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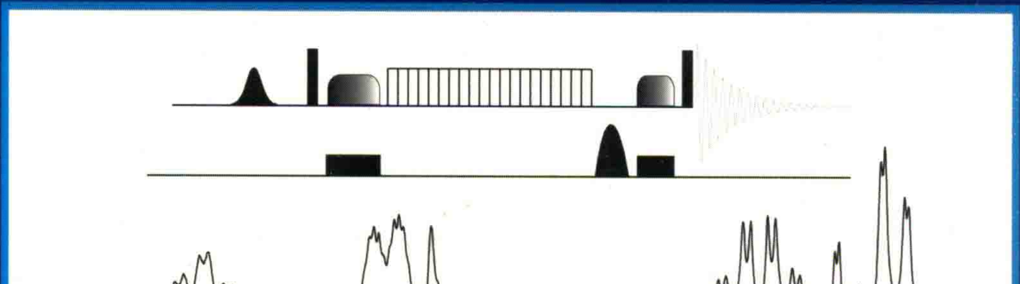
Second Edition



TIMOTHY D W CLARIDGE

High-Resolution NMR Techniques in Organic Chemistry

Second Edition

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Preface to Second Edition

It is 9 years since the publication of the first edition of this book and in this period the discipline of NMR spectroscopy has continued to develop new methodology, improve instrumentation and expand in its applications. This second edition aims to reflect the key developments in the field that have relevance to the structure elucidation of small to mid-sized molecules. It encompasses new and enhanced pulse sequences, many of which build on the sequences presented in the first edition, offering the chemist improved performance, enhanced information content or higher-quality data. It also includes coverage of recent advances in NMR hardware that have led to improved instrument sensitivity and thus extended the boundaries of application. Many of the additions to the text reflect incremental developments in pulsed methods and are to be found spread across many chapters, whereas some of the more substantial additions are briefly highlighted below.

Chapters 1 and 2 provide the background to NMR spectroscopy and to the pulse methods presented in the following chapters and thus have been subject to only minor modification. As with the first edition, no attempt is made to introduce the basic parameters of NMR spectroscopy and how these may be correlated with chemical structure since these topics are adequately covered in many other texts. Chapter 3 again provides information on the practical aspects of NMR spectroscopy and on how to get the best out of your available instrumentation. It has been extended to reflect the most important hardware developments, notably the array of probe technologies now available, including cryogenic, microscale, and flow probes and, indeed, those incorporating combinations of these concepts. The latest methods for instrument calibrations are also described. Chapter 4, describing one-dimensional methods, has been extended a little to reflect the latest developments in spectrum editing methods and to approaches for the quantification of NMR spectra using an external reference. Chapter 5 again provides an introduction to 2D NMR and describes homonuclear correlation methods, and has been enhanced to include advances in methodology that lead to improved spectrum quality, such as the suppression of zero-quantum interferences. It also contains an extended section on methods for establishing carbon–carbon correlations, notably those benefiting from proton detection, which gain wider applicability as instrument sensitivity improves. Chapter 6, presenting heteronuclear correlation techniques, has been extended substantially to reflect developments in methods for establishing long-range proton–carbon correlations in molecules, one of the more versatile routes to identifying molecular connectivities. These include new approaches to improved filtering of one-bond responses, to enhanced sampling of long-range proton–carbon coupling constants, for overcoming spectral crowding, and to methods for differentiating two-bond from three-bond correlations. It also briefly considers the measurement of the magnitudes of heteronuclear coupling constants themselves. The section on triple-resonance methods for exploiting correlations between two heteronuclides (termed X–Y correlations, where neither is a proton) has also been extended to include the more recent methods utilising proton detection. Chapter 7 on *J*-resolved methods has only a single addition in the form of an absorption-mode variant of the homonuclear method that has potential application for the generation of 'proton-decoupled proton spectra' and in the separation of proton multiplets. Rather like Chapter 5, that covering the NOE (Chapter 8) reflects incremental advances in established methods, such as those for cleaner NOESY data. It also extends methods for the observation of heteronuclear NOEs, a topic of increasing interest. Chapter 9 presents a completely new chapter dedicated to diffusion NMR spectroscopy and 2D diffusion-ordered spectroscopy (DOSY), mentioned only briefly in the first edition. These methods have become routinely available on modern instruments equipped with pulsed field gradients and have found increasing application in many areas of chemistry. The principal techniques are described and their practical implementation discussed, including the detrimental influence of convection and how this may be recognised and dealt with. A range of applications are presented and in the final section methods for editing or extending the basic sequences are briefly introduced. Descriptions of the components that make up modern NMR experiments are to be found in Chapter 10. This also contains the more recent developments in experimental methodologies such as the application of adiabatic frequency sweeps for pulsing and decoupling and the suppression of unwelcome artefacts through zero-quantum dephasing. The chapter concludes by considering some 'emerging methods', technologies that are presently generating significant interest within the NMR community and may have significant impact in the future, at least in some branches of chemistry, but remain subject to future development. These include techniques for acquiring 2D spectra in only a single scan, potentially offering significant time savings; those for generating highly polarised NMR samples, thus greatly enhancing detection sensitivity; and those for exploiting residual dipolar couplings between nuclei in weakly aligned samples as an alternative means of providing stereochemical information. The book again concludes with an extensive glossary of the many acronyms that permeate the language of NMR spectroscopy.

The preparation of the second edition has again benefited from the assistance and generosity of many people in Oxford and elsewhere. I thank my colleagues in the NMR facility of the Oxford Department of Chemistry for their support and assistance, namely Dr Barbara Odell, Tina Jackson, Dr Guo-Liang Ping and Dr Nick Rees, and in particular I acknowledge the input of Barbara and Nick in commenting on draft sections of the text. I also thank Sam Kay for assistance with the HMBC data fitting routines used in Chapter 6 and Dr James Keeler of the University of Cambridge for making these available to us. I am grateful to Bruker Biospin for providing information on magnet development and probe performance and to Oxford Instruments Molecular Biotools for proving the dynamic nuclear polarisation (DNP) data for Chapter 10. I also thank Deirdre Clark, Suja Narayana and Adrian Shell of Elsevier Science for their input and assistance during the production of the book.

Finally, I again thank my wife Rachael and my daughter Emma for their patience and support during the revision of this book. I apologise for sacrificing the many evenings and weekends that the project demanded and look to avoid this in the future. Until, perhaps, the next time.

Tim Claridge
Oxford, September 2008
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Preface to First Edition

From the initial observation of proton magnetic resonance in water and in paraffin, the discipline of nuclear magnetic resonance (NMR) has seen unparalleled growth as an analytical method and now, in numerous different guises, finds application in chemistry, biology, medicine, materials science and geology. Despite its inception in the laboratories of physicists, it is in the chemical laboratory that NMR spectroscopy has found the greatest use and, it may be argued, has provided the foundations on which modern organic chemistry has developed. Modern NMR is now a highly developed, yet still evolving, subject that all organic chemists need to understand, and appreciate the potential of, if they are to be effective in and able to progress their current research. An ability to keep abreast of developments in NMR techniques is, however, a daunting task, made difficult not only by the sheer number of available techniques but also by the way in which these new methods first appear. These are spread across the chemical literature in both specialised magnetic resonance journals and those dedicated to specific areas of chemistry, as well as the more general entities. They are often referred to by esoteric acronyms and described in a seemingly complex mathematical language that does little to endear them to the research chemist. The myriad of sequences can be wholly bewildering for the uninitiated and can leave one wondering where to start and which technique to select for the problem at hand. In this book I have attempted to gather together the most valuable techniques for the research chemist and to describe the operation of these using pictorial models. Even this level of understanding is perhaps more than some chemists may consider necessary, but only from this can one fully appreciate the *capabilities* and (of equal if not greater importance) the *limitations* of these techniques. Throughout, the emphasis is on the more recently developed methods that have, or undoubtedly will, establish themselves as the principal techniques for the elucidation and investigation of chemical structures in solution.

NMR spectroscopy is, above all, a practical subject that is most rewarding when one has an interesting sample to investigate, a spectrometer at one's disposal and the knowledge to make the most of this (sometimes alarmingly!) expensive instrumentation. As such, this book contains a considerable amount of information and guidance on how one implements and executes the techniques that are described and thus should be equally at home in the NMR laboratory as at the chemist's or spectroscopist's desk.

This book is written from the perspective of an NMR facility manager in an academic research laboratory and as such the topics included are naturally influenced by the areas of chemistry I encounter. The methods are chosen, however, for their wide applicability and robustness, and because, in many cases, they have already become established techniques in NMR laboratories in both academic and industrial establishments. This is not intended as a review of all recent developments in NMR techniques. Not only would this be too immense to fit within a single volume, but the majority of the methods would have little significance for most research chemists. Instead, this is a distillation of the very many methods developed over the years, with only the most appropriate fractions retained. It should find use in academic and industrial research laboratories alike, and could provide the foundation for graduate level courses on NMR techniques in chemical research.

The preparation of this book has benefited from the cooperation, assistance, patience, understanding and knowledge of many people, for which I am deeply grateful. I must thank my colleagues, both past and present, in the NMR group of the Dyson Perrins Laboratory, in particular Elizabeth McGuinness and Tina Jackson for their first-class support and assistance, and Norman Gregory and Dr Guo-Liang Ping for the various repairs, modifications and improvements they have made to the instruments used to prepare many of the figures in this book. Most of these figures have been recorded specifically for the book and have been made possible by the generosity of various research groups and individuals who have made their data and samples available to me. For this, I would like to express my gratitude to Dr Harry Anderson, Prof. Jack Baldwin, Dr Paul Burn, Dr John Brown, Dr Duncan Carmichael, Dr Antony Fairbanks, Prof. George Fleet, Dr David Hodgson, Dr Mark Moloney, Dr Jo Peach and Prof. Chris Schofield, and to the members of their groups, too numerous to mention, who kindly prepared the samples; they will know who they are and I am indebted to each of them. I am similarly grateful to Prof. Jack Baldwin for allowing me to use the department's instrumentation for the collection of these illustrative spectra.

I would like to thank Drs Carolyn Carr and Nick Rees for their assistance in proofreading the manuscript and for being able to spot those annoying little mistakes that I overlooked time and time again but that still did not register. Naturally I accept responsibility for those that remain and would be grateful to hear of these, whether factual or typographical. I also thank Eileen

Morrell and Sharon Ward of Elsevier Science for their patience in waiting for this project to be completed and for their relaxed attitude as various deadlines failed to be met.

I imagine everyone venturing into a career in science has at some time been influenced or even inspired by one or a few individual(s) who may have acted as teacher, mentor or perhaps role model. Personally, I am indebted to Dr Jeremy Everett and to John Tyler, both formerly from (what was then) Beecham Pharmaceuticals, for accepting into their NMR laboratory for a year a "sandwich" student who was initially supposed to gain industrial experience elsewhere as a chromatographer analysing horse urine! My fortuitous escape from this and the subsequent time at Beecham Pharmaceuticals proved to be a seminal year for me and I thank Jeremy and John for their early encouragement that ignited my interest in NMR. My understanding of what this could really do came from graduate studies with the late Andy Derome, and I, like many others, remain eternally grateful for the insight and inspiration he provided.

Finally, I thank my wife Rachael for her undying patience, understanding and support throughout this long and sometimes tortuous project, one that I'm sure she thought, on occasions, she would never see the end of. I can only apologise for the neglect she has endured but not deserved.

Tim Claridge
Oxford, May 1999

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Chapter 1

Introduction

From the initial observation of proton magnetic resonance in water [1] and in paraffin [2], the discipline of nuclear magnetic resonance (NMR) has seen unparalleled growth as an analytical method and now, in numerous different guises, finds application in chemistry, biology, medicine, materials science and geology. The founding pioneers of the subject, Felix Bloch and Edward Purcell, were recognised with a Nobel Prize in 1952 'for their development of new methods for nuclear magnetic precision measurements and discoveries in connection therewith'. The maturity of the discipline has since been recognised through the awarding of Nobel prizes to two of the pioneers of modern NMR methods and their application, Richard Ernst (1991, 'for his contributions to the development of the methodology of high-resolution NMR spectroscopy') and Kurt Wüthrich (2002, 'for his development of NMR spectroscopy for determining the three-dimensional structure of biological macromolecules in solution'). Despite its inception in the laboratories of physicists, it is in the chemical and biochemical laboratories that NMR spectroscopy has found greatest use. To put into context the range of techniques now available in the modern organic laboratory, including those described in this book, we begin with a short overview of the evolution of high-resolution (solution-state) NMR spectroscopy and some of the landmark developments that have shaped the subject.

1.1. THE DEVELOPMENT OF HIGH-RESOLUTION NMR

It is now over 16 years since the first observations of NMR were made in both solid and liquid samples, from which the subject has evolved to become the principal structural technique of the research chemist, alongside mass spectrometry. During this time, there have been a number of key advances in high-resolution NMR that have guided the development of the subject [3–5] (Table 1.1) and consequently the work of organic chemists and their approaches to structure elucidation. The seminal step occurred during the early 1950s when it was realised that the resonant frequency of a nucleus is influenced by its chemical environment and that one nucleus could further influence the resonance of

Table 1.1. A summary of some key developments that have had a major influence on the practice and application of high-resolution NMR spectroscopy in chemical research

Decade	Notable advances
1940s	*First observation of NMR in solids and liquids (1945)*
1950s	*Development of chemical shifts and spin–spin coupling constants as structural tools*
1960s	*Use of signal averaging for improving sensitivity* *Application of the pulse-FT approach* *The NOE employed in structural investigations*
1970s	*Use of superconducting magnets and their combination with the FT approach* *Computer controlled instrumentation*
1980s	*Development of multipulse and two-dimensional NMR techniques* *Automated spectroscopy*
1990s	*Routine application of pulsed field gradients for signal selection* *Development of coupled analytical methods, e.g. LC-NMR*
2000–	*Use of high-sensitivity cryogenic probes* *Routine availability of actively shielded magnets for reduced stray fields* *Development of microscale tube and flow probes*
2010+	*Adoption of fast and parallel data acquisition methods . . . ?*

FT, Fourier transformation; LC-NMR, liquid chromatography and nuclear magnetic resonance.

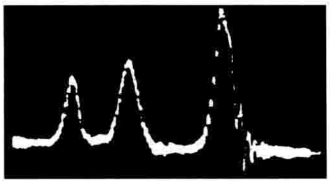

Figure 1.1. The first 'high-resolution' proton NMR spectrum, recorded at 30 MHz, displaying the proton chemical shifts in ethanol (reprinted with permission from [6], Copyright 1951, American Institute of Physics).

another through intervening chemical bonds. Although these observations were seen as unwelcome chemical complications by the investigating physicists, a few pioneering chemists immediately realised the significance of these chemical shifts and spin–spin couplings within the context of structural chemistry. The first high-resolution proton NMR spectrum (Fig. 1.1) clearly demonstrated how the features of an NMR spectrum, in this case chemical shifts, could be directly related to chemical structure, and it is from this that NMR has evolved to attain the significance it holds today.

The 1950s also saw a variety of instrumental developments that were to provide the chemist with even greater chemical insight. These included the use of sample spinning for averaging to zero field inhomogeneities, which provided a substantial increase in resolution, so revealing fine splittings from spin–spin coupling. Later, spin decoupling was able to provide more specific information by helping the chemists understand these interactions. With these improvements, sophisticated relationships could be developed between chemical structure and measurable parameters, leading to realisations such as the dependence of vicinal coupling constants on dihedral angles (the now well-known Karplus relationship). The inclusion of computers during the 1960s was also to play a major role in enhancing the influence of NMR on the chemical community. The practice of collecting the same continuous wave spectrum repeatedly and combining them with a CAT (computer of average transients) led to significant gains in sensitivity and made the observation of smaller sample quantities a practical realisation. When the idea of stimulating all spins simultaneously with a single pulse of radio frequency, collecting the time-domain response and converting this to the required frequency-domain spectrum by a process known as Fourier transformation (FT), was introduced, more rapid signal averaging became possible. This approach provided an enormous increase in signal-to-noise ratio and was to change completely the development of NMR spectroscopy. The mid-1960s also saw the application of the nuclear Overhauser effect (NOE) to conformational studies. Although described during the 1950s as a means of enhancing the sensitivity of nuclei through the simultaneous irradiation of electrons, the Overhauser effect has since found widest application in sensitivity enhancement between nuclei, or in the study of the spatial proximity of nuclei, and remains one of the most important tools of modern NMR. By the end of the 1960s, the first commercial FT spectrometer was available, operating at 90 MHz for protons. The next great advance in field strengths was provided by the introduction of superconducting magnets during the 1970s, which were able to provide significantly higher fields than the electromagnets previously employed. These, combined with the FT approach, made the observation of carbon-13 routine and provided the organic chemists with another probe of molecular structure. This also paved the way for the routine observation of a whole variety of previously inaccessible nuclei of low natural abundance and low magnetic moment. It was also in the early 1970s that the concept of spreading the information contained within the NMR spectrum into two separate frequency dimensions was proposed in a lecture. However, because of instrumental limitations, the quality of the first two-dimensional (2D) spectra was considered too poor to be published, and not until the mid-1970s, when instrument stability had improved and developments in computers made the necessary complex calculations feasible, did the development of 2D methods begin in earnest. These methods, together with the various multipulse one-dimensional (1D) methods that also became possible with the FT approach, did not have significant impact on the wider chemical community until the 1980s, from which point their development was nothing less than explosive. This period saw an enormous number of new pulse techniques presented that were capable of performing a variety of 'spin gymnastics', thus providing the chemist with ever more structural data, on smaller sample quantities and in less time. No longer was it necessary to rely on empirical correlations of chemical shifts and coupling constants with structural features to identify molecules, but instead a collection of spin interactions (through-bond, through-space and chemical exchange) could be mapped and used to determine structures more reliably and more rapidly. The evolution of new pulse methods continued throughout the 1990s, alongside which has emerged a fundamentally different way of extracting the desired information from molecular systems. Pulsed field gradient selected experiments have now become routine structural tools, providing better quality spectra, often in shorter times, than was previously possible. These came into widespread use not so much from a theoretical breakthrough (their use for signal selection was first demonstrated in 1980) but again as a result of progressive technological developments defeating practical difficulties. Similarly, the emergence of coupled analytical methods, such as liquid chromatography and NMR (LC-NMR), has come about after the experimental complexities of interfacing these very different techniques have been overcome, and these methods have established themselves for the analysis of complex mixtures. Developments in probe technologies over the last decade have led to the wider adoption of cryogenically cooled coils in probe heads that reduce significantly system noise and so enhance signal-to-noise ratios. Probe coil miniaturisation has also provided a boost in signal-to-noise for mass-limited samples, and the marrying of this with cryogenic technology nowadays offers one of the most effective routes to higher detection sensitivity. Instrument miniaturisation has been a constant theme in recent years leading to smaller and more compact consoles driven by developments in solid-state electronics. Likewise, new generations of actively shielded superconducting magnets with significantly reduced stray fields are now commonplace, making the siting of instruments considerably easier and far less demanding on space. For example, the first generation unshielded magnets operating at 500 MHz possessed stray fields that would extend to over 3 m horizontally from the magnet centre when measured at the 0.5 mT (5 gauss) level, the point beyond which disturbances to the magnetic field are not considered problematic. Nowadays, the latest generation shielded magnets have this line sited at somewhat less than 1 m from the centre and only a little beyond the magnet cryostat itself. This is achieved through the use of compensating magnet coils that seek to counteract the stray field generated outside of the magnet assembly. Other developments are seeking to recycle the liquid cryogens needed to maintain the superconducting state of the magnet through the reliquification of helium and nitrogen. Recycling of helium in this manner is already established for imaging magnets but poses considerable challenges in the context of high-resolution NMR measurements.

Modern NMR spectroscopy is now a highly developed and technologically advanced subject. With so many advances in NMR methodology in recent years, it is understandably an overwhelming task for the research chemist, and even the dedicated spectroscopist, to appreciate what modern NMR has to offer. This text aims to assist in this task by presenting the principal modern NMR techniques and exemplifying their application.

1.2. MODERN HIGH-RESOLUTION NMR AND THIS BOOK

There can be little doubt that NMR spectroscopy now represents the most versatile and informative spectroscopic technique employed in the modern chemical research laboratory and that an NMR spectrometer represents one of the largest single investments in analytical instrumentation the laboratory is likely to make. For both these reasons, it is important that the research chemist is able to make the best use of the available spectrometer(s) and to harness modern developments in NMR spectroscopy in order to promote their chemical or biochemical investigations. Even the most basic modern spectrometer is equipped to perform a myriad of pulse techniques capable of providing the chemist with a variety of data on molecular structure and dynamics. Not always do these methods find their way into the hands of the practising chemist, remaining instead in the realms of the specialist, obscured behind esoteric acronyms or otherwise unfamiliar NMR jargon. Clearly, this should not be so, and the aim of this book is to gather up the most useful of these modern NMR methods and present them to the wider audience who should, after all, find greatest benefit from their applications.

The approach taken throughout is non-mathematical and is based firmly on using pictorial descriptions of NMR phenomena and methods wherever possible. In preparing and updating this work, I have attempted to keep in mind what I perceive to be the requirements of three major classes of potential readers:

1. those who use solution-state NMR as tool in their own research, but have little or no direct interaction with the spectrometer,
2. those who have undertaken training in directly using a spectrometer to acquire their own data, but otherwise have little to do with the upkeep and maintenance of the instrument, and
3. those who make use spectrometers and are responsible for the day-to-day upkeep of the instrument. This may include NMR laboratory managers, although in some cases the user may not consider themselves dedicated NMR spectroscopists.

The first of these could well be research chemists and students in an academic or industrial environment who need to know what modern techniques are available to assist them in their efforts, but otherwise feel they have little concern for the operation of a spectrometer. Their data is likely to be collected under fully automated conditions or provided by a central analytical facility. The second may be a chemist in an academic environment who has hands-on access to a spectrometer and has his or her own samples that demand specific studies that are perhaps not available from fully automated instrumentation. The third class of reader may work in a smaller chemical company or academic chemistry department who have invested in NMR instrumentation but may not employ a dedicated NMR spectroscopist for its upkeep, depending instead on, say, an analytical or synthetic chemist for this. This, it appears (in the UK at least), is often the case for new