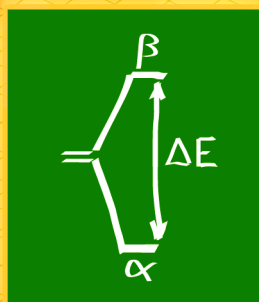
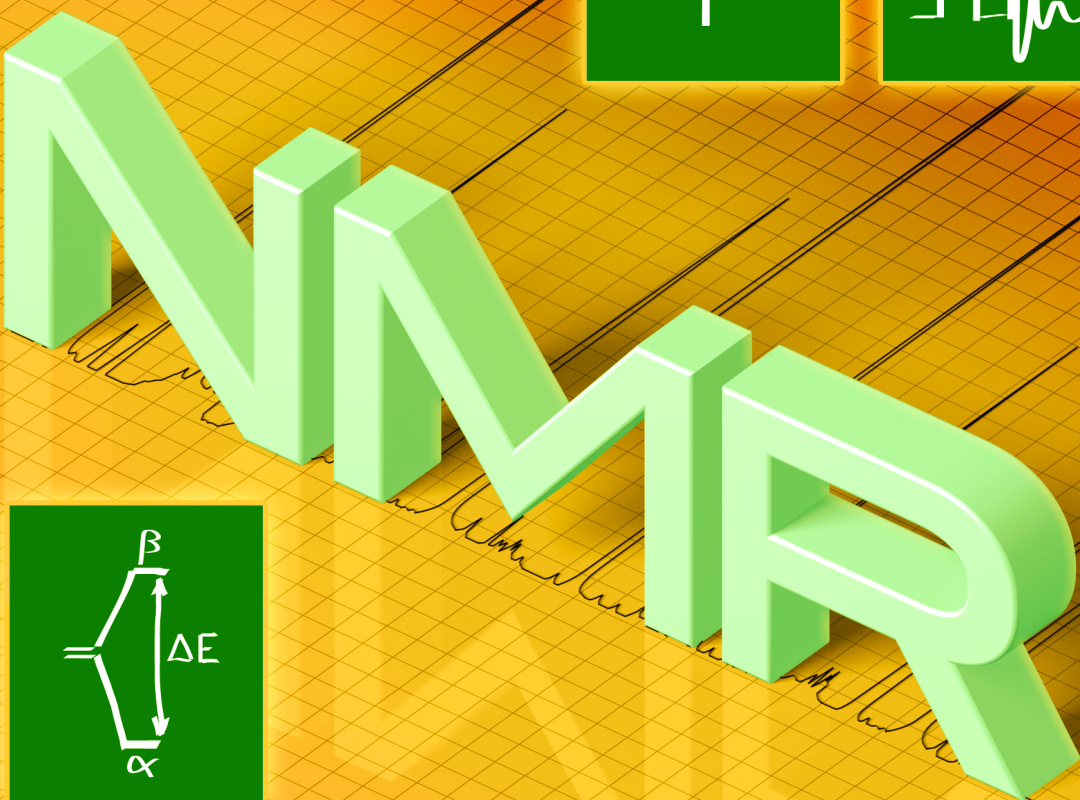
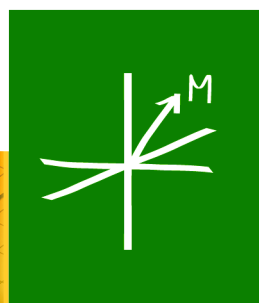


Oliver Zerbe and Simon Jurt

Applied NMR Spectroscopy for Chemists and Life Scientists



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Chemists and Life Scientists**

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Preface

NMR spectroscopy has developed very successfully from its early beginnings in the 1940s, at which time it was mainly subject to research in the labs of a few physicists, to its present frequent use by a broad community. Widespread use of NMR started in the 1960s when instruments moved into the laboratories of chemists to support analytics of synthesized products. The progress of modern chemistry only became possible with the advent of powerful analytical instrumental methods, with NMR spectroscopy playing a very pivotal role amongst them. To understand the importance of NMR, we only need to look back on natural product synthesis prior to the advent of NMR, where all intermediates had to be compared to known compounds through chemical transformations. Today, NMR is not only used by chemists, but also by researchers working in material science, structural biologists, the pharmaceutical industry, in product quality control as well as in many more fields of application.

Considering the importance of NMR in many branches of chemistry basic NMR knowledge is traditionally taught in the chemistry curriculum, and this is often done in combination with other spectroscopic techniques such as IR, UV, or MS. The content of these courses primarily aims at providing the student with practical skills of how to elucidate the structure of small (usually organic) molecules from simple spectra, mostly 1D and simple 2D spectra. Accordingly, the necessary empirical knowledge for example typical chemical shifts for important compound classes are taught, whereas the physicochemical background on the nature of the chemical shifts is less frequently explained. A reader interested in these topics is faced with a plethora of very good NMR books. However, these books generally aim at a readership with more advanced knowledge in physical chemistry and quantum mechanics, and as a result the reader may have difficulty understanding the presented topics.

NMR has rapidly moved into adjacent branches of science and today it is not only chemists that come into contact with NMR. Modern molecular biology makes heavy use of NMR to understand the structure and dynamics of biological macromolecules such as proteins, nucleic acids, or oligosaccharides. Today, some of the top Bio-NMR groups are hosted in the biological sector. NMR is also being increasingly applied in pharmaceutical sciences, both in the academic as well as in the industrial environment. Physicists also use NMR, often solid-state techniques, to

probe for properties of materials; and last but not least NMR experiments are performed in industrial labs worldwide.

We have written this book as an introduction to NMR for scientists in the above-mentioned fields. A guiding principle of the book is to introduce a topic first in very simple terms, and then to reexamine the topic at more elevated levels of theory. Thereby we hope to provide the reader with a source of knowledge that bridges the gap to the more advanced NMR books. We feel that the taught content and level of theoretical detail should be sufficient for a chemistry student at all levels, including those undertaking a PhD thesis unless the thesis topic is directly related to NMR. Of course, the reader is strongly encouraged to consult more advanced NMR textbooks, since we cannot cover all theoretical details in this book.

Twenty years ago samples were usually handed over to an NMR department and the spectroscopist would have returned processed and often also interpreted spectra. Since that time the situation has changed significantly to one where all these steps are performed by the students themselves. At the University of Zurich students are taught how to record their own NMR spectra, and they have hands-on experience of the spectrometers from the second year of their studies onwards. The stability of modern NMR spectrometer equipment and software has enabled nonexpert users to use NMR and easily perform more advanced 2D or even 3D NMR experiments. We feel, however, that it is important that the technical aspects of NMR are properly understood. The first steps in setting up an experiment are usually locking, shimming, probehead tuning etc., and although these steps are now often done automatically by the spectrometer we feel that it is unsatisfactory if users do not properly understand the actual meaning of these steps. Also of tremendous importance is correct spectra processing, and again, this is currently mostly done by the students themselves.

The book begins with a short basic introduction to solution NMR for the novice and explains the meaning of chemical shift and scalar couplings whilst also demonstrating how a small organic compound is readily identified from simple 1D spectra. The basics of NMR are then covered in the next part of the book with the second chapter reexamining the basic topics in more detail while also describing practical aspects of sample preparation, referencing etc. The third chapter provides an in-depth account of proton NMR spectroscopy, containing much of the empirical knowledge required for proton spectra interpretation. Following on from this we provide a similar account for ^{13}C and other X nuclei.

The second part of the book then presents the theory of NMR at a more advanced level, from single spins to macroscopic magnetization. It also describes the origin of the chemical shift and scalar couplings, and introduces the product operator formalism which is currently the most common technique to describe NMR experiments. This part finishes with a brief introduction to the quantum-mechanical description of NMR, and whilst this may prove too advanced for the novice reader, we considered it important for those readers that would like to consult the primary literature on NMR. The chapter introduces the meaning of many technical terms used in the field and may help in bridging the gap to the more advanced NMR books. Should students feel that they can successfully read the classical NMR liter-

ature after having read our book then we would certainly be very happy. Particularly in this last chapter we have excluded a lot of material for which the interested reader is referred to the more advanced NMR books or the original literature.

The third part of the book is devoted to the technical aspects of NMR, providing an overview of the instrument, spectra processing methods, and going into detail on spectra acquisition. Important experiments are described as well as features of pulses, gradients etc. For readers looking for more detail on the NMR experiments we have also added a chapter on the architecture of pulse programs.

The fourth part is devoted to special topics in NMR. It introduces important topics such as relaxation, the nuclear Overhauser effect, exchange phenomena, two-dimensional NMR, solid-state NMR, and the detection of intermolecular interactions by NMR (often referred to as screening in industry).

A good understanding of basic theory and the available set of experiments is certainly required, however the prime goal of NMR is still to correctly elucidate the chemical structure of a compound and this requires solid knowledge of empirical rules and an overview of the available NMR methods and experiments. Often the set of experiments that are most helpful for a particular task depend on the class of compound, and will be different, for example, for a peptide compared to an alkaloid. In this regard we present in the fifth part of the book a few important classes of natural products (carbohydrates, steroids, peptides, and nucleic acids). Each chapter begins with a brief summary of important chemical and structural features of the molecules concerned, provides summaries of typical chemical shifts, and suggests suitable strategies to most efficiently assign compounds from that class. Finally, an interpretation of a representative example from the class in question is provided on the basis of 1D and 2D spectra. PDF files of all spectra for enlargement are available under www.chem.uzh.ch/static/nmrbook. We will also publish corrections under this link.

This book was written with the invaluable help of many friends, who provided advice on the content of chapters and helpful criticism on how the material is presented. Any remaining errors are entirely our fault. We are particularly thankful to Stefan Berger, Sebastian Benz, Marcel Blommers, Fred Damberger, Marc-Olivier Ebert, Matthias Ernst, Thomas Fox, Gerd Gemmecker, Roland Hany, Erhard Haupt, Jan Helbing, Bernhard Jaun, Henning Jacob Jessen, Silke Johannsen, Ishan Calis, Wiktor Kozminski, Andrea Mazzanti, Frank Löhr, Detlef Moskau, Kerstin Möhle, David Neuhaus, Bernhard Pfeiffer, Daniel Rentsch, Alfred Ross, Markus Vöhler, Reto Walser, and Gerhard Wider. Nadja Bross helped with the preparation of the figures, measuring spectra, and critical reading of the chapters. Finally, we would like to thank our families for their patience.

Zurich, August 2013

Simon Jurt and Oliver Zerbe

1

Introduction to NMR Spectroscopy

Tremendous progress has been made in NMR spectroscopy with the introduction of multidimensional NMR spectroscopy and pulse Fourier transform NMR spectroscopy. For a deeper understanding of the experiment, a little knowledge of quantum physics is required. We will summarize the physical foundations of NMR spectroscopy in more detail in the following chapter. In this chapter, we will introduce the novice reader to the field of NMR spectroscopy in a simple way like we ourselves were introduced to it a long time ago. We will show some simple 1D spectra, and briefly describe what kind of information we can extract from these. For the moment we will assume that the spectra have been recorded by “someone,” and we will skip the technical aspects. Later in the book we will discuss all aspects of NMR spectroscopy – experimental, technical, and theoretical – to make you an NMR expert, who can run your own spectra and interpret them skillfully. You should then also have obtained the necessary knowledge for troubleshooting problems during data acquisition. Throughout the book we will introduce you to a subject first in a simple way, and then extend the discussion to more specialized topics and provide a more rigorous explanation.

1.1

Our First 1D Spectrum

Let us jump right into cold water and have a first glimpse at the spectrum of a simple organic compound. As an example we will choose an aromatic compound that is a natural product but produced synthetically on a large scale, called vanillin. So, let us have a first look at the proton spectrum (Figure 1.1).

We notice a number of signals at various places. The signals seem to be of different intensity. If we look a bit more closely, we recognize that lines are split into multiplets (see the expansion). Below the spectrum we find a scale which roughly runs from 0 to 10 ppm. The signals indicated by an arrow belong to the solvent (the signal at 2.5 ppm is from residual dimethyl sulfoxide and the signal at 0 ppm is from the tetramethylsilane standard used for referencing). Otherwise we can count six signals, corresponding to six different types of protons in vanillin. The

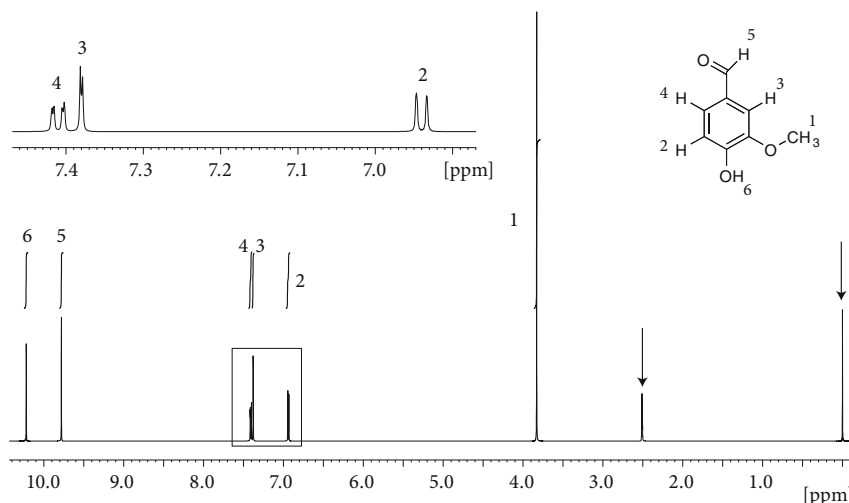


Figure 1.1 Proton NMR spectrum of a simple organic compound. The two arrows point to the standard for referencing (the tetramethylsilane signals) and the solvent line (the

dimethyl sulfoxide signal). Integral traces are depicted above the signals. The expansion shows the aromatic protons.

region from 6.9 to 7.5 ppm is expanded in the top panel. To start, let us learn a bit of nomenclature first

1.2

Some Nomenclature: Chemical Shifts, Line Widths, and Scalar Couplings

The phenomenon that the resonance frequency of a nucleus depends on the chemical environment is called *chemical shift*.¹⁾ The chemical shift is largely determined by the electron density around the nucleus. For practical reasons the chemical shift is given in parts per million relative to a standard. Chemical shifts, in general, are an invaluable source of information for the interpretation of spectra. In principle, they can be computed fairly precisely nowadays using quantum mechanical methods such as density functional theory. What makes chemical shifts really useful is that they are influenced by the presence of functional groups, double bonds, aromatic ring systems, and so on. This has led to elaborate tables of chemical shifts empirically derived from databases. You will find many of these tables in our chapters on proton and heteronuclear NMR, or in textbooks dedicated to that purpose. As a chemist, however, you will need to “memorize” some basic values. If you are

1) The chemical shift was discovered in 1950 by W.G. Proctor and F.C. Yu when they measured the magnetic moment of different types of nuclei. To their surprise they observed two distinct ¹⁴N lines for a solution of NH₄NO₃. The same observation was made almost simultaneously by W.C. Dickinson in the case of ¹⁹F nuclei.

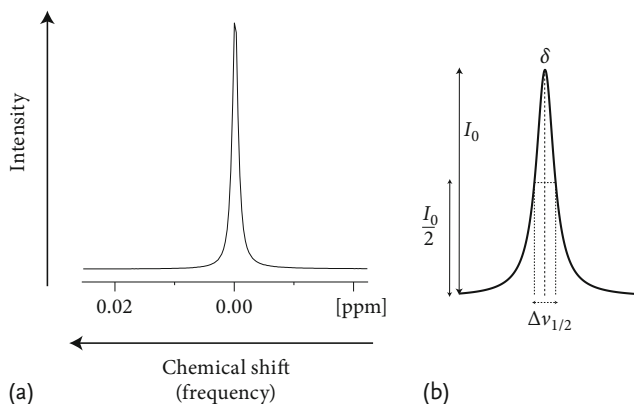


Figure 1.2 (a) A single resonance line. The frequency scale runs from the right to the left. A line with typical Lorentzian shape is depicted in (b).

working on a certain class of compounds, you will become an expert on chemical shifts for these molecules.

Let us now look more closely at a single line (Figure 1.2).

The line has a certain shape, a *Lorentzian* lineform. The signal is symmetric, and the highest intensity denotes the chemical shift position δ . The *line width* of the signal usually refers to the width at half height. Increasing values of chemical shift or frequency are plotted to the left for traditional reasons (note this is different from how it is usually done in physics or mathematics). Although the signals occur at certain frequencies, the frequency scale itself is not drawn, because it depends on the strength of the magnet. Instead, the values are presented in parts per million, which is the difference in frequency from a standard normalized by the frequency of the standard (do not worry, we will see how this scale is derived in more detail later).

Often signals are split into a number of lines (Figure 1.3), sometimes as many as nine or even more. These splittings are called *scalar couplings*, and originate from an interaction of the corresponding proton with neighboring protons, either on the same carbon or on the adjacent carbon(s) or heteroatom.

The center of the multiplet corresponds to the chemical shift δ of that signal. The separation of adjacent lines is called the *scalar coupling constant*, often abbreviated as J . Depending on whether the neighboring carbons are separated by rotatable bonds or whether the bond is sterically fixed, the number of lines due to scalar coupling is $N + 1$ (free rotation about the C–C bond) or 2^N (defined dihedral angle), where N denotes the number of neighboring protons. J is independent of the magnetic field strength and is specified in hertz. The individual lines often have different intensities (see Figure 1.3). Shown on the right of Figure 1.3 is a singlet, a doublet, a triplet, and a quartet. In the case of the quartet, the line intensities are 1 : 3 : 3 : 1. Since the number of lines follows simple rules, it helps us to establish the environment of the proton.

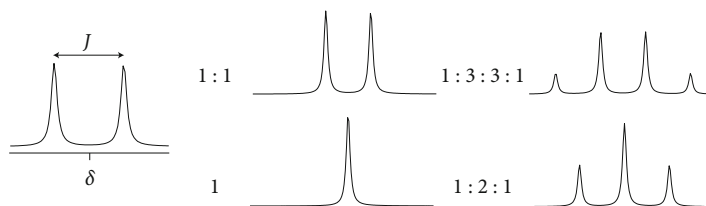


Figure 1.3 Scalar J couplings. Typical multiplet patterns for doublets, triplets and quartets are shown.

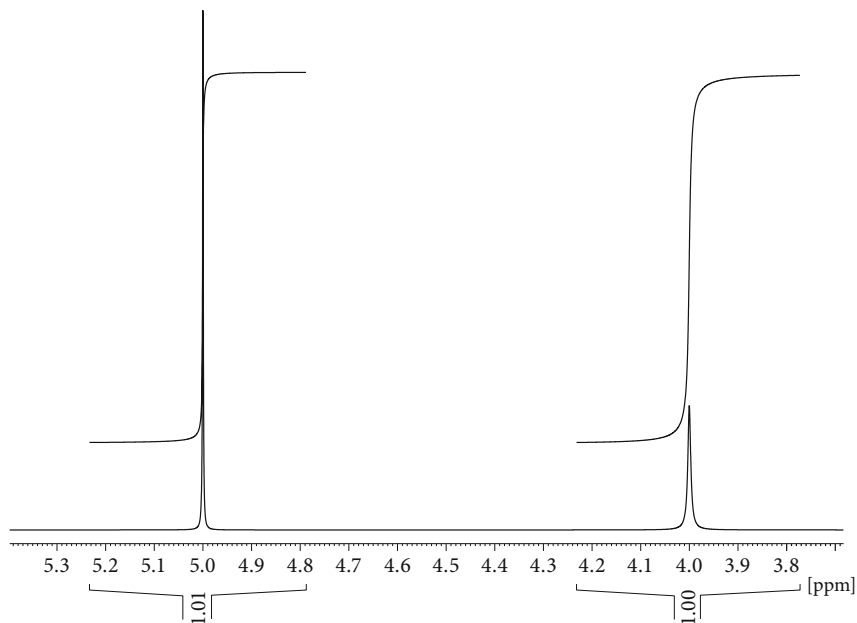


Figure 1.4 The effect of variable line widths. Two lines of very different intensity but the same integral are shown.

The intensity of the signals can be determined by integrating the spectra, and the *integrals* will tell us whether a certain signal is due to one, two, three, or more protons (Figure 1.4).

Integrals can be drawn as integral trails (usually directly on top of the signal) or their value can be plotted below the signal. Figure 1.4 displays two signals of identical integral but very different line width, with the signal at the lower frequency (the one on the right) being less intense. The line width has diagnostic value that is often underappreciated. Some lines become broader than others because the lifetime of the proton in a certain environment is short, a phenomenon due to either chemical or conformational exchange.

Spectra often also contain lines that do not belong to the molecule under study; some of them are referred to as *artifacts*. Such signals can belong to the solvent. In

Fourier transform NMR spectroscopy deuterated solvents are mandatory, but the degree of deuteration is never 100% and residual signal from the nondeuterated form is present. Another signal that is almost always present in proton spectra is the signal due to water, either from residual water in the solvent or because the compound has not been dried completely. Thirdly, a standard is often added for calibrating spectra. In most organic solvents tetramethylsilane is used because the signal usually occurs at one end of the spectrum and does not overlap with the signals of interest. Two-dimensional spectra contain other artifacts that are due to incomplete removal of unwanted coherence pathways, and we will deal with them later.

1.3

Interpretation of Spectra: A Simple Example

To get used to interpreting spectra, and to illustrate the strength of NMR spectroscopy, let us try to elucidate the structure of a small organic molecule. Its ^1H spectrum is shown in Figure 1.5.

The spectrum displays a number of signals, and the particular location of the signals, the chemical shift, already tells us a lot about the chemical nature of this molecule. For example, the signals at 7 ppm appear in a range that is typical for aromatic protons. Or, the signal around 3.6 ppm is most likely from a proton in the vicinity of some heteroatom. The signals around 1 ppm are most likely from methyl protons, which is also supported by the integral values of 3 and 6, respectively. Even

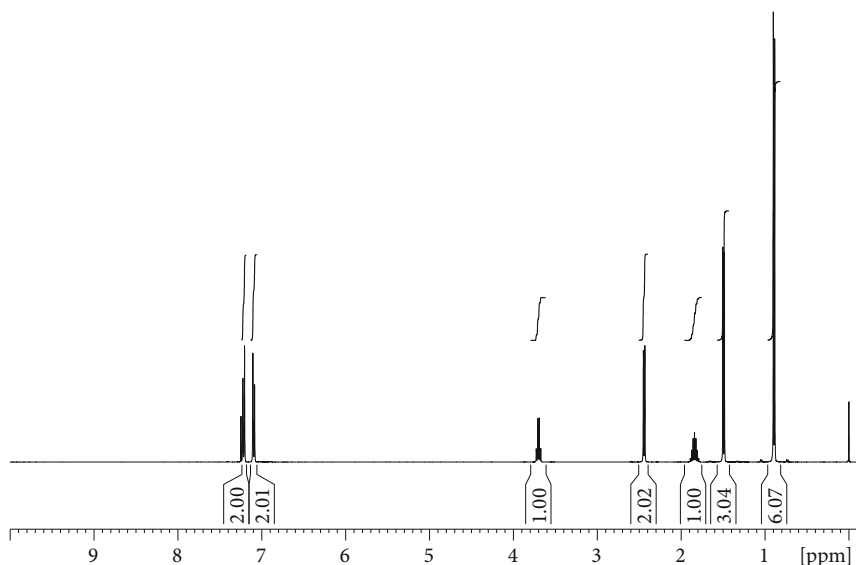


Figure 1.5 Proton NMR spectrum of ibuprofen.

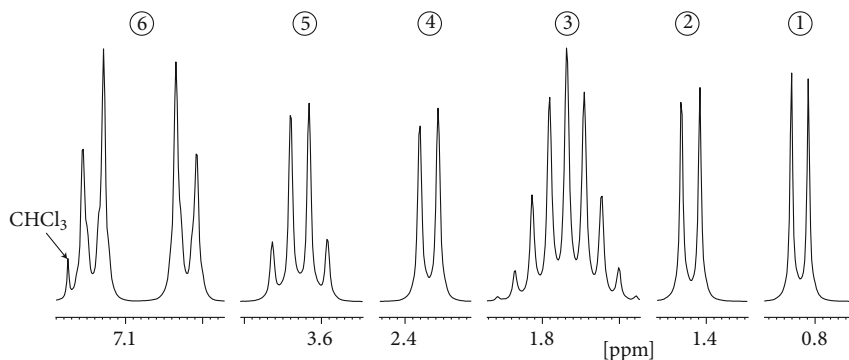


Figure 1.6 Expansions of the proton NMR spectrum revealing the multiplet fine structure of the signals.

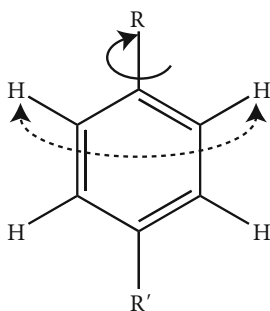


Figure 1.7 Our first fragment. Due to the symmetry of a para distributed benzene only two signals are observed for the four protons.

more helpful is the fine structure of the signals. To see that, let us zoom in a bit on the spectrum (Figure 1.6).

Most of the signals display the usual $(N + 1)$ multiplet pattern expected for protons in freely rotatable chains. The signal group labeled with 6 in Figure 1.6 consists of two doublets, which however, for reasons which will be explained in Section 3.4.2, are somewhat skewed. So let us begin building up the molecule.

We start with the signal group 6 in the range from 7–7.2 ppm. As mentioned before, this is the range typically observed for aromatic protons. The integral of these signals corresponds to 4. Although we do not know much about the chemical nature of the aromatic ring, we assume that it does not contain a heteroatom for the moment, and therefore is most likely derived from benzene. Four aromatic protons (instead of six) therefore indicates that the compound is a *disubstituted* benzene. The next question is whether the π system is 1,2-, 1,3-, or 1,4-disubstituted. In our case it is easy to determine this. We see only two peaks (two doublets). Since we have four aromatic protons, this is only possible if the substitution is such that two protons each become identical because of symmetry (see Figure 1.7). The aromatic ring therefore must be para disubstituted.

We will now try to identify the structure of the two substituents. Let us start with signal 1 at 0.8 ppm. It corresponds to six protons, likely two methyl groups. The

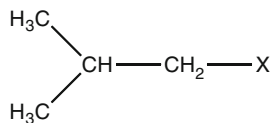


Figure 1.8 Our second fragment, an isobutyl group.

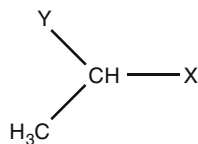


Figure 1.9 Our third fragment.

signal is due to either two distinct methyl groups at quaternary carbons (hence two singlets) or two identical methyl groups bound to a common carbon possessing *one* additional proton (hence two doublets with identical chemical shift). The latter case corresponds to an isopropyl group, for which we expect at least a septet (6 + 1 lines) for the CH proton. We say “at least” because the isopropyl group is connected to the remainder of the molecule, and other couplings may be due to the protons from the connecting carbon. In addition, the signal must integrate for *one* proton.

Indeed, if we look very carefully, we see that signal 3 at 1.8 ppm is split into nine lines (the outer lines are fairly weak and can easily escape our attention). This greatly supports the presence of an isopropyl group. Nine lines corresponds to eight protons on neighboring carbons. Since we have identified six already, the isopropyl group must be connected to a methylene (CH_2) group. The methylene signal must display an integral equal to 2, and the only signal that is left with such an integral is the one at 2.4 ppm (4). Since this signal is a doublet, and one of the connected carbons is a CH (from the isopropyl group), there cannot be any other CH carbons attached. Maybe this isobutyl fragment (Figure 1.8) is directly linked to the aromatic ring, a guess that must be verified later.

So far we have “explained” the presence of signals 1, 3, 4, and 6, and there remain two more signals (2 and 5). Obviously signal 2, which integrates for three protons, corresponds to a methyl group. Again, the doublet nature tells us that the methyl group is connected to a CH carbon. That proton signal must have at least four lines and an integral of 1, establishing the quartet 5 at 3.6 ppm as the neighbor. Since the signal has a multiplicity of four, no other CH is connected to that carbon.

If we again assume that this is the other fragment (Figure 1.9) linked to the aromatic ring, we are however missing one substituent, because one carbon has so far only three neighbors. The chemical shift of the proton at that carbon is 3.6 ppm, fairly low and indicating that a heteroatom is close. The full spectrum in addition displays a very broad signal around 10 ppm (we do not see it in Figure 1.5 because it is too broad), possibly from a hydroxyl proton. However, it could also be from a carboxyl group, and we will not be able to distinguish the two possibilities on the basis of the proton NMR spectrum. To resolve this ambiguity, let us have a look at the ^{13}C spectrum (Figure 1.10).

The signal at 180 ppm is due to a carboxyl group. The four signals in the range 125–142 ppm are due to the aromatic ring (two carbons each correspond to one sig-

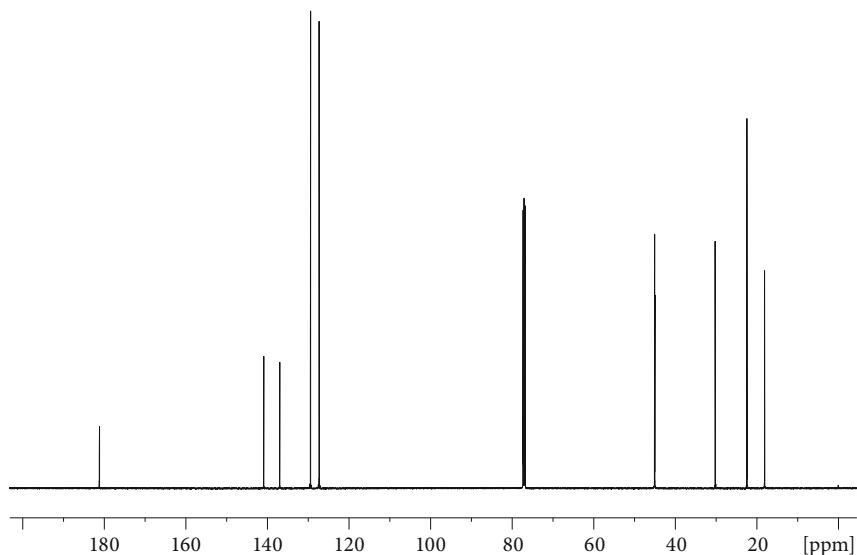


Figure 1.10 The ^{13}C NMR spectrum of ibuprofen.

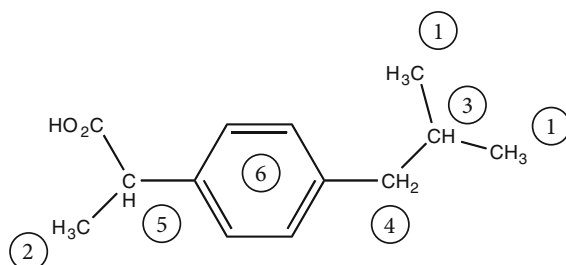


Figure 1.11 The molecular structure of ibuprofen and assignments of the proton signals.

nal due to the symmetry of the para-disubstituted ring). The signal around 77 ppm is from the chloroform solvent, and the four lines are from the other five carbons (the two isopropyl methyl carbons give rise to one signal). The missing fragment is therefore a carboxyl group and the structure of the compound is therefore unambiguously established as 2-[4-(2-methylpropyl)phenyl]propanoic acid (Figure 1.11), also known as ibuprofen, a painkiller that is produced on a multiton scale worldwide.

Of course, this is a very simple case, without any signal overlap. Moreover, the information on couplings and integrals always made the assignments unambiguous, and this is mostly not the case. However, we will see later that with the help of modern methods, in particular 2D NMR spectroscopy, fairly complicated molecules can still be identified unambiguously. However, we need to learn a few things before then so that we can exploit the power of NMR methods fully.

1.4

Two-Dimensional NMR Spectroscopy: An Introduction

The success of modern Fourier transform NMR spectroscopy is intimately linked to the development of multidimensional NMR spectroscopy. Protein structure determination by solution NMR spectroscopy or the elucidation of the structure of complex natural products is impossible without resorting to such methods. In the example of ibuprofen described above, the assignment was only possible in a straightforward fashion using 1D spectra, because at each point only a single resonance could be connected that had the right number of couplings and the correct integral. As soon as the molecules become larger, many ambiguous cases will arise, so further connectivities become unclear. The power of 2D shift-correlation spectroscopy is that the correct correlations can be directly extracted from the spectrum.

Two-dimensional spectra contain two frequency dimensions, and usually these correspond to chemical shifts. In the case of homonuclear spectra (the two frequency axes belong to the same type of nucleus, e.g., two proton frequencies), a diagonal runs through the 2D map, where the frequencies are the same in both dimensions. The really interesting information, however, resides in the off-diagonal, the so-called *cross peaks*. These peaks correspond to different chemical shifts and directly connect coupled nuclei. The exact type of experiment will determine which type of couplings (scalar or dipolar) have been used to establish the correlation. The

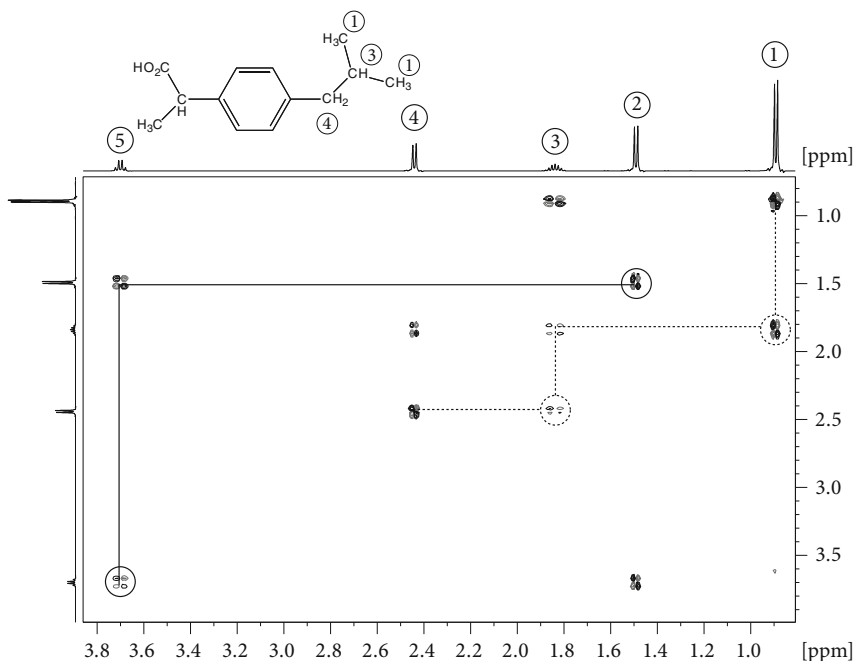


Figure 1.12 Two-dimensional correlation spectroscopy (COSY) spectrum of ibuprofen.

2D spectra are 3D objects, with two frequency dimensions, and the third dimension corresponding to the intensity of the signals. Usually, 2D spectra are displayed in the form of *contour plots*, quite similar to topographic maps, in which different heights (mountains) are indicated by contour lines that connect places of similar height. One of the simplest 2D experiment is the COSY experiment, a shift–shift correlation experiment in which correlations occur through scalar (usually vicinal) couplings. In the COSY spectrum in Figure 1.12 we have traced through correlations of the substituents in ibuprofen; the cross peaks are encircled, and the path for the isopropyl fragment is shown by dotted lines.