UCI NMR MANUAL

DRX400
GN500
CRYO500
AVANCE600

Please do not attempt to print this manual directly from the NMR workstations.

The latest version can be downloaded from **nmrserver.ps.uci.edu** in the directory **/v/NMRmanuals**. There is a link to this directory in each user's home directory, select **NMRmanuals**.

Disclaimer

This manual is intended as an operational guide to the spectrometers in the Department of Chemistry NMR Facility at the University of California, Irvine, USA.

Some of the techniques described will work on standard Bruker instruments elsewhere, but many rely on the presence of parameter files, automation programs, macros or pulse programs specific to the UCI facility. For example, in the case of the automation program 'xiref' there is now a Bruker program with the same name, which is used for a similar purpose.

No experiments are guaranteed to work in other facilities, and no responsibility is accepted for any damage caused by attempting to follow these instructions. It is possible that directors of other facilities may use the same filenames as used here for completely different purposes.

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"… if you're lost and you want to stay lost, I could be your guide" - Colorblind James

1 Introduction

This document is intended to be a useful operational guide. The UCI Department of Chemistry NMR Facility is currently comprised of four Bruker solution state instruments. Three are DRX spectrometers (DRX400, GN500 and CRYO500), and the fourth is an AVANCE spectrometer (AVANCE600). The instrument names used are for historical reasons and in order to distinguish between the two 500s. In addition to the spectrometer computers there are dedicated workstations for data processing.

The facility is divided between two locations: room B106 in the basement of Reines Hall, and room 1403B in the basement of Natural Sciences 1. Most instruments are in Reines Hall, but the DRX400 is located in Natural Sciences 1.

Most operation (400 and 500MHz spectrometers) is via Bruker *XwinNMR* software, running under *Irix* on Silicon Graphics O2 computers. The AVANCE600 instrument is operated via Bruker *TopSpin* software, running under CentOS Linux. The majority of this manual is therefore concerned with data acquisition and processing using *XwinNMR*, but it has been endeavored to include *TopSpin* alternatives. Chapter 29 is dedicated to data acquisition and processing using *TopSpin* as well as extra options that are available on the AVANCE600.

Data can be processed within the NMR facility using *XwinNMR* or *TopSpin*, or on users' own computers. *TopSpin* or *SpinWorks* can be installed free of charge, or users can provide their own copies of *MestReNova*. The facility provides processing computers running *XwinNMR* in B106 Reines Hall and rooms 1403B and 4302G in Natural Sciences 1, and CentOS computers running *TopSpin* in B106 Reines Hall.

There are comprehensive online manuals within *XwinNMR* and *TopSpin*, as PDF files, accessed from the **Help** menu. These can also be copied by FTP from **nmrserver.ps.uci.edu**, by following the link to **NMRmanuals** in the user home directory. The commands in this manual are based on version 3.5 of *XwinNMR* and version 3.2 of *TopSpin*.

The majority of commands can either be typed or accessed from on-screen menus. When possible, typing is usually more convenient and so is used in most of the following examples. Similarly, the BSMS keypad functions can be accessed through the computer keyboard or via a software panel, but these methods will not be described here.

The latest version of this manual can be found in printed form in the laboratory, or viewed on-line via the SGI Irix *Toolchest*:

NMR → UCI NMR Manual

On the Linux computers there is a link to this manual in the panel on the left edge of the screen.

1.1 Safety in the NMR facility

There are no known health problems caused by exposure to strong static magnetic fields. However, there are some simple rules to be observed for safe operation:

Do not enter the NMR facility if you have any medical implants which could be affected by magnetic fields, such as heart pacemakers, hearing aids or prosthetic devices.

Do not take any metallic or electronic devices close to the magnets unless they are known to be made of non-magnetic materials. Any phones, watches, tools, etc, should be placed close to the spectrometer computer whilst the owner is using the NMR instrument. Credit cards, photocopying cards and any other devices which use magnetic storage of information can be erased by the strong magnetic fields.

Compressed gas cylinders should not be moved within the facility without the supervision of the NMR Facility Director. The forces of attraction between such heavy magnetic objects and a superconducting magnet can cause the magnet to shift internally and sustain permanent, irreparable damage.

If any metallic device should become attached to a magnet, do not attempt to move the object but inform the facility director immediately.

Superconducting magnets are very stable devices which routinely operate for many years without problems. However, a quench can occur at any time. A quench is a rapid boil-off of cryogens caused by loss of vacuum within the magnet cryostat or by a sudden change to the magnetic field. This can be triggered by a failure of an O-ring seal on the magnet, or by a large magnetic object moving in the close vicinity of the magnet. The liquid helium and/or nitrogen will boil very rapidly which will produce a large cloud above the magnet and a loud rushing sound. Room B106 is a large space, has a high ceiling and good ventilation, so there should be no danger of asphyxiation if all users exit the room quickly. In extreme cases, the large quantities of helium and nitrogen produced displace the air in the room and cause condensation of oxygen due to their low temperatures.

If a quench should occur, the facility should not be re-entered until it has been inspected by the Facility Director. It should be ensured that the ventilation systems are working normally and that any helium and nitrogen exhausted from the magnet(s) has been safely removed from the laboratory atmosphere. Room B106 is equipped with a wall-mounted oxygen meter which constantly monitors the atmosphere. The Facility Director also owns a portable meter which can be used to test the air in both laboratories.

1.2 Reporting operational problems

There is a dedicated troubleshooting chapter of this manual which contains information which should be sufficient to solve many problems with the NMR spectrometers and computers. If there is a problem which cannot be solved by the current user, they should perform the following two tasks:

1. Leave a note by the keyboard of the computer explaining that the instrument is either not working or has some limitation of capabilities.

2. Write a clear explanation of the problem and the likely cause for the facility director. Please sign the note with a clear name and date/time. Note that this is so that further information can be gained later, not for apportioning blame. Please leave the note in the 'in' box by the facility director's office door. Problems can also be reported by email, particularly during evenings and weekends, and serious matters by telephone. A note by the office door will be found and acted upon more quickly at the start of the working day than notes left by instruments or by email.

Complex error messages can be photographed using a smart phone and emailed to the facility director. On the CentOS computers the current screen display can be saved as a file by pressing **Print Screen** on the keyboard.

In addition, the log book by each instrument should be used to record experiences such as problems shimming, spinning samples, parameter sets or automation programs not performing as expected, etc. Always clearly sign error reports so that follow up information can be easily obtained, both in person and from the software logging files.

1.3 Choice and care of NMR tubes

There are several manufacturers of NMR tubes and a wide range of grades of tube from each. Most manufacturers compare their tubes to the equivalent grade from Wilmad. For use in the Chemistry NMR facility, NMR tubes should be of equivalent quality to a Wilmad 526PP tube (Kontes 897230-0000/New Era NE-MP5-7/Norrell S-5-300-7), or better. The 800MHz instrument in the Biomolecular NMR facility requires the use of 528, 535, 542 (essential for sample spinning) or equivalent tubes. Some NMR catalogues are available in the **NMRmanuals** directory on **nmrserver.ps.uci.edu**.

Low quality NMR tubes will lead to poor peak shapes in spectra and possible probe damage.

NMR tubes are very thin walled, and so require careful handling. Tubes should never be dried in ovens, as the glass will bend. A curved NMR tube can cause probe damage and poor quality spectra. Inspect NMR tubes carefully before use, both for dirt and damage. Any marks or cracks on the tube may cause the glass to break inside the NMR probe.

NMR tubes with Young's taps attached can be used to control the atmosphere above samples, but these tubes require careful handling. The extra weight of the tap means that the tubes can only be spun slowly, between 5Hz and 15Hz. If the tubes are repaired, great care must be taken that the total length of the tube remains straight. The extra weight of the tube can also lead to problems ejecting samples.

1.4 NMR sample preparation

First select a good quality NMR tube as described above.

An ideal sample will contain a **4cm depth of solution** in the NMR tube, which is approximately 0.7ml of solution. The center of the coil in the 5mm NMR probe is 2cm from the bottom of the NMR tube. A 4cm deep sample will be symmetrical about the center of the coil, and the ends of the sample will be far enough from the coil not to distort the magnetic field. A larger volume of sample can yield excellent spectra, but uses an excess of solvent and dilutes the sample unnecessary. For variable temperature experiments 4cm sample depth should be used, as otherwise temperature gradients can develop along the length of the sample.

The NMR tube should be clean on the inside before loading the sample, and perfectly clean on the outside before loading into the NMR probe. Any dirt on the outside of the tube will contaminate the current spectrum and potentially transfer to the inside of the probe and contaminate subsequent spectra from other users' samples.

1.5 Choice of NMR spectrometer

The four solution state NMR spectrometers have different capabilities which should be considered when selecting the most appropriate instrument to use to run a particular sample or experiment:

1.5.1 DRX400: room 1403B Natural Sciences 1

Bruker DRX400 spectrometer with switchable QNP (quad nucleus probe) probe as standard. This can be used for ^{1}H, ^{13}C, ^{31}P, ^{19}F and ^{2}H NMR. No manual adjustments are required, the probe will automatically switch nuclei as necessary when experiments are started.

Standard parameter set names end with '**.q**', standard shim file name is **qnp**.

Sensitivity: ^{1}H - 276:1, ^{13}C - 174:1

Variable temperature range -150°C (123K) to +180°C (453K). Note that low temperature accessories are not normally available in this location.

If the standard switchable probe needs to be removed for servicing, a manually tunable (BBO) probe with similar performance will be substituted.

XwinNMR software/SGI computer/*Irix* operating system.

Equipped with a 120 holder sample changer for automatic operation under *IconNMR*. This is not in routine use, but can be requested by any user with a large number of samples to run, for example on behalf of a teaching lab.

1.5.2 GN500: room B106 Frederick Reines Hall

Bruker DRX500 spectrometer with BBO (broadband observe) probe as standard. This can be used for ^{1}H, ^{2}H and most other nuclei except ^{19}F. The probe should always be left tuned to ^{13}C, and so will require manual tuning for other nuclei (except for ^{2}H which is observed via the lock channel).

Standard parameter set names end with '**.s**', standard shim file name is **bbo**.

Sensitivity: ^{1}H - 465:1, ^{13}C - 235:1

Variable temperature range -150°C (123K) to +180°C (453K). Low temperature accessories are connected as standard, and a cylinder of nitrogen gas is available for spinning samples at temperatures below -30°C.

XwinNMR software/SGI computer/*Irix* operating system.

1.5.3 CRYO500: room B106 Frederick Reines Hall

Bruker DRX500 spectrometer with TCI (three channel inverse) cryoprobe as standard. This can only be used for ^{1}H, ^{13}C and ^{2}H NMR.

Standard parameter set names end with '**.c**', standard shim file name is **cryo**.

Sensitivity: ^{1}H - 5453:1, ^{13}C - 662:1

Variable temperature range 0°C (273K) to 50°C (323K) only. A pre-cooling accessory is connected as standard for temperature control down to 0°C.

If the cryoprobe needs to be removed for servicing, a manually tunable (BBO) probe with similar performance to the GN500 will be substituted.

XwinNMR software/SGI computer/*Irix* operating system.

1.5.4 AVANCE600: room B106 Frederick Reines Hall

Bruker AVANCE600 spectrometer with CBBFO (broadband, including fluorine, observe) cryoprobe as standard. This can observe nuclei in the range ^{15}N to ^{31}P, plus ^{19}F and ^{1}H, except for those in the frequency range from ^{77}Se to ^{153}Eu.

Standard parameter set names end with '**.c**', standard shim file name is **cryo**.

Sensitivity: ^{1}H - 2375:1, ^{13}C - 1309:1

Variable temperature range 0°C (273K) to +135°C (408K). A pre-cooling accessory is connected as standard for temperature control down to 0°C.

If the cryoprobe needs to be removed for servicing, a manually tunable (TBI) inverse probe will be substituted.

TopSpin software/HP computer/*Linux* operating system.

1.5.5 Biomolecular NMR Facility - Varian UnityInova 800MHz spectrometer

This instrument is not part of the Chemistry NMR facility, but is included here for comparison purposes.

Proton observation only, using one of two three channel, inverse, fixed nuclei probes. Either ^{1}H observation with ^{13}C and ^{15}N decoupling, or ^{1}H observation with ^{13}C and ^{31}P decoupling.

Sensitivity: ^{1}H - 1900:1

1.5.6 Spectrometer comparison table

Instrument (Probe)	**^{1}H sensitivity**	**^{13}C sensitivity**
DRX400 (QNP)	276:1	174:1
GN500 (BBO)	465:1	235:1
CRYO500 (TCI)	5453:1	662:1
AVANCE600 (CBBFO)	2375:1	1309:1
VARIAN UnityInova 800	1900:1	n/a

1.6 *XwinNMR* and *TopSpin*

Three of the UCI NMR spectrometers (DRX400, GN500 & CRYO500) are operated via Bruker *XwinNMR* software, whereas the AVANCE600 is operated via *TopSpin*. The hardware present in the spectrometer console determines the latest version of software which can be used for instrument control.

The three oldest instruments were produced when *XwinNMR* was the current software. Version 3.5 is in use on all of the Silicon Graphics O2 computers, this was the last version released for these systems. It would be possible to operate these three spectrometers via *TopSpin*, but only using a rather early version, 1.3. This would not offer any advantages over *XwinNMR* as it does not include 'topshim'.

The AVANCE600 is a much newer instrument, but is no longer compatible with the latest versions of *TopSpin*. The version in use is 3.2, which is the last that the instrument supports. Bruker have released free versions of *TopSpin* to academics for data processing, details of these can be found in Chapter 25. With each update the free processing version becomes more different to the software that is still be in use on the AVANCE600.

In both *XwinNMR* and *TopSpin* there are several different ways to access acquisition and processing operations, some via the command line and some via the menus and on-screen buttons. In many cases, command line operation is simpler, and is much easier to describe in a manual. In *XwinNMR*, the on-screen options can easily be ignored as they are accessed via the menus at the top of the screen. In *TopSpin* there are tabs, buttons and icons that can be used in place of typed commands.

In most cases, *XwinNMR* commands can be typed in *TopSpin*, provided that an equivalent operation exists. For example, typing **eda** or **edp** in *XwinNMR* open the acquisition or processing parameter editors, and in *TopSpin* will open the tabs for those parameters. However, there is no standard *TopSpin* equivalent for the *XwinNMR* command **edg**, because the plot systems are so different. At UCI, this command will open the spectra display preferences in *TopSpin*.

The basic method of interacting with the NMR data differs between the two programs. In *XwinNMR*, pressing the ***LMB*** (left mouse button) typically attaches the mouse pointer to the spectrum, and pressing the ***MMB*** (middle mouse button) typically defines the end points for data expansion. In *TopSpin* there is a vertical-line cursor on the screen whenever the mouse is over the data window. Expansion is performed by dragging the ***LMB*** to create a second cursor.

Some of the screen icons in *TopSpin* are a little obscure in appearance. Hovering the mouse pointer over a button for a second or two will produce an explanatory help message.

1.7 Style conventions

this font, bold :typed keyboard input, case sensitive

this font, normal :questions asked by software, or program status statements

this font, italicized :typed input, replace text with relevant number, login ID, etc

<italicized> :press particular key on keyboard, e.g. ***<enter>***

underlined :menu item or on-screen button to be selected

LMB, ***MMB***, ***RMB*** :left, middle, right mouse button

[BRACKETED] :BSMS keypad hardware button

1.8 Logging on to a computer

Login name: type your login name, followed by ***<enter>***

Password: type your password, followed by ***<enter>***

Note that both login and password are case sensitive.

1.9 Starting the NMR software

1.9.1 *XwinNMR*

Move the mouse over the *Toolchest* in the top left corner of the screen and use the ***LMB*** to select **NMR** followed by **XwinNMR**.

The *NMRterm* iconized window will appear first, followed automatically a few seconds later by the opening of the *XwinNMR* window.

The lock display and temperature controller windows will also open automatically on the spectrometer computers.

By default, *XwinNMR* will display the last dataset accessed on that particular computer. This is fine on the spectrometer computers, but on the processing workstations it is often more desirable to load data that has been recently acquired on one of the spectrometers. Therefore, on the workstation computers there are extra options in the *Toolchest* menu to load the most recently accessed datafiles from the spectrometer computers.

From the *Toolchest*, select:

NMR → **XwinNMR with latest data from:** → **DRX400**, **GN500**, or **CRYO500**

To load a different dataset, use the *XwinNMR* **search** command as described below.

1.9.2 *TopSpin*

The blue TopSpin icon appears both on the desktop and on the panel and the left-hand side of the screen. Either double-click on the desktop icon or single-click on the panel icon with the ***LMB***.

A terminal window will appear first, followed by the main *TopSpin* window and data browser, and on the spectrometer computer the lock display window.

On the spectrometer computer, the temperature display will open as an internal window and will appear at the front. A dataset window must be brought to the front before data can be loaded.

The last dataset previously accessed should appear on-screen automatically. Various methods can be used to load a different dataset from the data browser of software menus.

1.10 Using the NMR software for the first time

1.10.1 *XwinNMR*

Each computer has its own copy of the *XwinNMR* program, while the data is all stored on a central server. The first time a copy of the program is run, a default *XwinNMR* banner will be displayed. Subsequently each time the program is started the last dataset viewed on that computer will be displayed unless it was subsequently deleted via a different computer*. There are many ways of finding a dataset; this one is quick and easy:

mydata ***<enter>***

The Portfolio Editor is opened and will show data belonging to the user currently logged on to the computer. Select the *Name*, *Expno* and *Procno* of the desired dataset, and if the final selection is with the ***RMB***, the dataset will be loaded into the *XwinNMR* window.

*In this case the *XwinNMR* banner is displayed, but the **edcp** menu will contain the path name of the deleted experiment.

Instructions for using the *XwinNMR* program can be found in subsequent sections of this manual.

1.10.2 *TopSpin*

TopSpin will initially start with an empty blue data window. An existing dataset should be loaded into this window from the data browser or program menus before creating a new experiment. If this is not performed, then unexpected options will appear in the **edcp** menu.

1.11 Ending an NMR session

If applicable, close the lock display window with ***LMB*** over **quit** in the bottom right corner. Exit from *XwinNMR* by either typing **exit** or selecting **exit** from the **File** menu.

Log out from the computer by either holding down the ***RMB*** over the screen background and selecting **Log Out** from the resultant menu or by selecting **Log Out** from the *Toolchest* **Desktop** menu.

1.12 Changing your NMR password

The initial password allocated to each user is a computer generated random string of letters and numbers. This can be changed by the user, but the new password will only be accepted by the system if it also appears to be a random string of characters. A space can be included in the password.

To change your password, first open a unix shell from the SGI Irix *Toolchest*:

Desktop → **Open Unix Shell**

Or on a Linux computer use the **Terminal** icon or ***RMB*** → **Open Terminal**

Then type:

yppasswd ***<enter>***

The computer will respond as follows and request the current password and then a new password:

Changing YP password for *username* on chem.ps.uci.edu

Please enter old password:

Changing YP password for *username*

Please enter new password:

Please retype new password:

If the new password would be too easy for a hacking program to guess it will be rejected and a more random seeming combination of characters will be required.

1.13 User accounts

Every user has their own account on the NMR computers. Login details are shared by NIS, and each operating system shares a home directory. One home directory is shared across the SGI Irix computers, and another is shared across the Linux CentOS computers. The directories are linked so that postscript and PDF files, etc, can be easily accessed from either operating system.

When users leave UCI, their accounts are removed after they have been inactive for about a year. Data is deleted from the primary data server, but will remain on the two backup server drives. Old data can easily be reinstated from the backup servers by the facility director if required by current researchers, or for publication. The data backup system is described further in Chapter 22.

1.14 Maintenance

Each week the superconducting magnets are filled with liquid nitrogen. This is normally performed between 10:00 and 10:40 on Wednesdays in Reines Hall and 11:00-11:40 in Natural Sciences 1, but is sometimes moved to accommodate other tasks such as filling liquid helium at the same time. Other minor maintenance is also performed during these periods, which may require re-booting of the spectrometer or processing computers. Remote access to the facility computers may be interrupted during these times.

2 Basic 1D data acquisition

Most of the commands in this chapter can be used in *TopSpin* as well as *XwinNMR*. Where this is not possible, the *TopSpin* alternative is included.

2.1 Load sample

A 'blank' sample (normally a black nylon rod) is usually left in the magnet when the spectrometer is not in use, but the procedure is the same if the magnet is left empty. To confirm the sample status, check the LED indicators on the BSMS keypad, either *sample up*, *sample missing*, or *sample down* will be illuminated.

Each NMR tube must be carefully cleaned, the sample spinner positioned correctly using the depth gauge, tube and spinner wiped clean once more, before loading into the magnet.

It is a good idea to turn off **[LOCK]** and **[SPIN]** before changing samples.

[LIFT ON-OFF] (top left button on BSMS keypad) turns on sample eject air.

Position sample at top of magnet bore, supported by air flow.

Do not release your sample unless you are sure that the air flow will support it and it will not drop into the magnet.

If no sample was left in the magnet, there will be a short delay before the air flow comes on, and it is occasionally necessary to turn the air off and on again.

[LIFT ON-OFF] turns off eject air to lower sample.

When the sample is fully loaded into the probe, *sample down* will be illuminated on the keypad.

[SPIN ON-OFF] turns on sample spinning, the button LED will flash until the set speed is reached. Normally the speed is set at 20Hz. If necessary, this can be altered by selecting **[SPIN RATE]** and turning the knob. The sample spin rate can be monitored by pressing the orange **[2nd]** button followed by **[SPIN RATE]** to access **SPIN MEAS.**

2.2 Create a new dataset

edcp ***<enter>***

This command runs a macro which executes two commands: **edc** followed by **par**, which ensures that the new parameters selected will always be read into the new experiment number. Enter a new experiment name and/or experiment number. The experiment name becomes a Unix directory name, and so cannot contain special characters like *, /, (,) and &. It is advisable to only use alphanumeric characters and the following symbols: **.**, **+**, **−**, **=** and _. Note that the Unix file system is fully case sensitive, so filenames can be created which differ only in the case of characters. Care should be taken entering upper or lower case characters as desired to avoid confusion. A maximum of 14 characters is recommended.

Normally it will only be necessary to enter the experiment name and/or experiment number, however the very first time a dataset is created, **USER** must be set to your user ID (the default is guest) and **DU** must be set to /v (the default is /u).

After editing the entries as required, click on **SAVE** with the ***LMB*** to create the new files.

Note that if the filename specified already exists, then **edc** will recall that file.

Next a menu of all parameter sets suitable for the standard configuration of each instrument is produced. This menu can also be produced by the **par** command.

In *TopSpin* there is an option to turn on 'comments' which give a short description of each parameter file. This also displays the menu as a single vertical column which is more readable. To achieve this, select **Options → Show Comment** at the top of the parameter menu window. This setting will remain active during the current *TopSpin* setting, but is not retained on exit.

Select the required parameter set, e.g. **h1.q**, then select **copy all**.

If the **edc** command is used, then the parameter set name and copy option can be stated explicitly, e.g.

rpar h1.s all ***<enter>***

2.3 Read standard shim file

The shim file name is based upon the probe is use in the instrument. Most instruments use a single probe almost all of the time. Typical filenames are: **qnp** (DRX400), **cryo** (CRYO500), **bbo** (GN500), **cryo** (AVANCE600), e.g.

rsh bbo ***<enter>***

2.4 Lock field onto deuterium signal from solvent

The lock display window may have opened automatically on starting the NMR software. If it did not, or has been closed, type:

lockdisp ***<enter>***

to open the lock display window. Adjust shape and position of window if desired. The **mode** button switches between a single white trace and red and white traces.

Either:

lock ***<enter>***

Then select solvent name from the resultant menu.

Or:

lock followed by solvent name, e.g. **lock cdcl3** ***<enter>***

Note that the correct solvent abbreviation must be used, but in this instance, it is not case sensitive. Solvent names with a 'T' appended are for use when TMS or DSS are present; the software automatic calibration command will then search for a reference peak at 0ppm, instead of searching for the signal from the protonated component of the solvent.

Wait for lock : finished to appear on the status line, at the bottom of the window.

Optimize lock phase:

[LOCK PHASE] Turn knob and observe the height of the lock signal on the screen. Adjust for maximum height.

If necessary, adjust the lock sweep width (amplitude) and sweep rate on the BSMS keypad. Standard values are:

[SWEEP AMPL.] = 2.0 and **[SWEEP RATE] = 0.4**

2.5 Shim magnetic field

On the AVANCE600 this can be performed automatically using **optshim**. See Chapter 29 for more details.

Ensure that the **[ONAXIS]** button LED is illuminated on the BSMS keypad. For most solvents it is advisable to ensure that **[FINE]** is selected, but for chloroform the initial adjustment can be performed with this off.

[z] Turn knob and observe the height of the lock signal on the screen. Adjust for maximum height.

[z2] Turn knob until maximum signal height is achieved.

Re-adjust **[z]** and **[z2]** in turn until no further improvement is observed.

[z3] Adjust knob for maximum lock signal height.

Cycle through adjusting **[z3]**, **[z2]** and **[z]** in turn until no further improvement is achieved.

If the sample is not spinning, the X and Y shims can also be adjusted. To select X, press **[x]** followed by **[z0]** and to select Y, press **[y]** followed by **[z0]**.

[LOCK PHASE] Check that the lock phase is optimized.

[STD BY] Stand-by mode, knob is inactive.

If at any stage the lock signal level increases such that it rises out of the lock window:

[LOCK GAIN] Reduce level until the signal is about three squares below the top of the window.

2.6 Set receiver gain and acquire data

acqu ***<enter>***

Display acquisition screen.

ns ***<enter>***

If required adjust number of scans (use a multiple of 8).

rga ***<enter>***

Set receiver gain.

During the receiver gain adjustment, the number of scans is temporarily set to 100 which will be reflected in the experiment time displayed. Wait for rga : finished to appear at the bottom of the screen.

zg ***<enter>***

Note that these two commands can be combined by typing **rgazg**.

Note also that **go** can be used to add **ns** more scans to the current experiment if no other parameters have been changed and no lock or shim adjustments have been made.

2.7 Observe data from experiment in progress

tr ***<enter>***

Transfers data to disk at the end of the current scan.

XwinNMR only: **trat** *n* ***<enter>*** will transfer and process the data at the next multiple of *n* scans. The data is actually transferred after each scan until the requested number is reached, then **efp** is executed.

TopSpin only: **tr** *n* ***<enter>*** will transfer the data at the next multiple of *n* scans.

2.8 Interrupt experiment if necessary

If desired, the current experiment can be interrupted and the data saved or discarded:

halt ***<enter>***

Interrupts the experiment at the end of the next scan and saves the data.

XwinNMR only: **haltat** *n* ***<enter>*** will halt the experiment and save the data at the next multiple of *n* scans.

TopSpin only: **halt** *n* ***<enter>*** can be used.

stop ***<enter>***

Interrupts the experiment and discards the data. Any transferred scans will be preserved.

2.9 Process data quickly to check peak shapes

ft ***<enter>***

Fourier transform (or **fp** to also apply phase correction currently stored in file).

apks ***<enter>***

Automatic phase correction.

In *TopSpin*, **apk** ***<enter>*** is a better command to use. This option exists in *XwinNMR*, but is much slower than **apks**.

Expand spectrum and check that peaks are narrow and symmetrical. If they are not, adjust shim settings and repeat experiment.

2.10 Optimize parameters

For the best quality spectrum, optimize the acquisition parameters as described in section 3.

2.11 Run another experiment (if required)

If the sample is to be changed, then return to section 2.1. If more experiments are to be performed on the same sample, continue as follows:

edcp ***<enter>***

Create a new dataset. If the same sample is to be used simply increment the experiment number, then select the desired parameters from the **par** menu. Customize the parameters if required.

solvent ***<enter>***

Enter the current solvent name to enable automatic referencing and correct parameter printing.

Continue with section 2.6.

3 Optimization of 1D acquisition parameters

Most of the commands in this chapter can be used in *TopSpin* as well as *XwinNMR*. Where this is not possible, the *TopSpin* alternative is included.

The instructions in the preceding section will be sufficient to obtain a spectrum under most circumstances, but it is preferable to optimize the acquisition parameters to obtain a more accurate result.

3.1 Acquire initial spectrum

Use the instructions in section 2 to obtain an initial spectrum of the standard spectral range, e.g. a proton spectrum from +15ppm to -1ppm. Typically, the optimal range will be smaller than this. To find peaks outside this range proceed to section 3.5.

3.2 Make a copy of the data

It can be as important to have a spectrum showing where there are no peaks as it is to have an accurate spectrum containing the peaks. Therefore, it is a good idea to keep the original wide spectrum and make a copy to optimize.

cop ***<enter>***

The computer will offer the next experiment number, to select this type:

y ***<enter>*** or **yes** ***<enter>***

or type: **cop** *newexpno* ***<enter>***

3.3 Select the region of interest

Select and expand the required spectral region by pressing ***LMB*** to attach the mouse pointer to the spectrum and pressing ***MMB*** to define left and right end points for the expansion. In *TopSpin*, drag the ***LMB***.

For 1D experiments it is a good idea to include the reference peaks and at least 0.5ppm of baseline at each end of the spectrum to make data processing easier. Typically, an optimized proton spectrum might cover the range 10.5ppm to -0.5ppm, or to a higher shift if peaks are present above 10.5ppm.

When optimizing parameters for use in 2D experiments, only the sample peaks of interest need be included, the resolution should be maximized by reducing the spectral range as much as possible. For 2D experiments it is a good idea to ensure that no peaks are exactly at the center of the spectrum. Display the fixed grid (the left of the two grid buttons) and check that no peaks are on the center line. If necessary, re-define the expanded region to move peaks away from the center.

sw-sfo1 (Note that in *XwinNMR* 3.5 this must be pressed at least 3 times before the parameters are updated correctly.)

Sets the acquisition parameters to correspond to the displayed spectral region and displays the new values for **sw** and **o1** at the bottom of the screen.

In *TopSpin* this function is accessed via the  button

3.4 Re-run experiment

rgazg ***<enter>***

Reset receiver gain and acquire new FID.

3.5 Finding peaks outside the initial range

The default parameters may not be sufficient for locating all signals from unusual samples. For example, the presence of metals can result in very large shifts.

par ***<enter>***

Load standard experimental parameters.

sw ***<enter>***

Increase spectral width to a large value, e.g. 40ppm for protons. The center of the spectrum will not move, so that if the standard proton spectral range is +15ppm to -1ppm, then the new range will be +27ppm to -13ppm. If necessary, the parameter **o1p**, the chemical shift of the center of the spectrum, can also be adjusted.

rgazg ***<enter>***

Record new fid and then process data as usual.

Note that the acquisition time will now be much too short for accurate measurement of the FID, so completing the following steps is **essential**.

Expand the required spectral region by pressing ***LMB*** to attach mouse pointer to spectrum and pressing ***MMB*** to define the end points. In *TopSpin*, drag the ***LMB***.

sw-sfo1 (Note that in *XwinNMR* 3.5 this must be pressed at least 3 times before the parameters are updated correctly.)

Updates the acquisition parameters to correspond to the desired region and displays the new values for **sw** and **o1**.

In *TopSpin* this function is accessed via the  button

aq *<enter>*

Check the acquisition time. If the new spectral width is too large, then the acquisition time will be too short to record accurate data. This must be increased by acquiring more data points. For accurate proton experiments, the acquisition time should be about **6** seconds. If the current value of **aq** is less than this, proceed as follows:

aq 6 *<enter>*

Record accurate spectrum:

rgazg *<enter>*

Before processing the data, you must now ensure that all the datapoints will be Fourier transformed. The number of data points acquired is **td** and the number of data points transformed is **si**. The parameter **si** is normally used to improve the appearance of the data by zero-filling. If **si** equals **td** then **td** zeros are added to the data. This is because **td** refers to complex pairs of data points, whereas **si** refers just to real data points. The minimum value for **si** is therefore **td/2** , which results in no zero filling. A further restriction is that **si** must be an integral power of 2 (2^n). In most 1D parameter sets, **si** will be set to **64k**. If the new value of **td** is between 64k (65536) and 128k (131072), then **si** can be set to either 64k or 128k, if **td** is between 128k (131072) and 256k (262144), then **si** can be set to 128k or 256k.

td *<enter>*

Check the value of **td**. If necessary, increase **si**, e.g.:

si 128k *<enter>*

Now the data can be processed in the normal way.

4 Basic 1D data processing

Most of the data processing commands in this chapter can be used in *TopSpin* as well as *XwinNMR*. Where this is not possible, the *TopSpin* alternative is included. The method for printing spectra in *TopSpin* is very different to *XwinNMR*, however. See Chapter 29 and the on-screen help for more information.

Note that if data has been processed in the past, but the spectrum has subsequently been deleted, the processing parameters will be saved. See the end of this chapter for re-processing information.

4.1 Read the required dataset

search ***<enter>***

Open the *Portfolio Editor* and select the required dataset using the ***RMB*** which reads the data directly into the *XwinNMR* window. Use **mydata** instead if the currently displayed data belongs to a different user, or the banner screen is displayed.

In *TopSpin*, use the data browser or one of the menu options.

If the selected dataset has previously been processed, then suitable processing parameters will already be stored. A combination of **efp** (or **fp** if line broadening is not required), followed by **bcm** or **abs n** will re-generate the previous spectrum and integration. Processed data is automatically deleted from the server when the files are between one and two weeks old. The command **efpb** can be used to regenerate the spectrum, including baseline correction, provided that this has been performed accurately previously.

4.2 Exponential line broadening

Line broadening is used to smooth the spectrum, but broadens peaks as well as noise, and so may reduce the resolution of fine couplings.

Typical line broadening factors are 0.3Hz for ^{1}H data, or 1Hz for ^{13}C data. These values should already be set in the standard parameter sets, and so adjustment will not normally be necessary. If a different value is required, proceed as follows:

lb *new line broadening factor* ***<enter>*** e.g. **lb 0.5** ***<enter>***

Note that in *XwinNMR* the command **winfunc** can be used to interactively adjust the window function applied. This functionality can be accessed in *TopSpin* 4 by typing **wm** and then selecting **Manual window adjustment**.

4.3 Fourier transformation

efp ***<enter>***

This command is a combination of **em**, **ft** and **pk**, and applies exponential line broadening followed by Fourier transformation and phase correction. The phase correction applied is read from the dataset processing parameters and so will only be correct if the data has previously been processed.

Or:

fp ***<enter>***

If exponential line broadening is not required.

If the phase correction applied above causes subsequent automatic phasing to fail, then **ef** or **ft** can be used to Fourier transform with and without line broadening, with no phase correction.

4.4 Automatic phase correction

apks ***<enter>***

Note that under some conditions this process will fail. If **apks** produces errors, type **kill**, select **apks** from the menu and continue with manual phasing. If **apks** produces a very badly phased spectrum, try re-transforming the data using **ef** (or **ft** if no line broadening is desired) and then either re-try **apks** or phase manually.

In *TopSpin*, **apk** is a superior automatic phasing option. This is much slower in *XwinNMR*.

Expand the spectrum vertically. If the automatic phasing is good continue below with section 4.6, otherwise adjust manually:

4.5 Manual phase correction

phase

Enter the phase correction screen.

biggest (*XwinNMR* only, in *TopSpin* the biggest peak is automatically highlighted and defined as the pivot point.)

Marks the pivot point (reference peak for zero-order phase correction) and performs automatic zero-order correction.

PH0 (use the **0** button in *TopSpin*)

Hold down ***LMB*** and move the mouse to phase correct the peak labeled with a vertical dotted line.

PH1 (use the **1** button in *TopSpin*)

Hold down ***LMB*** and move the mouse to phase correct peaks far from the largest peak.

return → **save & return** (use the disk icon button in *TopSpin*)

4.6 Baseline correction

Note that the automatic baseline command, **abs**, will integrate the spectrum as well as perform baseline correction. This can be avoided by using the option **abs n**. If data has been previously manually baseline corrected, the same correction can be re-applied by typing **bcm** ***<enter>***.

abs n ***<enter>***

Expand the spectrum vertically and see if the automatic correction has produced a flat baseline. If not, perform a manual correction instead as below.

Manual baseline correction, if required:

efp ***<enter>***

Re-process and remove the effect of the poor automatic correction.

basl ***<enter>*** (In *TopSpin* use the **Baseline** tab.)

Enter the baseline correction screen.

A blue line is drawn across the center of the spectrum, described by the polynomial function in the info box.

Hold ***LMB*** over the **A**, **B**, **C**, **D**, and **E** boxes and move the mouse up and down as required to match the shape and position of the blue line to the spectrum baseline.

diff (use the triangle icon difference button in *TopSpin*)

Perform baseline subtraction.

undo (use the triangle icon difference button in *TopSpin*)

Remove the subtraction and modify the baseline shape, if required.

diff (use the triangle icon difference button in *TopSpin*)

Subtract the new baseline.

return → **save & return** (use the disk icon button in *TopSpin*)

4.7 Spectrum calibration

Typically, proton and carbon spectra are calibrated by referencing a peak in the spectrum which represents either the solvent or a reference compound. Chemical shifts are defined as relative to the proton frequency of TMS (tetramethylsilane), any other method represents an approximation. If your spectrum does not include a reference peak, proceed to section 4.7.3.

4.7.1 Automatic

This command reads the solvent name from the status parameters and then searches for the relevant reference peak. In general, if the solvent name has a 'T' appended then TMS will be referenced, and if not the residual proton signal from the solvent will be referenced. For example, solvent name CDCl3T will reference a TMS signal at 0ppm, and CDCl3 will reference a $CHCl_3$ signal at 7.27ppm.

Note that the software will search for the largest peak in a small region around the expected shift, and so if a larger peak is present from the sample then this may be referenced instead. It is a good idea to expand the spectrum horizontally and identify the solvent signal before trying the automatic calibration, as the un-calibrated spectrum is likely to be within 0.05ppm of the correct values, but if the automatic calibration selects the wrong signal this may introduce a larger error. For solvents with characteristic deuterium-proton coupling patterns, and for TMS, this is generally not a problem, but a small $CHCl_3$ peak can be harder to identify if the referencing has been incorrectly applied.

The solvent name is automatically stored by the lock command when the first experiment is set up, but may not be correct in subsequent experiments.

The solvent reference values are taken from the Cambridge Isotopes Ltd table (see Appendix A).

To check the solvent name (a prefix of **2s** is required to modify a status parameter in *XwinNMR* or **s** in *TopSpin*)):

2s solvent *<enter>*

Note that for very old data (acquired before August 2000) the instrument name status parameter may not be correct. This can be inspected by typing:

2s instrum *<enter>*

and should be either **drx400**, **gn500**, **cryo500** or **av600** (Prior to August 2000 the three DRX instruments had the same name: spect).

sref *<enter>*

Expand the spectrum and check that the referencing is now correct. If it is not, proceed as follows.

4.7.2 Manual

Expand a small region of the spectrum containing the peak to be referenced:

LMB to attach cursor to the spectrum.

MMB to define both ends of the region for expansion, repeat this if necessary, to zoom in closer to the desired peak.

calibrate

The cursor is attached to the spectrum and positioned in the center. Move the mouse sideways until the cursor is at the top of the desired peak.

MMB then type the relevant reference value in ppm.

In *TopSpin* use the **Calibrate** tab and then the ***LMB***.

Note that in *XwinNMR* referencing is restricted to discrete data points. In *TopSpin* any position on screen can be selected which may allow more accurate positioning in the center of peaks.

4.7.3 Use separate proton spectrum to calculate referencing

A ^{13}C spectrum recorded in a non-carbon containing solvent, like D_2O will not contain a reference peak, nor will spectra from most other nuclei. These spectra should be referenced by calculation based on the frequency of the TMS peak in the proton spectrum.

The proton spectrum should be recorded in the same operating session as the spectrum to be referenced, at the same temperature and under the same lock and shim conditions, with the same experiment name.

To reference the unknown spectrum, type:

xiref *<enter>*

The computer will request the experiment number and process number of the proton spectrum and calculate the referencing for the current spectrum.

4.8 Integration

Note 1: If automatic baseline correction (**abs**) has been used, then the spectrum will also have been automatically integrated. This can be loaded from the **File** menu within the integration screen: **File** → **Read ´intrng´**, but is normally ignored and replaced by the manual integration described below.

Note 2: If required, several spectra can be integrated with the same integral scaling. To achieve this, the **lastscal** on-screen button must be pressed immediately after entering the integrate screen.

First expand a region at the left hand end of the spectrum to a size suitable for integration with the mouse, ***LMB***, ***MMB***, ***MMB***.

Integrate (in *TopSpin* use the **Integrate** tab and then the ***LMB*** and on-screen buttons to perform the equivalent operations to those listed below.)

Enter the integration screen.

LMB to attach the cursor to the spectrum.

MMB, ***MMB*** to define the end points for the integral for each peak on the screen.

LMB to detach the cursor from the spectrum.

← to move the spectrum to the left, half a screen at a time, to find the next peak(s) to integrate.

Integrate all the peaks of interest in the spectrum before making any modifications. If the spectrum is baseline corrected accurately then there is normally no need to alter the integral shapes. This can be useful however if there are underlying

broad peaks in the spectrum. The default integral calibration is that the first integral has an area of 1. This can also be altered if necessary.

Optional:

 (from the **all:** menu section) Hold the ***LMB*** and move the mouse upwards to raise the integral trails above the spectrum baseline.

LMB to attach the cursor to the spectrum.

Move the mouse until the pointer is under the integral of interest.

LMB to define this integral as the 'current integral' for modification, labeled with a small arrow at the right hand end.

calibrate

Alter the area of the current integral, if desired.

The shape of the integral trail can be corrected by altering the **slope** and **bias**. Adjust the **bias** first (using the ***LMB***) to make the beginning of the integral trail horizontal, then adjust the **slope** (using the ***LMB***) to make the end of the trail horizontal. If necessary, the mouse sensitivity can be increased by reducing the adjustment increment with the ***LMB*** over the **mouse**: **/2** button.

When integration is complete:

return → **save as 'intrng' & return**

4.9 Plotting

The following instructions apply to *XwinNMR*. In *TopSpin*, use the **Plot** tab and on-screen buttons to plot spectra. More details can be found in Chapter 29.

There is a separate plotting program within *XwinNMR* called *XwinPlot*. This is suitable for producing complex annotated spectra and is described in section 20. There is also a thorough manual accessible via the *XwinPlot* **Help** menu. The instructions below are concerned with plotting spectra via the standard *XwinNMR* plot routines, which is generally easier for standard spectra.

setti *<enter>*

Enter a title (the text editor invoked can be defined by **setres**).

Expand the spectrum (if desired) with the ***LMB*** and ***MMB*** then:

dp1

Define the plot limits in ppm. The default limits offered by the dialogue are those currently displayed.

The computer will ask:

Change y-scaling on display, according to PSCAL?

Normally **n *<enter>*** is the most appropriate response.

vplot *<enter>*

Preview the plot in a window on screen.

Quit to close the previewer, then press ***LMB*** over **OK** to plot or **Cancel** to quit and continue.

If necessary, adjust plot scaling:

Plot scaling is controlled by two parameters: **cy** and **pscal**. **cy** is the scaling factor in cm. The peak that this is applied to depends on the setting of **pscal**. The most useful settings for **pscal** are **preg**, **psreg** and **global**.

preg: The tallest peak in the current plot region is plotted **cy** cm high.

psreg: The tallest peak in the plot region which is not a solvent peak is **cy** cm high. Correct operation of this option depends on the position of the solvent peak and its separation from neighboring signals. The solvent region file is controlled by the parameter **sreglst**. If **sreglst** is set from within the **edp** menu, then the correct file can be selected from a list. **pscal** can also be set from this menu; scroll the window down to find both parameters. If the solvent region filename has a 'T' appended, e.g. **1H.CDCl3T**, the region around 0ppm is ignored in addition to the region around the solvent peak.

global: The tallest peak in the entire spectrum is scaled to be **cy** cm high, irrespective of whether this peak is in the current plot region.

For proton spectra, **cy** is normally set to **15** cm to fill the available space in the standard plot layout.

The standard setting for **pscal** is **preg** will normally be suitable for plotting both full spectra and expansions. To ignore solvent peaks, change **pscal** to **psreg** and select the appropriate **sreglst**.

Note that the height in centimeters displayed on screen by the y-axis and the mouse 'Info' box correspond to a **pscal** *setting of* **global**. *If a different setting is in use, e.g.* **preg**, *the peak heights are only adjusted for the plotted output.*

An exception to the above method is when it is desirable to plot a region of a spectrum containing a wide range of peak heights, when the vertical scaling must be increased in order to see the smaller peaks clearly. In these cases the scaling can be adjusted in three different ways:

4.9.1 Scaling method 1 (manual):

Manually adjust the displayed vertical scaling to mimic the output of the **view** command; e.g. if **pscal** is currently set to **preg** adjust the spectrum scaling so that the tallest peak in the displayed region fills the height of the window.

Use the ***2** or ***8** button to increase the vertical scaling until the peaks of interest can be clearly seen, and keep track of the total increase. For example, pressing ***2** twice is equivalent to scaling the spectrum by a factor of 4. Then simply multiply **cy** by this factor, in this case increasing the value from **15** cm to **60** cm.

Alternatively, use the ***LMB*** to read the current height of both the tallest peak on screen and of the peak that is desired to fill the page.

Type **cycalc** and the computer will request the two peak heights and calculate an appropriate new value for **CY**. If pscal was set to **global** it will automatically be changed to **preg**.

4.9.2 Scaling method 2 (interactive):

Adjust the vertical scaling as required using the buttons on the left of the screen.

utilities

Enter the utilities screen.

CY

This function will only work if the y-axis units are set to cm and if **pscal** is set to **global**. If error messages indicate that the current settings are different, then change them as follows:

YU

Change the y-axis units from absolute intensity (abs) to centimeters (cm).

pg ***<enter>***

Set **pscal** to **global**. To reset **pscal** to **preg** or **psreg** afterwards, either type **pscal** ***<enter>*** and select the desired setting, or type **pscal** followed by that setting, e.g. **pscal psreg** ***<enter>***.

A horizontal blue line will appear on the screen at the 1cm level, or at the top of the screen if the maximum y-axis value is less than 1cm. Move the mouse to position this line level with the peak to be used for plot scaling.

Use the ***MMB*** to set this height to the current **cy** setting (normally 15cm), or the ***LMB*** to define a new height in cm.

Note that this operation works by calculating a new **cy** value for the tallest peak in the entire spectrum, which will result in the smaller peak being the required height. It is a good idea to set **cy** back to its original value afterwards, so that other spectral regions can be easily plotted using the standard settings for **pscal**, **preg** or **psreg**.

4.10 Adjust peak labeling (if necessary)

To inspect the current peak labeling, use either **view** ***<enter>*** to preview the plot on screen, or **pps** ***<enter>*** to list peak labels on screen. Peak labeling is controlled by two main parameters: **mi**, and **pc**.

mi is the minimum intensity for peal labeling, normally set to a small value, e.g. 0.5cm.

pc is the peak picking sensitivity factor, which controls the differentiation between peaks and noise. A default value of 4 works well for clean proton spectra. **pc** can be reduced for labeling of tiny peaks and peak shoulders, and for small peaks in carbon spectra. Try a value of 1 first, then reduce further if necessary. To change **pc** simply type a new value, e.g. **pc 1** ***<enter>***.

If reducing **pc** results in too many small peaks in the baseline being labeled, then increase **mi** until only the desired peak labels are seen. **mi** can be changed manually via the keyboard, or interactively via **utilities** → **mi**.

By default, the units for peak labels are ppm. This can be changed to Hz if desired:

plunit ***<enter>*** and select **Hz**; or **plunit Hz** ***<enter>***

Similarly, the units can be reset to ppm by:

plunit ***<enter>*** and selecting **ppm**; or **plunit ppm** ***<enter>***

Note that a list of peak labels for the current plot region, in both Hz and ppm, can be printed by:

pps ***<enter>*** and selecting **Print** in the bottom right corner.

When the desired peak labeling is achieved plot the spectrum.:

plot ***<enter>*** or **vplot** ***<enter>*** to check preview before plotting.

4.11 Adjust plot layout (if necessary)

The standard plot parameters will print a spectrum 22.8cm wide with parameters on the right. If you do not desire parameters to be printed, then the full page can be devoted to the spectrum. Set **cx** to **25** cm and set **PARAM** to **no** in the **edg** menu. Similarly, the **edg** menu can be used to turn off any other options not required, e.g. peak labels, integral trails, integral labels etc.

4.12 Automatic plot expansions

It is possible to automatically print expanded regions of a spectrum. Each integrated region of the spectrum will be printed separately, using as many pages as necessary. The peak labels are automatically displayed in Hz instead of ppm, and the default setting is that each spectral expansion is scaled to be 10Hz/cm. These settings can be changed using the **edgx** menu.

To preview the expansions on screen:

viewx ***<enter>***

Use **Next Page** at the top of the preview window to view the next expanded region(s).

To print the expansions to the currently defined plotter:

plotx ***<enter>***

The first time either **plotx** or **viewx** is executed on a particular dataset, the current integral region file, **intrng**, is copied with the filename **reg**. If this file already exists, however, it is not recreated. So, if the integral regions (in ppm) for the current spectrum are changed, for example following re-referencing of the spectrum, then the **reg** file is not automatically updated. Both **plotx** and **viewx** will display the previous ppm ranges which will no longer match the integrated regions. To overcome this, a new **reg** file must be saved via **File** → **Save as 'reg'** in the **integrate** screen after re-integrating the spectrum.

4.13 Re-processing old data

To save space on the data server, every Sunday spectrum files which are more than a week old are deleted. All of the processing parameters are retained, and so if the data is re-processed there is no need to re-apply most of the commands above.

The commands **efpb** or **fpb** will reprocess the data with or without exponential line broadening. Spectrum phasing, referencing, integration and baseline correction will be applied exactly as performed when the data was previously processed. If a manual baseline correction was used previously the same curve will be subtracted, and if not, automatic baseline correction without integration is used.

The automation program **multiefpb** can be used to automatically re-process a sequence of experiments.

The latest version of *TopSpin* has a 'serial processing' option. This can be used to re-process many datasets automatically. This could be used to create a PDF of every dataset on a predefined list, or even every dataset belonging to a particular user. The PDFs can then be combined into a single file for ease of access. The format of the output can be modified to match a user's requirements, see the facility director for further information.

5 Basic 2D data acquisition

5.1 Homonuclear experiments (e.g. gcosy)

Note that for best results probe tuning should be checked (see section 10), and some complex experiments may require optimization of parameters. See sections on individual experiments for more information.

Create a new experiment and acquire a 1D spectrum as described in section 2 (e.g. proton spectrum).

Process data, phase correct and calibrate spectrum.

5.1.1 *XwinNMR* only

Use ***LMB*** and ***MMB*** to expand display to region of interest. Ensure that the fixed grid (left of the two grid buttons) is displayed on screen and check whether a peak is in the exact center of the spectrum. If necessary, reselect the expanded region.

dp1

This saves the displayed spectrum as the plot region, which will be used for the 2D experiment.

edcp ***<enter>***

Set up a new experiment number, keeping the same experiment name, then select the required 2D parameters.

copypars ***<enter>***

The program will request the experiment number for the 1D spectrum created above. The acquisition region for the 2D experiment will be matched to the plot region in the 1D spectrum. The referencing parameters and solvent name will also be copied. A summary of the updated parameters in the 2D experiment will be presented on screen.

expt ***<enter>***

Check the experiment time and adjust the number of scans, **ns**, if necessary.

Note that the resolution in the indirect (F1) dimension can be increased by increasing the number of experiments. This is most easily performed in the acquisition parameter editor: Type **eda** and increase **TD** in the right (F1) column from 256 to 512. This will double the duration of the experiment.

[SPIN ON/OFF] to turn off sample spinning.

rgazg ***<enter>***

Set the receiver gain and run the experiment.

Note that for some advanced 2D experiments, e.g. TOCSY, the receiver gain must be set manually. See the chapter on individual experiments.

5.1.2 *TopSpin* only

edcp ***<enter>***

Set up a new experiment number, keeping the same experiment name, then select the required 2D parameters.

copypars ***<enter>***

Load the desired reference spectrum using the command line, data browser or menus.

Use the ***LMB*** to expand the data displayed to be the region of interest. If a 2D experiment is to be run using this region, make sure that there is not a peak exactly in the center of the spectrum.

Copy Parameters to load this region into the new experiment. The program will search the new experiment for all relevant parameters which may take several seconds to complete.

If the new acquisition time is more than twice that in the initial parameters, a list of choices will appear on screen:

Warning: AQ has increased by more than a factor of 2!

⊙ Keep new AQ

O Keep old AQ and reduce TD accordingly

O Keep AQ+D1 constant and (reduce TD if necessary)

The first option is the default and will be appropriate in most cases.

A summary of the new parameters will then appear on-screen.

expt ***<enter>***

Check the experiment time and adjust the number of scans, **ns**, if necessary.

Note that the resolution in the indirect (F1) dimension can be increased by increasing the number of experiments. This is most easily performed in the acquisition parameter editor: Type **eda** and increase **TD** in the right (F1) column from 256 to 512. This will double the duration of the experiment.

[SPIN ON/OFF] to turn off sample spinning.

rgazg ***<enter>***

Set the receiver gain and run the experiment.

Note that for some advanced 2D experiments, e.g. TOCSY, the receiver gain must be set manually. See the chapter on individual experiments.

5.2 Heteronuclear (e.g. ghmqc)

Note that for best results probe tuning should be checked, and some complex experiments may require optimization of parameters. See sections on individual experiments for more information.

For clarity it is assumed here that the first experiment will be a proton spectrum as experiment number 1, but in practice any sequence of experiment numbers can be used.

Create a new experiment and acquire a 1D spectrum for the observe nucleus, as described in section 2 (e.g. proton spectrum, experiment number 1).

edcp ***<enter>***

Create experiment number 2 and acquire a 1D spectrum for the second nucleus, as described in section 2 (e.g. carbon spectrum).

re 1 ***<enter>***

Process data, phase correct spectrum and calibrate spectrum. This can be performed while experiment 2 is in progress.

5.2.1 *XwinNMR* only

Use ***LMB*** and ***MMB*** to expand display to region of interest. Ensure that the fixed grid (left of the two grid buttons) is displayed on screen and check whether a peak is in the exact center of the spectrum. If necessary, reselect the expanded region.

dp1

This saves the displayed spectrum as the plot region, which will be used for the 2D experiment.

5.2.2 *XwinNMR* and *TopSpin*

re 2 ***<enter>***

Process data, phase correct spectrum and calibrate spectrum.

5.2.3 *XwinNMR* only

Use ***LMB*** and ***MMB*** to expand display to region of interest. Ensure that the fixed grid (left of the two grid buttons) is displayed on screen and check whether a peak is in the exact center of the spectrum. If necessary, reselect the expanded region.

dp1

This saves the displayed spectrum as the plot region, which will be used for the 2D experiment.

edcp ***<enter>***

Set up a new experiment number, keeping the same experiment name, then select the required 2D parameters.

5.2.4 *XwinNMR* only

copypars ***<enter>***

The program will request the experiment numbers for the two 1D spectra created above. The acquisition region for the 2D experiment will be matched to the plot regions in the 1D spectra. The referencing parameters and solvent name will also be copied. A summary of the updated parameters in the 2D experiment will be presented on screen.

For HMQC and HSQC experiments, if the proton spectral width (**sw**) is less than **10**, the relaxation delay (**d1**) should be increased from **1** to **2**. This is to reduce sample heating effects caused by the carbon decoupling during the acquisition.

expt ***<enter>***

Check the experiment time and adjust the number of scans, **ns**, if necessary.

Note that the resolution in the indirect (F1) dimension can be increased by increasing the number of experiments. This is most easily performed in the acquisition parameter editor: Type **eda** and increase **TD** in the right (F1) column from 256 to 512. This will double the duration of the experiment.

[SPIN ON/OFF] to turn off sample spinning.

rgazg ***<enter>***

Set the receiver gain and run the experiment.

5.2.5 *TopSpin* only

edcp ***<enter>***

Set up a new experiment number, keeping the same experiment name, then select the required 2D parameters.

copypars ***<enter>***

Load the desired reference spectrum for the observe dimension, usually ^{1}H, using the command line, data browser or menus.

Use the ***LMB*** to expand the data displayed to be the region of interest. If a 2D experiment is to be run using this region, make sure that there is not a peak exactly in the center of the spectrum.

Copy Parameters to load this region into the new experiment. The program will search the new experiment for all relevant parameters which may take several seconds to complete.

If the new acquisition time is more than twice that in the initial parameters, a list of choices will appear on screen:

Warning: AQ has increased by more than a factor of 2!

⊙ Keep new AQ

O Keep old AQ and reduce TD accordingly

O Keep AQ+D1 constant and (reduce TD if necessary)

The first option is the default and will be appropriate in most cases.

A summary of the new parameters will then appear on-screen.

copypars ***<enter>***

Load the desired reference spectrum for the indirect dimension, commonly ^{13}C, using the command line, data browser or menus.

Use the ***LMB*** to expand the data displayed to be the region of interest. If a 2D experiment is to be run using this region, make sure that there is not a peak exactly in the center of the spectrum.

Copy Parameters to load this region into the new experiment. The program will search the new experiment for all relevant parameters which may take several seconds to complete.

expt ***<enter>***

Check the experiment time and adjust the number of scans, **ns**, if necessary.

Note that the resolution in the indirect (F1) dimension can be increased by increasing the number of experiments. This is most easily performed in the acquisition parameter editor: Type **eda** and increase **TD** in the right (F1) column from 256 to 512. This will double the duration of the experiment.

[SPIN ON/OFF] to turn off sample spinning.

rgazg ***<enter>***

Set the receiver gain and run the experiment.

6 Processing 2D data

There are two types of 2D experiment: magnitude mode and phase sensitive. Processing of magnitude mode data is much simpler as no phase correction is necessary.

Note that if data has been processed in the past, but the spectrum has subsequently been deleted, the processing parameters will be saved. See section 6.3 for re-processing information.

6.1 Magnitude mode data (e.g. gcosy, ghmqc, ghmbc)

6.1.1 Select required datasets

It is necessary to process both the 2D data and the 1D data for the projection spectra. For homonuclear 2D experiments, e.g. gcosy, the two projection spectra will be from the same experiment, for heteronuclear experiments, e.g. ghmqc, they will be from different experiments.

If the 1D processing has been performed already, prior to using 'copypars', then skip the next two sections.

6.1.2 Set up F2 dimension 1D projection

The F2 dimension is the 'observe' dimension, and so will be a proton spectrum in most cases, e.g. gcosy, ghmqc and ghmbc.

re *F2expno* ***<enter>***, e.g. **re 1** ***<enter>***

Read the experiment number for the F2 projection, substituting the actual experiment number for *F2expno*.

Process the 1D data as described in section 4. If the 1D spectrum is not to be plotted separately then only phase correction, baseline correction and calibration are required.

XwinNMR only: **edp** ***<enter>***, then set **pscal** to **psreg** and **sreglst** to the relevant nucleus and solvent region file. Other settings for **pscal** can be used if preferred.

6.1.3 Set up F1 dimension 1D projection

This step can be ignored for homonuclear experiments, as both projections will be the same.

For heteronuclear experiments:

re *F1expno* ***<enter>***, e.g. **re 2** ***<enter>***

Read the experiment number for the F1 projection, substituting the actual experiment number for *F1expno*.

Process the 1D data as described in section 4. If the 1D spectrum is not to be plotted separately then only phase correction, baseline correction and calibration are required.

XwinNMR only: **edp** ***<enter>***, then set **pscal** to **psreg** and **sreglst** to the relevant nucleus and solvent region file. Other settings for **pscal** can be used if preferred.

6.1.4 Set up projection data in 2D dataset plot parameters

If 'copypars' was used to set up the 2D experiment there is no need to use 'getproj', these operations will already have been performed.

getproj ***<enter>***

This program will request the details of the projection spectra and load them into the 2D plot parameters and calibrate the 2D spectrum. Note that it is assumed here that the same username and experiment name were used for both the 1D and 2D experiments.

XwinNMR only: If this is not the case it is necessary to enter the details manually by the opening graphics editor, **edg**, and then selecting **ed** for **EDPROJ2** (F2 projection) and **ed** for **EDPROJ1** (F1 projection). This should be performed after executing getproj, or the same values as for the for 2D experiment will be re-inserted.

Enter the experiment number for the F2 projection, as in *F2expno* above.

Enter the experiment number for the F1 projection, as in *F1expno* above.

6.1.5 Process 2D data

xfb ***<enter>***

Fourier transform the 2D data in both dimensions.

absb ***<enter>***

Baseline correct in the F2 and F1 dimensions.

6.1.6 Adjust data display intensity levels

+/-

Use ***LMB*** to select positive intensity levels only (this should be set by default).

XwinNMR only: **#colors**, hold the ***LMB*** down and drag the mouse up or down until 8 different colors are displayed on the bar graph at the bottom right of the screen. More colors can be selected if preferred.

Hold the ***LMB*** down and drag the mouse up or down to adjust the intensity threshold. Adjust for a clear display of signals with a minimum of noise.

6.1.7 Select plot region

The following instructions apply to *XwinNMR*. In *TopSpin*, use the **Plot** tab and on-screen buttons to plot spectra. More details can be found in Chapter 29.

This section can be skipped if the entire 2D spectrum is to be printed. An expanded region can be selected either interactively on screen or by typing in the desired ppm limits. If the region is selected interactively then this region must also be separately defined as the plot region (see below), whereas if limits are entered manually the plot definition follows automatically.

There are three ways of expanding 2D data.

Either:

Position the mouse pointer within the spectrum window and press the ***LMB***.

If the *Info* window obscures the spectrum, re-position it by grabbing the title bar with the ***LMB***.

Position the mouse pointer at the lower left corner of the desired region, then:

MMB to define the lower left corner for the expansion region.

Move the pointer to the upper right corner of the desired region.

MMB to define the upper right corner for the expansion region.

LMB to remove the cursors.

To expand the selected region.

Or:

Enter the ppm limits for the region manually. This is particularly useful when expanding a square region of a homonuclear experiment. Position the pointer at the lower left and upper right of the desired region, read the ppm limits from the *Info* window and note them for manual entry.

Limits

Enter the limits of the desired region in ppm as requested, and answer the plot setup questions as described below. An advantage of this method is that the spectrum expansion is both displayed on screen and stored as the plot region. The other methods require separate definition of the plot region.

Or:

Hold the ***LMB*** down whilst dragging a box to select the expansion region. When the ***LMB*** is released the position and shape of the box on the screen can adjusted via the small squares in the corners and in the middle of the sides using the ***LMB***. To expand the spectrum, press the ***RMB*** with the pointer anywhere on the screen.

6.1.8 Define plot region

This section can be skipped if the **Limits** option was used above, as this operation will have already defined the plot region.

DefPlot

Define the current display settings as the plot settings. Answer the questions as follows:

Change levels?

y ***<enter>*** or simply ***<enter>*** as this is the default option.

Change the contour levels to match the intensities on the display.

Please enter number of pos. levels (max. n): (n will be the number currently displayed)

8 ***<enter>***

Select 8 contour levels (recommended, but any number can be used which is less than or equal to the number of color intensities currently displayed).

Display contours?

n ***<enter>*** or simply ***<enter>*** as this is the default option.

6.1.9 Enter a title

setti ***<enter>***

Type the required title and then save the file.

6.1.10 Preview and plot spectrum

vplot ***<enter>***

Preview plot on screen, check the contour settings and the projection scaling.

Quit to close the previewer, then press ***LMB*** over **OK** to plot or **Cancel** to quit and re-adjust plot parameters.

6.2 Phase sensitive data (e.g. ghsqc, noesyp, roesyp)

For NOESY and ROESY data, the diagonal peaks can be phase corrected to be positive, which results in the NOESY or ROESY cross-peaks as negative signals. For HSQC and TOCSY spectra all peaks should be phase corrected to be positive.

6.2.1 Select required datasets

It is necessary to process both the 2D data and the 1D data for the projection spectra. For homonuclear 2D experiments, e.g. noesyp, the two projection spectra will be from the same experiment, for heteronuclear experiments, e.g. ghsqcp, they will be from different experiments.

6.2.2 Set up F2 dimension 1D projection

The F2 dimension is the 'observe' dimension, and so will be a proton spectrum in most cases, e.g. noesyp and ghsqcp.

re *F2expno* ***<enter>***, e.g. **re 1** ***<enter>***

Read the experiment number for the F2 projection, substituting the actual experiment number for *F2expno*.

Process the 1D data as described in section 4. If the 1D spectrum is not to be plotted separately then only phase correction, baseline correction and calibration are required.

edp ***<enter>***

Set **pscal** to **psreg** and **sreglst** to the relevant nucleus and solvent region file. Other settings for **pscal** can be used if preferred.

6.2.3 Set up F1 dimension 1D projection

This step can be ignored for homonuclear experiments, as both projections will be the same.

For heteronuclear experiments:

re *F1expno* ***<enter>***, e.g. **re 2** ***<enter>***

Read the experiment number for the F1 projection, substituting the actual experiment number for *F1expno*.

Process the 1D data as described in section 4. If the 1D spectrum is not to be plotted separately then the integration stage can be ignored.

edp ***<enter>***

Set **pscal** to **psreg** and **sreglst** to the relevant nucleus and solvent region file. Other settings for **pscal** can be used if preferred.

6.2.4 Set up projection data in 2D dataset plot parameters

re *2Dexpno* ***<enter>***, e.g. **re 3** ***<enter>***

Read the 2D dataset, substituting the actual experiment number for *2Dexpno*.

getproj ***<enter>***

This automation program will request the details of the projection spectra and load them into the 2D plot parameters and calibrate the 2D spectrum. Note that it is assumed here that the same username and experiment name were used for both the 1D and 2D experiments. If this is not the case it is necessary to enter the details manually by the opening graphics editor, **edg**, and then selecting **ed** for **EDPROJ2** (F2 projection) and **ed** for **EDPROJ1** (F1 projection). This should be performed after executing **getproj**, otherwise the same values as for the 2D experiment will be re-inserted by the program.

Enter the experiment number for the F2 projection, as in *F2expno* above.

Enter the experiment number for the F1 projection, as in *F1expno* above.

6.2.5 Process 2D data

edp ***<enter>***

Ensure that **PH_mod** is set to **no** in both F2 and F1 dimensions.

xfb ***<enter>***

Fourier transform 2D data in both dimensions.

6.2.6 Optimize spectrum display

+/-

Use the ***LMB*** to display both positive and negative intensity levels.

XwinNMR only: **#colors**, hold the ***LMB*** down and drag the mouse up or down until 8 different colors are displayed on the bar graph at the bottom right of the screen. More colors can be selected if preferred.

Hold the ***LMB*** down and drag the mouse up or down to adjust the intensity threshold. Adjust for a clear display of signals with a minimum of noise.

The rest of section 6.2.5 applies to *XwinNMR* only. In *TopSpin* use the 'save the contour levels to disk' icon.

Defplot

Define the current display settings as the plot settings. Answer the questions as follows:

Change levels?

y ***<enter>*** or simply ***<enter>*** as this is the default option.

Change the contour levels to match the intensities on the display.

Please enter number of pos. levels (max. n): (n will be the number currently displayed)

8 ***<enter>***

Select 8 contour levels (recommended, but any number can be used which is less than or equal to the number of color intensities currently displayed).

Please enter number of neg. levels (max. n): (n will be the number currently displayed)

8 ***<enter>***

Select 8 contour levels (recommended, but any number can be used which is less than or equal to the number of color intensities currently displayed).

Display contours?

n ***<enter>*** or simply ***<enter>*** as this is the default option.

6.2.6 Phase correct 2D spectrum

phase

Enter phase correction screen.

6.2.6.1 *XwinNMR* only

The 2D spectrum is now displayed in a small window in the top left of the screen. Phase correction requires the extraction of 1D spectra from three representative regions of the 2D array, firstly as rows and secondly as columns. Suitable signals to select depend on the type of data being processed. For NOESY and ROESY data large peaks on the diagonal can be selected, as phasing these as positive signals will result in the nOe cross-peaks appearing as negative signals. For TOCSY data all peaks should be phased as positive signals.

The entire 2D spectrum is heavily compressed to appear in the small window, so it can be helpful to expand regions of the spectrum before selecting rows or columns.

Either:

Position the mouse pointer within the spectrum window and press the ***LMB***.

Position the mouse pointer at the lower left corner of the desired region, then:

MMB to define the lower left corner for the expansion region.

Move the pointer to the upper right corner of the desired region.

MMB to define the upper right corner for the expansion region.

LMB to remove the cursors.

 To expand the selected region.

Or:

Hold the ***LMB*** down whilst dragging a box to select the expansion region. When the ***LMB*** is released the position and shape of the box on the screen can adjusted via the small squares in the corners and in the middle of the sides using the ***LMB***. To expand the spectrum, press the ***RMB*** with the pointer anywhere on the screen.

row

Position the cursor on the first peak to phase, e.g. a large diagonal peak in the lower left of the spectrum for NOESY or ROESY.

MMB

Select this row.

mov: 1

Move the selected row to window 1.

If necessary, reset the display to the whole 2D spectrum by pressing **all** and select a new expanded region as described above.

row

Position the cursor on the second peak to phase, e.g. a large diagonal peak near the center of the spectrum for NOESY or ROESY.

MMB

Select this row.

mov: 2

Move the selected row to window 2.

If necessary, reset the display to the whole 2D spectrum by pressing **all** and select a new expanded region as described above.

row

Position the cursor on the third peak to phase, e.g. a large diagonal peak in the upper right of the spectrum for NOESY or ROESY.

MMB

Select this row.

mov: 3

Move the selected row to window 3.

Adjust the scaling of the selected rows if necessary, by selecting each window in turn and using the buttons on the bottom left of the screen.

Decide which of the three extracted rows contains the largest peak, and select **big: 1**, **big: 2** or **big: 3** accordingly, for automatic zero-order phase correction.

Use **ph0** and **ph1** to phase correct all three extracted spectra simultaneously.

return → save → Start xf2p ? OK

Wait for this operation to be completed.

If necessary, expand the 2D spectrum as described at the beginning of the previous section.

col

Position the cursor on the first peak to phase, e.g. a large diagonal peak in the lower left of the spectrum for NOESY or ROESY.

MMB

Select this column.

mov: 1

Move the selected column to window 1.

If necessary, reset the display to the whole 2D spectrum by pressing **all** and select a new expanded region as described above.

col

Position the cursor on the second peak to phase, e.g. a large diagonal peak near the center of the spectrum for NOESY or ROESY.

MMB

Select this column.

mov: 2

Move the selected column to window 2.

If necessary, reset the display to the whole 2D spectrum by pressing **all** and select a new expanded region as described above.

col

Position the cursor on the third peak to phase, e.g. a large diagonal peak in the upper right of the spectrum for NOESY or ROESY.

MMB

Select this column.

mov: 3

Move the selected row to window 3.

Adjust the scaling of the selected columns if necessary, by selecting each window in turn and using the buttons on the bottom left of the screen.

Decide which of the three extracted columns contains the largest peak, and select **big: 1**, **big: 2** or **big: 3** accordingly, for automatic zero-order phase correction.

Use **ph0** and **ph1** to phase correct all 3 extracted spectra simultaneously.

return → save & return → Start xf1p ? OK

When this operation is complete the display will revert to the main processing window.

To retain this phase correction for re-processing the data in future:

edp *<enter>*

Open the processing parameter editor and set **PH_mod** to **pk** in both the F2 and F1 columns.

The phase correction stored will automatically be applied as part of the **xfb** command when the data is next processed.

6.2.6.2 *TopSpin* only

Typically, three 2D correlation signals will be selected for phase correction of rows followed by columns. Adjust horizontal rows first as they have finer resolution than the vertical columns.

Use the on-screen buttons to expand a region of the spectrum containing a correlation near the top of the screen. Select the center of this peak using the ***RMB*** followed by **Add**.

Expand a region of the spectrum containing a correlation about half way up the screen, and select the center of this peak using the ***RMB*** followed by **Add**.

Expand a region of the spectrum containing a correlation towards the bottom of the screen, and select the center of this peak using the ***RMB*** followed by **Add**.

R To start the phase correction. Three spectra will be displayed, extracted as rows across the 2D spectrum. The tallest peak is highlighted with the red cursor. Use **0** and **1** to phase correct the three spectra together, in the same way as a standard 1D spectrum.

Use the disk button to save the phase correction and return to the previous screen. The spectrum will be re-phased to incorporate the new adjustment.

Next phase correct the columns in a similar way. Either keep the peaks selected above, or use ***RMB*** followed by **Remove All** to clear the selection and then choose new peaks as before. Select correlations close to the left side of the spectrum, near the middle and close to the right side.

C To start the phase correction. Three spectra will be displayed, extracted as rows across the 2D spectrum. The tallest peak is highlighted with the red cursor. Use **0** and **1** to phase correct the three spectra together, in the same way as a standard 1D spectrum.

Use the disk button to save the phase correction and return to the previous screen. The spectrum will be re-phased to incorporate the new adjustment.

Use the return button to exit from phase correction mode.

Open the **ProcPars** tab and ensure that **PH_mod** is set to **pk** in both columns. This will ensure that the new phase correction will be automatically re-applied if the data is reprocessed.

6.2.7 Baseline correction

absb ***<enter>***

Baseline correct in the F2 and F1 dimensions.

6.2.8 Re-optimize spectrum display and define plot parameters

The following instructions apply to *XwinNMR*. In *TopSpin*, use the **Plot** tab and on-screen buttons to plot spectra. More details can be found in Chapter 29.

Hold the ***LMB*** down and drag the mouse up or down to adjust the intensity threshold. Adjust for a clear display of signals with a minimum of noise.

Defplot

Define the current display settings as the plot settings. Answer the questions as follows:

Change levels?

y ***<enter>*** or simply ***<enter>*** as this is the default option.

Change the contour levels to match the intensities on the display.

Please enter number of pos. levels (max. n): (n will be the number currently displayed)

8 ***<enter>***

Select 8 contour levels (recommended, but any number can be used which is less than or equal to the number of color intensities currently displayed).

Please enter number of neg. levels (max. n): (n will be the number currently displayed)

8 ***<enter>***

Select 8 contour levels (recommended, but any number can be used which is less than or equal to the number of color intensities currently displayed).

Display contours?

n ***<enter>*** or simply ***<enter>*** as this is the default option.

6.2.9 Select plot region

This section can be skipped if the entire 2D spectrum is to be printed. An expanded region can be selected either interactively on screen or by typing in the desired ppm limits. If the region is selected interactively then this region must also be separately defined as the plot region (see below), whereas if limits are entered manually the plot definition follows automatically.

There are three ways of expanding 2D data.

Either:

Position the mouse pointer within the spectrum window and press the ***LMB***.

If the *Info* window obscures the spectrum, re-position it by grabbing the title bar with the ***LMB***.

Position the mouse pointer at the lower left corner of the desired region, then:

MMB to define the lower left corner for the expansion region.

Move the pointer to the upper right corner of the desired region.

MMB to define the upper right corner for the expansion region.

LMB to remove the cursors.

 To expand the selected region.

Or:

Enter the ppm limits for the region manually. This is particularly useful when expanding a square region of a homonuclear experiment. Position the pointer at the lower left and upper right of the desired region, read the ppm limits from the *Info* window and note them for manual entry.

Limits

Enter the limits of the desired region in ppm as requested, and answer the plot setup questions as described below. An advantage of this method is that the spectrum expansion is both displayed on screen and stored as the plot region. The other methods require separate definition of the plot region.

Or:

Hold the ***LMB*** down whilst dragging a box to select the expansion region. When the ***LMB*** is released the position and shape of the box on the screen can adjusted via the small squares in the corners and in the middle of the sides using the ***LMB***. To expand the spectrum, press the ***RMB*** with the pointer anywhere on the screen.

6.2.10 Define plot format

Normally spectra are printed in black and white, and different line types can be used to differentiate between positive and negative contour levels. For example, for a ROESY spectrum, solid lines can be used for the real negative cross-peaks and dotted lines for any positive peaks. This may be already set up as required in the experiment parameter file, and need only be set once for each experiment. Instructions for color printing can be found in section 16.

edg *<enter>*

Open the graphics editor window.

edcontp ed

Edit contour plot parameters. Scroll the window down to the bottom.

CPLIN linetypes

Ensure different linetypes (and not colors) are selected.

CPDIFCL yes

Ensure that different types for positive and negative levels are selected.

CPLTYPP ** Array **

Set all values to **1.01** for all positive contours to be drawn as dotted lines, if required. See below for the option to select solid lines.

DONE

CPLTYPN ** Array **

Set all values to **0** for all negative contours to be drawn as solid lines, if required. See above for the option to select dotted lines. Note that any characters after the decimal point are ignored if it is preceded by zero.

DONE

SAVE

Save changes and exit from **edg**.

6.2.11 Enter title

setti ***<enter>***

Type the required tile and then save the file.

6.2.12 Preview and plot spectrum

vplot ***<enter>***

Preview plot on screen, check the contour settings and the projection scaling.

Quit to close the previewer, then press ***LMB*** over **OK** to plot or **Cancel** to quit and re-adjust plot parameters.

6.3 Re-processing old data

If the selected dataset has previously been processed, then suitable processing parameters will already be stored. A combination of **xfb**, followed by **absb** will re-generate the previous spectrum. Note that phase sensitive spectra will only be automatically phased correctly if the parameter **PH_mod** has been set to **pk** in both the F2 and F1 dimensions, as described in section 6.2.6. Processed data is automatically deleted from the server when the files are four days old.

Spectral referencing and experiment names and numbers of 1D projection spectra are retained. Note that it may also be necessary to re-process the 1D datasets if the spectra no longer exist.

7 Importing parameters from one experiment to another

It is often desirable to use parameters from one experiment in another. The Bruker philosophy has been to keep separate, independent, parameter files for different types of experiments, so some extra programs are available to make it easier to extract certain parameters from one experiment and load them into another.

In every case the initial and optimized experiments must be run on the same instrument without changing any sample conditions, such as lock, shimming and temperature.

Three different options are described below. The first is designed to import a spectral window (observation frequency and spectral width) from one experiment to another. Two examples of use would be to optimize the spectral window for the best resolution in a two dimensional experiment, or to use exactly the same window for one dimensional experiments with and without proton decoupling. The second is designed to import just the observation frequency to another experiment. This might be used to set up nOe experiments irradiating a particular peak, or for solvent suppression experiments. The third is designed for optimizing 2D experiments when the indirect nucleus cannot be directly observed and the peak positions are initially unknown.

All of these options are available both in *XwinNMR* and *TopSpin*, but usage is slightly different in each case.

7.1 Import spectral window - 'copypars'

This command can be used to load a spectral region from one experiment into another.

7.1.1 *XwinNMR*

This program is provided to replicate most of the functionality of the *TopSpin* equivalent, but some preparation is required. This will only work if the reference and new experiments have different experiment numbers but share the same experiment name. The source experiment(s) must be 1D.

First process the 1D spectrum (or spectra) as normal. At this stage phasing does not need to be perfect, but referencing is important as this, and the solvent name, will also be imported to the new experiment.

Set the spectrum display to the desired region using ***LMB*** and ***MMB***.

If a 2D experiment is to be run using this region, make sure that there is not a peak exactly in the center of the spectrum.

Use **dp1** to save the display as the plot region, or edit the limits as required during the resultant dialog.

If a second spectrum is required, for example for a heteronuclear 2D experiment, then repeat the above using that dataset.

Set up a new experiment in the usual way, **edcp** (changing ONLY the experiment number) followed by selection of the desired parameter set.

Type **copypars** ***<enter>***

Initially an information box will appear on screen to remind the user that the spectral regions must be saved in the previous spectra as described above. If this has not been done, select **Cancel** and process those files. If all is well, press **OK**.

The responses from the computer will depend on whether the new experiment is 1D, homonuclear 2D or heteronuclear 2D:

7.1.1.1 New 1D experiment

The computer will respond:

Enter experiment number for reference spectrum:

The default answer is **1**, change this if required.

The computer will now extract several parameters from the reference experiment: the plot region, referencing, acquisition time (**aq**) and relaxation delay (**d1**). The user will next have the choice of whether to load **aq** and **d1** from the previous experiment or to keep the default values from the new parameter file.

For example, if a ^{19}F spectrum has been acquired with proton decoupling, the initial wide spectral width spectrum will normally be followed by an optimized experiment over a narrower window. Narrowing the spectral width will automatically increase the acquisition time which will lead to a more accurate measurement of the FID. In some cases, **d1** will be reduced to compensate for the longer **aq**, or it might be increased to record a more quantitative spectrum.

aq is now *newaq* s, it was *oldaq* s, change now if desired:

The default value will be that from the reference experiment.

In the ^{19}F example, 'oldaq' might be an optimized value of 2.4 s, whereas the default value in the wide spectral width parameter file will be 0.4 s. The larger value will produce a much better spectrum.

d1 is now *newd1* s, it was *oldd1* s, change now if desired:

The default value will be that from the reference experiment.

'oldd1' is the value from the reference experiment and 'newd1' is that present in the newly loaded parameter file.'

Finally, an information box will appear on screen summarizing the parameters loaded into the new experiment. These can be checked and modified if required before running the experiment.

7.1.1.2 New homonuclear 2D experiment

The computer will respond:

Enter experiment number for reference spectrum:

The default answer is **1**, change this if required.

The computer will now load the plot region and referencing from the reference experiment and save the details of that experiment as the projection spectrum for the 2D layout.

Finally, an information box will appear on screen summarizing the parameters loaded into the new experiment. These can be checked and modified if required before running the experiment.

7.1.1.3 New heteronuclear 2D experiment

The computer will respond:

Enter experiment number for F2 (observe) reference spectrum:

The default answer is **1**, change this if required.

Does an indirect dimension reference spectrum exist? (yes/no)

The default answer is yes, in which case the following questions will appear next. Typing **n** or **no** will result in just the direct dimension being optimized.

Enter experiment number for F1 (indirect) reference spectrum:

The default answer is **2**, change this if required.

The computer will now load the plot region and referencing from the reference experiments and save the details of those experiments as the projection spectra for the 2D layout.

Finally, an information box will appear on screen summarizing the parameters loaded into the new experiment. These can be checked and modified if required before running the experiment.

7.1.2 *TopSpin*

In *TopSpin* this is a fully interactive program, no preparation is necessary. Operation is the same for 1D and 2D experiments. If a 2D experiment is heteronuclear then execute **copypars** twice, once for each reference spectrum. The source for both dimensions can also be a single 2D spectrum.

If the same source regions are to be used for more than one experiment, they can be saved within the source files. Use the ***RMB*** over the spectrum and select **Save Display Region To…** then select **Parameters F1/2** followed by **OK**. To reuse the same region for a subsequent experiment, load the spectrum then ***RMB*** over the spectrum and select **Save Restore Display Region From Params. F1/2**. This method can be used for both 1D and 2D spectra.

Set up a new experiment in the usual way, **edcp** followed by selection of the desired parameter set.

Type **copypars** ***<enter>***

A grey window will open, with instructions at the top which can be followed.

Load the desired reference spectrum using the command line, data browser or menus.

Use the ***LMB*** to expand the data displayed to be the region of interest. If a 2D experiment is to be run using this region, make sure that there is not a peak exactly in the center of the spectrum.

Copy Parameters to load this region into the new experiment. The program will search the new experiment for all relevant parameters which may take several seconds to complete.

If the new acquisition time is more than twice that in the initial parameters, a list of choices will appear on screen:

Warning: AQ has increased by more than a factor of 2!

⊙ Keep new AQ

O Keep old AQ and reduce TD accordingly

O Keep AQ+D1 constant and (reduce TD if necessary)

The first option is the default and will be appropriate in most cases.

A summary of the new parameters will then appear on-screen.

7.2 Optimize 2D region from an initial 2D spectrum

If a 1D spectrum is not available for one or both dimensions of a 2D experiment, wide spectral windows can be used initially and then optimized based on the initial spectrum. For example, ^{1}H-^{15}N correlation experiments are normally performed without a 1D ^{15}N spectrum as ^{15}N is difficult to observe directly.

7.2.1 *XwinNMR*

Set up and acquire the initial 2D experiment as normal, then process the data.

Type **optimize2d** ***<enter>***

Next define the desired new acquisition region as the plot region within the current experiment. Any of the three methods described in section 6.1.7 and 6.1.8 can be used.

Press **OK**

The computer will respond:

enter new number for optimized experiment:

The default answer is the next experiment number. Select this or type the desired number. If that experiment exists already the computer will ask whether or not it should be over-written.

The new experiment will be created and then the **eda** page will open. This is necessary to ensure all of the parameters are internally consistent, but no changes are required. For a homonuclear 2D experiment the spectral widths in Hz (swh) can be made the same if desired. They may initially be very slightly different as one is calculated in Hz and the other in ppm. Press **OK** to close the page.

Finally an information box will appear summarizing the parameters for the new experiment.

7.2.2 *TopSpin*

Use **copypars** as described in the previous section.

7.3 Import observation frequency - 'keepsfo1'

This command can be used to load an observation (irradiation) frequency (**sfo1**) from one experiment into another. Operation is very similar in *XwinNMR* and *TopSpin*, but the latter program requires one extra step.

7.3.1 *XwinNMR*

First process the initial 1D spectrum as normal, including referencing.

Use **cop** *newexpno* ***<enter>*** to create a copy with a new experiment number.

Now set the irradiation frequency onto the peak of interest. This will be either the peak chosen for an nOe experiment, or a solvent signal which is to be suppressed.

If the desired peak is the largest in the spectrum, for example a water peak, then the program **setsfo1** can be used to set the observation frequency automatically in place of the following manual method.

Expand the spectrum with ***LMB*** and ***MMB*** to show a small region around the peak of interest.

Go to the **utilities** window, then press **O1**.

The mouse pointer will attach to the spectrum, move it to the center of the desired signal and press ***MMB***.

return back to the main data processing window.

Type **keepsfo1** ***<enter>***

Select the desired new experiment from the parameter menu.

The new parameters will be loaded and the irradiation frequency (**sfo1**) will be replaced with the value from the previous experiment. The spectrum referencing and solvent name will also be copied. The irradiation frequency will be displayed on-screen in both MHz and ppm, as well as the solvent name.

Finally, there is a reminder to check that the spectral width (**sw**) is appropriate for the new central frequency.

7.3.2 *TopSpin*

First process the initial 1D spectrum as normal, including referencing.

Use **cop** *newexpno* ***<enter>*** to create a copy with a new experiment number.

Now set the irradiation frequency onto the peak of interest. This will be either the peak chosen for an nOe experiment, or a solvent signal which is to be suppressed.

If the desired peak is the largest in the spectrum, for example a water peak, then the program **setsfo1** can be used to set the observation frequency automatically in place of the following manual method.

Expand the spectrum with ***LMB*** to show a small region around the peak of interest.

At the top of the screen press the Set RF from cursor button: 

Position the cursor over the required peak and press ***LMB***.

Select **O1** and then **OK** to save the frequency.

Type **keepsfo1** ***<enter>***

A message box will appear:

Load new parameters

BEFORE pressing 'Close' to continue

Select the desired new experiment from the parameter menu and then press **Close**.

If 'close' is pressed before loading the new parameters then the observation frequency will not be updated.

The new parameters will be loaded and the irradiation frequency (**sfo1**) will be replaced with the value from the previous experiment. The spectrum referencing and solvent name will also be copied. The irradiation frequency will be displayed on-screen in both MHz and ppm, as well as the solvent name.

Finally, there is a reminder to check that the spectral width **(sw**) is appropriate for the new central frequency.

7.4 Fetch projection information - 'getproj'

If a 2D dataset was acquired without optimization using **copypars**, described above, the referencing and projection information can be imported using the command **getproj**. Operation is the same in both *XwinNMR* and *TopSpin*.

Type **getproj** ***<enter>***

For a homonuclear 2D experiment, the computer will request a single experiment number for the 1D projection. For a heteronuclear experiment, the experiment number for the observe dimension projection will be requested, followed by a question about the existence of a projection spectrum for the indirect dimension. If one exists, then details can be entered.

The referencing parameter(s) **sr** will be copied and details of the 1D file(s) will be loaded into the plot parameters as projections for the 2D spectrum.

7.5 Fetch referencing information - 'getref'

Spectral referencing for a 1D or 2D spectrum can be imported from a separate 1D spectrum. Typical applications might be to reference a series of X-nucleus spectra without executing **xiref** every time, or if **copypars** was used to set up a new experiment before the initial spectrum was accurately calibrated. Operation is the same in both *XwinNMR* and *TopSpin*.

Type **getref** ***<enter>***

For a 1D or a homonuclear 2D experiment, the computer will request a single experiment number for the source of referencing. For a heteronuclear experiment the experiment number for the observe dimension referencing will be requested, followed by a question about the existence of a reference spectrum for the indirect dimension. If one exists, then details can be entered.

The referencing parameter(s) **sr** will be copied and then summarized on-screen.

8 Performing multiple experiments

8.1 Series of identical experiments

The most common applications are kinetic or stability studies, using a delay between each experiment.

Set up experiment in the usual way, using **edc**, **par** etc.

expt ***<enter>***

Check the experiment time and adjust the number of scans (**ns**) if necessary.

rga ***<enter>***

Set receiver gain.

multizgwt ***<enter>***

Enter number of experiments :

The computer requests the number of experiments to be run, the default setting is 10.

Enter delay between experiments in seconds :

The computer requests a 'wait' time in seconds between experiments, the default is 300 seconds (≡ 5 minutes).

The computer will calculate the total experiment duration, including wait delays.

Note that if a large number of experiments is requested, e.g. 100, then there will be a significant delay (30-90 seconds) before the first experiment is started while the **multizgwt** program sets up all the files, checks disk space etc. An alternative is to type **multizgqwt** which runs a similar program which has none of the advance file setup or checking. The first experiment will start much more quickly and each subsequent experiment will only be created as it is run. For results to be meaningful the 'wait' time must be significantly longer than the individual experiment durations.

If a time delay is not required between experiments use the automation programs **multizg** or **multizgq**. These operate in the same way as the above programs but without the 'wait' delay.

8.2 Sequence of different experiments

Set up a series of experiments with incremented experiment numbers, with **edc**, **par**, etc., and set the receiver gain in each with **rga**. Adjust the number of scans as required by using **expt** to check the duration of each experiment.

If a mixture of 1D and 2D experiments is to be performed, then sample spinning should be turned off for the 2D experiments. For this to work correctly, the parameter **ro** must be set correctly. Most parameter files should contain a suitable value. To check this, follow the instructions below for each experiment:

Note that typing **ro** will **not** set this, but will only control the current spin rate.

eda ***<enter>***

Open the acquisition parameter editor.

Type **ro** in the **Parameter** box at the bottom of the editor, to find and highlight the **ro** parameter.

Set this to **20** for normal spinning, or to **0** for no spinning.

SAVE

re *first experiment number* ***<enter>***

Read the first experiment of the sequence to be run.

Either:

multizg ***<enter>***

To keep the current spin rate as set manually via the BSMS keypad.

Or:

multizgro ***<enter>***

To read the spin rate from each experiment and adjust it automatically.

If desired, the programs **multizgwt** or **multizgrowt** can be used to introduce a 'wait' delay between experiments, as described in section 8.1.

8.3 Repeat a group of different experiments with a delay

A series of different experiments can be defined and then repeated with a delay between each group. The only limitation is that for a kinetic study the duration of the experiments should be short compared to the rate of change of the sample.

First set up the series of experiments in the usual way, for example a ^{1}H experiment followed by a ^{31}P experiment, including setting the receiver gain.

Load the first experiment of the series onto the screen.

Type:

multizggrwt ***<enter>***

Clear the information box if the correct file is on-screen, then respond to the following questions:

Enter number of expts in group :

The computer requests the number of experiments to be run in each group, the default setting is 2.

Enter number of cycles of group (including first cycle) :

The computer requests the number of times to run the group of experiments, the default setting is 10.

Enter delay between experiments in seconds :

The computer requests a 'wait' time in seconds between the groups of experiments, the default is 300 seconds (≡ 5 minutes).

The computer will calculate the total experiment duration, including wait delays, and display the information.

To interrupt the program and change the options, type **kill** and select the line containing **multizggrwt** from the resultant menu.

8.4 Performing multiple experiments with temperature control

There are several variations on the **multizg*** programs which allow control of the sample temperature:

multizgrt, **multizgrort** - request a 'reset temperature' (**rt**) before starting the experiments. For use when all the multiple experiments are to be run at the same temperature, but the sample temperature should be set to a new value at the end. The actual sample temperature is stored at the end of each experiment. Particularly useful to reset the temperature to room temperature at the end of a sequence of experiments. The desired temperature for the experiments must be set manually before starting the automation program.

multizgrtwt, **multiqrtwt**, **multizgrortwt** - as above but adding a 'wait' delay between experiments.

multizgvtrt, **multizgrovtrt** - request a 'reset temperature' (**rt**) before starting the experiments, and the temperature parameter **te** is read from the parameters of each experiment. The temperature is set and allowed to equilibrate for 5 minutes. Before each experiment is run the z1 and z2 shims will be optimized. At the end of all the experiments the sample temperature is set to the 'reset' value.

multizgvtl - reads temperatures from a variable temperature list (**vtlist**) so that only one experiment has to be set up in advance. The temperature equilibration time will be requested. A **vtlist** is a text file which contains the list of temperatures to be used in the sequence of experiments and can be created as follows:

edlist vt ***<enter>***

This will produce a menu of all the existing variable temperature list files. To ensure you will have permission to edit the file, use your username as the filename. If the file exists already, select it with the ***LMB***. To create a new file, select the **Type New Name** entry box and type your username.

Edit the file so that it contains the desired list of temperatures and save it.

The **multizgvtl** program will request the name of the **vtlist** before running the experiments.

For further information on variable temperature operation see Chapter 9.

8.5 Processing multiple datasets

There are several automation commands to enable automatic processing of a sequence of experiment numbers, employing differing degrees of processing. In many cases the same phase correction will be valid throughout a series of experiments, but sometimes adjustments are required. A good test is to phase correct the first spectrum and then use **getref** to important the phase parameters into a representative selection of the datasets. Manually process these with **efp** and check the phasing of the spectra. It may be possible to identify batches of experiments that can be processed with the same phase correction.

8.5.1 Applying the same phase correction to a sequence of experiments

The first experiment number should be processed in the usual way. A series of subsequent experiments can then be processed with the same phase correction. The computer will request the experiment number of the first experiment, and the total number of experiments to be processed. This total includes the first experiment which has been processed manually.

multifp - Fourier transform all experiments with the same phase correction (no line broadening).

multiefp - Apply exponential line broadening, Fourier transform all experiments with the same phase correction.

8.5.2 Phase correct and calibrate a sequence of experiments

multiefpd - As above, but the spectrum referencing parameter **sr** is copied from the first to all subsequent experiments.

8.5.3 Phase, baseline correct and calibrate sequence of experiments

multiefpabs - As above, with automatic baseline correction (with no integration) of each spectrum.

It is a good idea to check the phasing of a selection of the experiments before using this option, to avoid baseline correcting badly phased spectra. Process the first spectrum, then load a selection of spectra individually, using **getref** followed by **efp** to load phase parameters then process the datasets.

8.5.4 Automatically phase, baseline correct and calibrate sequence of experiments

multiproc - As above, but instead of copying the phase correction from one experiment to the next, each spectrum is phased automatically using the command 'apk'.

8.5.5 Integrating the same regions of a series of spectra

The data must first be processed using one of the methods above. Good phasing and baseline correction is essential for accurate integration. It may be necessary to employ some manual processing. There are currently three different versions of this program which handle the integral values in slightly different ways.

For all the programs, the integral regions must first be saved as a file. Manually integrate the first spectrum, then **return & save** from the integration screen.

Type **wmisc** ***<enter>***

then select **intrng**

Next, either select a file name from the resultant menu, or enter a new name into the **Type New Name** box at the bottom. Note that the file will be written to a directory shared by all users, but a user cannot overwrite a file created by a different user. It is therefore a good idea to use your own NMR login ID as the filename.

Finally type the name of the automation program appropriate for the method of integral calibration desired:

(i)

multi_integ – Integral values use absolute intensity scaling. Peak areas for subsequent spectra are relative to the first spectrum. The computer will request the following information:

Use EXPNOs (0) or PROCNOs (1) :

Type **0** if your spectra are a sequence of experiment numbers, or **1** if they are a sequence of processed data numbers. The default is **0**.

Enter first experiment number :

Respond with the number of the first spectrum in the sequence. If the answer to the previous question was **1**, the first processed number will be requested.

Enter number of experiments :

Respond with the total number of spectra, including the first one.

Enter name of intrng file :

Type the filename used above when saving the **intrng** file.

When the program has finished, an information box will appear on the screen with the location of the file which contains the integration information for all the spectra, which is inside the processed data directory of the first dataset:

/v/data/*username***/nmr/***expname***/***expno***/pdata/***procno***/intall.txt**

Integral data for the separate spectra are saved within the individual processed data directories with the filename **integrals.txt**. These files can be fetched from nmrserver to your own computer using any FTP program.

(ii)

multi_integ2 - Integral values are calibrated internally within every spectrum. A nominated integral is calibrated with area '1' in each spectrum. When integrating the first spectrum it is necessary to decide which integral should have an area of 1, and not which integral this is, if the left-most is number one. The computer will request the following information:

Use EXPNOs (0) or PROCNOs (1) :

Type **0** if your spectra are a sequence of experiment numbers, or **1** if they are a sequence of processed data numbers. The default is **0**.

Enter first experiment number :

Respond with the number of the first spectrum in the sequence. If the answer to the previous question was **1**, the first processed number will be requested.

Enter number of experiments :

Respond with the total number of spectra, including the first one.

Enter name of intrng file :

Type the filename used above when saving the **intrng** file.

Enter number of reference integral :

Respond with the number of the integral which you desire to be calibrated as an area of 1, counting from the left with the first integral being number 1.

When the program has finished, an information box will appear on the screen with the location of the file which contains the integration information for all the spectra, which is inside the processed data directory of the first dataset:

/v/data/*username***/nmr/***expname***/***expno***/pdata/***procno***/intall.txt**

Integral data for the separate spectra are saved within the individual processed data directories with the filename **integrals.txt**. These files can be fetched from nmrserver to your own computer using any FTP program.

(iii)

multi_integ2cal - Operation is similar to option (ii) above, except that the selected integral can have any chosen value.

First there is a reminder that the integral region file must be saved before continuing.

Use EXPNOs (0) or PROCNOs (1) :

Type **0** if your spectra are a sequence of experiment numbers, or **1** if they are a sequence of processed data numbers. The default is **0**.

Enter first experiment number :

Respond with the number of the first spectrum in the sequence. If the answer to the previous question was **1**, the first processed number will be requested.

Enter number of experiments :

Respond with the total number of spectra, including the first one.

Enter name of intrng file :

Type the filename used above when saving the **intrng** file.

Enter number of integral to be referenced (leftmost is 1) :

Respond with the number of the integral which you desire to be calibrated, counting from the left with the first integral being number 1.

Reference value for selected integral:

Enter the desired area for the chosen integral, the default is 1.

When the program has finished, an information box will appear on the screen with the location of the file which contains the integration information for all the spectra, which is inside the processed data directory of the first dataset:

/v/data/*username***/nmr/***expname***/***expno***/pdata/***procno***/intall.txt**

Integral data for the separate spectra are saved within the individual processed data directories with the filename **integrals.txt**. These files can be fetched from nmrserver to your own computer using any FTP program.

8.5.6 Re-processing multiple datasets

The automation program **multiefpb** can be used to reprocess multiple datasets. A sequence of experiments will be processed using the **efpb** command. This combines Fourier transformation with the saved phase correction, and a repeat of the same baseline correction.

9 Variable temperature operation

Note that the displayed temperatures are in Kelvin; **0°C ≡ 273.15K**.

The standard settings for the four instruments are as follows:

DRX400:	Gas Flow **535** l/h Heater **On**	Maximum Power **30%**	Target temp. **298.0** K
GN500:	Gas Flow **535** l/h Heater **On**	Maximum Power **30%**	Target temp. **298.0** K
CRYO500:	Gas Flow **670** l/h Heater **On**	Maximum Power **15%**	Target temp. **298.0** K
AVANCE600:	Gas Flow **670** l/h Heater **On**	Maximum Power **30%**	Target temp. **298.0** K

The spectrometers should always be left with the above settings when variable temperature experiments are complete. The Maximum Power is set via the **Set max…** button and should not normally be changed. When the heater is turned on it will initially briefly be at maximum power, if the maximum setting is too high the temperature can overshoot the required value.

Your sample should be as close as possible to the ideal depth in the NMR tube: 4cm. An excess can lead to temperature gradients along the length of the sample, and hence poor resolution. In extreme cases this can lead to a very unstable lock.

Variable temperature operation is possible within the range –150°C and +180°C for the standard 5mm probes, 0°C and +50°C for the 500MHz cryoprobe, 0°C and +135°C for the 600MHz cryoprobe -120°C to +150°C for the 2.5 mm probe, and –50°C to +80°C for the 5mm inverse probe.

There are sample spinners made of three different materials:

The standard beige plastic sample spinners can be used in the range 123K(-150°C) to 453K(+180°C), there are normally two of these by each instrument. The blue plastic sample spinners can be used within the range 223K(-50°C) to 323K(+50°C), these are normally used for the dummy samples. The white or translucent plastic spinners can be used in the range 233K(-40°C) to 313K(+40°C), these are normally used for automation on the DRX400.

The above temperature limits apply for short term operation only, there are also constraints on the allowed magnet and shim system temperature. See the NMR facility director for advice if you need to run a long experiment outside the range -50°C to +50°C.

Ensure that your sample/solvent combination can withstand the desired temperature. Due to possible errors in the calibration of the VT system do not operate within 10°C of the solvent boiling point or freezing point.

Allow sufficient time within your booking period for the system to return to room temperature for the next user.

Using *XwinNMR* 3.5 or *TopSpin* the correct sample temperature will automatically be stored at the end of each experiment.

Normally the sample is loaded at room temperature and a test spectrum is run before cooling or heating both probe and sample together. If the sample is unstable at room temperature then it is possible to set the probe temperature first, but it will be necessary to allow more time for the sample temperature to equilibrate. Several automation programs are available to ease the recording of spectra at different temperatures, some of which automatically reset the sample to room temperature when experiments are complete. See section 8 for full details.

9.1 High temperature operation

Ensure that the correct sample spinner is used if the target temperatures is over 50˚C, beige plastic.

Load sample, set up experiment, lock and shim at room temperature.

Record a proton spectrum to check line-shapes and sample integrity.

If the temperature control window has not opened automatically, or has been closed, type:

edte ***<enter>***

to open the temperature editor. The **Gas Flow** will normally be set to **535 l/h** or **670 l/h**

Target temp. Change...

Enter the desired temperature, then **OK** to close the window or **Apply** to keep it open.

Note that if the target temperature is within 20˚C of the solvent boiling point or melting point, it is advisable to initially set the temperature 10˚C lower to reduce the risk of the sample temperature over-shooting.

If the heater is not already turned on:

Heater Off

Turn on the probe heater, **Off** will change to **On**.

Wait for the target temperature to be attained. If necessary **Gas Flow** can be increased, but note that a high flow rate may cause the sample to rise from the probe. If this occurs, the **[SPIN ON-OFF]** light will flash and the lock level will fall.

Wait for 5-10 minutes for sample equilibration after the desired temperature has been reached.

If the lock signal appears unstable there may be a temperature gradient inside the NMR tube. This is a common problem if the sample depth is much more than 4cm. A solution is to increase the **Gas Flow** by one or two increments which should result in more even heating of the NMR tube. Too much air flow can cause the sample to rise from the probe. Monitor the spin light on the BSMS keypad - if the air flow is too high then the spin rate will be reduced and the light will flash.

Adjust shim settings and, if necessary, lock settings.

For all experiments apart from simple proton, check the probe tuning on both channels (see section 10 for instructions).

When the experiment is complete, either select a new temperature for a subsequent experiment, or turn off the heater to allow the system to cool to room temperature.

Heater On

Turn off the heater, **On** will change to **Off**.

Cooling can be accelerated by temporarily increasing the **Gas Flow**, which should be reset to the original value (535) afterwards.

When the sample temperature is close to ambient, set the target temperature to **298.00** and turn the heater on:

Target temp. Change... → 298.00

Heater Off

Turn on the heater, **Off** will change to **On**

File → Exit

Close the temperature editor window.

Note that the automation program **multizgrt** *can be used to automatically reset the sample temperature when the experiment is complete. When requested for the number of experiments, enter* **1**.

9.2 Low temperature operation (ambient to 273K, AVANCE600 & CRYO500)

The AVANCE600 and CRYO500 are equipped with pre-cooling accessories which chill the ambient compressed air supply before it enters the spectrometer probe. These units are automatically turned on whenever the probe heater is switched on in the **edte** window. This protects against accidental sample freezing, as if the probe heater is not on, the incoming compressed air is at ambient temperature.

The cooling units only turned on when required to prevent ice blockages from moisture in the compressed air supply. The orange power switch is labeled on each unit and should be used to turn the unit on before low temperature experiments and off again afterwards. It will take a short time for the cooling unit to cool down before the sample temperature starts to fall.

9.2.1 CRYO500

The standard air flow of **670 l/h** allows access to the full temperature range of 273-323K.

9.2.2 AVANCE600

The standard air flow is **670 l/h**. It may be necessary to increase the flow rate to **800 l/h** to reach the extremes of the temperature range, which is 273-408K.

9.3 Low temperature operation (ambient to 123K, GN500)

Ensure that the correct sample spinner is in use for temperatures below -50°C, beige plastic. If the 25 liter liquid nitrogen dewar is to be used, fill and position this before proceeding.

Load sample, set up experiment, lock and shim at room temperature.

Record a proton spectrum to check line-shapes and sample integrity.

It is easiest to operate without sample spinning. The normal compressed air supply can be used for sample spinning for temperatures down to approximately 233K (-40°C), but below this temperature a separate nitrogen gas supply is necessary

to avoid condensation and ice forming from the air supply. The GN500 is normally equipped with a nitrogen cylinder for this purpose. **Use the beige plastic (to -150˚C) sample spinner**.

Load sample as normal using compressed air.

For sample spinning below 243K, connect a nitrogen gas cylinder to the spectrometer console and switch the input valve to use this supply instead of compressed air.

[SPIN ON/OFF]

Turn sample spinning on or off according to the conditions above.

edte *<enter>*

Open temperature control window.

Check that the probe **Heater** is **Off**

Gas Flow -

Reduce the gas flow by pressing the *minus* button until it is **0 l/h**. (This is the room temperature gas supply which will not be used for low temperature experiments.)

Disconnect the air supply from the base of the probe at the ball and socket joint.

Place the rubber o-ring on the neck of the nitrogen dewar. Check that the black bayonet valve is open and the screw top from the refill port is removed on the boil-off heater unit. Slowly lower the heater unit into the dewar, be careful of spraying liquid nitrogen during this procedure, particularly during the last stage when the heater unit fills the neck of the dewar.

Connect the insulated transfer line to the ball and socket joint at the base of the probe. There should be a plastic loop attached the magnet leg to support the weight of the transfer tubing. Ensure that the transfer line is not under stress and clamp the top of the heater unit to the neck of the nitrogen dewar.

Cooling Off

Turn on nitrogen boil-off heater, **Off** will change to **On**.

Change…

Adjust the cooling power to around **15**.

Target temp. Change...

Enter the desired temperature, then **OK** to close the window or **Apply** to keep it open.

Heater Off

Turn on the probe heater, **Off** will change to **On**.

Allow 5-10 minutes after the desired temperature has been attained for equilibration before commencing experiments.

If necessary, adjust the cooling power setting. Start with about 15, and increase this if temperature fails to reduce to the value desired. Too much nitrogen flow will cause the sample to rise from the probe, and cause the dewar to empty more quickly. If the temperature reduction is small a relatively low power, e.g. 10, may be sufficient. Periodically check that the green 'sample down' LED is illuminated on the BSMS keypad to ensure that the sample is still in position. Adjust shim settings and, if necessary, lock settings.

For all experiments apart from simple proton, check the probe tuning on both channels (see section 10 for instructions).

When your low temperature experiments are complete it is necessary to warm the system carefully to avoid condensation which would result from introducing the standard air flow whilst the system is too cold.

Target temp. Change...

Set the target temperature to a normal room temperature value of 298K, and allow the probe to warm.

If the nitrogen gas cylinder has been used for sample spinning, wait until the sample temperature has warmed to at least 243K before switching the supply back to compressed air.

When the temperature has reached at least 273K:

Cooling On

Turn off nitrogen boil-off heater, **On** will change to **Off**.

Heater On

Turn off the probe heater, **On** will change to **Off**.

Disconnect the insulated nitrogen supply from the probe and replace it with the standard room temperature air supply. If the ball and socket joint is frozen, gently melt the ice by hand or by directing the room temperature air flow over the joint.

Gas Flow +

Increase the air flow by pressing the *plus* button until it is **535 l/h** or **670 l/h**

Heater Off

Turn on the probe heater, **Off** will change to **On**.

Finally remove the nitrogen boil-off heater from the nitrogen dewar. Place this unit tidily in the corner of the laboratory and move the liquid nitrogen dewar to the edge of the room.

Ensure that the sample temperature has reached the target temperature and that the standard sample spins normally before logging off the system.

9.4 NMR sample temperature calibration

The following techniques can be used to accurately measure the current temperature in the NMR probe. This can be performed either before or after recording data from the sample being studied. The same time (5-10 minutes) should be used to allow the temperature to equilibrate for both the real and standard samples.

If the SGI calculator icon is not displayed on the desktop, it can be accessed via:

Toolchest → Find → Icon Catalog: Desktop Tools → xcalc

9.4.1 Low temperature calibration

Temperature range: 175 to 310K (-98 to +37°C)

Sample: 100% methanol (available in cupboard close to AVANCE600)

Insert the methanol sample into the magnet and turn on sample spinning.

Ensure that **[LOCK]** is turned off, and then turn off **[SWEEP]**.

Allow the sample temperature to equilibrate for 5-10 minutes.

Set up a new experiment using **edcp** and load the standard proton parameter set, normally **h1.s**.

Set **ns** to **1**, **ds** to **0**. Record data, process and phase as usual (**rgazg**, **efp**, **apks** etc).

Use ***LMB*** and ***MMB*** to measure the difference in chemical shift (ppm) between the two peaks in the methanol spectrum.

The magnitude of the difference is $\Delta\delta$ in the equation below.

Use the following equation to calculate the actual sample temperature (K):

$$T_{calibrated}(K) = 403.0 - (29.53 \times \Delta\delta) - (23.87 \times \Delta\delta^2)$$

9.4.2 High temperature calibration

Temperature range: 300 to 400K (+27 to +127°C)

Sample: 100% ethylene glycol (available in cupboard close to AVANCE600)

Insert the ethylene glycol sample into the magnet and turn on sample spinning.

Ensure that **[LOCK]** is turned off, and then turn off **[SWEEP]**.

Allow the sample temperature to equilibrate for 5-10 minutes.

Set up a new experiment using **edcp** and load the standard proton parameter set, normally **h1.s**.

Set **ns** to **1**, **ds** to **0**. Record data, process and phase as usual (**rgazg**, **efp**, **apks** etc).

Use ***LMB*** and ***MMB*** to measure the difference in chemical shift (ppm) between the two peaks in the ethylene glycol spectrum.

The magnitude of the difference is $\Delta\delta$ in the equation below.

Use the following equation to calculate the actual sample temperature (K):

$$T_{calibrated}(K) = 466.0 - (101.6 \times \Delta\delta)$$

10 Probe tuning and probe changing

The standard (BBO) probe in use on the GN500 has two coils. One is tunable to a range of X-nuclei (^{13}C, ^{29}Si, etc) and the other is tuned for ^{1}H observation (and doubly tuned to ^{2}H for the lock channel). The X-channel should normally be left tuned to ^{13}C and so will need to be re-tuned for observation of a different X-nucleus. Both ^{1}H and ^{13}C coils should be fine-tuned before performing complex experiments - those requiring accurate 90 degree pulse settings, e.g. 2D experiments. Tuning should also be adjusted during variable temperature experiments, as the probe electronics will change temperature as well as the sample.

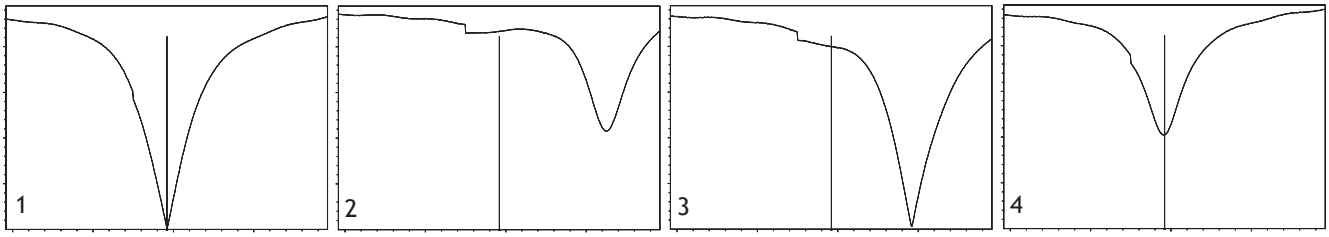
The DRX 400 has a switchable probe where the X-coil automatically switches to observe ^{13}C, ^{31}P or ^{19}F and no manual tuning is necessary. The characteristics of the probe result in good tuning and matching for ^{13}C as this is the least sensitive of the four nuclei, and less good tuning for ^{31}P and ^{19}F. However, this is compensated for in the pulse length settings in the parameter files and so is not a problem. DO NOT ADJUST THE ^{13}C, ^{31}P OR ^{19}F TUNING ON THE DRX400.

Other probes are available for each instrument: inverse 5mm, 2.5mm, 10mm and MAS probes at 500MHz and a tunable probe at 400MHz. Check with the NMR facility director to see if an alternative probe is suitable for your experiments, and whether suitable shim and parameter files are available.

The CRYO500 probe has four fixed frequencies - ^{1}H, ^{2}H, ^{13}C and ^{15}N. Only the first three of these are normally in use. Each channel can be fine-tuned by use of color-coded tune and match adjustor screws. The colors of the screws match the colors printed around the RF cable connectors on the probe. The ^{15}N channel is only suitable for ^{15}N decoupling, not direct observation.

The AVANCE600 instrument is normally fitted with a BBFO cryoprobe. This is a conventional geometry probe where the outer coil is tuned to ^{1}H and ^{2}H. The inner coil can be tuned for observation of most X-nuclei, including ^{19}F. This probe can be tuned automatically using the command **atma** which is described in Chapter 29. Alternative room temperature probes are TBI (inverse probe, ^{1}H, ^{2}H, X observation with optional ^{13}C decoupling), (TXI (inverse probe, ^{1}H or ^{2}H observation with ^{13}C and ^{15}N decoupling) and BBO (standard geometry, inner coil tunable to most X-nuclei, outer coil tuned to ^{1}H and ^{2}H).

The principle of probe tuning is similar on all instruments, the probe circuit must be tuned to the desired frequency and matched to an impedance of 50 Ohms. This is performed by monitoring either the acquisition screen display on the computer or the LED display on the top of the preamplifier housing, close to the magnet. The following graphics show the screen display in different states of good and bad tuning and matching:



1 – Good tuning and matching, the probe is perfectly adjusted.

2 – Bad tuning and bad matching.

3 – Good matching but bad tuning.

4 – Good tuning but bad matching.

The depth of the dip on the screen represents how well the NMR coil is matched to 50 Ohms. When well adjusted, the dip should reach the baseline. This is also indicated by the vertical LEDs on the preamplifier housing. When well adjusted, only green LEDs should be illuminated.

The position of the dip from left to right represents the frequency to which the probe is tuned. The vertical line in the center represents the observation frequency for the selected nucleus. When well adjusted, the dip should be centered on this line. This is also indicated by the horizontal LEDs on the preamplifier housing. When well adjusted, only green LEDs should be illuminated.

Note that the LEDs on the preamplifier housing indicate the point of lowest intensity of the trace on the computer screen. If the matching is badly set, the lowest point may be at the edge of the screen rather than the dip. The easiest way to resolve this is to observe the trace on the screen, as well as the LEDs, while making adjustments. If necessary, turn the computer display so that it can be seen.

10.1 X-coil tuning (GN500)

edcp ***<enter>***

Create a new experiment and read parameters for the desired nucleus. Note that if an experiment which use ^{1}H decoupling is selected, then the proton channel can be tuned from the same file.

acqu ***<enter>***

Display acquisition screen

wobb ***<enter>***

Start wobble function

Close to the magnet there is a card with a list of nuclei and numbers for 'tune' and 'match'. At the base of the probe there are numbered metal sliders which should be carefully adjusted with the attached tool to the values listed for the desired nucleus. Be very careful not to apply any pressure to the part of the probe which extends downwards next to the sliders as this contains a delicate glass dewar.

The acquisition screen display will show a line across the screen containing a V-shaped 'dip', and a vertical line in the center representing the irradiation frequency. When the probe is tuned correctly the 'dip' will be centered on the vertical line, and will descend to the bottom of the screen. 'Tuning' adjusts the horizontal position of the 'dip' and 'matching' adjusts the depth of the 'dip'.

There are LEDs on the top of the pre-amplifier which also display the tuning and matching, and it is easier to observe these while fine-tuning the probe. A well tuned probe should have only 2, 3 or 4 green LEDs at the bottom of the 'match' scale, and only 2 or 3 green LEDs at the center of the 'tune' scale. In some cases, yellow LEDs will show, but there should be no red LEDs illuminated.

First adjust the right-most match slider for a minimum on the display, then adjust the right-most tune slider to center the tune display. If necessary, further adjust the match control and then the tune control, until the best result is achieved.

stop ***<enter>***

Stop wobble function.

10.2 X-channel switching (DRX400 only)

The standard QNP probe will switch automatically between ^{13}C, ^{31}P and ^{19}F.

Do not attempt to adjust the probe tuning.

No connection changes are necessary - simply load the required parameters and run the experiment.

10.3 Proton coil tuning (GN500 and DRX400)

If an X-nucleus experiment with ^{1}H decoupling was selected above, then the 'CHANNEL SELECTOR' button on the pre-amplifier housing can be used to switch directly to ^{1}H channel tuning. Otherwise proceed as follows:

edcp ***<enter>***

Create a new experiment and read parameters for a proton experiment, e.g. **h1.s**.

acqu ***<enter>***

Display acquisition screen.

wobb ***<enter>***

Start wobble function.

The acquisition screen display will show a line across the screen containing a V-shaped 'dip', and a vertical line in the center representing the irradiation frequency. When the probe is tuned correctly the 'dip' will be centered on the vertical, and will descend to the bottom of the screen. 'Tuning' adjusts the horizontal position of the 'dip' and 'matching' adjusts the depth of the 'dip'.

There are LEDs on the top of the pre-amplifier which also display the tuning and matching, and it is easier to observe these while fine-tuning the probe. A well tuned probe should have only 2, 3 or 4 green LEDs at the bottom of the 'match' scale, and only 2 or 3 green LEDs at the center of the 'tune' scale. In some cases yellow LEDs may show, but there should be no red LEDs illuminated.

The ^{1}H tuning is adjusted by two brass screws with yellow labels, marked 'T' for tune and 'M' for match, on the base of the probe. There is a red metal non-magnetic screwdriver provided for easy adjustment of these.

First adjust the match screw for a minimum on the display, then adjust the tune screw to center the tune display. If necessary, further adjust the match screw and then the tune screw, until the best result is achieved.

stop ***<enter>***

Stop wobble function.

10.4 Proton coil tuning (CRYO500)

edcp ***<enter>***

Create a new experiment and load proton parameters.

acqu ***<enter>***

Display acquisition screen

wobb ***<enter>***

Start wobble function.

Adjust the yellow tune and match screws as described above in section 10.3. Due to the width of this magnet the controls can be difficult to access. Move the steps if necessary. Note that it is easier to load dedicated proton parameters than to switch the preamplifier channel on this instrument.

10.5 Carbon coil tuning (CRYO500)

edcp ***<enter>***

Create a new experiment and load carbon parameters.

acqu ***<enter>***

Display acquisition screen

wobb ***<enter>***

Start wobble function.

Adjust the blue tune and match screws as described above in section 10.3. Due to the width of this magnet the controls can be difficult to access. Move the steps if necessary. Note that it is easier to load dedicated carbon parameters than to switch the preamplifier channel on this instrument.

10.6 Changing probes (GN500)

Probe changes should only be attempted by specially trained users with the approval of the facility manager. Do not attempt to change the probe in the DRX400 as this is complicated by the extra controls for switching the X-nucleus channel.

Check the LEDs on the BSMS unit for sample condition (up, missing or down).

[LIFT ON-OFF] to ensure no that sample is loaded in the probe.

[LIFT ON-OFF] to turn off lift air.

edte ***<enter>***

Ensure that the probe heater is turned off.

At the base of probe, disconnect the following cables: ^{2}H channel, ^{1}H channel, X/BB channel, and Gradient cable (large connector).

Remove the probe heater: This is a bayonet fitting, push it up and turn it, then lower it from the probe dewar. Be very careful, the glass probe dewar is fragile. If the heater has been switched on recently it may still be warm, so be careful not to lay it over any cables.

Remove the thermocouple connector: hold the probe end of the connector in place whilst disconnecting to avoid moving the thermocouple.

Disconnect the temperature control air supply, this is ball and socket joint on the back of probe base, held together with a metal clip.

Make a mental note of the probe orientation, it must be replaced in exactly the same position later, and the new probe must be inserted with the same orientation.

The probe is held in place by two brass screws. On most probes one screw is long, the other is short. There is a red metal screwdriver, as is used for ^{1}H channel tuning, for turning these.

Unscrew one screw until it is loose, and the hold the probe in position with one hand, supporting the weight, whilst carefully unscrewing the second screw.

Gently lower the probe from the magnet, holding it with both hands. Note that the probe is partially supported by the effects of eddy currents whilst it is in the magnet bore, it will suddenly feel heavier when it reaches the bottom of the magnet.

Place the probe in a safe place, e.g. the box from which the replacement probe is to be removed.

Carefully insert the new probe into the magnet. Ensure that the orientation is the same as for the previous probe. Support the weight of the probe by hand whilst tightening the brass screws. Do not use the tightening of the screws to raise the probe.

Re-connect all the cables, etc., which were disconnected from the previous probe. Note that all probes are equipped for gradient pulses.

edhead ***<enter>***

Define current

Select the probe now in use.

save

If the 2.5 mm probe is being used, ensure that the adjustable sample depth gauge is in use and is set to the correct position.

rsh ***<enter>***

Select a shim file appropriate for the new probe.

Load a sample and then tune the channels which are to be used.

When experiments are complete, replace the original probe in the magnet, redefine the current probehead using **edhead**, load a standard $CDCl_3$ sample and check the tuning of both channels. It is very easy to accidentally move the tuning controls when changing the probe. Setting the sliders to the values on the card is **not** sufficient.

11 Manual locking

For the standard automatic locking functions to work, the initial shim settings must produce a reasonably clear and sharp lock signal. Normally this is achieved by reading the standard shim file before issuing the lock command. This will usually be successful, but some samples will require sufficiently different lock or shim settings that the lock command will fail. Possible causes of this include very shallow samples, mixed solvents, unusual solvents, very concentrated samples or very viscous samples.

This procedure can also be used if automatic locking results on the 'wrong' signal being found from a solvent with more than one deuterated environment, for example toluene-d8 or methanol-d4. The most problematic solvent is toluene-d8, as the large separation of the aromatic and methyl signals can result in a spectrum shifted by approximately 5ppm.

11.1 Kill or interrupt automatic locking

First ensure that the automatic locking processes are no longer running:

kill ***<enter>***

Select the 'lock' line from the resultant window with the ***LMB***.

If the lock light is flashing or is on constantly on the BSMS keypad, press the **[LOCK ON/OFF]** button to turn it off.

11.2 Load solvent lock parameters

lopoi *solventname* ***<enter>*** (In *TopSpin* the command **lopo** should be used instead of **lopoi**.)

e.g. **lopoi cdcl3** ***<enter>***

Or:

lopoi ***<enter>*** and select the name of the solvent in use.

This will set standard values for lock frequency, power, phase and gain for the selected solvent.

Enlarge the lock display window so that there are at least six grid boxes across, and that there is a central vertical grid line.

The desired deuterium signal from the solvent should now be visible in the lock display window. If no signal is visible, or if you are unsure which signal is being displayed, then increase the lock sweep amplitude by adjusting **[SWEEP AMPL.]** until all the expected solvent signals are displayed in the lock window.

Press **[FIELD]** and turn the knob on the BSMS unit to adjust the magnetic field until the desired lock signal is exactly in the center of the lock display window.

If it was altered above, return the **[SWEEP AMPL.]** to the standard setting of **2.0** and make further fine adjustments to the **[FIELD]** if the lock signal is no longer centered.

Press **[LOCK GAIN]** and increase the amplification until the lock signal fills the window vertically.

Press **[LOCK]** to lock onto the selected signal, then reduce **[LOCK GAIN]** until the signal is within the top three squares of the lock display window.

If lock fails, adjust the shim settings until a longer decaying beat pattern is observed, then try again.

If all attempts to lock the sample fail, turn off the **[SWEEP]** button and run the sample unlocked. The following procedure can be used to shim the field by observing the shape of the free induction decay.

12 Samples which cannot be shimmed using the deuterium lock

There are several situations where the deuterium lock signal does not give an adequate response for optimizing the homogeneity of the magnetic field. The most common scenarios are described below; firstly when the deuterium lock signal is insensitive to shimming, and secondly when a non-deuterated solvent is used.

12.1 Samples where the lock signal is insensitive to shimming

It is much more difficult to optimize the shim settings by observing the shape of the free induction decay than by observing the height of the lock signal. This technique can be used if the lock signal is too broad or weak to be adjusted easily, as can be the case with H_2O/D_2O mixtures or with very viscous samples.

Set up a new experiment and load the sample in the usual way.

Load the standard shim file for the instrument in use:

rsh *shimfile* ***<enter>***

Lock the sample in the usual way. If this fails, lock manually as follows:

lopoi *solventname* ***<enter>*** (on the AVANCE600 use **lopo** instead of **lopoi**)

Press **[FIELD]** and adjust the lock signal until it is centered in the lock display window.

Press **[AUTO LOCK]** to lock the sample. If this is unsuccessful, increase **[LOCK GAIN]** and repeat. If it proves impossible to lock the sample at all, follow the instructions in 12.2 below.

Acquire data under the standard conditions. Inspect the peak shapes. If all the peaks are broad, then it is unlikely that the spectrum can be improved by shimming, and the cause of the broadening is probably a characteristic of the sample, for example paramagnetism. If the peaks look fairly sharp but are asymmetric then shimming will probably improve the spectrum.

cop ***<enter>***

Create a copy of the current experiment with a new experiment number.

It is easiest to optimize the shape of the FID if the spectrum contains one dominant signal, and if this signal is off-resonance (not at the center of the spectrum). If necessary TMS can be added to the sample for this purpose - suck some vapor from a TMS bottle into a pipette, add to the NMR tube and shake to mix.

The following instructions apply to *XwinNMR*. The equivalent operations are available in *TopSpin*, just select the buttons that correspond to the listed commands.

Select a suitable region of the spectrum using the ***LMB*** and ***MMB*** and update the acquisition parameters to observe this region using **sw-sfo1**. If the fixed grid is displayed on screen, aim to position the peak within the second square from one of the edges of the spectrum.

aq 2 ***<enter>***

Reduce the acquisition time to 2 seconds.

acqu ***<enter>***

Change to the acquisition screen.

gs ***<enter>***

'Go setup' repeat pulse mode where each FID replaces the previous one. Use the **Ush** button to separate the two components of the FID and observe the shape of the decay more clearly.

If the FID is very small, even after expanding the displayed signal vertically, then increase the receiver gain as follows:

The **XwinNMR-gs** window will be open but iconized behind the *XwinNMR* window. Open this window and use the slider to increase **rg** until the signal can be clearly seen.

Adjust the shim settings, **[z1]**, **[z2]**, and **[z3]** until a smooth and long decay is observed.

Periodically re-run the previous experiment and inspect the peak shapes until they are narrow and symmetrical.

Alternatively, the peak shape can be observed in **gs** mode, by switching the display from the time domain to the frequency domain:

Display → **Phasing** → **Pk phasing**

Note that a spectrum must have been acquired and processed under the current acquisition conditions for the phase correction to be applicable.

12.2 Samples in non-deuterated solvents

If a suitable deuterated sample is not available, samples can be run in protonated solvents. The disadvantages are that a deuterium lock signal is not available for shimming, and if a proton spectrum is acquired it will be dominated by the large solvent signal.

First ensure that **[LOCK]** is off, and that the field sweep is off by pressing **[SWEEP]** (the LED should be off).

Set up a new experiment and load the sample in the usual way.

Load the standard shim file for the instrument in use:

rsh *shimfile* ***<enter>***

Acquire data under the standard conditions.

cop ***<enter>***

Create a copy of the current experiment with a new experiment number.

It is easiest to optimize the shape of the FID if the spectrum contains one dominant signal, and if this signal is off-resonance (not at the center of the spectrum). If necessary TMS can be added to the sample for this purpose - suck some vapor from a TMS bottle into a pipette, add to the NMR tube and shake to mix.

The following instructions apply to *XwinNMR*. The equivalent operations are available in *TopSpin*, just select the buttons that correspond to the listed commands.

Select a suitable region of the spectrum using the ***LMB*** and ***MMB*** and update the acquisition parameters to observe this region using **sw-sfo1**. If the fixed grid is displayed on screen, aim to position the peak within the second square from one of the edges of the spectrum.

aq 2 ***<enter>***

Reduce the acquisition time to 2 seconds.

acqu ***<enter>***

Change to the acquisition screen.

gs ***<enter>***

'Go setup' repeat pulse mode where each FID replaces the previous one. Use the **Ush** button to separate the two components of the FID and observe the shape of the decay more clearly.

If the FID is very small, even after expanding the displayed signal vertically, then increase the receiver gain as follows:

The **XwinNMR-gs** window will be open but iconized behind the *XwinNMR* window. Open this window and use the slider to increase **rg** until the signal can be clearly seen.

Adjust the shim settings, **[z1]**, **[z2]**, and **[z3]** until a smooth and long decay is observed.

Periodically re-run the previous experiment and inspect the peak shapes until they are narrow and symmetrical.

Alternatively, the peak shape can be observed in **gs** mode, by switching the display from the time domain to the frequency domain:

Display → **Phasing** → **Pk phasing**

Note that a spectrum must have been acquired and processed under the current acquisition conditions for the phase correction to be applicable.

13 Pulse length calibration

It is necessary to use a sample which will give a clear signal quickly, ideally from a single scan. When possible, the real sample to be observed should be used. This is normally easy for ^{1}H calibration, and for other sensitive nuclei, or if the sample is very concentrated. Often, however, a separate sample will be used, which might be isotopically enriched. For example a ^{15}N enriched sample of formamide can be used for ^{15}N calibration.

The purpose is normally to determine accurate values for 90 degree and 180 degree pulses for use in NMR experiments. It is generally easiest to determine the length of the 360 degree pulse and divide this value as necessary. This is because a 360 degree pulse produces a 'null' signal and the signal is changing rapidly each side of the 'null'. The 90 degree maximum signal is rather a broad maximum and so it is difficult to accurately determine the precise maximum. An advantage of this approach is that a 360 degree pulse returns the system to equilibrium, and so relaxation delays can be minimized.

Several calibrations may be necessary, as experiments often use both high power and low power pulses.

First set up a new experiment and select suitable parameters for the desired nucleus using **edcp**.

The instructions below apply to XwinNMR. Procedures are similar in TopSpin, but automated options are different and the pulse power levels are handled in a different way.

13.1 Initial setup

Tune the probe, either the ^{1}H channel for proton observation, or both channels for X-nucleus observation, using the procedures described in section 10.

Acquire a spectrum under the standard conditions.

Use the **cop** command to make a copy with a new experiment number.

pulprog ***<enter>***

If the current pulse program is **zg30**, change it to **zg0**; if it is **zgdc30** change it to **zg0dc**.

Expand the spectrum, by selecting a small region around a single peak to be observed during calibration.

sw-sfo1 (Note that in *XwinNMR* 3.5 this must be pressed at least 3 times before the parameters are updated correctly.)

This will update the acquisition parameters to observe this region of the spectrum, which will result in a long acquisition time. This should be reduced manually, but set to a long enough value to allow full relaxation, e.g. 16 seconds.

aq 16 ***<enter>***

Alternatively, a shorter acquisition time can be used in conjunction with a longer relaxation delay, **d1**.

Set the number of scans to 1 and the number of dummy scans to 0:

ns 1 ***<enter>***

ds 0 ***<enter>***

13.2 High power pulse calibration

If the approximate 90° pulse length is known, set **p0** to this value. The parameter **p1** can be inspected, as this is normally set to be a high power 90° pulse. If the 90 degree pulse length is not known, choose a short initial value, such as **3** us. The power level used, **pl1**, should already be set to the standard high power level for the nucleus under observation. This is likely to be in the range **0 dB** to **-6 dB**.

rgazgefp ***<enter>***

Acquire data, process and adjust phase to produce a positive peak.

13.2.1 Automatic calibration

If the 90° pulse length is completely unknown, then an automation program can be used to find the approximate value. The precise value can then be measured manually.

This procedure generates a composite spectrum from a sequence of experiments, using the plot region for the current spectrum. The plot region must be defined to be a small region (1-2ppm) around the peak of interest, using **dp1**.

It is very important that the total time between pulse (**aq** plus **d1**) is sufficient for full relaxation, i.e. $5xT_1$.

paropt ***<enter>***

To start the automation program. The computer will ask the following questions:

Enter parameter to modify:

Type **p0** ***<enter>***

Enter initial parameter value:

Choose a small value, e.g. **3** (units of microseconds are assumed)

Enter parameter increment:

Normally this can be the same as the initial value, e.g. **3** (units of microseconds are assumed)

Enter # of experiments:

The default is 16, press ***<enter>*** to select this.

The program will generate a composite spectrum, in process number 999 of the current experiment number, comprised of a series of peaks, one for each incremented value of **p0**. The largest peak can be used as an estimate of a 90° pulse, or the first null as an estimate of a 180° pulse. Overall the peak intensities should form a sine wave. To interrupt the paropt program before it has finished, use the **kill** command.

Once an approximate 90° pulse length has been determined, an accurate measurement can be performed manually.

13.2.2 Manual calibration

If this is following on from an automatic approximate calibration, as described above, start by recalling process number one for the current experiment:

rep 1 ***<enter>***

Multiply the estimated 90° pulse length by four to calculate an approximate 360° pulse, and set **p0** to this value.

zgefp ***<enter>***

Inspect the resultant spectrum. If the peak is positive then the current pulse length is greater than a 360° pulse, if the peak is negative the current pulse length is less than a 360° pulse. If there is no signal, or a small anti-phase signal, then the current setting is a 360° pulse.

If necessary, alter the pulse length slightly and repeat until a 360° pulse is determined.

13.3 Low power pulse calibration

The process is similar as described above for high power pulses, but the power level will also be adjusted. There are two slightly different techniques. Sometimes a particular power level is desired, but more often a particular 90° pulse length is required, and the power level must be adjusted to achieve this. First follow the initial setup in section 13.1.

13.3.1 Pulse length determination

Set the power level **pl1** to the desired value and then proceed as described in section 13.2 above.

13.3.2 Power level determination

It is common to require a particular 90° pulse length, for example 80 microseconds for proton decoupling, and thus necessary to find the power level required to achieve this. Note that power levels are set in decibels of attenuation, so for a lower power pulse, the attenuation must be increased.

First determine the high power 90° pulse length as described above. Set pulse **p1** to the length of the 90° pulse determined, and set **pl1** to the power level used. The automation program **pulse** can be used as follows to estimate pulse lengths at other power levels.

pulse ***<enter>***

Type the pulse length required, for example **80us**, and the computer will estimate the power level required to produce a 90° pulse of that duration.

Set power level **pl1** to this calculated value, and set pulse **p0** to the required 90° pulse length.

rgazgefp ***<enter>*** to acquire a one scan spectrum, and phase correct for a positive peak.

Change **p0** to be an approximate 360° pulse, i.e. four times the 90°.

Repeat the experiment using the same acquisition and processing parameters:

zgefp ***<enter>***

If a positive peak is observed, then the current power setting is producing a pulse that is longer than 360°. Increase the value of **pl1** and repeat. If a negative peak is observed, then the current power setting is producing a pulse that is shorter than 360°. Decrease the value of **pl1** and repeat.

When the correct setting is achieved for a 360° pulse no signal should be observed. In many cases, a small anti-phase signal will be seen.

14 Quantitative experiments

In theory NMR is a quantitative technique, but in practice compromises are used in order to quickly acquire reasonable quality data. For a fully quantitative spectrum, care must be taken to ensure full relaxation between scans.

A carefully acquired spectrum will yield quantitative information within that spectrum and is described in section 14.1. Gaining concentration information between samples requires the more complex technique described in section 14.2.

14.1 Acquiring a single quantitative spectrum

14.1.1 High sample concentration (proton)

If there is sufficient sample, a good quality proton spectrum can be obtained from one scan, and saturation problems can be avoided. It is necessary to ensure that there is a long delay between setting the receiver gain and acquiring the data.

Optimize the spectral region as normal, then:

d1 20 ***<enter>***

Increase the relaxation delay to **20** seconds.

ds 0 ***<enter>***

Set the number of dummy scans to **0**.

ns 1 ***<enter>***

Set the number of scans to **1**.

rgazg ***<enter>***

Set the receiver gain and acquire data. Careful processing, especially phasing and baseline correction, will yield a good quantitative spectrum.

14.1.2 Standard sample concentration (proton)

For an accurate quantitative proton spectrum, it is necessary to allow 20 seconds between scans for full relaxation.

Optimize the spectral region as normal, then:

d1 20 ***<enter>***

Increase the relaxation delay to 20 seconds. Adjust the number of scans, **ns**, if necessary.

rgazg ***<enter>***

Set the receiver gain and acquire data. Careful processing, especially phasing and baseline correction, will yield a good quantitative spectrum.

14.1.3 Other nuclei

Some nuclei can easily yield quantitative spectra, for others this is very difficult. Suitable parameters will depend on the system under investigation. ^{13}C spectra are only quantitative with either no proton decoupling or inverse gated decoupling, and with a long (several tens of seconds) relaxation delay. ^{31}P, ^{19}F or ^{11}B spectra should be reasonably quantitative under standard conditions, provided that the acquisition time and spectral region are optimized to obtain well defined lineshapes.

14.2 Calculating sample concentrations from spectra

In order to quantify data between two spectra, it is necessary to use special techniques to match the performance of the spectrometer when recording data from different samples. These methods replace the need to add a known quantity of a standard material to each sample. Several techniques are possible, such as ERETIC, PULCON and QUANTAS. The method described here is DIGITAL ERETIC, which uses software to add a simulated reference signal to very carefully acquired data from both a known standard sample and an unknown.

This method is currently available on the CRYO500 and AVANCE600 only, and data can be processed using any of the datastations. Eretic is built-in to *TopSpin3.0* on the AVANCE600, the following programs are not required.

Some experiment parameters must be kept the same between running the standard and the unknown sample, so the standard sample should have the same order of concentration as the unknown sample, and should be at the high end of the range of sample concentrations. For example, a 100mM standard sample would be suitable for measuring samples in the range 10-100mM.

The probe **must** be tuned for every sample, **and** the 90° pulse length **must** be calibrated.

14.2.1 Setup using a sample of known concentration

Load the sample as normal and set up a new experiment with suitable parameters to observe the required nucleus. Next optimize both the tuning and matching of the probe for that nucleus. For proton observation the tuning and matching controls are yellow.

Lock and shim the sample, and acquire a spectrum using the standard parameters.

Next calibrate the high power 90° pulse as described in Chapter 13.

Recall the standard spectrum acquired initially for this reference sample.

Use **cop** to copy to a new experiment number. The experiment used to calibrate the pulse lengths above can be re-used.

Use **sw-sfo1** to optimize the spectral region, remember to press the button 3-4 times. On the AVANCE600, use the equivalent button towards the top right of the screen.

For this experiment a 90° observe pulse will be used. Type **pulprog** and change the pulse program from **zg30** to **zg**, or from **zgdc30** to **zgdc**.

Type **p1** and enter the 90° pulse length measured above, and if the power level was changed, type **pl1** and enter the new value.

Type **aq** and ensure that its value is at least **6** seconds.

The time between scans is the sum of **aq** and **d1**. This total should be at least **40** seconds. Type **d1** and increase the value appropriately, i.e. if aq=6, then set d1=34.

Type **rga** to set the receiver gain.

All subsequent experiments that will use this calibration **must** be run using the same receiver gain setting. Therefore, to increase the available concentration range, it is a good idea to type **rg** and set a value that is half of the original.

Set **ds** to **0** and **ns** to **1** and run the experiment with **zg**.

Set **lb** to **1** and use **efp** to process the data. If the signal to noise level is not good enough, set **ds** to **2** and **ns** to a multiple of **8** and then re-run the experiment.

Data processing and calibration can be performed now or later after acquiring data from the unknown sample.

Very carefully phase correct and baseline correct the spectrum. Calibrate the spectrum.

Choose the calibration peak. This should be well separated from neighboring signals, and the assignment must be known, i.e. the number of nuclei in the sample molecular structure represented by the peak. Expand the spectrum around this peak and note the ppm values which you would use to start and finish an integral to fully cover the peak. These limits can be saved automatically by expanding the region on screen and then pressing **dp1**.You do not need to actually integrate the spectrum at this stage.

Type **quant-calib** to run the calibration program. The computer will ask a series of questions:

Left limit of reference integral (ppm):

Right limit of reference integral (ppm):

Number of nuclei in reference peak:

Sample concentration (mM):

Add synthetic peak to spectrum? (y/n)

If the answer is **y** the next questions will follow:

Concentration of synthetic peak (mM):

Position of synthetic peak (ppm):

14.2.2 Measurement of unknown sample

Load the unknown sample, lock and shim as normal, and acquire a standard spectrum.

Tune the probe, and calibrate the high power 90° pulse as described above.

Set up the quantitative experiment in the same way as described above, except that the **rga** step **must** be omitted. Type **rg** and set this to the same value as used above for the known sample.

Acquire data as above, using either one scan or an appropriate number for the sample concentration. Ensure that **lb** is set to **1** to apply the same line broadening as above when processing with **efp**.

Very carefully phase correct and baseline correct the spectrum. Calibrate the spectrum.

Integrate the peaks in the spectrum for which quantitation is desired.

Either

Type **quant-addpeak** to place a peak of concentration 10mM at -0.5ppm.

Or

Type **quant-addpeak** *X Y* to place a peak of concentration XmM at Yppm.

The integrals for the sample peaks will be re-scaled appropriately. In *TopSpin* the integrals can be displayed on screen in the main spectrum window. In *XwinNMR*, either print the spectrum to see the integrals, or type **li** to print an integral list. This command also saves the integral list as a file within the current dataset, as
/v/data/*username***/nmr/***expname***/***expno***/pdata/***procno***/int1d**

15 Working with small or unstable samples

15.1 Concentrating small samples within the NMR coil

A standard NMR sample is prepared to have a 4cm depth of solution in the NMR tube. This is so that the ends of the sample are outside the coil in the probe and good resolution data can be obtained. One disadvantage is that the sample is dispersed throughout the solution, and so not all of the species present will be 'seen' by the probe. A second disadvantage is that the solvent signal can be large relative to the sample signal if the sample quantity is very small. This can particularly be a problem when recording ^{13}C spectra in carbon-rich solvents.

One solution is to use Doty Susceptibility Plugs inside the NMR tube. These consist of an upper and lower piece. One fits into the bottom of the NMR tube and the other sits above the sample, held in place by a positioning rod. The plugs are made of material designed to match the magnetic susceptibility of the solvent. Thus, the volume of solvent used is much reduced and all of the sample is contained within this small volume of sample, in the center of the NMR coil.

The NMR facility currently possesses three sets of plugs: PPS (p-phenylene sulfide) for use with chloroform or water; zirconia for use with benzene, CCl4, chloroform, DMSO or water; G-10 (60% e-glass epoxy composite) for use with acetone and methanol.

The total length of the two plugs is approximately 26mm, so a sample depth of 14mm between the plugs should be used. This must be carefully centered about the probe coil using the depth gauge.

Contact the NMR facility director for loan of the plugs and positioning rod.

Another solution is to use Shigemi NMR microtubes. These have a solid region of glass below the sample, and a plunger with a solid section that is positioned above the sample. The principle is similar to the Doty plugs, the glass material is susceptibility matched to the solvent in use. These tubes can be easier to use and shim than the Doty plugs, but are more expensive and much more fragile. Shigemi tubes must be purchased by users.

15.2 Saving data periodically during a long acquisition

If a very long experiment is required to accumulate sufficient signal-to-noise it can be advantageous to save the data regularly during the experiment. This provides protection against both sample degradation and instrument breakdown.

Two methods are available. The first runs a series of experiments and the second makes a series of copies of an experiment that is already running.

15.2.1 Run a series of cumulative experiments

A series of datasets can be acquired. At the end of the first experiment **ns** scans are stored on disk. The data is then copied to the next experiment number and **ns** more scans are added to the existing data. This process can be repeated as many times as required, creating a series of datasets containing **ns** scans, **2 x ns** scans, **3 x ns** scans, etc.

If the final dataset is fine, then the previous experiments can be disregarded. If the final spectrum shows evidence of sample degradation or instrument performance problems, then the previous experiments can be inspected to select the best quality data acquired before the problem occurred. If only instrument problems are to be guarded against then each sub-experiment can safely be run for several hours. If sample instability is suspected, then a suitable experiment length must be estimated from known properties of the sample.

For example, a very long carbon experiment could be safeguarded by saving data after every 4000 scans. The first dataset would then contain 4000 scans, the second would contain 8000, the third would contain 12000, etc.

Two different automation programs are available and can be used as follows:

First set up the first experiment in the usual way. Read standard parameters for the required experiment, and optimize as required. Set the receiver gain using **rga**. Decide how long each sub-experiment should take, and set an appropriate number of scans, **ns**. Use the **expt** command to check the experiment duration.

Decide how many sub-experiments will be run, and ensure that the required sequence of experiment numbers has not already been used.

Type:

multisave ***<enter>***

The computer will request a number of experiments to be run, with a default option of ten.

Enter the required number of experiments and the computer will calculate the total experiment time and start the first experiment.

To make any changes, abort the automation program using the **kill** menu, change **ns** or the number of experiments and restart.

This automation program will create the required empty datasets before starting the first experiment. If a large number of experiments is to be performed, the program **multisaveq** can be used. Each experiment will be created only when it is required to be run. This program is therefore also suitable for use when the precise timings have not been calculated and a long sequence of experiments is to be interrupted.

15.2.2 Make a series of copies of data from a running experiment

A single experiment is run, and data is saved and copied to new experiment numbers at regular time intervals. The user needs to set the time interval to save data and the desired multiple of number of scans.

Set an experiment running as normal.

Type:

multicopy ***<enter>***

The computer will respond as follows:

Enter wait time in minutes:

The default value is 60, and does not have to be an integer.

Enter number of data copies:

The number of times to copy the data, the default value is 10.

Enter multiple of scans to copy at:

The multiple number of scans at which to copy the data, the default is 16.

For example, if an experiment is running as experiment number 1, and the default settings above are used, the following actions will occur. After one hour, the data will be transferred at the next multiple of 16 scans. When that has happened, the data will be copied to experiment number 2. Experiment number 1 is still running. After another hour, the data will be transferred at the next multiple of 16 scans and copied to experiment number 3. This will continue until 10 hours has elapsed and the last copy is made as experiment 11. Note that experiment 1 will continue to run until it is halted or stopped.

If it is desired to interrupt **multicopy**, then type **kill** and select the relevant entry in the resultant menu.

16 Network & color printing (*XwinNMR*)

16.1 Plotting spectra to networked printers from the NMR facility

A selection of automation programs is available to ease access to remote printers. These work only for plotting spectra. See below for general access to remote printers for printing text. A complete list of customized automation programs is in the dedicated section.

netvplot ***<enter>***

Produces a list of all available printers for selection, previews plot, then prompts for the spectrum to be plotted or cancelled. The output device 'current plotter' is temporarily changed during the program to the remote device, then restored to the original setting.

netplot ***<enter>***

As above, but will plot the spectrum immediately with no preview.

netvplot2 and **netvplot2** work as above but also save the spectrum as a postscript file.

16.2 General access to different printers from *XwinNMR*

The copies of the *XwinNMR* program in the NMR facility are configured so that printed output will normally be produced by the default printer for the workstation in use. This is the nearest printer and may be either a directly connected parallel port printer or a network printer.

Within each dataset there is an output devices menu, accessed by typing **edo** ***<enter>***.

There is a distinction in *XwinNMR* between plotting graphics (spectra) and printing text (peak lists, etc). To plot spectra to a different printer, select the desired printer from the **CURPLOT** menu, and to print text to a different printer, select the desired printer from the **CURPRIN** menu. Note that if a plotter is defined in the user interface (**setres**), then this must be removed before selecting a different plotter in **edo**.

The GN500 and DRX400 have parallel printers, both plotter names **hplj5l** and **hplj6l** will output to these devices. The GN500 and DRX400 can also access the two NMR facility network printers. The nearer one is named **NMRnet_def** (**hplj4mv** and **hplj4mvA4** will also output to this printer), and the further one is **NMRnet_alt**.

The three workstations can only output to the network printers. On these computers, all the following names will output to the nearest network printer: **NMRnet_def**, **hplj5l**, **hplj6l**, **hplj4mv** and **hplj4mvA4**; and **NMRnet_alt** will output to the further network printer.

The remaining printer names will either output to remote network printers, e.g. **VanVranken_lab**, or print to a file; **pscript_file**. See section 18 for more information on printing to files.

16.3 Color printing

There is currently no color printer accessible from *XwinNMR* within the NMR Facility, but there is one in Natural Sciences I. Within the NMR facility, color spectra should be saved as postscript files. These files can be converted to PDFs and then printed elsewhere.

In *XwinPlot*, colors can be selected using the editing tools.

In *XwinNMR*, colors must be selected via the **edg** menu. There are 10 predefined colors: black, blue, violet, magenta, red, orange, yellow, green, turquoise and brown. For 1D data it is straightforward to select different colors for different elements of the spectrum: integrals, peak labels, etc. For 2D data there is a choice between plotting positive and negative contour levels either in different line types or in different colors, so the latter must also be selected in addition to defining colors. If all positive (or all negative) contours are to be printed the same color, then only the first level needs to be defined, as the color list will be looped as many times as is necessary to draw the selected number of contours. If different colors are to be used for each level, then users must be careful to match the number of levels printed with the number of colors in the list.

Colors can also be used when saving spectra as postscript files.

17 Remote access to *XwinNMR* and *TopSpin*

The information in this chapter may be regarded as redundant, now that *TopSpin* can be installed on a user's own computer free of charge. See Chapter 25 for installation instructions.

It is possible to remotely access the NMR facility workstations and run *XwinNMR* or *TopSpin*. The remote computer must be equipped with 'X-server' software. There are four SGI processing workstations running *XwinNMR*: **bragg.ps.uci.edu**, **parker.ps.uci.edu**, **psqe.ps.uci.edu** and **tilbrook.ps.uci.edu**; and two Linux workstations running *TopSpin*: **costello.ps.uci.edu** and **heaton.ps.uci.edu**. It may not be necessary to include the *uci.edu* part of the domain name when connecting from within the university.

XwinNMR requires that the X-server must be running in 256 (8 bit) colors, but there are no restrictions for *TopSpin*.

If you are using a wireless connection at UCI, using 'UCInet Mobile Access', you may need to use a VPN program in order to connect to the NMR computers. If you have a static IP address configured on either of the NMR subnets (128.200.31.* or 128.200.228.*), but this is not active, you may also not be able to connect wirelessly unless you deactivate the ethernet connection in your network preferences. The UCI VPN must be used for off-campus access to all NMR computers except for nmrserver.

17.1 Host keys

The first time you make a secure connection to a remote workstation using **ssh**, your local computer will store a 'host key' for that workstation, and a warning message will appear on screen. During subsequent connections, the relevant host key will be found from a list and used.

The host key for a particular computer will change if the operating system is upgraded. If the host key has changed an attempt to log in remotely will produce an error message. To continue logging in, the local *known_hosts* host key file must either be edited to remove the entry for the remote computer in question, or deleted completely.

The error message will specify the file name and path of the host key file. This will normally be located in the local user home directory: *userhomedirectory*/.ssh/known_hosts

17.2 Connecting from a remote Unix computer

These instructions should be applicable to any Unix computer running X-windows or an equivalent, such as SGI and Sun computers.

Open a Unix shell from the Toolchest (or equivalent if the local computer is not Silicon Graphics) and type:

ssh -X *nmrusername*@*nmrhostname*.**ps.uci.edu**

e.g. **ssh -X jsmith@psqe.ps.uci.edu**

The first time a connection is made a 'secure host key' must be save by answering 'yes' (not simply 'y') to the question which appears.

If the remote computer runs *XwinNMR*, type:

tool ***<enter>*** to produce a Toolchest titled with the name of the remote computer, *XwinNMR* can be run as normal.

If the remoter computer runs *TopSpin*, type:

topspin ***<enter>***

17.3 Connecting from an Apple computer

From MacOS 10.4 (Tiger) to 10.7 (Lion), an X11 server was been included with each computer, but it was not always installed by default.

If the standard Apple package causes problems, an alternative is to use *XQuartz*. This is a free package which can be found at https://www.xquartz.org/

XQuartz can safely be installed in parallel with the Apple *X11*. The Apple *X11* supplied with OS 10.5 onwards has different color handling to the version supplied with 10.4. The older version is more compatible with the color handling used by *XwinNMR*. A legacy version is available for download, and it may be possible to install this on later MacOS versions. Some manual adjustment will be required to make it work, and it may be necessary to fully delete the standard Apple X11 first. The safest way to do this is to reinstall the OS from DVD and deselecting the X11 package. The legacy version can be found here:
http://static.macosforge.org/xquartz/downloads/Leopard/X11_Legacy-1.1.4.1.pkg
http://static.macosforge.org/xquartz/downloads/Leopard/X11_Legacy.README

17.3.1 Using *X11* under MacOS 10.8 (Mountain Lion) and newer

X11 is no longer supplied by Apple, *XQuartz* must be installed as described above. Usage is the same as for 10.6 and 10.7, described below.

17.3.2 Using *X11* under MacOS 10.6 (Snow Leopard) or 10.7 (Lion)

The Apple *X11* package is installed by default into the Utilities directory under MacOS 10.6. This version does not fully support the color handling model used by the Silicon Graphics computers.

Load *X11* or *XQuartz* and, if *XwinNMR* is to be accessed, use the *Preferences* menu to configure the display to 256 colors:

X11 → **Preferences…** → **Output** → **Colors** → **256 Colors**

To activate this change, *X11* must be restarted.

Log on to the NMR SGI workstation by typing the following commands in the *X11* terminal window:

ssh -Y *nmrusername@nmrhostname*.**ps.uci.edu**

e.g. **ssh -Y jsmith@psqe.ps.uci.edu** (Note that **ssh -X** can be used in MacOS 10.3 but not 10.4)

The first time a connection is made a 'secure host key' must be save by answering 'yes' to the question which appears.

If the remote computer runs *XwinNMR*, type:

tool ***<enter>***

Produces a Toolchest titled with the name of the remote computer, *XwinNMR* can be run as normal.

The *XwinNMR* window will initially open the same size as when *XwinNMR* was last run on the SGI computer which the Apple computer is connected to. If the Apple computer monitor is set to a lower resolution than 1280x1024 then the window will be too initially too large but can be re-sized with the green window button.

Several circumstances will cause the *X11* server to crash:

If the *XwinNMR* window was maximized using the window button, so that the help/status bar is off the bottom of the screen, then a crash seems to be caused by the initial attempt by *X11/XQuartz* to shrink the window to fit the Mac screen.

If an *XwinNMR* menu is scrolled the server will often crash. Avoid using menus during remote access, except for the **search** data browser which does not cause a crash,

The *XwinNMR* window will be displayed with some incorrect colors, black and white are used instead of some shades of grey. Some text will also appear as black-on-black, for example the message window when using the command **pstopdf**.

It is not possible to open directory views from the Toolchest, for example for renaming postscript files. These should be renamed from the command line in a Unix shell:

Desktop → **Open Unix Shell**

Then type:

cd postscript ***<enter>***

ll <enter> to list files, then

mv *oldfilename newfilename* ***<enter>*** to rename (move) file.

If the remoter computer runs *TopSpin*, type:

topspin ***<enter>***

17.3.3 Using *X11* under MacOS 10.5 (Leopard)

The Apple *X11* package is installed by default into the Applications directory under MacOS 10.5. This version does not fully support the color handling model used by the Silicon Graphics computers, see section 17.3.2 above for details of the limitations.

17.3.4 Using *X11* under MacOS 10.4 (Tiger)

X11 is an optional install which is supplied on the operating system disks and must be manually installed, it will not be installed by default. Simply load the OS X installation disk and select the package for installation. Note that it may be necessary to scroll the installer *Finder* window to the bottom to see the package. This version of X11 fully supports the graphics from the Silicon Graphics computers, all window functions should be available. X11 is installed into the Utilities directory.

Load *X11* and use the *Preferences* menu to configure the display to 256 colors.

Log on to the NMR SGI workstation by typing the following commands in the *X11* terminal window:

ssh -Y *nmrusername@nmrhostname*.**ps.uci.edu**

e.g. **ssh -Y jsmith@psqe.ps.uci.edu** (Note that **ssh -X** can be used in MacOS 10.3 but not 10.4 or later)

The first time a connection is made a 'secure host key' must be save by answering 'yes' to the question which appears.

If the remote computer runs *XwinNMR*, type:

tool *<enter>*

Produces a Toolchest titled with the name of the remote computer, *XwinNMR* can be run as normal.

The *XwinNMR* window will initially open the same size as when *XwinNMR* was last run on the SGI computer which the Apple computer is connected to. If the Apple computer monitor is set to a lower resolution than 1280x1024 then the window will be too initially too large but can be re-sized with the green window button.

If the remoter computer runs *TopSpin*, type:

topspin *<enter>*

17.4 Connecting from a PC

There are commercial X-server packages which run under Microsoft *Windows*. All should work fine for 1D data processing but some may not be able to display 2D spectra. Note that the exact procedure required by these packages may change with different program versions.

17.4.1 PC running Microsoft *Windows*

The free *Windows* X-server, Xming, can be used to access *TopSpin* remotely, but does not work in 256 color mode and so cannot be used for *XwinNMR*.

One commercial X-server, *Xmanager 6.0,* does offer 8-bit color depth and can be used for 30 days for evaluation. This has been tested using Windows 7 running as a virtual machine on a Mac. The evaluation instructions are supplied by email, and so the program cannot be simply reinstalled for further use. It must be purchased for $149 (educational price as at 2020-05-11).

https://www.netsarang.com/en/xmanager

After installation, select **Xstart**. Then complete the options in the resultant window:

New session

Enter the hostname of the computer you wish to connect to.

Set protocol to **SSH**

Enter your User Name

Set Authentication to **Password**

In the Command menu select **xterm(Unix)**

Save if desired and then **Run**.

After a few seconds a terminal window will open with a command line prompt. Type **tool** for an SGI ToolChest, or **xwinnmr** to start XwinNMR.

17.4.2 PC running Linux

Access to *TopSpin* is simple by logging on using:

ssh -X *nmrusername*@**costello.ps.uci.edu *<enter>***

then typing:

topspin *<enter>*

For *XwinNMR*, a remote X-session must be started and configured to 8-bit color. The exact command required will depend of the desktop environment currently in use. It may be necessary to stop the current X-server first, if it is running at a different color depth.

To stop X:

sudo service gdm stop *<enter>* (This works in Ubuntu)

Re-start in 256 colors:

startx -- :1 -depth 8 *<enter>*

Then log on to the NMR workstation:

ssh -X *nmrusername*@*nmrhostname*.**ps.uci.edu**

The first time a connection is made a ‘secure host key’ must be saved by answering ‘yes’ to the question which appears.

18 Stack plotting several spectra

Stacked plots can be created using either *XwinNMR* or *XwinPlot*. The former method will be described here, the latter method is described in the *XwinPlot* section. In *TopSpin* the **plot** tab offers this functionality as well as the multiple display screen described below.

18.1 Displaying multiple datasets on screen

18.1.1 *XwinNMR* dual display

In *XwinNMR*, two datasets can be displayed on screen but cannot be directly printed together. This mode is designed for comparing or for adding/subtracting data.

Dual display mode is entered by selecting **dual** on the left of the screen. The default setting is that a second copy of the same dataset will be displayed over the top of the first in a different number. The experiment number or process number of the second dataset can be incremented or decremented using the buttons on the left of the screen.

Alternatively, the second and third datasets can be defined manually in the **edc2** dialog. Save this screen and then select **dual** to display the files.

Within dual display mode it is also possible to add or subtract files. This is a useful method for background subtraction. The second dataset can be added to or subtracted from the first, and the result saved with the filename defined by the third dataset.

18.1.2 *TopSpin* 4 multiple display

In *TopSpin*, multiple datasets can be displayed in the same *TopSpin* window, and the result can be printed using the 'print active window' option.

Multiple display mode can be entered by selecting the button which has a red spectrum drawn above a black spectrum, or by typing **.md**. Any command that is subsequently used to load datasets will load more spectra into the same window. The spectra can be stacked on top or above each other, and moved and scaled relative to each other. The result can be printed, and the array of datasets will be remembered if multiple display is exited and then re-entered. Any changes to scaling or positioning are not saved.

See below for creating stack plots using the *TopSpin* plot editor.

18.2 Stack plot of similar spectra

18.2.1 Using automation in *XwinNMR*

If the series of spectra share the same chemical shift range, then this is an easy method for stacking the spectra. If the spectra are different, for example proton and carbon spectra from the same sample, then it is easier to create the composite plot using *XwinPlot*.

The source files for the series of spectra to be stacked must be an incremented sequence of either experiment numbers or process numbers. A convenient method is to make copies of the spectra with a new experiment name, e.g. **stack**, with a sequence of experiment numbers starting from **1**.

Stacked spectra can be scaled in two ways:

Fixed scaling - all the spectra in the stack will be scaled to be a fixed height in centimeters, according to the current settings of **pscal** and **cy**.

Relative scaling - the first spectrum will be scaled according to the current settings of **pscal** and **cy**, and subsequent spectra will be scaled relative to the first spectrum. Care must therefore be taken over the stacking order of the spectra and of the scaling factor used for the first spectrum.

The setup process is the same for each method, but the final automation program used is different.

18.2.1.1 Prepare the data files

Choose the spectra to be stacked, and select the stacking order. The stack plot will be created from the bottom upwards.

Open the data portfolio using **search** and select the first dataset with the ***RMB***.

Copy the spectrum to a new, temporary, experiment name, e.g. **stack**:

wrpa stack 1 ***<enter>***

Select the second dataset from the portfolio using the ***RMB***.

Copy the spectrum to a new, temporary, experiment name, e.g. **stack**:

wrpa stack 2 ***<enter>***

Repeat the above commands until all the spectra to be stack plotted are in a sequence of experiment numbers, with the same experiment name, e.g. **stack**.

18.2.1.2 Plan the stack plot layout

There are several choices to be made about the layout of the stack plot:

What kind of stack is desired? Spectra can be either stacked above each other vertically with no horizontal shift, or shifted both horizontal and vertically.

Which page orientation is most appropriate? A horizontal (landscape) layout allows for the width of each spectrum to be maximized but reduces vertical space, whereas a vertical (portrait) layout allows for greater height but narrower spectra.

Can all the spectra be fitted onto a single page, and if not how many spectra should be printed per page?

Calculate dimensions for each spectrum using the following information as a guide.

Horizontal (landscape) layout: The width of a standard spectrum, **cx**, is **22.8** cm, but as parameter printing is not normally appropriate for a stack plot, this can be increased to **25** cm. If spectra are to offset horizontally a smaller value must be used. The x-axis is normally printed at the bottom, which leaves approximately **18** cm of vertical space for the spectra. The height of each spectrum, **cy**, can be calculated by dividing this figure by the number of spectra to be plotted. Normally it is also necessary to allow for a small space between spectra, e.g. **0.5** cm, but this may not be necessary if peaks are broad.

Vertical (portrait) layout: The maximum width of a spectrum is **19** cm, and the available vertical space is approximately **25** cm.

18.2.1.3 Set up the layout for the first spectrum

The basic plot settings must be loaded into the first spectrum, and will automatically be copied to the subsequent spectra in the stack.

Read the first spectrum onto the screen:

re 1 ***<enter>*** (or **re stack 1** ***<enter>*** if a different experiment name is currently loaded)

The standard format is to plot the first spectrum with a title and an x-axis, and to subsequently plot each additional spectrum only.

Open the graphics editor:

edg ***<enter>***

Turn off all undesired options: **INTEGR**, **ILABELS**, **PARAM**, **PLABELS**, etc, by clicking on the **yes** box to change it to **no**. With the standard settings, a horizontal (landscape) stack plot will be produced. If a vertical (portrait) layout is required, then change the setting for **WROT** from **0** to **270**. If **WROT** has been changed to create a vertical layout, then the position of the title window must also be changed (if a title is to be printed, if not change **TITLE** to **no**). **EDTITLE** → **ed**, then increase **TYLLEFT** from **18.7** to **24**.

SAVE to save the settings.

Create a suitable title for the stack plot (if desired):

setti ***<enter>***

18.2.1.4 Create the stack plot

There are several choices of automation program which can be used, and these fall into two categories depending on whether fixed or relative vertical scaling is used for the spectra.

stack1df - fixed scaling, no preview

stack1dfv - fixed scaling, preview before plot

stack1dfv2 - fixed scaling, preview before plot, print to paper and save as postscript file

stack1dfvnet - fixed scaling, preview before plot, choice of any network printer

stack1dfvps - fixed scaling, preview before plot, print to postscript file

stack1dr - relative scaling, no preview

stack1drv - relative scaling, preview before plot

stack1drv2 - relative scaling, preview before plot, print to paper and save as postscript file

stack1drvnet - relative scaling, preview before plot, choice of any network printer

stack1drvps - relative scaling, preview before plot, print to postscript file

Operation is the same for all the above programs, except that the ***net** options include an extra menu to select the printer.

To use:

stack1dfv ***<enter>*** (or **xau stack1d*** ***<enter>*** to produce a menu of the different programs)

Click **OK** to continue with the program or **Cancel** to quit and make changes in **edg** etc.

Plot expnos (e) or procnos (p) :

Choose whether to plot a series of experiment numbers or process numbers. Press ***<enter>*** to keep the default setting of experiment numbers, or **p** ***<enter>*** to select process numbers.

Enter first expno/procno to plot :

Press ***<enter>*** to keep the default setting of **1** or enter the desired experiment number/process number.

Enter number of expnos/procnos to plot :

Enter the total number of spectra to be included in the stack plot.

Enter expno/procno increment :

Enter the numerical increment between the experiment numbers or process numbers to be plotted. Note that a negative increment can be used to plot experiment numbers or process numbers in descending order.

Enter number of expnos/procnos per page :

The default number offered is the total number entered previously. A smaller number should be used if the stack plot will be spread over several pages.

Enter width for each plot :

Enter the width in cm for each spectrum. A maximum of **25** can be used for a landscape layout, or **19** for a portrait layout. A smaller value must be used if the spectra are to be offset horizontally as well as vertically.

Optimum x-increment ('Enter' if OK) :

The default value is **0** which is the standard setting if spectra are to be stacked vertically only.

Plot height for largest peak in spectrum :

Calculate a suitable scaling factor for the first spectrum so that the desired number of spectra will fit on the page in the chosen layout.

Enter offset to X-axis (increase for -ve peaks) :

The default value is **0.5** cm. This should be increased if the spectra contain negative peaks.

Optimum y-increment ('Enter' if OK) :

The y-increment is the vertical distance between baselines for successive spectra in the stack. The default value is the sum of the peak height and offset to the x-axis entered above. This can be reduced to overlap spectra if required.

If a ***net** program is being used, at this point a menu of available printers will appear, and the required network printer should be selected.

The stack plot will be previewed on screen. Examine this and decide if the desired layout has been achieved.

Quit to exit from the previewer, then click **OK** to print the stack plot or **Cancel** to delete it from the plot queue.

To modify the layout, calculate new parameters and repeat the program.

18.2.2 Using the plot tab in *TopSpin* 4

Load the first spectrum, that will be at the bottom of the stack, into the *TopSpin* window.

Select the **PLOT** tab, or type **plot**, to enter the plot editor.

Select and delete anything that is not required to be present in the stack plot, including the current spectrum. The window will now probably be empty, unless the title has been retained.

Below the data browser there should now be the *Plot Portfolio* window, which will contain the details of the current dataset. All of the spectra required for the stack plot should be added, by dragging them to this window from the browser. If the set of spectra have already been displayed in the *TopSpin* multiple display screen, this list can be imported by clicking on the down pointing arrow and selecting **Load collection from multiple display mode**.

Pressing the ***RMB*** gives the option to rearrange or delete items from the list.

On the plot editor sidebar, select **NMR** → **Stacked 1D spectra**

Drag the ***LMB*** to create a window which will contain the first spectrum. Size and position this as required.

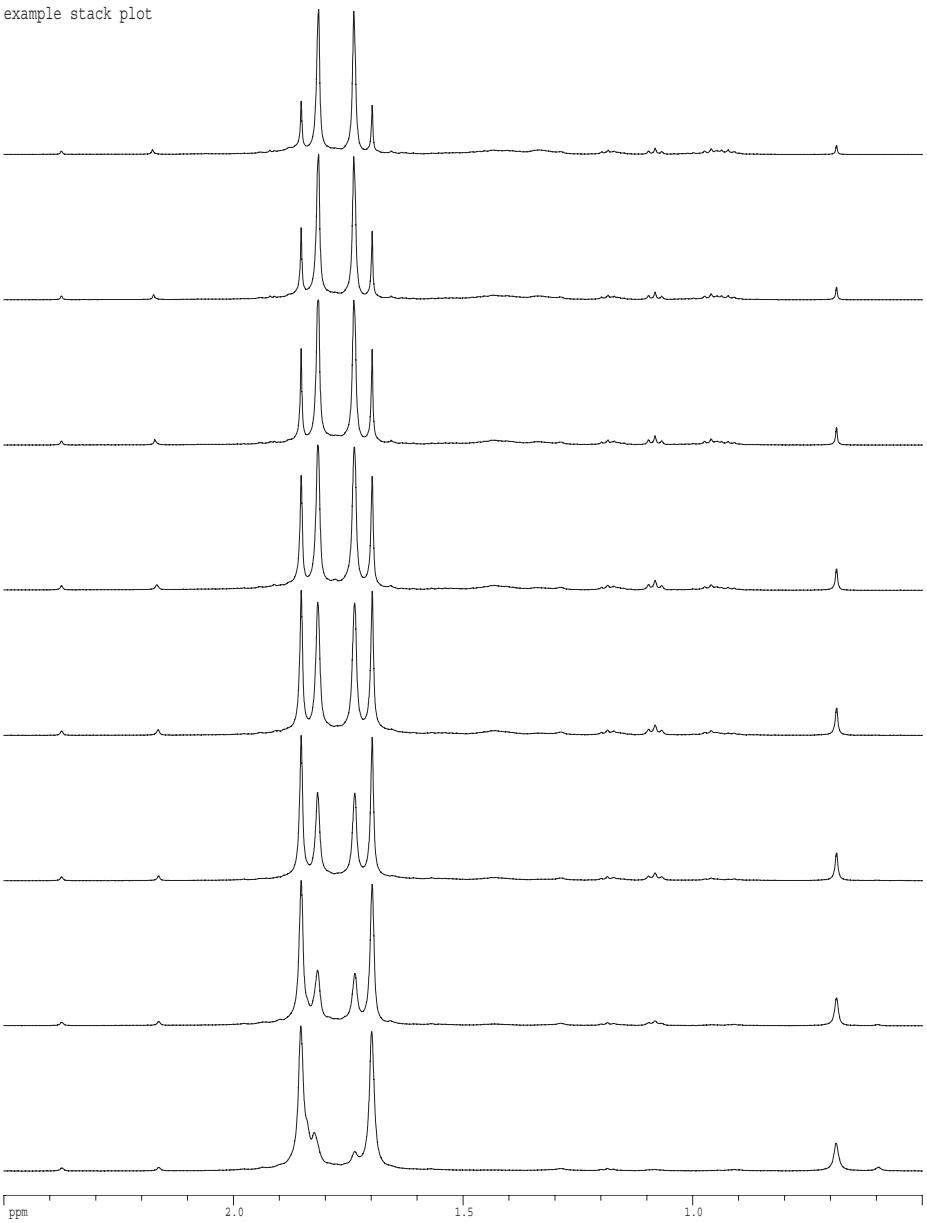
Click in the spectrum window to select it, then click on **Stacked Arrangeme…** in the left side panel.

Set the desired **Number of spectra**, **White Wash** and **X** & **Y** offsets, then the stack of spectra will appear.

There are currently two undesired features of the resultant spectrum. The values for the number of spectra and state of the White Wash option are printed on screen to the right of the plot. However, these can easily be positioned outside the active area. A 'Y' axis is also displayed on the right hand side of the stack. This is more difficult to position off-screen, but can be removed by editing the PDF file after export.

Titles and labels can be added to the stack plot as desired. In the plot editor side bar, select **Standard → Text**, then type and position as required. Other objects can be added in the same way.

To change the spectrum region, set the **Number of spectra** back to 1, use the **Expand Tool** as desired, then set the **Number of spectra** back to the number used for the stack. The stack plot will then be re-drawn with the new region.

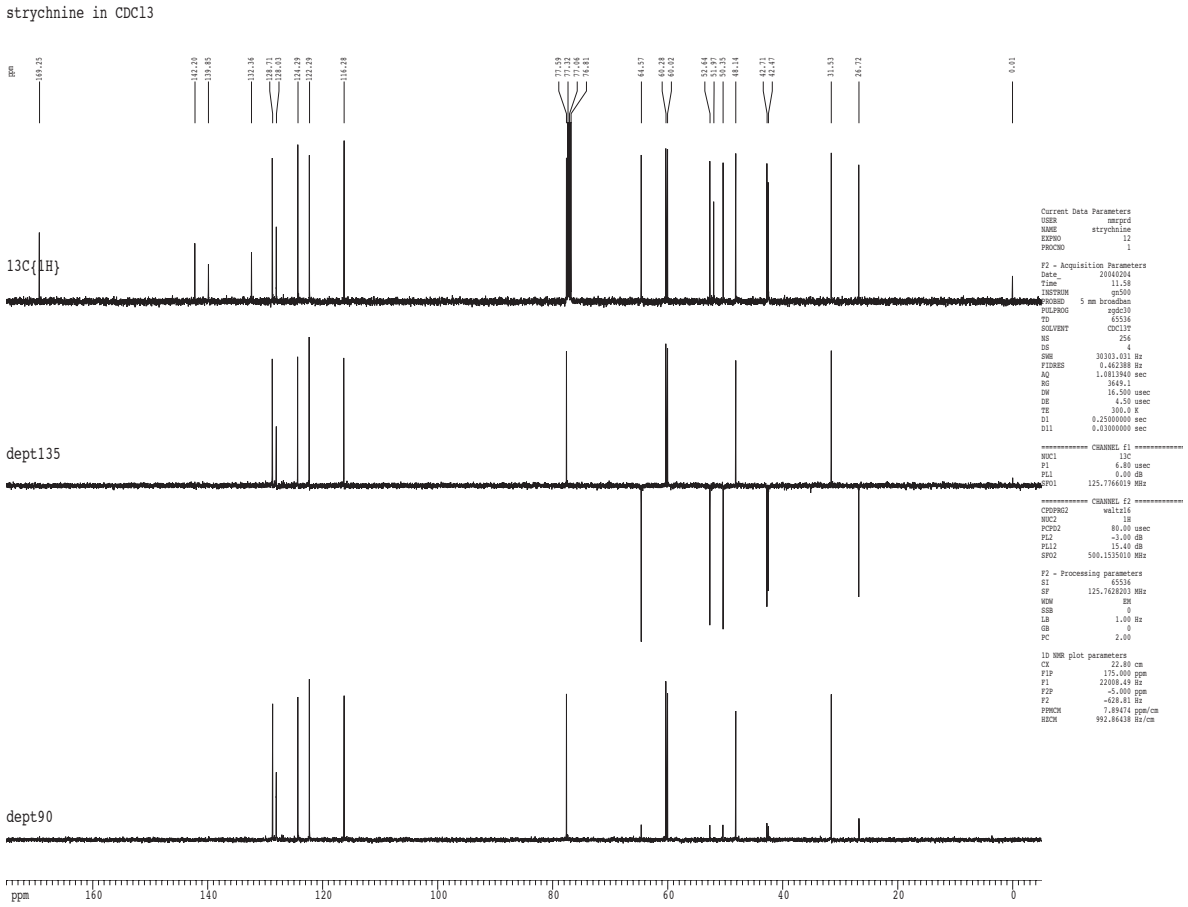


18.3 DEPT stack plot

A special case of stack plotting can be used to clarify the information obtained from DEPT experiments. Three different experiments are presented on one page: carbon-13 spectrum with proton decoupling, DEPT135 and DEPT90. Note that the parameters printed are those from the first experiment of the three: carbon-13 spectrum with proton decoupling.

18.3.1 Prepare files and process data

The experiments must be stored as consecutive experiments in the order listed above. If the data was not acquired in this sequence the files can be copied using either **copy** to copy to new experiment numbers with the same experiment name,



or **wrpa** to copy to new experiment name and numbers.

Ensure that the three spectra are phased correctly and have the same referencing. See the section on running DEPT experiments towards the end of this manual for tips.

18.3.2 Select plot region and modify parameters

Recall the first of the three experiments, the carbon-13 spectrum with proton decoupling.

Decide whether or not parameters are required on the stack plot. If not, turn these off in the **edg** menu and increase **cx** from **22.8** to **25**.

Edit the plot title using **setti**. Each of the three spectra will be automatically labeled with the type of experiment, and so the title need only contain sample details.

Expand the spectrum to the desired region on screen using the ***LMB*** and ***MMB***, then define this as the plot region using **dp1**.

18.3.3 Plot the stack of three spectra

There are three variants on the automation program:

d3vplot - previews the stack plot on screen, which can then be plotted or cancelled

d3psvplot - previews the stack plot on screen, which can then be saved as postscript or cancelled

d3netvplot - offers a selection of printers, after which the stack plot is previewed and can then be plotted or cancelled

18.4 Plotting different spectra on the same page

18.4.1 *XwinNMR*

Multiple spectra can be plotted manually on the same page using *XwinNMR*, simply set up the plot parameters and type **plots** instead of **plot**. When all the spectra have been included, type **flplot** to print the composite spectrum. Care must be taken so that they do not overlap with each other. If a mistake is made, the whole composite plot must be deleted with **rmplot** and recreated.

The current composite plot can be previewed with the command **viewmg**.

A popular example is to plot a portrait format page containing a proton spectrum above a carbon spectrum:

This can be easily achieved by replacing the *XwinNMR* plot parameters. The sequence of operations is as follows:

Load the first dataset, in this case the proton spectrum.

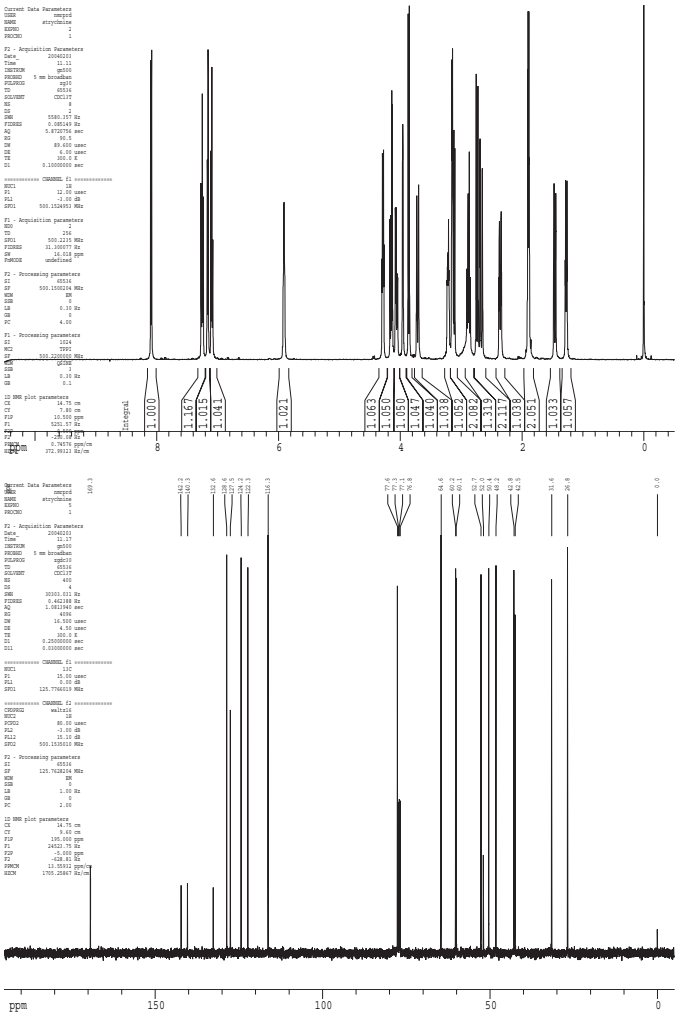
Modify the plot parameters to achieve the desired effect. In this case, **rpar top1H.plot all** will load plot parameters to produce a small spectrum in the top half of the page, with parameters on the left and integral labels but no integral trails. Alternatively, individual plot parameters can be varied in the graphics editor, **edg**.

Plot the spectrum to the queue: **plots**

Load the next dataset, in this case the carbon spectrum.

Modify the plot parameters to achieve the desired effect. In this case, **rpar bottom13C.plot all** will load plot parameters to produce a small spectrum in the bottom half of the page, with parameters on the left. Alternatively, individual plot parameters can be varied in the graphics editor, **edg**.

Plot the spectrum to the queue: **plots**



Add further datasets if required.

If desired, preview the composite plot: **viewmg**

Finally, either plot the composite spectrum to paper using either **flplot** or **netflplot**, or save as a postscript file using **psflplot**,

Note that the composite plot is removed when it is plotted. If both paper and postscript versions are required, then the above process must be performed twice.

Standard plot parameters can be restored to the individual experiments by selecting the most appropriate items from the **plotpar** menu.

18.4.2 *TopSpin 4*

Any combination of spectra can be created within the plot tab. On entering the plot tab, a suitable layout for plotting the single spectrum should be automatically loaded, for example **@h1.xwp** for a proton spectrum. These may not be present on a user's own computer unless they have correctly installed all of the UCI extra files.

One approach to creating a more complex plot is to start by loading a different layout file. Clicking on the 'down' arrow next to **Layout:** then selecting **Open…** will produce a menu.

The custom UCI files all have names starting with **@**. The subdirectories called **layouts.*** contain templates for various types of composite plots. These will provide suitable page positions for each element, and the desired spectra can be loaded by adding them to the **Plot Portfolio** and then by dragging them to the desired windows on the page.

Alternatively, custom composite spectra can be created by inserting more elements into an existing layout. Click outside the spectrum area to ensure that no elements of the current layout are selected. The left pane should now show **Click here to insert new elements**, with menus for **Standard** or **NMR** objects. These can be used to add more spectra or text labels, etc.

19 Exporting spectra as files for word processing *(XwinNMR)*

It is straightforward to save spectra in an electronic format which can be incorporated into documents. It is important that a vector graphics format is used, so that spectra can be re-sized and retain full resolution. Bitmap graphic formats, such as JPEG and TIFF should not be used unless there is no alternative.

Note that although postscript is a vector format and so is re-scalable, there is still a limit on how much of the spectral resolution is incorporated into the file. Not every data point from the NMR spectrum is included, so there is still a 'resolution' limit. For example, if a standard full page proton spectrum is saved as a postscript file and includes a spectral range of 12ppm, approximately 1 in 6 data points are included in the postscript file. Thus, if a spectral range of greater than 2ppm is used it is likely that the postscript file will not contain the full resolution of the original spectrum. The consequence is that it is much better to create separate postscript expansion spectra, rather than to expect to expand the postscript or PDF file to see expanded regions.

The most convenient format is PDF, which can be readily generated from postscript files produced by *XwinNMR*. Microsoft *Office 2008* (Mac) and *Office 2007* (Windows) programs, or newer versions, can import and display PDF files.

PDF files can be manipulated using Adobe *Illustrator*, or the free alternative *Inkscape*, on the NMR Facility Apple computers. Content can be edited, structures can be imported from *ChemDraw*, etc.

19.1 Saving spectra as postscript files from *XwinNMR*

It is necessary to change the output device 'current plotter' to the postscript file generator. An automation program can be used in place of the normal **plot**/**view**/**vplot** command:

Either:

psvplot ***<enter>***

The current plotter is changed to **pscript_file** automatically, and the plot is previewed. **Quit** from the previewer then click on **OK** to save as postscript, or **Cancel** to quit. The current plotter is then reset to the original setting.

Or:

psplot ***<enter>***

Similar to **psvplot**, but the preview stage is omitted.

The spectrum can be saved as either monochrome or color. The 'plot request message' box which appears following the above commands will contain the filename with which the postscript will be saved, e.g. ps_file-1234. This file is saved in the user's home directory on **nmrserver.ps.uci.edu**, in the subdirectory **postscript**.

To keep track of the contents of each postscript file it is a good idea to change the names to something more meaningful as they are created. A menu will appear listing all existing postscript files. Rename the file as necessary. When the name has been changed, press **Cancel** at the bottom of the menu.

plot2 can be used to print the spectrum to paper and then save a postscript file.

19.2 Batch conversion of postscript files to PDF

It is possible to automatically convert all postscript files in the **postscript** directory to Adobe Portable Document Format.

Type:

pstopdf ***<enter>***

either within *XwinNMR* or in a unix shell.

The output files are saved in the within the user's home directory, in the directory **PStoPDFfiles**, sub-directories **pdf** and **postscript**. The postscript files are moved to prevent repeat conversions. The postscript files retain their original filenames and the PDF files have **.pdf** appended.

It is important to retain the postscript files as well as the PDFs, as *Microsoft Word* renders postscript diagrams much better than it does PDFs. If necessary, PDFs can be converted to postscript files using *Adobe Illustrator* or *Corel Draw*.

19.3 Saving spectra as files from *XwinPlot*

It is possible to save spectra as PostScript, PDF, EPSI (encapsulated postscript) or TIFF (uncompressed bitmap) files from *XwinPlot*. PostScript is selected by default, and should normally be fine. If a TIFF file is desired this can be exported from *Adobe Illustrator* using LZW compression to produce a high quality image with a relatively small file size.

It is only* necessary to change the Printer Setup if a PDF, EPSI or TIFF file is desired, or to change output from monochrome to color:

* *Note:* Normally spectra from *XwinPlot* are printed via postscript, and so the same printer driver can be used both to print to paper and save to a postscript file. However, there is an incompatibility between the *XwinPlot* postscript output and some of the newer Hewlett Packard laser printers. It may be necessary to change the printer setup as described here. Change the printer type from **HP LaserJet 1100** to **PostScript**.

Options → **Printer Setup…**

Printer Type: → **PostScript**

Output Format: Change check box from Black & White to Color, if required.

OK

To print to a file:

File → **Print…** → **To File…**

The user's home directory appears by default, and can be changed if desired, for example to the subdirectory **postscript** to save a postscript file (this is necessary if the batch conversion to PDF described above is to be used).

Selection → *Enter a filename* → **OK**

See section 19.2 above for automatic conversion of postscript files in the **postscript** directory to PDF files.

The PDF or postscript file can then be loaded into *Adobe Illustrator* or *CorelDraw* for further manipulation and to re-save incorporating a bitmap preview.

19.4 Fetching PDF and postscript files

Any SFTP program can be used to transfer the PDF or postscript files to a personal computer. *Fetch* is a good FTP program for Apple computers, and *WinSCP* works well on PCs.

Hostname: **nmrserver.ps.uci.edu**

Use your normal NMR login ID and password.

Your home directory will be opened on nmrserver. To access PDF files, double click on the pdf link. Fetching peak list files

The *XwinNMR* command **pps** lists peaks in a window on screen and also creates a file containing the same information within the current dataset, called **peak.txt**. Note that this file only contains peaks contained within the current *XwinNMR* plot region as defined using **dp1**. The file is also created if a spectrum is plotted using *XwinPlot*.

To fetch this file to your own computer, connect to your home directory on nmrserver as described above.

Then double-click on **NMRdata** to navigate to your NMR data directory and navigate to the processed data directory for the required experiment: *expname*/*expno*/**pdata/***procno*

19.5 Creating diagrams from spectra

High quality PDF spectra can be created from both *XwinNMR* and *TopSpin*. These can be improved further to include structures or annotations or by removing unwanted parameters. It is important to use a program that will edit vector graphics, converting the spectrum to a bitmap graphic will greatly reduce the future editing options.

The most powerful program for editing vector graphics is Adobe *Illustrator*, which is available on the Apple computers within the NMR facility but is expensive to purchase. A free multi-platform alternative package is *Inkscape*.

The NMR Facility Apple computers have *ChemDraw* available for adding structures to spectra. These can be pasted into spectra before saving as PDF/postscript/EPS/svg files.

It is also possible to add postscript objects when printing spectra using *XwinPlot*, if files are first uploaded to nmrserver.

20 Printing spectra via *XwinPlot*

XwinPlot is a comprehensive, WYSIWYG printing package, useful for producing complex overlays and annotated spectra.

Operation is made relatively simple by careful choice of a 'layout' file. This can either be selected via the **edo** menu before starting *XwinPlot,* or by selecting **Open** from the **File** menu within *XwinPlot*.

There are standard Bruker layouts and a selection of custom UCI files. The custom UCI filenames all start with **@**. These will give similar results to the standard *XwinNMR* **plot** formats but allow for more user modifications.

The program can be started from within *XwinNMR* by typing:

xwp ***<enter>***

For a manual containing full information, consult the 'help' menu. The manual can also be downloaded from the ftp server on **nmrserver.ps.uci.edu**.

Data processing should be completed before starting *XwinPlot*. This includes adjustment, if necessary, of **mi** and **pc** to ensure that the desired peak labels are generated.

20.1 1D spectra

20.1.1 Select layout and start *XwinPlot*

Select a suitable plot layout using the **LAYOUT** section of the output devices menu, **edo**. An appropriate layout should already be loaded into this section if the dataset was generated from a standard experiment parameter file.

For a standard proton spectrum select **@h1.xwp**, or for a standard carbon spectrum select **@c13.xwp**. These can either be modified for more complex layouts, or a different file can be selected.

Set the desired plot region using **dp1**.

xwp ***<enter>***

The selected spectral region will appear in the main window. If a proton layout was selected then integral trails and labels will be displayed, if they exist. The title will be shown at the top left.

20.1.2 Enable peak labels and parameters

If *XwinPlot* is started with the command **xwp**, peak labels and parameters should be converted automatically. If this does not happen, or if the peak labels or parameters need to be updated, follow the instructions below.

XwinPlot uses a different format to *XwinNMR* for peak labels and parameters, and so these objects may not initially appear. If these options are desired, they can be enabled via the **XWIN-NMR** menu at the top of the main *XwinPlot* window.

XWIN-NMR → XWIN-NMR Interface…

Select the required command from the row of boxes: **Create Parameter List (xwp_lp)**, **Create Peak List (xwp_pp)** etc. Note that the commands in parentheses, **xwp_pp** etc, can be used within *XwinNMR* to generate these files.

The peak list generated above will only include peaks within the current plot region as defined in *XwinNMR*. Therefore, if peak labels do not appear for the whole of the desired region, redefine the *XwinNMR* plot region with **dp1** in the *XwinNMR* window, then repeat **xwp_pp**. It might also be necessary to modify **pc** or **mi** to ensure the desired peaks are labeled.

Note that if the spectrum has previously been plotted using *XwinNMR*, a parameter list file will exist but will include the plot parameters used for the previous printout. Parameter list files generated for *XwinPlot* do not include the plot parameters.

20.1.3 Select plot region

The desired plot region can either be selected using the mouse or by entering the ppm limits.

With the mouse:

Select 'expand' mode by pressing the ***LMB*** over the **EXPAND** button at the top left of the screen, then drag a box over the spectrum with the ***LMB*** to define the region. To revert to the whole spectrum use **Undo** from the **Edit** menu at the top left of the window. Note that the **ZOOM** button operates in a similar way, but enlarges a region of the layout on screen without affecting the plot settings.

Entering ppm limits:

Position the mouse over the spectrum and press the ***RMB***. This highlights the spectrum window and produces a menu of editing functions. Select **Edit…** with the ***LMB***. The plot limits can be entered as **Xmin, Xmax** in the 'Graph Object

Editor' section. Pressing the ***LMB*** over **OK** will close the editor window and re-draw the spectrum, pressing the ***LMB*** over **Apply** will re-draw the spectrum and leave the editor window open.

20.1.4 Adjust vertical scaling

Position the mouse over the spectrum and press the ***RMB***. This highlights the spectrum window and produced a menu of editing functions. Select **1D/2D-Edit…** with the ***LMB***.

The initial setting is that both spectrum and integral (if applicable) are selected at the top of the window, and so both will be scaled together. It is easier to scale the two objects individually, so first deselect one of them using the ***LMB***. The buttons can then be used to scale and/or move the spectrum or integrals. Next deselect the currently selected object and select the other object using the ***LMB*** then use the menu buttons to scale and/or move the object. Finally press the ***LMB*** over **Close** to close the editor window.

20.1.5 Further adjustments

Both the **Edit…** and **1D/2D-Edit…** menu windows can also be used to turn the axes, peak labels and integral labels on or off. The **Edit…** menu window can be used to change the formats for peak labels and integrals, font types and sizes and line widths etc for all components of the plot layout.

20.1.6 Print spectrum

If the default local printer is to be used:

File → Print… → Print

To print to a postscript file:

File → Print… → To File…

Select **postscript** from the 'Directories' menu, then type the desired filename in the 'Selection' box. Note that if a file with that name exists already, it will be overwritten without warning.

Note: Normally spectra from *XwinPlot* are printed via postscript, and so the same printer driver can be used both to print to paper and save to a postscript file. However, there is an incompatibility between the *XwinPlot* postscript output and the newest Hewlett Packard laser printers (HP LJ2420 series, darker colored). On computers equipped with these printers it is necessary to change the printer setup as described below. Change the printer type from **HP LaserJet 1100** to **PostScript**.

To print to a PDF file:

Options → Printer Setup…

Select **Adobe PDF** from the 'Printer Type:' menu, then:

File → Print… → To File…

Type the desired filename in the 'Selection' box.

To print to a remote printer:

Options → Printer Setup…

The 'Print Command' is normally:

lp –c –s oraw –n1 %s

To print to a remote printer, add the command **–d***printername*. For example, to print to the printer called 'Rychnovsky_lab', change the 'Print Command' to:

lp –c –s oraw –n1 –dRychnovsky_lab %s

Press the ***LMB*** over **OK**, then:

File → Print… → Print

20.1.7 Exit from *XwinPlot*

File → Close All

20.2 2D spectra

20.2.1 Select layout and start *XwinPlot*

Select a suitable plot layout using the **LAYOUT** section of the output devices menu, **edo**. An appropriate layout should already be loaded into this section if the dataset was generated from a standard experiment parameter file.

For a proton-proton 2D spectrum select **@h1h1-2D.xwp**, or for a proton-carbon 2D spectrum select **@h1c13-2D.xwp**. These can either be modified for more complex layouts, or a different file can be selected.

Ensure that processed data exists for the 2D spectrum and the 1D spectra required for the projections, and that all the spectra are correctly calibrated. If **copypars** was used to set up the 2D experiment then the reference parameters should have been set already, if not they can be updated using the **getproj** program. This program assumes that all of the spectra share the same experiment name, and will automatically set the 1D projection spectra experiment names the same as that of the 2D spectrum.

If the experiment name for either (or both) of the 1D projection spectra is different to the experiment name of the 2D spectrum, then should be entered using the command **edc2**. In this dialogue the second dataset corresponds to the F2 (observe) projection in the 2D spectrum, and the third dataset corresponds to the F1 (indirect) dimension. If necessary, update the experiment numbers also, but these should have been stored correctly by **getproj**.

Select the desired number of colors using the ***LMB*** over **#colors** then select an appropriate intensity range. Define the displayed settings as the current plot settings using **DefPlot**.

xwp ***<enter>***

The **xwp** command should ensure that both the 2D spectrum and the correct 1D projection spectra should be loaded automatically. If not, use the commands below.

20.2.2 Select 1D and 2D datasets

Position the mouse over the spectrum and press the ***RMB***. This highlights the spectrum window and produces a menu of editing functions. Select **Edit…** with the ***LMB***.

Scroll to the bottom of the window and then press the ***LMB*** over **Select from Portfolio** to open the 'Data Set Selector'.

Press the ***LMB*** over **Edit** to open the 'Portfolio Editor'. The portfolio is the same as that used within *XwinNMR*, but now the lower section is used for data selection.

Navigate the portfolio to find the required datasets, and select each one in turn by double clicking the ***LMB***. When the 'Portfolio:' section contains the required 1D and 2D datasets, press the ***LMB*** over **Apply** to load these into the *XwinPlot* 'Data Set Selector'.

The spectra may be automatically loaded into the appropriate parts of the *XwinPlot* layout. If necessary, load as follows:

At the bottom of the window: press the ***LMB*** over **Select from Portfolio** to open the 'Data Set Selector' and then highlight the 2D spectrum filename with the ***LMB*** then press the ***LMB*** over **OK**.

In the 'Projection Object Editor' section, press the ***LMB*** over **Top Data Set** to open the 'Data Set Selector' and then highlight the required 1D spectrum filename with the ***LMB*** then press the ***LMB*** over **OK**. Repeat this process for the **Left Data Set**.

20.2.3 Select spectral region

The desired plot region can either be selected using the mouse or by entering the ppm limits.

With the mouse:

Select 'expand' mode by pressing the ***LMB*** over the **EXPAND** button at the top left of the screen, then drag a box over the spectrum with the ***LMB*** to define the region. Both the 2D spectrum and the 1D projections will expand together. To revert to the whole spectrum use **Undo** from the **Edit** menu at the top left of the window. Note that the **ZOOM** button operates in a similar way, but enlarges a region of the layout on screen without affecting the plot settings.

Entering ppm limits:

Position the mouse over the spectrum and press the ***RMB***. This highlights the spectrum window and produces a menu of editing functions. Select **Edit…** with the ***LMB***. The plot limits can be entered as **Xmin, Xmax** and **Ymin, Ymax** in the 'Graph Object Editor' section. Pressing the ***LMB*** over **OK** will close the editor window and re-draw the spectrum, pressing the ***LMB*** over **Apply** will re-draw the spectrum and leave the editor window open.

20.2.4 Adjust contours and projections

Position the mouse over the spectrum and press the ***RMB***. This highlights the spectrum window and produces a menu of editing functions. Select **1D/2D-Edit…** with the ***LMB***.

Edit the three spectra as desired.

20.2.5 Print spectrum

If the default local printer is to be used:

File → Print… → Print

To print to a postscript file:

File → Print… → To File…

Select **postscript** from the 'Directories' menu, then type the desired filename in the 'Selection' box.

Note: Normally spectra from *XwinPlot* are printed via postscript, and so the same printer driver can be used both to print to paper and save to a postscript file. However, there is an incompatibility between the *XwinPlot* postscript output and the newest Hewlett Packard laser printers (HP LJ2420 series, darker colored). On computers equipped with these printers it is necessary to change the printer setup as described below. Change the printer type from **HP LaserJet 1100** to **PostScript**.To print to a PDF file:

Options → Printer Setup…

Select **Adobe PDF** from the 'Printer Type:' menu, then:

File → Print… → To File…

Type the desired filename in the 'Selection' box.

To print to a remote printer:

Options → Printer Setup…

The 'Print Command' is normally:

lp –c –s oraw –n1 %s

To print to a remote printer, add the command **–d***printername*. For example, to print to the printer called 'Rychnovsky_lab', change the 'Print Command' to:

lp –c –s oraw_–n1 –dRychnovsky_lab %s

Press the ***LMB*** over **OK**, then:

File → Print… → Print

20.2.6 Exit from XwinPlot

File → Close All

20.3 Complex plots

There are many ways of adding extra features to *XwinPlot* spectra. Examples are adding an expanded region to a 1D plot, creating a stacked plot, or overlaying two different 2D spectra.

20.3.1 Adding an expanded region of a 1D spectrum

First setup the layout of the main spectrum as described above.

There are two ways of adding a second spectrum to the layout. The simplest would be to press the ***LMB*** over the 1D spectrum mode button on the left of the screen and drag a new window to add a second spectrum. This is not the best method though, because the new spectrum will initially have the *XwinPlot* default settings for line width, character size, etc, which may be different for the settings currently loaded for the initial spectrum. A better method is to make a copy of the first spectrum then expand and move it as required.

Select the spectrum with the ***LMB***, then copy using **Edit → Copy**

A copy of the spectrum is now overlaid on the original. This can be resized and repositioned by dragging the green boxes. To expand a region in the second spectrum, click on **EXPAND** and then drag the required region using the ***LMB***.

20.3.2 1D stack plot

First identify the spectra to be stacked and ensure that all necessary data processing has been performed.

Start *XwinPlot* by typing **xwinplot**.

If required, change the page format from landscape to portrait: **Options** → **Printer Setup…** → **Output Format**

Press the ***LMB*** over the button labeled **Data** at the left hand end of the row across the top of the window.

Edit the 'Data Set Selector' until it includes all the spectra required for the stack plot, and ensure that the datasets are listed in ascending order for the stack. Highlight the first spectrum using the ***LMB***.

Press the ***LMB*** over the stack plot mode selector button - the small illustration of a stack plot on the button at the left side of the window.

Drag a box using the ***LMB*** to a suitable size for the first spectrum. The first spectrum will appear inside this window. Use the ***RMB*** to highlight the window and re-size if necessary. Use the **Edit** menu window to adjust parameters as necessary, then scroll down to the 'Stacked Spectrum Object Editor' section which is near the bottom. Set appropriate values for the number of spectra to be stacked and the x and y offsets. Also press the ***LMB*** over the box at the bottom left to 'Set Curve Attributes to "White Wash"'. This draws the stack such that if spectra overlap, only the front spectrum is shown.

Press the ***LMB*** over **Apply**, and the stack plot will be drawn. If modifications are necessary, adjust the relevant parameters in the **Edit** window and press **Apply** again to update the plot. Finally press **OK** to close the window.

Print the composite spectrum as described above.

20.3.3 Overlaying two 2D spectra

It is possible to overlay two 2D spectra and then print them in different colors or with different line types. Examples could be COSY and NOESY spectra, or HMBC and HMQC spectra.

Set up the plot layout for the first 2D spectrum as described above.

Select the 2D spectrum using the ***RMB*** then press the ***LMB*** over **To Clipboard** from the resultant menu.

From the **Edit** menu at the top left of the screen, press the ***LMB*** over **Get From Clipboard**.

There should now be two identical 2D spectra, one exactly on top of the other. The next step is to change the dataset for one of these to the second experiment.

Select one of the 2D spectra using the ***RMB*** then press the ***LMB*** over **Edit…** from the resultant menu. At the bottom of the menu, press the ***LMB*** over **Select from Portfolio** in the 'Basic NMR Object Editor'. Edit the 'Data Set Selector' as described above if necessary, then select the new 2D spectrum.

The two 2D spectra will now be overlaid, but both will be drawn in the same style. The style(s) can be modified in the '2D Spectrum Object Editor' section of the window. Change one of the 2D spectra to a different color or line style using the 'Level Attributes:' **Negative** and/or **Positive** sections.

Print the spectrum as described above.

21 Instrument specific notes

21.1 DRX400

The DRX400 is normally equipped with a switchable probe and so no tuning of the X-channel is required. The probe can be used to observe ^{1}H, ^{13}C, ^{31}P or ^{19}F. For the automatic switching to work, the probe controller must be set to automatic mode. This is indicated by a light at the bottom of the preamplifier housing, next to the magnet. In automatic mode the light is off, but in the three manual positions the light is on. If the light is on, then press the button repeatedly until it turns off.

If the instrument is in automation mode using *IconNMR* and the automatic sample changer, then manual operation is not possible.

The only parameter sets calibrated for this system are those with names ending in **.q**. Typing **par *<enter>*** will produce a menu of these parameter sets, or they can be selected manually, e.g. by typing **rpar h1.q all *<enter>***

The standard shim file is **qnp**, load using: **rsh qnp *<enter>***

Interference 'spikes' are sometime seen in ^{13}C spectra on this instrument close to 122.3 and 70.3ppm.

The airflow used to eject samples on this instrument is sometimes insufficient to eject NMR tubes equipped with Young's taps. To add extra air flow, open the temperature control window with **edte** and increased the **Gas Flow** using the **+** button. Increase the flow incrementally until the sample is ejected and then return the value to the standard **535** l/h.

21.2 GN500

The magnet on the GN500 has a high rate of field drift. This means that approximately every 13 months the operating frequency of the instrument must be reduced slightly, and that approximately every 4 years the magnetic field must be boosted to its original value.

The field/frequency change requires all the experiment parameter sets to be updated, which means that only the standard, current parameter sets can be guaranteed to work. Users should not save their own parameter files unless they are useful additions to the standard list with the agreement of the facility director.

The only parameter sets calibrated for this system are those with names ending in **.s**. Typing **par *<enter>*** will produce a menu of these parameter sets, or they can be selected manually, e.g. by typing **rpar h1.s all *<enter>***

The GN500 is the only instrument routinely equipped with a nitrogen gas cylinder for sample spinning at low temperatures.

The standard shim file is **bbo**, load using: **rsh bbo *<enter>***

There is currently (June 2009) an intermittent problem where the spectrometer does not switch frequencies correctly for ^{13}C (or other X-nucleus) operation. The symptoms are very similar to when the probe has been left tuned to another nucleus, very poor or no signals are observed.

The most reliable method to achieve correct switching of the carbon channel is as follows:

Set up a new experiment and load the **deptq.s** parameters. Run this experiment, and check that signals are obtained. Then reload the standard carbon experiment and run if required. If this fails the first time, try again. If it still fails check the probe is tuned to carbon.

21.3 CRYO500

This instrument employs a high sensitivity helium cooled cryoprobe. It is an extremely delicate instrument and can only be used to observe ^{1}H, ^{13}C and ^{2}H.

Only use very good quality NMR tubes - the minimum quality is Wilmad 526PP or equivalent (Kontes 897230-0000/New Era NE-MP5-7/Norrell S-5-300-7). This is both the safeguard the instrument and to ensure good data quality. The high performance of the cryoprobe will reveal any defects in the sample and tube.

Check **every** NMR tube for cleanliness and straightness before loading into the magnet.

Exercise extreme care when changing samples. A broken sample means a **minimum of one day of down time** to warm up the probe, remove the sample and cool down the probe. If any sample or glass fragments stick in the probe it will need to be returned to Bruker for servicing. This process could take several weeks.

Try to avoid touching the magnet when changing samples. It is finely balanced on pneumatic supports.

The variable temperature range of the cryoprobe is **0°C to +50°C (273-323K) only**. An air chiller is permanently connected to cool the compressed air supply to access temperatures between 273K and 298K. The chiller should only be turned on when needed, otherwise moisture in the air supply freezes and the ice collected forms a blockage. The full temperature range is accessible with the standard gas flow setting of **670 l/h**.

The cryoprobe has strict power limits. All experiments are automatically checked and an error message will appear if a power level is set too high.

The shim file is called **cryo**, load using: **rsh cryo *<enter>***

The only parameter sets calibrated for this system are those with names ending in **.c**. Typing **par *<enter>*** will produce a menu of these parameter sets, or they can be selected manually, e.g. by typing **rpar h1.c all *<enter>***

All of the most common proton and carbon experiments are currently available.

There is a bug on this system which occasionally results in the lock signal disappearing out of the top of the lock display window, and it cannot be brought back by adjusting the Lock Gain. The problem is that the lock DC offset gets set to +100, whereas the standard value is -71. To fix this: press **[LOCK DC]** on the BSMS keypad and reduce the value from **+100** to **-71** using the knob. The lock signal should now be visible in the normal position towards the bottom of the lock window.

The spectrometer console is only equipped with two radiofrequency channels, whereas the TCI cryoprobe has three. There is a ^{15}N channel which is not normally used. This is not sensitive enough for direct observation but can be used for decoupling or as the indirect dimension in 2D experiments. Special training is required for running these experiments as it involves disconnecting the normal ^{13}C connection and replacing this with ^{15}N. It is vital that the system is always left configured normally for ^{13}C operation.

21.4 AVANCE600

This instrument employs a high sensitivity helium cooled cryoprobe. It is an extremely delicate instrument and can be used to observe almost any nucleus, including ^{19}F, except those in the frequency range from ^{77}Se to ^{153}Eu.

Only use very good quality NMR tubes - the minimum quality is Wilmad 526PP or equivalent (Kontes 897230-0000/New Era NE-MP5-7/Norrell S-5-300-7). This is both the safeguard the instrument and to ensure good data quality. The high performance of the cryoprobe will reveal any defects in the sample and tube.

Check **every** NMR tube for cleanliness and straightness before loading into the magnet.

Exercise extreme care when changing samples. A broken sample means a **minimum of one day of down time** to warm up the probe, remove the sample and cool down the probe. If any sample or glass fragments stick in the probe it will need to be returned to Bruker for servicing. This process could take several weeks.

Try to avoid touching the magnet when changing samples. It is finely balanced on pneumatic supports.

The variable temperature range of the cryoprobe is **0°C to +135°C (273-408K)**. An air chiller is permanently connected to cool the compressed air supply to access temperatures between 273K and 298K. The chiller should only be turned on when needed, otherwise moisture in the air supply freezes and the ice collected forms a blockage. The full temperature range is accessible with the standard gas flow setting of **670 l/h**.

The cryoprobe has strict power limits. All experiments are automatically checked and an error message will appear if a power level is set too high.

The shim file is called **cryo**, load using: **rsh cryo *<enter>***

See Chapter 29 on operation using *TopSpin* and the advanced features of this instrument.

22 Data archiving and management

The NMR data server was upgraded in August 2017. The new server has much higher capacity and so processed data is no longer regularly deleted. Each spectrum contains 'real' and 'imaginary' data files. These are both required until the spectrum is phase corrected, but the 'imaginary' files are then no longer needed. These files are deleted when they are four weeks old. Therefore, it may occasionally be necessary to re-process data to recreate these files if phase correction needs to be updated.

All raw data is automatically copied to a backup server every night, and a secondary, incremental, backup is performed weekly. The primary backup is located in Reines Hall, the secondary backup is in Natural Sciences 1.

When users leave UCI, their accounts are removed and their data is archived. Files are deleted from the main data server but remain both backup systems. Data can easily be reinstated if it is required by current researchers or for publication. Older files may be compressed into archives, see the Facility Director for assistance with these.

22.1 Deleting processed data

Both spectra (processed data) and FIDs (raw data) are stored on disk by *XwinNMR*, and so it is necessary to ensure that the spectra are deleted before archiving the data. Until 2017, spectra were automatically regularly, and so old data directories (experiment names) may only contain raw data.

delp ***<enter>***

Delete processed data. Either select **delete_all** (this is slow, as all files are checked) or highlight files to delete with the ***LMB*** then delete with **execute**.

Note that wildcards can be used to produce a smaller menu more quickly, e.g. **delp a*** ***<enter>*** will list all experiments with names starting with 'a'.

22.2 Deleting raw data

Note that deleted files cannot be recovered, so data will be lost unless it has previously been copied to the backup server. Either complete experiment names, or individual acquired data sets can be deleted as follows:

del ***<enter>***

Delete dataset.

Select the required experiment name(s) for deletion with the ***LMB*** followed by **execute**. Wildcards can be used to produce a smaller menu more quickly, e.g. **del a*** ***<enter>*** will list all experiment names starting with 'a'. In this case, **delete_all** can be used to delete all experiment names on the current menu.

dela ***<enter>***

Delete acquired data.

The default option is to delete data but to preserve parameters.

mode

Change to '**deleting data+param.**'

Select the required experiment name(s) for deletion with the ***LMB*** followed by **execute**.

Wildcards can be used to produce a smaller menu more quickly, e.g. **dela a*** ***<enter>*** will list all experiment names starting with 'a'. In this case, **delete_all** can be used to delete all the experiments names on the current menu.

22.3 Data transfer over the network

Data can be copied over the network by FTP (file transfer protocol). Many FTP programs are available for Macs and PCs. A good graphical interface is useful for selecting files, and it is essential that the program will fetch directories and their contents, rather than just individual files. A good program for an Apple computer is *Fetch*, and *WinSCP* is one of the better PC utilities.

Hostname: **nmrserver.ps.uci.edu**

Use your normal NMR login ID and password.

Your home directory will be opened on nmrserver. To access NMR data, double click on the link directory called **NMRdata**.

Ensure that secure FTP (SFTP) is selected in the transfer program, then select and copy NMR data as required. The reverse process can be used to return data to the NMR system, if necessary.

22.4 Archiving data

Note: *Archiving data to CDs or DVDs is no longer recommended as these disks can degrade over time. NMR files are relatively small and large disk drives are now inexpensive. The safest location for data is a current backup system on a hard drive or solid-state storage. Regular use of the backup device is likely to show warnings of any impending hardware failure.*

The information below has thus been largely superseded, but may be useful for reloading old data into the NMR system.

The most convenient medium for archiving data is CDROM. DVDs have a greater capacity, but the NMR workstations are not equipped with DVD readers, and so data can only be restored from DVD to the NMR server via a remote connection.

The standard CDROM format, ISO9660, places restrictions on filename length and case and the depth of the directory structure. There are extensions to the standard to work around these restrictions, but they are not platform independent. Unless a multi-format CDROM is deliberately made, the disk may only be readable on the type of computer on which it was created.

Many programs, for example *Toast*, allow the creation of Apple/PC hybrid disks, but these may not be accessed fully by Unix computers. The best solution is to create a fully multiplatform ISOimage file for the CDROM on a Silicon Graphics computer in the NMR facility which is then transferred to a computer with a fast CDROM writer to burn the disk. The NMR Facility Director can assist with this operation.

Note that CDROMs must be handled very carefully to maintain the integrity of the stored data. Only label disks with pens designed for the purpose, and never use self-adhesive labels.

22.5 Reinstating data on nmrserver

In order to re-process archived data, it must first be copied back onto the NMR data server. If the data is stored on a CDROM, then this operation can either be performed remotely over the network, or locally using the SGI computer CDROM drive.

Reinstating data is simpler remotely over the network rather than directly on the NMR facility SGI computers. This is because the Unix file permissions for data copied from CDROM are 'read only' because a fixated CDROM is a read only medium. The file permissions must be changed to 'read and write' before data can be processed. If data is replaced over the network the permissions will automatically be correct.

The CD drives on the SGI computers are also rather slow compared to drives on more modern personal computers.

22.5.1 Reinstating data remotely

Insert the NMR data CDROM into your own computer, or open the directory containing the NMR data if it is on a hard drive.

Open an SFTP connection to the NMR data server using a program such as *Fetch* (Apple) or *SmartFTP* (PC).

Hostname: **nmrserver.ps.uci.edu**

Use your normal NMR login ID and password.

Your home directory will be opened on nmrserver. To open your NMR data directory, double click on the link directory called **NMRdata**.

Select and copy NMR data as required.

22.5.2 Reinstating data locally

Load the CDROM into the drive on the top of one of the NMR facility SGI workstations.

Data can either be copied using typed commands in a Unix shell or using the Graphical User Interface. For simplicity the GUI will be described here.

Double click on the CDROM icon on the SGI desktop to view the CDROM contents and navigate to the required experiment name. Open a directory viewer for your data directory:

Either double click on a directory icon on the desktop or open a new directory from the **Toolchest**:

Desktop → Access Files → In my Home Directory

and change the pathname to **/v/data/***username***/nmr**.

Select the required experiment name(s) in the CDROM directory viewer. These can be copied by 'drag and drop' to the NMR data directory viewer. The default action when dragging files between viewers is to move the files. An error message will appear asking if the files should be copied instead, as they cannot be deleted from the CDROM. This error message can be avoided by holding down ***<Ctrl>*** when dragging the files to select copying rather than moving.

In order to process the data, the file permissions must be changed to read and write:

Select the imported data then choose **Change Permissions…** from the **Selected** menu.

Click on the **write** button in the **User** section and click on the button labeled **Apply changes to contents of directories** in the bottom left of the window. Finally click on **Apply**.

22.6 Renaming NMR data files

This can be performed within *XwinNMR*, but is much easier in a Unix shell.

First open a shell from the **Toolchest**:

Desktop → Open Unix Shell

Then type **cddata** to move to your data directory.

The command **ls** will list all of your experiment names, or wildcards can be used to select a range of names, in which case **ls -d** should be used, e.g. **ls -d a***

To rename (move) a dataset, simply type (or copy and paste using ***LMB*** and ***MMB***):

mv *oldexpno newexpno*

If a 2D dataset has been renamed with its corresponding 1D files, it will be necessary to re-run **getproj** in the 2D file to update the experiment names for the 1D projection spectra.

Within *XwinNMR* the command **ren** can be used to rename files.

Alternatively, the *Irix* GUI can be used. This method may be necessary if the current experiment name starts with, or contains characters which are problematic for the Unix file system.

Toolchest → Desktop → Access Files → In My Home Directory

Then double click on **NMRdata** to access your data directory.

A similar method can be used with the CentOS GUI on the *TopSpin* computers.

22.7 Data belonging to departed users

The directory on nmrserver.ps.uci.edu for current users is **/v/data/*username*/nmr**

Data belonging to all users, past and present, can be found in **/w/data/*username*/nmr**

Data from any user can be copied from the server, but only a user's own data can be modified. All data acquired since 1999 should be available, but some older accounts have been compressed into archives. The Facility Director can assist with accessing files as required.

23 Reserving time on the NMR instruments

The nmr booking program runs on the nmrserver computer, so it is necessary to open a secure connection to that computer and then run the program.

23.1 Connecting to nmrserver

If you are using a wireless connection at UCI, using 'UCInet Mobile Access', you may need to use a VPN program in order to connect to the NMR computers. If you have a static IP address configured on either of the NMR subnets (128.200.31.* or 128.200.228.*), but this is not active, you may also not be able to connect unless you deactivate the wired ethernet connection in your network preferences.

23.1.1 From a Silicon Graphics computer in the NMR facility

This is the simplest remote connection, as the same login ID is used on the NMR workstations and on nmrserver, and the computers share the same local network.

Toolchest → **NMR** → **Reserve NMR time**

Enter your password to connect to nmrserver.

23.1.2 From a remote Unix computer (Linux, SGI, Sun, etc)

Obtain a Unix command prompt as above for an SGI computer, or as appropriate for other systems. The local user ID is likely to be different from the NMR user ID, so the NMR user ID must be specified along with the full hostname of nmrserver.

ssh *NMRuserID*@**nmrserver.ps.uci.edu** ***<enter>***

It may not be necessary to include the '.uci.edu' part of the host name if connecting from within UCI.

23.1.3 From an Apple computer

23.1.3.1 Automatic connection

A script is available to automatically set up a clickable file to connect to nmrserver.

Use *Fetch* or an alternative sFTP client to connect to nmrserver.ps.uci.edu

Click on the link **MacScripts**, download the file **MacScripts.zip**, and decompress it to find **setup_ssh_to_nmrserver.command**

Place this file in the directory from where you would like to run it, for example your desktop.

Control-click on the file, then select **Open** to run it. Your NMR login ID will be requested and incorporated into the file that is created in the same directory. Double-click on the this file to connect to nmrserver by ssh. Your NMR password will be requested.

If necessary change the permissions as follows. Press **Command-I** over the file, then click the arrow next to **Sharing & Permissions** to expand the section. Click the pop-up menu next to your user name to see the permissions settings. If you're not logged in as an administrator, you may need to click the lock to unlock it, then enter an administrator name and password. Change the permissions to **Read & Write**.

The script will delete itself when it has completed. If you want to run it again you'll either need to keep a copy or repeat the download.

23.1.3.2 Manual connection

Open a Terminal window:

Finder → **Go** → **Utilities** → **Terminal**

Connect to nmrserver:

ssh *NMRuserID*@**nmrserver.ps.uci.edu** ***<enter>***

It may not be necessary to include the '.uci.edu' part of the host name if connecting from within UCI.

These settings can be saved as a command file as follows.

Create a plain text (not rich text) file containing the connection command using any text editor, e.g. *TextEdit*:

ssh *NMRuserID*@**nmrserver.ps.uci.edu**

If running a version of OS X prior to 10.4, save this file with the extension '.command', e.g. **nmrserver.command**

Using OS X 10.4, and newer, it is sufficient to press ***Command*** (Apple Key) and ***i*** together and specify that the file should be opened by the *Terminal* application. It is also not necessary to use the extension '.command'.

Finally change the file permissions so that it is executable. Open a Terminal window and navigate to the directory containing the new file, then change the file permissions to **-rwxr--r--**, e.g.

chmod 744 nmrserver.command *<enter>*

A simple script file can also be used to allow access by several users. Contact the Facility Director for details.

23.1.4 From a *Windows* PC

An external program is required to provide a secure shell connection to a remote computer. The *cygwin* Linux-like package includes the **ssh** command, so if this is installed the commands listed in section 23.1.2 can be used. Alternatively, a *Windows* program called *putty* can be used.

Putty is a free download from: http://www.chiark.greenend.org.uk/~sgtatham/putty/download.html or search at *Google* if it has moved. It is a stand-alone program and so very easy to install.

The important settings are as follows:

Host name: **nmrserver.ps.uci.edu**

Protocol: **SSH**

Once entered, these settings can be saved to a file and simply double-clicked on in future. It is also possible to save settings for window size and color, font, etc.

Press **Open** to open a connection and then enter login ID and password into the dialogue box.

23.1.5 From a mobile device

Any phone or tablet device that runs a secure shell (ssh) terminal application can be used to connect to nmrserver. The available options change frequently, so a good approach is to search the relevant App Store for 'ssh client'.

The following are examples:

iOS apps: *Terminus*, *Shelly*, *Mobile Admin*, *WebSSH*, *Reflection*

Android apps: *Terminus, ConnectBot*, *JuiceSSH*

23.2 Running the UCI booking program

Type **go *<enter>*** from a desktop computer, or **mo *<enter>*** from a mobile device, to start the program.

The first screen displays information about the current status of the NMR spectrometers (**go** only).

Press ***<enter>*** twice to reach a menu of NMR spectrometers.

Select an instrument number, then press ***<enter>*** or type **0** to start the scheduler display with the current hour and skip the next two questions, or type **1** to specify start day and hour. (**go** only)

Type **0 *<enter>*** (or just ***<enter>***), or **1 *<enter>*** to select today or tomorrow (**go** only).

Enter a starting hour for the schedule display, any number from **0** to **23** (**go** only, **mo** starts with the current hour).

The display should now display reservations for the selected instrument for an 8 hour (if **go** was used) or 2 hour (if **mo** was used) period, starting at the requested hour. If another user is currently accessing this same time period for the same instrument the following message will appear:

File in use; Auto-try in 10 sec Or ctrl/C to exit

Only one user can access a particular booking period at any time. If no keys are pressed for 60 seconds the program will timeout to stop other users from being obstructed.

Press ***r*** to start a reservation. Then enter the time for the first ten minute slot, followed by the number of slots desired. The chosen time will be labeled with the user's login ID on the schedule. If too much time is requested, either according to the booking rules for the selected instrument at the chosen time of day, or according to the user's weekly quota, then the maximum time allowed will be reserved.

Press ***d*** to delete a reservation. Then enter the time for the first ten minute slot and select whether to delete all or part of the reservation,

Press ***f*** to shift the period of time displayed forwards by eight hours.

Press ***b*** to shift the period of time displayed backwards by eight hours.

Press ***w*** to find out the full name, research group and email address for any login ID.

Press ***q*** to quit from the booking program.

The last two options both offer the opportunity to make further reservations on different instruments rather than exiting from the program.

It is essential that the program is exited via the ***q*** or ***w*** to ensure that reservations are stored in the system.

23.3 Booking Rules

The current rules for reserving time on spectrometers - how much time can be reserved on each machine at different times of the day, and how long in advance reservations can be made - can be downloaded from nmrserver. In the *NMRmanuals* directory there is a file called 'BookingRules.pdf'. The current rules should also be displayed on the wall in the NMR facility close to the door. The rules at the time of writing this manual are listed in Appendix J.

23.4 Checking instrument and computer status

Connect to nmrserver as described above and then type:

status *<enter>*

The display will list current local users of the spectrometer and datastation computers. Note that this is not a 'live' display, but is compiled from files saved every minute listing the current users of each workstation.

24 Processing data from the Biomolecular NMR Facility

Data recorded on the Varian 800MHz NMR spectrometer in the Biomolecular NMR Facility can be imported into *XwinNMR* and converted to Bruker format for processing. The methods described below work for most of the standard experiments, as currently performed. See the Chemistry facility manager for assistance in case of problems. Similar methods can probably be used to import data into *TopSpin*, but this has not been tested. One difference is that just **s** is required to inspect or change status parameters in *TopSpin*, compared to **2s** or **1s** in *XwinNMR*.

24.1 Importing data to the Chemistry NMR Facility

Data must be copied from the Biomolecular NMR Facility to the user's data directory in the Chemistry NMR Facility. This can be performed by the facility director, or by the user if they have login access to the Biomolecular NMR Facility. Secure file copying must be used rather than standard FTP.

For example, when logged onto the 800MHz spectrometer computer navigate to the directory containing the data to be transferred and then type:

scp -r *variandataset.fid username*@**nmrserver.ps:/v/data/***username***/nmr/. <enter>**

The italicized items should be replaced by the Varian dataset name and the user's login ID in the Chemistry NMR Facility. If desired, a new name can be specified for the copy of the dataset, instead of the '**.**' at the end of the command.

24.2 Conversion of data to *XwinNMR* format

The *XwinNMR* command **vconv** can be used to convert Varian data to Bruker format for processing using *XwinNMR*. If there is an *XwinNMR* parameter set name which matches the value of the Varian parameter 'seqfil', then those parameters are automatically loaded during data conversion. If not, default 1D or 2D parameters will be used. In both cases some manual adjustment of parameters is required.

The **vconv** command can either be used alone to produce a menu of datasets, or followed by the required dataset name. The Varian datasets often have rather long names with the extension **.fid**. Only the first fifteen characters of the name will be displayed in the menu produced by **vconv**, which can make it difficult to distinguish between a series of files with similar names. This problem can be avoided by specifying the dataset name following the **vconv** command. This can be copied from a unix shell to avoid typing the full name.

Open a unix shell:

Desktop → **Open Unix Shell**

Then type:

cddata *<enter>*

to navigate to your *XwinNMR* data directory. List only the Varian dataset names by typing:

ls -d *.fid *<enter>*

Highlight the required dataset using the ***LMB***, then move the mouse pointer back over the *XwinNMR* window. Type:

vconv then press the ***MMB*** to paste the dataset name before pressing ***<enter>***.

The computer will then request an *XwinNMR* experiment name for the converted data, followed by an experiment number, disk name and username. The default answers for the last two should be correct.

Note that **vconv** does not warn the user if the specified *XwinNMR* experiment name and/or number exist already. If the files do exist, then the existing files will be overwritten. The converted data is not displayed automatically, but must be loaded using the standard *XwinNMR* commands, e.g. via the portfolio using **search**.

24.2.1 1D proton data

If the Varian parameter 'seqfil' has one of the following values: 't2pul_lek_800', 'wgate_echo_pln', 'water' or 'presat'; then most parameters will automatically set to useful values during data conversion.

One parameter must be manually adjusted: **si** (the size of the Fourier transform). First check the value of **td** (the number of complex datapoints recorded). **si** must be a power of 2, and should be a minimum of **td/2**. Typical values for **si** are **64k** (65536) or **128k** (131072).

It is a good idea to check the value of the line broadening parameter, **lb**, which is normally set to **0.3** Hz.

To automatically reference the spectrum, the solvent status parameter must be manually set to the correct solvent name:

2s solvent *solventname* ***<enter>*** e.g. **2s solvent CDCl3T *<enter>***

Not all solvents will reference correctly. Note the initial value for **sr** before issuing the **sref** command so that it can be reset if referencing fails. So far, solvent names **C6D6**, **CDCl3**, **CDCl3T**, **D2O**, **D2OT**, **DMSO** and **THF** have been tested.

The Varian data includes a text file which is converted to the *XwinNMR* title file. This contains many experimental details, often including the Varian experiment type, 'seqfil'. The title file should be edited using **setti** so that only important details are retained and so that the text is on 2 or 3 lines only. *XwinNMR* shrinks the title characters to fit the required lines of text into the title window, so any more than three lines of text will be very small.

If the Varian parameter 'seqfil' does not have one of the values listed above, then several parameters will have unsuitable values, including the plotter device name. If a non-existent plotter is listed, then the **vplot** command will fail. Consult the Chemistry NMR Facility Director for assistance in converting the data. A new parameter set can be created to automatically convert future datasets of the same experiment type.

24.2.2 1D carbon data

If the Varian parameter 'seqfil' has the value 's2pul' most parameters will automatically set to useful values during data conversion.

One parameter must be manually adjusted: **si** (the size of the Fourier transform). First check the value of **td** (the number of complex datapoints recorded). **si** must be a power of 2, and should be a minimum of **td/2**. Typical values for **si** are **64k** (65536) or **128k** (131072).

To automatically reference the spectrum, the solvent status parameter must be manually set to the correct solvent name:

2s solvent *solventname* ***<enter>*** e.g. **2s solvent CDCl3T** ***<enter>***

Not all solvents will reference correctly. Note the initial value for **sr** before issuing the **sref** command so that it can be reset if referencing fails. So far, solvent names **C6D6** and **THF** have been tested.

The Varian data includes a text file which is converted to the *XwinNMR* title file. This contains many experimental details, often including the Varian experiment type, 'seqfil'. The title file should be edited using **setti** so that only important details are retained and so that the text is on 2 or 3 lines only. *XwinNMR* shrinks the title characters to fit the required lines of text into the title window, so any more than three lines of text will be very small.

If the Varian parameter 'seqfil' does not have the value 's2pul', then several parameters will have unsuitable values, including the plotter device name. If a non-existent plotter is listed, then the **vplot** command will fail. Consult the Chemistry NMR Facility Director for assistance in converting the data. A new parameter set can be created to automatically convert future datasets of the same experiment type.

24.2.3 2D COSY data

Most COSY experiments performed on the Varian 800MHz spectrometer of the double quantum filtered (DQF) type. If the Varian parameter 'seqfil' is either 'dqfcosyn_pln', 'gcosy' or 'DQCOSY' most parameters will automatically set to useful values during data conversion. The following adjustments must be made manually.

First ensure that the observation frequencies are correct in both dimensions. Type:

2s sfo1 ***<enter>***

The observation frequency will appear in the CPR window. Press ***<enter>***. Next type:

1s sfo1 ***<enter>***

This probably has a different value to **2s sfo1**, but it should be identical. Type the correct value, which should still be visible in the CPR window, and press ***<enter>***.

Next check how many datapoints were recorded in each FID, and how many experiments were recorded in the two dimensional array. Type:

2s td ***<enter>*** and write down the value. This is the number of complex datapoints in each FID. Next type:

1s td ***<enter>*** and write down the value. This is the number of FIDs in the 2D array.

The next changes should be made in the processing parameter editor. Type:

edp ***<enter>*** to open the editor.

First set the two values for **SI** to suitable values. Normally both F2 and F1 values should be set to half of the value recorded above for **2s td**.

Ensure that **SF** has the same value in both F2 and F1 columns. The F2 value should initially be correct.

If the experiment is not phase sensitive, change the setting for **PH_mod** to **no** in the F2 dimension and **mc** in the F1 dimension. If it is phase sensitive, set **PH_mod** to **no** in both columns.

SAVE the parameter settings.

The Varian data includes a text file which is converted to the *XwinNMR* title file. This contains many experimental details, often including the Varian experiment type, 'seqfil'. The title file should be edited using **setti** so that only important details are retained and so that the text is on 2 or 3 lines only. *XwinNMR* shrinks the title characters to fit the required lines of text into the title window, so any more than three lines of text will be very small.

Use **getproj** to set the 1D projection information, then process the 2D data in the usual manner.

If the Varian parameter 'seqfil' does not have either of the values listed above, then several parameters will have unsuitable values, including the plotter device name. If a non-existent plotter is listed, then the **vplot** command will fail. If the processing parameters are not set correctly, then the spectrum is likely to appear very distorted. Consult the Chemistry NMR Facility Director for assistance in converting the data. A new parameter set can be created to automatically convert future datasets of the same experiment type.

24.2.4 2D NOESY data

If the Varian parameter 'seqfil' has one of the following values: 'gte_noesy', 'noesyh2o_pfg_800_pln', 'wgnoesy' or 'wgnoesy_bn'; most parameters will automatically set to useful values during data conversion. The following adjustments must be made manually:

First ensure that the observation frequencies are correct in both dimensions. Type:

2s sfo1 ***<enter>***

The observation frequency will appear in the CPR window. Press ***<enter>***. Next type:

1s sfo1 ***<enter>***

This probably has a different value to **2s sfo1**, but it should be identical. Type the correct value, which should still be visible in the CPR window, and press ***<enter>***.

Next check how many datapoints were recorded in each FID, and how many experiments were recorded in the two dimensional array. Type:

2s td ***<enter>*** and write down the value. This is the number of complex datapoints in each FID. Next type:

1s td ***<enter>*** and write down the value. This is the number of FIDs in the 2D array.

The next changes should be made in the processing parameter editor. Type:

edp ***<enter>*** to open the editor.

First set the two values for **SI** to suitable values. Normally both F2 and F1 values should be set to half of the value recorded above for **2s td**.

Ensure that **SF** has the same value in both F2 and F1 columns. The F2 value should initially be correct.

Change the setting for **PH_mod** to **no** in both F2 and F1 columns.

SAVE the parameter settings.

The Varian data includes a text file which is converted to the *XwinNMR* title file. This contains many experimental details, often including the Varian experiment type, 'seqfil'. The title file should be edited using **setti** so that only important details are retained and so that the text is on 2 or 3 lines only. *XwinNMR* shrinks the title characters to fit the required lines of text into the title window, so any more than three lines of text will be very small.

Use **getproj** to set the 1D projection information, then process the 2D data in the usual manner.

The plot parameters will be initially set to print positive contour levels as broken lines and negative contour levels as solid lines. If the spectrum is phased to produce a positive diagonal, the sign of the NOESY cross-peaks depends on the molecular size. For small molecules, less than 1000 molecular weight, the NOESY cross-peaks are negative, whereas for large molecules, greater than 2000 molecular weight, the NOESY cross-peaks are positive. For large molecules it will therefore be necessary to change the plot parameters. The line type for solid lines is **0** and the standard broken line type is **1.01**. Full instructions can be found in chapter 6.

If the Varian parameter 'seqfil' does not have any of the values listed above, then several parameters will have unsuitable values, including the plotter device name. If a non-existent plotter is listed, then the **vplot** command will fail. If the processing parameters are not set correctly, then the spectrum is likely to appear very distorted. Consult the Chemistry NMR Facility Director for assistance in converting the data. A new parameter set can be created to automatically convert future datasets of the same experiment type.

24.2.5 2D ROESY data

If the Varian parameter 'seqfil' has the following value: 'wgroesy'; most parameters will automatically set to useful values during data conversion. The following adjustments must be made manually:

First ensure that the observation frequencies are correct in both dimensions. Type:

2s sfo1 ***<enter>***

The observation frequency will appear in the CPR window. Press ***<enter>***. Next type:

1s sfo1 ***<enter>***

This probably has a different value to **2s sfo1**, but it should be identical. Type the correct value, which should still be visible in the CPR window, and press ***<enter>***.

Next check how many datapoints were recorded in each FID, and how many experiments were recorded in the two dimensional array. Type:

2s td ***<enter>*** and write down the value. This is the number of complex datapoints in each FID. Next type:

1s td ***<enter>*** and write down the value. This is the number of FIDs in the 2D array.

The next changes should be made in the processing parameter editor. Type:

edp ***<enter>*** to open the editor.

First set the two values for **SI** to suitable values. Normally both F2 and F1 values should be set to half of the value recorded above for **2s td**.

Ensure that **SF** has the same value in both F2 and F1 columns. The F2 value should initially be correct.

Change the setting for **PH_mod** to **no** in both F2 and F1 columns.

SAVE the parameter settings.

The Varian data includes a text file which is converted to the *XwinNMR* title file. This contains many experimental details, often including the Varian experiment type, 'seqfil'. The title file should be edited using **setti** so that only important details are retained and so that the text is on 2 or 3 lines only. *XwinNMR* shrinks the title characters to fit the required lines of text into the title window, so any more than three lines of text will be very small.

Use **getproj** to set the 1D projection information, then process the 2D data in the usual manner.

If the Varian parameter 'seqfil' does not have the value listed above, then several parameters will have unsuitable values, including the plotter device name. If a non-existent plotter is listed, then the **vplot** command will fail. If the processing parameters are not set correctly, then the spectrum is likely to appear very distorted. Consult the Chemistry NMR Facility Director for assistance in converting the data. A new parameter set can be created to automatically convert future datasets of the same experiment type.

24.2.6 2D TOCSY data

If the Varian parameter 'seqfil' has the value 'wgtocsy', 'wgtocsy_bn' or 'pswgtocsy_bn', most parameters will automatically set to useful values during data conversion. The following adjustments must be made manually:

First ensure that the observation frequencies are correct in both dimensions. Type:

2s sfo1 ***<enter>***

The observation frequency will appear in the CPR window. Press ***<enter>***. Next type:

1s sfo1 ***<enter>***

This probably has a different value to **2s sfo1**, but it should be identical. Type the correct value, which should still be visible in the CPR window, and press ***<enter>***.

Next check how many datapoints were recorded in each FID, and how many experiments were recorded in the two dimensional array. Type:

2s td ***<enter>*** and write down the value. This is the number of complex datapoints in each FID. Next type:

1s td ***<enter>*** and write down the value. This is the number of FIDs in the 2D array.

The next changes should be made in the processing parameter editor. Type:

edp ***<enter>*** to open the editor.

First set the two values for **SI** to suitable values. Normally both F2 and F1 values should be set to half of the value recorded above for **2s td**.

Ensure that **SF** has the same value in both F2 and F1 columns. The F2 value should initially be correct.

Change the setting for **PH_mod** to **no** in both F2 and F1 columns.

SAVE the parameter settings.

The Varian data includes a text file which is converted to the *XwinNMR* title file. This contains many experimental details, often including the Varian experiment type, 'seqfil'. The title file should be edited using **setti** so that only important

details are retained and so that the text is on 2 or 3 lines only. *XwinNMR* shrinks the title characters to fit the required lines of text into the title window, so any more than three lines of text will be very small.

Use **getproj** to set the 1D projection information, then process the 2D data in the usual manner.

If the Varian parameter 'seqfil' does not have any of the values listed above, then several parameters will have unsuitable values, including the plotter device name. If a non-existent plotter is listed, then the **vplot** command will fail. If the processing parameters are not set correctly, then the spectrum is likely to appear very distorted. Consult the Chemistry NMR Facility Director for assistance in converting the data. A new parameter set can be created to automatically convert future datasets of the same experiment type.

24.2.7 2D HMQC data

If the Varian parameter 'seqfil' has the value 'gHMQC' most parameters will automatically set to useful values during data conversion. The following adjustments must be made manually:

First check how many datapoints were recorded in each FID, and how many experiments were recorded in the two dimensional array. Type:

2s td ***<enter>*** and write down the value. This is the number of complex datapoints in each FID. Next type:

1s td ***<enter>*** and write down the value. This is the number of FIDs in the 2D array.

The next changes should be made in the processing parameter editor. Type:

edp ***<enter>*** to open the editor.

First set the two values for **SI** to suitable values. Normally both F2 and F1 values should be set to half of the value recorded above for **2s td**.

Ensure that the setting for **PH_mod** is **no** in both F2 and F1 columns.

SAVE the parameter settings.

The Varian data includes a text file which is converted to the *XwinNMR* title file. This contains many experimental details, often including the Varian experiment type, 'seqfil'. The title file should be edited using **setti** so that only important details are retained and so that the text is on 2 or 3 lines only. *XwinNMR* shrinks the title characters to fit the required lines of text into the title window, so any more than three lines of text will be very small.

Use **getproj** to set the 1D projection information, then process the 2D data in the usual manner. Note that this is a phase-sensitive HMQC experiment, and all peaks should be phased to be positive.

Manual calibration can be performed using **calibrate**. The F2 (proton) dimension may already be correct, but the F1 (carbon) dimension will probably need correction.

If the Varian parameter 'seqfil' does not have the value listed above, then several parameters will have unsuitable values, including the plotter device name. If a non-existent plotter is listed, then the **vplot** command will fail. If the processing parameters are not set correctly, then the spectrum is likely to appear very distorted. Consult the Chemistry NMR Facility Director for assistance in converting the data. A new parameter set can be created to automatically convert future datasets of the same experiment type.

24.2.8 2D HMBC data

If the Varian parameter 'seqfil' has the value 'gHMBC' most parameters will automatically set to useful values during data conversion. The following adjustments must be made manually:

First check how many datapoints were recorded in each FID, and how many experiments were recorded in the two dimensional array. Type:

2s td ***<enter>*** and write down the value. This is the number of complex datapoints in each FID. Next type:

1s td ***<enter>*** and write down the value. This is the number of FIDs in the 2D array.

The next changes should be made in the processing parameter editor. Type:

edp ***<enter>*** to open the editor.

First set the two values for **SI** to suitable values. Normally both F2 and F1 values should be set to half of the value recorded above for **2s td**.

Ensure that the setting for **PH_mod** is **no** in the F2 and **mc** in the F1 column.

SAVE the parameter settings.

The Varian data includes a text file which is converted to the *XwinNMR* title file. This contains many experimental details, often including the Varian experiment type, 'seqfil'. The title file should be edited using **setti** so that only important

details are retained and so that the text is on 2 or 3 lines only. *XwinNMR* shrinks the title characters to fit the required lines of text into the title window, so any more than three lines of text will be very small.

Use **getproj** to set the 1D projection information, then process the 2D data in the usual manner.

Manual calibration can be performed using **calibrate**. The F2 (proton) dimension may already be correct, but the F1 (carbon) dimension will probably need correction.

If the Varian parameter 'seqfil' does not have the value listed above, then several parameters will have unsuitable values, including the plotter device name. If a non-existent plotter is listed, then the **vplot** command will fail. If the processing parameters are not set correctly, then the spectrum is likely to appear very distorted. Consult the Chemistry NMR Facility Director for assistance in converting the data. A new parameter set can be created to automatically convert future datasets of the same experiment type.

25 Processing data using local software

Various software packages are available for processing NMR data, and they should all be able to import data in the Bruker *XwinNMR* format. Data can be imported to a personal computer by secure FTP (SFTP) connection to **nmrserver.ps.uci.edu**. Follow the **NMRdata** link in your home directory. Step-by-step instructions for *TopSpin* installation can be found in Appendix K.

25.1 Downloading data to a user's own computer

A secure FTP client is required, below are some recommended options.

25.1.1 MacOS

UCI has a license for an FTP program called *Fetch*. The method for obtaining this has recently changed.

Download the latest version from the Fetch website: **http://www.fetchsoftworks.com/**

Install as instructed, and it will function for 15 days without a license.

Run *Fetch* and then follow these steps.

Hostname: costello.ps.uci.edu

User name: NMR loginID

Connect using: **SFTP**

Password: NMR password

OK

Navigate to **TopSpinDownloads/Fetch**

Then download the license information. This should be valid for Fetch version 5. If you have problems, you can obtain the license from OIT by opening a ticket at - https://uci.service-now.com/sp?id=kb_article&sys_id=d7552e49dbfaba048427fb671d96192b&sysparm_category=7987752cdb67a700712f389f9d961995

To fetch NMR data use these settings:

Hostname: nmrserver.ps.uci.edu

User name: NMR loginID

Connect using: **SFTP**

Password: NMR password

OK

Click on **NMRdata** link.

25.1.2 Windows or Mac

Cyberduck can be downloaded from https://cyberduck.io/

Install and run *Cyberduck*, then follow these steps:

Open Connection

SFTP (SSH File Transfer Protocol)

Server: nmrserver.ps.uci.edu

Port: 22

Username: NMR loginID

Password: NMR password

Connect

Click on **NMRdata** link.

25.1.3 Windows

WinSCP can be downloaded from **https://winscp.net**

Install and run *WinSCP*, then follow these steps.

Select **New Site**, then

File protocol: SFTP

Host name: nmrserver.ps.uci.edu

Port number: 22

User name: NMR loginID

Password: NMR password

Save and give these settings a name if desired.

Login to make the connection.

Click on **NMRdata** link.

FileZilla is a very capable alternative, but is more complicated to use.

25.2 Bruker *TopSpin* (MacOS, Linux and Windows)

Use of *TopSpin* for data processing is free for academia:

Local copies of the Bruker installation files for Mac, Linux and Windows are available for download from **costello.ps.uci.edu**. The UCI VPN must be used for off-campus access to **costello.ps.uci.edu**

Connect by secure FTP (SFTP) and follow the link to **TopSpinDownloads** from your home directory. This contains two subdirectories, **BrukerDownloads** contains the program files and **UCIfiles** contains the same local extra files as mentioned below. Within **BrukerDownloads** there are subdirectories for the currently available versions of TopSpin. See below for the operating system compatibilities of these versions.

The NMR facility *TopSpin* installations include several files which have been added locally. These add standard plot layouts and extra processing commands like **efpb** and **absb**. These can be added to your own *TopSpin* installation by downloading them from the **TopSpinFiles** directory on nmrserver. There is a link to this directory from all users' home directories. These files can also be loaded together by using the method below.

The *TopSpin* command **nmrsave** can be used to save and load any user-modifications. If upgrading an existing installation, first use this command to **Save installation files**. Within the new installation, use **nmrsave** to **Restore installation files**. If an active *TopSpin* version is not available, the extra UCI files can be installed by copying the file 'nmr_backup_201700926-1728.zip' (or similar file with a newer date) and using **nmrsave** with the **Restore installation files** option.

Installation of the 'nmr_backup' file will only succeed if the file is present as a 'zip archive'. If the file is automatically uncompressed when it is copied, change the import/download preference settings for the FTP program in use to prevent automatic decoding.

The following versions of *TopSpin* are currently available, with different operating system requirements (the precise version numbers may change as new versions are released):

25.2.1 *TopSpin* 3.6.3

Compatible with Linux (CentOS 5.11 or 7.1(64bit)), MacOS (10.12 or newer) and Windows (7, 8.1 or 10).

The program files can be downloaded from **costello.ps.uci.edu**, but a *CodeMeter* license must be obtained from Bruker. You must first register an account at Bruker via https://www.bruker.com/about-us/register.html and then the license can be obtained via https://www.bruker.com/service/support-upgrades/software-downloads/nmr/free-topspin-processing/nmr-topspin-license-for-academia.html

If the license fails to activate on your computer, try using a different web browser.

The license is valid for 15 years and can be installed to a USB device for use on multiple computers or to a specific computer.

The first time *TopSpin* is run, the license should be installed via the **Install License Ticket** option.

25.2.2 *TopSpin* 4.1.1

Compatible with Linux (CentOS 7.1(64bit)), MacOS (10.12 or newer) and Windows (7(64bit) or 10(64bit)).

The program files can be downloaded from **costello.ps.uci.edu**, but a *CodeMeter* license must be obtained from Bruker as described above.

25.2.3 *TopSpin* 4.1.4

Compatible with Linux (CentOS 7.1(64bit)), MacOS (10.14 or newer) and Windows 10(64bit)).

The program files can be downloaded from **costello.ps.uci.edu**, but a *CodeMeter* license must be obtained from Bruker as described above.

Important notes for MacOS Catalina (10.15), Big Sur (11) or Monterey (12) users: Changes to the MacOS file system mean that *TopSpin* cannot open data files from within a user's home directory. The simplest workaround is to move your data to within the directory structure used by *TopSpin*. *TopSpin* installs into /opt, so create a new data directory there, for example /opt/NMRdata. An alternative method is to alter the security settings for your computer as described below.

This is not within your normal account files, and so will need extra permissions to create, for which you will be prompted. If you cannot see **/opt** in a Finder window, press **shift**+**command**+**g** and type **/opt** into the goto window. Alternatively, if you open a Finder window showing your hard drive's root directory, you can press **Command** + **Shift** + **.** to show all normally hidden items.

A script is available to automatically set up the data directory in /opt:

Use *Fetch* or an alternative sFTP client to connect to nmrserver.ps.uci.edu

Click on the link **MacScripts**, download the file **MacScripts.zip**, and decompress it to find the file **setup_NMRdatadir_in_opt.command**

Control-click on the file, then select **Open** to run it, you will be asked for your *MacOS* password. A new directory called **NMRdata** will be created in /opt, for you to store data for processing in *TopSpin*. A link to /opt/NMRdata, called NMRdata, will also be created on your desktop. You can move that link to somewhere else if you prefer.

The script will delete itself at the end. So if you want to run it again you'll either need to keep a copy or repeat the download.

When downloading the nmr_backup file, make sure that this is stored in a **/opt/topspin***version***/nmr_backup**. Use **Go → Go to Folder… → /opt** to navigate to that location.

To alter Mac security settings so that *TopSpin* can access any files:

If *TopSpin* fails to start and produces an error about the hostname, then ensure your computer's hostname does not include any apostrophes or spaces. Changing this in System Preferences is not enough. Open a Terminal window and type:

sudo scutil --set HostName *newname*

25.2.4 Altering Mac security settings so that *TopSpin* can access any files

Open **System Preferences → Security & Privacy**

Select the **Privacy** tab, if it is not already open.

Click on the lock in the bottom left in order to make changes, and enter your password.

Select **Full Disk Access** in the left column and click on **+**. A window should open.

Separately, in the Mac Finder, select **Go → Go to Folder…** from the menu bar. Type **/bin** and press ***<return>***.

Scroll the window until you see the entry **sh**. Drag this to the open security preferences window and then press **Open**.

You should now be able to use *TopSpin* to process data files located anywhere on your computer.

25.3 *MestReNova* (MacOS, Linux and Windows)

Available from:

http://mestrelab.com/software/mnova/

An advanced package which will handle both 1D and 2D data. A license is required to stop the program expiring after 45 days.

An old version, 5, is installed on one of the Apple computers in FRH and the iMac in NS1. *MestReNova* has a very sophisticated automatic baseline correction function, using the Whittaker smoother.

25.4 *SpinWorks*

Freely available from:

https://home.cc.umanitoba.ca/~wolowiec/spinworks/

A full manual is included, and this will also process both 1D and 2D data.

The original program is Windows only, but a multi-platform Java version is under development. An early version is available for download.

25.5 GNAT (General NMR Analysis Toolbox)

A software package intended primarily for the processing of multicomponent high resolution NMR datasets such as those acquired in diffusion, relaxation and kinetic experiments. Under development at the University of Manchester and available here: https://www.nmr.chemistry.manchester.ac.uk/?q=node/430

25.6 *iNMR* (MacOS and Windows)

Available from:

http://www.inmr.net

Without a license some functions are disabled and the program quits after one hour of use. Some overlap with development of *MestReNova*.

25.7 *NMRPipe* (Sun, SGI, Linux, MacOS)

Freely available from:

https://www.ibbr.umd.edu/nmrpipe/index.html

A complex and comprehensive program mainly aimed at interpretation of Biological NMR data.

NMRpipe is installed on the SGI workstations in the NMR Facility, but is not as user-friendly as *XwinNMR*.

25.8 ACD Labs (Windows only)

ACD Labs software is no longer free for academics, and will only run under Microsoft Windows:

http://www.acdlabs.com/

25.9 *SwaN-MR* (Mac OS 9)

Freely available from:

http://www.inmr.net/swan/

Not available for Mac OS X or later.

25.10 *matNMR* for *MATLAB* (all platforms)

Matlab is a commercial program (https://www.mathworks.com/products/matlab.html) for which there is a free NMR plugin available from http://matnmr.sourceforge.net/

26 Common NMR experiments

The basic *XwinNMR* and *TopSpin* techniques for all experiments listed below is to:

Create a new experiment name and/or number - **edc** or **edcp** etc

Load parameters for the desired experiment - **par** or **rpar** *parametersetname* **all** etc

Read standard shim file for the instrument in user - **rsh** *shimfile* (**bbo**, **qnp** etc)

Lock and shim before running the first experiment on a sample - **lock** *solventname*, shim via BSMS keypad etc

Set receiver gain and run standard experiment - **rgazg**

Check data quality and re-adjust shims if necessary - **ft**, **apks** etc

Optimize parameters - expand data, **sw-sfo1** etc

Re-set receiver gain and run experiment - **rgazg**

Process and plot data - **efp**, **apks** or **phase**, **abs** or **basl**, **sref** or **calibrate**, **integrate**, **setti**, **dp1**, **vplot**

Full details of each of these operations can be found in the preceding chapters.

Experiment parameters can be modified as required in three ways: by typing the parameter name with or without the desired value as an argument; via the **ased** acquisition setup editor; or via the **eda** acquisition parameter editor. **eda** lists all the *XwinNMR* acquisition parameters, whereas **ased** only lists those parameters used by the current experiment. Note that **ased** does not list all variants of parameters, for example the total frequency **sfo1** is listed, but not the offsets **o1** or **o1p**, and the spectral width is only included in Hz as **swh**, not in ppm as **sw**.

The following sections describe the most common NMR experiments and the parameters or commands which are most important in obtaining good quality data. Other, more specialized or complex, experiments are also available.

If the desired experiment is not listed, please consult the NMR Facility Director for assistance.

26.1 Proton spectrum (1D)

Purpose: Obtain proton peak positions and peak areas

Standard parameter set: **h1.***

Phase cycling used: **ns = 8 x n** (not important if ns is large, >~256)

Typical experiment duration: **1 minute for 8 scans**

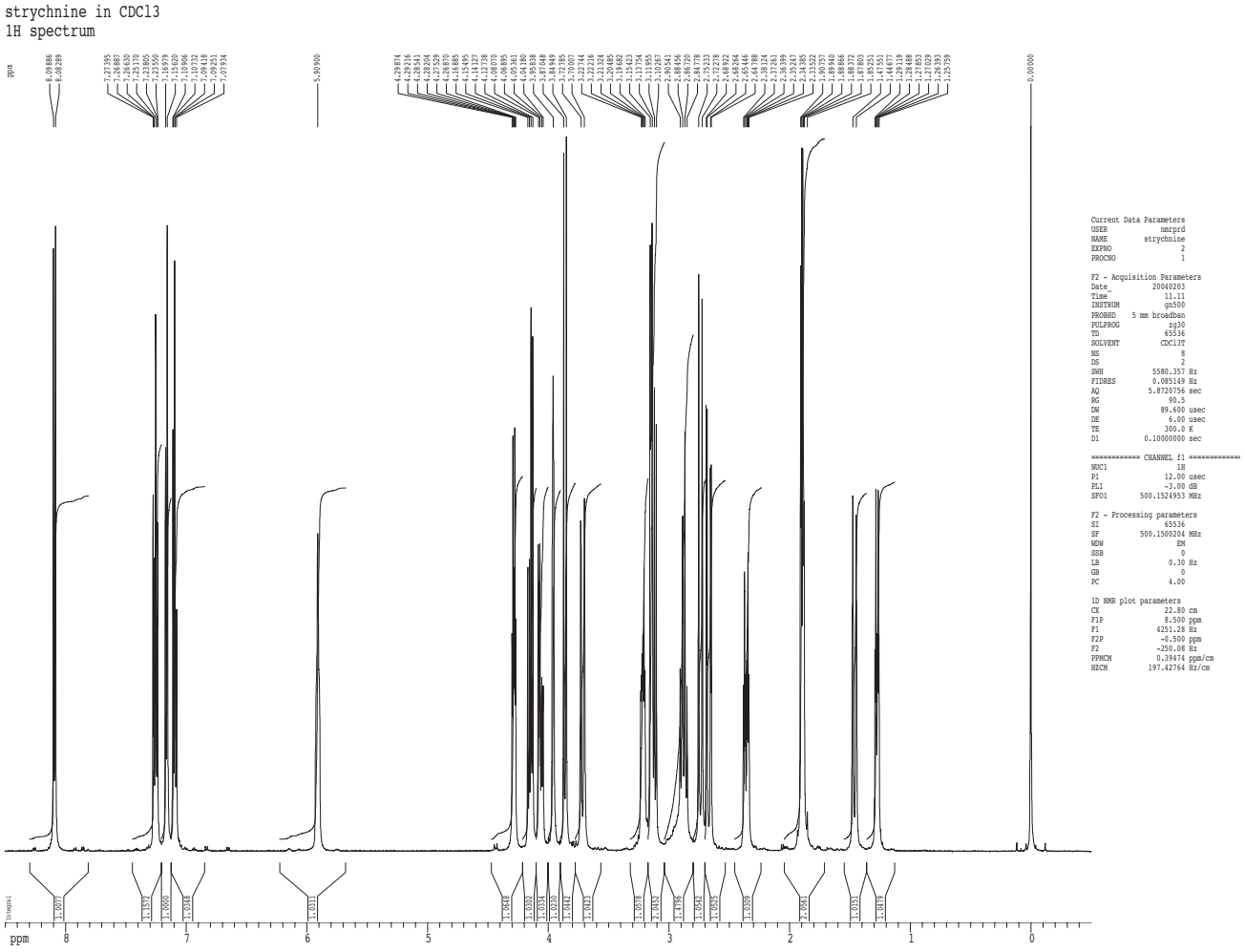
The default parameters produce a spectrum from 15ppm to -1ppm, i.e. a spectral width of 16ppm (**sw = 16**), centered at 7ppm (**o1p = 7**).

Each FID is acquired for 5.1s (**aq = 5.1**) with a short relaxation delay of 100ms (**d1 = 0.1**) between scans.

2 dummy scans (**ds = 2**) are performed before 8 scans (**ns = 8**) of recording data.

The total time between pulses is **aq + d1**. For a reasonably quantitative spectrum this should be a minimum of **5** seconds. If **sw** is increased to find peaks outside the standard range, **aq** will be reduced (the two parameters are inversely proportional). Therefore, to ensure that the time between pulses is still at least 5 seconds, either **aq** or **d1** should be increased. Increasing **aq** is best for recording a long FID accurately, but will measure more datapoints (**td**). When processing data, the size of the real part of the Fourier transform (**si**) should be a minimum of half the number of complex datapoints (**td**). So, if **aq** has been increased dramatically it may be necessary to increase **si**. Standard values for **td** are 65536 (at 400MHz) and 81728 (at 500MHz) and **si** is normally set at 64k (65536).

Data processing: Typically use line broadening to smooth data, using the command **efp**. Line broadening factor can be varied from the standard setting of **0.3** Hz via the parameter **lb** or interactively via the command **winfunc**. If line broadening is not desired use **fp** (line broadening broadens noise and peaks, which reduces resolution slightly).



26.2 Carbon spectrum with proton decoupling (1D)

Purpose: Obtain carbon peak positions

Standard parameter set: **c13.***

Phase cycling used: **ns = 8 x n** (not important if ns is large, >~256)

Typical experiment duration: **23 minutes for 1024 scans**

The default parameters produce a spectrum from 230ppm to -10ppm, i.e. a spectral width of 240ppm (**sw = 240**), centered at 110ppm (**o1p = 110**).

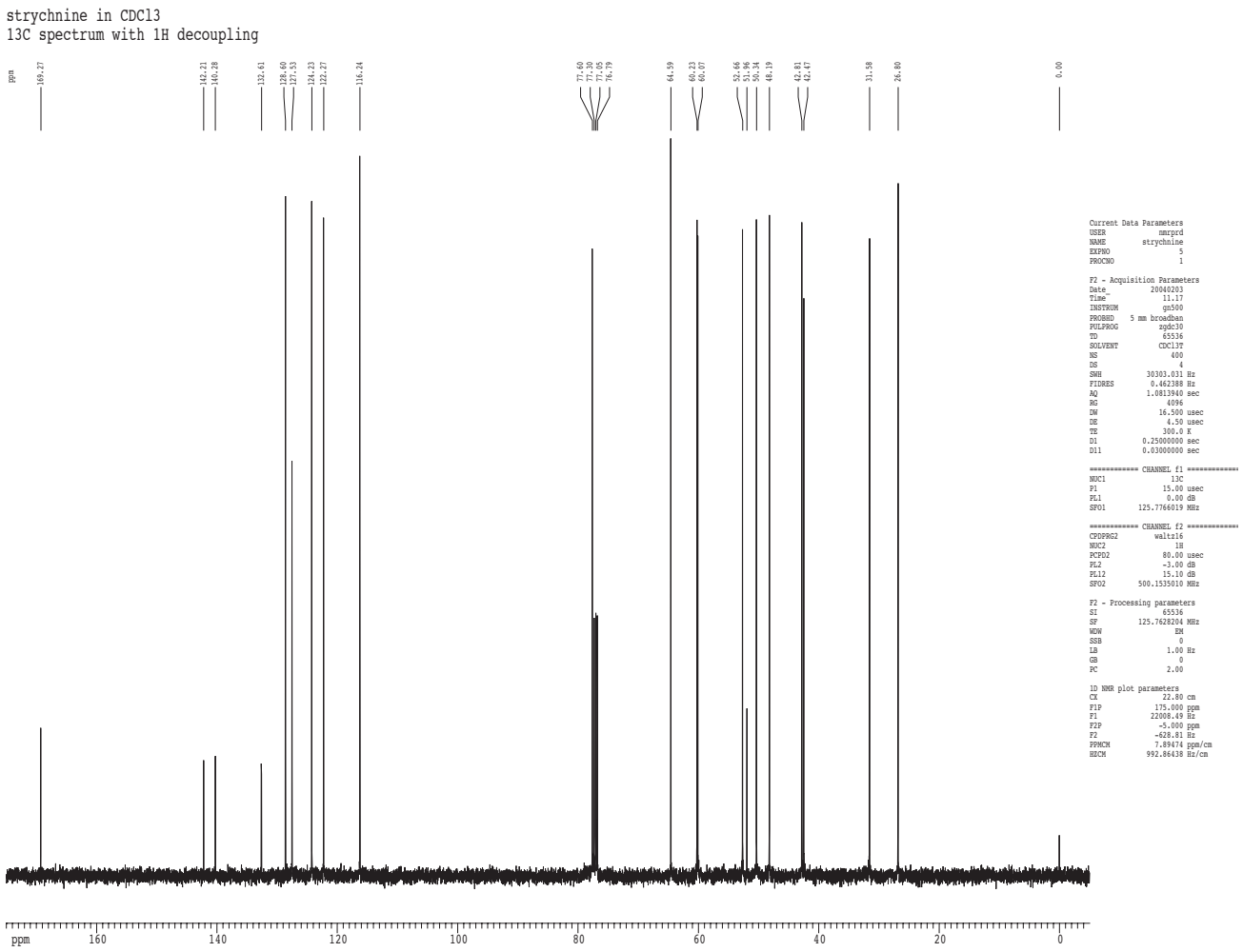
The acquisition time (**aq**) is followed by a short delay (**d1**) giving a total time of c. 1.33s between pulses.

4 dummy scans (**ds = 4**) are performed before 1024 scans (**ns = 1024**) of recording data, but the experiment can be interrupted at any time.

The standard parameters include a short relaxation delay, **d1**, which while generally suitable can lead to some quaternary carbons with long T_1s to be saturated and disappear from the spectrum. If necessary, the value of **d1** can be increased to improve detection of these carbons. There are two additional parameter sets, **c13d1=1.s** and **c13d1=1.5.s** which have **d1** increased to **1s** and **1.5s** respectively. Alternatively, **d1** can be increased manually. The default units are seconds.

If a long experiment is to be performed on a very small sample it is a good idea to use a **d1** of at least **1** second as a precaution against needing to repeat the experiment.

Data processing: Typically use line broadening to smooth data, using the command **efp**. Line broadening factor can be varied from the standard setting of **1** Hz via the parameter **lb** or interactively via the command **winfunc**. If line broadening is not desired use **fp**. This will improve resolution but beat patterns may be observed next to strong signals due to truncation of the FID.



26.3 Proton-proton correlation through bonds (COSY, TOCSY, homodecoupling)

26.3.1 Basic gradient enhanced COSY

Purpose: Establish which protons are coupled through bonds. Cross peaks are obtained for spin-spin coupling constants of ~3Hz to ~15Hz.

Standard parameter set: **gcosy.***

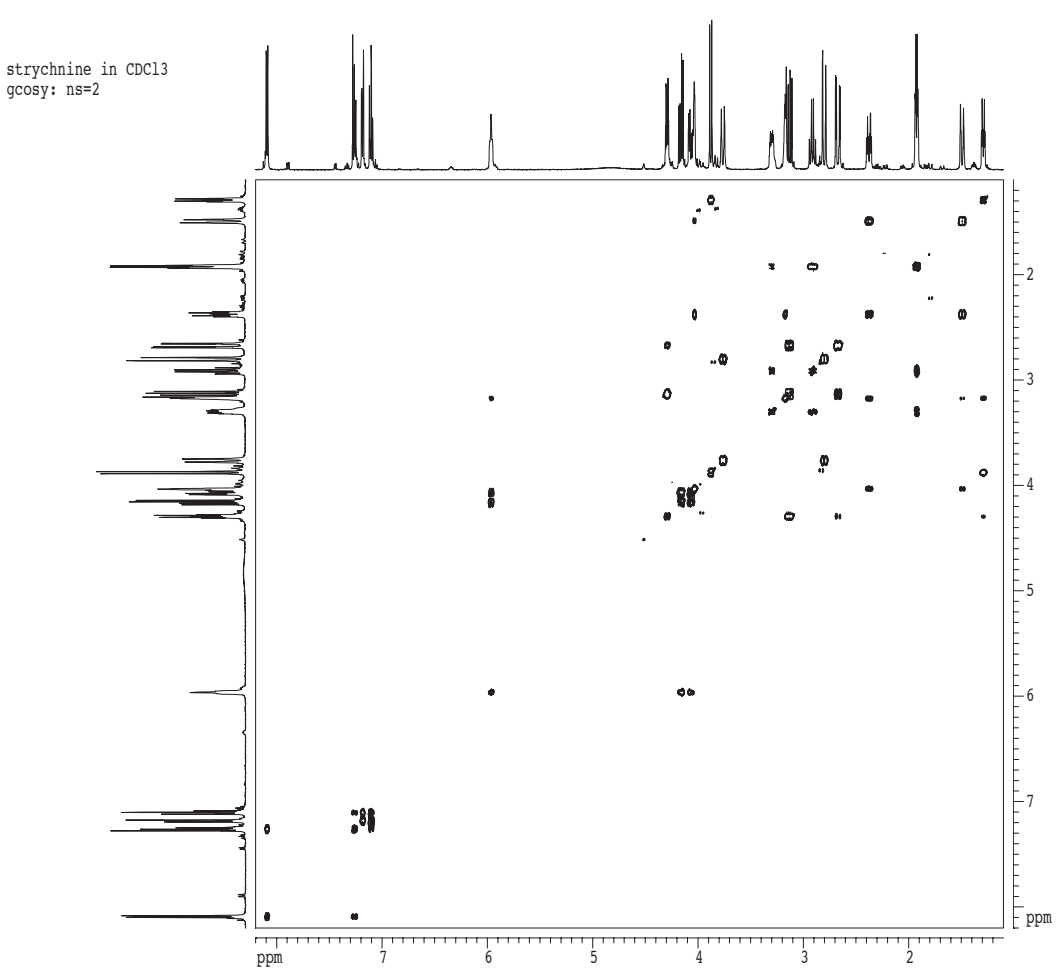
Gradient experiment: **ns = 1 x n**

Typical experiment duration: **11 minutes for 2 scans and 256 experiments**

The default parameters produce a spectrum from 15ppm to -1ppm, i.e. a spectral width of 16ppm (**sw = 16**), centered at 7ppm (**o1p = 7**). To maximize the resolution of the 2D experiment it is important to optimize the spectral region as described in chapter 5 using **copypars**. Resolution in the indirect dimension (F1) can be increased by acquiring more experiments. This parameter can most easily be adjusted in the acquisition parameter editor **eda**, by changing **td** in the F1 (right) column. On most instruments the standard value is **256**, but on the CRYO500 it is **512**.

The basic COSY experiment is very rugged as it involves few pulses. Closely coupled protons give the strongest cross-peaks, longer range couplings give weaker signals.

Data processing: Obtain referencing information from the parameter **sr** in the 1D proton spectrum, then use **getproj** to set up projection information. Standard window functions in **edp** are unshifted sine: **WDW = SINE** and **SSB = 0** in both F2 and F1 dimensions. Use **xfb** to Fourier transform, followed by **absb** for baseline correction.




```
        Current Data Parameters
USER              nmrprd
NAME           strychnine
EXPNO                 52
PROCNO                 1

      F2 - Acquisition Parameters
Date_           20040209
Time               17.04
INSTRUM           qn500
PROBHD    5 mm broadban
PULPROG          cosygp
TD                 2048
SOLVENT           CDCl3
NS                    2
DS                   16
SWH             4045.307 Hz
FIDRES          1.975248 Hz
AQ              0.2531828 sec
RG                161.3
DW               123.600 usec
DE                 6.00 usec
TE                 300.0 K
D0              0.00000300 sec
D1              1.00000000 sec
D13             0.00000300 sec
D16             0.00025000 sec
IN0             0.00024720 sec

 ============ CHANNEL f1 ============
NUC1                  1H
P0                 11.50 usec
P1                 11.50 usec
PL1                -3.00 dB
SFO1         500.1525565 MHz

============ GRADIENT CHANNEL =======
GPNAM1          SINE.100
GPNAM2          SINE.100
GPX1                0.00 %
GPX2                0.00 %
GPY1                0.00 %
GPY2                0.00 %
GPZ1               17.00 %
GPZ2               17.00 %
P16              1000.00 usec

      F1 - Acquisition parameters
ND0                   1
TD                  256
SFO1          500.1526 MHz
FIDRES        15.801982 Hz
SW                8.088 ppm

      F2 - Processing parameters
SI                 1024
SF          500.1500186 MHz
WDW                SINE
SSB                   0
LB                 0.00 Hz
GB                    0
PC                 1.40

      F1 - Processing parameters
SI                 1024
MC2                  QF
SF          500.1500186 MHz
WDW                SINE
SSB                   0
LB                 0.00 Hz
GB                    0

      2D NMR plot parameters
CX2               15.00 cm
CX1               15.00 cm
F2PLO             8.200 ppm
F2LO            4101.23 Hz
F2PHI             1.100 ppm
F2HI             550.16 Hz
F1PLO             8.200 ppm
F1LO            4101.23 Hz
F1PHI             1.100 ppm
F1HI             550.16 Hz
F2PPMCM        0.47333 ppm/cm
F2HZCM       236.73767 Hz/cm
F1PPMCM        0.47333 ppm/cm
F1HZCM       236.73767 Hz/cm
```

26.3.2 Double quantum filtered COSY

Purpose: Establish which protons are coupled through bonds. Cross peaks are obtained for spin-spin coupling constants of ~3Hz to ~15Hz.

Standard parameter set: **gcosydqf.***

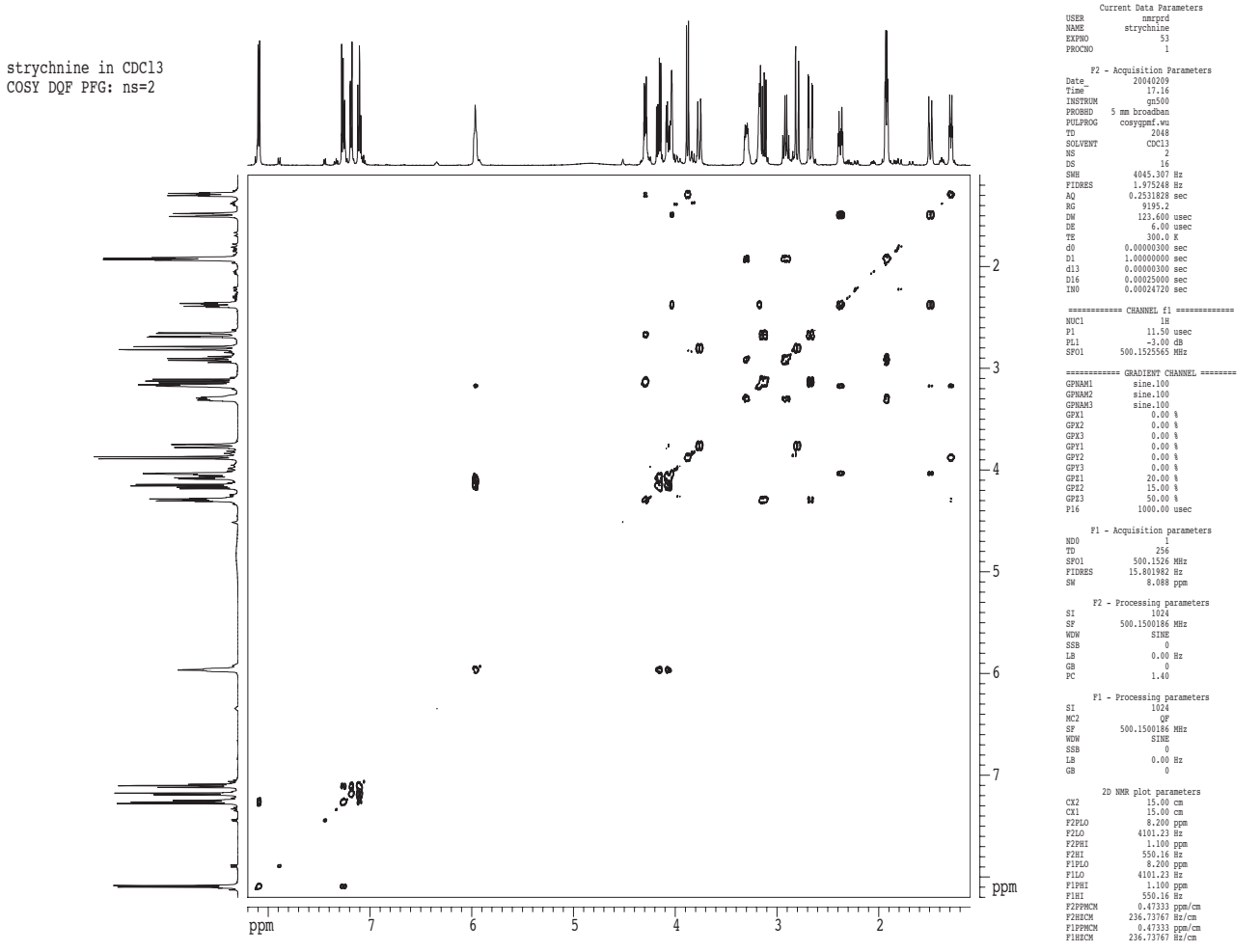
Gradient experiment: **ns = 1 x n**

Typical experiment duration: **11 minutes for 2 scans and 256 experiments**

The default parameters produce a spectrum from 15ppm to -1ppm, i.e. a spectral width of 16ppm (**sw = 16**), centered at 7ppm (**o1p = 7**). To maximize the resolution of the 2D experiment it is important to optimize the spectral region as described in chapter 5 using **copypars**. Resolution in the indirect dimension (F1) can be increased by acquiring more experiments. This parameter can most easily be adjusted in the acquisition parameter editor **eda**, by changing **td** in the F1 (right) column. On most instruments the standard value is **256**, but on the CRYO500 it is **512**.

This experiment produces slightly lower signal-to-noise, so more scans may be required than the simple COSY above, but results in narrower, less intense diagonal signals. Thus correlations closer to the diagonal can be resolved.

Data processing: Use **getproj** to update projection information if necessary. Standard window functions in **edp** are unshifted sine: **WDW = SINE** and **SSB = 0** in both F2 and F1 dimensions. Use **xfb** to Fourier transform, followed by **absb** for baseline correction.



26.3.3 Long range COSY

Purpose: Establish which protons are coupled through several bonds. Cross peaks are obtained for very small spin-spin coupling constants.

Standard parameter set: **cosylr.***

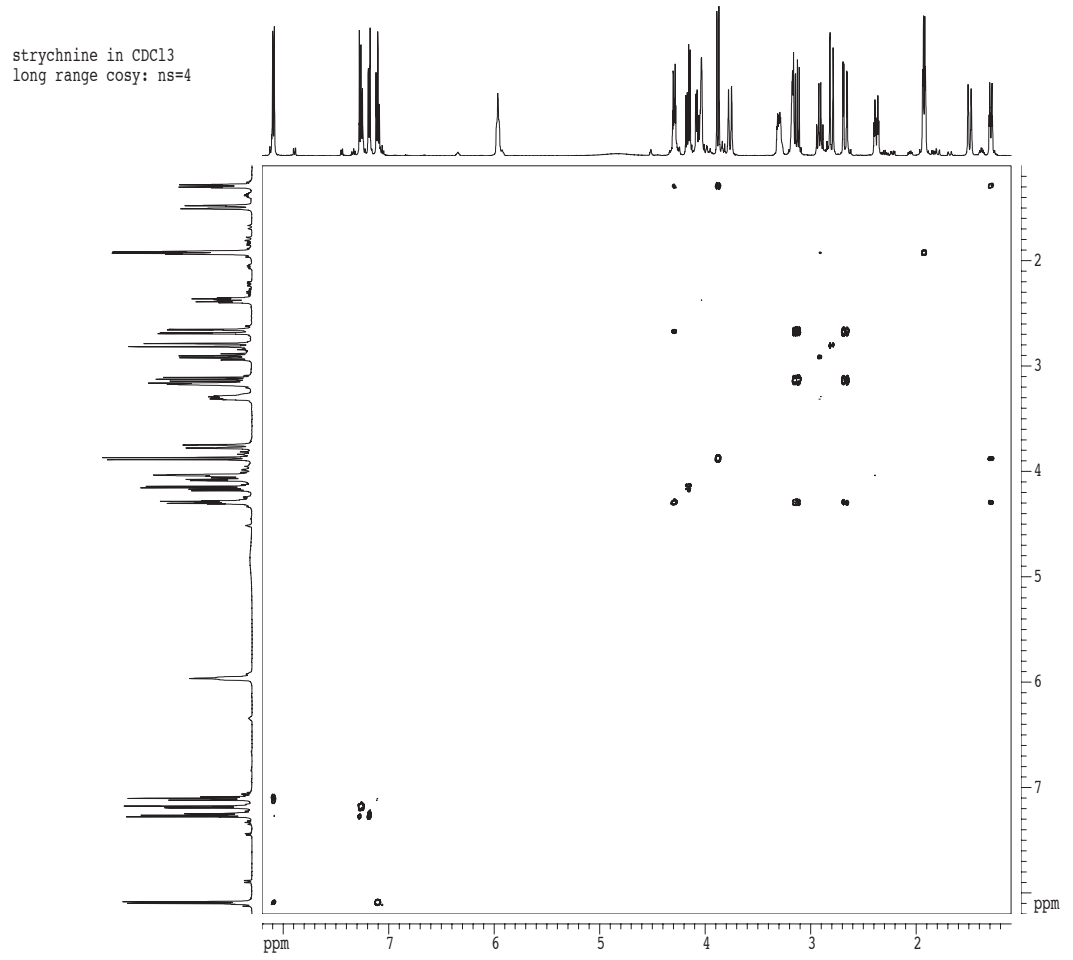
Phase cycling used: **ns = 4 x n**

Typical experiment duration: **22 minutes for 4 scans and 256 experiments**

The default parameters produce a spectrum from 15ppm to -1ppm, i.e. a spectral width of 16ppm (**sw = 16**), centered at 7ppm (**o1p = 7**). To maximize the resolution of the 2D experiment it is important to optimize the spectral region as described in chapter 5 using **copypars**. Resolution in the indirect dimension (F1) can be increased by acquiring more experiments. This parameter can most easily be adjusted in the acquisition parameter editor **eda**, by changing **td** in the F1 (right) column. On most instruments the standard value is **256**, but on the CRYO500 it is **512**.

The standard parameters use a delay of 0.3s (**d6**) for evolution of long range couplings. This can be varied in the range 0.1s to 0.4s.

Data processing: Use **getproj** to update projection information if necessary. Standard window functions in **edp** are unshifted sine: **WDW = SINE** and **SSB = 0** in both F2 and F1 dimensions. Use **xfb** to Fourier transform, followed by **absb** for baseline correction.




```
        Current Data Parameters
USER              nmrprd
NAME           strychnine
EXPNO                 55
PROCNO                 1

      F2 - Acquisition Parameters
Date_           20040209
Time              17.40
INSTRUM           qn500
PROBHD    5 mm broadban
PULPROG           cosylr
TD                  2048
SOLVENT            CDCl3
NS                     4
DS                    16
SWH             4045.307 Hz
FIDRES          1.975248 Hz
AQ              0.2531828 sec
RG                  3251
DW                123.600 usec
DE                  6.00 usec
TE                300.0 K
D0             0.00000300 sec
D1             1.00000000 sec
D6             0.30000001 sec
IN0            0.00024720 sec

 ============ CHANNEL f1 =============
NUC1                   1H
P1                  11.50 usec
PL1                -3.00 dB
SFO1         500.1525565 MHz

      F1 - Acquisition parameters
ND0                    1
TD                   256
SFO1            500.1526 MHz
FIDRES          15.801982 Hz
SW                  8.088 ppm

      F2 - Processing parameters
SI                  1024
SF           500.1500186 MHz
WDW                 SINE
SSB                    0
LB                  0.00 Hz
GB                     0
PC                  1.40

      F1 - Processing parameters
SI                  1024
MC2                   QF
SF           500.1500186 MHz
WDW                 SINE
SSB                    0
LB                  0.00 Hz
GB                     0

        2D NMR plot parameters
CX2                15.00 cm
CX1                15.00 cm
F2PLO              8.200 ppm
F2LO             4101.23 Hz
F2PHI              1.100 ppm
F2HI              550.16 Hz
F1PLO              8.200 ppm
F1LO             4101.23 Hz
F1PHI              1.100 ppm
F1HI              550.16 Hz
F2PPMCM          0.47333 ppm/cm
F2HZCM         236.73767 Hz/cm
F1PPMCM          0.47333 ppm/cm
F1HZCM         236.73767 Hz/cm
```

26.3.4 Total correlation spectroscopy -TOCSY/HOHAHA

26.3.4.1 Gradient TOCSY

Purpose: Total correlation of all protons within a chain. Correlations are shown between all protons which reside in a single spin system but which may not share mutual couplings. This version is preferable over the phase sensitive experiment (described next) if sample quantity is sufficient to acquire good data using less than eight scans.

Standard parameter set: **gtocsy.***

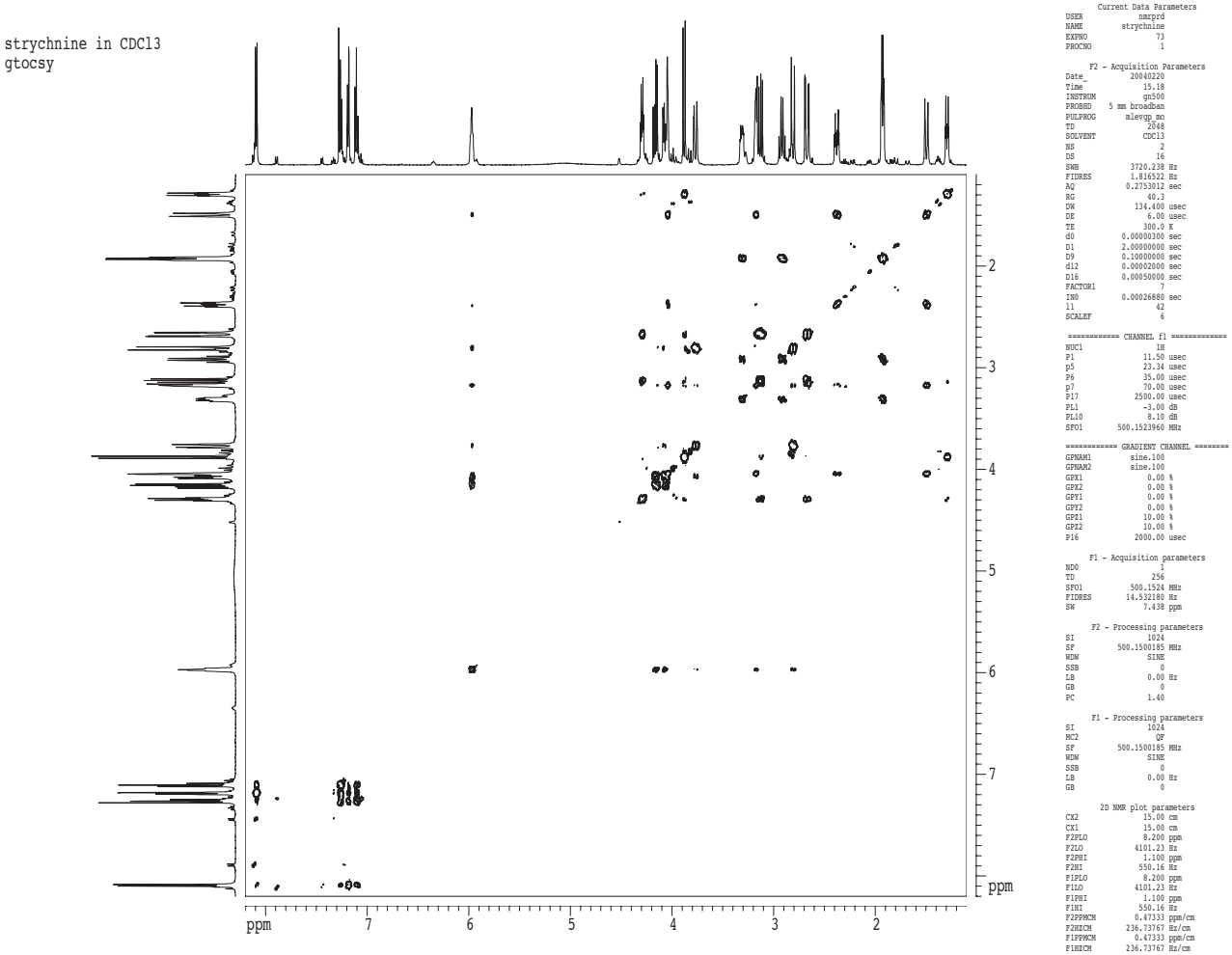
Gradient experiment: **ns = 1 x n**

Typical experiment duration: **20 minutes for 2 scans and 256 experiments**

The default parameters produce a spectrum from 15ppm to -1ppm, i.e. a spectral width of 16ppm (**sw = 16**), centered at 7ppm (**o1p = 7**). To maximize the resolution of the 2D experiment it is important to optimize the spectral region as described in chapter 5 using **copypars**. Resolution in the indirect dimension (F1) can be increased by acquiring more experiments. This parameter can most easily be adjusted in the acquisition parameter editor **eda**, by changing **td** in the F1 (right) column. On most instruments the standard value is **256**, but on the CRYO500 it is **512**.

Automatic receiver gain adjustment (**rga**) should **not** be used, as the first FID of the TOCSY experiment can be much smaller than subsequent signals. Manually set the receiver gain, **rg**, to the same value as used in the 1D proton experiment. It is also important to choose an appropriate mixing time, **d9**, which is normally in the range 50ms to 200ms.

Data processing: Use **getproj** to update projection information if necessary. Standard window functions in **edp** are unshifted sine: **WDW = SINE** and **SSB = 0** in both F2 and F1 dimensions. Use **xfb** to Fourier transform, followed by **absb** for baseline correction.




```
Current Data Parameters
USER                nmrprd
NAME             strychnine
EXPNO                    73
PROCNO                    1

F2 - Acquisition Parameters
Date_              20040220
Time                  15.18
INSTRUM             qnp500
PROBHD    5 mm broadban
PULPROG          mlevgp_mo
TD                     2048
SOLVENT              CDCl3
NS                        2
DS                       16
SWH                3720.238 Hz
FIDRES             1.816522 Hz
AQ                 0.2753012 sec
RG                      40.3
DW                   134.400 usec
DE                     6.00 usec
TE                    300.0 K
d0                 0.00000300 sec
D1                 2.00000000 sec
D9                 0.10000000 sec
d12                0.00002000 sec
D16                0.00050000 sec
FACTOR1                   7
IN0                0.00026880 sec
l1                       42
SCALEF                    6

============ CHANNEL f1 ============
NUC1                     1H
P1                    11.50 usec
p5                    23.34 usec
P6                    35.00 usec
p7                    70.00 usec
P17                 2500.00 usec
PL1                   -3.00 dB
PL10                   8.10 dB
SFO1            500.1523960 MHz

============ GRADIENT CHANNEL =======
GPNAM1              sine.100
GPNAM2              sine.100
GPX1                   0.00 %
GPX2                   0.00 %
GPY1                   0.00 %
GPY2                   0.00 %
GPZ1                  10.00 %
GPZ2                  10.00 %
P16                 2000.00 usec

F1 - Acquisition parameters
ND0                       1
TD                      256
SFO1                500.1524 MHz
FIDRES             14.532180 Hz
SW                     7.438 ppm

F2 - Processing parameters
SI                      1024
SF                500.1500185 MHz
WDW                     SINE
SSB                        0
LB                      0.00 Hz
GB                         0
PC                      1.40

F1 - Processing parameters
SI                      1024
MC2                       QF
SF                500.1500185 MHz
WDW                     SINE
SSB                        0
LB                      0.00 Hz
GB                         0

2D NMR plot parameters
CX2                    15.00 cm
CX1                    15.00 cm
F2PLO                  8.200 ppm
F2LO                4101.23 Hz
F2PHI                  1.100 ppm
F2HI                 550.16 Hz
F1PLO                  8.200 ppm
F1LO                4101.23 Hz
F1PHI                  1.100 ppm
F1HI                 550.16 Hz
F2PPMCM            0.47333 ppm/cm
F2HZCM           236.73767 Hz/cm
F1PPMCM            0.47333 ppm/cm
F1HZCM           236.73767 Hz/cm
```

26.3.4.2 Phase sensitive TOCSY

Purpose: Total correlation of all protons within a chain. Correlations are shown between all protons which reside in a single spin system but which may not share mutual couplings.

Standard parameter set: **tocsyp.***

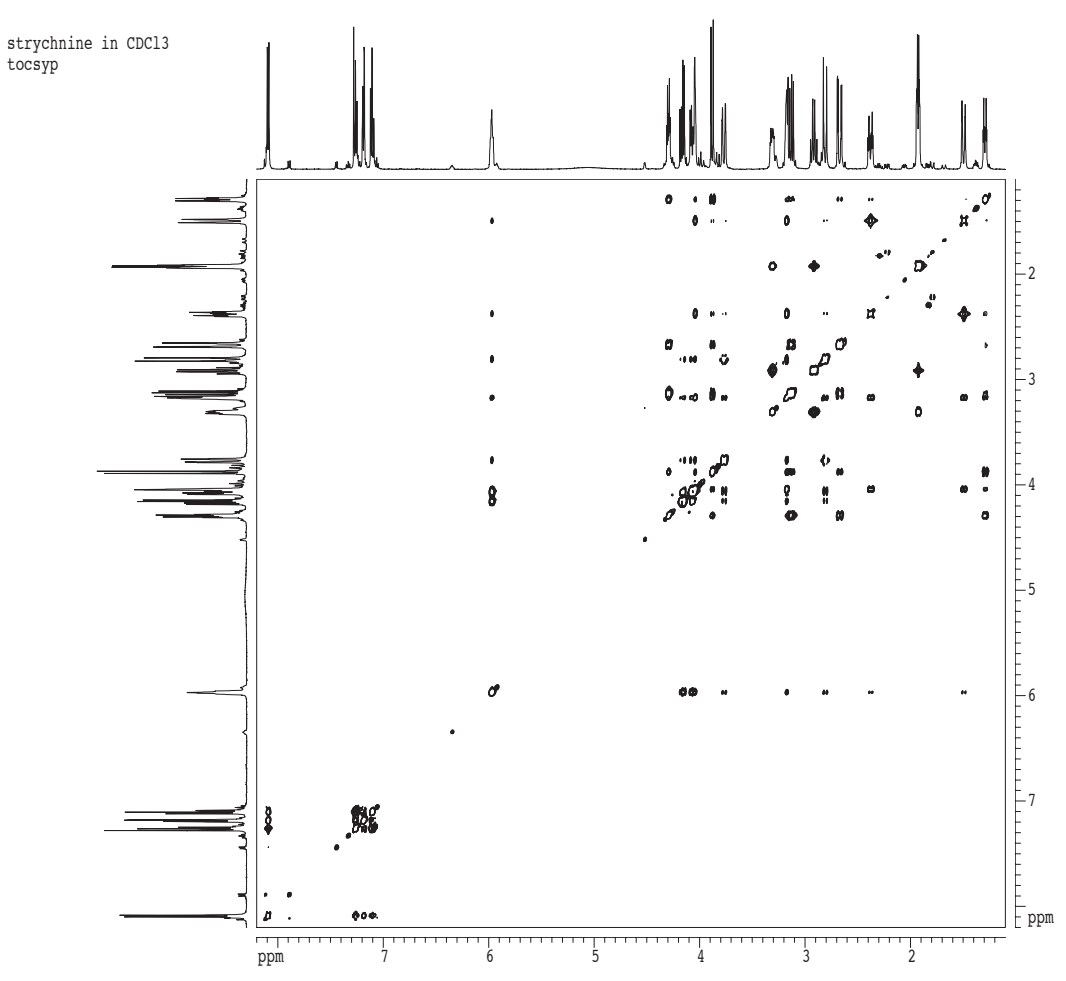
Gradient experiment: **ns = 8 x n**

Typical experiment duration: **1 hour and 26 minutes for 8 scans and 256 experiments**

The default parameters produce a spectrum from 15ppm to -1ppm, i.e. a spectral width of 16ppm (**sw = 16**), centered at 7ppm (**o1p = 7**). To maximize the resolution of the 2D experiment it is important to optimize the spectral region as described in chapter 5 using **copypars**.

Automatic receiver gain adjustment (**rga**) should **not** be used, as the first FID of the TOCSY experiment can be much smaller than subsequent signals. Manually set the receiver gain, **rg**, to the same value as used in the 1D proton experiment. It is also important to choose an appropriate mixing time, **d8**, which is normally in the range 50ms to 200ms.

Data processing: Use **getproj** to update projection information if necessary. Standard window functions in **edp** are shifted sine-squared: **WDW = QSINE** and **SSB = 2** in both F2 and F1 dimensions. Use **xfb** to Fourier transform, followed by phase correction. All peaks, both on and off diagonal, should be phased to be positive. Finally use **absb** for baseline correction.




```
      Current Data Parameters
USER              nmrprd
NAME          strychnine
EXPNO                 72
PROCNO                 1

      F2 - Acquisition Parameters
Date_           20040220
Time              13.42
INSTRUM             gn500
PROBHD    5 mm broadban
PULPROG       tocsytp.wu
TD                  2048
SOLVENT             CDCl3
NS                     8
DS                    16
SWH             3720.238 Hz
FIDRES          1.816522 Hz
AQ              0.2753012 sec
RG                  40.3
DW                134.400 usec
DE                  6.00 usec
TE                300.0 K
d0              0.00000300 sec
D1              2.00000000 sec
d8              0.19964767 sec
d12             0.00002000 sec
IN0             0.00013440 sec
L1                     86

 ============ CHANNEL f1 ============
NUC1                   1H
P1                  11.50 usec
p5                  23.34 usec
P6                  35.00 usec
p7                  70.00 usec
P17               2500.00 usec
PL1                 -3.00 dB
PL10                 8.10 dB
SFO1         500.1523960 MHz

      F1 - Acquisition parameters
ND0                    2
TD                   256
SFO1           500.1524 MHz
FIDRES         14.532180 Hz
SW                 7.438 ppm

      F2 - Processing parameters
SI                  1024
SF           500.1500185 MHz
WDW                 QSINE
SSB                    2
LB                  0.00 Hz
GB                     0
PC                  4.00

      F1 - Processing parameters
SI                  1024
MC2                 TPPI
SF           500.1500185 MHz
WDW                 QSINE
SSB                    2
LB                  0.00 Hz
GB                     0

       2D NMR plot parameters
CX2                15.00 cm
CX1                15.00 cm
F2PLO               8.200 ppm
F2LO             4101.23 Hz
F2PHI               1.100 ppm
F2HI              550.16 Hz
F1PLO               8.200 ppm
F1LO             4101.23 Hz
F1PHI               1.100 ppm
F1HI              550.16 Hz
F2PPMCM         0.47333 ppm/cm
F2HZCM        236.73767 Hz/cm
F1PPMCM         0.47333 ppm/cm
F1HZCM        236.73767 Hz/cm
```

26.3.5 1D proton spectrum with homonuclear decoupling

Purpose: Selectively remove couplings resulting from a single proton and observe the effect on the other peaks in the spectrum.

Standard parameter set: **h1hd.***

Gradient experiment: **ns = 8 x n**

Typical experiment duration: **1 minute and 20 seconds for 8 scans**

The default parameters produce a spectrum from 15ppm to -1ppm, i.e. a spectral width of 16ppm (**sw = 16**), centered at 7ppm (**o1p = 7**). First record a standard proton spectrum for comparison, optimizing the spectral region using **sw-sfo1**. Record the new values for **sw** and **o1** (the offset frequency of the center of the spectrum in Hz). Set up the homonuclear decoupling experiment using the standard parameter set and then enter the new values for **sw** and **o1** noted above.

Recall the standard proton spectrum, and expand a small region around the signal to be irradiated. Change to the **utilities** screen and using the ***MMB*** record the absolute frequency (in MHz) of the signal of interest. If this is a multiplet, choose the center of the splitting pattern. Recall the homonuclear decoupling experiment and set the decoupler frequency **sfo2** to this value.

The low power selective decoupling is applied during the relaxation delay **d1**, immediately before the observe pulse. This delay is normally set to **1s**, and the irradiation power, **pl24**, is normally set to **40dB**.

If the irradiated signal is not reduced to a singlet, increase the power by reducing **pl24** to **35dB** and repeat. If neighboring signals are also being decoupled, reduce the power by increasing **pl24** to **45dB** and repeat.

Data processing: Typically use line broadening to smooth data, using the command **efp**.

26.4 J-Resolved 1H spectrum (2D)

Purpose: Separate chemical shift and spin-spin coupling information to aid interpretation of overlapping multiplets.

Standard parameter set: **hjres2D.***

Phase cycling used: **ns = 4 x n**

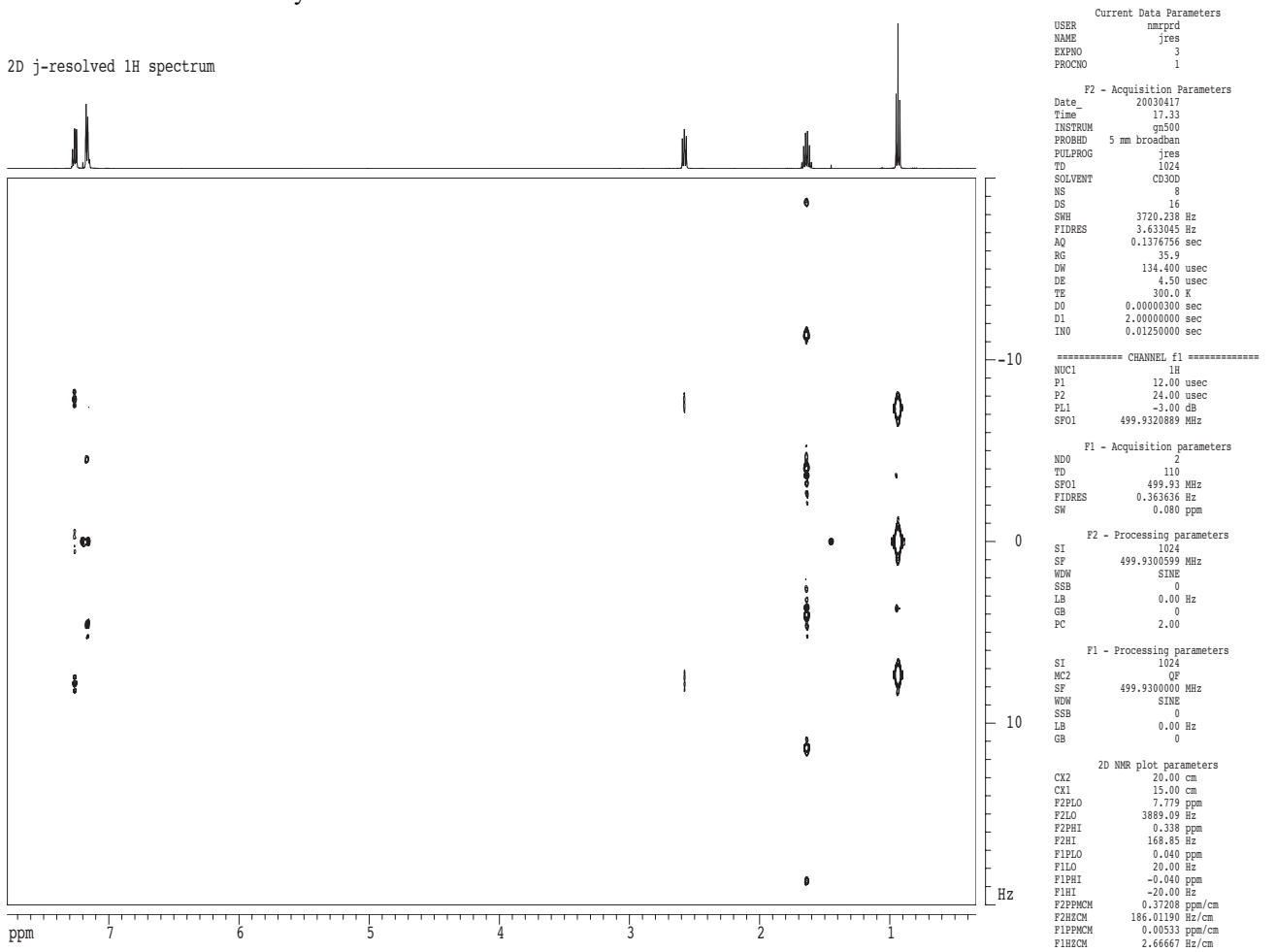
Typical experiment duration: **32 minutes for 4 scans and 128 experiments**

The default parameters produce a spectrum from 15ppm to -1ppm in the F2 domain, with a range of 40Hz to display spin-spin couplings in the F1 domain. The F2 domain can be optimized by manually changing the parameters **sw** and **o1**. If a wider frequency range is required to observe all couplings in the F1 domain, this can be changed manually by typing:

1 swh *<enter>*

Do **not** use an automation program to set up the spectral window for this experiment.

Data processing: Use **getproj** to update projection information if necessary. Do **not** change the **sr** value in the F1 domain. No projection will be printed in the F1 domain. Standard window functions in **edp** are unshifted sine functions in both dimensions: **WDW = SINE** and **SSB = 0**. Use **xfb** to Fourier transform, followed by **tilt** to apply a slight geometrical correction. Finally use **absb** for baseline correction.




```
Current Data Parameters
USER              nmrprd
NAME                jres
EXPNO                  3
PROCNO                 1

F2 - Acquisition Parameters
Date_           20030417
Time                17.33
INSTRUM             qn500
PROBHD    5 mm broadban
PULPROG             jres
TD                  1024
SOLVENT            CD3OD
NS                     8
DS                    16
SWH             3720.238 Hz
FIDRES          3.633045 Hz
AQ              0.1376756 sec
RG                  35.9
DW                134.400 usec
DE                  4.50 usec
TE                300.0 K
D0              0.00000300 sec
D1              2.00000000 sec
IN0             0.01250000 sec

============ CHANNEL f1 =============
NUC1                  1H
P1                 12.00 usec
P2                 24.00 usec
PL1                -3.00 dB
SFO1         499.9320889 MHz

F1 - Acquisition parameters
ND0                    2
TD                   110
SFO1              499.93 MHz
F1DRES          0.363636 Hz
SW                0.080 ppm

F2 - Processing parameters
SI                  1024
SF           499.9300599 MHz
WDW                 SINE
SSB                    0
LB                  0.00 Hz
GB                     0
PC                  2.00

F1 - Processing parameters
SI                  1024
MC2                   QF
SF           499.9300000 MHz
WDW                 SINE
SSB                    0
LB                  0.00 Hz
GB                     0

2D NMR plot parameters
CX2                20.00 cm
CX1                15.00 cm
F2PLO               7.779 ppm
F2LO             3889.09 Hz
F2PHI               0.338 ppm
F2HI              168.85 Hz
F1PLO               0.040 ppm
F1LO               20.00 Hz
F1PHI              -0.040 ppm
F1HI               -20.00 Hz
F2PPMCM          0.37208 ppm/cm
F2HZCM         186.01190 Hz/cm
F1PPMCM          0.00533 ppm/cm
F1HZCM           2.66667 Hz/cm
```

26.5 Proton-carbon correlation through bonds (2D)

26.5.1 Direct correlation - HMQC

Purpose: Establish which protons and carbons are directly bonded to each other. The best experiment to use for small to medium sized molecules.

Standard parameter set: **ghmqc.***

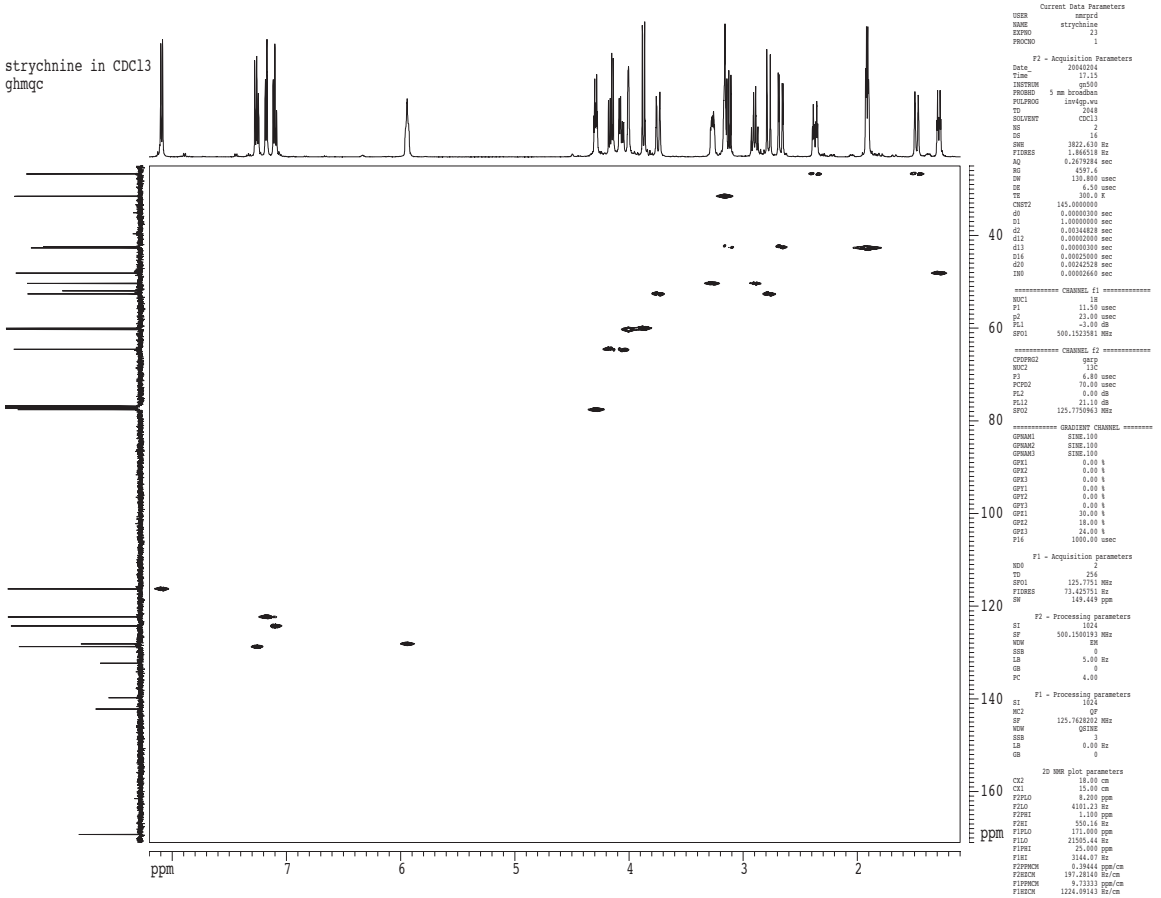
Gradient experiment: **ns = 1 x n**

Typical experiment duration: **11 minutes for 2 scans and 256 experiments**

The default parameters produce a spectrum from 15ppm to -1ppm in the proton domain and 230ppm to -10ppm in the carbon domain. To maximize the resolution of the 2D experiment it is important to optimize the spectral regions for both dimensions as described in chapter 5 using **copypars**. Resolution in the indirect dimension (F1) can be increased by acquiring more experiments. This parameter can most easily be adjusted in the acquisition parameter editor **eda**, by changing **td** in the F1 (right) column. On most instruments the standard value is **256**, but on the CRYO500 it is **512**.

Note: Reducing the width of the proton domain improves resolution and increases the acquisition time (**aq**). This experiment involves ^{13}C decoupling during data acquisition. If **aq** is too long the ^{13}C decoupling will result in sample heating which may cause chemical shifts to change so that the 1D and 2D spectra are no longer aligned. These effects can be reduced by increasing the relaxation delay between scans, **d1**. This delay is normally set at **1** second and should be increased to **2** seconds if a proton spectral width (**sw**) of less than **10ppm** is used.

Data processing: Use **getproj** to update projection information if necessary. Standard window functions in **edp** are exponential line broadening of 5Hz in the F2 dimension: **WDW = EM** and **LB = 5**, and shifted sine-squared in the F1 dimension: **WDW = QSINE** and **SSB = 3**. Use **xfb** to Fourier transform, followed by **absb** for baseline correction.



26.5.2 Direct correlation - HSQC

Purpose: Establish which protons and carbons are directly bonded to each other. Correlation peaks are smaller than HMQC, resulting in better resolution, but 2D spectrum requires phase correction.

Standard parameter set: **ghsqcp.***

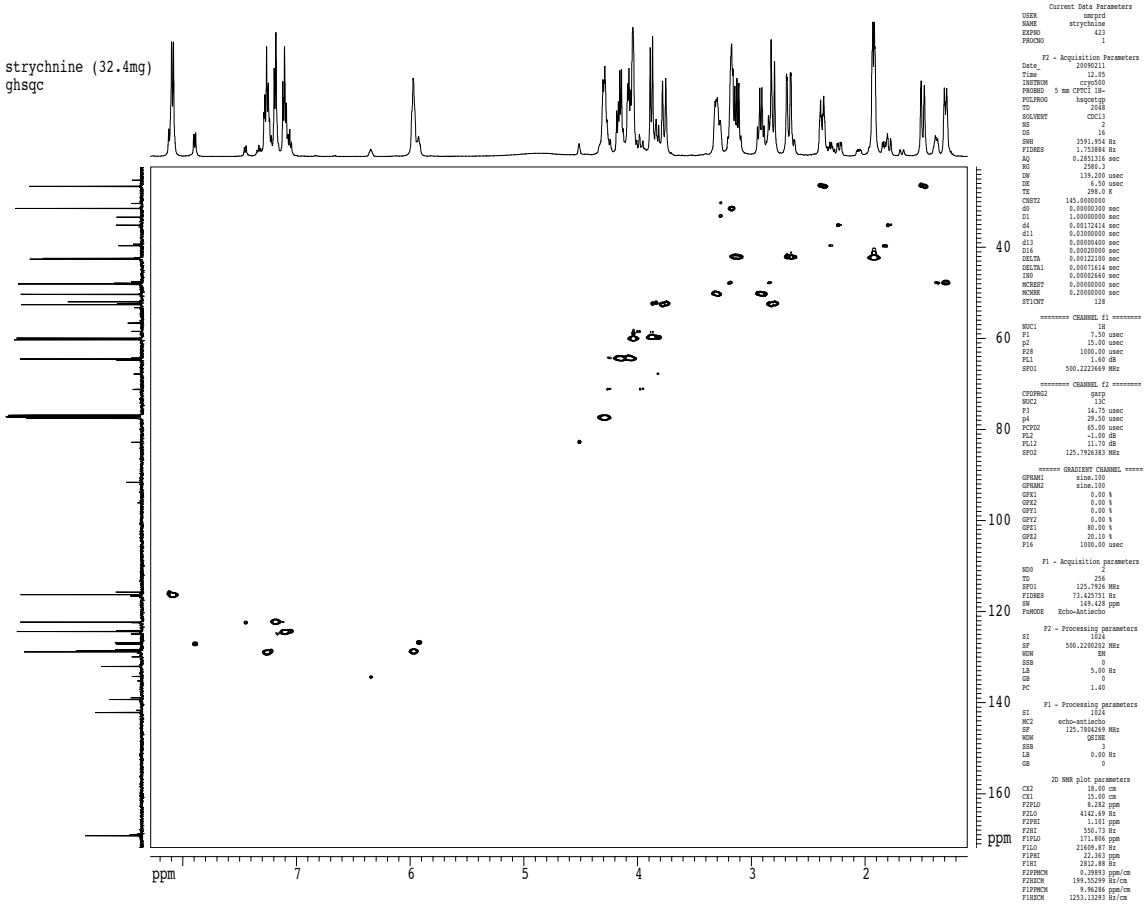
Gradient experiment: **ns = 2 x n**

Typical experiment duration: **11 minutes for 2 scans and 256 experiments**

The default parameters produce a spectrum from 15ppm to -1ppm in the proton domain and 230ppm to -10ppm in the carbon domain. To maximize the resolution of the 2D experiment it is important to optimize the spectral regions for both dimensions as described in chapter 5 using **copypars**. Resolution in the indirect dimension (F1) can be increased by acquiring more experiments. This parameter can most easily be adjusted in the acquisition parameter editor **eda**, by changing **td** in the F1 (right) column. On most instruments the standard value is **256**, but on the CRYO500 it is **512**.

Note: Reducing the width of the proton domain improves resolution and increases the acquisition time (**aq**). This experiment involves ^{13}C decoupling during data acquisition. If **aq** is too long the ^{13}C decoupling will result in sample heating which may cause chemical shifts to change so that the 1D and 2D spectra are no longer aligned. These effects can be reduced by increasing the relaxation delay between scans, **d1**. This delay is normally set at **1** second and should be increased to **2** seconds if a proton spectral width (**sw**) of less than **10ppm** is used.

Data processing: Use **getproj** to update projection information if necessary. Standard window functions in **edp** are exponential line broadening of 3Hz in the F2 dimension: **WDW = EM** and **LB = 3**, and Gaussian multiplication in the F1 dimension: **WDW = GM** and **GB = 0.3**. Use **xfb** to Fourier transform. The 2D spectrum must be phase corrected using the interactive **phase** screen. All peaks should be phased to be positive. Finally, baseline correct via **absb**.



26.5.3 Direct correlation – HETCOR

Purpose: Establish which protons and carbons are directly bonded to each other. Direct detection through carbon-13, resulting in lower sensitivity than the inverse experiments above. The advantage is better resolution in the carbon domain, so this can be a good choice when the carbon spectrum contains many close peaks.

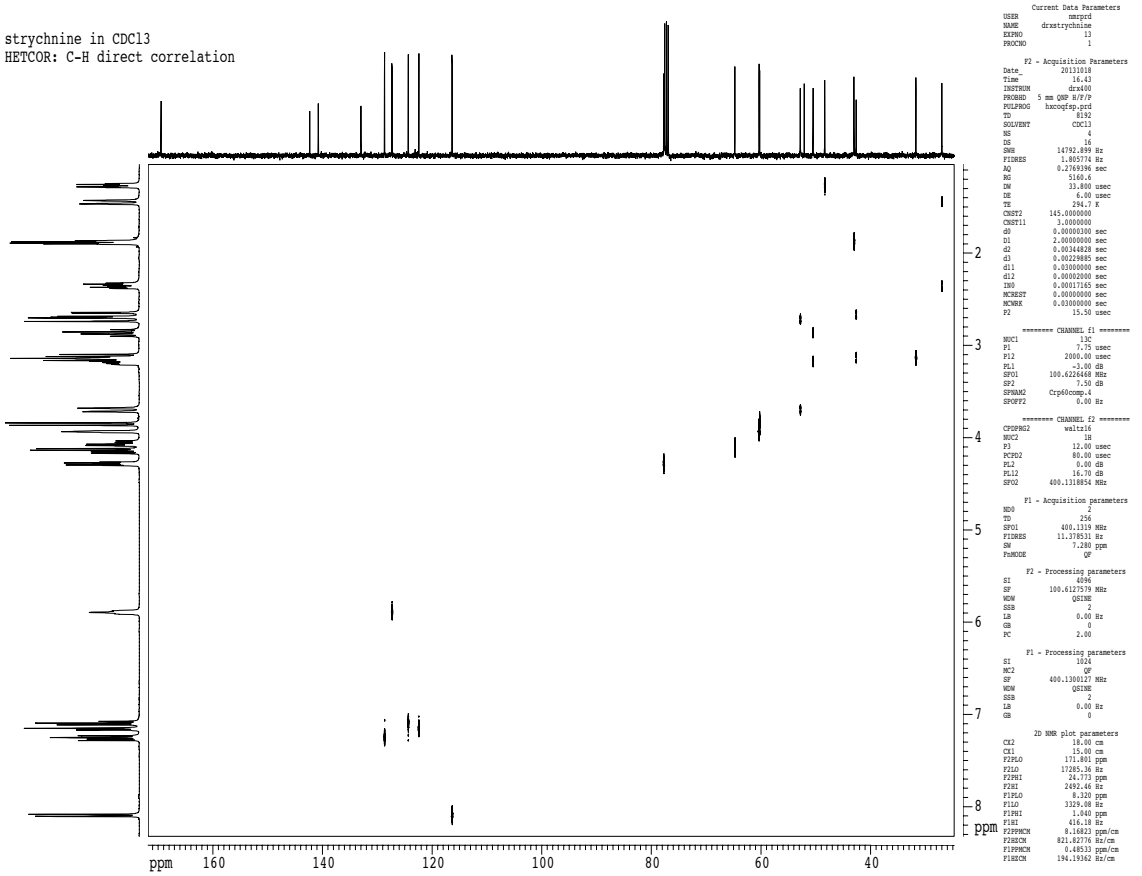
Standard parameter set: **hetcor.***

Gradient experiment: **ns = 4 x n**

Typical experiment duration: **41 minutes for 4 scans and 256 experiments**

The default parameters produce a spectrum from 230ppm to -10ppm in the carbon domain and from 15ppm to -1ppm in the proton domain. To maximize the resolution of the 2D experiment it is important to optimize the spectral regions for both dimensions as described in chapter 5 using **copypars**. Resolution in the indirect dimension (F1) can be increased by acquiring more experiments. This parameter can most easily be adjusted in the acquisition parameter editor **eda**, by changing **td** in the F1 (right) column. The standard value is **256**.

Data processing: Use **getproj** to update projection information if necessary. Standard window functions in **edp** are shifted sine-squared: **WDW = QSINE** and **SSB = 2** in both F2 and F1 dimensions. Use **xfb** to Fourier transform, followed by **absb** for baseline correction.



26.5.4 Long range correlation - HMBC

Purpose: Establish which protons and carbons are connected via multiple bonds.

Standard parameter sets: **ghmbc10Hz.* , ghmbc2Hz.***

Gradient experiment: **ns = 2 x n**

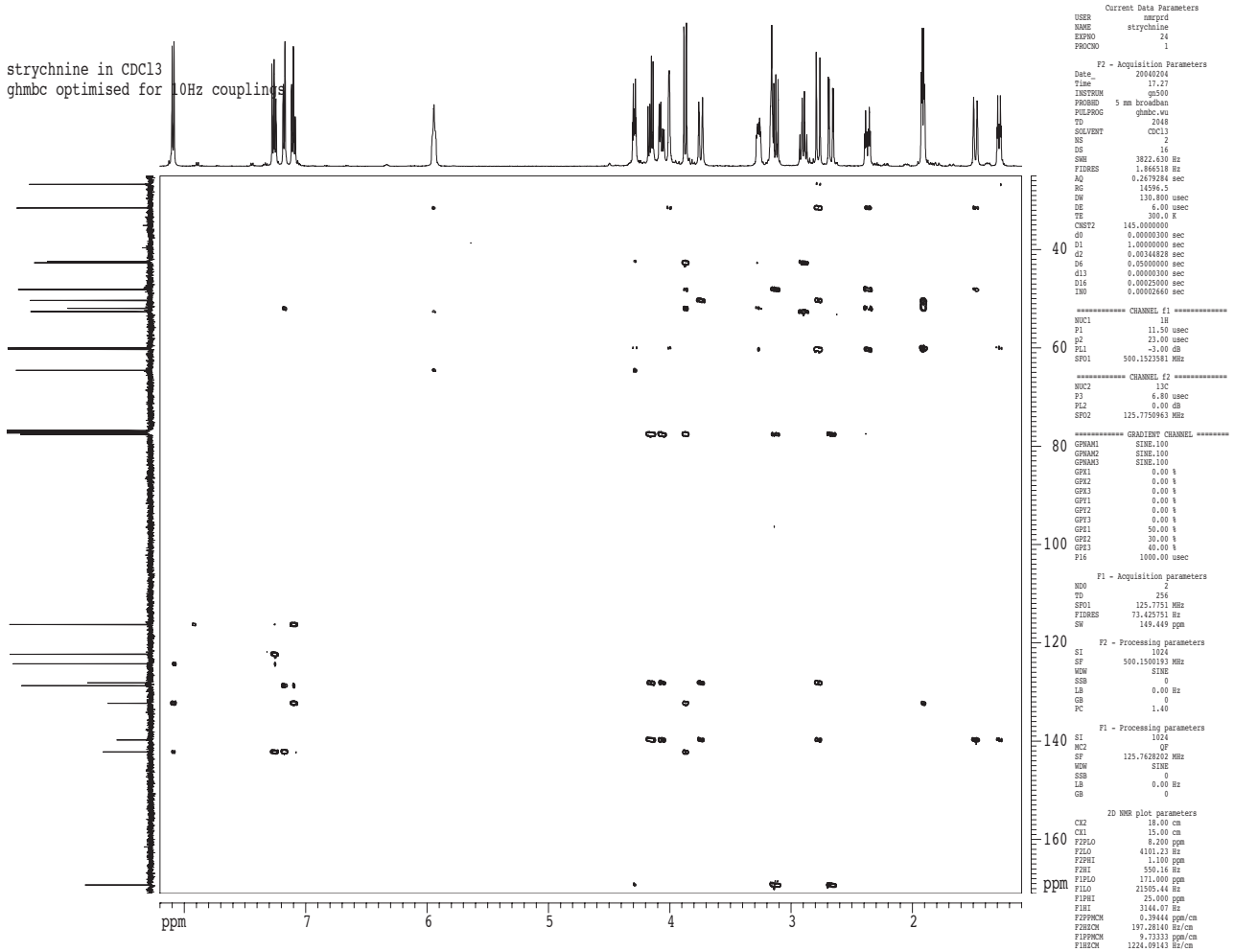
Typical experiment duration: **14 minutes for 2 scans and 256 experiments**

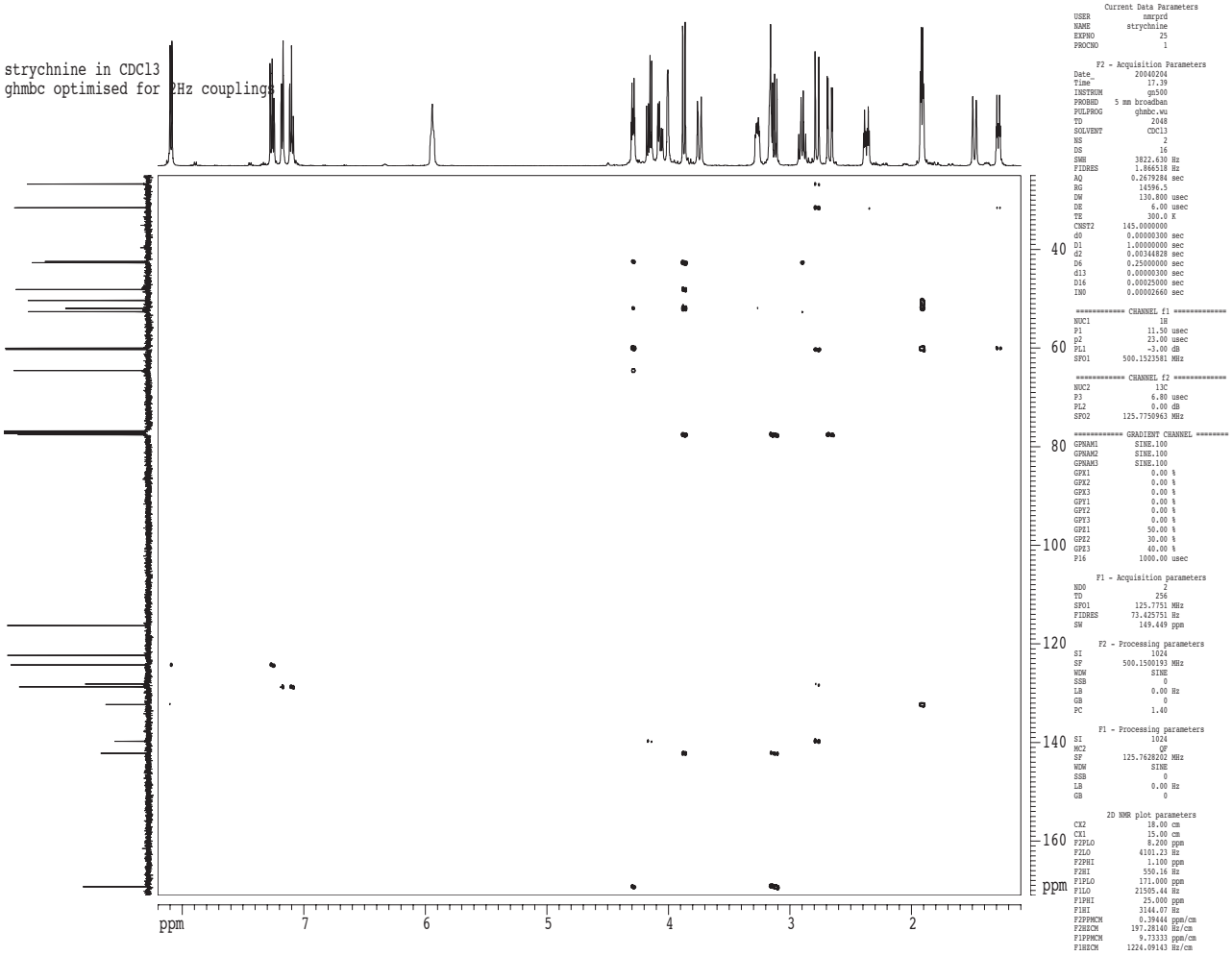
The default parameters produce a spectrum from 15ppm to -1ppm in the proton domain and 230ppm to -10ppm in the carbon domain. To maximize the resolution of the 2D experiment it is important to optimize the spectral regions for both dimensions as described in chapter 5 using **copypars**. Resolution in the indirect dimension (F1) can be increased by acquiring more experiments. This parameter can most easily be adjusted in the acquisition parameter editor **eda**, by changing **td** in the F1 (right) column. On most instruments the standard value is **256**, but on the CRYO500 it is **512**.

This experiment is selective via coupling constants. The **ghmbc10Hz.s** parameter set will yield correlations over 2 or 3 bonds and the **ghmbc2Hz.s** parameter set will yield correlations over 3 or 4 bonds. Selectivity can be adjusted by changing delay **d6** which is set to be 1/2J where J is the chosen coupling constant.

The experiment does not employ decoupling during data acquisition. This means that for strong signals direct proton-carbon couplings can also be observed, but they appear as doublets in the proton dimension positioned symmetrically about the proton peaks.

Data processing: Use **getproj** to update projection information if necessary. Standard window functions in **edp** are unshifted sine: **WDW = SINE** and **SSB = 0** in both F2 and F1 dimensions. Use **xfb** to Fourier transform, followed by **absb** for baseline correction.





Current Data Parameters

USER	nmrprd
NAME	strychnine
EXPNO	25
PROCNO	1

F2 - Acquisition Parameters

Date_	20040204
Time	17.39
INSTRUM	qn500
PROBHD	5 mm broadban
PULPROG	ghmbc.wu
TD	2048
SOLVENT	CDCl3
NS	2
DS	16
SWH	3822.630 Hz
FIDRES	1.866518 Hz
AQ	0.2679284 sec
RG	14596.5
DW	130.800 usec
DE	6.00 usec
TE	300.0 K
CNST2	145.0000000
d0	0.00000300 sec
D1	1.00000000 sec
d2	0.00344828 sec
D6	0.25000000 sec
d13	0.00000300 sec
D16	0.00025000 sec
IN0	0.00002660 sec

========== CHANNEL f1 ==========

NUC1	1H
P1	11.50 usec
p2	23.00 usec
PL1	-3.00 dB
SFO1	500.1523581 MHz

========== CHANNEL f2 ==========

NUC2	13C
P3	6.80 usec
PL2	0.00 dB
SFO2	125.7750963 MHz

========== GRADIENT CHANNEL =======

GPNAM1	SINE.100
GPNAM2	SINE.100
GPNAM3	SINE.100
GPX1	0.00 %
GPX2	0.00 %
GPX3	0.00 %
GPY1	0.00 %
GPY2	0.00 %
GPY3	0.00 %
GPZ1	50.00 %
GPZ2	30.00 %
GPZ3	40.00 %
P16	1000.00 usec

F1 - Acquisition parameters

ND0	2
TD	256
SFO1	125.7751 MHz
FIDRES	73.425751 Hz
SW	149.449 ppm

F2 - Processing parameters

SI	1024
SF	500.1500193 MHz
WDW	SINE
SSB	0
LB	0.00 Hz
GB	0
PC	1.40

F1 - Processing parameters

SI	1024
MC2	QF
SF	125.7628202 MHz
WDW	SINE
SSB	0
LB	0.00 Hz
GB	0

2D NMR plot parameters

CX2	18.00 cm
CX1	15.00 cm
F2PLO	8.200 ppm
F2LO	4101.23 Hz
F2PHI	1.100 ppm
F2HI	550.16 Hz
F1PLO	171.000 ppm
F1LO	21505.44 Hz
F1PHI	25.000 ppm
F1HI	3144.07 Hz
F2PPMCM	0.39444 ppm/cm
F2HZCM	197.28140 Hz/cm
F1PPMCM	9.73333 ppm/cm
F1HZCM	1224.09143 Hz/cm

26.6 Proton-proton correlation through space (NOE-1D/NOESY-2D/ROESY-2D)

All of the following experiments utilize the nuclear Overhauser effect. Nuclei which are close in space exhibit dipolar coupling and selective irradiation of one nucleus will affect the signal from a coupled nucleus.

26.6.1 DPFGSE-NOE experiment (1D)

Purpose: Establish which protons are close in space but not connected through bonds by observation of NOE enhancements. The Double Pulsed Field Gradient Spin Echo technique involves selective irradiation of a single proton and observation of the effect on the rest of the spectrum. Each signal of interest must be irradiated separately, and approximately eight times as many scans as required for a standard 1D experiment will be needed to acquire sufficient signal to noise from the very small NOE enhancements.

If a sample has many NOE interactions, performing a 2D NOESY experiment will be more efficient.

Standard parameter set: **gnoe.***

Phase cycling used: **ns = 8 x n**

Typical experiment duration: **25 minutes for 256 scans**

The NOE measurement requires the recording of a standard proton spectrum which is used both to determine the frequencies of the peaks of interest and in calculating the NOE enhancements. The procedure is as follows:

Record a standard proton spectrum as described previously.

It is necessary to load the exact frequencies of the peaks to be irradiated into the NOE experiment as follows.

Make a copy of the proton spectrum with a new experiment number, e.g. **cop 2** (if the initial experiment is experiment number 1).

Expand the spectrum on screen so that the peak to be irradiated is displayed clearly.

XwinNMR: **utilities** → **O1**, then move the mouse over the spectrum and press ***MMB*** when it is centered on the desired peak or multiplet.

TopSpin: At the top of the screen press the Set RF from cursor button:  , click on the desired peak or multiplet and then select **O1**.

Type **keepsfo1** to retain this frequency and load new parameters for the gnoe experiment, e.g. **gnoe.s**. In *TopSpin* do NOT close the keepsfo1 message box until AFTER loading the parameters.

The spectral width, **sw**, must be set to an appropriate value. The selected signal for irradiation is now at the center of the spectrum, so **sw** must be large enough to include the whole spectrum. The chemical shift of the center of the spectrum can be checked via the parameter **o1p**.

A suitable mixing time, **d8**, must be chosen. The standard parameter set contains a value of **0.5**, and typically this will lie between 0.1s and 2s.

Turn off sample spinning using **[SPIN ON/OFF]** then start the experiment with **rgazg**.

To run further NOE experiments on the same sample, load the standard proton again and repeat the steps above.

Data processing and calculation:

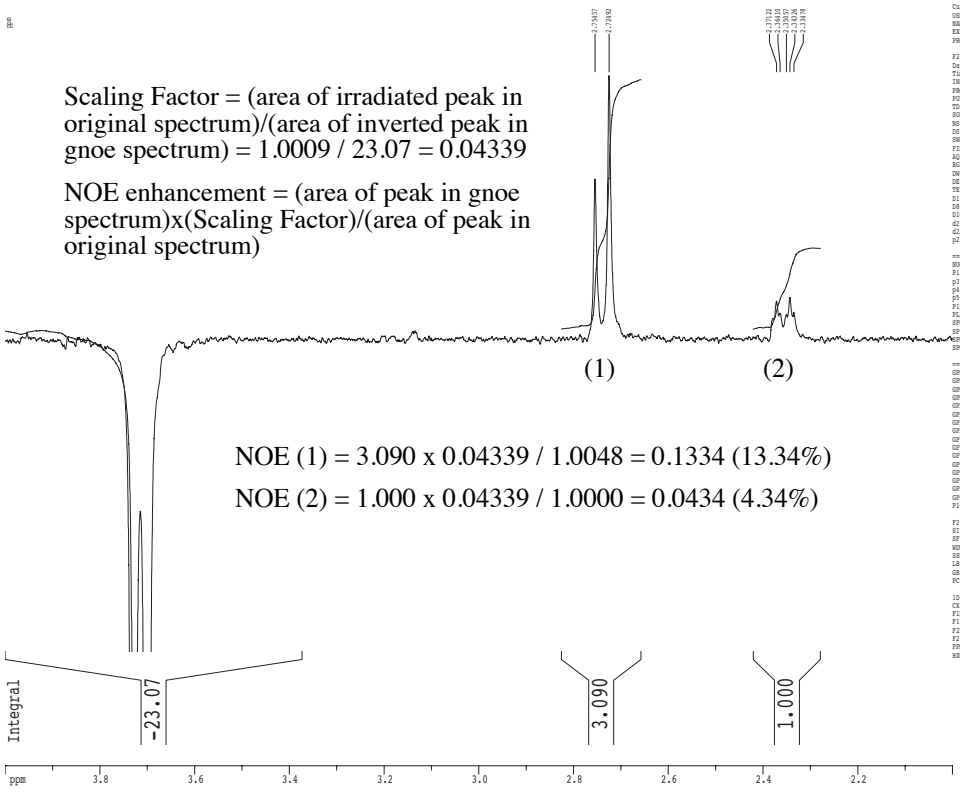
Process the two datasets using the standard techniques, paying particular attention to accurate phasing and baseline correction as the NOE enhancements may be very small. The gnoe spectrum should be phased so that the irradiated peak is negative and all other peaks are positive. If automatic phasing is used, the irradiated peak will be phased to be positive because it is the largest peak in the spectrum. The entire spectrum can be inverted using the command **invert**.

Integrate the inverted peak in the gnoe spectrum as well as the NOE signals. Recall the standard proton spectrum and carefully integrate the same peaks as were integrated in the gnoe spectrum. It is possible to do this automatically by saving the integral regions to a file and reading them into the first experiment, but this is not recommended as the standard spectrum will contain more peaks than the gnoe spectrum, and there is a risk of including parts of neighboring peaks into the integrals.

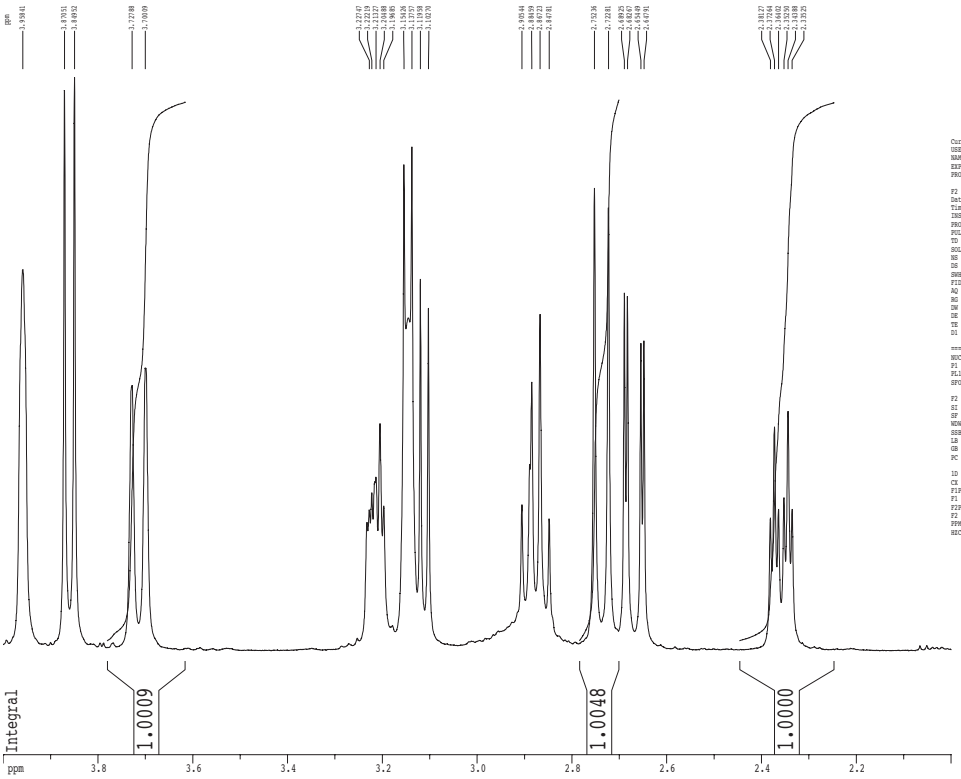
The integral values from the two experiments can be used to calculate the NOE enhancement for each peak. Note that this experiment is a transient method, rather than a steady-state technique, and so results may differ from NOE difference spectra.

The following example calculation shows an expanded region of the strychnine spectrum.

strychnine in CDCl3
gnoe



strychnine in CDCl3
1H spectrum



26.6.2 NOESY (2D)

26.6.2.1 Gradient NOESY

Purpose: Establish which protons are close in space but not connected through bonds by observation of NOE enhancements. This experiment is advantageous compared to the non-gradient version if sample quantity is sufficient to acquire good quality data with less than eight scans. For samples with a molar mass greater than 1000, ROESY should be used instead.

Standard parameter set: **gnoesyp.***

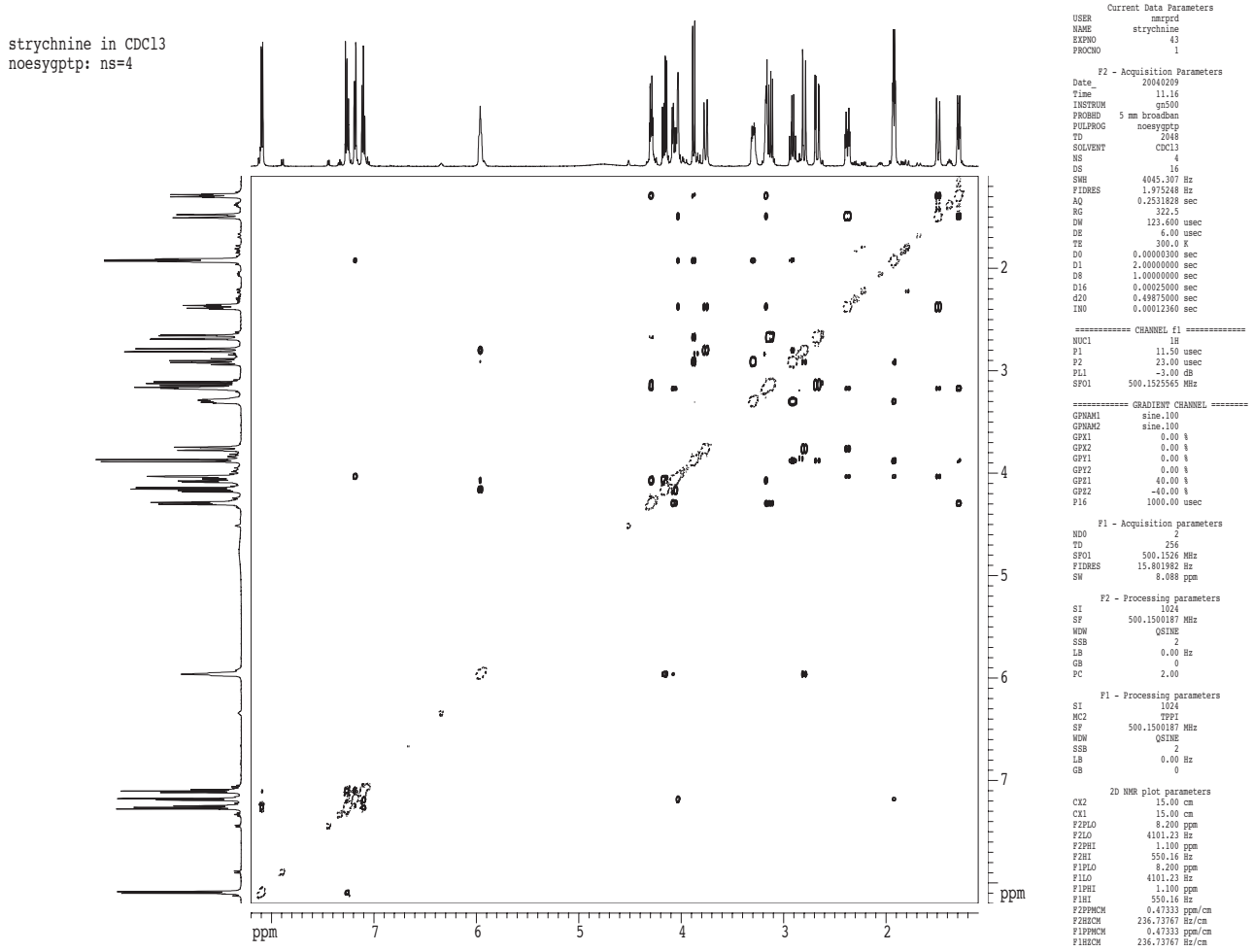
Gradient phase sensitive experiment: **ns = 2 x n**

Typical experiment duration: **58 minutes for 4 scans and 256 experiments**

The default parameters produce a spectrum from 15ppm to -1ppm, i.e. a spectral width of 16ppm (**sw = 16**), centered at 7ppm (**o1p = 7**). To maximize the resolution of the 2D experiment it is important to optimize the spectral region as described in chapter 5 using **copypars**. Resolution in the indirect dimension (F1) can be increased by acquiring more experiments. This parameter can most easily be adjusted in the acquisition parameter editor **eda**, by changing **td** in the F1 (right) column. On most instruments the standard value is **256**, but on the CRYO500 it is **512**.

It is important to choose an appropriate mixing time, **d8**. For small organic molecules, a value in the range 0.5s to 2s is appropriate.

Data processing: Use **getproj** to update projection information if necessary. Standard window functions in **edp** are shifted sine-squared: **WDW = QSINE** and **SSB = 2** in both F2 and F1 dimensions. Use **xfb** to Fourier transform, followed by phase correction. Phase the diagonal peaks to be positive. Positive NOE correlation signals will then be negative if the sample has a molar mass of less than 1000, negative NOE and chemical exchange signals will be positive. Finally use **absb** for baseline correction. The standard plot parameters are set up to print negative signals as solid lines and positive signals as dotted lines. The entire spectrum can be inverted using the command **invert**.




```
      Current Data Parameters
USER             nmrprd
NAME          strychnine
EXPNO                43
PROCNO                1

      F2 - Acquisition Parameters
Date_          20040209
Time              11.16
INSTRUM           qn500
PROBHD    5 mm broadban
PULPROG       noesygptp
TD                2048
SOLVENT           CDCl3
NS                   4
DS                  16
SWH            4045.307 Hz
FIDRES         1.975248 Hz
AQ             0.2531828 sec
RG                322.5
DW              123.600 usec
DE                6.00 usec
TE                300.0 K
D0           0.00000300 sec
D1           2.00000000 sec
D8           1.00000000 sec
D16          0.00025000 sec
d20          0.49875000 sec
IN0          0.00012360 sec

============ CHANNEL f1 ============
NUC1                 1H
P1                11.50 usec
P2                23.00 usec
PL1               -3.00 dB
SFO1        500.1525565 MHz

=========== GRADIENT CHANNEL =======
GPNAM1          sine.100
GPNAM2          sine.100
GPX1               0.00 %
GPX2               0.00 %
GPY1               0.00 %
GPY2               0.00 %
GPZ1              40.00 %
GPZ2             -40.00 %
P16             1000.00 usec

      F1 - Acquisition parameters
ND0                  2
TD                 256
SFO1          500.1526 MHz
FIDRES        15.801982 Hz
SW                8.088 ppm

      F2 - Processing parameters
SI                1024
SF          500.1500187 MHz
WDW               QSINE
SSB                  2
LB                0.00 Hz
GB                   0
PC                2.00

      F1 - Processing parameters
SI                1024
MC2               TPPI
SF          500.1500187 MHz
WDW               QSINE
SSB                  2
LB                0.00 Hz
GB                   0

      2D NMR plot parameters
CX2              15.00 cm
CX1              15.00 cm
F2PLO            8.200 ppm
F2LO           4101.23 Hz
F2PHI            1.100 ppm
F2HI           550.16 Hz
F1PLO            8.200 ppm
F1LO           4101.23 Hz
F1PHI            1.100 ppm
F1HI           550.16 Hz
F2PPMCM        0.47333 ppm/cm
F2HZCM       236.73767 Hz/cm
F1PPMCM        0.47333 ppm/cm
F1HZCM       236.73767 Hz/cm
```

26.6.2.2 Non-gradient NOESY

Purpose: Establish which protons are close in space but not connected through bonds by observation of NOE enhancements. For samples with a molar mass greater than 1000, ROESY should be used instead.

Standard parameter set: **noesyp.***

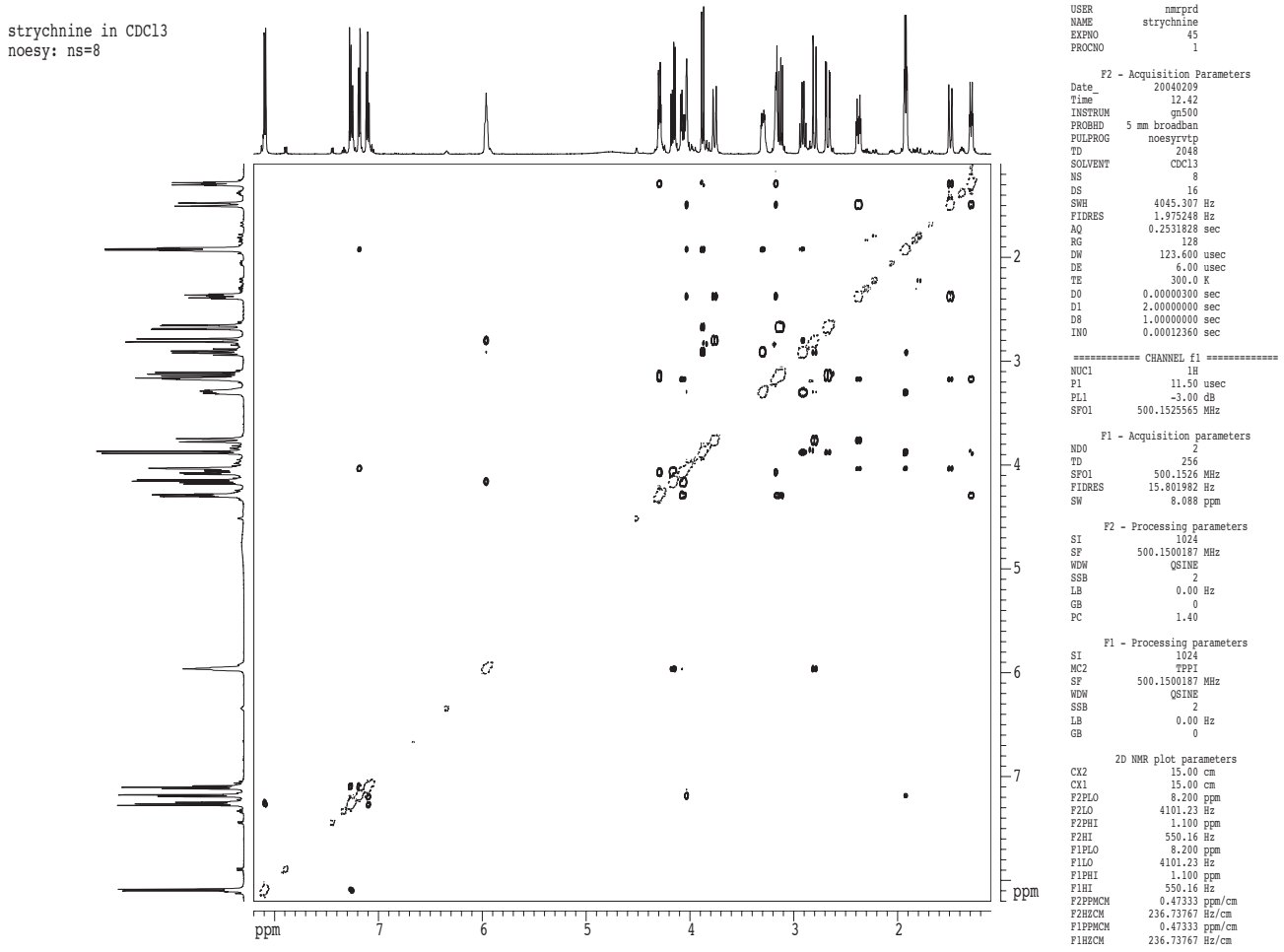
Phase sensitive experiment: **ns = 8 x n**

Typical experiment duration: **1 hour and 53 minutes for 8 scans and 256 experiments**

The default parameters produce a spectrum from 15ppm to -1ppm, i.e. a spectral width of 16ppm (**sw = 16**), centered at 7ppm (**o1p = 7**). To maximize the resolution of the 2D experiment it is important to optimize the spectral region as described in chapter 5 using **copypars**.

It is important to choose an appropriate mixing time, **d8**. For small organic molecules a value in the range 0.5s to 2s is appropriate.

Data processing: Use **getproj** to update projection information if necessary. Standard window functions in **edp** are shifted sine-squared: **WDW = QSINE** and **SSB = 2** in both F2 and F1 dimensions. Use **xfb** to Fourier transform, followed by phase correction. Phase the diagonal peaks to be positive. Positive NOE correlation signals will then be negative if the sample has a molar mass of less than 1000, negative NOE and chemical exchange signals will be positive. Finally use **absb** for baseline correction. The standard plot parameters are set up to print negative signals as solid lines and positive signals as dotted lines. The entire spectrum can be inverted using the command **invert**.




```
      Current Data Parameters
USER              nmrprd
NAME           strychnine
EXPNO                 45
PROCNO                 1

      F2 - Acquisition Parameters
Date_           20040209
Time              12.42
INSTRUM             qn500
PROBHD    5 mm broadban
PULPROG         noesyrvtp
TD                  2048
SOLVENT             CDCl3
NS                     8
DS                    16
SWH             4045.307 Hz
FIDRES          1.975248 Hz
AQ              0.2531828 sec
RG                   128
DW                123.600 usec
DE                  6.00 usec
TE                 300.0 K
D0            0.00000300 sec
D1            2.00000000 sec
D8            1.00000000 sec
IN0           0.00012360 sec

 ============ CHANNEL f1 ============
NUC1                  1H
P1                 11.50 usec
PL1                -3.00 dB
SFO1         500.1525565 MHz

      F1 - Acquisition parameters
ND0                    2
TD                   256
SFO1            500.1526 MHz
F1DRES          15.801982 Hz
SW                  8.088 ppm

      F2 - Processing parameters
SI                  1024
SF           500.1500187 MHz
WDW                 QSINE
SSB                    2
LB                  0.00 Hz
GB                     0
PC                  1.40

      F1 - Processing parameters
SI                  1024
MC2                 TPPI
SF           500.1500187 MHz
WDW                 QSINE
SSB                    2
LB                  0.00 Hz
GB                     0

      2D NMR plot parameters
CX2                15.00 cm
CX1                15.00 cm
F2PLO               8.200 ppm
F2LO             4101.23 Hz
F2PHI               1.100 ppm
F2HI              550.16 Hz
F1PLO               8.200 ppm
F1LO             4101.23 Hz
F1PHI               1.100 ppm
F1HI              550.16 Hz
F2PPMCM          0.47333 ppm/cm
F2HZCM         236.73767 Hz/cm
F1PPMCM          0.47333 ppm/cm
F1HZCM         236.73767 Hz/cm
```

26.6.3 ROESY (2D)

Purpose: Establish which protons are close in space but not connected through bonds by observation of NOE enhancements. Measuring the NOE in the rotating frame ensures that enhancements are always positive, irrespective of molecular weight. Results are similar to the NOESY experiment, except that under some circumstances small signals can result from TOCSY correlations. The ROESY experiment can also be quicker to perform as a shorter spin-lock period is used compared to the NOESY mixing time.

Standard parameter set: **roesyp.***

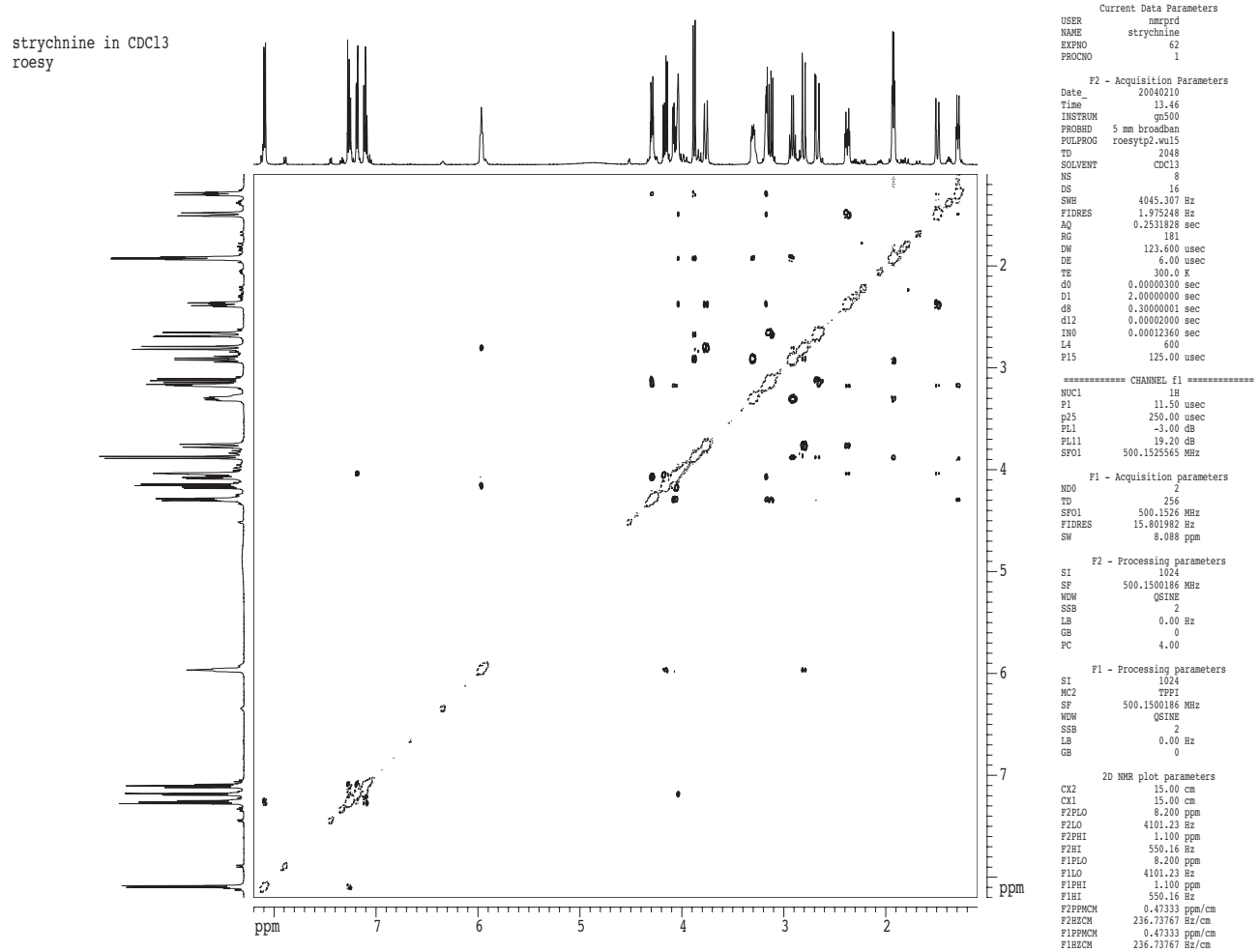
Phase sensitive experiment: **ns = 8 x n**

Typical experiment duration: **1 hour and 28 minutes for 8 scans and 256 experiments**

The default parameters produce a spectrum from 15ppm to -1ppm, i.e. a spectral width of 16ppm (**sw = 16**), centered at 7ppm (**o1p = 7**). To maximize the resolution of the 2D experiment it is important to optimize the spectral region as described in chapter 5 using **copypars**.

It is important to choose an appropriate spin-locking pulse, **p15**, in microseconds. This should be changed in the acquisition setup editor: **ased**. For convenience and backwards compatibility, this time is also displayed as **d8**, in seconds. Note For small organic molecules a value in the range 0.3s to 0.6s is appropriate.

Data processing: Use **getproj** to update projection information if necessary. Standard window functions in **edp** are shifted sine-squared: **WDW = QSINE** and **SSB = 2** in both F2 and F1 dimensions. Use **xfb** to Fourier transform, followed by phase correction. Phase the diagonal peaks to be positive. Positive NOE correlation signals will then be negative if the sample has a molar mass of less than 1000, negative NOE and chemical exchange signals will be positive. Finally use **absb** for baseline correction. The standard plot parameters are set up to print negative signals as solid lines and positive signals as dotted lines. The entire spectrum can be inverted using the command **invert**.



Current Data Parameters

USER	nmrprd
NAME	strychnine
EXPNO	62
PROCNO	1

F2 - Acquisition Parameters

Date_	20040210
Time	13.46
INSTRUM	qn500
PROBHD	5 mm broadban
PULPROG	roesytp2.wu15
TD	2048
SOLVENT	CDCl3
NS	8
DS	16
SWH	4045.307 Hz
FIDRES	1.975248 Hz
AQ	0.2531828 sec
RG	181
DW	123.600 usec
DE	6.00 usec
TE	300.0 K
d0	0.00000300 sec
D1	2.00000000 sec
d8	0.30000001 sec
d12	0.00002000 sec
IN0	0.00012360 sec
L4	600
P15	125.00 usec

========== CHANNEL f1 ==========

NUC1	1H
P1	11.50 usec
p25	250.00 usec
PL1	-3.00 dB
PL11	19.20 dB
SFO1	500.1525565 MHz

F1 - Acquisition parameters

ND0	2
TD	256
SFO1	500.1526 MHz
FIDRES	15.801982 Hz
SW	8.088 ppm

F2 - Processing parameters

SI	1024
SF	500.1500186 MHz
WDW	QSINE
SSB	2
LB	0.00 Hz
GB	0
PC	4.00

F1 - Processing parameters

SI	1024
MC2	TPPI
SF	500.1500186 MHz
WDW	QSINE
SSB	2
LB	0.00 Hz
GB	0

2D NMR plot parameters

CX2	15.00 cm
CX1	15.00 cm
F2PLO	8.200 ppm
F2LO	4101.23 Hz
F2PHI	1.100 ppm
F2HI	550.16 Hz
F1PLO	8.200 ppm
F1LO	4101.23 Hz
F1PHI	1.100 ppm
F1HI	550.16 Hz
F2PPMCM	0.47333 ppm/cm
F2HZCM	236.73767 Hz/cm
F1PPMCM	0.47333 ppm/cm
F1HZCM	236.73767 Hz/cm

26.7 Attached proton tests (1D)

Various experiments have been devised where an acquired carbon-13 spectrum has positive or negative peaks, according to the number of protons attached to each carbon. These methods have largely been superseded by 2D techniques like HMQC, which in general are quicker to perform and yield more information. A disadvantage of these 1D experiments is that selectivity is based on an average value for the proton-carbon coupling constant. Species with unusually small couplings can therefore yield peaks which are of the opposite sign to that expected for that proton multiplicity. The potential advantage of these experiments is higher resolution than is normally achieved in the carbon domain of 2D experiments.

26.7.1 DEPTQ

Purpose: Assign C, CH, CH_2 and CH_3 carbon peak positions. This is single experiment which yields almost as much information as the DEPT sequence. The spectrum will contain C and CH_2 peaks in one direction and CH and CH_3 peaks in the other. The deuterated solvent signals appear as C peaks and can be used as a reference for phase correction and calibration. Sensitivity is good for the non-protonated carbons.

Standard parameter sets: **c13deptq.***

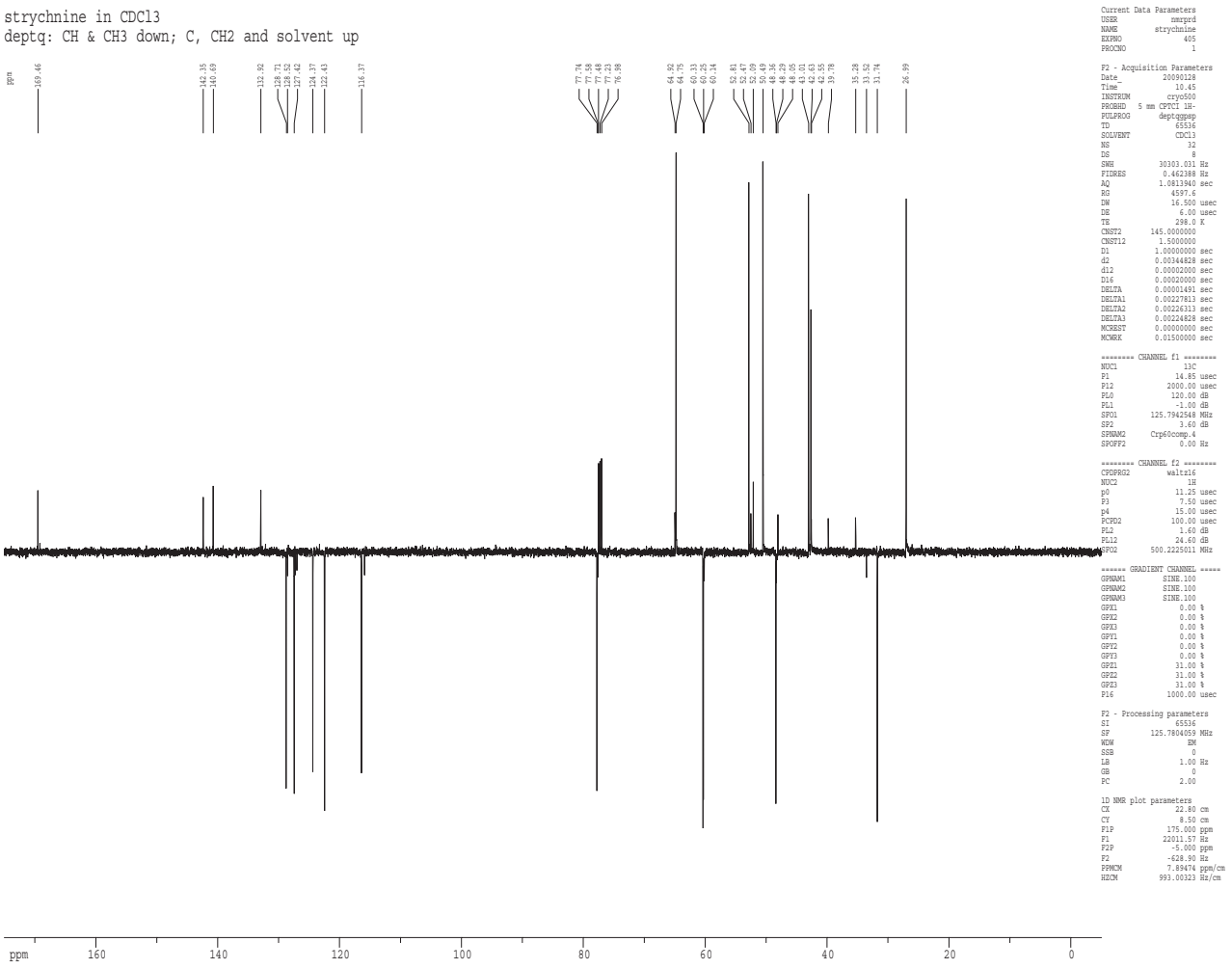
Phase cycling used: **ns = 8 x n** (not important if ns is large, >~256)

Typical experiment duration: **37 minutes for 1024 scans**

The default parameters produce a spectrum from 230ppm to -10ppm, i.e. a spectral width of 240ppm (**sw = 240**), centered at 110ppm (**o1p = 110**). The standard relaxation delay is one second (**d1 = 1**) which should be sufficient for observation of all signals. If some expected peaks are very small or do not appear, then **d1** can be increased.

This experiment uses 90 degree and 180 degree pulses, and so for best results both channels of the probe should be tuned.

Data processing: Process as for a standard carbon spectrum. The automatic phasing command (**apks**) will normally ensure that the tallest peak is positive. If the opposite phasing is required, the command **nm** can be used to invert the spectrum. The standard plot parameters are adjusted so that the spectrum baseline is positioned in the center of the page. This can be adjusted by altering **szero**.



Current Data Parameters
USER nmrprd
NAME strychnine
EXPNO 405
PROCNO 1

F2 - Acquisition Parameters
Date 20090128
Time 10.45
INSTRUM cryo500
PROBHD 5 mm CPTCI 1H-
PULPROG deptqqpep
TD 65536
SOLVENT CDCl3
NS 32
DS 8
SWH 30303.031 Hz
FIDRES 0.462388 Hz
AQ 1.0813940 sec
RG 4597.6
DW 16.500 usec
DE 6.00 usec
TE 298.0 K
CNST2 145.0000000
CNST12 1.5000000
D1 1.00000000 sec
d2 0.00344828 sec
d12 0.00002000 sec
D16 0.00020000 sec
DELTA 0.00001491 sec
DELTA1 0.00227813 sec
DELTA2 0.00226313 sec
DELTA3 0.00224828 sec
MCREST 0.00000000 sec
MCWRK 0.01500000 sec

======== CHANNEL f1 ========
NUC1 13C
P1 14.85 usec
P12 2000.00 usec
PL0 120.00 dB
PL1 -1.00 dB
SFO1 125.7942548 MHz
SP2 3.60 dB
SPNAM2 Crp60comp.4
SPOFF2 0.00 Hz

======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
p0 11.25 usec
P3 7.50 usec
p4 15.00 usec
PCPD2 100.00 usec
PL2 1.60 dB
PL12 24.60 dB
SFO2 500.2225011 MHz

====== GRADIENT CHANNEL =====
GPNAM1 SINE.100
GPNAM2 SINE.100
GPNAM3 SINE.100
GPX1 0.00 %
GPX2 0.00 %
GPX3 0.00 %
GPY1 0.00 %
GPY2 0.00 %
GPY3 0.00 %
GPZ1 31.00 %
GPZ2 31.00 %
GPZ3 31.00 %
P16 1000.00 usec

F2 - Processing parameters
SI 65536
SF 125.7804059 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 2.00

1D NMR plot parameters
CX 22.80 cm
CY 8.50 cm
F1P 175.000 ppm
F1 22011.57 Hz
F2P -5.000 ppm
F2 -628.90 Hz
PPMCM 7.89474 ppm/cm
HZCM 993.00323 Hz/cm

26.7.2 Conventional DEPT

Purpose: Assign C, CH, CH_2 and CH_3 carbon peak positions. For full assignment three separate datasets must be acquired. The DEPT experiments acquire signal which is transferred from the protons to the carbons, and so only carbons with protons attached are detected. A standard carbon spectrum must also be acquired in order to detect the quaternary carbons and the solvent signal for calibration. DEPT135 produces a spectrum with CH and CH_3 peaks positive and CH_2 peaks negative (by convention), and DEPT90 produces a spectrum containing only the CH peaks.

Standard parameter sets: **c13.* , c13dept135.* , c13dept90.***

Phase cycling used: **ns = 8 x n** (not important if ns is large, >~256)

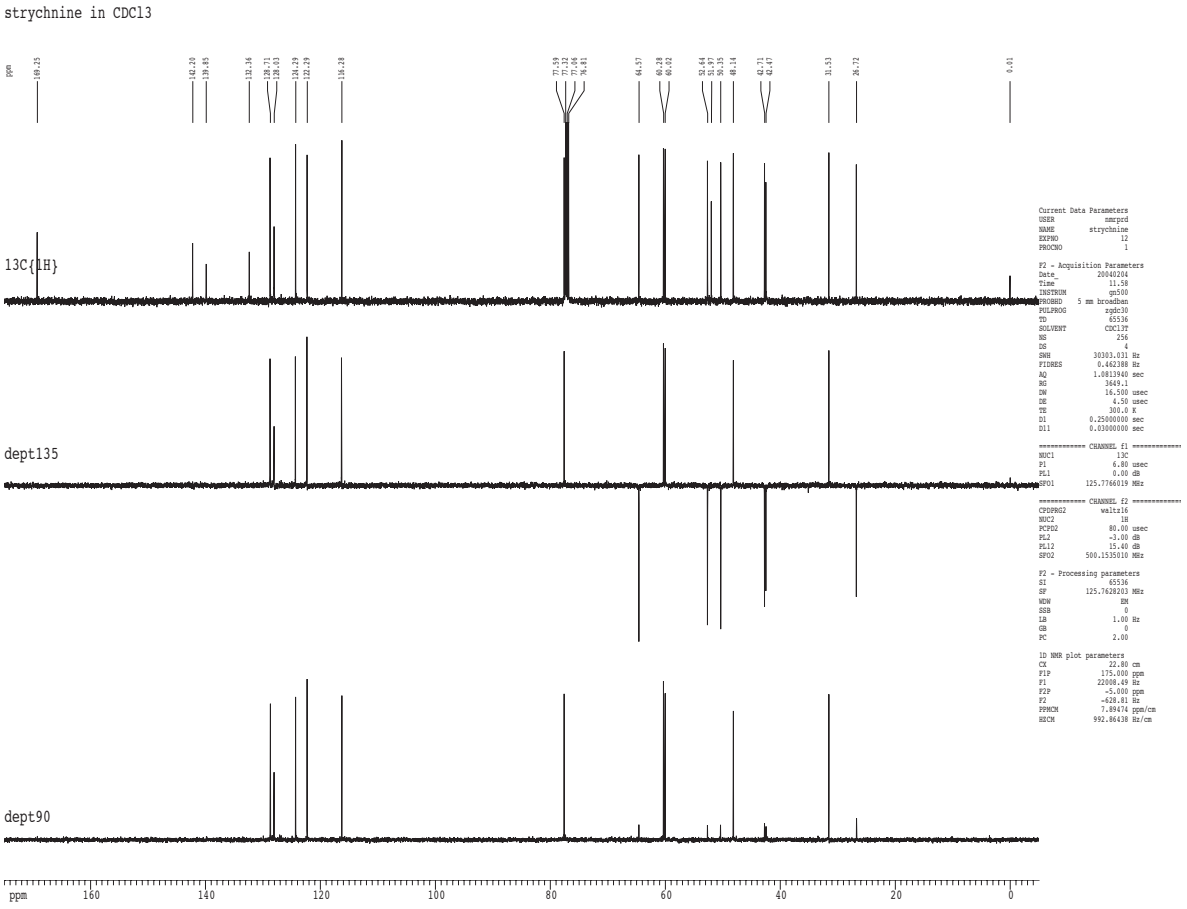
Typical experiment duration: **23 minutes for 1024 scans (c13.*), 27 minutes for 512 scans (c13dept135.*), 27 minutes for 512 scans (c13dept90.*)**

The default parameters produce a spectrum from 230ppm to -10ppm, i.e. a spectral width of 240ppm (**sw = 240**), centered at 110ppm (**o1p = 110**).

The standard carbon experiment, **c13.s**, is as described previously. The two DEPT experiments use a relaxation delay of 2 seconds (**d1 = 2**) as standard. The carbon peaks in the DEPT spectra are enhanced by signal transfer from the protons, and so in general good data will be acquired from half as many scans as is required for a good standard carbon spectrum.

The DEPT experiments use 90 degree and 180 degree pulses, and so for good results both channels of the probe should be tuned. If calibration is not correct, small residual signals will appear in the DEPT90 spectrum.

Acquire the standard carbon spectrum first using the standard method, **rga**, **zg**, etc. Set up the DEPT135 and DEPT90 experiments as the next two experiment numbers. Manually set the receiver gain (**rg**) in both experiments to the same value as was used in the standard carbon spectrum.



Data processing: Process the standard carbon spectrum as normal, using the command **efp**, followed by **apks** or manual phasing, and finally calibration. The two DEPT experiments can be automatically processed using the command **multiefpd**. The DEPT spectra will receive the same phase correction and referencing as the standard carbon spectrum. If the receiver gain was set for the DEPT experiments as described above the phasing of the spectra should be good, although it might require very small adjustments.

The spectra can be automatically plotted on one page. First set the plot limits, title, etc, for the standard carbon spectrum and then use one of the following programs: **d3vplot** (to preview and print to the local printer), **d3netvplot** (to preview and print to a network printer), and **d3psvplot** (to preview and save as postscript).

26.8 Solvent suppression techniques

There are several different methods for reducing the intensity of unwanted peaks from solvents in NMR spectra. Suitable peaks are those from H_2O/HOD in D_2O, and from water as an impurity in hygroscopic solvents like DMSO. The choice of technique is dependent on the size of the signal which is to be suppressed and on the kind of information which is to be extracted from the spectrum.

The pre-saturation technique uses a long low power pulse to saturate the solvent signal and remove it from the spectrum. The disadvantage of this method is that sample signals close to the solvent signal will also be reduced in intensity. The advantage of this method is that a clear quantitative spectrum can be obtained from all peaks separated from the solvent peak by approximately 1 or more ppm. Typical parameters are **h1presat.***. The width of the region of the spectrum affected by the pre-saturation pulse can be reduced by increasing the length and decreasing the power. The acquisition time should also be optimized to avoid recovery of the solvent signal during measurement of the FID.

Pulsed field gradients can also be used to remove the solvent signal. This method can give a more complete removal of the solvent signal, but frequently slight distortion of sample peaks will be observed across the spectrum. Typical parameters are **h1sup.***.

For both techniques it is necessary to accurately set the irradiation frequency at the frequency of the peak to be removed. This should be done in two stages:

First record a normal ^1H spectrum. Process as normal, the use **cop** to copy the spectrum to a new experiment number. Use **setsfo1** and **keepsfo1** as described in chapter 7 to set the observation frequency from this spectrum and then load it into the suppression experiment.

Next adjust this value accurately as follows:

Use a short acquisition time (**aq**), e.g. **1.5**, and a long relaxation delay (**d1**), e.g. **5**.

Go to the acquisition screen (**acqu**), then type **gs** ***<enter>*** to start 'go setup' mode. A single pulse is repeated, with each new FID replacing the previous one. Press **Ush** to separate the two components of the FID. Expand the data vertically and horizontally so that the FID shape can be observed.

The **XwinNMR-gs** window will be open but iconized behind the *XwinNMR* window. Open this window and use the slider to adjust **o1** in steps of 0.1Hz (the default setting).

Adjust **o1** to minimize the size of the FID, select **save** to save this frequency, then **stop** to stop pulsing.

Start the experiment with **rgazg**.

26.9 Observing and referencing X-nuclei other than carbon-13

Standard proton and carbon NMR is relatively easily because the whole of the expected chemical shift range can be observed in a single experiment. Many other nuclei have a much larger chemical shift range for which this is not possible, and it is necessary to find the signals of interest using approximate settings and then acquire an accurate spectrum of the region of interest. Two parameters must be considered during this process: the acquisition time (**aq**) and the relaxation delay (**d1**). The acquisition time is inversely proportional to the spectral width (**sw**). Thus an initial approximate experiment with a large spectra width may require a long relaxation delay, but when the spectral region is reduced to the signals of interest the acquisition time will increase and it may be possible to reduce the relaxation delay.

Spectral referencing is also easy for proton and carbon NMR, as a signal will usually be observed from the solvent or from TMS. This is internal referencing - the reference signal is part of the same solution as the sample signals. For most other nuclei this is not possible. One method is to record data from an separate standard sample, called external referencing. This can be a sample sealed within a capillary inside the NMR tube, but for 5mm sample tubes is more commonly a completely separate sample. The separate reference sample must be run in exactly the same magnetic field as the unknown sample.

The IUPAC approved method for referencing all NMR spectra is to use the absolute frequency of 0ppm in the proton frequency to calculate the reference frequency for spectra from all other nuclei. For the best accuracy the proton spectrum should be referenced using TMS (or DSS for aqueous samples). This can be applied automatically as follows.

The proton spectrum must be acquired in the current session, with the same lock and shim conditions as the X-nucleus spectrum. Calibrate the proton spectrum, then type:

xiref ***<enter>***

The computer will request the experiment number and process number for the proton spectrum. Messages will appear on screen relating to the nuclei being referenced and the equivalent reference standard. The command can be used to reference both 1D and 2D data. Carbon-13 spectra can also be referenced by this method, and the computer will ask if the sample is in an organic solvent for TMS referencing, or aqueous for DSS referencing. The calibration is slightly different in these two cases.

This method is called the Ξ (pronounced 'xi' scale) and should be described as such when reporting data. If a secondary method, such as the solvent signal instead of TMS/DSS, is used for the proton calibration, then this should also be reported. A table of Ξ values for various nuclides can be found in Appendix C.

If the GN500 is used to observe another nucleus it is essential that the probe is carefully retuned to carbon-13 afterwards. A blank sample of deuterated chloroform is provided by the GN500 and should be loaded into the probe before retuning.

26.9.1 Deuterium

Deuterium can be observed using the lock channel on all the spectrometers, so probe tuning is not required. The chemical shift range is the same as for protons, and the same signals can be used for spectrum calibration. However, experiment times can be long as the relative sensitivity is only 0.61 compared to carbon-13. Deuterium is a quadrupolar nucleus with spin 1, and so peaks will tend to be broad. Short acquisition times, **aq**, can therefore be used to accurately measure the FID, but the time between pulses should be maintained at a minimum of approximately 6 seconds by increasing the relaxation delay **d1**. Spectra can be acquired with or without proton decoupling using the parameter sets **h2.*** or **h2h1dec.***.

The design of the inverse cryoprobe used on the CRYO500 can result in a large 'spike' at the beginning of the deuterium FID. Use **rgac** to set the receiver gain to ignore the 'spike' and set a value appropriate to the sample signal.

Observation via the lock channel requires long pulses and so will not be suitable for all experiments. Consult the facility director before performing critical experiments to determine the most suitable technique. Occasionally the lock switching hardware is problematic.

Observation via the probe X-channel requires that the lock must be disconnected and that the ^{2}H filter must be removed from the preamplifier input.

If deuterium has been observed using the lock channel the lock will be disabled until it is reset. Load parameters for a standard experiment, such as ^{1}H or ^{13}C, then type **ii** ***<enter>*** to reset the lock. This is particularly important if the deuterium experiment is the last one performed, as the lock will be disabled for the next user.

26.9.2 Boron-11

Boron-11 is an easy nucleus to observe as sensitivity is high (10.69 relative to carbon-13) and spin-lattice relaxation is rapid. The full chemical shift range, +100ppm to -100ppm, can easily be observed in a single experiment. Spectra can be acquired with or without proton decoupling, and the standard parameter set **b11.*** uses decoupling. The GN500 BBO probe (manual tuning) and AVANCE600 BBFO cryoprobe (automatic tuning) can be used to observe boron-11.

There is a very broad background signal from boron nitride used in the construction of the NMR probe, and if a standard borosilicate glass NMR tube is used, a broad signal from this will also be observed. The **b11bs** parameters can be used to dramatically reduce the probe signal, and quartz NMR tubes can be used which do not contain boron. Alternatively, a 'blank' experiment can be performed and subtracted from the sample spectrum.

26.9.3 Nitrogen-15

Nitrogen-15 is a difficult nucleus to observe. Sensitivity is low (0.065 relative to carbon-13) and the chemical shift range is very large, 1000ppm. This range is too wide to observe accurately in a single experiment. A high sample concentration is required, particularly if the molecular weight is high. An additional complication is spectrum calibration, as historically two different reference samples have been used, both assigned as 0ppm. Chemists have traditionally used neat nitromethane (CH_3NO_2), and biologists have traditionally used liquid ammonia. The chemical shift of nitromethane relative to liquid ammonia is +380.2ppm, so care should be exercised when attempting to reproduce published data.

The Ξ scale method is equivalent to referencing using liquid ammonia.

Proton decoupling can be used, but peak intensities can be decreased by a negative nOe effect resulting from the negative gyromagnetic ration of ^{15}N. A series of regularly spaced artifact 'spikes' normally appear the spectrum, which should not be confused with real signals. Transfer the data regularly to check for real signals. The artifacts are very narrow spikes and have random phase and so they appear and disappear as the experiment progresses. The GN500 BBO probe (manual tuning) and AVANCE600 BBFO cryoprobe (automatic tuning) can be used to observe nitrogen-15.

There are no general parameter sets due to the wide chemical shift range. Consult the facility director about your requirements.

26.9.4 Fluorine-19

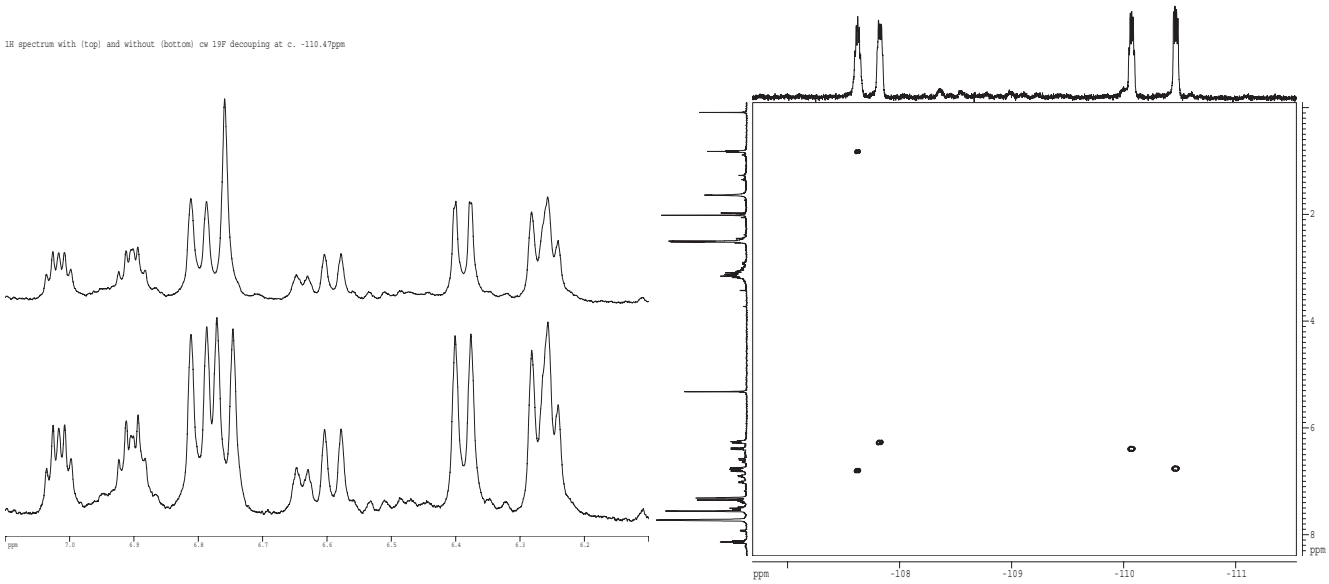
Fluorine-19 has a much higher resonance frequency than most other commonly observed X-nuclei, and so cannot be observed using the standard tunable probes. Fluorine-19 data can only be obtained on the DRX400 spectrometer using the switchable probe and the AVANCE600 using the cryoprobe. Sensitivity is nearly as good as for protons, but the chemical shift range is much wider. Most organic compounds will yield signals in the range 0ppm to -300ppm, but shifts can be as high as +600ppm. Proton decoupling can be used if desired. Sensitivity is high, 52.20 relative to carbon-13.

The AVANCE600 can observe the full standard ppm range of +10 to -300 in a single experiment, but the DRX400 is limited to a maximum spectral width for ^{19}F of 200ppm. On the DRX400 there are parameter sets for two different ranges, 0ppm to -200ppm and -100ppm to -300ppm, with and without proton decoupling. Filenames are **f19_0to-190.q** ,

f19_to-300.q, **f19dec0-190.q**, and **f19decto-30.q**. On the AVANCE600 the choices are **@f19.c** and **@f19dec.c** for the full range.

Peaks in fluorine spectra can be very narrow, and so it is often necessary to optimize the spectral region to record peak shapes accurately. This particularly important if coupling patterns are to be interpreted. First use one of the parameter sets above to find the same peaks. Expand the spectrum using ***LMB*** and ***MMB***, then use **sw-sfo1** to update the acquisition parameters to the displayed region. Reducing the spectral width will result in an extended acquisition time, **aq**. Press **Fid** to observe the length of decay of the signal and estimate a suitable acquisition time to record the data accurately. Normally an acquisition time in the range 1s to 2s is suitable. Press **Re** to return to the spectrum display.

Fluorine-proton interactions can be examined further via one or two dimensional experiments.



For the top spectrum, the parameter set **h1f19cwdec.q** was used, with the parameter **SFO2** set to the frequency in MHz of the desired fluorine resonance.

2D correlation using the parameter set **f19h1cor.q**. Spectral regions should be optimized and the parameter **cnst2** set to a typical H-F coupling constant. Here 10Hz was used.

26.9.5 Silicon-29

Silicon-19 has low sensitivity (0.49 relative to carbon-13) and spin-lattice relaxation is slow. The full chemical shift range, +200ppm to -400ppm, can be observed in a single experiment. Inverse gated proton decoupling is normally used to enhance signals. A broad background signal will be observed from the glass in the probe and from the NMR tube between -80ppm and -300ppm. This can be reduced by narrowing the region of interest around the sample signal. The standard parameter file **si29.*** has a relaxation delay of two seconds.

Spectra can be acquired with or without proton decoupling. The GN500 BBO probe (manual tuning) and AVANCE600 BBFO cryoprobe (automatic tuning) can be used to observe silicon-29.

26.9.6 Phosphorus-31

Phosphorus-31 can be observed on all three spectrometers and has high sensitivity (4.17 relative to carbon-13). The DRX400 probe will automatically switch to 31P when required, the AVANCE600 cryoprobe will tune automatically, but the GN500 BBO probe must be manually tuned. Phosphorus-31 is relatively easy to observe. Sensitivity is approximately four times that of carbon-13, and the full spectral range can be observed in a single experiment. Standard parameters are available without proton decoupling, **p31.***, and with proton decoupling, **p31dec.***. Spin-lattice relaxation times can be long, a time of a minute or more between pulses may be required for quantitative spectra.

Peaks are generally very narrow and so better resolution can be obtained by narrowing the spectral region to the area of interest. This is particularly important if proton couplings are being measured. Two dimensional proton-phosphorus correlation experiments can also be performed.

26.9.7 Other nuclei

Most NMR active nuclei can be observed using the GN500 and AVANCE600 instruments. The GN500 BBO probe can be tuned to any nucleus between silver-109 and phosphorus-31. The AVANCE600 BBFO cryoprobe will tune automatically to any nucleus in the range from nitrogen-15 to phosphorus-31, plus fluorine-19, except for the frequency range between europium-153 and mercury-199. If a parameter set already exists for the nucleus of interest, then the procedure is as described above. If parameters are needed for a new nucleus, discuss your requirements with the facility director. Normally

a sample of a standard reference compound will be required in order to check the spectrometer frequency is set correctly and to calibrate pulse lengths.

26.10 Diffusion ordered spectroscopy (DOSY)

Pulsed field gradient NMR can be used to measure translational diffusion of molecules. A series of experiments can be performed and combined as a pseudo-2D experiment for measurement of diffusion constants. Good sample temperature stability is essential, otherwise convection currents within the sample will disrupt the measurement. The best solvents for DOSY experiments are D_2O and DMSO, where convection is not a problem.

The following experiments are available on the CRYO500 and AVANCE600 only. The AVANCE600 has a much better gradient amplifier than the CRYO500. Both instruments can be used to differentiate between compounds by diffusion, but diffusion constants will be more accurate when measured on the AVANCE600.

If measurements are made in H_2O or D_2O, external calibration is not necessary. The known diffusion constant for water can be used.

26.10.1 Optimizing parameters

Load sample and tune the proton channel of the probe. Allow the sample temperature to equilibrate, load standard proton parameters, lock and shim.

Acquire a proton spectrum using the standard parameters, then use **edcp** to create a new DOSY experiment by loading the parameters **dosy1D.c** (CRYO500) or **@dosy1D.c** (AVANCE600).

Use **copypars** as described in chapter 7 to update the spectral window.

The pulse program included in these parameters is **ledbpgp2s1d**. This is a 1D version of the Longitudinal Encode-Decode sequence with Bipolar Gradient pulse Pair. If desired a different pulse sequence can be used, but it should be the 1D version of the 2D sequence that will be used later.

During the diffusion experiment the gradient strength will be varied. Suitable values must be chosen for the diffusion time, Δ, and the diffusion gradient length, δ, so that experiments are performed which will accurately map the decay curve. The parameters corresponding to Δ and δ may be pulse program dependent. This can be checked by listing the pulse program with **edcpul**. In the pulse program **ledbpgp2s1d**, Δ is **d20** and δ is **p30**. These parameters can be easily accessed using the acquisition setup editor, **ased**.

Set Δ to **75ms** and δ to **1ms**, and set the gradient strength, **gpz6** to **2%**.

Run the experiment, then type **iexpno** to set up an identical experiment with the next experiment number.

Change **gpz6** to **95%** and then run the second experiment.

Load the first experiment and then use **dual** display mode (*XwinNMR*) or multiple dataset display (*TopSpin*) to compare the intensities of the two spectra. The signal intensity of the second spectrum should be approximately 5% of the first, i.e. the signal has been attenuated by 95% due to diffusion. The smallest signal in the second spectrum should still be clearly detectable above the noise. If it is not, repeat both experiments with more scans.

If the signal in the second spectrum has disappeared completely, reduce the gradient strength. If the signal is too big, either increase Δ or δ. It is better to increase δ, as the effect is greater.

26.10.2 Set up and run the 2D experiment

Use **makedosy2d** to set up a new experiment, which will be converted into the pseudo two dimensional experiment, with the incremented gradient strength creating the second dimension. The same command works in both *XwinNMR* and *TopSpin*, but slightly different operations are performed.

The new experiment number for the 2D DOSY will be suggested and can be changed if necessary. In *TopSpin* the program will complete with no user intervention, except to specify the projection experiment number, but in *XwinNMR* the following is required:

Press **Seen** on the message box stating: Existing parameterfiles and PARMODE inconsistent. eda changed to EDA2D

Save the edpar eda screen.

OK to Delete the 'meta.ext' files?

The above program should convert the new experiment to 2D, set the **FnMODE** to **QF**, remove the last two characters from the pulse program name (to change it from 1D to 2D), change **PH_mod** in F2 column to **pk**, and change **SI** in F1 to **256** (suitable if 16 experiments are being acquired). If any of these settings are not desired, they can be manually changed.

The automation program **dosy** is used to define the gradient ramp and generate the 2D dataset. Various options can be selected and added as arguments to the command: first gradient value, last gradient value, number of gradient steps, type of ramp (linear **l**, squared **q** or exponential **e**), whether to start the experiment immediately, whether to execute **rga**.

For example, typing: **dosy 2 95 16 l y y**

uses a first value of 2%, last value of 95%, 16 steps, linear ramp, start acquisition, execute rga first.

26.10.3 Processing DOSY data

The phase parameters should be correct as they were inherited from the 1D experiments above, provided the receiver gain setting is the same for both experiments. The 2D phasing can be checked. and updated if necessary, by the extracting the first FID from the 2D dataset.

rser 1 *<enter>*

The first FID is extracted as a new 1D file with the experiment name **~TEMP**.

Process this dataset as a normal 1D spectrum, e.g. with **efp**, then enter the manual phase screen. Optimize the phasing carefully, then save the resultant phase parameters directly to the 2D DOSY experiment:

XwinNMR: Exit the phase screen with **return** → **Save as 2D & return**, then **File** → **Recall last** → **Last 2D data set** to reload the 2D DOSY experiment onto the screen.

TopSpin: Exit the phase screen with **Save for nD spectrum**, then use any method to reload the 2D DOSY experiment onto the screen.

If **makedosy2d** above was used to set up the pseudo 2D experiment, then the following settings should already be correct. If not, type **edp** and change **PH_mod** in the F2 column to **pk**. Use zero filling in the F1 dimension for data processing. If 16 experiments have been performed, change **SI** in the F1 column to **256**. Then **SAVE** to exit from the editor. Type **getproj** to load information about the F2 projection spectrum.

The F1 projection will be printed as a calculated spectrum from the 2D DOSY experiment. This can be turned off if it is not desired.

It is important that the previous steps are performed before processing the data.

Type **xf2** to Fourier transform all the spectra in the F2 dimension, followed by **abs2** to baseline correct.

Type **setdiffparm** to prepare the DOSY parameters for processing.

Open the DOSY parameter editor with **eddosy**.

Various settings can be adjusted in this window. The most important initially are **Imode**, which switches the intensity mode between peak areas or heights, and **Scale**, which switches the scaling between linear and logarithmic.

The lower and upper display limits can also be entered here, or calculated automatically. For automatic calculation, **SAVE** the editor settings, then type **dosy2d setup**.

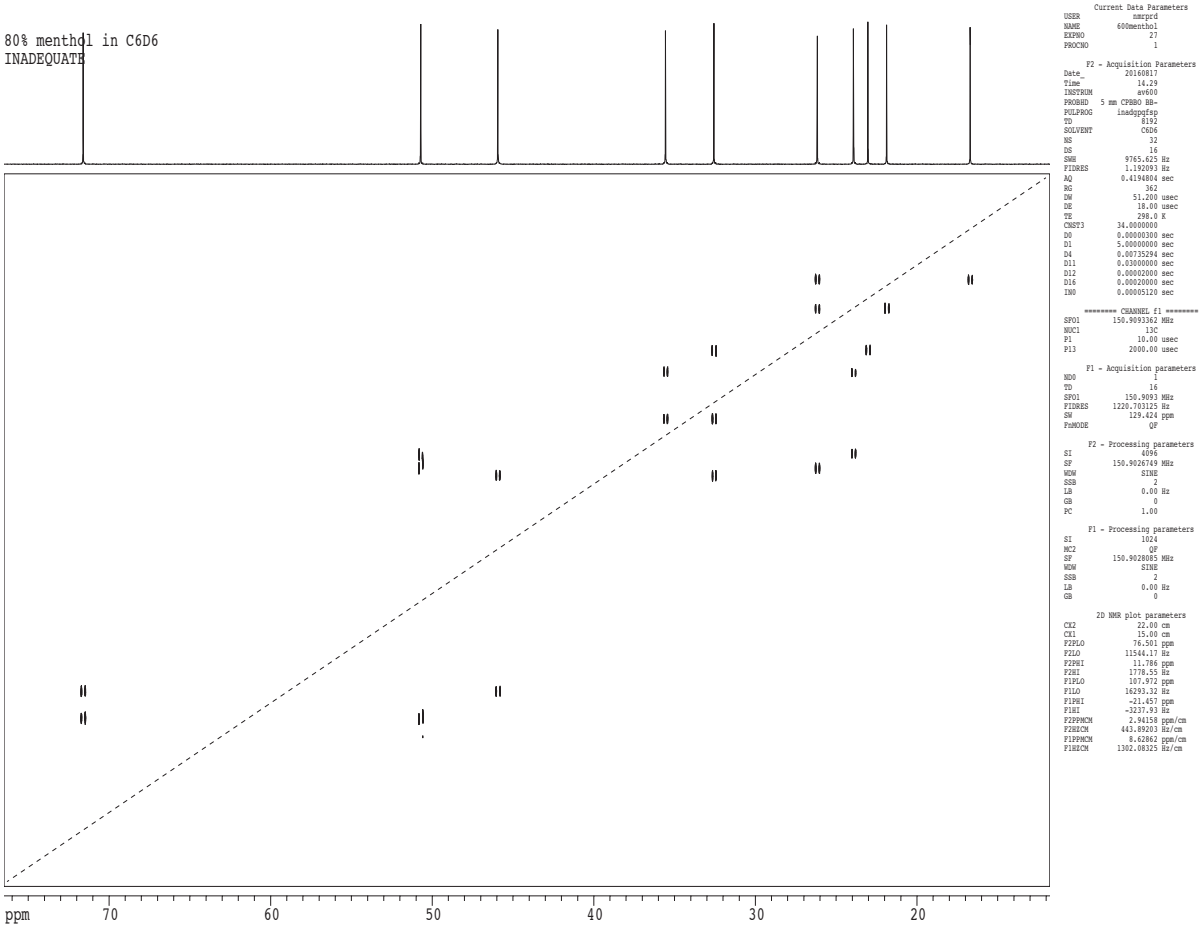
Process the DOSY data by typing **dosy2d**.

26.11 Carbon-carbon correlation through bonds (INADEQUATE)

INADEQUATE is inherently an insensitive experiment, as only ^{13}C-^{13}C pairs are observed. This represents 0.01% of the carbon in a natural abundance sample. Two modern advances make this experiment possible with standard sample concentrations. One is the high sensitivity of a cryoprobe. The other is non-uniform sampling (NUS). NUS enables measurement of a fraction, typically 25%, of the datapoints in a 2D array and the prediction by calculation of the remaining data.

The AVANCE600 is the only instrument equipped both with a cryoprobe and software capable of running NUS experiments.

Careful preparation is necessary. Initially to check that sample concentration is sufficient and then to measure the ^{13}C T_1s to determine the required relaxation delay.



This example spectrum was acquired in only 48 minutes on the AVANCE600, but used a very concentrated sample, an 80% solution of menthol in benzene. The carbon-13 T_1s are short, allowing a relaxation delay of only 5 seconds, and the chemical shift range is small. Only 16 F1 increments were employed, and NUS was used to calculate 64 experiments to generate the indirect dimension.

To generate a complete spectrum as above, the spectral width in the indirect dimension (F1) must be double that in the observation dimension (F2). This requires a significant number of increments to provide sufficient resolution and usually results in considerable empty space in the spectrum. It does, however, allow a diagonal line to be drawn across the spectrum which bisects the horizontal pairs of signals that show the carbon-carbon couplings. This is illustrated by the dashed line above. This can be used to deduce couplings if the signal-to-noise level does not allow clear observation of both peaks.

An alternative approach is to reduce the number of increments performed and to record a narrower spectrum in F1. The advantage is a reduction in experiment time (or increase in signal-to-noise), the disadvantage is the loss of the diagonal as peaks can be 'reflected' back into the observed region. Use of NUS can make this method unnecessary. It is important to acquire at least as many real increments as there are peaks in the carbon spectrum to calculate the remainder accurately. With a 25% sampling rate it is thus easy to generate 64 or 128 increments by acquiring 16 or 32 FIDs.

The next sections describe the procedure for a more realistic sample, 88mg of aspirin in acetone-d6.

26.11.1 Check carbon-13 signal-to-noise ratio

Acquire a single scan ^{13}C spectrum using a 90° pulse and measure the signal-to-noise ratio:

Load the parameter set: **@c13p90.c**

Do not adjust the receiver gain. This would take a long time as d1=60s. The maximum value of 2050 is already set in the parameter file.

Run experiment with **zg**.

If a signal size error is displayed on running the experiment, reduce **rg** to half the current value and repeat.

Process data with **ft** and **apk**.

Manually adjust phase if necessary and then baseline correct either with **abs n** or manually.

Define the 'signal' area of the spectrum:

Identify the smallest sample peak in the spectrum and expand the display to show a small region around just this peak.

Press ***RMB***, select **Save Display Region To…** then **Parameters SIG1,2 (signal region)(used by 'sino')**.

Define the 'noise' area of the spectrum:

Expand a small region of typical baseline of the spectrum, containing just noise.

Press ***RMB***, select **Save Display Region To…** then **Parameters NOISF1,2 (noise region)(used by 'sino')**.

Type **sino** to calculate the signal-to-noise for the spectrum, which will appear in a window on screen.

*The resultant value should be at least **30** for an INADEQUATE experiment to be recorded in a reasonable time.*

26.11.2 Measure carbon-13 T_1

It is necessary to measure the ^{13}C T_1 values in order to determine an appropriate relaxation delay (d1) for the INADEQUATE experiment.

In the inversion-recovery T1 experiment, the magnetization is inverted and then allowed to recover (relax) for a delay period. For a very short delay all of the signals are inverted and for a very long delay are all positive. The spin-lattice relaxation time (T_1) for each peak can be estimated from the delay after which the signal passes through zero. This is known as the null time (τ_n).

$T_1 = \tau_n / \ln(2) = 1.443 \times \tau_n$

The next page shows a set of spectra from a sample of aspirin in acetone-d6, recorded with a series of delays from 0.1s to 16s. It can be seen that the sample peaks in the region 150-170ppm all pass through zero at around 8 seconds. The behavior of the deuterated solvent is unimportant, it will often have long T_1s.

The longest T_1 can be estimated as 1.443 x 8 = 11.54 seconds.

Proceed as follows to perform the T_1 measurement:

Load the parameter set: **@c13decT1ir1D.c**.

Do not adjust **rg** unless it was necessary in the previous step, in which case change it to that value.

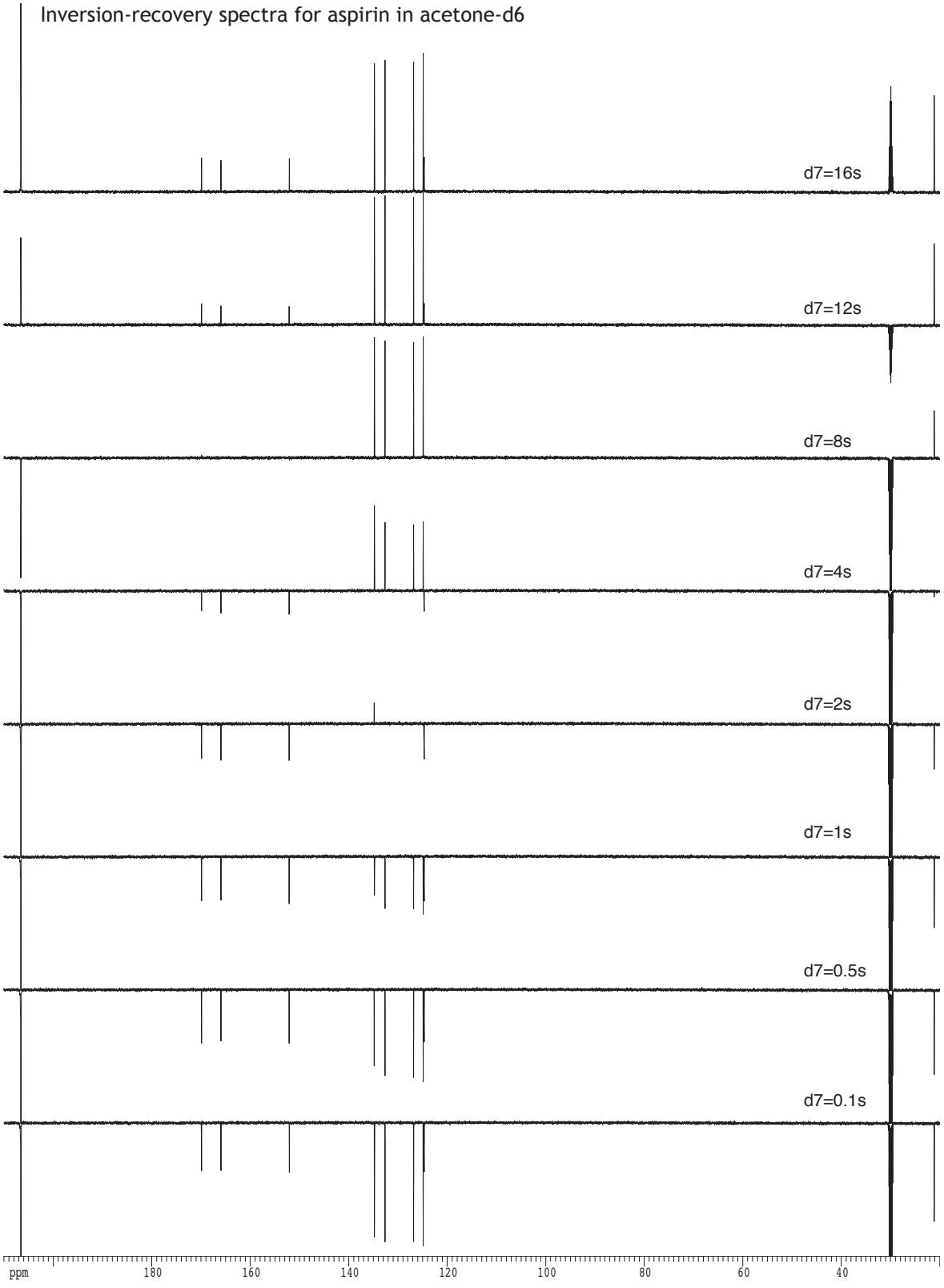
Set the recovery delay **d7** to **0.1**.

Run the experiment with **zg**, process with **ef** and manually phase correct (use **0** and **1** in the **phase** tab) to produce an inverted spectrum.

Type **iexpno** to create an identical experiment with the next experiment number.

Increase **d7** to **5**, and type **zgefp** to repeat the experiment and process the new data using the same phasing as used previously.

Repeat and either increase or decrease **d7** until a spectrum is obtained where the slowest relaxing peak is missing and all other sample peaks are positive. Use this value to calculate T_1 as shown above.


Inversion-recovery spectra for aspirin in acetone-d6
d7=16s
d7=12s
d7=8s
d7=4s
d7=2s
d7=1s
d7=0.5s
d7=0.1s
ppm
180
160
140
120
100
80
60
40

26.11.3 Acquire 2D INADEQUATE spectrum

Acquire INADEQUATE data with optimized parameters:

Use **edcp** to set up a new experiment and load the 2D INADEQUATE parameters **@c13inad2D.c**.

Use **copypars** to update the spectral region using one if the 1D spectra recorded above.

Set **d1** to the longest T_1 value calculated in the previous section.

The parameter **cnst 3** must be set to a typical value for J_{cc}, the carbon-carbon coupling constant. If this is not known for the current sample, typical values are 35-45Hz for a single bond and around 65Hz for a double bond. It is possible to observe satellite peaks for the ^{13}C-^{13}C couplings in a ^{13}C spectrum acquired with ^{1}H decoupling, if a very high single-to-noise ratio is achieved.

Three more settings will determine the experiment duration and data quality. Open the **AcquPars** tab to adjust these parameters.

Indirect spectral width:

As mentioned at the beginning of this section, a 'complete' spectrum will be obtained if the spectral width in the second dimension (F1) is double that in the observe dimension (F2). This allows some prediction of correlation positions by use of the diagonal, but results in significant empty space in the spectrum. The width in F1 can be reduced to 'concentrate' the correlations in a smaller area, with the loss of the diagonal.

A useful compromise is to set **SW (F1) = SW (F2)**.

Number of experiments:

NUS is selected by default with a ratio of 25%, this should be appropriate in most cases. It is necessary to record a significant amount of data in order for the prediction process to be accurate. A good principle is to record at least as many experiments as there are peaks in the carbon-13 spectrum, including solvent peaks. In practice this means recording 16 or 32 experiments in most cases.

Note that the displayed number of experiments, **TD** in the right (F1) column, represents the total number after NUS reconstruction. If the NUS ratio is 25%, only a quarter of these experiments will actually be acquired.

Typical values for **TD (F1)** are **64** or **128**.

Number of scans in each experiment:

The number of scans, **ns**, must be a multiple of **32**.

Set a suitable value for **ns** then type **expt** or use the clock icon to calculate the experiment duration. Adjust **ns** to match the time available for the experiment.

If **rg** was reduced from the maximum value in the setup experiments then use the same value, otherwise leave it at the maximum value of **2050**.

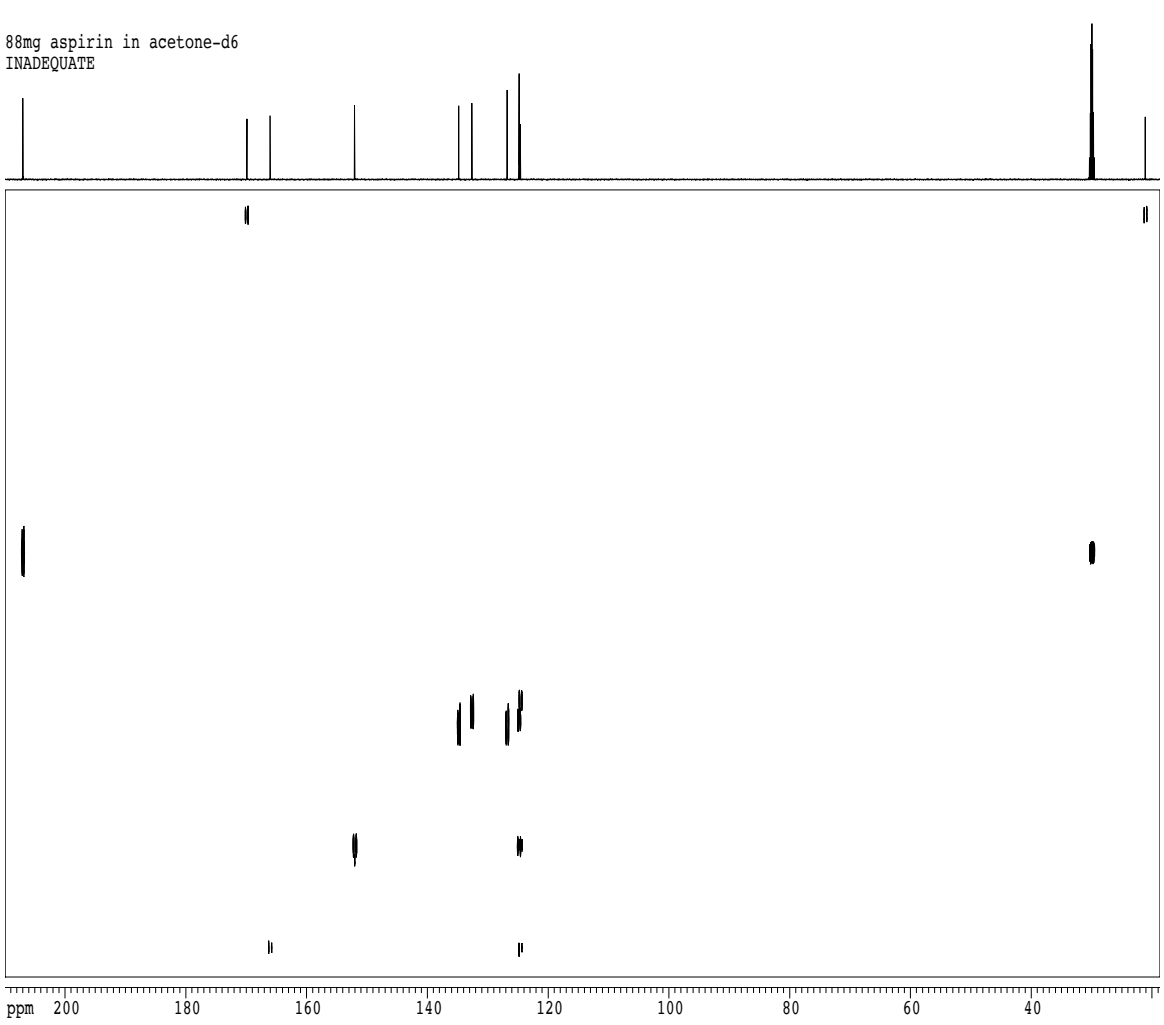
Run the experiment by typing **zg**.

The AVANCE600 computer does not have a license for NUS, so data must be processed either using the facility *TopSpin* workstation costello.ps.uci.edu, or using a local version of *TopSpin* 3.5 on the user's own computer. Note that the NUS calculation takes significantly more time than conventional 2D data processing.

Use **getproj** to enter the experiment number for the 1D carbon spectrum which will be used as the F2 projection.

Type **xfb** to Fourier transform, and when this is complete, **absb** to baseline correct.

The spectrum on the next page was acquired in 17 hours using a sample of 88mg of aspirin in acetone-d6. The spectrum is cropped to show the region of interest. 320 scans were acquired in 16 experiments, using the same spectral width in both dimensions. Fortuitously in this case there is no 'folding' of the spectrum.



Current Data Parameters

USER	nmrprd
NAME	600aspirin
EXPNO	93
PROCNO	1

F2 - Acquisition Parameters

Date_	20160914
Time	19.14
INSTRUM	av600
PROBHD	5 mm CPBBO BB-
PULPROG	inadgpgfsp
TD	16384
SOLVENT	Acetone
NS	320
DS	16
SWH	29761.904 Hz
FIDRES	1.816522 Hz
AQ	0.2753012 sec
RG	2050
DW	16.800 usec
DE	18.00 usec
TE	298.0 K
CNST3	57.0000000
D0	0.00000300 sec
D1	11.53999996 sec
D4	0.00438596 sec
D11	0.03000000 sec
D12	0.00002000 sec
D16	0.00020000 sec
IN0	0.00003360 sec

======= CHANNEL f1 =======

SFO1	150.9198507 MHz
NUC1	13C
P1	10.00 usec
P13	2000.00 usec

F1 - Acquisition parameters

ND0	1
TD	16
SFO1	150.9199 MHz
FIDRES	1860.119019 Hz
SW	197.203 ppm
FnMODE	QF

F2 - Processing parameters

SI	4096
SF	150.9026790 MHz
WDW	SINE
SSB	2
LB	0.00 Hz
GB	0
PC	1.00

F1 - Processing parameters

SI	1024
MC2	QF
SF	150.9028085 MHz
WDW	SINE
SSB	2
LB	0.00 Hz
GB	0

2D NMR plot parameters

CX2	22.00 cm
CX1	15.00 cm
F2PLO	209.902 ppm
F2LO	31674.81 Hz
F2PHI	18.695 ppm
F2HI	2821.17 Hz
F1PLO	179.961 ppm
F1LO	27156.57 Hz
F1PHI	72.873 ppm
F1HI	10996.79 Hz
F2PPMCM	8.69123 ppm/cm
F2HZCM	1311.52930 Hz/cm
F1PPMCM	7.13916 ppm/cm
F1HZCM	1077.31897 Hz/cm

27 Useful macros and automation programs

Several of the commands listed in previous sections of this manual are actually automation programs or macros which execute a combination of *XwinNMR* commands. This normally will not concern the user, but it does mean that commands available on UCI systems will not necessarily be available elsewhere, and users who have used *XwinNMR* elsewhere may find that some commands that they are used to using are unavailable. Commands which run locally written macros and automation programs will not be found in the Bruker *XwinNMR* manuals.

Note that some of the macros and automation programs which are concerned only with data processing or network printing may only be found on the data processing workstations and not on the spectrometer computers. Similarly, programs controlling data acquisition will only be found on the spectrometer computers.

Some of the following are available in both *XwinNMR* and *TopSpin*. Several are *XwinNMR* only, either because they use the *XwinNMR* plotting system, or are designed to replicate behavior included as standard in *TopSpin*.

27.1 Macros

Macros provide a shorthand way of entering a series of commands, or abbreviations for commands. All available macros can be examined by typing: **edmac** ***<enter>***

Examples:

rgazg, **zgfp**, **zgefp**, **rgazgfp**, **rgazgefp** - execute **rga** followed by **zg**; **zg** followed by **fp**; etc

absb - execute **abs2** followed by **abs1**

edcp - execute **edc** followed by **par**

ones - **one** followed by **saveshims**

par - abbreviation for **rpar** ***.n*** to produce a parameter menu appropriate to the current spectrometer configuration

pargn500, **parcryo500**, **pardrx400** - on workstations will produce a menu of the standard parameters on the specified spectrometer, these files are automatically updated monthly.

plotpar - produce a menu of plot and output device parameters for the standard range of experiments. Use when importing *TopSpin* or Varian data into *XwinNMR* or to simply restore standard parameters

pstopdf - converts all postscript files in /nmr/*username*/plot.code to landscape format PDF files

rgac - sets receiver gain on CRYO500 for carbon experiments, ignoring the spike at the beginning of the FID

roll - uses reverse linear prediction to correct carbon data recorded on the CRYO500

saveshims - save current shim settings inside the current dataset

27.2 Automation programs

Automation (au) programs can be more complex than macros and are compiled programs. Before they can be used by typing the program name alone, they must be compiled. If the required program fails to run it can be re-compiled by prefixing the name with the command **xau**, e.g. **xau swo1hom** ***<enter>***

Examples**:**

cop - copy current data to a different experiment number

copypars - use plot parameters from 1D spectra to set observation regions for 1D or 2D experiments

d3vplot - plot carbon spectrum, DEPT135 and DEPT90, with preview

d3psvplot - plot carbon spectrum, DEPT135 and DEPT90 to a postscript file, with preview

d3netvplot - plot carbon spectrum, DEPT135 and DEPT90 to a network printer, with preview

dexpno - decrement experiment number (read existing or create new experiment)

dta - create an analog copy of a dataset

efpb - for re-processing previously processed and baseline corrected data, executes **efp** followed by either **bcm** or **abs n** as appropriate

fpb - for re-processing previously processed and baseline corrected data, executes **fp** followed by either **bcm** or **abs n** as appropriate

getphase - get phase and reference parameters from a previous experiment and load into the current experiment. Source and destination can be 1D or 2D

getproj - get projection information and referencing from 1D files to load into current 2D dataset

getref - get referencing from separate 1D file(s) to load into current 1D or 2D dataset

haltat n - halt experiment at next multiple of n scans. The following multiple of n may be used if the target is too close to the current number. This program can be over-ridden by manually halting the experiment if necessary. *XwinNMR* only, '**halt** n' can be used in *TopSpin*.

iexpno - increment experiment number (read existing or create new experiment) (This is a standard Bruker program included here for completeness)

invert - invert the phase of a 1D or 2D spectrum

keepsfo1 - create a new experiment and load the observation frequency from the previous experiment

makedosy2d - create a 2D DOSY experiment from a 1D DOSY experiment

multidta - create analog copies of a series of experiment numbers

multiefpabs - process a series of experiment numbers using the same phase correction and calibration, followed by automatic baseline correction with no integration

multiefpd - performs **efpb** (see above) on a sequence of experiments

multiefpd - process a series of experiment numbers using the same phase correction and calibration

multi_integ - integrate a series of spectra using the same regions and relative intensities, and create a single file containing all the results

multi_integ2 - integrate a series of spectra using the same regions and with a chosen integral set to 1 in all spectra, and create a single file containing all the results

multi_integ2cal - as above but reference integral area can be defined

multiproc - process a series of experiment numbers using automatic phase correction and previous calibration, followed by automatic baseline correction with no integration

multisave - acquire **ns** scans, copy to next experiment, add **ns** more, copy again, etc

multisaveq - quick version of above program without advance creation of empty datasets

multisr - copy the calibration from one spectrum to a series of subsequent experiment numbers (1D data only)

multizg - perform a series of experiments

multizggrwt - repeat a group of experiments with a delay between each group

multizgq - quick version of above program without advance creation of empty datasets

multizgqwt - perform a series of experiments without advance file creation, with a 'wait' delay between experiments

multizgrort - perform a series of experiments at the current temperature controlling spin rate and reset sample temperature

multizgrortwt - perform a series of experiments at the current temperature controlling spin rate and reset sample temperature, with a 'wait' delay between experiments

multizgro - perform a series of experiments and control sample spin rate

multizgrovtrt - perform a series of experiments, load temperature and spin rate for each and reset temperature at end

multizgrowt - perform a series of experiments and control sample spin rate, with a 'wait' delay between experiments

multizgqrtwt - perform a series of experiments at the current temperature and reset the temperature afterwards, with a 'wait' delay between experiments, datafiles are created as required

multizgrt - perform a series of experiments at the current temperature and reset the temperature afterwards

multizgrtwt - perform a series of experiments at the current temperature and reset the temperature afterwards, with a 'wait' delay between experiments

multizgvtl - perform a series of experiments at different temperatures, reading the temperatures from a list

multizgvtrt - perform a series of experiments, load temperature from each experiment and reset temperature at end

multizgwt - perform a series of experiments, with a 'wait' delay between experiments

mydata - starts the 'search' browser and automatically loads the current user's data

netplot - plot spectrum to a network printer

netplot2 - plot spectrum to a network printer and also as a postscript file

netplots - plot suppress using a network printer

netvplot - plot spectrum to a network printer with preview

netvplot2 - plot spectrum to a network printer with preview and also as a postscript file

netflplot - flush plot composite spectrum to a network printer

one - temporarily sets **ds** to **0** and **ns** to **1**, runs experiment, then resets **ds** and **ns** to original values

ones - as above , but also save the shim settings within the current dataset

optimize2D – optimize a 2D spectral region based on an initial wider spectrum

plot2 - plot spectrum to paper and also as a postscript file

psplot - plot spectrum to a postscript file

psplots - plot suppress using postscript driver

psflplot - flush plot composite spectrum to a postscript file

psvplot - plot spectrum to a postscript file with preview

quant-calib - calibration program for Digital Eretic quantitative experiments

quant-addpeak - add a Digital Eretic peak to a quantitative spectrum

rshdata - load shim settings from inside the current dataset, if a shim file exits. Warns and gives the chance to quit of the shims were saved using a different probe to the one in use.

setproj - enter 1D projection and calibration parameters into 2D experiment. Note that there is also a standard Bruker au program called 'setproj'. This program is only used in the background when starting *XwinPlot* via the command 'xwp', when it is invoked by the au program 'viewxwinplot'. On the UCI systems the Bruker program has been renamed 'setproj.bruker' to avoid conflict.

setsfo1 - automatically set the observation frequency on the largest peak in the current plot region.

setsfo1full - automatically set the observation frequency on the largest peak in the current spectrum.

setsfo2 - automatically set SFO2 on the largest peak in the current plot region.

setsfo2full - automatically set SFO2 on the largest peak in the current spectrum.

stack1df - stack plot with fixed scaling, no preview

stack1dfv - stack plot with fixed scaling, preview before plot

stack1dfv2 - stack plot with fixed scaling, preview before plot, also save as postscript file

stack1dfvnet - stack plot with fixed scaling, preview before plot, choice of any network printer

stack1dfvps - stack plot with fixed scaling, preview before plot, print to postscript file

stack1dr - stack plot with relative scaling, no preview

stack1drv - stack plot with relative scaling, preview before plot

stack1drv2 - stack plot with relative scaling, preview before plot, also save as postscript file

stack1drvnet - stack plot with relative scaling, preview before plot, choice of any network printer

stack1drvps - stack plot with relative scaling, preview before plot, print to postscript file

swo1 - enter values for **sw**, **o1** and **sr** for any 2D experiment, equivalent to either **swo1het** or **swo1hom** followed by **setproj**

swo1het - enter values for **sw** and **o1** for heteronuclear 2D experiment

swo1hom - enter values for **sw** and **o1** for homonuclear 2D experiment

swo1inad - enter values for **sw** and **o1** for INADEQUATE 2D experiment

trat n - transfer and process at next multiple of n scans. The following multiple of n may be used if the target is too close to the current number. *XwinNMR* only, '**tr** n' can be used in *TopSpin*. Note that data is actually transferred after every scan following this command, but only processed when the target is reached.

vplot - preview spectrum then plot if required

vplot2 - preview spectrum then plot if required and also save as a postscript file

xiref - reference a non-proton spectrum by calculation from the reference frequency of a contemporaneous proton spectrum

28 Troubleshooting

The suggestions below should provide a cure for most common error situations. If problems persist, consult the NMR facility director.

28.1 Common software problems (some specific to *XwinNMR*)

28.1.1 General computer issues?

28.1.1.1 Silicon Graphics O2 computers

A range of computer errors can occur due to users omitting to exit from programs or other issues. Symptoms are errors from the "File Alteration Manager", missing icons on the right hand side of the desktop, or a grey window on screen instead of the normal login screen. This step should only be performed if no help is available.

Shut down the SGI workstation by pressing the small blue button on the lower left of the front of the O2 computer. The LED will flash and finally turn off as the computer shuts down. Press the button again and wait for the system to restart.

When the computer restarts, select the "Start System" icon at the top of the list on the left of the screen. If the computer fails to reboot to the login screen, repeat the shutdown and restart process.

28.1.1.2 CentOS Linux computers

Log out and select the "reboot" option from the login screen. The position varies with operating system versions.

28.1.2 Screen frozen?

[Esc]

Press Escape key.

If this fails to cure the problem proceed as follows:

If the *NMRterm* window is visible on the screen, try to open it with the ***LMB*** and press ***<Ctrl>*** and ***<\>*** (control and back slash keys together) and answer **y** to restart the graphics. This will not affect a running experiment.

If all else fails, try:

<Ctrl> <Shift> <F12> </>

The 'Vulcan death grip': (press the left hand Control and Shift keys, the F12 key and the forward slash key on the numeric keypad simultaneously). This will kill all active processes and exit from the current login.

As a very last resort press the power button on the computer to turn it off, wait for complete shutdown (the LED will go out), then press it again to turn the computer back on.

28.1.3 Mouse frozen?

Log out using the 'Vulcan death grip': (press and **hold down** the left hand Control and Shift keys, the F12 key and the forward slash key on the numeric keypad simultaneously) and then log back in again.

<Ctrl> <Shift> <F12> </>

This restarts the computer graphics.

28.1.4 Mouse moving slowly?

Log off the computer and log back in again.

28.1.5 Command fails (e.g. apks, halt)?

kill ***<enter>***

Select the offending command from the resultant menu with the ***LMB*** to kill it.

28.1.6 Lock display window not open?

Type **lockdisp** ***<enter>*** to open the lock display window.

If **checking rs-channel** appears at the bottom of the *XwinNMR* window for several seconds, followed by error boxes containing messages like **xtlib: error** and **connection to " :0.0" refused by server, xlib: maximum number of clients reached** then it is necessary to re-boot the computer.

Press the small blue button on the lower left of the front of the Silicon Graphics O2 computer. The LED will flash and finally go off when the computer has shut down. Press the button again to restart the computer and wait for it to reboot to the login screen.

28.1.7 All peaks shifted by a few ppm? Automatic lock has found the 'wrong' signal

If the solvent in use has more than one deuterium signal, for example toluene-d8 or methanol-d4, then there is a chance that the automatic locking processes will select the undesired signal. The most problematic solvent is toluene-d8, as the large separation of the aromatic and methyl signals can result in a spectrum shifted by approximately 5ppm.

Turn off the lock by pressing **[LOCK]**.

Enlarge the lock display window so that there are at least six grid boxes across, and that there is a central vertical grid line.

Reload the lock parameters for the current solvent, e.g. **lopoi tol** ***<enter>*** (on AVANCE600 use **lopo tol** ***<enter>***)

The desired deuterium signal from the solvent should now be visible in the lock display window. If no signal is visible, or if you are unsure which signal is being displayed, then increase the lock sweep amplitude adjusting **[SWEEP AMPL]** until all the expected solvent signals are displayed in the lock window.

Adjust the magnetic field using **[FIELD]** until the desired lock signal is exactly in the center of the lock display window.

If it was altered above, return the **[SWEEP AMPL]** to the standard setting of **2.0** and adjust the **[FIELD]** further if the lock signal is no longer centered.

Adjust **[LOCK GAIN]** until the lock signal fills about 75% of the height of the lock display window.

Press **[LOCK]** to lock onto the selected signal, then reduce **[LOCK GAIN]** until the signal is within the top three squares of the lock display window.

28.1.8 Lock command fails?

There are several reasons why a sample may fail to lock.

If an error message appears: Error while locking sample: LOCKAH No FFASignal found, then no deuterium lock signal has been detected.

First ensure that the correct deuterated solvent has been used.

If the previous user used the lock channel to acquire a deuterium spectrum, then the lock will be disabled. Ensure that standard proton (or carbon, etc) parameters are loaded and then type **ii** ***<enter>*** to re-initialize the electronics.

If the **[LOCK]** light is flashing, press the button to turn off the lock. **[SWEEP]** should now be on.

The lock signal should now show a beat pattern with a symmetrical display. If this looks OK, then repeat the **lock** command.

If the lock signal is noisy and broad with a very short decay, then the current shim settings are inappropriate for the sample in use. First re-read the standard shim file for the system, e.g. **rsh bbo** ***<enter>***

If the signal still looks poor, then eject the sample and check that the spinner is at the correct height on the NMR tube, and that the sample is at least 4cm deep. Try shaking the sample to ensure there is no concentration gradient.

If it is essential to examine a shallower sample, then position the spinner so that the sample is symmetrical about the center of the NMR coil, as indicated by the horizontal line around the depth gauge.

If the sample still fails to lock, with **[LOCK]** off, adjust the **[FIELD]** until the lock signal is at the center of the window. If necessary, adjust **[z1]** and **[z2]** to lengthen the decay of the beat pattern and increase **[LOCK GAIN]** until the signal fills the window vertically. Finally, **[LOCK]** and the adjust **[LOCK GAIN]** as required before shimming.

If the system still fails to detect a deuterium lock signal, then the shim system (BSMS) can be reconfigured. This should only be necessary on the GN500, where the lock frequency is changed periodically with the main operating frequency.

cfbsms ***<enter>***

Then press ***<enter>*** to confirm the correct serial connection.

If all attempts to lock fail, turn off **[LOCK]** and **[SWEEP]** and shim using the FID.

28.1.9 Error entering command?

<Delete>

Most dialogue windows in *XwinNMR* can be terminated by pressing the 'Delete' key if mistakes are made.

28.1.10 Very distorted spectrum following apks?

Sometimes the **apks** command results in a spectrum with a rolling baseline, most often if there is a broad peak close to the edge of the spectrum.

First remove the erroneous large phase correction by re-Fourier transforming the FID. Use **ef** ***<enter>*** if line broadening is to be used, or **ft** ***<enter>*** if no line broadening is desired.

Next either try **apks** ***<enter>***, and repeat the above procedure if it fails, or change to the **phase** screen and apply manual phase correction. Alternatively, the slower automatic phase command **apk** can be tried.

28.1.11 Automation program fails to run?

Precede the command with **xau**, e.g. **xau swo1hom** ***<enter>***, to re-compile the automation program.

28.1.12 File permission errors?

If an error message appears concerning file permissions, check that the correct settings are made in **edc** for your dataset. The disk unit must be **/v**, the username must be your **username**, and the data type must be **nmr**. Also check that you do not have any disallowed characters in your experiment name, e.g. /, (,), &, *,\ or spaces.

28.1.13 Error when trying to print a spectrum with peak labels?

Reduce **cy** by a small amount, e.g. 0.5cm, or reduce the number of characters in the peak labels: **edg** ***<enter>*** → **edplabl** → **pldigit**, or type **pldigit** ***<enter>***.

28.1.14 Spectrum won't plot?

Try turning off **integral trails** and **integral labels** in the **edg** menu. If the spectrum will now print, then there is an error in the integral file. Re-integrate the spectrum, turn the integrals back on, and re-plot.

28.1.15 *XwinNMR* fails to start?

Open the *NMRterm* window and look for error messages. The most common result of a previous crash is the need to issue the 'shmrm' command before re-starting the program.

Toolchest → **Desktop** → **Open Unix Shell**

Open a unix shell.

shmrm ***<enter>***

28.1.16 Spectrum chemical shift scale does not match data?

It is likely that the experiment parameters have accidentally been overwritten by those for a different experiment, for example carbon parameters may have been read into an existing proton experiment by mistake, instead of into a new experiment.

The easiest solution is to re-read the parameters for the original experiment into the file. This can be performed either on the original spectrometer or on one of the workstations. On a workstation, typing **pargn500** ***<enter>***, **parcryo500** ***<enter>***, or **pardrx400** ***<enter>*** will produce a menu of parameters from the relevant spectrometer. These parameter lists are updated monthly, and the standard parameter set names have **.gn500**, **.cryo500** or **.drx400** appended.

28.1.17 Printed spectrum is extremely compressed horizontally?

The user has probably accidentally pressed the **dp2** screen button instead of **dp1** when setting up the plot parameters. This retains the plot scaling in Hz/cm and changes the width in cm (**cx**). Change **cx** manually back to the standard value, either **22.8** if parameters are to be plotted to the right of the spectrum, or **25** if parameters are not required.

28.1.18 Error message including rcuerror or digitizer warning?

If an error message appears which states that a requested hardware unit like a receiver (**rcuerror**) or digitizer (**DIGTYP**) is not present, or is unavailable, then the currently loaded parameters are not configured for the instrument in use. It is likely that the user has used **edc** to generate the current experiment from the previously displayed experiment, and that that experiment was recorded on a different spectrometer. Use **par** to load parameters for the current instrument.

28.1.19 Spectrum integrals are shifted relative to peaks?

When a spectrum is integrated, the integral regions are saved in ppm. If the spectrum is subsequently re-calibrated the peak chemical shifts are changed, but the integral regions are not changed and so no longer match the peak positions. Re-integrate the spectrum.

28.1.20 Plotx/viewx expansions do not match peak positions?

The commands **plotx** and **viewx** create a file called **reg** to define the expanded plot regions for each integral. If the spectrum is re-calibrated and **plotx** or **viewx** re-executed, this file is not automatically updated even if the spectrum is re-integrated. Thus the expansion regions no longer match the integrated regions. The **reg** file must be manually updated in the **integrate** screen (after creating or reading the new integral file), via **File → Save as 'reg'**.

28.1.21 Spectrum is shifted vertically, scaled strangely or is off-screen?

If a spectrum is scaled strangely, or shifted wildly either vertically or horizontally, then a sequence of three buttons will always reposition the data so that it fills the *XwinNMR* window:


Resets the horizontal scaling so that the spectrum fills the window.


Moves the spectrum baseline to the bottom of the window.


Resets the vertical scaling so that the spectrum fills the window.

28.1.22 Can't find data in portfolio or menus?

If a dataset does not seem to appear in the menus, the experiment name used may have included disallowed characters and may have been truncated by *XwinNMR*. There are two easy options to find the data sorted by the date on which it was created. Within *XwinNMR* the command **dirdat** can be used to sort data by date or select date ranges, but the resultant list is limited to 512 entries. An alternative is to list the data in a unix shell:

Open a shell from the Toolchest: **Toolchest → Desktop → Open Unix Shell**

Change to the NMR data directory: **cddata *<enter>***

List all datasets in chronological order: **ls -alrt *<enter>***

This should reveal the experiment name with which the data has actually been saved.

If the original erroneous experiment name contained a forward slash character (/) there is likely to be a further problem. The forward slash is likely to have been interpreted as a directory separator by the file system, and the data will then be contained by one or more extra nested directories. The NMR software will not be able to find the data unless the standard directory structure is restored. Contact the facility director for assistance unless you are familiar with moving files in Unix shells.

28.1.23 Final 2D spectrum looks poor compared to spectrum observed during experiment

If a 2D experiment is interrupted using **halt** instead of **stop**, the resulting spectrum may be distorted. This happens if the last experiment saved does not contain the same number of scans as all of the previous experiments.

The fix for this to only process the complete experiments, and ignore the last one.

In *XwinNMR*, type:

1s td *<enter>*, then reduce the value by one, e.g. if the computer responds with 193, change this to 192.

In *TopSpin*, type:

td *<enter>*, then reduce the value in the right hand column by one.

Then re-process the data using **xfb** etc.

28.1.24 Observe and lock module are identical error

If the following error is seen:

HPPR: Error, observe and lock module are identical! Change or turn off locnuc!

The most common cause is that the parameters for the current experiment are from a different instrument to the one being used. Update the parameters for the current instrument by typing **par *<enter>***.

28.1.25 Tune and Match LEDs don't correspond to dip on screen

The LEDs on the preamplifier housing indicate the point of lowest intensity of the 'wobb' trace on the computer screen. If the matching is badly set, the lowest point may be at the edge of the screen rather than the dip. The easiest way to resolve this is to observe the trace on the screen, as well as the LEDs, while making adjustments. If necessary, turn the computer display so that it can be seen.

28.2 Common hardware problems

If an instrument has been re-booted, it can be necessary to issue extra commands to ensure all components are communicating correctly. The following options can be tried without requiring entry of any special passwords:

28.2.1 Communication failure?

If a command fails with a message about a communication problem with the CCU, try the following:

Open the left hand door of the spectrometer cabinet. Inside, at the top right, press the red 'reset' button on the CCU unit. The spectrometer computer will reload software from the Silicon Graphics computer. After approximately two minutes this process should be complete and the spectrometer should be operational.

28.2.2 CRYO500 or AVANCE600 won't lock or tune?

If the cryoprobes are not cold, then nothing will work. The first obvious symptoms will be that the probe won't tune or lock. Check that the cryoprobes have not warmed up. Are the cryoplatforms making the normal noises? Look at the status lights on the cryoplatform cabinet. On the AVANCE600 these are easily seen, on the CRYO500 the lights are on the end of the cabinet facing the corner of the room. In normal operation the green "Cold" light should be on. An "Error" light in addition to this means a problem has occurred but operation is currently normal. If the "Cold" light is not on, then there is a problem. There are many possible causes and no user actions that can correct this without establishing the reason. Inform the facility director and leave a note on the instrument that it is out of use.

28.2.3 BSMS keypad buttons won't work?

The buttons on the keypad on the AVANCE600 tend to stick, so sometimes need to be pressed firmly.

If none of the buttons work to load samples, etc, then the BSMS keypad can be reset via the computer.

On the *Irix/XwinNMR* systems open a unix shell from the Toolchest: **Desktop** → **Open Unix Shell** or on the Linux system open a Terminal window by clicking on the icon, then type:

bsms ***<enter>***

Type **y** to run the program and then select option **1** to initialize. When completed, press ***<enter>***, followed by **q** to quit, and finally **y** to confirm quitting.

28.2.4 No nmr signal?

ii ***<enter>***

Initializes electronic interfaces for the current experiment. Use if no signals are obtainable. If no change is observed, try setting up a second experiment to observe a different nucleus, e.g. ^{13}C if the first experiment was ^{1}H, try **ii** on that experiment, then re-read the first experiment and try **ii** again.

If this command produces an error, reset communication with the spectrometer as described above.

28.2.5 Sample won't load?

If the sample lift compressed air fails to come on, switch it off and on again.

If the 'sample down' LED fails to light ('missing' remains on): First check that lift air is off. Next eject the sample and check the positioning of the sample spinner on the NMR tube. If this is OK, check that the NMR tube is straight using the tube tester.

If the problem persists it is probably due to debris or damage in the probe. Report to the facility director.

28.2.6 Sample won't spin?

Remove sample and check that the NMR tube is straight using the tube tester, that both tube and spinner are clean, and that the spinner is correctly positioned on the NMR tube.

If the problem persists it is probably due to dirt on the spinner bearings, or debris or damage in the probe. Report the problem to the facility director.

28.2.7 Sample won't load or spin?

There is probably a problem with the gas supply to the spectrometer console. Normally the console is supplied with compressed air, but for low temperature experiments the supply may be switched to a nitrogen gas cylinder. If the compressed air supply has failed then all instruments will be affected - there will be no air for sample loading, spinning or temperature control.

A more common problem on the GN500 is that the gas supply has not been switched back from the nitrogen cylinder to air, following low temperature experiments. In this case the nitrogen gas cylinder is quickly exhausted and then no pressure is

available for sample loading etc. The nitrogen gas cylinder is located against the wall, behind the computer monitor, as seen from the operator's chair. There is a valve on the floor to switch between the two supplies. It is a 'T' shaped connection. The transparent tube connects to the nitrogen cylinder and the red tube opposite connects to the compressed air supply. The red tube at the base of the 'T' connects to the spectrometer console. The handle of the valve points towards the selected gas supply - if this is pointing towards the nitrogen cylinder, then rotate it by 180° to select compressed air.

28.2.8 Sample won't eject?

There are various possibilities, but there is a common problem on the DRX400. First check that you can hear some air flow after pressing **[LIFT ON/OFF]**. If the NMR tube is equipped with a Young's tap, the airflow on the DRX400 is sometimes insufficient to lift the sample. To add extra air flow, open the temperature control window with **edte** and increased the **Gas Flow** using the **+** button. Increase the flow incrementally until the sample is ejected and then return the value to the standard **535** l/h or **670** l/h.

If the air flow doesn't not come on at all after pressing **[LIFT ON/OFF]**, two options can be tried:

Within *XwinNMR*, type **ej *<enter>*** to eject a sample, or **ij *<enter>*** to load (inject) a sample. These commands will often restore communication to the keypad.

If the above commands do not work, open a Unix shell from the Toolchest and type:

bsms *<enter>*

Type **y** to run the program and then select option **1** to initialize. When completed, press ***<enter>***, followed by **q** to quit, and finally **y** to confirm quitting.

If the **bsms** command is used, the lock frequency may be changed. This **MUST** be reset within *XwinNMR* by using the command **cfbsms**.

28.2.9 TRANS. P-DOWN error light on BSMS keypad?

This error can be reset in *XwinNMR* by typing:

acbdisp *<enter>*

then select **Controls** → **ACB Reset**

If this command produces an error or fails, first reset communication with the spectrometer as described above (27.2.1).

28.2.10 Lock signal displays sine wave interference?

This is currently a common problem on the GN500, and appears only when the spectrometer electronics are switched for X-nucleus operation, e.g. ^{13}C. Thus, whether or not the interference is seen depends on the type of experiment performed last by the previous user. A workaround is as follows:

First load the proton parameter set as usual before locking, e.g. **rpar h1.s all <enter>**.

Before locking, type **ii *<enter>***. This will set up the electronics for ^{1}H observation, without actually starting the experiment, and should remove the interference.

Finally lock and shim as normal.

28.2.11 Lock signal out of window on CRYO500?

There is a bug in the CRYO500 which occasionally results in the lock signal disappearing out of the top of the lock display window, and it cannot be brought back by adjusting the Lock Gain. The problem is that the lock DC offset gets set to +100, whereas the standard value is -71. To fix this: press **[LOCK DC]** on the BSMS keypad and reduce the value from **+100** to **-71** using the knob. The lock signal should now be visible in the normal position towards the bottom of the lock window.

28.2.12 No carbon-13, phosphorus-31 or fluorine-19 data on DRX400?

For automatic probe switching to work, the probe controller must be set to automatic mode. This is indicated by a button at the bottom of the preamplifier housing, next to the magnet. This has four settings, manual selection of the position for each of the three nuclei, and automatic switching. In the three manual positions the button light is on, but in automatic mode the light is off. If the spectrometer has been restarted after being powered down, this controller is initially in manual mode. Press the button repeatedly to cycle through the settings until the light turns off.

28.2.13 No carbon-13 data on CRYO500?

The standard configuration of the CRYO500 allows observation of ^{1}H, ^{2}H and ^{13}C. The TCI cryoprobe also includes a ^{15}N channel, which is normally not in use. This is not sensitive enough for direct observation, but can be used for decoupling or for the indirect dimension of a 2D experiment. Select users have been trained for this, which involves changing a cable connection. The system should always be left setup for ^{13}C observation, but if no signals are obtainable the cable should be

checked. On the back of the preamplifier housing, at the bottom, are cable connectors labeled ^{13}C and ^{15}N. Only the ^{13}C connector should be in use, if the cable is currently connected to ^{15}N it should be moved to ^{13}C.

28.3 Complete system restart

This operation should not be undertaken unless absolutely necessary. Some standard restart routines cannot be undertaken without access to administrative passwords.

28.3.1 DRX400, GN500 and CRYO500

28.3.1.1 Shut down SGI workstation

This should only be performed if no help is available. Press the small blue button on the lower left of the front of the O2 computer. The LED will flash and finally turn off as the computer shuts down.

The operations described below will restart all parts of the computer and spectrometer and will clear many error situations. However, if the current problem is caused by a hardware failure then one or more of the following procedures will fail.

28.3.1.2 Shutdown spectrometer console

Open both doors of the spectrometer cabinet.

Turn off the acquisition processor using the key at the top of the left rack.

Turn off the power button on the BSMS unit at the bottom of the left rack.

Turn off the power button on the variable temperature controller at the top of the right rack.

Turn off the power button on the AQR rack in the center of the right rack.

Turn off the power button on the BLAXH amplifier at the bottom of the right rack.

Turn off the power button on the back of the Gradient Amplifier which is on top of the spectrometer cabinet.

Finally press the red button at the top of the center panel between the doors to turn off all power to the spectrometer.

28.3.1.3 Wait

DRX400 and GN500 - Wait for a minimum of three minutes before proceeding.

CRYO500 - Press the green button at the top of the center panel between the doors to turn on power to the spectrometer, and then turn on the power button on the variable temperature controller at the top of the right rack. If the air flow through the cryoprobe is absent for too long, the probe will cool down beyond its operating range. This will trigger the probe to warm up.

28.3.1.4 Turn on SGI workstation

Press the small blue button on the lower left of the front of the O2 computer. The LED will flash and finally turn green as the computer restarts.

Wait until the login screen appears before proceeding.

28.3.1.5 Turn on spectrometer console

DRX400 and GN500 - Press the green button at the top of the center panel between the doors to turn on power to the spectrometer. Wait 5-10 seconds between each of the following operations.

Turn on the power button on the back of the Gradient Amplifier which is on top of the spectrometer cabinet.

Turn on the power button on the BLAXH amplifier at the bottom of the right rack.

Turn on the power button on the AQR rack in the center of the right rack.

DRX400 and GN500 - Turn on the power button on the variable temperature controller at the top of the right rack.

Turn on the power button on the BSMS unit at the bottom of the left rack.

Turn on the acquisition processor using the key at the top of the left rack.

Close the cabinet doors.

28.3.1.6 Check communication

Wait 30 seconds for communication to be established between the console and computer.

Log in to the SGI computer.

Open a unix shell and type:

ping spect *<enter>*

If there is no response from the acquisition computer, the computer will hang with the message:

PING spect (149.236.99.99): 56 data bytes

kill the command by pressing **Ctrl-c**.

Repeat the above command until the computer produces a list of response messages and times. Note that the 'up arrow' key allows access to previous commands, so it is not necessary to re-type the whole command.

When communication is established the output will be of the form:

64 bytes from 149.236.99.99: icmp_seq=0 ttl=255 time=2.730 ms

64 bytes from 149.236.99.99: icmp_seq=1 ttl=255 time=4.267 ms

Stop this process with **Ctrl-c**.

Next try to log in to the acquisition computer:

telnet spect *<enter>*

If an error appears, kill with **Ctrl-c** and repeat the command until the login prompt appears (this may take up to one minute):

Trying 149.236.99.99...

Connected to spect.

Escape character is '^]'.

RISC/os (spect)

login:

Press **Ctrl-d** to kill this process.

28.3.1.7 Restart spectrometer components

Open a Unix shell and then type:

bsms *<enter>*

Type **y** to run the program and then select option **1** to initialize. When completed, press ***<enter>***, followed by **q** to quit, and finally **y** to confirm quitting.

acb *<enter>*

Type **y** to run the program and then select option **1** to initialize. When completed, press ***<enter>***, followed by **q** to quit, and finally **y** to confirm quitting. (This program can also be used to clear the 'ACB powerdown' error on the BSMS keypad).

DRX400 and GN500 only: **hppr *<enter>***

Type **y** to run the program and then select option **1** to initialize. When completed, press ***<enter>***, followed by **q** to quit, and finally **y** to confirm quitting.

CRYO500 only: **UniTool *<enter>***

Type **hppr** to select the preamplifiers followed by ***<enter>*** to confirm the address. Press ***<enter>*** again to proceed and then **1** to initialize. There may be a message to clear about power having been lost. Finally, **x** to exit from UniTool.

28.3.1.8 DRX400 ONLY

The QNP probe on the DRX400 can be used to observe ^{1}H, ^{13}C, ^{31}P or ^{19}F. For the automatic switching to work, the probe controller must be set to automatic mode. This is indicated by a button at the bottom of the preamplifier housing, next to the magnet. This has four settings, manual selection of each position and automatic switching. In the three manual positions the button light is on, but in automatic mode the light is off. Press this button repeatedly until the light turns off.

28.3.1.9 Start *XwinNMR*

Restart operation as normal. When an experiment is started it is likely that a couple of messages will appear in connection with units being reset after a power shutdown. Ideally the system would be reconfigured before use, but this requires the nmr superuser password.

28.3.2 AVANCE600

28.3.2.1 Restart computer

Use the restart option from the login screen.

28.3.2.2 Shutdown spectrometer console

Open the cabinet door and turn off the BSMS/2, the BLAX and BLAXH amplifiers and the AQS/2.

Press the red button at the top left on the outside of the cabinet to turn off all power. Wait 5-10 seconds and then press the green button to restore power. On this instrument the variable temperature controlled is hidden in the back of the console and restoring power ensures that air will flow through the probe to prevent the temperature from falling out of range.

28.3.2.3 Turn on spectrometer components

Turn on the BLAXH, BLAX, BSMS/2 and AQS/2, waiting 5-10 seconds between each one.

28.3.2.4 Check communication

Wait 30 seconds for communication to be established between the console and computer.

Logon to the Linux computer.

Open a terminal window and type:

ping spect *<enter>*

If there is no response from the acquisition computer, the computer will hang with the message:

```
PING spect (149.236.99.99): 56 data bytes
```

kill the command by pressing **Ctrl-c**.

Repeat the above command until the computer produces a list of response messages and times. Note that the 'up arrow' key allows access to previous commands, so it is not necessary to re-type the whole command.

When communication is established the output will be of the form:

```
64 bytes from 149.236.99.99: icmp_seq=0 ttl=255 time=2.730 ms
64 bytes from 149.236.99.99: icmp_seq=1 ttl=255 time=4.267 ms
```

Stop this process with **Ctrl-c**.

Next try to log in to the acquisition computer:

telnet spect *<enter>*

If an error appears, kill with **Ctrl-c** and repeat the command until the login prompt appears (this may take up to one minute):

```
Trying 149.236.99.99...
Connected to spect.
Escape character is '^]'.
RISC/os (spect)
login:
```

Press **Ctrl-d** to kill this process.

28.3.2.5 Restart spectrometer components

Open a Unix shell and then type:

bsms *<enter>*

Type **y** to run the program and then select option **1** to initialize. When completed, press ***<enter>***, followed by **q** to quit, and finally **y** to confirm quitting.

acb *<enter>*

Type **y** to run the program and then select option **1** to initialize. When completed, press ***<enter>***, followed by **q** to quit, and finally **y** to confirm quitting. (This program can also be used to clear the 'ACB powerdown' error on the BSMS keypad).

UniTool *<enter>*

Type **hppr** to select the preamplifiers followed by ***<enter>*** to confirm the address. Press ***<enter>*** again to proceed and then **1** to initialize. There may be a message to clear about power having been lost. Finally, **x** to exit from UniTool.

28.3.2.6 Start *TopSpin*

Restart operation as normal. When an experiment is started it is likely that a couple of messages will appear in connection with units being reset after a power shutdown. Ideally the system would be reconfigured before use, but this requires the NMR superuser password.

29 Data acquisition and processing using *TopSpin*

29.1 Introduction

The AVANCE600 spectrometer uses a new generation of Bruker software, called *TopSpin*, on a computer running *CentOS* Linux. This instrument contains new hardware which is not compatible with *XwinNMR*. The current *TopSpin* version is 3.2 on the spectrometer computer but the processing computer costello.ps.uci.edu uses the most recent version, 3.5.

It is difficult to write comprehensive instructions for using *TopSpin* as there are several ways of performing most operations, as well as a choice of user interfaces. When the 600MHz cryoprobe is in use, both probe tuning and field shimming can be performed automatically.

The *TopSpin* User Manual can be found via the **Help** or **?** menus and is a fairly comprehensive guide.

Some commands take time to execute. Error messages will appear if the system is busy when a new operation is requested. Active commands can be listed using the command **show**. If a command appears to have hung up, the resultant menu offers a **kill** option.

29.2 *Topspin* compared to *XwinNMR*

There are many similarities between the two programs. The majority of commands are the same, although the user interface looks very different. There is greater use of graphical icons on the on-screen buttons instead of text, although commands can also be accessed via the command line.

TopSpin has the ability to display several datasets on screen at once, or several views of the same dataset. The windows quickly get rather small, however, so normally they are stacked behind each other on the screen.

Every button has a help message which is displayed if the mouse is positioned over the button for a few seconds. Every open window also has a small icon, either at the top right of the *TopSpin* window, or on the bar at the top of the screen. A help message is available via the mouse to describe each of these.

The standard *XwinNMR* method of plotting spectra does not exist in *TopSpin*. The *TopSpin* plot editor is a development of *XwinPlot*, and it is also possible to perform a simpler printout of the current data display window. This latter option includes the ability to quickly print a multiple dataset display.

Raw data recorded on the AVANCE600 is incompatible with *XwinNMR* as it has a new generation of digitizer. If data has been Fourier transformed in *TopSpin*, all subsequent processing can be performed in *XwinNMR*, but the spectrum cannot be initially created from the FID by Fourier transform. The spectrometer computer or the *TopSpin* processing computer can be used to perform the Fourier transform. Full processing can also be performed on a user's own *TopSpin* installation.

Alternatively, an analog copy of the digital data can be created using *TopSpin* using the command **dta** for a single dataset or **multidta** for a series of experiments. The analog data can be fully processed using *XwinNMR*.

29.3 Using the Linux workstation

Use the same login ID and password as on the other NMR systems.

Start *TopSpin* by single clicking on the blue icon on the panel at the left of the screen, or by double clicking on the desktop icon.

If *TopSpin* fails to start, open a terminal shell and type **shmrm**

If *TopSpin* still will not start, then log off, and reboot the computer. There is a **restart** button on the login screen, no password is required.

29.4 Acquisition command summary

edcp Set up new dataset and load desired parameters.

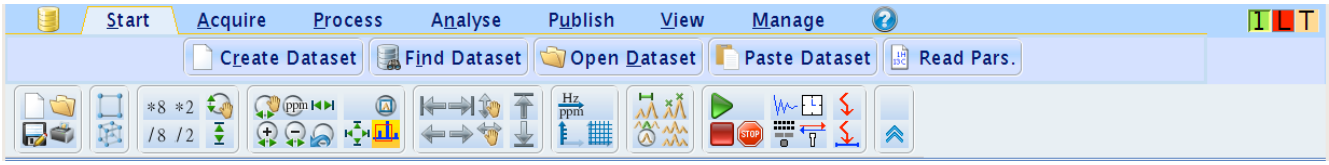
lock or **lock *solvent*** Lock field onto solvent signal in the normal way.

atma Automatically tune and match probe for all nuclei required by the current experiment.

optshim Optimize lock, shim field and then re-optimize lock.

If a subsequent experiment on the same sample irradiates a new nucleus, execute **atma** again.

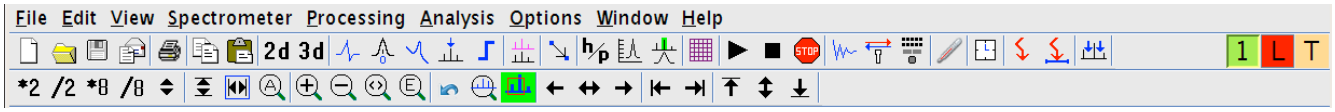
29.5 User interfaces



There are many user preferences within *TopSpin*, and two main interface choices. The 'new' appearance is the 'flow interface' (above). This is the only option in *TopSpin* versions 3.5 onwards. The hardware of the AVANCE600 is not compatible with software newer than version 3.2, so the instrument setup will not change in the near future. The processing computers now run version 4, which has an improved user interface. User's own free installations of *TopSpin* will also be version 3.6 or newer.

It is possible to select a clearer version of the button icons. Press ***RMB*** over an empty area of the icon bar and select **Toggle Icon Style**. The button array can also be made more compact by selecting **Toggle Icon Border**.

A more 'traditional' interface (below) is available in *TopSpin* 3.2, with an appearance more similar to *XwinNMR*. It is possible to switch between the two interfaces as follows:



29.5.1 Change from 'flow' to 'traditional' interface

Manage → **Preferences** → **Window settings**

Enable TopSpin 3 Flow User Interface	uncheck box
Use TopSpin 2.1 icons instead of TopSpin 3.0 icons	check box

Quit and restart *TopSpin*.

Options → **Preferences** → **Window settings**

Enable TopSpin 3.1 Color/Toolbar Scheme	uncheck box

29.5.2 Change from 'traditional' to 'flow' interface

Options → **Preferences** → **Window settings**

Enable TopSpin 3 Flow User Interface	check box
Enable TopSpin 3.1 Color/Toolbar Scheme	check box

Quit and restart *TopSpin*.

29.6 Loading a dataset

There are several methods of loading a dataset, either into the current data window or a new window. Unfortunately, none are as easy to use as the *XwinNMR* portfolio editor.

There is an 'NMR Data Browser' which opens in a separate window when *TopSpin* is started. There will be a corresponding icon on the panel at the top of the screen. This can be identified by holding the mouse over the labels for a couple of seconds until the help messages appear.

Scroll through the browser to find your user name. Either press the ***LMB*** over the small circle to the left of the username folder or double click on the folder to open the next level. Navigate until the experiment number(s) are displayed for the desired experiment name. The dataset can be loaded into the *TopSpin* window by double clicking on the folder icon for the experiment number, or by dragging into the data window.

After the system has been used for the first time, the software will automatically load the last previously viewed dataset when starting *TopSpin*. In addition, the data browser will automatically scroll to the current dataset. However, this operation will take a few seconds to complete.

29.7 Sample loading, locking, probe tuning and shimming

The main lock display window should open automatically and, if necessary, can be opened manually by the command **lockdisp**.

The BSMS keypad is exactly the same as those on the other instruments. Sample spinning is not normally used.

Lock the sample first and the tune the probe by typing **atma**. This will tune all channels required by the current experiment. The starting point for ^{1}H tuning is determined by the solvent name used for the lock command, so it is important that this is correct. If the proton channel has been tuned already and, for example, a carbon spectrum is required, some time can be saved by temporarily loading parameters that do not use proton decoupling, just to tune the probe. Update the parameters appropriately before running the experiment.

Occasionally automatic probe tuning fails if the sample in use is very different to a 'standard' sample in the same solvent. For example, if a sample in D_2O is unusually salty. The probe can be tuned by manually controlling the motor unit, but the standard tuning window is non-linear and narrow. Use the command **mantune** to access manual control via a wider and clearer window. In this example of a salty sample, it will be necessary to check pulse calibrations to acquire anything more than a simple proton spectrum.

Shimming can be performed manually as on the other instruments, or automatically by typing **topshim**. Do not change to the acquisition screen while this is in progress. Either do so before typing 'topshim', or when the status messages show that it has finished. If you see an error about filenames, reload the current dataset from the browser. The lock phase and gain can be adjusted automatically with the commands **autogain** and **autophase**. This can also be performed together by typing **algp**.

The operations above can be combined by using the command **optshim**. This will optimize the lock phase and gain, shim automatically, and then re-optimize the lock phase and gain. Only the axial (z) shims will be optimized automatically. The X and Y shims can be adjusted manually. To select X, press **[x]** followed by **[z0]** and to select Y, press **[y]** followed by **[z0]**.

29.8 Acquisition

Most *XwinNMR* commands can be used. To avoid confusion, it is best to display the acquisition window before starting an experiment. If the processing window is at the front of the screen, *TopSpin* may ask for confirmation of which experiment should be run. This is because several experiments can be open simultaneously in different processing windows.

The best method to create a new dataset is the command **edcp**. The **edc** window includes the option to load new experiment parameters instead of inheriting the current parameters, but the menu includes all parameter sets in the system, not just those set up for the current configuration of the instrument. Thus, it is better to ignore this option keeping the default of inheriting the current parameters and then selecting new parameters from the next menu produced by **edcp**. This menu will only contain calibrated parameter sets for the current probe.

There is an option to turn on 'comments' which give a short description of each parameter file. This also displays the menu as a single vertical column which is more readable. To achieve this, select **Options → Show Comment** at the top of the parameter menu window. This setting will remain active during the current *TopSpin* setting, but is not retained on exit.

Acquisition or processing parameter menus can be accessed either via the tabs across the top of the data window, or using the same commands as *XwinNMR*, **eda**, **ased**, **edp** etc.

Parameters can be optimized in a similar way to *XwinNMR*, but there is no 'sw-sfo1' button. The equivalent function is accessed via a button at the top of the screen. This is the second button from the right-hand end, and has a red jagged arrow over a horizontal line. The help message will confirm its function if the mouse is hovered. Note that if this is used to extract parameters for a separate experiment, four different numbers will be listed on-screen, ensure that the correct ones are noted. **copypars** removes the need to write these down in most cases.

The **calibrate** tab can be used to obtain the exact frequency of a peak for an nOe experiment, or **keepsfo1** can be used to load this automatically into a new experiment.

In addition to the standard usage of **halt** and **tr**, the commands **halt** n and **tr** n can be used. These will halt the experiment or transfer the data at the next multiple of n scans.

29.9 Optimizing 2D experiments

The python program **copypars** can be used to interactively set up observation regions for 2D experiments by selecting the desired sections of 1D spectra.

First acquire 1D data as normal, process and reference the spectra.

Load the default parameters for the desired 2D experiment into a new dataset. Next type **copypars**.

You will be prompted to load the appropriate 1D spectrum, zoom to the desired region, and then save that region to the 2D experiment. The parameters will be loaded to all dimensions of the new 2D experiment relevant to the observe nucleus in the 1D experiment.

For a heteronuclear 2D experiment, run **copypars** a second time and select the 1D file for the second nucleus.

The program is a little slow as it performs a lot of checks, so wait for it to finish.

Referencing information as well as acquisition information will be saved into the 2D file, so there will be no need to run 'getproj'.

If the acquisition time for the 2D experiment increases more than two fold, compare to the default, an extra question will appear. You can select whether to accept the new **aq** or reduce it. Usually the new value will be appropriate. This situation will occur if a spectral width of less than 8ppm is chosen for the proton observe dimension of the 2D experiment.

29.10 Non-uniform sampling

Non-uniform sampling (NUS) is a method for improving data quality using mathematical prediction and is only available in *TopSpin*. Its primary use is to improve the resolution of multi-dimensional experiments. A spectrum can be acquired in less time, a higher resolution spectrum can be acquired in the same time, or a combination of both. If a conventional 2D experiments acquires 256 experiments with 8 scans in each, then NUS can either be used to predict 1024 experiments, or to only acquire 64 experiments and predict 256. The latter case would reduce the experiment time by a factor of 4, allowing for a quicker experiment or for the number of scans to be increased to 32 to take the same time and gain signal-to-noise.

In principle NUS can be applied to any multi-dimensional experiment, but there are some restrictions for accurate results:

(i) Sufficient 'real' data must be acquired. In practice this means at least one experiment in the indirect dimension for every expected signal in the 2D spectrum. Normally this number will represent 25% of the calculated number of indirect experiments.

(ii) All expected correlation signals should be of similar intensity, including signals from impurities or small components of mixtures. NUS is ideally suited to HSQC, INADEQUATE and similar experiments. HMBC spectra often show a wide intensity range, with very strong correlations from methyl signals and so are less suitable. NOESY and ROESY experiments produce strong diagonal signals and much weaker correlation peaks and so NUS should only be used with caution.

29.10.1 Data acquisition using NUS

Open the **AcquPars** tab and select the **NUS** section in the left column.

Set **NusAMOUNT [%]** to the desired value, usually **25**.

Scroll to the top of the **AcquPars** tab and set **FnTYPE** to **non-uniform_sampling**

Run the experiment as normal. **SI** in the F1 dimension now represents the total number of experiments following reconstruction, only **NusAMOUT [%]** of these will be measured.

29.10.2 Processing data acquired using NUS

The software used to control the AVANCE600 spectrometer, *TopSpin 3.2*, does not have a license for NUS processing. A license has only been supplied as standard with *TopSpin 3.5* onwards. These later versions are available on the dedicated *TopSpin* processing station costello.ps.uci.edu, or users' own *TopSpin* installations. Recent versions of *MestReNova* should also handle NUS data.

There are two modes for data processing, **mdd** and **cs**. **cs** will always be used unless a separate NUS license has been purchased. Processing involves more intensive calculations than standard Fourier transformation, therefore it will be noticeably slower.

Magnitude mode data can be processed in the normal way. However, NUS data does not acquire all of the files required for phase correction. A Hilbert transform must be applied to calculate the missing data. Type **xht2** to achieve this.

29.11 Processing

Most *XwinNMR* commands can be used. For automatic phasing, **apk** should be used instead of **apks**. For manual spectrum calibration, the reference position can be anywhere on-screen, it is not restricted to discrete datapoints. The easiest way to access functions for phasing, baseline correction, calibration and integration is via the tabs across the top of the spectrum window.

Peak picking must be performed explicitly in *TopSpin*. There is a tab for interactive peak picking, or this can be performed automatically using the command **ppf**. **mi** and **pc** can be adjusted if required, as in *XwinNMR*.

Peak labels, integral trails, integral labels, etc, can be displayed on the spectrum in the current spectrum window, if desired. These options can be selected or de-selected via the ***RMB*** menu item **Spectra Display Preferences**.

29.12 Plotting

The standard *XwinNMR* method of plotting spectra does not exist in *TopSpin*. There are three options:

(i) Use the plot tab. This will display the spectrum with interactive plot options. Vertical scaling can be adjusted via the buttons on the normal spectrum toolbar. **Automation Actions** provides some options for automatic scaling spectra, similar to those used in *XwinNMR*.

(ii) The '+', '-' and '<>' magnifier icons can be used to change the horizontal scaling, but the data cannot be directly expanded with the mouse. A simple solution is to switch to the Spectrum tab to select a new region, then return to the

Plot tab. Options for printing this window are different to those above. CTRL-P, or the printer icon, produces a dialog to select a printer. Within the NMR Facility the default printer is best, unless color is required. For remote access, a network printer can be easily selected here. To print to a file, use the **File** menu at the top of the window, and select **Export…** Note that is necessary to navigate to a suitable directory, e.g. your PDF directory, and to append an extension to the filename to define the file type, e.g. **.pdf**. The default PDF resolution is rather low at 300 dpi. This can be increased, e.g. to 1200, to generate higher quality output.

(iii) Print the entire spectrum window, exactly as it appears on screen. This will include multiple datasets if they are currently displayed. Use the Print tab, printer icon or CTRL-P; then select 'Print active window'.

To save as a file, use the **File** menu at the top of the window, and select **Export…**.

It is necessary to append an extension to the filename to define the file type, e.g. **.pdf**., then to save the file navigate to pdf or postscript subdirectory in your home directory.

(iv) *This method is not available in Mac versions of TopSpin :* Use the old *TopSpin* Plot Editor for full control, similar to *XwinPlot*. Type **plot0**. A suitable layout should be loaded, this can be changed if desired. Use the same method as *XwinPlot* to print to a file. If a non-default network printer is required, this should be selected in the initial Print window. Click on the box beside **Override plotter saved in Plot Editor:** then select the required printer in the menu.

29.13 Using *XwinNMR* to process and plot *TopSpin* data

There are two important factors when processing *TopSpin* data using *XwinNMR*. The first is that *XwinNMR* cannot Fourier transform digital data generated using *TopSpin*. If a spectrum already exists, then it can be processed further and plotted, but it cannot be created by Fourier transform. If it is necessary to recreate the spectrum by Fourier transform either a *TopSpin* processing computer (costello.ps.uci.edu or heaton.ps.uci.edu) or spectrometer computer can be used.

A good option is to Fourier transform, phase and baseline correct using *TopSpin*, then if preferred use *XwinNMR* for the subsequent processing operations.

An analog dataset can be created which can be fully processed by *XwinNMR*. This MUST be carried out in *TopSpin* (either on the spectrometer computer or a processing station):

dta ***<enter>***

The computer will request a new experiment number for the analog version of the data. The automation program **multidta** can be used to convert a series of experiment numbers.

The second factor is that *TopSpin* does not employ the standard *XwinNMR* method of plotting spectra, and so a *TopSpin* dataset does not contain the plot and output device parameters that *XwinNMR* requires. When a *TopSpin* dataset is first opened in *XwinNMR* a message will appear that plot and output device parameters were missing, and that standard parameters have been loaded. These standard parameters will not be suitable for the UCI configuration, and should be replaced as follows:

plotpar ***<enter>***

Select the most appropriate parameter set from the menu.

Note that these parameter sets only contain plot and output parameters, so **copy all** can safely be used. It may also be necessary to update the following parameters: **pc**, **mi**, and **pscal**. Some *TopSpin* title files contain empty lines that will cause *XwinNMR* to use very small characters - these can be removed by editing the title with **setti**.

If a 2D dataset is being plotted it is also necessary to update the parameters for the 1D projection spectra:

getproj ***<enter>***

In *Topspin3.n* there are changes to the parameters used to control pulse lengths and power levels. These parameters will cause error messages when plotting using *XwinNMR* or *TopSpin1.3*. These can be safely ignored. They are caused by the generation of the parameter listing for the plot, and the result is that some acquisition parameters will be missing.

29.14 Temperature control

The temperature controller window should open automatically on starting *TopSpin*, and the current temperature is also displayed at the bottom of the main window. It can be opened manually by typing **edte**.

29.15 Common *TopSpin* problems

29.15.1 Wrong user's data is displayed

Occasionally when *TopSpin* is started, the main display will show a dataset belonging to the previous user of the system. The default option of **edcp** is to create a new experiment in the current data directory. This will not be allowed in the data directory of another user, and a permissions error will appear. The solution is to manually load a dataset from your own data directory, either from the data browser, or from the **File** menu.

29.15.2 Commands seem slow to complete

In some situations, automated commands can be slow to complete. Automatic tuning consists of small motors adjusting the same kinds of controls that are set manually on the other instruments. Initial settings can take up to ten minutes to be established if the spectrometer console has been restarted.

If typing a command produces an unexpected error message, a previous command is probably still active. Cancel the error and try typing the command again.

Current activity can be checked with the command **show**. This will list all active commands with the option to kill them. Alternatively, exiting and re-starting *TopSpin* can resolve issues.

30 *XwinNMR* and *TopSpin* file locations

The file structures for *XwinNMR* and *TopSpin* installations are quite complex. Files such as pulse programs, automation programs and plot layouts are stored within the program structure so that they are available to all datasets. Files such as peak lists, multiplet analyses and integral regions are dataset specific and so are stored within the directory of the current experiment.

The relative locations of the files are similar under all operating systems, but the absolute locations vary.

30.1 NMR data files

Traditionally, under a Unix file system, Bruker NMR data has been stored in the following structure:

expname/*expno*/pdata/*procno*/

The expno directory contains the raw data, fid (1D) or ser (2D) files), parameters and associated files. The pdata subdirectory contains all processed files created from that raw data. Normally procno will be 1 unless multiple spectra have been generated from the same raw data. The procno directory contains the spectrum data files, 1r and 1i (1D) or 2rr and 2ii (2D), parameter files, plot format files and any other files generated by processing such as integrals and peak lists.

When copying data to your own computer, it is advisable to copy the experiment name directory and all of the files it contains. Not all of the files will be required, but the above structure will be expected by whichever program is importing the data.

At UCI, the location of data files on nmrserver is:

/v/data/*username*/nmr/*expname*

this can be found via a link in each user's home directory:

NMRdata/*expname*

A user's own installation of *TopSpin* does not have to follow this convention. Data can be stored anywhere as long as the structure within each experiment name is preserved. It is often convenient to keep all experiment names within the same directory for easy access from *TopSpin*, but other options may be preferred.

30.2 Output files from data processing

Operations such as integration, peak picking, multiplet analysis etc, generate output files which may be required for external use. These files will normally be stored within the processed data directory of the current dataset:

expname/*expno*/pdata/*procno*/

30.3 *TopSpin* program files

30.3.1 Linux

The standard *TopSpin* installation directory is:

/opt/topspin*version*

There may be a link to the current installation from /opt/topspin for easy access.

Automation programs can be found in:

/opt/topspin*version*/exp/stan/nmr/au/src (Bruker files)

/opt/topspin*version*/exp/stan/nmr/au/src/user (user added or modified files)

Macro files can be found in:

/opt/topspin*version*/exp/stan/nmr/lists/mac (Bruker files)

/opt/topspin*version*/exp/stan/nmr/lists/mac/user (user added or modified files)

Pulse program files can be found in:

/opt/topspin*version*/exp/stan/nmr/lists/pp (Bruker files)

/opt/topspin*version*/exp/stan/nmr/lists/pp/user (user added or modified files)

Plot layouts can be found in:

opt/topspin*version*/plot/layouts

30.3.2 MacOS

File locations are the same as Linux (above), but there is an extra Application file which is used to start *TopSpin*, typically /Applications/TopSpin*version*

A complication for users wishing to add/change/inspect files, is that the /opt directory is 'hidden' in a standard Finder window and so cannot easily be opened.

The Finder preferences can be changed to show 'hidden' files as follows.

MacOS Sierra (10.12) or newer:

Press **CMD SHIFT** and **.** together to toggle the preference on and off.

On older versions of MacOS, the following command in a Terminal window will turn on display of 'hidden' files:

```
defaults write com.apple.finder AppleShowAllFiles YES
```

then restart the Finder.

To reverse this, repeat the above but use the command:

```
defaults write com.apple.finder AppleShowAllFiles NO
```

30.3.3 Microsoft *Windows*

A desktop shortcut will normally automatically be setup to start the *TopSpin* program. The program file structure will normally be created as for Unix operating systems above, but inside the directory C:\Bruker\TopSpin*version*\

30.4 History, error files etc

Files relating to a user's most recent *XwinNMR* or *TopSpin* session are stored within that user's 'current directory'. These are located as follows:

XwinNMR: /u/prog/curdir/*username*

TopSpin: topspin*version*/prog/curdir/*username* (see above for location of topspin*version*)

31 DRX400 automation using *IconNMR*

31.1 Introduction

The DRX400 is equipped with a 120 position automatic sample changer. This can be used via the *IconNMR* automation interface of *XwinNMR*. If *IconNMR* is running, then manual operation is not available unless the automation run is stopped by the Facility Director.

The current standard state is that this instrument is operated manually using *XwinNMR*, similarly to the 500MHz spectrometers. Automation sessions can be arranged for any user who needs to run many samples in a short period of time, contact Dr Dennison.

31.2 Choice of NMR tube

The automatic sample changer has a robot arm that grabs the top of the NMR tube from above and grips the top end of the tube. It is very important that the arm grabs the glass tube and not the cap, as the cap will come off and the tube will be dropped on the floor.

There are two restrictions on the NMR tubes that can be used with the automatic changer:

The **minimum length of tube is 17.8cm (7 inches)**.

Only **standard plastic caps or rubber septum caps** can be used. NMR tubes with vacuum connectors such as Young's taps cannot be used.

Tubes that are longer than **20.3cm (8 inches)**, including the cap, can only be used in the inner sample positions, 1-60. If a tube exceeds this length in an outer holder it will obstruct the loading of a sample from the adjacent inner position.

See Appendix D for dimensions of a typical sample.

31.3 Sample labeling

Loose paper labels must **not** be used. The ideal labeling method is to write directly onto the side of the NMR tube, close to the open end, using a fine-pointed permanent marker pen. The tube can be cleaned using an organic solvent. An alternative is to tape a paper label to the tube, but the label must be tightly wrapped round the tube above the spinner position, with no loose flaps.

31.4 Sample loading

Clean the NMR tube, check that it is straight, and position a spinner turbine in the normal way. Most spinner turbines for the sample changer are white in color and can be used in the temperature range -40°C (243K) to +40°C (313K).

The automatic sample changer has 120 sample positions. Holders numbered 1-60 are on the inside track and 61-120 are on the outside track. Load the sample into a holder and note the holder number. If necessary, the sample carousel can be rotated using the control buttons on the side of the sample changer to access an empty holder.

It is important to ensure that the sample tube is sitting vertically in the holder. If the tube is inclined the robot arm may hit the top of the tube and break it as it lowers onto the sample.

31.5 Logging on

The *IconNMR* automation software should already be running.

Each user must log on using their standard NMR user ID and password. This ensures that data is stored in the correct user directory and so is available for re-processing. The current user will be logged out automatically after two minutes of inactivity. Alternatively, the large green **Change User** button can be used. The menu can be used to select a username, or it can be typed into the **User ID** box.

The top right of the automation panel should show the approximate duration of the currently queued experiments.

31.6 Setting up experiments

31.6.1 Initial setup

Select the holder position and double click on it to activate data entry. The holder number can be selected by scrolling the list, or via a button containing a pattern of four squares. This button will open a window containing a grid of numbered circles representing the holders. If the sample holder number contains details of completed experiment(s), these can be deleted.

A row of empty boxes will appear which must be filled to define the desired experiment(s).

Name Enter an experiment name.

No Enter an experiment number - the first experiment will be numbered '1' by default.

Solvent Select the required solvent name from the drop down menu.

Experiment Select the required experiment from the drop down menu. Experiments labeled 'N' are single experiments, those labeled 'C' are composite experiments.

Composite experiments consist of two or more related experiments. For example, a COSY experiment requires a proton 1D experiment as a precursor, and a spectral width optimized ^{31}P experiment requires a standard wide spectral width experiment.

If a composite experiment is selected, then the required series of experiments is generated automatically. These experiments can be adjusted to avoid repetition of the same experiment. For example, the composite **ghmqc** experiment requires a proton (**h1**) and carbon (**c13**) experiment as references, and will generate them if they do not already appear in the queue for the current sample. However, if a different, but similar experiment has already been requested, then this can be used instead. In this example, if the **c13sn** experiment is already queued, then the automatically generated **c13** experiment can be deleted (using the **Delete** button) and the F1 reference experiment number under the **ghmqc** experiment can be changed to that of the **c13sn** experiment. The experiment number of the **ghmqc** experiment can also be decremented.

See Appendix G for the current list of available experiments.

Par Parameters **ns**, **d1** and **te** can be modified via this dialogue. Click on the yellow on blue **=** box to access these parameters. After modifying one of these parameters the icon changes to a yellow on red **!**. All other parameters can be accessed via the **Parameters** menu at the top of the window. The sample temperature will be controlled at 298K unless the parameter **te** is changed. Samples can be heated but not cooled. The standard white sample spinner can only be used up to +40°C (313K).

Orig/Title Enter a title. Choosing **Set & Copy Title** will copy the title to all experiments for the current sample holder.

31.6.2 Adding further experiments on the same sample

Highlight a single entry for the current holder with the ***LMB*** and press **Add** to append a new experiment entry line.

31.6.3 Experiment submission

Highlight the experiment(s) with the ***LMB*** and then press **Submit** to add the experiments to the automation queue. Samples will be loaded in the order in which they appear in the

31.6.4 Modifying queued experiments

Experiments can be modified at any time before they are started. Highlight the line(s) containing the experiment(s) to be modified using the ***LMB***, then press **Cancel** followed by **Edit**.

31.6.5 Experiment execution and priority

Normally, samples will be loaded and experiments executed in the chronological order in which they were added to the queue, but there are two priority settings for experiments. Quick experiments, for example 1D proton, gcosy, 1D ^{31}P and 1D ^{19}F experiments can be run at any time, but longer duration experiments can only be run during the off-peak period - 05:00 to 07:00.

31.6.6 Experiment status

At the bottom of the Acquisition Setup window there is a history list containing details of recent experiments.

31.6.7 Observing experiments in progress

When an experiment is in progress, the Acquisition Controls Window should be displayed on the left of the screen. If it is not visible, this window can be turned on via the **View** menu, or brought to the front of the window stack using the large **i** button.

The buttons in the Acquisition Controls Window allow display of the current data, and also allow the experiment to be terminated before it has finished.

31.7 Progress notification and data processing

Progress is notified to the user via email. At the end of the experiment the data is automatically processed and a PDF of the spectrum dispatched by email.

The automatic output should be regarded as a rough guide to the spectrum. Most data will require re-processing for accurate integration and interpretation. There is also a limit to the 'resolution' of the PDF spectrum. Although PDF is a vector format, there is a limit on how many datapoints can be included in creating the file. Therefore, expanding the display of the PDF spectrum on-screen will not reveal as much spectral information as expanding the original data. A more accurate PDF can be created from the expanded section, if required.

Data can be accessed using *XwinNMR* in the usual manner for re-processing. Note that data is not available until the *IconNMR* data acquisition and processing has been completed. During an experiment data can be examined from within *IconNMR*, but data is not copied to the owner's NMR data directory until the end of the experiment.

31.8 Sample retrieval

Finally, collect your sample(s) from the sample changer so that the position(s) become available to other users. Do not leave empty spinner turbines in the carousel, but return them to the box. Samples should be retrieved promptly. There are currently not enough sample spinners to fill every sample position in the carousel simultaneously. Note that the sample holder number is incorporated into the spectrum title.

31.9 Troubleshooting

The automation run is 'owned' by the user who started *IconNMR* during an *XwinNMR* session. This user will normally be the NMR Facility Director or the user 'auto'. The run cannot be stopped by another user, but some error situations can be corrected.

31.9.1 Automation is still running, but individual sample failed

If an error has occurred during the automation run and a sample has not been run, the sample will be labeled as 'Failed' instead of 'Finished' in the main automation window. The following checks should be performed:

31.9.1.1 Correct holder position?

Check that the sample is in the correct holder position - remember that the inside track contains holders 1-60 and the outer track contains holders 61-120.

31.9.1.2 Tube in correct position and correct length?

Check that the NMR tube is positioned correctly in the spinner and is long enough to be grabbed by the robot arm, and is sitting vertically in the carousel holder.

31.9.1.3 Tube broken?

If the tube was not originally positioned correctly, the top part may have been broken by the robot arm. If the spinner is still present in the carousel then the arm will repeatedly try and fail to pick up the sample. An empty spinner, or spinner with a broken tube, will appear as a sample to the sensors in the carousel. If the position of the robot arm obstructs the removal of the sample or spinner, see the instructions below.

31.9.2 Automation appears to be frozen

If a series of errors occurs then the automation program will refuse to run any samples, producing a series of error messages. Any new samples added to the queue are likely to be rapidly labeled 'Failed'.

31.9.2.1 Check error messages

Error messages can appear either on the computer screen or on the sample changer unit. On the side farthest from the magnet there is an LCD display surrounded by buttons. The buttons on the right side of the display are labeled **CONT** (continue) and **RESET**, and have LEDs inset which may be illuminated. If the red LED is flashing on the **CONT** button, then press this button to try and continue the run.

If the error message 'spin rate read error' appears on the computer screen, then the run must be restarted by the Facility Director.

31.9.2.2 Clear broken tube or empty spinner

If the system is repeatedly trying to load an empty spinner or broken tube, then this must be removed from the carousel. The robot arm is likely to be positioned just above the holder position, making it impossible to remove the spinner. Press the **RESET** button on the carousel. The robot arm will move away from the carousel so that the holder can be emptied.

31.9.2.3 Reset the sample changer

If the red LED is flashing, then press the **CONT** button. Cancel and resubmit a sample and see if the automation runs successfully. If this fails then try pressing the **RESET** and **CONT** buttons again before canceling and resubmitting a sample.

Appendix A NMR solvent data

The data below is modified slightly from the table supplied by Cambridge Isotopes Limited. The current PDF file on their website lists the chemical shift for the residual proton signal from deuterated chloroform as 7.24ppm. This has been changed to the more common value of 7.27ppm. The chemical shift values listed below are used by the **sref** command in *XwinNMR*.

Note that most chemical shift values are temperature, concentration and pH dependent. TMS should always be used for precise calibration.

SOLVENT (*XwinNMR* abbreviation)	^{1}H chemical shift: ppm (multiplicity)	J_{HD}: Hz	^{13}C chemical shift: ppm	J_{CD}: Hz	^{1}H chemical shift of HOD: ppm	density at 20°C (293K): gcm^{-3}	melting point: °C (K)	boiling point: °C (K)	dielectric constant
acetic acid-d4 (Acetic, AceticT)	11.65 (1) 2.04 (5)	2.2	178.99 (1) 20.0 (7)	2.0	11.5	1.12	16 (289)	115 (389)	6.1
acetone-d6 (Aceton, AcetonT, Acetone, AcetoneT)	2.05 (5)	2.2	206.68 (1) 29.92 (7)	0.9 19.4	2.8	0.87	-94 (179)	57 (330)	20.7
acetonitrile-d3 (CD3CN, CD3CNT)	1.94 (5)	2.5	118.69 (1) 1.39 (7)	21	2.1	0.84	-45 (228)	82 (355)	37.5
benzene-d6 (C6D6, C6D6T)	7.16 (1)		128.39 (3)	24.3	0.4	0.95	5 (278)	80 (353)	2.3
chloroform-d (CDCl3, CDCl3T)	7.27 (1)		77.23 (3)	32.0	1.5	1.50	-64 (209)	62 (335)	4.8
cyclohexane-d12 (C6D12)	1.38 (1)		26.43 (5)	19	0.8	0.89	6 (279)	81 (354)	2.0
deuterium oxide (D2O, D2OT)	4.80 (DSS) 4.81 (TSP)				4.8	1.11	3.8 (277)	101 (374)	78.5
N,N-dimethyl-formamide-d7 (DMF, DMFT)	8.03 (1) 2.92 (5) 2.75 (5)	 1.9 1.9	163.15 (3) 34.89 (7) 29.76 (7)	29.4 21.0 21.1	3.5	1.04	-61 (212)	153 (426)	36.7
dimethyl sulfoxide-d6 (DMSO, DMSOT)	2.50 (5)	1.9	39.51 (7)	21.0	3.3	1.18	18 (291)	189 (462)	46.7
1,4-dioxane-d8 (Dioxan, DioxanT)	3.53 (m)		66.66 (5)	21.9	2.4	1.13	12 (285)	101 (374)	2.2
ethanol-d6 (EtOH)	5.29 (1) 3.56 (1) 1.11 (m)		56.96 (5) 17.31 (7)	22 19	5.3	0.89	<-130 (<143)	79 (352)	24.5
methanol-d4 (CD3OD, CD3ODT, MeOH)	4.87 (1) 3.31 (5)	 1.7	49.15 (7)	21.4	4.9	0.89	-98 (175)	65 (338)	32.7
methylene chloride-d2 (CD2Cl2, CD2Cl2T)	5.32 (3)	1.1	54.00 (5)	27.2	1.5	1.35	-95 (178)	40 (313)	
pyridine-d5 (C5D5N, C5D5NT, Pyr, PyrT)	8.74 (1) 7.58 (1) 7.22 (1)		150.35 (3) 135.91 (3) 123.87 (3)	27.5 24.5 3	5	1.05	-42 (231)	116 (389)	12.4
tetrahydrofuran-d8 (THF, THFT)	3.58 (1) 1.73 (1)		67.57 (5) 25.37 (5)	22.2 20.2	2.4-2.5	0.99	-109 (164)	66 (339)	7.6
toluene-d8 (Tol, TolT, Toluene, TolueneT)	7.09 (m) 7.00 (1) 6.98 (m) 2.09 (5)	 2.3	137.86 (1) 129.24 (3) 128.33 (3) 125.49 (3) 20.4 (7)	 23 24 24 19	0.4	0.94	-95 (178)	111 (384)	2.4
trifluoroacetic acid-d (TFA)	11.50 (1)		164.2 (4) 116.6 (4)		11.5	1.50	-15 (258)	72 (345)	
trifluoroethanol-d3	5.02 (1) 3.88 (4x3) 2 (9)		126.3 (4) 61.5 (4x5)	22	5	1.41	-44 (229)	75 (348)	

Appendix B Chemical shifts of solvents as impurities

Tables taken from: Fulmer et al., *Organometallics* **2010**, 29, 2176-2179, supplementary information.

Table S1. [1]H NMR Data[2]

	proton	mult	THF-d_8	CD_2Cl_2	$CDCl_3$	toluene-d_8	C_6D_6	C_6D_5Cl	$(CD_3)_2CO$	$(CD_3)_2SO$	CD_3CN	TFE-d_3	CD_3OD	D_2O
solvent residual signals			1.72 3.58	5.32	7.26	2.08 6.97 7.01 7.09	7.16	6.96 6.99 7.14	2.05	2.50	1.94	5.02 3.88	3.31	4.79
water	OH	s	2.46	1.52	1.56	0.43	0.40	1.03	2.84[3]	3.33[3]	2.13	3.66	4.87	-
acetic acid	CH_3	s	1.89	2.06	2.10	1.57	1.52	1.76	1.96	1.91	1.96	2.06	1.99	2.08
acetone	CH_3	s	2.05	2.12	2.17	1.57	1.55	1.77	2.09	2.09	2.08	2.19	2.15	2.22
acetonitrile	CH_3	s	1.95	1.97	2.10	0.69	0.58	1.21	2.05	2.07	1.96	1.95	2.03	2.06
allyl acetate	C***H***CH_2	ddt	5.90	5.92	5.93	5.67[4]	5.68[4]	5.77	5.92	5.91	5.93	5.93	5.94	5.99
	CHC***H***$_2$(1)	ddt	5.27	5.31	5.32	5.05	5.06	5.15	5.29	5.29	5.29	5.32	5.30	5.37
	CHC***H***$_2$(2)	ddt	5.15	5.22	5.24	4.94	4.94	5.04	5.18	5.20	5.21	5.25	5.21	5.30
	CH_2	ddd	4.50	4.55	4.57	4.34	4.38	4.44	4.53	4.52	4.53	4.58	4.56	4.62
	CH_3	s	1.98	2.05	2.09	1.63	1.63	1.80	2.02	2.03	2.02	2.07	2.05	2.13
benzaldehyde	HCO	s	9.98	10.01	10.03	9.57	9.64	9.77	10.05	10.02	10.01	9.88	10.00	9.96
	CH(2,6)	m	7.86–7.88	7.87–7.89	7.88–7.91	7.45–7.47	7.49–7.53	7.59–7.61	7.92–7.94	7.91–7.93	7.89–7.91	7.90–7.92	7.90–7.93	7.97–7.99
	CH(3,5)	m	7.51–7.55	7.53–7.57	7.51–7.57	6.95–6.99	6.93–6.99	7.15–7.19	7.59–7.63	7.61–7.67	7.57–7.61	7.56–7.59	7.56–7.60	7.57–7.66
	CH(4)	m	7.60–7.64	7.63–7.67	7.61–7.65	7.03–7.07	7.01–7.07	7.24–7.28	7.69–7.73	7.69–7.75	7.67–7.71	7.68–7.72	7.66–7.70	7.76–7.80
benzene	CH	s	7.31	7.35	7.36	7.12	7.15	7.20	7.36	7.37	7.37	7.36	7.33	-
tert-butyl alcohol	CH_3	s	1.15	1.24	1.28	1.03	1.05	1.12	1.18	1.11	1.16	1.28	1.40	1.24
	OH	s[5]	3.16	-	-	0.58	0.63	1.30	-	4.19	2.18	2.20	-	-
BHA	ArH	s	6.68	6.73	6.76	6.83	6.93	6.83	6.72	6.62	6.73	6.87	6.71	-
	OH	s[5]	5.64	4.76	4.76	4.45	4.53	4.62	5.65	6.52	4.98	-	4.85	-
	$ArOCH_3$	s	3.68	3.73	3.77	3.48	3.48	3.61	3.72	3.66	3.72	3.79	3.72	-
	$ArC(CH_3)_3$	s	1.40	1.42	1.44	1.34	1.41	1.37	1.41	1.36	1.40	1.44	1.41	-
BHT	ArH	s	6.92	6.97	6.98	6.99	7.05	6.97	6.96	6.87	6.97	7.06	6.92	-
	OH	s[5]	5.81	5.00	5.01	4.72	4.79	5.50	-	6.65	5.20	-	-	-
	$ArCH_3$	s	2.21	2.25	2.27	2.23	2.24	2.20	2.22	2.18	2.22	2.24	2.21	-
	$ArC(CH_3)_3$	s	1.40	1.42	1.43	1.36	1.38	1.37	1.41	1.36	1.39	1.43	1.40	-
chloroform	CH	s	7.89	7.32	7.26	6.10	6.15	6.74	8.02	8.32	7.58	7.33	7.90	-
18-crown-6	CH_2	s	3.57	3.59	3.67	3.36	3.39	3.41	3.59	3.51	3.51	3.64	3.64	3.80
cyclohexane	CH_2	s	1.44	1.44	1.43	1.40	1.40	1.37	1.43	1.40	1.44	1.47	1.45	-
cyclohexanone	CH_2(2,6)	t	2.24	2.29	2.33	1.95	1.98	2.08	2.27	2.25	2.27	2.38	2.34	2.40
	CH_2(3,5)	m	1.77–1.82	1.81–1.87	1.84–1.86	1.33–1.39	1.28–1.37	1.48–1.53	1.79–1.83	1.74–1.78	1.79–1.84	1.87–1.92	1.85–1.87	1.85–1.90
	CH_2(4)	m	1.68–1.71	1.69–1.72	1.71–1.73	1.16–1.20	1.08–1.16	1.33–1.37	1.70–1.74	1.64–1.66	1.67–1.72	1.75–1.78	1.74–1.76	1.70–1.75
diallyl carbonate	C***H***CH_2	ddt	5.92	5.95	5.94	5.63	5.65	5.75	5.96	5.93	5.96	5.92	5.94	5.99
	CHC***H***$_2$(1)	ddt	5.31	5.35	5.37	5.09	5.09	5.17	5.35	5.33	5.34	5.35	5.34	5.40
	CHC***H***$_2$(2)	ddt	5.19	5.26	5.27	4.92	4.92	5.03	5.23	5.25	5.25	5.28	5.25	5.32
	CH_2	ddd	4.58	4.61	4.64	4.34	4.38	4.46	4.62	4.61	4.61	4.62	4.61	4.69
1,2-dichloroethane	CH_2	s	3.77	3.76	3.73	2.91	2.90	3.26	3.87	3.90	3.81	3.71	3.78	-
dichloromethane	CH_2	s	5.51	5.33	5.30	4.32	4.27	4.77	5.63	5.76	5.44	5.24	5.49	-
diethyl ether	CH_3	t, 7	1.12	1.15	1.21	1.10	1.11	1.10	1.11	1.09	1.12	1.20	1.18	1.17
	CH_2	q, 7	3.38	3.43	3.48	3.25	3.26	3.31	3.41	3.38	3.42	3.58	3.49	3.56
diglyme	CH_2	m	3.43	3.57	3.65	3.43	3.46	3.49	3.56	3.51	3.53	3.67	3.61	3.67
	CH_2	m	3.53	3.50	3.57	3.31	3.34	3.37	3.47	3.38	3.45	3.62	3.58	3.61
	OCH_3	s	3.28	3.33	3.39	3.12	3.11	3.16	3.28	3.24	3.29	3.41	3.35	3.37
1,2-dimethoxyethane	CH_3	s	3.28	3.34	3.40	3.12	3.12	3.17	3.28	3.24	3.28	3.40	3.35	3.37
	CH_2	s	3.43	3.49	3.55	3.31	3.33	3.37	3.46	3.43	3.45	3.61	3.52	3.60
dimethylacetamide	CH_3CO	s	1.94	2.02	2.09	1.59	1.60	1.74	1.97	1.96	1.97	2.09	2.07	2.08
	NCH_3	s	2.95	2.97	3.02	2.56	2.57	2.65	3.00	2.94	2.96	3.05	3.31	3.06
	NCH_3	s	2.82	2.87	2.94	2.11	2.05	2.42	2.83	2.78	2.83	2.94	2.92	2.90
dimethyl carbonate	CH_3	s	3.69	3.75	3.79	3.31	3.30	3.48	3.72	3.69	3.72	3.77	3.74	3.69
dimethyl malonate	CH_3	s	3.65	3.72	3.75	3.24	3.23	3.41	3.68	3.65	3.68	3.76	3.72	3.78
	CH_2	s	3.35	3.37	3.40	2.92	2.97	3.15	3.42	3.53	3.38	3.41	3.44	3.60
dimethylformamide	CH	s	7.91	7.96	8.02	7.57	7.63	7.73	7.96	7.95	7.92	7.86	7.97	7.92
	CH_3	s	2.88	2.91	2.96	2.37	2.36	2.51	2.94	2.89	2.89	2.98	2.99	3.01
	CH_3	s	2.76	2.82	2.88	1.96	1.86	2.30	2.78	2.73	2.77	2.88	2.86	2.85
dimethyl sulfoxide	CH_3	s	2.45	2.55	2.62	1.64	1.68	2.03	2.52	2.54	2.50	2.63	2.65	2.71
1,4-dioxane	CH_2	s	3.56	3.65	3.71	3.33	3.35	3.45	3.59	3.57	3.60	3.76	3.66	3.75

	proton	mult	THF-d_8	CD_2Cl_2	$CDCl_3$	toluene-d_8	C_6D_6	C_6D_5Cl	$(CD_3)_2CO$	$(CD_3)_2SO$	CD_3CN	TFE-d_3	CD_3OD	D_2O
solvent residual signals			1.72 3.58	5.32	7.26	2.08 6.97 7.01 7.09	7.16	6.96 6.99 7.14	2.05	2.50	1.94	5.02 3.88	3.31	4.79
ethane	CH_3	s	0.85	0.85	0.87	0.81	0.80	0.79	0.83	0.82	0.85	0.85	0.85	0.82
ethanol	CH_3	t, 7	1.10	1.19	1.25	0.97	0.96	1.06	1.12	1.06	1.12	1.22	1.19	1.17
	CH_2	q, 7[6]	3.51	3.66	3.72	3.36	3.34	3.51	3.57	3.44	3.54	3.71	3.60	3.65
	OH	s[5,6]	3.30	1.33	1.32	0.83	0.50	1.39	3.39	4.63	2.47	-	-	-
ethyl acetate	CH_3CO	s	1.94	2.00	2.05	1.69	1.65	1.78	1.97	1.99	1.97	2.03	2.01	2.07
	CH_2CH_3	q, 7	4.04	4.08	4.12	3.87	3.89	3.96	4.05	4.03	4.06	4.14	4.09	4.14
	CH_2CH_3	t, 7	1.19	1.23	1.26	0.94	0.92	1.04	1.20	1.17	1.20	1.26	1.24	1.24
ethyl methyl ketone	CH_3CO	s	2.03	2.09	2.14	1.59	1.58	1.78	2.07	2.07	2.06	2.16	2.12	2.19
	CH_2CH_3	q, 7	2.39	2.43	2.46	1.82	1.81	2.06	2.45	2.43	2.43	2.49	2.50	3.18
	CH_2CH_3	t, 7	0.96	1.00	1.06	0.84	0.85	0.89	0.96	0.91	0.96	1.05	1.01	1.26
ethylene	CH_2	s	5.36	5.40	5.40	5.25	5.25	5.29	5.38	5.41	5.41	5.40	5.39	5.44
ethylene glycol	CH_2	s[7]	3.48	3.66	3.76	3.36	3.41	3.58	3.28	3.34	3.51	3.72	3.59	3.65
furan	CH(2,5)	dd	7.48	7.46	7.45	7.10	7.13	7.24	7.56	7.67	7.52	7.44	7.49	7.57
	CH(3,4)	dd	6.37	6.41	6.40	6.07	6.08	6.19	6.43	6.47	6.44	6.42	6.40	6.51
H grease[8]	CH_3	m	0.85–0.91	0.84–0.90	0.84–0.87	0.89–0.96	0.90–0.98	0.86–0.92	0.90	0.82–0.88	-	0.88–0.94	0.86–0.93	-
	CH_2	br s	1.29	1.27	1.25	1.33	1.32	1.30	1.29	1.24	-	1.33	1.29	-
hexamethylbenzene	CH_3	s	2.18	2.20	2.24	2.10	2.13	2.10	2.17	2.14	2.19	2.24	2.19	-
hexamethyldisiloxane	CH_3	s	0.07	0.07	0.07	0.10	0.12	0.10	0.07	0.06	0.07	0.08	0.07	0.28
n-hexane	CH_3	t, 7	0.89	0.89	0.88	0.88	0.89	0.85	0.88	0.86	0.89	0.91	0.90	-
	CH_2	m	1.29	1.27	1.26	1.22	1.24	1.19	1.28	1.25	1.28	1.31	1.29	-
HMPA	CH_3	d, 9.5	2.58	2.60	2.65	2.42	2.40	2.47	2.59	2.53	2.57	2.63	2.64	2.61
hydrogen	H_2	s	4.55	4.59	4.62	4.50	4.47	4.49	4.54	4.61	4.57	4.53	4.56	-
imidazole	CH(2)	s	7.48	7.63	7.67	7.30	7.33	7.53	7.62	7.63	7.57	7.61	7.67	7.78
	CH(4,5)	s	6.94	7.07	7.10	6.86	6.90	7.01	7.04	7.01	7.01	7.03	7.05	7.14
methane	CH_4	s	0.19	0.21	0.22	0.17	0.16	0.15	0.17	0.20	0.20	0.18	0.20	0.18
methanol	CH_3	s[9]	3.27	3.42	3.49	3.03	3.07	3.25	3.31	3.16	3.28	3.44	3.34	3.34
	OH	s[5,9]	3.02	1.09	1.09	-	-	1.30	3.12	4.01	2.16	-	-	-
nitromethane	CH_3	s	4.31	4.31	4.33	3.01	2.94	3.59	4.43	4.42	4.31	4.28	4.34	4.40
n-pentane	CH_3	t, 7	0.89	0.89	0.88	0.87	0.87	0.84	0.88	0.86	0.89	0.90	0.90	-
	CH_2	m	1.31	1.30	1.27	1.25	1.23	1.23	1.27	1.27	1.29	1.33	1.29	-
propane	CH_3	t, 7.3	0.90	0.90	0.90	0.89	0.86	0.84	0.88	0.87	0.90	0.90	0.91	0.88
	CH_2	sept, 7.3	1.33	1.32	1.32	1.32	1.26	1.26	1.31	1.29	1.33	1.33	1.34	1.30
2-propanol	CH_3	d, 6	1.08	1.17	1.22	0.95	0.95	1.04	1.10	1.04	1.09	1.20	1.15	1.17
	CH	sept, 6	3.82	3.97	4.04	3.65	3.67	3.82	3.90	3.78	3.87	4.05	3.92	4.02
propylene	CH_3	dt, 6.4, 1.5	1.69	1.71	1.73	1.55	1.55	1.58	1.68	1.68	1.70	1.70	1.70	1.70
	CH_2(1)	dm, 10	4.89	4.93	4.94	4.92	4.95	4.91	4.90	4.94	4.93	4.93	4.91	4.95
	CH_2(2)	dm, 17	4.99	5.03	5.03	4.98	5.01	4.98	5.00	5.03	5.04	5.03	5.01	5.06
	CH	m	5.79	5.84	5.83	5.70	5.72	5.72	5.81	5.80	5.85	5.87	5.82	5.90
pump oil	CH_3	m	0.86–0.90	0.84–0.89	0.83–0.89	0.88–0.96	0.91–0.97	0.88–0.91	0.87	0.74	0.85	0.99	0.86–0.91	-
	CH_2	br s	1.29	1.27	1.26	1.30	1.37	1.31	1.29	1.15	1.27	1.41	1.29	-
pyridine	CH(2,6)	m	8.54	8.59	8.62	8.47	8.53	8.51	8.58	8.58	8.57	8.45	8.53	8.52
	CH(3,5)	m	7.25	7.28	7.29	6.67	6.66	6.90	7.35	7.39	7.33	7.40	7.44	7.45
	CH(4)	m	7.65	7.68	7.68	6.99	6.98	7.25	7.76	7.79	7.73	7.82	7.85	7.87
pyrrole	NH	br t	9.96	8.69	8.40	7.71	7.80	8.61	10.02	10.75	9.27	-	-	-
	CH(2,5)	m	6.66	6.79	6.83	6.43	6.48	6.62	6.77	6.73	6.75	6.84	6.72	6.93
	CH(3,4)	m	6.02	6.19	6.26	6.27	6.37	6.27	6.07	6.01	6.10	6.24	6.08	6.26
pyrrolidine[10]	CH_2(2,5)	m	2.75	2.82	2.87	2.54	2.54	2.64	-	2.67	2.75	3.11	2.80	3.07
	CH_2(3,4)	m	1.59	1.67	1.68	1.36	1.33	1.43	-	1.55	1.61	1.93	1.72	1.87
silicone grease	CH_3	s	0.11	0.09	0.07	0.26	0.29	0.14	0.13	–0.06	0.08	0.16	0.10	-
tetrahydrofuran	CH_2(2,5)	m	3.62	3.69	3.76	3.54	3.57	3.59	3.63	3.60	3.64	3.78	3.71	3.74
	CH_2(3,4)	m	1.79	1.82	1.85	1.43	1.40	1.55	1.79	1.76	1.80	1.91	1.87	1.88
toluene	CH_3	s	2.31	2.34	2.36	2.11	2.11	2.16	2.32	2.30	2.33	2.33	2.32	-
	CH(2,4,6)	m	7.10	7.15	7.17	6.96–7.01	7.02	7.01–7.08	7.10–7.20	7.18	7.10–7.30	7.10–7.30	7.16	-
	CH(3,5)	m	7.19	7.24	7.25	7.09	7.13	7.10–7.17	7.10–7.20	7.25	7.10–7.30	7.10–7.30	7.16	-
triethylamine	CH_3	t, 7	0.97	0.99	1.03	0.95	0.96	0.93	0.96	0.93	0.96	1.31	1.05	0.99
	CH_2	q, 7	2.46	2.48	2.53	2.39	2.40	2.39	2.45	2.43	2.45	3.12	2.58	2.57

Table S2. $^{13}C\{^{1}H\}$ NMR Data[2]

	carbon	THF-d_8	CD_2Cl_2	$CDCl_3$	toluene-d_8	C_6D_6	C_6D_5Cl	$(CD_3)_2CO$	$(CD_3)_2SO$	CD_3CN	TFE-d_3	CD_3OD	D_2O
solvent signals		67.21 25.31	53.84	77.16	137.48 128.87 127.96 125.13 20.43	128.06	134.19 129.26 128.25 125.96	29.84 206.26	39.52	1.32 118.26	61.50 126.28	49.00	-
acetic acid	CO	171.69	175.85	175.99	175.30	175.82	175.67	172.31	171.93	173.21	177.96	175.11	177.21
	CH_3	20.13	20.91	20.81	20.27	20.37	20.40	20.51	20.95	20.73	20.91	20.56	21.03
acetone	CO	204.19	206.78	207.07	204.00	204.43	204.83	205.87	206.31	207.43	32.35	209.67	215.94
	CH_3	30.17	31.00	30.92	30.03	30.14	30.12	30.60	30.56	30.91	214.98	30.67	30.89
acetonitrile	CN	116.79	116.92	116.43	115.76	116.02	115.93	117.60	117.91	118.26	118.95	118.06	119.68
	CH_3	0.45	2.03	1.89	0.03	0.20	0.63	1.12	1.03	1.79	1.00	0.85	1.47
allyl acetate	CO	170.14	170.83	170.81	169.44	169.67	169.59	170.61	169.97	171.32	175.98	172.41	174.78
	$\boldsymbol{C}HCH_2$	133.90	132.94	132.33	132.98	132.90	132.69	133.76	132.71	133.83	133.33	133.71	132.48
	$CH\boldsymbol{C}H_2$	117.58	118.00	118.34	117.49	117.64	117.63	117.81	117.64	118.06	119.39	118.22	119.03
	CH_2	65.31	65.36	65.28	64.87	64.92	64.86	65.28	64.32	65.55	67.61	66.14	66.52
	CH_3	20.45	21.06	21.02	20.21	20.37	20.40	20.68	20.54	21.02	21.10	20.71	21.00
benzaldehyde	HCO	191.95	192.61	192.67	191.09	191.43	191.24	192.95	193.08	193.64	197.63	194.11	191.67
	C(1)	137.78	136.98	136.58	137.12	137.05	136.78	137.66	136.20	137.62	137.84	137.96	136.11
	CH(2,6)	129.98	129.98	129.91	129.61	129.65	129.49	130.23	129.45	130.42	131.78	130.64	130.09
	CH(3,5)	129.56	129.42	129.16	128.68	128.95	128.87	129.90	129.10	130.07	130.82	130.12	129.48
	CH (4)	134.67	134.79	134.64	133.88	133.95	134.02	135.14	134.52	135.40	137.17	135.60	134.70
benzene	CH	128.84	128.68	128.37	128.57	128.62	128.38	129.15	128.30	129.32	129.84	129.34	-
tert-butyl alcohol	$(CH_3)_3\boldsymbol{C}$	67.50	69.11	69.15	68.12	68.19	68.19	68.13	66.88	68.74	72.35	69.40	70.36
	$(\boldsymbol{C}H_3)_3C$	30.57	31.46	31.25	30.49	30.47	31.13	30.72	30.38	30.68	31.07	30.91	30.29
BHA	C(1)	154.07	153.05	152.57	153.50	153.62	153.19	153.97	152.53	154.02	153.74	154.34	-
	C(2,6)	148.62	148.06	147.85	148.06	148.13	147.87	148.48	147.44	148.39	150.52	149.04	-
	CH(3,5)	110.94	110.93	110.69	110.99	111.15	110.84	111.00	109.80	111.35	112.90	111.30	-
	C(4)	140.07	137.77	137.36	137.34	137.50	137.29	140.32	141.16	140.20	140.23	141.36	-
	CH_3O	55.39	55.88	55.70	55.04	55.27	55.08	55.51	54.89	55.94	57.55	55.96	-
	$(\boldsymbol{C}H_3)_3C$	30.65	30.37	30.32	30.30	30.35	30.21	30.64	30.30	30.55	30.80	30.82	-
	$(CH_3)_3\boldsymbol{C}$	35.51	34.91	34.72	34.69	34.72	34.56	35.45	34.76	35.48	36.07	35.83	-
BHT	C(1)	152.48	151.92	151.55	152.06	152.05	151.69	152.51	151.47	152.42	153.46	152.85	-
	C(2,6)	137.93	136.32	135.87	136.12	136.08	135.92	138.19	139.12	138.13	138.59	139.09	-
	CH(3,5)	125.71	125.84	125.55	125.79	125.83	125.58	126.03	124.85	126.38	127.11	126.11	-
	C(4)	128.64	128.73	128.27	128.44	128.52	128.26	129.05	127.97	129.61	130.62	129.49	-
	CH_3Ar	21.21	21.27	21.20	21.42	21.40	21.10	21.31	20.97	21.23	21.34	21.38	-
	$(\boldsymbol{C}H_3)_3C$	31.55	30.54	30.33	31.39	31.34	30.19	31.61	31.25	31.50	31.01	31.15	-
	$(CH_3)_3\boldsymbol{C}$	34.91	34.56	34.25	34.39	34.35	34.11	35.00	34.33	35.05	35.69	35.36	-
carbon dioxide	CO_2	125.69	125.26	124.99	124.86	124.76	126.08	125.81	124.21	125.89	126.92	126.31	-
carbon disulfide	CS_2	193.37	192.95	192.83	192.71	192.69	192.49	193.58	192.63	193.60	196.26	193.82	197.25
carbon tetrachloride	CCl_4	96.89	96.52	96.34	96.57	96.44	96.38	96.65	95.44	96.68	97.74	97.21	96.73
chloroform	CH	79.24	77.99	77.36	77.89	77.79	77.67	79.19	79.16	79.17	78.83	79.44	-
18-crown-6	CH_2	71.34	70.47	70.55	70.86	70.59	70.55	71.25	69.85	71.22	70.80	71.47	70.14
cyclohexane	CH_2	27.58	27.38	26.94	27.31	27.23	26.99	27.51	26.33	27.63	28.34	27.96	-
cyclohexanone	CO	208.79	211.82	212.57	208.60	209.10	209.30	210.36	210.63	211.99	221.30	214.69	221.22
	$CH_2(2,6)$	42.17	42.31	41.97	41.78	41.83	41.79	42.24	41.32	42.44	43.16	42.61	42.02
	$CH_2(3,5)$	27.69	27.47	27.00	27.05	27.00	27.02	27.68	26.46	27.80	28.56	28.16	27.50
	$CH_2(4)$	25.76	25.42	24.97	25.15	25.03	25.07	25.59	24.32	25.62	26.00	25.86	24.77
diallyl carbonate	CO	155.36	155.15	154.88	155.15	155.24	154.87	155.48	154.16	155.66	157.39	156.28	157.78
	$\boldsymbol{C}HCH_2$	133.08	132.24	131.58	132.30	132.18	131.93	133.16	132.18	133.20	132.72	133.25	132.76
	$CH\boldsymbol{C}H_2$	117.70	118.75	118.96	118.04	118.22	118.22	118.53	118.32	118.86	120.15	118.74	118.75
	CH_2	68.58	68.76	68.55	68.20	68.28	68.19	68.78	67.86	69.09	70.69	69.35	68.81
1,2-dichloroethane	CH_2	44.64	44.35	43.50	43.40	43.59	43.60	45.25	45.02	45.54	45.28	45.11	-
dichloromethane	CH_2	54.67	54.24	53.52	53.47	53.46	53.54	54.95	54.84	55.32	54.46	54.78	-
diethyl ether	CH_3	15.49	15.44	15.20	15.47	15.46	15.35	15.78	15.12	15.63	15.33	15.46	14.77
	CH_2	66.14	66.11	65.91	65.94	65.94	65.79	66.12	62.05	66.32	67.55	66.88	66.42
diglyme	CH_3	58.72	58.95	59.01	58.62	58.66	58.42	58.77	57.98	58.90	59.40	59.06	58.67
	CH_2	71.17	70.70	70.51	70.92	70.87	70.56	71.03	69.54	70.99	73.05	71.33	70.05
	CH_2	72.72	72.25	71.90	72.39	72.35	72.07	72.63	71.25	72.63	71.33	72.92	71.63
1,2-dimethoxyethane	CH_3	58.72	59.02	59.08	58.63	58.68	58.31	58.45	58.03	58.89	59.52	59.06	58.67
	CH_2	72.58	72.24	71.84	72.25	72.21	71.81	72.47	71.17	72.47	72.87	72.72	71.49
dimethylacetamide	CH_3	21.15	21.64	21.53	21.05	21.16	21.03	21.51	21.29	21.76	21.40	21.32	21.09
	CO	169.77	171.05	171.07	169.65	169.95	169.79	170.61	169.54	171.31	175.74	173.32	174.57
	NCH_3	34.60	35.23	35.28	34.58	34.67	34.59	34.89	34.42	35.17	36.28	35.50	35.03
	NCH_3	37.56	38.22	38.13	36.98	37.03	37.13	37.92	37.38	38.26	39.06	38.43	38.76

	carbon	THF-d_8	CD_2Cl_2	$CDCl_3$	toluene-d_8	C_6D_6	C_6D_5Cl	$(CD_3)_2CO$	$(CD_3)_2SO$	CD_3CN	TFE-d_3	CD_3OD	D_2O
solvent signals		67.21 25.31	53.84	77.16	137.48 128.87 127.96 125.13 20.43	128.06	134.19 129.26 128.25 125.96	29.84 206.26	39.52	1.32 118.26	61.50 126.28	49.00	-
dimethyl carbonate	CO	156.91	156.73	156.45	156.61	156.71	156.36	157.04	155.76	157.26	159.04	157.91	163.96
	CH_3	54.58	55.09	54.89	54.13	54.30	54.23	54.95	54.63	55.39	56.17	55.25	55.81
dimethyl malonate	CO_2	167.14	167.32	167.18	166.49	166.66	166.51	167.58	166.91	168.07	170.88	168.70	170.12
	CH_3	52.07	52.75	52.57	51.76	51.86	51.89	52.47	52.08	52.95	54.00	52.83	53.65
	CH_2	41.15	41.48	41.11	40.88	41.04	40.93	41.43	40.72	41.77	42.13	41.60	42.13
dimethylformamide	CH	161.96	162.57	162.62	161.93	162.13	162.01	162.79	162.29	163.31	166.01	164.73	165.53
	CH_3	35.65	36.56	36.50	35.22	35.25	35.45	36.15	35.73	36.57	37.76	36.89	37.54
	CH_3	30.70	31.39	31.45	30.64	30.72	30.71	31.03	30.73	31.32	30.96	31.61	32.03
dimethyl sulfoxide	CH_3	41.21	41.33	40.76	40.41	40.03	40.27	41.23	40.45	41.31	40.06	40.45	39.39
1,4-dioxane	CH_2	67.65	67.47	67.14	67.17	67.16	66.95	67.60	66.36	67.72	68.52	68.11	67.19
ethane	CH_3	6.79	6.91	6.89	6.94	6.96	6.91	6.88	6.61	6.99	7.01	6.98	-
ethanol	CH_3	18.90	18.69	18.41	18.78	18.72	18.55	18.89	18.51	18.80	18.11	18.40	17.47
	CH_2	57.60	58.57	58.28	57.81	57.86	57.63	57.72	56.07	57.96	59.68	58.26	58.05
ethyl acetate	$\boldsymbol{C}H_3CO$	20.45	21.15	21.04	20.46	20.56	20.50	20.83	20.68	21.16	21.18	20.88	21.15
	CO	170.32	171.24	171.36	170.02	170.44	170.20	170.96	170.31	171.68	175.55	172.89	175.26
	CH_2	60.30	60.63	60.49	60.08	60.21	60.06	60.56	59.74	60.98	62.70	61.50	62.32
	CH_3	14.37	14.37	14.19	14.23	14.19	14.07	14.50	14.40	14.54	14.36	14.49	13.92
ethyl methyl ketone	$\boldsymbol{C}H_3CO$	28.92	29.55	29.49	28.74	28.56	28.82	29.30	29.26	29.60	29.64	29.39	29.49
	CO	207.05	209.57	209.56	206.31	206.55	206.87	208.30	208.72	209.88	218.31	212.16	218.43
	$\boldsymbol{C}H_2CH_3$	36.59	37.01	36.89	36.32	36.36	36.39	36.75	35.83	37.09	38.23	37.34	37.27
	$CH_2\boldsymbol{C}H_3$	7.87	7.94	7.86	7.89	7.91	7.79	8.03	7.61	8.14	8.29	8.09	7.87
ethylene	CH_2	123.09	123.20	123.13	122.92	122.96	122.95	123.47	123.52	123.69	124.08	123.46	-
ethylene glycol	CH_2	64.35	64.08	63.79	64.29	64.34	64.03	64.26	62.76	64.22	64.87	64.30	63.17
furan	CH(2,5)	143.26	142.98	142.71	142.65	142.73	142.49	143.49	142.82	143.74	144.22	143.68	143.57
	CH(3,4)	109.88	109.86	109.57	109.63	109.67	109.64	110.24	109.62	110.49	111.06	110.33	110.23
H grease[8]	CH_2	30.45	30.14	29.71	30.31	30.22	30.11	-	-	-	-	-	-
hexamethylbenzene	C	131.88	132.09	132.21	131.72	131.79	131.54	132.22	131.10	132.61	134.04	132.53	-
	CH_3	16.71	16.93	16.98	16.84	16.95	16.68	16.86	16.60	16.94	17.04	16.90	-
hexamethyldisiloxane	CH_3	1.83	1.96	1.97	1.99	2.05	1.92	2.01	1.96	2.07	2.09	1.99	2.31
n-hexane	CH_3	14.22	14.28	14.14	14.34	14.32	14.18	14.34	13.88	14.43	14.63	14.45	-
	CH_2(2,5)	23.33	23.07	22.70	23.12	23.04	22.86	23.28	22.05	23.40	24.06	23.68	-
	CH_2(3,4)	32.34	32.01	31.64	32.06	31.96	31.77	32.30	30.95	32.36	33.17	32.73	-
HMPA[11]	CH_3	36.89	36.99	36.87	36.80	36.88	36.64	37.04	36.42	37.10	37.21	37.00	36.46
imidazole	CH(2)	135.72	135.76	135.38	135.57	135.76	135.50	135.89	135.15	136.33	136.58	136.31	136.65
	CH(4,5)	122.20	122.16	122.00	122.13	122.16	121.96	122.31	121.55	122.78	122.93	122.60	122.43
methane	CH_4	–4.90	–4.33	–4.63	–4.34	–4.29	–4.33	–5.33	–4.01	–4.61	–5.88	–4.90	-
methanol	CH_3	49.64	50.45	50.41	49.90	49.97	49.66	49.77	48.59	49.90	50.67	49.86	49.50[12]
nitromethane	CH_3	62.49	63.03	62.50	61.14	61.16	61.68	63.21	63.28	63.66	63.17	63.08	63.22
n-pentane	CH_3	14.18	14.24	14.08	14.27	14.25	14.10	14.29	13.28	14.37	14.54	14.39	-
	CH_2(2,4)	23.00	22.77	22.38	22.79	22.72	22.54	22.98	21.70	23.08	23.75	23.38	-
	CH_2(3)	34.87	34.57	34.16	34.54	34.45	34.26	34.83	33.48	34.89	35.76	35.30	-
propane	CH_3	16.60	16.63	16.63	16.65	16.66	16.56	16.68	16.34	16.73	16.93	16.80	-
	CH_2	16.82	16.63	16.37	16.63	16.60	16.48	16.78	15.67	16.91	17.46	17.19	-
2-propanol	CH_3	25.70	25.43	25.14	25.24	25.18	25.14	25.67	25.43	25.55	25.21	25.27	24.38
	CH	66.14	64.67	64.50	64.12	64.23	64.18	63.85	64.92	64.30	66.69	64.71	64.88
propylene	CH_3	19.27	19.47	19.50	19.32	19.38	19.32	19.42	19.20	19.48	19.63	19.50	-
	CH_2	115.74	115.70	115.74	115.89	115.92	115.86	116.03	116.07	116.12	116.38	116.04	-
	CH	134.02	134.21	133.91	133.61	133.69	133.57	134.34	133.55	134.78	136.00	134.61	-
pump oil	CH_2	30.63	30.13	29.84	30.33	30.24	30.11	30.36	29.33	30.86	31.85	31.35	-
pyridine	CH(2,6)	150.57	150.27	149.90	150.25	150.27	149.93	150.67	149.58	150.76	149.76	150.07	149.18
	CH(3,5)	124.08	124.06	123.75	123.46	123.58	123.49	124.57	123.84	127.76	126.27	125.53	125.12
	CH(4)	135.99	136.16	135.96	135.17	135.28	135.32	136.56	136.05	136.89	139.62	138.35	138.27
pyrrole	CH(2,5)	118.03	117.93	117.77	117.61	117.78	117.65	117.98	117.32	118.47	119.61	118.28	119.06
	CH(3,4)	107.74	108.02	107.98	108.15	108.21	108.03	108.04	107.07	108.31	108.85	108.11	107.83
pyrrolidine[10]	CH_2(2,5)	45.82	47.02	46.93	47.12	46.86	46.75	-	46.51	47.57	47.43	47.23	46.83
	CH_2(3,4)	26.17	25.83	25.56	25.75	25.65	25.59	-	25.26	26.34	25.73	26.29	25.86
silicone grease	CH_3	1.20	1.22	1.19	1.37	1.38	1.09	1.40	-	-	2.87	2.10	-
tetrahydrofuran	CH_2(2,5)	68.03	68.16	67.97	67.75	67.80	67.64	68.07	67.03	68.33	69.53	68.83	68.68
	CH_2(3,4)	26.19	25.98	25.62	25.79	25.72	25.68	26.15	25.14	26.27	26.69	26.48	25.67
toluene	CH_3	21.29	21.53	21.46	21.37	21.10	21.23	21.46	20.99	21.50	21.62	21.50	-
	C(1)	138.24	138.36	137.89	137.84	137.91	137.65	138.48	137.35	138.90	139.92	138.85	-
	CH(2,6)	129.47	129.35	129.07	129.33	129.33	129.12	129.76	128.88	129.94	130.58	129.91	-
	CH(3,5)	128.71	128.54	128.26	128.51	128.56	128.31	129.03	128.18	129.23	129.79	129.20	-
	CH(4)	125.84	125.62	125.33	125.66	125.68	125.43	126.12	125.29	126.28	126.82	126.29	-
triethylamine	CH_3	12.51	12.12	11.61	12.39	12.35	11.87	12.49	11.74	12.38	9.51	11.09	9.07
	CH_2	47.18	46.75	46.25	46.82	46.77	46.36	47.07	45.74	47.10	48.45	46.96	47.19

Appendix C Recommended values of Xi for various nuclides

Isotope	Frequency ratio, Ξ/%	Reference compound	Reference compound conditions[a]
^{1}H	100.000 000	Me_4Si	$CDCl_3$, φ = 1 %
	100.000 000[b]	DSS methyl signal	D_2O
^{2}H	15.350 609	$(CD_3)_4Si$	neat
^{3}H	106.663 974	Me_4Si-t_1	
^{3}He	76.178 976[c]	He	gas
^{6}Li	14.716 086	LiCl	D_2O, 9.7 mol/kg
^{7}Li	38.863 797	LiCl	D_2O, 9.7 mol/kg
^{9}Be	14.051 813	$BeSO_4$	D_2O, 0.43 mol/kg
^{10}B	10.743 658	$BF_3.Et_2O$	$CDCl_3$, φ = 15 %
^{11}B	32.083 974	$BF_3.Et_2O$	$CDCl_3$, φ = 15 %
^{13}C[d]	25.145 020	Me_4Si	$CDCl_3$, φ = 1 %
	25.144 953[b]	DSS methyl signal	D_2O
^{14}N	7.226 317	$MeNO_2$	neat/$CDCl_3$[e]
^{15}N	10.136 767	$MeNO_2$	neat/$CDCl_3$[e]
	10.132 912[b]	NH_3 (liquid)	external
^{17}O	13.556 457	D_2O	neat
^{19}F	94.094 011	CCl_3F	
^{21}Ne	7.894 296	Ne	gas, 1.1 MPa
^{23}Na	26.451 900	NaCl	D_2O, 0.1 mol/dm^3
^{25}Mg	6.121 635	$MgCl_2$	D_2O, 11 mol/dm^3
^{27}Al	26.056 859	$Al(NO_3)_3$	D_2O, 1.1 mol/kg
^{29}Si	19.867 187	Me_4Si	$CDCl_3$, φ = 1 %
^{31}P	40.480 742	H_3PO_4	external
	40.480 864[b]	$(MeO)_3PO$	internal
^{33}S	7.676 000	$(NH_4)_2SO_4$	D_2O, satd.
^{35}Cl	9.797 909	NaCl	D_2O, 0.1 mol/dm^3
^{37}Cl	8.155 725	NaCl	D_2O, 0.1 mol/dm^3
^{39}K	4.666 373	KCl	D_2O, 0.1 mol/dm^3
^{40}K	5.802 018	KCl	D_2O, 0.1 mol/dm^3
^{41}K	2.561 305	KCl	D_2O, 0.1 mol/dm^3
^{43}Ca	6.730 029	$CaCl_2$	D_2O, 0.1 mol/dm^3
^{45}Sc	24.291 747	$Sc(NO_3)_3$	D_2O, 0.06 mol/dm^3
^{47}Ti	5.637 534	$TiCl_4$	neat/C_6D_{12}[e]
^{49}Ti	5.639 037	$TiCl_4$	neat/C_6D_{12}[e]
^{50}V	9.970 309	$VOCl_3$	neat/C_6D_6[e]
^{51}V	26.302 948	$VOCl_3$	neat/C_6D_6[e]
^{53}Cr	5.652 496	K_2CrO_4	D_2O, satd.
^{55}Mn	24.789 218	$KMnO_4$	D_2O, 0.82 mol/kg
^{57}Fe	3.237 778	$Fe(CO)_5$	C_6D_6, φ = 80 %
^{59}Co	23.727 074	$K_3[Co(CN)_6]$	D_2O, 0.56 mol/kg
^{61}Ni	8.936 051	$Ni(CO)_4$	neat/C_6D_6, φ = 80 %
^{63}Cu	26.515 473	$[Cu(MeCN)_4][ClO_4]$	CH_3CN, satd./C_6D_6[e]
^{65}Cu	28.403 693	$[Cu(MeCN)_4][ClO_4]$	CH_3CN, satd./C_6D_6[e]
^{67}Zn	6.256 803	$Zn(NO_3)_2$	D_2O, satd.
^{69}Ga	24.001 354	$Ga(NO_3)_3$	D_2O, 1.1 mol/kg
^{71}Ga	30.496 704	$Ga(NO_3)_3$	D_2O, 1.1 mol/kg
^{73}Ge	3.488 315	Me_4Ge	neat
^{75}As	17.122 614	$NaAsF_6$	CD_3CN, 0.5 mol/dm^3
^{77}Se	19.071 513	Me_2Se	neat/C_6D_6[e]
^{79}Br	25.053 980	NaBr	D_2O, 0.01 mol/dm^3
^{81}Br	27.006 518	NaBr	D_2O, 0.01 mol/dm^3
^{83}Kr	3.847 600	Kr	Gas
^{85}Rb	9.654 943	RbCl	D_2O, 0.01 mol/dm^3
^{87}Rb	32.720 454	RbCl	D_2O, 0.01 mol/dm^3
^{87}Sr	4.333 822	$SrCl_2$	D_2O, 0.5 mol/dm^3
^{89}Y	4.900 198	$Y(NO_3)_3$	H_2O/D_2O
^{91}Zr	9.296 298	$Zr(C_5H_5)_2Cl_2$	CH_2Cl_2, satd./C_6D_6[e]
^{93}Nb	24.476 170	$K[NbCl_6]$	CH_3CN, satd./CD_3CN[e]
^{95}Mo	6.516 926	Na_2MoO_4	D_2O, 2 mol/dm^3
^{97}Mo	6.653 695	Na_2MoO_4	D_2O, 2 mol/dm^3
^{99}Tc	22.508 326	NH_4TcO_4	H_2O/D_2O
^{99}Ru	4.605 151	$K_4[Ru(CN)_6]$	D_2O, 0.3 mol/dm^3
^{101}Ru	5.161 369	$K_4[Ru(CN)_6]$	D_2O, 0.3 mol/dm^3
^{103}Rh	3.186 447	$Rh(acac)_3$	$CDCl_3$, satd.
^{105}Pd	4.576 100	K_2PdCl_6	D_2O, satd.
^{107}Ag	4.047 819	$AgNO_3$	D_2O, satd.
^{109}Ag	4.653 533	$AgNO_3$	D_2O, satd.
^{111}Cd	21.215 480	Me_2Cd	neat
^{113}Cd	22.193 175	Me_2Cd	neat
^{113}In	21.865 755	$In(NO_3)_3$	D_2O, 0.1 mol/dm^3[f]
^{115}In	21.912 629	$In(NO_3)_3$	D_2O, 0.1 mol/dm^3[f]
^{115}Sn	32.718 749	Me_4Sn	neat/C_6D_6[e]
^{117}Sn	35.632 259	Me_4Sn	neat/C_6D_6[e]
^{119}Sn	37.290 632	Me_4Sn	neat/C_6D_6[e]
^{121}Sb	23.930 577	$KSbCl_6$	CH_3CN, satd./CD_3CN[e]
^{123}Sb	12.959 217	$KSbCl_6$	CH_3CN, satd./CD_3CN[e]
^{123}Te	26.169 742	Me_2Te	neat/C_6D_6[e]
^{125}Te	31.549 769	Me_2Te	neat/C_6D_6[e]
^{127}I	20.007 486	KI	D_2O, 0.01 mol/dm^3
^{129}Xe	27.810 186	$XeOF_4$	neat
^{131}Xe	8.243 921	$XeOF_4$	neat
^{133}Cs	13.116 142	$CsNO_3$	D_2O, 0.1 mol/dm^3
^{135}Ba	9.934 457	$BaCl_2$	D_2O, 0.5 mol/dm^3
^{137}Ba	11.112 928	$BaCl_2$	D_2O, 0.5 mol/dm^3
^{138}La	13.194 300	$LaCl_3$	D_2O/H_2O
^{139}La	14.125 641	$LaCl_3$	D_2O, 0.01 mol/dm^3
^{177}Hf	(4.007)	–	
^{179}Hf	(2.517)	–	
^{181}Ta	11.989 600	$KTaCl_6$	CH_3CN, satd.
^{183}W	4.166 387	Na_2WO_4	D_2O, 1 mol/dm^3
^{185}Re	22.524 600	$KReO_4$	D_2O, 0.1 mol/dm^3
^{187}Re	22.751 600	$KReO_4$	D_2O, 0.1 mol/dm^3
^{187}Os	2.282 331	OsO_4	CCl_4, 0.98 mol/dm^3
^{189}Os	7.765 400	OsO_4	CCl_4, 0.98 mol/dm^3
^{191}Ir	(1.718)	–	
^{193}Ir	(1.871)	–	
^{195}Pt	21.496 784	Na_2PtCl_6	D_2O, 1.2 mol/dm^3
^{197}Au	(1.729)	–	
^{199}Hg	17.910 822	Me_2Hg[g]	neat
^{201}Hg	6.611 583	Me_2Hg[g]	neat
^{203}Tl	57.123 200	$Tl(NO_3)_3$	
^{205}Tl	57.683 838	$Tl(NO_3)_3$	
^{207}Pb	20.920 599	Me_4Pb	neat/C_6D_6[e]
^{209}Bi	16.069 288	$Bi(NO_3)_3$	$HNO_3/D_2O/H_2O$

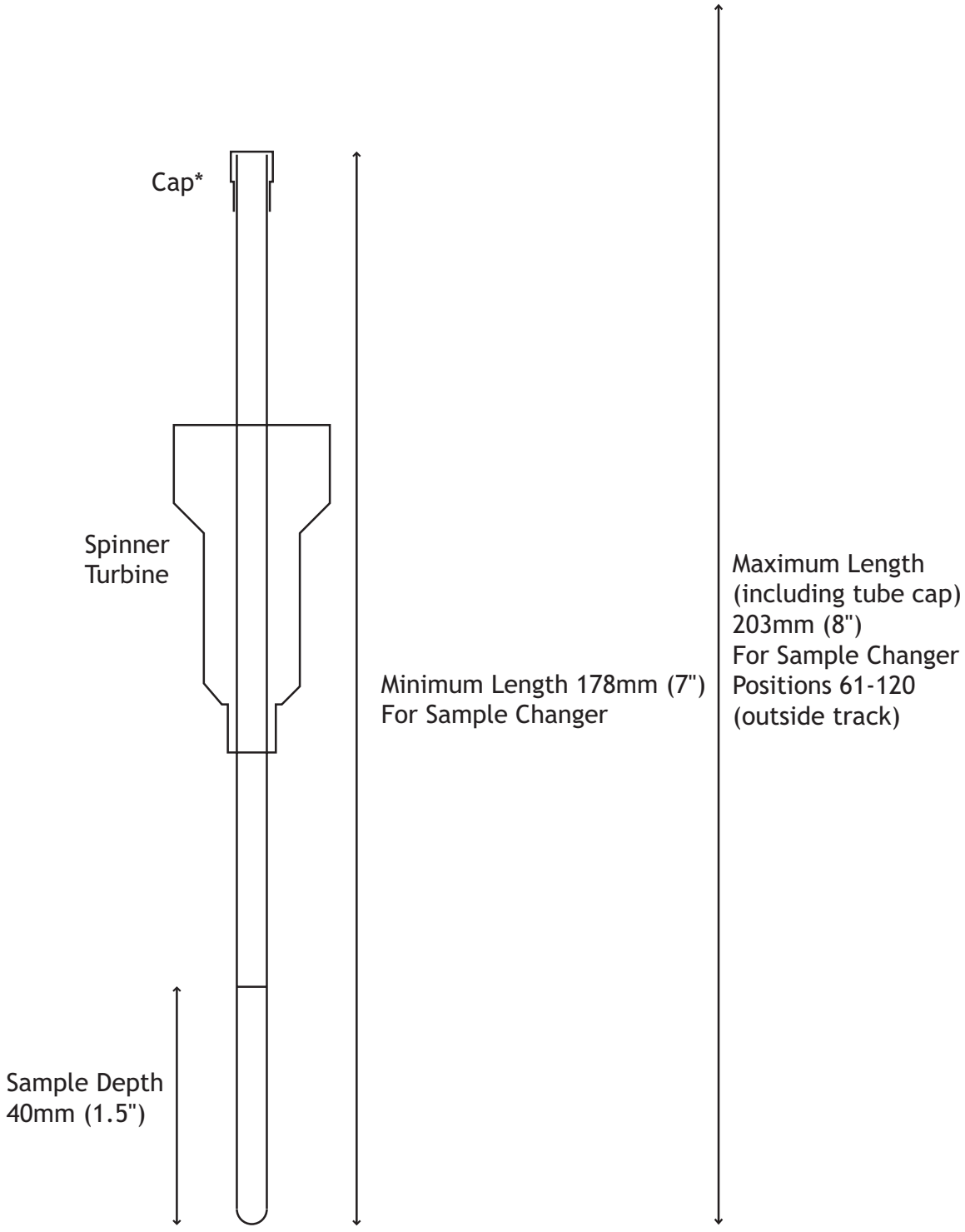
Table taken from:

Further conventions for NMR shielding and chemical shifts (IUPAC Recommendations 2008)

Robin K. Harris, Edwin D. Becker, Sonia M. Cabral de Menezes, Pierre Granger, Roy E. Hoffman and Kurt W. Zilm

Pure Appl. Chem., Vol. 80, No. 1, pp. 59–84, 2008.

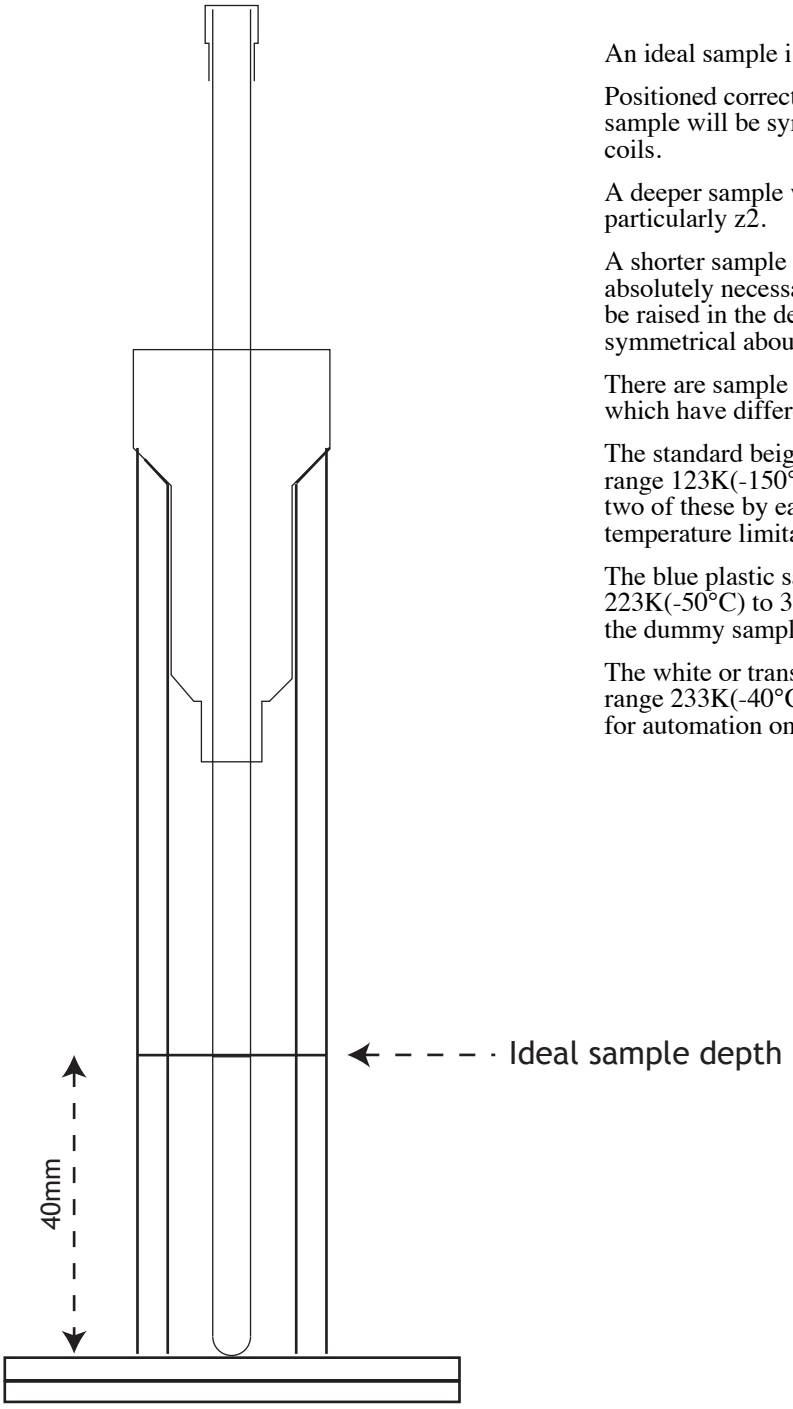
Appendix D Anatomy of an NMR sample



*Standard plastic tube caps are preferred for automatic sample changer operation. Rubber septum caps can be used, but only if the NMR tube is no longer than 203mm (8 inches).

Young's vacuum tap tubes should be used in manual operation only.

Appendix E Correct positioning of the sample spinner



An ideal sample is 4cm deep in the NMR tube.

Positioned correctly at the bottom of the depth gauge the sample will be symmetrical about the center of the NMR coils.

A deeper sample will require greater shim adjustments, particularly z2.

A shorter sample will result in poor peak shapes. If it is absolutely necessary to use a shallow sample, the tube should be raised in the depth gauge so that the center of the sample is symmetrical about the 2cm line.

There are sample spinners made of three different materials which have different temperature limitations:

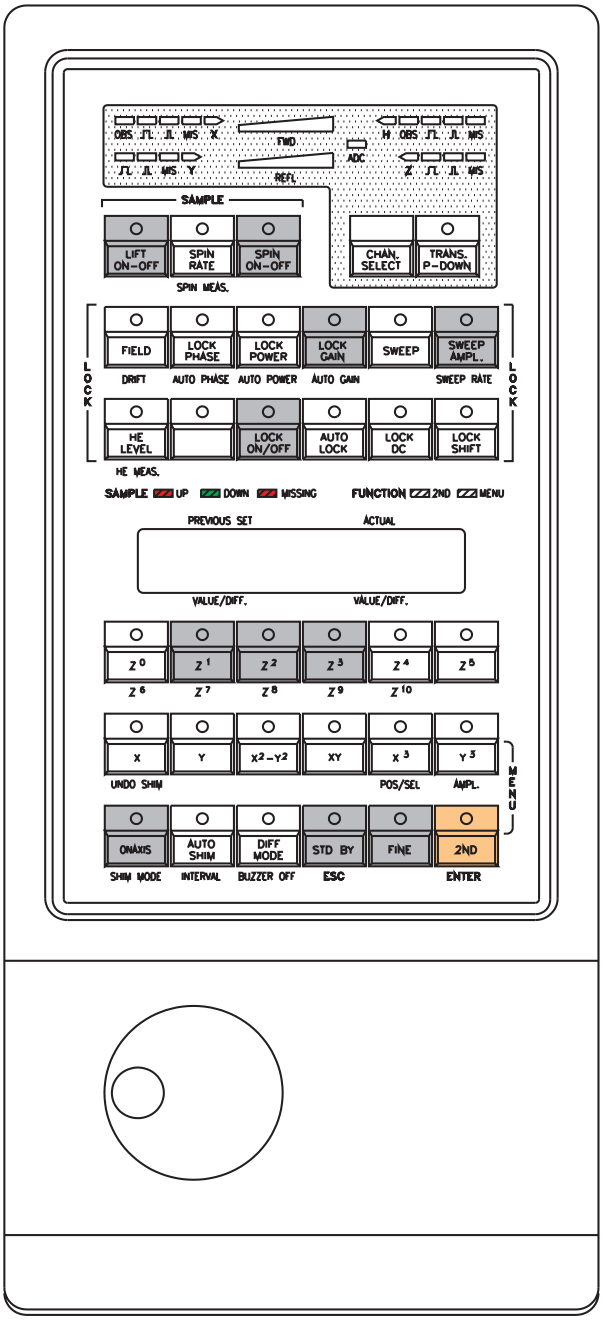
The standard beige plastic sample spinners can be used in the range 123K(-150°C) to 453K(+180°C), there are normally two of these by each instrument. For practical purposes the temperature limitation is the type of probe in use.

The blue plastic sample spinners can be used within the range 223K(-50°C) to 323K(+50°C), these are normally used for the dummy samples.

The white or translucent plastic spinners can be used in the range 233K(-40°C) to 313K(+40°C), these are normally used for automation on the DRX400.

Appendix F BSMS keypad layout

With usage the button labels on the BSMS keypads wear off. The diagram below highlights the most commonly used buttons on the type of keypad in the UCI facility.



Appendix G List of experiments available in *IconNMR* on DRX400

Unless stated otherwise, experiments can be run at any time of day. 'Normal experiments' acquire a single dataset, 'Composite experiments' acquire a series of related datasets. Current definitions of peak and off-peak times will be posted close to the instrument.

Normal experiments

h1 - Standard proton spectrum. Data will be processed and plotted from 11.5 to -0.5ppm, with an insert of the region from 16 to 11ppm if any peaks are present within that range.

c13 - Standard carbon-13 spectrum with proton decoupling, **aq = 1.36** and **d1= 0.1**. Full spectrum will be plotted. *Off-peak experiment only*.

c13maxns=256 - As above but limited to a maximum **ns** of **256**.

c13d1=1 - As above except that **d1= 1**. *Off-peak experiment only*.

c13d1=1.5 - As above except that **d1= 1.5**. *Off-peak experiment only*.

c13sn - Carbon-13 spectrum with signal-to-noise test, default **d1= 0.1**. Data will be accumulated up to a maximum of **ns** scans, with a test of the signal-to-noise ratio every 128 scans. *Off-peak experiment only, .*

Composite experiments

gcosy - Gradient assisted COSY experiment with automatic spectral range optimization. A 1D proton spectrum is acquired using the **h1** experiment and the peak range extracted for use in the 2D experiment.

ghmqc - Gradient assisted HMQC. 1D proton and carbon spectra are acquired using the **h1** and **c13** experiments and both peak ranges are extracted for the 2D experiment. *Off-peak experiment only*.

ghmbc10Hz - Gradient assisted HMBC, optimized for 10Hz couplings. 1D proton and carbon spectra are acquired using the **h1** and **c13** experiments and both peak ranges are extracted for the 2D experiment. *Off-peak experiment only*.

ghmbc2Hz - Gradient assisted HMBC, optimized for 2Hz couplings. 1D proton and carbon spectra are acquired using the **h1** and **c13** experiments and both peak ranges are extracted for the 2D experiment. *Off-peak experiment only*.

gnoesyp - Gradient assisted NOESY with default mixing time **d8 = 1**. A 1D proton spectrum is acquired using the **h1** experiment and the peak range extracted for use in the 2D experiment. *Off-peak experiment only*.

cosylr - Long range COSY experiment with automatic spectral range optimization. A 1D proton spectrum is acquired using the **h1** experiment and the peak range extracted for use in the 2D experiment. *Off-peak experiment only*.

h1,c13,cosy,hmqc - Sequence of proton, carbon, gradient COSY and gradient HMQC acquired as listed above. *Off-peak experiment only*.

p31dec - Phosphorus-31 spectrum with proton decoupling, with both spectral range optimization and signal-to-noise tests. The initial experiment is over the full spectral range of +230ppm to -230ppm. The signal-to-noise ratio is tested every 64 scans until a value of 100 is attained. The peak range is extracted from this experiment and used to run a more accurate experiment over a narrower spectral region. A minimum spectral range of 68ppm is used, corresponding to **aq = 3**. The second experiment is run until a signal-to-noise ratio of 200 is achieved, testing every 64 scans. A proton experiment is run first from which referencing is calculated. If the proton spectrum calibration is incorrect, update it and then reference the ^{31}P spectra using **xiref**.

p31 - Phosphorus-31 spectrum (without proton decoupling), with both spectral range optimization and signal-to-noise tests. The initial experiment is over the full spectral range of +230ppm to -230ppm. The signal-to-noise ratio is tested every 64 scans until a value of 100 is attained. The peak range is extracted from this experiment and used to run a more accurate experiment over a narrower spectral region. A minimum spectral range of 68ppm is used, corresponding to **aq = 3**. The second experiment is run until a signal-to-noise ratio of 200 is achieved, testing every 64 scans. A proton experiment is run first from which referencing is calculated. If the proton spectrum calibration is incorrect, update it and then reference the ^{31}P spectra using **xiref**.

f19dec+10to_190 - Fluorine-19 spectrum with proton decoupling, with both spectral range optimization and signal-to-noise tests. The initial experiment is over the spectral range of +10ppm to -190ppm. The signal-to-noise ratio is tested every 32 scans until a value of 100 is attained. The peak range is extracted from this experiment and used to run a more accurate experiment over a narrower spectral region. A minimum spectral range of 30ppm is used, corresponding to **aq = 3**. The second experiment is run until a signal-to-noise ratio of 200 is achieved, testing every 32 scans. A proton experiment is run first from which referencing is calculated. If the proton spectrum calibration is incorrect, update it and then reference the ^{19}F spectra using **xiref**.

f19dec_100to_300 - Fluorine-19 spectrum with proton decoupling, with both spectral range optimization and signal-to-noise tests. The initial experiment is over the spectral range of -100ppm to -300ppm. The signal-to-noise ratio is tested every 32 scans until a value of 100 is attained. The peak range is extracted from this experiment and used to run a more

accurate experiment over a narrower spectral region. A minimum spectral range of 30ppm is used, corresponding to **aq = 3**. The second experiment is run until a signal-to-noise ratio of 200 is achieved, testing every 32 scans. A proton experiment is run first from which referencing is calculated. If the proton spectrum calibration is incorrect, update it and then reference the ^{19}F spectra using **xiref**.

f19+10to_190ppm - Fluorine-19 spectrum (without proton decoupling), with both spectral range optimization and signal-to-noise tests. The initial experiment is over the spectral range of +10ppm to -190ppm. The signal-to-noise ratio is tested every 32 scans until a value of 100 is attained. The peak range is extracted from this experiment and used to run a more accurate experiment over a narrower spectral region. A minimum spectral range of 30ppm is used, corresponding to **aq = 3**. The second experiment is run until a signal-to-noise ratio of 200 is achieved, testing every 32 scans. A proton experiment is run first from which referencing is calculated. If the proton spectrum calibration is incorrect, update it and then reference the ^{19}F spectra using **xiref**.

f19_100to_300ppm - Fluorine-19 spectrum (without proton decoupling), with both spectral range optimization and signal-to-noise tests. The initial experiment is over the spectral range of -100ppm to -300ppm. The signal-to-noise ratio is tested every 32 scans until a value of 100 is attained. The peak range is extracted from this experiment and used to run a more accurate experiment over a narrower spectral region. A minimum spectral range of 30ppm is used, corresponding to **aq = 3**. The second experiment is run until a signal-to-noise ratio of 200 is achieved, testing every 32 scans. A proton experiment is run first from which referencing is calculated. If the proton spectrum calibration is incorrect, update it and then reference the ^{19}F spectra using **xiref**.

Appendix H A quick guide to proton and carbon NMR (*XwinNMR*)

The operations listed here are explained in more detail in Chapters 2-4 of the main manual. See section 1 for style conventions.

Startup:

> Log on to the computer and start *XwinNMR* from the *Toolchest*. If a dataset is not displayed, load one using **mydata**.

Prepare and load sample:

> Clean the NMR tube carefully with a tissue (and solvent if necessary). Remove any labeling and glue residue.

> Check that the NMR tube is straight by inserting fully into the glass tube tester.

> Clean the sample spinner with a tissue, and then insert the bottom of the NMR tube into the top (larger end). Use the depth gauge to position the spinner by gently pushing the NMR tube through the spinner until it reaches the base plate.

> On the BSMS keypad, check that lock and sample spinning are turned off.

> Turn on the sample lift air by pressing the top left button on the BSMS keypad.

> Collect the current (dummy or previous user's) sample from the magnet and replace with the new sample.

> Press the top left button on the BSMS keypad to turn off the lift air and load the sample.

> If required, press **[SPIN]** to turn on sample spinning.

Proton acquisition:

> Use **edcp** to set up a new experiment name and set the experiment number to **1**, then select standard proton parameters (**h1.s**, **h1.c** or **h1.q** depending on instrument).

> Load the standard shim file for the instrument in use: **rsh bbo** (GN500), **rsh qnp** (DRX400) or **rsh cryo** (CRYO500).

> **lock** and select the solvent name in use, e.g. **CDCl3**, wait for lock : finished before proceeding.

> Optimize **[LOCK PHASE],** then adjust shim controls to maximize the signal height in the lock display window. Start with **[z1]**, then **[z2]**, then go back and forth between **[z1]** and **[z2]** until no further improvement is seen. Then **[z3]**, **[z2]**, **[z1]** and repeat until there is no further change. If signal trace moves out of the top of the window, reduce **[LOCK GAIN]**.

> **acqu** to go to the acquisition screen, then **rgazg** to set receiver gain and start experiment.

> Standard experiment takes about 52 seconds.

> **ft** followed by **apks** to process data.

> Expand spectrum using ***LMB*** and ***MMB*** and check peak shapes - re-shim and repeat experiment if necessary.

> **cop 2** to copy data to experiment number 2, use ***LMB*** and ***MMB*** and to select narrower spectral range, either from **10.5** to **-0.5ppm**, or include 1ppm of baseline each end of the spectrum if a wider range is needed.

> Press **sw-sfo1** three or four times to update the acquisition region (new values for SW and O1 appear at the bottom of the screen).

> If better signal-to-noise is required, increase the number of scans, **ns** (use a multiple of 8).

> **acqu** to go to the acquisition screen, then **rgazg** to run experiment 2. You may need to confirm overwriting of the data.

Carbon acquisition:

> Use **edcp** to set up experiment number **3**, then select carbon parameters (**c13.s**, **c13.c** or **c13.q** depending on instrument).

> Type **solvent** and update the solvent name if necessary.

> **acqu** to go to the acquisition screen, then **rgazg** to set receiver gain and start experiment.

> The standard experiment will take about 24 minutes to acquire 1024 scans, check data during experiment as follows:

> Type **tr** and wait for the on-screen signal to shrink back to zero, before processing.

> **efp** followed by **apks** to process data.

> To interrupt the experiment when the signal-to-noise ratio is good enough, type **haltat 8**.

> If some peaks are missing or very small, increase relaxation delay **d1** (seconds), or choose the parameter set with a 1s or a 1.5s delay, and repeat experiment.

Proton processing:

> Either use **re 2** to load file onto the acquisition computer, or first start *XwinNMR* with the 'latest data from…' option if using a processing workstation.

> **efp** to Fourier transform with line broadening, or **fp** without.

> **apks** to phase spectrum automatically.

> Expand spectrum vertically and check phasing; use **phase** screen to improve if necessary by adjusting **PH0** and **PH1**.

> **abs n** for automatic baseline correction.

> Expand spectrum vertically and check baseline shape. If it is not a flat line, re-process with **efp**, enter manual baseline correction screen with **basl** and adjust coefficients **A**, **B**, **C** and **D** as necessary. Subtract baseline curve and **return** → **save & return**.

> Expand spectrum horizontally using the ***LMB*** and ***MMB*** and locate the reference peak, either the solvent or TMS.

> If the peak is taller than neighboring signals, reference automatically with **sref**. Otherwise use the **calibrate** screen followed by the ***MMB*** for manual calibration.

> Expand the left most peaks using the ***LMB*** and ***MMB*** then **integrate** to enter integration screen. Use the ***LMB*** and ***MMB*** to define integral regions. When all peaks have been integrated adjust slope, bias and calibration as necessary. **return** → **save & return**.

> **setti** to edit the spectrum title.

> **dp1** to define plot region.

> **vplot** to preview spectrum and then plot.

> Adjust peak labeling if necessary. Reduce **pc** to label small features on larger peaks, or reduce **mi** to label very small peaks.

> Expand spectral regions and re-plot if necessary. To ignore solvent peaks, type **edp**, change **pscal** to **psreg**, and check the value of **sreglst**. To interactive change vertical scaling, type **pg** then enter the **utilities** screen and adjust **CY**.

Carbon processing:

> Either **re 3** to load file onto the acquisition computer, or first start *XwinNMR* with the 'latest data from…' option if using a processing workstation.

> **efp** to Fourier transform with line broadening.

> **apks** to phase spectrum automatically.

> **abs n** for automatic baseline correction.

> Expand spectrum horizontally using the ***LMB*** and ***MMB*** and locate the reference peak, either the solvent or TMS.

> If the peak is taller than neighboring signals reference automatically with **sref**, otherwise use the **calibrate** screen followed by the ***MMB*** for manual calibration.

> **setti** to edit the spectrum title.

> **dp1** to define plot region.

> **vplot** to preview spectrum and then plot.

> Adjust peak labeling if necessary. Reduce **pc** to label small features on larger peaks, or reduce **mi** to label very small peaks.

> Expand spectral regions and re-plot if necessary. To ignore solvent peaks, type **edp**, change **pscal** to **psreg**, and check the value of **sreglst**. To interactive change vertical scaling, type **pg** then enter the **utilities** screen and adjust **CY**.

Appendix I Use of the 500MHz and 600MHz cryoprobes

1. Only use very good quality NMR tubes - the minimum quality is Wilmad 526PP or equivalent (Kontes 897230-0000/New Era NE-MP5-7/Norrell S-5-300-7).

2. Check **every** NMR tube for cleanliness and straightness before loading into the magnet.

3. **Exercise extreme care when changing samples**. A broken sample means a **minimum of one day of down time** to warm up the probe, remove the sample and cool down the probe. If any sample or glass fragments stick in the probe it will need to be returned to Bruker for servicing. This process could take several weeks.

4. Try to avoid touching the magnet when changing samples. It is finely balanced on pneumatic supports.

5. The variable temperature range of the 500MHz cryoprobe is **0°C to +50°C (273-323K) only**. A chiller is permanently connected to cool the compressed air supply to access temperatures between 278K and 298K. The full temperature range is accessible with the standard gas flow setting of **670 l/h**. The variable temperature range of the 600MHz cryoprobe is **0°C to +135°C (273-408K)**. A chiller is permanently connected to cool the compressed air supply to access temperatures between 273K and 298K. The full temperature range is accessible with the standard gas flow setting of **670 l/h**

6. Both cryoprobes have strict power limits. All experiments are automatically checked and an error message will appear if a power level is set too high.

Appendix J NMR booking rules

1. Reserve time for yourself only: Users may only reserve time that they will actually use and may only reserve time for themselves. A user may not reserve time on more than one NMR instrument simultaneously.

2. Maximum limits for advance booking: Each user can reserve *in advance* up to four hours per week per instrument, *plus* one overnight run.

3. Non-advance booking: Within the next hour, a user may reserve any vacant time slots (in compliance with the other rules) without counting against their four-hours-per-week-per-instrument limit. The four-hour quota is reset at midnight on Saturdays, and so if a user has used up their four hours on a particular instrument for that week, then they can only sign up for more slots on that instrument less than one hour in advance.

4. Walk-up periods: On the GN500 there are periods of one hour each when no reservations can be made. The following rules apply: **(a)** Each user can use a maximum of 10 minutes examining 1 or 2 samples but should use less time if their experiment(s) are complete. **(b)** No data processing should be performed on the spectrometer computers, the dedicated workstations must be used. **(c)** Users should form an orderly queue and are responsible for enforcing the previous rules on their fellow users.

5. Overnight runs: from midnight to 0700 the next day. When making reservations, users may not break the overnight block into smaller sections until 1600 the previous day. After 1600, users can reserve any slots between midnight and 0700 the next day.

6. Exceptional requirements: If a user requires more instrument time than the rules allow, then they should approach the Facility Manager to make reservations on their behalf.

7. Cancellation: Users are required to cancel *in advance* if their reserved blocks are no longer required. If the slots are not cancelled and the time is not used, see the **Penalties** section.

8. No-show slots: become available to others 10 minutes after the scheduled starting time. Any user can delete the remaining slots and claim them (in compliance with other rules) for use.

9. Weekends and holidays: On the 500MHz and 600MHz instruments, the above rules are effective from Monday to Saturday in a week. On Sunday up to 4 hours can be reserved at any time, from 24 hours in advance. There are no exceptions for public holidays. The DRX400 has the same booking rules every day of the week except for when automation is in use.

10. Maintenance: Instrument maintenance and repair take precedence over all user scheduling. If there is a problem with an instrument, reservations may be cancelled.

11. Penalties: The NMR Facility is not operated in punitive ways. Normally a "guilty" user will receive a warning. However, if serious or repeated violations of the booking rules are found, a user may be deleted from the system until an understanding is reached with the facility manager. Also, any scheduled blocks in violation of the rules will be deleted without prior notice.

DRX400: Restrictions

Time Period	Booking Commences	Maximum Allowance
0000 - 0700	1200 the previous day	7 hours
0700 - 0900	24 hours in advance	1 hour
0900 - 1900	24 hours in advance	3 separate 10 minute slots
1900 - 0000	1200 the same day	4 hours

(except when automation is in use)

GN500: Restrictions

Time Period	Booking Commences	Maximum Allowance
0000 - 0700	1200 the previous day	7 hours
0700 - 1200	24 hours in advance	1 hour
1200 - 1900	24 hours in advance	3 separate 10 minute slots
(except for walkup periods)		
1900 - 0000	1200 the same day	4 hours

CRYO500: Restrictions

Time Period	Booking Commences	Maximum Allowance
0000 - 0700	1200 the previous day	7 hours
0700 - 0900	24 hours in advance	1 hour
0900 - 1900	24 hours in advance	30 minutes
1900 - 0000	1200 the same day	4 hours

AVANCE600: Restrictions

Time Period	Booking Commences	Maximum Allowance
0000 - 0700	1200 the previous day	7 hours
0700 - 0900	24 hours in advance	1 hour
0900 - 1900	24 hours in advance	1 hour
1900 - 0000	1200 the same day	4 hours

Last updated 20140415

WALKUP OPERATION

The ‘walkup’ scheme now covers: 1400-1500 and 1600-1700 on the GN500

1. During the periods specified above, there will be no advance bookings, usage will be by “walkup” only.

2. Users can use a maximum of 10 minutes examining 1 or 2 samples but should use less time if their experiment(s) are complete.

3. No data processing should be performed on the spectrometer computers, the dedicated workstations must be used.

4. Users should form an orderly queue and will be responsible for enforcing the above rules on their fellow users.

AUTOMATED OPERATION OF DRX400

Starting from Wednesday 3 July 2019, *IconNMR* automated operation will no longer be regularly scheduled. This change is due to lack of use.

The automatic sample changer and software remain available, but usage must now be requested when required. This could be for a batch of research samples or for a set of teaching lab samples.

Contact Dr Dennison to schedule a suitable period of operation as needed.

Appendix K Installing *Topspin*

This appendix will explain the various steps required to get *TopSpin* running on a Mac or Windows computer. Both computer platforms require a free license to be obtained from Bruker, but the program files can be downloaded locally instead of from the Bruker server.

To download files from UCI computers you will need an FTP (file transfer protocol) client that supports secure FTP, sFTP. The UCI VPN must be used for off-campus access to **costello.ps.uci.edu**

Mac:

UCI has a license for an FTP program called *Fetch*. The method for obtaining this has recently changed.

Download the latest version from the Fetch website: http://www.fetchsoftworks.com/

Install as instructed, and it will function for 15 days without a license.

Run *Fetch* and then follow these steps.

Hostname: costello.ps.uci.edu

User name: NMR loginID

Connect using: **SFTP**

Password: NMR password

OK

Navigate to **TopSpinDownloads/Fetch**

Then download the license information. This should be valid for Fetch version 5. If you have problems, you can obtain the license from OIT by opening a ticket at - https://uci.service-now.com/sp?id=kb_article&sys_id=d7552e49dbfaba048427fb671d96192b&sysparm_category=7987752cdb67a700712f389f9d961995

Mac or Windows:

Cyberduck can be downloaded from https://cyberduck.io/

Install and run *Cyberduck*, then follow these steps.

Open Connection

SFTP (SSH File Transfer Protocol)

Server: costello.ps.uci.edu

Port: 22

Username: NMR loginID

Password: NMR password

Connect

Windows:

WinSCP can be downloaded from https://winscp.net

Install and run *WinSCP*, then follow these steps.

Select **New Site**, then

File protocol: SFTP

Host name: costello.ps.uci.edu

Port number: 22

User name: NMR loginID

Password: NMR password

Save and give these settings a name if desired.

Login to make the connection.

Use of any of the above options should result in a file browser window opening and displaying the contents of the home directory of the NMR account in use.

Navigate as follows to download the *TopSpin* files.

TopSpinDownloads → BrukerDownloads

Three versions of *TopSpin* are available. Version 4.1.4 is best and runs on Linux (CentOS 7.1(64bit)), MacOS (10.14 or newer) or Windows 10(64bit)). Version 4.1.1. is very similar and runs on Linux (CentOS 7.1(64bit)), MacOS (10.12 or newer) or Windows (7(64bit) or 10(64bit)). Version 3.6.3 supports older operating systems, Linux (CentOS 5.11 or 7.1(64bit)), MacOS (10.12 or newer) and Windows (7, 8.1 or 10).

Download the required software version by opening the directory and dragging the relevant file to a suitable location on your own computer.

Next download the UCI extra files, by opening

TopSpinDownloads → UCIfiles

All of the extra components are stored in the file with a name starting with **nmr_backup**

Drag this to a convenient location on your computer.

Install *TopSpin* on your computer. The installation varies a little between different operating systems. Several questions will be asked, and the default option can generally be used. You will be requested to enter a password for *TopSpin* administration. This can be anything you like, it does not have to match any other password, but must be entered.

Under MacOS Monterey, and possibly earlier versions, it is necessary to install *Xcode* from the Mac App store in order to compile and run automation programs, including 'nmrsave', used below. *Xcode* must also be run in order to accept the licensing terms. This may proceed automatically as part of the *TopSpin* installation.

You will be prompted to obtain a *CodeMeter* license from Bruker, which you will then install via the **Install License Ticket** option. Linux and Windows installations must be configured before use, this is not necessary under MacOS.

To obtain a *CodeMeter* license from Bruker, first register an account at Bruker via https://www.bruker.com/about-us/register.html and then the license can be obtained via https://www.bruker.com/service/support-upgrades/software-downloads/nmr/free-topspin-processing/nmr-topspin-license- for-academia.html

If the license fails to activate on your computer, try using a different web browser.

Under MacOS Monterey, and possibly earlier versions, the **nmr_backup** file (downloaded above) must be placed in **/opt/topspin***version***/nmr_backup**. Use **Go → Go to Folder… → /opt** to navigate to that location.

Next install the UCI extras into *TopSpin*.

At the *TopSpin* command line, type **nmrsave *<enter>***

At the top of the resultant window, select **Restore installation files**

Either type the **Location of backup file:** into the box, or **Browse** to find it. This should be the directory where you saved the 'nmr_backup' file above, not the file itself.

Click **Restore** to install the files.

To copy NMR data for processing in *TopSpin*, use the sFTP instructions above, substituting nmrserver.ps.uci.edu as the host name and the ID and password for the account you are accessing. When you have connected, select **NMRdata** link and copy the files to your own computer.

The directory where you place the NMR data must be accessible to *TopSpin*. On most computers this can be anywhere. It is a good idea to put all of your NMR data into the same directory.

For MacOS Catalina (10.15), Big Sur (11) or Monterey (12), and newer, users: Changes to the MacOS file system mean that *TopSpin* cannot open data files from within a user's home directory. The simplest workaround is to move your data to within the directory structure used by *TopSpin*. *TopSpin* installs into /opt, so create a new data directory there, for example /opt/data.

This is not within your normal account files, and so will need extra permissions to create, for which you will be prompted. If you cannot see **/opt** in a Finder window, press **shift+command+g** and type **/opt** into the goto window. Alternatively, if you open a Finder window showing your hard drive's root directory, you can press **Command + Shift + .** to show all normally hidden items.

A script is available to automatically set up the data directory in /opt:

Use *Fetch* or an alternative sFTP client to connect to nmrserver.ps.uci.edu

Click on the link **MacScripts**, download the file **MacScripts.zip**, and decompress it to find the file **setup_NMRdatadir_in_opt.command**

Control-click on the file, then select **Open** to run it, you will be asked for your *MacOS* password. A new directory called **NMRdata** will be created in /opt, for you to store data for processing in *TopSpin*. A link to /opt/NMRdata, called NMRdata, will also be created on your desktop. You can move that link to somewhere else if you prefer. /opt/NMRdata will also be loaded into the *TopSpin* data browser. The script will delete itself when completed. So if you want to run it again you'll either need to keep a copy or repeat the download.

When downloading the nmr_backup file, make sure that this is stored in **/opt/topspin***version***/nmr_backup**. Use **Go** → **Go to Folder…** → **/opt** to navigate to that location.

Appendix L Changes since the last bound version of this manual

20220407 – Corrected typo on page 102.

20220411 – Added to section 26.9.1 on using ‘rgac’.

20220503 – Added phasing 2D spectrum from 1D to DOSY processing.

20220623 – Clarified *TopSpin* installation section.