

Two Dimensional Heteronuclear Correlation Spectroscopy

Gradient HMQC

William D. Wheeler, Ph.D.
Department of Chemistry
University of Wyoming

Revised September 7, 2006

INTRODUCTION

Correlation Spectroscopy

Correlation experiments reveal connections between spin coupled nuclei. Spin, or scalar, coupling between nuclei is established by the appearance of cross peaks (peaks off the diagonal) in the two-dimensional spectrum. Since spin coupled nuclei are usually separated by one to three bonds, the correlation experiment, by revealing this connectivity within the compound, is often enough to establish the chemical structure. Resonances which are coupled through four and five bonds will occasionally show cross peaks also. Although most correlation experiments are designed to emphasize short range coupling, there are experiments designed to emphasize long range correlations as well.

Two-dimensional spectra can be acquired in either of two modes. In the magnitude, or absolute value mode, only the "real" part of the data is collected since the final spectrum is not phased. In the phase sensitive mode, both the "real" and "imaginary" parts of the data are acquired so that the spectrum can be phased. An advantage of a phase sensitive spectrum is that the peaks are much narrower and the resolution greater. A disadvantage of a phase sensitive spectrum is that twice as much data must be recorded in order to extract the phase information. Thus, it takes twice the time to record a spectrum in phase sensitive mode.

Gradient HMQC

The gradient Heteronuclear Multiple Quantum Correlation experiment described here shows connections between carbon atoms and the protons directly bonded to them. The experiment also furnishes information about carbon types. Quaternary carbons, for instance, have no attached protons and consequently, show no correlations. Methine carbons, can show one and only one peak. Methylene carbons can show one or two peaks depending on the chemical shifts of the two protons and methyl groups generally show a single intense peak or a single multiplet. A two dimensional spectrum usually has quite low resolution so the fine structure due to multiplets is minimal. HMQC spectra can be acquired in either magnitude or phase sensitive mode.

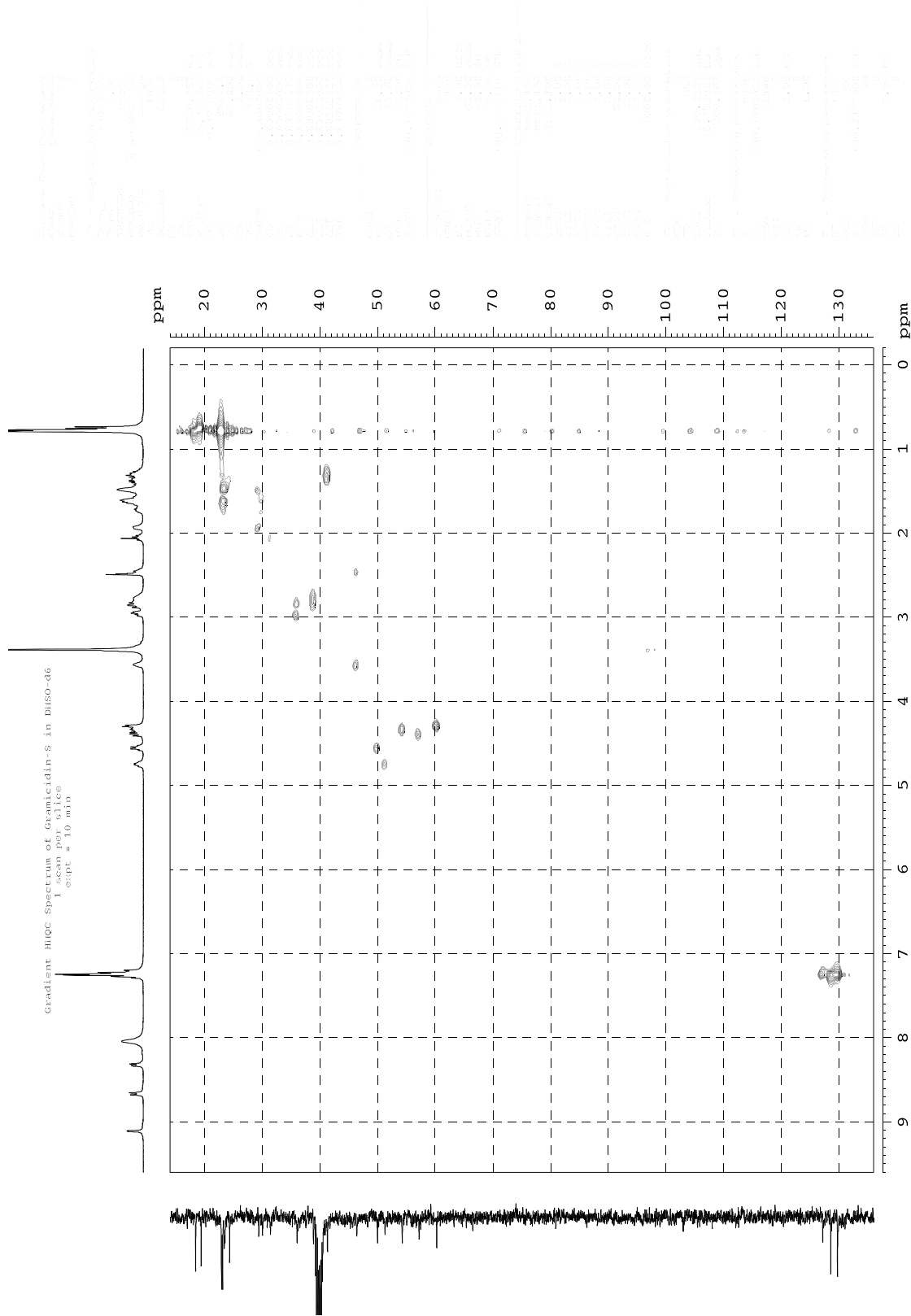
HMQC is known as an "inverse" experiment, because the ^{13}C chemical shift information is encoded into the ^1H signal. The data set for the example spectrum, however, was collected on a "direct" probe. In general, direct experiments detect the X nucleus, where the coil for the X channel is the closest to the sample. Inverse experiments detect the ^1H nucleus, where the coil for the ^1H channel is the closest to the sample. In this experiment, we are detecting the ^1H nucleus where the ^1H (decoupling) coil is NOT the closest coil to the sample. The S/N ratio is reduced, but the loss is not as great as switching to "direct" detection. This makes it possible to get ^{13}C - ^1H correlation data in a relatively short time without having to change to an inverse probe.

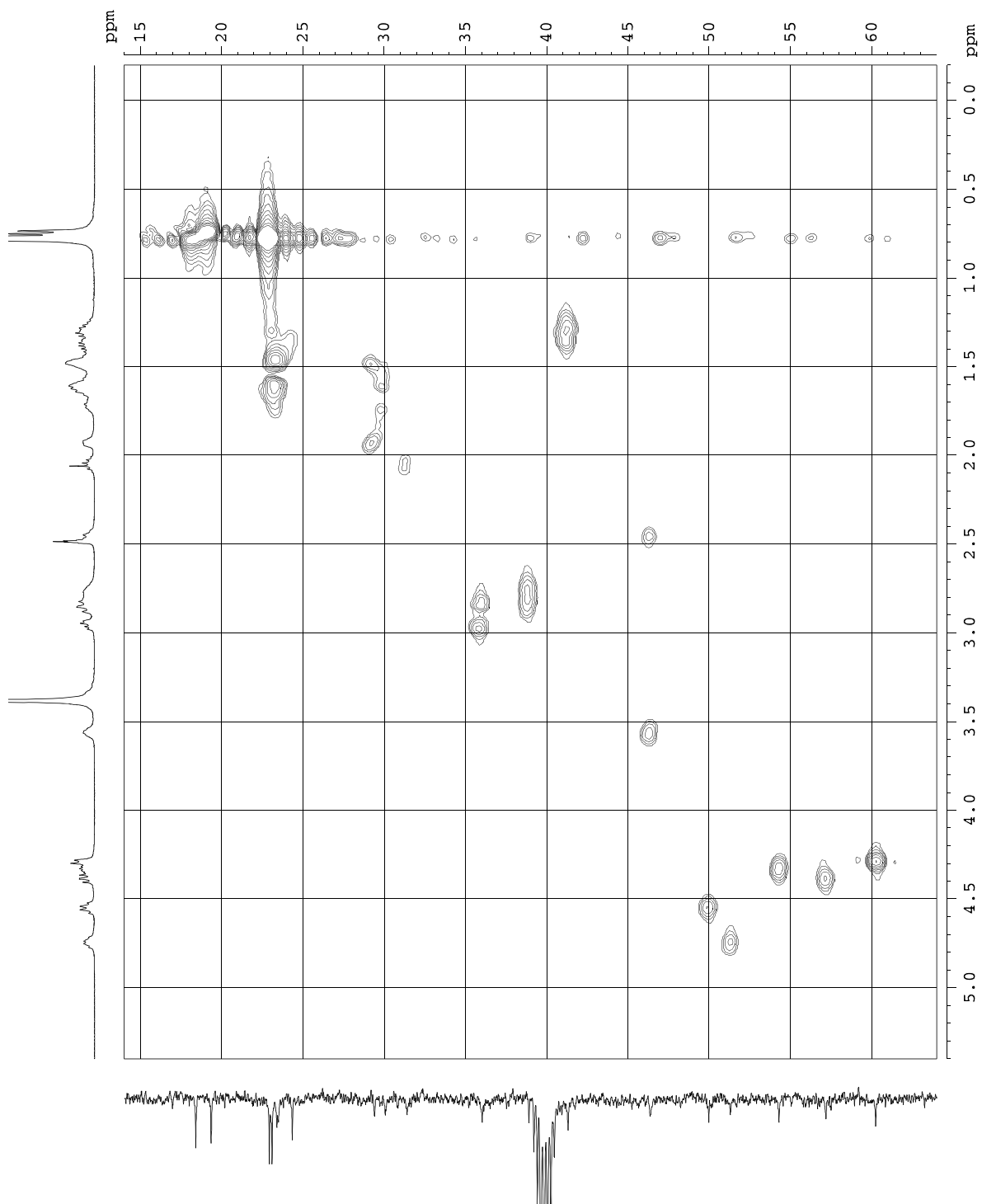
The gradient HMQC spectrum of Gramicidin S is shown on a following page. Gramicidin S is a cyclic deca-peptide consisting of the five amino acid pairs of Leucine, Valine, Proline, Ornithine (one L and one D), Phenylalanine (one L and one D). Notice, that the peaks between 7.6 and 9.6 ppm in the proton spectrum have no correlations to the carbon spectrum. These are amine protons. The peak at 3.3 ppm is water. The solvent (99% deuterated) shows a small sharp peak at 2.5 ppm in the proton spectrum which does correlate to the very large peak at 39.5 ppm in the carbon spectrum. The lowest contour level is above the top of this peak so that it does not appear in the spectrum as shown, but it is there. Other features include: The peak for the phenyl group of Phe at {7.2,128}; the five alpha C-H's between {4.0,50} and {5.0,60} ppm and the methyl groups of Val and Leu near {0.8,18-23}. Also evident in this spectrum are several sets of methylene groups where the two protons are non-equivalent, thus showing two peaks with different ^1H chemical shifts, but a single ^{13}C chemical shift.

The Gramicidin S spectrum required about 10 minutes to collect.

References.

- 1) Avance Users Guide, Bruker Instruments, Chapter 16.
- 2) 150 and More Basic NMR Experiments, A practical Course, S. Braun, H.-O. Kalinowski, S. Berger, Wiley-VCH, 1998, pages 485-488.
- 3) Basic One- and Two-Dimensional NMR Spectroscopy, Second, enlarged edition, Horst Friebolin, VCH Publishers, 1993, pages 239-247.
- 4) Modern NMR Spectroscopy, A Guide for Chemists, Jeremy K.M. Saunders and Brian K. Hunter, Oxford University Press, 1987, pages 100-108.
- 5) Biochemistry, Second Edition, Albert L. Lehninger, Worth Publishers, Inc., 1975, pages 73-75.





Inverse heteronuclear ^1H - ^{13}C correlation of Gramicidin S

EXPERIMENTAL SETUP

The concentration of the sample should be about 50 mM to obtain results similar to those described here for Gramicidin S. This is about 16 mg of a sample of molecular weight 400 in 0.8 ml of solvent. If it is not possible to obtain this concentration, then the number of scans for the 2-D experiment may need to be increased. If you halve the concentration, you will need to quadruple the number of scans in order to achieve the same signal to noise ratio.

Record a ^1H spectrum.

NEW (or EDC)	Create a new data set for your sample.
NAME name	Data set name.
EXPNO 1	Experiment number (must be 1).
PROCNO 1	Process data number (must be 1).
[SAVE]	Save the data set.
RPAR +proton all	Read in the standard proton parameters.
NS, etc.	Adjust NS and other parameters as necessary.
RGA, ZG	Acquire some data.
FT, APK, ref	Fourier transform, phase and reference the spectrum.
Zoom	Zoom in on the region of interest. The expanded region need not contain a reference peak.
[sw-sfo1]	Set the sweep width and spectrometer frequency to cover the zoomed region. Reduce TD if the acquisition time (AQ) is unnecessarily large.
RGA, ZG	Acquire a spectrum of the zoomed region.
FT, etc.	Fourier transform, phase etc.

Record a ^{13}C spectrum. (Optional)

You do not need a ^{13}C NMR spectrum to complete the HMQC.

NEW (or EDC)	Create a new data set for your sample.
NAME name	Data set name.
EXPNO 13	Experiment number (suggest 13).
PROCNO 1	Process data number (must be 1).
[SAVE]	Save the data set.

If you have already collected a ^{13}C spectrum, skip to the "Zoom" section to set up a spectrum of a zoomed region, and acquire that.

RPAR +carbon all NS, etc. RGA, ZG FT, APK, ref Zoom	Read in the standard carbon parameters. Adjust NS and other parameters as necessary. Acquire some data. Fourier transform, phase and reference the spectrum. Zoom in on the region of interest. The expanded region need not contain a reference peak.
[sw-sfo1]	Set the sweep width and spectrometer frequency to cover the zoomed region. Reduce TD if the acquisition time (AQ) is unnecessarily large.
RGA, ZG FT, etc.	Acquire a spectrum of the zoomed region. Fourier transform, phase etc.

Set up the ^{13}C - ^1H correlation experiment.

The following AU program sets the parameters for gradient HMQC.

setup2d	Pick the [GRASP-HMQC] button and then press [Setup]. You should have experiment number 1 displayed (re 1) before executing setup2d.
16	The experiment number where the HMQC spectrum will be stored. The suggested number is 16. Zero and any number from 2 to 997 is valid.
[OK]	Answer "Delete `meta.ext` files ?" with [OK].
13	The experiment number where the ^{13}C spectrum is stored. Enter 0 (zero) if you did not collect a ^{13}C spectrum. Any number from 2 to 997 is valid.
[Seen]	Answer "Error: Routing for channel 2 is not complete." with [Seen].
[Default]	Press the [Default] button in the edasp window. This sets up the signal routing for the spectrometer.
[Save]	Press the [Save] button to close the edasp window.

The setup2d program suggests the following file parameters.

File parameter		
EXPNO	1 13 16	Experiment number 1, for 1-D proton. (Required) Experiment number 13, for 1-D carbon. (Suggested) Experiment number 16, for 2-D HMQC. (Suggested)

The setup2d program sets the following acquisition parameters. Use EDA to display the results.

Acquisition Parameters		
Time domain 2 (F2)		
PULPROG	hmqcgpqf	PULse PROGram for HMQC.
TD	2048	Time Domain points.
NS	2	Number of Scans (integer multiple of 1)
DS	16	number of Dummy Scans for 1st row.
D1	1.5 sec	relaxation Delay.
CNST2	145.0	One bond C-H coupling constant, J_{CH}
P16	0.00150	homospoil / gradient pulse
D16	0.000150	delay for homospoil / gradient recovery
GPZ1	50.0	Z gradient 1
GPZ2	30.0	Z gradient 2
GPZ3	40.1	Z gradient 3
Time domain 1 (F1)		
TD	256	Time Domain points.
ND0	2	Number of D0 periods per cycle.
SW	120	SW of ^{13}C spectrum collected in experiment 13 (120 if no spectrum).
O1P	60	Center frequency (in ppm) of ^{13}C spectrum collected in experiment 13 (60 if no spectrum).

ASED	Check that acquisition parameters are set correctly. A brief description of other parameters, not described above, is given in the pulse program at the end of this document.
[SAVE]	Save the acquisition parameters.
<u>EXPT</u>	Calculates the approximate length of time to do the experiment. This may help you to decide if you want to collect more or fewer slices or scans or points etc. The Gramicidin spectrum required about 10 minutes.

DATA ACQUISITION

[Spin on/off]	TURN THE SPINNER OFF (press the Spin On/Off button on the BOSS keyboard).
RGA	Set the receiver gain. This will take a few seconds.
ZG	Start the acquisition.

DATA PROCESSING

The setup2d program sets the following processing parameters. Use EDP to check or modify the values.

Processing Parameters		
Time/frequency domain 2 (F2)		
SI	1024	the Slze in F2 (zero fill rows).
WDW	QSINE	Sine squared multiplication.
SSB	2	90° shifted sine squared bell.
PH_mod	NO	No phase correction.
PKNL	TRUE	Required with digital filter.
BC_mod	NO	Background correct quadrature data.
SF		Spectrum reference frequency for ¹ H.
Time/frequency domain 1 (F1)		
SI	512	the Slze in F1 (zero fill columns).
WDW	QSINE	Sine multiplication. (Or EM with LB 2-5)
SSB	2	90° shifted sine bell.
PH_mod	MC	Magnitude calculation.
BC_mod	NO	No background correction.
MC2	QF	Forward quadrature FFT.
SF	100.6127290	Spectrum reference frequency for ¹³ C.
OFFSET	120	Offset (in ppm) of ¹³ C spectrum collected in experiment 13 (120 if no spectrum).

XFB

Background correct, window, zero fill, Fourier transform, phase and reference. The whole enchilada in one command. It is OK to execute this command on a partial data set, while acquisition is in progress.

2-D CONTOUR DISPLAY

X-Y Expansion the spectrum.

[Limits]	Set the limits of the plot region.
[PlotReg]	Forces XWinNMR to display the plot region.

Setting the intensity scale.

[DefPlot]	Sets the intensity scale for plotting to be the same as the currently displayed intensity scale.
[contours]	Displays the equal-intensity contours of the data.
[intensities]	Displays ranges of intensity as a color map.

PLOTTING

SETTI Set the title for the 2D spectrum.

The setup2d program sets the following plotting parameters. Use xwinplot to plot the spectrum. The parameter names are not visible in xwinplot, but you should get the idea. If you did not collect a carbon spectrum, you will need to either remove the F1 projection area, or create a projection to use in the F1 projection area.

Plotting Parameters		
Projection for Frequency domain 2		
PF2DU	/opt/xwinnmr	Disk Unit of the data set.
PF2USER	username	Your user name.
PF2NAME	name	Name of the data set.
PF2EXP	1	Experiment number of the ¹ H spectrum.
PF2PROC	1	Process number of the ¹ H spectrum.
Projection for Frequency domain 1		
PF1DU	/opt/xwinnmr	Disk Unit of the data set.
PF1USER	username	Your user name.
PF1NAME	name	Name of the data set.
PF1EXP	13	Experiment number of the ¹³ C spectrum.

Plotting Parameters		
PF1PROC	1	Process number of the ¹³ C spectrum.
Additional plotting parameters		
PF1CY	2.5	The value of CY for the F1 projection. This can be used to increase or decrease the height of the spectrum displayed in the F1 projection. (PF1CY = 5.0 will double the height of the 1-D spectrum).
PF2CY	2.5	The value of CY for the F2 projection.

Additional Processing Parameters		
Frequency domain		
F1LO	bbb	Set to the value for the bottom of the spectrum (ppm).
F1HI	ttt	Set to the value for the top of the spectrum (ppm).
F2LO	lll	Set to the value for the left side of the spectrum (ppm).
F2HI	rrr	Set to the value for the right side of the spectrum (ppm).

Additional Processing Commands	
Frequency domain	
ABS1	Automatic Baseline Subtraction for F1 (uses F1LO, F1HI).
ABS2	Automatic Baseline Subtraction for F2 (uses F2LO, F2HI).

Pulse Program for gradient HMQC

```
;hmqcgpqf
;avance-version (02/05/31)
;HMQC
;2D H-1/X correlation via heteronuclear zero and double quantum
; coherence
;with decoupling during acquisition
;using gradient pulses for selection
;use pulseprogram 'inv4gpnd1d' for setup
```

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

```
"p2=p1*2"
"d0=3u"
"d2=1s/(cnst2*2)"
"d12=20u"
"d13=4u"
"DELTA1=d2-p16-d16-d13-d12"
```

```
1 ze
2 d1 do:f2
3 p1 ph1
  d2 p12:f2 UNBLKGRAD
  p3:f2 ph3
  d0
  p16:gp1
  d16
  p2 ph2
  d13
  p16:gp2
  d16
  d0
  p3:f2 ph4
  d13
  p16:gp3
  d16
  DELTA1
  d12 p12:f2 BLKGRAD
  go=2 ph31 cpd2:f2
  d1 do:f2 mc #0 to 2 F1QF(id0)
exit
```

```

ph1=0
ph2=0
ph3=0 2
ph4=0 0 2 2
ph31=0 2 2 0

```

```

;p11 : f1 channel - power level for pulse (default)
;p12 : f2 channel - power level for pulse (default)
;p12: f2 channel - power level for CPD/BB decoupling
;p1 : f1 channel - 90 degree high power pulse
;p2 : f1 channel - 180 degree high power pulse
;p3 : f2 channel - 90 degree high power pulse
;p16: homospoil/gradient pulse
;d0 : incremented delay (2D)          [3 usec]
;d1 : relaxation delay; 1-5 * T1
;d2 : 1/(2J)XH
;d12: delay for power switching       [20 usec]
;d13: short delay                     [4 usec]
;d16: delay for homospoil/gradient recovery
;cnst2: = J(XH)
;in0: 1/(2 * SW(X)) = DW(X)
;nd0: 2
;NS: 1 * n
;DS: 16
;td1: number of experiments
;FnMODE: QF
;cpd2: decoupling according to sequence defined by cpdprg2
;pcpd2: f2 channel - 90 degree pulse for decoupling sequence

```

```

;use gradient ratio:                gp 1 : gp 2 : gp 3
;                                  50 : 30 : 40.1 for C-13
;                                  70 : 30 : 50.1 for N-15

```

```

;for z-only gradients:
;gpz1: 50% for C-13, 70% for N-15
;gpz2: 30%
;gpz3: 40.1% for C-13, 50.1% for N-15

```

```

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

```

```

;$Id: hmqcgpqf,v 1.1 2002/06/12 09:04:42 ber Exp $

```