Two Dimensional Homonuclear Correlation Spectroscopy

DQF-COSY

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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 two or three bonds, the correlation experiment, by revealing the 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 valve mode, only the “real” part of the data is collected since the 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.

Double Quantum Filtered COSY (DQF-COSY).

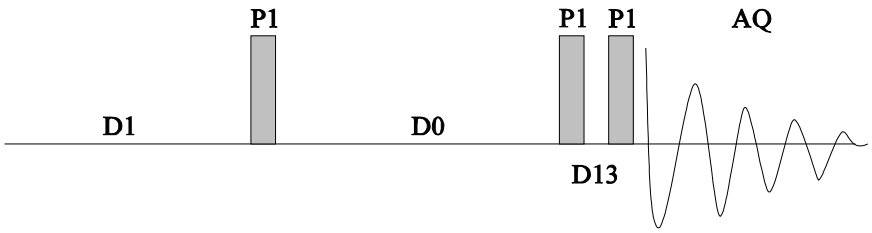
The double quantum filtered experiment detects a phenomenon known as a double (or two) quantum coherence. Since two (and higher) quantum coherence's can only be observed in first and higher order spin systems, the resulting spectra are somewhat simplified. Peaks on the diagonal are greatly reduced in intensity (since they represent single quantum transitions) with the consequent clarification in this region and much reduced t_1 noise.

A further advantage of DQF-COSY, is that in the phase sensitive mode, both diagonal and cross peaks can be adjusted to have a pure absorption line shape.

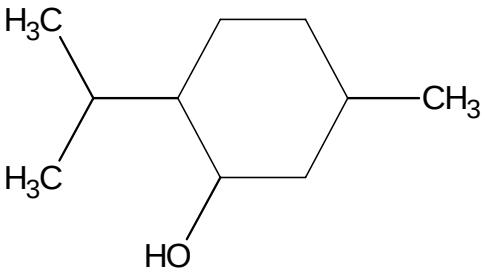
A phase sensitive DQF-COSY spectrum of Menthol is shown on a following page. Since this spectrum was recorded with a frequency range of only 2003 Hz (5.0 ppm) the resolution (2.0 Hz/point) is quite high for a 2-D spectrum and the coupling patterns are apparent in both the diagonal and cross peaks. As the frequency range becomes larger and the resolution smaller, the structure of the coupling may not be resolved and it is more common for the cross peaks to look like single spots. This is especially true with magnitude mode (non phase sensitive) spectra where the intensity is positive everywhere. The spectrum of Menthol is an example of a very complicated spin system. In a phase sensitive

experiment, the phase relationships within the cross peaks can sometimes be studied to determine which coupling partner a particular cross peak is due to.

The pulse sequence for DQF-COSY is shown below.



The phase sensitive DQF-COSY spectrum of Menthol is shown on a following page. The spectrum required 2 hours and 20 minutes to collect.

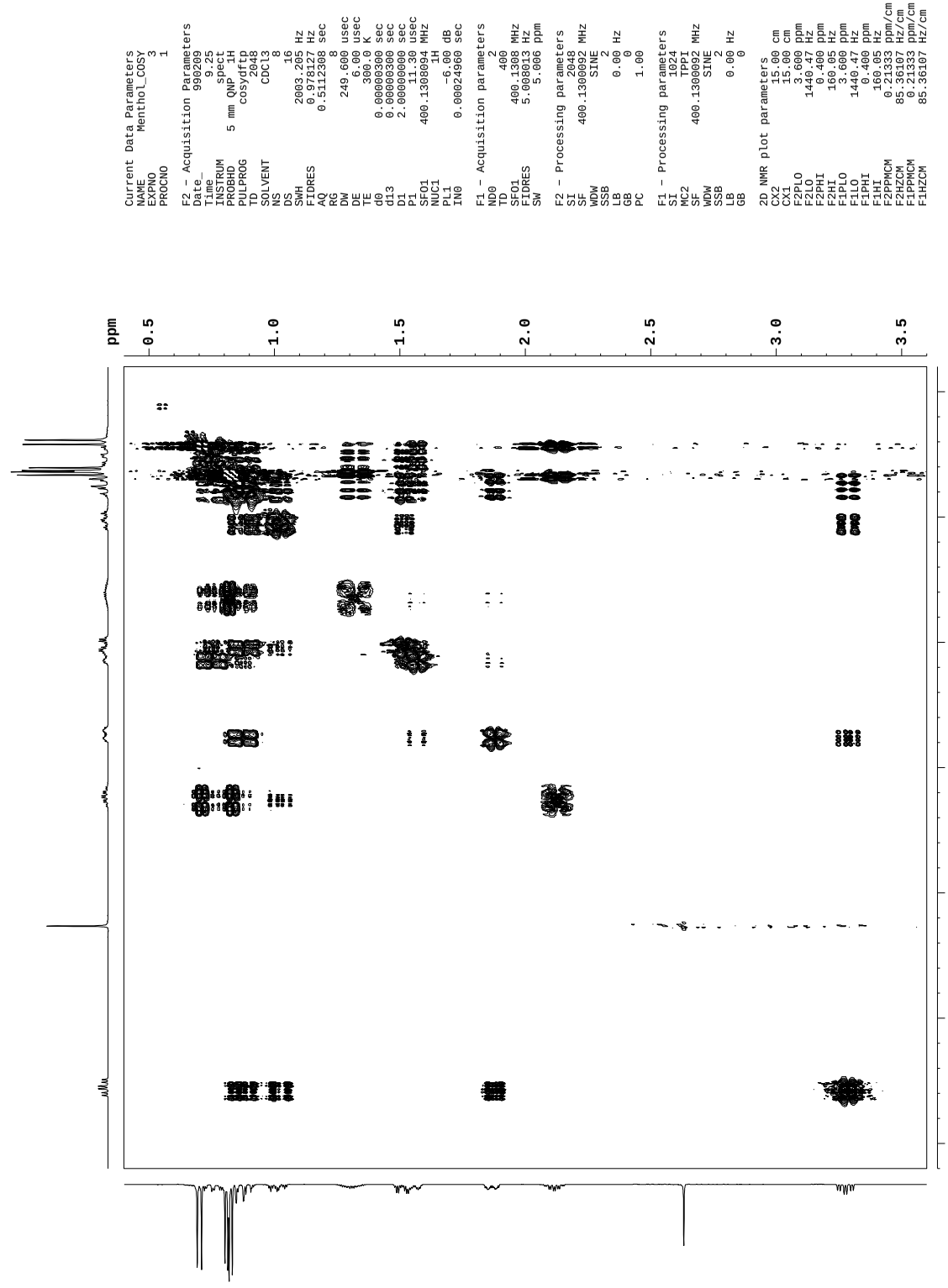


Menthol

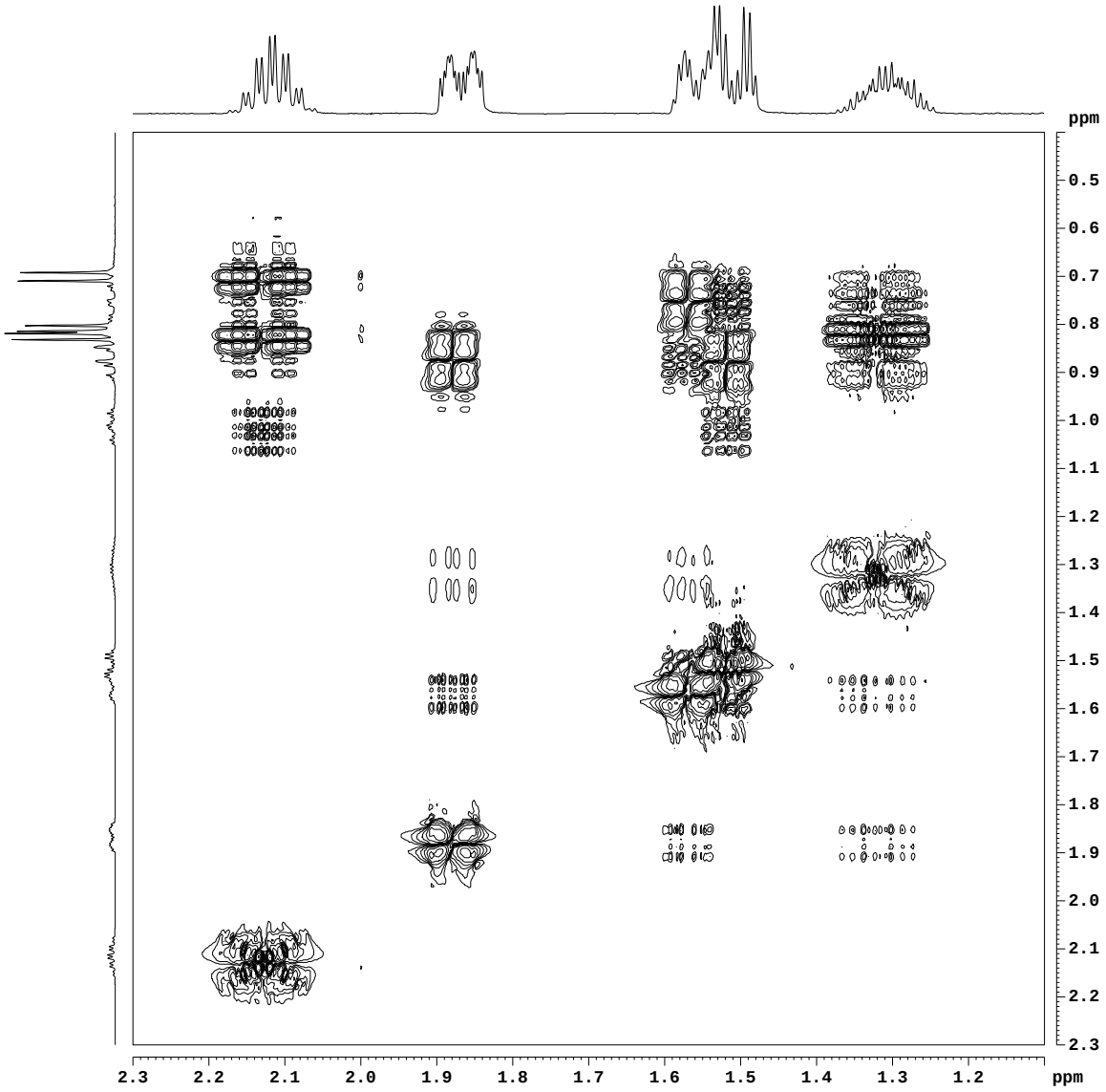
References.

1) **150** and More Basic NMR Experiments, A practical Course, S. Braun, H.-O. Kalinowski, S. Berger, Wiley-VCH, 1998, pages 368-371.

2) Modern NMR Spectroscopy, A Guide for Chemists, Jeremy K.M. Saunders and Brian K. Hunter, Oxford University Press, 1987, pages 126-127.



Double Quantum Filtered COSY of Menthol



Double Quantum Filtered COSY of Menthol - Expanded region

```
Current Data Parameters
NAME        Menthol_COSY
EXPNO                  3
PROCNO                 1

F2 - Acquisition Parameters
Date_             990209
Time                9.25
INSTRUM            spect
PROBHD     5 mm QNP  1H
PULPROG         cosydftp
TD                 2048
SOLVENT            CDCl3
NS                    8
DS                   16
SWH             2003.205 Hz
FIDRES         0.978127 Hz
AQ             0.5112308 sec
RG                    8
DW              249.600 usec
DE                6.00 usec
TE                300.0 K
d0           0.00000300 sec
d13          0.00000300 sec
D1           2.00000000 sec
P1                11.30 usec
SFO1        400.1308094 MHz
NUC1                1H
PL1               -6.00 dB
IN0          0.00024960 sec

F1 - Acquisition parameters
ND0                   2
TD                  400
SFO1            400.1308 MHz
FIDRES          5.008013 Hz
SW                 5.006 ppm

F2 - Processing parameters
SI                 2048
SF          400.1300092 MHz
WDW                SINE
SSB                   2
LB                 0.00 Hz
GB                    0
PC                 1.00

F1 - Processing parameters
SI                 1024
MC2                TPPI
SF          400.1300092 MHz
WDW                SINE
SSB                   2
LB                 0.00 Hz
GB                    0

2D NMR plot parameters
CX2               15.00 cm
CX1               15.00 cm
F2PLO              3.600 ppm
F2LO            1440.47 Hz
F2PHI              0.400 ppm
F2HI             160.05 Hz
F1PLO              3.600 ppm
F1LO            1440.47 Hz
F1PHI              0.400 ppm
F1HI             160.05 Hz
F2PPMCM         0.21333 ppm/cm
F2HZCM         85.36107 Hz/cm
F1PPMCM         0.21333 ppm/cm
F1HZCM         85.36107 Hz/cm
```

EXPERIMENTAL SETUP

The concentration of the sample should be high enough that you can record a high signal to noise ^{1}H NMR spectrum in 64 - 128 scans.

Record a ^{1}H 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.

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

The following AU program sets up all of the parameters for dqf-cosy.

XAU su_dqfcosy	Set up double quantum filtered cosy (dqf-cosy).
[OK]	Answer “Delete `meta.ext` files ?” with [OK].

The AU program sets the following file parameters. Use EDC to display the results.

File parameter		
EXPNO	1 3	Experiment number 1, for 1-D proton. Experiment number 3, for dqf-cosy.

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

Acquisition Parameters		
Time domain 2 (F2)		
PULPROG	cosydftp	PULse PROGram for dqf-cosy.
TD	2048	Time Domain points.
NS	16	Number of Scans (integer multiple of 8).
DS	16	number of Dummy Scans for 1st row.
D1	2 sec	relaxation Delay.
Time domain 1 (F1)		
TD	512	Time Domain points.
ND0	2	Number of D0 periods per cycle.
IN0	1 / SW	Increment in t1 (calculated).
SW		SW of ^{1}H spectrum (same as for F2).
SFO1		^{1}H frequency (same as for F2).

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.

EXPT — 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 Menthol spectrum required 2 hours and 20 minutes.

DATA ACQUISITION

[Spin on/off] — TURN THE SPINNER OFF (press the Spin On/Off button on the BOSS keyboard).

ZG — Start the acquisition.

DATA PROCESSING

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

Processing Parameters		
Time/frequency domain 2 (F2)		
SI	2048	the SIze in F2 (zero fill rows).
WDW	SINE	Sine multipication.
SSB	2	90° shifted sine bell.
PH_mod	PK	Phase correct.
PKNL	TRUE	Required with digital filter.
BC_mod	QUAD	Background correct quadrature data.
Time/frequency domain 1 (F1)		
SI	1024	the SIze in F1 (zero fill columns).
WDW	SINE	Sine multipication.
SSB	2	90° shifted sine bell.
PH_mod	PK	Phase correct.
BC_mod	NO	No background correction.
MC2	TPPI	Forward real FFT.
OFFSET		Frequency offset (same as for F2).
SF		Spectrometer frequency (same as for F2).

XFB

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

2-D CONTOUR DISPLAY

X-Y Expansion the spectrum.

[Limits]	Set the limits of the plot region. NOTE: For homo-nuclear correlation experiments, (COSY, NOESY, etc.) set F1LO = F2LO and F1HI = F2HI so the display will be symmetric and the diagonal peaks will appear on the diagonal line.
[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 equi-intensity contours of the data.
[intensities]	Displays ranges of intensity as a color map.

PHASING DQF-COSY SPECTRA

This is the hard part of the experiment, both to describe and accomplish. The idea, is to first phase correct 1-3 rows (or columns) from the data set, and apply this phase correction to the rest of the spectrum. In the case of NOESY and ROESY spectra, the first row (second for ROESY) of the spectrum can be phased like a 1D spectrum. This phase correction is used to phase correct the rest of the data set. Unfortunately, there is no such magic for DQF-COSY.

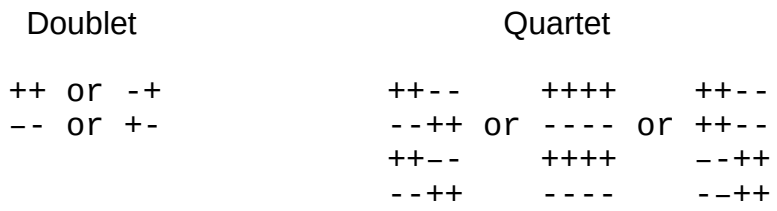
Phase Correct 2D rows or columns.

[phase]	Enter the 2D phase correction window.
[row] or [col]	Prepare to copy a row (or column) to a “mini” window. Note: you can copy either rows or columns, but you can not mix the two.

Move the cross hairs to the row (or column) that you want to copy. You should pick a row (or column) that has an off diagonal peak. When you are satisfied that the cross hairs are in position, click the MIDDLE mouse button. Move the cross hairs slightly and click the LEFT mouse button (this turns the cross hairs off). Click the mov[1] button. This copies the row (or column) to the #1 mini window. Repeat this for the #2 and #3 mini windows. Phase the spectra in the three mini windows using [ph0] and [ph1]. Note: the [big| 1 | 2 | 3] buttons will attempt to phase the biggest peak in the respective mini

window. Repeat the above procedure on the columns or until you are satisfied with the results.

A properly phased DQF-COSY spectrum will contain peaks that are "antiphase". This means that a doublet (in a 1D spectrum) will contain four peaks in the DQF-COSY spectrum, two with positive intensity and two with negative intensity. A quartet will show 16 peaks.



When you are finished, click [return]. The pop up window gives you several choices. The "save" button, applies the phase correction to the spectrum and then redisplays the phase window. "Save and return" button, applies the phase correction to the spectrum and then returns you to the normal display window.

PLOTTING

The AU program sets the following plotting parameters. Use EDG to check or modify the values. Once EDG has started, press either the [EDPROJ2] or [EDPROJ1] buttons.

Plotting Parameters		
Projection for Frequency domain 2		
PF2DU	z	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	z	Disk Unit of the data set.
PF1USER	username	Your user name.
PF1NAME	name	Name of the data set.

Plotting Parameters		
PF1EXP	1	Experiment number of the ^{1}H spectrum.
PF1PROC	1	Process number of the ^{1}H 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.

SETTI — Set the title for the 2D spectrum.

PLOT — Plot the contours of the 2D spectrum along with the hi-resolution ^{1}H spectrum in experiment #1.

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).
SYM	Symmetrize the spectrum. This “averages” the upper and lower triangles of the matrix. CAUTION! This is an easy way to introduce artifacts into your spectrum.
SYMA	For antiphase (phase sensitive) spectra.

Pulse Program for Double Quantum Filtered COSY

```
;cosydftp
;avance-version
;2D homonuclear shift correlation
;phase sensitive using TPPI
;with double quantum filter
;phasecycle: A. Derome & M. Williamson, J. Magn. Reson. 88,
;               177 - 185 (1990)

#include <Avance.incl>

"d0=3u"
"d13=3u"

1 ze
2 d1
3 p1 ph1
  d0
  p1 ph2
  d13
  p1 ph3
  go=2 ph31
  d1 wr #0 if #0 id0 ip1 zd
  lo to 3 times td1
exit

ph1=1 1 1 1 0 0 0 0
ph2=0 0 0 0 1 1 1 1
ph3=1 2 3 0 2 3 0 1
ph31=0 3 2 1 3 2 1 0

;pl1 : f1 channel - power level for pulse (default)
;p1 : f1 channel -  90 degree high power pulse
;d0 : incremented delay (2D)                          [3 usec]
;d1 : relaxation delay; 1-5 * T1
;d13: short delay                                     [3 usec]
;in0: 1/(2 * SW) = DW
;nd0: 2
;NS: 8 * n
;DS: 16
;td1: number of experiments
;MC2: TPPI
```