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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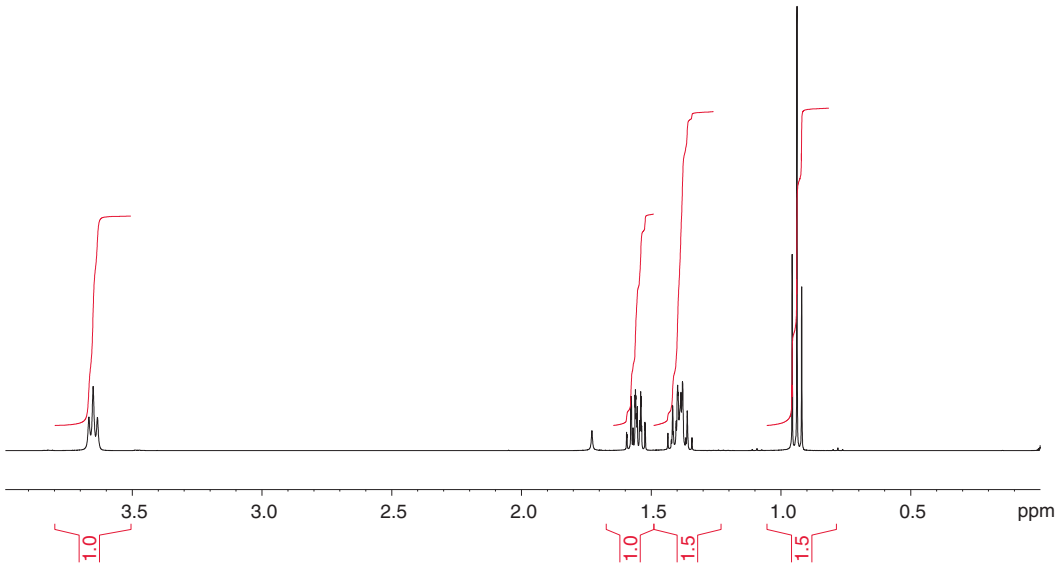
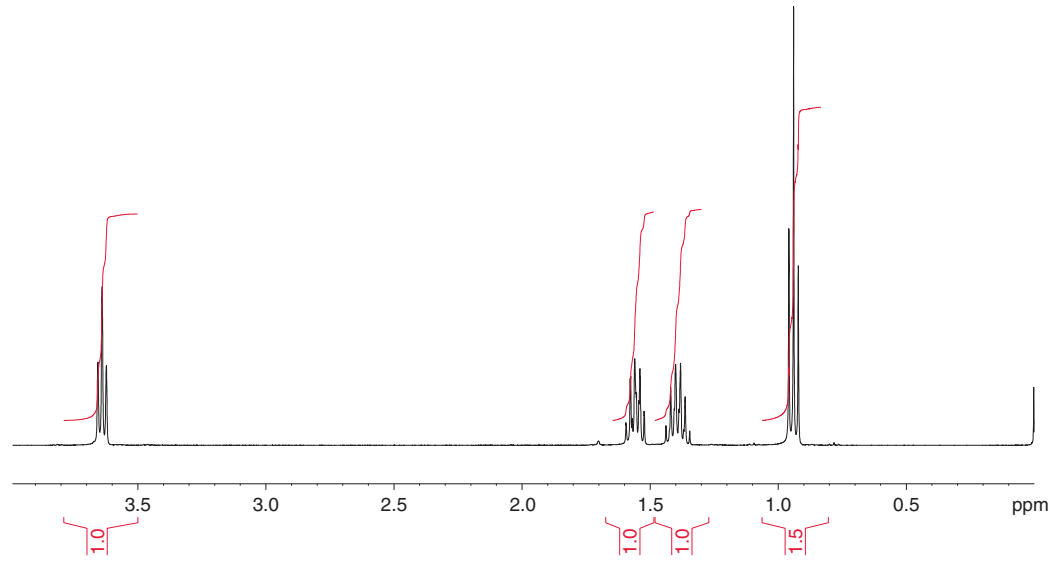
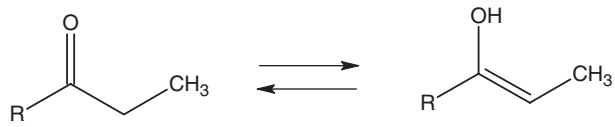


Spectrum 7.1 *n*-Butanol in $CDCl_3$ with -OH obscured by multiplet at 1.39 ppm.

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).

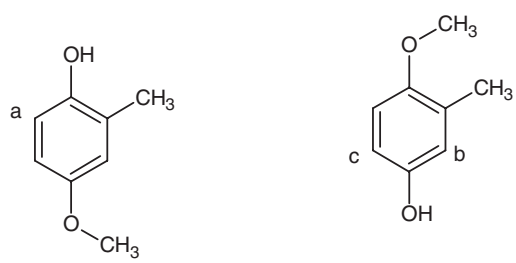
Note that deuterium exchange of the -OH leads to incorporation of deuterium alpha to the carbonyl in the ketone form. This may happen, even if there is no evidence of any enol signals in the spectrum initially, i.e., it can occur even when the equilibrium is heavily in favour of the ketone. Aromatic 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.

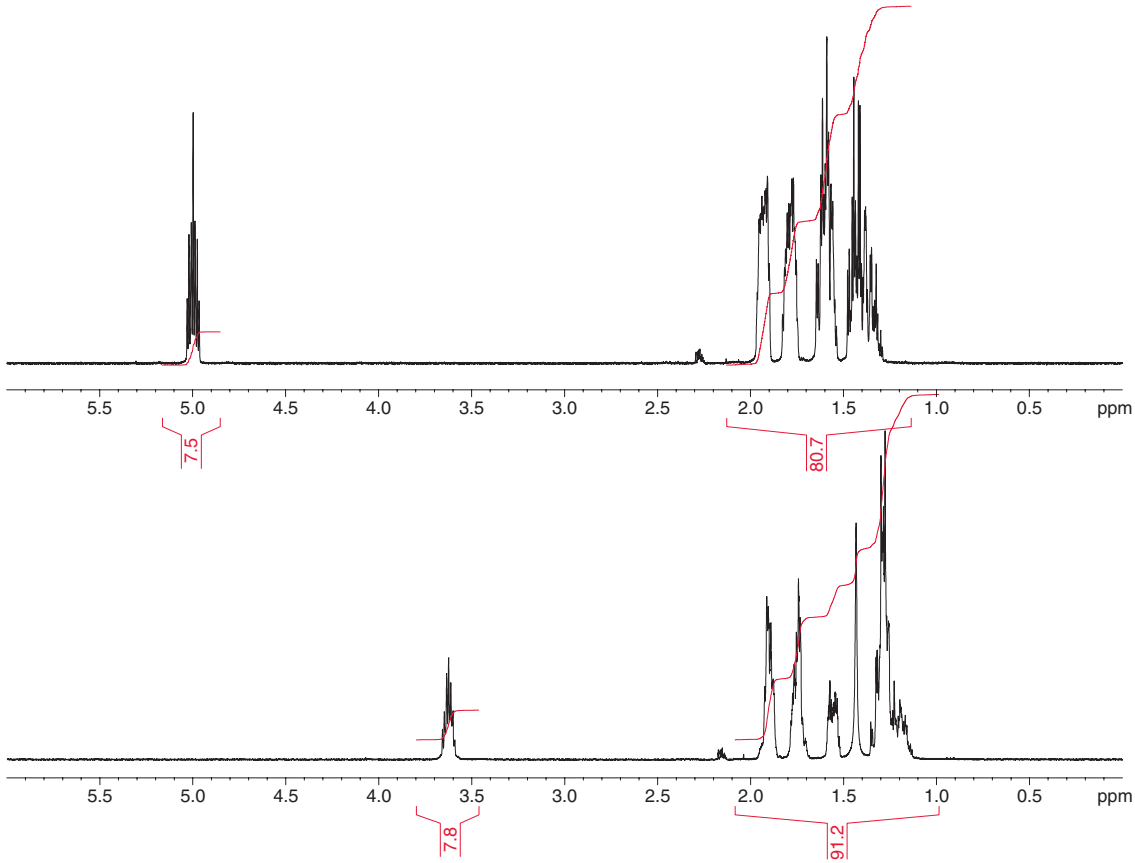
Consider Spectrum 7.3. The bottom trace shows the ordinary spectrum of cyclohexanol, run in $CDCl_3$. Distinguishing it from chlorocyclohexane is not easy (without the use of ^{13}C NMR) – the chemical shift of the proton alpha to the functional group would be similar in both compounds, and in the case 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.

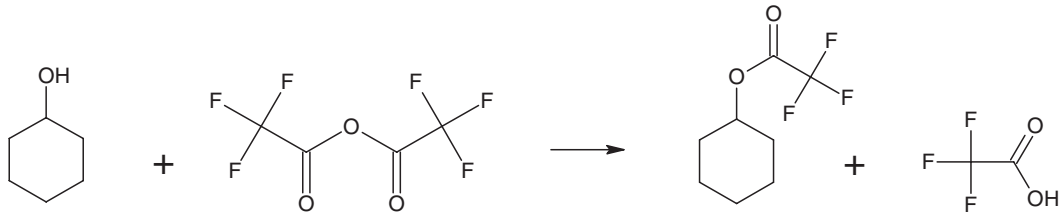
This is a relatively quick and convenient technique, the reagent reacting quite readily (assuming no great steric hindrance of course) with alcohols, primary and secondary amines. (Note the possibility of complication if you react a secondary amine with TFAA – it will yield a tertiary amide!) If the 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.

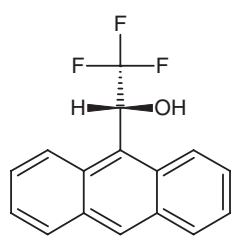
If, however, you are still soldiering on with a spectrometer of 100 MHz or less, then by all means try using them to 'stretch' a spectrum out – if your compound is suitable. They work by coordinating with an atom that has a lone pair of electrons available for donation. The more available the lone pair, 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

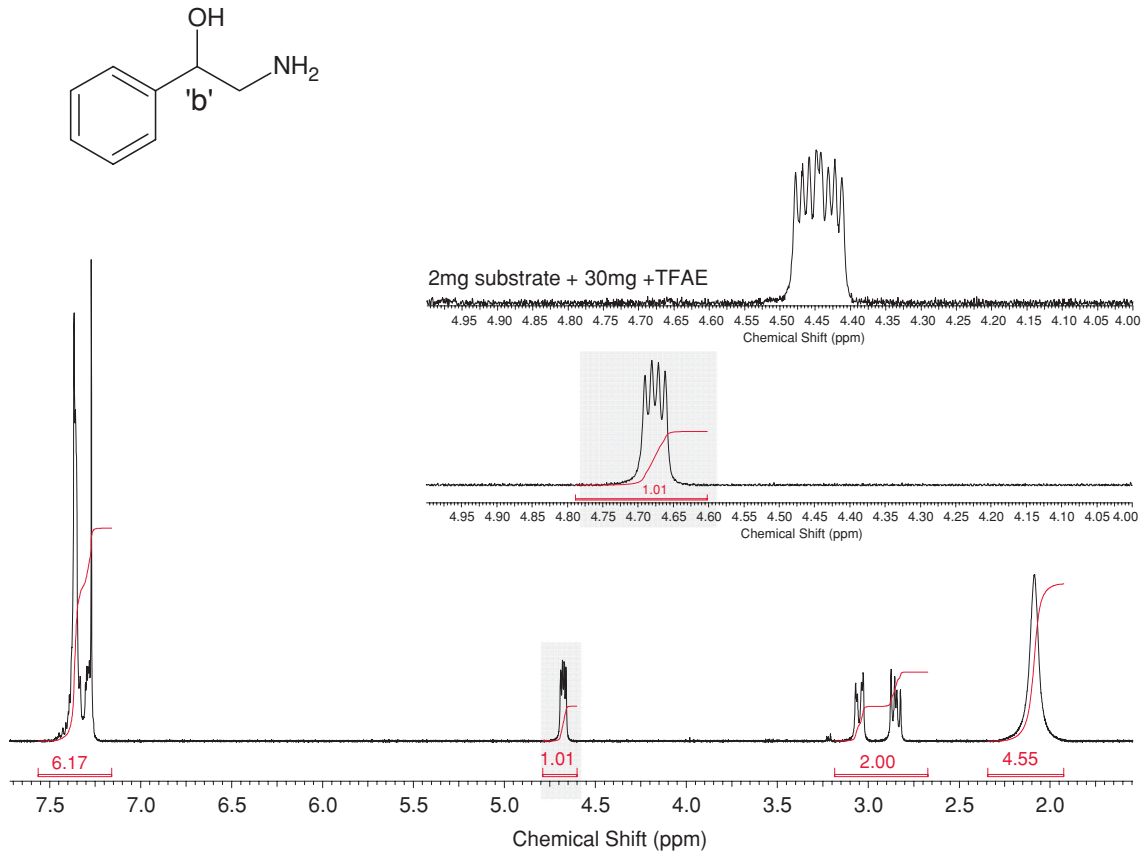
We have seen that the spectra of enantiomers, acquired under normal conditions, are identical. The NMR spectrometer does not differentiate between optically pure samples, and racemic ones. The wording is carefully chosen, particularly 'normal conditions', because it is often possible to distinguish enantiomers, 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.

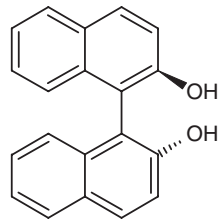


Spectrum 7.4 The use of TFAE as a chiral resolving agent.

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

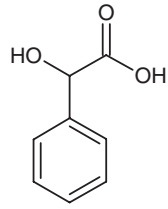
This is a member of an interesting class of compounds which are chiral, without actually containing a defined chiral centre. They are chiral because their mirror images are non-superimposable. In the case of this molecule, there is no rotation about the bond between the two naphthol rings because of 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'. . .