7.4: Two Dimensional Heteronuclear NMR Spectroscopy
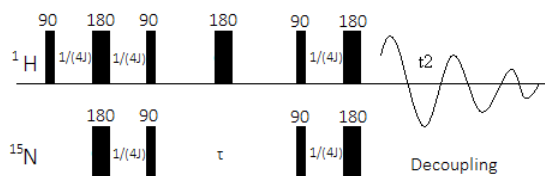
> **◖▶ Learning Objectives**
>
> - Understand what HSQC is and when to use it
> - Understand what HMBC is and when to use it

The previous section discussed the correlation of ^{1}H to ^{1}H, but those are not the only NMR active nuclei in a molecule. Heteronculear 2-D NMR is the correlation between different nuclei, such as a ^{1}H to ^{13}C and heteronuclear 2-D NMR is especially important in biological chemistry, especially in the elucidation of the three-dimensional structure of proteins.
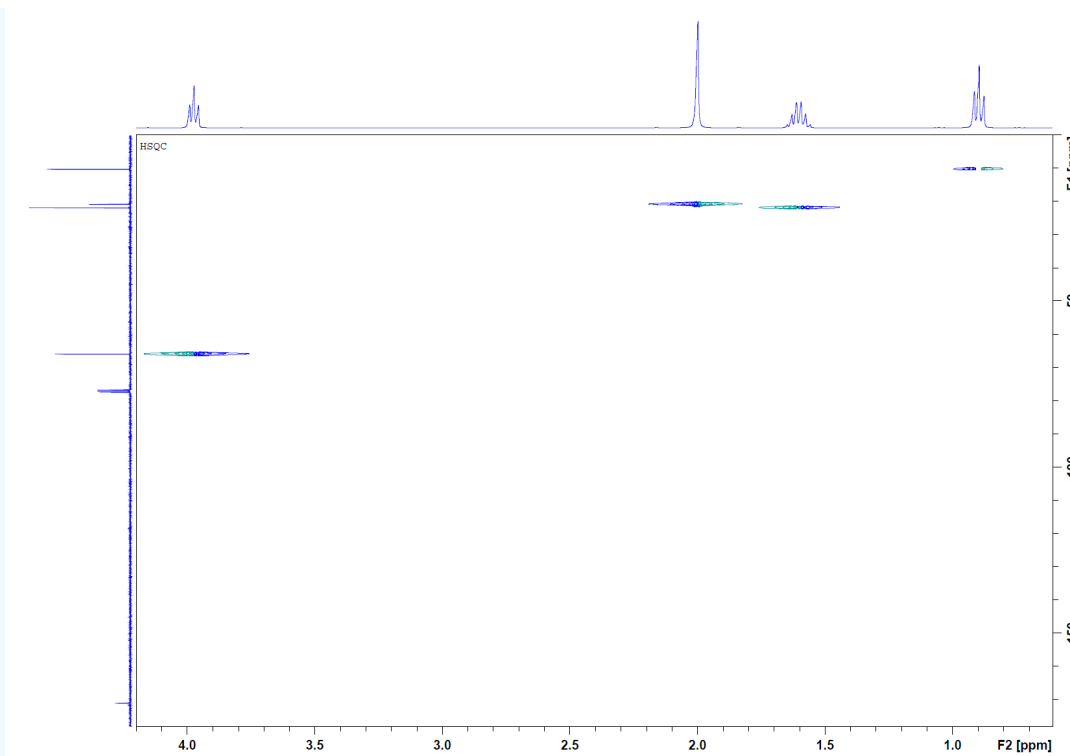
Heteronuclear Single Quantum Coherence

Hetereonuclear Single Quantum Coherence (HSQC) is used to determine the proton to carbon or heteroatom (often nitrogen) single bond correlations. It is also known as heteronuclear multiple quantum coherence (HMQC). While the DEPT may give the same information for ^{13}C to ^{1}H correlations, HSQC is more sensitive, therefore, it may be more advantageous to use this type of experiment in complex situations. In an HSQC experiment, polarization is transferred from a ^{1}H nuclei to a neighboring heteroatom (^{13}C or ^{15}N). This polaraization is then transferred back to the ^{1}H nuclei. The signal from the ^{1}H nuclei is recorded. The pulse sequence for a typical HSQC experiment is detailed below involving ^{1}H and ^{15}N nuclei.

In an HSQC spectrum, a ^{13}C or heteroatom spectrum is displayed on one axis and a ^{1}H spectrum is displayed on the other axis. Cross-peaks show which proton is attached to which carbon or heteroatom. The purpose of a HSQC is to determine which protons are coupled to what other specific carbon or heteroatom in the molecule through bonds.

> **✔ Example 7.4.1**
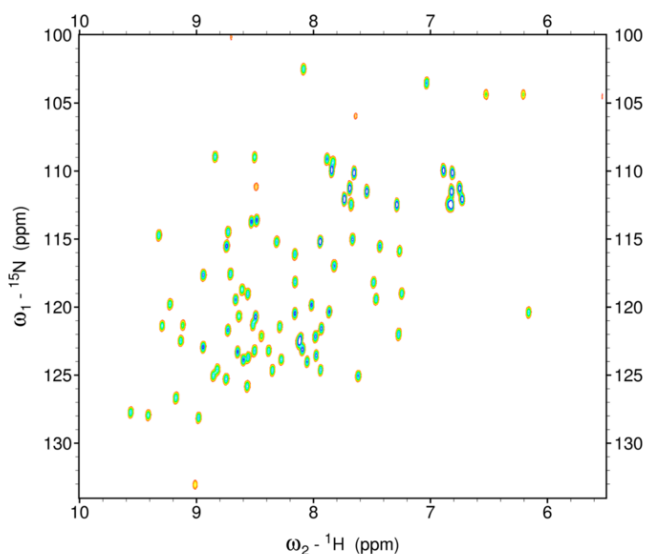>
> The HSQC of propyl acetate is below:

Assign which hydrogens are attached to which carbons.

Solution

An HSQC experiment spreads things out into two dimensions just like the homonuclear experiments did in the previous section. Looking along the x axis, we the ^{1}H NMR spectrum and along the y axis we see the ^{13}C NMR spectrum displayed. The peak at 171 ppm in the ^{13}C has no cross peak. This means that this carbon does not have any hydrogens attached, therefore, this is our carbonyl carbon. The peak at 77 ppm is residual solvent, in this case CDCl$_3$. The cross peak at (3.9, 66) is a methylene attached to a carbon that is attached to an electron-withdrawing group. If you look at a ^{13}C data table, then you can see where different carbon groups show up. In propyl acetate, oxygen is the most electron withdrawing group, so the piece we have at this cross peak is -CH$_2$-O. The cross peak at (2.0, 21.8) is the methyl group that has no coupling neighboring hydrogens. The next cross peak at (1.6, 20.8) is a methylene group, The final cross peak is (0.9, 10.2) is a methyl group.

Another example HSCQ spectrum from ubiquitin is shown below.
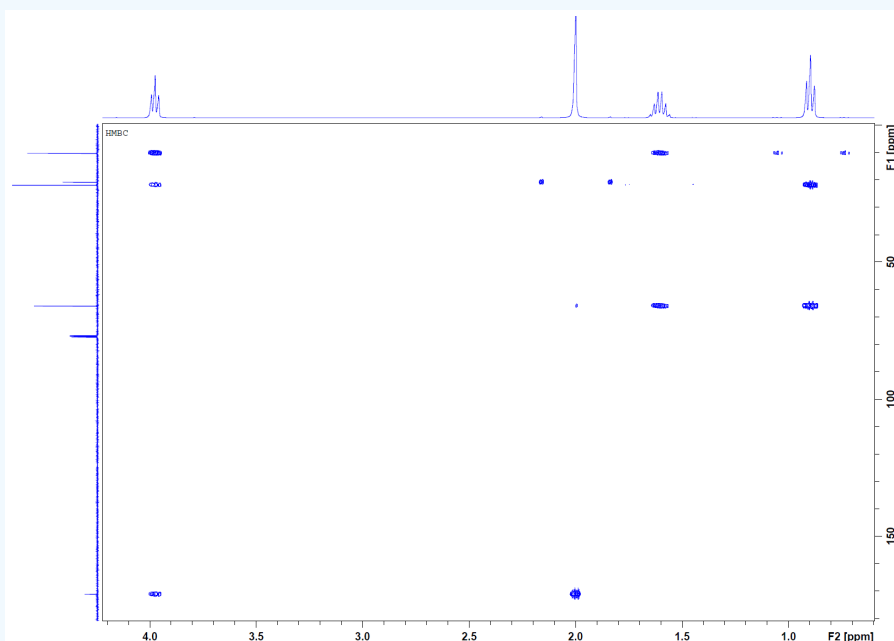
$^1H^{15}N$ HSQC spectrum of ubiquitin

Notice the greater clarity of spectra of the HSQC experiment. This is a strong advantage of heteronuclear NMR. In this diagram, each peak corresponds to a cross peak, showing coupling between sets of ^1H and ^{15}N nuclei. Each peak represents the ^{15}N—^1H of a unique amino acid along the backbone of the amino acid.

Heteronuclear Multiple Bond Correlation (HMBC)

Hetereonuclear Multiple Bond Correlation (HMBC) is used to determine long range ^1H to ^{13}C connectivity. This experiment gives the correlation between ^1H and ^{13}C when separated by two, three, and even four (if through a conjugated system) bonds away. In an HSQC experiment, direct one bond correlations are suppressed as part of the sequence and like HSQC is proton detected. The time delay in the pulse sequence can be optimized for different coupling constants, J. The signal from the ^1H nuclei is recorded. The spectra of HSQC give rise to cross peaks that correlate one nuclei to another. These correlations help determine which protons are coupled to what other specific heteroatom more than one bond away.
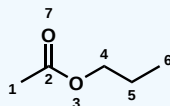
✔ Example 7.4.2

Below is the HMBC of propyl acetate:

Does the HMBC corroborate with the structure of propyl acetate?
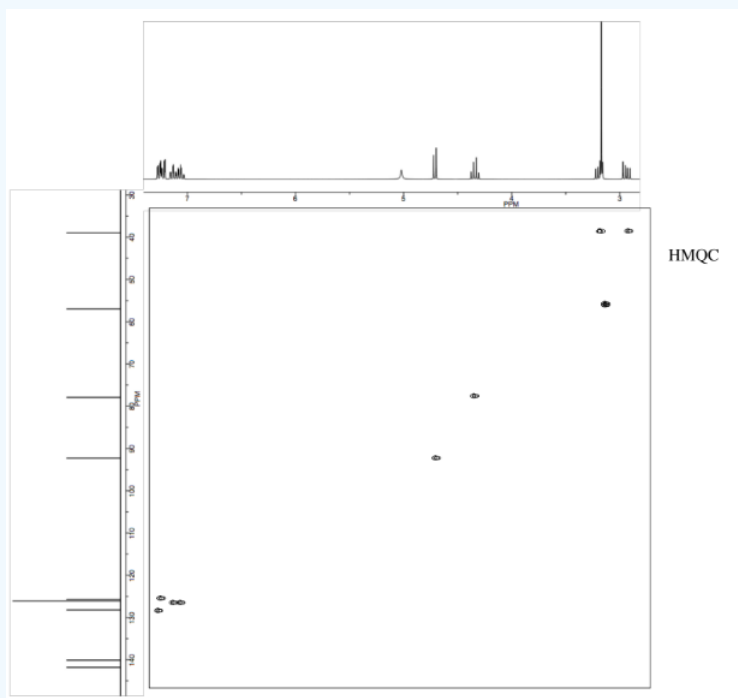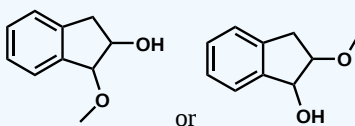
Solution

Yes! While propyl acetate is a simpler structure than typically analyzed by 2D NMR spectroscopy, it helps illuminate how to read the spectra and what information to gather from it. By using the HSQC (above), we already know which hydrogen(s) are directly attached to which carbons. The cross peaks here indicate what other carbons are attached beyond one bond away. Below is propyl acetate with the atoms labeled:
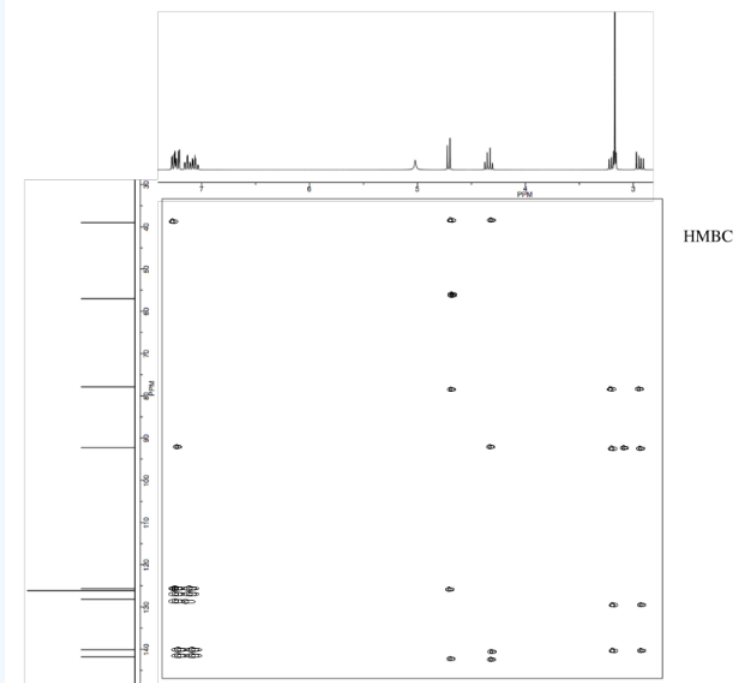

Focus on a column from one hydrogen peak. If we start with the 0.9 ppm methyl group (6), there are two cross peaks in the column (09., 20.8) and (0.9, 65.9). These correspond to a methylene group (5) and the methylene group attached to the oxygen (4). If we move the the methylene (5) at 1.6 ppm, then it has two cross peaks that correspond to C6 and C4. The methyl (1) at 2 ppm has not shown any correlations in previous spectra, but in HMBC there is a cross peak. The methyl (1) is attached to the carbonyl carbon (2). Finally, the methylene at 3.9 ppm has 3 cross peaks. It shows that it is connected through bonds to a methyl (6), a methylene (5), and the carbonyl carbon (2). All of this does match the structure for propyl acetate.
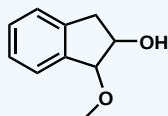

? Exercise 7.4.1

Analyze the data to determine which of the two isomers (below) we are dealing with.

or

HMQC

HMBC

Answer

? Exercise 7.4.2

What type of information do you get from:

1. HSQC?
2. HMBC?

Answer

1. HSQC determines the proton-carbon single bond correlations. It could also be other heteroatoms to hydrogen single bonds.

2. HMBC gives the correlations between carbon and hydrogen separated by 2 to 4 bonds away. This gives more information about connectivity within the molecule.

References

Silverstein, R.M, Webster, F.X, and Kiemle D.J. Spectrometric Identification of Organic Compounds. 7th ed. John Wiley & Sons, Inc. 2005.