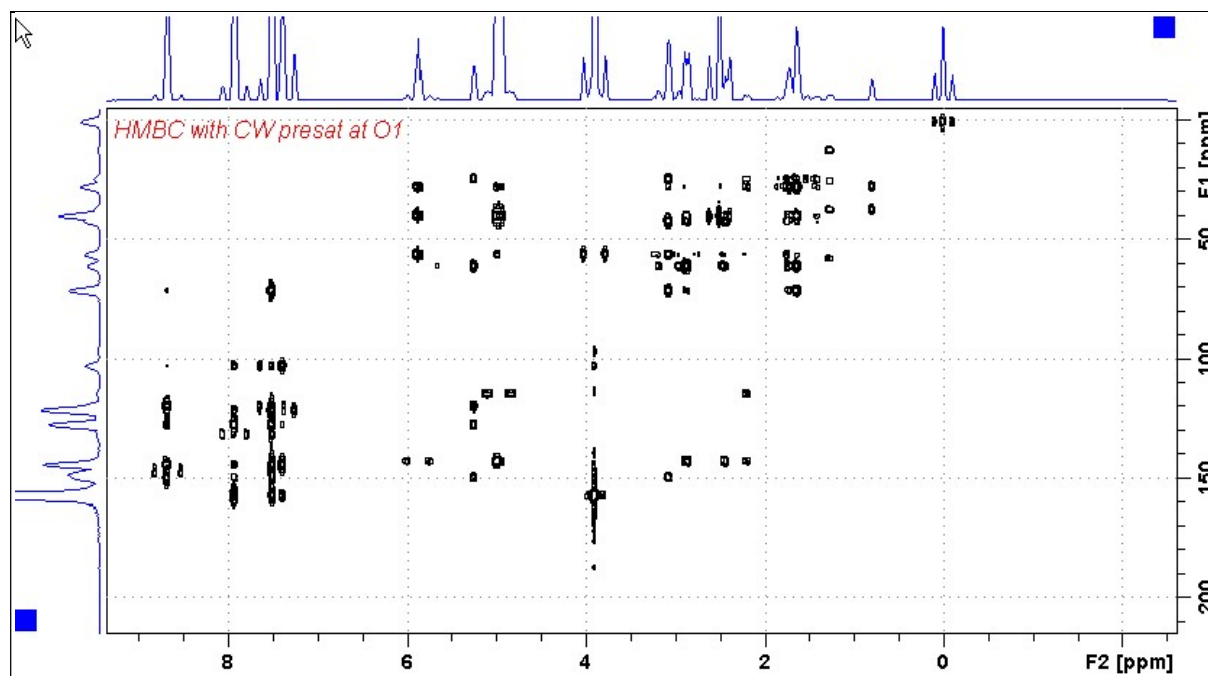Type **ased** (enter) and review parameters used in the job.
Check gradients are OK.

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

Process with: **SI(F2) = SI(F1) = 1K** or **2K**
                WDW(F1) = WDW(F2) = SINE
                SSB(F2) = SSB(F1) = 0
                xfb, abs1 and abs2

AVII-600 **HMBCPR** spectrum of quinine in D$_6$-DMSO with CW presaturation of the HOD signal at 3.37 ppm.

2.14 HMBCL2

Parameter set: **awhmbcl2 (+ getprosol)**
Pulse programme: **hmbcgpl2ndqf**

Type **eda** (enter) and enter **SW (^{1}H)** and **SW (^{13}C)** in ppm.
Enter **O1P** = ^{1}H spectral window midpoint in ppm.
Enter **O2P** = ^{13}C spectral window midpoint in ppm.
TD(F2) = 1K or 2K, **TD(F1)** = 128-256 (your choice).

NS = 4, 8, 16 (multiple of 4 or 8 recommended), **DS** = 8 or 16.
D1 = repetition delay = **1.5 sec** or other time of your choice.
CNST6 = min.^1J coupling constant = **125** Hz or other value of your choice.
CNST7 = max.^1J coupling constant = **165** Hz or other value of your choice.
CNST13 = ^nJ selection filter = **8Hz** or other value of your choice

Type **ased** (enter) and review parameters used in the job.
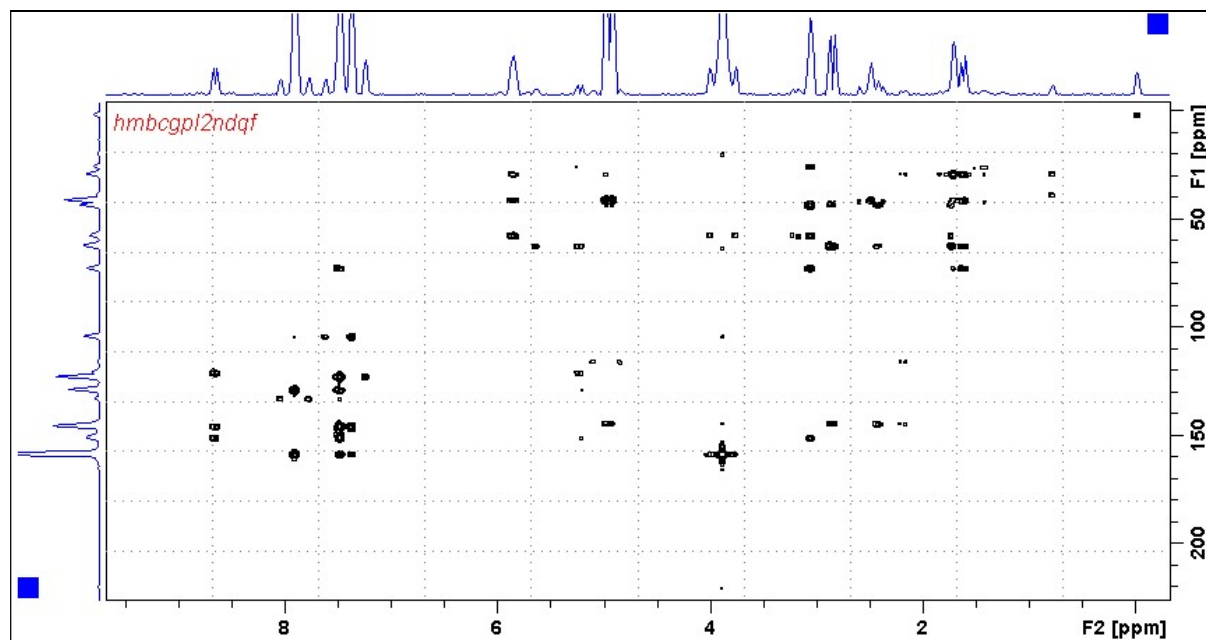Check gradients are OK.

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

Process with: **SI(F2) = SI(F1) = 1K or 2K**
                WDW(F1) = WDW(F2) = SINE
                SSB(F2) = SSB(F1) = 0
                xfb, abs1 and abs2

AVII-600 **HMBCL2** spectrum of quinine in D$_6$-DMSO with a two stage ^1J filter.

2.15 HMBC-CIGAR

Parameter set: **awhmbc-cigar (+ getprosol)**
Pulse programme: **hmbcacgplpqf**
        Spectrum is acquired with ^{13}C decoupling

Type **eda** (enter) and enter **SW (^{1}H)** and **SW (^{13}C)** in ppm.
Enter **O1P = ^{1}H** spectral window midpoint in ppm.
Enter **O2P = ^{13}C** spectral window midpoint in ppm.
TD(F2) = 1K or 2K, **TD(F1)** = 128-256 (your choice).

NS = 4, 8, 16 (multiple of 4 or 8 recommended), **DS** = 8 or 16.
D1 = repetition delay = **1.5 sec** or other time of your choice.
CNST6 = 125 Hz, **CNST7** = 160 Hz = min/max ^1J selection filter range.
CNST14 = 4 Hz, **CNST15** = 12 Hz min/max ^nJ selection filter range.
CNST16 = 1.0 = J scale factor.

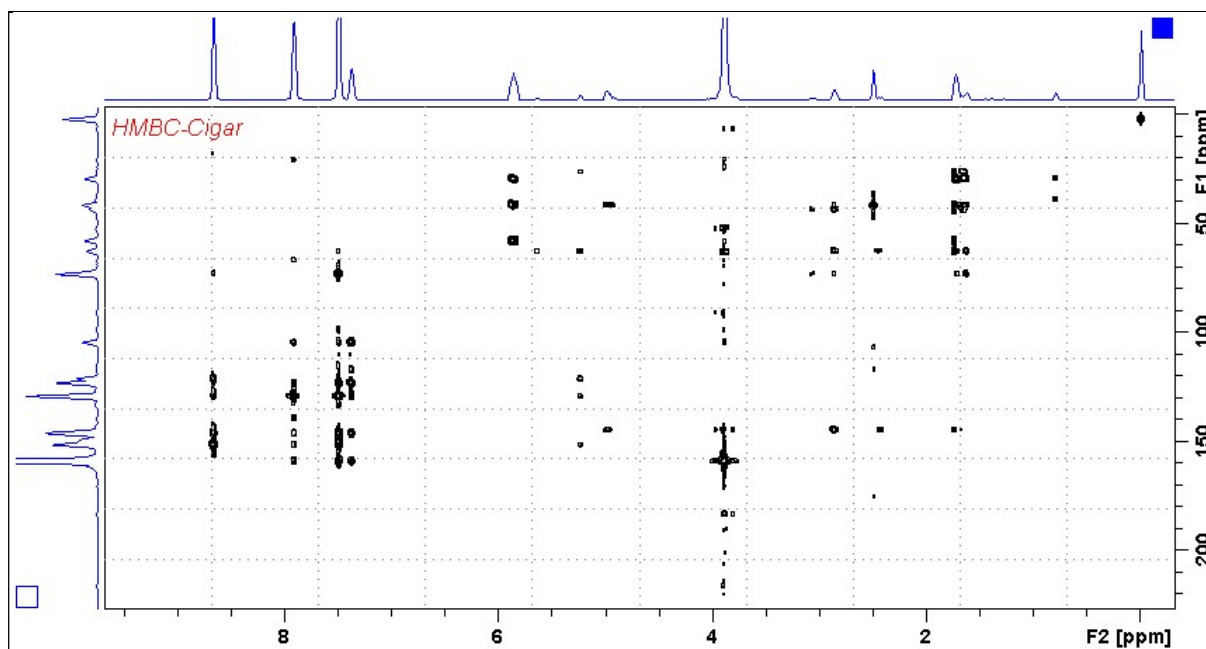Type **ased** (enter) and review parameters used in the job.
Check gradients are OK.

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

Process with: **SI(F2) = SI(F1) = 1K or 2K**
            WDW(F1) = WDW(F2) = SINE
            SSB(F2) = SSB(F1) = 0
            xfb, abs1 and abs2

AVII-600 **HMBC-CIGAR** spectrum of quinine in D$_6$-DMSO with ^{13}C decoupling.

2.16 H2BC spectrum (+ getprosol)

Parameter set: **awh2bc (+ getprosol)**
Pulse programme: **h2bcetgpl3**

Type **eda** (enter) and enter **SW (^{1}H)** and **SW (^{13}C)** in ppm.
Enter **O1P** = ^{1}H spectral window midpoint in ppm.
Enter **O2P** = ^{13}C spectral window midpoint in ppm.
TD(F2) = 1K or 2K, **TD(F1)** = 128-256 (your choice).

NS = 4, 8, 16 (multiple of 4 or 8 recommended), **DS** = 8 or 16.
D1 = repetition delay = **1.5 sec** or other time of your choice.
CNST6 = 125 Hz, **CNST7** = 165 Hz = min/max ^1J selection filter range.

Type **ased** (enter) and review parameters used in the job.
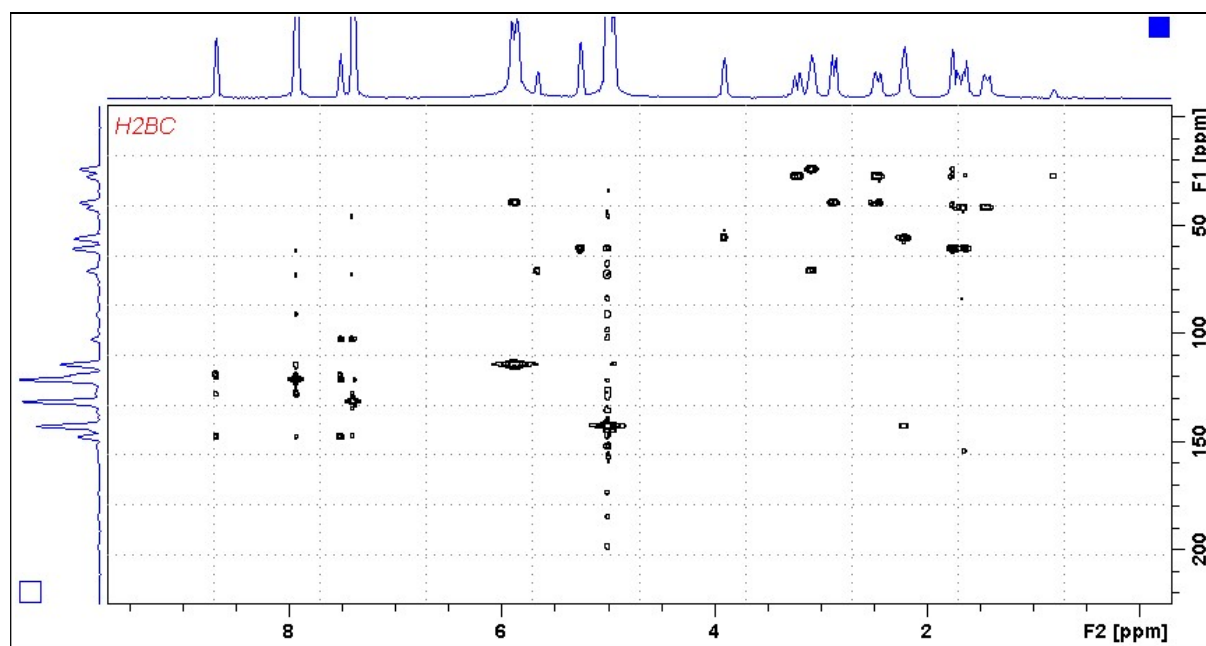Check gradients are OK.

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

Process with: **SI(F2) = SI(F1) = 1K or 2K**
WDW(F1) = WDW(F2) = SINE
SSB(F2) = SSB(F1) = 0
xfb, xf2m, abs1 and abs2

AVII-600 **H2BC** spectrum of quinine in D$_6$-DMSO.