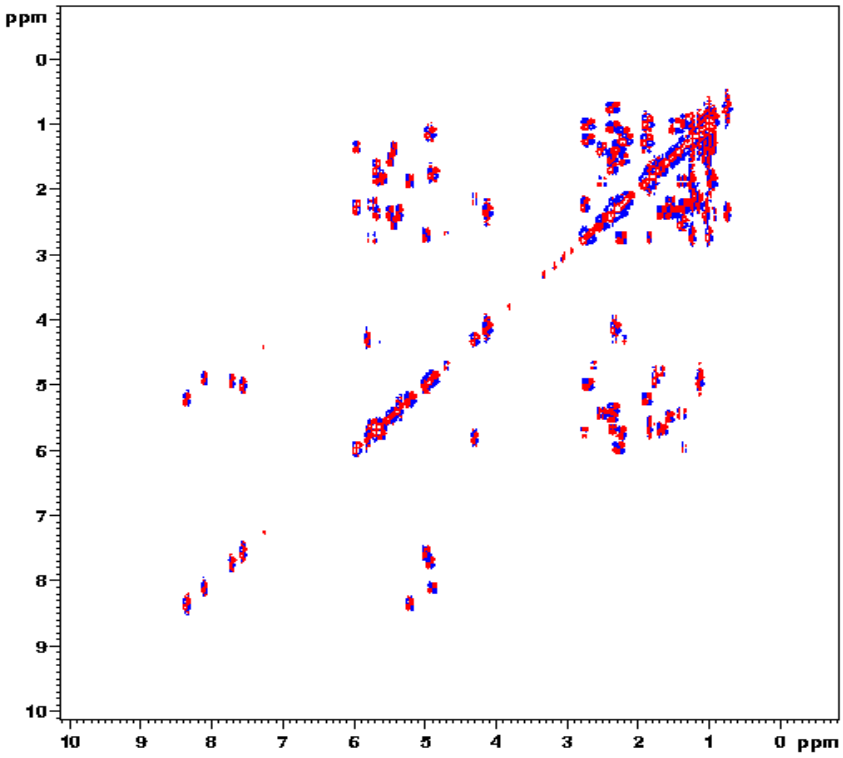
Avance 1D and 2D Course



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1 Introduction

This manual gives an introduction into basic one- and two-dimensional nuclear magnetic resonance (NMR) spectroscopy. After a short introduction the acquisition of basic 1D ^{1}H and ^{13}C NMR spectra is described in the Chapters 3 to 7. Homonuclear 2D [^{1}H,^{1}H] correlation spectra are described in Chapter 8 (COSY), 9 (TOCSY), 10 (ROESY) and 11 (NOESY). Heteronuclear 2D [^{13}C,^{1}H] correlation experiments are described in Chapter 12 (XHCORR), 13 (COLOC), 14 (HMQC) and 15 (HMBC). The Chapter 16 contains the description of inverse 2D [^{13}C,^{1}H] correlation experiments using pulsed field gradients, and some special NMR experiments are described in chapters 17 to 20. A brief introduction to NMR automation with the IconNMR program is given in chapter 21.

1.1 An Important Note on Power Levels

Several times throughout this manual, the user is asked to set the power levels **pl**1, **pl**3, etc. to the “high power” level for the corresponding channel (f1 or f2). ***In order to avoid damaging the probehead or other hardware components***, the user is advised to use only the power levels indicated in Table 1 below, if no other information (e.g. final acceptance tests) is available.

Note that these “power levels” are really attenuation levels, and so a higher value corresponds to a lower power. Also note that these power levels pertain ***only*** to the specific spectrometers and amplifiers listed below, which correspond to the AVANCE instruments as of July 2000. It is assumed that no correction tables (CORTAB) are existing.

Table 1: Suggested “Proton and Carbon High Power” Levels for Avance Instruments

Nucleus	Spectrometer	Amplifier	Power Level
^{1}H	Avance	BLA2BB	≥ + 3dB
		BLARH100	≥ + 3dB
		BLAXH300/50	≥ 0dB
	Avance DPX	BLAXH20	= - 6dB
		BLAXH40	= - 3dB
		BLAXH100/50	≥ 0dB
	Avance DRX	BLAXH150/50	≥ 0dB
		BLAXH300/50	≥ 0dB
	Avance DMX	BLARH100	≥ + 3dB

Nucleus	Spectrometer	Amplifier	Power Level
^{13}C	Avance	BLA2BB	≥ + 6dB
		BLAX300/50	≥ + 6dB
		BLAX300	≥ + 6dB
		BLAX500	≥ + 9dB
	Avance DPX	BLAXH20	= - 6dB
		BLAXH40	= - 6dB
		BLAXH100/50	≥ - 3dB
	Avance DRX	BLAXH40	≥ - 3dB
		BLAXH150/50	≥ 0dB
		BLAXH300/50	≥ 6dB
	Avance DMX	BLAX300	≥ + 6dB
		BLAX500	≥ + 9dB

1.2 NMR Spectrometer

The NMR spectrometer consists of three major components: (1) The superconducting magnet with the probe, which contains the sample to be measured; (2) The console, which contains all the electronics used for transmission and reception of radio frequency (rf) pulses through the pre-amplifier to the probe; (3) The computer, from where the operator runs the experiments and processes the acquired NMR data.

1.3 Classical Description of NMR

A more complete theoretical description of NMR is given in chapter 22.

Among the various atomic nuclei, about a hundred isotopes possess an intrinsic angular momentum, called spin and written $\hbar I$. They also possess a magnetic moment m which is proportional to their angular momentum:

$$m = g\hbar I$$

where g is the gyromagnetic ratio.

The Larmor theorem states that the motion of a magnetic moment in a magnetic field B_0 is a precession around that field, where the precession frequency is given by:

$$w_0 = -gB_0 \quad \text{Larmor frequency}$$

By convention, the external static field (B_0) is assumed to be along the z-axis and the transmitter/receiver coil along either the x- or y-axis. After the sample has been inserted into the magnetic field it shows a magnetization vector $\vec{M}$ along the z-axis. In this state, no NMR signal is observed, as we have no tranverse rotating magnetization.

By application of an additional rotating magnetic field B_1 in the x-y-plane, the orientation of $\vec{M}$ can be tilted into the x-y plane where it precesses around the total magnetic field, e.g. the vector sum of B_0 and B_1. Such a rotating magnetic field is obtained by applying rf-pulses, and the components of $\vec{M}$ are described by the Bloch equations:

$$\frac{d}{dt} M_x^r = 0$$

$$\frac{d}{dt} M_y^r = gB_1 M_z$$

$$\frac{d}{dt} M_z = -M_y^r gB_1$$

Assuming the magnetization at time 0 to be along the z-axis with amplitude M_0, we find the following solution to the above equation:

$$M_y^r(t) = M_0 \sin(gB_1 t)$$

$$M_z(t) = M_0 \cos(gB_1 t)$$

The magnetization vector is precessing around the B_1 axis which is aligned with the x-axis of the reference system. If we choose the time t of suitable duration, we obtain:

$$b = gB_1 t = \frac{p}{2}$$

which is defined as the 90 degree pulse creating maximum y-magnetization, which in turn yields maximal signal intensity.

1.4 Spin Operators of a One-Spin System

All NMR experiments start from the thermal equilibrium. In thermal equilibrium, the classical description gives a magnetic moment parallel to the static field, M_z. In the Spin Operator formalism, this is described by:

$$s_{eq} = I_z$$

where σ_{eq} is the equilibrium density matrix representing the state of the spin system under investigation.

Now there are only two basic types of evolutions: (1) An external perturbation, e.g. a rf-pulse, or (2) an unperturbed evolution which will eventually bring the system back to the thermal equilibrium.

1.4.1 Effect of rf-Pulses

The effect of an rf-pulse is that of a rotation along the pulse axes according to the following calculus rules:

$$I_z \xrightarrow{b_x} I_z \cos b - I_y \sin b$$
$$I_z \xrightarrow{b_y} I_z \cos b + I_x \sin b$$
$$I_x \xrightarrow{b_x} I_x$$
$$I_y \xrightarrow{b_y} I_y$$
$$I_x \xrightarrow{b_y} I_x \cos b - I_z \sin b$$
$$I_y \xrightarrow{b_x} I_y \cos b + I_z \sin b$$

If the flip angle β = 90° then:

$$\mathrm{I_z} \xrightarrow{90_{y,x}} \pm \mathrm{I_{x,y}}$$
$$\mathrm{I_{x,y}} \xrightarrow{90_{y,x}} \mp \mathrm{I_z}$$

We find the expected result, that a 90° pulse will generate transverse magnetization. The rest of this chapter will be concerned with finding out about the fate of this transverse magnetization in time.

We introduced tacitly the arrow notation, where we find on the left side the system before and on the right side after the specific evolution under the operator noted above the arrow. This notation is simple, very convenient and not only limited to the description of rf-pulses. We will discuss this notation in more details in the next section.

1.4.2 Effect of Chemical Shift Evolution

The so-called chemical shift Hamiltonian is given by:

$$\mathrm{H} = d \cdot I_z$$

where d is the chemical shift of the corresponding nucleus in the NMR spectrum ($d = w_0 - w$ where w_0 is the Larmor frequency of the spin and ω the carrier frequency of the interaction frame).

The calculus rules for the chemical shift evolution are the following:

$$\mathrm{I_z} \xrightarrow{\delta \cdot \mathrm{I_z} \cdot \mathrm{t}} \mathrm{I_z}$$
$$\mathrm{I_x} \xrightarrow{\delta \cdot \mathrm{I_z} \cdot \mathrm{t}} \mathrm{I_x} \cos(\delta\, \mathrm{t}) + \mathrm{I_y} \sin(\delta\, \mathrm{t})$$
$$\mathrm{I_y} \xrightarrow{\delta \cdot \mathrm{I_z} \cdot \mathrm{t}} \mathrm{I_y} \cos(\delta\, \mathrm{t}) - \mathrm{I_x} \sin(\delta\, \mathrm{t})$$

The time *t* is the period, during which the Hamiltonian is valid. The Hamiltonian of a spin system can change with time, for example if the experimental setup prescribes first a rf-pulse and then a period of unperturbed evolution. For the calculus rules it is mandatory, that each Hamiltonian is time independent during the time *t*.

What's the general idea? The whole NMR experiment is divided into time intervals, during which the Hamiltonian can be made time independent by

choice of a suitable interaction frame. Typical experiments are divided in pulse intervals and free evolution times.

During the pulses, the chemical shift and scalar coupling interaction is ignored. Only the applied B_1 field is considered. This approach is justified for pulses with $t_{pulse} \ll T_1, T_2$.

1.4.3 Effect of Scalar Coupling

Apart from the chemical shift, there is a second very import interaction between spins, the scalar coupling. The scalar depends on the mediation of electrons, which are confined in orbitals around both nuclei. The scalar coupling is expressed in Hz and noted as J. The operator expression for the scalar coupling is:

$$2\pi J_{12} I_{1z} I_{2z}$$

The above Hamiltonian expresses the scalar coupling between spin 1 and spin 2 with a coupling constant J_{12}. The evolution Hamiltonian for this spin system is then:

$$H = \delta_1 I_{1z} + \delta_2 I_{2z} + 2\pi J_{12} I_{1z} I_{2z}$$

To calculate the effect of this Hamiltonian, it is divided into 3 parts:

$$\delta_1 I_{1z}$$
$$\delta_2 I_{2z}$$
$$2\pi J_{12} I_{1z} I_{2z}$$

which are applied in sequence, where this sequence is arbitrary. After a 90° pulse has been applied to the two spins, we first calculate the two chemical shift terms:

$$\begin{aligned}\sigma_{eq} = I_{1z} + I_{2z} &\xrightarrow{\delta_1 \cdot I_{1z} \cdot t} I_{1x}\cos(\delta_1 t) + I_{1y}\sin(\delta_1 t) + I_{2z} \\ &\xrightarrow{\delta_2 \cdot I_{2z} \cdot t} I_{1x}\cos(\delta_1 t) + I_{1y}\sin(\delta_1 t) \\ &\qquad + I_{2x}\cos(\delta_2 t) + I_{2y}\sin(\delta_2 t) \Rightarrow \sigma_1\end{aligned}$$

The next step will be to compute the evolution under the scalar coupling.

The scalar coupling term can be evaluated with a simple set of rules:

$$\begin{aligned}
I_{1z} &\xrightarrow{2\pi J_{12} I_{1z} I_{2z} t} I_{1z} \\
I_{1x} &\xrightarrow{2\pi J_{12} I_{1z} I_{2z} t} I_{1x}\cos(\pi J_{12} t) + 2 I_{1y} I_{2z}\sin(\pi J_{12} t) \\
I_{1y} &\xrightarrow{2\pi J_{12} I_{1z} I_{2z} t} I_{1y}\cos(\pi J_{12} t) - 2 I_{1x} I_{2z}\sin(\pi J_{12} t) \\
2 \cdot I_{1x} I_{2z} &\xrightarrow{2\pi J_{12} I_{1z} I_{2z} t} 2 I_{1x} I_{2z}\cos(\pi J_{12} t) + I_{1y}\sin(\pi J_{12} t) \\
2 \cdot I_{1y} I_{2z} &\xrightarrow{2\pi J_{12} I_{1z} I_{2z} t} 2 I_{1y} I_{2z}\cos(\pi J_{12} t) - I_{1x}\sin(\pi J_{12} t) \\
2 \cdot I_{1x} I_{2y} &\xrightarrow{2\pi J_{12} I_{1z} I_{2z} t} 2 I_{1x} I_{2y}
\end{aligned}$$

which can then be applied to the various terms of s_1 above:

$$
\begin{aligned}
\sigma_1 \xrightarrow{2\pi J_{12}\, I_{1z} I_{2z}\, t} & \{I_{1x}\cos(\pi J_{12} t) + 2\, I_{1y} I_{2z} \sin(\pi J_{12} t)\} \cdot \cos(\delta_1 t) \\
& + \{I_{1y}\cos(\pi J_{12} t) - 2\, I_{1x} I_{2z} \sin(\pi J_{12} t)\} \cdot \sin(\delta_1 t) \\
& + \{I_{2x}\cos(\pi J_{12} t) + 2\, I_{1z} I_{2y} \sin(\pi J_{12} t)\} \cdot \cos(\delta_2 t) \\
& + \{I_{2y}\cos(\pi J_{12} t) - 2\, I_{1z} I_{2x} \sin(\pi J_{12} t)\} \cdot \sin(\delta_2 t) \\
& = \sigma_2
\end{aligned}
$$

References: O. W. Sørensen, G.W. Eich, M. H. Levitt, G. Bodenhausen, R. R. Ernst, *Progres in NMR Spectroscopy*, **16**, 163 (1983).

1.5 Sensitivity of NMR Experiments

The sensitivity of NMR experiments is given by the signal to noise ratio:

$$S/N = \frac{N g_{exc} T_2 \left(g_{det} B_0\right)^{3/2} \sqrt{ns}}{T}$$

S/N	=	signal to noise ratio
N	=	number of spins in the system (sample concentration)
g_{exc}	=	gyromagnetic ratio of the excited nucleus
g_{det}	=	gyromagnetic ratio of the detected nucleus
ns	=	number of scans
B_0	=	external magnetic field
T_2	=	transverse relaxation time (determines the line width)
T	=	sample temperature

(Comment: here we can already see that it might be useful for a better signal to noise ratio to excite one kind of nuclei and detect another kind with a better gyromagnetic ratio in the same experiment. This is done in inverse experiments which are described in sections 14 to 16).

1.6 Useful Coupling Constants

Many NMR constants such as chemical shift ranges, sensitivities, common NMR solvent properties etc. can be found in the Bruker Almanac. Here we added the values of some common coupling constants that are used more often as parameters (**`cnst1`** – **`cnst5`**) in some pulse programs.

1.6.1 Coupling Constants: $^nJ_{CH}$

As a rule of thumb it is possible to estimate the $^1J_{CH}$ coupling constant from the following equation: $^1J_{CH}$ ~ 500*(fractional CH s character). That is: 125Hz < $^1J_{CH}$ < 250Hz, so that $^1J_{CH}$ = 145Hz is a good approximation in most cases.

The values of $^2J_{CH}$ coupling constants increase with increasing HCαCβ angles and with the electronegativity of the Cβ substituent. They vary between –5 and 50Hz.

The $^3J_{CH}$ coupling constants are mostly positive and are maximal at CCCH angles of 0° and 180°. The values for *trans* couplings are larger as for *cis* couplings (Karplus relation).

Table 2: Useful CH Coupling Constants

Compound	**$^1J_{CH}$ in Hz**
Ethane	124.9
Acetonitrile	136.0
Ethene	156
Benzene	159
Dichloromethane	178.0
Chloroform	209.0
Formaldehyde	222.0

System	**$^2J_{CH}$ in Hz**
$C(sp^3)C(sp^3)H$	-10 to +6
$C(sp^3)C(sp^2)H$	0 to +30
$C(sp^2)C(sp^3)H$	-7 to -4
$C(sp^2)C(sp^2)H$	-4 to +14

System	**$^3J_{CH}$ in Hz**
$C(sp^3)C(sp^3)C(sp^3)H$	0 to 8
$C(sp^3)C(sp^2)\ C(sp^2)H$	0 to 20
$C(sp^2)C(sp^2)\ C(sp^3)H$	0 to 20
	$J_{trans} > J_{cis}$

References: H.-O. Kalinowski, S. Berger, S. Braun; *^{13}C-NMR-Spektroskopie;* Georg Thieme Verlag; Stuttgart, New York.

1.6.2 Coupling Constants of Hydrocarbons: $^nJ_{HH}$

Usually $^2J_{HH}$ coupling constants are negative and vary in a range between -0.5Hz and -15Hz in hydrocarbons. $^3J_{HH}$ coupling constants are mostly positive and usually range from 2 up to 18Hz. The $^{n>3}J_{HH}$ coupling is positive or negative with smaller absolute values, that range from 0 to 3Hz. The Karplus relation is also valid: $J_{trans} > J_{cis}$.

Table 3: Useful HH Coupling Constants

System	**$^2J_{HH}$ in Hz**
$HC(sp^3)H$	-12 to -15
$HC(sp^2)H$	-0.5 to -3

System	**$^3J_{HH}$ in Hz**
$HC(sp^3)C(sp^3)H$	2 to 9
$HC(sp^3)C(sp^2)H$	4 to 10
$HC(sp^2)C(sp^2)H$	6 to 18
$HC(sp^3)CHO$	1 to 3
$HC(sp^2)CHO$	2 to 4

System	**$^4J_{HH}$ (abs. value) in Hz**
$HC(sp^3)C(sp^3)C(sp^3)H$	0
$HC(sp^3)C(sp^2)C(sp^2)H$	0 to 3
$HC(sp)C(sp)C(sp^3)H$	2 to 3

Heteroatoms with considerable I or M effect can shift the J values dramatically.

2 Preparing for Acquisition

2.1 Sample Preparation

The sample quality can have a significant impact on the quality of the NMR spectrum. The following is a brief list of suggestions to ensure high sample quality:

- Always use clean and dry sample tubes to avoid contamination of the sample.
- Always use high quality sample tubes to avoid difficulties with shimming.
- Filter the sample solution.
- Always use the same sample volume or solution height (recommended values: 0.6 ml or 4 cm of solution for 5 mm sample tubes, 4.0 ml or 4 cm of solution for 10 mm sample tubes). This minimizes the shimming that needs to be done between sample changes.
- Use the depth gauge to position the sample tube in the spinner. This is discussed further in Chapter 5 ‘Sample Positioning' of the BSMS User's Manual.
- Check that the sample tube is held tightly in the spinner so that it does not slip during an experiment.
- Wipe the sample tube clean before inserting it into the magnet.
- For experiments using sample spinning, be sure that the spinner, especially the reflectors, are clean. This is important for maintaining the correct spinning rate.

2.2 Bruker NMR software

There are three major tasks that are controlled by the NMR software: acquisition, processing and plotting. The XWinNMR program is the user interface for all of these tasks. The commands can either be called up by selecting the menu items or by typing the appropriate command in the command line followed by RETURN. There are many parameters that are important for each job and they can be accessed and edited by the user. These parameters and the measured data as well as the processed spectra are stored in datasets which are specified by names, experiment numbers (expno) and processing numbers (procno).

Each parameter can be accessed directly by entering it's name in the command line followed by RETURN or in the **eda**, **edp** or **edg** window for acquisition-, processing- or plotting parameters respectively. Since these panels contain all possible parameters and are rather large, it is often more convenient to use somewhat more reduced parameter editor interfaces. The **ased** command opens the panel for the acquisition parameters that are of importance only for the selected pulse program. Here the parameters are also commented on.

2.2.1 Predefined Parameter Sets

The XWinNMR philosophy is to work with predefined parameter sets that are suitable for most of the NMR tasks and experiments you are facing. These parameter sets include the pulse program, acquisition and processing AU programs as well as all other necessary parameters except spectrometer specific values for pulse lengths and power levels. These standard parameter sets usually have the same base name as the corresponding pulse program. Each parameter set can be called up into a dataset of your choice by the command **rpar**. You can modify the parameters and save the new parameter set by the command **wpar**. Bruker predefined parameter sets are written in capital letters, and we recommend that you do not change them but rather create new ones that you can use just as well.

Therefore the most simple way to run a certain experiment is to create a new dataset with a specific name, using the command **edc**. Then you would read the corresponding parameter set by **rpar** (i.e. **rpar PROTON all**), set the pulse lengths and power levels by **getprosol** and type **xaua** to start the acquisition. (It is assumed that the sample is shimmed and the probe is matched and tuned for the specific nuclei). If you are using the Bruker predefined parameter sets, you can always process the data by typing **xaup**.

The following list is a short summary of the most commonly used experiments and the corresponding parameter sets. The emphasis is on the spectroscopic information that you will get from the experiments rather than on the type of experiment. (For the experiments in this table, it is always recommended to use the gradient version of the experiment if you have the required z-gradient hardware. These experiments usually require less time than the ones without gradients).

Table 4: Short List of Typical Experiments, Parameter Sets and What They Do

Atom / Group	Information (1D Experiments)	a.k.a.	Parameter Set
H	^{1}H chemical shift and coupling	1D^{1}H	PROTON
C	^{13}C chemical shift, ^{1}H decoupled (signal enhancement, integration not possible)	1D^{13}C	C13CPD
C	^{13}C chemical shift, ^{1}H coupled (signal enhancement, integration not possible)	1D^{13}C	C13GD
CH, CH_2, CH_3	^{13}C chemical shift, select CH, CH_2 and CH_3 signals only (same phase)	DEPT45	C13DEPT45
CH	^{13}C chemical shift, select CH signals only	DEPT90	C13DEPT90
CH, CH_3	^{13}C chemical shift, select CH and CH_3 signals only (opposite phase)	DEPT135	C13DEPT135

Correlation	Information (2D Experiments)	a.k.a.	Parameter Set
H–H	^{1}H/^{1}H nearest neighbor, through bond chemical shift correlation	COSY	COSYGPSW[1] COSY45SW
H–H	^{1}H/^{1}H nearest neighbor, through bond chemical shift correlation plus coupling constants	DQF-COSY	COSYGPDFPHSW[1] COSYDQFPHSW
H–(–)$_n$H	^{1}H/^{1}H total spin system through bond chemical shift correlation	TOCSY	MLEVPHSW
C–H	Sensitive ^{1}H/^{13}C directly bound chemical shift correlation (one bond), lower resolution in ^{13}C dimension	HSQC HMQC	HSQCGPPH[1] HMQC
C–H	Sensitive ^{1}H/^{13}C directly bound chemical shift correlation (one bond), lower resolution in ^{13}C dimension (small molecules, solemnly select ^{13}C/^{1}H not ^{12}C/^{1}H)	BIRD-HMQC	HMQCBI
C–H	Insensitive ^{1}H/^{13}C directly bound chemical shift correlation (one bond), high resolution in ^{13}C dimension	HETCOR	HCCOSW
C–(–)$_n$H	Sensitive ^{1}H/^{13}C long range chemical shift correlation (more than one bond), lower resolution in ^{13}C dimension	HMBC	HMBCGPLPND[1] HMBCLPND
C–(–)$_n$H	Insensitive ^{1}H/^{13}C long range chemical shift correlation (one and more bonds), high resolution in ^{13}C dimension	COLOC	HCCOLOCSW
H···H	^{1}H/^{1}H non bound structural neighbor, through space chemical shift correlation (small molecules, low fields)	ROESY	ROESYPHSW
H···H	^{1}H/^{1}H non bound structural neighbor, through space chemical shift correlation (large molecules, proteins)	NOESY	NOESYPHSW

In most of the 2D parameter sets there is a spectral width optimization implemented (*PULSEPROGRAM*SW). So if you acquire the corresponding 1D experiments in the previous experiment number the spectral width for the 2D will be optimized according to the 1D information.

A complete list of parameter sets can be called up by typing **`rpar`** without a following name. The nomenclature of the parameter sets follows the rules for

[1] z-gradient hardware required

the nomenclature of the pulse programs. They can be found in the file: $XWinNMRHome/exp/stan/nmr/lists/pp/Pulprog.info

However in this manual, we focus on the manual setup of the experiments from scratch and the optimization of the corresponding parameters. therefore the **rpar** command will not be used throughout this text.

2.2.2 XWinNMR parameters and commands

A list of commonly used acquisition and processing commands and parameter names as well as a description of the corresponding command or parameter is given in short in the tables below.

Table 5: General Commands and AU Programs

setres	customize the XwinNMR interface
edmac	edit or create an XWinNMR macro
edau	edit or create an XWinNMR AU program
edpul	edit or create an XWinNMR pulse program
xau listall_au	create a file called “listall” in your home directory with a list of all available AU programs including short descriptions
edcpul	edit the current pulse program

Table 6: Data Set Related Commands

edc, new	create a new data set, experiment number or processing number
xau iexpno	copy the current experiment number including all parameters to the consecutive experiment number
wrpa	copy of the current data set including the spectra
re	move to a specific experiment number within the data set
rep	move to a processing number within the experiment number
browse	browse the data set directories
search	find a specific data set
wpar	save the current parameters
rpar	select and read a predefined parameter set

Table 7: Acquisition Parameters

ns	number of scans
ds	number of dummy scans
td	Time domain, number of acquired data points
sw	sweep width in ppm
aq	acquisition time
o1p	transmitter frequency of f1 channel in ppm
o2p	transmitter frequency of f2 channel in ppm
rg	receiver gain
pulprog	definition of the pulse program
aunmp	definition of the acquisition AU program

Table 8: Acquisition and Pre-acquisition Commands

edhead	define the current probehead
edprosol	define probehead specific pulse lengths and power levels
getprosol	use probehead specific pulse lengths and power levels in the

	current pulse program
xau pulse	calculate the power level from pulse lengths and vice versa
edsp, edasp	configure the routing of the spectrometer
edcpul	open the current pulse program in a text editor window
eda	edit all acquisition parameters
ased, as	edit the acquisition parameters that are relevant for the current pulse program
ppg	graphical display of the current pulse program
spdisp	open the graphical pulse program editor
dpa	display all status parameters for the acquisition

wbchan	select the wobbling channel for tuning and matching
wobb	tuning and matching the probe
atma	automatic tune and match the ATM probe
atmm	manually tune and match the ATM probe
edsolv	define solvent parameters
edlock	define lock parameters for probhead and solvent
lock	Automatically lock on solvent (parameters defined in edlock)
lockdisp	open the lock display window
rsh	select and read shim values
gradshim	start the gradient shimming subprogram
wsh	save the current shim values

edte	open the temperature control window
edau	select or edit AU programs
stdisp	open the shape tool

expt	estimate the experiment time
rga	Automatically adjust the receiver gain
zg	start acquisition
xaua	start the acquisition AU program (this also starts the acquisition)
gs	Interactive adjustment of acquisition parameters
tr	data transfer during acquisition

halt, stop	stop the acquisition
kill	kill a specific process

Table 9: Processing Parameters

si	size of the real spectrum
phc0, phc1	Parameters for zero order and first order phase corrections
lb	line broadening factor for **em**
aunmp	definition of the processing AU program

Table 10: Processing Commands

edp	edit all processing parameters
dpp	display all status parameters for processing
ft	Fourier transform the current data
em	apply exponential window function
ef	combined command of **ft** and **em**
phase	set the phase correction defined by **phc0** and **phc1**
apk	Automatically phase correct the spectrum
abs	Automatically baseline correct and integrate the spectrum
efp	combined command of **ft**, **em** and **phase**

sr	spectral referencing
sref	Automatically calibrate the spectrum

edc2	select a second and a third data processing number
dual	invoke the dual display

edo	select an output device
edg	edit all graphics and plotting parameters
view	plot preview
xwinplot	start the plot program

Table 11: Pulse Program Specific Parameters

pl1	f1 channel – power level for pulse (default)
pl2	f2 channel – power level for pulse (default)
pl9	f1 channel – power level for presaturation
pl10	f1 channel – power level for TOCSY-spinlock
pl11	f1 channel – power level for ROESY-spinlock
pl12	f2 channel – power level for CPD/BB decoupling
pl14	f2 channel – power level for cw saturation
pl15	f2 channel – power level for TOCSY-spinlock

sp1	f1 channel – shaped pulse for selective excitation or f1 channel - shaped pulse for water flipback
sp2	f1 channel – shaped pulse 180 degree or f2 channel - shaped pulse 90 degree (on resonance)
sp7	f2 channel – shaped pulse 180 degree (off resonance2) or f2 channel – shaped pulse 180 degree (adiabatic) or f1 channel - shaped pulse for wet

p0	for different applications i.e. f1 channel - variable flip angle high power pulse in DEPT
p1	f1 channel - 90 degree high power pulse
p2	f1 channel – 180 degree high power pulse
p3	f2 channel - 90 degree high power pulse
p4	f2 channel – 180 degree high power pulse
p6	f1 channel - 90 degree low power pulse
p11	f1 channel - 90 degree shaped pulse (selective excitation or water flipback/watergate or wet)
p15	f1 channel – pulse for ROESY spinlock
p16	homospoil/gradient pulse
p17	f1 channel – trim pulse at pl10 or pl15
p18	f1 channel – shaped pulse (off resonance presaturation)

d0	incremented delay (2D) [3 usec]
d1	relaxation delay 1-5 * T1
d2	1/(2J)
d3	1/(3J)
d4	1/(4J)
d6	delay for evolution of long range couplings
d7	delay for inversion recovery
d8	NOESY mixing time
d9	TOCSY mixing time
d11	delay for disk I/O [30 msec]
d12	delay for power switching [20 usec]
d14	delay for evolution after shaped pulse

d16	delay for homospoil/gradient recovery
d17	delay for DANTE pulse-train
d18	delay for evolution of long range couplings
d19	delay for binomial water suppression
d20	for different applications

cnst0	for different applications
cnst1	J (HH)
cnst2	J (XH)
cnst3	J (XX)
cnst4	J (YH)
cnst5	J (XY)
cnst11	for multiplicity selection
cnst12	for multiplicity selection

vc	variable loop counter, taken from vc-list
vd	variable delay, taken from vd-list

l1	loop for MLEV cycle (((p6*64) + p5) * l1) + (p17*2) = mixing time
l2	loop for GARP cycle l2 * 31.75 * 4 * p9 => AQ
l3	loop for phase sensitive 2D or 3D using States et al. or States-TPPI method l3 = td1/2
l4	for different applications i.e. noediff

Note that the default units for pulses are microseconds (u), the units for delays are seconds (s), but one can always enter a value combined with a unit to define a time slot in XWinNMR. The nomenclature here is: **s** = seconds, **m** = milliseconds and **u** = microseconds. For example To set the value of **d1** to **500m** would define **d1** to last for half a second.

The complete information on the nomenclature and default usage of the pulse program parameters can be found in:
$XWinNMRHome/exp/stan/nmr/lists/pp/Param.info

The nomenclature and description of the standard pulse programs and predefined parameter sets can be found in:
$XWinNMRHome/exp/stan/nmr/lists/pp/Pulprog.info

Acquisition, processing and plotting commands can be given either in the XWinNMR command line or via menu selection. Examples are **zg**, which starts the acquisition, **ft** which performs a Fourier transformation on the current data or **apk** which invokes the automatic phase correction.

Another possibility to manage different task in XWinNMR are AU programs. They handle many routine jobs an can be selected or edited by the **edau** command. AU programs have to be compiled before first usage. Compile and start AU Programs by entering **xau** followed by the program name.

XWinNMR also offers extensive online documentation, which can be accessed via the help menu in the XWinNMR menu bar.

2.2.3 Changes for XWinNMR 3.5

XWinNMR version 3.5 is shipped with new systems now. There are some new commands and the handling of some pulse programs have changed from the software version 3.1.

- In XWinNMR 3.5 the names of pulse program and parameter files have been adjusted to the general NMR nomenclature. For recording HSQC, HMQC and HMBC spectra pulse program and parameter files starting with the 4 letter code hsqc, hmqc, and hmbc, respectively, have to be given in the **`pulprog`** line in the **`eda`** table.
- A new parameter **`TD0`** is now available in the **`eda`** table. This parameter brings about a storage of your 1D data after recording ns/TD0 scans. This is especially useful for very long 1D experiments.

For more information on general changes, please refer to the release letter of your software packet. Information for pulse program specific changes can be found in: $XWinNMRHome/exp/stan/nmr/lists/pp/Update.info

2.3 Tuning and Matching the Probe

In a probehead there are resonant circuits for each nucleus indicated on the probehead label (e.g., one for ^{1}H and one for ^{13}C in a dual ^{1}H/^{13}C probehead; one for ^{1}H and one for a wide range of nuclei in BBO or BBI probeheads). There is also a resonant circuit for the lock nucleus, but the standard user will never need to adjust this, so we will ignore it in the following. Each of the circuits has a frequency at which it is most sensitive (the resonance frequency). Once the sample is inserted, the probehead should be tuned and matched for these individual frequencies.

Tuning is the process of adjusting this frequency until it coincides with the frequency of the pulses transmitted to the circuit. For example, the frequency at which the ^{1}H resonant circuit is most sensitive must be set to the carrier frequency of the ^{1}H pulses (which is **`sfo1`** if the ^{1}H circuit is connected to the f1 channel, **`sfo2`** if it is connected to the f2 channel, etc.). Matching is the process of adjusting the impedance of the resonant circuit until it corresponds with the impedance of the transmission line connected to it. This impedance is 50 Ω. Correct matching maximizes the power that is transmitted to the coil. A probehead is said to be tuned and matched when all of its resonant circuits are tuned and matched. Once a probehead has been tuned and matched, it is not necessary to retune or rematch it after slight adjustments of the carrier frequency, since the probehead is generally tuned and matched over a range of a couple of hundred kHz. On the other hand, large adjustments to the carrier frequency, necessary when changing nuclei, warrant retuning and rematching of the probehead. Thus, a broadband probe needs to be retuned and rematched each time the heteronucleus is changed.

If you have an ATM probe, enter **edsp** and set the spectrometer parameters for the channels that should be matched and tuned. For 1H on channel F1 and 13C on channel F2 enter the following values:

NUC1	1H
NUC2	13C
NUC3	OFF

This automatically sets **sfo1** to a frequency appropriate for ^{1}H and **sfo2** to the corresponding ^{13}C frequency for tuning and matching. Exit **edsp** by clicking **SAVE**.

Type **atma**. This will invoke the automatic match and tune program for all nuclei that were selected previously in **edsp**. Therefore it is not necessary to tune and match manually.

2.4 Tuning and Matching ^{1}H (non ATM Probes)

When the NMR experiments to be performed are ^{1}H homonuclear experiments (e.g., ^{1}H 1D spectroscopy, COSY, NOESY, or TOCSY), only the ^{1}H circuit of the probehead has to be tuned and matched.

Make sure that the sample is in the magnet, and the probehead is connected for standard ^{1}H acquisition. Note that there is no special configuration for tuning and matching. Also, it is recommended to tune and match *without* sample spinning.

2.4.1 Set the Parameters

In XWIN-NMR, enter **edsp** and set the following spectrometer parameters:

NUC1	1H
NUC2	OFF
NUC3	OFF .

This automatically sets **sfo1** to a frequency appropriate for ^{1}H tuning and matching. There is no need to adjust **sfo1** carefully now. Exit **edsp** by clicking **SAVE**.

2.4.2 Start Wobbling

Tuning and matching are carried out simultaneously using XWIN-NMR. During wobbling, a low power signal is transmitted to the probehead. This signal is swept over a frequency range determined by the parameter **wbsw** (the default value is 4 MHz) centered around the carrier frequency (i.e., **sfo1**, **sfo2**, etc., depending on which nucleus is being tuned/matched). Within the preamplifier (High Performance Preamplifier Assembly or HPPR), the impedance of the probe over this frequency range is compared to the impedance of a 50 Ω resistor. The results are shown both on the LED display of the HPPR and in the acquisition submenu of XWIN-NMR. Both displays show the reflected power of the probehead versus the frequency of the

signal. The user observes either one or both of these displays while tuning and matching the probehead.

Before starting the wobbling procedure, ensure that no acquisition is in progress, e.g., enter **`stop`**.

Enter **`acqu`** to switch to the acquisition window of XWIN-NMR, if it is desired to use this to monitor the tuning and matching. Notice that being in the acquisition window slows down the wobbling procedure, so if the HPPR LED display will be used to monitor tuning and matching, it is best to remain in the main XWIN-NMR window and not to switch to the acquisition window.

Start the frequency sweep by typing **`wobb`**. The curve that appears in the acquisition window is the reflected power as a function of frequency. Unless the probehead is quite far from the correct tuning and matching, there will be a noticeable dip in the curve. When the ^{1}H circuit is properly tuned, the dip will be in the center of the window, denoted by the vertical marker; and when the circuit is properly matched, the dip will extend all the way down to the *x* axis. Similar information is conveyed by the LED display on the HPPR. The horizontal row of LED's indicates tuning and the vertical row matching. When the circuit is properly tuned and matched, the number of LEDs is minimized. This usually means that only green LED, are lit in both the horizontal and vertical displays.

2.4.3 Tune and Match

Adjust the tuning and matching screws (labeled T and M) at the base of the probehead. Note that the screws are color coded and those for the ^{1}H circuit are usually yellow. *Also note that the screws have a limited range and attempting to turn them beyond this range will damage the probehead*.

Since there is an interplay between tuning and matching, it is generally useful to adjust the T and M screws in an iterative fashion. Turn the M screw until the dip is well matched at some frequency (the dip extends to the *x* axis and the number of LEDs lit in the vertical HPPR display is minimized). Most likely this will ***not*** be the desired frequency. Adjust the T screw slightly to move the dip toward the center of the window, or equivalently, to reduce the number of LEDs lit in the horizontal HPPR display. Rematch the dip by adjusting the M screw again. Note that it is possible to run out of range on the M screw. If this happens, return M to the middle of its range, adjust T to get a well matched dip at some frequency, and walk the dip towards the correct frequency as described above.

As mentioned above, ideal tuning and matching is when the dip is centered in the window and extends to y = 0 (the *x* axis) on the acquisition window, or equivalently, when the number of LED's lit on the preamplifier is minimized in both the vertical and horizontal display.

When the ^{1}H circuit is tuned and matched, exit the wobble routine by typing **`stop`**. Click on **`return`** to exit the acquisition window and return to the main window.

2.5 Tuning and Matching ^{13}C (non ATM Probes)

Since most ^{13}C experiments make use of ^{1}H decoupling, besides ^{13}C the ^{1}H should be tuned and matched as well. When tuning and matching a probehead with multiple resonant circuits, it is best to tune and match the lowest frequency circuit first. Thus, when tuning and matching a probehead for both ^{1}H and ^{13}C, first do the ^{13}C and then the ^{1}H adjustments.

Make sure that the sample is in the magnet, and the probehead is connected for the appropriate experiment. Also, it is recommended to tune and match *without* sample spinning.

2.5.1 Set the Parameters

In XWIN-NMR, enter **edsp** and set the following spectrometer parameters:

NUC1	13C
NUC2	OFF
NUC3	OFF .

This automatically sets **sfo1** to a frequency appropriate for ^{13}C tuning and matching. Exit **edsp** by clicking **SAVE**.

2.5.2 Start Wobbling, Tune and Match

Ensure that no acquisition is in progress, enter **stop**.

Enter **acqu** to switch to the acquisition window, if this will be used to monitor the tuning and matching.

Start the frequency sweep by typing **wobb**. The curve that appears in the acquisition window is for ^{13}C. Adjust the tuning and matching following the guidelines given above for ^{1}H. Notice that some probeheads (e.g., broadband probeheads) have sliding bars instead of screws, one set labeled tuning and another labeled matching. Set the tuning and matching sliding bars to the values indicated for ^{13}C on the menu. Adjust the tuning and matching bars until the dip is well tuned and matched at some frequency as described above for ^{1}H.

Once the ^{13}C circuit is tuned and matched, the ^{13}C wobbling must be stopped before the ^{1}H wobbling. Exit the wobble routine by typing **stop**. Enter **edsp**, change NUC1 to 1H, and exit by clicking **SAVE**. Start the ^{1}H frequency sweep by typing **wobb**. After a few seconds the ^{1}H curve appears in the acquisition window and the ^{1}H circuit can be tuned and matched as described above.

Alternatively, if the user already has a data set in which NUC1 = 1H and NUC2 = OFF, there is no need to redo **edsp** for the current data set. The user may simply read in the ^{1}H data set and then type **wobb**.

Once the probehead is tuned and matched for ^{13}C and ^{1}H, exit the wobble routine by typing **stop**.

Click on [return] to exit the acquisition window and return to the main window.

2.6 Locking and Shimming

Before running an NMR experiment, it is necessary to lock and shim the magnetic field.

2.6.1 Locking

To display the lock signal enter **lockdisp**. This opens a window in which the lock trace appears.

The most convenient way to lock is to use the XWIN-NMR command **lock**. To start the lock-in procedure, enter **lock** and select the appropriate solvent from the menu. Alternatively, enter the solvent name together with the lock command, e.g., **lock cdcl3**. During lock-in, several parameters such as the lock power, the field value, and the frequency shift for the solvent are set according to the values in the lock table. This table can be edited using the command **edlock**. Note that the lock power listed in this table is the level used once lock-in has been achieved. The field-shift mode is then selected and autolock is activated. Once lock-in is achieved, the lock gain is set so that the lock signal is visible in the lock window. At this point the message "lock: finished" appears in the status line at the bottom of the window.

The lock-in procedure outlined above sets the frequency shift to the exact frequency shift value for the given solvent as listed in the **edlock** table. It also sets the field value to the value listed in the **edlock** table and then adjusts it slightly to achieve lock-in (the absolute frequency corresponding to a given ppm value no longer depends on the lock solvent). Following this lock-in procedure, the **solvent** parameter in the **eda** table is set automatically, which is important if you wish to use the automatic calibration command **sref** (see "Spectrum Calibration and Optimization").

The lock-phase adjustment by monitoring the sweep wiggles (i.e., while the field is not locked but is being swept) is recommended each time the probehead is changed, because autolock may fail. If the original phase is reasonably close to the correct value, lock-in can be achieved and the phase can be adjusted using autophase. Note that the lock phase for each probehead is stored in the **edlock** table. In some cases, the lock power level listed in the **edlock** table is set too high leading to a saturation of the lock signal. Usually, lock-in can be achieved, but the signal oscillates due to saturation. A quick fix is simply to reduce the lock power manually after lock-in. However, it is better to change the power level in the **edlock** table. Note that the appropriate lock power level depends on the lock solvent, the field value, and the probehead.

2.6.2 Shimming

If the sample has been changed, the first step after locking is shimming the magnetic field. Enter **rsh** and select an appropriate shim file from the menu. Usually, only the Z and Z^2 shims (and probably the X and Y) must be adjusted while observing the lock signal. The best shim values correspond to the highest lock level (height of the lock signal in the window). For further

discussion of shimming see Chapter 6 ‘Shim Operation' of the BSMS User's Manual.

If you have a gradient probe, you can also use the gradient shimming tool, which can be started by the command **`gradshim`**. For more Information, please refer to the gradient shimming installation and users guide which is available online in the XWinNMR help menu.

2.6.3 Optimize lock settings (optional)

Once the magnetic field has been locked and shimmed, the user may wish to optimize the lock settings as described below. It is strongly recommended to follow this procedure before running any experiment requiring optimal stability (e.g., NOE difference experiments).

After the field is locked and shimmed, start the auto-power routine from the BSMS keyboard (see Chapter 2 ‘Key Description' of the BSMS User's Manual). For lock solvents with long T_1 relaxation times (e.g., $CDCl_3$), however, auto power may take an unacceptably long time and the lock power should be optimized manually. Simply increase the lock power level until the signal begins to oscillate (i.e., until saturation), and then reduce the power level slightly (approximately 3 dB). For example, if the lock signal begins to oscillate at a power of –15 dB, the optimal magnetic field stability can be expected when a level of approximately –18 dB (or even –20 dB) is used. The field stability will be significantly worse if a power level of, say, –35 dB is used instead.

When the lock power is optimized, start the auto-phase routine, and finally the auto-gain routine. Take note of the gain value determined by auto gain. Using this value, select the appropriate values for the loop filter, loop gain, and loop time as shown below in Table 12.

Table 12: Lock Parameters (BSMS Firmware Version 980930)

Lock RX Gain (after auto gain) [dB]	**Loop Filter [Hz]**	**Loop Gain [dB]**	**Loop Time [sec]**
120	20	–17.9	0.681
	30	–14.3	0.589
110	50	–9.4	0.464
	70	–6.6	0.384
	100	–3.7	0.306
	160	0.3	0.220
	250	3.9	0.158
	400	7.1	0.111
90	600	9.9	0.083
	1000	13.2	0.059
	1500	15.2	0.047
	2000	16.8	0.041

So, for example if auto gain determines a lock gain of 100 dB, the user should set the loop filter to 160 Hz, the loop gain to 0.3 dB, and the loop time to 0.220 sec (see Chapter 4 ‘Menu Description' of the BSMS User's Manual for how to set these parameters from the BSMS keyboard).

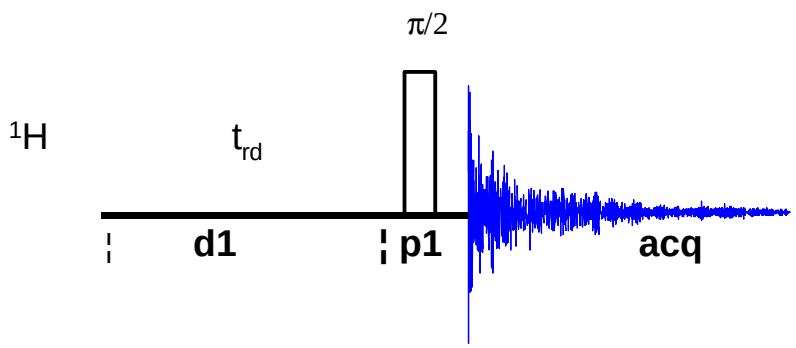
3 Basic ^{1}H Acquisition and Processing

3.1 Introduction

This chapter describes the acquisition and processing of a 1D ^{1}H NMR spectrum using the simple one-pulse NMR experiment shown in Figure 1. The pulse sequence consists of the recycling delay, t_{rd}, the radio-frequency (RF) pulse, and the acquisition time during which the signal is recorded. The pulse angle is shown to be $\pi/2$, although, in practice, it is often chosen less. The two parameters, **d1** and **p1**, correspond to the length of the recycle delay, t_{rd}, and the length of the RF pulse, respectively.

Note that the time intervals depicted in the pulse sequence diagrams are not drawn to scale. For example, **d1** is typically a few seconds while **p1** is typically a few microseconds in length.

Figure 1: 1D ^{1}H NMR One-Pulse Sequence



3.1.1 Sample

The sample used for demonstrating the basic 1D ^{1}H experiment is 100 mg Cholesterylacetate in $CDCl_3$ with 0.5 % TMS.

3.1.2 Preparation

Make sure that you have done the following steps (see also Chapter 2 'Preparing for Acquisition'):

- Insert a suitable probehead
- Read in the corresponding shim file
- Insert the sample
- Lock the spectrometer

- Optimize the Z and Z^2 (and probably X and Y) shims
- Tune and match the probehead for ^1H

3.2 Spectrometer and Acquisition Parameters

Before the acquisition of a spectrum a new data set must be created. All the spectrometer and acquisition parameters are entered within the new data set.

The spectrometer parameters are responsible for the hardware settings necessary for configuring the spectrometer for a particular experiment. The command **edsp** calls up a window in which the spectrometer parameters for the observe and the decoupler channel(s) are set.

The acquisition parameters include all pulse sequence parameters, the number of data points, number of scans, receiver gain, and many others. These may be displayed and edited by entering **eda**. Notice that the spectrometer parameters are also listed in the **eda** table. It is important to set the spectrometer parameters before setting the acquisition parameters, because the values from **edsp** automatically overwrite the corresponding ones from the **eda** table.

3.3 Create a New File Directory for the Data Set

To create a new data set, type **edc** in the command line of the XWIN-NMR window. This calls up a small window entitled “Current Data Parameters”. Enter a data set name (NAME), an experiment number (EXPNO), a processed data number (PROCNO), the disk unit (DU) where the data is stored, the user id (USER), and the data type (TYPE). Change the parameters as follows:

NAME	proton
EXPNO	1
PROCNO	1

Click on **SAVE**. This exits **edc** and creates the data set proton/1/1. The message “NEW DATA SET” should now appear on the screen.

3.4 Set Up the Spectrometer Parameters

Enter **edsp** and set the following spectrometer parameters:

NUC1	1H
NUC2	off
NUC3	off

Since there is no decoupling, the only relevant spectrometer parameters are SFO1. Click on **SAVE** to save the spectrometer parameters and return to the main window. The spectrometer is now prepared to pulse and detect at the ^{1}H frequency.

3.5 Set Up the Acquisition Parameters

Enter **eda** and set the acquisition parameters as shown in Table 13, where only the relevant parameters are listed. Note that the parameters **d1**, **p1**, and **pl1** are included in the parameter arrays D, P and PL in the **eda** table, respectively. These parameters can be edited within **eda**, by clicking the ‘ **Array**-button next to the corresponding parameter

Table 13: Basic ^{1}H Spectrum Acquisition Parameters

Parameter	Value	Comments
PULPROG	zg	see Figure 1 for the pulse sequence diagram
AQ_mod	DQD	If DQD is not available, use qsim
TD	32 k	32 k is a standard value for a high-resolution 1D spectrum
PARMODE	1D	One-dimensional experiment
NS	1	one scan is recorded for parameter optimization
DS	0	no dummy scans are recorded
D1	2	the default unit for delays is seconds; entering “2” sets a delay of 2 seconds (click the D**Array** button)
P1	3	the default unit for pulse lengths is microseconds; entering “3* sets a pulse length of 3 microseconds (µs) (click the P**Array** button)
PL1	PL1 =	power level for the p1 pulse see also “An Important Note on Power Levels” on page 3 (click the P**Array** button)
SW	50	for the first spectrum of an unknown sample use a large spectral width; when you enter “50” the registered value is slightly different
RG	64	suggested receiver gain
NUC1	1H	observe nucleus
O1P	15	position of the carrier frequency is 15 ppm

Click on **SAVE** to save the acquisition parameters and return to the main window. Click on **DONE** to save the changes and return to the **eda** table. As with most acquisition parameters, however, **d1**, **p1**, and **pl1** can also be edited by typing them in the command line of the main XWIN-NMR window. As mentioned before, most of the acquisition parameters for the current pulse program can also be entered in the **ased** table.

3.6 Acquisition

Enter **acqu** to switch to the acquisition window. While it is possible to acquire a spectrum from the main window, the buildup of the FID can only be observed in the acquisition window.

Enter the command **rga**, which performs several acquisitions and sets a suitable value for the receiver gain (**rg**). Enter **zg**, which deletes any previous data (zero') and starts the experiment (go'). The message **scan 1/1** indicates that the spectrometer is performing the first scan and that only one scan will be performed.

If, at any time, a submenu is entered accidentally, click on the return button located on the button bar and then enter **acqu** to switch back to the acquisition window.

If, at some point the message “DATA OUT OF WINDOW” appears, or if the scaling is unsuitably large or small, click on the and buttons located on the button bar.

3.7 Processing

After the FID has been acquired the next step is to process the acquired data. The processing parameters are displayed and edited by entering **edp**. First, Fourier transformation is performed by entering the command **ft**. The number of points used to resolve the resulting spectrum is determined by the processing parameter **si** (size). The spectrum consists of **si** real points and **si** imaginary points, and thus the default setting of **si** is **td**/2, where **td** is the acquisition parameter indicating the number of time domain data points. In general, **td**/2 and **si** are numbers described by powers of 2 (2, 4, 8, 16, 32, 64, 128, ...). If **si** < **td**/2 not all the time domain data is used for the Fourier transformation, and if **si** > **td**/2 the time domain data is zero-filled with 2(**si**) before the Fourier transformation. In 1D spectroscopy, it is often recommended to zero-fill once, i.e., to set **si** = **td**.

Check the value of **si**. Enter **si** and when prompted enter 32k (appropriate for **td** = 32 k). Enter **ft**: The display automatically switches from the acquisition window to the main window and displays the. The FID can still be viewed by returning to the acquisition window. If the *x* axis of the Fourier transformed spectrum is displayed in Hz, click on Hz/ppm to convert into a ppm scale. If necessary, use the buttons as described above to scale the spectrum.

You can zoom into a part of the spectrum by defining the appropriate 1D plot range. Move the cursor into the display window and press the left mouse button to tie the cursor to the spectrum. Move the cursor to one side of the desired zoom region and click the middle mouse button to define it. Move the cursor to the other side of the desired plot region and click the middle mouse button again to zoom into this region. To display the whole spectrum push the button.

3.8 Phase Correction

Once the spectrum is Fourier transformed it must be phase corrected. Click on phase to enter the phase correction submenu. Click on biggest for setting the reference for the 0th-order phase correction to the position of the biggest peak in the spectrum and adjusts its phase. To adjust the 0th-order phase manually, place the cursor on PH0 and hold down the left mouse button. Move the mouse until the reference peak is positive and the baseline on either side is as flat as possible.

Most likely, the peaks on either side of the reference peak are not yet phased correctly and require a 1st-order phase correction. To adjust the 1st-order phase correction, place the cursor on PH1 and hold down the left mouse button, and move the mouse until the peaks far from the reference point are also in-phase.

Note that it is advisable to select the reference peak for the 0th-order phase correction near one edge of the spectrum. However, for some samples the biggest peak will be located in the middle of the spectrum. In such cases, click on cursor and define the reference peak by moving the cursor onto the desired peak and clicking with the middle mouse button.

Once the spectrum is phased correctly, click on **return** to exit the submenu and save the phase corrections by selecting **Save & return**. The 0th- and 1st-order phase correction values are stored as processing parameters **phc0** and **phc1**, respectively. To quit the phase correction submenu without saving the corrections, simply click on **return** and select **return**. In either case, the display returns to the main menu and the spectrum appears on the screen.

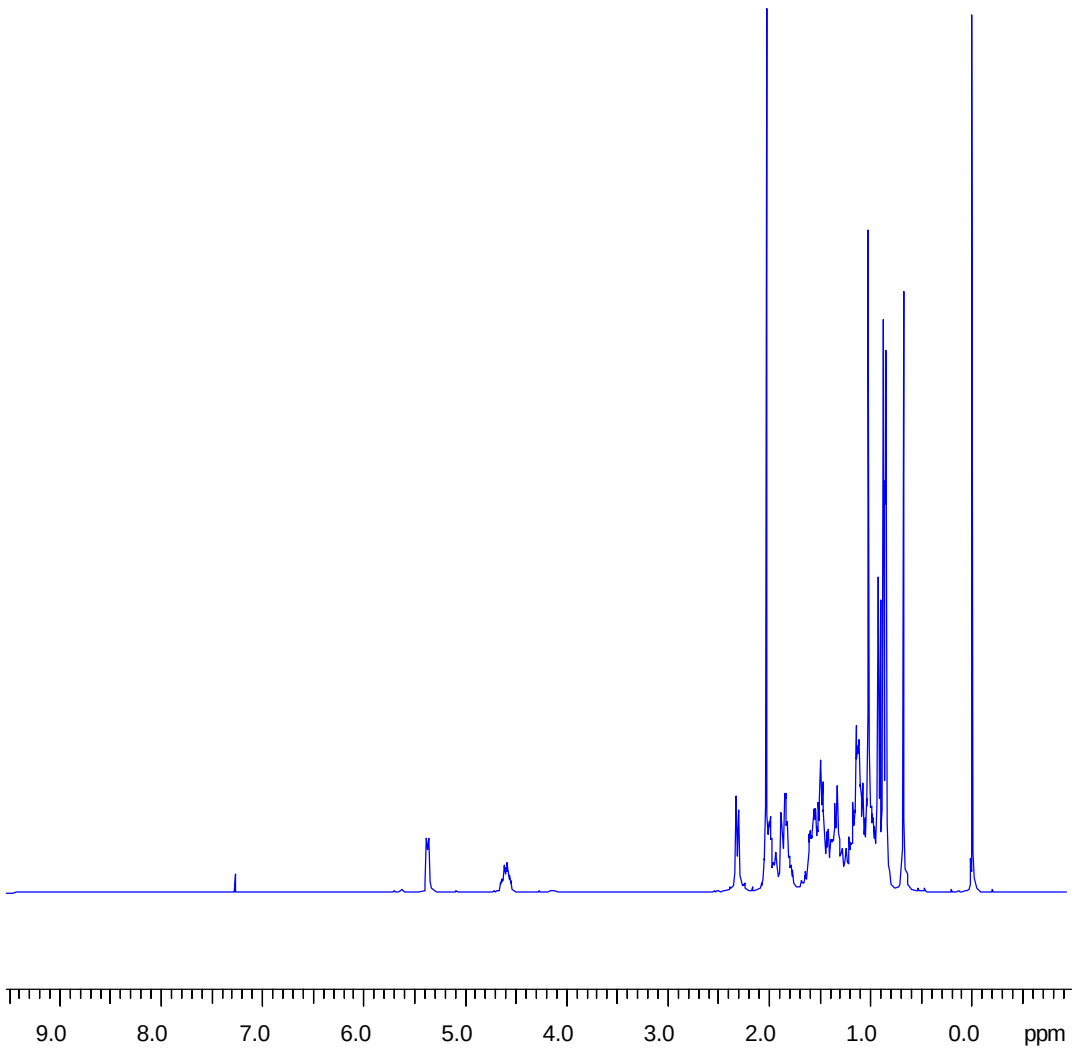
Note that once suitable values of **phc0** and **phc1** have been stored it is possible to use them for phase correcting subsequent spectra by typing the command **pk**. In addition, the Fourier transformation (**ft**) and the phase correction (**pk**) can be performed within one step using the command **fp.**

3.9 Windowing

Before the Fourier transformation is performed, it is common to apply a window (or filter) function to the time domain data. The main reason for this is the improvement of either signal-to-noise, or resolution. Usually, for a simple 1D spectrum as described here, the signal-to-noise ratio is improved by multiplying the FID with a simple exponential function achieved by the command **em**.

The decay rate of the exponential function determines the amount of line broadening. This rate is determined by the processing parameter **lb** (in Hz). Enter **lb** and set the value to 0.3, which corresponds to an appropriate line broadening for high-resolution ^{1}H spectra. Enter **em** to perform the multiplication, and then enter **fp** to Fourier transform and phase correct the filtered data. You can also use the combined command **efp**, which performs the windowing, Fourier transformation and the phasing with the previously determined phase correction. The final spectrum should look like the one shown in Figure 2.

Figure 2: ^{1}H 1D Spectrum of 100 mg Cholesterylacetate in CDCl3



3.10 Integration

To quantitatively analyze an observed signal, the integrated intensity of the peaks are compared within each other.

Click integrate to enter the integration submenu. To integrate a peak, first move the cursor into the spectral window and click the left mouse button. Next, click the middle mouse button once at each end of the range of interest; the integral appears automatically. Click the left mouse button again to release the cursor from the spectrum. An asterisk or a vertical arrow appears next to the right end of the integral (if not, select the integral with the left mouse button). Correct the baseline of the integral with the slope bias buttons. Integrate the other areas or peaks in the same way.

For the calibration, select an integral (asterisk/arrow) and click on calibrate Enter 100 to calibrate this integral to 100%. Upon return select **Save & store 'intrng'** to save the integral and normalization constant and return to the main 1D processing window.

It is also possible to compare integral values of spectra located in different data sets: Integrate both spectra and calibrate the integral(s) in one of them, e.g. to 100 as described above. Enter the integration mode in the second spectrum, select the corresponding integral (asterisk/arrow) and click on the lastscal button to display the integral value compared to the calibrated 100% of the other signal.

4 Pulse Calibration: Protons

4.1 Introduction

This chapter describes pulse calibration procedures for ^{1}H and ^{13}C. It is assumed that the user is already familiar with acquisition and processing of simple 1D NMR spectra. Appendix A (Data Sets and Selected Parameters), which lists all data sets generated throughout this course, and Appendix B (Pulse Calibration Results), which provides all the pulse lengths and power levels determined during this course, maybe useful in this context.

4.2 Proton Observe 90° Pulse

For the calibration of a ^{1}H 90° pulse on the observe channel (F1), the one-pulse sequence described in Chapter 3 is used. The carrier frequency (**o1p**) is set onto the resonance frequency of a peak in the ^{1}H spectrum of an appropriate sample. This peak is monitored while the length (**p1**) and/or the strength (**pl1**) of the RF pulse is adjusted to determine the exact conditions for a 90° pulse.

A common sample used for the ^{1}H pulse calibration is 0.1% Ethylbenzene in $CDCl_3$. Ethylbenzene shows a simple ^{1}H spectrum with well-separated signals, which facilitates the selection of a single resonance line. However, due to the relatively long spin-lattice *or* longitudinal relaxation time (T_1) of Ethylbenzene, a long recycle delay time must be used.

4.2.1 Preparation

Insert the sample and lock the spectrometer (**lock**). Readjust the Z and Z^2 shims until the lock level is maximal (use **lockdisp**). Tune and match the probehead for ^{1}H observation (*see* Chapter 2.3).

First, create a new data set. Since this will be a ^{1}H observe experiment, it is best to start out from a previous ^{1}H data set, e.g., proton/1/1: Enter **re proton 1 1**, then enter **edc** and change the following parameters:

NAME	test1h
EXPNO	1
PROCNO	1

Click on **SAVE** to create the data set test1h/1/1.

Enter **eda** and set the acquisition parameter values as shown in Table 14.

Table 14: 1D ^{1}H one-pulse Acquisition Parameters

Parameter	Value	Comments
PULPROG	zg	see Figure 1 for the pulse sequence diagram.
TD	4 k	
NS	1	number of scans
DS	0	no dummy scans
D1	10	interscan delay (10s, because of long T_1)
P1	3	start with 3µs, which should correspond to less than a 90° pulse
PL1		power level for the p1 pulse see “An Important Note on Power Levels” on page 3
SW	20	start with a large spectral width of 20ppm; which will be optimized lateron
o1p	5	will be optimized lateron

Enter **rga** to perform an automatic receiver gain adjustment, then enter **zg** to acquire the FID, and **edp** to set the processing parameters as shown in Table 15.

Table 15: 1D ^{1}H one-pulse Processing Parameters

Parameter	Value	Comments
SI	2 k	
LB	1 Hz	
PSCAL	global	

Fourier transform the spectrum with the command **ef** and phase the spectrum according to Chapter 3.8. Type **sref** to calibrate the spectrum and confirm the message “no peak found in ‘sref' default calibration done”.

4.2.2 Optimize the Carrier Frequency and the Spectral Width

The carrier position (**o1p**) should now be set to the signal used for monitoring the 90° pulse calibration, which is the quartet signal of the Ethylbenzene ^{1}H spectrum. Expand the spectrum so that only the quartet at 2.6 ppm is displayed. Click on utilities to enter the calibration submenu. Click on O1 with the left mouse button, move the cursor to the center of the quartet and click the middle mouse button to assign **o1p** to this frequency. Click on return to exit the calibration submenu and return to the main window. Reduce the spectral width by entering **swh** and change the value to 1000 (Hz).

Enter **zg** to acquire a new FID using the new values for **o1p** and **swh** and process the spectrum using the command **ef**.

4.2.3 Define the Phase Correction and the Plot Region

The phase correction and the spectral region plotted in the output file must be optimized before the automation program for the pulse calibration is executed. Phase correct the spectrum according to Chapter 3.8 in a way that the quartet signal is positive. Expand the spectrum so that the quartet covers approximately the central quarter of the screen. Click on dp1 with the left mouse button and hit return for the following three prompts, or answer them as follows:

F1	2.8 ppm
F2	2.4 ppm
change y-scaling on display according to PSCAL?	y

The preparations are now completed and the calibration experiment can be executed as described in the next section.

4.2.4 Calibration: High Power

For the 90° pulse calibration an automation program called **paropt** is used. (Since the execution of this automation is time consuming, it is not the best choice if the correct pulse times and power levels are already known approximately. In such cases, the correct values are usually just checked by acquiring 1D spectra with different pulse widths to check for maximal signal.)

The automation program is started by typing **xau paropt** and answering the appearing questions as follows:

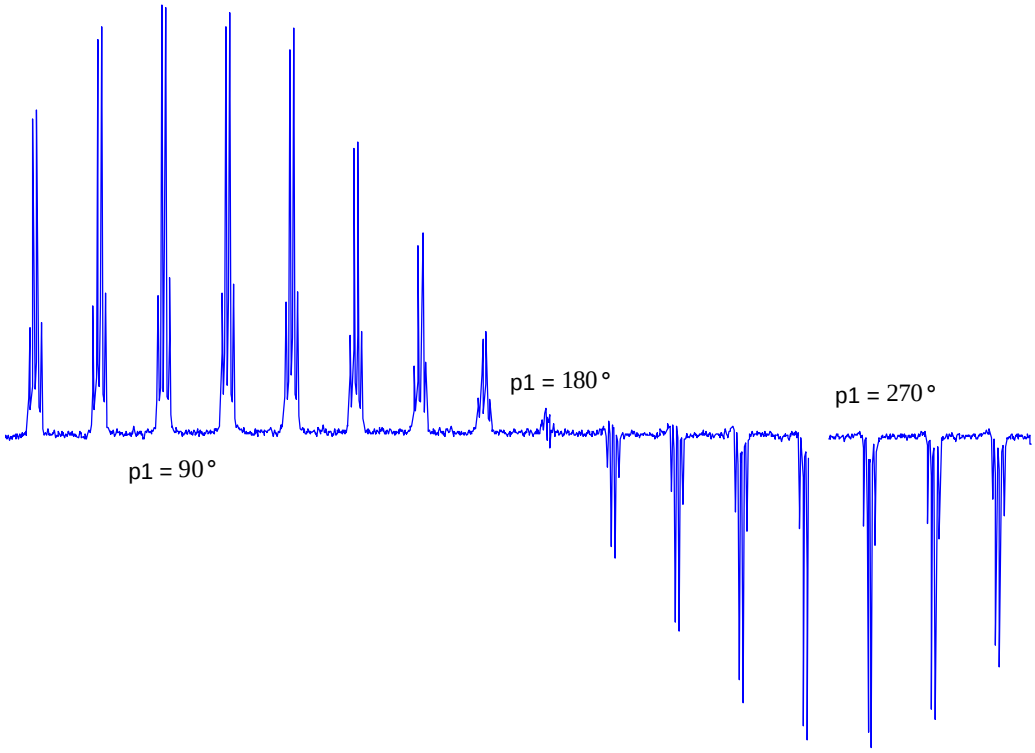
Enter parameter to modify:	p1
Enter initial parameter value:	2
Enter parameter increment:	2
Enter # of experiments:	16

The spectrometer acquires and processes 16 spectra with incrementing the parameter **p1** from 2 µsec by 2 µsec to a final value of 32 µsec. For each of the 16 spectra, only the spectral region defined above is plotted, and all the spectra are plotted side-by-side in the file test1h/1/999 as shown in Figure 3. At the end of the experiment, the message “paropt finished” and a value for the parameter **p1** is displayed, which corresponds to the 90° pulse length of the ^{1}H transmitter with the power level as defined by **pl1**. Write this value down and follow the procedure described below to obtain a more accurate 90° pulse measurement.

Return to the data set test1h/1/1 by entering **re 1 1**. Type **p1** and enter a value which corresponds to a 360° pulse (i.e., four times the 90° value determined by paropt before). Acquire and process a new spectrum by typing **zg** and **efp** (*see* Chapter 3.9) respectively. Change **p1** slightly and acquire and process a spectrum again, until the quartet undergoes a zero crossing as expected for an exact 360° pulse. Note that the quartet signal is negative for pulse angles slightly less than 360° and positive when the pulse angle is slightly more than 360°.

The 360° pulse length divided by four yields the accurate ^{1}H 90° transmitter pulse length for the actual power level **pl1**.

Figure 3: Paropt Results for 1H 90° Pulse Calibration



4.2.5 Calibration: Low Power for MLEV Pulse Train (TOCSY)

The ^{1}H 90° pulse for the MLEV pulse train used during the spinlock period of a TOCSY sequence is between 30 µsec to 40 µsec. The procedure outlined below uses the paropt routine to determine the corresponding power level. However, the power level can be estimated roughly by using a rule of thumb: The pulse length doubles for an additional 6 dB increase of the power level. For example, the 90° pulse length (**p1**) was determined 8 µsec for **pl1** = 0 dB. Thus, the **p1** = 16 µsec for **pl1** = 6 dB, or the **p1** = 32 µsec for **pl1** = 12 dB.

For performing the exact determination of the low power pulse, return to the file test1h/1/1 (**re 1 1**). Enter **p1** and change the value to 35 (µsec), type **xau paropt** and answer the questions as follows:

Enter parameter to modify:	pl1
Enter initial parameter value:	0
Enter parameter increment:	1
Enter # of experiments:	16 .

Again, the 16 spectra will be displayed in the file test1h/1/999 and at the end of the experiment, the message “paropt finished” and a value for **pl1** is displayed. This value corresponds to the ^{1}H transmitter power level for a 90° pulse length of 35 µsec. Write down this value and follow the procedure described below to obtain a more accurate 90° pulse measurement.

Return to test1h/1/1 (**re 1 1**), type **p1** and change the value to 140 µsec (= 360° pulse). Acquire and process a spectrum (**zg**, **efp**) by using the power level **pl1** determined by paropt above. Change **pl1** slightly until the quartet undergoes a zero-crossing indicating the accurate 360° pulse. Divide this 360° pulse time by four to get the 90° pulse length.

Note that the parameters used by the TOCSY sequence are **p6** for the 90° pulse length and **pl10** for the power level, rather than **p1** and **pl1**.

4.2.6 Calibration: Low Power for ROESY Spinlock

The power level required for the cw spinlock pulse used with ROESY experiments corresponds to a 90° pulse length of 100 µsec to 120 µsec. As described for the 90° pulse determination of the MLEV pulse above in Chapter 4.2.5, the power level can again be estimated using the rule of thumb, or measured using the paropt automation.

When using paropt, return to the file test1h/1/1 (**re 1 1**), enter **p1** and change the value to 110 (µsec), and type **xau paropt**. Answer the questions as follows:

Enter parameter to modify:	pl1
Enter initial parameter value:	10
Enter parameter increment:	1
Enter # of experiments:	16 .

The results are displayed in the file test1h/1/999, and at the end of the experiment, the message “paropt finished” and a value for **pl1** corresponding to the ^{1}H transmitter power level for a 90° pulse length of 110 µsec are displayed. Follow the same procedure as described in Chapters 4.2.4 and 4.2.5 for a more accurate determination of the power level.

Note that since ROESY uses cw spinlock, only the power level determination is important here, but not the actual 90° pulse length. The power level parameter used with the ROESY sequence is **pl11**, rather than **pl1**.

5 Basic ^{13}C acquisition and processing

5.1 Introduction

This chapter describes the acquisition and processing of a ^{13}C spectrum acquired with a one-pulse sequence with and without ^{1}H decoupling.

5.1.1 Sample

Since NMR is much less sensitive to ^{13}C nuclei than to ^{1}H, it is advisable to replace the 100 mg sample of cholesterylacetate used in chapter 3 'Basic ^{1}H Acquisition and Processing' with a 1g sample of cholesterylacetate in $CDCl_3$.

5.1.2 Prepare a New Data Set

Create a new data set starting from setup1h/1/1 created in the last chapter. Enter **edc** and change the following parameters:

NAME	setup13c
EXPNO	1
PROCNO	1

Click on **SAVE** to create the data set setup13c/1/1. Enter **edsp** and set the following spectrometer parameters:

NUC1	13C
NUC2	1H

Click on **SAVE**. The spectrometer is now ready to pulse and detect at the ^{13}C frequency and to pulse and decouple at the ^{1}H frequency.

Lock the spectrometer (**lock cdcl3**), adjust the Z and Z^2 shims until the lock level is optimized (use **lockdisp**), tune and match the probehead for ^{13}C and ^{1}H.

5.2 One-Pulse Experiment without ^{1}H Decoupling

The one-pulse sequence without decoupling is identical to the one used in Chapter 3 (Figure 1) except that the RF pulse is applied at the ^{13}C frequency. Enter **eda** and set the acquisition parameters values as shown in Table 16.

Table 16: ^{13}C Basic Acquisition Parameters

Parameter	Value	Comments
PULPROG	zg	see Figure 1 for pulse sequence diagram.
TD	64 k	
NS	1	
DS	0	
D1	2	
P1	3	the default unit for pulse lengths is micro-seconds; entering “3” sets a pulse length of 3 microseconds
PL1		high power level on F1 channel (^{13}C) see also “Important note on power levels”
SW	250	^{13}C spectra cover a much broader spectral range than ^{1}H spectra
O1P	100	will be optimized later

Enter **rga** to start the automatic receiver gain adjustment and then **zg** to acquire the FID.

Type **si** and enter a value of **32k**. Type **lb** and enter 3. Enter **ef** to add line broadening and Fourier transform the data. Manually phase correct the spectrum and store the correction. Subsequent ^{13}C spectra can now be processed with the command **efp**, which combines the exponential multiplication, Fourier transformation, and phase correction using the stored **phc0** and **phc1** values.

The processed spectrum is very noisy and most likely only one single peak is visible arising from the $CDCl_3$ solvent as shown in Figure 4. Type **sref** to calibrate the spectrum correctly. Note that the **sref** command works properly only if the parameter **solvent** is set to the correct solvent in the **eda** table.

The signal-to-noise ratio is improved by acquiring more than one scan.

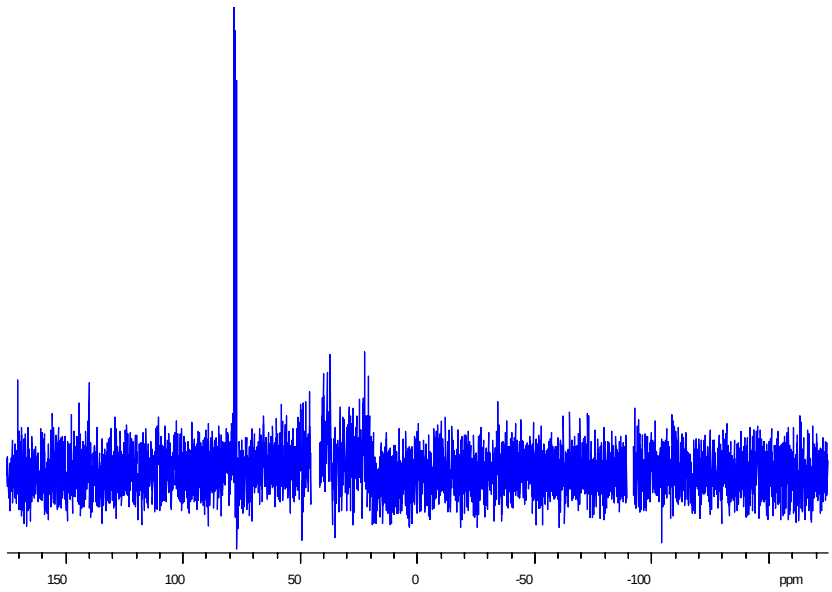
Enter **edc** and set EXPNO to 2. Click on **SAVE** to create the data set setup13c/2/1.

Enter **ns** (number of scans) and change the current value to 64. Enter **ds** (dummy scans) and change the current value to 4. The four dummy scans ensure that the system reaches steady state conditions before any spectra are added together. Enter **zg** to acquire the FID and **efp** to add line broadening, Fourier transform, and phase correct the data after the acquisition is completed. As shown in Figure 5, more peaks are visible now. However, the signal-to-noise ratio still is unsatisfactory.

The ^{13}C carrier frequency must be adjusted and set to the center of the spectrum. To do so, click on the button utilities to enter the calibration

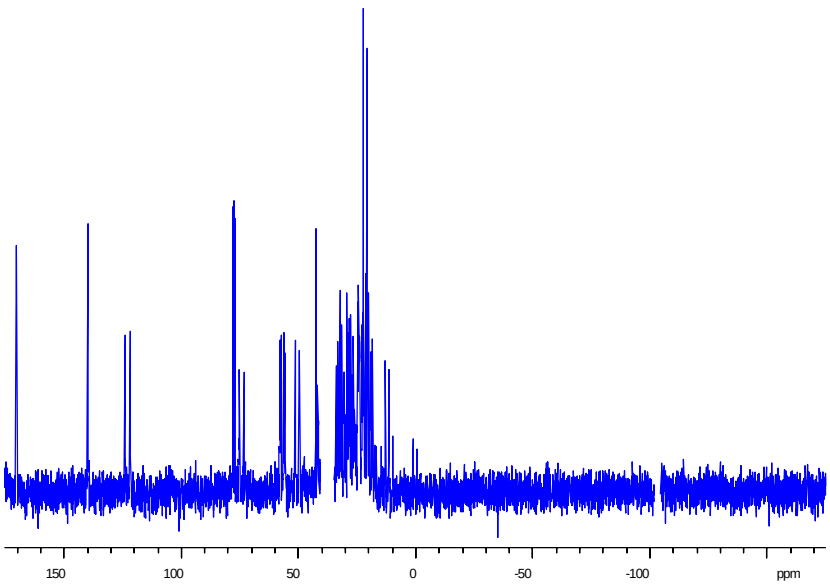
submenu, and then O1 with the left mouse button to select **o1p** calibration. Tie the cursor to the spectrum, move it to the Chloroform peak and press the middle mouse button to set **o1p** to this frequency. Click on return. Acquire and process another spectrum with this new **o1p** (**zg**, **efp**).

Figure 4: ^{13}C spectrum of 1 g cholesterylacetate in CDCl3



The signal-to-noise ratio can be improved further by the application of ^{1}H decoupling as shown in the next section.

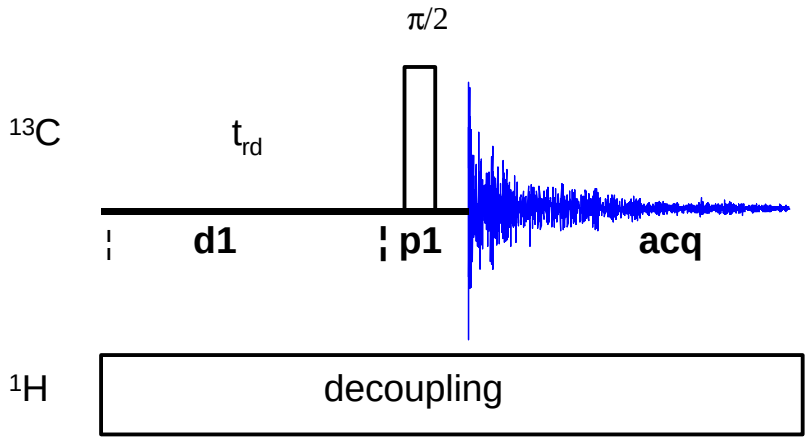
Figure 5: ^{13}C spectrum of 1 g cholesterylacetate in CDCl3



5.3 One-Pulse Experiment with ^{1}H Decoupling

The one-pulse sequence with ^{1}H decoupling is shown in Figure 6. In addition to the one-pulse sequence used before, ^{1}H is decoupled throughout the entire length of the pulse program.

Figure 6: ^{13}C One-Pulse Sequence with 1H Decoupling



Before acquiring a ^{1}H-decoupled ^{13}C spectrum, the frequency of the ^{1}H signals in must be determined. Figure 2 (Section 3.9) shows a ^{1}H spectrum of cholesterylacetate (or look it up with **`re setup1h 1 1`**): Most ^{1}H signals lie in the range of 0.5 to 5.5 ppm. An appropriate frequency for ^{1}H decoupling would therefore be 3 ppm. *In general, 5 ppm is a safe frequency to select for ^{1}H decoupling if no 1H spectrum is available.*

In case you looked up the ^{1}H spectrum (data set proton/1/1), return to the previous carbon spectrum by entering **`re setup13c 3 1`**. Enter **edc**, set EXPNO to 3 and click on **SAVE** to create the data set setup13c/3/1.

Enter **eda** and set the acquisition parameters as shown in Table 17.

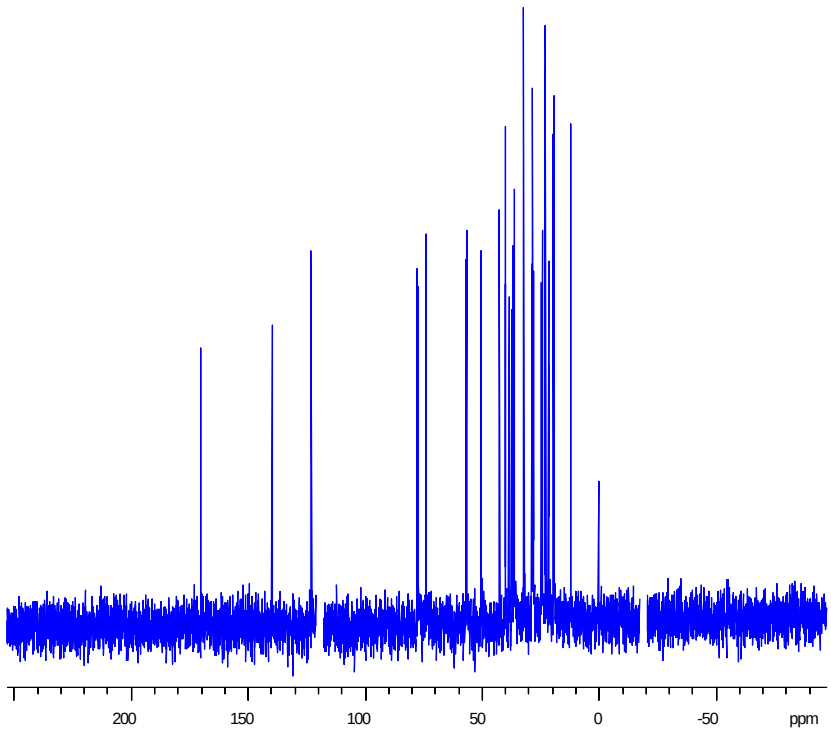
Table 17: ^{13}C Acquisition Parameters for 1H Decoupled Spectrum

Parameter	Value	Comments
PULPROG	zgdc	see Figure 6 for pulse sequence diagram.
TD	32k	
NS	1	
DS	0	
D1	2	should be 1–5*T_1(^{13}C).
P1	3	the default unit for pulse lengths is micro-seconds; entering “3” sets a pulse length of 3 microseconds
PL1		power level for the p1 pulse (^{13}C) See also “Important note on power levels”
Pl12		low power level on F2 channel (^{1}H) as determined in Section 4.2.6 for ROESY spin lock
PCPD2		^{1}H 90° pulse for cpd sequence as determined in Section 4.2.6
SW	350 ppm	
O1P		frequency of ^{13}C carrier as optimized in Section 5.2
O2P	3	frequency of ^{1}H carrier
CPDPRG2	waltz16	^{1}H decoupling sequence

Enter **`zg`** to acquire an FID.

Enter **`efp`** to perform line broadening, Fourier transformation, and phase correction. A ^{1}H decoupled ^{13}C spectrum is shown in Figure 7. Note the improved signal-to-noise ratio.

Figure 7: ^{13}C spectrum of 1 g cholesterylacetate in $CDCl_3$ with ^{1}H decoupling



6 Pulse Calibration: Carbon

6.1 Carbon Observe 90° Pulse

The ^{13}C observe pulse calibration experiment requires a sample with a strong ^{13}C signal, e.g., 80% Benzene in Acetone-d6. If no appropriate sample is available, the inverse mode ^{13}C pulse calibration procedure described in Section 6.3 can be used instead.

6.1.1 Preparation

Insert the sample and lock the spectrometer (**`lock`**). Readjust the Z and Z^2 shims until the lock level is maximal (use **`lockdisp`**). Tune and match the probehead for ^{13}C observation and ^{1}H decoupling (*see* Chapter 2.5). Create a new data set: Enter **`re proton 1 1`**, enter **`edc`** and change the following parameters:.

NAME	test13c
EXPNO	1
PROCNO	1 .

Click on **SAVE** to create the data set test13c/1/1.

Enter **`edsp`** and set NUC1 to 13C, turn off NUC2 and press the DEFAULT button, then click on SAVE.

Enter **`eda`** and set the acquisition parameters as shown in Table 18.

Table 18: 1D ^{1}H one-pulse Acquisition Parameters

Parameter	Value	Comments
PULPROG	zg	see Figure 1 for pulse sequence diagram.
TD	4 k	
NS	1	
DS	0	
D1	20	interscan delay (20s, because of long T_1)
P1	3	start with 3µs, which should correspond to less than a 90° pulse
PL1		power level for the p1 pulse see “An Important Note on Power Levels” on page 3
SW	350	start with a large spectral width of 350ppm; which will be optimized lateron
o1p		start with 100ppm, it will be optimized later

Enter **rga** to perform an automatic receiver gain adjustment; enter **zg** to acquire the FID, and **edp** to set the processing parameters as shown in Table 19.

Table 19: 1D ^{13}C one-pulse Processing Parameters

Parameter	Value	Comments
SI	2 k	
LB	3 Hz	
PSCAL	global	

Add line broadening and Fourier transform the spectrum with the command **ef**. Manually phase correct the spectrum and store the correction.

Type **sref** to calibrate the spectrum and confirm the message “no peak found in ‘sref' default calibration done”.

6.1.2 Optimize the Carrier Frequency and the Spectral Width

The carrier frequency should now be set to the signal used to calibrate the 90° pulse: Expand the spectrum until only the doublet at 130 ppm is displayed. Enter the calibration submenu by clicking utilities . Click on O1 with the left mouse button and move the cursor to the center of the doublet. Click the middle mouse button to assign **o1p** to this frequency. Click return to exit the calibration submenu and return to the main window.

Reduce the spectral width by entering **swh** and change the value to 1000 (Hz). Acquire and Fourier transform another spectrum (**zg**, **ef**).

6.1.3 Define the Phase Correction and the Plot Region

Now it is necessary to define the phase correction and spectral region that will be plotted in the output file produced by paropt. Phase correct the spectrum so that the doublet is positive. Expand the spectrum so that the doublet covers approximately the central third of the screen. Click on dp1 with the left mouse button and answer the three questions as follows:

F1	133 ppm
F2	127 ppm
change y-scaling on display according to PSCAL?	y

6.1.4 Calibration: High Power

As for the ^{1}H 90° calibration (Chapter 4.2.4), the automation program paropt is used. Type **xau paropt** and answer the questions as follows:

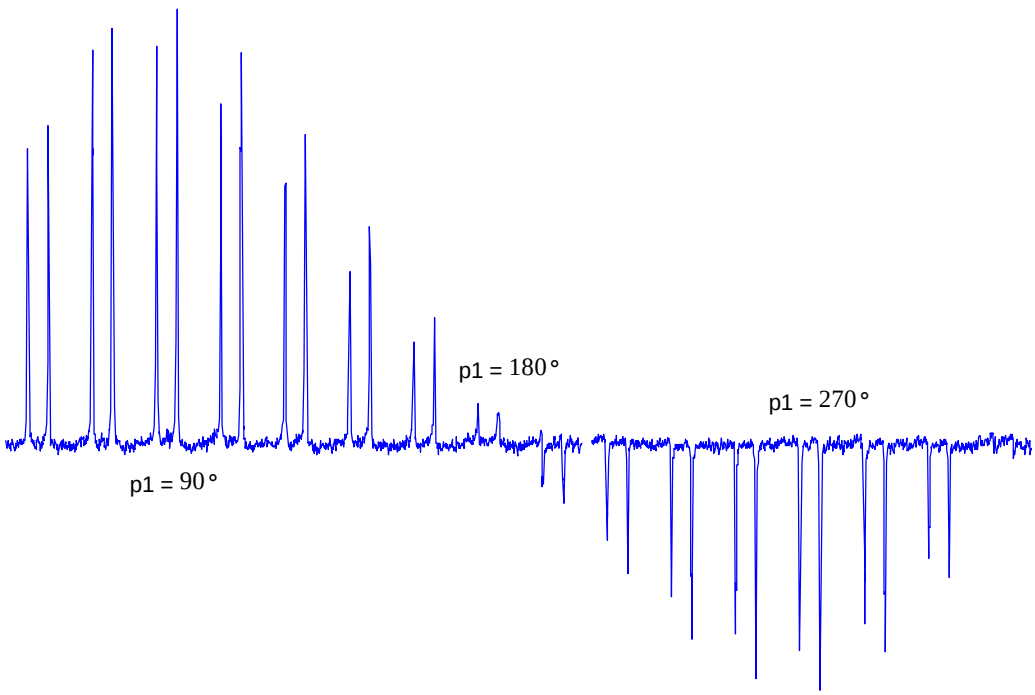
Enter parameter to modify:	p1
Enter initial parameter value:	2
Enter parameter increment:	2
Enter # of experiments:	16

Paropt acquires and processes 16 spectra while incrementing the parameter **p1** from 2 µsec to 32 µsec. The result is displayed side-by-side in test13c/1/999 and should resemble Figure 8. At the end of the experiment, the message “paropt finished” appears and a value for **p1** is displayed, which corresponds to the 90° pulse length of the ^{13}C transmitter using the current power level **pl1**. Write this value down and follow the procedure below to obtain a more accurate 90° pulse measurement.

Return to the data set test13c/1/1 by entering **re 1 1**. Type **p1** and enter a value corresponding to a 360° pulse (i.e., four times the 90° value determined by paropt above). Acquire and process another spectrum (**zg**, **efp**). Change **p1** slightly, acquire and process a spectrum again, until the doublet shows a zero-crossing indicating the 360° pulse. The 360° pulse time divided by 4 is the exact 90° pulse length for the ^{13}C transmitter for the power level **pl1**.

Note that the probe may arc, if the 90° pulse length is less than 5 µsec for 5 mm probes and less than 10 µsec for 10 mm probes. In this case the **pl1** must be set to a higher value (increase the attenuation on the transmitter) and the corresponding 90° pulse length must be determined again.

Figure 8: Paropt Results for ^{13}C 90° Pulse Calibration



6.2 Proton Decoupling 90° Pulse During ^{13}C Acquisition

Ideally, this procedure is carried out immediately following the ^{13}C observe pulse calibration described above, since the magnet is then locked and shimmed, and the probehead is tuned and matched for both ^{13}C and ^{1}H.

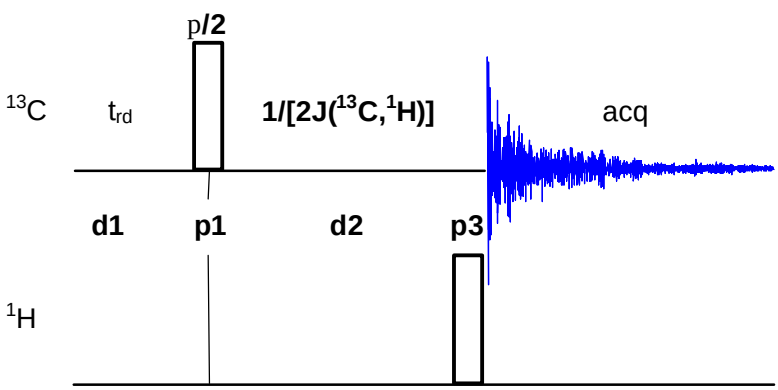
6.2.1 Sample

For the ^{1}H decoupling pulse calibration, the sample must yield a ^{13}C signal of reasonable intensity with a detectable scalar ^{13}C–^{1}H coupling: Therefore, 80% Benzene in Acetone-d6 is a good choice.

6.2.2 Pulse Sequence

The pulse sequence used is called DECP90 and it is shown in Figure 9. The sequence consists of a recycle delay (t_{rd}, **d1**) followed by a 90° ^{13}C pulse (**p1**), a delay 1/[2J(^{13}C,^{1}H)] (**d2**, **cnst2**) for the evolution of antiphase ^{13}C–^{1}H magnetization, a ^{1}H pulse (**p3**), and the ^{13}C detection period (acq, **aq**). For the calibration of the 90° ^{1}H decoupling pulse, the length (and/or the strength) of the ^{1}H pulse **p3** (and/or the power level **pl2**) is adjusted. No signal is acquired for an exact ^{1}H 90° pulse **p3**, since only undetectable multiple quantum coherence ^{13}C magnetization is present.

Figure 9: DECP90 Pulse Sequence



6.2.3 Set the ^{1}H Carrier Frequency

The first spectrum will be a ^{1}H observe experiment to determine the correct frequency for the DECP90 ^{1}H decoupling pulse. Create a new data set starting from e.g., proton/1/1 (**re proton 1 1**), enter **edc** and change the following parameters:

NAME	testdec
EXPNO	1
PROCNO	1

Click on **SAVE** to create the data set testdec/1/1 and enter **eda** to set the acquisition parameters as shown in Table 20.

Table 20: 1D ^{1}H one-pulse Acquisition Parameters

Parameter	Value	Comments
PULPROG	zg	see Figure 1 for the pulse sequence diagram.
TD	4 k	
NS	1	
DS	0	
D1	10	interscan delay (10s, because of long T_1)
P1	3	start with 3µs, which should correspond to less than a 90° pulse
PL1		high power level on F1 channel (^{1}H) as determined in Section 4.2.4
SW	20	start with a large spectral width of 20ppm; which will be optimized lateron
o1p	5	

Enter **`rga`**, **`zg`** and **`edp`** to perform an automatic receiver gain adjustment, acquire an FID, and set the processing parameters as shown in Table 21.

Table 21: 1D ^{1}H one-pulse Processing Parameters

Parameter	Value	Comments
SI	2 k	
LB	1	
PSCAL	global	

Type the command **`ef`** to perform line broadening and Fourier transformation, and phase correct the spectrum. Type **`sref`** to calibrate the spectrum and confirm the message “no peak found in ‘sref' default calibration done”.

Click the left mouse button with the cursor placed in the spectral field of the main window. Move the cursor to the top of the Benzene peak at around 7.3 ppm and note the exact ppm-value of the cursor position in the small “Info” window. Click the left mouse button to release the cursor from the spectrum.

6.2.4 Set the ^{13}C Carrier Frequency and the Spectral Width

These parameters were already determined for the data set test13c/1/1. To transfer all the parameters from test13c/1/1 to the new data set, enter **`re test13c 1 1`**, enter **`edc`** and change the following parameters:

NAME	testdec
EXPNO	2
PROCNO	1

Click on **SAVE** to create the data set testdec/2/1.

Since now ^{1}H decoupling is required, enter **edsp** and set NUC2 to 1H so that the spectrometer parameters are as follows:

NUC1	13C
NUC2	1H
(NUC3	off)

Enter **eda** and set the acquisition parameters values as shown in Table 22. The parameters **o1p** and **swh** should be set as used in test13c/1/1. Set **o2p** to the exact ^{1}H offset frequency determined in the previous section (around 7.3ppm). *The DECP90 experiment will not work as described below unless both **o1p** and **o2p** are set correctly.*

Table 22: DECP90 Acquisition Parameters

Parameter	Value	Comments
PULPROG	decp90	see Figure 9 for pulse sequence diagram.
TD	4 k	
NS	1	
DS	0	
PL1		high power level on F1 channel (^{13}C) as determined in Section 6.1.4
PL2		high power level on F2 channel (^{1}H) as determined in Section 4.2.4
P1		^{13}C 90° pulse as determined in Section 6.1.4
P3	3	start with 3µs, which should correspond to less than a 90° pulse
D1	5	interscan delay (5s, because of long T_1)
CNST2	160 Hz	heteronuclear scalar J(^{13}C,^{1}H) coupling
D2	3.125 msec	1/[2J(^{13}C,^{1}H)] (calculated automatically from cnst2 above)
SWH	1000 Hz	
o1p		^{13}C offset as determined in Section 6.1.2
o2p		^{1}H offset as determined in Section 6.2.3

Enter **zg** to acquire the FID (the receiver gain should already be set appropriately), enter **edp** and verify the processing parameters as shown in Table 23.

Table 23: DECP90 Processing Parameters

Parameter	Value	Comments
SI	2 k	
LB	1 Hz	
PSCAL	global	

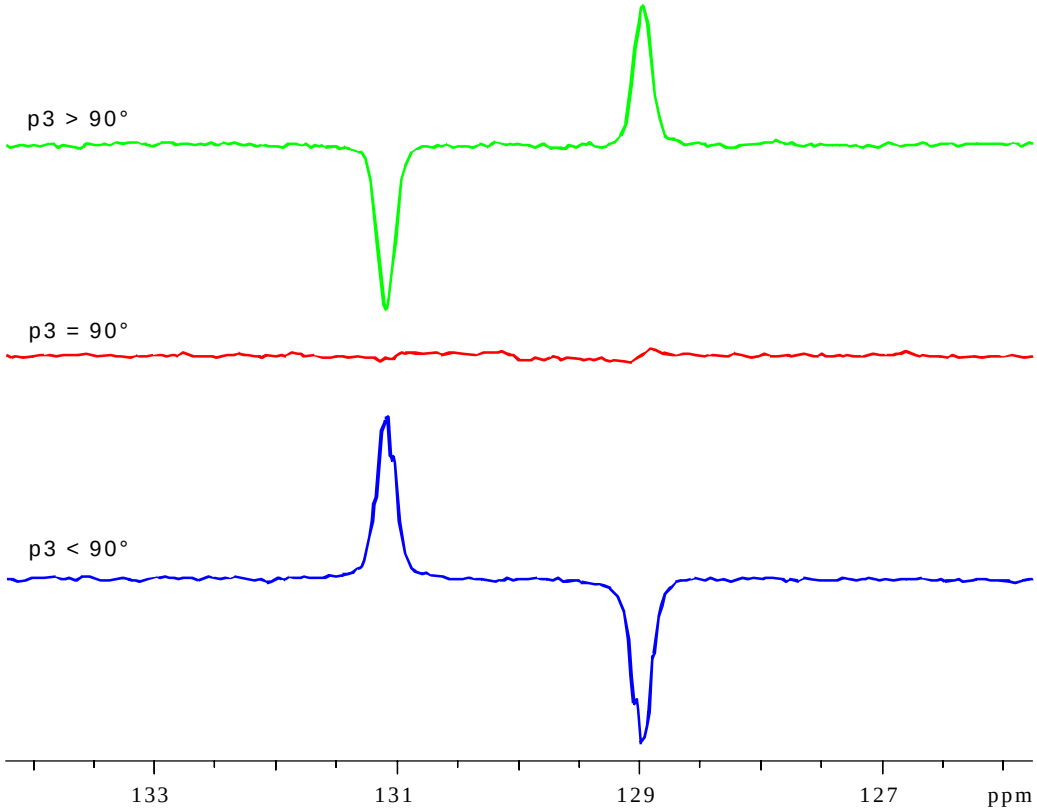
Fourier transform the spectrum with line broadening by the command **ef**. Manually phase correct the spectrum so that the left peak is positive and the right peak is negative, and store the correction.

The spectrum is already calibrated if the current data set was created from test13c/1/1. Since paropt is not used here, neither the phase correction nor the plot region have to be defined.

6.2.5 Calibration: High Power

The ^{1}H 90° decoupling pulse length should be close to the ^{1}H 90° observe pulse length for the same power level: Set **p3** to the value found in Section 4.2.4 and acquire and process a spectrum (**zg**, **efp**). If the ^{1}H 90° pulse (**p3**) is less than 90°, the left peak will be positive and the right peak negative. If the pulse angle is between 90° and 270°, the phase of the two peaks will be opposite. If the **p3** pulse length corresponds to a 90° pulse, the signals show a zero-crossing as shown in Figure 10.

Figure 10: 1H Decoupling 90° Pulse Calibration



6.2.6 Calibration: Low Power for WALTZ-16 Decoupling

The WALTZ-16 composite pulse decoupling (cpd) sequence requires a ^{1}H 90° decoupling pulse length of 80 to 100 µsec. Adjust **pl2** and **p3** to determine the 90° pulse length in this range. Make use of the rule of thumb: 90° pulse length approximately doubles for an additional 6 dB increase in attenuation.

Note that the parameters used by cpd sequences are **pcpd2** for the 90° pulse length and **pl12** for the decoupler power level, rather than **p3** and **pl2** as used here.

6.3 Carbon Decoupler 90° Pulse (Inverse Mode)

This calibration procedure should yield approximately the same pulse length as for the ^{13}C observe 90° pulse (Section 6.1.4). However, the method described here is more convenient because of the more sensitive ^{1}H detection instead of ^{13}C. In addition, the T_1 of ^{1}H is shorter than for ^{13}C, which allows the selection of shorter interscan delays.

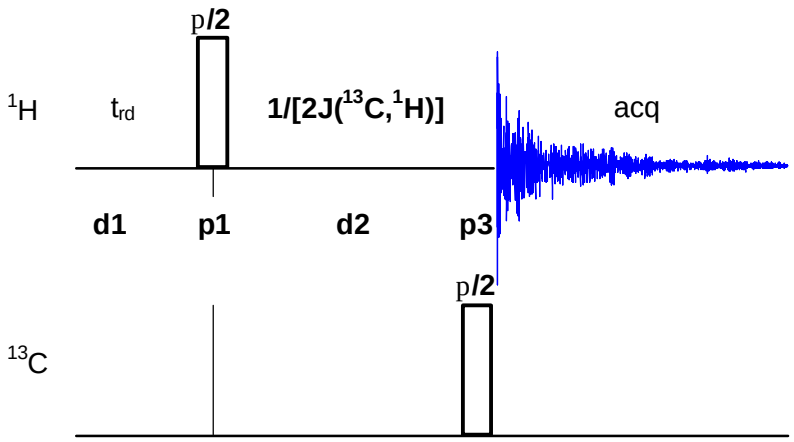
6.3.1 Sample

For these so-called inverse experiments, the detected nucleus is ^{1}H, but ^{13}C satellites must be visible. Therefore, a convenient sample is the ^{1}H Lineshape Sample (3% Chloroform in Acetone-d6 for frequencies between 300 MHz and 500 MHz, and 1% Chloroform in Acetone-d6 for frequencies

>600 MHz). Here the procedure is described for this readily available standard sample. However, use the “Pulse Calibration” sample containing 0.1M ^{13}C-methanol and 0.1M ^{15}N-urea in DMSO-d_6, if this is available.

The same DECP90 pulse sequence as used for the ^{1}H 90° decoupling pulse determination in Section 6.2 is used here, except that the ^{1}H and ^{13}C channels are interchanged as shown in Figure 11.

Figure 11: DECP90 Pulse Sequence



6.3.2 Preparation

Insert the sample, lock the spectrometer, readjust the Z and Z^2 shims until the lock level is optimized, tune and match the probehead for ^{1}H observation and ^{13}C decoupling.

6.3.3 Set the ^{13}C Carrier Frequency

First, a ^{13}C observe experiment is recorded to determine the correct ^{13}C carrier frequency (which is **o1p** here but will be **o2p** in the inverse calibration experiment).

Create a new data set starting from a previous ^{13}C data set, e.g., carbon/1/1 (**re carbon 1 1**), enter **edc** and change the following parameters:

NAME	testinv
EXPNO	1
PROCNO	1

Click on **SAVE** to create the data set testinv/1/1. Type **edsp** and set the parameters as follows for observing ^{13}C and decoupling ^{1}H:

NUC1	13C
NUC2	1H
(NUC3	off)

Click on **SAVE** to save the spectrometer parameters and return to the main window and enter **eda** to set the acquisition parameters as shown Table 24.

Table 24: 1D ^{13}C One-pulse Acquisition Parameters

Parameter	Value	Comments
PULPROG	zgdc	
TD	4 k	
NS	1	
DS	0	
PL1		high power level on F1 channel (^{13}C) as determined in Section 6.1.4
PL12		low power level on F2 channel (^{1}H) for cpd as determined in Section 6.2.6
P1	3	start with less than a 90° pulse
PCPD2		^{1}H 90° pulse for cpd sequence as determined in Section 6.2.6
D1	5	
SWH	1000	
RG	4 k	
o1p	77ppm	Chloroform resonance
o2p	5	
CPDPRG2	waltz16	cpd sequence for the ^{1}H decoupling

Notice that the spectral width (**swh**) is much smaller than usually used for ^{13}C spectra, because only the chloroform signal must be recorded and **o1p** is set to almost the correct frequency in this data set.

Enter **zg** to acquire the FID. Enter **edp** and set the processing parameters as shown in Table 25.

Table 25: 1D ^{13}C One-pulse Processing Parameters

Parameter	Value	Comments
SI	2 k	
LB	1 Hz	
PSCAL	global	

Fourier transform the data (**ef**) and phase the spectrum manually. Type **sref** to calibrate the spectrum and confirm the message “no peak found in ‘ sref' default calibration done”.

Expand the spectrum until only the chloroform signal at 77 ppm is displayed. Enter the calibration submenu by clicking utilities . Click on O1 with the left mouse button and move the cursor to the center of the signal. Click the middle mouse button to assign **o1p** to this frequency. Click return to exit

the calibration submenu and return to the main window. This **o1p** value will be the ^{13}C **o2p** value for the DECP90 pulse sequence below.

6.3.4 Set the ^{1}H Carrier Frequency and the Spectral Width

Now, a ^{1}H observe experiment to determine the correct offset for ^{1}H (**o1p**) must be recorded: Create a new ^{1}H data set starting from a previous one, e.g., proton/1/1 (**re proton 1 1**). Enter **edc** and change the following parameters:

NAME	testinv
EXPNO	2
PROCNO	1

Click on **SAVE** to create the data set testinv/2/1, then enter **eda** and change the acquisition parameters as shown in Table 26.

Table 26: 1D ^{1}H One-pulse Acquisition Parameters

Parameter	Value	Comments
PULPROG	zg	see Figure 1 for pulse sequence diagram.
TD	8 k	
NS	1	
DS	0	
PL1		high power level on F1 channel (^{1}H) as determined in Section 4.2.4
P1	3	^{1}H pulse (less than a 90° pulse)
D1	5	
SW	20 ppm	
o1p	5	

Enter **rga** to perform an automatic receiver gain adjustment, **zg** to acquire the FID and **edp** to set the processing parameters as shown in Table 27.

Table 27: 1D ^{1}H One-pulse Processing Parameters

Parameter	Value	Comments
SI	4k	
LB	0.3 Hz	
PSCAL	global	

Fourier transform the data (**ef**) and phase correct the spectrum. Type **sref** to calibrate the spectrum and confirm the message “no peak found in ‘sref' default calibration done”.

Expand the spectrum until only the chloroform signal at 7.2ppm is displayed and set the **o1p** value exactly on the methanol peak in the utilities menu (follow the same procedure as described in Section 4.2.2). The spectral width (**swh**) can now be reduced to 1000 Hz.

6.3.5 Preparations for the Inverse Pulse Calibration

The correct ^{1}H and ^{13}C frequencies have now been determined. Next, a DECP90 spectrum is acquired to determine the appropriate phase correction values. Create a new data set by entering **edc** and change EXPNO to 3. Click **SAVE** to create the data set testinv/3/1.

Enable ^{13}C decoupling by entering **edsp** and set the following spectrometer parameters:

NUC1	1H
NUC2	13C
NUC3	off

Click on **SAVE** to save the spectrometer parameters and return to the main window. Set **o2p** to the **o1p** value of the ^{13}C determined in Section 6.3.3.

Enter **eda** and set the acquisition parameters as shown in Table 28.

Table 28: DECP90 Acquisition Parameters

Parameter	Value	Comments
PULPROG	decp90	see Figure 11 for pulse sequence diagram.
TD	8 k	
NS	1	
DS	0	
PL1		high power level on F1 channel (^{1}H) as determined in Section 4.2.4
PL2		high power level on F2 channel (^{13}C) as determined in Section 6.1.4
P1		^{1}H 90° pulse as determined in Section 4.2.4
P3	0	for the phase correction, do not use p3
D1	5	relaxation delay; should be 1–5 * $T_1(^{1}H)$.
CNST2	214	heteronuclear scalar $J(^{13}C,^{1}H)$ coupling [Hz]
D2	3.34ms	$1/[2J(^{13}C,^{1}H)]$ (calculated automatically from cnst2 above)
SWH	1000 Hz	
RG		use value from testinv/2/1.
o1p	~7.24ppm	^{1}H offset frequency of chloroform peak as determined in Section 6.3.4
o2p	~77ppm	^{13}C offset frequency of chloroform peak as determined in Section 6.3.3

Enter **zg** to acquire the FID and enter **edp** to set the processing parameters as shown in Table 29.

Table 29: DECP90 Processing Parameters

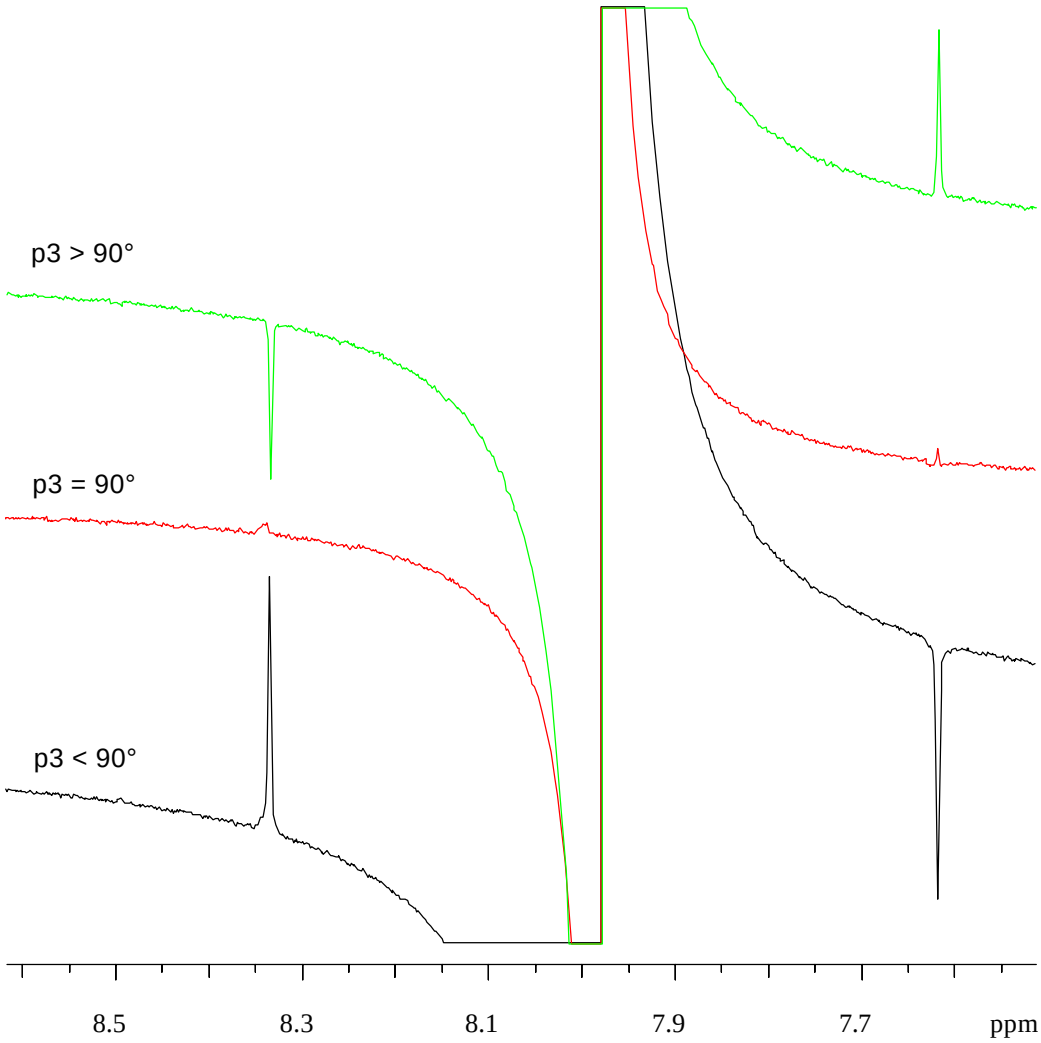
Parameter	Value	Comments
SI	4 k	
LB	0.3 Hz	
PSCAL	global	

Fourier transform the spectrum (**ef**). Expand the spectrum to display the region between 8.5 to 7.5 ppm, in the region of the chloroform peak with it's two ^{13}C satellites. Correct the phase in a way that the left satellite is positive and the right satellite is negative.

6.3.6 Calibration: High Power

Set **p3** to the value obtained with the direct method in Section 6.1. Acquire and process another spectrum (**zg**, **efp**). If the pulse is less than 90°, the left satellite will remain positive and the right satellite negative. If the pulse angle is between 90° and 270°, the two satellite signals will show opposite phase. If the **p3** pulse corresponds to 90°, the satellites show a zero-crossing (Figure 12).

Figure 12: ^{13}C Decouple 90° Pulse Calibration Results on the chloroform sample



6.3.7 Calibration: Low Power for GARP Decoupling

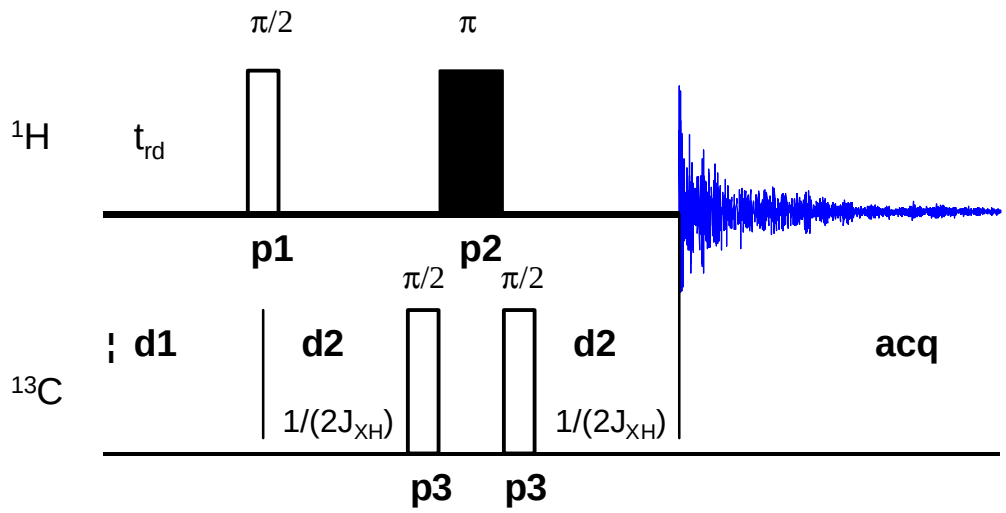
The GARP composite pulse decoupling (cpd) sequence requires a 90° decoupling pulse length of 60 to 70 µsec. Adjust **pl2** and **p3** to determine the combination that yields a 90° pulse length in this range. Use the rule of thumb: The pulse length doubles for a 6 dB increase in attenuation.

Note that the parameters used by cpd sequences are **pcpd2** for the 90° pulse length and **pl12** for the decoupler power level, rather than **p3** and **pl2** as used here.

6.4 1D Inverse Test Sequence

The 1D HMQC pulse sequence shown in Figure 13 is used to check the parameters for inverse experiments. This experiment detects only signals of protons directly attached to ^{13}C nuclei, whereas the signal arising from protons attached to ^{12}C is suppressed by phase cycling. Thus, the 1D HMQC spectrum of the current sample (10% Chloroform in Acetone-d6) yields only signal from the ^{13}C satellites without the large central peak.

Figure 13: 1D HMQC Pulse Sequence



Create a new data set starting from testinv/3/1 (**re testinv 3 1**), enter **edc** and set EXPNO to 4. Click **SAVE** to create the data set testinv/4/1. Enter **eda** and set the acquisition parameters as shown in Table 30.

Table 30: 1D HMQC Acquisition Parameters

Parameter	Value	Comments
PULPROG	inv4ndrd1d	see Figure 13 for pulse sequence diagram.
TD	8 k	
NS	16	the number of scans should be 4 * n in order for the phase cycling to work properly.
DS	16	number of dummy scans.
PL1		high power level on F1 channel (^{1}H) as determined in Section 4.2.4
PL2		high power level on F2 channel (^{13}C) as determined in Section 6.1.4
P1		^{1}H 90° pulse as determined in Section 4.2.4
P2		^{1}H 180° pulse: set to 2*P1
P3		^{13}C 90° pulse as determined in Section 6.3.6
D1	20 sec	relaxation delay; should be 1–5 * T_1(^{1}H).
CNST2	214	heteronuclear scalar J(^{13}C,^{1}H) coupling [Hz]

Enter **zg** to acquire the FID and **edp** to set the processing parameters as shown in Table 31.

Table 31: 1D HMQC Processing Parameters

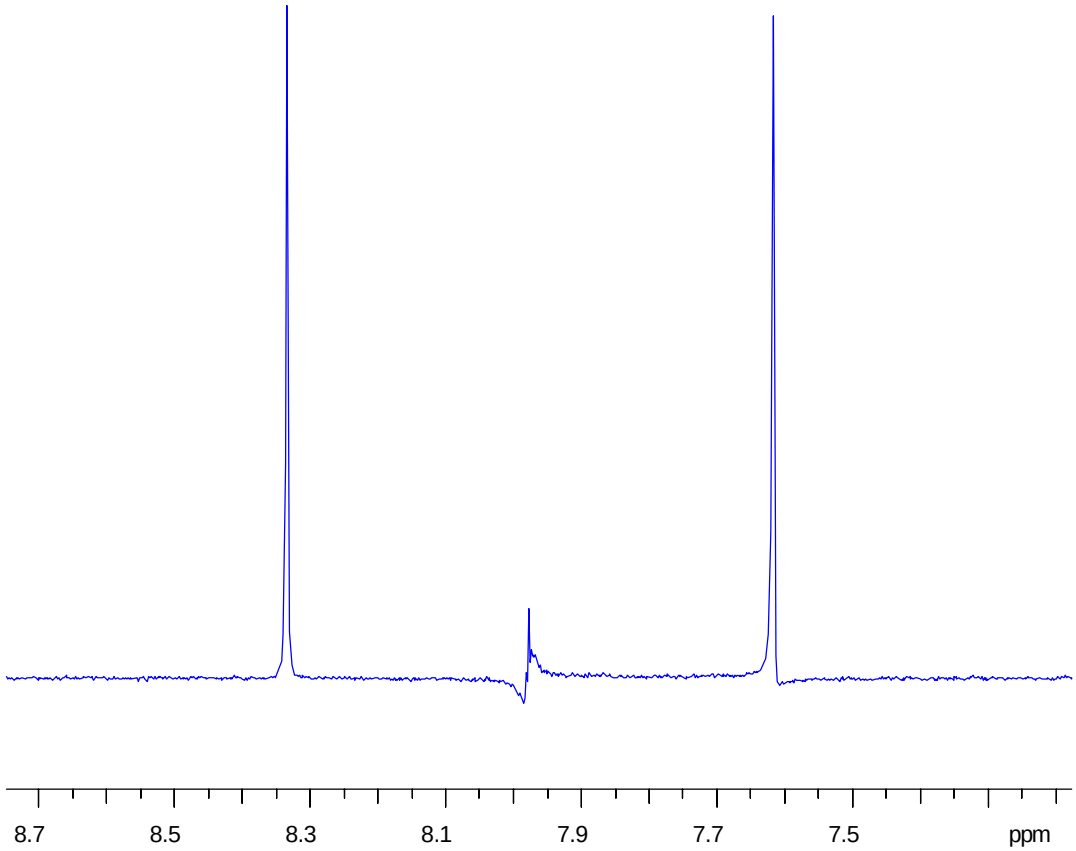
Parameter	Value	Comments
SI	4 k	
WDW	EM	
LB	0.30 Hz	
PKNL	TRUE	necessary when using the digital filter.

Fourier transform the data (**ef**) and phase correct the spectrum.

A 1D HMQC spectrum of Chloroform is shown in Figure 14. Note that the phase cycling is not as perfect to completely suppress the main signal arising from ^{1}H directly attached to ^{12}C, due to technical limitations

.

Figure 14: 1D HMQC Spectrum of Chloroform



7 Advanced 1D ^{13}C Experiments

7.1 Carbon Experiments with Gated ^{1}H-Decoupling

In principle, the values for scalar couplings between ^{1}H and ^{13}C, and the signal multiplicity can give additional information for the structure determination. The disadvantages of not decoupling ^{1}H in ^{13}C spectra are the decreased sensitivity due to the distribution of the signal intensity into the different lines of the multiplet, the signal overlap, and the missing NOE effect. This chapter describes the acquisition and processing of ^{13}C spectra acquired with commonly used ^{1}H decoupling techniques called 'gated-decoupling' and 'inverse gated-decoupling'.

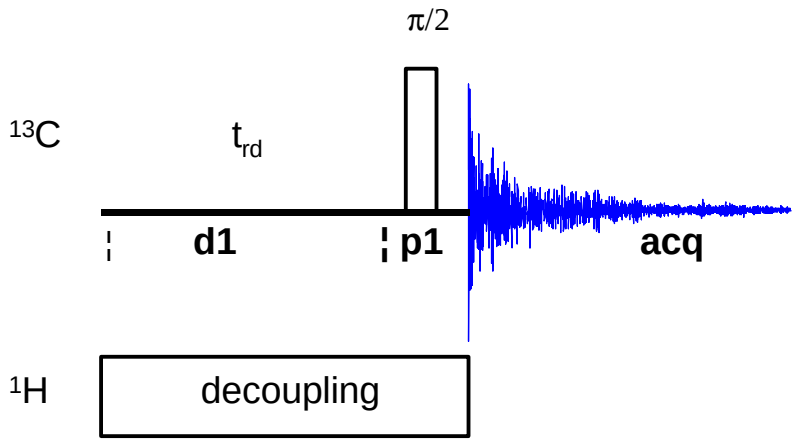
The gated-decoupled ^{13}C spectrum is recorded with ^{1}H decoupling during the relaxation delay, whereas the decoupling is switched off during ^{13}C acquisition.

For the inverse gated-decoupling experiment the ^{1}H decoupling is active during the acquisition, whereas it is switched off during the relaxation delay. The sensitivity improvement due to the NOE-effect is suppressed for this experiment and thus the acquired spectrum can be integrated. Note that there is a build up of the NOE-effect during the acquisition period when decoupling is active. In order to suppress this NOE-effect the relaxation delay must be 10-times the T_1 relaxation time for ^{13}C.

The pulse sequences for the gated-decoupling and the inverse gated-decoupling experiments are shown in Figure 15 and Figure 16, respectively.

However, before acquiring a ^{1}H-decoupled ^{13}C spectrum, the frequencies of the cholesterylacetate ^{1}H signals must be determined. See Section 5.3 for the determination of the exact ^{1}H carrier frequency. As a rule of thumb, 5 ppm is a safe frequency to select for ^{1}H decoupling when no optimized ^{1}H spectrum is available.

Figure 15: ^{13}C Pulse Sequence with Gated 1H Decoupling



Return to the carbon/3/1 data set by entering **`re carbon 3 1`**. Enter **`edc`** and set EXPNO to 4. Click on SAVE to create the data set carbon/4/1 for the ^{1}H-gated-decoupled ^{13}C spectrum.

Enter **`edsp`** and set NUC2 to 1H. Set **`o1p`** to the value determined from the ^{1}H spectrum or to 5 ppm. Enter **`eda`** and set the acquisition parameters as shown in Table 32.

Table 32: ^{13}C Acquisition Parameters with Gated and Inverse Gated-1H Decoupling

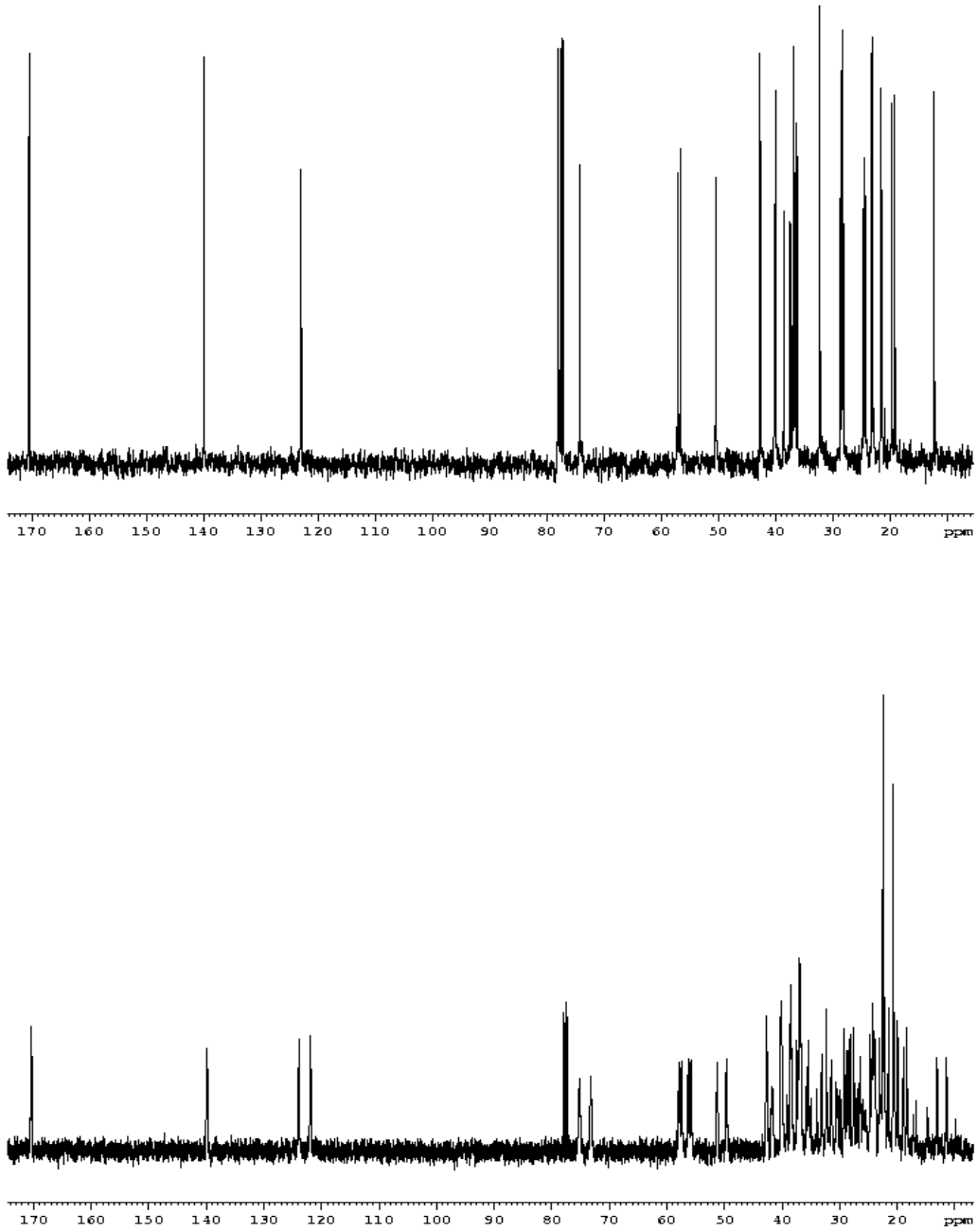
Parameter	Value	Comments
PULPROG	zggd zgig	zggd for gated-decoupling zgig for inverse gated-decoupling
TD	32 k	
NS	16	
DS	4	
PL1		high power level on F1 channel (^{13}C) as determined in Section 6.1.4
PL2	120	no high power pulses on F2 channel (^{1}H)
PL12		low power level on F2 channel (^{1}H) for CPD as determined in Section 6.2.6
D1	2 60	2s for gated-decoupling 60s for inverse gated-decoupling
P1		^{13}C 90° pulse as determined in Section 6.1.4
SW	250	250 ppm
RG	8 k	or use **`rga`**
o1p	120	frequency of the ^{13}C carrier (120 ppm)
o2p		frequency of the ^{1}H carrier (see text)
PCPD2		^{1}H 90° pulse for cpd sequence as determined in Section 6.2.6
CPDPRG2	waltz16	^{1}H decoupling sequence

Enter **`rga`** to start the automatic receiver gain adjustment and **`zg`** to acquire the FID.

Enter **`si`** and set it to **`32k`**, set **`lb`** to 3 and Fourier transform the data using **`ef`**. Phase correct the spectrum and store the correction.

The resulting spectrum is noisy compared to the regularly decoupled ^{13}C spectrum. The most intense peak arises from the Chloroform solvent, which actually is a triplet.

Figure 16: ^{13}C Spectrum of 1g Cholesterylacetate in CDCl3 using (a) Gated-Decoupling, and



7.1.1 Plotting 1D ^{13}C Spectra

1D ^{13}C spectra are most easily plotted using the standard plot parameter file, which sets most of the plotting parameters to appropriate values. Type **`rpar`** and select **`standard1D`** from the list of parameter file names. Select **`plot`**

from the menu of parameter file types. Equivalently, simply enter **`rpar standard1D plot`**.

To select the spectral region (full or expanded) to be plotted, make sure the spectrum appears on the screen as desired, and then type **`defplot`**. Hit return in response to the following three questions:

F1 =	<return>
F2 =	<return>
Change y-scaling on display according to PSCAL?	<return>

Unless special precautions are taken to deal with the long ^{13}C T_1 relaxation times and potential NOE build-up during ^{1}H decoupling, the integrated intensities in 1D ^{13}C-NMR spectra do not reflect the correct numbers of different types of ^{13}C nuclei in a given molecule. Thus, standard ^{13}C spectra are usually not integrated and the integrals are therefore not plotted: Type **edg** and click the button next to the parameter INTEGR so that it toggles to no. Click SAVE to exit the **edg** menu.

Finally, create a title for the spectrum by entering **`setti`** and write a title. Save the file and simply enter **`plot`** (provided the correct plotter is selected in **edo**) to plot the spectrum.

7.2 DEPT

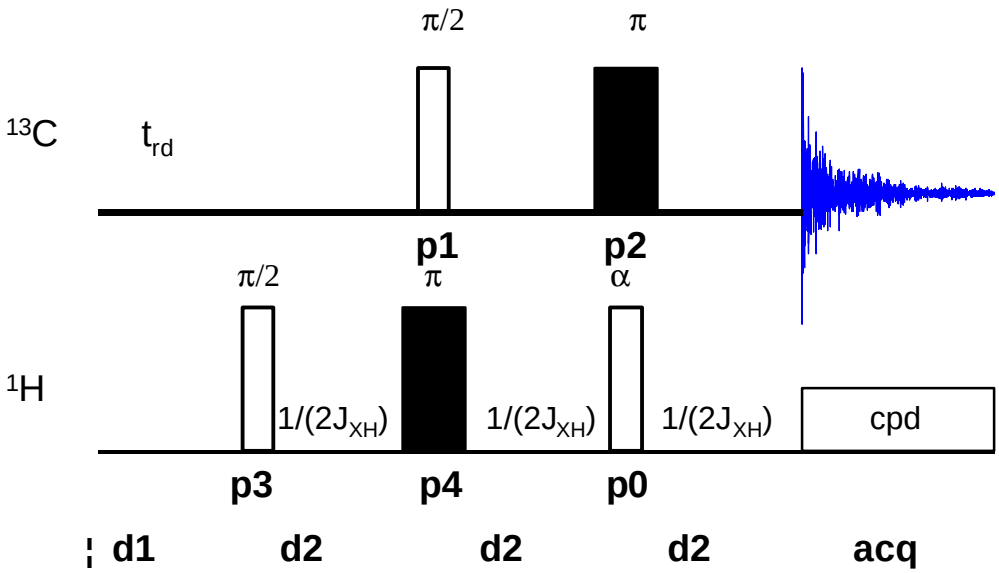
DEPT (**D**istortionless **E**nhancement by **P**olarization **T**ransfer) is a polarization transfer technique used for the observation of nuclei with a small gyromagnetic ratio, which are J-coupled to ^{1}H (most commonly ^{13}C). DEPT is a spectral editing sequence, that is, it can be used to generate separate ^{13}C subspectra for methyl (CH_3), methylene (CH_2), and methine (CH) signals. DEPT makes use of the generation and manipulation of multiple quantum coherences to differentiate between the different types of ^{13}C signals. Quaternary carbons are missing from DEPT spectra because the large one-bond heteronuclear J-coupling (J_{XH}) is used for polarization transfer.

DEPT may be run with or without ^{1}H-decoupling and it is relatively insensitive to the precise matching of delays with coupling constants, and so is much easier to use than the closely related INEPT sequence. DEPT, on the other hand, is more sensitive to pulse imperfections than INEPT.

The sample used in this chapter is 1 g Cholesterylacetate in $CDCl_3$.

The DEPT pulse sequence is shown in Figure 17. The final ^{1}H pulse with flip angle α selects for the CH_3, CH_2 or CH signals. This angle is set to 45° in the DEPT-45 sequence, which yields spectra with positive CH, CH_2, and CH_3 signals; to 90° in DEPT-90, which yields spectra with only CH signals; and to 135° in DEPT-135, which yields spectra with positive CH and CH_3 signals and negative CH_2 signals.

Figure 17: DEPT Pulse Sequence



7.2.1 Acquisition and Processing

Insert the sample in the magnet. Lock the spectrometer. Readjust the Z and Z^2 shims until the lock level is optimized. Tune and match the probehead for ^{13}C observation and ^{1}H decoupling.

7.2.2 Reference Spectra

Since DEPT is a ^{13}C-observe experiment with ^{1}H-decoupling experiment, a reference ^{1}H spectrum of the sample must be recorded to determine the correct **o2p** for ^{1}H decoupling. Then, a ^{1}H-decoupled ^{13}C spectrum must be recorded to determine the correct **o1p** and **sw** for the DEPT experiments. However, both steps were already carried out in Section 5.3 (a ^{1}H-decoupled ^{13}C reference spectrum of this sample can be found in carbon/3/1).

7.2.3 Create a New Data Set

Enter **re carbon 3 1** to call up the reference spectrum. Enter **edc** and change the following parameters:

NAME	dept
EXPNO	1
PROCNO	1

Click SAVE to create the data set dept/1/1.

7.2.4 Spectrum Acquisition

Enter **eda** and set the acquisition parameters as shown in Table 33.

Table 33: DEPT Acquisition Parameters

Parameter	Value	Comments
PULPROG	dept	*or* dept45, dept90, dept135
TD	32k	
NS	4	the number of scans must be 4 * ns
DS	8	number of dummy scans.
PL1		high power level on F1 channel (^{13}C) as determined in Section 6.1.4
PL2		high power level on F2 channel (^{1}H) as determined in Section 4.2.4
PL12		low power level on F2 channel (^{1}H) for CPD as determined in Section 6.2.6
P0		^{13}C α^{o} pulse as 90° determined in Section 6.1.4 (α = 45°, 90°, 135°)
P1		^{13}C 90° pulse as determined in Section 6.1.4
P2		^{13}C 180° pulse, calculated from P1
P3		^{1}H 90° pulse as determined in Section 4.2.4
P4		^{1}H 180° pulse, calculated from P3
PCPD2		^{1}H 90° pulse for cpd sequence as determined in Section 6.2.6
D1	2	relaxation delay; should be 1–5 * T_1(^{13}C).
CNST2	145	heteronuclear scalar J(^{13}C,^{1}H) coupling 145 Hz is a good intermediate value
D2	3.45 msec	1/[2J(^{13}C,^{1}H)] (calculated automatically from cnst2 above)
CPDPRG2	waltz16	cpd sequence for the ^{1}H decoupling

Acquire a DEPT-45 spectrum by either selecting the pulse program dept45 (type **`pulprog dept45`**) or set **`p0`** to the length of a 45° pulse pulse (type **`p0`** and enter the value of 0.5*__`p1`__ at the prompt). Enter **`zg`** to acquire the data (the receiver gain should already be set correctly if this data set was created from carbon/3/1).

7.2.5 Processing of the Spectrum

Enter **edp** and set the processing parameters as shown in Table 34.

Table 34: DEPT Processing Parameters

Parameter	Value	Comments
SI	16k	
WDW	EM	exponential multiplication
LB	2	2 Hz line broadening.
PKNL	TRUE	necessary when using the digital filter

Add line broadening and Fourier transform the time domain data with the command **ef**. Manually phase correct the spectrum so that all peaks are positive. The signals in this spectrum arise from the ^{13}C nuclei in CH, CH_2, and CH_3 groups.

7.2.6 Other spectra

To obtain a DEPT-90 spectrum create the data set dept/2/1 and either select the pulse program dept90 (type **pulprog dept90**) or set **p0** to the length of a 90° pulse (type **p0** and enter the value of **p1** at the prompt). Acquire (**zg**) and process (**efp**) the data. Only signals from CH groups are visible in this experiment.

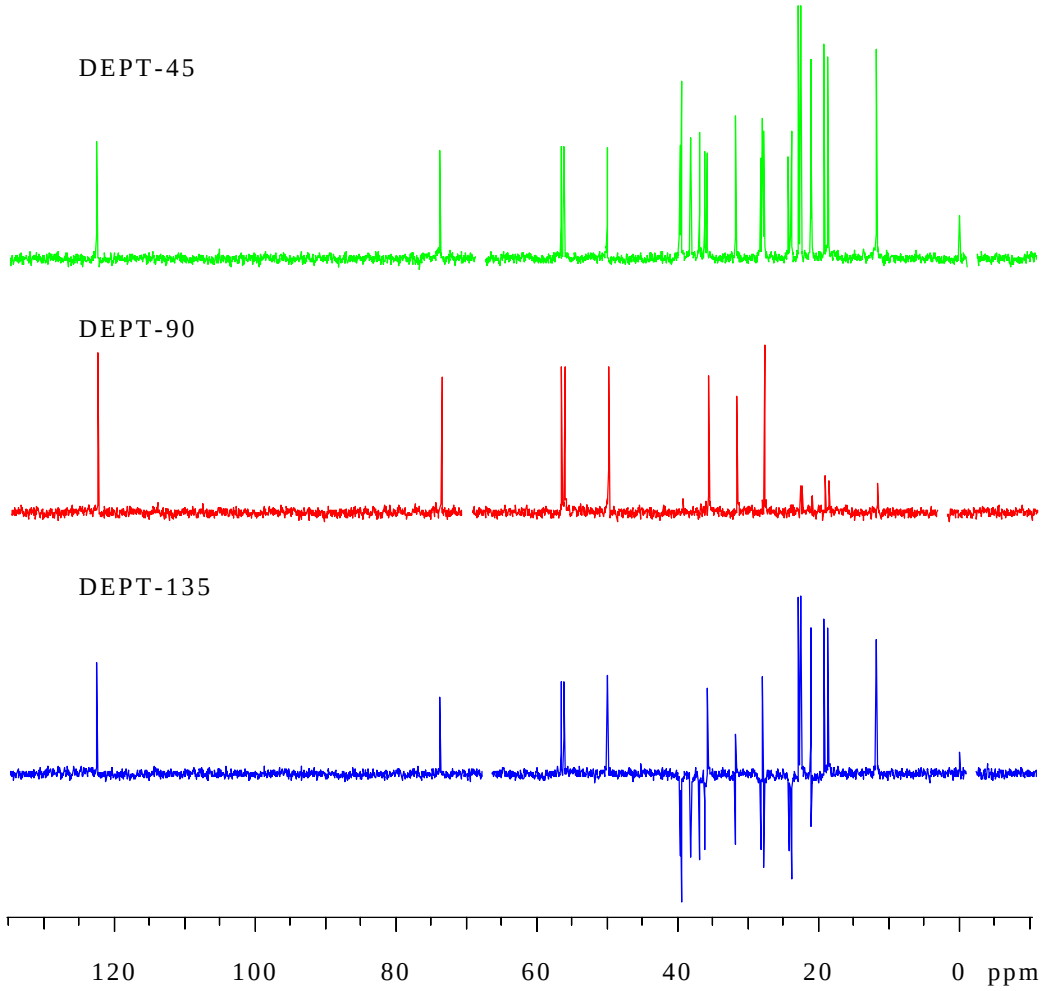
To obtain a DEPT-135 spectrum, create the data set dept/3/1 and either select the pulse program dept135 (type **pulprog dept135**) or set **p0** to the length of a 135° pulse (type **p0** and enter the value of 1.5***p1** at the prompt). Acquire (**zg**) and process (**efp**) the data. Only signals from CH and CH_3 groups are visible in this experiment.

7.2.7 Plot the spectra

See Section 7.1.1 for instructions on how to plot the acquired spectra. DEPT-45, DEPT-90, and DEPT-135 spectra of 1 g Cholesterylacetate in $CDCl_3$ are shown in Figure 18.

The DEPT results can be compared with the standard ^{1}H-decoupled ^{13}C spectrum in carbon/3/1 (see Section 5.3). Note that the signals from the quaternary ^{13}C in the carbon/3/1 do not appear in any of the DEPT spectra. From the combination of standard ^{1}H-decoupled, and DEPT-45, -90, and -135 spectra, it is possible to determine which signals arise from primary, secondary, tertiary, and quaternary ^{13}C's.

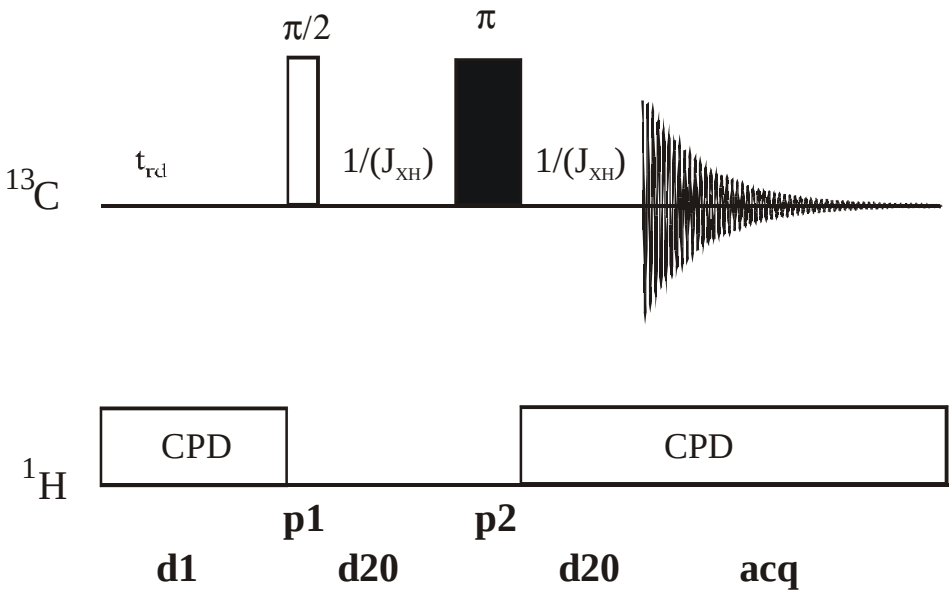
Figure 18: DEPT Spectra of 1 g Cholesterylacetate in CDCl3



7.3 APT (Attached Proton Test)

The APT (**A**ttached **P**roton **T**est) is a simple experiment for assigning multiplicities in ^{13}C NMR spectroscopy. The APT pulse sequence is shown in Figure 19. The first 90 degree pulse creates transverse magnetisation followed by a 180 degree pulse in the middle of the evolution period (spin echo sequence). During the evolution period the different components of the carbon multiplets precess at their individual frequencies. During the half of the evolution period the decoupler is OFF to introduce J-modulation in the spectrum. The length of the evolution period controls the amplitude of the carbon signal. Normally the evolution period is set to $1/(J_{CH})$ then the CH and CH3 groups appear as positive peaks while those from CH2 and quarternary carbons are negative. Compared to the DEPT experiment all carbon nuclei are visible in one spectrum.

Figure 19: APT Pulse Sequence



References: D.W. Brown, T.T. Nakashima, and D.L. Rabenstein, *J. Magn. Res.*, **45**, 302 (1981); S.L. Patt and N. Shoorly, *J. Magn. Reson.*, **46**, 535 (1982); A.M. Torres, T. T. Nakashima, and R.E.D. McClung, *J. Magn. Reson.*, **101**, 285 (1993).

7.3.1 Acquisition and Processing

Insert the sample in the magnet. Lock the spectrometer. Readjust the Z and Z^2 shims until the lock level is optimized. Tune and match the probehead for ^{13}C observation and ^{1}H decoupling.

7.3.2 Reference Spectra

Since APT is a ^{13}C-observe experiment with ^{1}H-decoupling, a reference ^{1}H spectrum of the sample must be recorded to determine the correct **o2p** for ^{1}H

decoupling. Then, a ^{1}H-decoupled ^{13}C spectrum must be recorded to determine the correct **o1p** and **sw** for the APT experiments. However, both steps were already carried out in Section 5.3 (a ^{1}H-decoupled ^{13}C reference spectrum of this sample can be found in carbon/3/1).

7.3.3 Create a New Data Set

Enter **re carbon 3 1** to call up the reference spectrum. Enter **edc** and change the following parameters:

NAME	apt
EXPNO	1
PROCNO	1

Click SAVE to create the data set apt/1/1.

7.3.4 Spectrum Acquisition

Enter **eda** and set the acquisition parameters as shown in Table 35.

Table 35: APT Acquisition Parameters

Parameter	Value	Comments
PULPROG	jmod	*spin echo experiment*
TD	32k	
NS	4	the number of scans must be 4 * ns
DS	4	number of dummy scans.
PL1		high power level on F1 channel (^{13}C) as determined in Section 6.1.4
PL2		high power level on F2 channel (^{1}H) as determined in Section 4.2.4
PL12		low power level on F2 channel (^{1}H) for CPD as determined in Section 6.2.6
P1		^{13}C pulse as 90° determined in Section 6.1.4
P2		^{13}C 180° pulse, calculated from P1
PCPD2		^{1}H 90° pulse for cpd sequence as determined in Section 6.2.6
D1	2	relaxation delay; should be 1–5 * T_1(^{13}C).
CNST2	140	heteronuclear scalar J(^{13}C,^{1}H) coupling 140 Hz is a good intermediate value
CNST11	1 2	X, XH2 positive, XH, XH3 negative only X
D20	7.14 msec	1/[1J(^{13}C,^{1}H)] (calculated automatically from cnst2 * cnst11 above)
CPDPRG2	waltz16	cpd sequence for the ^{1}H decoupling

7.3.5 Processing of the Spectrum

Enter **edp** and set the processing parameters as shown in Table 36.

Table 36: APT Processing Parameters

Parameter	Value	Comments
SI	16k	
WDW	EM	exponential multiplication
LB	2	2 Hz line broadening.
PKNL	TRUE	necessary when using the digital filter

Add line broadening and Fourier transform the time domain data with the command **ef**. Manually phase correct the spectrum so that CH and CH_3 groups are positive and C and CH_2 groups are negative..

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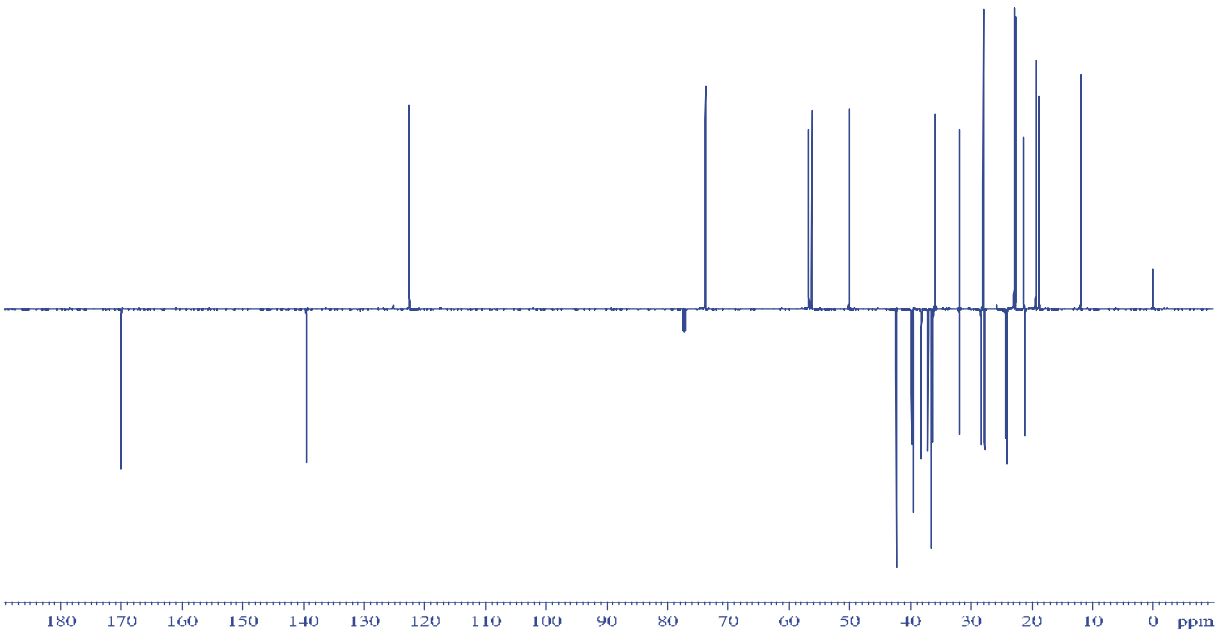
7.3.6 Plot the spectra

See Section 7.1.1 for instructions on how to plot the acquired spectra. The APT spectrum of 1 g Cholesterylacetate in $CDCl_3$ is shown in Figure20.

The APT results can be compared with the standard ^{1}H-decoupled ^{13}C spectrum in carbon/3/1 (see Section 5.3) and with the DEPT experiments. Note that the signals from the quaternary ^{13}C is visible in the APT experiment.

Figure 20: APT Spectrum of 1 g Cholesterylacetate in CDCl3



8 COSY

8.1 Introduction

COSY (**CO**rrelation **S**pectroscop**Y**) is a homonuclear 2D technique that is used to correlate the chemical shifts of ^{1}H nuclei which are J-coupled to one another. In this chapter, two types of COSY sequences, magnitude COSY and double-quantum filtered DQF-COSY with and without pulsed field gradients, will be discussed. The different pulse sequences are quite simple and can be explained as follows: The first pulse creates transverse magnetization components which evolve chemical shift and homonuclear J-coupling during the evolution period t_1. The second pulse mixes the magnetization components among all the transitions that belong to the same coupled spin systems. The final distribution of labeled magnetization components is detected by measuring their precession frequencies during the detection period t_2. The COSY spectrum is processed by a 2D Fourier transform with respect to t_1 and t_2, and the cross peaks indicate which ^{1}H nuclei are J-coupled.

The sample used to demonstrate magnitude and DQF-COSY in this chapter is 50 mM Cyclosporin in benzene-d6.

8.2 Magnitude COSY

Several simple two-pulse programs can be used to record a magnitude mode COSY spectrum, e.g., cosy, cosy45, and cosy90. These vary with respect to the angle of the final pulse. Any value between 20° and 90° may be chosen for the final pulse angle. However, a pulse angle of 45° is recommended because this yields the best signal-to-noise ratio together with a simple cross peak structure in the final spectrum.

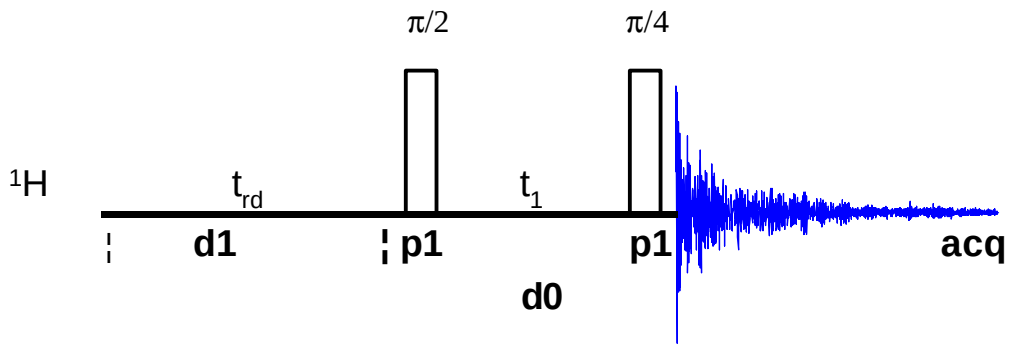
The signals acquired with one of these experiments have absorptive and dispersive lineshape contributions in both F1 and F2 dimensions. This means that it is impossible to phase the spectrum with all peaks purely absorptive, and, as a consequence, the spectrum must be displayed in magnitude mode. A typical spectral resolution of 3 Hz/pt is sufficient for resolving large scalar couplings. In order to resolve small J-couplings fine digital resolution is required, which significantly increases the experimental time. In general, the DQF-COSY experiment is recommended if a higher resolution is desired.

References: W. P. Aue, E. Bartholdi, and R. R. Ernst, *J. Chem. Phys.*, **64**, 2229 (1976); K. Nagayama, A. Kumar, K. Wüthrich, and R. R. Ernst, *J. Magn. Reson.*, **40**, 321 (1980).

8.2.1 Pulse Sequence

The COSY-45 pulse sequence is shown in Figure 21. The pulse **p1** must be set to the appropriate 90° pulse length found in Chapter 4.2.4

Figure 21: COSY-45 Pulse Sequence



8.2.2 Acquisition of the 2D COSY Spectrum

Insert the sample in the magnet. Lock the spectrometer. Readjust the Z and Z^2 shims until the lock level is optimized. Tune and match the probehead for ^{1}H observation.

It is recommended to run all 2D experiments without sample spinning.

Record a ^{1}H reference spectrum to obtain the correct carrier frequency (**o1p**) and spectral width (**sw**) values: Enter **re proton 1 1** to call up the data set proton/1/1; enter **edc** and change the following parameters

NAME	cosy
EXPNO	1
PROCNO	1

Click SAVE to create the data set cosy/1/1.

Enter **rga** to perform an automatic receiver gain adjustment. Acquire and process a standard ^{1}H spectrum. Calibrate the spectrum, and optimize **sw** and **o1p** so that the ^{1}H signals cover almost the entire spectral width. Acquire an optimized spectrum.

Type **xau iexpno** (increment experiment number) to create the data set cosy/2/1.

Enter **eda** and set PARMODE to 2D. Click on SAVE and ok the message "Delete 'meta.ext' files?". The window now switches to a 2D display and the message "NEW 2D DATA SET" appears.

Enter **eda** and set the acquisition parameters as shown in Table 37. The F2 parameters **o1p** and **sw** should be identical to the values used in the optimized ^{1}H reference spectrum (cosy/1/1). Note that **in0** and sw(F1) are not independent from each other.

Table 37: COSY Acquisition Parameters

F2 Parameters		
Parameter	**Value**	**Comments**
PULPROG	cosyqf	see Figure 21 for pulse sequence diagram
TD	1k	
NS	8	the number of scans should be 4 * n
DS	16	number of dummy scans.
PL1		high power level on F1 channel (^{1}H) as determined in Section 4.2.4
P1		^{1}H 90° pulse as determined in Section 4.2.4
P0	P1*0.5	^{1}H 45° pulse
D0	3	incremented delay (t_1); predefined
D1	3	relaxation delay; should be about 1.25 * T_1(^{1}H).
F1 Parameters		
Parameter	**Value**	**Comments**
TD	256	number of experiments
FnMODE	QF	absolute value mode
ND0	1	there is one d0 period per cycle
IN0		t_1 increment: equal to 2 * DW used in F2
SW		sw of the optimized ^{1}H spectrum (cosy/1/1): same as for F2.
NUC1		select ^{1}H frequency for F1; same as for F2.

The receiver gain is already set correctly. Enter **zg** to acquire the data, which requires about 1.4 hours. (This can be estimated previously by entering **expt** into the command line).

8.2.3 Processing of the 2D COSY Spectrum

Enter **edp** and set the processing parameters as shown in Table 38.

Table 38: COSY Processing Parameters

F2 Parameters		
Parameter	**Value**	**Comments**
SI	512	
SF		spectrum reference frequency (^{1}H)
WDW	SINE	multiply data by phase-shifted sine function
SSB	0	choose pure sine wave
PH_mod	no	this is a magnitude spectrum
PKNL	TRUE	necessary when using the digital filter
BC_mod	quad	
F1 Parameters		
Parameter	**Value**	**Comments**
SI	512	
SF		spectrum reference frequency (^{1}H)
WDW	SINE	multiply data by phase-shifted sine function.
SSB	0	choose pure sine wave
PH_mod	mc	this is a magnitude spectrum
BC_mod	no	
MC2	QF	determines type of FT in F1; QF results in a forward quadrature complex FT

Enter **`xfb`** to perform the 2D Fourier transformation.

For the magnitude COSY, sine-type window functions are selected to suppress the diagonal peaks relative to the cross peaks. Such a window function is also resolution enhancing, which is appropriate for a magnitude mode 2D spectrum. Adjust the threshold level by placing the cursor on the button, holding down the left mouse button and moving the mouse up and down.

Since this is a magnitude spectrum, click on +/- with the left mouse button until only the positive peaks are displayed.

The region can be expanded with the button followed by choosing the desired spectral region with the left mouse button depressed. The full spectrum is displayed again by clicking the all button.

The optimum may be saved by clicking on DefPlot and confirming the appearing questions as follows

```
Change levels?                          y
Please enter number of positive levels? 6
Display contours?                       n
```

8.2.4 Plotting the Spectrum

Read in the plot parameter file standard2D (**`rpar standard2D plot`**), which sets most of the plotting parameters to appropriate values.

Enter **edg** to edit the plotting parameters: Click the **ed** next to the parameter EDPROJ1 to enter the F1 projection parameters submenu. Edit the parameters as follows:

PF1DU	u
PF1USER	(name of user for file cosy/1/1)
PF1NAME	cosy
PF1EXP	1
PF1PROC	1

Click SAVE to save these changes and return to the **edg** menu.

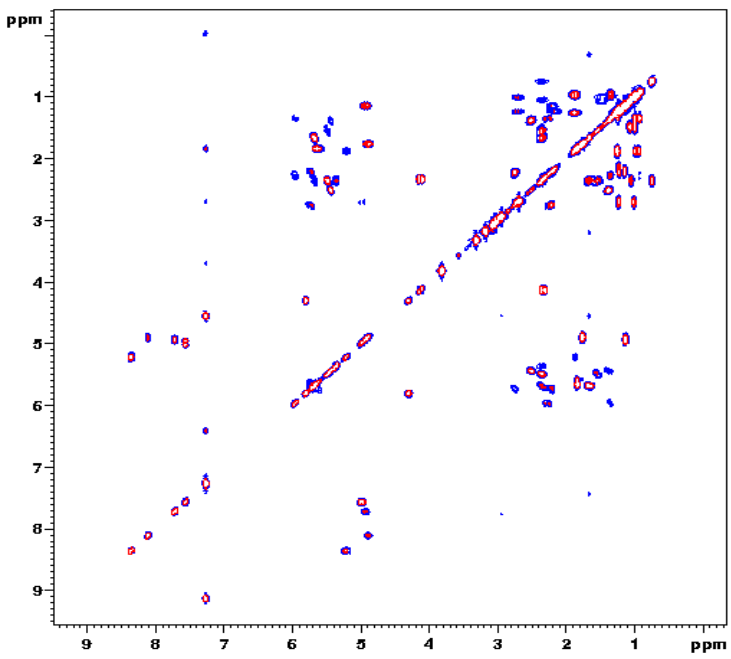
Click the **ed** next to the parameter EDPROJ2 to enter the F2 projection parameters submenu. Edit the parameters as follows:

PF2DU	u
PF2USER	(name of user for file cosy/1/1)
PF2NAME	cosy
PF2EXP	1
PF2PROC	1 .

Click SAVE to save these changes and return to the **edg** menu, and again to exit the **edg** menu.

Create a title for the spectrum (**`setti`**) and plot the spectrum (**`plot`**). A magnitude COSY spectrum of 50 mM Cyclosporin in C_6D_6 is shown in Figure 20.

Figure 22: COSY Spectrum of 50 mM Cyclosporin in C6D6



8.3 Double-Quantum Filtered (DQF) COSY

The DQF-COSY pulse sequence consists of three pulses, where the third pulse converts part of the multiple quantum coherence into observable single-quantum coherence, which is detected during the acquisition period.

One advantage of the DQF-COSY experiment is the phase-sensitivity, i.e., the cross peaks can be displayed with pure absorption lineshapes in both the F1 and the F2 dimension. In general, a phase-sensitive spectrum has a higher resolution than an otherwise equivalent magnitude spectrum because the magnitude lineshape is broader than the pure absorption lineshape.

Another advantage is the partial cancellation of the diagonal peaks in a DQF-COSY spectrum: Thus, the diagonal ridge is much less pronounced in a DQF-COSY spectrum than in a normal COSY spectrum.

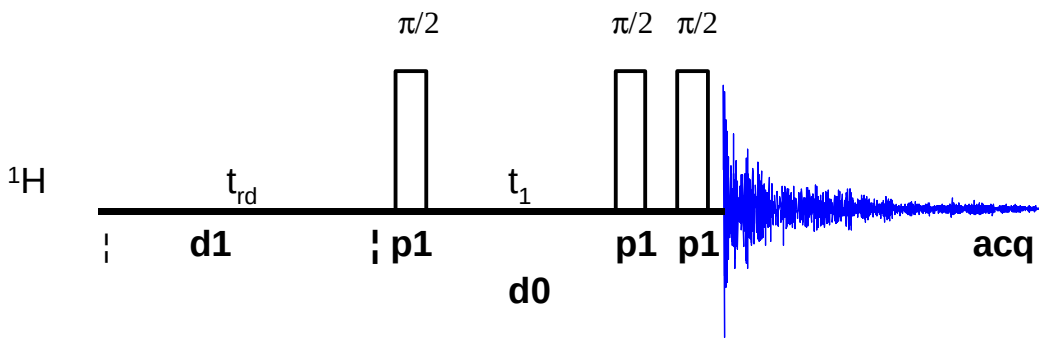
A third advantage of the double quantum filter is the elimination of strong signals, e.g., the solvent ^{1}H which do not experience homonuclear J-coupling.

References: M. Rance, O. W. Sørensen, G. Bodenhausen, G. Wagner, R. R. Ernst, and K. Wüthrich, *Biochem. Biophys. Res. Commun.*, **117**, 479 (1984); A. Derome and M. Williamson, *J. Magn. Reson.*, **88**, 117 (1990).

8.3.1 Pulse Sequence

The DQF-COSY pulse sequence is shown in Figure 23. The pulse **p1** must be set to the appropriate 90° pulse length found in Chapter 4.2.4. Note that the DQF-COSY experiment is sensitive to high pulse-repetition rates, i.e., it is important to choose a long recycle delay time **d1** in order to avoid multiple-quantum artifacts in the spectrum. A suitable value for this sample is **d1** = 3 sec.

Figure 23: DQF-COSY Pulse Sequence



8.3.2 Acquisition and Processing

From the data set cosy/2/1, enter **edc** and change EXPNO to 3.

Click SAVE to create the data set cosy/3/1.

Enter **eda** and change the following acquisition parameters: It is recommended to use a larger value of **td** in both F1 (type **1 td 512**) and F2 (type **td 2k**) and a larger number of scans (**ns 16**) for a DQF-COSY experiment than for a magnitude COSY experiment. The pulse program must be set by typing **pulprog cosydfph** and the **FnMODE** in the F1 parameter list in the **eda** table must be set to “States-TPPI”.

Enter **zg** to acquire the data. The approximate experiment time for the DQF-COSY using the acquisition parameters above can be estimated by the command **expt** and should be 5.5 hours.

Enter **edp** and set the processing parameters as shown in Table 39.

Table 39: DQF-COSY Processing Parameters

F2 Parameters		
Parameter	**Value**	**Comments**
SI	2k	
SF		spectrum reference frequency (^{1}H)
WDW	SINE	multiply data by phase-shifted sine function
SSB	2	choose pure sine wave
PH_mod	pk	determine 0- and 1st-order phase correction with phasing subroutine
PKNL	TRUE	necessary when using the digital filter.
BC_mod	no	if aq_mod=DQD
F1 Parameters		
Parameter	**Value**	**Comments**
SI	1k	
SF		spectrum reference frequency (^{1}H)
WDW	SINE	multiply data by phase-shifted sine function
SSB	2	choose pure sine wave
PH_mod	pk	determine 0- and 1st-order phase correction with phasing subroutine
BC_mod	no	
MC2	States-TPPI	States-TPPI results in a forward complex FT

Enter **xfb** to perform the 2D Fourier transformation and adjust the displayed spectrum as described in Section 8.2.3.

8.3.3 Phase correct the spectrum

The phase correction of DQF-COSY spectra is best performed while examining the cross peaks rather than the diagonal peaks. When the

spectrum is phased properly, the cross peaks will be purely absorptive (i.e., they will not have the slowly decaying wings characteristic of dispersion peaks). However, since DQF-COSY peaks are antiphase (i.e., each multiplet has adjacent positive and negative peaks), it is not possible to phase the spectrum so that all peaks are positive.

Generally, a 2D spectrum is first phase corrected in the F2 dimension (rows), and then in the F1 dimension (columns). To phase correct the spectrum in F2, three rows each with a cross peak should be selected. The cross peak of one row should be to the far left of the spectrum, the cross peak of the second row should be close to the middle, and the one of the third row should be to the far right of the spectrum.

Enter the phase correction menu by clicking on the phase button. Select one row by clicking on row with the left mouse button to tie the cursor to the 2D spectrum appearing in the upper left corner of the display. Move the mouse until the horizontal cross hair is aligned with a row that has a cross peak. Select the row by clicking the middle mouse button. If the selected row does not intersect the most intense portion of the cross peak, click + -

with the left mouse button until it does. Once the desired row is selected, click on nov 1 with the left mouse button to move the row to window 1 appearing in the upper right hand corner of the display.

Repeat the selection of rows described above for a row with a cross peak in the middle and another row with a cross peak at the right edge of the spectrum and move them to window 2 and 3, respectively.

Now that three rows have been selected, the 0th- and 1st-order phase corrections in F2 are determined by hand exactly as described for the 1D spectrum in Section 3.8:

Click on the big: 1 or the cur: 1 button to tie the cursor to the biggest peak of the row in window 1. Phase Correct this row using the 0th-order phase correction ph0 button ph1 . Correct the 1st-order phase correction for the other two rows using the button and observe the rows in window 2 and 3, respectively.

Save the phase correction by returning to the main window (select **Save & return** at the prompt).

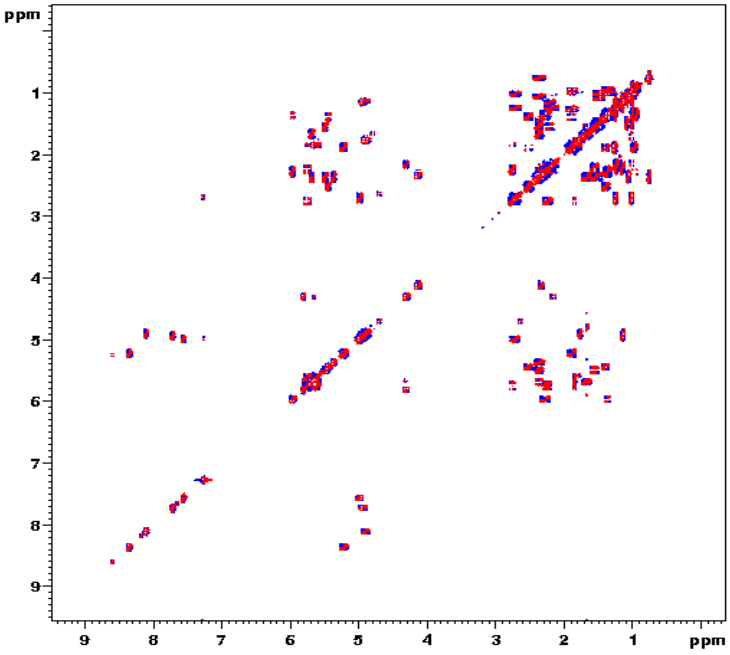
To phase correct the spectrum in F1, repeat the above procedure by selecting three columns rather than rows.

8.3.4 Plot the spectrum

See the plotting instructions given for the magnitude COSY spectrum in Section 8.2.4.

A DQF-COSY spectrum of 50 mM cyclosporin in C_6D_6 is shown in Figure 24.

Figure 24: DQF-COSY Spectrum of 50 mM Cyclosporin in C6D6



8.4 Double-Quantum Filtered COSY using Pulsed Field Gradients (GRASP-DQF-COSY)

The first high-resolution NMR experiments using pulsed field gradients (PFG) were the COSY experiments mainly to demonstrate that the application of PFGs can replace phase cycling. The quality in selecting a desired coherence pathway by PFGs turned out to be more efficient than phase cycling. In contrast to phase cycling, which requires several scans for each t_1 increment for coherence selection, field gradients allow coherence selection with only a single scan for each t_1 increment.

There are mainly two common PFG applications with COSY experiments:

1. Quadrature detection in the ω_1 dimension. The experiment time for such a COSY is in the order of a few minutes.
2. Double-quantum filter: the quality of the double-quantum filter using field gradients is very efficient. Therefore, solvent signals without homonuclear ^{1}H coupling (like water) can be suppressed very efficiently without additional solvent suppression techniques.

In this chapter we will describe the phase-sensitive double-quantum filtered COSY experiment and the pulse sequence is shown in Figure 25.

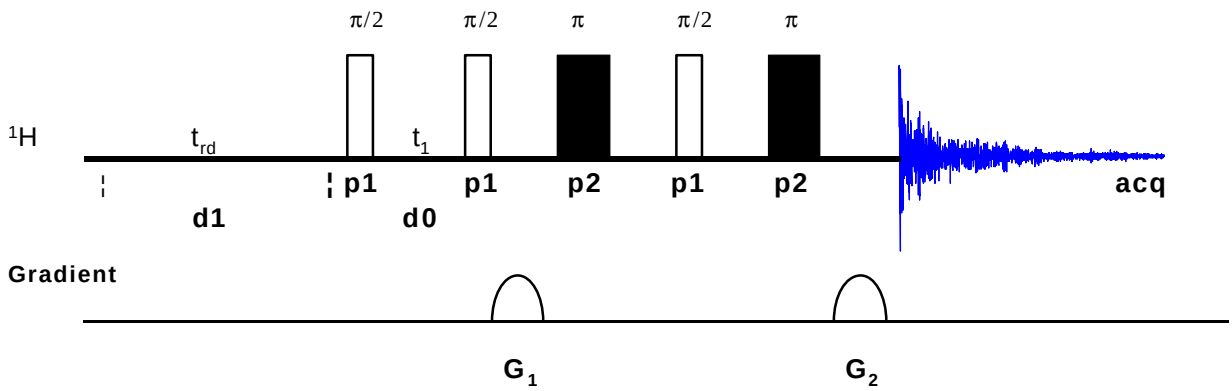
8.4.1 Pulse Sequence

The GRASP-DQF-COSY pulse sequence is very similar to the conventional DQF-COSY pulse program. After the second pulse, the spin system exhibits multiple-quantum coherence and the application of a PFG G_1 yields complete

dephasing of all coherences. In order to obtain a phase sensitive spectrum later on, the effect of chemical shift evolution during G_1 has to be eliminated by a spin echo. The third 90° pulse converts part of the multiple quantum coherence into observable single-quantum coherence, which is rephased by the PFG G_2 of proper intensity. All the unwanted magnetization stays dephased and can not be observed during the acquisition: Only spins J-coupled to at least one other spin are detected and solvent signals, especially water, are suppressed very efficiently.

The intensity ratio of the PFGs G_1:G_2 is 2: 1 for a double-quantum filter, and 3:1 for a triple quantum filter:

Figure 25: GRASP-DQF-COSY Pulse Sequence



8.4.2 Acquisition and Processing

Follow the instructions given in Sections 8.3.2 to 8.3.4 for the conventional DQF-COSY and create the data set cosy/4/1 starting out from the DQF-COSY data set (cosy/3/1).

Three parameters related to the PFGs G_1 and G_2 must be defined: The length of the PFG (**p16**), the recovery delay after the PFG (**d16**), and the shape and the intensity of the individual gradients.

Table 40: GRASP DQF-COSY Acquisition Parameters

F2 Parameters		
Parameter	**Value**	**Comments**
PULPROG	cosygpmfph	
TD	2K	
NS	4	
DS	16	
PL1		high power level on F1 channel (^{1}H) as determined in Section 4.2.4
P1		^{1}H 90° pulse as determined in Section 4.2.4
D0	3u	incremented delay (t_1); predefined
D1	3	relaxation delay; should be about 1.25 * T_1(^{1}H).

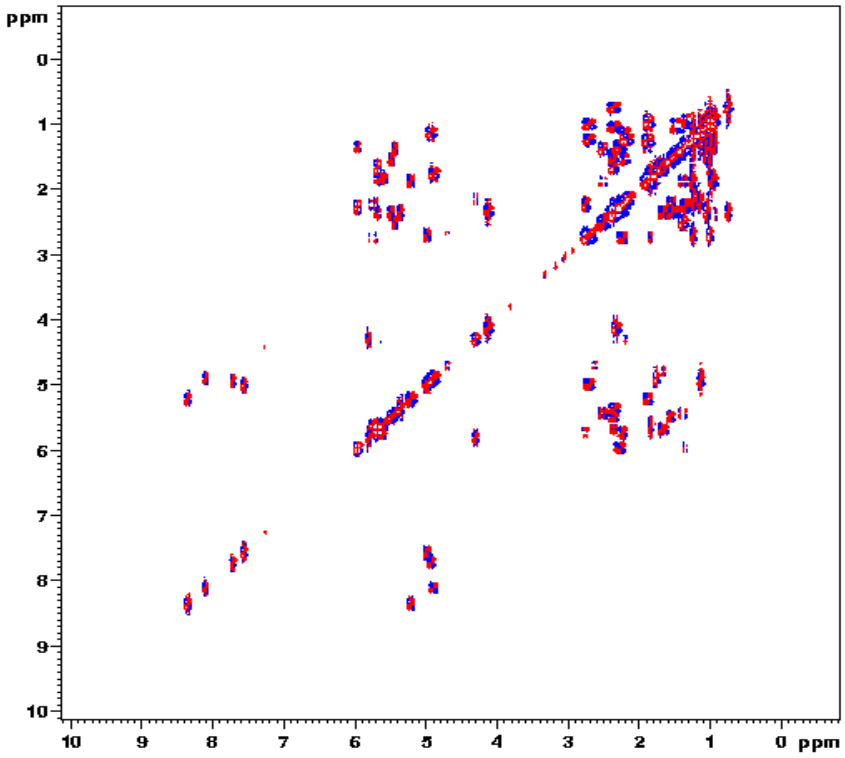
Gradient Parameters for the gp-syntax		
Parameter	**Value**	**Comments**
P16	1.5m	Length of gradient pulses
D16	150u	Gradient recovery delay
gpz1	10	% of the maximum gradient amplitude
gpz2	20	% of the maximum gradient amplitude 20 for double-quantum selection, 30 for triple-quantum selection
gpnam1	SINE.100	Gradient shape
gpnam2	SINE.100	Gradient shape
F1 Parameters		
Parameter	**Value**	**Comments**
TD	512	number of experiments
FnMODE	TPPI	
ND0	1	there is one d0 period per cycle
IN0		t_1 increment: equal to 2 * DW used in F2
SW		sw of the optimized ^{1}H spectrum (cosy/1/1): same as for F2.
NUC1		select ^{1}H frequency for F1; same as for F2

Enter **zg** to start the DQF-COSY experiment. With the acquisition parameters shown above, the approximate experiment time is 1h.

Enter **edp** and set the processing parameters as shown in Table 39 for the conventional DQF-COSY except that the F1 parameter MC2 must be set to TPPI instead of States-TPPI.

Enter **xfb** to multiply the time domain data by the window functions and to perform the 2D Fourier transformation, adjust the threshold level, set the phase correction and plot the spectrum. A GRASP-DQF-COSY spectrum of 50 mM Cyclosporin in C_6D_6 is shown in Figure 26.

Figure 26: GRASP-DQF-COSY experiment of 50mM Cyclosporin in C6D6



9 TOCSY

9.1 Introduction

TOCSY (**TO**tal **C**orrelation **S**pectroscop**Y**) provides a different mechanism of coherence transfer than COSY for 2D correlation spectroscopy in liquids. In TOCSY, cross peaks are generated between all members of a coupled spin network. An advantage is that pure absorption mode spectra with positive intensity peaks are created. In traditional COSY, cross peaks have zero integrated intensity and the coherence transfer is restricted to directly spin-coupled nuclei. In TOCSY, oscillatory exchange is established which proceeds through the entire coupling network so that there can be net magnetization transfer from one spin to another even without direct coupling. The isotropic mixing which occurs during the spin-lock period of the TOCSY sequence exchanges all in-phase as well as antiphase coherences.

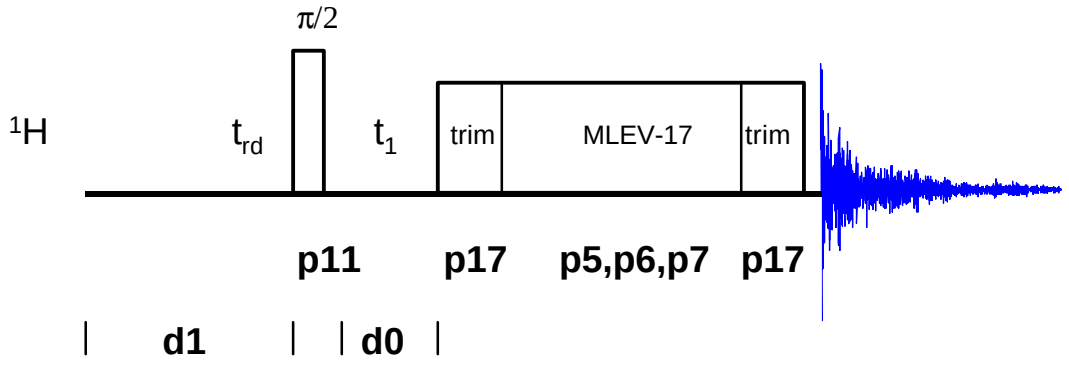
The coherence transfer period of the TOCSY sequence occurs during a multiple-pulse spin-lock period. The multiple-pulse spin-lock sequence most commonly used is MLEV-17. The length of the spin-lock period determines how far the spin coupling network will be probed. A general rule of thumb is that $1/(10\ J_{HH})$ should be allowed for each transfer step, and five transfer steps are typically desired for the TOCSY spectrum.

The sample used to demonstrate TOCSY in this chapter is 50 mM Cyclosporin in C_6D_6.

References: L. Braunschweiler and R. R. Ernst, *J. Magn. Reson.*, **53**, 521 (1983); A. Bax and D. G. Davis, *J. Magn. Reson.*, **65**, 355 (1985).

The TOCSY pulse sequence is shown in Figure 27. The pulse **p1** must be set to the appropriate 90° time found Section 4.2.4 and the MLEV-17 sequence used during the spinlock period requires the calibrated 90° time **p6** as determined in Section 4.2.5.

Figure 27: TOCSY Pulse Sequence



9.2 Acquisition

Insert the sample in the magnet. Lock the spectrometer. Readjust the Z and Z^2 shims until the lock level is optimized. Tune and match the probehead for ^{1}H observation.

It is recommended to run 2D experiments without sample spinning.

Record a ^{1}H reference spectrum to determine the correct values for **o1p** and **sw**. A ^{1}H reference spectrum of this sample was already created for the magnitude COSY experiment (Section 8.2.2). This spectrum is found in the data set cosy/1/1.

The TOCSY data set can be created from the data set of any of the previous homonuclear 2D experiments run on this sample. For example, enter **re cosy 2 1** to call up the data set cosy/2/1. Enter **edc** and change the following parameters:

NAME	tocsy
EXPNO	1
PROCNO	1

Click SAVE to create the data set tocsy/1/1.

Enter **eda** and set the acquisition parameters as shown in Table 41.

The parameter **l1** determines the number of cycles of the MLEV spinlock sequence, and thus determines the length of the “mixing period”. The mixing period typically lasts 20 to 100 msec, and so **l1** should be chosen so that the quantity [(**p6** * 64) + **p5**) *__11__ + (**p17** * 2)] is 20 to 100 msec. The general rule of thumb is that a mixing time of $1/2J_{HH}$ or approximately 75 msec should be used.

The parameter **p17** determines the length of the trim pulses at the beginning and end of the mixing period. A good value for **p17** is 2.5 msec. The trim pulses are used to ensure that the final 2D spectrum can be phased. Note, however, that for aqueous samples only the first trim pulse should be used, in which case **l1** should be adjusted so that [(**p6** * 64) + **p5**) *__11__ + **p17**] is 20 to 100 msec.

Table 41: TOCSY Acquisition Parameters

F2 Parameters		
Parameter	**Value**	**Comments**
PULPROG	mlevph	
TD	1k	
NS	8	the number of scans should be 8 * n
DS	16	number of dummy scans
PL1		high power level on F1 channel (^{1}H) as determined in Section 4.2.4
PL10		low power level on F1 channel (^{1}H) for MLEV-mixing as determined in Section 4.2.5

P1		^{1}H 90° pulse as determined in Section 4.2.4
P5		^{1}H 60° pulse, calculated from p6
P6		^{1}H 90° pulse as determined in Section 4.2.5
P7		^{1}H 180° pulse, calculated from p6
P17	2.5m	2.5 msec trim pulse
D1	2	relaxation delay; should about 1.25 * T_1 (^{1}H)
D9	80ms	TOCSY mixing time
L1	~ 30	loop for MLEV cycle ((p6 * 64) + p5) *11 + (p17 * 2) = mixing time); calculated internally
F1 Parameters		
Parameter	**Value**	**Comments**
TD	256	number of experiments
FnMODE	States-TPPI	
ND0	1	one d0 period
IN0		t_1 increment: equal to 2 * DW used in F2
SW		sw of the optimized ^{1}H spectrum (cosy/1/1): same as for F2
NUC1		select ^{1}H frequency for F1; same as for F2.

Type **rga** to set the receiver gain and **zg** to acquire the time domain data. The approximate experiment time for the TOCSY with the acquisition parameters set as shown above is 1.3 hours.

9.3 Processing

Enter **edp** and set the processing parameters as shown in Table 42.

Table 42: TOCSY Processing Parameters

F2 Parameters		
Parameter	**Value**	**Comments**
SI	512	
SF		spectrum reference frequency (^{1}H)
WDW	SINE	multiply data by phase-shifted sine function
SSB	2	choose pure cosine wave
PH_mod	pk	
PKNL	TRUE	
BC_mod	no	
F1 Parameters		

Parameter	Value	Comments
SI	512	
SF		spectrum reference frequency (^{1}H)
WDW	SINE	multiply data by phase-shifted sine function
SSB	2	choose pure cosine wave
PH_mod	pk	
BC_mod	no	
MC2	States-TPPI	

Enter **`xfb`** to multiply the time domain data by the window functions and to perform the 2D Fourier transformation.

The threshold level can be adjusted by placing the cursor on the button, holding down the middle mouse button, and moving the mouse back and forth. The optimum may be saved by typing **`defplot`** and answering the questions which appear.

9.4 Phase Correction

To simplify the phasing of the 2D TOCSY spectrum, it helps to first phase the second row. Enter **`rser 2`** to transfer the second row to the 1D data set ~TEMP/1/1. Enter **`sinm`** to apply the sine-bell windowing function, and enter **`ft`** to Fourier transform the data. Manually phase correct the spectrum as any 1D spectrum *except that* when you are finished, click return and select **Save as 2D & return** to save the corrections **`phc0`** and **`phc1`** to the 2D data file tocsy/1/1. Click 2D to return to the 2D data set tocsy/1/1.

Now enter **`xfb`** to Fourier transform the TOCSY spectrum again, this time applying the appropriate phase correction to F2. The spectrum should now require additional phase correction only in F1, and this can be accomplished in the 2D phasing subroutine.

Click on phase to enter the phase correction submenu.

Click on col with the left mouse button to tie the cursor to the 2D spectrum appearing in the upper left hand corner of the display. Move the mouse until the vertical cross hair is aligned with a column towards one end of the spectrum. Once the desired column is selected, move it to window 1, appearing in the upper right hand corner of the display (see Section 8.3.3).

Repeat the above procedure to select two further columns, one with a diagonal peak in the middle and one with a peak at the other end of the spectrum. Move these columns to window 2 and 3, respectively.

Now that three columns have been selected, the 0 - and 1st-order phase corrections in F1 are determined manually exactly as for the DQF-COSY spectrum (see Section 8.3.3). When the phase correction is satisfactory, click on and select **Save & return** to save the results and confirm the xf1p option to apply this phase correction to the spectrum.

At this point, the spectrum should be phased correctly. If, however, the user wishes to make further adjustments, the above procedure can be repeated to adjust the F1 phasing. To further phase correct the spectrum in F2, repeat the above procedure for rows rather than columns. Phase correct as described above and confirm the xf2p option.

It should be possible to phase correct the spectrum so that all TOCSY peaks are positive.

9.5 Plot the Spectrum

Set the region, the threshold and peak type (positive and/or negative) to be used for plotting the spectrum. Make sure the spectrum appears as desired on the screen, type **`defplot`** and answer the following questions:

Change levels?	y
Please enter number of positive levels?	6
Please enter number of negative levels?	3
Display contours?	n

Enter **`edg`** to edit the plotting parameters.

Click the **`ed`** next to the parameter EDPROJ1 to enter the F1 projection parameters submenu. Edit the parameters from PF1DU to PF1PROC as follows:

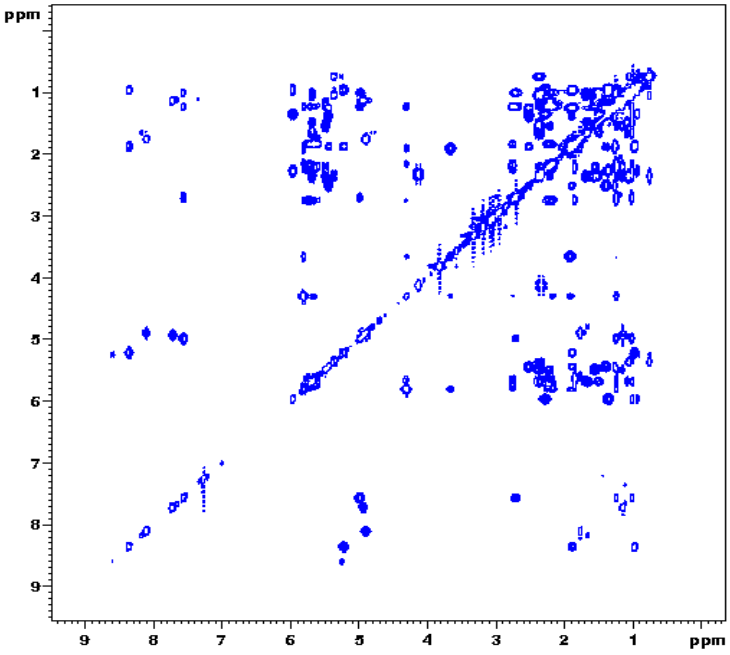
PF1DU	u
PF1USER	(name of user for file cosy/1/1)
PF1NAME	cosy
PF1EXP	1
PF1PROC	1

Click SAVE to save these changes and return to the **`edg`** menu.

Click the **`ed`** next to the parameter EDPROJ2 to enter the F2 projection parameters submenu as described for EDPROJ1 above. Click SAVE to save all the above changes and exit the **`edg`** menu. Enter **`setti`** to open the title file and enter a title.

To plot the spectrum, simply enter **`plot`**. A TOCSY spectrum of 50 mM Cyclosporin in C_6D_6 is shown in Figure 28.

Figure 28: TOCSY Spectrum of 50 mM Cyclosporin in C6D6


ppm
1
2
3
4
5
6
7
8
9
9
8
7
6
5
4
3
2
1
ppm

10 ROESY

10.1 Introduction

ROESY (**R**otating-frame **O**verhauser **E**ffect **S**pectroscop**Y**) is an experiment in which homonuclear Nuclear Overhauser effects (NOEs) are measured under spin-locked conditions. ROESY is especially suited for molecules with motional correlation times (τ_c) such that $\omega\tau_c \sim 1$, were ω is the angular frequency $\omega=\gamma B$. In such cases the laboratory-frame NOE is nearly zero, but the rotating-frame NOE (or ROE) is always positive and increases monotonically for increasing values of τ_c. In ROESY the mixing time is the spin-lock period during which spin exchange occurs among spin-locked magnetization components of different nuclei (recall that spin exchange in NOESY occurs while magnetization is aligned along the z axis). Different spectral density functions are relevant for ROESY than for NOESY and these cause the ROE to be positive for all values of τ_c.

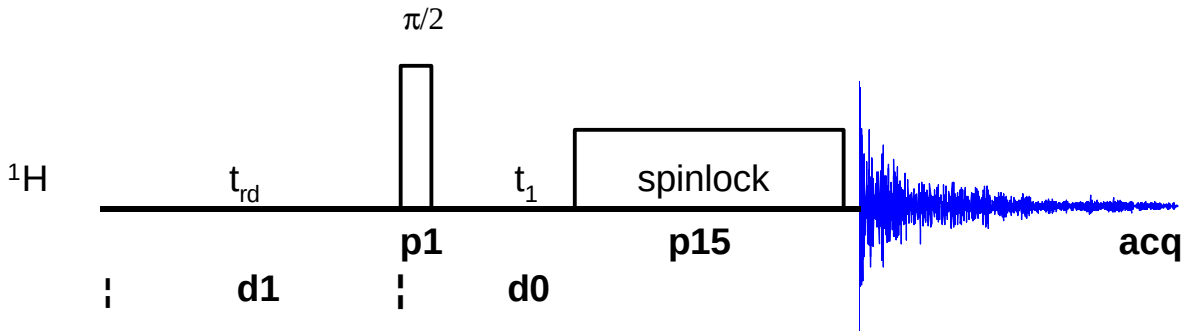
ROESY spectra can be obtained in 2D absorption mode. This is also useful for the identification of certain artifacts. Spurious cross peaks, both COSY-type and TOCSY-type, can be observed due to coherence transfer between scalar coupled spins. COSY-type artifacts (anti-phase) arise when the mixing pulse transfers anti-phase magnetization from one spin to another. TOCSY-type artifacts (which have the same phase as the diagonal peaks, while ROESY cross peaks have opposite phase) arise when the Hartmann-Hahn condition is met (e.g., when spins A and B have opposite but equal offsets from the transmitter frequency or when they have nearly identical chemical shifts). In general, to minimize these artifacts, it is suggested to limit the strength of the spin-locking field.

Reference: A. Bax and D. G. Davis, *J. Magn. Reson.*, **63**, 207 (1985).

The sample used to demonstrate ROESY in this chapter is 50 mM Cyclosporin in C_6D_6.

The ROESY pulse sequence is shown in Figure 29.

Figure 29: ROESY Pulse Sequence



10.2 Acquisition

Insert the sample in the magnet. Lock the spectrometer. Readjust the Z and Z^2 shims until the lock level is optimized. Tune and match the probehead for ^{1}H observation.

It is recommended to run 2D experiments without sample spinning.

Record a ^{1}H reference spectrum to determine the correct values for **o1p** and **sw**. A ^{1}H reference spectrum of this sample was already created for the magnitude COSY experiment (Section 8.2.2). This spectrum is found in the data set cosy/1/1.

The ROESY data set can be created from the data set of any of the previous homonuclear 2D experiments run on this sample. For example, enter **re cosy 2 1** to call up the data set cosy/2/1. Enter **edc** and change the following parameters:

NAME	roesy
EXPNO	1
PROCNO	1

Click SAVE to create the data set roesy/1/1.

Enter **eda** and set the acquisition parameters as shown in Table 43.

The pulse **p15** at **pl11** sets the length of the cw spinlock pulse. The value listed in Table 43 is appropriate for this sample. For other samples with different relaxation properties, optimal results may be achieved with slightly different values. The typical range for **p15** is from 50 to 300 msec. A good rule of thumb is that **p15** for the ROESY experiment of a molecule should be about the same as **d8** for the NOESY experiment of that molecule.

Table 43: ROESY Acquisition Parameters

F2 Parameters		
Parameter	**Value**	**Comments**
PULPROG	roesyph	
TD	1k	
NS	32	the number of scans must 8 * n
DS	16	number of dummy scans.
PL1		high power level on F1 channel (^{1}H) as determined in Section 4.2.4
PL11		low power level on F1 channel (^{1}H) for spinlock as determined in Section 4.2.6
P1		^{1}H 90° pulse as determined in Section 4.2.4
P15	200m	spinlock pulse
D1	2	

F1 Parameters		
Parameter	**Value**	**Comments**
TD	256	number of experiments.
FnMODE	States-TPPI	
ND0	1	one d0 period per cycle
IN0		t_1 increment: equal to 2 * DW used in F2
SW		sw of the optimized ^{1}H spectrum (cosy/1/1): same as for F2
NUC1		select ^{1}H frequency for F1; same as for F2

Enter **zg** to acquire the time domain data. The approximate experiment time for ROESY with the acquisition parameters set as shown above is 5.5 hours.

10.3 Processing

Enter **edp** and set the processing parameters as shown in Table 44.

Table 44: ROESY Processing Parameters

F2 Parameters		
Parameter	**Value**	**Comments**
SI	512	
SF		spectrum reference frequency (^{1}H)
WDW	SINE	multiply data by phase-shifted sine function
SSB	2	choose pure cosine wave
PH_mod	pk	
PKNL	TRUE	
BC_mod	no	
F1 Parameters		
Parameter	**Value**	**Comments**
SI	512	
SF		spectrum reference frequency (^{1}H)
WDW	SINE	multiply data by phase-shifted sine function
SSB	2	choose pure cosine wave
PH_mod	pk	
BC_mod	no	
MC2	States-TPPI	

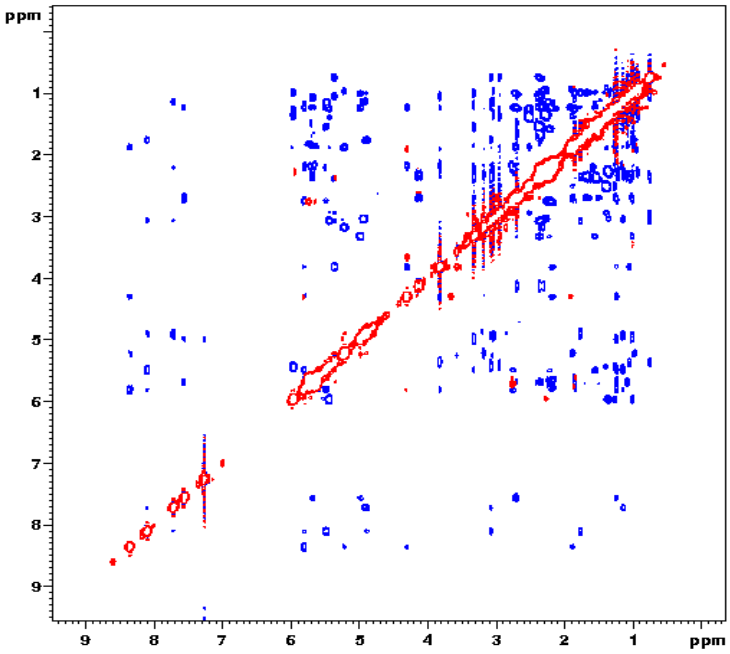
Enter **xfb** to multiply the time domain data by the window functions and to perform the 2D Fourier transformation.

The threshold level can be adjusted by placing the cursor on the button, holding down the middle mouse button, and moving the mouse back and forth. The optimum may be saved by typing **defplot** and answering the questions which appear.

10.4 Phase Correction and Plotting

For the phase correction procedure and the plotting procedure please follow the instructions given for the TOCSY spectrum in Sections 9.4 and 9.5, respectively.

Figure 30: ROESY Spectrum of 50 mM Cyclosporin in C6D6



11 NOESY

11.1 Introduction

NOESY (**N**uclear **O**verhauser **E**ffect **S**pectroscop**Y**) is a 2D spectroscopy method whose aim is to identify spins undergoing cross-relaxation and to measure the cross-relaxation rates. Most commonly, NOESY is used as a homonuclear ^{1}H technique. In NOESY, direct dipolar couplings provide the primary means of cross-relaxation, and so spins undergoing cross-relaxation are those which are close to one another in space. Thus, the cross peaks of a NOESY spectrum indicate which protons are close to each other in space. This can be distinguished from COSY, for example, which relies on J-coupling to provide spin-spin correlation, and whose cross peaks indicate which ^{1}H's are close to which other ^{1}H's through the bonds of the molecule.

The basic NOESY sequence consists of three $\pi/2$ pulses. The first pulse creates transverse spin magnetization. This precesses during the evolution time t_1, which is incremented during the course of the 2D experiment. The second pulse produces longitudinal magnetization equal to the transverse magnetization component orthogonal to the pulse direction. Thus, the basic idea is to produce an initial situation for the mixing period τ_m Note that, for he basic NOESY experiment, τ_m is kept constant throughout the 2D experiment. The third pulse creates transverse magnetization from the remaining longitudinal magnetization. Acquisition begins immediately following the third pulse, and the transverse magnetization is observed as a function of the time t_2. The NOESY spectrum is generated by a 2D Fourier transform with respect to t_1 and t_2.

Axial peaks, which originate from magnetization that has relaxed during τ_m, can be removed by the appropriate phase cycling.

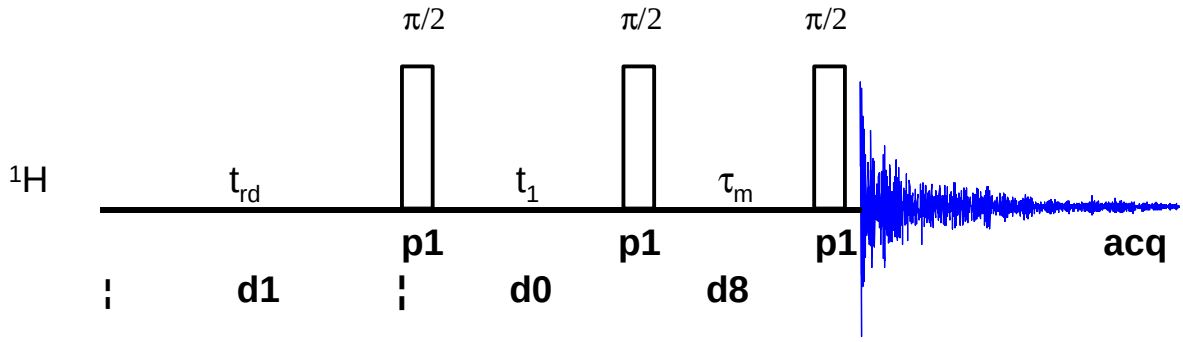
NOESY spectra can be obtained in 2D absorption mode. Occasionally, COSY-type artifacts appear in the NOESY spectrum; however, these are easy to identify by their anti-phase multiplet structure.

References: J. Jeener, B. H. Meier, P. Bachmann, R. R. Ernst, *J. Chem. Phys.*, **69**, 4546 (1979); G. Wagner and K. Wüthrich, *J. Mol. Biol.*, **155**, 347 (1982).

The sample used to demonstrate NOESY in the chapter is 50 mM Cyclosporin in C_6D_6.

The NOESY pulse sequence is shown in Figure 31. The delay **d8** determines the length of the mixing period, during which NOE buildup occurs.

Figure 31: NOESY Pulse Sequence



11.2 Acquisition and Processing

Insert the sample in the magnet. Lock the spectrometer. Readjust the Z and Z^2 shims until the lock level is optimized. Tune and match the probehead for ^{1}H observation.

It is recommended to run 2D experiments without sample spinning.

Record a ^{1}H reference spectrum to determine the correct values for **o1p** and **sw**. A ^{1}H reference spectrum of this sample was already created for the magnitude COSY experiment (Section 8.2.2). This spectrum is found in the data set cosy/1/1.

The NOESY data set can be created from the data set of any of the previous homonuclear 2D experiments run on this sample. For example, enter **re cosy 2 1** to call up the data set cosy/2/1. Enter **edc** and change the following parameters:

NAME	noesy
EXPNO	1
PROCNO	1

Click SAVE to create the data set noesy/1/1.

Enter **eda** and set the acquisition parameters as shown in Table 45.

Table 45: NOESY Acquisition Parameters

F2 Parameters		
Parameter	**Value**	**Comments**
PULPROG	noesyph	
TD	1k	
NS	32	the number of scans must 8 * n
DS	16	number of dummy scans.
PL1		high power level on F1 channel (^{1}H) as determined in Section 4.2.4
P1		^{1}H 90° pulse as determined in Section 4.2.4
D8	350m	Mixing time
D1	2	
F1 Parameters		
Parameter	**Value**	**Comments**
TD	256	number of experiments.
FnMODE	States-TPPI	
ND0	1	one d0 period per cycle
IN0		t_1 increment: equal to 2 * DW used in F2
SW		sw of the optimized ^{1}H spectrum (cosy/1/1): same as for F2
NUC1		select ^{1}H frequency for F1; same as for F2

11.2.1 Optimize Mixing Time

The parameter **d8** determines the length of the mixing period during which NOE buildup occurs. This should be on the order of T_1. The value listed in Table 45 is appropriate for this sample at 300 MHz and room temperature. If no appropriate value of **d8** is available the following quick and easy procedure can be used.

Create a 1D data set from the NOESY 2D data set: Enter **edc**, set EXPNO to 2, and click SAVE to create the data set noesy/2/1. Enter **eda**, set PARMODE to 1D, click and ok the requests to delete a number of files.

In **eda** set PULPROG to zg (or enter **pulprog zg**). Set **ns** to 1 and **ds** to 0. Use **zg** and **ef** to acquire and process a 1D ^{1}H spectrum. Manually phase correct the spectrum and store the correction.

In **eda** change PULPROG to the pulse program t1ir1d (or enter **pulprog t1ir1d**). This is a so-called inversion recovery sequence. Set **d7** to

approximately 1 msec (**d7 1m**), record and process a spectrum using **zg** and **efp**. The signals should all be negative.

To set **d7** to 1 sec, enter **d7 1** and record and process another spectrum using **zg** and **efp**. The signals should all be positive. Now find a value for **d7** in the range of 300-600ms, where all the signals are minimal. This length of time is sufficient for NOE buildup in small molecules (in order to avoid spin diffusion in macromolecules, it may be necessary to use a shorter length of time).

Return to the NOESY data set by typing **re 1**. Enter **d8** and set this to the value of **d7** determined above.

11.2.2 Acquire the 2D data set

Enter **zg** to acquire the time domain data. The approximate experiment time for NOESY with the acquisition parameters set as shown above is 5.8 hours.

11.3 Processing

Enter **edp** and set the processing parameters as shown in Table 46.

Table 46: NOESY Processing Parameters

F2 Parameters		
Parameter	**Value**	**Comments**
SI	512	
SF		spectrum reference frequency (^{1}H)
WDW	SINE	multiply data by phase-shifted sine function
SSB	2	choose pure cosine wave
PH_mod	pk	
PKNL	TRUE	
BC_mod	no	
F1 Parameters		
Parameter	**Value**	**Comments**
SI	512	
SF		spectrum reference frequency (^{1}H)
WDW	SINE	multiply data by phase-shifted sine function
SSB	2	choose pure cosine wave
PH_mod	pk	
BC_mod	no	
MC2	States-TPPI	

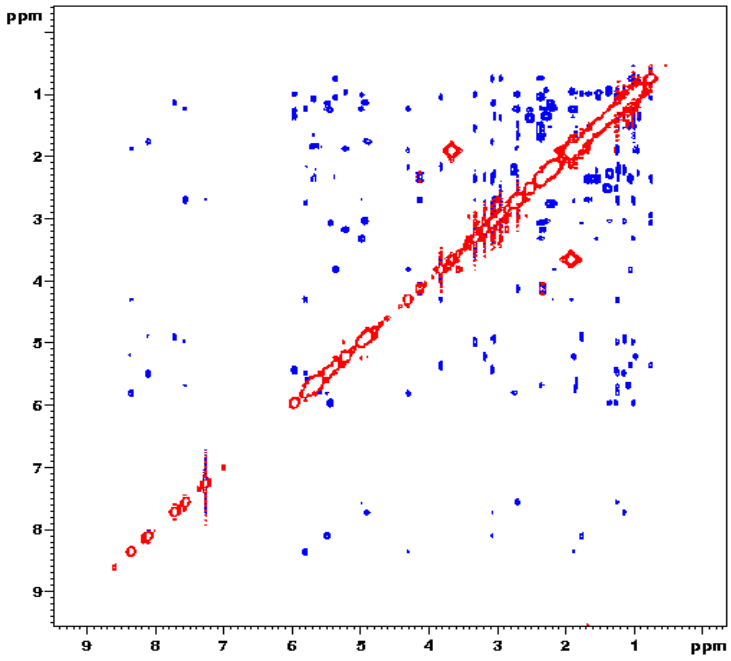
Enter **xfb** to multiply the time domain data by the window functions and to perform the 2D Fourier transformation.

The threshold level can be adjusted by placing the cursor on the button, holding down the middle mouse button, and moving the mouse back and forth. The optimum may be saved by typing **defplot** and answering the questions which appear.

11.4 Phase Correction and Plotting

For the phase correction procedure and the plotting procedure please follow the instructions given for the TOCSY spectrum in Sections 9.4 and 9.5, respectively. Note that for the NOESY spectrum recorded here, the first serial file should be chosen for the F2 phase correction: type **rser 1** instead of **rser 2**, as for the TOCSY and ROESY spectra.

Figure 32: NOESY Spectrum of 50 mM Cyclosporin in C_6D_6



12 XHCORR

12.1 Introduction

Heteronuclear (**X**, **H**) shift **CORR**elation spectroscopy is a 2D technique that can be used to determine which ^{1}H of a molecule are bonded to which ^{13}C nuclei (or other X nuclei). Like DEPT, XHCORR makes use of the large one-bond heteronuclear J-coupling (J_{XH}) for polarization transfer, and thus only ^{13}C bonded directly to ^{1}H's are detected. For ^{13}C and directly attached ^{1}H, J_{XH} = 100 to 200 Hz, while for more distant ^{1}H, J_{XH} = 5 to 20 Hz.

The final 2D XHCORR spectrum has a projection onto the F2 axis which is the usual ^{1}H-decoupled ^{13}C spectrum with all quaternary carbons missing, and a projection onto the F1 axis which is the normal ^{1}H spectrum with reduced signal to noise since only ^{1}H directly attached to ^{13}C contribute to the signal. The XHCORR experiment is not phase-sensitive, and so the final 2D spectrum must be displayed in magnitude mode.

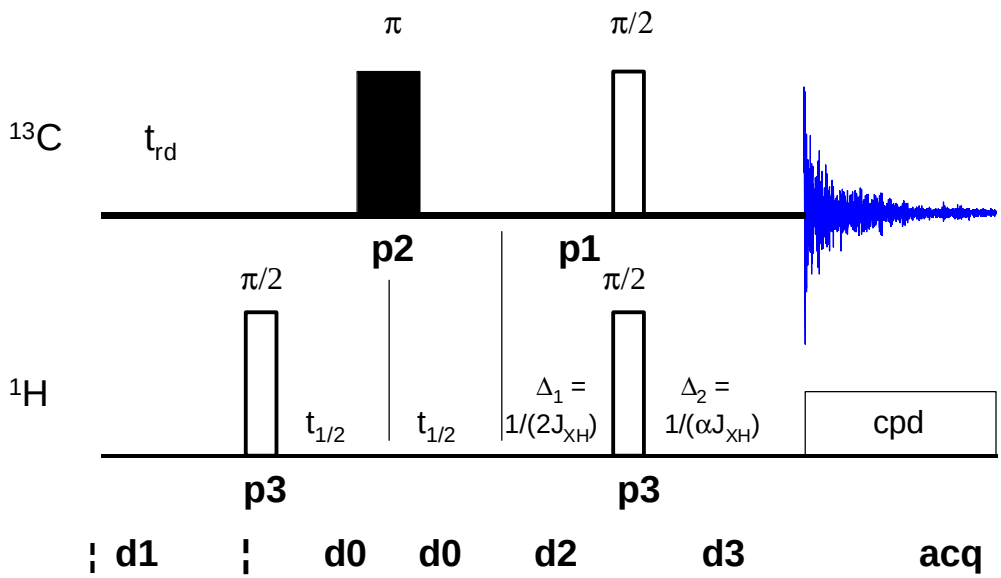
Reference: A. Bax and G. A. Morris, *J. Magn. Reson.*, **42**, 501 (1981).

The sample used to demonstrate XHCORR in this chapter is 1 g Cholesterylacetate in $CDCl_3$, which was used to demonstrate DEPT.

The XHCORR pulse sequence is shown in Figure 33. The short delay between the final ^{13}C pulse and the start of acquisition is a refocusing period so that the ^{13}C lines do not have opposite phase and thus do not cancel one another when ^{1}H-decoupling is applied. The optimal refocusing time (Δ_2) depends on whether the ^{13}C belongs to a CH, CH_2 or CH_3 group. Generally a compromise value of $\Delta_2=1/(3J_{XH})$ is chosen. ^{13}C couplings during t_1 are removed by adding a ^{13}C π pulse in the middle of t_1, so that there is refocusing by the end of t_1. To enable maximum polarization transfer, a fixed delay $\Delta_1=1/(2J_{XH})$ is added after t_1. This delay allows anti-phase magnetization to be re-established.

In this pulse sequence, the delay time **d2** determines the length of the delay for the creation of anti-phase magnetization ($\Delta_1=1/(2J_{XH})$), and the time **d3** determines the length of the refocusing delay ($\Delta_2=1/(\alpha J_{XH})$), were α is usually chosen to be 3.

Figure 33: XHCORR Pulse Sequence



12.2 Acquisition

Insert the sample in the magnet. Lock the spectrometer. Readjust the Z and Z^2 shims until the lock level is optimized. Tune and match the probehead for ^{13}C observation and ^{1}H decoupling.

It is recommended to run 2D experiments without sample spinning.

12.2.1 Proton Reference Spectrum

Record a ^{1}H reference spectrum to obtain the correct ^{1}H carrier frequency (**o1p**) and spectral width (**sw**) values: Enter **re proton 1 1** to call up the data set proton/1/1; enter **edc** and change the following parameters

NAME	xhcorr
EXPNO	1
PROCNO	1

Click SAVE to create the data set xhcorr/1/1.

Enter **rga** to perform an automatic receiver gain adjustment. Acquire and process a standard ^{1}H spectrum. Calibrate the spectrum, and optimize **sw** and **o1p** so that the ^{1}H signals cover almost the entire spectral width. Acquire an optimized spectrum.

12.2.2 Carbon Reference Spectrum

A ^{1}H-decoupled ^{13}C reference spectrum to determine the correct carrier frequency (**o1p**) and spectral width (**sw**) values for ^{13}C: Since XHCORR detects only ^{13}C directly bonded to ^{1}H, a DEPT-45 spectrum is typically used

as a ^{13}C reference spectrum. Enter **`re dept 1 1`** to call up the data set dept/1/1; enter **`edc`** and change the following parameters

NAME	xhcorr
EXPNO	2
PROCNO	1

Click SAVE to create the data set xhcorr/2/1.

Enter **`rga`** to perform an automatic receiver gain adjustment. Acquire and process a ^{13}C spectrum. Calibrate the spectrum, and optimize **`sw`** and **`o1p`** so that the ^{13}C signals cover almost the entire spectral width. Acquire an optimized spectrum.

12.2.3 Acquire the 2D Data Set

Type **`xau iexpno`** (increment experiment number) to create the data set xhcorr/3/1.

Enter **`eda`** and set PARMODE to 2D. Click on SAVE and ok the message "Delete 'meta.ext' files?". The window now switches to a 2D display and the message "NEW 2D DATA SET" appears.

Enter **`eda`** and set the acquisition parameters as shown in Table 47.

Table 47: XHCORR Acquisition Parameters

Parameter	Value	Comments
PULPROG	hxcoqf	
TD	1k	
NS	8	the number of scans must be 4 * ns
DS	16	number of dummy scans.
PL1		high power level on F1 channel (^{13}C) as determined in Section 6.1.4
PL2		high power level on F2 channel (^{1}H) as determined in Section 4.2.4
PL12		low power level on F2 channel (^{1}H) for CPD as determined in Section 6.2.6
P1		^{13}C 90° pulse as determined in Section 6.1.4
P2		^{13}C 180° pulse, calculated from P1
P3		^{1}H 90° pulse as determined in Section 4.2.4
PCPD2		^{1}H 90° pulse for cpd sequence as determined in Section 6.2.6
D1	2	relaxation delay; should be 1–5 * $T_1(^{13}C)$
CNST2	145	heteronuclear scalar J(^{13}C,^{1}H) coupling 145 Hz is a good intermediate value
D2	3.45 msec	1/[2J(^{13}C,^{1}H)], calculated automatically from cnst2 above

CNST11	3	used to calculate d3; 3 for all multiplicities
D3	2.30 msec	calculated automatically from cnst11 above
CPDPRG2	waltz16	cpd sequence for the ^{1}H decoupling
F1 Parameters		
Parameter	**Value**	**Comments**
TD	256	number of experiments.
FnMODE	QF	
ND0	2	two d0 periods per cycle
SW		sw of the optimized ^{1}H spectrum (xhcorr/1/1)
IN0		t_1 increment, calculated from SW above
NUC1		selects ^{1}H frequency for F1

Since this data set was created from the DEPT-45 reference spectrum, the receiver gain is already set correctly.

Enter **zg** to acquire the spectrum; the approximate experiment time for XHCORR with the acquisition parameters set as shown above is 2.5 hours.

12.3 Processing

Enter **edp** and set the processing parameters as shown in Table 48.

Table 48: XHCORR Processing Parameters

F2 Parameters		
Parameter	**Value**	**Comments**
SI	1k	
SF		spectrum reference frequency (^{13}C)
WDW	EM	
LB	3	a value of 2-5 Hz is appropriate
PH_mod	no	this is a magnitude spectrum.
PKNL	TRUE	necessary when using the digital filter
BC_mod	quad	

F1 Parameters		
Parameter	**Value**	**Comments**
SI	512	
SF		spectrum reference frequency (^{1}H)
WDW	QSINE	multiply data by squared sine function
SSB	1	choose pure sine bell
PH_mod	mc	this is a magnitude spectrum.
BC_mod	no	
MC2	QF	

Enter **xfb** to perform the 2D Fourier transformation.

The threshold level can be adjusted by placing the cursor on the button, holding down the left mouse button, and moving the mouse up and down. The #colors button is used to set the number of levels. The user can choose to display positive peaks only, negative peaks only, or both positive and negative peaks by clicking on +/- with the left mouse button. Since this is a magnitude spectrum, only positive peaks need to be displayed.

Since this is a magnitude spectrum, no phase adjustment can be made.

When the spectrum appears as desired on the screen, click DefPlot and answer the following questions.

Change levels?	y
Please enter number of positive levels?	6
Display contours?	n

12.4 Plotting the Spectrum

Read in the plot parameter file standard2D by entering **rpar standard2D plot** to set most of the plotting parameters to values which are appropriate for this 2D spectrum.

Enter **edg** to edit the plotting parameters. Click the **ed** next to the parameter EDPROJ1 to enter the F1-projection parameters submenu. Edit the parameters from PF1DU to PF1PROC as follows:

PF1DU	u
PF1USER	(name of user for file xhcorr/1/1)
PF1NAME	xhcorr
PF1EXP	1
PF1PROC	1

Click SAVE to save these changes and return to the **edg** menu.

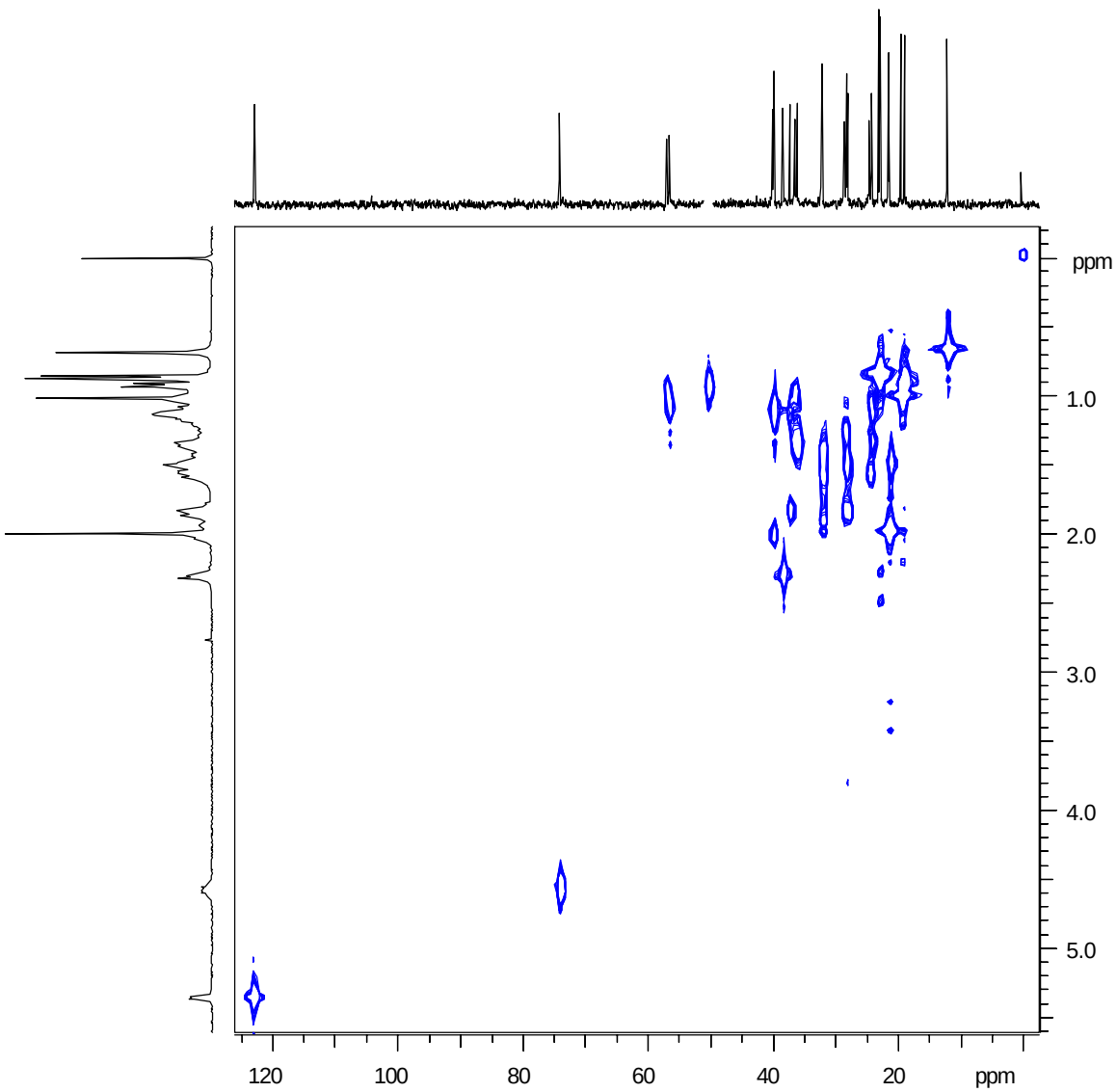
Click the **ed** next to the parameter EDPROJ2 to enter the F2-projection parameters submenu. Edit the parameters from PF2DU to PF2PROC as follows:

PF2DU	u
PF2USER	(name of user for file dept/4/1)
PF2NAME	xhcorr
PF2EXP	2
PF2PROC	1

Click twice SAVE to save these changes and return to main menu.

Create a title for the spectrum (**setti**) and plot the spectrum (**plot**). An XHCORR spectrum of 1 g Cholesterylacetate in $CDCl_3$ is shown in Figure 34.

Figure 34: XHCORR Spectrum of 1g Cholesterylacetate in $CDCl_3$



13 COLOC

13.1 Introduction

COLOC (**CO**rrelation spectroscopy via **LO**ng-range **C**oupling) is a 2D heteronuclear correlation technique very similar to the XHCORR experiment described in the previous Section 12. However, unlike XHCORR, COLOC makes use also of small long-range heteronuclear J-couplings ($^nJ_{XH}$, n > 1) for the polarization transfer, and detects all ^{13}C, even those which are not directly bonded to ^1H.

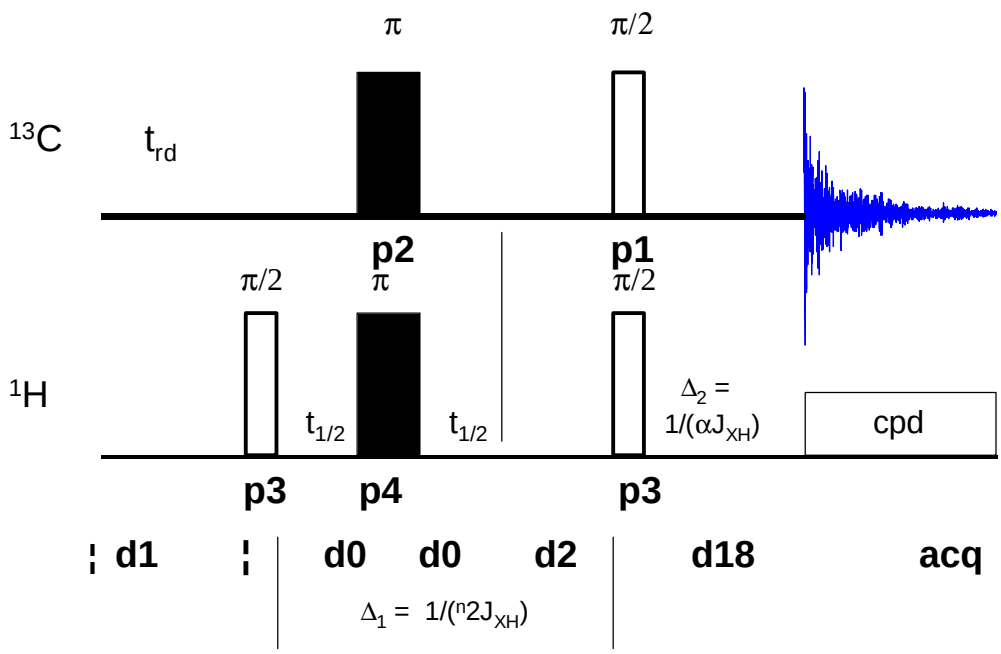
Because of the close similarity to the XHCORR, the COLOC experiment is only described in brief here.

Reference: H. Kessler, C. Griesinger, J. Zarbock, and H. R. Loosli, *J. Magn. Reson.*, **57**, 331 (1984).

The sample used to demonstrate COLOC in this chapter is 1 g Cholesterylacetate in $CDCl_3$ as already used for the DEPT and XHCORR experiments.

The COLOC pulse sequence is shown in Figure 35. The evolution time t_1 is incorporated in the polarization transfer period $\Delta_1=1/(2\ ^nJ_{XH})$: Since the long-range heteronuclear coupling constants are small (e.g. $^nJ_{CH}$=5 to 20 Hz), the time period Δ_1 is rather long and serious sensitivity losses due to transverse relaxation are inevitable.

Figure 35: COLOC Pulse Sequence



13.2 Acquisition and Processing

Start out from the xhcorr/3/1 data set (**`re xhcorr 3 1`**) and create the data set coloc/1/1 (type **`edc`** and change the name to coloc and the experiment number to **`1`**). The acquisition parameters are shown in Table 49.

In this pulse sequence, the delay time **`d6`** determines the length of the delay for the creation of anti-phase magnetization ($\Delta_1 = 1/(2\ {}^nJ_{CH})$), and the time **`d18`** determines the length of the refocusing ($\Delta_1 = 1/(a\ {}^nJ_{CH})$), where a is generally chosen to be 3. To ensure that the pulses occur during Δ_1, the user must make sure that **`d6`** = **`d0`** + (**`td`**(F1) * **`in0`**) + (**`p2`** or **`p4`**); in other words, that **`d6`** is at least as long as the maximum evolution time (t_1) plus the length of the longest pulse (**`p2`** or **`p4`**).

Table 49: COLOC Acquisition Parameters

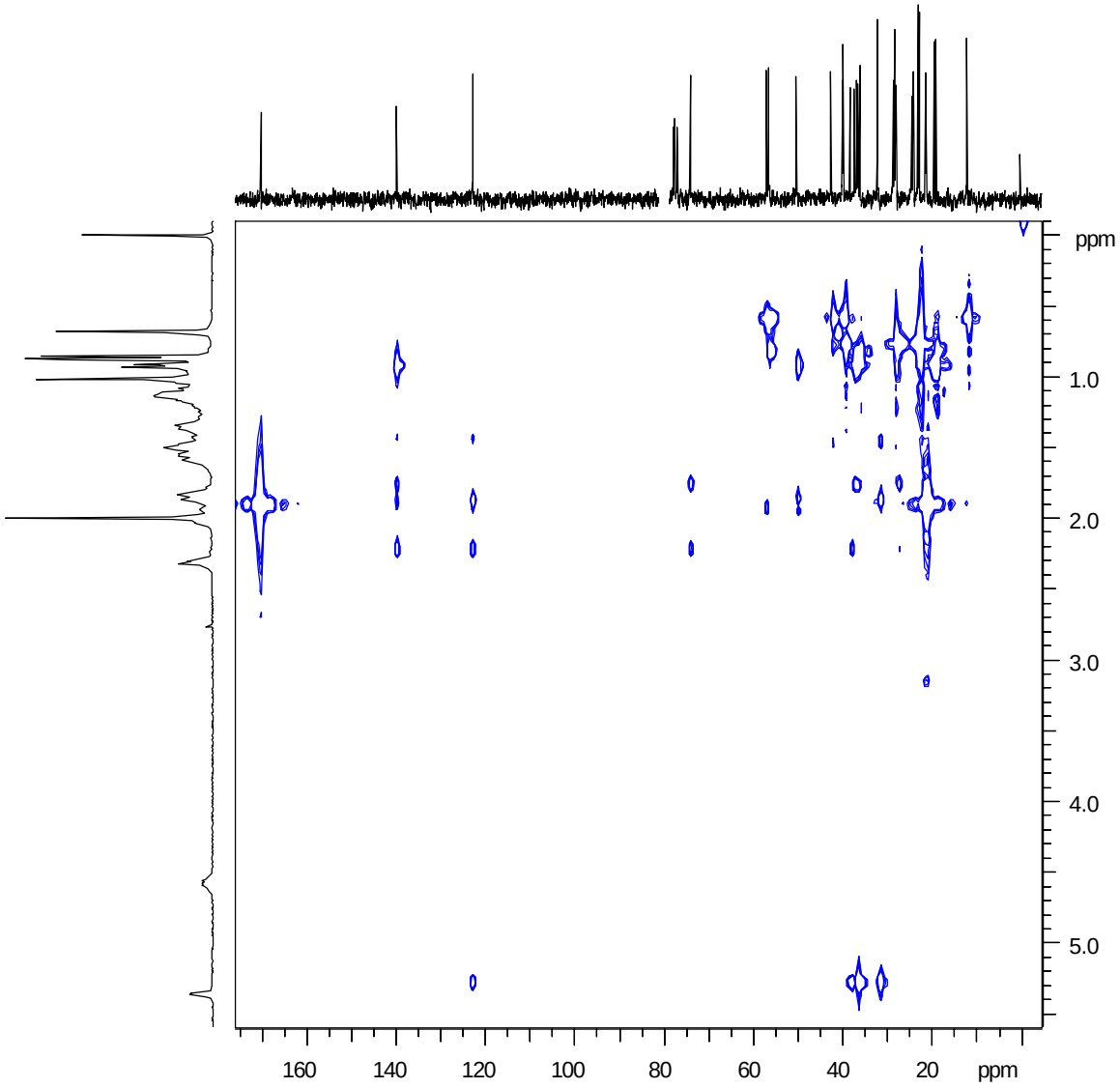
Parameter	Value	Comments
PULPROG	colocqf	
TD	1k	
NS	8	the number of scans must be 4 * ns
DS	16	number of dummy scans.
PL1		high power level on F1 channel (^{13}C) as determined in Section 6.1.4
PL2		high power level on F2 channel (^{1}H) as determined in Section 4.2.4
PL12		low power level on F2 channel (^{1}H) for CPD as determined in Section 6.2.6
P1		^{13}C 90° pulse as determined in Section 6.1.4
P2		^{13}C 180° pulse, calculated from P1
P3		^{1}H 90° pulse as determined in Section 4.2.4
P4		^{1}H 180° pulse, calculated from P3
PCPD2		^{1}H 90° pulse for cpd sequence as determined in Section 6.2.6
D1	2	relaxation delay; should be 1–5 * $T_1(^{13}C)$
D6	50m	Delay for evolution of heteronuclear scalar long-range $J(^{13}C,^{1}H)$ couplings
D18	33.3m	Delay for evolution of heteronuclear scalar long-range $J(^{13}C,^{1}H)$ couplings
CPDPRG2	waltz16	cpd sequence for the ^{1}H decoupling

F1 Parameters		
Parameter	**Value**	**Comments**
TD	256	number of experiments.
FnMODE	QF	
ND0	2	two d0 periods per cycle
SW		sw of the optimized ^{1}H spectrum (xhcorr/1/1)
IN0		t_1 increment, calculated from SW above
NUC1		selects ^{1}H frequency for F1

The ^{1}H- and the ^{13}C-reference spectra have already been recorded for the XHCORR experiment. Follow the instructions given there for the acquisition and the processing of the 2D COLOC experiment.

A COLOC spectrum of 1g Cholesterylacetate in $CDCl_3$ is shown inFigure 36.

Figure 36: COLOC Spectrum of 1g Cholesterylacetate in $CDCl_3$



14 HMQC

14.1 Introduction

HMQC (**H**eteronuclear **M**ultiple **Q**uantum **C**orrelation) spectroscopy is an inverse chemical shift correlation experiment that yields exactly the same information as the XHCORR. The advantage of HMQC is that the nucleus with the highest γ (^{1}H) is detected, and so it is possible to obtain the highest sensitivity. The challenge of an inverse chemical shift correlation experiment, however, is that the large signals from ^{1}H not coupled directly to a ^{13}C nucleus must be suppressed in a difference experiment. This poses a dynamic range problem: the signal of interest is that of ^{1}H coupled directly to ^{13}C nuclei; however, the signal detected is dominated by the contribution of ^{1}H bonded directly to ^{12}C nuclei. HMQC minimizes this dynamic range problem while optimizing the sensitivity of the experiment. The resonance frequency of low γ spins can be detected with enhanced sensitivity by the creation and ^{1}H detection of ^{1}H-^{13}C (or other X nucleus) multiple-quantum coherence.

References: A. Bax, R. H. Griffey, and B. L. Hawkins, *J. Magn. Reson.*, **55**, 301 (1983); A. Bax and S. Subramanian, *J. Magn. Reson.*, **67**, 565 (1986).

The sample used to demonstrate HMQC in this chapter is 50 mM Cyclosporin in C_6D_6. This is the same sample that was used to demonstrate COSY, NOESY, ROESY, and TOCSY.

The HMQC pulse sequence is shown in Figure 37, which should be used on samples consisting of proteins and other macromolecules. The first ^{1}H pulse creates transverse magnetization, some of which evolves into anti-phase magnetization at the end of the first $1/(2J_{XH})$ delay. This anti-phase magnetization is converted into multiple-quantum coherence by the $(\pi/2)_X$ pulse and evolves chemical shift during t_1. In analogy with XHCORR a delay $1/(2J_{XH})$ is inserted between the final 90° pulse after t_1 and the start of the acquisition so that ^{13}C decoupling can be used during acquisition. Without this delay, the ^{1}H magnetization components would be anti-phase at the start of the acquisition and so ^{13}C decoupling would result in mutual cancellation of the ^{1}H signals.

Note that since it is the longitudinal ^{1}H magnetization present before the first $(\pi/2)_H$ pulse that is converted into heteronuclear multiple-quantum coherence, it is the ^{1}H T_1 which determines the appropriate recycle delay. Thus, it is possible to use a shorter recycle delay for HMQC than for XHCORR.

For small molecules, it is useful to use a BIRD preparation period in conjunction with the HMQC experiment (Figure 38). The basic idea of this preparation period is to saturate all ^{1}H not directly attached to a ^{13}C nucleus.

HMQC is a phase-sensitive experiment, and after a 2D Fourier transform with respect to t_1 and t_2, the 2D spectrum can be phased so that all peaks are purely absorptive.

Figure 37: HMQC Pulse Sequence

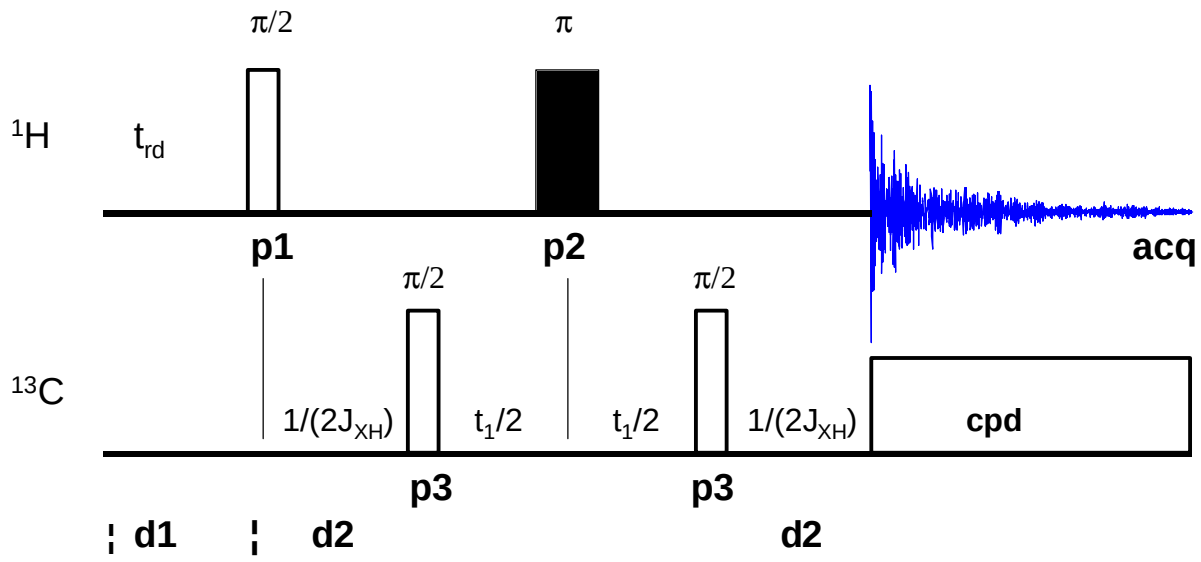
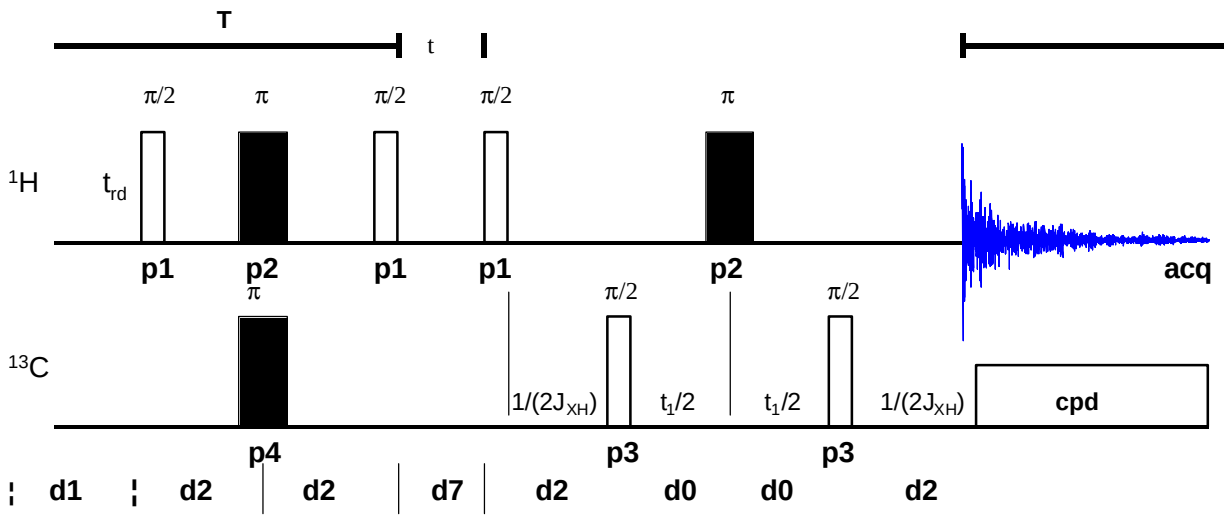


Figure 38: HMQC with BIRD Pulse Sequence



14.2 Acquisition

Insert the sample in the magnet. Lock the spectrometer. Readjust the Z and Z^2 shims until the lock level is optimized. Tune and match the probehead for ^{1}H observation and ^{13}C decoupling.

It is recommended to run 2D experiments without sample spinning

As for the XHCORR experiment, both ^{1}H and ^{13}C reference spectra of this sample must be recorded; see Sections 12.2.1 and 12.2.2 for the

corresponding instructions. Use **wrpa** to store the reference spectra as data sets hmqc/1/1 (for the ^{1}H spectrum) and hmqc/2/1 (for the ^{13}C spectrum).

Enter **re hmqc 1 1** to return to the optimized ^{1}H spectrum. Create the data set hmqc/3/1 by using **edc**.

Enter **edsp** and set NUC2 to 13C.

Set **o2p** to the value found for the optimized ^{13}C spectrum in hmqc/2/1.

Enter **eda** and set PARMODE = 2D. Click on SAVE and ok the message "Delete 'meta.ext' files?". The window now switches to a 2D display and the message "NEW 2D DATA SET" appears.

Enter **eda** and set the acquisition parameters as shown in Table 50.

Table 50: HMQC with BIRD Acquisition Parameters

F2 Parameters		
Parameter	**Value**	**Comments**
PULPROG	hmqcbiph	HMQC with BIRD for HMQC without BIRD choose hmqcph
TD	1k	
NS	8	the number of scans should be 4 *
DS	16	number of dummy scans.
PL1		high power level on F1 channel (^{1}H) as determined in Section 4.2.4
PL2		high power level on F2 channel (^{13}C) as determined in Section 6.1.4
PL12		low power level on F2 channel (^{13}C) for CPD as determined in Section 6.3.7
P1		^{1}H 90° pulse as determined in Section 4.2.4
P2		^{1}H 180° pulse, calculated from P1
P3		^{13}C 90° pulse as determined in Section 6.1.4
P4		^{13}C 180° pulse, calculated from P3
PCPD2		^{13}C 90° pulse for cpd sequence as determined in Section 6.3.7
D1	1.5	relaxation delay; should be 1–5 * T_1(^{1}H)
CNST2	145	heteronuclear scalar J(^{13}C,^{1}H) coupling 145 Hz is a good intermediate value
D2	3.45 msec	1/[2J(^{13}C,^{1}H)], calculated automatically from cnst2 above
CPDPRG2	garp	cpd sequence for the ^{13}C decoupling
D7	100 msec	delay for inversion recovery (optimize)

F1 Parameters		
Parameter	**Value**	**Comments**
TD	256	number of experiments.
FnMODE	States-TPPI	
ND0	2	there are two d0 periods per cycle
IN0		t_1 increment.
SW	190	sw of the ^{13}C spectrum ,typically 190 ppm
NUC1		select ^{13}C frequency for F1

14.2.1 Optimize d7 (only for HMQC with BIRD)

Set the acquisition parameters as shown above and choose a starting value of 400 msec for **d7**. Enter **acqu** to enter the acquisition window. Enter **gs** to start the go setup routine. Click the left mouse button to fix the acquisition-gs window somewhere on the screen, and then click on the box in the upper right hand corner of the window to iconize it. While monitoring the intensity of the time domain data, adjust the value of **d7** (simply enter **d7** and then a new value at the prompt). The optimum value of **d7** corresponds to the minimum signal intensity. Once the optimum value of **d7** is found and stored, enter **rga** to optimize the receiver gain for this minimum signal.

14.2.2 Acquire the 2D data set

Enter **zg** to start the HMQC experiment. With the acquisition parameters shown above, the approximate experiment time is 1.2 hours.

14.3 Processing

Enter **edp** and set the processing parameters as shown in Table 51.

Table 51: HMQC with BIRD Processing Parameters

F2 Parameters		
Parameter	**Value**	**Comments**
SI	1k	
SF		spectrum reference frequency (^1H)
WDW	QSINE	sine-squared window function
SSB	2	pure cosine-squared wave
PH_mod	pk	apply 0- and 1st-order phase correction determined by phase correcting the first row
PKNL	TRUE	necessary when using the digital filter.
BC_mod	no	

F1 Parameters		
Parameter	**Value**	**Comments**
SI	512	
SF		spectrum reference frequency (^{13}C)
WDW	QSINE	sine-squared window function
SSB	2	pure cosine-squared wave
PH_mod	pk	apply 0- and 1st-order phase correction determined by automation program calcphinv
BC_mod	no	
MC2	States-TPPI	

Enter **`xfb`** to multiply the time domain data by the window functions and to perform the 2D Fourier transformation.

The threshold level can be adjusted by placing the cursor on the button, holding down the middle mouse button, and moving the mouse back and forth. Both positive and negative peaks can be displayed by clicking on the +/- button. The optimum may be saved by typing **`defplot`** and answering the questions which appear.

14.4 Phase Correction

Enter **`rser 1`** to transfer the first row to the 1D data set ~TEMP/1/1. Enter **`sinm`** to apply the sine-bell windowing function, and enter **`ft`** to Fourier transform the data. Manually phase correct the spectrum. Click return and select **Save as 2D & return** to save the corrections **`phc0`** and **`phc1`** to the corresponding F2 parameters in the 2D data file hmqc/3/1. Click 2D with the left mouse button to return to the 2D data set hmqc/3/1.

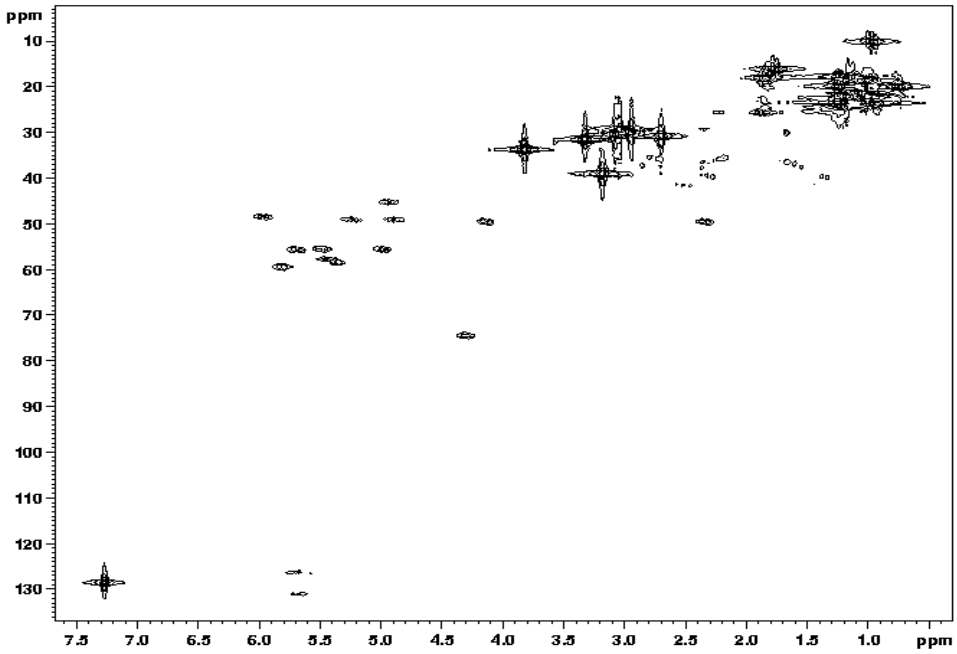
It is convenient to use an automation program to determine the F1 phase correction. From the data set hmqc/3/1, simply enter **`xau calcphinv`**. Note that this automation program is designed specifically for HMQC-type experiments.

Now enter **`xfb`** to Fourier transform the HMQC spectrum again using the appropriate phase correction to F1 and F2. The spectrum should be phased correctly and all peaks should be positive. Further adjustments can be made in the 2D phase subroutine, as described in previous chapters.

14.5 Plotting

Follow the instructions given for the previous experiments, i.e., XHCORR. An HMQC spectrum of 50 mM Cyclosporin in C_6D_6 is shown in Figure 39.

Figure 39: HMQC Spectrum of 50 mM Cyclosporin in C_6D_6



15 HMBC

15.1 Introduction

HMBC (**H**eteronuclear **M**ultiple **B**ond **C**orrelation) spectroscopy is a modified version of HMQC suitable for determining long-range ^{1}H-^{13}C connectivities. Since it is a long-range chemical shift correlation experiment, HMBC provides basically the same information as COLOC but it has a higher sensitivity, since it is an inverse experiment.

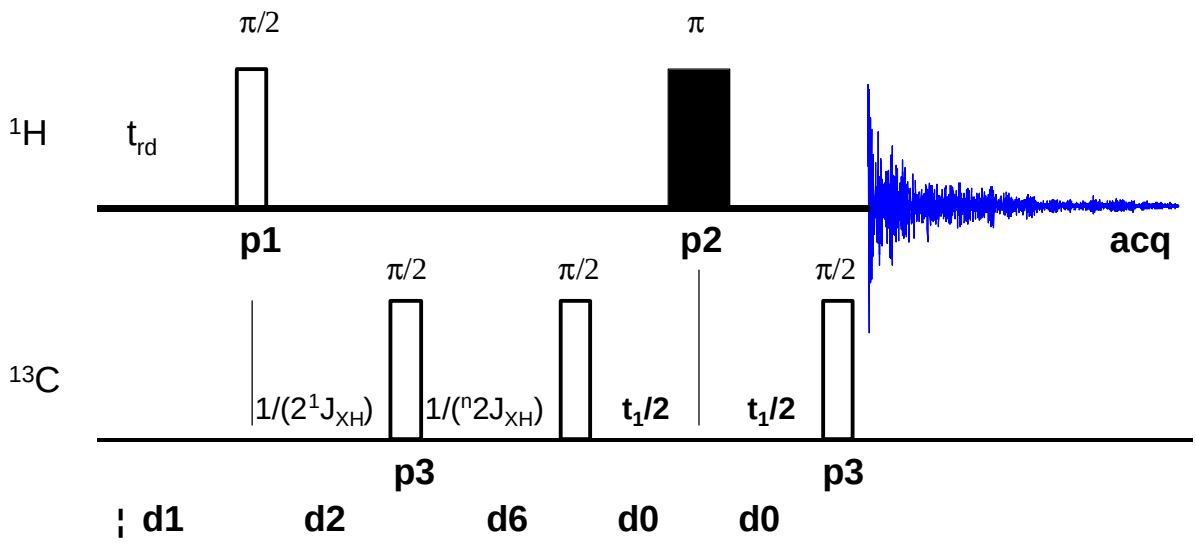
Reference: A. Bax and M. F. Summers, *J. Am. Chem. Soc.*, **108**, 2093 (1986).

The sample used to demonstrate HMBC in this chapter is 50 mM cyclosporin in C_6D_6 as was used to demonstrate HMQC.

The HMBC pulse sequence is shown in Figure 40. The first ^{13}C 90° pulse, which is applied $1/(2\ ^{1}J_{XH})$ after the first ^{1}H 90° pulse, serves as a low-pass J-filter to suppress one-bond correlations in the 2D spectrum by creating ^{1}H-^{13}C heteronuclear multiple quantum coherence. This unwanted coherence is removed by phase cycling the first ^{13}C 90° pulse with respect to the receiver. After the delay Δ_2 of about 60msec, the second ^{13}C 90° pulse creates the desired heteronuclear multiple-quantum coherence for long-range ^{1}H-^{13}C J-couplings. Phase cycling of the second ^{13}C 90°pulse removes signals from ^{1}H without long-range coupling to ^{13}C. The final ^{13}C 90°pulse after the t_1 evolution period is followed immediately by the detection period t_2. The signal detected during t_2 is phase modulated by the homonuclear ^{1}H J-couplings. The 2D spectrum is generated by a Fourier transform with respect to t_1 and t_2. If more than one long-range ^{1}H-^{13}C connectivity is detected for one particular proton, the relative intensities of the corresponding resonances are directly related to the magnitude of the coupling constant.

Because of phase modulation the spectrum has peaks with a combined absorptive and dispersive lineshape. It is not possible to phase correct the spectrum so that the peaks are purely absorptive, and so the spectrum must be presented in magnitude mode.

Figure 40: HMBC Pulse Sequence



15.2 Acquisition and Processing

Follow the instructions given for the HMQC experiment (Section 14), since the HMBC is very similar to the HMQC experiment.

Create the data set hmbc/1/1 and set the parameters as described in Section 14.2 except the pulse program, number of scans and the delay d6, which are to set as described in Table 52.

Table 52: HMBC Acquisition Parameters

F2 Parameters		
Parameter	**Value**	**Comments**
PULPROG	hmbclpndqf	
TD	4k	
NS	64	the number of scans should be 16 * n
DS	32	
CNST2	145	heteronuclear scalar J(^{13}C,^{1}H) coupling 145 Hz is a good intermediate value
D6	50m	delay for evolution of long range couplings (1/ (^{n} J_{XH}))
F2 Parameters		
Parameter	**Value**	**Comments**
TD	256	
FnMODE	QF	

Enter **zg** to start the HMBC experiment. With the acquisition parameters shown above, the approximate experiment time is 13.5 hours.

Enter **edp** and set the processing parameters as shown in

Table 53.

Table 53: HMBC Processing Parameters

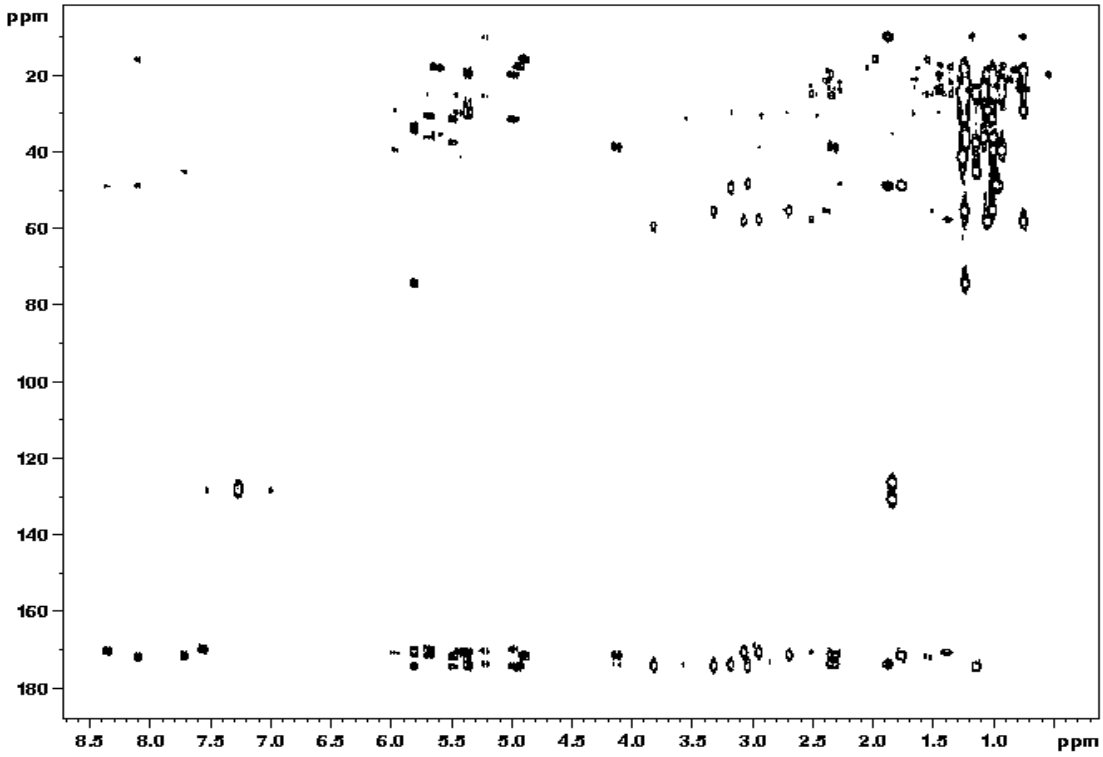
F2 Parameters		
Parameter	**Value**	**Comments**
SI	2k	
SF		spectrum reference frequency (^{1}H)
WDW	QSINE	sine-squared window function
SSB	0	pure sine-squared wave
PH_mod	no	
PKNL	TRUE	necessary when using the digital filter.
BC_mod	quad	
F1 Parameters		
Parameter	**Value**	**Comments**
SI	512	
SF		spectrum reference frequency (^{13}C)
WDW	SINE	sine window function
SSB	0	pure cosine wave
PH_mod	mc	
MC2	QF	

Enter **xfb** to multiply the time domain data by the window functions and to perform the 2D Fourier transformation.

Adjust the display as described for the HMQC spectrum.

An HMBC spectrum of 50 mM Cyclosporin in C_6D_6 is shown in Figure 41.

Figure 41: HMBC spectrum of 50 mM Cyclosporin in C_6D_6


ppm
20
40
60
80
100
120
140
160
180
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm

16 Proton-Carbon Inverse Shift Correlation- Experiments using Pulsed Field Gradients

16.1 Introduction

The three most common inverse chemical shift correlation experiments are **HSQC**, **HMQC** and **HMBC**, which are used to determine which ^{1}H of a molecule are bonded to which ^{13}C nuclei (or other X nuclei). The advantage of inverse experiments over X detection experiments is that with inverse experiments the nucleus with the highest γ (usually ^{1}H) is detected yielding the highest sensitivity. The challenge of an inverse chemical shift correlation experiment, however, is that the large signals from ^{1}H not coupled directly to a ^{13}C nucleus must be suppressed in a difference experiment, which poses a dynamic range problem. Common techniques for the suppression of ^{1}H bound to ^{12}C are the BIRD-sequence in **HMQC** experiments and a trim pulse of 1-2ms during the first INEPT in **HSQC** experiments. However, the suppression is still imperfect and usually additional phase cycling is required. The introduction of pulsed field gradients in high-resolution NMR greatly improved the problem of suppressing signals from ^{1}H bonded to ^{12}C: The suppression is almost perfect without additional phase cycling.

In general, NMR experiments using PFGs are called **GRASP** experiments: **GRASP-HSQC**, **GRASP-HMQC**, **GRASP-HMBC** etc.

Details on the GRASP technique are covered by the *GRASP course* and will not be discussed here.

16.2 GRASP-HMQC

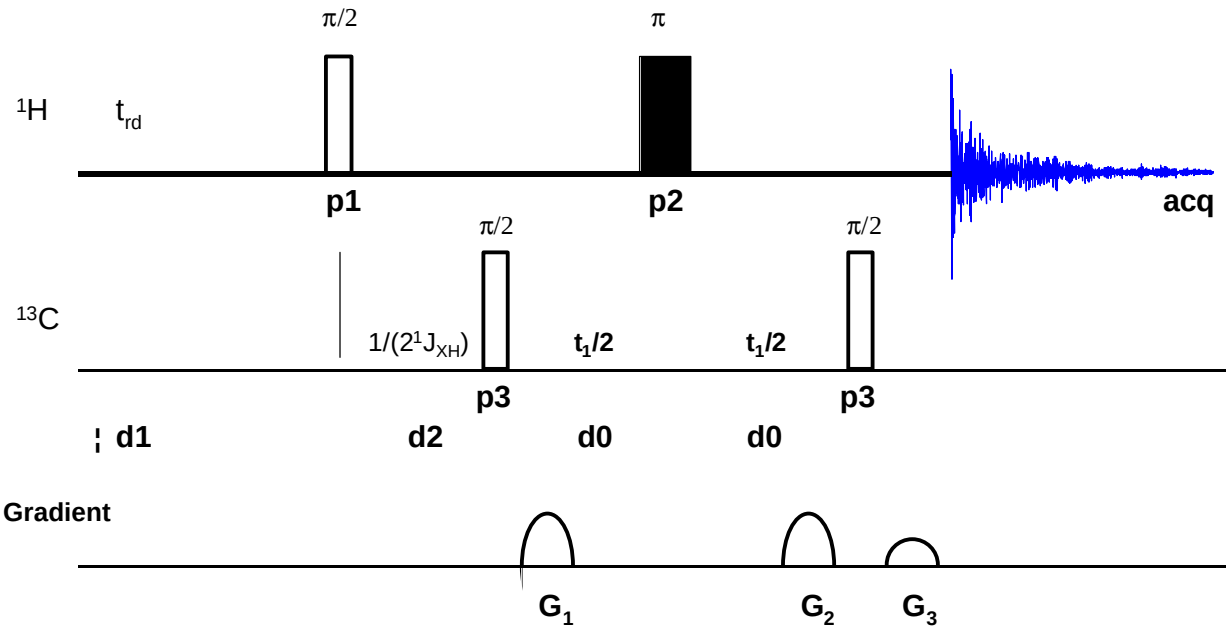
See Section 14 for information about the HMQC experiment.

The GRASP-HMQC pulse sequence is shown in Figure 42. The PFGs in the experiment are used for coherence selection and the quadrature detection in the ω_1 dimension. The two gradients applied during t_1 (gradients G_1 and G_2) dephase all ^{1}H magnetization, while the third gradient G_3 rephases the magnetization of interest.

The gradient ratio $G_1+G_2:G_3$ for a GRASP-HMQC is 2:1 for ^{13}C and 5:1 for ^{15}N.

This version of the GRASP-HMQC experiment is not phase sensitive.

Figure 42: GRASP-HMQC Pulse Sequence



Note that all correlation peaks of any HMQC experiment are splitted along the ω_1 dimension due to evolution of the homonuclear ^{1}H-^{1}H coupling, which cannot be refocussed by the 180° ^{1}H pulse applied during t_1. Therefore, the resolution of the HMQC experiment along the ω_1 dimension is limited. For a better resolution in the ω_1 dimension the HSQC experiments must be done.

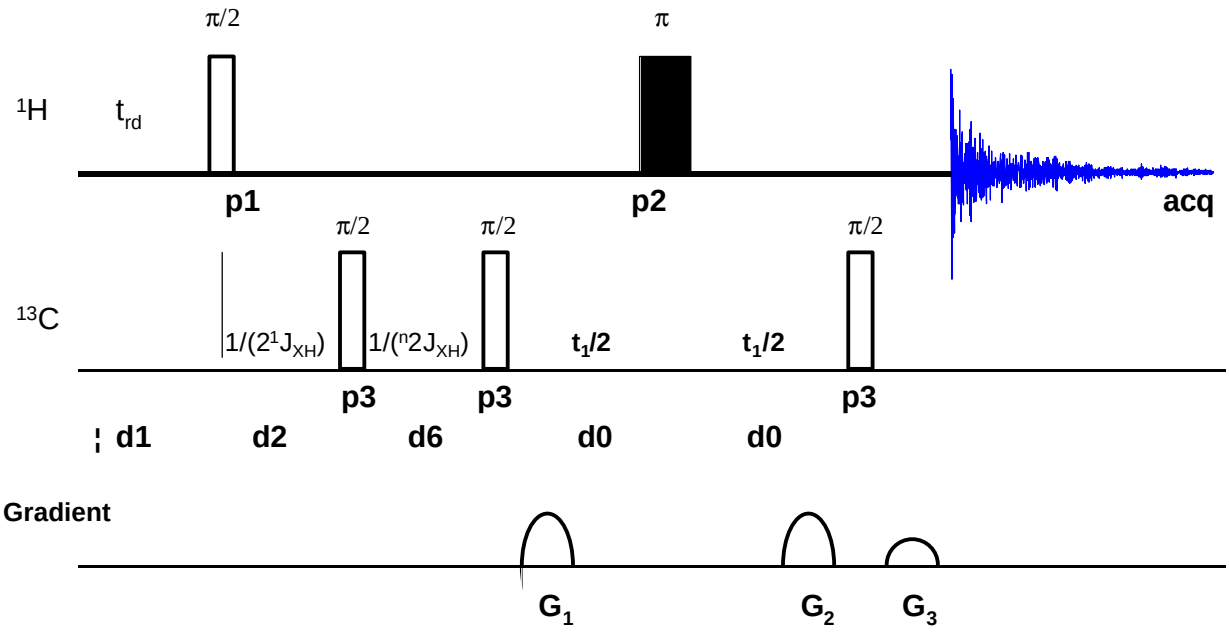
References: A. Bax, R. H. Griffey, and B. L. Hawkins, *J. Magn. Reson.*, **55**, 301 (1983); A. Bax and S. Subramanian, *J. Magn. Reson.*, **67**, 565 (1986).

16.3 GRASP-HMBC

See Section 15 for information about the HMBC experiment.

The GRASP-HMBC pulse sequence is shown in Figure 43. Identical gradient ratios are used for the GRASP-HMBC as for the GRASP-HMQC (see Section 16.2).

Figure 43: GRASP-HMBC pulse sequence



16.4 GRASP-HSQC

HSQC (***H**eteronuclear **S**ingle **Q**uantum **C**orrelation*) yields the same spectrum as HMQC but is based on single-quantum NMR. In the HSQC sequence, the pulse scheme prior the t_1 evolution period represents a so called INEPT sequence and creates transverse single-quantum magnetization on the X-nucleus, e.g., ^{13}C or ^{15}N, which evolves X chemical shift during t_1. The G_1 gradient dephases all the transverse magnetization. This gradient is located in a spin echo in order to refocus chemical shift evolution during the gradient. Then, a second INEPT segment transfers the magnetization to ^{1}H, where it is detected after it has been rephased by a second gradient G_2.

The field gradients in this version of a GRASP-HSQC experiment are solely used for the coherence selection. The gradient ratio G_1:G_2 for a GRASP-HSQC is 4:1 for ^{13}C and 10:1 for ^{15}N.

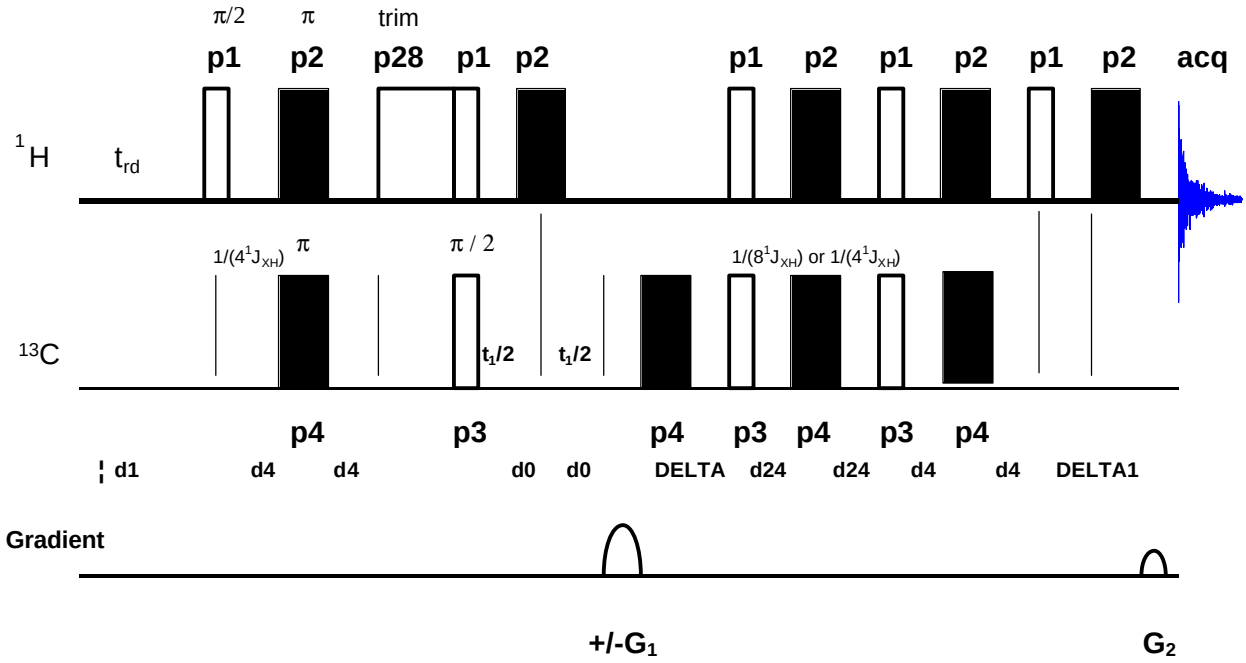
This version of the GRASP-HMQC experiment is phase sensitive.

Compared to the HMQC experiment no line broadening along the ω_1 dimension appears as only ^{13}C single-quantum magnetization is present during the t_1 evolution period.

References: A. Bax, R. H. Griffey, and B. L. Hawkins, *J. Magn. Reson.*, **55**, 301 (1983); A. Bax and S. Subramanian, *J. Magn. Reson.*, **67**, 565 (1986).

Pulse sequence for GRASP-HSQC is shown in Figure 44.

Figure 44: GRASP-HSQC Pulse Sequence



16.5 Acquisition and Processing

The GRASP experiments are best started from a corresponding data set without gradients, e.g. for the GRASP-HMQC and GRASP-HSQC from the HMQC data set (**`re hmqc 3 1`**) (Section 14) or HMBC (Section 15).

Table 54: $^{1}H,^{13}C$ HMQC, HMBC and HSQC Acquisition Parameters

F2 Parameters		
Parameter	**Value**	**Comments**
PULPROG	hmqcgpqf hmbcgplpndqf hsqcetgpsi	For GRASP-HMQC For GRASP-HMBC For GRASP-HSQC.
TD	2k	
NS	2 8	For GRASP-HMQC GRASP-HSQC For GRASP-HMBC
DS	16	number of dummy scans, has to be ns*2*n
P28	0.5u	Trim pulse in HSQC ***Do not set it to a longer value***
D4	1.72msec	For HSQC only: 1/[2 $^{1}J(^{13}C,^{1}H)$],

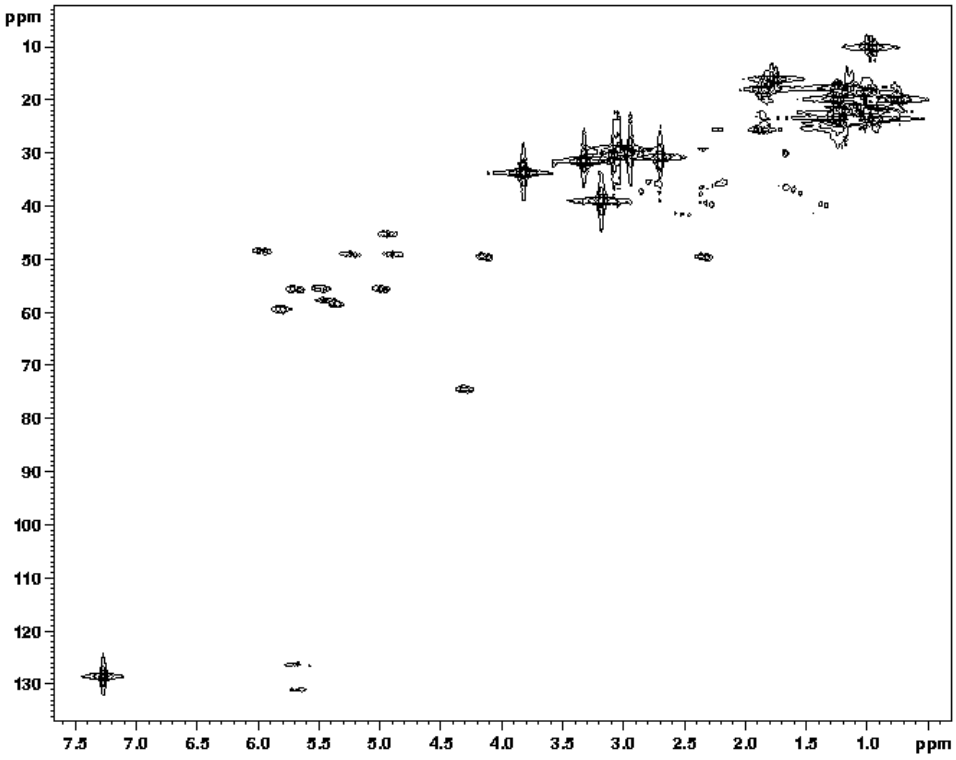
		calculated automatically from cnst2 above
D24	0.86msec	For HSQC only: delay for refocussing J_{CH}. For all multiplicities set the value to: $1/[8\ ^1J(^{13}C,^1H)]$ Only for CH-groups set the value to: $1/[4\ ^1J(^{13}C,^1H)]$ = D4
F1 Parameters		
Parameter	**Value**	**Comments**
TD	256	number of experiments.
FnMODE	QF echo-antiecho QF	*For GRASP-HMQC* *For GRASP-HSQC* *For GRASP-HMBC*
ND0	2	
IN0		t_1 increment.
SW	190ppm 260ppm	For HSQC and HMQC For HMBC
NUC1		select ^{13}C frequency for F1

Gradient Parameters for the gp-syntax		
Parameter	**Value**	**Comments**
P16	1.5ms	Length of gradient pulses
D16	150u	Gradient recovery delay
gpz1	50 80	For HMQC and HMBC For HSQC
gpz2	30 20.1	For HMQC and HMBC For HSQC
gpz3	40.1	For HMQC and HMBC
gpnam1	SINE.100	gradient shape
gpnam2	SINE.100	gradient shape
gpnam3	SINE.100	gradient shape

With the acquisition parameters shown above, the approximate experiment times are 0.3h for HMQC and HSQC, and 1.2 hours for HMBC.

Process the data according to Sections 14.3 and 15.2, respectively, except that for the HSQC the parameter MC2 must be set to echo-antiecho. Figures 43-45 show GRASP-HMQC, GRASP-HMBC and GRASP-HSQC spectra of Cyclosporin in benzene.

Figure 45: $^{1}H,^{13}C$ GRASP-HMQC experiment of 50mM Cyclosporin in C_6D_6



7

Figure 46: $^{1}H,^{13}C$ GRASP-HMBC experiment of 50mM Cyclosporin in C_6D_6

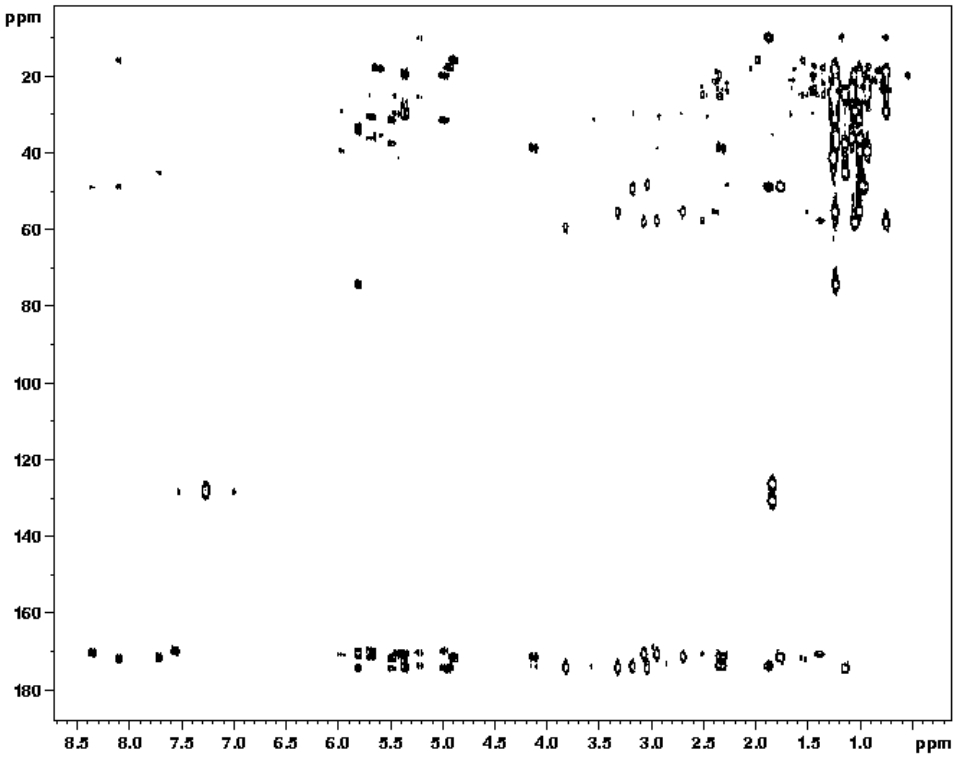
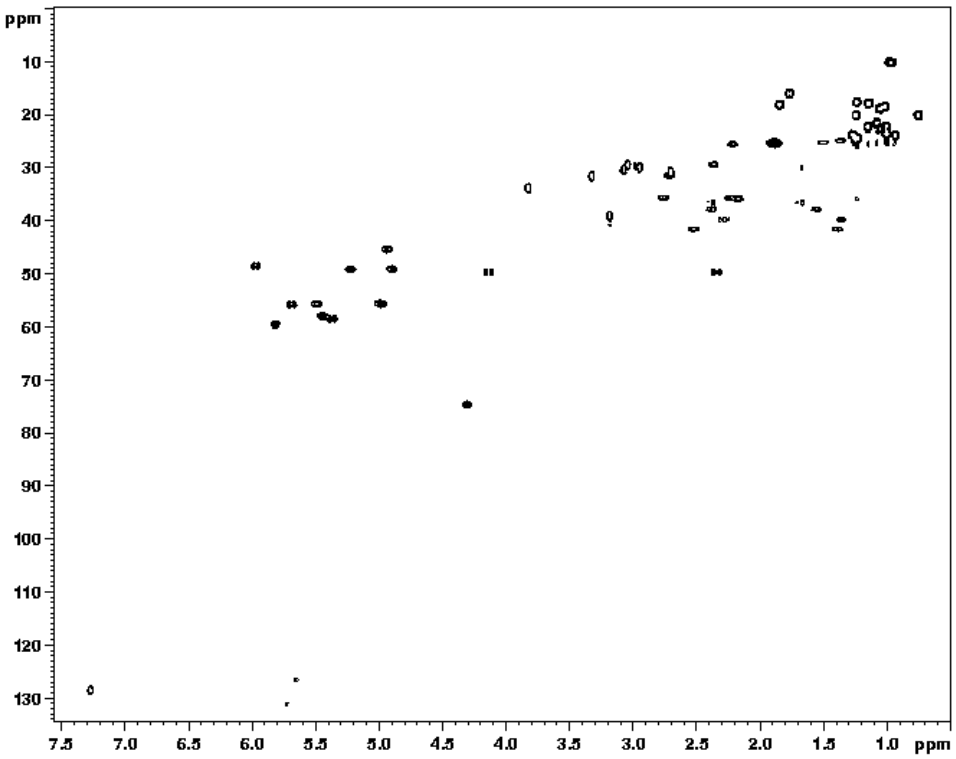


Figure 47: $^{1}H,^{13}C$ GRASP-HSQC experiment of 50mM Cyclosporin in C_6D_6



17 1D NOE Difference

17.1 Introduction

The **N**uclear **O**verhauser **E**ffect (NOE) is a net change of the signal intensity from one spin due to the relaxation of a saturated spin that is dipole-dipole coupled to the first spin. NOE's develop due to through-space rather than through-bond interactions, and thus contain information on the distances between spins.

The rate or efficiency of the NOE buildup depends on the rate or efficiency of the dipole-dipole relaxation, which itself depends on the strength and frequency of the fluctuating fields. These fluctuating fields depend on the distance between the nuclei involved, the tumbling rate of the molecule, and the characteristics of the nuclei themselves. The presence of paramagnetic molecules (e.g., metal ions, rust, or dissolved oxygen) distorts any NOE experiment, since they dominate T_1 relaxation processes.

In an NOE difference experiment, a ^{1}H resonance is selectively preirradiated until saturation is achieved. During the preirradiation period, NOE buildup occurs at other ^{1}H nuclei close in space. A $\pi/2$ pulse then creates observable magnetization, which is detected during the acquisition period. The experiment is repeated using different preirradiation frequencies, including one which is off-resonance. The latter is used to obtain a reference or control spectrum. The final spectra are displayed as the difference between a spectrum recorded with on-resonance preirradiation and the reference spectrum.

Very small phase or frequency shifts between two spectra will give rise to imperfect signal subtraction. To minimize subtraction artifacts an efficient signal averaging and maximal acceptable line broadening should be used. Other artifacts from temperature instability or magnetic field drift may be minimized by acquiring the preirradiated and the reference data in an interleaved manner.

Reference: D. Neuhaus and M. P. Williamson, “The Nuclear Overhauser Effect in Structural and Conformational Analysis,” New York: VCH Publishers, Inc., 1989.

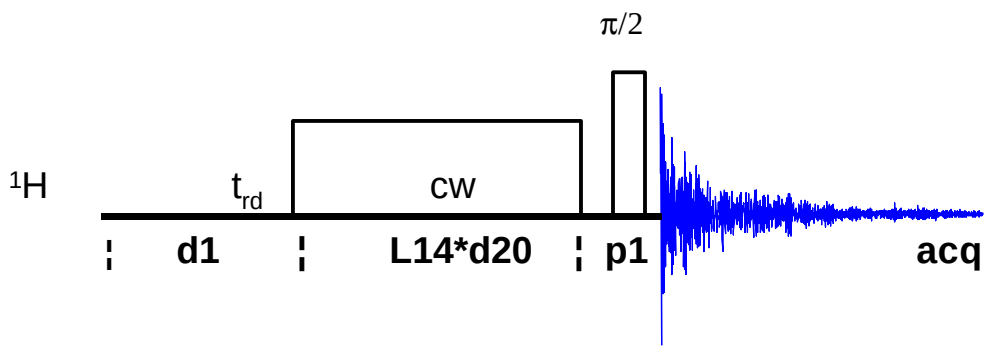
The sample used to demonstrate a 1D NOE difference experiment in this chapter is 100 mM Pamoic Acid in DMSO-d6.

The NOE difference pulse sequence is shown in Figure 48. The pulse sequence begins with the recycle delay time **`d1`**. This is followed by the cw irradiation period of total time **`l4*d20`**, where **`d20`** is the irradiation time for one particular frequency. The pulse program makes use of a frequency list (**`fq2list`**) to determine the frequencies for cw irradiation. The final 90°

pulse **p1** creates the observable magnetization and is followed by the acquisition period.

Several spectra are acquired during an NOE difference experiment, and for each spectrum a different **fq2list** is used. For the reference spectrum, the cw pulse is applied off-resonance and the au program **noemult** is used to acquire the spectra in an interleaved manner.

Figure 48: 1D NOE Difference Pulse Sequence



17.2 Acquisition

Insert the sample in the magnet. Lock the spectrometer. Readjust the Z and Z^2 shims until the lock level is optimized. Tune and match the probehead for ^{1}H observation.

For best results it is recommended to optimize the lock parameters as described. NOE difference experiments should be run without sample spinning.

The parameters and spectra shown below are from a 300 MHz spectrometer. The signal enhancements for Pamoic Acid in DMSO-d6 at other field strengths will be different than those shown here. If an NOE response is difficult to obtain, it may be necessary to change the sample temperature or solvent. In particular for this sample at 400 MHz, it is recommended to use a temperature of 40°C.

17.2.1 Create a new file directory

Enter **re proton 1 1** to call up the data set proton/1/1. Enter **edc** and create the data set noediff/1/1.

Enter **edasp** and set both NUC1 and NUC2 to 1H. The f2 channel is used for cw irradiation during the NOE experiment.

17.2.2 Proton reference spectrum

Enter **rga** to perform an automatic receiver gain adjustment. Acquire and process a standard ^{1}H spectrum, as described in Chapter 3. Calibrate the

spectrum and optimize **sw** and **o1p**. Keep in mind that the control spectrum should be irradiated well off-resonance (in this case –2 ppm). Acquire and process an optimized spectrum.

17.2.3 Select the resonances for irradiation

The frequencies used by the f2 channel during the preirradiation periods of the NOE experiment must be written to the corresponding **fq2list**. A separate list must be created for each resonance to be irradiated, where a given list may contain several frequencies if irradiation of a resonance at several points is needed. One of the lists must contain a frequency well off-resonance for generating the reference spectrum.

Here, we will create lists with frequencies for the resonances at 4.8 ppm and 8.5 ppm, and one with the off-resonance frequency –2 ppm. The frequency lists are defined using the [frqlist] routine, which is found in the submenu . [utilities] Note that if it is necessary to expand the spectrum in order to accurately define the irradiation points this must be done before entering the routine. Display the peak at 4.8 ppm and enter the [frqlist] routine and answer the questions as shown below:

Please enter type of list (f1, f2, f3):	f1
Please enter name of f1 list:	noedif.1
Write name of f1 list to acqu parameters?	n

The following option appears if a f1 frequency list with the same name already exists:

Frequency list exists, append (a), overwrite (o) or quit (q):

Answer **a** if you wish to add new frequencies to the existing list, **o** if you wish to overwrite the existing list, or **q** if you wish to quit the routine and keep the old list.

Once the questions have been answered, move the mouse until the cursor is tied to the spectrum. Click on the peak at 4.8 ppm with the middle mouse button. Finish the list by clicking the left mouse button. Remember that for a given list, multiple irradiation points should all be part of the same multiplet. A separate frequency list should be generated for each multiplet irradiated.

Note: The “type of list (f1, f2, f3)” refers to the *directory* where the frequency list is stored and not to the spectrometer channel for which the list will be used). In any acquisition parameter set it is possible to define eight separate frequency lists (**fq1list**, **fq2list**, etc.). The pulse program **noemul** uses only one frequency list: **fq2list**. Therefore, set the parameter **fq2list** to the appropriate list name “noedif.1” within the **eda** menu, The automation program **noemult** redefines **fq2list** each time **noemul** is to be run with a different frequency list.

Repeat this procedure for the peak at 8.5 ppm and for the off-resonance frequency of –2 ppm. Write the lists to the files noedif.2 and noedif.3, respectively. Note that the automation program **noemult** requires that all frequency lists have the same base name and increasing extension numbers.

Click on [return] to leave the utilities submenu and return to the main menu.

17.2.4 Set up the acquisition parameters

Enter **edc** and change EXPNO to 2 to create the data set noedif/2/1.

Enter **eda** and set the acquisition parameters as shown in Table 55. Verify that NUC2 has been set to 1H in **edasp**.

Table 55: 1D NOE Difference Acquisition Parameters

Parameter	Value	Comments
PULPROG	noemul	
TD	64k	
NS	8	the number of scans must be 8 * n
DS	2	
PL1		high power level on F1 channel (^{1}H) as determined in Section 4.2.4
PL14	70	power level for NOE buildup
P1		^{1}H 90° pulse as determined in Section 4.2.4
D1	3.5	relaxation delay
D20	50m	50 msec irradiation time
L4	T_1 / D20	loop counter to determine overall irradiation time (T_1 = L4 * D20)
FQ2LIST	noedif.1	frequency list for f2 frequency of selective irradiation

The pulse program **noemul** operates such that **o2** is set to the first frequency of the **fq2list** and the selected multiplet is irradiated with this frequency for a time **d20**. Then **o2** is set to the next frequency (if there is one) of the **fq2list** and the selected multiplet is irradiated with this frequency for a time **d20**. This process continues until the multiplet is irradiated for a total of **l4** times.

17.2.5 Optimize the irradiation power and duration

For the NOE difference experiment, the au program **noemult** will run the pulse program **noemul** with successive fq2lists. However, the optimization of irradiation power and duration can be performed for a single resonance. Thus, the au program is not necessary and the pulse program **noemul** can be started with the command **zg**.

Make sure that **fq2list** is set to **noedif.1** (or **noedif.2**, i.e., make sure that the cw irradiation will be applied on resonance for one of the multiplets). Start the acquisition with **zg**, process the spectrum with **ef** (see the processing parameters listed below in Table 56), and manually phase correct the spectrum.

Compare this spectrum with the reference spectrum noedif/1/1 by using the dual mode. From the current data set (noedif/2/1), enter **edc2** to define the

second data set to be shown in the dual display. Set EXPNO2 to 1 and PROCNO2 to 1 and click SAVE .

Click dual to enter the dual display mode. Both the spectra from noedif/1/1 and noedif/2/1 appear in the window. When the comparison of the spectra is finished, click to return to the main 1D processing window.

Ideally, the target resonance is completely saturated by the selective irradiation, while all other signals are unaffected by the irradiating field. In practice, the chemical shift difference between signals is often too small, so that neighboring resonances may be saturated as well.

It is almost always preferable to use low-power (and hence selective) irradiation rather than unwanted saturation of nearby resonances. However, partial saturation of a multiplet leads to selective population transfer which may obscure NOE effects. To avoid this, the individual components of a target multiplet are irradiated in an interleaved manner during the preirradiation period before each scan.

If needed, adjust **pl14** to change the power level of the cw irradiation.

Note that the total cw irradiation time (**l4*d20**) should be approximately equal to T_1 of the irradiated peak, but with the au program **noemult**, it is necessary to use the same total irradiation time for each peak irradiated. Thus, the irradiation time should be chosen based on the longest T_1. Here a total irradiation time of 2.5 sec is used, which is longer than the T_1 of the peak at 8.5 ppm.

17.2.6 Perform the multiple NOE experiment

To start the NOE difference experiment, type **xau noemult** and answer the questions as follows:

base name of all frequency lists:	noedif
# of frequency lists:	3
# of cycles through each list:	L4
# of average cycles:	8

The number of frequency lists is the number of fq2lists written above and it will be the number of spectra acquired. The number of cycles through each list is the loop counter **l4**. The number of average cycles controls the total number of scans for each frequency list. For each frequency list (and hence, for each spectrum) the total number of scans is **ns**, where **ns** should be as small as possible (e.g., 8) and then the signal-to-noise ratio is improved by increasing the number of average cycles (to, e.g., 10).

The au program automatically starts the pulse program **noemul** using the acquisition parameters defined in the current data set (noedif/2/1) and the **o2** frequencies defined in the first fq2list (noedif.1). Next, an experiment is performed using the **o2** frequencies defined in the second fq2list (noedif.2) and the results are written to the next data set (noedif/3/1) and so on. Note, that new data sets created by **noemult** have the same name as the original data set, but increasing EXPNO. Here, the spectrum irradiated at 4.5 ppm is noedif/2/1, that at 8.5 ppm is noedif/3/1, and that at –2 ppm is noedif/4/1.

The entire cycle is repeated until the experiment is finished. The number of times this cycle is performed is determined by the value entered for the number of average cycles.

17.3 Processing

Enter **edp** and set the processing parameters as shown in Table 56.

Table 56: 1D NOE Difference Processing Parameters

Parameter	Value	Comments
SI	8k	
WDW	EM	
LB	0.3	
PKNL	TRUE	

17.3.1 Perform the Phase Correction

To process the spectra acquired by **noemult** it is necessary to define the phase correction parameters first.

Read in the first file (**re 2 1**). Apply the window function and Fourier transformation with the command **ef**. Manually phase correct the spectrum and store the correction (see Section 3.8).

All spectra are processed identically by entering **xau multiefp** and answer the questions as follows:

Enter first expno to process: 2
Enter number of expnos: 3

Here, the “first expno to process” indicates the spectrum that is already Fourier transformed and phase corrected. The au program **multiefp** reads all processing parameters (including 0th- and 1st-order phase corrections) from this data set and uses them to process the following three spectra with the same data set name

At this point the data consists of a series of spectra with various saturated resonances and one reference spectrum. The procedure for creating the difference spectra is outlined below.

17.3.2 Create NOE Difference Spectra

The NOE difference spectra are created by subtracting the reference spectrum from each of the preirradiated spectra. Within the data set of each preirradiated spectrum, the second and third data sets are defined by using the **edc2** command, where the second data set refers to the reference spectrum and the third data set refers to the data set where the difference spectrum is stored.

Display the first preirradiated spectrum (**re 2 1**). Enter **edc2** and set EXPNO2 and PROCNO2 to 4 and 1, respectively (reference spectrum). Set EXPNO3 and PROCNO3 2 and 2, respectively, so that the difference spectrum will be stored with the same experiment number and processing number 2. Click on SAVE to the main menu.

Enter the dual submenu by clicking on dual . Both the current spectrum and the reference spectrum appear on the screen. Click on diff and select **Save & return** to subtract the reference spectrum from the current preirradiated spectrum. The difference spectrum appears automatically in the window.

Click return to save the results and return to the main menu. The message “result will be put into: DU = u, USER = <username>, NAME = noedif, EXPNO = 2, PROCNO = 2, click OK if ok” appears. Click **OK** and notice that the current data set is now noedif/2/2.

Move to the next preirradiated spectrum (**re 3 1**) and repeat the above procedure (set EXPNO2 = 4, PROCNO2 = 1, EXPNO3 = 3 and PROCNO2 = 2 to store the difference spectrum in noedif/3/2).

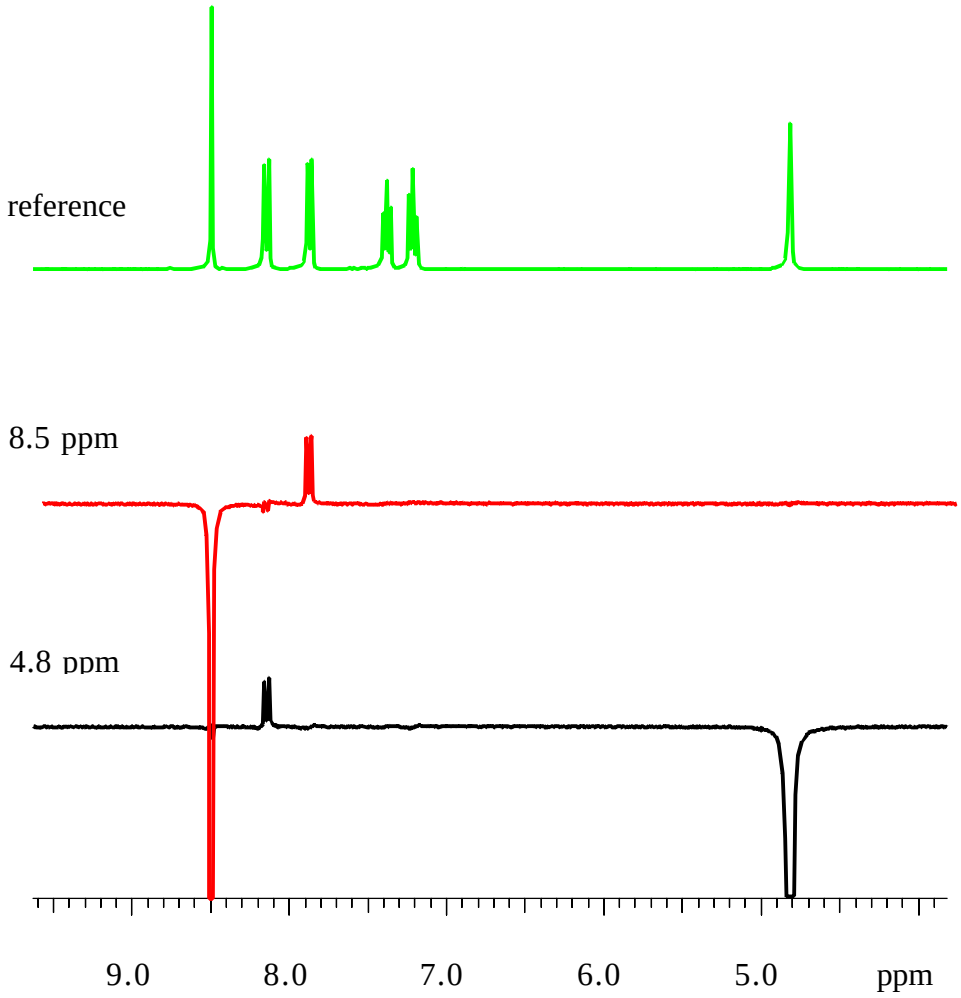
Two NOE difference spectra (with cw irradiation on-resonance at 8.5 ppm and 4.8 ppm) and the reference spectrum (with cw irradiation off-resonance at –2 ppm) of 100 mM Pamoic Acid in DMSO-d6 are shown in Figure 49.

In both the difference spectra with cw irradiation at 8.5 ppm and 4.8 ppm, the large negative peak is the irradiated resonance and the small positive doublet is the NOE. Note that these spectra were recorded on a DPX300 at 298 K. Experiments recorded at 500 MHz and 298 K will have negative NOE peaks, while those recorded at 400 MHz and 298 K may show no NOE peaks at all.

17.3.3 Quantitate the NOE

To quantitate an observed NOE, the integrated intensity of the NOE peak in the difference spectrum is compared with the integrated intensity of the peak that was irradiated. However, this latter intensity should be measured in the reference spectrum. Thus, it is necessary to integrate peaks in both the control and the difference spectrum, and to use the same normalization constant for the integrals in both spectra (see Section 3.10).

Figure 49: NOE Difference Spectra of 100 mg Pamoic Acid in DMSO



18 Homonuclear decoupling

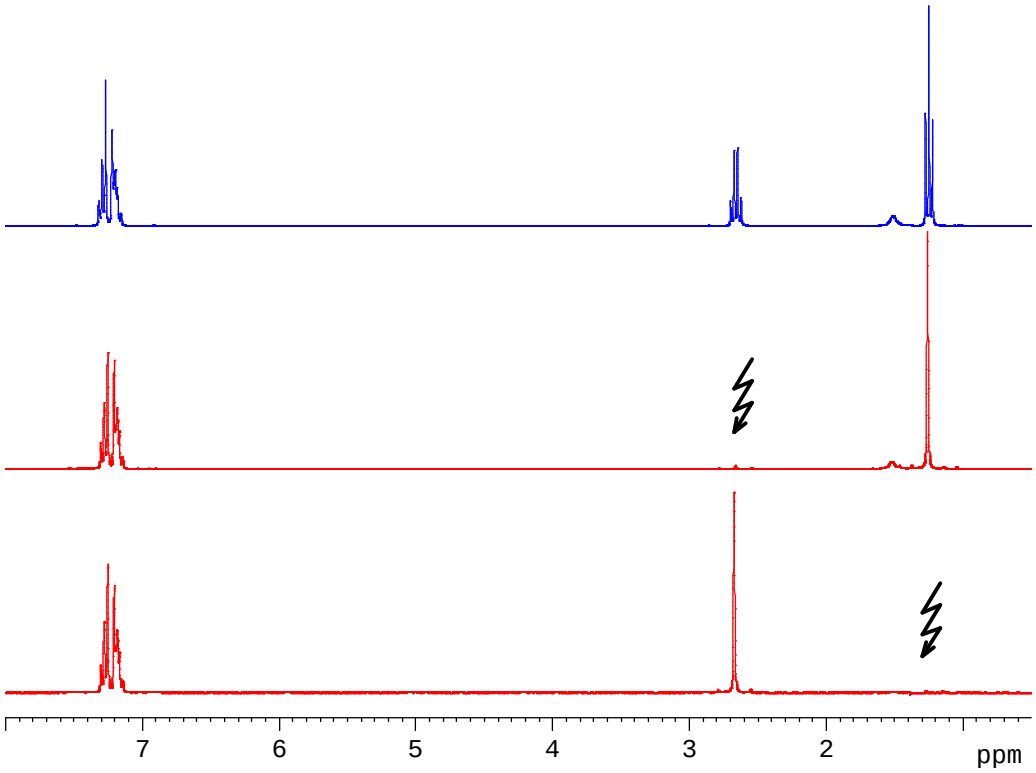
18.1 Introduction

The technique of homonuclear decoupling (double resonance, spin decoupling, homo decoupling) was established long before routine pulsed FT spectroscopy became popular. In those days the usual NMR experiment consisted of applying a variable field B_1 to the sample for the observation of the absorption spectrum (so-called continuous wave method). Double resonance or homonuclear decoupling is the term used for experiments in which a field B_2 in addition to B_1 is applied to the sample.

On modern FT spectrometers equipped with a single coil probe-head, this decoupling requires a special mode in order to apply the decoupling energy (B_2) during the acquisition. The so-called hd-mode is applying the energy in pulsed mode within the duty cycle of the dwell time and the preamplifier is switched off during the decoupler pulses.

The underlying principles of homonuclear decoupling can be illustrated by considering e.g. the molecule ethyl benzene and coupling pattern of the ethyl group. Irradiation of the methylene group will result in the collapse of the methyl group to a singulet and vice versa. (Figure 50).

Figure 51: Spectra of ethyl benzene without and with homo decoupling



Suitable samples to setup homo decoupling are the proton sensitivity sample 0.1% ethyl benzene in $CDCl_3$ or 100mM pamoic acid in DMSO-d_6.

18.2 Acquisition

Insert the sample in the magnet. Lock the spectrometer. Readjust the Z and Z^2 shims until the lock level is optimized. Tune and match the probehead for ^{1}H observation. This experiment can be run with sample rotation.

18.2.1 Create a new file directory

Enter **`re proton 1 1`** to call up the data set proton/1/1. Enter **`edc`** and create the data set homodec/1/1.

Enter **`edasp`** and set both NUC1 and NUC2 to 1H. The f2 channel is used for cw irradiation during the NOE experiment.

18.2.2 Proton reference sepctrum

Enter **`rga`** to perform an automatic receiver gain adjustment. Acquire and process a standard ^{1}H spectrum, as described in Chapter 3. Calibrate the spectrum and optimize **`sw`** and **`o1p`**. Acquire the standard spectrum using the parameters outlined in the table :

Table 57: Acquisition Parameters for ^{1}H Reference Spectrum

Parameter	Value	Comments
PULPROG	zg30	One pulse acquisition with 30° flip angle
NS	8	number of scans
DS	2	number of dummy scans

Process the FID with **`em`**, **`ft`** and phase correct it.

18.2.3 Selection of irradiation frequency

The frequency used by the f2 channel for the irradiation of the multiplet can be defined by entering the submenu utilities and clicking on the button for selecting the **`o2`** frequency O2 . The cursor is now bound to the spectrum and changed his shape (vertical arrow). The mouse buttons in this mode do have the functions

left=return

middle=define frequency

right=unused.

Move the cursor to the position of interest and press the middle mouse button to define SFO2/O2 frequency. Leave the utilities submenu with the return button. The determined value for **`o2`** is now stored in the current data set.

18.2.4 Setting up the homo decoupling parameters

Enter **edc** and change EXPNO to 2 to create the data set homodec/2/1.

Setup the relevant parameters according to the table.

Table 58: Acquisition Parameters for homo decoupling

Parameter	Value	Comments
PULPROG	zghd.2	Pulse program for homo decoupling
CPDPRG2	hd	Decoupling sequence during relaxation
PL 24	~ 50	Needs to be optimized for good decoupling
DIGMOD		For AV instruments: digital For D..X instruments: homodecoupling-digital
HDDUTY	20%	

Optimise **pl24** until the multiplet of interest collapses completely to e.g. a single line. Be careful when increasing the power, values below 40dB should be avoided!

The phase correction values of a homo decoupled spectrum is different to the reference spectrum and must therefore be adjusted for each irradiated signal.

19 T_1 Measurement

19.1 Introduction

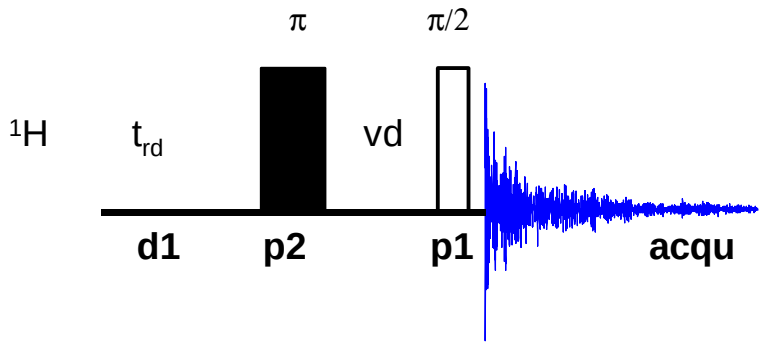
The spin-lattice (T_1) relaxation time of the various ^{1}H nuclei of a molecule may be determined by using an inversion recovery experiment. The pulse sequence begins with a recycle delay (t_{rd}) that is sufficiently long to ensure that all magnetization returns to equilibrium (i.e., pure z-magnetization). A 180° pulse is applied for the inversion of the whole magnetization. During the recovery delay the magnetization is allowed to recover to a certain amount and the final 90° pulse then converts the residual z-magnetization into observable transverse magnetization, which is detected during the acquisition period. Note that for a very short recovery delay time the pulse sequence is equivalent to a 270° pulse, and the detected signal has full, negative intensity; if the delay is very long, full T_1 relaxation occurs between the 180° and 90° pulses, and the detected signal has full positive intensity. T_1 can be determined by repeating the experiment with different recovery delay values. The resulting curve is an exponential with rate $1/T_1$. Note that for some intermediate value of the recovery delay, the peak intensity is zero and $T_1 = t_{null}/\ln(2)$.

The procedure described in this chapter is for determining ^{1}H T_1 values. A similar procedure may be used for measuring ^{13}C T_1 values. However, for measuring ^{13}C T_1's, it is important to use inverse-gated ^{1}H decoupling to improve the spectral signal-to-noise ratio without selectively enhancing peak intensities through NOE effects. It is also important to use a sufficiently long recycle delay (recall that ^{13}C T_1 can be much longer than ^{1}H T_1).

The sample used to demonstrate a T_1 experiment in this chapter is 100 mM Pamoic Acid in DMSO-d6.

The inversion recovery pulse sequence is shown in Figure 52. The 180° pulse **p2** is followed by the recovery delay **vd**. The value of **vd** is determined by the delays contained in the appropriate vdlist, and is varied over the course of the experiment. A 1D spectrum is obtained for each value of **vd**, and the results are stored in a 2D data set. The 2D data set is used by the T_1 calculation routine, which allows the user to determine T_1 for any number of peaks of the 1D spectrum.

Figure 52: Inversion Recovery Pulse Sequence



19.2 Acquisition

Insert the sample in the magnet. Lock the spectrometer. Readjust the Z and Z^2 shims until the lock level is optimized. Tune and match the probehead for ^{1}H observation.

Create the data set t1data/1/1 starting out from noedif/1/1 and record a ^{1}H reference spectrum (see Section 17.2).

From the data set t1data/1/1, enter **`xau iexpno`** to create the data set t1data/2/1. This data set will be used for the inversion recovery experiment. Although inversion recovery is not technically a 2D experiment, it does generate an array of 1D spectra which are most easily handled as one 2D file. Thus, t1data/2/1 must be changed into a 2D data set as described below.

Enter **`parmode`** and select 2D. The window now switches to a 2D display and the message “NEW 2D DATA SET” appears.

19.2.1 Write the variable delay list

The inversion recovery experiment requires a variable delay list to provide all the values of the recovery time **`vd`**. To create the variable delay list enter **`edlist`**. A menu of list types appears. Select **`vd`** from this menu. This calls up a menu of existing vdlist filenames and gives the user the option of creating a new file (‘Type new name'). Type the name **`t1delay`** to call up the editor. Enter the delays (in [s]) as listed below:

10
5
4
3
2
1
0.5
0.25
0.1
0.01

Save the file and exit the editor. It is recommended to begin and end the list with the longest **vd** value and to scramble the order of the intermediate values.

19.2.2 Set up the acquisition parameters

Enter **eda** and set the acquisition parameters as shown in Table 59.

Table 59: Inversion Recovery Acquisition Parameters

F2 Parameters		
Parameter	**Value**	**Comments**
PULPROG	t1ir	see Figure 52 for pulse sequence diagram.
TD	16k	
NS	8	the number of scans must be 8 * n
DS	4	number of dummy scans
PL1		high power level on F1 channel (^{1}H) as determined in Section 4.2.4
P1		^{1}H 90° pulse as determined in Section 4.2.4
P2		^{1}H 180° pulse (2*P1)
D1	10	10s relaxation delay
L4	10	loop counter; set to number of entries in vdlist
VDLIST	t1delay	vdlist with various recovery delays
F1 Parameters		
Parameter	**Value**	**Comments**
TD	10	number of experiments; set to value of L4

19.2.3 Acquire the 2D data set

If this data set was created from the ^{1}H reference spectrum t1data/1/1, the receiver gain is already set correctly. Enter **zg** to acquire the time domain data. The approximate experiment time for the inversion recovery experiment with the acquisition parameters set as shown above is 30 minutes.

19.3 Processing

Enter **edp** and set the processing parameters as shown in Table 60.

Table 60: Inversion Recovery Processing Parameters

F2 Parameters		
Parameter	**Value**	**Comments**
SI	8k	
SF		spectrum reference frequency (^{1}H)
WDW	EM	
LB	1	
PH_mod	no	
PKNL	TRUE	necessary when using the digital filter
BC_mod	quad	
F1 Parameters		
Parameter	**Value**	**Comments**
SI	16	select a power of two greater than or equal to the number of delays in vdlist
BC_mod	no	
MC2	QF	

The spectra will be processed by the automation program **proc_t1**. If desired, however, the spectra may be processed manually. Simply enter **xf2** to multiply the time domain data by the window function and also perform the Fourier transformation in F2 only. The 2D data set is displayed automatically.

19.3.1 Write the integral range file and baseline point file

The automation program **proc_t1** which will be used to calculate T_1 for the defined peaks requires a predefined integral range file and baseline point file. These files must exist before running the automation program.

From the 2D data set, move to a 1D data set containing the row for which **vd** is a maximum, e.g. the first spectrum here with 10s recovery delay. This may be accomplished by entering **rser 1**, which copies the FID of the first row into the data set ~TEMP/1/1.

Enter **ef** to apply line broadening. Manually phase correct the spectrum and store the correction.

Click integrate to enter the integration mode (see Section 3.10) and integrate each peak for which T_1 should be calculated. Click the left mouse

button to release the cursor from the spectrum. Click on and select **Save as ' intrng' and return** to store the regions and return to the main 1D window. Enter **wmisc** to call up the menu of miscellaneous list types. Select **intrng** to select the integral range file type. This calls up the list of possible files. Simply type the new name **t1reg**. Now the integral regions selected above are written to the integral range file **t1reg**.

Enter **basl** to enter the baseline submenu and from here click on def-pts to enter the baseline point subroutine. In this subroutine, the cursor is tied to the spectrum. Use the middle mouse button to select the points for which T_1 will be calculated. One and only one point must be selected for each integral region defined above. Take care to select the point of maximum intensity for each peak (region). When finished, click the left-hand mouse button to release the cursor from the spectrum and store the baseline points. Next enter **wmisc** to call up the menu of miscellaneous list types. Select **baslpnts** to select the baseline point file type. This calls up the list of possible files. Simply type the new name **t1bas**. Now the baseline points selected above are written to the baseline point file **t1bas**.

Click on **return** to return to the main 1D window. From here, click on 2D to return to the 2D data set.

19.4 T_1 Calculation

Once the T_1 data has been acquired and the integral range and baseline point files have been defined, the data may be processed and the T_1 calculation is carried out using the automation program **proc_t1**. This program first Fourier transforms and phase corrects the rows of the 2D T_1 data set. It then performs a T_1 calculation on all the peaks indicated by the integral range and baseline point files.

Start the automation program from the 2D data set by entering **xau proc_t1**. Answer the questions as follows:

Enter fid no. for phase determination:	1
Enter left limit for baseline correction:	1000
Enter right limit for baseline correction:	–1000
Enter no. of drift points:	20
Enter name of baseline point file:	t1bas
Enter name of integral range file:	t1reg
Enter name of VD list to use:	t1delay
Enter calc. type (T1=1, T2=2):	1

The FID corresponding to the largest value of **vd** (i.e., full relaxation between the 180° and 90° pulses) should be used for the phase determination. The values ±1000ppm are suggested merely to ensure that the whole spectrum is corrected. The automation program applies a baseline correction in F2 (**abs2**) between these two limits, and it is important to baseline correct the entire spectral width.

The number of drift points accounts for the fact that the maximum of a peak selected for a T_1 calculation is usually not at exactly the same position for

each of the 1D spectra. The number of drift points specifies how many digital points the peak maximum may vary. This parameter may need some optimization. It is important to select the number of drift points large enough so that you are always sure to find the peak maximum, yet small enough so that the maximum is always of the *same* peak. If the number of drift points is chosen incorrectly, peak picking will not work properly and the T_1 curves will not be smooth exponential curves.

When **proc_t1** is finished, the message "T1 result stored in t1r" appears. The pathname of this file is t1data/2/pdata/1/t1r (i.e., it is in the same directory as the processed NMR data).

The peak intensity *vs*. **vd** time data are also gathered and plotted for each resonance. To view these results type **t1/t2** to enter the T_1/T_2 routine. The first T_1 curve appears automatically in the window. Enter **nxtp** to view the T_1 curves for successive peaks.

19.4.1 Check T_1 curves

The T_1 curve for each selected resonance must be verified that the **vd** values were chosen so that all curves are clearly defined. If any T_1 curve is not well defined, it is necessary to edit the vdlist **t1delay** and rerun the experiment so that reliable T_1 measurements for those resonances may be obtained as well.

Also check all T_1 curves to be smoothly exponential. If not, the T_1 calculation can be redone with bad points eliminated for the calculation. Points may be removed from a curve one at a time by typing **elim** and then selecting a point with the middle mouse button (click the left mouse button to quit without choosing a peak). Eliminated points may be restored by entering **rstp** (this restores all eliminated points to all T_1 curves). Once the bad points have been removed from a curve, enter **ct1** to begin the T_1 calculation for that resonance. Enter **nxtp** to call up the next curve, remove the bad points, enter **ct1** to calculate T_1 for that peak, and so on. Alternatively, remove the unwanted points from all curves and then enter **dat1** to begin the T_1 calculation for all selected peaks. (Note that unless CURPRIN is changed *before* using **ct1** or **dat1** to recalculate T_1, the numerical results from **proc_t1** will be overwritten as discussed below.)

If there are too many bad points for a given T_1 curve to be a reliable fit, **proc_t1** should be rerun. It may be necessary to use a different number of drift points, or to redefine the integral range and baseline point files.

19.4.2 Check numerical results

The numerical results generated by the T_1 calculation routine may be stored in a file, displayed on the monitor, or sent directly to the printer. The automation program **proc_t1** automatically stores the results in the file t1r in the processed data subdirectory. (After running **proc_t1** enter **edo** to call up the plotter options menu and note that CURPRIN is set to t1r). Each time a T_1 calculation is run with CURPRIN set to t1r, this file is overwritten. However, before using **ct1** or **dat1**, the user also has the option to set CURPRIN to $screen or to the appropriate printer.

To display the numerical results on the screen, set CURPRIN to $screen as follows: enter **edo**, click the box next to CURPRIN with the left mouse button and enter $screen. Click SAVE to exit the **edo** menu.

The numerical results consist of a table for each selected peak. These tables indicate TAU (i.e., **vd** value), CURSOR, FREQ, PPM, INTEGRAL, and INTENSITY for each point. Below each table is the statement "[n] intensities fit" or "[n] areas fit". This is an indication of how well the peak picking worked. For example, if peak picking worked well for the 10 **vd** values, 10 intensities should have been fit for each peak selected. If 0 or very few intensities were fit for one or more peaks, it is a good idea to redefine the integral range and baseline point files and rerun **proc_t1**.

Finally, for each selected peak there is a table indicating the T_1 and standard deviation.

19.4.3 T_1 parameters

If necessary, the user may edit a number of parameters used in the T_1 calculation routine. In the T_1/T_2 menu (type **t1/t2**) enter **edt1**: Some appropriate values are indicated in Table 61.

Table 61: T_1 Parameters

Parameter	Value	Comments
NUMPTS	10	number of delays in vdlist.
FITTYPE	intensity	T_1 will be calculated from peak intensity (rather than integrated area).
CURSOR	1	start with the first peak chosen.
CONV	e^{-5}	convergence criterion for the fit algorithm.
DRIFT	20	allowed peak drift for peak picking.
START	1	starting spectrum for peak picking.
INC	1	increment for next spectrum used in peak picking.
NUMTERM	3	number of variables used in fitting routine.

19.5 Create a Stacked Plot

This section describes a method for obtaining a stacked plot of the 2D data set. The plot is created by the au program **stack2d**, which uses the plot parameter set **stackplot**. Note that **stackplot** is a 1D plot parameter set.

To create the 1D parameter set, first return to the reference spectrum (enter **re 1 1**) and select an appropriate region for plotting. Next create the plot parameter set to be used by the au program. Enter **edg** to call up the plot parameter menu. Make sure that SPECT is set to YES, but that XAXIS, YAXIS, TITLE, INTEGR, and PARAM are set to NO. Click on the ED which

appears next to the option EDSPECT to call the submenu “Spectrum Plot Parameters”. The following selected parameter values are suggested for A4 (8.5" ∞ 11") paper.

Table 62: Spectrum Plot Parameters for Stacked Plot

Parameter Name	Value (A4)	Comments
SXLLEFT	2.0 cm	
SYLLEFT	1.0 cm	
CX	20.0 cm	
SHEI	20.0 cm	
F1P		These parameters were set when **DP1** was used to define the plot region.
F1		
F2P		
F2		
PPMCM		
HZCM		
DHEI	17.5 cm	
SZERO	2.0 cm	
CY	6.0 cm	Size of each individual spectrum.

Click **SAVE** to save these changes and return to the main **edg** menu. There, click **SAVE** to save all changes and exit **edg**.

Next, save these parameters as the plot parameter file by entering **wpar stackpar plot**.

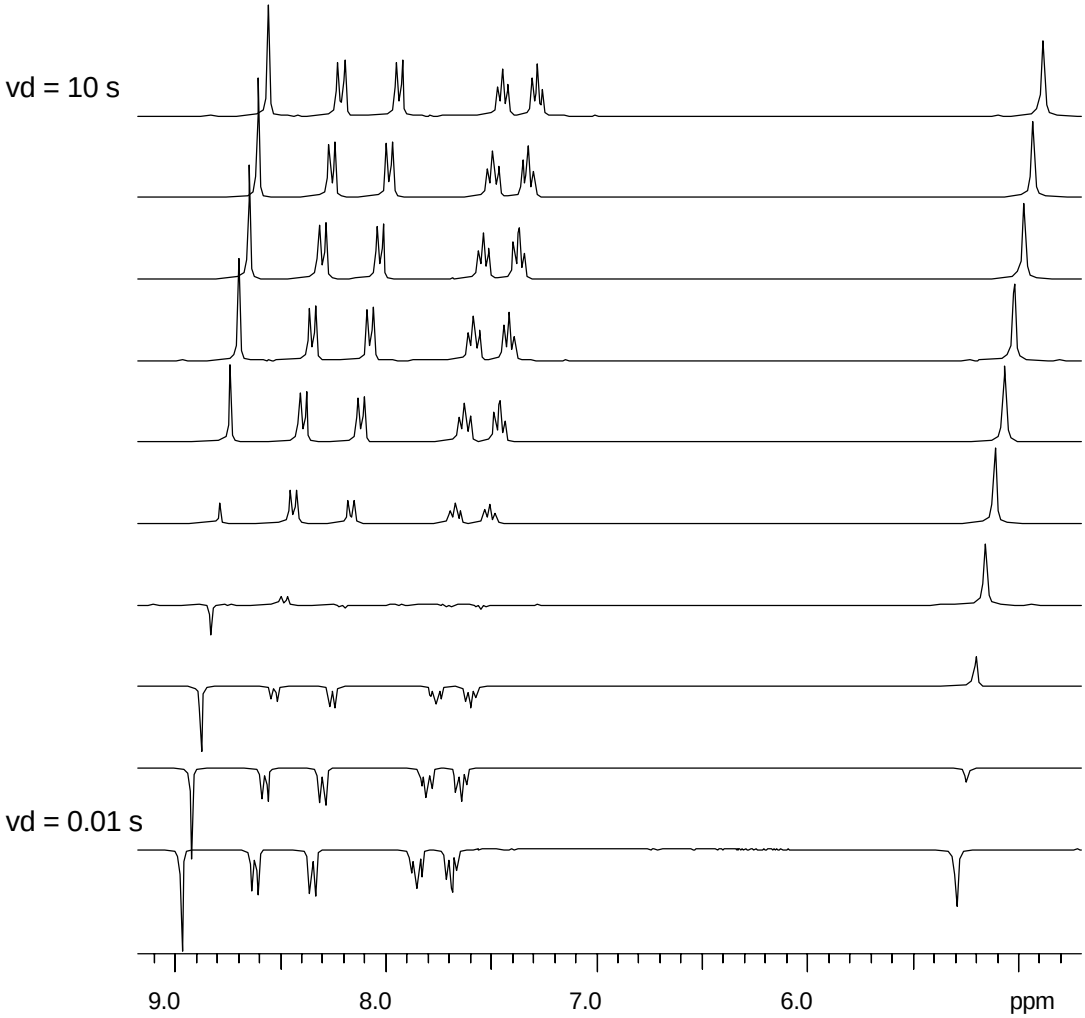
Return to the 2D processing menu (enter **`re 2 1`**) and start the stacked plot automation program by entering **`xau stack2d`**. Answer the questions as follows:

Enter NAME:	t1data
Enter EXPNO:	2
Enter PROCNO:	1
Enter USER:	[user name]
Enter DISK:	u
Repeat dialog (r) or continue (c):	c
Enter first row to plot:	10
Enter row increment:	–1
Enter number of rows:	10
Enter row for scaling:	1
Enter x increment [cm]:	0.2
Enter y increment [cm]:	1.2 .

The resulting stacked plot is sent to the plotter specified by the parameter CURPLOT. To check or change this parameter, enter **edo** to call up the output device parameter menu and click on the box next to CURPLOT to open the menu of plotter options. Select one of these with the left-hand mouse button and exit the **edo** menu.

A stacked plot of the results of the inversion recovery sequence run on 100 mM Pamoic Acid in DMSO-d6 is shown in Figure 53.

Figure 53: Inversion Recovery Spectra of 100 mM Pamoic Acid in DMSO-d6



20 Selective Excitation

20.1 Introduction

The hard pulses used in all the experiments from the previous chapters are used to uniformly excite the entire spectral width. This chapter introduces soft pulses which selectively excite only one multiplet of a ^{1}H spectrum. Important characteristics of a soft pulse include the shape, the amplitude, and the length. The selectivity of a pulse is measured by its ability to excite a certain resonance (or group of resonances) without affecting near neighbors. Since the length of the selective pulse affects its selectivity, the length is selected based on the selectivity desired and then the pulse amplitude (i.e., power level) is adjusted to give a 90° (or 270°) flip angle.

Note that the transmitter offset frequency of the selective pulse must be set to the frequency of the desired resonance. This transmitter frequency does not have to be the same as **o1p** (the offset frequency of the hard pulses), but for reasons of simplicity, they are often chosen to be identical.

Most selective excitation experiments rely on phase cycling, and thus subtraction of spectra, to eliminate large unwanted signals. It is important to minimize possible sources of subtraction artifacts, and for this reason it is generally suggested to run selective experiments non-spinning.

References: C. J. Bauer, R. Freeman, T. Frenkiel, J. Keeler, and A. J. Shaka, *J. Magn. Reson.*, **58**, 442 (1984); H. Kessler, H. Oschkinat, C. Griesinger, and W. Bermel, *J. Magn. Reson.*, **70**, 106 (1986); L. Emsley and G. Bodenhausen, *J. Magn. Reson.*, **82**, 211 (1989).

The sample used to demonstrate selective pulse experiments in this chapter is 50 mM Cyclosporin in C_6D_6.

20.2 Selective Pulse Calibration

Insert the sample in the magnet. Lock the spectrometer. Readjust the Z and Z^2 shims until the lock level is optimized. Tune and match the probehead for ^{1}H observation. Selective experiments are measured without sample spinning.

Before performing selective excitation experiments, it is necessary to calibrate the selective pulse. First, a ^{1}H reference spectrum must be recorded and the resonance frequency of the desired resonance is determined; second, define the shaped pulse; and third, perform the pulse calibration experiment.

20.2.1 Proton reference spectrum

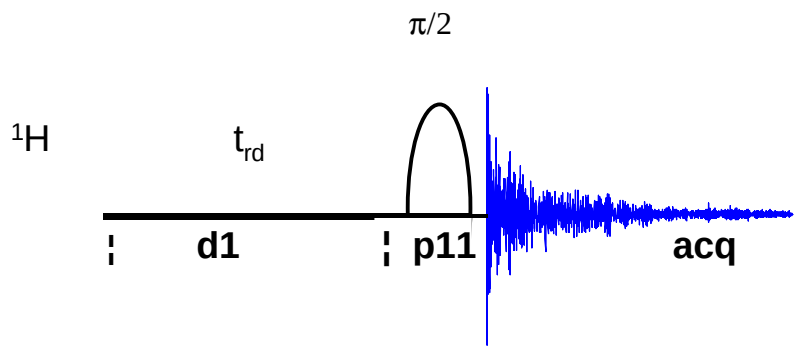
Acquire and process a standard ^{1}H spectrum in the data set selex/1/1. Optimize **o1p** and **sw**. Click on utilities to enter the calibration submenu. Click on O1 with the left mouse button to select the **o1p** calibration. Move the cursor to the center of the doublet at 8.1ppm and click the middle mouse button to assign **o1p** to this frequency. Exit the utilities submenu and return to the main window.

Make sure **sw** is large enough to include the entire ^{1}H spectrum with this new **o1p** value. Acquire and process a ^{1}H spectrum.

20.2.2 Selective one-pulse sequence

The pulse sequence used to calibrate the selective pulse is shown in Figure 54. This sequence is identical to the standard one-pulse sequence shown in Figure 1, except for the pulse is applied with low-power and a shape. The pulse length **p11** and the pulse strength **sp1** must be adjusted so that the pulse is 90° or 270° (see below)

Figure 54: Selective One-Pulse Sequence



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20.2.3 Define the pulse shape

Shaped pulses are designed using the shape tool of XWIN-NMR (version 2.1 and higher). Enter **stdisp** in XWIN-NMR. Select ***Gauss*** from the pull-down menu ***Shapes***. A small window appears containing default parameters for the shapes pulse. Select **OK**. Store the shape by choosing ***Save As*** from the ***File*** menu and enter the filename **gauss1.1k**.

20.2.4 Acquire and process the selective one-pulse spectrum

Create the data set selex/2/1 for the 1D selective experiment by typing **xau iexpno** starting from the data set selex/1/1. Set up the acquisition parameters as shown in Table 63.

Table 63: Selective One-Pulse Acquisition Parameters

Parameter	Value	Comments
PULPROG	selzg	see Figure 54 for pulse sequence diagram.
TD	8k	
NS	1	no need for signal averaging yet.
DS	0	no need for dummy scans yet.
SP1	80	shaped pulse power level on f1 channel.
P11	80m	90° shaped pulse on f1 channel.
D1	10	relaxation delay
PL0	120dB	Sets power to zero before selective pulse.
SP	edit	enter this array to edit power level, offset, and filename for the shaped pulse

In the **eda** menu enter the power level, offset, and filename for the shaped pulse by clicking on the **edit** button next to the parameter **SP07**. This calls up the table “Power for shaped pulses”, which has four columns: one for the shaped pulse index number (Index), one for the power level (Power[dB]), one for the offset frequency (Offset-Frequency), and one for the filename of the shaped pulse (Filename). The pulse program **selzg** makes use of shaped pulse **1** only. In row 1, set the power level for the shaped pulse to 80 dB. (This parameter is also known as **sp1**). For on-resonance selective excitation, make sure that the offset frequency is set to 0 Hz. Click on the filename box with the right mouse button to call up the menu of possible shape files. From this list, select **gauss1.1k** with the left mouse button.

All other acquisition parameters should be the same as for the reference spectrum, in particular **td**, **o1**, **sw**, and **rg**.

Acquire and process a selective one-pulse spectrum. The spectrum should be processed with the command **efp** using the same phase settings as for the reference spectrum with hard pulse. The N-H resonance should appear in the middle of the window and no other peaks should be visible. Phase correct the N-H resonance at 8.1ppm using the 0th-order correction only. Note this value, but return to the main menu *without* storing the phase correction. This additional phase correction might to be applied to the shaped pulse only, not to the hard pulses (used in the pulse programs **selco** and **selmlzf** below): Type **2 phcor 1** and enter the phase correction value.

Now if the spectrum is reacquired and processed with **efp**, the peptide N-H should be phased properly.

Expand the spectrum so that the N-H doublet occupies approximately the center quarter of the window (e.g., so that the region from approximately 9.2 ppm to 8.1 ppm is displayed). Save this as a plotting region by clicking on dp1 with the left-hand mouse button and hit return in response to the questions. This plotting region will be used by the au program **paropt**, below.

20.2.5 Perform the pulse calibration

The au program **paropt** may is used to perform an automatic pulse calibration. Simply enter **xau paropt** and answer the questions as follows:

Enter parameter to modify:	sp1
Enter initial parameter value:	90
Enter parameter increment:	–2
Enter # of experiments:	20

At the end of the experiment, the message “paropt finished” and a value for **sp1** are displayed. This value is the approximate ^{1}H transmitter power level for a 90° pulse time of 80 msec.

To obtain a more accurate 90° pulse, repeat **paropt** using a smaller increment for **sp1**. (At this point it may be useful to repeat the above procedure for a range of **p11** pulse lengths.)

Paropt results of selective excitation of a N-H resonance is shown in Figure 55. A selective one-pulse ^{1}H spectrum of Cyclosporin, together with the reference spectrum, is shown in Figure 56.

Figure 55: Selective One-pulse Paropt Results

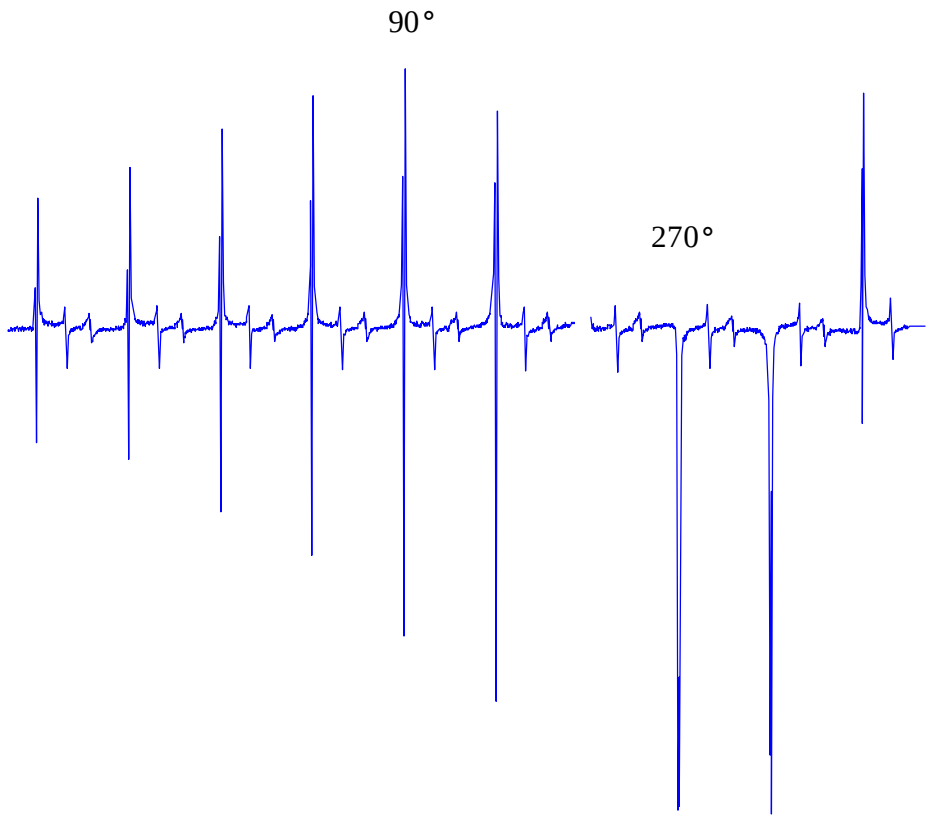
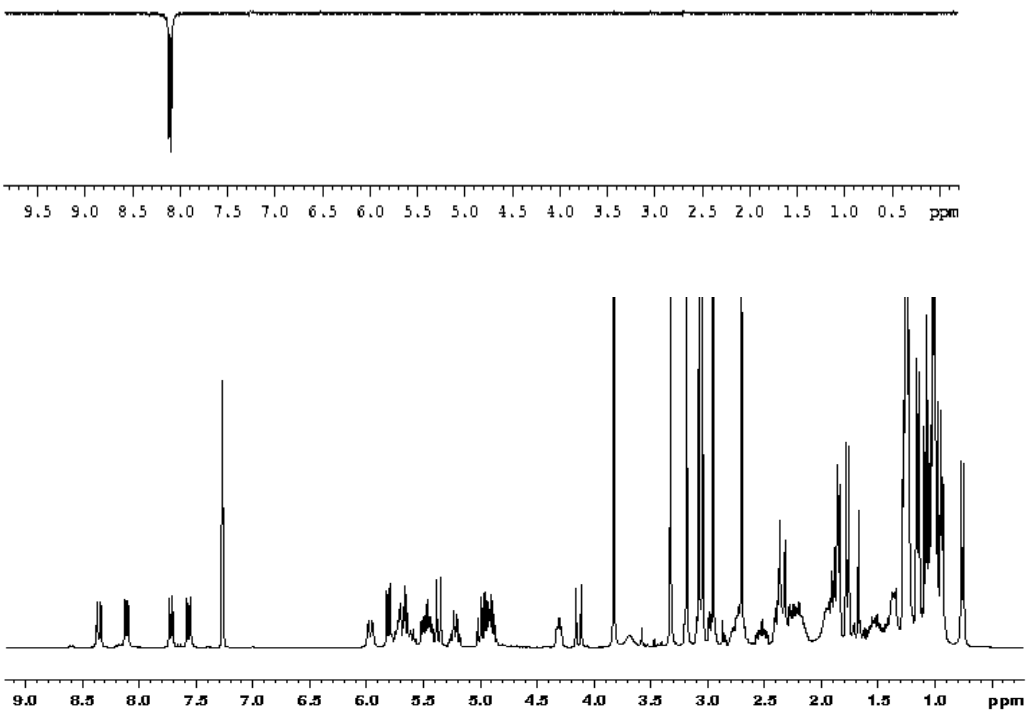


Figure 56: Selective One-pulse Spectrum of 50 mM Cyclosporin in C_6D_6



20.3 Selective COSY

Many 2D NMR experiments can be converted to analogous 1D experiments by using Gaussian pulses. A 1D sequence is advantageous when a limited amount of information is desired, which is often the case for medium-sized molecules. In such cases, the total experiment and data manipulation times are shorter for the 1D experiment than for the 2D experiment.

The 2D COSY experiment is very effective at indicating coupling except in cases where the ^{1}H chemical shifts are closely crowded together so that many cross-peaks overlap. Selective COSY gives the same ^{1}H coupling information at a time without involving a 2D Fourier transform. This is useful for probing regions of the spectrum where the ^{1}H shifts are densely packed, provided that some ^{1}H resonances are sufficiently well separated that they can be picked out for selective irradiation.

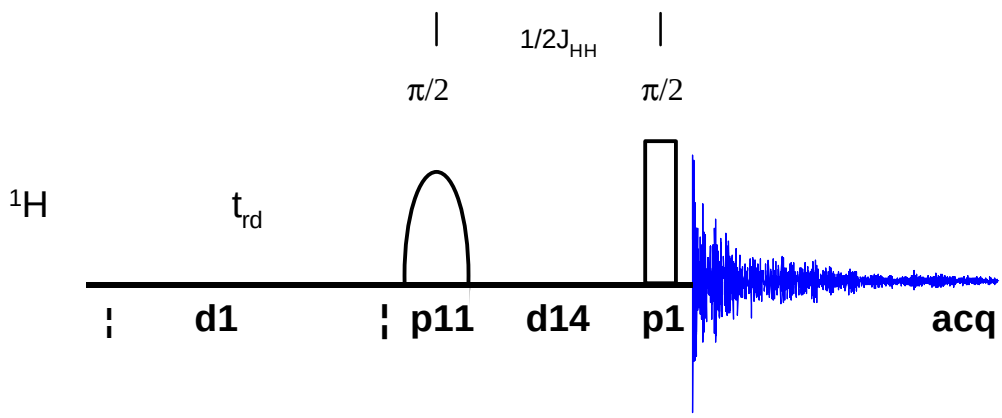
The selective COSY pulse sequence is shown in Figure 57. It is very similar to the standard COSY sequence shown in Figure 21, except that the first pulse is a frequency selective 90° excitation pulse and the delay between the two pulses (**d14**) is not incremented. The duration of this delay is measured from the middle of the Gaussian envelope. As with 2D COSY, the second (or coherence transfer) pulse is a hard 90° pulse. This pulse creates observable magnetization from the antiphase coherence present at the end of the fixed delay. The acquisition period follows immediately after the second pulse.

The frequency of the selective pulse is set to the chemical shift of a multiplet and the selectivity is chosen so that adjacent multiplets are unperturbed. The spectral width is set large enough to cover the entire chemical shift range whatever the transmitter offset. The intensity of the transferred signal depends on the magnitude of the appropriate coupling constant and on the length of the fixed delay, and varies in a sinusoidal fashion. There is a chance that a particular transfer falls accidentally at a null, in which case a coupling path would be overlooked. This risk can be minimized by selecting the precession interval short compared with the reciprocal of the largest expected coupling constant. The lower level of the delay is one half the Gaussian duration needed to get the required selectivity.

Since the final pulse gives coherence transfer to spins whose couplings are in antiphase to the selectively excited spin, 1D selective COSY gives rise to antiphase multiplets (which will unavoidably have adjacent positive and negative intensities). Thus, direct extraction of the coupling constants may be complicated due to annihilation of individual lines within the multiplet.

Note that the final pulse also converts any longitudinal magnetization into transverse magnetization. The resulting signals are intense for all ^{1}H other than the one excited by the selective pulse. These signals are eliminated by the same phase cycling as is used in 2D COSY; however, the corresponding signals in the 2D experiment are much weaker, and so are more easily eliminated by the phase cycling.

Figure 57: Selective COSY Pulse Sequence



20.3.1 Acquisition

For best results, run selective COSY experiments non-spinning.

Insert the Cyclosporin sample (see Section 20.2) and, starting from the data set selex/2/1, create the data set selco/1/1 and record a reference ^{1}H spectrum for the selective COSY experiment with **o1p** set to the N-H resonance at 8.1ppm.

Enter **eda** and set the acquisition parameters as shown in Table 64.

Table 64: Selective COSY Acquisition Parameters

Parameter	Value	Comments
PULPROG	selco	see Figure 57 for pulse sequence diagram
TD	32k	
NS	64	number of scans must be 8 * n
DS	16	
PL1		high power level on F1 channel (^{1}H) as determined in Section 4.2.4
P1		^{1}H 90° pulse as determined in Section 4.2.4
SP1		shaped pulse power level on F1 channel (^{1}H) as determined in Section 20.2
P11		^{1}H 90° shaped pulse as determined in Section 20.2
D1	2	
D14	35m	delay for evolution after shaped pulse ((p11)/2 + d14 = 1/(2 J_{HH}))
PHCOR(1)		additional phase correction applied to shaped pulse p11 (see Section 20.2)

Note that in this pulse sequence, the delay **d14** is to ensure that the magnetization is antiphase when the second pulse is applied. This is accomplished by choosing **d14** such that **p11**/2 + **d14** = 1/2J_{HH}.

Perform a routine acquisition with **zg**. The approximate experiment time for Selective COSY with the parameters set as shown above is 4 minutes.

20.3.2 Processing

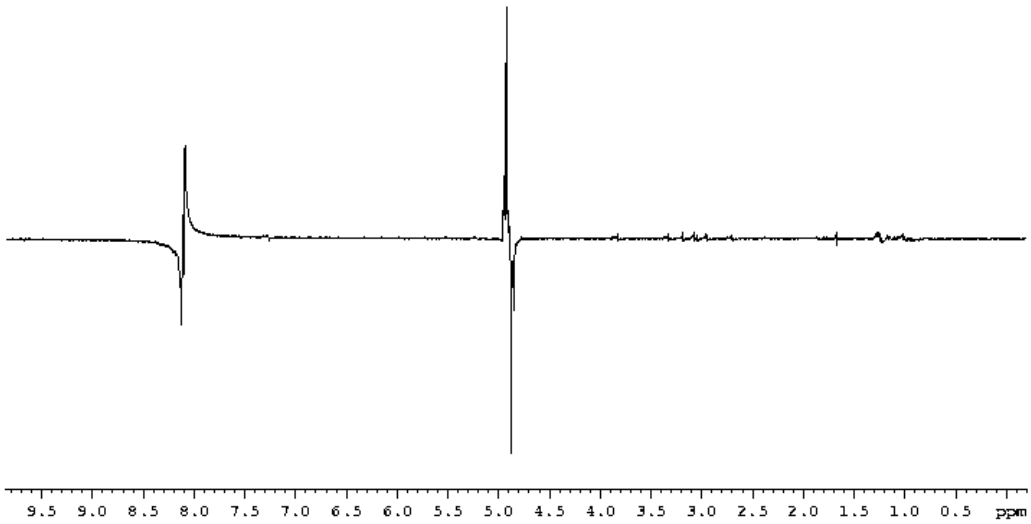
Enter **edp** and set the processing parameters as shown in Table 65.

Table 65: Selective COSY Processing Parameters

Parameter	Value	Comments
SI	16k	
WDW	EM	
LB	0.30	
PKNL	TRUE	necessary when using the digital filter.

Add line broadening and Fourier transform the time domain signal with the command **ef**. Manually phase correct the spectrum. The resulting spectrum should look like that inFigure 58.

Figure 58: Selective COSY Spectrum of 50 mM Cyclosporin in C_6D_6

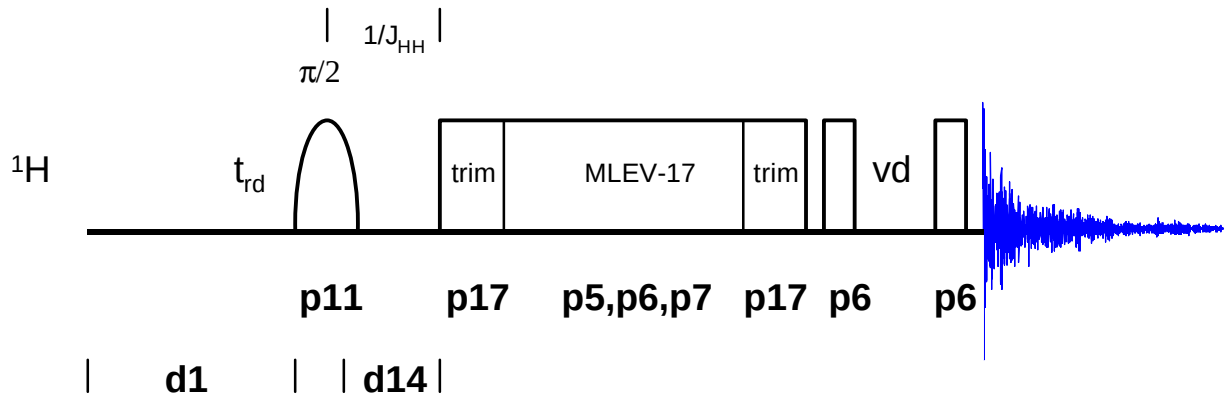


20.4 Selective TOCSY

Selective TOCSY gives the same ^{1}H coupling information as 2D TOCSY without a 2D Fourier transformation. The selective TOCSY pulse sequence is shown in Figure 59. It is very similar to the standard TOCSY sequence shown in Figure 27, except that the first pulse is a low-power shaped pulse, the following delay (**d14**) is not incremented, and the spin-lock period is followed by a z-filter. As for the COSY, the selective TOCSY sequence begins with a 90° frequency selective excitation pulse. This is followed by a fixed delay (rather than the variable evolution period of the 2D TOCSY sequence) during which in-phase coherence is created by evolution due to J-coupling. The duration of this delay is measured from the middle of the Gaussian envelope. Next, the coherence transfer occurs during the multiple-pulse spin-lock period. The multiple-pulse spin-lock sequence most commonly used is MLEV-17. The length of the spin-lock period determines how “far” the spin coupling network will be probed. A general rule of thumb is that 1/(10 J_{HH}) should be allowed for each transfer step, and five transfer steps are typically desired for the TOCSY spectrum.

Immediately after the spin-lock period a z-filter is introduced, which allows easier phase correction of the final spectrum. Since the TOCSY correlation peaks arise from magnetization that was in-phase during the fixed delay, they can be phase corrected to be positive and absorptive.

Figure 59: Selective TOCSY Pulse Sequence



20.4.1 Variable Delay List

The z-filter in the selective TOCSY experiment requires a variable delay list. To create the variable delay list, enter **`edlist`**. A menu of list types appears. Select **`vd`** from this menu. This calls up a menu of existing vdlist filenames and gives the user the option of creating a new file (‘Type new name'). Simply type the name **`zf`**. This calls up the editor. Enter the delays desired, some appropriate values are listed below:

0.004
0.016
0.010
0.006
0.004
0.010
0.017
0.011
0.018
0.012

When the list is complete, save the file and exit the editor.

20.4.2 Acquisition

From the data set selco/1/1 create the data set seltoc/1/1 and record a ^{1}H reference spectrum of Cyclosporin exactly as described for the selective COSY in Section 20.3.

Enter **eda** and set the acquisition parameters as shown in Table 66.

Table 66: Selective TOCSY Acquisition Parameters

F2 Parameters		
Parameter	**Value**	**Comments**
PULPROG	selmlzf	
TD	32k	
NS	32	the number of scans should be 16 * n
DS	16	number of dummy scans
PL1		high power level on F1 channel (^{1}H) as determined in Section 4.2.4
PL10		low power level on F1 channel (^{1}H) for MLEV-mixing as determined in Section 4.2.5
SP1		shaped pulse power level on F1 channel (^{1}H) as determined in Section 20.2
P11		^{1}H 90° shaped pulse as determined in Section 20.2
P5		^{1}H 60° pulse, calculated from p6
P6		^{1}H 90° pulse as determined in Section 4.2.5
P7		^{1}H 180° pulse, calculated from p6
P17	2.5m	2.5 msec trim pulse
D1	2	relaxation delay; should about 1.25 * T_1 (^{1}H)
D14		delay for evolution after shaped pulse ((p11)/2 + d14 = $1/J_{HH}$).
L1	30	loop for MLEV cycle ((p6 * 64) + p5) *11 + (p17 * 2) = mixing time)
L4	10	number of delays in vdlist.
VDLIST	zf	name of vdlist used for z-filter
PHCOR(1)		phase correction applied to shaped pulse P11

Perform a routine acquisition with **zg**.

20.4.3 Processing

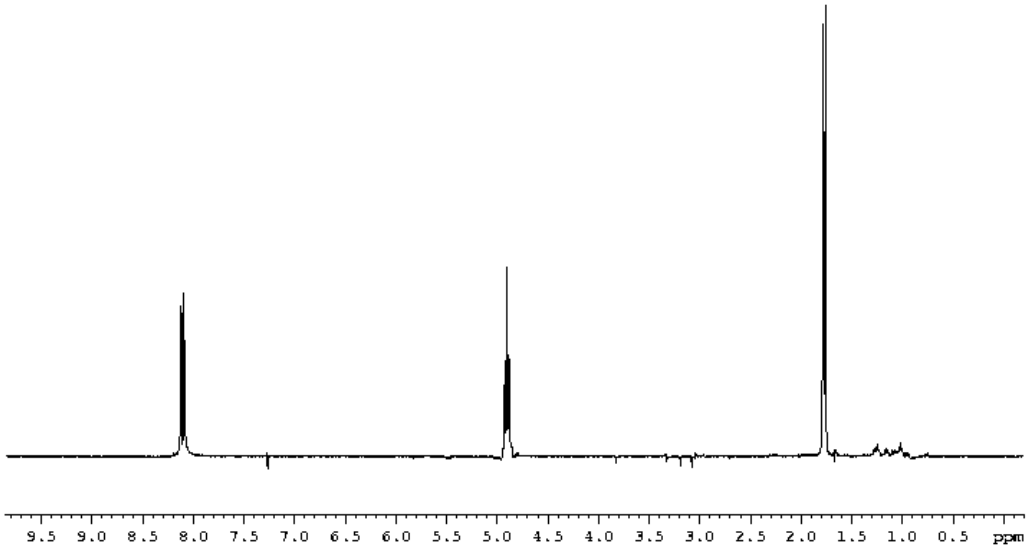
Enter **edp** and set the processing parameters as shown in Table 67.

Table 67: Selective TOCSY Processing Parameters

Parameter	Value	Comments
SI	16 k	
WDW	EM	
LB	0.30	
PKNL	TRUE	necessary when using the digital filter.

Add line broadening and Fourier transform the time domain signal with the command **ef**. Manually phase correct the spectrum using the 0th-order phase correction. The resulting spectrum should look like that in Figure 60.

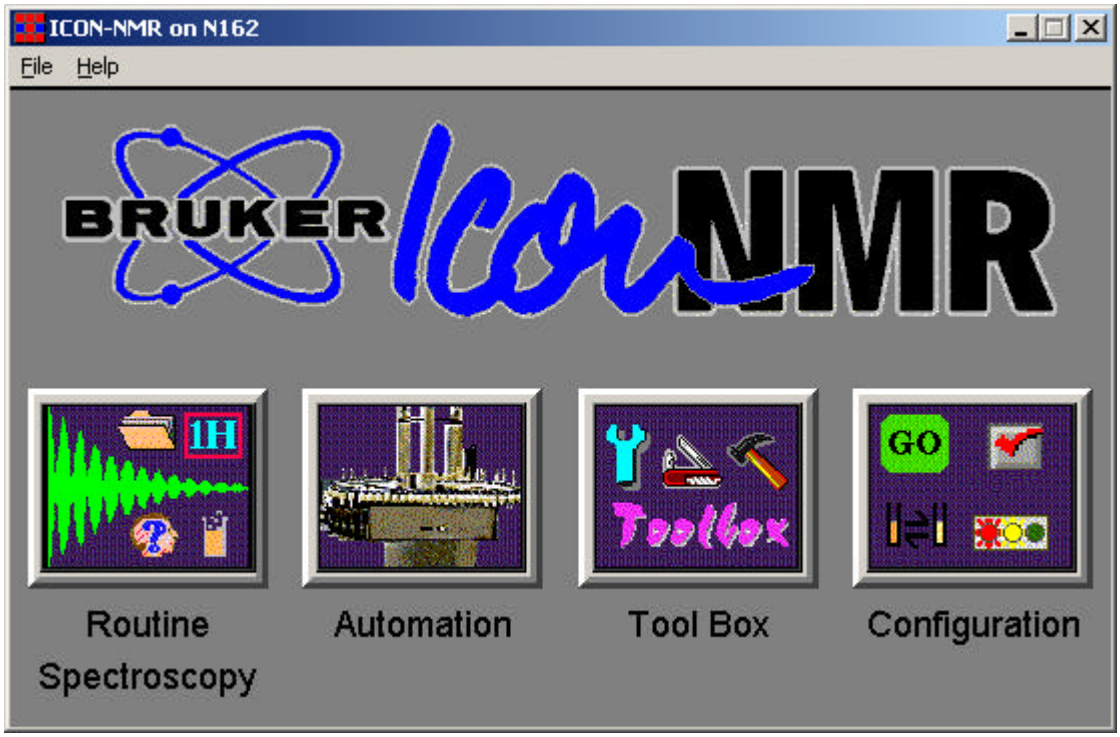
Figure 60: Selective TOCSY Spectrum of 50mM Cyclosporin in C_6D_6



21 IconNMR: NMR Automation

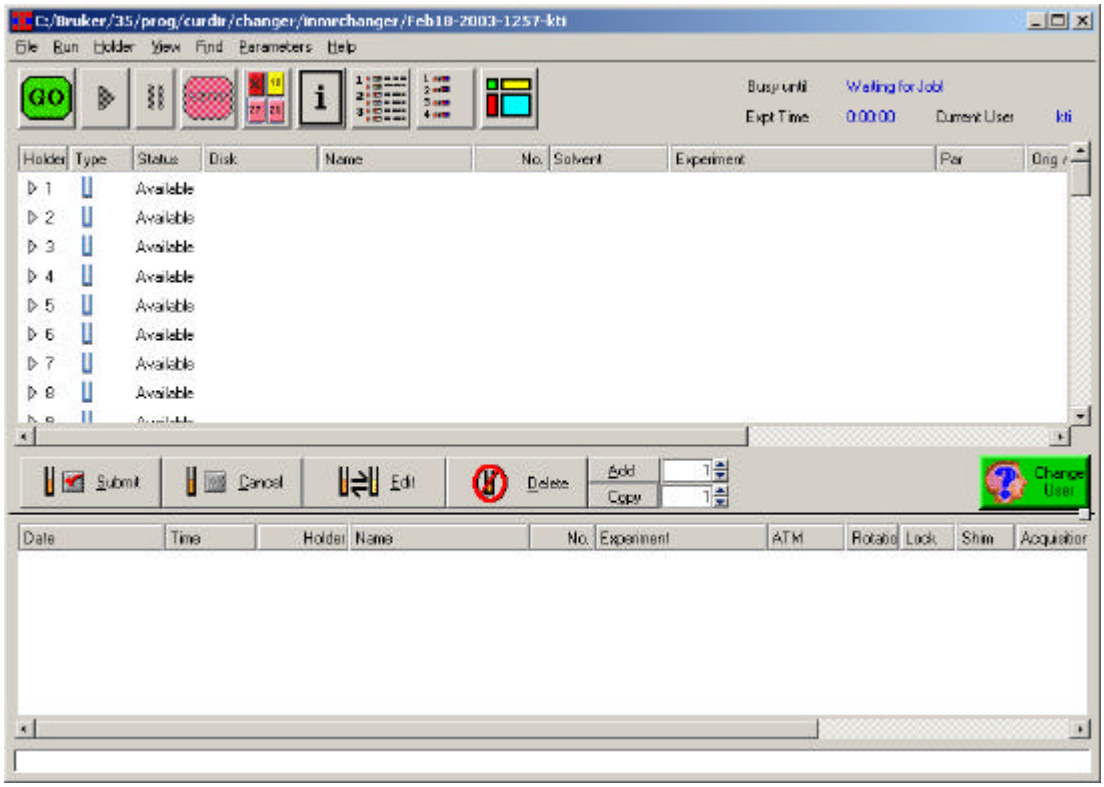
IconNMR is the Bruker NMR Automation Software tool. It offers a quick and simple approach to NMR also for unexperienced users via the consequent use of predefined parameter sets.

To start IconNMR, type **`iconnmr`** in the XWinNMR command line and click on the “Automation” button.



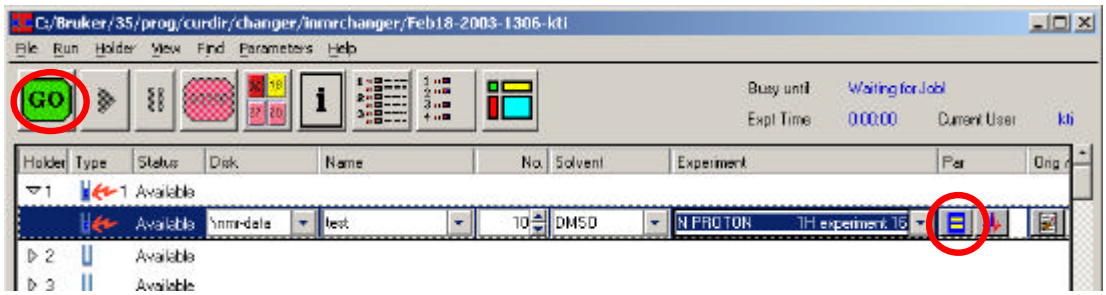
You need an IconNMR user account in order to run NMR experiments under the IconNMR control. Such an account can be created, activated and administrated in the “Configuration” window of IconNMR under “User Manager”. For a detailed description of the IconNMR configuration and administration please refer to the IconNMR software manual.

After the login into the automation window, the following setup window will appear. Here you can setup your experiments.



If you run IconNMR with a sample changer you can setup several experiments for different sample holders at once. If you do not have a sample changer, just setup the desired experiments on the first “virtual” holder and you will be prompted for the sample insert.

In order to set up the experiments, just double click on the line with the respective holder and choose an experiment name, accept the suggested experiment number, select your solvent and select the experiment that you want to run. Then press the green “Go” button to start the automation run.



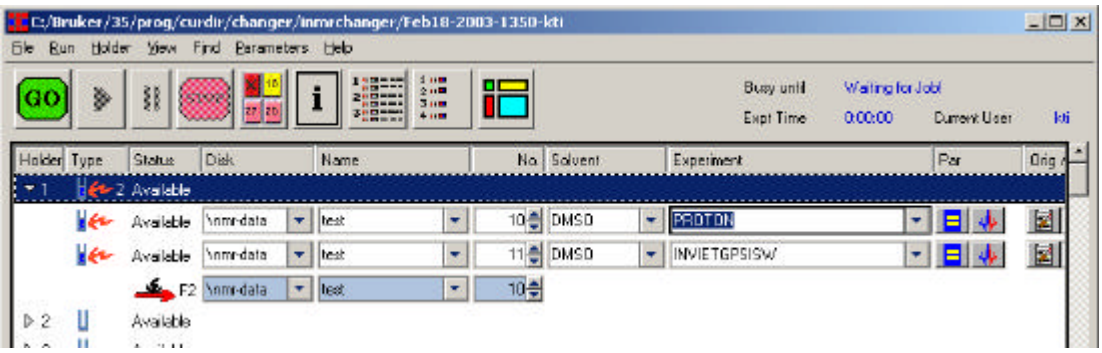
By clicking on the “Par” button in the setup window (2nd red circle in the image above), you can modify the parameters that are defined for this user in the IconNMR user specific configuration.

To submit the experiment, press the submit button in the automation window:

This will either start the sample changer automation or you will be prompted to insert the respective sample manually, depending on your configuration.

According to your settings in the IconNMR software configuration, IconNMR will then perform the automatic tuning and matching routine, lock the spectrometer on the selected solvent, shim the sample, run the data acquisition and do the spectrum processing automatically. It will also generate a plot of the spectrum.

Certain experiments also require a so called preparation experiment. These are, for instance, 2D experiments where a 1D proton experiment is run as a preparation experiment for the sweep width optimization in the direct dimension. These experiments are called composite experiments. If you select one of those, the preparation experiment will automatically be set up to be run before the 2D experiment. In the example below, a HSQC experiment was selected and the proton experiment was automatically set up in front:



You can also setup several experiments on the same sample. Therefore, press the “Add” button before you submit the experiment and you will get a new entry line where you can enter the new experiment for the same sample.

If you click on the “Copy” button all the parameters you have entered for one sample will be copied to the next sample(s).

The automation is parameter-set driven and therefore it is very simple to setup your own experiments for the automation in IconNMR. The only requirement is a working parameter set for your experiment with AU programs for the data acquisition and processing. These AU programs have to be defined under the **aunm** and **aunmp** parameters, respectively.

22 Appendix A: Artifacts in 2D-NMR Experiments

22.1 Introduction

22.1.1 Why do artifacts occure?

In general, an artifact simply is an artificial signal in the spectrum. It cannot be correlated to the chemical structure and therefore can mislead the chemist, who tries to determine a structure. We therefore have to have at least a basic background about typical artifacts occuring in NMR spectroscopy.

While artifacts in 1D-NMR spectra are quite well known, many spectroscopists have a very little knowledge about artifacts in 2D-NMR spectra. Modern NMR spectrometer allow to start even complex 2D-NMR experiments – like ROESY, TOCSY and the inverse experiments HSQC and HMBC – with single button push, even without the need of any knowledge of the theory behind the experiment. We therefore like to give an introduction to common artifacts in 2D-NMR spectra, allowing a more reliable interpretation of the spectra.

There are three classes of artifacts. First, those artifacts which are a result of the spectrometer hardware. With modern NMR spectrometer that source can be neglected. Second, artifacts can be a simple result of the spin system under investigation. One example is the J-Resolved experiment, were second-order effects of the scalar coupling introduce additional peaks. Finally, artifacts can be introduced by missettings of the experiment conditions. In this small overview we will focus on artifacts which are generated by missettings of acquisition parameters.

22.2 The Double-Quantum Filtered COSY Experiment

22.2.1 Rapid Scanning Artifacts

The T_1 relaxation rate of protons differs from less than 1sec for large molecules to values above 5sec for small organic molecules. If the relaxation rate is not taken into account and a standard repetition rate of e.g. 2sec is used, so-called multiple-quantum diagonales will be observed. The artifacts are additional peaks which can be placed on those additional diagonales.

The multiple-quantum diagonales can be quite easily found in the spectrum. They have either twice, three-times, four-times ... the slope of the regular diagonal.

Figure 61: Reference spectrum. DQF-COSY experiment of pamoic acid, recorded with a repetition rate of 5s. No artifacts are visible.

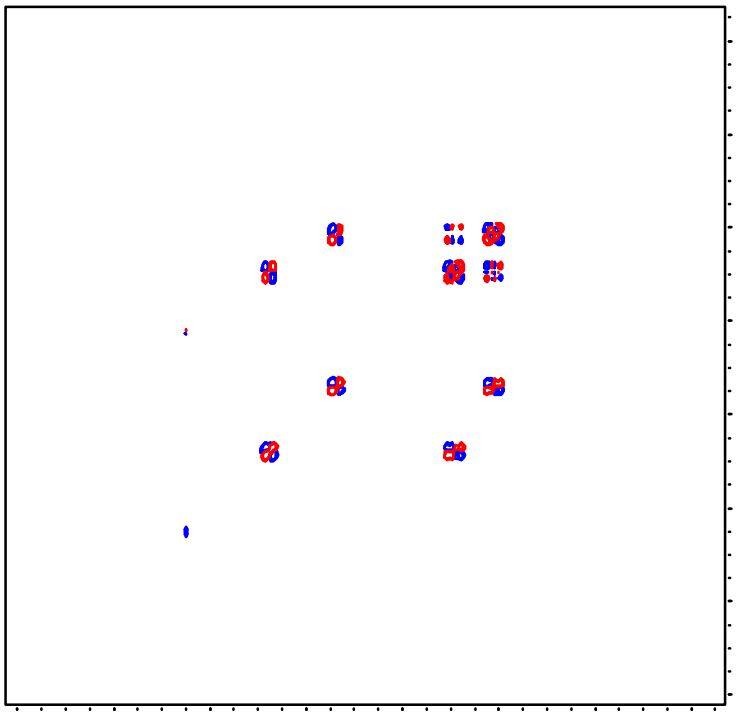
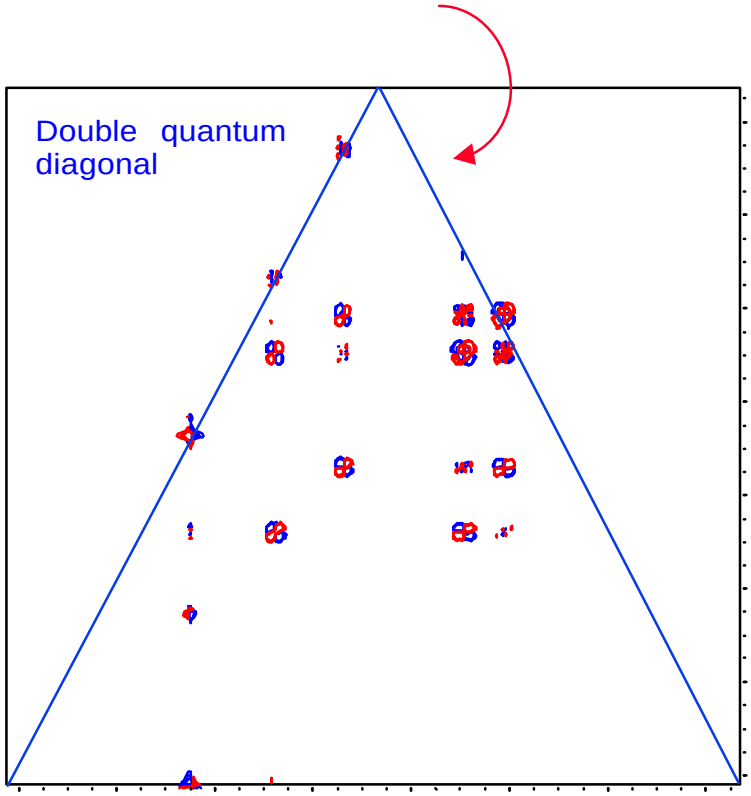


Figure 62: DQF-COSY experiment of pamoic acid, recorded with a repetition rate of 1s. Artifacts appear on the double quantum diagonal.

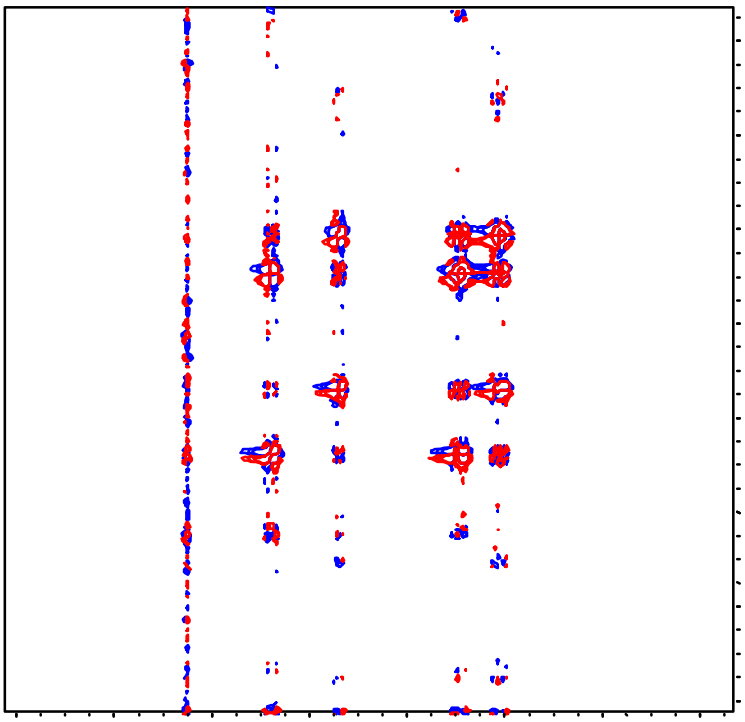


22.2.2 Overload of the receiver

With the introduction of field gradients in high resolution NMR spectroscopy the spectral quality of 2D experiments was improved dramatically. With field gradients just the magnetisation of interest can be selected.

For the gradient assisted DQF-COSY experiment the intensity of the first t_1 FID is zero, as no magnetisation transfer can occur at that state. As a consequence, the automated receiver gain adjustment will fail, and the receiver gain will be set to a too large value. With the receiver overloaded the phase cycling of the receiver will not work properly anymore, leading to errors like quadrature artifacts along the F1 dimension, e.g. a diamond pattern. In addition, the baseline will be distorted and high t_1-noise can appear. Please note, that care has to be taken for the automated adjustment of the receiver gain for ROESY and TOCSY experiments, as well.

Figure 63: DQF-COSY experiment of pamoic acid. The receiver is overloaded and additional t_1-noise appears



22.2.3 The ‘diamond pattern’

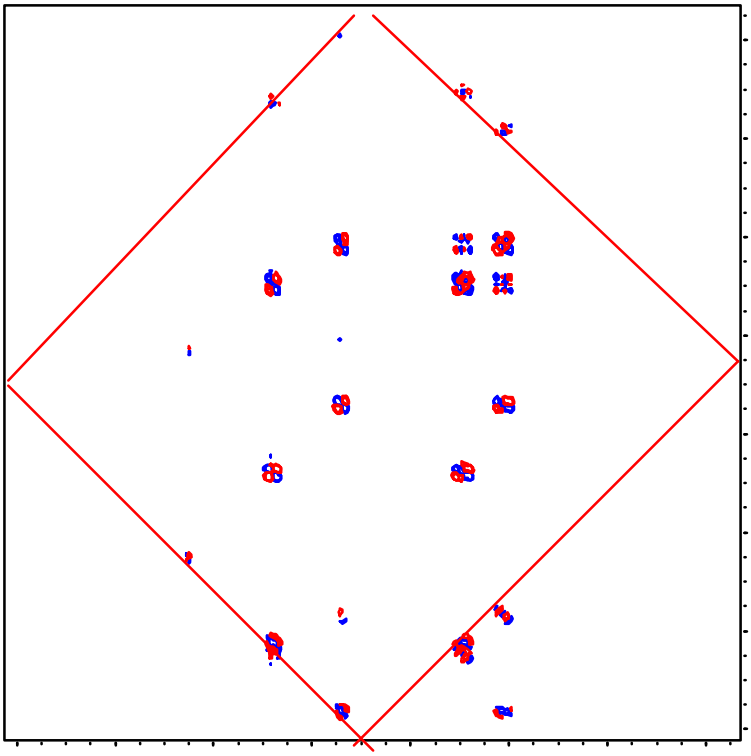
The so-called ‘diamond pattern’ forms an either quadratic or rectangular arrangement of additional peaks. In literature this artifact is explained by errors in the phase of that pulse, on which the TPPI phase cycle is done in an homonuclear experiment. This is true, if:

- The phase preset times of the spectrometer is set to a values which are too short,
- The pulse width of the proton pulse is set to a short value.

We recommend to use proton pulses in the order of e.g. 10µsec. In addition the phase preset time for the F1 channel of the spectrometer can be edited and increased with the XWIN-NMR command *edscon*.

A diamond pattern can also be caused by temperature oscillation, either of the sample or of the room temperature. Note, that too high values for the receiver gain also can cause a diamond pattern in homonuclear 2D experiment.

Figure 64: DQF-COSY experiment of pamoic acid. Artifacts are aligned on the ‘diamond pattern’, which is shown in red.

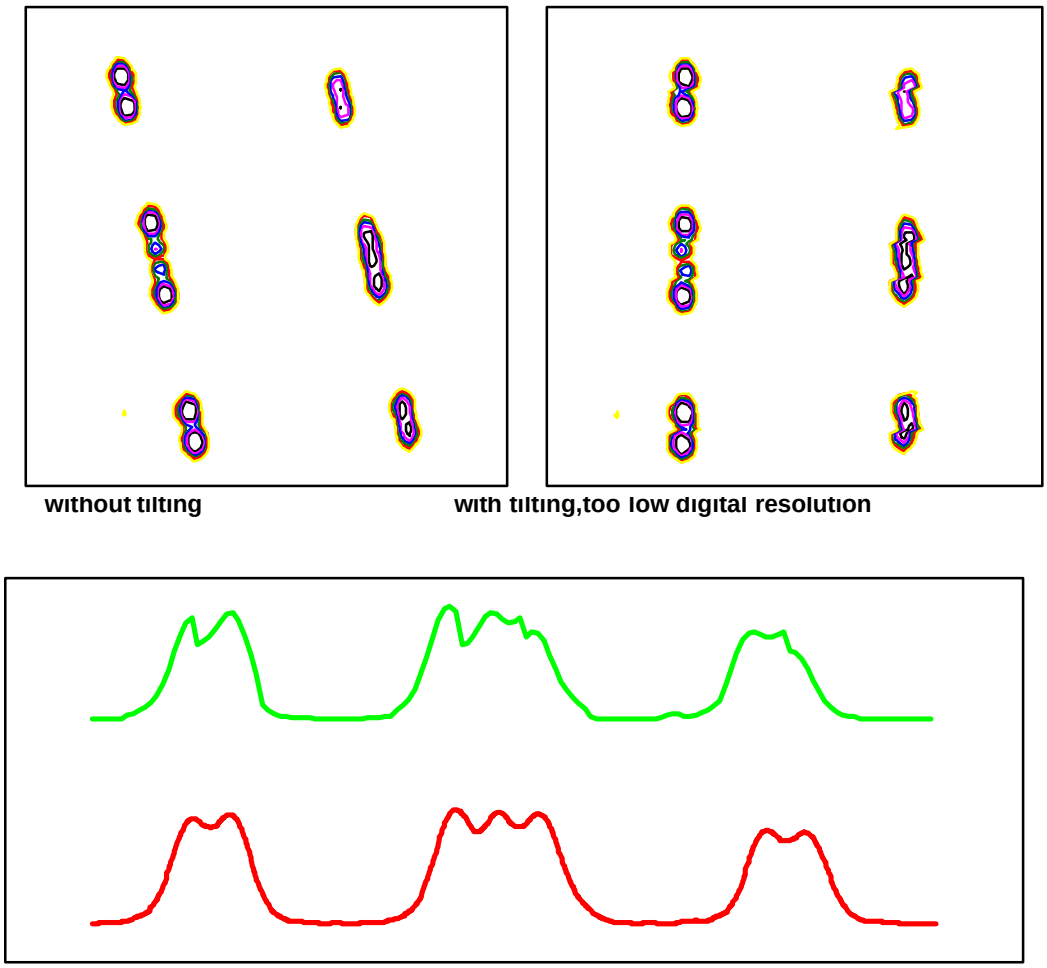


22.3 The Homonuclear J-Resolved Experiment

22.3.1 The Effect of digital resolution and tilting of the spectrum

The homonuclear J-Resolved experiment requires a tilting of the spectrum, which is applied after the 2D Fourier transformation. In order to get well-resolved multiplets along the F1-dimension of that experiment, a high digital resolution is required in the F1-dimension. It might be necessary to do a zero-filling in the F1-dimension of a factor 16-32.

Figure 65: Homonuclear J-Resolved experiment of pamoic acid. The processing size was set to 2048 points for an experiment were 64 points were collected in the F1-dimension (red trace). The spectrum obtained with a processing size of 256 points is shown in green.



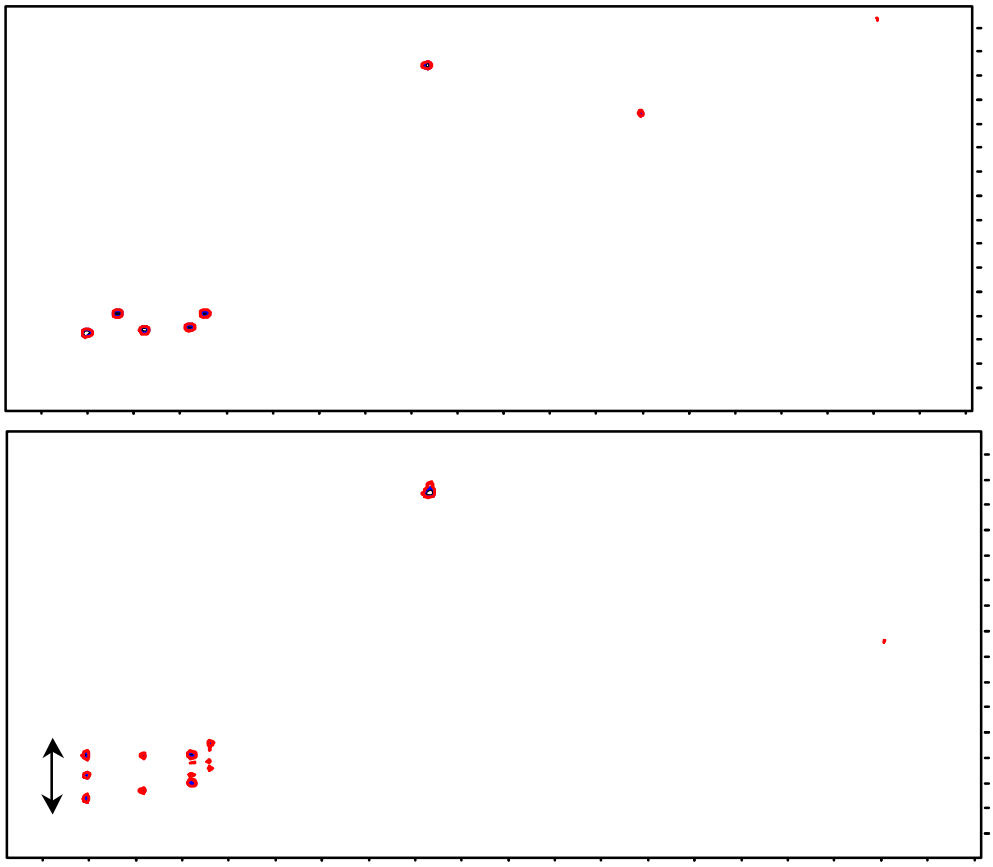
22.4 Inverse Experiments

22.4.1 Incorrect proton pulses

A common artifact of the inverse experiments HMQC and HMBC is caused by an incorrect proton pulse. The 180° proton pulse in those experiments is used to refocus the chemical shift evolution of protons. If the proton pulses are set incorrectly, the chemical shift of protons during the t1 evolution period is not refocussed. As a result, additional peaks will show up along the F1-dimension.

Those artifacts can easily assigned, as their distance from the correct correlation signal increased with the distance to the centre of the spectrum, which is O1P.

Figure 66: HMQC experiment with pamoic acid. Top spectrum is the reference, the spectrum on the bottom shows artifacts along the F1-dimension due to incorrect proton pulses.

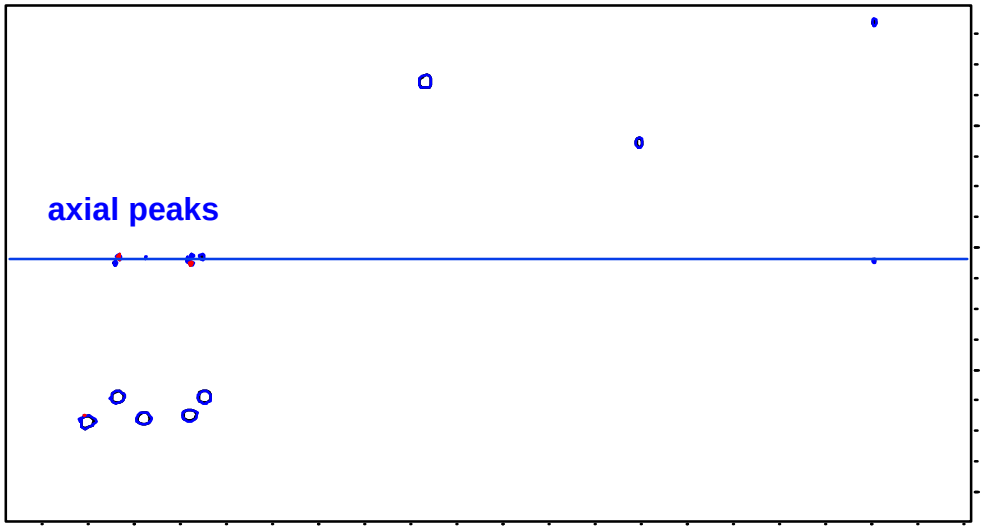


22.4.2 Rapid scanning artifacts

The HSQC experiment can show axial peaks as rapid scanning artifacts. The axial peaks can be found in the centre of the F1-dimension.

Increase the repetition delay to avoid these artifacts.

Figure 67: HMQC experiment with pamoic acid. Axial peaks are due to rapid scanning.



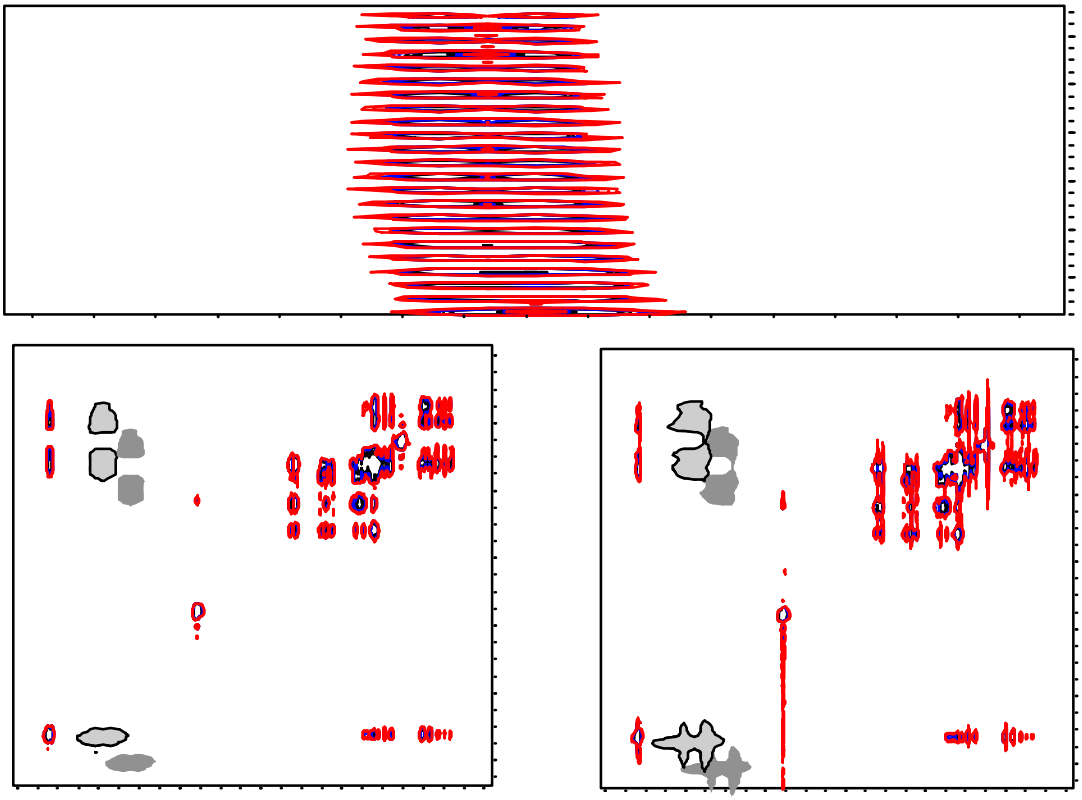
22.5 The TOCSY experiment

22.5.1 Sample heating due to the spin lock sequence

For the spin lock sequence of the TOCSY experiment rather short proton pulses are applied for a duration of 50-100ms. This can cause heating of the sample, especially if water or salty solutions are used. The artefact appears as baseline distortion of the peaks along the F1-dimension.

The number of dummy scans has to be sufficiently high to avoid artifacts caused by heating. In addition, the gas flow for temperature regulation can be increased.

Figure 68: Top: A zoom of a TOCSY experiment, after Fourier transformation along the acquisition dimension. For this experiment the number of dummy scans was insufficiently low, therefore a shift of the NMR signal is observed. Bottom: the right spectrum shown artifacts due to sample heating, the left spectrum was recorded with a sufficient number of dummy scans.



22.5.2 Solvent suppression and trim pulses

Commonly the TOCSY experiment contains two trim pulses: one located after the t_1 evolution delay, and one directly following the spin lock. Both trim pulses defocus magnetisation which has dispersive phase, and therefore the trim pulses improve the phase of the signals in the TOCSY experiment.

One has to take into account that trim pulses act as B_1 gradient pulses. This means, that the second trim pulse might partially refocus magnetisation which has been defocused by the first trim pulse. This effect can be observed in TOCSY experiment with aqueous solutions, were a presaturation is used for water suppression.

The artefact, which is caused by the partial refocusing described above, results in a poor solvent suppression in the TOCSY spectrum. In the FID it appears as an echo signal, which is delayed by the length of the spin lock.

By removing the first spin lock pulse, this artefact can be avoided.

Figure 69: First serial file of a TOCSY experiment. Top: two trim pulses. Bottom: one trim pulse

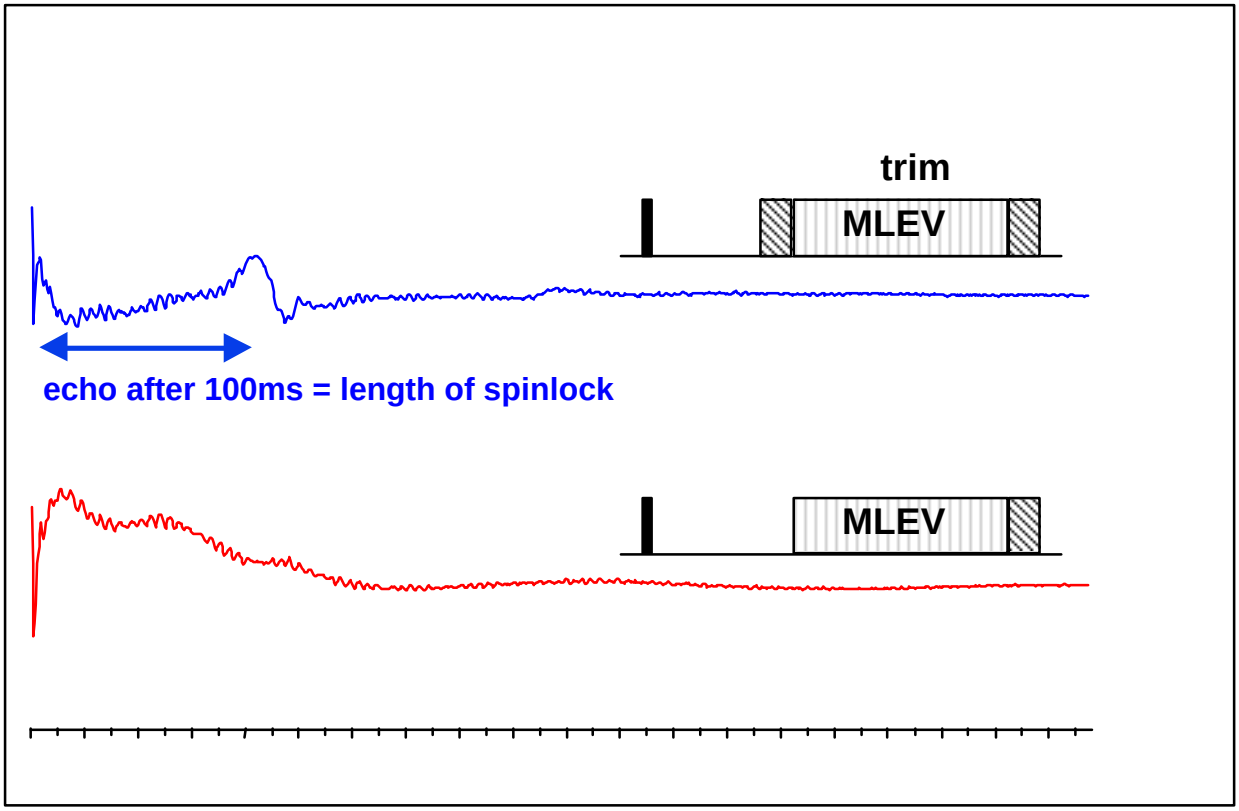
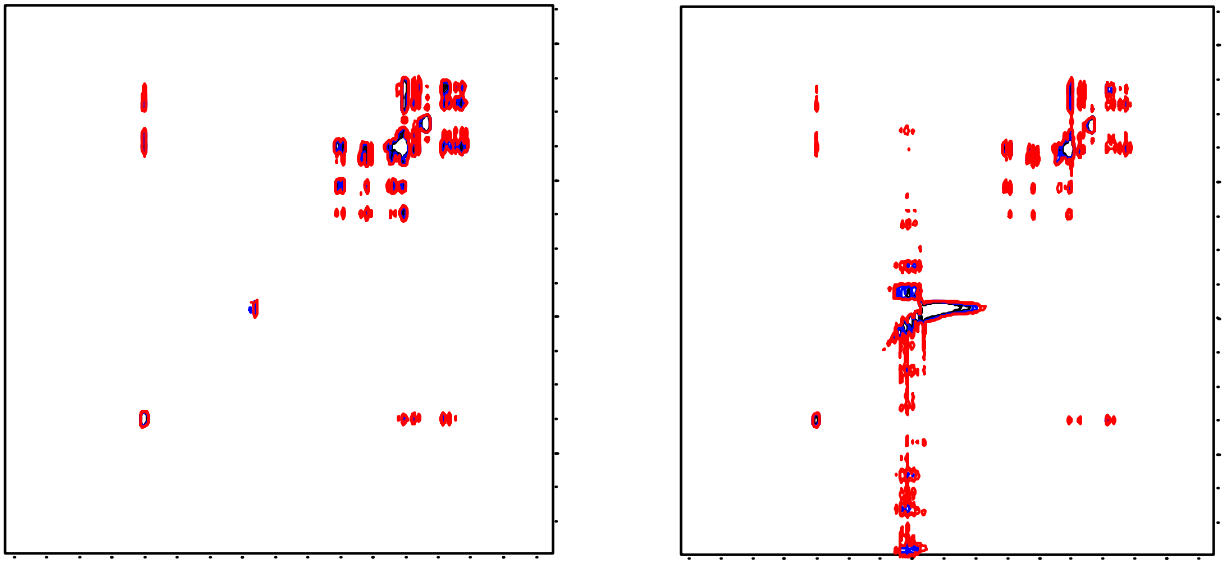


Figure 70: Two TOCSY experiments of 2mM sucrose in 90% H_2O, 10% D_2O. The spectrum shown on the left side was recorded using one trim pulse, the spectrum shown on the right was recorded using two trim pulses and has a poor solvent suppression.



23 Appendix B: Theoretical Background of NMR

23.1 Introduction

In the next few paragraphs, an attempt will be made to introduce the spin operator as a handy tool for understanding more or less involved NMR experiments. However, at the same time, we will try to limit the mathematical and purely academic sides of this formalism to an absolute minimum. In other words: you should not need a degree in mathematical science to be able to use and understand the spin operator formalism as a tool for a better understanding of the experiments covered during this course.

Also being strictly correct, this introduction will try to avoid as many tricky or complicated issues as possible. The goal will be to enable the use of the spin operator formalism as a tool and not to give an introduction to quantum mechanics.

In order to make the first contact with the subject a bit smoother, we will introduce the first concepts in analogy to the Bloch equations. The Bloch equations are very intuitive and convenient to explain relatively simple 1D experiments. But coupled 2-spin systems are already a challenge in this model, while a 3-spin system becomes impossible to describe. The spin operator formalism for a one-spin system is very similar to the Bloch equations and we will use this similarity to ease the first contact. However it should be kept in mind, that while the Bloch formalism is concerned with macroscopic magnetization only, the spin operator formalism describes the full state of the spin system, including non-observable terms. Those non-observable terms however, ignored in the Bloch equations, are the basis of most modern experiments!

23.2 Classical Description of NMR

Among the various atomic nuclei, about a hundred isotopes possess an intrinsic angular momentum, called spin and written $\hbar I$. They also possess a magnetic moment μ which is proportional to their angular momentum:

$$m = g\hbar I$$

where γ is the gyromagnetic ratio.

The Larmor theorem states that the motion of a magnetic moment $\vec{M}$ (where $\vec{M}$ represents the bulk magnetic moment of a collection of identical nuclei) in

a magnetic field B_0 is a precession around that field. The precession frequency is given by:

$$w_0 = -gB_0 \quad \text{Larmor frequency}$$

By convention, the external static field (B_0) is assumed to be along the z-axis and the transmitter/receiver coil along either the x- or y-axis. After the sample has reached its thermal equilibrium (in this context: the equilibrium magnetic polarization!), the system shows a magnetization vector $\vec{M}$ along the z-axis. In this state, no NMR signal is observed, as we have no transverse rotating magnetization.

By application of an additional rotating magnetic field B_1 in the x-y-plane, the orientation of $\vec{M}$ can be tilted into the x-y plane as the precession of $\vec{M}$ is always around the total magnetic field, e.g. the vector sum of B_0 and B_1. A rotating magnetic field is obtained by using RF-pulses. To describe the motion of $\vec{M}$ in the presence of the rotating B_1, it is convenient to use a rotating coordinate system instead of a static one. By convention, B_1 is assumed to be along the x-axis of a coordinate system rotating around the z-axis. The rotating coordinate system is chosen to rotate at the same frequency than B_1, thus making both B_0 and B_1 time independent in this reference system. The Bloch equations in this coordinate system are then:

$$\frac{d}{dt} M_x^r = M_y^r \{gB_0 + w\}$$

$$\frac{d}{dt} M_y^r = M_x^r \{gB_0 + w\} + gB_1 M_z$$

$$\frac{d}{dt} M_z = -M_y^r gB_1$$

ω is the rotational frequency of the coordinate system. The relaxation during the rf-pulse is neglected, as the pulse is assumed to be very short compared with the relaxation time. By assuming an effective magnetic field:

$$B_{eff} = B_0 + \frac{w}{g}$$

we recognize the Bloch equation from the static coordinate system. The magnetization precessess in the rotating frame around B_{eff} instead of B_0. By choosing ω to be:

$$w = -gB_0$$

B_{eff} vanishes and the Bloch equation simplifies to:

$$\frac{d}{dt} M_x^r = 0$$

$$\frac{d}{dt} M_y^r = gB_1 M_z$$

$$\frac{d}{dt} M_z = -M_y^r gB_1$$

Assuming the magnetization at time 0 to be along the z-axis with amplitude M_0, we find the following solution to the above equation system:

$$M_y^r(t) = M_0 \sin(gB_1 t)$$
$$M_z(t) = M_0 \cos(gB_1 t)$$

This means, that the magnetization vector is precessing around $B_{eff} = B_1$, e.g. the magnetization is rotating around the B_1 axis which is aligned with the x-axis of the reference system. If we choose the time t of suitable duration, we obtain:

$$b = gB_1 t = \frac{p}{2}$$

which is defined as the 90 degree pulse. As we can see, the 90° creates a maximum of y-magnetization which in turn yields a maximal signal intensity.

This results will now be presented in the quantum mechanical notation.

23.3 Spin Operators of a One-Spin System

In the spin operator formalism, the state of a spin is represented by a linear combination of four operators: I_x, I_y, I_z and ½ E. The first three can be understood as M_x, M_y and M_z respectively, also this is not strictly correct, as M_x refers to a macroscopic magnetization while I_x refers to a single spin. For all practical purposes, this detail can be neglected. The fourth operator, ½ E or unity operator, is added for reasons of mathematical consistency and is usually omitted in the notation. We will also follow this convention and omit ½ E.

The operators form a basis in the so called Liouville space, which is the mathematical frame work, in which the spin system is described. But we don't need to worry about this for the moment.

23.4 The Thermal Equilibrium State

All NMR experiments start from the thermal equilibrium. In thermal equilibrium, the classical description gives rise to a magnetic moment parallel to the static field. This is due to the fact, that the energy level for spins in a parallel orientation with the external field is slightly lower than the one for the antiparallel spins. According to Boltzman, the lower energy level will have a higher population than the high energy level, the difference being proportional to the energy difference. The energy difference between these two "Zeeman levels" being very small, the resulting population difference is in the order of $6.5*10^{-3}$%! Following the convention of the static field being aligned with the z-axis of the reference frame, the equilibrium magnetization is also called M_z.

In the spin operator formalism, this result has to be derived from statistical quantum mechanics considerations using ensemble averages and population probabilities. For us, it will be good enough to know the following result:

$$s_{eq} = I_z$$

σ_{eq} is the equilibrium density matrix. The density matrix represents the state of the spin system under investigation and is represented as a linear combination of the basis spin operators. For a one-spin system in thermal equilibrium, the coefficients of all but the I_z basis operator vanish. To understand, what happens during an NMR experiment, we will have to evaluate the changes in the density matrix during the experiment, starting from the equilibrium matrix. These changes are also referred to as evolution of the system.

There are two basic type of evolutions: under the effect of an external perturbation, e.g. a RF-pulse or the unperturbed evolution which will eventually bring the system back to the thermal equilibrium.

23.5 Effect of RF-Pulses

Let us first consider the evolution under an RF-pulse. In modern spectrometers, pulses are only applied in the x-y- or transverse plane. Pulses in-between the x- and y-axis are calculated by a combination of a rotation around the z-axis followed by an x- or y-pulse.

In the classical description, we moved to a rotating coordinate system to describe the effect of the rf-pulse. In the Spin Operator formalism, a similar approach is taken, although with a slightly different vocabulary. The “rotating coordinate system” is called rotating frame or interaction frame.

For the same reason then in the classical approach, the rotational axis is chosen along the z-axis, parallel to the static field B_0 and the B_1 field is assumed along the x-axis. The interaction frame rotates by definition with the frequency of the rf-pulse (or the reference frequency of the detector, which is identical to the former) and is called the carrier frequency. As a consequence, all Larmor frequencies are changed into chemical shift frequencies, defined by:

$$d = w_0 - w$$

The pulse is assumed to be of very short duration, such that chemical shift evolution and relaxation during the pulse can be ignored. Then the effect of an rf-pulse is that of a rotation along the pulse axes according to the following calculus rules:

$$I_z \xrightarrow{b_x} I_z \cos b - I_y \sin b$$
$$I_z \xrightarrow{b_y} I_z \cos b + I_x \sin b$$
$$I_x \xrightarrow{b_x} I_x$$
$$I_y \xrightarrow{b_y} I_y$$
$$I_x \xrightarrow{b_y} I_x \cos b - I_z \sin b$$
$$I_y \xrightarrow{b_x} I_y \cos b + I_z \sin b$$

If the flip angle β = 90° then:

$$I_z \xrightarrow{90_{y,x}} \pm I_{x,y}$$
$$I_{x,y} \xrightarrow{90_{y,x}} \mp I_z$$

We find the expected result, that a 90° pulse will generate transverse magnetization. The rest of this chapter will be concerned with following the fate of this transverse magnetization in time.

We introduced tacitly the arrow notation, where we find on the left side the system before and on the right side after the specific evolution under the operator noted above the arrow. This notation is simple, very convenient and not only limited to the description of rf-pulses. We will discuss this notation in more detail in the next section.

23.6 The Hamiltonian: Evolution of Spin Systems in Time

The arrow notation, which was introduced like a *deus ex machina* in the previous section, needs some more explanation. First, let us introduce a new type of operator, the Hamiltonian H. Each quantum mechanical system has its associated H which describes the possible changes of energy of the system. Once the H is known, the evolution of the density matrix of the corresponding system can be described by:

$$s(t) = \exp(-i \cdot \mathrm{H} \cdot t) s(0) \exp(i \cdot \mathrm{H} \cdot t)$$

under the condition that H by itself is time independent. The above equation in the arrow notation will be:

$$s(0) \xrightarrow{\mathrm{H}\ t} s(t)$$

In other words, the arrow notation is a compact an elegant way of describing the different steps of a time evolution under different Hamiltonians. The Hamiltonian corresponding to an rf-pulse, neglecting relaxation and chemical shift, is given by:

$$\mathrm{H} = g \cdot B_1 \cdot I_x$$

which describes a precession around I_x with frequency γB_1. The corresponding flip angle β equals $\beta = \gamma B_1 t$, where t is the duration of the pulse:

$$H \quad t = g \cdot B_1 \cdot I_x \cdot t = b \cdot I_x$$

This result illustrates, that for a given flip angle β, one can either use a high B_1-field or a long pulse duration t. Furthermore, the gyromagnetic ratio γ also strongly influences the behavior of the flip angle. This explains the need for specific rf power for different nuclei.

23.6.1 Effect of Chemical Shift Evolution

So far, we discussed the Hamiltonian corresponding to an system under perturbation by an rf-pulse and neglecting chemical shift and relaxation at the same time. In this simple introduction, relaxation will always be neglected. The chemical shift Hamiltonian of the unperturbed system will have to describe a precession around the static field. We have to remember, that for convenience, all operations are done in the interaction frame, e.g. that all Larmor frequencies are replaced by the chemical shift or precisely by the difference between the Larmor- and the carrier frequency.

Under this condition, the chemical shift Hamiltonian is given by:

$$H = d \cdot I_z$$

where δ is: $d = w_0 - w$, where ω_0 is the Larmor frequency of the spin and ω the carrier frequency of the interaction frame. In case that the Larmor frequency is different from the carrier frequency, this is a rotation around I_z in the rotating frame. If there is no relaxation shifting the system back to thermal equilibrium, this is the expected result.

The calculus rules for the chemical shift evolution are the following:

$$I_z \xrightarrow{d \cdot I_z \cdot t} I_z$$
$$I_x \xrightarrow{d \cdot I_z \cdot t} I_x \cos(d\ t) + I_y \sin(d\ t)$$
$$I_y \xrightarrow{d \cdot I_z \cdot t} I_y \cos(d\ t) - I_x \sin(d\ t)$$

The time t is the period, during which the Hamiltonian is valid. The Hamiltonian of a spin system can change with time, for example if the experimental setup prescribes first a rf-pulse and then a period of unperturbed evolution. For the calculus rules given to be valid, it is mandatory, that each Hamiltonian is time independent during the time t.

This means, that chemical shift can evolve only in the state of magnetization within the x/y plane a.k.a. “transversal magnetization”.

Thus, the whole experiment is divided into time intervals, during which the Hamiltonian can be made time independent by choice of a suitable interaction frame. Typical experiments are divided in pulse intervals and free evolution times.

During the pulses, the chemical shift and scalar coupling interaction is ignored. Only the applied B_1 field is considered. This approach is justified for pulses with t_{Pulse}<<T1,T2.

The question now is how to interpret this quantum mechanical result in terms of macroscopic measurements. To answer this question, we will need to discuss the difference between operators and physical observables. This will be the subject of the next paragraph.

23.7 Observable Signals and Observable Operators

Not all operators correspond to physical forces or fields. In fact, only a minority gives rise to detectable energy changes of any kind. In our particular case, only I_x, I_y and I_z are physical observables, e.g. they correspond to physical phenomena, which can be measured. In a one spin system, obviously only ½ E is not a physical observable (we neglected this operator already in the beginning). But as we will see in paragraph 23.8.1, a two spin system exhibits 16 operators but only 6 of them are physically observable.

So what are the “unobservable” operators good for?

- First, they describe quantum mechanical interactions in the system and
- second, they can evolve into observable magnetization!

A typical example for this is the scalar coupling, which is described in paragraph 23.8.1.

How is the FID obtained from these physical observables? The trick is to introduce another operator with the same qualities as the physical detector.

In the spectrometer, quadrature detection is used, that is we observe the magnetic flux along the x- and along the y-axis in the rotating frame and combine the results into one complex valued number. The corresponding operators are:

$$I_+ = I_x + i \cdot I_y$$
$$I_- = I_x - i \cdot I_y$$

In principle, we have the choice of selecting either I_+ or I_-, depending how we combine the physical measurements. By convention, I_+ is used as the detection operator. It should be noted, that I_+ as the detector selects the I component of the signal. To calculate the physical value of an operator at a given time, the trace of this operator is multiplied by the relevant density operator:

$$\langle I_+ \rangle (t) = \mathrm{Tr}\{I_+ \cdot s\,(t)\}$$

It is convenient to express σ in terms of the operators I_+ and I_- to evaluate this expression. Let’s continue with the example of the one spin system: during detection (t_2), we get the following expression for our density operator:

$$s\,(t_2) = I_x \cos(d \cdot t_2) + I_y \sin(d \cdot t_2)$$

After rewriting the equation for I_+ and I_-:

$$I_x = \frac{1}{2}(I_+ + I_-)$$

$$I_y = -\frac{i}{2}(I_+ - I_-)$$

we can substitute I_x and I_y in:

$$\begin{aligned} s(t_2) &= I_x \cos(d \cdot t_2) + I_y \sin(d \cdot t_2) \\ &= \frac{1}{2}(I_+ + I_-) \cdot \cos(d \cdot t_2) + (-\frac{i}{2})(I_+ - I_-) \cdot \sin(d \cdot t_2) \\ &= \frac{1}{2} I_+ [\cos(d \cdot t_2) - i \cdot \sin(d \cdot t_2)] + \frac{1}{2} I_- [\cos(d \cdot t_2) + i \cdot \sin(d \cdot t_2)] \\ &= \frac{1}{2}\left(I_+ \cdot e^{-i \cdot d \cdot t_2} + I_- \cdot e^{i \cdot d \cdot t_2}\right) \end{aligned}$$

When calculating the expectation value of I_+ (the observable signal, we find:

$$\begin{aligned} \langle I_+ \rangle(t_2) &= \mathrm{Tr}\{I_+ \cdot s(t_2)\} \\ &= \mathrm{Tr}\{I_+ \cdot \frac{1}{2}\left(I_+ \cdot e^{-i \cdot d \cdot t_2} + I_- \cdot e^{i \cdot d \cdot t_2}\right)\} \\ &= \frac{1}{2} \cdot e^{-i \cdot d \cdot t_2} \cdot \mathrm{Tr}\{\underbrace{I_+ I_+}_{=0}\} + \frac{1}{2} \cdot e^{i \cdot d \cdot t_2} \cdot \mathrm{Tr}\{\underbrace{I_+ I_-}_{=I_0}\} \\ &= \frac{1}{2} \cdot I_0 \cdot e^{i \cdot d \cdot t_2} \end{aligned}$$

The signal function is an oscillation with the frequency δ and the amplitude ½ I_0. The amplitude ½ I_0 is in fact an elegant way to hide a bunch of quantum mechanical constants and it is ignored most of the time. Normally one is interested in relative signal intensities rather than in absolute values.

One might object, that δ is not the Larmor frequency, which one might have expected, but only the chemical shift relative to the rotation frequency (carrier frequency) of the interaction frame. Remember, that also the detection operator is defined in the rotating frame, e.g. is also rotating with the carrier frequency.

The technical realization of this “rotating detector” is achieved by mixing the signal from the probe - which is in the MHz range - with the carrier frequency, which is also in the MHz range. The mixing process yields the difference frequency between the two oscillations and is of the order of few 10 kHz. The mixing process can be understood as comparing the signal at any time with a rotating reference vector, which is exactly what we have done in the interaction frame with a fixed detector on the x- or y- axis.

For all practical purposes, ½ I_0 is assumed to be one. and the signal function is assumed to:

$$\begin{aligned} F(t_2) &= e^{i \cdot d \cdot t_2} \\ &= e^{i \cdot 2 \cdot p \cdot n' \cdot t_2} \end{aligned}$$

The radial frequency δ in radians was replaced by the frequency ν' in Hz. This is the unit in which the spectra are expressed finally.

The time domain function $F(t_2)$, needs to be Fourier transformed to obtain the spectral function $S(\nu)$.

$$F(t_2) = e^{i \cdot 2 \cdot p \cdot n' \cdot t_2} \xrightarrow{FT} S(n) = d_{Dirac}(n - \nu')$$

The function $S(\nu)$ is zero except for the point $\nu=\nu'$, where it is infinite. This is a so called stick spectrum, as the intensities are meaningless and only the frequency information is relevant. The signal function $e^{i \cdot 2 \cdot p \cdot n \cdot t_2}$ will come up frequently during NMR calculations, so that it is worthwhile to remember its Fourier transformation. Note that, while the time domain function is complex valued, the frequency domain function is strictly real.

Things get more complex once relaxation is taken into account. To obtain correct line shape information, relaxation becomes vitally important.

At this point, we have successfully evaluated the outcome of a simple NMR experiment consisting of a 90º excitation pulse followed by the detection period and the evaluation of the detection operator I_+. In the next paragraph, we are going to extend our example from one to two spins and calculate the outcome of the same experiment.

23.8 Observing Two and More Spin Systems

A one-spin system does indeed not show much complexity. So let us then proceed to a two-spin system. Traditionally there are two notations widely used to distinguish different spins: I_1 and I_2 as indices or I and S with different "names". For protons, we use the indices notation and for heteronuclei we use S or S_1.

As a first two-spin system, let us consider at a homonuclear system with two ^{1}H nuclei. The number of operators in the basis of a spin system is given by 4^N, where N is the number of spins in the system. Fortunately, it is very simple to construct such a basis. The basis for two single spins (compare section 23.3) are multiplied to yield the needed 16 operators:

$$\left\{I_{1x}, I_{1y}, I_{1z}, \frac{1}{2}E_1\right\} \otimes \left\{I_{2x}, I_{2y}, I_{2z}, \frac{1}{2}E_2\right\} \Rightarrow \left\{\begin{array}{l} \frac{1}{2}E_{1,2}, \quad I_{1z}, \quad I_{2z}, \quad 2I_{1z}I_{2z}, \\ I_{1x}, \quad I_{1y}, \quad 2I_{1x}I_{2z}, \quad 2I_{1y}I_{2z}, \\ I_{2x}, \quad I_{2y}, \quad 2I_{1z}I_{2x}, \quad 2I_{1z}I_{2y}, \\ 2I_{1x}I_{2x}, 2I_{1y}I_{2y}, 2I_{1x}I_{2y}, 2I_{1y}I_{2x} \end{array}\right\}$$

Note, that ½ E_1 and ½ E_2 were consistently omitted from the notation. In section 23.7, we discussed observable vs. non-observable operators. In general, only single spin operators along x, y or z are observable operators and only those along x or y will give rise to a NMR signal. In this particular case the operators that relevant for NMR are I_{1x}, I_{2x}, I_{1y} and I_{2y}.

In a two-spin system, the thermal equilibrium density operator now includes also the second spin and is given by:

$$s_{eq} = I_{1z} + I_{2z}$$

When applying a rf-pulse, e.g. a 90º pulse, the same rules still apply. However the corresponding Hamiltonian has changed to include also the operator from spin 2:

$$\mathrm{H} = g \cdot B_1 \cdot (I_{1x} + I_{2x})$$

The above Hamiltonian can be split into two Hamiltonians

$$\mathrm{H} = g \cdot B_1 \cdot (I_{1x}) \text{ and } \mathrm{H} = g \cdot B_1 \cdot (I_{2x})$$

being applied one after the other. The first acts only on operators of spin 1 and is ignored by all spin 2 operators. The second applies accordingly only to spin 2 operators.

Accordingly, a selective rf-pulse could be realized by applying e.g. a pulse with the respective Hamiltonian to achieve a selective pulse on a certain spin. This issue will be discussed again, when the theory of the inverse experiments is discussed. In the arrow notation, if not explicitly mentioned otherwise, the rf-pulse always applies to all spins in the system.

In our example, we apply a 90º pulse to the equilibrium density matrix:

$$s_{eq} \xrightarrow{90_y} s(0) = I_{1x} + I_{2x}$$

The next step will be to evaluate the free evolution during the acquisition time. But before we can do so, we need to have a look at the corresponding Hamiltonian and there we will find a new phenomenon: the scalar coupling!

23.8.1 Effect of Scalar Coupling

Apart from the chemical shift, there is a second very import interaction between spins, the scalar coupling. The scalar depends on the mediation of electrons, which are confined in orbitals around both nuclei. The scalar coupling is expressed in Hz and noted as J. The operator expression for the scalar coupling is:

$$2p\ J_{12}\ I_{1z} I_{2z}$$

The above Hamiltonian expresses the scalar coupling between spin 1 and spin 2 with a coupling constant J_{12}. The evolution Hamiltonian for this spin system is then:

$$\mathrm{H} = d_1\ I_{1z} + d_2\ I_{2z} + 2p\ J_{12}\ I_{1z} I_{2z}$$

To calculate the effect of this Hamiltonian, it is divided into 3 parts:

$$d_1\ I_{1z}$$
$$d_2\ I_{2z}$$
$$2p\ J_{12}\ I_{1z} I_{2z}$$

which are applied in sequence, where this sequence is arbitrary. After a 90° pulse has been applied to the two spins, we first calculate the two chemical shift terms:

$$
\begin{aligned}
s_{eq} = I_{1x} + I_{2x} &\xrightarrow{d_1 \cdot I_{1z} \cdot t} I_{1x}\cos(d_1\, t) + I_{1y}\sin(d_1\, t) + I_{2z} \\
&\xrightarrow{d_2 \cdot I_{2z} \cdot t} I_{1x}\cos(d_1\, t) + I_{1y}\sin(d_1\, t) \\
&\qquad + I_{2x}\cos(d_2\, t) + I_{2y}\sin(d_2\, t) \Rightarrow s_1
\end{aligned}
$$

The next step will be to calculate the evolution under the scalar coupling.

23.8.2 Evolution under Weak Coupling

To apply the last part of the Hamiltonian, we need some new calculus rules. The scalar coupling term can be evaluated with a simple set of rules:

$$
\begin{aligned}
I_{1z} &\xrightarrow{2p\, J_{12}\, I_{1z} I_{2z}\, t} I_{1z} \\
I_{1x} &\xrightarrow{2p\, J_{12}\, I_{1z} I_{2z}\, t} I_{1x}\cos(pJ_{12}t) + 2\, I_{1y} I_{2z}\sin(pJ_{12}t) \\
I_{1y} &\xrightarrow{2p\, J_{12}\, I_{1z} I_{2z}\, t} I_{1y}\cos(pJ_{12}t) - 2\, I_{1x} I_{2z}\sin(pJ_{12}t) \\
2 \cdot I_{1x} I_{2z} &\xrightarrow{2p\, J_{12}\, I_{1z} I_{2z}\, t} 2\, I_{1x} I_{2z}\cos(pJ_{12}t) + I_{1y}\sin(pJ_{12}t) \\
2 \cdot I_{1y} I_{2z} &\xrightarrow{2p\, J_{12}\, I_{1z} I_{2z}\, t} 2\, I_{1y} I_{2z}\cos(pJ_{12}t) - I_{1x}\sin(pJ_{12}t) \\
2 \cdot I_{1x} I_{2y} &\xrightarrow{2p\, J_{12}\, I_{1z} I_{2z}\, t} 2\, I_{1x} I_{2y}
\end{aligned}
$$

From the above equation, we immediately recognize a very important fact: the scalar coupling can generate observable operators from non-observable ones through free evolution! This is the reason, why we can not neglect the non-observable operators until we apply the detection operator (signal acquisition)!

Befitted with the above equations, we can now evaluate the last part of the Hamiltonian:

$$
\begin{aligned}
s_1 \xrightarrow{2p\, J_{12}\, I_{1z} I_{2z}\, t} &\{I_{1x}\cos(pJ_{12}t) + 2\, I_{1y} I_{2z}\sin(pJ_{12}t)\} \cdot \cos(d_1 t) \\
&+ \{I_{1y}\cos(pJ_{12}t) - 2\, I_{1x} I_{2z}\sin(pJ_{12}t)\} \cdot \sin(d_1 t) \\
&+ \{I_{2x}\cos(pJ_{12}t) + 2\, I_{1z} I_{2y}\sin(pJ_{12}t)\} \cdot \cos(d_2 t) \\
&+ \{I_{2y}\cos(pJ_{12}t) - 2\, I_{1z} I_{2x}\sin(pJ_{12}t)\} \cdot \sin(d_2 t) \\
&= s_2
\end{aligned}
$$

after some rearrangement, this leads to:

$$
\begin{aligned}
s_2 = \; & I_{1x}\cos(p\cdot J_{12}\cdot t)\cdot\cos(d_1\cdot t) + I_{1y}\cos(p\cdot J_{12}\cdot t)\cdot\sin(d_1\cdot t) \\
& + I_{2x}\cos(p\cdot J_{12}\cdot t)\cdot\cos(d_2\cdot t) + I_{2y}\cos(p\cdot J_{12}\cdot t)\cdot\sin(d_2\cdot t) \\
& + 2\cdot I_{1y}I_{2z}\sin(p\cdot J_{12}\cdot t)\cdot\cos(d_1\cdot t) - 2\cdot I_{1x}I_{2z}\sin(p\cdot J_{12}\cdot t)\cdot\sin(d_1\cdot t) \\
& + 2\cdot I_{1z}I_{2y}\sin(p\cdot J_{12}\cdot t)\cdot\cos(d_2\cdot t) - 2\cdot I_{1z}I_{2x}\sin(p\cdot J_{12}\cdot t)\cdot\sin(d_2\cdot t) \\
= \; & (I_{1x}\cos(d_1\cdot t) + I_{1y}\sin(d_1\cdot t))\cdot\cos(p\cdot J_{12}\cdot t) \\
& + (I_{2x}\cos(d_2\cdot t) + I_{2y}\sin(d_2\cdot t))\cdot\cos(p\cdot J_{12}\cdot t) \\
& + (2\cdot I_{1y}I_{2z}\cos(d_1\cdot t) - 2\cdot I_{1x}I_{2z}\sin(d_1\cdot t))\cdot\sin(p\cdot J_{12}\cdot t) \\
& + (2\cdot I_{1z}I_{2y}\cos(d_2\cdot t) - 2\cdot I_{1z}I_{2x}\sin(d_2\cdot t))\cdot\sin(p\cdot J_{12}\cdot t)
\end{aligned}
$$

Again, the final step is the calculation of the expectation value of the detection operator. Of course, the detection operator also needs to include spin 2. We introduce a detection operator F_+:

$$F_+ = I_{1+} + I_{2+}$$

or more general for a N spin system:

$$F_+ = \sum_i^N I_{i+}$$

In the previous section, we were rewriting the density operator in terms of I_+ and I_- to calculate the detection results. While this is very elegant, it is also very tedious. We know from those equations that F_+ is going to select F_- in the density operator and we know also, that the coefficient of F_- is obtained by using the coefficients of $F_x=\Sigma I_{ix}$ minus $F_y=\Sigma I_{iy}$ times the complex constant. Furthermore, we elegantly disposed of the $½I_0$ factors in a constant, which here we can replaced by F_0 or simply be omitted altogether. While neither being elegant nor exactly correct, we can obtain very useful results much faster then going through all the details.

23.8.3 The Signal Function of a Coupled Spectrum

By omitting F_0, we obtain the following signal function for our coupled two-spin system:

$$
\begin{aligned}
\mathrm{Tr}\{F_+\cdot s_2\} = \; & (\cos(d_1\cdot t) + i\cdot\sin(d_1\cdot t))\cdot\cos(p\cdot J_{12}\cdot t) \\
& + (\cos(d_2\cdot t) + i\cdot\sin(d_2\cdot t))\cdot\cos(p\cdot J_{12}\cdot t) \\
= \; & \cos(p\cdot J_{12}\cdot t)\cdot e^{i\cdot d_1\cdot t} + \cos(p\cdot J_{12}\cdot t)\cdot e^{i\cdot d_2\cdot t} \\
= \; & \frac{1}{2}(e^{i\cdot p\cdot J_{12}\cdot t} + e^{-i\cdot p\cdot J_{12}\cdot t})\cdot e^{i\cdot d_1\cdot t} + \frac{1}{2}(e^{i\cdot p\cdot J_{12}\cdot t} + e^{-i\cdot p\cdot J_{12}\cdot t})\cdot e^{i\cdot d_2\cdot t} \\
= \; & \frac{1}{2}(e^{2\cdot p\cdot i\cdot(n_1+\frac{J_{12}}{2})\cdot t} + e^{2\cdot p\cdot i\cdot(n_1-\frac{J_{12}}{2})\cdot t} + e^{2\cdot p\cdot i\cdot(n_2+\frac{J_{12}}{2})\cdot t} + e^{2\cdot p\cdot i\cdot(n_2-\frac{J_{12}}{2})\cdot t})
\end{aligned}
$$

To get the results above, we made extensive use of the Euler relation. The form of the signal function should look familiar: it describes a frequency spectrum with four signals at the frequencies $\nu_1+J_{12}/2$, $\nu_1-J_{12}/2$, $\nu_2+J_{12}/2$ and

ν_2-$J_{12}/2$. We recognize a spectrum with two doublet signals, each doublet having two lines of equal intensity that are separated by J_{12} Hz.

At this point, we are able two handle a two-spin system in a 1D experiment. Most of the calculations using the spin operator formalism will never include a spin system larger then two, as the number of operators quickly become too cumbersome to handle. Nevertheless, let us take a look at a simple 3-spin system in order to introduce some important simplification schemes for handling such large system.

23.9 Simplification Schemes on A Three-Spin System

Our spin system shall include three spins of the same type, all three being coupled with each other and two coupling constants should be identical:

$$H = d_1 I_{1z} + d_2 I_{2z} + d_3 I_{3z} + 2 \cdot p \cdot J_{12} \cdot I_{1z} I_{2z} + 2 \cdot p \cdot J_{13} \cdot I_{1z} I_{3z} + 2 \cdot p \cdot J_{23} \cdot I_{2z} I_{3z}$$

with $J_{12}=J_{13}=J$.

The full set of spin operators includes $4^3=64$ elements and we certainly don't want to mess with that many operators! The first simplification consists in only considering one spin, e.g. instead of using the full equilibrium density matrix we use only a reduced form. In this way, we will obtain the signal originating from that particular spin only. Most of the time, this is absolutely sufficient. In our example, we will look at spin 1 only.

$$s_{eq} = I_{1z} \xrightarrow{90_y} s_0 = I_{1x}$$

Again, the Hamiltonian is split it into chemical shift terms and scalar coupling terms which are the applied subsequently. But this time, we will only keep the terms including $I_{1\{x,y,z\}}$, knowing that the other terms will not have any effect on σ_0:

$$\begin{aligned} & d_1 I_{1z} \\ & 2 \cdot p \cdot J_{12} \cdot I_{1z} I_{2z} \\ & 2 \cdot p \cdot J_{13} \cdot I_{1z} I_{3z} \end{aligned}$$

Applying this reduced Hamiltonian to σ_0 yields:

$$\begin{aligned} s_0 \xrightarrow{H \cdot t} \; & I_{1x} \cos(p \cdot J_{13} \cdot t) \cdot \cos(p \cdot J_{12} \cdot t) \cdot \cos(d_1 \cdot t) \\ + \; & I_{1y} \cos(p \cdot J_{13} \cdot t) \cdot \cos(p \cdot J_{12} \cdot t) \cdot \sin(d_1 \cdot t) \\ + \; & 2 I_{1y} I_{3z} \sin(p \cdot J_{13} \cdot t) \cdot \cos(p \cdot J_{12} \cdot t) \cdot \cos(d_1 \cdot t) \\ - \; & 2 I_{1x} I_{3z} \sin(p \cdot J_{13} \cdot t) \cdot \cos(p \cdot J_{12} \cdot t) \cdot \sin(d_1 \cdot t) \\ + \; & 2 I_{1y} I_{2z} \cos(p \cdot J_{13} \cdot t) \cdot \sin(p \cdot J_{12} \cdot t) \cdot \cos(d_1 \cdot t) \\ - \; & 2 I_{1x} I_{2z} \cos(p \cdot J_{13} \cdot t) \cdot \sin(p \cdot J_{12} \cdot t) \cdot \sin(d_1 \cdot t) \\ - \; & 4 I_{1x} I_{2z} I_{3z} \sin(p \cdot J_{13} \cdot t) \cdot \sin(p \cdot J_{12} \cdot t) \cdot \cos(d_1 \cdot t) \\ - \; & 4 I_{1y} I_{2z} I_{3z} \sin(p \cdot J_{13} \cdot t) \cdot \sin(p \cdot J_{12} \cdot t) \cdot \sin(d_1 \cdot t) \\ = \; & s_1 \end{aligned}$$

The corresponding signal function is:

$$\begin{aligned}\mathrm{Tr}\{I_{1+}\cdot s_1\} &= \cos(p\cdot J_{13}\cdot t)\cdot\cos(p\cdot J_{12}\cdot t)\cdot\cos(d_1\cdot t)\\ &\quad + i\cdot\cos(p\cdot J_{13}\cdot t)\cdot\cos(p\cdot J_{12}\cdot t)\cdot\sin(d_1\cdot t)\\ &= \frac{1}{4}(e^{2\cdot p\cdot i\cdot(n_1+\frac{J_{13}}{2}+\frac{J_{12}}{2})\cdot t}+e^{2\cdot p\cdot i\cdot(n_1-\frac{J_{13}}{2}+\frac{J_{12}}{2})\cdot t}\\ &\quad + e^{2\cdot p\cdot i\cdot(n_1+\frac{J_{13}}{2}-\frac{J_{12}}{2})\cdot t}+e^{2\cdot p\cdot i\cdot(n_1-\frac{J_{13}}{2}-\frac{J_{12}}{2})\cdot t})\end{aligned}$$

In the case, where $J_{12}=J_{13}=J$, this simplifies to:

$$\mathrm{Tr}\{I_{1+}\cdot s_1\} = \frac{1}{4}(e^{2\cdot p\cdot i\cdot(n_1+J)\cdot t}+2\cdot e^{2\cdot p\cdot i\cdot n_1\cdot t}+e^{2\cdot p\cdot i\cdot(n_1-J)\cdot t})$$

which is the well known 1:2:1 triplet we expected! Note, that the J_{23} is totally irrelevant for the signal of the spin 1.

We have now seen 3 examples of a 1D-experiment. Let us now turn to 2D-experiments. As an example for all 2D-experiments, we will study the most fundamental 2D, the magnitude COSY experiment.

23.10 The COSY Experiment

The minimum spin system size for a COSY is a two spin system, as we need at least two coupled spins. The pulse sequence of a COSY is very simple: first, we use a 90º excitation pulse from the y-direction, followed by a free evolution time t_1 and a second 90º pulse around y just before the acquisition time t_2. As you may have noticed, the number of operator terms has a tendency to dramatically increase during free evolution periods. Therefore we will discuss more simplifications in order to keep the problem within reasonable size.

For the COSY, we use a two-spin system with the Hamiltonian:

$$\mathrm{H} = d_1\cdot I_{1z}+d_2\cdot I_{2z}+2\cdot p\cdot J_{12}\cdot I_{1z}I_{2z}$$

For the equilibrium density operator, again we use the reduced version from the previous section:

$$s_{eq}=I_{1z}\xrightarrow{90_y}s_0=I_{1x}$$

The evolution of σ_0 under the above Hamiltonian therefore yields:

$$\begin{aligned}s_0\xrightarrow{H\cdot t_1} &\{I_{1x}\cos(p\cdot J_{12}\cdot t_1)+2\cdot I_{1y}I_{2z}\sin(p\cdot J_{12}\cdot t_1)\}\cdot\cos(d_1\cdot t_1)\\ &+\{I_{1y}\cos(p\cdot J_{12}\cdot t_1)-2\cdot I_{1x}I_{2z}\sin(p\cdot J_{12}\cdot t_1)\}\cdot\sin(d_1\cdot t_1)\\ = &\ I_{1x}\cos(p\cdot J_{12}\cdot t_1)\cdot\cos(d_1\cdot t_1)\\ &+I_{1y}\cos(p\cdot J_{12}\cdot t_1)\cdot\sin(d_1\cdot t_1)\\ &+2\cdot I_{1y}I_{2z}\sin(p\cdot J_{12}\cdot t_1)\cdot\cos(d_1\cdot t_1)\\ &-2\cdot I_{1x}I_{2z}\sin(p\cdot J_{12}\cdot t_1)\cdot\sin(d_1\cdot t_1)\Rightarrow s_1\end{aligned}$$

The first evolution period is identical to what we know from the 1D-example. The 2D-experiment starts now by applying a second pulse after the first evolution period during t_1:

$$
\begin{aligned}
s_1 \xrightarrow{90_y} & -I_{1z}\cos(p\cdot J_{12}\cdot t_1)\cdot\cos(d_1\cdot t_1)\\
& + I_{1y}\cos(p\cdot J_{12}\cdot t_1)\cdot\sin(d_1\cdot t_1)\\
& + 2\cdot I_{1y}I_{2x}\sin(p\cdot J_{12}\cdot t_1)\cdot\cos(d_1\cdot t_1)\\
& + 2\cdot I_{1z}I_{2x}\sin(p\cdot J_{12}\cdot t_1)\cdot\sin(d_1\cdot t_1)\Rightarrow s_2
\end{aligned}
$$

We now could simply apply the Hamiltonian again for the evolution during t_2 and battle through 16 operators with countless coefficients just to realize, that in fact very few of the original operators contribute to the observable magnetization. It's probably more rewarding however, to consider σ_2 for a while and try to figure out, which operators will evolve into observable magnetization and which will just keep us busy.

I_{1z} is a clear cut case, as we can see from our calculus table: it will not evolve at all. The same is true for $I_{1y}I_{2x}$. The reduced density matrix relevant for the observable magnetization σ_2' is then:

$$
\begin{aligned}
s_2' = \; & I_{1y}\cos(p\cdot J_{12}\cdot t_1)\cdot\sin(d_1\cdot t_1)\\
& + 2\cdot I_{1z}I_{2x}\sin(p\cdot J_{12}\cdot t_1)\cdot\sin(d_1\cdot t_1)
\end{aligned}
$$

Applying the chemical shift part of the Hamiltonian yields:

$$
\begin{aligned}
s_2' \xrightarrow{(d_1\cdot I_{1z}+d_2\cdot I_{2z})t_2} \; & [I_{1y}\cos(d_1\cdot t_2)-I_{1x}\sin(d_1\cdot t_2)]\cos(p\cdot J_{12}\cdot t_1)\cdot\sin(d_1\cdot t_1)\\
& + 2\cdot I_{1z}[I_{2x}\cos(d_2\cdot t_2)+I_{2y}\sin(d_2\cdot t_2)]\sin(p\cdot J_{12}\cdot t_1)\cdot\sin(d_1\cdot t_1)\\
= & -I_{1x}\sin(d_1\cdot t_2)\cdot\cos(p\cdot J_{12}\cdot t_1)\cdot\sin(d_1\cdot t_1)\\
& + I_{1y}\cos(d_1\cdot t_2)\cdot\cos(p\cdot J_{12}\cdot t_1)\cdot\sin(d_1\cdot t_1)\\
& + 2\cdot I_{1z}I_{2x}\cos(d_2\cdot t_2)\cdot\sin(p\cdot J_{12}\cdot t_1)\cdot\sin(d_1\cdot t_1)\\
& + 2\cdot I_{1z}I_{2y}\sin(d_2\cdot t_2)\cdot\sin(p\cdot J_{12}\cdot t_1)\cdot\sin(d_1\cdot t_1)\Rightarrow s_3
\end{aligned}
$$

Finally, the coupling Hamiltonian is applied:

$$s_3 \xrightarrow{2p\cdot J_{12}\cdot I_{1z}I_{2z}\cdot t_2} s_4$$

$$\begin{aligned}
s_4 = &-\left(I_{1x}\cos(p\cdot J_{12}\cdot t_2)+2\cdot I_{1y}I_{2z}\sin(p\cdot J_{12}\cdot t_2)\right)\sin(d_1\cdot t_2)\cdot\cos(p\cdot J_{12}\cdot t_1)\cdot\sin(d_1\cdot t_1)\\
&+\left(I_{1y}\cos(p\cdot J_{12}\cdot t_2)-2\cdot I_{1x}I_{2z}\sin(p\cdot J_{12}\cdot t_2)\right)\cos(d_1\cdot t_2)\cdot\cos(p\cdot J_{12}\cdot t_1)\cdot\sin(d_1\cdot t_1)\\
&+\left(2\cdot I_{1z}I_{2x}\cos(p\cdot J_{12}\cdot t_2)+I_{2y}\sin(p\cdot J_{12}\cdot t_2)\right)\cos(d_2\cdot t_2)\cdot\sin(p\cdot J_{12}\cdot t_1)\cdot\sin(d_1\cdot t_1)\\
&+\left(2\cdot I_{1z}I_{2y}\cos(p\cdot J_{12}\cdot t_2)-I_{2x}\sin(p\cdot J_{12}\cdot t_2)\right)\sin(d_2\cdot t_2)\cdot\sin(p\cdot J_{12}\cdot t_1)\cdot\sin(d_1\cdot t_1)\\
=&-I_{1x}\cos(p\cdot J_{12}\cdot t_2)\cdot\sin(d_1\cdot t_2)\cdot\cos(p\cdot J_{12}\cdot t_1)\cdot\sin(d_1\cdot t_1)\\
&+I_{1y}\cos(p\cdot J_{12}\cdot t_2)\cdot\cos(d_1\cdot t_2)\cdot\cos(p\cdot J_{12}\cdot t_1)\cdot\sin(d_1\cdot t_1)\\
&-I_{2x}\sin(p\cdot J_{12}\cdot t_2)\cdot\sin(d_2\cdot t_2)\cdot\sin(p\cdot J_{12}\cdot t_1)\cdot\sin(d_1\cdot t_1)\\
&+I_{2y}\sin(p\cdot J_{12}\cdot t_2)\cdot\cos(d_2\cdot t_2)\cdot\sin(p\cdot J_{12}\cdot t_1)\cdot\sin(d_1\cdot t_1)\\
&-2\cdot I_{1x}I_{2z}\sin(p\cdot J_{12}\cdot t_2)\cdot\cos(d_1\cdot t_2)\cdot\cos(p\cdot J_{12}\cdot t_1)\cdot\sin(d_1\cdot t_1)\\
&-2\cdot I_{1y}I_{2z}\sin(p\cdot J_{12}\cdot t_2)\cdot\sin(d_1\cdot t_2)\cdot\cos(p\cdot J_{12}\cdot t_1)\cdot\sin(d_1\cdot t_1)\\
&+2\cdot I_{1z}I_{2x}\cos(p\cdot J_{12}\cdot t_2)\cdot\cos(d_2\cdot t_2)\cdot\sin(p\cdot J_{12}\cdot t_1)\cdot\sin(d_1\cdot t_1)\\
&+2\cdot I_{1z}I_{2y}\cos(p\cdot J_{12}\cdot t_2)\cdot\sin(d_2\cdot t_2)\cdot\sin(p\cdot J_{12}\cdot t_1)\cdot\sin(d_1\cdot t_1)
\end{aligned}$$

The corresponding signal function therefore is:

$$\begin{aligned}
\mathrm{Tr}\{F_+\cdot s_4\} = &-\cos(p\cdot J_{12}\cdot t_2)\cdot\sin(d_1\cdot t_2)\cdot\cos(p\cdot J_{12}\cdot t_1)\cdot\sin(d_1\cdot t_1)\\
&+i\cdot\cos(p\cdot J_{12}\cdot t_2)\cdot\cos(d_1\cdot t_2)\cdot\cos(p\cdot J_{12}\cdot t_1)\cdot\sin(d_1\cdot t_1)\\
&-\sin(p\cdot J_{12}\cdot t_2)\cdot\sin(d_2\cdot t_2)\cdot\sin(p\cdot J_{12}\cdot t_1)\cdot\sin(d_1\cdot t_1)\\
&+i\cdot\sin(p\cdot J_{12}\cdot t_2)\cdot\cos(d_2\cdot t_2)\cdot\sin(p\cdot J_{12}\cdot t_1)\cdot\sin(d_1\cdot t_1)\\
= &\ \frac{i}{2}\cdot\cos(p\cdot J_{12}\cdot t_1)\cdot\sin(d_1\cdot t_1)\cdot(e^{2p\cdot i\cdot(n_1+\frac{J_{12}}{2})\cdot t_2}+e^{2p\cdot i\cdot(n_1-\frac{J_{12}}{2})\cdot t_2})\\
&+\frac{i}{2}\cdot\sin(p\cdot J_{12}\cdot t_1)\cdot\sin(d_1\cdot t_1)\cdot(e^{2p\cdot i\cdot(n_2+\frac{J_{12}}{2})\cdot t_2}-e^{2p\cdot i\cdot(n_2-\frac{J_{12}}{2})\cdot t_2})\\
= &\ \frac{1}{4}\cdot\cos(p\cdot J_{12}\cdot t_1)\cdot(e^{2p\cdot i n_1\cdot t1}-e^{-2p\cdot i n_1\cdot t_1})\cdot(e^{2p\cdot i\cdot(n_1+\frac{J_{12}}{2})\cdot t_2}+e^{2p\cdot i\cdot(n_1-\frac{J_{12}}{2})\cdot t_2})\\
&+\frac{1}{4}\cdot\sin(p\cdot J_{12}\cdot t_1)\cdot(e^{2p\cdot i n_1\cdot t1}-e^{-2p\cdot i n_1\cdot t_1})\cdot(e^{2p\cdot i\cdot(n_2+\frac{J_{12}}{2})\cdot t_2}+e^{2p\cdot i\cdot(n_2-\frac{J_{12}}{2})\cdot t_2})\\
= &\ \frac{1}{8}\cdot(e^{2p\cdot i\cdot(n_1+\frac{J_{12}}{2})\cdot t1}+e^{2p\cdot i\cdot(n_1-\frac{J_{12}}{2})\cdot t1}-e^{2p\cdot i\cdot(-n_1+\frac{J_{12}}{2})\cdot t_1}-e^{2p\cdot i\cdot(-n_1-\frac{J_{12}}{2})\cdot t_1})\\
&\quad\cdot(e^{2p\cdot i\cdot(n_1+\frac{J_{12}}{2})\cdot t_2}+e^{2p\cdot i\cdot(n_1-\frac{J_{12}}{2})\cdot t_2})\\
&-\frac{i}{8}\cdot(e^{2p\cdot i\cdot(n_1+\frac{J_{12}}{2})\cdot t1}-e^{2p\cdot i\cdot(n_1-\frac{J_{12}}{2})\cdot t1}-e^{2p\cdot i\cdot(-n_1+\frac{J_{12}}{2})\cdot t_1}+e^{2p\cdot i\cdot(-n_1-\frac{J_{12}}{2})\cdot t_1})\\
&\quad\cdot(e^{2p\cdot i\cdot(n_2+\frac{J_{12}}{2})\cdot t_2}-e^{2p\cdot i\cdot(n_2-\frac{J_{12}}{2})\cdot t_2})
\end{aligned}$$

This signal function is not quiet what we expected: first the signals are mirrored in the t_{1t} dimension, and second the cross peak is in the imaginary part of the spectrum. What is the problem?

To be able to distinguish between positive and negative signals, we need both the sine and the cosine modulation. This is true for the t_2 domain in the above signal function, but not for the t_1 part, where we only have the sine modulation of the chemical shift. What can be done?

In case of the two pulse COSY, fortunately this is quite simple: we repeat the experiment but apply the second pulse now around the x-axis instead of the y-axis. After a lot of painstaking manipulations, we finally find for the sequence 90_y-t_1-90_x-t_2:

$$\begin{aligned} s'_4 = \; & I_{1x}\cos(p\cdot J_{12}\cdot t_2)\cdot\cos(d_1\cdot t_2)\cdot\cos(p\cdot J_{12}\cdot t_1)\cdot\cos(d_1\cdot t_1) \\ & + I_{1y}\cos(p\cdot J_{12}\cdot t_2)\cdot\sin(d_1\cdot t_2)\cdot\cos(p\cdot J_{12}\cdot t_1)\cdot\cos(d_1\cdot t_1) \\ & + I_{2x}\sin(p\cdot J_{12}\cdot t_2)\cdot\cos(d_2\cdot t_2)\cdot\sin(p\cdot J_{12}\cdot t_1)\cdot\cos(d_1\cdot t_1) \\ & + I_{2y}\sin(p\cdot J_{12}\cdot t_2)\cdot\sin(d_2\cdot t_2)\cdot\sin(p\cdot J_{12}\cdot t_1)\cdot\cos(d_1\cdot t_1) \\ & + \cdots \end{aligned}$$

compared with the sequence 90_y-t_1-90_y-t_2:

$$\begin{aligned} s_4 = & -I_{1x}\cos(p\cdot J_{12}\cdot t_2)\cdot\sin(d_1\cdot t_2)\cdot\cos(p\cdot J_{12}\cdot t_1)\cdot\sin(d_1\cdot t_1) \\ & + I_{1y}\cos(p\cdot J_{12}\cdot t_2)\cdot\cos(d_1\cdot t_2)\cdot\cos(p\cdot J_{12}\cdot t_1)\cdot\sin(d_1\cdot t_1) \\ & - I_{2x}\sin(p\cdot J_{12}\cdot t_2)\cdot\sin(d_2\cdot t_2)\cdot\sin(p\cdot J_{12}\cdot t_1)\cdot\sin(d_1\cdot t_1) \\ & + I_{2y}\sin(p\cdot J_{12}\cdot t_2)\cdot\cos(d_2\cdot t_2)\cdot\sin(p\cdot J_{12}\cdot t_1)\cdot\sin(d_1\cdot t_1) \\ & + \cdots \end{aligned}$$

If we sum up the signal functions of both experiments, we find:

$$\begin{aligned} \mathrm{Tr}\{F_+\cdot s_4\} + \mathrm{Tr}\{F_+\cdot s'_4\} = & -\cos(p\cdot J_{12}\cdot t_2)\cdot\sin(d_1\cdot t_2)\cdot\cos(p\cdot J_{12}\cdot t_1)\cdot\sin(d_1\cdot t_1) \\ & + i\cdot\cos(p\cdot J_{12}\cdot t_2)\cdot\cos(d_1\cdot t_2)\cdot\cos(p\cdot J_{12}\cdot t_1)\cdot\sin(d_1\cdot t_1) \\ & - \sin(p\cdot J_{12}\cdot t_2)\cdot\sin(d_2\cdot t_2)\cdot\sin(p\cdot J_{12}\cdot t_1)\cdot\sin(d_1\cdot t_1) \\ & + i\cdot\sin(p\cdot J_{12}\cdot t_2)\cdot\cos(d_2\cdot t_2)\cdot\sin(p\cdot J_{12}\cdot t_1)\cdot\sin(d_1\cdot t_1) \\ & + \cos(p\cdot J_{12}\cdot t_2)\cdot\cos(d_1\cdot t_2)\cdot\cos(p\cdot J_{12}\cdot t_1)\cdot\cos(d_1\cdot t_1) \\ & + i\cdot\cos(p\cdot J_{12}\cdot t_2)\cdot\sin(d_1\cdot t_2)\cdot\cos(p\cdot J_{12}\cdot t_1)\cdot\cos(d_1\cdot t_1) \\ & + \sin(p\cdot J_{12}\cdot t_2)\cdot\cos(d_2\cdot t_2)\cdot\sin(p\cdot J_{12}\cdot t_1)\cdot\cos(d_1\cdot t_1) \\ & + i\cdot\sin(p\cdot J_{12}\cdot t_2)\cdot\sin(d_2\cdot t_2)\cdot\sin(p\cdot J_{12}\cdot t_1)\cdot\cos(d_1\cdot t_1) \\ = & \frac{i}{2}\cdot\cos(p\cdot J_{12}\cdot t_1)\cdot\sin(d_1\cdot t_1)\cdot(e^{2\cdot p\cdot i\cdot(n_1+\frac{J_{12}}{2})\cdot t_2} + e^{2\cdot p\cdot i\cdot(n_1-\frac{J_{12}}{2})\cdot t_2}) \\ & + \frac{i}{2}\cdot\sin(p\cdot J_{12}\cdot t_1)\cdot\sin(d_1\cdot t_1)\cdot(e^{2\cdot p\cdot i\cdot(n_2+\frac{J_{12}}{2})\cdot t_2} - e^{2\cdot p\cdot i\cdot(n_2-\frac{J_{12}}{2})\cdot t_2}) \\ & + \frac{1}{2}\cdot\cos(p\cdot J_{12}\cdot t_1)\cdot\cos(d_1\cdot t_1)\cdot(e^{2\cdot p\cdot i\cdot(n_1+\frac{J_{12}}{2})\cdot t_2} + e^{2\cdot p\cdot i\cdot(n_1-\frac{J_{12}}{2})\cdot t_2}) \\ & + \frac{1}{2}\cdot\sin(p\cdot J_{12}\cdot t_1)\cdot\cos(d_1\cdot t_1)\cdot(e^{2\cdot p\cdot i\cdot(n_2+\frac{J_{12}}{2})\cdot t_2} - e^{2\cdot p\cdot i\cdot(n_2-\frac{J_{12}}{2})\cdot t_2}) \end{aligned}$$

Which can also be expressed as:

$$\begin{aligned}\mathrm{Tr}\{F_+ \cdot s_4\} + \mathrm{Tr}\{F_+ \cdot s'_4\} = \; & \frac{1}{2}\big(\cos(p \cdot J_{12} \cdot t_1) \cdot \cos(d_1 \cdot t_1) + i \cdot \cos(p \cdot J_{12} \cdot t_1) \cdot \sin(d_1 \cdot t_1)\big) \\ & \cdot (e^{2 \cdot p \cdot i \cdot (n_1 + \frac{J_{12}}{2}) \cdot t_2} + e^{2 \cdot p \cdot i \cdot (n_1 - \frac{J_{12}}{2}) \cdot t_2}) \\ & + \frac{1}{2}(\sin(p \cdot J_{12} \cdot t_1) \cdot \cos(d_1 \cdot t_1) + i \cdot \sin(p \cdot J_{12} \cdot t_1) \cdot \sin(d_1 \cdot t_1)) \\ & \cdot (e^{2 \cdot p \cdot i \cdot (n_2 + \frac{J_{12}}{2}) \cdot t_2} - e^{2 \cdot p \cdot i \cdot (n_2 - \frac{J_{12}}{2}) \cdot t_2})\end{aligned}$$

The final result will then lead to:

$$\begin{aligned}\mathrm{Tr}\{F_+ \cdot s_4\} + \mathrm{Tr}\{F_+ \cdot s'_4\} = \; & \frac{1}{4}(e^{2 \cdot p \cdot i \cdot (n_1 + \frac{J_{12}}{2}) \cdot t_1} + e^{2 \cdot p \cdot i \cdot (n_1 - \frac{J_{12}}{2}) \cdot t_1}) \cdot (e^{2 \cdot p \cdot i \cdot (n_1 + \frac{J_{12}}{2}) \cdot t_2} + e^{2 \cdot p \cdot i \cdot (n_1 - \frac{J_{12}}{2}) \cdot t_2}) \\ & + \frac{1}{4}(e^{2 \cdot p \cdot i \cdot (n_1 + \frac{J_{12}}{2}) \cdot t_1} - e^{2 \cdot p \cdot i \cdot (n_1 - \frac{J_{12}}{2}) \cdot t_1}) \cdot (e^{2 \cdot p \cdot i \cdot (n_2 + \frac{J_{12}}{2}) \cdot t_2} - e^{2 \cdot p \cdot i \cdot (n_2 - \frac{J_{12}}{2}) \cdot t_2})\end{aligned}$$

After Fourier transform, we find an all positive diagonal peak multiplet and an anti-phase cross peak multiplet of four peaks each.

23.11 Summary and Useful Formulae

23.11.1 Effects on Spins in the Product Operator Formalism

Effect of pulses on magnetization:

$$I_z \xrightarrow{b_x} I_z \cos b - I_y \sin b$$
$$I_z \xrightarrow{b_y} I_z \cos b + I_x \sin b$$
$$I_x \xrightarrow{b_x} I_x$$
$$I_y \xrightarrow{b_y} I_y$$
$$I_x \xrightarrow{b_y} I_x \cos b - I_z \sin b$$
$$I_y \xrightarrow{b_x} I_y \cos b + I_z \sin b$$

If the flip angle β = 90° then:

$$I_z \xrightarrow{90_{y,x}} \pm I_{x,y}$$
$$I_{x,y} \xrightarrow{90_{y,x}} \mp I_z$$

Effect of chemical shift on magnetization:

$$I_z \xrightarrow{d \cdot I_z \cdot t} I_z$$
$$I_x \xrightarrow{d \cdot I_z \cdot t} I_x \cos(d\ t) + I_y \sin(d\ t)$$
$$I_y \xrightarrow{d \cdot I_z \cdot t} I_y \cos(d\ t) - I_x \sin(d\ t)$$

Effect of scalar coupling on magnetization:

$$I_{1z} \xrightarrow{2p\ J_{12}\ I_{1z} I_{2z}\ t} I_{1z}$$
$$I_{1x} \xrightarrow{2p\ J_{12}\ I_{1z} I_{2z}\ t} I_{1x} \cos(pJ_{12}t) + 2\ I_{1y} I_{2z} \sin(pJ_{12}t)$$
$$I_{1y} \xrightarrow{2p\ J_{12}\ I_{1z} I_{2z}\ t} I_{1y} \cos(pJ_{12}t) - 2\ I_{1x} I_{2z} \sin(pJ_{12}t)$$
$$2 \cdot I_{1x} I_{2z} \xrightarrow{2p\ J_{12}\ I_{1z} I_{2z}\ t} 2\ I_{1x} I_{2z} \cos(pJ_{12}t) + I_{1y} \sin(pJ_{12}t)$$
$$2 \cdot I_{1y} I_{2z} \xrightarrow{2p\ J_{12}\ I_{1z} I_{2z}\ t} 2\ I_{1y} I_{2z} \cos(pJ_{12}t) - I_{1x} \sin(pJ_{12}t)$$
$$2 \cdot I_{1x} I_{2y} \xrightarrow{2p\ J_{12}\ I_{1z} I_{2z}\ t} 2\ I_{1x} I_{2y}$$

23.11.2 Mathematical Relations

The Euler relations where used extensively in the previous paragraphs:

$$e^{i \cdot w \cdot t} = \cos(w \cdot t) + i \cdot \sin(w \cdot t)$$

$$e^{-i \cdot w \cdot t} = \cos(w \cdot t) - i \cdot \sin(w \cdot t)$$

$$\cos(w \cdot t) = \frac{1}{2}(e^{i \cdot w \cdot t} + e^{-i \cdot w \cdot t})$$

$$\sin(w \cdot t) = -\frac{i}{2}(e^{i \cdot w \cdot t} - e^{-i \cdot w \cdot t})$$

Frequently used simplifications in 2D:

$$\begin{aligned} -i \cdot \cos a + \sin a &= -i \cdot \cos a - i^2 \cdot \sin a \\ &= -i(\cos a + i \cdot \sin a) \\ &= -i \cdot e^{i \cdot a} \end{aligned}$$

$$\begin{aligned} \cos a \cdot e^{i \cdot b} &= \frac{1}{2}(e^{i \cdot a \cdot t} + e^{-i \cdot a \cdot t}) \cdot e^{i \cdot b} \\ &= \frac{1}{2}(e^{i(b+a) \cdot t} + e^{i(b-a) \cdot t}) \end{aligned}$$

$$\begin{aligned} \sin a \cdot e^{i \cdot b} &= -\frac{i}{2}(e^{i \cdot a \cdot t} - e^{-i \cdot a \cdot t}) \cdot e^{i \cdot b} \\ &= -\frac{i}{2}(e^{i(b+a) \cdot t} - e^{i(b-a) \cdot t}) \end{aligned}$$

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