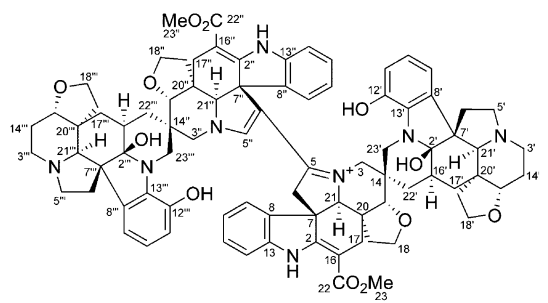
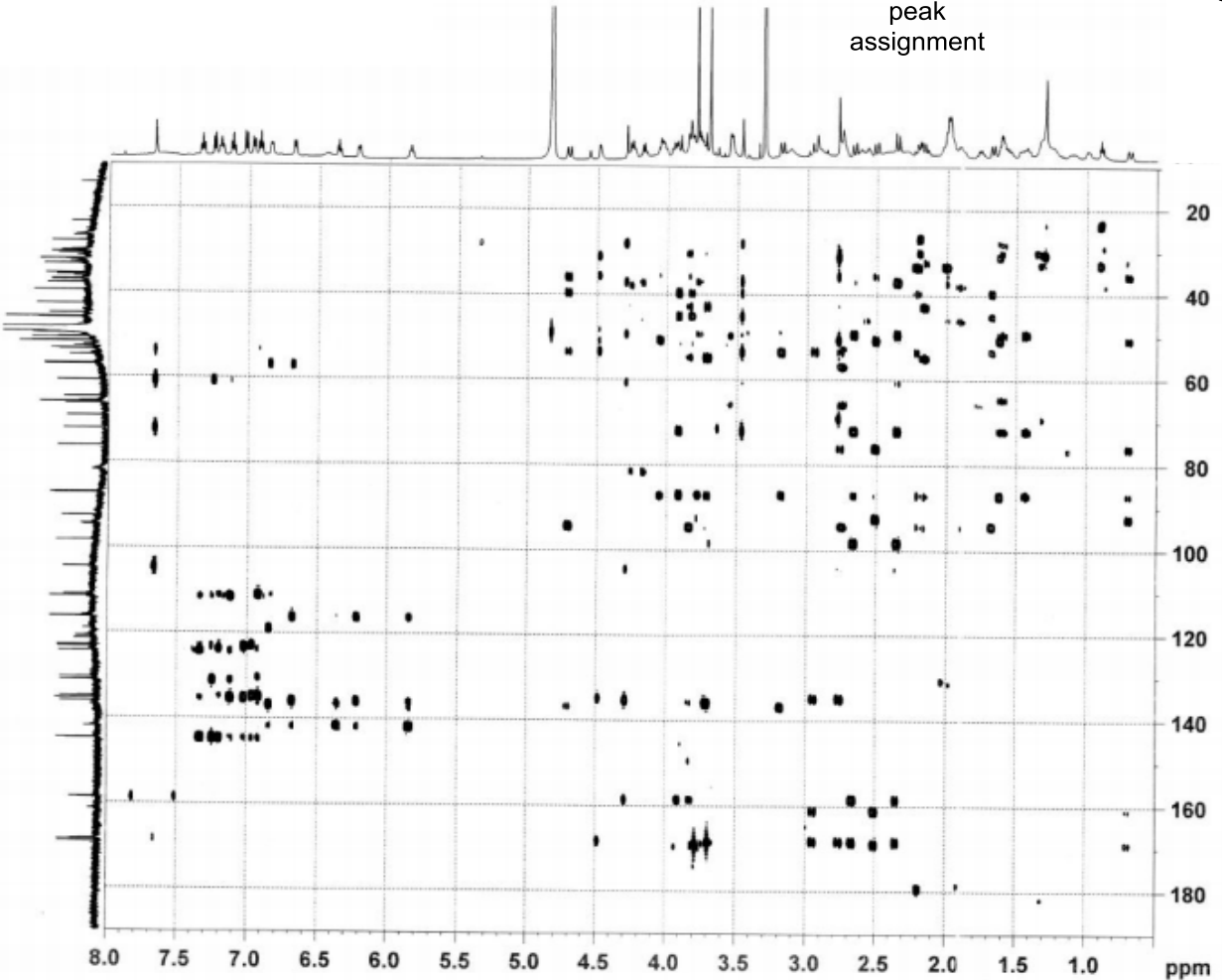
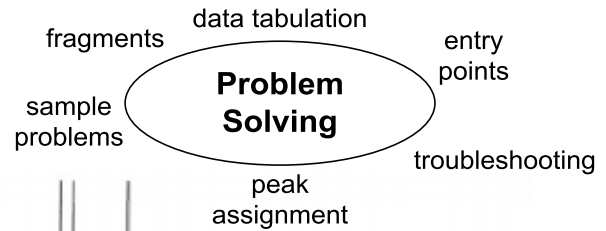


Nuclear Magnetic Resonance III

Eugene E. Kwan



Summary of 2D NMR Experiments

- HSQC: 1-bond C,H - all experiments are **proton-detected**
- COSY: 2,3-bond H,H
- HMBC: 2,3-bond C,H

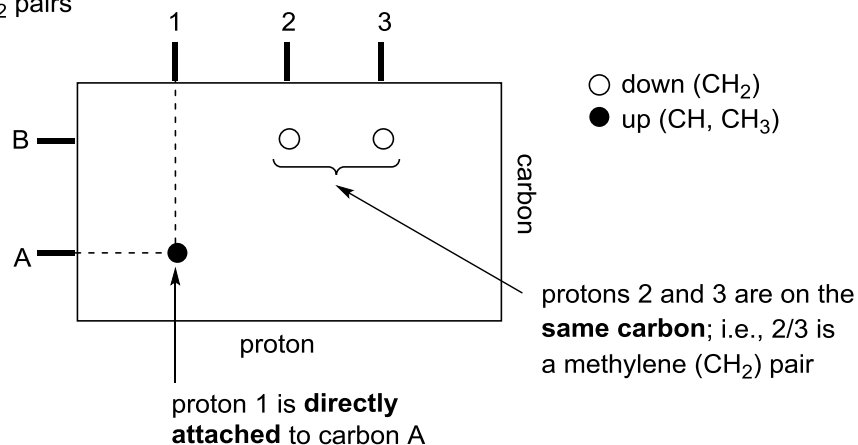
HSQC

By far the simplest spectrum to understand, begin here.

axes: proton, carbon

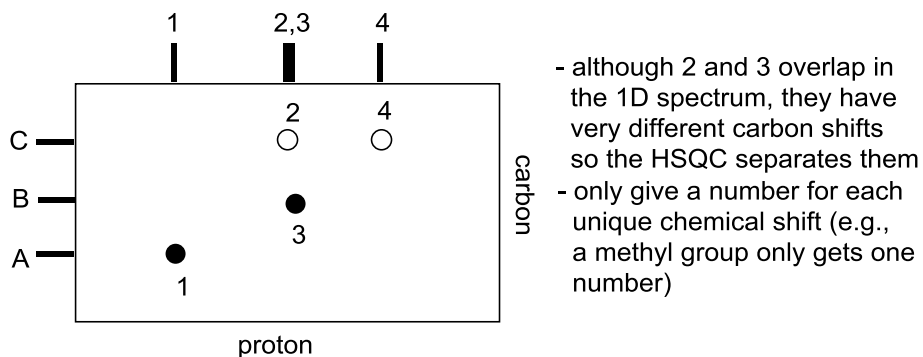
correlations: 1-bond C,H couplings

purpose: number proton spectrum, match each proton with a carbon, identify CH₂ pairs



Numbering the Spectrum

Even if the proton spectrum overlaps, HSQC will usually separate the peaks enough so they can be numbered (convention: left to right).



COSY

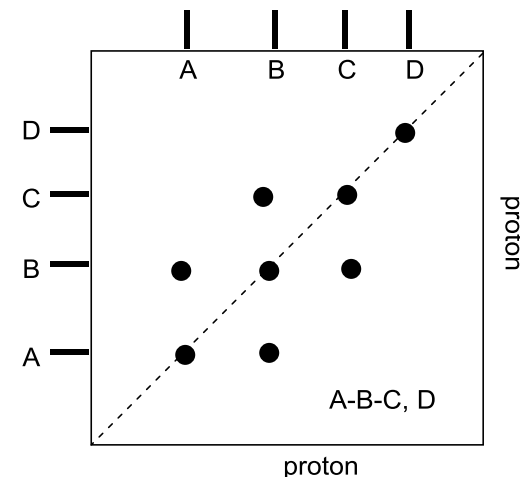
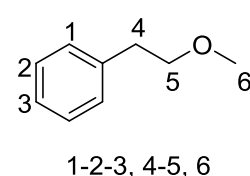
Next, use COSY to determine the composition of each spin system.

axes: proton, proton

correlations: off-diagonal peaks are 2-3 bond couplings between protons

purpose: assign protons to spin systems

spin system: a set of protons sharing through-bond (*J*) couplings

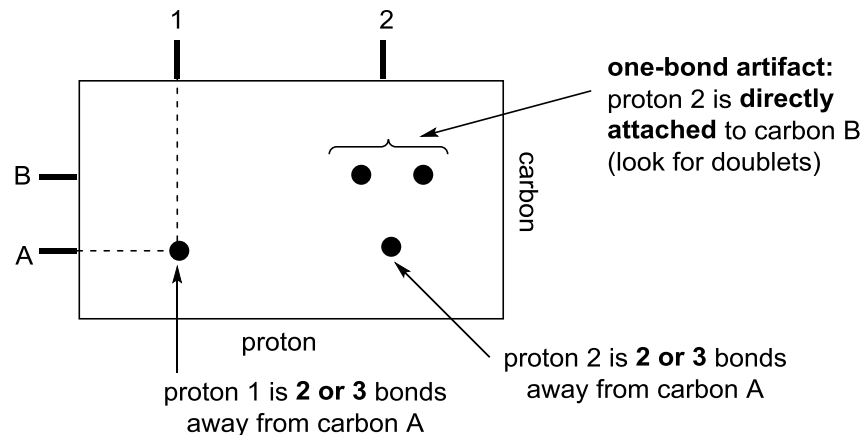


HMBC

axes: proton, carbon

correlations: 2-3 bond couplings between protons

purpose: connect spin systems



Types of Structural Determination Problems

(1) Spectral Assignment

- given a structure and a spectrum, verify that the spectrum matches the compound and assign all of the peaks to atoms in the molecule
- real world scenario: reaction product analysis

(2) Unknown Structural Elucidation

- given the spectra of an unknown compound, determine its structure and then assign of its peaks
- real world scenario: novel natural product, unexpected outcome of a reaction

Workflow

These steps apply to both problems.

(0) Compound Purity

Has the compound been purified to the best of your ability? Is the baseline of the proton spectrum clean? Dirty spectra are hard to interpret and can make conclusions difficult to draw. Consider semi-preparative HPLC or preparative TLC prior to analysis. (Elemental analysis remains the gold standard of purity. A high resolution mass "hit" does *not* guarantee any level of purity.)

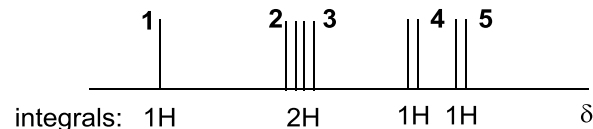
(1) Data Acquisition

- 1D spectra: proton, carbon (optional)
- 2D spectra: COSY-45, HSQC, HMBC
- of course, not all of these data will be necessary for simpler compounds

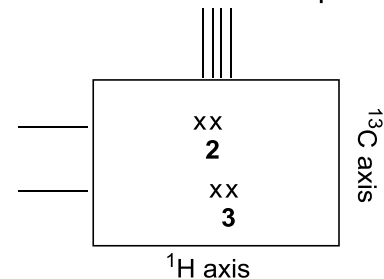
(2) Tabulate Data

- make it a habit to tabulate the data before analysis to avoid confirmation bias

Here is a hypothetical spectrum:

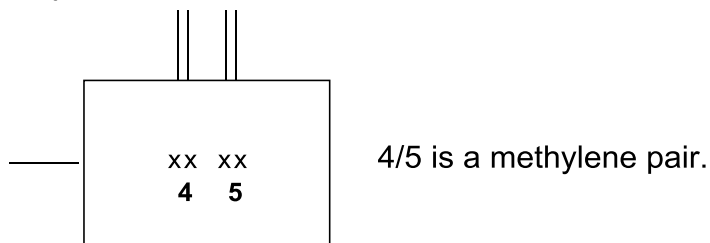


- Adopt the convention of labeling the peaks from left to right. This gives you a unique name for every resonance.
- Mass Balance: Are there the right number of protons and carbons?
- Start with the HSQC: Sometimes, the peaks will overlap. Use the HSQC to uniquely label every proton, as well as determine direct attachments between protons and carbons:



Here, peaks **2** and **3** overlap in the proton spectrum, but the greater chemical shift range of the carbon axis disperses the peaks so that they can be distinguished.

- Multiplicity: HSQC phase gives DEPT information. Red/up=CH or CH₃; blue/down=CH₂. Write down all the methylene pairs for reference. These will help you distinguish geminal coupling from vicinal or long-range couplings in the COSY.
- Quaternary carbons: These do not appear in the HSQC, but should appear in the carbon or HMBC. Isolated, but clearly resolved peaks are often excellent "entry points" for analysis. Make a list of these carbons.
- Be methodical and double-check everything.**

(2) Tabulate Data**(d) Multiplicity**

(g) Bookkeeping: Here is the format I use. A similar format is used in many natural products papers.

ID	$\delta(^1\text{H})$	$\delta(^{13}\text{C})$	Hs	type	J (Hz)	COSY	HMBC
1	5.76	145.23	1	d	5.1	2	152.12
2	3.76	72.45	1	d	5.1	1,3	32.47
3	3.47	--	1	br s	--	--	202.57
... etc ...							

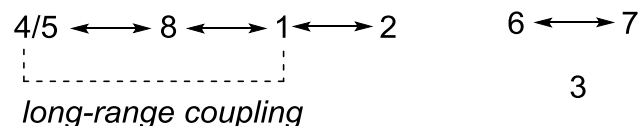
Quaternary Carbons: 35.57, 54.32, 202.57...

CH₂ pairs: 4/5, ...

- peaks are listed by number from high to low chemical shift
- HSQC: connect each proton to its directly attached carbons; find methylene pairs
- COSY: if 1 is coupled to 2, then check that 2 is coupled to 1; however, both partners of a methylene pair may not show couplings to a common partner (peak 3)
- exchangeable protons do not appear in the HSQC
- quaternary carbons can be found from HMBC or the 1D spectrum carbon
- HMBC: watch for one-bond peaks; more intense peaks like methyl groups are more likely to show long-range correlations; sp^2 systems: 2J is small, but 3J is large (bigger for anti than syn)

(3) Generate Spin Systems

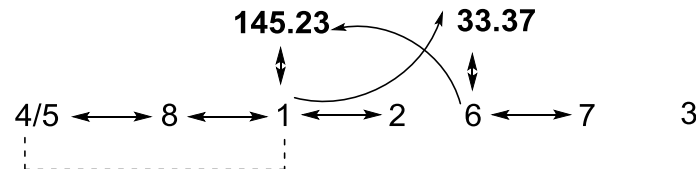
Use COSY to build up spin systems. Each "component" of the spin system is a methyl group, a methylene pair, or a methine (from the HSQC). Double-headed arrows represent vicinal couplings, with dashed lines for long-range couplings:



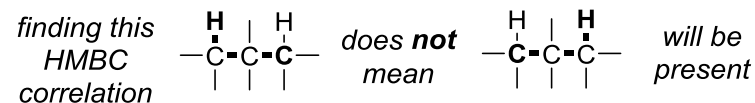
(You might not know which ones are long-range. Use your chemical intuition and look at the peak intensities and asymmetry about the diagonal. You might have to change your diagram if you find it to be inconsistent later.)

(4) Connect Spin Systems

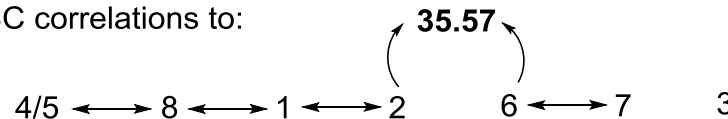
Look for HMBC correlations that connect a proton in one spin system to a carbon in another spin system:



Curved arrows indicate HMBC correlations. If you find such a connection, that tells you that the spin systems must be adjacent in the head-to-tail sense shown. Note that these do not have to be mutual like COSY couplings:

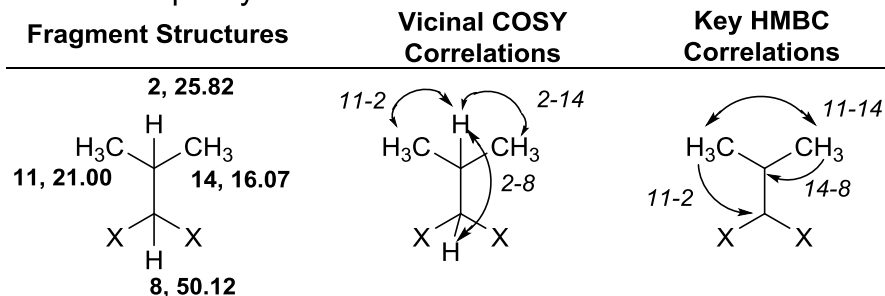


If that doesn't work, you can look for a carbon, possibly quaternary, that protons in both spin systems have common HMBC correlations to:



(5) Generate Fragments

In many cases, complex, overlapping signals will prevent you from drawing out all the spin systems of the molecule. In particular, unknown structural elucidations will require you to generate and connect *fragments*, which are tied together by what is visible in a spin system and HMBC. Here is how I put together menthol, which has some overlapping signals and one continuous spin system:



Key Evidence

- methyls 11, 14: COSY to 2
- 2: COSY to 8
- HSQC: 2, 8 = CH
- 11, 14: mutual HMBC
- 11, 14: HMBC to 2, 8

- HMBC: double-headed arrows to indicate mutual correlations; single-headed arrows to indicate a one-way correlation
- if you feel comfortable, you can write "11-2" instead of "11-25.82" to speed things up, but remember, HMBC correlations are from protons to carbons
- Xs represent non-protons

Write down your reasoning (even though it's boring). By their nature, these problems are very complicated, and there's a 99.9% chance you will not remember what peak is at 16.07 ppm several years from now. It's also likely you will make some mistakes, which will be easier to track down this way.

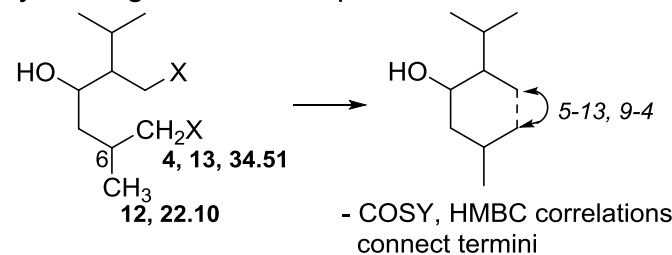
(6) Entry Points

When you look at a COSY or HMBC spectrum, you will see a lot of peaks. Where should you start generating fragments from? In general: clearly resolved peaks with unambiguous or characteristic chemical shifts. Here are some ideas:

- carbonyl region
- aromatic rings and olefins
- methyl groups
- quaternary carbons

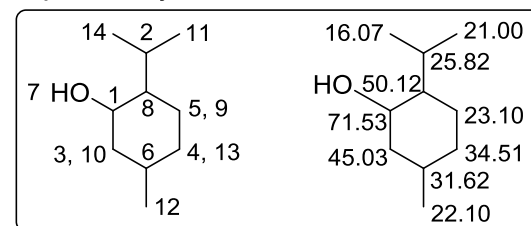
(7) Expand and Connect Fragments

Don't bite off too much at a time. Make a small fragment with correlations you feel confident in assigning. Then, move on to another entry point and generate another fragment. Fragments with uncertain correlations are hard to use. Once you have exhausted all the easy data, work on what's left to expand your fragments, and if possible, connect them.



(8) List Full Assignments

This is self-explanatory:



You should also file all the FIDs and your "good" notes of what you've done in the same place. If you feel the compound will go to publication, take the time to write it out in journal format.

(9) Check Answer

- Is your flat structure consistent with *all* of the NMR data?
- Are there any other possibilities?

(10) Interpret Relative Stereochemistry

- H,H coupling constants
- nOe/rOe correlations
- (C,H) coupling constants
- more on this next lecture

Troubleshooting**My proton spectrum looks bad.**

Do you have paramagnetic ions, particulates, or other impurities? Purify your compound by flash chromatography or HPLC, or at least filter it through some cotton. **A very pure 0.5 mg is preferable to a dirty 5 mg.**

Is the sample volume correct? Samples that are too short are difficult to shim.

Is this a S/N issue? If you have <0.5 mg, a detailed 2D NMR analysis with standard equipment will be very difficult. You will save time by going back to make more sample first. Remember, a lot of these experiments depend on the observation of carbon-13 satellites, which are very small!

Are you in the intermediate exchange regime? Try changing the temperature or field strength (higher or lower). Try changing from CDCl₃ to d₆-DMSO.

Is this a shimming issue? Is your NMR tube bad? Try another tube. Try recalling the default shims and manually shimming. Try using the gradient autoshim on deuterium-gradient-enabled spectrometers, followed by simplex autoshimming. Turn the spin off and adjust the XY shims as well.

My spectrum looks nice, but the peaks overlap.

Are any multiplets or even parts of multiplets exposed? Try changing the solvent (benzene titration?) first. Then, try 1D-TOCSY with a variety of mixing times to deconvolute the spectrum.

I don't have a lot of sample.

Did you set the experimental parameters correctly? Did you tune the probe and calibrate the pulse widths? These will make a substantial difference.

Try a Shigemi tube. Tubes with plugs of different magnetic susceptibilities are available. Samples in these expensive tubes will be hard to shim, but have about double the S/N. These are nice for getting carbon-13 spectra. You can also try a smaller diameter tube (but you have to have the right spinner and probe.)

Desperate? Try a cryo-probe at higher field strengths.

My sample has a lot of solvent in it.

Did you use a deuterated solvent? (Duh.) Co-evaporation of your sample with (regular) chloroform helps. If that's not enough, try heptane or toluene (may require higher vacuum).

I have nice data, but I don't understand it.

Did you check your data table? One mistake can make the data unintelligible or give a ridiculous answer. Is everything consistent?

Try another angle. Have a coffee (or a beer) and start from a new entry point. Try having someone else look at the data. Just don't try the same strategy over and over. Rethink your assumptions. Above all, **think about the chemistry**. Where did the compound come from? What is its reactivity?