

TopSpin

Basic NMR Experiments
 User Manual
 Version 004

Innovation with Integrity

NMR

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1 About This Manual

This manual enables safe and efficient handling of the device.

This manual is an integral part of the device, and must be kept in close proximity to the device where it is permanently accessible to personnel. In addition, instructions concerning labor protection laws, operator regulations tools and supplies must be available and adhered to.

Before starting any work, personnel must read the manual thoroughly and understand its contents. Compliance with all specified safety and operating instructions, as well as local work safety regulations, are vital to ensure safe operation.

The figures shown in this manual are designed to be general and informative and may not represent the specific Bruker model, component or software/firmware version you are working with. Options and accessories may or may not be illustrated in each figure.

1.1 Policy Statement

It is Bruker's policy to improve products as new techniques and components become available. Bruker reserves the right to change specifications at any time.

Every effort has been made to avoid errors in text and figure presentation in this publication. In order to produce useful and appropriate documentation, we welcome your comments on this publication. Field Service Engineers are advised to check regularly with Bruker for updated information.

Bruker is committed to providing customers with inventive, high-quality, environmentallysound products and services.

1.2 Symbols and Conventions

Safety instructions in this manual and labels of devices are marked with symbols.

The safety instructions are introduced using indicative words which express the extent of the hazard.

In order to avoid accidents, personal injury or damage to property, always observe safety instructions and proceed with care.



DANGER indicates a hazardous situation, which, if not avoided, will result in death or serious injury.

This is the consequence of not following the warning.

- 1. This is the safety condition.
- ► This is the safety instruction.



WARNING indicates a hazardous situation, which, if not avoided, could result in death or serious injury.

This is the consequence of not following the warning.

- 1. This is the safety condition.
- ► This is the safety instruction.



CAUTION indicates a hazardous situation, which, if not avoided, may result in minor or moderate injury or severe material or property damage.

This is the consequence of not following the warning.

- 1. This is the safety condition.
- ► This is the safety instruction.

NOTICE

NOTICE indicates a property damage message.

This is the consequence of not following the notice.

- 1. This is a safety condition.
- ► This is a safety instruction.

SAFETY INSTRUCTIONS

SAFETY INSTRUCTIONS are used for control flow and shutdowns in the event of an error or emergency.

This is the consequence of not following the safety instructions.

- 1. This is a safety condition.
- ► This is a safety instruction.



This symbol highlights useful tips and recommendations as well as information designed to ensure efficient and smooth operation.

1.3 Font and Format Conventions

Type of Information	Font	Examples	
Shell Command, Commands, "All that you can enter"	Arial bold	Type or enter fromjdx zg	
Button, Tab, Pane and Menu Names "All that you can click"	Arial bold, initial letters capitalized	Use the Export To File button. Click OK . Click Processing	
Windows, Dialog Windows, Pop-up Windows Names	Arial, initial letters capitalized	The Stacked Plot Edit dialog will be displayed.	
Path, File, Dataset and Experiment Names Data Path Variables Table Column Names Field Names (within Dialog Windows)	Arial Italics	\$tshome/exp/stan/nmr/ lists expno, procno,	
Parameters	Arial in Capital Letters	VCLIST	
Program Code Pulse and AU Program Names Macros Functions Arguments Variables	Courier	go=2 au_zgte edmac CalcExpTime() XAU(prog, arg) disk2, user2	
AU Macro	Courier in Capital Letters	REX PNO	

Table 1.1: Font and Format Conventions

2 Introduction

2.1 Limitation of Liability

All specifications and instructions in this manual have been compiled taking account of applicable standards and regulations, the current state of technology and the experience and insights we have gained over the years.

The manufacturer accepts no liability for damage due to:

- Failure to observe this manual.
- Improper use.
- Deployment of untrained personnel.
- · Unauthorized modifications.
- Technical modifications.
- Use of unauthorized spare parts.

The actual scope of supply may differ from the explanations and depictions in this manual in the case of special designs, take-up of additional ordering options, or as a result of the latest technical modifications.

The undertakings agreed in the supply contract, as well as the manufacturer's Terms and Conditions and Terms of Delivery, and the legal regulations applicable at the time of the conclusion of the contract shall apply.

2.2 Copyright

All rights reserved. This manual is protected by copyright and intended solely for internal use by customers.

This manual must not be made available to third parties, duplicated in any manner or form – whether in whole or in part – and the content must not be used and/or communicated, except for internal purposes, without the written consent of the manufacturer.

Product names used are trademarks[™] or registered trademarks[®] of their respective holders.

Violation of the copyright will result in legal action for damages. We reserve the right to assert further claims.

2.3 Warranty Terms

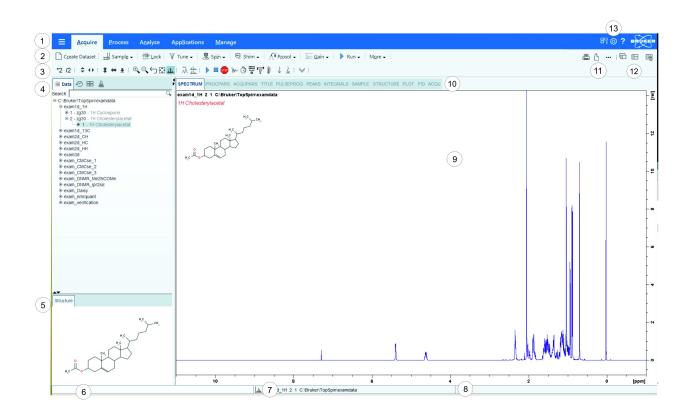
The warranty terms are included in the manufacturer's Terms and Conditions.

2.4 Customer Service

Our customer service division is available to provide technical information. See the chapter *Contact* [> 127] for contact information.

In addition, our employees are always interested in acquiring new information and experience gained from practical application; such information and experience may help improve our products.

The TopSpin Interface 3



1	Menu bar	8	Status display bar
2	Workflow button bar	9	Dataset window
3	Tool bar	10	Dataset window tabs
4	Browser and search window	11	Print, export and publish
5	Structure window	12	Viewing options
6	Command line	13	Window switcher, setup preferences and help
7	Current dataset bar		

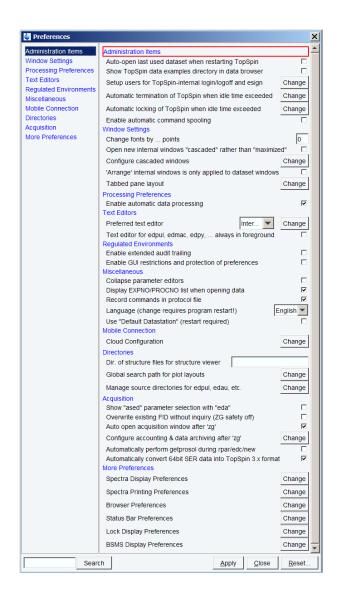


Setup Preferences

For all changes in the TopSpin appearance use the global Setup preferences button in the menu bar.

TopSpin can be tailored to your preference in many respects. This ranges from startup options to spectrum objects, menu settings, remote connections, colors and fonts etc. Every standard user can create his own set of preferences.

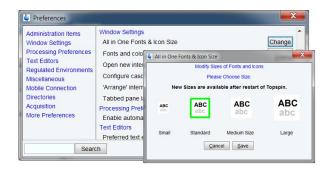
A dialog box will appear with, at the left side, the categories that can be tailored. Click the category you want to view or change. It will become highlighted and the corresponding objects will be displayed at the right part of the dialog box.



TopSpin on High Resolution Screens

To adapt the font and icon size to a small/standard/medium or large screen resolution

• click Setup Preferences and in the category Window Settings | All In One Fonts & Icon Size click Change.



Help/About TopSpin/Version and Licence Information

This button gives information about the TopSpin documentation, software version and licence.

A hint in the manuals like

Click Help | Manuals | Acquisition Application Manuals | Dosy

will open the DOSY_and_Diffusion.pdf document.

Alternatively enter help or docs on the command line.

	Please click on a manual title to open the document!
General	
User Manual	A description of the TopSpin user interface and its functionality
Control & Function Keys	A list of predefined Control and Function keys.
Release Letter	Describes the changes and new features of this TopSpin version and the spectrometer hardware requirements
Beginner Guide	For Avance Spectrometers With SGU Based Frequency Generation:
boginner odide	A basic description of the Bruker NMR spectrometer, its main components, functionality and usage.
CodeMeter License Management	Installing and Managing Software Licenses.
Acquisition - User Guides	installing and managing contract accross.
1D and 2D Step-by-Step - Basic	A step-by-step tutorial of setting up and running the most frequently used 1D and 2D experiments.
1D and 2D Step-by-Step - Dasic 1D and 2D Step-by-Step - Advanced	A step-by-step tutorial of setting up and running DOSY, Inverse and 19F experiments.
Basic 1D and 2D Experiments	A step-by-step futorial of setting up and running BOS1, inverse and 191 experiments. A theoretical and practical description of setting up and running the most frequently used 1D and 2D experiments
3D/Triple-Resonance experiments	How to set up and run common 3D/triple-resonance experiments for isotope labeled proteins
Acquisition - Application Manuals	
Eretic2	Introduction into NMR Quantification using the Eretic 2 method
Solids Introduction	A basic introduction into the NMR of solids.
Solids	A description of setting up and running Solids experiments.
TopSolids	Assisted Biological Solid State NMR.
Cross Polarization Dynamics	An introduction into Cross Polarization Dynamics experiments.
SB/MAS	A description of setting up and running SB/MAS experiments.
BEST-NMR	A description of setting up and running BEST-NMR experiments.
LC-NMR	A description of setting up and running LC-NMR experiments.
Dosy	A description of setting up and running Dosy experiments.
Diffusion	A description of setting up and running Diffusion experiments.
Shapetool	A description of creating, analyzing and manipulating RF- and gradient Shapes.
Gradient Shimming	A description of the gradient shimming interface.
TopShim	User manual for the automatic shimming tool.
CMCQ	Complete molecular confidence for quality assurance
APSY	Automated Projection Spectroscopy: Get N-dim. correlations via low-dimensional projections.
SmartDriveNMR	The smart spectrometer for structure verification.
NMR Thermometer	Introduction into NMR Thermometer.
WaveMaker	Pulse Shaping Software.
Acquisition & Processing Referer	nces
Acqu. Commands & Parameters	A description of all acquisition and acquisition related commands and parameters.
Proc. Commands & Parameters	A description of all processing and analysis commands and parameters.
Edprosol Manual	How to set up probe and solvent dependent parameters
Edlock Guide	A description of how to setup solvent and lock dependent parameters.
Pulse Program Catalogue, 1D/2D	A graphical presentation of the Bruker supplied pulse programs, 1D and 2D experiments.
Pulse Program Catalogue, BIO	A graphical presentation of the Bruker supplied pulse programs, biomolecular experiments.
NUS Parameters	A description of the parameter setup for Non Uniform Sampling
Automation and Data Publishing	·······
•	III

Tooltips

Pointing to a button with the mouse in the various menus opens a tooltip that describes the button functionality. Example:



Start acquisition (zg) Uses the acquisition parameters of the currently active dataset. If a dataset exists, zg will ask to override it, unless this feature was disabled (command "set", ZG safety off). You may also enable "auto-archiving" so as to avoid inadvertent data data loss. (command "set", Configure accounting and data archiving).

Workflow Button Bar

The workflow-based interface with its arrangement of all working processes allows the user to control the workflow intuitively.

Clicking one of the menu buttons opens the corresponding workflow. It contains an horizontal feature list which stays open and provides all functionality for this workflow with one mouse-click.

Furthermore, some of the buttons on the Workflow button bar include a **drop-down** arrow. Click the **drop-down** arrow to see more options.

 Proc. Spectrum ▼

 Compute Spectrum from raw data (proc1d y)

 Configure Standard Processing (proc1d)

 Window Multiplication (wm)

 Fourier Transform (ft)

 Eourier Transform Options ... (ftf)

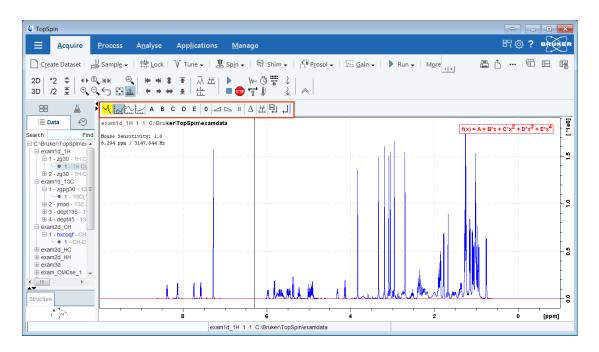
 Start Automation AU Program (xaup)

Dataset Toolbar

Depending on which dataset window tab is selected, an individual dataset toolbar is displayed, in the example the ACQU toolbar:



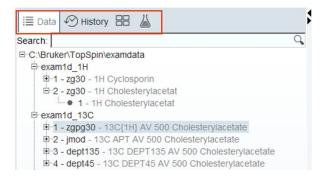
Note: Sometimes the dataset toolbar is displayed without dataset window tabs, e.g. entering a command as **.basl** will display the baseline correction toolbar:



Browse and Search Window

The Browser window provides tabs as:

- Data browser and Search
- History browser
- · Dataset Switcher
- Experiment Selector library



The window can be toggled On or Off with a click on the black left or right arrow.

Alternatively, the **Toggle Perspective** button on the top-right of the TopSpin window can be clicked.

Х

The data tree tab includes a the search field. Enter a search phrase, for example:

Search: pulprog=nsgc	Search:	pulprog=hsqc	
----------------------	---------	--------------	--

To limit the search results, select or deselect the data trees with SHIFT click or CTRL click.

⊡C:\Bruker\TopSpin\examdata
⇔exam1d_1H
🕀 1 - zg30 - 1H Cyclosporin
🖻 <mark>2 - zg30</mark> - 1H Cholesterylacetat
1 - 1H Cholesterylacetat
⇔exam1d_13C
1 - zgpg30 - 13C{1H} AV 500 Cholesterylacetate
2 - jmod - 13C APT AV 500 Cholesterylacetate
1 - 13C APT AV 500 Cholesterylacetate
3 - dept135 - 13C DEPT135 AV 500 Cholesterylacetate
4 - dept45 - 13C DEPT45 AV 500 Cholesterylacetate
⊕ exam2d_CH
⊕ exam2d_HC
⊕ exam2d HH

History Tab

The History tab displays the last opened datasets in a list.

i≣ Data 🐼 History ⊞ 👗
Show Spectra Thumbnails
 exam1d_13C 1 1 C:\Bruker\TopSpin\examdata - zgpg30, 13C{1H} exam1d_1H 2 1 C:\Bruker\TopSpin\examdata - zg30, 1H Cholestery exam1d_1H 1 1 C:\Bruker\TopSpin\examdata - zg30, 1H Cholestery exam1d_13C 2 1 C:\Bruker\TopSpin\examdata - jmod, 13C APT , exam2d_HC 3 1 C:\Bruker\TopSpin\examdata - hsqcedetgpsp.3, ec exam2d_CH 1 1 C:\Bruker\TopSpin\examdata - hxcoqf, CH-CO Cycl exam1d_13C 3 1 C:\Bruker\TopSpin4.0.b.20161103pl0\examdata - exam1d_14 2 1 C:\Bruker\TopSpin4.0.b.20161103pl0\examdata - exam1d_14 2 1 C:\Bruker\TopSpin4.0.b.20161103pl0\examdata - z exam2d_CH 1 1 C:\Bruker\TopSpin4.0.b.20161103pl0\examdata - z

Dataset Switcher Tab

This tab has a similar function as the Window Switcher a quick overview of all currently opened data sets.

i≣ Data ∮	History 🔠 👗
®⊠ ±	-
	LL exam1d_13C
	H Multiple Display Mode

Experiment Library Tab

The Experiment library tab provides easy access to the vast experiment library of standard experiments that is available in the standard release of TopSpin. It is also a tool that allows the user to personalize his most frequently used experiments into a separate library.

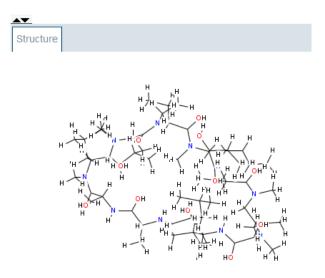
82

, see below, but only provides

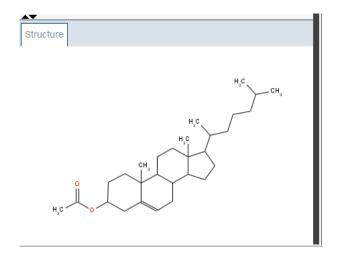
i≣ Data 🔗 History 🔠 👗	\$
🖓 TopSpin Library	
Small_Molecule_Experiments	
Protein_Experiments	
RNA_Experiments	
Drug_Discovery	
. LC-NMR	
Metabonomics	
E Semi-Solids	
Personal Library	

Structure Window

Molecular structures as *.mol* or *.pdb* files can be displayed here and are freely resizable. The structure window can be toggled On or Off with a click on the black **up** or **down** arrow \blacktriangle .



• Drag the vertical or horizontal split bar to resize the structure window.





The **Window Switcher** button indicates the number of all opened dataset windows plus all opened acquisition control windows like the BSMS Control Suite and the Temperature Control Suite.

• Click the Window Switcher button to switch between these windows.

™ ™ I	BSMS Control Suite
∬i Temperature Control Suite	BSMS Control Suite
exam2d_HC	exam1d_13C
exam1d_1H Expno: 1 Procno: 1	

A dataset can be	closed with	the Close	button	

Viewing Options

• Switch the perspective, e.g. full screen or side-by-side, compare open datasets or change spectra view options in the Viewing Options bar.



• Click **Spectrum display properties** to toggle the visibility of integrals, peaks and other spectra components. They can only be displayed when available.



Print, Export and Publish

The work can be shared from every point in the working routine with colleagues, sent to printer, export in optional data formats.



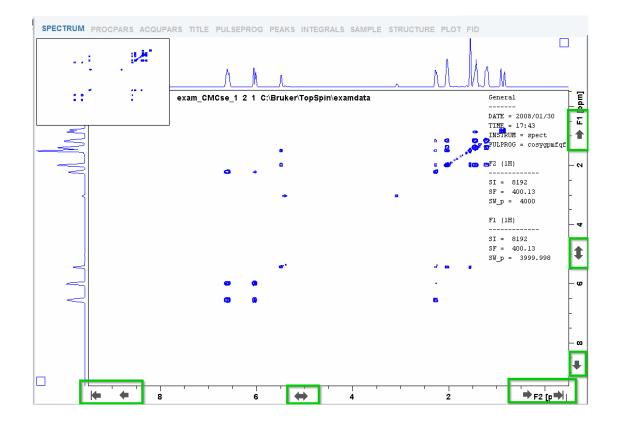
• Click **Show More publishing options**, e.g. to copy and paste, E-mail or use shared cloud directories.



Shift, Scroll and Zoom Spectra Axes

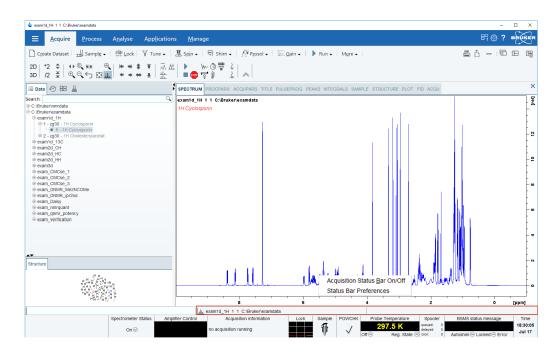
Clicking and dragging the spectrum axis allows intuitive scrolling through the spectrum.

- Single Clicks on the end regions of the axes or a click and dragging the mouse shift through the axes depending on the mouse position, see the next figure.
- Double click in the middle of the spectra axes switches to full axes region.
- Double click in the middle of the spectra itself maximizes all axes regions.
- · Click on axes to shift left, right, up or down.
- Turn mouse wheel to zoom in or out.

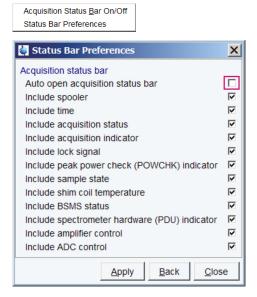


Acquisition Status Bar

 Right-click the Status display or Current dataset bar to toggle the Acquisition status bar On or Off.



• Click **Status Bar Preferences** to set the *Auto-open the acquisition status bar* option as default.



The acquisition status bar contains the new Spectrometer Status area to turn the spectrometer On and Off. This functionality is also available in the menu with a click on **Manage | Spectrometer | Spectrometer power On/Off** or the command **pdudisp**.

Liu_exam1d_1H 1 1 C\Brukeriexamdata										
	Spectrometer Status	Amplifier Control	Acquisition information	Lock	Sample	POWCHK	Probe Temperature	Spooler	BSMS status message	Time
	On⊘		no acquisition running		ŧ	\checkmark	297.5 K ofr⊙ Reg. State:⊖	queued: 0 delayed: 0 cron: 0	Autoshim C Locked Error	18:30:0 Jul 17

4 Spectrometer Basics

4.1 Magnetic Safety

A Magnetic Field surrounds the magnet in all directions. This field (known as the stray field) is invisible, hence the need to post warning signs at appropriate locations. Objects made of ferromagnetic materials, e.g. iron, steel etc. will be attracted to the magnet. If a ferromagnetic object is brought too close, it may suddenly be drawn into the magnet with surprising force. This may damage the magnet, or cause personal injury to anybody in the way! Of critical importance is that people fitted with cardiac pacemakers or metallic implants should never be allowed near the magnet.

Because the strength of the stray field drops significantly as one moves away from the magnet, it is still useful to discuss safety to work around magnets. Details of stray fields for various magnets can be found in the Site Planning Guides delivered with the BASH CD.

4.2 Cryogenic Safety

The magnet contains relatively large quantities of liquid Helium and Nitrogen. These liquids, referred to as cryogens, serve to keep the magnet core at a very low temperature.

Because of the very low temperatures involved, **gloves**, **a long sleeved shirt or lab coat and safety goggles** should always be worn when handling cryogens. Direct contact with these liquids can cause frostbite. The system manager should regularly check and make sure that evaporating gases are free to escape from the magnet, i.e. the release valves must not be blocked. Do not attempt to refill the magnet with Helium or Nitrogen unless you have been trained in the correct procedure.

Helium and Nitrogen are non-toxic gases. However, because of a possible **magnet quench**, whereupon the room may suddenly fill with evaporated gases, adequate ventilation must always be provided.

4.3 Electrical Safety

The spectrometer hardware is no more or less hazardous than any typical electronic or pneumatic hardware and should be treated accordingly. Do not remove any of the protective panels from the various units. They are fitted to protect you and should be opened by qualified service personnel only. The main panel at the rear of the console is designed to be removed using two quick release screws, but again, this should only be done by trained personnel.

4.4 Chemical Safety

Users should be fully aware of any hazards associated with the samples they are working with. Organic compounds may be highly flammable, corrosive, carcinogenic etc.

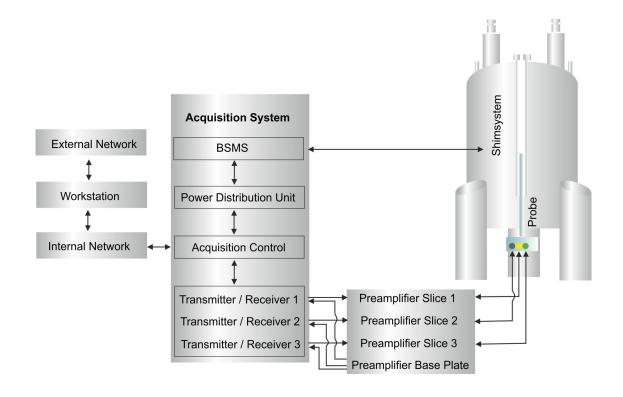
4.5 **CE Certification**

All major hardware units housed in the AVANCE NEO consoles as well as peripheral units such as the HPPR, shim systems, probe and BSMS keyboards comply with the CE Declaration of Conformity. This includes the level of any stray electromagnetic radiation that might be emitted as well as standard electrical hazards.



To minimize electromagnetic radiation leakage, the doors of the console should be closed and the rear paneling mounted.

4.6 AVANCE Architecture Overview

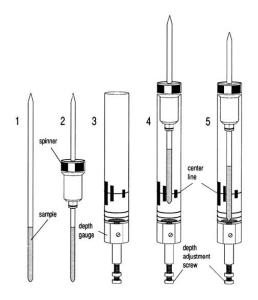


i

Please use the BASH (**B**ruker **A**dvanced **S**ervice **H**andbook) for further information about the AVANCE system and hardware.

4.7 Sample Preparation

- Use clean and dry sample tubes.
- Use medium to high quality sample tubes.
- Always filter the sample solution.
- · Always use the same sample volume or solution height.
- Filling volume of a 5 mm tubes is 0.6 ml or 5 cm.
- Filling volume of a 10 mm tubes is 4 ml or 5 cm.
- Use the sample depth gauge to adjust the sample depth (1.8 cm for older style probes, 2.0 cm for newer style probes).



- The sample tube should sit tightly inside the spinner.
- Wipe the sample tube clean before inserting into magnet.
- Turn on lift air to insert the sample into the magnet.

4.8 Inserting the Sample Plus Spinner into the Magnet

The raising and lowering of the sample is controlled by a stream of pressurized air. Make sure that the air flow is present (it is quite audible) before placing a sample onto the top of the bore.

4.9 Spinning the Sample

A second function of pressurized air is to enable the sample to rotate. The spinning of the sample serves to *even-out* some of the inhomogeneities that may exist in the magnetic field at the center of the magnet.



Sample tubes with a diameter of less then 5mm and samples to be investigated using inverse probes are normally not rotated.

Suggested spin rates are:

- 20 Hz for a 5 mm probe
- 12 Hz for a 10 mm probe

4.10 Tuning and Matching the Probe

The sensitivity of any probe will vary with the frequency of the signal transmitted to it and there exists a frequency at which the probe is most sensitive. Furthermore this frequency may be adjusted over a certain range using tuning capacitors built into the probe circuitry. **Tuning** involves adjusting the probe circuitry so that the frequency at which it is most sensitive is the relevant transmission frequency (SFO1, SFO2 etc.) Each coil in the probe will be tuned (and matched) separately.

If the probe has been changed or the transmission frequency altered significantly, it may be necessary to retune the probe. For routine work in organic solvents with selective probes, the value of the transmitted frequencies are unlikely to vary greatly. Hence, once the probe has been initially tuned, slight variations in frequency will not warrant retuning. Typically the transmitted frequency would need to be altered by at least 100kHz to warrant retuning. However for broadband probes the frequencies transmitted will vary greatly from nucleus to nucleus and so the probe will need to be tuned each time the selected nucleus is altered.

Whenever a probe is tuned it should also be matched. **Matching** involves ensuring that the maximum amount of the power arriving at the probe base is transmitted up to the coil which lies towards the top of the probe. This ensures that the minimum amount of the power arriving at the probe base is reflected back towards the amplifiers (and consequently wasted).



Bruker offers two different types of Tuning and Matching adjustments. In addition to the manual adjustments of the tuning and matching capacitors, the probes can be equipped with an Automatic Tuning Module (ATM). Follow the steps below for either option.

4.10.1 Probes Equipped with ATM

4.10.1.1 Automatic Tuning

- Create a new data set, see also Experiment Setup [> 31].
- On the menu bar, click Acquire.
- On the Workflow button bar, click Tune.

or

• On the command line, type atma.



The display will switch automatically to the acquisition window and displays the wobble curve. The tuning and matching is performed automatically. If multiple frequencies are used in a parameter set such as C13CPD, HNCACOGP3D etc., ATMA will start adjusting the lowest frequency first and will switch in the order of increasing frequency automatically.

4.10.1.2 Manual Tuning

- Create a new data set, see also *Experiment Setup* [> 31].
- On the menu bar, click Acquire.
- On the **Tune** button, click the **drop-down** arrow to see more options.
- In the list, select Tune/match ATM probe manually.

or

• On the command line, type **atmm**.

The ATMM control window appears and the display will switch automatically to the acquisition window and displays the wobble curve, see the next figure.

Bruker TopSpin 4.0.3.b.5 on av4600 as demo						- D X
<u> </u>	<u>M</u> anage				昂@?	BRUKER
Λ Pro <u>c</u> . Spectrum - \land Adjust Phase - \land Baseline - \land Cal	ib. A <u>x</u> is 🗸 A <u>d</u> vanced 🗸			d	5 A 1 M	E B
2D *2 \$ \$ \$	*8					
	CQUPARS TITLE PULSEPROG PEAKS	INTEGRALS SAMPLE STRUCTUR	E PLOT FID ACQU			×
🗌 Show Spectra Thumbnails 🛛 🖓 🖓 🖓						
P belocknow 1 1 /u/data/demo/nmr - za A	demo/nmr	Ĭ I			Wobble	5
Information					TUNENUC = 15	
Probe: PA TXI 600S3 H-C/N-D-05 Z					TUNEFREQ = 6 CENTERFREQ =	0.813365
Solvent: H2O+D2O					WBSW - 1 WBST = 1024	V
State: Idle						-8
Channel/Nucleus					~	
Channel/Nucleus selection: 0 13C (13C)			Comple	x wobble display		×
© 150 (150)						
○ 1H (1H)						1
Complex wobble display						-9
Show complex wobble display			1			
Automatic adjustment (ATMA)						l+
Start ATMA, probe does not support storing positions for future use Start						-
User defined WBSW and WBST	- · · · · · · · · · · · · · · · · · · ·					
Wobble sweep width [MHz] 1.0 Set						
Number of wobble steps 1024 Set						-6
Tuning						
Fine						
<<< << < Stop > >> >>>			/			1
Matching				{		If .
Fine				1	1	-2
<<< << < Stop > >> >>>				Ň	/	t
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60.4	60.6	60.8	61.0		61.2	[MHz]
	the beubi_cn3.15 201 1 /w/data/demo/n		taking wobble data			
	Spectrometer Status Amplifier Control	Acquisition information Tune nucleus: 15N	Fid Flash Lock POWCHK	Spooler BSMS gueued: 0	5 status message	Time 10:27:38
	On⊘	Tune frequency: 60.813365 Center frequency:60.8163		delayed: 0	ΔZ -1 ⊙ Locked ⊙ Error(Feb 01

- In the ATMM control window, click the **Tuning** buttons to move and display the wobble curve centered.
- In the ATMM control window, click the **Matching** buttons to adjust the dip of the wobble curve to the lowest position.

It is very convenient to look at the complex wobble display. If the circle with the red dot is too big or too small, then adjust matching first. Once the circle runs through the center of the coordinate system, bring the red dot into the center by adjusting the tuning. In essence, the curve tells you in which order to adjust tuning and matching.

Note: The T/M settings will be saved with a click on the Start button.



Since the Tuning and Matching adjustment interact with each other, a repeat of all steps is necessary for a perfect tune and match. If multiple frequencies are used in a parameter set such as C13CPD, use the **Nucleus Selection** radio buttons in the ATMM control window to switch to another nucleus and repeat the tuning and matching.

4.11 Locking the Sample

Deuterated solvents are used to generate the signal to be detected and monitored by the lock system. The frequency and strength of this signal will depend on the solvent used. The main feature of the Topspin lock routine is that it sets parameters such as the lock power, gain and frequency to a value appropriate to the solvent. With these default values set close to that which would be expected for that solvent, the BSMS can quickly locate and lock onto the solvent signal by sweeping through a range of frequencies or magnetic field values. The solvent dependent parameters are taken from the **edlock** table.

4.12 Shimming the Sample

Shimming is a process in which minor adjustments are made to the magnetic field until the field homogeneity (uniformity) is optimized. Improving the homogeneity will result in better spectral resolution. It will be necessary to re-shim each time a probe or sample is changed. The system manager has stored appropriate shim values (in so called shim files) for each probe that will greatly reduce the shimming time required whenever a probe is changed.

4.12.1 Shimming on the Lock Signal

When the spectrometer is locked, the vertical offset of the lock trace on the graphics display corresponds to the amplitude of the lock substance signal, assuming constant lock DC, gain, and power levels. The lock level, then, serves as useful guide for basic shim adjustment. The goal in shimming on the lock signal is to adjust the shims so that the lock trace appears as high on the graphics display as possible. This lock level corresponds to the highest possible lock substance signal amplitude.

4.12.2 Shimming on the FID (Free Induction Decay)

The shape of the FID, and especially the beginning of the FID, indicates the shape of the transformed signal line, while the length of the FID tail is important to the overall resolution. For good line shape and high resolution, the shim controls must be adjusted so that the FID envelope is truly exponential with the longest possible decay time.

4.12.3 Shimming Using the Tune File

This method of shimming is useful when gradients are not available. A simple text file is edited to give the BSMS the instructions to shim the sample automatically. A default shim file *example* can be edited using the **edtune** command and then stored with a new name in

<TopSpin-home>/exp/stan/nmr/lists/group.

The file can be executed with the command tune. The figure shows an example of a tune file.

SHIMMIT spin DELAY 3 MAXLOCK 0.4 TIMEOUT 1800 LOCKDWELL 3 # Shim_name Maximum_Step_Size Number_of_Iterations 30 z 3 Z2 30 3 Ζ 5 5 _ Z2 5 5 5 5 Z3

4.12.4 Shimming Using TopShim

This is routine shimming and should be carried out at the beginning of every NMR session, and whenever the sample in the magnet is changed. Routine shimming involves making fine adjustments to the Z, Z2, Z3, Z4 and Z5 shims. Some higher field magnets may require higher order Z shims. The system administrator has programed TopShim to achieve the best homogeneity on each sample and it is fully automatically.

The core method of TopShim is gradient shimming. A quality criterion for the final line-shape ensures best results for all situations.

TopShim is using for all deuterated solvents the ${}^{2}H$ gradient shimming method and for other solvents especially $H_{2}O$, the ${}^{1}H$ gradient shimming method.

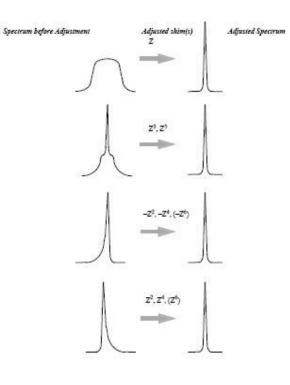
4.13 Optimizing Resolution and Line Shape

The standard sample for measuring the proton line shape and resolution specifications is, $CHCI_3$ in Acetone-d6. The concentration of $CHCI_3$ depends on the field strength of the magnet and the probe and can vary from 3% down to 0.1%.

For measuring the ^{13}C resolution and line shape test the standard sample ASTM (60% Dioxane in 40% C6D6) sample may be used.

For both tests the line shape is measured at 50%, 0.55% and 0.11% of the peak. The Bruker standard parameter sets to use for this tests are PRORESOL and C13RESOL.

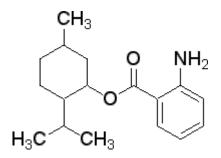
The figure below illustrates the influence of the On-axis shims on the line shape.



5 1D Proton Experiment

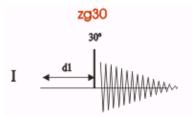
5.1 Sample

The sample of **30 mg Menthyl Anthranilate in DMSO-d6** is used for all experiments in this chapter.



5.2 1D Proton Experiment

This chapter describes the acquisition and processing of a one-dimensional ¹H NMR spectrum using the standard Bruker parameter set **PROTON**. The pulse sequence **zg30** consists of the recycling delay, the radio-frequency (RF) pulse, and the acquisition time during which the signal is recorded. The pulse angle is shown to be 30°. The two parameters, D1 and P1, correspond to the length of the recycle delay and the length of the 90° RF pulse, respectively.



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.

5.2.1 Experiment Setup

Create a new dataset

 On the menu bar, click Acquire | Create Dataset to open the Create New Dataset window.

initializing its NMR For multi-receiver	parameter: experiment	t by creating a new da s according to the sel s several datasets ar receivers in the Optic	ected experiment e created.	type.
Dataset				
NAME	Proton	_exp		
EXPNO	1			
Directory	C:\Dat	а	•	
Dpen in new wind	wot			
Set solvent Additional action	DMS	30 •	•	
_				
Do nothing				
Execute getprose			_	
Keep parameters	P 1, 01,	PLW 1 Change	}	
Advanced Number of datasets (rece	ivers)	1		-
Title				
Menthyl Anthranilate in D Proton	MSO			

• In the Create New Dataset window, enter or select:

NAME = **Proton_exp** EXPNO = **1** Directory = e.g. *C:\Data*

Directory, **NAME** and **EXPNO** define where the new experiment dataset will be stored on the computer:

Directory/NAME/EXPNO/ In the example: C:\Data\proton_exp\1

Directory can be selected in the drop-down list, or a new location can be typed in the text field. **NAME** is a subdirectory of **Directory**, and **EXPNO** is a subdirectory of **NAME**. **EXPNO** must be a positive integer. It provides a way to store multiple related datasets under the same **NAME**.

• In the Parameters group Parameters click **Read parameterset** and **Select** to open the rpar window.

a				×			
File Options Help		Source = C:\Bruker\TopSpin4.0.3.a\exp\stan\nmr\par					
Find file names v enter any string, *, ? Exclude: Clear			C:\Bruker\TopSpin4.0.3.a\exp\stan\nmr\par\user				
Class = Any V Dim = Any V Show Recommended							
	Type = Any 🔻 SubTypeB =						
C13CPD	C13DEPT135	C13DEPTQ135	C13UDEFT	COSYGPDFPHSW			
OSYGPSW	HMBCETGPL3ND	HMBCGP	HMBCGP 15N	HSQC TOCSY			
ISQC_TOCSY_ADIA	HSQCEDETGPSISP	HSQCEDETGPSISP_ADIA	HSQCETGP_15N	HSQCETGPSISP			
ISQCETGPSISP_ADIA	MLEVPHPR	MLEVPHSW	NOESYPHPR	NOESYPHSW			
	ROESYPHPR	ROESYPHSW	WATERSUP				

- Enable **Show Recommended** to get the list of the most common small molecule experiments.
- Ensure that the source directory is

<Topspin>\exp\stan\nmr\par

and not

<Topspin>\exp\stan\nmr\par\user

- In the table select **PROTON** as experiment and click **Set selected item in editor**.
- In the Create New Dataset window check **Set solvent** and in the drop-down list select **DMSO**.
- In the TITLE field enter a text stating the experiment, sample, the solvent and any other useful information. The title information can be used to search for a dataset.
- In the New Dataset window, click **OK**.
- On the menu bar, click Acquire.

To acquire a spectrum, use the Workflow buttons in the Workflow button bar from left to right (see steps below). Alternatively, commands which are displayed in brackets of the various popup windows, can also be typed at the TopSpin command prompt (e.g. **ej**, **ij**, **edte** etc.).

- On the **Sample** button, click the **drop-down** arrow to see more options.
- · In the list, select Eject sample manually (ej).



Wait until the sample lift air is turned on and remove the sample which may be in the magnet.

Load the sample

- · Place the sample with the spinner onto the top of the magnet.
- On the Sample button, click the drop-down arrow to see more options.
- In the list, select Insert sample manually (ij).



Wait until the sample is lowered down into the probe and the lift air is turned off. A clicking sound may be heard.

Lock the solvent

- On the Workflow button bar, click Lock.
- In the Solvents table window, select the solvent, e.g. DMSO. Click OK.

🤹 Solvents table	1						
Solvent	Description						
Acetic	acetic acid-d4						
Acetone	acetone-d6						
C6D6	benzene-d6						
CD2Cl2	dichlormethane-d2						
CD3CN	acetonitrile-d3						
CD3CN_SPE	LC-SPE Solvent (Acetonitrile)						
CD3OD_SPE	LC-SPE Solvent (Methanol-d4)						
CDCI3	chloroform-d						
CH3CN+D2O	HPLC Solvent (Acetonitril/D2O)						
CH3OH+D2O	HPLC Solvent (Methanol/D2O)						
D2O	deuteriumoxide						
D2O_salt	deuteriumoxide with salt						
Dioxane	dioxane-d8						
DMF	N,N-dimethylformamide-d7						
DMSO	dimethylsulfoxide-d6						
EtOD	ethanol-d6						
H2O+D2O	90%H2O and 10%D2O						
H2O+D2O_salt	90%H2O and 10%D2O with salt						
HDMSO	90%DMSO and 10%DMSO-d6						
Juice	fruit juice						
MeOD	methanol-d4						
Plasma	blood plasma						
Pyr	pyridine-d5						
T_H2O+D2O+Me4NCI	(CD3)4NCI in 90%H2O and 10%D2O, for NMR the	rmometer					
T_H2O+D2O+NaAc	sodium acetate in 90%H2O and 10%D2O, for NMF	R thermometer					
T_H2O+D2O+Pivalate	pivalate-d9 in 90% H2O and 10% D2O, for NMR thermometer						
T_MeOD	methanol-d4, for NMR thermometer						
TFE	trifluroethanol-d3						
THF	tetrahydrofuran-d8						
Tol	toluene-d8						
Urine	urine						
Lock nucleus: 2H 🗸		OK Cancel					

Tune and match the probe

• On the Workflow button bar, click Tune.

This performs an **atma (automatic tuning and matching)** and requires a probe equipped with an automatic tuning module. For more options, click the **drop-down** arrow on the **Tune** button.

Spin the sample (optional)

- On the **Spin** button, click the **drop-down** arrow to see more options.
- In the list, select Turn sample rotation on (ro on).



Rotation may be turned **OFF** for probes such as **BBI**, **TXI**, **TBI** and for small sample probes.

Shim the sample

• On the Workflow button bar, click Shim.

This executes the command **topshim**. The shimming starts momentarily and should take less then a minute. On the **Shim** button, click the **drop-down** arrow to see more options.

Load the prosol parameters

• On the Workflow button bar, click **Prosol**.

This will load the pulse widths and power levels into the parameter set.

5.2.2 Acquisition

Set the receiver gain

• On the Workflow button bar, click Gain.

or

• On the Gain button, click the drop-down arrow to adjust the receiver gain manually.

Start the data acquisition

- On the Workflow button bar, click **Run**.
- or
 - On the **Run** button, click the **drop-down** arrow to see more options.

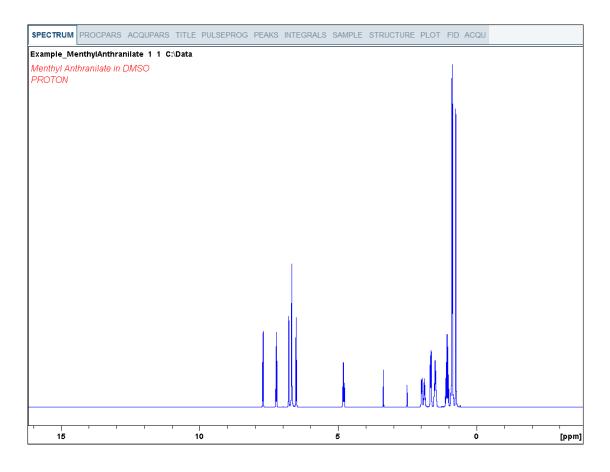
5.2.3 Processing

- When the acquisition has finished, click **Process** on the menu bar.
- On the **Proc Spectrum** button, click the **drop-down** arrow to see more options.
- · In the list, select Configure Standard Processing (proc1d).
- In the proc1d window, enable the following options:
 - Exponential Multiply (em)
 - Auto Phasing (apk)
 - Auto Baseline Correction (absn)

🖕 proc1d				×
Press 'Execute' to process the curre Press 'Save' to just change the proc Changed options will be effective wh one-click 'Proc. Spectrum' button.	essir	ng options.		
Exponential Multiply (em)	\checkmark	LB [Hz] =	0.3	
Fourier Transform (ft)				
Auto - Phasing (apk)	\checkmark			
Set Spectrum Reference (sref)				
Auto - Baseline Correction (absn)	\checkmark	Include integration =	no	~
Plot (autoplot)		LAYOUT =	+/1D_H.xwp	~
Warn if processed data exist	\checkmark			
			Save Execute	Cancel

- If TMS is added to the sample for referencing, enable Set Spectrum Reference (sref).
- In the proc1d window, click **Execute** and then click **Save** to save the selected processing settings.

Now all future datasets can be processed with the defined actions with a click on **Proc Spectrum.**

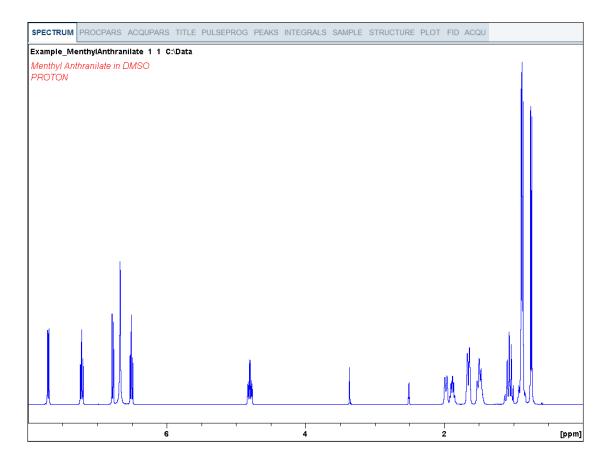


5.2.4 Integration

To quantitatively analyze an observed proton signal, the integrated intensity of the peaks is compared within each other. It is common to integrate a proton spectrum to account for the number of protons in the analyzed molecule.

To get more precise quantitative integration results, please refer to the **Quantitative NMR** manual.

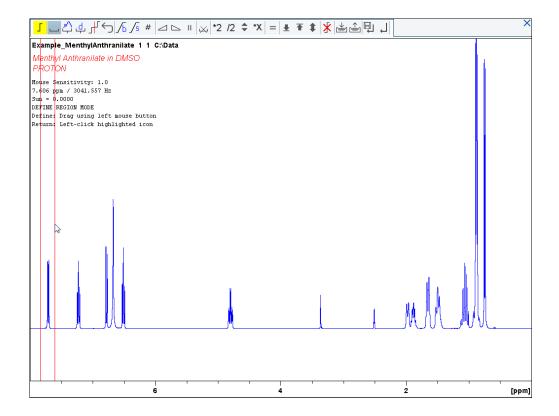
• Expand the spectrum to include all peaks.



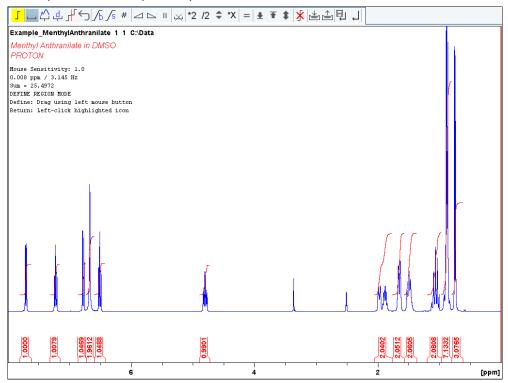
• On the menu, click Analyse | Integrate.

This enters the manual Integration mode. **The Dataset** tabs bar is replaced by the **Integration Tool** bar.

- Select the **Define new region using cursor** button.
- Set the cursor line to the left of the first peak to be integrated. Click the left mouse button and drag the cursor line to the right of the peak and then release the mouse button.



· Repeat the last step for all peaks of interest.



On the Integration Tool bar, click **Return, save region** to save the integration regions.

5.2.5 Plotting the 1D Proton Spectra

- Expand the spectrum to include all peaks.
- On the toolbar, click Retain expansion and scale.

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The spectrum can be printed as it is displayed in the SPECTRUM tab with a click on **Print** active window

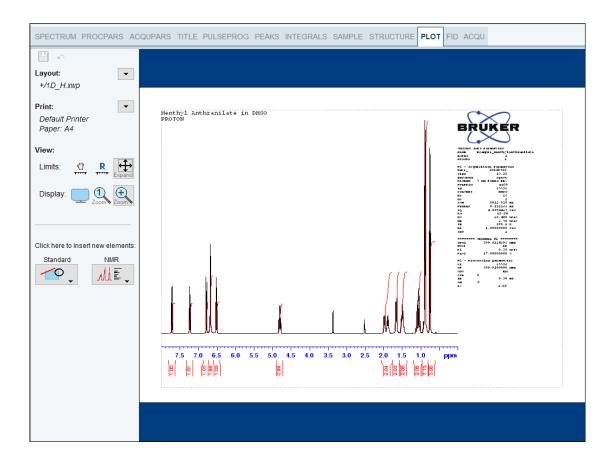
The spectrum can also be printed with a predefined layout with a click on **Export active data**

or plot window as PDF

Additional options are available with the Show more Publish Options button on the menu

bar and Switch to plot editor $\bigcirc \bigcirc \bigcirc \bigcirc \bigcirc \bigcirc \bigcirc \bigcirc \bigcirc$. You may also switch to the interactive plot editor by clicking on the **Plot** tab in the Dataset window tabs bar (see figure below).

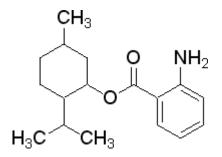
For details of working with the plot editor and modifying layouts see the **Data Publishing** manual in the help menu ?. Click **Help | Manuals | Automation and Data Publishing | Data Publishing**.



6 1D Selective Experiments

6.1 Sample

The sample of **30 mg Menthyl Anthranilate in DMSO-d6** is used for all experiments in this chapter.



6.2 1D Selective COSY

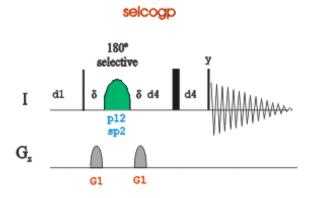
The hard pulses used in all the experiments from the previous chapters are used to uniformly excite the entire spectral width. This chapter introduces soft pulses which selectively excite only one multiplet of a ¹H spectrum. Important characteristics of a soft pulse include the shape, the amplitude, 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.



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 pulse), but for reasons of simplicity, they are often chosen to be identical.

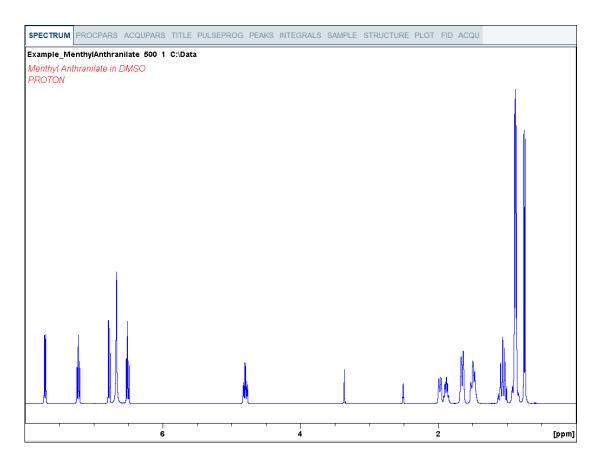
Most selective excitation experiments rely on phase cycling, and thus subtraction of spectra, to eliminate large unwanted signals. It is important to minimize possible sources of subtraction artifacts, and for this reason it is generally suggested to run selective experiments using pulse field gradients and non-spinning.

This chapter describes the acquisition and processing of a one-dimensional ¹H selective gradient COSY experiment. The standard Bruker parameter set is SELCOGP and includes the pulse sequence **selcogp** shown in the next figure. It consists of the recycling delay, four radio-frequency (RF) pulses and the acquisition time during which the signal is recorded. The first RF pulse is a 90° pulse, followed by a 180° shaped pulse, a 180° hard pulse and finally a 90° pulse. The delay between the 180° and 90° pulse is 1/4*J(H,H). The gradient pulses are applied before and after the shape pulse.



6.2.1 Reference Spectrum

• Run a **1D Proton** spectrum, following the instructions in the chapter *1D Proton Experiment*, *Experiment Setup* [▶ 31] through *Processing* [▶ 35].



6.2.2 Selective Excitation Region Setup



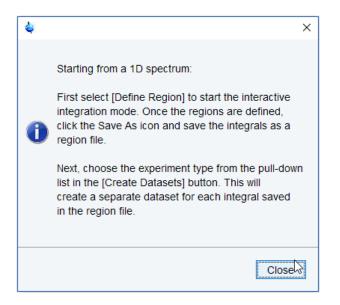
The selective pulse regions are set up using the integration tools. Power and duration of the shape pulses are calculated using the hard 90° pulse in the prosol table.

• On the menu bar, click Acquire.

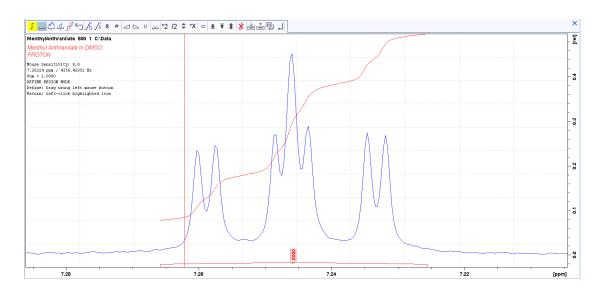
- On the More button, click the drop-down arrow to see more options.
- In the list, select Setup Selective 1D Expts.

The Workflow button bar changes for setting up the 1D selective experiment.

- On the Workflow button bar, click **1D Selective Experiment Setup.**
- In the message window, click Close.



- Expand the peak at 7.2 ppm.
- On the Workflow button bar, click **Define Regions**.
- Integrate the multiplet at 7.2 ppm.



If desired, other peaks can be integrated and a separate dataset will be created for each saved integral.

· On the toolbar, click Save/Export Integration Region.



- In the list, select Save the Region to 'reg'.
- On the toolbar, click Return do NOT save regions! to exit the integration mode.
- In the message window, click No.

4	×
0	Save Changes?
	Yes No

- On the Create Dataset button, click the drop-down arrow to see more options.
- In the list, select Selective gradient COSY.
- Click Accept.

To change the Gaus1_180r.1000 pulse, in the SELCOGP window click Change Shape.

🥌 SELCOGP 🛛 🔀		
1D Selective Gradient COSY		
Shape = Gaus	s1_180r.1000	
D 4 (sec)	0.031250	mixing time
NS	8	
first EXPNO	501	
Accept	Change Shape	Cancel

• In the SELCOGP message window, click Accept.

The new dataset is created and all parameters are automatically calculated and set.

• In the sel1d message window, click **OK** to start the acquisition.

🖕 sel1d	8
?	1D Selective Gradient COSY: SELCOGP Dataset created in expno 501. total experiment time will be 1 min 13 sec OK: starts acquisition CANCEL: creates data sets only.
	OK Cancel

6.2.3 Acquisition

• On the Workflow button bar, click Gain.

or

- On the Gain button, click the drop-down arrow to adjust rg manually.
- On the Workflow button bar, click **Run**.

or

• On the **Run** button, click the **drop-down** arrow to see more options.

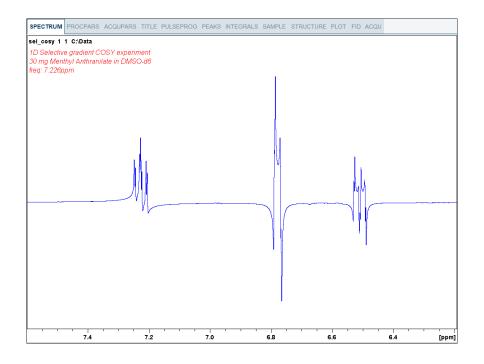
6.2.4 Processing

When the acquisition is finished:

- On the menu bar, click **Process**.
- On the Proc Spectrum button, click the drop-down arrow to see more options.
- In the list, select Configure Standard Processing (proc1d).
- In the proc1d window, deselect the following options:
 - Auto-Phasing (apk)
 - Set Spectrum Reference (sref)
 - Auto-Baseline correction (abs)
 - Warn if Processed data exist

🖕 proc1d				×
Press 'Execute' to process the curre Press 'Save' to just change the pro- Changed options will be effective will one-click 'Proc. Spectrum' button.	cessir	ng options.		
Exponential Multiply (em)	\checkmark	LB [Hz] =	0.1	
Fourier Transform (ft)				
Auto - Phasing (apk)				
Set Spectrum Reference (sref)				
Auto - Baseline Correction (absn)		Include integration =	no	\sim
Plot (autoplot)		LAYOUT =	+/1D_H.xwp	~
Warn if processed data exist	\checkmark			
			Save Execute Cance	4

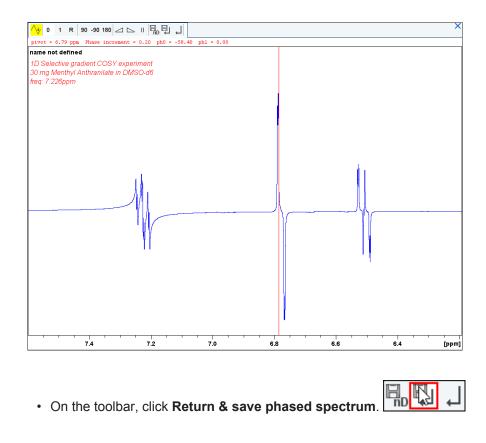
- In the proc1d window, click Execute.
- Expand the spectrum from 7.6 ppm to 6.2 ppm.



• On the Workflow button bar, click Adjust Phase.

The Dataset tabs are replaced by the Adjust Phase tool bar.

• Adjust the **0** order correction on the peak at **6.8 ppm** to display an antiphase pattern.

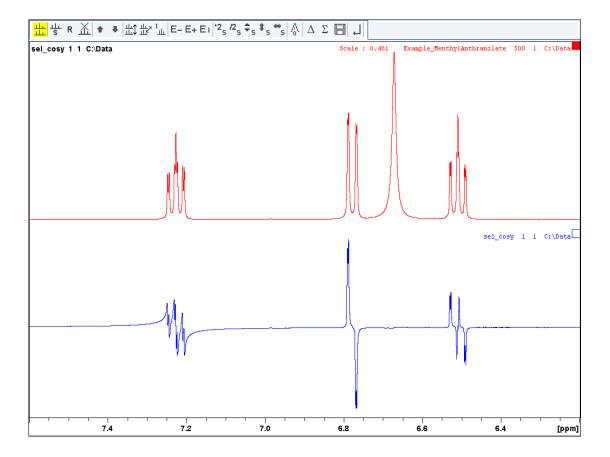


6.2.5 Plotting Two Spectra on the Same Page

Display the selective COSY spectrum.



- On the toolbar, click Multiple display.
- Drag the **Reference spectrum** (1D Proton) into the spectral window.



Click the small box in the upper right corner of the spectrum display to select the reference spectrum.

s *s

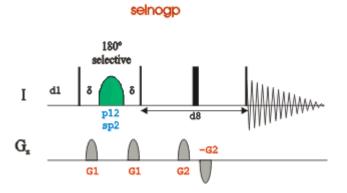
...

- Adjust the spectra for best fit with the tools:
- On the Workflow button bar, click **Print active window**

This will print the active window with the colors displayed in the TopSpin window.

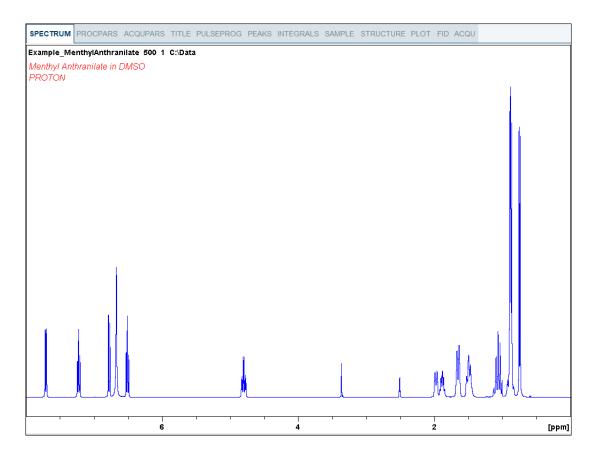
6.3 1D Selective NOESY

This chapter describes the acquisition and processing of a one-dimensional ¹H selective gradient NOESY experiment. The standard Bruker parameter set is SELNOGP and includes the pulse sequence **selnogp** shown in the next figure. It consists of the recycling delay, five radio-frequency (RF) pulses and the acquisition time during which the signal is recorded. The first RF pulse is a 90° pulse, followed by a 180° shaped pulse, a 90 degree pulse, a 180 degree pulse and finally a 90 degree pulse. The mixing time **D8** is applied before and after the 180° pulse. There are four gradient pulses applied, one each between the RF pulses.



6.3.1 Reference Spectrum

Run a **1D Proton** spectrum, following the instructions in chapter 1D Proton Experiment, *Experiment Setup* [> 31] through *Processing* [> 35].



6.3.2 Selective Excitation Region Setup

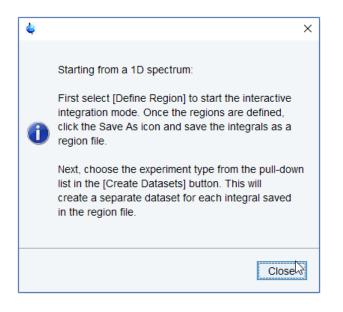


The selective pulse regions are set up using the integration tools. Power and duration of the shape pulses are calculated using the hard 90° pulse in the prosol table.

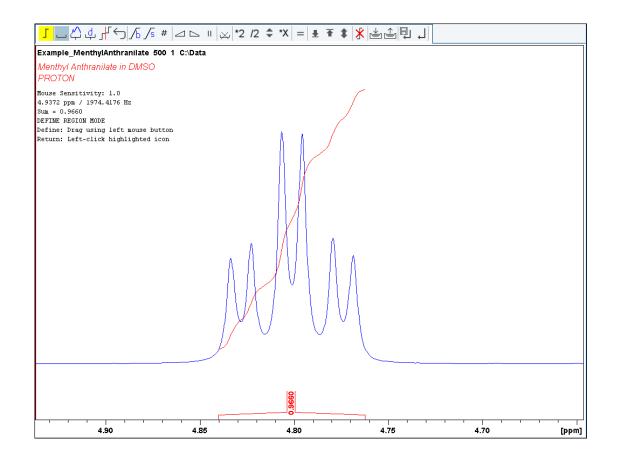
- On the menu bar, click Acquire.
- On the More button, click the drop-down arrow to see more options.
- In the list, select Setup Selective 1D Expts.

The Workflow button bar changes for setting up the 1D selective experiment.

- On the Workflow button bar, click 1D Selective Experiment Setup.
- In the message window, click Close.



- Expand the peak at 4.8 ppm.
- On the Workflow button bar, click **Define Regions**.
- Integrate the multiplet at **4.8 ppm**.



If desired, other peaks can be integrated and a separate dataset will be created for each saved integral.

- On the toolbar, click Save/Export Integration Region.
- In the list, select Save the Region to 'reg'.
- On the toolbar, click Return do NOT save regions! to exit the integration mode.

 Image: Ima
- In the message window, click No.

4	×
•	Save Changes?
	Yes No

- On the **Create Dataset** button, click the **drop-down** arrow to see more options.
- In the list, select Selective gradient NOESY.
- Click Accept.

The default parameters are taken from the standard parameter set SELNOGP. The mixing time **D8** is dependent on the size of the molecule and the magnetic field strength. It can vary from **100 ms** to **800 ms** for a large molecule to a small molecule.

- To change the **Gaus1_180r.1000** pulse, in the SELNOGP window click **Change Shape**.
- In the SELNOGP window, enter
 D8 = 0.450
 NS = 32

🖕 SELNOGPZS.2 🛛 🗙			
1D Selective Gradient NOESY			
Shape = Gaus1_180r.1000			
D 8 (sec)	0.450	mixing time	
NS	32		
first EXPNO	2		
Accept	Change Shape	Cancel	

• In the SELNOGP message window, click Accept.

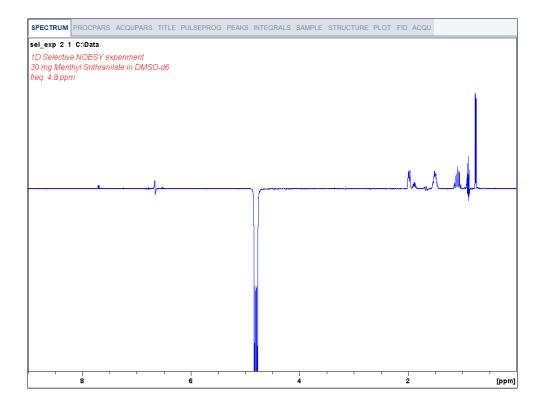
The new dataset is created and all parameters are automatically calculated and set.

• In the sel1d message window, click **OK** to start the acquisition.

🖕 sel1o	1	×
2	1D Selective Gradient NOESY: SELNOGPZS.2 Dataset created in expno 4009. total experiment time will be 3 min 56 sec OK: starts acquisition CANCEL: creates data sets only.	
	OK Cancel	

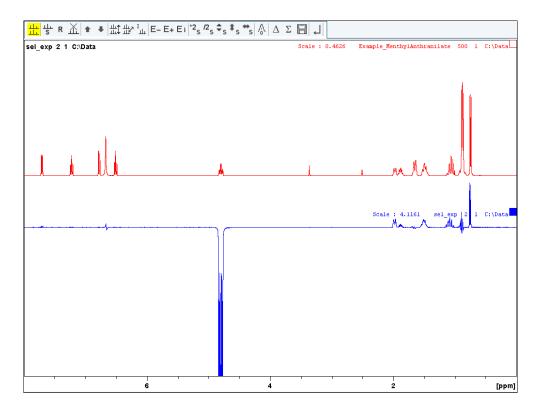
6.3.3 Processing

- Follow the first processing instructions in the chapter 1D Selective Experiments *Processing* [▶ 45].
- Manually adjust the phase of the irradiation peak at 4.8 ppm to show negative absorption and phase the peaks between 3 ppm and 1 ppm dependent on the field strength, to be either positive or negative.



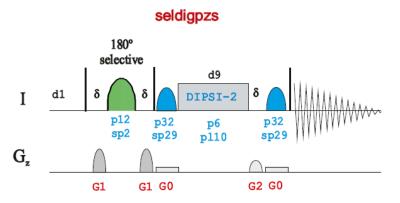
6.3.4 Plotting Two Spectra on the Same Page

- Display the selective NOESY spectrum.
- Follow the plotting instructions in chapter *Plotting Two Spectra on the Same Page* [47] for the Selective COSY.



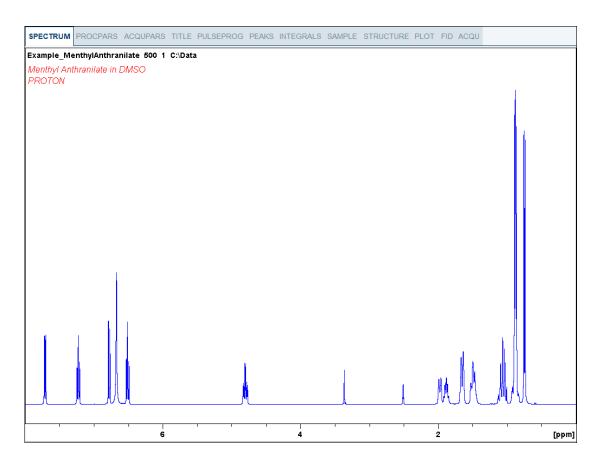
6.4 1D Selective TOCSY

This section describes the acquisition and processing of a one-dimensional ¹H selective gradient TOCSY experiment. The standard Bruker parameter set is SELDIGPZS and includes the pulse sequence **seldigpzs** shown in the figure below. It consists of the recycling delay, a radio-frequency (RF) pulse, a DIPSI2 sequence for mixing and the acquisition time during which the signal is recorded.



6.4.1 Reference Spectrum

Run a **1D Proton** spectrum, following the instructions in chapter 1D Proton Experiment, *Experiment Setup* [> 31] through *Processing* [> 35].



6.4.2 Selective Excitation Region Set Up



The selective pulse regions are set up using the integration tools. Power and duration of the shape pulses are calculated using the hard 90° pulse in the prosol table.

- On the Workflow button bar, click **Define Regions** to define the excitation region. See detailed instructions in chapter 1D Selective Experiments, Selective Excitation Region Setup [> 49] up to Selective Excitation Region Setup [> 50].
- On the Create Dataset button, click the drop-down arrow to see more options.
- In the list, select Selective gradient TOCSY.
- · Click Accept.

The default parameters are taken from the standard parameter set SELDIGPZS. If desired, click **Change Shape** to modify the **Gaus1_180r.1000** pulse. A mixing time of **0.06 s** to **0.08 s** is typical for the TOCSY experiment.

In the SELDIGPZS window, enter

D9 = **0.08** NS = **8**

🖕 SELDIGPZS 🛛 🗙				
1D Selective	1D Selective Gradient TOCSY			
Shape = Gaus	\$1.1000			
D 9 (sec)	0.080000	mixing time		
NS	8			
first EXPNO	3			
Accept	Change Shape	Cancel		

• Click Accept.

The new dataset is created and all parameters are automatically calculated and set.

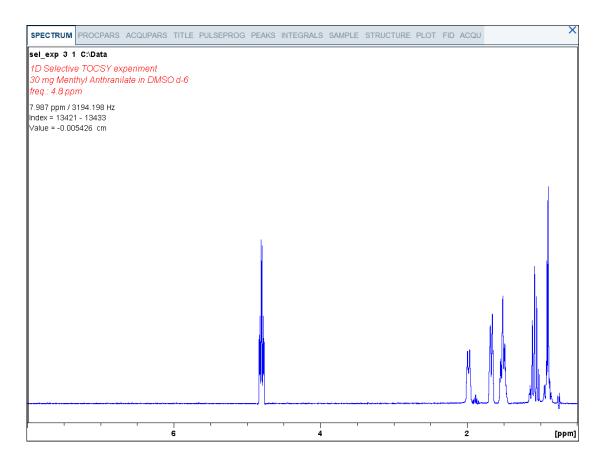
• In the sel1d message window, click **OK** to start the acquisition.

🖕 sel1o	I	×
0	1D Selective Gradient TOCSY: SELDIGPZS Dataset created in expno 3. total experiment time will be 1 min 17 sec OK: starts acquisition CANCEL: creates data sets only.	
	OK Cancel	

6.4.3 Processing

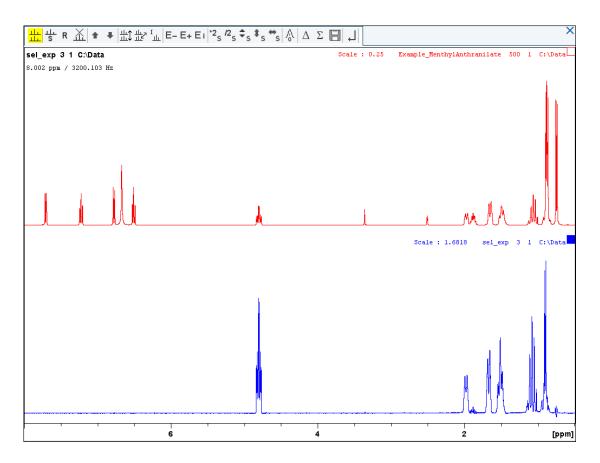
Follow the first processing instructions in the chapter 1D Selective Cosy Processing [45] up to Processing [45].

• Manually adjust the phase on all peaks for positive absorption.



6.4.4 Plotting Two Spectra on the Same Page

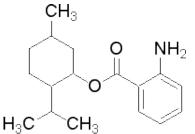
- Display the selective TOCSY spectrum.
- Follow the plotting instructions in chapter *Plotting Two Spectra on the Same Page* [47] for the Selective COSY.



7 2D Homonuclear Experiments

7.1 Sample

The sample of **30 mg Menthyl Anthranilate in DMSO-d6** is used for all experiments in this chapter.



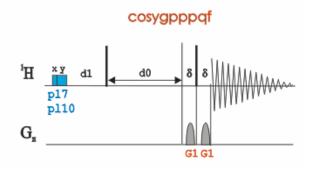
7.2 2D Gradient COSY

The COSY experiment relies on the J-couplings to provide spin-spin correlations, and its cross peaks indicate which 1H atoms are close to other 1H atoms through the bonds of the molecule. Typically, protons that are separated by up to 3 bonds can be observed.

The signals acquired with one of these experiments have absorptive and dispersive line shape 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.

Using pulsed field gradients (PFG), the coherence pathway selection and the axial peak suppression can be achieved with only one scan per time increment. Thus, if enough substance is available, a typical gradient COSY experiment with 128 time increments can be recorded in 5 minutes.

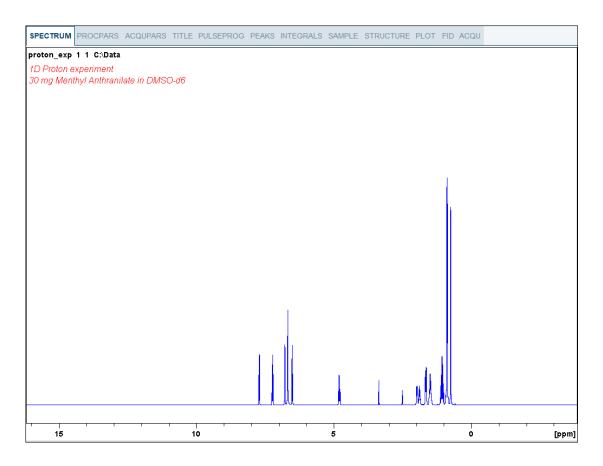
This chapter describes the acquisition and processing of a two-dimensional 1H gradient COSY. The standard Bruker parameter set is COSYGPSW and includes the pulse sequence **cosygpppqf** shown in the next figure. It consists of the recycling delay, two radio-frequency (RF) pulses, separated by the increment delay D0 and the acquisition time during which the signal is recorded. Both pulses have a 90° flip angle. Two gradient pulses are applied before and after the second pulse in the sequence. Purge pulses are applied before d1.



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.

7.2.1 Preparation Experiment

• Run a **1D Proton** spectrum, following the instructions in chapter *1D Proton Experiment*, *Experiment Setup* [▶ 31] through *Processing* [▶ 35].



7.2.2 Setting up the COSY Experiment

- On the menu bar, click Acquire | Create Dataset to open the Create New Dataset window.
- In the New Dataset window, enter or select:

NAME = cosy_exp

EXPNO = 1

Directory = e.g. C:\Data

- In the Parameters group Parameters click **Read parameterset** and select the experiment **COSYGPSW**.
- In the Create New Dataset window check Set solvent and in the drop-down list select DMSO.
- In the TITLE field enter a text stating the experiment, sample, the solvent and any other useful information. The title information can be used to search for a dataset.
- In the New Dataset window, click OK.
- On the **Spin** button, click the **drop-down** arrow to see more options.
- In the list, select Turn sample rotation off.

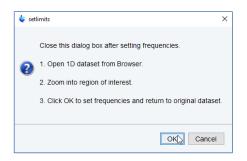
2D experiments should be run non-spinning.

• On the Workflow button bar, click **Prosol**.

This will load the pulse widths and power levels into the parameter set.

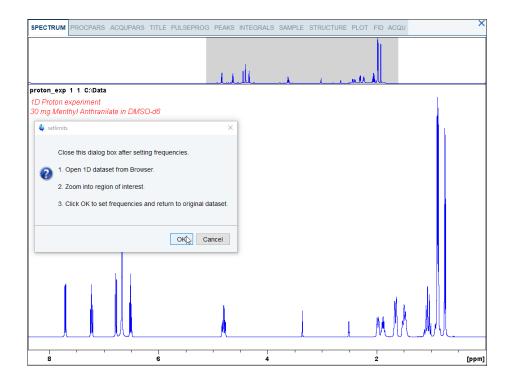
7.2.3 Limit Setting

• On the Workflow button bar, click SetLimits.



- To open the 1D Proton spectrum, right click on the dataset name in the browser window (e.g. proton_exp) and select **Display** or drag the 1D Proton dataset to the spectrum window.
- Expand the spectrum to display all peaks, leaving ca. 0.2 ppm of baseline on either side of the spectrum.

The solvent peak may be excluded if it falls outside of the region of interest. Digital filtering however is only applied in F2 and the solvent peak will be folding in F1.



- In the setlimits message window, click **OK** to assign the new limits to the 2D dataset.
- In the message window, click Close.

۵	×
1	1H spectral limits copied for F1 and F2 dimensions. SW: 9.1261 ppm O1P: 4.196 ppm
	Close

The display changes back to the 2D dataset.

7.2.4 Acquisition

• On the Workflow button bar, click Gain.

or

- On the Gain button, click the drop-down arrow to adjust the receiver gain manually.
- On the Workflow button bar, click Run.

or

• On the Run button, click the drop-down arrow to see more options.

7.2.5 Processing

When the acquisition is finished:

- On the menu bar, click **Process**.
- On the Workflow button bar, click **Proc Spectrum**.

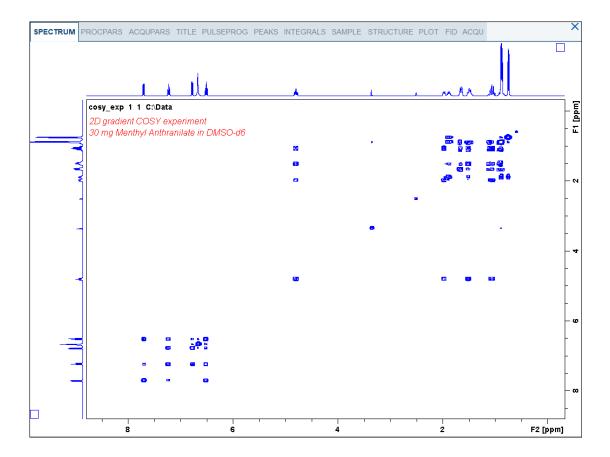
This executes a standard processing program proc2d.

The apk2d message window is displayed in case of a magnitude 2D experiment and when the **apk2d** option is enabled and the processing of the magnitude COSY it not affected.

• In the apk2d window, just click **Close**.

🍐 apk2d	×
8	Spectrum has no imaginary part: MC2[F1]=QF PH_mod[F1]=mc. Could not phase real spectrum
	Close

To disable the **apk2d** option, on the **Proc. Spectrum** button click the **drop-down** arrow to configure the Standard Processing (**proc2d**) program.



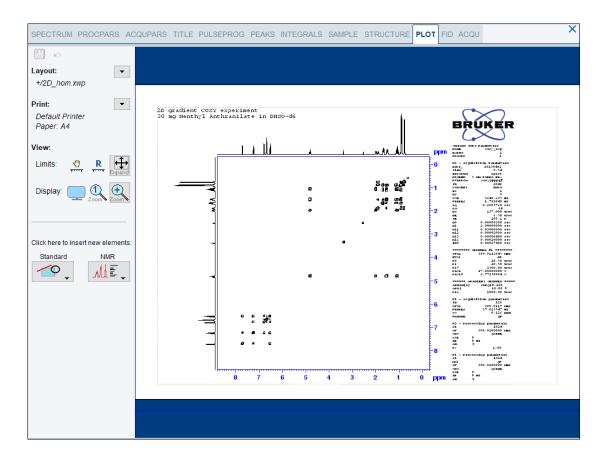
2D Homonuclear Experiments

7.2.6 Plotting the COSY Spectrum

- Use the **Smaller/larger** buttons to adjust for a suitable contour level. ^{★2} /2 | 🕄 *X 🝷 🖳
- Type .Is or click Contour levels to disk. *2 /2 | * *X



• Select Switch to plot editor (plot).



If desired, any changes can be administered by using the tools on the left side of the display.

- Click the down arrow button in the left Print section.
 Print:
- In the list, select **Print** ...

-

7.3 2D Gradient NOESY Experiment

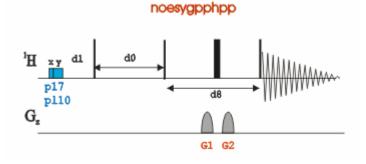
NOESY (Nuclear Overhauser Effect SpectroscopY) is a 2D spectroscopy method used to identify spins undergoing cross-relaxation and to measure the cross-relaxation rates. Most commonly, NOESY is used as a homonuclear 1H technique. In NOESY, direct dipolar couplings provide the primary means of cross-relaxation, 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 its cross peaks indicate which 1H atoms are close to other 1H atoms 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 t1, 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 d8. Note that, for the basic NOESY experiment, d8 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 t2. The NOESY spectrum is generated by a 2D Fourier transform with respect to t1 and t2.

Axial peaks, which originate from magnetization that has relaxed during tmix, 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.

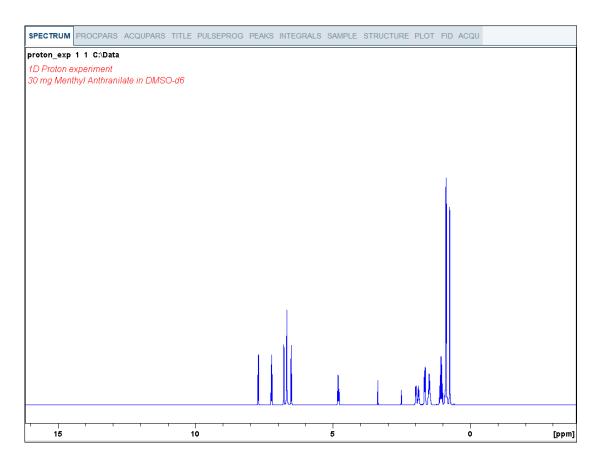
This section describes the acquisition and processing of a two-dimensional 1H phase sensitive NOESY. The standard Bruker parameter set is NOESYPHSW and includes the pulse sequence **noesygpphpp** shown in the next figure. It consists of the recycling delay, three radio-frequency (RF) pulses, separated by the increment delay D0 between the first and second pulse, a mixing time D8 between the second and third 90 degree pulses and the acquisition time during which the signal is recorded. All three pulses are of 90°.



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.

7.3.1 Preparation Experiment

• Run a **1D Proton** spectrum, following the instructions in chapter *1D Proton Experiment*, *Experiment Setup* [▶ 31] through *Processing* [▶ 35].



7.3.2 Setting up the NOESY Experiment

- On the menu bar, click Acquire | Create Dataset to open the Create New Dataset window.
- In the New window, enter or select:

NAME = noesy_exp

EXPNO = 1

Directory = e.g. C:\Data

- In the Parameters group Parameters
 click Read parameterset and select the
 experiment NOESYGPPHSW
- In the Create New Dataset window check **Set solvent** and in the drop-down list select **DMSO**.
- In the TITLE field enter a text stating the experiment, sample, the solvent and any other useful information. The title information can be used to search for a dataset.
- In the New Dataset window, click OK.

Follow the instructions in the chapter *Setting up the COSY Experiment* [> 58] for performing **Prosol** and **SetLimits**. If you know what you're doing, this should give you all the necessary information. If you need more details, you're referred to those details from the COSY experiment.

• In the Dataset window, select the **AcquPars** tab.



• In the Field D8[sec] enter 0.450.

D8 [sec] 0.450	Mixing time
----------------	-------------



The mixing time depends on the size of the molecule. The range for Bio-molecules is typically from 0.05 s to 0.2 s, medium size molecules from 0.1 s to 0.5 s and for small molecules 0.5 s to 0.9 s.

• In the Dataset window, select the Spectrum tab.

7.3.3 Acquisition

• On the Workflow button bar, click Gain.

or

- On the Gain button, click the drop-down arrow to adjust the receiver gain manually.
- On the Workflow button bar, click Run.

or

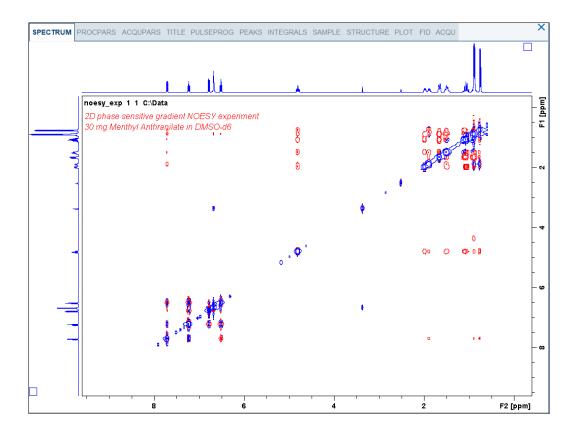
• On the **Run** button, click the **drop-down** arrow to see more options.

7.3.4 Processing

When the acquisition is finished:

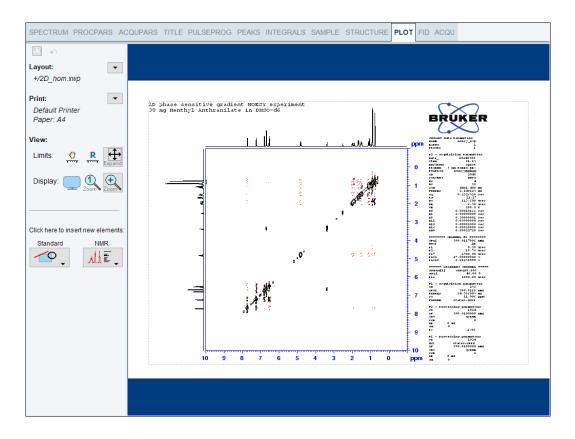
- On the menu bar, click **Process**.
- On the Workflow button bar, click **Proc Spectrum**.

This executes a standard processing program **proc2d**. The **apk2d** option has to be enabled. To enable the **apk2d** option, on the **Proc. Spectrum** button click the **drop-down** arrow and configure the **Standard Processing (proc2d)** program.



7.3.5 Plotting the NOESY Spectrum

• Follow the plotting instructions in the chapter *Plotting the COSY Spectrum* [> 62].

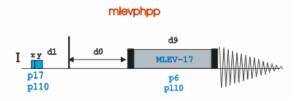


7.4 2D Phase Sensitive TOCSY Experiment

TOCSY (TOtal Correlation SpectroscopY) 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 coherence.

The coherence transfer period of the TOCSY sequence occurs during a multiple-pulse spinlock 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 JHH) should be allowed for each transfer step, and five transfer steps are typically desired for the TOCSY spectrum.

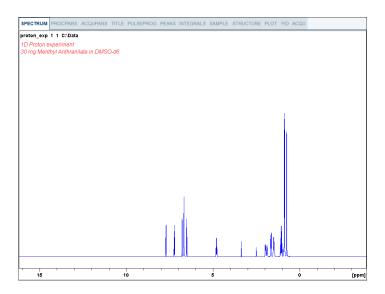
This section describes the acquisition and processing of a two-dimensional ¹H phase sensitive TOCSY. The standard Bruker parameter set is MLEVPHSW and includes the pulse sequence **mlevphpp** shown in the next figure. It consists of the recycling delay, two radio-frequency (RF) pulses, separated by the increment delay **D0** and the acquisition time during which the signal is recorded. The first RF pulse is a 90° pulse, the second pulse is the mlev spinlock pulse.



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.

7.4.1 Preparation Experiment

• Run a **1D Proton** spectrum, following the instructions in chapter *1D Proton Experiment*, *Experiment Setup* [▶ 31] through *Processing* [▶ 35].



7.4.2 Setting up the TOCSY Experiment

- On the menu bar, click Acquire | Create Dataset to open the Create New Dataset window.
- In the New window, enter or select:

NAME = tocsy_exp EXPNO = 1

Directory = e.g. C:\Data

- In the Parameters group Parameters click Read parameterset and select the experiment MLEVPHSW
- In the Create New Dataset window check **Set solvent** and in the drop-down list select **DMSO**.
- In the TITLE field enter a text stating the experiment, sample, the solvent and any other useful information. The title information can be used to search for a dataset.
- In the New Dataset window, click OK.

Follow the instructions in the chapter *Setting up the COSY Experiment* [> 58] for performing **Prosol** and **SetLimits**. If you know what you're doing, this should give you all the necessary information. If you need more details, you're referred to those details from the COSY experiment.

• In the Dataset window, select the AcquPars tab.



- In the AcquPars tab toolbar click Show pulse program parameters
- In the Field D9[sec] enter 0.08.

D9 [sec] 0.08	TOCSY mixing time
---------------	-------------------



A mixing time of 0.06 s to 0.08 s is typical for the TOCSY experiment.

• In the Dataset window, select the Spectrum tab.

7.4.3 Acquisition

• On the Workflow button bar, click Gain.

or

- On the Gain button, click the drop-down arrow to adjust the receiver gain manually.
- On the Workflow button bar, click **Run**.

or

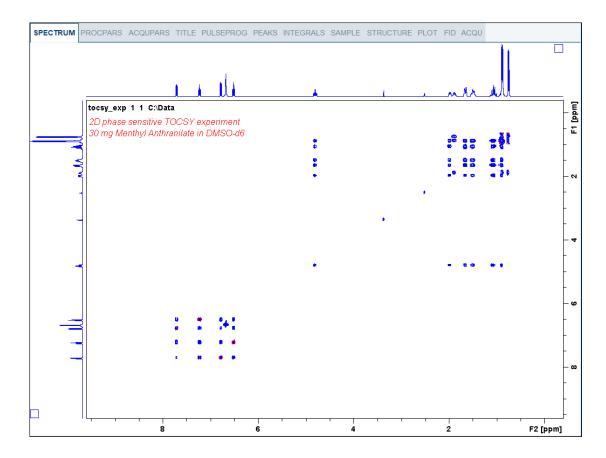
• On the Run button, click the drop-down arrow to see more options.

7.4.4 Processing

When the acquisition is finished:

- On the menu bar, click **Process**.
- On the Workflow button bar, click **Proc Spectrum**.

This executes a standard processing program **proc2d**. The **apk2d** option has to be enabled. To enable the **apk2d** option, on the **Proc. Spectrum** button click the **drop-down** arrow and configure the **Standard Processing (proc2d)** program.



7.4.5 Plotting the TOCSY Spectrum

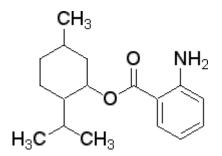
• Follow the plotting instructions in the chapter *Plotting the COSY Spectrum* [> 62].

yout:					
+/2D_110111.xwp					
rint: 💌	2D phase sensitive	TOCSY experiment			
Default Printer	30 mg Menthyl Anth	ranilate in DMSO-d6		h	BRUKER
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iew:		1.111			ORIENTE ENGAFARMATERO PPDM EXEMPS 1
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8 1D Carbon Experiments

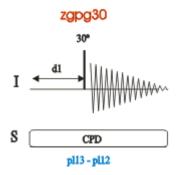
8.1 Sample

The sample of **30 mg Menthyl Anthranilate in DMSO-d6** is used for all experiments in this chapter.



8.2 1D Carbon Experiment

This chapter describes the acquisition and processing of a one-dimensional 13C NMR spectrum. The standard Bruker parameter set C13CPD, includes the pulse sequence **zgpg30**, shown in the figure below. The ¹³C channel consists of the recycling delay, a RF pulse, and the acquisition time during which the signal is recorded. The pulse angle is shown to be 30°. The two parameters, D1 and P1, correspond to the length of the recycle delay, and the length of the 90° RF pulse, respectively. The ¹H channel consists of two decoupling pulses which can be power gated. The first pulse, an NOE build up pulse during the recycle delay may be of lower power then the second pulse on during the acquisition which is the true decoupling pulse. This can be useful to avoid RF heating on salty samples or probes where a higher decoupling power can be problematic.



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.

8.2.1 Experiment Setup

- On the menu bar, click Acquire | Create Dataset to open the Create New Dataset window.
- In the New Dataset window, enter or select:

NAME = carbon_exp EXPNO = 1

Directory = e.g. C:\Data

- In the Parameters group Parameters
 click Read parameterset and select the
 experiment C13CPD
- In the Create New Dataset window check Set solvent and in the drop-down list select DMSO.

Create New Dataset - new		— ×
	w experiment by creating a new data set and R parameters according to the selected experiment type.	
	er experiments several datasets are created.	
Please define the	e number of receivers in the Options.	
Dataset		
NAME	carbon_exp	
EXPNO	1	
Directory	C:\Data	-
Open in new window	N	
Set solvent Additional action Do nothing Execute getprosol	DMSO •	
Keep parameters	P 1, 01, PLW 1 Change	
Advanced		
Number of datasets (r	receivers) 1	
Title		
THUE	MEO	
Menthyl Anthranilate in D C13CPD	10150	

- In the TITLE field enter a text stating the experiment, sample, the solvent and any other useful information. The title information can be used to search for a dataset.
- In the New Dataset window, click OK.
- In the Dataset window, select the AcquPars tab.
- Make the following change:

NS = 128

• On the menu bar, click Acquire.



To aquire a spectrum, use the Workflow buttons from left to right.

- On the Sample button, click the drop-down arrow to see more options.
- In the list, select **Eject sample manually (ej)**. The sample lift is turned on.

j

Wait until the sample lift air is turned on and remove any sample which may have been in the magnet.

- Place the sample plus the spinner on top of the magnet bore.
- On the Sample button, click the drop-down arrow to see more options.
- In the list, select Insert sample manually (ij).



Wait until the sample is lowered down into the probe and the lift air is turned off. A clicking sound may be heard.

- On the Workflow button bar, click Lock.
- In the Solvents table list, select DMSO and click OK.

🖕 Solvents table		×					
△ Solvent Description							
Acetic	acetic acid-d4						
Acetone	acetone-d6						
C6D6	benzene-d6						
CD2Cl2	dichlormethane-d2						
CD3CN	acetonitrile-d3						
CD3CN_SPE	LC-SPE Solvent (Acetonitrile)						
CD3OD_SPE	LC-SPE Solvent (Methanol-d4)						
CDCI3	chloroform-d						
CH3CN+D2O	HPLC Solvent (Acetonitril/D2O)						
CH3OH+D2O	HPLC Solvent (Methanol/D2O)						
D2O	deuteriumoxide						
D2O_salt	deuteriumoxide with salt						
Dioxane	dioxane-d8						
DMF	N,N-dimethylformamide-d7						
DMSO	dimethylsulfoxide-d6						
EtOD	ethanol-d6						
H2O+D2O	90%H2O and 10%D2O						
H2O+D2O_salt	90%H2O and 10%D2O with salt						
HDMSO	90%DMSO and 10%DMSO-d6						
Juice	fruit juice						
MeOD	methanol-d4						
Plasma	blood plasma						
Pyr	pyridine-d5						
T_H2O+D2O+Me4NCI	(CD3)4NCI in 90%H2O and 10%D2O, for NMR therr	nometer					
T_H2O+D2O+NaAc	sodium acetate in 90%H2O and 10%D2O, for NMR	thermometer					
T_H2O+D2O+Pivalate	pivalate-d9 in 90% H2O and 10% D2O, for NMR the	ermometer					
T_MeOD	methanol-d4, for NMR thermometer						
TFE	trifluroethanol-d3						
THF	tetrahydrofuran-d8						
Tol	toluene-d8						
Urine	urine						
Lock nucleus: 2H V		OK Cancel					

• On the Workflow button bar, click Tune.



This performs an **atma** (automatic tuning and matching) and requires a probe equipped with an automatic tuning and matching module. The tuning always starts with the lowest frequency, in this case carbon, and then switches over to tune the higher frequencies, in this case proton. On the **Tune** button, click the **drop-down** arrow to see more options.

- On the **Spin** button, click the **drop-down** arrow to see more options.
- In the list, select Turn sample rotation on (ro on).



Rotation may be turned off for probes such as **BBI**, **TXI**, **TBI** and for small sample probes.

• On the Workflow button bar, click Shim.

This executes the command **topshim**. On the **Shim** button click the **drop-down** arrow to see more options.

• On the Workflow button bar, click **Prosol**.

This will load the pulse widths and power levels into the parameter set.

8.2.2 Acquisition

- On the Workflow button bar, click Gain.
- or
 - On the Gain button, click the drop-down arrow to adjust the receiver gain manually.
- On the Workflow button bar, click **Run**.

or

• On the **Run** button, click the **drop-down** arrow to see more options.

8.2.3 Processing

When the acquisition is finished:

- On the menu bar, click **Process**.
- On the Proc Spectrum button, click the drop-down arrow to see more options.
- In the list, select Configure Standard Processing (proc1d).
- In the proc1d window, select the options:
 - Exponential Multiply (em)
 - Auto Phasing (apk)

Set Spectrum Reference (sref)

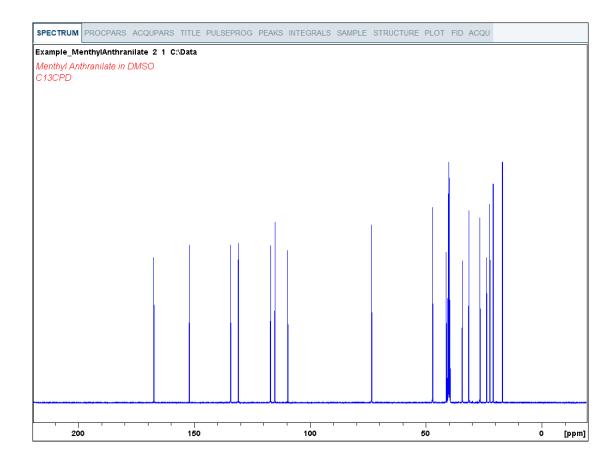
Auto - Baseline Correction (absn)

Press 'Execute' to process the curre Press 'Save' to just change the proc			
Changed options will be effective wh			
one-click 'Proc. Spectrum' button.			
Exponential Multiply (em)	\checkmark	LB [Hz] =	1
Fourier Transform (ft)	\checkmark		
Auto - Phasing (apk)	v		
Set Spectrum Reference (sref)	v		
Auto - Baseline Correction (absn)	v	Include integration =	no
Plot (autoplot)		LAYOUT =	+/1D_X.xwp
Warn if processed data exist	v		

- In the proc1d window, click **Execute**.
- In the proc1d window, click **Save** to save the selected processing settings.

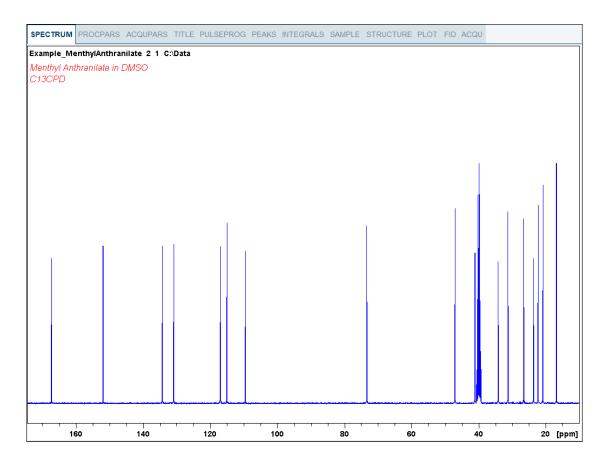


Now all future datasets can be processed with the defined actions with a click on **Proc Spectrum**.



8.2.4 **Peak Picking**

· Expand the spectrum to include all peaks.



- On the menu bar, click Analyse.
- On the Workflow button bar, click Pick Peaks.

or

• On the Pick Peaks button, click the drop-down arrow to see more options.

This enters the manual peak picking mode.

The Dataset tabs are replaced by the Peak Picking toolbar.

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By default the **Define new peak picking range** button is enabled.

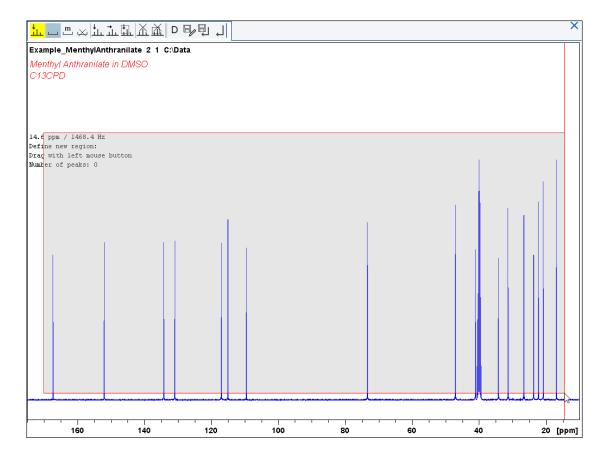
 Click left and drag the cursor line from left to the right side of the spectrum, drawing a rectangular box.

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The Modify existing peak picking range button allows raising the bottom and/or lowering the top of the peak picking box to exclude noise or solvent.

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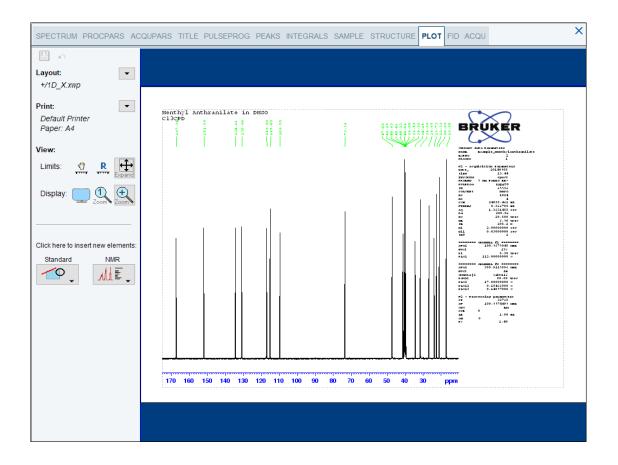
- On the Peak Picking toolbar, click **Return, save region** to store the peak values.
- To display the peak picking labels, right click in the spectrum window and select **Spectra Display Preferences**. In the Spectrum components enable **Peak labels** and **Peak annotations**. Click **Apply** and **Close**.

8.2.5 Plotting the 1D Carbon Spectrum

- Expand the spectrum to include all peaks.
- On the toolbar, click **Retain expansion and scale**. ④ ⑤ 🖸 🔐 点 壯
- On the menu bar, click Show more Publishing Options.

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Select Switch to plot editor (plot).





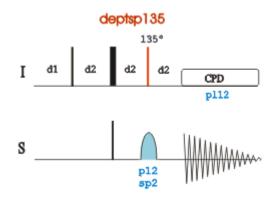
If desired, any changes can be administered with the tools on the left side of the display.

- In the left **Print** section, click the **drop-down** arrow to see more options.
- In the list, select **Print**.

8.3 DEPT-135 Experiment

DEPT (Distortionless Enhancement by Polarization Transfer) is a polarization transfer technique used for the observation of nuclei with a small gyro magnetic ratio, which are J-coupled to 1H (most commonly 13C). DEPT is a spectral editing sequence, that is, it can be used to generate separate 13C sub spectra for methyl (CH_3), methylene (CH_2), and methine (CH) signals. DEPT makes use of the generation and manipulation of multiple quantum coherence to differentiate between the different types of 13C signals. Quaternary carbons are missing a direct bond proton, and as a result are absent from all DEPT spectra.

This chapter describes the acquisition and processing of a one-dimensional 13C-DEPT135 NMR spectrum. The standard Bruker parameter set C13DEPT135, includes the pulse sequence **deptsp135**, shown in the figure below. The 13C channel consists of the recycling delay, a 90° RF pulse, an editing delay D2 followed by a 180° shaped pulse and the acquisition time during which the signal is recorded. The editing delay D2 is 1/2*J(XH). The 1H channel consists of three pulses, a 90°, a 180°, followed by a 135° RF pulse and are separated by the editing delay D2. The final 135° 1H pulse selects the CH₃, CH₂ or CH signals. The protons are decoupled during the acquisition period.



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.

8.3.1 Experiment Setup

This experiment usually follows a regular ¹H decoupled ¹³C experiment. The result of a DEPT-135 experiment shows only the protonated carbons with the CH and CH₃ as positive and the CH₂ as negative signals.

- On the menu bar, click Acquire | Create Dataset to open the Create New Dataset window.
- · In the New Dataset window, enter or select:

NAME = carbon_exp

EXPNO = 2

Directory = e.g. C:\Data

- In the Parameters group Parameters click Read parameterset and select the experiment C13DEPT135
- In the Create New Dataset window check Set solvent and in the drop-down list select DMSO.
- In the TITLE field enter a text stating the experiment, sample, the solvent and any other useful information. The title information can be used to search for a dataset.
- In the New Dataset window, click OK.
- In the Dataset window, select the AcquPars tab.
- Enter:

NS = 64

- On the menu bar, click Acquire.
- On the Workflow button bar, click **Prosol**.

This will load the pulse widths and power levels into the parameter set.

8.3.2 Acquisition

• On the Workflow button bar, click Gain.

or

- To adjust the receiver gain manually, on the Gain button click the drop-down arrow.
- On the Workflow button bar, click Run.

or

• On the **Run** button, click the **drop-down** arrow to see more options.

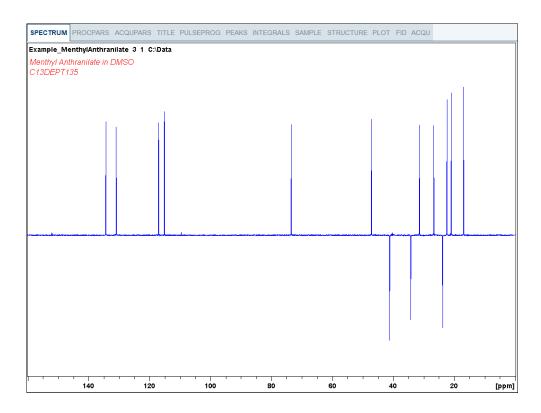
8.3.3 Processing

When the acquisition is finished:

- On the menu bar, click Process.
- On the Workflow button bar, click Proc Spectrum.



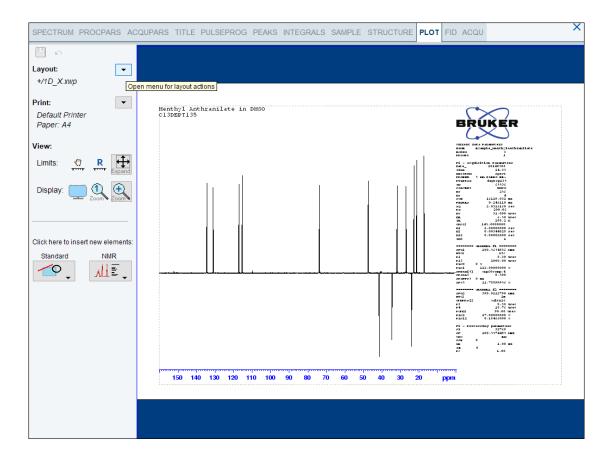
Proc. Spectrum executes a processing program including commands such as an exponential window function **em**, Fourier transformation **ft**, an automatic phase correction **apk** and a baseline correction **abs**. On the **Proc. Spectrum** button, click the **drop-down** arrow to see more options. In the list, select **Configure Standard Processing (proc1d)**. Due to the fact that a DEPT135 spectrum contains negative and positive peaks, there is the possibility of getting phase results that are 180 degrees off from the convention of -CH and - CH₃ peaks phased positively and -CH₂ peaks phased negatively. In this case, click **Adjust Phase** to enter the manual phase routine and reverse the spectrum by clicking on the **180** icon.



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8.3.4 Plotting the DEPT-135 Spectrum

- Expand the spectrum to include all peaks.
- On the toolbar, click **Retain expansion and scale**.
- On the menu bar, click Show more Publishing Options.



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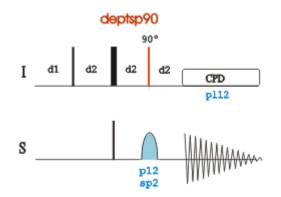
If desired, any changes can be administered with the tools on the left side of the display.

- In the left **Print** section, click the **drop-down** arrow to see more options.
- In the list, select **Print**.

8.4 DEPT-90 Experiment

This section describes the acquisition and processing of a one-dimensional 13C-DEPT90 NMR spectrum. The standard Bruker parameter set C13DEPT90, includes the pulse sequence **dept90**, shown in the next figure. The 13C channel consists of the recycling delay, a 90° RF pulse, an editing delay D2 followed by a 180° shaped pulse and the acquisition time during which the signal is recorded. The editing delay D2 is 1/2*J(XH). The 1H channel consists of three pulses, a 90 degree, a 180 degree, followed by a 90° RF pulse and are separated by the editing delay D2. The final 90° 1H pulse selects the CH signals only. The

protons are decoupled during the acquisition period. The filtering of the CH_2 and CH_3 resonances is aided by having accurate 90 degree pulse calibrations although small CH_2 and/ or CH_3 peaks may *bleed through* because of their J_{CH} value.



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.

8.4.1 Experiment Setup



The DEPT90 experiment usually follows a regular ¹H decoupled ¹³C experiment and a DEPT-135 experiment. It is used to assign the methine (CH) signals.

- On the menu bar, click Acquire | Create Dataset to open the Create New Dataset window.
- · In the New Dataset window, enter or select:

```
NAME = carbon_exp
EXPNO = 3
```

```
Directory = e.g. C:\Data
```

- In the Parameters group Parameters click Read parameterset and select the experiment C13DEPT90
- In the Create New Dataset window check Set solvent and in the drop-down list select DMSO.
- In the TITLE field enter a text stating the experiment, sample, the solvent and any other useful information. The title information can be used to search for a dataset.
- In the New Dataset window, click OK.
- In the Dataset window, select the AcquPars tab.
- · Make the following change:
 - NS = 64
- On the menu bar, click Acquire.
- On the Workflow button bar, click **Prosol**.

This will load the pulse widths and power levels into the parameter set.

8.4.2 Acquisition

• On the Workflow button bar, click Gain.

or

- To adjust the receiver gain manually, on the **Gain** button click the **drop-down** arrow.
- On the Workflow button bar, click Run.

or

• On the Run button, click the drop-down arrow to see more options.

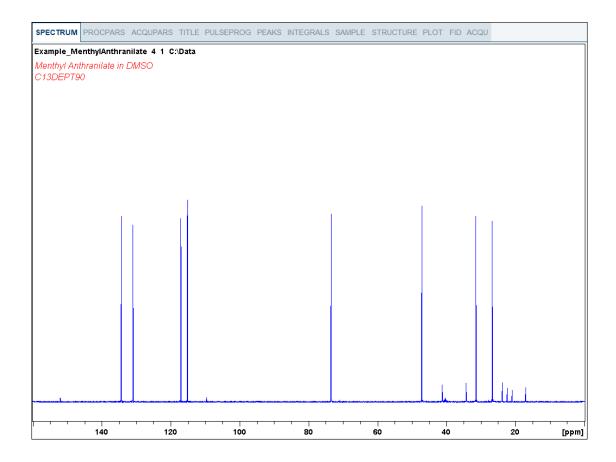
8.4.3 Processing

When the acquisition is finished:

- On the menu bar, click Process.
- On the Workflow button bar, click Proc Spectrum.



Proc. Spectrum executes a processing program including commands such as an exponential window function **em**, Fourier transformation **ft**, an automatic phase correction **apk** and a baseline correction **abs**. On the **Proc. Spectrum** button, click the **drop-down** arrow to see more options. In the list, select **Configure Standard Processing (proc1d)**.



8.4.4 Plotting the DEPT-90 Spectrum

- · Expand the spectrum to include all peaks.
- On the toolbar, click **Retain expansion and scale**.
- On the menu bar, click Show more Publishing Options.
- Select Switch to plot editor (plot).

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<u>н</u> ю	QUPARS TITLE PULSEPROG PEAKS INTEGRALS SAMPLE STRUCTURE PLOT FID ACQU
+/1D_X.xwp Print: Default Printer Paper: A4 View: Limits: ① R	Menthyl Anthranilate in DHSO Cl3DEF190 www.dersachte combinet.combinet.combinet.com combinet.combinet.combinet.combinet.com combinet.combinet.combinet.combinet.combinet.combinet.combinet.com combinet.combine
Click here to insert new elements:	150 140 130 120 110 100 90 80 70 60 50 40 30 20 ррт

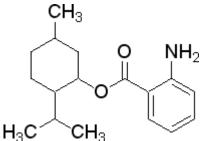
If desired, any changes can be administered with the tools on the left side of the display.

- In the left **Print** section, click the **drop-down** arrow to see more options.
- In the list, select Print.

9 2D Heteronuclear Experiments

9.1 Sample

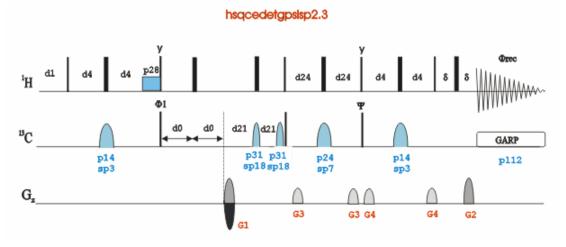
The sample of **30 mg Menthyl Anthranilate in DMSO-d6** is used for all experiments in this chapter.



9.2 2D Edited HSQC

The **HSQC** (Heteronuclear Single Quantum Coherence) experiment performs an H,C-correlation spectrum via the ¹³C chemical shift evolution of the single-quantum coherence. This method is superior to other heteronuclear experiments in the case of a crowded ¹³C NMR spectrum.

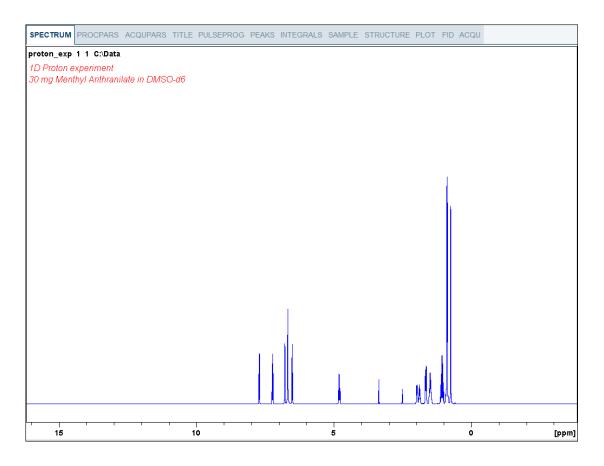
In the sequence shown in the next figure, the signals are not broadened by homonuclear H,H coupling in F1. It is possible to obtain a complete editing of inverse recorded 1D H,X correlation spectra. This kind of multiplicity determination has been achieved by including an editing period within HSQC. In the experiment shown here the standard Bruker parameter set HSQCEDETGPSISP2.3_ADIA is used and the graphical display of the pulse program **hsqcedetgpsisp2.3** is shown in the figure below.



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.

9.2.1 **Preparation Experiment**

• Run a **1D Proton** spectrum, following the instructions in Chapter 1D Proton Experiment, *Experiment Setup* [▶ 31] through *Processing* [▶ 35].



9.2.2 The HSQC Experiment Setup

- On the menu bar, click Acquire | Create Dataset to open the Create New Dataset window.
- In the New Dataset window, enter or select:

NAME = hsqc_exp

EXPNO = 1

Directory = e.g. C:\Data

- In the Parameters group Parameters
 click Read parameterset and select the
 experiment HSQCEDETGPSISP2.3_ADIA
- In the Create New Dataset window check Set solvent and in the drop-down list select DMSO.
- In the TITLE field enter a text stating the experiment, sample, the solvent and any other useful information. The title information can be used to search for a dataset.
- In the New Dataset window, click OK.
- On the menu bar, click Aquire.
- On the **Spin** button, click the **drop-down** arrow to see more options.
- In the list, select Turn sample rotation off.

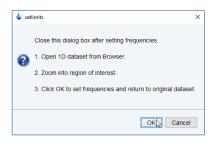
2D experiments should be run non-spinning.

• On the Workflow button bar, click **Prosol**.

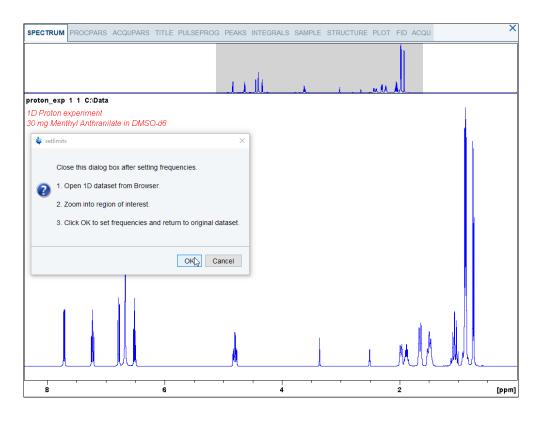
This will load the pulse widths and power levels into the parameter set.

9.2.3 Limit Setting

• On the Workflow button bar, click SetLimits.



- To open the 1D Proton spectrum, right click on the dataset name in the browser window (e.g. proton_exp) and select **Display** or drag the 1D Proton dataset to the spectrum window.
- Expand the spectrum to display all peaks, leaving ca. 0.2 ppm of baseline on either side of the spectrum.



· In the setlimits message window, click OK to assign the new limit.

• In the message window, click Close.

0	1H spectral limits copied for F2 dimension. SW: 10.1051 ppm O1P: 4.462 ppm
	Close

The display changes back to the 2D dataset.

The parameter set HSQCEDETGPSISP2.3_ADIA has a fixed F1 sweep width of **160 ppm** and it is big enough to cover the protonated resonances for a broad range of samples. If desired, changes to the F1 sweep width can be done by using the **SetLimits** button for a second time. In this case a 1-D **C13DEPT45** or **C13DEPT135** experiment on the same sample has to be observed. Be aware, if the acquisition time is increased do to making the sweep width smaller (e.g. no aromatic peaks), there may be a risk of heating the sample. As an example to set the F1 limit, follow the steps below.

• On the Workflow button bar, click SetLimits.

🖕 set	limits	×
?	Close this dialog box after setting frequencies.1. Open 1D dataset from Browser.2. Zoom into region of interest.3. Click OK to set frequencies and return to original dataset	
	OK Cancel	

- To open the 1D C13DEPT135 spectrum, right click on the dataset name in the browser window (e.g. carbon_exp 2) and select Display or drag the 1D C13DEPT135 dataset to the spectrum window.
- Expand the spectrum to display all peaks, leaving ca. 5 ppm of baseline on either side of the spectrum.

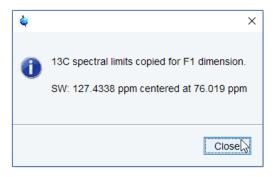


The solvent peak may be excluded if it falls outside of the region of interest. Digital filtering however is only applied in F2 and the excluded solvent peak will be folded in F1.

2D Heteronuclear Experiments

SPECTRUM PROCPARS ACQUPARS	S TITLE PULSEPROG	PEAKS INTEGRALS SAMP	PLE STRUCTURE F	PLOT FID ACQU	×
carbon_exp 2 1 C:\Data					
1D Carbon Dept135 experiment 30 mg Menthyl Anthranilate in DMS	SO-d6				
	 Open 1D da Zoom into re 	og box after setting frequencie ataset from Browser. egion of interest. set frequencies and return to o			
120	100	80		40	 20 [ppm]

- In the setlimits message window, click OK to assign the new limit.
- In the message window, click Close.



9.2.4 Acquisition

- On the Workflow button bar, click Gain.
- On the Workflow button bar, click Run.

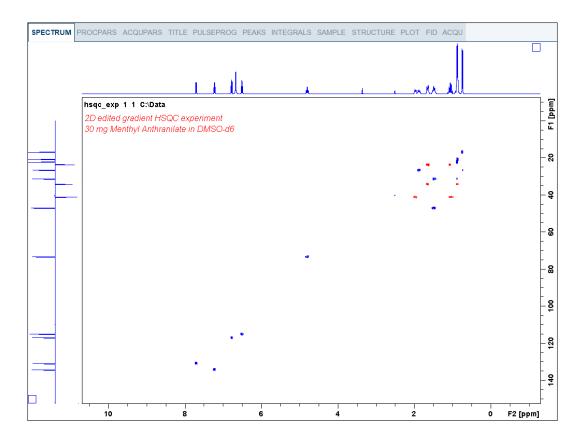
9.2.5 Processing

When the acquisition is finished:

- On the menu bar, click **Process**.
- On the Workflow button bar, click **Proc Spectrum**.

This executes a standard processing program proc2d.

The **apk2d** option has to be enabled. To enable **the apk2d** option, on the Workflow button bar click the **drop-down** arrow in the Proc. Spectrum button and configure the **Standard Processing (proc2d)** program. By default, the baseline of the F1 projection will be at the bottom, cutting off the negative peaks of the DEPT135 spectrum. Right click inside the F1 projection window and change the setting to display the baseline at the center. The CH and CH₃ groups will phase the same and CH₂ groups will phase oppositely. The convention is to phase CH,CH₃ positive and CH₂ negative. The **apk2d** command may invert this.



9.2.6 Plotting the 2D HSQC Spectrum

- On the toolbar, click **Smaller/larger** and move the mouse up or down to adjust a suitable contour level. ^{★2} /2 | →★ 至 →
- Type .Is or click Save the contour levels to disk. ^{*2} /2 | ♀ *X 至[]
- On the menu bar, click **Print active window**.

This will print the active window with the colors displayed in the TopSpin window showing both the F2 and F1 projections.



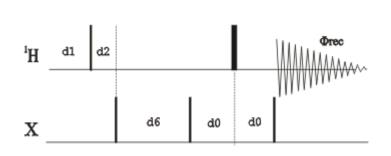
The default layout is designed not to show the F1 projection and the negative peak colors are not assigned. A new layout has to be created to add the F1 projection (in this example, red).

9.3 2D HMBC Experiment

The basic 2D HMBC pulse sequence (see the figure below) is closely related to the HMQC pulse sequence but incorporating the following modifications:

- An optional low-pass J-filter (consisting of a delay-90⁰(13C) cluster) can be included after the initial 90⁰ 1H pulse to minimize direct response.
- The defocusing period is optimized to 1/2*ⁿJ(CH) (5-10Hz).
- The refocusing period is usually omitted.
- Proton acquisition is performed without X decoupling.

Using this experiment qualitative heteronuclear long-range connectivity, including quaternary carbons or through heteronuclei can be extracted.



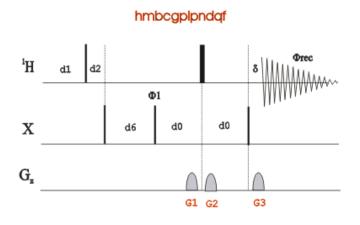
hmbclpndqf

The non-gradient 2D HMBC spectrum of Menthyl Anthranilate in DMSO-d6 shows considerable artifacts. Additionally a minimum number of 8 scans is required for the full phase cycling.

The main advantages of using gradients in high resolution NMR experiments include:

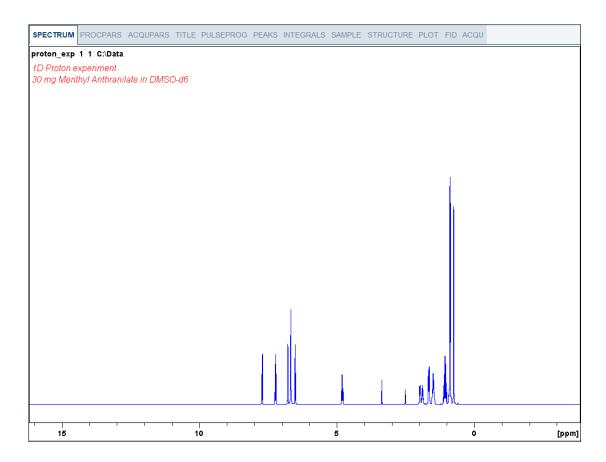
- Coherence selection and frequency-discrimination in the indirect dimension (F1) can be achieved with a single scan per t1 increment.
- A reduction in the number of required phase cycle steps for the suppression of undesired coherences.
- An important decrease in the total acquisition times for sufficiently concentrated samples.
- The obtaining of higher quality spectra with an important reduction in T1 noise.
- An efficient suppression of undesired signals such as, for instance, the intense solvent signal in H2O solution and the 1H-12C (1H-14N) magnetization in proton detected heteronuclear experiments at natural abundance. In these inverse experiments, the starting BIRD cluster or spin-lock pulse are no longer needed.
- A much easier data processing and therefore more accurate spectral analysis.
- A decrease of dynamic-range limitation.

The figure below shows the gradient HMBC pulse sequence.



9.3.1 Preparation Experiment

• Run a **1D Proton** spectrum, following the instructions in Chapter 1D Proton Experiment, *Experiment Setup* [▶ 31] through *Processing* [▶ 35].



9.3.2 The HMBC Experiment Setup

- On the menu bar, click **Acquire** | **Create Dataset** to open the Create New Dataset window.
- In the New window, enter or select:

NAME = hmbcgp_exp EXPNO = 1

Directory = e.g. C:\Data

- In the Parameters group Parameters
 click Read parameterset and select the
 experiment HMBCGPND
- In the Create New Dataset window check **Set solvent** and in the drop-down list select **DMSO**.
- In the TITLE field enter a text stating the experiment, sample, the solvent and any other useful information. The title information can be used to search for a dataset.
- In the New Dataset window, click **OK**.
- On the menu bar, click Acquire.
- On the **Spin** button, click the **drop-down** arrow to see more options.
- In the list, select Turn sample rotation off.



2D experiments should be run non-spinning.

• On the Workflow button bar, click **Prosol**.

This will load the pulse widths and power levels into the parameter set.

9.3.3 Limit Setting

• On the Workflow button bar, click SetLimits.

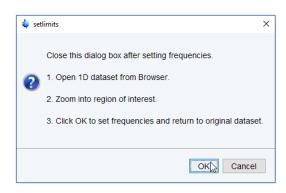
🖕 set	limits	×
?	Close this dialog box after setting frequencies.1. Open 1D dataset from Browser.2. Zoom into region of interest.3. Click OK to set frequencies and return to original dataset	-
	OK Cancel	

 To open the 1D Proton spectrum, right click on the dataset name in the browser window (e.g. proton_exp) and select Display or drag the 1D Proton dataset into the spectrum window.

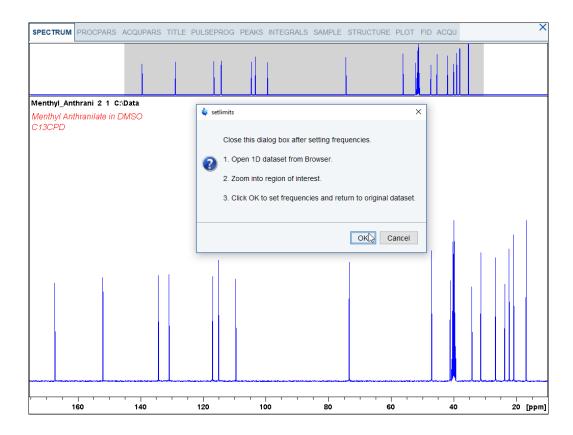
- Expand the spectrum to display all peaks, leaving ca. **0.2 ppm** of baseline on either side of the spectrum.
- Click OK in the setlimits message window to assign the new limit.

The display changes back to the 2D data set. The parameter set HMBCGP has a fixed F1 sweep width of 222 ppm and it is big enough to cover all Carbon resonances for a broad range of samples. If desired, changes to the F1 sweep width can be done with the **Set_limits** button for a second time. In this case a 1D C13CPD experiment on the same sample has to be selected. As an example to set the F1 limit, follow the steps below.

• On the Workflow button bar, click **SetLimits**.



- To open the 1D C13 spectrum, right click on the dataset name in the browser window and select **Display** or drag the 1D C13 dataset into the spectrum window.
- Expand the spectrum to display all peaks, leaving ca. **5 ppm** of baseline on either side of the spectrum.



- Click **OK** in the setlimits message window to assign the new limit.
- In the message window, click Close.

۷	×
0	13C spectral limits copied for F1 dimension. SW: 165.4795 ppm centered at 92.847 ppm
	Close

9.3.4 Acquisition

- On the Workflow button bar, click Gain.
- On the Workflow button bar, click **Run**.

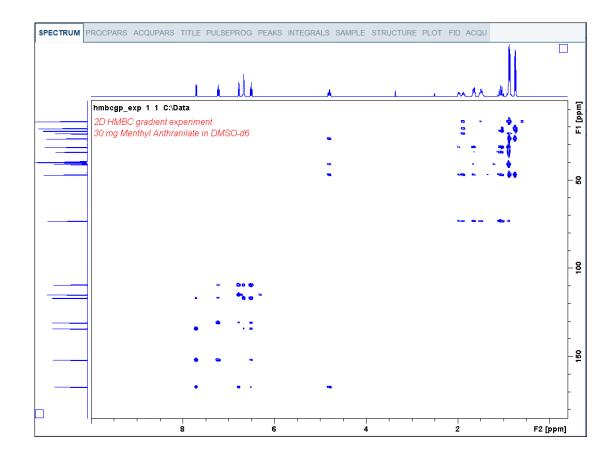
9.3.5 Processing

- On the menu bar, click **Process**.
- On the Workflow button bar, click **Proc Spectrum**.

🍦 apk2d	×
3	Spectrum has no imaginary part: MC2[F1]=QF PH_mod[F1]=mc. Could not phase real spectrum
	Close

This executes a standard processing program **proc2d**. The message shown in the figure above pops up in case of a magnitude 2D experiment and the **apk2d** option is enabled. The message appears because **apk2d** requires phase sensitive data and the HMBC is a magnitude mode experiment. Nevertheless, the magnitude mode calculation is still performed and the message is just an information message. To disable the **apk2d** option, on the **Proc. Spectrum** button click the **drop-down** arrow and configure the **Standard Processing (proc2d)** program.

• In the apk2d message window, click Close.



9.3.6 Plotting the 2D HMBC Spectrum

Follow the instructions in chapter *Plotting the 2D HSQC Spectrum* [> 90].

10 Determination of 90 Degree Pulses

This chapter describes pulse calibration procedures for 1H and 13C. It is assumed that the user is already familiar with acquisition and processing of simple 1D NMR spectra, see chapter 1D Proton Experiment [> 31] and chapter 1D Carbon Experiments [> 71].



This chapter is intended as a guide for calibrating the 90° pulse of a probe or verifying the values observed using ATP.

10.1 Proton 90 Degree Transmitter Pulse

Standard Test Sample: 0.1% Ethylbenzene in CDCI3

10.1.1 Parameter Setup

- On the menu bar, click Acquire | Create Dataset to open the Create New Dataset window.
- · In the New Dataset window, enter or select:

NAME = proton_90

EXPNO = 1

Directory = e.g. C:\Data

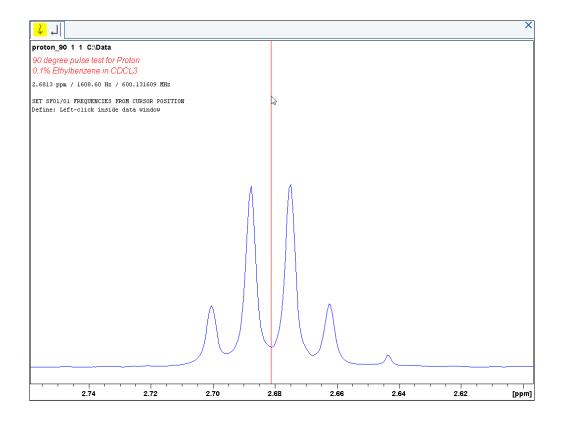
- In the Parameters group Parameters
 click Read parameterset and select the
 experiment PROTON
- In the Create New Dataset window check **Set solvent** and in the drop-down list select **CDCI3**.
- In the TITLE field enter a text stating the experiment, sample, the solvent and any other useful information. The title information can be used to search for a dataset.
- In the New Dataset window, click OK.
- Run a 1D Proton spectrum, following the step *Parameter Setup* [▶ 99] in chapter 1D Proton Experiment through Processing *Processing* [▶ 35] described in this manual.

SPECTRUM	PROCPARS	ACQUPARS	TITLE	PULSEPROG	PEAKS	INTEGRALS	SAMPLE	STRUCTURE	PLOT	FID	ACQU		
proton_90 1 90 degree p 0.1% Ethylb	1 C:\Data oulse test for oenzene in C	Proton DCL3											
								1					
									 ^				
	8		6	1	, , , , , , , , , , , , , , , , , , ,	4			:		1	 0	[ppm]

\$\$\$

- Expand the peak at **2.7 ppm**.
- On the toolbar, click Set RF from cursor.

The Dataset tabs are replaced by the Set RF tool bar.



- Move the cursor line to the center of the multiplet.
- Left click to set the frequency.
- In the O1/O2/O3 window, click O1.

e 01/02/03	×
Define SFO1/O1 fre	equencies
SFO1 [MHz] = O1/2/3 [Hz] =	600.131609 1608.97
01 02	O3 Cancel

- In the Dataset window, select the **AcquPars** tab.
- Enter:

```
PULPROG = zg
TD = 8192
SW [Hz] =1000
D1 [sec] = 30
DS = 0
NS = 1
```

- In the Dataset window, select the **ProcPars** tab.
- Enter or select:

SI = **4096** LB [Hz] = **1** PH_mod = select **pk**

- On the menu bar, click Acquire.
- On the **Spin** button, click the **drop-down** arrow to see more options.
- In the list, select Turn sample rotation off.



This test should be run non spinning.

10.1.2 Acquisition

- On the menu bar, click Acquire.
- On the Workflow button bar, click Gain.
- On the Workflow button bar, click Run.

10.1.3 Processing

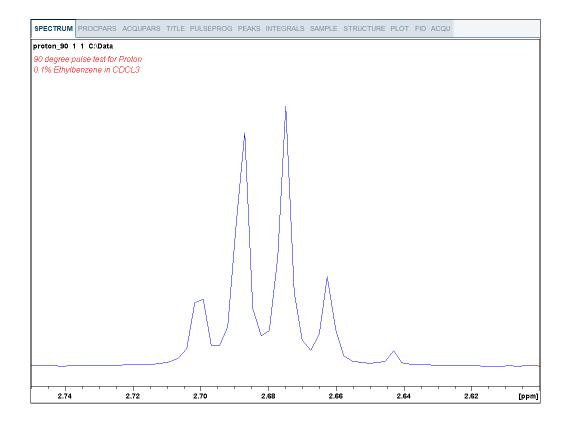
When the acquisition is finished:

• On the menu bar, click **Process**.

- On the Proc Spectrum button, click the drop-down arrow to see more options.
- In the list, select Configure Standard Processing (proc1d).
- Enter or select the following options:
 - Exponential Multiplay (em)
 - LB [Hz] **= 1**
 - Auto Phasing (apk)
- Deselect the following options:
 - Set Spectrum Reference (sref)
 - Auto-Baseline correction (abs)
 - Warn if Processed data exist

🖕 proc1d				×
Press 'Execute' to process the curre Press 'Save' to just change the proc Changed options will be effective will one-click 'Proc. Spectrum' button.	essir	ng options.		
Exponential Multiply (em)	\checkmark	LB [Hz] =	1	
Fourier Transform (ft)				
Auto - Phasing (apk)	\checkmark			
Set Spectrum Reference (sref)				
Auto - Baseline Correction (absn)		Include integration =	no	~
Plot (autoplot)		LAYOUT =	+/1D_H.xwp	~
Warn if processed data exist				
			Save Execute Cancel	

- In the proc1d window, click Execute.
- Expand the spectrum from 2.75 ppm to 2.6 ppm.



- Right-Click in the spectral window.
- In the list, select Save Display Region to ...
- In the Save Display Region to... window, select Parameters F1/2.
- Click OK.

🖕 Save display region to	×			
Options				
Parameters F1/2 (e.g. used by 'restore display',) [dp	ŋ			
O Parameters ABSF1/2 (e.g. used by 'absf, apkf')				
○ Parameters STSR/STSI (used by strip ft)				
O Parameters SIGF1,2 (signal region) (used by 'sino')				
O Parameters NOISF1,2 (noise region) (used by 'sino')				
○ A text file for use with other programs				
OK Cancel				

- In the command line, type **wpar** to store the parameter for future use.
- In the Parameter Sets: wpar window, select the user parameter directory.

Source = C:\Bruker\TopSpin4.0.0.b.20\exp\stan\nmr\par\user

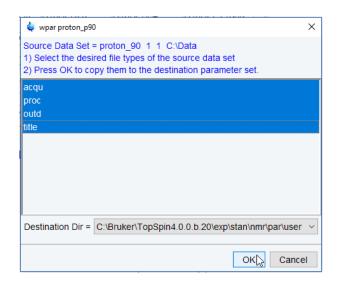
· Click Write New.

File Options He	p Source =	C:\Bruker\TopSp	in4.0.0.b.20\exp\s	tan\nmr\par\user 🚿
Find file names	V PROTON*	Exclu	ude:	Clear
Class = 🔍 Di	m = 🔍 🗌 Sh	ow Recommended		
Type = 📉 Su	bType = 🔍 S	ubTypeB =	Reset Filters	
1H 256	C13CPD128.m	C13CPD2K	C13CPD64	C13DEPT135N
C13DEPT135p	carbon_p90	COSYGP.fixsw	F19_mod	F19COSYGP.t
H1p90_urea	HCCHCOGP3D	HMBCEDETGP	HSQCEDETGP	hsqcedetgpsis
HSQCETGPN15	MebiasF19	media_fast	MLEVETGPSW	NOESYGPPH
nov simplex 60c	nov test3 60c	novatia title	NP 1H	NP C13CPD
NP COSY	NP DEPTQ	NP HMBC	NP HSQC	NP JRES
NP ZG30	PRO128PP	PROTON	PROTON 1K	PROTON 3exp
proton_p90	Proton16	ROESYETGPSW	SELCOGP.pz	SELMLGP.pz
SELNOGP.mod	SELNOGP.pz	SELROGP.pz	SI29IGSW	SOLVSUP_WET
test				

• In the popup window, type proton_p90. Click OK.



• In the *wpar proton_p90* window, select all parameter options. Click **OK**.



• In the Parameter Sets: wpar window, click Close.

🖕 Parameter Sets:	wpar			×
File Options Hel	p Source =	C:\Bruker\TopSp	in4.0.0.b.20\exp\s	stan\nmr\par\user ~
Find file names	V PROTON*	Exclu	ude:	Clear
Class = 💎 Di	m = 🔍 🗌 Sh	ow Recommended	I	
Type = 💛 Su	bType = 💛 S	ubTypeB =	Reset Filters	
1H_256	C13CPD128.m	C13CPD2K	C13CPD64	C13DEPT135N
C13DEPT135p	carbon p90	COSYGP.fixsw	F19 mod	F19COSYGP.t
H1p90 urea	HCCHCOGP3D	HMBCEDETGP	HSQCEDETGP	hsqcedetgpsis
HSQCETGPN15	MebiasF19	media fast	MLEVETGPSW	NOESYGPPH
nov simplex 60c	nov test3 60c	novatia title	NP 1H	NP C13CPD
NP COSY	NP DEPTQ	NP HMBC	NP HSQC	NP JRES
NP ZG30	PRO128PP	PROTON	PROTON 1K	PROTON 3exp
proton p90	Proton16	ROESYETGPSW	SELCOGP.pz	SELMLGP.pz
SELNOGP.mod	SELNOGP.pz	SELROGP.pz	SI29IGSW	SOLVSUP_WET
test				
		W	/rite Write	New Close

10.1.4 Determine the 90 Degree Pulse

- On the menu bar, click Acquire.
- On the Run button, click the drop-down arrow to see more options.
- In the list, select Optimize Acquisition Params (popt).
- In the proton_90 window, enter:

```
OPTIMIZE = Step by step
PARAMETER = p1
OPTIMUM = POSMAX
STARTVAL = 2
NEXP = 20
VARMOD = LIN
INC = 2
```

Click Save.

The ENDVAL parameter has been updated.

• Click Start Optimize.

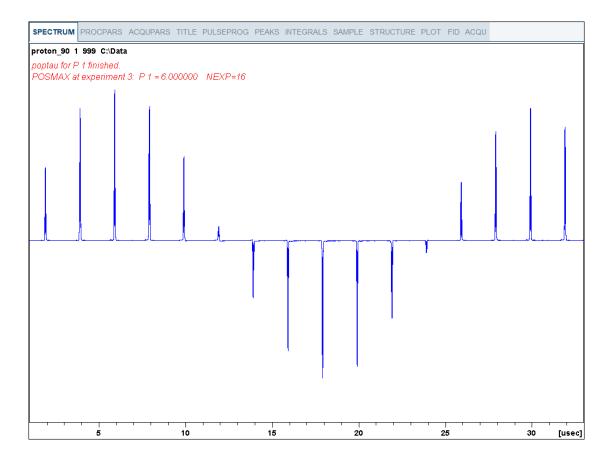
store as 2D data (store as 2D data (ser file)					×			
The AU program s	ram specified in AUNM will be executed					WDW= EM			
Perform automatic	automatic baseline correction (ABSF)					PH_mod= pk			
Overwrite existing	files (disable cont	firmation Me	ssage)		FT_mod= fs	с			
Stop sample spinn	ning at the end of	optimization	(mash)						
Run optimization in	n background								
No display of estin	nated running time	e							
Calculate optimum	after POPT has	finished, but	do not store in da	ataset					
Correlate 2D Cont	tainer with experin	nent							
OPTIMIZE	GROUP PA	RAMETER	OPTIMUM	STARTVAL	ENDVAL	NEXP	VARMOD	INC	
Step by step	p1		POSMAX	2	40.0	20	LIN	2	
Start optimize	Skip current op	otimiz	Show protocol	Add paramet	er R	estore	Save 🔓	Read array file	
Save array file as	Stop optimiza	ation D	elete parameter	Display Datas	set Updat	e ProcPars	Help		

• In the poptau window, enter y and click OK.

🖕 poptau	×
Number of experiments: 20 total experiment time will be: 10 min -0 sec Continue ? [y n]	
У	
OK Cance	el



The parameter optimization starts. The spectrometer acquires and processes 20 spectra with incrementing the parameter p1 from 2 usec by 2 usec to a final value of 40 usec. For each of the 20 spectra, only the spectral region defined above is plotted, and all the spectra are plotted side-by-side in the file proton_90/1/999 as shown in the figure below.



The POSMAX value of **p1** is displayed in the title window which is the 90° pulse, along with the experiment number and the NEXP value. Write this value down. To obtain a more accurate 90° pulse measurement, follow the steps below.

- Close the popt setup window. At the command prompt:
- Enter **rep 1**. Note, that there is a space between **rep** and **1**.
- Enter **p1**.
- Enter the value which corresponds to a 360^o pulse (four times the POSMAX value).
- Enter zg.
- Enter efp.
- Change **p1** slightly and repeat the last 2 steps, until the quartet undergoes a zero crossing as expected for an exact 360° pulse.



The quartet signal is negative for a pulse angle slightly less then 360^o and positive when the pulse angle is slightly more then 360^o.

• Simply divide the determined 360° pulse value by 4. This will be the exact 90° pulse length for the proton transmitter on the current probe.

10.2 Carbon 90 Degree Transmitter Pulse

Standard Test Sample: ASTM (60% C6D6 / 40% p-Dioxane)

10.2.1 Parameter Setup

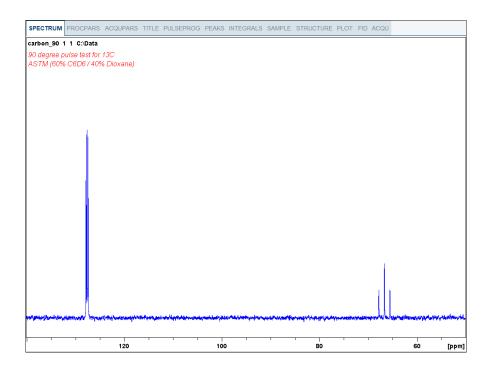
- On the menu bar, click Acquire | Create Dataset to open the Create New Dataset window.
- · In the New Dataset window, enter or select:

NAME = carbon_90

EXPNO **= 1**

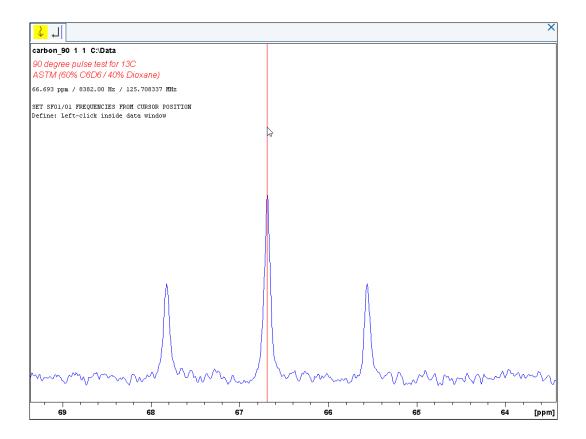
Directory = e.g. C:\Data

- In the Parameters group Parameters click **Read parameterset** and select the experiment **C13CPD**.
- In the Create New Dataset window check **Set solvent** and in the drop-down list select **C6D6**.
- In the TITLE field enter a text stating the experiment, sample, the solvent and any other useful information. The title information can be used to search for a dataset.
- In the New Dataset window, click OK.
- Run a 1D Carbon spectrum, following the instructions in chapter *Experiment Setup* [▶ 72] and chapter *Acquisition* [▶ 74].
- · Enter the following acquisition parameters:
 - PULPROG = zg
 - DS = 0
 - NS = 1
- Continue with chapter *Processing* [> 74].

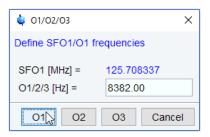


- Expand the peak at 67 ppm.
- On the toolbar, click Set RF from cursor.

The Dataset tabs are replaced by the Set RF toolbar.



- · Move the cursor line into the center peak of the triplet.
- Left click to set the frequency.
- In the O1/O2/O3 window, click O1.



- In the Dataset window, select the AcquPars tab.
- Enter: TD = 8192
 D1 [sec] = 60

- In the Dataset window, select the **ProcPars** tab.
- Enter or select:

SI **= 4096** LB [Hz] **= 3.5**

PH_mod = select pk

- On the menu bar, click Acquire.
- On the **Spin** button, click the **drop-down** arrow to see more options.
- In the list, select Turn sample rotation off.



This test should be run non spinning.

10.2.2 Acquisition

- On the menu bar, click Acquire.
- On the Workflow button bar, click Gain.
- On the Workflow button bar, click **Run**.

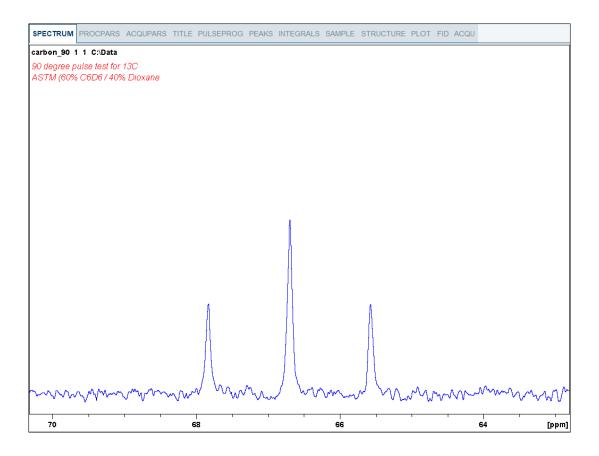
10.2.3 Processing

When the acquisition is finished:

- On the menu bar, click **Process**.
- On the Proc Spectrum button, click the drop-down arrow to see more options.
- In the list, select Configure Standard Processing (proc1d).
- · Select the following options:
 - Exponential Multiplay (em)
 - LB [Hz] = 3.5
 - Auto Phasing (apk)
- Deselect the following options:
 - Set Spectrum Reference (sref)
 - Auto-Baseline correction (abs)
 - Warn if Processed data exist

🍦 proc1d					×
Press 'Execute' to process the curre Press 'Save' to just change the pro Changed options will be effective will one-click 'Proc. Spectrum' button.	essir	ng options.			
Exponential Multiply (em)	\checkmark	LB [Hz] =	3.5		
Fourier Transform (ft)					
Auto - Phasing (apk)	\checkmark				
Set Spectrum Reference (sref)					
Auto - Baseline Correction (absn)		Include integration =	no		~
Plot (autoplot)		LAYOUT =	+/1D_X.xwp		~
Warn if processed data exist					
			Save	xecute	Cancel

- Click Execute.
- Expand the spectrum from 71 ppm to 63 ppm.



- In the spectral window click right.
- In the list select Save Display Region To...
- In the Save Display Region To... window, enable Parameters F1/2 and click OK.

🖕 Save display region to 🛛 🗙				
Options				
Parameters F1/2 (e.g. used by 'restore display',) [dpl]				
O Parameters ABSF1/2 (e.g. used by 'absf, apkf')				
O Parameters STSR/STSI (used by strip ft)				
O Parameters SIGF1,2 (signal region) (used by 'sino')				
O Parameters NOISF1,2 (noise region) (used by 'sino')				
\bigcirc A text file for use with other programs				
OK Cancer				

- In the command line, type **wpar** to store the parameter for future use.
- In the Parameter Sets: wpar window, select the user source parameter directory.

Source = C:\Bruker\TopSpin4.0.0.b.20\exp\stan\nmr\par\user >

· Click Write New.

File Options He	lp Source =	C:\Bruker\TopSp	in4.0.0.b.20\exp\s	tan\nmr\par\user 🚿
Find file names	V C13CPD*	Exclu	ude:	Clear
Class = 💛 Di	m = 🔍 🗌 Sh	ow Recommended	l	
Type = 📉 Su	bType = S	ubTypeB = 🔍	Reset Filters	
1H 256	C13CPD128.m	C13CPD2K	C13CPD64	C13DEPT135N
C13DEPT135p	carbon p90	COSYGP.fixsw	F19 mod	F19COSYGP.t
H1p90_urea	HCCHCOGP3D	HMBCEDETGP	HSQCEDETGP	hsqcedetgpsis
HSQCETGPN15	MebiasF19	media fast	MLEVETGPSW	NOESYGPPH
nov simplex 60c	nov test3 60c	novatia title	NP 1H	NP C13CPD
NP COSY	NP DEPTQ	NP HMBC	NP HSQC	NP JRES
NP ZG30	PRO128PP	PROTON	PROTON 1K	PROTON 3exp
proton_p90	Proton16	ROESYETGPSW	SELCOGP.pz	SELMLGP.pz
SELNOGP.mod	SELNOGP.pz	SELROGP.pz	SI29IGSW	SOLVSUP_WET
test				

• In the popup window, enter **carbon_p90** and click **OK**.

4	×
Please enter the new name	
carbon_p90	
OK Cancel	

• In the *wpar carbon_p90* window, select all parameter options and click **OK**.

🖕 wpar carbon_p90	Х
Source Data Set = carbon_90 1 1 C\Data 1) Select the desired file types of the source data set 2) Press OK to copy them to the destination parameter set.	
acqu proc outd title	
Destination Dir = C:\Bruker\TopSpin4.0.0.b.20\exp\stan\nmr\par\user	· ~
OK Canc	el

• In the Parameter Sets: wpar window click Close.

File Options He	lp Source =	C:\Bruker\TopSp	in4.0.0.b.20\exp\s	tan\nmr\par\user					
Find file names	Find file names V C13CPD* Exclude: Clear								
Class = 💎 Di	m = 🔍 🗌 Sh	ow Recommended	1						
Type = 💛 Su	bType = 💛 S	ubTypeB = 🔍	Reset Filters						
1H_256	C13CPD128.m	C13CPD2K	C13CPD64	C13DEPT135N					
C13DEPT135p	cabon p90 📐	carbon_p90	COSYGP.fixsw	F19_mod					
F19COSYGP.t	H1p90_urea	HCCHCOGP3D	HMBCEDETGP	HSQCEDETGP					
hsqcedetgpsis	HSQCETGPN15	MebiasF19	media_fast	MLEVETGPSW					
NOESYGPPH	nov_simplex_60c	nov_test3_60c	novatia_title	NP_1H					
NP_C13CPD	NP_COSY	NP_DEPTQ	NP_HMBC	NP_HSQC					
NP JRES NP ZG30		PRO128PP	PROTON	PROTON_1K					
PROTON 3exp proton p90		Proton16	ROESYETGPSW	SELCOGP.pz					
SELMLGP.pz SELNOGP.mod SE		SELNOGP.pz	SELROGP.pz	SI29IGSW					
SOLVSUP WET	toot								

10.2.4 Determine the 90 Degree Pulse

- On the menu bar, click Acquire.
- On the **Run** button, click the **drop-down** arrow to see more options.
- In the list, select Optimize Acquisition Params (popt).
- In the carbon_90 window, enter: OPTIMIZE = Step by step PARAMETER = p1 OPTIMUM = POSMAX STARTVAL = 2 NEXP = 13

VARMOD = LIN INC = 2

• Click Save.

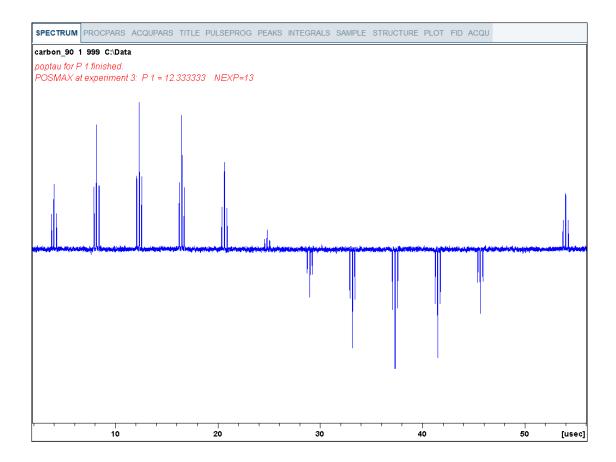
Image: Stat optimize Stap optimizet WDW= EM WDW= Em WDW= EM Perform automatic baseline correction (ABSF) PH_mod= pk Overwrite existing files (disable confirmation Message) FT_mod= fqc Stop sample spining at the end of optimization (mash) FT_mod= fqc Ren optimization in background Stop sample spining at the end of optimization (mash) Calculate optimum after POPT has finished, but do not store in dataset Stop sample spining Correlate 20 Container with experiment OPTIMIZE GROUP PARAMETER OPTIMUM STARTVAL ENDVAL NEXP VARMOD INC Step by step 0 p1 POSMAX 4 54 13 LIN 4	store as 2D dat	a (ser file)							×	
Start optimize Skip current optimiz Show protocol Add parameter Read array file	The AU program specified in AUNM will be executed WDW= EM									
Stop sample spinning at the end of optimization (mash) Run optimization in background No display of estimated running time Calculate optimum after POPT has finished, but do not store in dataset Correlate 2D Container with experiment OPTIMIZE GROUP PARAMETER OPTIMUM Step by step 0 p1 POSMAX 4 54 13 LIN 4 54 Start optimize Skip current optimiz Show protocol Add parameter Read array file	Perform automatic baseline correction (ABSF)					PH_mod= pk				
Start optimize Skip current optimiz Show protocol Add parameter Restore Save () Read array file	Overwrite existin	Overwrite existing files (disable confirmation Message)					fqc			
Start optimize Skip current optimiz Show protocol Add parameter Restore Save > Read array file	Stop sample sp	inning at the e	nd of optimizatio	n (mash)						
Start optimize Skip current optimiz Show protocol Add parameter Restore Save > Read array file	Run optimization	n in backgrour	hd							
Orrelate 2D Container with experiment OPTIMIZE GROUP PARAMETER OPTIMUM STARTVAL ENDVAL NEXP VARMOD INC Step by step 0 p1 POSMAX 4 54 13 LIN 4	No display of es	timated runnir	ng time							
OPTIMIZE GROUP PARAMETER OPTIMUM STARTVAL ENDVAL NEXP VARMOD INC Step by step 0 p1 POSMAX 4 54 13 LIN 4	Calculate optim	um after POPT	F has finished, bi	ut do not store in da	itaset					
Step by step 0 p1 POSMAX 4 54 13 LIN 4	Correlate 2D Co	ontainer with e	experiment							
Start optimize Skip current optimiz Show protocol Add parameter Restore Save 🔉 Read array file	OPTIMIZE	GROUP	PARAMETER	OPTIMUM	STARTVAL	ENDVAL	NEXP	VARMOD	INC	
	Step by step	0	p1	POSMAX	4	54	13	LIN	4	
Save array file as Stop optimization Delete parameter Display Dataset Update ProcPars Help	Start optimize	Skip curr	ent optimiz	Show protocol	Add parameter	er	Restore	Save 🔓	Read array file	
	Save array file as	Stop o	ptimization	Delete parameter	Display Datas	et Upd	ate ProcPars	Help		

The ENDVAL parameter has been updated.

- Click Start optimize.
- In the poptau window, enter **y** and click **OK**.

🝦 poptau	×
Number of experiments: 13 total experiment time will be: 13 min 0 sec Continue ? [y n]	
у	
ОК 🖓 Са	ncel

The parameter optimization starts. The spectrometer acquires and processes 13 spectra with incrementing the parameter p1 from 4 usec by 4 usec to a final value of 40 usec. For each of the 13 spectra, only the spectral region defined above is plotted, and all the spectra are plotted side-by-side in the file carbon_90/1/999 as shown in the figure below.



The POSMAX value of **p1** is displayed in the title window which is the 90° pulse, along with the experiment number and the NEXP value. Write this value down. To obtain a more accurate 90° pulse measurement, follow the steps below.

- Close the popt setup window. At the command prompt:
- Enter rep 1. Note, that there is a space between rep and 1.
- Enter p1.
- Enter the value which corresponds to a 360° pulse (four times the POSMAX value).
- Enter zg.
- Enter efp.
- Change **p1** slightly and repeat the last 2 steps, until the quartet undergoes a zero crossing as expected for an exact 360° pulse.



The quartet signal is negative for a pulse angle slightly less then 360^o and positive when the pulse angle is slightly more then 360^o.

• Simply divide the determined 360° pulse value by 4. This will be the exact 90° pulse length for the proton transmitter on the current probe.

11 Sensitivity Tests

This chapter describes the sensitivity test procedures for 1H and 13C. It is assumed that the user is already familiar with acquisition and processing of simple 1D NMR spectra, see chapter 1D Proton Experiment [> 31] and chapter 1D Carbon Experiments [> 71] in this manual.

Also the 90[°] pulses have to be properly calibrated, see chapter *Determination of 90 Degree Pulses* [> 97].



This chapter is intended as a guide for running the 1H and 13C Signal to Noise test on a probe or verifying the values observed using ATP.

11.1 ¹H Sensitivity Test

Standard Test Sample:

0.1% Ethylbenzene in CDCI3

11.2 Experiment Setup

- On the menu bar, click **Acquire** | **Create Dataset** to open the Create New Dataset window.
- In the New Dataset window, enter or select:

NAME = proton_sensitivity

EXPNO = 1

Directory = e.g. C:\Data

- In the Parameters group Parameters
 click Read parameterset and select the experiment PROSENS.
- In the Create New Dataset window check **Set solvent** and in the drop-down list select **CDCI3**.
- In the TITLE field enter a text stating the experiment, sample, the solvent and any other useful information. The title information can be used to search for a dataset.
- In the New Dataset window, click **OK**.
- On the menu bar, click Acquire.

For the following steps, use the Workflow button bar.

- Click Sample and eject the sample, if there is one inserted, and insert the new sample.
- Click Lock and select CDCI3 solvent.
- To tune the probe, click **Tune**.
- Click Spin and select Turn sample rotation on.



The Proton sensitivity test should be run with the sample spinning. Rotation may be turned off for probes such as **BBI**, **TXI**, **TBI** and for small sample probes.

- On the Workflow button bar, click Shim.
- For best homogeneity use TopShim.

To load the probehead/solvent depended parameters:

• On the Workflow button bar, click **Prosol**.

11.3 Acquisition

To adjust the receiver gain:

• On the Workflow button bar, click Gain.



The relaxation time **D1** is by default in this parameter set **60 s** and therefore the adjustment of the receiver gain will take some time.

To start the acquisition:

• On the Workflow button bar, click Run.

11.4 Processing

When the acquisition has finished:

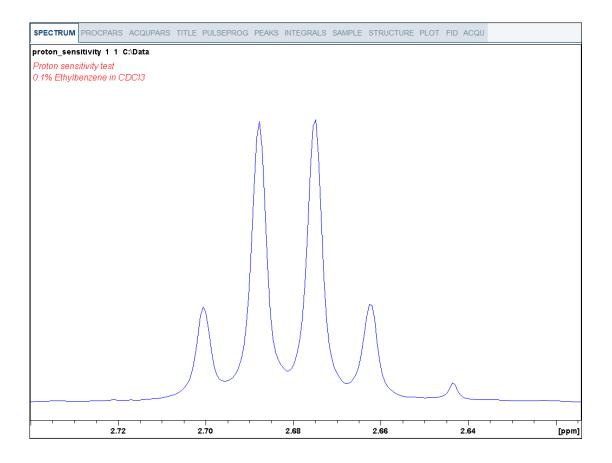
- On the menu bar, click Process.
- On the Workflow button bar, click Proc Spectrum.



Proc. Spectrum executes a processing program including commands such as an exponential window function **em**, Fourier transformation **ft**, an automatic phase correction **apk** and a baseline correction **abs**. On the **Proc. Spectrum** button, click the **drop-down** arrow to see more options. In the list, select **Configure Standard Processing (proc1d)**.

11.5 Calculating the Signal to Noise Ratio

The signal to noise ratio is determined on the intensity of the **quartet** lines between **2 ppm** and **3 ppm**. It is calculated by AU-program sinocal over a range of **2 ppm** between **2.8 ppm** and **7 ppm**. The s/n ratio is strongly dependant on good resolution and line shape. The splitting between the two central lines of the methyl quartet should go lower than 15% of the signal height (with LB=1Hz), see the figure below.



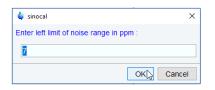
- At the command prompt, type **sinocal**.
- In the sinocal window, enter 3 for the left limit of the signal range. Click OK.

🖕 sinocal	×
Enter left limit of signal range ir	ppm :
3	
	OK Cancel

• In the sinocal window, enter 2 for the right limit of the signal range. Click OK.

🖕 sinocal	×
Enter right limit of signal range in ppm :	
2	
ОКЪ	Cancel

• In the sinocal window, enter 7 for the left limit of the noise range. Click OK.



• In the sinocal window, enter 2.8 for the right limit of the noise range. Click OK.

🎍 sinocal	×
Enter right limit of noise range i	n ppm :
2.8	
	OK Cancel

• In the sinocal window, enter 2 for the noise width. Click OK.

🖕 sinocal		×
Enter noise width in ppm :		
2		
	окЪ	Cancel

SPECTRUM	PROCPARS ACQUE	PARS TITLE	PULSEPROG PEAKS INTEGR	ALS SAMPL	E STRUCTI	JRE PLOT FI	D ACQU		
	sitivity 1 1 C:\Data								
Proton sens 0.1% Ethylb	sitivity test enzene in CDCl3								
		🖕 sinocal		×					
			Best sino value: 783.0 Signal from 3.00 to 2.00 ppm						
		_	Noise from 5.07 to 3.07 ppm						
				ОК					
		L							
	Ь								
	l.				A	٨			
-					/ _		Nu		
	8	6	4	1	1 1	2	1	, i	[ppm]

11.6 ¹³C Sensitivity Test with ¹H Decoupling

Standard Test Sample: 10% Ethylbenzene in CDCI3

11.6.1 Experiment Setup

- On the menu bar, click Acquire | Create Dataset to open the Create New Dataset window.
- · In the New Dataset window, enter or select:

NAME = Carbon_sensitivity_ETB

EXPNO = 1

Directory = e.g. C:\Data

- In the Parameters group Parameters click **Read parameterset** and select the experiment **C13SENS**.
- In the Create New Dataset window check **Set solvent** and in the drop-down list select **CDCI3**.
- In the TITLE field enter a text stating the experiment, sample, the solvent and any other useful information. The title information can be used to search for a dataset.
- In the New Dataset window, click **OK**.
- On the menu bar, click Aquire.

For the following steps, use the Workflow button bar.

- Click Sample and eject the sample, if there is one inserted, and insert the new sample.
- Click Lock and select CDCI3 solvent.
- To tune the probe, click **Tune**.
- · Click Spin and select Turn sample rotation on.



The Carbon sensitivity test should be run with the sample spinning. Rotation may be turned off for some probes. Please refer to the probe specification sheet for more details.

- On the Workflow button bar, click **Shim**.
- For best homogeneity use TopShim.

To load the probehead/solvent depended parameters:

• On the Workflow button bar, click **Prosol**.

11.6.2 Acquisition

To adjust the receiver gain:

• On the Workflow button bar, click Gain.



The relaxation time **D1** is by default in this parameter set **300 s** and therefore the adjustment of the receiver gain will take some time.

To start the acquisition:

• On the Workflow button bar, click **Run**.

11.6.3 Processing

When the acquisition has finished:

- On the menu bar, click Process.
- On the Workflow button bar, click Proc Spectrum.



Proc. Spectrum executes a processing program including commands such as an exponential window function **em**, Fourier transformation **ft**, an automatic phase correction **apk** and a baseline correction **abs**. On the **Proc. Spectrum** button, click the **drop-down** arrow to see more options. In the list, select **Configure Standard Processing (proc1d)**.

11.6.4 Calculating the Signal to Noise Ratio

The signal to noise ratio is determined on the highest peak of the **aromatic** part between **127 ppm** and **129 ppm**, see the figure below. It is calculated by AU-program sinocal over a range of **40 ppm** between **30 ppm** and **125 ppm**. The s/n ratio is strongly dependent on good resolution and line shape.

SPECTRUM	PROCPARS	ACQUPARS	TITLE PULSEPROG	PEAKS INTEGRALS	SAMPLE STRUCTURE	PLOT FID ACQU		
carbon_sen								
13C sensiti 10% Ethylb	vity test with enzene in Cl	1H decouplii DCI3	ng					
					Д			
		~~~~~						
128	3.8	128.6	128.4	128.2	128.0	127.8	127.6	[ppm]

- At the command prompt, type **sinocal**.
- In the sinocal window, enter 128 for the left limit of the signal range. Click OK.

🖕 sinocal	×
Enter left limit of signal range in	ppm :
128	
	OK Cancel

• In the sinocal window, enter **127** for the right limit of the signal range. Click **OK**.

🖕 sinocal	×
Enter right limit of signal range in	n ppm :
127	
	OK Cancel

• In the sinocal window, enter **125** for the left limit of the noise range. Click **OK**.

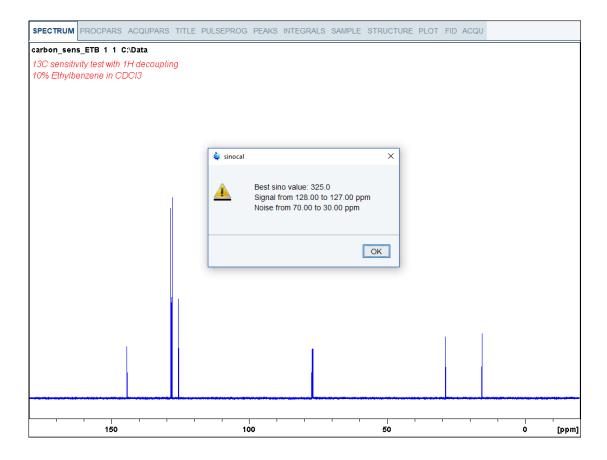
🖕 sinocal	×
Enter left limit of noise range in	ppm :
125	
	OK Cancel

• In the sinocal window, enter **30** for the right limit of the noise range. Click **OK**.

🖕 sinocal	×
Enter right limit of noise r	ange in ppm :
30	
	OK Cancel

• In the sinocal window, enter 40 for the noise width. Click OK.

🖕 sinocal	×
Enter noise width in ppm :	
40	
	OK Cancel



# 11.7 ¹³C Sensitivity Test without ¹H Decoupling

Standard Test Sample: **ASTM** (60% C6D6 / 40% p-Dioxane)

## 11.7.1 Experiment Setup

- On the menu bar, click Acquire | Create Dataset to open the Create New Dataset window.
- · In the New Dataset window, enter or select:

#### NAME = Carbon_sensitivity_ASTM

EXPNO = 1

Directory = e.g. C:\Data

- In the Parameters group Parameters click Read parameterset and select the experiment C13SENS.
- In the Create New Dataset window check **Set solvent** and in the drop-down list select **C6D6**.
- In the TITLE field enter a text stating the experiment, sample, the solvent and any other useful information. The title information can be used to search for a dataset.
- In the New Dataset window, click OK.
- On the menu bar, click Aquire.

For the following steps, use the Workflow button bar.

- Click Sample and eject the sample, if there is one inserted, and insert the new sample.
- Click Lock and select C6D6 solvent.
- To tune the probe, click Tune.
- · Click Spin and select Turn sample rotation on.

j

The Carbon sensitivity test should be run with the sample spinning. Rotation may be turned off for probes such as **BBI**, **TXI**, **TBI** and for small sample probes.

- On the Workflow button bar, click Shim.
- For best homogeneity use TopShim.

To load the probehead/solvent depended parameters:

- On the Workflow button bar, click **Prosol**.
- In the Dataset window, select the **AcquPars** tab.
- Make the following changes:

PULPROG = zg

TD = 65536

SW [ppm] = 200

O1p = **100** 

- In the Dataset window, select the **ProcPars** tab.
- Make the following changes: SI = **32768**

LB [Hz] = 3.5

• In the Dataset window, select the **Spectrum** tab.

## 11.7.2 Acquisition

To adjust the receiver gain:

• On the Workflow button bar, click Gain.



The relaxation time **D1** is by default in this parameter set **300 s** and therefore the adjustment of the receiver gain will take some time.

To start the acquisition:

• On the Workflow button bar, click Run.

### 11.7.3 Processing

When the acquisition has finished:

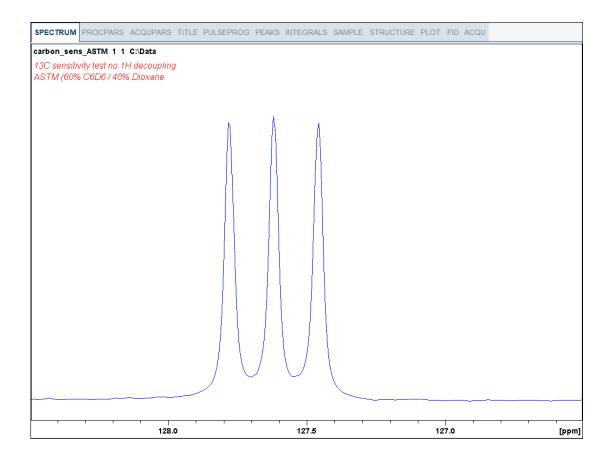
- On the menu bar, click Process.
- On the Workflow button bar, click Proc Spectrum.



**Proc. Spectrum** executes a processing program including commands such as an exponential window function **em**, Fourier transformation **ft**, an automatic phase correction **apk** and a baseline correction **abs**. On the **Proc. Spectrum** button, click the **drop-down** arrow to see more options. In the list, select **Configure Standard Processing (proc1d)**.

## 11.7.4 Calculating the Signal to Noise Ratio

The signal to noise ratio is determined on the triplet of the **deuterated benzene** between **127 ppm** and **129 ppm**. It is calculated by AU-program sinocal over a range of **40 ppm** between **70 ppm** and **125 ppm**. The s/n ratio is strongly dependant on good resolution and line shape. The splitting of the 1:1:1 triplet should go lower than 9% of the signal height for **5mm** probes and 10% for **10mm** probes, see the figure below.



- At the command prompt, type **sinocal**.
- In the sinocal window, enter **129** for the left limit of the signal range. Click **OK**.

🖕 sinocal	×
Enter left limit of signal range	e in ppm :
129	
	OK Cancel

• In the sinocal window, enter 127 for the right limit of the signal range. Click OK.

🖕 sinocal	×
Enter right limit of signal range	in ppm :
127	
	OK Cancel

• In the sinocal window, enter 125 for the left limit of the noise range. Click OK.

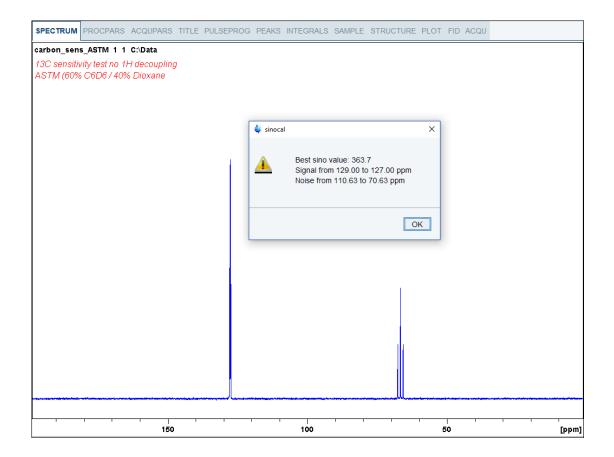
🖕 sinocal	×
Enter left limit of noise rang	ge in ppm :
125	
	OK Cancel

• In the sinocal window, enter 70 for the right limit of the noise range. Click OK.

🖕 sinocal	×
Enter right limit of noise range	in ppm :
70	
	OK 🔓 Cancel

• In the sinocal window, enter 40 for the noise width. Click OK.

🖕 sinocal	×
Enter noise width in ppm :	
40	
	OK Cancel



# **12 Additional Information**

A list of all standard parameter sets is available with the command **rpar**, see figure below. The list varies according to the number of channels of your spectrometer.

# 12.1 Standard Parameter Set List

• On the command line, enter rpar.

<u>File</u> Options <u>H</u> elp			Source = C:\Bruker\Top	Spin4.0.3.a\exp\stan\nmr\par	
Find file names 👻 enter a	any string, *, ? Exclude:	Clear			
Class = Any	Dim = Any V Show Re	commended			
Type = Any 🔻 Sul	oType = Any 🔻 SubTypeB =	Any  Reset Filters			
AL27ND	ASSURE 13C	ASSURE 19F	ASSURE 1H	ASSURE 31P	
B HNCACBGP3D	B HNCACBIGP3D	B HNCACOGP3D	B HNCACOGP4D	B HNCAGP3D	-
B HNCAIGP3D	B HNCOCACBGP3D	B HNCOCACBGP4D	B HNCOCAGP3D	B HNCOCAGP4D	
B HNCOGP3D	B HNCOIGP3D	B HSQCETF3GPSI	B TRHNCACBGP3D	B TRHNCACBIGP3D	
B TRHNCACOGP3D	B TRHNCAGP3D	B TRHNCAIGP3D	B TRHNCOCACBGP3D	B TRHNCOCAGP3D	
B TRHNCOGP3D	B TRHNCOIGP3D	B TROSYETF3GPSI	B TROSYF3GPPH	B117G	
BESTPROFILE	C CACO	C CACO IA	C CACO S3	C CAN IASQ	
C CAN MQ	C CAN MQ.2	C CANCO IA3D	C CANCO IA3D.2	C CANCOL IA3D	-
C CBCACO IA3D	C CBCACO S33D	C CBCACON JA3D	C CBCANCO IA3D	C CCCO IA3D	
C CCCO S33D	C CCCON IA3D	C CCFLOPSY16	C CCFLOPSY16 CT	C CCFLOPSY16 CTIA	
C CCFLOPSY16 IA	C CCNOESY	C CCNOESY CT	C CCNOESY2	C COCA	
C COCA IA	C COCA MQ	C COCA MQ.2	C CON IASQ	C CON MQ	
C CON MQIA	C CON SQ	C COSY	C COSY CT	C COSY2 CT	
C HACACO 3D	C HCACO IA3D	C HCACO S33D	C HCAN IA3D	C HCANCO IA3D	
C HCANCOL IA3D	C HCBCA IA3D	C HCBCACO IA3D	C HCBCACO S33D	C HCBCAN IA3D	
C HCCFLOPSY16 3D	C HNCA IA3D	C HNCACO IA3D	C HNCACO S33D	C HNCO IA3D	
C_HNCOCA_IA3D	C_HNCOCA2_IA3D	C13APT	C13CPD	C13CPD32	
C13CPDSN	C13DE45SN	C13DEPT135	C13DEPT135p	C13DEPT45	
C13DEPT90	C13DEPTQ135	C13GD	C13HUMP	C13IG	
C13MULT	C13MULT135	C13MULT90	C13MULTCOMP	C130FF	
C13PPTI	C13RESOL	C13SENS	C13UDEFT	CBCACONHGP3D	
CBCACONHGPWG3D	CBCACONHGPWG3D.2	CBCACONHGPWG4D	CBCANHGP3D	CBCANHGPWG3D	
CCACONHGP2H3D	CCACONHGP3D	CCACONHGP3D.2	CCANHGP2H3D	CCANHGP3D	
CCANHGP3D.2	CCCONHGP2H3D	CCCONHGP3D	CD111ZG	CD113ZG	
CL35ZG	CL37ZG	CMC_13C	CMC_HMBC	CMC_HSQC	
CMC_PROTON	CMC_SINGLE	CMC_SINGLE_H2O	CMC_WET	CMCse_13C	
CMCse 15NHMBCf2	CMCse 15NHSOCf2	CMCse 1H	CMCse ADEO	CMCse COSY	

• Click **Show Recommended** to reduce the list to the most frequently used parameter sets as recommended by Bruker.

File Options Help			Source = C:\Bruker\To	pSpin4.0.3.a\exp\stan\nmr\par
Find file names 👻 enter an	ny string, *, ? Exclude:	Clear		
Class = Any	Dim = Any 🔻 📝 Show Reco	ommended		
Type = Any Sub	Type = Any V SubTypeB = A	Reset Filters		
C13CPD	C13DEPT135	C13DEPTQ135	C13UDEFT	COSYGPDFPHSW
COSYGPSW	HMBCETGPL3ND	HMBCGP	HMBCGP 15N	HSQC TOCSY
HSQC TOCSY ADIA	HSQCEDETGPSISP	HSQCEDETGPSISP ADIA	HSQCETGP 15N	HSQCETGPSISP
HSQCETGPSISP ADIA	MLEVPHPR	MLEVPHSW	NOESYPHPR	NOESYPHSW
PROTON	ROESYPHPR	ROESYPHSW	WATERSUP	

Ensure that the source directory is
 <Topspin>\exp\stan\nmr\par
 and not <Topspin>\exp\stan\nmr\par\user

# 12.2 Pulse Program Information

A list of all standard pulse programs is available with the command **edpul**, see figure below.

- The list can be reduced by specifying text fragments as argument for the **edpul** command, for example **edpul hmbc***.
- Click **Show Recommended** to reduce the list to the most frequently used pulse programs as recommended by Bruker.

File Options Help			Source = C:\Bruker\TopSp	in4.0.3.a\exp\stan\nmr\lists\pp
			Source - C. Braker (10pop	in4.0.5.alexplataritini inatalpp
Find file names 🔻 ente	r any string, *, ? Exclude:	Clear		
Class = Any	▼ Dim = Any ▼ Show	Recommended		
Type = Any	✓ SubType = Any ✓	SubTypeB = Any	set Filters	
cosygpirppqf	cosygpmfphpp	cosygpppqf	deptsp135	hmbcetgpl3nd
hmbcgplpndqf	hsqcdietgpsisp.2	hsqcedetgpsisp2.3	hsqcetgpsisp2.2	mlevphpp
mlevphpr.2	noesygpphpp	noesygpphpr	noesygppr1d	roesyphpp.2
roesyphpr.2	zg30	zgpg30		
reesphpr.z	,2900	rahdaa		
			Edit Graphical E	Edit Set PULPROG Close

Find further information about pulse programs in the following files which can be accessed with the command **edpul *.info** :

- Pulprog.info (Topspin two letter code naming conventions)
- Param.info (Standard parameter assignments consistent with prosol & relations files)
- Relations.info (Prosol to parameter translation files)
- Update.info (Additions, changes, corrections to sequences)

🖕 Pulse Programs				×
<u>File</u> Options <u>H</u> elp			Source = C:\Bruker\TopSp	in4.0.3.a\exp\stan\nmr\lists\pp
Find file names  .info	Exclude:	Clear		
Class = Any	Dim = Any  Show	Recommended		
Type = Any 🔻	SubType = Any 🔻	SubTypeB = Any 🔻	Reset Filters	
Param.info	Pulprog.info	Relations.info	Update.info	
			Edit Craphical I	
			Edit Graphical B	Edit Set PULPROG Close

## 12.3 Standard Test Samples

For a list of all commercially available standard reference samples go to the Bruker webshop *https://bruker-labscape.store/* 

# **13 Contact**

#### Manufacturer

Bruker BioSpin GmbH Silberstreifen 4 D-76287 Rheinstetten Germany http://www.bruker.com

WEEE DE43181702

#### **NMR Hotlines**

Contact our NMR service centers.

Bruker BioSpin NMR provides dedicated hotlines and service centers, so that our specialists can respond as quickly as possible to all your service requests, applications questions, software or technical needs.

Please select the NMR service center or hotline you wish to contact from our list available at:

https://www.bruker.com/service/information-communication/helpdesk.html

Phone: +49 721-5161-6155 E-mail: nmr-support@bruker.com

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