Problem of the Month:

October 2020

Solution

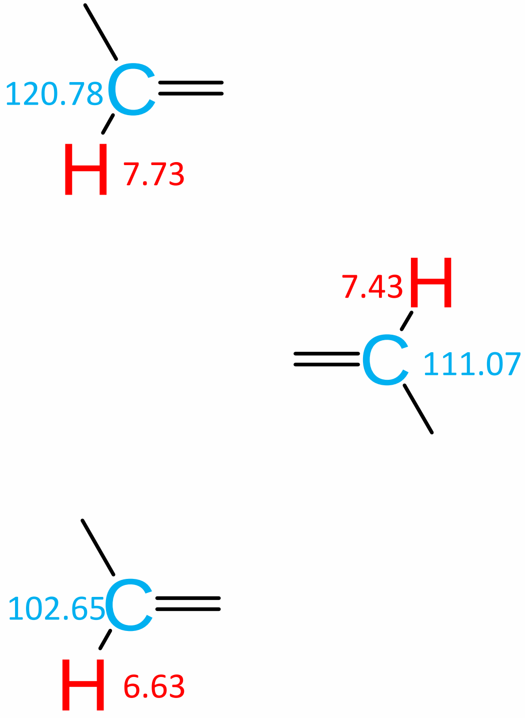
If available, in most cases the HSQC is an ideal point to start a structure elucidation.

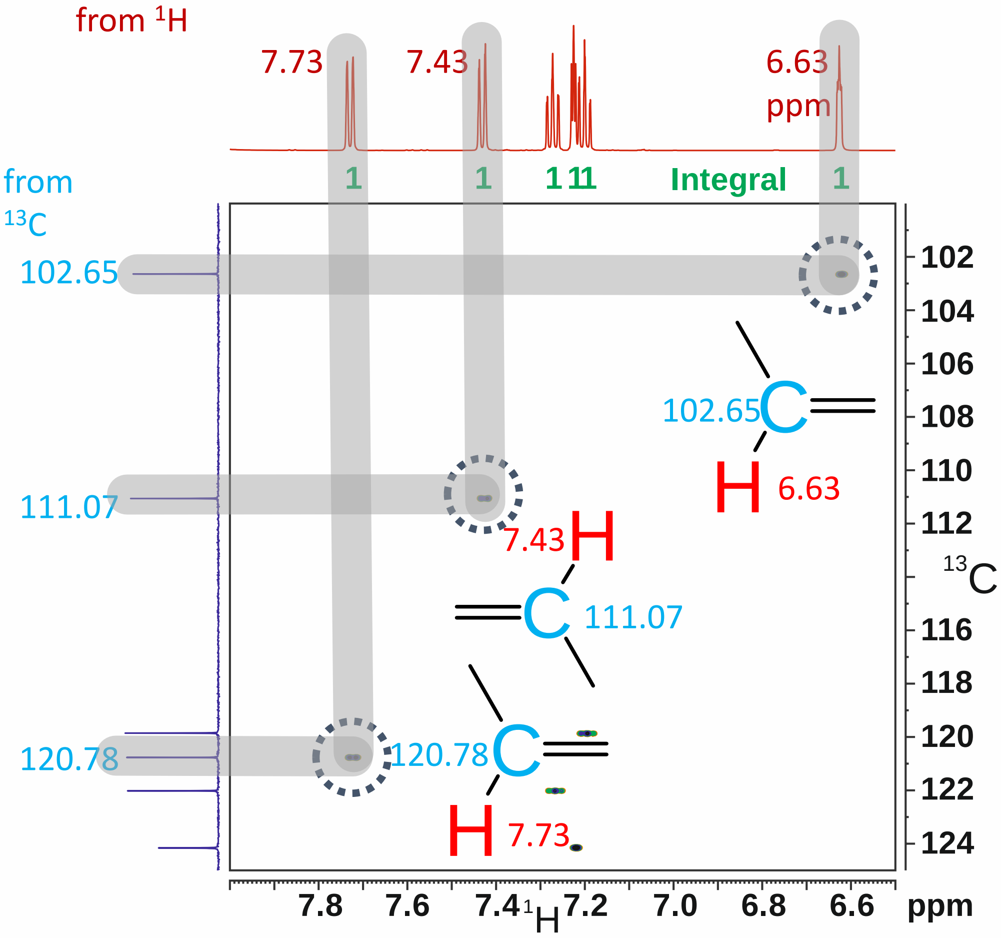
For the molecular formula C_8H_7N, a number of 6 double bond equivalents (degree of unsaturation) can be figured. Roughly, this makes up for one aromatic unit plus 2 more dbe.

The evaluation of an HSQC is a bit like picking out the right Lego bricks from the big box.

Let's start with three well separated cross peaks.

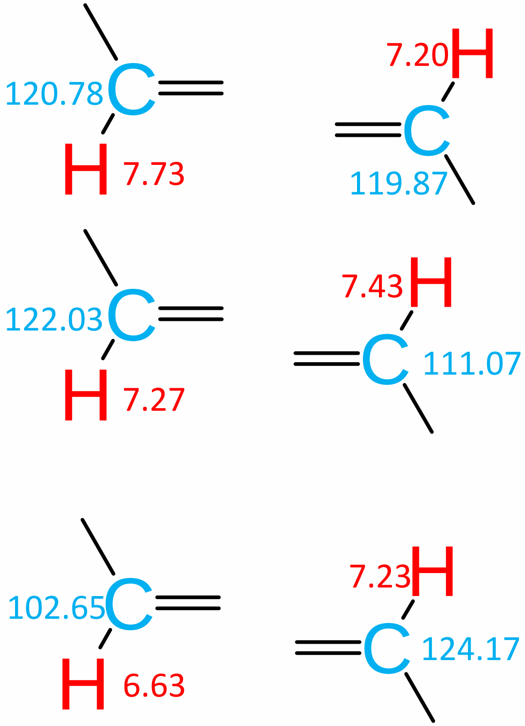
As a first step we extract the chemical shifts from the one-dimensional spectra.

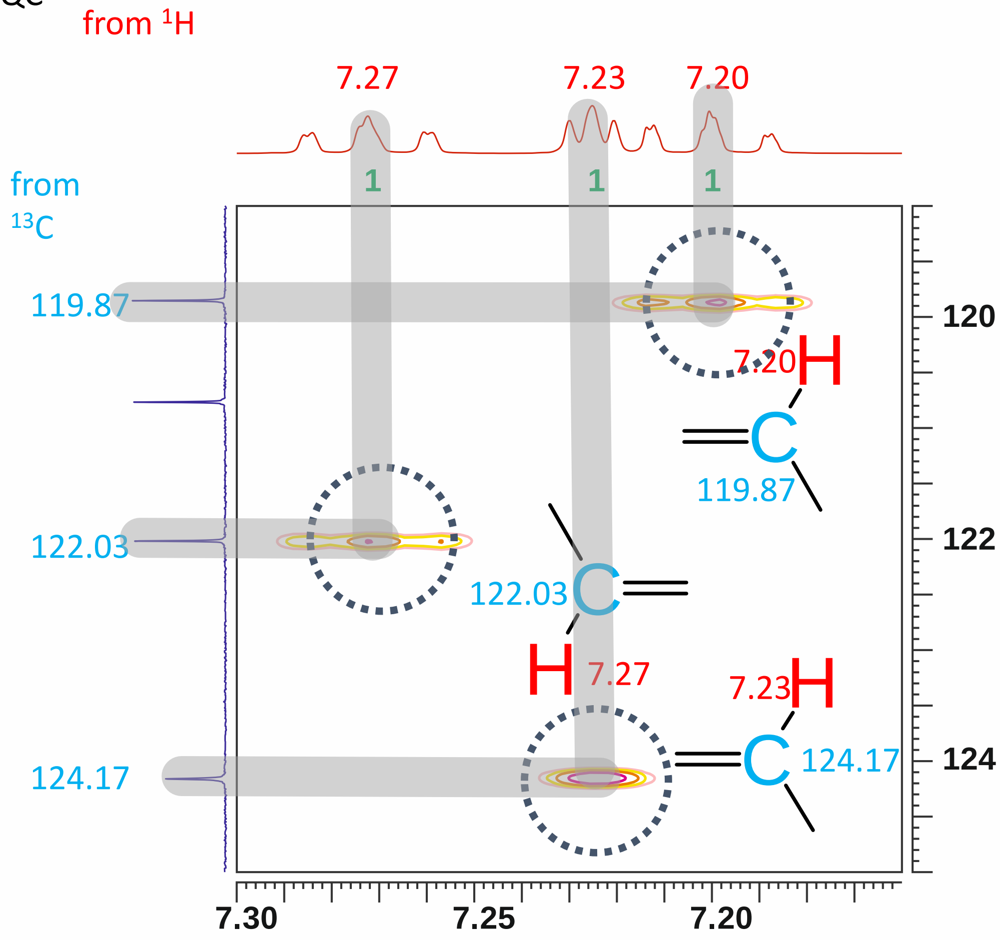




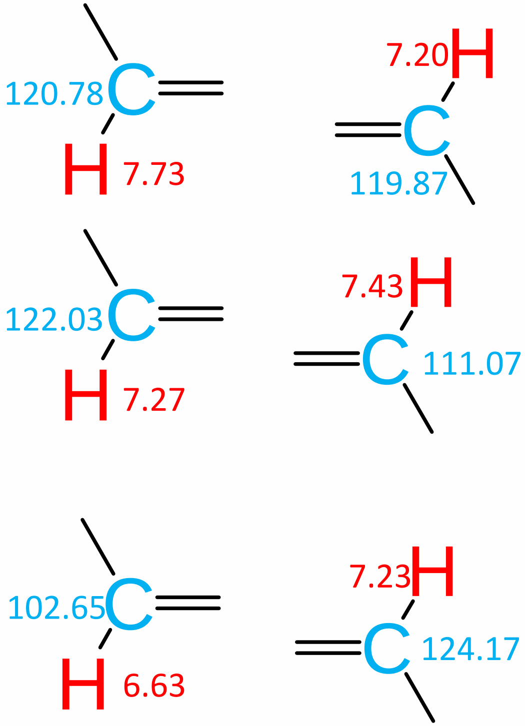
We continue with the peaks in the lower third of the HSQC with very similar proton chemical shifts.

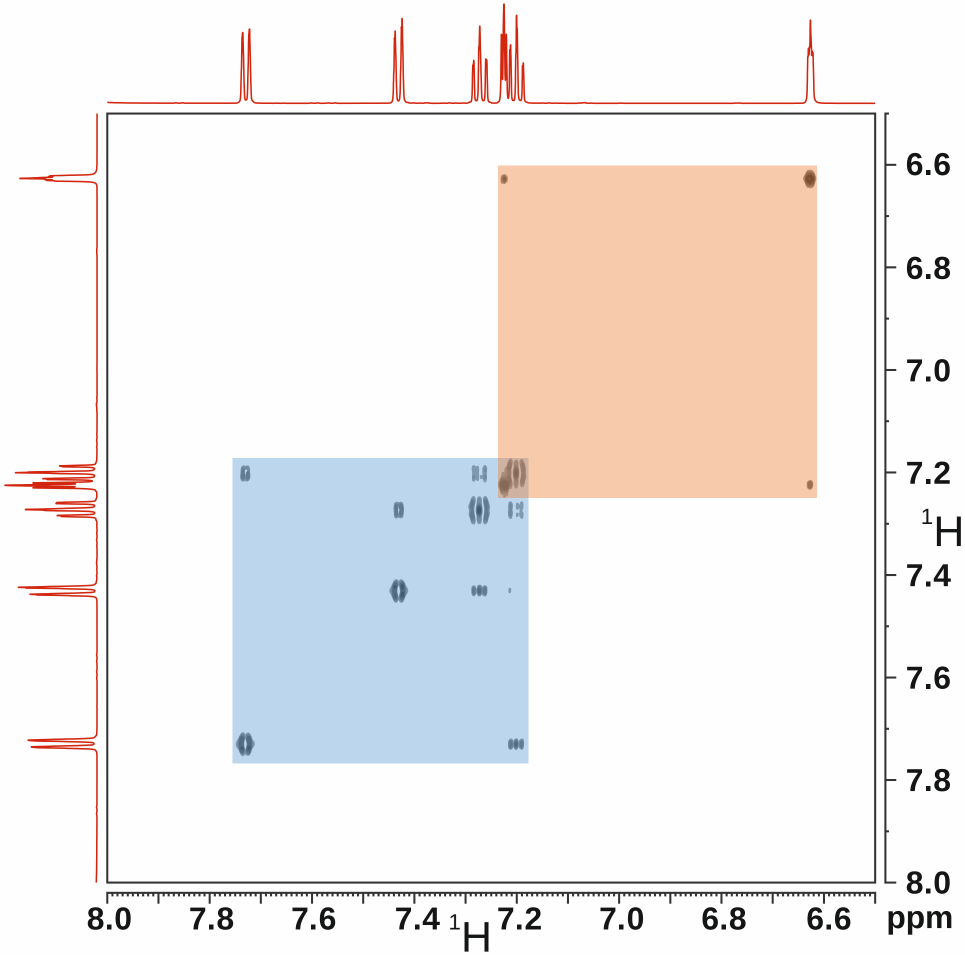
The procedure for the next three building blocks is the same as seen before.





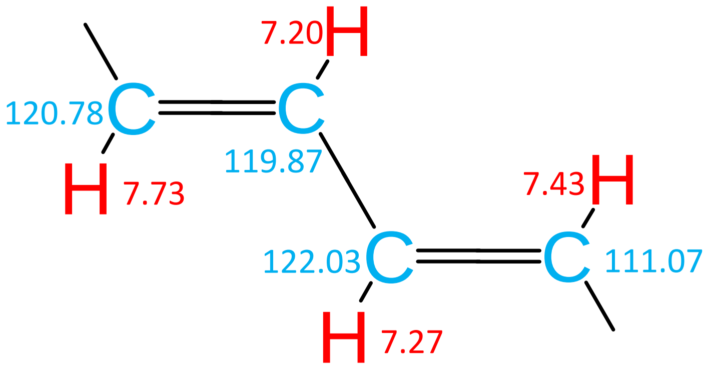
Now let‘s connect the building blocks via the TOCSY. Fortunately two well separated spin systems are easily visible

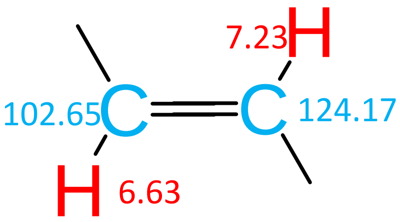




a short interim result

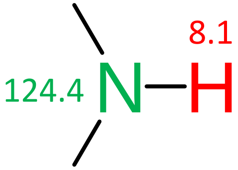
molecular formula	**C_8H_7N**
fragment 1	C_4H_4
fragment 2	C_2H_2
unassigned	C_2HN



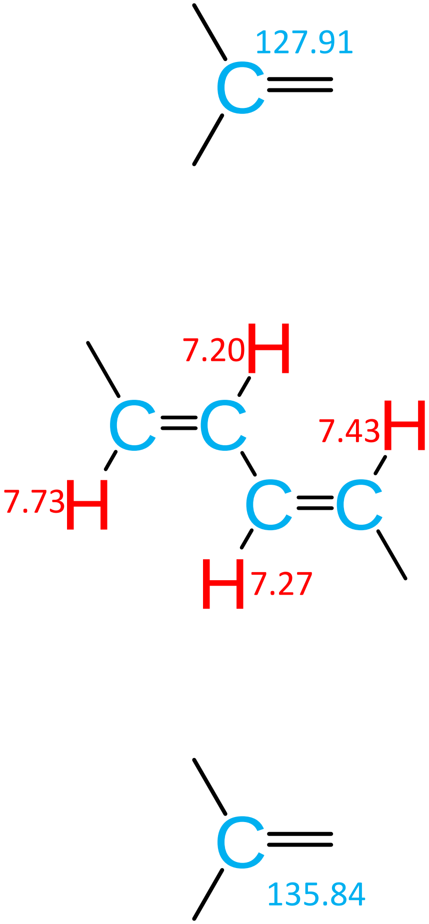


The two missing quaternary carbons may be extracted from the one dimensional carbon spectrum, since there are just two unassigned peaks remaining.

An the very last missing fragment is …

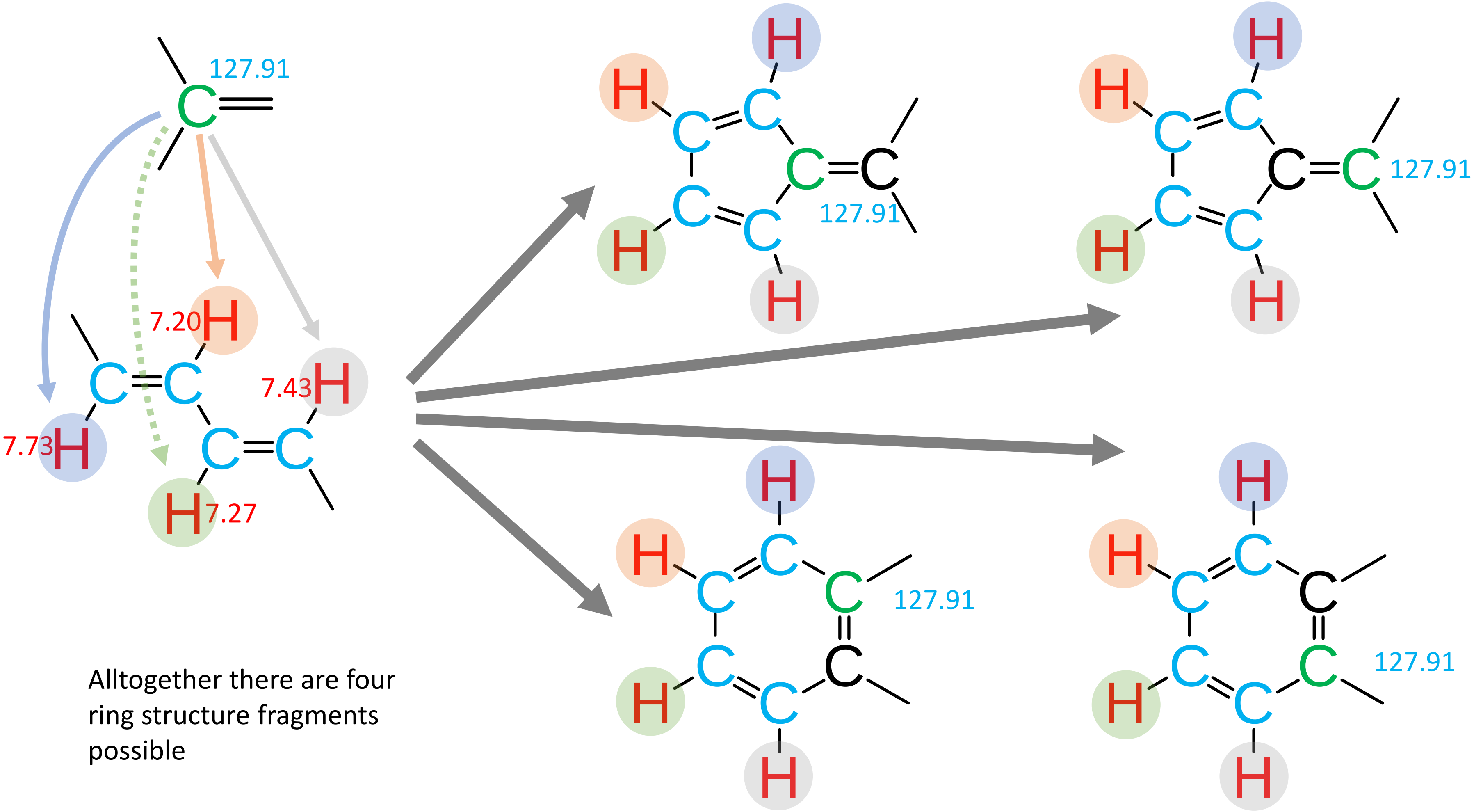


Now the “bricks” have to be put together



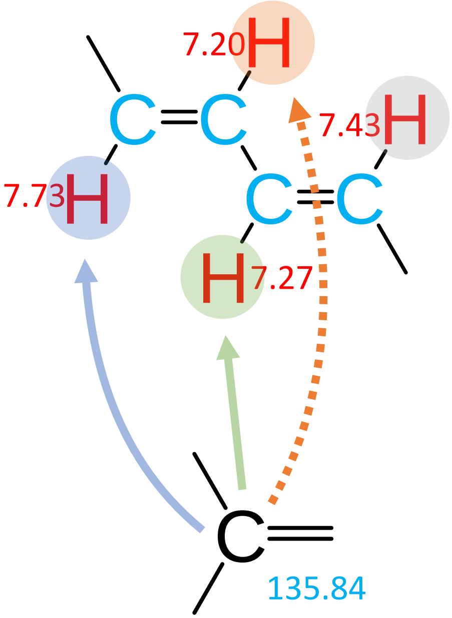
The two quaternary carbon atoms have to be the bridges between the three protoned fragments.

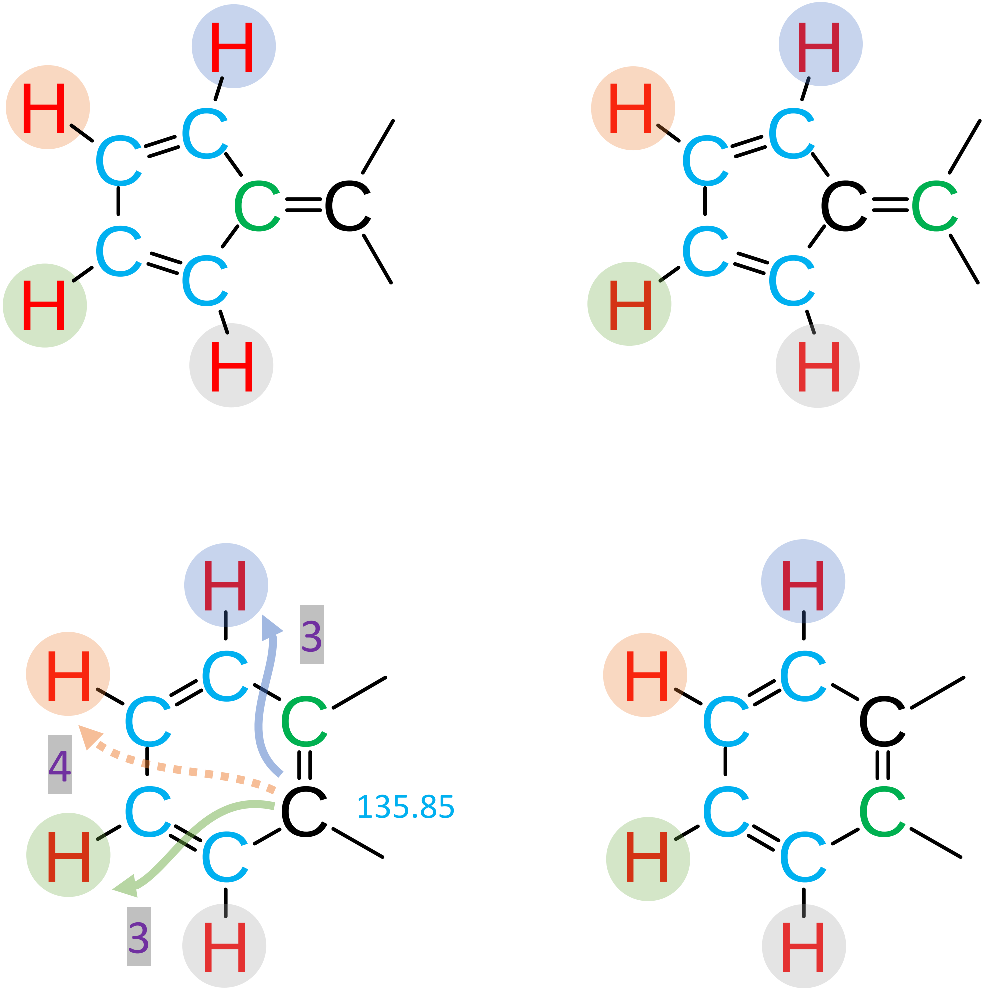
There have to be correlations betwen these carbons and the four membered fragment, visible in the HMBC.



Alltogether there are four ring structure fragments possible

Let us neglect the order of the quaternary carbons for the moment and check, whether the five membered ring might be a reasonable partial structure or not.





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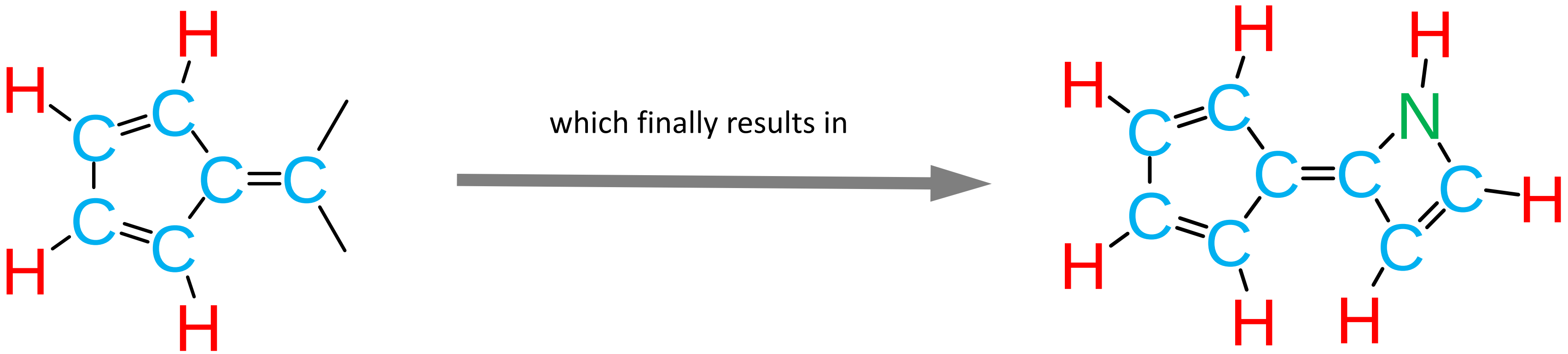
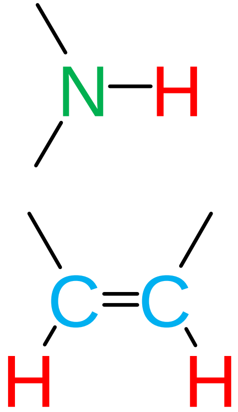
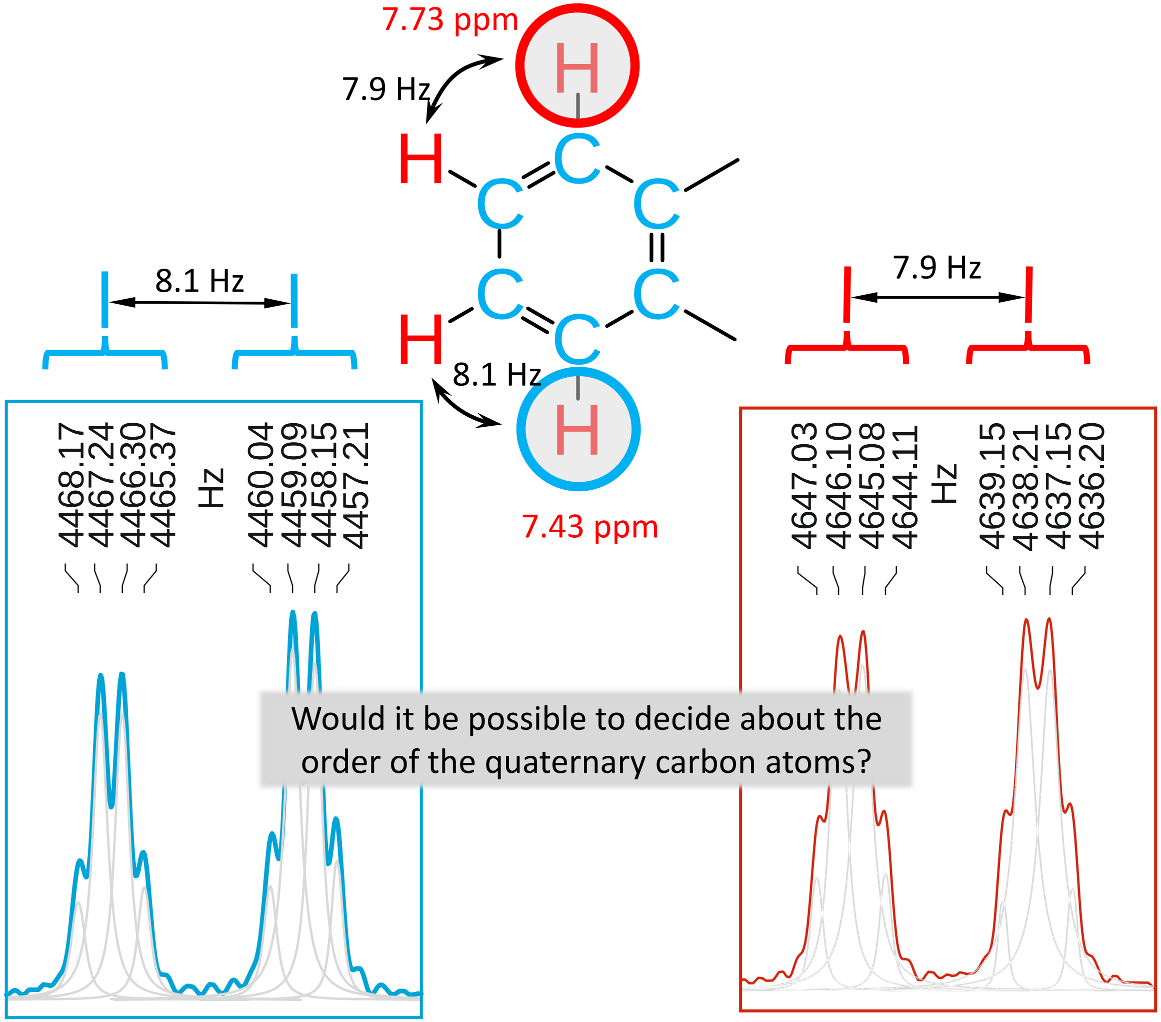


which finally results in

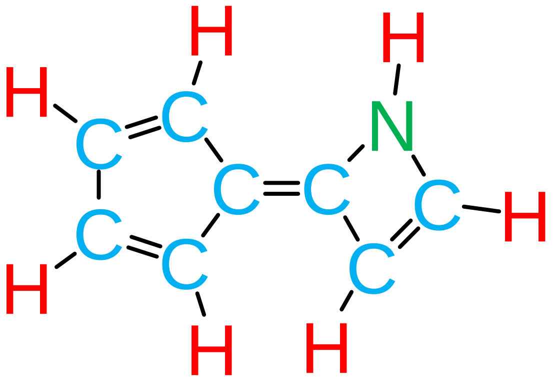
Somewhat earlier
we already found
these structural
fragments



Remark:
A total of eight permutations are possible regarding the allocation of the quaternary carbon atoms, the stereochemistry of the >NH-group relative to the five membered ring and the assignment of the -CH=CH-fragment. But the constitution is **always the same**.



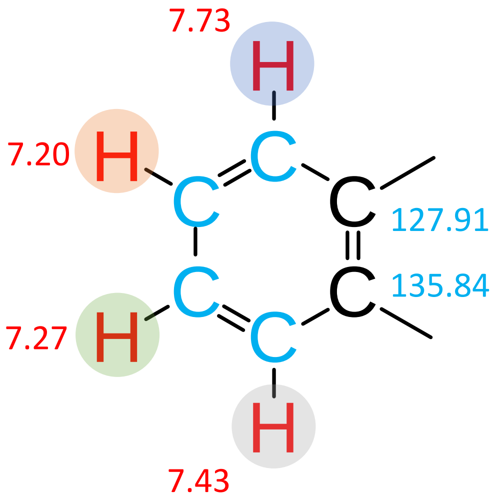
Would it be possible to decide about the order of the quaternary carbon atoms?

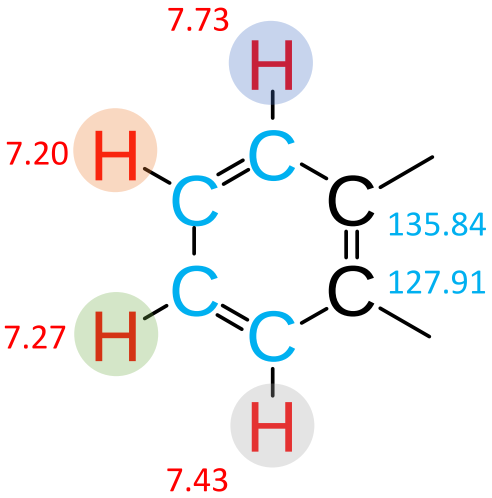


A strange unsaturated heterocyclic four membered ring system

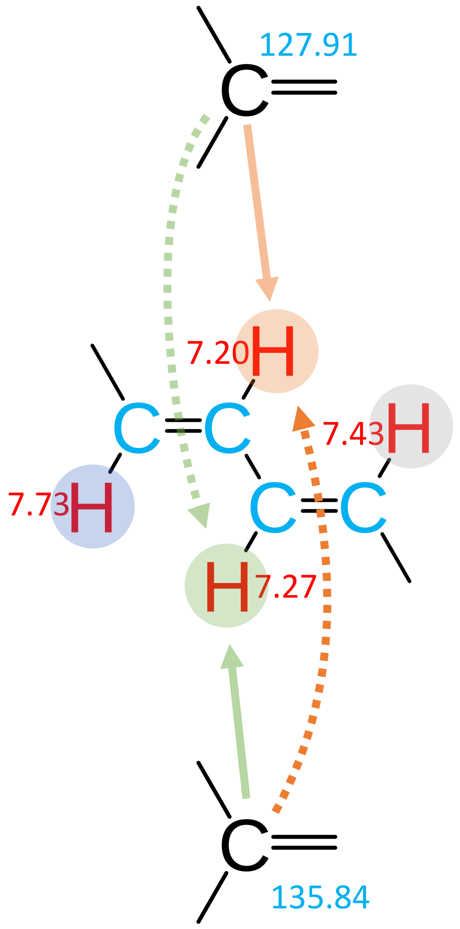
Of course ***strange*** is no scientific argument.

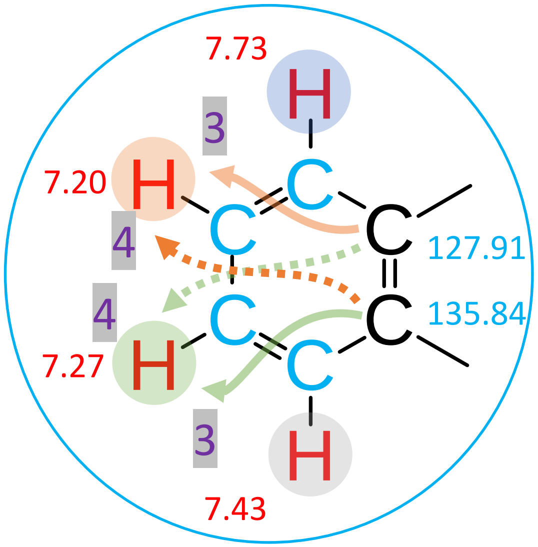
But if we take a six membered ring as a starting point, we find two coupling constants very characteristic for six membered aromatic ring systems.

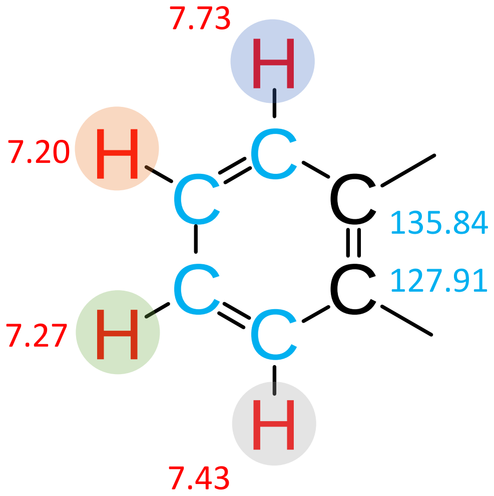




We have to distinguish between two possibilities.







Within the partial structure given above the **intense** cross peaks correlate with a coupling over **three** bonds.

The **weak** cross peaks correlate with a coupling over **four** bonds.

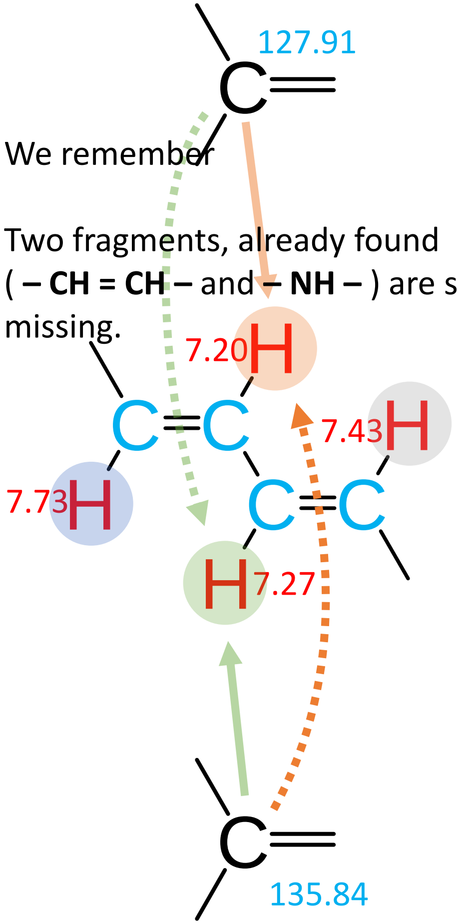
As a crude rule of thumb, the intensity of the cross peaks decreases with the number of bonds between the coupling nuclei.

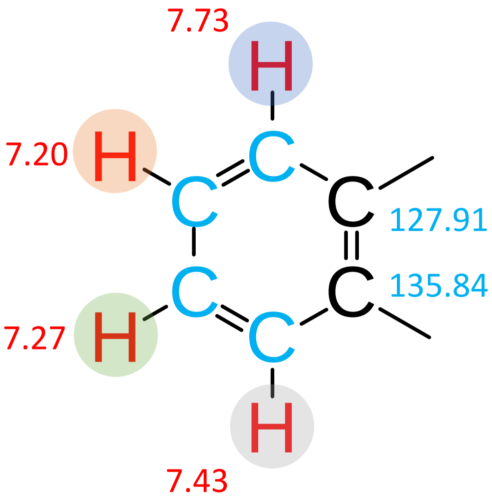
Solid lines mean intensive, dashed lines mean weak cross peaks.

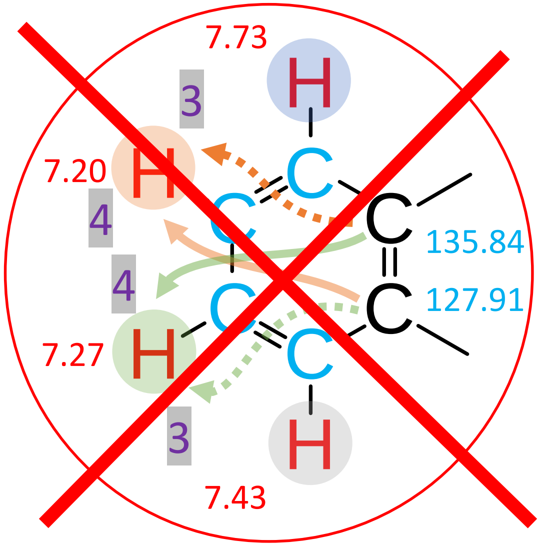
The partial structure seems to be reasonable.

We remember

Two fragments, already found (**– CH = CH** – and **– NH –**) are still missing.







Within the partial structure given below the **intense** cross peaks correlate with a coupling over **four** bonds.

The **weak** cross peaks correlate with a coupling over **three** bonds.

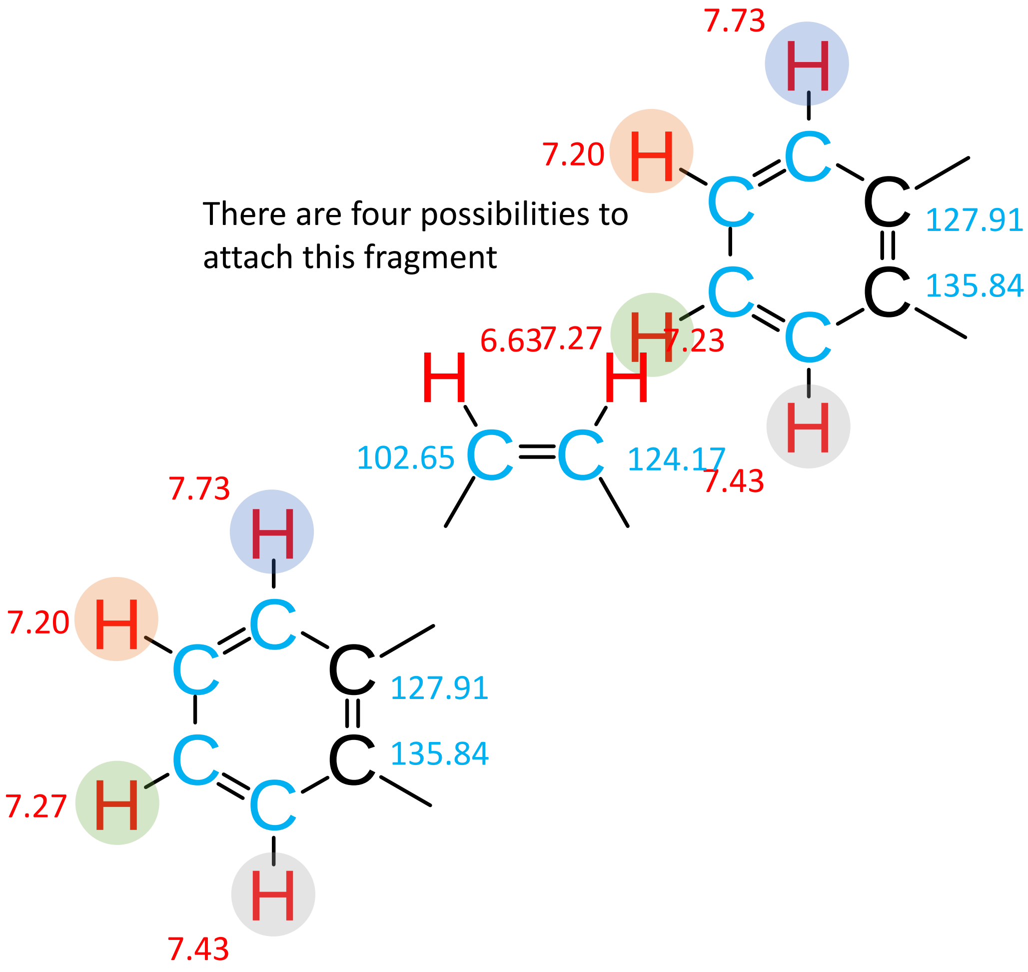
✘

As a crude rule of thumb, the intensity of the cross peaks decreases with the number of bonds between the coupling nuclei.

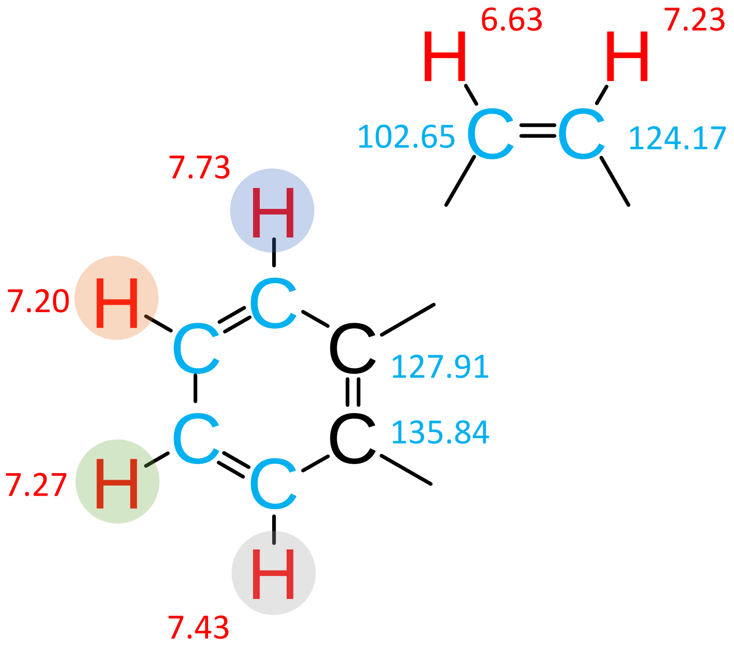
This is not completely impossible, but very unlikely.

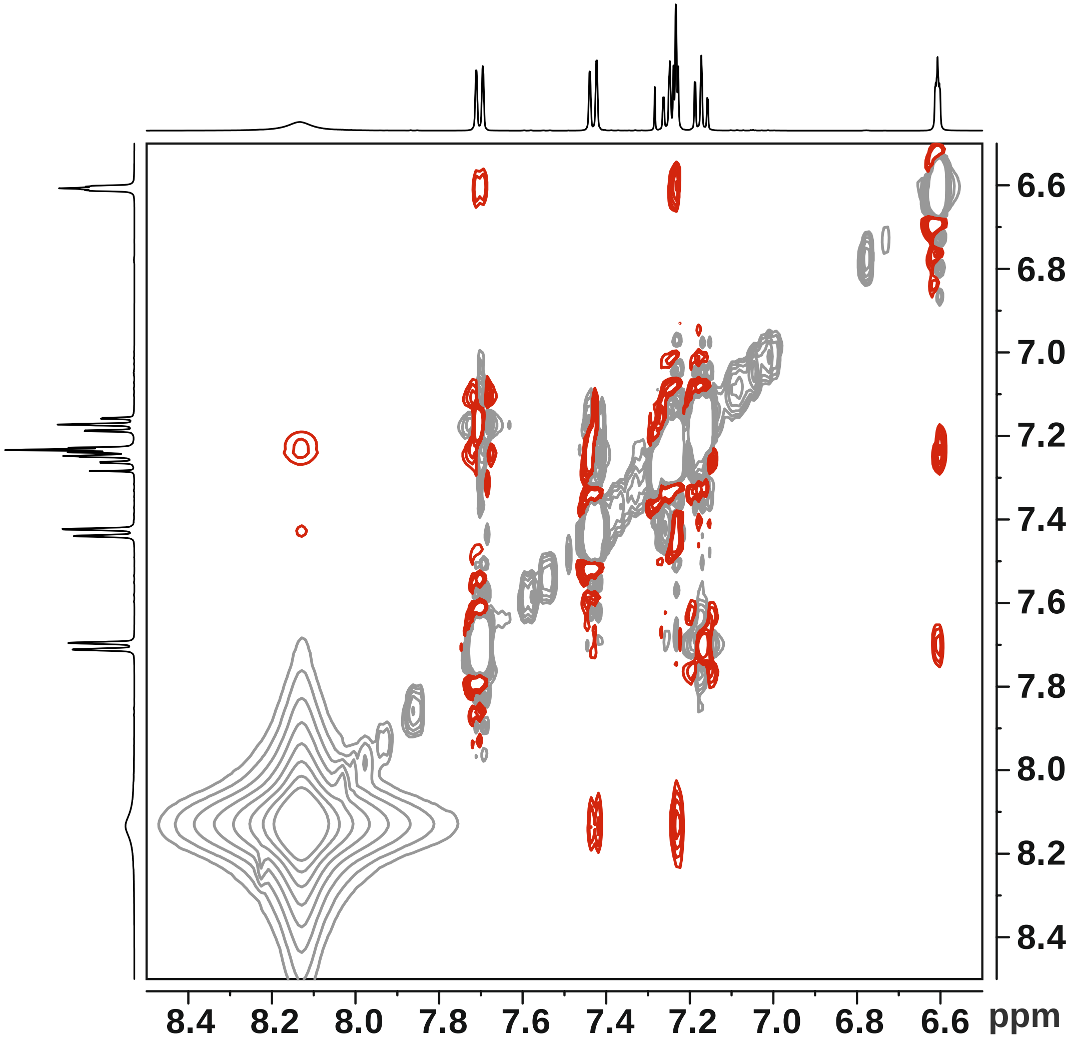
Unless you have good reason for doing otherwise, you should go with simplest (most probable) explanation.

There are four possibilities to attach this fragment



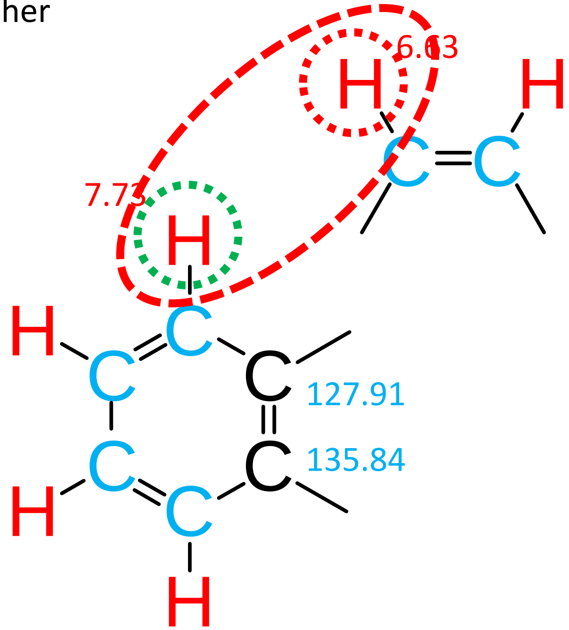
The NOESY is **one way** to see the connectivity.

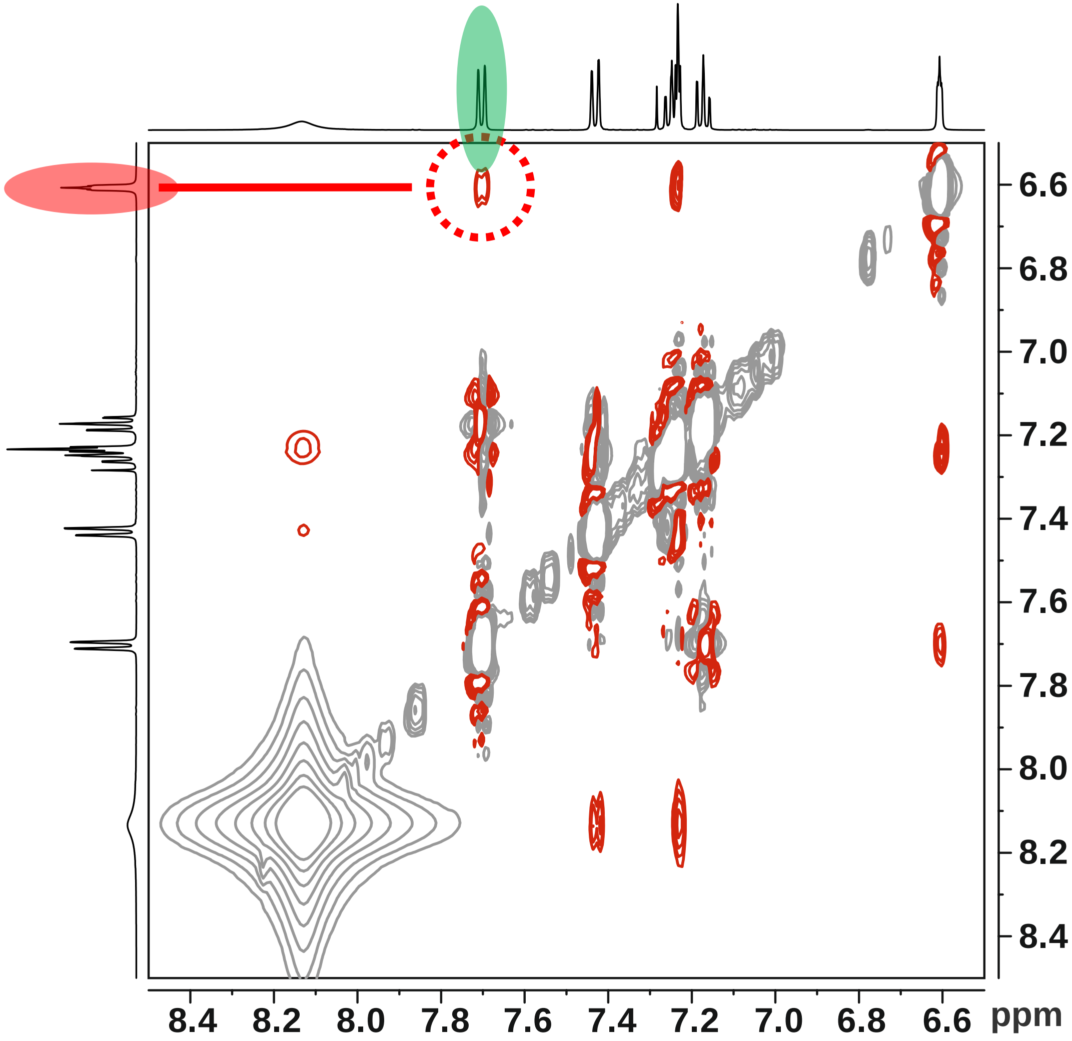




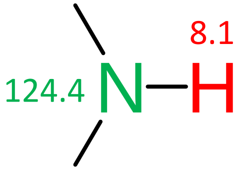
In the crowded NOESY just one clearly separated peak is very helpful.

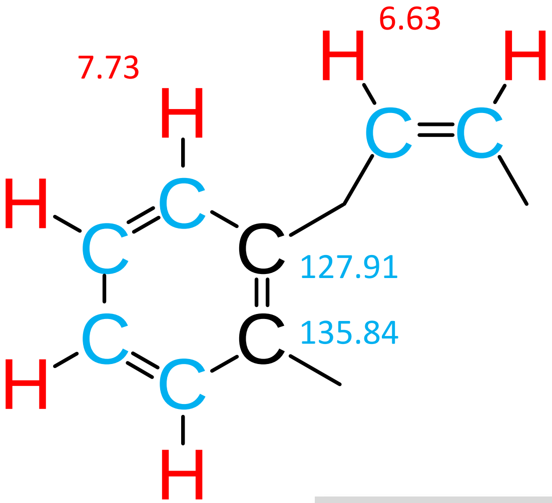
These protons have to be close in space to each other



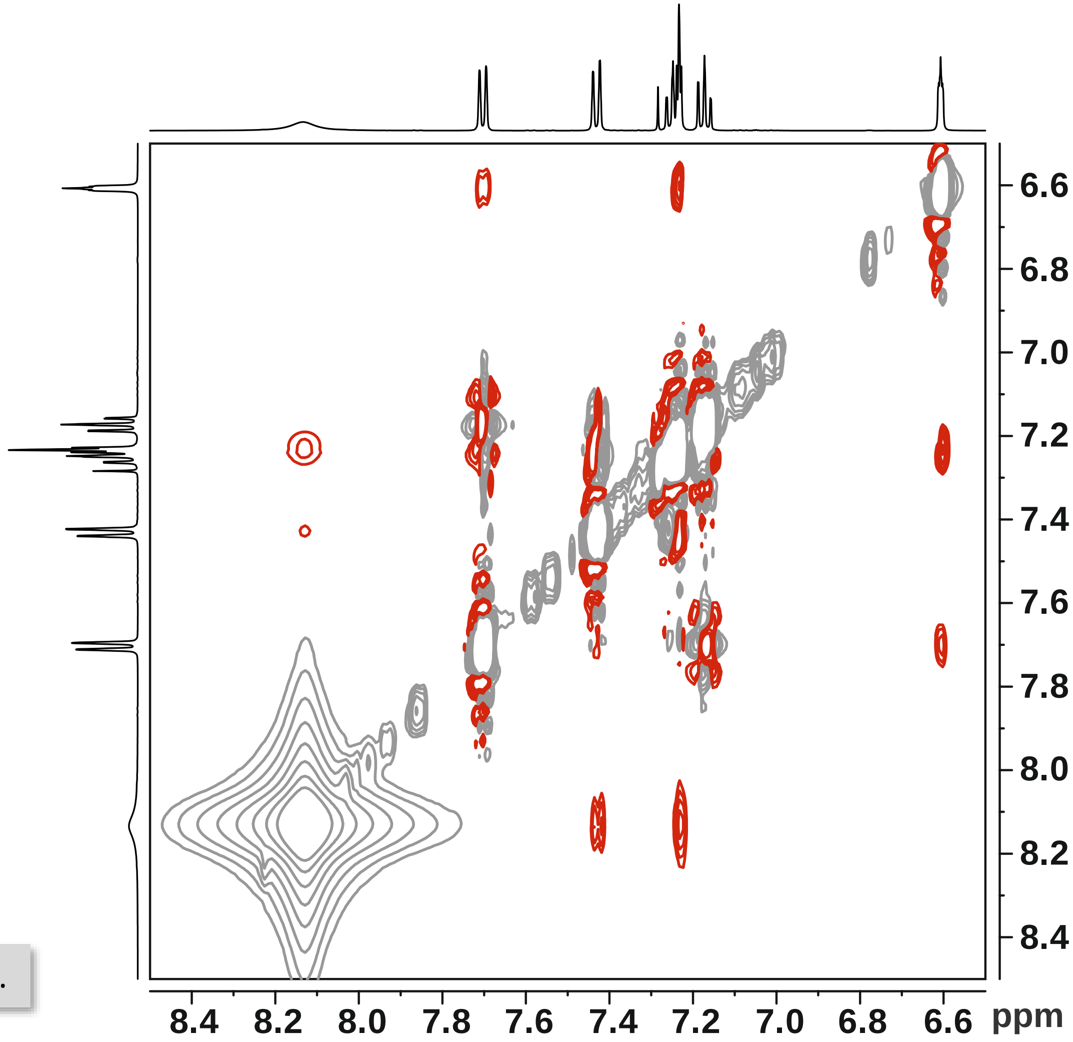


There is then effectively only one possibility for inserting the last missing building block

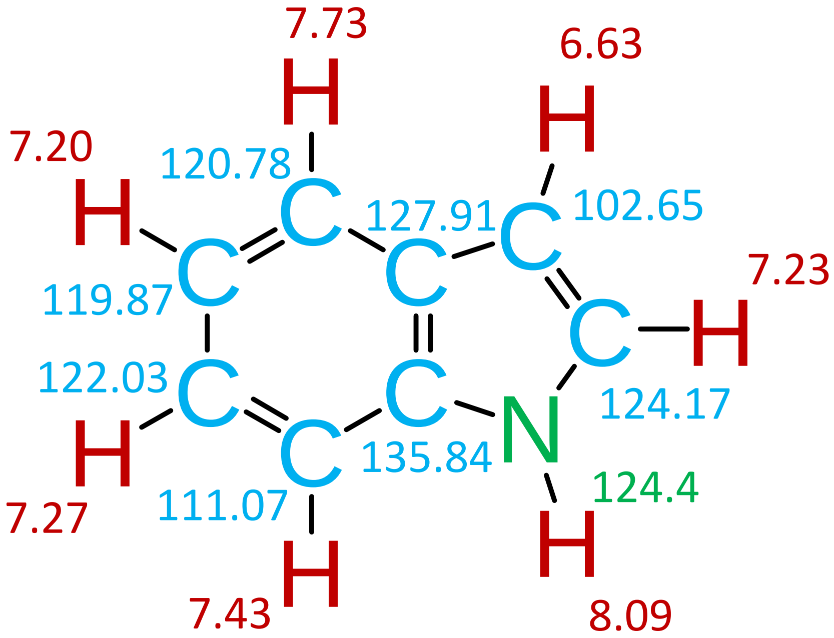




Finally this results in …

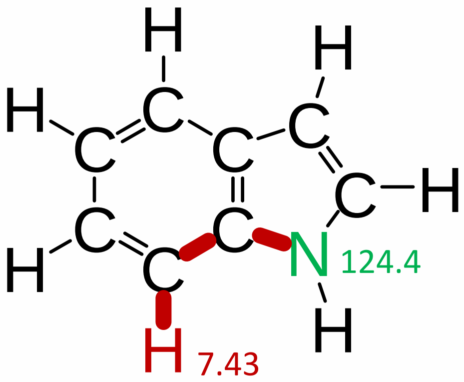


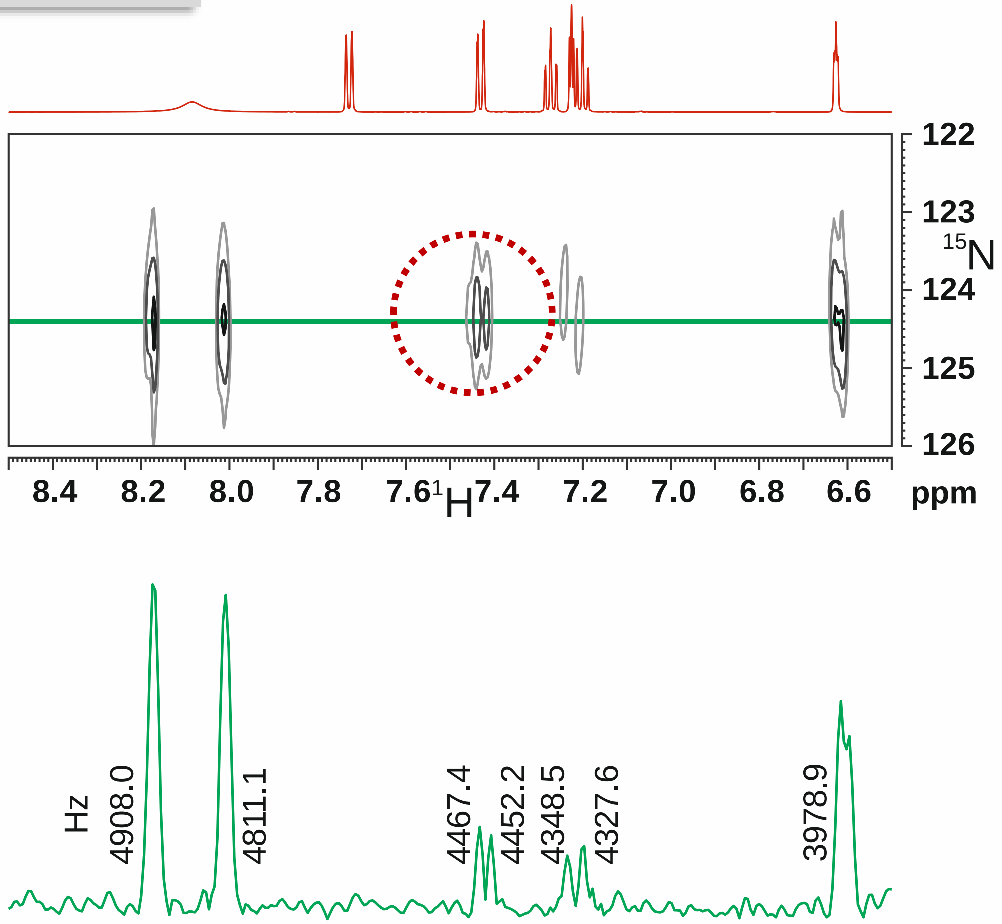
Indole


7.73
6.63
7.20
120.78
102.65
127.91
7.23
119.87
122.03
124.17
135.84
124.4
7.27
111.07
7.43
8.09
C
H
N

Let us check the structure with some information not yet used.

First some long-range ^{15}N-^{1}H correlations





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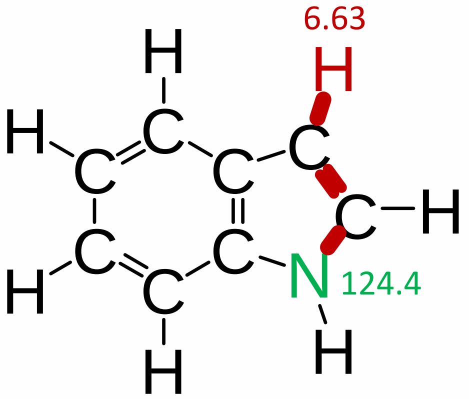

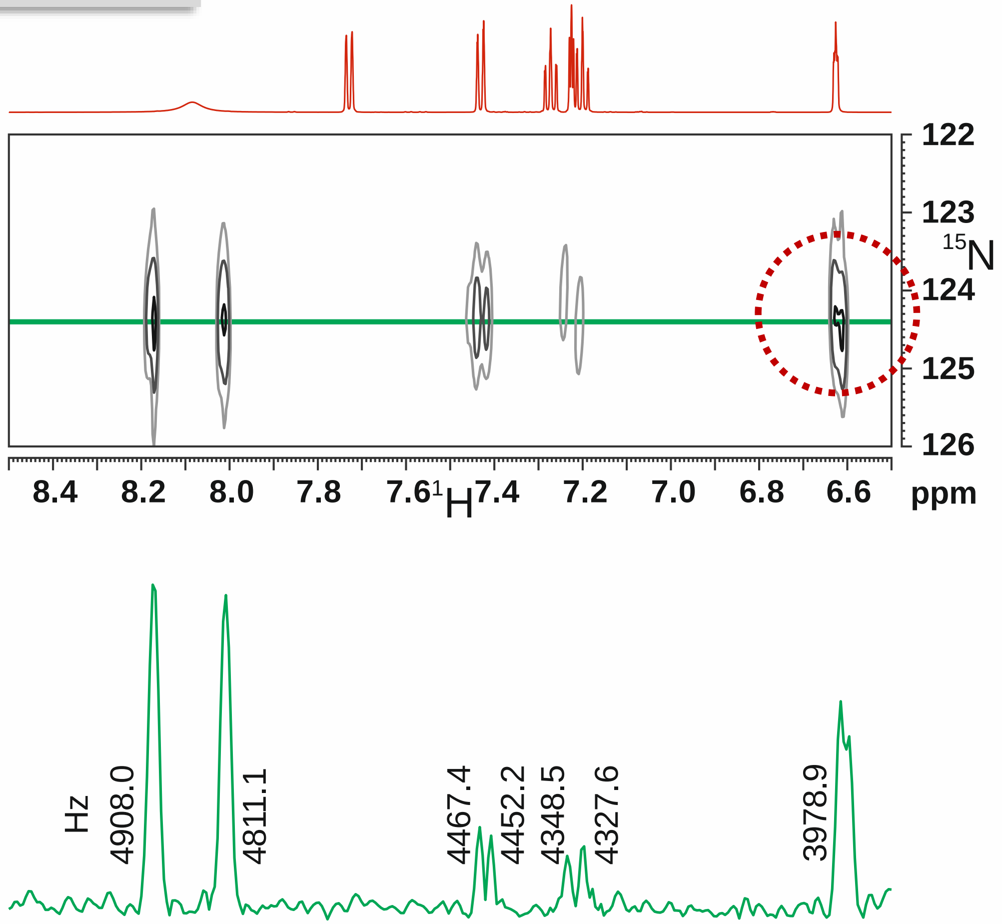

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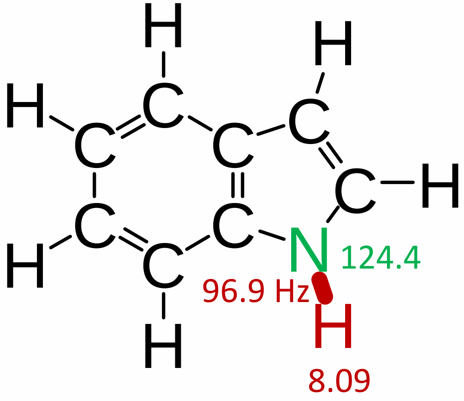
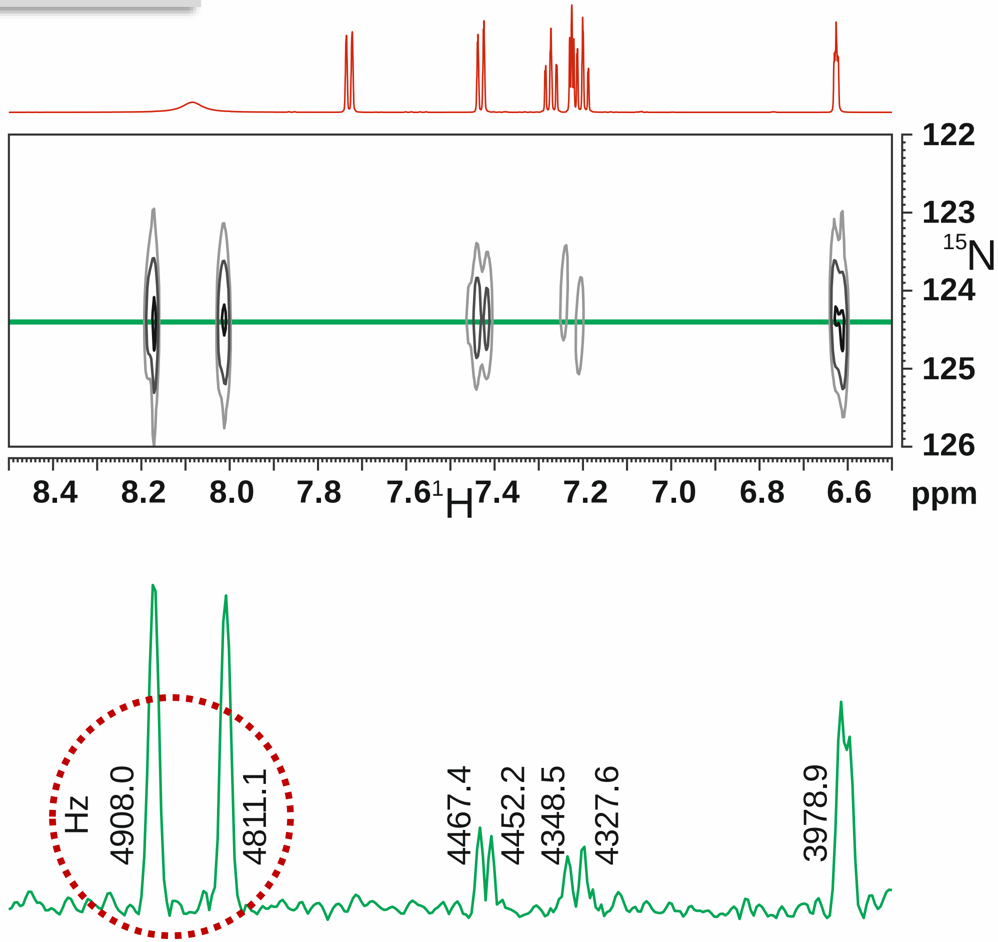
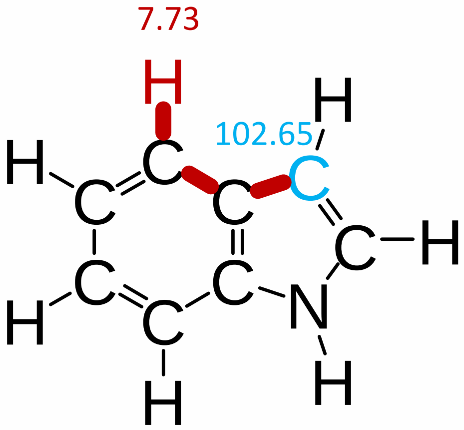
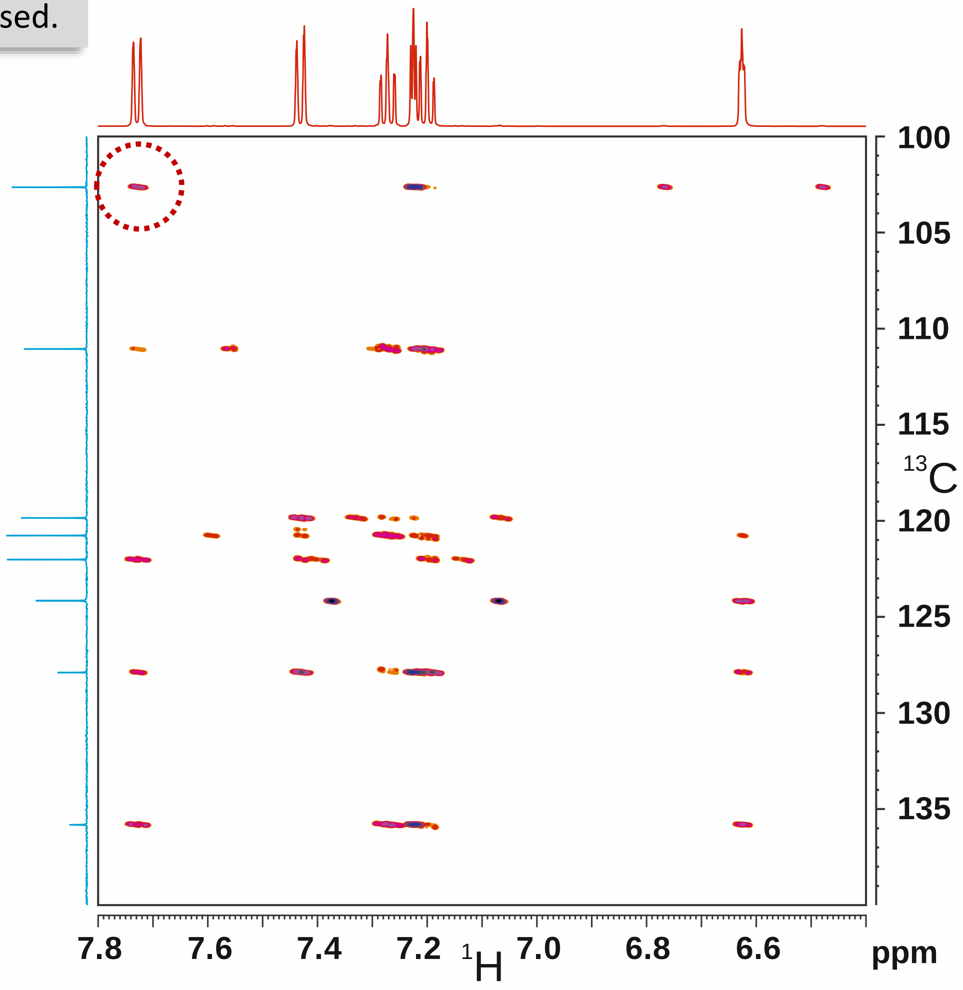




Let us check the structure with some information not yet used.

Now using long-range ^{13}C-^{1}H correlations





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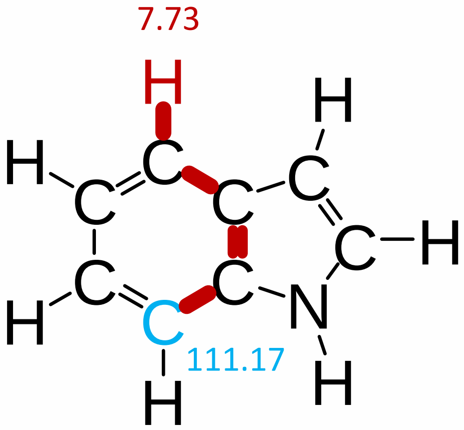
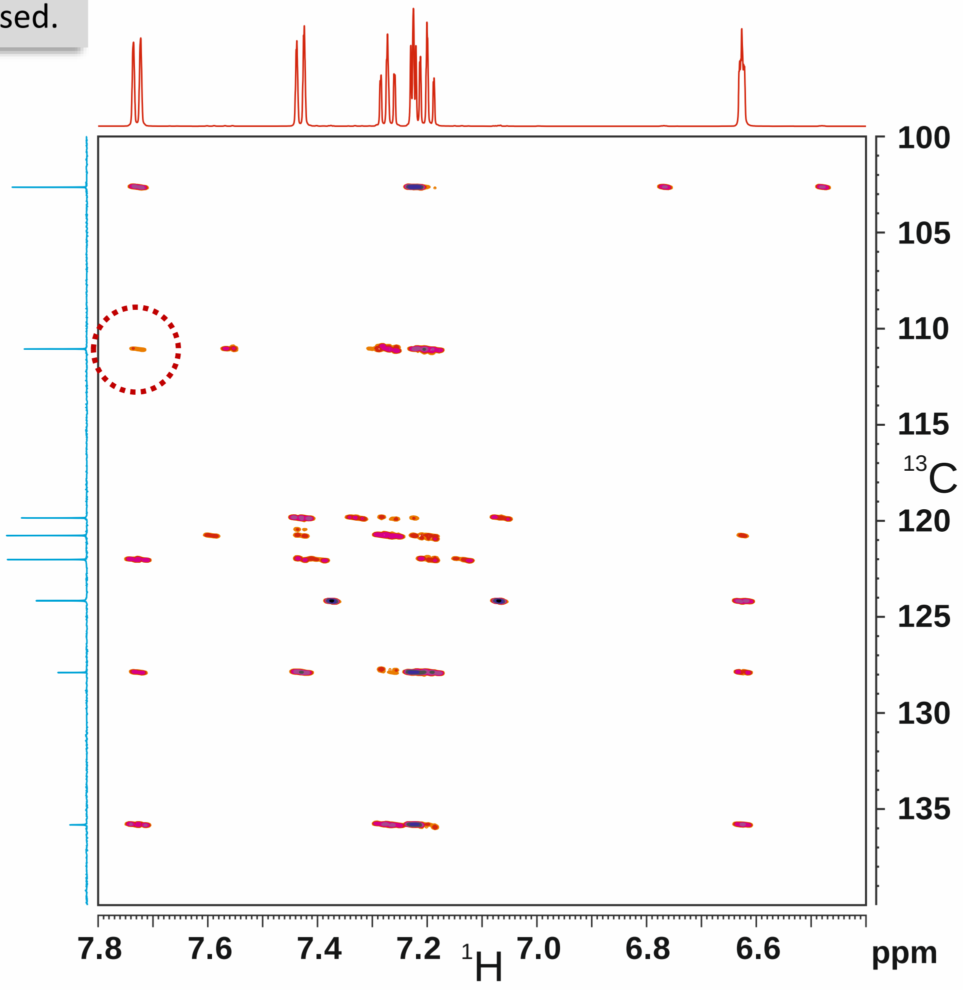
Now using long-range ^{13}C-^{1}H correlations





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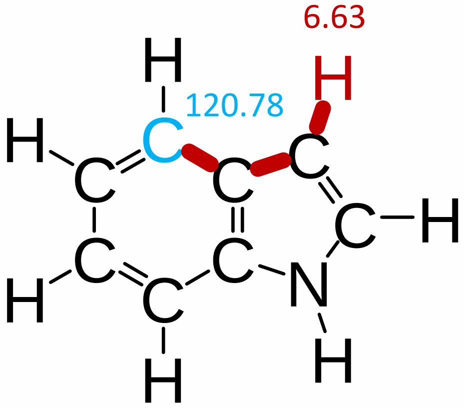
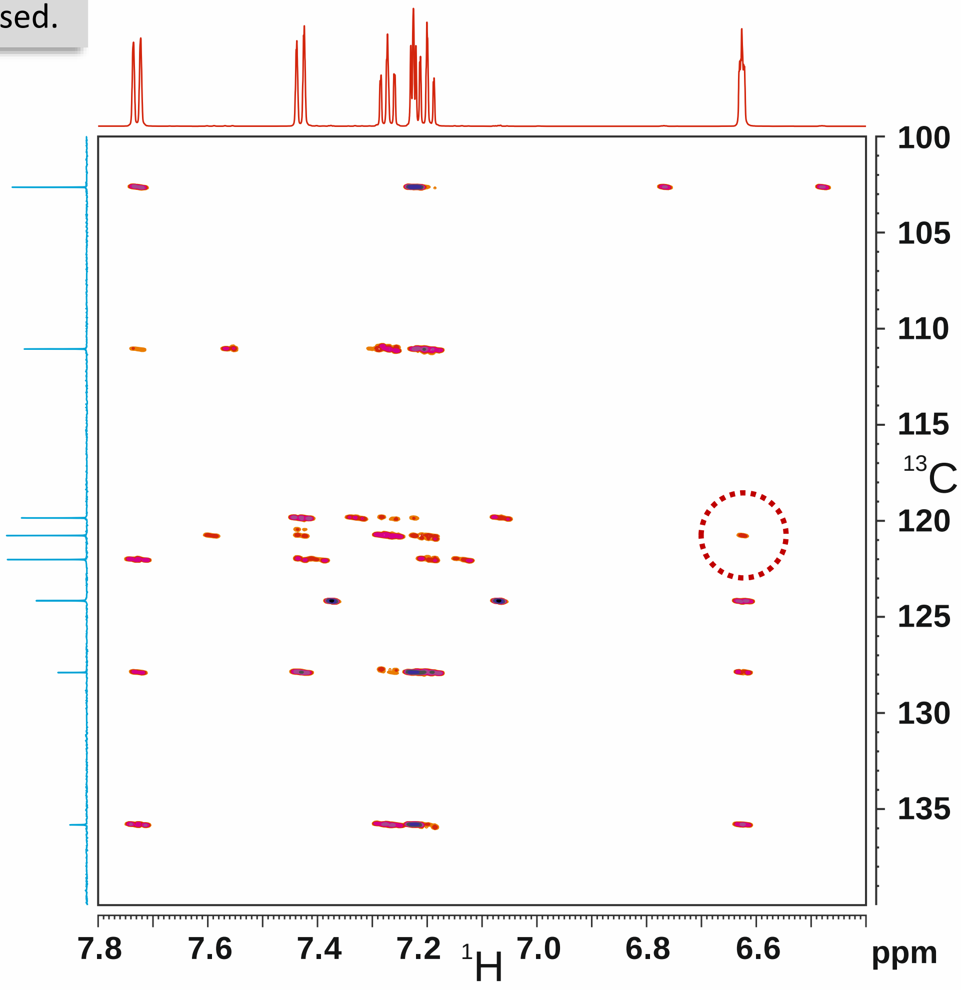
Now using long-range ^{13}C-^{1}H correlations





The analysis of the homonuclear coupling constants

A basic consideration about the multiplet structure

There are no equivalent protons in this molecule. As a result, every coupling pattern without any exception - completely independent of its apparent structure - can only be a doublet, a doublet of doublets, a doublet of doublets of doublets, ...

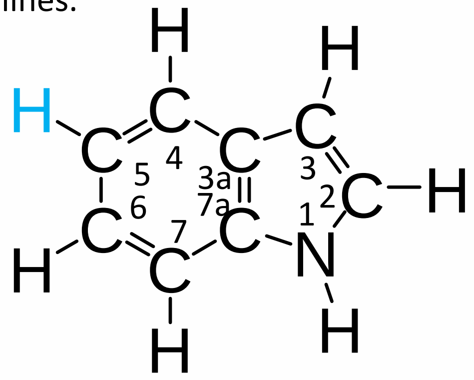
Each multiplet consists of 2, 4, 8, or generally 2^n lines of equal intensity. Deviations from this general rule can only be explained by overlapping lines.

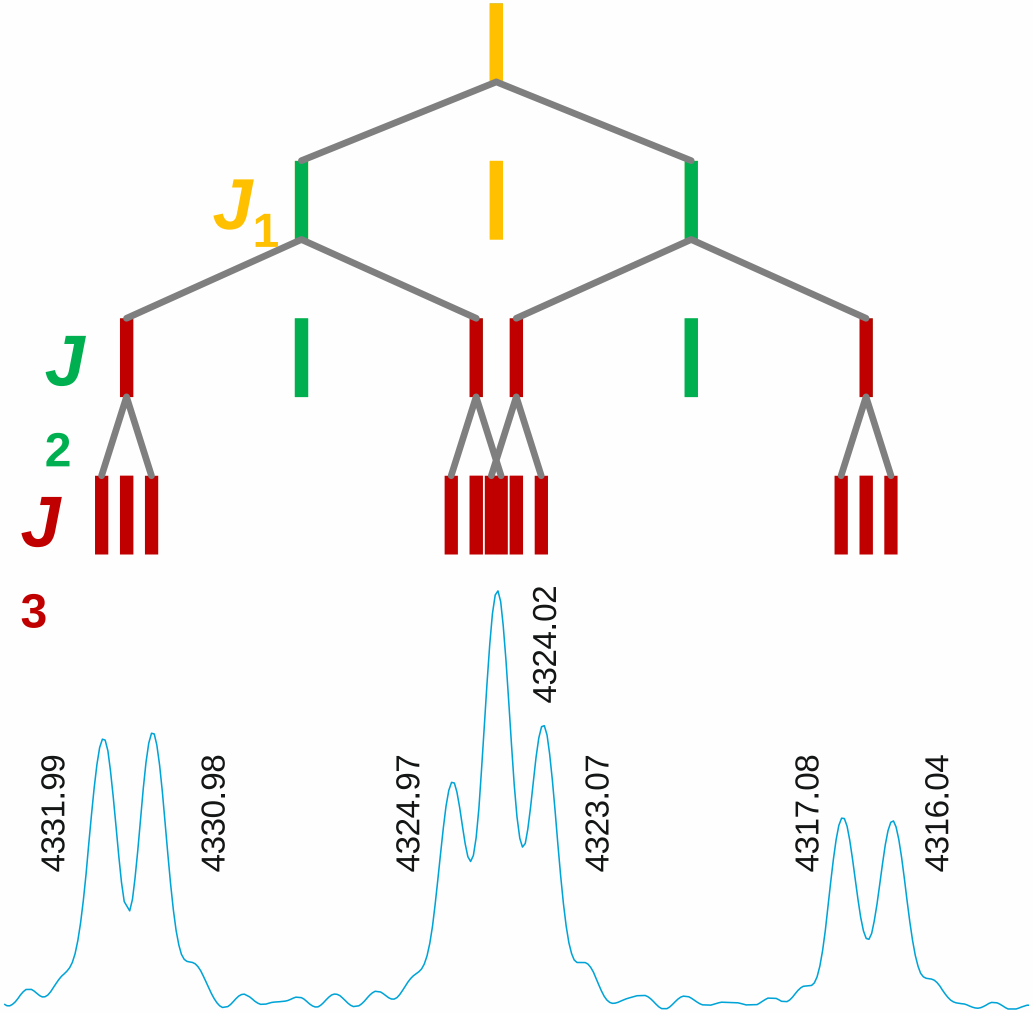
Let us start with H-5

One neighbouring proton (which one is unknown so far) causes a splitting into a doublet

Another different neighbour splits each individual line into a doublet

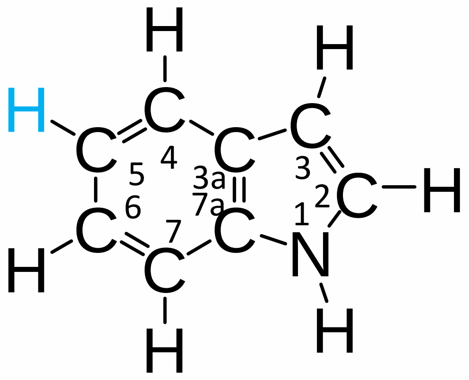
And the third, different, neighbouring hydrogen atom does the same with each of the four lines.

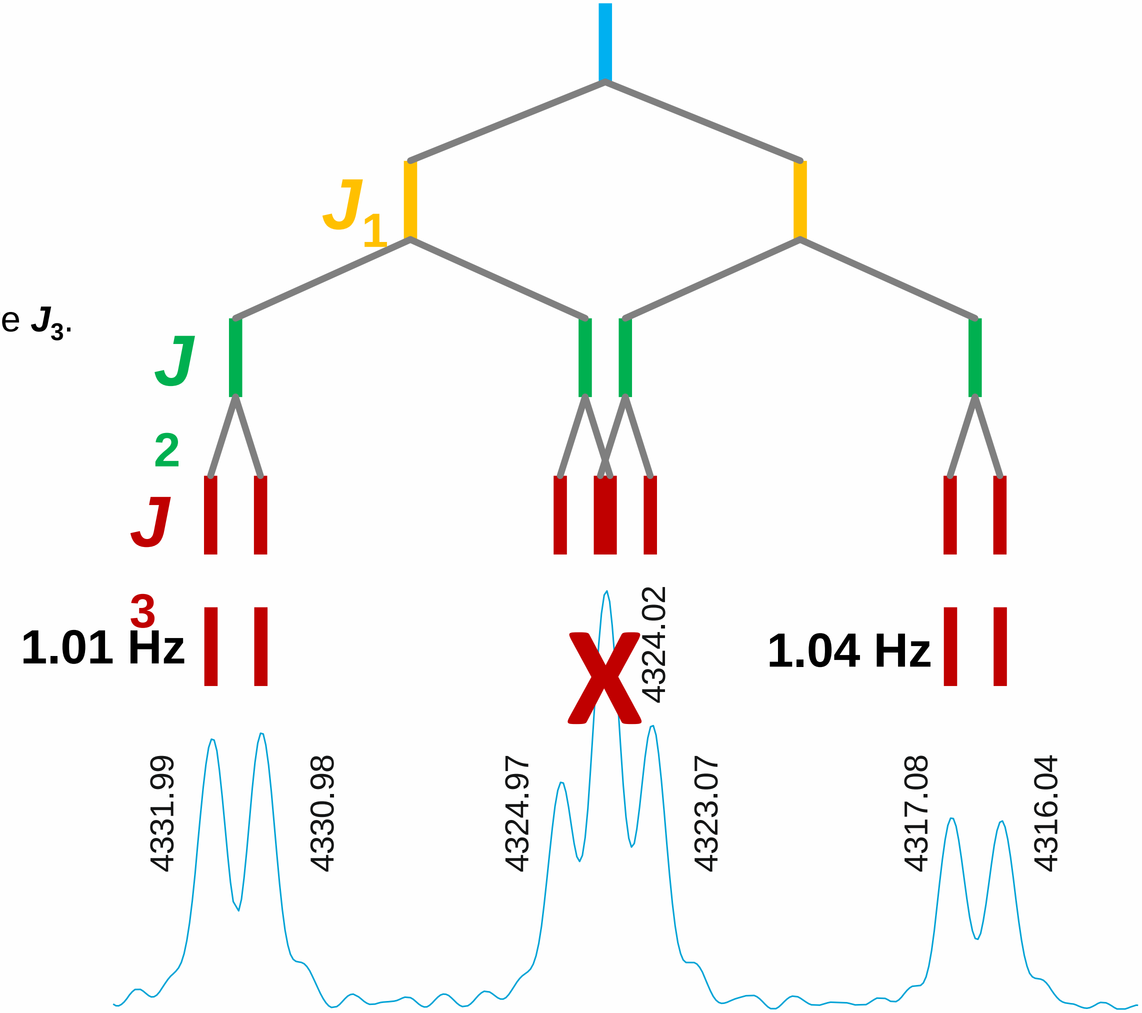




To measure J_3 we cannot use the area of the two overlapping peaks.

But we have two other choices to measure J_3.



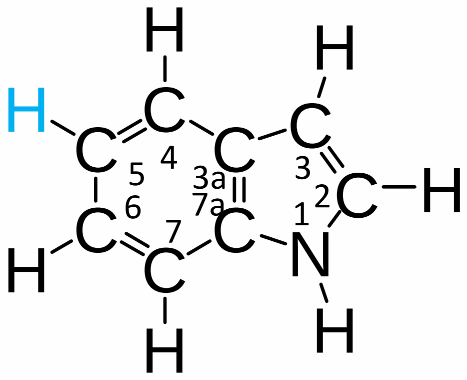


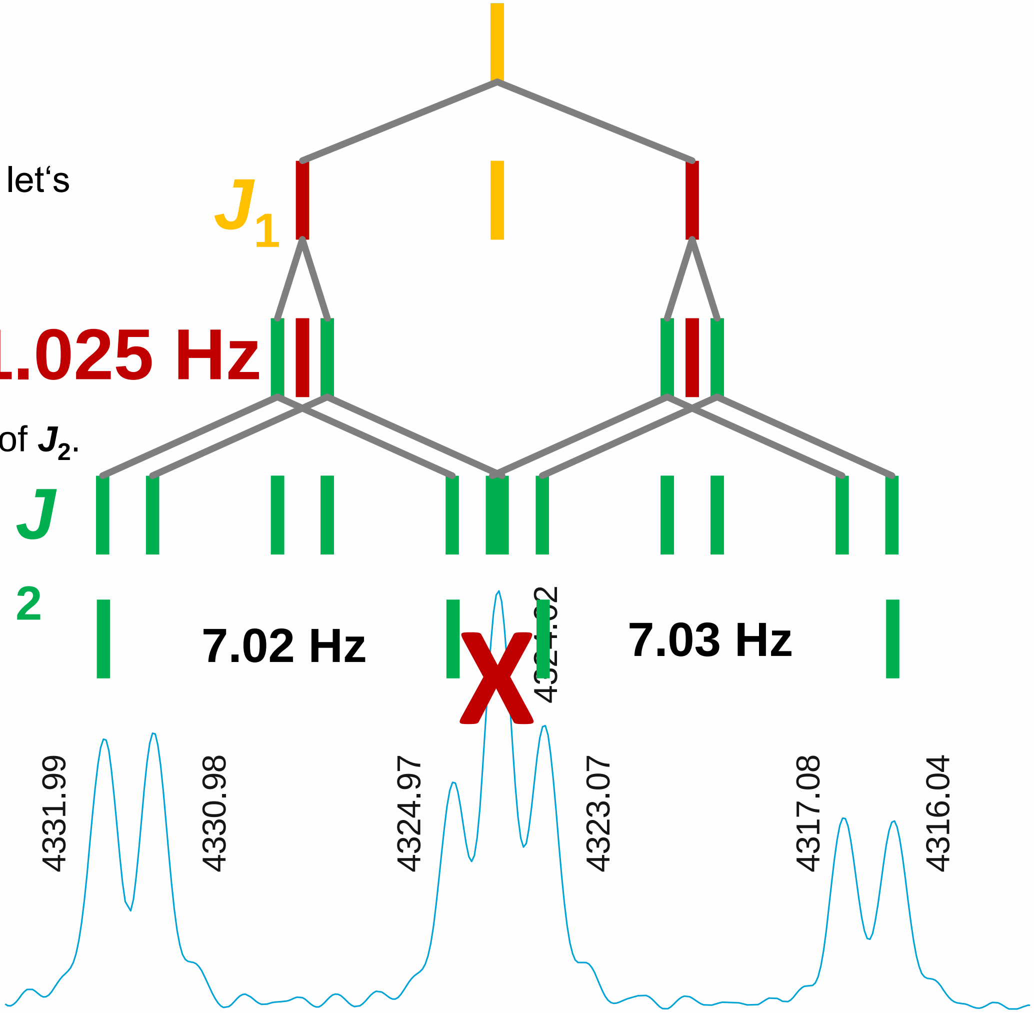
How to get J_2?

The couplings are 100% commutative, so simply let's change the order.

J_3 = 1.025 Hz

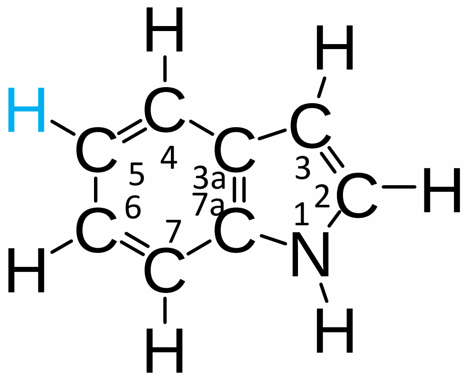
There are two easy possibilities to get the value of J_2.

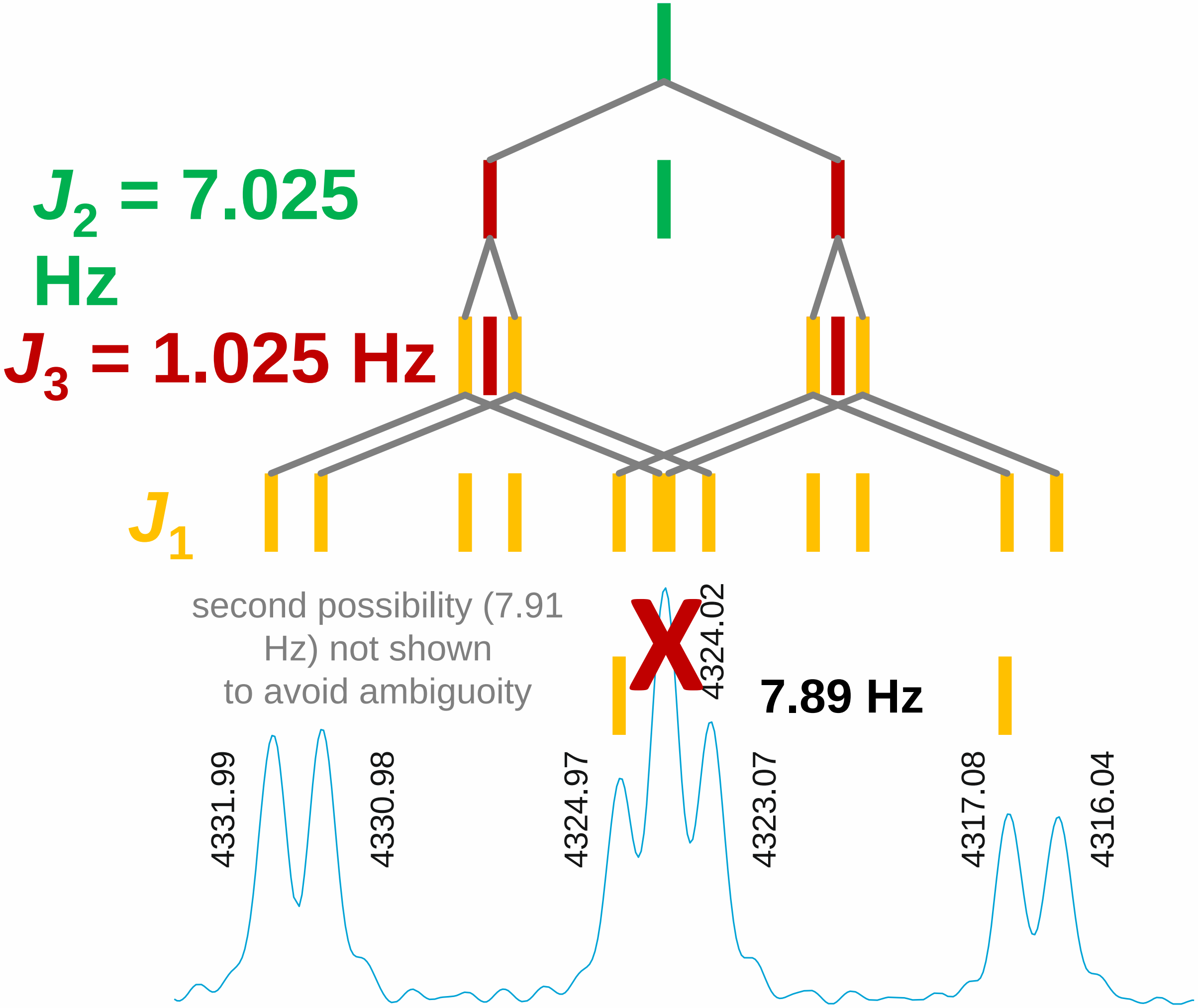




And J_1?

Let us shuffle a last time.





J_2 = 7.025 Hz

J_3 = 1.025 Hz

J_1

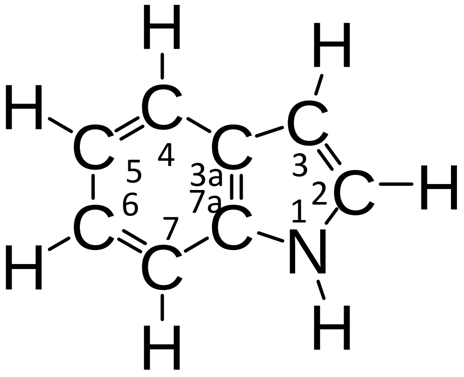
second possibility (7.91 Hz) not shown to avoid ambiguoity

7.89 Hz

At the moment the three coupling partners are unknown. For the moment we note the three coupling constants next to the corresponding proton.

By the way: the sum of the three coupling constants should be equally to the frequency difference between the two outermost lines of the multiplet.

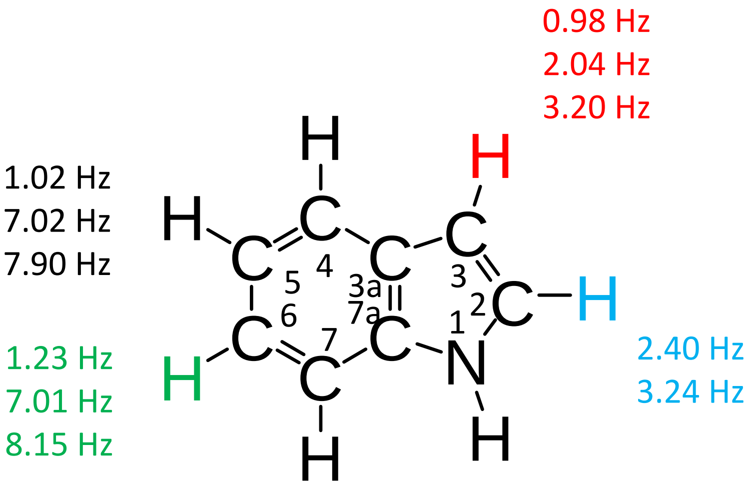
1.02 Hz
7.02 Hz
7.90 Hz

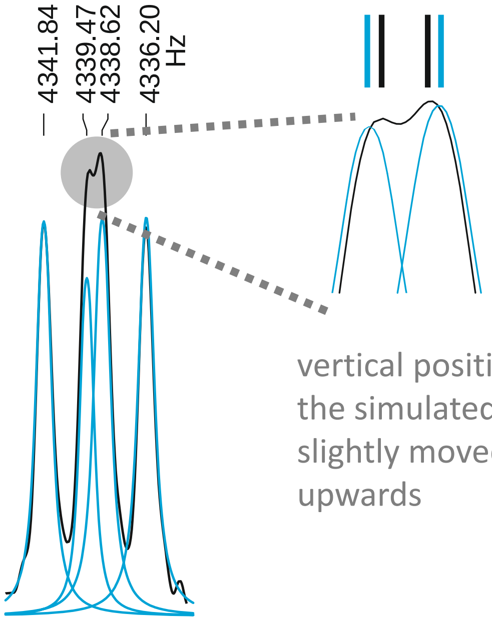


To determine the coupling constants it is not necessary to construct the coupling tree. Here, the representation served as a tool to visualize the selection of the correct peaks from the multiplet.

The multiplets of H-3 and H-6 resemble the multiplet of H-5 almost perfectly. Three coupling constants can be determined from each multiplet.

In the multiplet of H-2 two lines heavily overlap. A simple search of the peak maxima leads to wrong values, but fitting the whole multiplet using a sum of Lorentzian functions provides useful values. Actual software to process NMR spectra is able to do this job.





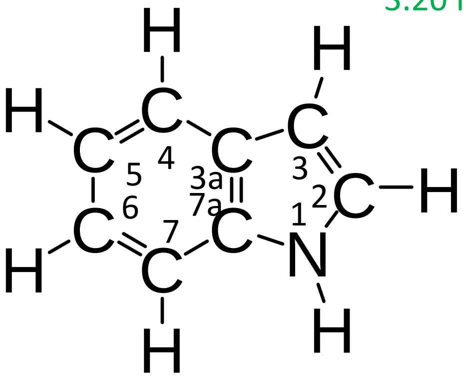
vertical position of the simulated lines slightly moved upwards

real

apparent

peak maximum

Two vicinal couplings (7.01/7.02 Hz and 3.20/3.24 Hz) are visible immediately due to identical coupling constants within two different multiplets.



0.98 Hz
2.04 Hz
3.20 Hz

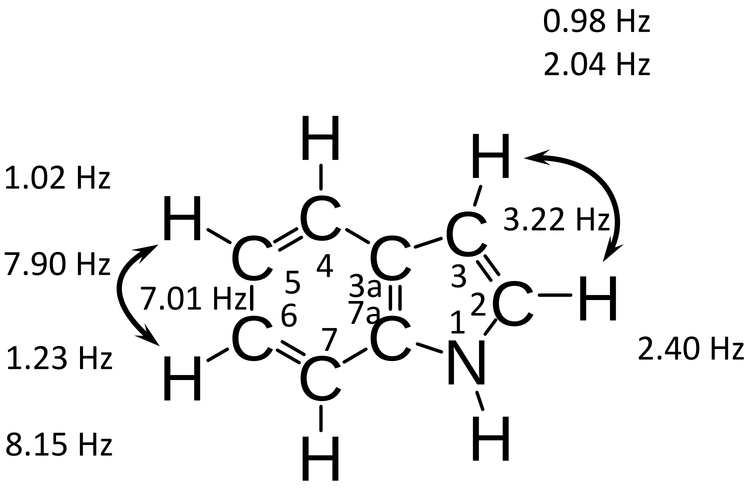
1.02 Hz
7.02 Hz
7.90 Hz

1.23 Hz
7.01 Hz
8.15 Hz

2.40 Hz
3.24 Hz

Two vicinal couplings (7.01/7.02 Hz and 3.20/3.24 Hz) are visible immediately due to identical coupling constants within two different multiplets.

7.90 Hz and 8.15 Hz are typical vicinal coupling constants between protons in six membered aromatic rings. In both cases there is only one vicinal coupling partner available.



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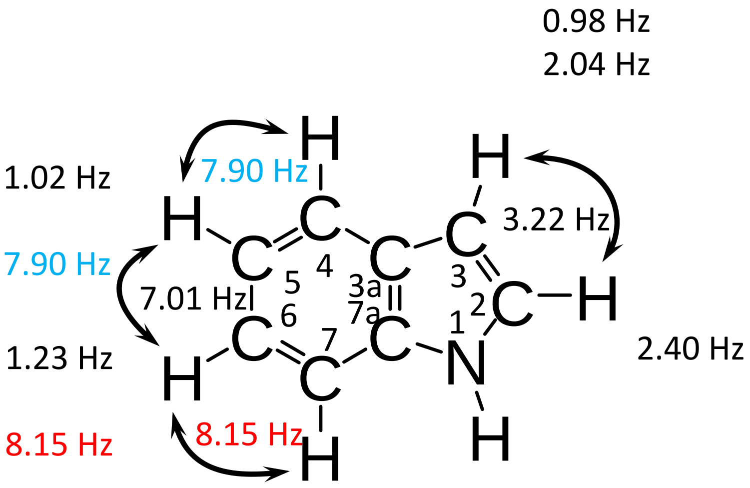

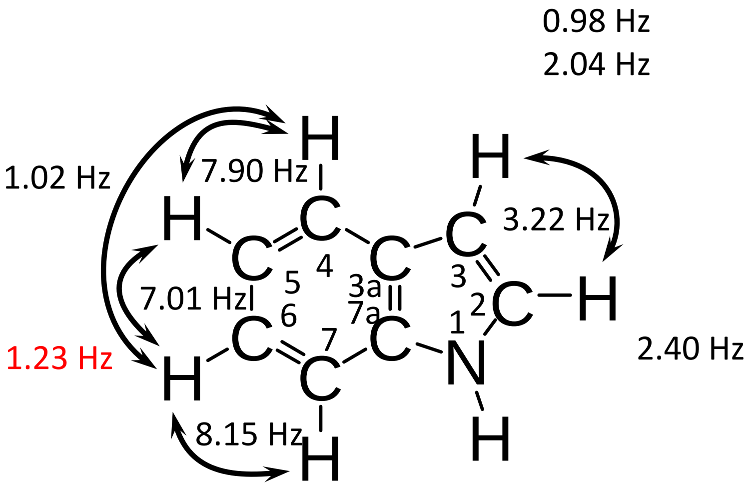

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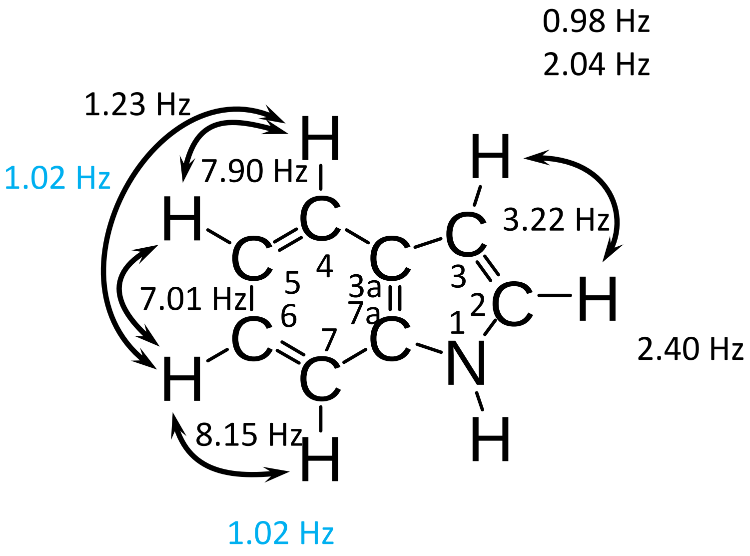

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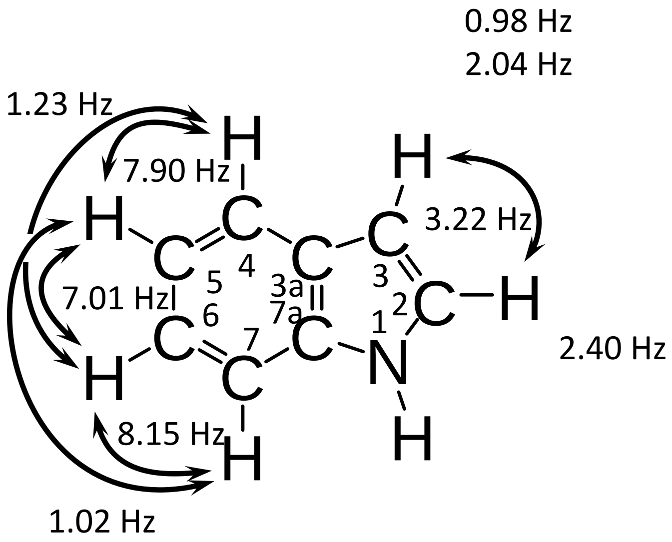

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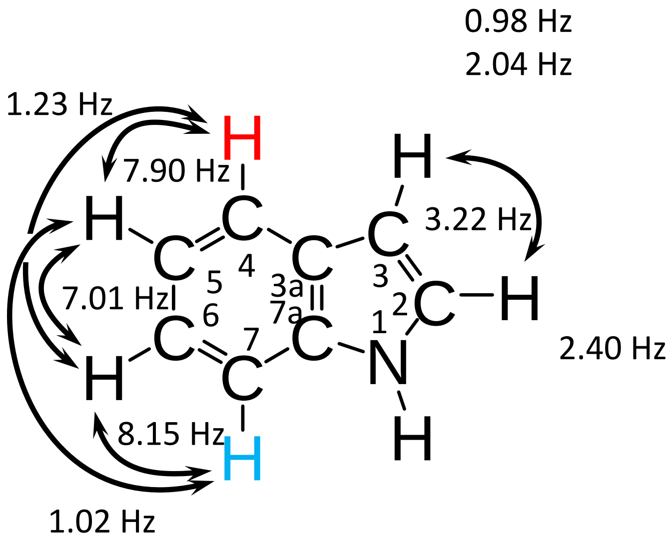
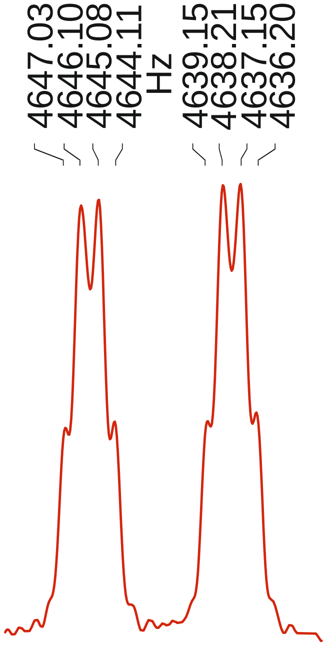
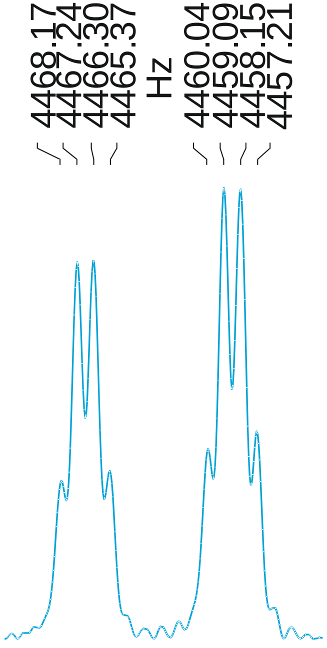
H-4 and H-6





$J_{\text{Doublet}} = 7.91\text{ Hz}$

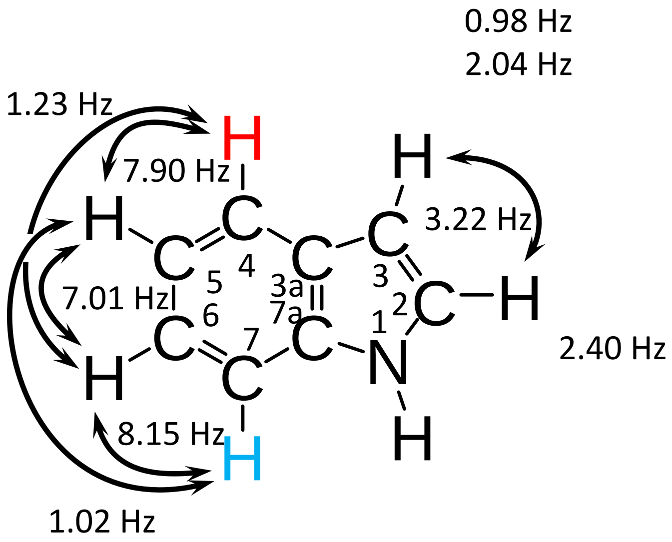


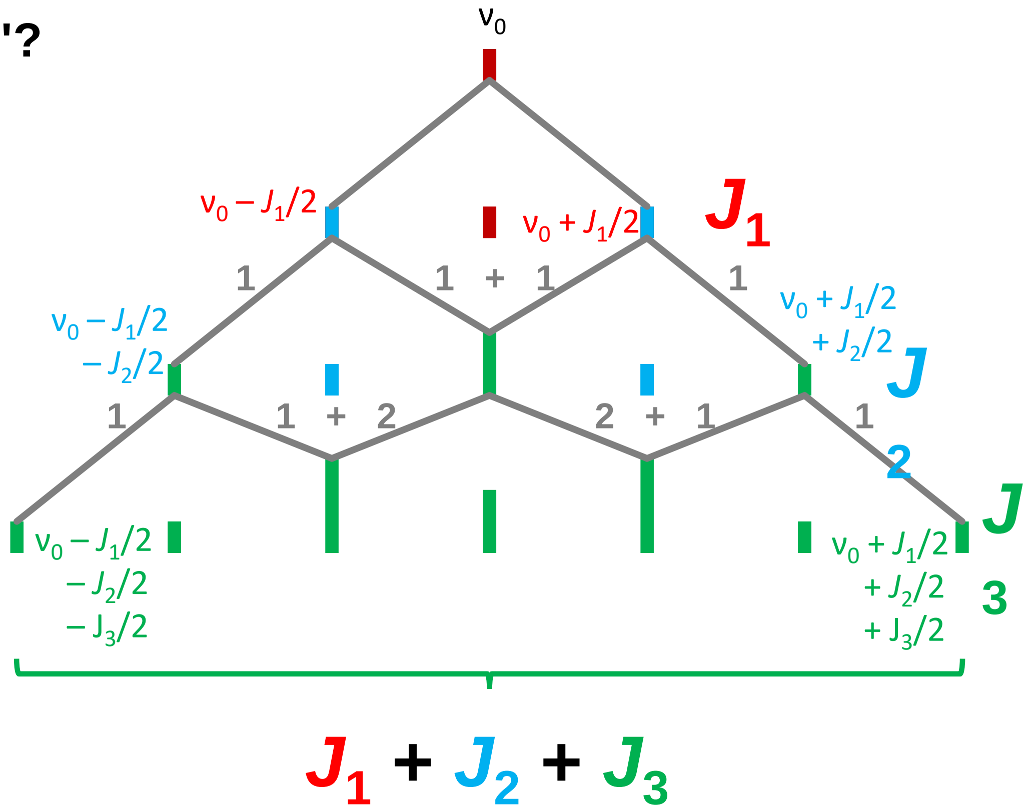
$J_{\text{Doublet}} = 8.15\text{ Hz}$

The multiplets of both H-4 and H-6 seem to be doublets of quartets.
That is **not possible**!
All multiplets have to be cascades of doublets somehow.
The coupling constants of the clear visible doublet structure in both multiplets is easy to calculate, they confirm the values already calculated from the multiplets of H-5 and H-6.

But, how to explain the "quartets"?

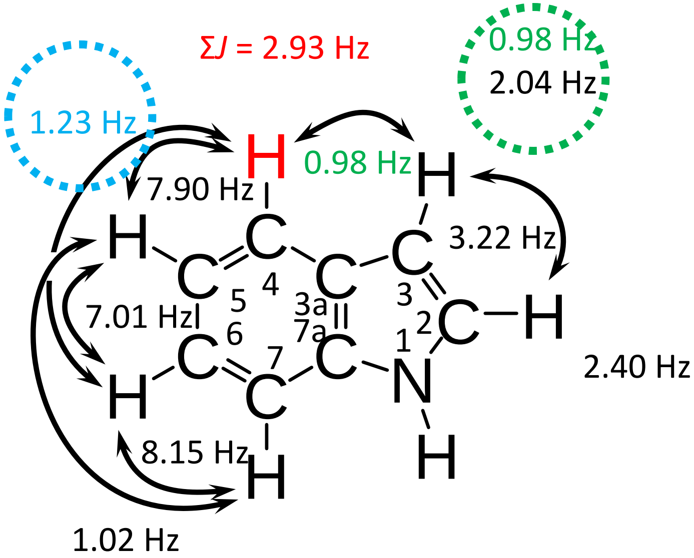
Think about three consecutive doublet splittings with the coupling constants J_1, J_2 and J_3.
The differences between the values of J_1, J_2 and J_3 should be parts of the natural linewidth only.

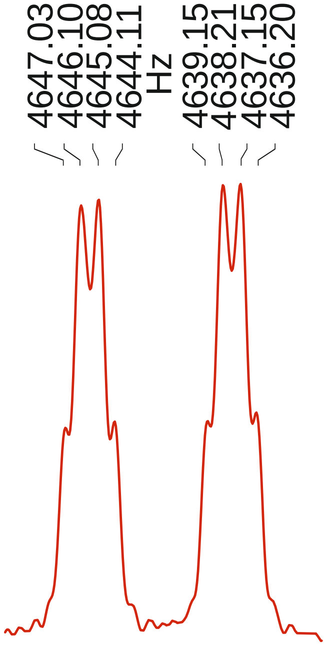




Because of strong overlap within the limit of the natural linewidth it is impossible to extract the values of J_1, J_2 and J_3 directly.
But at least the sum of all three coupling constants can be measured.

Let us return to H-4





H-4

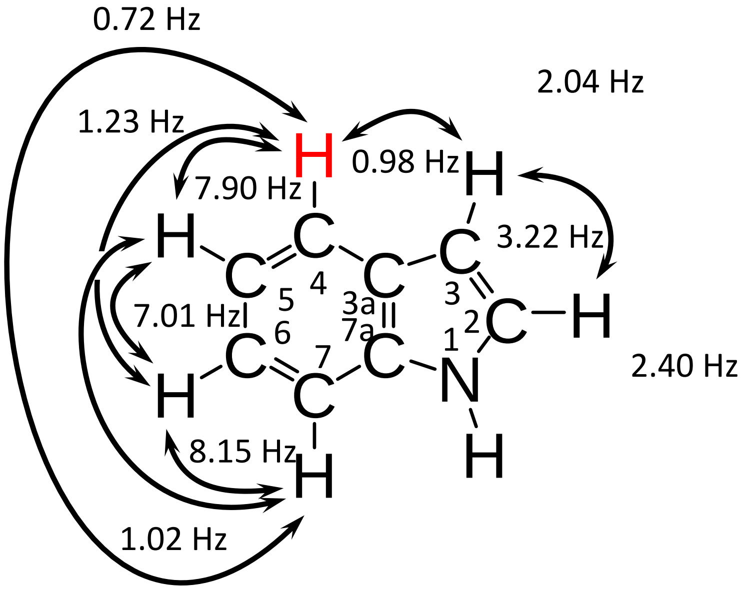
The sum of J_1, J_2 and J_3 in the pseudo-quartets is 2.93 Hz, so the value of J_1, J_2 and J_3 is about 1 Hz each.

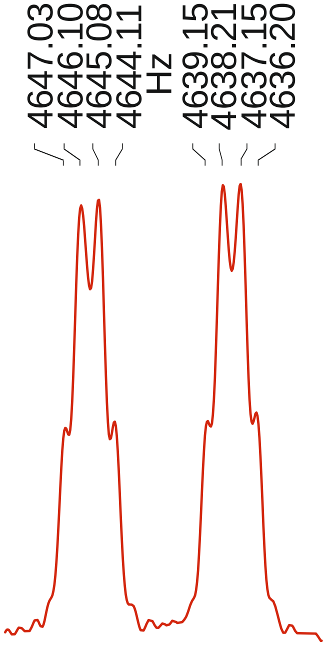
J_1 (1.23 Hz) is already known.

With a very high amount of probability J_2 is the already know coupling constant with a value of 0.98 Hz found in the doublet of doublet of doublets of H-3.

As a consequence we end with J_3 = 0.72 Hz.

Let us return to H-4





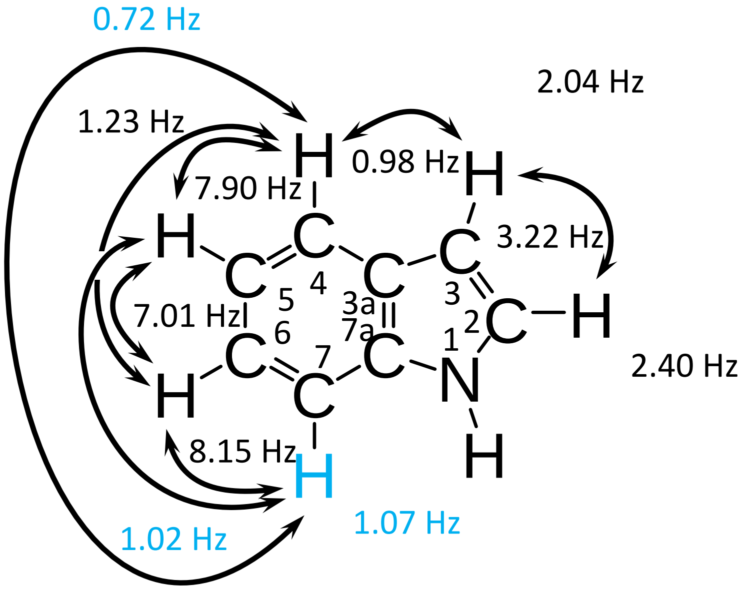
H-4

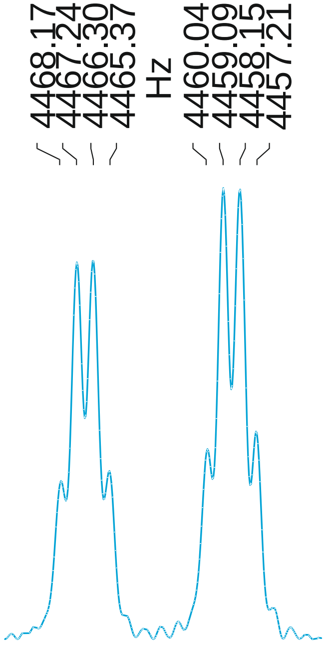
This might be a five bond coupling between H-4 and H-1 or between H-4 and H-7

There is no final proof with the given data, but the more probable coupling path is between H-4 and H-7.

As a consequence we end with J_3 = 0.72 Hz.

And now H-7



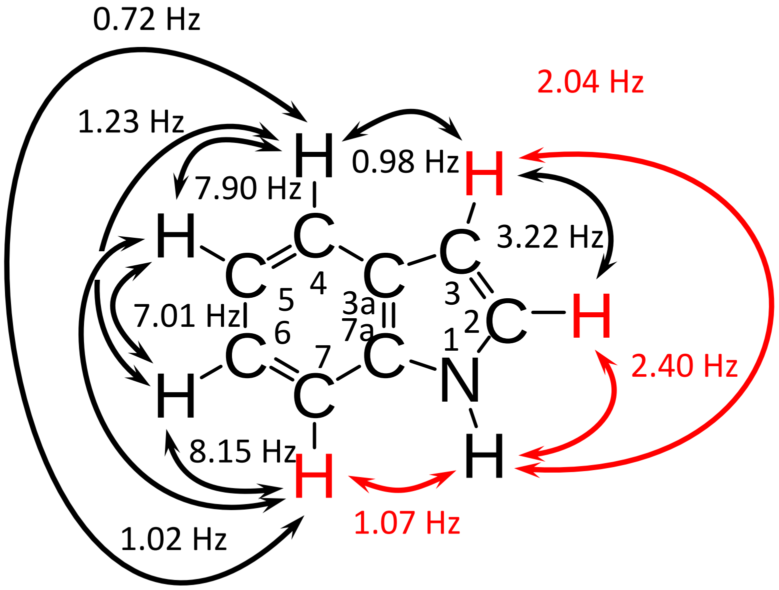


H-7

The sum of J_1, J_2 and J_3 in the pseudo-quartet structure of H-7 is 2.81 Hz.

J_1 (0.72 Hz) and J_2 (1.02 Hz) are already known, resulting in J_3 = 1.07 Hz.

Three unassigned coupling constants



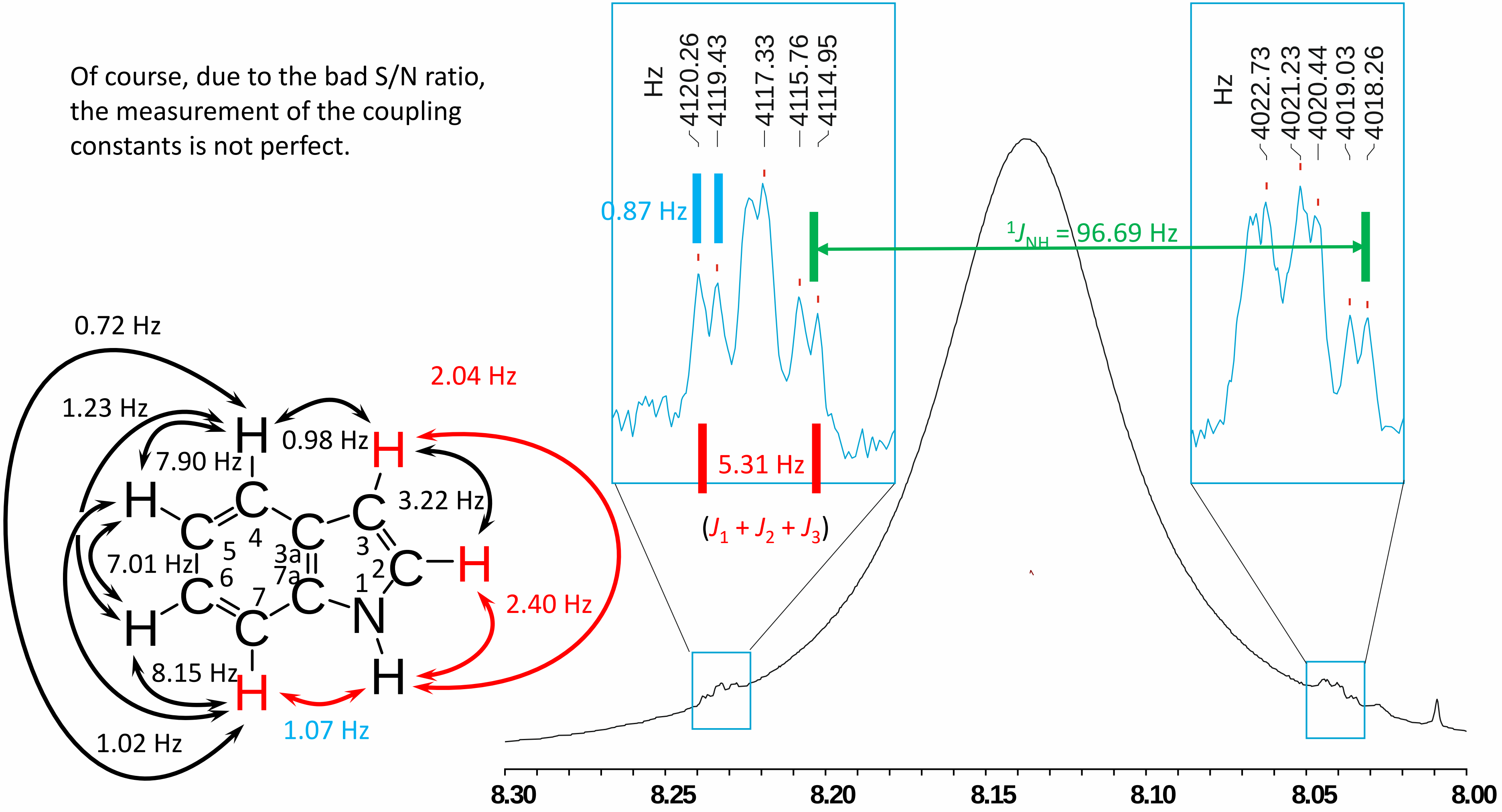
At its current state, each multiplet has been completely analyzed - with one exception: the multiplet of the NH proton.

The three couplings not yet assigned should lead to the NH proton.

Unfortunately the NH-signal is very wide due to the adjacent ^{14}N-quadrupole.

But we have a chance. If we analyze the broad signal very carefully, we see the signals of the ^{15}NH proton, of course only with 0.3% integral. Additionally there is another coupling: the ^{15}N-^{1}H on bond coupling of about 90 Hz.

Of course, due to the bad S/N ratio, the measurement of the coupling constants is not perfect.



Some final remarks

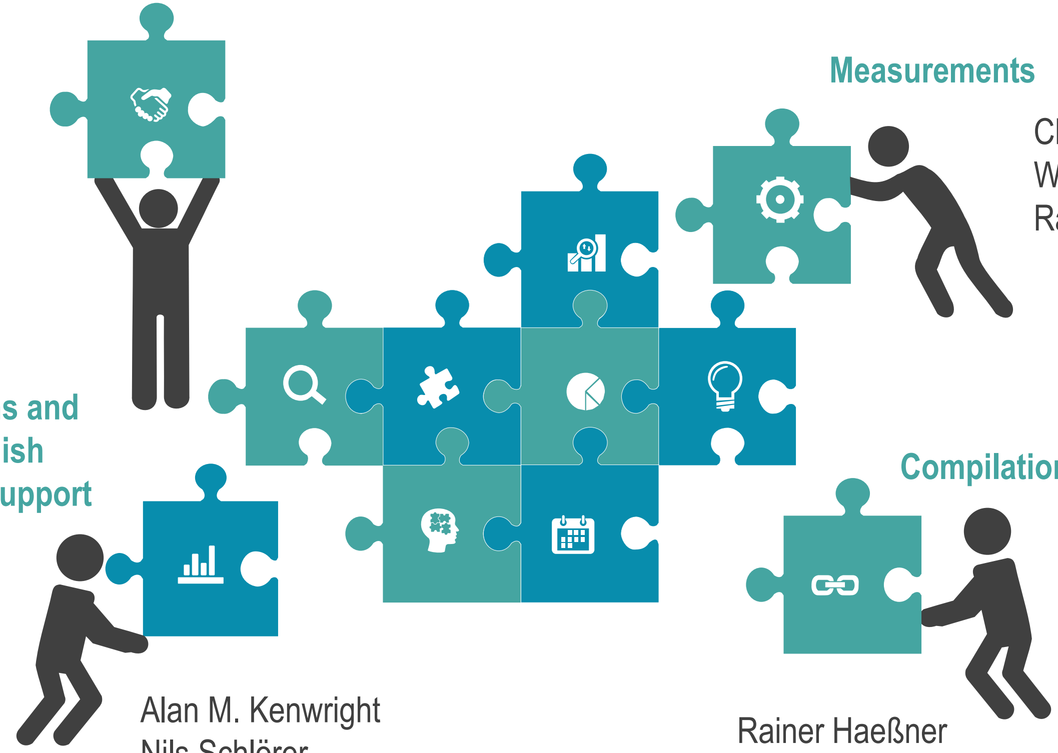
The methods described here for assigning all signals and the coupling constants are not fully bullet proof.

For the intensity of the cross peaks in the HMBC, not the number of bonds between the coupling partners is responsible, but the so-called **transfer function.**The simplified way shown here nevertheless led to the correct result.

Special experiments, e.g. selective decoupling, are necessary for the unambiguous assignment of the coupling constants. Compared to literature reference, two of the long-range coupling constants given here are interchanged. (Of course, the literature reference might be wrong as well...).

DOI in nmrshiftdb: 10.18716/nmrshiftdb2/10006007/nmredata_pom_bmrz_cdcl3

Contributions



Spectrometer time

TU Munich

Measurements

Christine Schwarz,
Wolfgang Eisenreich,
Rainer Haeßner

Discussions and native English language support

Alan M. Kenwright
Nils Schlörer

Compilation

Rainer Haeßner