# 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.

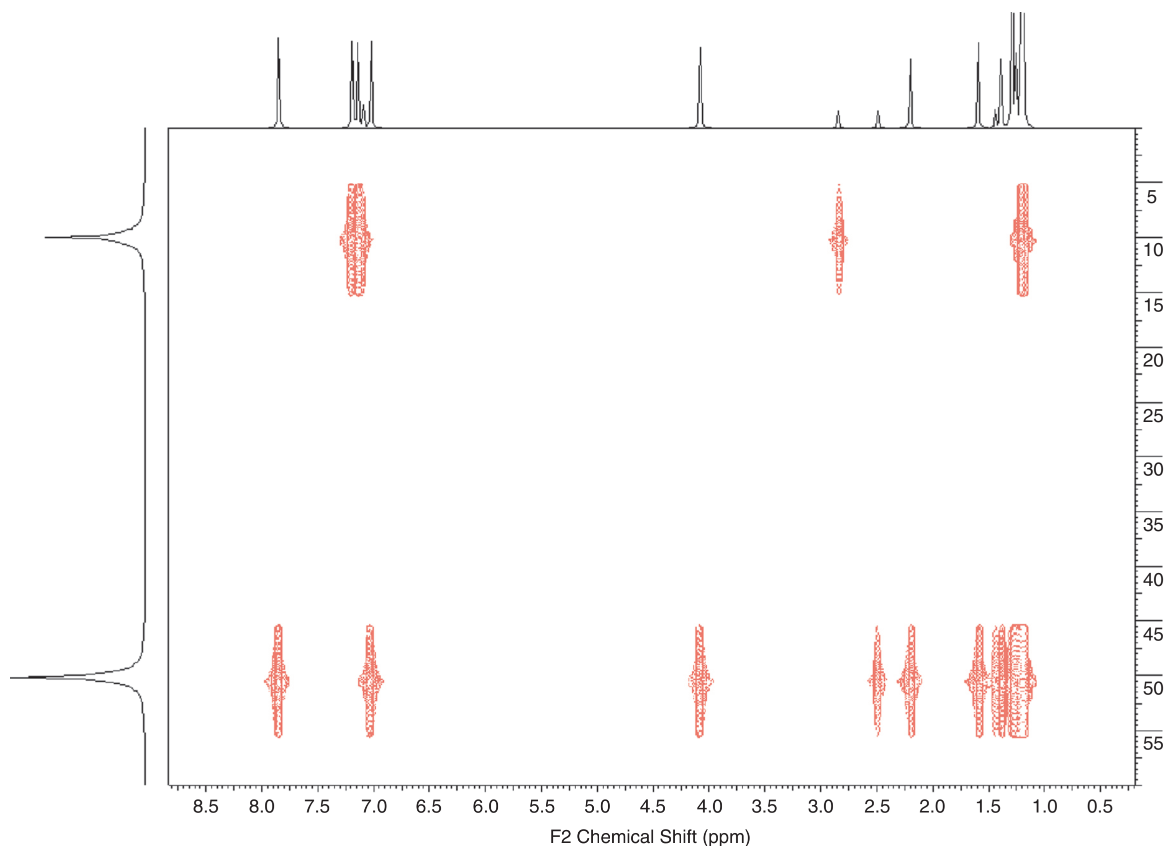
### 10.5.2 J-Resolved

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.