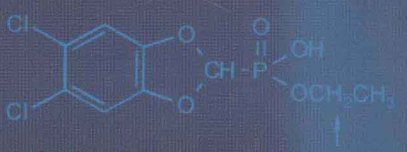
Terence N. Mitchell
Burkhard Costisella

NMR – From Spectra to Structures

An Experimental Approach



Springer

Terence N. Mitchell · Burkhard Costisella

NMR – From Spectra to Structures

An Experimental Approach

Springer

Terence N. Mitchell
Universität Dortmund
– Fachbereich Chemie –
44221 Dortmund
Germany
e-mail: mitchell@chemie.uni-dortmund.de

Burkhard Costisella
Universität Dortmund
– Fachbereich Chemie –
44221 Dortmund
Germany
e-mail: Costi@chemie.uni-dortmund.de

ISBN 3-540-40695-6 Springer-Verlag Berlin Heidelberg New York

Cataloging-in-Publication Data applied for
Bibliographic information published by Die Deutsche Bibliothek
Die Deutsche Bibliothek lists this publication in the Deutsche Nationalbibliografie;
detailed bibliographic data is available in the Internet at <http://dnb.ddb.de>.

Springer-Verlag Berlin Heidelberg New York
a member of BertelsmannSpringer Science + Business Media GmbH

http://www.springer.de

Printed in Germany

Typesetting: medio Technologies AG, Berlin
Cover design: Künkel/Lopka, Heidelberg

Printed on acid-free paper 5 4 3 2 1 0

Springer

Berlin
Heidelberg
New York
Hong Kong
London
Milan
Paris
Tokyo

dedicated to Reiner Radeglia
an NMR pioneer in a then divided Germany

Preface

Why write another NMR book? Most of the many already available involve theoretical approaches of various kinds and levels of complexity. Few books deal with purely practical aspects and a handful are slanted towards problem-solving. Collections of problems of different complexity are invaluable for students, since theory of itself is not very useful in deducing the structure from the spectra.

However, there is now a huge variety of NMR experiments available which can be used in problem-solving, in addition to the standard experiments which are a "must". We start by providing an overview of the most useful techniques available, as far as possible using one single molecule to demonstrate which information they bring. The problems follow in the second part of the book.

Readers can obtain a list of answers to the problems on application (by mail or e-mail) to the authors.

We thank Annette Danzmann, Christa Nettelbeck and Bernhard Griewel for their invaluable help in recording the spectra and our wives Karin and Monika for their patience and support during the writing of the book. We also thank Bernd Schmidt for reading the manuscript and giving us valuable tips on how it could be improved. Finally we thank the staff at Springer for turning our manuscript into the finished product you now have in your hands.

Terence N. Mitchell
Universität Dortmund
– Fachbereich Chemie –
44221 Dortmund
Germany
e-mail:
mitchell@chemie.uni-dortmund.de

Burkhard Costisella
Universität Dortmund
– Fachbereich Chemie –
44221 Dortmund
Germany
e-mail:
Costi@chemie.uni-dortmund.de

Table of Contents

Introduction

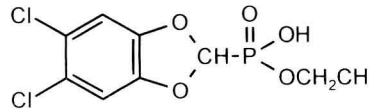
NMR spectroscopy is arguably the most important analytical method available today. The reasons are manifold: it is applied by chemists and physicists to gases, liquids, liquid crystals and solids (including polymers). Biochemists use it routinely for determining the structures of peptides and proteins, and it is also widely used in medicine (where it is often called MRI, Magnetic Resonance Imaging). With the advent of spectrometers operating at very high magnetic fields (up to 21.1 T, i.e. 900 MHz proton resonance frequency) it has become an extremely sensitive technique, so that it is now standard practice to couple NMR with high pressure liquid chromatography (HPLC). The wide range of nuclei which are magnetically active makes NMR attractive not only to the organic chemist but also to the organometallic and inorganic chemist. The latter in particular often has the choice between working with liquid or solid samples; the combination of high resolution and magic angle spinning (HR/MAS) of solid samples provides a wealth of structural information which is complementary to that obtained by X-ray crystallography. The same suite of techniques, slightly adapted, is now available to those working in the field of combinatorial chemistry. This is only a selection of the possibilities afforded by NMR, and the list of methods and applications continues to multiply.

No single monograph can hope to deal with all the aspects of NMR. In writing this book we have concentrated on NMR as it is used by preparative chemists, who in their day-to-day work need to determine the structures of unknown organic compounds or to check whether the product obtained from a synthetic step is indeed the correct one.

Previous authors have taught the principles of solving organic structures from spectra by using a combination of methods: NMR, infrared spectroscopy (IR), ultraviolet spectroscopy (UV) and mass spectrometry (MS). However, the information available from UV and MS is limited in its predictive capability, and IR is useful mainly for determining the presence of functional groups, many of which are also visible in carbon-13 NMR spectra. Additional information such as elemental analysis values or molecular weights is also often presented.

It is however true to say that the structures of a wide variety of organic compounds can be solved using only NMR spectroscopy, which provides a huge arsenal of measurement techniques in one to three dimensions. To determine an

organic structure using NMR data is however not always a simple task, depending on the complexity of the molecule. This book is intended to provide the necessary tools for solving organic structures. However, it does not just consist of a series of problems. These form Part 2 of the book, and in Part 1 a relatively simple organic compound (**1**) is used as an example to present the most important 1D and 2D experiments.

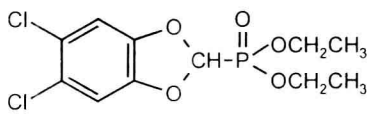


1

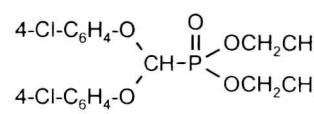
All the magnetic nuclei present in the molecule (^{1}H, ^{13}C, ^{31}P, ^{17}O, ^{35}Cl) are included in the NMR measurements, and the necessary theory is discussed very briefly: the reader is referred to suitable texts which he or she can consult in order to learn more about the theoretical aspects.

The molecule which we have chosen will accompany the reader through the different NMR experiments; the "ever-present" structure will make it easier to understand and interpret the spectra.

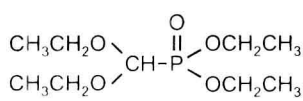
Our standard molecule is however not ideally suited for certain experiments (e.g. magnetic non-equivalence, NOE, HPLC-NMR coupling). In such cases other simple compounds of the same type, compounds **2-7,** will be used:



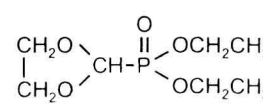
2



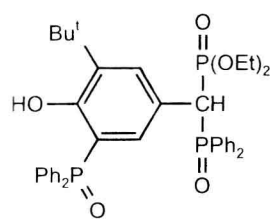
3



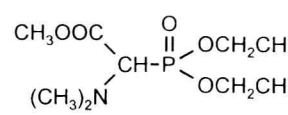
4



5



6



7

Part 1: NMR Experiments

This book is not intended to teach you NMR theory, but to give you a practical guide to the standard NMR experiments you will often need when you are doing structure determination or substance characterisation work, and (in Part 2) to provide you with a set of graded problems to solve. At the end of Part 1 we shall recommend some books which you will find useful when you are working on the problems.

Thus we shall try to take you through Part 1 without recourse to much theory. We shall of course use many terms which will be unfamiliar to you if you have not yet had a course in NMR theory, and these will be emphasised by using **bold** lettering when they appear. You can then, if you wish, go to the index of whatever theory book you have available in order to find out exactly where you can read up on this topic. From time to time, when we feel it advisable to say one or two words about more theoretical aspects in our text, we shall do so using *italic*.

The Appendix at the end of Part 1 contains a list of recommended texts for theoretical and experimental aspects of NMR as well as for solving spectroscopic problems.

1
1D Experiments

1.1
^{1}H, D (^{2}H): Natural Abundance, Sensitivity

Hydrogen has two NMR-active nuclei: ^{1}H, always known as "the proton" (thus "proton NMR"), making up 99.98%, and ^{2}H, normally referred to as D for deuterium.

These absorb at completely different frequencies, and since deuterium and proton chemical shifts are identical (also because deuterium is a **spin-1 nucleus**), deuterium NMR spectra are hardly ever measured.

However, NMR spectrometers use deuterium signals from deuterium-labelled molecules to keep them stable; such substances are known as **lock substances** and are generally used in the form of solvents, the most common being deuterochloroform $CDCl_3$.

1.1.1
Proton NMR Spectrum of the Model Compound 1

Before we start with the actual experiment it is very important to go through the procedures for preparing the sample. The proton spectra are normally measured in 5 mm sample tubes, and the concentration of the solution should not be too high to avoid line broadening due to viscosity effects. For our model compound we dissolve 10 mg in 0.6 mL $CDCl_3$: between 0.6 and 0.7 mL solvent leads to optimum **homogeneity**. It is vital that the solution is free from undissolved sample or from other insoluble material (e.g. from column chromatography), since these cause a worsening of the homogeneity of the magnetic field. Undesired solids can be removed simply by filtration using a Pasteur pipette, the tip of which carries a small wad of paper tissue.

The sample is introduced into the spectrometer, locked onto the deuterated solvent (here $CDCl_3$) and the homogeneity optimised by **shimming** as described by the instrument manufacturer (this can often be done automatically, particularly when a sample changer is used).

The proton experiment is a so-called **single channel experiment**: the same channel is used for sample irradiation and observation of the signal, and the irradiation frequency is set (automatically) to the resonance frequency of the protons at the magnetic field strength used by the spectrometer.

Although some laboratories have (very expensive) spectrometers working at very high fields and frequencies, routine structure determination work is generally carried out using instruments whose magnetic fields are between 4.6975 Tesla (proton frequency 200 MHz) and 14.0296 Tesla (600 MHz). *The NMR spectroscopist always characterises a spectrometer according to its proton measuring frequency!*

The precise measurement frequency varies slightly with solvent, temperature, concentration, sample volume and solute or solvent polarity, so that exact adjustment must be carried out before each measurement. This process, known as **tuning and matching**, involves variation of the capacity of the circuit. Modern spectrometers carry out such processes under computer control.

The measurement procedure is known as the **pulse sequence**, and always starts with a delay prior to switching on the irradiation pulse. The irradiation pulse only lasts a few microseconds, and its length determines its power. The NMR-active nuclei (here protons) absorb energy from the pulse, generating a signal.

*To be a little technical: the magnetisation of the sample is moved away from the y-axis, and it is important to know the length of the so-called 90° pulse which, as the name suggests, moves it by 90°, as this is needed in other experiments. In the experiment we are discussing now, a shorter pulse (corresponding to a pulse angle of 30-40°, the so-called **Ernst angle**) is much better than a 90° pulse.*

When the pulse is switched off, the excited nuclei return slowly to their original undisturbed state, giving up the energy they had acquired by excitation. This

process is known as **relaxation**. The detector is switched on in order to record the decreasing signal in the form of the **FID** (free induction decay). You can observe the FID on the spectrometer's computer monitor, but although it actually contains all the information about the NMR spectrum we wish to obtain, it appears completely unintelligible as it contains this information as a function of time, whereas we need it as a function of frequency.

This sequence, delay-excitation-signal recording, is repeated several times, and the FIDs are stored in the computer. The sum of all the FIDs is then subjected to a mathematical operation, the **Fourier transformation**, and the result is the conventional NMR spectrum, the axes of which are frequency (in fact chemical shift) and intensity. Chemical shift and intensity, together with coupling information, are the three sets of data we need to interpret the spectrum.

Figure 1 shows the proton spectrum of our model compound, recorded at a frequency of 400 MHz.

All signals are assigned to the corresponding protons in the molecular formula: this is made easier by prediction programmes. Table 1 presents the result of a prediction compared with the actual values.

If you do not have a prediction programme available, look on the Internet to see whether you can find freeware or shareware there. Otherwise use tables such as those you will find in the book by Pretsch et al. (see Appendix).

We shall now consider these signals and demonstrate the correctness of the assignment using different NMR techniques.

Before continuing, remind yourself of the rules for **spin-spin coupling**, i.e. for determining the number of lines in a multiplet (with the help of the **"n+1 rule"**) and their intensities (using **binomial coefficients**).

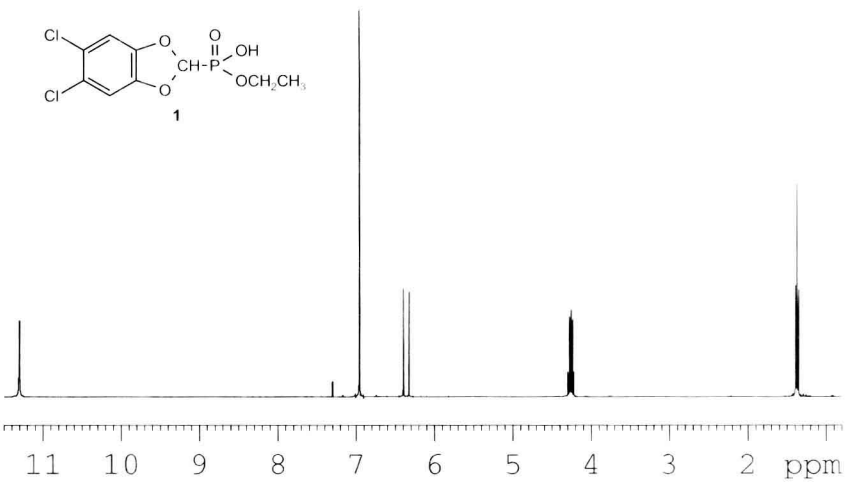


Fig. 1 Proton spectrum of compound 1 at 400 MHz. Signal assignment (from left to right): OH proton (singlet), aromatic protons (singlet), methine proton (doublet), OCH_2 protons (apparently a quintet), CH_3 protons, triplet. The small signal at 7.24 ppm is due to $CHCl_3$.

Table 1

Chemical shift (ppm)	J_{HP} (Hz)	Chemical shift (calc.)	J_{HP} (calc.)	Assignment
11.58	0	10.6	0	OH
6.92	not observed	7.0	0.3	CH_{arom}
6.32	28.7	6.6	16.9	CH-P
4.20	8.0	4.2	8.4	CH_2
1.33	0.6	1.3	1.0	CH_3

Let us start with the one-line signal on the left, the singlet, at 11.58 ppm. Our standard, tetramethylsilane TMS, gives a one-line signal whose chemical shift is defined as 0.00 ppm. Signals to its left are said to absorb at low field, those to its right (quite unusual in fact) at high field of TMS. Thus the signal at 11.58 ppm is that which absorbs at the lowest field, and we have assigned this as being due to the OH proton. This proton is acidic, the O-H bond being relatively weak, and can thus undergo fast chemical exchange with other water molecules or with deuterated water, D_2O. Thus if our sample is treated with 1-2 drops of D_2O and shaken for a few seconds the OH signal will disappear when the spectrum is recorded again: a new signal due to HOD appears at 4.7 ppm.

This technique works for any acidic proton present in a compound under investigation and is very useful in structure determination.

The next signal is a very small one at 7.24 ppm and comes from the small amount of $CHCl_3$ present in the $CDCl_3$.

The singlet at 6.92 ppm is due to the two aromatic protons: these have identical environments and thus show no coupling with other protons. They are too far from the phosphorus atom to show measurable coupling to it.

The two lines between 6.25 and 6.40 ppm are in fact a doublet due to the methine (CH) proton, which absorbs at relatively low field because it is bonded to two electronegative oxygen atoms. This proton is very close (separated by only two bonds) to the phosphorus, which is a **spin-1/2 nucleus** (there is only one isotope, phosphorus-31). The proton is also a spin-1/2 nucleus, so that H-H and H-P coupling behaviour is analogous. The distance between the two lines in the doublet is the coupling constant J, or to be exact $^2J_{P\text{-}C\text{-}H}$ and must be given in Hz, *not* ppm! The actual J value is 28.7 Hz.

How can we show that the two lines are due to a coupling? We need to carry out a so-called **decoupling** experiment, which "eliminates" couplings. Since two different nuclei are involved here, we do a **heterodecoupling** experiment (as opposed to **homodecoupling** when only one type of nucleus is involved, most commonly the proton). Decoupling is a 2-channel experiment in which we excite (and observe) the protons with channel 1 and excite the phosphorus nuclei with channel 2, which we call the decoupling channel. Channel 2 is set to the phosphorus resonance frequency, which we can obtain from tables; the excitation of the phosphorus eliminates the coupling. Figure 2 shows the sig-

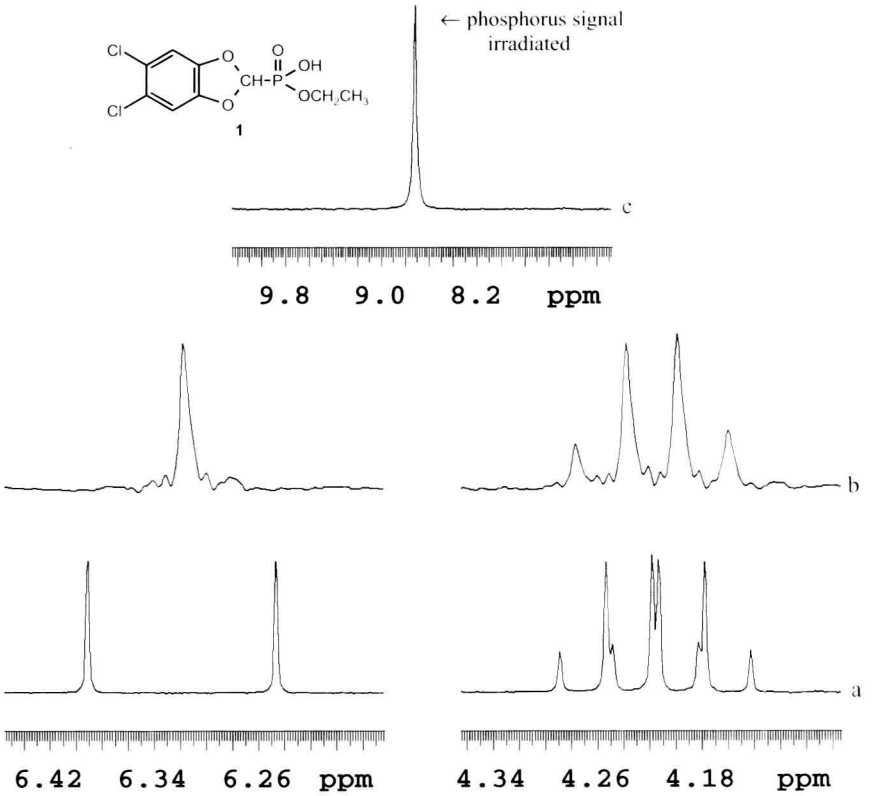


Fig. 2a–c Heterodecoupling experiment on compound **1** (at 200 MHz). **a** undecoupled methine and methylene signals; **b** signals after decoupling of the phosphorus; **c** ^{31}P spectrum, showing the signal which is irradiated using the decoupling channel (channel 2)

nals due to the CH proton (ca. 6.3 ppm) and the OCH_2 protons (ca. 4.2 ppm) before (lower traces) and after (upper traces) decoupling. The top trace shows the ^{31}P signal which is irradiated. On irradiation, the methine doublet is transformed to a singlet, the chemical shift of which lies exactly at the centre of the initial doublet.

The OCH_2 signal at ca. 4.2 ppm in the undecoupled spectrum consists of 8 lines and is due to those methylene protons which have only one oxygen atom in their neighbourhood rather than two. Heterodecoupling reduces the number of lines to 4; we now have a quartet with line intensities 1:3:3:1; thus phosphorus couples with these methylene protons across 3 bonds ($^3J_{P\text{-}O\text{-}C\text{-}H}$). The quartet in the decoupled spectrum (upper trace) is due to coupling of the CH_2 protons with the three equivalent CH_3 protons ($^3J_{H\text{-}C\text{-}C\text{-}H}$): this can be demonstrated by a homodecoupling experiment, a further 2-channel experiment where the second channel is used for *selective* irradiation of the methyl proton signal (a triplet, intensity 1:2:1) at 1.33 ppm (the only signal we have not yet discussed). The

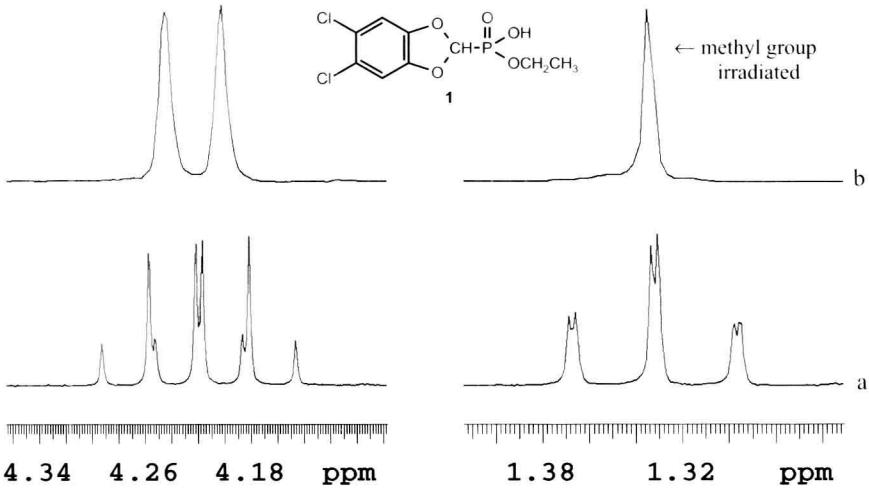


Fig. 3a,b Homodecoupling experiment on compound **1** (at 200 MHz). **a** undecoupled methylene and methyl signals; **b** signals after irradiation of the methyl group

result is now the elimination of ($^3J_{H\text{-}C\text{-}C\text{-}H}$). leading to a doublet signal, the distance between the lines being equal to ($^3J_{P\text{-}O\text{-}C\text{-}H}$).

Thus the original 8-line multiplet is a doublet of quartets (dq).

We can now use a homodecoupling experiment to show that in the methyl signal (triplet, with each line split into a doublet) at 1.33 ppm, the distances between lines 1 and 3, 2 and 4, 3 and 5 or 4 and 6 are equal to ($^3J_{H\text{-}C\text{-}C\text{-}H}$): we irradiate the methylene protons and observe the methyl protons. The result of this experiment is shown in Fig. 3.

There we see the signals due to OCH_2CH_3 on the left and OCH_2-CH_3 on the right. After decoupling (above), the 8-line OCH_2CH_3 signal becomes a doublet due to the P-H coupling, which is of course still present. The 6-line OCH_2-CH_3 signal, the one which is irradiated, becomes one single line. This experiment was carried out on a state-of-the-art spectrometer: earlier spectrometers would more likely have shown the decoupled OCH_2-CH_3 signal in a highly-distorted form.

Homo- and heterodecoupling experiments such as those described here are used routinely in structural analysis and can be carried out very rapidly. In the present case they have provided exact proof that the signal assignments were correct.

1.1.2
Field Dependence of the Spectrum of 1

The decoupling experiments which we have just discussed showed that the multiplet (doublet of quartets) due to the OCH_2 group arises from the presence of