KJM 5250 and KJM 9250

HSQC, HMBC, HMBCCT and H2BC NMR spectra with and without solvent peak pre saturation on the AVneo400 spectrometer.

Version 3.1

Topspin 4.3

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AVneo400 HSQC, HMBC, HMBCCT and H2BC Experiments

1.0 Introduction

^{1}H detected aw coded **HSQC, HMBC, HMBCT** and **H2BC** parameter sets are set up with 1K or 2K acquired ^{1}H points and 128 to 256 increments. ^{1}H and ^{13}C spectral windows and their midpoints should be determined before setting up **HSQC, HMBC, HMBCCT** or **H2BC** experiments.

The **getprosol** command will read in **prosol Table** saved pulse time, powers and types into an experiment. After using the **getprosol** command the **pulsecal** and **pulscal 13C** commands can be used to adjust the ^{1}H and ^{13}C 90 degree pulse times and **prosol Table** linked pulse powers to take account of solvent and/or buffer matrix effects which influence a samples 90 degree pulse time.

Presaturation (PR) can be used to suppress an HOD or solvent signal. The midpoint of spectral window should be set to the frequency in Hz of the HOD or solvent line to be suppressed. **HSQC135** and **HMBC** experiments with **Excitation Sculptured (ES)** peak suppression are described in a separate document.

Presaturation is applied at power level **PLW9(db)** on F1. The presaturation power level can be *decreased by adding 3-6 db* or *increased by subtracting 3-6 db* respectively from their prosol Table linked values. *6 db = a factor 2.*

1.1 Processing

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

HMBC experiments are absolute value experiments. Phasing is not required.

The **HMBCCT** and **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 have been set up on the **Neo -400** spectrometer.

2.1	**hsqcetgpsisp2.2-45**	not multiplicity edited, DEPT45 like
2.2	**hsqcetedgpsisp2.3-135**	multiplicity edited, DEPT135 like
2.3	**hsqcetedgpsisp2.3-135pr**	multiplicity edited, with presaturation
2.4	**hsqc-dipsi2**	DEPT135-like variant
2.5	**hsqc-noesy**	
2.6	**hsqc-roesy**	CW spin locked variant
2.7	**hsqc-roesy2**	pulsed spin locked variant
2.8	**hmbc**	with ^{n}J selection
2.9	**hmbcpr**	with ^{n}J selection and presaturation
2.10	**hmbcct**	constant time HMBC expt
2.11	**hmbcctpr**	constant time expt with presaturation

2.12 h2bc for ^{2}J correlations

2.1 HSQCEDETGPSISP2.2-45

Parameter set: **awhsqcetgpsisp2.2-45 (+ getprosol)**
Pulse programme: **awhsqcetgpsisp2.2-45**
d24 is automatically calculated from **cnst2**

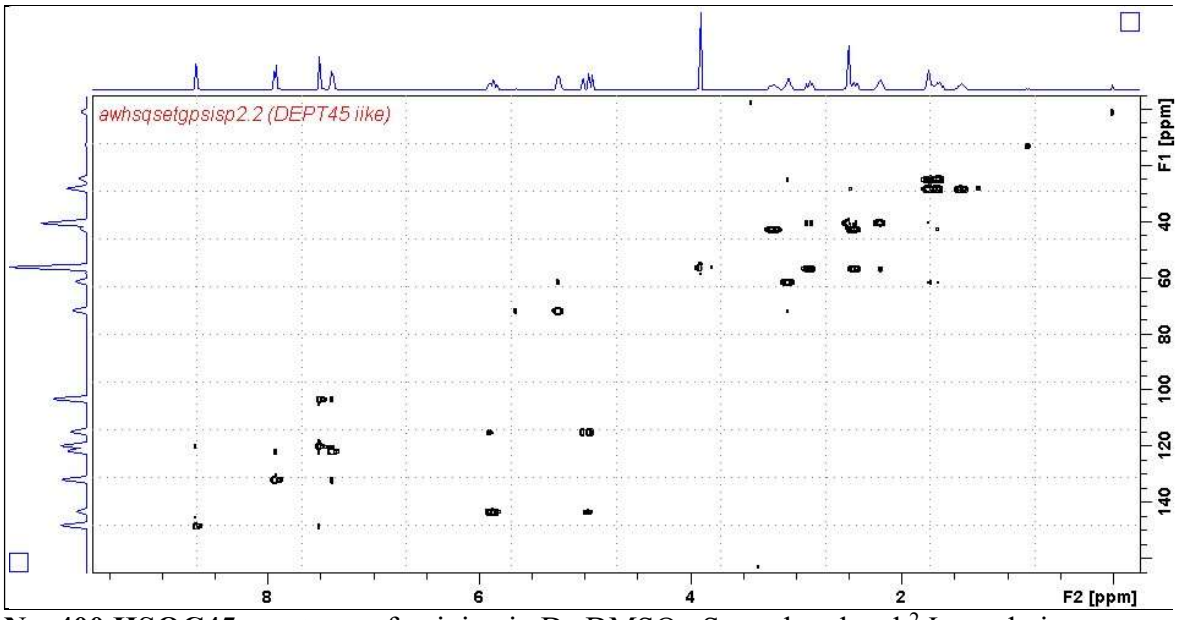
Type **eda** (enter) and enter **SW (^{1}H)** and **SW(^{13}C)** in ppm.
Enter **O1P = ^{1}H** spectral window midpoint in ppm.
Enter **O2P = ^{13}C** spectral window midpoint in ppm.
TD(F2) = 1K or 2K, **TD(F1)** = 128-256 (your choice).

NS = multiple of 4, 8 or 16, **DS** = 8 or 16.
D1 = repetition delay = **1.5 sec** or other time of your choice.
CNST2 = ^{1}J 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



Neo400 HSQC45 spectrum of quinine in D_6-DMSO. Some low level ^{2}J correlations are visible in this spectrum.

2.2 HSQCEDETGPSISP2.3-135

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

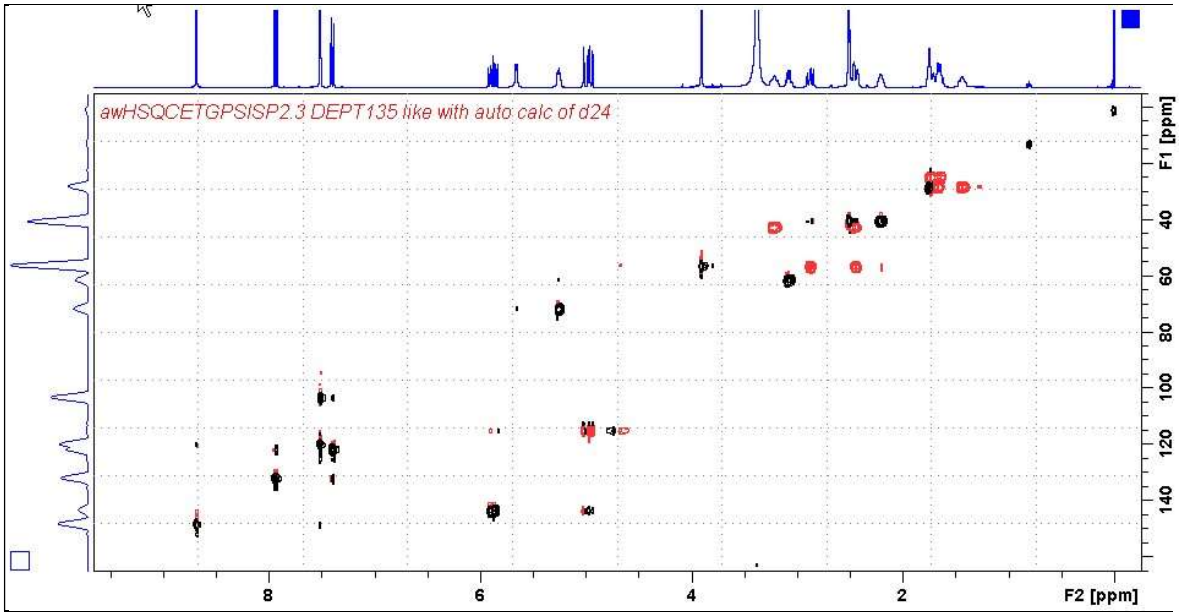
Type **eda** (enter) and enter **SW (^{1}H)** and **SW(^{13}C)** in ppm.
Enter **O1P = ^{1}H** spectral window midpoint in ppm.
Enter **O2P = ^{13}C** spectral window midpoint in ppm.
TD(F2) = 1K or 2K, **TD(F1)** = 128-256 (your choice).

NS = multiple of 4, 8 or 16, **DS** = 8 or 16.
D1 = repetition delay = **1.5 sec** or other time of your choice.
CNST2 = ^{1}J 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



Neo400 HSQC135 spectrum of quinine in D_6-DMSO with positive CH and CH_3 correlations (black) and negative CH_2 correlations (red). Some low level ^{2}J correlations are visible in this spectrum.

2.3 HSQCEDETGPSISP2.3-135PR

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

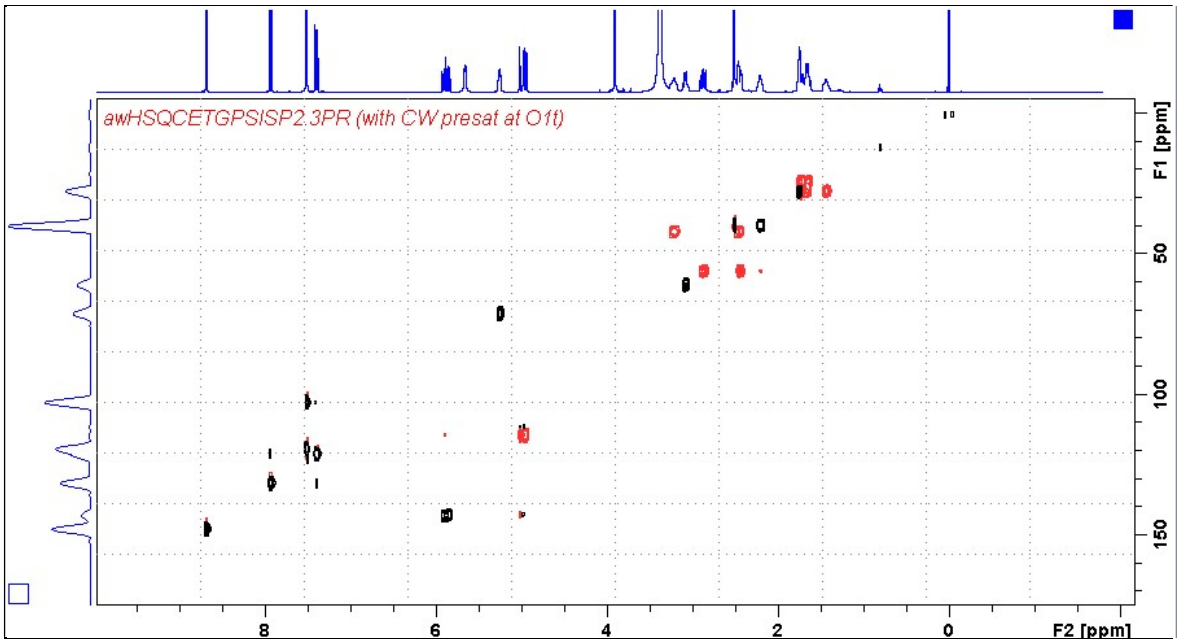
Type **eda** (enter) and enter **SW (^{1}H)** and **SW (^{13}C)** in ppm.
Enter **O1 in Hz** of the signal to be presaturated.
O1 = spectral window midpoint. Check **SW (^{1}H)** is wide enough.
Enter **O2P = ^{13}C** spectral window midpoint in ppm.
TD(F2) = 1K or 2K, **TD(F1)** = 128-256 (your choice).

NS = multiple of 4, 8 or 16, **DS** = 8 or 16.
D1 = repetition delay = **2 sec** or other time of your choice.
CNST2 = ^{1}J coupling constant = **145 Hz** or other value of your choice (eg: 125-160 Hz).

Type **ased** (enter) and review parameters used in the job.
The **PL9(db)** prosol linked presaturation power level can be adjusted if required.
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



Neo400 HSQC135PR spectrum of quinine in D_6-DMSO with its 3.91 ppm OCH_3 signal suppressed

2.4 HSQC-DIPSI2

Parameter set: **awhsqc-dipsi2 (+ getprosol)**
Pulse programme**: hsqcdiedetgpsisp.2**

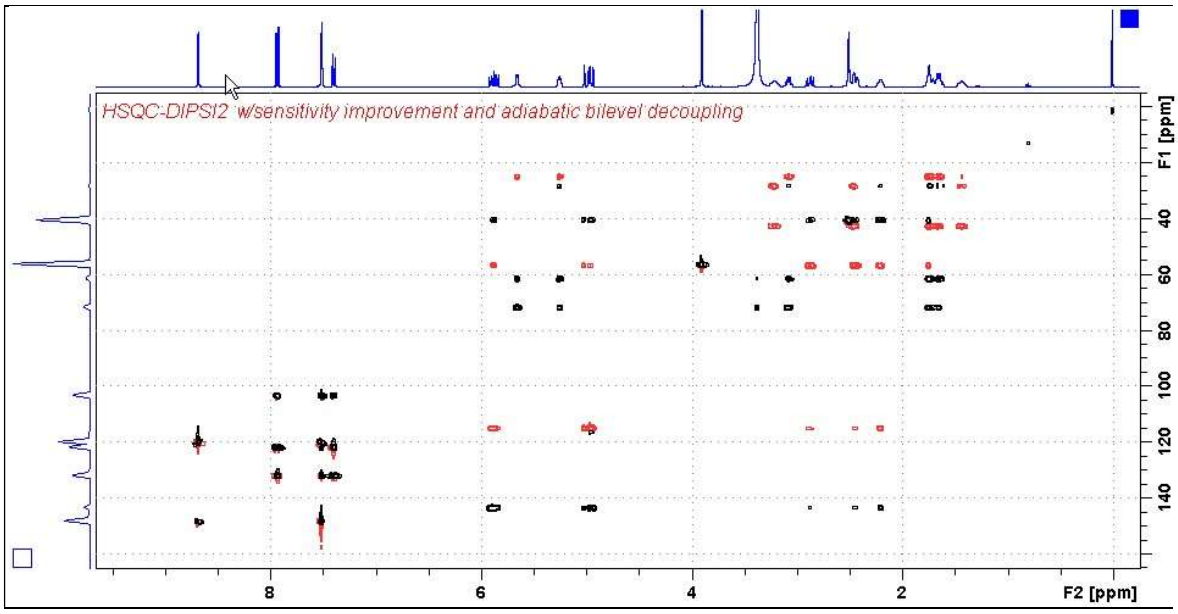
Type **eda** (enter) and enter **SW (**^{1}**H)** and **SW (**^{13}**C i**n ppm.
Enter **O1P =** ^{1}**H** spectral window midpoint in ppm.
Enter **O2P =** ^{13}**C** spectral window midpoint in ppm.
TD(F2) = 1K or 2K, **TD(F1)** = 128-256 (your choice).

NS = multiple of 4, 8 or 16, **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 = ^{1}J 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 **D21** = 1/**2*J*** (~ 3.45 msec), **D24** = 1/8*J* (~ 0.89 msec).
These values are not auto-calculated using Topspin's hsqcdiedetgpsisp.2 pp.
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



Neo400 HSQC-DIPSI2 spectrum of quinine in D_6-DMSO.

2.5 HSQC-NOESY

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

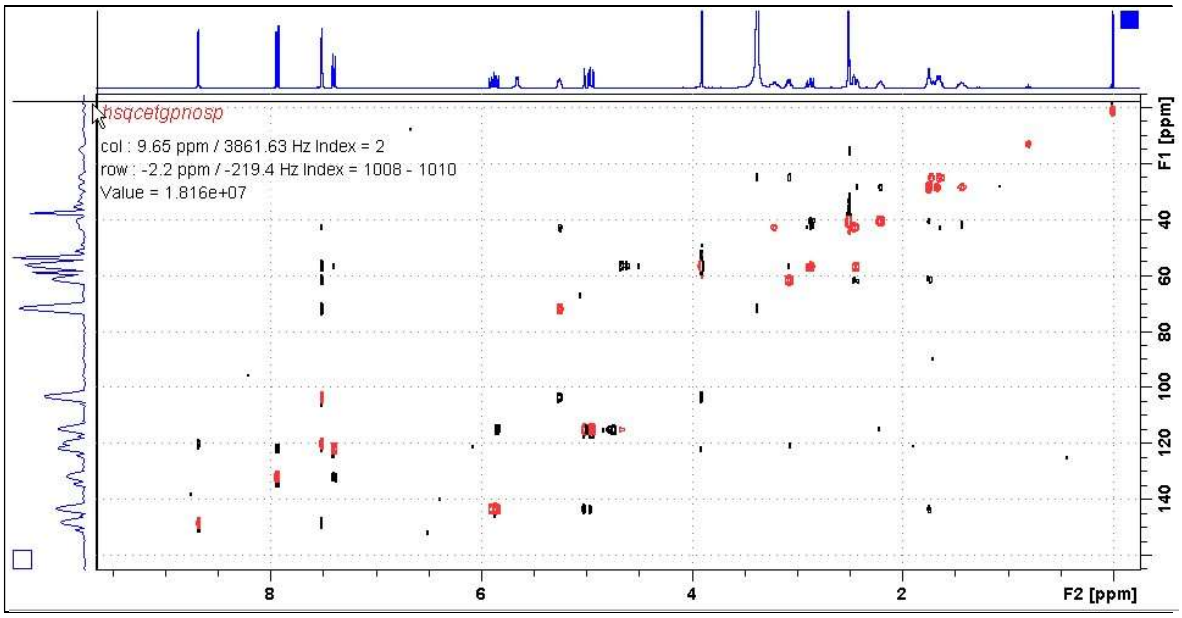
Type **eda** (enter) and enter **SW (^{1}H)** and **SW (^{13}C)** in ppm.
Enter **O1P = ^{1}H** spectral window midpoint in ppm.
Enter **O2P = ^{13}C** spectral window midpoint in ppm.
TD(F2) = 1K or 2K, **TD(F1)** = 128-256 (your choice).

NS = multiple of 4, 8 or 16, **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 = ^{1}J 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



Neo400 HSQC-NOESY spectrum of quinine in D_6-DMSO

2.6 HSQC-ROESY

Parameter set: **awhsqc-roesy (+ getprosol)**
Pulse programme: **hsqcetgprosp**

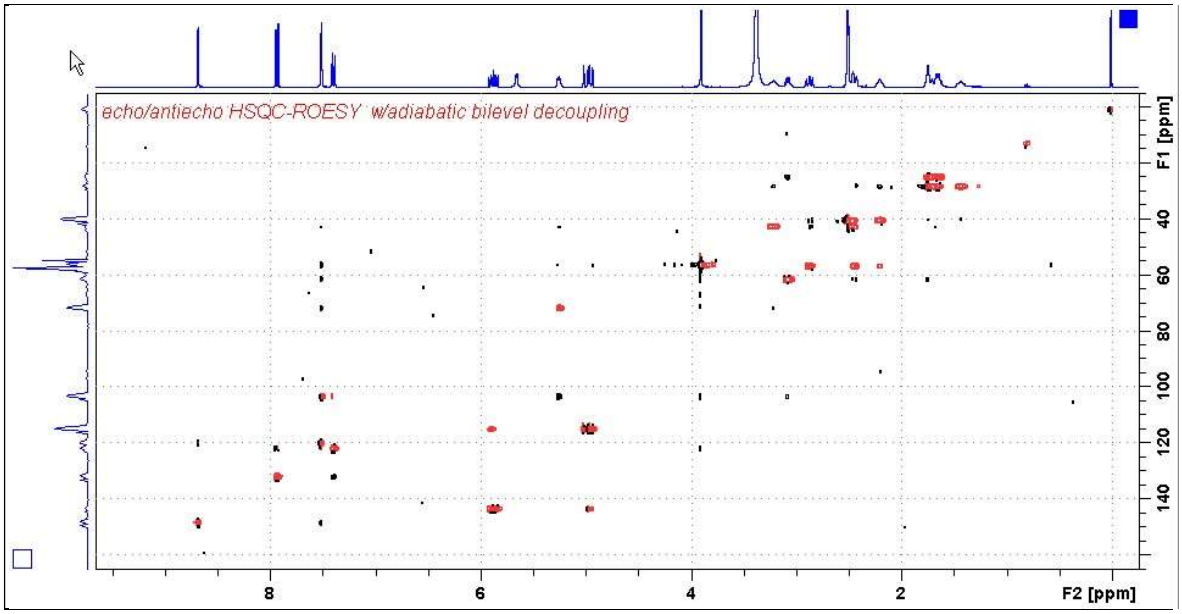
Type **eda** (enter) and enter **SW (^{1}H)** and **SW (^{13}C)** in ppm.
Enter **O1P = ^{1}H** spectral window midpoint in ppm.
Enter **O2P = ^{13}C** spectral window midpoint in ppm.
TD(F2) = 1K or 2K, **TD(F1)** = 128-256 (your choice).

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 = ^{1}J 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



Neo400 HSQC-ROESY spectrum) of quinine in D6-DMSO.

2.7 HSQC-ROESY2

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

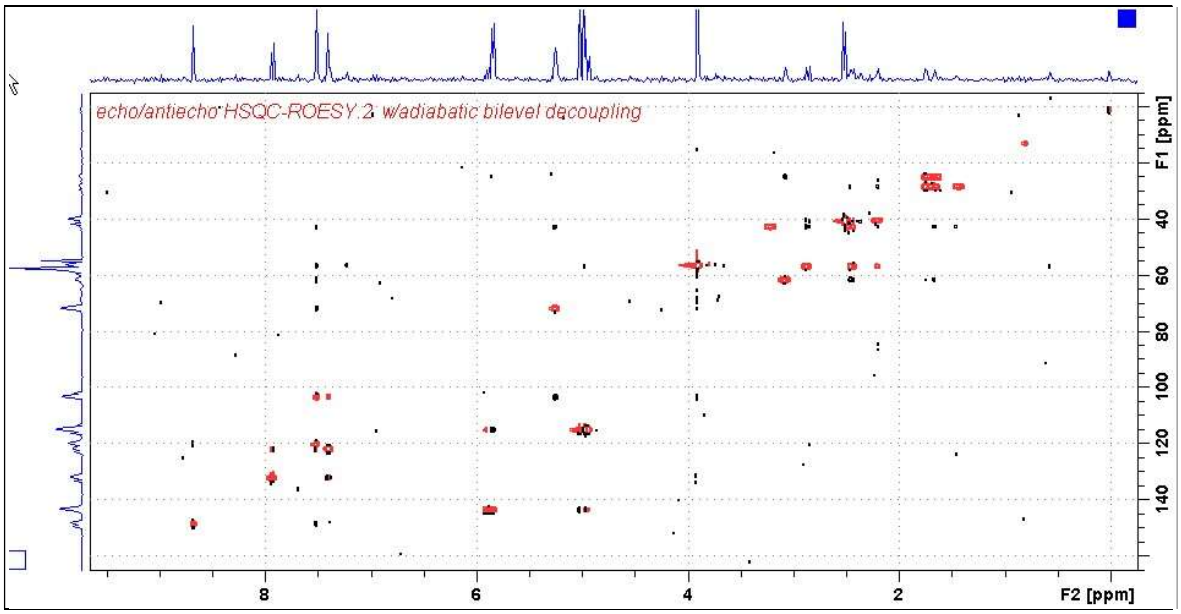
Type **eda** (enter) and enter **SW (^{1}H)** and **SW (^{13}C)** in ppm.
Enter **O1P = ^{1}H** spectral window midpoint in ppm.
Enter **O2P = ^{13}C** spectral window midpoint in ppm.
TD(F2) = 1K or 2K, **TD(F1)** = 128-256 (your choice).

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 = ^{1}J 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



Neo400 HSQC-ROESY2 spectrum of quinine in D_6-DMSO.

2.8 HMBC

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

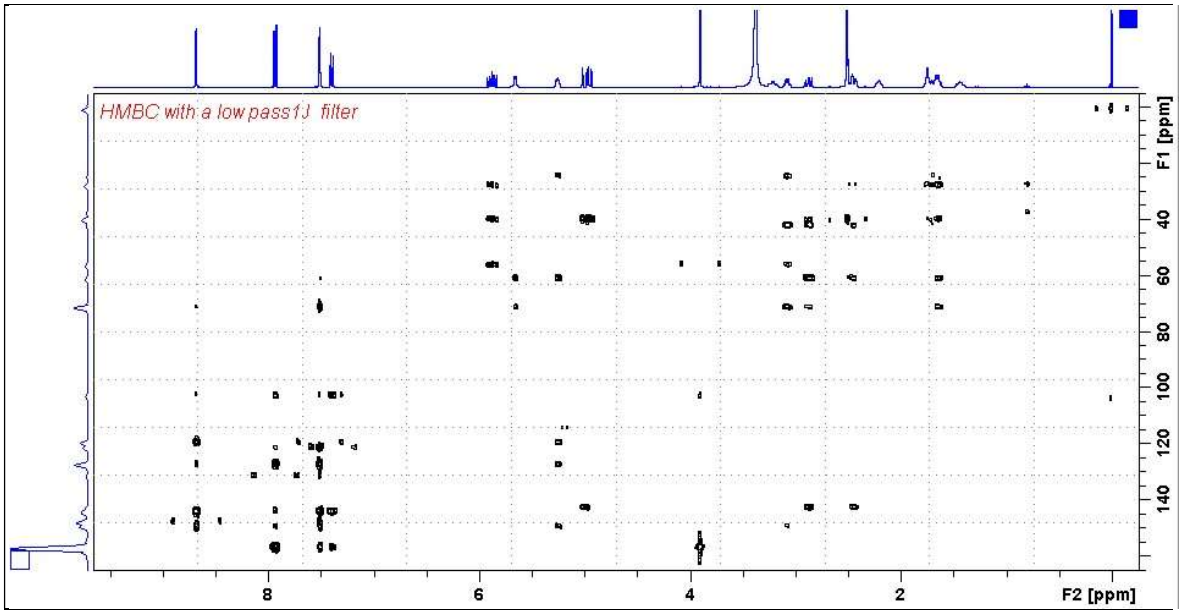
Type **eda** (enter) and enter **SW (^{1}H)** and **SW (^{13}C)** in ppm.
Enter **O1P = ^{1}H** spectral window midpoint in ppm.
Enter **O2P = ^{13}C** spectral window midpoint in ppm.
TD(F2) = 1K or 2K,**TD(F1)** = 128-256 (your choice).

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 = ^{1}J coupling constant = **145 Hz** or other value of your choice.
CNST13 = ^{n}J selection filter = **8 Hz** or other value of your choice.

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) = SINE
SSB(F2) = SSB(F1) = 0
xfb, abs1 and abs2



Neo400 HMBC spectrum of quinine in D_6-DMSO.

2.9 HMBCPR

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

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

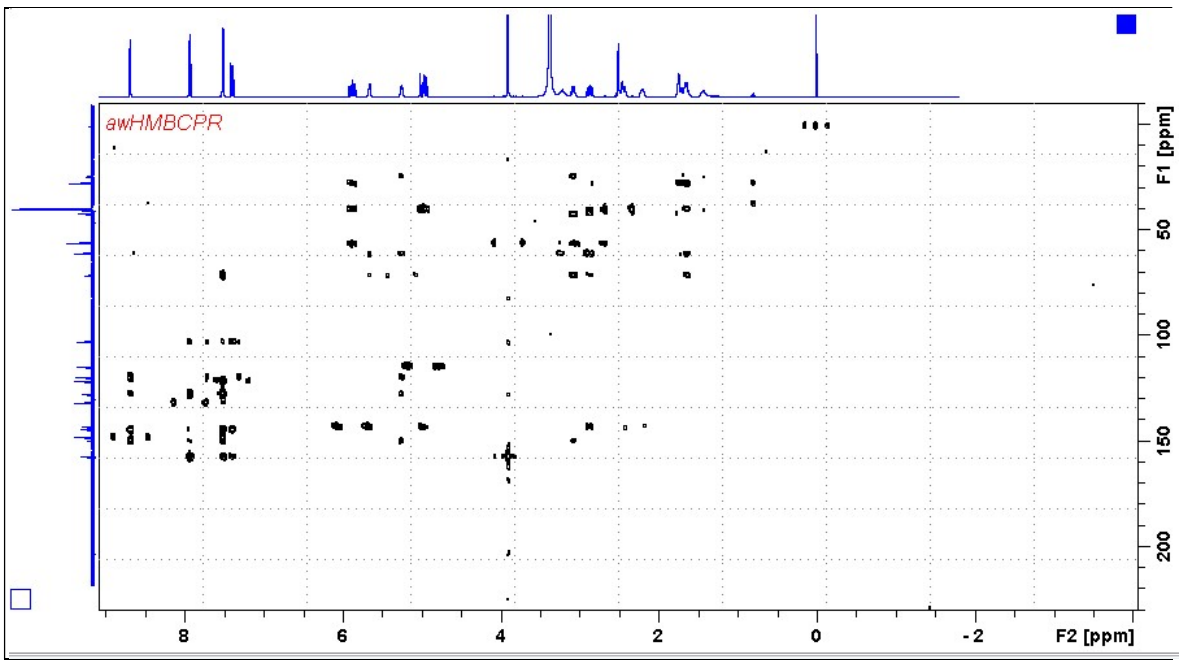
NS = multiple of 4, 8 or 16, **DS** = 8 or 16.
D1 = repetition delay = **2 sec** or other time of your choice.

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 = ^{1}J coupling constant = **145 Hz** or other value of your choice.
CNST13= ^{n}J selection filter = **8 Hz** or other value of your choice.

Type **ased** (enter) and review parameters used in the job.
The **PL9** prosol linked presaturation power level can be adjusted if required.
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) = SINE
SSB(F2) = SSB(F1) = 0
xfb, abs1 and abs2



Neo400 HMBCCTPR spectrum of quinine in D_6-DMSO with CW presaturation of the DMSO signal at 2.5 ppm.

2.10 HMBCCT

Parameter set: **awhmbcct (+ getprosol)**
Pulse programme: **hmbcetgpl2nd**

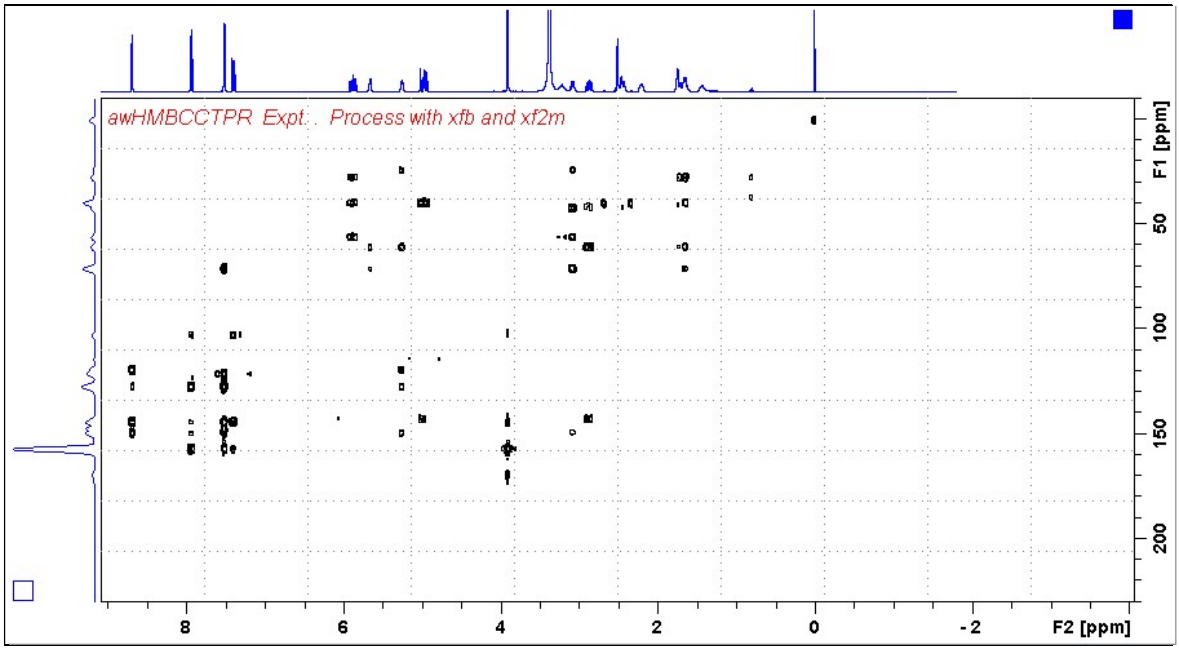
Type **eda** (enter) and enter **SW (^{1}H)** and **SW (^{13}C)** in ppm.
Enter **O1P = ^{1}H** spectral window midpoint in ppm.
Enter **O2P = ^{13}C** spectral window midpoint in ppm.
TD(F2) = 1K or 2K **TD(F1)** = 128-256 (your choice).

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 ^{1}J coupling constant = **120 Hz** or other value of your choice.
CNST7 = max ^{1}J coupling constant = **170 Hz** or other value of your choice.
CNST13 = ^{n}J selection filter = **8 Hz** or other value of your choice.

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) = SINE
SSB(F2) = SSB(F1) = 0
xfb, xf2m, abs1 and abs2



Neo400 HMBCCTPR spectrum of quinine in D_6-DMSO with CW presaturation of the DMSO signal at 2.5 ppm.

2.11 HMBCCTPR

Parameter set: **awhmbcct (+ getprosol)**
Pulse programme: **hmbcetgpl2nd**

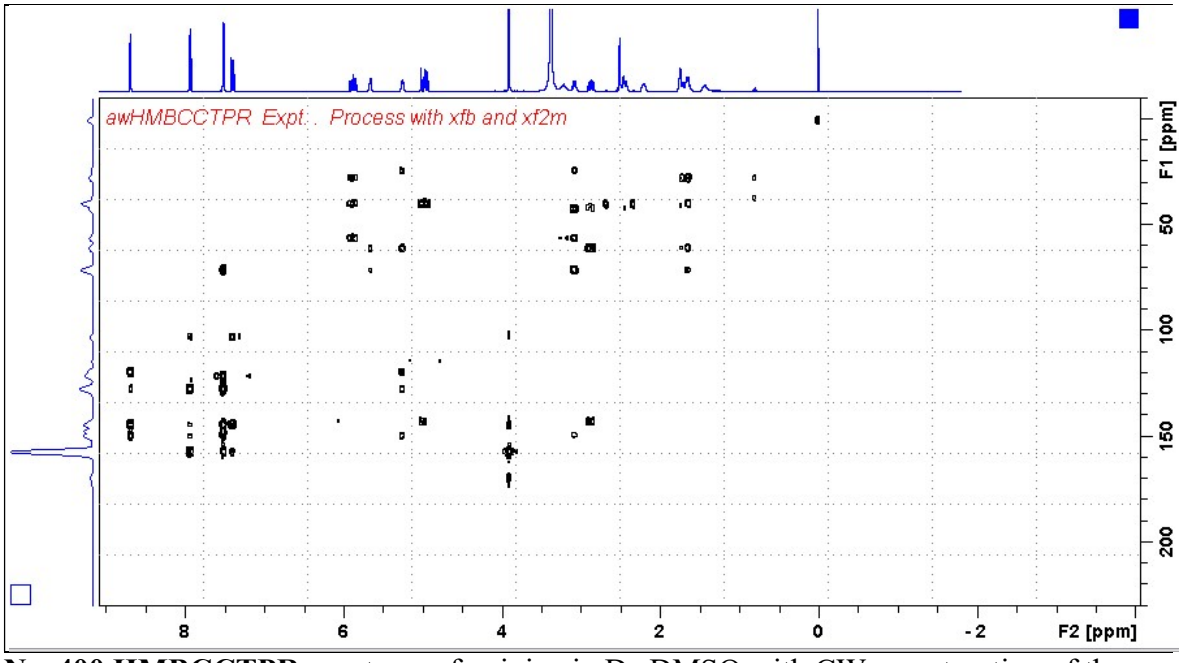
Type **eda** (enter) and enter **SW (^{1}H)** and **SW (^{13}C)** in ppm.
Enter **O1 in Hz** of the signal to be presaturated.
O1 = spectral window midpoint. Check **SW (^{1}H)** is wide enough.
Enter **O2P = ^{13}C** spectral window midpoint in ppm.
TD(F2) = 1K or 2K, **TD(F1)** = 128-256 (your choice).

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.
CNST6 = min ^{1}J coupling constant = **120 Hz** or other value of your choice.
CNST7 = max ^{1}J coupling constant = **170 Hz** or other value of your choice.
CNST13 = ^{n}J selection filter = **8 Hz** or other value of your choice.

Type **ased** (enter) and review parameters used in the job.
The **PL9** prosol linked presaturation power level can be adjusted if required.
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) = SINE
SSB(F2) = SSB(F1) = 0
xfb, xf2m, abs1 and abs2



Neo400 HMBCCTPR spectrum of quinine in D_6-DMSO with CW presaturation of the DMSO signal at 2.5 ppm.

2.12 H2BC spectrum

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

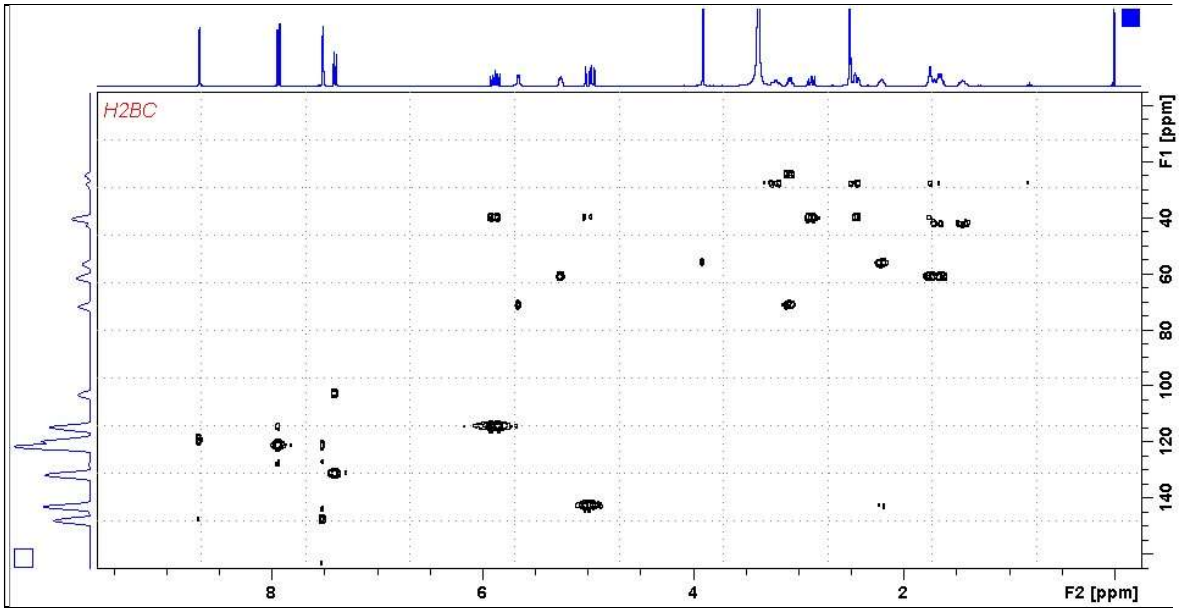
Type **eda** (enter) and enter **SW (^{1}H)** and **SW (^{13}C)** in ppm.
Enter **O1P = ^{1}H** spectral window midpoint in ppm.
Enter **O2P = ^{13}C** spectral window midpoint in ppm.
TD(F2) = 1K or 2K, **TD(F1)** = 128-256 (your choice).

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 ^{1}J selection filter range.

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, xf2m, abs1 and abs2



Neo400 H2BC spectrum of quinine in D_6-DMSO.