

## 2D NMR: HMBC Assignments and Publishing NMR Data Using MNova

**Use Artemis (Av-400) or Callisto (Av-500) for this week's HW.  
Best to use the same sample as in HW#9+10.**

- Reading**
- HMBC in Claridge section 6.4 (esp. 6.4.2)
  - Assignments in MNova are best learned by doing them. Even so, there are a couple useful tutorials/presentations available on-line: a short introduction for [Assignments](#)<sup>1</sup> at the MestreLabs website, and a [longer version](#)<sup>2,3</sup> available on the UWChemNMR website. In both presentations (and elsewhere on the MestreLabs website), ignore discussions about predictions and automated assignments. These require the NMRPredict license, which is not available under our MNova site license (and automated assignments for academic research are not at all a good idea).
- Goals**
- Learn about common setup and use issues with HMBC.
  - Combine 1D and 2D spectra to provide assignments, and how to publish NMR data using MNova.

### I. HMBC: Heteronuclear multiple-bond correlation spectroscopy

HMBC is both similar and different from HSQC in setup and use. Key points are:

- HMBC experiments are not as sensitive as HSQC: a common rule-of-thumb is HMBC takes 4× as long as HSQC. Note that often the minimum (good: NS=2) HSQC has more than sufficient signal-to-noise. In such cases, NS=8 may be more than needed for the HMBC.
- Since quaternary carbons are observed in HMBC, the <sup>13</sup>C spectral range is almost always larger than with HSQC: 240ppm as default in HMBC vs 180ppm in HSQC.
- Multiple-bond <sup>1</sup>H-<sup>13</sup>C couplings have the <sup>1</sup>H bound > 99% of the time to <sup>12</sup>C. Because <sup>13</sup>C nuclei assist in relaxing protons, the protons detected in HSQC relax faster than in HMBC. Short D1 values therefore cause more trouble (artifacts) in HMBC experiments: resist making **D1** too small for HMBC.
- There are a large number of HMBC variants in the literature. For small molecules, the recommended experiment on our Bruker spectrometers, HMBCEtGPL3ND, is best for most situations. The number of variants raises the issue why so many: problems crop up on regular occasions, and awareness of them is important. Two are particularly prevalent:
  - HMBCEtGPL3ND has a three-fold 1-bond filter that is very good at removing 1-bond crosspeaks. We typically do not want to observe these in an HMBC; the HSQC provides these 1-bonds correlations.
  - Important crosspeaks may not show up with the default settings for an HMBC spectrum: these will be correlations involving small <sup>n</sup>J<sub>HC</sub>. The default for <sup>n</sup>J<sub>HC</sub> is **CNST13** = 8 Hz. The

<sup>1</sup> <http://mestrelab.com/resources/assignments/>

<sup>2</sup> [http://www.chem.wisc.edu/~cic/nmr/Guides/Other/Mnova\\_NMR\\_Training\\_for\\_chemists\\_on\\_1D\\_and\\_2D\\_NMR-Version\\_8.0.pdf](http://www.chem.wisc.edu/~cic/nmr/Guides/Other/Mnova_NMR_Training_for_chemists_on_1D_and_2D_NMR-Version_8.0.pdf)

<sup>3</sup> This tutorial was written for version 8, so there will be a few differences if you are using the newer version 9 software.

most common solution to this issue is to run a second experiment with a smaller setting for  ${}^nJ_{\text{HC}}$ , e.g., **CNST13** = 3 Hz.

Critical parameters for HMBC, those that should always be checked, are:

$$\mathbf{D1} \geq 1.5 \times T_1(\text{longest})$$

$$\mathbf{NS} \geq 2 \quad (\text{often good to use } 4 \times \mathbf{NS} \text{ as used for the HSQC})$$

$$\mathbf{TD1} = 256, 512 \dots \text{ up to perhaps } 2048$$

$$\mathbf{CNST13} = 8 \quad \text{often run again at } 3\text{Hz to pick up small/missing crosspeaks}$$




1. Acquire a good quality HMBC on the sample you've been using for the 2D labs. Make sure **NS**  $\geq 2$  (**NS**=8 is often best for research samples; but for facility-provided samples **NS**=4 will likely be ok), and set **TD** = 4096 and **TD1**  $\geq 256$ . Use IconNMR on the 400 or Callisto; your submission will be forced into the nightqueue.

## II. Publishing NMR Data Using MNova

- Data for sections A–C below are located at: \\castor\fry\public\_html\Chem636\datatables.

MNova setup reminder:

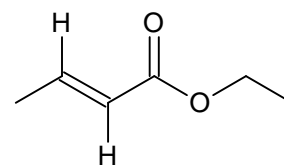
To perform multiplet analysis in MNova, the following settings are recommended:

	Peak Pick	→ Options	→ Method: <b>GSD</b>
	Integration	→ Options	→ Calculation Method: <b>Sum</b>
	Multiplet Analysis	→ Options	→ Calculation method: <b>Peaks</b>


*These settings can have a profound effect on the processing speed, and more importantly, the quantitative behavior of data analysis in MNova. We'll discuss these issues in lecture, but it is very important to be aware of their potential impact on your data analysis.*

### A. Generating publishable ${}^1\text{H}$ data tables for ethyl crotonate.

Process the  ${}^1\text{H}$  spectrum of ethyl crotonate in acetone- $d_6$ : expno 13. This is a spectrum acquired on Callisto that is fairly concentrated, using the pulse sequence **zg**. Perform normal processing tasks including apodization, phasing, baseline correction, and referencing. *Do not perform peak picks or integrations (or if you have, delete them).* The Multiplet Analysis tool will provide these for you.

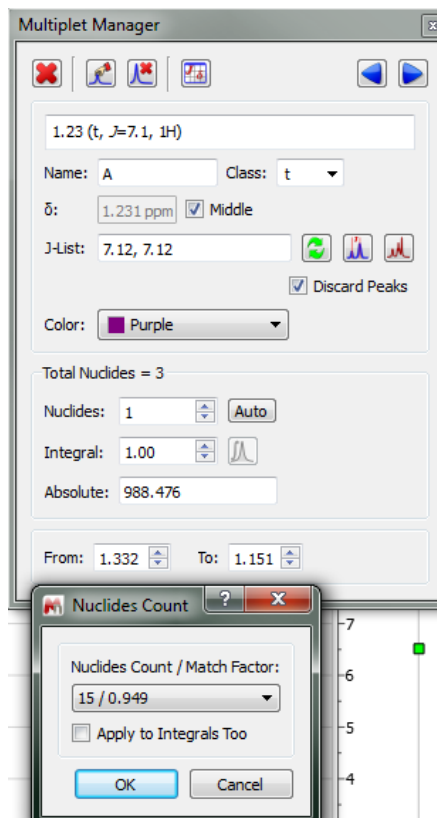


**Ethyl crotonate**  
**C<sub>6</sub>H<sub>10</sub>O<sub>2</sub>**

Use the Manual Multiplets Analysis tool in manual mode,  [**J**], to create the five multiplets for this molecule. Since the Multiplet tool performs an integration, use normal region placements when creating each multiplet [this isn't too important because the **Integration** method has been set to **Peaks**; even so, best to make habits that won't cause problems if you switch this option back to **Sum**]. MNova has an easy time with portions of this small molecule, extracting J couplings, chemical shifts, etc. The two downfield peaks, on the other hand, will likely be defined as *m* multiplets. [What would you expect for these? More in lecture...]

But there is at least one problem! Always carefully inspect the report when you generate it:

1. First, check that you correctly referenced the spectrum. If you didn't, you will have to remove all multiplets, delete all peak picks (which were done automatically by the multiplet tool), do the reference, and redo the multiplet selections.
2. Now look again at the report, and check the proton counts [I prefer **View** → **Tables** → **Multiplets**]. Depending on the direction you made the multiplets (but in either case for this molecule), the proton count will be wrong. To correct the count, use the Nuclides setting in the Multiplet Manager (double click on a multiplet box to open this) and click on Auto. MNova tries to figure out the best count: for ethyl crotonate there are 10 protons, not 15, as shown on the right. Amazingly, the Auto button may not provide a 10 count, so a manual correction 3, 3, 2, 1, 1 had to be done to the multiplets from upfield to downfield. If you placed your multiplets the other way around, starting downfield (on the left), you may have a 10 option. *In any event, it is imperative that the report listing show the correct proton counts when you add the listing to your paper/supplemental!*



2. Do everything above and get a good report onto the  $^1\text{H}$  1D spectrum. Move the report so it can be read, and upload the .mnova and .pdf files.


So what's up with this spectrum? Ethyl crotonate is small enough and sparse enough in protons such that relaxation varies considerably across the molecule. The ethyl and methyl protons relax relatively fast, in  $< 10$  seconds. But the vinyl protons, especially when degassed to remove  $\text{O}_2$  (as was done here), relax very slowly, taking 20 to 40s. If one doesn't increase **d1** enough (remember that on Athena the parameter is **RD**), the vinyl protons will be much smaller than expected. To exasperate the problem, this spectrum was acquired with a  $90^\circ$  flip angle using the pulse program **zg**. [Why is that a problem?] That throws off MNova's attempt to estimate the number of nuclides.


*The main point is that automated procedures can produce obviously incorrect results. Make sure you check you data and analyses carefully before you publish: don't allow embarrassing oversights to end up in your publications!!*

**D1** Think of a few other ways spectra acquisition parameters or processing steps might mess up MNova as you work through this HW. We'll talk about this in class in one of the lectures.

## B. Generate a publishable $^{13}\text{C}$ data table for ethyl crotonate

Process the  $^{13}\text{C}$  spectrum of ethyl crotonate in acetone- $d_6$ , expno 12. You need only properly phase and check the reference of this spectrum.

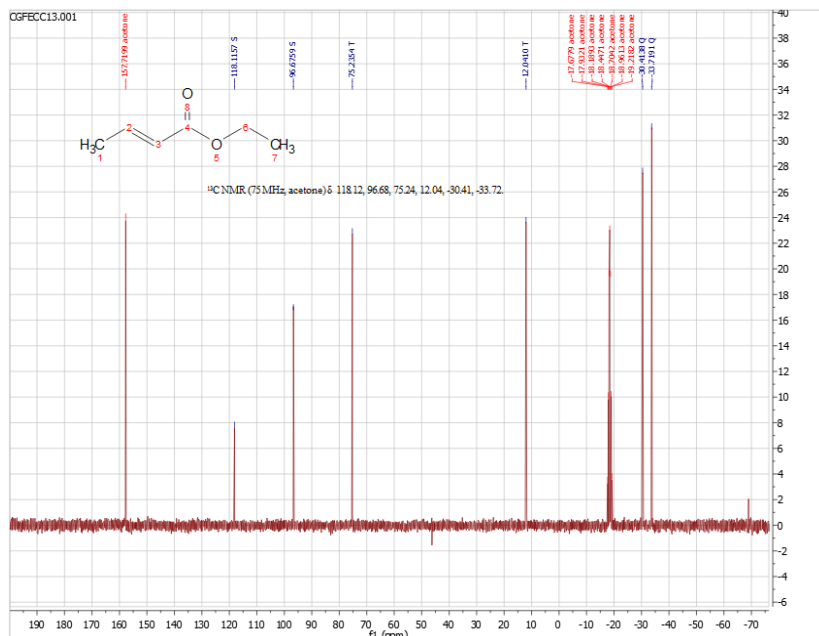
**Note:** The Annotations feature in the referencing tool  in MNova has a bug introduced with version 9.0, so don't use it (i.e., uncheck it).


To generate a  $^{13}\text{C}$  report, do a Peak Pick . Multiplet analysis in a  $^{13}\text{C}$  1D spectrum is overkill — it would work, but would define a bunch of singlets. Either remove the peak picks that are solvent or impurity, or label them as such in the Peaks Table. **Peak Pick** → **Auto Classify Peaks** will do at least part of the job for you. **Peak Pick** → **Report Peaks** will now produce the needed table for publications, almost....

The report may not contain the solvent — normally reported. Use **View** → **Tables** → **Parameters** and add or correct the solvent cell if needed. More commonly, the Auto Classify will miss some solvent peaks: likely here it missed the downfield ketone carbon at 206 ppm. It may have also have not label some impurities as impurities, or labeled actual peaks as impurities. In any event, clean up the labeling and peak picks using the Peak Table [easily gotten to by a single left-click on any peak in the spectrum]; double-click on the **Type** and correct there. The final check on a  $^{13}\text{C}$  spectrum is to make sure the peak count for the compound matches the carbon count for the molecule: here the carbon and peak count is 6. Once done, delete the current report and generate a new one. All should be well.

**Note:** You can label the multiplicity in the Annotation column in the Peaks Table, but I can't see how to get those to list in the Peaks Reports. The labels will show, however, on the spectrum itself above the chemical shifts.

**Note:** You can of course add a structure to the plot from ChemDraw, but remember that to get numbering the way you want, copy-and-paste from ChemDraw to ChemSketch, and then into MNova may be required. To the right is a plot with the structure included.



3  Do everything above and get a good report onto the spectrum. Move it so it can be read, and upload the .mnova and .pdf files as normal.

**C. Repeat the demonstration in you lab session that separated the two multiplets, quartet and triplet, for 3-heptanone in the spectrum: D1402121048\_Av400-3hep-1H/10.**

This procedure is reasonably straightforward once you work through it once or twice.


4☞ Produce a good report of the  $^1\text{H}$  spectrum. Upload mnova and .pdf files as normal.

**D. Generate  $^1\text{H}$  and  $^{13}\text{C}$  reports from the 1D spectra of your assigned compound.**

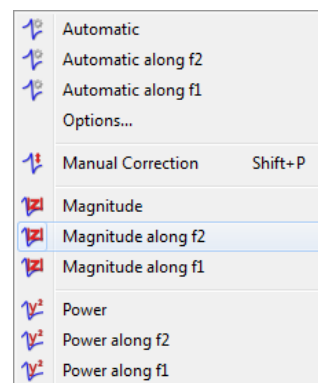
5+6☞ Produce good reports of the  $^1\text{H}$  and  $^{13}\text{C}$  spectra. Upload mnova and .pdf files as normal.

### III. Data Assignments Using MNova

Continue in the manner started in last week's HW to perform assignments in MNova, but now of course include your HMBC data. Assignments from HMBC crosspeaks will be similar to HSQC. It is now the most common method for assigning quaternary carbons chemical, and for confirming the more difficult assignments in a structure, especially those linking different portions of a structure connected via non-carbon atoms (e.g., via an ether or amide bond).

7☞ Work up the HMBC spectrum. These spectra should be magnitude mode in f2. If different colors are displayed, the magnitude mode has not been set by MNova: choose the dropdown for  and "Magnitude along f2".

Make a reasonable number of assignments using all of the data sets you have obtained:  $^1\text{H}$  1D,  $^{13}\text{C}$  1D, COSY, TOCSY, HSQC, and HMBC. Involve at least on quaternary carbon. Save the assignments as a .mnova file and upload.



**Plot HMBC spectra. Provide as many assignments as you can via compound structure. Upload 1 plot as .pdf and upload. Save a .mnova file containing five spectra, the compound structure, and your assignments.**