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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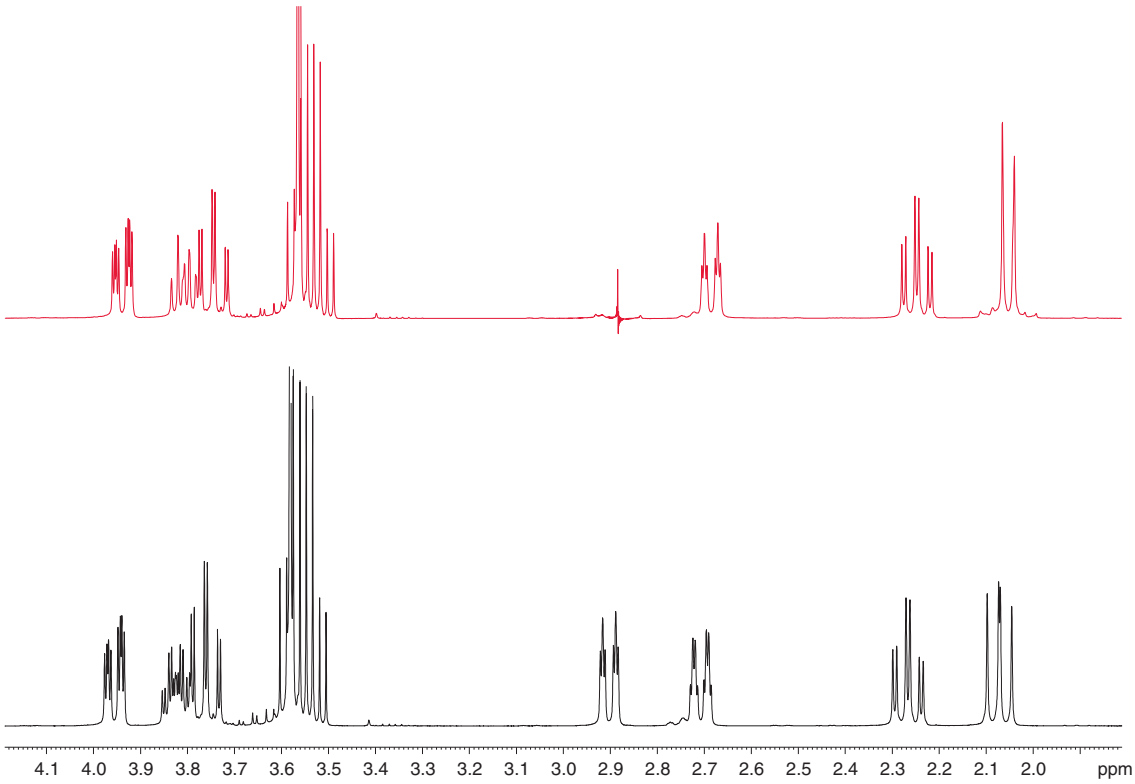


Spectrum 8.1 1-D spin decoupling experiment (decoupled at 2.9 ppm).

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

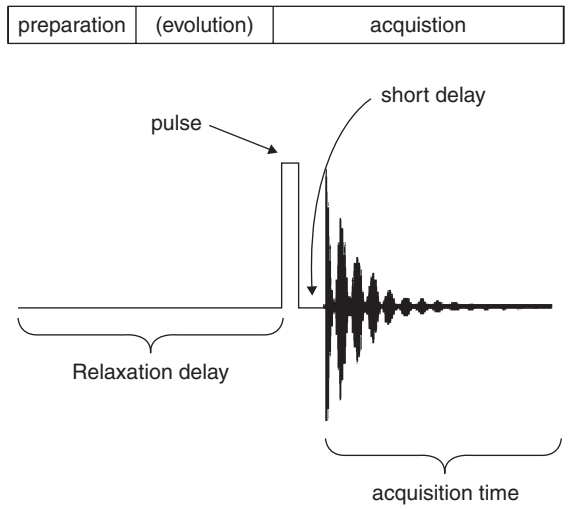


Figure 8.1 A typical 1-D pulse sequence.

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.

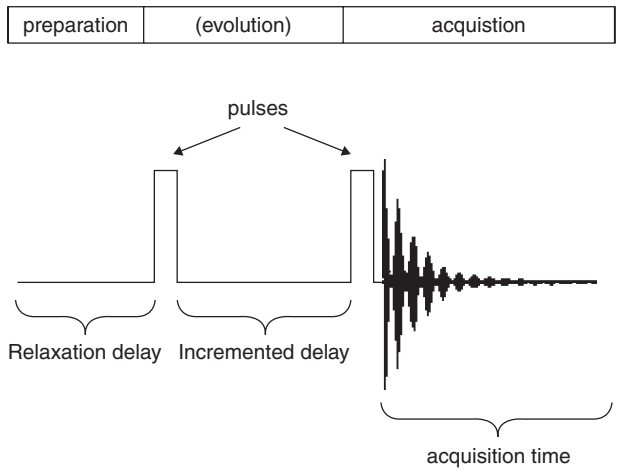


Figure 8.2 A simple COSY pulse sequence.

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

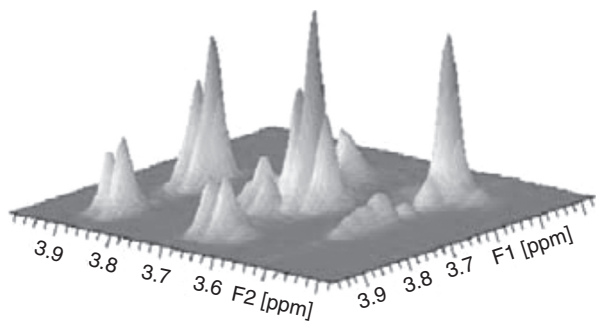
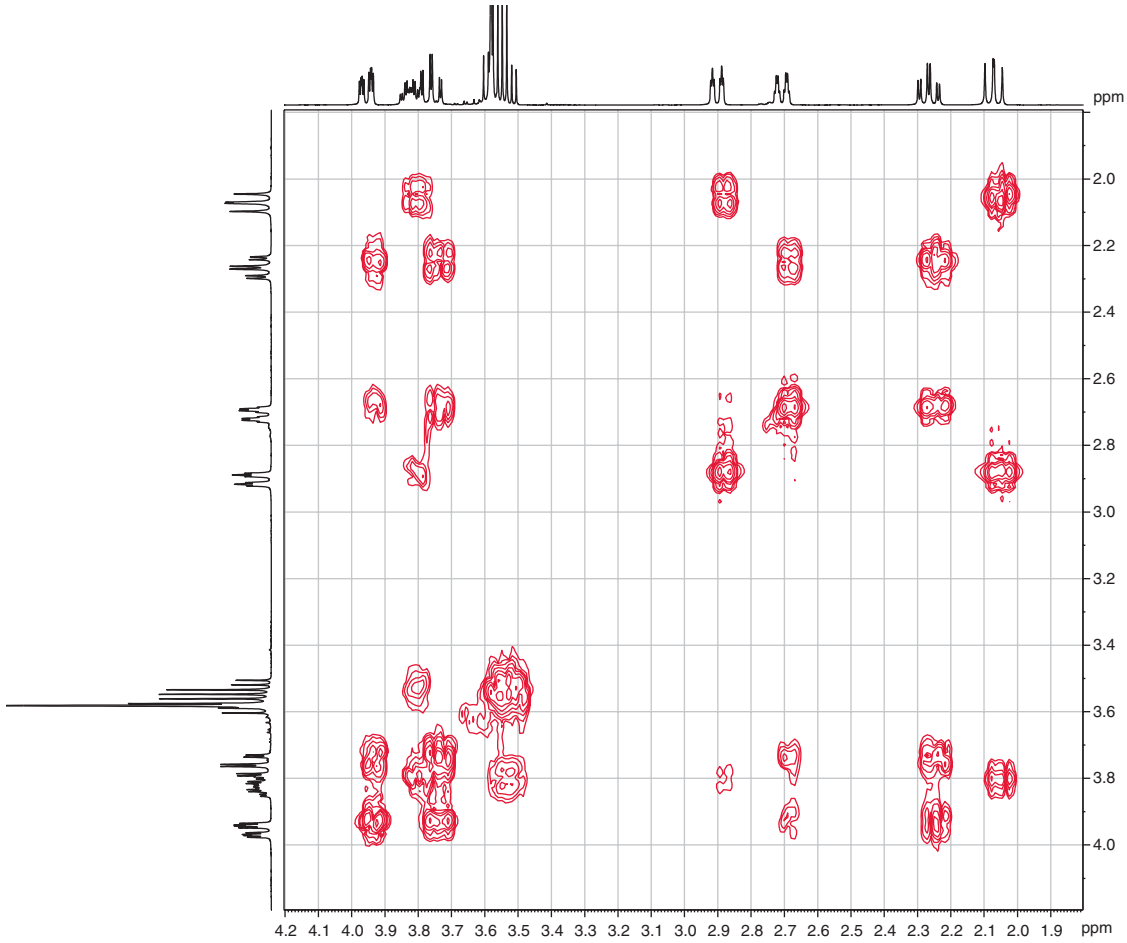


Figure 8.3 A COSY data set.



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!

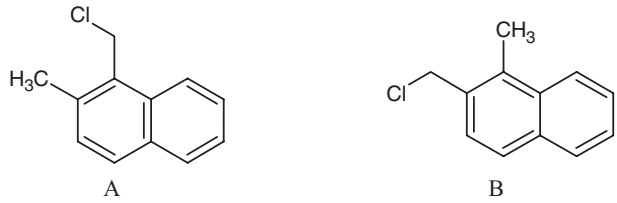
In the definition above, the term 'neighbouring nucleus' was used. The NOE is highly distance dependant – so much so that it falls off with the sixth power of the distance separating the nuclei in question. This very sharp distance dependency makes the effect a very useful tool for probing inter-atomic 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

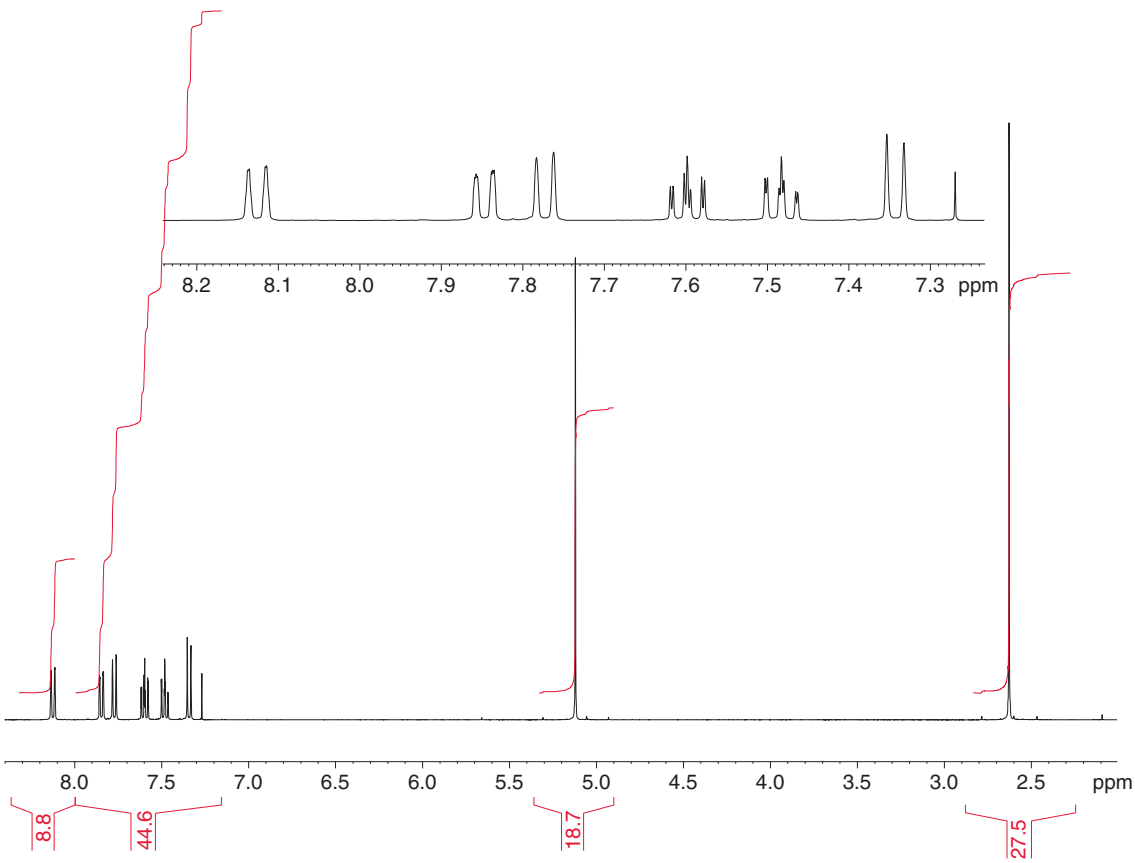
These two compounds would give very similar proton (and carbon) spectra and though an educated estimate of the -CH_2- and -CH_3 chemical shifts would give a good indication of identity *if both compounds were available*, we would never entertain such liberties if we had only one of the compounds in isolation. (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



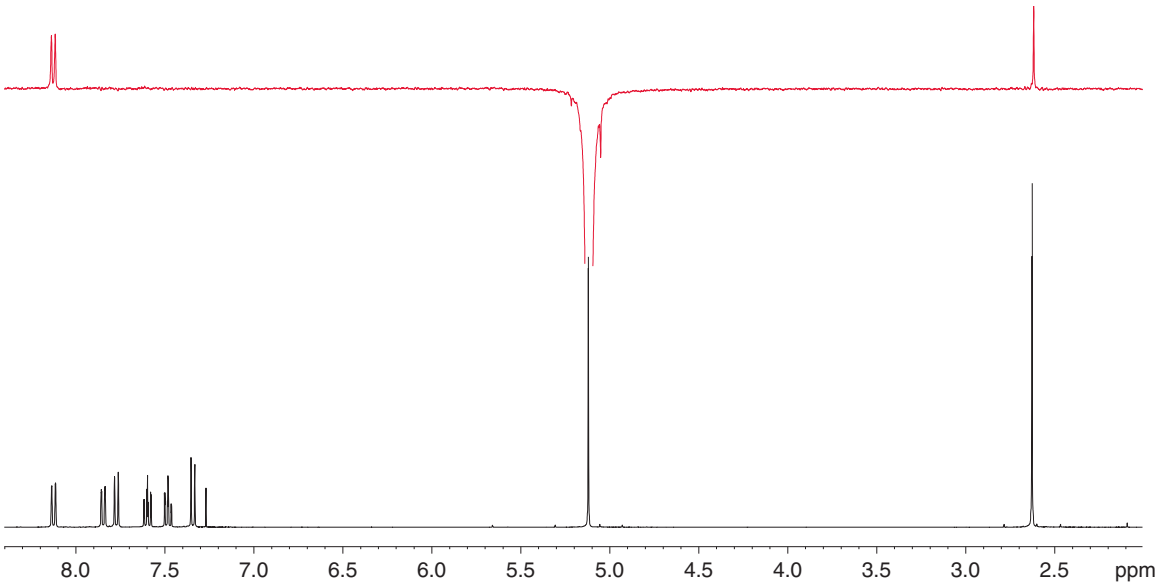
Structure 8.1 Two naphthalene structures which can be distinguished by NOE.

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.



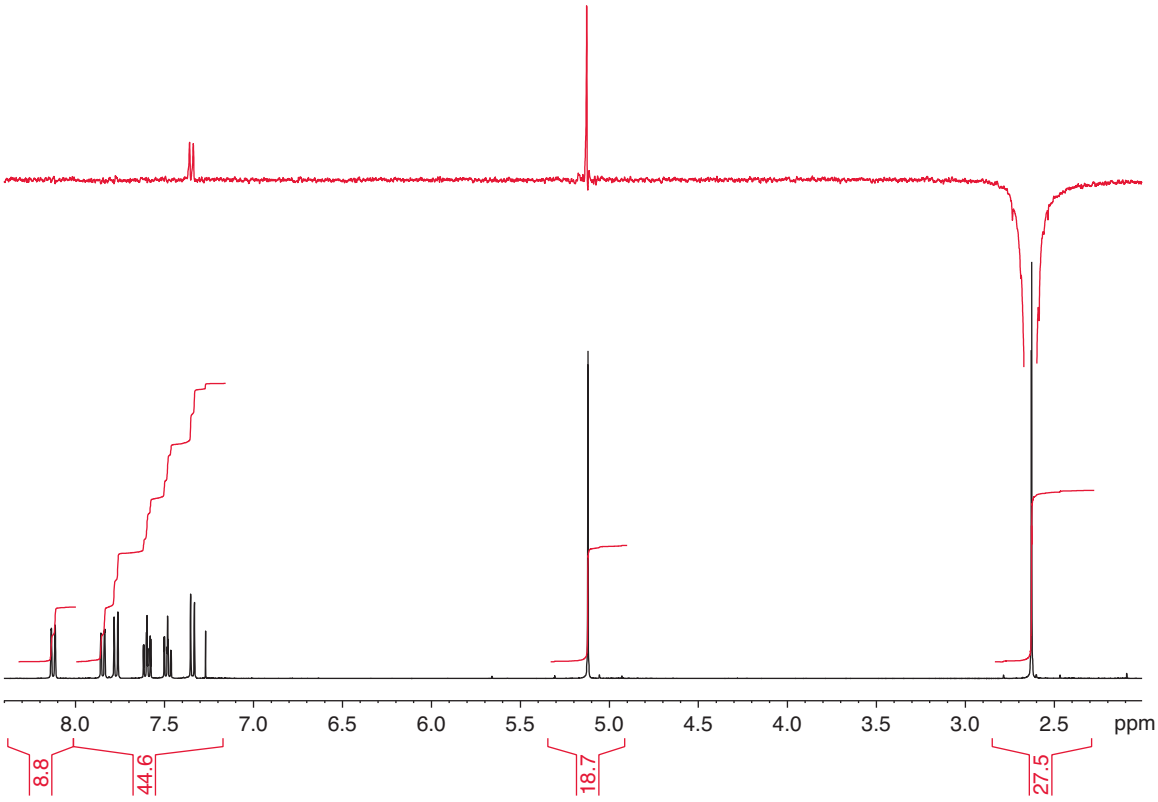
Spectrum 8.4 NOE experiment with irradiation of -CH_2- at 5.1 ppm.

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

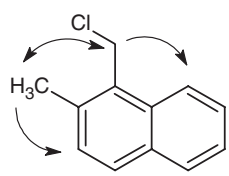


Spectrum 8.5 NOE experiment with irradiation of -CH_3 at 2.63 ppm.

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.



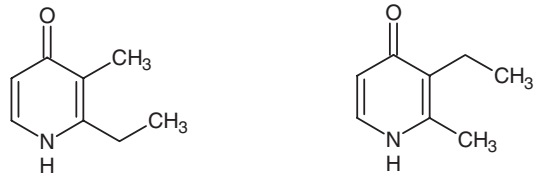
Structure 8.2 Correct naphthalene structure showing key NOEs observed.

Structure 8.3 Isomeric molecules.

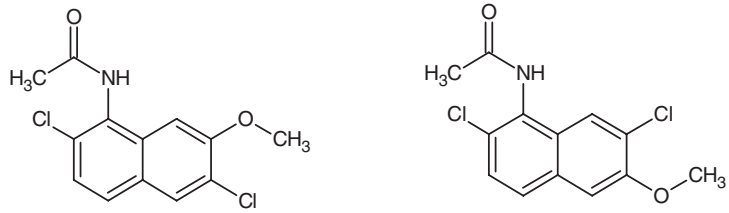
This phenomenon could give no end of potential problems with an NOE experiment. The molecules may exist in solution, predominantly as either hydroxy pyridines or as pyridones, depending to some extent on solute concentration, choice of solvent, its water content, pH and temperature. Irradiation of the 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.

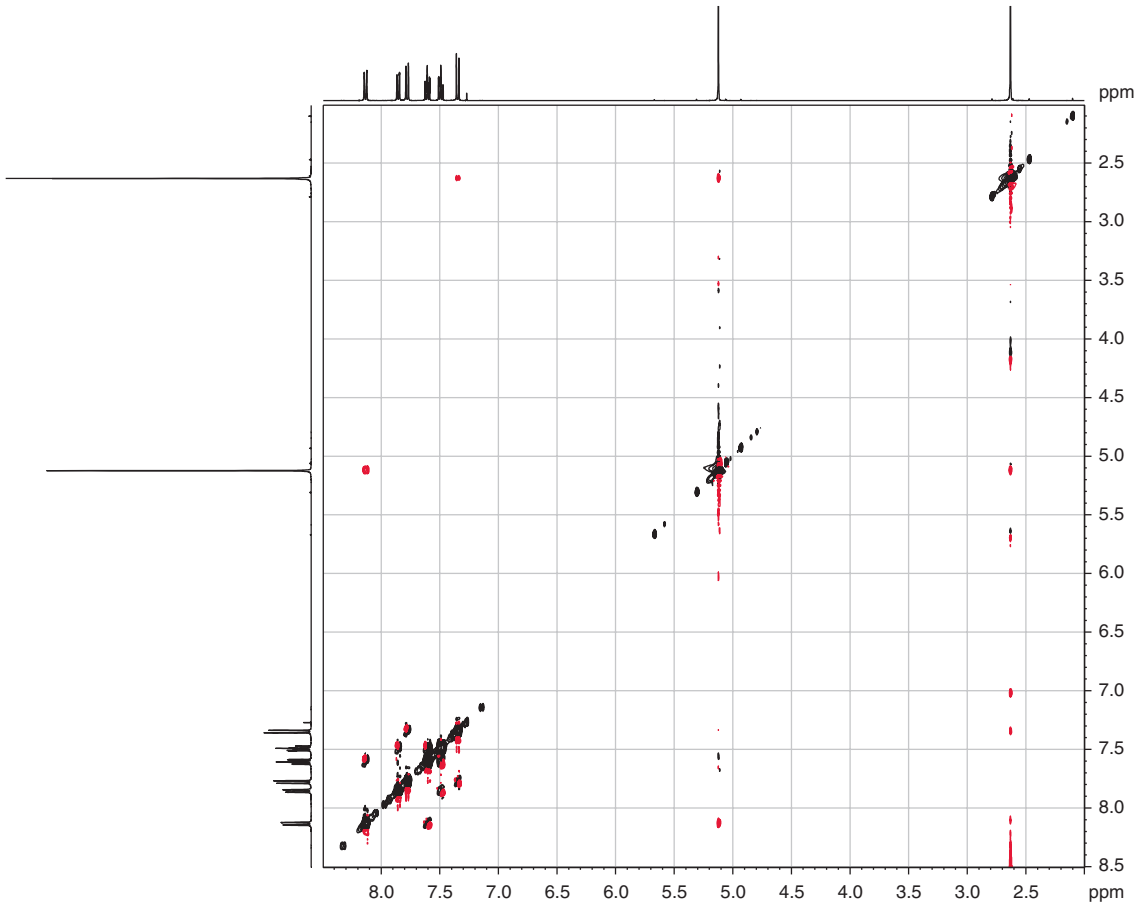
In this case, a better method might be to work from the aromatic signals of the AB pair to establish connectivity *to* the -OCH_3 rather than *from* it.

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

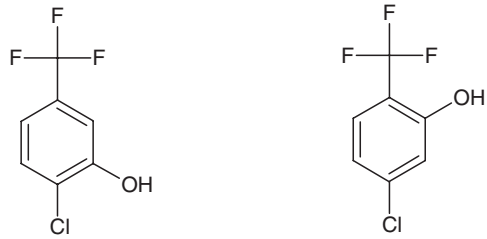


Spectrum 8.6 2-D ROESY spectrum of the naphthalene compound.

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.

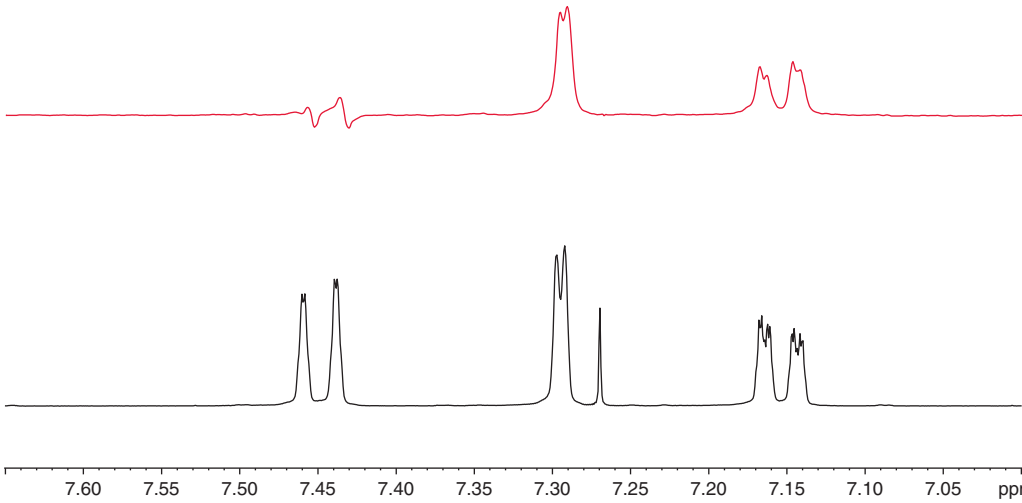
At the beginning of this section, we listed 1- and 2-D GOESY as an alternative method of collecting NOE data. This technique (*g*radient enhanced *O*verhauser *e*ffect *s*pectroscop*y*) is broadly similar to conventional NOE in terms of the results you achieve. In the 1-D case, there are no subtraction 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.)



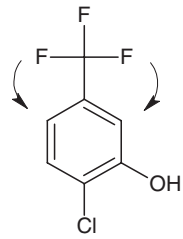
Spectrum 8.7 An NOE experiment with irradiation of the -CF_3 group at –62.85 ppm in the ^{19}F domain.

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.