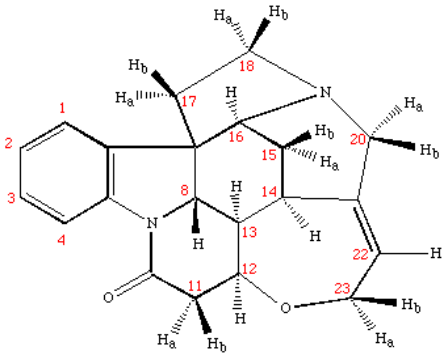
2D Experiments

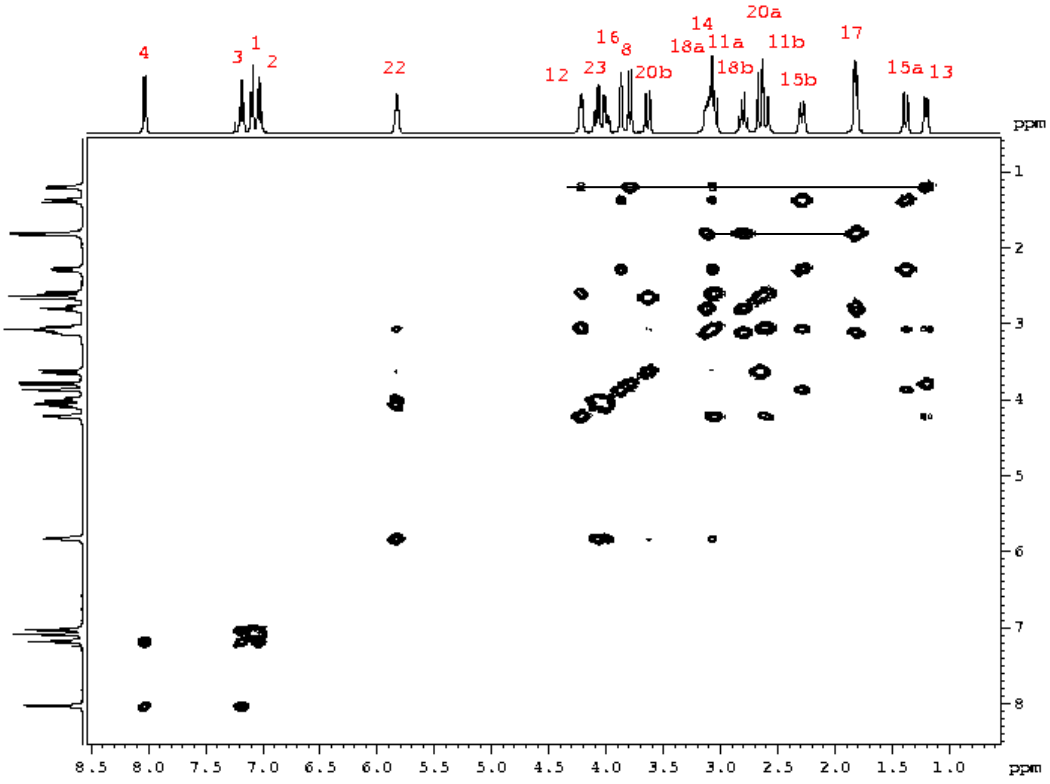
Much information can be obtained by doing 2D experiments as almost all of them are "inverse detection" experiments where the nucleus actually being observed is ^{1}H so the sensitivity is high enough that 2D spectra can be obtained in a matter of minutes. However, while these experiments can be performed with the click of a few buttons, there are some practical points to consider. On the following pages I outline some of these.

The best instruments for these experiments are the two 500 MHz instruments and the Bruker AV400, although the Varian 300 with autosampler does a good job on small molecules when the sample is not too dilute. Unlike the two Bruker instruments, the Varian 500 does not have autotune hardware (which ensures the NMR probe is accurately tuned to the right frequencies), but manual probe tuning on the Varian 500 is easy (and absolutely necessary when taking all 2D spectra or ^{13}C 1D spectra) and I can show you how to do that any time. There are 2D manuals beside the Varian 500 and the Varian 300 with the autosampler describing which version of the corresponding experiments you should use. The Bruker instruments automatically select the correct pulse sequence.

^{1}H - ^{1}H COSY

This experiment reveals which protons are J-coupled to each other through intervening bonds and only takes ~3-5 minute to perform. The diagonal is the normal ^{1}H spectrum; the off-diagonal peaks are the correlations. The spectrum is symmetric around the diagonal so the information in the bottom right corner of the spectrum is the same as the upper left corner. In the example below H17 is coupled to H18a and H18b and H13 is coupled to H14, H8 and H12.

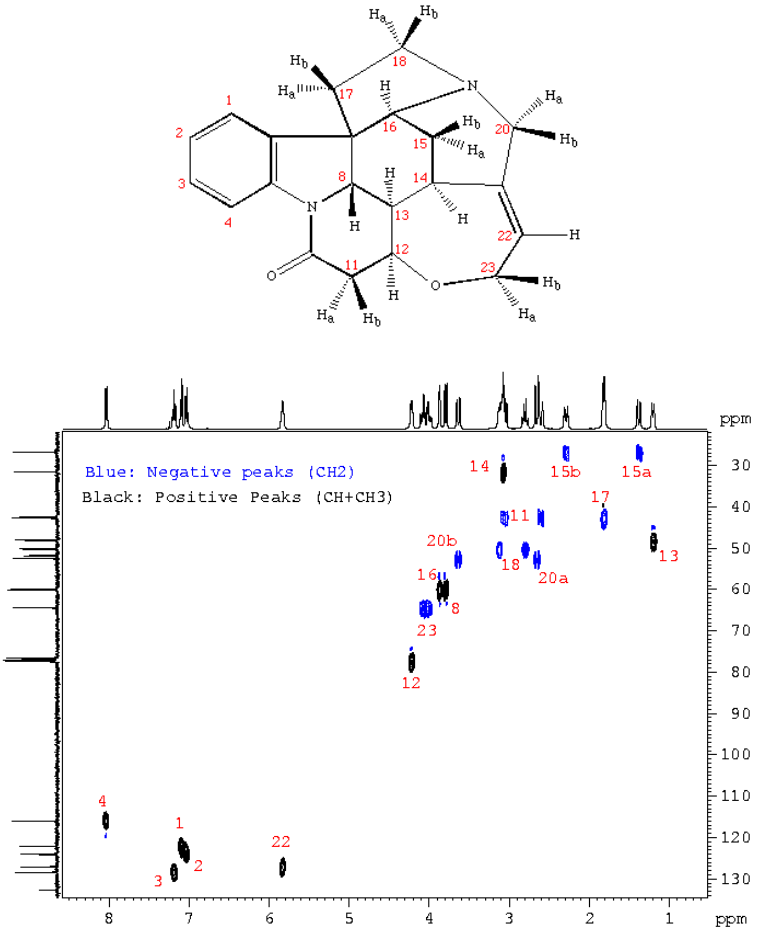




^{1}H - ^{13}C HSQC experiment:

This experiment is quick to do and easy to analyze. It is usually said that this experiment shows correlations between a ^{13}C and directly attached protons. To be more accurate, this experiment is optimized to show correlations between a ^{13}C and protons when the one bond coupling constant between them ($^{1}J_{CH}$) is about 120-160 Hz. Values of $^{1}J_{CH}$ vary from ~120 Hz for aliphatic carbons to about ~160 Hz for aromatic (and olefinic) carbons. Any carbon-proton pair whose one bond coupling constant falls outside this range might not be observed with the default parameters used in an HSQC experiment. What kinds of carbon-proton pairs fall outside this range? Carbons that are part of a three or four-membered ring will also have larger than normal $^{1}J_{CH}$ values and might not be observed or show very weak correlations. Terminal acetylenes carbons will have $^{1}J_{CH}$ values of up to 200 Hz. If your molecule possesses one of these moieties you may have to run the HSQC experiment a second time, changing the default value assumed for the $^{1}J_{CH}$.

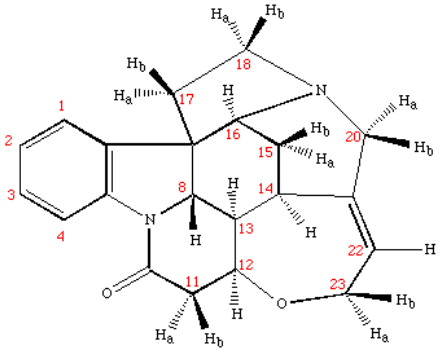
Since the HSQC spectrum only reveals correlations between ^{13}Cs and directly attached protons, both the Varian and Bruker experiments reduce the ^{13}C dimension to the range in which the vast majority of protonated ^{13}Cs will appear. This means that, for example, many aldehyde carbons will not appear. If you expect that you have an aldehyde carbon in your molecule you will have to increase the ^{13}C spectral width in the 2D HSQC experiment before acquiring the data. I can show you how to make these changes to the assumed $^{1}J_{CH}$ value or the spectral width in the ^{13}C dimension of an HSQC experiment.

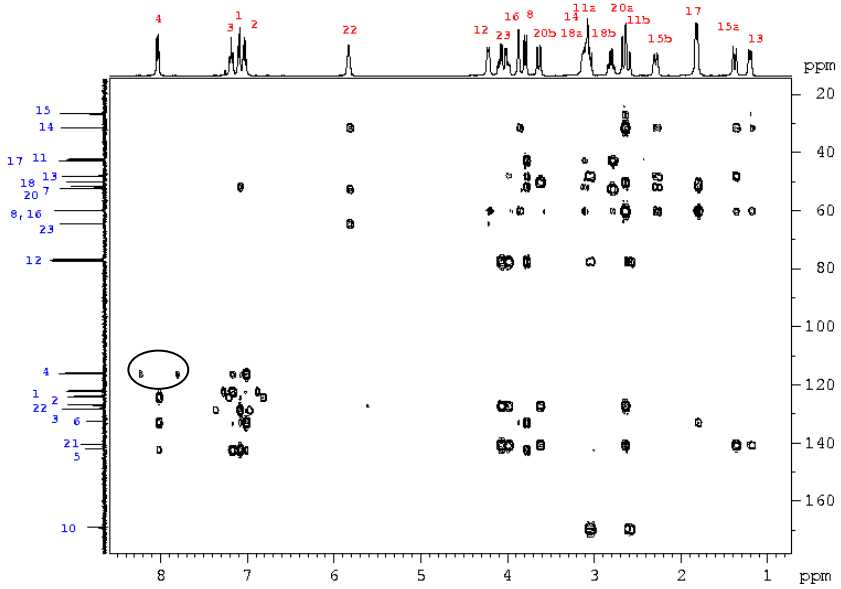


^{1}H - ^{13}C HMBC experiment:

It is usually said that this experiment shows correlations between a ^{13}C and protons that are two or three bonds away. To be more accurate, this experiment is optimized to show correlations between a ^{13}C and protons when the two or three bond coupling constant between them ($^{2}J_{CH}$ or $^{3}J_{CH}$) is approximately 8 Hz. Again, if the coupling between them is very different from this value (say 2 or 15 Hz) the correlation in the HMBC spectrum might be of weak intensity or not observed at all. Unlike the $^{1}J_{CH}$ values, there is no straightforward relationship with structure that describes what the value of the two or three coupling constant will be. It will depend on internal bond angles and whether there are nearby heteroatoms for instance. The assumed value of 8 Hz can be changed and the HMBC spectrum run a second time if you feel that some correlations are missing from your HMBC spectrum.

Note that the ^{1}H-^{13}C HMBC experiment is specifically designed to filter out and <u>NOT</u> show the one bond correlations between carbons and directly attached protons that one observes in the HSQC experiment. However, some one bond correlations may occasionally appear (see circle in the 2D plot below). They are identifiable because the correlations will not line up with any peak in the ^{1}H 1D spectrum.



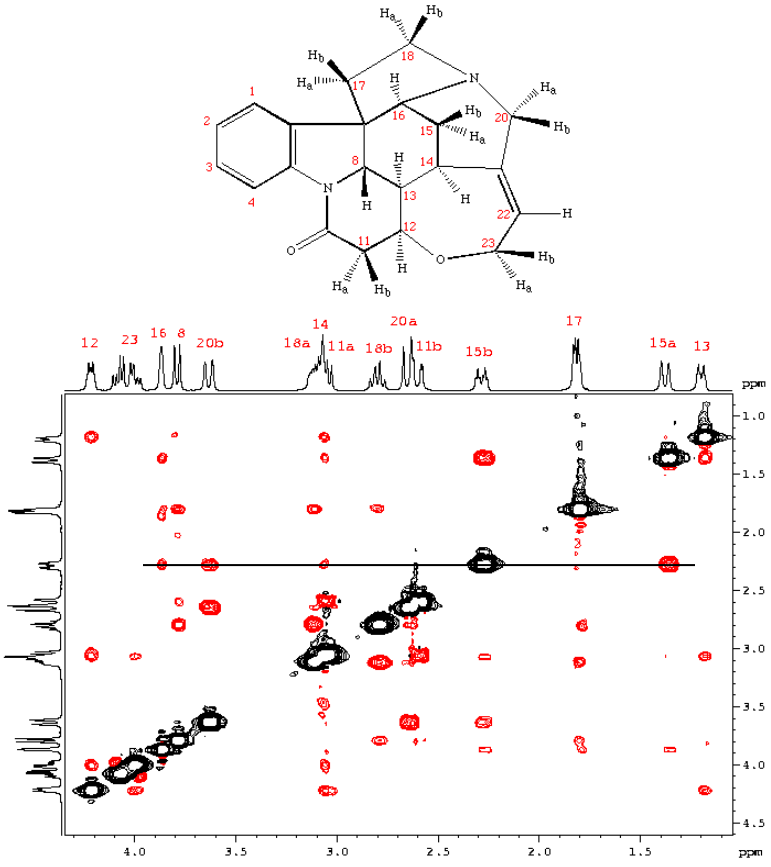


^{1}H - ^{1}H NOESY

This spectrum is helpful for determining conformations because it reveals protons which are near each other in space (generally ≤ 5 Å). It is not necessary that they be coupled to each other. The NOEs (nuclear Overhauser effects) are small so this experiment takes much longer than a COSY experiment, typically more than one hour. For molecules with molecular weight ~700-2000 (very approximately), NOE effects are near zero and you need to do a **ROESY** experiment instead. The "Mixing time" parameter in the NOESY experiment should be set to 0.7s (700 ms); the "Spinlock Mixing time" parameter in the ROESY experiment should be set to 200 ms.

When you do NOESY 2D experiments it is important to remember that any correlations (the off diagonal peaks) that are true NOESY correlations must be of the opposite phase to the diagonal peaks, i.e. if the diagonal peaks are positive, the NOESY correlations are negative (or vice versa). In other words, the diagonal peaks and the off diagonal peaks will be displayed in different colours. In the NOESY 2D experiment it is possible to see what are called "COSY-type" cross peaks that are artifacts due to strong through bond couplings rather than due to through space NOESY interactions and they do not necessarily mean the two protons are close in space. These "COSY-type" correlations will be of the same phase as the diagonal and therefore the same colour as the diagonal. They can also be caused by chemical exchange.

If there is one proton in particular you are interested in you can do a **1D version** that is much faster (5 minutes vs > 1 hour for the 2D experiment).



The natural abundance of ^{15}N is very low and prevents the observation of ^{15}N 1D spectra in a reasonable amount of time. ^{15}N chemical shift information can, however, be obtained by doing 2D experiments.

^{1}H - ^{15}N HSQC experiment:

This experiment is only available on the Bruker 400 and 500. I have found that the ^{1}H-^{15}N experiment often fails to show any correlations (other than sometimes two artifacts near the edges in the ^{15}N dimension) in molecules with amines. This may be due to rapid chemical exchange involving the $amine_x$ protons. However, ^{15}N chemical shift information should be observable in the HMBC experiment (see below).

^{1}H - ^{15}N HMBC experiment:

This experiment is only available on the Bruker 400 and 500. As in the ^{1}H-^{13}C HMBC, this experiment shows correlations between ^{15}N and protons which are typically two or three bonds away from each other. The assumed coupling constant is 5 Hz. Unlike the ^{1}H-^{13}C HMBC, the one bond correlations between ^{15}N and directly attached protons are NOT filtered out and you MIGHT also observe them (if they are observable in the HSQC experiment). This is due to the way the pulse programs are written. Therefore, currently I see no real reason to run a ^{1}H-^{15}N HSQC spectrum since in the HMBC you will not only observe correlations for protons to nonprotonated nitrogens , you will likely observe a correlation due to any amine nitrogen if there is at least one proton two or three bonds away from it. However, as with the ^{1}H-^{13}C HMBC, if $^{2}_{JNH}$ or $^{3}J_{NH}$ is far from 5 Hz, you might not see correlations.

^{1}H - ^{31}P HMBC experiment:

On the Bruker instruments there is also a ^{1}H - ^{31}P HMBC 2D experiment which will tell you which protons are two or three bonds from ^{31}P (actually also directly bonded although that structure is rather rare.)

DEPT-90

While not a 2D experiment, DEPT-type experiments can be useful. One advantage of the ^{1}H - ^{13}C HSQC experiment is that the carbons are essentially separated into two groups, CH_2s and (CHs and CH_3s). I see some people also running a DEPT-135 experiment which does the same thing so is redundant and unnecessary. The HSQC spectrum is more useful than the DEPT-135 spectrum because it also helps in the assignment of the ^{13}C peaks. I would suggest that you run an HSQC experiment and if you then wish to run a DEPT, run the **DEPT-90**. The DEPT-90 reveals only the CHs in the sample and therefore when combined with the HSQC data will complete the assignments of the CH, CH_2 and CH_3 carbons. Also, of course, any peak in a ^{13}C spectrum then remaining unidentified will be a nonprotonated carbon.