Application Note 21

Structural Elucidation by Benchtop NMR Spectroscopy: Ibuprofen

Introduction

One of the major uses of nuclear magnetic resonance (NMR) spectroscopy is for the structural elucidation of unknown chemical compounds, and the structural confirmation of known compounds.

In this application note, a series of one- and two-dimensional NMR spectra obtained on the **Oxford Instruments X-Pulse Broadband Benchtop NMR Spectrometer** are analysed, showing how the information obtained by NMR spectroscopy corresponds to structural features in a known molecule. In this case the chosen molecule is Ibuprofen, and the spectra were obtained of a 1 mol/ℓ solution in $CDCl_3$.

Ibuprofen

Ibuprofen, 2-(4-(2-methylpropyl)phenyl)propanoic acid, is an anti-inflammatory drug that is commonly used to treat pain, fever, and inflammation. Ibuprofen was originally discovered in 1961 by Dr Stewart Adams and Dr John Nicholson, working at Boots UK Ltd., in Nottingham, U.K. It is on the World Health Organisation's List of Essential Medicines, and is available without prescription worldwide. It was chosen as an example here due to it's similarity in molecular weight and structural complexity to a wide range of common production pharmaceuticals that are readily analysable by benchtop NMR.

The molecular structure of Ibuprofen is shown in figure 1, where the eight unique proton chemical environments (1, 3, 4, 6, 7, 9 - 11), and the ten unique carbon environments (2 - 11) can be clearly identified.

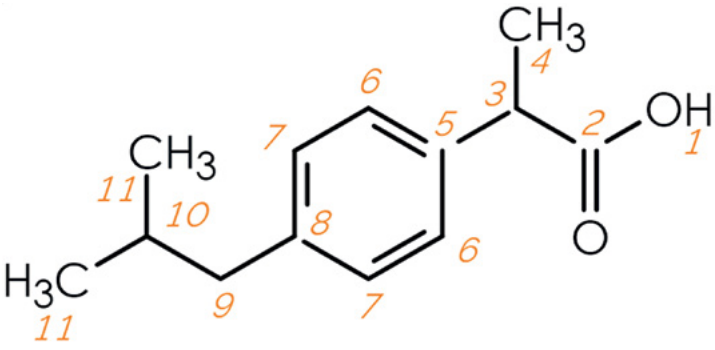

Figure 1 Molecular Structure of Ibuprofen.

Structural Elucidation

One-dimensional NMR Spectra

Simple one-dimensional NMR spectroscopy entails the excitation of nuclei of a single isotope to get information about those atoms only. This produces spectra with signal intensity against frequency. The chemical shift (δ) is plotted on the horizontal axis, in ppm (parts per million). The plotting of chemical shift in ppm rather than Hertz (Hz) ensures comparability between data measured by different spectrometers.

Proton (Hydrogen-1) NMR

The first NMR spectrum obtained of most known or unknown samples, is usually a simple proton (hydrogen-1) NMR spectrum, which for most samples can be obtained in under 5 minutes. The ^{1}H NMR spectrum of Ibuprofen is shown in figure 2.

Signals in an NMR spectrum comprise one or more individual peaks, for example while the signal at δ_H 11.9 ppm is a single peak, the signal centred at δ_H 3.7 ppm comprises four peaks (and commonly would be described as a quartet). There are three major features that should be considered for each signal in a ^{1}H NMR spectrum:

1. The chemical shift, δ_H, of the signal in the spectrum, which corresponds to the local chemical environment of the nuclei giving rise to the signal.
2. The (integrated) area of each signal, which corresponds to the number of nuclei associated with each signal. The integral value shown in green below each signal in figure 2.
3. The multiplicity of the signal, which comprises the number of peaks in the signal, their relative intensities, and the separation between the peaks. It also provides information on other nearby NMR-active nuclei.

By initially considering only the chemical shifts and integrated areas of the signals, an initial assignment can be made as follows:

- one carboxylic acid proton.
- four aromatic protons.
- five signals, arising from thirteen aliphatic protons.

Since there is only a single carboxylic acid proton in Ibuprofen, 1, that can be assigned to the signal at δ_H 11.9 ppm. The signal for four aromatic protons centred around δ_H 7.2 ppm, can be assigned to protons 6 & 7.[1]

To further assign the five alkyl signals, their multiplicity should be considered alongside their integrations and chemical shifts.

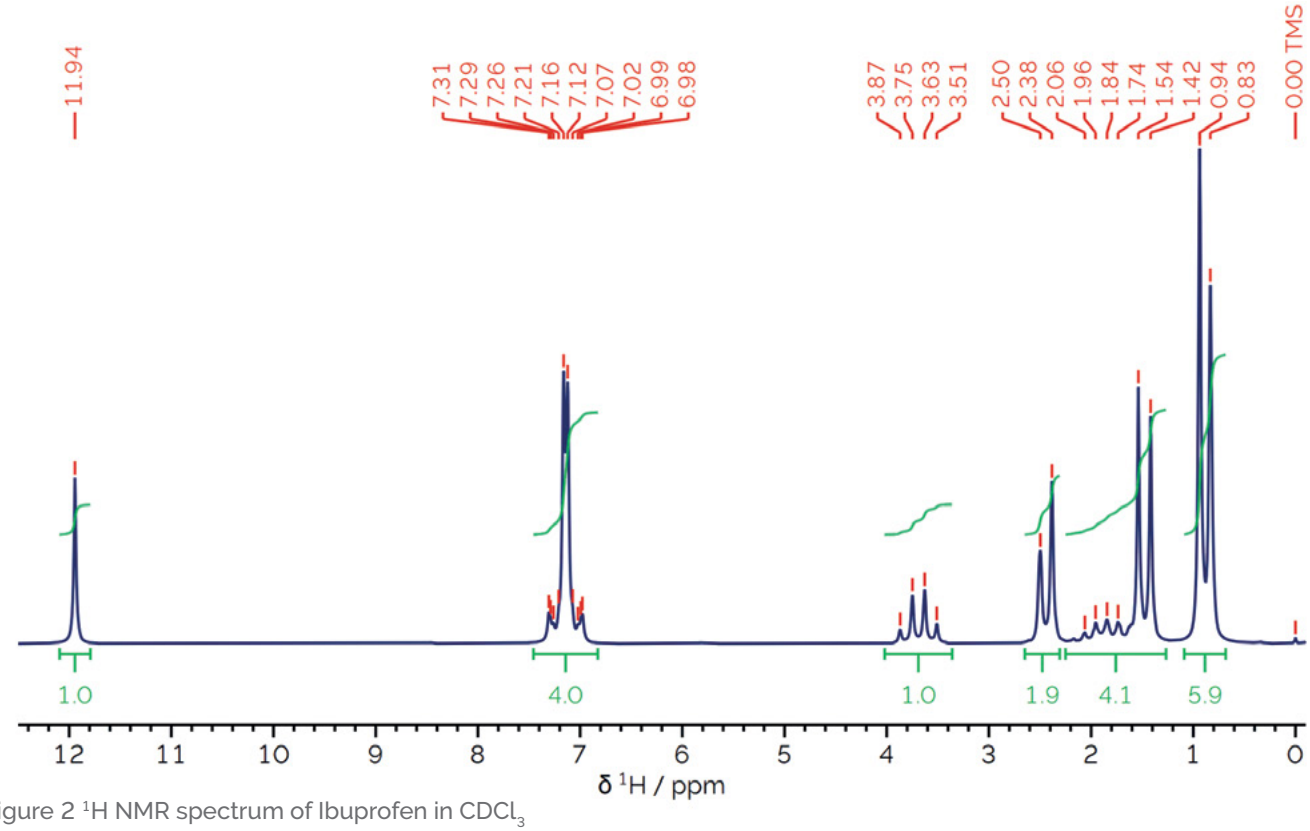

Figure 2 ^{1}H NMR spectrum of Ibuprofen in $CDCl_3$

[1] The multiplicity of this signal is consistent with a para substituted aromatic ring, however an explanation of this is beyond the scope of this Application Note.

In simple cases, the number of peaks that make up an ^{1}H NMR signal correlate to the number of protons bound to adjacent carbon atoms, with one more peak that the number of carbon atoms. It is therefore possible to identify the following facts for the remaining unassigned signals.

The signal at δ_H 3.7 ppm is made up of four peaks, and integrates as a single proton, implying a CH group adjacent to a CH_3 group, the only proton in Ibuprofen consistent with this is 3.

There are three signals at δ_H 2.4, 1.5 & 0.9 ppm, each made up of two peaks (a doublet) implying they're immediately adjacent to a single CH group. The signal at δ_H 0.9 ppm integrates as six protons, suggesting two CH_3 groups, with the two methyl groups 11, as the only possible protons in Ibuprofen it could be. The signal at δ_H 2.4 ppm integrates as two protons, thereby corresponding to a CH_2 bound to a CH, and consistent with 9 in Ibuprofen. The remaining doublet at δ_H 1.5 ppm overlaps with the multiplet (meaning a signal with a complex multiplicity), at δ_H 1.9 ppm, however close inspection of the integrations, identifies the doublet as corresponding to three protons, hence as a CH_3 bound to CH, and 3 in Ibuprofen.

By a process of elimination, the multiplet at δ_H 1.9 ppm, can be assigned to the CH group 10, which is bound to two CH_3 groups and one CH_2 explaining the more complex coupling pattern.

While unnecessary to assign the proton NMR spectrum in this case, there's one more piece of information which may be obtained from a ^{1}H NMR spectrum to aid in the assignment of signals.

When couplings occur between nuclei, resulting in observation of multiplets in the NMR spectrum, the magnitude of that coupling can be measured (in Hz) from the separation of the peaks in the multiplet. Since the coupling is a constant, the same value will be measured whichever signal from a pair is chosen. This can be applied to pair up signals in the NMR spectrum that are directly coupled. For example, the quartet at δ_H 3.7 ppm, which has been assigned as 3, has a coupling constant, $^{3}J_{HH}$ of 7.1 Hz, while the doublets at δ_H 2.4, 1.5 & 0.9 ppm, have coupling constants of $^{3}J_{HH}$ 6.7, 7.2, & 6.3 Hz respectively, confirming that the signal at δ_H 3.7 ppm, is coupled with that at δ_H 1.5 ppm (which has already been assigned as 4). [2] This potentially time-consuming process of measuring and pairing couplings to aid assignment can be avoided with the use of two-dimensional spectra, as shown later for the ^{1}H-^{1}H COSY spectrum.

Carbon-13 NMR

While proton is a high gyromagnetic ratio nucleus with near 100% natural abundance, making it the 'best' nuclei for NMR spectroscopy, the situation for carbon is more complex. The most abundant isotope of carbon, carbon-12, is NMR inactive. Instead carbon NMR spectra are measured using carbon-13, this has only 1.1% natural abundance along with a lower gyromagnetic ratio than proton. Therefore, carbon-13 is 1/5870th the receptivity of the proton; hence requiring higher sample concentrations and longer measurement times.

The carbon-13 NMR spectrum is shown in figure 3, the spectrum appears simpler than the proton spectrum with all signals appearing as a single sharp peak. This is because all carbon-proton couplings have been removed by applying proton-decoupling.

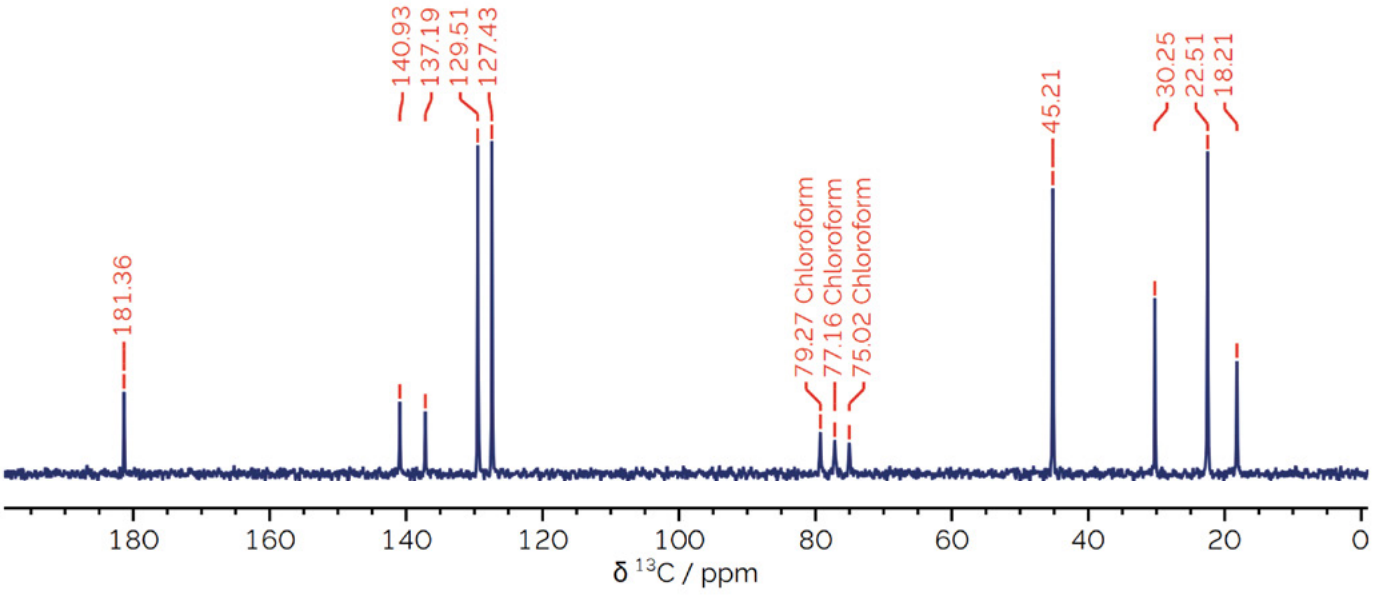

Figure 3 $^{13}C\{^{1}H\}$ NMR spectrum of Ibuprofen in $CDCl_3$.

[2] The magnitude of the J-coupling can also provide information about the relative positions of the nuclei; however there are no suitable examples in Ibuprofen, putting this beyond the scope of this Application Note.

This spectrum is referred to as a proton-decoupled carbon-13 NMR spectrum, simplified to $^{13}C\{^{1}H\}$ NMR spectrum.[3]

However, as previously noted there are ten chemically unique carbon environments in Ibuprofen, yet there are only nine signals observed in the NMR spectrum due to the Ibuprofen, suggesting that two signal overlap appearing as a single peak.

Were this a proton NMR spectrum, it would be easy to identify any overlapping signals, by considering the integrated areas of the peaks. In general a proton-decoupled carbon-13 NMR spectrum is an exception to the rule that integration of peaks/signals directly corresponds to the number of nuclei giving rise to that signal.[4] There is some information which can be inferred from the intensity of signals in a $^{13}C\{^{1}H\}$ NMR spectrum. In general signals of carbon atoms not directly bound to any protons (quaternary carbons), are considerably less intense than if the carbon atom is directly bound to one or more protons.

This can be observed for the signals at δ_C 181.4, 140.9 & 137.2 ppm, along with the signal (a 1:1:1 triplet) arising for the deuterated chloroform solvent.

By considering the chemical shift of these carbon signals, it's possible to assign the signal at δ_C 181.4 ppm as a carbonyl group, and hence to the carboxylic acid carbon 2.

While the signals at δ_C 140.9 & 137.2 ppm are consistent with aromatic carbons, and therefore the quaternary carbons 5 & 8.

The two considerably more intense signals in the aromatic region, at δ_C 129.5 & 127.4 ppm, can therefore be assigned as the aromatic CH carbons 6 & 7.

The remaining four signals in the carbon-13 NMR spectrum, are all consistent with alkyl, CH_x carbons, with those at a lower chemical shift likely to correspond with the terminal methyl, CH_3 groups. A complete assignment of these signals requires additional information which cannot be obtained from a simple one-dimensional $^{13}C\{^{1}H\}$ NMR spectrum.

[3] Carbon-carbon couplings are generally not observed, due to the low natural abundance of carbon-13, and hence the weak signals that would result.

[4] It is possible to obtain carbon-13 spectra that can be integrated, however that requires non-standard sequences, and even longer experiment durations.

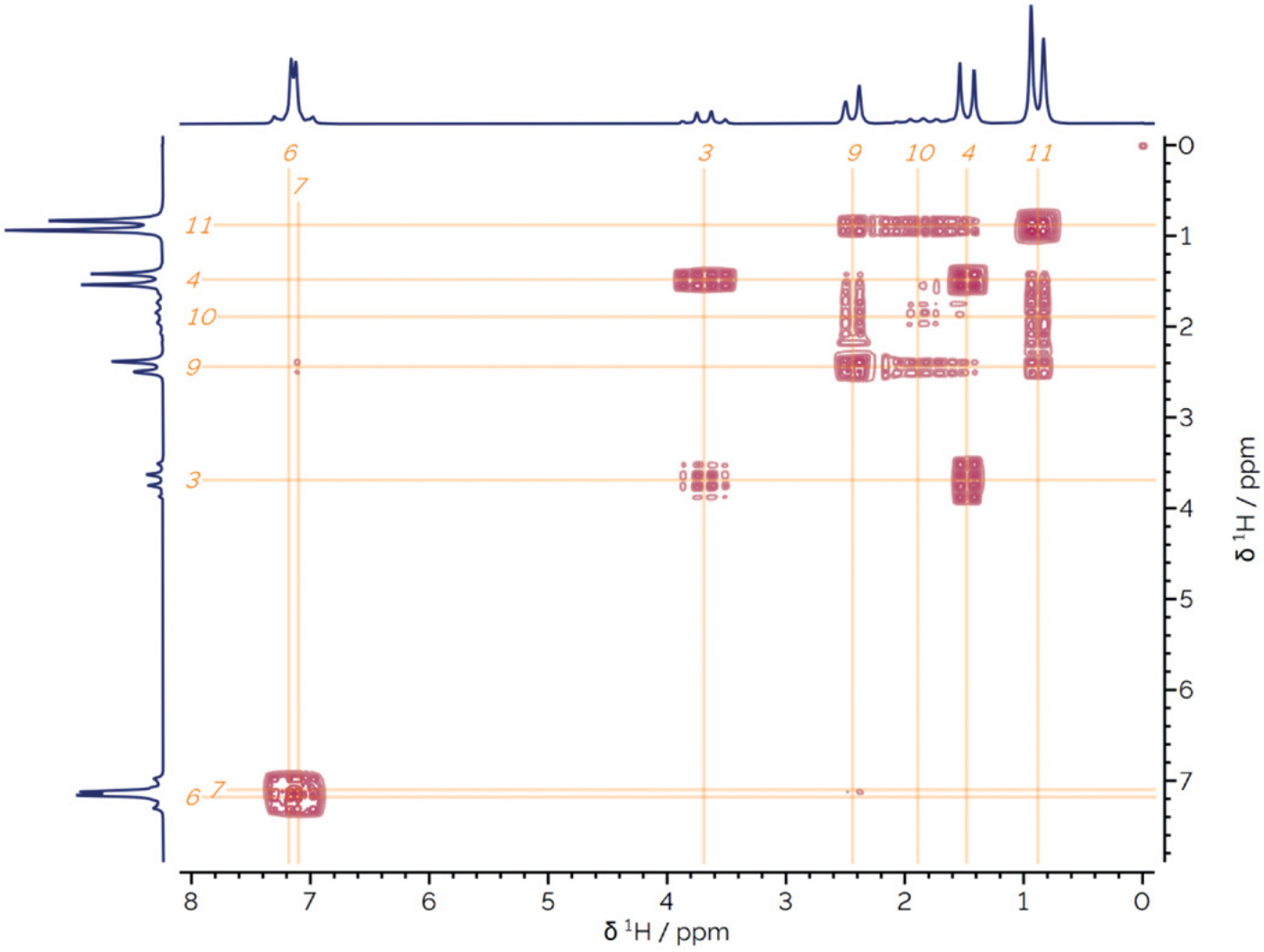

Figure 4 ^{1}H-^{1}H gradient-selective **Co**rrelation **S**pectroscop**y** NMR spectrum of Ibuprofen in $CDCl_3$.

Two-dimensional NMR Spectra

So far, only simple one-dimensional NMR spectra have been considered. However, one of the great strengths on NMR spectroscopy, is the wide range of different pulse sequences available, and the different spectra which may be obtained. One major group of sequences are those that give two-dimensional correlation spectra. In these cases, cross-peaks are observed corresponding to interactions (such as through *J*-couplings) between signals observed in the corresponding one-dimensional spectra. The following examples demonstrate how the introduction of a second dimension can resolve complications due to overlap, significantly simplify the assignment process, and result in rapid identification of complex molecules.

^{1}H-^{1}H Correlation Spectroscopy

The first two-dimensional spectrum to be considered in the proton-proton **Co**rrelation **S**pectroscop**y** (COSY) spectrum, which is shown in figure 4 for Ibuprofen.

Note that on the *x* and *y* axis, the simple proton spectrum discussed previously is displayed. The actual two-dimensional spectrum consists of cross-peaks shown in red in figure 4.

As a homonuclear correlation spectrum, a COSY is symmetric along the diagonal, with off-diagonal cross-peaks present when signals directly couple. In general, cross-peaks will be observed in the COSY, for signal pairs where *J*-coupling is observed in the one-dimensional spectrum.

In the case of Ibuprofen, cross-peaks are observed in the COSY spectrum for 4-CH_3 & 3-CH; additionally cross peaks are observed between the three signals 9-CH_2, 10-CH & 11-CH_3. This is also an example of where COSY cross peaks can be observed when the *J*-coupling is not observed in the one-dimensional spectrum, as in the case for the four-bond interaction between 9-CH_2 & 11-CH_3.

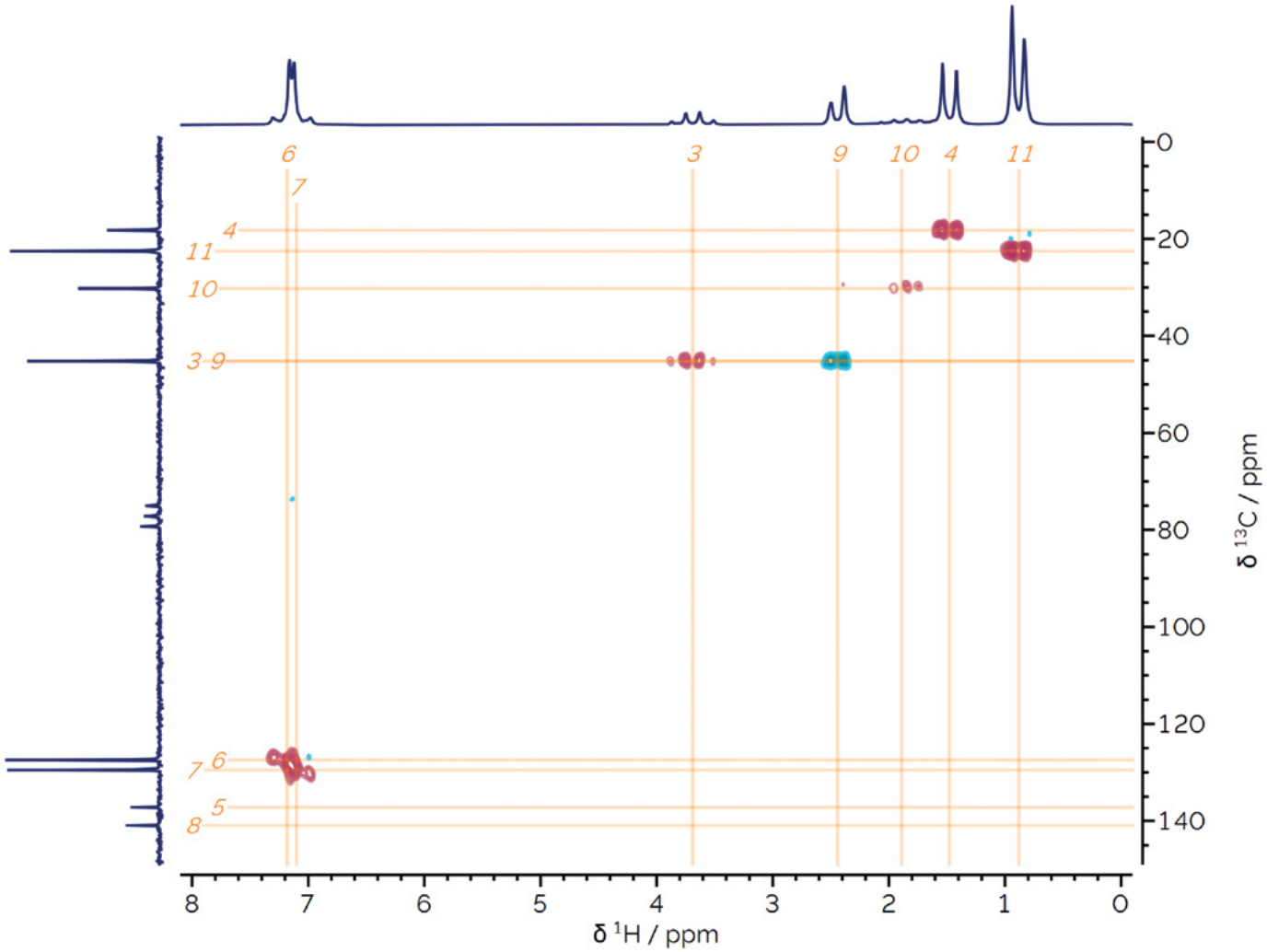

Figure 5 ^{1}H-^{13}C gradient-selective **H**eteronuclear **S**ingle-**Q**uantum **C**orrelation with multiplicity editing NMR spectrum of Ibuprofen in $CDCl_3$.

^{1}H-^{13}C Correlation Spectra

In addition to proton-proton correlation experiments like the COSY, pulse sequences which allow for the observation of proton-carbon correlations also aid the assignment process. Two of the most commonly used are the **H**eteronuclear **S**ingle **Q**uantum **C**oherence with **M**ultiplicity **E**diting (HSQC-ME) sequence which allow for the observation of single bond proton-carbon correlations, and the **H**eteronuclear **M**ultiple **B**ond **C**orrelation (HMBC) sequence which allows for the observation of multiple bond proton-carbon correlations.

The ^{1}H-^{13}C HSQC-ME and ^{1}H-^{13}C HMBC spectra (figures 5 & 6) both show the proton spectrum in the horizontal dimension, while the carbon spectrum is displayed in the vertical dimension. The cross peaks in figure 5 for the HSQC-ME spectrum of Ibuprofen correspond to the single bond proton-carbon correlations.

This spectrum was also obtained multiplicity edited, which provides information on the multiplicity of the CHx environments. With CH and CH_3 giving cross-peaks with positive phase (red in figure 5), and CH_2 giving cross-peaks with negative phase (blue in figure 5).

The first thing that should be noted from the HSQC-ME spectrum is it explains the nine signals that are observed in the one-dimensional $^{13}C\{^{1}H\}$ spectrum when ten signals were expected. The signal at δ_C 45.2 ppm, correlates with two proton signals: 3-CH (δ_H 3.69 ppm) & 9-CH_2 (δ_H 2.44 ppm), confirming that the two carbon atoms directly bound to the aromatic ring have indistinguishable ^{13}C chemical shifts.

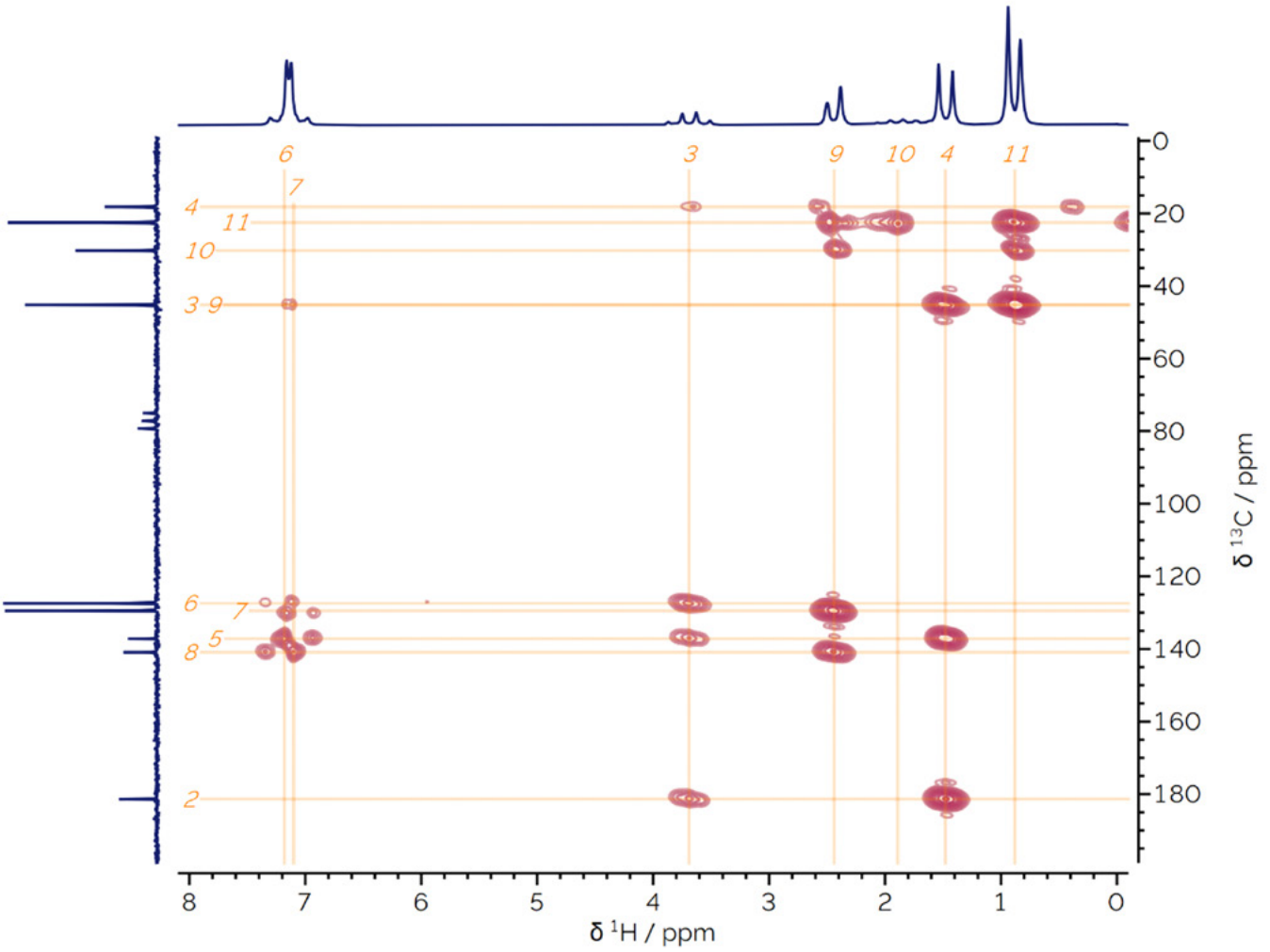

Figure 6 ^{1}H-^{13}C gradient-selective **H**eteronuclear **M**ultiple-**B**ond **C**orrelation NMR spectrum of Ibuprofen in $CDCl_3$.

The ^{1}H-^{13}C HMBC spectrum of Ibuprofen is shown in figure 6, with the observed cross-peaks corresponding to two or three bond proton-carbon correlations. The HMBC spectrum allows for the full structural assignment / elucidation for Ibuprofen to be completed.

For example, the signal at δ_H 3.69 ppm for 3-CH, correlates with an aromatic CH at δ_C 127.4 ppm and a quaternary aromatic carbon at δ_C 137.2 ppm, identifying the source of those signals as 6 and 5 respectively. By systematically applying this for all the signals, the carbon-connectivity can be deduced, and the full spectrum assigned / structure elucidated.

Additional Two-dimensional Correlation Spectra

The COSY, HSQC and HMBC are not the only two-dimensional pulse sequences available on the **X-Pulse** which are useful for the assignment of NMR spectra, and elucidation of chemical compounds. The **T**otal **C**orrelation **S**pectroscop**y** (TOCSY) sequence allows for the observation of not only the direct proton-proton couplings observed in the COSY, but also indirect proton-proton couplings through the spin system; and is discussed in our Application Note "Spin Locking: Total Correlation Spectroscopy (TOCSY)".

While the **N**uclear **O**verhauser **E**nhancement **S**pectroscop**y** (NOESY) shows cross-peaks arising from a through-space interaction,[5] and allows for the three-dimensional structure of molecules to be determined.

[5] Rather than the through-bond interactions observed by COSY, TOCSY, HSQC & HMBC.

Ibuprofen Spectral Assignments

By analysing all these spectra, it's possible to fully assign the signals in the one-dimensional spectra. The complete assignment of the proton and carbon-13 NMR spectra of Ibuprofen are detailed as follows.

δ_H (60 MHz, 40°C, $CDCl_3$): 11.94 (1H, s, 1-OH), 7.46 – 6.83 (4H, m, 6,7-C_6H_4), 3.69 (1H, q, $^3J_{HH}$ 7.1, 3-CH), 2.44 (2H, d, $^3J_{HH}$ 6.7, 9-CH_2), 1.89 (1H, qt, $^3J_{HH}$ 6.7, $^3J_{HH}$ 6.3, 10-CH), 1.48 (3H, d, $^3J_{HH}$ 7.2, 4-CH3), 0.88 (6H, d, $^3J_{HH}$ 6.3, 11-CH_3).

δ_C (15 MHz, 40°C, $CDCl_3$): 181.36 (1C, s, 2-C=O), 140.93 (1C, s, 8-CH), 137.19 (1C, s, 5-CH), 129.51 (2C, s, 7-CH), 127.43 (2C, s, 6-CH), 45.21 (2C, s, 3-CH & 9-CH2), 30.25 (1C, s, 10-CH), 22.51 (2C, s, 11-CH_3), 18.21 (1C, s, 4-CH_3).

Summary

Using a range of one- and two-dimensional proton and carbon-13 NMR pulse sequences, the **Oxford Instruments X-Pulse Broadband Benchtop NMR Spectrometer**, complete structural assignment / elucidation can be performed on a wide range of small molecules. Acquisition of the experiments demonstrated here can be automated for up to 25 samples at once when X-Pulse is used in conjunction with the X-Auto autosampler.

If you have any questions about this application note, please contact our experts:
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