JPP Journal of Pharmacy and Pharmacology

JPP 2007, 59: 225–239

Received August 2, 2006
Accepted August 30, 2006
DOI 10.1211/jpp.59.2.0009
ISSN 0022-3573

Applications of solid-state NMR to pharmaceutical polymorphism and related matters*

Robin K. Harris

Department of Chemistry, University of Durham, South Road, Durham, DH1 3LE, UK

Robin K. Harris

Correspondence: R. K. Harris, Department of Chemistry, University of Durham, South Road, Durham, DH1 3LE, UK. E-mail: r.k.harris@durham.ac.uk

Acknowledgements: Many people have been involved in the work described herein, including my colleagues Drs P. Hodgkinson and D. C. Apperley, together with other Durham participants (A. King, A. Muruganantham, R. Fournier and Drs L. H. Merwin, P. Jackson, E. A. Christopher and A. M. Kenwright). Collaborators from elsewhere are Dr R. B. Hammond, Dr C.-Y. Ma and Professor K. Roberts (University of Leeds), Dr S. Cadars and Professor L. Emsley (ENS de Lyon), Drs S. Joyce, J. R. Yates and C. Pickard (University of Cambridge), Dr R. Lancaster (GSK, Stevenage), Dr M. Kinns and colleagues (Pfizer, Sandwich) and Professor U. Griesser (University of Innsbruck). I am particularly grateful to Dr P. Y. Ghi, now at the National School of Pharmacy, University of Otago, Dunedin, New Zealand. She played a key role in several of the projects whose results are briefly discussed in this article.

*This article is based on lectures given by RKH at the tenth Arden House European Conference organised by the Royal Pharmaceutical Society in London in March 2005 and at the 6th International Conference and Exhibition organised by Scientific Update LLP in Windermere in October 2005.

Abstract

Magic-angle spinning NMR is now making a significant contribution to our understanding of the structure of polymorphs and solvates of pharmaceutical significance. This personal review article discusses a range of applications, with particular emphasis on information about crystallography, for which NMR can address problems that cannot be readily solved by diffraction techniques (such as dynamic disorder and non-stoichiometric hydration). Unlike diffractograms, NMR spectra yield immediate chemical information. Moreover, heterogeneous samples can be investigated and amorphous content provides no significant barrier to studies. Furthermore, NMR can be an effective technique for quantitation (down to the level of ca. 1%). Additional strength is being derived from computation of chemical shifts in solids, using a code that takes account of the spatial repetition inherent in crystalline materials.

Introduction

Many tools have been brought to bear in investigations of chemical and crystallographic structure in the solid state, with diffraction methods and the various forms of spectroscopy appearing prominently. In principle (and frequently in practice), diffraction experiments on single crystals produce detailed maps of atomic positions in crystallographic unit cells and such work is therefore regarded as the gold standard of solid-state characterization. However, there are more weaknesses in diffraction techniques than is sometimes realised, so that other measurements are often desirable. Indeed, the best strategy for any study of the solid state clearly involves a combination of a wide range of methods. The problem of obtaining single crystals can sometimes be overcome by full analysis of diffraction patterns of powders (i.e. microcrystalline material) using recently developed analysis techniques (Harris et al 2001). Molecular structure is usually known before diffraction work, the most important technique in this context being solution-state NMR. However, it has to be recognised that sometimes aspects of chemical structure change with phase (e.g. conformation, tautomeric form, etc.). Obviously, if solid-state NMR can give spectral resolution approaching that obtained for solutions, it can act as a bridge between solution-state NMR and single-crystal diffraction. This possibility has been realisable since the pioneering experiments of Schaefer & Stejskal (1976), which combined cross polarisation (CP), magic-angle spinning (MAS) and high-power heteronuclear decoupling. Such methods are required both to improve the sensitivity of NMR for solids and to overcome problems associated with the fact that molecular mobility is relatively low in the solid state. By these means, properties such as the dipolar interaction, which is orientation-dependent, are averaged so that they do not generally influence the resonance frequencies (a process which occurs automatically for solutions because of molecular tumbling). The techniques of CPMAS NMR are now routinely applied (Duer 2004) but they have been expanded to give a series of highly sophisticated experiments, involving a variety of pulse sequences, to address a range of solid-state issues, including questions of molecular-level mobility and phase transformations as well as chemical structure. In the early years of high-resolution solid-state NMR, the emphasis was on applications to polymers and catalysts (especially zeolites) and, although some work was carried out on organic and pharmaceutical compounds, it is only in the last decade that the power of solid-state NMR in this area has become widely recognised. It is particularly in questions of polymorphism and solvate formation that the technique is now heavily used, as has recently been reviewed (Harris 2006). Such matters are ubiquitous

in pharmaceutical chemistry and cause many headaches for industry (Bernstein 2002). Traditionally, powder X-ray diffraction (PXRD) and vibrational spectroscopy have been the tools used to recognise various solid forms of a given compound and thermal methods have been used to study their transformations. However, it is now arguable that solid-state NMR is the technique par excellence for such work. Moreover, it is becoming recognised that NMR has a strong contribution to make to crystallography per-se (Harris 2004).

This article arises from invited lectures the author has recently given at international conferences and it gives an overview of some of the work in the author's laboratory in the recent past, to exemplify the contributions solid-state NMR can make to questions that arise in pharmaceutical polymorphism, solvate formation, etc. Of course, further examples from a wide range of laboratories are to be found in the review article mentioned above (Harris 2006), together with the references therein. In fact, three interlocking themes run through our recent research, namely NMR crystallography, understanding chemical shifts for solids, and organic/pharmaceutical polymorphism.

NMR crystallography is an emerging discipline to add to the recognised areas of X-ray crystallography, neutron crystallography and electron crystallography. Its power lies partly in its complementary nature to diffraction methods, and of course it will frequently be necessary, in solving solid-state structural problems, to use the two types of technique together (and also with other approaches). It is worthwhile, at this point, to emphasise some of the weaknesses of diffraction experiments, in particular those of single-crystal X-ray work. These may be summarised as follows:

- Diffraction relies on long-range order, so that crystals of a reasonable size are needed (though recent advances mean that for X-ray work on a medium-sized organic molecule a crystal of 0.1 mm^3 should be suitable — and the minimum size may be 0.01 mm^3 if synchrotron radiation is used).
- Electron density is the determining factor rather than positions of nuclei (though neutron diffraction does depend on nuclear positions — but this requires significantly larger crystals).
- Powder diffraction is less powerful than single-crystal work, in spite of recent advances.
- Difficulties may be encountered in cases of twinning or significant amounts of defects.
- Disorder can also cause problems, and it is generally not feasible to distinguish spatial from dynamic disorder.
- In fact, in cases of disorder even the definition of a space group is problematic since translational symmetry does not strictly speaking pertain.
- Molecular-level mobility may cause complications and it is not possible to recognise or obtain information about mutual site exchange which occurs by jumps (e.g. 180° flips of phenyl groups). The timescale of diffraction experiments is the exposure time to the X-ray or neutron beam.
- The precise location of hydrogen atoms can still be difficult for X-rays (though not for neutrons), especially in the presence of heavy atoms. This is particularly important for hydrogen bonds.
- Little or no information can be obtained for amorphous solids.
- Heterogeneous materials cannot be studied by single-crystal methods.

By contrast, some of the characteristics of high-resolution NMR of solids (NMR crystallography), which will be explored in later sections of this article, are:

- The spectra depend on the local environment of the nucleus in question, not on long-range order.
- Nuclei are specifically targeted, and the technique is isotope-specific, so that it is always clear which element is being studied and multinuclear work can build up the information content.
- Detailed information is available from microcrystalline (powdered) samples. Indeed, such samples are the norm.
- NMR information can be combined with powder diffraction results to improve the efficiency of determination of crystal structures.
- Immediate information is available on the crystallographic asymmetric unit (i.e. the atomic group which, by the full repetition arising from the symmetry, gives the total structure) — see below. In some cases one obtains further information about molecular symmetry in the crystalline environment and it is even feasible to determine space groups (King 2001).
- Direct internuclear distances are available from dipolar coupling constants (Bennett et al 1994) when special pulse sequences are used.
- Molecular conformations and intermolecular interactions can be assessed from chemical shifts.
- Information on hydrogen atoms is accessible from proton or deuteron NMR.
- Spatial disorder can be examined and dynamic disorder investigated if it occurs with lifetimes of the order of a few ms.
- Molecular-level mobility can also be obtained by measurement of relaxation times.
- Amorphous materials can be studied readily, giving nearly as much information as crystalline systems.
- Heterogeneous systems are also readily examined, and, if suitable experimental precautions are observed, components can be quantified down to less than 1% of the total sample.
- Polymorphs can be identified by inspection of the spectra.
- Solvates can also be recognised, whether they are stoichiometric or not.

Clearly, NMR has some disadvantages, the principal one of which is the relatively large amount of sample required (ca. 200 mg for ^{13}C in natural abundance and ca. 20 mg for ^{1}H). Even with these amounts, some experiments require long times.

Examples of each of the three themes, taken from work by the Durham group, will be discussed briefly below, though in practice the cases presented invariably involve at least two of the three themes. Emphasis will be placed on the value of isotropic chemical shifts (Harris 2004).

Theme 1 — Polymorphism and related phenomena

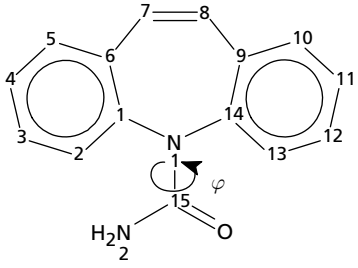
As mentioned in the Introduction, problems involving polymorphism of organic and pharmaceutical compounds are widespread and cause great concern to industry (Bernstein 2002). Metastable

forms are often produced but not always recognised as such. Preparations of particular modifications are sometimes not robust and do not work on scale-up. Unexpected transformations occur during processing and storage, and reproducibility of production can be a problem, giving so-called disappearing polymorphs (Dunitz & Bernstein 1995). Such matters are vital in questions of patent establishment and protection, resulting in many high-profile cases of litigation. All this means that tools for recognising and tracking polymorphs and solvates are a vital requirement. The following are some examples of ways in which solid-state NMR can address various problems of this nature.

Distinguishing polymorphs and solvates

Figure 1 shows the ^{13}C magic-angle spinning spectra of the four anhydrous forms of carbamazepine (**I**) (Harris et al 2005c). It is worth noting that, although most of the signals occur over a narrow chemical shift range (causing substantial spectral overlap), the spectra are all noticeably different, so that NMR can readily be used for identification.

Figure 2 illustrates the ^{13}C spectrum of the cycloheptanone solvate of sulfathiazole (**II**) (Portieri 2001). The signals arising from the incorporated solvent molecules are easily distinguished and it can be shown that the host–solvent molecular ratio is 1:1.



Carbamazepine **(I)**

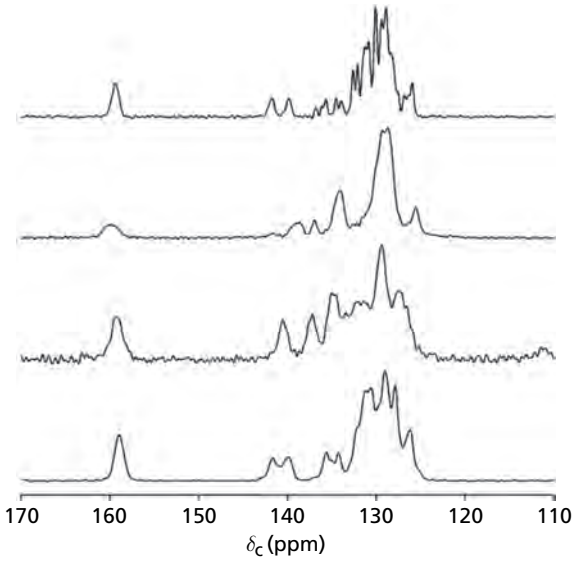
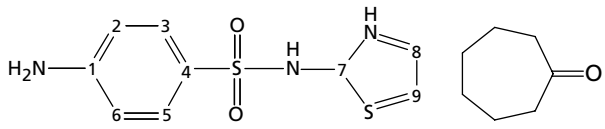


Figure 1 Carbon-13 CPMAS spectra of various polymorphs of carbamazepine (**I**). Top to bottom: trigonal form, P-monoclinic form, C-monoclinic form, triclinic form.



Sulfathiazole: cycloheptanone adduct **(II)**

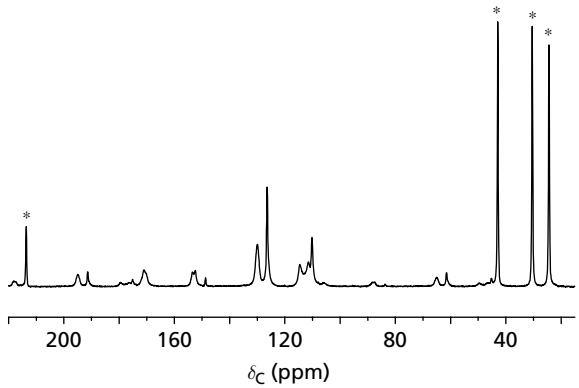


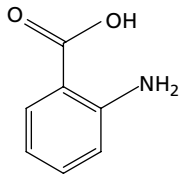
Figure 2 Carbon-13 CPMAS spectrum of the cycloheptanone adduct of sulfathiazole (**II**). The signals given by the cycloheptanone moiety are indicated by asterisks. Small peaks outside the region 90–180 ppm are spinning sidebands.

Detecting tautomeric form in polymorphs

Whether different tautomeric forms are considered as polymorphs is a moot point, but certainly their detection is of some importance. The system 2-aminobenzoic acid (**III**) involves at least three forms, and questions of tautomerism arise: do they contain neutral molecules or zwitterions? Carbon-13 MAS NMR supplies a ready answer (Harris & Jackson 1987), since the anticipated chemical shifts differ widely. It turns out that two of the forms contain only neutral molecules, whereas the third has an asymmetric unit consisting of one neutral molecule and one zwitterion, as can be clearly seen from Figure 3. In fact, ^{1}H MAS NMR shows that the NH_2 and NH_3^+ protons are rapidly exchanging on the NMR timescale under ambient conditions, a feature that became explicable from the complete crystal structure (Brown 1968; Brown & Ehrenberg 1985), which shows them to be close in space but remote from the unique carboxylic proton.

Transitions between polymorphs

It is a simple matter to monitor phase transitions by solid-state NMR so that kinetics can also be readily determined on timescales from minutes to days (and longer if samples are held at relevant temperatures outside the spectrometer between recording sessions). For rapid transitions, it may be difficult to measure the kinetics, but in such cases it is becomes easy to determine transition temperatures. Figure 4 shows ^{13}C spectra (Rubin-Preminger et al 2004) of two forms of [S,S]-ethambutol hydrochloride (**IV**). These interconvert by a single-crystal to single-crystal mechanism, which is very rapid. The figure shows spectra obtained around the transition temperature (ca. 74°C), with a deliberate temperature gradient across the sample so that the two forms co-exist in the



2-Aminobenzoic acid (neutral molecule form) (**III**)

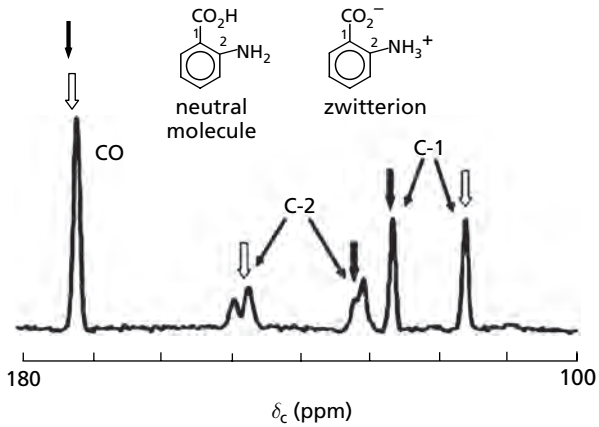
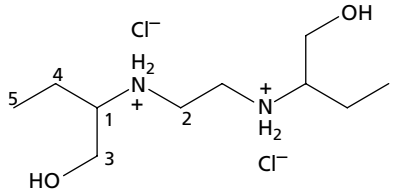


Figure 3 Carbon-13 CPMAS spectrum of form I of 2-aminobenzoic acid (**III**). This was obtained using the dipolar dephasing pulse sequence, so that only signals arising from quaternary carbons are observed. The unit cells of this polymorph contain both neutral molecules and zwitterions, the resonances of which are separately indicated. The signals from the neutral molecules are indicated by open arrows; those from zwitterions are indicated by the closed arrows.



[S,S]Ethambutol hydrochloride (**IV**)

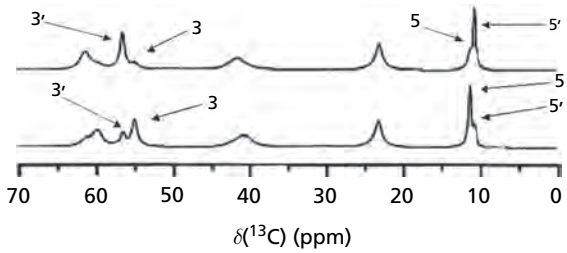
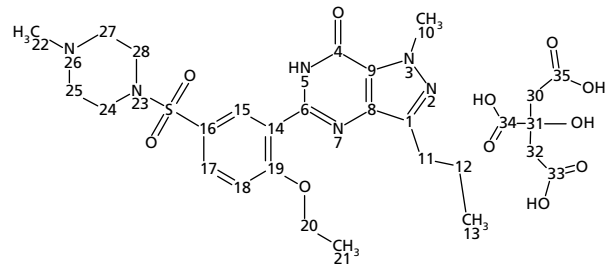


Figure 4 Carbon-13 CPMAS spectra of [S,S]-ethambutol hydrochloride (**IV**). These were recorded in the vicinity of the transition temperature (74°C) between form II (indicated by X) and form I (indicated by X′), where X is the atomic numbering displayed in (**IV**). A deliberate temperature gradient was applied across the sample, the average temperature being somewhat higher in the upper spectrum than in the lower.

probe but with concentration ratios that differ between the two spectra. This illustrates the spectral changes caused by the transition.

Ingress of water and interactions with the host system

Many crystal structures of important pharmaceutical compounds take up water non-stoichiometrically (and often reversibly), which is of great concern for industry, especially with respect to questions of storage. It is difficult to investigate this phenomenon by XRD because the unit cell dimensions may not change significantly when water is absorbed, and also because the water molecules may be mobile. However, NMR is well placed to give detailed information. Figure 5 shows ^{13}C spectra of sildenafil citrate (**V**) (Apperley



Sildenafil citrate (**V**)

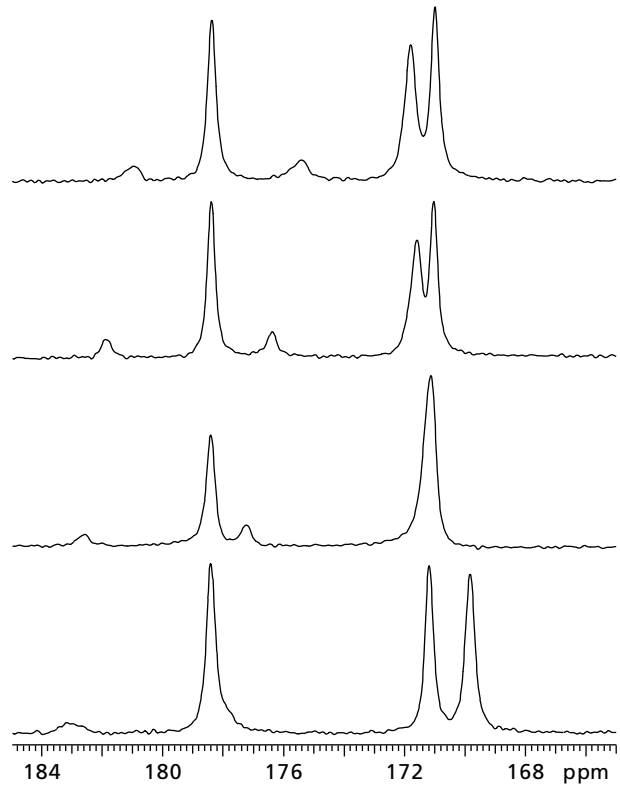


Figure 5 Expansions for the carboxyl region of the ^{13}C CPMAS spectra of sildenafil citrate (**V**) stored under different conditions of humidity. Top to bottom: 88% r.h. for 7 days, 88% r.h. for 3.5 days, as received, 0% r.h. for 7 days.

et al 2005b), the active principle of Viagra, for samples stored under various conditions of relative humidity. It is obvious that the water molecules are associated with one (and only one) of the citrate carboxyl groups, and also interact with the propyl side-chain. Since separate resonances are not observed for the active carboxyl group when hydrated and non-hydrated, it is also clear that the water molecules are mobile on the NMR timescale.

Amorphous forms

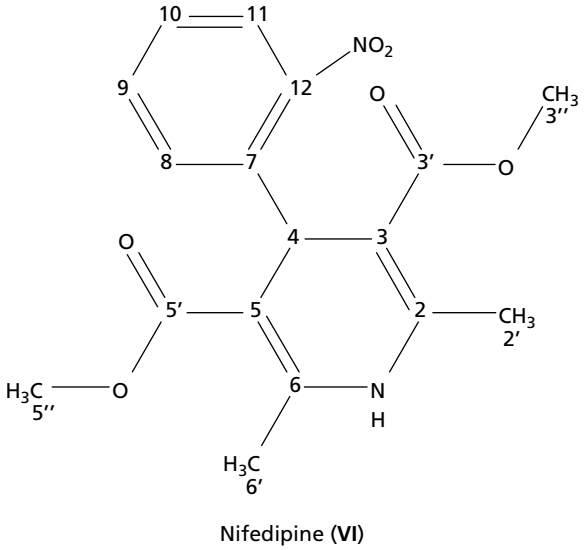
Unlike the situation for diffraction studies, solid-state NMR still provides chemical information about amorphous samples. Admittedly, the peaks are somewhat broader than for crystalline materials, but resolution of signals for chemically different carbon atoms, for example, is still moderately good. This can be seen (Fournier 2006) from Figure 6, which shows ^{13}C spectra for samples of nifedipine (**VI**). It may be noted that when glassy forms are prepared by flash-freezing liquids, the range of conformations present may be distinctly different from those locked into a crystal structure. Thus it is common to see differences in chemical shifts between amorphous samples of this type and crystalline material. Such differences may not be apparent when amorphous samples are prepared by grinding crystalline material.

Linewidths can provide direct information about the amorphous/crystalline nature of samples, so that MAS NMR can be used to detect crystallisation during processing operations. Grinding can cause such recrystallisation, as illustrated (Apperley et al 2005a) in Figure 7 which shows recrystallisation of amorphous nifedipine (**VI**) but not of amorphous indometacin (**VII**).

Quantitation

As is well known, NMR (of both solutions and solids) can yield information on relative concentrations of chemical species — but only provided the experimental conditions are carefully controlled. Since solid-state NMR can give chemical information about heterogeneous solids, quantitation experiments can be carried out on formulated drug systems. Indeed, whole tablets can be inserted into MAS rotors, as shown (Harris et al 2005a) in Figure 8. There are a number of different ways of carrying out quantitation experiments. One of the most reliable is to use an internal standard, since then many factors influencing absolute intensities are eliminated or at least minimised. Figure 9 illustrates (Harris et al 2005a) the spectrum of a model formulation of the drug bambuterol hydrochloride (**VIII**) (BHC), at 5% w/w level, in a matrix including a related drug (terbutaline sulfate), magnesium stearate and lactose. The arrowed peak at the left arises from BHC and has been used for quantitation studies by comparison with the peak arrowed on the right, which arises from magnesium stearate. It was shown that for this system the limit of quantitation was 1% w/w and the limit of detection was 0.5% w/w when a moderately low field spectrometer (4.7 T) was used for a reasonable accumulation time (3 h).

In a similar fashion, the polymorphic content of a drug formulation can be monitored, as shown (Apperley et al 2003) in Figure 10, from which a molar concentration ratio of 43%:57% can be obtained for formoterol fumarate (**IX**) in admixture with its dihydrate when the total drug concentration was 2% in lactose.



Nifedipine (**VI**)

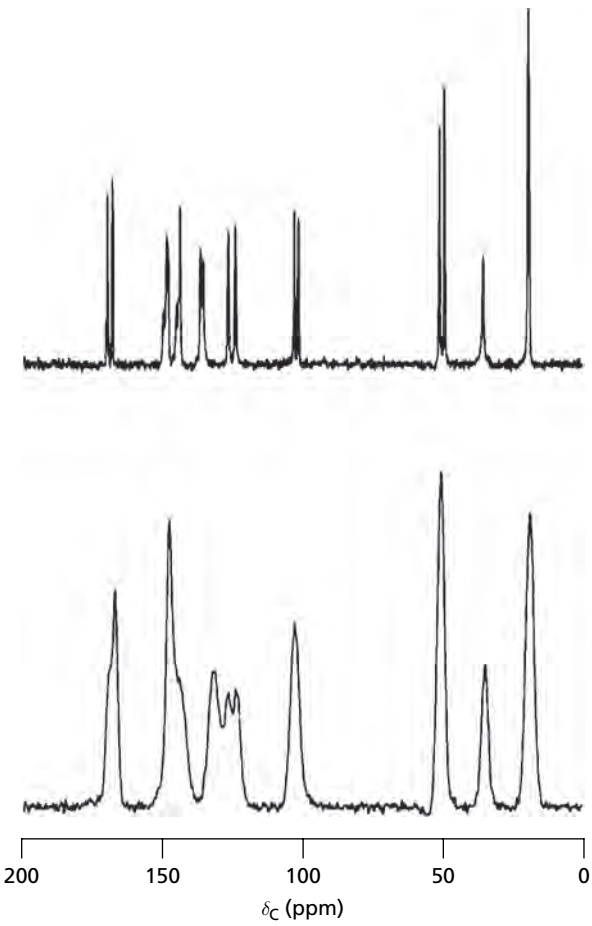
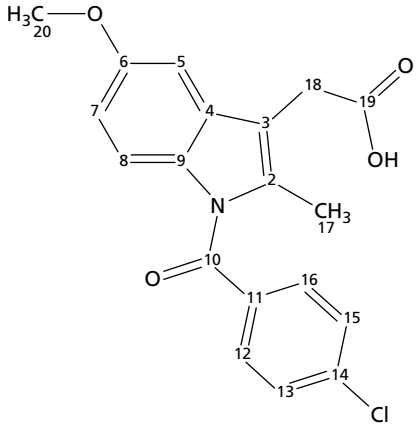


Figure 6 Carbon-13 CPMAS spectra of nifedipine (**VI**). Top: crystalline form I. Bottom: amorphous (obtained from the melt by rapid cooling).



Indometacin (**VII**)

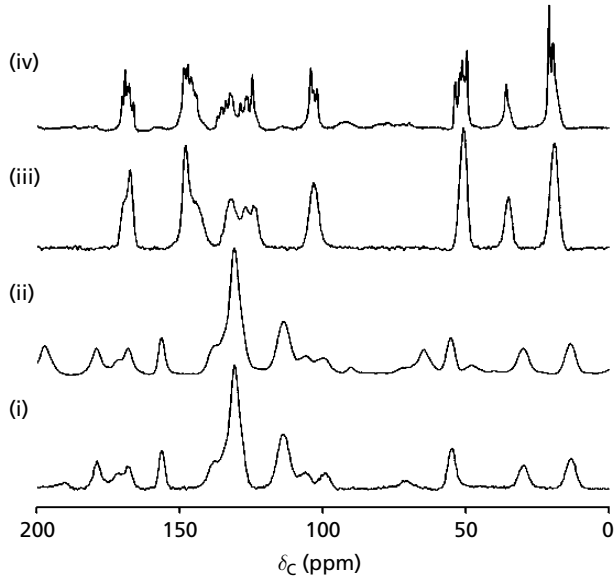
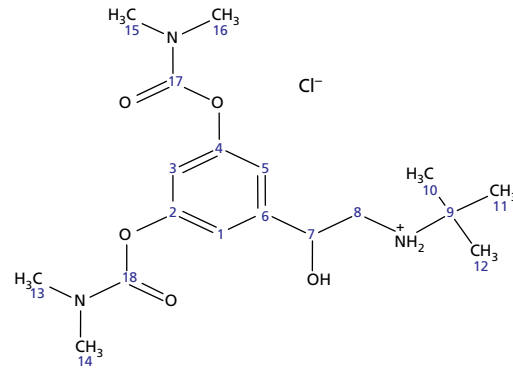


Figure 7 Carbon-13 CPMAS spectra of indometacin (**VII**) (i and ii) and nifedipine (**VI**) (iii and iv), obtained for quench-cooled melts at ambient probe temperature before (i and iii) and after (ii and iv) grinding.

Figure 8 View of the rotor and tablets used to obtain spectra of formulated bambuterol hydrochloride (**VIII**).



Bambuterol hydrochloride (**VIII**)

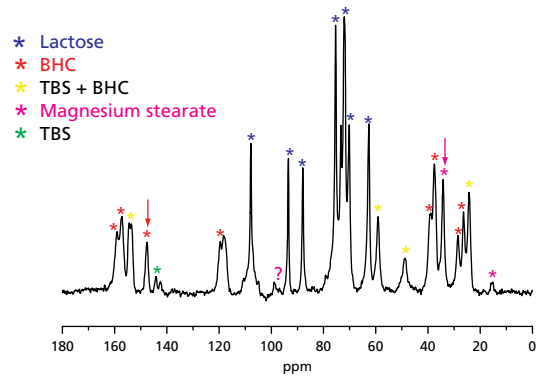


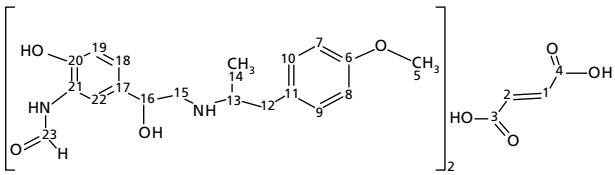
Figure 9 Carbon-13 CPMAS spectrum of a simulated drug formulation containing 5% w/w bambuterol hydrochloride (**VIII**) in lactose, together with terbutaline sulfate and magnesium stearate. The signals used to quantify the bambuterol hydrochloride with reference to magnesium stearate are indicated by arrows.

Theme 2 — NMR crystallography

The Introduction to this article stressed both the value and the limitations of diffraction methods in investigating solid-state structure. It also indicated the characteristics of solid-state NMR that make it suitable for use as a complementary technique to diffraction. NMR crystallography is increasingly recognised as a discipline in its own right, as the following examples should testify.

Determination of the asymmetric unit and molecular symmetry

The high-frequency region of the ^{13}C spectra of two polymorphs of oxybuprocaine hydrochloride (**X**) are contained in Figure 11. Signals from four chemical sites are observed (Harris et al 2007), though those for one (C-4) are somewhat obscured by special effects arising from the directly-bonded nitrogen atom. Clearly, each carbon gives two signals of



Formoterol fumarate (**IX**)

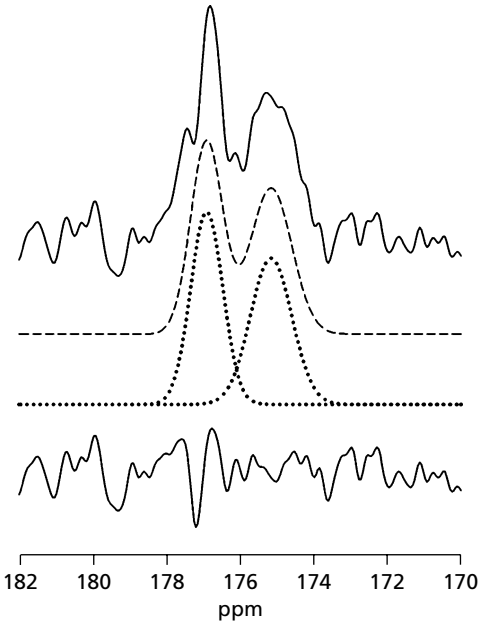
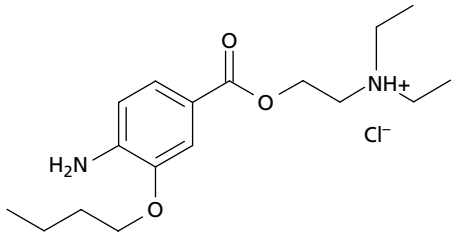


Figure 10 Carbon-13 CPMAS dipolar-dephased spectra (fumarate carboxyl region only) of formoterol fumarate (**IX**) and its dihydrate at a total level of 2% in lactose. Top to bottom: observed spectrum, deconvoluted simulated spectrum, dihydrate peak and anhydrate peak, difference between simulated and observed spectrum.



Oxybuprocaine hydrochloride (**X**)

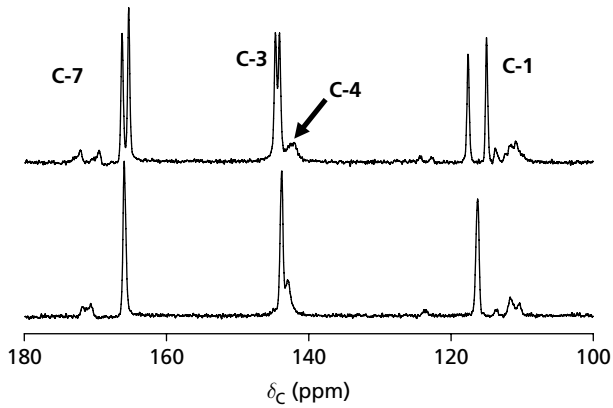


Figure 11 Carbon-13 CPMAS spectra (high-field region only) of two polymorphs of oxybuprocaine hydrochloride (**X**). Bottom: Form I. Top: Form II. The doubling of peaks for Form II shows that the crystallographic asymmetric unit consists of two molecules.

equal intensity for Form II but only one for Form I. It is therefore obvious that while Form I has a single molecule in the asymmetric unit, Form II has two independent molecules.

Figure 12 takes this matter further (Harris & Sebald 1987, 1989) for two (non-pharmaceutical) molecular systems, namely the tris(dimethyltin chalcogenides) (**XI**), which are isomorphous. Thus, Figure 12A (for the selenide) shows there are two ^{77}Se signals in 1:2 ratio, which proves that the asymmetric unit consists of half a trimeric molecule, while Figure 12B (for the sulfide) obviously contains three equal-intensity ^{13}C peaks, demonstrating that the molecule must have a two-fold rotation axis passing through opposing Sn and S(Se) atoms rather than the alternative mirror plane (which would give four signals with 2:2:1:1 intensity ratios).

Disorder in polymorphs and intramolecular mobility

There are three known polymorphs of the disperse diazo dyestuff DR278 (**XII**). Carbon-13 NMR (Figure 13) reveals (McGeorge et al 1996) that one of them (Form C) has two signals for each carbon in the -$CH_2CH_2CO_2CH_3$ side chains indicating that they are non-equivalent in the solid state, as expected. The four CH carbons of the phenylene ring give separate peaks for this form, showing that rotation of the ring about the *para* substituents is slow in the solid (in contrast to the liquid state, as demonstrated by the observation of only 10 peaks for the aromatic carbons; Figure 13 (d)) — again not unexpected. Otherwise, this form gives a simple spectrum. However, the other two forms are remarkable in that they show two signals (of unequal intensity) for each of the carbons C2′ and C6′ (arrowed in Figure 13 (a) and (b)). This suggests that two conformations are present, presumably giving a disordered structure, as was later confirmed (McGeorge et al 1998) by full structural characterisation by diffraction methods (the solution of which was significantly aided by the NMR finding). Even more remarkably, variable temperature ^{15}N NMR indicated (McGeorge et al 1998) that the two conformations were exchanging on the NMR timescale, and the thermodynamic parameters of the exchange process were determined (from bandshape changes and selective population inversion experiments) by an Eyring plot to be: $\Delta H^* = 63\,\text{kJ mol}^{-1}$; $\Delta S^* = -6\,\text{J mol}^{-1}\,\text{K}^{-1}$. At first sight, this seems to require movement of significantly large groups. However, it was later realised that a relatively small motion, mostly involving the azo nitrogen atoms, sufficed for the interchange (Figure 14). The vertical arrows on Figures 13(c) and 13(d) show that the conformation of the ring containing C2′ and C6′ is very different in Form C from that in the liquid state, presumably because of substantial averaging by mobility in the liquid.

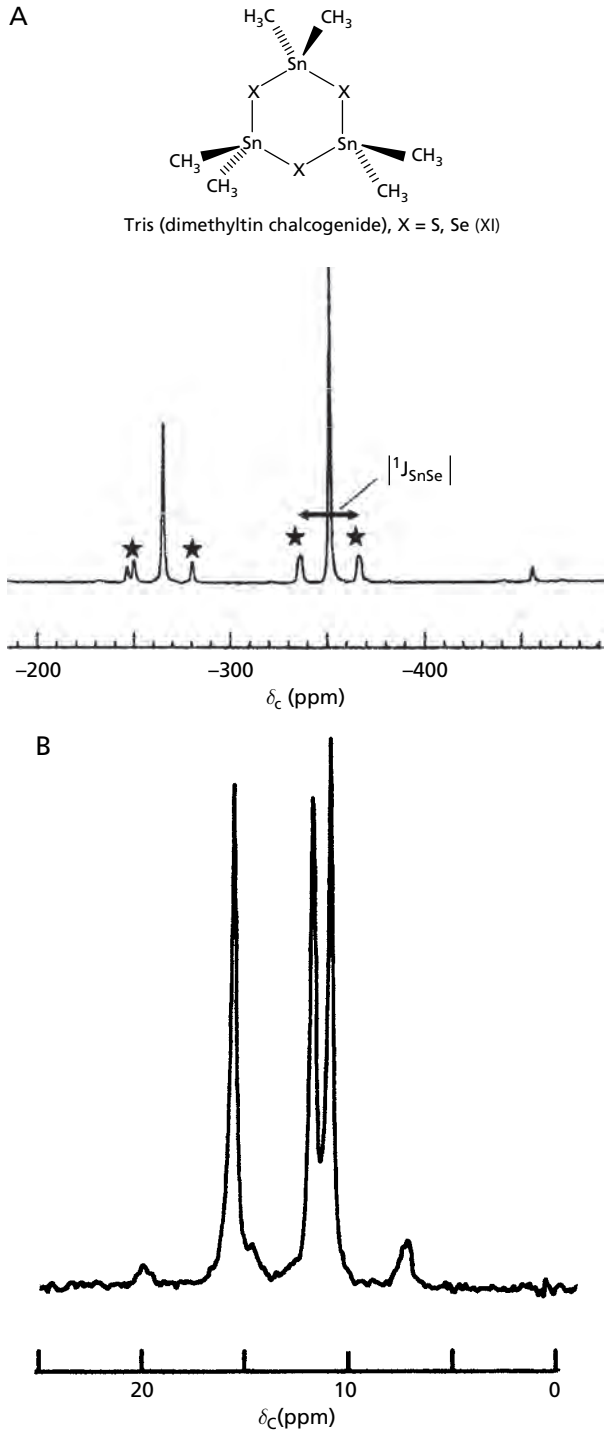


Figure 12 A. Selenium-77 CPMAS spectrum of $[Me_2SnSe]_3$ (**XI**, X=Se), showing the existence of two-fold molecular symmetry in the crystal environment. B. Carbon-13 CPMAS spectrum of $[Me_2SnS]_3$ (**XI**, X=S), giving further information about the molecular symmetry.

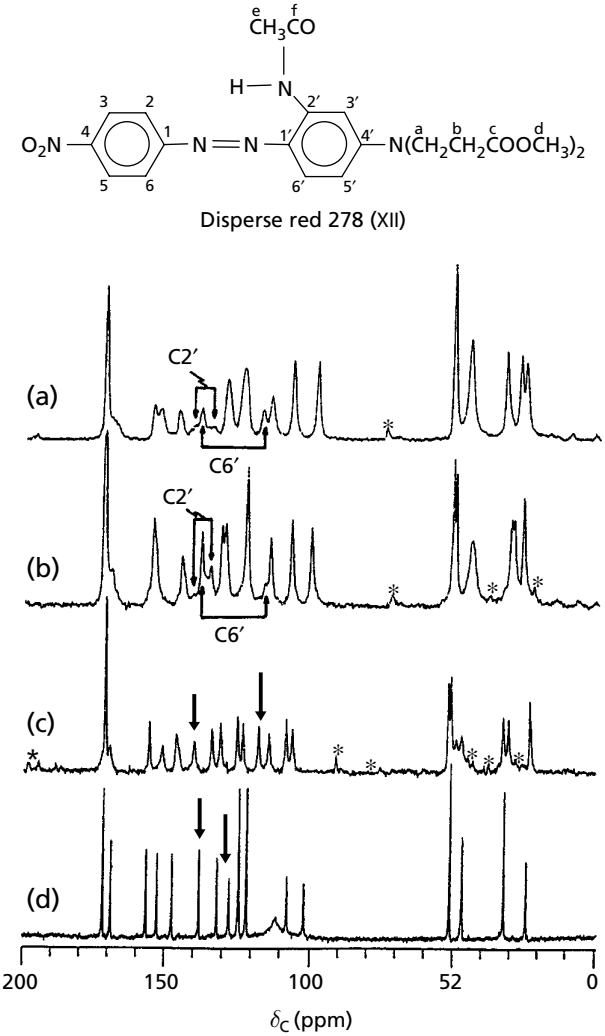


Figure 13 Carbon-13 CPMAS spectra of various forms of the dyestuff DR278 (**XII**). Top to bottom: polymorph A, polymorph B, polymorph C, melt (spectrum obtained at 150°C). The significance of the arrowed resonances is discussed in the text. The asterisks indicate spinning sidebands.

Locating hydrogen atoms in hydrogen bonds

Traditionally, hydrogen atoms have been difficult to locate by XRD because of their low electron density so that neutron diffraction, a technique not very readily available, may be required to accurately pinpoint them. Of course, advances in XRD techniques have improved the situation but it is still desirable to bring other techniques to bear. Location is of particular interest for hydrogen bonds and this is where NMR chemical shifts can be helpful, since they are well known to depend sensitively on the geometry of hydrogen bonds. This fact has been used for the white form of 4-methyl-2-nitroacetanilide (**XIII**), which has intermolecular hydrogen bonding from the NH group to the CO group. The reported crystal structure (Moore et al 1983) (admittedly rather old) simply places hydrogens at chemically reasonable positions. Fast MAS experiments readily provide (Harris et al 2003) the chemical shift of the hydrogen in the H-bond (at 10.6 ppm). However, computation using the position of the hydrogen from the XRD results gives a wildly different value. Therefore computations were carried out by varying the position of the hydrogen between the heavy atoms (themselves kept at the XRD-derived positions). Figure 15 shows the result – a

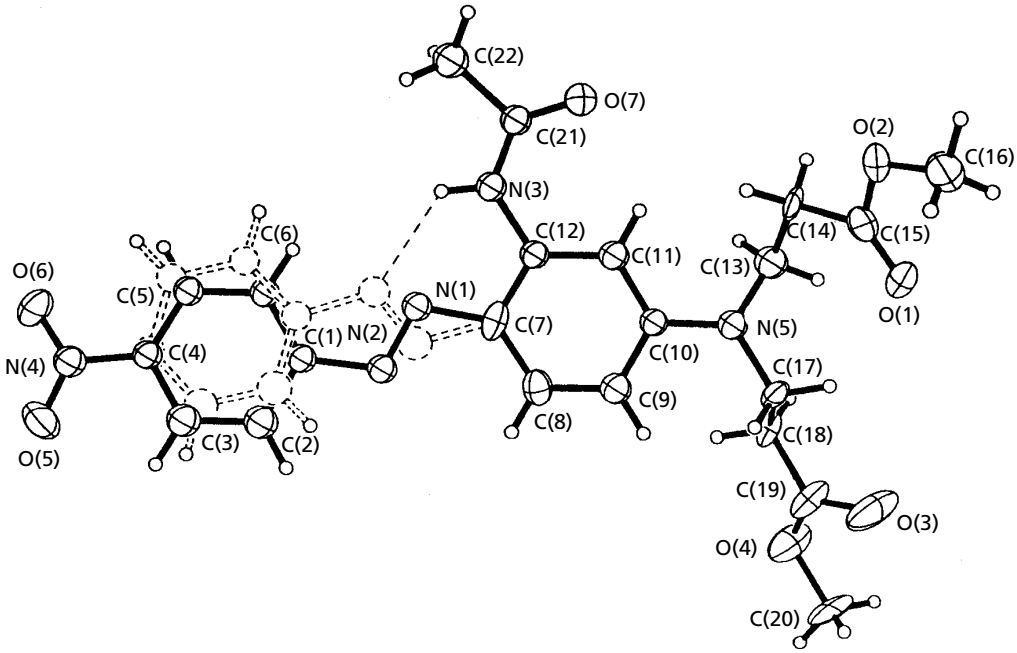


Figure 14 Molecular structure of the dyestuff DR278 (**XII**), polymorph A, showing the disorder of the diazo group and neighbouring phenylene group. The occupancy of the two sites of the disorder is 53%:47%.

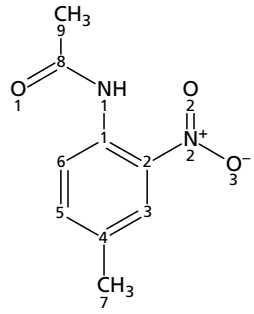
very strong dependence. Matching the experimental shift to the computed curve gave a N....H distance of 1.033Å, contrasting strongly with the value 0.917 Å assumed in the XRD report. This method of locating hydrogens was validated (Harris et al 2003) using L-histidine hydrochloride monohydrate as a model compound.

Solving crystal structures from powder diffraction data using restraints based on NMR measurements

Powerful techniques are now available to solve crystal structures using X-ray or neutron diffraction data from microcrystalline powders rather than single crystals. However, the computational requirements are substantial and it is not always possible to find a structure solution. The key difficulty is reaching a structure that is sufficiently close to the correct solution for Rietveld refinement to be employed. Any information that can assist in this procedure is to be welcomed. NMR provides crystallographic information that has frequently been used in parallel with the diffraction analysis. However, there is clearly an advantage if the NMR information can form part of the protocol for analysing the powder diffraction results. This has now been shown to be feasible. A computer program for this procedure, incorporating NMR information in the form of restraints in the simulated annealing algorithm for structure solution, has been written and tested (Hammond et al 2003, 2006; Harris et al 2006b). The structure of cortisone acetate (**XIV**) form II, already known (Declercq et al 1972) from single-crystal X-ray work, has been solved from powder XRD data in this fashion (Hammond et al 2006) and compared with both the single-crystal result and the structure from powder data without the NMR restraint. In this proof of concept study, only two very simple pieces of information from ^{13}C CPMAS NMR were incorporated, namely the asymmetric unit (one molecule) and the atoms linked by intermolecular hydrogen bonding (three carboxyl/carbonyl groups could in principle participate). Table 1 shows the results. The powder analysis with the restraints saves computer time and gets generally closer to the single-crystal result than the case without the NMR data. However, clearly more NMR information is really needed to tie down the conformation of the side chain, and especially for the angle 1. Such information could come, in principle, from measurements of dipolar coupling constants. The basic methodology of using NMR restraints in the powder XRD analysis protocol has, nonetheless, been implemented successfully.

Theme 3 — Computing chemical shifts

The most characteristic feature of NMR spectroscopy is its high resolution, which enables signals from different chemical sites to be readily resolved and thus the spectra to be directly related to chemical structure. However, for the direct relationship to be properly evaluated, it is essential that the factors influencing chemical shifts should be fully understood so that assignments of resonances to specific nuclear sites can be firmly established. Empirical correlations have their place, but theoretical approaches are desirable. This subject has been developed over many years, but, until recently, the approach has been a molecular one. Computer programs such as Gaussian, based on quantum mechanics, have been used to calculate shielding constants (and hence chemical shifts) and much information thus obtained. However, this approach has been largely limited to isolated molecules and therefore is not directly relevant to crystalline compounds. This situation is now changing and some recent developments are exemplified below.



4-Methyl-2-nitroacetanilide (**XIII**)

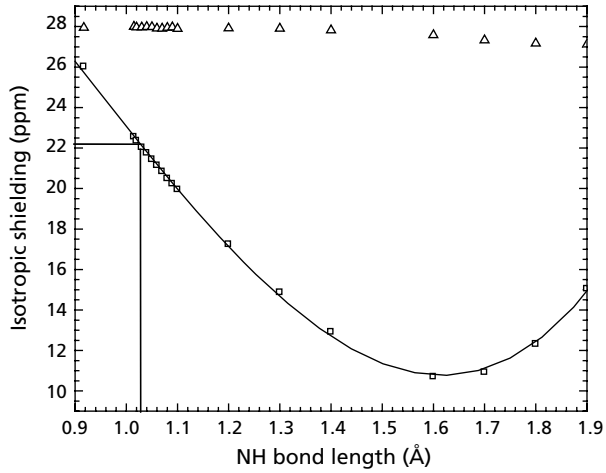


Figure 15 Computed dependence of the isotropic ^{1}H shielding for the hydrogen-bonded proton in the white form of 4-methyl-2-nitroacetanilide (**XIII**) as a function of the N,H distance within the bond. The distance between the relevant heavy atoms was kept constant at the value obtained by X-ray diffraction analysis. A cluster of two molecules was used for the computations. The horizontal line corresponds to the observed chemical shift, so the vertical line gives the derived N,H distance as 1.033 Å. The triangles show the dependence of the shielding on N,H distance in the absence of hydrogen bonding.

Polymorphic form identified by a combination of powder XRD, NMR and molecular modelling

The molecule theophylline (**XV**) has aroused much interest. A few years ago, we were presented with a problem arising from attempts to determine the crystal structure of an available polymorph by analysis of its powder XRD pattern. Computations showed that two structures could exist with very similar energies within the same unit cell. The two differed in their hydrogen-bonding motifs, one involving N-H...O bonds and the other N-H...N bonds. We were asked whether NMR could ascertain which was the correct solution. The ^{15}N MAS spectrum contained four peaks (Smith et al 2001), as expected. These were readily assigned to the four nitrogen sites on general chemical shift grounds. Computations were undertaken to predict the chemical shifts on three structural assumptions: an isolated molecule; the central molecule in a trimeric cluster involving N-H...N bonds; and the central molecule in a trimeric cluster with N-H...O bonds. The results are given in Table 2. It is clear that the computations for an isolated molecule do not fit the observed shifts for two of the nitrogens, as expected (since hydrogen bonds are not accounted for). The third case (central molecule in a trimeric cluster with N-H...O bonds) has even worse discrepancies from the observed shifts, whereas the second case (central molecule in a trimeric cluster with N-H...N bonds) has good agreement between observed and computed shifts. Clearly the correct structure involves N-H....N hydrogen bonds. Actually, the computations were not really essential to this conclusion, since the effects of hydrogen bonding on chemical shifts are well documented, but in other cases computer predictions prove to be vital.

Chemical shift differences between polymorphs

Traditionally, shielding constants (and thus chemical shifts) have been computed using programs such as Gaussian. Until recently, these have been based on a molecular approach, so that the only way of carrying out such computations for crystalline solids was to model the total structure by using a cluster of molecules with the geometry of the crystalline arrangement. Because shielding is a relatively local effect, this approach works well for many organic molecular crystals, but it suffers from two disadvantages: firstly, it is never quite clear, a-priori, how big a cluster is needed (and one might require different clusters to compute chemical shifts in different parts of the molecule); and, secondly, large clusters require big cpu times. These problems are particularly acute when the molecules contain many atoms and when there is more than one molecule in the asymmetric unit. The obvious answer is to make use of the full symmetry inherent in the crystalline state, with its translational repetition. Recently, computer programs are utilising this approach. One such program code is CASTEP, which has been modified to calculate shielding parameters (Pickard & Mauri 2001), using the fully periodic Gauge Including Projector Augmented Wave (GIPAW) method. This method is now undergoing extensive testing and a number of papers describing applications are already in the literature (Gervais et al 2004; Yates et al 2004, 2005a, b; Harris et al 2005c, 2006). Polymorphism provides a critical test, as demonstrated by the example of testosterone (**XVI**). This steroid exists in several forms, among them the α-anhydrate and the β-monohydrate. The former has two molecules in the asymmetric unit (Roberts et al 1973), while the latter has only one (Precigoux et al 1973) (Figure 16). The experimental and computed shift differences between the α-form (averages for the two independent molecules) and the β-form are listed (Harris et al 2005b) in Table 3. While quantitative agreement is not particularly good, it is encouraging to note that the correct sign is predicted by the computation for 9 out of the 10 shift differences which are more than 1 ppm.

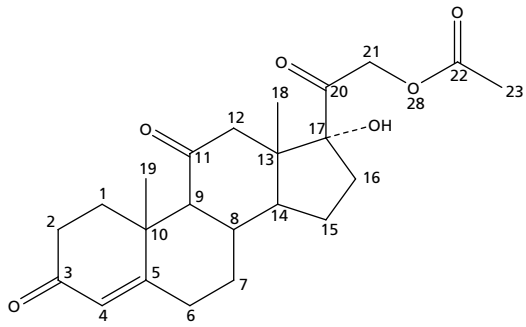
Assigning signals to crystal structure environments

When there is only one molecule in the crystallographic asymmetric unit, assignment of resonances to, say, particular carbon atoms automatically connects the chemical shifts to the environments of the atoms in the crystal structure. However, this is not the case when there is more than

Table 1 Structural parameters for the best trial models of cortisone acetate (**XIV**) form II: without and with a distance restraint (Hammond et al 2006), and the reported crystal structure (Declercq et al 1972)

Trial Model	Torsion Angle (°)[a]				H-Bond distance (Å)		Angle (°)
	1 (13–17–20–21)	2 (17–20–21–28)	3 (20–21–28–22)	4 (21–28–22–23)	H-O	O-O	O-H-O
Without restraint	–91.5	115.0	110.9	55.0	2.96	3.84	150.9
With restraint	–71.9	–177.3	–103.7	111.0	2.18	3.06	151.8
Single crystal	–94.4	168.5	–82.0	178.1	1.92	2.86	164.5

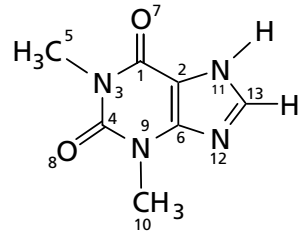
[a]For the atomic numbering, see the molecular structure (**XIV**).



Cortisone acetate (**XIV**)

Table 2 Experimental and computed ^{15}N chemical shifts (in ppm) for theophylline (**XV**) (Smith et al 2001)

	Expt.	Calc.	Expt. – Calc.	Calc.	Expt. – Calc.	Calc.	Expt. – Calc.
N3	–222	–221	–1	–222	0	–222	0
N9	–264	–261	–3	–262	–2	–254	–10
(NH)	–214	–239	+25	–218	+4	–226	+12
N12	–158	–139	–19	–157	–1	–120	–38
	MAS	Monomer		NH . . . . N trimer		NH . . . . O trimer	



Theophylline **(XV)**

one independent molecule in the structure since, a-priori, we do not know how to connect resonances for the same independent molecule and, even if we succeed in doing that, we cannot link one set of resonances with a particular independent molecule without further work. However, connectivities can be established via the solid-state INADEQUATE experiment (Lesage et al 1999) (though this takes a lot of spectrometer time if natural-abundance samples are used). The hope must then be that computation can provide the link between a set of resonances and a given independent molecule. This has now been achieved for the α-form of testosterone (**XVI**), which has two molecules in the asymmetric unit (Roberts et al 1973). Figure 17 shows part of the one-dimensional ^{13}C spectrum of this compound,

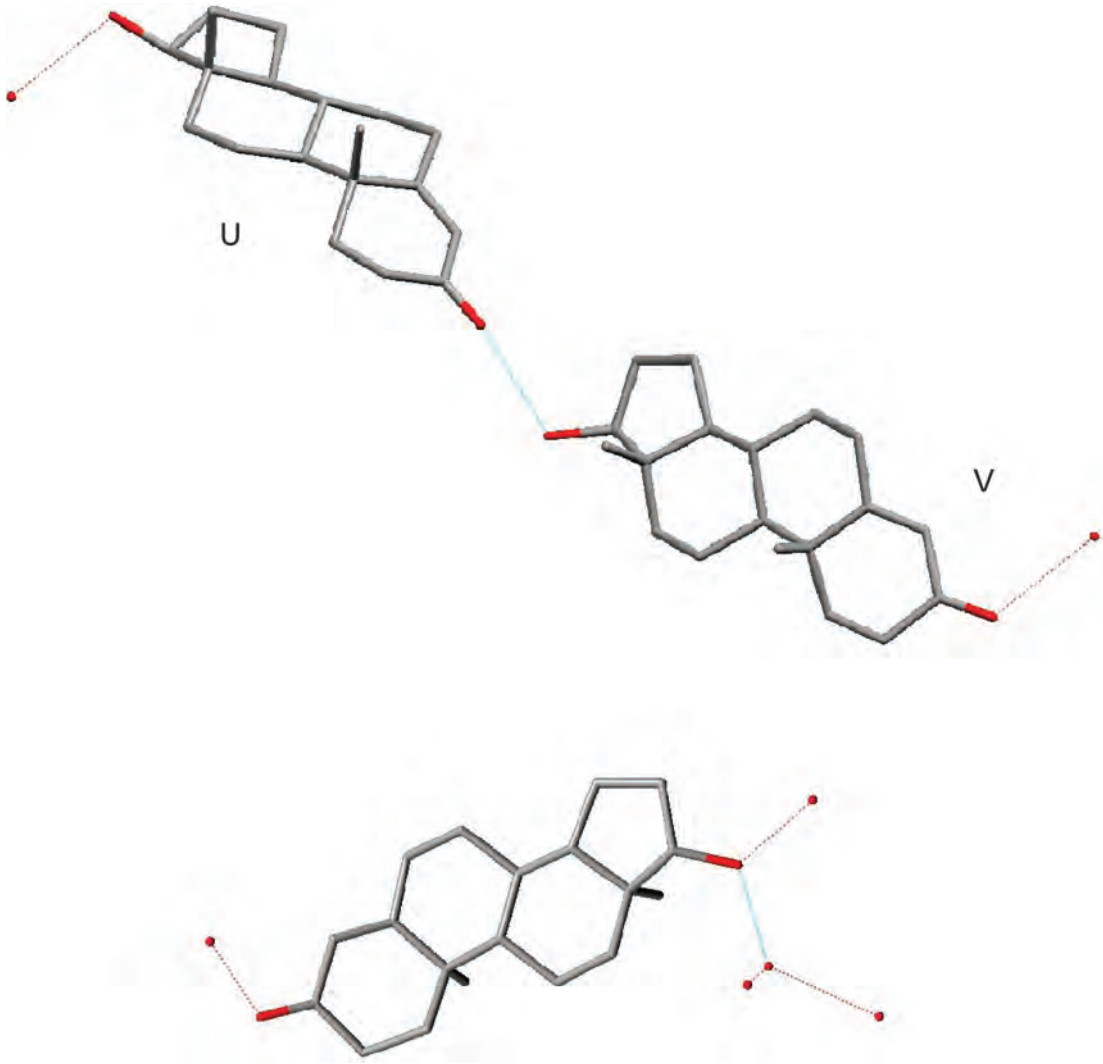


Figure 16 The crystallographic asymmetric units for the α-form (anhydrous) (Roberts et al 1973) and β-form (monohydrate) (Precigoux et al 1973) of testosterone (**XVI**).

Table 3 Computed and experimental[a] shift differences (Harris et al 2005b) between the α-and β-forms of testosterone (**XVI**), given as $\alpha - \beta$

Carbon No.	**Shift (ppm)**		**Carbon No.**	**Shift (ppm)**	
	Computed	Experimental		Computed	Experimental
1	3.02	1.4	11	1.72	1.5
2	−1.12	−1.2	12	2.80	2.3
3	1.16	1.8	13	−0.04	0.0
4	0.70	0.7	14	0.13	−0.1
5	−5.21	−2.4	15	1.81	0.3
6	1.29	0.1	16	−0.04	1.2
7	−1.46	−1.2	17	−1.11	0.8
8	1.84	1.4	18	0.76	−0.7
9	−0.69	0.0	19	2.35	1.3
10	−0.20	0.4			

[a]The published experimental shifts for the β-form have been increased by 0.50 ppm to account for a systematic difference between the new and old values for the α-form.

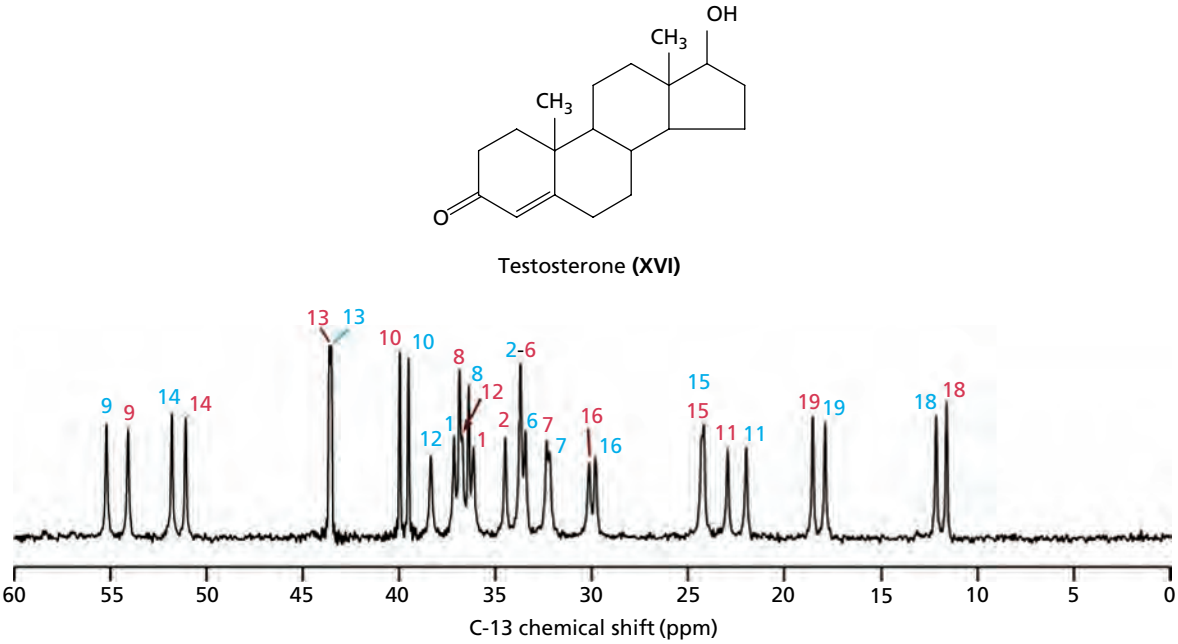


Figure 17 The low-frequency region of the ^{13}C CPMAS spectrum for the α-form of testosterone (**XVI**), showing in different colours the assignments to the two independent molecules. The red numbers correspond to the molecule U described by Harris et al (2005b), whereas the blue numbers are for molecule V (see Table 4).

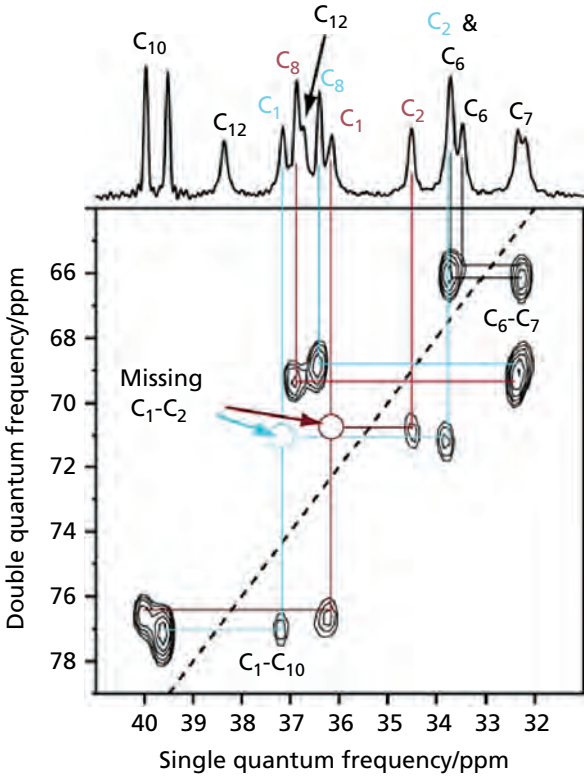


Figure 18 Partial INADEQUATE ^{13}C two-dimensional spectrum for the α form of testosterone (**XVI**), showing the links between resonances for each independent molecule.

while Figure 18 illustrates part of the two-dimensional INADEQUATE spectrum (Harris et al 2005b), showing linkages between signals. Table 4 lists the values of the crystallographic splittings (Harris et al 2005b), including signs, as determined from the INADEQUATE experiment, together with the results of CASTEP computations. While agreement between experimental and theoretical values is far from perfect, the assignments given produce the correct signs for the four largest known experimental splittings. This has enabled resonances to be attributed to the two independent molecules separately, as shown in Figure 17. Note that only isotropic chemical shifts were used. Facelli & Grant (1993) have shown that chemical shift tensor components can provide an even more powerful link between NMR spectra and crystal structures.

Conclusions

The examples given above demonstrate that solid-state NMR, aided by quantum mechanical computations, contributes to our understanding of crystal structures. It also allows us to investigate a number of matters of importance in pharmaceutical chemistry, including:

- Polymorph detection and characterisation.
- Detecting the existence of solvates.
- Examining phase transitions.
- Monitoring ingress of water.
- Studying amorphous forms.
- Examining heterogeneous (formulated) systems.
- Quantitation.

Table 4 Computed and experimental crystallographic splittings (Harris et al 2005b) for α-testosterone (**XVI**)[a]

Carbon No.	Splitting (ppm)		Carbon No.	Splitting (ppm)	
	Computed	Experimental		Computed	Experimental
1	−0.10	−1.01	11	−1.23	+0.97
2	1.74	+0.79	12	−0.50	−1.63
3	−0.33	±1.50[b]	13	−0.19	+0.10[c]
4	1.69	±0.50[b]	14	−0.00	−0.72
5	−0.16	−1.45	15	0.50	±0.10[b]
6	1.22	+0.26	16	0.17	+0.33[c]
7	0.50	+0.15[c]	17	−3.40	−2.34
8	1.59	+0.48	18	0.14	−0.54
9	−0.13	−1.12	19	0.73	+0.64
10	1.11	+0.46			

[a]Signs given as U – V. [b]Signs not accessed experimentally. [c]The splitting sign is not certain on the simple basis of the experimental data, although suggested. Of course, changing the sign of these entries would affect other pairs, including C17.

References

Apperley, D. C., Harris, R. K., Larsson, T., Malstrom, T. (2003) Quantitative NMR analysis of solid formoterol fumarate. *J. Pharm. Sci*. **92**: 2496–2503

Apperley, D. C., Forster, A. H., Fournier, R., Harris, R. K., Hodgkinson, P., Lancaster, R. W., Rades, T. (2005a) Characterisation of indomethacin and nifedipine using variable-temperature solid-state NMR. *Magn. Reson. Chem*. **43**: 881–892

Apperley, D. C., Basford, P. A., Dallman, C. I., Harris, R. K., Kinns, M., Marshall, P. V., Swanson, A. G. (2005b) NMR investigation of the interaction of water vapour with sildenafil citrate in the solid state. *J. Pharm. Sci*. **94**: 516–523

Bennett, A. E., Griffin, R. G., Vega, S. (1994) Recoupling of homo- and heteronuclear dipolar interactions in rotating solids. *NMR Basic Princ. Prog*. **33**: 1–77

Bernstein, J. (2002) *Polymorphism in molecular crystals*. Oxford University Press, Oxford

Brown, C. J. (1968) Crystal structure of anthranilic acid. *Proc. R. Soc. A* **302**: 185

Brown, C. J., Ehrenberg, M. (1985) Anthranilic acid-I, $C_7H_7NO_2$, by neutron diffraction. *Acta Cryst. C* **41**: 441–443

Declercq, J. P., Germain, G., Van Meerssche, M. (1972) 21β-Acetoxy-17α-hydroxy-4-pregnene-3,11,20-trione, $C_{23}H_{30}O_6$. *Cryst. Struct. Commun*. **1**: 59–62

Duer, M. J. (2004) *Introduction to solid-state NMR spectroscopy*. Blackwell Publishing Ltd, Oxford

Dunitz, J. D., Bernstein, J. (1995) Disappearing polymorphs. *Acc. Chem. Res*. **28**: 193–200

Facelli, J. C., Grant, D. M. (1993) Determination of molecular symmetry in crystalline napthalene using solid-state NMR. *Nature* **365**: 325–327

Fournier, R. (2006) *Probing amorphous and crystalline pharmaceutical systems using NMR*. PhD Thesis, University of Durham

Gervais, C., Profeta, M., Lafond, V., Bonhomme, C., Azais, T., Mutin, M., Pickard, C. J., Mauri, F., Babonneau, F. (2004) Combined ab initio computational and experimental multinuclear solid-state magnetic resonance study of phenylphosphonic acid. *Magn. Reson. Chem*. **42**: 445–452

Hammond, R. B., Ma, C., Roberts, K. J., Ghi, P. Y., Harris, R. K. (2003) Systematic approach to identifying molecular adducts with two moieties in crystallographic asymmetric units: application to co-crystals of urea with dihydroxy benzene isomers. *J. Phys. Chem. B* **107**: 11820–11826

Hammond, R. B., Ma, C., Pencheva, K., Roberts, K. J., Ghi, P. Y., Harris, R. K. (2006) Crystal structure determination of cortisone acetate polymorphs using a simulated-annealing approach with combining information for solid-state NMR and powder diffraction. *Solid State Sci*. In press

Harris, R. K. (2004) NMR crystallography: the use of chemical shifts. *Solid State Sci*. **6**: 1025–1037

Harris, R. K. (2006) NMR studies of organic polymorphs & solvates. *The Analyst* **131**: 351–373

Harris, R. K., Jackson, P. (1987) High-resolution 1H and 13C NMR of solid 2-aminobenzoic acid. *J. Phys. Chem. Solids* **48**: 813–818

Harris, R. K., Sebald, A. (1987) Experimental methodology for high-resolution solid-state NMR of heavy-metal spin-1/2 nuclei. *Magn. Reson. Chem*. **25**: 1058–1062

Harris, R. K., Sebald, A. (1989) High-resolution solid-state 119Sn and 207Pb NMR study of organotin and organolead chalcogenides: observation of J-coupling in solids. *Magn. Reson. Chem*. **27**: 81–87

Harris, K. D. M., Tremayne, M., Kariuki, M. (2001) Advances in the use of powder X-ray diffraction for structure determination. *Angew. Chem. Int. Ed*. **40**: 1626–1651

Harris, R. K., Ghi, P. Y., Hammond, R. B., Ma, C.-Y., Roberts, K. J. (2003) Refinement of hydrogen atomic position in a hydrogen bond using a combination of solid-state NMR and computation. *Chem. Comm*. 2834–2835

Harris, R. K., Hodgkinson, P., Larsson, T., Muruganatham, A. (2005a) Quantification of bambuterol hydrochloride in a formulated product. *J. Pharm. Biomed. Anal*. **38**: 858–864

Harris, R. K., Joyce, S. A., Pickard, C. J., Cadars, S., Emsley, L. (2005b) Assigning carbon-13 NMR spectra to crystal structures by the INADEQUATE pulse sequence and first principle computation: a case study of two forms of testosterone. *Phys. Chem. Chem. Phys*. **8**: 137–143

Harris, R. K., Ghi, P. Y., Puschmann, H., Apperley, D. C., Griesser, U. J., Hammond, R. B., Ma, C.-Y., Roberts, K. J., Pearce, G. J., Yates, J. R., Pickard, C. J. (2005c) Structural studies of the polymorphs of carbamazepine, its dihydrate, and two solvates. *Org. Process Res*. **9**: 902–910

Harris, R. K., Ghi, P. Y., Hammond, R. B., Ma, C. Y., Roberts, K. J., Yates, J. R., Pickard, C. J. (2006) Solid-state NMR and computational studies of 4-methyl-2-nitroacetanilide. *Magn. Reson. Chem*. **44**: 325–333

Harris, R. K., Cadars, S., Emsley, L., Yates, J. R., Pickard, C. J., Jetti, R. K. R., Griesser, U. J. (2007) NMR crystallography of oxybuprocaine hydrochloride, Modification II. *Phys. Chem. Chem. Phys*. **9:** 360–368

King, I. J., Fayon, F., Massiot, D., Harris, R. K., Evans, J. S. O. (2001) A space group assignment of ZrP_2O_7 by ^{31}P solid state NMR. *Chem. Comm.* 1766–1767

Lesage, A., Bardet, M., Emsley, L. (1999) Through-bond carbon-carbon connectivities in disordered solids by NMR. *J. Am. Chem. Soc*. **121**: 10987–10993

McGeorge, G., Harris, R. K., Chippendale, A. M., Bullock, J. F. (1996) Conformational analysis for a series of polymorphs of a disperse azobenzene dyestuff. *JCS Perkin* **2**: 1733–1738

McGeorge, G., Harris, R. K., Batsanov, A. S., Churakov, A. V., Chippendale, A. M., Bullock, J. F., Gan, Z. (1998) Analysis of a solid-state conformational rearrangement using 15N NMR and X-ray crystallography. *J. Phys. Chem. A* **102**: 3505–3513

Moore, J. C., Yeadon, A., Palmer, R. A. (1983) Crystal and molecular structures of two polymorphs of 4-methyl-2-nitroacetanilide (MNA). *J. Cryst. Spect. Res*. **13**: 279–292

Pickard, C. J., Mauri, F. (2001) *Phys. Rev. B* **63**: article no. 245101

Portieri, A. (2001) *Solid state NMR of sulfa-drugs*. PhD Thesis, University of Durham

Precigoux, G., Hospital, M., Vanden Bosche, G. (1973) Testosterone hydrate, $C_{19}H_{28}O_2.H_2O$. *Cryst. Struct. Commun*. **3**: 435

Roberts, P. J., Pettersen, R. C., Sheldrick, G. M., Isaacs, N. W., Kennard, O. (1973) Crystal and molecular structure of 17β-hydroxyandrost-4-en-3-one (testosterone). *J. Chem. Soc. Perkin* **II** 1978–1984

Rubin-Preminger, J. M., Bernstein, J., Harris, R. K., Evans, I. R., Ghi, P. Y. (2004) Variable temperature studies of a polymorphic system comprising two pairs of enantiotropically-related forms: [S,S]-ethambutol dihydrochloride. *Crystal Growth Des*. **4**: 431–439

Schaefer, J., Stejskal, E. O. (1976) Carbon-13 NMR of polymers spinning at the magic angle. *J. Am. Chem. Soc*. **98**: 1031–1032

Smith, E. D. L., Hammond, R. B., Jones, M. J., Roberts, K. J., Mitchell, J. B. O., Price, S. L., Harris, R. K., Apperley, D. C., Cherryman, J. C., Docherty, R. (2001) The determination of the crystal structure of anhydrous theophylline by X-ray powder diffraction with a systematic search algorithm, lattice energy calculations and 13C and 15N solid-state NMR: a question of polymorphism in a given unit cell. *J. Phys. Chem. B* **105**: 5818–5826

Yates, J. R., Pickard, C. J., Payne, M. C., Dupree, R., Profeta, M., Mauri, F. (2004) Theoretical investigation of oxygen-17 NMR shielding and electric field gradients in glutamic acid polymorphs. *J. Phys. Chem. A* **108**: 6032–6037

Yates, J. R., Dobbins, S. E., Pickard, C. J., Mauri, F., Ghi, P. Y., Harris, R. K. (2005a) A combined first principles computational and solid-state NMR study of a molecular crystal: flurbiprofen. *Phys. Chem. Chem. Phys*. **7**: 1402–1407

Yates, J. R., Pham, T. N., Pickard, C. J., Mauri, F., Amado, A. M., Gil, A. M., Brown, S. P. (2005b) An investigation of weak CH . . . . O hydrogen bonds in maltose anomers by a combination of calculation and experimental solid-state NMR spectroscopy. *J. Am. Chem. Soc*. **127**: 10216–10220