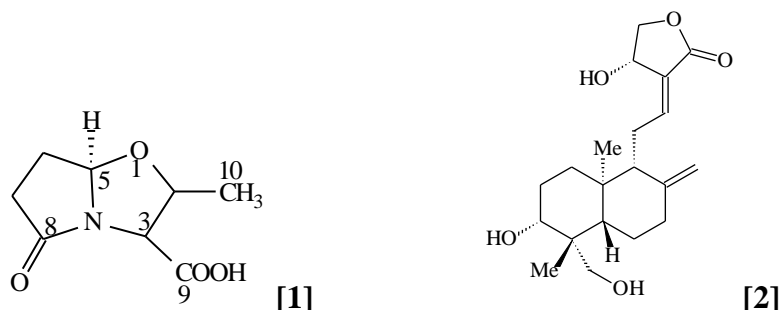


NMR Techniques in Organic Chemistry: *a quick guide*

The NMR spectrum proves to be of great utility in structure elucidation because the properties it displays can be related to the chemist's perception of molecular structure. The *chemical shift* of a particular nucleus can be correlated with its chemical environment, the *scalar coupling* (or *J-coupling*) indicates an indirect interaction between individual nuclei, mediated by electrons in a chemical bond, and, under suitable conditions, the area of a resonance is related to the number of nuclei giving rise to it. Thus, when we speak of "assigning a spectrum" we are really trying to identify a chemical structure that is consistent with the spectrum; we may have a fairly good idea of what this structure is likely to be, or we may have very little. There are a number of techniques in common use that help with this aim, as discussed below. This handout is not intended to provide descriptions of *how* the experiments work, rather it suggests *why* they are useful to the practising chemist. Compounds [1] and [2] below are used to illustrate these applications.



1D ¹H NMR

The ¹H nucleus is the most commonly observed nucleus in NMR spectroscopy. Hydrogen is found throughout most organic molecules and, fortunately for chemists, the proton has high intrinsic sensitivity as well as being almost 100% abundant in nature, all of which make it a favourable nucleus to observe. The proton spectrum contains a wealth of chemical shift and coupling information and is the starting point for most structure determinations.

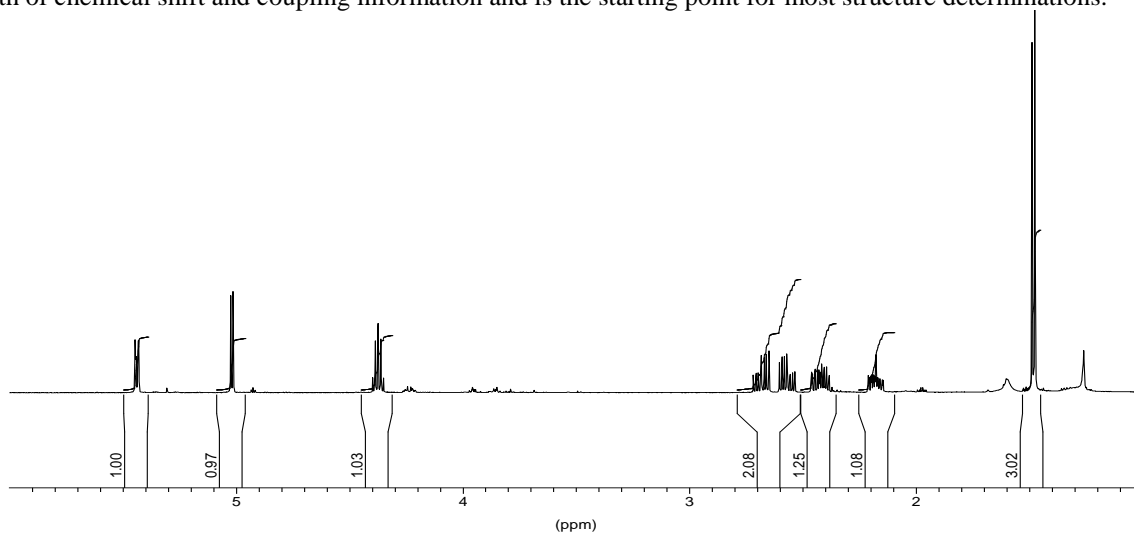


Figure 1: ¹H NMR spectrum of [1] with relative integrals.

In addition to these data, the area of a resonance, usually presented as its relative integral, relates to the number of protons giving rise to the signal, giving further information as to a possible structural fragment. However, for most routine acquisitions, the accuracy of such integrals is not high and may commonly show errors of 10% or more. This level of accuracy is usually sufficient if you wish to decide whether it is one or two protons that are giving rise to a particular resonance, but are unsuitable if you wish to get an *accurate* determination of, say, the relative proportion of two isomers in a mixture. Suitable adjustment of the acquisition parameters are required for the latter, but a discussion of these is not included here.

Homonuclear decoupling in ^1H NMR

The appearance of multiplet fine structure on NMR resonances is due to the presence of scalar coupling with another nucleus. In proton spectroscopy, such coupling will usually occur over two or three bonds (geminal or vicinal coupling respectively) and its presence provides direct evidence of a bond within a structure; it indicates *connectivity*. If the connectivity between all atoms in a structure is known, the gross structure is, therefore, defined. Coupling partners can often be identified by direct analysis of multiplet fine structure; if proton A shows a coupling to proton B of 7 Hz, then the coupling from B to A is also 7 Hz. However, it is often not possible to identify coupling partners in this way; the multiplet may be too complex to determine all coupling constants; one, or both, protons may be hidden under another resonance or many multiplets may display a coupling similar in magnitude to the one of interest. Homonuclear decoupling (which belongs to a class of experiments known as *double-resonance* experiments) offers a simple and effective means to identify coupled protons. The idea is to selectively saturate one multiplet in the spectrum with a radio-frequency during acquisition. This causes a loss of all couplings with the saturated proton and, hence, the multiplet structure of its partners will change. The example below illustrates this point. The spectrum of compound [1] shows a double-doublet at 5.44 ppm due to H5 and its coupling to the C6 protons, which resonate in the 2-3 ppm region. The multiplet structures of the resonances in this area are quite complex and do not lend themselves to direct analysis. A simple homonuclear decoupling experiment with saturation of the C5 proton unambiguously identifies the C6 protons by removal of the vicinal 5-6 couplings. The 2D COSY experiment (described below) is now more widely used for mapping couplings, although homonuclear decoupling is a useful method for simplifying multiplet structures to better enable their analysis or measure J values in some cases.

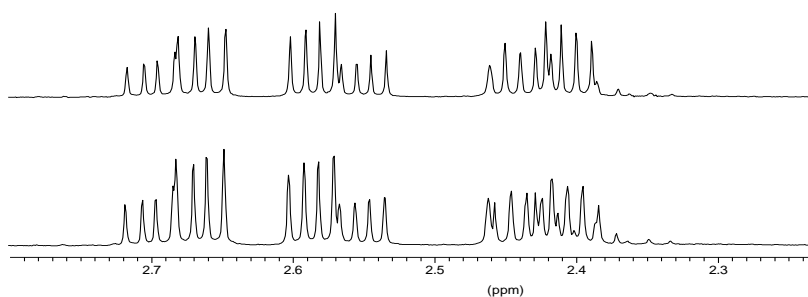


Figure 2: Homonuclear decoupling. The lower trace shows a section of the proton spectrum of [1]. The upper trace shows the same region when the proton at 5.44 ppm (H5) is decoupled. The change in the multiplet structure in one of the resonances identifies this as a C6 proton.

1D ^{13}C NMR

The ^{13}C spectrum offers further characterisation of the molecule as it relates directly to the carbon skeleton. We are forced to observe the carbon-13 nucleus as carbon-12 has no nuclear spin and is "NMR silent". Unfortunately, ^{13}C has a lower intrinsic sensitivity than the proton and has only 1.1 % natural abundance. The low abundance means that direct ^{13}C - ^{13}C coupling is usually never seen in the spectrum so we are unable to assign carbon-carbon connectivities directly. Each carbon may be coupled to a number of protons in the molecule, typically over one, two or three bonds such that the resulting carbon resonances have complex structure which further reduces signal intensity by spreading the resonance. The spectrum is, therefore, usually recorded with broadband decoupling of all protons. This removes multiplicity in carbon resonances, so that the doublet, triplet and quartet patterns indicative of CH, CH₂ and CH₃ groups, respectively, are not seen and each carbon resonance appears as a singlet (so increasing sensitivity). Typically one line is observed for each carbon atom in the molecule, as resonance overlap is rare. The broadband decoupling produces saturation of the proton resonances and this generates a nuclear Overhauser enhancement of the carbon signal, further increasing signal intensity.

The chemical shift of each resonance is again indicative of environment, and it is possible to identify certain functional groups for which there is no direct evidence in the proton spectrum e.g. carbonyls.

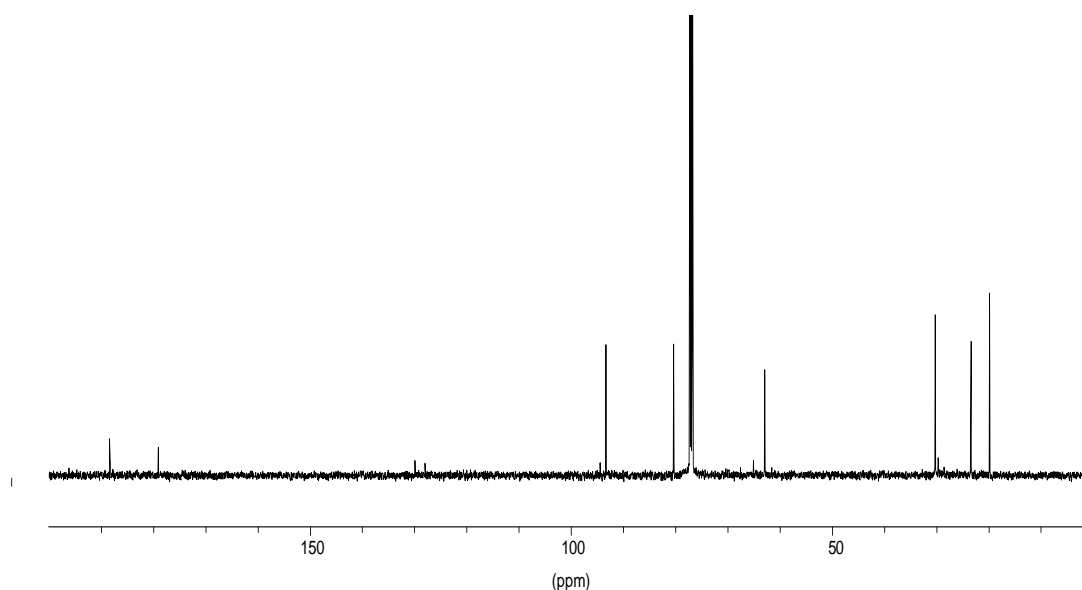


Figure 3: ^{13}C NMR spectrum of [1]. The weak resonances at 180 and 190 ppm are due to the non-protonated carbonyl carbons. The intense signal at 77 ppm is due to the solvent, CDCl_3 .

Unlike proton spectra, the routine carbon spectrum is unsuitable for integration, for a number of reasons relating to relaxation times, the nOe and the way in which the data are digitised. However, this is not usually a limitation when attempting to identify a molecular structure as precise integral data would be of limited use. Despite this, it is often possible to distinguish non-protonated carbon resonances from their low intensity relative to protonated carbons. This effect is due principally to long carbon relaxation times which result from the lack of a directly-bonded proton. This is apparent in figure 3 for the carbonyls at 180 and 190 ppm.

1D ¹³C DEPT

DEPT is "Distortionless Enhancement by Polarisation Transfer" and is used as a means of enhancing signal intensity and for editing spectra. As stated above, broadband proton decoupling removes multiplicity in carbon resonances, but the DEPT sequence allows one to establish the nature of the carbon atom whilst still acquiring broadband decoupled spectra, by making use of changes in signal intensities under differing experimental conditions. Three DEPT spectra are required for a full analysis and are termed DEPT-45, DEPT-90 and DEPT-135 (the number indicates the flip angle of the editing proton pulse in the sequence) The signal intensities in these spectra are as follows:

	CH	CH ₂	CH ₃
DEPT-45	+ve	+ve	+ve
DEPT-90	+ve	zero	zero
DEPT-135	+ve	-ve	+ve

Non-proton-bearing carbons are not seen in DEPT spectra because the technique relies on polarisation transfer, that is, in this case, the transfer of proton magnetisation onto the *directly bound* carbon. Analysis of the three spectra reveals the carbon multiplicities directly, or they may be combined appropriately to yield sub-spectra containing CH or CH₂ or CH₃ resonances only. Often, it is not necessary to acquire all three experiments to assign multiplicities. Methyl resonances are often easily identified as they frequently resonate at lower frequency, so a DEPT-135 alone may be sufficient to distinguish between methine and methylene protons. DEPT spectra for compound [1] are shown below, and should be compared with the carbon spectrum of figure 3. A recent variant DEPT-Q also retains signals from quaternary (non-protonated) centres, although these can be rather weak.

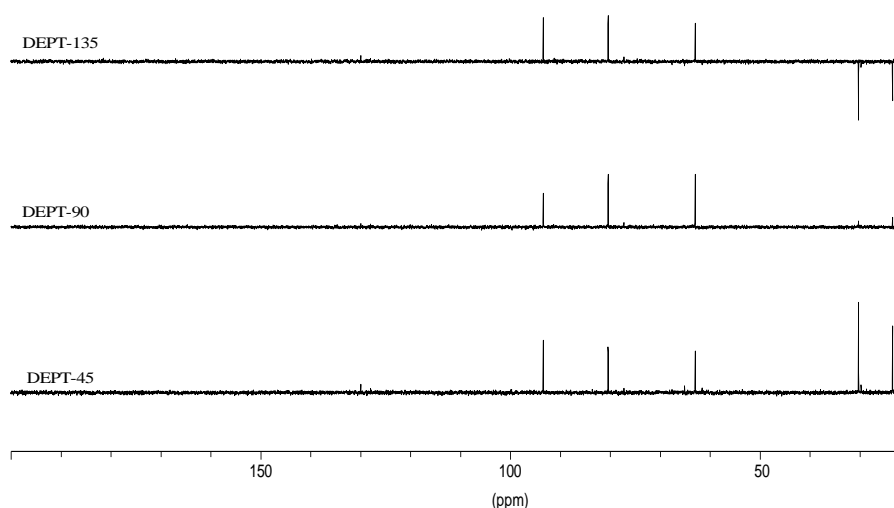


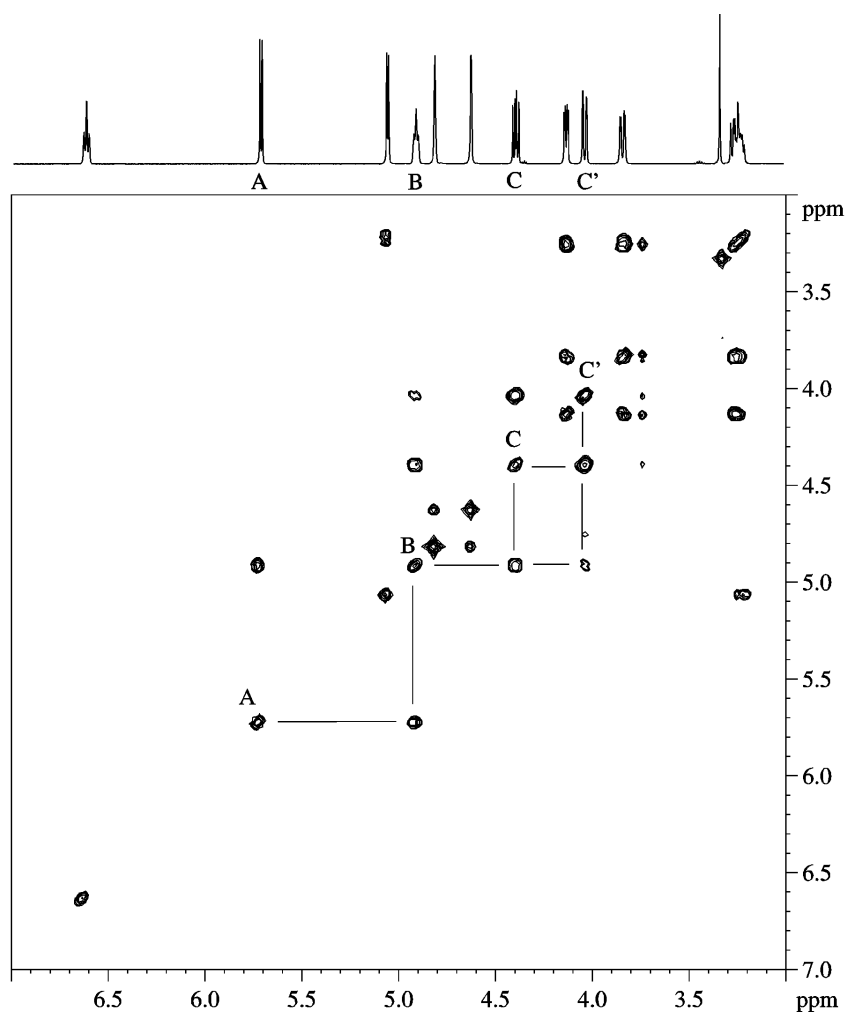
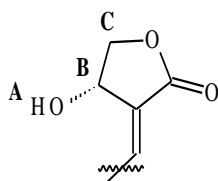
Figure 4: ¹³C DEPT spectra of [1]. Note that small signals remain for the methylene and methyl carbons in the DEPT-90 experiment; this is quite common but is not problematic as an unambiguous analysis is possible with all three spectra.

2D ^1H - ^1H COSY

The COSY experiment (COrelation SpectroscopY) provides a means of identifying mutually coupled protons and is the most widely used 2D experiment. It finds use when the homonuclear decoupling experiment is unsuitable, for example in complex spectra, where selective decoupling is not possible because of resonance overlap. The COSY experiment is a very efficient way of establishing connectivities when a large number of coupling networks need to be identified, as it maps all correlations with a single experiment and is now more frequently used than homonuclear decoupling.

The experiment presents a two-dimensional contour map, each dimension representing proton chemical shifts and the contours representing signal intensity (just as contours are used to map mountains heights). The diagonal (running bottom left to top right) shows peaks that correspond with those in the usual 1D spectrum, and contain no new information. The peaks of interest are the off-diagonal or *crosspeaks*. Each of these represents a coupling between the protons that are correlated by the crosspeak. The spectrum is symmetrical about the diagonal as a coupling from proton A to B will always be matched with one from B to A. A portion of the COSY spectrum of [2] is shown in figure 5 and the coupling pathway from the hydroxyl proton (which is slow to exchange in this dry DMSO sample) to neighbouring protons is illustrated. This experiment allows one to identify possible structural fragments, such as OH-CH-CH₂ in [2].

Figure 5: A section of the ^1H - ^1H COSY spectrum of [2]. Correlations between coupled protons are mapped out in a step-wise manner, as shown for the fragment below.



2D ^1H - ^{13}C HSQC (and HMQC)

The HSQC (Heteronuclear Single-Quantum Correlation) experiment is a *heteronuclear* correlation technique that offers a means of identifying 1-bond H-C connectivities within a molecule. The results are displayed in a similar manner to those from COSY, with one dimension of the 2D map representing ^{13}C chemical shifts and the other representing ^1H chemical shifts. Crosspeaks in the contour plot define to which carbon a particular proton (or group of protons) is attached, and it is therefore possible to map ^{13}C assignments from known ^1H assignments. The technique relies on magnetisation transfer from the proton to its directly attached carbon atom, and back onto the proton (for higher sensitivity) and so no responses are to be expected for non-protonated carbons (for example the carbonyl group at 170 ppm in figure 6) or for protons bound to other heteroatoms (for example the OH resonances in figure 6). Usually, one peak is observed at the frequency of each protonated-carbon resonance, although occasionally two are seen and this is indicative of a diastereotopic CH_2 group. Such an example is seen in figure 6 for the ^{13}C resonance of the terminal alkene at 110 ppm.

The HSQC experiment is also useful in the analysis of complex ^1H spectra as it provides dispersion of the proton spectrum along the ^{13}C dimension. Thus, a mass of overlapping multiplets in the proton spectrum can be spread apart by differences in ^{13}C chemical shifts, so allowing the ^1H chemical shift of each multiplet to be recognised. ^1H - ^1H COSY and ^1H - ^{13}C HSQC represent the primary 2D techniques of organic chemistry (the HMQC experiment provides equivalent data to HSQC but is now a less used alternative experiment).

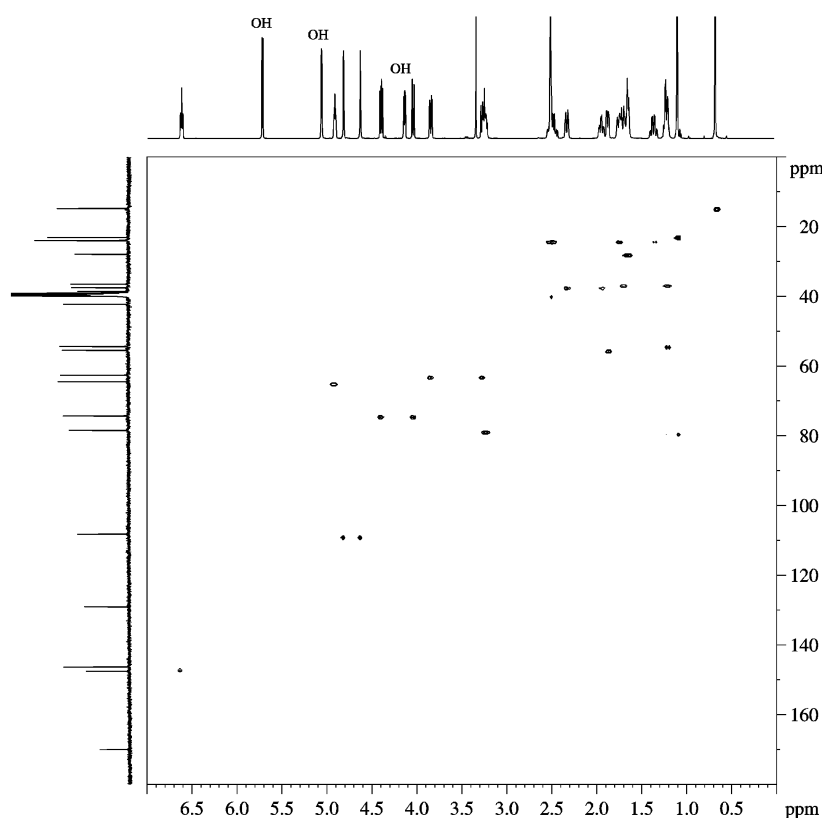


Figure 6: The HSQC heteronuclear correlation spectrum of [2]. One dimension represents ^1H chemical shift, the other ^{13}C chemical shifts.

Crosspeaks indicate one-bond ^1H - ^{13}C connectivity. The spectrum disperses the proton multiplets according to carbon shifts and thus can aid interpretation of the proton spectrum itself, in addition to providing carbon assignments.

2D ^1H - ^{13}C HMBC

This experiment (Heteronuclear Multiple-Bond Correlation) is closely related to HSQC and operates in essentially the same manner. In this case, however, the sequence timings are optimised for much smaller coupling constants and therefore seeks the correlations across more than one bond that arise from so-called *long-range* couplings. These ^1H - ^{13}C couplings typically occur with significant intensity over only 2 and 3 bonds ($^n\text{J}_{\text{CH}}$ usually <10 Hz), but may be apparent over 4 bonds in conjugated systems. Such experiments contain a mass of data on the molecular skeleton and can be extremely powerful tools in structure elucidation. The sequence also produces correlations across heteroatoms other than carbon (notice those from the hydroxyl protons below). Those prove useful when attempting to link fragments identified, for example, from ^1H - ^1H correlation spectra, and which show no ^1H - ^1H couplings between the fragments themselves. Correlations can also be observed to quaternary centres and can be used, for example, in the assignment of carbonyl resonances. In the example below a clear correlation can be observed between the lone alkene proton at 6.6 ppm and the carbonyl carbon on the adjacent lactone ring at 170 ppm. Note however, that as this experiment relies on correlations through small couplings, it is significantly less sensitive than the HSQC experiment.

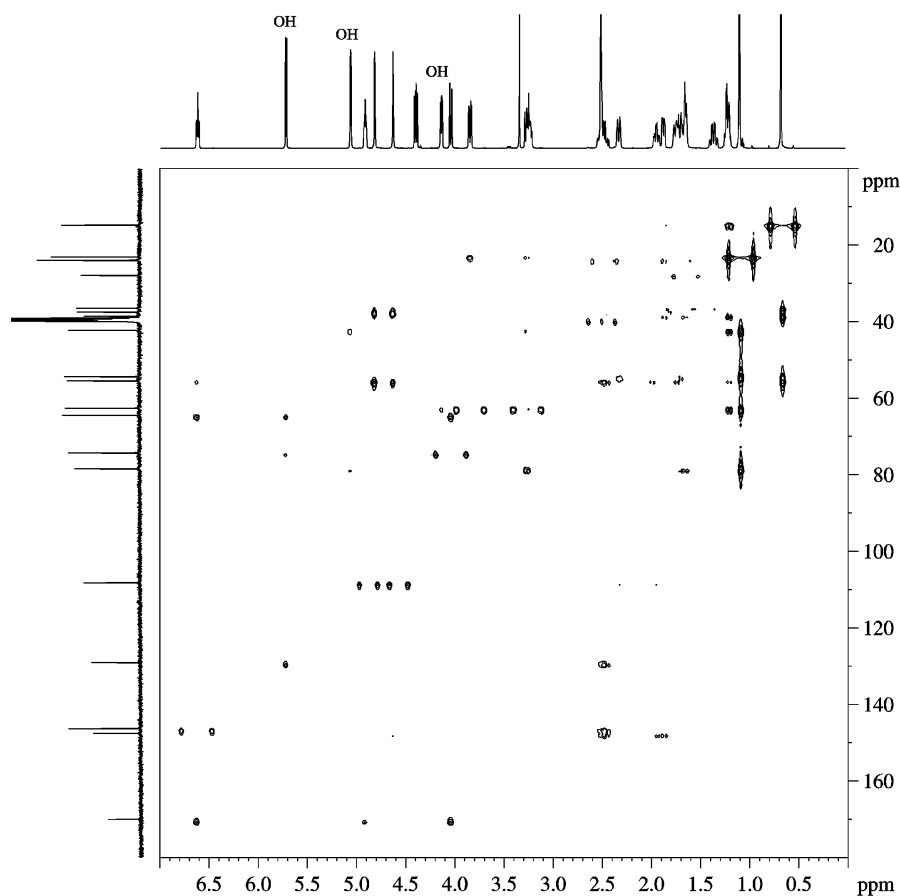


Figure 7: The HMBC spectrum of [2]. Numerous 2- and 3-bond correlations can be observed throughout the molecule and these provide a wealth of information on the molecular skeleton.

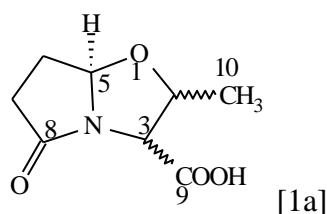
1D and 2D ^1H - ^1H nOe experiments

The nOe (nuclear Overhauser effect) experiment is used, primarily, to define the stereochemistry within a molecule. Unlike all the above techniques, its mode of operation relies on the direct, through-space interaction between nuclei, and is independent of the presence of through-bond couplings. This through-space effect is akin to the forces experienced between two bar magnets when brought towards each other. The conventional experiment was the nOe difference technique, but a better and quicker alternative now used widely is the gradient 1D NOESY experiment (see below).

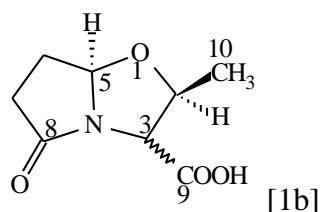
The 1D NOESY experiment involves the application of a radio-frequency field to a single resonance in the spectrum, such that the corresponding protons become inverted (that is, the population *differences* between their high and low energy levels are inverted). The system no longer has the initial population distribution between its energy levels, as defined by the Boltzmann distribution, and like any chemical system that has been perturbed from its equilibrium conditions, it will attempt to adjust itself so as to counteract the change. In the context of the nOe, this adjustment takes the form of changes in population differences for the energy levels of nuclei that are 'close in space' to the saturated proton. Recording of the proton spectrum after the period of change (allowing so-called cross-relaxation) may, therefore, show changes in signal intensities for those protons in the vicinity of the irradiated proton, due to these population changes. The observed intensity changes build with time but then decay through spin-relaxation so termed *transient nOes* and are usually rather small (often much less than 10%).

In practice, the nOe only operates over a short range ($< 4 \text{ \AA}$) thus providing evidence of spatial proximity of two protons. In transient nOe experiments, the initial build-up rates correlate with internuclear distances r (as $1/r^6$), providing a qualitative means of assessing relative distances, with the nOes usually interpreted as being small, medium or large and compared on a relative basis in routine studies.

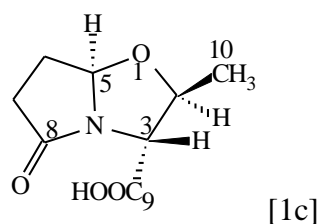
Consider the use of the nOe experiment on [1], figure 8. From the previous techniques, and by application of other analytical techniques, we may have arrived at structure [1a], below.



Although the overall structure has been established, we have no information on the stereochemistry at positions 2 and 3, although that at 5 is known from the chemistry involved in synthesising [1]. Irradiating H5 shows enhancements to H2 and to the H6 protons but nothing to the methyl proton. Irradiating H2 shows enhancements to H5, to H3 and, not surprisingly, to the methyl. These data so far suggest the H2 proton to be in the vicinity of H5, and is therefore down from the plane of the paper. This implies the methyl is up, irradiation of which shows no enhancement to H5 and likewise there is none from H5 to the methyl. All these results are consistent with structure [1b].



Irradiating H3 gives enhancement to H2 and to the methyl, but none to H5. Likewise, irradiating H5 has only a tiny influence on H3, and irradiating the methyl has a large effect on H3, all of which suggests H3 is up from the plane of the paper. The stereochemistry of [1] is, therefore, as shown in [1c]. It is important that all data are self-consistent and that they are consistent with only one stereoisomer.



When the nOe is used in this manner it is an immensely powerful tool; none of the other techniques discussed so far would have been able to provide the data needed to define the stereochemistry of [1]. However, it must be used thoughtfully and cautiously to arrive at an unambiguous assignment.

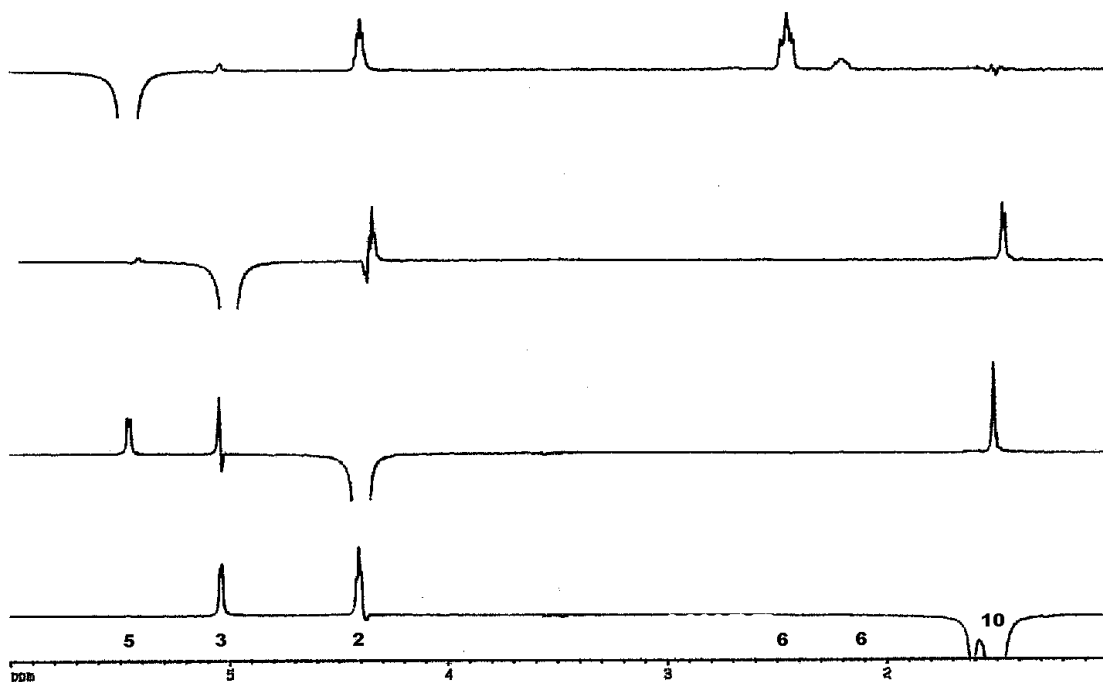


Figure 8: 1D nOe spectra for [1] (compare with figure 1). The negative-going signals represent the irradiated proton, whereas the positive signals are nOe enhancements caused by the saturation. Proton assignments are shown at the bottom.

The 2D NOESY experiment operates in a similar manner to the 1D version above and is interpreted in a similar way, but provides a complete map of (transient) nOes within a molecule in a single experiment, and resembles in appearance the COSY spectrum. This comparison between the 1D and 2D experiments parallels that between the homonuclear decoupling and COSY experiments described above for mapping J-coupling.

The greatest drawback to any nOe experiment is the fact that it has very low sensitivity, (meaning they are relatively long experiments to run so should be used wisely) and requires cautious interpretation to avoid erroneous conclusions being drawn.

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