1

Getting Started

1.1 The Technique

This book is not really intended to give an in-depth education in all aspects of the NMR effect (there are numerous excellent texts if you want more information) but we will try to deal with some of the more pertinent ones.

The first thing to understand about NMR is just how insensitive it is compared with many other analytical techniques. This is because of the origin of the NMR signal itself.

The NMR signal arises from a quantum mechanical property of nuclei called 'spin'. In the text here, we will use the example of the hydrogen nucleus (proton) as this is the nucleus that we will be dealing with mostly. Protons have a 'spin quantum number' of $^1/_2$. In this case, when they are placed in a magnetic field, there are two possible spin states that the nucleus can adopt and there is an energy difference between them (Figure 1.1).

The energy difference between these levels is very small, which means that the population difference is also small. The NMR signal arises from this population difference and hence the signal is also very small. There are several factors which influence the population difference and these include the nature of the nucleus (its 'gyromagnetic ratio') and the strength of the magnetic field that they are placed in. The equation that relates these factors (and the only one in this book) is shown here:

$$\Delta E = \frac{\gamma h B}{2\pi}$$

γ = Gyromagnetic ratio
h = Planck's constant
B = Magnetic field strength

Because the sensitivity of the technique goes up with magnetic field, there has been a drive to increase the strength of the magnets to improve sensitivity.

Unfortunately, this improvement has been linear since the first NMR magnets (with a few kinks here and there). This means that in percentage terms, the benefits have become smaller as development has continued. But sensitivity has not been the only factor driving the search for more powerful magnets. You also benefit from stretching your spectrum and reducing overlap of signals when you go to higher fields. Also, when you examine all the factors involved in signal to noise, the dependence on field is to

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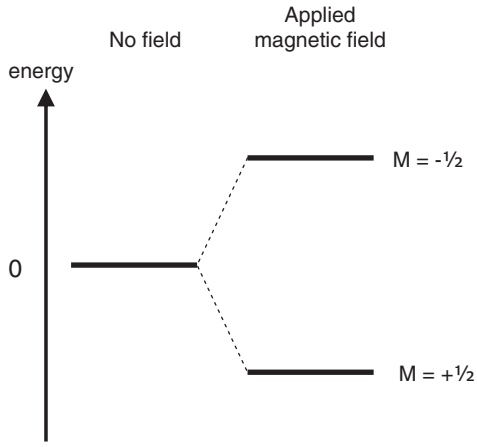


Figure 1.1 Energy levels of spin $^1/_2$ nucleus.

the power of 3/2 so we actually gain more signal than a linear relationship. Even so, moving from 800 to 900 MHz only gets you a 20 % increase in signal to noise whereas the cost difference is about 300 %.

In order to get a signal from a nucleus, we have to change the populations of each spin state. We do this by using radio frequency at the correct frequency to excite the nuclei into their higher energy state. We can then either monitor the absorption of the energy that we are putting in or monitor the energy coming out when nuclei return to their low energy state.

The strength of the NMR magnet is normally described by the frequency at which protons resonate in it – the more powerful the magnet, the higher the frequency. The earliest commercial NMR instruments operated at 40 megacycles (in those days, now MHz) whereas modern NMR magnets are typically ten times as powerful and the most potent (and expensive!) machines available can operate at fields of 1 GHz.

1.2 Instrumentation

So far, we have shown where the signal comes from, but how do we measure it? There are two main technologies: continuous wave (CW) and pulsed Fourier transform (FT). CW is the technology used in older systems and is becoming hard to find these days. (We only include it for the sake of historical context and because it is perhaps the easier technology to explain). FT systems offer many advantages over CW and they are used for all high field instruments.

1.3 CW Systems

These systems work by placing a sample between the pole pieces of a magnet (electromagnet or permanent), surrounded by a coil of wire. Radio frequency (r.f.) is fed into the wire at a swept set of frequencies. Alternatively, the magnet may have extra coils built onto the pole pieces which can be used to sweep the field with a fixed r.f. When the combination of field and frequency match the resonant frequency of each nucleus r.f. is emitted and captured by a receiver coil perpendicular to the transmitter

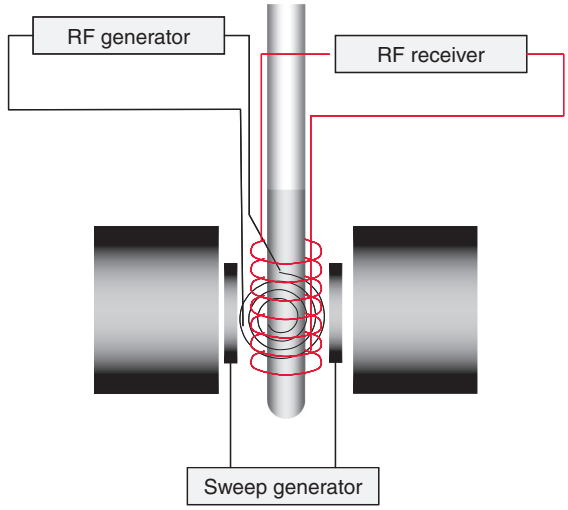


Figure 1.2 Schematic diagram of a CW NMR spectrometer.

coil. This emission is then plotted against frequency (Figure 1.2). The whole process of acquiring a spectrum using a CW instrument takes typically about 5 min. Each signal is brought to resonance sequentially and the process cannot be rushed!

1.4 FT Systems

Most spectrometers used for the work we do today are Fourier transform systems. More correctly, they are pulsed FT systems. Unlike CW systems, the sample is exposed to a powerful polychromatic pulse of radio frequency. This pulse is very short and so contains a spread of frequencies (this is basic Fourier theory and is covered in many other texts). The result is that all of the signals of interest are excited simultaneously (unlike CW where they are excited sequentially) and we can acquire the whole spectrum in one go. This gives us an advantage in that we can acquire a spectrum in a few seconds as opposed to several minutes with a CW instrument. Also, because we are storing all this data in a computer, we can perform the same experiment on the sample repeatedly and add the results together. The number of experiments is called the number of scans (or transients, depending on your spectrometer vendor). Because the signal is coherent and the noise is random, we improve our signal to noise with each transient that we add. Unfortunately, this is not a linear improvement because the noise also builds up albeit at a slower rate (due to its lack of coherence). The real signal to noise increase is proportional to the square root of the number of scans (more on this later).

So if the whole spectrum is acquired in one go, why can't we pulse really quickly and get thousands of transients? The answer is that we have to wait for the nuclei to lose their energy to the surroundings. This takes a finite time and for most protons is just a few seconds (under the conditions that we acquire the data). So, in reality we can acquire a new transient every three or four seconds.

After the pulse, we wait for a short whilst (typically a few microseconds), to let that powerful pulse ebb away, and then start to acquire the radio frequency signals emitted from the sample. This exhibits itself as a number of decaying cosine waves. We term this pattern the 'free induction decay' or FID (Figure 1.3).

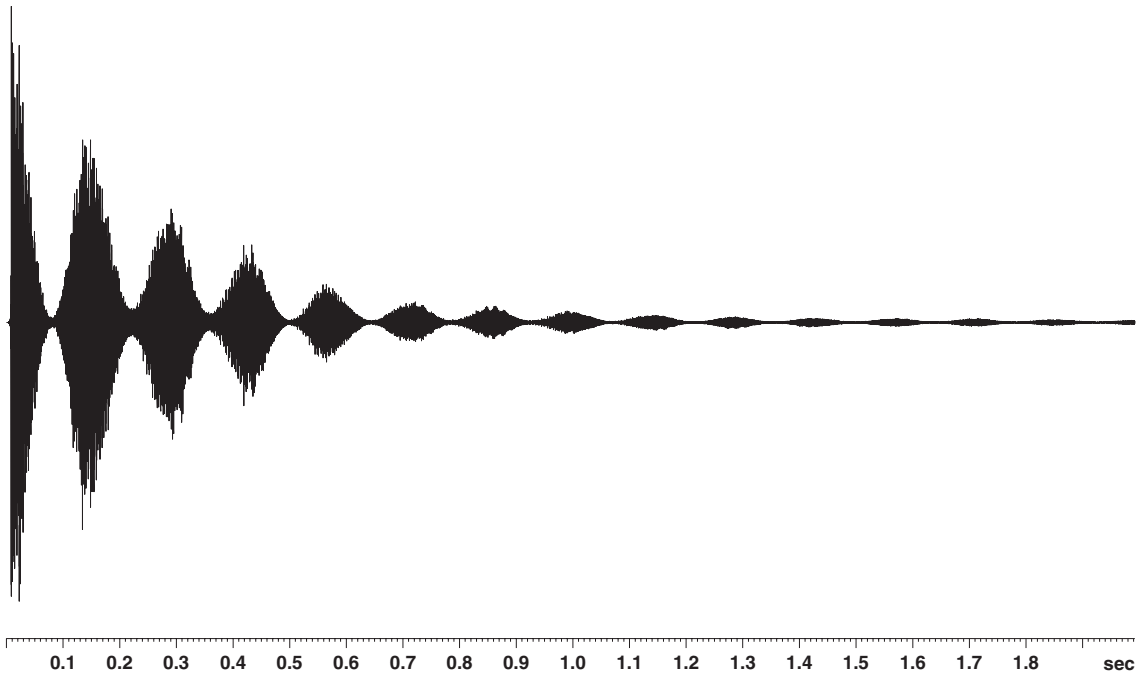


Figure 1.3 A free induction decay.

Obviously this is a little difficult to interpret, although with experience you can train yourself to extract all the frequencies by eye . . . (only kidding!) The FID is a 'time domain' display but what we really need is a 'frequency domain' display (with peaks rather than cosines). To bring about this magic, we make use of the work of Jean Baptiste Fourier (1768–1830) who was able to relate time-domain to frequency-domain data. These days, there are superfast algorithms to do this and it all happens in the background. It is worth knowing a little about this relationship as we will see later when we discuss some of the tricks that can be used to extract more information from the spectrum.

There are many other advantages with pulsed FT systems in that we can create trains of pulses to make the nuclei perform 'dances' which allow them to reveal more information about their environment. Ray Freeman coined the rather nice term 'spin choreography' to describe the design of pulse sequences. If you are interested in this area, you could do much worse than listen to Ray explain some of these concepts or read his book: *Spin Choreography Basic Steps in High Resolution NMR* (Oxford University Press, ISBN 0-19-850481-0)!

Because we now operate with much stronger magnets than in the old CW days, the way that we generate the magnetic field has changed. Permanent magnets are not strong enough for fields above 90 MHz and conventional electromagnets would consume far too much electricity to make them viable (they would also be huge in order to keep the coil resistance low and need cooling to combat the heating effect of the current flowing through the magnet coils). The advent of superconducting wire made higher fields possible.

(The discovery of superconduction was made at Leiden University, by Heike Kamerlingh Onnes back in 1911 whilst experimenting with the electrical resistance of mercury, cooled to liquid helium temperature. His efforts were recognised with the Nobel Prise for Physics in 1913 and much later, a

crater on the dark side of the moon was named after him. The phenomenon was to have a profound effect on the development of superconducting magnets for spectrometers years later when technologies were developed to exploit it.)

Superconducting wire has no resistance when it is cooled below a critical temperature. For the wire used in most NMR magnets, this critical temperature is slightly above the boiling point of liquid helium (which boils at just over 4 K or about –269 °C). (It should be noted that new superconducting materials are being investigated all the time. At the time of writing, some ceramic superconductors can become superconducting at close to liquid nitrogen temperatures although these can be tricky to make into coils.) When a superconducting magnet is energised, current is passed into the coil below its critical temperature. The current continues to flow undiminished, as long as the coil is kept below the critical temperature. To this end, the magnet coils are immersed in a Dewar of liquid helium. Because helium is expensive (believe it or not, it comes from holes in the ground) we try to minimise the amount that is lost through boil off, so the liquid helium Dewar is surrounded by a vacuum and then a liquid nitrogen Dewar (temperature –196 °C). A schematic diagram of a superconducting magnet is shown in Figure 1.4. Obviously, our sample can't be at –269 °C (it wouldn't be very liquid at that temperature) so there has to be very good insulation between the magnet coils and the sample measurement area.

In the centre (room temperature) part of the magnet we also need to get the radiofrequency coils and some of the tuning circuits close to the sample. These are normally housed in an aluminium cylinder with some electrical connectors and this is referred to as the 'probe'. The NMR tube containing the sample is lowered into the centre of the magnet using an air lift. The tube itself is long and thin (often 5 mm outside diameter) and designed to optimise the filling of the receive coil in the probe. We would call such a probe a '5 mm probe' (for obvious reasons!). It is also possible to get probes with different diameters and the choice of probe is made based on the typical sample requirements. At the time of writing, common probes go from 1 mm outside diameter (pretty thin!) to 10 mm although there are some other special sizes made.

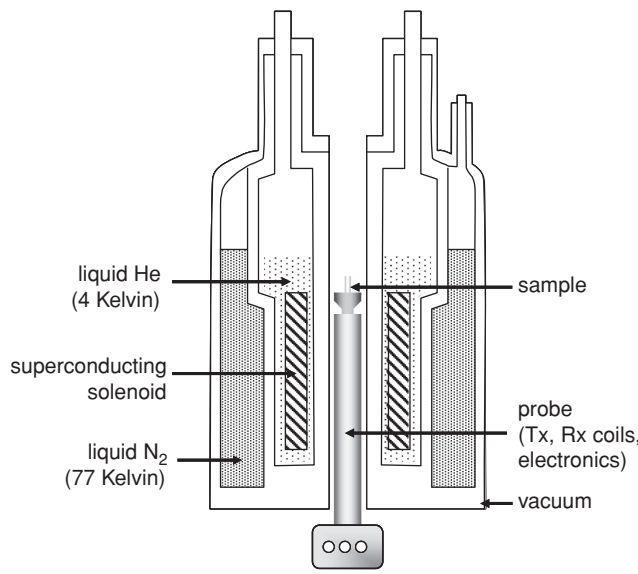


Figure 1.4 Schematic diagram of a superconducting NMR magnet.

Probes are designed to look at a specific nucleus or groups of nuclei. A simple probe would be a proton, carbon dual probe. This would have two sets of coils and tuning circuits, one for carbon the other for proton. Additionally there would be a third circuit to monitor deuterium. The reason for using a deuterium signal is that we can use this signal to 'lock' the spectrometer frequency so that any drift by the magnet will be compensated by monitoring the deuterium resonance (more on this later).

There is a vast array of probes available to do many specialist jobs but for the work that we will discuss in this book, a proton–carbon dual probe would perform most of the experiments (although having a four nucleus probe is better as this would allow other common nuclei such as fluorine or phosphorus to be observed).

The last thing to mention about probes is that they can have one of two geometries. They can be 'normal' geometry, in which case the nonproton nucleus coils would be closest to the sample or 'inverse' geometry (the inverse of normal!). We mention this because it will have an impact on the sensitivity of the probe for acquiring proton data (inverse is more sensitive than normal). Most of the time this shouldn't matter unless you are really stuck for sample in which case it is a bigger deal . . .

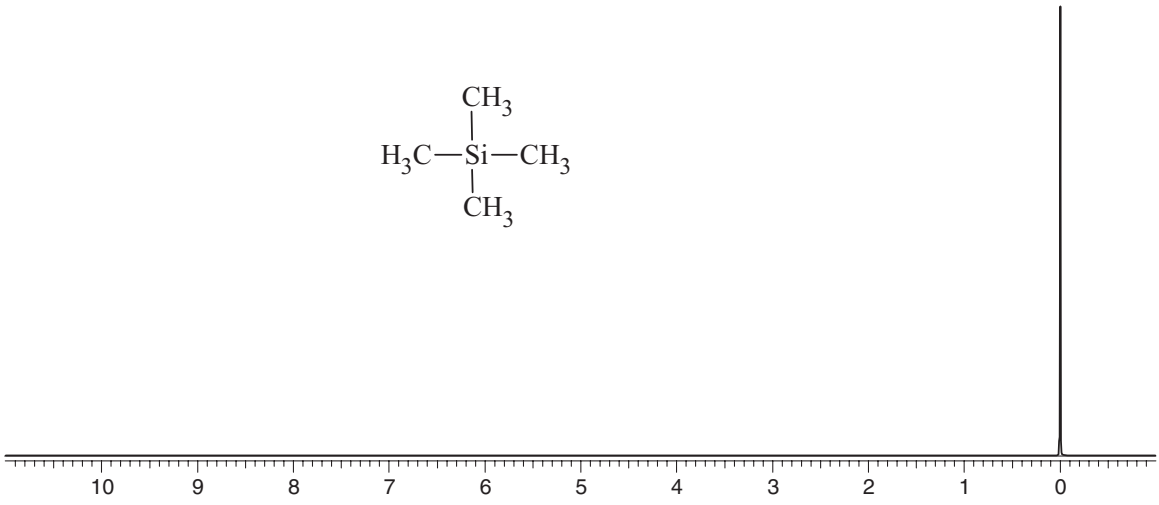
1.4.1 Origin of the Chemical Shift

Early NMR experiments were expected to show that a single nucleus would absorb radio frequency energy at a discrete frequency and give a single line. Experimenters were a little disconcerted to find instead, some 'fine structure' on the lines and when examined closely, in some cases, lots of lines spread over a frequency range. In the case of proton observation, this was due to the influence of surrounding nuclei shielding and deshielding the close nuclei from the magnetic field. The observation of this phenomenon gave rise to the term 'chemical shift', first observed by Fuchun Yu and Warren Proctor in 1950. There were some who thought this to be a nuisance but it turned out to be the effect that makes NMR such a powerful tool in solving structural problems.

There are many factors that influence the chemical shift of an NMR signal. Some are 'through bond' effects such as the electronegativity of the surrounding atoms. These are the most predictable effects and there are many software packages around which do a good job of making through bond chemical shift predictions. Other factors are 'through space' and these include electric and magnetic field effects. These are much harder things to predict as they are dependant on the average solution conformation of the molecule of interest.

In order to have a reliable measure of chemical shift, we need to have a reference for the value. In proton NMR this is normally referenced to tetramethyl silane (TMS) which is notionally given a chemical shift of zero. Spectrum 1.1 shows what a spectrum of TMS would look like.

You will notice that the spectrum runs 'backwards' compared with most techniques (i.e., '0' is at the right of the graph). This is because the silicon in TMS shields the protons from the magnetic field. Most other signals will come to the left of TMS. For some years, there was a debate about this and there were two different scales in operation. The scale shown here is the now accepted one and is called 'δ'. The older scale (which you may still encounter in old literature) is called 'τ' and it references TMS at 10, so you need a little mental agility to make the translation between the two scales. The scale itself is quoted in parts per million (ppm). It is actually a frequency scale, but if we quoted the frequency, the chemical shift would be dependant on the magnetic field (a 400 MHz spectrometer would give different chemical shifts to a 300 MHz spectrometer). To get around this, the chemical shift is quoted as a ratio compared with the main magnet field and is quoted in ppm.



Spectrum 1.1 Proton NMR spectrum of TMS.

Finally, we have an issue with how we describe relative chemical shifts. Traditionally (from CW NMR days) we describe them as 'upfield' (to lower delta) and 'downfield' (to higher delta). This is not strictly correct in a pulsed FT instrument (because the field remains static) but the terminology continues to be used. We still use these terms in this book as the alternatives are a bit cumbersome.

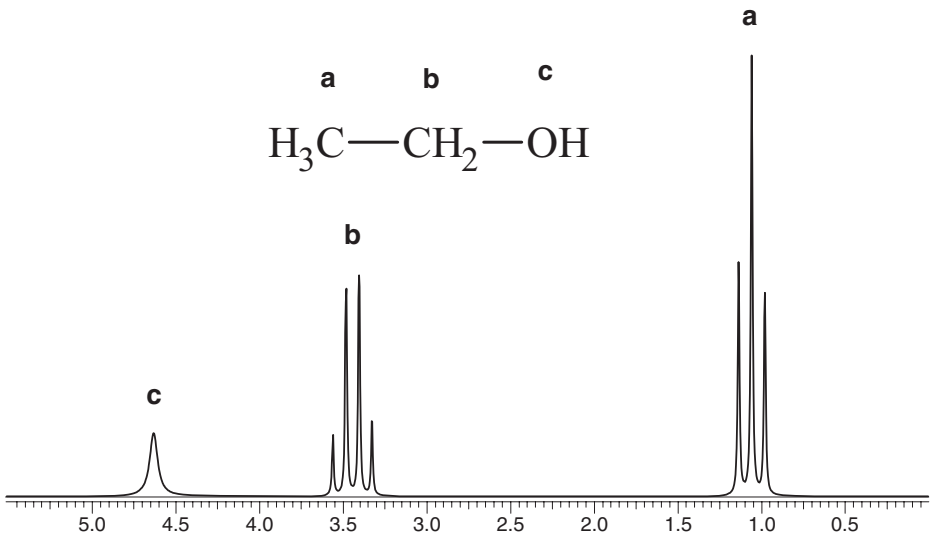
1.4.2 Origin of 'Splitting'

So far, we have seen where NMR signals come from, and touched on why different groups of protons have different chemical shifts. In addition to the dispersion of lines due to chemical shift, if you look closely, the individual lines may be split further. If we take the example of ethanol, this becomes obvious (Spectrum 1.2). We now have to understand why some signals appear as multiple lines rather than just singlets. Protons that are chemically and magnetically distinct from each other interact magnetically if they are close enough to do so by the process known as 'spin–spin coupling'. 'Close enough' in this context means 'separated by two, three, or occasionally four bonds.' Let us consider an isolated ethyl group such as found in ethanol. (We will assume no coupling from the -OH proton for the moment).

On examining Spectrum 1.2, you will notice that the -CH_2- protons appear as a 4-line quartet, whilst the -CH_3 protons give a 3-line triplet. Furthermore, the relative intensities of the lines of the quartet are in the ratio, 1:3:3:1, whilst the triplet lines are in the ratio 1:2:1.

We'll consider the methyl triplet first. Whilst the signal is undergoing irradiation, the methylene protons are, of course, aligned either with, or against the external magnetic field as discussed earlier. Note that as far as spin-spin coupling is concerned, we may consider the two states to be equally populated. If we call the methylene protons H_A and H_B, then at any time, H_A and H_B may be aligned with the external magnetic field, or against it. Alternatively, H_A may be aligned with the field, whilst H_B is aligned against it, or vice versa, the two arrangements being identical as far as the methyl protons are concerned.

So the methyl protons experience different magnetic fields depending on the orientation of the methylene protons. The statistical probability of one proton being aligned with and one against the magnetic field is twice as great as the probability of both being aligned either with, or against the field.



Spectrum 1.2 90 MHz proton spectrum of ethanol.

This explains why the relative intensity of the methyl lines is 1:2:1. Spin–spin coupling is always a reciprocal process – if protons '*x*' couple to protons '*y*', then protons '*y*' must couple to '*x*'. The possible alignments of the methyl protons (which we will call H_C, H_D and H_E) relative to the methylene protons are also shown in Spectrum 1.2. Think about the orientations of protons responsible for multiplet systems as we meet them later on.

There are two other important consequences of spin–spin coupling. First, *n* equivalent protons will split another signal into $n + 1$ lines (hence three methyl protons split a methylene CH_2 into $3 + 1 = 4$ lines). Second, the relative sizes of peaks of a coupled multiplet can be calculated from Pascal's triangle (Figure 1.5).

We have often found that students have a touching but misplaced faith in Mr. Pascal and his triangle and this can lead to no end of angst and confusion! It is very important to note that you will only come across this symmetrical distribution of intensities within a multiplet when the signals coupling

Splitting pattern	Number of adjacent protons	*Description*
1	0	*singlet*
1 1	1	*doublet*
1 2 1	2	*triplet*
1 3 3 1	3	*quartet*
1 4 6 4 1	4	*quintet*
1 5 10 10 5 1	5	*sextet*
1 6 15 20 15 6 1	6	*septet*

Figure 1.5 Using Pascal's triangle to calculate relative peak sizes.

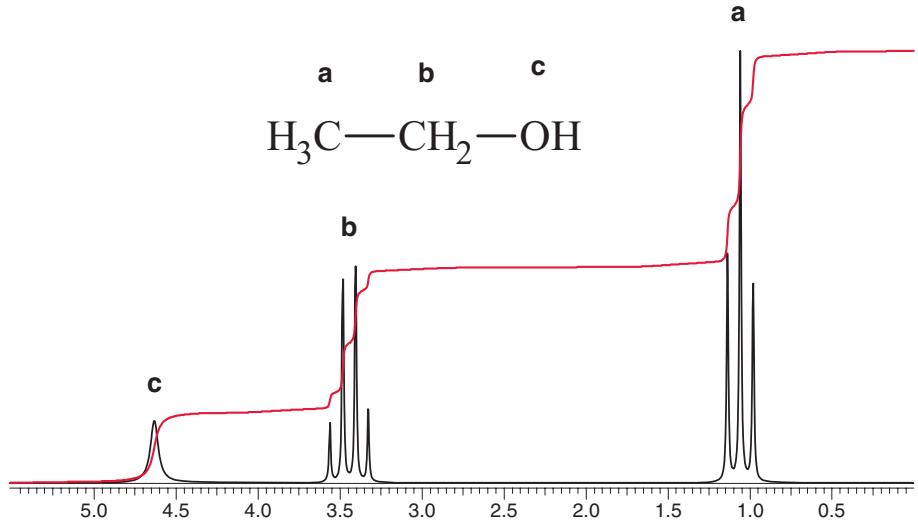
to each other *all share the same coupling constant* – as soon as a molecule gains a chiral centre and couplings from neighbouring protons cease to be equivalent, Pascal's triangle ceases to have any value in predicting the appearance of multiplets. Also, coupled signals must be well separated in order to approximately adhere to Pascal's distribution. This obviously begs the question: 'How well separated?' Well, this is a tricky question to answer. It is not possible to put an absolute figure on it because the further away the coupling signals are from each other in the spectrum, the better will be the concord between the theoretical distribution of intensity and the actual one. We will talk about this problem again later. Well separated coupled signals give rise to 'first order' spectra, and poorly separated ones give rise to 'non-first-order' spectra. We'll see examples of both types in due course.

The separations between the lines of doublets, triplets and multiplets are very important parameters, and are referred to as 'coupling constants', though the term is not strictly accurate. 'Measured splittings' would be a better description, since true coupling constants can only be measured in totally first order spectra, (which implies infinite separation between coupled signals) which never exist in practise. However, the differences between true coupling constants and measured splittings are so small for reasonably first order spectra, that we shall overlook any discrepancies which are vanishingly small anyway.

We measure coupling constants in Hz, since if we measured them in fractions of ppm, they would not be constant, but would vary with the magnetic field strength of the spectrometer used. This would obviously be most inconvenient! Note that 1 ppm = 250 Hz on a 250 MHz spectrometer and 400 Hz on a 400 MHz spectrometer, etc.

1.4.3 Integration

The area of each signal is proportional to the number of nuclei at that chemical shift. If we look at the previous example, the signal for the methyl group in ethanol should have an area with the ratio of 3 : 2 compared with the methylene signal. When we plot proton NMR data, we usually also plot the integral as well. This will show us the relative areas under the curves. Spectrum 1.3 shows the spectrum of ethanol with integrals.



Spectrum 1.3 90 MHz proton spectrum of ethanol with integrals.

Often, the integrals are broken up to maximise their size on the display and make them easier to measure. Integrals are often tricky to measure exactly, especially if the signal to noise of the spectrum is low or if the baseline rolls. Overlapping signals also make it difficult to integrate accurately and so other tools are available to perform peak fitting and use the peak parameters to back-calculate the integrals.

2

Preparing the Sample

Whilst sample preparation may not be the most interesting aspect of NMR spectroscopy, it is nonetheless *extremely* important as it will have a huge bearing on the quality of the data obtained and therefore on your ability to make logical deductions about your compounds. This is particularly true when acquiring the most straightforward 1-D proton spectra. The most typical manifestation of sub-standard sample preparation is poor line shape. It is worth remembering that in terms of 1-D proton NMR, 'the devil' can be very much 'in the detail'. 'Detail', in this context, means 'fine structure' and fine structure is always the first casualty of poor sample preparation.

The reason for this can best be appreciated by considering just how small the differences in chemical shifts of signals really are – and indeed, just how small (but significant!) a long-range coupling can be. Consider for example, a 3-7 coupling in an indole. (Structure 2.1).

Being able to see this coupling is reassuring in that it ties the 3 and 7 protons together for us. It might seem a trifling matter, but observing it, even if it appears only as a slight but definite broadening, helps underpin the credentials of the molecule because we know it should be there. Such a five-bond coupling will be small – comparable in fact with the natural line width of a typical NMR signal. Let's say we are looking for a coupling of around 1 Hz, for the sake of argument. 1 Hz, on a 400 MHz spectrometer corresponds to only 1/400 of a part per million of the applied magnetic field (since 1 ppm = 400 Hz in a 400 MHz spectrometer). So in order to observe such a splitting, we will need resolution of better than 0.5 Hz, which corresponds to one part in $10^6/(0.5/400)$, or ideally, better than one part in 10^9! To achieve such resolution requires corresponding levels of magnetic field homogeneity through your sample but this can only be achieved in extremely clean solutions of sufficient depth. We will be dealing with this issue in detail later on. In real terms, establishing first class magnetic field homogeneity means that molecules of your compound will experience exactly the same field no matter where they are in the NMR tube – therefore, they will all resonate in unison – rather than in a fragmented fashion. Any factor which adversely effects field homogeneity will have a corresponding deleterious effect on line shape. We will see this more clearly later.

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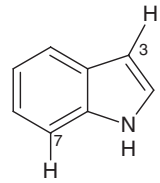


Structure 2.1 An indole with 3-7 coupling.

2.1 How Much Sample Do I Need?

This section might be alternatively titled, 'How long is a piece of string?' There is no simple answer to this question which we have been asked many, many times. What you need in solution is sufficient material to produce a spectrum of adequate signal/noise to yield the required information but this is no real answer as it will vary with numerous factors. How powerful is the magnet of the spectrometer you are using? What type of probe is installed in it? What nucleus are you observing? What type of NMR acquisition are you attempting? How pure is your sample? What is the molecular weight of your sample? Is it a single compound or is it a mixture of diastereoisomers? These are just some of the relevant questions that you should consider.

And there are others. If you are using a walk-up system, there will probably be some general guidelines posted on it. Assume that these are useful and adhere to them as far as possible. They will be by their very nature, no more than a guide, as every sample is unique in terms of its molecular weight and distribution of signal intensity. Also, a walk-up system is likely to be limited in terms of how much time (and therefore how many scans) it can spend on each sample.

If you are fortunate enough to be 'driving' the spectrometer yourself, you can of course compensate for lack of sample by increasing the number of scans you acquire on your sample – but this is not a licence to use vanishingly small amounts. It is worth remembering that in order to double the signal/noise ratio, you have to acquire four times the number of scans. Think about it. If your sample is still giving an unacceptably noisy spectrum after five minutes of acquisition, how long will you have to leave it acquiring in order for the signal/noise to become acceptable? Doubling the S/N is likely to do little. If you improve it by a factor of four (probably a worthwhile improvement) you will have to acquire for an hour and twenty minutes (16 × 5 minutes)! The law of diminishing returns operates here and makes its presence felt very quickly indeed.

All that having been said, we will attempt to draw up a few rough guidelines below.

If you are unfortunate enough to be struggling away with some old continuous-wave museum piece, then in all probability, you will only be looking at proton spectra. Even though the proton is THE most sensitive of all nuclei, you will still be needing *at least* 15 mg of compound, assuming a molecular weight of about 300 (if it's a higher molecular weight, you will need more material, lower and you may get away with a little less)

It's more likely these days that you will be using a 250 or 400 MHz Fourier transform instrument with multi-nuclei capability. If such an instrument is operating in 'walk up' mode so that it can acquire >60 samples in a working day, then it will probably be limited to about 32 scans per sample (a handy number – traditionally, the number of scans acquired has always been a multiple of eight but we won't go into the reasons here. If you want more information, take a look at the term 'phase cycling' in one of the excellent texts available on the more technical aspects of NMR). This means that for straightforward

Table 2.1 A rough guide to the amount of sample needed for NMR.

	Comfortable amount of material needed (mg)	
Field (MHz)	^{1}H	^{13}C
90	20	Lots!
250	5	30
400	2	10
600	1	5

1-D proton acquisition, you will need about 3 mg of compound as above, though you may get away with as little as 1 mg with a longer acquisition time, assuming a typical 5 mm probe. The same 3 mg solution (sticking with the approx. 300 mol. wt throughout) would also get you a reasonable fluorine spectrum, if available, since the ^{19}F nucleus is a 100 % abundant and is therefore, a relatively sensitive nucleus.

If you are looking for a ^{13}C spectrum, then you will probably find that they will only be available overnight. This is because the ^{13}C nucleus is extremely insensitive and acquisition will take hours rather than minutes (only 1.1 % natural abundance and relatively low gyromagnetic ratio – see Glossary). Whilst the signal to noise available for ^{13}C spectra will be highly dependant on the type of probe used (i.e., 'normal' geometry or 'inverse' geometry – see Glossary), about 10 mg of compound will be needed for a typical acquisition, which will probably entail about 3200 scans and run for about 2 h. Even then, the signal/noise for the least sensitive quaternary carbons may well prove marginal. (Note that the inherently low sensitivity of the ^{13}C nucleus can to some extent be addressed by acquiring various inverse-detected 2-D data such as HMQC/HSQC and HMBC, all of which we will discuss later).

Operating at 500 or 600 MHz and using a 3 mm probe should yield an approximate threefold improvement in signal/noise which can be traded for a corresponding reduction in sample requirement.

Various technologies do exist to give still greater sensitivities – perhaps even an order of magnitude greater, e.g., 'nano' probes, 1 mm probes and cryoprobes, but they are currently unusual in a 'routine' NMR environment. These tools tend to be the preserve of the NMR specialist.

Table 2.1 gives a very rough guide to the amount of sample you need, given all the previous provisos. Of course, if you are prepared to wait a long time and don't have a queue of people waiting to use the instrument, you can get away with less material. Generally, more is better (as long as the solution is not so gloopy that it broadens all the lines!).

2.2 Solvent Selection

The first task when running any liquid-phase NMR experiment is the selection of a suitable solvent. Obvious though this sounds, there are a number of factors worth careful consideration before committing precious sample to solvent. A brief glance at any NMR solvents catalogue will illustrate that you can purchase deuterated versions of just about any solvent you can think of but we have found that there is little point in using exotic solvents when the vast majority of compounds can be dealt with using one of four or five basic solvents.

Your primary concern when selecting a solvent should be the *complete* dissolution of your sample. Again, this might seem an unduly trivial observation, but if your sample is not in solution, then it

will remain 'invisible' to the spectrometer. Consider for a moment a hypothetical sample – a mixture of several components, only one of which being soluble in your chosen solvent. Under these circumstances, your spectrum may flatter you (your desired compound is preferentially soluble in solvent of choice), or alternatively, it may paint an unduly pessimistic view of your sample (one or more of the undesired components is preferentially soluble in solvent of choice). Either way, there are possibilities for being mislead here so the primary objective in selecting a solvent should be the total dissolution of your sample. In general, we advise adhering to the simple old rule that 'like dissolves like'. In other words, if your sample is nonpolar, then choose a nonpolar solvent and vice versa.

2.2.1 Deutero Chloroform ($CDCl_3$)

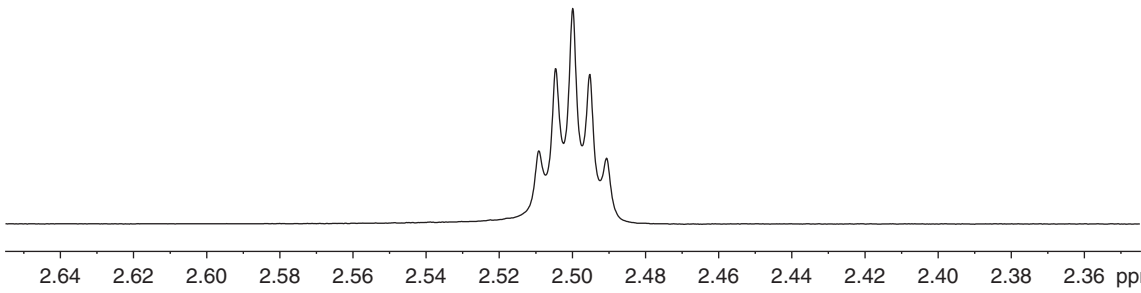
This is a most useful NMR solvent. It can dissolve compounds of reasonably varying polarity, from nonpolar to considerably polar, and the small residual $CHCl_3$ signal at 7.27 ppm seldom causes a problem. $CDCl_3$ can easily be removed by 'blowing off' should recovery of the sample be necessary. Should a compound prove only sparingly soluble in this solvent, deutero dimethyl sulfoxide can be added drop by drop to increase the polarity of the solvent – but see cautionary notes below! This may be preferable to running in neat D_6-DMSO due to the disadvantages of D_6-DMSO outlined below. It should be noted that D_6-DMSO causes the residual $CHCl_3$ signal to move downfield to as low as 8.38 ppm, its position providing a rough guide to the amount of D_6-DMSO added. The main disadvantage of using a mixed solvent system is the difficulty of getting reproducible results, unless you take the trouble of measuring the quantities of each solvent used!

It should also be noted that $CDCl_3$ is best avoided for running spectra of salts, even if they are soluble in this solvent. This is because deutero chloroform is an 'aprotic' solvent that does not facilitate fast transfer of exchangeable protons. For this reason, spectra of salts run in this solvent are likely to be broad and indistinct as the spectrometer 'sees' two distinct species of compound in solution; one with a proton attached and another with it detached. As the process of inter-conversion between these two forms is slow on the NMR timescale (i.e., the time taken for the whole process of acquiring a single scan to be completed in), this results in averaging of the chemical shifts and consequent broadening of signals – particularly those near the site of protonation.

2.2.2 Deutero Dimethyl Sulfoxide (D_6-DMSO)

Deutero dimethyl sulfoxide (D_6-DMSO) is undoubtedly very good at dissolving things. It can even dissolve relatively insoluble heterocyclic compounds and salts, but it does have its drawbacks. Firstly, it's relatively viscous, and this causes some degree of line-broadening. In cases of salts, where the acid is relatively weak (fumaric, oxalic, etc.), protonation of the basic centre may well be incomplete. Thus, salts of these weak acids may often look more like free bases! It is also a relatively mild oxidising agent, and has been known to react with some compounds, particularly when warming the sample to aid dissolving, as is often required with this solvent.

Problems associated with restricted rotation (discussed later) also seem to be worse in D_6-DMSO, and being relatively nonvolatile (it boils at 189 °C, though some chemical decomposition occurs approaching this temperature so it is always distilled at reduced pressure), it is difficult to remove from samples, should recovery be required. This nonvolatility however, makes it the first choice for high temperature work – it could be taken up to above 140 °C in theory, though few NMR probes are capable of operating



Spectrum 2.1 Residual solvent signal in DMSO.

at such high temperatures. At the other end of the temperature scale it is useless, freezing at 18.5 °C. In fact, if the heating in your NMR lab is turned off at night, you may well find this solvent frozen in the morning during the cold winter months!

The worst problem with DMSO, however, is its affinity for water, (and for this reason, we recommend the use of sealed 0.75 ml ampoules wherever possible) which makes it almost impossible to keep dry, even if it's stored over molecular sieve. This means that bench D_6-DMSO invariably has a large water peak, which varies in shape and position, from sharp and small at around 3.46 ppm, to very large and broad at around 4.06 ppm in wetter samples. This water signal can be depressed and broadened further by acidic samples! This can be annoying as the signals of most interest to you may well be obscured by it. One way of combating this, is to displace the water signal downfield by adding a few drops of D_2O, though this can also cause problems by bringing your sample crashing out of solution. If this happens, you've got a problem! You could try adding more D_6-DMSO to re-dissolve it. The residual CD_2HSOCD_3 signal occurs at 2.5 ppm, and is of characteristic appearance (caused by ^2H–^1H coupling). Note that the spin of deuterium is 1, which accounts for the complexity of the signal (see Spectrum 2.1). Even so-called 100 % isotopic D_6-DMSO has a small residual signal so you can't totally negate the problem by using it – just lessen it.

Extreme care should be taken when handling DMSO solutions, as one of its other characteristics is its ability to absorb through the skin taking your sample with it! This can obviously be a source of extreme hazard. Wash off any accidental spillages with plenty of water – immediately! (This goes for all other solvents too).

2.2.3 Deutero Methanol (CD_3OD)

This is a very polar solvent, suitable for salts and extremely polar compounds. Like DMSO it has a very high affinity for water and is almost impossible to keep dry. Its water peak is sharper and occurs more predictably at around 4.8 ppm. The residual CD_2HOD signal is of similar appearance to the D_6-DMSO residual signal and is observed at 3.3 ppm.

Its main disadvantage is that it will exchange ionisable protons in your sample for deuterons, and hence they will be lost from the spectrum, e.g., -OH, -NH and even -$CONH_2$, though these can often be relatively slow to exchange. Also, protons α to carbonyl groups may exchange through the enol mechanism. The importance of losing such information should not be underestimated. Solving a structural problem can often hinge on it!

2.2.4 Deutero Water (D_2O)

D_2O is even more polar than D_4-methanol and rather limited in its use for that reason – usually for salts only. Like deutero methanol, it exchanges all acidic protons readily and exhibits a strong HOD signal at about 4.9 ppm. Samples made up in D_2O often fail to dissolve cleanly and benefit from filtration through a tight cotton wool filter (cf. Section 2.4.1).

2.2.5 Deutero Benzene (C_6D_6)

D_6-Benzene is a rather specialised solvent and not normally used in 'routine' work. It is often added to $CDCl_3$ solutions, though it can of course be used neat, when it may reveal hidden couplings or signals by altering chemical shifts of your compound. It does this because it can form collision complexes with sample molecules by interactions of the pi electrons. This can bring about changes in the chemical shifts of the sample peaks because benzene is an anisotropic molecule, i.e., it has non-uniform magnetic properties (shielding above and below the plane of the ring, and deshielding in the plane of the ring). This is really an extreme example of a solvent shift. Whenever you change the solvent, expect a change in the spectrum! C_6D_6 shows a residual C_6D_5H signal at 7.27 ppm. *Cautionary note*: benzene is of course a well known carcinogen and due care should be taken when handling it – particularly if used in combination with DMSO!

With these five solvents at your disposal, you will be equipped to deal with virtually any compound that comes your way but it might be worth briefly mentioning two others.

2.2.6 Carbon Tetrachloride (CCl_4)

This would be an ideal proton NMR solvent, (since it is aprotic and cheap) were it better at dissolving things! Its use is now very limited in practise to very nonpolar compounds. Also, it lacks any deuterated signal that is required for locking modern Fourier transform spectrometers – (an external lock would be necessary making it inconvenient – see Section 2.3). Carbon tetrachloride is very hydrophobic, so any moisture in a sample dissolved in this solvent will yield a milky solution. This might impair homogeneity of the solution and therefore degrade resolution, so drying with anhydrous sodium sulfate can be a good idea. Carbon tetrachloride does have the advantage of being non-acidic, and so can be useful for certain acid-sensitive compounds. Take care when handling this solvent, as like benzene, it is known to be carcinogenic. Not recommended.

2.2.7 Trifluoroacetic Acid (CF_3COOH)

Something of a last resort this one! It seems to be capable of dissolving most things, but what sort of condition they're in afterwards is rather a matter of chance! It has been useful in the past for tackling extremely insoluble multicyclic heterocyclic compounds. If you have to use it, don't expect wonders. Spectra are sometimes broadened. It shows a very strong -COOH broad signal at about 11 ppm. Again, the lack of a deuterated signal in this solvent makes it less suitable for FT making an external lock necessary – see above. Not recommended unless no alternative available.

Well, that just about concludes our brief look at solvents. If you can't dissolve it in one of the common solvents, you've got problems. If in doubt, try a bit first, before committing your entire sample. Use nondeuterated solvents for solubility testing if possible, as they are much cheaper.

2.2.8 Using Mixed Solvents

Whilst it is perfectly possible to use a mixed solvent system ($CDCl_3$/DMSO is always a popular example as chemists have a tendency to opt for $CDCl_3$ out of habit or in the hope that it will dissolve their samples, only to find that solubility is not as good as expected), we advise against it, particularly if you are running your spectra on a 'walk up' automated system. Remember that the spectrometer uses the deuterated signal for frequency locking and if it has more than one to chose from, things can go wrong and you might find yourself the proud owner of a spectrum that has been offset by several ppm as the spectrometer locks onto the D_6-DMSO signal and sets about its business in the belief that it has in fact locked onto $CDCl_3$! Furthermore, it is very difficult to reproduce exact solvent conditions if you are required to re-make a compound. Using a suitable single solvent will prevent these issues ever troubling you.

2.3 Spectrum Referencing (Proton NMR)

NMR spectroscopy differs from other forms of spectroscopy in many respects, one of which is the need for our measurement to be referenced to a known standard. For example, considering infra red spectroscopy for a moment, if a carbonyl group stretches at 1730 cm^{-1}, then as long as we have a suitably calibrated spectrometer, we can measure this, confident in the knowledge that we are measuring an absolute value associated with that molecule.

In NMR spectroscopy, however, the chemical shift measurement we make takes place in an environment of our making that is both entirely artificial and arbitrary (i.e., the magnet!). For this reason, it is essential to reference our measurements to a known standard so that we can all 'speak the same language,' no matter what make or frequency of spectrometer we use.

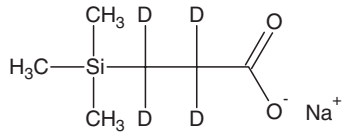
The standard is usually added directly to the NMR solvent and is thus referred to as an 'internal' standard, though it is possible to insert a small tube containing standard in solvent into the bulk of the sample so that the standard does not come into direct contact with the sample. This would be referred to as an 'external' standard. We recommend an internal standard wherever possible for reasons of convenience and arguably superior shimming.

Apart from some very early work in the field which was performed using water as a standard (It would be difficult to imagine a worse reference standard as the water signal moves all over the place in response to changing pH!) the historical reference standard of choice has always been TMS (Tetra Methyl Silane), as mentioned earlier. TMS has much to recommend it as a standard. It is chemically very inert and is volatile (b.p. 26–28 °C) and so can easily be removed from samples if required. Furthermore, only a tiny amount of it is needed as it gives a very strong twelve proton singlet in a region of the spectrum where other signals seldom occur.

TMS is not ideally suited for use in all solvents, however. As you can see from the structure, it is extremely nonpolar and so tends to evaporate from the more polar solvents (D_6-DMSO and D_4-MeOD). For this reason, a more polar derivative of TMS [3-(Trimethylsilyl) propionic-2,2,3,3-D_4 acid; TSP – see Structure 2.2] is often used with these solvents.

Note that the side chain is deuterated so that the only signal observed in the proton NMR spectrum is the trimethyl signal.

Deuterated solvents can be purchased with these standards already added if required and this would be our recommendation because so little standard is actually needed that it is very difficult to add



Structure 2.2 3-(Trimethylsilyl) propionic-2,2,3,3-D_4 acid, sodium salt.

little enough to a single sample without overdoing it! (An enormous standard peak, apart from looking amateurish, is to be avoided since it will limit signal/noise ratio as the spectrometer scales the build up of signals according to the most intense peak in a spectrum.) Of course, TMS and TSP do not have *exactly* the same chemical shifts so to be totally meticulous, you should really quote the standard you are using when recording data.

Of course, you don't have to use either of the above standards at all. In the case of samples run in deutero chloroform/methanol and dimethyl sulfoxide, it is perfectly acceptable, and arguably preferable, to reference your spectra to the residual solvent signal (e.g., CD_2HOH) which is unavoidable and always present in your spectrum (see Table 2.2). These signals are perfectly solid in terms of their shifts (in pure solvent systems) though the same cannot be said for the residual HOD signal in D_2O and for this reason, we would advise adhering to TSP for all samples run in D_2O.

We will discuss referencing issues with respect to other nuclei in later chapters.

2.4 Sample Preparation

Note that sample depth is important! When using a typical 5 mm probe, a sample depth of about 4 cm (approx. 0.6 ml) is necessary, though this varies slightly from instrument to instrument. There should be guidance available to you in this respect on each individual spectrometer. If you try to get away with less than this, magnetic field homogeneity, and therefore, shimming (see Section 3.8) will be compromised as the transmitter and receiver coils in the probe must be covered to a sufficient depth to avoid the problems of 'edge effects' (see Figure 2.1).

Of course, there is no point in overfilling your NMR tubes. This *can* make shimming more difficult (but certainly not impossible as in the case of too low a sample depth) but more importantly, it merely wastes materials and gives rise to unduly dilute samples giving reduced signal/noise. Any sample outside the receiver coils does not give rise to signal.

If your sample is reluctant to dissolve in the chosen solvent, avoid adding more solvent for the reasons outlined above. Instead, try warming the sample vial carefully on a hotplate or with a

Table 2.2 Residual solvent signals.

Solvent	Chemical shift of residual signal (ppm)
$CDCl_3$	7.27
CD_3SOCD_3	2.50
CD_3OD	3.30

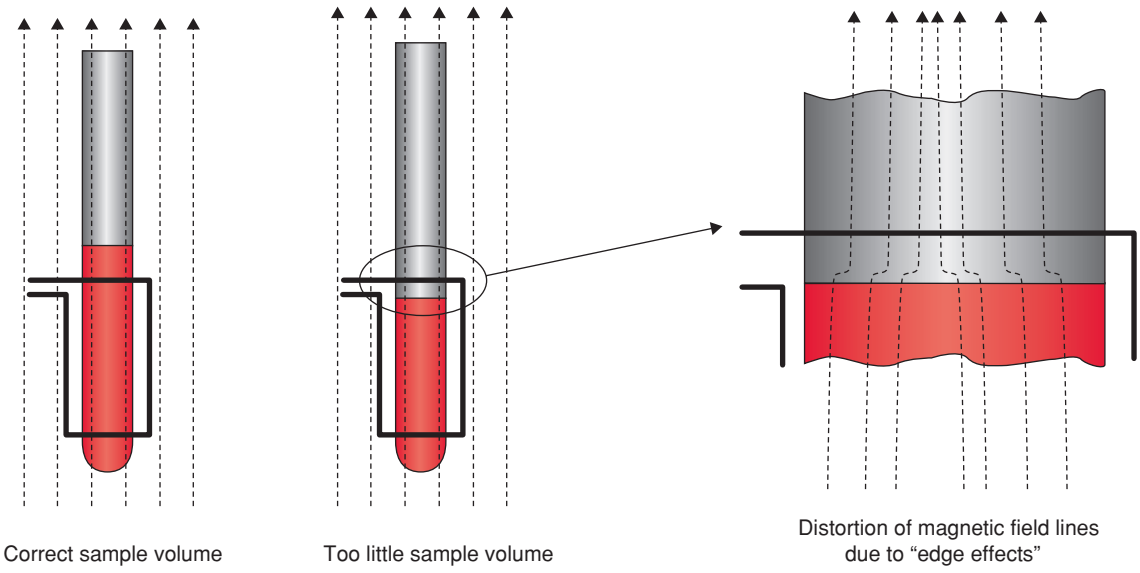


Figure 2.1 Sample depth and magnetic field homogeneity.

hairdryer – sometimes a bit of thermal agitation will be all that is required to assist the dissolving process. This is particularly true in the case of highly crystalline samples which can be slow to dissolve. Another useful approach is to use an ultrasonic bath. These provide very powerful agitation and are even more effective when used in combination with a heat source.

2.4.1 Filtration

Of course, there are always samples that refuse to dissolve completely even after ample exposure to heat and prolonged dunking in an ultrasonic bath. Samples that appear in any way cloudy when held up to the light, simply *must* be filtered. Any particulate matter held in suspension will severely compromise field homogeneity and thus line shape (Figure 2.2). Suspended material (of whatever origin) is *the* major cause of substandard line shape in NMR spectroscopy.

The whole filtration issue is perhaps a little confusing. Earlier in this section we were stressing the importance of dissolving the *whole* sample and yet here we are, now advocating filtration? On the face of it, there might seem to be an inherent contradiction in this – and perhaps there is. We can only say that in an ideal world, samples would dissolve seamlessly to give pristine clear solutions without even a microscopic trace of insoluble material in suspension. Samples in the real world are often not quite so obliging! Filtration is very much the lesser of the two evils. If you *know* that you have filtered something from your solution, you are at least aware of the fact that the spectrum is not entirely representative of the sample. But if you *don't* filter, the resultant spectrum may be so poor as to fail to yield any useful information at all – the choice is as simple as that.

Be warned that very small particle size material, that may even be invisible to the naked eye, is the worst in terms of ruining line shape. The big stuff quite often floats or sinks and therefore doesn't interfere much with the solution within the r.f. coils.

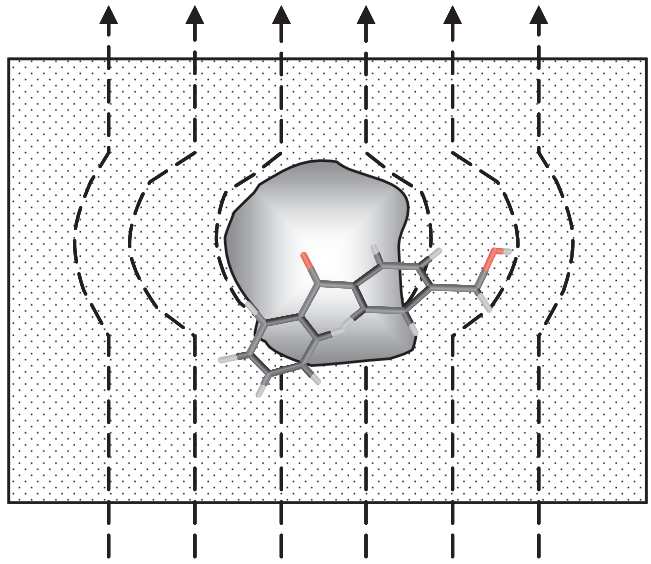


Figure 2.2 Undissolved material causing loss of magnetic field homogeneity.

A convenient method for the filtration of small volumes of liquids is shown in Figure 2.3.

The filter can in a sense be 'customised' as required. A tight plug of cotton wool (rammed hard into the neck of a pipette using a boiling stick) alone is often enough to remove fairly obvious debris from your solution but the addition of a layer of a similarly compacted 'hyflo' on top of the cotton wool makes for a very tight filter which will remove all but the most microscopic of particles. Note that using a pipette bulb to force the liquid through the filter is an excellent idea as it speeds the whole process considerably. Even so, if you are using D_6-DMSO as a solvent, be prepared for a long squeeze as the viscosity of this solvent makes it reluctant to pass through a tight filter. If you suspect that your sample is wet (usually, cloudy $CDCl_3$ solutions with no obvious particulate matter present), you can take this opportunity to dry it at this stage by introducing a layer of anhydrous sodium sulfate to the filter. This will remove most (but not all) of the water present.

A couple of final observations on line shape – just occasionally, we have encountered samples that give very broad lines even after the most stringent filtering. This can be caused by contamination by a tiny amount of paramagnetic material in solution. In one memorable case, a chemist had been stirring a sample around in an acidic solution with a nickel spatula. The tiny quantity of nickel leached from the spatula was sufficient to flatten the entire spectrum. The reason for this is that the ions of any of the transition (d-block) elements provide a *very* efficient relaxation pathway for excited state nuclei, enabling them to relax back to their ground state very quickly. Fast relaxation times give rise to broad lines and vice versa, so to summarise, keep NMR solutions well away from any source of metal ions! Should you find yourself in this situation, your only course of action is to run your sample down a suitable ion exchange column.

One other (very rarely encountered) situation is that of the stabilised free radical. It is possible for certain conjugated multi-ring heterocyclic compounds to support and stabilise a delocalised, free electron in their pi clouds. Such a free electron again provides an extremely efficient relaxation pathway for all

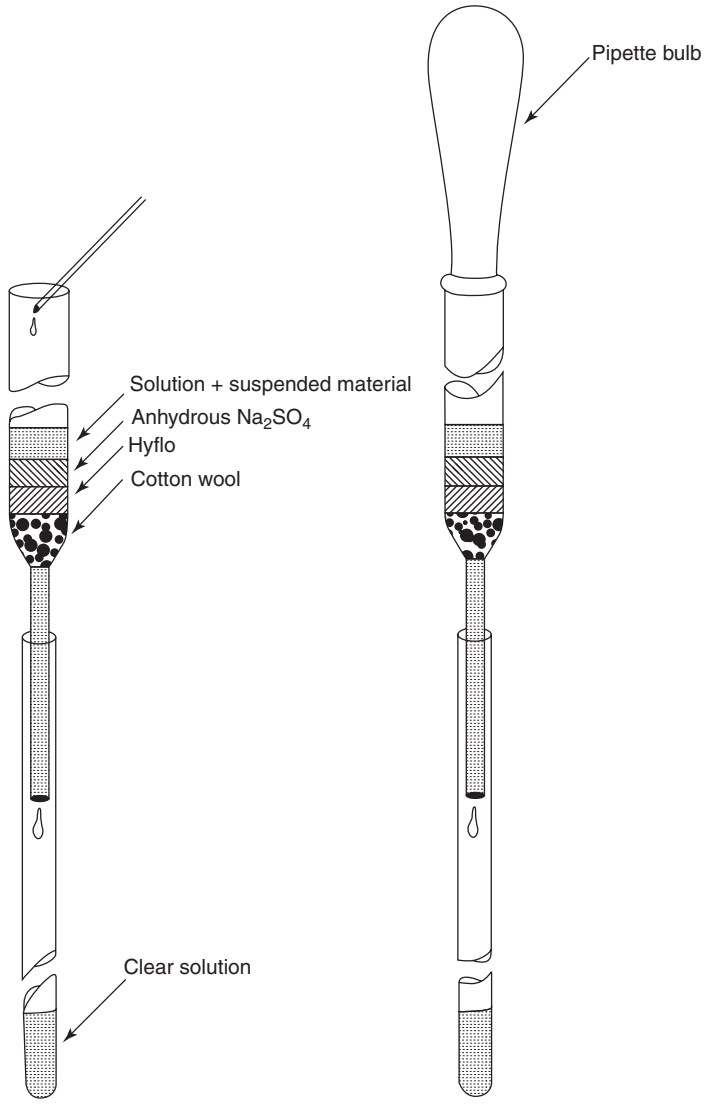


Figure 2.3 A convenient method of filtering NMR solutions.

nuclei in such a molecule and would give rise to an almost entirely flat spectrum. Such compounds usually give a clue to their nature by being intensely coloured (typically very dark blue) Filtration would do little to improve such a situation but running in the presence of a suitable radical scavenger such as dichloro, dicyano quinone can provide the solution. The scavenger mops up the lone electron and a spectrum can be obtained as normal.

3

Spectrum Acquisition

This was probably the most difficult chapter to put together in this book. For many people who use NMR spectrometers, there will be little (or no) choice about parameters for acquisition – they will probably have been set up by a specialist to offer a good compromise between data quality and amount of instrument time used. This could make this chapter irrelevant (in which case you are welcome to skip it). But if you do have some control over the acquisition and/or processing parameters, then there are some useful hints here. This brings us on to the next challenge for the section – hardware (and software) differences. You may operate a Bruker, Varian, Jeol or even another make of NMR spectrometer and each of these will have their own language to describe key parameters. We will attempt to be 'vendor neutral' in our discussions and hopefully you will be able to translate to your own instrument's language.

The first thing to note is that there are many, many parameters that need to be set correctly for an NMR experiment to work. Some are fundamental and we don't play with them. Some are specific to a particular pulse sequence and determine how the experiment behaves. It is difficult to deal with all of these here so we will look at some of the parameters that affect nearly all experiments and are often the ones that you will be able to control in an open access facility. Many of these parameters affect each other and we will try to show where this is the case.

This area is actually quite complex. The descriptions here are not necessarily scientifically complete or rigorous. Hopefully they will help you understand what will happen when you change them (and in which direction to move them!).

3.1 Number of Transients

Probably the most basic parameter that you will be able to set is the number of spectra that will be co-added. This is normally called the 'number of transients' or 'number of scans'. As mentioned elsewhere in the book, the more transients, the better the signal to noise in your spectrum. Unfortunately, this is not a linear improvement and the signal to noise increase is proportional to the square root of the number of transients. As a result, in order to double your signal to noise, you need four times the number of scans. This can be shown graphically in Figure 3.1.

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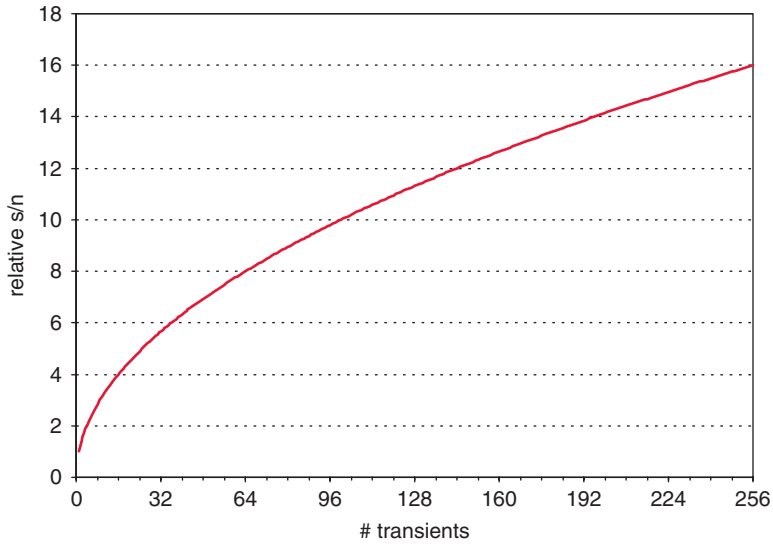

Figure 3.1 Relative signal to noise versus number of transients.

There are several implications of this relationship, the main one being that if you use double the amount of sample, you can acquire the same signal to noise spectrum in a quarter of the time. This is particularly apparent if you are acquiring data on insensitive nuclei like ^{13}C where you might be acquiring data for several hours and this can be cut down dramatically if you can spare a little more sample. Don't forget, NMR is a nondestructive technique and you can always get your sample back afterwards (even from DMSO – it just takes a little longer than $CDCl_3$ or MeOD).

Note that you can't just use any number of transients. Many experiments require a multiple of a base number of transients to work correctly. This is due to the needs of phase-cycling which we won't describe here – once again, check other text books if you want to find out more about this. Generally you will be safe if you choose a multiple of eight as this covers most of the commonly used phase cycles although there are many experiments that can use multiples of two or even one. If in doubt, check the pulse programme or ask someone who knows.

3.2 Number of Points

Because the acquisition is digital, you will need to specify how many points you are going to collect the data into. This figure is related to the field that you are operating at – the higher the field, the more points that you are going to need. This parameter relates to the spectral width observed and the acquisition time through sampling theory. The Fourier transform algorithm demands that the number of points is a power of two so we tend to use the computer term of 'k' to describe the number of points (where 1 k = 1024 points). If we acquire 20 ppm at 400 MHz, this has a spectral width of 8000 Hz. If we then want to have a digital resolution of 0.5 Hz we would need 16 k to achieve this. Because we are acquiring both real and imaginary data, we would need to double this so we would need 32 k points to achieve this resolution. We can improve the appearance a little by using 'zero filling' and this is described later.

3.3 Spectral Width

Sampling theory states that you must sample a waveform at least twice per cycle otherwise you will observe a lower-frequency signal than the true signal (you often see this effect in old cowboy films where waggon wheels speed up and then appear to stop and move backwards). In NMR we set the sampling rate by choosing the spectral width of the spectrum. If we chose too narrow a spectral width, then signals outside that range will 'fold back' into the spectrum (normally with strange phase). Older spectrometers use electronic filters to try to avoid this but even the best ones don't cut off frequencies exactly; they attenuate signals close to the filter edge. More modern spectrometers use oversampling and digital filters which treat the spectrum computationally to produce a very sharp filter. This means that folding ('aliasing') is seldom seen in one dimensional spectra. This is not the case in 2-D spectra though, as the indirect dimension cannot benefit from these filters. In this case, setting too narrow a spectral width in the second dimension will result in folded peaks in the 2-D spectrum.

3.4 Acquisition Time

This parameter is not normally set directly but is a function of the values that you set for spectral width and number of points. The narrower the spectral width, the longer will be the acquisition time and the greater the number of points, the longer the acquisition time.

3.5 Pulse Width/Pulse Angle

When we excite the nuclei of interest, we use a very short pulse of radiofrequency. Because the pulse is very short, we generate a spread of frequencies centred about the nominal frequency of the radiation. The longer this pulse, the more power is put into the system and the further that it tips the magnetisation from the z axis. We call this the 'flip angle'. A 90° flip angle gives rise to the maximum signal (you can picture it as the projection on the x–y plane, where z is the direction of the magnetic field. This is shown diagrammatically in Figure 3.2.

The other consequence of the pulse width is the spread of frequencies generated. The shorter the pulse, the wider will be the spread of frequencies. Because we often want to excite a wide range of frequencies, we need very short pulses (normally in the order of a few microseconds). This gives rise to a so-called sinc function (Figure 3.3).

At first sight, this may appear to be a lousy function to excite evenly all the frequencies in a spectrum but because we use such a short pulse, we only use the bit of the function around $x = 0$. The first zero-crossing point is at 1/(2× pulse width) – this would be at about 150 kHz for a 3 μs pulse. For a 400 MHz spectrometer, we need to cover a bandwidth of about 8 kHz for a proton spectrum. As Figure 3.4 shows, there is minimal power fall off for such a small pulse.

Of course, it is quite easy to solve the bandwidth needs of proton spectra – they only have a spread over about 20 ppm (8 kHz at 400 MHz). Things get a bit more difficult with nuclei such as ^{13}C where we need to cover up to 250 ppm (25 kHz) spread of signals and we do notice some falloff of signal intensity at the edge of the spectrum. This is not normally a problem as we seldom quantify by ^{13}C NMR. However, it can be a problem for some pulse sequences that require all nuclei to experience 90°

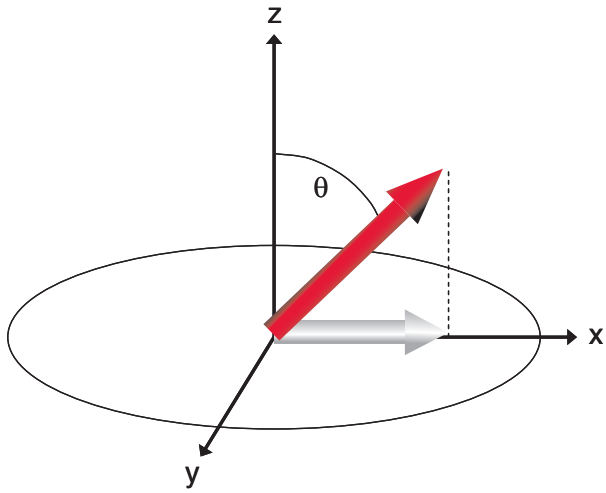

Figure 3.2 'Flip angle.'

or 180° pulses. This is particularly true at higher fields but we now have access to different ways of generating these transitions using so-called 'adiabatic pulses'.

One last comment about pulse widths; it is important that we know what the 90° pulse width is for the nuclei that we observe as accurate pulse widths are required for many pulse sequences (as mentioned previously). Failure to set these correctly may give rise to poor signal to noise or even generate artifacts in the spectrum. When instruments are serviced, these pulse widths are measured and entered into a table to ensure that the experiments continue to work in the future.

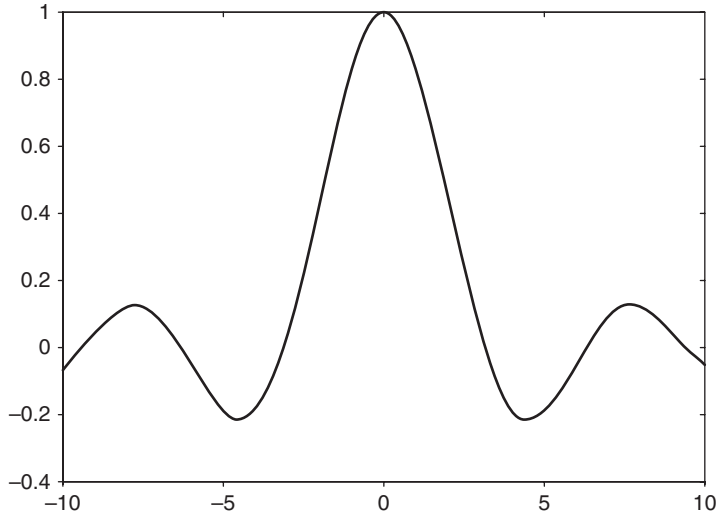

Figure 3.3 The 'sinc' function.

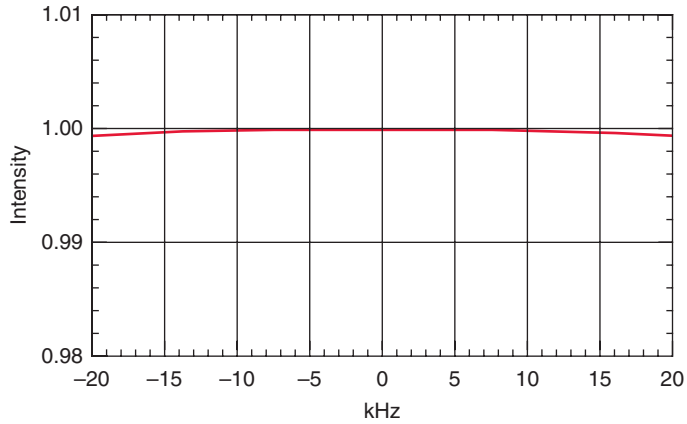

Figure 3.4 Falloff of power with frequency from central pulse frequency.

3.6 Relaxation Delay

This is the amount of time included in a pulse sequence to allow all the spins to lose their energy. Failure to let this happen will cause signals to integrate less than they should (or may cause artifacts in some experiments). The amount of time that you leave depends on the amount that you have tipped the spins with your excitation pulse (see 'pulse width'). If you have made a 90° pulse then you will have to wait for about 30–50 s between pulses to allow the spins to re-equilibrate. The exact length of time is specific to the environment of the nuclei that you are observing. Generally, singlets are the slowest signals to relax and will tend to under-integrate if you have too short a relaxation delay. The spins have the total time from when they were excited until their next excitation to relax. This means that the value that you set for the relaxation delay also depends on the acquisition time.

For most 1-D proton experiments we tend to use a pulse angle of about 30° and an acquisition time of about 3 s – so a relaxation delay of about 2 s is normally fine for most proton work. If you need super-accurate integrals you can play safe and give a relaxation time of 10 s; and this should cover most eventualities. So why not just set a relaxation delay of 1 min? This would obviously cover every eventuality. The problem is that this delay is inserted into every pulse cycle so your experiment would take a long time to complete. It ends up that you have a compromise of how much you tip the spins, how long you acquire for and how long you wait for. For example, if you get maximum signal by using a 90° pulse you may have to wait such a long time for the spins to relax that you don't achieve the throughput that you were after. It turns out that the optimum flip angle (in terms of rate of data collection) is about 30° and this is what we use for most 1-D proton spectra.

3.7 Number of Increments

For 2-D experiments, not only will you need to set the number of points for your direct detection dimension, you will also need to set the number of experiments in the second dimension as this will determine what resolution you have in that dimension. There is no simple answer to help here – it

depends on the experiment that you are performing, what information you need and what frequency you are operating at. For a COSY experiment, we probably need quite a few increments because we are often interested in signals that can be quite overlapped. In this case, 256 increments would be quite reasonable at 400 MHz. If you were operating at 800 MHz then you would need double this (512 increments) to get the same resolution. There are some mathematical tricks that we can perform with the data to improve this situation and these are described in the next chapter. By the way, you have to be a bit careful with the name for the second dimension – Bruker call it 'f1' and Varian call it 'f2'. In this book we will stick with the term 'the indirect dimension'.

3.8 Shimming

When we are looking at NMR data, we need to be able to resolve 0.5 Hz (or better) in a few hundred MHz. This means our field must be homogenous to better than one part per billion! Magnets are created to exacting standards and produce highly linear fields in the sample area. This is achieved through very precise engineering and the addition of coils which can tweak the field to make it even more precise (by passing a current through them). These coils are called 'shim coils' and they come in two different flavors: cryoshims and room temperature (RT) shims. The cryoshims are at liquid helium temperatures and are set up when the magnet is energised. The cryoshims are capable of getting the field homogeneity to better than 5 ppm and once they are set up they are not normally altered. To get the field to the desired homogeneity we use the RT shims and these are adjusted by passing different amounts of current through them. Changes in the environment due to the sample or other external factors may cause this field to be distorted. To get the field to the ultimate homogeneity, the RT shims are adjusted so that they contribute field to add or take-away from the main field. There are a large number of these shim coils (up to 40 on some magnets) and they each have a particular influence on the magnetic field. They are named after the mathematical function of the field that they supply. The basic ones are somewhat obviously called 'X', 'Y' and 'Z'. That is, they have a linear effect on the field in the X, Y and Z directions. The more complex shaped ones have esoteric names such as X2Y2Z4 ($X^2Y^2Z^4$).

So, given this frightening range of coils, how do you go about shimming a system? The answer is: 'with lots of experience'. To be able to shim a system from scratch is a highly skilled job and requires huge patience. Fortunately, you may never have to do it. Once a system is set up, the shim values (how much current is passing through each shim coil) for most of the shims remain relatively static. We normally only have to tweak the 'low order' Z shims in daily use. This means 'Z' (nearly always), 'Z2' (nearly always), 'Z3' (quite often) and 'Z4' (sometimes). The rest, we can normally ignore. Modern spectrometers will go even further to help you and will shim your sample automatically. This normally uses a 'simplex' approach and takes about a minute or two. In addition to this, there is the more recent development of 'gradient shimming'. Unlike the simplex method (which gives the coil a tweak and looks at the result and then decides what to do next), gradient shimming acquires a map of the field and then works out which functions will make it more homogeneous. It then sets the values in the coils and doesn't have to go through the iterative process of the simplex method. The simplex method takes longer for each shim that you optimise whereas the gradient approach will take the same length of time to do all of the 'Z' coils.

Manual shimming is not yet a 'thing of the past' but it is certainly less of a badge of honour for budding spectroscopists.

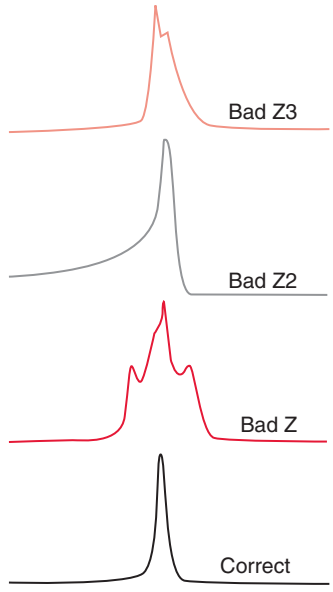

Figure 3.5 Correct line shape and some typical distortions caused by poor shimming.

Sometimes the (automatic) shimming process goes wrong and the instrument is unable to generate the field homogeneity that is needed. You will need to spot this otherwise you may make the wrong judgement about your compound. So how can you tell? Well, the key is to understand what physically happens if the field is not homogeneous. Your sample should experience the same strength field wherever it is in your sample tube. If it doesn't, then molecules in different parts of the tube will resonate at slightly different frequencies. This will give rise to line broadening and, depending on the shim which is out, may give rise to distinctive line shapes. Some of the common distortions are shown in Figure 3.5.

As mentioned earlier, poor lineshape may be due to a number of different factors and these are covered in the sample preparation chapter. It is important to know whether the poor lineshape is due to sample or spectrometer – after all, you don't want to spend time playing with your sample when the spectrometer was the problem all along and conversely, you don't want to spend time fruitlessly shimming the spectrometer when the problem lies with your sample. Dynamic sample effects can be identified because the sample signals will be broad but the solvent (and impurity signals should there be any) will be sharp. It is more difficult to distinguish sample preparation effects from shimming effects as they both affect all signals in the spectrum. The smoking gun for an instrumental problem is if the samples before or after yours also look bad. If they are fine, it's probably your sample. If they are bad then it's probably the instrument (unless you prepared them, in which case it could be your technique!). In the case of the 'Z' shim, you may end up with multiple peaks for each of your real peaks. If you don't realise that this is a shimming problem then you might assume that your sample is impure when it is not. Note, however, that bad shimming is unusual so don't use it as an excuse to pretend that your compound is pure when it is really a mess. You can always check – look at the solvent peaks in the spectrum. If they are split too, then it is shimming – if they aren't, it's your sample! Note that no amount of shimming, manual or automatic, can compensate for undissolved material in solution, or incorrect sample depth!

In conclusion, shimming is best left to the experts (or the instrument) but it is important to be able to spot shimming problems so that you don't misjudge your sample.

3.9 Tuning and Matching

The NMR probe is a tuned radiofrequency circuit. When we insert a sample in the coils of the probe, we affect the circuit and can change its resonant frequency. If the circuit becomes de-tuned, it becomes less efficient at transmitting the radiofrequency to the sample. This often results in pulses that do not tip the magnetisation as much as we were hoping to achieve. As mentioned earlier, this can have a detrimental effect on complex pulse sequences and create artifacts in the spectrum (or decrease the signal to noise for simple pulse sequences). Tuning and matching allow us to tweak the circuit to compensate for the sample load on the coils. In older systems (many of which are still in use), tuning and matching is carried out on the probe using tuning knobs. In more modern systems this is done under automation by the instrument. Differences in probe tuning can be seen when running different solvents after each other (e.g., $CDCl_3$ followed by DMSO) or if you have 'lossy' samples which are highly conductive (e.g., salt solutions).

3.10 Frequency Lock

Because the magnetic field of an NMR spectrometer can drift slowly over time, it is necessary to 'lock' the spectrometer frequency to something that drifts at the same rate. This is achieved by monitoring the deuterium signal in your solvent. As the magnet field drifts, so does the deuterium signal and this moves the spectrometer frequency at the same time. Normally you don't need to think about this but it becomes important when you are using a mixed solvent as the instrument may lock onto the wrong solvent signal. If this is the case, your chemical shifts will be incorrect. You can check whether this has happened by looking at your residual solvent signals (or TMS if you have any in your sample).

Obviously, if you are running in a nondeuterated solvent you will not be able to lock your sample. In this case there are a few options:

3.10.1 Run Unlocked

If your experiment is short, you don't need to worry about field drift. Modern magnets are quite stable and can be used for at least a few minutes without drifting too far. The disadvantage with this approach is that shimming is normally performed on the deuterium signal and you will need to shim your sample differently if there is no deuterium in the sample.

3.10.2 Internal Lock

You can keep the spectrometer happy by adding a deuterium source to the sample. On the other hand, you probably don't want to do this otherwise you would have selected a deuterated solvent in the first place! Nonetheless it is still an option in some cases. Note that if you only add a small amount of the deuterium source, you may struggle to achieve lock because the signal is too weak.

3.10.3 External Lock

If you don't want to contaminate your sample, you can use a small tube, filled with a deuterated solvent, inside your main tube, as mentioned earlier. This is particularly useful if you are running a neat liquid or if the deuterated solvent is immiscible with your sample. This is probably the most common approach to this problem.

Finally, it has been noted that some people think that they need TMS in their samples to enable them to lock. This is not the case! On modern spectrometers, TMS is used for referencing only. There was a time when it was used for locking CW instruments (in an early form of spectrum averaging) but it is not used in that way for FT instruments now.

3.11 To Spin or Not to Spin?

In the early days of NMR, spinning the sample was seen as essential. The reason for spinning is to average out inhomogeneity in the magnetic field which can be caused by the sample or poor shimming. By rotating the sample tube, molecules will experience an average field. This can improve the resolution of the signals which is obviously a good thing. With modern NMR systems, however, this is seldom necessary. Magnetic field homogeneity has improved considerably over the years due to better magnet design, shim system design and shimming software. Spinning is not without its problems, particularly in very sensitive probes, and can introduce its own artifacts such as Q-modulation sidebands in 1-D spectra (antiphase peaks either side of the main peak) and other artifacts in 2-D spectra.

The advice for most modern spectrometers is not to spin. A little time spent in decent sample preparation makes this unnecessary. From experience in the real world, we have found that sample preparation is not always of the highest standard and spinning may help to correct this to some extent. In the end, for a workhorse 400 MHz system with an ordinary probe, it is a pragmatic decision based on your individual needs. If you are lucky enough to have a high performance probe then it is best not to spin.

4

Processing

4.1 Introduction

Acquiring your data is just the first step in producing a useful spectrum. Fortunately, systems are normally set up so that they perform the processing steps automatically. Most of the time they do an excellent job and your data is fine. Sometimes you may have special requirements and other processing will be required. This chapter looks at some of the things that can be altered to improve the appearance of the data for you. Note that most of the examples are for 1-D proton spectra but all of the sections are valid for certain types of 2-D experiment.

4.2 Zero Filling and Linear Prediction

Because we are always in a hurry (so many samples, so little spectrometer time) we always try to acquire that little bit faster than we should. This is particularly true with 2-D acquisitions which can be very time-consuming. As discussed previously, we try to minimise the number of increments to save time. This gives rise to highly truncated data sets and poor resolution. This can be made to look a little prettier by adding a load of zeros to the experiment before Fourier transforming it. We call this (somewhat obviously) 'zero filling'. Note that this doesn't add any information but it does make the result look nicer.

Linear prediction works in a different way by predicting what the missing (future) values would be, based on the existing (past) values. This approach is more powerful than mere zero filling but it also brings with it some risks (artefacts). You can't linear predict infinitely and so we tend to advise that one degree of linear prediction is about all the data can reliably take without going into the realms of fantasy. If we take the example of our COSY spectrum, we would probably linear predict out once (to double its size to 512) and then zero fill once or twice to take the final size to 1024 or 2048 points (in the indirect dimension). It is also possible to 'backward linear predict'. This allows us to reconstruct the first part of the FID which we can't collect because we have to wait a finite time for the transmit pulse signal to die away. This effect is known as 'ring down' and causes baseline distortion. Backward linear prediction allows us to throw these points away and replace them with what might have been there.

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4.3 Apodization

Sometimes the FID doesn't behave as we would like. If we have a truncated FID, Fourier transformation (see Section 4.4) will give rise to some artefacts in the spectrum. This is because the truncation will appear to have some square wave character to it and the Fourier transform of this gives rise to a Sinc function (as described previously). This exhibits itself as nasty oscillations around the peaks. We can tweak the data to make these go away by multiplying the FID with an exponential function (Figure 4.1).

This has the effect of smoothing the FID away to zero, thus yielding lovely peaks. We call this 'exponential multiplication' for obvious reasons!

It is also possible to play other mathematical tricks with the FID. For example, we may want to make our signals appear sharper so we can see small couplings. In this case, we want our FID to continue for longer (an infinite FID has infinitely thin lines when Fourier transformed). To do this we use 'Gaussian multiplication'. This works exactly the same way as exponential multiplication but uses a different mathematical function (Figure 4.2).

It should be noted that Gaussian multiplication can severely distort peaks and also reduce signal-to-noise of the spectrum so it is not a good idea to do this if you have a very weak spectrum to start with. Spectrum 4.1 shows a real case where Gaussian multiplication has been able to resolve a triplet. Note that it is possible to just make out the triplet nature of the peak in the unmultiplied spectrum – Gaussian multiplication helps verify this and also allows us to measure the splitting pattern.

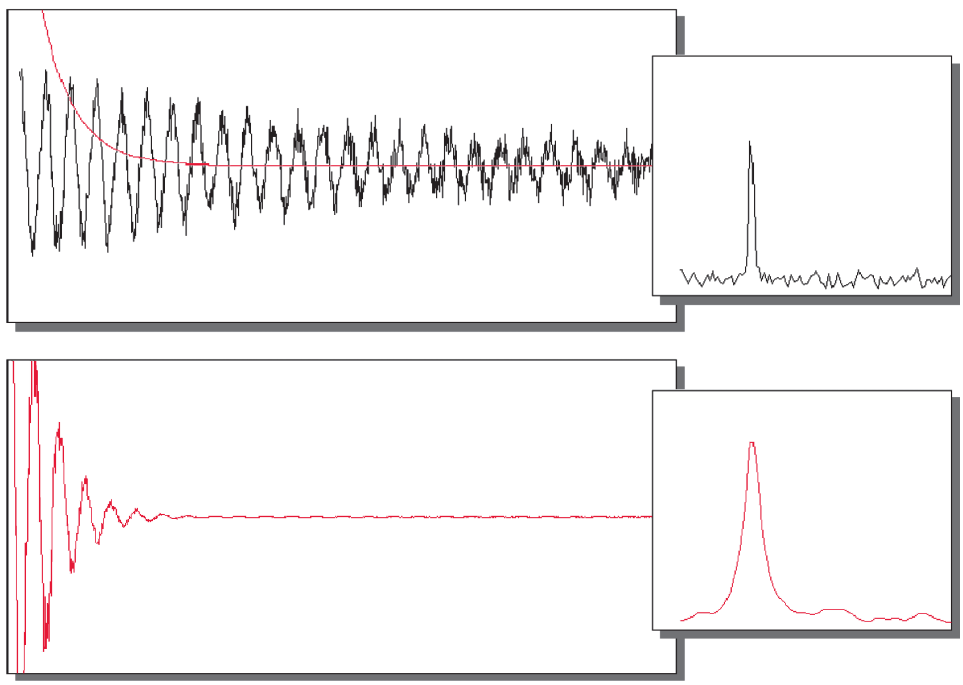


Figure 4.1 Exponential multiplication.

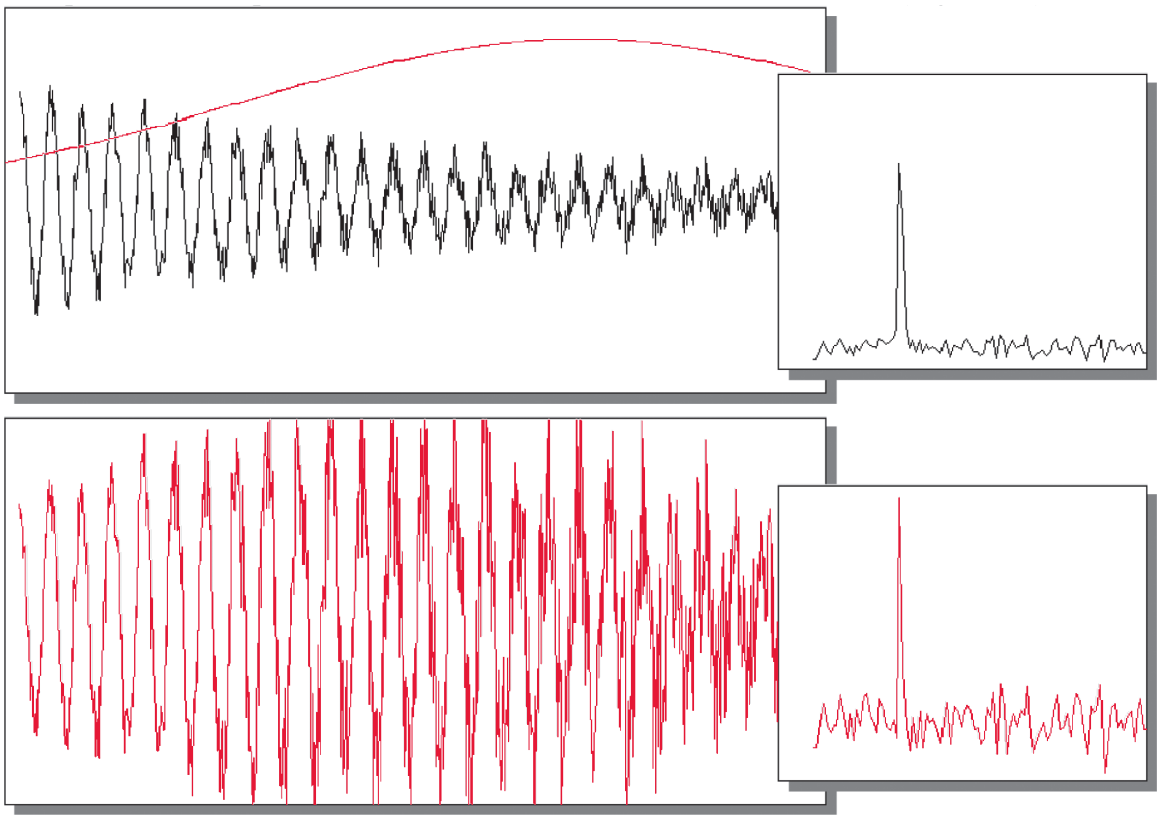
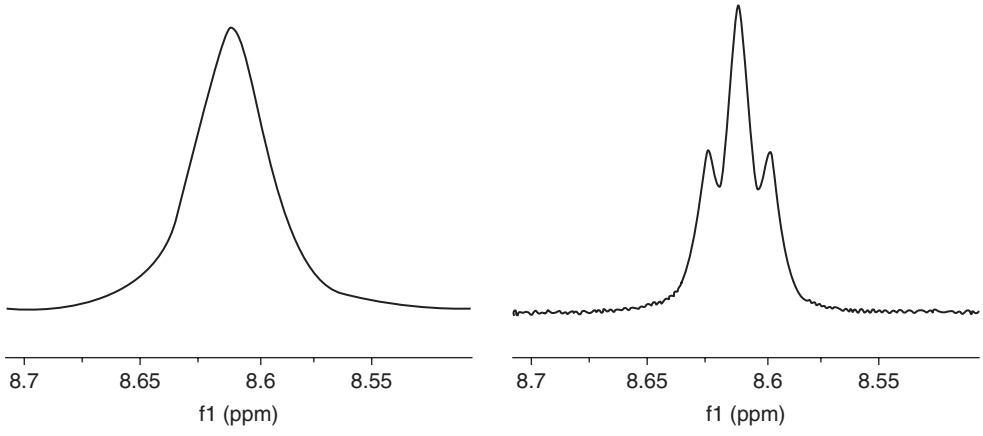


Figure 4.2 Gaussian multiplication.



Spectrum 4.1 Gaussian multiplication in action.

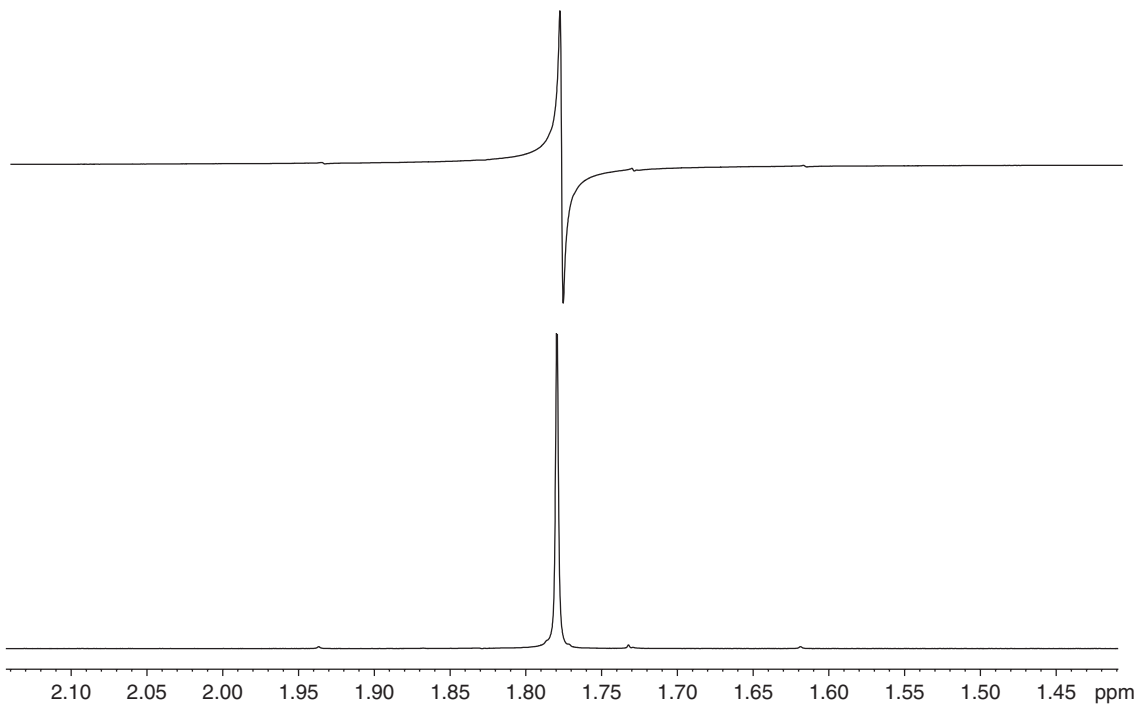
There are many other apodization functions which are used for specific types of NMR data. In fact you can make up your own if you want to but for most data sets, the 'canned' ones that are shipped with the instrument are more than adequate.

4.4 Fourier Transformation

As mentioned earlier, we acquire data in the 'time domain' but to make sense of it, we need to view it in the 'frequency domain.' This is where the Fourier transformation comes in. There is not too much to do here – there are no parameters to change. It is a necessary step but the automatic routines will perform this for you with no input.

4.5 Phase Correction

For several technical reasons, it is not possible to acquire NMR data with perfect phase. One reason is the inability to detect XY magnetisation correctly; another is the fact that we are unable to collect the data as soon as the spins are excited. These limitations mean that we have to phase correct our spectrum so that we end up with a pure absorption spectrum. What we *don't* want is a dispersion signal (see Spectrum 4.2).



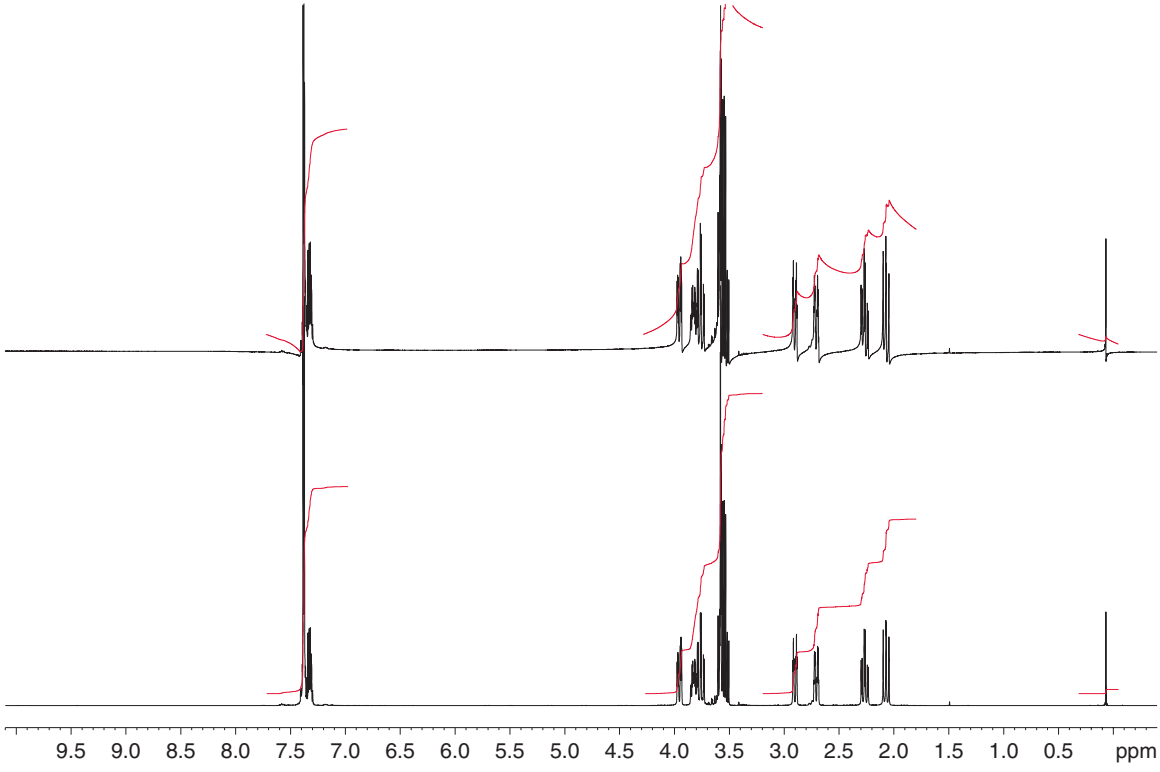
Spectrum 4.2 An absorption signal (below) and dispersion signal (above).

The XY problem gives rise to a constant phase error across the spectrum, the delay problem gives a linear phase error. To correct for this, we have two phase adjustment parameters at our disposal: zero and first order.

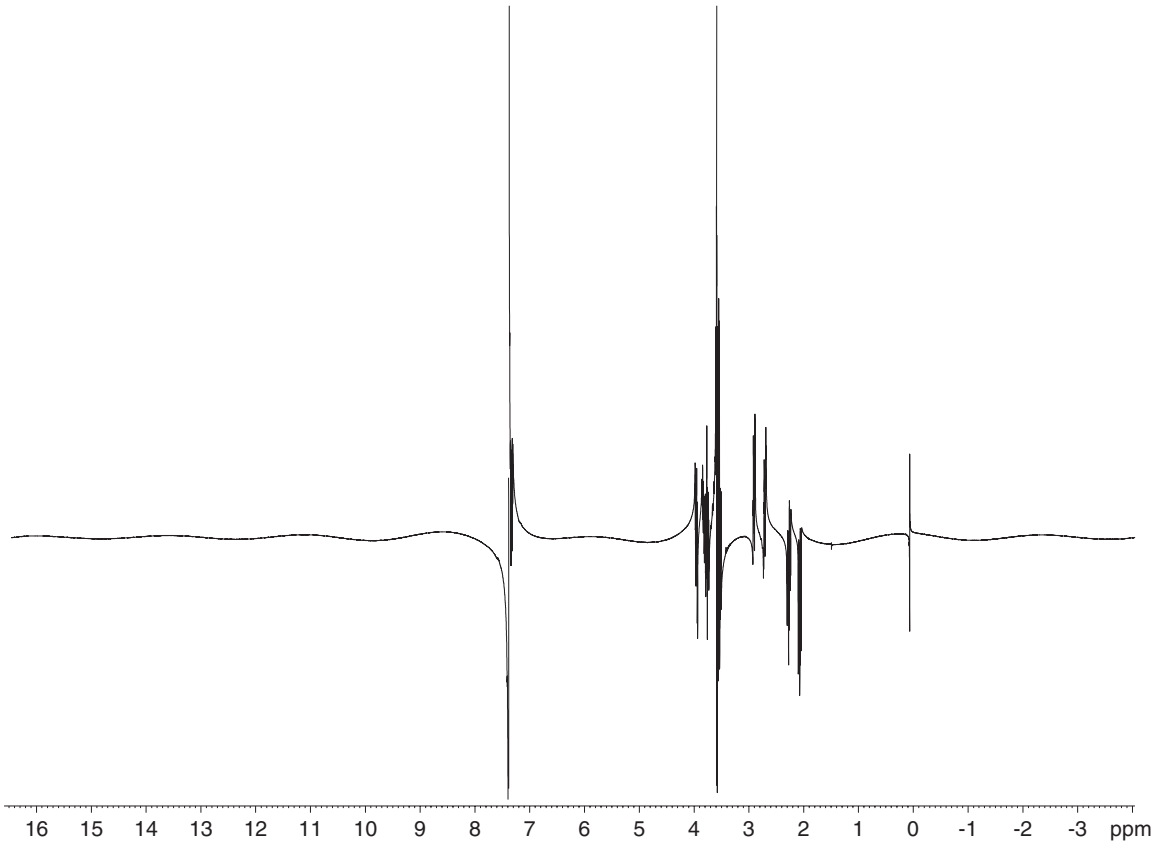
Modern NMR software comes with very good automatic phase routines so most of the time you should end up with a beautifully phased spectrum. Sometimes, however, the software doesn't quite perform and you may need to tweak the phase manually. It can take a bit of familiarity to get this right but it is just a matter of practise. If you remember that the zero order adjustment works constantly across the spectrum and that the first order doesn't, it is quite easy to see what is going on. Normally the software gives you an option of setting the 'pivot point' of the first order adjustment (i.e., the frequency in the spectrum where there is no effect from the first order adjustment). This pivot point is normally set to the largest peak.

Spectrum 4.3 shows how the phase can be improved with a manual tweak. Note that in a poorly phased spectrum, the integrals will be distorted such that they are pretty much unusable.

So far, we have talked about phasing 1-D spectra but this is also valid for some 2-D experiments. Phase-sensitive 2-D experiments also require phasing in one or both dimensions. Similar approaches are used as described here. Note that this is not necessarily the case for all 2-D experiments as some of them are collected in 'magnitude mode' where we look at only the intensity of the signals, not their sign.



Spectrum 4.3 A well phased spectrum with reliable integrals (below) and a badly phased spectrum with unusable integrals (above).



Spectrum 4.4 Too much first order phase!

One last cautionary note: the first order phase can be increased beyond +/– 360° – but shouldn't be! If this happens, you will end up with a distorted, 'wavy' baseline. A sine wave is in effect superimposed on the spectrum, so if you see a wavy baseline, check that you haven't wrapped the phase too far. Spectrum 4.4 shows what happens when you go a bit mad with first order phase! If you end up in this position, do not attempt any kind of baseline correction as this will add to your problems. Just set both your phase parameters back to zero and start again. . .

4.6 Baseline Correction

There are many reasons why your baseline may not be as flat as you would like. Many of them are hardware-related; some are brought about by having a distortion in the early points in the FID. They can also be caused by background in the probe (this is often the case for Fluorine spectra due to PTFE in the probe). Whatever their cause, bad baselines not only make the spectrum look poor, they also give rise to poor integrals. Whilst there has been a lot of work at improving the hardware, there is still a need to massage the baseline to make it look good. There are numerous algorithms to help with your

baseline and these will generally be applied automatically by the software that was used to acquire your data. These poor baselines are particularly noticeable when you have a very weak sample (for example, carbon spectra). It is also possible to manually correct your baseline if the automatic algorithms fail. In this case, you tell the software where the baseline should be and it then performs a spline-fit to level it.

4.7 Integration

As you are no doubt aware, integrals are one of the key parameters in the interpretation of proton spectra and are pivotal in quantification. They measure the area under a peak and this is directly proportional to the number of protons (in the case of proton NMR) in that environment. Most software will automatically try to identify the peaks in your spectrum and integrate them for you. If you need to do it yourself, then it is a fairly trivial matter of defining the start and end point of the integrals of interest. The only complication is that you may need to tweak the slope and bias of the integral. This should be unnecessary if you have got the phase and baseline of your spectrum correct. If you find that you need to adjust slope and bias, we suggest that you go back and try to sort out baseline and phase a bit better.

Integrals may appear low on signals that have a long relaxation time (see Chapter 3). If this is the case, then you should acquire your data with a longer relaxation delay. This is likely to be most noticeable on singlets and isolated protons as these tend to have quite long relaxation times. If you have poor signal to noise, this will also affect the accuracy of your integrals.

4.8 Referencing

As mentioned in Chapter 3, we standardise our reporting of chemical shifts with reference to TMS or the residual solvent peak. Your spectrometer software should do this for you automatically. If it gets it wrong (which is possible if you have a mixed solvent or a spurious peak near TMS), then you can set it manually using your software.

4.9 Peak Picking

If you want accurate chemical shifts or splittings, peak picking can help. However, it is worth issuing a health warning here! The accuracy of your chemical shifts and your splittings is limited by the digital resolution of your spectrum. This means that whilst the computer is happy to spout figures to four decimal places, in reality you may not be able to measure to better than +/– 0.5 Hz. Always check your digital resolution before trying to quote things too accurately. Don't forget, your chemical shifts will be influenced by concentration, temperature and pH so it is probably pointless quoting chemical shifts to a greater accuracy than 0.05 ppm except in special circumstances. Also, be warned that measured splitting is influenced by line width, so very broad peaks (or very close peaks) may show a smaller value than the real value.

5

Interpreting Your Spectrum

We should perhaps make a few important points before going any further – the title of this chapter is highly ambitious! We certainly cannot promise to turn you, the reader into expert interpreters in the time it takes you to read this section. Experience is essential and to become really proficient in this area, you need to critically examine literally *thousands* of spectra. However, be that as it may, by establishing some sound principles and cultivating a critical approach to the spectra you encounter, this book should prove useful in helping you along the way.

It might be worth considering at this stage, what we really mean by the term 'spectral interpretation'. What do we consider to be acceptable criteria for the interrogation of spectral data? Is a cursory glance sufficient if you are also holding a mass spectrum in your other hand that shows a parent ion of the correct mass for your desired compound? Or should you throw every known NMR technique at all your compounds irrespective of how trivial the chemical change being attempted? These questions should be pondered in the light of the fact that an NMR spectrum should never be regarded in itself as an *absolute* proof of structure. If this is what is required, then you had better practise your crystal-growing skills because you will be needing the services of an X-ray crystallography department. That having been said, NMR data can certainly provide the next best thing – in the right hands.

Our initial observations are aimed at improving your understanding of 1-D proton spectra, though many of the principles we will try to establish will be equally applicable to other nuclei too. We will discuss issues specific to ^{13}C interpretation in Chapter 9.

As we mentioned in the Introduction, it is ironic that one of the major problems encountered when dealing with NMR spectra, is the sheer quantity of information that you are presented with. Unless you are practiced in the art of interpretation, you may find yourself swamped by it. Clearly, a methodical and universally applicable approach would be advantageous. There is not necessarily a 'right' or a 'wrong' way to approach a spectrum, but some ways are probably better than others! These are our 'top ten' recommendations, for what they are worth.

1. Take a moment to survey the spectrum and ask yourself if it is fit for purpose? Of course, if you have run it yourself, then it should be fine but this may not always be so with walk-up systems. Is the line shape and resolution up to standard? Has the spectrum been phased correctly? Is the vertical scale well adjusted so that you can see the tops of all the peaks (except perhaps, obvious

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singlets)? Are the integrals well displayed? If the horse is dead, don't flog it – get a new one. Note: a good walk-up system will run day and night, producing quality results for the vast majority of samples. However, the occasional spectrum may 'come off the rails' for no obvious reason, but remember that there are dozens of processes that must run correctly in the background before a high quality spectrum drops into the collection tray and a slight hiccup in any of them can spoil the end result. Some of these problems (vertical scaling, phasing and integration) can be rectified by reprocessing the acquired data and some cannot as the 'raw' data itself may have been corrupted (poor signal/noise and sub-standard shimming).

2. If the spectrum is satisfactory, you can get to work on it. Can you identify any obvious impurities or solvents that might be present? Crossing them off at this stage is a valuable exercise in data reduction and clears the way ahead so that you can concentrate on the important peaks.
3. Does your proposed structure exhibit any special features likely to have a significant effect on your spectrum? (e.g., chiral centres, sites of potential restricted rotation, abnormal stereochemistry, etc.)
4. Can you identify a signal that gives a clear integration for a known number of protons?
5. Now work from left to right, assigning each signal, or groups of signals that you observe, to protons in your proposed structure. (If there is logic in starting at the left of the spectrum, it is that most molecules have some aromatic or heterocyclic core, to which, various alkyl functions are attached. If there is a problem with the core, then you will at least discover it promptly and be able to relate it to the alkyl components of the molecule.)
6. Interrogate each and every signal in your spectrum to check that they conform to the expected values for the *three* crucial NMR parameters: (1) chemical shift, (2) coupling pattern and (3) integration. And this, in a very real sense, must form the basis of our working definition of 'interpretation.' In the words of the song by Meatloaf 'two out of three ain't bad.' In NMR, however, two out of three isn't good enough! Obviously, in order for you to match your observed values for chemical shifts and couplings, to expected values, you will need a great deal of data at your disposal and this will be provided in the following chapters.
7. If you note an obvious mismatch between observed and predicted data, might you have overlooked something in (3) above? Interpretation is essentially an iterative process. Try to maintain a degree of flexibility in your approach – without being *too* flexible! Achieving this balance takes practise! If there is still no way of reconciling observations with predictions, you must accept the strong probability that your proposed structure is incorrect.
8. If so, propose an alternative structure and start the whole process again. Alternatively, could your sample be a mixture? If so, might your sample benefit from a chromatographic investigation at this stage or is it possible to qualify and quantify the components directly?
9. If you have any reasonable cause for doubt (e.g., because some key signals in your spectrum are obscured, etc.), would the acquisition of more NMR data be helpful? If so, consider exactly what you wish to achieve and select the appropriate technique and gather the data.
10. Re-evaluate all data again and again until you are as happy as possible with all aspects of your spectrum. Guard against complacency! Is it watertight? Check on this by asking yourself if you would be happy to stand up in a court of law and defend your efforts.

Adherence to all these points might seem to make the whole process of interpretation incredibly convoluted and unappealing but in reality, it should eventually becomes 'second nature'. Developing the theme further, you will hopefully soon get used to mentally synthesising the spectra of compounds you look at and matching these against what you see before you. The degree of deviation between the two

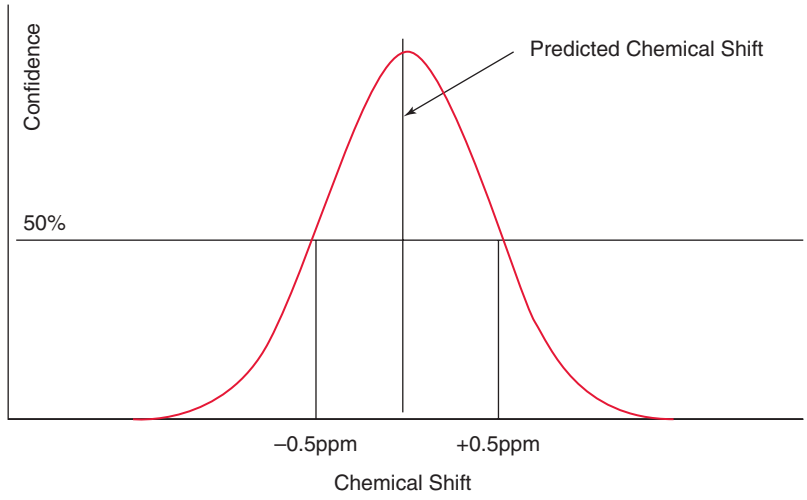


Figure 5.1 The confidence curve.

will be critical and we will explore this a little later. Should you be lucky enough to have a job which involves you looking at literally, *thousands* of spectra, then mental shortcuts will evolve and you will find yourself taking-in and digesting certain patterns almost subliminally in the same way that you read the words on this page. This is perfectly valid but *always* be on your guard against complacency! Take pride in your craft. *Make* yourself revisit that splitting pattern. There is nothing worse than falling into an obvious trap!

Of course, we do not mean to imply any kind of moral imperative here. For example, if you were checking out some dubious looking starting material that supposedly contained a given functionality (e.g., Ar-$\mathbf{CH_2}$-Br with no ortho substituent on the aryl ring and no other obvious steric clash between this aryl system and any other), then it would be quite acceptable – and even desirable – to exploit some obvious 'handle' in the form of a 'cutdown interpretation'. In this case for example, if there is no two-proton singlet (or AB system if the molecule is chiral) at around 4.6–4.3 ppm, then you can reasonably conclude that the stuff is not what you wanted. Job done. Move on.

We discussed earlier the concept of mentally synthesising a spectrum and trying to match it with real, observed data. This might sound like a task that a computer would be very good at – but this is certainly not the case in the field of proton NMR! We will discuss this in detail in a later chapter dealing with software issues. Our synthesised spectrum will need to be based on hard data. For example, in the case above, just how much leeway can you allow yourself in predicting the chemical shift of the Ar-$\mathbf{CH_2}$-Br protons? +/– 0.1 ppm? +/– 0.2 ppm? More? Instinctively, of course, the closer our observed signals are to our predicted ones, the better we will feel about them and vice versa. We have tried to portray this in Figure 5.1. "The Confidence Curve".

Whilst this might seem to state the obvious, it begs a number of very important questions. Ultimately, and perhaps after the expenditure of a great deal of mental effort, the bottom-line of an interpretation boils down to a simple question: Does this spectrum support this putative structure or doesn't it? Yes or no? The question is clearly black or white but the answer has plenty of scope for shades of grey! The yes/no question will have to be answered on the back of a great many other questions regarding the 'goodness of fit' of all the signals in the spectrum. Let's return to the simple case of Ar-$\mathbf{CH_2}$-Br above,

and consider how far the observed shift can be allowed to deviate from our predicted position. At what stage must we reject the structure? After all, how can we be sure that it isn't Ar-**CH_2**-Cl instead? Or what if the aryl ring is further substituted? What would you expect to see if there was a -NO_2 ortho to the -CH_2-Br? Or what about a para -NR_2?

Hopefully, we make our point. The confidence curve is NOT fashioned out of granite – it has to be applied with understanding and circumspection. It will always have the same basic shape but we have to be prepared to take a view on how wide it should be in every individual situation! Matters become a great deal more complex when we come up against structures that are sterically crowded (i.e., structures where bond constraints force various moieties into close proximity with one another).

Note also that the concept of the 'confidence curve' is equally applicable when considering coupling data. That is: What size coupling should I be looking for in this system or that? Is it too big? Too small?

Unfortunately, it is impossible to cover all the potential pitfalls that wait for the unwary. Many more will come to light in the following chapters but for now we will concentrate on supplying you with useful proton NMR chemical shift data . . . We have done this by collating various types of protons into convenient 'groups,' but first, let's clear the wood from the trees and deal with commonly encountered solvents and impurities in the regularly used NMR solvents.

5.1 Common Solvents and Impurities

As we pointed out earlier, it is a good idea if you can eliminate peaks from common solvents and impurities before getting into the real interpretation (note how chemical shifts can vary in different solvents – another factor which helps define the breadth of the confidence curve). Table 5.1 can be very helpful in this regard.

Table 5.1 The proton chemical shifts of common solvents and impurities.

Impurities	$CDCl_3$	DMSO	D_2O	MeOD
Acetic acid	2.13	1.95	2.16	1.99
Acetone	2.17	2.12	2.22	2.15
Acetonitrile	1.98	2.09	2.05	2.03
Benzene	7.37	7.40	7.44	7.33
Bromoform	6.85	7.75	insoluble	7.42
n-Butanol	3.67(t,6)	3.41(t,6)	3.60(t,6)	3.54(t,6)
	0.94(t,7)	0.89(t,7)	0.89(t,7)	0.93(t,7)
t-Butyl alcohol	1.28	1.14	1.23	–
Chloroacetic acid	4.14	4.28	4.25	–
Chloroform	7.27	8.35	Insoluble	7.88
Cyclohexane	1.43	1.42	Insoluble	1.45
1,2-Dibromoethane	3.63	3.84	3.79	3.72
Dichloroacetic acid	5.98	6.68	6.21	–
1,2-Dichloroethane	3.73	3.93	3.92	3.78
Dichloromethane	5.30	5.79	Insoluble	5.48
Diethyl ether	3.48(q,7)	3.42(q,7)	3.56(q,7)	3.48(q,7)
	1.20(t,7)	1.13(t,7)	1.17(t,7)	1.17(t,7)

Table 5.1 (Continued)

Impurities	$CDCl_3$	DMSO	D_2O	MeOD
Diisopropyl ether	1.12(d,6)	1.04(d,6)	1.12(d,6)	–
Dimethylacetamide	3.01	2.99	3.05	3.05
	2.94	2.82	2.89	2.91
	2.08	1.99	2.08	2.07
Dimethylformamide	8.01	7.98	7.91	7.98
	2.95	2.92	3.00	2.99
	2.88	2.76	2.86	2.85
Dimethyl sulfoxide	2.62	2.52	2.70	2.65
Dioxan	3.70	3.61	3.75	3.65
Ethanediol	3.76	3.42	3.66	–
Ethanol	3.72(q,7)	3.49(q,7)	3.64(q,7)	3.60(q,7)
	1.24(t,7)	1.09(t,7)	1.16(t,7)	1.17(t,7)
Ethyl acetate	4.12(q,7)	4.08(q,7)	4.14(q,7)	4.09(q,7)
	2.04	2.02	2.08	2.01
	1.25(t,7)	1.21(t,7)	1.23(t,7)	1.23(t,7)
Ethyl formate	8.04	8.23	8.16	–
	4.22(q,7)	4.17(q,7)	4.28(q,7)	–
	1.29(t,7)	1.24(t,7)	1.29(t,7)	–
Formic acid	8.02	8.18	8.22	–
Isobutyl methyl ketone	2.12	2.08	2.19	2.11
	0.92(d,6)	0.88(d,6)	0.88(d,6)	0.91(d,6)
Isopropyl acetate	2.02	2.00	Insoluble	1.99
	1.22(d,6)	1.21(d,6)		1.22(d,6)
Isopropyl alcohol	1.2(d,6)	1.06(d,6)	1.18(d,6)	1.14(d,6)
	4.03(m)			3.92(m)
Methanol	3.48	3.20	3.35	3.35
Methyl acetate	3.67	3.61	3.68	–
	2.05	1.92	2.09	
Methyl iodide	2.16	2.21	Insoluble	2.15
Morpholine	3.69(m)	3.52(m)	3.70(m)	3.64(m)
	2.85(m)	2.68(m)	2.79(m)	2.79(m)
Nitromethane	4.32	4.44	4.41	–
Petroleum spirit	1.28	1.28	Insoluble	1.30
(60°–80°)	0.90	0.89		0.88
Potassium Acetate	Insoluble	1.60	1.91	–
Propanol	3.60(t,7)	1.45(m)	3.61(t,7)	3.49(t,7)
	1.60(m)	0.87(t,7)	1.57(m)	1.54(m)
	0.93(t,7)		0.89(t,7)	0.92(m)
Proprionic acid	2.42(q,7)	2.26(q,7)	2.47(q,7)	–
	1.18(t,7)	1.03(t,7)	1.10(t,7)	–
Pyridine	8.60(m)	8.61(m)	8.50(m)	8.53(m)
	7.69(m)	7.83(m)	7.90(m)	7.84(m)
	7.28(m)	7.40(m)	7.46(m)	7.43(m)
Succinimide	2.75	2.63	2.78	–
Tetrahydrofuran	3.74(m)	3.63(m)	3.75(m)	3.72(m)
	1.85(m)	1.78(m)	1.88(m)	1.87(m)

Note: The peaks listed are singlets, unless described as doublets (d), triplets (t), quartets (q), or multiplets (m). Coupling constants (in Hz) are given in parentheses.

5.2 Group 1 – Exchangeables and Aldehydes

Of all the protons you may encounter in an NMR spectrum, exchangeables (any protons that exist in a state of dynamic equilibrium with any free water that might be present in the solvent, i.e., -OH, -NHR, -SH, -COOH, etc.) can be the least predictable – both with regard to their shape, and their position. A guide to their typical chemical shift ranges and any notable features is given in Table 5.2. An alkyl -OH or -NHR, for example, may be sharp and uncoupled, sharp and coupled, or broad and partially coupled. In a molecule with numerous exchangeables, they may appear distinct, or they may combine with each other, and with any water present – watch out for it particularly in D_6-DMSO solutions! Remember also that exchangeable protons will not be present in spectra of compounds run in D_4-MeOH, or D_2O solutions because they will have exchanged for deuterium. This forms the basis of a useful method for the identification of exchangeable protons which we will discuss in Chapter 7.

The origin of this unpredictability lies in the fact that they are relatively acidic, and can undergo exchange in solution. The appearance of the signals we observe is governed by the rate at which this process occurs, the rate being greatly influenced by the nature of the solvent, its water content, pH, temperature, and concentration of the compound.

Table 5.2 Typical exchangeable protons.

Exchangeable	Typical shift (ppm)	Remarks
Alkyl-OH	5–1	Can appear sharp and are capable of coupling to adjacent protons in dry aprotic solvents. Easily exchanged by shaking with D_2O.
Phenolic-OH	11–5	Often broad. Easily exchanged.
Phenolic-OH (H-bonded)	17–11	Can be broad but more usually sharp as proton exchange is slowed by need to break both bonds. Can therefore be more difficult exchange. Warm if necessary.
Alkyl-COOH	12–6	Usually broad but can be extremely broad! Very easily exchanged.
Aryl-COOH	14–8	As for alkyl-COOH.
Alkyl-NH_2/NHR	5–1	Generally similar to alkyl-OH but maybe somewhat broader even in dry solutions and less likely to couple to adjacent protons. Ability to protonate nitrogen tends to broaden protons and displace to lower field. Easily exchanged.
Aryl-NH_2/NHR	11–6	Usually broad. Easily exchanged.
Alkyl-$CONH_2$/-CONHR	9–7	Often broad but frequently couple. Primary amides often appear as two broad signals due to partial double bond character of amide bond. Often slow to exchange and may require warming/mild base
Aryl-$CONH_2$/-CONHR	13–7	As for alkyl-$CONH_2$/-CONHR
Alkyl-SH	5–1	Usually sharp and couple to adjacent protons. May need mild base to exchange (e.g., drop of $NaHCO_3/D_2O$ solution) Beware easy oxidation to -S-S-. Therefore important to locate!
Aryl-SH	7–3	Somewhat broader and easier to exchange than alkyl-SH. Again, an important one to find!
Alkyl/aryl-SO_3H	14–6	Similar to corresponding -COOH

A discussion of the kinetics of the process is outside the scope of this book because it won't help you to interpret your spectrum, but it is worth considering the two extremes of exchange, and the all-important region which lies between these extremes, as this might give you an insight into the seemingly fickle behaviour of exchangeable protons.

If you take a pure sample of ethanol, and run its NMR spectrum in dry $CDCl_3$, the hydroxyl proton will appear as a well-defined triplet, which couples to the adjacent -CH_2-, rendering it a multiplet. This is because the hydroxyl proton remains on the oxygen for relatively long periods of time, as there is nothing in the solution to entice it off, i.e., exchange (if any) is said to be very slow on the NMR timescale (less than about 1 s).

The presence of a trace of acid and water however, causes collapse of the hydroxyl-OH to a singlet (at lower field), the proton can now protonate, and de-protonate the oxygen very rapidly, as the process is catalysed by the acid, i.e., exchange is said to be fast on the NMR timescale (less than about 10^{-6} s).

In practise, one often encounters exchangeable protons which are exchanging at an intermediate rate, which leads to broadening of their signals, and only partial coupling (which can manifest itself as a mere broadening of the exchangeable proton and any they couple to). The actual position of the centre of a broad exchangeable signal, is dependant on how much water (or alternative exchange site) is present, and on the difference in the chemical shifts of the proton in the two environments. For example, a carboxylic acid proton, in a very dry solvent, may occur at about 12 ppm. A similar molar quantity of water in DMSO would absorb at around 3.5 ppm, and in such a solution, the carboxylic acid proton may well appear as a very broad signal, centred between these positions.

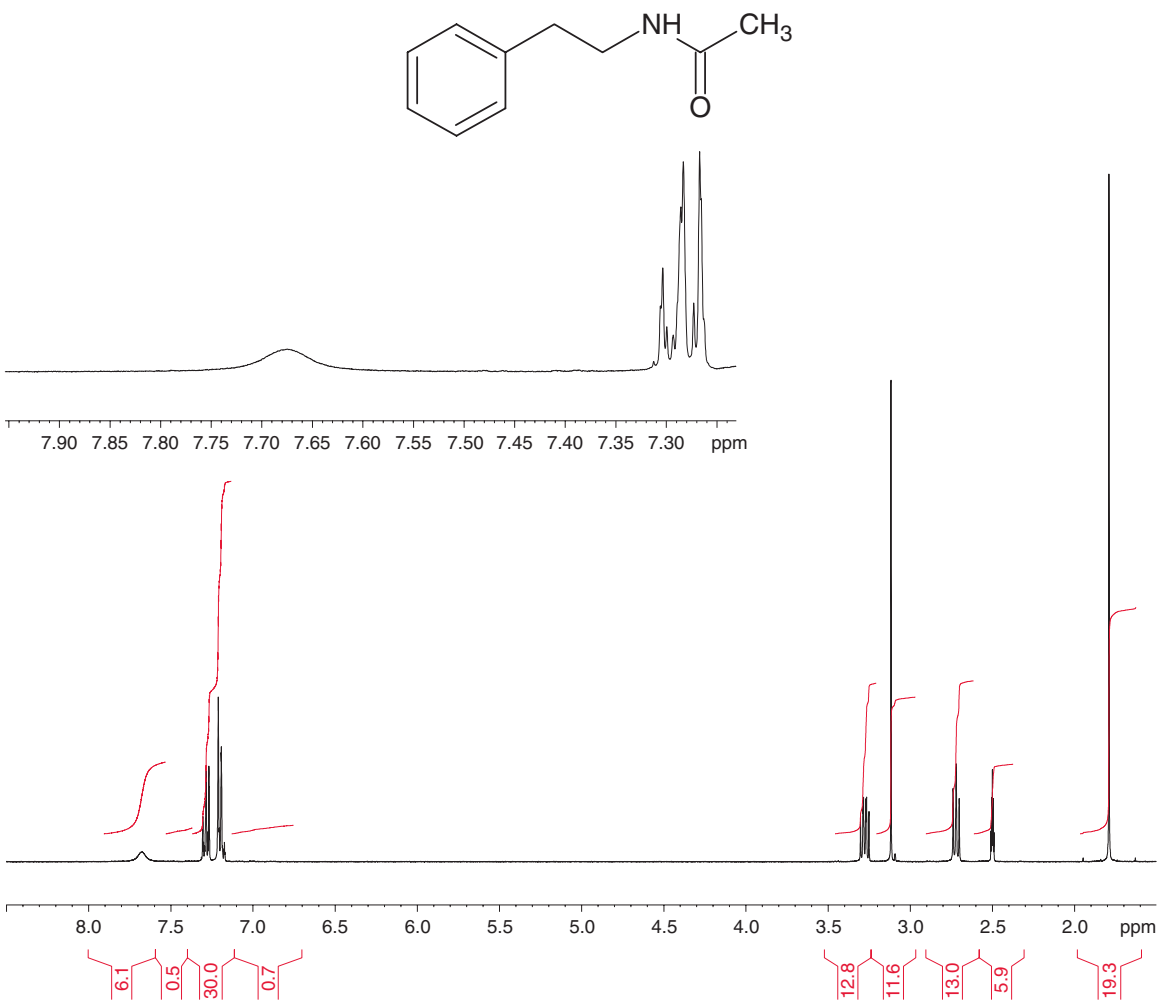
It is worth mentioning that >NH protons may often appear somewhat broader than their -OH counterparts, for another reason: >NH protons have another relaxation mechanism available to them (quadrupole relaxation) because the ^{14}N nucleus has an electric quadrupole moment. This extra relaxation capability can lead to a shorter relaxation time for >NH protons, and since the natural linewidth of a peak is inversely proportional to the relaxation time of the proton(s) giving rise to it, a shorter relaxation time will give rise to a broader peak.

This can lead to cases where an >NH of an amide, for example, couples to a -CH_2- adjacent to it, without *appearing* to show a reciprocal coupling itself, which as we know, is impossible! What happens is that its coupling becomes lost in the broadness of the signal – consider the compound shown with its spectrum (Spectrum 5.1).

Should your spectrum contain a very broad signal, such as the carboxylic acid proton of 4-fluoro benzoic acid shown in Spectrum 5.2 below and you aren't sure whether it's there at all, or whether your eyes are deceiving you, try looking along the baseline. Any slight lump which could be a signal will be seen more easily in this way. Of course if you are operating the spectrometer yourself, you only have to turn up the vertical gain but if you are looking at a walk-up spectrum, this trick might be useful.

As we have mentioned already, a very useful tool when trying to identify exchangeables is to exchange them for deuterium, which removes them from the spectrum. This will be covered in detail in Chapter 7 but don't be in too much of a hurry to do this – they are part of the spectrum and hold valuable information. If they are sharp enough, they may become potentially useful targets for NOE experiments which we will discuss later.

Finally, a brief word about aldehydes. They are included at the end of this group for convenience only and should be spotted easily. Aldehydes bound to aromatic rings give sharp singlets at 10.2–9.9 ppm, whilst in alkyl systems, they give sharp signals at 10.0–9.7 ppm, which couple to adjacent alkyl protons with a relatively small coupling constants (2–4 Hz).



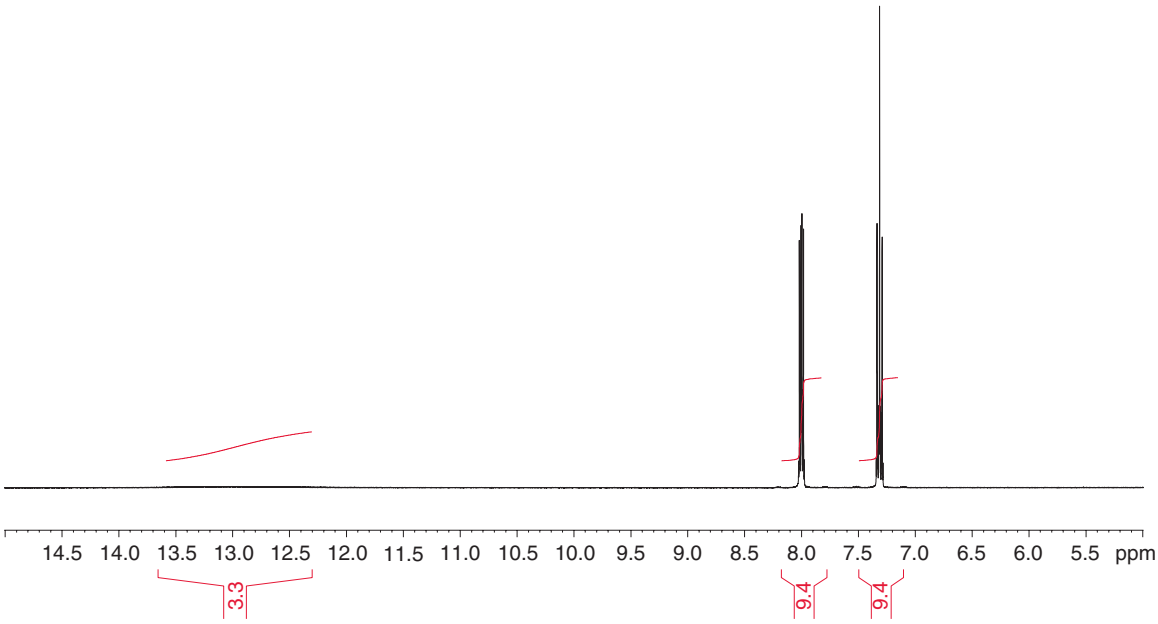
Spectrum 5.1 An amide NH (7.68 ppm) appearing to show no coupling to -CH_2 (3.28 ppm).

5.3 Group 2 – Aromatic and Heterocyclic Protons

Protons on aromatic rings are generally fairly predictable, both as regards to their position, and shape. The effects of substituents on a benzene ring are shown in Table 5.4.

They are applicable to compounds in the common NMR solvents – but not in D_6-benzene (or D_5-pyridine). The substituent effects are additive, but don't place too much reliance on chemical shifts predicted using the table, in compounds where more than two groups are substituted next to each other, as steric interactions between them can cause large deviations from expected values. Note that Table 5.4, like all others, does not cater for solvent shifts, etc!

A number of features become apparent on running an eye over these figures. Firstly, one saturated carbon in a substituent between the benzene ring and another group (e.g., -CH_2-OH) is sufficient to



Spectrum 5.2 A very broad carboxylic acid signal.

virtually isolate the ring from the influence of that other group, i.e., in this case, the -OH. This assumes that there are no abnormal 'through space' effects, of course, which we'll touch on later.

Secondly, groups which withdraw electrons (e.g., -NO_2, -COR, -COOR) cause shifts of the aromatic protons to lower field, to varying extents around the ring (the ortho-protons are generally the most influenced by a substituent, followed by the para-protons, and the meta-protons being the least influenced). But some groups which are known to be electron-withdrawing in alkyl systems, such as -OH, -OR, -NR actually bring about upfield shifts in aromatic systems. This is because, whilst these groups withdraw electrons inductively, they more than make up for this by supplying electrons, mesomerically. This effect is almost exactly balanced in the case of -Cl, which has very little influence on aromatic protons.

As for spin coupling around the benzene ring, Table 5.3 shows the expected ranges and typical values.

Note that: (1) in saturated systems proton–proton couplings are seldom observed beyond three bonds, but (2) in aromatic and heterocyclic systems, four- and even five-bond coupling is commonplace. This is because spin coupling is transferred by electrons. Where you have extended conjugation, you can expect to observe coupling over a greater number of bonds.

Table 5.3 Spin coupling around the benzene ring.

Position	Range (Hz)	Typical value
Ortho	6.0–9.4	About 8.0 Hz
Meta	1.2–3.1	About 2.5 Hz
Para	0.2–1.5	Negligible!

Table 5.4 Aromatic protons – the common substituent effects.

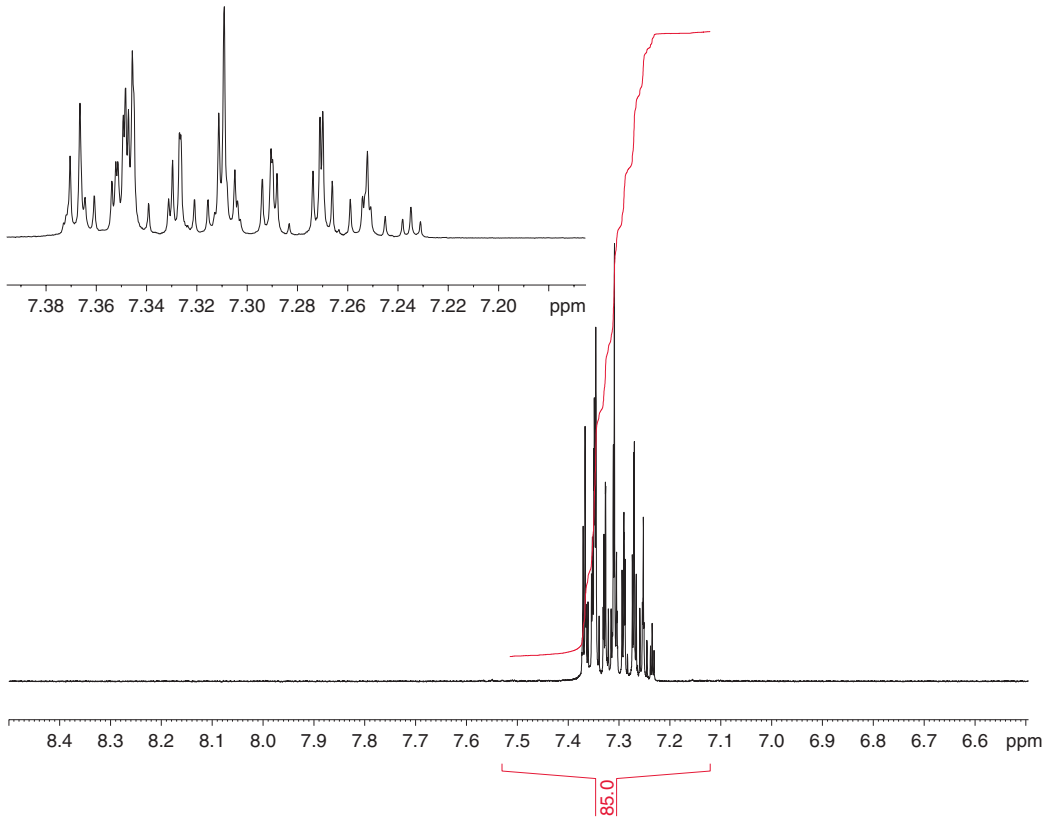
	Change in chemical shift (in ppm) relative to benzene (7.27)		
Substituent	Ortho	Meta	Para
-NO_2	0.95	0.17	0.33
-CHO	0.58	0.21	0.27
-COCl	0.83	0.16	0.30
-COOH	0.80	0.14	0.20
-$COOCH_3$	0.74	0.07	0.20
-$COCH_3$	0.64	0.09	0.30
-CN	0.27	0.11	0.30
-Ph	0.18	0.00	−0.08
–CCl_3	0.80	0.20	0.20
–$CHCl_2$	0.10	0.06	0.10
–CH_2Cl	0.00	0.01	0.00
–CH_3	−0.17	−0.09	−0.18
–CH_2CH_3	−0.15	−0.06	−0.18
–$CH(CH_3)_2$	−0.14	−0.09	−0.18
–$C(CH_3)_3$	0.01	−0.10	−0.10
–CH_2OH	−0.10	−0.10	−0.10
–CH_2NH_2	0.00	0.00	0.00
–F (couples!)	−0.30	−0.02	−0.22
–Cl	0.02	−0.06	−0.04
–Br	0.22	−0.13	−0.03
–I	0.40	−0.26	−0.03
–OCH_3	−0.43	−0.09	−0.37
–$OCOCH_3$	−0.21	−0.02	0.00
–OH	−0.50	−0.14	−0.40
–NH_2	−0.75	−0.24	−0.63
–SCH_3	−0.03	0.00	0.00
–$N(CH_3)_2$	−0.60	−0.10	−0.62

Note: A positive sign denotes a *downfield* shift (i.e., a shift to larger delta number; signal moves to the left).

In the light of this information, we can now consider a few examples of frequently encountered benzene-substitution patterns.

5.3.1 Monosubstituted Benzene Rings

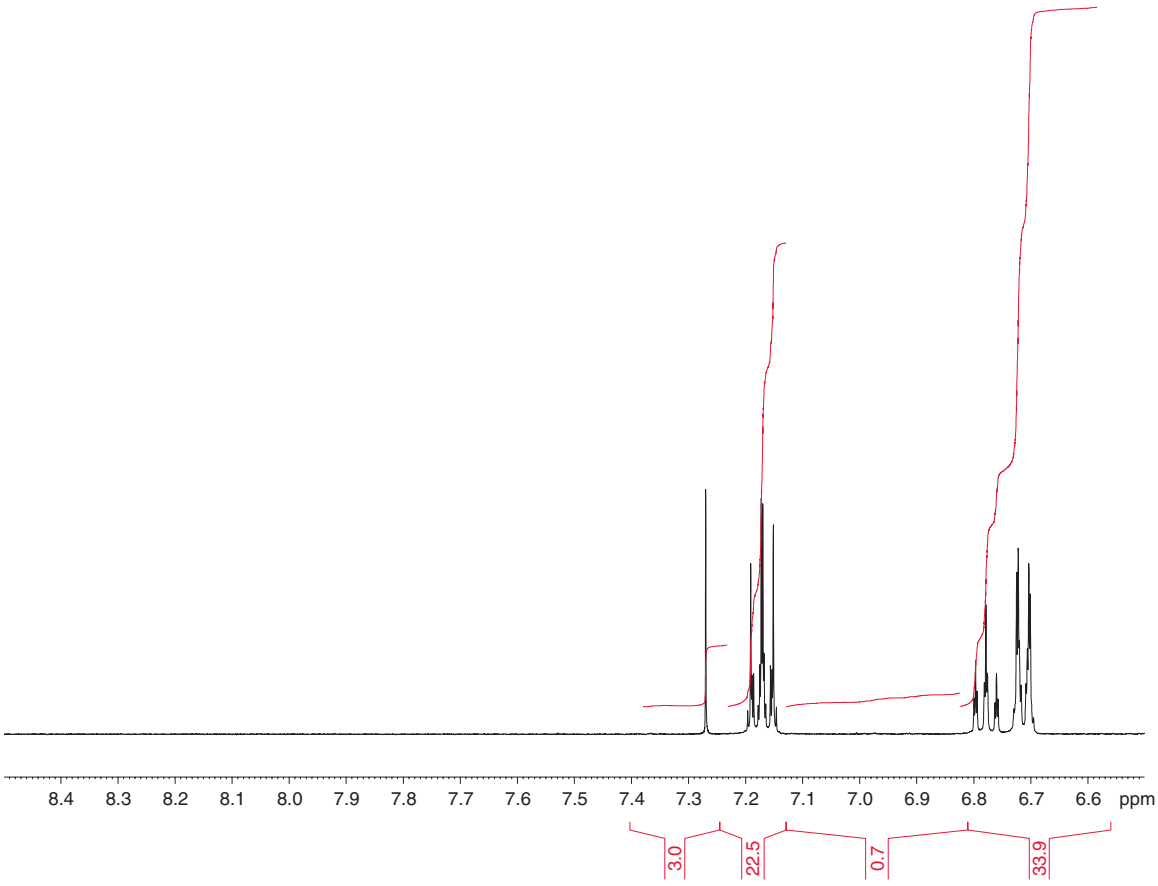
Spectrum 5.3 shows a typical pattern for a benzene ring monosubstituted with a relatively 'electron neutral' group. In this case, it's plain old chlorobenzene but similar patterns can be expected for any relatively neutral substituents, i.e., groups that neither donate nor withdraw electrons to or from the ring to any great extent (e.g., alkyl substituents). As the group has only a slight effect on the aromatic protons, they all resonate at quite close chemical shifts, giving anything from what can essentially be a singlet with small 'fringy bits' at its base, through to broader, heavily roofed multiplets as in this case though the exact appearance will of course vary considerably with spectrometer frequency. The complexity of the multiplet observed is dependant on two phenomena. The first, non-first-order behaviour, we will



Spectrum 5.3 A benzene ring bearing a single fairly neutral substituent.

discuss below. The second, magnetic non-equivalence, we will discuss in Section 5.3.2, which covers multisubstituted benzene rings.

Splitting patterns of signals are nice and predictable, only as long as the protons coupling to each other are separated by a chemical shift which is large relative to the size of the coupling between them. Notice for example, how the triplet and quartet of an ethyl signal are almost perfectly symmetrical. However, when signals coupled to each other are much closer together in the spectrum so that the difference between their chemical shifts and the size of their coupling is comparable, non-first-order effects can be expected. The closer they are, the more distorted (more non-first-order) they will be. In cases where signals are *very* close together, energy levels become mixed, and to quote L.M. Jackman: 'We find multiplicity rules no longer hold. Usually, more lines appear, and simple patterns of spacings and intensities are no longer found.' Such complex patterns can, in some cases, be subjected to mathematical analysis and the coupling information they contain extracted, but this practise has thankfully virtually died out with the advent of high-field spectrometers, or at least become a job for the computer! Our chlorobenzene example would look far less resolved (and thus more complex) than it does had it been run on an old 90 MHz instrument! Understanding, or at least recognising 'non-first-orderness' is very important and relevant to interpreting the spectra you will encounter.

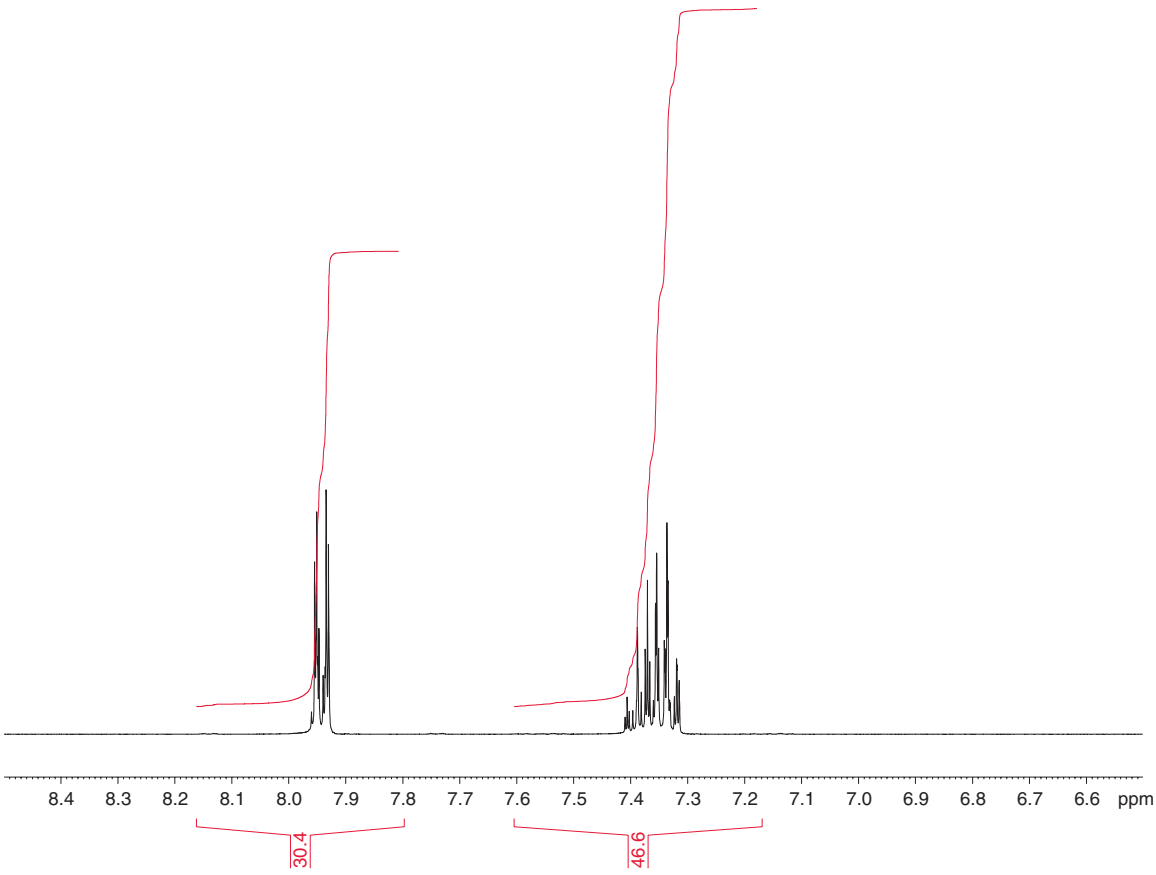


Spectrum 5.4 A benzene ring bearing a single electron-donating substituent.

Spectrum 5.4 shows a typical pattern of a benzene ring monosubstituted with an electron donating group (in this case, it's $-NH_2$).

Of the five aromatic protons, notice from the integration that two of them are below 7 ppm, occupying a position only slightly upfield of benzene itself, whilst the other three have been shifted upfield, above 7 ppm. A glance at Table 5.4 will show that these high-field signals can be assigned to the ortho, and para protons. The meta protons have been 'left behind', as it were. This shielding at the ortho and para positions is characteristic of simple electron-donating substituents.

These observations are of course underpinned by spin coupling observations. The meta protons both experience two ortho couplings of about 8 Hz which should yield a triplet (or more correctly, a doublet of doublets – note that a proton coupled to two other protons, which are different from each other, gives a doublet of doublets – when the two couplings are the same size, the signal appears as a triplet). What we actually observe is a very distorted 'triplet', the intensity of its lines being nothing like the 1:2:1 you might expect if you took Pascal's triangle too seriously! This distortion is referred to as 'roofing', and is the initial manifestation of the non-first-order behaviour just discussed.



Spectrum 5.5 A benzene ring bearing a single electron-withdrawing substituent.

The ortho- protons are shielded to the greatest extent and appear as a 'roofed' doublet of doublets whilst the less shielded para- proton presents as a triplet of triplets constructed from two large ortho- couplings and two small meta- ones. In cases where the electron donating substituent is oxygen-based (i.e., -OH or -OR), para- shielding can be as large as the ortho- shielding so that the ortho- and para- protons may have very similar chemical shifts. The consequence of this will be explored further in the next section.

Spectrum 5.5 shows the effect of a single deshielding substituent (carboxylic acid) on the benzene ring.

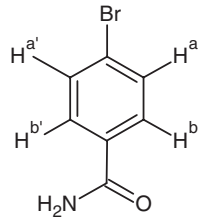
This time, we observe a pronounced downfield shift of the protons ortho- to the deshielding substituent and note that the signal is dominated by the large ortho-coupling and that it also bears a smaller meta- one. The signal is however both 'roofed' and is composed of more lines than you might naively expect.

The meta and para protons themselves appear as one ill defined multiplet, but on closer inspection, you can see that they are just resolved from each other. The para proton is slightly more deshielded than the meta protons and is centred at 7.39 ppm and is in fact a heavily 'roofed' triplet of triplets.

Note that Spectra 5.3, 5.4 and 5.5 are all plotted on the same scale to give you a feeling for the range of shifts that are typically encountered when looking at aromatic protons.

5.3.2 Multisubstituted Benzene Rings

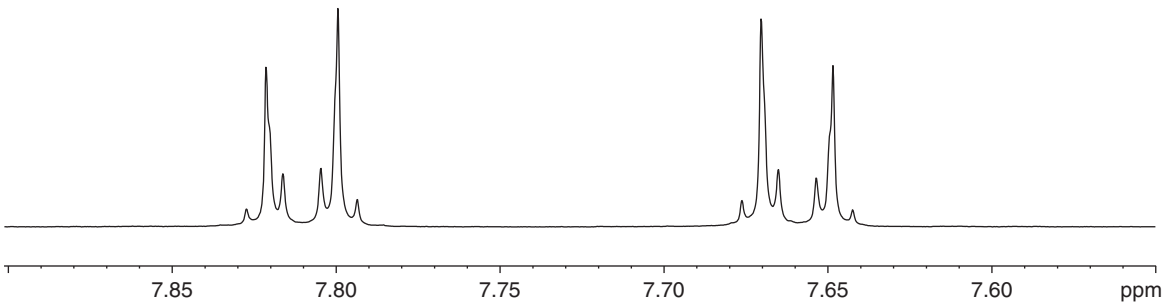
Moving on to multisubstituted aromatic systems, the real value of Table 5.4 soon becomes apparent. In dealing with such systems, it will not be long before you encounter a 1,4 di-substituted benzene ring. This substitution pattern (along with the 1,2 symmetrically di-substituted systems) gives rise to an NMR phenomenon that merits some explanation – that of *chemical* and *magnetic* equivalence and the difference between them. Consider the 1,4 di-substituted aromatic compound shown in Structure 5.1.



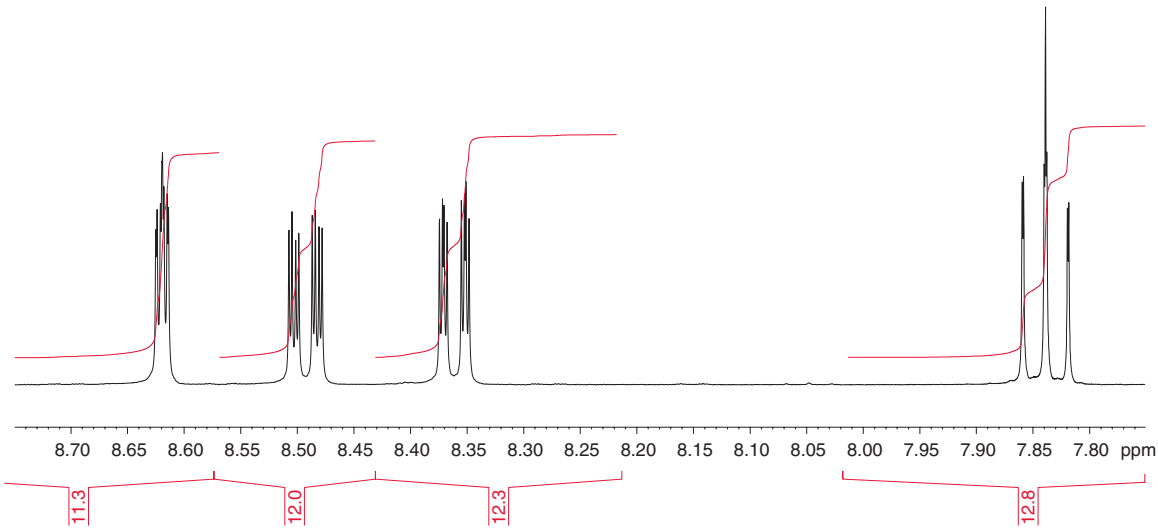
Structure 5.1 4-Bromobenzamide.

In terms of *chemical* equivalence, (or more accurately, *chemical shift* equivalence) clearly, H^a is equivalent to $H^{a'}$. But it is not *magnetically* equivalent to $H^{a'}$ because if it was, then the coupling between H^a and H^b would be the same as the coupling between $H^{a'}$ and H^b. Clearly, this cannot be the case since H^a is ortho to H^b but $H^{a'}$ is para to it. Such spin systems are referred to as AA′BB′ systems (pronounced *A-A dashed B-B dashed*). The dashes are used to denote magnetic non-equivalence of the otherwise chemically equivalent protons. What this means in practise is that molecules of this type display a highly characteristic splitting pattern which would be described as a pair of doublets with a number of minor extra lines and some broadening at the base of the peaks (Spectrum 5.6).

These extra lines are often mistakenly thought to be impurity peaks. An in-depth understanding of how they may arise is not really necessary for the purpose of interpretation. What is important is that you instantly recognise the appearance of such spin systems. Check that the system integrates correctly and check that the two halves of the system are symmetrical. *Note*: This phenomenon has nothing whatsoever



Spectrum 5.6 A typical aromatic AA′BB′ system (4-bromobenzamide).



Spectrum 5.7 Methyl 3-nitrobenzoate.

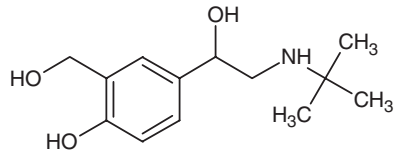
to do with chiral centres and is purely a function of the spatial arrangement of the protons involved as described above.

Spectrum 5.6 also shows a good example of 'roofing', which we touched on earlier. If you imagine the simple case of a pair of doublets well separated from each other, then all four of their lines will be of almost equal intensity. But when coupled doublets get closer together, they become distorted so that their inner lines become more intense, and their outer lines less intense. This is the onset of 'non-first-orderness'. The closer a pair of coupled doublets are to each other, the more extreme the effect becomes. It is worth noting that the phenomenon can sometimes be a useful interpretive tool, as the roofing can indicate which doublet is coupled to which other one, in spectra where you encounter two or more systems of this type: doublets which are coupled to each other, always roof towards a point between them, as shown.

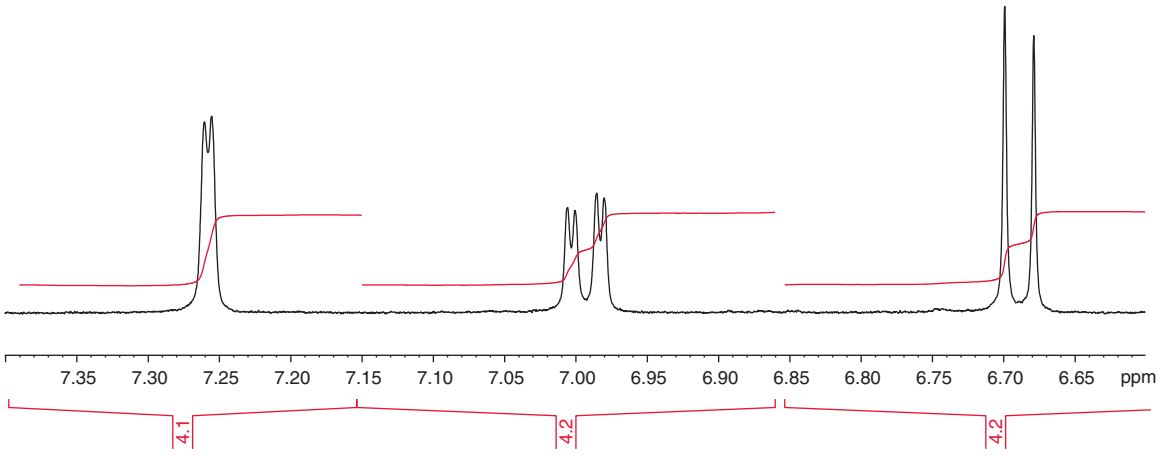
Obviously, there are too many possible combinations of groups for us to show a comprehensive collection of them but Spectrum 5.7 shows a nice example of a 1,3 di-substituted pattern featuring two strongly deshielding groups (a nitro group and a methyl ester) and serves to demonstrate the limitations of Table 5.4.

Predicting the chemical shift of the proton between the two substituents using Table 5.4 suggests a figure of 8.96 ppm. The observed figure is in fact 8.62 ppm. Very low field for sure, but significantly not as low as predicted. We find that this sort of error is quite commonplace in ring systems containing two or more very deshielding groups. Naively, it's as if the first group withdraws so much electron density from the ring that there is not much left for the second group to withdraw so the combined effect is less than expected. Be that as it may, Table 5.4 at least succeeds in predicting the correct *relative* positions of the protons, even if the actual values are a little off the mark.

And finally, Structure 5.2 and Spectrum 5.8 show a classic example of a 1,2,4 tri-substituted benzene ring, (a well known anti-asthma drug, salbutamol). Obviously, the scope for variation in these systems is vast!



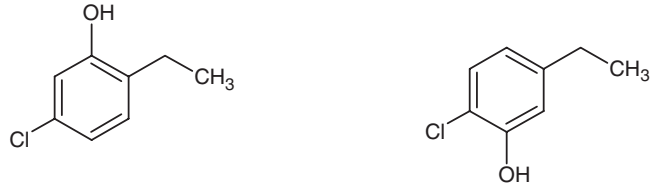
Structure 5.2 Salbutamol.



Spectrum 5.8 Aromatic region of salbutamol.

As a closing observation, it is difficult to say just how close you can reasonably expect predicted and observed values to be, even discounting highly sterically interactive systems mentioned earlier. A crude observation would be that the more substituents on the ring, the less accurate your predictions are likely to be. For what it's worth however, a rough working guide would be an expectation of, shift predictions within 0.3 ppm for multi-substituted rings in the absence of strong steric interactions between groups.

A final word of caution on aromatic systems – the electron donating groups (notably those in which oxygen is the shielding entity) can cause problems, because their ortho- and para- effects are so similar. Consider the following example – you are presented with a sample known to be one of the two isomers shown in Structure 5.3:



Structure 5.3 Two possible isomers.

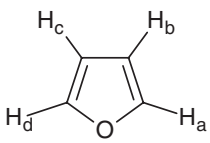
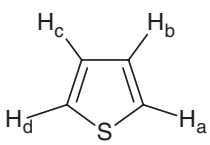
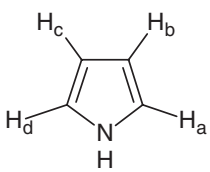
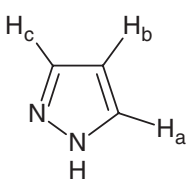
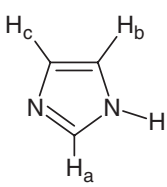
The two compounds will give very similar spectra and you would not be able to tell which isomer your sample is without an authentic spectrum of at least one of the isomers, for comparison. The only unambiguous way to tell these isomers apart, in the absence of an authentic spectrum, would be

by a nuclear Overhauser experiment (NOE), which we'll discuss later. Without performing such an experiment, you'd be ill-advised to chance your arm! Any chemical shift differences would be far too small to exploit with any certainty whatsoever!

5.3.3 Heterocyclic Ring Systems (Unsaturated) and Polycyclic Aromatic Systems

Heterocyclic systems resemble aromatic systems in some respects, but are more varied and interesting. We'll outline a few of these interesting features and then provide some useful chemical shift and coupling data in Table 5.5. It is not really feasible to provide information as in Table 5.4, as every heterocycle would need its own specific table and there are a great many heterocycles out there!

Table 5.5 Chemical shifts and couplings in some common heterocyclic and polycyclic aromatic systems.

Compound	Chemical shift (ppm)	Typical couplings (Hz) in parent *or* derivative
	a/d 7.4 b/c 6.3	a-b 1.8 b-c 3.5 a-c 0.8 a-d 1.6
	a/d 7.19 b/c 7.04	a-b 4.7 b-c 3.4 a-c 1.0 a-d 2.9
	a/d 6.62 b/c 6.05	a-b 2.6 b-c 3.4 a-c 1.1 a-d 2.2
	a/c* 7.55 b 6.25 * Note that tautomerism renders 'a' and 'c' equivalent in the parent NH compound.	a-b* 2.9 b-c* 1.6 a-c* 0.7 * Couplings measured in nontautomeric alkylated derivatives.
	a 7.7 b/c* 7.14 * Note that tautomerism renders 'b' and 'c' equivalent in the parent NH compound.	b-c 1.6 a-b ≈ a-c 0.8-1.5

(*continued*).

Table 5.5 (Continued)

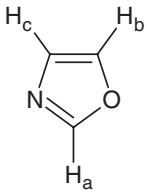
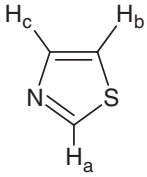
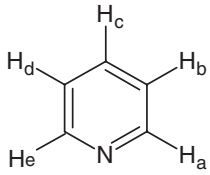
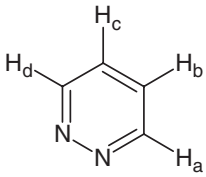
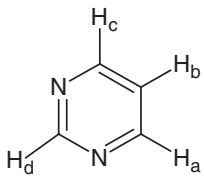
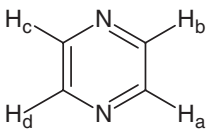
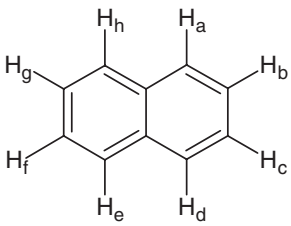
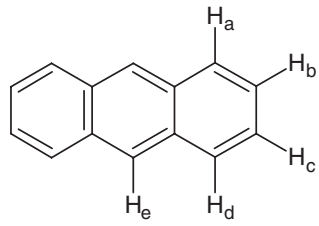
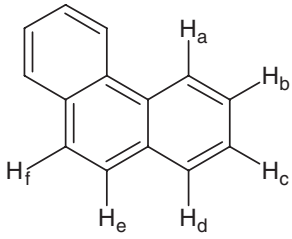
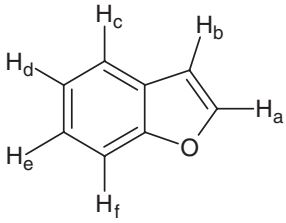
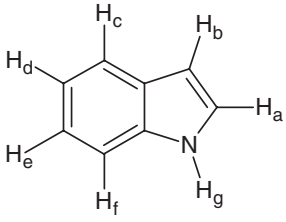
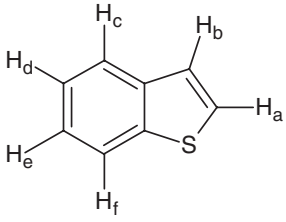
Compound	Chemical shift (ppm)	Typical couplings (Hz) in parent *or* derivative
	a 7.95 b 7.69 C 7.09	b-c 0.8 a-b 0.5 a-c 0.0
	c 8.88 b 7.41 C 7.98	b-c 3.1–3.6 a-b 1.9 a-c 0.0
	a/e 8.6 b/d 7.28 c 7.69	a-b 4.0–6.0 b-d 7.0–9.0 a-c 0–2.5 a-e 0–0.6 b-d 0.5–2.0 a-d 0–2.5
	a/d 9.17 b/c 7.68	a-b 5.0 b-c 8.4 a-c 2.0 a-d 3.5
	a/c 8.6 b 7.1 d 9.15	a-b 5.0 a-d 0 a-c 2.5 b-d 1.5
	a/b/c/d 8.5	a-b 1.8–2.0 a-d 0.5 a-c 1.5
	a/d 7.67 b/c 7.32	a-b 8.0–9.0 b-c 5.0–7.0 a-c 1.0–2.0 a-d ≈ 1.0 a-e ≈ 1.0

Table 5.5 (Continued)

Compound	Chemical shift (ppm)	Typical couplings (Hz) in parent *or* derivative
	a/d 7.98 b/c 7.44 e 8.40	Very similar to naphthalene above.
	a 8.65 b 7.61 c 7.57 d 7.86 e 7.70	a-b 8.4 b-c 7.2 e-f 9.0 a-c ≈ b-d 1.2 a-d ≈ 0.7
	a 7.5 b 6.66 c 7.5 d 7.13 e 7.2 f 7.4	a-b 2.5 c-d 8.0 d-e 7.3 e-f 8.4 c-f 0.8 b-f ≈ 1.0
	a 7.26 b 6.45 c 7.55 d 7.0 e 7.1 f 7.4 g 9.0–12.0 (very solvent dependant!)	a-b 3.0 c-d ≈ e-f 8.0 d-e 7.0 c-e ≈ d-f 1.3 b-f 0.7 c-f ≈1 b-f 0.7 g-a 2.5 g-b 2
	a 7.44 b 7.34 c 7.83 d 7.36 e 7.34 f 7.9	a-b 5.5 c-d ≈ d-e ≈ e-f 7.0–8.0 c-e ≈ e-f ≈1.0 b-f 0.8

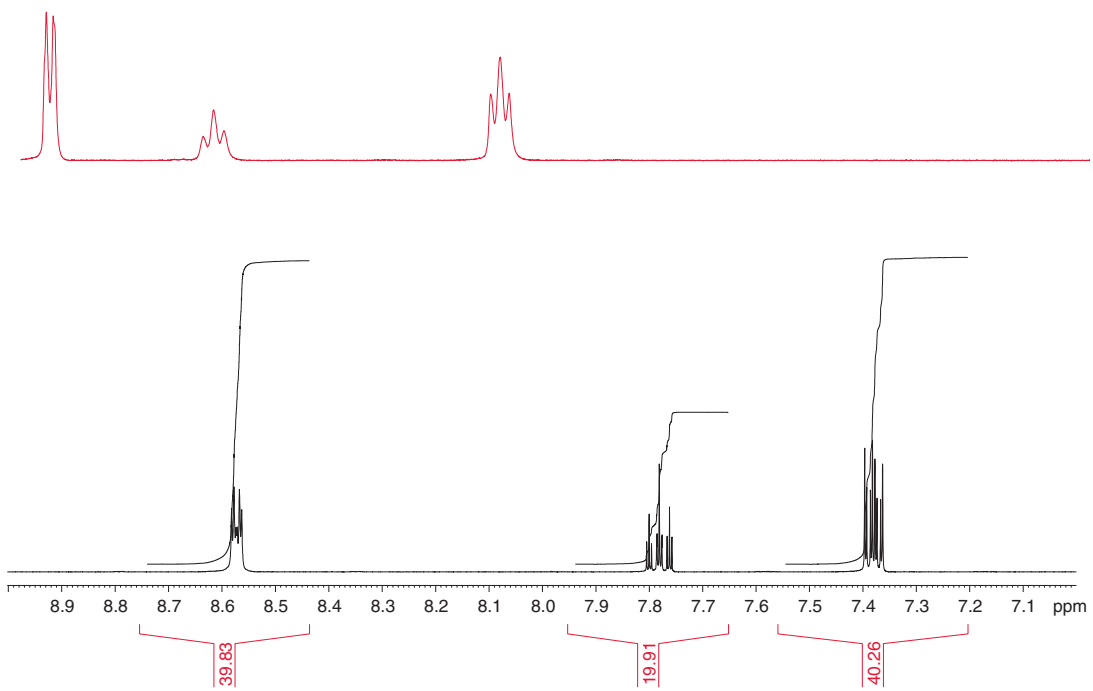
So when confronted with a problem involving an unsaturated heterocycle, our advice is to make yourself aware of the shifts and couplings of the parent compound (Table 5.5) and then use the known effects of substituents from Table 5.4 and 'superimpose' them. This will give you a rough guide only and your 'confidence curve' will need to be adjusted accordingly as the magnitude of the induced shifts are usually somewhat different and may vary within the heterocycle. In a 3-substituted furan, or thiophene, for example, the magnitudes of the ortho effects to the 2′, and 4′ protons are different – sometimes, considerably so!

One other, perhaps even more dramatic and common example concerns compounds like 2′ and 4′ hydroxy- and amino-pyridines. These compounds exhibit tautomeric behaviour and tend to exist in solution as the corresponding pyridone and imine. This reduces the familiar pyridine-like properties of the ring system, accentuating the effects of these substituents (in terms of induced chemical shifts) and at the same time, radically increasing the expected couplings 2′–3′ couplings.

The size of couplings around heterocyclic rings can also vary dramatically. Ortho- couplings in five-membered heterocycles such as furan and thiophene for example, are much smaller than in normal aromatic rings. Note also that even within a given heterocycle, there can be substantial variation in the size of ortho couplings themselves! As with any spectroscopic phenomenon, this should not be regarded as just another complication, but as an important part of your spectroscopic armour, or indeed, as part of your spectroscopic offensive weaponry for attacking problems of substitution etc.

Nitrogen-containing heterocycles are sometimes basic enough to protonate and form salts in acidic conditions and this leads to substantial changes in chemical shifts of their protons – see Spectrum 5.9 (pyridine alone, pyridine + DCl)

Note also that fluorine couplings to protons in heterocyclic systems can be well outside intuitive expectations! See Section 6.5.2 for an example!



Spectrum 5.9 Pyridine in DMSO solution (bottom) and with one drop DCl (top).

One last word on heterocycles. Very small couplings (<1 Hz) have been found to exist between some protons on *different* rings of bicyclic heterocycles. For example, in indole, there is a 3-7 coupling of about 0.7 Hz. In practise however, these very small couplings may only manifest themselves as a broadening of the signals concerned.

Obviously, this table is far from exhaustive but it establishes the typical shifts and couplings found in some of the more commonly encountered heterocycles.

5.4 Group 3 – Double and Triple Bonds

In this section, we will look at alkene, imine, enol ether and alkyne protons. It's convenient to consider the first three at this stage as they usually absorb in the 8-5 delta region and the alkyne is included here for convenience.

Alkene chemical shifts can be estimated using Table 5.6. Use this table with the same circumspection as you would all other tables of this type. It's a useful guide, not gospel.

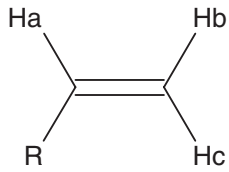
Substitute the additive values in Table 5.6 into the following equation:

$$\text{Approximate chemical shift of proton (ppm)} = 5.25 + Z_{\text{gem}} + Z_{cis} + Z_{trans}$$

Table 5.6 Estimation of chemical shifts for alkene protons.

R	Z_{gem} (ppm)	Z_{cis}	Z_{trans}
-H	0.00	0.00	0.00
-Alkyl	0.45	−0.22	−0.28
-CH_2-OR	0.64	−0.01	−0.02
-CH_2-SR	0.71	−0.13	−0.22
-CH_2-halogen	0.70	0.11	−0.04
-CH_2NR_2	0.58	−0.10	−0.08
>C=C< (isolated)	1.00	−0.09	−0.23
>C=C< (conjugated)	1.24	0.02	−0.05
-CN	0.27	0.75	0.55
-C ≡ C-R	0.47	0.38	0.12
>C=O (isolated)	1.10	1.12	0.87
>C=O (conjugated)	1.06	0.91	0.74
-COOH	0.97	1.41	0.71
-COOR	0.80	1.18	0.55
-CHO	1.02	0.95	1.17
-$CONR_2$	1.37	0.98	0.46
-COCl	1.11	1.46	1.01
-OR	1.22	−1.07	−1.21
-OCOR	2.11	−0.35	−0.64
-CH_2-Ar	1.05	−0.29	−0.32
-Cl	1.08	0.18	0.13
-Br	1.07	0.45	0.55
-I	1.14	0.81	0.88
-NR_2	0.80	−1.26	−1.21
-NRCOR	2.08	−0.57	−0.72
-Ar	1.38	0.36	−0.07
-SR	1.11	−0.29	−0.13
-SO_2R	1.55	1.16	0.93

Figure 5.2 shows typical couplings found in alkenes.:



J_{a-b} (*cis* coupling)	7–13 Hz, typically 11 Hz
J_{a-c} (*trans* coupling)	11–18 Hz, typically 16 Hz
J_{b-c} (geminal coupling)	Approximately 1–2 Hz

Figure 5.2 Typical couplings found in alkenes.

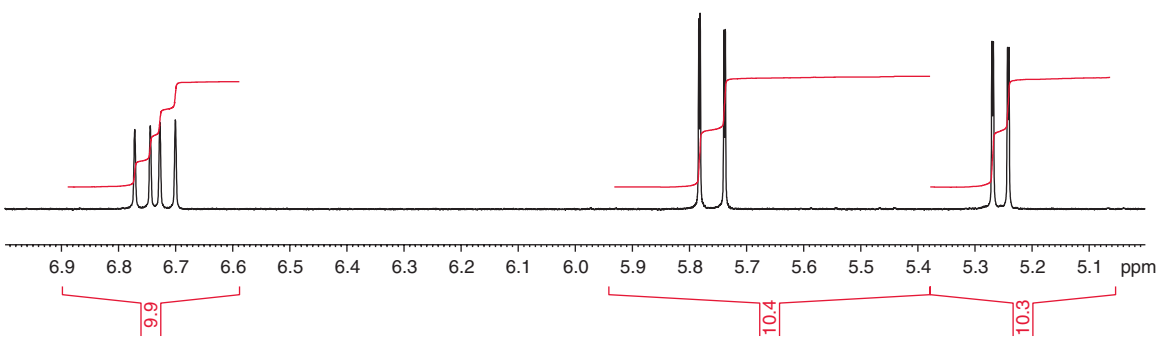
These couplings are exemplified below with reference to styrene (Spectrum 5.10).

Note that small couplings (approx. 1.0–2.5 Hz) would also be expected between the first CH_2 of any alkyl group (R) and both Hb and Hc.

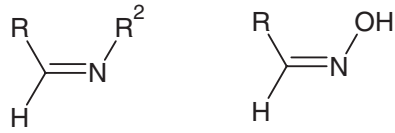
The actual sizes of the observed *cis*- and *trans*- couplings are influenced by the electronegativity of the substituents attached to the double bond. In general, the more electronegative the substituents, the smaller the observed couplings. (There is actually an approximately linear relationship between the size of the coupling and the sum of the electronegativities of the substituents).

It is interesting to note that in cases where an alkene is joined directly to an aromatic ring, the alkene proton geminal to the aromatic ring is invariably at the lowest field of the alkene protons. This is because the alkene bond tends to lie in the same plane as the aryl ring and for this reason, the geminal proton is held in the deshielding zone of the aromatic ring, as is the alkene proton cis to the aromatic ring. This is an example of anisotropy which we will discuss in some detail later on.

Determining whether an alkene is *cis* or *trans* in cases where the alkene is in the middle of a long alkyl chain is usually not possible by ^{1}H NMR as both cis and trans protons have very similar shifts in such circumstances, as do the -CH_2s attached to the alkene. Such a problem can however be dealt with using ^{13}C NMR where the shifts of these CH_2s are diagnostic.



Spectrum 5.10 The alkene protons of styrene (Ph-CH=CH2).

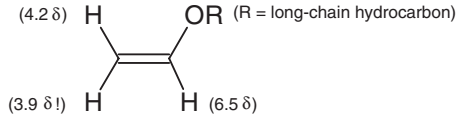


Structure 5.4 Double-bond moieties.

Other double-bond moieties which are often encountered include the imines and oximes (Structure 5.4).

Note that the lack of rotation about the double bond means that 'E' and 'Z' isomers are distinct entities in the same way that *cis* and *trans* isomers are distinct in conventional alkenes. It is not really feasible to give a comprehensive guide to the chemical shifts of these protons but expect them to be somewhat lower field (approx. 1 ppm) than for comparable alkenes, with chemical shifts being driven largely by the anisotropy of the substituents.

Enol ether protons are interesting in that their chemical shifts are unusually high field in comparison with other alkenes on account of lone pair donation into the double bond from oxygen (Structure 5.5). No special precautions are necessary when dealing with them as this is reflected in the values obtained using Table 5.6.



Structure 5.5 An example of an enol ether showing typical shifts.

Whereas alkene protons are relatively de-shielded by the overlapping p electrons of the double bond, alkyne protons are fairly shielded by their electronic environment. In common with alkenes, however, is the possibility of small, long-range coupling through the triple bond. The chemical shifts of alkyne protons are highly influenced by the electronegativity of groups attached to the other end of the triple bond as can be seen from the examples in Table 5.7. It is worth bearing in mind that alkyne protons may exchange in strongly basic solutions.

Table 5.7 The chemical shifts of alkyne protons.

Alkyne	Chemical shift (ppm)	Comments
R-C≡C-**H**	1.9	(R = long-chain hydrocarbon)
Ph-C≡C-**H**	3.1	
Ph-Ph-C≡C-**H**	4.2	(Ph-Ph = biphenyl rings)

5.5 Group 4 – Alkyl Protons

This section must necessarily be brief and general on account of the size of the category and the vast number of case studies we could dissect in detail.

For now, the discussion will be restricted to straightforward systems (open-chain and containing no chiral centres) and adhere to previous practise by supplying chemical shift data (Table 5.8) which will enable you to estimate the chemical shifts of methyl, methylene and methane protons you will typically encounter. Typical three-bond couplings in such systems can be expected in the region of 7–9 Hz, what variations there are being attributable to electronic effects of the substituents. These small variations can sometimes be exploited as a means of verifying which signal is coupled to which other, e.g., in cases where you are up against a molecule with two different -CH_2CH_2- systems. Perhaps we should mention at this stage that the single most significant factor in determining the magnitude of a three-bond (vicinal) coupling is the dihedral angle Φ, between the protons in question (Figure 5.3).

In open chain compounds that lack any chiral centre of course, rotation about all single bonds can be assumed to be both relatively 'free' and fast on the NMR timescale and the 7–9 Hz range quoted is the result of averaging of this angle. The same is of course not true in cyclic systems where structures are rigid and bond angles constrained. We will deal with this topic thoroughly in Section 6.6.5.

Let's stop for a moment, and reflect on what we have dealt with so far. In fact, we've covered quite a lot of ground already. We started by considering some basic theory and background to the

Table 5.8 Estimation of chemical shifts for alkyl protons.

X	C	X	C
-CH_3	0.5	-NR,COR	2.4
-Alkyl	0.6	-NR_2	1.6
>=<	1.3	-$^+NR_3$	2.4
-C≡C-Ar	1.7	-N=C=S	2.9
-C≡C-R	1.4	-Ar	1.8
-CN	1.7	-OCOAr	3.5
-COAr	1.9	-OCOR	3.1
-COCl	1.8	-OAr	3.2
-$CONR_2$	1.6	-OH	2.6
-COOR	1.5	-OR	2.4
-COR	1.6	-OSO_2Ar	3.4
-Cl	2.5	-SAr	2.1
-Br	2.3	-SR	1.9
-I	2.1	-CF_3 *	1.1
-NO_2	3.7	-F *	3.6

*Note: both show coupling to neighbouring alkyl protons.
For methine protons, approx. chemical shift (ppm) will be: 0.1 + CX + CX1 + CX2
For methylene protons, approx. chemical shift will be: 0.3 + CX + CX1
For methyl protons, approx. chemical shift will be: 0.5 + CX
Note: the values 0.1, 0.3 and 0.5 are just 'fudge factors' to give better estimates.
For more extensive shift and coupling data on a wider variety of compounds, we would recommend *Structure Determination of Organic Compounds* by E. Pretsch, P. Bűhlmann and C. Affolter (Springer, ISBN 3-540-67815-8)

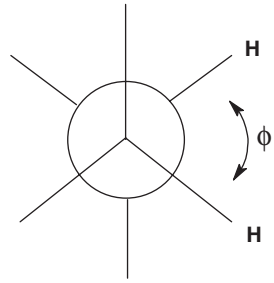


Figure 5.3 Dihedral angle Φ between protons.

subject. We've looked at the very important issues of sample preparation and skimmed the surface of spectrometer set up. We've established a good standard method of dealing with spectra, by partitioning the information available into coherent segments – both with respect to the nature of the information (chemical shift, multiplicity and integration) and also, with respect to the various classes of proton commonly encountered. And finally, we've spent a good deal of time examining these different types of protons in some detail. In fact, it might be tempting to wonder what more needs to be said on the subject of spectral interpretation. After all, you now have in your grasp some pretty powerful tools – tables and so on, which will, if used prudently, give you a good idea of what to expect from relatively simple spectra.

Unfortunately, it's not quite as simple as that. In the next chapter, we'll delve a little deeper and have a look at some possible pitfalls you may encounter in more complex spectra.

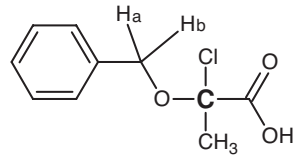
6

Delving Deeper

6.1 Chiral Centres

It's fair to say, that if all molecules were flat and lacked chiral centres, the interpretation of their NMR spectra would be far easier than it actually is but it would be a whole lot less fun too! In moving on to discuss more interesting chiral compounds, we have an opportunity to deal with some commonly held misconceptions and urban myths that can severely limit understanding of the subject.

A good working knowledge of stereo-chemistry is certainly a big advantage when looking at the spectra of chiral molecules. Let's start by considering Structure 6.1.



Structure 6.1 A chiral molecule.

Clearly, the highlighted carbon is a chiral centre (it has four different groups attached to it). For this reason, the two protons Ha and Hb can never be in the same environment. The fact that there is free rotation around all the single bonds in the molecule is irrelevant. This can best be appreciated by building a model of the molecule. Having done so, look down the molecule from left to right as drawn and rotate the C-O bonds so that Ha and Hb rotate. It should now be clear why these two protons can never occupy the same space and are therefore not equivalent.

Now for the next big step forward: if they are not equivalent, then there is no reason for them to have the same chemical shift. Another big step: and if they have different chemical shifts, they will couple to each other. In fact, in molecules of this type (i.e., that have an isolated CH_2 in the region of a chiral centre) the likelihood is that the CH_2 will be observed as a pair of doublets (see Spectrum 6.1).

How close they are to each other, or how far apart, is not something that can be easily estimated as it depends on the through-space interactions (anisotropies) of both protons with all the other groups in the molecule. That having been said, the two doublets are likely to be within 1 ppm of each other and

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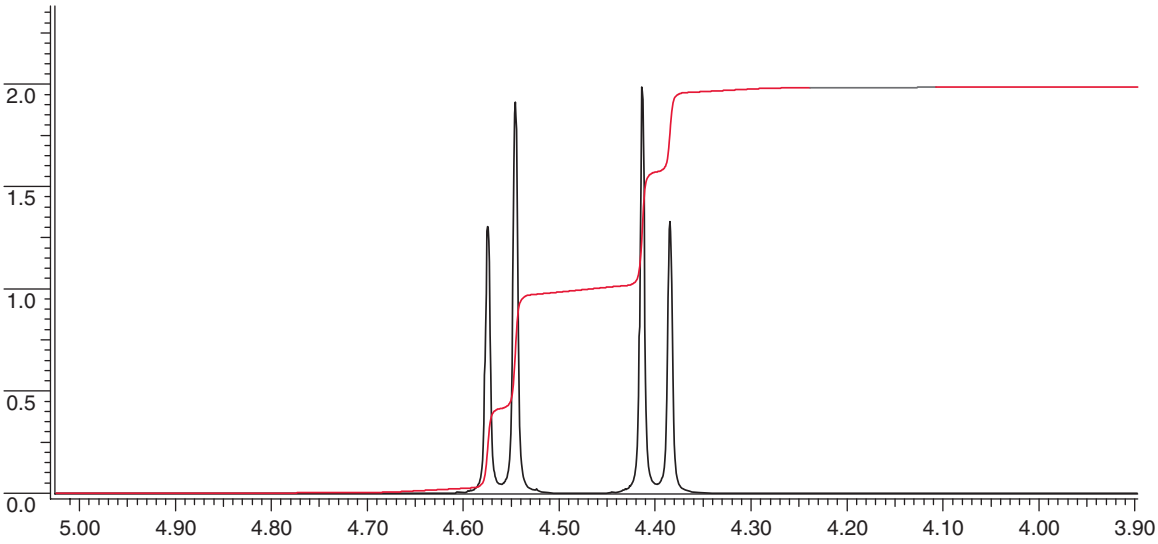
 ISBN: 978-0-470-71092-0



Spectrum 6.1 An AB system.

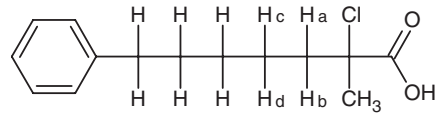
are therefore likely to be clearly 'roofed' to each other. Spectroscopists use the term 'AB system' to describe this type of arrangement. All it means is that the spin system contains two protons which are relatively close to each other in chemical shift terms, (but not equivalent to each other), HA and HB, and they couple to each other and nothing else.

Geminal couplings of this type are typically in the region of 12–14 Hz, though interestingly, they can be as large as 19 Hz between protons that are alpha to an alkene or carbonyl function. This can be a useful interpretive 'handle' if you are looking for a starting point in a complex assignment. (Take a look at a spectrum of camphor if you need convincing! Spot any huge geminal couplings?)

Of course, it is quite possible, though statistically unlikely, that you might encounter a molecule of this type in which the chemical shifts of the two protons, Ha and Hb just happen to be identical. Under these circumstances, there will be no splitting observed and you will just observe a singlet as if there were no chiral centre in the molecule at all. But beware! Should you run the sample in a different solvent, or even in the same solvent but at a different concentration, the singlet would be likely to re-present itself as an AB system. *Note*: The degree of separation between Ha and Hb reflects the anisotropic influences the different groups on the chiral centre exert on the two protons. If these groups were all very similar in nature (e.g., an ethyl, propyl and butyl) there would be very little 'difference' engendered in Ha and Hb, and for this reason, we could reasonably expect the chemical shift difference between these two protons to be small.

You might consider there to be an issue in predicting the chemical shift of a signal that is split into an AB system in this way but in reality, we have found it safe to treat the prediction as the midpoint between the two doublets of the AB.

So in summary, the presence of a chiral centre in a molecule can render nearby geminal pairs of protons non-equivalent. 'Nearby' is not an exact term and varies according to circumstance. Let's consider our molecule again, but this time, replace the -CH_2- with an alkyl chain (Structure 6.2).



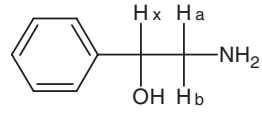
Structure 6.2 A chiral molecule with an alkyl chain.

In this case, it should be clear that Ha and Hb are just as non-equivalent as before. And because they are non-equivalent, it stands to reason that the next pair of protons, Hc and Hd must also be non-equivalent – and the next pair and the next pair. And so it is. In terms of the spectral lines observed, complexity will certainly be the name of the game! Not only will Ha and Hb couple to each other but they will obviously both couple to Hc and Hd. What will not necessarily be so obvious is that the size of the splittings between Ha and Hc and between Ha and Hd will very likely be different! This is because although there is free rotation about all single bonds, the chiral centre will place certain steric constraints upon the molecule such that it will tend to adopt a conformation that will minimise these constraints. This means that the time-averaged dihedral angles between H_a and H_c and H_a and H_d will not be the same – and neither will be the corresponding couplings. All of a sudden, in this welter of complex, overlapped, heavily roofed multiplets, Pascal's triangle starts to look woefully inadequate, doesn't it?

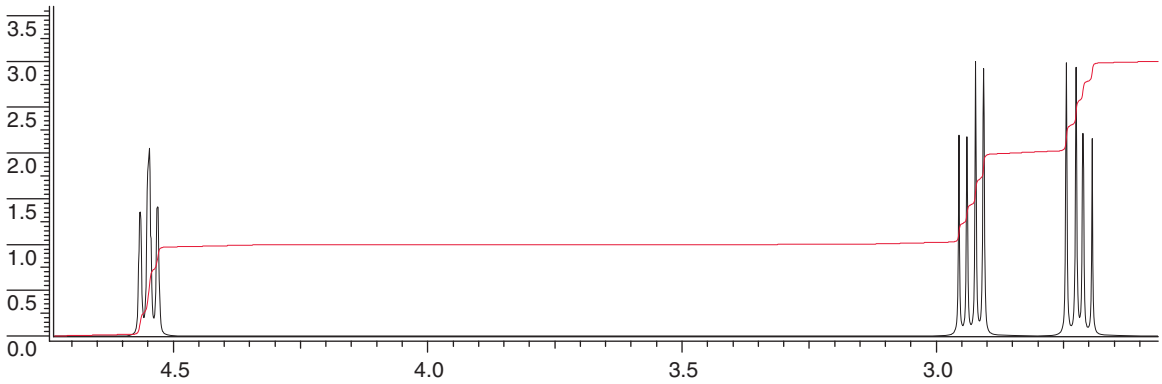
In practise, of course, we find that the further away from the chiral centre we go, the smaller the difference in chemical shift between corresponding geminal protons is likely to be. By the time we move three or four carbons down the chain, the likelihood is that corresponding pairs will be approximately equivalent, so for example, in the case above, we might expect the $-CH_2-$ next to the phenyl ring to be just a fairly normal, slightly broadened, roofed triplet rather than a pair of complex multiplets. It is not impossible, however for a molecule to wrap itself up in certain conditions such that the a geminal pair of protons are brought near to a chiral centre in the molecule – even though they may be many, many bonds away from it. It is important to remember that this is a 'through space' effect rather than a 'through bond' effect.

The convention of appending letters of the alphabet to protons in order to describe spin systems is commonly used in two more important cases. Structure 6.3 shows a molecule likely to exhibit a classic ABX system (see Spectrum 6.2).

As before, the chiral centre renders H_a and H_b non-equivalent and for the reasons already covered, H_x will couple to both with all three couplings (H_a-H_b, H_a-H_x and H_b-H_x) likely to be different. So the classical presentation of an ABX system is that of three multiplets, each of four lines. (Note that in Spectrum 6.2, the size of the H_a-X and the H_b-X couplings are almost identical so the X proton appears as an approximate triplet. This is quite common.). The AB part indicates that the geminal pair are likely to be relatively close in terms of chemical shift, whilst the X proton is someway distant from both. Obviously, the scope for variation in the appearance of ABX systems is enormous. The difference in chemical shift between H_a and H_b is a major factor in this but we have also come across ABX systems



Structure 6.3 A molecule likely exhibiting a classic ABX system.



Spectrum 6.2 A typical ABX system.

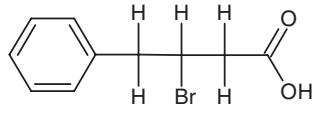
constrained within five-membered rings where all three splittings happen to be the same size. In such cases, we observe three triplets. Another possibility is for A and B to be accidentally equivalent in which case we observe something approximating to a simple doublet for H_a and H_b and a triplet for H_x.

It is also quite common to see molecules in which the X proton is actually the X of two distinct ABX systems. Structure 6.4 and Spectrum 6.3 show an example of such a molecule.

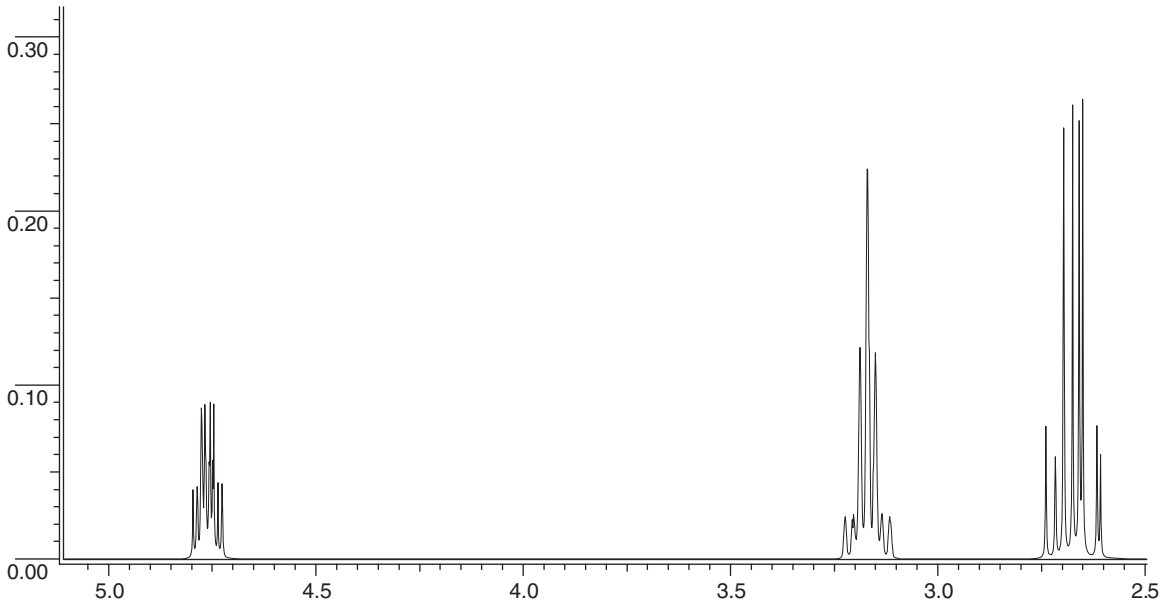
In a molecule like this, it would be theoretically possible for H_x to present as a 16-line multiplet but it is extremely unlikely that you would be able to count this many as there would almost certainly be a considerable overlap between them. Then of course, it is would be quite possible for the two AB parts to overlap. Be flexible in your approach and alert to the possibilities . . .

Moving on to some wider stereochemical considerations, just as enantiomers are indistinguishable as far as their physical and chemical properties are concerned (except, of course, as regards their reactions with other optically active reagents) so their spectra, acquired under normal conditions, are identical. The NMR spectrometer does not differentiate between optically pure samples and racemic ones. *Note*: there is a way of differentiating between enantiomers by NMR but it involves using certain chiral reagents which we'll discuss in detail later.

So much for one chiral centre. The problems really begin when you come up against molecules which have two or more chiral centres! With two chiral centres, we can construct four possible stereoisomers. These can be separated into two enantiomeric pairs (indistinguishable by NMR). But, (*key sentence coming up*) if we compare one member of each of these enantiomeric pairs, we will find that they may be distinguished from each other by NMR, because they are diastereoisomers. Diastereoisomers are stereoisomers which are not mirror images of each other – they are different compounds with distinct physical and chemical properties. See Figure 6.1 if this isn't clear.



Structure 6.4 X proton belonging to two distinct ABX systems.



Spectrum 6.3 A complex double ABX system.

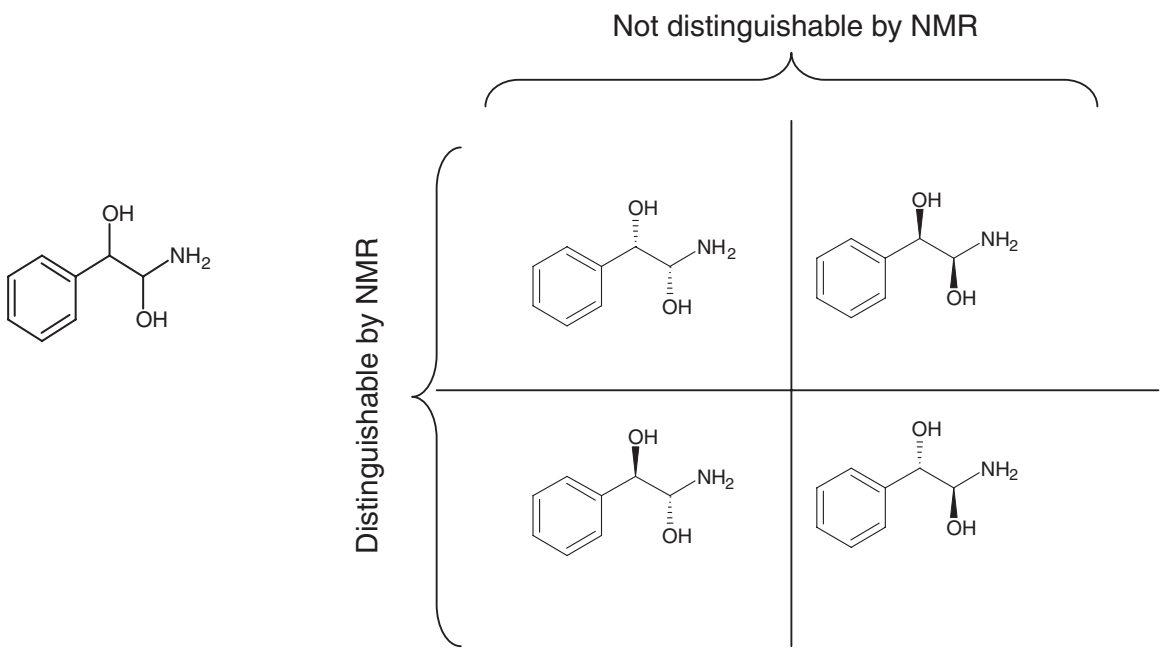
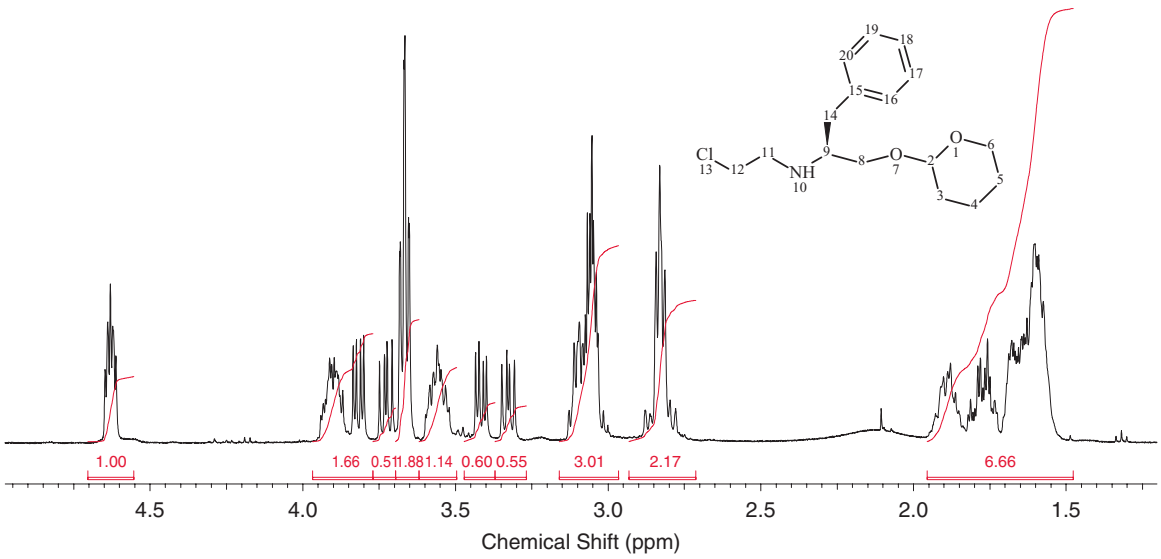


Figure 6.1 NMR and the relationship between enantiomers and diastereoisomers.



Spectrum 6.4 A mixture of diastereoisomers.

Differences in the spectra of diastereoisomers are generally most noticeable in the region of the chiral centres. Spectrum 6.4 shows a typical example.

Note how two sets of signals are clearly visible, for the protons labelled '8' above. These present as two pairs of protons, i.e., two AB parts of two ABX systems at 3.30–3.45 and 3.70–3.85 ppm, each integrating for approximately half a proton with respect to the unresolved parts of the spectrum. You certainly wouldn't expect *all* the signals of a pair of diastereoisomers to resolve (e.g., protons 3, 4 and 5 in the example above) but some will almost certainly do so. In some cases, the differences in the spectra of diastereoisomers can be quite spectacular, with chemical shift differences of 0.5 ppm or more.

With more than two unspecified chiral centres, problems multiply rapidly – three chiral centres yield eight stereoisomers, and thus four possible sets of signals and so on. From this, it follows that n chiral centres give rise to 2^n chiral entities of which $2^n/2$ will be distinguishable by NMR.

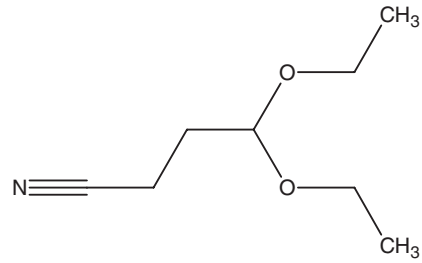
A final point on this phenomenon – nitrogen can sometimes act as a chiral centre. This topic is explored in some detail in Section 6.6.6.

6.2 Enantiotopic and Diastereotopic Protons

Consider ethanol (*key sentence coming up*). If you were to replace each of the methylene protons in turn with some other group, Z, you would end up with a pair of enantiomers. We call this, 'the Z test.' For this reason, the protons (or whatever groups may be involved, in molecules of the type: X-CA_2-Y) are described as *enantiotopic*. This is of no consequence in the spectrometer, because as we have mentioned, enantiomers are not distinguishable by NMR under normal conditions.

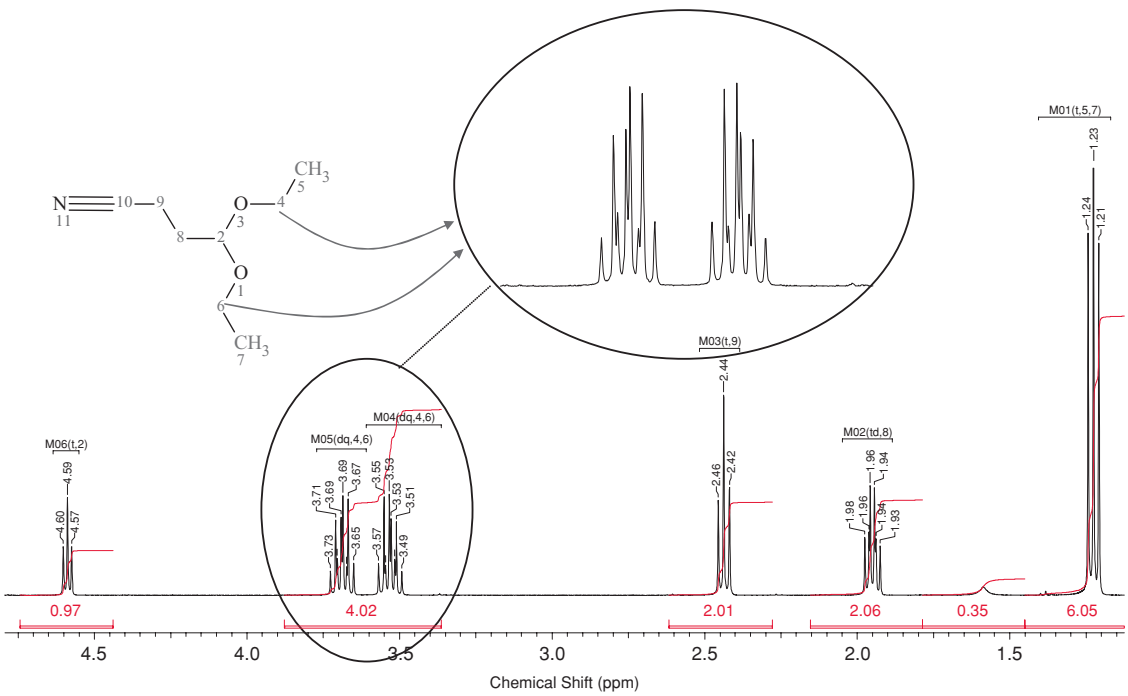
So far so good. Now consider the molecule in Structure 6.5.

The molecule clearly does not contain any chiral centres and so should give a perfectly straightforward spectrum. Now take a look at the Spectrum 6.5.



Structure 6.5 A diastereotopic molecule.

On close examination, it is clear that the methylene protons of the -OEt groups (H4, H6), do not give the nice simple quartet which we might reasonably expect. Close examination of the methylene signal shows it to be a complex multiplet. But why? Try applying the 'Z test' to the methylene protons. Straight away, the difference between this molecule and ethanol becomes apparent. Whereas ethanol would yield a pair of enantiomers in response to the test, this molecule would yield a pair of diastereoisomers as a second chiral centre would be generated at the branch point (C2)! For this reason, the methylene protons in this molecule would be described as *diastereotopic*. Such protons are not equivalent and therefore exhibit further splittings as they couple to each other – hence the complexity.

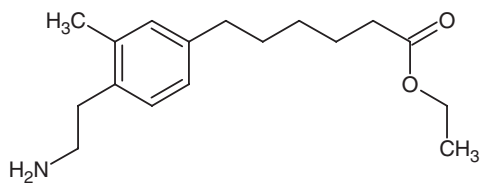


Spectrum 6.5 Diastereotopic protons.

Some confusion can arise over use of the term 'prochiral' to describe various sites within molecules and is perhaps best avoided for this reason. The term means literally, one step removed from being chiral (i.e., swap one of the protons for 'Z' and you have a full chiral centre). The methylene in ethanol for example, would be a good example. What we have in the di-ethoxy molecule above is one prochiral centre acting in combination with another to render a pair of protons non-equivalent.

6.3 Molecular Anisotropy

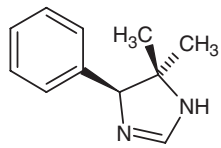
There are two factors that determine chemical shifts – electron distribution and molecular anisotropy. We have already seen how electronics define chemical shifts in previous sections. When we use Table 5.4 to estimate shifts around an aromatic ring, for example, the predictions we arrive at are based on the known electron withdrawal or supply of the various substituents on the ring. No allowance is made for unusual anisotropy. Similarly, predictions of chemical shifts of alkyl protons using Table 5.8 will be calculated on the basis of electronic factors only as it would be impossible to vector anisotropy into the prediction since it varies in each individual molecule. They will be reasonably accurate in molecules where electronic factors predominate and molecular anisotropy has little or no influence. A typical example of such a molecule is shown in Structure 6.6. Note the lack of steric crowding in the structure.



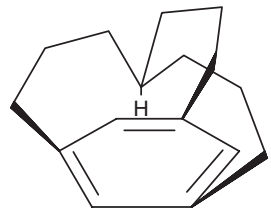
Structure 6.6 Typical molecule where electronic factors predominate.

However, in molecules where groups are constrained for whatever steric reasons, molecular anisotropy can play a large part in determining chemical shifts. Take for example, the molecule in Structure 6.7.

When confronted by a molecule like this, we can be sure that whatever conformation it adopts in solution, the likelihood is that the two methyl groups will not be equivalent! The driving force for their non-equivalence will of course be the aromatic ring. One of the methyl groups will be on the same face of the five-membered ring as the phenyl group and the other will not (once again, building a model is a good idea). In terms of through-bond electronics, both methyls enjoy much the same environment but the magnetic field that each will experience in terms of their proximities to the phenyl ring will be very different. And this, in essence, is what molecular anisotropy is all about – non-uniform distribution of electrons within groups, inducing significant chemical shift changes in parts of molecules by the



Structure 6.7 Molecule displaying molecular anisotropy.



Structure 6.8 An extreme example of anisotropy.

introduction of localised magnetic fields. In Structure 6.7 above, for example, it would be likely that the phenyl ring would interact with the methyl group *cis* to it (i.e., on same face of five-membered ring) in such a way as to minimise contact. In order to do this, it would probably spend most of its time at right-angles to the plane of the paper, i.e., sticking up vertically out of the page. This would position the *cis* methyl over the phenyl ring's pi-cloud which would induce an upfield shift in this methyl and cause it to be higher field than you might expect. The *trans* methyl would be relatively unaffected.

Structure 6.8 demonstrates a most extreme example of anisotropy. In this unusual metacyclophane, the predicted chemical shift (Table 5.8) of the methine proton that is suspended above the aromatic ring would be 1.9 ppm. In fact, the observed shift is –4 ppm, i.e., 4 ppm *above* TMS! The discrepancy between these values is all down to the anisotropic effect of the benzene ring and the fact that the proton in question is held very close to the delocalised 'p' electrons of the pi cloud.

All groups have a certain measure of anisotropy associated with them so that any protons forced abnormally close to *any* group are likely to exhibit some deviation from expected chemical shifts but the most notable are the aromatic/heterocyclic groups, carbonyls and alkenes. Expect abnormal shifts in molecules where steric crowding forces groups into close contact with each other. Build models and try to envisage the likely (lowest energy) conformations of your molecules. How will various groups within your molecules align themselves with respect to the anisotropic moieties? Remember that aromatic/heterocyclic rings shield groups that are held *above* or *below* their plane but de-shield groups that are held *in* their plane and that groups held near the 'oxygen end' of a carbonyl group will be de-shielded.

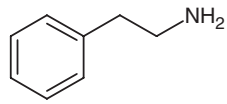
It is anisotropy that is the ultimate cause of the chemical shift differences between the geminal protons in AB and ABX systems. And indirectly, it is changes in anisotropy that bring about differences in observed chemical shifts for the same sample that is run in different solvents. The unknown extent of the anisotropy term in defining chemical shifts make it difficult (or perhaps impossible?) to devise a prediction tool, computer-based or otherwise, that can accurately predict the shifts of all protons regardless of environment. You may be wondering why the extent of the anisotropy term should be unknown. This is because in order to calculate it, we would first need to know the exact shape of the molecule in question – in solution. Molecular modelling packages deal with single molecules in a vacuum. This is nothing like variable concentrations in a variety of organic solvents with varying water content!

Molecular anisotropy affects proton chemical shifts to a far greater extent than ^{13}C chemical shifts. This is because the protons occupy the outer extremities of a molecule whilst the carbon framework is far more internal and to a large extent, removed from the influences of anisotropy.

Always be aware of anisotropy but as with all NMR phenomena, avoid becoming obsessed with it!

6.4 Accidental Equivalence

Accidental equivalence is a fairly self explanatory term used to describe situations where different signals happen to be coincidental. In most cases, this will come as no great surprise, and should cause no great problem. As you run through the mental exercise of estimating the chemical shifts of all the protons in your spectrum in order to create a hypothetical spectrum, don't forget to consider the possibilities of signals sharing exactly the same chemical shifts. There is no mysterious force acting to 'repel' chemical shifts away from each other and it is quite possible for chemical shifts to be coincidental. Take for example, the simple molecule of the type shown in Structure 6.9.



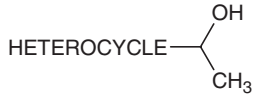
Structure 6.9 An example of accidental equivalence.

Checking the chemical shifts for the alkyl chain *as a free base*, would lead us to conclude that the -CH_2- next to the aromatic ring should absorb at a slightly lower field (approx. 2.9 ppm, making allowance for the short chain length and slight beta de-shielding effect of the -NH_2 group) than the -CH_2- next to the -NH_2 function (should be approx. 2.6 ppm). However, should the nitrogen be protonated by an acid, then the -CH_2- next to the -NH_3^+ would have the lower chemical shift (approx. 3.4 ppm) and those next to the aromatic ring, approx. 3.1 ppm (due to enhanced beta de-shielding). The act of protonation causes the shift of one -CH_2- to 'overtake' the other, as they both move downfield. But at a certain intermediate acid concentration, both CH_2-s will have exactly the same chemical shift and will present as a four-proton singlet. If confronted with a situation like this, your first thought might be: 'This cannot be right!' But your second thought should be: 'Ah! Maybe the nitrogen has been partially protonated by exposure to some acid?' And your third thought should be: 'OK. So what am I going to do to prove this?'

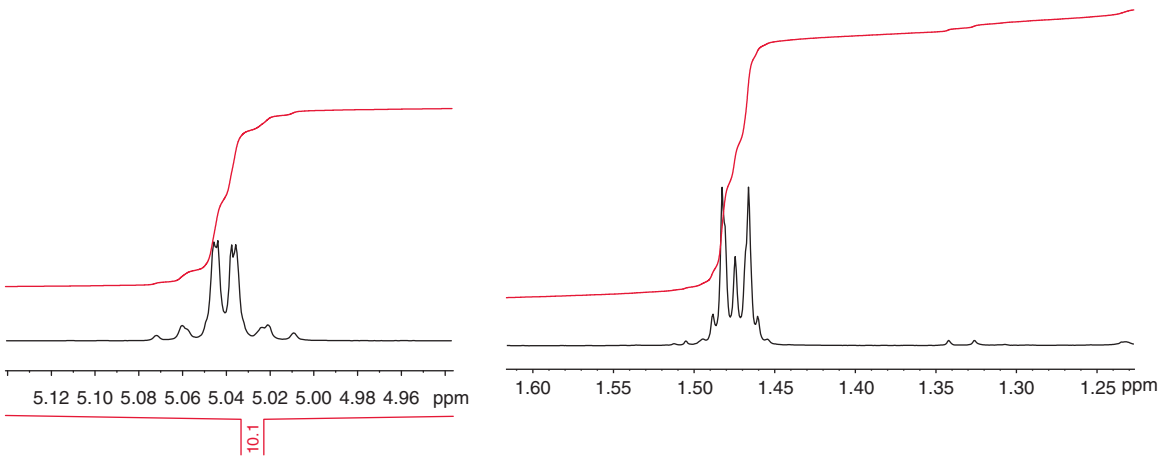
As we have already seen, accidental equivalence could be responsible for the theoretically non-equivalent protons of an AB system presenting as a singlet and for the more complex ABX system presenting as a simple doublet and triplet. But occasionally, even more interesting manifestation of accidental equivalence can be observed. Consider the molecule below (Structure 6.10) and its spectrum (Spectrum 6.6) which shows only the regions of interest to us – expanded and with the intervening region removed.

The complex multiplet centred at 5.04 ppm results from the overlap of the methine and -OH protons (i.e., they are 'accidentally equivalent') whilst the equally complex methyl signal is centred at 1.48 ppm. Because of this overlap, their lines are indistinguishable and so the -OH is said to be 'virtually coupled' to the methyl group. Virtual coupling is another potential consequence of non-first order behaviour.

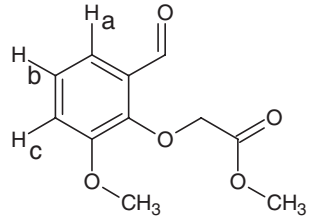
And for a final example, consider the molecule in Structure 6.11 and Spectrum 6.7. Please note: Spectrum 6.7 has been simulated on account of no compound being available at the time of writing. The



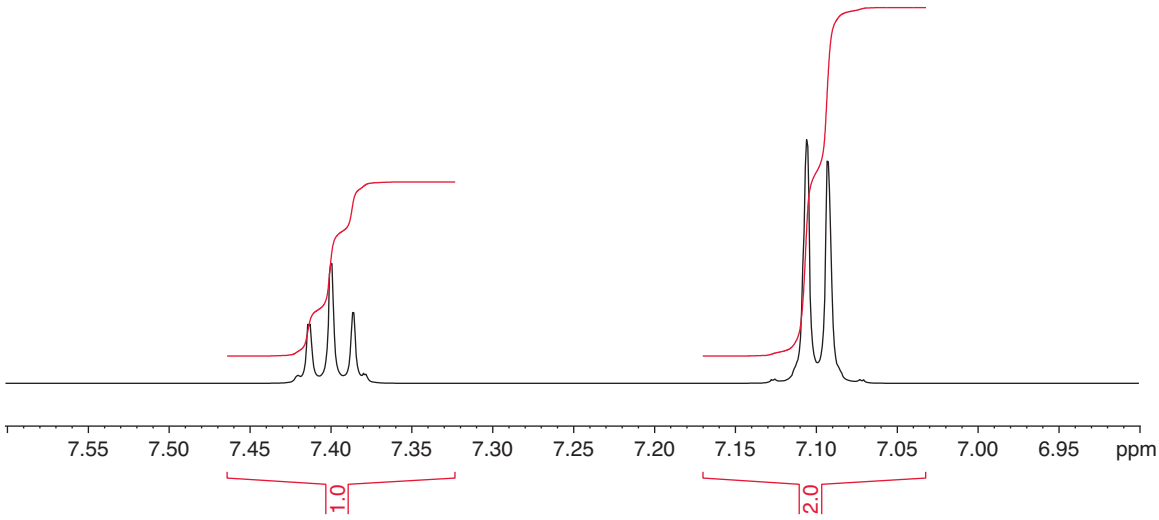
Structure 6.10 An example of vitrual coupling.



Spectrum 6.6 Virtual coupling.



Structure 6.11 A deceptively simple molecule.



Spectrum 6.7 Deceptive simplicity.

chemical shifts and splitting values were taken from an actual spectrum published in *Laboratory Guide to Proton NMR Spectrocopy* (see Introduction).

When we look at this 'deceptively simple' spectrum, it soon becomes clear that two of the aromatic protons must be isochronous since we see only two multiplets with the appropriate integration of 2 : 1 for the three protons. The lowest field of the aromatic protons must be 'Ha' as it is ortho to the de-shielding aldehyde function and therefore it must be the slightly higher-field protons 'Hb' and 'Hc' which are accidentally equivalent to each other as they are either ortho or para to the electron-donating (upfield-shifting) oxygen atoms. Were it not for the fact that 'Hb' and 'Hc' share the same chemical shift, we would expect to see them couple to 'Ha' with couplings of about 7.5 and 2.5 Hz, respectively. What we see in reality is an approximate triplet/doublet structure with an apparent splitting of about 5 Hz! This is clearly too large to be a meta- coupling and too small to be an ortho- coupling. Note that the small additional lines flanking the doublet and triplet are real and part of the signals in question. They can be explained by the magnetic non-equivalence of 'Hb' and 'Hc' and are a manifestation of non-first order behaviour.

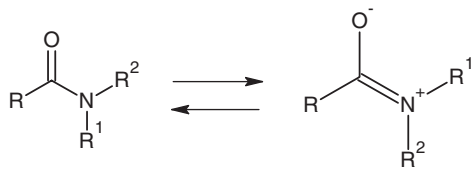
It is in effect a hybrid splitting; literally, an average of the two expected couplings. The two protons become indistinguishable from each other and both appear to exist in some hybrid ortho/meta state! The term 'deceptive simplicity' is quite apt to describe such a spin system. It might look simple, but it isn't. It's non-first order splitting at its most beguiling! Don't bother trying to find this sort of thing in your spectra. It is a rare phenomenon (and the more powerful your magnet, the rarer it is) and you won't find it. But it's good to be aware of it because if you look at enough spectra, one day *it* might find *you*.

6.5 Restricted Rotation

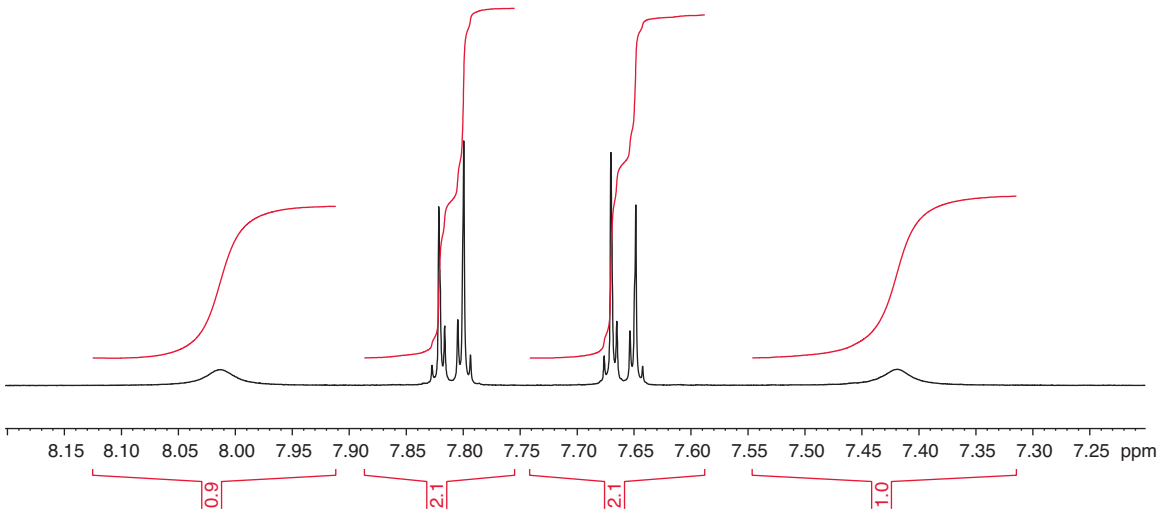
Certain types of bond, whilst nominally being considered as 'single', have in fact, sufficient 'double bond character', to render rotation about their axis, 'restricted'. The one you are most likely to encounter, is the amide bond. Partial double bond character exists between the carbonyl, and the nitrogen, and may be represented as in Structure 6.12:

This can lead to problems in NMR spectra. The magnitude of the energy barrier to the rotation determines what the effect on the spectrum will be. (For the thermodynamically-minded, we are talking about energy barriers of the order of 9–20 Kcal mol.)

Should the energy barrier be substantially lower than this, then restriction will be slight, and rotation will be relatively fast on the NMR time scale, and therefore, we may only see a slight broadening of signals in the region of the site of restricted rotation. Conversely, should the energy barrier be relatively high, rotation will be slow enough for us to see two distinct sets of signals. The worse case scenario



Structure 6.12 Partial double bond character.



Spectrum 6.8 4-Bromobenzamide showing typical appearance of primary amide protons as two non-equivalent broad signals separated by about 0.6 ppm.

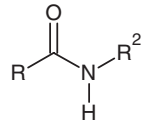
is that of rotation which is of intermediate pace on the NMR timescale, as this gives rise to broad semi-coalesced signals that are impossible to interpret.

Let's return to our amides. In primary amides, where R′ and R″ are both just protons, we can expect to see them as two, distinct, broad signals (Spectrum 6.8).

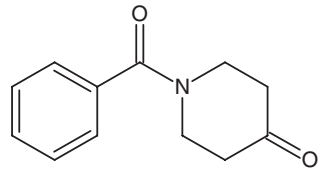
This is because the two protons do not occupy the same environment. Though they do exchange their positions with each other, the process is 'slow on the NMR timescale.' This means that during the time in which a single transient is acquired, there will have been relatively little exchange and for this reason, the spectrometer will 'see' the two amide protons in two distinct environments and you will observe two distinct broad humps separated typically by about 0.6 ppm. Anisotropy of the carbonyl group ensures that the lower-field of the two humps corresponds to the proton that is *cis* to the carbonyl oxygen at the time of the acquisition and the higher field hump, to the proton *trans* to the carbonyl oxygen. No other signals in the spectrum of a primary amide will be broadened by restricted rotation about the primary amide bond.

Secondary amides, on the other hand, generally do not exhibit two rotametric forms (that is not to say that rotation about the amide bond in secondary amides doesn't occur at all – just that secondary amides spend most of their time with the two large groups, R and R^2, *trans* to each other (Structure 6.13).

For this reason, secondary amides do not generally cause any spectroscopic headaches.



Structure 6.13 A secondary amide.



Structure 6.14 First example of restricted rotation.

It is the tertiary amides that tend to be the most problematic in terms of proton NMR. They usually exhibit two rotametric forms, the relative proportion of each being determined by both electronic factors and by the relative sizes of the two groups, R^1 and R^2. *Note*: this in no way implies that the rotameric forms of a tertiary amide could ever be physically separated as the inter-conversion rate between the two forms is generally in the order of seconds. A 50/50 ratio of rotamers is only guaranteed where $R^1=R^2$ (as in the case of a primary amide where $R^1=R^2=H$). Consider the two compounds in Structures 6.14 and 6.15.

In the case of the molecule in Structure 6.14, only the protons of the piperidone ring would be affected by restricted rotation about the amide bond. As far as the aromatic protons are concerned, there is no anisotropic difference in the environment they experience, because the piperidone has a plane of symmetry through it.

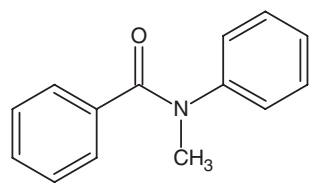
Now consider Structure 6.15. In this case, there is no such symmetry and so *all* the signals of the spectrum of this compound would be expected to be broadened or duplicated! Always consider the symmetry of the molecule in anticipation of the extent of rotameric complexity.

We will see later on, that we can often overcome rotational energy barriers (providing they are not too high) and thus simplify our spectra by running our samples at high temperature. Note that in cases where there is a large difference in the ratio of the rotamers, the coalescence point will not just be midway between the positions of the two rotamers, but will be closer to the position of the major rotamer. Note also that in cases where the amide function is sterically constrained, rotamers may *not* be observed as one rotameric form might be of significantly lower energy than the other and therefore may predominate, perhaps totally in a molecule like the one in Structure 6.16.

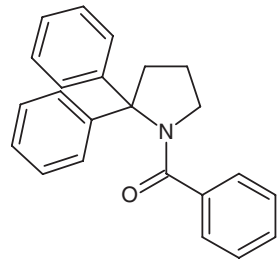
Another group which is well known for restricted rotation is the nitrovinyl group (Structure 6.17).

This time, the alkene nominal double bond has sufficient single bond character to permit a certain amount of rotation, as resonance forms can be drawn (e.g., Structure 6.18).

Another group that frequently – and perhaps surprisingly, in view of secondary amide characteristics – exhibits rotameric behaviour is the secondary carbamate (R-COO-NHR^1), though the energy barrier to rotation tends to be a little lower than in the amide case.



Structure 6.15 Second example of restricted rotation.



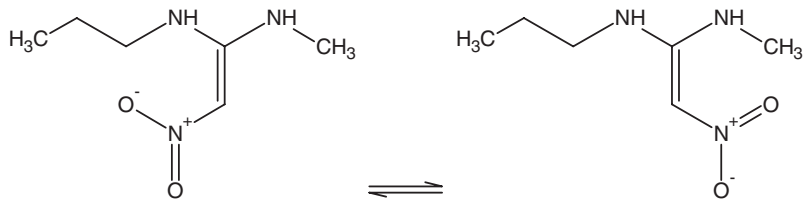
Structure 6.16 Amide function sterically constrained.

Finally, it's worth mentioning the formamide group. Although this looks like a special case of a secondary amide, rotamers of different intensity are often seen. Compounds with a formamide attached to an aromatic ring can give particularly complex spectra. Not only does the NH proton couple to the CHO proton, with a coupling of about 2–3 Hz in the *cis* isomer, and 8–9 Hz in the *trans* isomer, but, any aromatic protons ortho to the formamide are also split out in the rotamers!

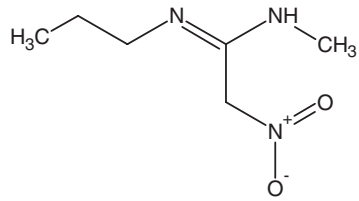
So to sum up, we've seen that restricted rotation can give rise to considerable complexity by broadening or duplication of signals. Indeed, overlap of signals from rotameric pairs is commonplace and can cause further ambiguity. As with any other phenomenon, if it is recognised for what it is, and the spectrum can be interpreted in terms of it, then all well and good. If however, the quality of your spectral information is diminished as a result of it, (and remember that you may have more than one site of restricted rotation in a molecule) to the point where you cannot be confident about determining the structure of your compound, then further action must be taken! (like running your sample hot, or perhaps trying it in D_4-methanol for example – this solvent can reduce rotational energy barriers, probably by eliminating intramolecular H-bonding.)

But don't assume that just because your compound exhibits restricted rotation, you must run it hot, to do it justice. Not so! Sometimes, the barrier to rotation is just too high to allow simplification by heating. Remember – it is easier to deal with a spectrum of two, sharp rotamers than a broad semi-coalesced mess!

It is worth noting that whilst we have restricted discussion in this section to conformational interconversion based on the slow rotation of bonds, the concept of 'the NMR timescale' is equally applicable to other types of interconversion, such as can sometimes be seen in cyclic systems which may exist in two different conformational forms.



Structure 6.17 Nitrovinyl group.



Structure 6.18 Resonance form.

6.6 Heteronuclear Coupling

So far, we've considered spin coupling in considerable detail, but only proton–proton coupling. There are in fact, over 60 elements having nuclei of one or more of their naturally occurring isotopes which have magnetic moments. This means that they not only have their own NMR spectra (e.g., ^{19}F, ^{31}P, which can be recorded with a suitable spectrometer) but also the capability of coupling with protons. The most notable and obvious feature of heteronuclear coupling, is that no reciprocal coupling is observed in the proton spectrum – because it exists in the spectrum of the heteroatom, of course. In this section, we'll have a look at the hetero-atoms of importance, which you are quite likely to encounter, and one or two others, which are less commonly encountered. It might be tempting to think that if your compound contains a heteroatom there should be an imperative to acquire a spectrum for that specific nucleus – but this is not so. The proton spectrum often contains all the confirmation of the hetero atom that you need, as the size and nature of the couplings observed can be quite specific.

We will deal with the spectroscopy of a few of these nuclei in later sections but for now, we will restrict ourselves to the consequences of hetero atoms seen in proton spectroscopy.

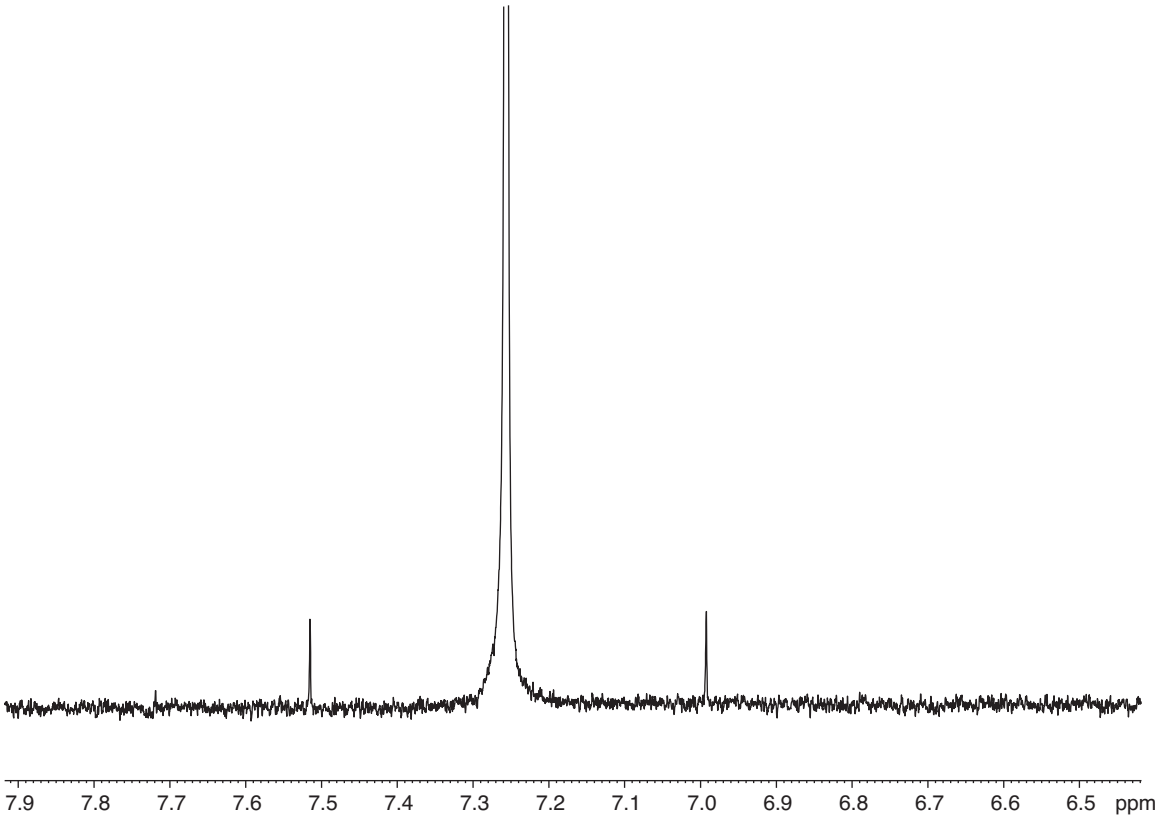
6.6.1 Coupling between Protons and ^{13}C

Consider Spectrum 6.9 which shows a $CHCl_3$ singlet plotted at very high intensity.

On each side of the signal, a number of minor peaks may be seen, one pair of which are the '^{13}C satellites.' (We'll discuss spinning side bands a little later). Since the ^{13}C nucleus has a magnetic moment, it couples to proton signals, but as its natural abundance is only 1.1 %, the ^{13}C satellites are very small, each satellite accounting for only 0.55 % of the intensity of the peak to which it belongs. The only time you might notice them, is when you have a very strong singlet in your spectrum, such as a tertiary butyl.

The ^{13}C nucleus, like the proton, has a nuclear spin quantum number (I) of 1/2, so there are only two permitted energy states of the nucleus with respect to the external magnetic field. This means of course, that there are only two satellite peaks, i.e., the 1.1 % of the protons that are attached to ^{13}C nuclei are split by the ^{13}C nucleus into a doublet (and the 98.9 % that are attached to ^{12}C, are not). If you measure the coupling (from satellite to satellite), you'll find that it's 210 Hz – though the size of ^{13}C-H couplings vary considerably, depending on the type of function the carbon is incorporated into. This coupling may seem very large, but don't forget it is a one-bond coupling.

These days, improvements in magnet design and consequent greater field homogeneity have made it quite common practise to run NMR experiments, nonspinning. Indeed, many of the two-dimensional experiments should definitely *not* be run spinning (see Chapter three for more discussion about spinning vs. non spinning). However, for one-dimensional spectra, the best resolution is likely to be obtained



Spectrum 6.9 $CHCl_3$ singlet plotted at high intensity.

by spinning your samples at about 20 Hz. If you do this, you *may* encounter spinning side bands. These should never be a problem in a well shimmed instrument operating to record spectra at typical levels of gain but it is possible to observe them occasionally as small peaks on either side of very strong peaks (most notable singlets) such as t-butyl singlets. Their relative intensities are not fixed as with ^{13}C satellites but can vary with the state of the high-order shims and with the quality of the NMR tubes you use. *Note*: Should spinning side-bands ever exceed the size of the ^{13}C satellites, you should seriously consider a major shim of your instrument! Should you be looking for some very minor constituent of your sample, ^{13}C satellites, and spinning side bands may get in the way. Spinning side bands can be moved by altering the spin rate of the sample tube but you can't do anything about the satellites. Notice that the separation of the first spinning side band, (if seen) from the main peak, when measured in Hz, gives the spinning speed (also in Hz of course). Notice too, that the phase of a second spinning sideband, if present, is always 'out' with respect to all the other peaks – a useful diagnostic feature.

^{13}C satellites can actually be quite useful sometimes, as they give a ready-made visual comparator for the quality of spectrometer high-order shimming and for trace impurities that you may be trying to quantify, since we know that each satellite will have an intensity of 0.55 % of the peak it is associated with.

Two final interesting points relating to ^{13}C satellites . . . Whilst they are generally, evenly spaced on either side of the major peak, they do not have to be *exactly* symmetrically disposed about it. It is

quite possible to observe a small isotopic shift so that the proton chemical shift of the ^{13}C species is fractionally different from the major ^{12}C species. Also, if you do observe ^{13}C satellites, they will only ever be the product of one-bond ^{13}C-proton coupling. Two- and three-bond couplings between ^{13}C and protons certainly exist (and indeed are pivotal in the HMBC technique as we will see later) but such couplings do not generally manifest themselves in 1-D proton spectra as any satellites thus produced would be too close to the major peak to observe. ^{13}C satellites themselves are never seen to be split further by ^{13}C–^{13}C coupling simply because the statistical chance of finding two ^{13}C atoms next to each other is extremely small in terms of NMR sensitivity.

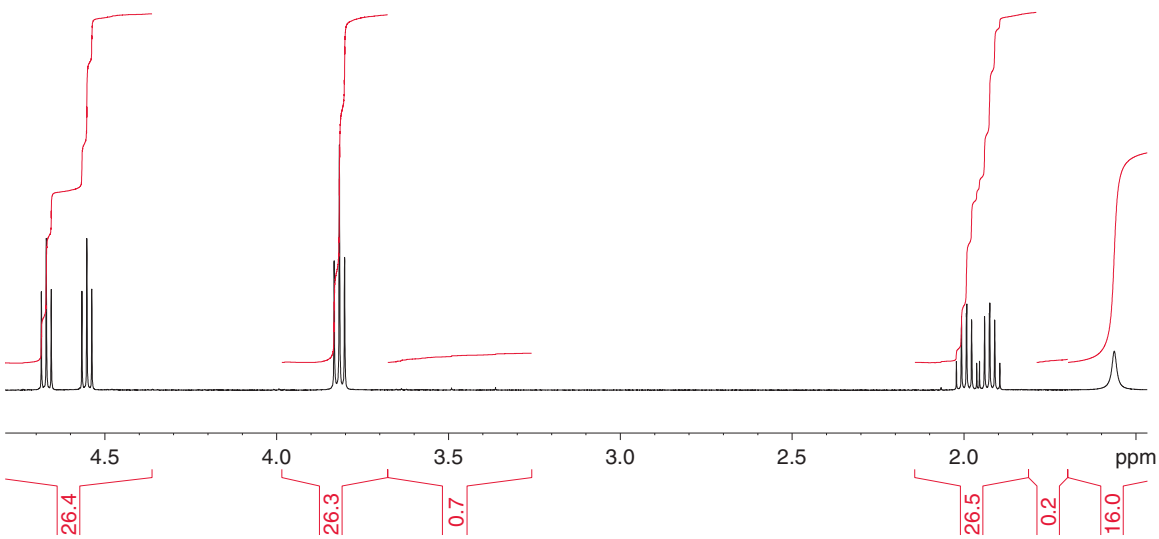
^{13}C coupling has very little significance in everyday proton NMR interpretation, though it has been used in the past to crack specific problems by means of selective enrichment of a specific carbon during synthesis, with a greater than normal percentage of ^{13}C isotope, which makes detection easy.

6.6.2 Coupling between Protons and ^{19}F

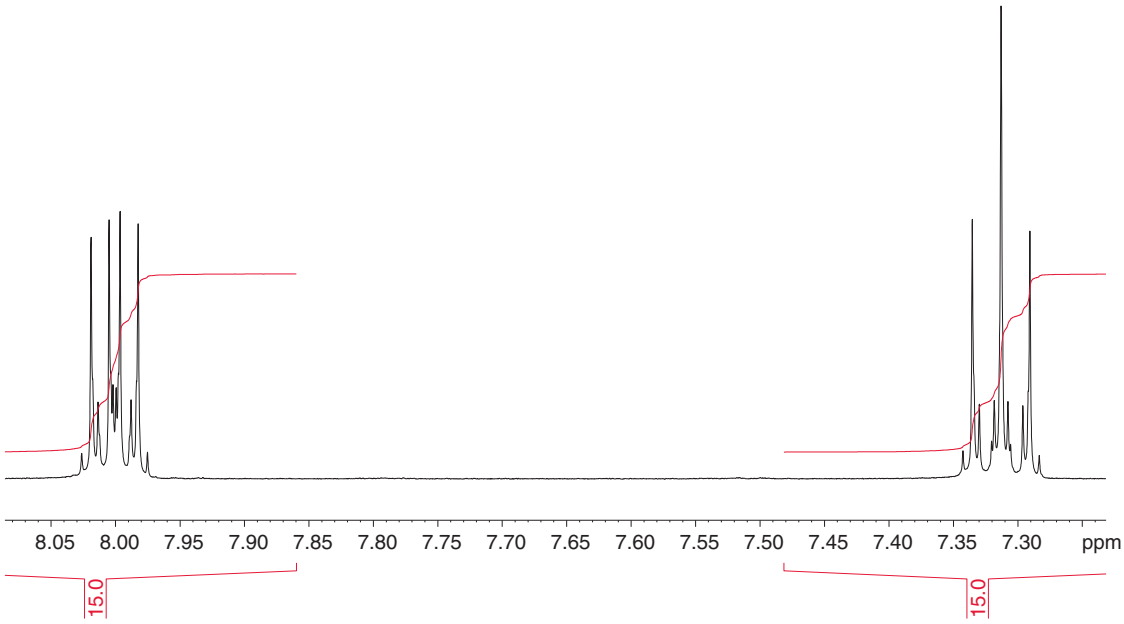
Fluorine usually makes its presence felt in a fairly spectacular fashion, when it is present in a molecule. Once again, I = 1/2, so we only have two allowed states to worry about. Unlike ^{13}C however, fluorine has only one isotope, ^{19}F, and as this of course, has 100 % natural abundance, we see the whole proton signal split, instead of a couple of tiny satellites on either side of our signals!

This point is well illustrated with a spectrum of 3-fluoro propanol (Spectrum 6.10), which shows a fairly dramatic example of fluorine coupling. The F-C**H$_2$**- coupling is about 47 Hz, and the F-CH$_2$-C**H$_2$**- coupling, is 27 Hz. The coupling to the third methylene group is non-existent in this example but can be seen sometimes (0–3 Hz).

Another example of ^{19}F coupling, this time in an aromatic system, (4-fluoro benzoic acid) is shown in Spectrum 6.11. Note how the ^{19}F couplings to the aromatic protons give the AA′BB′ system an



Spectrum 6.10 3-Fluoro propanol.



Spectrum 6.11 4-Fluoro benzoic acid.

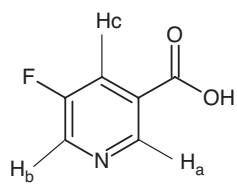
asymmetric appearance. The actual values in this case are 9.0 Hz (ortho) and 5.6 Hz (meta) which are fairly typical.

More useful ^{19}F coupling data is given in Table 6.1.

Fluorine can sometimes throw up some unexpected couplings in certain situations and spectra need to be handled with care! Sometimes, fluorine can be seen to couple over an unfeasible number of bonds (we have seen a seven bond coupling in the past). This is because fluorine is so electron hungry that it can couple through space as well as through-bond!

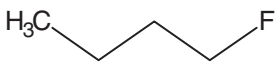
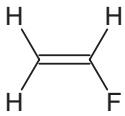
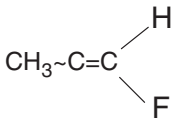
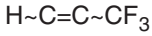
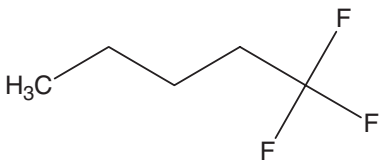
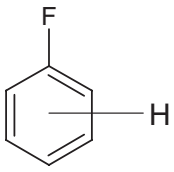
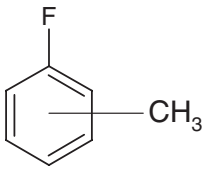
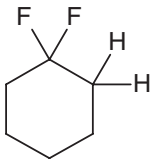
We have also noted some strange behaviour with fluorinated pyridines, for example, 3-fluoro nicotinic acid (Structure 6.19 and Spectrum 6.12). The signal for H_c (approx. 8.1 ppm) clearly shows couplings of 9.1, 2.9 and 1.7 Hz. The 9.1 Hz coupling must be from the fluorine as it does not appear anywhere else in the spectrum and its chemical shift distinguishes it from either of the other two protons.

Of the other two protons, the signal at 8.82 ppm, (H_b) shows only a 2.9 Hz coupling which is also found in H_c, whilst Ha exhibits two small couplings (2.0 and 1.7 Hz), the smallest of these also appearing in H_c. These observations lead to the conclusion that the fluorine–proton couplings in this molecule are as given in Table 6.2.



Structure 6.19 3-Fluoro nicotinic acid.

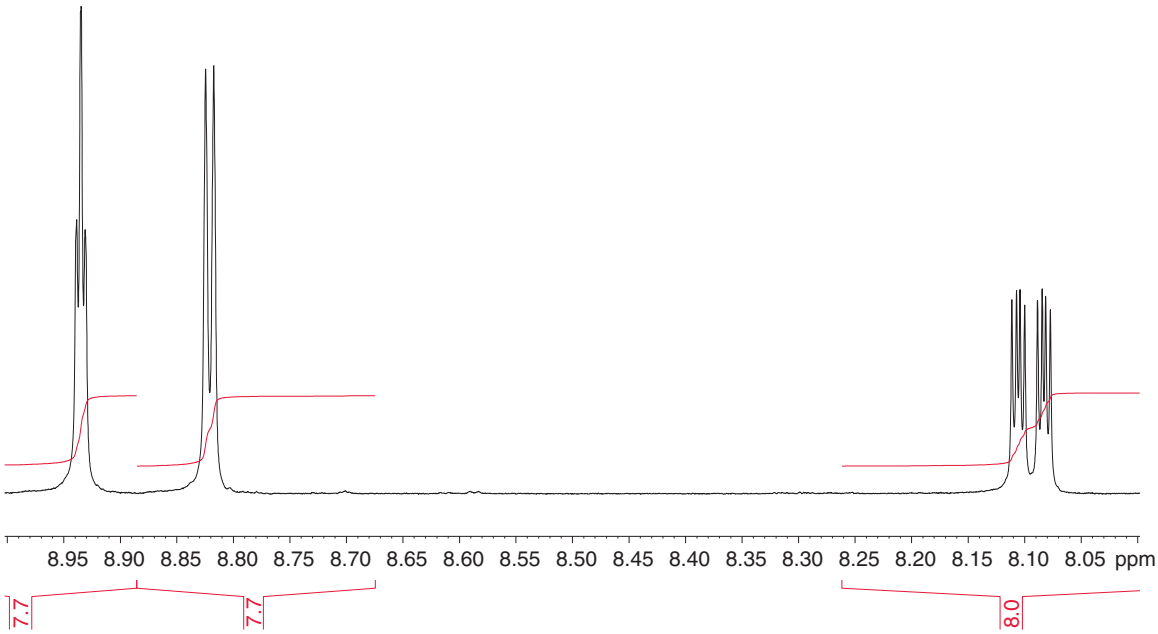
Table 6.1 Some typical ^{19}F–proton couplings.

Structure	^{19}F-^{1}H position	Typical ^{19}F-^{1}H coupling (Hz)
	F-**CH_2**- F-CH_2-**CH_2**- F-CH_2-CH_2-**CH_2**-	45 24 0–3
	F-H (geminal) F-H (*cis*) F-H (*trans*)	85 20 50
	F-CH_3	2–4
	F_3C-H	0-1
	F_3C-CH_2-	8–10
	F-H (ortho) F-H (meta) F-H (para)	6.2–10.3 3.7–8.3 0–2.5
	F-CH_3 (ortho) F-CH_3 (meta) F-CH_3 (para)	2.5 0 1.5
	F_{axial}–H_{axial} F_{axial}–$H_{equatorial}$ $F_{equatorial}$–$H_{equatorial}$	34 11.5 5–8

F-H_c coupling did not surprise and neither did F-H_a coupling. But the F-H_b coupling of less than a single Hz is totally baffling and defies obvious logic!

Having learnt the lessons from this simple little compound, it would seem reasonable to expect similarly surprising couplings in other fluorinated heterocycles.

Tread carefully!



Spectrum 6.12 3-Fluoro nicotinic acid.

Table 6.2 Fluorine–proton couplings in 3-fluoro nicotinic acid.

Position	Coupling (Hz)
F-H_a	2.0
F-H_b	very small, <1.0!
F-H_c	9.1

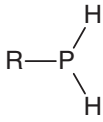
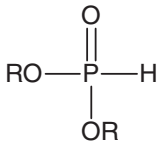
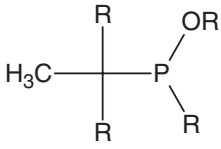
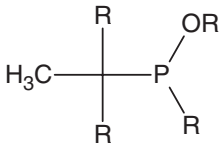
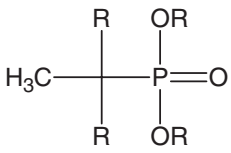
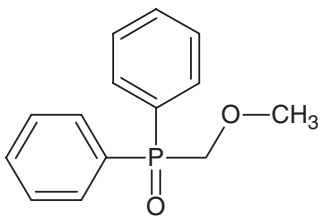
6.6.3 Coupling between Protons and ^{31}P

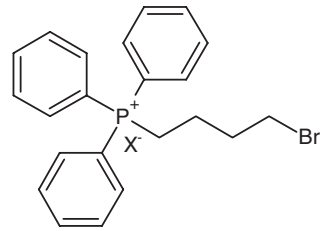
Phosphorus is the other heteroatom of major coupling importance to the organic chemist. Like ^{19}F, ^{31}P has a spin of $^1/_2$ and a 100 % natural abundance, so you know what to expect! The actual size of the couplings observed with ^{31}P can vary considerably, depending on the oxidation state of the ^{31}P atom. You'll find some useful examples in Table 6.3.

^{31}P shows one particularly interesting feature. The size of couplings normally decreases dramatically with the number of intervening bonds, but this is not always the case with ^{31}P (Table 6.3).

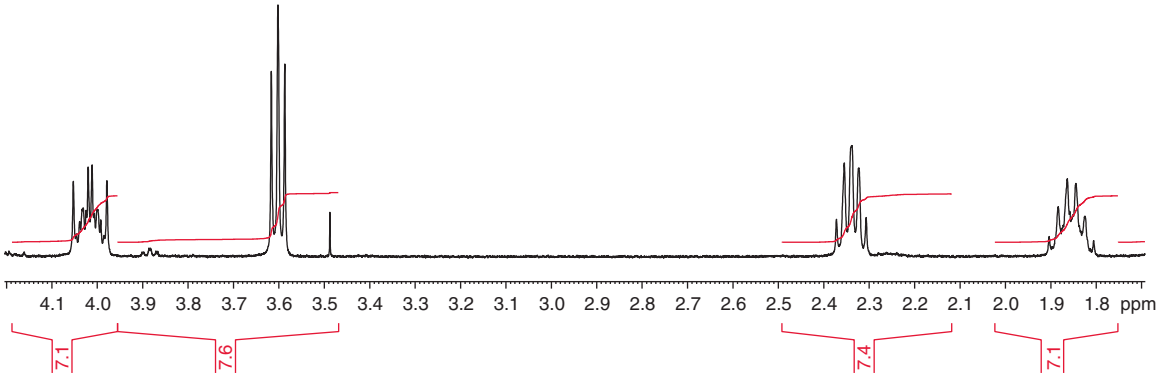
A proton directly bonded to a ^{31}P atom can be split by an enormous coupling of as much as 700 Hz (depending on the oxidation state of the phosphorus)! That means that the two parts of such a signal would be separated by almost 3 ppm at 250 MHz! So huge is this coupling that you could easily fail to recognise or accept it as a coupling at all, if you came across it. Structure 6.20 and Spectrum 6.13 show an example of ^{31}P-^{1}H coupling.

Table 6.3 Some typical ^{31}P–proton couplings.

Structure	^{31}P-^{1}H relative position	Typical ^{31}P-^{1}H coupling (Hz)
$(CH_3)_3P$	P-CH_3	2.7
$(CH_3)_3P{=}O$	P-CH_3	13.4
$(CH_3)_4P^+I^-$	P-CH_3	14.4
$(CH_3\text{-}CH_2)_3P$	P-$\mathbf{CH_2}$-CH_3 P-CH_2-$\mathbf{CH_3}$	0.5! 13.7!
$(CH_3\text{-}CH_2)_3P{=}O$	P-CH_2- P-CH_3	11.9 16.3
	P-H	180–200
	P-H	630–710
	P-CR_2-$\mathbf{CH_3}$	10.5–18.0
	P-CR_2-$\mathbf{CH_3}$	10.5–18.0
	P-CR_2-$\mathbf{CH_3}$	10.5–18.0
	P-$\mathbf{CH_2}$-	6



Structure 6.20 Compound showing ^{31}P-^{1}H coupling.



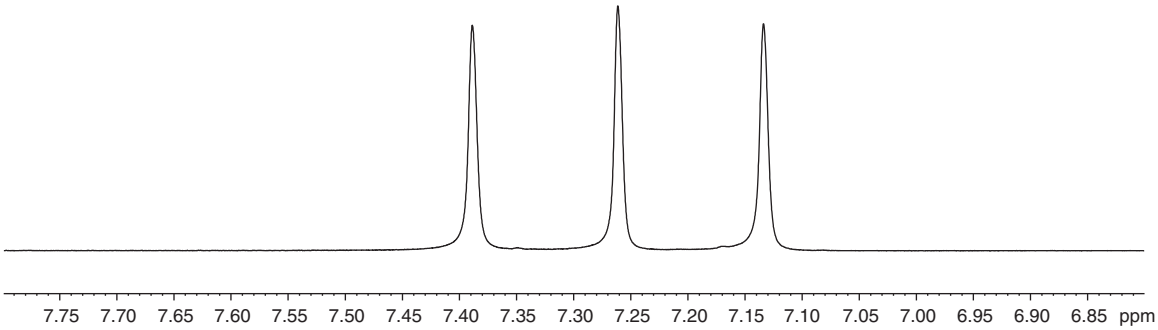
Spectrum 6.13 ^{31}P-^{1}H coupling.

The complex multiplet at 4.02 ppm shows a 13 Hz 2-bond ^{31}P coupling to the first -CH_2 in the chain and spin decoupling enables the 3-bond ^{31}P coupling to the next -CH_2 in the chain (1.86 ppm) to be measured (8 Hz).

6.6.4 Coupling between ^{1}H and other Heteroatoms

If you ever run a sample which is contaminated with an ammonium salt, in DMSO, you will see ^{14}N–proton coupling, as shown in Spectrum 6.14. Note that the three lines of the multiplet are of equal intensity (the middle line is a little bit taller than the outer ones, but this is because of the width of the peaks at their bases. The central signal is reinforced because it stands on the tails of the outer two). This is because ^{14}N has a spin of I = 1, and the allowed states are therefore –1, 0 and +1. This three line pattern with its 51 Hz splitting is highly characteristic and once seen, should never be forgotten.

^{14}N coupling is only observed when the nitrogen atom is quaternary. In all other cases, any coupling is lost by exchange broadening, or quadrupolar broadening, both of which we've discussed before. Two-bond couplings, [e.g., $^{14}\mathbf{N}^{+}$-($\mathbf{CH_2}$)$_4$] are not observed, even when the nitrogen is quaternary, in 'quat salts' such as (*n*-butyl)$_4$N^{+-} Br^{-}, presumably because the coupling is very small. So the phenomenon is only ever observed in the $^{+}$$NH_4$ ion! *Note*: The -CH_2- attached to the quaternary nitrogen in compounds like tetra N-butyl ammonium chloride **does** present as a distorted

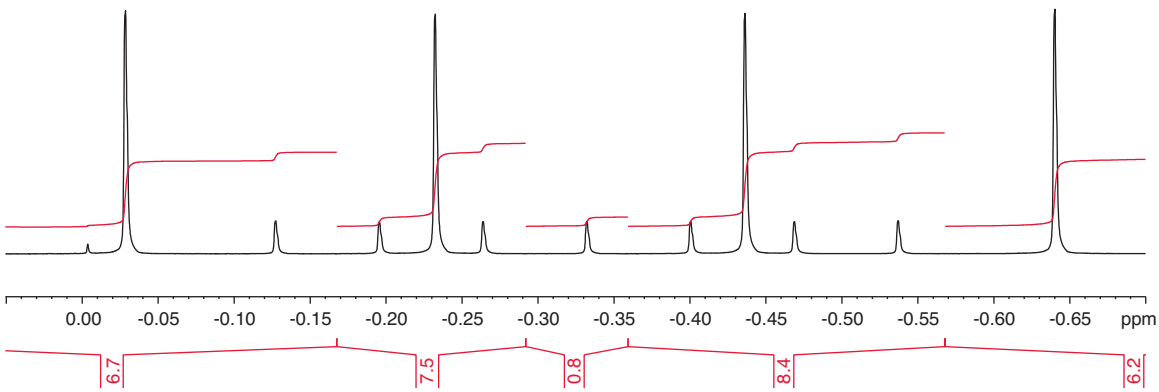


Spectrum 6.14 Typical appearance of $^{+}NH_4$ ion in DMSO.

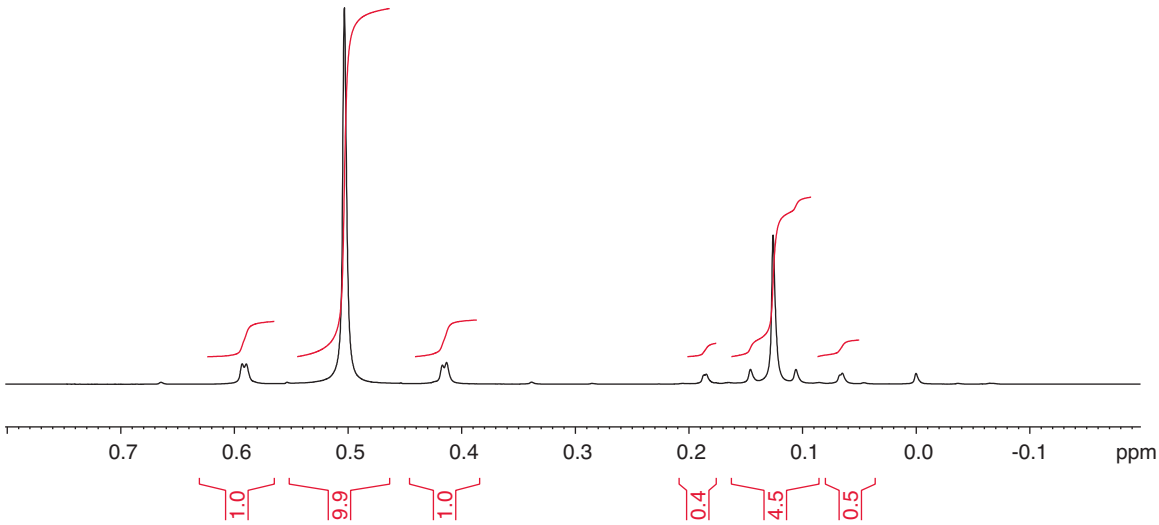
triplet with its central line split into a narrow triplet but this has nothing to do with ^{14}N coupling as the same distortion can sometimes be seen in -CH_2- groups next to certain other moieties, e.g., -SO_2R. It is a non-first order phenomenon caused by slight non-equivalence of the two protons in question.

Boron has two isotopes, both of which have spin! ^{10}B has a natural abundance of 18.8 %, and a spin of I = 3 (allowed spin states –3, –2, –1, 0, +1, +2, +3; i.e., one signal will be split into seven lines of equal intensity), whilst ^{11}B has a natural abundance of 81.2%, and a spin of I = 3/2 (allowed spin states –3/2, –l/2, +1/2, and +3/2; i.e., one signal will be split into four lines of equal intensity).

This gives rise to amazing effects in the borohydride, BH_4^- ion (Spectrum 6.15), which can sometimes be formed accidentally during borohydride reductions. Note that the ^{10}B-H couplings are of a different size to the ^{11}B-H couplings. All 11 lines of the BH_4^- ion are to be found between 0 and –0.7 ppm. Note that, like ^{14}N, ^{11}B has a quadrupolar nucleus, but once again the symmetrical environment of the borohydride ion negates the relaxation pathway that would otherwise cause significant line broadening. Boron coupling is not generally seen in asymmetric environments or over multiple bonds.



Spectrum 6.15 Boron–proton coupling in the borohydride ion.



Spectrum 6.16 Mixture of two organotin compounds.

One other heteroatom worth mentioning is tin as organotin compounds are significant in organic synthesis. Tin has no fewer than ten naturally occurring isotopes, but fortunately, only three of them have nuclear spin. ^{115}Sn has a natural abundance of a mere 0.32 %, which makes it spectroscopically insignificant, of course. The only isotopes of tin that need concern us, are ^{117}Sn (natural abundance 7.67 % and I=1/2), and ^{119}Sn (natural abundance 8.68 %, and also, I=1/2).

These two isotopes are both capable of two-bond and three-bond couplings in alkyl organotin compounds. This is demonstrated in Spectrum 6.16 which shows a mixture of two organotin compounds. The compound with a strong central peak at 0.5 ppm is thought to be $(CH_3)_3$-Sn-OH. The inner satellites result from a ^{117}Sn-CH_3 coupling of 69 Hz and the outer satellites to a ^{119}Sn-CH_3 coupling of 72 Hz.

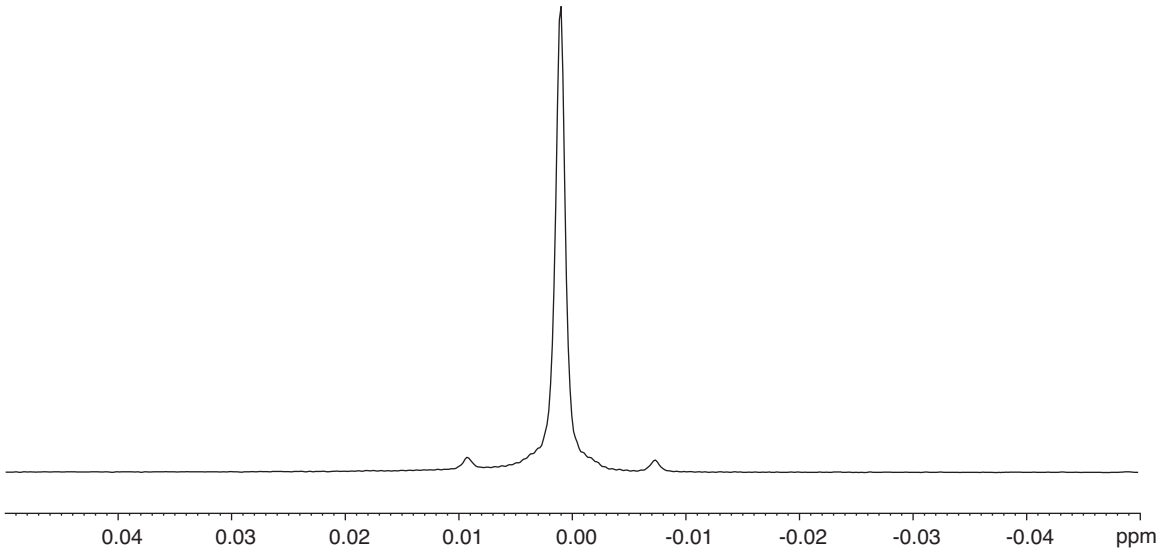
The second compound with the major signal centred at 0.13 ppm is $(CH_3)_3$-Sn-Sn-$(CH_3)_3$. In this case, we see once again, satellites resulting from two-bond couplings but also a second set of inner satellites resulting from smaller three-bond couplings of about 16 Hz for both ^{117}Sn and ^{119}Sn (i.e., **Sn**-Sn-**CH_3**).

Note too from the chemical shifts of these methyl groups that tin has quite a strong shielding effect.

Finally, ^{29}Si is an isotope that you should be aware of – every time you acquire a well prepared sample using TMS as a standard! ^{29}Si satellites (accounting for about 4.7 % of the total signal, J ^{29}Si – -CH_3, 6.6 Hz) should be visible at the base of your TMS peak. The small coupling provides a good test of shimming quality (Spectrum 6.17).

6.6.5 Cyclic Compounds and the Karplus Curve

As we have already mentioned, chemical shifts and couplings are heavily influenced by molecular constraint and for this reason, some guidance in dealing with cyclic (saturated) compounds might well prove useful. We have already seen that in straightforward open-chain alkyl systems, the size of proton-proton couplings is governed by the electronegativity of neighbouring atoms. But the most



Spectrum 6.17 TMS showing ^{29}Si satellites.

important factor which governs the size of couplings between vicinal protons is the dihedral angle between them.

In open-chain systems, this angle is usually averaged by rotation about the C-C single bond, and so is not normally of significance. But in carbocyclic systems, dihedral angles are usually fixed, since the structures are generally rigid. It is therefore vital that we understand how the size of vicinal couplings varies with dihedral angle. This data can be obtained by using the Karplus equation but the information derived from this equation (or equations as there are various versions of it) is more usefully portrayed graphically. A family of curves thus constructed makes additional allowance for factors other than dihedral angles which influence vicinal proton couplings, e.g., localised electronegativities (Figure 6.2) but we have opted for a simplified graph showing only three curves.

Selection of the best curve for a given situation is perhaps rather a matter of trial and error, but is best approached by positively identifying an axial–axial coupling, since this arrangement ensures (in six-membered rings at least) a dihedral angle of 180° between the protons. Choose the curve that best fits the value that you observe for an axial–axial coupling in your molecule. Note that in the absence of any extreme electronic effects, this should give rise to a coupling of about 12 Hz. Similarly, a dihedral angle of 90° gives rise to a coupling of approximately 0 Hz, and where the angle is 0°, we may expect a coupling of about 10 Hz. Making a model of the molecule becomes very important in the case of carbocyclic compounds, as it is important to be able to make fairly accurate estimates of dihedral angles.

Now let us consider Structure 6.21 and Spectrum 6.18 and see how the Karplus curve can be used to aid assignment of the spectrum. (This compound will be referred to from now on as *the* morpholine compound as we will use it to demonstrate several different techniques) Note that the aromatic region has been omitted as it contains little of interest and we wish to concentrate on the carbocyclic region of the spectrum. It was acquired in $CDCl_3$.

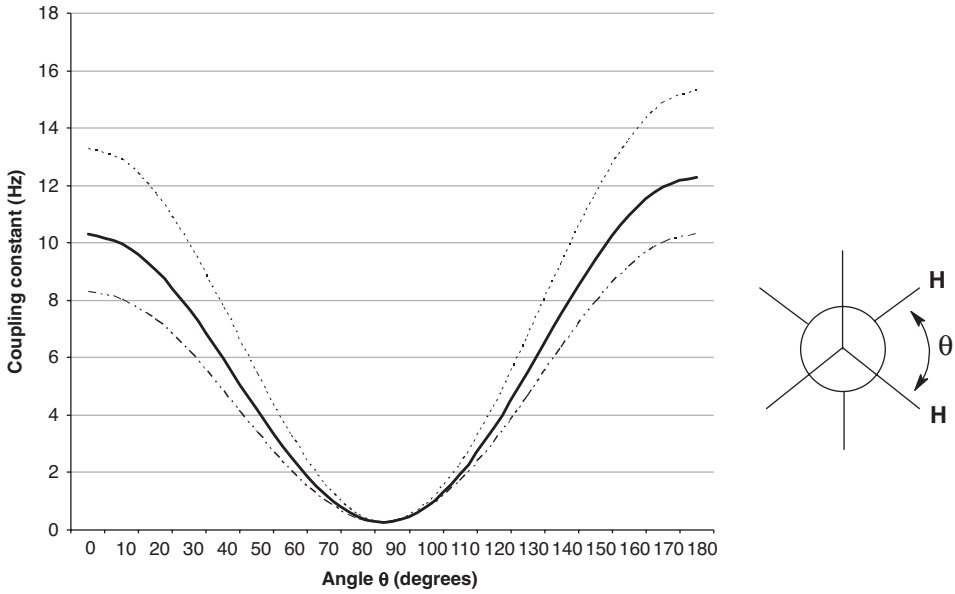
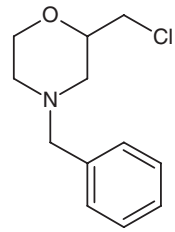


Figure 6.2 The Karplus curve – for relating the observed splitting between vicinal protons to their dihedral angle, θ.

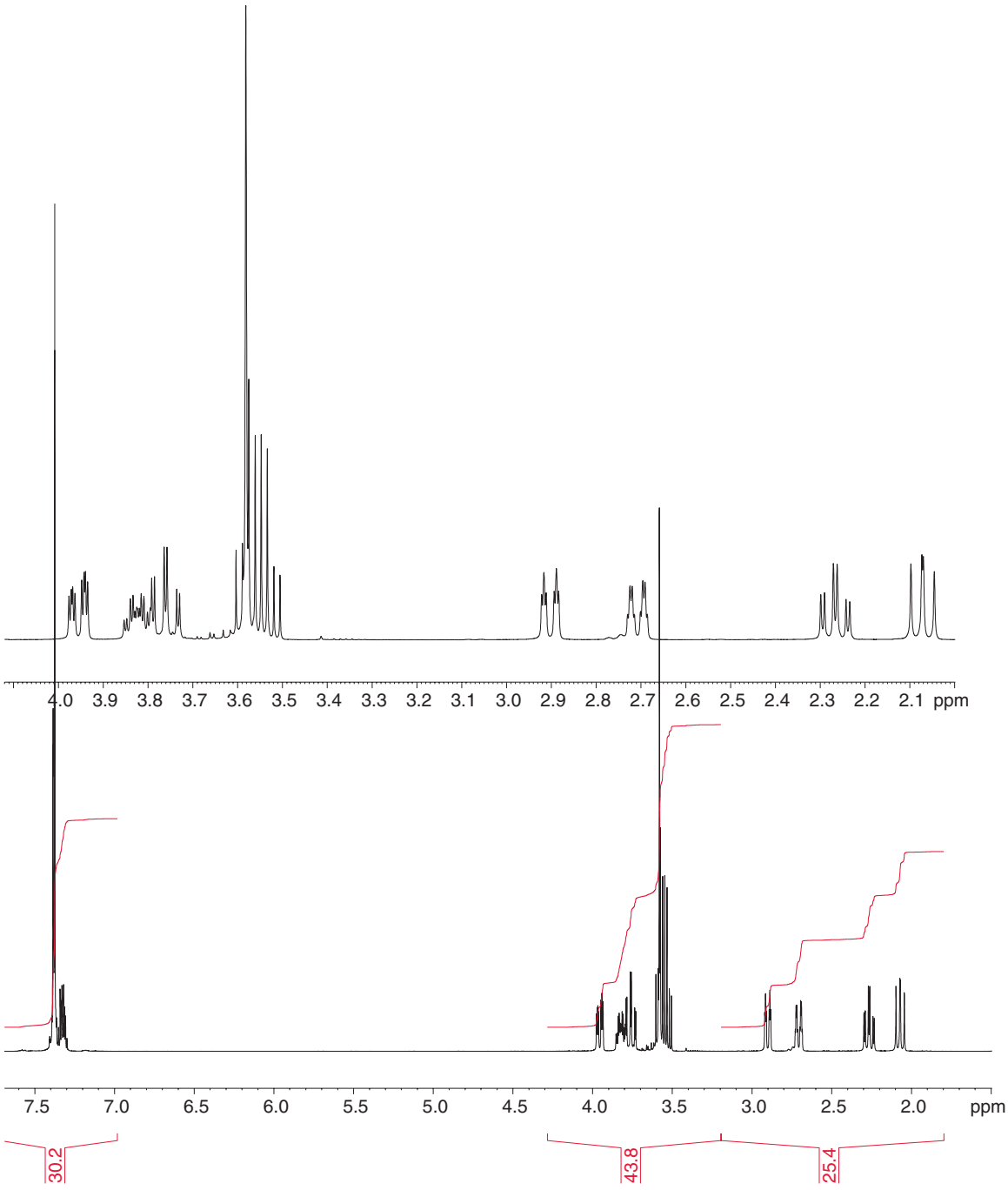
To derive maximum benefit from this exercise, we recommend that you make a model of this molecule, and refer to it as we go through the spectrum. Note that the morpholine ring falls naturally into a 'chair' conformation. Note also that in this example, the -CH_2-Cl function will seek to minimise the morpholine ring energy by occupying an equatorial environment as this minimises steric interactions between it, and protons, and other substituents on the ring. All groups do this. The benzyl function will do likewise by inversion of the nitrogen stereochemistry.

It is also worth noting that nine times out of ten, equatorial protons absorb at somewhat lower field than the corresponding axial protons. This can be reversed in certain cases where the specific anisotropies of the substituents predominate over the anisotropies of the rings themselves but this is relatively rare. The difference is typically 0.5–1.0 ppm, but may be more.

The structure is depicted as a Newman projection below (Figure 6.3). Aromatic protons aside, (they give the expected five-proton multiplet centred at around 7.3–7.4 ppm) the first signal we encounter as



Structure 6.21 *The* morpholine compound.



Spectrum 6.18 *The* morpholine compound in $CDCl_3$ with expansion.

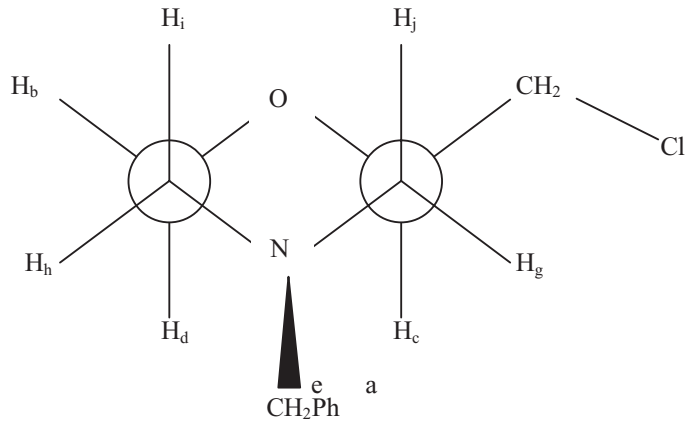


Figure 6.3 *The* morpholine compound shown as a Newman projection.

we work from left to right is a complex multiplet – which is actually, a doublet of doublet of doublets [ddd] – at 3.95 ppm. Careful measurement of the couplings reveals them to be 11.4, 3.4 and 2.0 Hz. Since the multiplet is dominated by one large coupling, we can be safe in the knowledge that it must be an *equatorial* proton.

This is because the dihedral angles between equatorial protons and both their equatorial and axial vicinal partners are always such that they give rise to relatively small couplings (check model and the Karplus curves). The only large coupling (i.e., 10 Hz or more) an equatorial proton can have will always be to its geminal partner – if it has one. So in this case, the 11.4 Hz coupling is clearly a geminal coupling. If we now make the entirely reasonable deductions that the proton giving rise to this signal is likely to be alpha to oxygen rather than nitrogen (on the basis of chemical shift) and that as the -CH_2-Cl will be equatorial (as explained earlier), then this multiplet can only be assigned to the equatorial proton 'b' since there are no other equatorial protons that are alpha to oxygen in the molecule. The other two couplings can be rationalised in terms of the equatorial–axial coupling (3.4 Hz which is reciprocated in the ddd at 2.27 ppm) and the equatorial–equatorial coupling (2.0 Hz which is reciprocated in the dddd at 2.71 ppm). *Note*: methods of unpicking couplings will be discussed at length in later sections. Such methods are very useful when dealing with more complex spin systems like this one.

The degree of roofing of 'b' indicates that its geminal partner must be fairly close to it in terms of chemical shift and sure enough, the six-line multiplet (another ddd) centred at 3.76 ppm satisfies the requirements for this proton ('d'). Note that the second large coupling to this signal is also 11.4 Hz, the axial–axial coupling being the same size as the geminal coupling in this instance. The small remaining coupling (approx. 2 Hz) is reciprocated in the dddd at 2.71 ppm and is an axial–equatorial coupling.

Proton 'c' can be defined by the fact that it is *not* equatorial and it is highly coupled. The multiplet at 3.82 ppm satisfies these requirements. It is in the right ball park for chemical shift and is highly complex in that this proton is already the X part of an ABX system coupled to both protons alpha to the chlorine (the AB part). It is then further coupled with a 10 Hz, axial–axial coupling (reciprocated in the dd at 2.07 ppm) and with a 2 Hz axial–equatorial coupling which is reciprocated in the ddd at 2.90 ppm. Note that 'c' and 'd' are not fully resolved from each other. Such overlap inevitably complicates the issue.

The N-benzyl protons are accidentally equivalent, presenting as a singlet at 3.59 ppm and overlap with the two protons alpha to the chlorine atom which present as the heavily roofed AB part of an ABX system (i.e., eight lines) centred at 3.55 ppm.

Without slavishly dissecting the remaining four signals (2.90, 2.71, 2.27, 2.07 ppm), we hope that the principles of carbocyclic analysis have now been established. You should see at a glance that the 2.90 and 2.71 ppm signals must belong to equatorial protons because they are each dominated by only one large coupling and the remaining two must correspond to their axial partners. You should now be able to verify which equatorial proton belongs to which axial proton just by inspection.

There is one last coupling which we have not yet mentioned and that is the apparent extra small coupling that can be seen on the equatorial protons alpha to the nitrogen (2.90 and 2.71 ppm). These two signals are in fact coupled to each other by what is known as a W path coupling. These are 4-bond couplings (unusual in saturated systems) which can be seen in situations where all the intervening proton–carbon and carbon–carbon bonds lie in the same plane. You can see from the model which you have next to you (?) that by definition, such protons can only be equatorial. Note that whilst all the assignments in this section have been made purely on the basis of observations of couplings and multiplet appearance, this type of assignment is often simplified by having definitive knowledge of coupling pathways. We will discuss the options available for acquiring this type of data in a later chapter.

Whilst six-membered rings may often give rise to quite complex spectra, they are at least generally rigid and based on the 'chair' conformation. As we have seen, this means that dihedral angles can be relied on and the Karplus curve used with reasonable confidence. Unfortunately however, the same approach will end in tears if applied to other ring systems. Five-membered rings for example, are notoriously difficult to deal with as they have no automatic conformational preference. They are inherently flexible, their conformations driven by steric factors. *Cis* protons on five-membered rings can have dihedral angles ranging from approximately –30° to 0° to +30° and exhibit a range of couplings to match. *Trans* protons on the other hand can range from +90° to +150°. Deductions that can be made on the basis of observed vicinal couplings are therefore limited. If the observed coupling is *very* small, the two protons can only be trans to each other but if it is not, then they may be either *cis* or *trans*. We council against reliance on molecular modelling packages to produce a valid conformation of such structures. The energy difference between potential conformers is often small and could change in different solvents. Modelling packages consider molecules in isolation and thus make no allowances for solvent effects. Stereochemical assignments of such ring systems can only be confidently made on the basis of NOE experiments which we will cover in detail in Section 8.5.

6.6.6 Salts, Free Bases and Zwitterions

Sometimes, misunderstandings can arise when dealing with compounds containing protonatable centres. Hopefully, in this section, we will be able to clarify a few key issues that are relevant to such compounds.

As we have already mentioned, $CDCl_3$ should be avoided as a solvent for salts for two reasons. Firstly, salts are unlikely to be particularly soluble in this relatively nonpolar solvent but more importantly, spectral line shape is likely to be poor on account of relatively slow proton exchange at the protonatable centre. The remedy is simple enough – avoid using $CDCl_3$ and opt for one of the more polar options instead, e.g., deuterated DMSO or MeOH and you should obtain spectra every bit as sharp as those of free bases.

In practical terms, it is invariably a nitrogen atom that is protonated in salt formation. This always leads to a downfield shift for protons on carbons both alpha and beta to the nitrogen concerned. In alkyl amines, the expected shifts would be about 0.7 and 0.3 ppm respectively. Remember that some heterocyclic compounds (e.g., pyridine) contain nitrogen atoms that are basic enough to protonate and comparable downfield shifts can be expected (Spectrum 5.9).

A misconception that we commonly encounter is that a spectrum can be a 'mixture of the salt and the free base.' This is an excuse that is often used by chemists to explain an inconveniently messy looking spectrum! Don't be tempted by this idea – proton transfer is fast on the NMR timescale (or at least, it is when you use a polar solvent!) and because of this, if you have a sample of a compound that contains only half a mole-equivalent of an acid, you will observe chemical shifts which reflect partial protonation and *not* two sets of signals for protonated and free-base forms. It doesn't happen – ever!

Of course, whether a compound forms a salt or not depends on the degree of availability of the lone pair of electrons on any nitrogen atoms in the compound (i.e., their p*K*b values) and on how strong the acids involved in the salt formation (pKas). As a rough rule of thumb, alkyl and aryl amines *do* form salts whilst amides, ureas, most nitrogen-containing heterocycles and compounds containing quaternary nitrogen atoms *do not*.

It should always be remembered of course, that the NMR spectrum reflects a compound's behaviour *in solution*. It is quite possible for a compound and a weak acid to crystallise out as a stoichiometric salt and yet in solution, for the compound to give the appearance of a free base. For this reason, care should be taken in attempting to use NMR as a guide to the extent of protonation. If the acid has other protons that can be integrated reliably, e.g., the alkene protons in fumaric or maleic acid, then there should be no problem but if this is not the case, e.g., oxalic acid, then we would council caution! Do not be tempted to give an estimate of acid content based on chemical shift. With weak acids, protonation may not occur in a *pro rata* fashion though it is likely to in the case of strong acids.

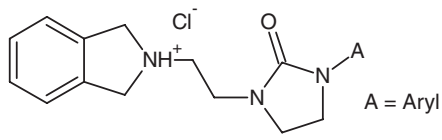
Sometimes, you may encounter compounds which have more than one protonatable centre. It is often possible to work out if either one or more than one are protonated in solution. A good working knowledge of pKbs is useful to help estimate the likely order of protonation with increasing acidity. Assume that the most basic centre will protonate first and assess the chemical shifts of the protons alpha to each of the potentially protonatable nitrogen atoms.

Zwitterionic compounds are worthy of special mention:

$$H_2N{-}R-COOH \leftrightarrow H_3N^+{-}R-COO^-$$

By their very nature, their partial charge separation can make them fairly insoluble and the degree of this charge separation (and hence resultant NMR spectra) tends to be highly dependant on concentration and pH. For these reasons, we recommend dealing with such compounds by 'pushing' them one way or the other, i.e., by adjusting the pH of your NMR solution so that the compound in question is either fully protonated (addition of a drop of DCl) or de-protonated (addition of a drop of saturated sodium carbonate in D_2O):

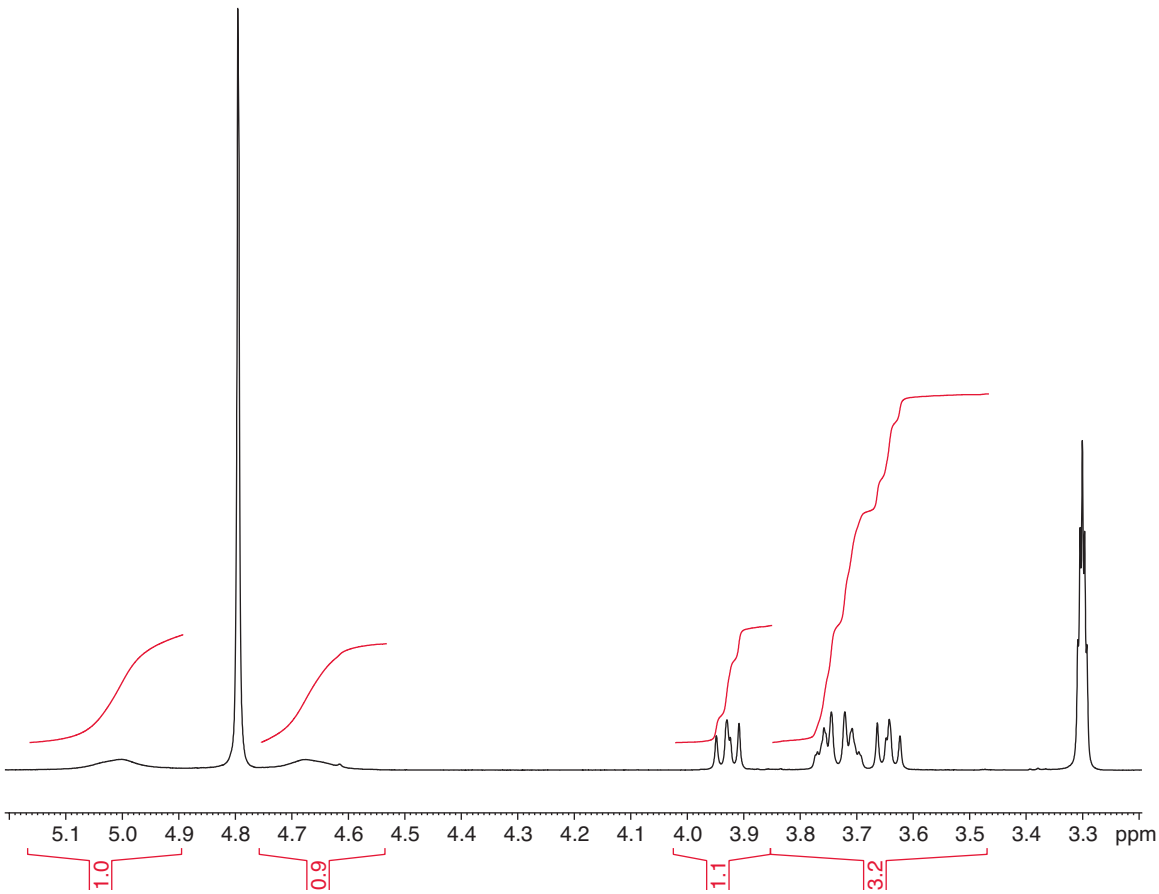
$H_3N^+{-}R-COOH$	$H_2N-R-COO^-$
Fully protonated	Fully de-protonated



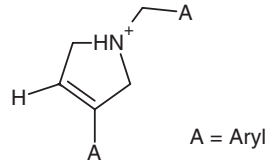
Structure 6.22 A protonation example.

Whilst dealing with protonation issues, it is well worth considering the time dependence of the process in the context of the NMR timescale. A compound of the type shown in Structure 6.22 provides an interesting example.

As a free base, the Ar-CH_2-N protons would present themselves as a simple singlet. The lone pair of electrons on the nitrogen invert very rapidly on the NMR timescale and so the environment of the two protons is averaged and is therefore identical. However, on forming a salt, the whole process of stereochemical inversion at the nitrogen is slowed down dramatically because the sequence of events



Spectrum 6.19 Slow inversion of a protonated tertiary amine nitrogen.



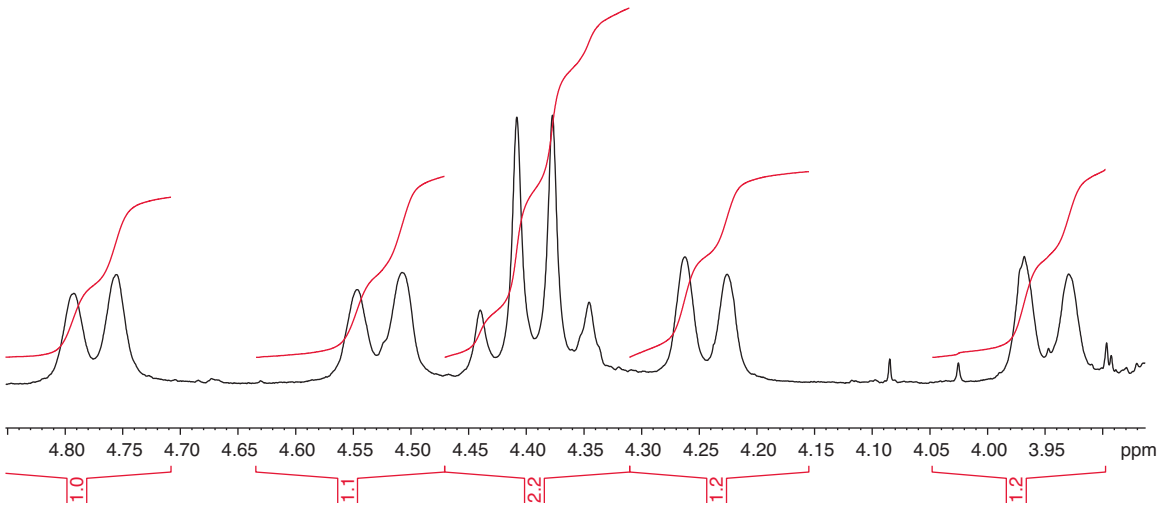
Structure 6.23 Compound showing 'pseudo enantiomeric' behaviour.

would be; de-protonation, inversion and re-protonation. Although as we said earlier, proton transfer is in itself a very fast process on the NMR timescale, it is the time taken for the *entire* process to occur that determines the nature of the spectrum that we observe.

What we actually observe for the Ar-CH_2-N protons of the salt in this molecule is a pair of broad, featureless signals at 4.6 and 5.0 ppm. The explanation for this is simple enough once the concept of time dependency for the inversion sequence has been appreciated. The protons in question find themselves in different environments (within the context of the NMR timescale) and therefore have distinct chemical shifts. The signals are broad because the dynamic exchange process is taking place with a time period comparable to the NMR timescale, the broadening masking the geminal coupling between them (see Spectrum 6.19).

A logical extension of these ideas will lead you to a recognition of the fact that a phenomenon of this type could yield species in solution which appear to behave as if they contain a chiral centre – even when they don't. We have seen 'pseudo enantiomeric' behaviour in compounds of the type shown in Structure 6.23 (when protonated).

All the protons of the CH_2s in a molecule of this type may be non-equivalent (i.e., you observe essentially three AB systems). Note that the coupling from the alkene CH is would be small to both of the cyclic CH_2s when the spectrum is acquired in the presence of HCl (see Spectrum 6.20). When the free



Spectrum 6.20 Protonated nitrogen of a tertiary amine acting as a 'chiral centre'.

base is liberated, all the AB systems collapse to give singlets. The explanation follows on logically from a consideration of the previous example. Protonation of the tertiary amine generates a chiral centre at the nitrogen atom, forcing all the geminal pairs of protons into different environments – hence the three AB systems. But this does not in any way imply that it would be possible to separate out enantiomers of the compound in salt form. These 'pseudo enantiomers' can only be differentiated within the context of a technique which has a timescale of a couple of seconds. Attempting to separate them on an HPLC column for example, would be unsuccessful as this technique has a timescale of several minutes (defined by how long compounds take to travel down the column and enter the detector). During this time, proton exchange and consequent 'enantiomer' interconversion would have occurred many times in the course of the analysis. The only manifestation of this might be a slightly broader than normal (single) peak.

This whole area of spectroscopy touches on many different topics and can only be approached confidently with a reasonable working knowledge of basic NMR, stereochemistry and certain aspects of physical chemistry.

7

Further Elucidation Techniques – Part 1

If a spectrum does not yield the definitive information that you require on inspection, there are many other 'tools of the trade' that we can use to further elucidate structures. Broadly speaking, these fall into two categories – chemical techniques and instrumental techniques.

7.1 Chemical Techniques

We will take a brief look at chemical techniques first. It is true to say that the development of more and more sophisticated instrumental techniques has to a considerable extent, rendered these less important in recent years but they still have their place and are worthy of consideration in certain circumstances.

Before embarking on any of these chemical techniques, however, be advised that they are not without a measure of risk as far as your sample is concerned! One of the great strengths of NMR is that it is a nondestructive technique but that can change quite rapidly if you start subjecting your compounds to large changes in pH or to potentially aggressive reagents like trifluoroacetic anhydride (TFAA)! Be sure that you can afford to sacrifice the sample as recovery may not be possible. In the case of precious samples, chemical techniques should be regarded as a last option rather than a first choice.

7.2 Deuteration

Deuteration is the most elementary of the chemical techniques available to us, but it is still useful for assigning exchangeable protons which are not obviously exchangeable, and for locating exchangeables masked by other signals in the spectrum. There are of course, other ways of identifying exchangeables. The signal can be scrutinised closely to see if it has any ^{13}C satellites associated with it, though this is not viable in the case of broad signals. Alternatively, irradiation of the water peak in an NOE experiment can be used as we'll see later. Nonetheless, deuteration does provide a quick and easy method of identification which is still perfectly valid.

Just to recap on the procedure, add a couple of drops of D_2O to your solution, and shake vigorously for a few seconds. Note that with $CDCl_3$ solutions, the best results are obtained by passing the resultant solution through an anhydrous sodium sulfate filter to remove as much emulsified D_2O as possible. (Note

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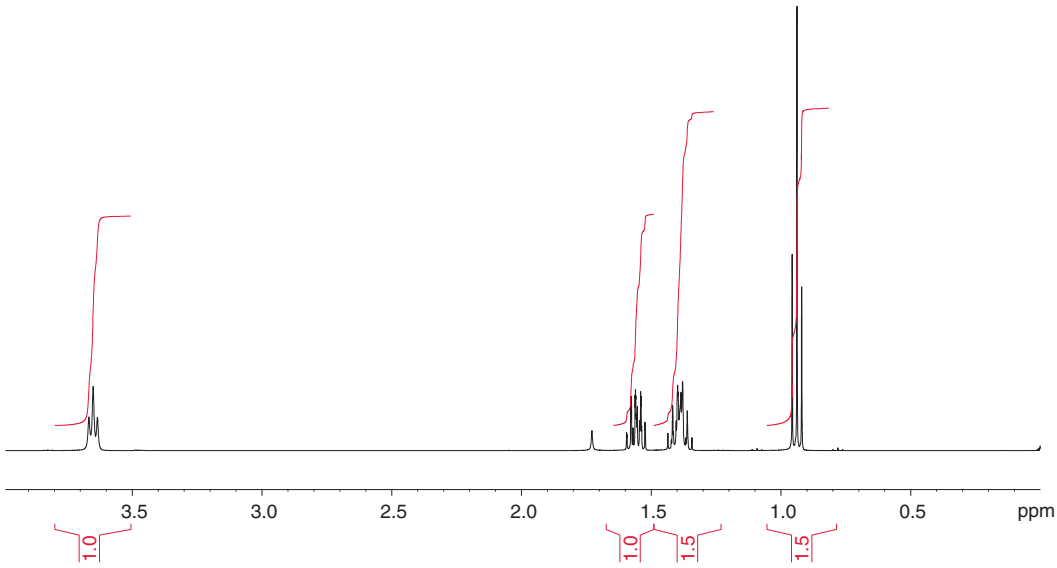
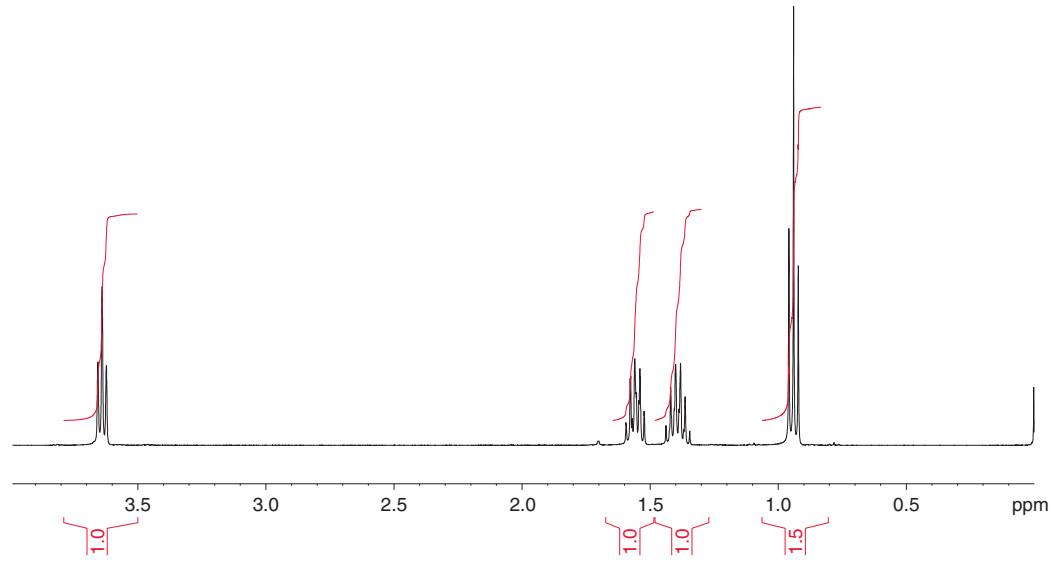
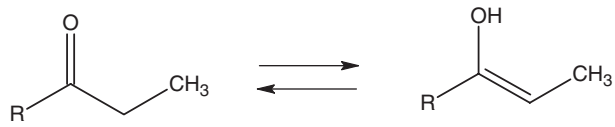


Spectrum 7.1 *n*-Butanol in $CDCl_3$ with -OH obscured by multiplet at 1.39 ppm.

also that $CDCl_3$ and D_2O are not miscible, the $CDCl_3$ forming the bottom layer as it is more dense than D_2O.) You then re-run the spectrum and check for the disappearance of any signals. Careful comparison of integrations before and after addition of D_2O should reveal the presence of any exchangeable protons buried beneath other signals in the spectrum. If they are slow to exchange, like amides, a solution of sodium carbonate in D_2O, or NaOD may be used. The technique is demonstrated using *n*-butanol in Spectra 7.1 and 7.2. Note the reduction in integration of the multiplet centred at 1.39 ppm.



Spectrum 7.2 *n*-Butanol in $CDCl_3$ after shaking with two drops of D_2O



Structure 7.1 Keto-enol exchange.

Remember that any proton which is acidic enough is prone to undergo deuterium exchange. Methylene protons alpha to a carbonyl for example, may exchange if left standing with D_2O for any length of time, as they can exchange via the keto-enol route (i.e., Structure 7.1).

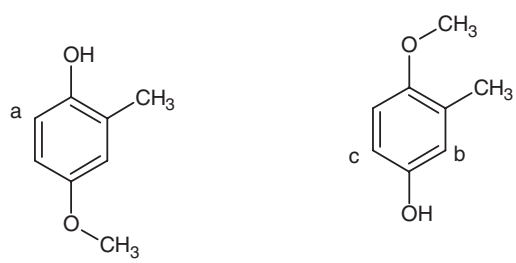
Note that deuterium exchange of the -OH leads to incorporation of deuterium alpha to the carbonyl in the ketone form. This may happen, even if there is no evidence of any enol signals in the spectrum initially, i.e., it can occur even when the equilibrium is heavily in favour of the ketone. Aromatic protons of rings which bear two or more -OH groups are also prone to undergo slow exchange, as are nitrovinyl protons.

7.3 Basification and Acidification

This topic has been dealt with quite extensively in Section 6.6.6 so we won't go over the material again but there is perhaps one other type of problem that may be worth looking at with a view to solving by a change of pH. Consider the two structures in Structure 7.2.

Whilst the preferred method of differentiating these structures would be by an NOE experiment, it would be possible to accomplish this by running them in DMSO and then adding a drop of base to each solution and re-running. (Note: DMSO is the preferred solvent for this experiment as both the neutral and the charged species would be soluble in it.) In both cases, the phenoxide ion ($Ar\text{-}O^-$) would be formed and the extra electron density generated on the oxygen would feed into the ring and cause a significant upfield shift of about 0.3–0.4 ppm in any protons ortho- or para- to the hydroxyl group. In the example above, the compound on the left would show such an upfield shift for only a single doublet (a), whilst the compound on the right would show an analogous upfield shift for both a narrow doublet (b) and a doublet of doublets (c).

Caution should be exercised if attempting any determination of this type as it is not the preferred method and it is always safest if *both* compounds to be distinguished are available for study in this way.



Structure 7.2 Compounds which show one or two upfield shifts.

7.4 Changing Solvents

If a signal of particular interest to you, is obscured by other signals in the spectrum, it is often worth changing solvent – you might be lucky, and find that your signal (or the obscuring signals) move sufficiently to allow you to observe it clearly. You might equally well be unlucky of course, but it's worth a try.

Running a sample in an anisotropic solvent like D6-benzene or D5-pyridine, can bring about some even more dramatic changes in chemical shifts. We tend to use benzene in a fairly arbitrary fashion, but in some cases, there is a certain empirical basis for the upfield and downfield shifts we observe.

For example, benzene forms collision-complexes with carbonyl groups, 'sitting' above and below the group, sandwich-style. When the carbonyl is held rigidly within the molecule, either because it forms part of a rigid system, or because of conjugation, we can generally expect protons on the oxygen side of a line drawn through the carbon of the carbonyl, and at right angles to the carbonyl bond to be deshielded. Conversely, those on the other side of the line are shielded.

7.5 Trifluoroacetylation

This is quite a useful technique which can give a rapid, positive identification of -OH, $-NH_2$, and -NHR groups in cases where deuteration would be of little value. Even though the technique can be a little time-consuming and labour-intensive in terms of sample preparation, it can nonetheless yield results in less time than it would take to acquire definitive ^{13}C data – particularly if your material is limited.

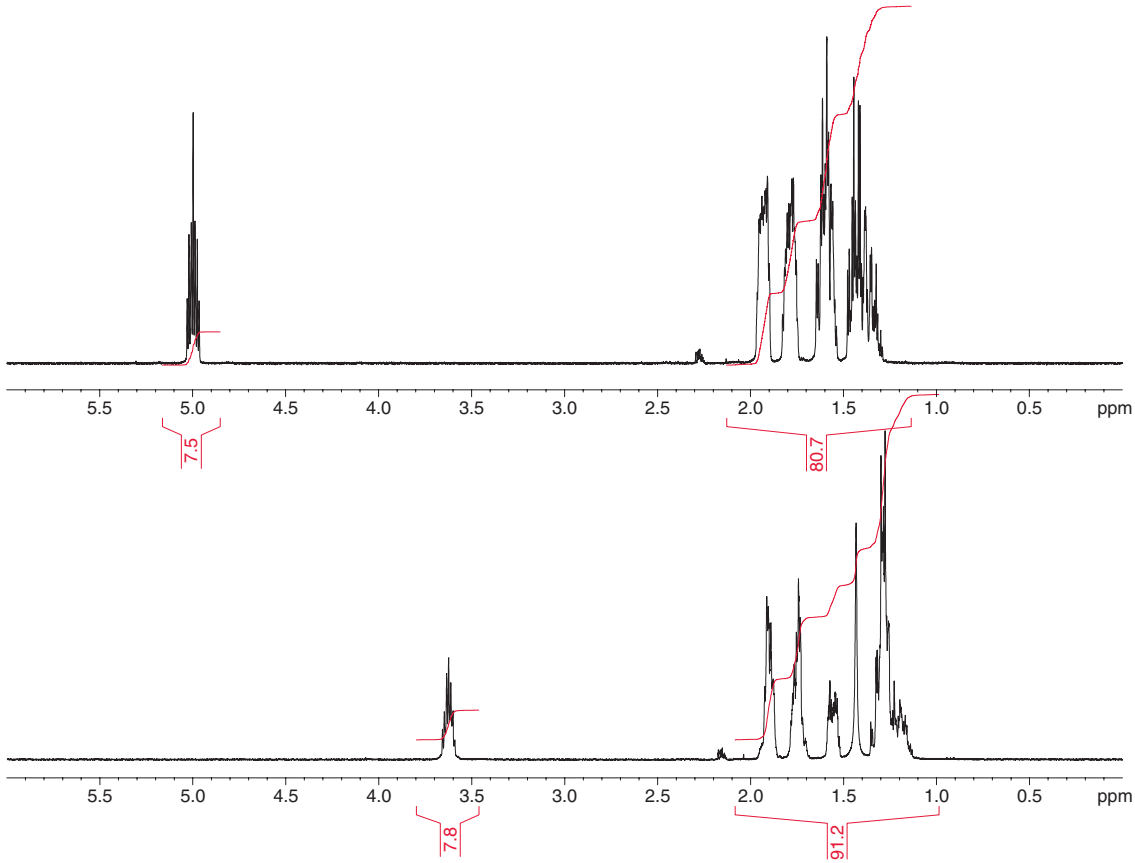
Consider Spectrum 7.3. The bottom trace shows the ordinary spectrum of cyclohexanol, run in $CDCl_3$. Distinguishing it from chlorocyclohexane is not easy (without the use of ^{13}C NMR) – the chemical shift of the proton alpha to the functional group would be similar in both compounds, and in the case of the alcohol, the -OH need not show coupling to it. Furthermore, in problems of this type, the -OH proton itself may well be obscured by the rest of the alkyl signals or combined with the solvent water peak. Integration of the alkyl multiplet before and after deuteration will not necessarily be very reliable, since looking for 1 proton in a multiplet of 10 or 11, will give only a relatively small change in integral intensity (and let us not forget that water in the $CDCl_3$ which will absorb in this region, along with any water that may be residual in the compound).

The top trace shows what happens when the sample is shaken for a few seconds with a few drops of TFAA. The reaction shown in Structure 7.3 occurs.

The resultant spectrum is clearly very different from the alcohol, as the trifluoroacetic ester function is far more deshielding with respect to the alpha proton than is the -OH group. A downfield shift of >1 ppm can be seen. This clearly distinguishes the alcohol from the analogous chloro compound which would of course give no reaction.

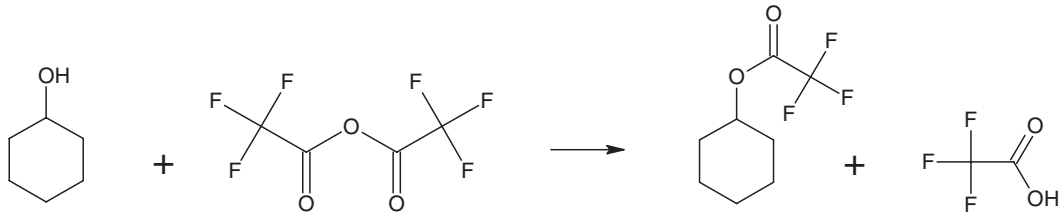
This is a relatively quick and convenient technique, the reagent reacting quite readily (assuming no great steric hindrance of course) with alcohols, primary and secondary amines. (Note the possibility of complication if you react a secondary amine with TFAA – it will yield a tertiary amide!) If the reaction is a little slow, as is often the case with phenolic -OH groups, you can 'speed it up a little' by gentle warming, more shaking, and even adding a drop of D5-pyridine to base-catalyse the reaction.

Use of this reagent is however, somewhat limited. You can only use it in solvents which don't react with it (D4-methanol, and D_2O are obviously out of the question), or contain a lot of water, i.e., D6-DMSO. Another slight drawback is that the cleaving of the anhydride liberates trifluoroacetic acid,



Spectrum 7.3 The use of TFAA to identify an -OH group.

which has nuisance value if your compound is very acid-sensitive, and will also protonate any unreacted amine function present. If this salt-formation is a problem, it is worth adding sodium bicarbonate in D_2O, dropwise, to neutralise the acid. You'll know when the excess TFA has been neutralised, as further additions of bicarbonate fail to produce any further effervescence (shake thoroughly, and don't forget to release built up pressure by releasing carbon dioxide!) Dry your solution through an anhydrous sodium sulfate filter before rerunning.



Structure 7.3 Using TFAA to identify an -OH group.

7.6 Lanthanide Shift Reagents

Unfortunately, the use of lanthanide shift reagents such as the europium compound, $Eu(fod)_3$ is a practise that has been largely consigned to the dustbin of history so we will say very little about them. The problem with trying to use them in high-field spectrometers is that the fast relaxation times of the collision complexes brought about by paramagnetic relaxation (courtesy of the europium or other lanthanide atom), leads to severe line broadening. This paramagnetic broadening is very much worse in high field spectrometers so if you are using a 250 MHz or above, don't bother trying.

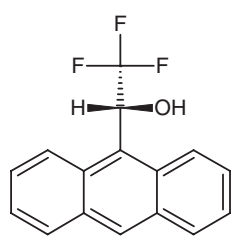
If, however, you are still soldiering on with a spectrometer of 100 MHz or less, then by all means try using them to 'stretch' a spectrum out – if your compound is suitable. They work by coordinating with an atom that has a lone pair of electrons available for donation. The more available the lone pair, the greater will be the affinity ($-NH_2$/NHR > -OH > >C=O > -O- > -COOR > -CN). Note that they will only work in dry solvents that don't contain available lone pairs. Good luck!

7.7 Chiral Resolving Agents

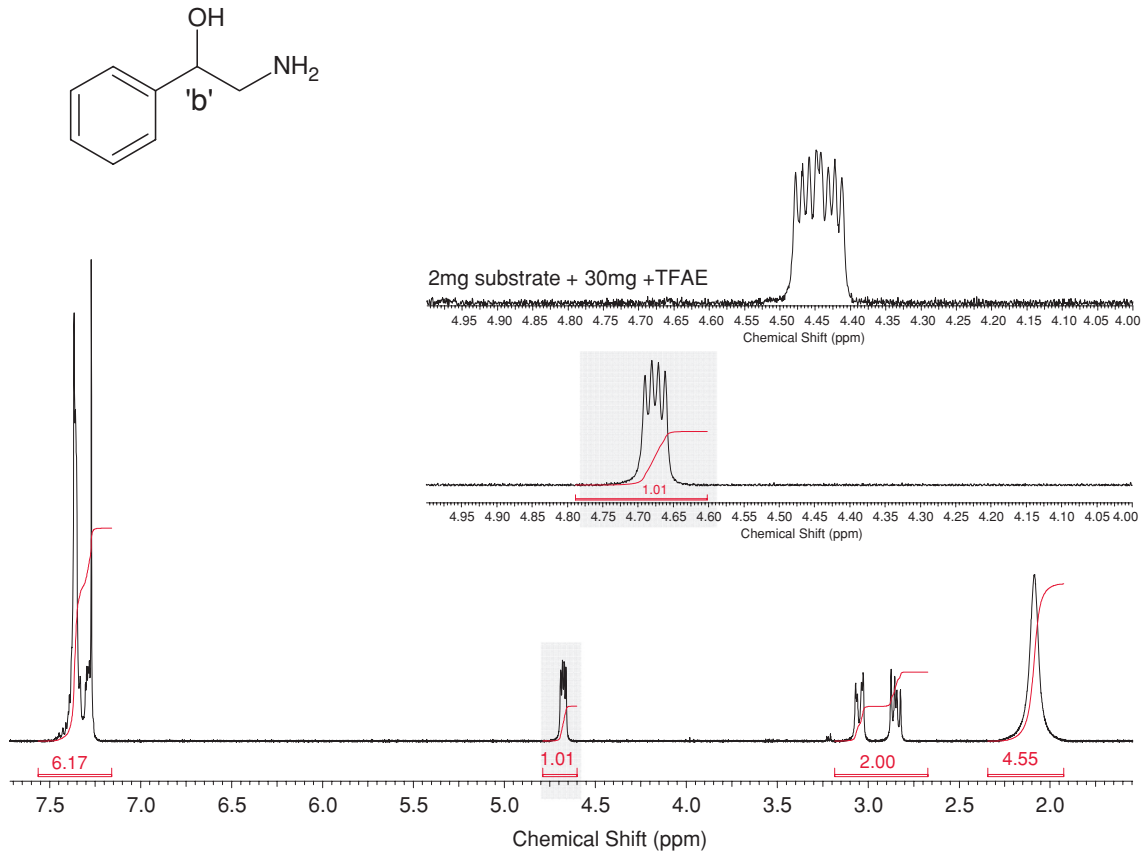
We have seen that the spectra of enantiomers, acquired under normal conditions, are identical. The NMR spectrometer does not differentiate between optically pure samples, and racemic ones. The wording is carefully chosen, particularly 'normal conditions', because it is often possible to distinguish enantiomers, by running their spectra in abnormal conditions – in the presence of a chiral resolving agent. Perhaps the best known of these is (–)2,2,2,trifluoro-l-(9-anthryl) ethanol, abbreviated understandably to TFAE. (W.H. Pirkle and D.J. Hoover, *Top. Stereochem.*, 1982, **13**, 263). Structure 7.4 shows its structure.

This reagent may form weak collision-complexes with both enantiomers in solution. As the reagent is itself optically pure, these collision-complexes become 'diastereomeric.' That is, if we use (–)TFAE (note that the (+) form can be used equally well), the complexes formed will be: (–)reagent – (+)substrate, and (–)reagent – (–)substrate. These complexes often yield spectra sufficiently different to allow both discrimination and quantification of enantiomers. This difference will be engendered largely by the differing orientations of the highly anisotropic anthracene moieties in the two collision complexes. You won't be able to tell which is which by NMR, of course – that's a job for polarimetry or circular dichroism, but if you know which enantiomer is in excess, you can get a ratio, even in crude samples which would certainly give a false reading in an optical rotation determination!

The use of TFAE is demonstrated in Spectrum 7.4, which shows the appearance of proton 'b', before and after the addition of 30 mg of (+)TFAE to the solution. (This is the region of interest – it is usually protons nearest the chiral centre, which show the greatest difference in chemical shifts in the pair of



Structure 7.4 Structure of TFAE.

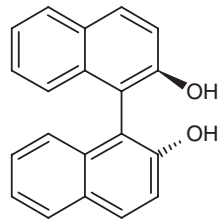


Spectrum 7.4 The use of TFAE as a chiral resolving agent.

complexes formed). The middle trace shows the expansion of proton 'b' prior to the addition of TFAE and the top trace, its appearance after the addition of the reagent. It is clear from this trace that proton 'b' is no longer a simple X-part of an ABX system. Apart from shifting upfield, it has broadened, and eight lines are apparent. This is because the 'b' protons in the two collision complexes have slightly different chemical shifts and we can now see them resolved from one another. (Clearly, this sample was a racemate as the ratio of the resolved 'X' multiplets is 50/50.) This difference is often quite small, and so as to exploit it to the full, experiments of this type are best performed at high field (e.g., 400 MHz or more).

TFAE is a very useful reagent for this type of work, as it is very soluble in $CDCl_3$, which is just as well, as a considerable quantity of it is often needed to produce useful separations – even at high field. Its other advantage is that its own proton signals are generally well out of the way of the sort of substrate signals you are likely to be looking at. It should be noted that the compound under investigation should have at least one, and preferably two potentially lone pair donating atoms to facilitate interaction with the reagent.

Another useful reagent of this type is 'chiral binaphthol' (see Structure 7.5).



Structure 7.5 (R)-(+)-1,1′-bi-2-naphthol

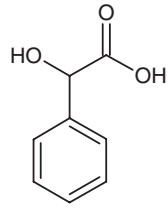
This is a member of an interesting class of compounds which are chiral, without actually containing a defined chiral centre. They are chiral because their mirror images are non-superimposable. In the case of this molecule, there is no rotation about the bond between the two naphthol rings because of the steric interaction between the two hydroxyl groups. 'd' and 'l' forms can be isolated and are perfectly stable (Optical purity determination by 'H NMR, D. P. Reynolds, J. C. Hollerton and S. A. Richards, in *Analytical Applications of Spectroscopy*, edited by C. S. Creaser and A. M. C. Davies, 1988, p346).

Optically pure mandelic acid (see Structure 7.6) can be a useful chiral resolving agent where the compound you are looking at has a basic centre, as it can form an acid-base pair with it, which is a stronger form of association. This compound is of sparing solubility in $CDCl_3$ however and can precipitate out your compound if, as is often the case, its protonated form is of low solubility in $CDCl_3$.

The technique of using resolving agents is obviously a useful one in following the synthesis of a compound of specified chirality. To summarise, we take our compound, which has a chiral centre of unknown rotation and form some sort of complex by introducing it to a reagent of known optical purity. The complexes we form have diastereoisoromeric character, which can give rise to a difference in the chemical shifts of one or more of the substrate signals. This enables us to determine the enantiomer ratio, either visually, or by integration, if we have sufficient signal separation.

In practise, if using one of these reagents to follow the course of a chiral separation, it is essential to determine whether resolution is possible, by performing a test experiment either on a sample of racemate, or at least a sample known to contain significant quantities of both enantiomers. Once useable resolution has been established, the technique can be used to monitor solutions of unknown enantiomer ratios with reasonable accuracy, down to normal NMR detection limits.

It is a good idea to keep the ratio of reagent to sample as high as possible. We recommend starting with about 1–2 mg of compound in solution with about 10 mg of reagent. In this way, you can minimise both the quantity of your sample and the amount of (expensive) reagent used. Keeping the initial sample small has another advantage – it avoids line broadening associated with increased viscosity of



Structure 7.6 Mandelic acid.

very concentrated solutions, whilst at the same time leaving the option open for further increasing the concentration of reagent, if needed.

One final point – the use of chiral resolving agents is restricted to nonpolar solvents, i.e., $CDCl_3$ and C_6D_6, though combining these can sometimes augment separation. That just about concludes the 'Chemical techniques' section. As we've seen, some important types of problem can be tackled using them, and if your sample is scarce, all is not lost – it can often be recovered, though this might take some effort on your part. Deuterated samples can be back-exchanged by shaking with an excess of water, trifluoroacetylated samples can be de-acetylated by base hydrolysis, and shift reagents can be removed by chromatography. Now, we shall have a look at some of the most important 'Instrumental techniques'. . .

8

Further Elucidation Techniques – Part 2

8.1 Instrumental Techniques

Much of the research in NMR spectroscopy has been in the field of devising new and improved techniques for extracting ever more information from samples. Nowadays, the plethora of available techniques can be daunting for the relative newcomer to NMR. In the following sections, we shall endeavour to guide you through the veritable forest of acronyms by describing the most important and useful techniques and demonstrate how they can be used to solve real-world problems.

Before entering the forest, we would advise you to step back a moment and pause for thought. What information do you require? Is it just a case of an aid to an assignment question, or do you need to discriminate between two or more possible structures? It is important to select the right tool for the job, as some of the experiments we will consider later on can take a significant time to acquire. Doing so will enable you to work more efficiently and have greater confidence in your handiwork.

Many of these instrumental techniques have a two-dimensional (2-D) counterpart, which have their own advantages and disadvantages. Rather than treat 2-D spectroscopy as a separate issue, we will include it where appropriate, interleaving it with the corresponding 1-D method. 2-D spectroscopy should perhaps be viewed as an interpretational aid for 1-D spectroscopy, rather than an end itself.

8.2 Spin Decoupling (Homonuclear, 1-D)

This is probably the oldest of the instrumental techniques but it is still very useful even today. It enables the user to determine which signals in a spectrum are spin-coupled to each other. It can be an extremely useful aid to assignment and can in some cases, even be used to facilitate conformational studies.

In practise, a powerful secondary radio frequency is centred on the signal of interest whilst the spectrum is reacquired. This causes the irradiated proton(s) to become saturated which effectively destroys any spin coupling from the protons giving rise to this signal. By comparing the resultant spectrum with its un-decoupled counterpart, it should be easy to work out which protons couple to the signal of interest. This is demonstrated in the following example. Note that this technique is applicable to both FT

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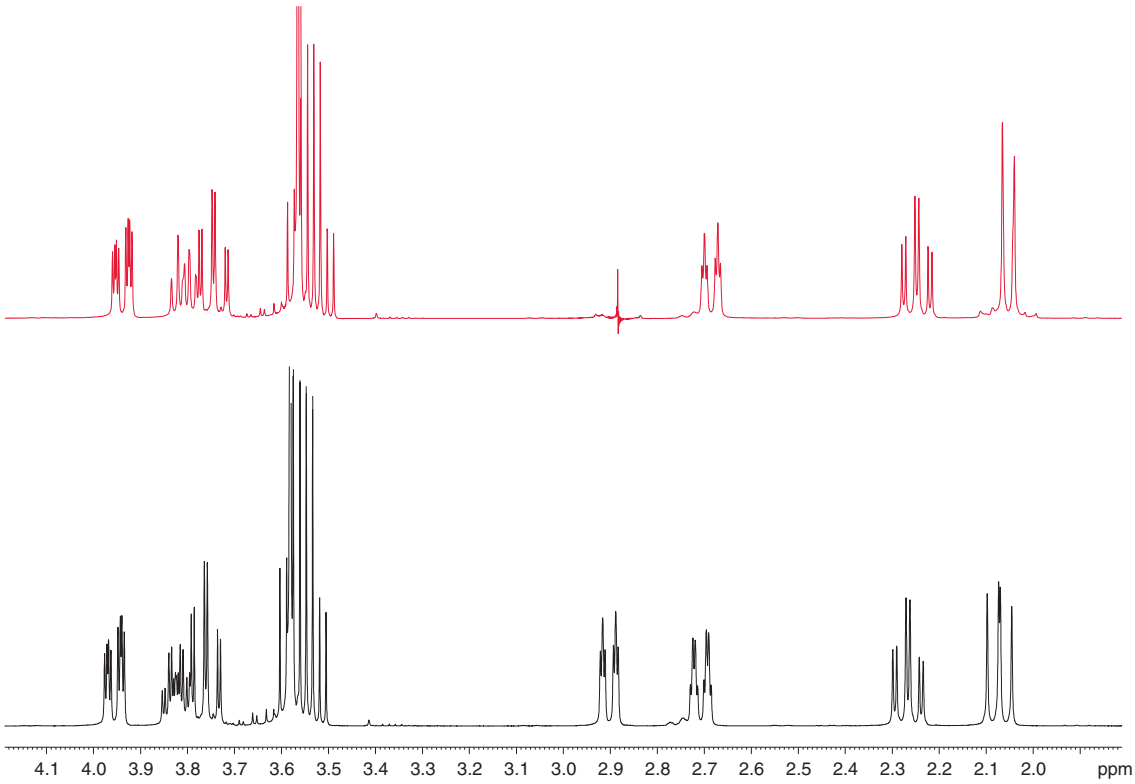


Spectrum 8.1 1-D spin decoupling experiment (decoupled at 2.9 ppm).

and the older CW instruments. The technique is demonstrated in Spectrum 8.1, using *the* morpholine compound.

For convenience and ease of interpretation, it is a good idea to plot the decoupled spectrum above the normal 1-D trace so that you can see at a glance which signals have been decoupled and which have not. The first thing that you'll notice is that the irradiated signal (2.90 ppm) has been obliterated by the decoupler. In our example, the loss of the major coupling from the multiplet at 2.07 ppm, a minor coupling from the multiplet at 2.71 ppm and another from the multiplet at 3.82 ppm are all clearly visible.

1-D decoupling is a very useful tool for unpicking spin systems in this way. You can work your way through your spectrum, decoupling one signal at a time and building up a picture of your structure as you go. Although hardly cutting edge, the 1-D decoupling can offer advantages over the 2-D COSY technique in circumstances where finding an actual value for a coupling is important as well as just establishing connectivity.

8.3 Correlated Spectroscopy (2-D)

Of course, you can find yourself looking at spectra that are complex enough to warrant numerous decoupling experiments for elucidation. In these circumstances, running a single correlated spectroscopy (COSY) 2-D experiment as an alternative might well be the answer. A full explanation of the theoretical

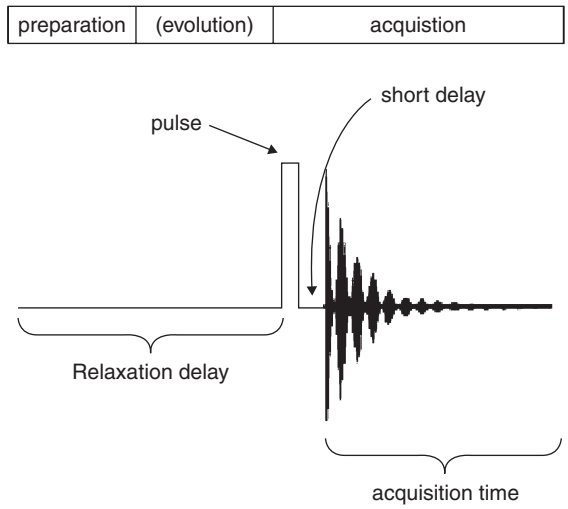


Figure 8.1 A typical 1-D pulse sequence.

considerations behind this and other 2-D techniques is well outside the scope of this book but in brief, it works something like this.

First, it is useful to understand what we mean by 1-D and 2-D experiments. If you consider a normal proton spectrum, it is plotted in two dimensions (chemical shift on the *x* axis and intensity on the *y*), so why is it called 1-D? In fact, when NMR started, it wasn't because there was no need to distinguish it from what we now call '2-D.' The dimensions that we are talking about are the number of frequency dimensions that the data set possesses. To try to understand we need to explain the basics of the pulse programme. If we take a simple example (e.g., 1-D proton) we can represent the pulse sequence in Figure 8.1.

This diagram shows that we wait for a certain time (the relaxation delay) and then generate a radiofrequency pulse. We then wait for a short whilst (to let that intense pulse purge itself from the circuits), switch on the receiver and start receiving the signal. In most experiments we then do it again and again, averaging the spectra that we receive. We generalise these pulse sequences into three components: preparation, evolution and acquisition. In our basic 1-D pulse sequence, there is no 'evolution' bit but this is the key part when we look at 2-D experiments.

What makes 2-D different is that it uses this 'evolution' time to allow something to happen to the spins in the molecule. This can be seen graphically in a simple COSY pulse sequence (Figure 8.2).

In this case we pulse at the beginning of the evolution time and then wait before doing our acquisition pulse. If we vary this wait by incrementing it for each successive cycle, we can change what we see in the FID. This is what generates our second dimension. In the case of the COSY experiment, we allow the coupling information to evolve during this period and then 'read' what has happened to it with the acquisition pulse.

Once we have acquired the data, we have two 'time domains' (one from the normal acquisition time, the other from the incremented delay, hence the data is now '2-D'). As with normal spectra, we need to look at the data in the frequency domain. We do this by Fourier transformation, first in one dimension and then in the other. The resultant data can be portrayed or plotted in one of two different formats.

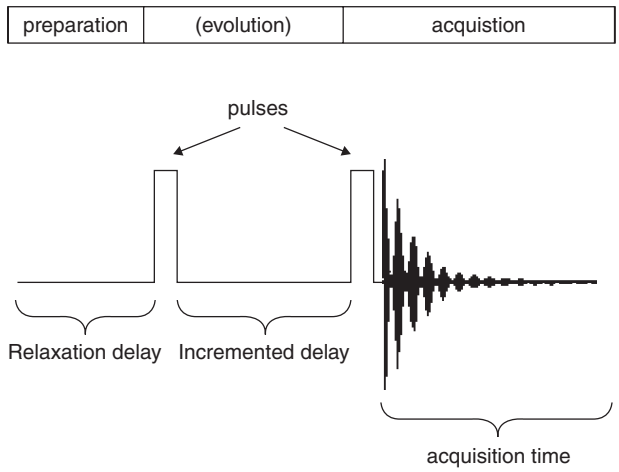


Figure 8.2 A simple COSY pulse sequence.

A typical 'stack plot' is shown below (Figure 8.3) and, whilst the intriguing appearance may conjure images of prog rock album covers, stack plots are not in the least user-friendly in terms of interpretation!

For this reason, COSY (and other 2-D spectra) are invariably plotted using a 'map' view or 'contour plot' where contours indicate the intensity of the peaks (Spectrum 8.2 shows a COSY spectrum of the interesting region of *the* morpholine compound). It is worth spending a little time familiarising yourself with the use of a COSY spectrum using this example of a familiar compound. Select, for example, the signal at 2.7 ppm and locate it on the diagonal. Now, using a ruler, project vertically from the diagonal at this point until you connect with a contour. From this contour, project horizontally back to the diagonal. These two signals are spin coupled to each other. Now return to the peak at 2.7 ppm again and project vertically downwards from it until you encounter two more contours . . .

It is worth noting that in order to observe all the small couplings, it might be necessary to plot the spectrum with varying intensities. Too low a level will sometimes fail to show all the small couplings whilst too high a level may cause an unacceptable spread of the diagonal and the stronger correlations.

The diagonal (bottom left to top right) shows the tops of the peaks, as if you were looking down on the peaks of a 1-D spectrum from above. The off-diagonal contours show the couplings between signals and are duplicated on both sides of the diagonal. This might appear strange as half the information portrayed

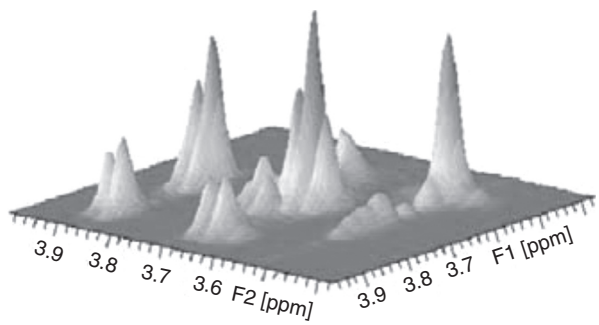
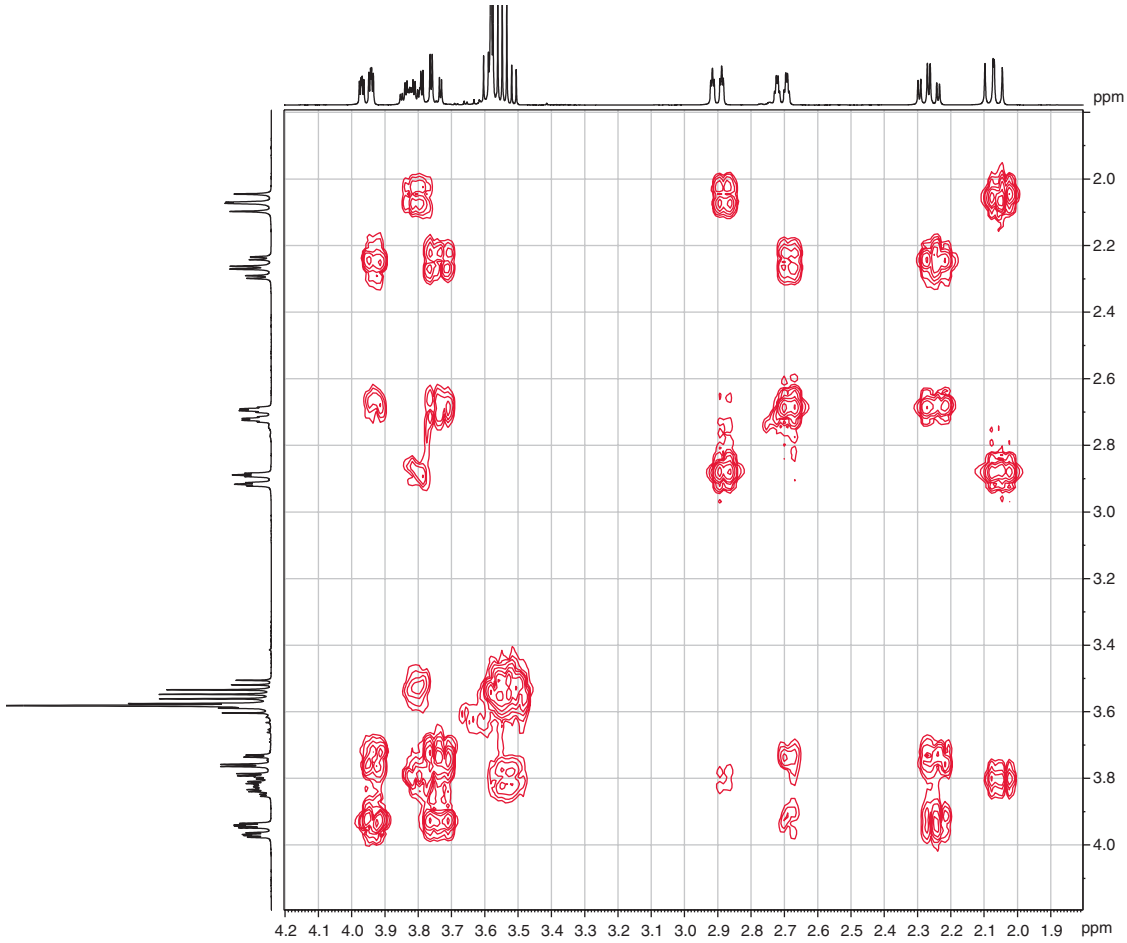


Figure 8.3 A COSY data set.



Spectrum 8.2 A COSY contour plot of *the* morpholine compound.

must be redundant but in fact this duplication can be useful as it enables us to tell true correlations from artifacts. This is particularly valuable when dealing with spectra where the signal/noise ratio is marginal or a coupling is weak – the coupling contours should always ideally feature in *both* halves of the spectrum.

As you can see, a major advantage of COSY over a conventional 1-D decoupling experiment is that ALL the couplings are displayed on the single plot. On the minus side, it takes a good deal longer to acquire a COSY spectrum as it is made up of typically, 256 or 512 individual 1-D spectra, each of at least four scans. For a typical 2–5 mg sample in a 400 MHz spectrometer with an ordinary probe (i.e., 5 mm normal or inverse geometry probe), a high quality spectrum will take about 30 min to acquire, though this can be reduced by cutting a few corners.

Another disadvantage is that for solving certain stereochemistry problems, it is necessary to be able to not only establish connectivity but to measure couplings fairly accurately so that the data can be used in conjunction with the Karplus curve. Whilst this is possible using a 'phase sensitive' COSY (Note –

there are numerous variants of this experiment using various modifications of the pulse sequence, each offering certain advantages/disadvantages), we certainly wouldn't recommend it because of the limited digital resolution available. (Note that in order to avoid collecting gigantic amounts of data, a typical COSY data matrix may be typically 2 k in one dimension and 256 points in the other. For a typical 10 ppm sweep width, this means that in a 400 MHz spectrometer, the digital resolution will be at best, 400 × 10/2048, or in other words, 2 Hz per point. This would obviously not be good enough to measure couplings to the accepted 0.1 Hz!).

8.4 Total Correlation Spectroscopy (1- and 2-D)

The total correlation spectroscopy (TOCSY) techniques, which come in both 1- and 2-D versions, offer an alternative to 1-D spin decoupling and COSY methods for establishing through-bond connectivities. The important difference between the two is that TOCSY methods allow easy identification of isolated spin systems. For example, using our trusty morpholine compound once more, you can see that it is possible to identify the -CH_2-CH_2- spin system between the nitrogen and the oxygen atoms, these hetero-atoms, effectively isolating the protons from all others in the molecule.

This ability to discriminate between protons of one spin system and those of another can be very useful in some cases but not in others. Imagine for example, a compound analogous to our morpholine but with the oxygen and nitrogen replaced by CH_2s. In this case, TOCSY experiments would be of little value, as there would be one continuous coupling pathway, right around the molecule and the resultant TOCSY would look much the same as a corresponding COSY.

In the 1-D experiment, you select a clear (i.e., not overlapped) signal for irradiation and after initiating the appropriate pulse sequence, the resultant spectrum will show only those protons that are in the same 'coupling network' as the selected proton(s). The intensity of the signals produced ultimately dies away with increasing number of bonds from the selected proton(s) but by varying one of the delays in the pulse sequence (the spin lock pulse), the experiment can be fine tuned for 'range.' A relatively short spin lock will give rise to shorter range (i.e., weaker or non-existent correlations to distant protons) whilst a relatively long spin lock will favour long range correlations though in this case, care must be taken not to damage the probe by pushing too much energy through it.

In the 2-D experiment, as in the COSY, no selection of any signal is required. The sequence is initiated and the data collected.

8.5 The Nuclear Overhauser Effect and Associated Techniques

Whereas spin decoupling, COSY and TOCSY techniques are used to establish connectivities between protons *through bonds*, techniques that make use of the nuclear Overhauser effect (NOE), such as 1-D NOE and NOESY, 1- and 2-D GOESY, 1- and 2-D ROESY, can establish connectivities *through space*. Before looking at these techniques in detail, it's worth spending a little time considering the NOE phenomenon itself – in a nonmathematical manner, of course!

A working definition of the nuclear Overhauser effect would be: 'A change in the intensity of an NMR signal from a nucleus, observed when a neighbouring nucleus is saturated.' Such changes in intensity may be positive or negative (depending upon how the observation is made, the tumbling rate of the molecule in solution and the frequency of the spectrometer used) and they can be observed in both the homonuclear and the heteronuclear sense. The maximum theoretical magnitude for such effects in

steady-state experiments (simple 1-D NOE – difference experiments) is 50 % (of the size of the original signal) but in reality, they tend to be a lot smaller, usually less than 10 % and often as small as 1 % but nonetheless, still relevant.

For this reason, they are best observed using a 'difference technique,' i.e., a pulse sequence which allows subtraction of two data sets, allowing only differences to be observed and unchanged signals to be edited out of the spectrum. The advantage to this approach should be clear if you consider attempting to observe a change in intensity of 2 % in a peak that is 100 mm in height. Would 102 mm look significant? Probably not – but the difference between a peak of 2 mm and no peak at all would be immediately apparent! Note that since you might be looking for an enhancement of less than 2 % (i.e., signal intensity of less than 2 % of the original spectrum), the signal to noise ratio may well be an issue and acquiring the data could take a significant time. If you were to investigate half a dozen different sites within a molecule, running the experiments overnight would be advantageous!

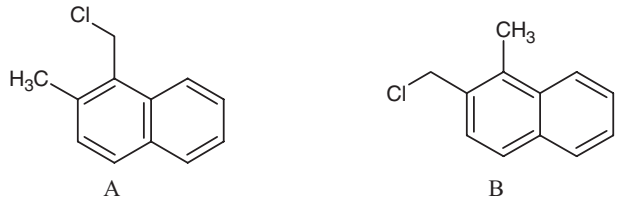
In the definition above, the term 'neighbouring nucleus' was used. The NOE is highly distance dependant – so much so that it falls off with the sixth power of the distance separating the nuclei in question. This very sharp distance dependency makes the effect a very useful tool for probing inter-atomic distances. Two nuclei separated by 3.5 Å should experience the effect between them, but should that distance be 4.0 Å, they will not normally be observed. Another important point to bear in mind is that in marked contrast to spin coupling, though proton (x) gives an NOE to another nucleus (y), there is no guarantee that (y) will give an NOE back to (x). This is because (y) might have more favourable relaxation pathways available to it.

The ability to devise experiments that can make use of the NOE gives us massively powerful tools which can be used to crack all manner of problems. For example, they can be used in the more trivial sense, as an assignment aid and to tackle problems of positional isomerism. But the area where NOE experiments really come into their own by offering information that no other NMR techniques can offer, is in the field of stereochemistry. Is this group up or down? Could this centre have epimerized? An NOE experiment could be just what you need.

In the basic 1-D NOE experiment, the spectrometer collects two sets of FIDs, one with a second r.f. source centred on the signal to be examined and a second set with the same r.f. source centred on a blank part of the spectrum. After a suitable number of both sets of scans have been acquired, (an equal number of both!) the two sets are subtracted from each other to leave a resultant spectrum which should only show signals of protons that have undergone enhancement because they were within approximately 3.5 Å of the proton(s) that was irradiated. Note that during the acquisition pulse, the decoupler is switched off and so the enhanced signals retain any coupling they may have. Note also that subtraction may not be perfect and that the enhanced spectrum may contain a few subtraction artifacts. Theses can usually be easily distinguished from genuine enhanced peaks as they cannot be phased, have intensity above and below the baseline and usually have no net integration associated with them. Note also that it is advisable to run NOE experiments with the sample *not* spinning. This helps minimise subtraction artifacts by broadening the peaks very slightly.

As an example of how useful an NOE experiment can be, consider the structures in Structure 8.1

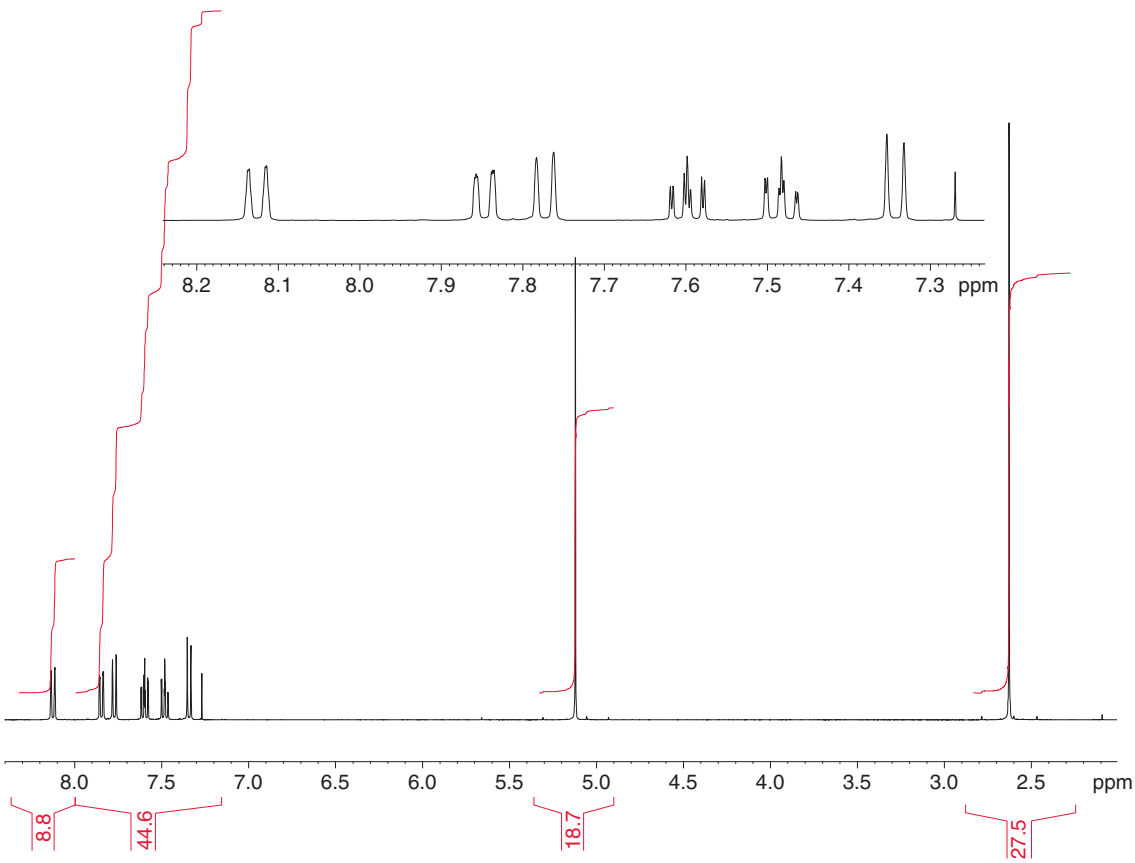
These two compounds would give very similar proton (and carbon) spectra and though an educated estimate of the -CH_2- and -CH_3 chemical shifts would give a good indication of identity *if both compounds were available*, we would never entertain such liberties if we had only one of the compounds in isolation. (Note that the chemical shifts of these substituents would be expected to be at slightly lower field when they are in the alpha positions. This is because alpha substituents are deshielded by two aromatic rings whereas those in beta environments are subjected to deshielding by only one of the aromatic rings. The



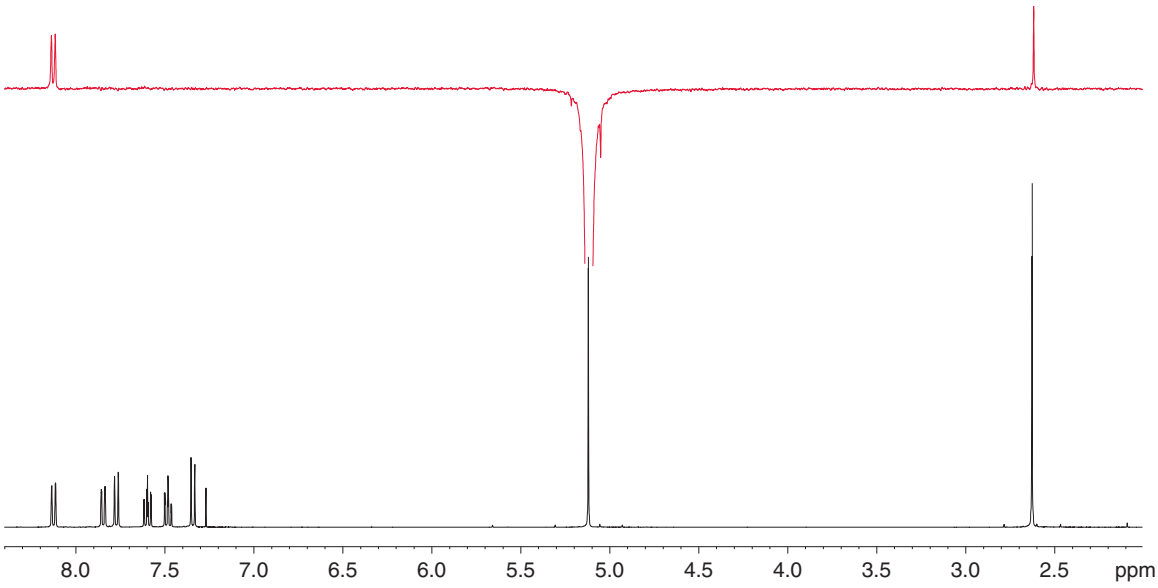
Structure 8.1 Two naphthalene structures which can be distinguished by NOE.

differences involved would only be of the order of 0.2 ppm.) An appropriate NOE experiment however, removes all speculation and in combination with relevant decoupling/COSY, rapidly yields a full and unambiguous assignment of the molecule.

In this example, both the -CH_2- and the -CH_3 would be excellent targets for irradiation and we would recommend making use of both of them. A brief inspection of the 1-D spectrum (Spectrum 8.3) is enough to confirm that the compound does have both substituents on one of the rings as four protons can easily be observed as one continuous spin system (8.13, 7.85, 7.6 and 7.48 ppm) whilst the remaining



Spectrum 8.3 Naphthalene substituted with -CH_3 and -CH_2-Cl groups and expansion.



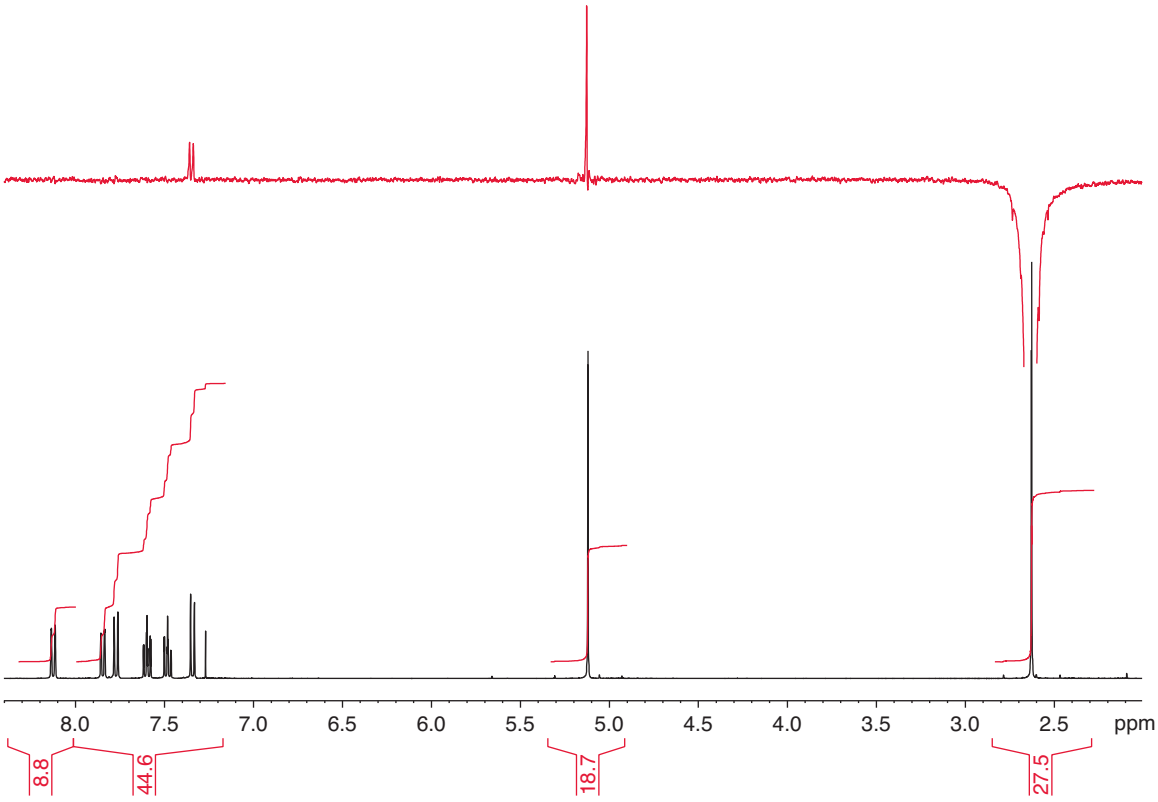
Spectrum 8.4 NOE experiment with irradiation of -CH_2- at 5.1 ppm.

two signals are a pair of ortho-coupled doublets at 7.77 and 7.34 ppm. This proves that both substituents are not only on the same ring but also that they must be either ortho- or para- to each other. The first NOE experiment (Spectrum 8.4) in which the -CH_2-Cl protons are irradiated gives a clear enhancement of the broad doublet at 8.13 ppm. The -CH_3 protons are also enhanced which shows that these substituents are ortho to each other. (Note that the NOE trace is plotted in red above the standard 1-D plot and on the same scale for convenience.) The enhancement of the broad doublet at 8.13 ppm is entirely consistent with structure 'A' above.

The second NOE targeting the methyl group (Spectrum 8.5) shows an enhancement of the doublet at 7.34 ppm which underpins the structure which is shown below with the enhancements depicted. The differentiation of the two structures is therefore unambiguous and the correct structure with enhancements is shown in Structure 8.2.

There are a number of pitfalls waiting for the unwary when setting up and interpreting the results of NOE experiments. For example, the signal that is being irradiated should not be too close to any other signal in the spectrum. This is because there is a danger of 'spill over' from the decoupler signal so that you might inadvertently saturate a nearby peak which could of course give rise to completely bogus enhancements. (Note that this is only a potential problem in the 1-D techniques where selective irradiation of a specific signal is used.) In the 1-D experiments, the irradiated signal always shows the opposite phase to the enhanced signals (as long as the NOE is positive, which is the case for most small molecules at medium field) and should be plotted so that it is negative. If, during the phasing of your NOE spectrum, any other signal which is close to your target signal phases negatively, then be advised that it has been at least partially saturated and spurious enhancements may be present!

Enhancements between signals that are strongly spin-coupled to each other are best ignored as they are prone to another competing phenomenon, that of Selective Population Transfer (SPT). This makes it difficult to decide if any observed enhancement is down to a genuine NOE, or is merely an SPT. SPT

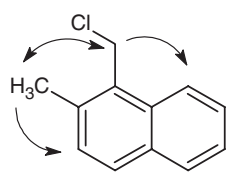


Spectrum 8.5 NOE experiment with irradiation of -CH_3 at 2.63 ppm.

signals are characterised by their inability to phase properly. In practise, it is not often that an NOE between coupled signals would be useful anyway so this is not a major problem . . . unless you are trying to work out whether the fusion of a saturated bicyclic system is *cis* or *trans*.

We have discussed the significance of the 'NMR timescale' in earlier sections and it is worth knowing that the 'NOE timescale' is somewhat longer and that this can have consequences for NOE experiments in molecules that have dynamic processes taking place within them. To give a more specific example, consider the isomers shown in Structure 8.3.

Differentiating these two compounds, particularly in isolation, would not be easy by proton NMR. The temptation to irradiate the −OH should be resisted (note: the irradiation of exchangeable protons in NOE experiments is not generally recommended, even if they give rise to sharp peaks) as these compounds can undergo tautomerism and exist in the forms shown in Structure 8.4.



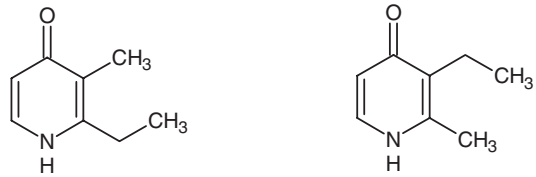
Structure 8.2 Correct naphthalene structure showing key NOEs observed.

Structure 8.3 Isomeric molecules.

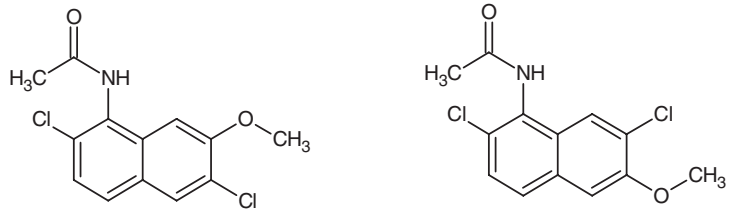
This phenomenon could give no end of potential problems with an NOE experiment. The molecules may exist in solution, predominantly as either hydroxy pyridines or as pyridones, depending to some extent on solute concentration, choice of solvent, its water content, pH and temperature. Irradiation of the exchangeable signal would therefore be an uncertain proposition as you could not be sure what exactly you were irradiating! Furthermore, it is quite possible that the two tautomers could both exist in solution simultaneously. Tautomerism is *generally* fast on the NMR timescale, i.e., we *usually* see only one set of signals that represent the average contributions of the chemical shifts of both tautomers. During an NOE experiment, it would be likely that both forms would effectively undergo irradiation because an irradiated -OH, undergoing chemical exchange to become an NH, *takes its irradiation with it (and vice versa of course)*! You would effectively be irradiating both sites at once. Should the proton transfer process turn out to be slow on the NMR timescale (i.e., you observe two distinct sets of signals for the two different tautomers) it would still be relatively fast on the NOE timescale. This is because the experiment requires a low power irradiation of the signal under investigation, which generally lasts for at least one second. During this time, chemical exchange inevitably occurs and both exchangeable sites would still be irradiated. This would obviously give rise to useless data and meaningless results.

This exchange process can also be a problem where the water in a solvent becomes unintentionally irradiated during an NOE experiment because the protons of the water are in constant chemical exchange with all exchangeable protons in the molecule being studied. Consider for example, the following hypothetical problem. You wish to distinguish between the two compounds shown in Structure 8.5.

A reasonable strategy might be to positively identify the proton ortho- to the -OCH_3 group by means of an NOE experiment and then use this proton as a further probe in a second NOE experiment to see if it enhanced either the NH or possibly acetyl methyl in one isomer, or the peri- aryl proton in the other. A problem could arise here, using DMSO as a solvent perhaps, if irradiation of the -OCH_3 accidentally irradiated the water present in the solvent. Don't forget – you only have to catch the edge of the peak to saturate it. The (irradiated) water could chemically exchange with the NH, passing irradiation on to it and in so doing, initiate a bogus enhancement from the NH to the proton peri- to it which would beg the question: 'Did the enhancement come from the -OCH_3 or from the NH (relayed from the water)?'



Structure 8.4 Tautomeric forms of compounds in Structure 8.3.



Structure 8.5 Two problematic compounds for NOE experiment.

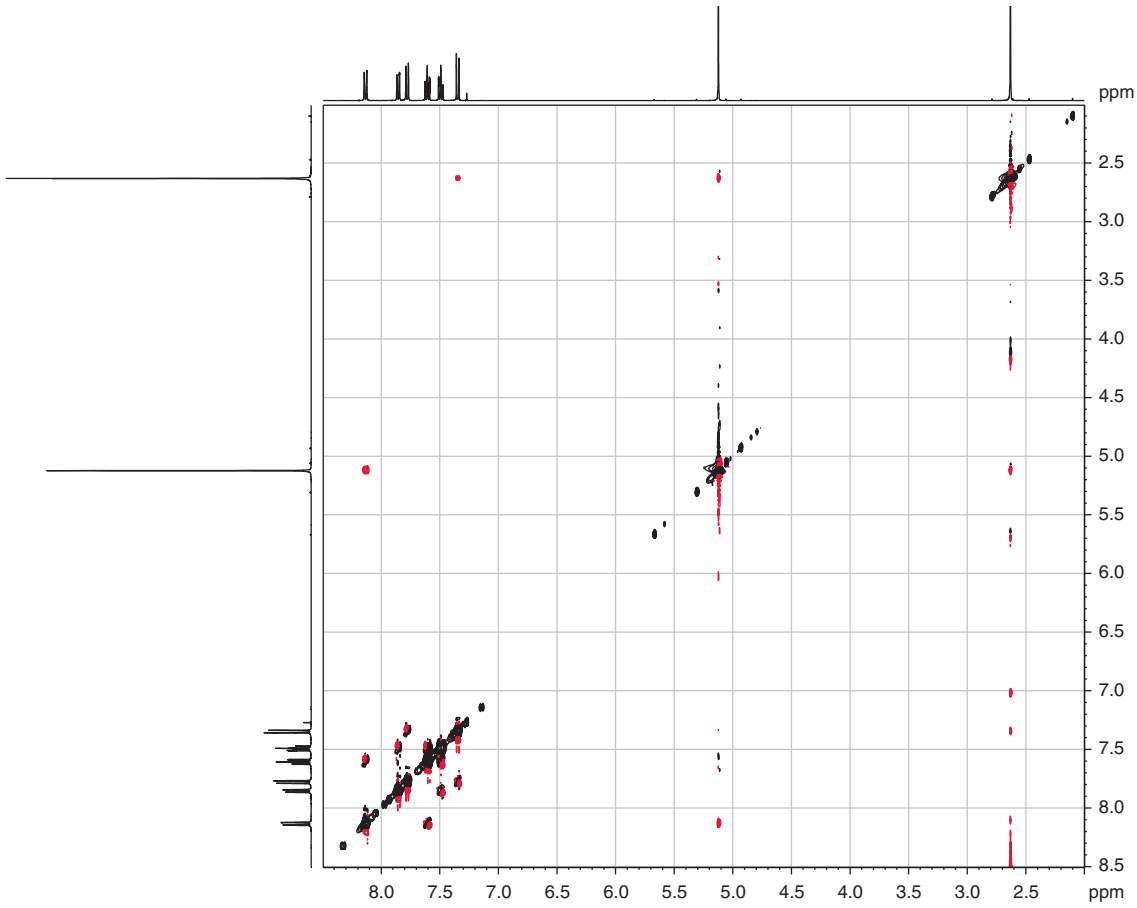
In this case, a better method might be to work from the aromatic signals of the AB pair to establish connectivity *to* the -OCH_3 rather than *from* it.

These are just a few examples of what could go wrong with an NOE experiment. NOE experiments are not 'boring' and 'all the same' as a chemist acquaintance once famously remarked. Quite the contrary in fact. Designing sensible experiments to make use of the NOE and dovetailing the results with other NMR data, can be quite challenging – and rewarding when you finally pull all the threads together to produce a sensible picture of a problem molecule.

At the beginning of this section, we listed the various experiments that are available which make use of the Nuclear Overhauser Effect but as yet, we have made no attempt to indicate the pros and cons of each of these and under what circumstances one may be preferable over another. It is virtually impossible to give cast iron advice regarding the selection of one NOE experiment over another as the decision has to be based on a huge number of considerations, and on the instrumentation and software available to you. Having said that, we shall now attempt to establish some broad guidelines.

Perhaps the first decision to be made is whether to select a 1-D or a 2-D technique. Note that in all the 2-D NOE experiments, the off-diagonal-peaks contours represent NOE connections between signals and are displayed on each side of the diagonal in exactly the same way that coupling connectivities are displayed in COSY spectra. Both have their advantages and disadvantages. If you are working on a relatively simple problem such as that of the -CH_2-Cl and -CH_3 groups on the naphthalene which we considered earlier, then a 1-D approach would be preferable since the problem could be cracked with a single NOE experiment, or two at the most and this could be achieved more quickly than by running a 2-D experiment. The simple 1-D NOE is a robust and trustworthy tool. For more complex problems, where you might benefit from having NOE data from multiple sites, a 2-D technique might be preferable as it should give you all the available NOE information about the molecule in one spectrum. Both 1- and 2-D techniques can suffer from artifacts (features in the spectra that are not genuine NOE signals). We have already mentioned subtraction errors in the basic 1-D method but perhaps some of the artifacts that can occur in 2-D spectra can be more serious. For example, 'T1 noise,' which manifests itself as a streak of cross-peaks running down the spectrum in a line with any strong peaks on the diagonal, can cause problems. This type of streak can obscure genuine correlations. The severity of T1 noise is an instrumental factor that is related to r.f. stability and thus varies from instrument to instrument.

One potential problem that can occur with slightly larger molecules (typically of m.w. $>$ 600) is that the NOE response in both NOE and 2-D (NOESY) experiments is related to the tumbling rate of molecules in solution. The larger the molecule, the slower it will tumble and at a certain point, all expected enhancements will be nullified. This null point depends not only on the tumbling rate (and therefore the size, or more accurately, the shape of the molecule) but also on the field strength of the

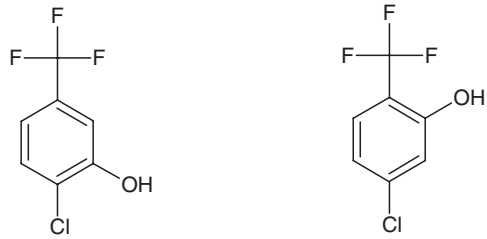


Spectrum 8.6 2-D ROESY spectrum of the naphthalene compound.

spectrometer being used. A molecule giving positive NOEs in a 400 MHz instrument may well not give NOEs in a 600 MHz machine – or maybe it will give negative NOEs.

In order to combat this, the *r*otating frame *O*verhauser *e*ffect *s*pectroscop*y* (ROESY) techniques can be employed. An in-depth discussion of how this technique works is outside the remit of this book but suffice to say, in the ROESY methods (1- and 2-D), NOE data is acquired as if in a weak r.f. field rather than in a large, static magnetic field and this assures that all NOEs are present and positive, irrespective of tumbling rate and magnet size. It is possible that some TOCSY correlations can break through in ROESY spectra but these will have opposite phase to the genuine ROESY correlations and so should therefore not be a problem – unless they should overlap accidentally with them. A 2-D ROESY spectrum of the naphthalene compound is shown below (Spectrum 8.6).

A comparison between the one- and two-dimensional data shown for this compound is interesting. As we have said, the 2-D ROESY does offer the advantage of displaying all enhancements occurring in the molecule simultaneously but against that, the data is probably more prone to artifacts than the corresponding 1-D technique. This can be particularly apparent in cases where the transmitter offset



Structure 8.6 Two possible positional isomers.

frequency (which defines the centre of the sweep width) happens to coincide with a signal in your spectrum! In terms of making optimum use of spectrometer time, the 1-D experiment would be the preferred choice in cases where you only have a few 'target' signals to irradiate whilst the 2-D method might be the best choice in cases where you need to look at four or more signals. The 1-D experiment also offers another advantage in that the enhanced signal is 'reconstructed.' This can be very useful if this signal is overlapped with other signals which do not enhance, as it provides us with a method of extracting coupling information not available in the standard 1-D spectrum.

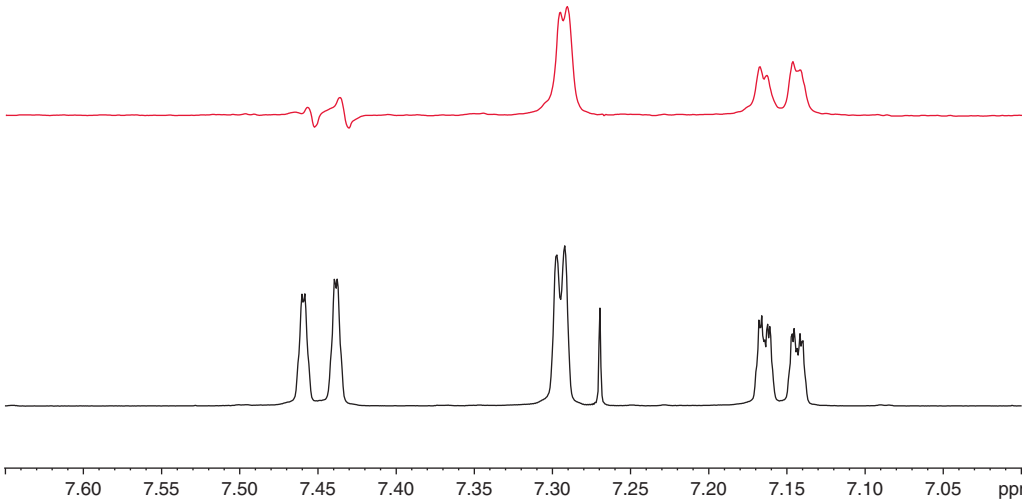
At the beginning of this section, we listed 1- and 2-D GOESY as an alternative method of collecting NOE data. This technique (*g*radient enhanced *O*verhauser *e*ffect *s*pectroscop*y*) is broadly similar to conventional NOE in terms of the results you achieve. In the 1-D case, there are no subtraction artifacts since the subtraction of data is handled by a phase cycle. Viewed pragmatically, GOESY spectra are generally cleaner but offer no notable advantage in terms of signal to noise. It would seem that the conventional NOE method might also be somewhat more robust – we have seen examples of problems that have not given an expected enhancement in a GOESY experiment but have given perfectly acceptable results in a conventional NOE experiment.

So to sum up, if you have a small molecule, a straightforward issue to resolve and a typical 250/400 MHz instrument at your disposal, use an ordinary 1-D NOE. If you have a more complex problem involving multiple sets of NOE data to consider, go for a 2-D method, and if you have a larger molecule and a more powerful spectrometer, go for a ROESY option.

We have concentrated on the proton–proton, homonuclear NOE experiments in this section but the potential use of analogous heteronuclear experiments should not be overlooked, if you have the appropriate hardware available to you. The ^{19}F– ^{1}H NOE experiment, for example, can be very useful in certain situations as demonstrated in the following example. You have one of two possible positional isomers (Structure 8.6).

How would you differentiate between them? This problem is not a good one for proton NMR as both compounds would give similar spectra (if you had both compounds, you might draw a reasonable conclusion on the basis of the -CF_3 group's ortho deshielding). Note that both compounds have protons that are ortho and para to the shielding -OH group and that they would exhibit the same multiplicities in both compounds. ^{13}C spectroscopy would give a good indication of identity on the basis of the chemical shifts of several of the aromatic carbons – but you would need access to a good data base to have confidence in solving the problem in this way.

But the most unambiguous and arguably the most elegant confirmation of structure would come in the shape of a hetero-nuclear NOE experiment. (First, you have to run a quick ^{19}F spectrum in order to determine the relevant ^{19}F resonance frequency and set the decoupler in the fluorine domain, of course.)



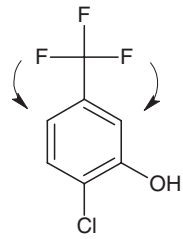
Spectrum 8.7 An NOE experiment with irradiation of the -CF_3 group at –62.85 ppm in the ^{19}F domain.

Irradiation of the -CF_3 group would yield an enhancement of the two protons in one case and to just the single de-shielded proton in the other (see Spectrum 8.7).

The enhancement of the two protons as shown in Structure 8.7 clearly defines the isomer.

Note that in cases where ^{19}F–^{1}H NOE experiments are attempted in molecules where fluorine is spin coupled to any of the protons within NOE range, SPT effects can be expected as described earlier!

There are several other extremely useful techniques for the elucidation of structures that we use regularly but, since these all make use of ^{13}C data, we'd better start a new chapter.



Structure 8.7 Identifying the positional isomer.

9

Carbon-13 NMR Spectroscopy

9.1 General Principles and 1-D ^{13}C

^{13}C NMR gives us a another vast area of opportunity for structural elucidation and is incredibly useful in many cases where compounds contain relatively few protons, or where those that are available are not particularly diagnostic with respect to the proposed structures. Before we delve into any detail, there are certain general observations which we need to make regarding ^{13}C NMR and the fundamental differences that exist between it, and proton NMR.

For a start, we must be mindful of the fact that ^{13}C is only present as 1.1 % of the total carbon content of any organic compound. This, in combination with an inherently less sensitive nucleus, means that signal to noise issues will always be a major consideration in the acquisition of ^{13}C spectra – particularly 1-D ^{13}C spectra which we will restrict the discussion to for the moment. (Note that the *overall* sensitivity of ^{13}C, probe issues aside, is only about 0.28 % that of proton because the nucleus absorbs at a far lower frequency – in a 400 MHz instrument, ^{13}C nuclei resonate at around 100 MHz.). So it takes a great deal longer to acquire ^{13}C spectra than it does proton spectra. More material is obviously an advantage but can in no way make up for a 350-fold inherent signal to noise deficiency!

Another important aspect of ^{13}C NMR is that the signals are never normally integrated. The reason for this is that some carbon signals have quite long relaxation times. In order to make NMR signals quantitative, acquisition must allow for a relaxation delay (delay period between acquisition pulses) of at least five times the duration of the slowest relaxing nuclei in the compound being considered. With relaxation times of the order of 10–20 s, it is therefore obvious why we cannot obtain quantitative ^{13}C data! The inherent insensitivity of the ^{13}C nucleus often demands thousands of scans to achieve acceptable signal to noise so we can ill afford 100 s relaxation delays between pulses! The only thing that we can say is that methine, methylene and methyl carbons *generally* appear to be more intense than quaternary carbons (see below for explanation).

Yet another significant difference with ^{13}C NMR is that we do not observe coupling between neighbouring nuclei as we do in proton NMR. This is not by virtue of any decoupling technology – it is purely a matter of statistics. As the natural abundance of ^{13}C is only 1.1 %, the chances of having two ^{13}C atoms sitting next to each other is statistically small (it would occur in only 1.1 % of that 1.1% in fact) and so ^{13}C–^{13}C coupling just isn't an issue. It should be noted that with the extra sensitivity of cryoprobes, it is

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becoming possible to observe ^{13}C–^{13}C couplings and these can be used to solve tricky regiochemistry problems.

^{1}H–^{13}C coupling however, *would* be a serious issue – if it were allowed to occur. 1-D ^{13}C spectra are always normally acquired with full proton decoupling. There are a number of good reasons for this. First, the already meagre signal/noise would be further eroded by splitting the signal intensities into doublets, triplets, etc. Furthermore, identifying individual signals would be extremely difficult in compounds having many carbons in a similar chemical environment – particularly in view of the large couplings that exist between protons and ^{13}C nuclei. The potential overlap of signals would make spectra horrifically complex.

Another good reason for fully decoupling protons from ^{13}C is that the ^{13}C sensitivity, to some extent benefits from Overhauser enhancement (from proton to ^{13}C which comes about as a result of decoupling the protons). This explains why quaternary carbons appear less intense than those attached to protons – they lack the Overhauser enhancement of the directly bonded proton.

So far, it might seem that ^{13}C spectroscopy is just a long list of disadvantages. Here we have a technique that is extremely insensitive and thus time-consuming to acquire. It is largely nonquantitative, since we can't integrate the signals and to gild the lily, we can't relate carbon to carbon by means of spin coupling as we have no coupling information to assist us in our assignments. Just about the only commodity we have left at our disposal is the chemical shift – but how do you go about interpreting a spectrum that is composed entirely of singlets? We will explore this a bit later. (Note that although all ^{1}H–^{13}C couplings are decoupled, couplings between ^{13}C and other hetero atoms such as fluorine and phosphorus will *not* be decoupled and splitting of ^{13}C signals will be observed in molecules where these hetero atoms are found in environments that are conducive to coupling.)

So if this all sounds a bit bleak, what's the *good* news? Well, strangely, there is quite a lot. For a start, let's not forget that had the ^{13}C nucleus been the predominant carbon isotope, the development of the whole NMR technique itself would have been held back massively and possibly even totally overlooked as proton spectra would have been too complex to interpret. Whimsical speculation aside, chemical shift prediction is far more reliable for ^{13}C than it is for proton NMR and there are chemical shift databases available to help you that are actually very useful (see Chapter 14). This is because ^{13}C shifts are less prone to the effects of molecular anisotropy than proton shifts as carbon atoms are more internal to a molecule than the protons and also because as the carbon chemical shifts are spread across approximately 200 ppm of the field (as opposed to the approx. 13 ppm of the proton spectrum), the effects are proportionately less dramatic. This large range of chemical shifts also means that it is *relatively* unlikely that two ^{13}C nuclei are exactly coincident, though it does happen.

Other good news comes in the shape of the ^{13}C nucleus having a spin quantum number of $^{1}/_{2}$. This means that ^{13}C signals are generally sharp as there are no line-broadening quadrupolar relaxation issues to worry about and we don't have to deal with any strange multiplicities.

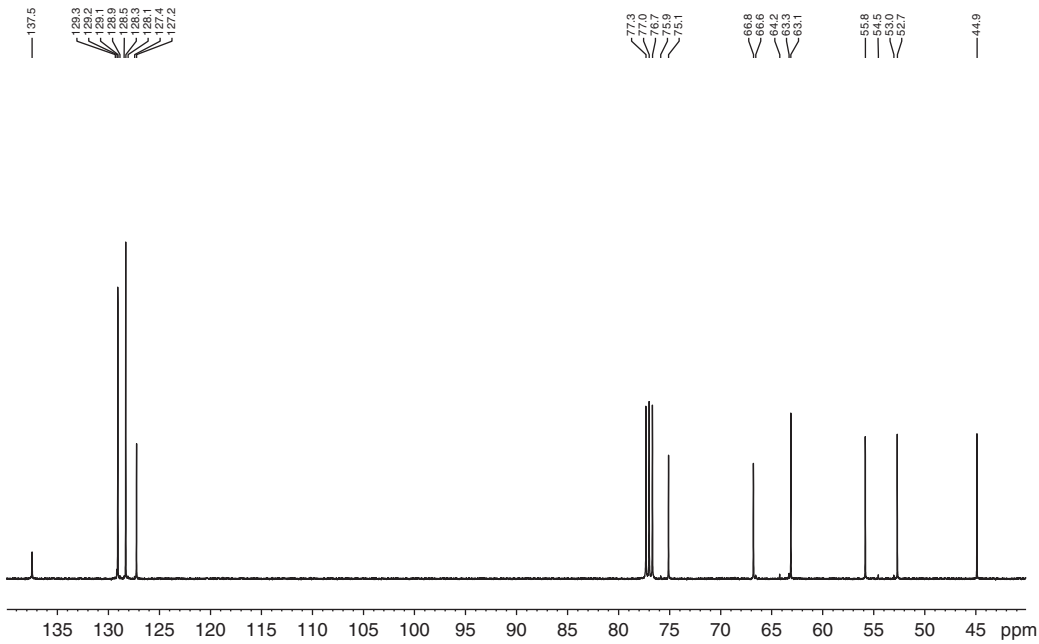
So to a large extent, 1-D ^{13}C NMR interpretation is a case of matching observed singlets to predicted chemical shifts. These predictions can be made by reference to one of the commercially available databases that we've mentioned, or it can be done the hard way – by a combination of looking up reference spectra of relevant analogues and using tables to predict the shifts of specific parts of your molecule (e.g., aromatic carbons). We have included some useful ^{13}C shift data at the end of the chapter but it is by necessity, very limited.

^{13}C prediction software is certainly the preferred option but it should always be used with circumspection. It generally works by using a combination of library data to generate an estimate of the chemical shifts of all the carbons in your proposed structure but it is inevitable that these estimates will be prone

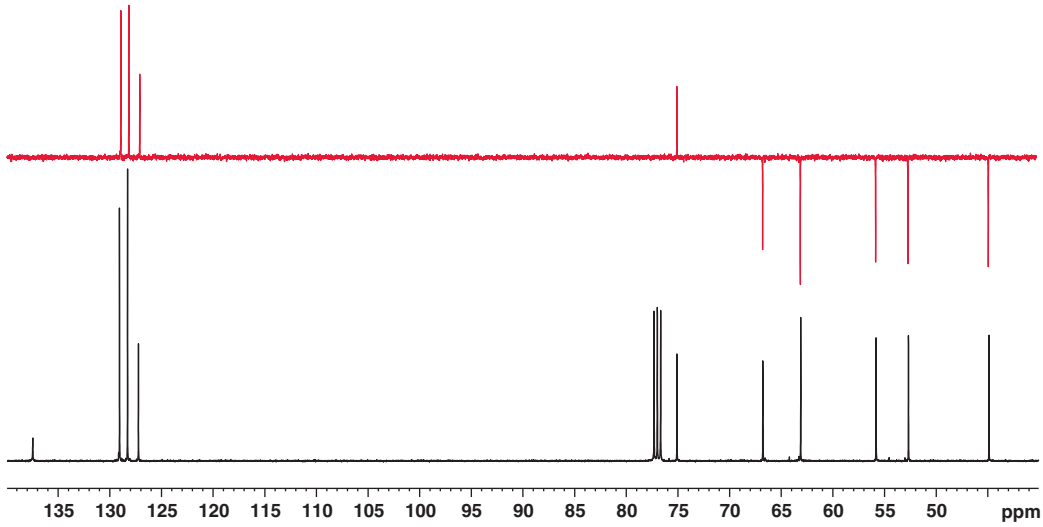
to error. It is important to realise that some shift estimates will be far better than others – even within the same molecule. It is also important to note that whilst these packages may give a measure of confidence with each prediction, these limits must be viewed critically as they may be either unduly pessimistic or (worse) unduly optimistic. We would always recommend that if your prediction software allows you to browse the *actual* compounds used in the predictions, you do so! This will enable you to 'personalise' the predictions to some extent as you will be able to lean more towards the shifts of the compounds in the database that are more similar to your proposed structure. For example, if you are working with steroids and you are trying to predict the shift of a certain carbon in your molecule, it would be wise to pay more heed to the shifts of carbons in similar environments *in other steroids* as opposed to analogous carbons in completely different types of molecule.

From a purely pragmatic point of view, and some purists may take issue with this, it is perhaps not essential that you unambiguously assign every carbon to a *specific* peak as this can be virtually impossible in cases where there are many carbons with similar shifts and all you have to guide you is a mediocre prediction. What *is* important, is that the total number of peaks observed match the number of carbons in your proposed structure and that all their chemical shifts are at least plausible. We shall see presently that there are other tools available which can be used to yield unambiguous assignments in many cases. Consider the carbon spectrum of our familiar morpholine compound (Spectrum 9.1) which demonstrates this point. The chemical shifts of the two carbons in the morpholine ring next to oxygen are pretty close. So too are the carbons next to the nitrogen.

The first of these tools is the *d*istortionless *e*nhancement by *p*olarization *t*ransfer (DEPT) pulse sequence. There are a number of versions of this experiment which can be very useful for distinguishing the different types of carbons within a molecule. Of these, we have found the DEPT 135 sequence to be the most useful. In this experiment, the quaternary carbons are edited out of the spectrum altogether.



Spectrum 9.1 1-D ^{13}C spectrum of *the* morpholine compound.



Spectrum 9.2 1-D ^{13}C spectrum of *the* morpholine compound with DEPT-135 plotted above it.

Methyl and methine protons naturally phase at 180° relative to the methylene carbons and the spectra are usually plotted with methyls and methines positive. (Note that should you encounter a signal that you cannot confidently assign to either a methyl or methine carbon, the DEPT 90 sequence may well be of use as it differentiates these carbons – methines appear positive and methyls are edited out of the spectrum but this technique can be considered obsolete if you have access to any of the 2-D proton–carbon correlated experiments discussed in Section 9.2.)

This is demonstrated once more with our familiar morpholine compound in Spectrum 9.2. The DEPT sequences are of course, still relatively insensitive, though they are probably a little more sensitive than the standard 1-D, fully decoupled ^{13}C spectrum. We find it convenient, particularly with complex molecules, to combine the 1-D ^{13}C spectrum with the DEPT-135 spectrum, which is plotted above it at the same expansion, of course! This enables you to differentiate the different types of carbon in your spectrum at a glance.

9.2 2-D Proton–Carbon (Single Bond) Correlated Spectroscopy

The most powerful techniques of all are undoubtedly the 2-D proton–carbon experiments (*H*eteronuclear *M*ultiple *Q*uantum *C*oherence/*H*eteronuclear *S*ingle *Q*uantum *C*oherence, or HMQC/HSQC; and *H*eteronuclear *M*ultiple *B*ond *C*orrelation, or HMBC) as they provide an opportunity to dovetail proton and carbon NMR data directly.

Taking the HMQC and HSQC first, both these techniques establish one-bond correlations between the protons of a molecule and the carbons to which they are attached. Both techniques are considerably more sensitive than a 1-D ^{13}C spectrum, which might seem strange when you consider that the whole 2-D matrix is composed of a considerable number of ^{13}C spectra. The secret of the superior signal/noise performance of these methods lies in the fact that they are both ‘indirect detection’ techniques. This means that the carbon signals are detected (indirectly) by the transfer of their magnetisation to the much more sensitive protons! A typical data matrix for an HMQC or HSQC might be composed of 256

increments in the carbon domain, each of 2 k points in the proton domain. For a 5–10 mg sample of typical 200–400 molecular weight, reasonable signal/noise could be achieved with about 16–32 scans per increment in a 400 MHz instrument which means that you could easily achieve a good quality spectrum in about 1–2 h.

In terms of choosing between the two, bear in mind that the choice available on the spectrometer you use may well be limited by the hardware itself. Historically, the HMQC looked like the better bet at first, as it was more robust. The HSQC technique was fine – but the large number of 180° pulses in the sequence, require accurate pulse calibration if severe cumulative errors are to be avoided. In other words, if the probe tuning was not optimised, you could expect very poor signal/noise or even no signal at all. Probe tuning and matching is not the sort of thing you can reasonably expect the average walk-up user to get involved with and for this reason, HSQC was a nonstarter. HMQC was the way to go but times and hardware move on and nowadays, most modern instruments *can* deal with HSQC routinely without the need for any poking around under the magnet with a nonmagnetic tweaking stick!

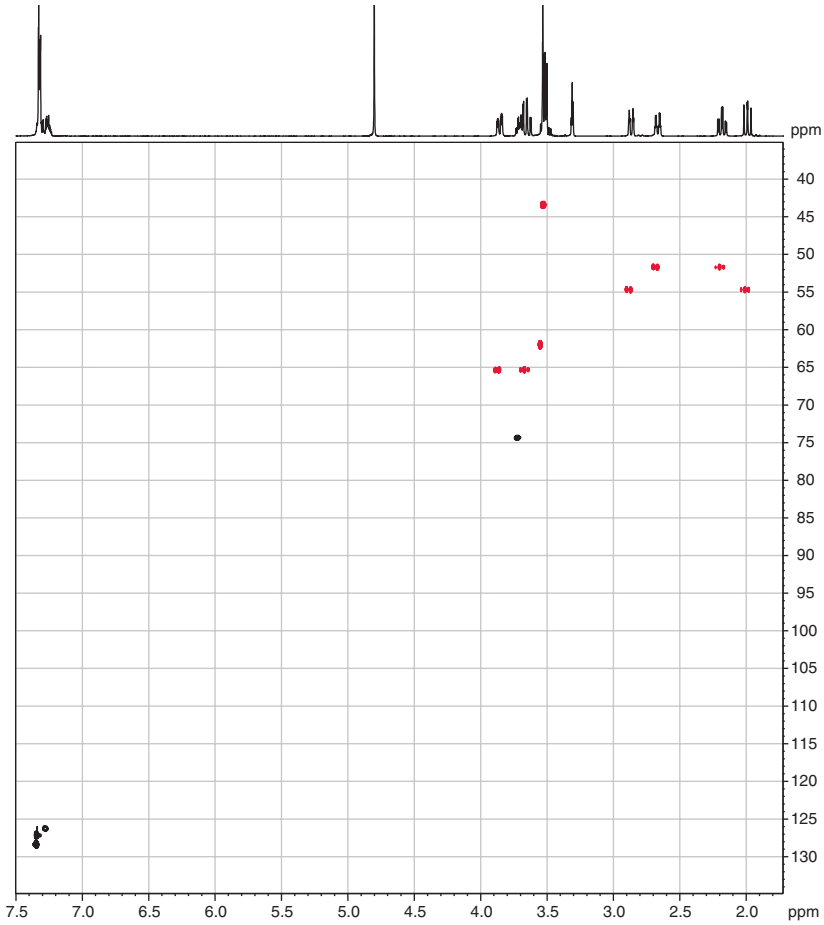
The two developments responsible for this are 'automatic probe tuning and matching', and 'adiabatic pulses.' Automatic probe tuning and matching enables optimal probe tuning to be achieved for every sample in an automated run, regardless of solvent. Adiabatic pulses solve the problem in a different way – by removing the need for accurate pulse calibration. Solving this problem enables us to routinely enjoy the benefits of HSQC over HMQC which include fewer spectral artefacts and slightly better resolution in the carbon domain.

So to sum up, if you have the luxury of modern equipment with all the go faster boxes at your disposal, go for HSQC. If you are stuck with an older instrument and you're not keen on grovelling around under the magnet, an HMQC is for you.

Our preferred experiment of this type is the so called 'DEPT-edited HSQC' which is both relatively artefact-free and sensitive. It also has one other major advantage up its sleeve. This experiment is not an 'absolute value' technique like most of the others, but it allows for discrimination between different types of carbons. Methyl and methine carbons give crosspeaks that are phased opposite to the methylene carbons and so the results are best plotted on a colour plotter which can portray this clearly by plotting positive and negative cross peaks in different colors.

A brief note on the phasing of the DEPT-edited HSQC spectra – because the technique is 'phase sensitive' (as opposed to 'absolute value'), these spectra require phasing. This is usually done under automation in walk-up systems and usually done well. (Note that phasing has to be performed in *both* dimensions) Sometimes, you may find a signal at one end of your spectrum which is clearly not phased, despite the fact that all the neighbouring signals appear perfectly phased. The likely reason for this will be that the size of the ^{1}H–^{13}C coupling for the carbon in question is abnormally large or small and there is not much that can be done about it. (Attempts to phase such signals correctly will result in the phasing of all the other signals suffering!) Instruments are typically set up to give a maximum sensitivity for couplings of around 145 Hz. This figure is a compromise between the generally smaller couplings found in alkyl systems and the slightly larger ones encountered in aryl systems. The larger the deviations of the one-bond ^{1}H–^{13}C couplings from this value, the greater will be the phasing inaccuracies encountered. Typical problem carbons are those of the nitrogen-bearing heterocycles where couplings approaching 200 Hz are quite common.

Interpreting HMQC/HSQC spectra is relatively straightforward as you can see from the HSQC spectrum of *the* morpholine compound (Spectrum 9.3). Basically, it's a case of lining up the proton signal with the contour, and reading off the ^{13}C chemical shift. The technique is extremely powerful – particularly when used in combination with HMBC as we'll see later. In examples like this one, it

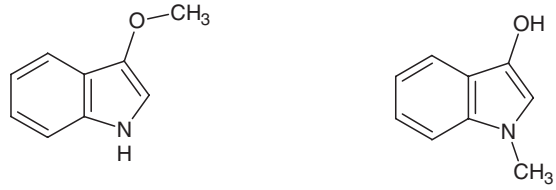


Spectrum 9.3 DEPT-edited HSQC of *the* morpholine compound.

enables you to identify geminal pairs of protons at a glance as you can see which protons are attached to the same carbons. As a philosophical aside, we should always be on our guard against using proton data to 'hammer' the ^{13}C data to fit. Although the 2-D techniques tie the data sets together, we must still interrogate them separately. In other words, if a correlation flags up an implausible shift in one domain or the other, the whole structure should be reconsidered.

HMQC/HSQC spectra can be extremely useful in resolving problems where there is a significant carbon chemical shift precedent that could be used to support one putative structure over another – for example, in dealing with cases of O- versus N- alkylation. Take for example the two methylated indoles in Structure 9.1.

How could you be certain of which site had alkylated? Any judgement based on proton chemical shift would be foolhardy as there would be little to choose between them. (Note that in molecules where the lone pair of electrons on a nitrogen are effectively 'removed' from the nitrogen for whatever reason – in this case, by donation into the aromatic ring - the nitrogen becomes more electron-deficient and thus more 'oxygen-like.' The chemical shift of alkyl groups substituted onto such nitrogens therefore become



Structure 9.1 Two methylated indoles.

very similar to those of analogous O-alkylated compounds and distinction between them on the basis of proton chemical shift becomes unreliable!) The methyl groups in both molecules might be expected to give Overhauser enhancements to their nearest aryl protons but in order to make use of this, you would have to be absolutely certain of the assignment of the aryl protons themselves! ^{13}C data would be unambiguous here. A methyl singlet with a carbon shift in the range, 35–45 ppm and you are looking at N-methylation. If the carbon shift of the methyl is in the region of 55–65 ppm, it's the oxygen that has been methylated.

If interpreting the single-bond correlation experiments is easy, the multiple bond experiment (HMBC) can be considerably less so . . .

9.3 2-D Proton–Carbon (Multiple Bond) Correlated Spectroscopy

Potentially even more useful, is the HMBC experiment. In this experiment, correlations are obtained between carbon atoms and protons that are separated by two and three bonds. Of course, the actual number of bonds separating the protons from the carbons is something of a red herring. What the spectrometer records are carbon-proton correlations for carbons that have protons couplings of specified magnitude. The sensitivity of the spectrometer to various sizes of proton-carbon coupling is controlled by one of the delays in the HMBC pulse sequence. This delay is selected on the basis of $1/2J$, where J is the coupling you wish to optimise for. A proton–carbon coupling of 10 Hz is a fairly typical value for the experiment, and thus the relevant delay would be set at $1/2 \times 10$, or 0.05 s. This means that the spectrometer sensitivity would be optimised for carbons with proton couplings of around 10 Hz. It does not mean that it will not detect carbons with smaller or larger proton couplings, just that the response shown will not be as intense.

In practise, 3-bond proton couplings tend to be nearer to this value than are the 2-bond couplings and for this reason, the HMBC sequence is usually more sensitive to 3-bond than to 2-bond correlations. This has of course to be viewed within the context of the overall signal/noise for the experiment. If the signal/noise for the whole experiment is less than excellent, it is quite possible for some 2-bond correlations to slip through the net altogether. If you are wondering why the value of $1/2J$ is not used to even-up the response to 2- and 3-bond correlations, there are two important factors to consider. If this value was optimised for, say, 5 Hz proton couplings, then the spectra we would obtain would be further greatly complicated by 4-bond couplings which would start to come through, since the J values for some 4-bond couplings are comparable with 2-bond values. Furthermore, as the value for J gets smaller, so the optimal delay required gets longer so that more and more signal gets lost to relaxation prior to acquisition and overall sensitivity for the experiment is lost. This incidentally partially explains why the technique is not as sensitive as HSQC in the first place (1-bond proton–carbon couplings are typically around 150 Hz, so the delay is extremely short and very little signal is lost.)

So, putting it bluntly, HMBC spectra are more difficult to unpick because there will inevitably be far more correlations recorded than in the corresponding HMQC/HSQC. The problems do not end there, however. For example, it is not immediately obvious by inspection, which are the 2-bond and which are the 3-bond correlations. This has to be reasoned out within the context of whatever molecule you are dealing with. Furthermore, whilst most 4-bond proton–carbon couplings are less than 2 Hz, some are not, allowing unwanted 4-bond correlations we've mentioned through into our spectra, even when we've optimised for 10 Hz couplings! This can be a problem particularly in the case of aromatic, heterocyclic and conjugated compounds where signal to noise is good. These need to be identified for what they are as soon as possible or they will cause confusion!

Unfortunately, the complexity does not stop there. One-bond couplings can also come through in the HMBC experiment, despite filters used to block them. This can be seen in our HMBC spectrum of *the* morpholine compound (Spectrum 9.4) with reference to one of the aromatic signals at 126 ppm. One-bond correlations are characterised by a pair of contours that are symmetrically displayed on either side of the 1-D (proton) projection they relate to, the separation between them giving the proton–carbon coupling, of course. Whilst this is generally not a problem for obvious isolated singlets, it certainly can be a problem in the crowded aromatic region of the spectrum where chemical shifts are relatively tightly packed in both proton and carbon dimensions. Problems can arise where 1-bond contours fall in positions where they line up exactly with peaks from the 1-D proton projection, giving rise to potentially very confusing bogus 'correlations.'

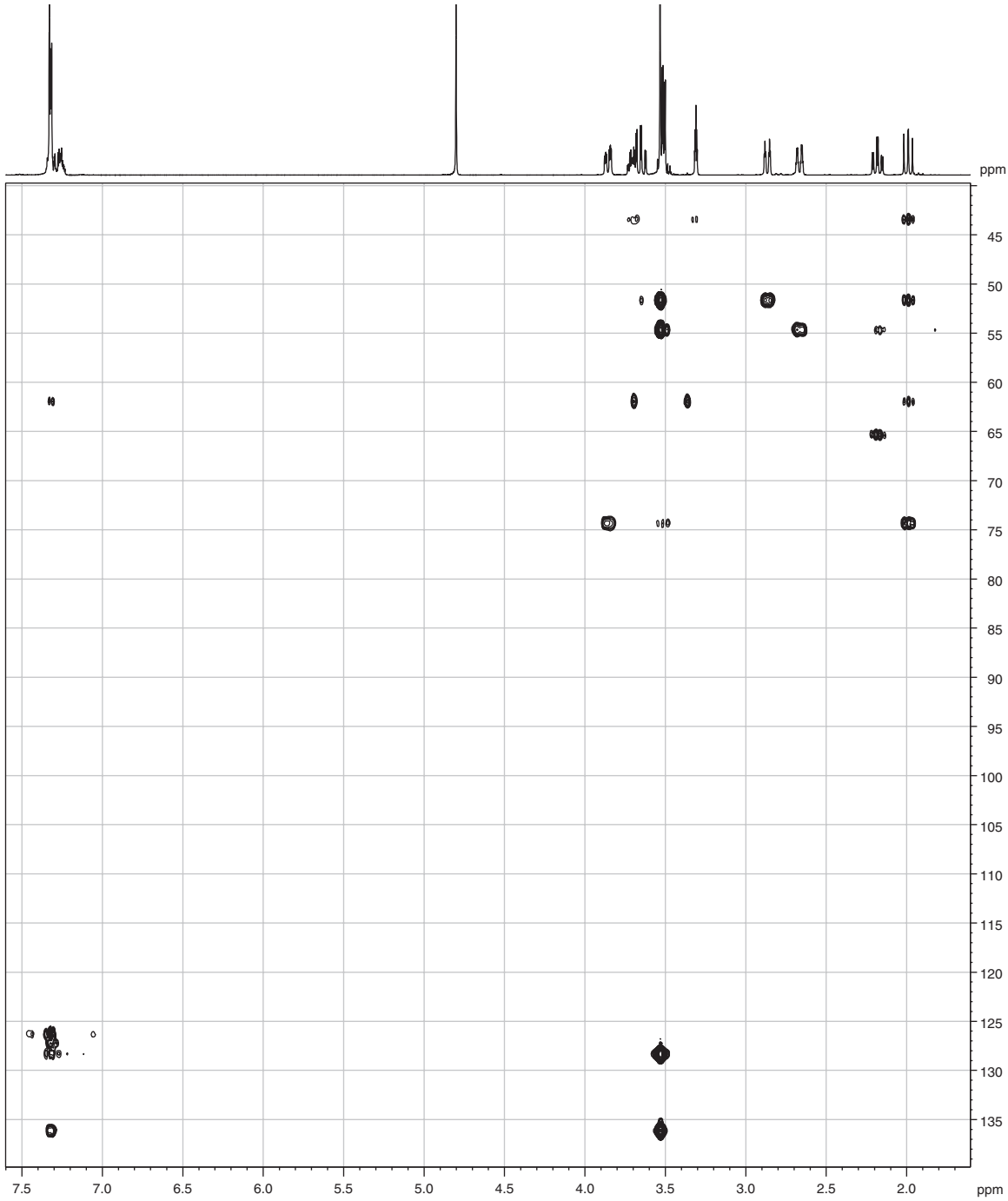
Another feature that is worth being aware of is the so-called 'auto-correlation' phenomenon. These can be observed in molecules which contain moieties such as -N-dimethyl or isopropyl groups. Such groups can give the initially confusing arrangement of three contours in a row. In such cases, the two outer contours are the 1-bond correlation, and the centre correlation, the genuine 3-bond HMBC correlation from the protons of one of the methyl groups to the carbon of the *other* methyl group. Once this pattern has been noted, you will recognise it easily and even make use of it as a quick identifier for these groups.

If all these cautionary notes make the technique sound like a complex nightmare, we're not done just yet . . . Just as an unwanted 4-bond correlation can come through to muddy the water and a 2-bond coupling can fail to materialize, so too can a 3-bond coupling fail to register for exactly the same reason – the size of the proton–carbon coupling may be too far from the optimised value to give a sufficient response to be recorded. There can be two possible reasons for this. First, it can just be a question of local electron distribution giving rise to an abnormal value for the 3-bond proton–carbon coupling. One that springs to mind is the lack of a correlation often observed between the 3′ proton and the 4′ carbon in indoles (Structure 9.2).

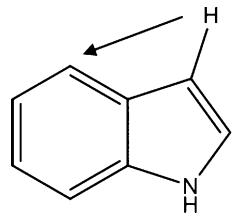
Another reason for not observing expected 3-bond couplings relates to the Karplus equation which we discussed at length in Section 6.6.5. Just as the size of proton–proton vicinal couplings are dependant on the dihedral angle between them, so too are proton–carbon couplings. You can come across molecules where the relevant angle suppresses the coupling and hence a 3-bond proton–carbon coupling can fail to show.

Our advice is that if it is vital to establish such a connectivity, then re-run the experiment optimising for a smaller coupling value (e.g., 5 instead of 10 Hz). Yes, you will take a hit on signal/noise and spectral complexity may well increase as 4-bond couplings start to come through but if you are chasing down one specific coupling, then these things don't really matter.

HMBC experiments are not limited to proton–carbon interaction. With suitable hardware, it is possible to acquire ^{1}H–^{15}N spectra which can be extremely useful for confirming the identity of nitrogen-containing heterocyclic compounds. The sensitivity of this technique is very low, probably only about



Spectrum 9.4 HMBC of *the* morpholine compound.



Structure 9.2 Expected 3-bond correlation often not observed.

one-tenth of the ^{1}H–^{13}C technique but sometimes it can provide that extra, vital piece of the jigsaw. We have provided some basic but useful ^{15}N shift data in Chapter 11.

After digesting this information and noting the many benefits of the 2-D proton–carbon techniques, (providing the pitfalls and complexity of the HMBC technique haven't put you off the idea!) you might be wondering why anybody would ever bother acquiring a simple 1-D ^{13}C spectrum any more. Well, there are two good reasons that spring to mind. First, it is quite possible to encounter a molecule with no protons within a 3-bond range of one or more of its carbon atoms. Such carbons will be 'invisible' to the HMBC technique and will only be visible in a 1-D spectrum.

Second, the resolution achieved in a 2-D experiment, particularly in the carbon domain is nowhere near as good as that in a 1-D spectrum. You might remember that we recommended a typical data matrix size of 2 k (proton) × 256 (carbon). There are two persuasive reasons for limiting the size of the data matrix you acquire – the time taken to acquire it and the shear size of the thing when you have acquired it! This data is generally artificially enhanced by linear prediction and zero-filling, but even so, this is at best equivalent to 2 k in the carbon domain. This is in stark contrast to the 32 or even 64 k of data points that a 1-D ^{13}C would typically be acquired into. For this reason, it is quite possible to encounter molecules with carbons that have very close chemical shifts which do not resolve in the 2-D spectra but will resolve in the 1-D spectrum. So the 1-D experiment still has its place.

9.4 Piecing It All Together

As we've mentioned before, the interpretation of NMR spectra is often made complex by the sheer quantity of information that you are confronted with. This is every bit as true for carbon NMR as it is for proton and when you combine the two, that huge pile of information just gets bigger. . . More important still then that you approach the pile in a logical, methodical manner.

Once your problem takes on a ^{13}C dimension, you are of course, obligated to examine the ^{13}C data with the same level of dispassionate scrutiny that you apply to the proton data. Chemical shifts cannot be fudged and unexpected peaks cannot be glossed over. You have to be able to account for everything you see to have confidence in your product.

We will assume that you have already been through the proton data with a fine-toothed comb and found it wanting in some way, or insufficient to give the level of reassurance that you require. So turning to carbon, a 1-D ^{13}C spectrum of adequate signal/noise would be a luxury, though not an absolute necessity. We'll assume you have one. Strike out the carbons for any known solvents etc. and then count the total number of carbon peaks in the spectrum. Do they match the requirements for your proposed structure? (Don't forget that a para-di-substituted aromatic ring gives four peaks for its six carbons on

Structure 9.3 Our naphthalene problem.

account of its symmetry.) Note also that knowing with certainty, the number of carbons in a structure can be very helpful in narrowing the search for a molecular formula by mass spectroscopy (accurate mass).

If you have a DEPT 135 spectrum, now is the time to use it. Categorise all peaks to one of the following types: quaternaries and carbonyls, methines, methylenes and methyls. Now get hold of plausible prediction data for the shifts of your proposed structure. Use HMQC/HSQC spectra to assign the proton-bearing carbons and if satisfactory, move on to assign all the quaternaries and carbonyls by using the HMBC spectrum. Do all the long-range connectivities from the HMBC make sense? Does it all hang together?

As with proton interpretation, this must be considered an iterative process. Try to shoot your proposed structure down. Don't be afraid to tear it up at any stage and start again if some glaring problem becomes apparent. Resist temptation – don't hammer the square peg into a round hole! This is why we do spectroscopy in the first place. If it crashes and burns then it was wrong so shed no tears. If it survives then it's got a good chance of being a winner. Finally, go back again and check that there is no mismatch between any carbon data and any supplementary proton data, e.g., NOE experiments.

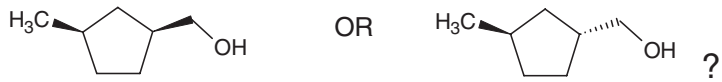
When it *all* sits happily or can at least be explained, the job is done as well as it can be. Not before.

9.5 Choosing the Right Tool

If you have successfully read this far, it might have occurred to you that some problems could well be solved by either an NOE-based approach, or by an HMBC approach and you might be wondering which technique would be preferable under such circumstances. In truth, there may not be a right or wrong answer to this question and each problem should be considered on its merits. The selection of experiment may even be down to personal preference or to the hardware available to you. Questions of positional isomerism can often be resolved by either approach. We have seen how our naphthalene problem could be resolved by using an NOE technique (Structure 9.3).

This problem could also have been resolved by an HMBC approach – you would expect to see a correlation from the protons of either the -CH_2-, or the -CH_3 to one of the quaternary carbons at the junction of the two rings. This same carbon should also show correlations to at least two, and ideally three of the protons on the unsubstituted aromatic ring and one of the protons on the substituted ring.

It is when questions of stereochemistry arise that the NOE techniques come into their own. For example, consider the compounds in Structure 9.4. There would be no chance of resolving these two structures by HMBC, but an NOE technique might well prove successful. (The methyl group would be expected to give an enhancement to either of the -C**H$_2$**-OH protons in one isomer or to the >C**H**-CH_2OH in the other, depending on which face of the ring the two substituents lie relative to each other.)



Structure 9.4 A question of stereochemistry.

As in the case of all NMR problem-solving, the issue is always one of using the most appropriate tool for the job. The two techniques are in no way mutually exclusive. Too much data is not a bad thing if the instrument time is available but taking a chance on insufficient data can be a costly mistake in the long run.

Tables 9.1–9.7 give a useful guide to ^{13}C chemical shifts.

Table 9.1 ^{13}C chemical shifts of some common heterocyclic and fused aryl compounds.

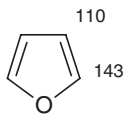
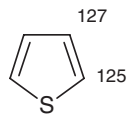
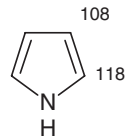
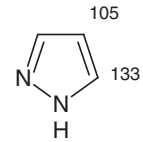
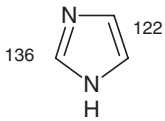
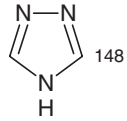
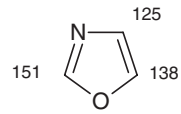
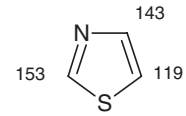
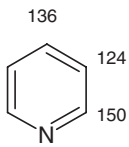
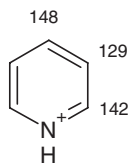
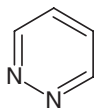
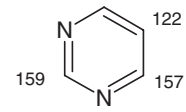
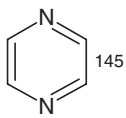
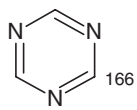
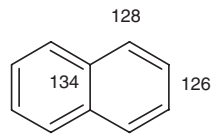
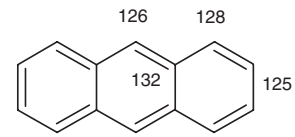
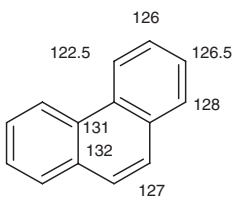
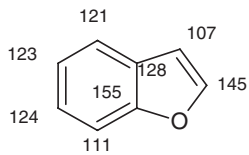
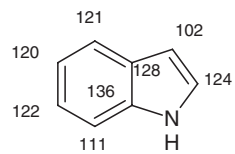
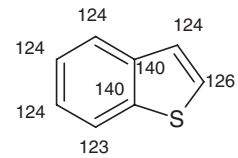
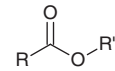
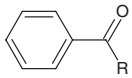
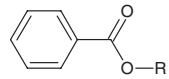
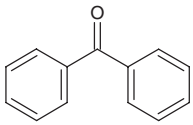
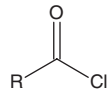
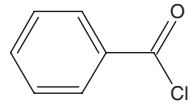
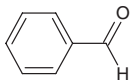
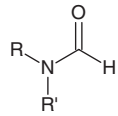
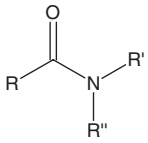
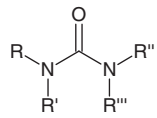
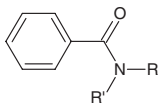
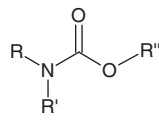
			
			
			
			
			

Table 9.2 ^{13}C chemical shifts of some common carbonyl functions.

Type of carbonyl	Typical shift	Type of carbonyl	Typical shift
	205–210		170–180
	195–200		165–170
	~195		170–175
	196–202		~168
	190–195		160–165
	167–173		160–165
	165–172		153–160

Note: thio-carbonyl analogues generally absorb at considerably lower field – sometimes by as much as 40 ppm.

Table 9.3 ^{13}C chemical shifts of some CN functions.

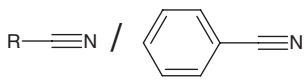
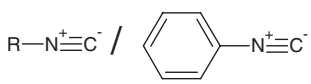
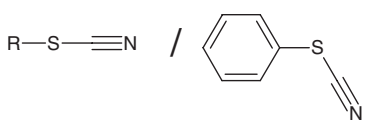
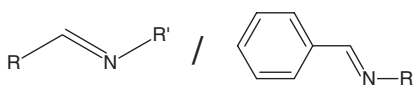
Type of CN function	Typical shift
	115–120
	156–166
	110–115
	155–170

Table 9.4 Data for the estimation of aryl ^{13}C chemical shifts.

Substituent X	C1	C2	C3	C4
-H	0.0	0.0	0.0	0.0
-CH_3	9.2	0.7	0	–3.0
-CH_2-any (approx.)	2–12	–2 (+)2	–2 (+)2	–2 (+)2
-CH=CH_2	9	–2	0	–0.8
-C&ΞC-R (approx.)	–6	4	0	0
-Phenyl	8	–1	0.5	–1
-F	34	–13	1.5	–4
-Cl	5	0	1	–2
-Br	–5	3	2	–1
-I	–31	9	2	–1
-OH/-OR (approx.)	30	–13	1	–7.5
-O-phenyl	28	–11	0	–7
-$OCOCH_3$	22	–7	0	–3
-NH_2/-NR_2 (approx.)	17	–14	1	–10
-NH_3^+/-NR_2H^+ (approx.)	3	–5	2	1
-NO_2	20	–5	1	6
-CN	–16	3.5	1	4
-NC	–2	–2	1	1
-SH/-SR (approx.)	7	0	0	–3.5
-S-phenyl	7	2.5	0.5	–1.5
-SOR	18	–5	1	2
-SO_2R	12	–1	1	5
-SO_2Cl	16	–2	1	7
-SO_3H	15	–2	1	4
-SO_2NH_2	11	–3	0	3
-CHO	8	1	0.5	6
-COR	9	0	0	4
-COOH/-COOR (approx.)	2	1.5	0	4.5
-$CONH_2$/-$CONR_2$ (approx.)	5.5	–1	0	2

Note: substitute values relative to benzene (128 ppm) as follows: chemical shift of C1–C4 = 128 + additive value for C1–C4 from table above.

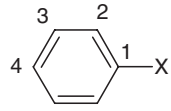


Table 9.5 Data for the estimation of alkene ^{13}C chemical shifts.

Substituent X	C1	C2	Substituent X	C1	C2
-H	0	0	-$OCOCH_3$	18	–27
-alkyl	10–20	–4 to –12	-NR_2	28	–32
-$CH{=}CH_2$	14	–7	-N^+R_3	20	–11
-CH&ΞCH	–6	6	-NO_2	22	–1
-Phenyl	12.5	–11	-CN	–15	14
-F	25	–34	-NC	–4	–3
-Cl	3	–6	-SR	9	–13
-Br	–9	–1	-CHO	15	15
-I	–38	7	-COR	14	5
-OR	28	–37	-COOR	5	10

Note: substitute values relative to ethene (123 ppm) as follows: chemical shift of C1 and C2 = 123 + additive value for C1/C2 from Table 9.4.

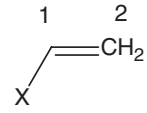


Table 9.6 ^{13}C chemical shifts for alkynes.

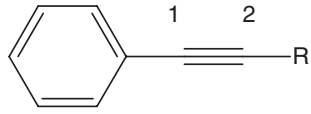
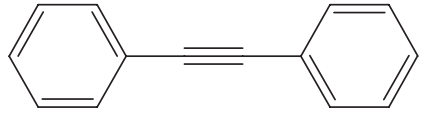
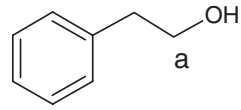
Type of Alkyne	Typical shift
	75–80
	~85(C1) ~80(C2)
	~90

Table 9.7 Data for the estimation of alkyl ^{13}C chemical shifts.

Substituent X	α	β	γ
-H	0	0	0
-alkyl	9	9	–3
-C=C-R_2	20	7	–2
-C≡C-R	4	6	–3
-Phenyl	22	9	–3
-F	70	8	–7
-Cl	31	10	–5
-Br	19	11	–4
-I	–7	11	–2
-OR	49	10	–6
-OCOR	57	7	–6
-NR_2	28	11	–5
-NR^+_3	28	6	–6
-NO_2	62	3	–5
-CN	3	2	–3
-SR	11	11	–4
-SO_2R	30	7	–4
-CHO	30	–1	–3
-COR	23	3	–3
-COOR	20	2	–3
-$CONR_2$	22	3	–3
-COCl	33	8	–3

Note: this table gives only very approximate shift estimates and is intended for use as a rough guide only. The presence of highly branched substituents and atoms bearing multiple halogens, multiple oxygen atoms etc. can cause even more serious deviations rendering the table of questionable value under such circumstances. It is used by summing the substituent effects at each carbon relative to methane (–2.3 ppm). For example, the shift of carbon 'a' below would be estimated as: $-2.3 + 49 + 9 + 9 = 65$ ppm (approx.). Actual value 63ppm.



10

Some of the Other Tools

If you have the facility to acquire good quality spectra of the types discussed in this chapter and the one preceding it, then you should be well positioned to tackle virtually any problem that comes your way. We have covered what in our view are the most important and relevant techniques – but there are many others you may have heard of, all with their own enticing sounding acronyms. We'll now take a look at some of them and try to outline some of their potential uses as well as their shortcomings.

10.1 Linking HPLC with NMR

Linking techniques together might seem like a good idea in theory but in practise, there can be as many problems as potential advantages. HPLC-NMR does have undeniable use in the field of bio-fluid NMR and in process control in a production environment but we feel that it has little to offer the organic chemist looking to monitor a reaction.

The two techniques don't really sit happily together. HPLC is essentially a dynamic technique – solvent and solute move continuously from injection port through column to detector. Data acquisition takes place rapidly as the various fractions pass through the detector. But NMR detection isn't like that. It takes time – and with dilute solutions, the sample just isn't resident in the flow cell (a flow cell replaces the conventional NMR tube in HPLC-NMR) for long enough for any useful acquisition to take place. In order to overcome this problem, it is possible to use a 'stop flow' technique where the flow is stopped and the fraction contributing to a peak is 'parked' in the probe for long enough to acquire some useful data. Of course, this can lead to serious chromatographic problems. It is quite possible that whilst the early-running peaks are in the flow cell, the later running ones are still on the column. Stopping the flow at this stage will inevitably lead to fractions broadening themselves by diffusion on the column.

Another serious problem is that of the chromatography solvent gradient. It is common practise for reverse-phase columns to be run using a solvent gradient system so that polarity of the solvent is gradually changed from polar to nonpolar throughout the separation by altering the ratio of two different solvents during the run. This has the benefit of extending the range of polarities that can be accommodated on one column within a sensible run time. The problem with this from an NMR perspective is that shimming, and indeed, probe tuning will be very much altered as the run progresses giving rise to line-shape and

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sensitivity issues. Note that even without this complication, resolution in a flow cell can never be as good as in a conventional tube as a flow cell can't spin! The solvents themselves are yet another issue. To run such a system on deuterated solvents would be prohibitively expensive and so normal nondeuterated ones are used. This of course gives rise to massive solvent peaks which must be suppressed, denying you access to potentially important parts of the spectrum.

There are numerous other problems associated with the technique. Such systems need very careful setting up to ensure that the fractions park accurately in the flow cell so as to maximise concentration and hence signal to noise. Other minor irritants can include; various plumbing problems, blockages causing capillaries to burst off, wet carpets etc.

There are several variations on the theme of instrument set up, which have been used in an attempt to overcome the shortcomings inherent in the concept. For example, as an alternative to the stop–flow method, the various fractions can be collected into sample 'loops' (small loops of capillary tubing) which can then be flushed into the flow cell and studied at leisure. After spectroscopic examination, each sample can then be returned to its loop and the next pumped in. Fractions suffer dilution in this way but this approach would seem to offer an advantage over stop–flow in that at least the chromatography is not compromised by diffusion on the column.

Another variation is that of trapping eluting compounds onto solid-phase cartridges and then washing them off as required using a suitable *deuterated* solvent. In this way, the problem of solvent suppression needed for dealing with nondeuterated solvents, is neatly side stepped. But all of a sudden, it would seem that the two techniques are becoming more and more segregated again. And there's the rub – HPLC is an excellent technique and so is NMR but what is good for one is bad for the other and vice versa. Perhaps then, it is best if we do not force them into an uneasy alliance. By analogy, various attempts have been made in the past to build an amphibious car but results have generally been characterised by mediocre performance on land and worse on water. Engineering to cater for many disparate requirements in any system can lead to compromises which adversely affect the performance of the whole system.

10.2 Flow NMR

In a sense, Flow NMR is like HPLC-NMR without the chromatography part. It has found use in the field of 'array chemistry' where 'libraries' of compounds, usually with a common motif, are made – or at least presented – in the wells of a 'plate.'

Systems have been developed by some of the major spectrometer manufacturers to deal specifically with this type of application. These systems are designed with automation very much a priority. Typically, an integrated robot adds a predetermined volume of solvent to each of the wells and then injects the resultant solution into a flow line that transfers it into the spectrometer's probe, which is of course fitted with a flow cell. Spectroscopy can then be performed without the time constraints of the HPLC-NMR system and the sample returned to the well on the plate where it came from, or into a fresh one if required.

With careful fettling, these systems can work quite well but they are not without their potential pitfalls. For example, there is always a danger that the samples will suffer some degree of cross-contamination as they are all being drawn up by the same automated syringe and transported through the same capillaries. Obviously, such systems use a flushing cycle between samples but it is not impossible for a particularly 'sticky' sample to hang around in some recess of the plumbing only to be gradually flushed out with the

passing of subsequent samples. So it is not reasonable to expect the re-formatted plate to be of as good a quality as it was before spectroscopic investigation. There is also the question of sample recovery and dilution. It would be unreasonable to expect 100 % sample recovery after shunting a solution through several meters of plumbing and recovery rates will vary with individual system set up. These factors can have implications if you wish to revisit one of the samples of the library for further investigation. Ideally, you would do any further investigation when the sample in question is in the probe but there's not much point in having an automated system if you have to stand over it all the time!

Obviously, flow NMR can generate a huge amount of data. Library plates can often hold 96 samples and an overnight run can easily present you with 96 spectra to look at the following morning! In other words, such a system could generate spectra faster than you could interpret them. In these circumstances, a rather cut down approach to interpretation is required. The chemistry under investigation might, for example, be that of the reaction of a specific amine with 96 different carboxylic acids. Rather than address every minute feature of every spectrum, you might have to make do with an indication that the reactants have reacted as desired and an overall impression of sample purity.

Flow NMR has recently been eclipsed by the advent of robotic sample handling systems capable of dealing with very small sample quantities and volumes. We now have a system operating in our laboratory that makes up samples directly into 1 mm NMR tubes, using only about 8 ul of solvent. These can be run under automation and the tubes emptied back into the plate wells by the same robot. This technology offers superior performance and largely gets around the problems of contamination and recovery.

10.3 Solvent Suppression

When dealing with high-quality samples, solvent suppression is not an issue that should ever cause concern. However, if for some reason, your sample is heavily laden with some solvent or water which cannot be easily removed from your sample, then you might need to consider some form of solvent suppression. Why? Because when acquiring the spectrum of a sample that contains a peak or peaks that are orders of magnitude larger than those of interest, the receiver gain requirements will be set to cater for the large peak(s) at the expense of the small ones. This would be analogous to acquiring with far too low a receiver gain and yield very poor signal to noise for the peaks of interest.

Such a problem can be addressed by artificially suppressing the huge unwanted peak(s) so that the smaller desired peaks can be acquired optimally. At its crudest, peak suppression is nothing more than a decoupler signal of suitable power, centred on the unwanted peak. Once the problem peak is saturated to oblivion, the rest of the spectrum can acquire normally. This method works well enough in most cases but far more subtle methods have been developed. It is possible, by using a simple macro programme, to move the decoupler signal rapidly back and forth over the peak to be suppressed. This can improve the resultant spectrum by minimising suppression artifacts.

Other even more cunning methods have been devised to suppress the water signal in samples that have a large water content (e.g., bio-fluid samples) such as the WET and the WATERGATE pulse sequences. Other sequences have been devised to cope with signals from carbon-bound hydrogens. Some of these actually collapse the ^{13}C satellites into the main ^{12}C peak prior to suppression. Such a sequence would be useful if you were forced to acquire a spectrum in a nondeuterated solvent.

10.4 Magic Angle Spinning NMR

Synthesis of compounds on solid-phase supports became quite popular in the late 1990s and, though interest might have waned a little more recently, there may still be a demand for it in some establishments. If monitoring reactions carried out on resins is what is required, then a magic angle spinning (MAS) probe is the only way to go.

The NMR of solids is a specialist field and as it is of little or no relevance to the organic chemist, is outside the scope of this book and so we will say very little about it. The main problem associated with solids is that the lattice relaxation is very efficient which causes NMR lines to be extremely wide. (Remember – the faster the relaxation time of a nucleus, the broader its NMR peak will be.) Spinning samples at very high frequency (2–6 kHz!) at the so-called 'magic angle' helps to minimise this broadening because it sharpens NMR lines by negating the effects of chemical shift anisotropy which arises (in solids and semisolids) as a result of the directional character of chemical shifts with respect to the applied magnetic field. Chemical shift anisotropy (and dipolar interactions, another source of broadening) varies with the term $3\cos^2 q–1$, where 'q' is the angle from the vertical axis of the applied field. This term becomes zero when q is $54^\circ 44'$ – the 'magic' angle.

The problem with trying to run spectra of solid-phase gels with organic compounds bound to them is that the materials are in NMR spectroscopy terms at least, 'solid-like.' Trying to run them in conventional probes is a complete waste of time. NMR line widths will be hundreds of hertz wide and no useful information will be forthcoming. Running them in a MAS probe can greatly improve matters – if the resin is of the right type. The key to achieving sharp lines is molecular mobility, or if you like, the removal of the very efficient lattice relaxation pathway mentioned above.

Numerous resin supports are commercially available for solid-phase synthesis and some allow the acquisition of quite reasonable quality spectra of compounds bonded to them – and some don't. The resins to avoid (if you intend trying to monitor your reactions by MAS-NMR) are any that are based purely on cross-linked polystyrene. These are too rigid and afford little or no mobility to any bound compound. These resins are relatively cheap and have high specific loadings but will give very poor spectra even in a MAS probe. We see little point in running spectra of compounds on these resins as the quality of the spectra make them virtually useless – and perhaps worse – potentially misleading.

Compounds bound to resins such as Tentagel and Argogel, on the other hand, give spectra that can yield useful results. These resins are still polystyrene-based but differ in that they have long polyethylene glycol (PEG) chains bonded to them and the compounds synthesised are bound to the end of these chains via a linker. These chains allow considerable freedom of movement at the end of the chain and thus the bound compound experiences something far more akin to a normal liquid environment. There are certain unique problems associated with the acquisition of spectra of compounds bound to solid-phase supports. One problem associated with these samples arises from the very long PEG chains which connect the 'linkers' bearing the synthesised molecule, to the beads themselves. The PEG chains are about fifty units long giving a most unwelcome 200 proton signal at about 3.5–4.0 ppm! This often completely obscures important signals in this region and nothing can be done about it.

The broad polystyrene signals of the support beads are another major problem and render integration of most regions of the spectra impossible. These broad signals can be suppressed by using the Carr–Purcell–Meiboom–Gill (CPMG) pulse sequence. (This sequence works by multiple de-focusing and re-focusing of the sample magnetisation. The sharp lines refocus and the broad ones do not.) Unfortunately, this means that the CPMG spectra cannot be integrated, as signal suppression occurs for all

broad signals – irrespective of their origin and of the nature of the broadening. We recommend acquiring both the ordinary spectrum and the CPMG spectrum and looking at them alongside each other.

In practical terms, very little material is needed, as the MAS probe is extremely sensitive. More material tends to be lost in handling than actually ends up in the tiny NMR tubes used in this probe (typical volume 40 ul). One mg of bead is usually ample. Note that this corresponds to far less than 1 mg of compound, of course! We have found that it is best to introduce resin-bound sample into the 'nanotubes' before adding the solvent as wet resin is extremely difficult to handle! $CDCl_3$ or CD_2Cl_2 is used as the 'solvent,' though its real purpose is to disperse the beads and cause them to swell, as well as to provide a source of deuterium for locking the spectrometer. This swelling function is important as it allows the PEG chains to move about more freely. The less restricted the motion of the chains, the sharper will be the signals from them.

Some MAS probes are single-coil, allowing proton-only acquisition, and some are dual-coil, allowing the acquisition of 2-D proton-carbon data. Note that MAS probes can be used for ordinary solution work and though very labour-intensive to use, can give excellent sensitivity where the available compound is limited and signal to noise is at a premium.

10.5 Other 2-D Techniques

10.5.1 INADEQUATE

High on any NMR spectrocopist's wish list would be a technique that could be used to establish connectivities directly between carbon atoms. Such a technique does exist and it goes by the name of INADEQUATE (*i*ncredible *n*atural *a*bundance *d*oubl*e* *qua*ntum *t*ransfer *e*xperiment). Whilst this might sound fantastic in theory, everything in the garden is far from rosy.

In order for this to work, it is necessary to have molecules where adjacent carbons are both ^{13}C! Given that only 1.1 % of the entire carbon content of any molecule (assuming no selective enrichment) is ^{13}C, then statistically, you will find adjacent ^{13}C atoms in only one molecule in about 10 000! And this is the real problem with the technique – inadequate sensitivity! Here, we are talking about a method which has sensitivity so low that we would be needing at least 100 mg of material and still need many hours of scanning to get anything like a useable signal to noise.

Research chemists in our experience seldom have this amount of material to play with but even if you are fortunate in this respect, solubility could well be an issue. Dissolving 100 mg of compound in 0.6 ml of solvent is seldom possible.

Practical constraints prevent this technique from living up to its potential, even in this, the era of the superconducting probe. Until sensitivity improves by at least another order of magnitude, the INADEQUATE experiment will remain just that – inadequate by name and by nature.

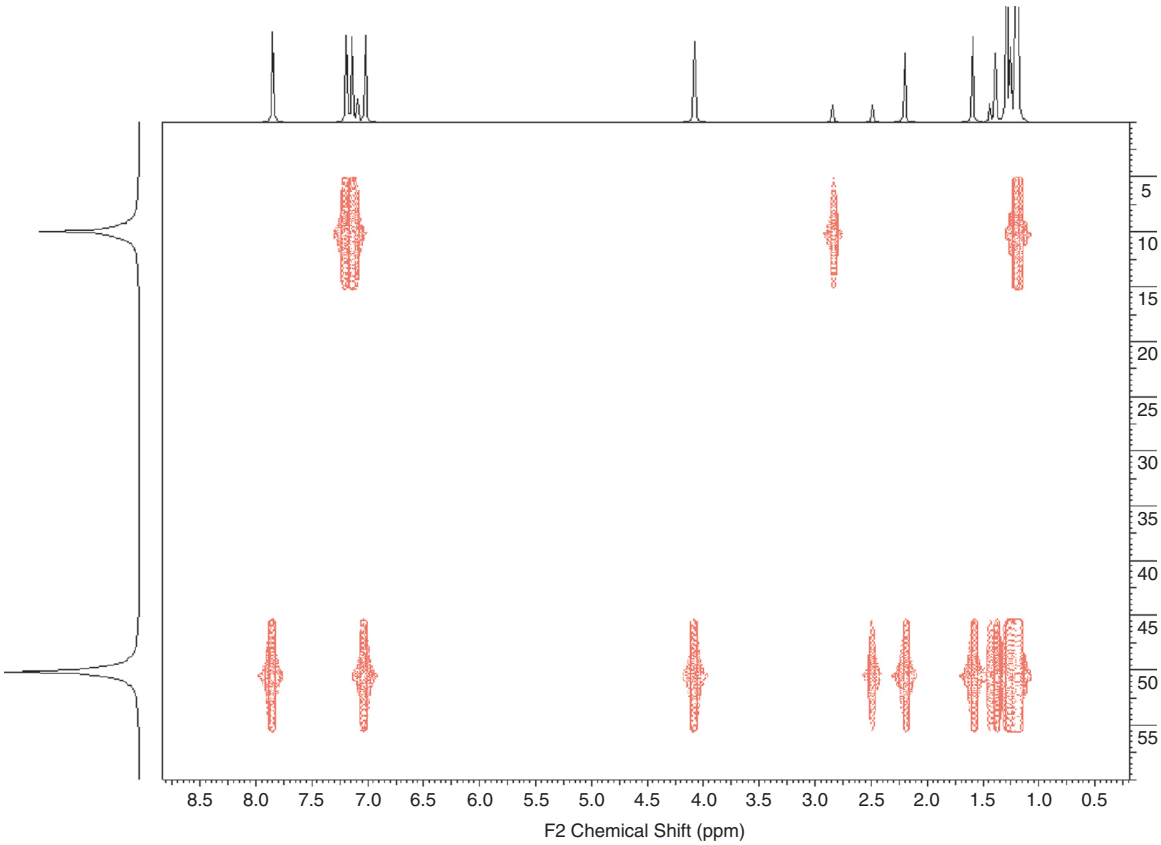
10.5.2 J-Resolved

Another useful-sounding technique is the proton *J*-resolved experiment in which chemical shift and coupling information are separated into two different dimensions. This is equivalent to turning the peaks sideways and looking down on them from above so that viewing them in the *x* direction, they all appear as singlets, but in the *y* direction, they reveal their multiplicities.

This would be very useful indeed, particularly where overlapping multiplets are concerned. Unfortunately, in the very circumstances where the technique would be most useful, it tends to fall over with strong artefacts becoming intrusive in strongly coupled systems.

10.5.3 Diffusion Ordered Spectroscopy

Most of the approaches we have seen rely on manipulations of nuclear spins. Diffusion ordered spectroscopy (DOSY) is a little different in that it is based on properties of the whole molecule. In this case, what we are measuring is the diffusion rate of a molecule. Normally this is used for mixtures so they can be resolved in the NMR tube. The technique works by using field gradients to make the sample experience a different field in different parts of the tube. If a molecule moves during the acquisition process, it will experience a different field. The more it moves, the more different the field it experiences. This has the effect of decreasing the intensity of the signals (the more they move, the more they are attenuated). If we change the strength of the gradient for each 2-D increment, acquire the data and FT the result, we end up with a typical 2-D plot where the chemical shift of the signals is shown on the



Spectrum 10.1 Simulated DOSY spectrum of two compounds.

x-axis and the diffusion rate on the *y*-axis (Spectrum 10.1). Obviously, because the diffusion rate is a whole molecule property, you see all the signals for the same molecule on the same horizontal line.

Fine? No need for chromatography then? Well, unfortunately it is not quite as easy as that. Whilst the experiment has improved over the years, it still struggles to resolve compounds of a similar size and mobility. This means that your mixture of regioisomers will probably not resolve using DOSY. That said, some recent work on using micelles and shift reagents looks promising to improve the technique further and it may figure more prominently in the future.

10.6 3-D Techniques

If 2-D NMR techniques are really useful then 3-D ones must be even more so . . . shouldn't they? A number of 3-D experiments have been devised which are in fact, produced by merging two, 2-D experiments together. The results could never be plotted in true 3-D format since etching them into an A3-sized block of glass would not be practical and viewing them as some sort of holographic projection, would probably not be feasible! In essence, 3-D spectra have to be viewed as 'slices through the block' which effectively yield a series of 2-D experiments. It is possible to combine techniques to yield experiments such as the HMQC-COSY and the HSQC-TOCSY.

Of course, what works well on a 10 % solution of ethyl benzene in 5 h may not be so good when you're confronted with an impure 1 mg of dubious origin! These techniques may well be useful in specialised circumstances but are probably outside the realm of what a practicing organic chemist would want to get involved with. They are, however, very useful in protein NMR.

We have tried to point you in the direction of the experiments that we have come to use and rely on, with good reason. There are dozens more out there that have been developed; some have evolved and are now generally known by another name (e.g., the ROESY experiment used to be known as CAMELSPIN) and some have been superseded and fallen by the wayside. If you have the instrument time and the inclination, by all means play but if time is of the essence, as it usually is, stick with the safe options.

11

Some of the Other Nuclei

As we have already pointed out in the section dealing with heteronuclear coupling that it is not always necessary to confirm the presence of a particular hetero atom by acquiring the NMR spectrum of that nucleus. More often than not, the hetero atom will have a clear signature in the proton or carbon spectrum. Fluorine and phosphorus are both examples of nuclei that couple to protons over two, three, four and even more bonds.

If you have suitable hardware, any of the 60+ NMR-sensitive nuclei can be observed, though some are more suitable than others and in terms of organic synthesis, many are largely irrelevant. The most suitable nuclei for observation have three characteristics in common – high natural abundance (ensuring good sensitivity), a spin quantum number of $^1/_2$ (ensuring that an uncoupled signal will appear as a singlet) and no quadrupole (ensuring that line shape will be naturally sharp). Both fluorine and phosphorus tick all three boxes, making them two of the more important 'also rans.'

11.1 Fluorine

There are cases where coupling from the hetero atom to neighbouring protons is not observed for some reason. Consider the examples in Structure 11.1.

In terms of proton NMR, these two compounds would give remarkably similar spectra. The solitary fluorine on the 1,4 di-substituted ring would certainly couple to the protons both ortho- and meta- to it but the fluorines of the -CF_3 group would not show any discernable coupling to the ring protons and neither would the fluorines of the fully substituted ring. Yes, you could certainly discriminate between the two by ^{13}C NMR but if you only had a mg. of each, you would be really struggling for sufficient signal/noise to observe the key carbons – even if you had a top of the range spectrometer at your disposal. By ^{19}F NMR, however, the distinction would be easily made.

There are a number of features regarding ^{19}F NMR that are worthy of special note. Firstly, spectra may be acquired that are either proton-decoupled, or un-decoupled. We recommend acquiring both if you can. A comparison between the two can yield valuable information regarding neighbouring protons and other fluorines. Secondly, the range over which fluorine resonances occur is very large indeed and a sweep width of about 400 ppm (–300 to +100 relative to $CFCl_3$) is required to capture all the organofluorine resonances that can be expected. When such a large sweep width is plotted on a single sheet, it effectively

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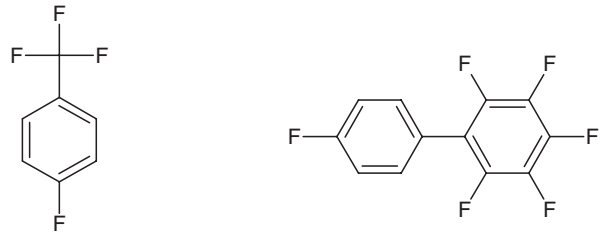


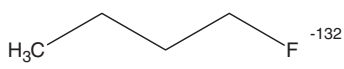
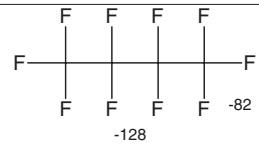
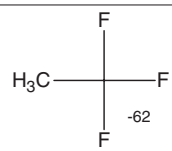
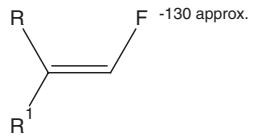
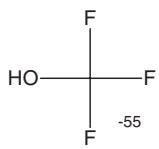
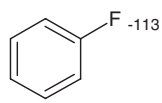
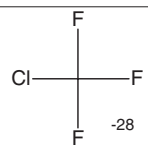
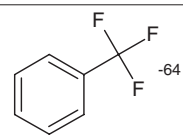
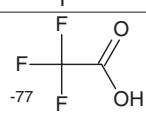
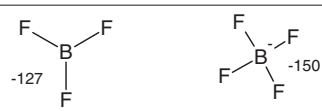
Structure 11.1 Fluorinated compounds.

compresses any couplings, making them almost unnoticeable. For this reason, expansion of peaks is recommended in coupled spectra.

Another consequence of the large sweep width needed for ^{19}F acquisition is that the electronics of the instrument are pushed to the limit: it is difficult to generate uniform r.f. irradiation over such a large frequency range and for this reason it may be necessary to acquire spectra in different spectral ranges, depending on the expected fluorine environment. This is particularly so in the case of high-field (>400 MHz) spectrometers.

There seems to be no universal reference standard in ^{19}F NMR as there is in proton NMR and this can cause confusion. Chemical shifts may be quoted relative to $CFCl_3$ or to CF_3COOH and there maybe a

Table 11.1 ^{19}F chemical shifts of some typical fluorinated compounds (relative to $CFCl_3 = 0$ ppm).

	
	
	 Note - aryl fluorines in range -100 - 190 ppm according to substituents
	
	

few other standards in use as well for all we know. If you are following some literature data, always check which reference standard was used. In Table 11.1, we try to give a brief overview of ^{19}F chemical shifts in some of the more commonly encountered fluorine environments. The tables we quote are relative to CF_3Cl where the chemical shift of the fluorine is set at 0 ppm. Note that all shifts have negative values when using this standard.

11.2 Phosphorus

^{31}P is another nucleus which can be useful to the organic chemist. Many of the comments we have made about ^{19}F are also relevant to ^{31}P. Once again, compounds containing phosphorus are likely to show a clear ^{31}P 'signature' in their proton and carbon spectra and once again the chemical shift range for this nucleus is extremely large – as you might imagine, given the variable oxidation state of the element – typically from –200 to +230 ppm.

The reference of choice for this nucleus is either H_3PO_4 or $(CH_3O)_3PO$. When H_3PO_4 is chosen, it is generally used as an external reference (80–85 % solution) on account of its high acidity. The number and variety of possible organophosphorus compounds combined with the large chemical shift range over which the nucleus resonates make the inclusion of any useful NMR data somewhat problematic. We would refer you to more specialised texts or articles and also to some useful web sites offering some enlightenment on the subject.

11.3 Nitrogen

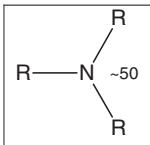
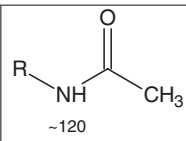
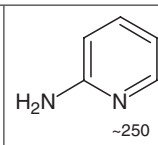
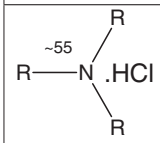
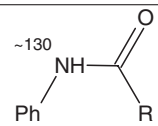
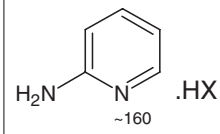
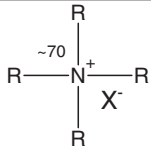
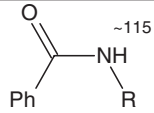
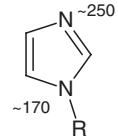
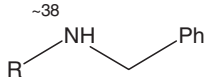
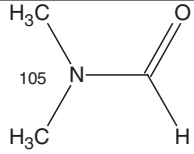
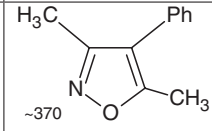
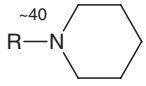
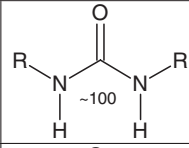
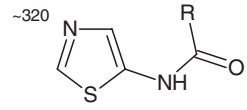
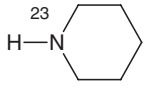
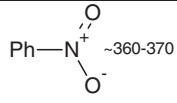
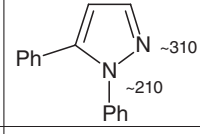
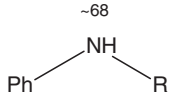
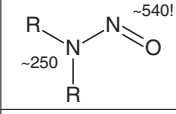
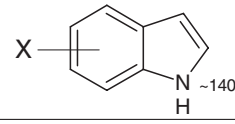
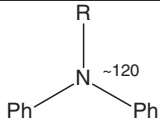
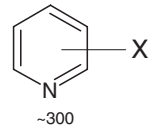
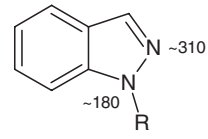
The ^{15}N nucleus is potentially extremely useful, particularly when dealing with nitrogen-rich heterocycles but as its natural abundance is so low (0.36 %) it is a nucleus that you would never chose to observe directly without a very good reason! It can really come into its own via the proton–nitrogen HMBC experiment that we have already mentioned. As the number of nitrogen atoms in a small organic molecule is likely to be relatively low – seldom more than half a dozen – in a sense, the ability to accurately predict nitrogen chemical shifts is perhaps a little less pivotal than you might think. Observed ^{1}H–^{15}N correlations should define which nitrogen is which in an unknown compound. The real power of the technique lies in using it to establish connectivities in unknown compounds to settle stereochemical issues. Nevertheless, we have included some useful chemical shift information in Table 11.2

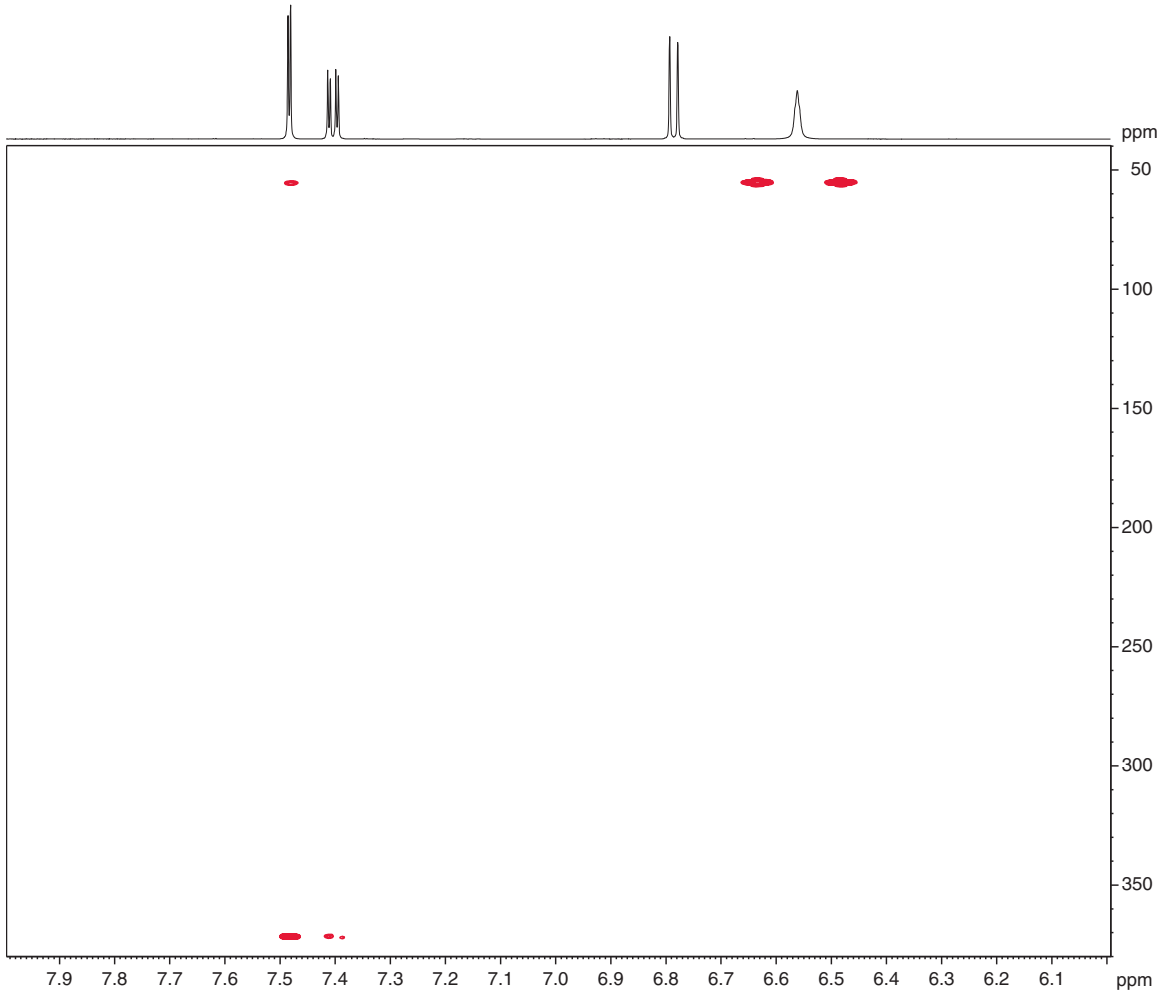
Spectrum 11.1, which was recorded on a 600 MHz instrument fitted with a cryoprobe, shows an example of a ^{15}N HMBC. The compound in question is of the type shown in Structure 11.2.

The chemical shift of the amine nitrogen is 55 ppm and shows a clear 3-bond correlation to the aromatic proton giving a fine doublet at 7.49 ppm. There is also a strong, and in this case, very useful, 1-bond correlation to this nitrogen from the amine proton itself. Note that whether or not you see 1-bond correlations depends largely on how broad the -NH signal is in the proton domain. The sharper the -NH, the more likely you are to see them. As with ^{13}C HMBC, 2-bond correlations can sometimes be quite weak and that is so in this case as there is no obvious correlation to be seen from the methylene protons adjacent to the amine.

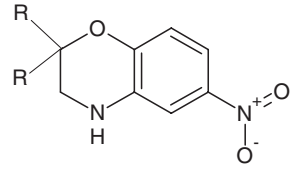
The nitrogen of the nitro group absorbs at 371 ppm and shows 3-bond correlations from both the aromatic protons flanking the group (7.49 and 7.41 ppm). The common correlation from the former signal to both nitrogens confirms the regiochemistry of the structure.

Table 11.2 ^{15}N chemical shifts of some common nitrogen-bearing compounds (relative to $NH_3 = 0$ ppm).



Spectrum 11.1 ^{15}N HMBC.



Structure 11.2 Example of a molecule examined by ^{15}N HMBC.

12

Quantification

12.1 Introduction

NMR offers us a great tool for quantification. This is because it offers a uniform response to the nucleus of interest (see caveats at the end of this chapter). We rely on this when we look at integrals in a proton NMR spectrum – a methyl group integrates for three protons, a methylene integrates for two protons, etc. As NMR spectroscopists, we get a little blasé about this – we just expect it. This is not true for all techniques though. For example, ultraviolet (UV) detection is often used on HPLC systems but its response depends on the degree of conjugation in the compound of interest. If we were to have a chromatogram with two different compounds in it, we would not be able to tell what their relative proportions were unless we knew their UV response at the wavelength (or wavelengths) being monitored. In NMR, this is not the case.

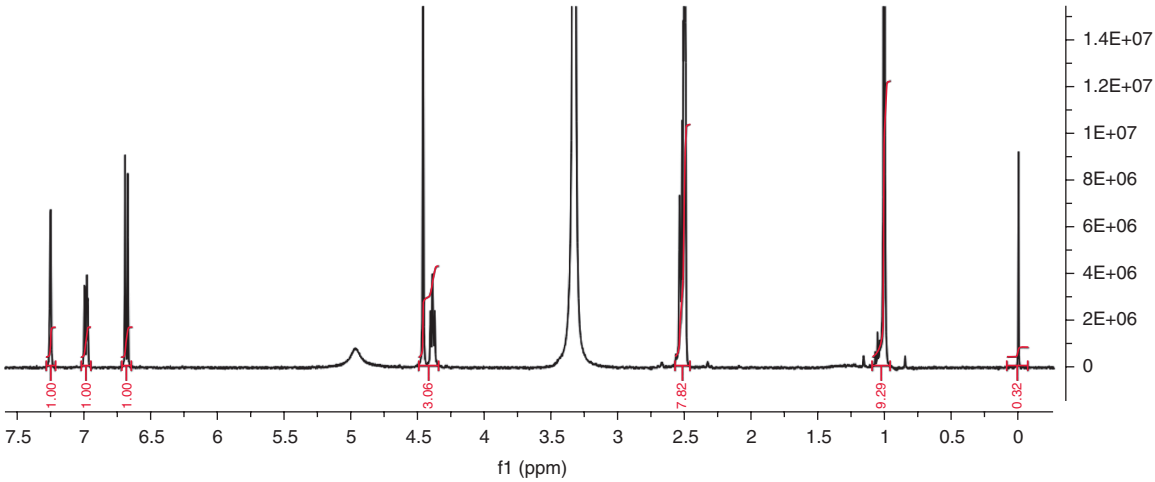
12.2 Relative Quantification

This is the easiest case for NMR (and other analytical techniques). What we are looking for is the relative proportion of compounds in a mixture. To do this, we identify a signal in one compound and a signal in the other. We then normalise these signals for the number of protons that they represent and perform a simple ratio calculation. This gives us the *molar* ratio of the two compounds. If we know the structure (or the molecular weight) of these compounds, then we can calculate their *mass* ratio.

Spectrum 12.2 shows a spectrum of salbutamol in D_6-DMSO with some TMS in it. As an exercise, we can easily quantify the TMS as follows. . .

The signal for TMS (0 ppm) is for 12 protons. The signals in the aromatic region are from salbutamol and represent one proton each. If we set the integral of the aromatic protons to equal 1.0 and assuming adequate relaxation time for the relevant protons of both salbutamol and TMS, then we see that the relative integral of the TMS is 0.32. Because this signal is for 12 protons we can calculate that we have $(0.32/12) \times 100 = 2.6$ mol% of TMS in the sample. The molecular weight of salbutamol is 239 and the molecular weight of TMS is 88 so their weight ratio is 0.36 which means that the weight ratio of TMS is $2.6 \times 0.36 = 0.96$ % w/w.

Essential Practical NMR for Organic Chemistry S. A. Richards and J. C. Hollerton
 ISBN: 978-0-470-71092-0



Spectrum 12.1 Salbutamol with TMS.

12.3 Absolute Quantification

The example shown before is fine if all you want to know is the relative proportions of compounds in your solution. If you know the *absolute* concentration of one of the components, then you can work out the absolute concentration of the other as a result.

12.3.1 Internal Standards

If we add a known amount of a compound to our solution, we can use it to quantify the material of interest. This is great except that we may not want to contaminate our material with some other compound. A number of people have looked at using standards that are volatile so that they can be got rid of later (TMS is an example that we have seen published). The problem with this approach is that if the sample is volatile then you need to run it quickly before it disappears. TMS disappears really quickly from DMSO so it is probably not a good idea in this case. TMS also suffers from the fact that it has a long relaxation time so you have to be very careful with your experiment to ensure that you do not saturate the signal. The last major problem with TMS is that it comes at the same part of the spectrum as silicon grease which can be present in samples. Choosing a standard so that it has a short relaxation time, is volatile and comes in a part of the spectrum free of interference is really tricky. In fact, we wouldn't recommend it at all.

12.3.2 External Standards

So how do we quantify if we don't have an internal standard? One way is to use an external standard. This is done by inserting a capillary containing the standard into the NMR tube (Figure 12.1).

Of course, we still have the problem of selecting a compound that doesn't interfere with the spectrum and that has a suitable relaxation time but we don't need to worry about its volatility. What would be really good is a standard that doesn't interfere with the sample at all. Something that has no relaxation

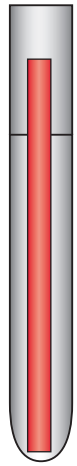


Figure 12.1 External standard placed in the NMR tube.

time to worry about and something that you could put in the spectrum in an area where there were definitely no signals.

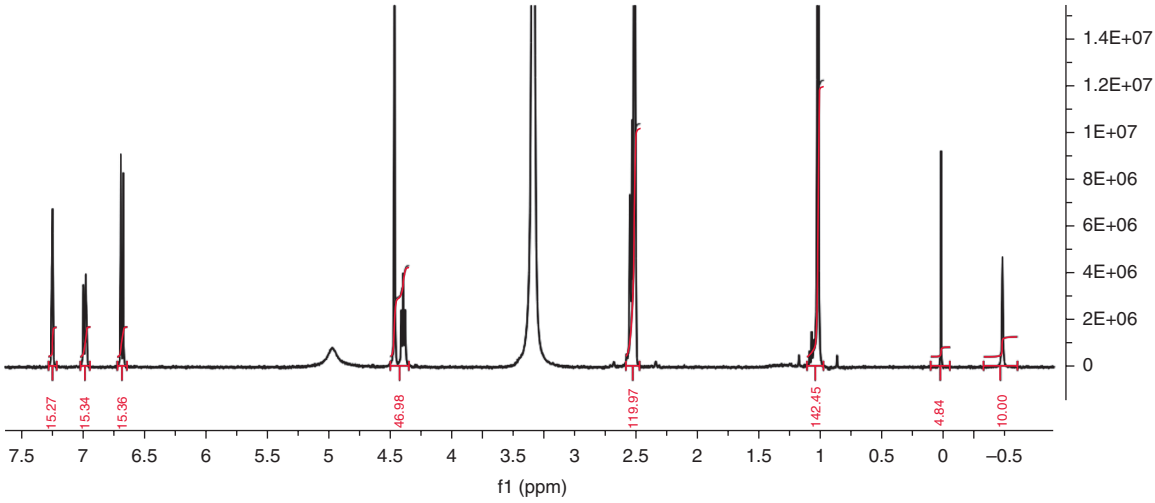
12.3.3 Electronic Reference

This problem was eventually solved in the magnetic resonance imaging world where they needed to be able to quantify things *in vivo*. The result was the use of an extra radio-frequency source during acquisition. It was called 'ERETIC' (***e***lectronic ***re***ference ***t***o access ***i****n vivo* ***c***oncentrations) and has been used extensively in recent years in the high-resolution liquid NMR areas. The great advantage of this approach is that you can make the signal as big or as small as you want and put it anywhere in the spectrum (–1.0 ppm is a favourite place). The way that you use it is to quantify the ERETIC signal against a sample of known concentration. Once this has been done, you can then insert the signal into your unknown concentration spectrum and integrate it against one of the signals in your compound.

There are some problems with the ERETIC approach. Firstly, it does not respond in the same way as the signals in your sample so if your probe tuning is not quite right, you will get an inaccurate answer. Secondly, it requires rewiring of your system so that you can introduce the signal (alternatively, you can rely on cross-talk in the system to let the signal bleed through – this too has some problems associated with it). Lastly, because the signal is generated in a different manner from those of the sample, it can suffer from phase-errors which give rise to inaccuracy when integrating the signal.

12.3.4 QUANTAS Technique

Given that the ERETIC approach has problems, why not introduce a defined intensity signal into your spectrum using software? This is the approach adopted in the QUANTAS technique (*quan*ification *t*hrough an *a*rtificial *s*ignal). No, not the Australian airline (which is QANTAS, by the way, short for *Q*ueensland and *N*orthern *T*erritory *A*erial *S*ervice). In this approach, a reference spectrum with a single signal is created using software. This is added to a spectrum of a sample of known concentration and a scaling factor is calculated to make the signal exactly the correct size for the concentration that it is to



Spectrum 12.2 Spectrum with QUANTAS signal.

represent. If this spectrum is added to any sample spectrum, using the calculated scaling factor, it will be able to represent a defined concentration in the unknown concentration spectrum. This is shown in the salbutamol spectrum used before (Spectrum 12.2).

Unlike ERETIC, this approach does not track receiver gain or number of scans (the signal is a fixed intensity). This doesn't cause a problem though – you can choose to run under identical conditions to your reference, or you can compensate for differences in acquisition condition. For example, the signal builds directly proportionally to the number of scans (note, not the square root of the number of scans. It is the signal to noise that builds with the square root of the number of scans). On modern spectrometers, receivers are linear and it is possible to compensate for receiver gain differences linearly too. The current implementation of the QUANTAS method uses a small programme to automatically take into account any changes in receiver gain and number of scans so you just end up with the signal at the correct level.

This approach offers by far the most simple and flexible way of quantifying samples and is even better because it can be run retrospectively on any sample (as long as the spectrometer is performing similarly to when the signal was standardised). It turns out that for most modern spectrometers, the spectrometer is stable over many months or even years.

12.4 Things to Watch Out For

It all seems so simple when you look at this example. Unfortunately this is not necessarily the case. We need to be a little careful about how we acquire the data if we are going to use it for quantification.

The first thing to look out for is the relaxation time (T_1) of the protons that you are going to measure. In order to get an accurate integral, the protons must return to their rest state each time before you pulse them. The recommendation for a 90° pulse is to wait for 3–5 × T_1. Obviously this assumes that you know the T_1 of all of your protons. It is possible to measure them (and this is indeed the 'right' thing to do) but you need to decide how accurate you need the result to be. If you want a fairly accurate result, it is sufficient to 'guesstimate' your T_1 values just by looking at the chemical structure. Small molecules

tend to have long T_1s. Methyl groups tend have longer T_1s than methylenes. Methines may have long T_1s if they are isolated from any other protons. Symmetrical molecules have slightly longer T_1s than unsymmetrical molecules. If you use a 30° pulse (which is more normal) then you can probably get away with using a relaxation delay of about 5 s if your acquisition time is about 3 s (hence a total recycle time of about 8 s).

On older spectrometers, it is important that the signal that you are measuring is not at the edge of the spectrum. This is because older spectrometers used hardware frequency filters and these start to decrease signal intensity at the edge of the spectrum. More modern spectrometers use digital filters that are capable of very sharp cutoffs that will not affect the intensity at the edge of the spectrum. Be warned, even here you may get problems with distortions in the baseline at the edge of the spectrum (so-called 'smileys'). In general, try to avoid your signal of interest being at the edge of the spectrum.

All quantification relies on being able to standardise against a known concentration standard. This is not a trivial thing to do as it requires an accurately weighed amount of a known purity compound, made up accurately to a precise volume. If your standard is wrong, all your measurements will be wrong so it is worth spending some time getting it right!

Ultimately, you will be measuring and comparing integrals so you need to be very careful about how you get these. Your signals of interest must be perfectly phased, clear of other signals and on a good baseline. The integrals must also have good slope and bias (which they should do if everything else is correct). Any problems with any of these variables will seriously degrade the accuracy of your result. In our experience, the biggest single error with any NMR quantification approach is the error in measuring the integral.

12.5 Conclusion

If you do manage to get everything right, NMR offers excellent quantification results. What's more is that it is free if you have acquired a 1-D spectrum. Note that you can use this approach to quantify other nuclei – it works just as well for ^{19}F. Note that it won't work very well for ^{13}C because we normally acquire ^{13}C data with NOE enhancement from the protons so the signals are not quantitative. (It is possible to collect carbon data in a quantitative way but it is not something that we would normally do. . .).

13

Safety

NMR systems are pretty safe if treated correctly but this short chapter outlines some of the things you may need to think about when using them. Note that we are not pretending to offer a full safety assessment but this should alert you to the major hazards associated with modern NMR systems. There are very good documents available from the major NMR manufacturers which cover this area in considerable detail.

NMR-associated risks fall into three categories: (1) magnetic fields, (2) cryogens and (3) sample-related risks. If you're picky, then you could also add things like risk from electrical shock, etc., but I'm sure that you're not!

13.1 Magnetic Fields

It might seem obvious but magnetic fields can attract magnetic materials towards them. It is something that we are all used to but most people haven't experienced the strength of field from an NMR magnet. Modern magnets are often shielded but they still have quite strong external fields, especially at the base of the magnet. Because the field is invisible it is easy to forget that it is there. We know of a case where a photographer forgot about the field and moved his tripod closer to the magnet to get a better shot. The next thing he knew, his tripod flew across the room and smashed into the magnet, damaging it in the process. It took nine months to get the magnet back to field and a lot longer to live down the embarrassment. Had someone been between the tripod and the magnet it could have been even more serious. There is also a story (maybe an urban myth?) about someone who had a metal ruler in their lab coat pocket whilst he did some work at the base of the magnet. The ruler flew out and sliced the end of his nose off.

As well as the obvious risks, there can be less obvious risks too. Heart pacemakers can be disrupted by strong magnetic fields so this needs to be pointed out to anyone who enters the area in case they are reliant on one. Another risk is for people who have certain metal prosthetics (e.g., hip joints) – you wouldn't want them stuck to the side of the magnet, would you? Another example that we have had is with metal breathing apparatus – someone was pulled back to the magnet when wearing it during a fire drill.

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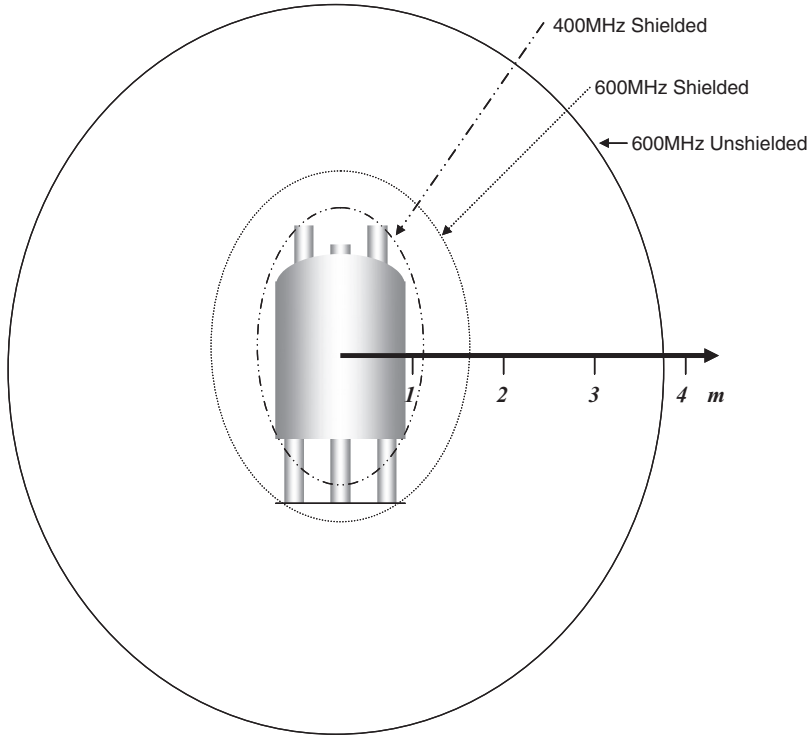

Figure 13.1 The 5 Gauss line for a range of magnets.

There can be other untoward effects which may not be exactly safety related but are inconvenient to say the least. Magnetic strips on credit cards can be erased, hard (and floppy) disks can become corrupted (watch your iPod near a magnet) and electrical equipment with relays can be affected. CRT monitors are particularly sensitive to stray magnetic fields and you end up with an artistic, if useless, monitor if it is too close to the magnet. Additionally, strong fields may saturate transformers so that they don't behave as expected – probably not a good thing if you are expecting a particular voltage out of them. Finally, analogue watches fare badly in strong magnetic fields. If you are lucky, the watch may just lose time whilst it is close to the magnet. If you are unlucky, it may stop and refuse to start again. As a rule of thumb, don't get close to the magnet when wearing a watch unless it doesn't have hands.

So how close is too close? Well it depends on the magnetic field, whether it is shielded or unshielded and whether it is a wide bore or normal magnet. The magnet manufacturer will be able to give you the figures. We measure the safe distance by the stray field at that point. Normally we take the 5 Gauss field line as a safe distance and this is normally marked in some way (tape on the floor or a physical barrier). For a 400 MHz shielded spectrometer, this distance is about 1.0–1.5 m from the centre of the magnet. The larger the magnet, the further the stray field (generally), although modern shielded magnets may have the 5 Gauss line at the edge of the magnet can. Figure 13.1 shows the 5 Gauss line for a set of magnets.

There are no known biological effects of static magnetic fields and it has been deemed that they do not cause problems to people working with them. This is always under review and there may be moves to limit time spent in strong magnetic fields at some time in the future.

13.2 Cryogens

Because the magnet is essentially a Dewar containing liquid nitrogen and liquid helium, the biggest risk is from those cryogens. The first risk is from the low temperatures of these liquids (liquid helium boils at about –270°C) which can cause serious burns. The second risk is of asphyxiation as the cryogens boil off.

The risk of burns is normally only experienced when filling a magnet with nitrogen or helium. You need to be protected in case the liquid spills or the transfer line breaks. Protection just means covering up any exposed skin (lab coat, visor and thick gloves are normally sufficient). At all other times, the cryogens are safely in their cans and should stay there unless something catastrophic happens.

Asphyxiation is another invisible hazard. If the oxygen level in a room decreases below a certain level you may become unconscious and die. In general, big laboratories are better than small labs because the natural volume of the room will help to dilute these effects. Likewise, an efficiently air conditioned room will change the air in the room fairly rapidly and this will also help keep oxygen levels up. If your NMR magnet is in a small room, it may be necessary to install oxygen depletion sensors. These will alert you should the oxygen level fall below a safe value.

When a magnet is not being filled, it will give off a steady stream of nitrogen and helium. The helium will normally sit near the ceiling whereas the nitrogen will tend to permeate the whole volume of the room. When your instrument is installed, a survey should have been carried out to evaluate the risks.

Lastly (on the subject of asphyxiation), when transporting Dewars of cryogens it is important that you and the Dewar are not in a small space together. This includes lifts (elevators). If you need to transport a Dewar up or down floors in a building, you should send the Dewar on its own and prevent people from joining it!

There is a special condition that can arise in an NMR magnet, called a ‘quench.’ This occurs if the magnet coils suddenly cease to be superconducting and all the energy stored within them is released as heat. This causes the helium in the can to boil off very rapidly. There are two major risks from this. The first is obviously asphyxiation; the second is the pressure that is generated by the increased gas volume. To minimise problems from the latter, the room should be constructed so that the gas can escape quickly. The other precaution is to ensure that the doors to the laboratory open outwards, otherwise the gas pressure may make it difficult to open them, trapping the occupants. The asphyxiation risk from a quench is quite low because the helium has a tendency to sit at the ceiling of the room and it also escapes very rapidly from wherever it can. Nonetheless, it is advisable to leave the room if a quench happens. You will know when a system is quenching – it makes a lot of noise and you get clouds forming in the ceiling!

The event that would cause the largest release of cryogens would be a catastrophic failure of the can. This would release the helium and the nitrogen very quickly. Fortunately, this is an unusual event and previously mentioned precautions should still work.

One last hazard with cryogens is that they may lead to local build-up of oxygen through liquefaction of air. When filling a magnet, it is possible to see liquid air condensing at the fill port. If this happens,

there is a risk of causing combustion of oil or other materials that are close to the liquefied air. This risk can be eliminated by keeping sources of combustion away from the magnet and Dewar.

13.3 Sample-Related Injuries

Whilst the potential hazards associated with powerful magnetic fields and cryogens are spectacular, it's the everyday hazards associated with the handling of NMR samples that are most likely to catch out the unwary! Standard 5 mm NMR tubes are very fragile (3 mm even more so!) and the thin-walled glass tube they are made from can cause nasty cuts. Pushing on the plastic tube tops is the most dangerous part of the process as considerable force is sometimes needed. We have found that it is safest to hold the tube in one hand and lay the knuckles of that same hand into the palm of the other which is used to push on the top. Locking your hands together in this way, minimises the chance of injury should the tube shatter, as there will be no danger of sudden violent movement of flesh towards broken glass!

Another source of danger relates to the samples themselves. In a research environment, many of the compounds made are of totally unknown toxicity and so should be handled with extreme caution. NMR solvents are obviously toxic in their own right but when they contain unknown organic compounds in solution, the hazards are far worse. Be warned that all organic solvents commonly used for NMR, can pass through skin and into the bloodstream but DMSO is particularly good at it. If this happens, it will take anything dissolved in it through as well so avoid spilling any solutions on your hands whilst making up or filtering samples. Don't be lulled into a false sense of security by wearing thin rubber gloves – they offer little protection because solvents can penetrate them too!

14

Software

There are many software tools available to help with the acquisition, processing and interpretation of NMR data. Attempts have been made to automate the verification process and even perform full structural elucidations of unknown compounds. As you might guess from the complexity of the interpretation chapters, these software solutions are not foolproof! It remains to be seen whether they ever will be good enough but there have certainly been some major steps forward in all of these areas.

In this chapter, we will look at the different types of software but be warned that software development is quite dynamic and the landscape may be very different when you come to read this section!

14.1 Acquisition Software

You seldom have much choice about this software. When you buy a spectrometer you will get some software from the manufacturer. The big manufacturers are Bruker, Varian and JEOL. Their software is called: Topspin, VNMRJ and Delta respectively. These pieces of software are quite complex as they have to perform all the spectrometer control as well as processing and some simulation. That said, all manufacturers have improved their software to make it more user-friendly in recent times and it is not the challenging beast that it used to be.

14.2 Processing Software

As mentioned above, the manufacturers provide software to process the data. These pieces of software are designed to process data created on that manufacturer's instrument although they can process most data from other vendors (sometimes this is not as easy as it could be). In addition to manufacturers' software, there are also third party software suppliers who offer software capable of processing data from all makes of NMR spectrometer (Figure 14.1). At the time of writing, there are a number of these companies, the most well known of these are probably Advanced Chemistry Development (ACD/Labs; http://www.acdlabs.com) and Mestrelab Research (http://www.mestrec.com). ACD's product for processing is called ACD/Specmanager (there are modules that you can purchase for 1-D or 2-D processing).

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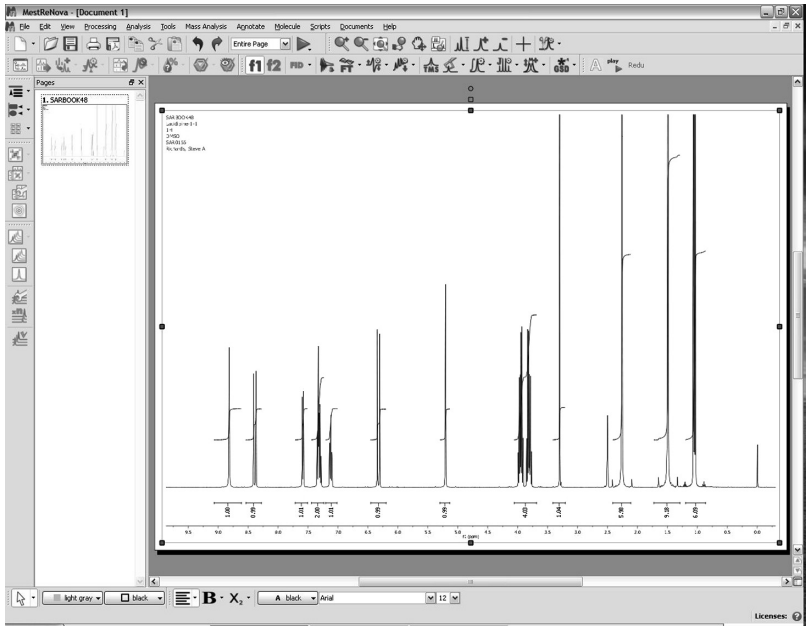


Figure 14.1 Third party processing software.

Mestrelab have a product called MestReNova which performs 1-D and 2-D processing. These products are focused on data processing and so tend to be a little less daunting than the spectrometer software.

There are many other pieces of processing software out there, some good, some bad. If you don't have any and you need some, it is worth having a look around to see what is available. In our experience, you tend to get what you pay for and the more expensive software is generally better. However, one of the cheaper packages may do just what you want, in which case you have a bargain! Table 14.1 lists some of the more well known packages.

Table 14.1 Processing software packages.

Name	Web site	Platform
NUTS	www.acornnmr.com	PC
NMRnotebook	www.nmrtec.com	Mac, PC, Linux
NMRPipe	spin.niddk.nih.gov	Unix, Linux
iNMR	www.inmr.net	Mac
MestreNova	www.mestrec.com	PC, Mac, Linux
ACD/SpecManager	www.acdlabs.com	PC
FELIX	www.felixnmr.com	PC, Linux, Unix

14.3 Prediction and Simulation Software

You will normally have access to the previous two categories of software as a minimum – they will be on the spectrometer itself. One thing that you don't get supplied is software to predict chemical shifts (although you may get some sort of simulation software). The desire to predict the chemical shift of a nucleus has been around since the first time that the chemical shift phenomenon was discovered. There are numerous papers going back to the earliest days of NMR trying to relate structural properties to chemical shift. Early work was concentrated on proton chemical shift prediction (because carbon data was so hard to get) but it was soon realized that the unpredictable nature of proton chemical shifts (their dependency on average solution conformation) made this job difficult. It was easy to get to within about 0.5 ppm of the correct shift but this is not too good when 90 % of your chemical shifts come within a 6 ppm range. Apart from generating additivity tables (as used in this book), proton chemical shift prediction was soon ignored.

14.3.1 ^{13}C Prediction

Once ^{13}C data was more readily available (with the advent of FT spectrometers), interest in chemical shift prediction was reborn. The reasons for this were that carbon spectra don't show carbon–carbon coupling information (unlike proton–proton coupling) and so knowledge of carbon chemical shifts was really important in the assignment of ^{13}C spectra. There were numerous efforts at carbon prediction but perhaps the first truly successful method was created by Wolfgang Bremser in 1977. He realized that you needed to have a standard way of naming and sorting carbon atoms so that you could look them up in a table. He also realized that if you were methodical about this and used a naming system that grew in 'shells' (Figure 14.2) from the atom of interest (atoms closest to the atom of interest come first in the name), you could predict the chemical shift of a similar compound by interpolating between entries in his table. He named this the HOSE code (*h*ierarchically *o*rdered *s*pherical description of *e*nvironment).

Bremser produced tables of these HOSE codes from NMR work that was carried out at BASF in Germany. Most modern carbon prediction routines still use this HOSE code today (albeit slightly modified from the original). Modern software hides all the HOSE code generation in the background so all you do is draw a structure and press the predict button and all the chemical shifts are calculated.

Modern carbon prediction software has hundreds of thousands of chemical structures to call on (Bremser had about 10 000 when he started). The more structures you have, the better the chance that something similar to your structure will be in the database - and the better the quality of the chemical shift

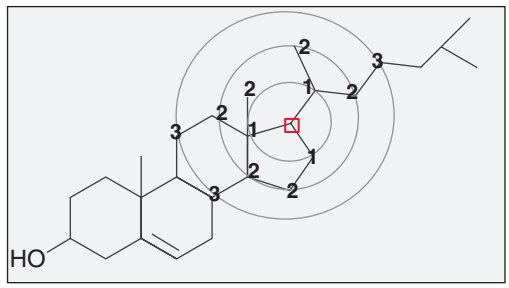


Figure 14.2 HOSE code in operation.

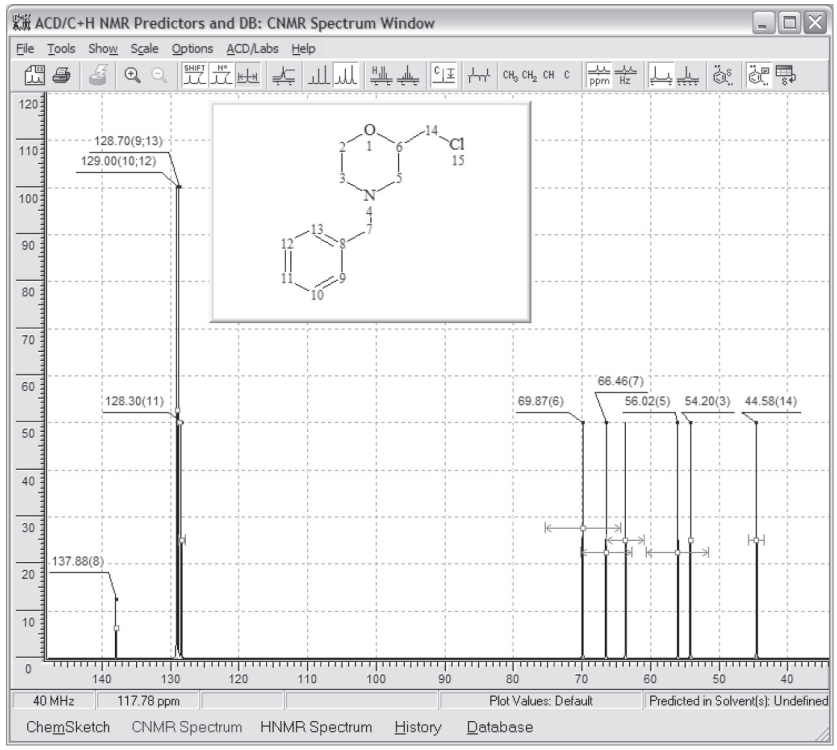


Figure 14.3 Carbon NMR prediction.

prediction. On the other hand, you may have something that has a fragment that just isn't represented in the database – in which case you cannot predict the chemical shift accurately.

There are a number of ^{13}C prediction packages that are commercially available. Once again, ACD/Labs offer a widely used prediction application called ACD/CNMR (Figure 14.3). This is well respected and has a large database with it. Another piece of software with a large database is NMRPredict from Modgraph. This software offers two different methods of ^{13}C prediction: a HOSE code method (with modification, designed by Wolfgang Robien) and a neural network approach (again from Wolfgang Robien). The HOSE code method works very well for well represented fragments but if you have an unusual fragment, the neural network offers better interpolation.

All in all, carbon prediction is really very good. This is partially due to the huge amount of carbon data in the public domain, partially due to the fact that carbon chemical shifts are distributed over 200 ppm (instead of 10 ppm for proton) and partially due to the fact that carbon chemical shift is mainly influenced by contributions through bond rather than through space (hence less dependence on conformation). Nonetheless, you need to be a little discriminating when assessing the chemical shift values that these systems come up with – if you have a carbon atom in an unusual environment and this is not covered in the database, the prediction bears very little weight and you must rely on other information to assign the atom.

14.3.2 ^{1}H Prediction

Despite having been the earliest attempted prediction, proton prediction remains relatively poor. The reasons for this have been alluded to earlier but to summarise; the proton chemical shift is often highly dependant on through-space effects (anisotropy) and has a very small distribution. There are four main commercial approaches to proton prediction currently: Incremental parameters, HOSE code databases, semi-empirical and *ab initio* methods.

14.3.2.1 Incremental Approaches

These are computerised versions of the tables in this book. Chemical shifts are calculated by adding together the contributions from the various functional groups attached to the core of interest. These are normally split into three types: aromatic, aliphatic and olefinic. Probably the best example of this approach is the Upsol predictor that was supplied with the book *Structure Determination of Organic Compounds* by Pretch *et al.* (Springer). This has found its way as an add-in to a few commercial systems and is currently used in the Modgraph NMRPredict package. The advantage of this approach is that calculations are quick and it is very easy to implement (and hence low cost). The disadvantage is that it is not very accurate and becomes progressively less accurate as more substituents are added to a core.

14.3.2.2 HOSE Code Databases

ACD/Labs have an extensive database which uses this approach. This approach works well except for anisotropic groups. Unlike carbon prediction this can have a massive effect on the chemical shift values and so can give rise to big errors in prediction, even for structural fragments that are well represented in the database.

14.3.2.3 Semi-Empirical Approaches

Currently there is only one product that adopts this approach and this is NMRPredict from Modgraph. It is based on the work by Prof. Ray Abraham at the University of Liverpool (UK). This approach calculates chemical shifts for a range of low energy conformers and averages them to give a net chemical shift. This approach seems to offer the most accurate prediction of chemical shift but the disadvantage is that it is very slow (particularly for conformationally flexible molecules).

14.3.2.4 **Ab Initio** *Approaches*

Another way to calculate chemical shifts is to use density functional theory (DFT). This quantum mechanical approach has been shown to predict chemical shifts well in certain cases. The disadvantage with this and semi-empirical approaches is that they rely on modelling the range of low energy conformers of the structure of interest. Not only is this time-consuming, it is also difficult to achieve in conformationally flexible molecules. Due to its slow performance, it is not a tool in regular use in solving structural problems although it has shown its value in specific cases, particularly where databases are not available for the structural feature/nucleus of interest.

14.3.3 Simulation

Spectral simulation is normally provided with proton prediction packages. This takes the predicted chemical shifts and coupling constants and uses them to simulate the appearance of the spectrum. This can be a little misleading as it gives rise to an authentic looking spectrum which may differ considerably in appearance to the experimental one. This is because even small errors in chemical shift or coupling constant prediction may give rise to significant differences in appearance of the signals. Simulation can be useful to try to mimic an observed signal to help calculate the coupling constants when they are not obvious by inspection. Simulation-only software is normally available as part of the NMR acquisition software and may be used to help understand complex splitting patterns observed in real spectra.

14.3.4 Structural Verification Software

Being able to verify a proposed chemical structure from its NMR spectrum automatically has been a goal for many years. This is particularly true recently since chemists have been making arrays of compounds (tens to thousands of compounds). It is possible to acquire data automatically on large numbers of compounds but it is still a major task to interpret all of the data. Verification software performs a prediction and simulation and then tries to fit the experimental data to the calculated data. Obviously this approach requires good prediction as well as good data extraction. As you will have seen in this book, these things are neither trivial nor reliable. The latest approaches use a combination of 1-D proton spectra and 2-D proton–carbon correlated spectra to try to use the strengths of ^{13}C prediction to aid the process. There appears to be some promise with this approach but it still has a way to go before it is truly reliable. The leaders in this area are currently ACD/Labs with a product called 'NMR Expert,' although a number of other companies are getting involved in this.

14.3.5 Structural Elucidation Software

Unlike structural verification software, this software is designed to propose structures that may fit the analytical data. The first requirement is for a molecular formula for the mystery compound. The more data that you have, the better, so you would normally need a proton–carbon correlated spectrum, plus any information that you can glean from the data (so if you can spot an ethyl group, you enter this information into the programme). You build up so-called 'good lists' and 'bad lists' of fragments that you might think are present and the software then calculates all the possible structures that could fit the data that you have entered. It then orders them by similarity between the experimental and calculated spectral data. This approach has been very useful in the area of natural product structural elucidation. Another area where this approach has potential is in spotting other possible structures that may fit the data. Even the best spectroscopists sometime become fixated with a structure that fits the data. This software can suggest other possible structures that are worthy of consideration.

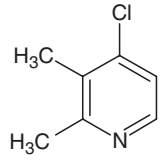
15

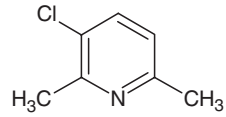
Problems

So there you have it. Our mission to enlighten draws to a close. If you would like to find out if we have been in any way successful, this chapter contains some problems to have a go at. Obviously, real-world problems will normally have other information about them, not just the NMR spectrum. The flow chart in Appendix A.1 gives some indication of useful reminder of the thought processes for real-world problems.

15.1 Ten NMR Problems

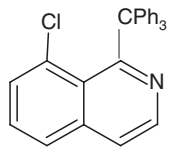
Q1. You are given a sample that is known to be one of the following compounds:

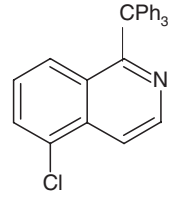




It is not clear whether the compound is a free base, a salt or a partial salt. What would you need to do to be confident beyond reasonable doubt that you could positively identify the compound?

Q2. What key observation might lead you to differentiate the following pair of compounds from nothing more than their proton spectra?





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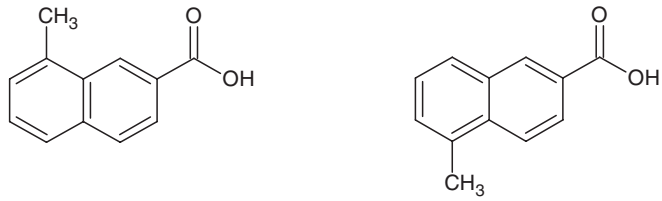
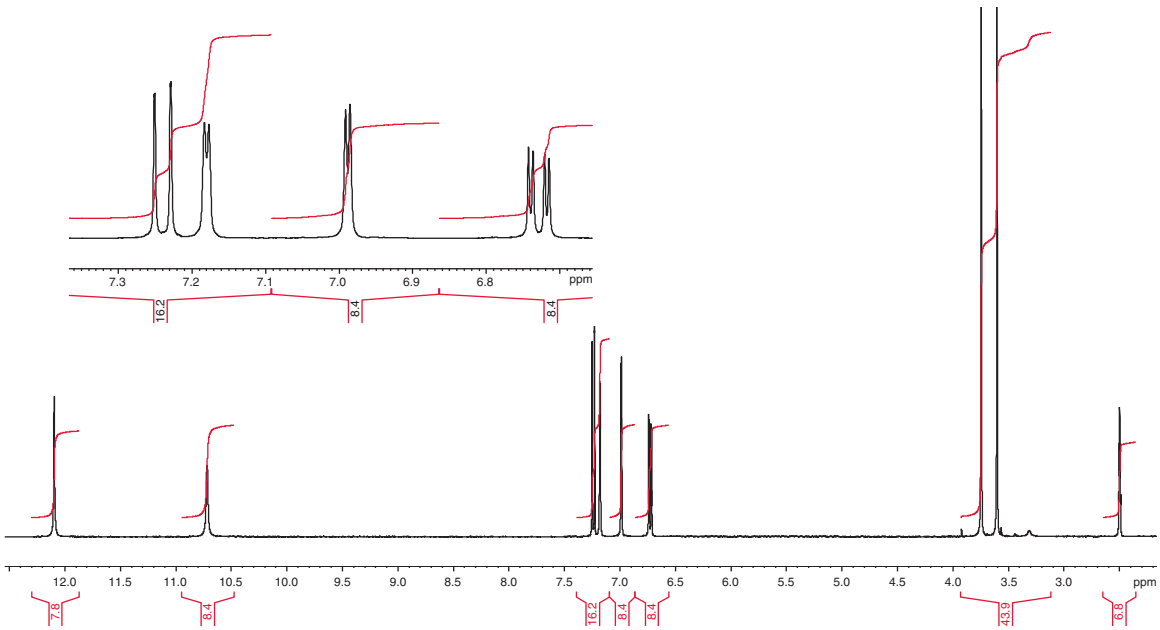
Q3. How would you differentiate this pair of isomers?

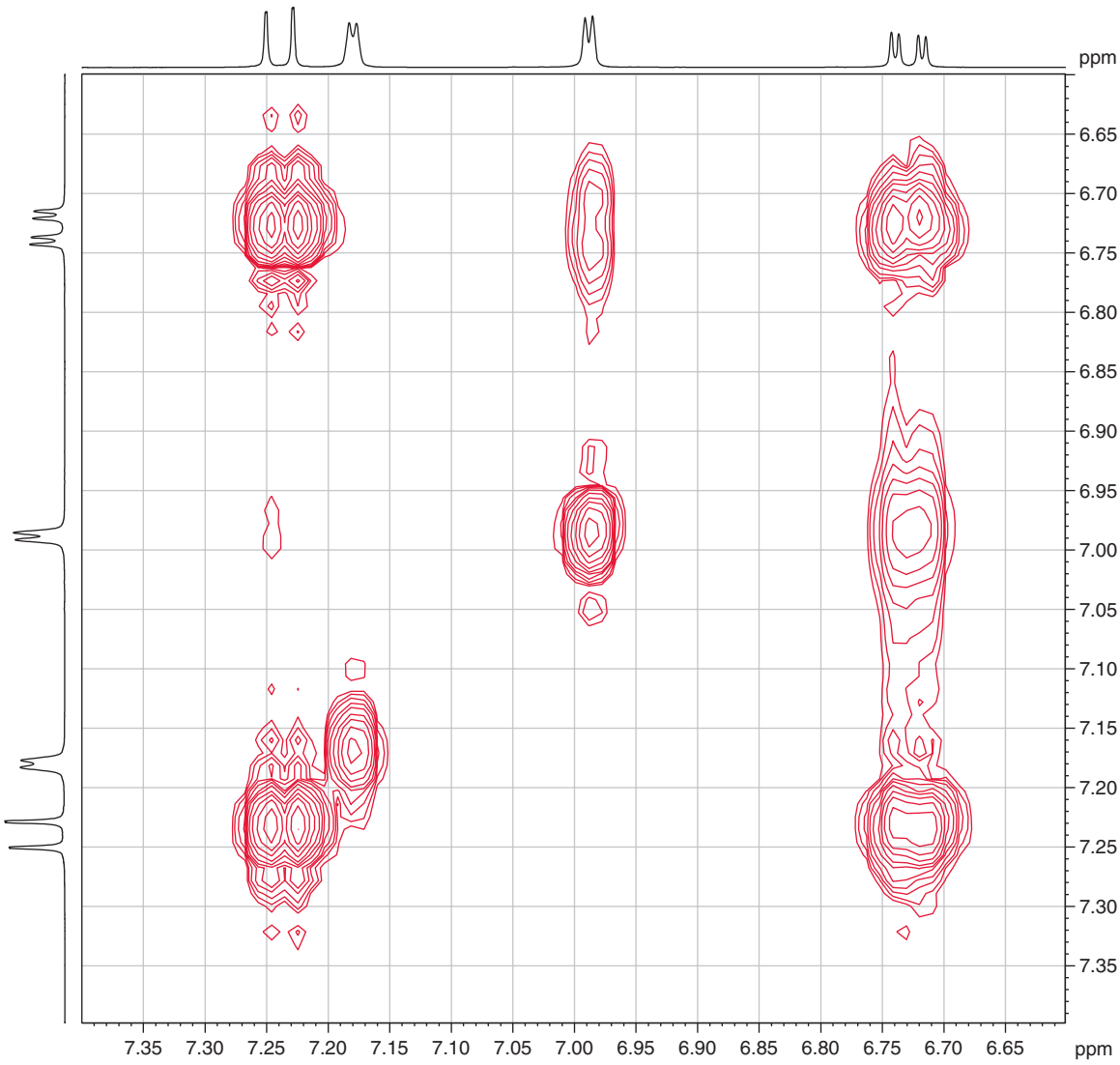


Q4. It's another case of distinguishing two compounds but this time they aren't isomers. (Unfortunately, the mass spec next door is out of action following a sub-optimal 'preventative maintenance' visit from the service engineer and the compounds probably wouldn't ionise anyway!)

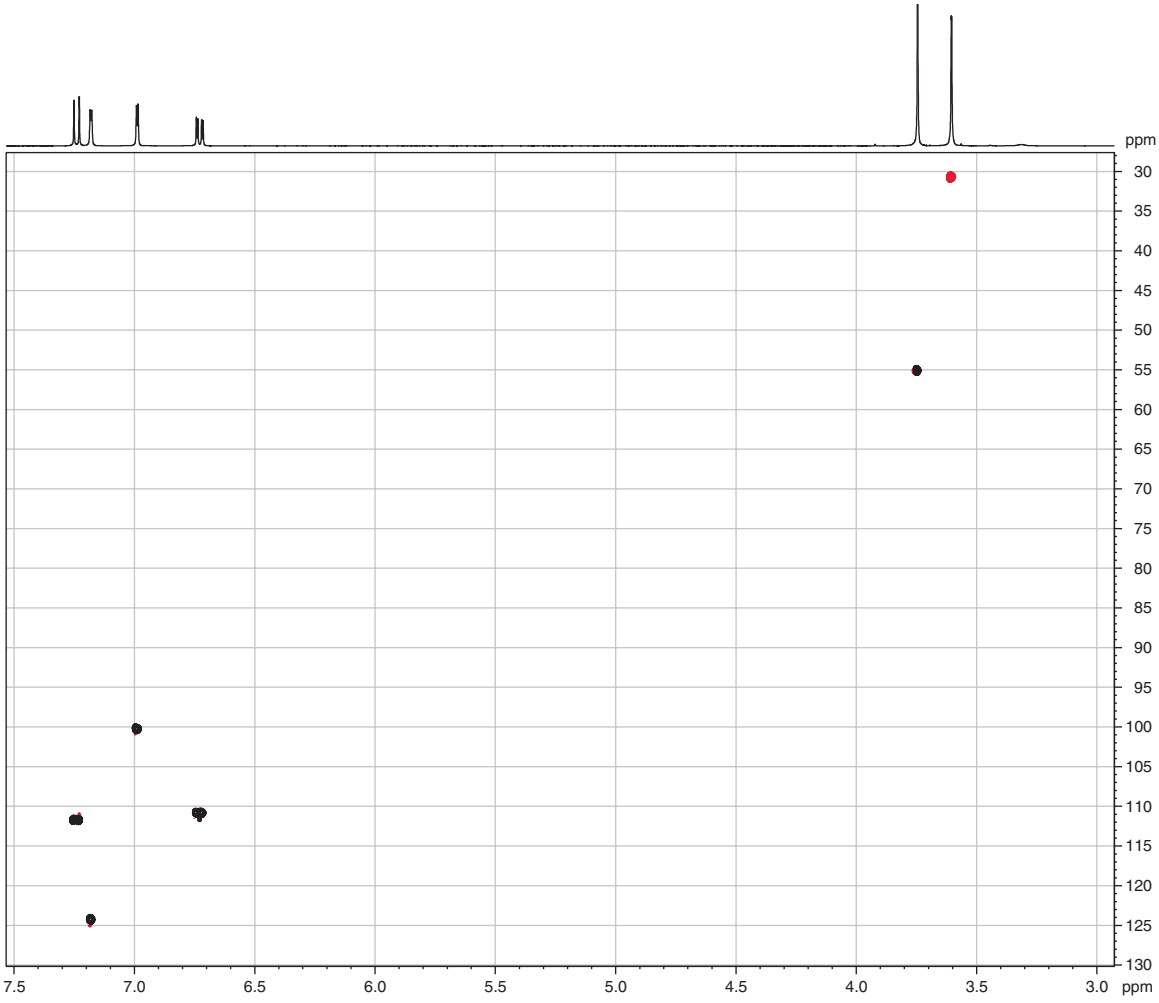
Q5. From Spectra 15.1–15.5, construct a plausible structure for the unknown compound which has a molecular formula: $C_{11}H_{14}NO_3$. (It is known to be an indole and to contain the following fragments: -OCH_3 and -CH_2-COOH.)



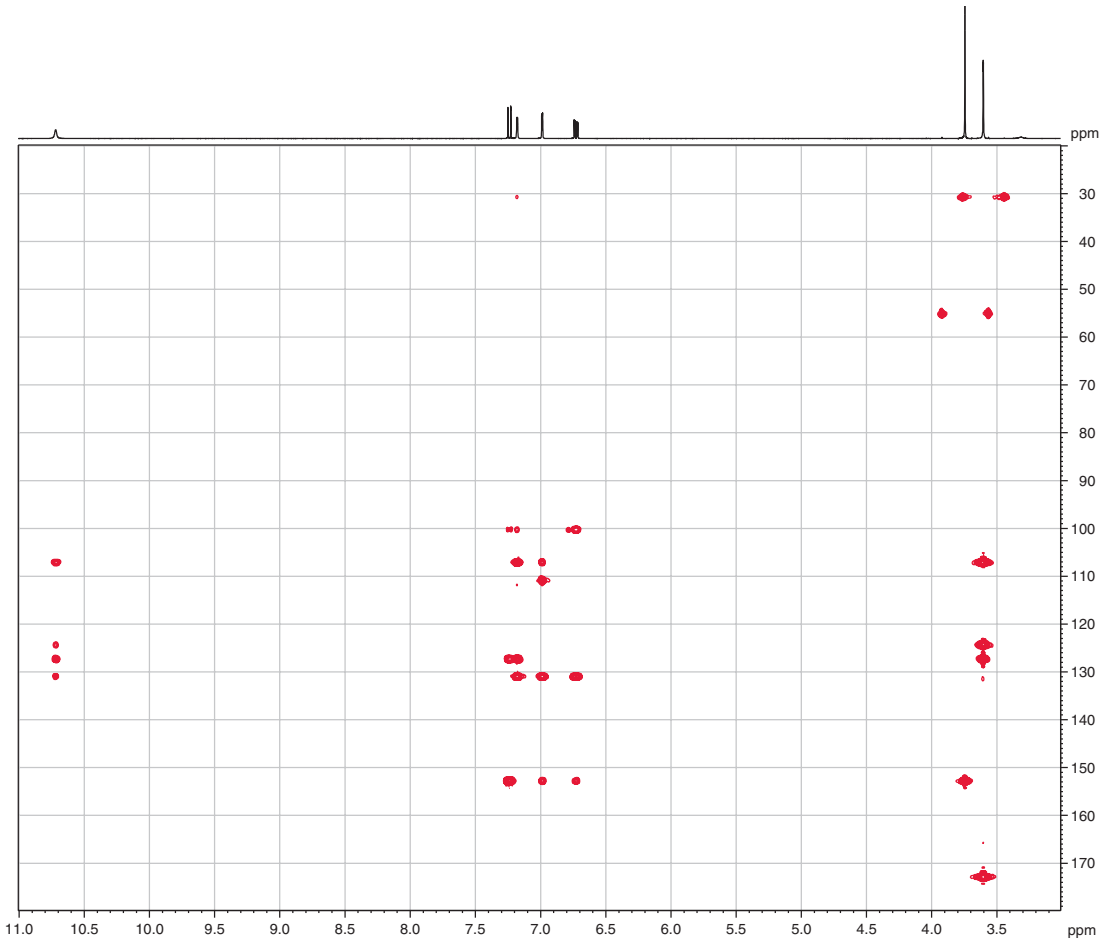
Spectrum 15.1 ^{1}H 1-D.



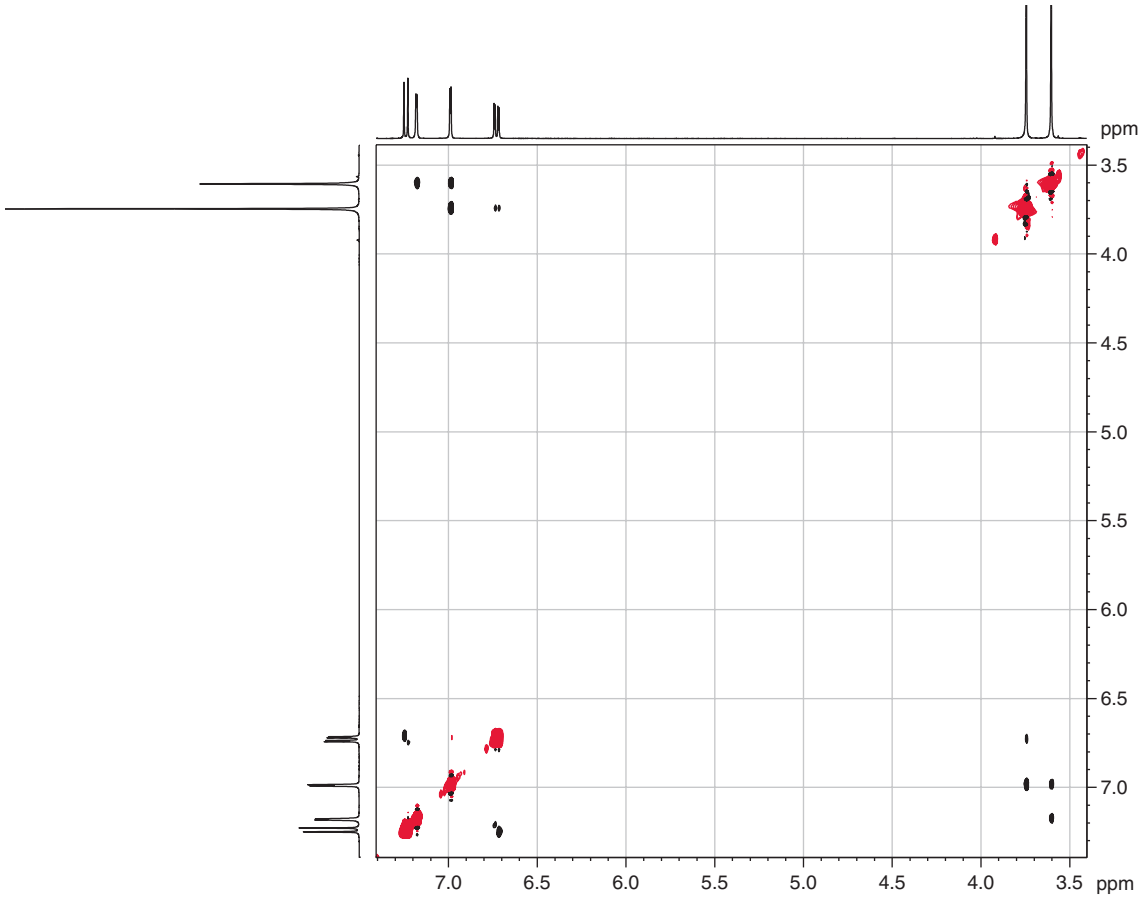
Spectrum 15.2 COSY.



Spectrum 15.3 DEPT-edited HSQC.

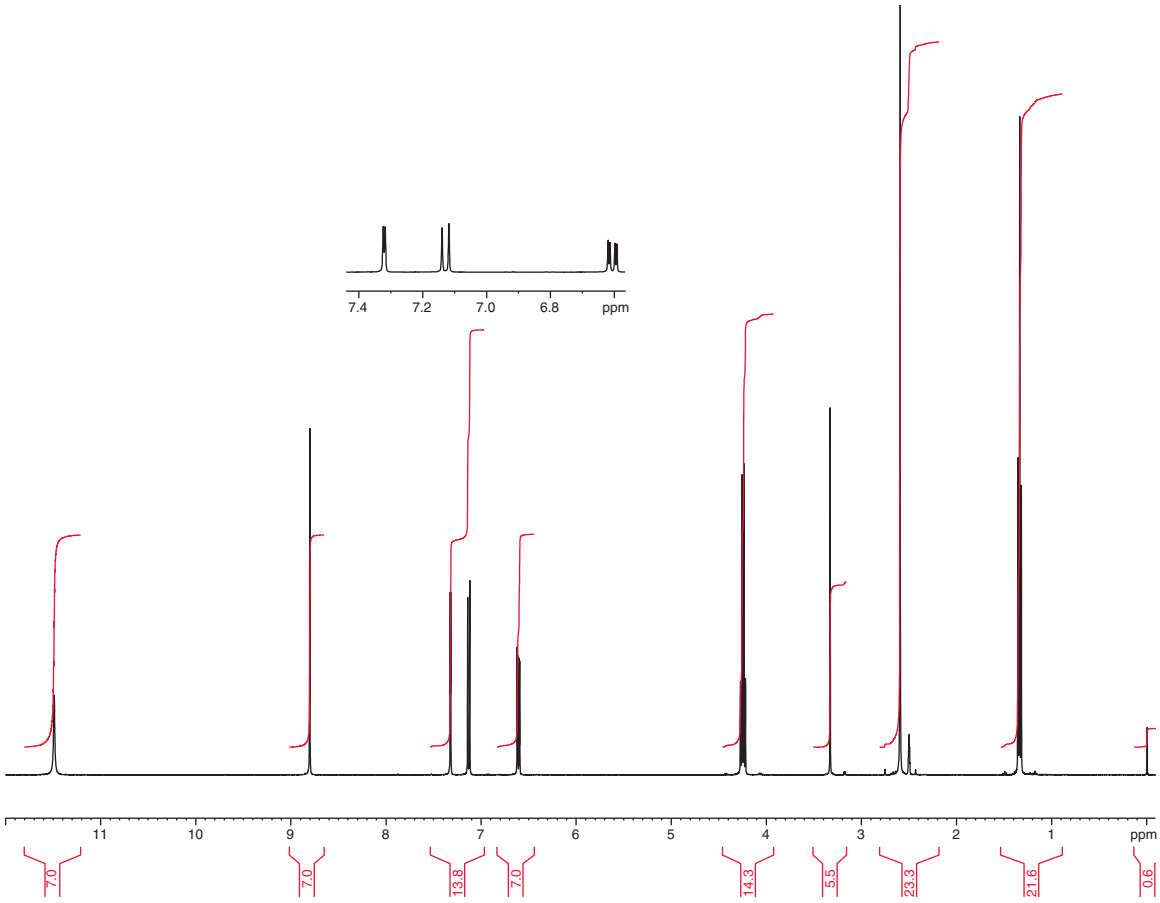


Spectrum 15.4 HMBC.

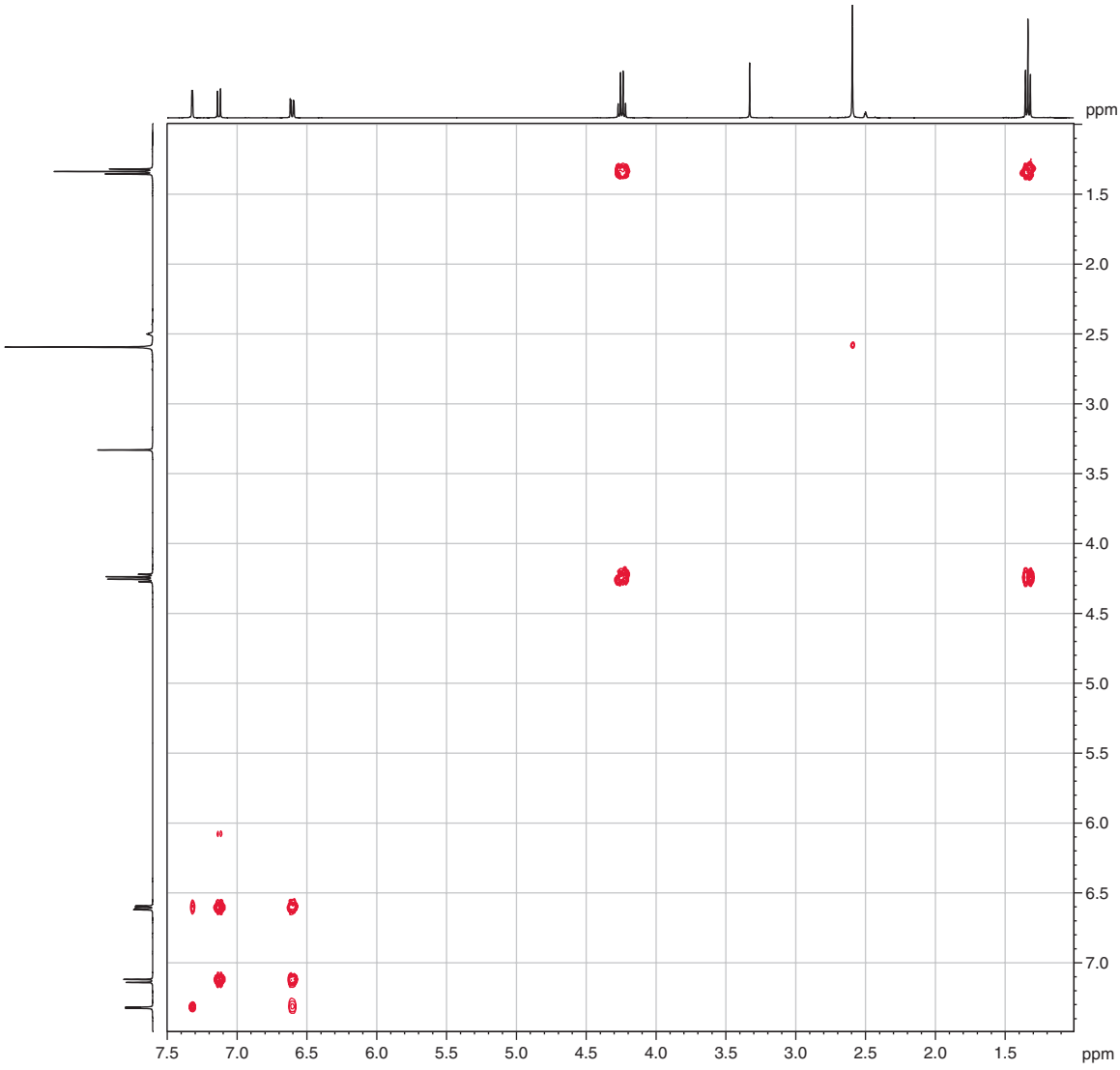


Spectrum 15.5 2-D ROESY.

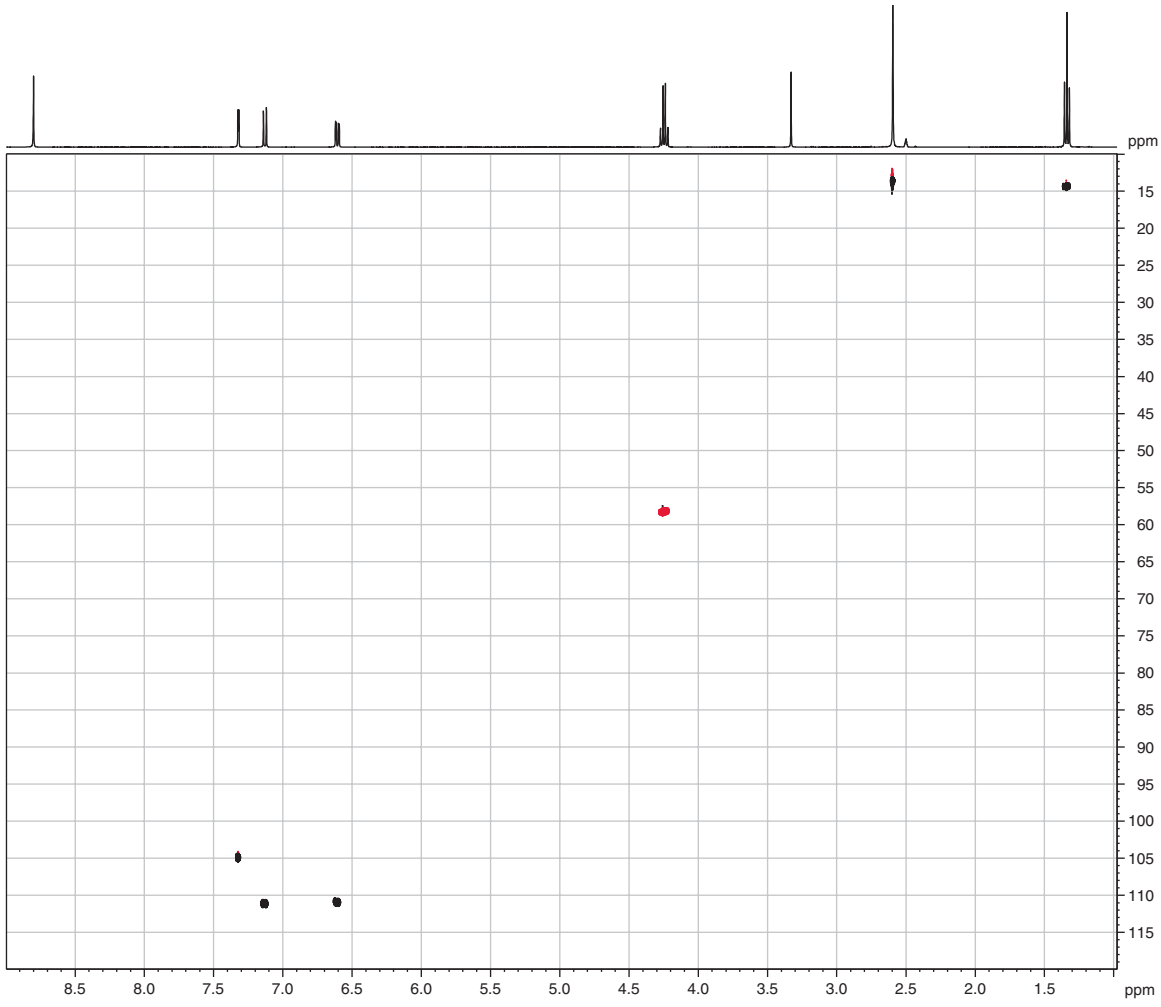
Q6. It's another indole problem but maybe a bit more difficult. It has a formula of $C_{12}H_{13}NO_3$. The spectra are given in Spectra 15.6–15.9. Enjoy!



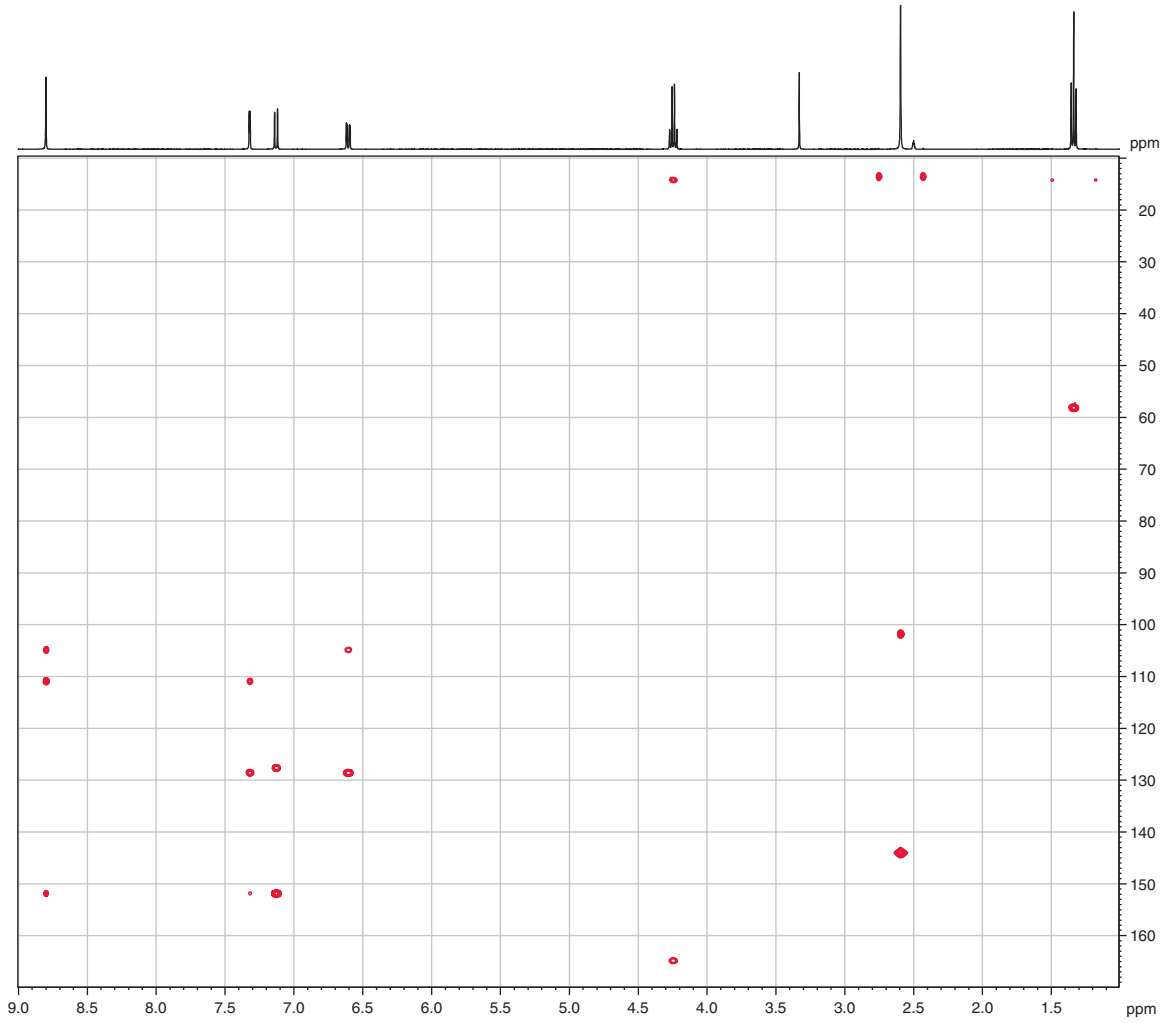
Spectrum 15.6 ^{1}H 1-D.



Spectrum 15.7 COSY.

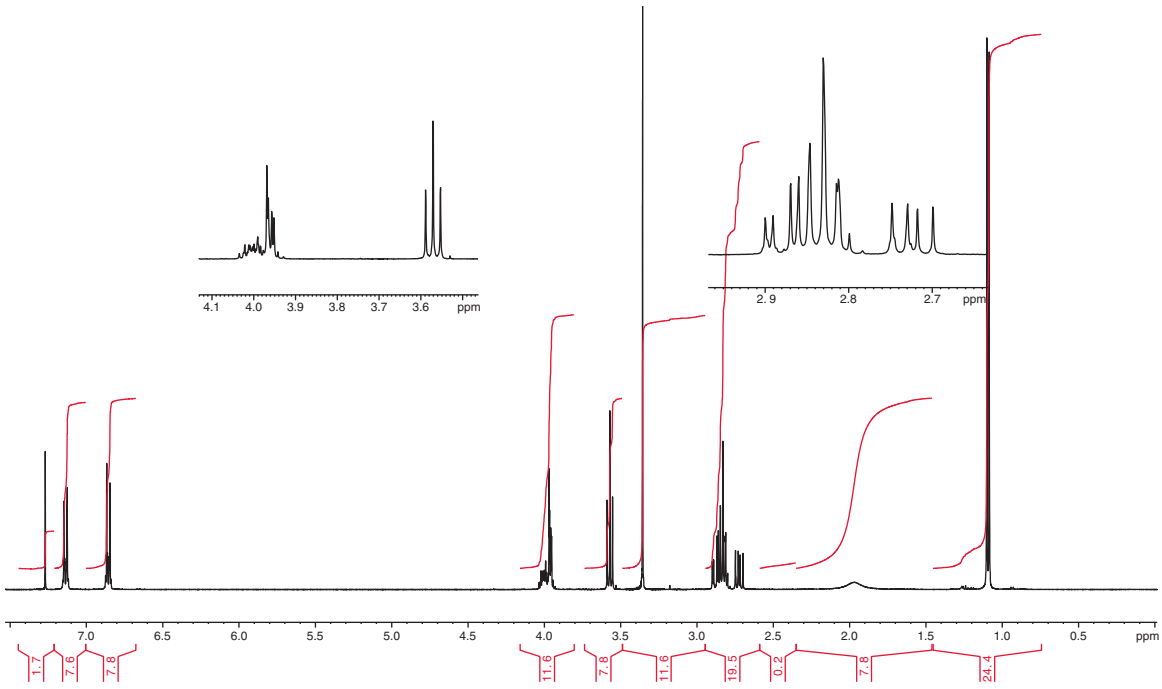


Spectrum 15.8 DEPT-edited HSQC.

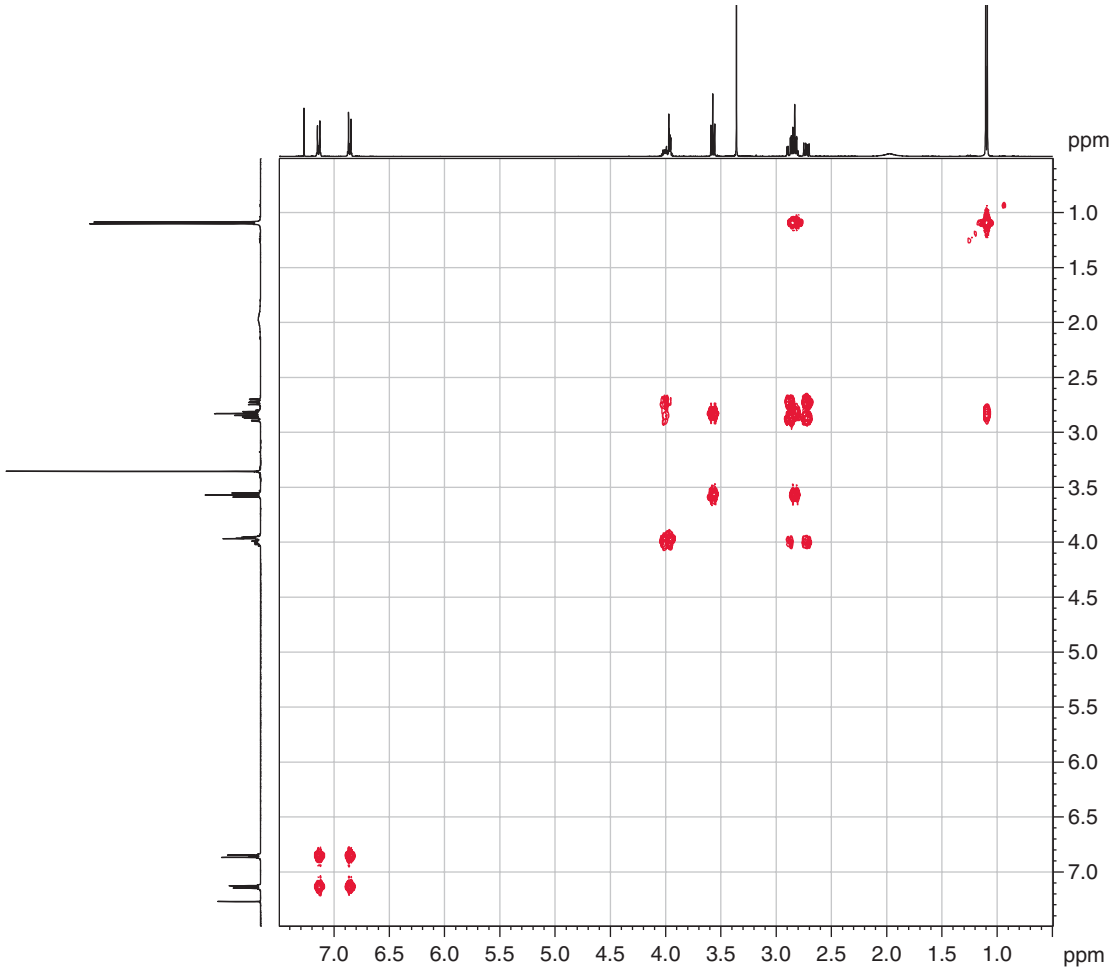


Spectrum 15.9 HMBC.

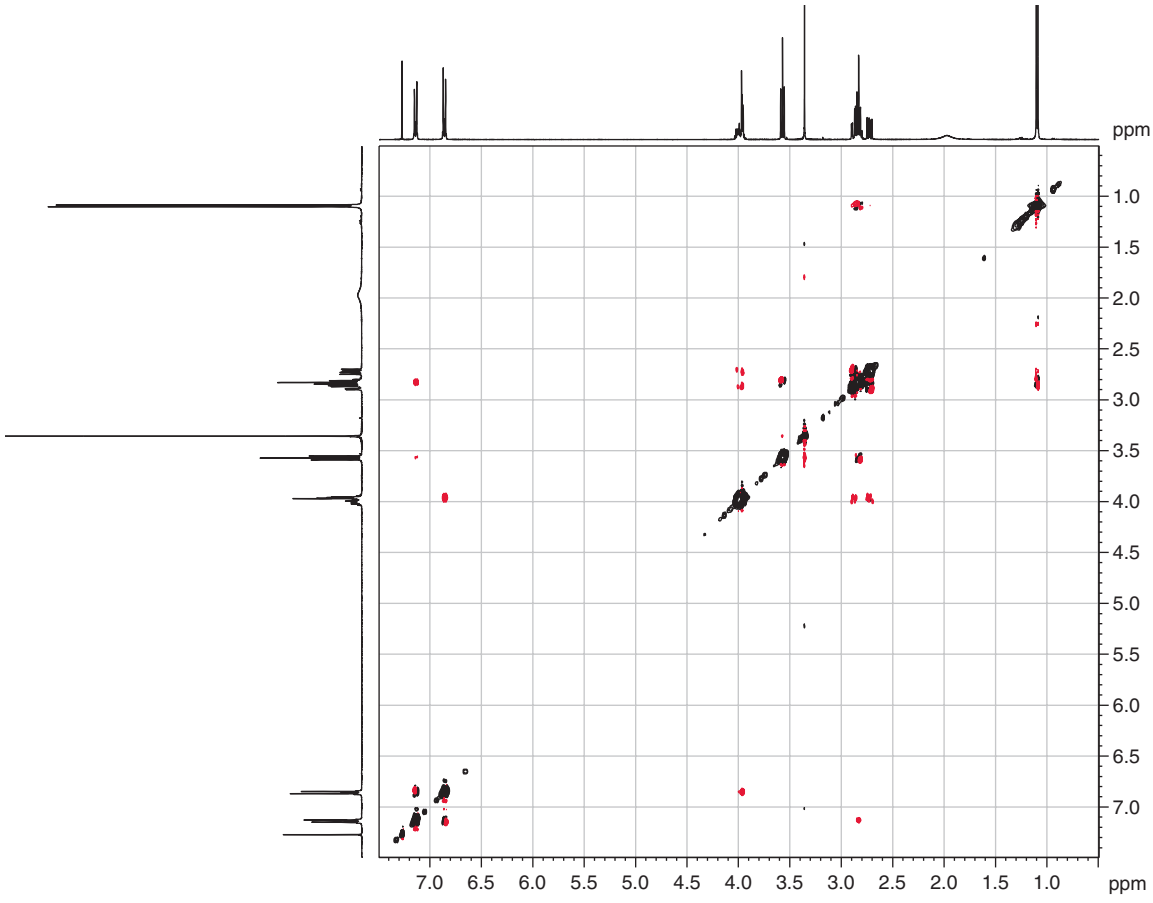
Q7. Propose a structure from Spectra 15.10–15.13. The compound is known to be a free base and is composed of only carbon, hydrogen, oxygen and nitrogen. It has a molecular weight of 267. To further complicate matters, the compound was extracted into $CDCl_3$ solution from D_2O/sodium carbonate so that no exchangeable protons can be observed.



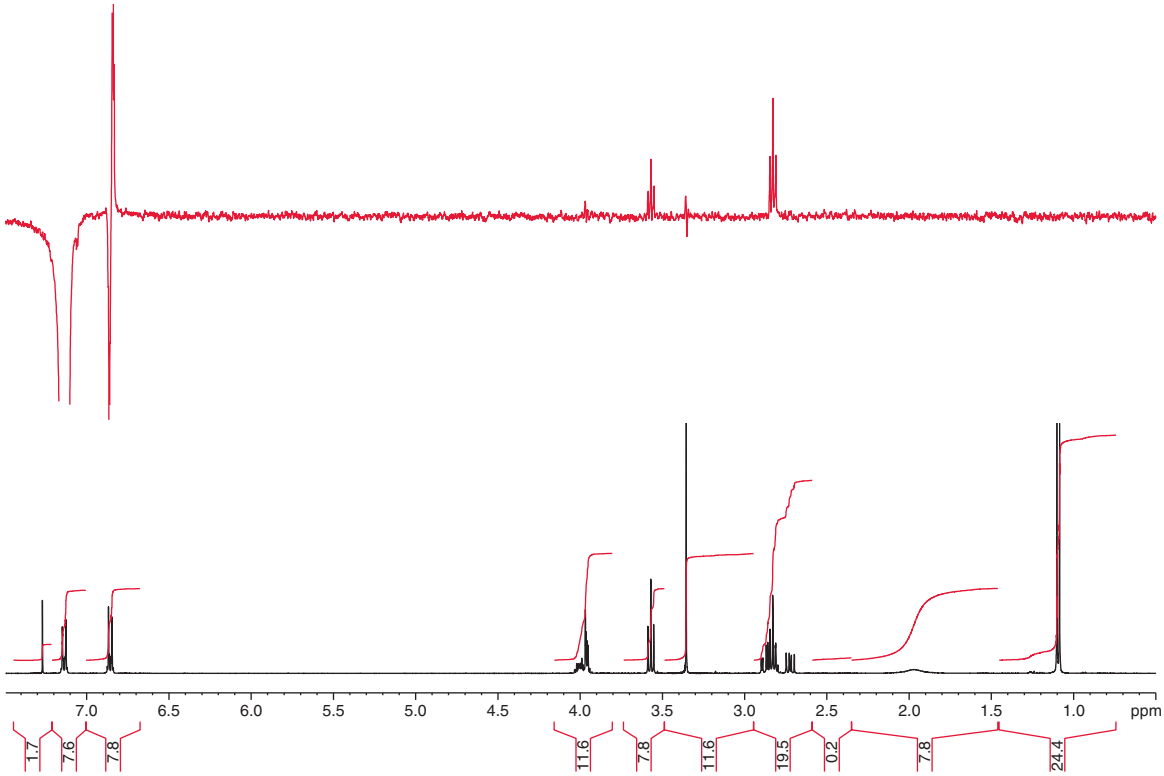
Spectrum 15.10 ^{1}H 1-D.



Spectrum 15.11 COSY.

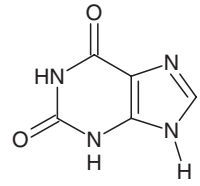


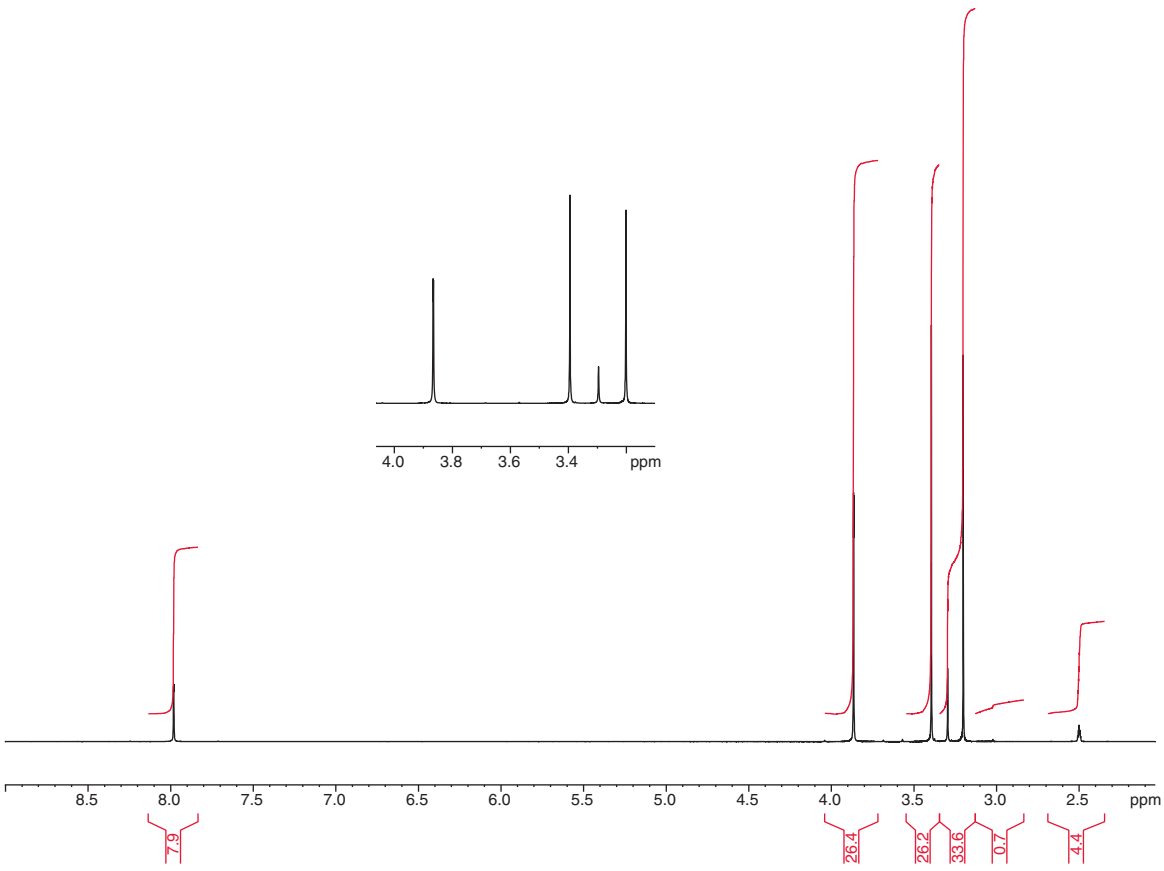
Spectrum 15.12 2-D ROESY.



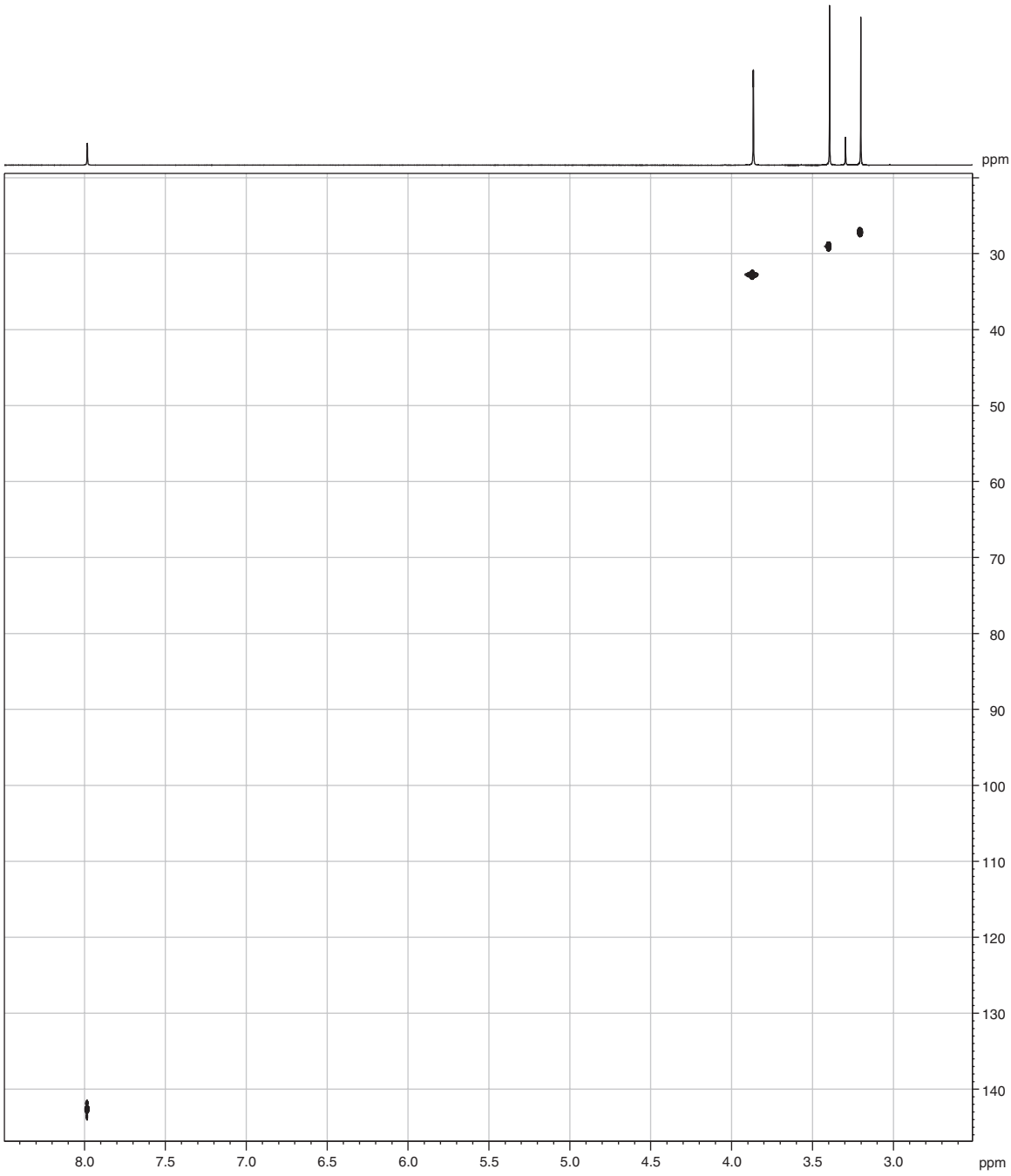
Spectrum 15.13 1-D ROESY (irradiation at 7.15 ppm).

Q8. The following heterocycle is known to have been methylated at three positions. How would you determine which they are? *Note*: Spectra 15.14–15.16 acquired in DMSO.

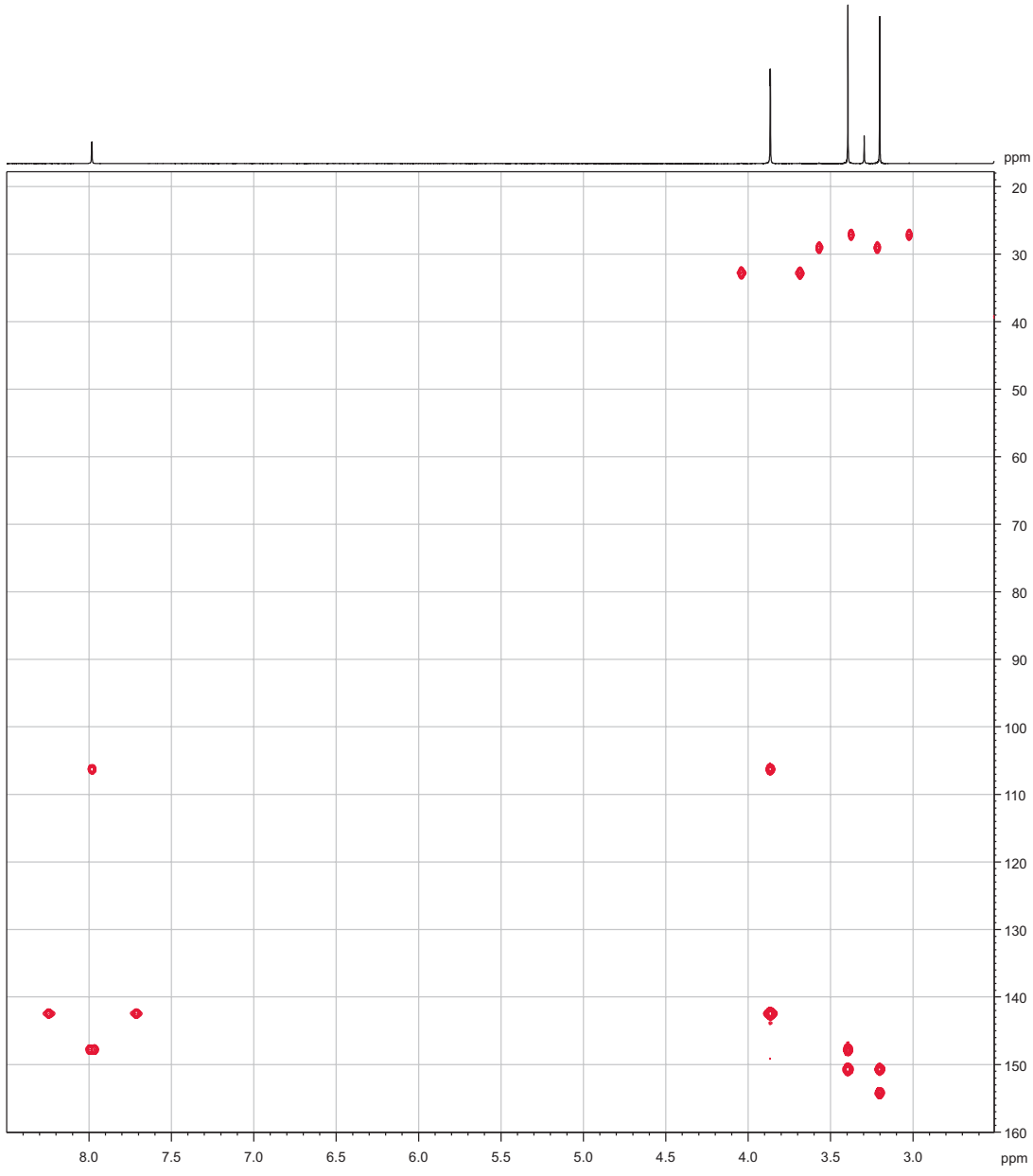




Spectrum 15.14 1H 1-D.



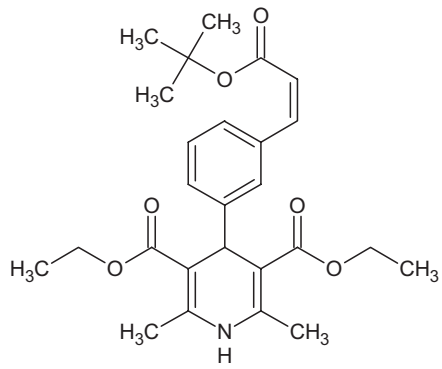
Spectrum 15.15 DEPT-edited HSQC.

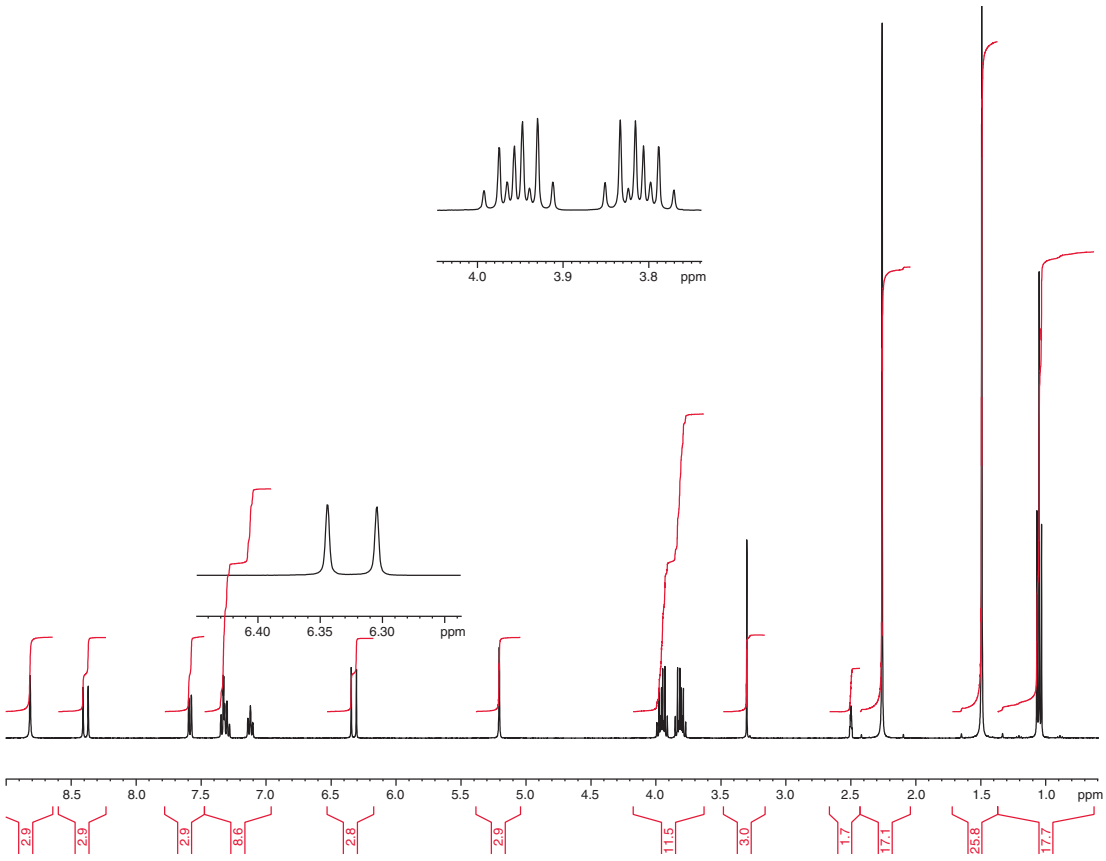


Spectrum 15.16 HMBC.

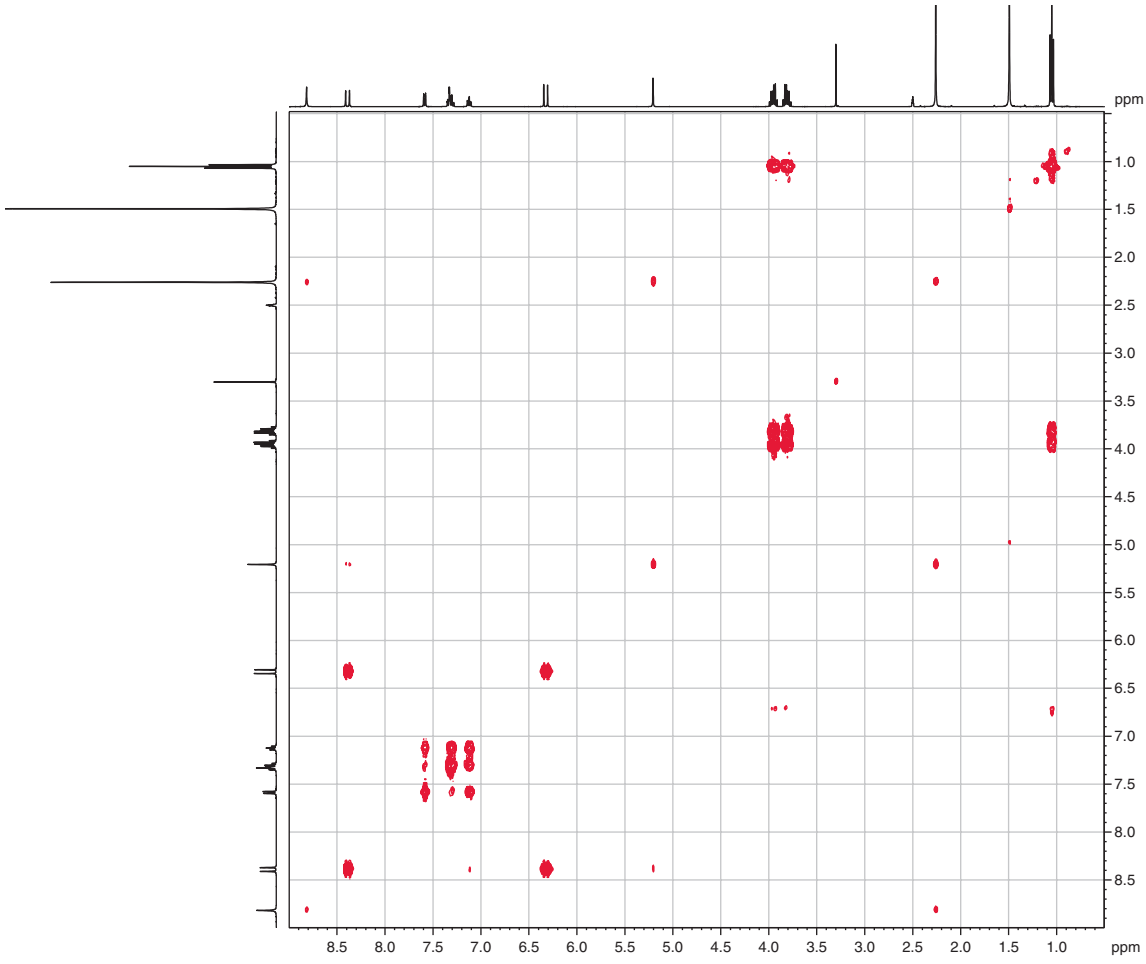
Q9. A compound comes to you for a routine check (Spectra 15.17–15.21). The alleged structure is shown below but previous experience of compounds from this source makes you naturally suspicious! Examine the proton spectrum (Spectrum 15.17) to see if these suspicions are justified and if so, can you suggest an alternative structure that better fits the data? What extra level of

reassurance would you like to see before you would be totally happy with the compound and what technique(s) would you employ to achieve this?

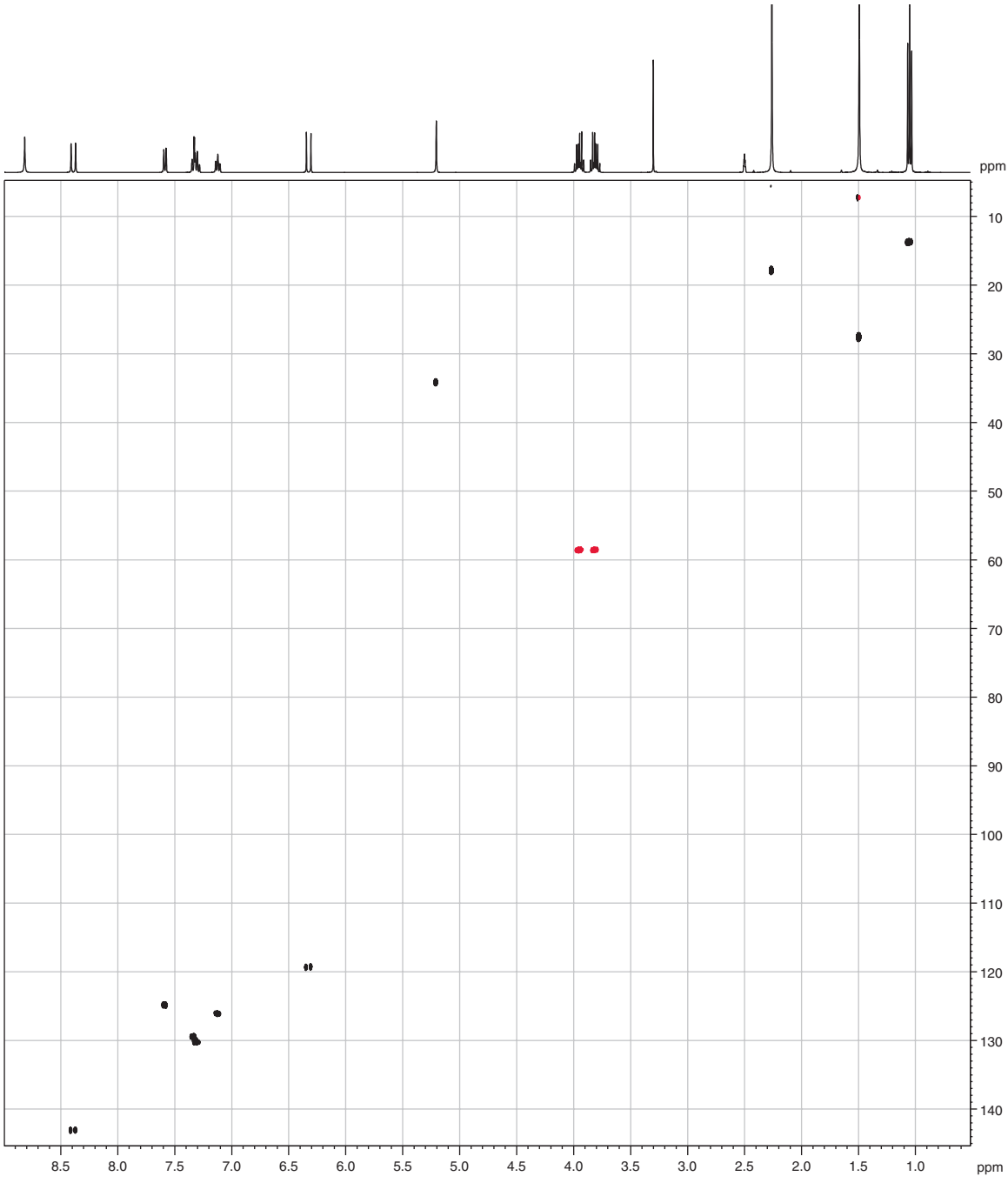




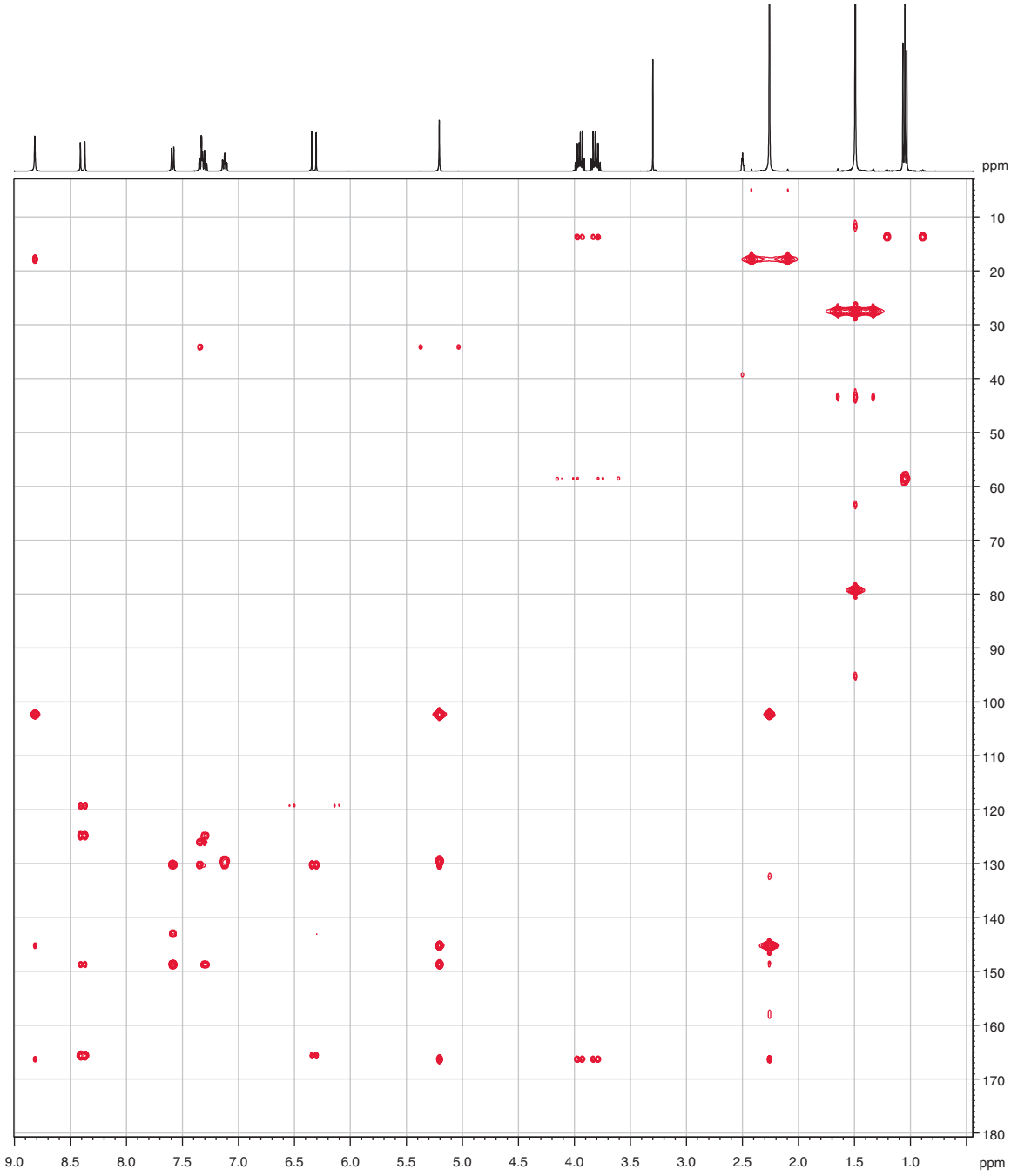
Spectrum 15.17 ^{1}H 1-D.



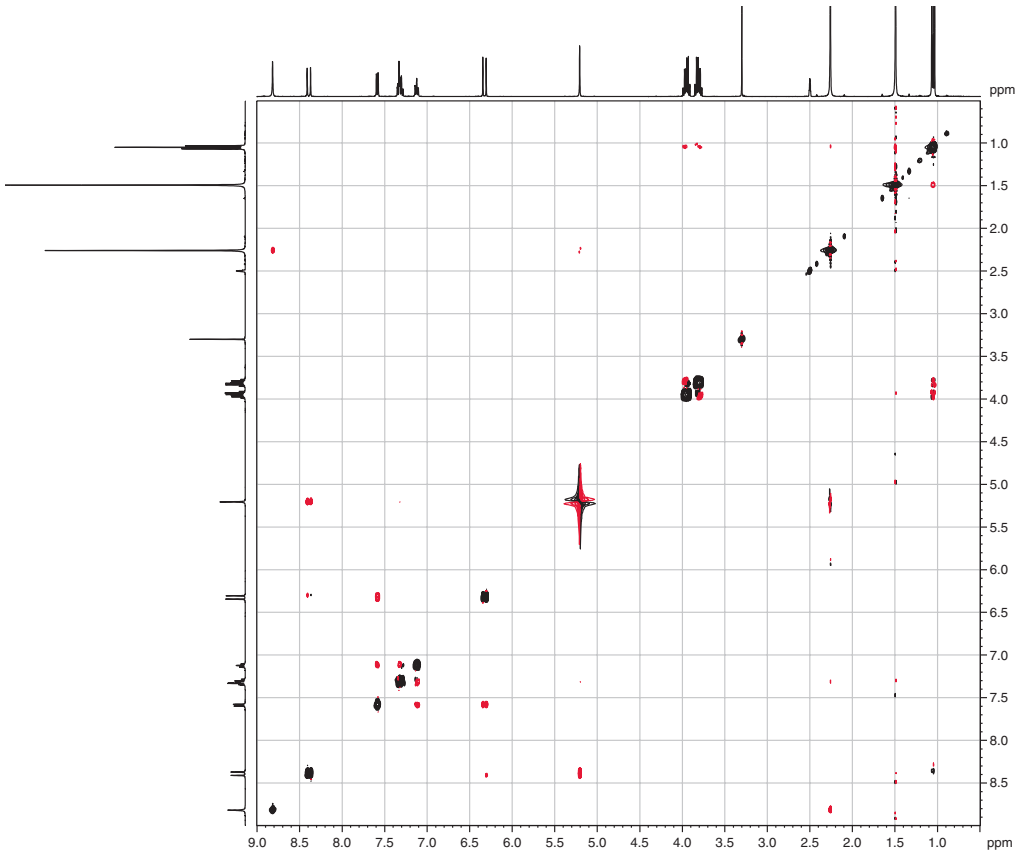
Spectrum 15.18 COSY.



Spectrum 15.19 DEPT-edited HSQC.



Spectrum 15.20 HMBC.



Spectrum 15.21 2-D ROSEY.

Q10. You have secured the post of 'Head of Structural Verification' in a small pharmaceutical company (by the strategic deployment of some particularly interesting pictures of the chairman at last year's Xmas party) and you have a capital budget of £ 350 000 for the year. What do you spend it on?

15.2 Hints

Q1. Run the proton spectrum in a suitable solvent. It's always the best way to begin! Stop and think about it. The answer may be right there in front of you. Maybe there is no need for any further experiments. As the state of protonation of the pyridine nitrogen is unknown so chemical shift information may be unreliable but the spin coupling should be relatively unaffected by this.

Q2. Think about through-space interactions.

Q3. This pair of isomers would indeed give similar proton spectra. There would be no obvious feature available from the basic proton spectrum to facilitate differentiating them. You need a further technique that can be used to exploit the different spatial relationships between the protons in both compounds.

Q4. Two very simple little molecules but they have no structural difference between them. It looks like it will have to be a question of discrimination on the basis of some predictable and *significant* chemical shift differences.

Q5. Check out the proton spectrum first! Extract as much information as possible before considering any of the other spectra. Given that the proton spectrum serves to eliminate most of the potential isomers, select the spectrum that yields the information required most directly and unambiguously.

Q6. Once again, wring all the information available from the proton spectrum first. The proton spectrum is your friend! What can you deduce about the number, nature and likely positions of the substituent(s)? What do you need to 'firm up?' What further techniques are needed?

Q7. This is a tricky one! Take your time and see if you can identify any 'special feature' in the 1-D proton spectrum which will help give you an important lead. Think about the consequences of protons having similar chemical shifts – both when they are coupled to each other and when they are not.

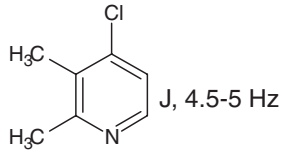
Q8. Take a look at the proton spectrum. Which site has not been methylated? So far so good. Can proton techniques help any further? If not, it could be a good idea to acquire some data for another nucleus.

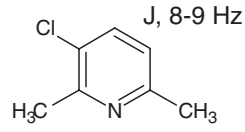
Q9. Check out the aromatic region. Are you happy with the splitting pattern? Now do the same with the alkene.

Q10. Hmm! Tricky one. But there can only be one answer.

15.3 Answers

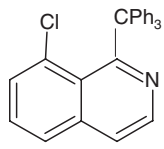
Q1. Measure the coupling between the two pyridine protons accurately. Now check the value against data quoted in Table 5.5 and all should become clear. . .

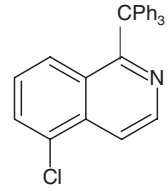




This data is solid. There are numerous other confirmatory experiments that could be performed of course but they would not be really necessary.

Q2.

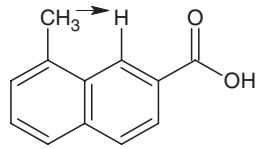


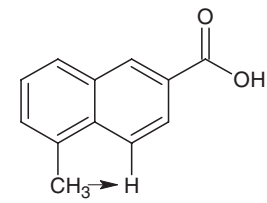


The compound on the left should give a relatively unremarkable proton spectrum as none of its protons are in a position to get anywhere near the highly anisotropic trityl (-CPh_3) group.

The proton para to the chlorine in the compound on the right however, would certainly be held very close to the trityl moiety and be likely to exhibit an unexpected chemical shift and would probably be shielded by a whole 1 ppm and maybe more.

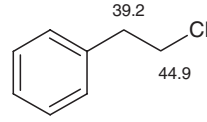
Q3.

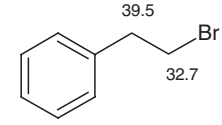




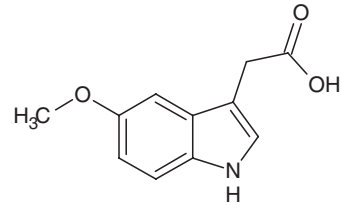
Whilst these two isomers could undoubtedly be differentiated by using HMBC, this is a problem that would be best solved by using an NOE technique. The aryl methyl would provide an ideal target for irradiation. Such an experiment would be expected to give strong enhancements in either case as shown above. As couplings of any enhanced signals are maintained in Overhauser experiments, the distinction between the two would be immediately obvious.

Q4. The proton spectra of these two compounds are very similar and so it would be unwise to try to discriminate between them in this way. The ^{13}C spectra however, would show differences in the alkyl chain which would be both significant and predictable.





Q5. The correct structure is shown below:

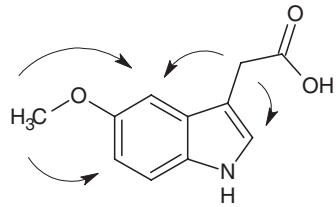


From the proton spectrum, it can be seen that there is one substituent on the 6-membered indole ring and because one of the signals on this ring (the doublet of doublets) has a chemical shift of 6.7 ppm, then it is a requirement for this substituent to be shielding in character. Check the shifts of indole itself in the relevant table! Given the choice of the two substituents, then it must be the -OCH_3 that is located on the six-membered ring. Given the observed coupling pattern for this ring, two positions for this substituent would be possible: the 5 and 6 positions. This will be defined later by reference to some further spectroscopic method.

The -CH_2-COOH substituent must therefore reside on the five-membered ring and it defines its position by the chemical shift of the proton that remains on this ring. This substituent has relatively little influence on the chemical shifts of protons ortho to it and so a shift of 7.18 ppm is immediately indicative of a proton in the 2 position and therefore the substituent in the 3

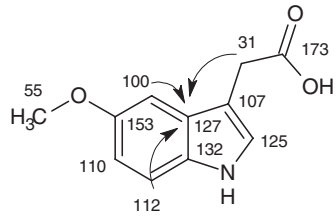
position. Note that all groups of the type -CH_2-X tend to be fairly neutral in terms of their shielding/deshielding characteristics on aromatic systems, with the exception of -CH_2-NR_3^+ which is moderately deshielding. (With the group in the 2 position and a proton in the 3 position, we would be looking for a chemical shift of about 6.7 ppm for this proton).

The final piece of the puzzle must be to define the position of the methoxy group and this is best performed in this case by use of an NOE-based experiment. The ROESY experiment shows clear enhancements, as indicated below:



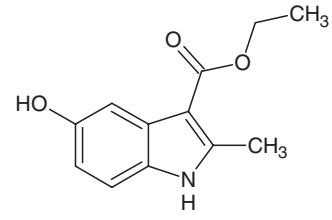
The common enhancements from both the substituents to the 4 proton is pivotal in defining the methoxy group as a 5 rather than a 6 substituent. Note the small coupling between the indole NH and the indole 2 proton that can be seen in the COSY spectrum. Observing this coupling is *not* proof that this proton is in the 2 rather than the 3 position as the 'zigzag' path between the indole NH and an indole 3 proton facilitates a similar sized, four-bond coupling between them! This is typical in indoles and analogous heterocyclic compounds.

So, full marks if you opted for the NOE-based approach to solving this problem. This of course, in no way implies that solving by the HMBC approach is wrong! A full carbon assignment with key correlations is shown below:



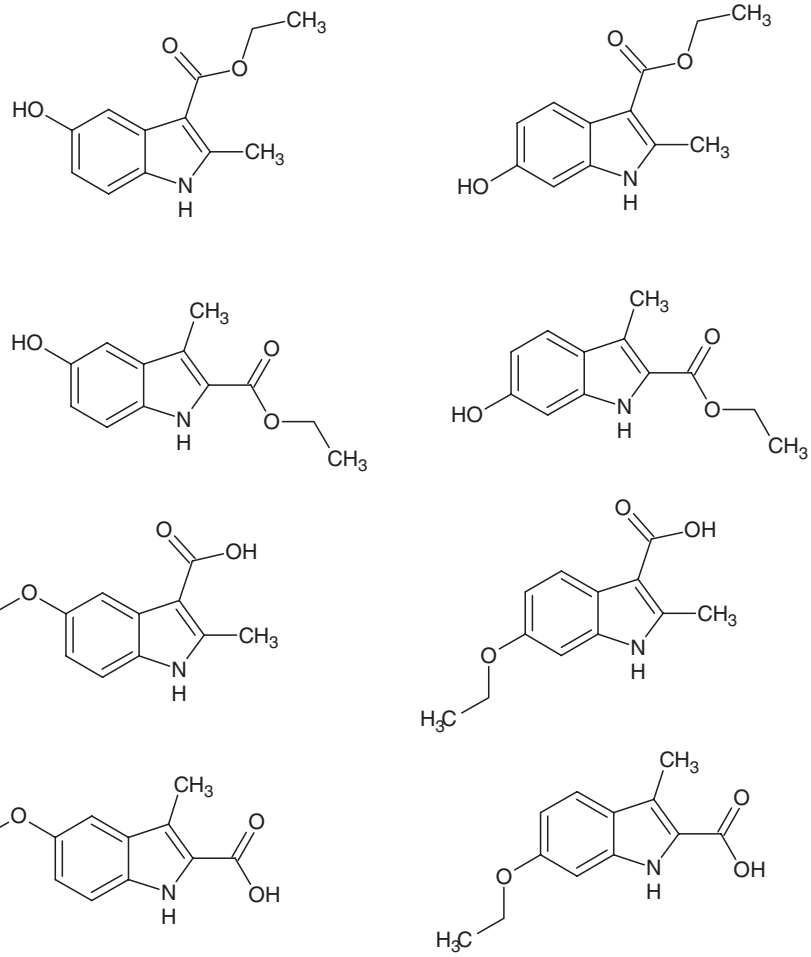
Use of the HSQC and HMBC spectra to assign the compound and establish key connectivities in this way, unambiguously establishes the substitution positions of both groups but is a lot more labour-intensive than the NOE-based approach.

Q6. The correct structure is shown below:



Once again, the proton spectrum reveals a shielding group attached to the six-membered ring of the indole in either the 5 or the 6 position and, given the molecular formula, this has to be oxygen-based. It is also clear that there is an ethyl group present and the shift of the -CH_2 of this group shows that it is either part of an aryl-ethyl ether, or that it is part of an ethyl ester (note that both systems would give similar shifts for the respective -CH_2s!). We also have evidence of a methyl singlet with a shift typical for a methyl attached to an aromatic moiety. Given that there are clearly three protons on the six-membered indole ring (and therefore only one substituent) and that no other heterocyclic protons are visible, it is logical to conclude that there must be two substituents on the five-membered ring, one of which being a methyl group and the other either a carboxylic acid or an ethyl ester, depending on whether the substituent on the six-membered ring is a phenol or an ethyl ether.

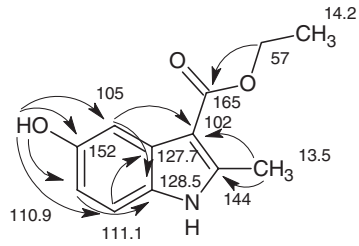
Given the molecular formula, this gives eight possible isomers which would all give perfectly plausible structures to fit the observed proton spectrum:



Note that any attempt to differentiate carboxylic acids from phenols on the basis of how broad their respective signals are is to be discouraged in the strongest possible terms! Whilst carboxylic acids *tend* to be broader than phenols, it is by no means guaranteed that this is always the case. Steric and electronic factors and hydrogen bonding can reverse this in certain situations.

Conclusive validation of the correct structure in this case takes a little thought. NOE-based experiments will tend to be less useful in these circumstances because in isomers where the methyl group is in the 2 position, no useful NOEs may be observed – note that an ethyl ester is inherently flexible and NOEs between the ethyl ester protons and any aromatic protons would be unlikely and unreliable. Furthermore, relying on exchangeable protons for the purpose of gathering NOE data is not recommended and is often unfeasible if such signals are broad. Attempting to gather NOE data from compounds which contain more than one exchangeable is even more ill advised. In this case, the possibility exists for bogus relayed NOEs from the phenol via the indolic NH!

HSQC/HMBC is the way to nail this problem. The full assignment with key correlations is shown below:

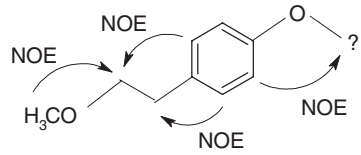


The first obvious deduction is that the compound must be an ethyl ester rather than an ethyl ether as the -CH_2 correlates to a carbonyl carbon at 165 ppm. No other protons correlate to this carbon so even without any ^{13}C prediction, the ethyl ester is confirmed. The relative positions of the ester and methyl substituents are confirmed as if they were reversed, a common correlation from both the methyl protons and the indole 7 proton to one of the ring junction carbons (127.7 ppm in this compound) would be expected. Finally, the position of the -OH is confirmed by comparison with ^{13}C prediction data for both the 5 and the 6 isomer and by the weak but significant correlation from the 4 proton to the 3 carbon at 102 ppm. Note that correlations from the -OH in this case are a bonus. Exchangeable signals are often too broad to give useful correlations.

Q7. Casting an eye over the proton spectrum, the AB part of an ABX system immediately presents itself at 2.88 and 2.73 ppm. This means the molecule contains a chiral centre!

Working methodically from left to right, it is clear from the proton spectrum that the compound has a single aromatic ring and that it is 1,4 disubstituted. One of the substituents is fairly neutral as one half of the aromatic AA′BB′ system has a chemical shift of about 7.14 ppm whilst the other is quite strongly shielding as the other half of the AA′BB′ is at about 6.8 ppm. Since the compound is known to contain both oxygen and nitrogen, it is quite reasonable to deduce that the shielding entity must be one of these two atoms. Further investigation will be required to determine which it is in due course.

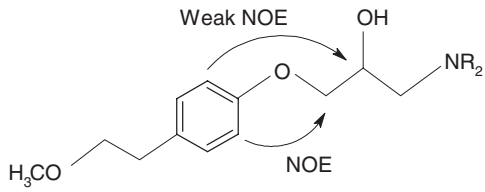
Now consider the ROESY experiment. It is clear that the aromatic protons at 7.14 ppm show an enhancement to the multiplet, or rather, one element of the multiplet, at around 2.83 ppm. This would be a typical shift for an alkyl substituent. Similarly, the aromatic protons at 6.8 ppm enhance protons between 4.1 and 3.9 ppm. This would be a typical shift for an aromatic O alkyl substituent and is important information. Remember that the compound has been base extracted from D_2O/sodium carbonate and for this reason, no exchangeable protons will be visible. The fact that both sets of aromatic protons show enhancements to different alkyl protons means that there must be alkyl substituents on both ends of the ring (as opposed to an -OH at one end and everything else attached at the other). The aromatic ring therefore conveniently splits the molecule into two segments that can be dealt with separately. The 2-D ROESY has certainly proven to be very useful so far but the severely overlapped nature of the alkyl protons makes it difficult to see *exactly* what is being enhanced. For this reason, specific 1-D ROESY experiments hold a big advantage as the enhanced multiplet is always reconstructed complete with all couplings. Two signals show enhancement from the aromatic protons at 7.15 ppm and they have the appearance of a pair of coupled triplets. By inspecting the ordinary 1-D proton spectrum, it becomes clear that this must be a $-CH_2-CH_2-$ system with the triplet at 2.83 ppm more intense in appearance than its coupled partner at 3.57 ppm. This shift looks good for another oxygen and in fact, a $-OCH_3$ as another 1-D ROESY irradiating the singlet at 3.35 ppm establishes the connection between this singlet and the triplet $-CH_2$ at 3.57 ppm. So piecing together what we have so far, we're looking at something like this:



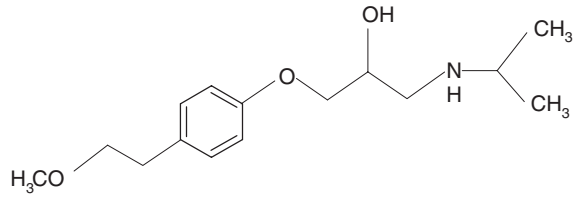
Concentrating now on the right hand side of the molecule and re-examining the signals which show enhancement from the aromatic protons at 6.8 ppm, it would seem that the entire multiplet (4.05–3.93 ppm) which integrates for three protons is part of a close-coupled non-first order spin system. The coupling between these protons is not at all clear from the COSY spectrum because the chemical shifts of the protons are so close. The coupling is more apparent from close scrutiny of the 1-D proton spectrum. The right hand side of this multiplet (3.98–3.93 ppm) consists of heavily roofed eight-line system which is characteristic of the AB part of an ABX system where the shifts of 'A' and 'B' are extremely close. The A-X and B-X couplings are not obvious from the COSY because the 'X' is extremely close to 'A' and 'B' and in fact is the left hand side of the multiplet (4.05–3.98 ppm)!

The complexity of this spectrum does not end there however as two key features of this spectrum must now be addressed. First, the 'X' part of the ABX system we have just discussed consists of far more than the normal four lines; and second, the four-line multiplets centred at 2.88 and 2.73 ppm are clearly 'A' and 'B' parts of a second ABX system! These features are linked in that the COSY spectrum clearly shows that the complex 'X' part (4.05–3.98 ppm) is in fact coupled to both the 'A' and 'B' parts of the second ABX system. Therefore, we can deduce that

the 'X' part is common to *both* ABX systems. Chemical shifts indicate that a likely arrangement of hetero atoms would give a right hand side for the molecule looking like this:



Almost home and dry now! Back to the COSY. The six-proton doublet at 1.1 ppm shows a coupling to something at 2.83 ppm. We know that the triplet at 2.83 ppm is part of the closed spin system on the left hand side of the molecule and therefore cannot in any way be responsible for this correlation. Measuring the integral from 2.91–2.78 ppm reveals the presence of *four* protons. One of them has already been assigned as part of the second ABX system and the triplet at 2.83 ppm accounts for two protons. Then, the implication must be that one proton is almost completely hidden from view beneath these two signals. In terms of chemical shifts, an isopropyl group attached to the nitrogen would fit perfectly. So fitting it all together, we have:



(This is the drug Metoprolol, a beta-blocker). Obviously, a great many deductions have to be made to arrive at a structure from scratch in this way and whilst each one in this example is valid in its own right and they all fit together perfectly well with no obvious conflicts, structural verification via the HMQC/HMBC route would be advisable!

Q8. A quick inspection of the proton spectrum for this compound confirms that a heterocyclic proton is present at 8.0 ppm so C-methylation cannot have taken place. Furthermore, the proton spectrum confirms the presence of three methyl signals at approximately 3.9, 3.4 and 3.2 ppm. There is little more to be gleaned from the proton spectrum except for the fact that the methyl at 3.9 ppm is slightly broader than the other two. This is indicative of a small long-range coupling to the heterocyclic proton though this information is only of limited value. It is clear that another nucleus must be examined.

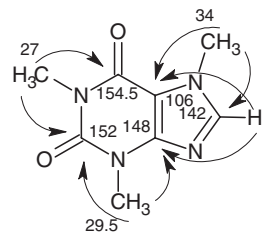
As the parent compound contains four nitrogen atoms, it might be tempting to opt for proton–nitrogen HMBC but the technique would be of limited value in this case. ^{13}C spectroscopy offers by far the most comprehensive solution. The HSQC spectrum shows that the chemical shifts for the methyls are approximately 33, 29 and 27 ppm. It is immediately clear that the methyl groups must therefore all be attached to the nitrogen atoms and not to any of the oxygens (which would give shifts in the 55–65 ppm range).

The information required to solve this problem will come from the HMBC experiment. After first discounting the one-bond couplings that have come through (either by reference to the HSQC experiment or just by observation) it can be seen that the heterocyclic CH shows two, three-bond correlations to carbons at 148 and 106 ppm. Since the carbon shifts of the methyl groups indicates that O-methylation is not an option, it is safe to assume that the oxygen atoms will still be in the form of conjugated amidic or urea carbonyl functions. The chemical shift of such carbonyls will always be in the 150–160 ppm range. We know the shift of the carbon bearing the solitary heterocyclic proton (142 ppm) and of the two remaining quaternary carbons, the one flanked by two nitrogens is likely to be far more de-shielded than the other so even without using ^{13}C prediction software, this problem should be relatively straightforward.

The salient features of the HMBC could be summarised as follows:

1. There is a common correlation from the methyl protons at 3.9 ppm and the heterocyclic proton (8.0 ppm) to a quaternary carbon at 106 ppm.
2. This proton also correlates to another quaternary carbon at 148 ppm.
3. The methyl protons at 3.2 ppm correlate to two quaternary (carbonyl) signals at 154.5 and 152.0 ppm.
4. The methyl protons at 3.4 ppm correlate to one of the carbonyls at 152 ppm and also to the quaternary carbon at 148 ppm (see item 2, above).

Putting all this information together we have: caffeine.



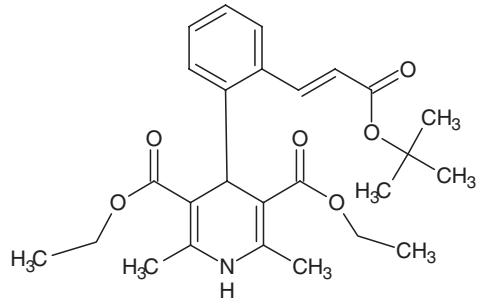
This summarizes the proton–carbon correlations and shows all the ^{13}C chemical shifts. Note that no other arrangement of the methyl groups would satisfy the observations made. For example, had one of the methyl groups been attached to the other nitrogen in the five-membered ring, then the correlation to a carbon anywhere near 106 ppm would have been replaced by one to a carbon nearer to 150 ppm.

Note also that though the methyl protons at 3.9 ppm correlate to the carbon at 142 ppm, there is no guarantee that the corresponding proton at 8.0 ppm will show a correlation to the carbon of this methyl group (34 ppm). In fact this correlation does exist but it is a lot weaker than the others and does not show up in the plot without turning up the gain to the point where the rest of the spectrum becomes difficult to understand. The apparent intensities of the observed correlations reflect the size of the proton–carbon couplings concerned. The (methyl) proton–heterocyclic carbon coupling must be significantly different from the C**H**-methyl (carbon) coupling.

Q9. At first glance, the proton spectrum for this compound looks excellent. The protons are, with the exception of two aromatic protons, well separated and this is always a bonus! The alkene protons draw immediate attention as they sit on either side of the aromatic protons and the doublet at about 8.4 ppm is definitely the alkene closest to the aromatic ring. Its coupling partner, closest to

the t-butyl ester is the doublet at approximately 6.32 ppm. The coupling between these two alkene protons looks large and measurement indicates that it is in fact 16 Hz. This is too large to support the proposed *cis* alkene and is far more in keeping with *trans* geometry! As an interesting footnote to this question of alkene configuration, a trans alkene on an aromatic ring will generally show NOEs between *both* alkene protons and the aromatic proton(s) ortho to the point of substitution, whilst the corresponding *cis* alkene can only show an NOE from *one* of the alkene protons and the ortho protons on the aromatic ring. This could provide useful back up information if the observed coupling was in any way doubtful.

Furthermore, scrutiny of the aromatic region shows coupling patterns that are not consistent with 1,3 substitution. Given that the aromatic protons are relatively well spread out – and this is an important point as little or nothing could be deduced about the substitution pattern if the substituents were such that all the aromatic protons were heavily overlapped – we should be looking to see two doublet of doublets, one with two small (meta) couplings and one with two larger (ortho) couplings. What we do observe is a pair of broad triplet structures, a broad doublet with one ortho coupling and a doublet of doublets dominated by an ortho coupling. This pattern can only occur in 1,2 disubstituted aromatic rings. Thus a far more plausible structure would be:



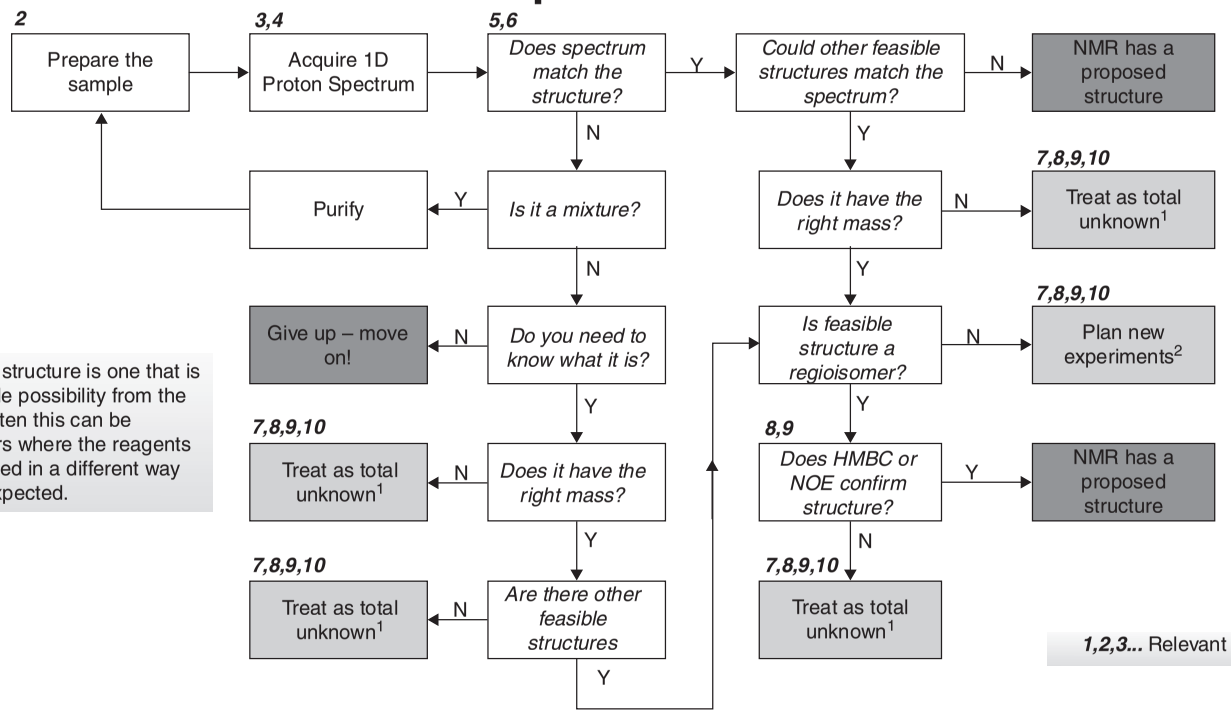
The ethyl ester protons are worthy of note in this molecule. Though there is no chiral centre present, these are non-equivalent by virtue of being diastereotopic (remember the 'Z test?').

In order to be as fully confident as possible with this compound, given the two errors already apparent, it would be advisable to check it out thoroughly with HSQC, HMBC and a ROESY. This would establish the relative positions of the ethyl ester and methyl groups. A mass spectrum might be a good idea as well!

Q10. Flippancy aside, there is at least a semiserious aspect to this tongue in cheek question. Without wishing to cause offence to any mass spectroscopist or devotee of any other form of spectroscopy, we hope that we've demonstrated (to some extent at least) the unrivalled power and flexibility of the NMR technique for elucidating chemical structures. The quality and depth of the information available is remarkable and the range of associated techniques gives the method huge versatility. If an organic compound can be dissolved then it *will* give NMR signals – no question about it. NMR may be used in a quantitative as well as qualitative manner and given the right hardware, can be applied to several key nuclei.

Spend the money wisely – on the best NMR system you can get your hands on – and don't forget to keep your camera handy at next year's office party – you might fancy an upgrade.

NMR Interpretation Flow Chart



A "feasible" structure is one that is a reasonable possibility from the reaction. Often this can be regioisomers where the reagents have attached in a different way from that expected.

[1] Treat as unknown
In the case of a total unknown it is a case of the more data, the better. Solving this sort of problem is like doing a jigsaw puzzle. You piece together information from a variety of sources to come up with a feasible structure. You then test that structure with more experiments to ensure you get a consistent answer. As a minimum you should consider COSY, HSQC, HMBC, 1D 13C. Don't forget – NMR is not the only technique so look at mass spectrometry (accurate mass in particular) and IR to help.

[2] Plan new experiments
If you have two or more possible structures that fit the data, you will need to look for differences that can be identified by NMR. Often this is HMBC and/or NOE (to identify key connectivities). Every problem is different so you need to use all your skills to look for tools that can help distinguish the putative structures. As in the case of total unknowns, don't use NMR to the exclusion of other techniques as they may be able to make the choice much easier.

Don't forget! NMR on its own cannot prove a structure.

Appendix A.1 Useful thought processes for tackling NMR problems.

Glossary

Note – This glossary is by no means exhaustive but it hopefully contains most of the more important terms you will come across in a typical 'NMR environment.' Some of the entries may not even have featured in the text itself. Whilst every effort has been made to make the entries scientifically valid, please note that it is sometimes difficult to condense a highly complex topic into a pithy three-line explanation, so some of the definitions are sketchy to say the least!

Acquisition Process of collection of NMR data.

Adiabatic pulse A type of pulse employing a frequency sweep during the pulse. This type of pulse is particularly efficient for *broadband decoupling* over large sweep widths.

Aliased signals Signals that fall outside the *spectral window* (i.e., those that fail to meet the *Nyquist condition*). Such signals still appear in the spectrum but at the wrong frequency because they become 'folded' back into the spectrum and are characterised by being out of phase with respect to the other signals.

Anisotropy Non-uniform distribution of electrons about a group which can lead to non-uniform localised magnetic fields within a molecule. The phenomenon leads to unexpected chemical shifts – particularly in ^{1}H NMR – in molecules where steric constraints are present.

Apodization The use of various mathematical functions which when applied to an FID, yield improvements in the resultant spectrum. These include *exponential multiplication* and *Gaussian multiplication.*

Bloch-Siegert shift A shift in resonant frequency of a signal which is in close proximity to a secondary applied r.f. The effect forces signals away from the applied r.f. and is only ever noticeable in homonuclear decoupling experiments where the applied r.f. and the observed signal can be very close.

Boltzmann Distribution The ratio of nuclei which exist in the *ground state* to those in the *excited state* for a sample introduced into a magnetic field - prior to any r.f. pulsing. This varies with probe temperature but primarily with magnet field strength.

Broadband decoupling Decoupling applied across a wide range of frequencies, e.g., the decoupling of all proton signals during the acquisition of 1-D ^{13}C spectra.

CAMELSPIN *C*ross-relaxation *a*ppropriate for *m*inimolecules *e*mulated by *l*ocked spins. Now known as *ROESY.*

Chemical shift Position of resonance in an NMR spectrum for any signal relative to a reference standard.

Chiral centre An atom in a molecule (usually but not exclusively carbon) which is bound to four different atoms or groups such that the mirror image of the whole molecule is not super-imposable on the molecule itself. A chiral centre in a molecule implies the possibility of the isolation of two distinct forms of the compound which are known as *enantiomers*.

Chirality Properties conferred by the presence of one or more *chiral centres*.

Essential Practical NMR for Organic Chemistry S. A. Richards and J. C. Hollerton
 ISBN: 978-0-470-71092-0

Composite pulses Use of a series of pulses of varying duration and phase in place of a single pulse. Such systems, when used in the pulse sequences of many modern NMR techniques, give improved performance as they are more tolerant to r.f. inhomogeneity.

Configuraton The arrangement of atoms and bonds in a molecule. The configuration of a molecule can be changed by breaking and re-forming bonds to yield different *regioisomer.*

Conformation The shape a molecule adopts by the rotation and deformation (but *not* the breaking and re-forming) of its bonds.

Continuous Wave (CW) Technology used initially in the acquisition of NMR data. The radiofrequency or the magnetic field was swept and nuclei of different *chemical shift* were brought to resonance sequentially.

COSY *Co*rrelative *sp*ectroscopy. Homonuclear (normally ^{1}H) 2-D spectroscopic technique which relates nuclei to each other by spin coupling.

Coupling The interaction between nuclei in close proximity which results in splitting of the observed signals due to the alignment of the neighbouring nuclei with respect to the magnetic field. Also referred to as *spin coupling.*

Coupling constant The separation between lines of a coupled signal measured in Hz.

CPMG pulse sequence *C*arr-*P*urcell-*M*eiboom-*G*ill pulse sequence. A pulse sequence used for removing broad signals from a spectrum by multiple defocusing and refocusing pulses.

Cryoprobe *Probe* offering greatly enhanced sensitivity by the reduction of thermal electronic noise achieved by maintaining probe electronics at or near liquid helium temperature.

Cryoshims Rough (*superconducting*) shim coils that are built into superconducting magnets and adjusted at installation of the instrument.

Decoupling The *saturation* of a particular signal or signals in order to remove spin coupling from those signals. Also referred to as *spin decoupling.*

DEPT *D*istortionless *e*nhancement by *p*olarization *t*ransfer. A useful one-dimensional technique which differentiates methyl and methine carbons from methylene and quaternary carbons.

Diastereoisomers *Stereoisomers* that are not *enantiomers*. Diastereoisomers are compounds that always contain at least two centres of chirality.

Diastereotopic proton/group A proton (or group) which if replaced by another hypothetical group (not already found in the molecule), would yield a pair of diastereoisomers.

Enantiomer A single form of an optically active compound. Optically active compounds usually (but not exclusively) contain one or more chiral centres. Enantiomers are defined by their ability to rotate the plane of beam of polarised light one way or the other and these are referred to as either 'D' or 'L', or alternatively '+' or '–', depending on whether the polarised light is rotated to the right (*D*extro) or the left (*L*evo) .

Enantiotopic proton/group A proton (or group) which if replaced by another hypothetical group (not already found in the molecule), would yield a pair of *enantiomers.*

Epimers Diastereoisomers related to each other by the inversion of only one of their *chiral centres*.

Epimerization Process of inter-conversion of one *epimer* to the other. The process is usually base-mediated as abstraction of a proton is often the first step in the process.

Excited state. Condition where nuclei in a magnetic field have their own magnetic fields aligned so as to oppose the external magnet, i.e., N-N-S-S. Also known as the high-energy state.

Exponential multiplication The application of a mathematical function to an *FID* which has the effect of smoothing the peak shape. Signal/noise may be improved at the expense of resolution.

First-order spin systems Not very specific term used to describe spin systems where the difference in *chemical shift* between coupled signals is very large in comparison to the size of the *coupling*. In reality, there is no such thing as a completely first-order system as the chemical shift difference is never infinite. See *Non-first order spin system.*

Folded signals See aliased signals.

Fourier Transformation. Mathematical process of converting the interference *free induction decay* into a spectrum.

Free Induction Decay (FID) Interference pattern of decaying cosine waves collected by Fourier Transform spectrometers, stored digitally prior to *Fourier Transformation.*

Gated decoupling A method of *decoupling* in which the decoupling is switched on prior to acquisition and turned off during it.

Gradient field A linear magnetic field gradient, deliberately imposed on a sample in, for example, the z-axis in order to defocus the magnetisation. This allows other refocusing gradient pulses to be used to selectively observe desired transitions. Only possible with appropriate hardware. Gradient fields improve the quality of many 2-D techniques and where used, replace the need for *phase cycling.*

Gradient pulse The application of a *gradient field* for a discrete period of time. Also referred to as *Pulsed field gradients (PFGs).*

Gaussian multiplication The application of a mathematical function to an *FID* to improve resolution (sharpen lines) at the expense of signal/noise.

GOESY *G*radient *O*verhauser *e*ffect *s*pectroscop*y*. An early version of a 1-D *NOESY* making use of gradients.

Gradient shimming A system of *shimming* based on mapping the magnetic field inhomogeneity using field gradients and calculating the required shim coil adjustments required to achieve homogeneity.

Ground state Condition where nuclei in a magnetic field have their own magnetic fields aligned *with* that of the external magnet, i.e., N-S-N-S. Also known as the low-energy state.

Gyromagnetic ratio A measure of how strong the response of a nucleus is. The higher the value, the more inherently sensitive will be the nucleus. ^{1}H has the highest value. Also known as *Magnetogyric ratio.*

Hard pulse A pulse which is equally effective over the whole chemical shift range. See *Soft pulse.*

HETCOR *Het*eronuclear *cor*relation. Early method of acquiring one-bond ^{1}H-^{13}C data. Not nearly as sensitive as *HMQC* and *HSQC* methods which have largely superseded it.

HMBC *H*eteronuclear *m*ultiple *b*ond *c*orrelation. A proton-detected, two-dimensional technique that correlates protons to carbons that are two and three bonds distant. Essentially, it is an HMQC that is tuned to detect smaller couplings of around 10 Hz.

HMQC *H*eteronuclear *m*ultiple *q*uantum *c*oherence. A proton-detected, 2-D technique that correlates protons to the carbons they are directly attached to.

HOHAHA *Ho*monuclear *Ha*rtmann *Ha*hn spectroscopy. See *TOCSY.*

HSQC *H*eteronuclear *s*ingle *q*uantum *c*oherence. As for *HMQC* but with improved resolution in the carbon dimension.

INADEQUATE Incredible *n*atural *a*bundance *d*oubl*e qua*ntum *t*ransfer *e*xperiment. Two-dimensional technique showing ^{13}C-^{13}C coupling. It should be the 'holy grail' of NMR methods but is in fact of very limited use due to extreme insensitivity.

Indirect detection Method for the observation of an insensitive nucleus (e.g., ^{13}C) by the transfer of magnetisation from an abundant nucleus (e.g., ^{1}H). This method of detection offers great improvements in the sensitivity of proton–carbon correlated techniques.

Inverse geometry Term used to describe the construction of a probe that has the ^{1}H receiver coils as close to the sample as possible and the X nucleus coils outside these ^{1}H coils. Such probes tend to give excellent sensitivity for ^{1}H spectra at the expense of X nucleus sensitivity in 1-D techniques. They offer a lot of compensation in terms of sensitivity of *indirectly detected* experiments.

J-resolved spectroscopy Two-dimensional techniques, both homo- and heteronuclear, that aims to simplify interpretation by separating chemical shift and coupling into the two dimensions. Unfortunately prone to artifacts in closely coupled systems.

Laboratory frame model A means of visualising the processes taking place in an NMR experiment by observing these processes at a distance, i.e., with a static coordinate system. See *Rotating frame model.*

Larmor frequency The exact frequency at which nuclear magnetic resonance occurs. At this frequency, the exciting frequency matches that of the precession of the axis of the spin of the nucleus about the applied magnetic field.

Larmor precession The motion describing the rotation of the axis of the spin of a nucleus in a magnetic field.

Linear prediction Method of enhancing resolution by artificially extending the *FID* using predicted valued based on existing data from the *FID.*

Longitudinal relaxation (T_1) Recovery of magnetisation along the 'z' axis. The energy lost manifests itself as an infinitesimal rise in temperature of the solution. This used to be called *spin-lattice relaxation*, a term which originated from solid-state NMR.

Magic Angle Spinning (MAS) 54° 44′ (from the vertical). Spinning a sample at this, the so-called 'magic angle' gives the best possible line shape as the broadening effects of chemical shift anisotropy and dipolar interactions are both minimised at this angle. Used in the study of molecules tethered to solid supports.

Meso compound A symmetrical compound containing two *chiral centres* configured so that the *chirality* of one of the centres is equal and opposite to the other. Such internal compensation means that these compounds have no overall effect on polarised light (e.g., meso tartaric acid).

Normal geometry Term used to describe the construction of a conventional dual/multi channel probe. Since the X nucleus is a far less sensitive nucleus than ^{1}H, a 'normal geometry' probe has the X nucleus receiver coils as close to the sample as possible to minimise signal loss and the ^{1}H receiver coils outside the X nucleus coils (i.e., further from the sample). This design of probe is thus optimised for X nucleus sensitivity at the expense of some ^{1}H sensitivity.

NOE *N*uclear *O*verhauser *e*ffect/*n*uclear *O*verhauser *e*nhancement. Enhancement of the intensity of a signal via augmented relaxation of the nucleus to other nearby nuclei that are undergoing saturation. See also:

NOE *N*uclear *O*verhauser *e*xperiment. Experiment designed to capitalise on the above. Such experiments (and related techniques, e.g., *NOESY*, etc.) are extremely useful for solving stereochemical problems by spatially relating groups or atoms to each other.

NOESY *N*uclear *O*verhauser *e*ffect *s*pectroscop*y*. Two-dimensional technique that correlates nuclei to each other if there is any *NOE* between them.

Non-first-order pattern Splitting pattern where the difference in chemical shift between coupled signals is comparable to the size of the coupling between them. These are characterised by heavy distortions of expected peak intensities and even the generation of extra unexpected lines.

Nyquist condition Sampling of all signals within an FID such that each is sampled at least twice per wavelength.

Phase The representation of an NMR signal with respect to the distribution of its intensity. We aim to produce a pure absorption spectrum (one where all the signal intensity is positive).

Phase cycling The process of repeating a pulse sequence with identical acquisition parameters but with varying r.f. phase. This allows real NMR signals to add coherently whilst artifacts and unwanted NMR transitions cancel.

Phasing The process of correcting the *phase* of a spectrum (either manually or under automation).

Probe Region of the spectrometer where the sample is held during the acquisition of a spectrum. It contains the transmitter and receiver coils and gradient coils (if fitted).

Pulse A short burst of radio frequency used to bring about some nuclear spin transition.

Pulsed field gradients (PFGs) See *Gradient pulse.*

Quadrature detection Preferred system of signal detection using two detection channels with reference signals offset by 90°.

Quadrupolar nuclei Those nuclei, which because of their *spin quantum number* (which is always >1/2), have asymmetric charge distribution and thus posses an electric quadrupole as well as a magnetic dipole. This feature of the nucleus provides an extremely efficient relaxation mechanism for the nuclei themselves and for their close neighbors. This can give rise to broader than expected signals.

Quadrupolar relaxation Rapid relaxation experienced by *quadrupolar nuclei.*

Racemate A 50/50 mixture of *enantiomers.*

Regiochemistry The chemistry of a molecule discussed in terms of the positional arrangement of its groups.

Regioisomers Isomeric compounds related to each other by the juxtaposition of functional groups.

Relaxation The process of nuclei losing absorbed energy after excitation. See *longitudinal relaxation* and *transverse relaxation.*

Relaxation time Time taken for *relaxation* to occur.

ROESY *R*otating-frame *O*verhauser *e*ffect *s*pectroscop*y*. A variation (one and two dimensional) on the nuclear Overhauser experiment (*NOE*). The techniques have the advantage of being applicable for all sizes of molecule. See *Laboratory frame model.*

Rotating frame model A means of visualising the processes taking place in an NMR experiment by observing these processes as if you were riding on a disc describing the movement of the bulk magnetisation vector.

Saturation Irradiation of nuclei such that the slight excess of such nuclei naturally found in the *ground state* when a sample is introduced into a magnet, is equalized.

Shim coils Coils built into NMR magnets designed to improve the homogeneity of the magnetic field experienced by the sample. Two types of shims are used: *cryoshims* and room temperature shims. Normal shimming involves the use of the room temperature shims.

Shimming The process of adjusting current flowing through the room temperature *shim coils* in order to achieve optimal magnetic field homogeneity prior to the *acquisition* of NMR data. The process may be performed manually or under automation.

Soft pulse Pulse designed to bring about irradiation of only a selected region of a spectrum. See *Hard pulse.*

Solvent suppression Suppression of a dominant and unwanted signal (usually a solvent) either directly by saturation or by use of a more subtle method such as the *WATERGATE* sequence.

Spectral Window The range of frequencies observable in an NMR experiment.

Spin coupling See *Coupling*.

Spin decoupling See *Decoupling* and *Broadband decoupling*.

Spin quantum number Number indicating the number of allowed orientations of a particular nucleus in a magnetic field. For example, ^{1}H has an I value of $^{1}/_{2}$, allowing for two possible orientations, whereas ^{14}N has an I of 1, allowing three possible orientations.

Spin-lattice relaxation See *Longitudinal relaxation*.

Spin-spin relaxation See *Transverse relaxation*.

Stereochemistry The chemistry of a molecule discussed in terms of its 3-D shape.

Stereoisomers Diastereoisomers related to each other by the inversion of any number of chiral centres.

Superconduction Conduction of electric current with zero resistance. This phenomenon occurs at liquid helium temperature and has made possible the construction of the very high powered magnets that we see in today's spectrometers.

TOCSY *To*tal *c*orrelation *s*pectroscop*y*. One and two-dimensional techniques that are analogous to *COSY* but which differ in that it shows couplings within specific spin systems.

Transverse relaxation (T_2) Relaxation by transfer of energy from one spin to another (as opposed to loss to the external environment as in *longitudinal relaxation*). This used to be referred to as *spin–spin relaxation.*

WATERGATE *Water* suppression through *gra*dient *t*ailored *e*xcitation.

Zero filling Cosmetic improvement of a spectrum achieved by padding out the *FID* with zeros.

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