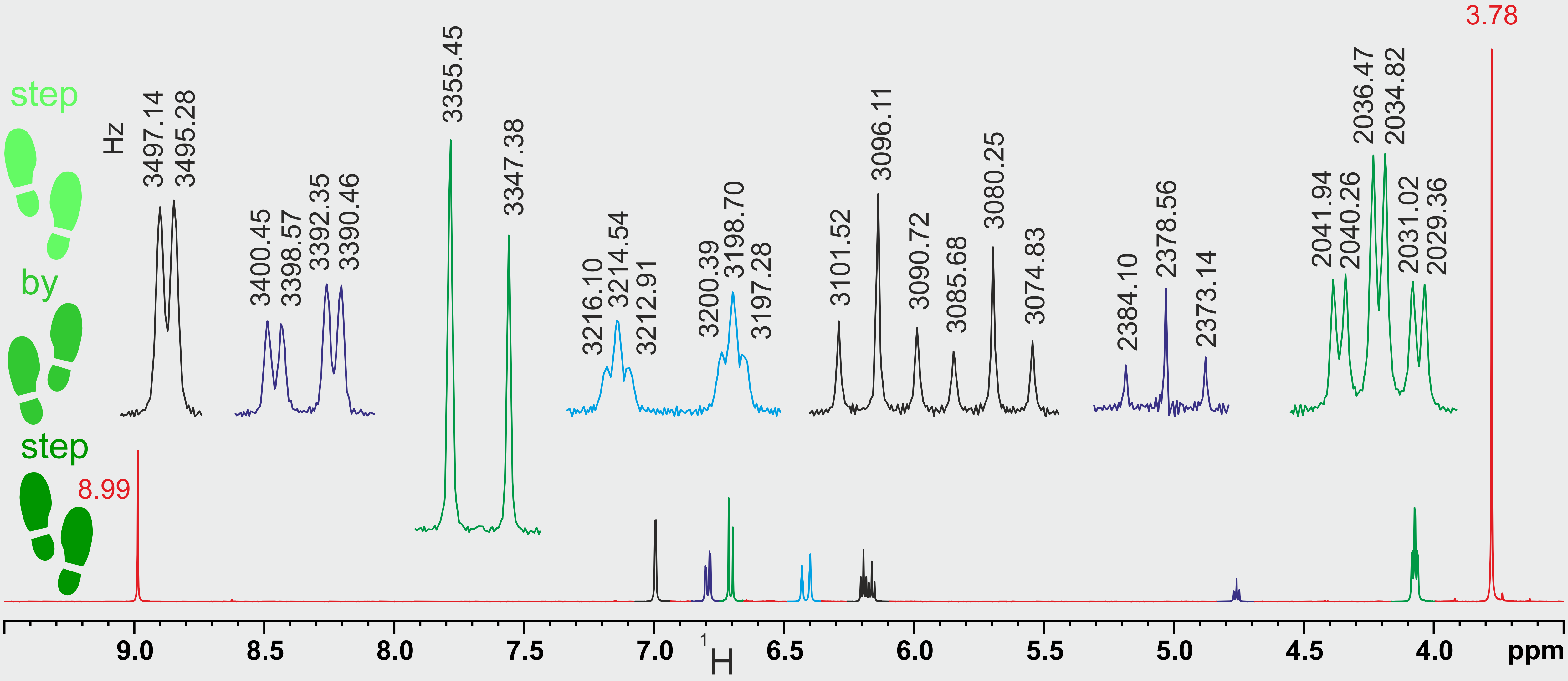
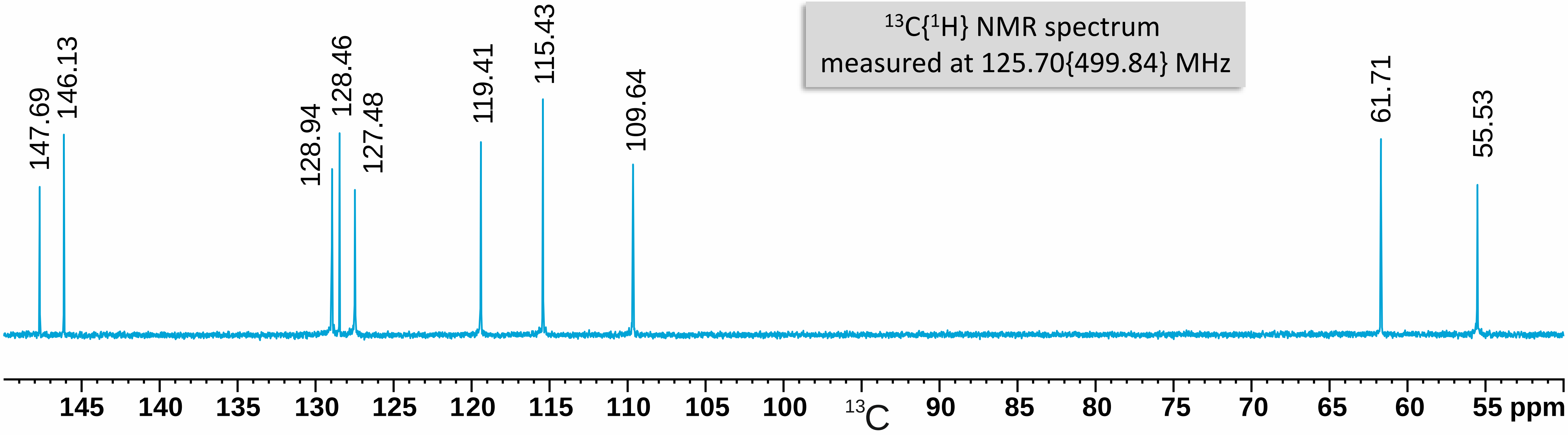
Exercise plus Solution – Quick PDF overview

It is recommended to use this PDF version only for a quick overview of the NMR challenge. All animations of the PowerPoint version are missing, under certain circumstances quality deficiencies may also occur.
The higher quality PowerPoint files are freely available for download at any time.

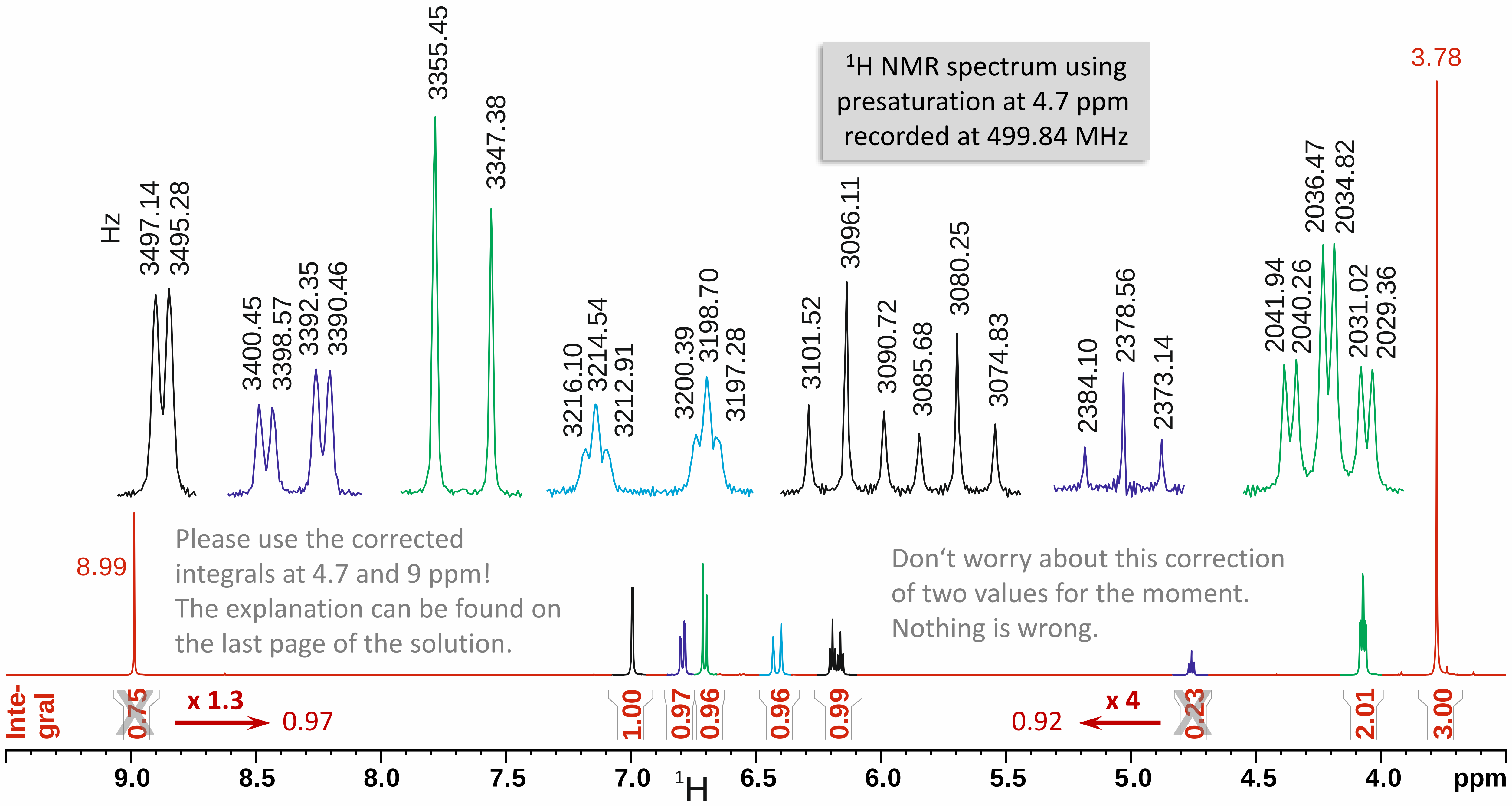


$C_{10}H_{12}O_3$ measured in DMSO-d_6

Coniferous wood contains significant amounts of this compound. Can you determine the structure of this natural compound?



$^{13}C\{^1H\}$ NMR spectrum measured at 125.70{499.84} MHz



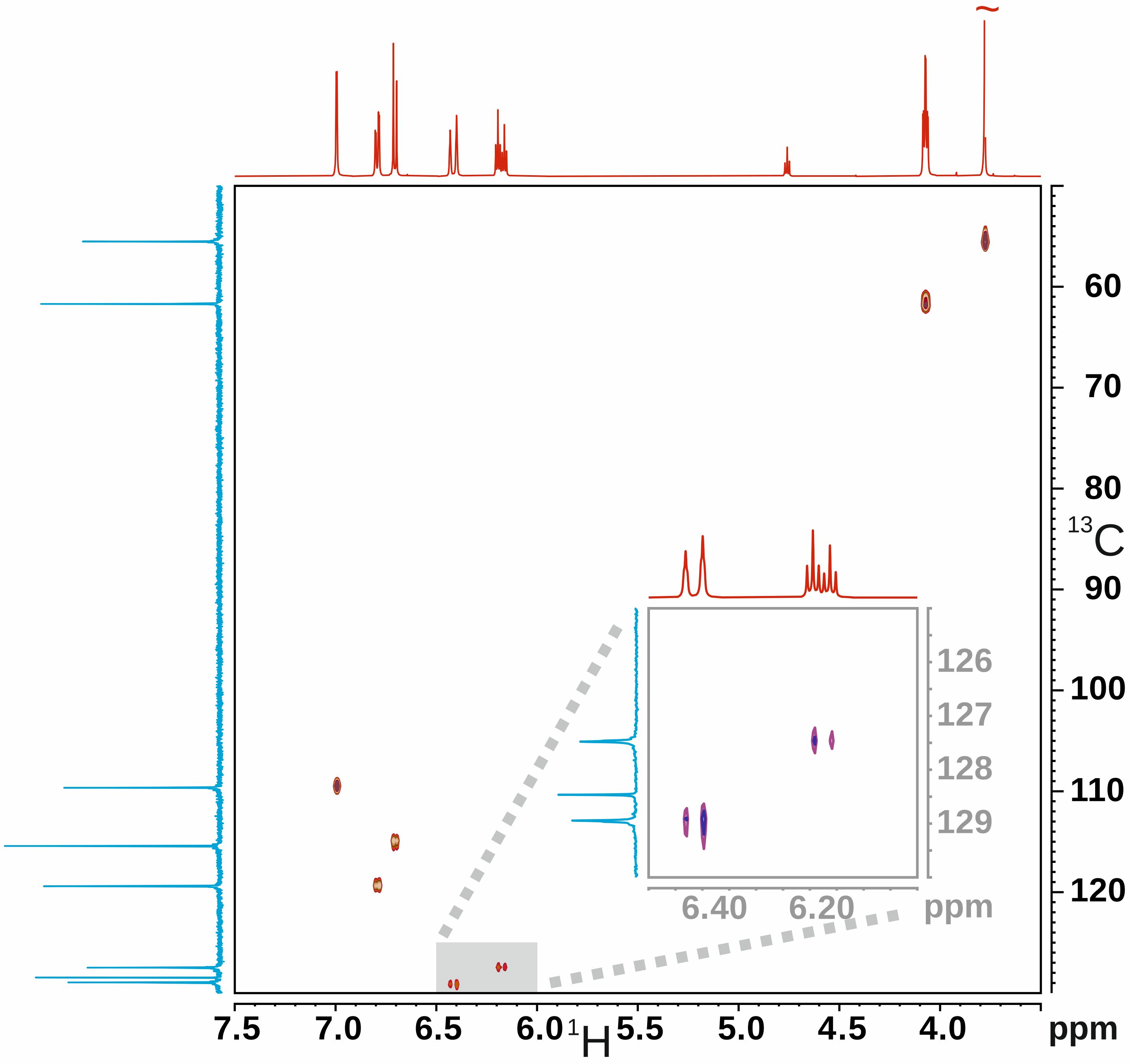
^{1}H NMR spectrum using presaturation at 4.7 ppm recorded at 499.84 MHz

Please use the corrected integrals at 4.7 and 9 ppm! The explanation can be found on the last page of the solution.

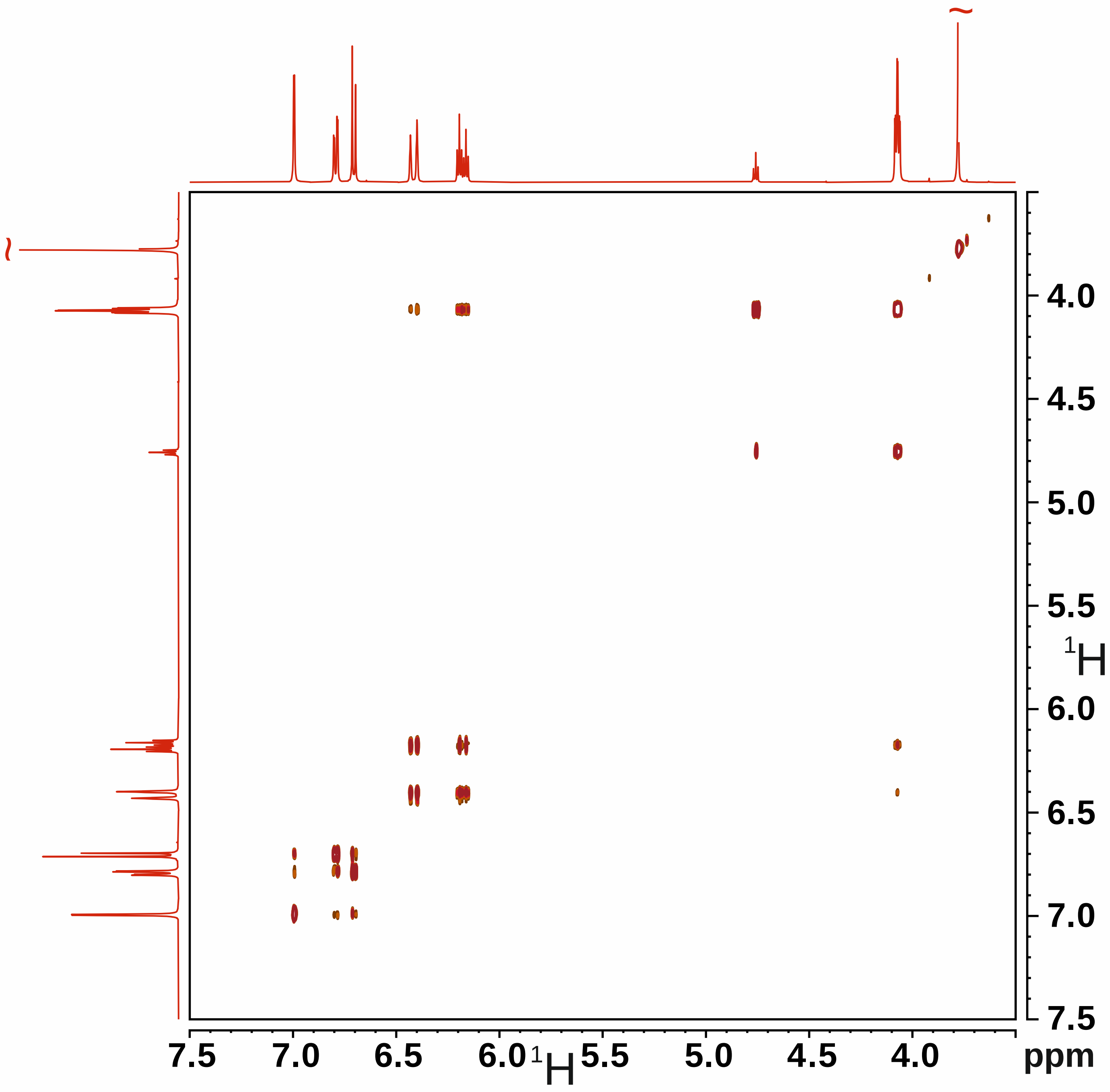
Don‘t worry about this correction of two values for the moment. Nothing is wrong.

$^1H/^{13}C$ HSQC
recorded at 499.84/125.70 MHz

There is no cross peak to the proton signal at 9 ppm in the HSQC.

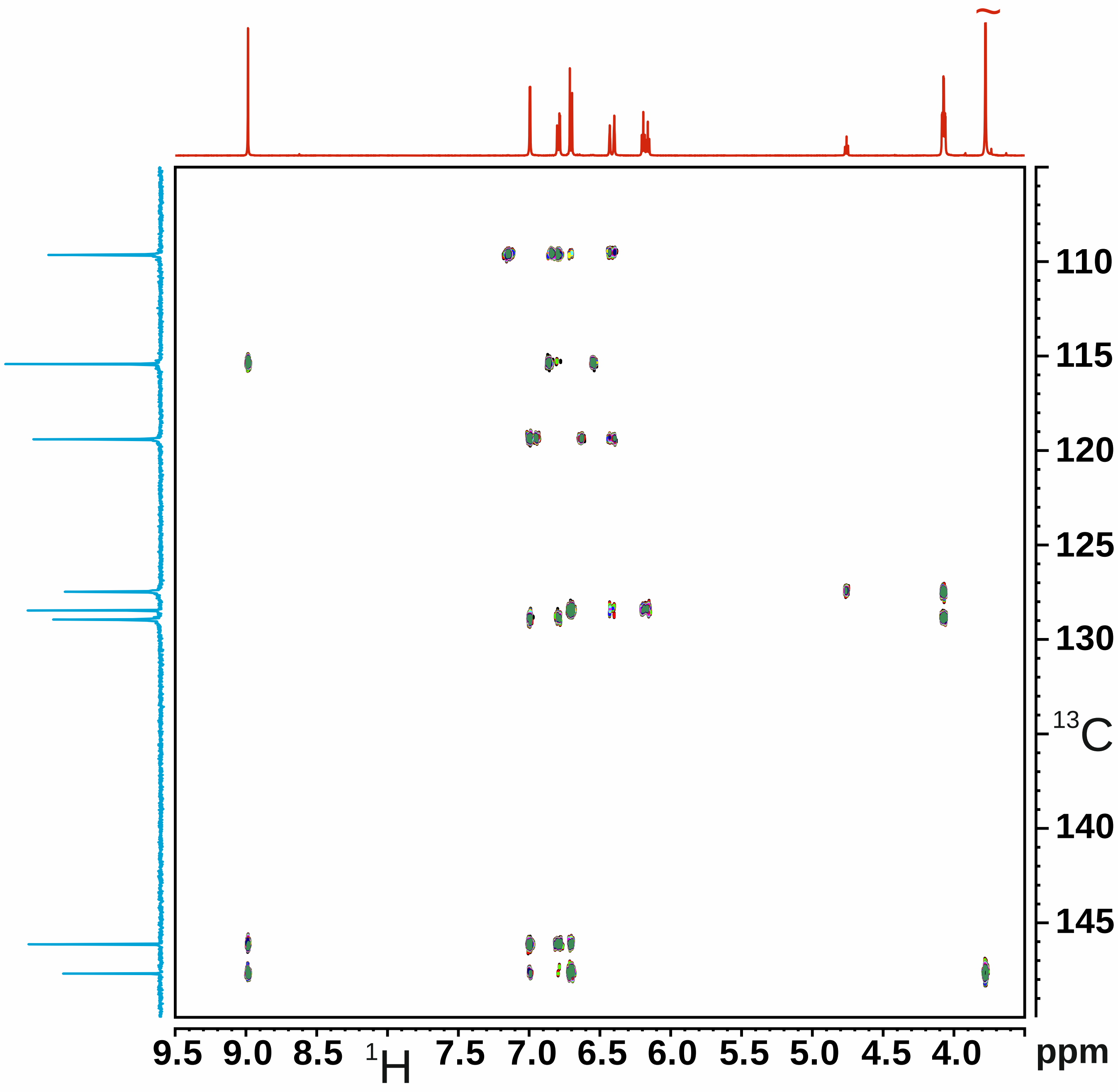


^{1}H TOCSY
measured at 499.84 MHz

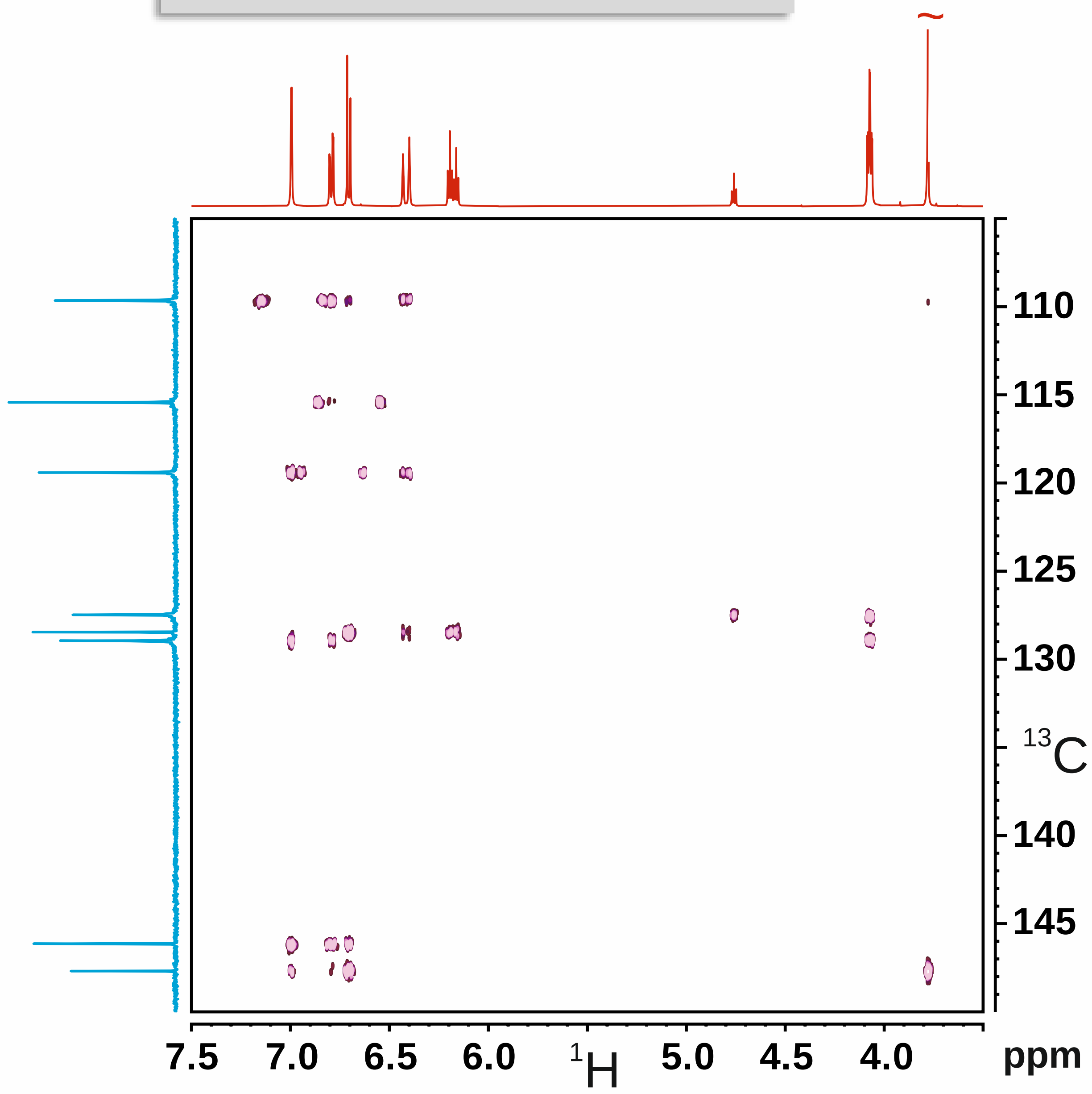


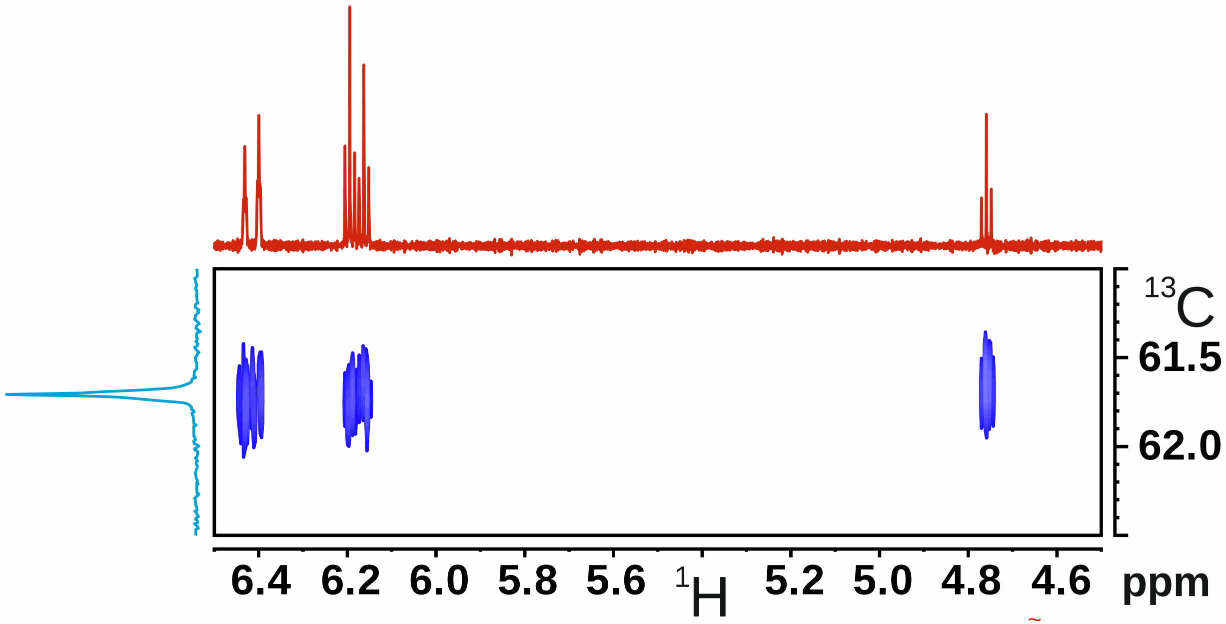
part of $^1H/^{13}C$ HMBC
measured at 499.84/125.70 MHz

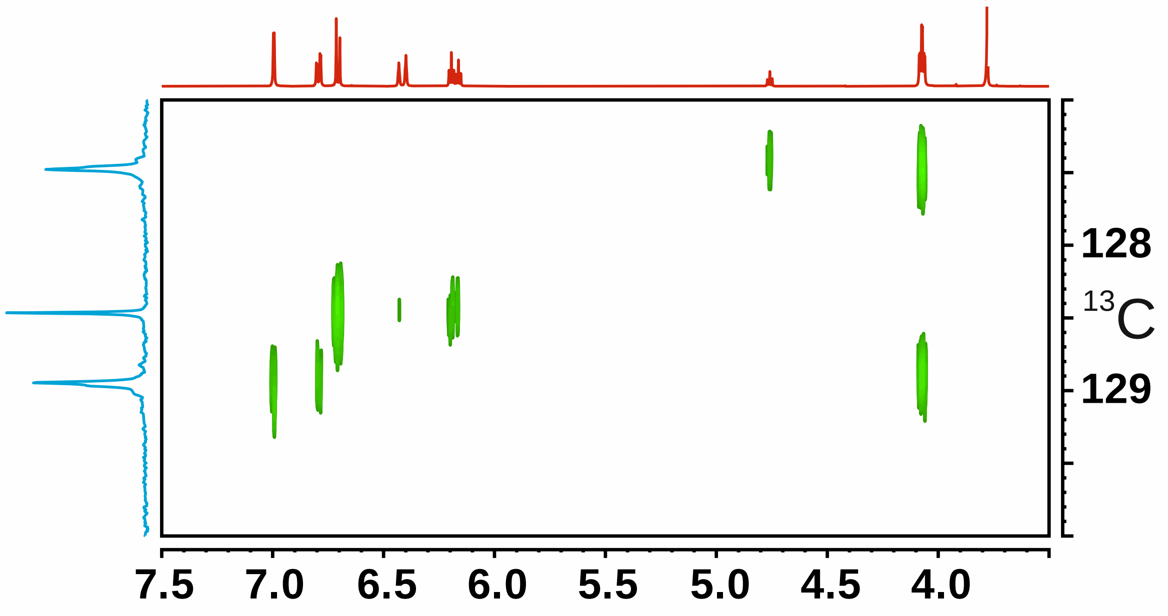
If the cross peaks are difficult to distinguish in some sections of the spectrum, please check whether there is a better resolved expansion of the same spectral region on the next page.



further parts of the $^{1}H/^{13}C$ HMBC
recorded at 499.84/125.70 MHz







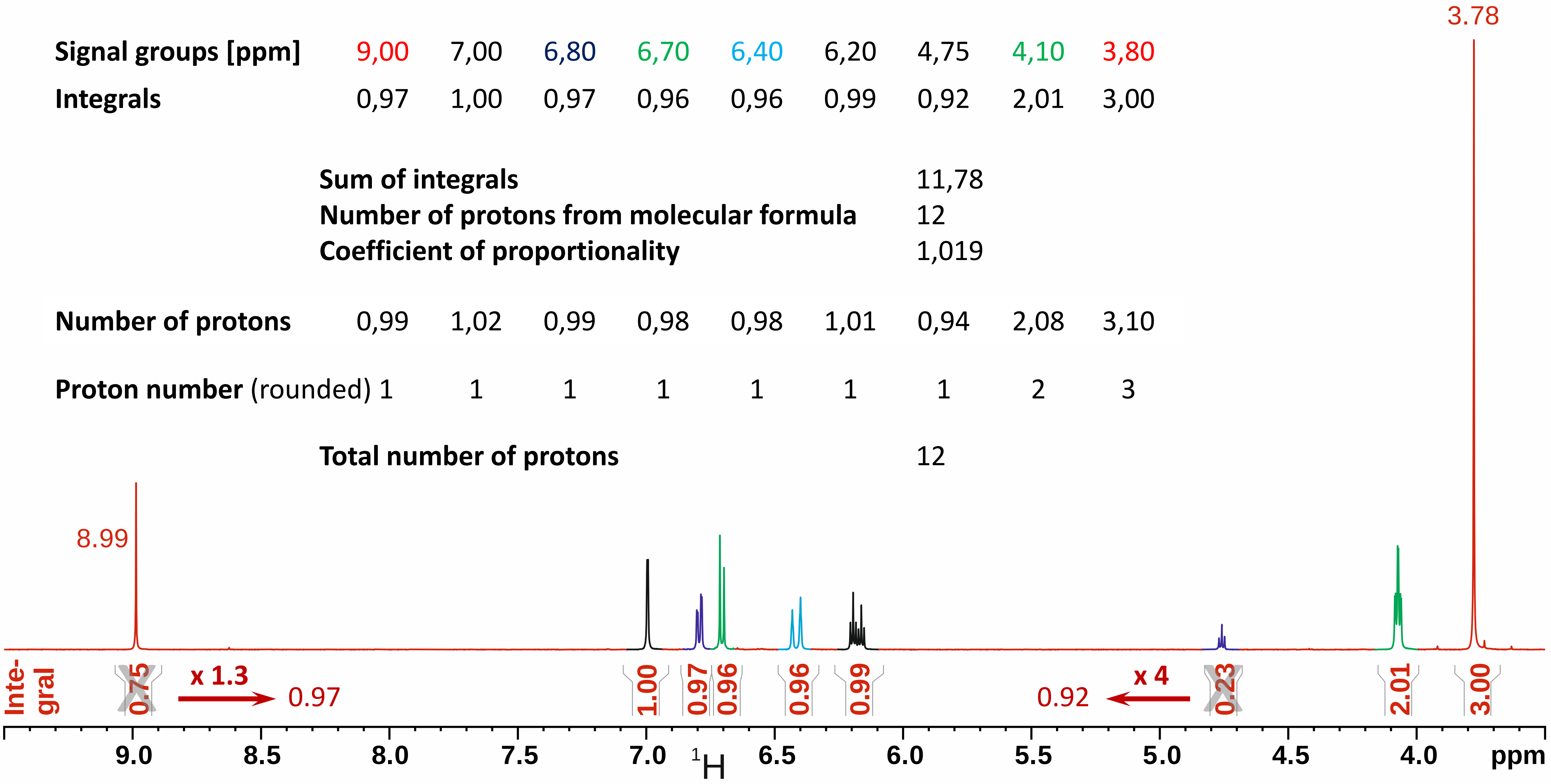
Integration

Signal groups [ppm]	9,00	7,00	6,80	6,70	6,40	6,20	4,75	4,10	3,80
Integrals	0,97	1,00	0,97	0,96	0,96	0,99	0,92	2,01	3,00

Sum of integrals	11,78
Number of protons from molecular formula	12
Coefficient of proportionality	1,019

Number of protons	0,99	1,02	0,99	0,98	0,98	1,01	0,94	2,08	3,10
Proton number (rounded)	1	1	1	1	1	1	1	2	3

Total number of protons 12



First partial structure

Spin system taken from TOCSY

The TOCSY contains three spin systems with

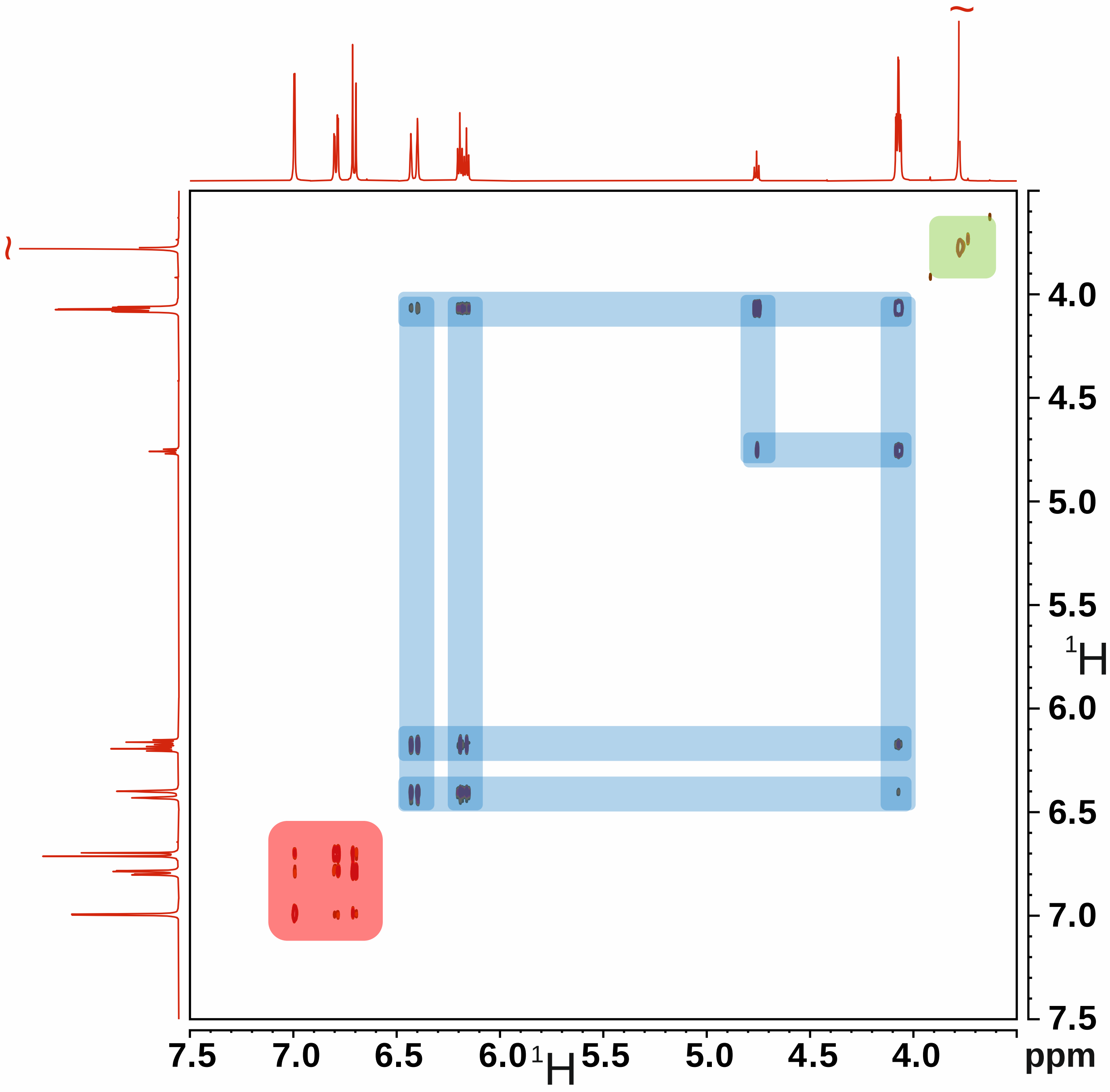
- three
- four

and

- one

proton multiplet(s).

Let us start with the most extensive spin system.



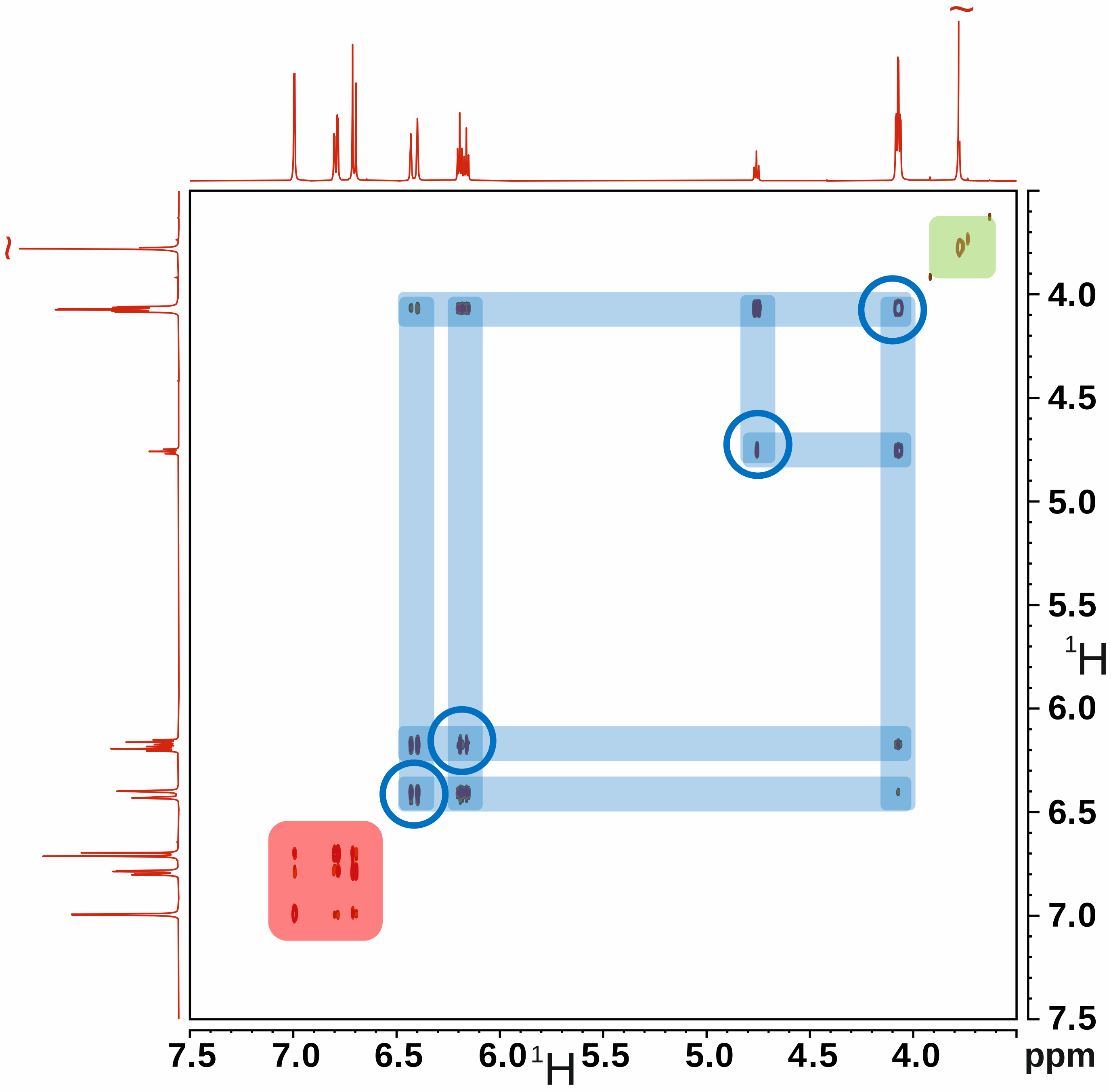
First partial structure

Spin system taken from TOCSY

Let us now determine the chemical shifts of the proton multiplets, which belong to this spin system.

In all subsequent 2D spectra, cross and diagonal peaks that are no longer needed for further evaluation after extracting the information they contain become deleted. The same procedure applies to the signals in the projections.

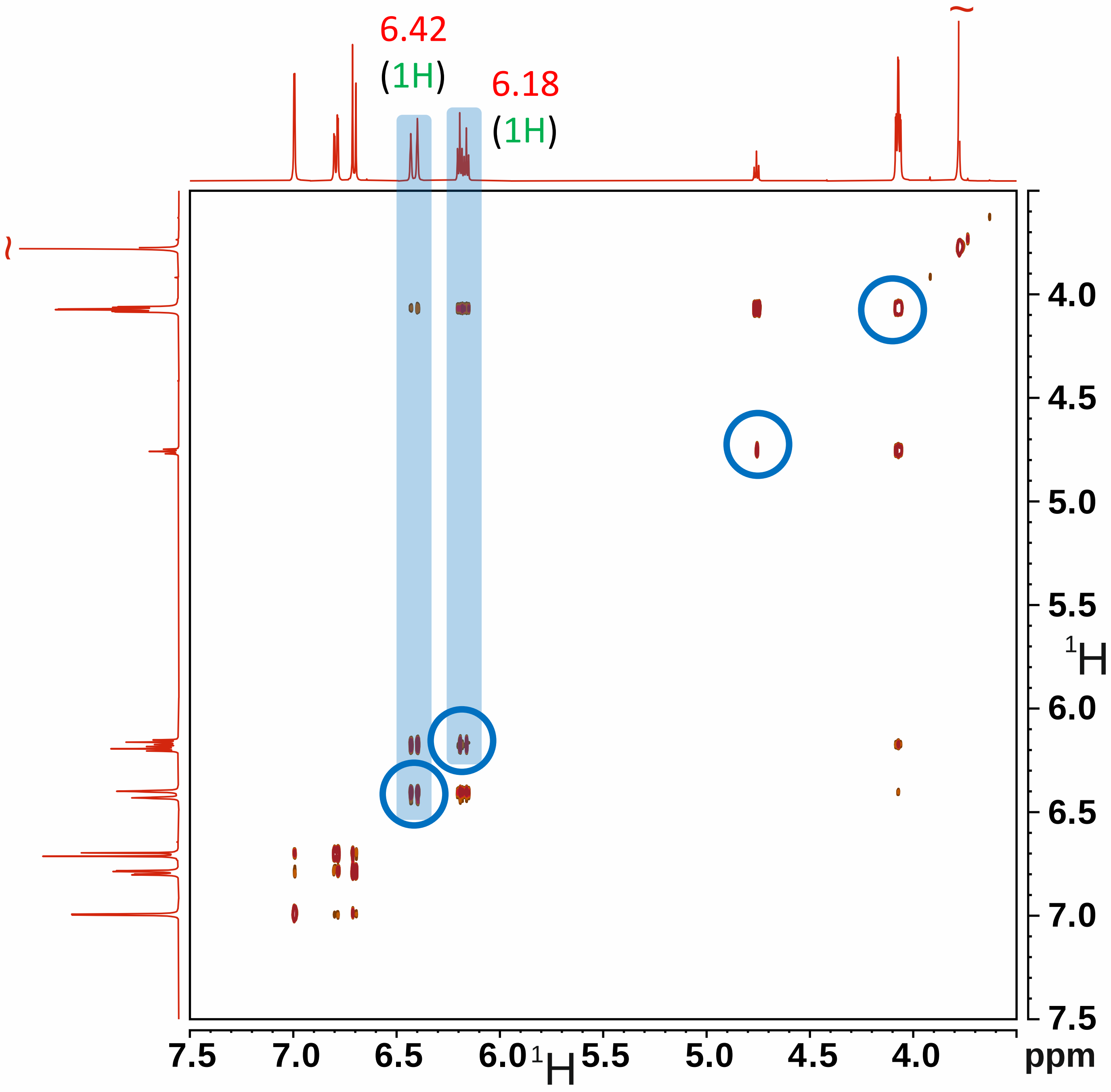
Thus signals that have already been evaluated cannot be used a second time by mistake. When using printed spectra on paper, simply cross out such signals.



First partial structure

Spin system taken from TOCSY

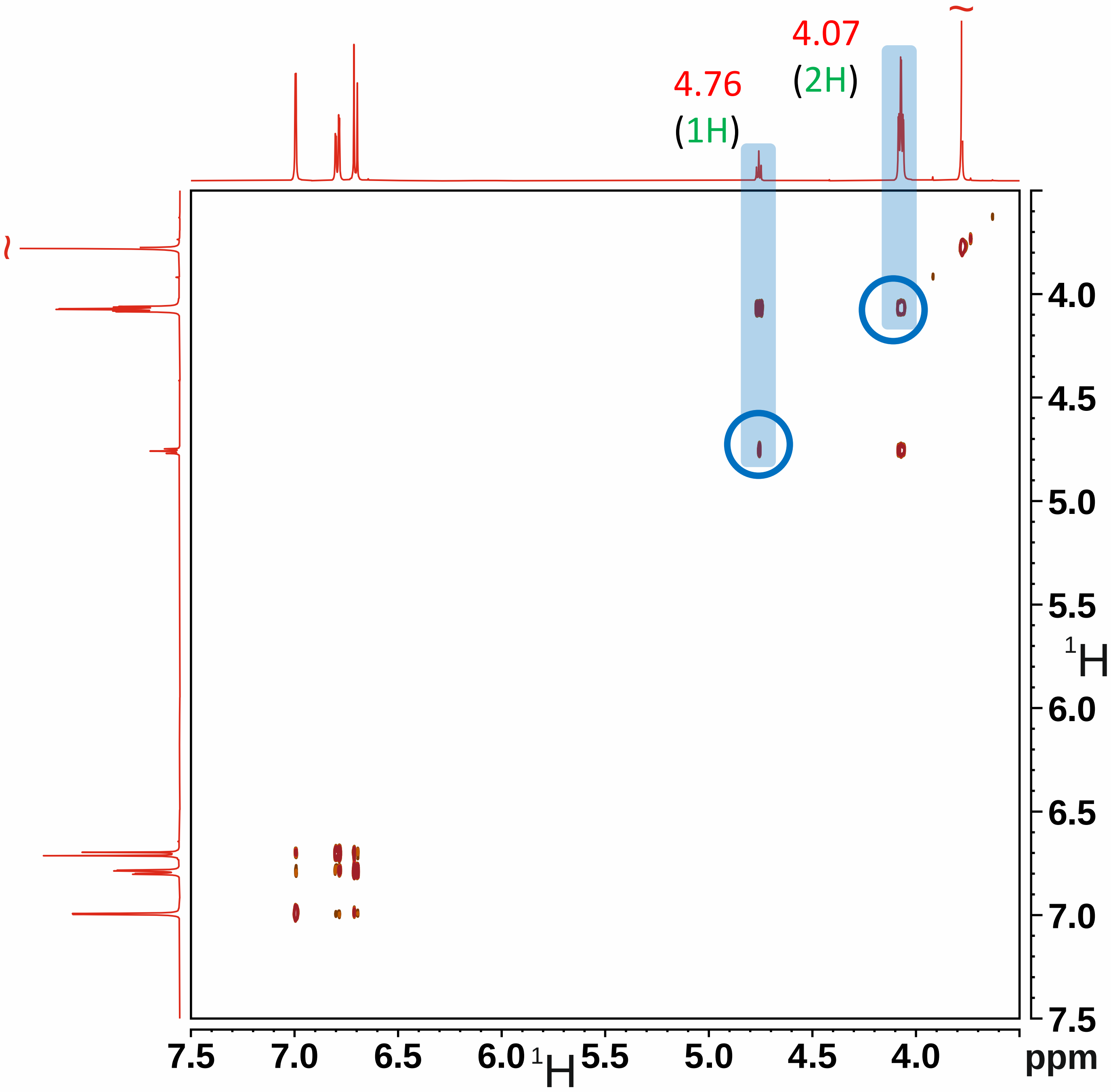
6.42 (1H)   6.18 (1H)



First partial structure

Spin system taken from TOCSY

6.42	6.18	4.76	4.07
(1H)	(1H)	(1H)	(2H)



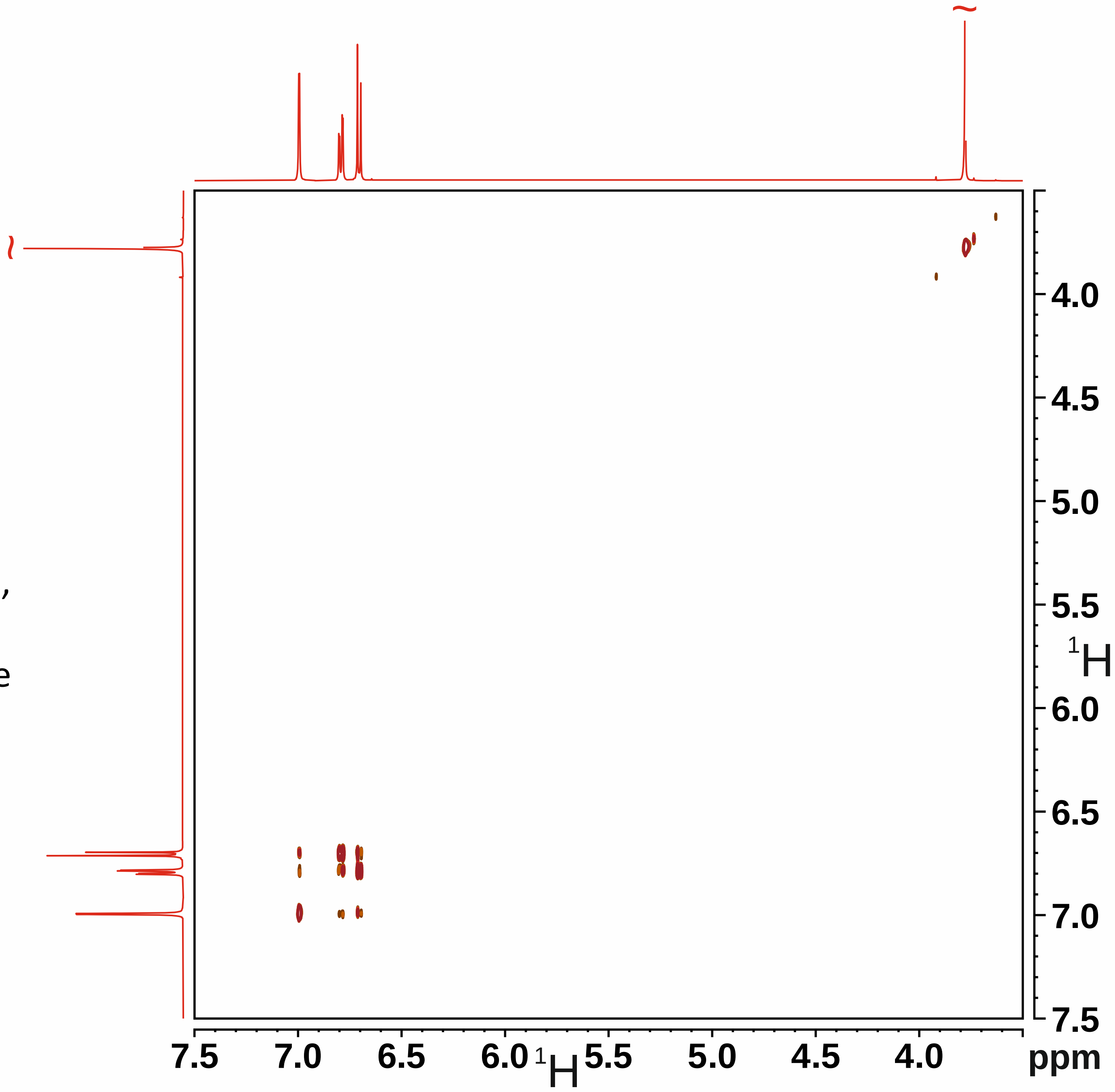
First partial structure

Spin system taken from TOCSY

6.42	6.18	4.76	4.07
(1H)	(1H)	(1H)	(2H)

Later we will need the TOCSY again. To simplify later use, all cross and diagonal peaks, as well as the associated projections of the four extracted proton multiplets, were removed. On a sheet of paper, you can simply cross out the signals using a pencil.

The multiplet structures of all four multiplets are rather simple, let us evaluate these multiplets as the next step.



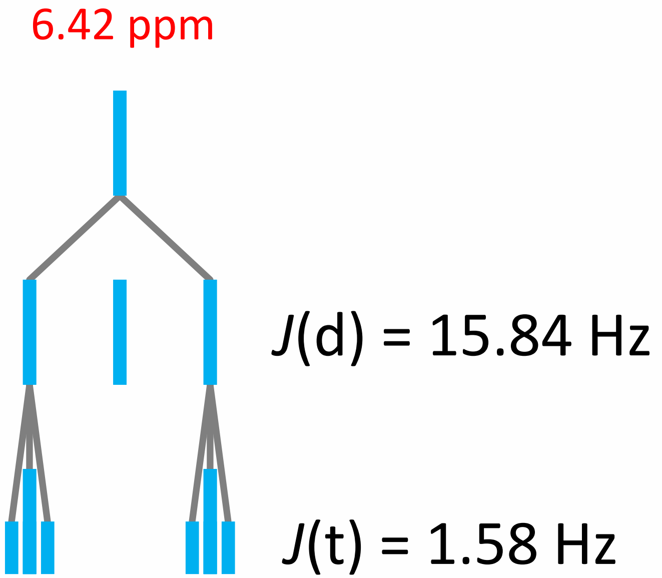
First partial structure

Multiplicity and coupling constants

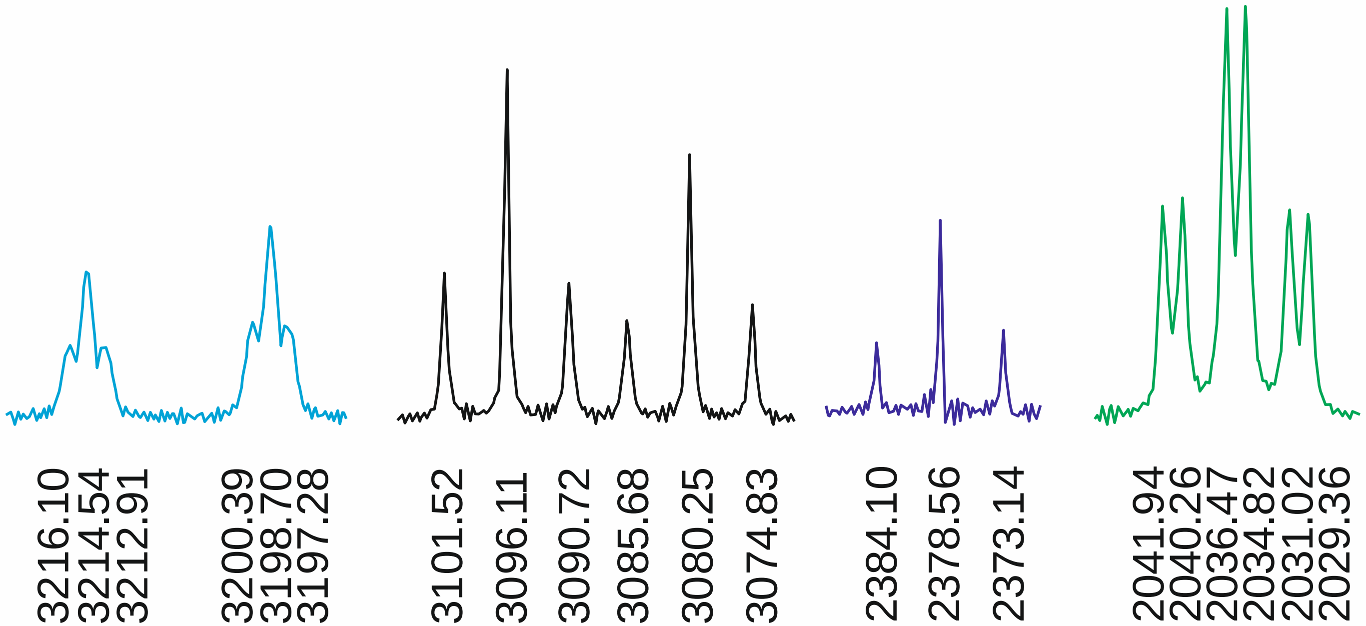
15.84 Hz(d)

1.58 Hz(t)

6.42	6.18	4.76	4.07
(1H)	(1H)	(1H)	(2H)



Let's start with the doublet of triplets at lowest field (6.42 ppm).



First partial structure

Multiplicity and coupling constants

15.84 Hz(d) 15.86 Hz(d)
1.58 Hz(t) 5.41 Hz(t)

6.42	6.18	4.76	4.07
(1H)	(1H)	(1H)	(2H)

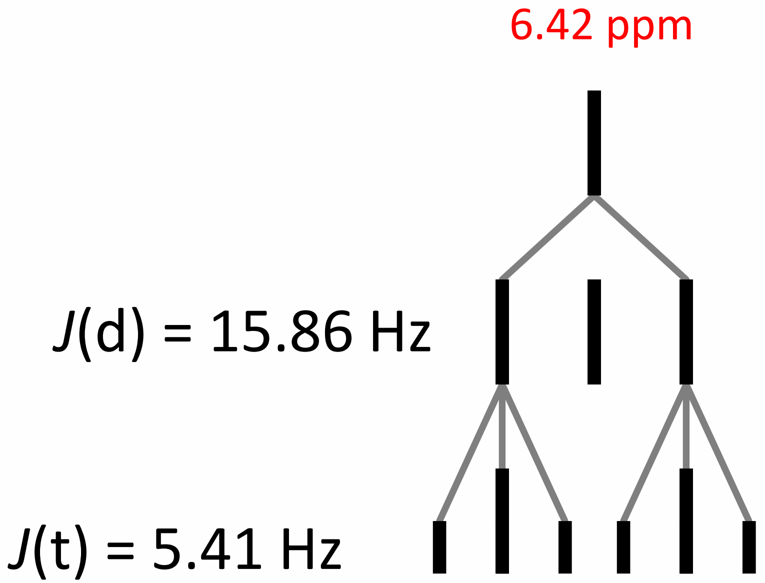
As an example for the second doublet of triplets (6.18 ppm) the detailed calculation of the coupling constants is shown here.

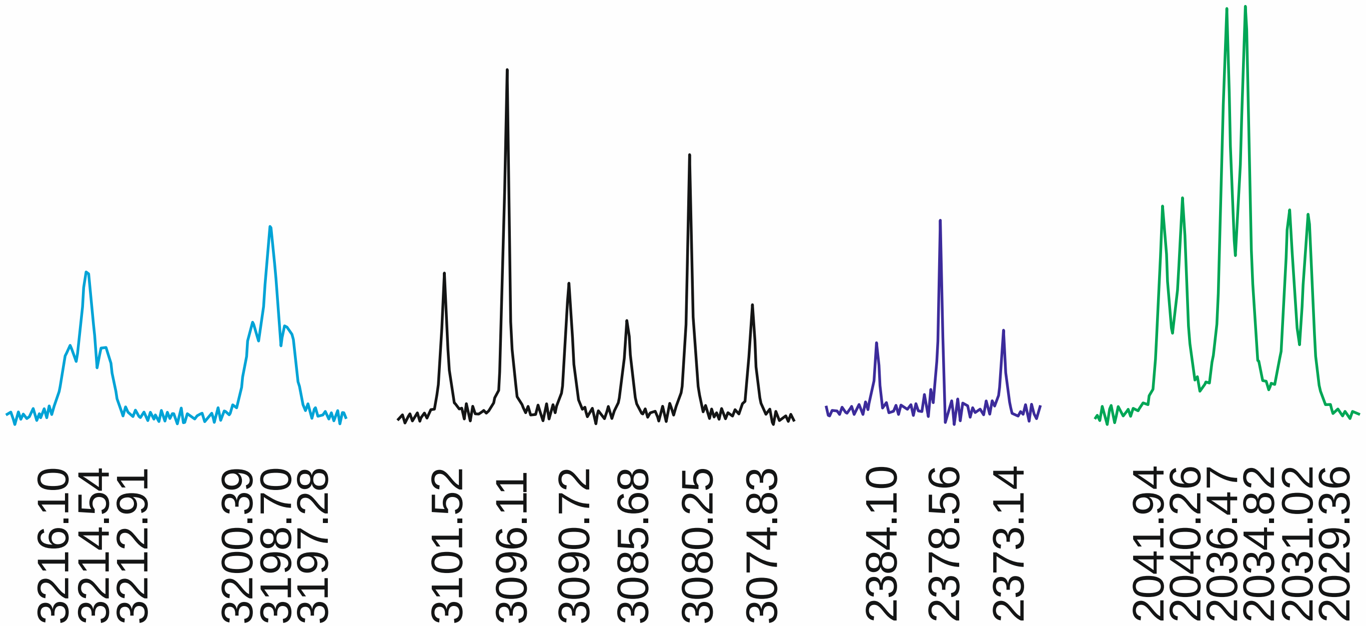
$$J(\mathrm{d}) = (3096.11\ \mathrm{Hz} - 3080.25\ \mathrm{Hz}) = \mathbf{15.86\ Hz}$$

$$J(\mathrm{t})_{\mathrm{a}} = \frac{(3101.52\ \mathrm{Hz} - 3090.72\ \mathrm{Hz})}{2} = \mathbf{5.4\ Hz}$$

$$J(\mathrm{t})_{\mathrm{b}} = \frac{(3085.68\ \mathrm{Hz} - 3074.83\ \mathrm{Hz})}{2} = \mathbf{5.42\ Hz}$$

$$J(\mathrm{t}) = \frac{J(\mathrm{t})_{\mathrm{a}} + J(\mathrm{t})_{\mathrm{b}}}{2} = \mathbf{5.41\ Hz}$$





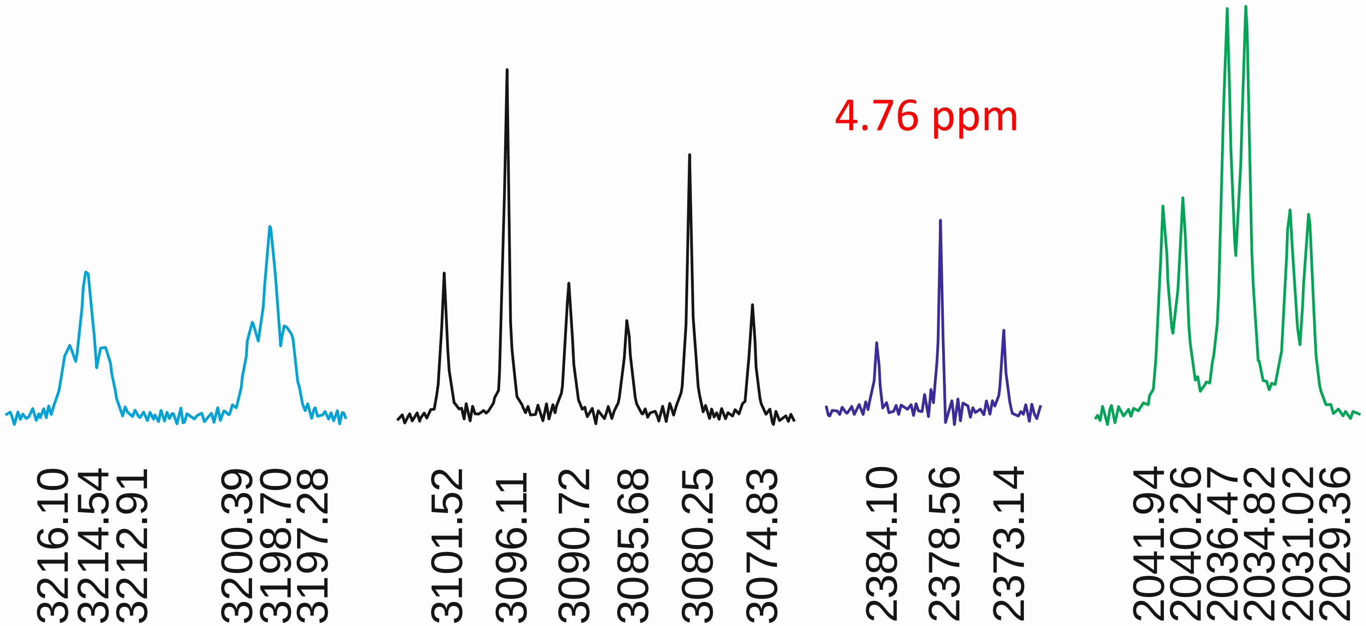
First partial structure

Multiplicity and coupling constants

15.84 Hz(d) 15.86 Hz(d)
1.58 Hz(t) 5.41 Hz(t) 5.48 Hz(t)
6.42 6.18 4.76 4.07
(1H) (1H) (1H) (2H)

The triplet at 4.76 ppm is simple.

$$J(\mathrm{t}) = \frac{(2384.10\ \mathrm{Hz} - 2373.14\ \mathrm{Hz})}{2} = \mathbf{5.48\ Hz}$$

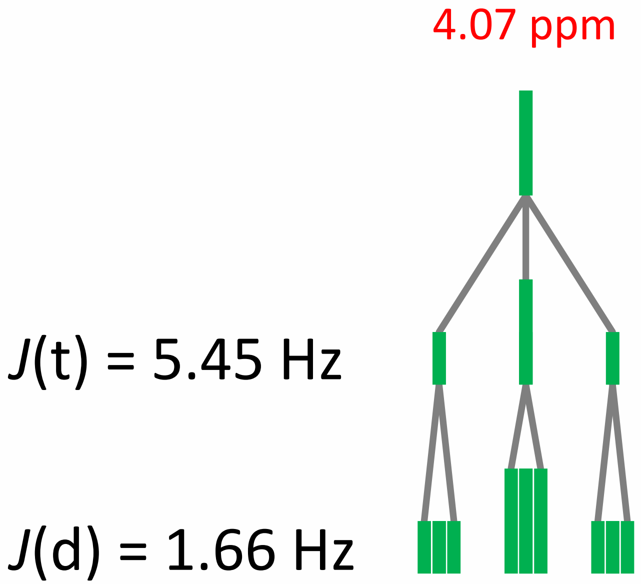


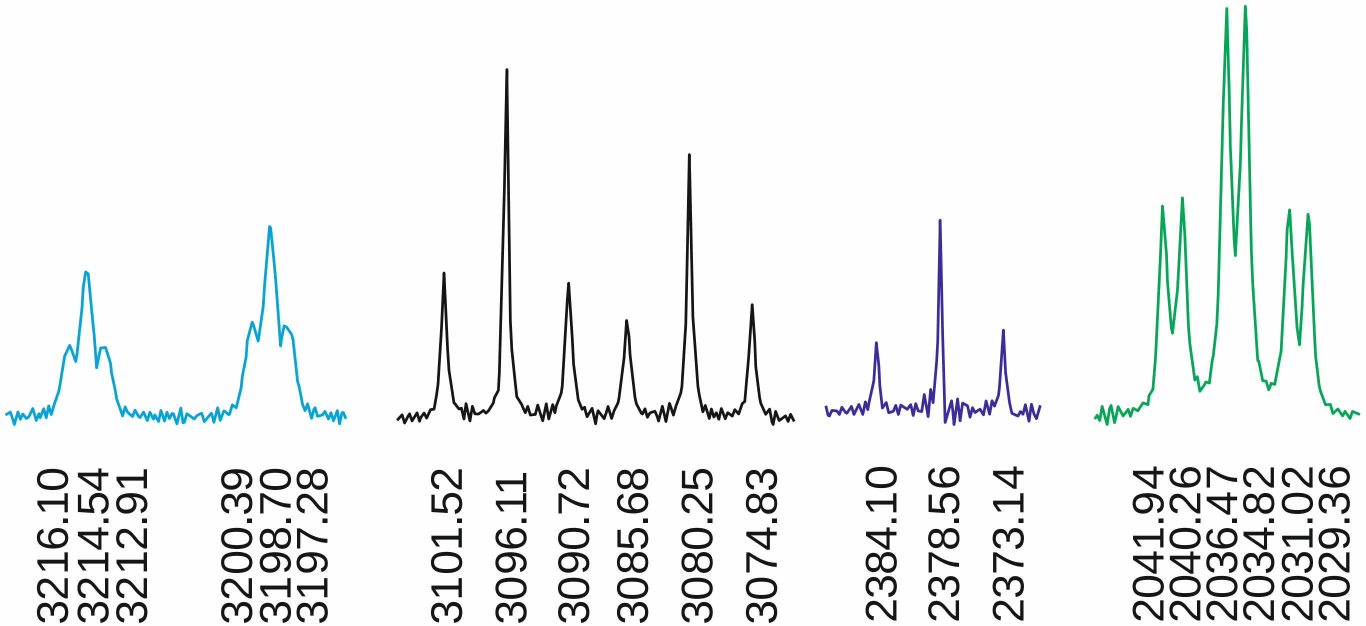
First partial structure

Multiplicity and coupling constants

15.84 Hz(d)	15.86 Hz(d)		1.66 Hz(d)
1.58 Hz(t)	5.41 Hz(t)	5.48 Hz(t)	5.45 Hz(t)
6.42	6.18	4.76	4.07
(1H)	(1H)	(1H)	(2H)

There is a last doublet of triplets at 4.07 ppm.





First partial structure

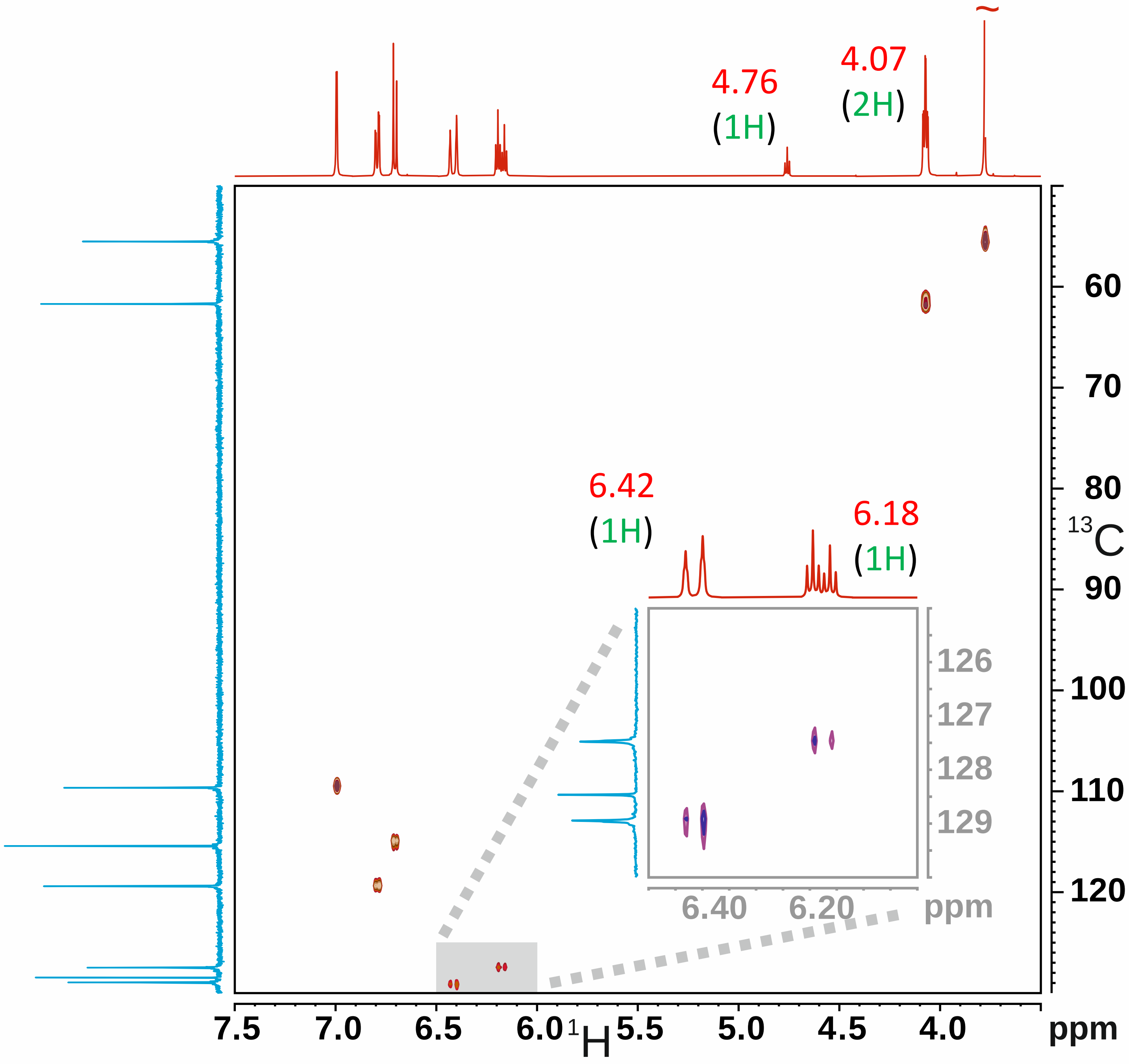
Building blocks

15.84 Hz(d)	15.86 Hz(d)		1.66 Hz(d)
1.58 Hz(t)	5.41 Hz(t)	5.48 Hz(t)	5.45 Hz(t)
6.42	6.18	4.76	4.07
(1H)	(1H)	(1H)	(2H)

We have four proton multiplets. The HSQC shows us the attached carbon atoms.

Let us label the projections with the chemical shifts of the known proton multiplets.

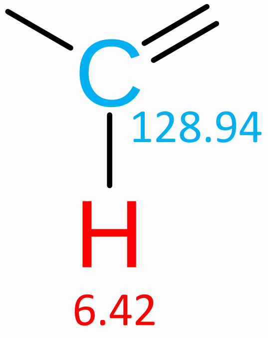
Due to close chemical shifts of three carbon signals there is an enlarged part of the HSQC for two cross peaks only.



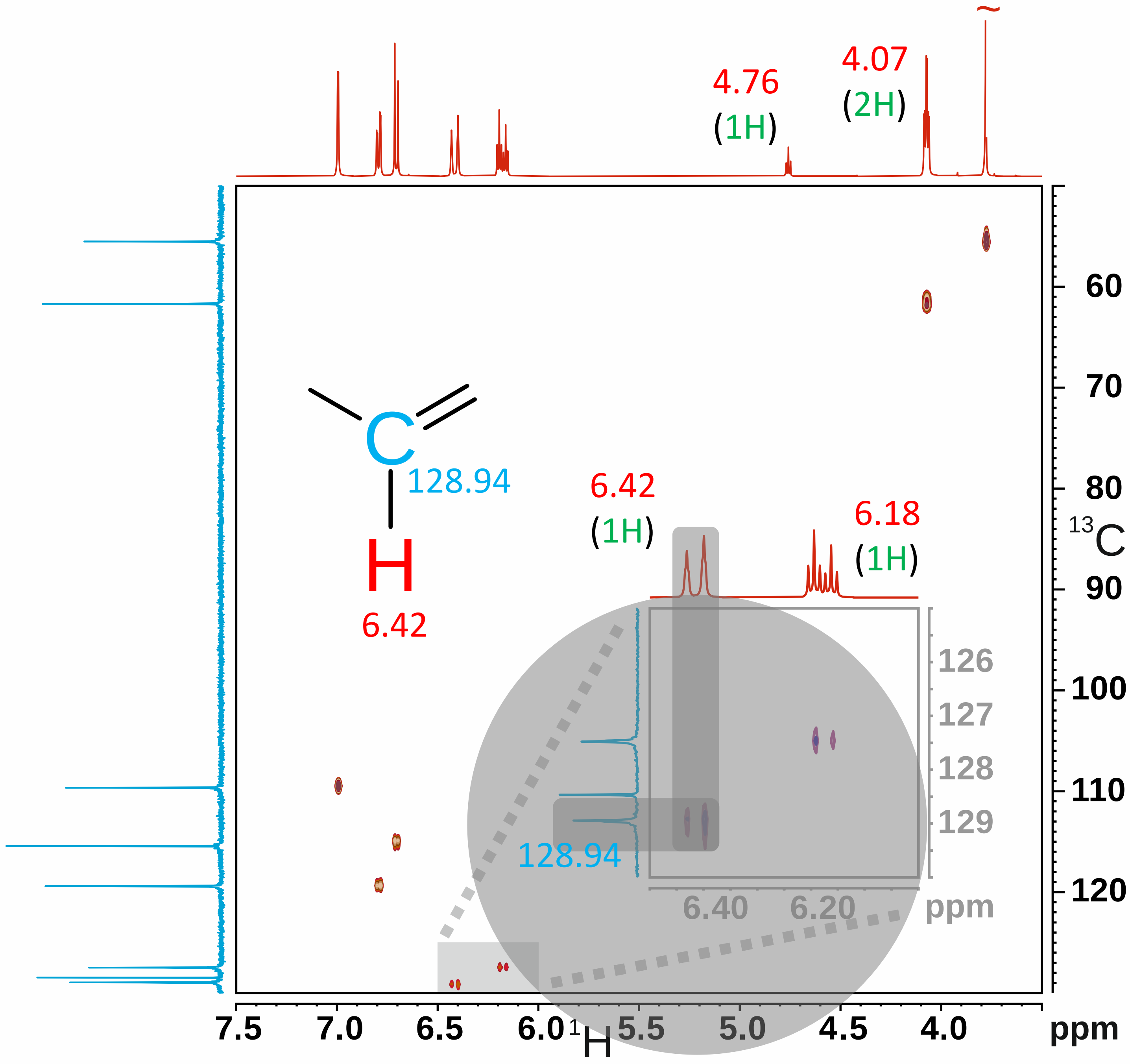
First partial structure

Building blocks

15.84 Hz(d)	15.86 Hz(d)		1.66 Hz(d)
1.58 Hz(t)	5.41 Hz(t)	5.48 Hz(t)	5.45 Hz(t)
6.42	6.18	4.76	4.07
(1H)	(1H)	(1H)	(2H)



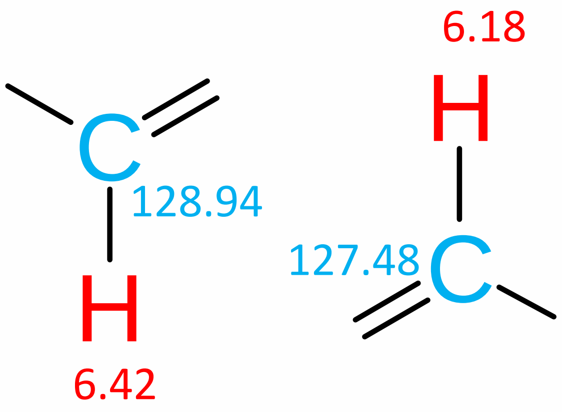
There is another cross peak inside this enlarged area.



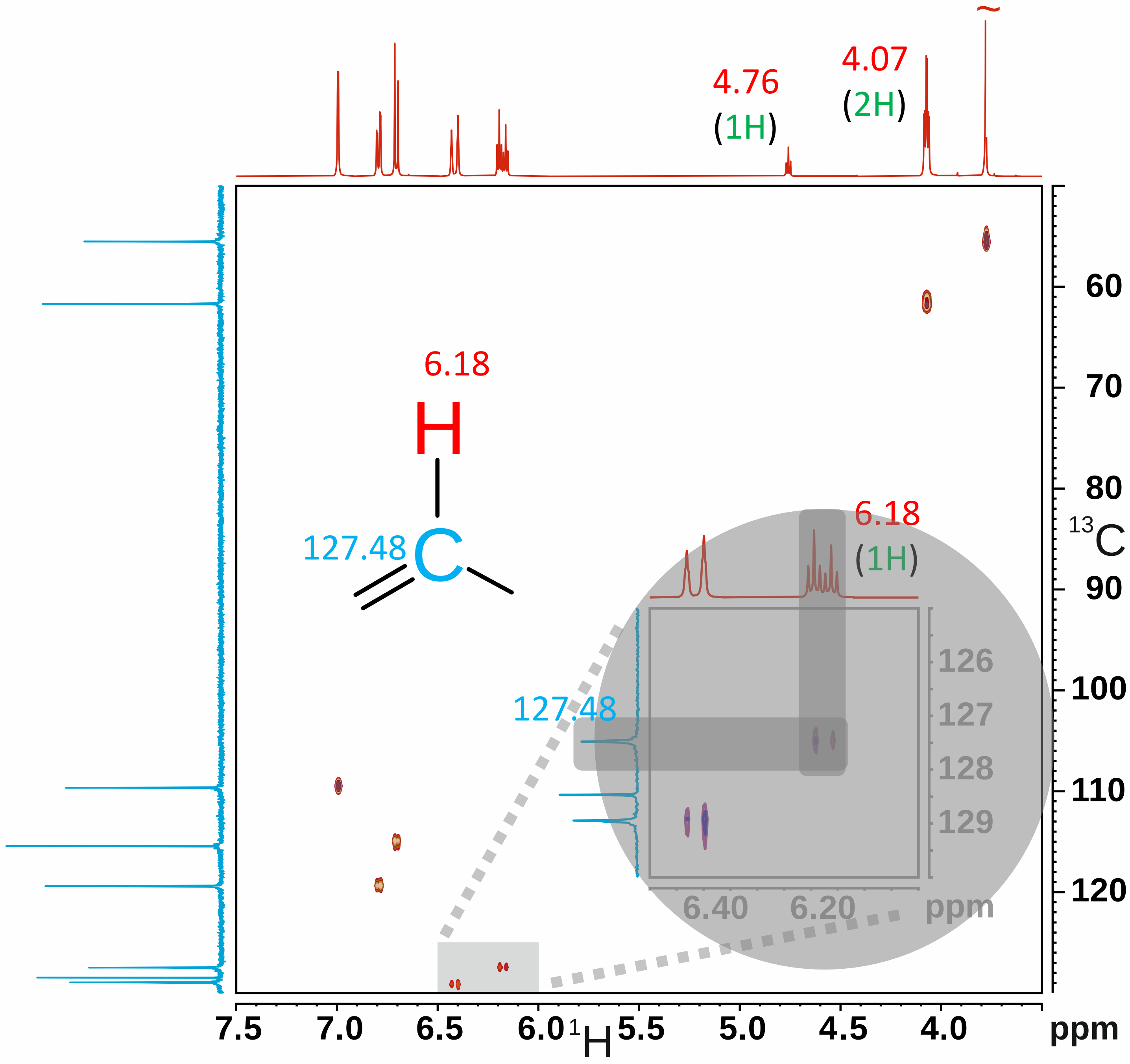
First partial structure

Building blocks

15.84 Hz(d)	15.86 Hz(d)		1.66 Hz(d)
1.58 Hz(t)	5.41 Hz(t)	5.48 Hz(t)	5.45 Hz(t)
6.42	6.18	4.76	4.07
(1H)	(1H)	(1H)	(2H)



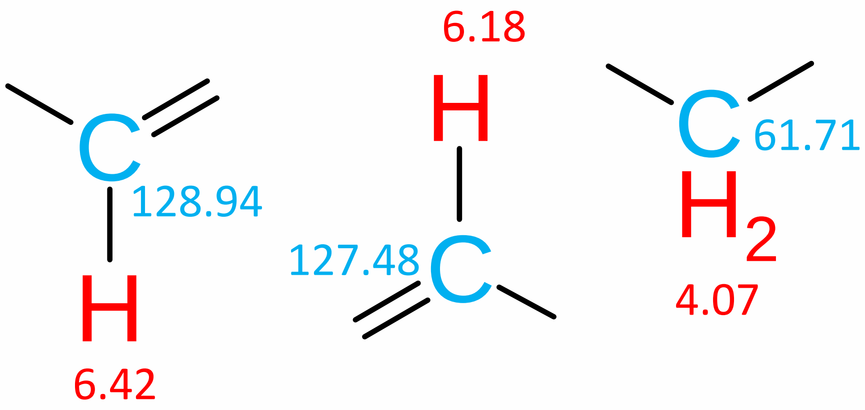
After removing all signals that are no longer needed from the HSQC for sake of clarity, we can turn to the proton signal at 4.07 ppm.



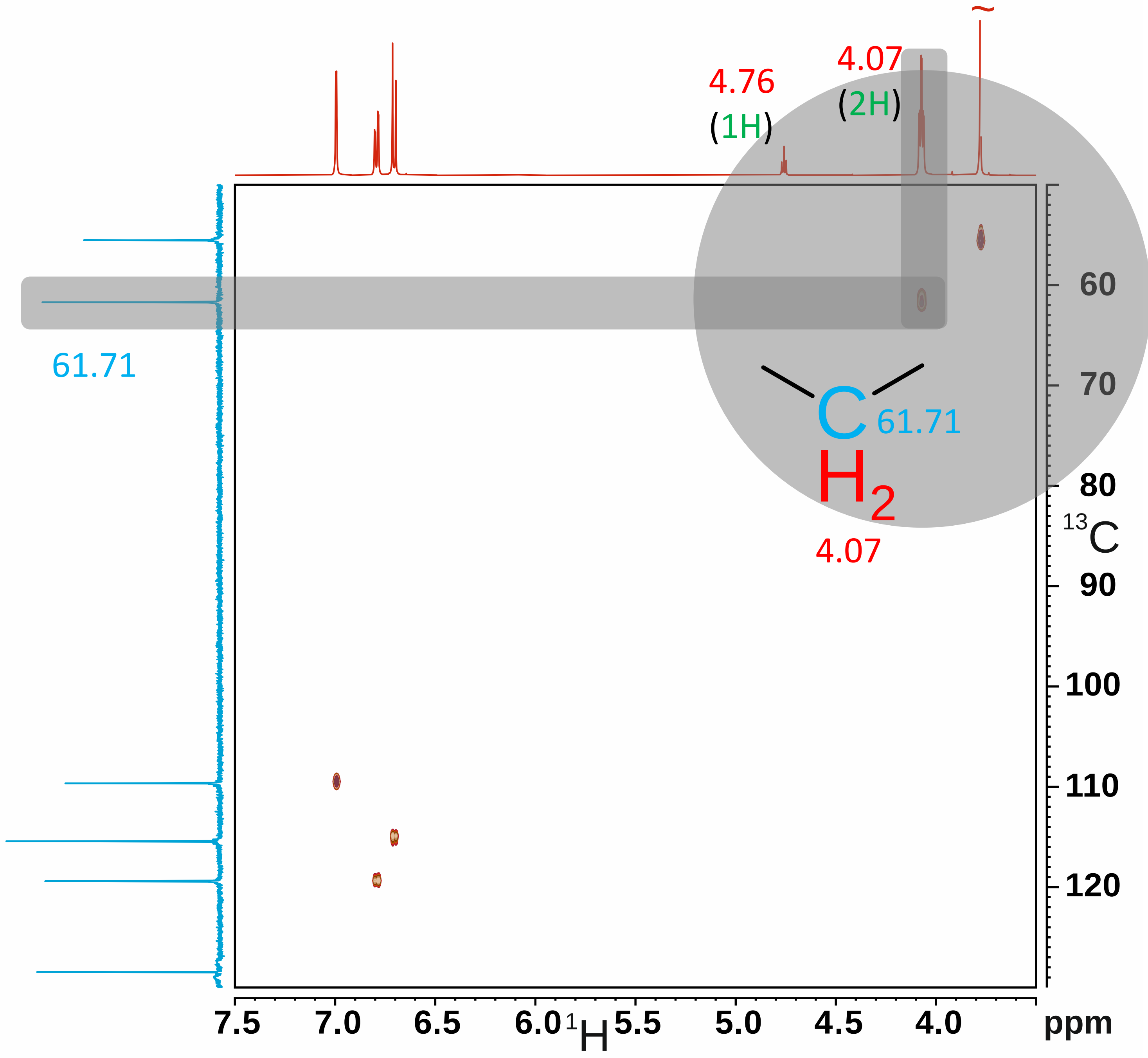
First partial structure

Building blocks

15.84 Hz(d)	15.86 Hz(d)		1.66 Hz(d)
1.58 Hz(t)	5.41 Hz(t)	5.48 Hz(t)	5.45 Hz(t)
6.42	6.18	4.76	4.07
(1H)	(1H)	(1H)	(2H)



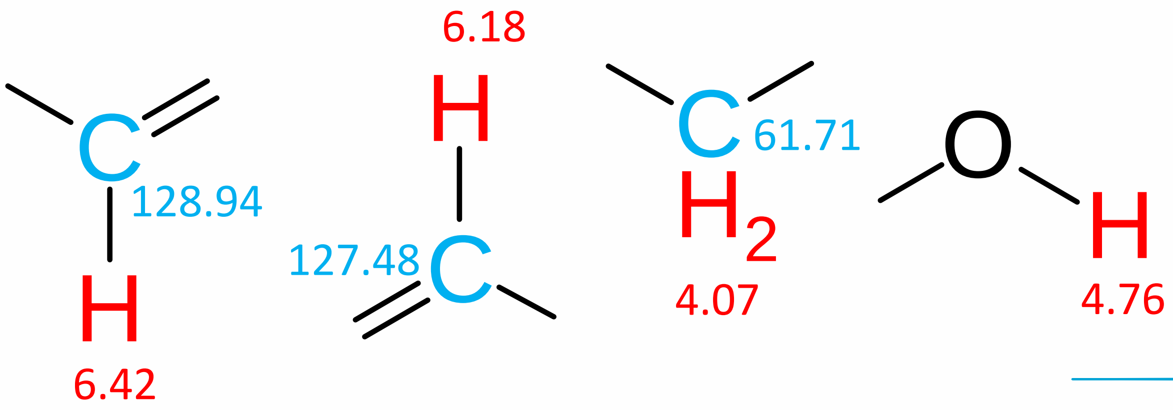
And the proton multiplet 4.76 ppm?



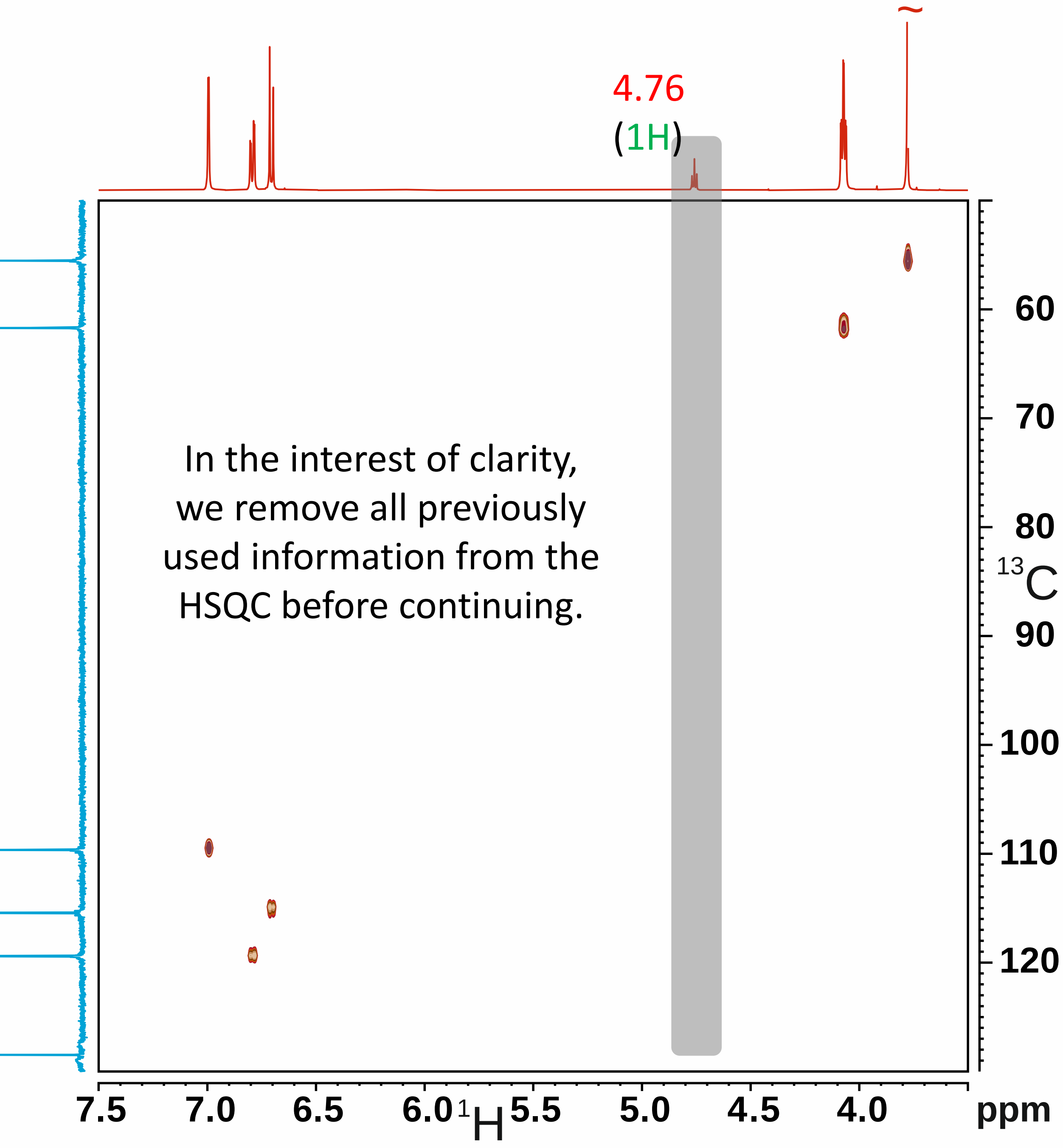
First partial structure

Building blocks

15.84 Hz(d)	15.86 Hz(d)		1.66 Hz(d)
1.58 Hz(t)	5.41 Hz(t)	5.48 Hz(t)	5.45 Hz(t)
6.42	6.18	4.76	4.07
(1H)	(1H)	(1H)	(2H)



There is no adjacent carbon atom. The proton at 4.76 ppm has to be an OH group.

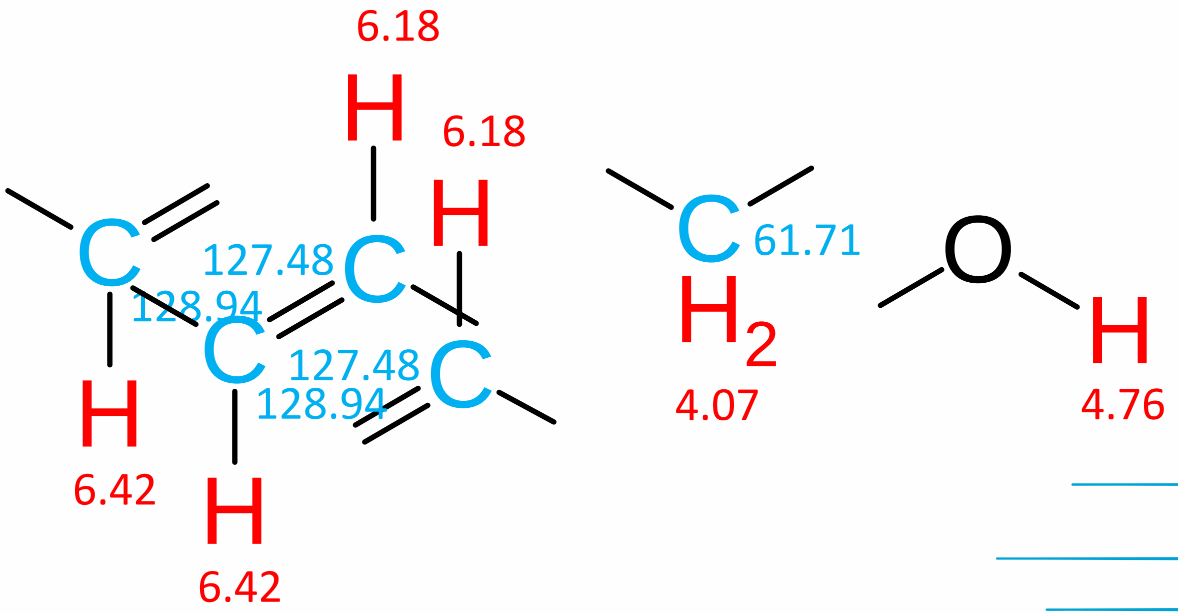


In the interest of clarity, we remove all previously used information from the HSQC before continuing.

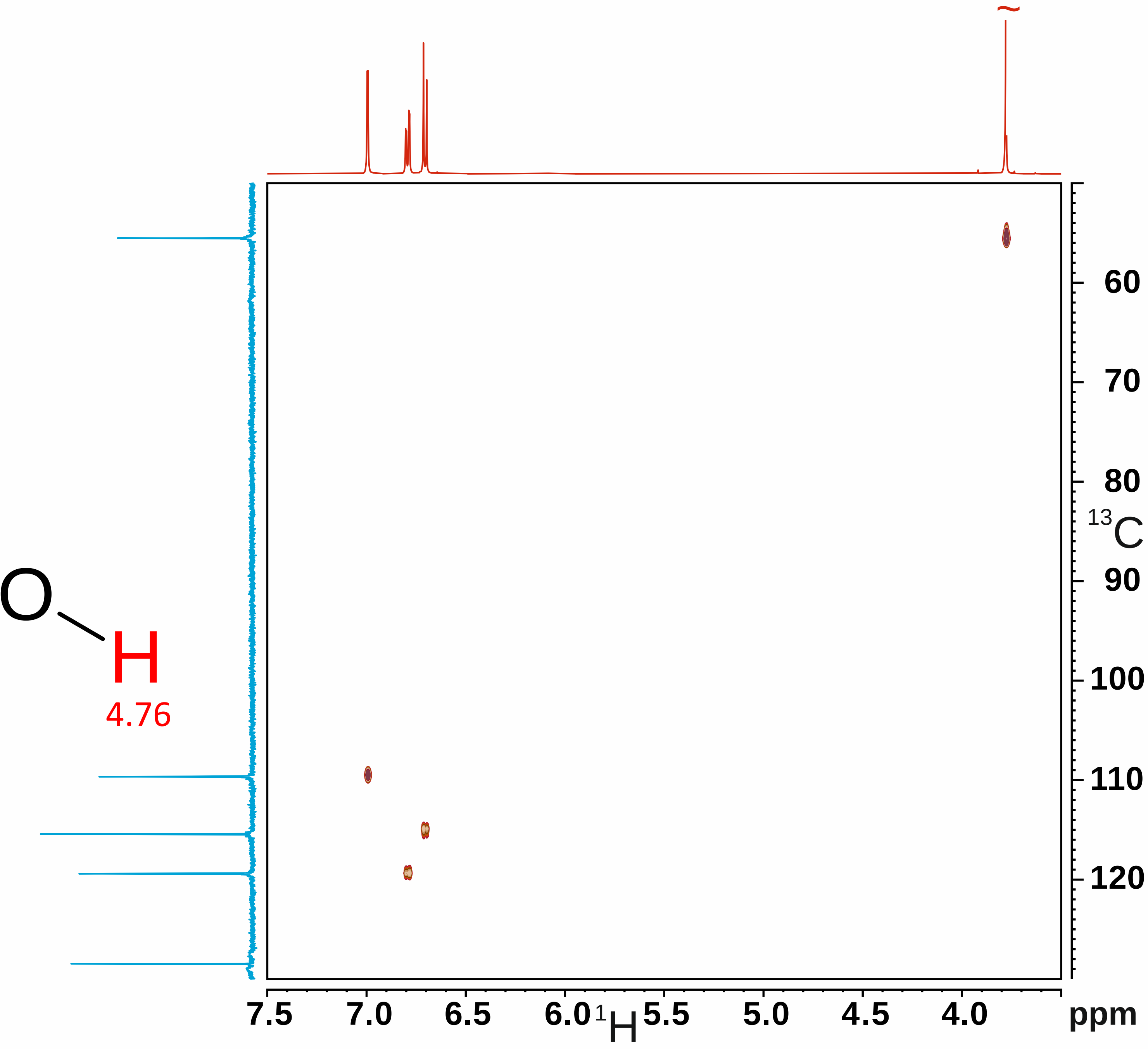
First partial structure

Combine the fragments

15.84 Hz(d)	15.86 Hz(d)		1.66 Hz(d)
1.58 Hz(t)	5.41 Hz(t)	5.48 Hz(t)	5.45 Hz(t)
6.42	6.18	4.76	4.07
(1H)	(1H)	(1H)	(2H)



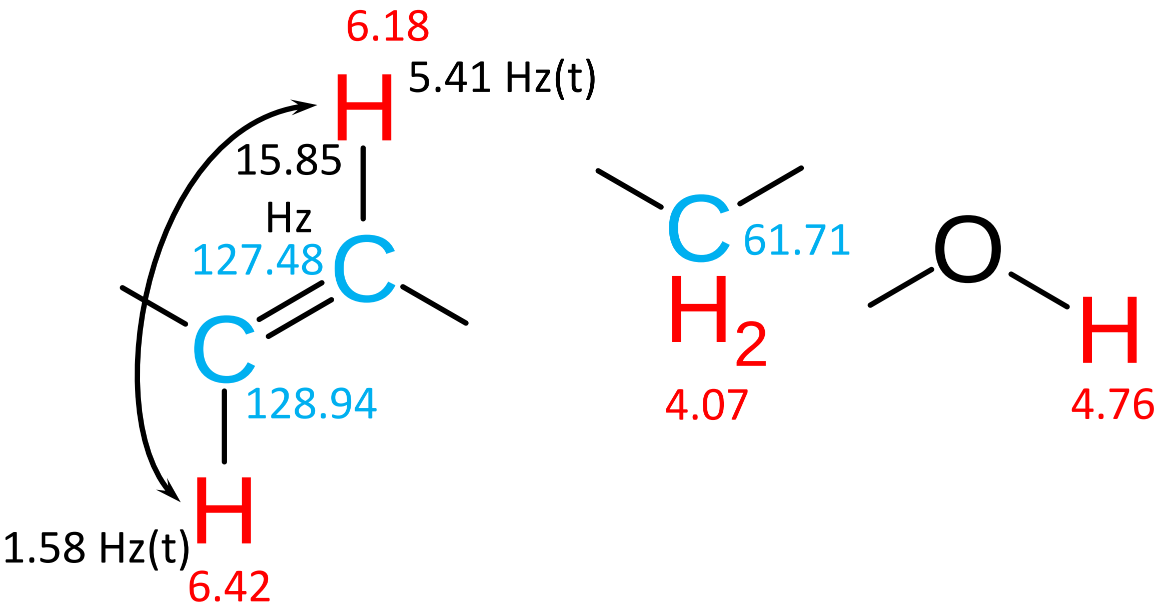
There is only one possible combination for the two **=CH-** fragments.



First partial structure

Combine the fragments

15.84 Hz(d)	15.86 Hz(d)		1.66 Hz(d)
1.58 Hz(t)	5.41 Hz(t)	5.48 Hz(t)	5.45 Hz(t)
6.42	6.18	4.76	4.07
(1H)	(1H)	(1H)	(2H)



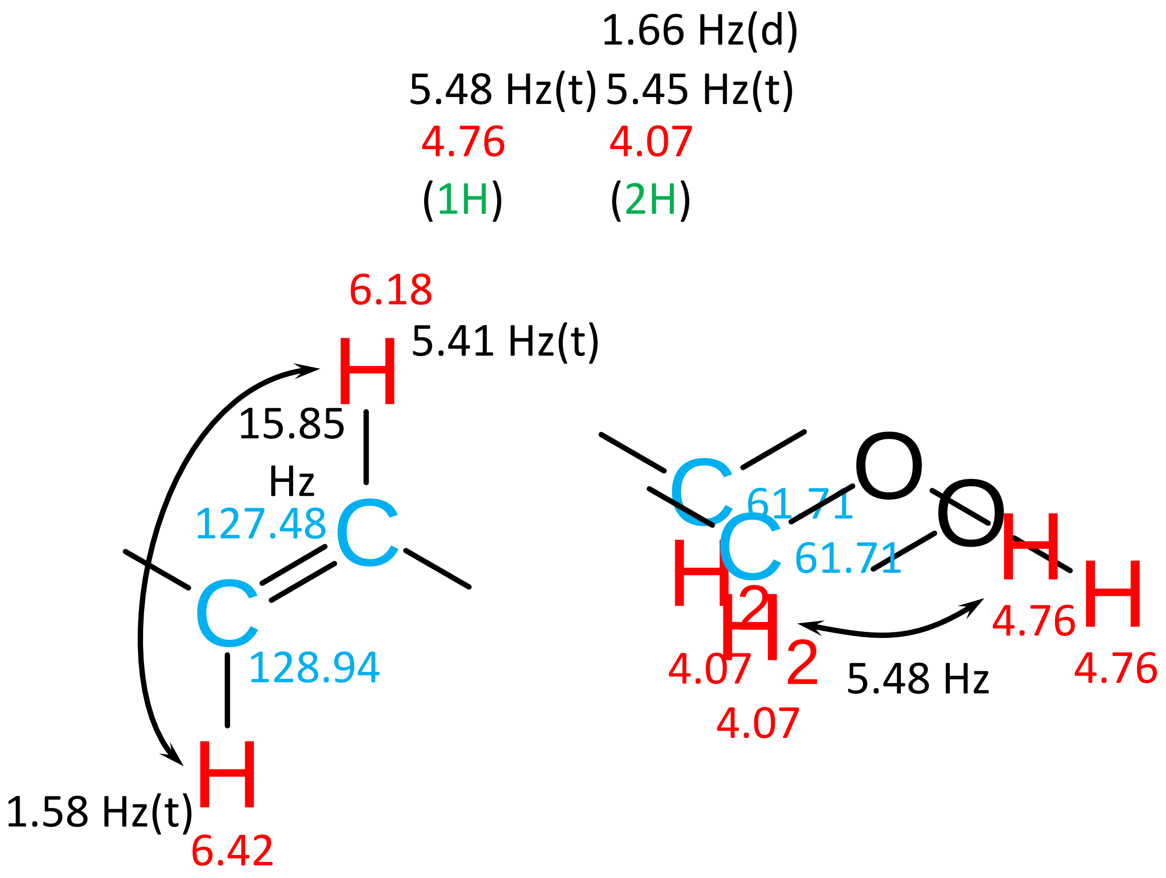
Both proton multipletts have a common coupling constant of **15.85 Hz** (averaged value). Thus the protons are trans to each other.

We note the other two coupling constants of **1.58 Hz** and **5.41 Hz** next to the protons for future use.

The multiplet of the OH proton is a clean triplet. The OH proton can only be bound to the methylene group..

First partial structure

Combine the fragments

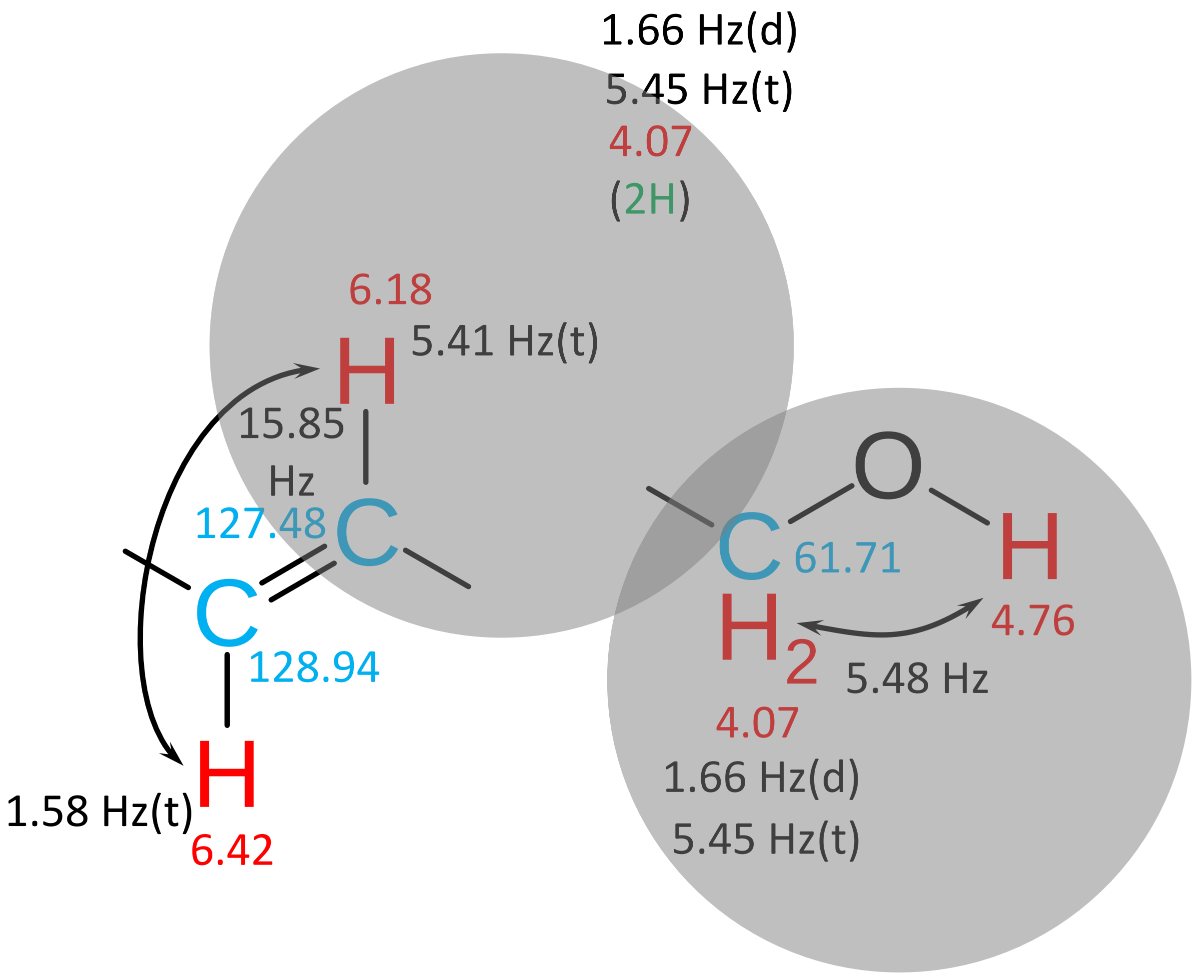


And what about the two coupling constants in the multiplet of methylene protons at 4.07 ppm?

A *triplet* with a coupling constant of **5.45 Hz** and a *doublet* with a coupling constant of **1.66 Hz**?

First partial structure

Combine the fragments



The multiplet of the methylene protons cannot possibly be a triplet. There are no two neighbouring protons that are equivalent. However, a doublet of doublets with the very similar coupling constants of **5.48 Hz** (OH group) and **5.41 Hz** (=CH- group) would be a good explanation.

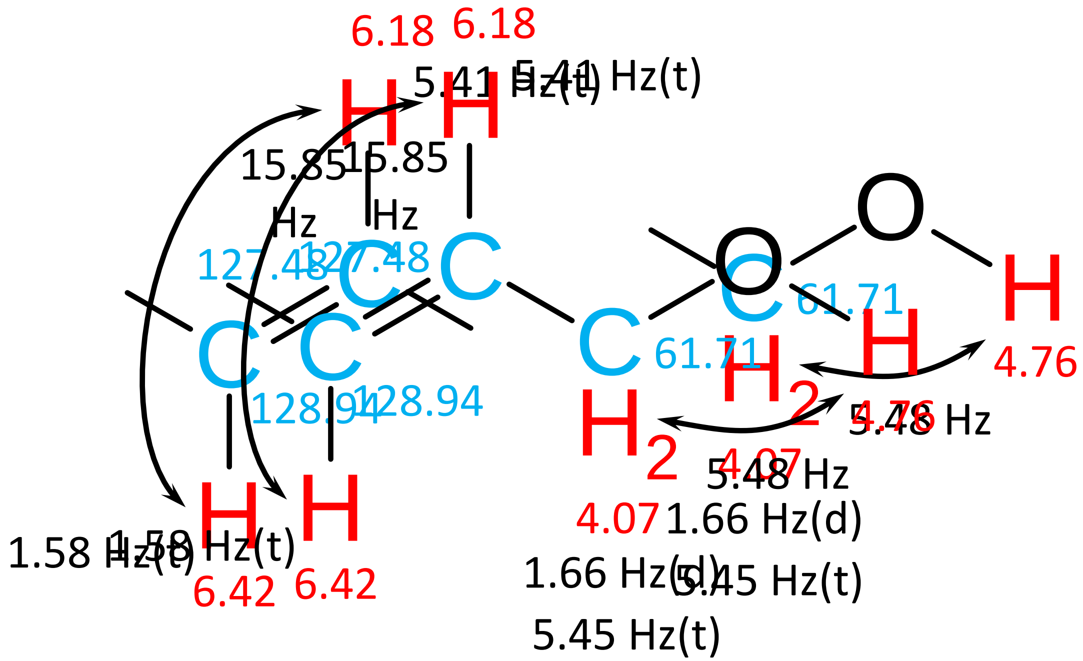
This results in a pseudo triplet with an apparent coupling constant that corresponds to the averaged value of the two values mentioned above.

We keep the doublet splitting of **1.66 Hz** for future use.

5.41 Hz is a typical vicinal coupling constant, the -CH$_2$-OH group is bound to the =CH- group with the proton signal at 6.18 ppm.

First partial structure

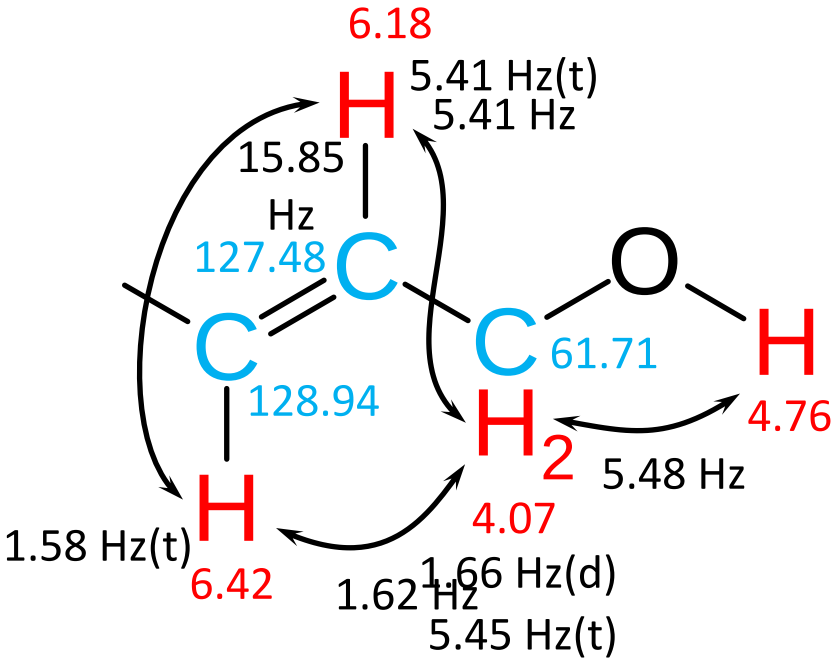
Combine the fragments



The coupling constants unassigned so far can now be easily explained.

First partial structure

Combine the fragments



At this point, it makes sense to temporarily "hide" the structural fragment found so far with the molecular formula C_3H_5O and to continue with the extraction of the remaining pieces of information from the HSQC.

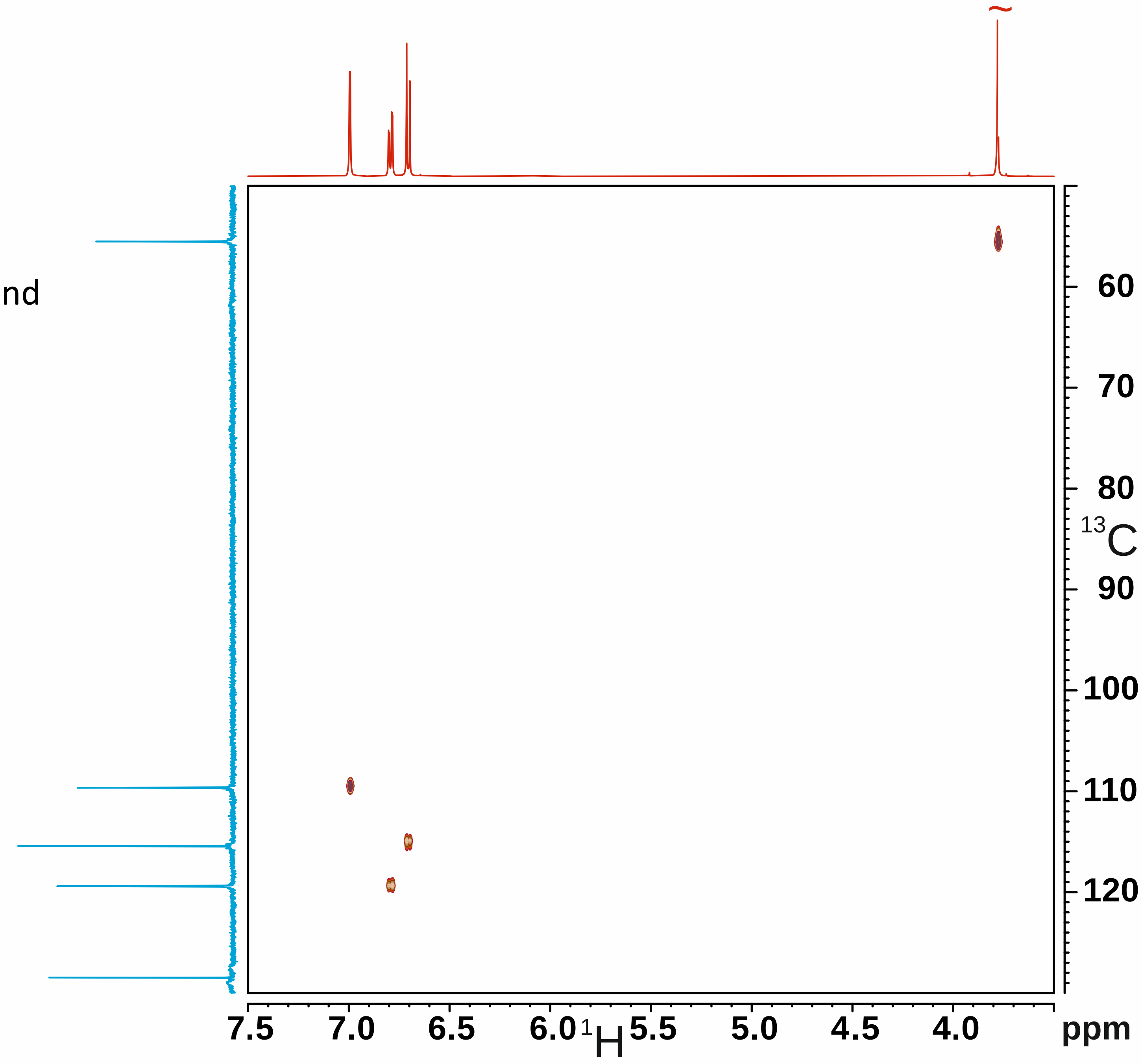
If we subtract C_3H_5O from the molecular formula, we still have to assign

- $C_7H_7O_2$ and
- four double bond equivalents.

More building blocks

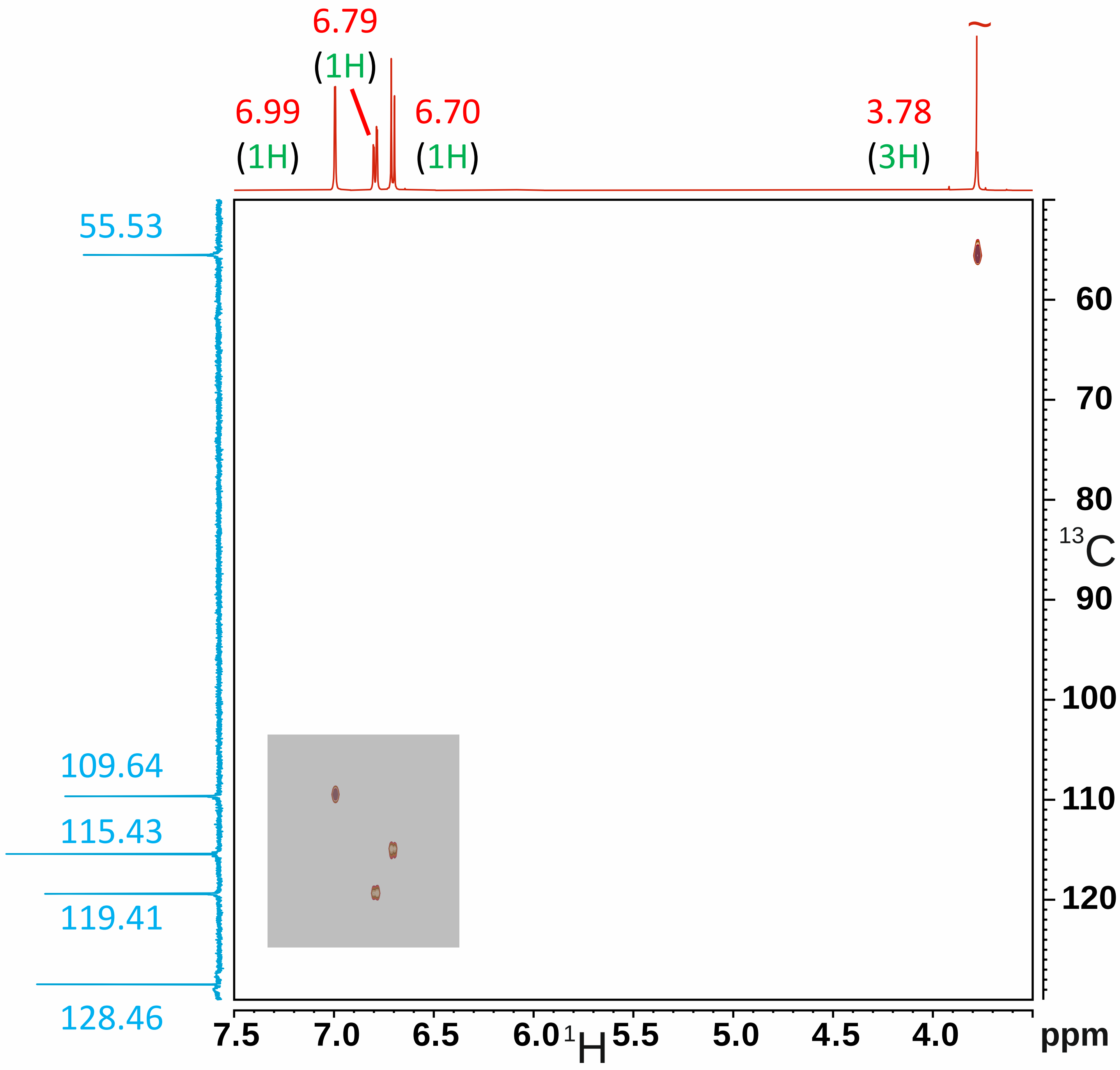
HSQC

First we take the chemical shifts from the proton and carbon one dimensional spectra, as well as the integrals from the proton spectrum and add those values to the HSQC projections.



More building blocks

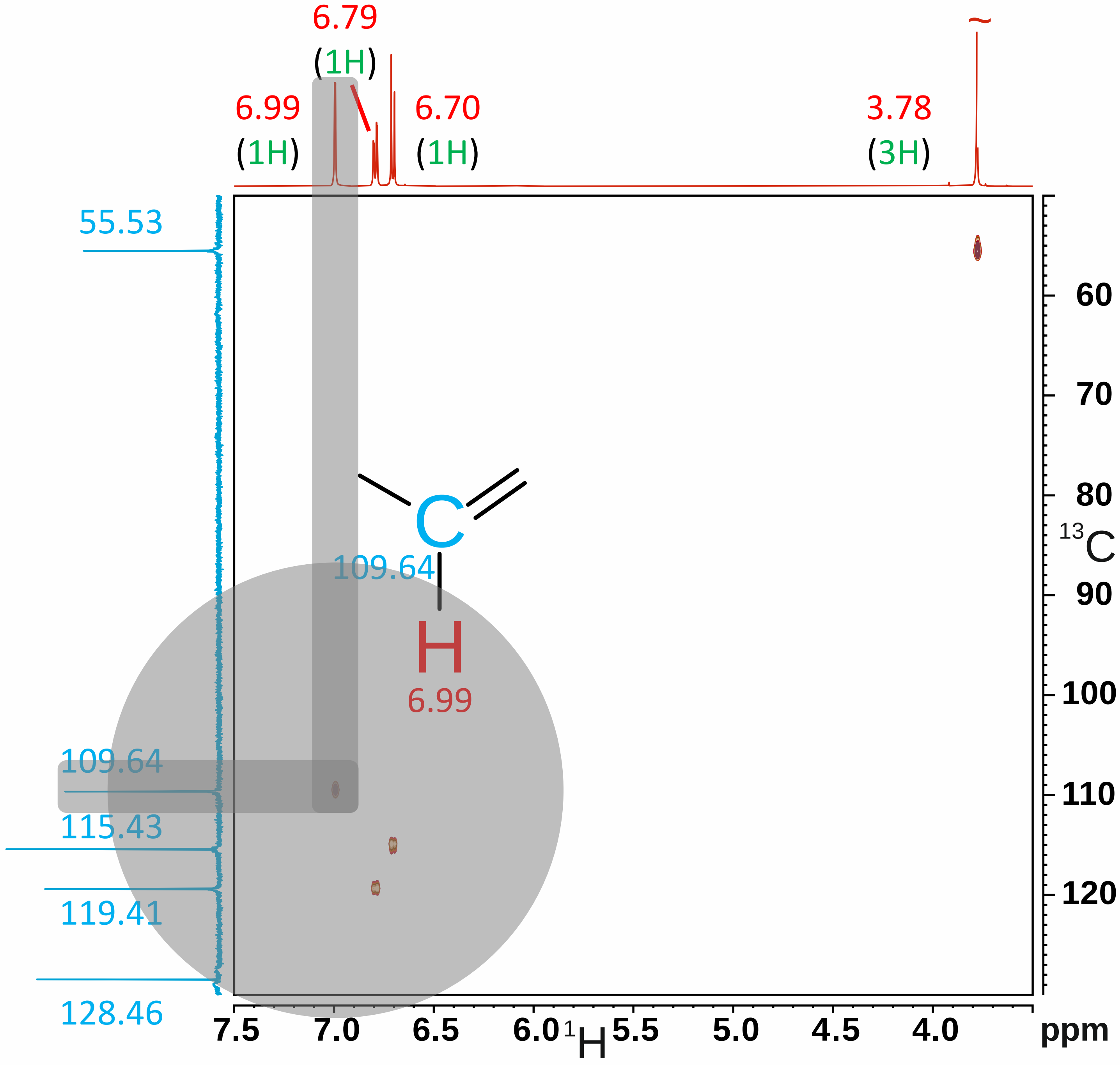
HSQC

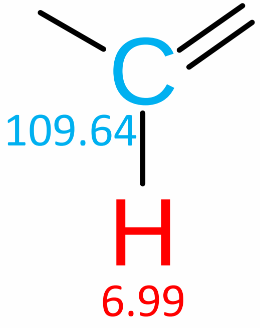


Three cross peaks clearly belong to three different =CH- groups.

More building blocks

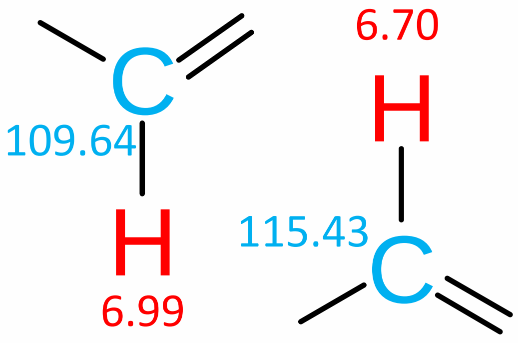
HSQC

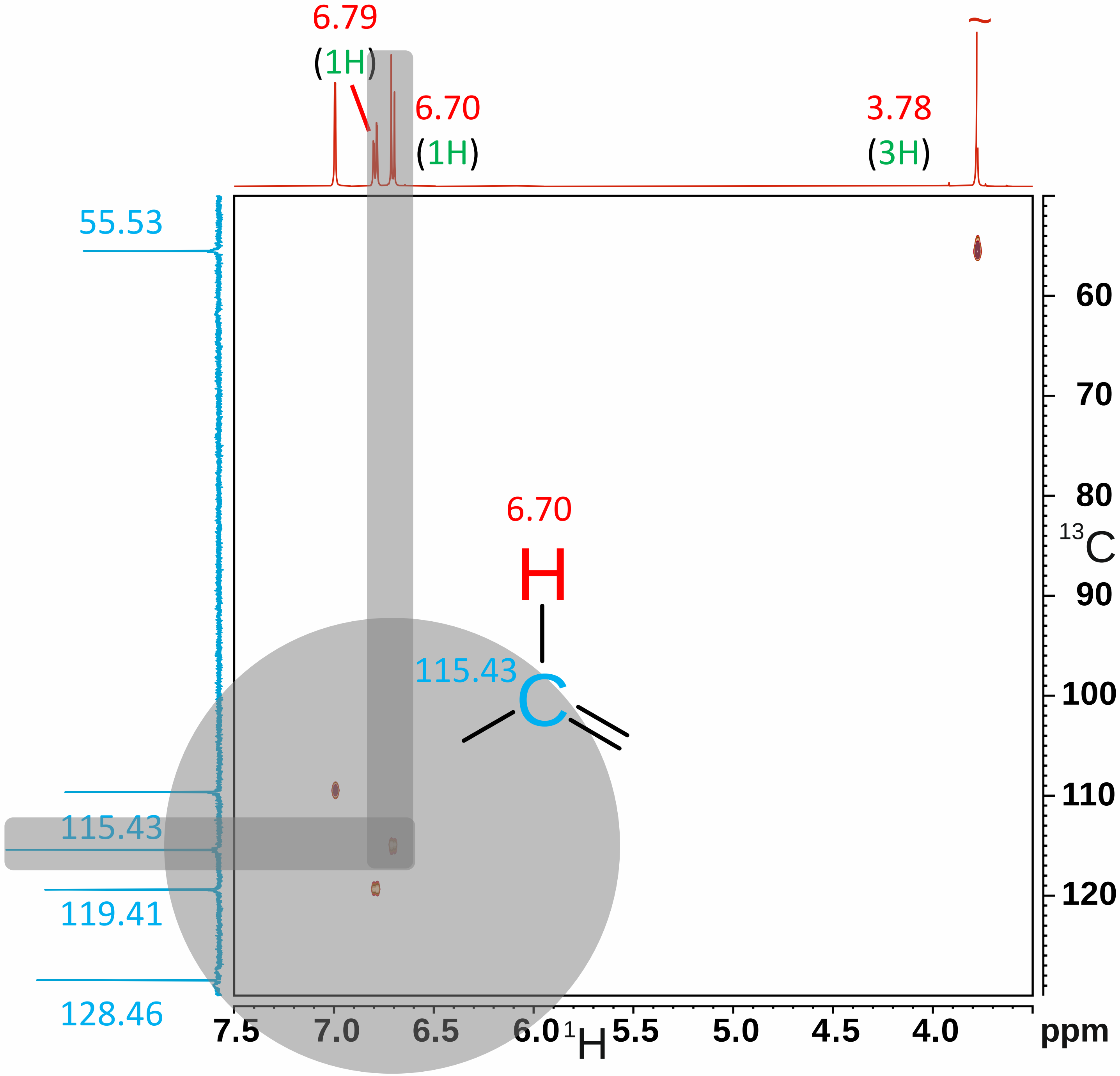




More building blocks

HSQC

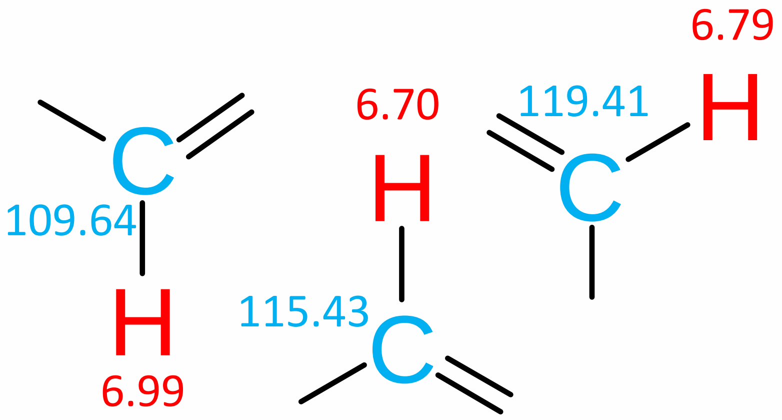


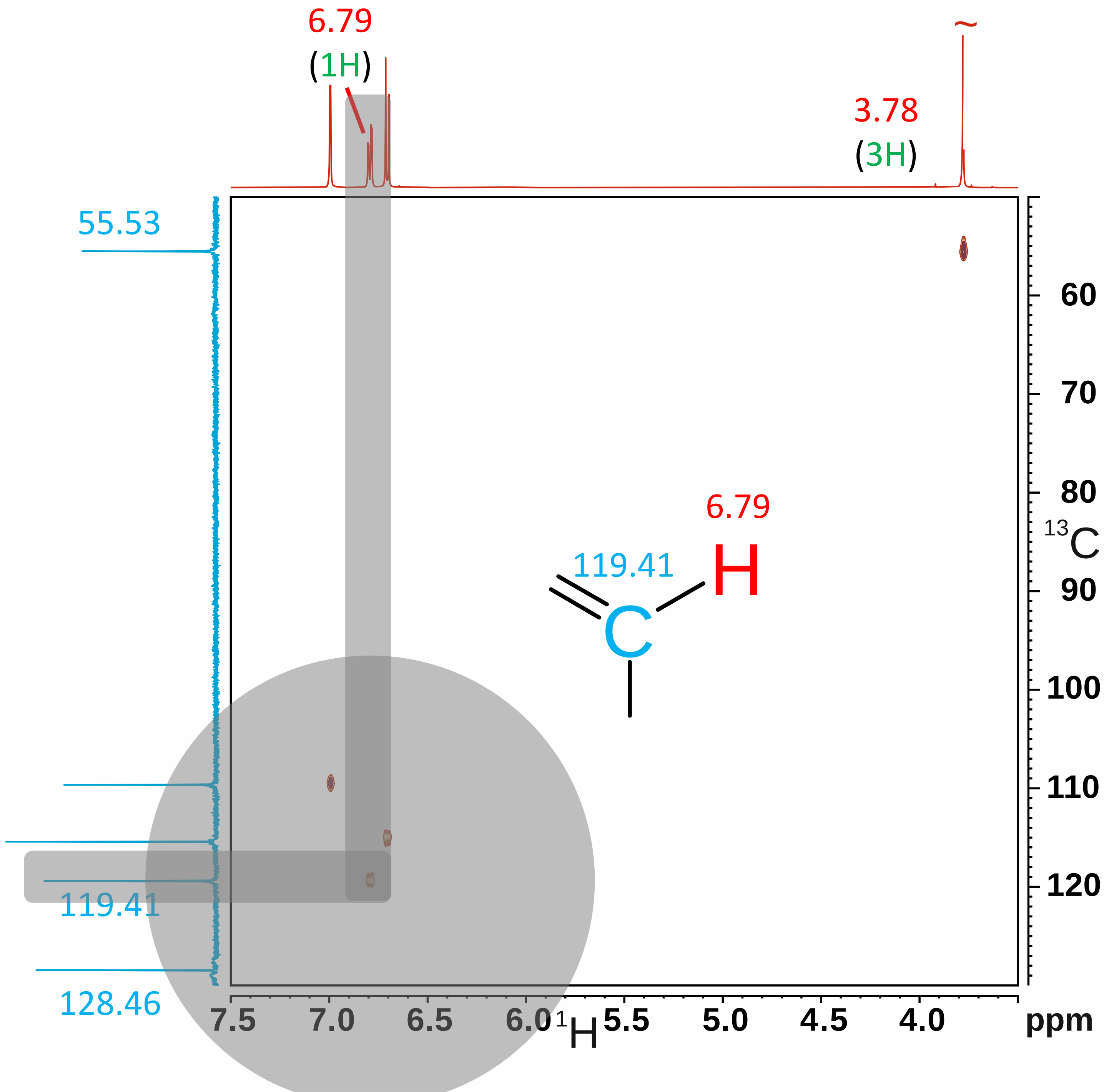


More building blocks

HSQC

Finally there is a methyl group.

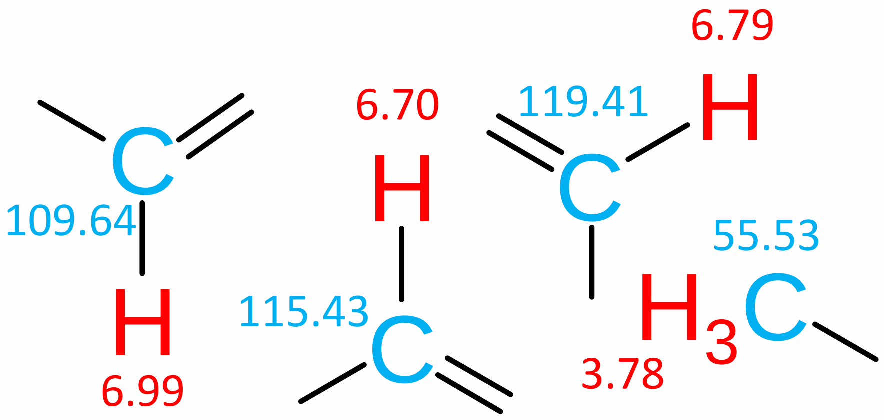


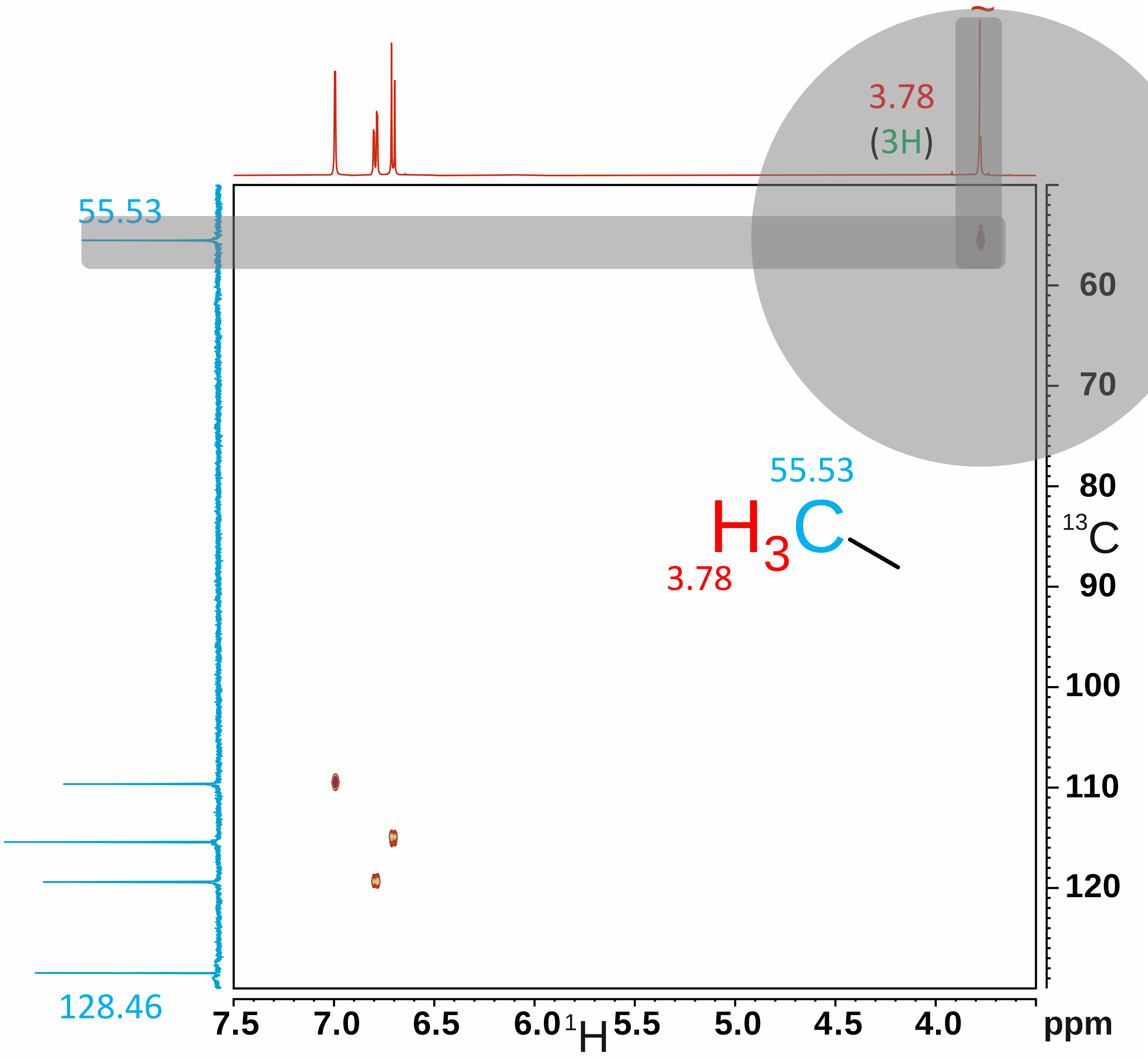


More building blocks

HSQC

And the carbon signal with the chemical shift of 128.46 ppm?





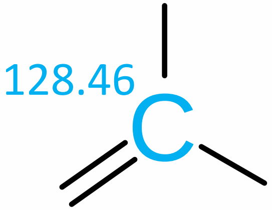
More building blocks

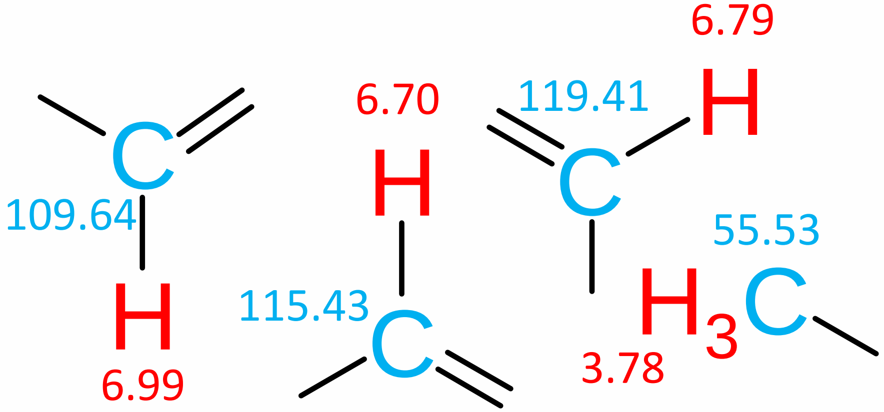
HSQC

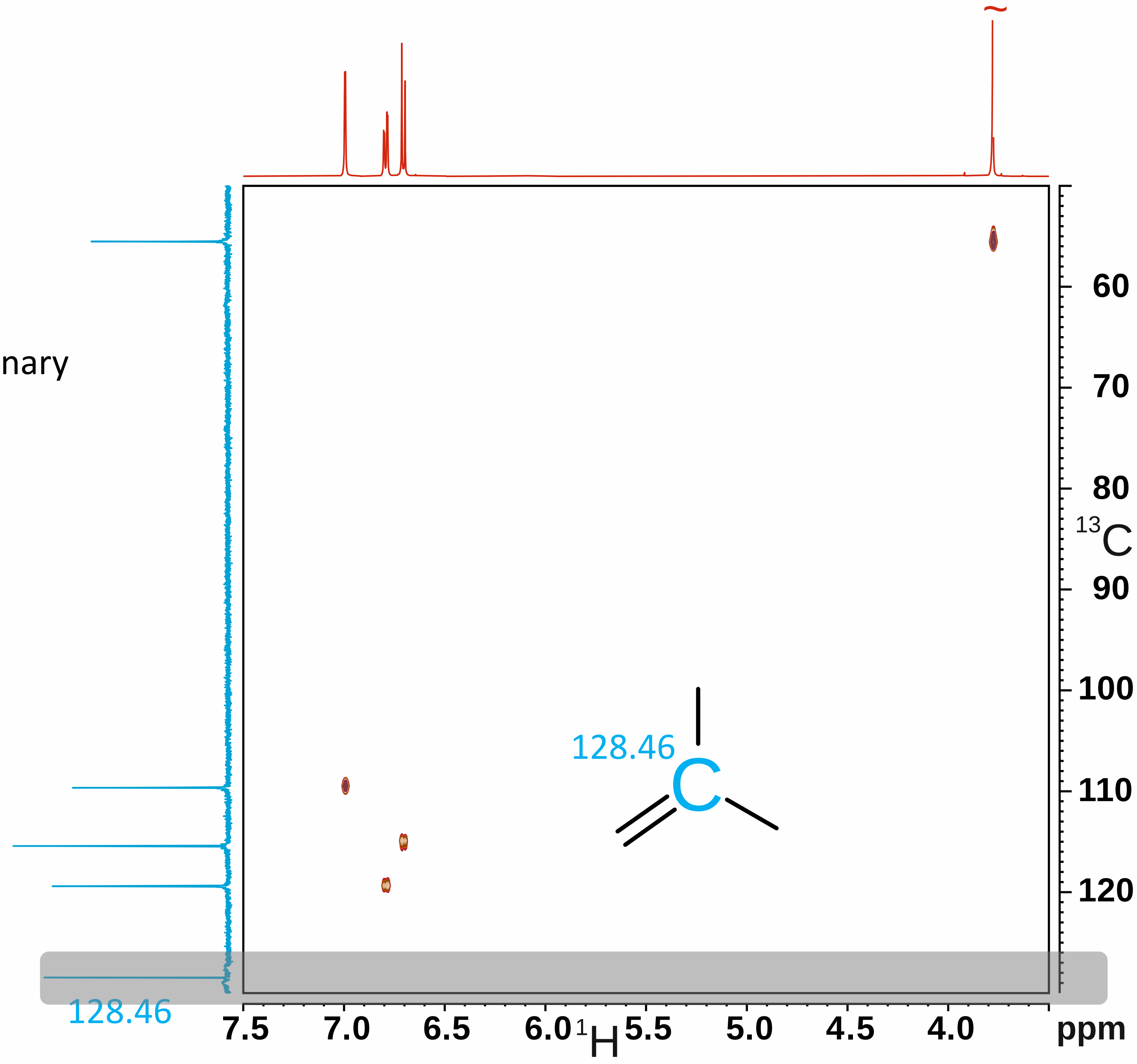
And the carbon signal with the chemical shift of 128.46 ppm?

There is no cross peak in the HSQC, that‘s a quaternary carbon atom.

What else is missing?



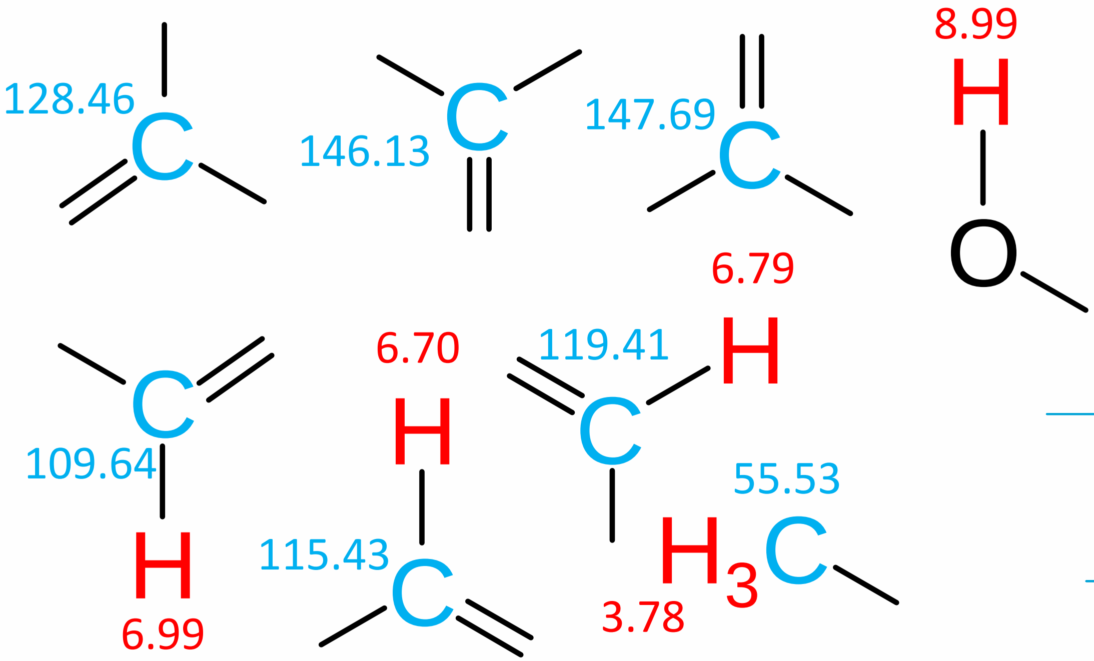


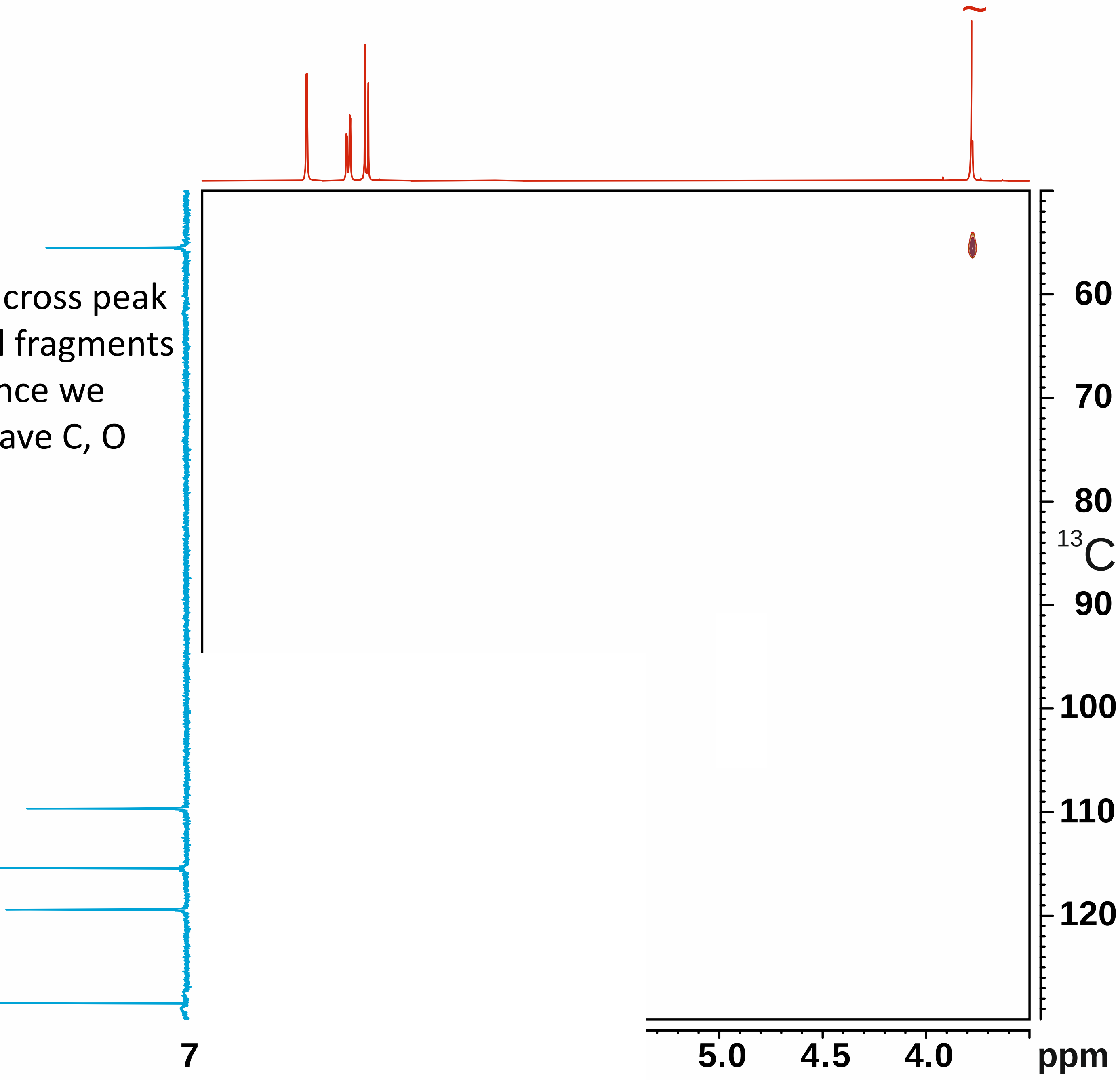


Last building blocks

1D signals without
corresponding HSQC cross peak

Three signals in the ^{1}H and ^{13}C spectra do not show any cross peak in the HSQC. This immediately results in three structural fragments (For the proton, the binding partner has to be oxygen once we exclude the possibility of a CH fragment since we only have C, O and H in the molecular formula).



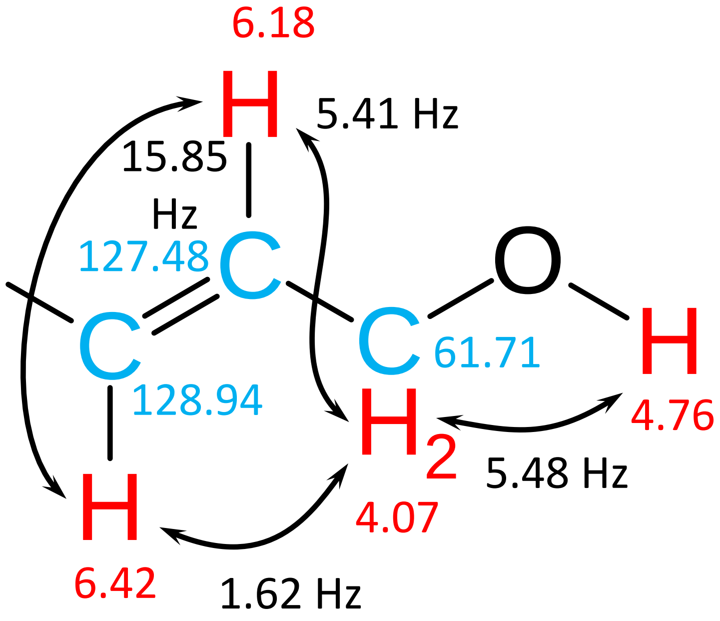


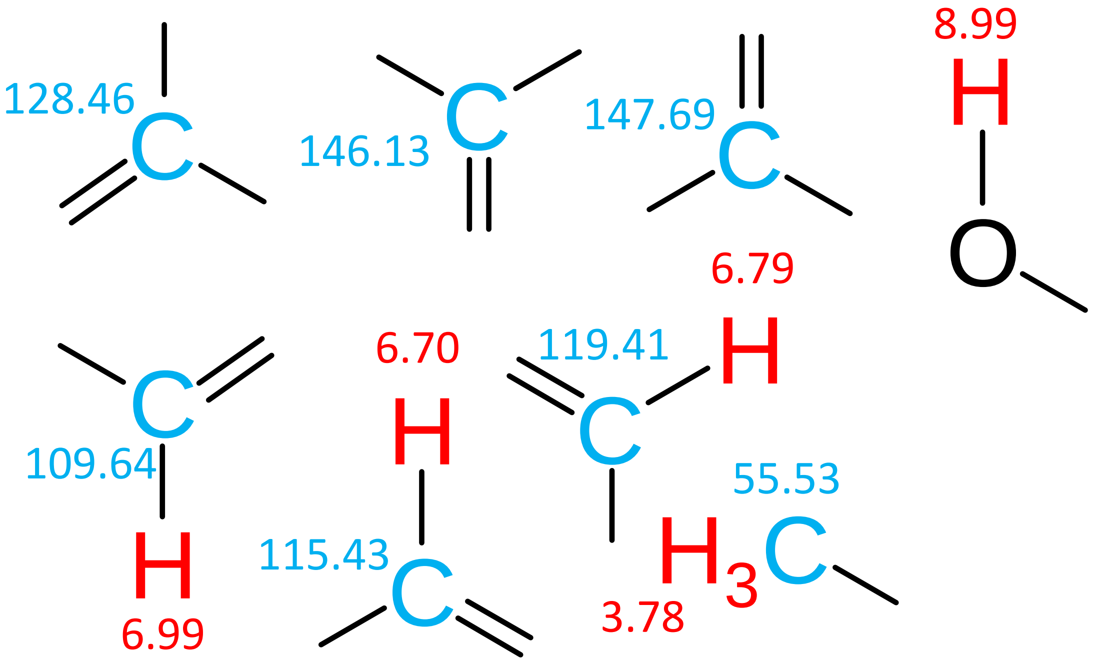
Final building blocks

A short inventory

still missing

- one oxygen atom
- one double bond equivalent



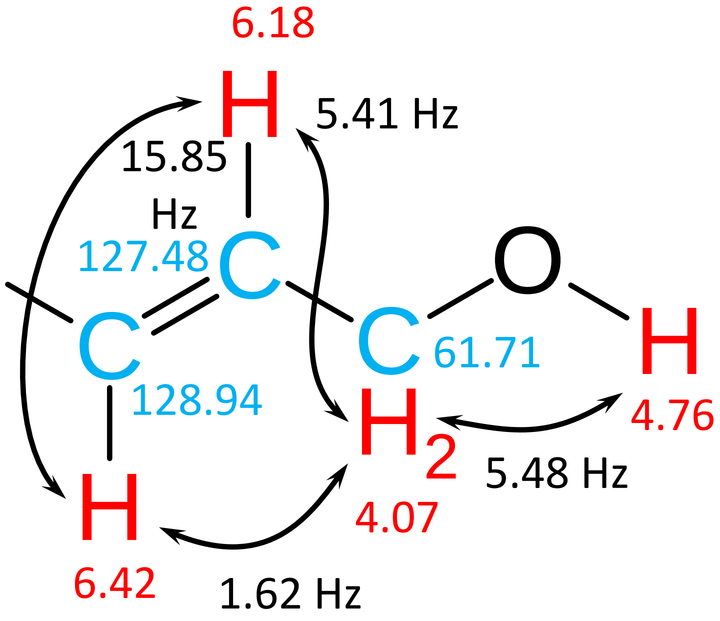


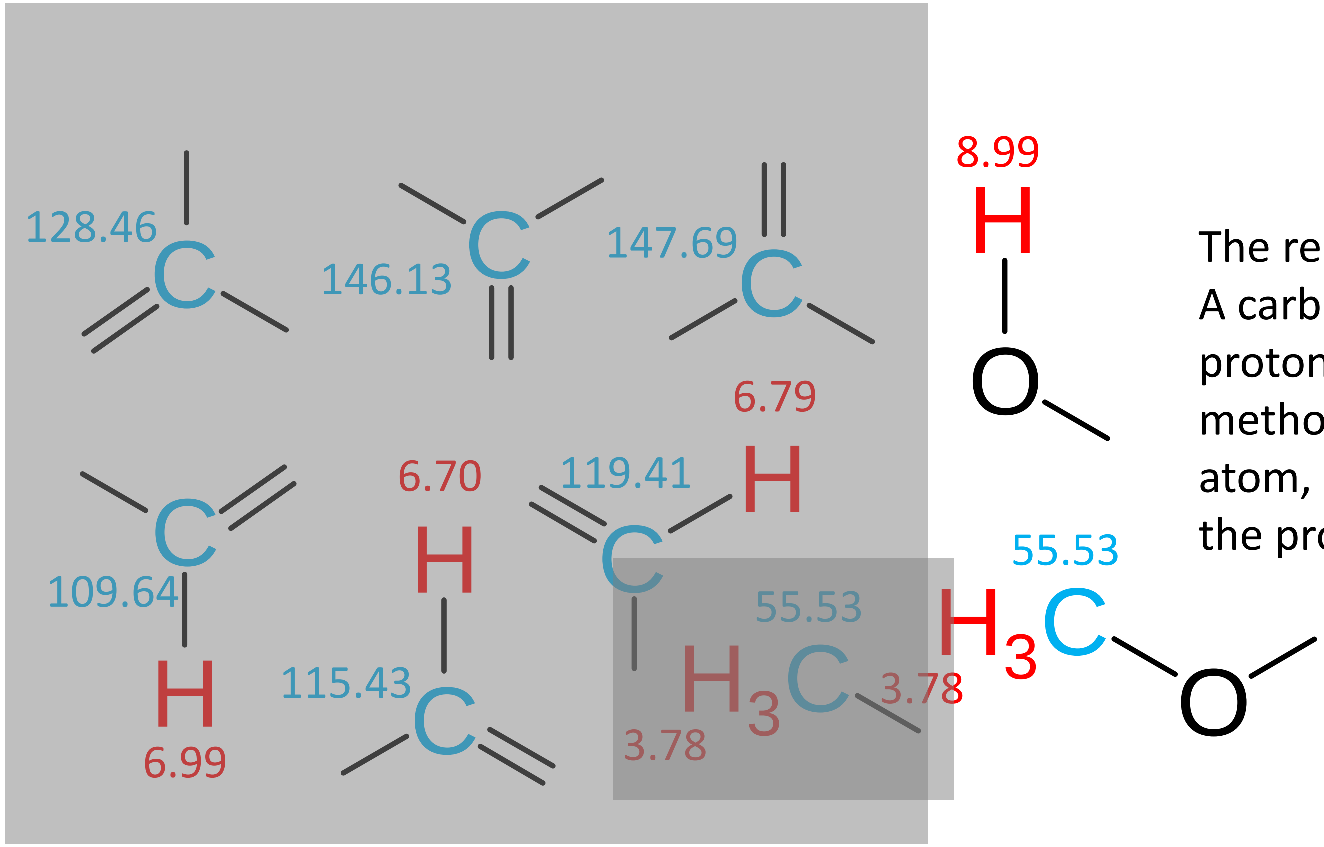
atoms found so far	$C_{10}H_{12}O_2$
double bond equivalents	4
molecular formula	$C_{10}H_{12}O_3$
resulting double bond equivalents from molecular formula	5

Final building blocks

A short inventory

And these six fragments, when put together, would make a nice phenyl ring with three free valences - matching the three remaining structural fragments - and the still missing double bond equivalent.



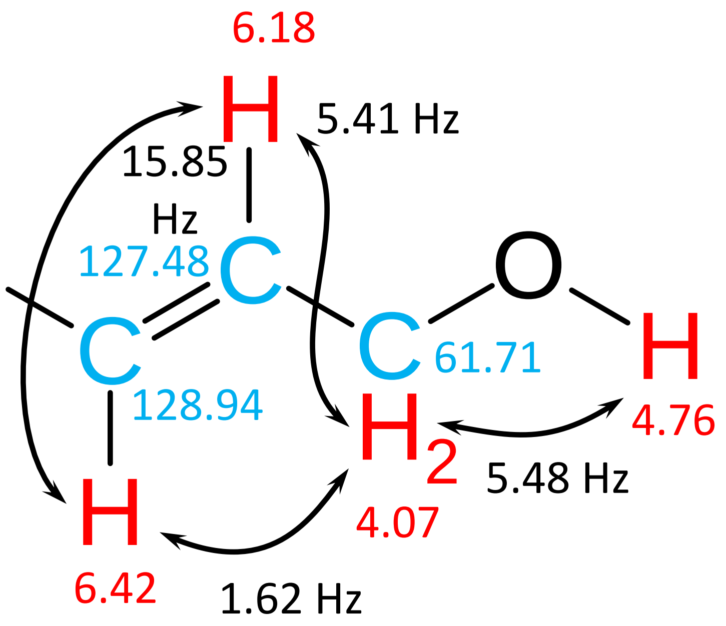


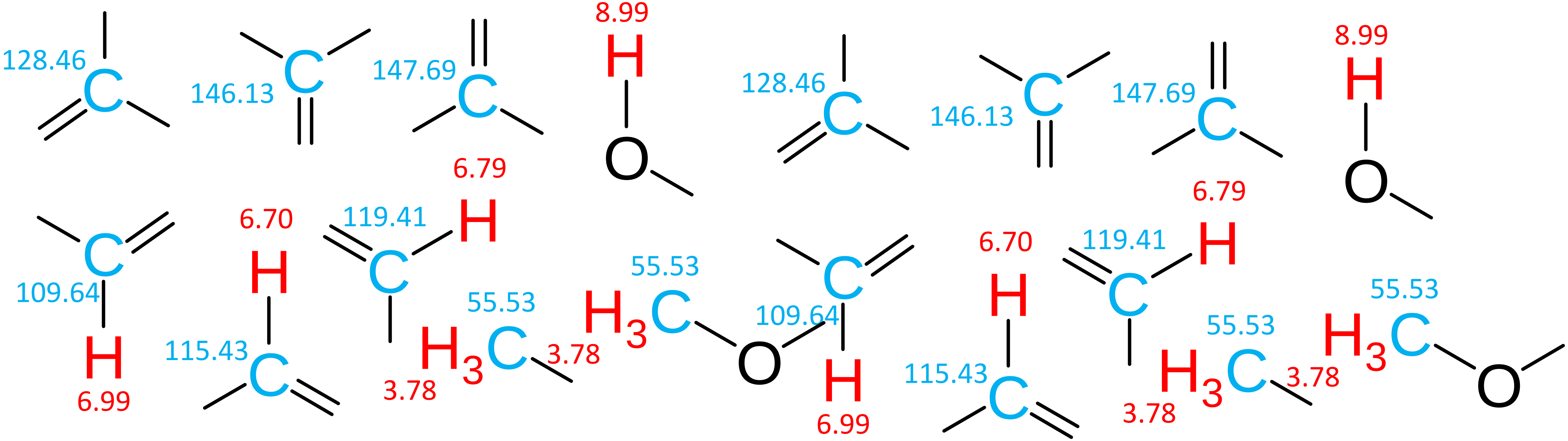
The remaining oxygen fits best next to the methyl group. A carbon signal with a chemical shift of about 60 ppm and a proton signal at about 4 ppm are very characteristic for a methoxy group. With the methyl group next to an sp^2 carbon atom, we would expect chemical shifts of about 2.5 … 3 ppm in the proton spectrum and 25 ppm in the carbon spectrum.

Final building blocks

A short inventory

Which patterns are possible, if we assume a triple substituted phenyl ring?



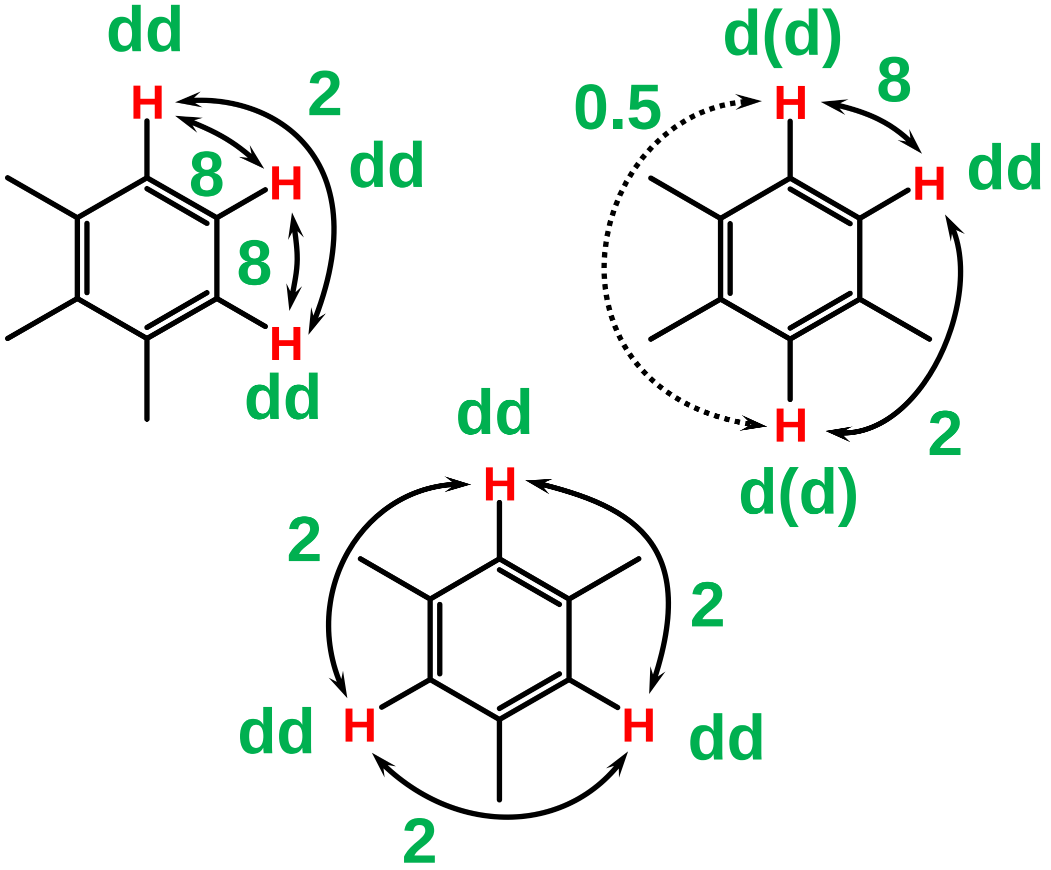


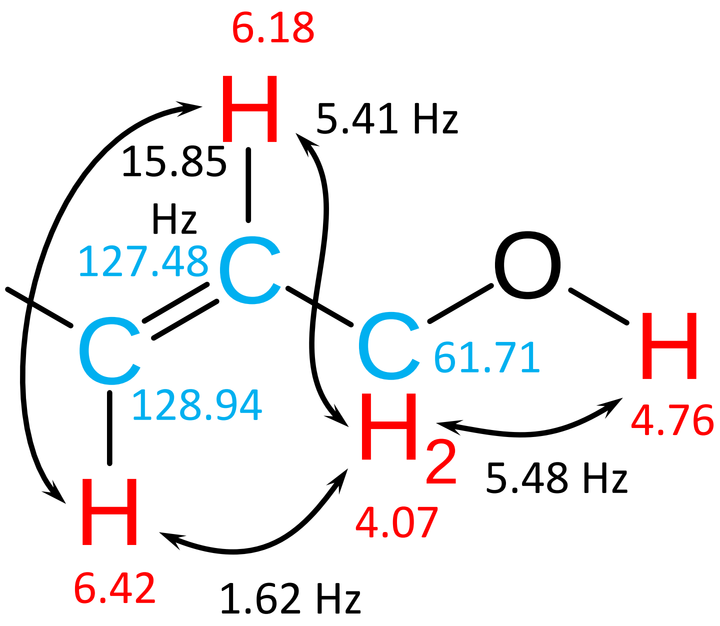
Phenyl ring

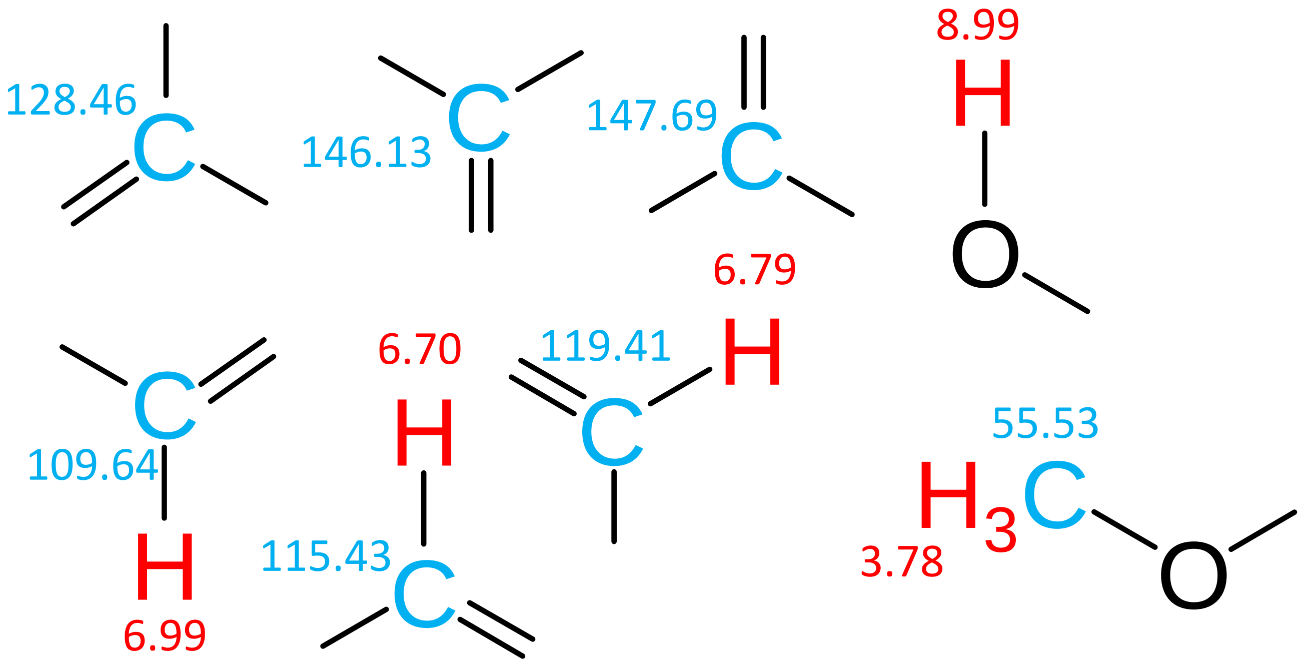
Three base structures

We expect the following coupling constants [**Hz**] and multiplets.

The dashed coupling path and the resulting multiplet structure might not be visible because of the very small coupling constant.



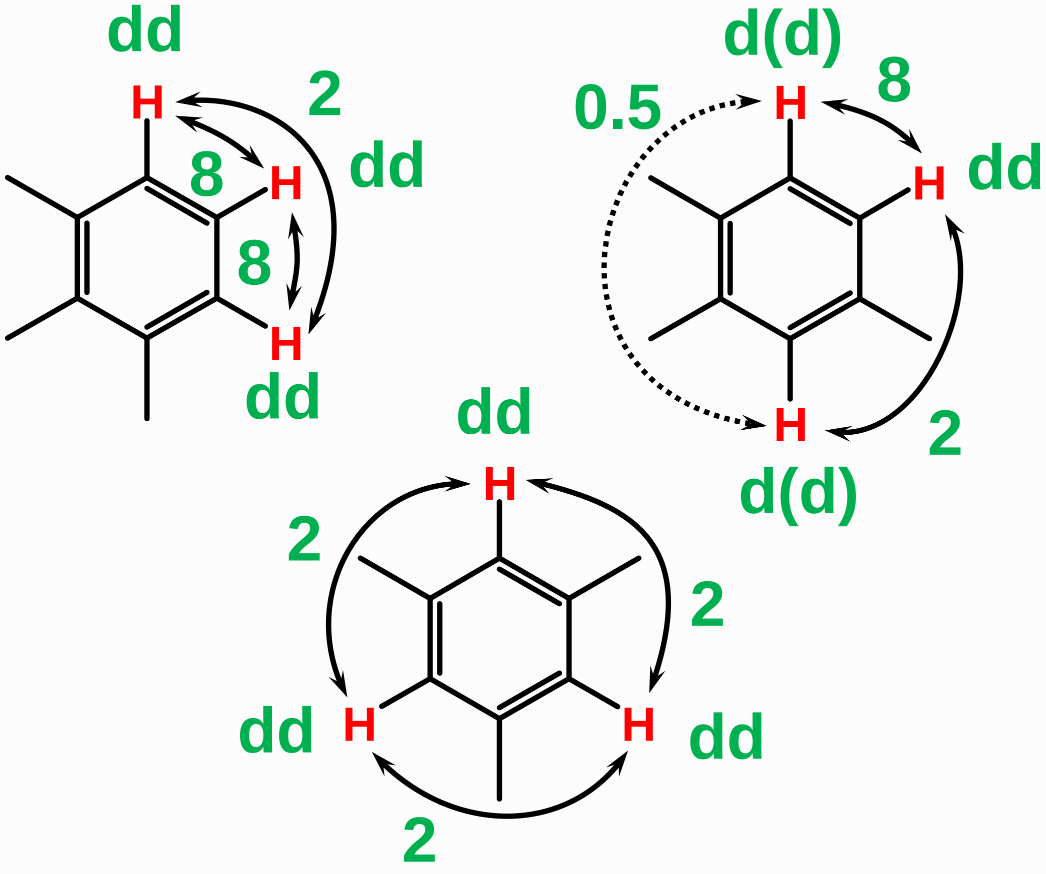


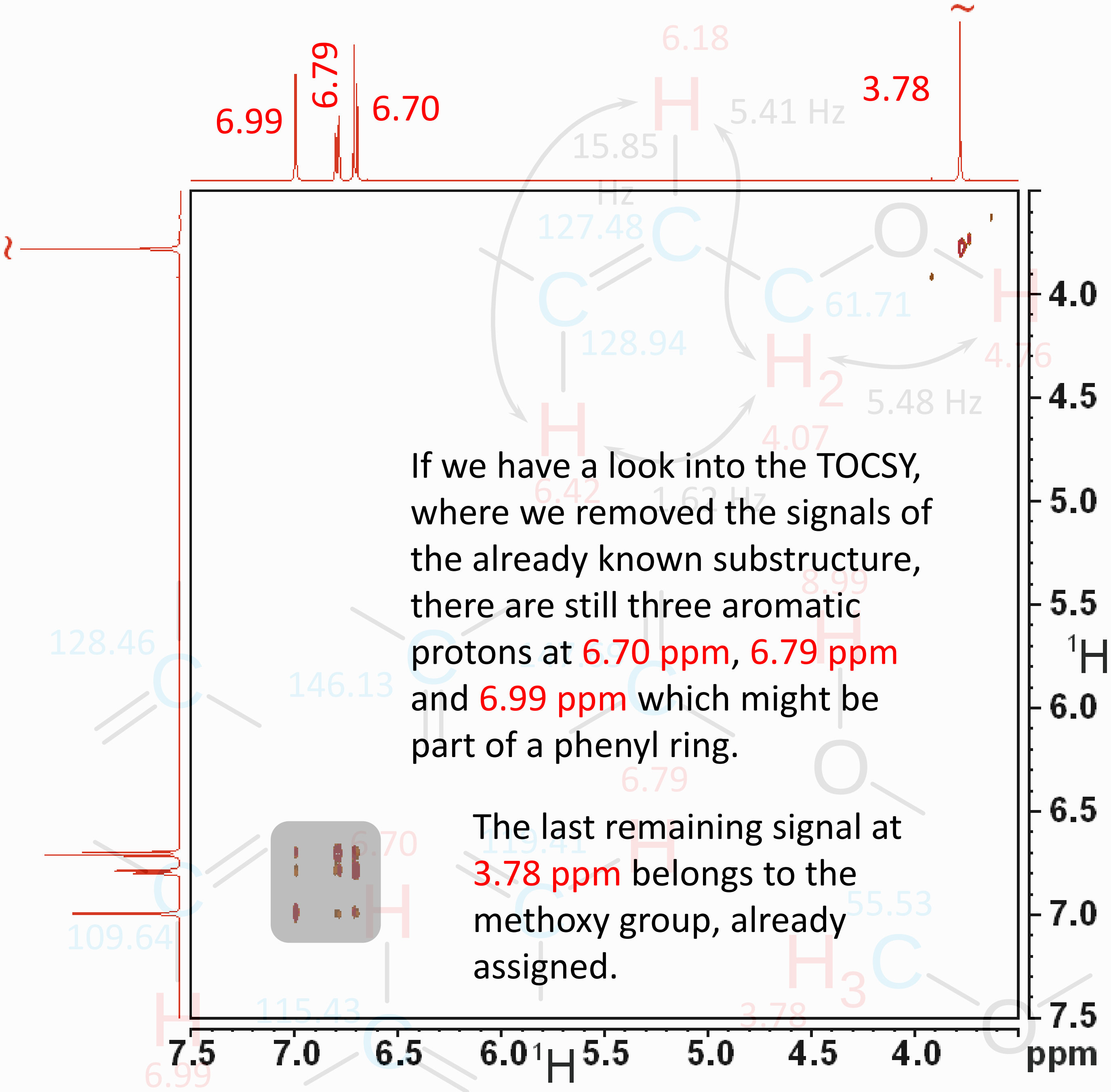


Phenyl ring

Three base structures

First lets have to look for three aromatic protons.





If we have a look into the TOCSY, where we removed the signals of the already known substructure, there are still three aromatic protons at 6.70 ppm, 6.79 ppm and 6.99 ppm which might be part of a phenyl ring.

The last remaining signal at 3.78 ppm belongs to the methoxy group, already assigned.

Phenyl ring

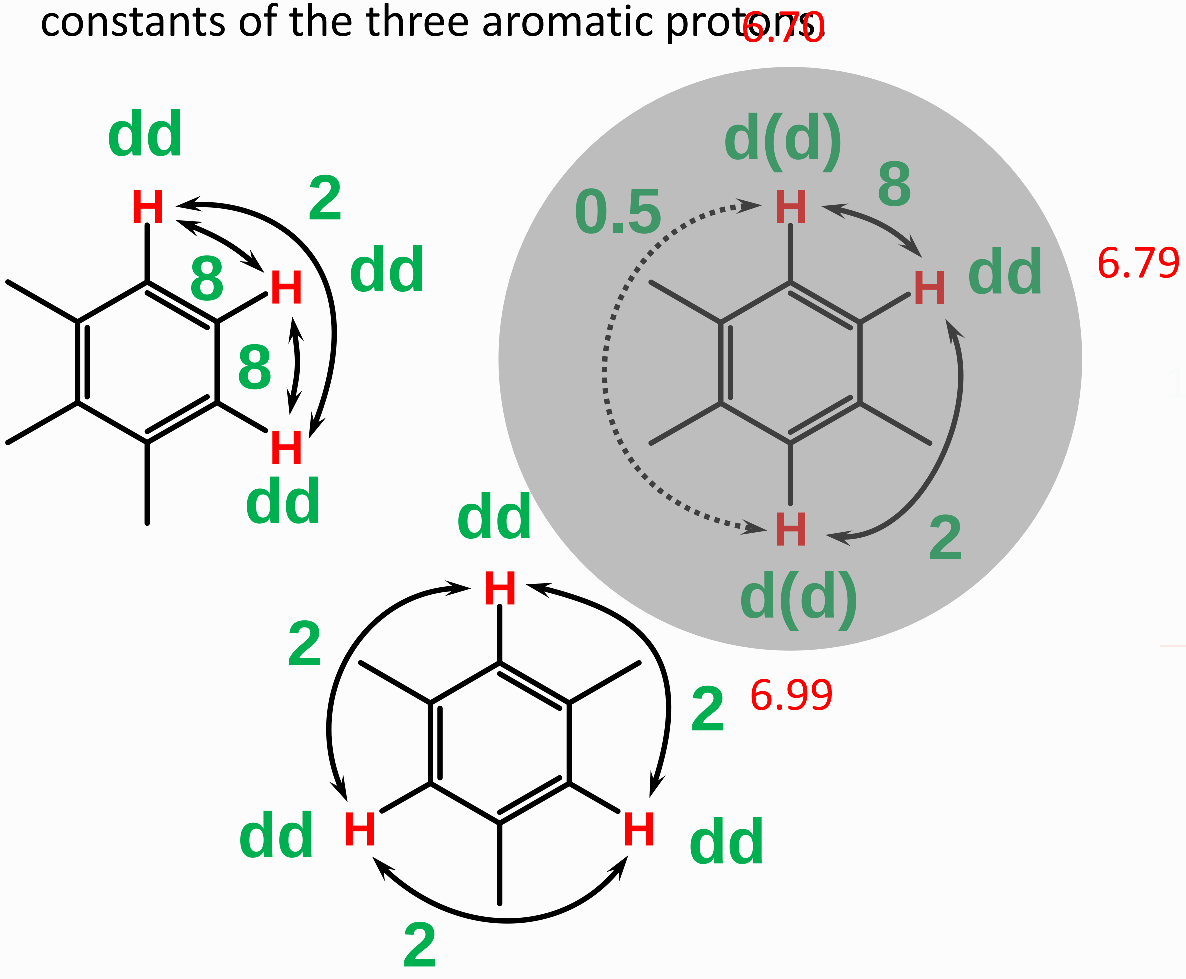
Multiplet structure of three
aromatic protons

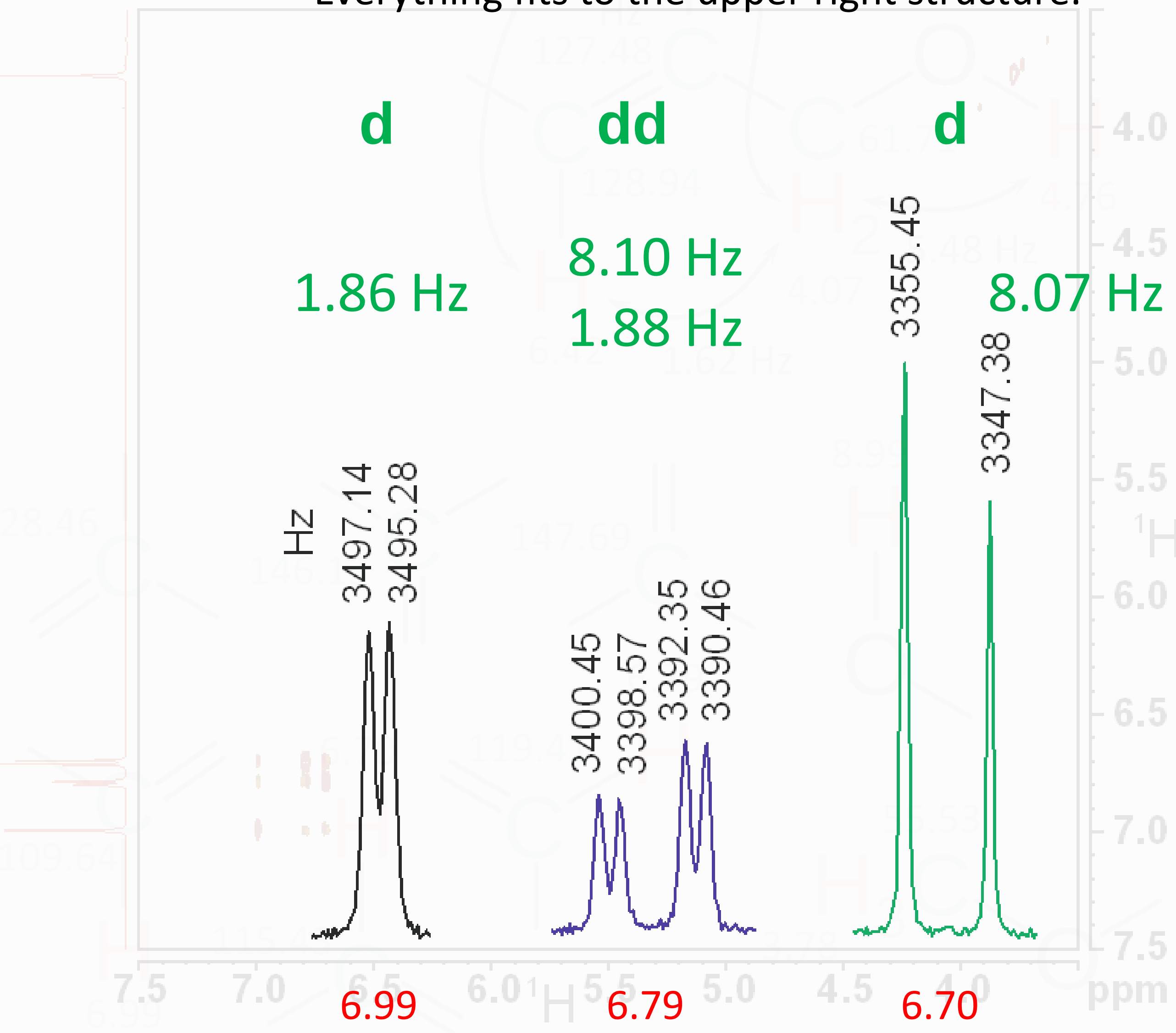
We no longer need the TOCSY.

We need the multiplet structure and the coupling constants of the three aromatic protons

It is easy to analyze the multiplets.

Everything fits to the upper right structure.

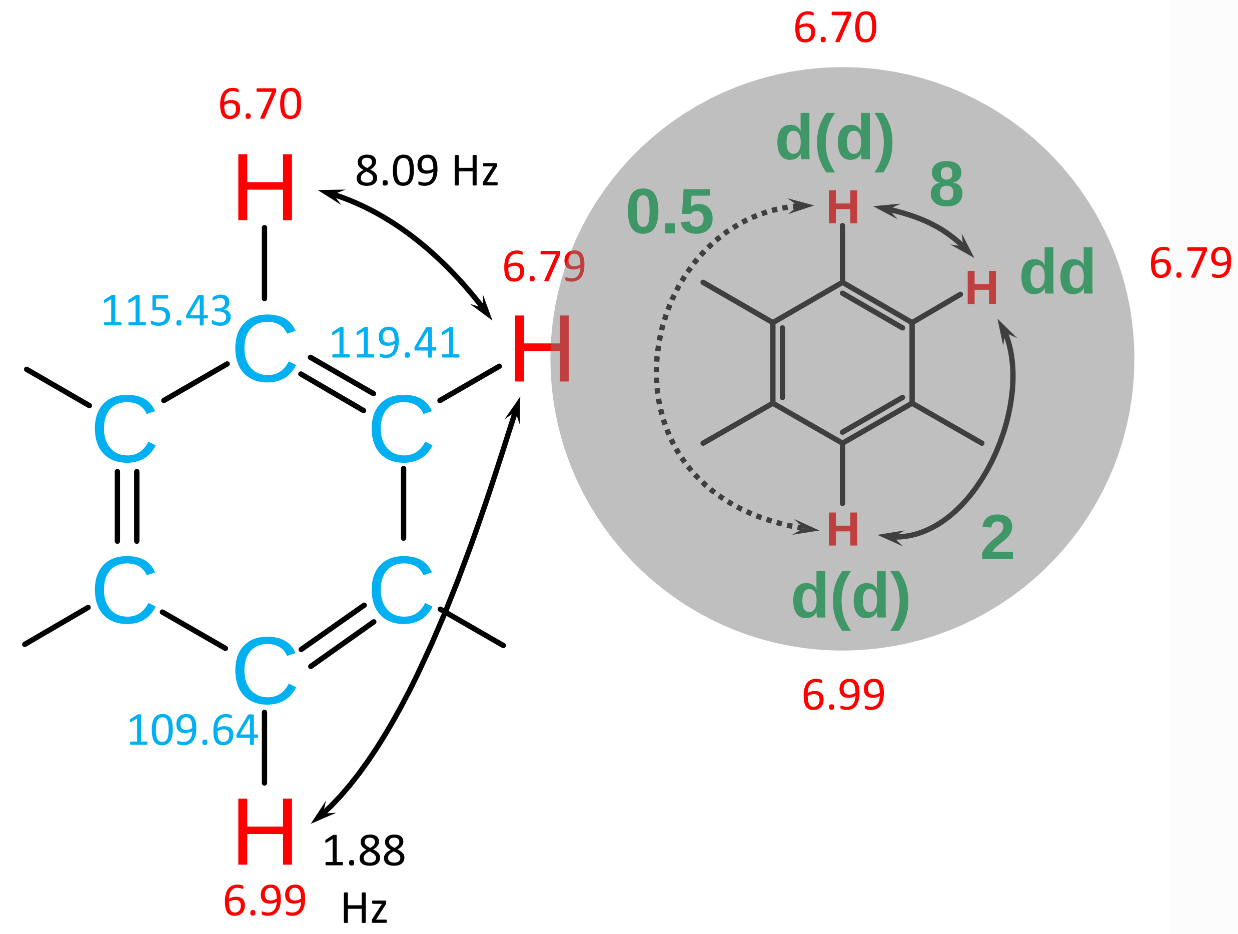


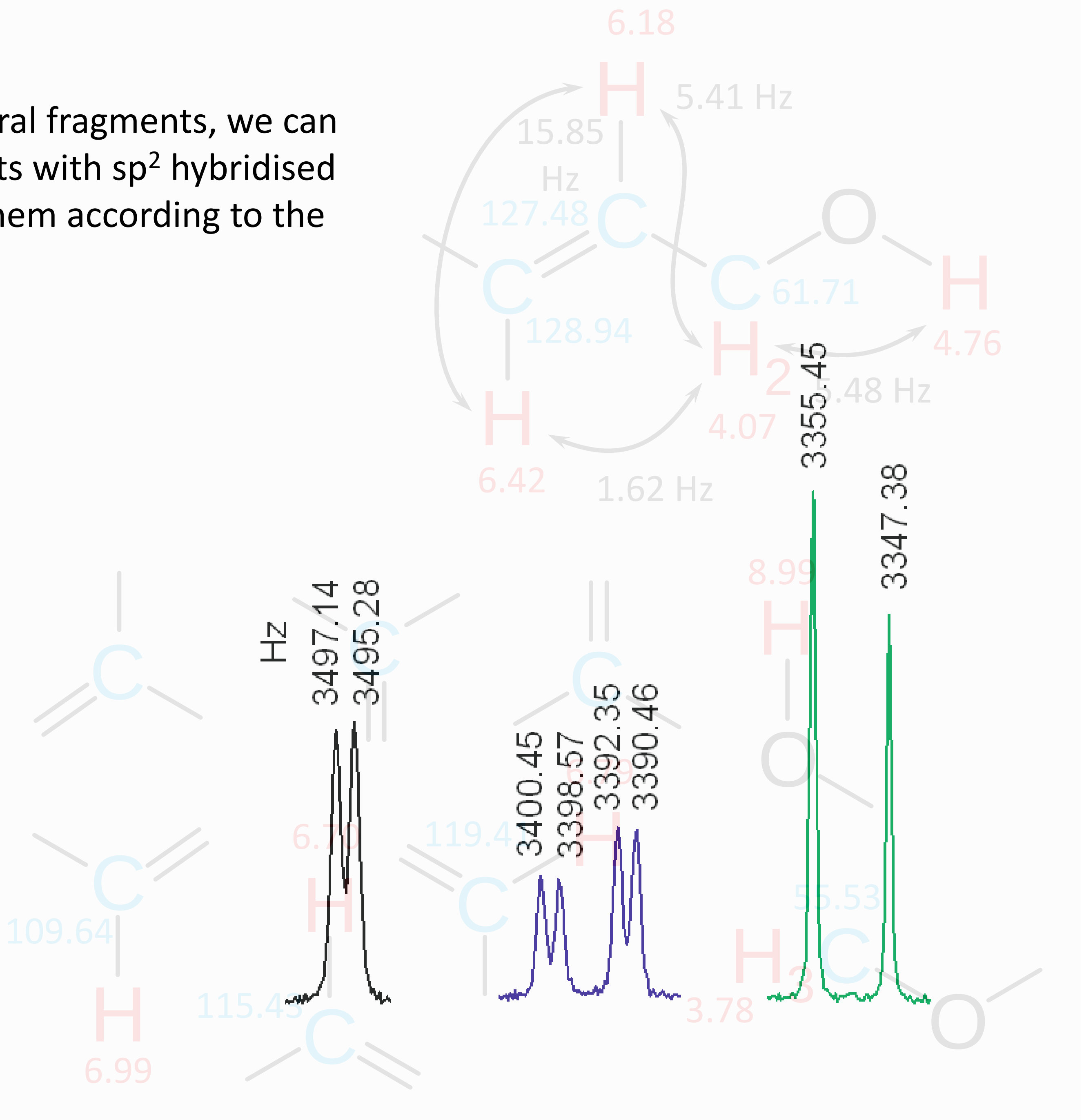


Phenyl ring

Multiplet structure of three aromatic protons

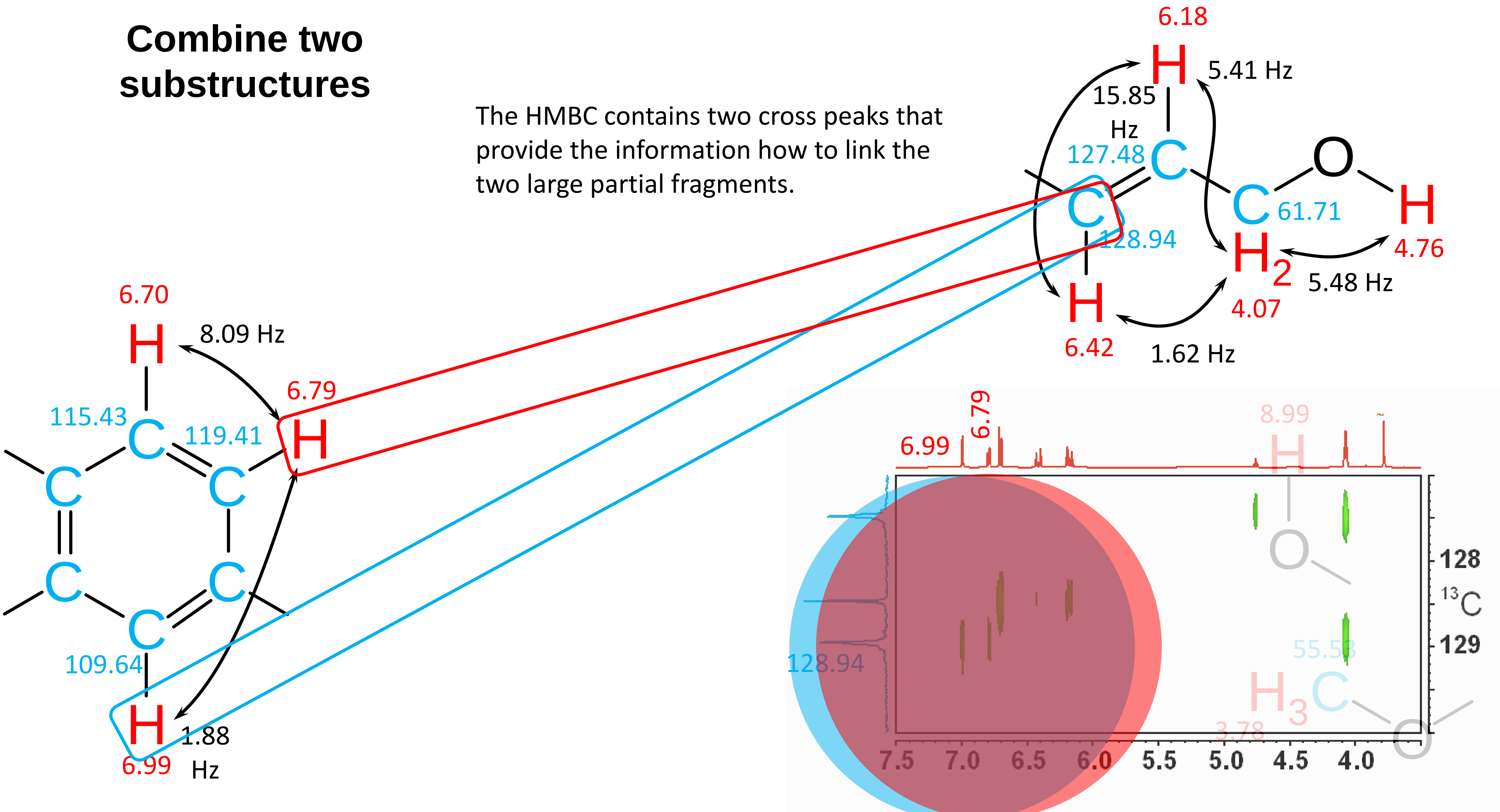
From our pile of structural fragments, we can now pick the 6 fragments with sp^2 hybridised C atoms and combine them according to the pattern.





Combine two substructures

The HMBC contains two cross peaks that provide the information how to link the two large partial fragments.

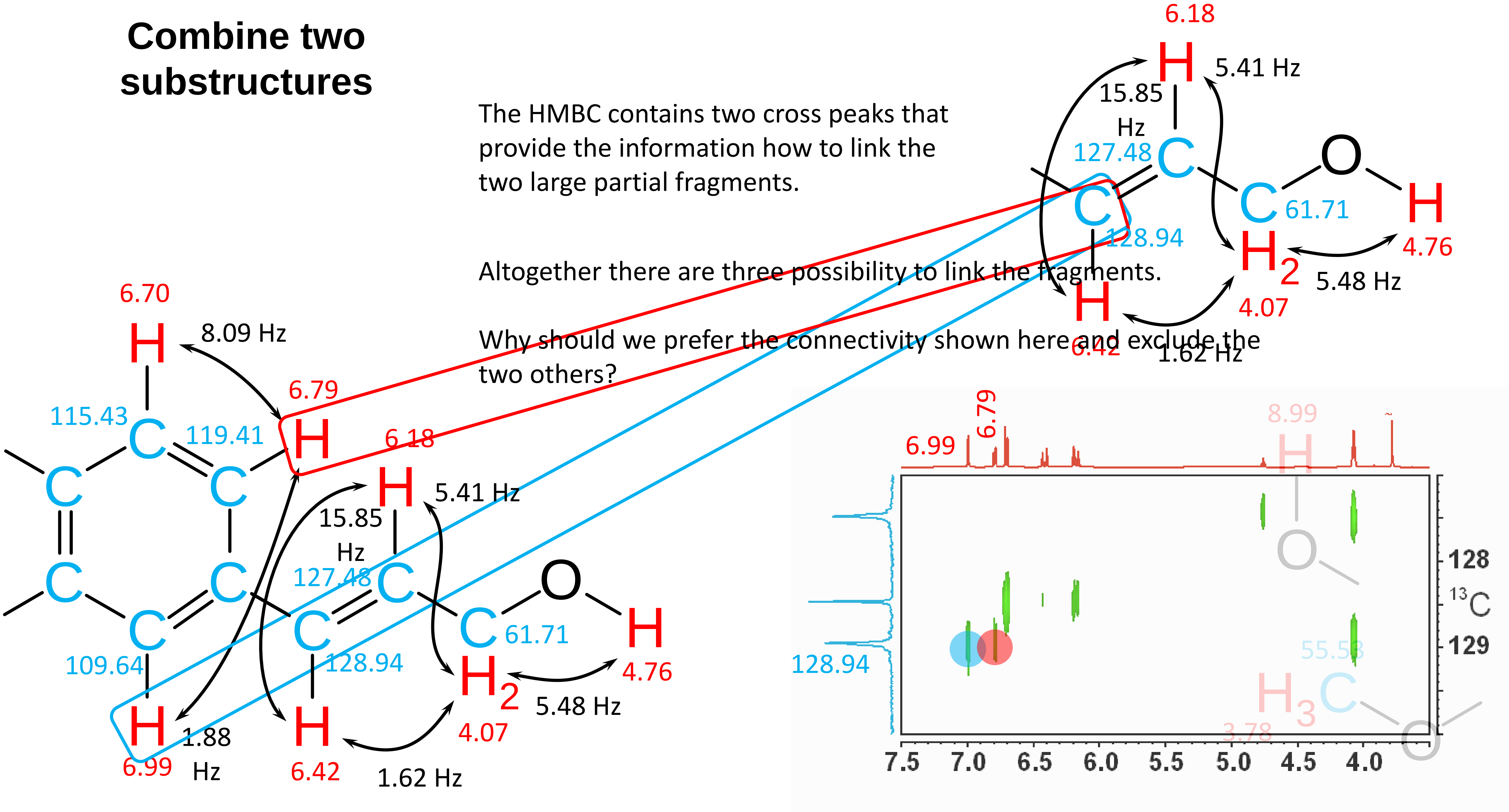


Combine two substructures

The HMBC contains two cross peaks that provide the information how to link the two large partial fragments.

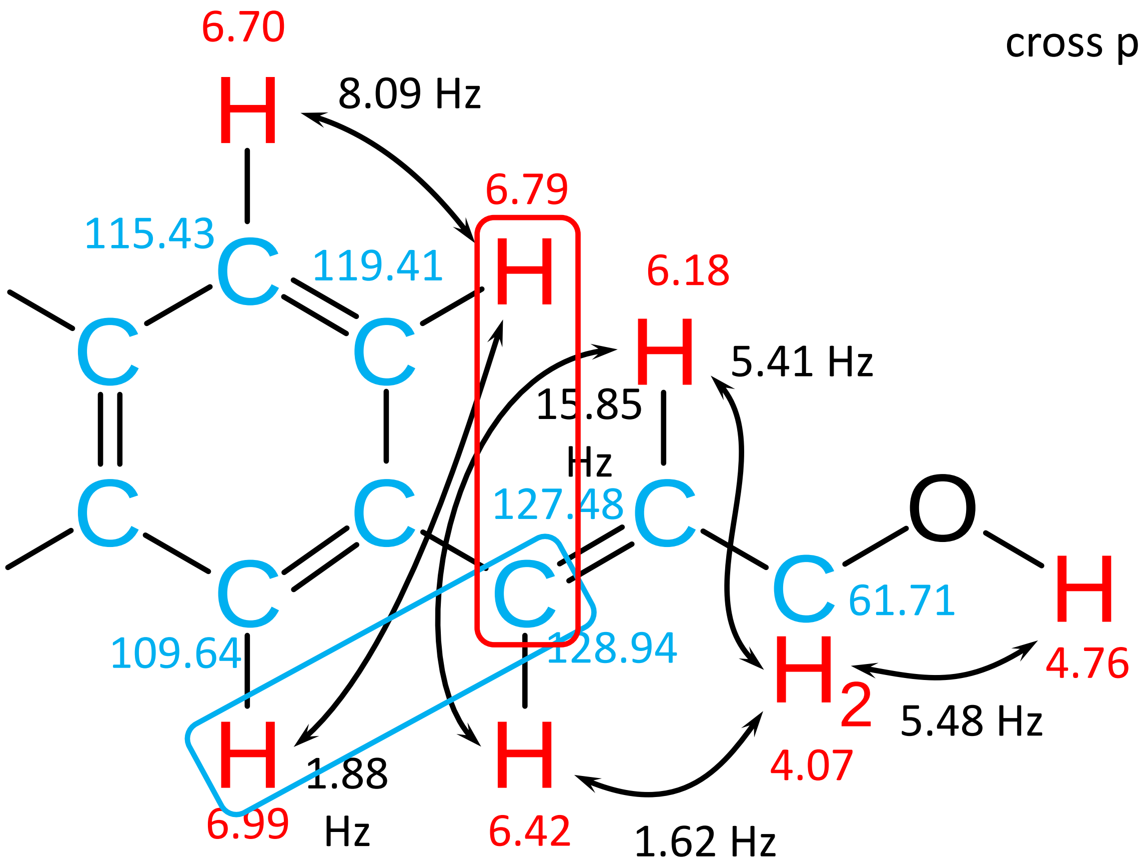
Altogether there are three possibility to link the fragments.

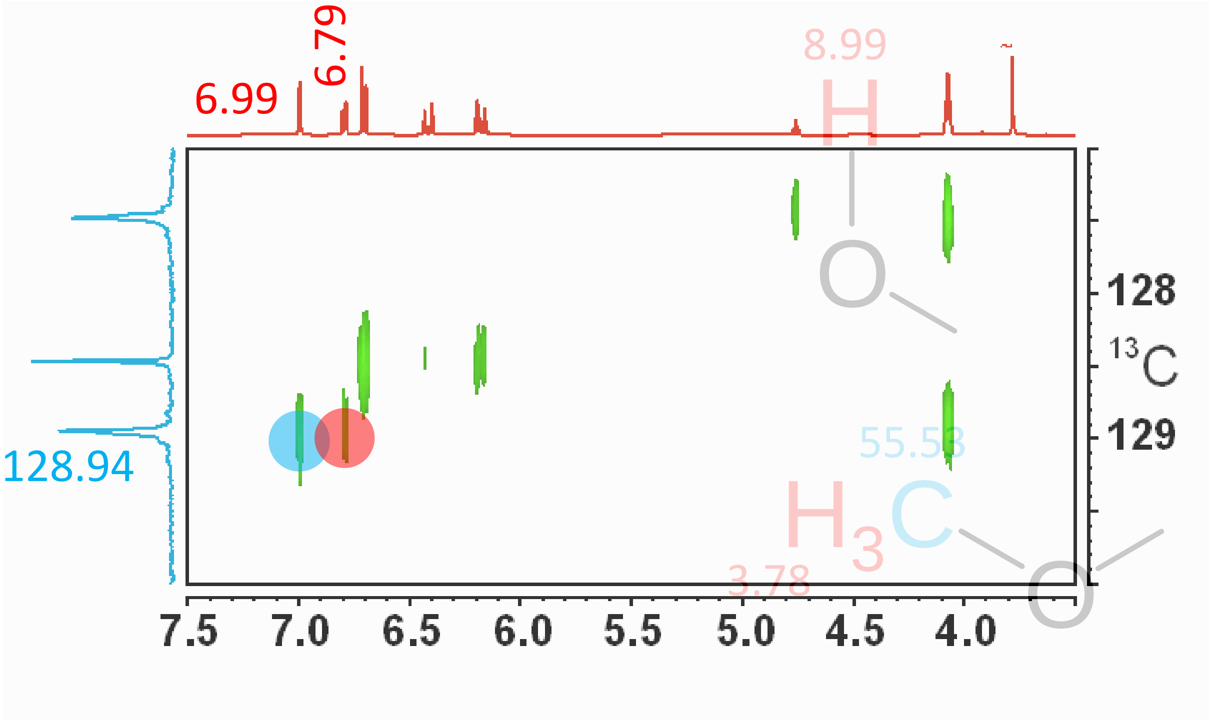
Why should we prefer the connectivity shown here and exclude the two others?



Combine two substructures

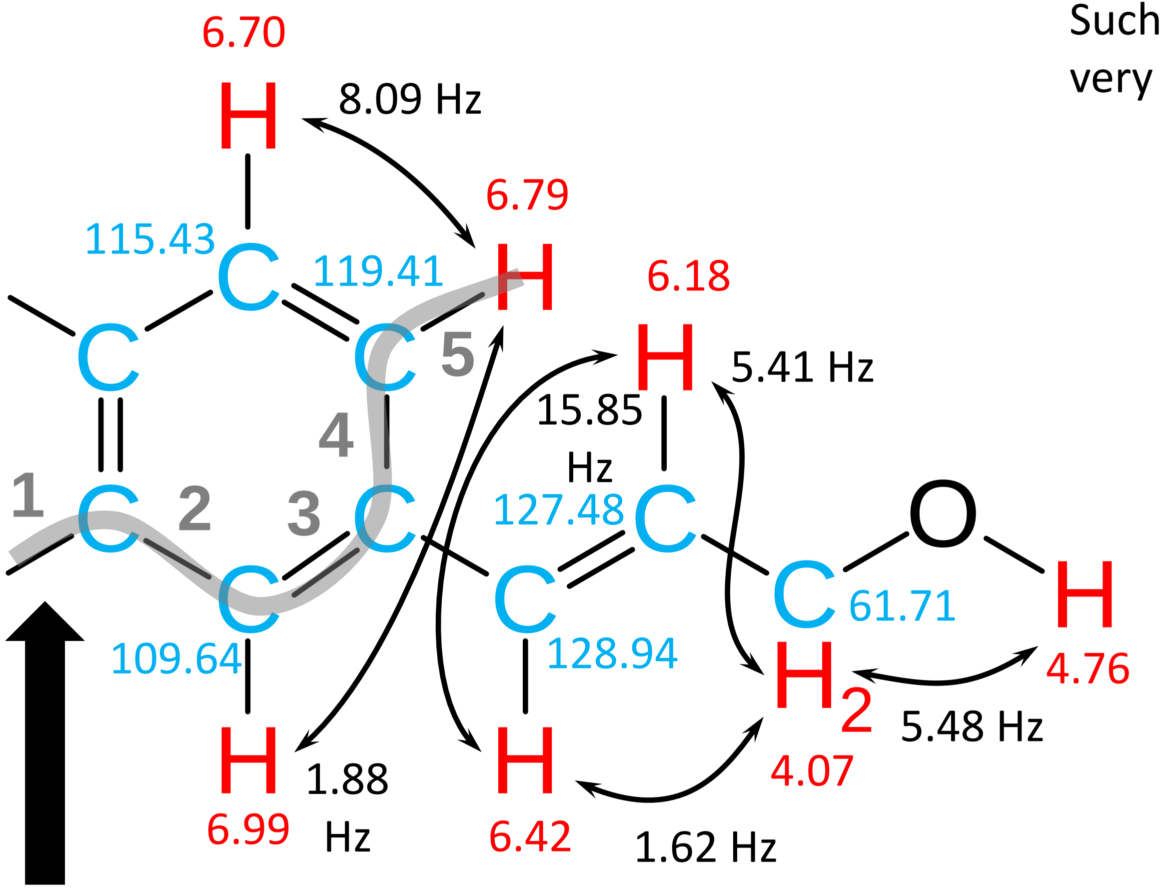
Starting from the C atom with the chemical shift of 128.94 ppm, the protons with the chemical shifts of 6.79 ppm and 6.99 ppm are comparable. Each of the protons is three bonds away from the carbon; moreover, the geometry of the coupling path is identical in both cases. Which means, at least in theory, we should expect two cross peaks with very similar intensity, as visible in the HMBC.





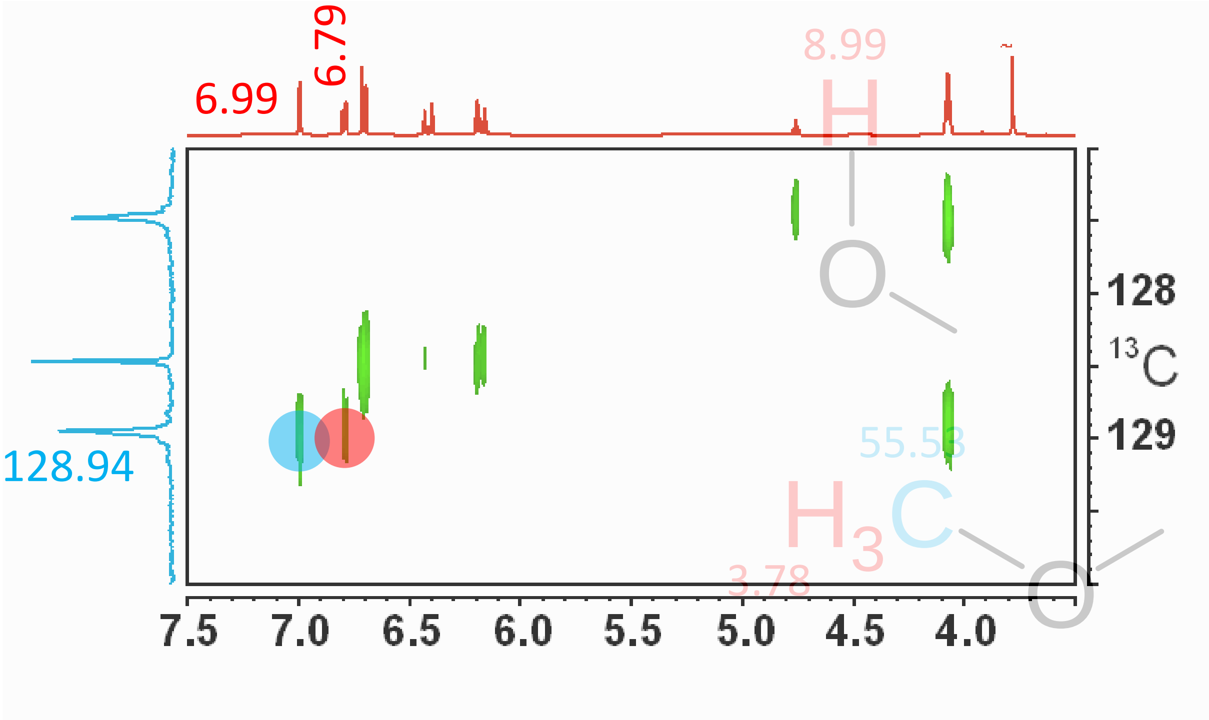
Combine two substructures

Exclude two other options



If we choose this C atom to combine the two fragments, there would be five bonds from the C atom with the chemical shift of 128.94 ppm to the proton with the chemical shift of 6.79 ppm.

Such five bond correlations are not totally impossible, but really very rare.

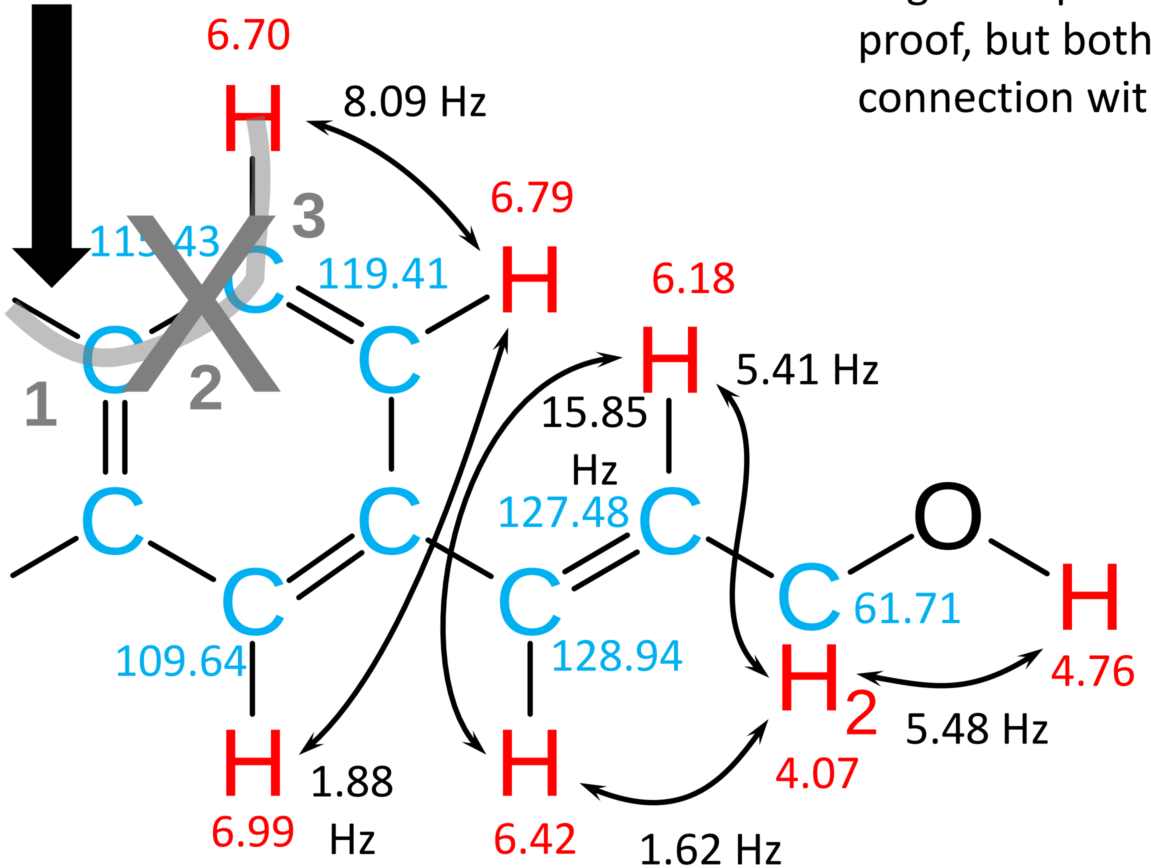


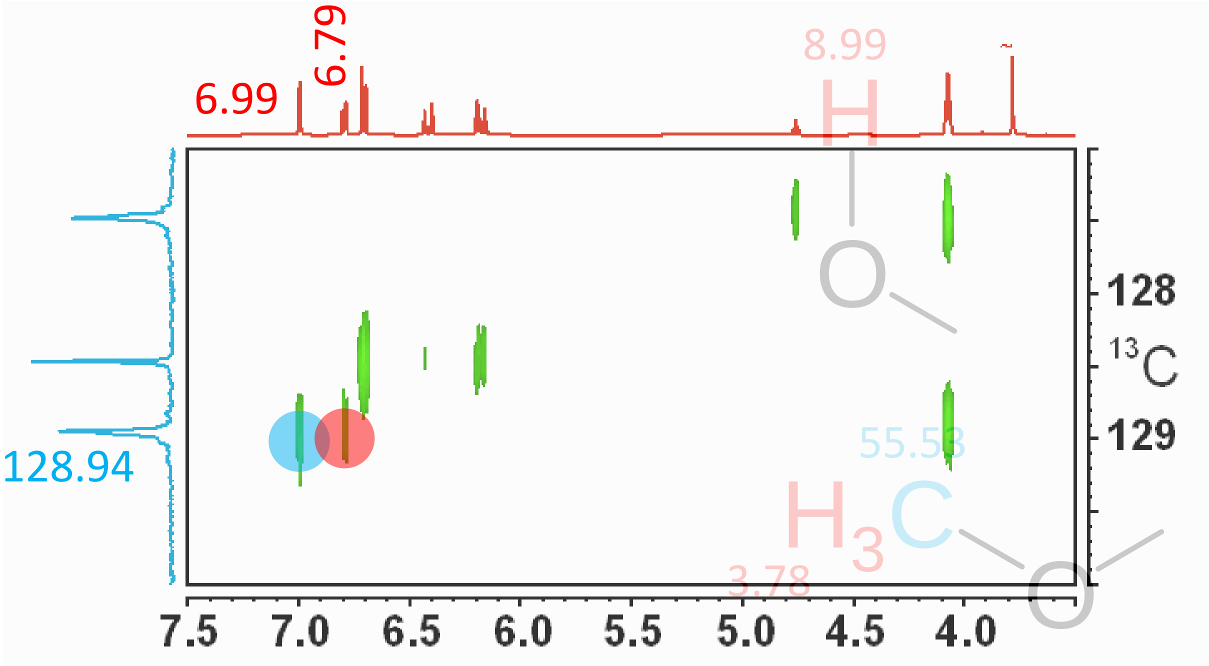
Combine two substructures

Exclude two other options

If we link our fragment to this C atom, there would be four bonds between the C atom with the chemical shift of 128.94 ppm and the proton with the chemical shift of 6.99 ppm. Such a 4-bond correlation is possible.

On the other hand, between the mentioned C atom (128.94 ppm) and the proton with the chemical shift of 6.70 ppm there are only three bonds. However, a cross peak linking those two is not visible even though there should be one with a high degree of probability. The absence of a cross peak in the HMBC is not definitive proof, but both arguments mentioned above taken together exclude this connection with a very high probability.





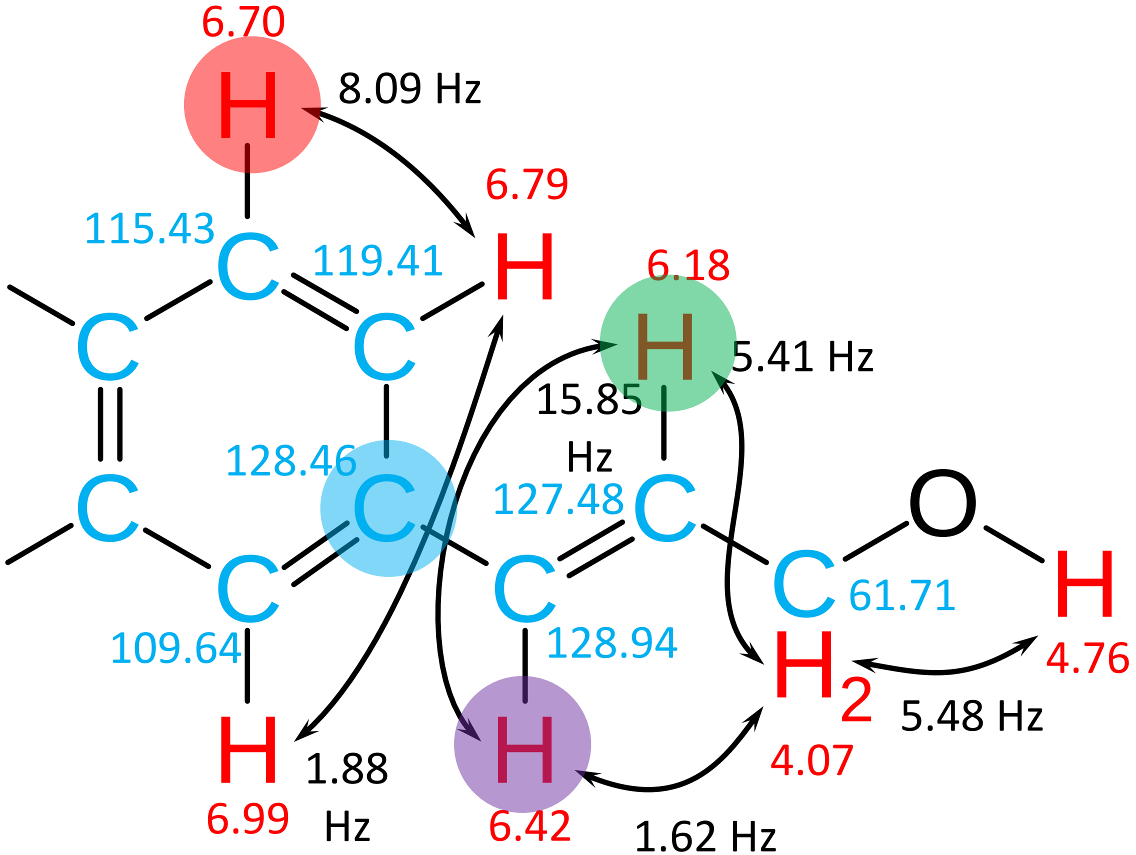
Combine two substructures

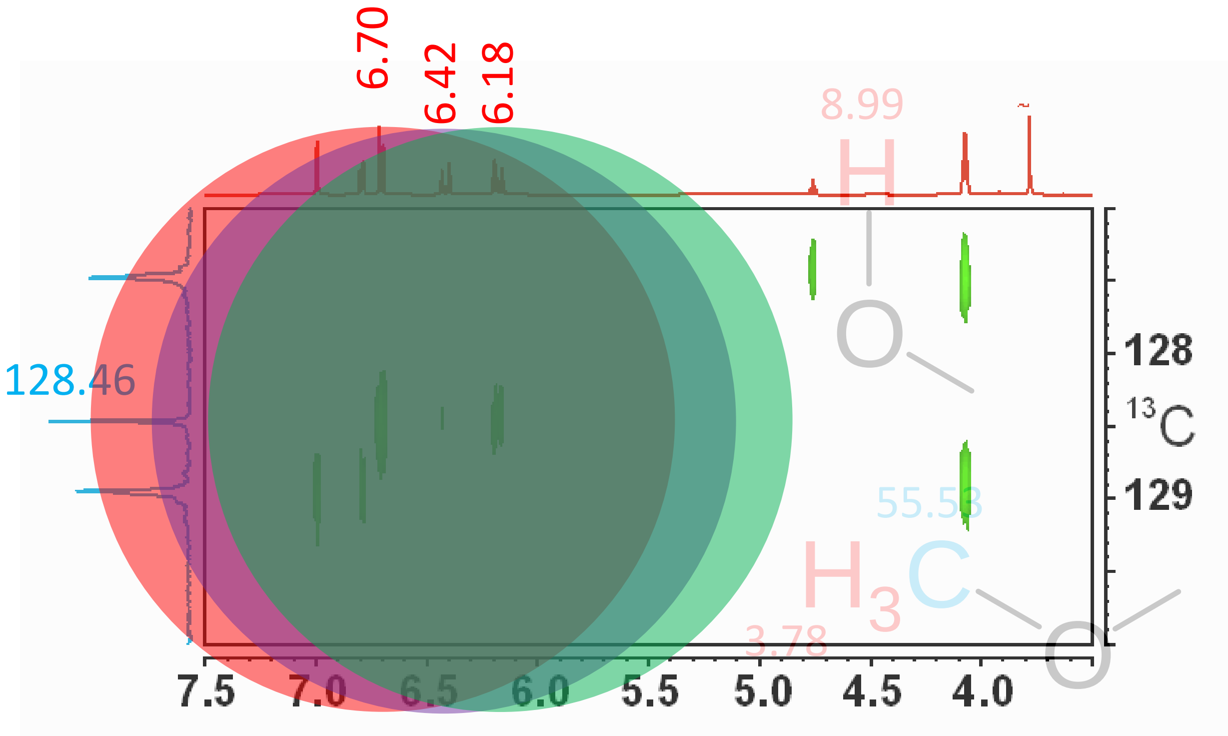
Assign the first quaternary carbon atom

Our pile of building blocks includes three quaternary **=C-** fragments with chemical shifts of 128.46, 146.13 and 147.69 ppm. Let’s inspect the signal at 128.46 ppm in the HMBC.

Two intense and one weak cross peaks belong to this carbon atom.

For assignment of the quaternary C atom at 128.46 ppm, only one position is possible..



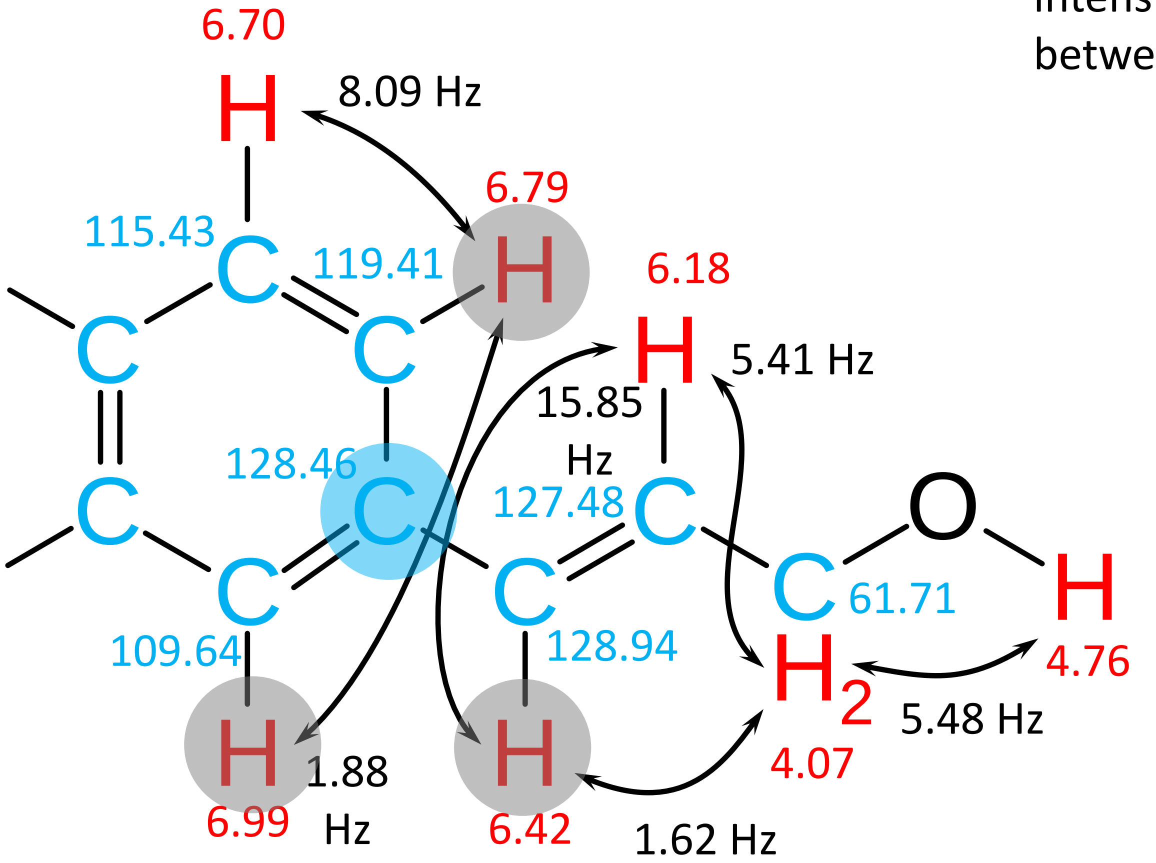


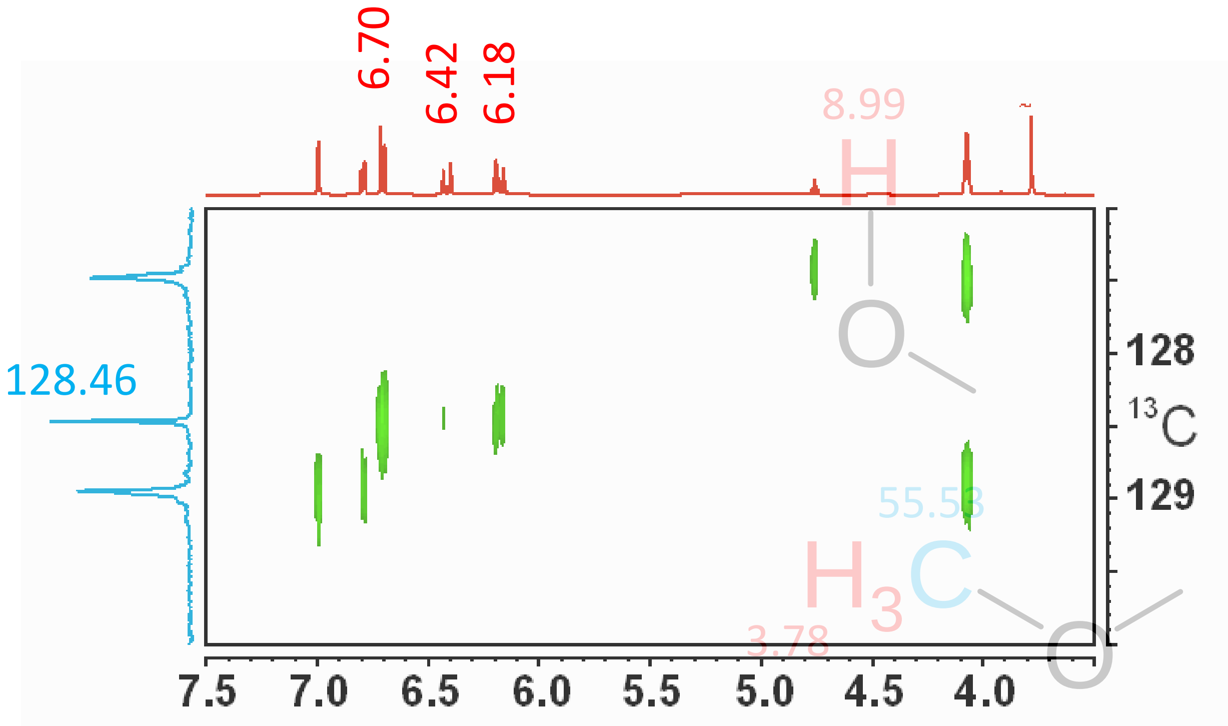
Combine two substructures

Assign the first quaternary carbon atom

But why is the intensity of the cross-peak to the proton at 6.42 ppm that weak and why are there no cross-peaks at all to the protons at 6.79 ppm and 6.99 ppm?

The size of coupling constants only tends to depend on the number of bonds between the coupling partners.
Specifically, geminal C-H coupling constants - and thus indirectly the intensity of cross peaks - are often very small if the bond angle between the coupling partners is **120°**.



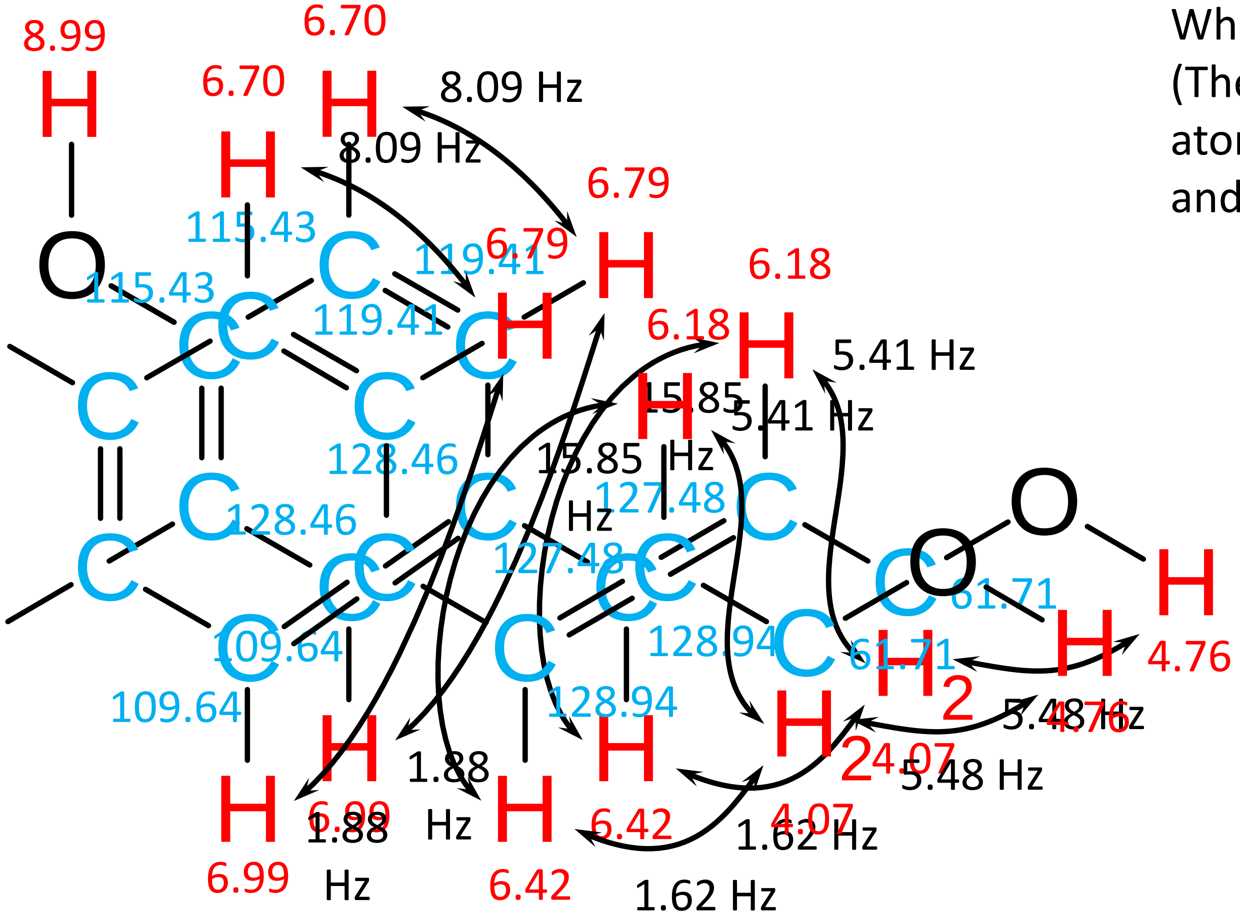


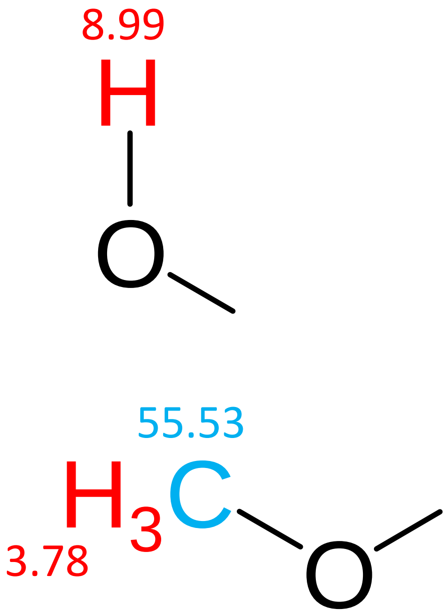
OH group

There are only two possibilities to attach the OH group.

Let's just try one of them.

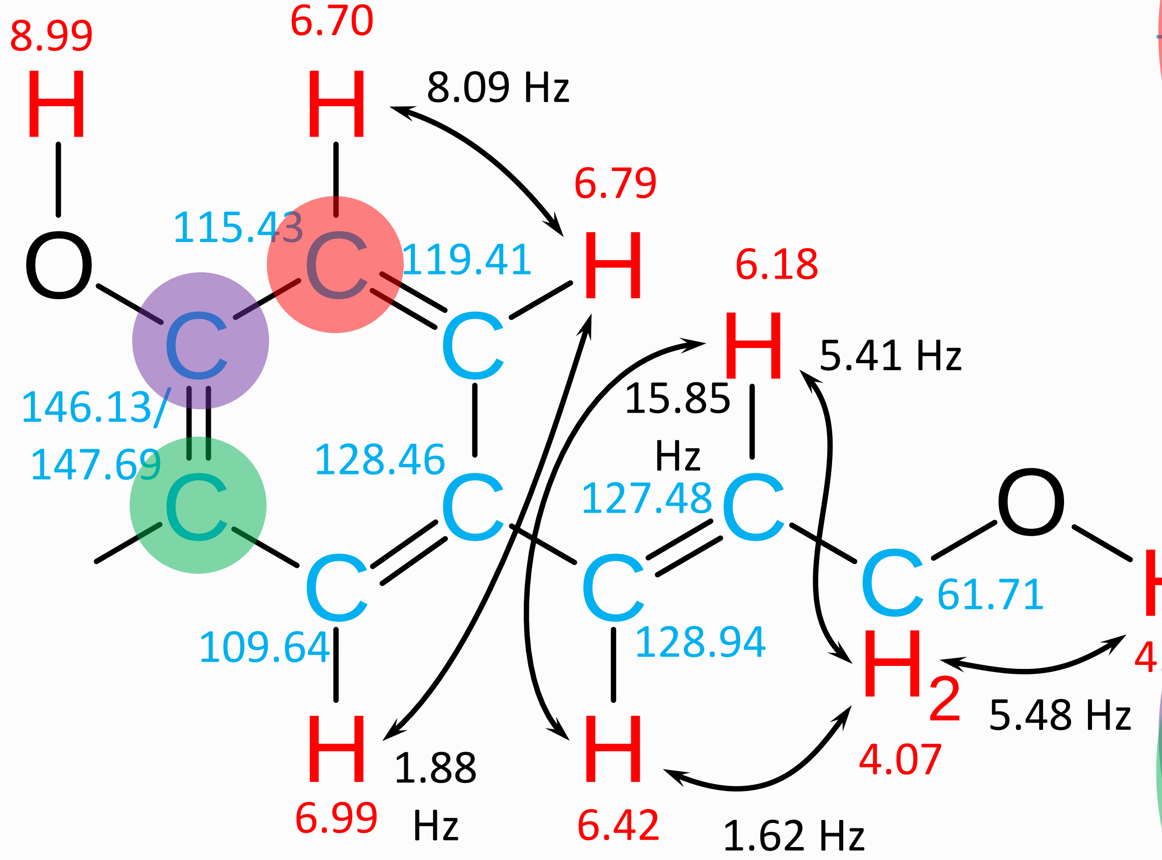
Which cross peaks are visible in the HMBC?
(The assignment of the two remaining quaternary **=C-** atoms is still uncertain. There are only two possibilities and maybe the uncertain assignment doesn’t matter).

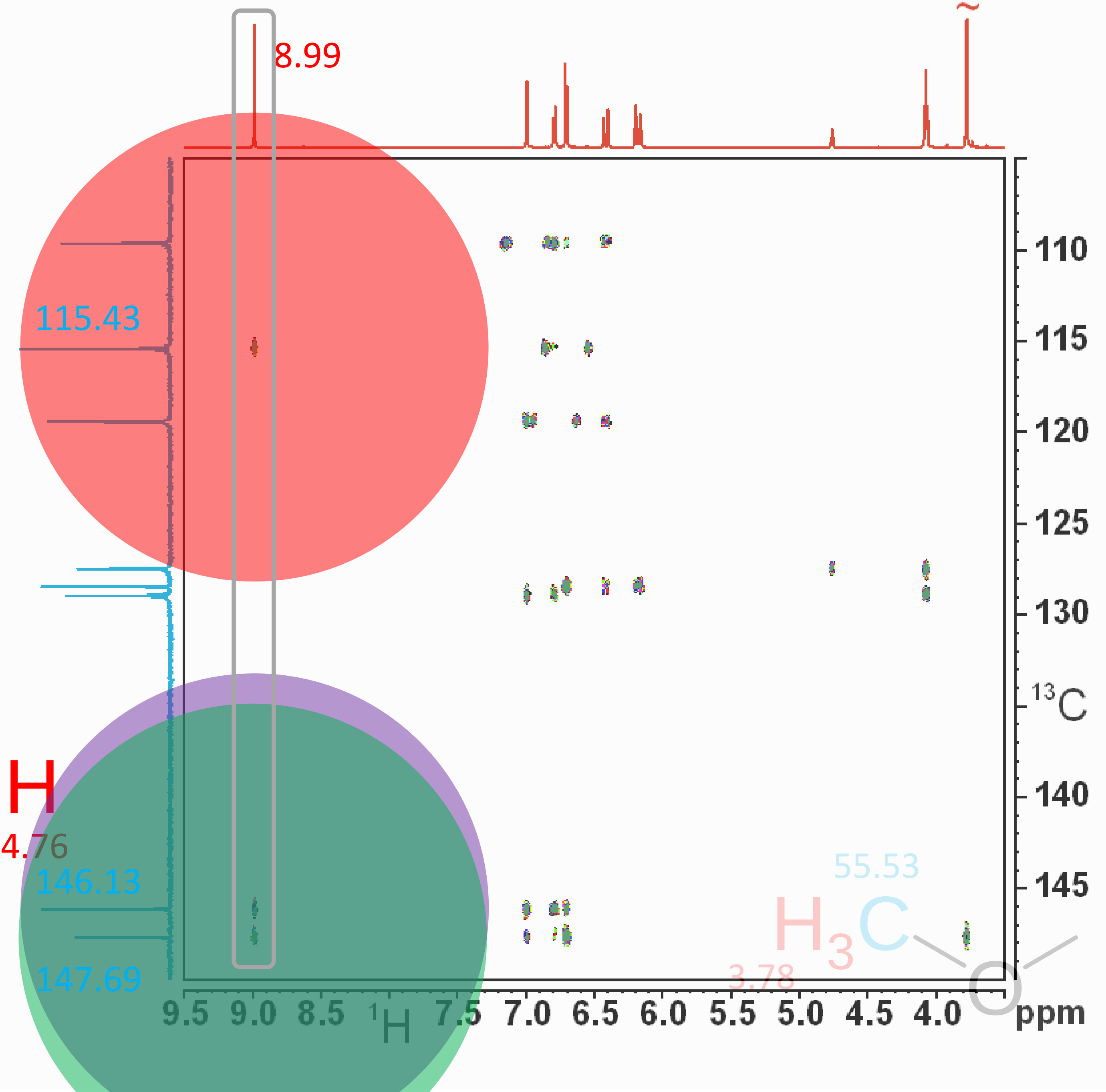




OH group

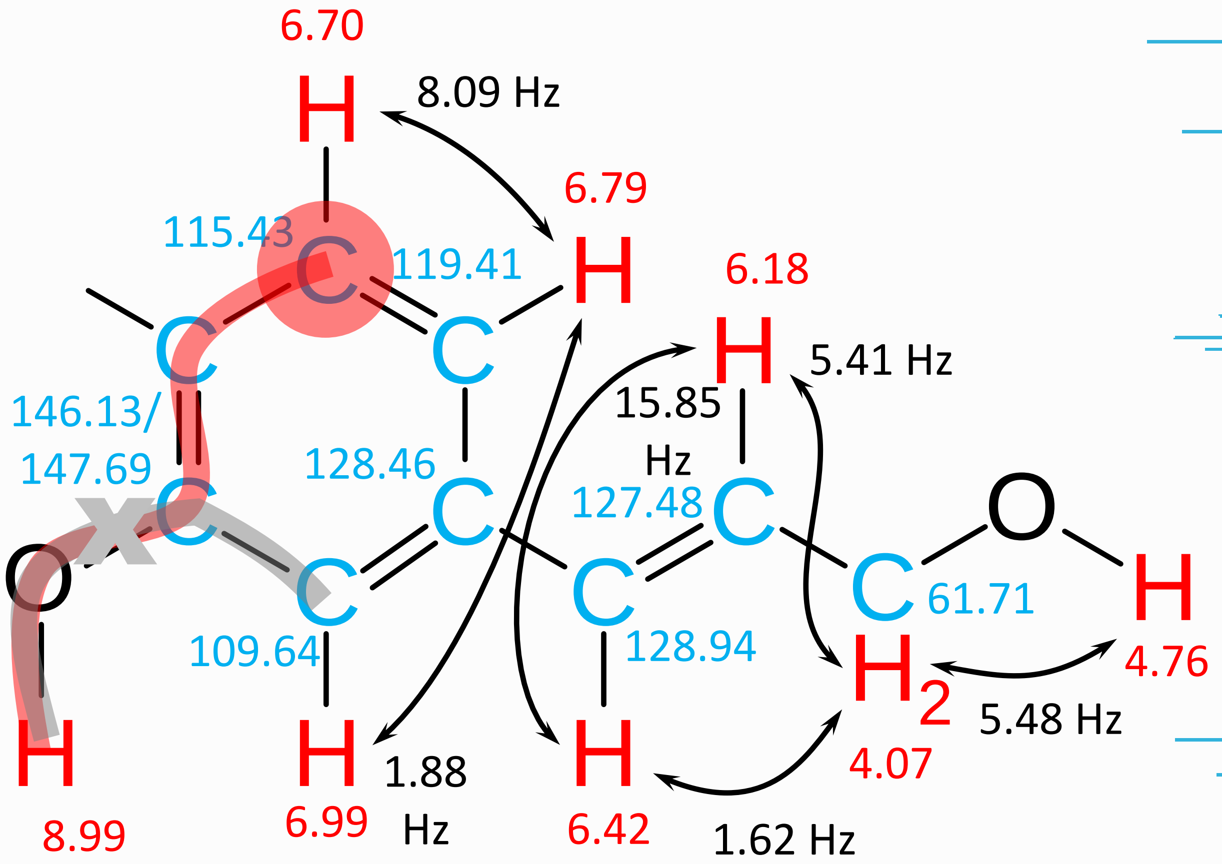
Starting from the signal of the OH group at 8.99 ppm, there are three cross peaks in the HMBC.
There are a maximum of three bonds between the proton and each of the carbon atoms, no matter how we assign the signals at 146.13/147.69 ppm.

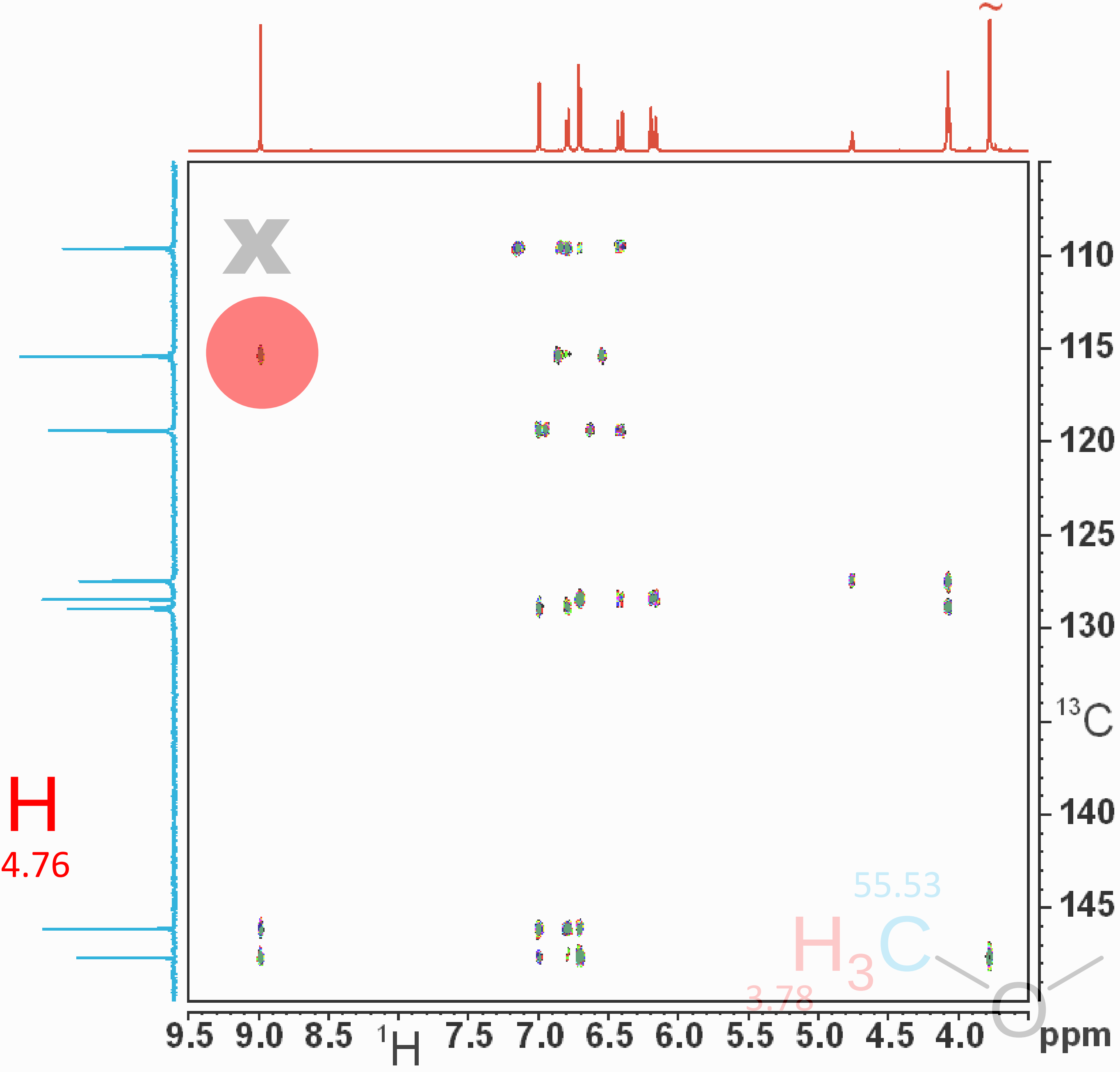




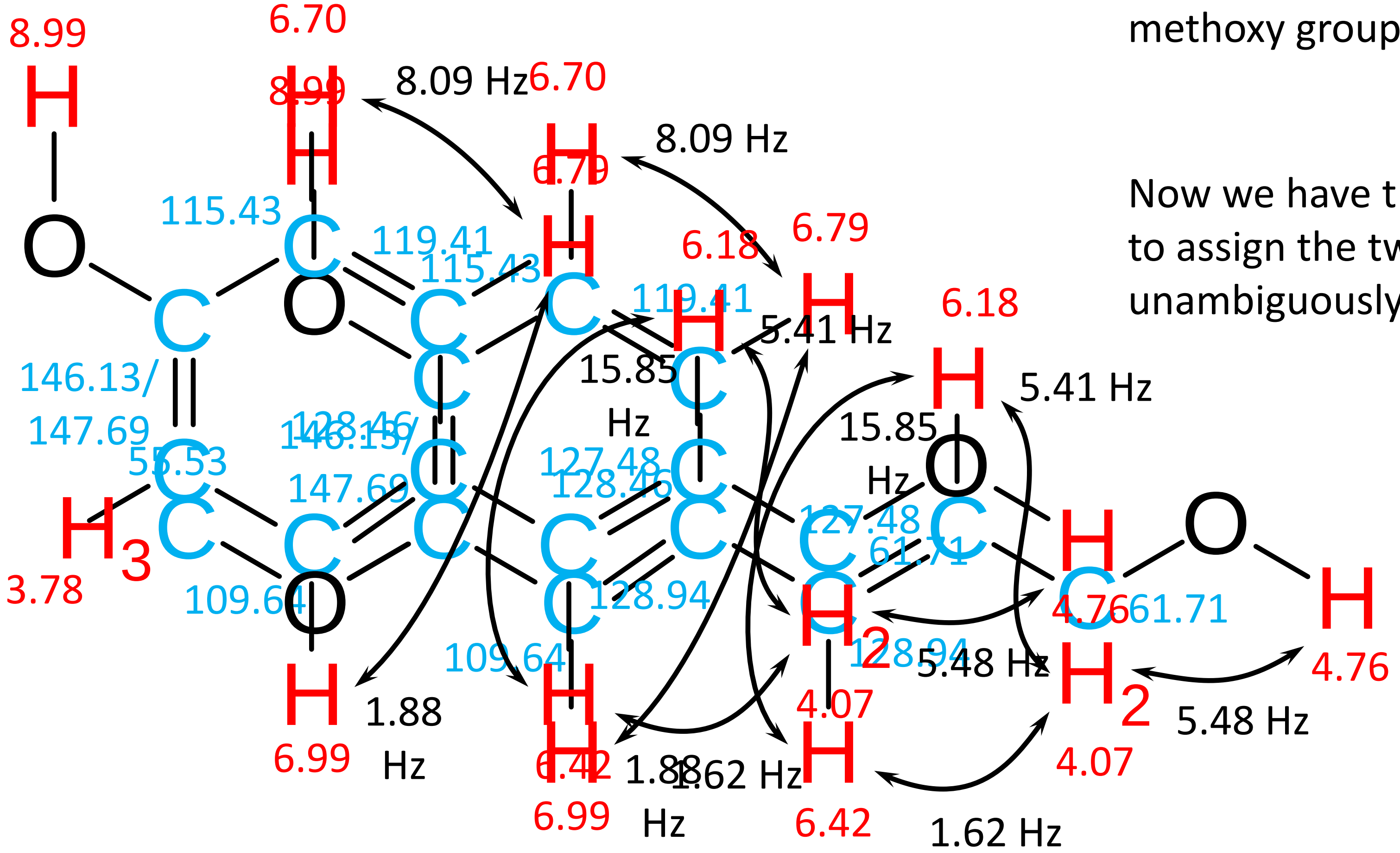
OH group

The second possible position of the OH group would require a four-bond correlation, while on the other hand an expected three bond correlation does not appear in the HMBC.





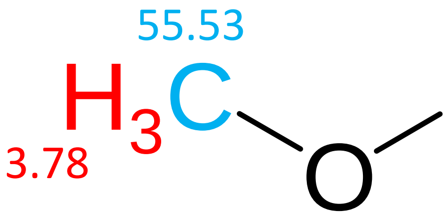
Final structure



Let's return to the first way of inserting the OH group.

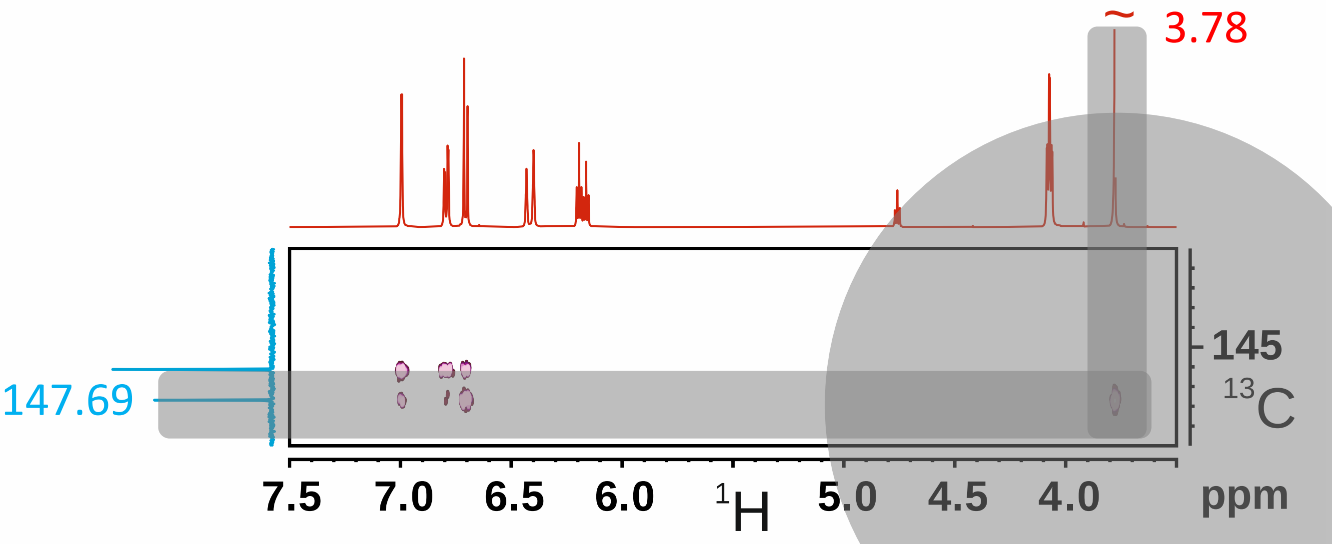
There is only one remaining possibility to attach the methoxy group.

Now we have the final constitution. We might try to assign the two quaternary carbon atoms unambiguously.

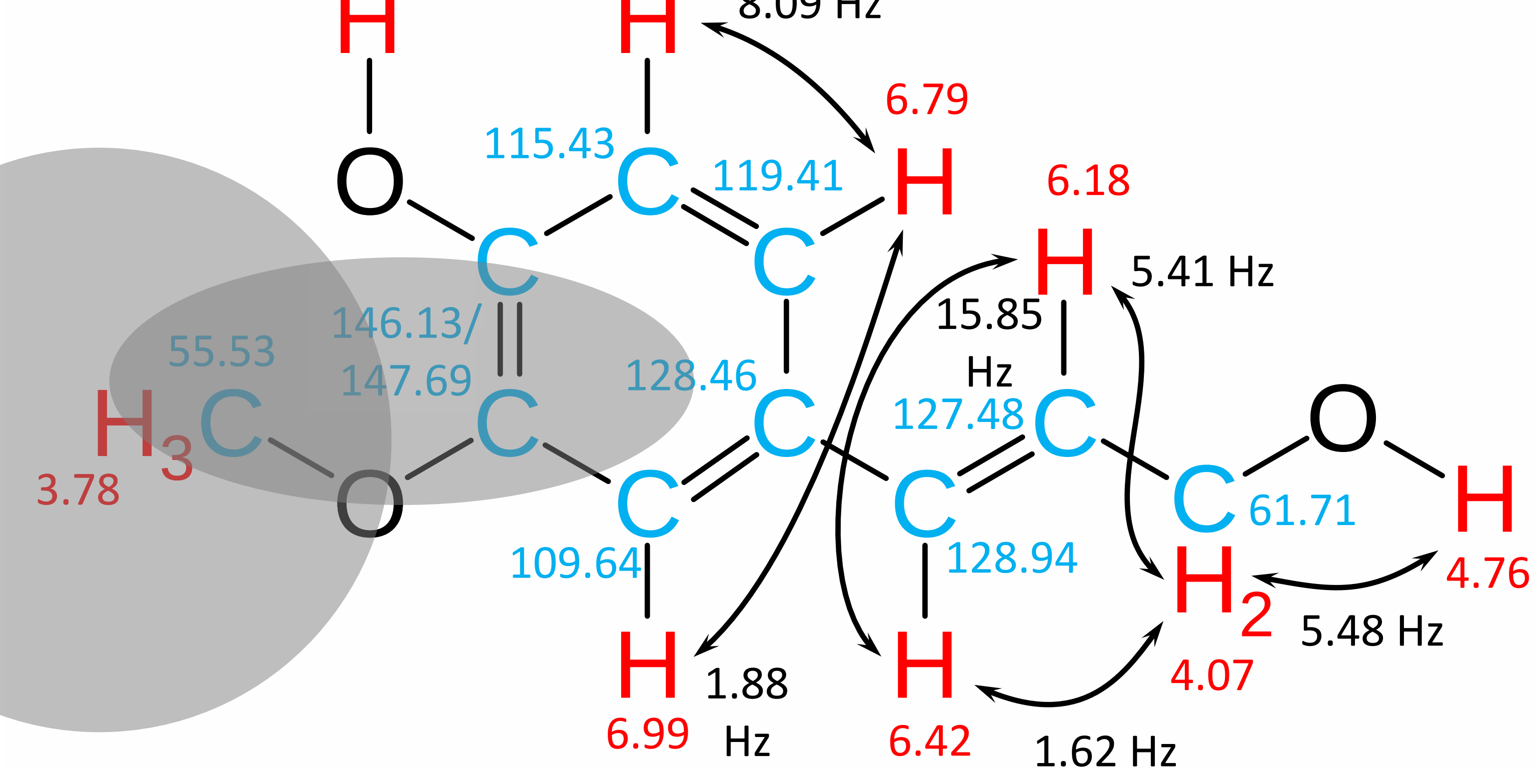


Final structure

Assign the two remaining quaternary carbon atoms



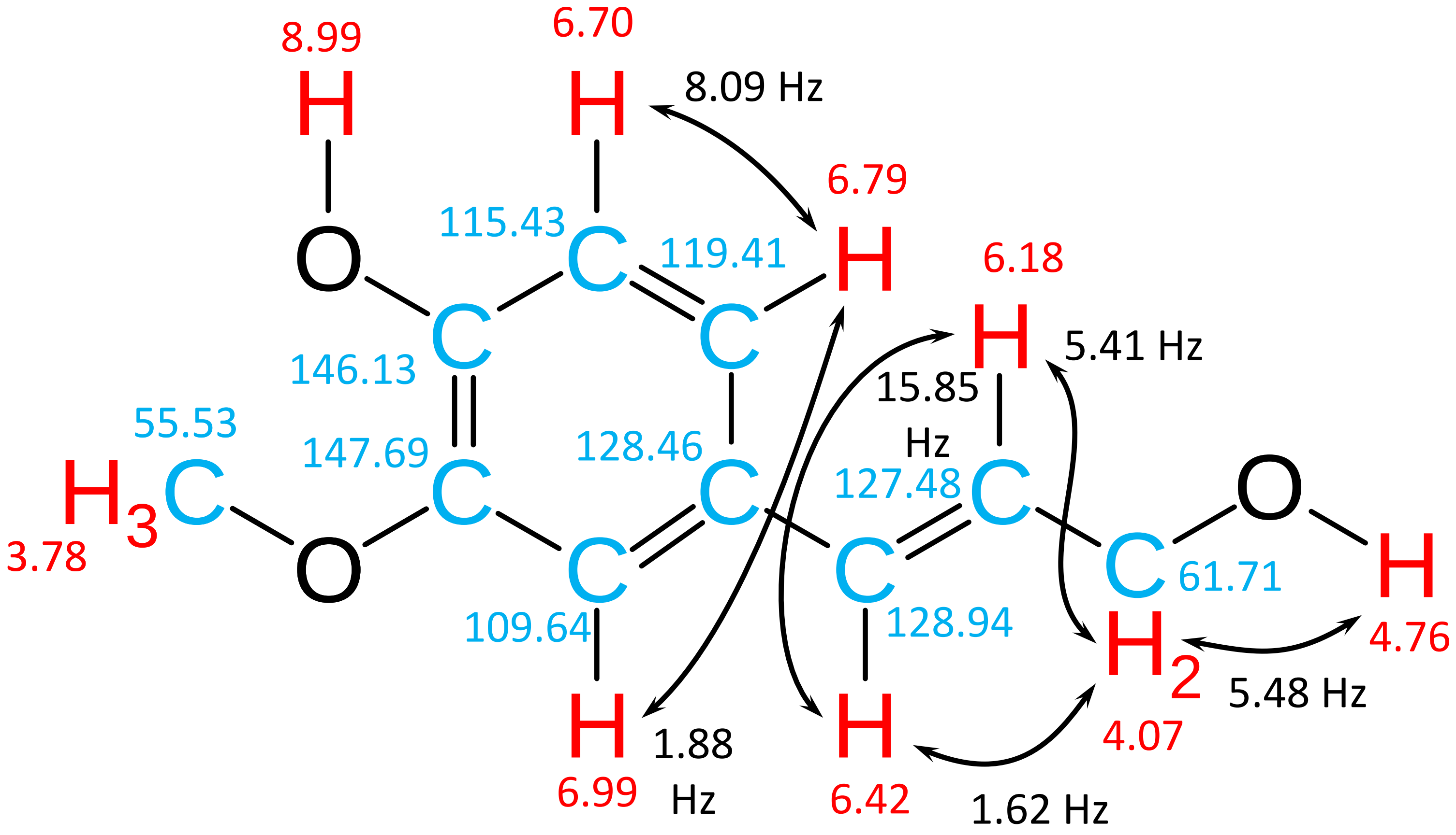
The cross peak is easily explained if the carbon atom with the chemical shift of 147.69 ppm is next to the methoxy group.



If the carbon with the chemical shift of 147.69 ppm were next to the OH group, the above cross-peak would correspond to a four-bond correlation. On the other hand, there would be three bonds between C(146.13ppm) and H(3.78 ppm). However, a corresponding cross-peak for that is not observed.

Final structure

But, there is still one open question ...



Some remarks:

From the given data, the structure can be deduced with a very high probability (> 99.99%), but not with 100% certainty.

To be absolutely sure, additional data would be necessary. In this example a NOESY might be very helpful.

The assignment of the quaternary carbon atoms at 146.13 ppm and 147.69 ppm is very close to perfection, but the ultimate proof would require the evaluation of ^{13}C-^{13}C coupling constants.

Alternatively, “external” pieces of information are possible, for example knowledge of the synthesis pathway.

Wrong integrals

The integral of the proton signal at 4.76 ppm was too small by a factor of **4** and the integral of the OH group at 8.99 ppm was too small by about **25%**.

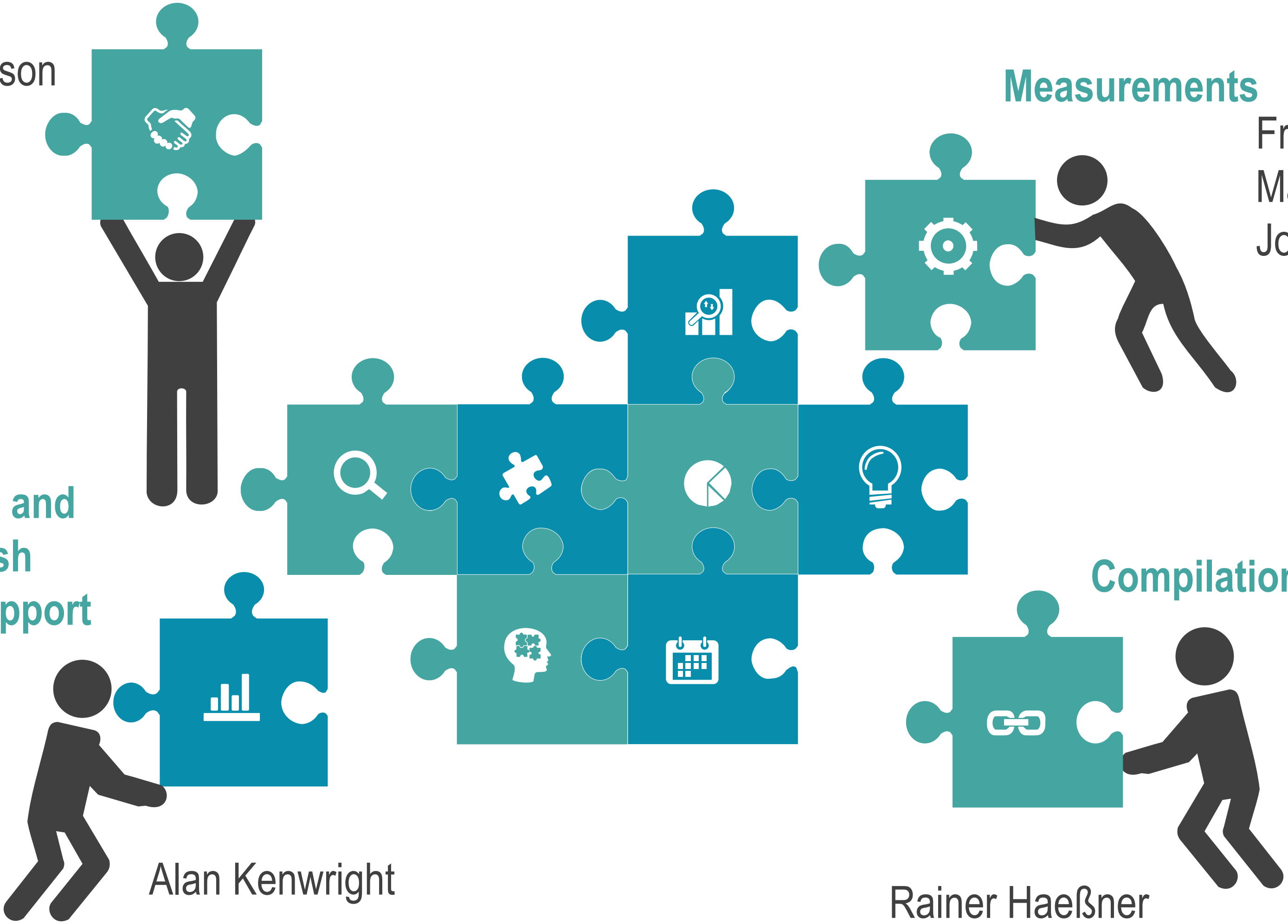
The solution to the riddle is hidden on page 2 of the exercise: " ***... using presaturation ...***".

Presaturation is used to suppress the water signal at **4.75 ppm** and as a side effect also almost extinguishes a closely neighbouring signal at 4.76 ppm. Through chemical exchange, the second OH group at 8.99 ppm is also indirectly influenced by the presaturation.

But why to use water suppression at 4.75 ppm for a sample measured in DMSO-d_6? We expect the water signal in DMSO at about 3.3 ppm. Even if we assume a strong contamination with water, the presaturation would have been done at the wrong chemical shift.

This nice measurement is a gift from a laboratory mainly dealing with aqueous samples. To see the spectrum of a substance with a concentration of about 2 mMol measured in an environment containing more than 100 Mol of protons coming from the solvent requires special techniques, such as presaturation.
Apparently accidentally one of such special technique was used (inappropriately) here. If there were no signals close to 4.75 ppm that would not matter. Unfortunately, correction of unfavourable measuring conditions post-acquisition is not possible.

Contributions



Spectrometer time

University of Wisconsin-Madison
(BioMagResBank)

Measurements

Francisca Jofre,
Mark E. Anderson,
John L. Markley

Discussions and native English language support

Alan Kenwright

Compilation

Rainer Haeßner

[More exercises …]