AVANCE Beginners Guide

User Guide

Version 006

Innovation with Integrity

NMR

This manual was written by

Bruker BioSpin END

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For further technical assistance for this product, please do not hesitate to contact your nearest BRUKER dealer or contact us directly at:

Bruker Corporation

am Silberstreifen

76287 Rheinstetten

Germany

Phone: +49 721 5161 6155

FAX:+49 721 5171 01

E-mail: nmr-support@bruker.de

Internet: www.bruker.com

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1 Introduction

The goal of this manual is to describe the basic components of a Bruker spectrometer, their functionality, and how they are addressed by the TopSpin software to acquire NMR data. This should enable a relatively inexperienced user to carry out a series of basic 1-D High Resolution (HR) NMR experiments. Menthyl Antranilate was selected as an example. Both proton observe and carbon observe with proton decoupling will be described. To assist the user, standard parameter sets delivered with every TopSpin software package will be used. This emphasis minimizes the time spent on the actual spectrometer itself, particularly in the case where relatively large numbers of undergraduate students are being trained. In this scenario processing can be easily carried out on a separate PC using the tutorials delivered with the spectrometer documentation.

For the purpose of instruction within this manual it is assumed that the user has:

- A basic knowledge of the TopSpin software package.
- A probe or probes capable of observing proton and observing carbon while decoupling protons.
- A basic knowledge of how use the flow tabs and buttons within the TopSpin window.

While every effort has been made to genuinely provide a step by step description, new users will invariably have some questions, and as such will require occasional assistance from a more experienced user. The goal of this manual is, where practicable, to enable users to work independently and acquire a basic understanding of how to operate the system. It is hoped that the time taken to train new users will be significantly reduced through the use of this manual.

1.1 Sources of Hazards

The safety chapter will deal with safety in more detail, but for now it is worth highlighting potential hazards that arise when operating an NMR spectrometer at this introductory stage. For such a sophisticated system there are surprisingly few opportunities for inexperienced users to damage the equipment, so only the most important ones are worth noting. With normal operation the most likely causes of damage are:

- Removing a sample from the magnet with the bore still plugged.
- Inserting a sample into the magnet without the presence of the supporting air cushion.
- Transmitting RF power along unconnected cables or poorly matched probes.

New users are advised to acquaint themselves with these potential hazards before commencing. System managers are advised to ensure that any new users understand the issues mentioned above.

1.2 Software Version and Command Syntax

This manual has been written for TopSpin version 3.2. Throughout the manual procedures to enter various commands will be outlined.

Z31633E_3_006

2   Safety

In terms of safety the presence of a relatively strong magnet is what differentiates NMR spectrometers from most other laboratory equipment. When designing an NMR laboratory, or training personnel who will work in or around the laboratory, no other feature is of greater significance. As long as correct procedures are adhered to, working in the vicinity of superconducting magnets is completely safe and has no known harmful medical side effects. Negligence however can result in serious accidents.

The magnet is potentially hazardous due to:

- The large attractive force it exerts on ferromagnetic objects.
- The large content of liquid nitrogen and helium.

2.1   Magnetic Safety

A magnetic field surrounds the magnet in all directions. This field (known as the stray field) is invisible and supervisors should have posted warning signs at appropriate locations. Objects made of ferromagnetic materials, e.g. iron, steel etc. will be attracted to the magnet. If a ferromagnetic object is brought too close, it may suddenly be drawn into the magnet with surprising force. This may damage the magnet, or cause personal injury to anyone in the way!

It is important that people working in the vicinity of the magnet fully understand the potential hazards. Of critical importance is that people fitted with cardiac pacemakers or metallic implants should never be allowed near the magnet.

Because the strength of the stray field drops significantly as one moves away from the magnet, it is important to discuss worksite safety around magnets with your supervisor.

2.1.1   Cryogenic Safety

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

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

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

2.2   Electrical Safety

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

2.3   Chemical Safety

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

2.4   CE Certification

All major hardware units housed in the AVANCE with SGU consoles, as well as peripheral units such as the HPPR, shim systems and probe comply with the CE Declaration of Conformity. This includes the level of any stray electromagnetic radiation that might be emitted as well as standard electrical hazards. Note that to minimize electromagnetic radiation leakage, the doors of the console should be closed and the rear paneling mounted.

Note: Additional safety information for AVANCE systems is available in the manual *AVANCE III Systems General Safety Considerations* (P/N Z31836) manual available on the BASH DVD.

3   Introductory Theory and Terminology

NMR is a technique used to analyze the structure of many chemical molecules, primarily organic compounds. A typical compound might consist of carbon, hydrogen and oxygen atoms.

In its simplest form, an NMR experiment consists of three steps:

1. Place the sample in a static magnetic field.
2. Excite nuclei in the sample with a radio frequency pulse.
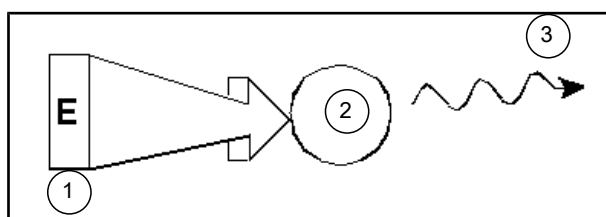3. Measure the frequency of the signals emitted by the sample.

Figure 3.1: Excitation and Response

1.	Excitation Pulse
2.	Atom
3.	Emitted Signal

From the emitted frequencies analysts can deduce information about the bonding and arrangement of the atoms in the sample. The NMR active nuclei in the sample resonate at different frequencies which are called **"resonance frequencies"**. These are the frequencies emitted by the nuclei when they are excited by the incoming radio frequency pulse. The value of a resonance frequency depends on two factors:

1) Type of Nucleus:

Every isotope has a particular combination of protons and neutrons in its nucleus. The nuclear structure largely determines the value of the resonance frequency. Thus every isotope displays a "basic resonance frequency". 13C nuclei will have a different basic resonance frequency compared to that of 1H etc. Note the large variation in basic resonance frequencies between different isotopes as listed in the following table:

Nucleus	NMR active	Basic resonance frequency (approx.) [MHz	Natural Abundance [%]
^1H	yes	500	99.98
^2H	yes	77	0.015
^3H	yes	533	Traces (ca 10^{-18})
^{12}C	no	---	98.89
^{13}C	yes	126	1.11

Nucleus	NMR active	Basic resonance frequency (approx.) [MHz	Natural Abundance [%]
^{35}Cl	yes	49	75.77
^{37}Cl	yes	41	24.23

Table 3.1: Table of Data for Various Isotopes (frequencies quoted for an 11.7T magnet)

2) Local Atomic Environment:

Superimposed on the basic resonance frequency is an effect due to the local atomic environment in which an isotope is situated. The precise value of the resonance frequency of a 1H nucleus in a particular compound will depend upon the atoms it is bonded to and surrounded by. The nucleus is surrounded by electrons which may be viewed as moving electrical charges with associated magnetic fields. These electrons act as a source of magnetic shielding for the nucleus. The extent of the shielding will depend on the precise local atomic environment. The size of typical local field variations (which will result in a frequency variation) will depend on the isotope and the strength of the magnetic field in which the sample is placed. The table below shows the typical frequency variation for two of the most widely used NMR nuclei, 1H and 13C. It is clear that the local atomic environment has a relatively small effect on the basic resonance frequency.

Nucleus	Typical variation in basic resonance frequency due to local atomic environment.
^{1}H	6 kHz
^{13}C	30 kHz

Table 3.2: Frequency Variations (quoted for an 11.7 T magnet)

NMR signals are usually plotted as spectra and analyzed with respect to two features, **frequency** and **intensity**. It is conventional in NMR to plot frequency on the horizontal axis and increasing towards the left.
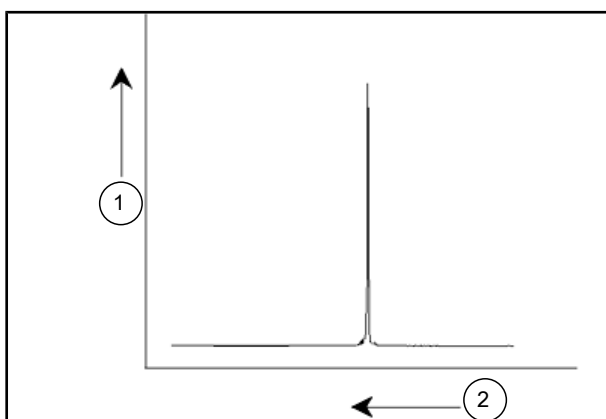

Figure 3.2: NMR Spectrum

1.	Intensity
2.	Frequency

As mentioned above, the frequency yields qualitative information regarding the local atomic environment. The **integrated intensity** of a signal is a **measure of signal strength** and is determined by integrating the area under the signal peak. The integral will be

directly proportional to the number of nuclei contributing to a signal at a particular frequency (if all nuclei are equally excited) and hence will provide quantitative information regarding chemical structure.

To excite a given nucleus in an NMR experiment, the frequency of the excitation pulse should closely match the resonance frequency of the nucleus. This frequency is referred to as the **carrier frequency**. Thus, if experiments are carried out using a 11.7 T magnet, the 1H nuclei would require a carrier frequency of approximately 500 MHz, whereas 13C nuclei would require a carrier frequency close to 126 MHz. The carrier frequency is specified by the parameter SFO1. The nucleus that is excited by this carrier frequency is referred to as the **observe nucleus**.

Note that there are experiments in which more than one nucleus gets excited, e.g. during polarization transfer or decoupling. In these cases one has more than one carrier frequency but still only one observe frequency.

Not all **isotopes** will respond to radio frequency pulses, i.e. not all are **NMR active**. Three isotopes of the element hydrogen are found in nature: ^{1}H (hydrogen), ^{2}H (deuterium), and ^{3}H (tritium, radioactive!). The natural abundance of these isotopes are 99.98%, 0.015%, and 0.005% respectively. All three are NMR active, although as can be seen in table 3.1, they all display a large variation in resonance frequency. To analyze a sample for hydrogen, the ^{1}H isotope is excited, as this isotope is by far the most abundant. Of the carbon isotopes found in nature, only one is NMR active. By far the most common isotope, ^{12}C (98.89% natural abundance) is inactive. Hence, NMR analysis of organic compounds for carbon rely on the signals emitted by the ^{13}C isotope, which has a natural abundance of only 1.11%. Obviously, NMR analysis for carbon is more difficult than that of, for example, ^{1}H (there are other factors which affect sensitivity, these will be discussed in the next sections of this chapter).

Using the brief introduction to NMR outlined above, it is a good exercise to consider how the technique could be used to analyze the composition of chloroform ($CHCl_3$).

3.1   NMR Analysis of Chloroform

Three separate experiments, as outlined in the figure below, can be performed corresponding to the three possible observe nuclei ^{1}H, ^{13}C and ^{35}Cl.
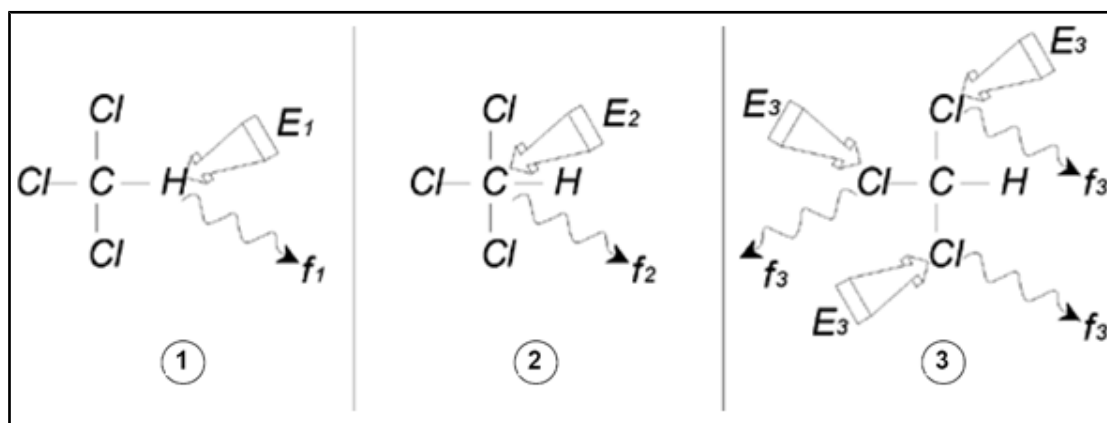

Figure 3.3: NMR Analysis of CHCl3

1.	Excitation E_1
2.	Excitation E_2
3.	Excitation E_3

Three excitation pulses (E_1, E_2, E_3) are directed at the sample at appropriate carrier frequencies. E_1 corresponds to the 1H resonance frequency, E_2 to the ^{13}C frequency, and E_3 to the ^{35}Cl frequency. Assuming the three isotopes were successfully excited, the sample will emit signals at three frequencies f_1, f_2 and f_3 which are recorded on three separate spectra. If the emitted signals are displayed in a single plot, the user can expect a spectrum similar to that in the figure below (note that the signal frequencies illustrated are for a 11.7 T magnet and that all signals have been plotted as singlets, i.e. single peaks).
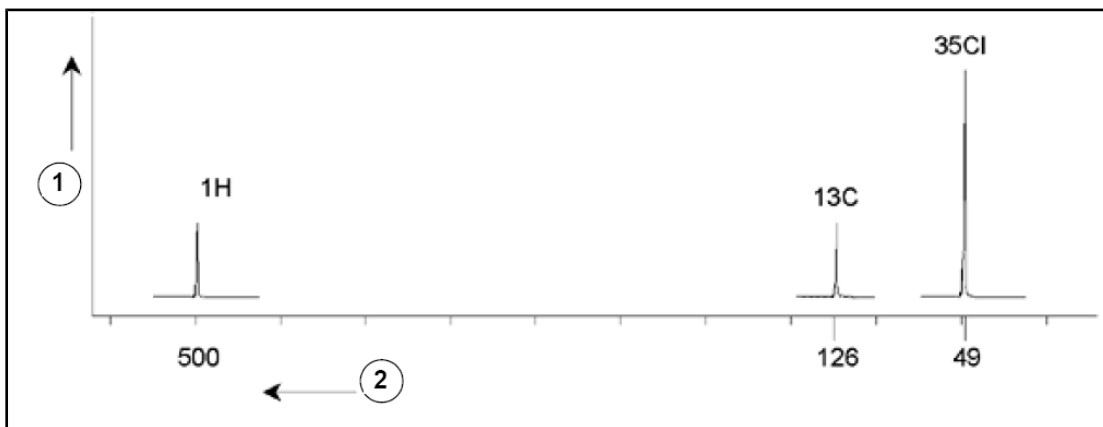

Figure 3.4: NMR Signals Emitted by CHCl3

1.	Intensity
2.	Frequency (MHz)

This artificial spectrum shows three peaks corresponding to the three isotopes. Taking the relative numbers of the three isotopes into account, one might expect that the intensity of the chlorine, hydrogen and carbon peaks would be in the ratio of 3:1:1. However, the natural abundance of the three isotopes must also be accounted for, resulting in a ratio of 227:100:1. The user will find that the experimentally determined peak intensity ratios will not agree with these values. The reason is that every isotope has an inherent sensitivity to the NMR technique. The ^1H is 63 times more sensitive to NMR than ^{13}C. Thus, even if a sample would contain exactly the same number of ^1H nuclei as ^{13}C, the intensity of the ^1H signals will be 63 times greater than the ^{13}C signals.

With a plot such as that in the figure above, any detailed information will be lost and precise determination of a particular frequency would be impossible. The spectrum would be said to display very poor resolution (the horizontal resolution of a spectrum is a measure of how well the spectrum differentiates between two signals that are close in frequency).

A further complication is the huge range in vertical scaling. The variation in inherent sensitivity to NMR, coupled with the variations in natural abundance, will often make the plotting of signals from different isotopes onto a single plot unfeasible. In fact, the vertical resolution of the spectrum will be very poor (the vertical resolution, i.e. the signal to noise ratio of a spectrum is a measure of sensitivity).

If our analysis of chloroform is proving rather complicated, it is because we are attempting to compare the signals from three different observe nuclei on a single spectrum (we are ignoring here any hardware/electronic restrictions). Therefore, in practice, NMR experiments are performed with a single observe nucleus. Although more than one isotope may be excited simultaneously, by using more than one carrier frequency (e.g. decoupling experiments), we only ever observe the signals from a single isotope. This greatly simplifies spectrum analysis.

It was mentioned earlier that variations in the basic resonance frequency due to the local atomic environment tend to be relatively small. Thus, large spectral ranges will not be encountered. Furthermore natural abundance and inherent sensitivity will always be the

same for a particular isotope. Hence, the relative intensity of say two signals emitted from [1] H isotopes on a single spectrum will depend only on the number of atoms contributing to the signal. This greatly simplifies analysis of spectra for quantitative information. Before proceeding further with a more detailed description of NMR the reader should become familiar with the concept of measuring signals in *ppm* (parts per million) with respect to a reference signal.

3.2   Reference Compounds, Hertz, ppm

It has already been stated that NMR signals are analyzed with respect to two characteristics, intensity and frequency. Absolute frequencies are measured in Hertz (Hz - cycles per second) or Megahertz (MHz). Reporting on measured signals is simplified if all frequency measurements are made with respect to a reference. For ^{1}H NMR the recommended reference is a chemical called tetramethylsilane (TMS). When a ^{1}H or a ^{13}C spectrum is acquired the presence of TMS gives rise to a single, easily identifiable peak. This peak is referenced to zero and the frequencies of all other peaks are given in terms of their frequency relative to the TMS frequency. Thus, we can talk of a signal being 2.5 kHz "above" the TMS peak. This is preferable to quoting the absolute frequency of the signal which might look like 500.1325 MHz.

Referencing signals to the TMS peak clearly cuts down the number of digits needed to describe the frequency of a signal. However, this can be even more simplified if the ppm unit is used instead of Hertz. The ppm unit represents frequencies as a fraction of the absolute resonance frequency which will depend on the strength of the magnet. The advantage of the ppm unit is that frequency measurements are independent of magnet strength. This greatly simplifies the comparison of spectra acquired on different spectrometers.

The conversion factors to be used when changing from Hertz to ppm and vice versa are shown in the diagram below.
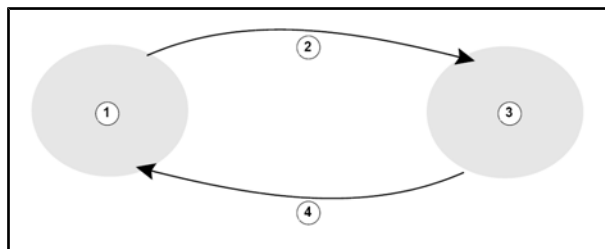

Figure 3.5: Converting Hertz and ppm

1.	Hertz
2.	Divide by the carrier frequency (SFO1) in MHz
3.	ppm
4.	Multiply by the carrier frequency (SF=1) in MHz

The advantages of using the ppm unit are best illustrated with a practical example.

Suppose a ^{1}H signal was observed at 2.5 kHz above TMS using a carrier frequency (SF01) of 500 MHz The frequency of any emitted NMR signal is directly proportional to the strength of the magnet. The same signal would appear at 3.0 kHz above TMS on a 600 MHz spectrometer and at 2.0 kHz above TMS on a 400 MHz machine. A single conversion may not be a great inconvenience but it must be done for every peak for each system. Now consider the same signal but quoted in the ppm unit.

Frequency in Hertz divided by SFO1 = Frequency in ppm

Examples:

```
2500 Hz / 500 MHz = 5 ppm
3000 Hz / 600 MHz = 5 ppm
2000 Hz / 400 MHz = 5 ppm
```

The ^{1}H signal may now be described as being located at 5 ppm "above", i.e. "downfield", the TMS peak regardless of the spectrometer frequency.

Experienced users will always deal in ppm, and spectra reproduced in scientific journals will have the horizontal scale graduated in ppm and not in Hertz.

The reader should be aware of some simplifications that have been made in the above example. The value of the ^{1}H carrier frequency on a 500 MHz spectrometer will not be exactly 500 MHz. The carrier frequency to be used in a ppm calculation should be the precise value assigned to the parameter SF01. Similarly for the 600 MHz and 400 MHz spectrometers, quoted above the ^{1}H carrier frequency, will not be exactly 600 MHz and 400 MHz, respectively.

Note also that a positive ppm value denotes a frequency higher TMS and is defined as "downfield" relative to TMS.

3.3 Proton NMR - Chemical Shift

Since ^{1}H is the most commonly observed isotope in NMR experiments, this shall now be dealt with in greater detail. A ^{1}H nucleus contains a single proton and spectra in which ^{1}H is the observe nucleus are normally referred to as proton spectra.

It was stated previously that a proton in an 11.7 T magnet will display a basic resonance frequency of approximately 500 MHz, but that the precise resonance frequency will depend upon the local atomic environment. A proton in a molecule of chloroform resonates at a slightly different frequency compared to a proton in benzene (C_6H_6). Therefore, the emitted frequency acts as a label which gives analysts qualitative information on the local atomic neighborhood in which a proton is located. This is the basis of NMR.

The variation in precise resonance frequency is referred to as the "*chemical shift*". The resonance frequency is shifted by the effect of neighboring atoms and in particular the extent of magnetic shielding from local electrons discussed earlier. The size of the shift is normally measured in ppm relative to the TMS peak which is referenced to 0 ppm.

Most protons, regardless of the organic compound to which they are bonded, display chemical shifts within 14 ppm of TMS.
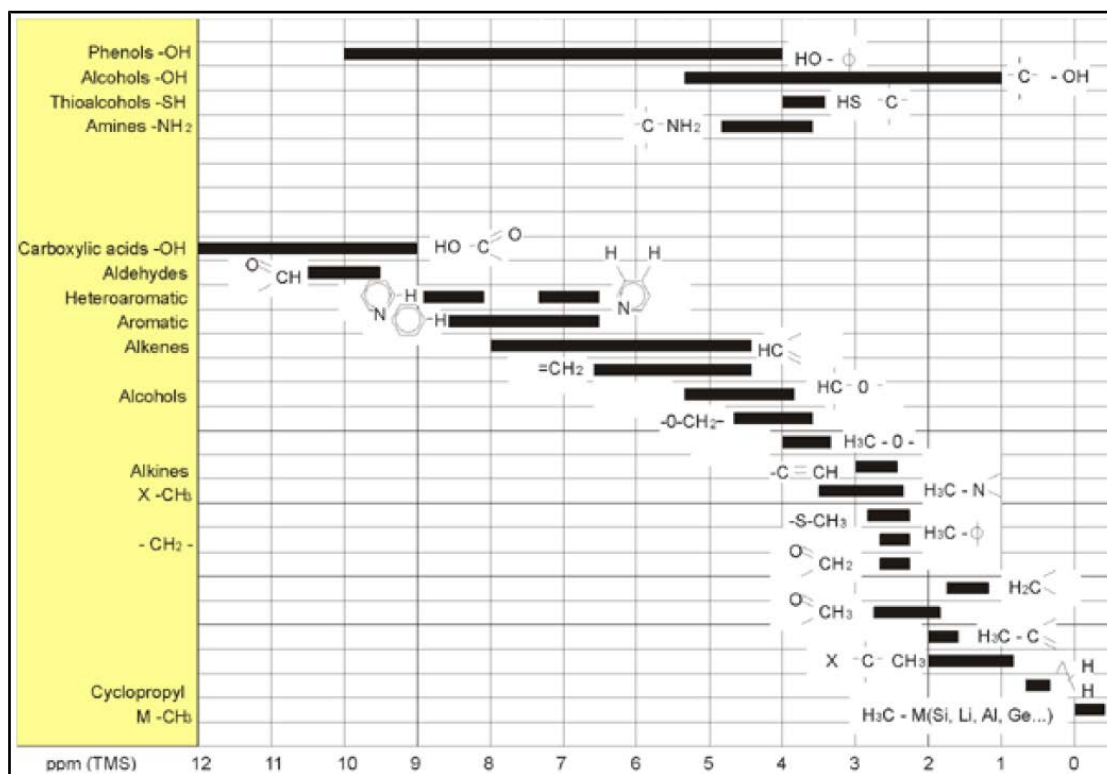
Figure 3.6: 1H Chemical Shifts in Organic Compounds

The figure above is an illustration of typical proton chemical shifts in organic compounds.

3.4 Proton Spectrum of Benzene

The structure of the benzene ring is illustrated in the following figure:
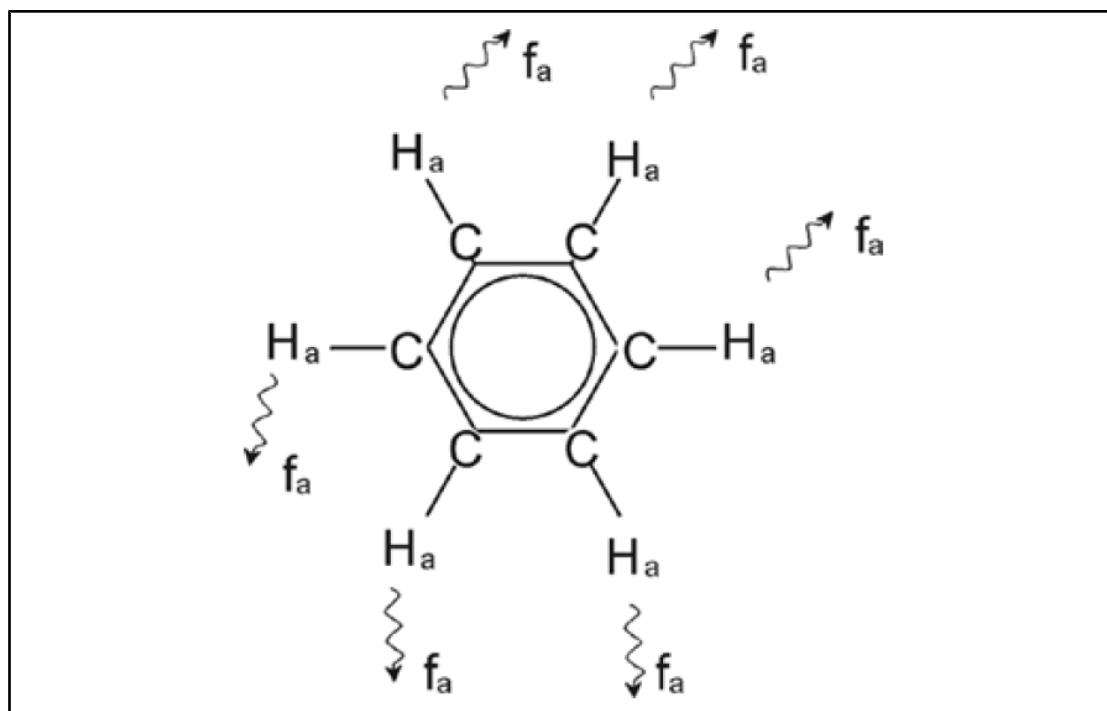

Figure 3.7: Benzene Ring

All six protons (labeled H_a) may be deemed identical. They are each single-bonded to one carbon atom. Each carbon atom in turn forms two aromatic bonds with its nearest carbon neighbors. Hence each of the six protons is in an identical chemical environment and is said to be "chemically equivalent", and in this case even "magnetically equivalent". They will all resonate at precisely the same frequency f_1 without showing a coupling pattern. Therefore, for pure benzene we can expect a single signal. The figure below is a spectrum of benzene in acetone - d6 and shows that the signal is located at 7.5 ppm.
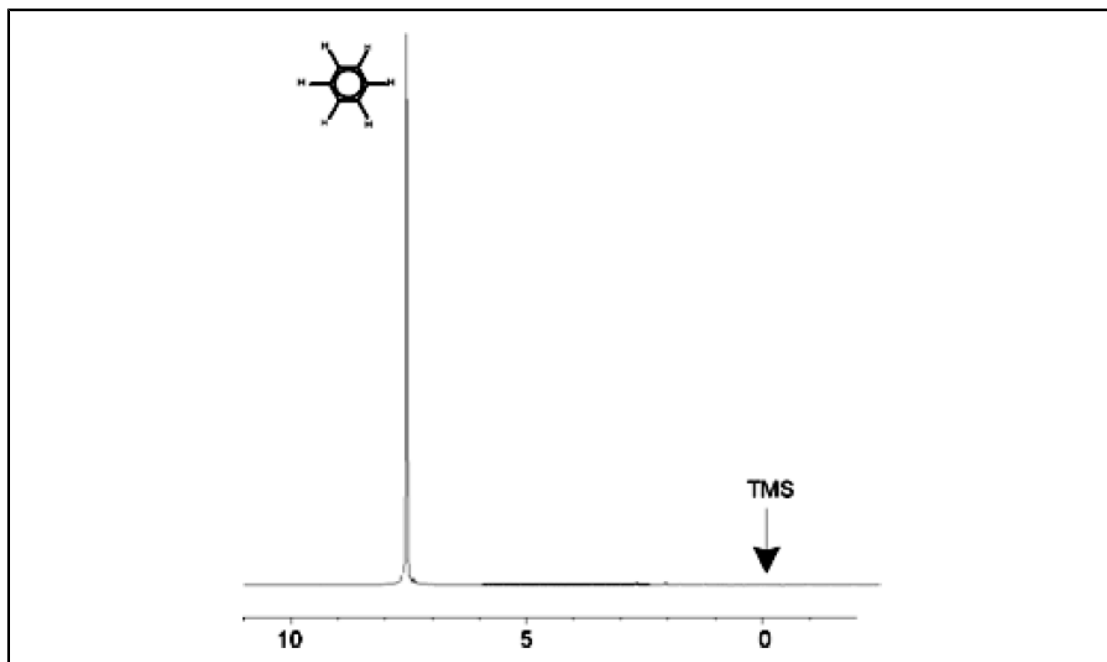
Figure 3.8: Spectrum of Benzene

3.5 Proton Spectrum of Benzylacetate

Benzylacetate (C_6H_5 - CH_2 - O - CO - CH_3) is a more complicated organic molecule whose structure is illustrated in the following figure:
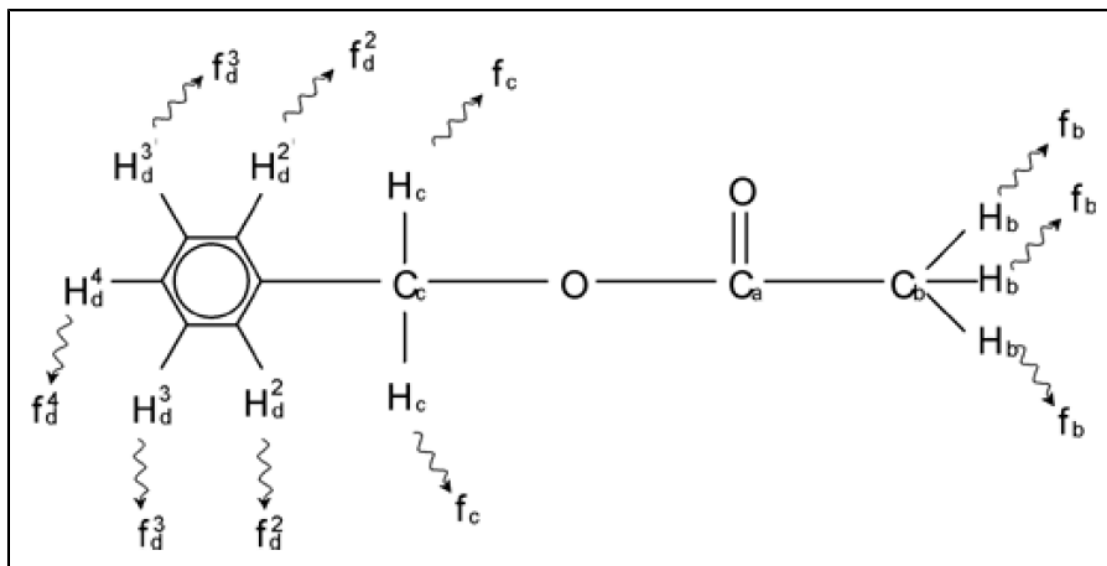

Figure 3.9: Benzylacetate

We can now distinguish between three different groups of protons that have been labeled accordingly. For example, the three protons labeled H_b clearly find themselves in a different atomic environment to that of the two protons labeled H_c.

The three H_b protons are bonded to the carbon C_b which is single-bonded to another carbon atom C_a. The two H_c protons are bonded to the carbon C_c which itself is single-bonded to the benzene ring and an oxygen atom. The third group of protons consists of the five protons H_d of the benzene ring itself. The figure above is a proton spectrum of benzylacetate in acetone - d6. In this spectrum we expect three signals corresponding to the three groups of protons.

Note that the position of signals from the benzene ring protons has been shifted slightly from 7.5 ppm (shown in the Spectrum of Benzene figure) to approximately 7.2 ppm (shown in the figure below).

The benzene ring protons are no longer magnetically equivalent, are to some extent not even chemically equivalent, and have been labeled accordingly. It is clear from figure 3.10 that the signal arising from the H_d protons is a multiplet, but such details will be omitted until the next section. The three proton peaks shown in this figure have clearly different intensities.

Quantitative analysis of the spectrum is relatively simple, since all the signals are emitted by the same ^1H isotope i.e. natural abundance and inherent sensitivity to the NMR technique is the same for each peak. Therefore the area under the benzene, CH_2 and CH_3 peaks should be in the ratio of 5:2:3 respectively corresponding to the contributing number of protons.
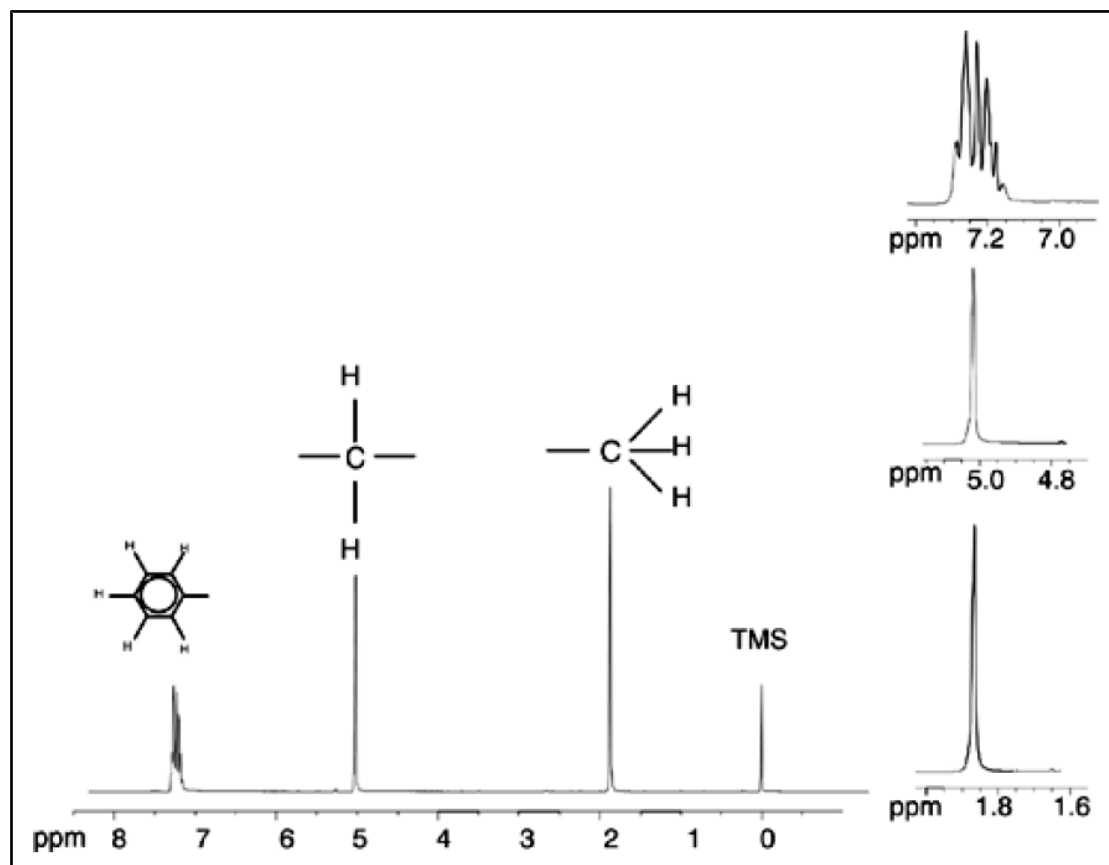

Figure 3.10: Proton Spectrum of Benzylacetate

3.6   Proton Spectrum of Ethylbenzene with Spin/Spin Coupling

The description of proton NMR spectra thus far has been greatly simplified by the fact that all signals, with the exception of those from the benzene ring in benzylacetate, have been singlets. The structure of the organic compound ethylbenzene and the corresponding proton spectrum are illustrated in the Ethylbenzene figure and the Ethylbenzene Spectrum figure respectively. As before, the protons have been labeled as three distinct groups corresponding to three basic atomic environments.

The most obvious difference between the signals in this spectrum and those of benzylacetate is the splitting into **multiplets**. The signal emitted by the CH3 protons is a **triplet** and the signal from the CH_2 protons is a **quartet**. Note also that the signal positions do not coincide. The CH_3 protons in benzylacetate emit a signal at 1.85 ppm, while the corresponding CH_3 protons in ethylbenzene emit the triplet signal at 1.25 ppm. This is hardly surprising, because the two CH_3 groups are in different chemical environments.

The cause of the multiplet splitting is due to an effect known as spin-spin coupling. A full account of this effect is beyond the scope of this manual and the reader should refer to a standard NMR text for details. For our purpose a brief outline of the spin-spin coupling should suffice.
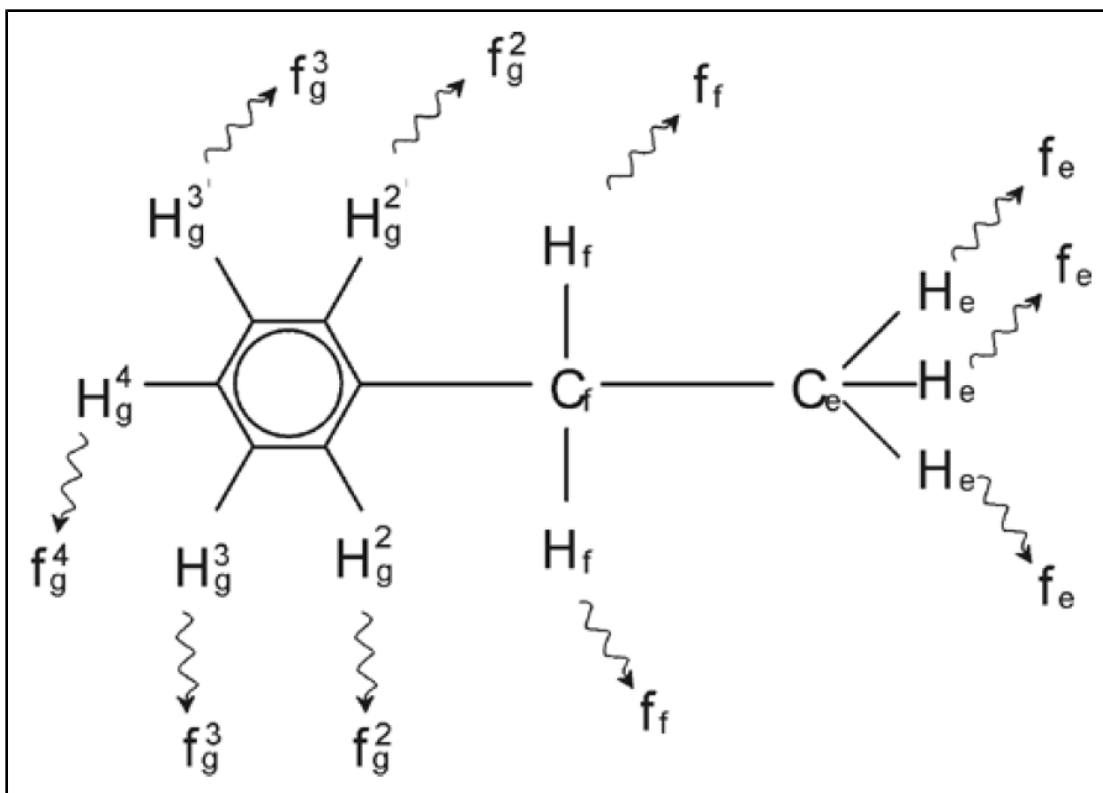

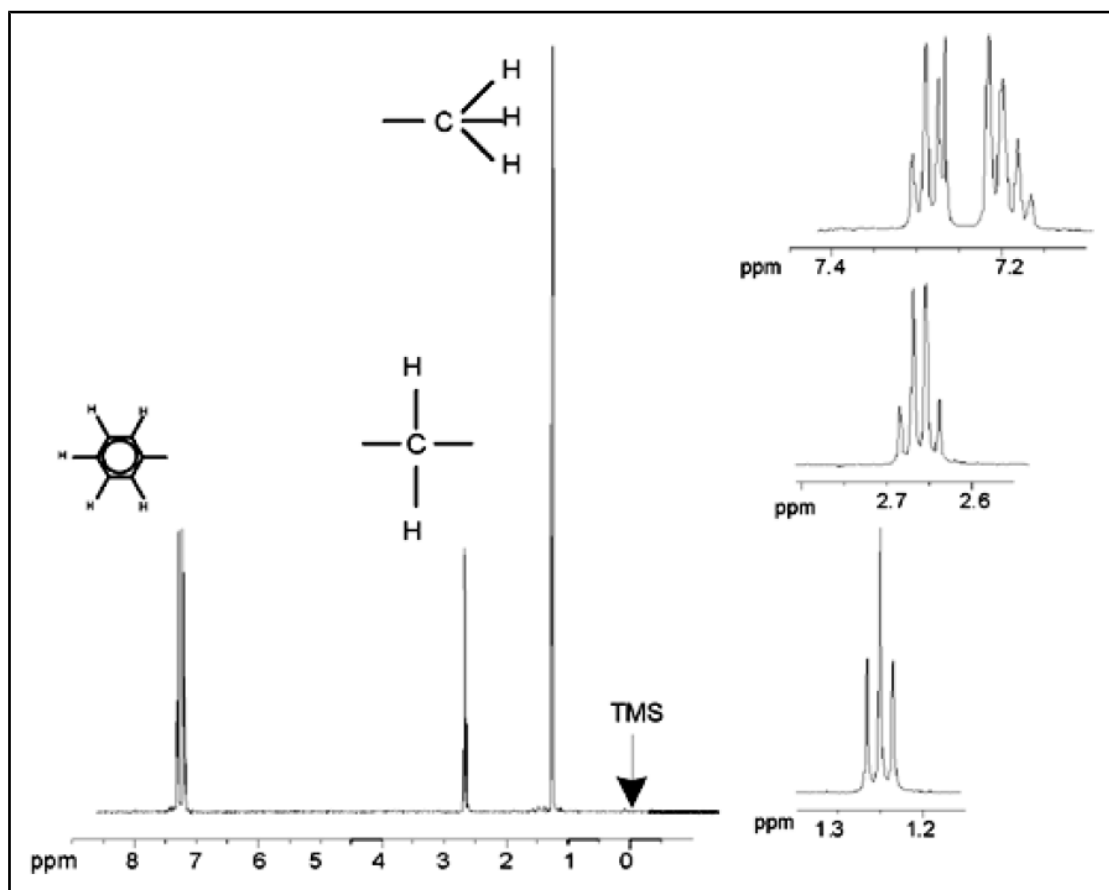*Figure 3.11:* Ethylbenzene

Figure 3.12: Ethylbenzene Spectrum

The splitting of NMR signals in the figure Ethylbenzene results from a magnetic interaction between neighboring protons. The two H_f protons are magnetically equivalent and do not interact with each other. Similarly, the three H_e protons are magnetically equivalent and do not affect each other. However, the two H_f protons and the three He protons are in different local environments and are "coupled" to each other via their bonding electrons. The net result of this coupling is that the two groups of protons interact with each other and cause the splitting of the NMR signals.

The two H_f protons can combine to exist in three possible magnetic states (this is a result of spin orientation and hence the term spin-spin coupling). As a result of coupling, the NMR signals emitted by the H_e protons resonate at three possible frequencies and a triplet is observed.

Similarly, the effect of the He protons is to split the H_f signals. The three He protons can combine to exist in four possible magnetic states. Consequently the H_f protons resonate at four possible frequencies, so the signal is split into a quartet.

The signals from the benzene protons have also been split as a result of magnetic non-equivalence and resulting spin-spin coupling. The question arises why the CH_2 and CH_3 protons of ethylbenzene interact with each other whereas the two comparable groups of protons in benzylacetate do not. The answer lies in the number of bonds separating the two groups. In ethylbenzene the two proton groups are attached to adjacent carbon atoms and may be expected to interact sufficiently with each other. In benzylacetate however, the two carbon atoms C_c and C_b are connected across two extra bonds between oxygen and another carbon atom. As a result the proton groups are too far away from each other to display significant spin-spin coupling.

3.7   Decoupling

The effect of spin-spin coupling can be removed by a technique called "decoupling". The effect of decoupling is to mask the presence of a particular proton group, e.g. the He protons in the Ethylbenzene figure. A spectrum is acquired as if the He protons were absent! This is achieved by transmitting a decoupling pulse sequence at the He resonance frequency f_e and thereby permanently changing the spin orientation of these protons. For the spectrum illustrated in the Ethylbenzene Spectrum figure the decoupling frequency would be 1.25 ppm above the TMS peak.

Decoupling pulses tend to be longer and of lower power than excitation pulses. The Decoupling Experiment figure below is a representation of a decoupling experiment, while the Ethylbenzene Spectrum with Homodecoupling figure shows the decoupled spectrum. The CH_2 quartet has now become a **singlet**. Spectroscopists speak of the quartet collapsing to a singlet. Furthermore, the area under the singlet should be equal to that of the original quartet (compare the relative heights of the CH_2 and benzene ring peaks in the two figures). The signal from the CH_3 group at 1.25 ppm is missing from the decoupled spectrum, because the decoupling pulses effectively removes the effects of the presence of the CH_3 protons.
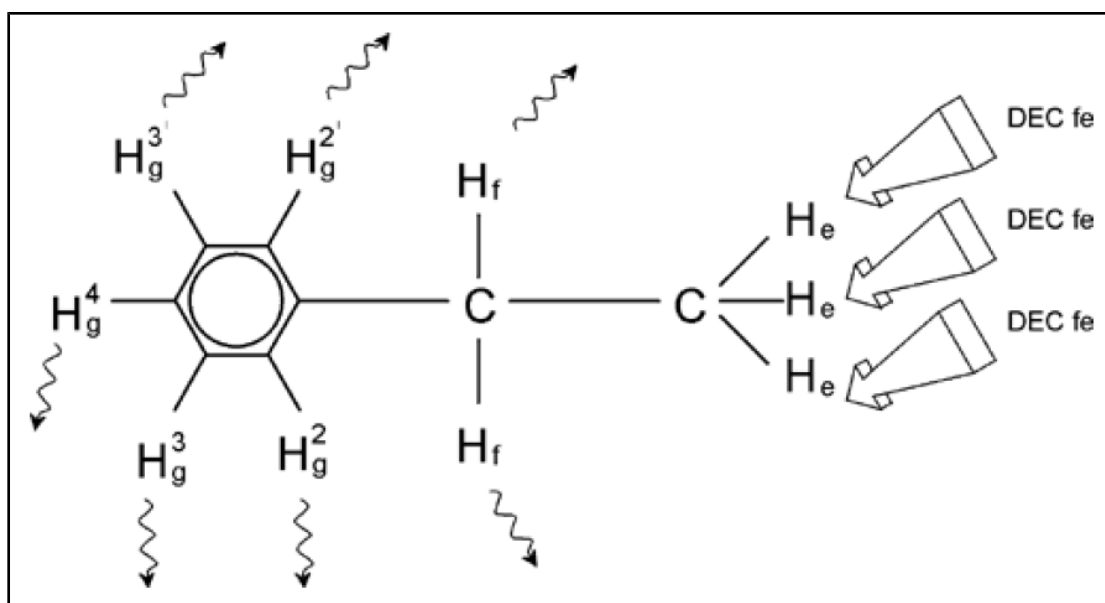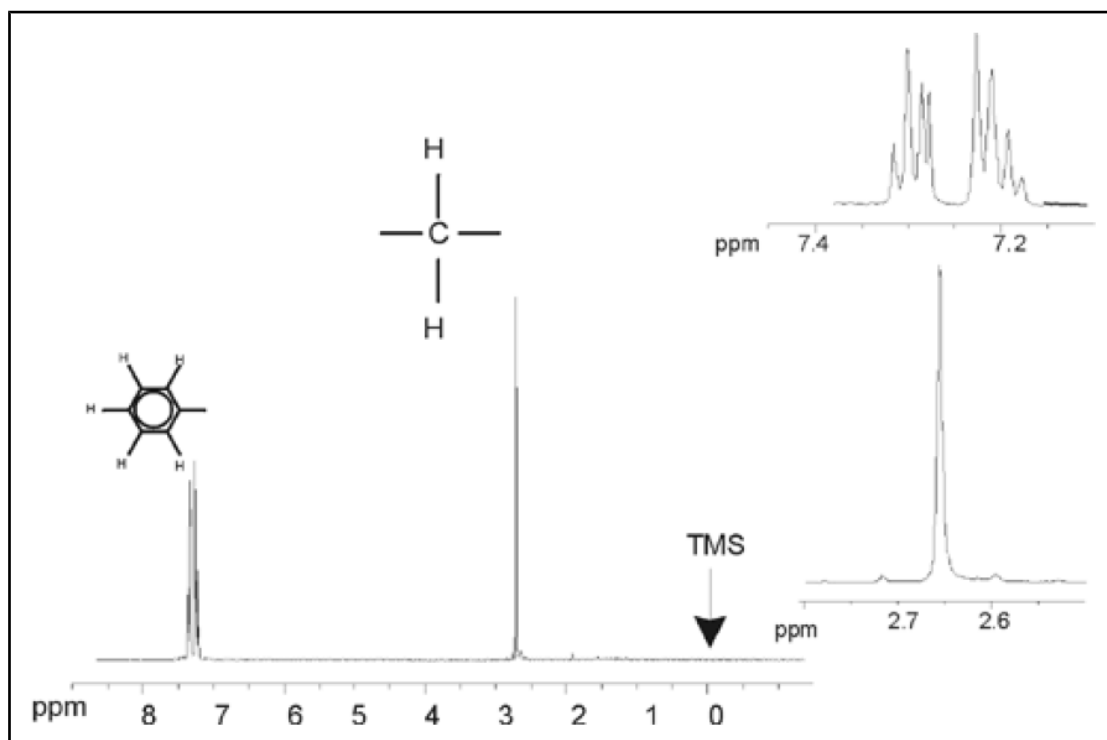

Figure 3.13: Decoupling Experiment

Figure 3.14: Ethylbenzene Spectrum with Homodecoupling

The experiment described above is an example of homodecoupling in that the same isotope, namely ^{1}H, is observed and decoupled. Heterodecoupling is where the isotope to be observed is different to that which is decoupled. In the chapter *13C Spectrum with Proton Decoupling [▶ 61]* of this manual you will perform a heterodecoupling experiment where you observe ^{13}C and decouple [1]H. AVANCE with SGU spectrometers have the ability to perform very complicated types of experiments depending upon the number of channels installed. A four channel spectrometer can be used to observe one nucleus and decouple three other nuclei. With up to eight independent channels the possible range of experiments is quite amazing. The user should be aware that currently the limiting factor is not in the generation of RF excitation and decoupling pulses but in transmitting these pulses to the sample via the probes and to some extent the preamplifiers. The signal routing of the experiment to be carried out is set up using the "edasp" menu. More details can be found in the manual "Acquisition Commands and Parameters" (P/N H9775SA3).

3.8   FID and Spectrum

The signals emitted by the excited atoms in the sample are received by the spectrometer and Fourier transformed by the software of the data station computer. The process of receiving the NMR signals is called an acquisition. Data is said to be acquired. A distinction should be made between the two terms; "FID" (time domain) and its associated "spectrum" (frequency domain).

When an acquisition is carried out, "raw" data is acquired and the received signal is called an FID (Free Induction Decay). A typical FID is illustrated in the figure below.
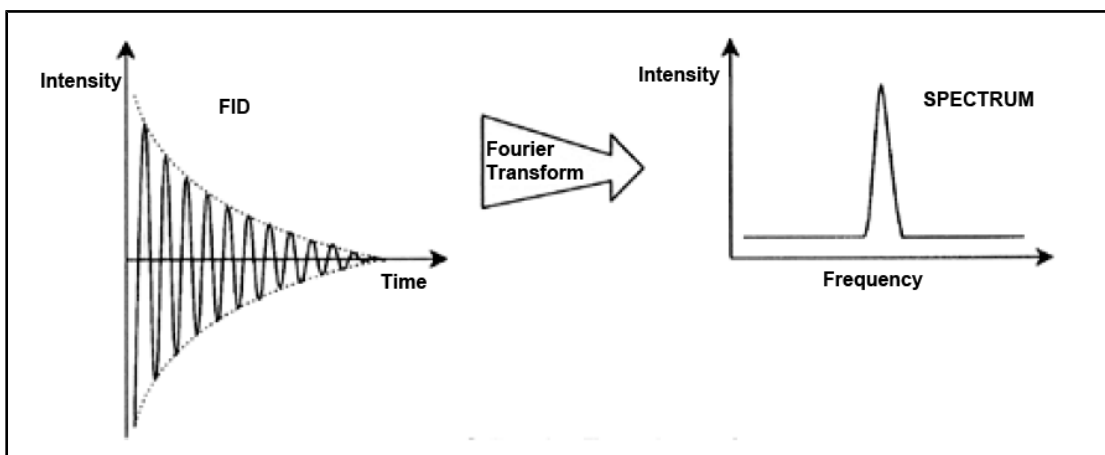
Figure 3.15: Fourier Transformation

Before an FID can be usefully analyzed it must first be transformed to the frequency domain. This is achieved by applying a Fourier transformation. A **Fourier transformation** is a mathematical operation which converts the FID into a frequency spectrum. An **FID** is a signal whose intensity varies with time whereas a spectrum displays how intensity varies with frequency. Fourier transformation is the most important one of several processing operations that is normally carried out on raw data.

4 System Description

The spectrometer consists of the following subunits:

- **Operator console** including the host computer, monitor and the keyboard.
- **Console** containing the electronic hardware.
- **Magnet system** including the shim system and the probe.

Figure 4.1: Magnet, Operator Console, and Console

4.1   AVANCE Architecture Overview

The picture below shows a simplified architecture overview of an AVANCE system. Please use the BASH (Bruker Advanced Service Handbook) for further information about the AVANCE system and hardware.
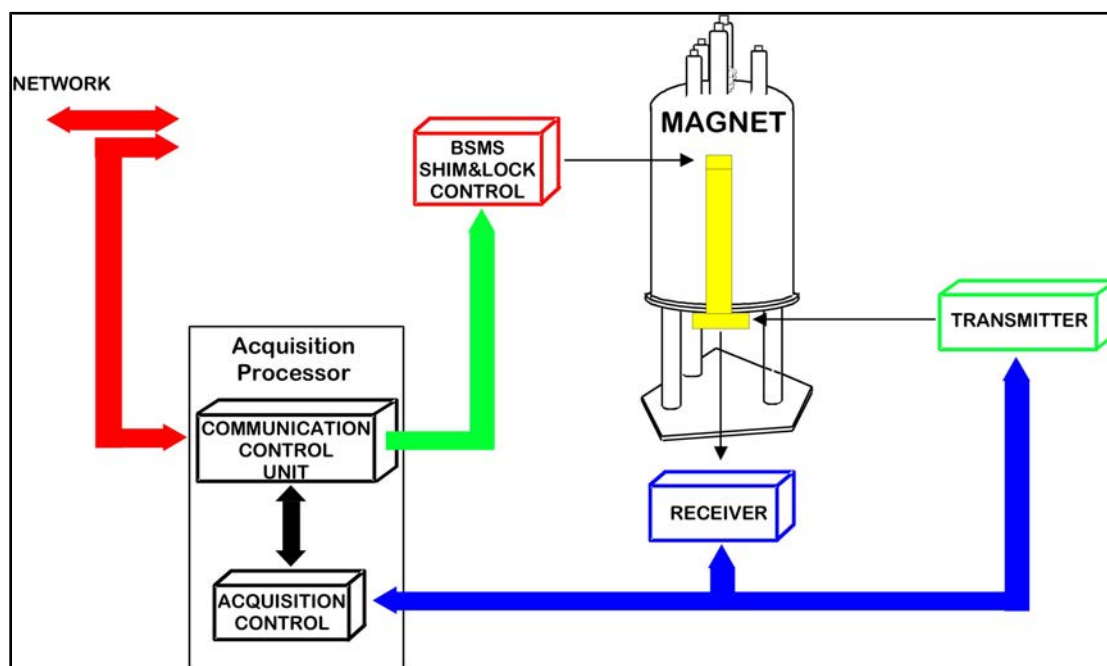
Figure 4.2: AVANCE Architecture Overview

4.1.1   Operator Console and Connections

All aspects of the spectrometer operation are controlled from the operator console. The design and implementation of experiments as well as the analysis of data are controlled by commands entered by the operator at the console. The sub components of the operator console are as follows:

Host computer: This is a Personal Computer. The host computer runs the TopSpin program and handles all the data analysis and storage. All operations relevant to data acquisition are controlled by a second computing system called the *IPSO* (Intelligent Pulse Sequence Organizer) housed within the console itself.

Ethernet connection from host computer to IPSO: This is used to transfer data and instructions between the host computer and the IPSO.

4.2   Console

This unit may be a NanoBay, OneBay, or TwoBay bay depending upon the system and houses most of the electronic hardware associated with a modern digital spectrometer. The principal units are the *IPSO* (Intelligent Pulse Sequence Organizer), the *BSMS* (Bruker Smart Magnet System), the *VTU* (Variable Temperature Unit) as well as various amplifiers.

IPSO: The various units within the IPSO generate the radio frequency pulses used to excite the sample and receive, amplify and digitize the NMR signals emitted by the sample. Once the data is received and digitized, the information is transferred to the host computer for further processing and storage. The main link with the host computer is via Ethernet. It is important to emphasize that the IPSO has total control over spectrometer operation within the duration of an experiment. This is to ensure uninterrupted operation and so

guarantee the integrity of the acquisition. The rack contains a set of digital and analogue slot-in type boards that prepare the signal to be transmitted and also receive, amplify and digitize the NMR signal. A detailed description of these boards is beyond the scope of this manual.

BSMS: This system is controlled via software using the `bsmsdisp` command) and is used to operate the lock and shim system as well as controlling the sample lift and spin.

VTU: Depending on the model the VTU may be a separate stand-alone unit or may be incorporated into the BSMS. Its function is to vary the sample temperature in a controlled manner or maintain it at a constant value.

Amplifiers, also known as **Transmitters**. Signals of relatively large amplitude are often required to excite the NMR sample and hence the need for amplifiers. Amplifiers can be internal (incorporated into the IPSO rack) or external (separate stand-alone units). Cables running directly from the amplifier outputs to the _HPPR_ (High Performance Preamplifier) carry the RF signal to the sample. Although there is a wide range of available amplifiers (including solid amplifiers) the two main categories are:

Selective amplifiers (also known as ^{1}H or proton amplifiers) are specifically designed to amplify the higher frequencies associated with ^{1}H and ^{19}F.

Broadband amplifiers (also known as X amplifiers) are designed to amplify a wide range of frequencies (excluding ^{1}H and ^{19}F).

4.3 Link between the Host Computer and the IPSO

Although during a typical TopSpin session this link is permanently in operation and effectively invisible to the user, the link is broken anytime either the host computer or the IPSO is switched off and must be re-established when they are switched back on. This will be done automatically.

See also

- Basic Troubleshooting [▶ 69]

4.4 Magnet, Shim System, HPPR and Probe

The **magnet** generates the magnetic field required to induce NMR transitions. To maintain a superconducting system the magnet core is cooled to very low temperatures using liquid nitrogen and helium (for more details see the section _The Magnet and Magnet Dewar [▶ 27]_).

The room temperature **shim system**, mounted into the lower end of the magnet, is a set of current carrying coils (known as shims) used to maximize field homogeneity by offsetting any existing in homogeneities. The currents in these room temperature shims (so called as they are not cooled by being immersed in a bath of liquid helium) are controlled by the BSMS and may be adjusted from the BSMS display to optimize the NMR signal. This has a major effect on signal resolution and sensitivity. This action of adjusting the currents in the room temperature shims is referred to as **shimming** the magnet.

Figure 4.3: Photo of Magnet, Shim System, Probe and HPPR

1.	Operator Console	5.	HPPR/2 Cover 2 Module with Connections to the Probe
2.	Console	6.	Probe
3.	Magnet	7.	Shim System
4.	HPPR/2 Cover 1 Module Top View	8.	Probe and Shim System

Although the **HPPR** (High Performance Preamplifier) carries the transmitted signal *to* the sample it is primarily concerned with magnifying the relatively weak signals emitted *from* the sample. It is located at the base of the magnet to amplify the NMR signal at the earliest possible opportunity and thus minimize losses along the cable. Once the signal has been amplified with the HPPR any subsequent losses in cabling are less critical. The HPPR also transmits and receives the deuterium (or fluorine) lock signals and is used in the wobble routine. Up to 5 (**HPPR**) or 8 (**HPPR/2**) individual modules (excluding the cover module which is always present) may be configured. A very common configuration consisting of three individual modules is a **Proton**, **X-BB** and **2H** along with a cover module.

The probe is inserted into the shim system at the base of the magnet and essentially consists of various coils used to transmit the excitation pulses to the sample as well as receive the emitted signal. The probe also transmits and receives the lock signal.

4.5 The Magnet and Magnet Dewar

A range of magnets are available with different strengths. The **strength of the magnet** is graded according to the frequency of the NMR signals emitted by hydrogen atoms. The stronger the magnet field, the higher this hydrogen frequency. For example, with a 500 MHz magnet (11.7 T), this means that when a chemical sample is placed in the magnet for analysis, the ^{1}H atoms in the sample will emit signals with a frequency very close to 500 MHz. Bruker magnets are available in the range of 200-1000 MHz.

Superconducting magnets are **electromagnets**, and as such make use of the fact that an electric current produces a magnetic field. The **magnet core** consists of a large coil of current carrying wire in the shape of a solenoid. At the center of the coil a very intense static magnetic field exists. The sample to be analyzed is placed inside this magnetic field.

At very low temperatures certain materials show the remarkable property of superconductivity. A superconducting wire carries electricity without the need for any driving energy (i.e. battery or mains supply). Once a current is started in a superconducting loop it will continue forever. Bruker magnets consist of such a superconducting loop. The only maintenance required on the magnet is to ensure that the coil is kept immersed in liquid helium.

The magnet consists of several sections. The outer casing of the magnet is evacuated and inner surfaces are silvered (this is the same principle as a Thermos). Next comes a bath of nitrogen which reduces the temperature to 77.35K (-195.8°C) and finally a tank of helium in which the superconducting coil is immersed in. This tank is thermally isolated against the nitrogen bath by a second evacuated section (see picture below).
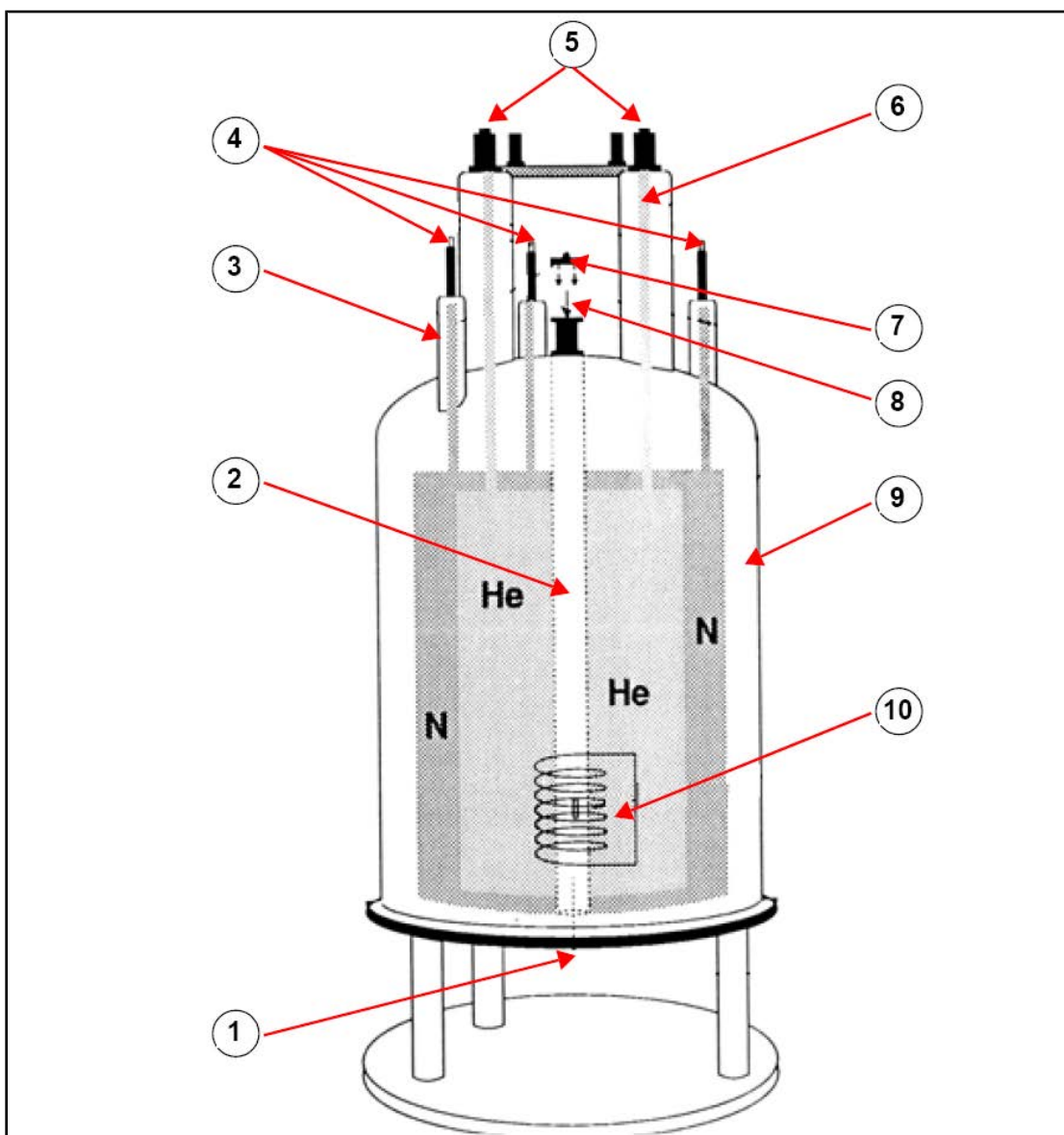
Figure 4.4: Superconducting Magnet

1.	Insert Probe here	6.	Helium Tower
2.	Bore	7.	Metal Plug
3.	Nitrogen Tower	8.	Insert sample here
4.	Nitrogen Ports	9.	Vacuum Chamber
5.	Helium Ports	10.	Magnet

4.5.1 Room Temperature Bore

The helium and nitrogen tanks are wrapped around a central column known as the magnet bore. A metal plug normally closes off the top of the bore. Magnets are available with either standard bore or wide bore. Samples to be analyzed are introduced into the magnet via the top of the bore. Probes, which hold the sample and carry signals to and from the sample, are inserted from the bottom.

4.5.2 Helium Tank

In a standard magnet the helium tank is suspended from two necks which extend high above the magnet. Access to the helium tank can be made via two ports. One of these ports permits refilling of the liquid helium and is also the entry for a **helium level sensor**. The other port is used only when the magnet is being charged or discharged. The helium necks may support several valves which control the release of the small quantities of helium that will inevitably evaporate.

> **i** **Note:** Handling of the valves and filling the magnet with liquid Helium should only be done by a trained person.

4.5.3 Nitrogen Tank

The three shorter necks extending above the magnet allow access to the nitrogen tank.

4.6 Introduction to the Lock System

This section has been included to give the user a basic understanding of the principles of the lock system. Practical aspects such as how to actually lock the sample will be dealt with in the section Locking the Sample.

The aim of the lock system is to ensure that the strength of the magnetic field surrounding the sample does not change during an experiment or that the field is not modulated by external disturbances. NMR analysis involves measuring the precise frequency of signals that are emitted by the sample. The frequencies of these signals are directly proportional to the magnetic field strength, i.e. if the field strength varies, so does the emitted frequency. Therefore, the user must be confident that the magnetic field strength is always maintained at precisely the same strength, which is referred to as "**locking**" the sample. The **lock system** is essentially a separate spectrometer designed to observe deuterium. It should be mentioned that the signals emitted by deuterium are normally far removed from frequencies of interest. However, if the deuterium frequency is unsuitable, then a **Fluorine** (19F) **lock** may be used. Since it is by far the most popular, only the deuterium lock will be dealt with here, but the reader should note that the principle of the deuterium and the fluorine lock are identical.

In AVANCE systems the BSMS provides the hardware required to implement the lock and a separate deuterium module in the HPPR transmits and receives the lock signals. Some deuterium must of course be introduced into samples that are under analysis. This can most easily be done by dissolving the sample in a deuterated solvent. A *deuterated solvent* is one in which a large percentage of the hydrogen atoms have been replaced by deuterium. Commonly used deuterated solvents are acetone-d6, benzene-d6, chloroform-d and DMSO-d6, although many other solvents are available. The sample which will be used to illustrate some basic NMR techniques in this manual is Menthyl Anthranilate in DMSO-d6.

The frequency of the signals emitted by deuterium for a particular size of magnet is precisely known. Therefore, if the magnetic field strength is correct, any deuterium nucleus in the sample should emit this exact frequency. If the magnet strength varies so will the deuterium frequency. The lock system uses a receiver (housed in the BSMS rack) to monitor this deuterium frequency and makes adjustments to the magnetic field strength accordingly.

The receiver in the lock system is designed so that when the field strength is correct (i.e. the correct deuterium frequency is detected), no adjustments are made to the field. However, should the field strength vary (drift), a current is changed in a special coil (the H0 coil) located within the shim system of the magnet, which has the effect of returning the

field strength to the correct value. The deuterium frequency is measured several thousand times per second. Hence, as long as the system is locked, the user can be confident that the field is maintained at a constant strength during acquisition.

4.7   Probes

The probe is designed to hold the sample, transmit radio frequency signals which excite the sample and receive the emitted response. The transmission and reception is achieved by using specially designed RF coils.

The probe is inserted into the bottom of the magnet and sits inside the room temperature shims. Coaxial cables carry the excitation signals from the console amplifiers to the probe and the NMR signal back from the sample to the receiver. The cables are routed through a set of preamplifiers (the HPPR) which are situated next to the base of the magnet or on NanoBay systems are built in to the console. The **preamplifiers** are needed to boost the NMR signals which are typically very weak.
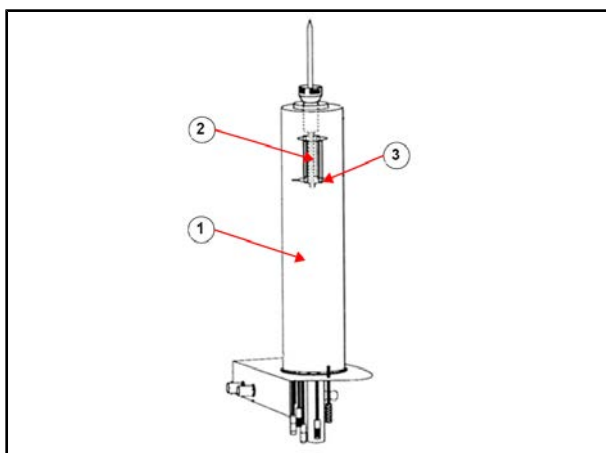
Figure 4.5: Sample in Probe

1.	Probe	3.	Coils
2.	Sample Volume		

Probes come in different sizes and types. The size of the probe is given in terms of the sample tube sizes it can hold, with 5mm and 10mm sample tube diameters the most popular. Different types of probes are used depending upon the type of experiment. **Selective probes** are specially designed to observe specific nuclei, e.g. 13C, while multinuclear (X-BB or broadband) probes may be used to analyze a wide range of nuclei. The number and design of the internal coils are what physically distinguishes one type of probe from another. In addition, the outer diameter and length of the probe is built to the specifications of the various magnets (wide bore or standard bore; different length from the bottom to the center of the magnetic field for magnets with different field strength).

Signals enter and leave the coils of the probe via connectors which are clearly labeled and located at the base of the probe. The same cable is used to carry the signal to and from the probe. Each probe has an inner coil (the **observe coil**). This coil is located closest to the sample volume to maximize sensitivity. The color coding of the inner coil BNC follows a simple rule. It always has the same color as the rectangular strip located directly above the BNC connectors. The following figure shows the labeling of a multinuclear probe. In this case the broadband coil is the inner coil.
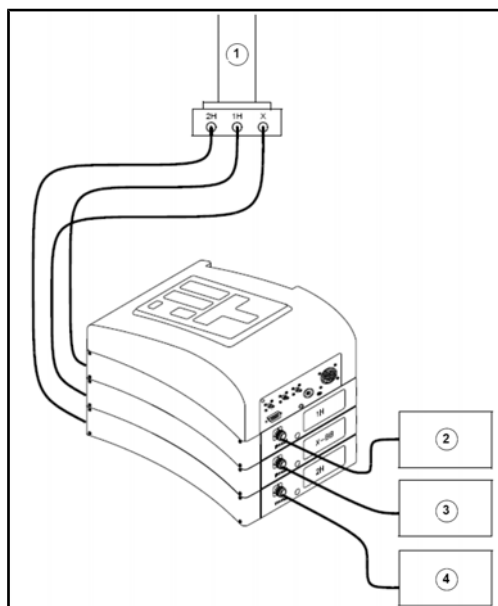
Figure 4.6: Typical HPPR Cabling

1.	Probe	3.	X-amplifier
2.	Proton amplifier	4.	BSMS 2H transmitter

4.8 Broadband Probe

One type of a probe, which will be described here as an example, is the 5 mm BBO Smart probe. As its name suggests this probe is designed for users who are interested in analyzing samples of various nuclei. The x-frequency range is typically from ^{15}N to ^{31}P including ^{19}F.

The left BNC is marked ^{2}H (see figure below) and is used to carry the lock signal. The other two connections are for the ^{1}H and x-nucleus signals and are labeled accordingly. The cables leaving the ^{1}H, X and ^{2}H BNCs are connected to the preamplifiers.

Figure 4.7: Example of a Broadband Probe

The probes are designed to enable the temperature of the NMR sample to be controlled. A **heater** is used in conjunction with an air/**N2 transfer line** to control the sample temperature. A **thermocouple** serves as a thermometer to monitor the sample

temperature. All these devices are attached to the base of the probe and are easily accessible. The Variable Temperature Unit (**VTU**) which is located inside the console constantly monitors the thermocouple reading and makes adjustments in the heater power to maintain the required temperature.

All but a few high resolution probes come equipped with gradient coils and the connector is found on the side of the probe base.

Finally, the black box on the underside of the probe base houses the tuning and matching equipment. These are used to make fine adjustments to the probe in order to optimize the performance. When a compound is analyzed it is excited with signals of a definite frequency (the resonance frequency). Different nuclei will be excited by different frequencies and tuning involves adjusting circuitry within the probe so that it is at its most sensitive for the frequency of interest. The probe is also matched to ensure that as little as possible of the excitation signals and the FID are reflected (i.e. wasted). Both tuning and matching are interactive in that they cannot be adjusted independently of each other.

The probe should be tuned and matched every time the sample in the magnet is changed. Each coil in the probe is tuned and matched separately using the 'atma' or 'atmm' routine which is described in *Tuning and Matching the Probe [▶ 38]*.

Changing a probe will require reconnecting the probe to the preamplifiers.

4.9 Changing a Probe

If the probe is to be changed, the procedure below should be followed. Probes are fragile and expensive, so you should check with the system manager before attempting to change the probe. The magnet, and in particular eddy currents, greatly affect the mechanical motion of the probe. As you remove the probe from the magnet expect it to accelerate suddenly as it reaches the lower end of the magnet bore. Also be prepared for some resistance when you place the probe into the magnet.

Procedure for Changing a Probe:

1. Ensure that no acquisition is running by clicking the STOP button in the upper toolbar of the TopSpin window or entering stop in the command line.
2. Turn off any **heating** or **cooling** from the system. Use the 'edte' command to switch off the heater. Allow the probe to settle to room temperature. Do not shut off the system itself!
3. Remove the air/N$_2$ connection which is attached to the base of the probe.
4. After making sure that the magnet bore is not plugged, remove any sample that may be present in the magnet by activating the **LIFT** button in the BSMS window.
5. Turn off the LIFT.
6. Disconnect all BNC cables from the probe base.
7. Disconnect the thermocouple, heater and any other gradient or PICS connection.
8. Using the attached screwdriver, loosen the two screws which secure the probe in the magnet.
9. Lower the probe straight down and out of the magnet.
10. Insert the new probe and secure using the two screws.
11. Reconnect the coaxial cables, thermocouple, heater, cooling lines and any other connections as appropriate.
12. Turn the heater back on.

5   Basic Procedures

This chapter will outline basic operations which are used every time a spectrum is acquired. The TopSpin software has an easy to use work flow-based interface which guides a user through all the necessary steps to obtain a spectrum. All operations are software controlled and can be selected with a mouse click on the work flow tabs (see 12. in the TopSpin Window figure), which opens up a row of work flow buttons (see 11. in the TopSpin Window figure).

The readers may skip this chapter if they are already familiar with these operations.

5.1   The TopSpin Window

The picture below shows the layout of the basic TopSpin window.
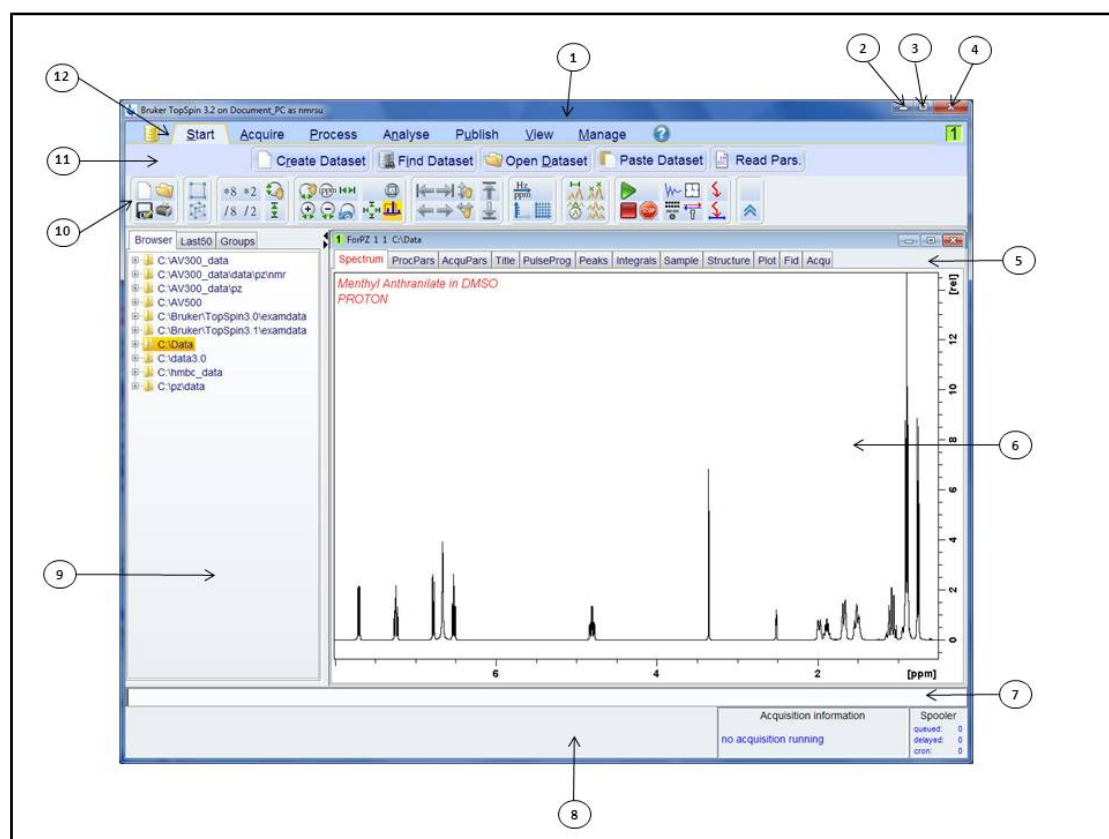
Figure 5.1: The TopSpin Window

1.	Title bar	7.	Command line
2.	Minimize button	8.	Status bar
3.	Maximize button	9.	Browser window
4.	Close button	10.	Tool bar
5.	Data Set tabs	11.	Workflow button bar
6..	Data Display window	12.	Workflow menu bar

5.1.1 Creating a New Data Set

Regular use of a spectrometer will soon result in the accumulation of large amounts of data. Users will want to store data in conveniently named files so that they can easily access their data later. This is particularly important in a multi-user environment. When a set of data is acquired, it can be stored in what is called a **data set**. Each data set must have a unique descriptor (identifier) so that different data sets can be distinguished. A full descriptor for a data set requires the use of four parameters: DIR, NAME, EXPNO, and PROCNO.

1. Click on the **Start** tab in the Workflow tab bar.
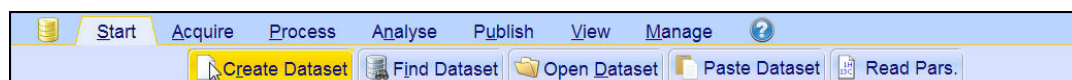
2. Select the **Create Dataset** button in the Workflow button bar by clicking on it.
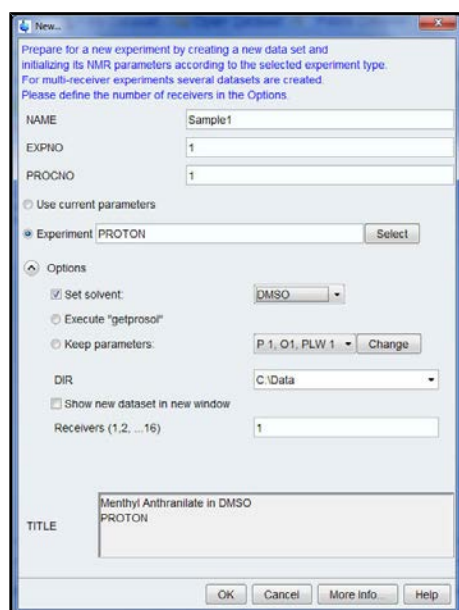
3. You can now create your own data set. For NAME you may enter a string of up to 13 characters. For both EXPNO and PROCNO enter '1'.

4. Choose the experiment you want to run by clicking on the **Select** button to the right of the Experiment window.

5. Enable **Show Recommended** inside the experiment selection window.
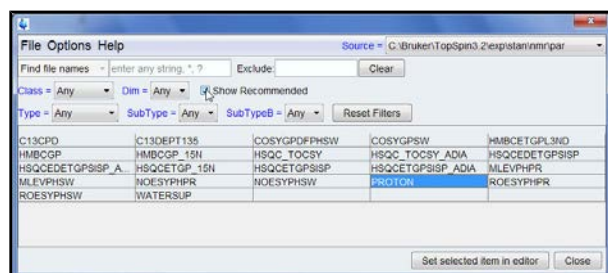
6. Select the experiment (e.g. Proton).

7. Click on the **Set selected item in editor** inside the experiment selection window.

8. Choose the solvent of your sample by clicking on the **down arrow** to the right of the Solvent window.
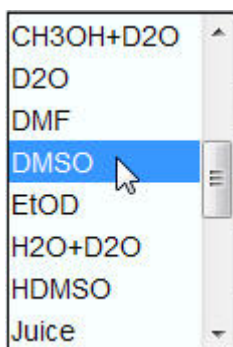
9. Select the solvent (e.g. DMSO) by clicking on it.

10. Choose a directory path where your data set is being stored on the computer's hard drive by clicking on the **down arrow** to the right of the DIR window.
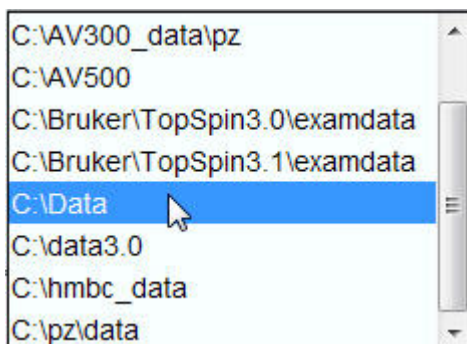
11. Select the directory path (e.g. C:\Data)

12. Enter a desrciption of your sample in to the **Title** window.

13. Click on the **OK** button inside the New Data set window.

Note: Your newly created data set is now the current data set and details of the descriptor will be stored on the harddrive in the folder: DIR\NAME\EXPNO, where each EXPNO corresponds to a completely independent data set. In this example, the data will be stored at **C:\Data\Sample1\1**. This path will also appear in the title bar of the data window of Topspin

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5.1.2   Sample Preparation

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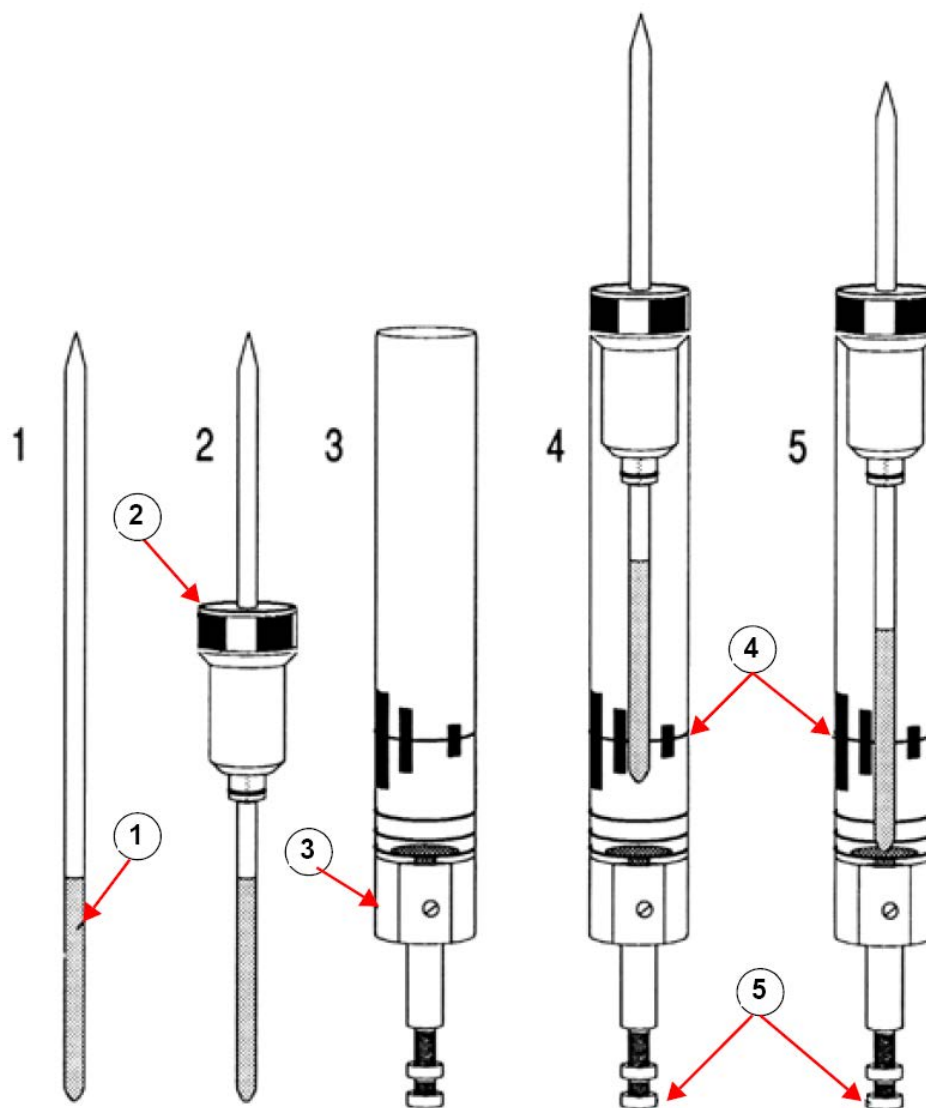
Inserting the Sample into the Spinner

1.	Sample	4.	Center Line
2.	Spinner	5.	Depth Adjustment Screw
3.	Depth Gauge		

- The sample tube should sit tightly inside the spinner.
- Wipe the sample tube clean before inserting into magnet.

5.2   Inserting the Sample plus Spinner into the Magnet

The raising and lowering of the sample is controlled by a stream of pressurized air. Before placing a sample onto the top of the bore, make sure that the air flow is present (it is quite audible) and remove any sample which may be rising to the top of the bore.

1. Click on the **Acquire** tab in the Workflow tab bar.
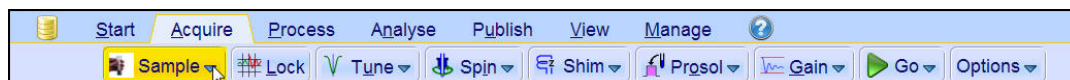
2. Click on the **down arrow** inside the **Sample** button in the Workflow button bar.

3. Select **Turn on sample lift air (ej)** by clicking on it.

4. Place the sample plus the spinner on to the top of the magnet bore.

5. Click on the **down arrow** inside the **Sample** button in the Workflow button bar.

6. Select **Turn off sample lift air (ij)** by clicking on it.

5.3   Locking the Sample

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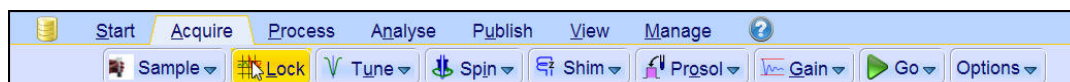
1. Select the **Lock** button in the Workflow button bar by clicking on it.
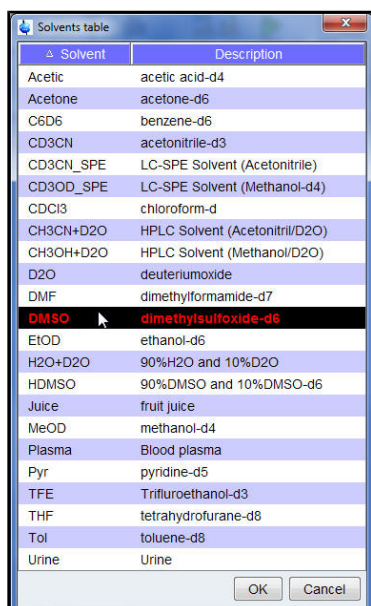
2. Select a solvent by clicking on it.

3. Click on the **OK** button inside the Solvent table window.

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i | **Note**: If lock has been achieved, the signal should be of the form of a horizontal line with some associated noise or ripples (see figure below). The height of this line is called the lock level.
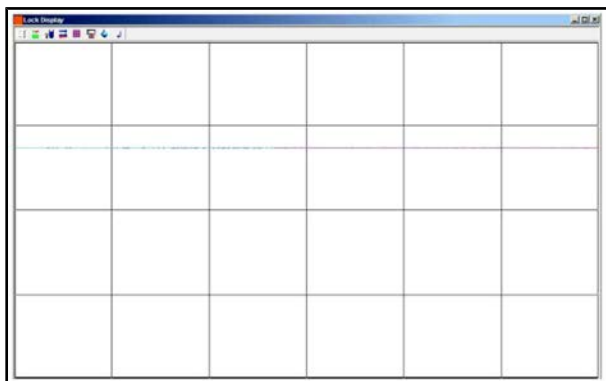
Figure 5.2: Lock Display after Locking the Sample

5.4 Tuning and Matching the Probe

The sensitivity of any probe will vary with the frequency of the signal transmitted to it and there exists a frequency at which the probe is most sensitive. Furthermore this frequency may be adjusted over a certain range using tuning capacitors built into the probe circuitry. **Tuning** involves adjusting the probe circuitry so that the frequency at which it is most sensitive is the relevant transmission frequency (SFO1, SFO2 etc.) Each coil in the probe will be tuned (and matched) separately. If the probe has been changed or the transmission frequency altered significantly, it may be necessary to retune the probe. For routine work in organic solvents with selective probes, the value of the transmitted frequencies are unlikely to vary greatly. Hence, once the probe has been initially tuned, slight variations in

frequency will not warrant retuning. Typically the transmitted frequency would need to be altered by at least 100kHz to warrant retuning. However for broadband probes the frequencies transmitted will vary greatly from nucleus to nucleus and so the probe will need to be tuned each time the selected nucleus is altered. Whenever a probe is tuned it should also be matched. **Matching** involves ensuring that the maximum amount of the power arriving at the probe base is transmitted up to the coil which lies towards the top of the probe. This ensures that the minimum amount of the power arriving at the probe base is reflected back towards the amplifiers (and consequently wasted).

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

5.4.1 Probes Equipped with ATM using the Automated Tuning Routine

- Select the **Tune** button in the Workflow button bar by clicking on it.

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

5.4.2 Probes Equipped with ATM Using the Manual Tuning Routine
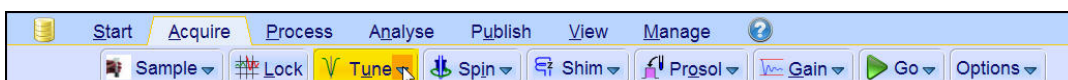
- Click on the **down arrow** inside the **Tune** button in the Workflow button bar.

- Select **Tune/match ATM probe manually (atmm)** by clicking on it.

> **i** **Note**: The ATMM Probe Tuning/Matching window (figure below) and a window displaying the wobble curve will be opened.
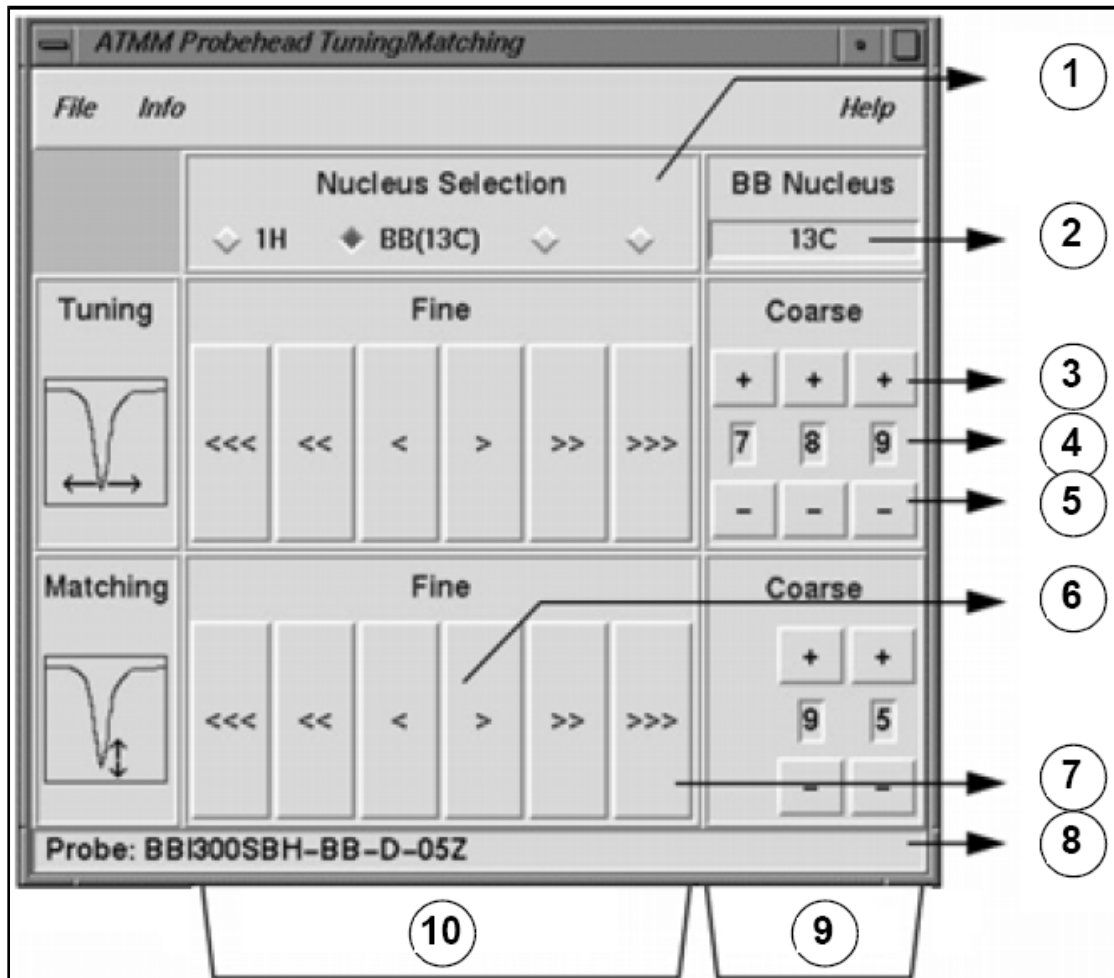
Figure 5.3: ATMM Probe Tuning/Matching Window

1.	Select next nucleus to wobble on (only routed channels are visible).
2.	Selected nucleus on broad-band channel.
3.	Move step up.
4.	Nucleus position.
5.	Move step down.
6.	Move slow (red, inactive button indicates no further change in this direction).
7.	Move fast.
8.	Probe type.
9.	Coarse tuning and matching on broadband probes.
10.	Fine tuning and matching on all probes.

Select a suitable nucleus for your experiment and then make the adjustment by clicking the buttons with the arrows. Always start with matching, then the tuning.

The **wobble** routine works by transmitting a weak signal to the probe and comparing the probe and cable impedance with a 50 Ohm reference within the HPPR. The transmitted frequency is centered on SFO1, SFO2 etc. but "swept" over a range determined by the size of the parameter WBSW (see below). The resultant curve is the familiar response curve of a resonance circuit and is simply a measure of the amplitude of the reflected signal (vertical axis) against frequency (horizontal axis).

Matching involves adjusting the probe so that the minimum of the wobble curve is at the base of the display (i.e. touching the horizontal frequency axis). This represents minimum reflection of the transmitted signal.

Tuning involves ensuring that this occurs at the frequency of transmission which will be at the center of the screen's horizontal scale. It will be seen however that the tune and match adjustments interact with each other and must be adjusted in tandem. When the wobble curve minimum is centered and at the base of the screen the probe has been optimally tuned and matched.
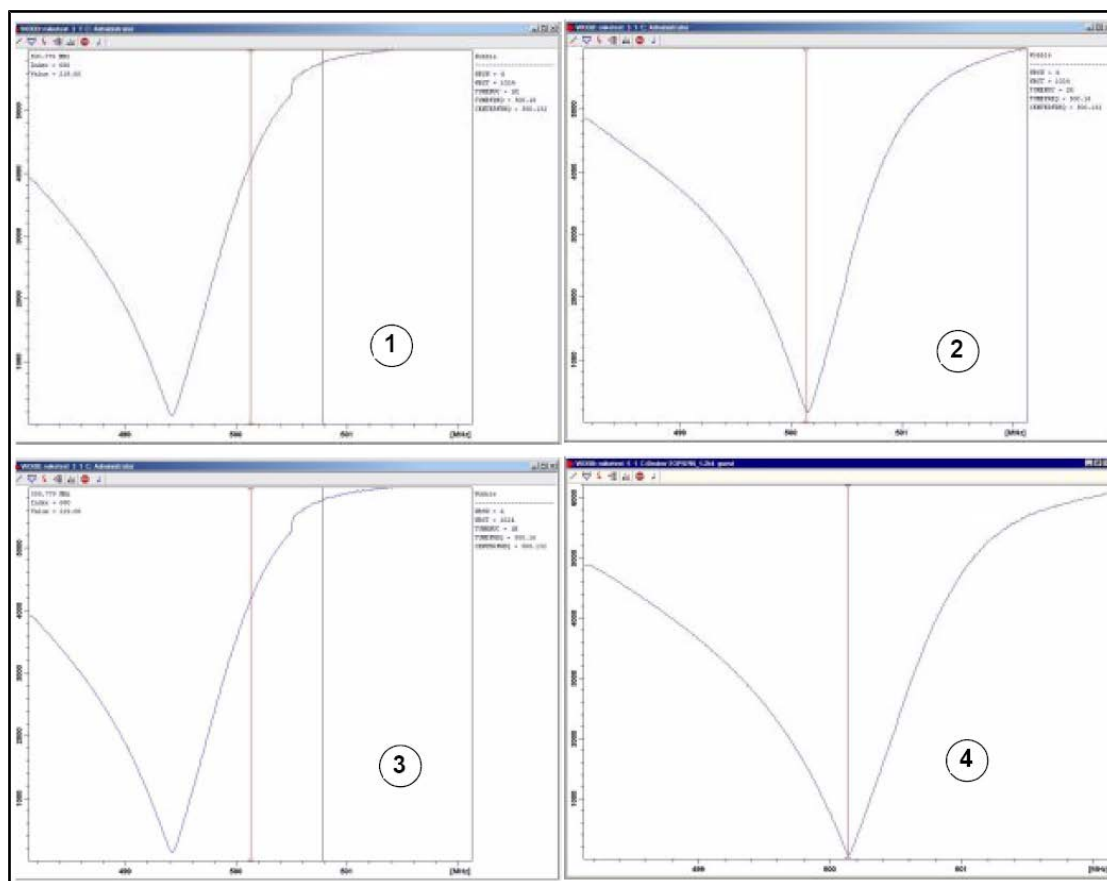
Figure 5.4: Examples of Wobble Curves with Different Tuning and Matching

1.	Bad matching and tuning.	3.	Good matching, bad tuning.
2.	Bad matching, good tuning.	4.	Good matching and tuning.

If you want to optimize the probe for several nuclei (e.g. decoupling experiments) you can choose the next nucleus in the ATMM Probe Tuning/Matching Window.

5.5 Spinning the Sample

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

> **Note**: Samples to be investigated using experiments such as 2-D, 3-D, SELECTIVE, NOEDIFF, T1 and all inverse probes are normally not rotated.

Suggested spin rates are:

- 20 Hz for a 5 mm probe
- 12 Hz for a 10 mm probe
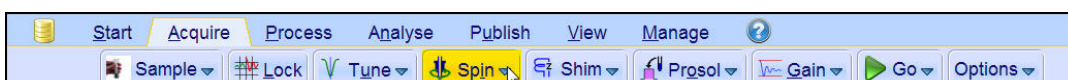
- Click on the **down arrow** inside the **Spin** button in the Workflow button bar.
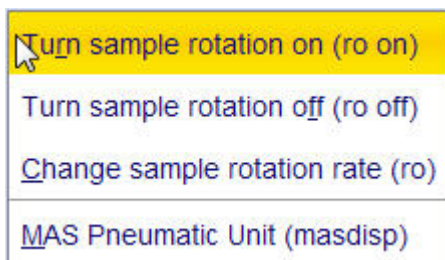
- Select Turn sample rotation on (**ro on**) by clicking on it.

5.6 Shimming

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

5.6.1 Routine Shimming Using TopShim

This is routine shimming and should be carried out at the beginning of every NMR session, and whenever the sample in the magnet is changed. Routine shimming involves making fine adjustments to the Z, Z^2, Z^3, Z^4 and Z^5 shims. Some higher field magnets may require higher order Z shims. The system administrator has programed TopShim to achieve the best homogeneity on each sample and it is fully automatically.

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

TopShim is using for all deuterated solvents the ^{2}H gradient shimming method and for other solvents especially H_2O, the ^{1}H gradient shimming method.
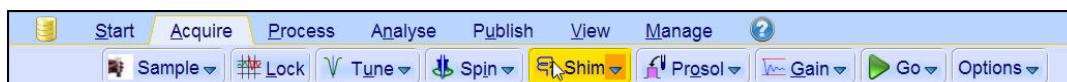
• Select the **Shim** button in the Workflow button bar by clicking on it. The shimming will start momentarily and should take less than a minute.

5.7 Setting the Probe/Solvent Dependent Parameters

Parameters, such as the 90^0 or the decoupler pulse length with associated power levels can be stored for any specific nucleus on any available channel. These values have been entered by the installation engineer or by the system administrator and are automatically loaded clicking on the **Prosol** button.
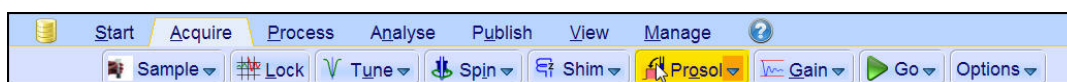
• Select the **Prosol** button in the Workflow button bar by clicking on it.

5.8 Adjusting the Receiver Gain

The receiver gain is a very important parameter that is used to match the amplitude of the FID to the dynamic range of the digitizer. The gain is automatically set clicking on the **Gain** button.
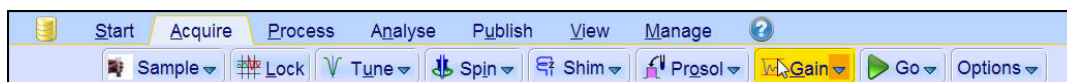
• Select the **Gain** button in the Workflow button bar by clicking on it. The receiver gain adjustment will start momentarily and it should take less than a minute.

5.9 Starting the Acquisition

The process of receiving the NMR signals is called an acquisition. Data is said to be acquired. When an acquisition is carried out, "raw" data is acquired and the received signal is called an FID (Free Induction Decay). The digital point size of the FID is called TD, Time Domain.
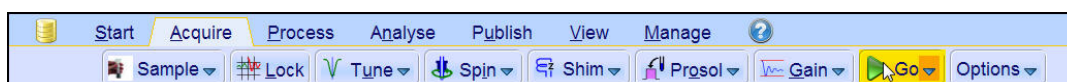
• Select the **Go** button in the Workflow button bar by clicking on it. As a default from the selected experiment, the spectrometer performs a number of dummy-scans before starting the actual number of scans.

5.10 Processing the Data

A Fourier Transformation is used to convert the FID to a frequency spectrum. The number of points used to form the resulting spectrum is determined by the parameter SI (size). The FID is transformed into a spectrum consisting of SI data points in the real part and SI data points in the imaginary part. The normal setting for SI is SI = TD/2. If you have loaded the parameter set "PROTON" you can easily verify that TD = 64K and Si = 32K.

1. Click on the **Process** tab in the Workflow tab bar.
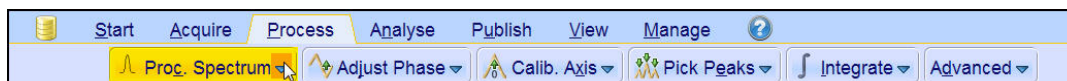
2. Click on the **down arrow** inside the **Proc. Spectrum** button in the Workflow button bar.
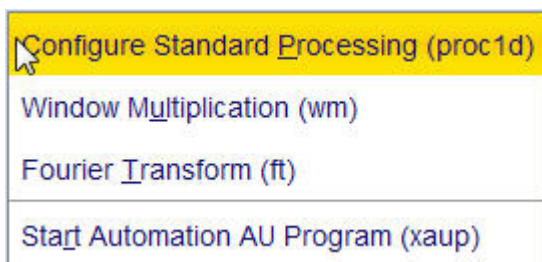
3. Select **Configure Standard Processing (proc1d)** by clicking on it.
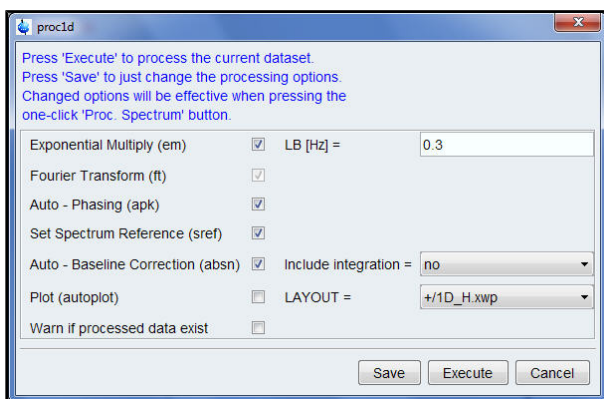
4. Enable the following options:

- **Exponential Multiply (em)**
- **Auto** – **Phasing (apk)**
- **Set Spectrum Reference (sref)**
- **Auto** – **Baseline Correction (absn)**

5. Click on **Execute** inside the proc1d window

Note: Use the **Save** button inside the proc1d window to save the processing settings.

6 Preparing for Acquisition, Frequency Related Parameters

This chapter will explain two of the most important parameters for observing a NMR spectrum. Before assigning values to various parameters, it is important that the user understands the concept of data sets, as groups of parameters are inextricably linked to these.

6.1 Frequency

The frequencies of the transmitted signals on channels one, two and three etc. are given by SFO1, SFO2 and SFO3, etc., respectively. However, these frequencies may not be set directly (you will notice that they cannot be highlighted using the mouse).

The transmitted frequencies are controlled by setting offsets to basic frequencies BF1, BF2 and BF3 etc.

For the observe channel:

- SFO1 = BF + offset (O1)

Transmitted frequency is automatically set by the user.

Similarly, for the next two decoupling channels:

- SFO2 = BF2 + offset (O2)
- SFO3 = BF3 + offset (O3)

When a specific nucleus is selected, the appropriate basic frequency is automatically set. After reading a standard parameter set, the basic frequency will be set correctly and only the offset values need to be adjusted.

Remember that SFOX is the most relevant parameter in that it is the frequency that is actually transmitted to the sample. Note also that the offsets can be set to zero in which case SFOX = BFX. A more detailed description is given in the next section.

6.2 Numerical Explanation of Transmitted, Basic and Offset Frequencies

Consider a 600 MHz spectrometer used to observe hydrogen. The spectrometer is configured to have a BF1 of 600.13 MHz (a 500 MHz spectrometer normally has a BF1 of 500.13 MHz, a 400 MHz a BF1 of 400.13 etc.).

If O1 is set to zero then: SFO1 = 600.13 + 0 = 600.13 MHz

Thus the spectrum center would lie at 600.13 MHz. If SWH was set to 20 kHz, the spectrum might look like in the picture below.
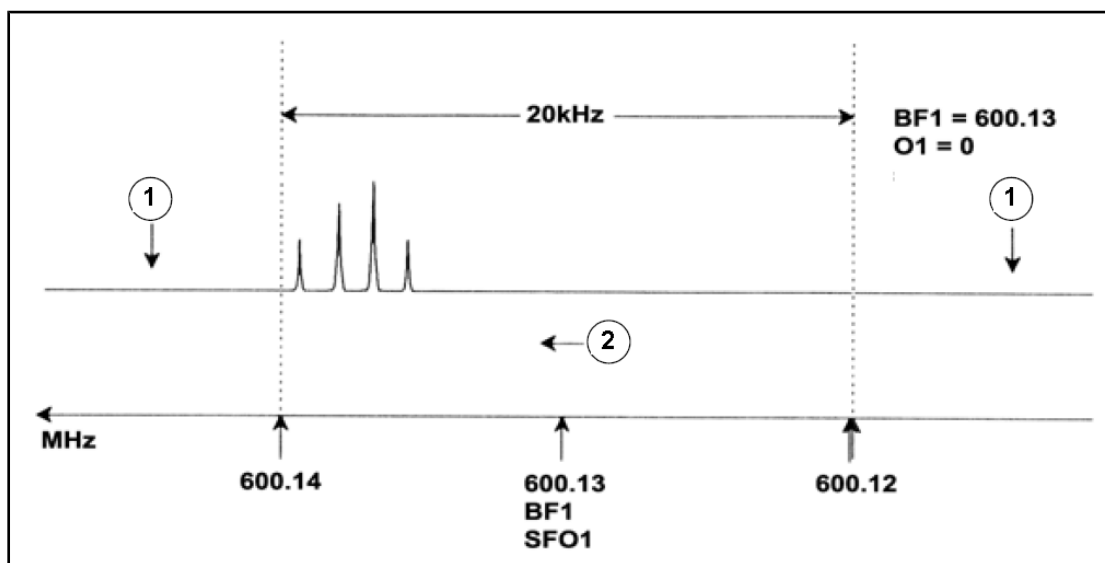
Figure 6.1: Spectrum with BF1 = 600.13 MHz, 01 = 0 Hz

1.	Signals filtered out	2.	Frequency

It is clear from our hypothetical spectrum that the NMR signals all appear towards the high frequency end of the spectral width. Furthermore it is possible that some signals may appear above 600.14 MHz and since these signals are outside the spectral window they have been filtered out and are not observed. To check for the presence of such signals two options are available:

- The spectral width could be increased to encompass any missing signal. This however has disadvantages such as increasing the FID resolution (the lower the value of FIDRES, the better the resolution).

- The preferred option would be to leave the spectral width unchanged, but assign a value to O1 to shift the center of the window.

In our example, the detected signals all appear in the region of 600.138 MHz and we wish to center the spectrum at this frequency.

=> SFO1 = 600.138 = BF1 + O1

=> 600.138 = 600.13 + O1

=> O1 = 0.008 MHz = 8 kHz

Hence if O1, the offset frequency, is set to 8 kHz, the window is shifted to look like that in the figure below.
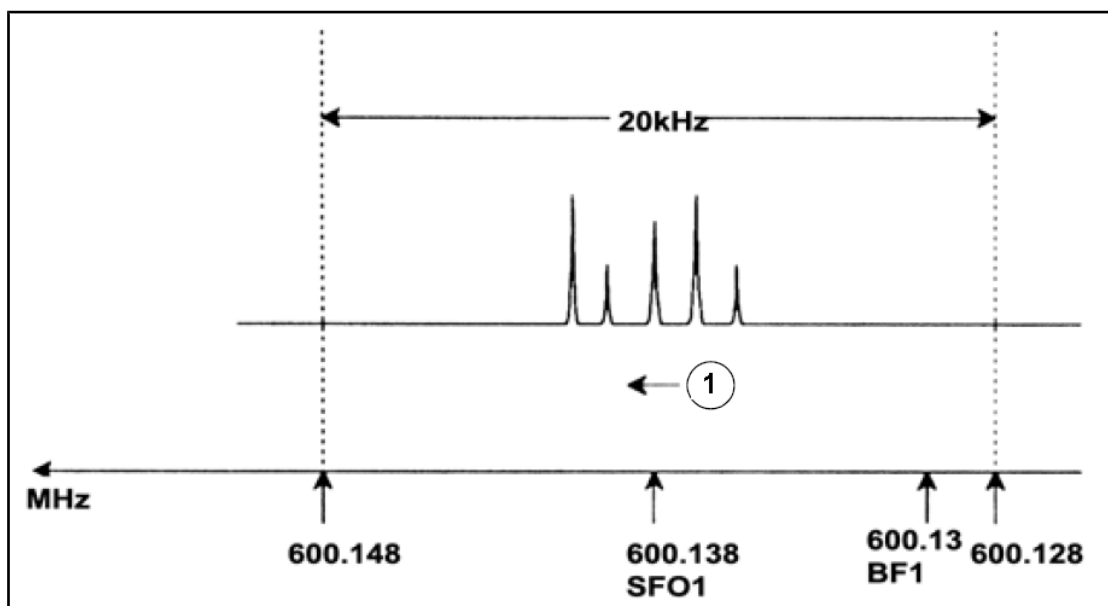
Figure 6.2: Spectrum with BF1 =600.13 MHz, 01= 8 kHz

1.	Frequency		

Finally it is clear from the figure above that the NMR signals emitted by the protons in our hypothetical sample occupy only part of the spectral width. Therefore the spectral width could be reduced without any loss of relevant data. One advantage of decreasing the SW is that the spectral resolution is improved. The disadvantage is that the time taken to acquire the data is proportionally increased.

In *Introductory Theory and Terminology [▶ 9]*, it was stated that the chemical shifts of protons rarely exceed 14 ppm. This corresponds to 8.4 kHz on a 600 MHz spectrometer. The figure below shows the hypothetical spectrum redrawn with the value assigned to SWH reduced from 20 kHz to 8.4 kHz.
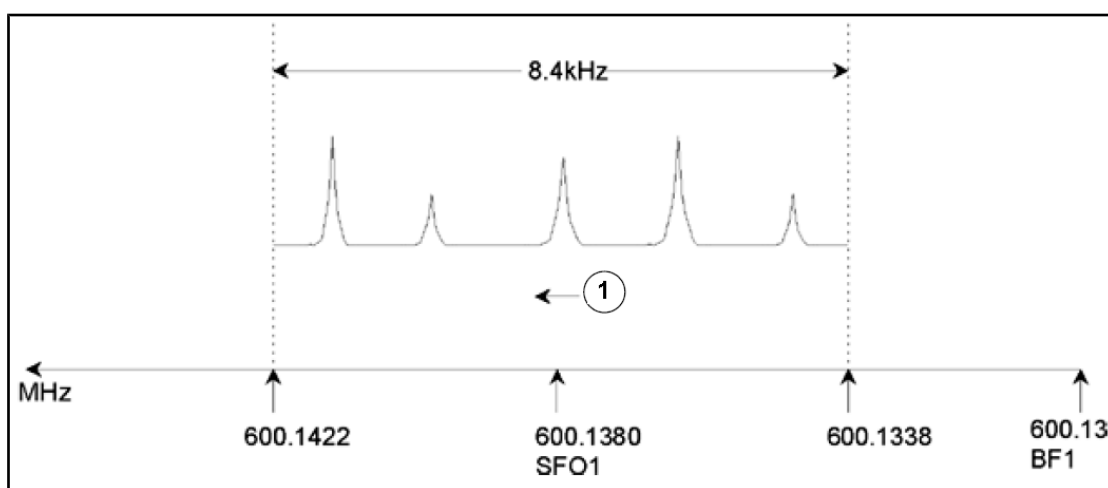
Figure 6.3: Spectrum with BF1 = 600.13 MHz, 01 = 8 kHz, SWH = 8.4 kHz

1.	Frequency		

It should be noted that the value of SWH used in any given experiment is determined only by the sample under analysis and the required spectral resolution. The value of 14 ppm for hydrogen spectra will ensure that most proton signals are detected. For a detailed study of a particular signal, however, much smaller values of SWH are used.

The figure below illustrates the general principles of how SFO1, BF1 and O1 interact (here shown with a new sample).
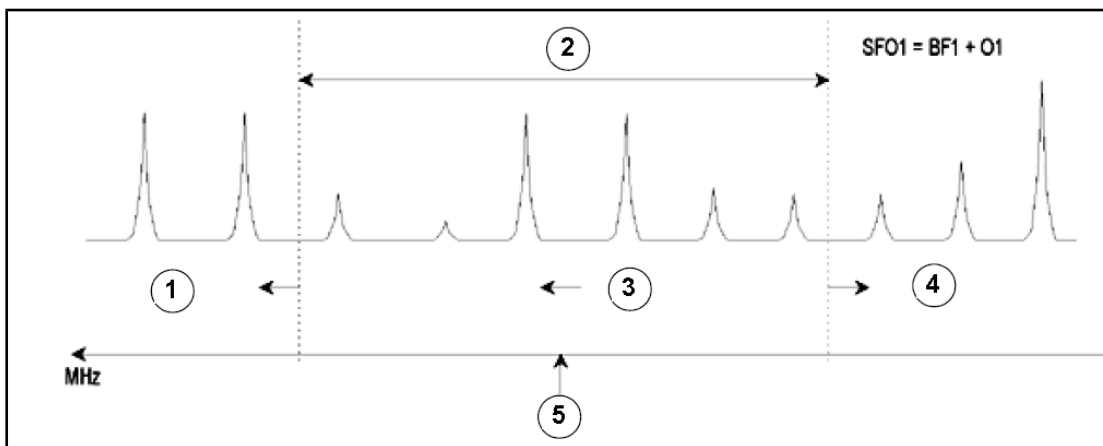
Figure 6.4: Interaction of SFO1, BF1 and O1

1.	Setting O1 to a positive value shifts the window to higher frequencies.
2.	SW determines the width of the window.
3.	Frequency.
4.	Setting O1 to a negative value shifts the window to lower frequencies.
5.	SFO1 is the center of the spectrum.

7 The NMR Sample

When a **solid** is investigated using the NMR technique, signals tend to be broad and the fine structure, which is of most interest to scientists, cannot be resolved. Consequently, solid samples are usually dissolved in a suitable solvent prior to acquisition. The same goes for **liquid samples**. In organic solvents, small amount of a reference compound may be added. However, to achieve best results the sample should be as pure as possible. Signals from **impurities** will at best make the spectrum unnecessarily complicated and at worst mask genuine signals. Particular care should be taken to ensure that the sample is free of **magnetic impurities** as these can distort the magnetic field and hence degrade the spectrum resolution. Solid impurities can be most easily removed by filtering. For samples in **organic solvents**, dissolved water can be removed as far as possible by thoroughly drying the sample prior to dissolution.

7.1 Solvent Selection

Once the sample has been sufficiently purified and dried, the next step is to choose a suitable solvent. Since deuterium is by far the most popular lock nucleus the sample is usually dissolved in a deuterated solvent (a deuterated solvent is one in which a large proportion, typically more than 99%, of the hydrogen atoms have been replaced by deuterium). Commonly used **deuterated solvents** are benzene-d6, acetone-d6, and chloroform-d though many other solvents are available. Factors to be considered when choosing a solvent are:

1. **Solubility**: Clearly the more soluble the sample is in the solvent the better. This maximizes the amount of sample within the sensitive volume which increases the sensitivity of the experiment. High solubility is particularly important if only small quantities of the sample are available.

2. **Interference of solvent signals with the sample spectrum**: The solvent itself will inevitably produce NMR signals which will obscure regions of the spectrum. These 'residual solvent peaks' should not overlap with signals from the sample.

3. **Temperature dependence**: For experiments above or below room temperature the solvent's melting and boiling points are also important factors. Furthermore the solubility of the sample is likely to vary with temperature.

4. **Viscosity**: The lower the solvent viscosity, the better the resolution of the experiment.

5. **Cost**: Clearly for routine NMR where many samples need to be measured, the solvent's cost is an important consideration. As a rule of thumb, the price increases with the number of deuterated atoms.

6. **Water content**: Almost all NMR solvents contain water traces. Also many are hygroscopic (they absorb water from the atmosphere) and hence the longer they are stored the more water they contain. The presence of a water (HDO) peak will only serve to degrade the quality of NMR spectra. The water level in the solvent can be greatly reduced by filtration through a drying agent or by storing the solvent by adding molecular sieves.

The choice of solvent for a particular sample will be the best compromise between the various advantages and disadvantages of each. Surge the web for precise details of specific solvents.

7.2   Sample Tube

When the sample is being analyzed it may be rotated, depending on the type of probe or experiment. **Spinning** the sample has the effect of cancelling out field inhomogeneities in the X and Y direction and consequently improves the spectral resolution. A disadvantage of spinning is that it may lead to the presence of **spinning sidebands**. These are spurious signals (i.e. peaks) that result from the modulation of the magnetic field at the spinning frequency. The peaks always appear on either side of any large genuine peak at a separation equal to the spinning rate. The intensity of these sidebands will be proportional to the intensity of the genuine peak. Thus if the spin rate is 20 revolutions/second (= 20 Hz), you would look for spinning side bands at frequencies 20 Hz above and below the resonance frequencies of genuine signals.
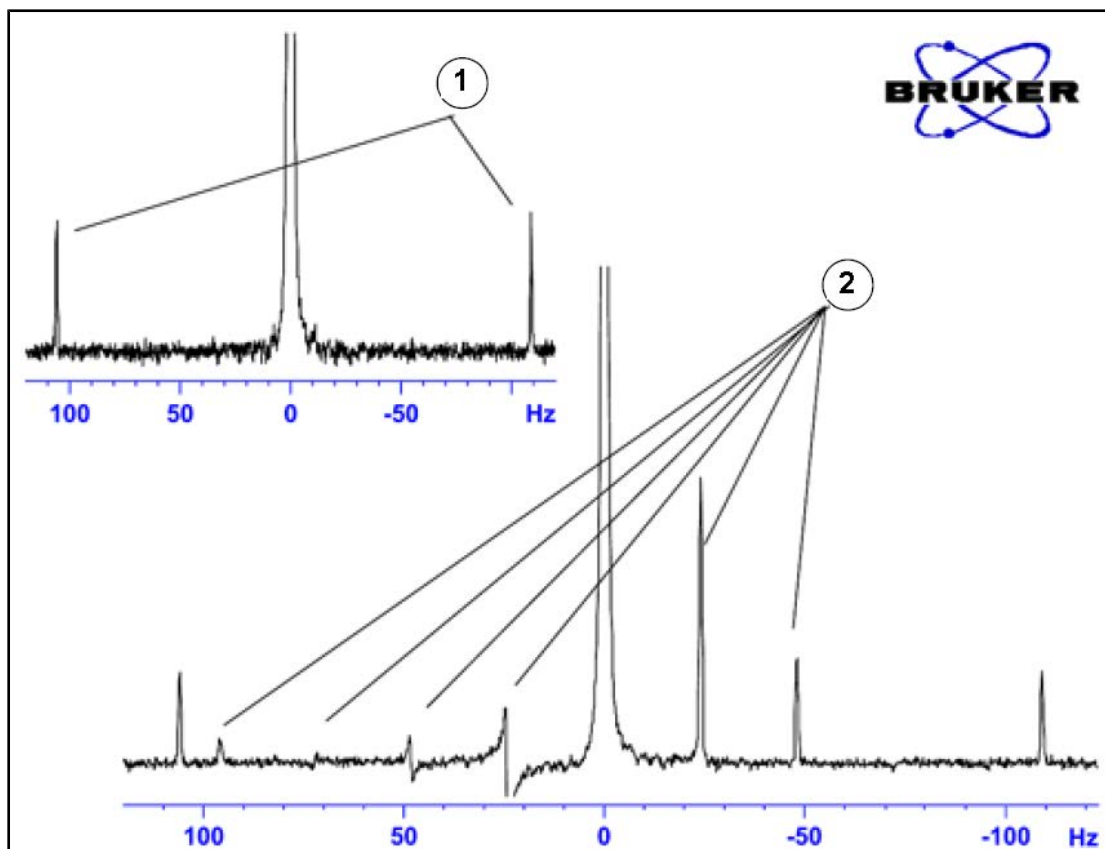
Figure 7.1: Spectrum Showing Spinning Sidebands

1.	13C Satellites	2.	Spinning Sidebands

While the presence of spinning sidebands may be inevitable, their size often depends on the sample tube quality. Ideally, the sample tube should have perfect cylindrical symmetry. Unusually large sidebands might suggest that the **tube symmetry** is inadequate and might warrant using tubes with higher specifications (and of course greater cost).

Sample tubes must always be kept clean and free from dust and scratches. Do not scrub the tubes with test tube brushes. Be aware that new NMR tubes cannot be assumed to be clean. The tubes may be cleaned by washing in Acetone or distilled water. Liquid detergent may be used as long as it is rinsed out within a few minutes to prevent etching of the tube. The tubes may also be cleaned ultrasonically in an appropriate solution. If all the above measures fail, the tubes should be soaked in AQUA REGIA for up to two days and then

rinsed thoroughly before being dried. NMR tubes can be oven dried but should not be heated above 100°C as they can become distorted and subsequently fail to spin as required. Drying is best achieved by passing filtered nitrogen through the tube.

7.3   Sample Handling

It is good practice to filter NMR solutions directly into the sample tube to keep the solution free from dust and other contamination.

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Note: The sample tube should always be held by the top!

Typical procedures to prepare a sample might be as follows:

1. For a solid sample using a 5 mm tube dissolve up to 20 mg of the sample in about 0.6 cm3 of the chosen solvent (for 10 mm tubes dissolve 80 mg in 2.5 cm^3). Typically for a liquid sample, and when observing protons, dissolve 20% sample in 80% deuterated solvent.

2. Add a small amount (~0.1%) of reference compound Tetramethylsilane (TMS). Make sure the TMS signal is smaller than the most intense sample or solvent signal (otherwise the signal-to-noise ratio is wasted because of low receiver gain).

3. Filter the solution into the sample tube through a Pasteur pipette containing a small plug of Kimwipe.

4. Filter 0.2 cm^3 of solvent through the filter into the tube. The resulting solution should have a depth of three to four centimeters.

5. Close the tube with a cap, seal the top with parafilm to reduce evaporation and label the tube near the top. Be careful to ensure that the cap, parafilm and label are concentric or otherwise they will adversely affect sample spinning.

i

Note: Some problems may occur when using glass fiber for filtering the sample, especially when you want to measure T_1.

8 Proton Spectrum

This chapter describes the acquisition and processing of a one-dimensional 1H NMR spectrum using the standard Bruker parameter set **PROTON**. The pulse sequence **zg30** (see figure below) consists of the recycling delay, the radio-frequency (RF) pulse, and the acquisition time during which the signal is recorded. The pulse angle is shown to be 30^0. The two parameters, **d1** and **p1**, correspond to the length of the recycle delay and the length of the 90^0 RF pulse, respectively.
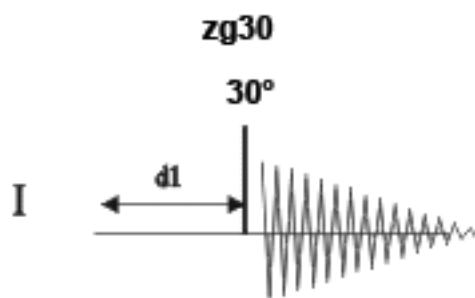
The time intervals depicted in the pulse sequence diagrams are not drawn to scale. For example, d1 is typically a few seconds while p1 is typically a few microseconds in length.

The sample to be used is **30 mg of Menthyl Antranilate in DMSO-d6**.
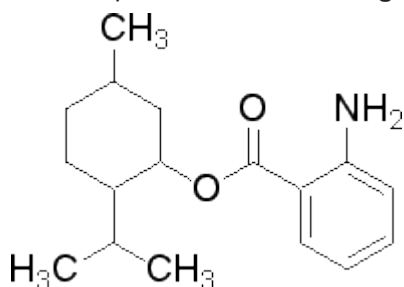

8.1 Obtaining the Proton Spectrum

The principal steps are described in the **Basic Procedures** chapter and are as follows:

1. Create a new data set.
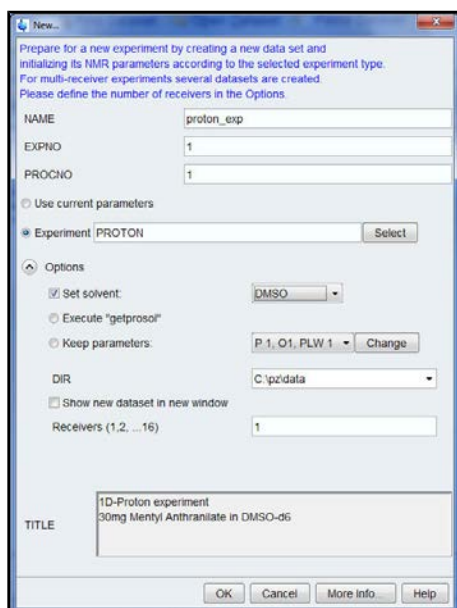
Note: The directory (DIR) is specific to how the data are stored and therefore may show different entries as the one in the figure above. Click on the down arrow button to browse for a specific directory.

2. Sample preparation.

3. Insert the sample plus spinner into the magnet.
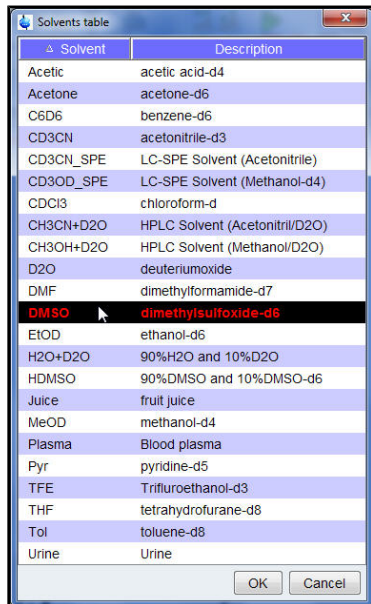
4. Locking the sample.

Note: Select **DMSO** from the solvents table by clicking on it.

5. Tuning and matching the probe.

6. Spinning the sample.

7. Shimming.

8. Setting the probe/solvent dependent parameters.

9. Adjust the receiver gain.

10. Starting the acquisition.

11. Processing the data.
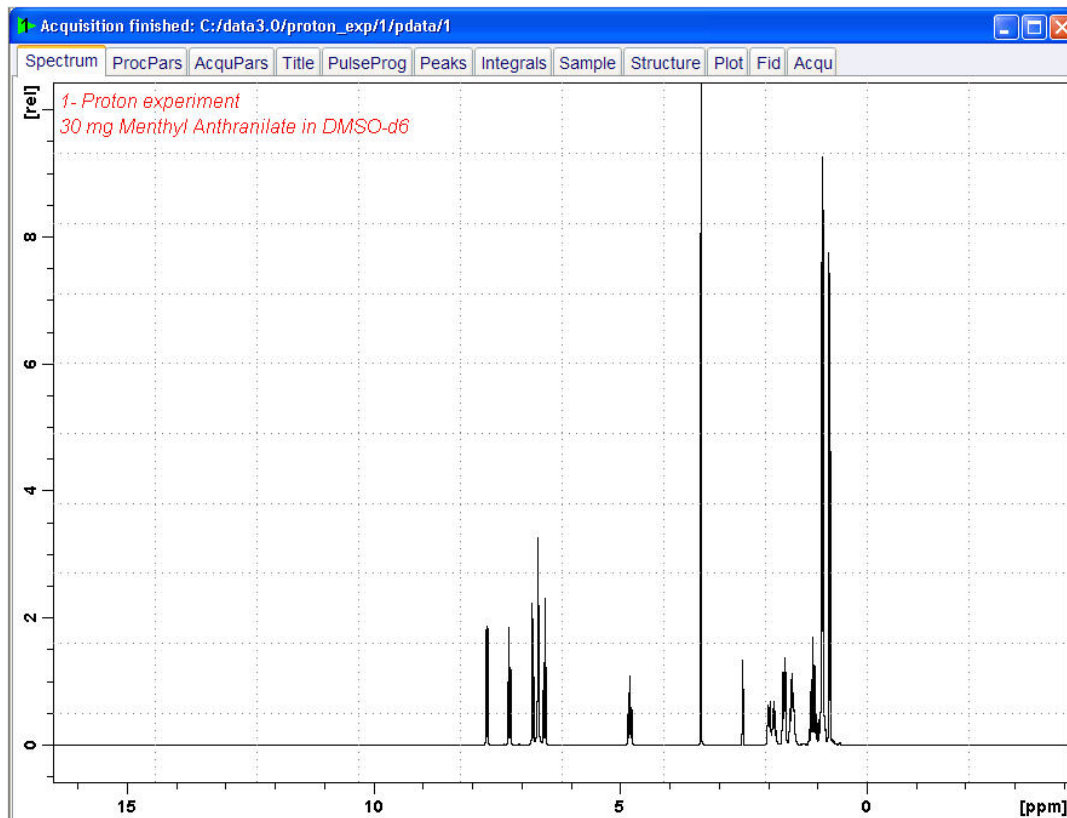
8.1.1 Optimizing the Sweep Width

Optimizing the sweep width will achieve a better resolution and separation of the peaks in the Proton spectrum.

1. Click on the **ppm (Exact zoom)** Icon in the **Tool bar**.
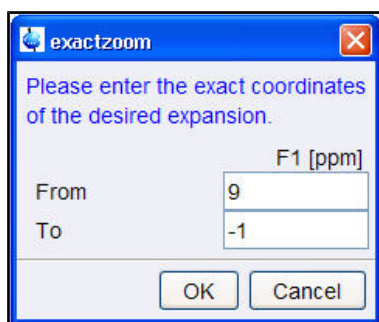
2. Enter the following F1 [ppm] values:

From = **9**

To = **-1**

3. Click on the **OK** button inside the "Exactzoom" window.
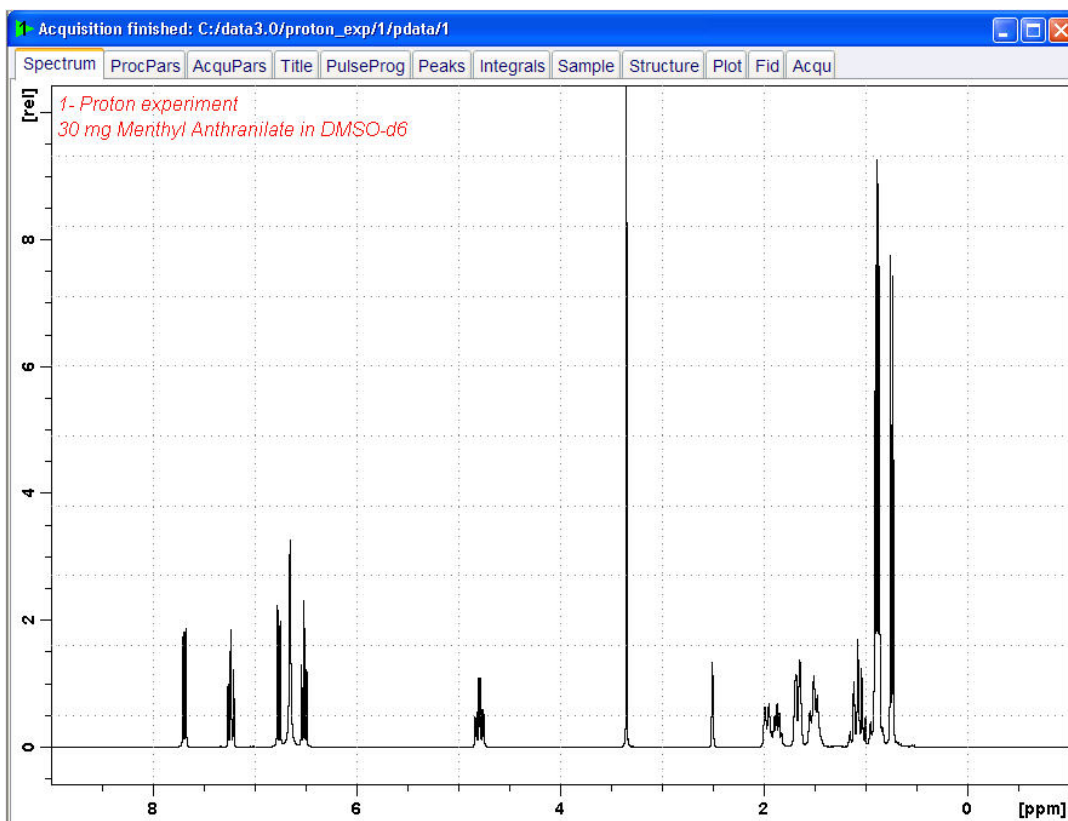
4. Click on the **Set SW to current region, and SFO1 to center of region** icon in the **Tool bar**.
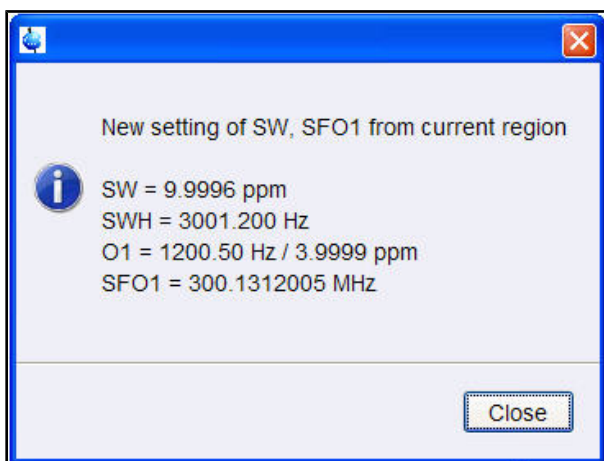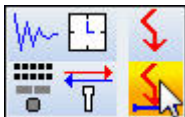
5. Click on the **Close** button inside the "New setting of SW" window.

6. Click on the **Start acquisition** icon in the Tool bar.

7. Click on the **Process** tab in the Workflow tab bar.

8. Select the **Proc. Spectrum** button in the Workflow button bar by clicking on it.

> **i**
>
> **Note**: This executes the saved processing parameters of the proc1d window.

8.2   Integration

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

1. Expand the spectrum to include all peaks.
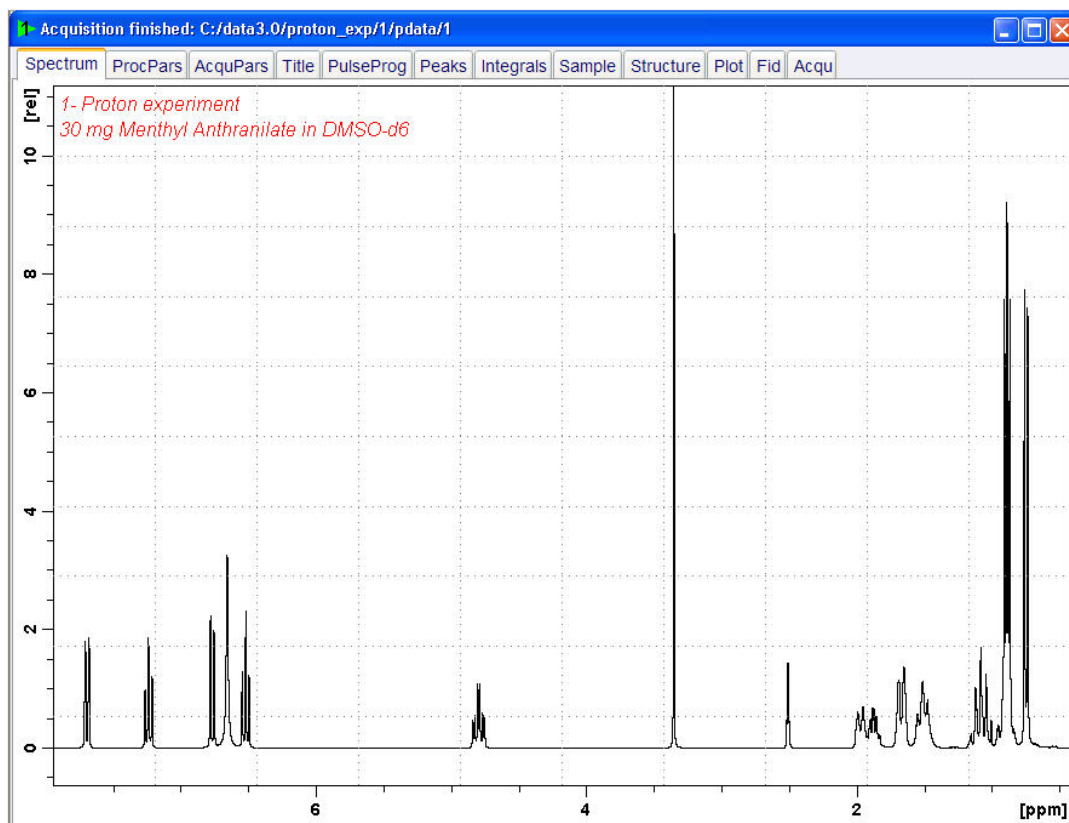
2. Click on the **Integrate** button in the Workflow button bar.

> **i**
>
> **Note**: This enters the manual Integration mode. Other options are available by clicking on the down arrow inside the 'Integrate' button.

The **Data Set tabs** are replaced by the **Integration Tool** bar:

3. Set the cursor line, starting at the left of the spectrum, to the left of the first peak to be integrated, click the left mouse button and drag the cursor line to the right of the peak, then release the mouse button.
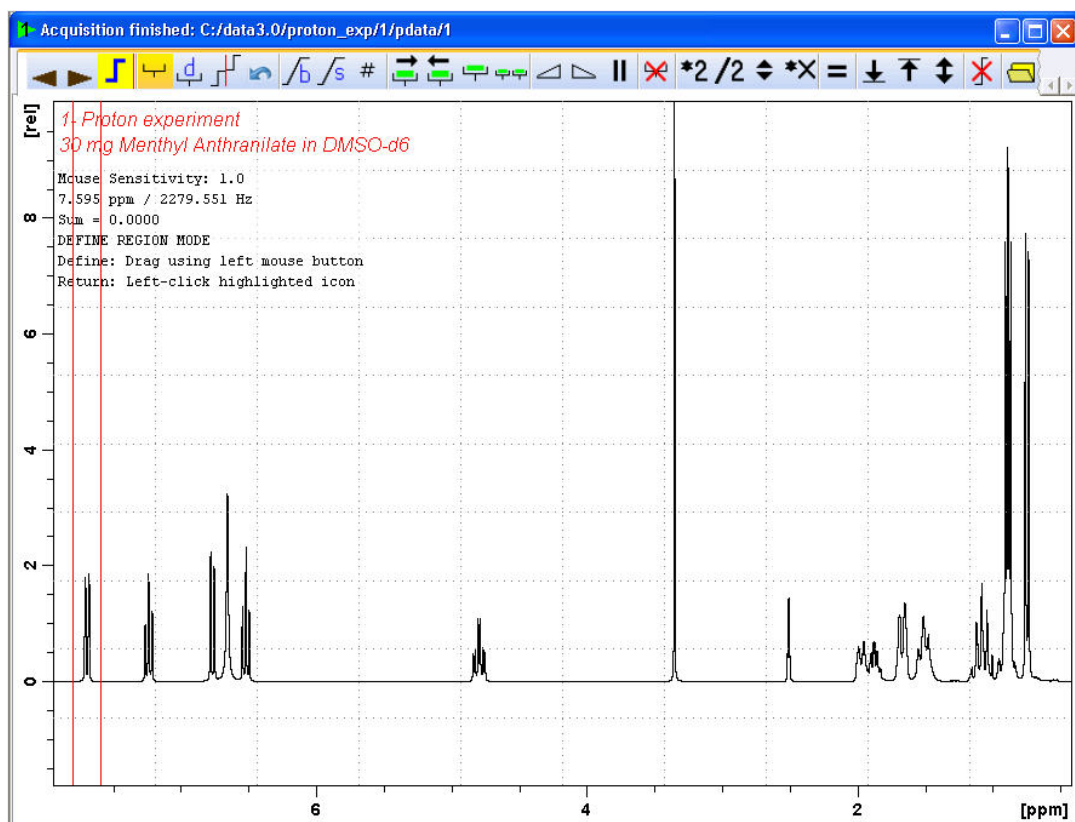
4. Repeat step 3 for the remainder of the peaks.
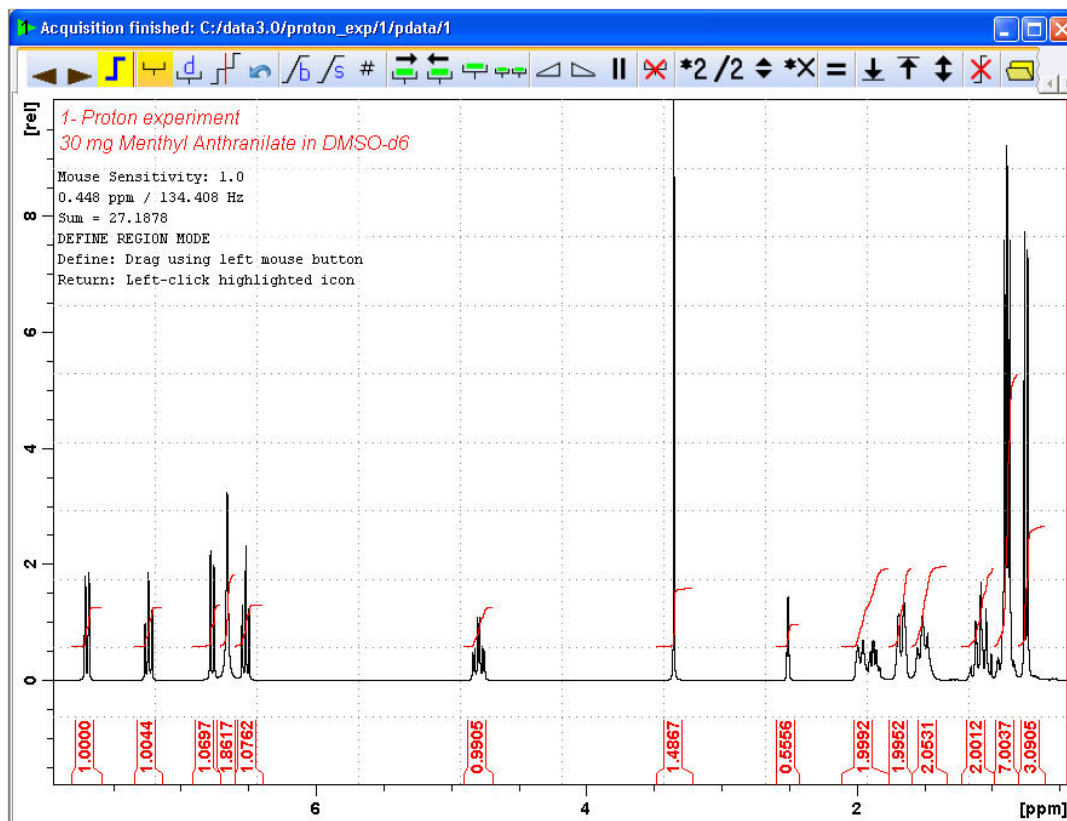
5. Click on the **Return, save region** icon in the Integration Tool bar.

8.3   Plotting the Proton Spectrum

1. Expand the spectrum to include all peaks.

2. Click on the **Retain expansion and scale** icon in the Tool bar.

3. Click on the **Publish** tab in the Workflow tab bar.

4. Select the **Plot Layout** button in the Workflow button bar by clicking on it.

> **i** **Note**: If desired, changes can be administered by using the tools on the left side of the display.
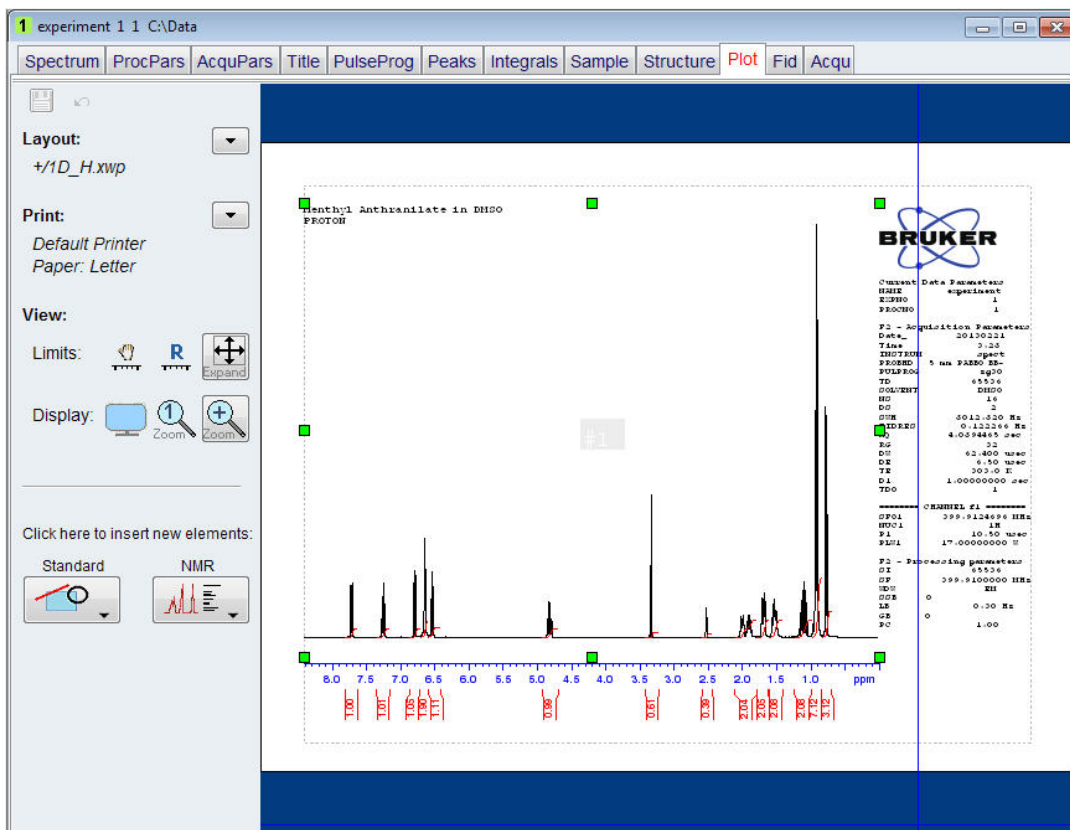
5. Click on the **down arrow** in the **Print** section of the Plot Layout window.
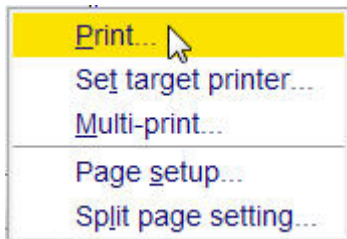
6. Select **Print** by clicking on it.

9 13C Spectrum with Proton Decoupling

This chapter describes the acquisition and processing of a one-dimensional 13C NMR spectrum. The standard Bruker parameter set **C13CPD**, includes the pulse sequence **zgpg30**, shown in the figure below. The 13C channel consists of the recycling delay, a RF pulse, and the acquisition time during which the signal is recorded. The pulse angle is shown to be 30°. The two parameters, **d1** and **p1**, correspond to the length of the recycle delay, and the length of the 90° RF pulse, respectively. The 1H channel consists of two decoupling pulses which can be power gated. The first pulse, an NOE build-up pulse during the recycle delay may be of lower power then the second pulse during the acquisition which is the true decoupling pulse. This can be useful to avoid RF heating on salty samples or probes where a higher decoupling power can be problematic.
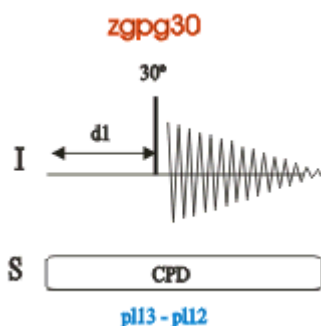
The time intervals depicted in the pulse sequence diagrams are not drawn to scale. For example, d1 is typically a few seconds, while p1 is typically a few microseconds in length.

The sample to be used is **30 mg of Menthyl Antranilate in DMSO-d6**.
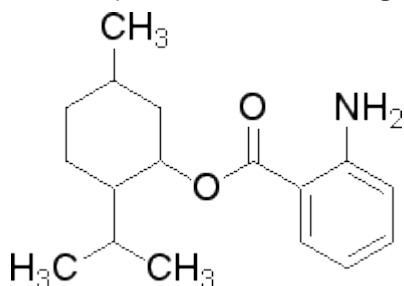
9.1 Obtaining the 13C Spectrum

The principal steps are described in the **Basic Procedures** chapter and are as follows:

1. Creating a new data set.
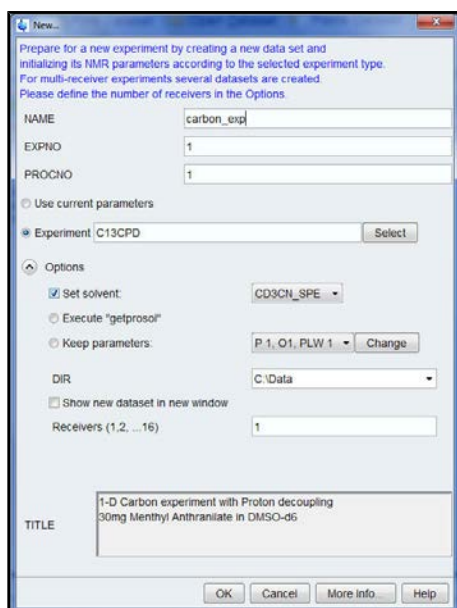
Note: The directory (DIR) is specific to how the data are stored and therefore may show different entries as the one in the figure above. Click on the down arrow button to browse for a specific directory.

2. Sample preparation.

3. Inserting the sample plus spinner into the magnet.
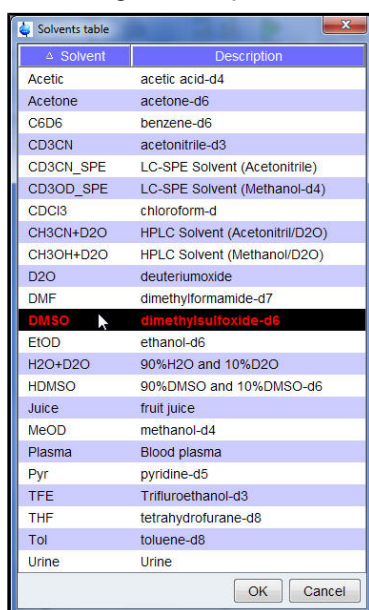
4. Locking the sample.

Note: Select **DMSO** from the solvents table by clicking on it.

5. Tuning and matching the probe.

6. Spinning the sample.

7. Shimming.

8. Setting the probe/solvent dependent parameters.

9. Adjust the receiver gain.

10. Starting the acquisition.

11. Processing the data.
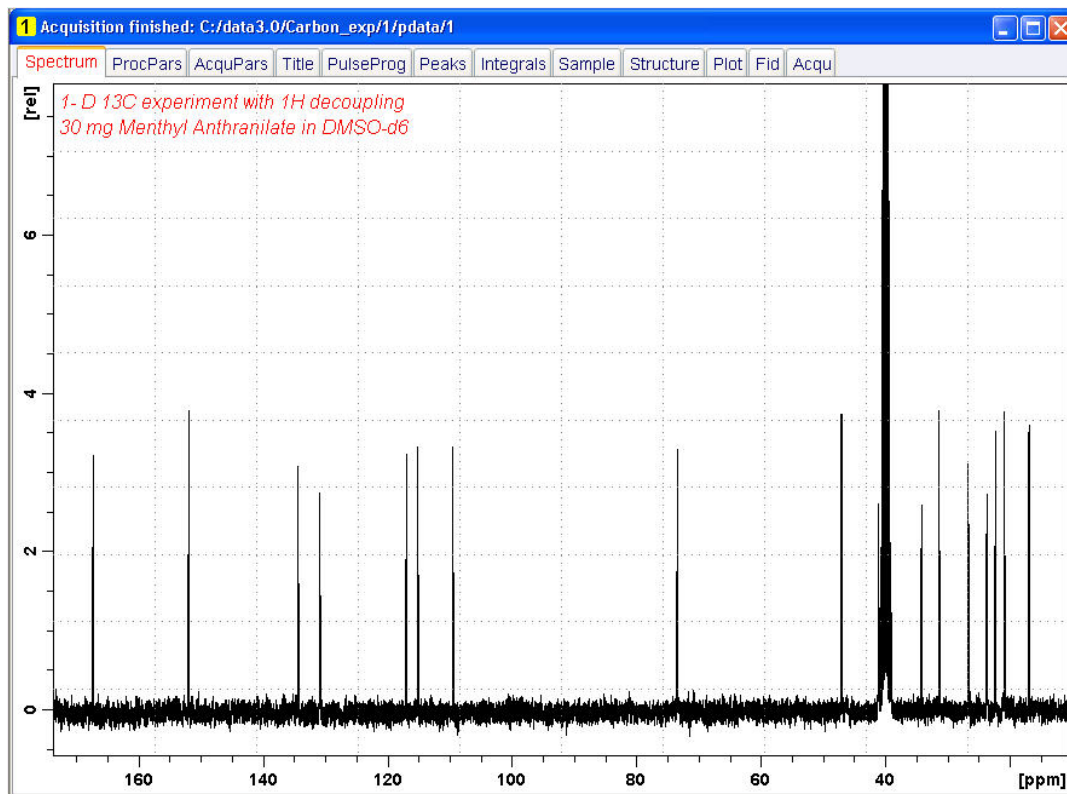
See also

⊟ Basic Procedures [▶ 33]

9.2 Peak Picking

To analyze an observed carbon spectrum, the chemical shift values of the peaks are compared within each other. It is common to perform a peak picking on a proton decoupled carbon spectrum to account for the various carbons in the analyzed molecule.

1. Expand the spectrum to include all peaks.

2. Click on the **Pick Peaks** button in the Workflow button bar.
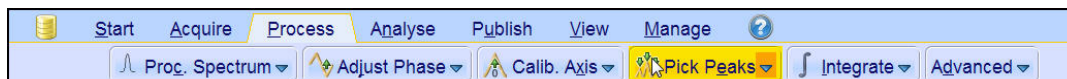
Note: This enters the manual peak picking mode. Other options are available by clicking on the down arrow inside the **Pick Peaks** button.

The **Data Set tabs** are replaced by the **Peak Picking Tool** bar:
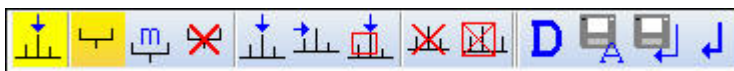
3. Start in the left upper corner inside the spectrum window and click the left mouse button. Drag the cursor line from left to the right side of the spectrum to draw a box, including all peaks.
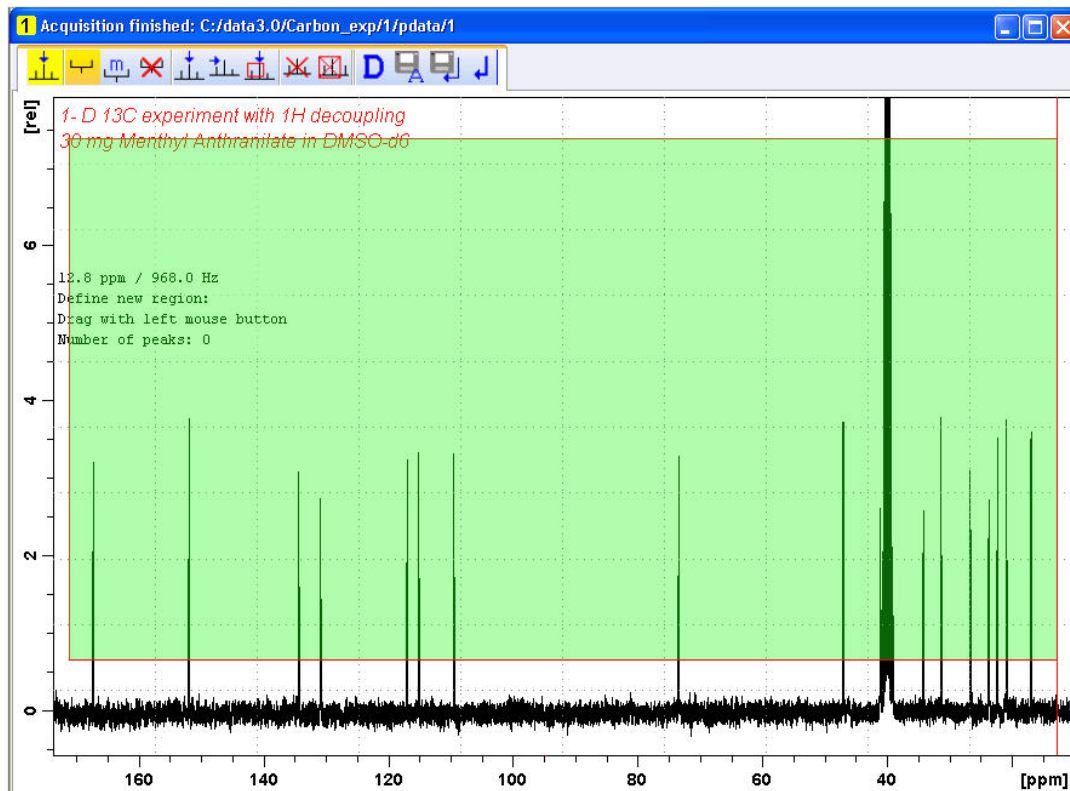
4. Click on the **Modify existing peak picking range** icon in the Peak Picking Tool bar.
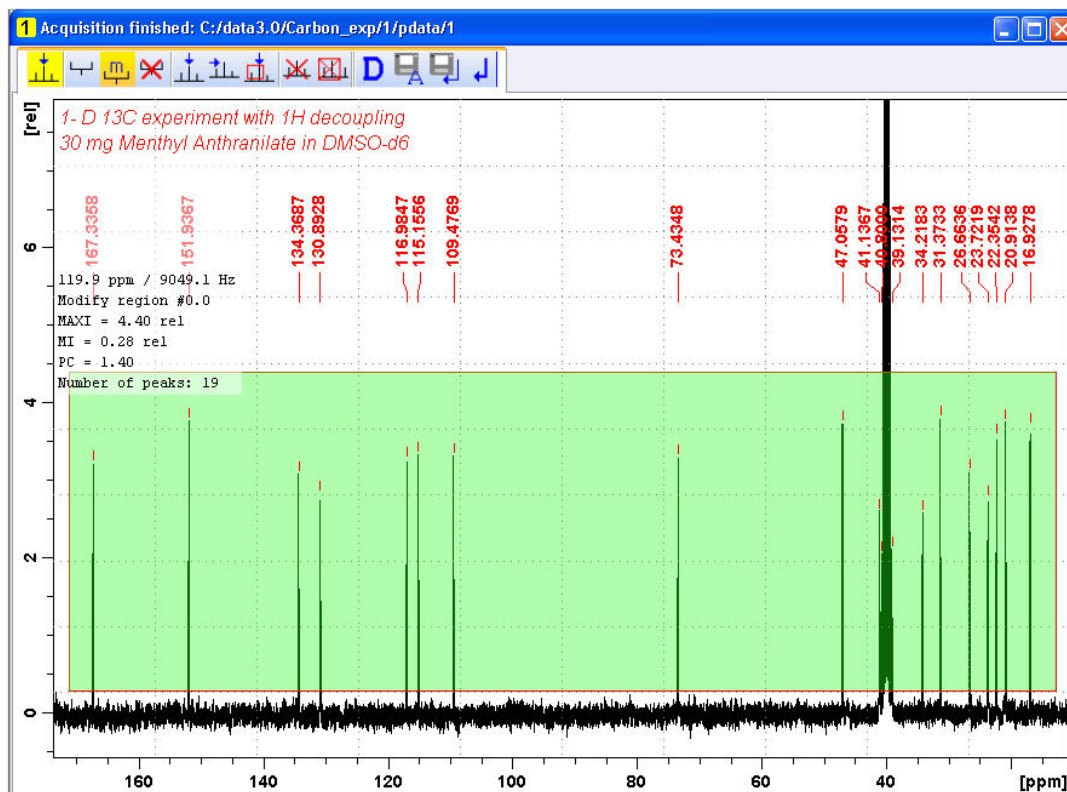
5. Click on the bottom line of the region box with the left mouse button and drag the line above the noise level, to set the minimum peak picking level.

6. Click on the top line of the region box with the left mouse button and drag the line below unwanted peaks e.g. solvent peaks, to set the maximum peak picking level.

7. Click on the **Return, save region** icon in the Peak Picking Tool bar.
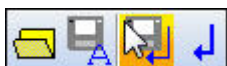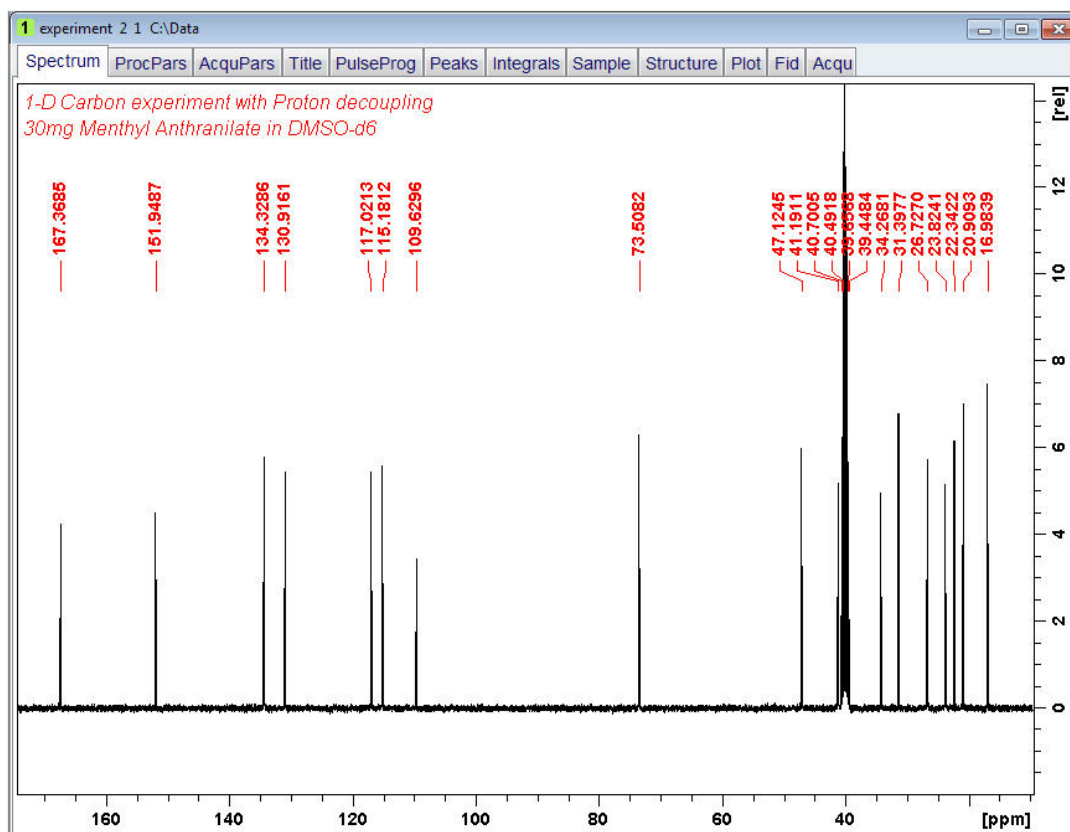
9.3   Plotting the 13C Spectrum

1. Expand the spectrum to include all peaks.

2. Click on the **Retain expansion and scale** icon in the Tool bar.

3. Click on the **Publish** tab in the Workflow tab bar.

4. Select the **Plot Layout** button in the Workflow button bar by clicking on it.
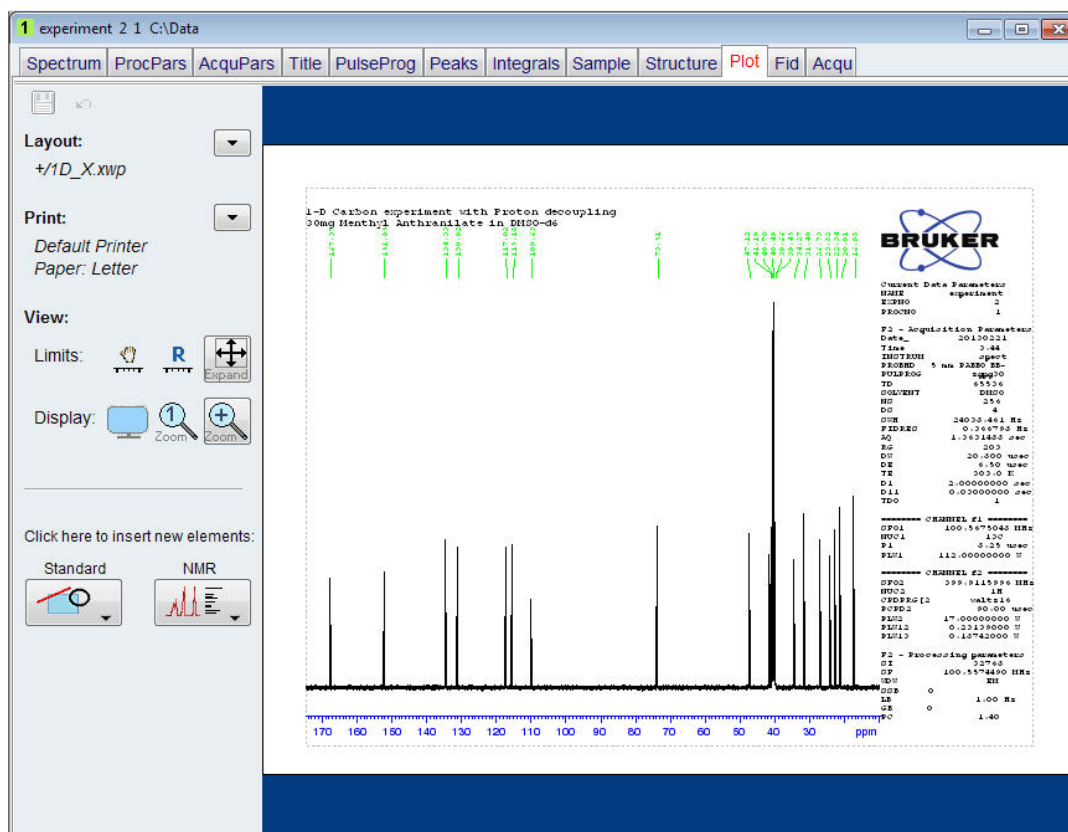
Note: If desired, any changes can be administered by using the tools on the left side of the display.

5. Click on the **down arrow** in the **Print** section of the Plot Layout window.
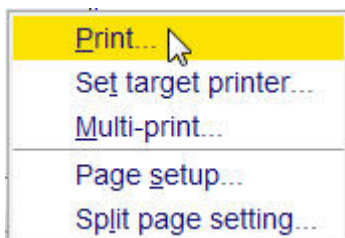
6. Select Print by clicking on it.

10 Basic Troubleshooting

For a beginner's guide it is unfeasible to describe any advanced level of troubleshooting. However, all users should at least be able to execute a complete power on and off of the system.

In case of a computer problem, this should also be known because the PC controls the DHCP server (which is responsible for the network addresses and most of the boards) and the Diskless Operating System for IPSO.

> **i** **Note**: The system manager should have shown all instrument users how to execute a complete power on and off of the system.
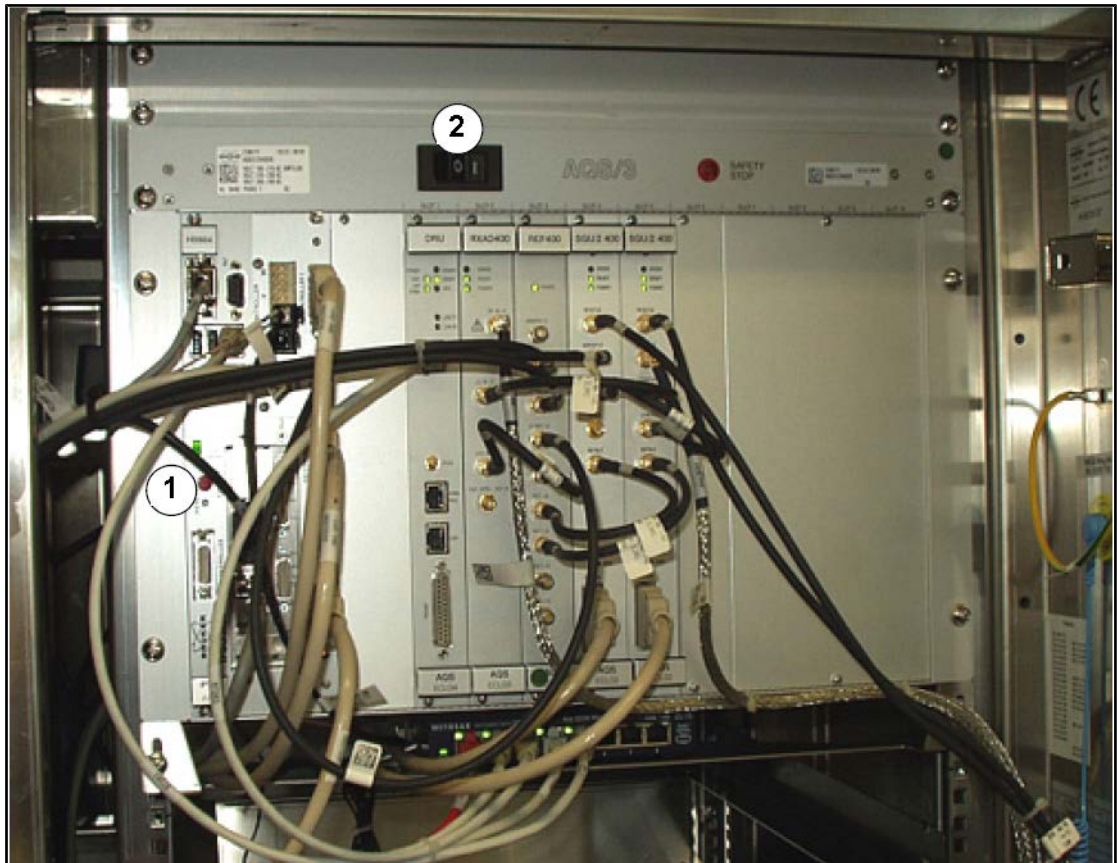
Figure 10.1: Location of the Mains Switches for AQS and IPSO

1.	IPSO mains switch	2.	AQS mains switch
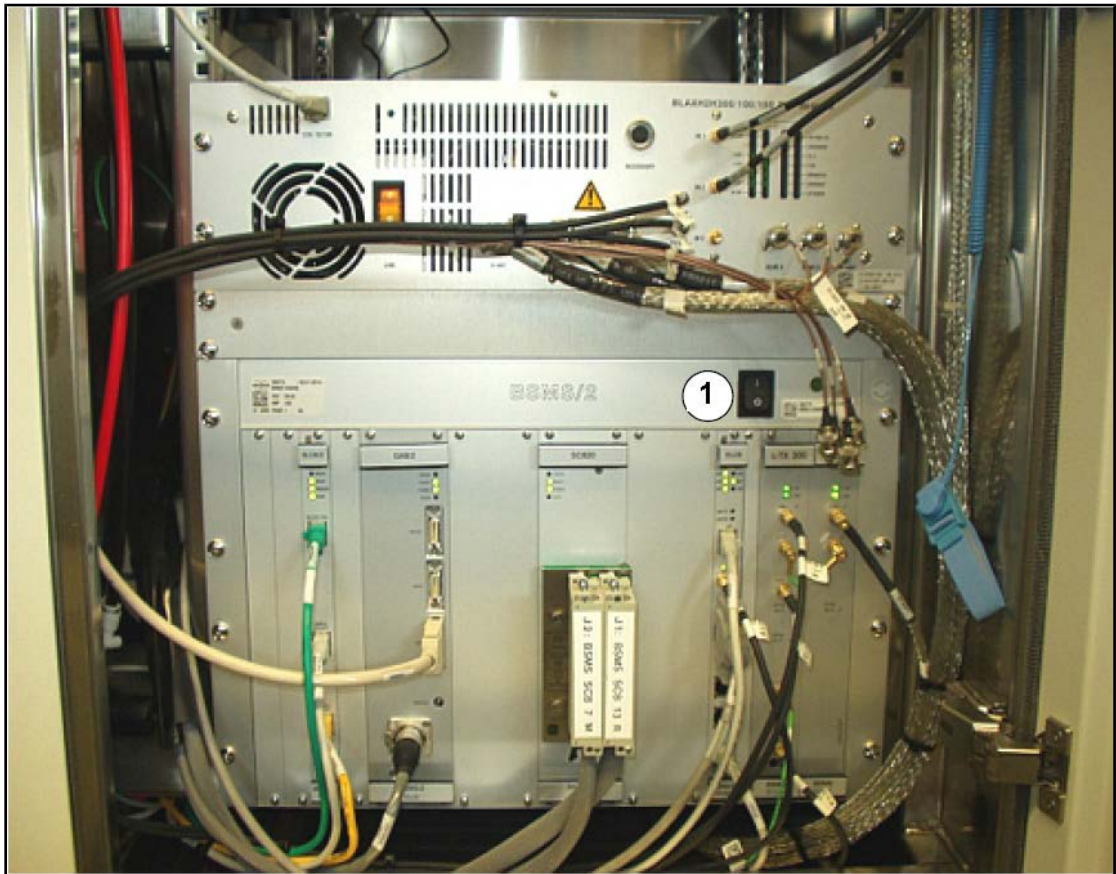
Figure 10.2: Position of the BSMS Main Switch

1.	BSMS mains switch		

11 Contact

Manufacturer:

Bruker BioSpin NMR
am Silberstreifen

D-76287 Rheinstetten

Germany

Phone: +49 721-5161-0

http://www.bruker-biospin.com

NMR Hotlines

Contact our NMR service centers. Bruker BioSpin NMR provide dedicated hotlines and service centers, so that our specialists can respond as quickly as possible to all your service requests, applications questions, software or technical needs. Please select the NMR service center or hotline you wish to contact from our list available at:

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Glossary

BSMS

The Bruker Smart Magnet control System (BSMS) provides computer control of various functions associated with the magnet, magnetic field, and sample.

chemical shift

The variation in precise resonance frequency.

deuterated solvent

A deuterated solvent deuterated solvent is one in which a large percentage of the hydrogen atoms have been replaced by deuterium. Commonly used deuterated solvents are acetone-d6, benzene-d6, chloroform-d and DMSO-d6, although many other solvents are available.

HPPR

High Performance Preamplifier

IPSO

Intelligent Pulse Sequence Organizer

ppm

parts per million

VTU

Variable Temperature Unit

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