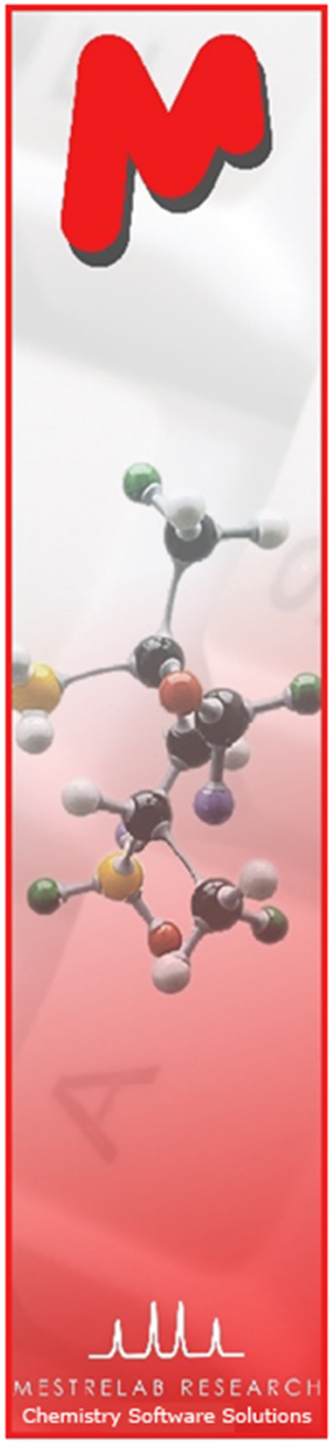

New Handy Tools in Mnova 6.3 for 1D and 2D NMR Assignment

Mnova 6.3.0
April 2011

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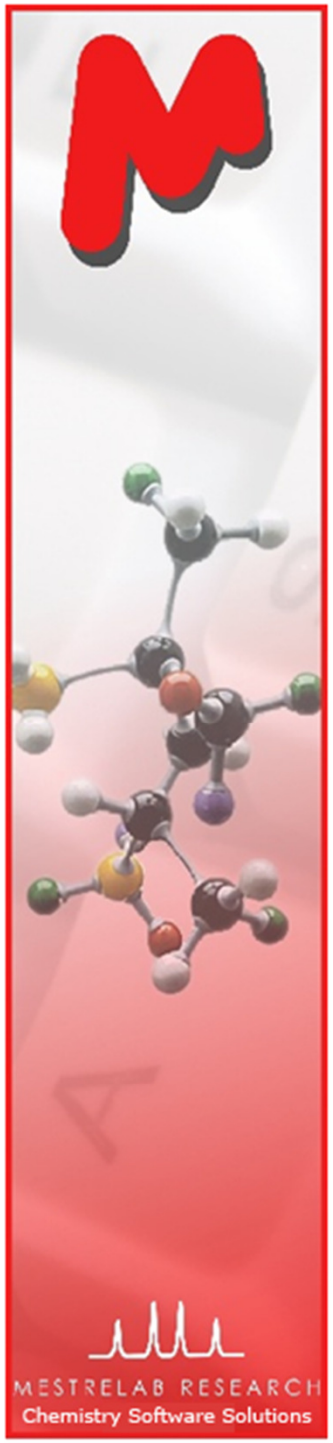


Contents

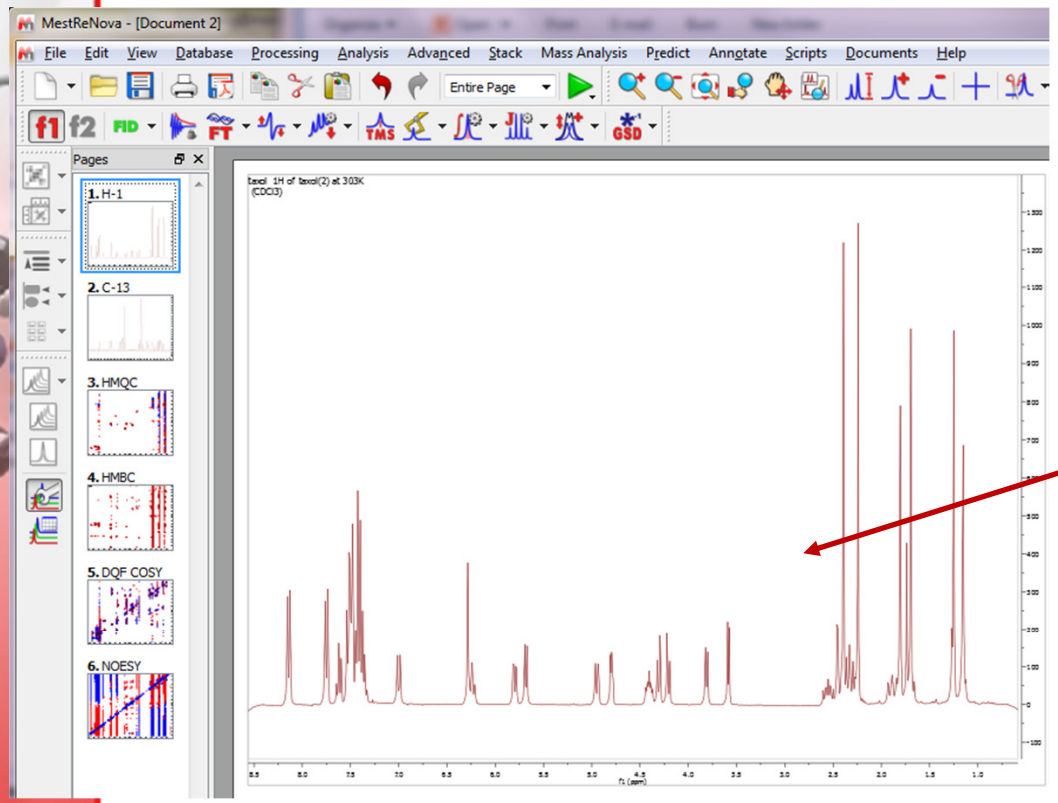
To use Mnova NMR and NMRPredict Desktop to assign the 1D and 2D peaks to a molecule, including:

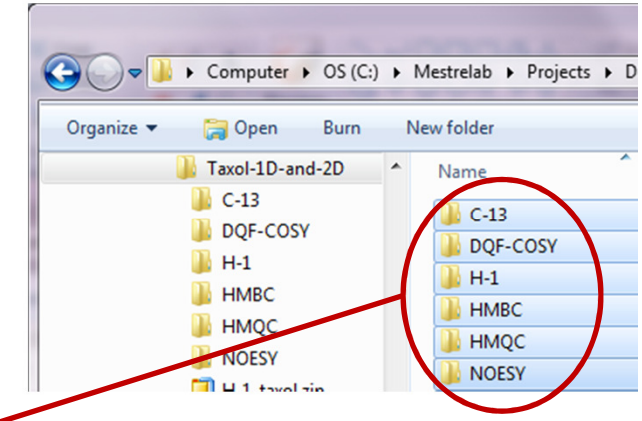
- Open the spectra and structure
- Assign 1D peaks (optionally assisted by NMR prediction)*
- Assign 1D and 2D spectra together
- Export and report assignment results
- Use your assignments to improve NMR prediction*

** A separate license for Mnova NMRPredict Desktop is needed.*

To open and transform your NMR data

- Choose **File | Open** to open the **fid** file for each spectrum, or
- Select one or more **folders** *containing the fid* files in a File Browser, and drag them to Mnova*
- Mnova automatically transforms the raw data into frequency domain (*including Windowing function, Fourier transform, phase correction etc) ***





Drag & drop

**Only folders that contain a fid file immediately under them will work. Use Scripts > Import > Multi-Open for more sophisticated multiple spectra opening.*

***Parameters from the raw data are used for processing. You can view or change the processing parameters by choosing **Processing | Processing Parameters.** See **Help > Contents > Processing Basics** for more details*

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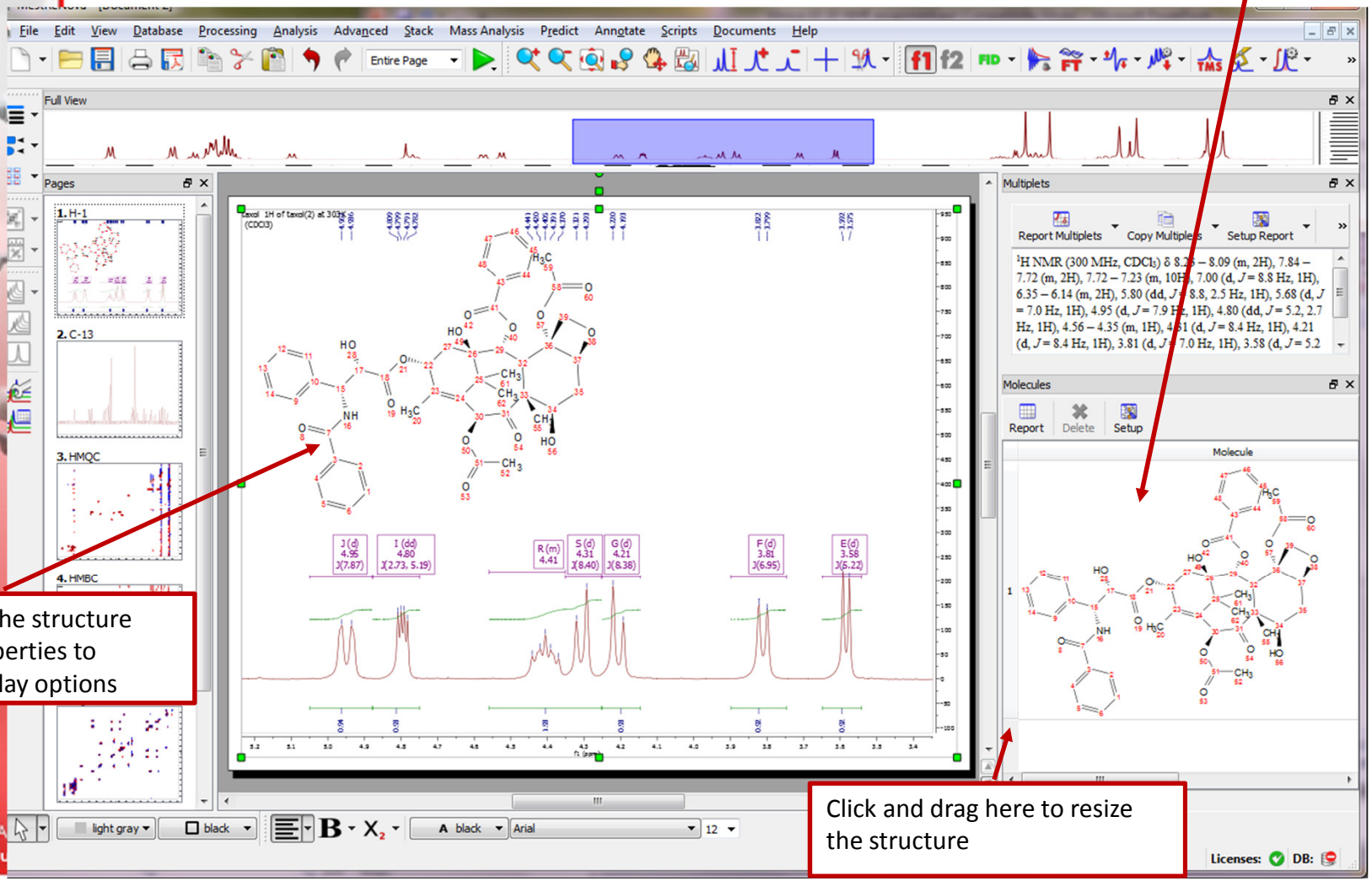
To open a structure

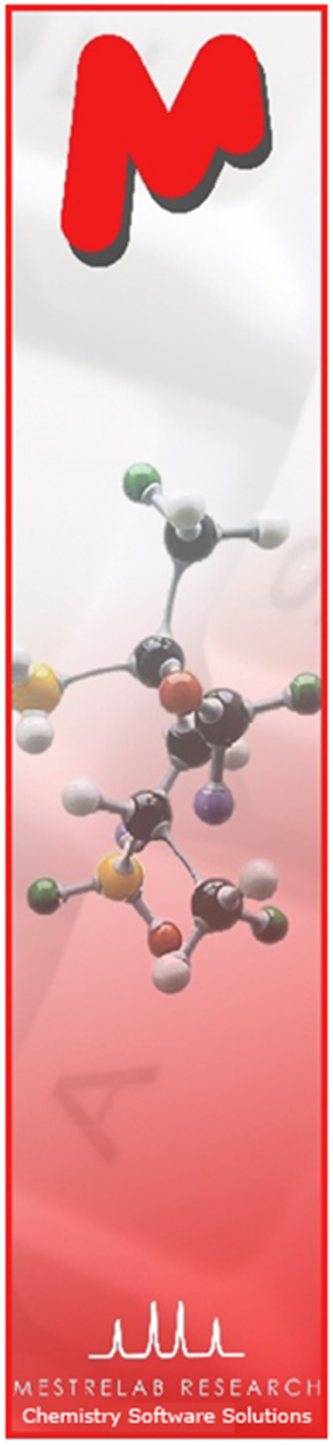
- Drag .MOL or .SDF file(s) to Mnova to open one or more structures
- Or copy a structure from ChemDraw®, Isis/Draw®, or ChemSketch®
- Choose **View | Tables | Molecules** to view the Molecules Table. *Use this Table to do assignment with multiple spectra*

Molecules Tables: Choose View | Tables | Molecules to open it



Right-click on the structure and select Properties to change its display options

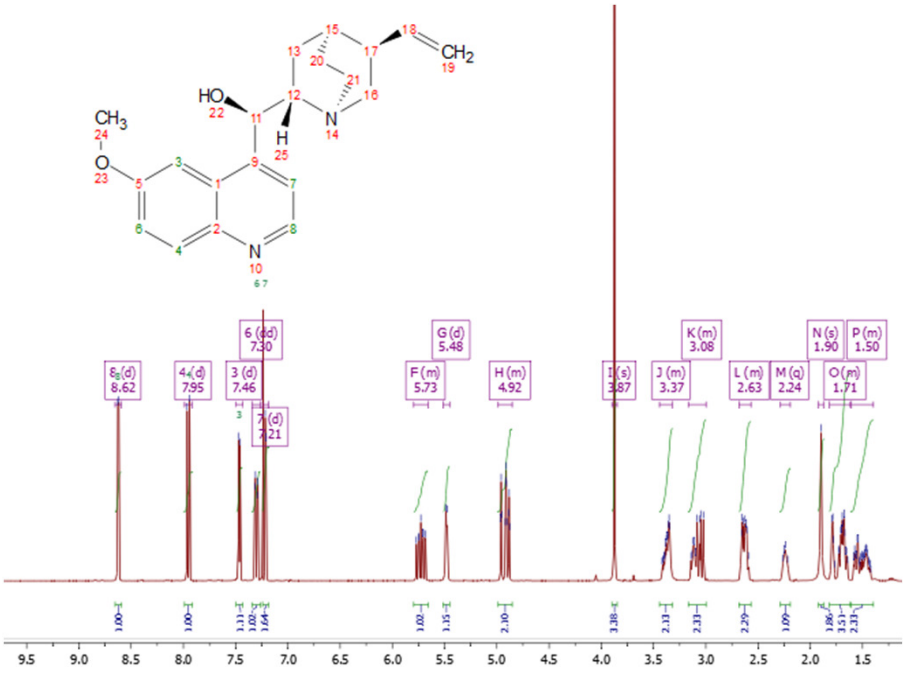
Click and drag here to resize the structure



To assign a single 1D ^{1}H spectrum

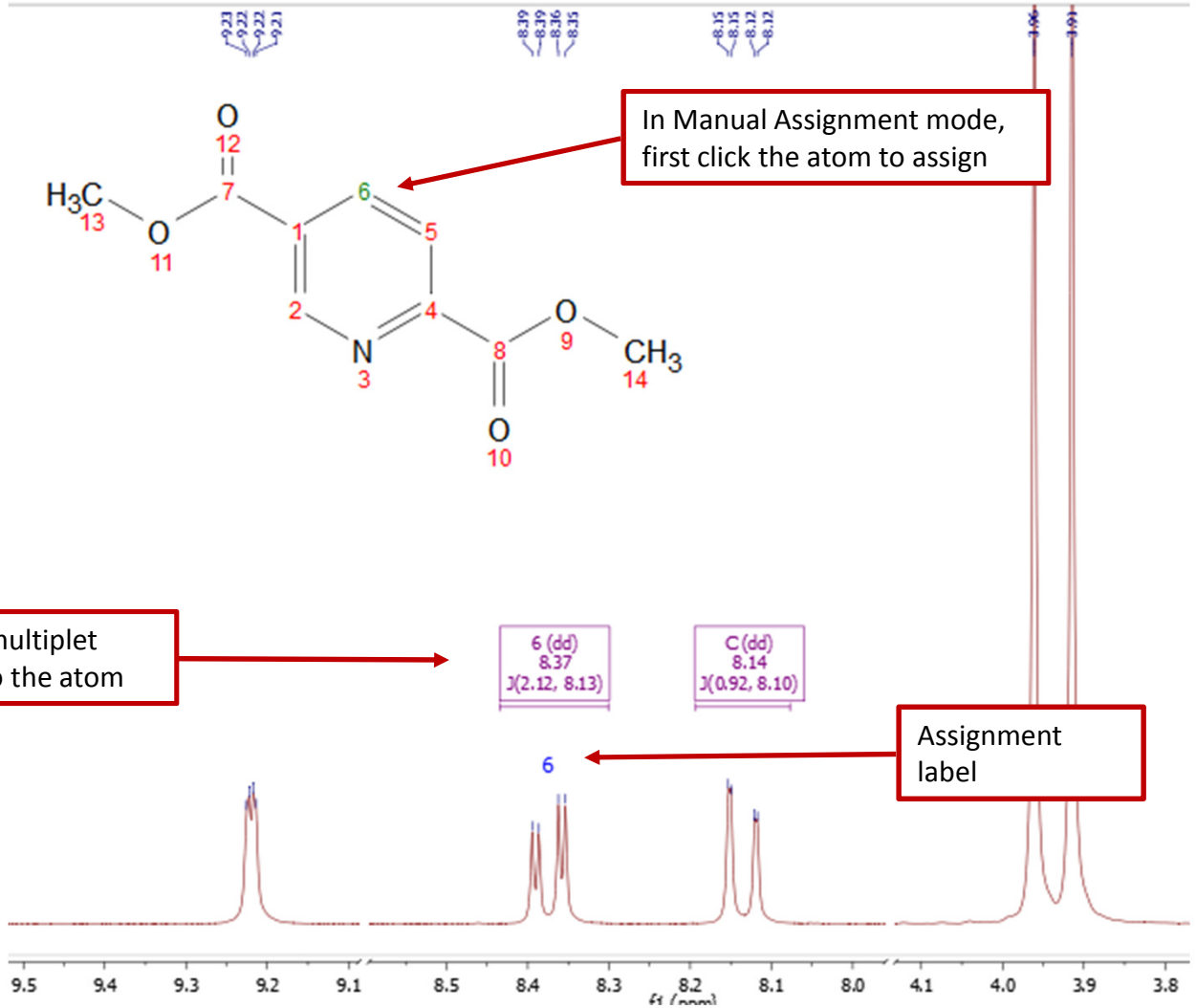
- Click **A** key (or choose **Analysis | Manual Assignment**) to enter Assignment mode.

- Click on an **atom** in the structure. Then choose the **peak** you want to assign. There are 3 ways to do it:
  - A picked **multiplet**, by clicking on the multiplet label, or
  - A **peak top**, or any point in the spectrum by clicking on it**, or
  - A **range** in the spectrum, by click-and-dragging to cover it
- If necessary, predict the ^{1}H spectrum to assist your assignment



**There are tools for automatic, semi-automatic or manual multiplet analysis in Mnova. See Help > Contents > Analysis Tools > Multiplet Analysis for details*

To assign a multiplet to an atom



Tip: After the assignment, the atom label is changed to green. The multiplet label shows the atom label. The multiplet label can be turn off by unchecking Analysis | Multiplet Analysis | Show Multiplets

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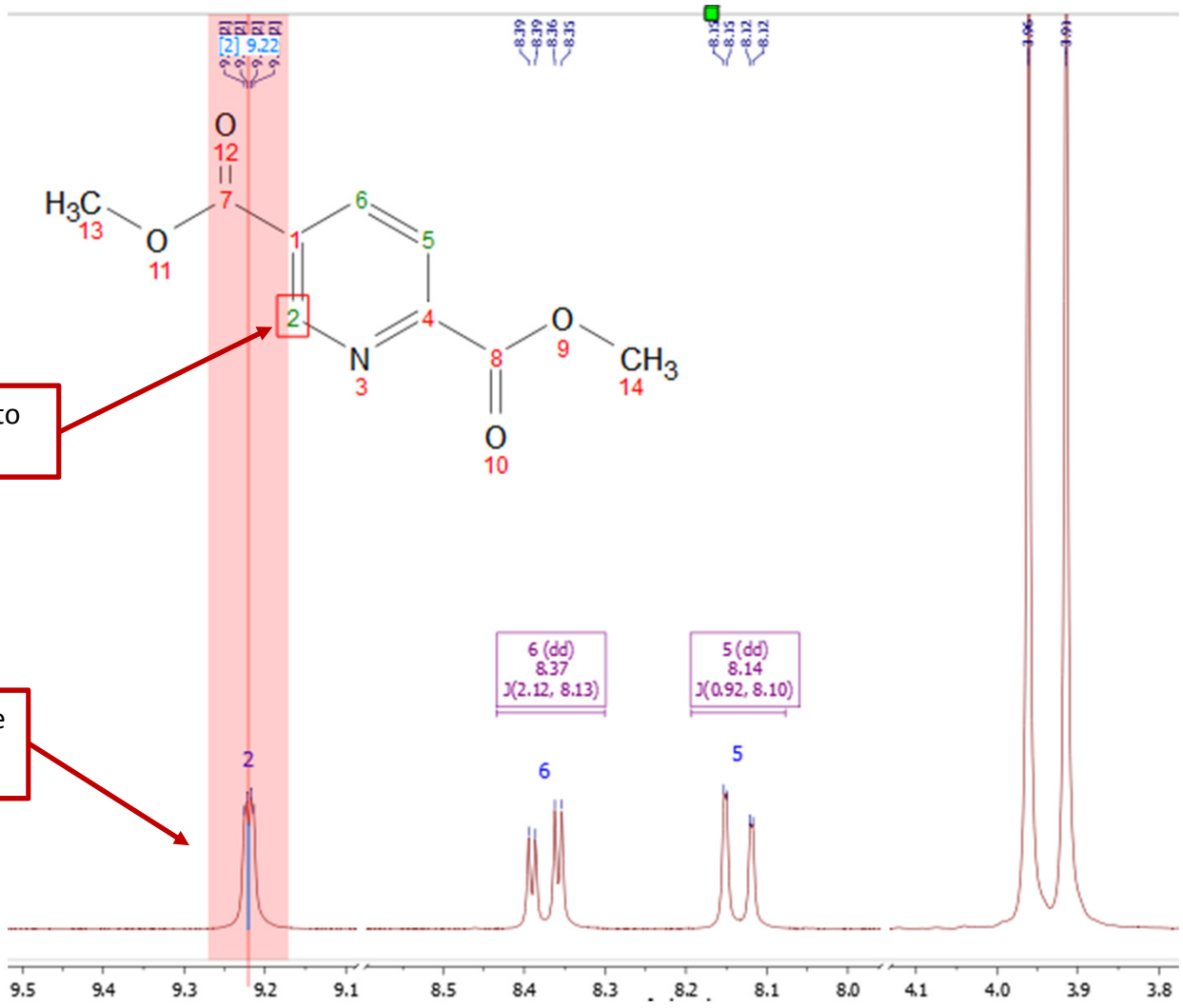
To assign a region to an atom



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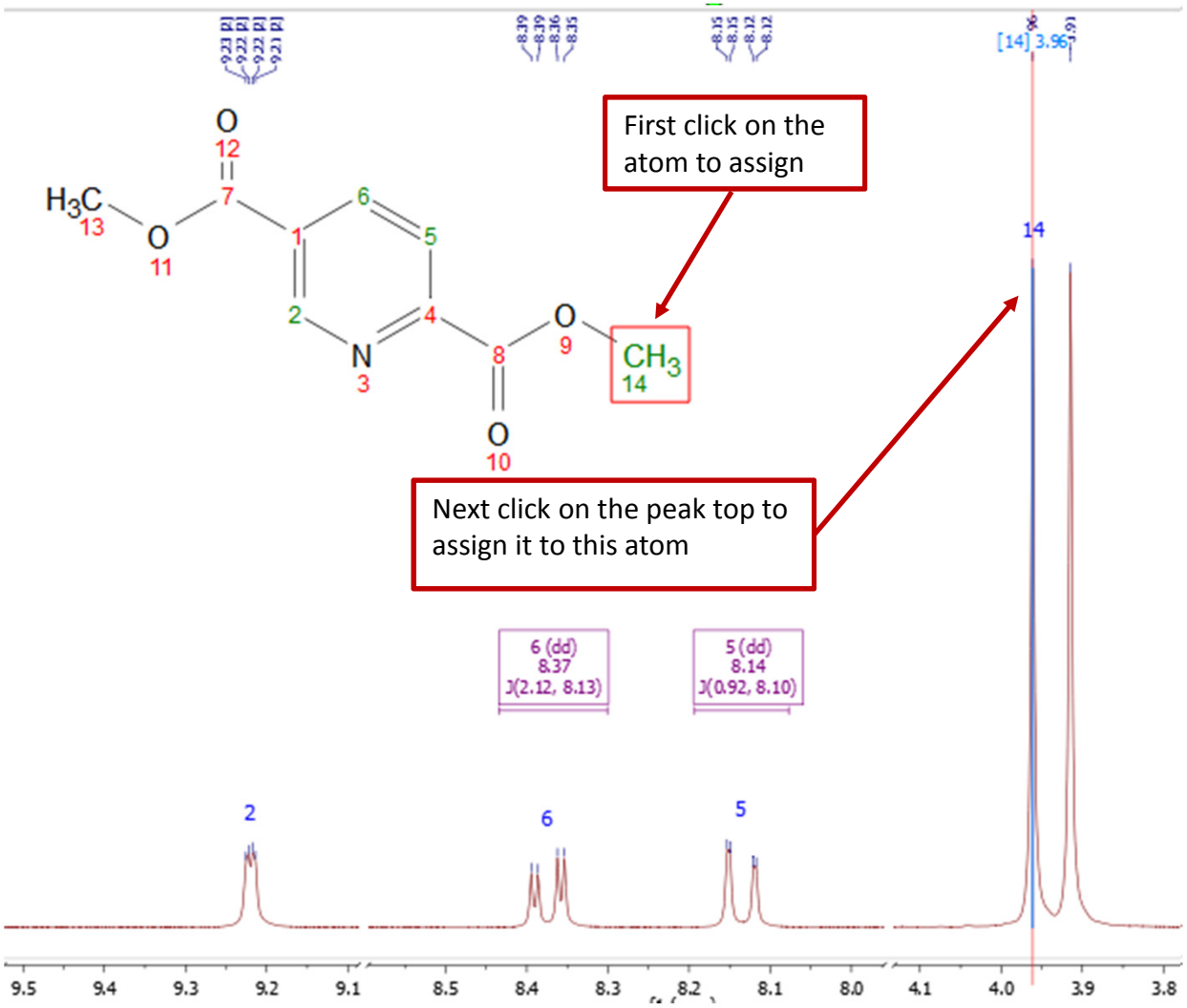
To assign a peak top to an atom



*Tip: By Default, Mnova automatically snaps to a peak top (with interpolation). Click **Shift** key one time to toggle it off if you want to choose a shoulder peak.*

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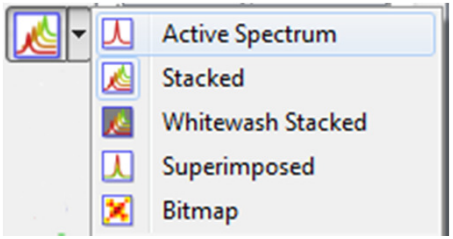
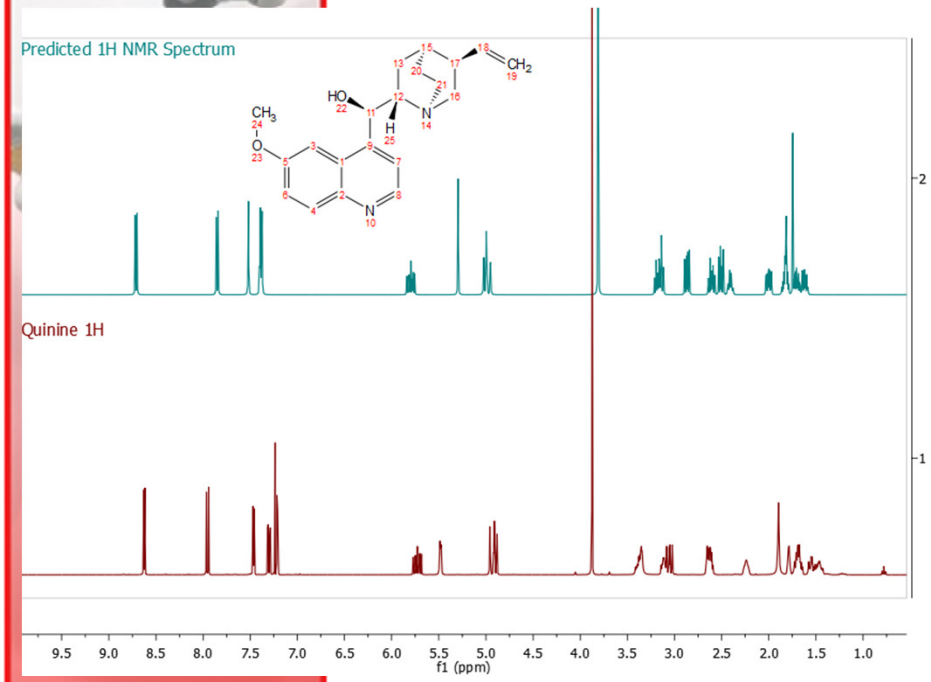
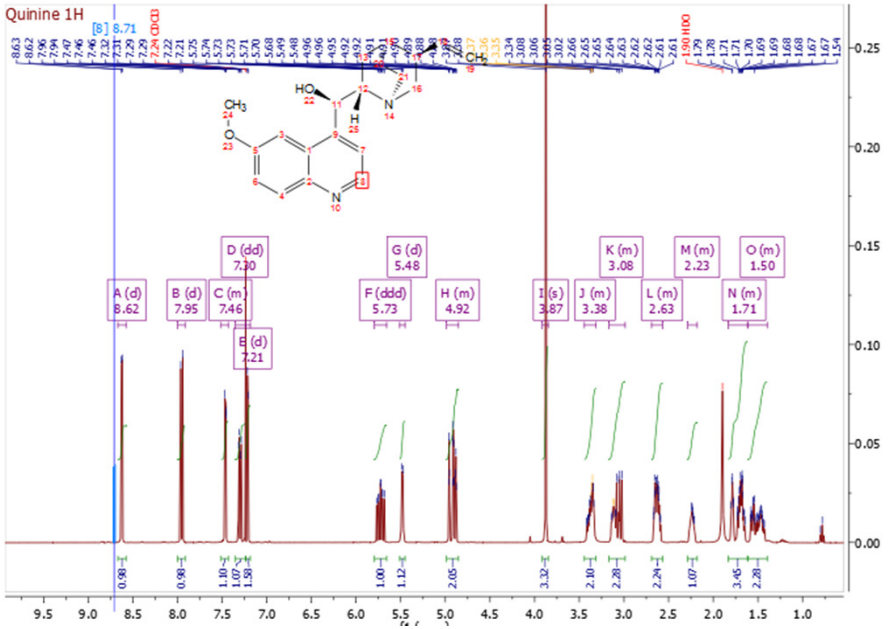
Predict NMR to assist your assignment

- Choose **Analysis | Predict & Compare** to predict a ^{1}H spectrum and stack it with the experimental one for comparison
- Next switch to **Active Spectrum mode** to do the assignment. When you hover your cursor on an atom, its predicted multiplet is displayed
- This can be done for other nuclei as well.







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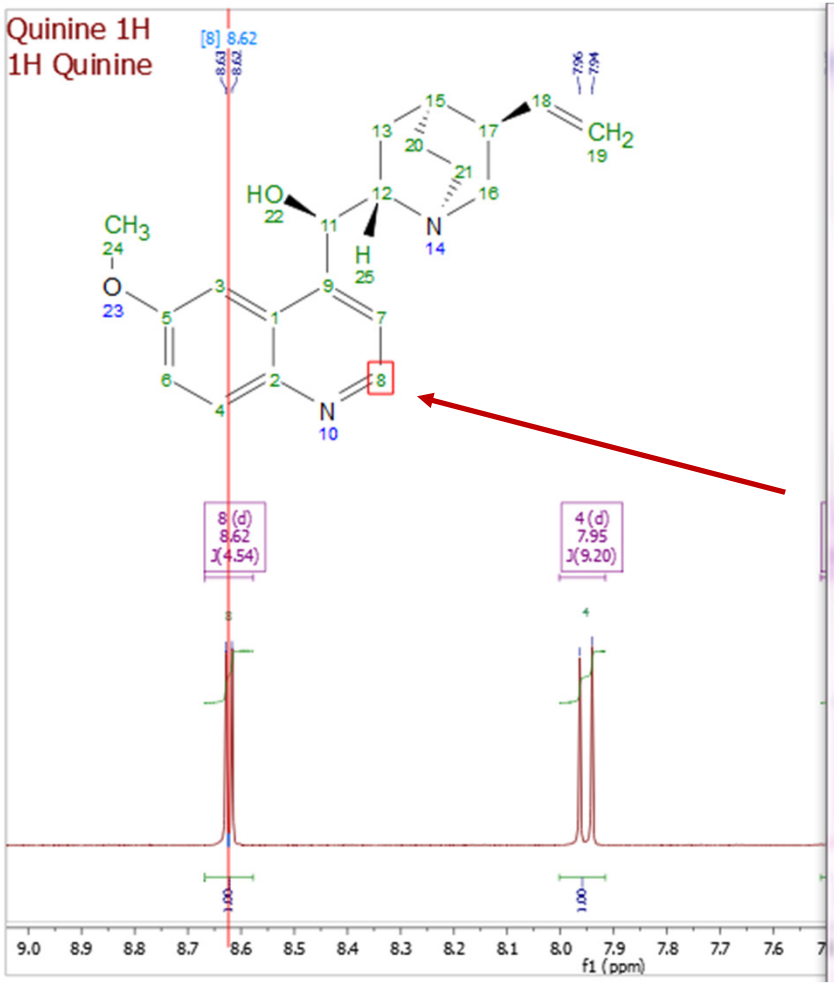
*Tip: When two or multiple spectra are superimposed, you can click **Shift+Up Arrow** keys to change the active spectrum. Make sure the experimental spectrum is the active one when you do the assignment*

To display and browse assignment results

- Choose **View | Tables | Assignments** to open the Assignments Table
- The Table and the structure are correlated: You can click a row to highlight the atom (and its assigned peak), and vice versa



Assignments

Report | Copy | Delete | Expand | Collapse

Atom	Chemical	Predicted	COSY
1 C	126.64	128.49	
2 C	144.26	143.12	
3 C	101.16	103.40	
H	7.21	7.52	
4 C	131.50	131.38	
H	7.95	7.85	
5 C	157.70	158.32	
6 C	118.25	119.87	
H	7.47	7.39	
7 C	121.52	121.28	
H	7.30	7.20	
8 C	147.42	147.32	
H	8.62	8.71	
9 C	147.51	148.25	
10 N			
11 C	72.14	70.97	
H	5.48	5.63	
12 C	59.73	62.31	
13 C	21.85	23.83	
H'	1.69	1.71, 2.02	
...	1.56	1.71, 2.02	
14 N			
15 C	27.73	27.31	
H	1.79	1.88	
16 C	57.06	55.57	
H'	3.05	2.50, 2.78	
...	2.63	2.50, 2.78	
17 C	39.83	40.36	
H	2.23	2.08	
18 C	141.75	139.85	
H	5.73	5.70	

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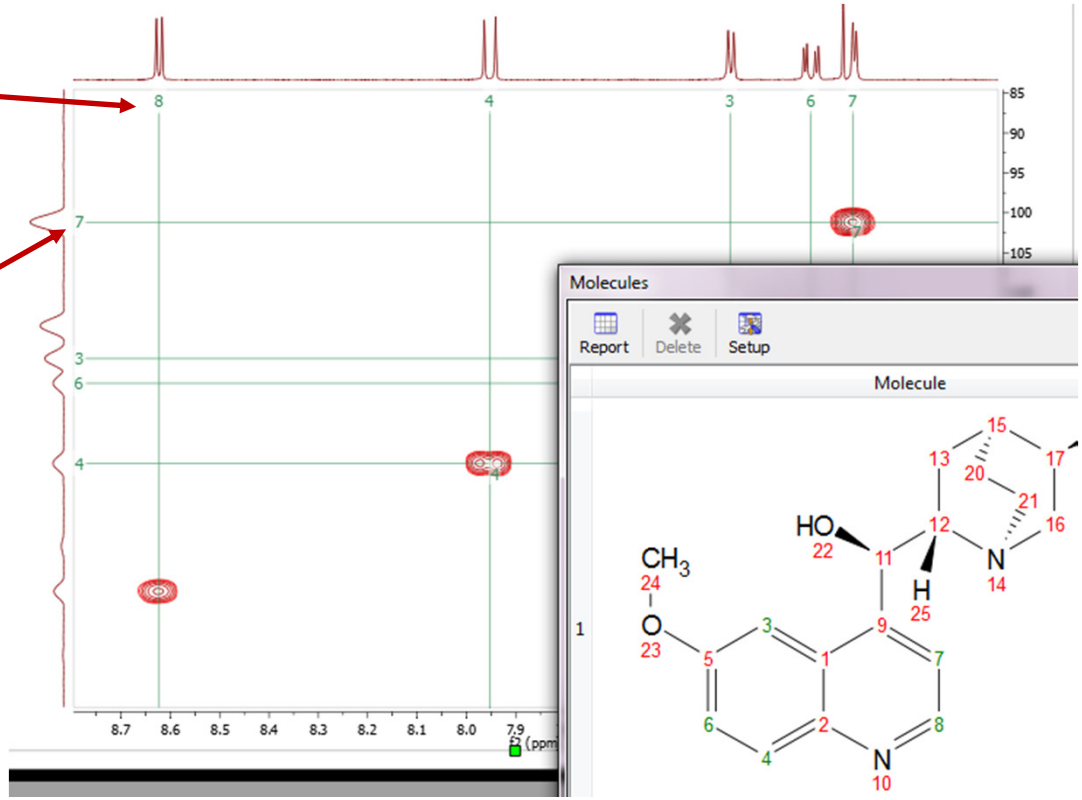
If you have 2D HSQC

- You can either first assign 1D H-1 peaks, and then assign HSQC cross peaks, or the opposite.
- Assignments in one spectrum is carried over to all other spectra in the same document
- To assign in HSQC, click **A** key to enter Assignment mode. Click on an **atom** in the structure. Next click on the cross peak to assign to it*

H-1 assignments from 1D spectrum or HSQC

C-13 assignments from HSQC



By Default, Mnova automatically snaps to a peak top (with interpolation). Click **Shift key one time to toggle it off if you want to manually locate the peak center.*

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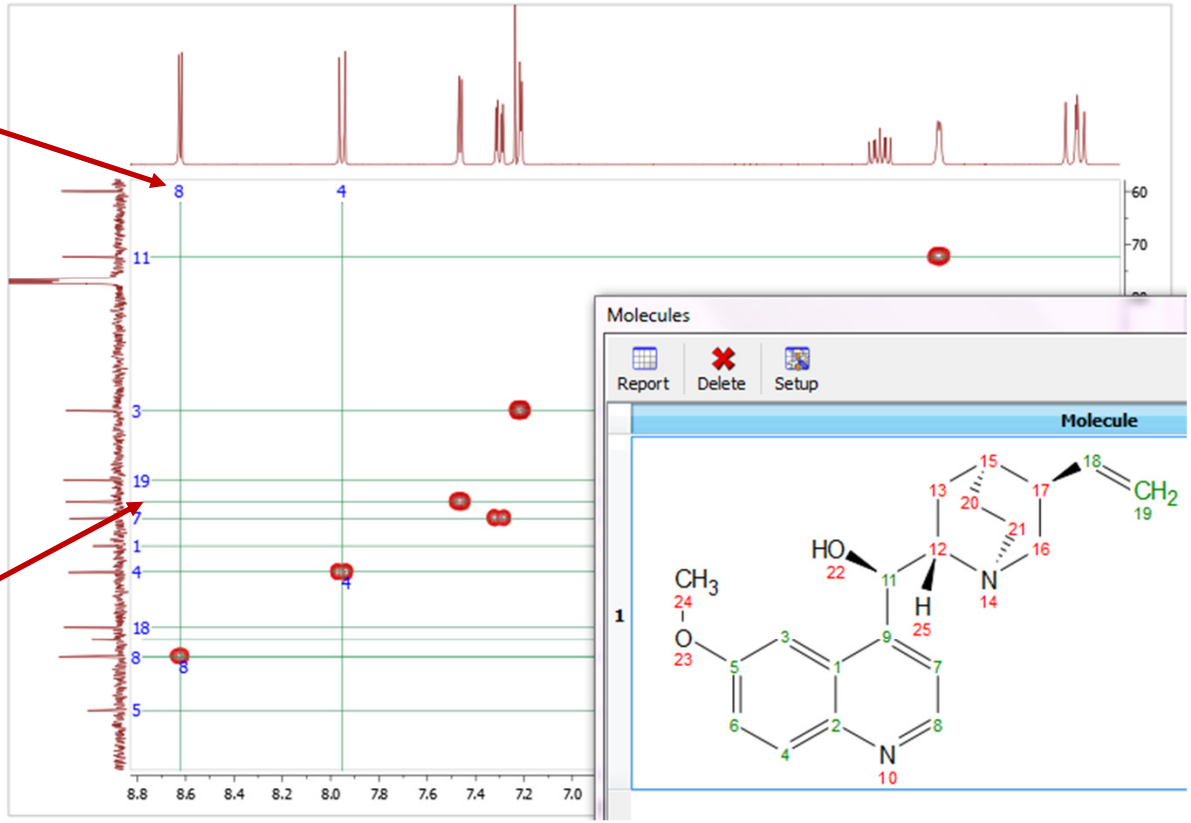
If you have a C-13 spectrum

- You can first assign the C-13 peaks, possibly with the help of Predict and Compare
- Next you can switch to the HSQC, and easily assign the HSQC peaks, and get most of the H-1 shift assigned.
- Finally you can switch to the H-1 spectrum, and assign all H-1 peaks *

H-1 assignments from HSQC peaks

C-13 assignments from C-13 spectrum



** If you assign a H-1 chemical shift to the same atom multiple times (e.g. first from HSQC, and then from H-1 spectrum), the last one is taken. It is possible to assign multiple atoms to the same peak. To remove an assignment, delete the assigned chemical shift from the Assignments Table*

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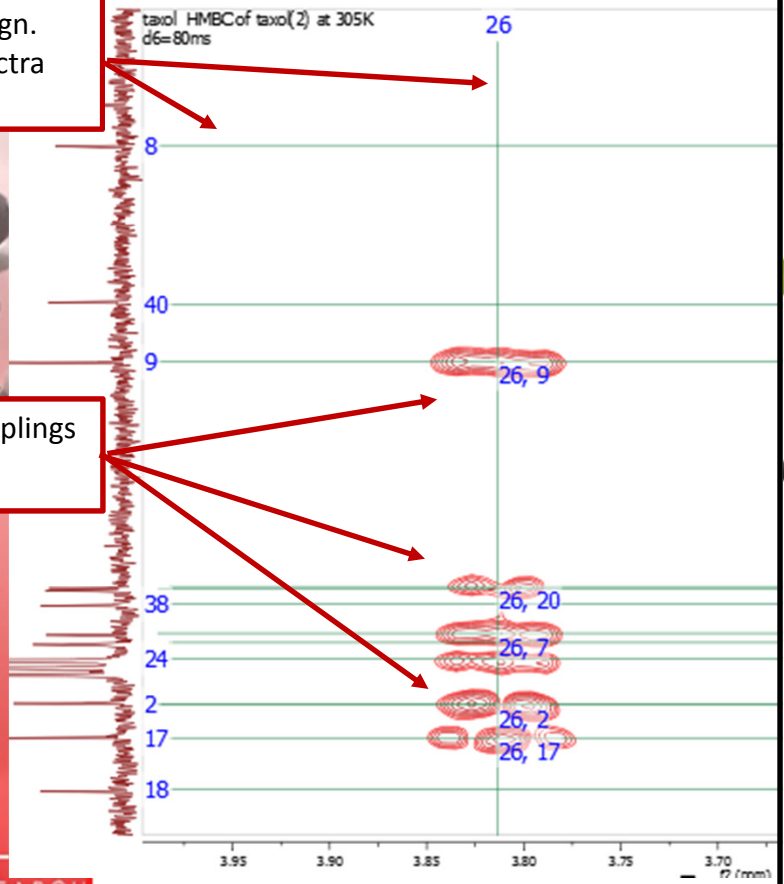
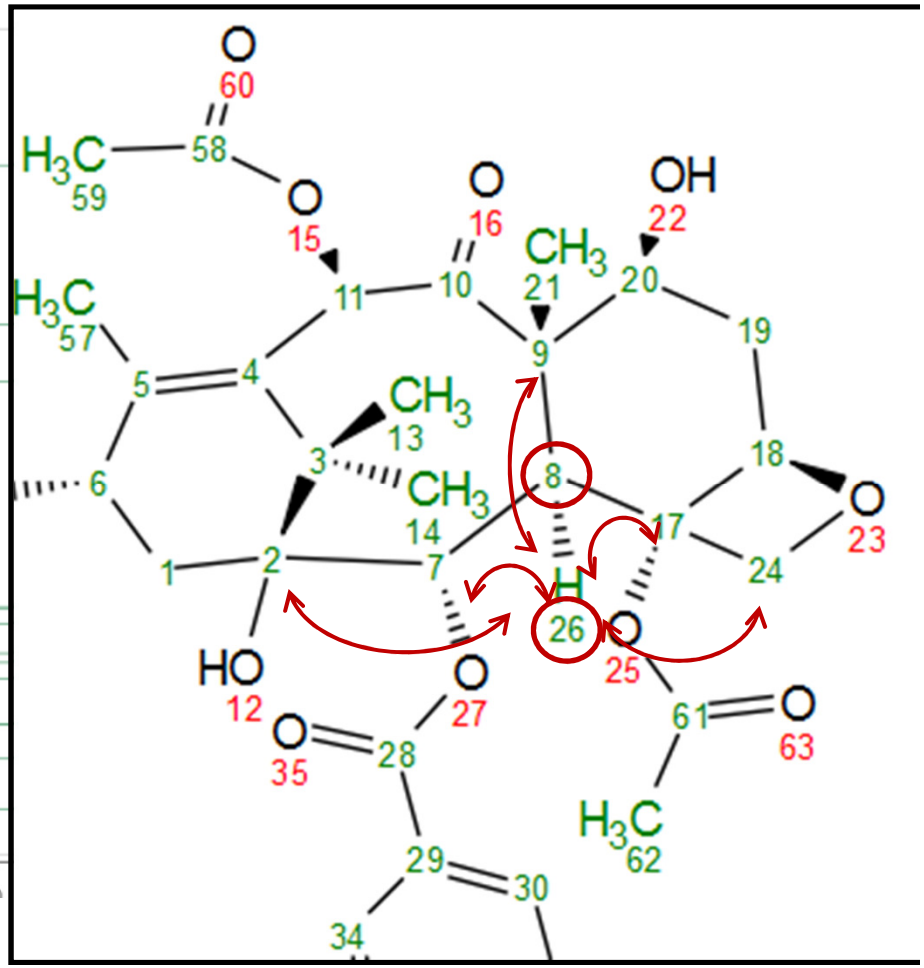
If you have 2D HMBC

- The assignments of both H-1 and C-13 shifts are displayed on HMBC, making it easy to identify 2-3-bond long-range correlations between them.
- To assign an HMBC peak, click on an **atom** in the structure, next click on the cross peak to assign. Choose the other atom from the dialog.

H-1 & C-13 assign. from other spectra for H26-C8

Long-range couplings to H26





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To superimpose HMBC and HSQC

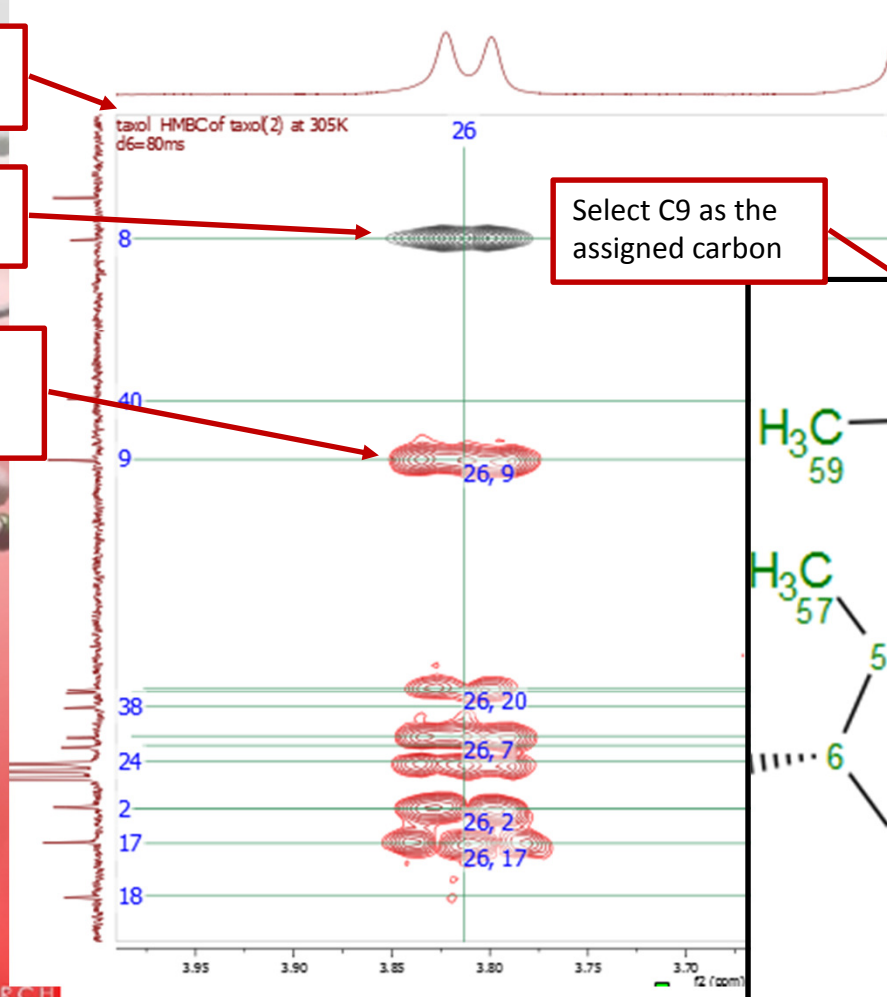
- Select both HMBC and HSQC from the Pages View, click  to superimpose them. Use **Shift+Up Arrow** keys to toggle the active spectrum. Change their contour colors (e.g. Grey for HSQC, Red for HMBC)*
- Make sure HMBC is the active one. In the assignment mode, click on a peak and then on one of the atoms. Choose the other atom from the dialog.

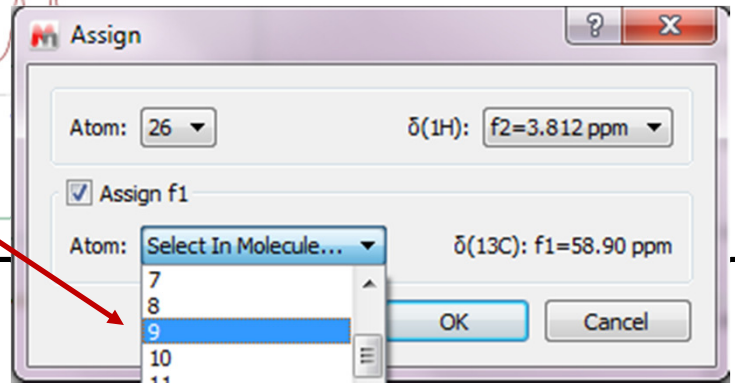
Title of the active spectrum

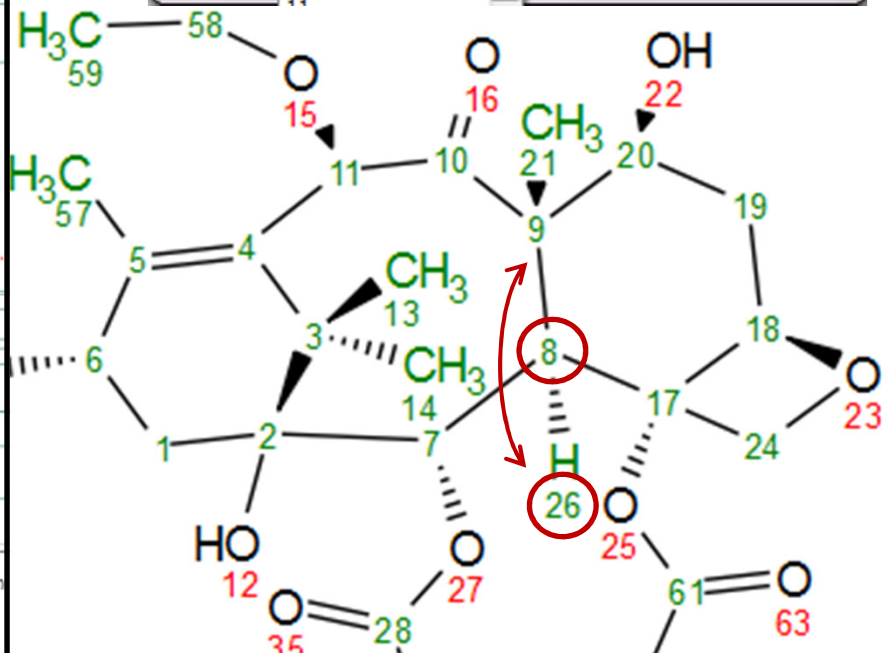
HSQC peak between C8-H26

Click on this peak and then click on H26 in structure

Select C9 as the assigned carbon

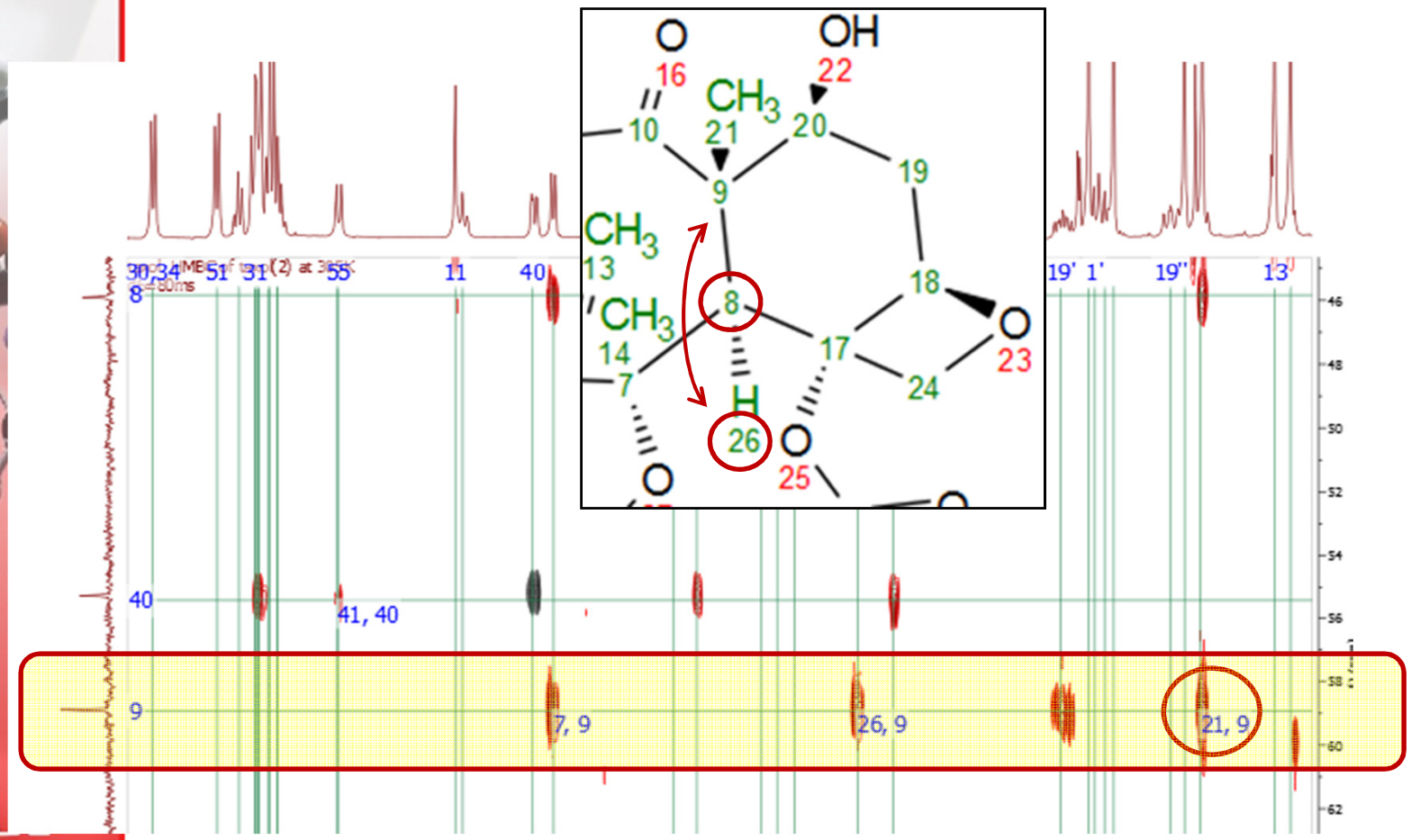






How did we know it's C9?

- From the superimposed spectra, it shows no HSQC cross peak – so it's a quaternary carbon
- It shows an HMBC peak with H21 – a methyl group – so it is close to CH_3(21)



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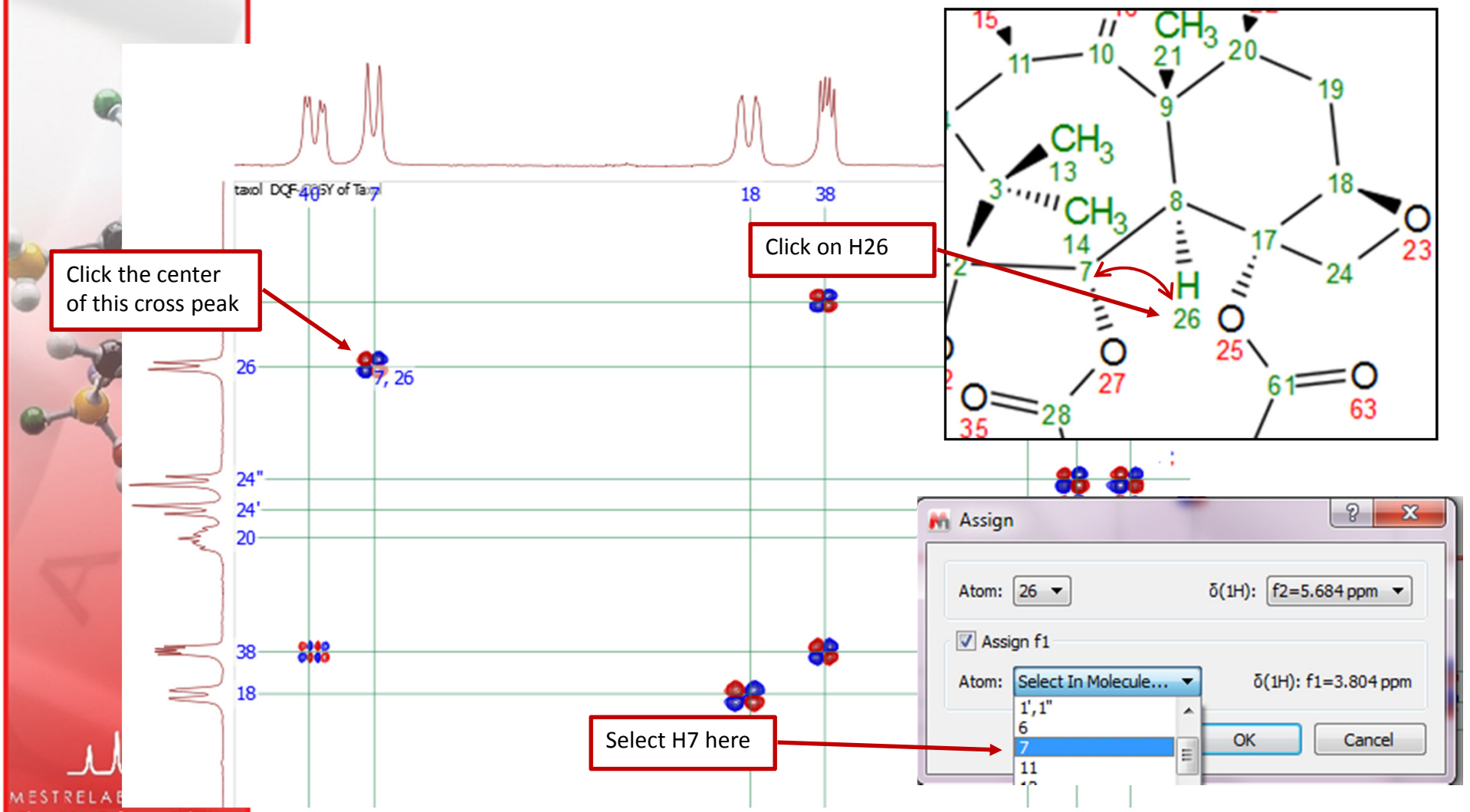
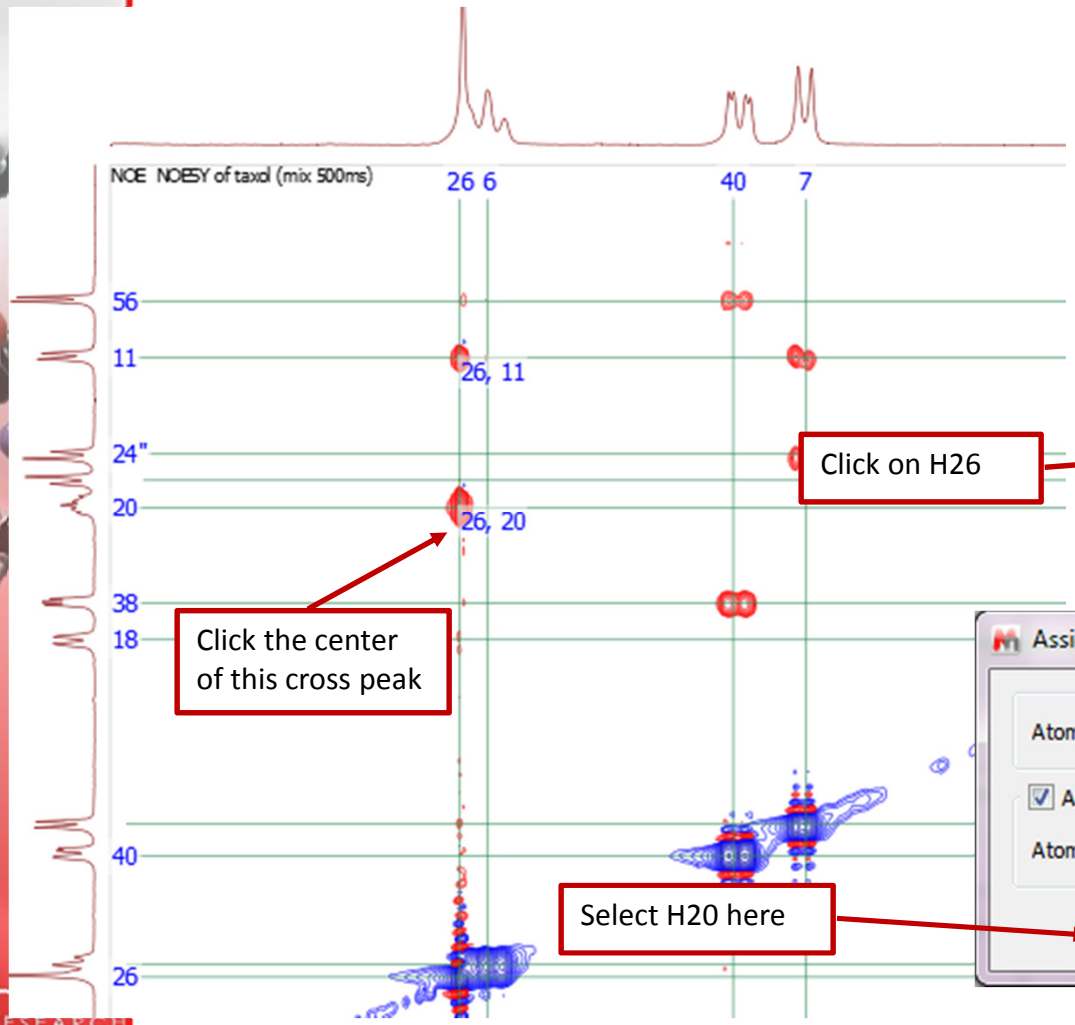
If you have COSY, TOCSY etc…

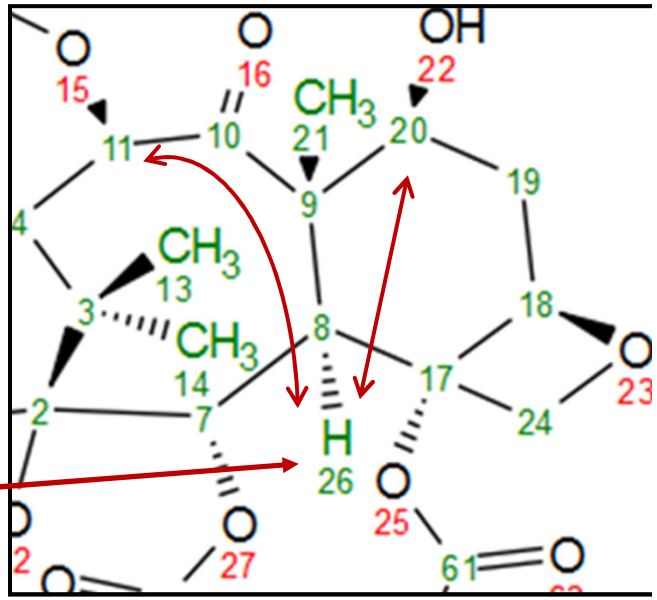
- H-1 assignments are displayed on such H-H J-correlated spectra. You can assign such cross peaks by clicking on a peak and a H atom, and then selecting the other proton. See example below:

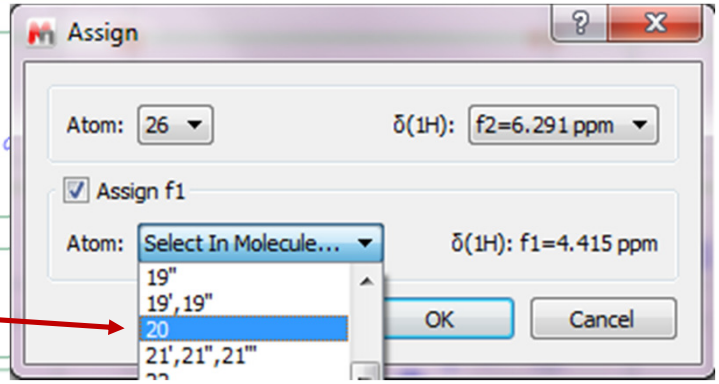


If you have NOESY, ROESY etc…

- Locate the cross peaks at the intersections of the assignment grids. Click on them and the corresponding atoms to do the assignments
- The assignment of two NOESY peaks are shown below







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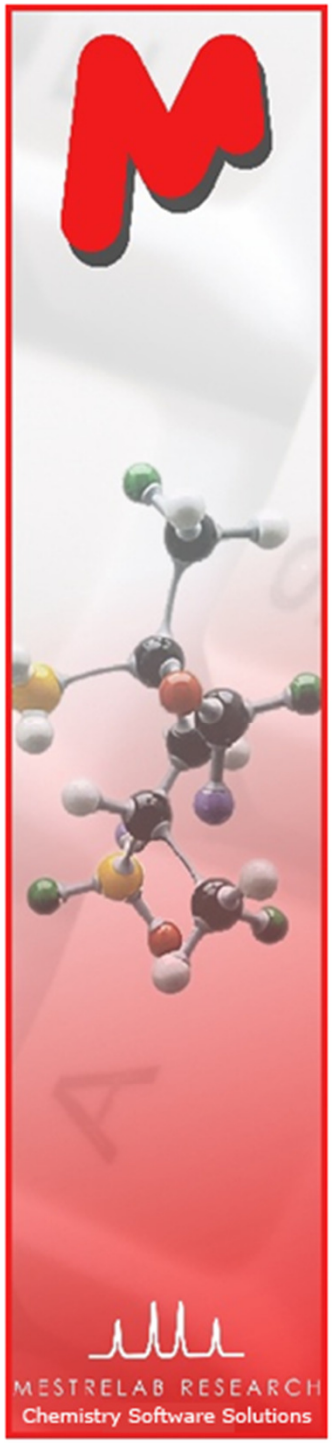


The Assignment Table for multiple spectra

- Choose **View | Tables | Assignments** to open the Assignments Table if not yet
- The Table lists all assignment results, which can be copied to other documents

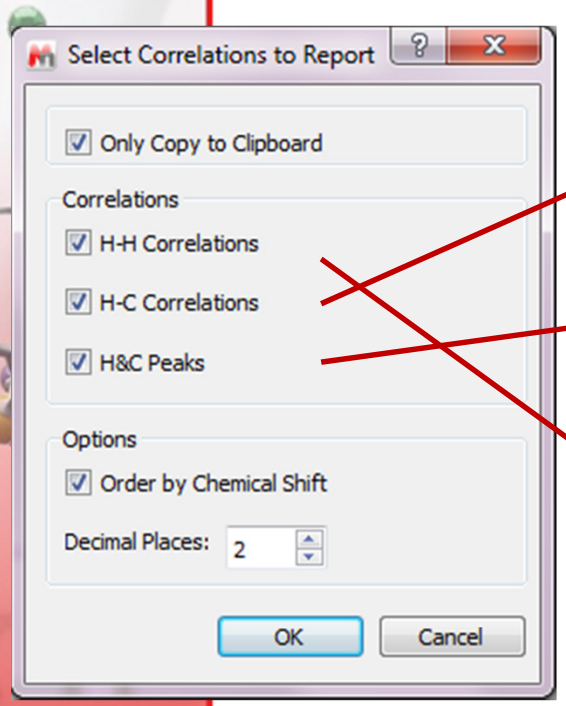
Assignments

Report | Copy | Delete | Expand | Collapse | Setup

Atom	Chemical Sl	Predicted S	COSY	TOCSY	HSQC	HMBC	H2BC	NOESY
1 C	36.08				1', 1"	7		
H'	2.35				1			
H"	2.29				1			
2 C	79.42					7, 26		
3 C	79.40					13', 13", ...		
4 C	133.54					57', 57", ...		
5 C	142.27					57', 57", ...		
6 C	72.46				6			
H	6.24				6			
7 C	75.13				7	26		
H	5.68		26		7	1, 2, 9, 28		
8 C	45.84				26			
9 C	58.92					7, 26, 21...		
10 C	203.84					11, 21', ...		
11 C	75.68				11			
H	3.82				11	10		26
12 O								
H								
13 C	27.02				13', 13", ...			
H3	1.25				13	3		
14 C	21.95				14', 14", ...			
H3	1.15				14			
15 O								
16 O								
17 C	81.46					24', 24", ...		

To export assignment results

- The **Assignment Table** can be copied to other documents such as MS Excel
- For more sophisticated reports, highlight the structure, then choose **Scripts | Report | Assignments**, and select the options. Next paste the reports to a MS Word or other documents directly



Number	δ (ppm)	HSQC ($^1J_{CH}$) Correlations (ppm)	HMBC Long Range ($^{1+n}J_{CH}$) Correlations (ppm)
3	7.22	101.16	
4	7.95	131.51	
6	7.46	118.25	
7			
8			

Number	^{1}H-Chemical Shift	Multiplicity (*J*-couplings)	^{13}C- Chemical Shift
8	8.62	d, *J*=4.54 Hz	147.42
4	7.95	d, *J*=9.20 Hz	131.50
6	7.47		118.25
7	7.30	dd, *J*=9.25,2.68 Hz	121.52

Number	δ (ppm)	COSY Correlations (ppm)	NOESY Correlations (ppm)	TOCSY Correlations (ppm)
30	8.14			
45	7.50			
43	7.49		7.01(41')	
47	7.49		5.80(38'), 4.80(40'), 3.59(56')	
44	7.43			
46	7.43			
52	7.38			
54	7.38			
41	7.01		7.49(43'), 7.01(55')	
55	7.01		7.01(41')	
26	6.29			
6	6.24			
38	5.80	3.59(56')	7.49(47')	
7	5.68			
18	4.95			

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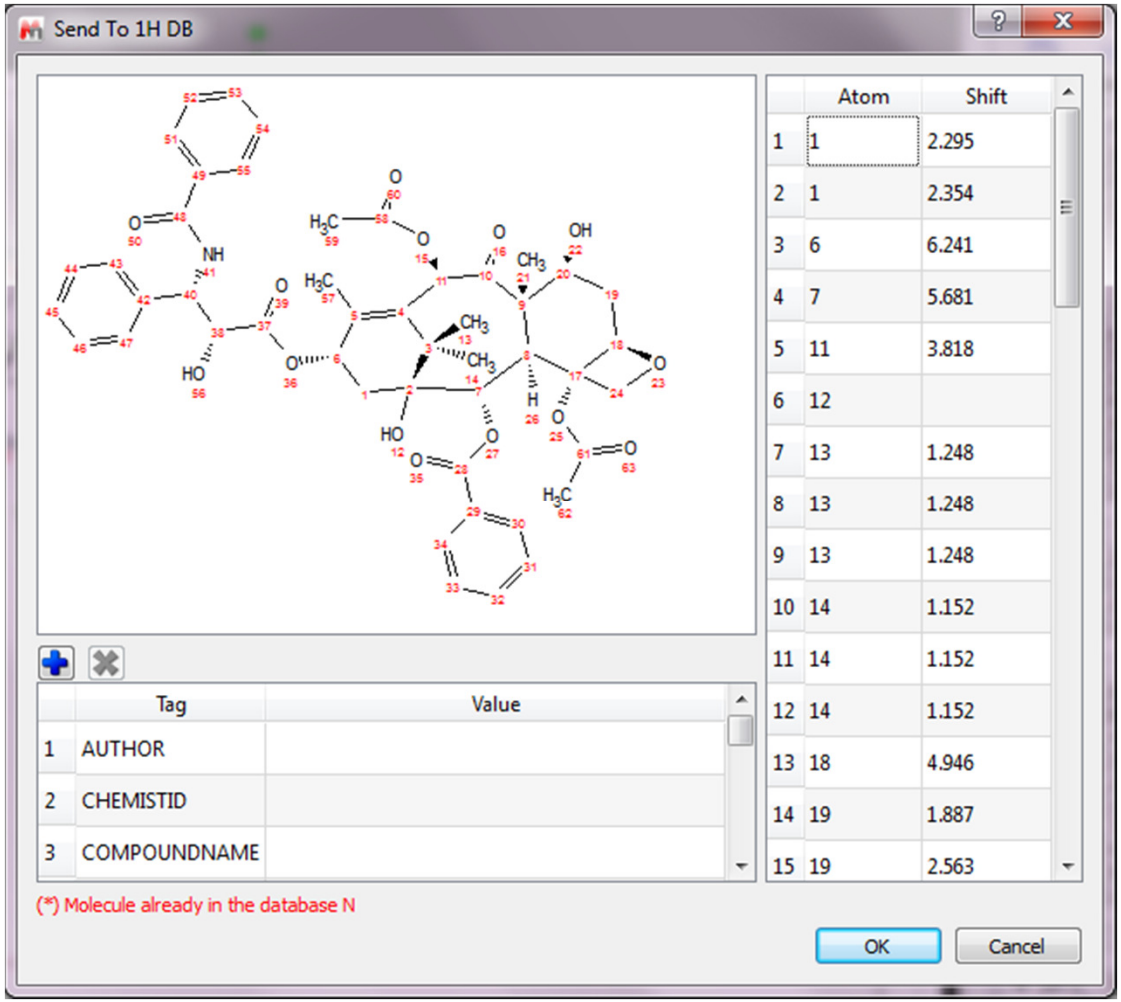
To improve NMR prediction using your assignments

- After you are done with the assignment of a 1D spectrum, choose **Predict | Update 1H User DB** to save it as a knowledgebase for H-1 prediction. This will improve the 1H prediction of similar structures



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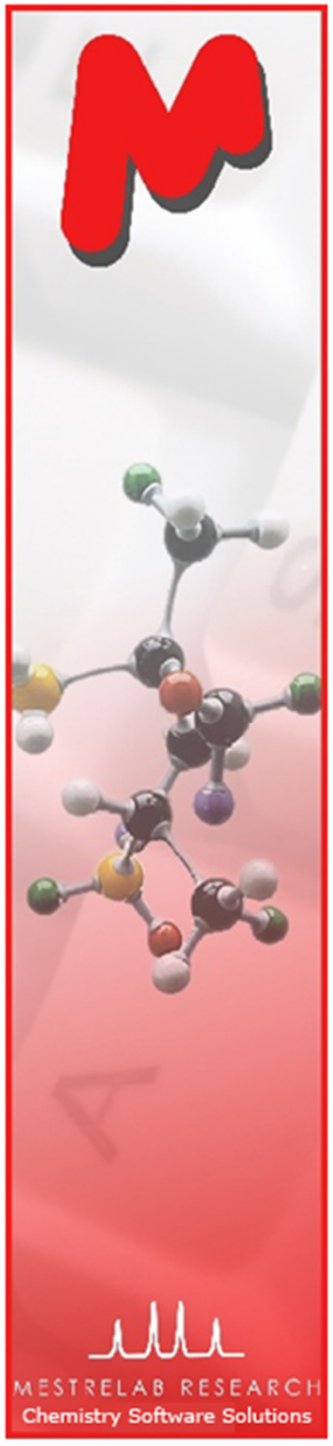
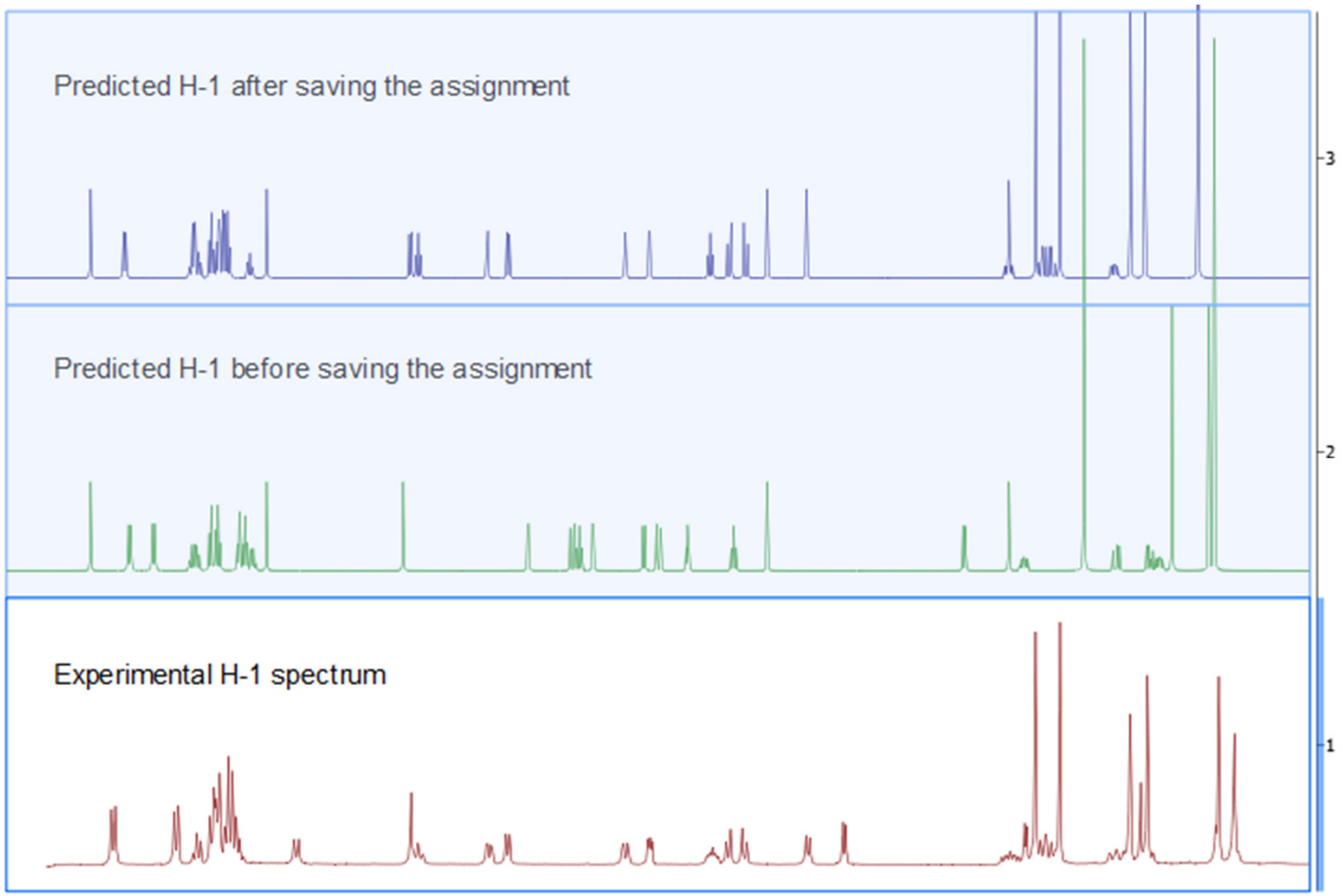
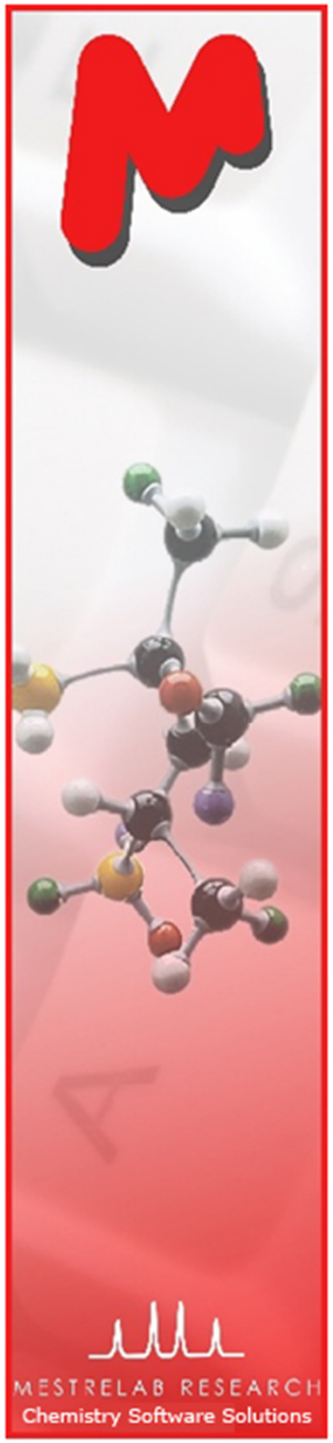


To improve NMR prediction using your assignments

- The prediction is usually improved after you save your assignments to Mnova NMRPredict Desktop





Summary

- Mnova NMR provides intuitive and easy-to-use tools for processing and assigning multiple 1D and 2D NMR spectra
- The relevant tables and script make it very easy to report and publish such results
- Mnova NMRPredict Desktop can be used to assist the assignment or verify your results. Your assignments can be used to improve the precision of NMR prediction
- We are working to automate the assignment of H-1 and HSQC
- For 45 day free trial of Mnova, go to www.mestrelab.com and download and install, or write to me at chen.peng@mestrelab.com