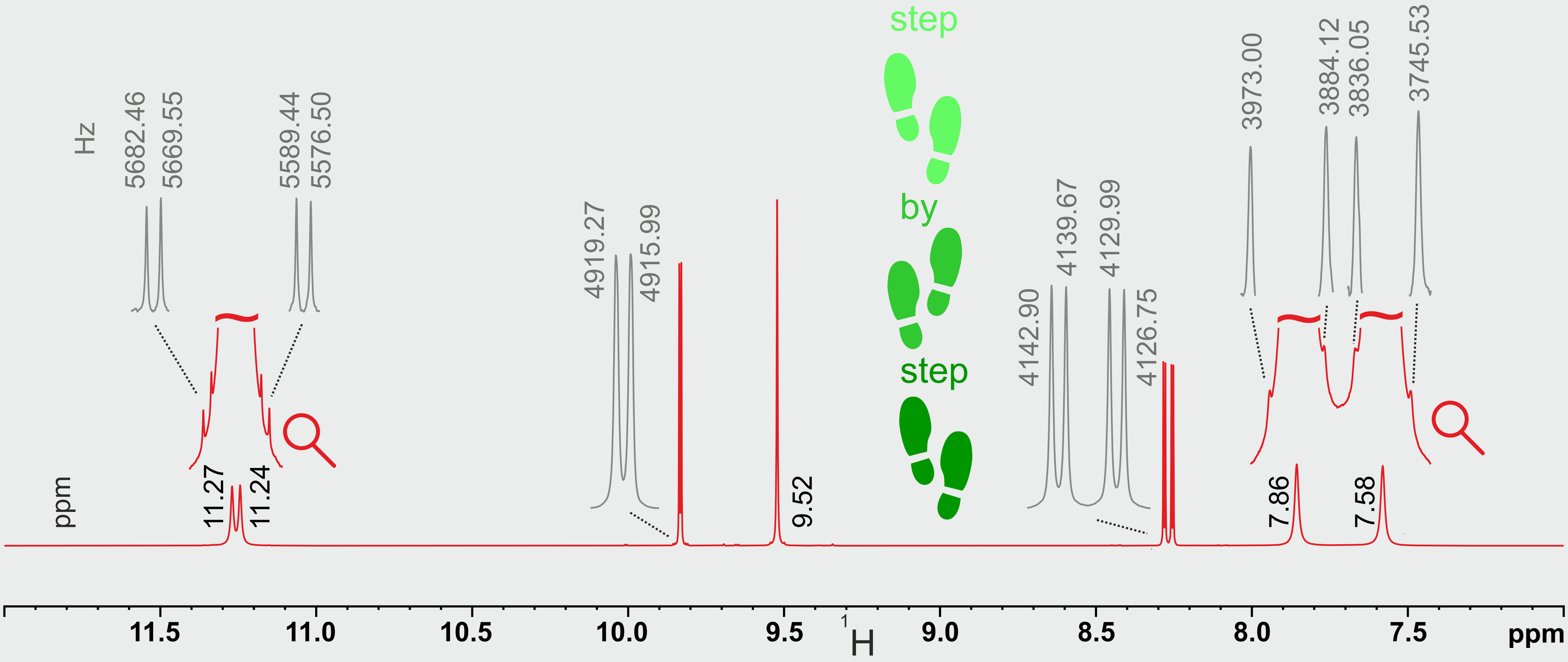
Exercise plus Solution – Quick PDF overview

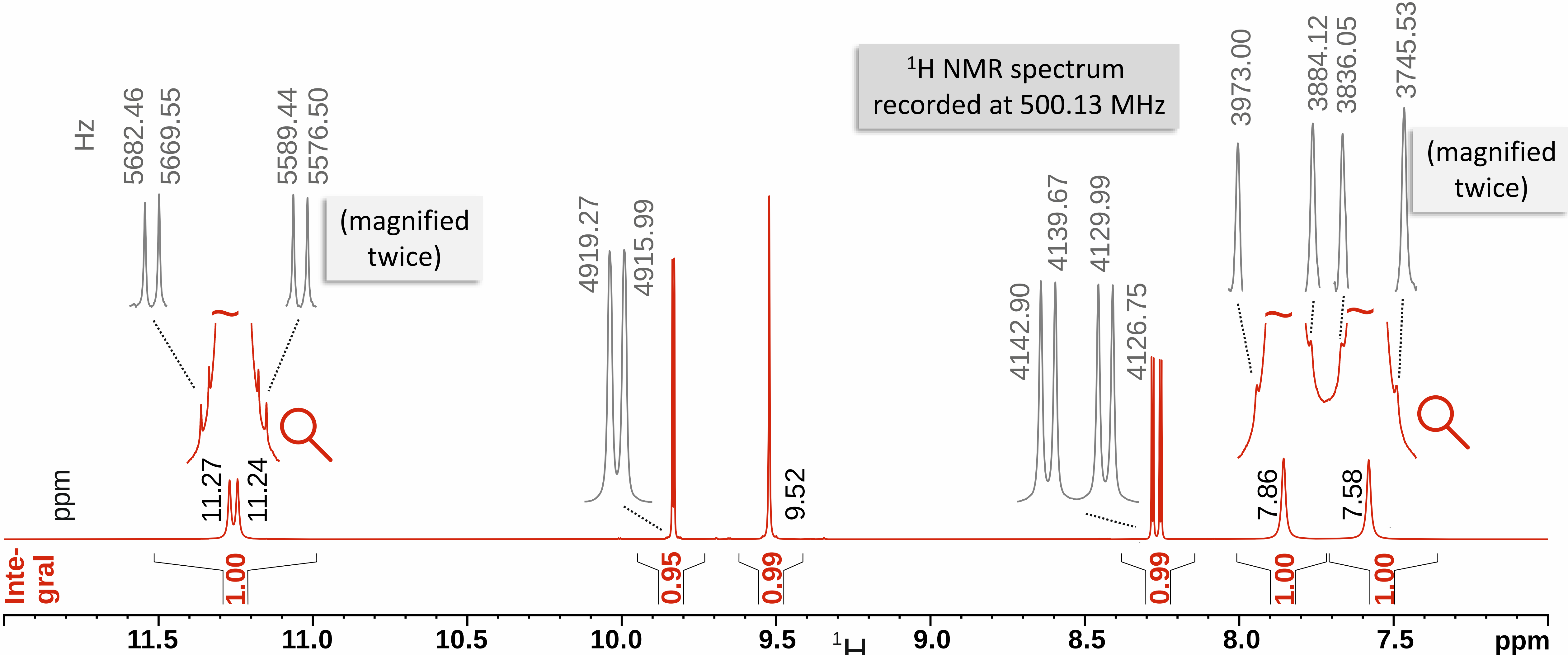
It is recommended to use this PDF version only for a quick overview of the NMR challenge. All animations of the PowerPoint version are missing, under certain circumstances quality deficiencies may also occur.
The higher quality PowerPoint files are freely available for download at any time.



Problem of the Month:

October 2021

$C_5H_6O_3N_2$ in DMSO-d_6

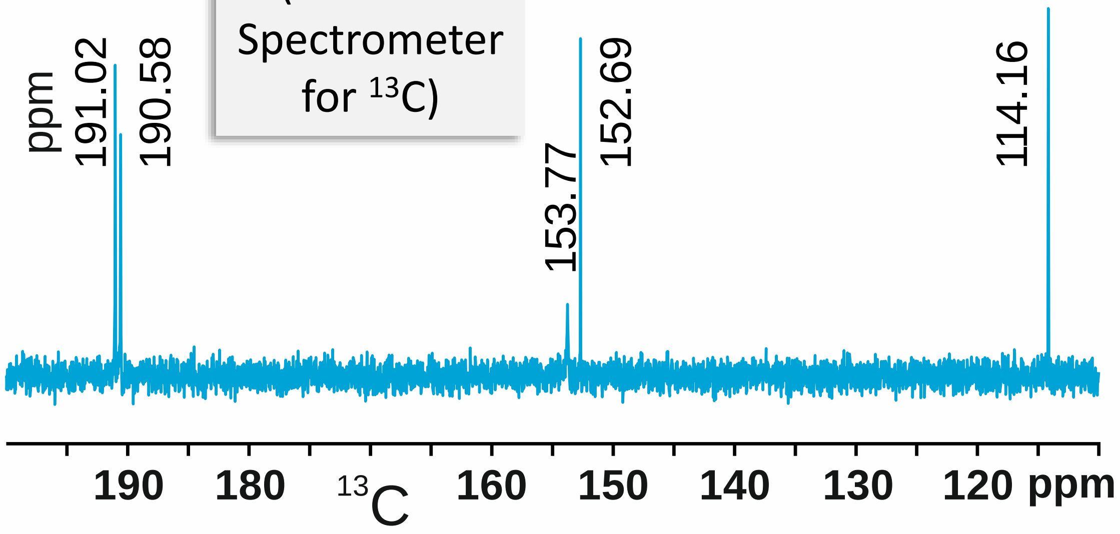


Deduce the structure of this small molecule. There is one ethylene fragment. Using all spectra presented here, you should be able to do a stereochemically correct assignment of all atoms.

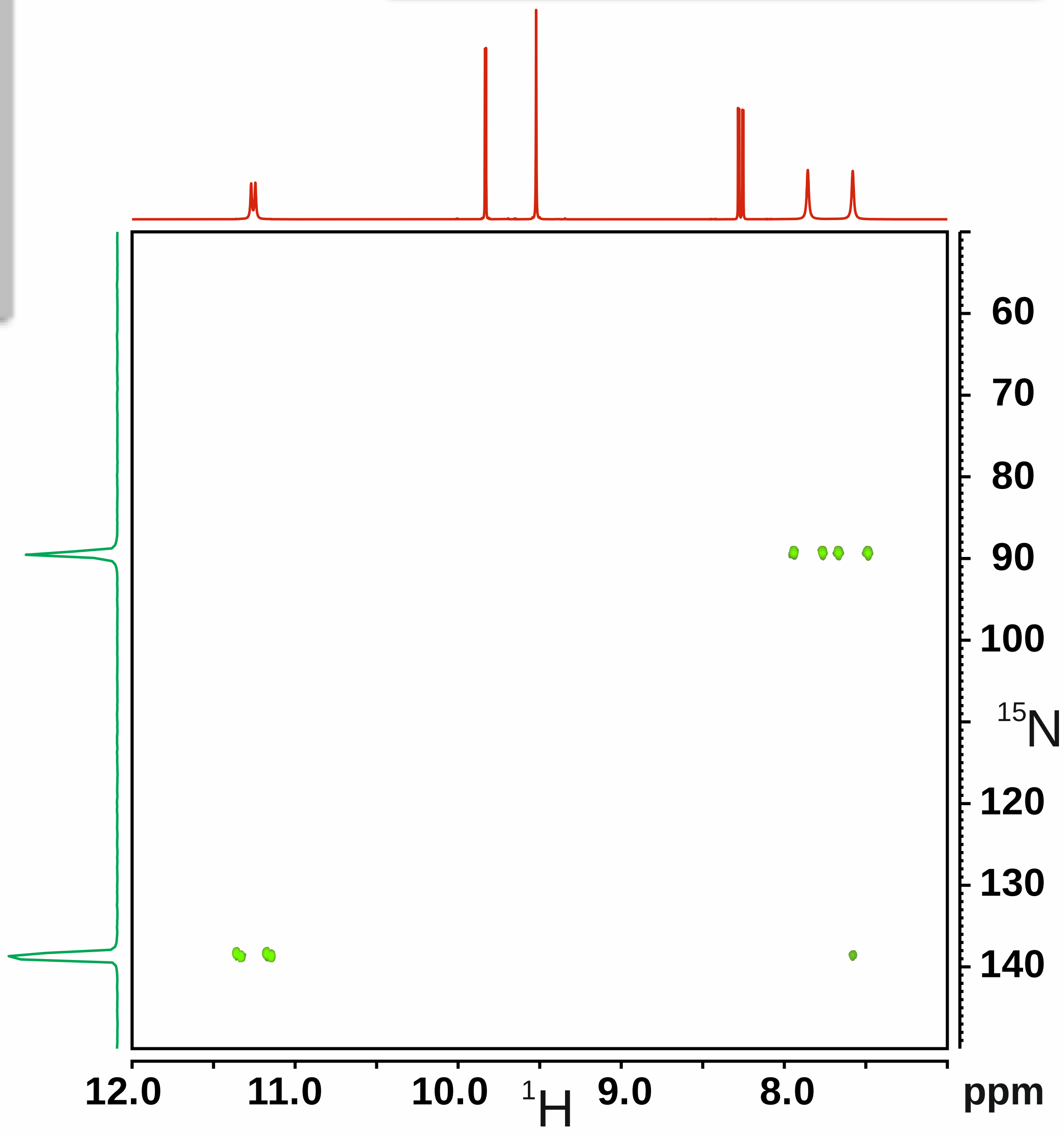
The proton spectrum contains a few strong magnified signals. Are you able to extract some helpful pieces of information from those signals?

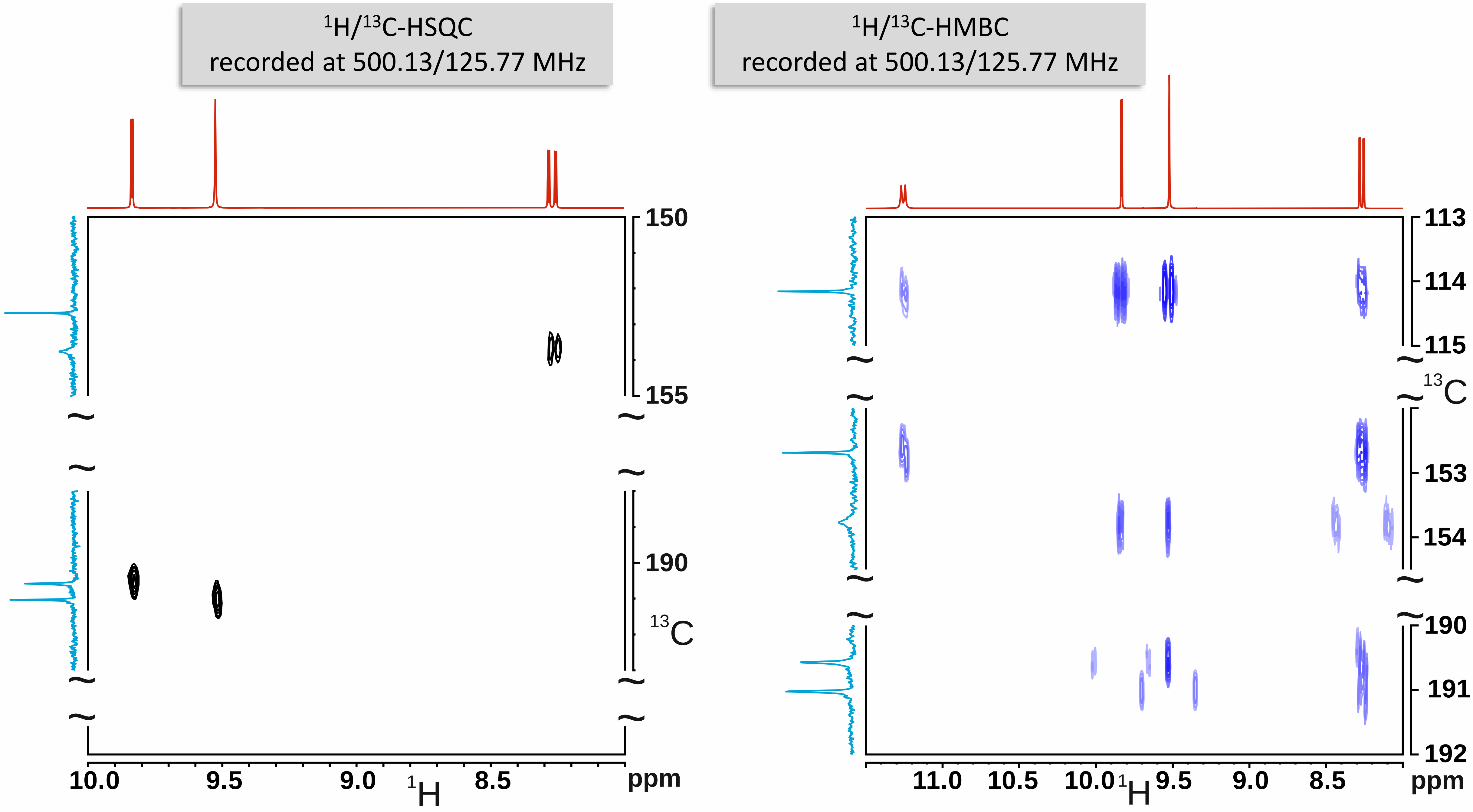
$^{13}C\{^1H\}$ NMR spectrum recorded at 90.56{360.13} MHz

(different Spectrometer for ^{13}C)

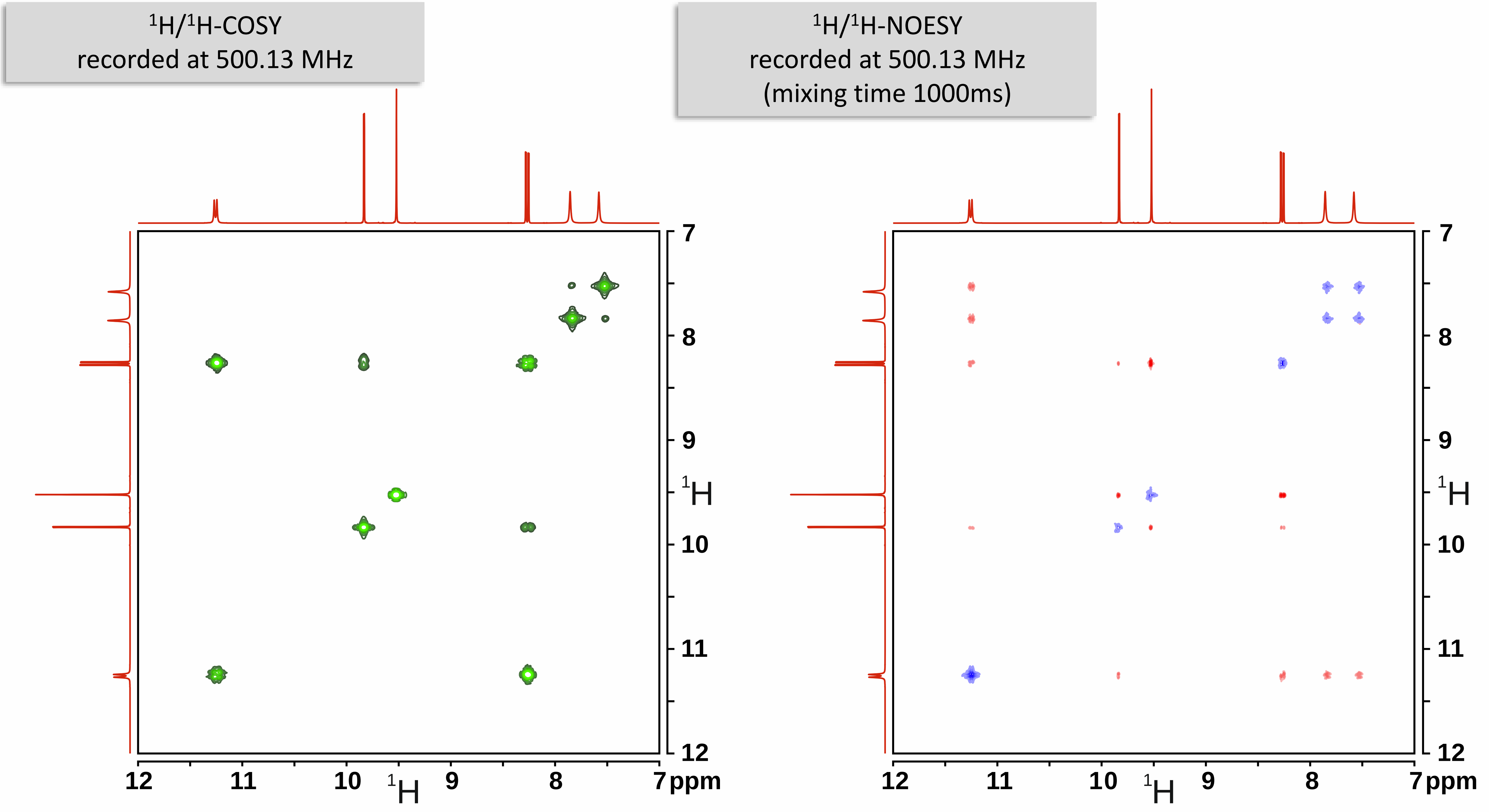


$^1H/^{15}N$-HMBC recorded at 500.13/50.60 MHz





1H/13C-HSQC
recorded at 500.13/125.77 MHz
1H/13C-HMBC
recorded at 500.13/125.77 MHz
150
155
190
13C
10.0
9.5
9.0
1H
8.5
ppm
113
114
115
13C
153
154
190
191
192
11.0
10.5
10.0
1H
9.5
9.0
8.5
ppm



$^1H/^1H$-COSY
recorded at 500.13 MHz
$^1H/^1H$-NOESY
recorded at 500.13 MHz
(mixing time 1000ms)
12
11
10
9
8
7
ppm
^1H

Problem of the Month:

October 2021

Solution

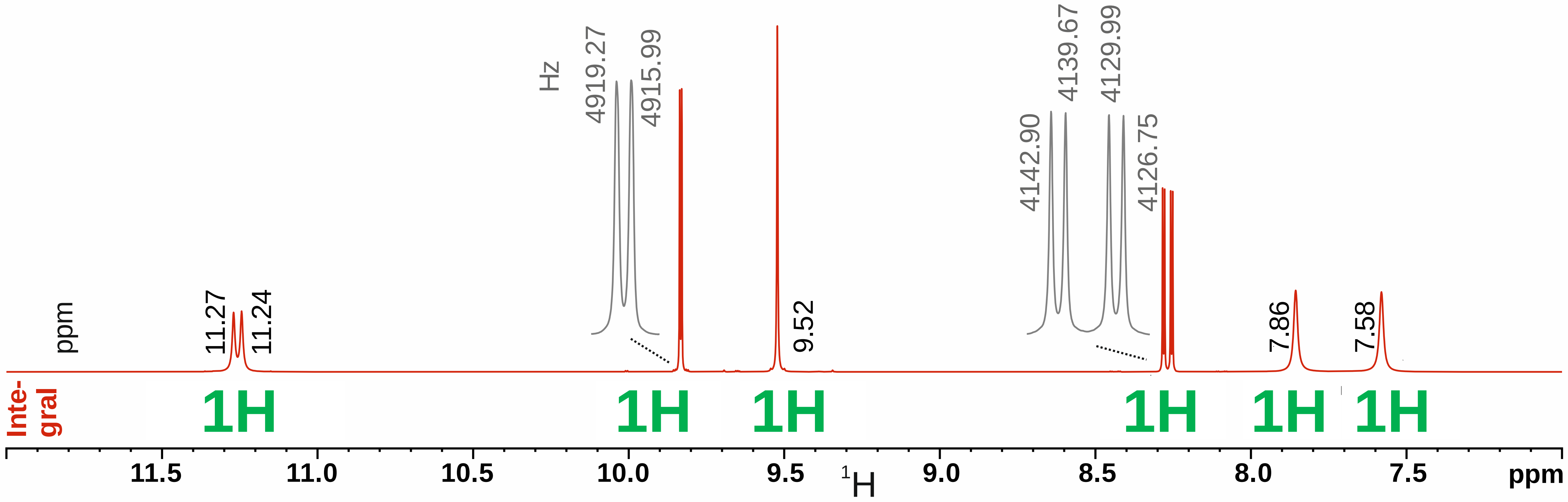
Basic considerations

Double bond equivalents, integral

$C_5H_6O_3N_2$ in DMSO-d_6

The nitrogen might be part of an amino group or a nitro group. The calculation of the double bond equivalents is different for the two cases. Let us postpone this calculation for the moment.

The distribution of the 6 protons from the molecular formula to the six signal groups is simple because the integrals of all signal groups are almost identical.



Building blocks

CH_n-fragments

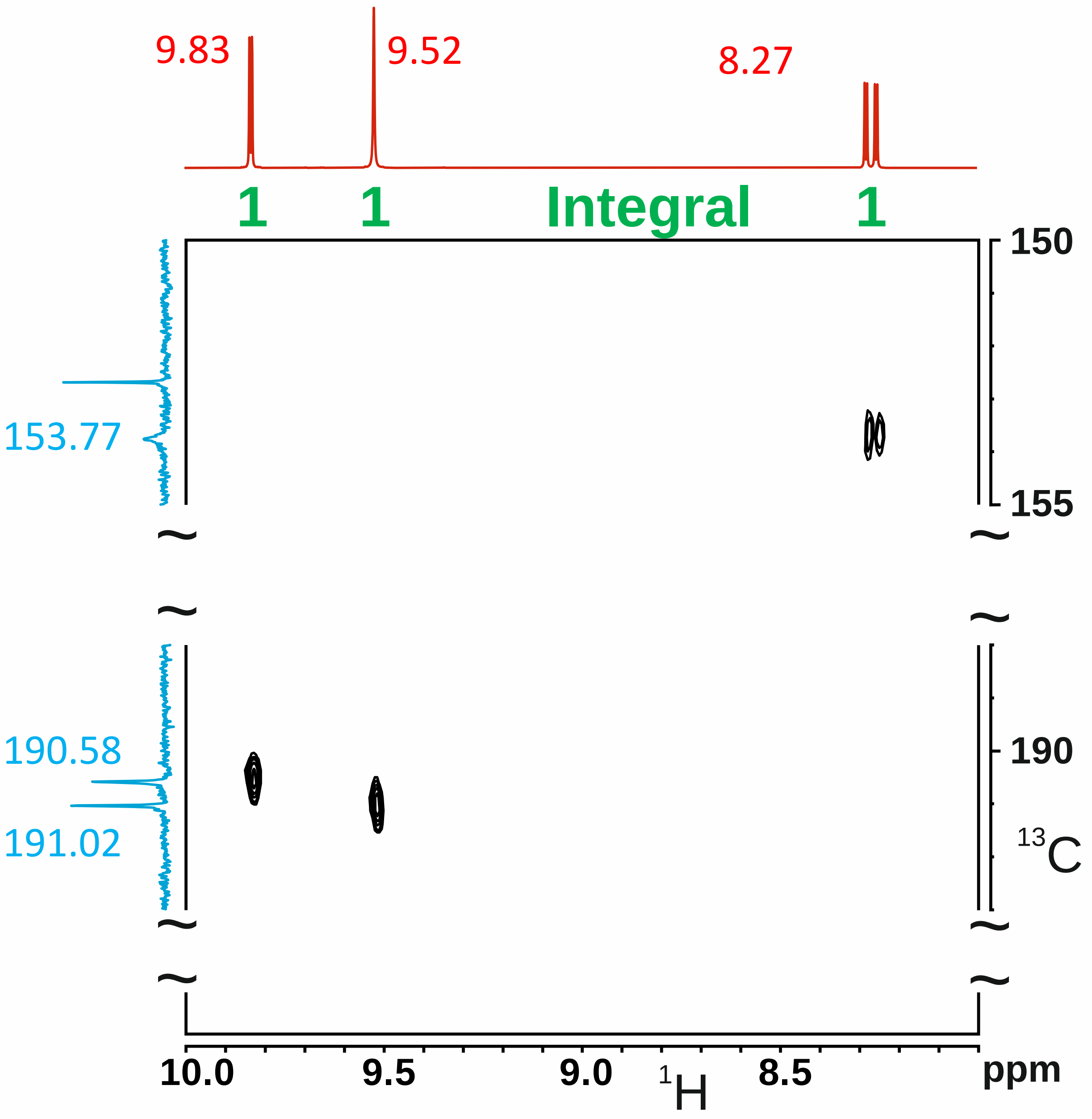
It is very easy to evaluate a HSQC. The sensitivity, of course, is less than the sensitivity of a one dimensional proton spectrum but much higher than a one dimensional carbon spectrum. Therefore, the measurement of an HSQC is always recommended, if possible.

We need some data for the projections, chemical shifts and integrals from the one dimensional proton spectrum and the carbon chemical shifts from the one dimensional carbon spectrum.

You need to calculate the chemical shifts [ppm] for some signals from the chemical shifts [Hz] as shown here for one multiplet.

$$\delta = \frac{(4142.90\ \text{Hz} + 4126.75\ \text{Hz})}{2 * 500.13\ \text{MHz}} = \mathbf{8.27\ ppm}$$

HSQC

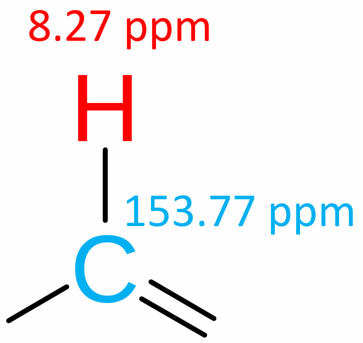


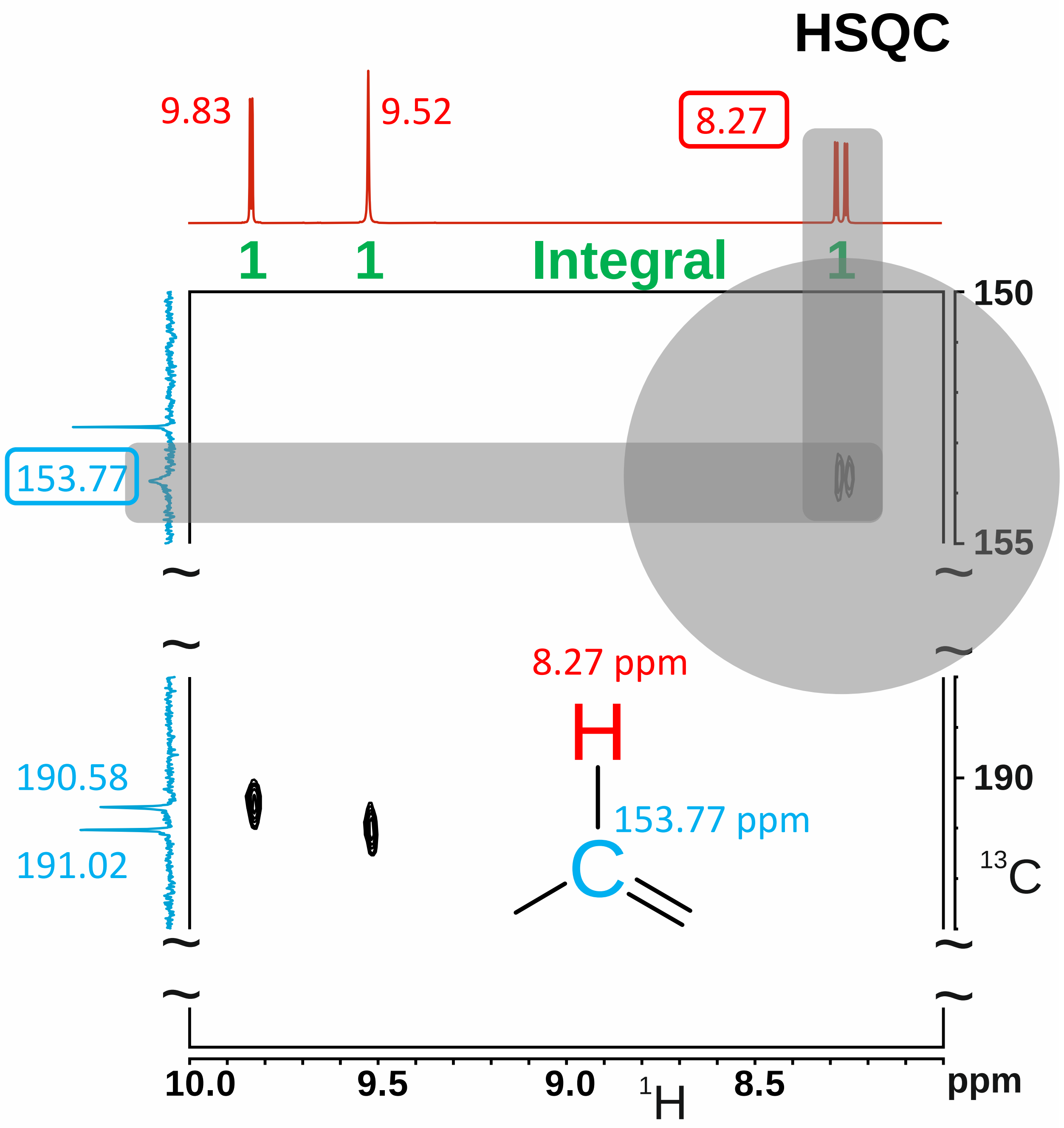
Building blocks

CH$_n$-fragments

The first fragment we can extract from the HSQC is a **=CH-** group with a sp^2 hybridized carbon atom.

Both the proton and the carbon chemical shift clearly indicate the sp^2 hybridization.



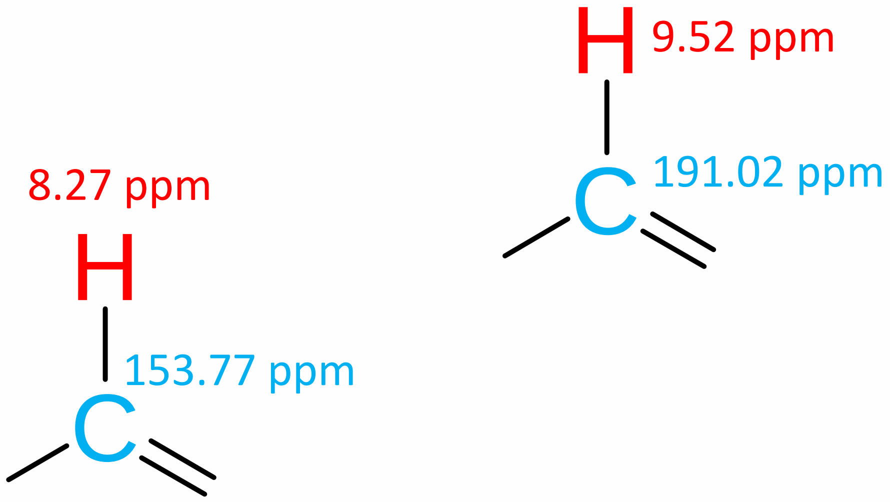


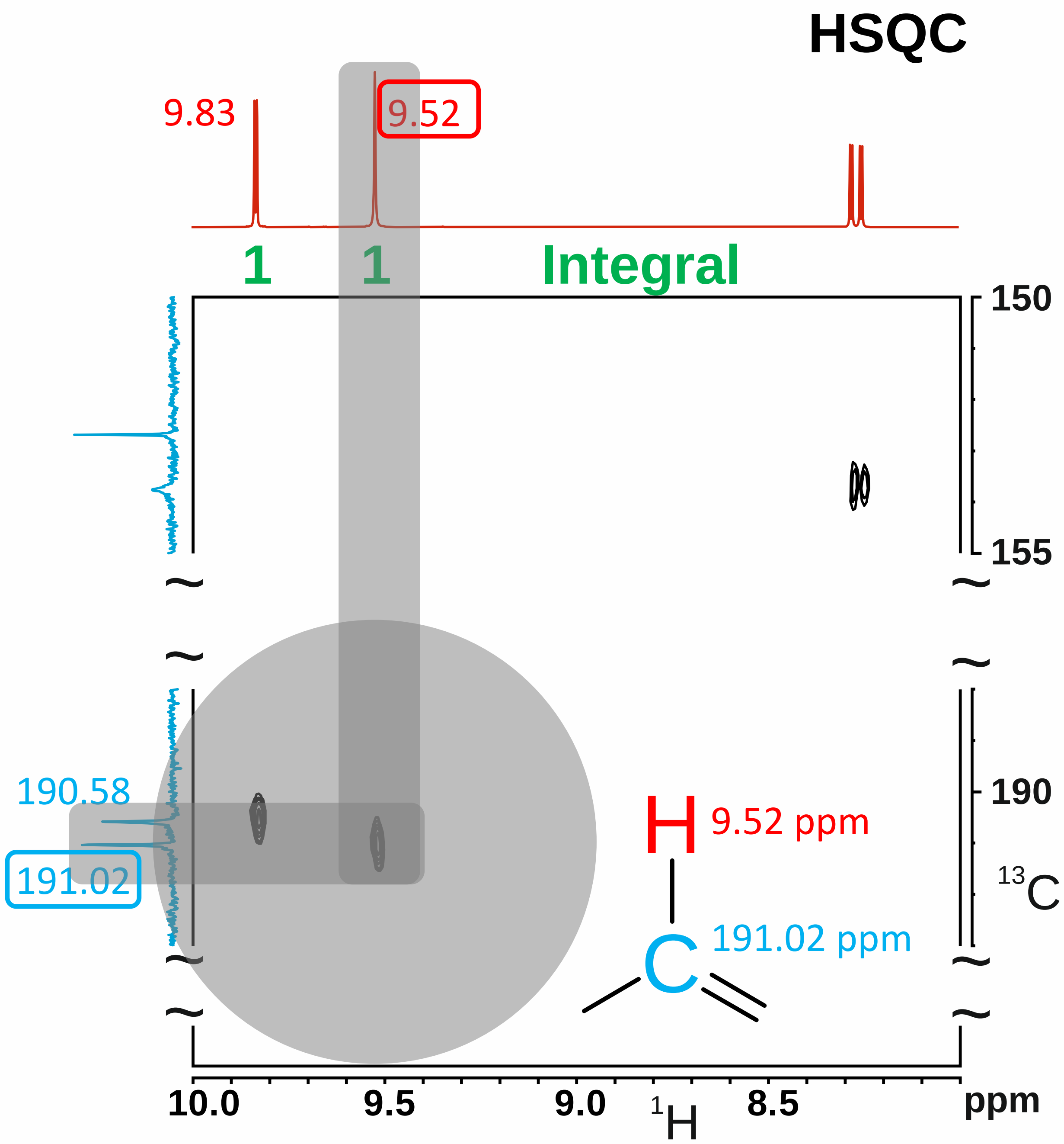
Building blocks

CH_n-fragments

The second fragment we can extract from the HSQC is another **=CH-** group with a sp[2] hybridized carbon atom.

But …



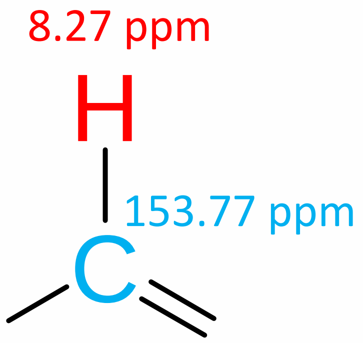


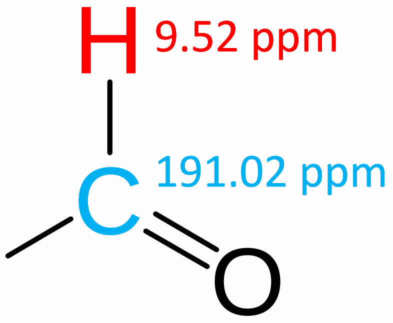
Building blocks

CH_n-fragments

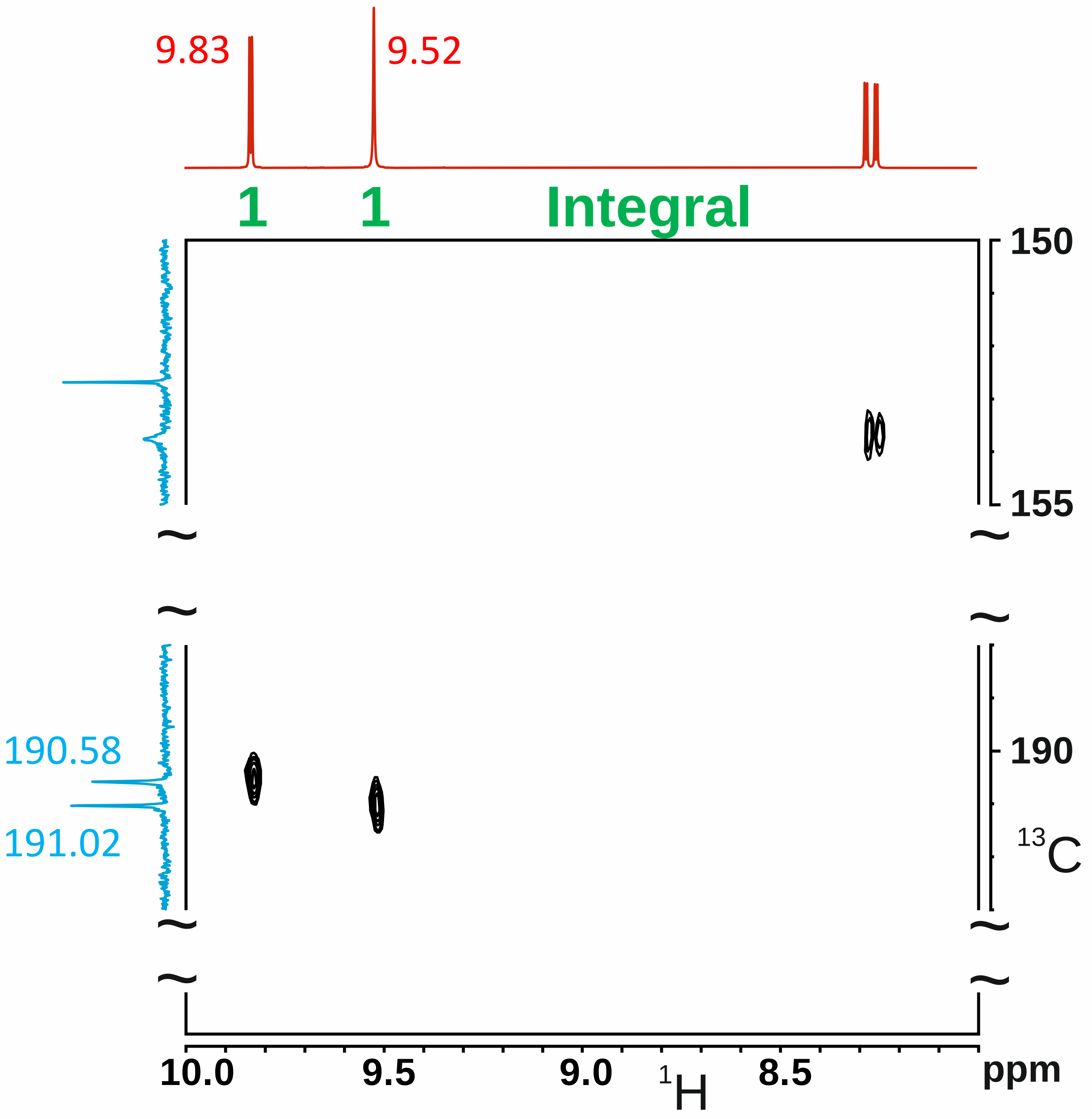
A chemical shift of 191.02 ppm for a carbon atom and of 9.52 ppm for a proton bound to this carbon is very characteristic.

That‘s an aldehyde group.





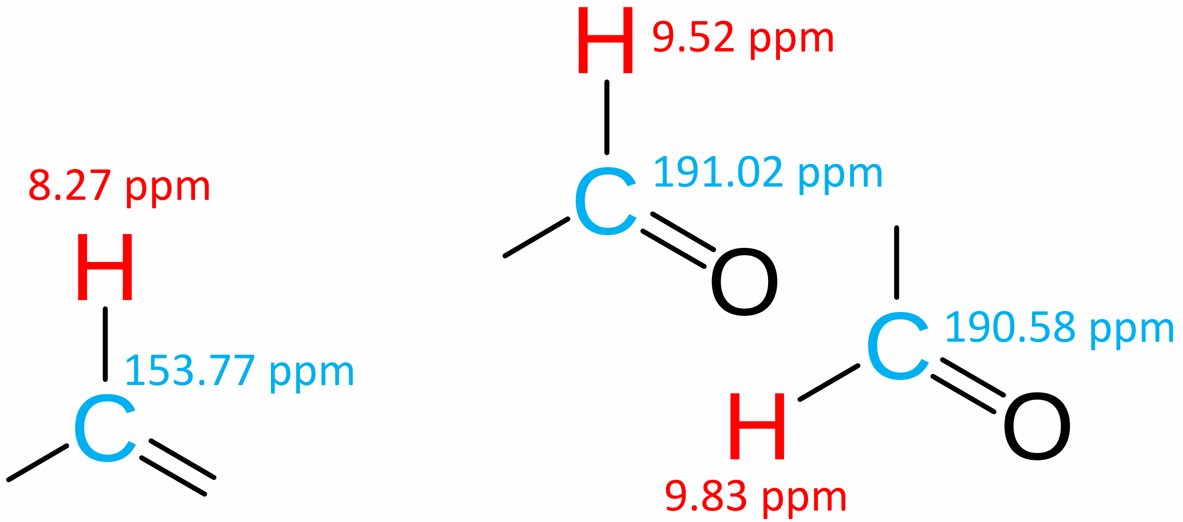
HSQC



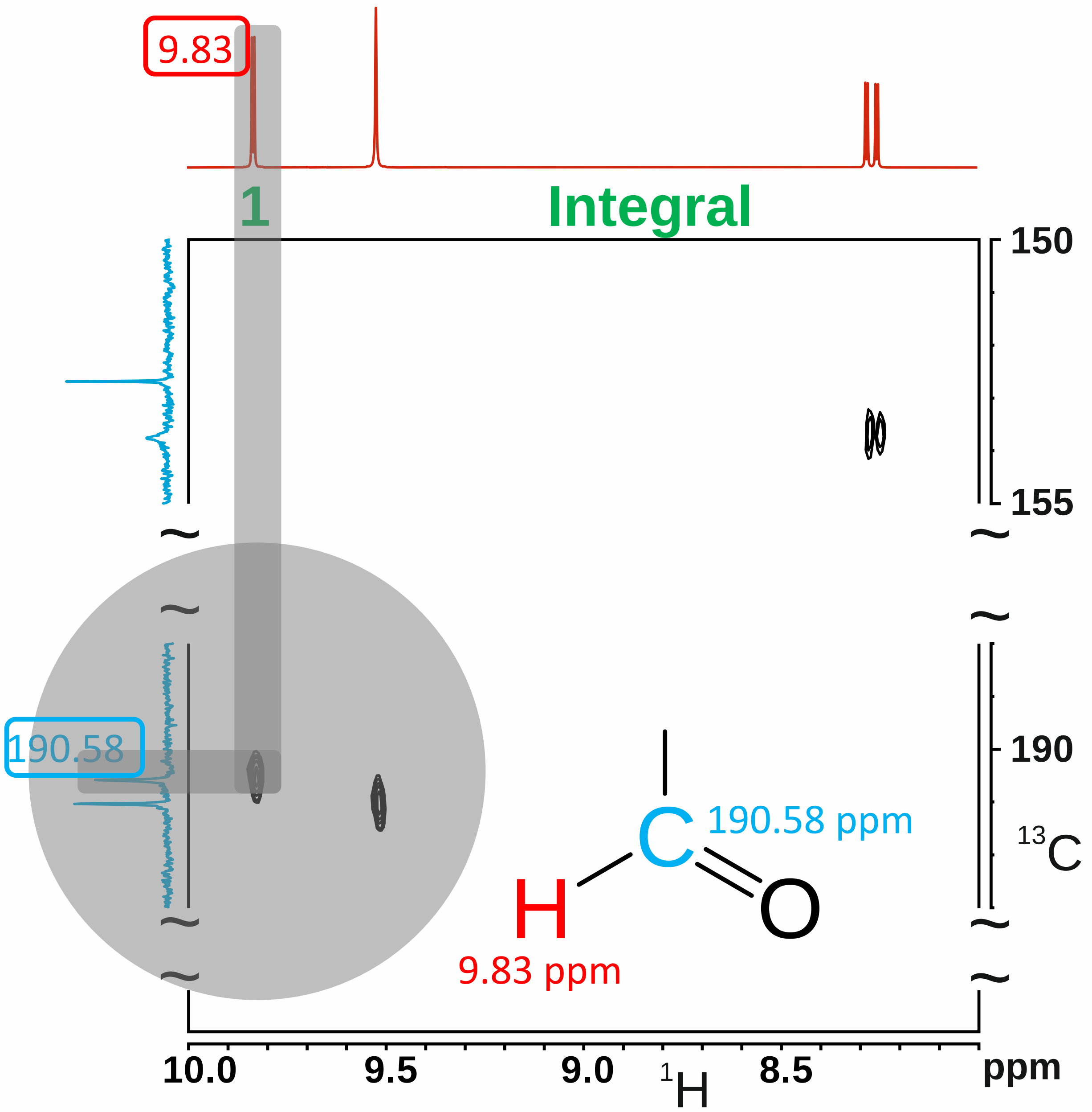
Building blocks

CH$_n$-fragments

There is another aldehyde group.



HSQC



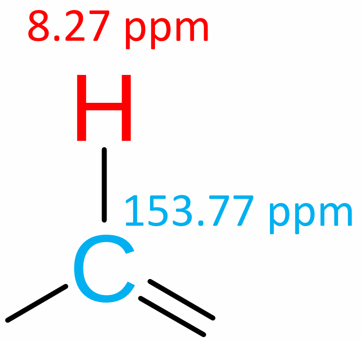
Building blocks

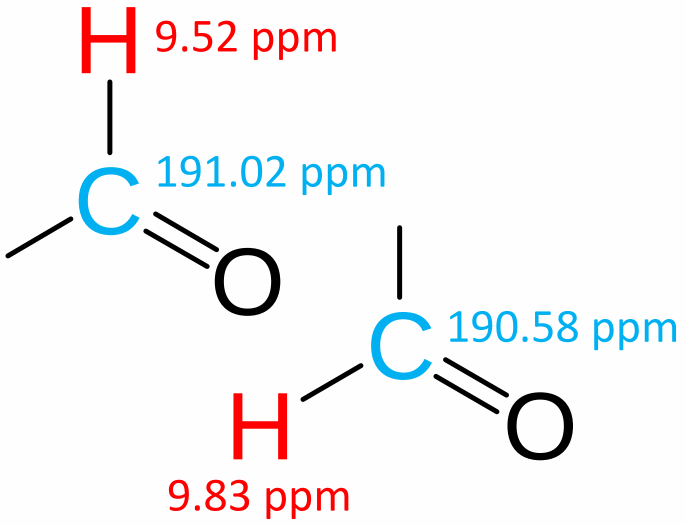
Quaternary carbon atoms

As seen in the HSQC there are no more protonated carbon atoms.

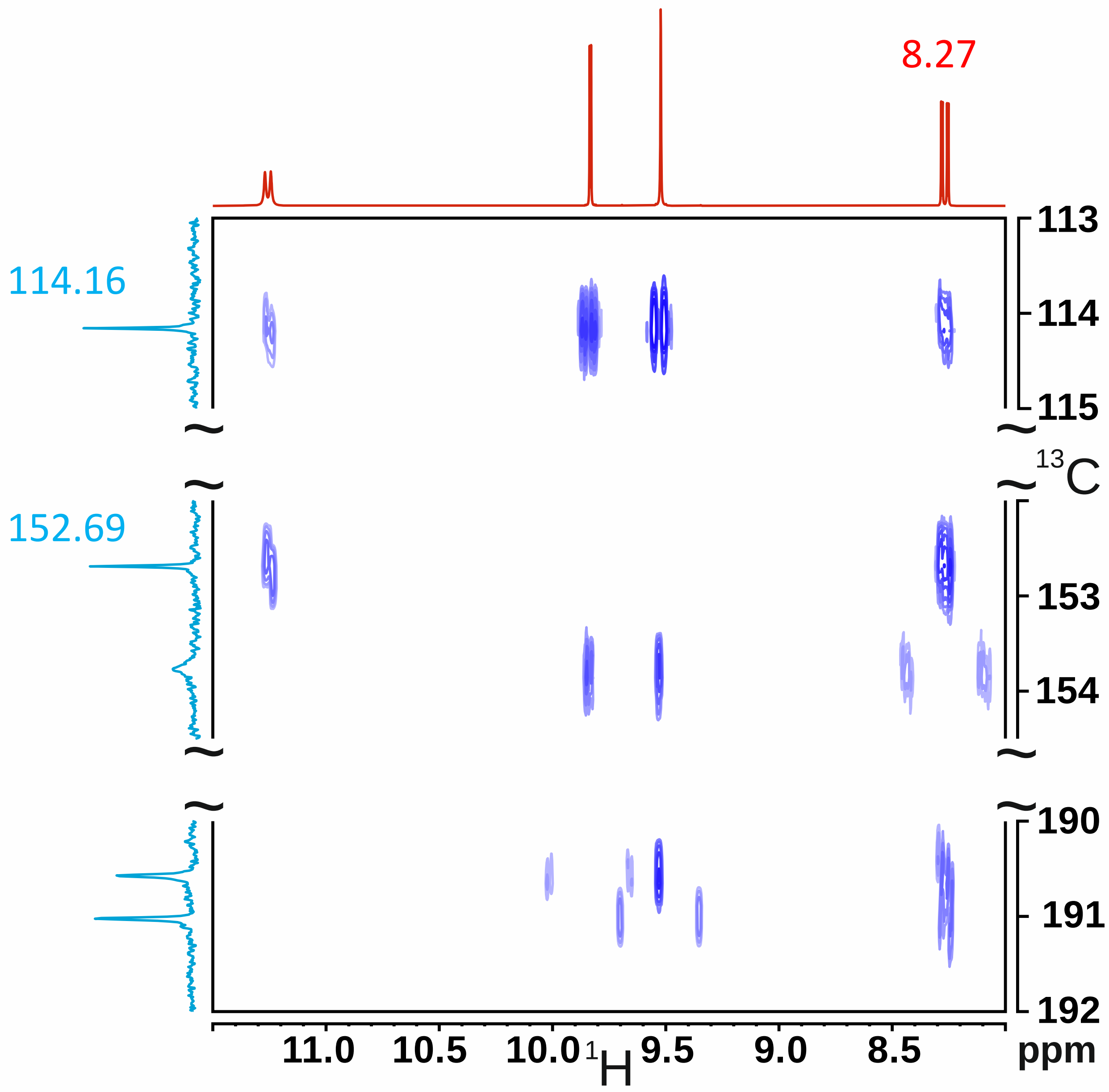
According to their chemical shifts the two quaternary carbon at 114.16 ppm and 152.69 ppm are sp^2 hybridized.

First let us investigate the carbon atom at 114.16 ppm in some more detail.





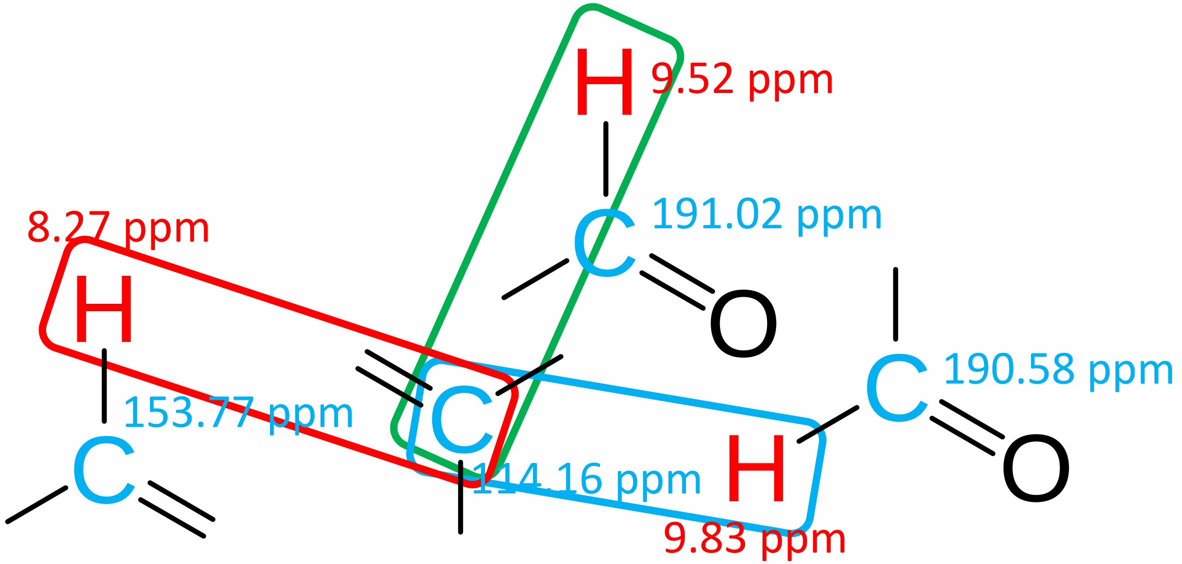
HMBC

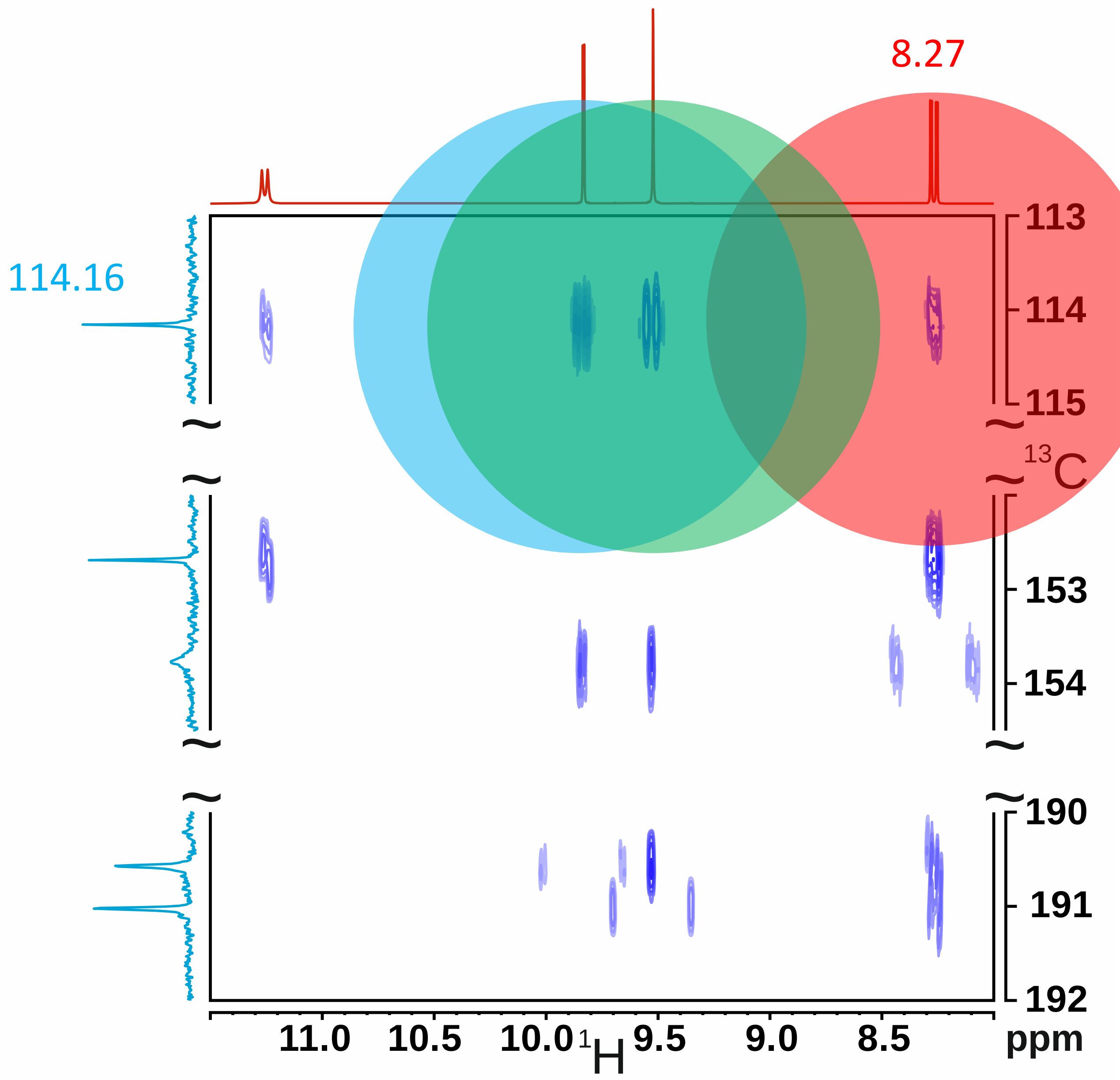


Building blocks

Quaternary carbon atoms

For the moment there are three correlations of interest between the sp^2 hybridized carbon atom at 114.16 ppm and the proton signals already known.

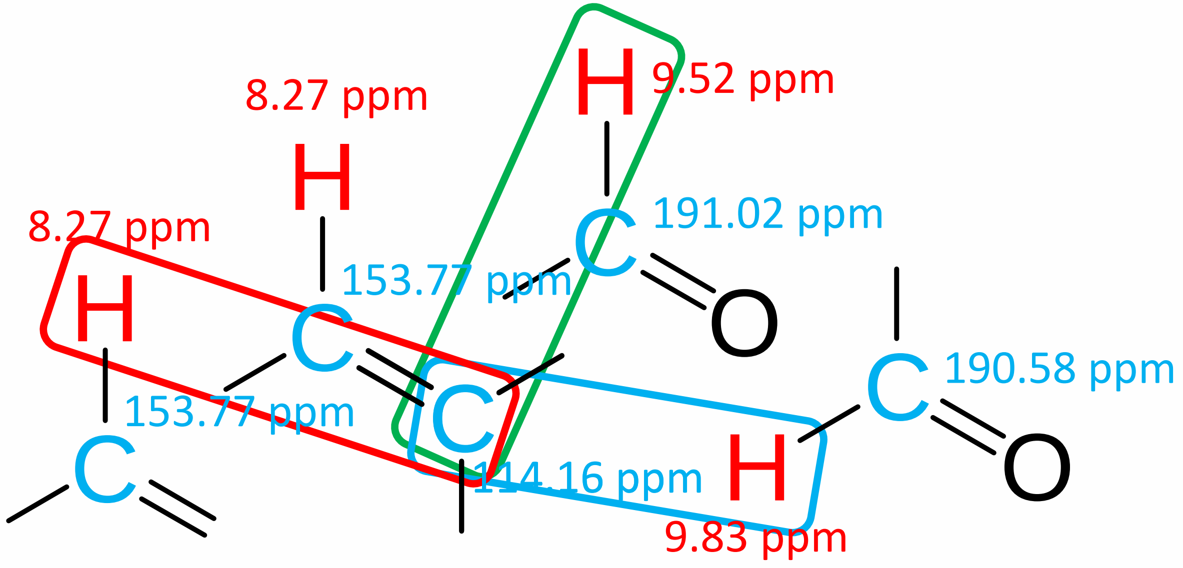


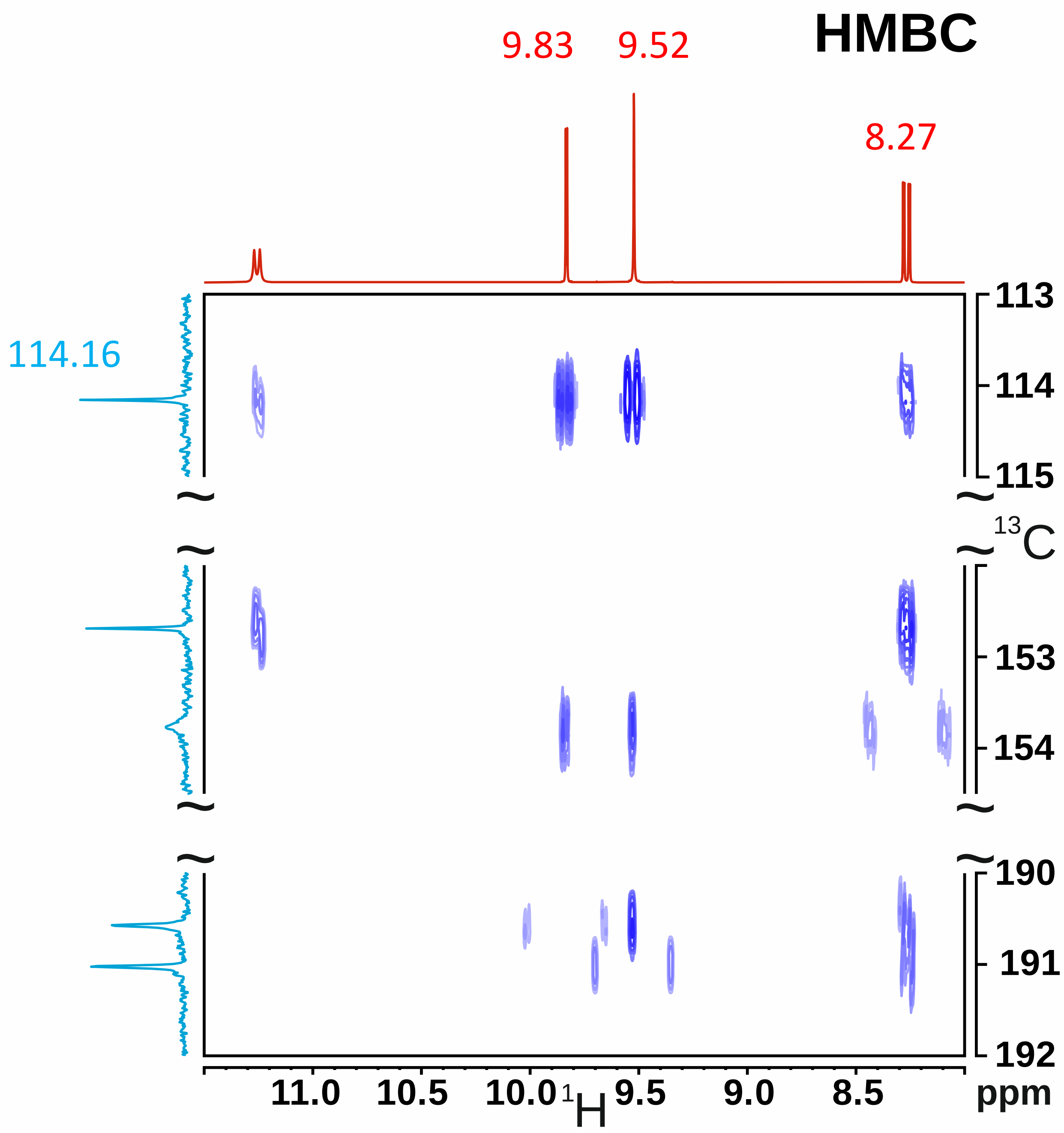


Building blocks

Quaternary carbon atoms

Let us try to get a partial structure step by step, which fulfills all correlations observed.





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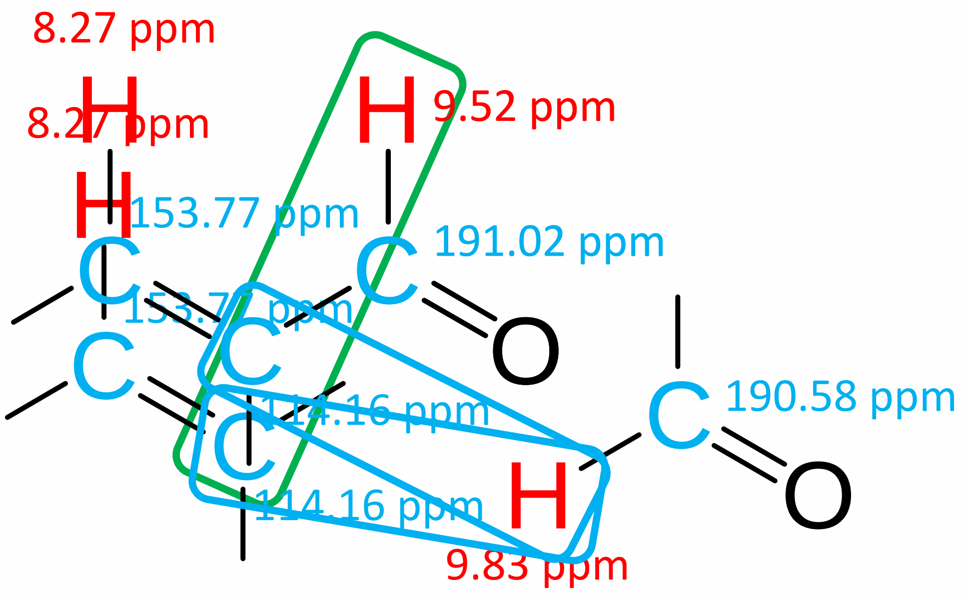

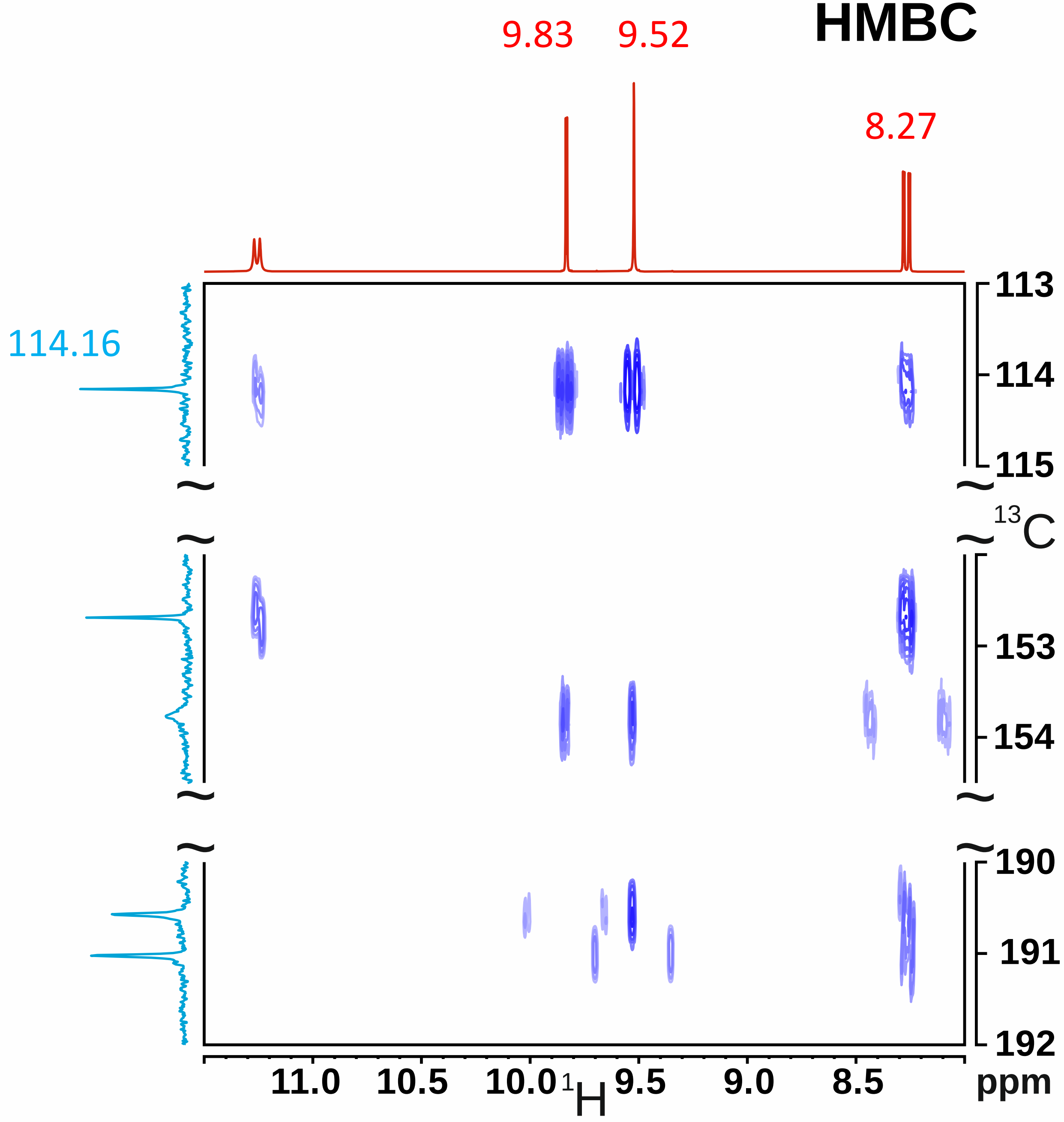

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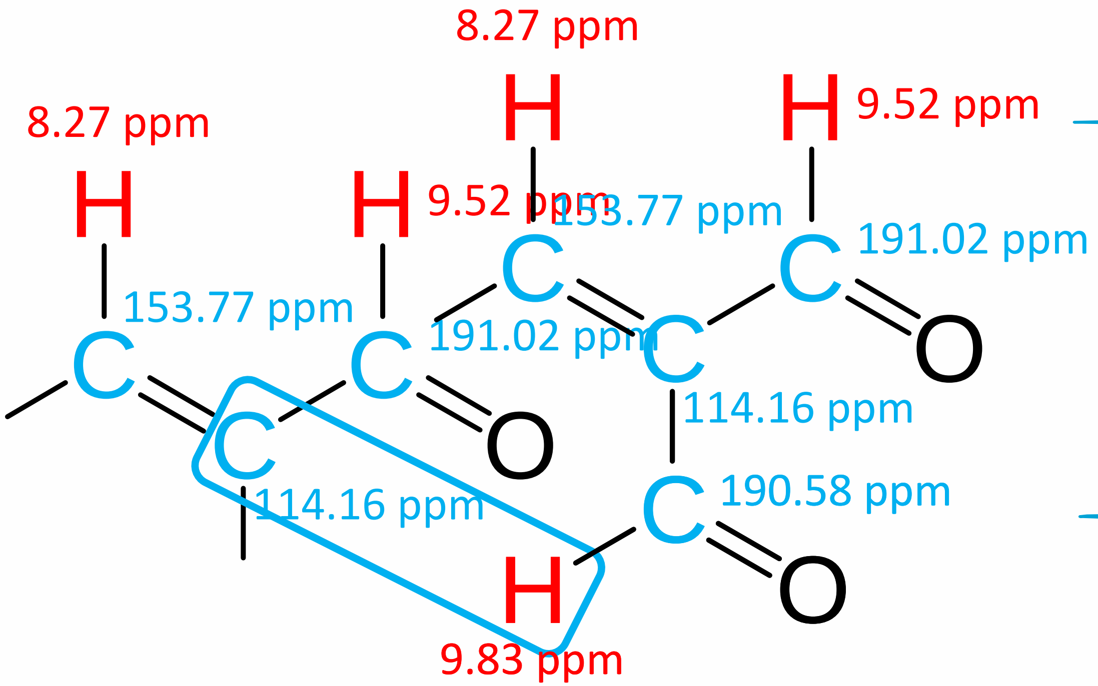
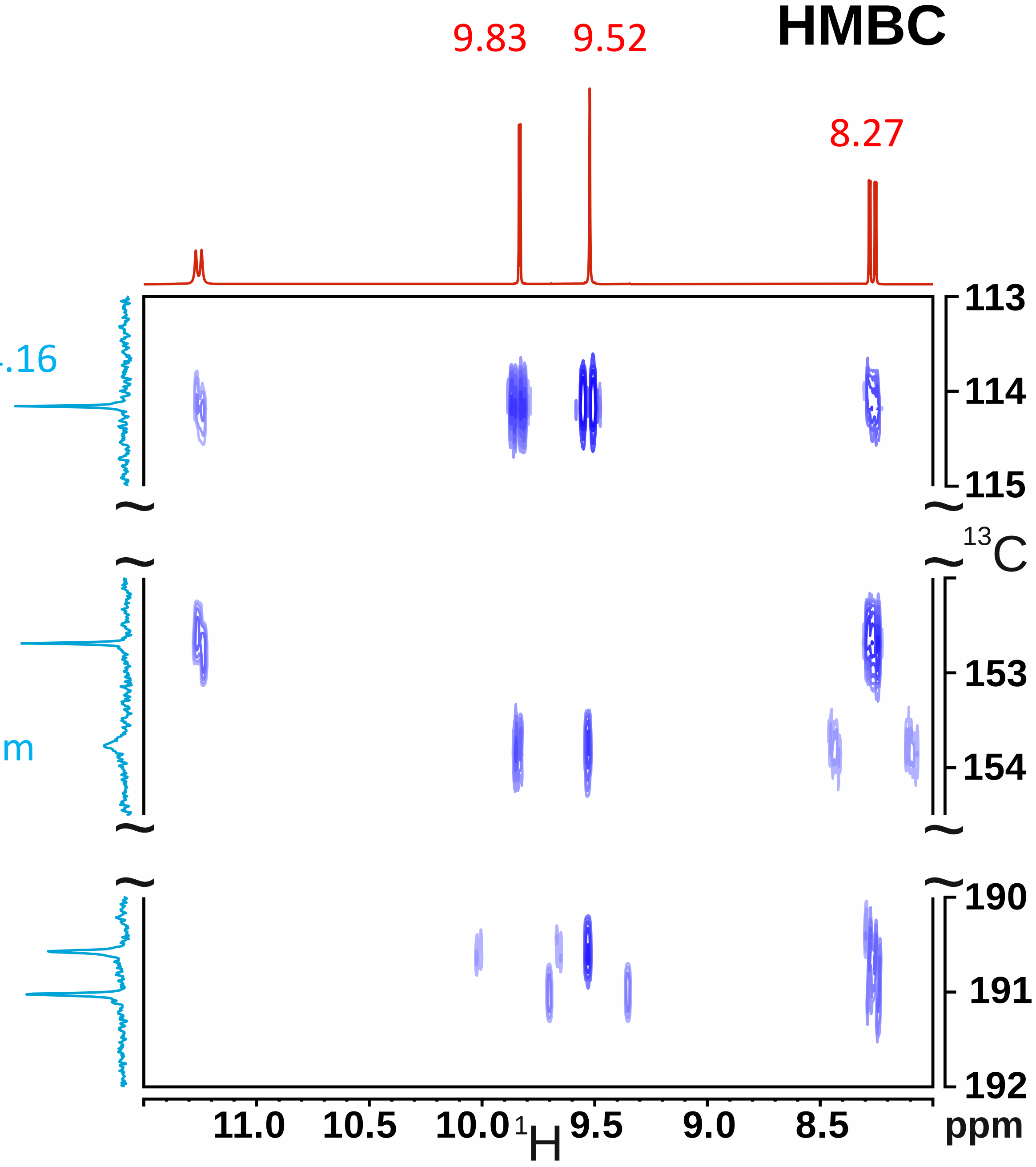
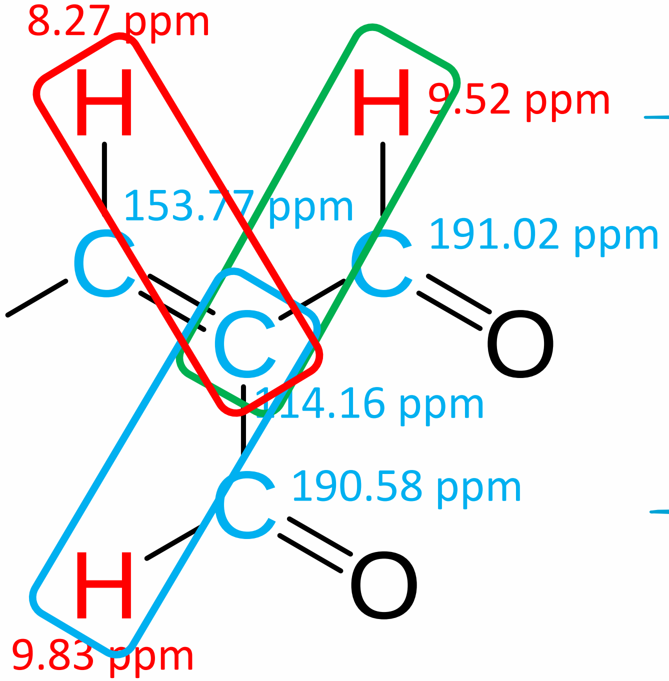


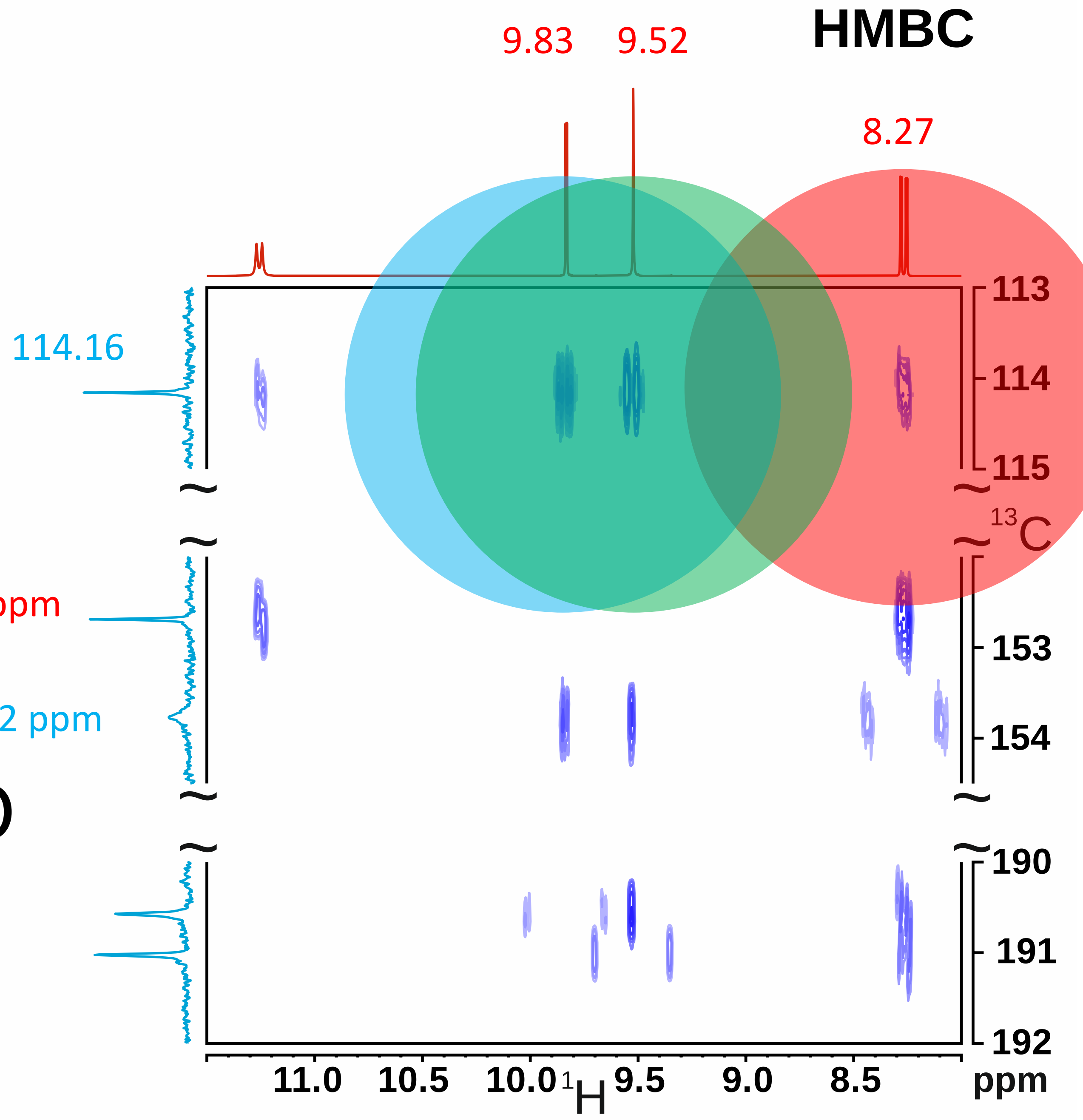


Building blocks

Quaternary carbon atoms

To be sure let us check the HMBC cross peaks again with the constructed structure fragment.



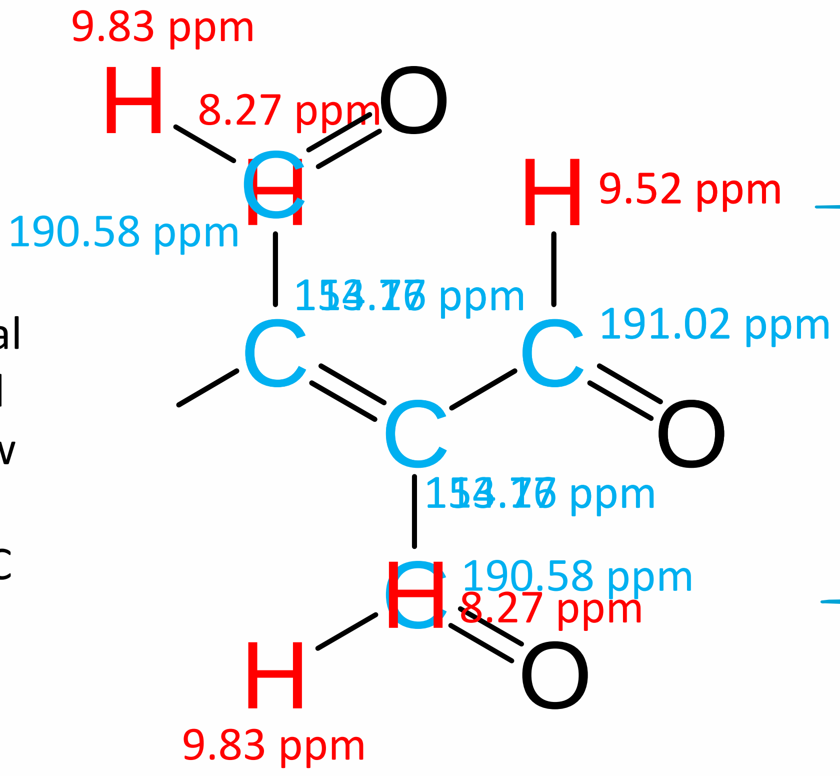


Building blocks

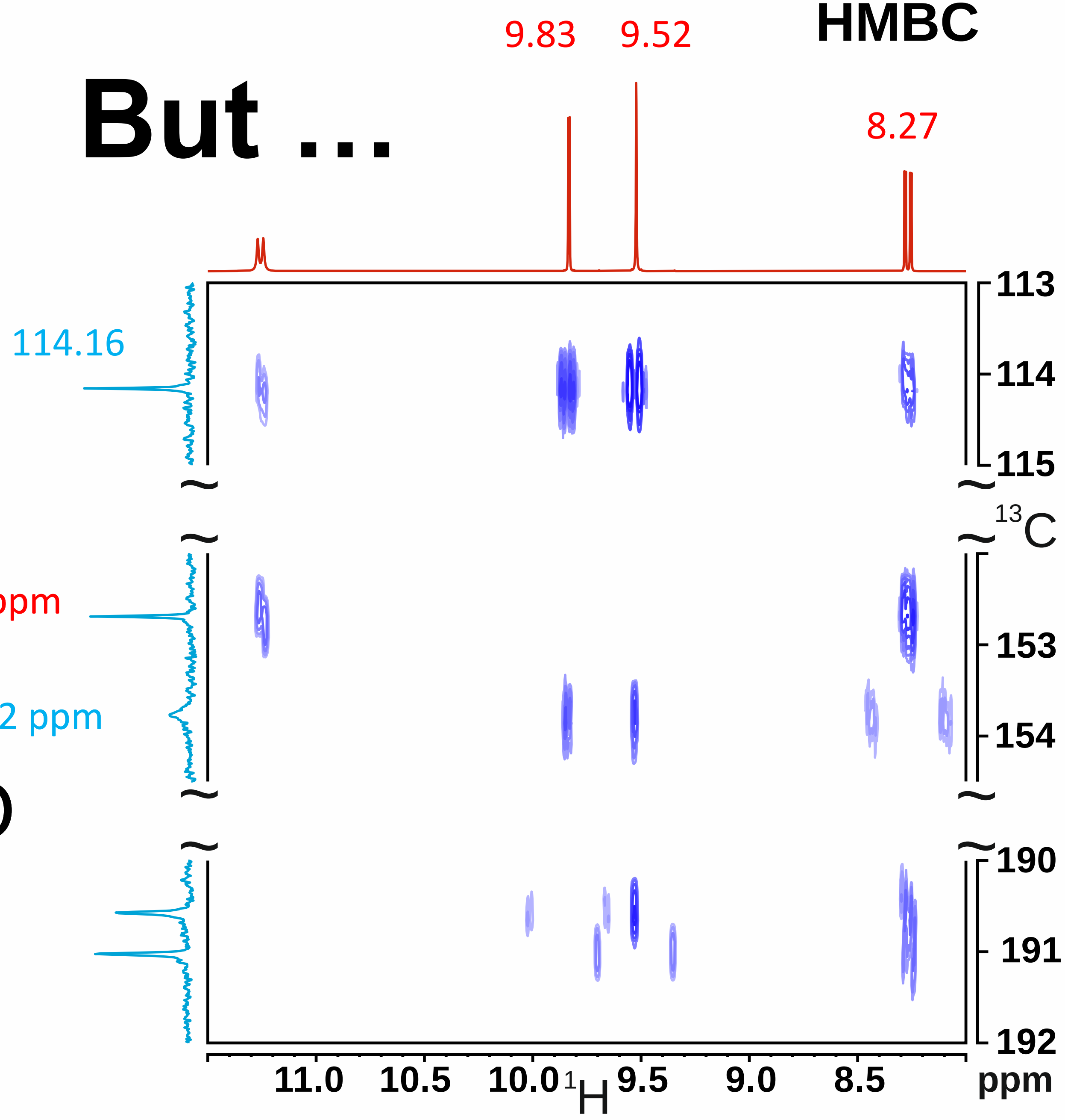
Quaternary carbon atoms

Is there maybe another combination of the four fragments, which is consistent with the HMBC cross peaks?

Let us modify the partial structure a little bit and check, whether the new partial structure might explain the three HMBC cross peaks.



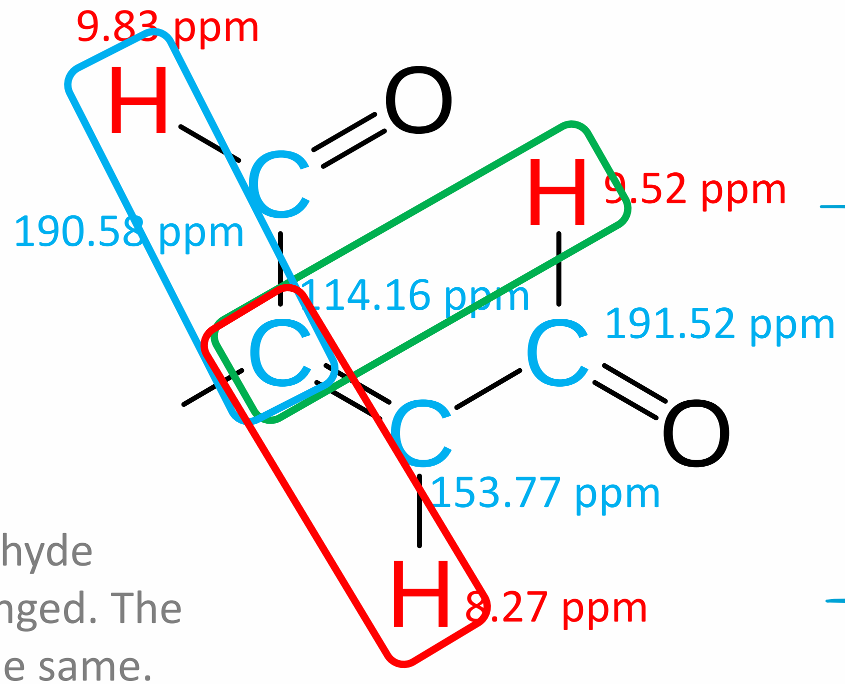
But …



Building blocks

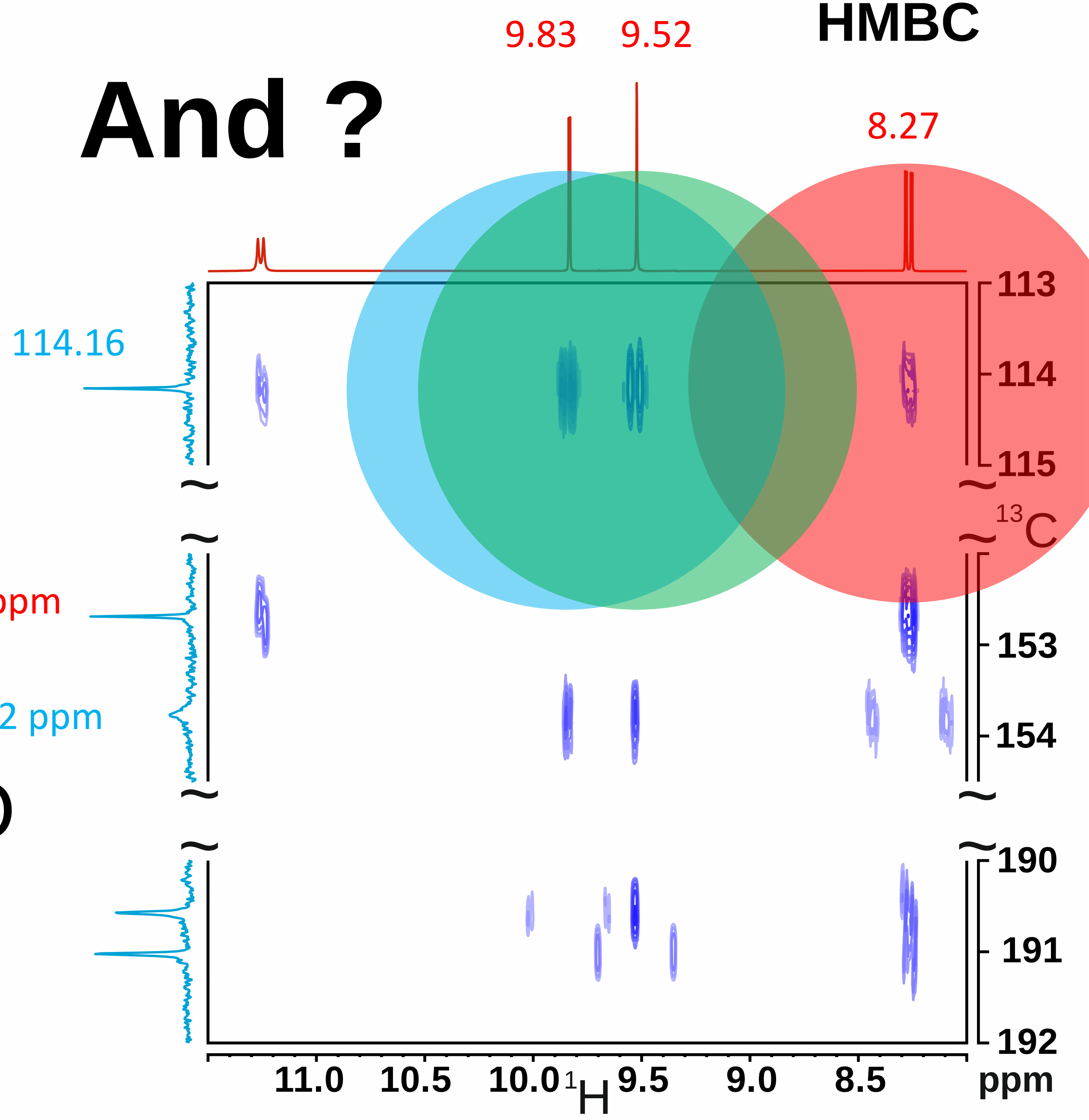
Quaternary carbon atoms

It is possible to explain **all three** HMBC cross peaks with this partial structure.



Please note: Both aldehyde groups might be exchanged. The final result would be the same.

And ?

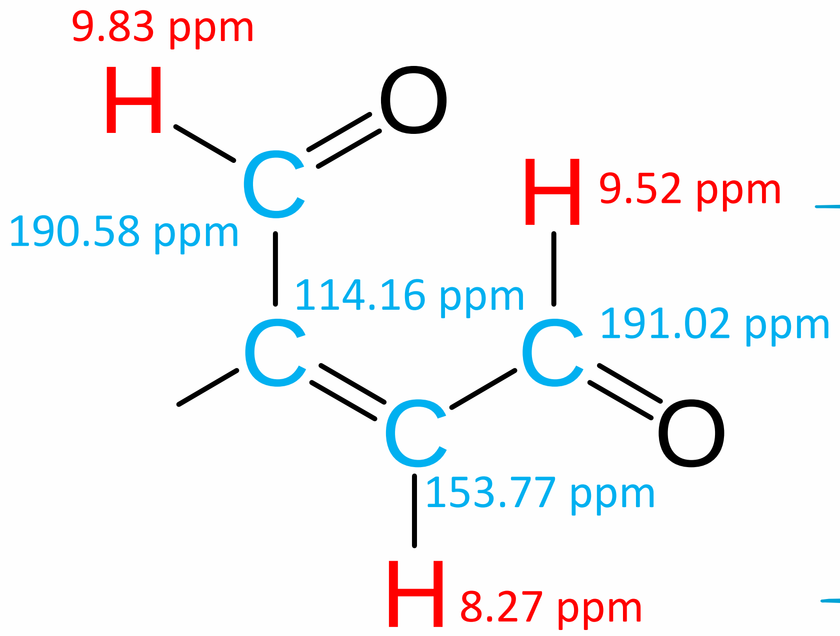


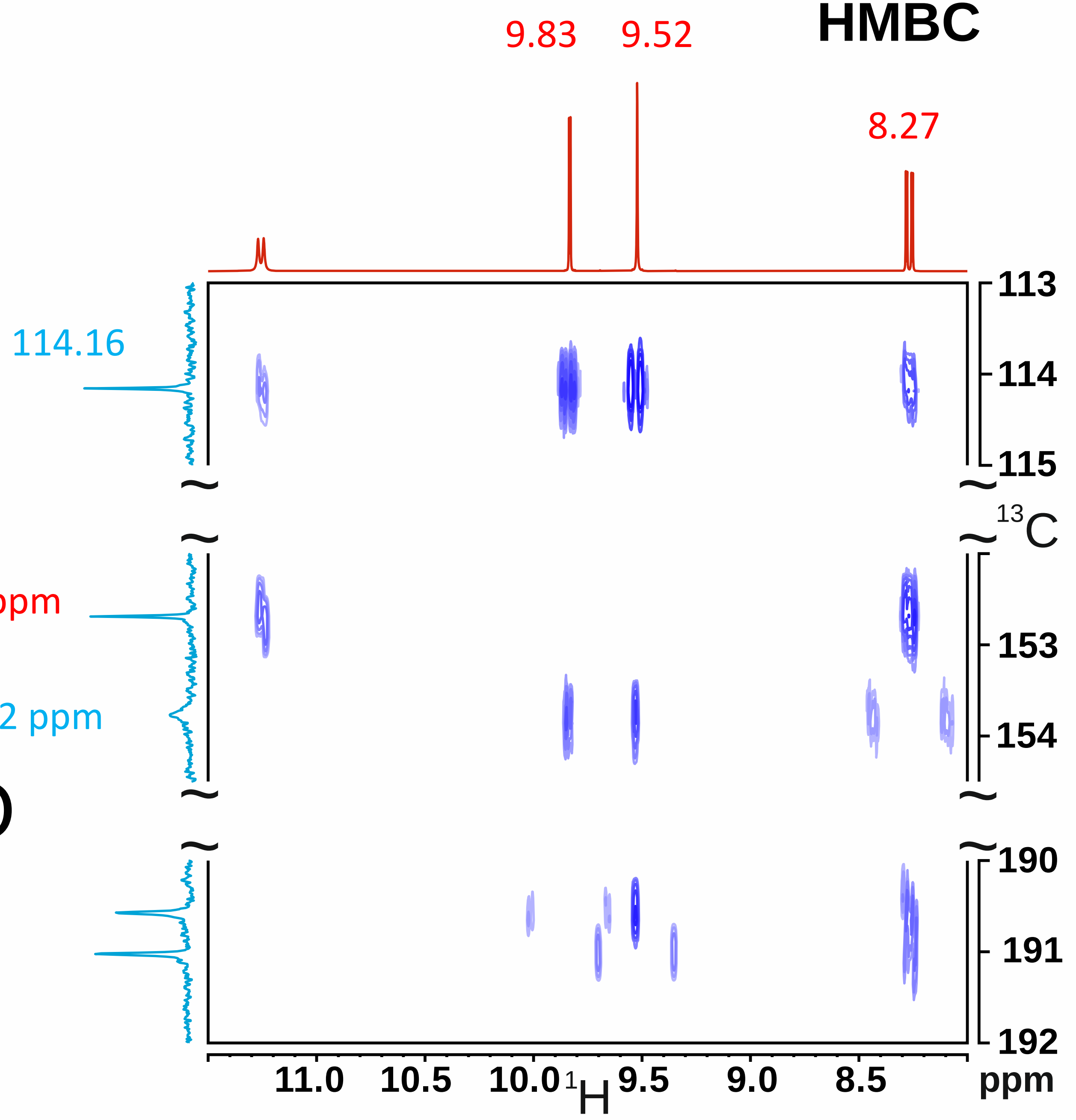
Building blocks

Exclude one partial structure

Is there any possibility to exclude this partial structure?

Let us investigate the multiplets of the three protons.



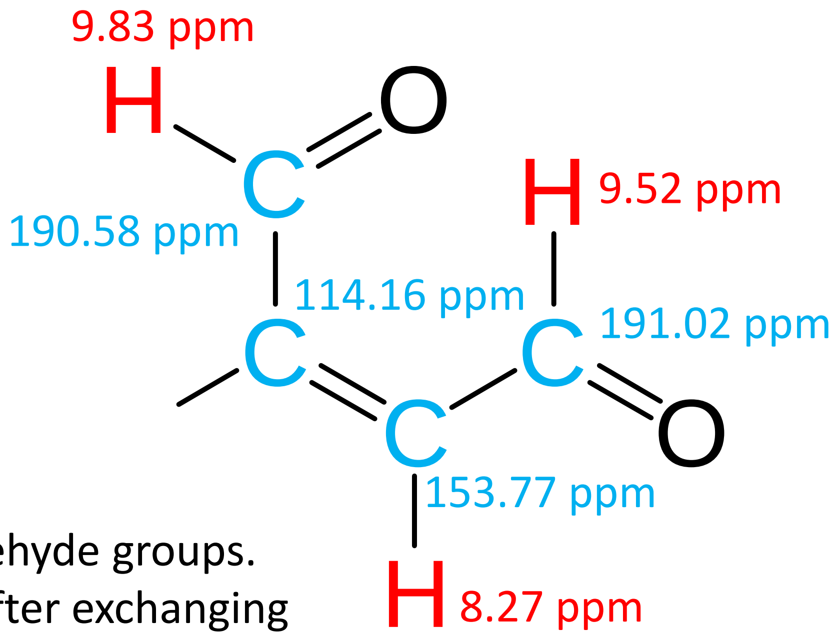


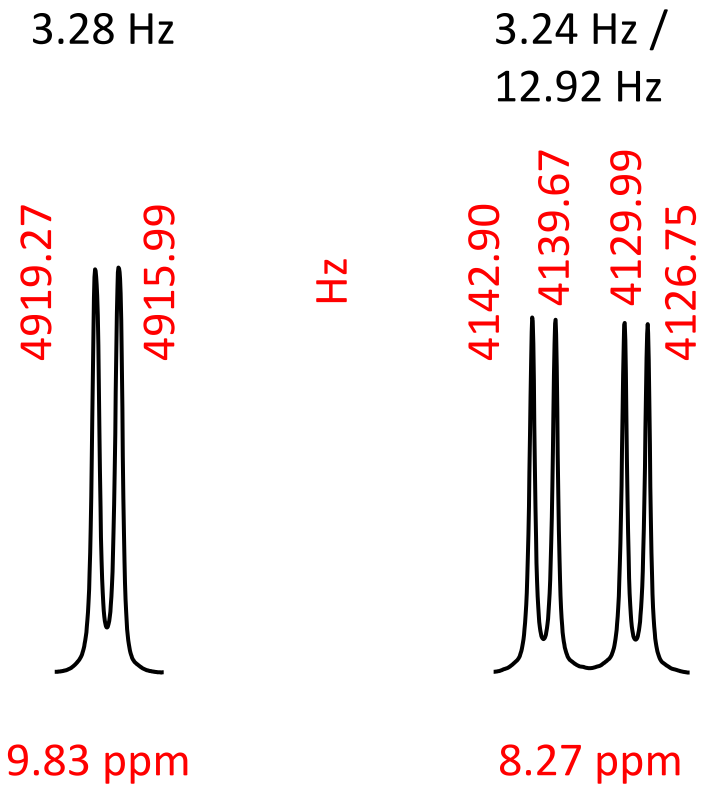
Building blocks

Exclude one partial structure

The proton signal at 9.52 ppm appears as **singlet** (not shown here), the signal at 9.83 ppm as **doublet** and the signal at 8.27 ppm as **doublet of doublets**.

Extracting the coupling constants should (hopefully) be no challenge.





Let us exchange both aldehyde groups. Please remember, even after exchanging the aldehyde groups we would be able to explain the HMBC peaks.

Building blocks

Exclude one partial structure

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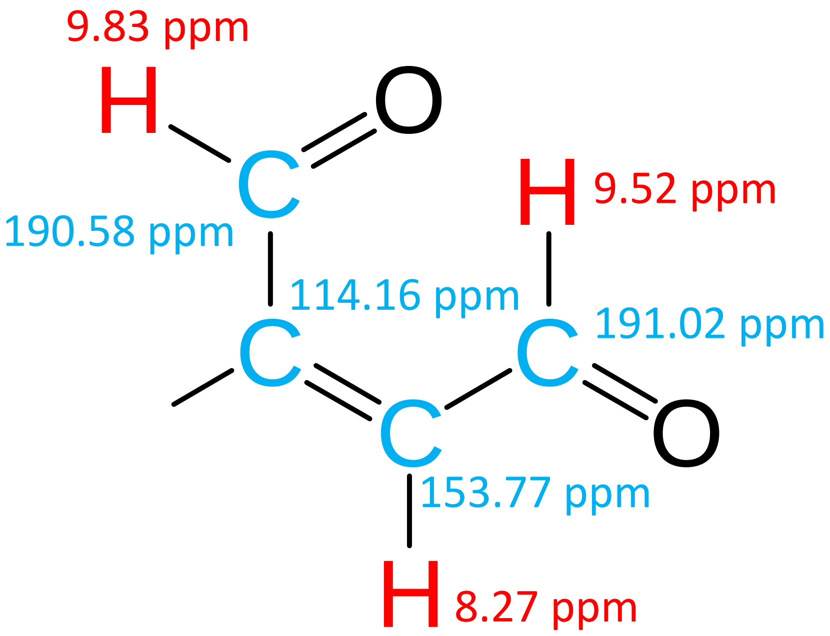

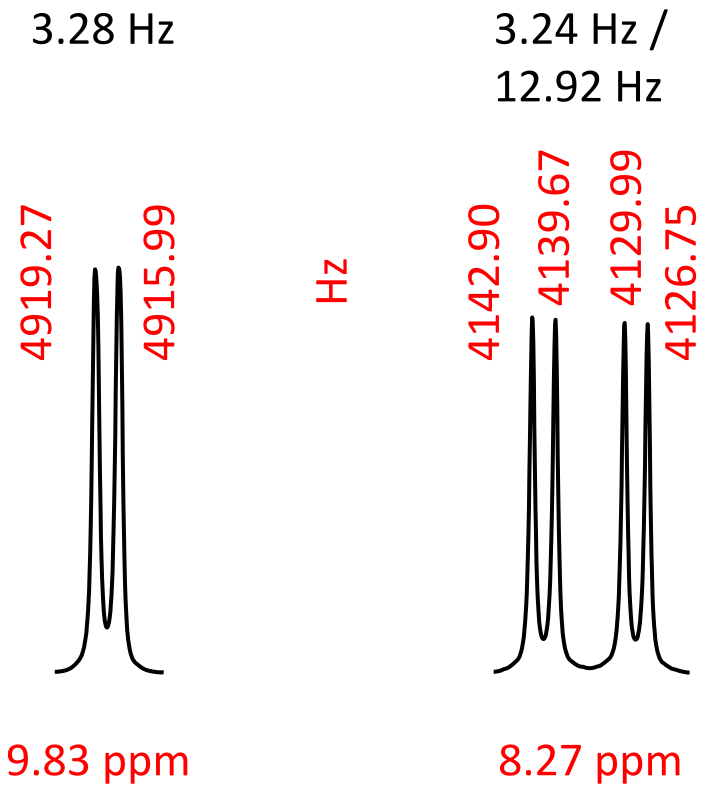

Building blocks

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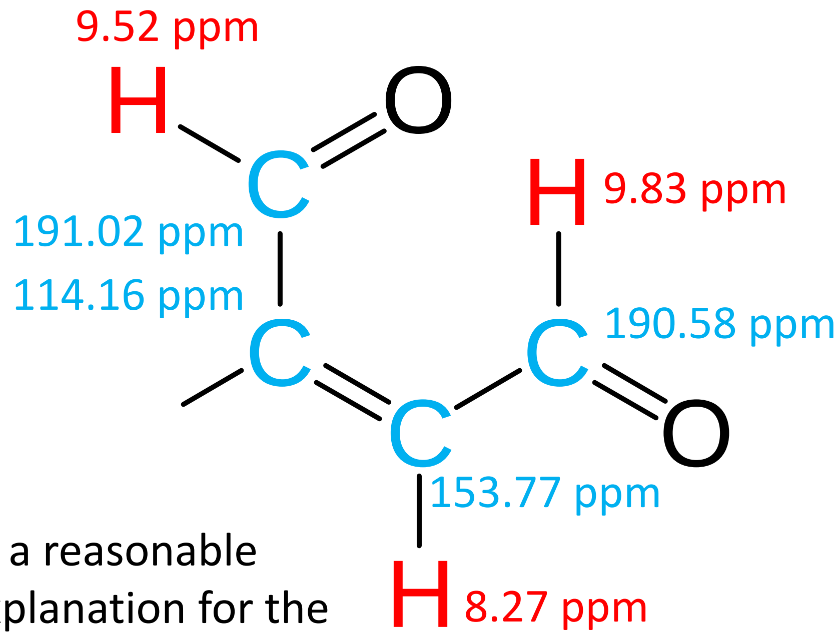
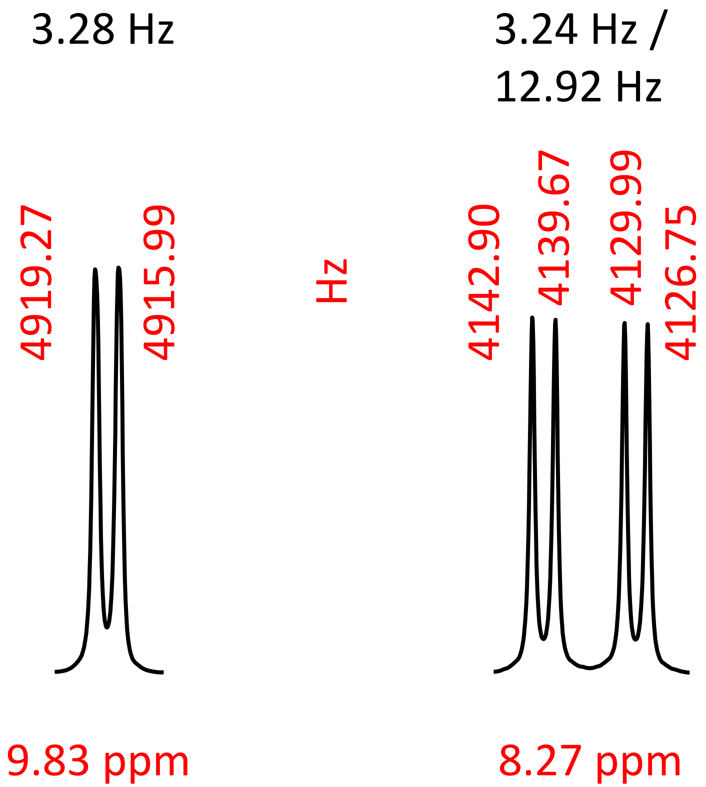




Now there seems to exist a reasonable (although not perfect) explanation for the smaller of both coupling constants (we take the average of 3.24 Hz and 3.28 Hz).

Building blocks

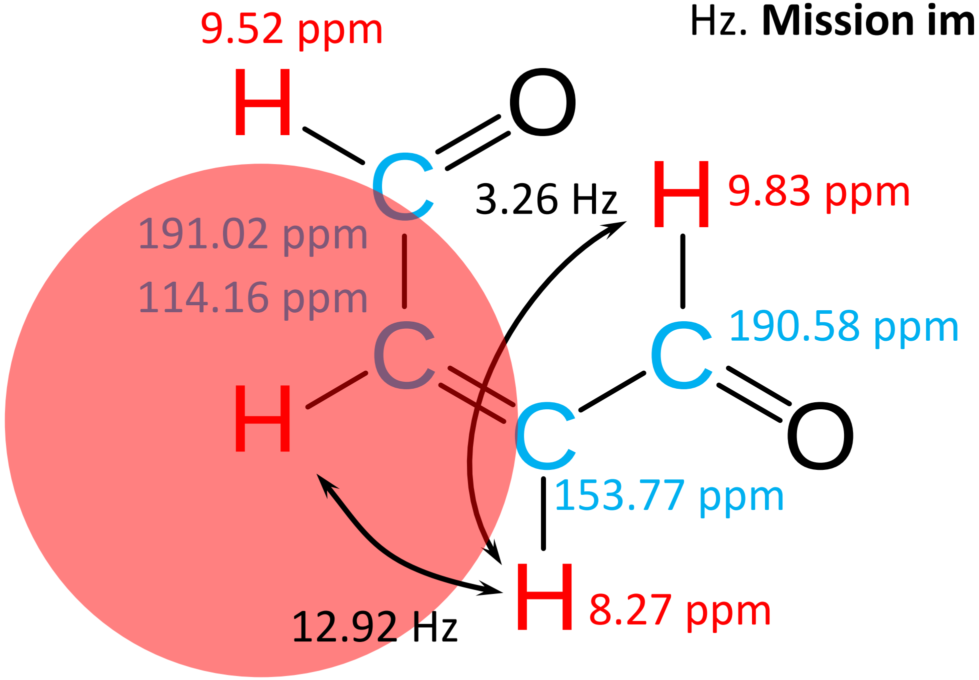
Exclude one partial structure

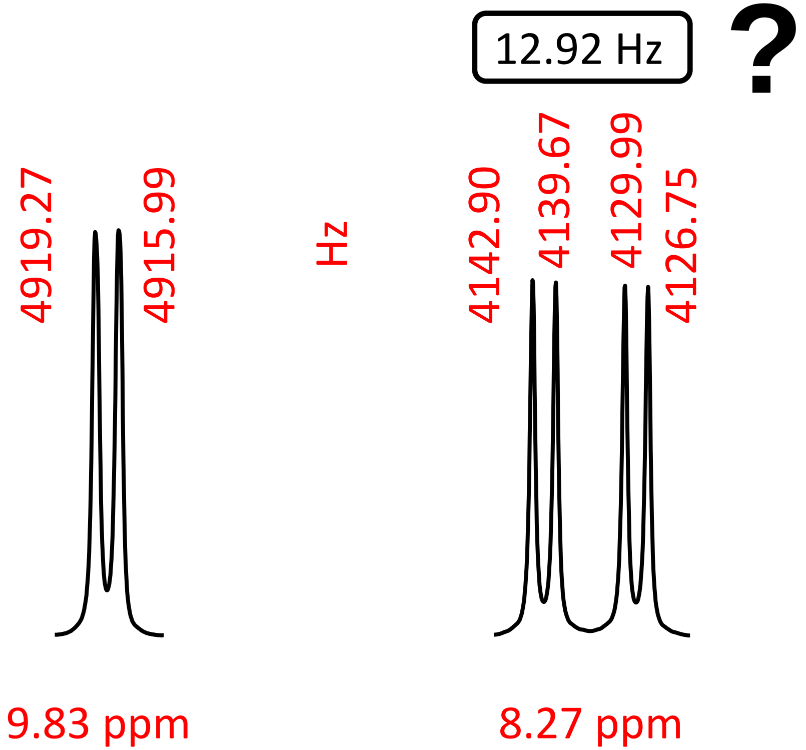
Let us return to our first partial structure.

But what about the second coupling constant of **12.92 Hz** visible in the multiplet of the proton at 8.27 ppm?

Of course we might think about an additional proton.

But the carbon atom at 114.16 ppm is not connected to a hydrogen as seen in the HSQC. Which means, we would have at least a four bond coupling constant with a value of 12.92 Hz. **Mission impossible**.





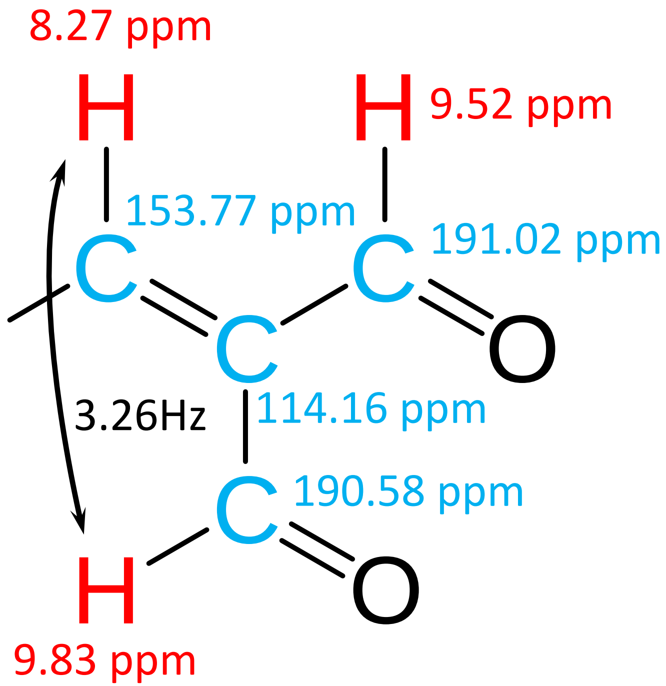
Building blocks

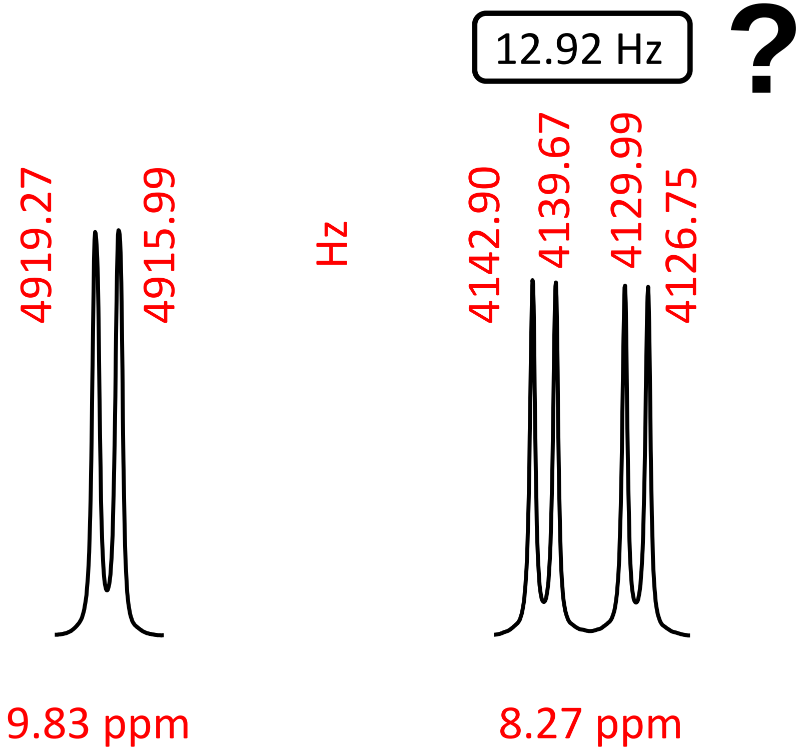
Back to first partial structure

If we return to the first partial structure the coupling constant of 3.26 Hz has to be a four bond coupling constant. There is no other possibility. That‘s rather common, as soon as the coupling path includes π electrons.

But what about the 11.92 Hz?

Let us introduce a hypothetical **X**H group next to the carbon with the chemical shift of 153.77 ppm.

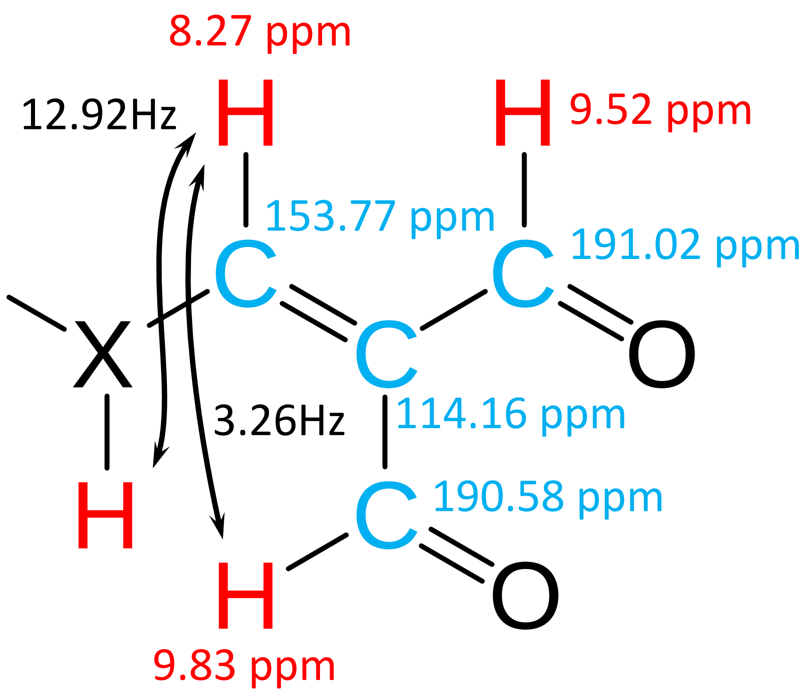


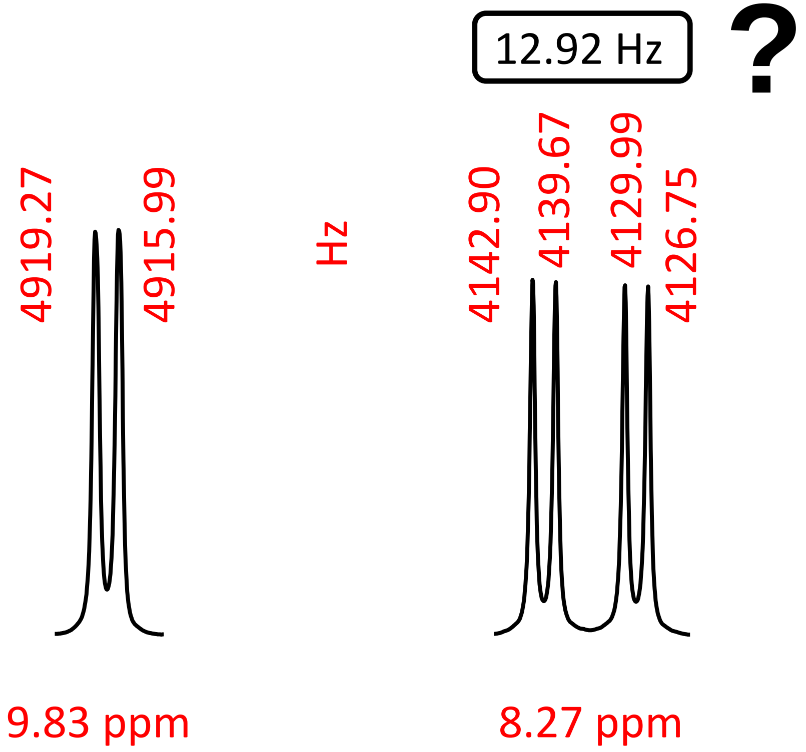


Building blocks

Extend the partial structure

Now it is easy to understand the value of 12.92 Hz as a common vicinal coupling constant.



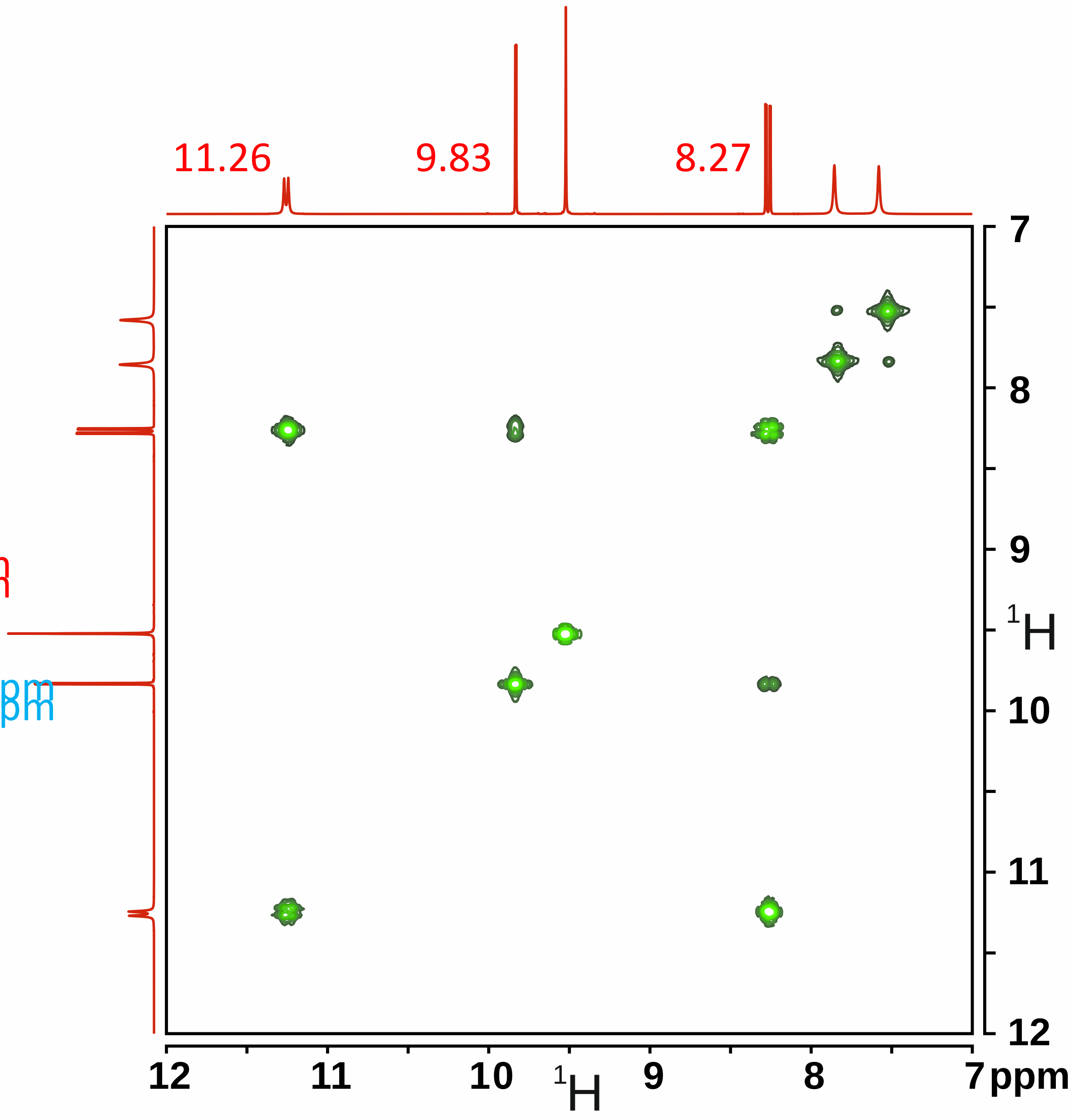


Let us introduce a hypothetical **XH** group next to the carbon with the chemical shift of 153.77 ppm.

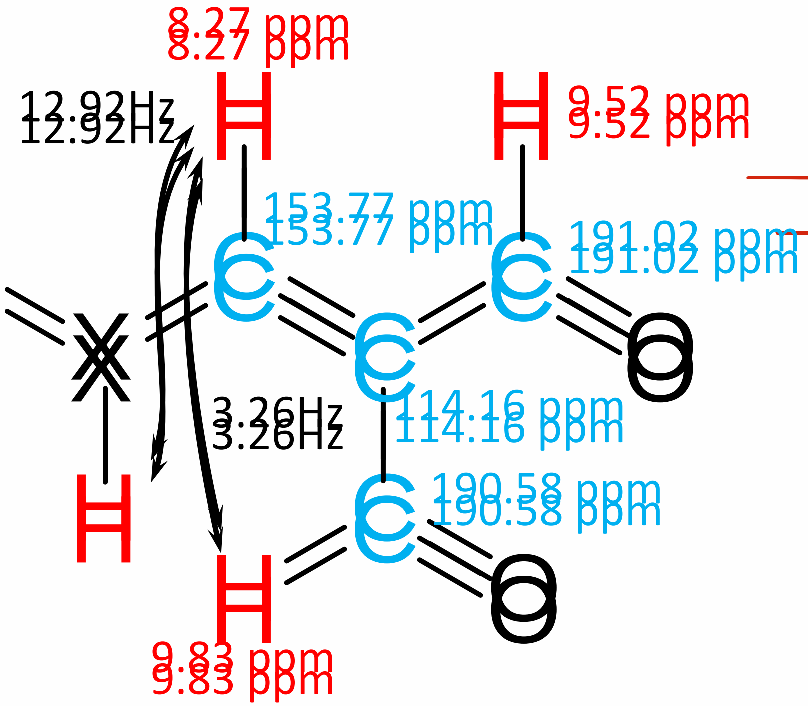
Building blocks

Extend the partial structure

COSY



Both coupling pathways should be visible in the COSY.



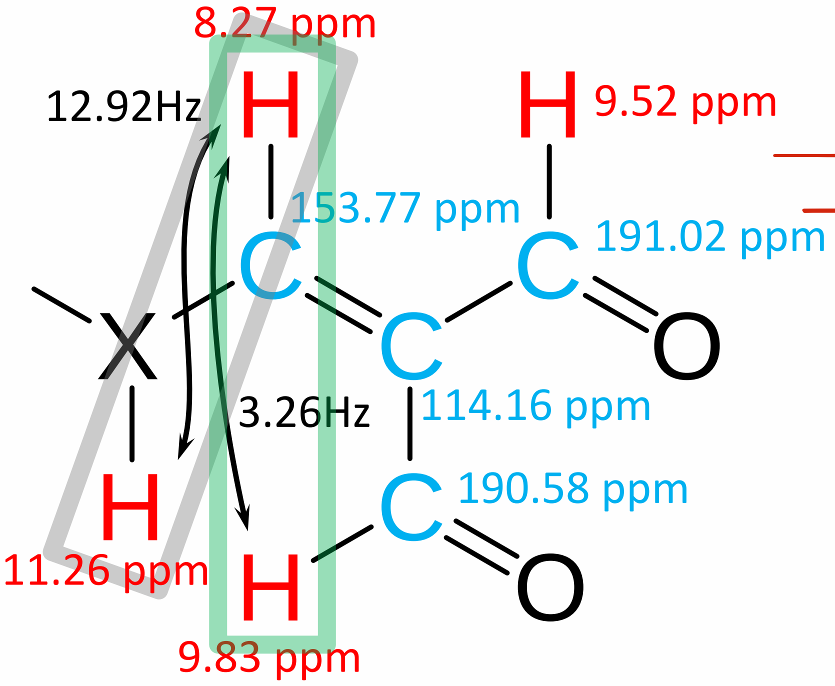
Building blocks

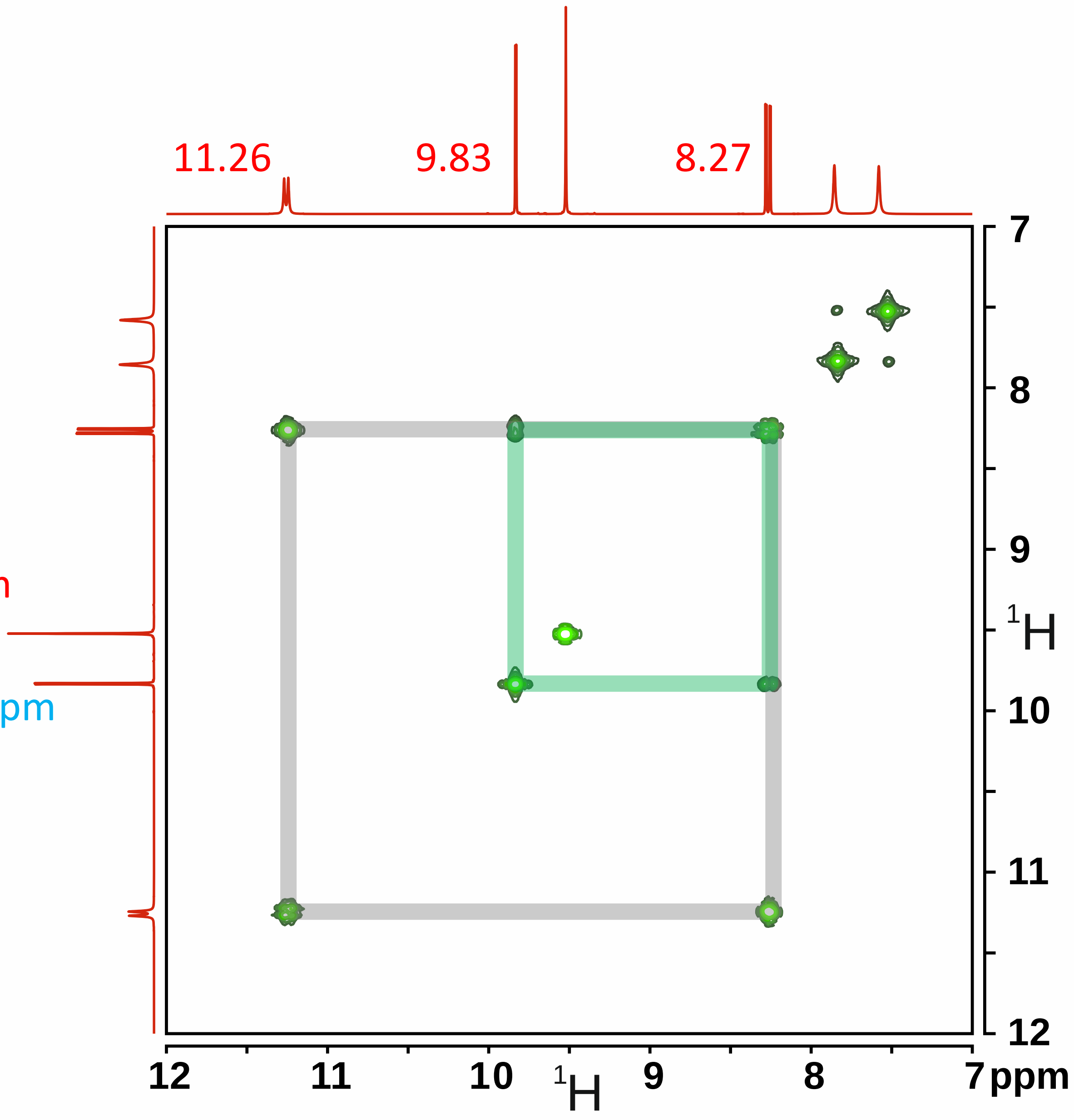
Check the extension

COSY

Let us check.

We found the chemical shift of our **XH** proton.





Building blocks

Replace X

$^1H/^{15}N$-HMBC

But what does **X** mean?

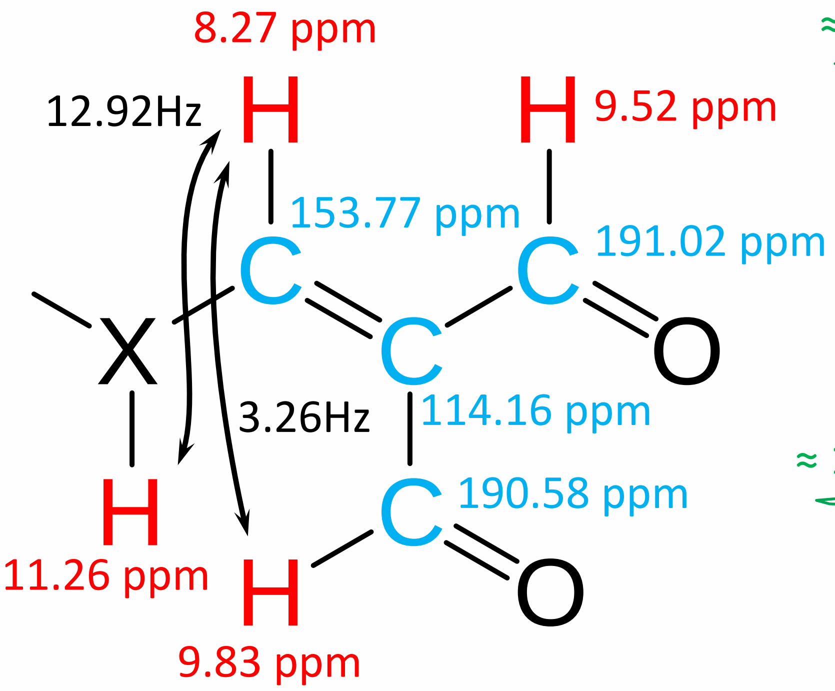
O is excluded, because this would mean the end of the molecule, but we still have some atoms to assign.

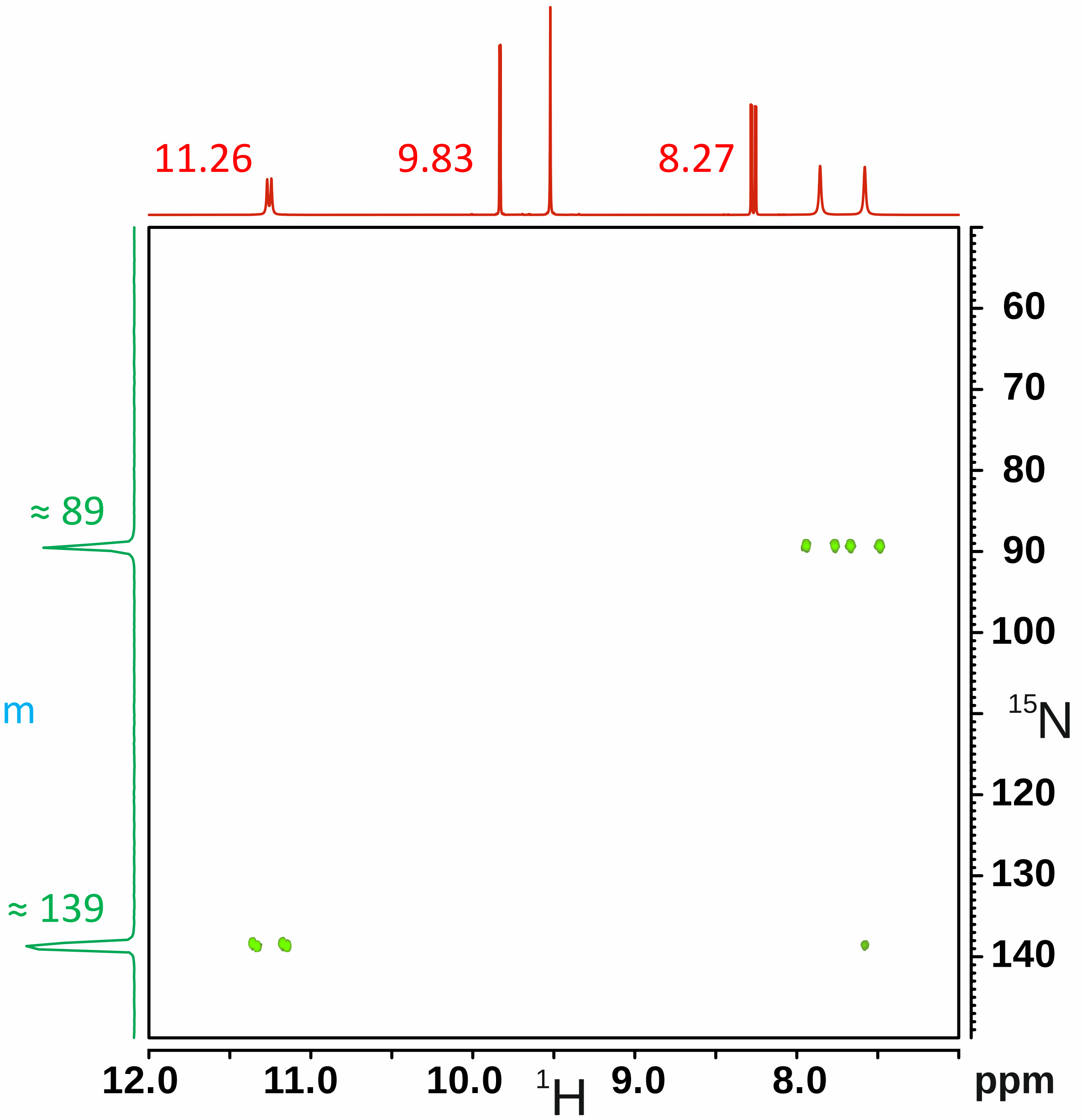
C is excluded as well, because there was no cross peak in the HSQC pointing to the proton signal at 11.26 ppm.

The remaining possibility is

X = **N**

Let us check in the $^1H/^{15}N$-HMBC.





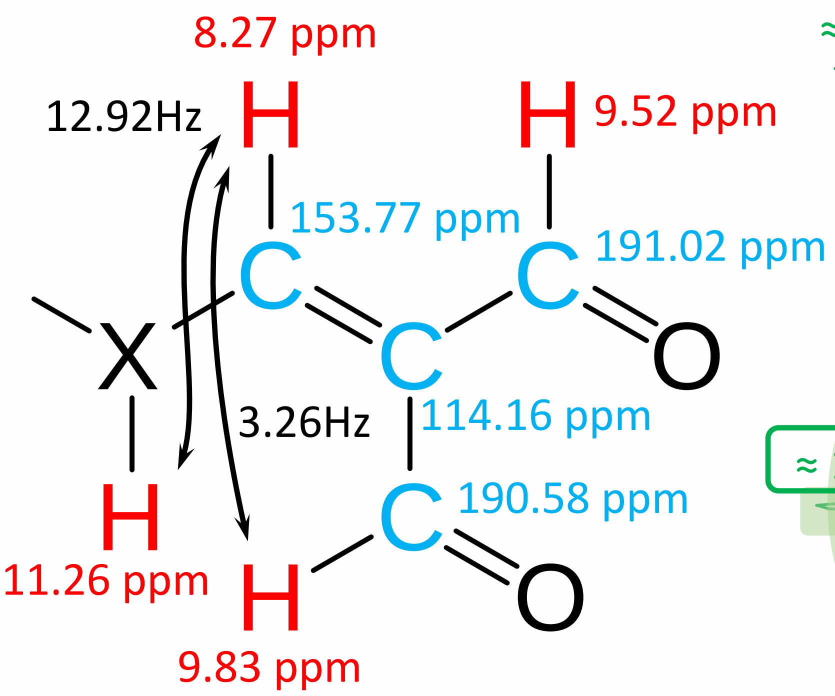
Building blocks

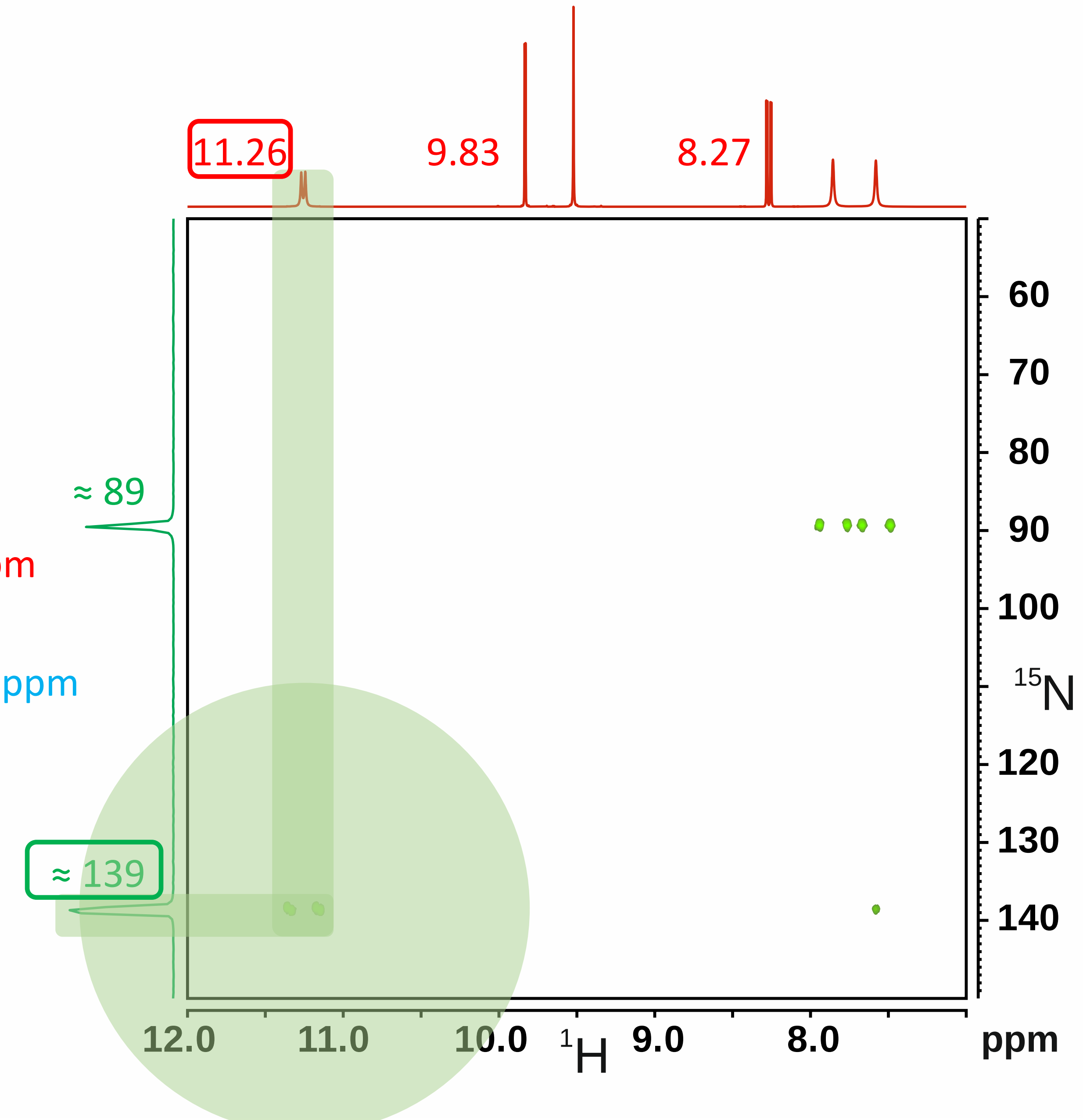
Replace X

$^1H/^{15}N$-HMBC

Indeed there is a cross peak between the proton with the chemical shift of 11.26 ppm and a nitrogen atom with a chemical shift of about 139 ppm.

But this is a HMBC and no HSQC?





Building blocks

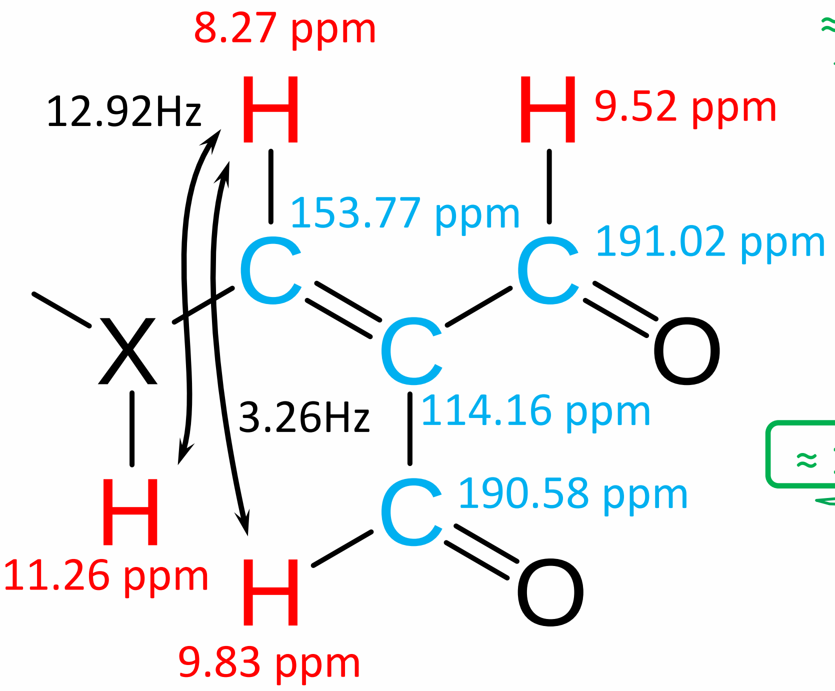
Replace X

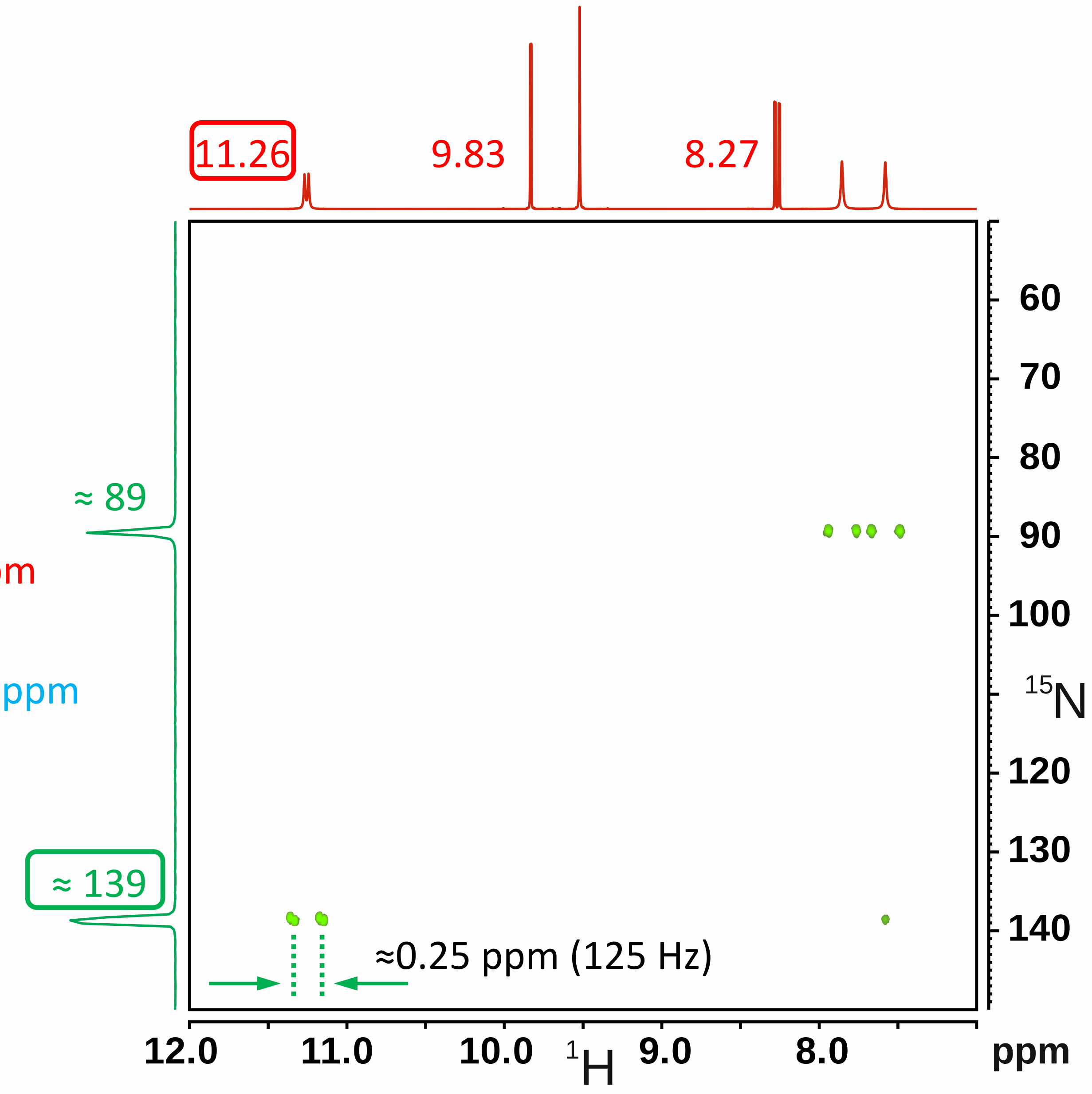
$^1H/^{15}N$-HMBC

Don‘t worry. Our cross peak consists of two parts separated by about 0.25 ppm (125 Hz) in the proton dimension. This rough measurement is close to the expected one bond coupling constant of about 90…95 Hz between a nitrogen atom and a proton.

Usually such HSQC artifacts are unwanted inside a HMBC but in our case we found a really helpfulpiece of information.

Let us replace **X** by **N**.



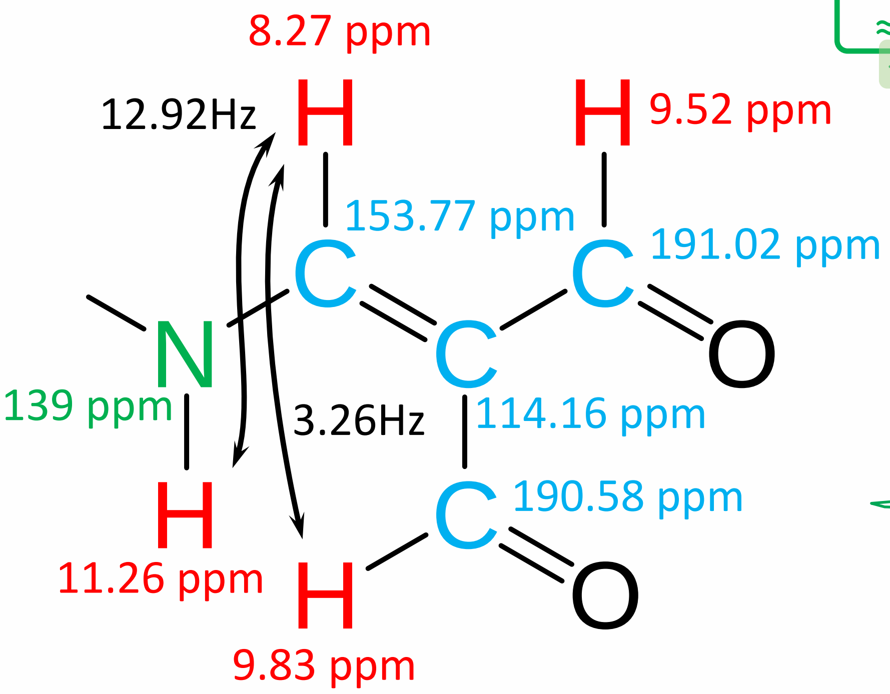


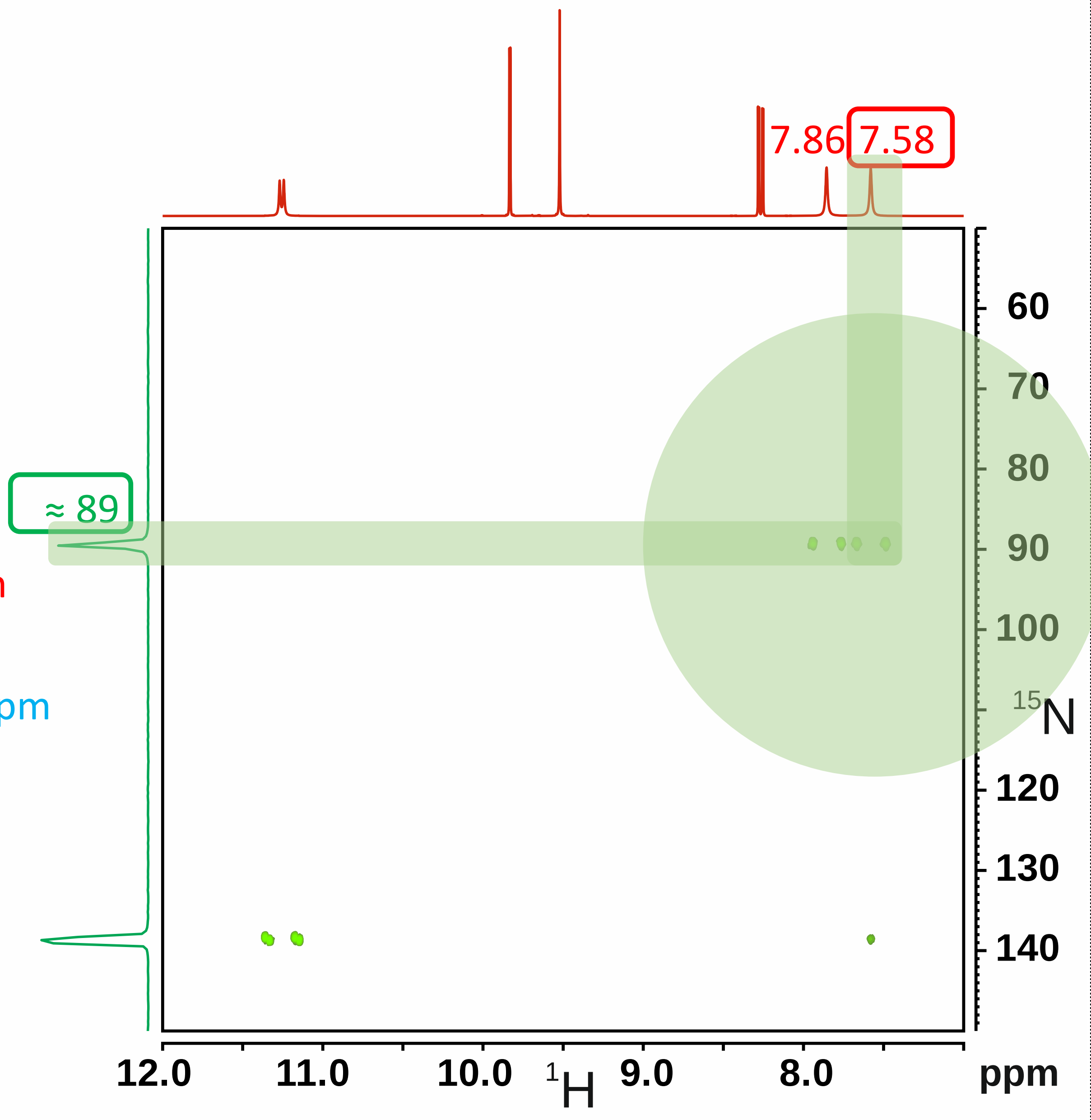
Building blocks

Replace X

$^{1}H/^{15}N$-HMBC

Inspecting our HMBC again we see two more of these „pseudo HSQC peaks“. One of them (between the nitrogen atom with the chemical shift of about 89 ppm and the proton with the chemical shift of 7.58 ppm) is shown here.

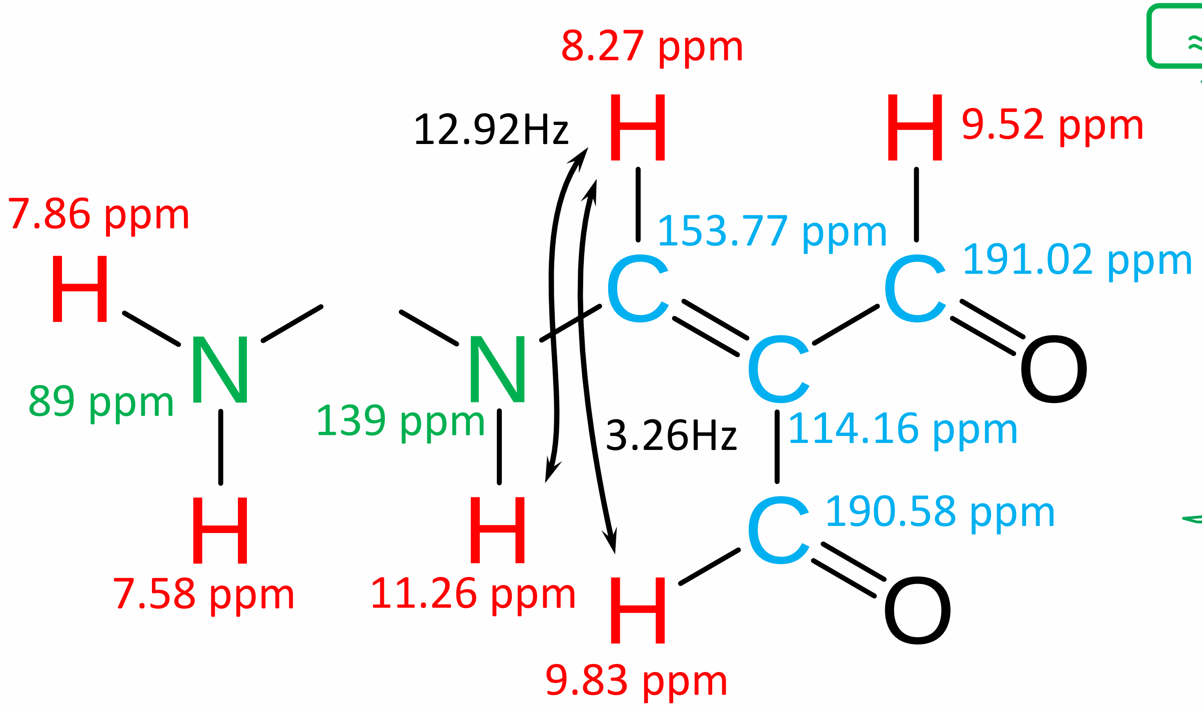




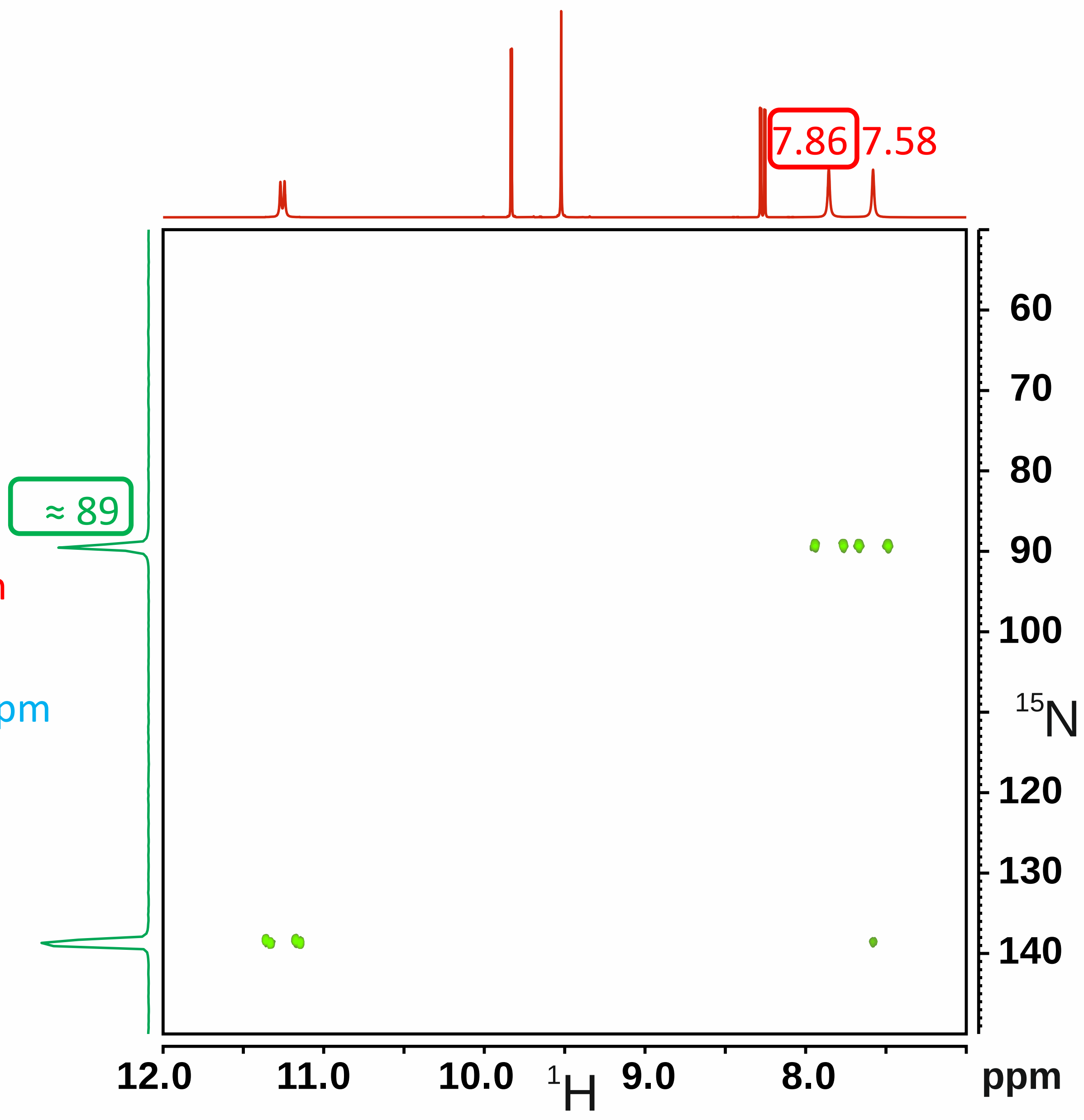
Building blocks

Another amino group

There is a second proton with the chemical shift of 7.86 ppm bound to the nitrogen atom with the chemical shift of about 89 ppm and we end in an NH_2 group.



$^1H/^{15}N$-HMBC



Final structure

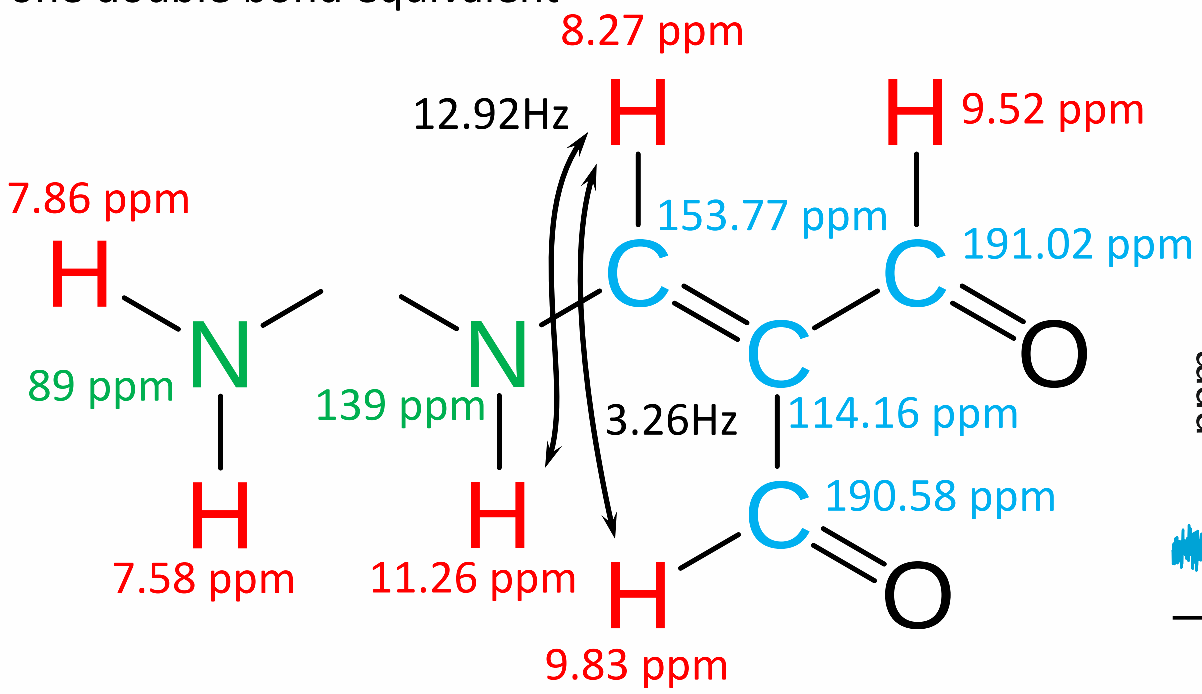
Add the missing parts

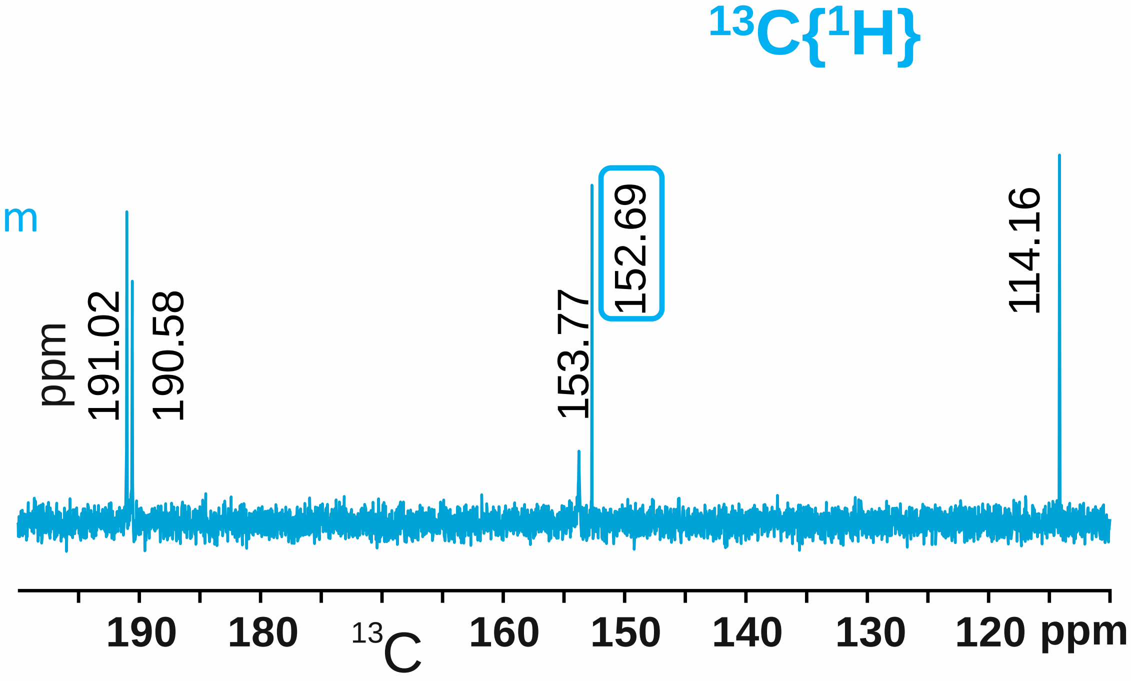
Now we are able to calculate the number of double bond equivalents (remember the first slide).

We still need

- one carbon atom (152.69 ppm)
- one oxygen atom
- one double bond equivalent

There is only one possibility ...





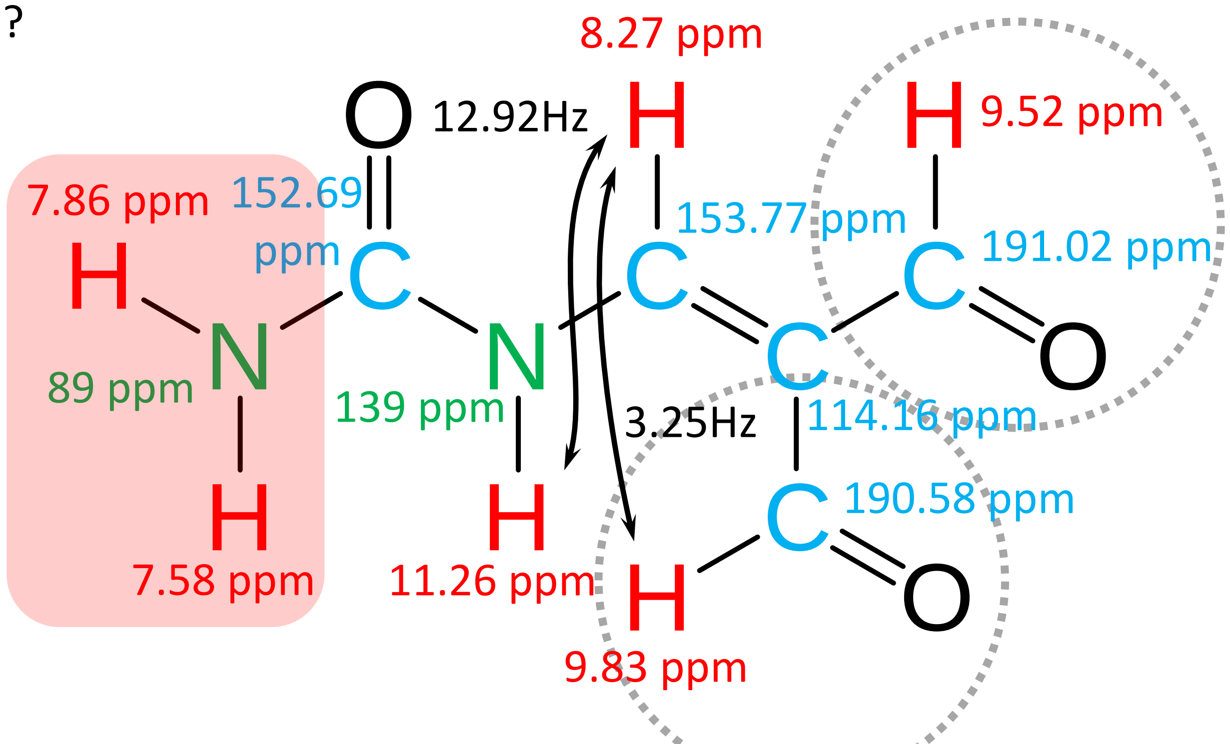
Final structure

But new questions

Even if we have our final structure, there are some open questions.

Why are these protons chemically not equivalent? There should be free rotation around the C-N single bond?

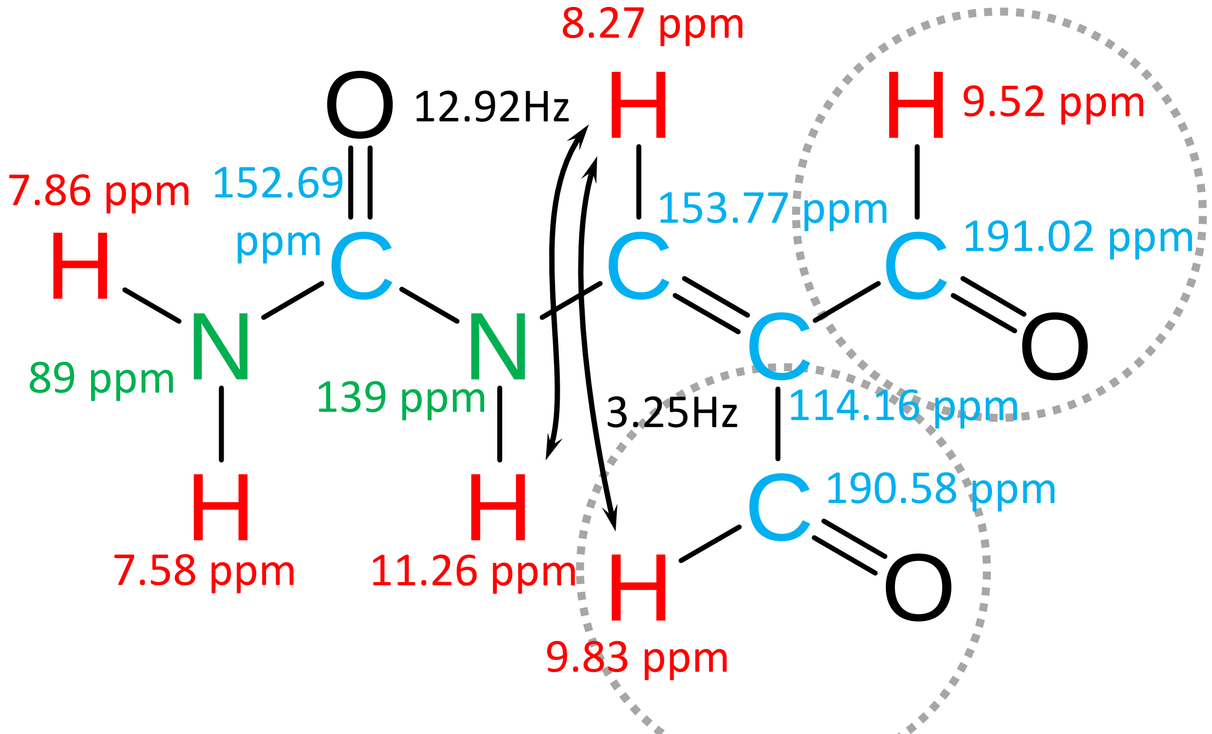
If we change the assignment of both aldehyde groups the structure remains the same. But which assigment is the correct one?



Final structure

Configuration

Let us start with the configuration of both aldehyde groups. For clarity let us remove some pieces of information not necessary to answer this specific question.

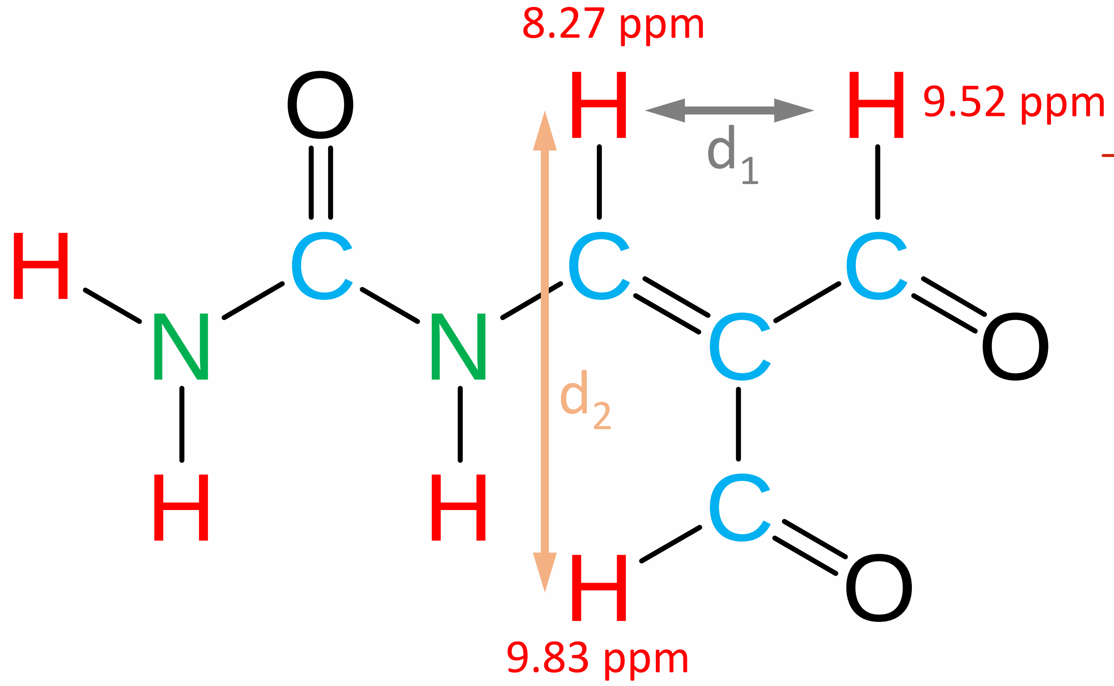


Final structure

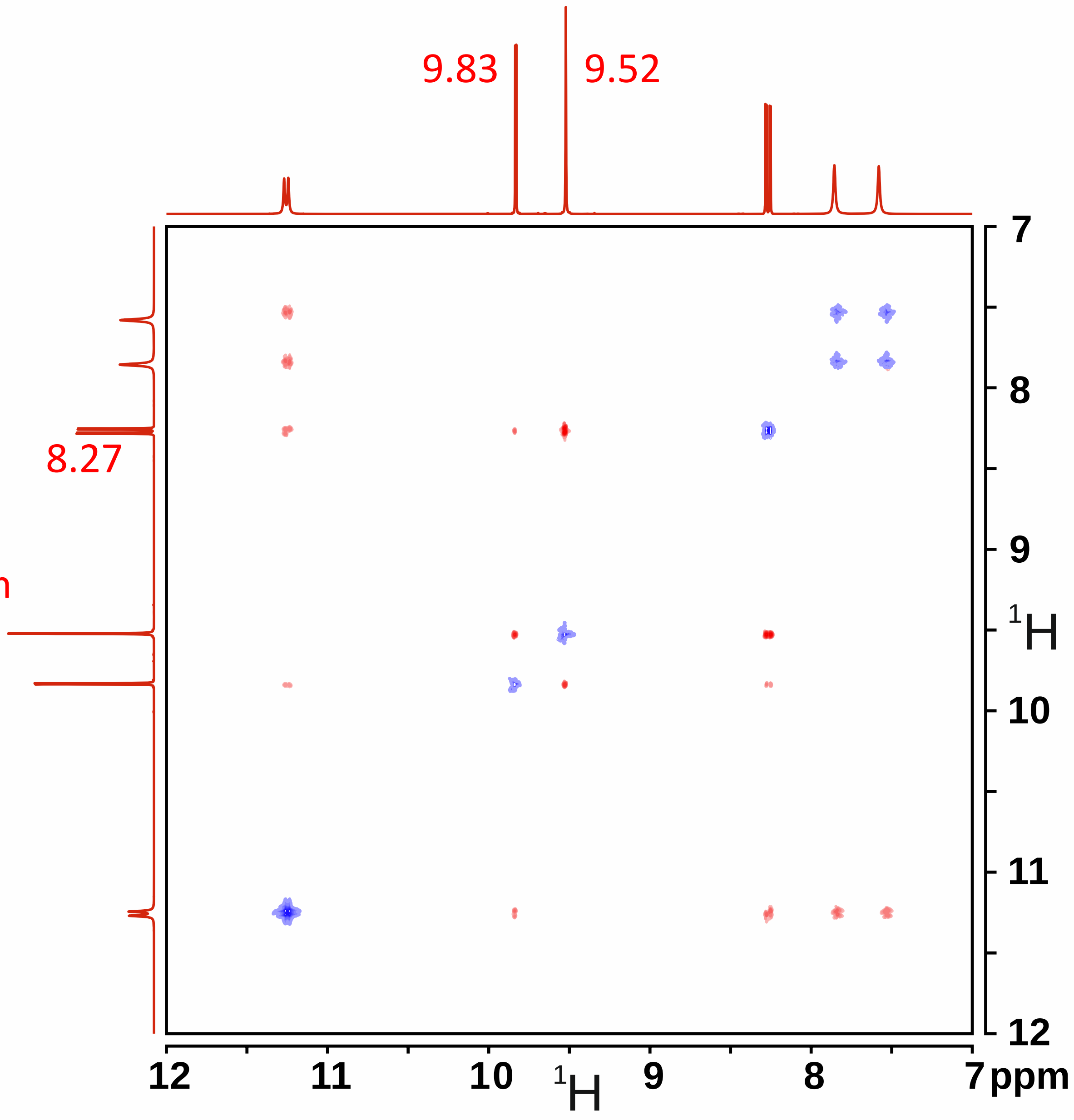
Configuration

If there is a possibility to measure distances, we should be able to assign both aldehyde groups unambiguously.

d_2 is clearly larger than d_1 and using an experiment to measure distances (NOESY) the cross peak at 8.27/9.52 ppm should be more intense than the cross peak at 8.27/9.83 ppm.
This, of course, is valid for the conformation presented here.



NOESY

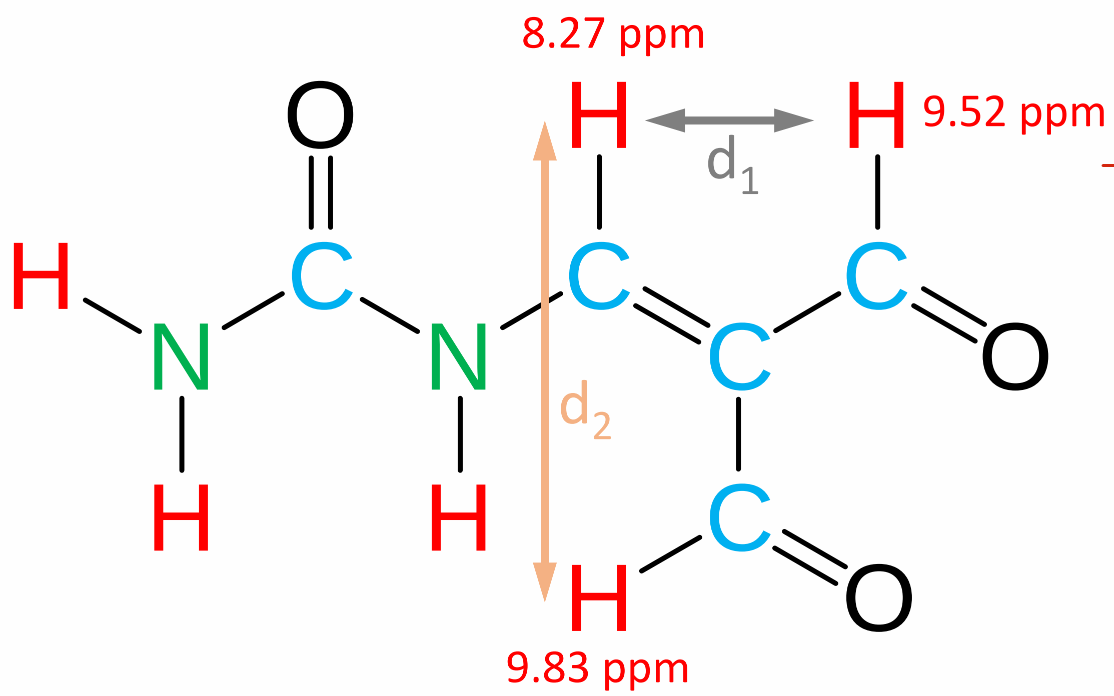


Final structure

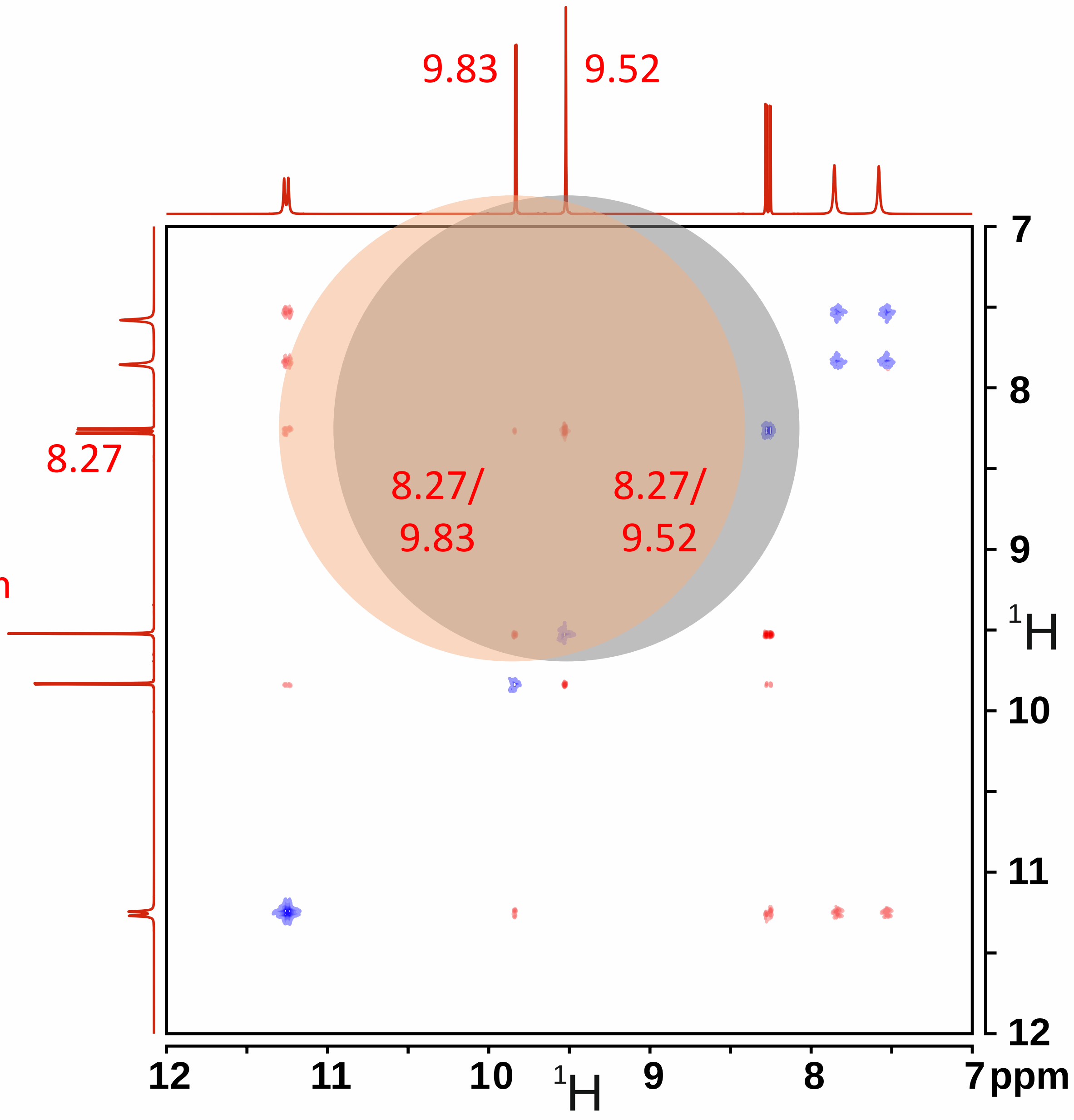
Configuration

Indeed the intensity of the cross peak betweeen the protons with the chemical shifts of 8.27 ppm and 9.52 ppm is significantly stronger than the intensity of the second second one, which is the result of the distance d_2.

Apparently we found the correct configuration by chance.



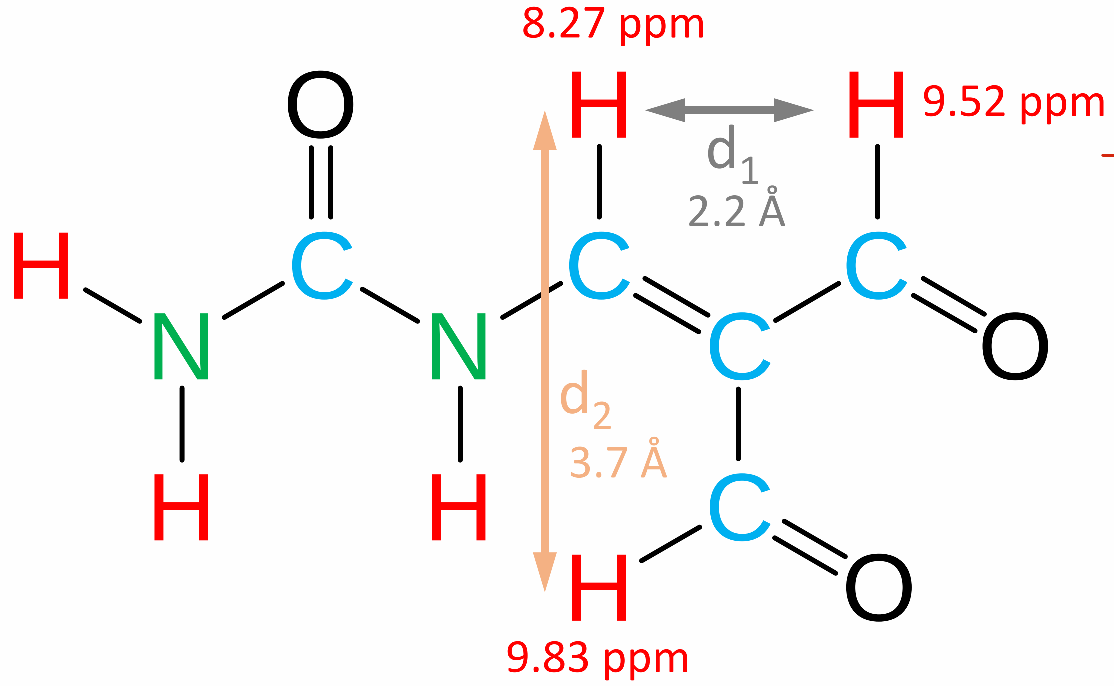
NOESY



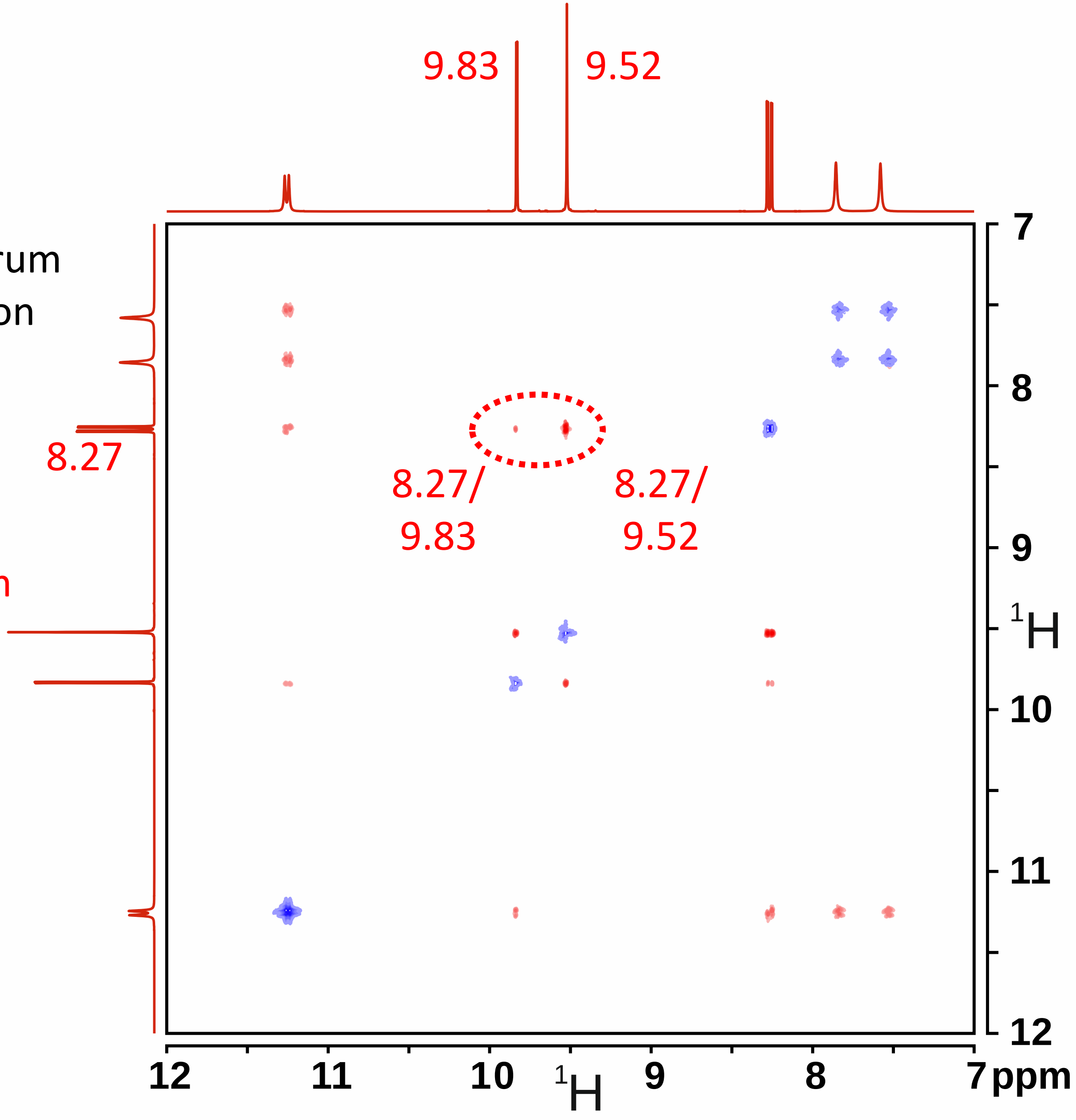
Final structure

Configuration

But we should be a little bit more careful. There is a second conformation for both aldehyde groups and in the second conformation the distances are different. From the NMR spectrum we cannot extract any piece of information about the population of these conformations.
Let us take a 3D modeling software and calculate the differences for this conformation first.



NOESY

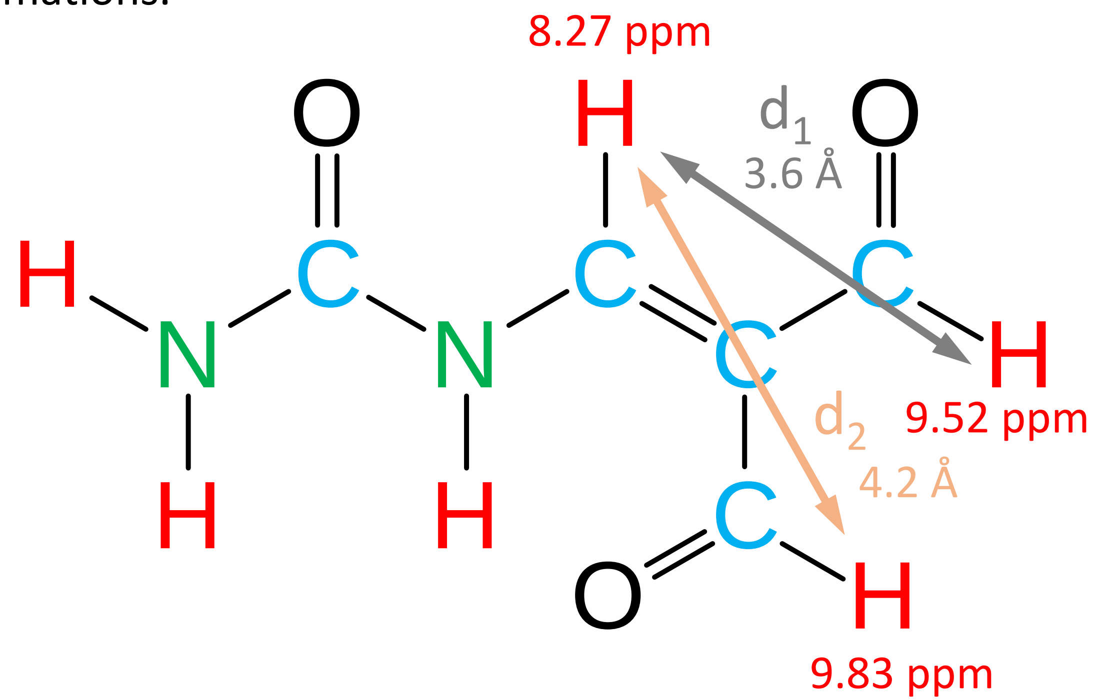


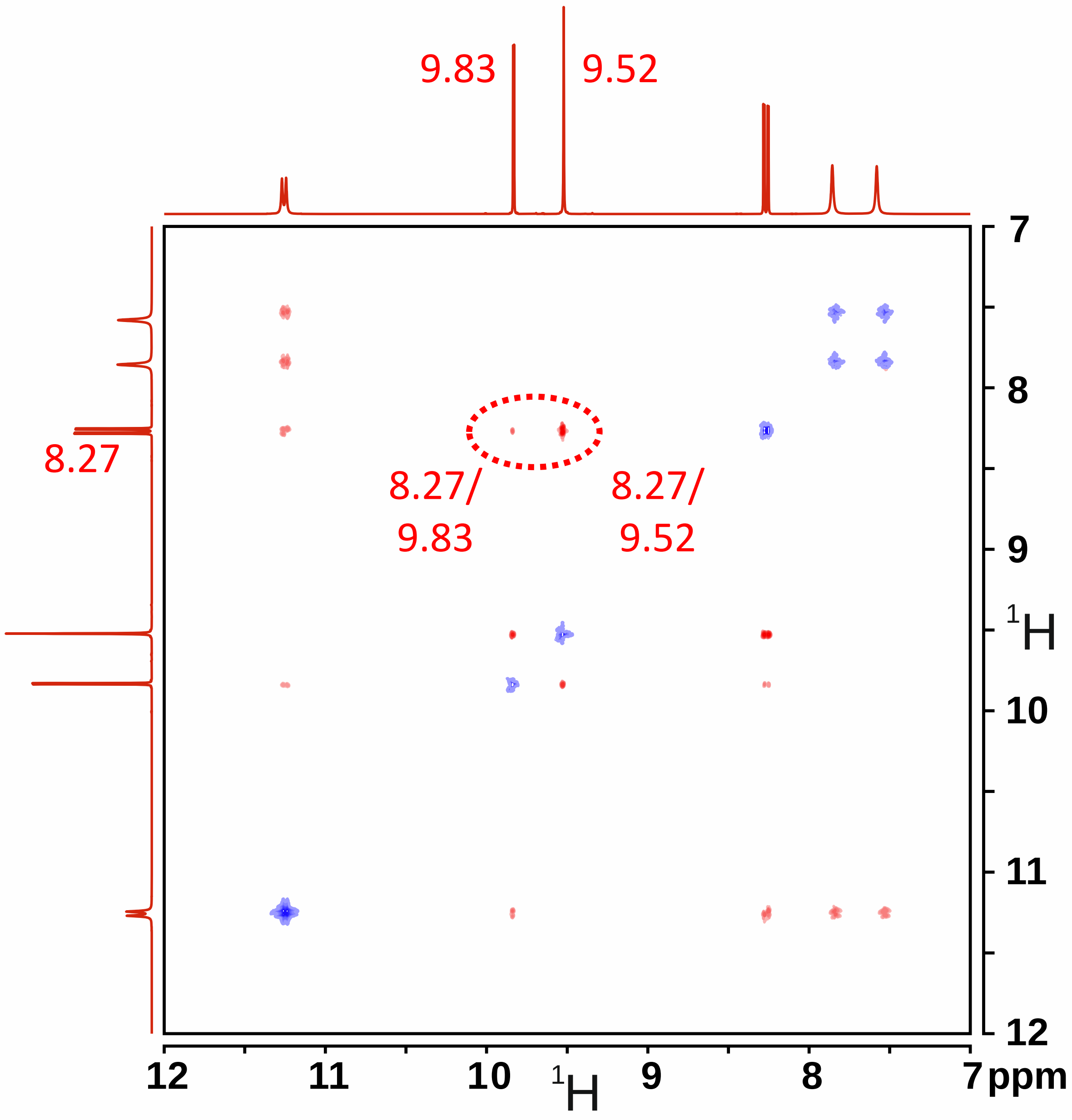
Final structure

Configuration

In the second conformation both distances are larger.

Fortunately d_1 (2.2/3.6Å) is always smaller than d_2 (3.7/4.2Å) independent on the conformation. This confirms our configuration without taking into account different populations of alltogether four possible combinations of conformations.

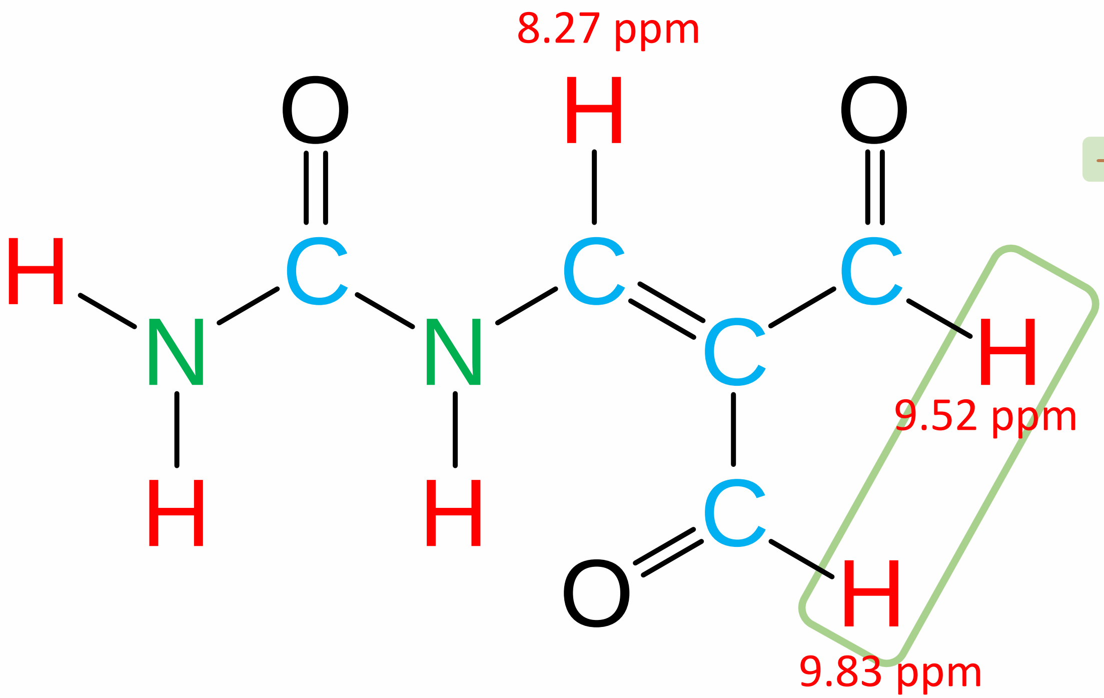


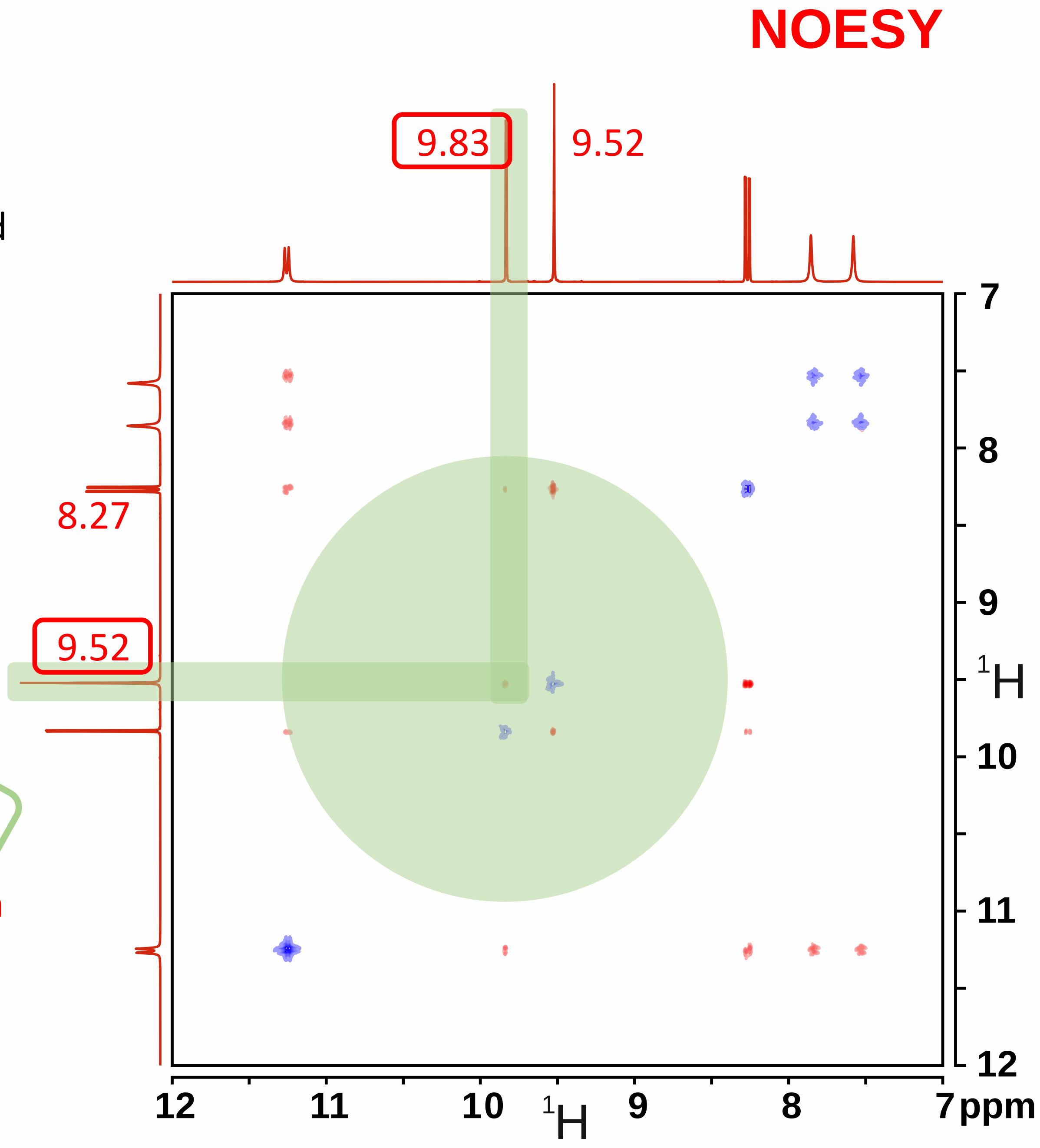


Final structure

Configuration

As a side effect, the conformation shown here provides a good explanation of one of the NOESY cross peak.





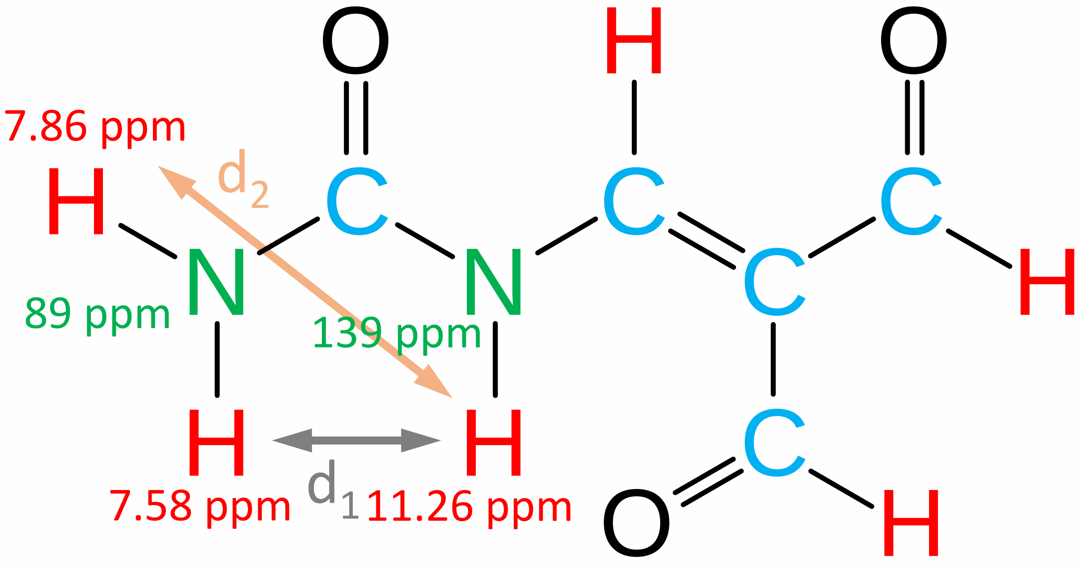
Final structure

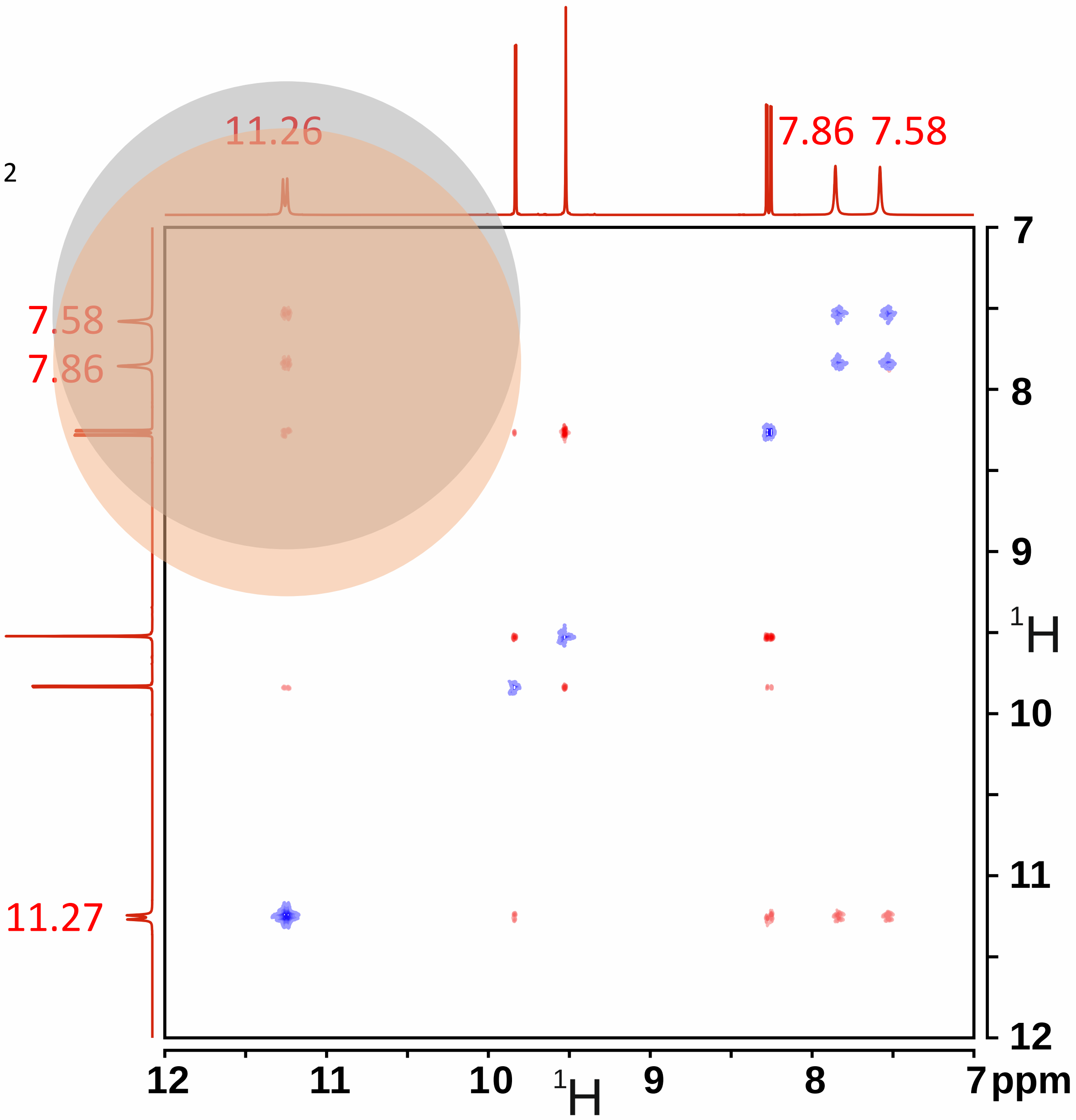
Two different NH_2 protons

Let us now return to the question of the two protons of the NH_2 group with different chemical shifts.

To make it a little bit more strange: the intensity of the cross peaks between the amino proton at 11.27 ppm and each of the different protons of the NH_2 group are apparently identical.

But there should be a difference!





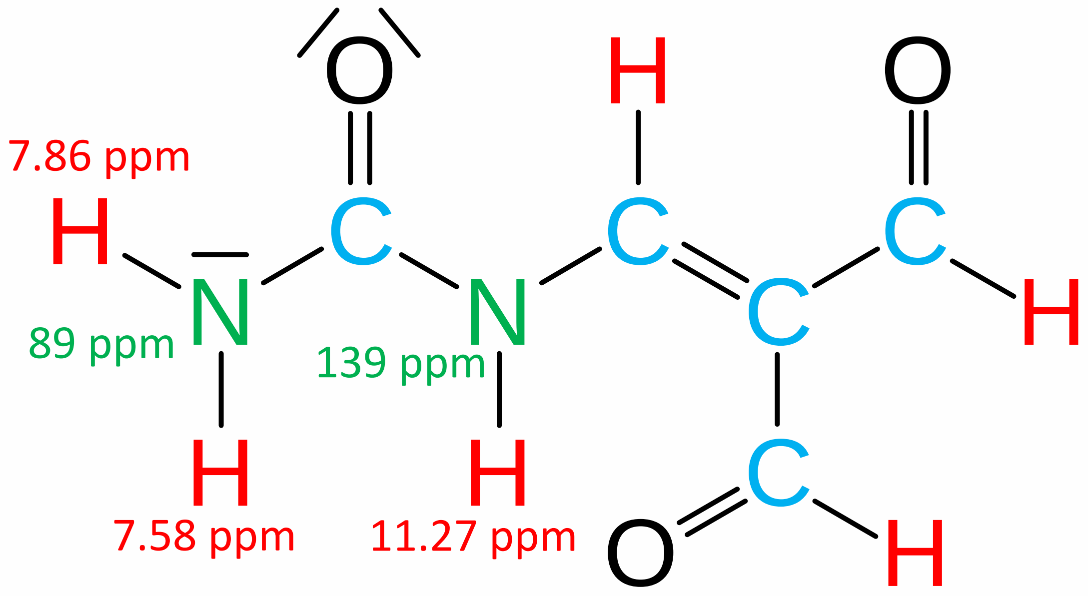
Final structure

Two different NH_2 protons

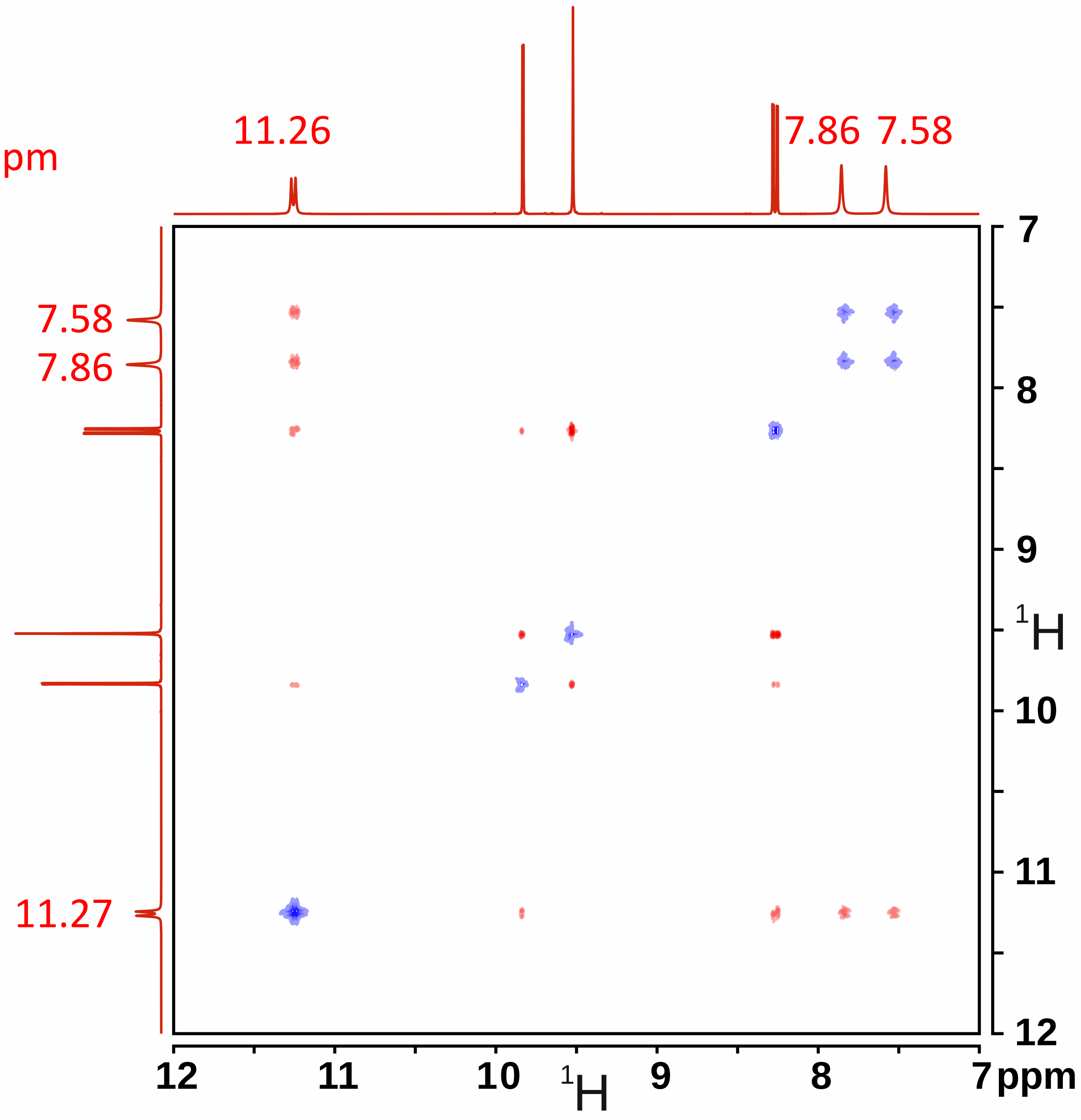
Explaining the different chemical shifts of 7.86 ppm and 7.58 ppm is not too challenging.

First let us note some of the lone pairs explicitly. There are more but for the explanation of the different chemical shifts we need these three pairs.

And now lets move some electron pairs a little bit.



NOESY

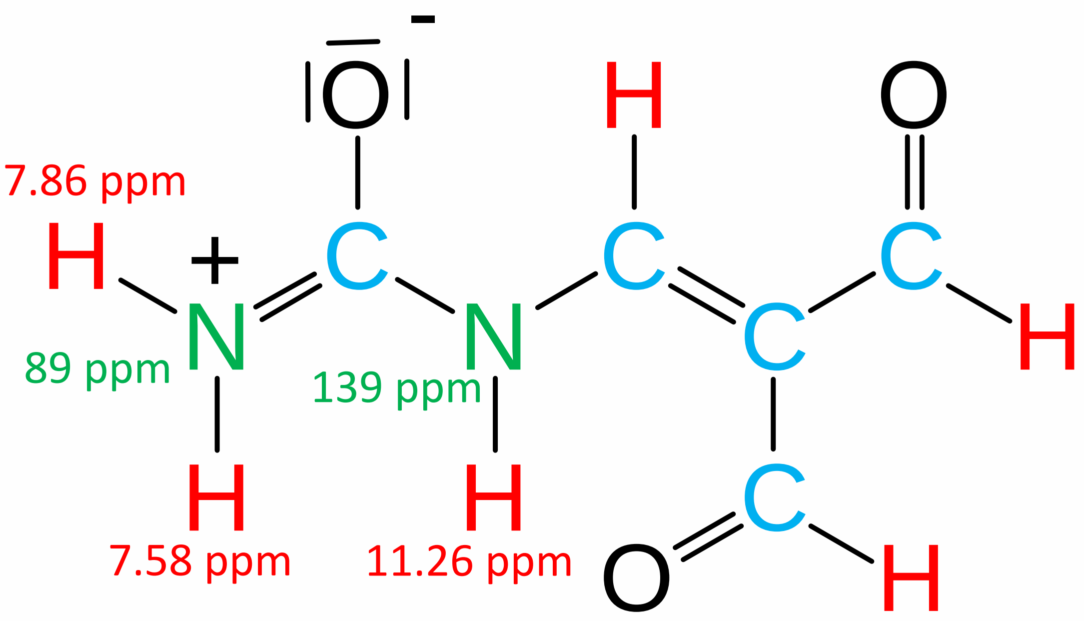


Final structure

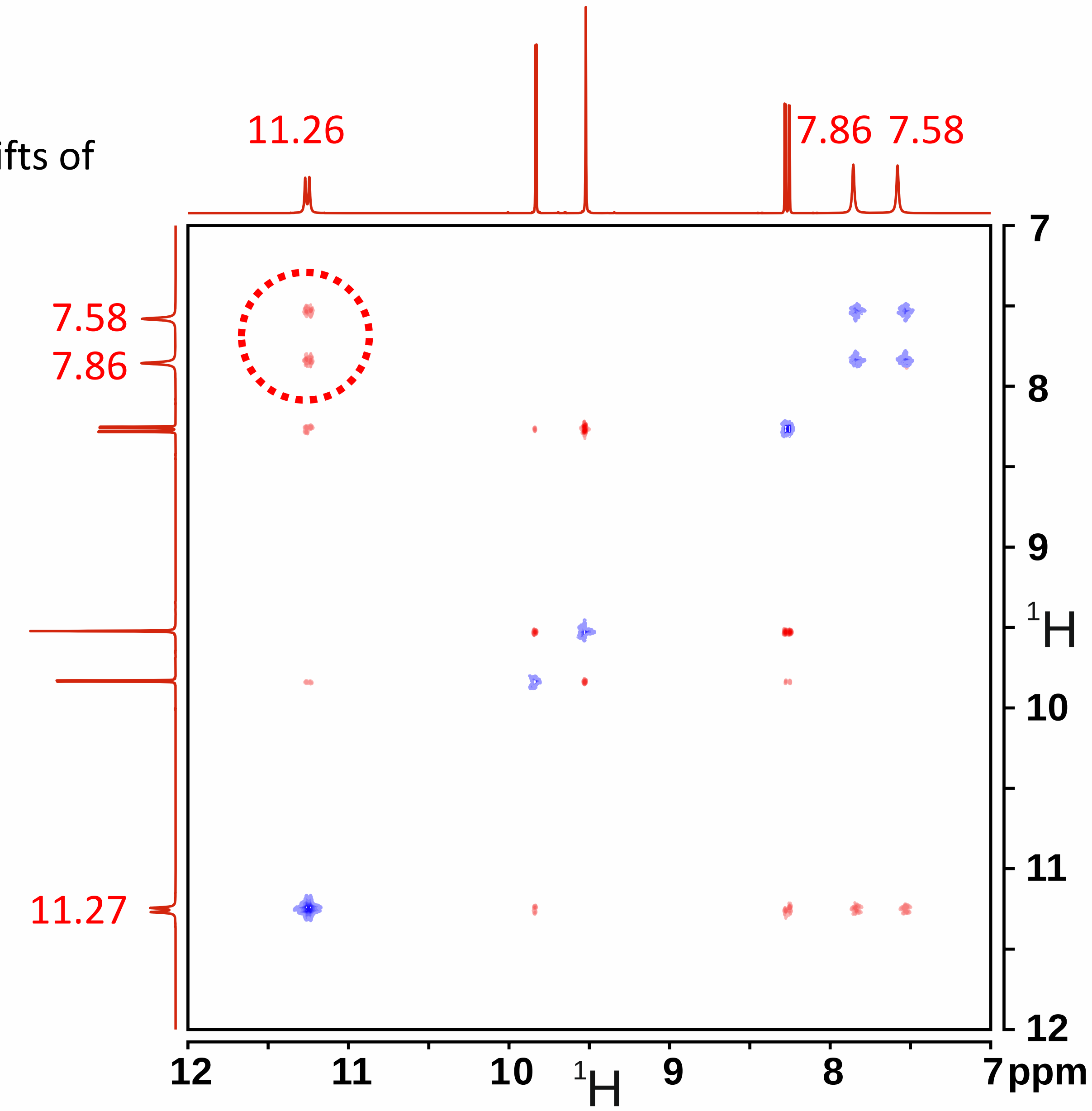
Two different NH_2 protons

Assuming this mesomeric structure, the different chemical shifts of the two NH_2 protons are easy to understand.

But the previously mentioned two NOESY cross peaks should still be of diffferent intensity.



NOESY

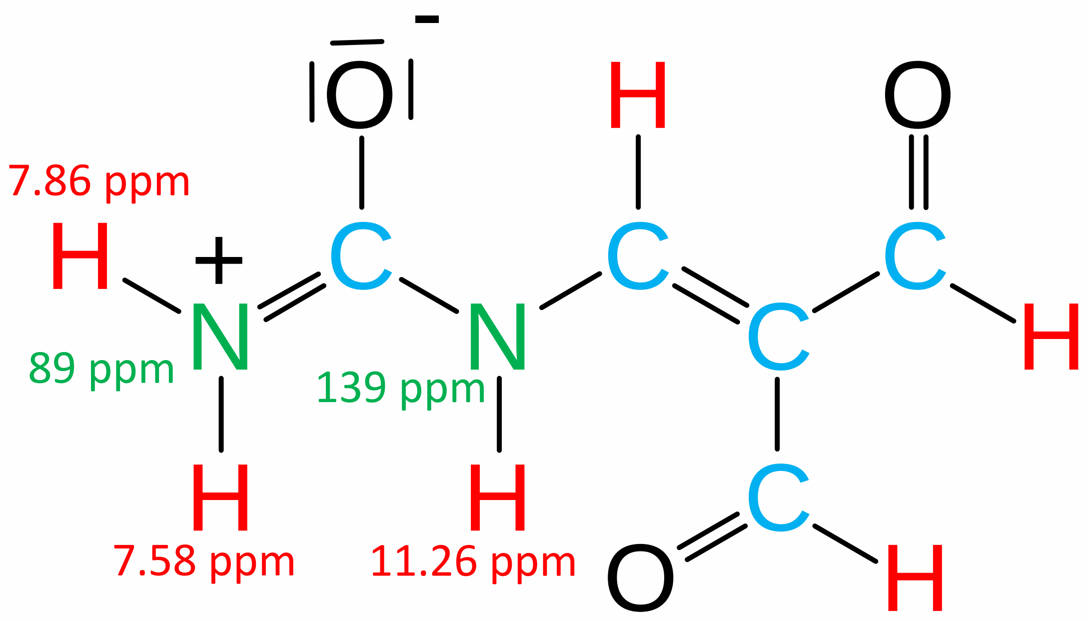


Final structure

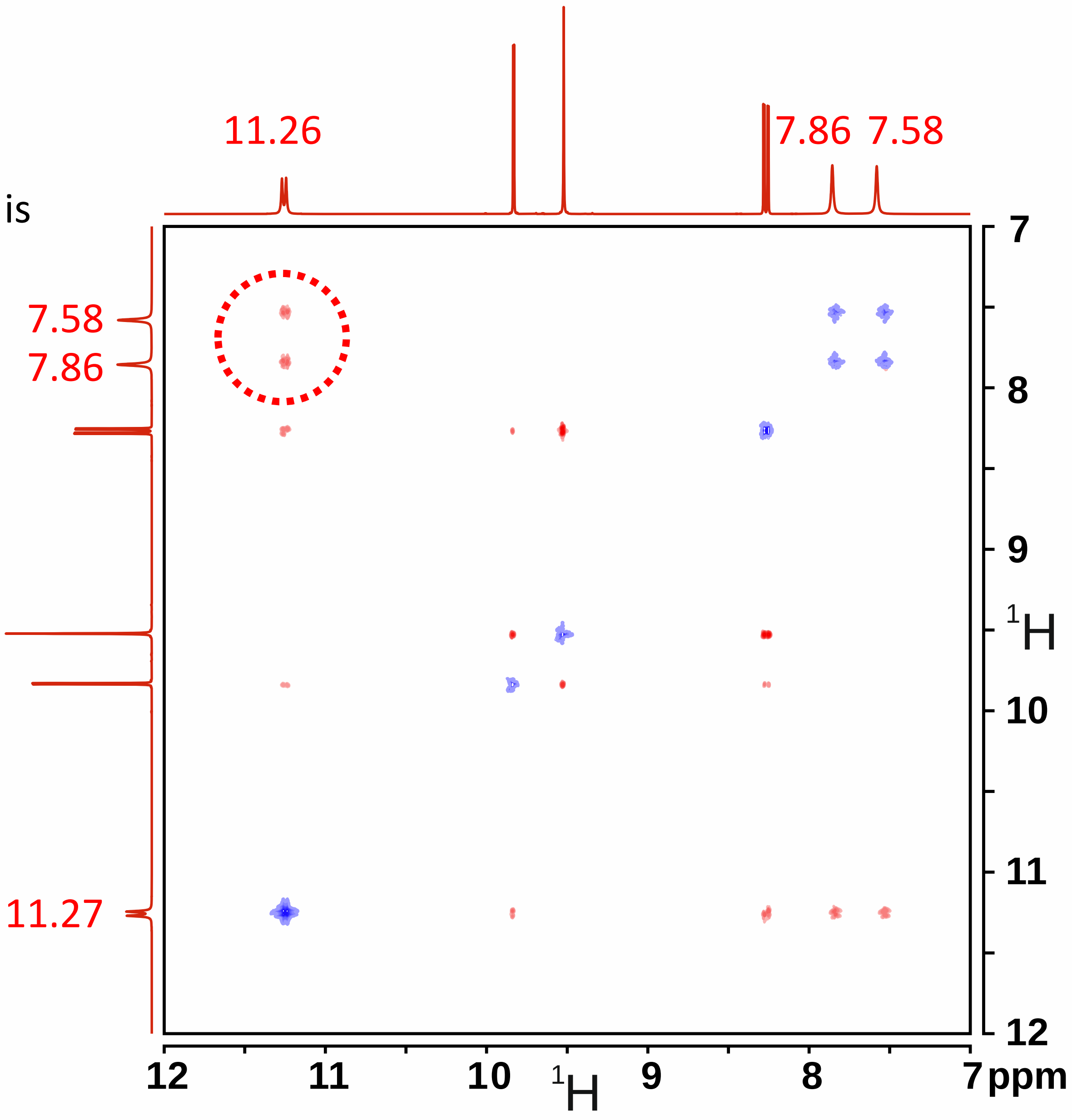
Two different NH_2 protons

Let us assume, the rotation around the C=N bond (this is only a partial double bond depending on the mesomeric equilibrium) is slow enough to show different chemical shifts for both NH_2 protons.

On the other hand the rotation has to be fast enough to average the NOESY effect (mixing time here is 1000ms).



NOESY

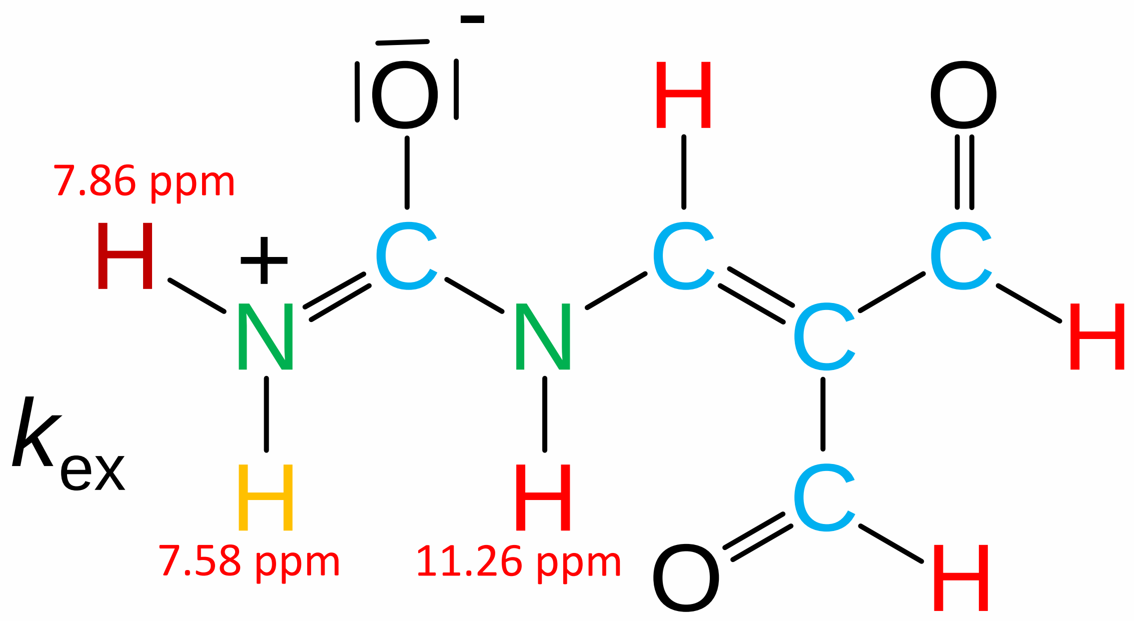


Final structure

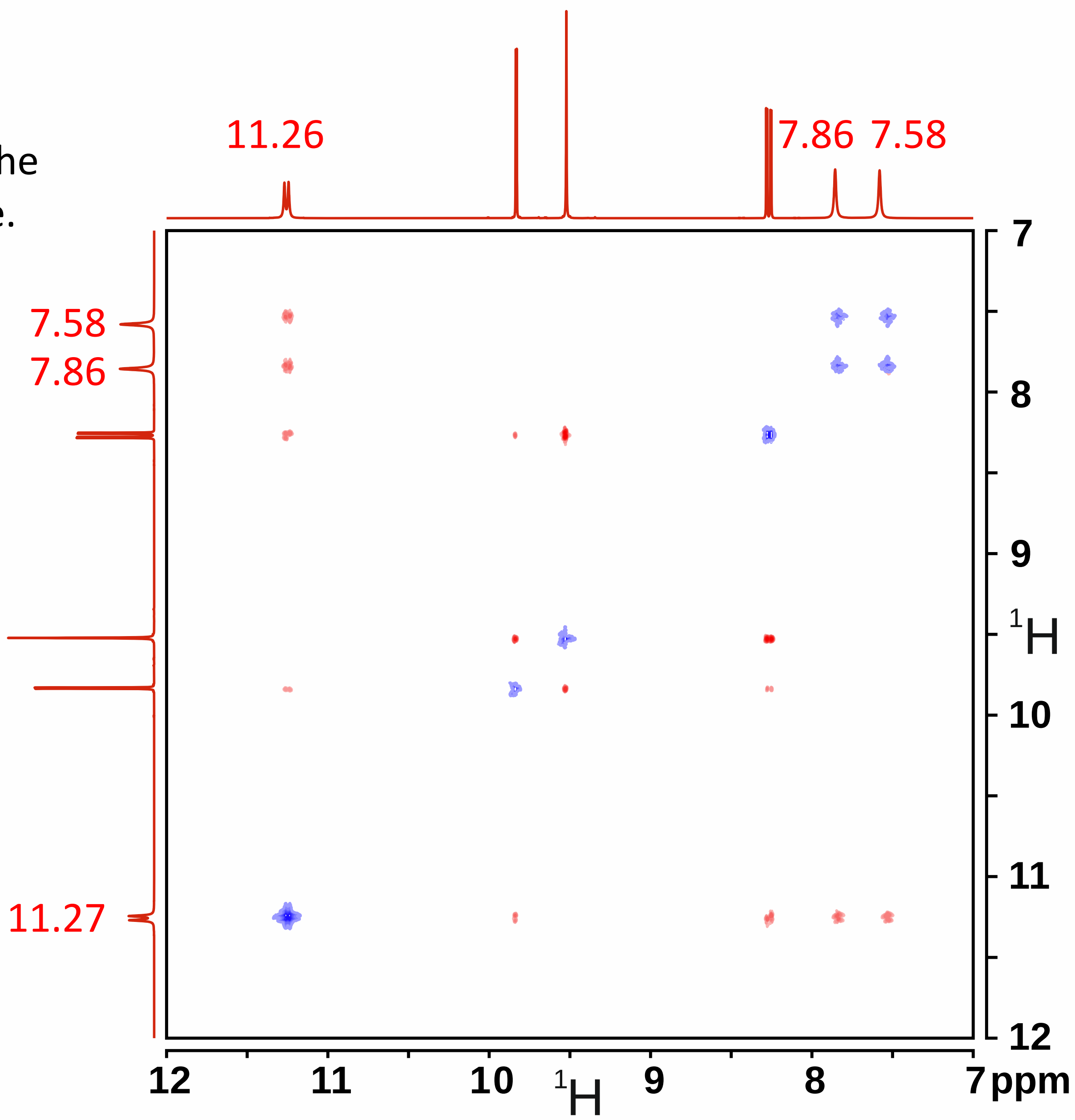
Two different NH_2 protons

Let us remove unnecessary pieces of information and change the colours of both NH_2 protons a bit to make them distinguishable.

What happens with the proton signals at 7.86 ppm and 7.58 ppm, if we change the position of the recoloured protons with a first order rate constant k_{ex}?



NOESY



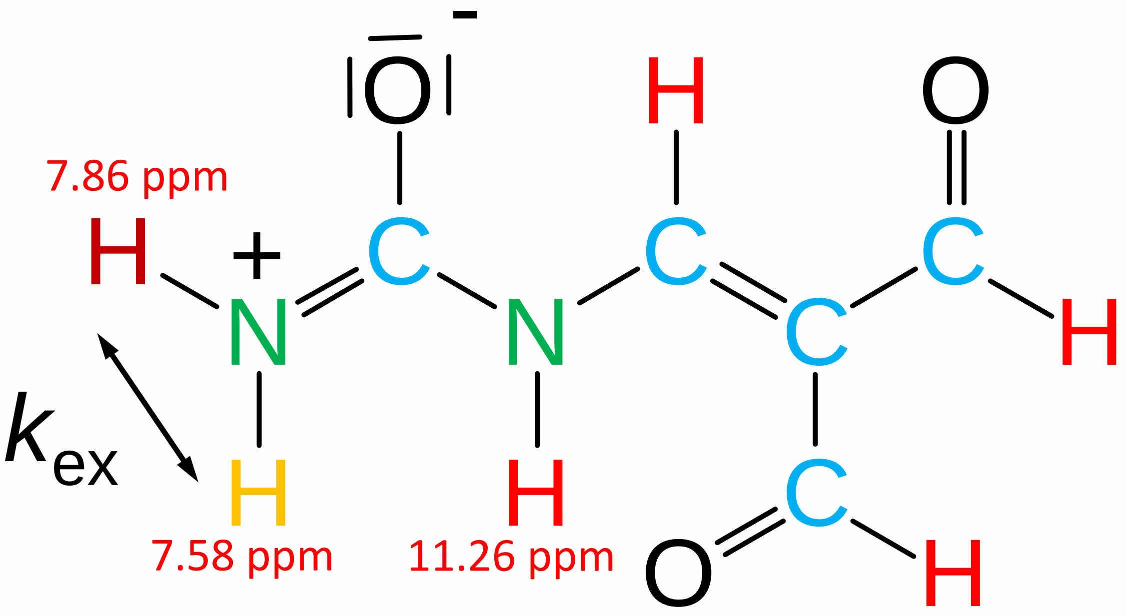
Final structure

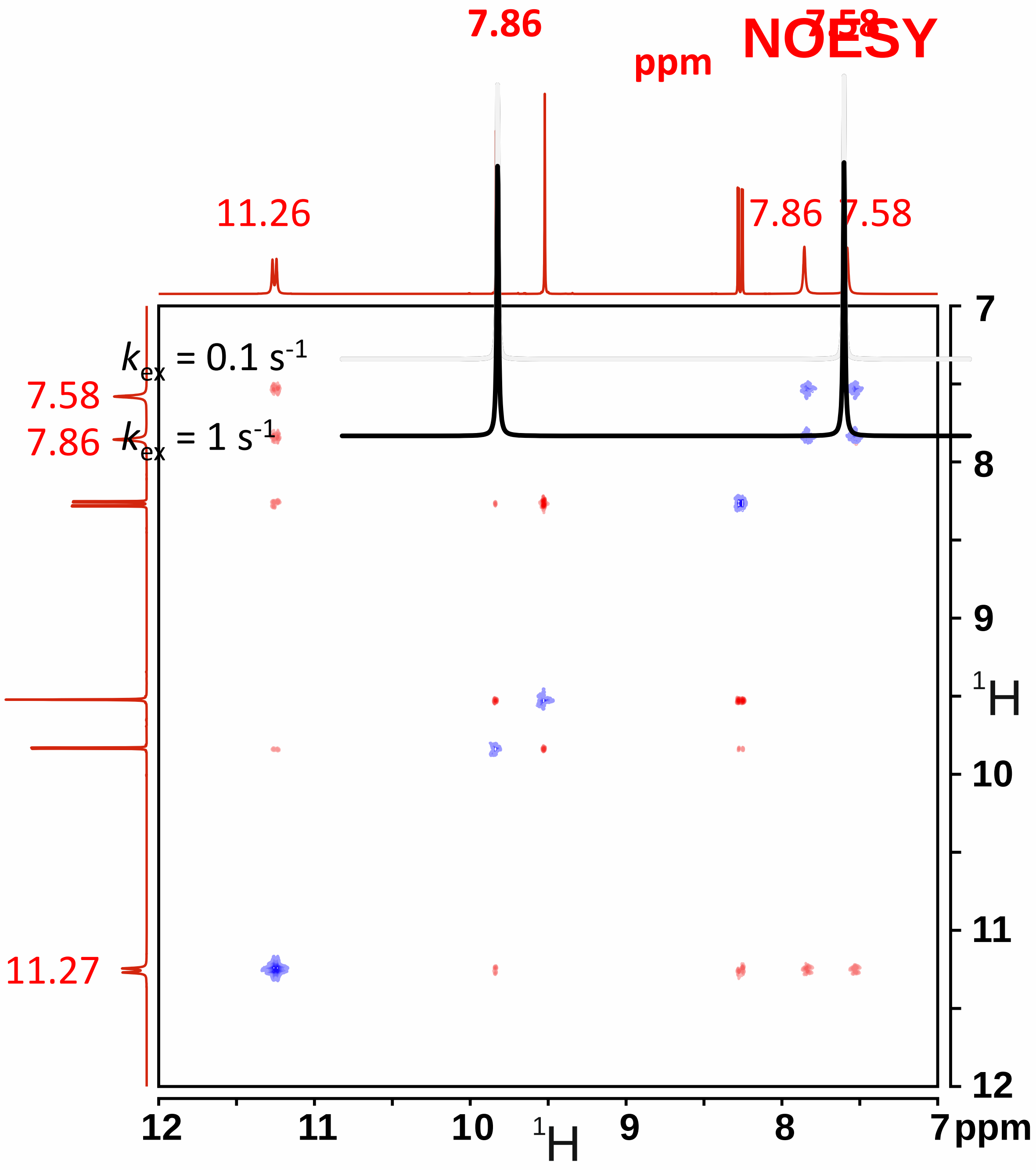
Two different NH_2 protons

Let us start with a very slow value of k_{ex}.

Increasing k_{ex} by a factor of 10 results in some line broadening but nearly no change in chemical shift.

Let us further increase k_{ex} step by step.



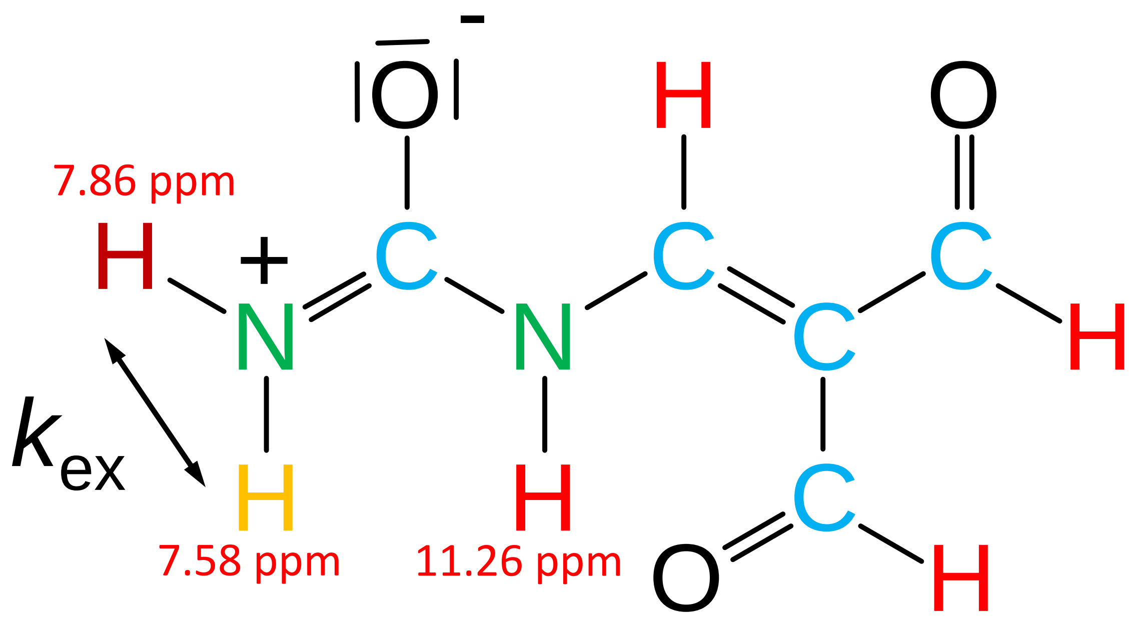


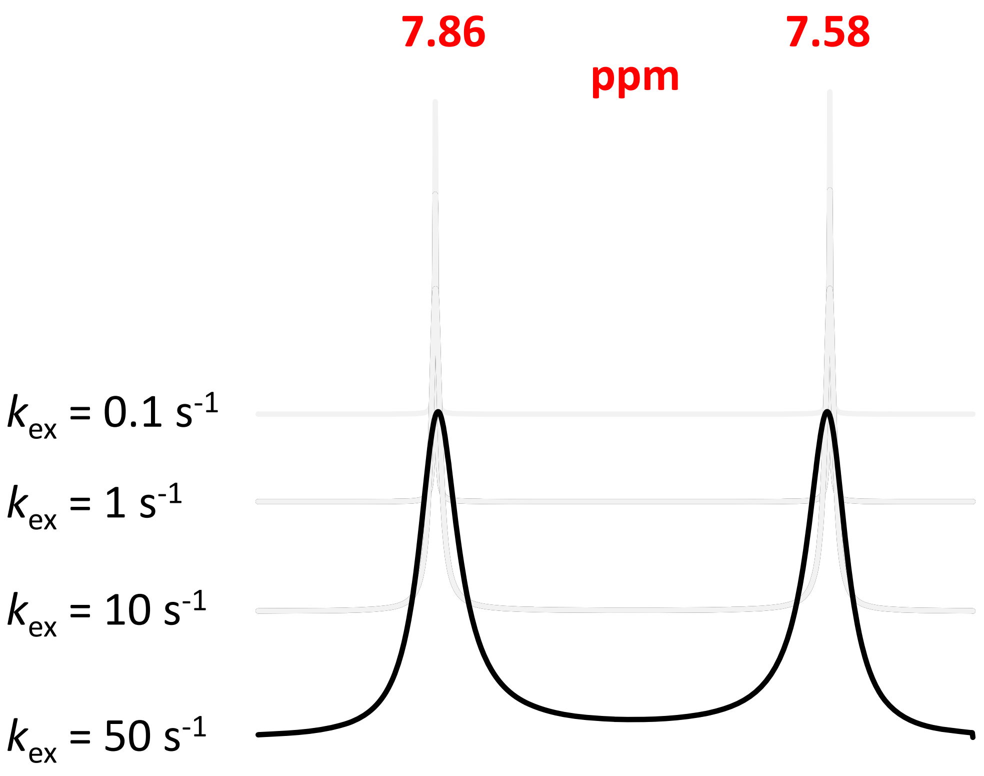
Final structure

Two different NH_2 protons

Changing the proton positions statistically every 100 milliseconds results in broader but still well separated lines.

The very first signs of chemical shift averaging become visible.



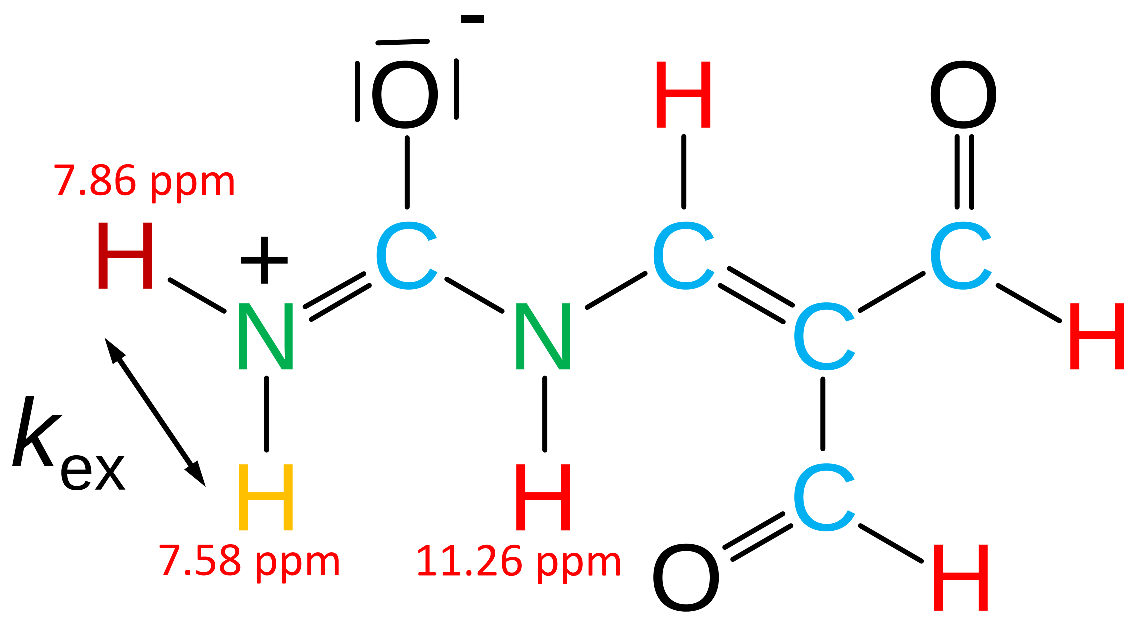


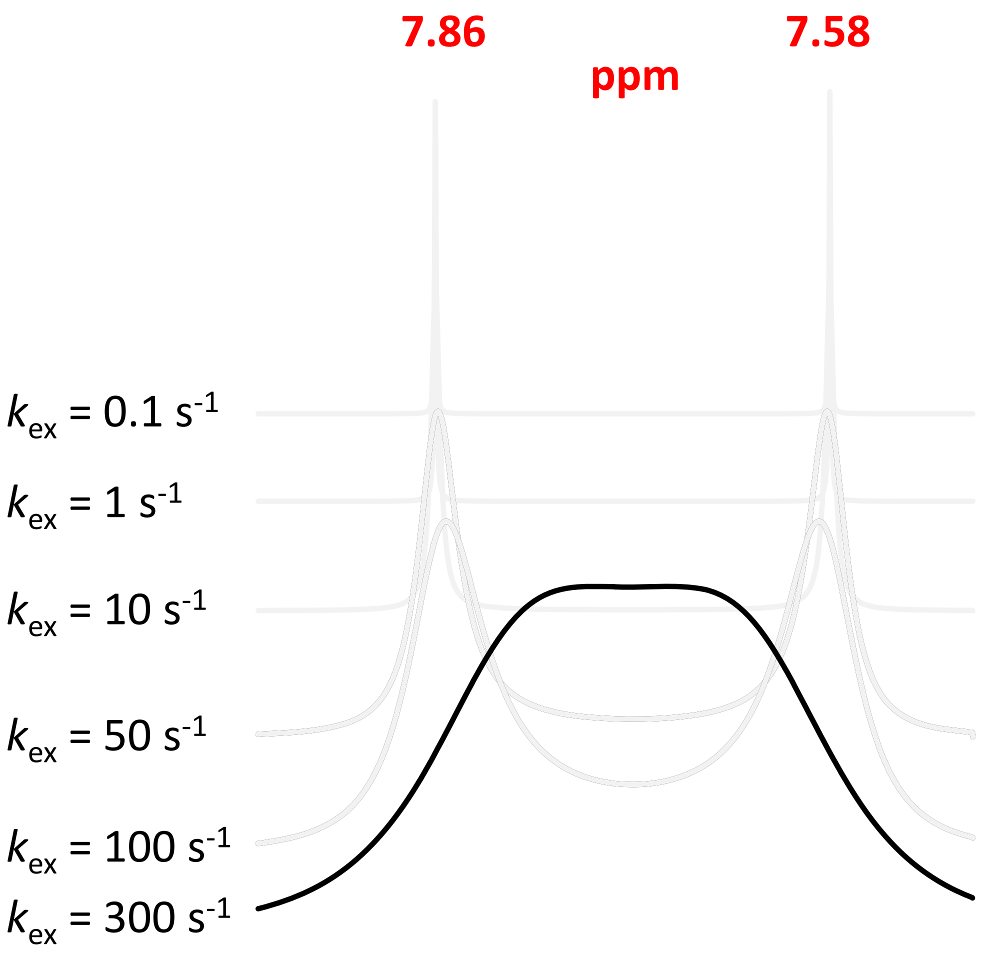
Final structure

Two different NH_2 protons

Now averaging the chemical shifts clearly continues.

If, increasing k_{ex}, for the first time there is nearly no minimum visible between the two signals. If the remaining tiny minimum vanishes, we speak about coalescence. In our case, according to the Gutowsky-Holm equation, coalescence would occur at k_{ex} = 306 s^{-1}.



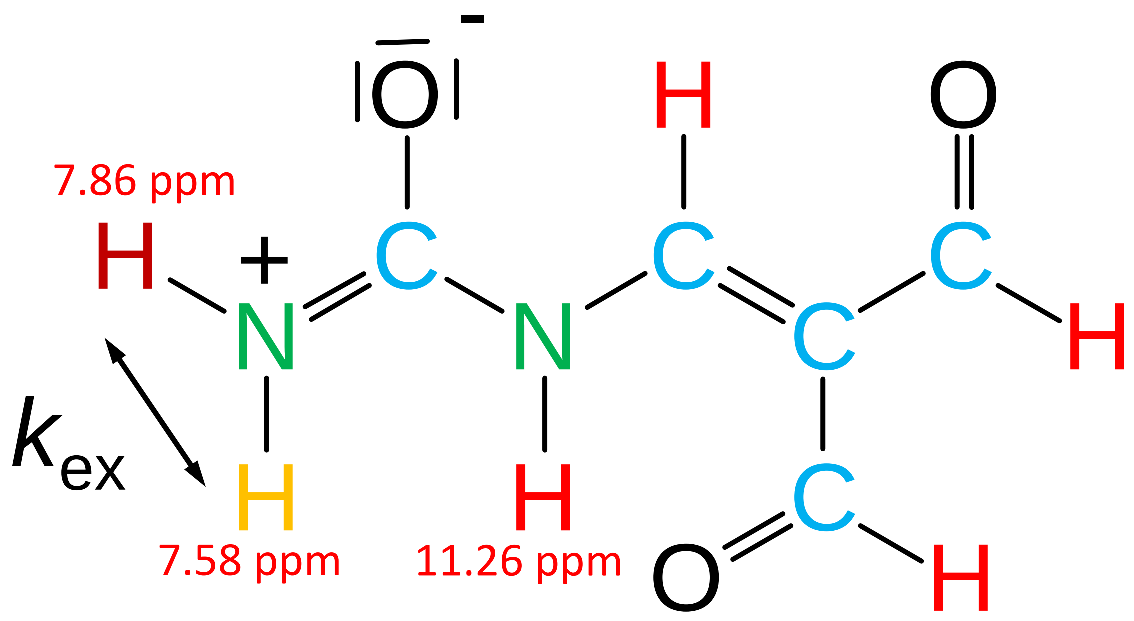


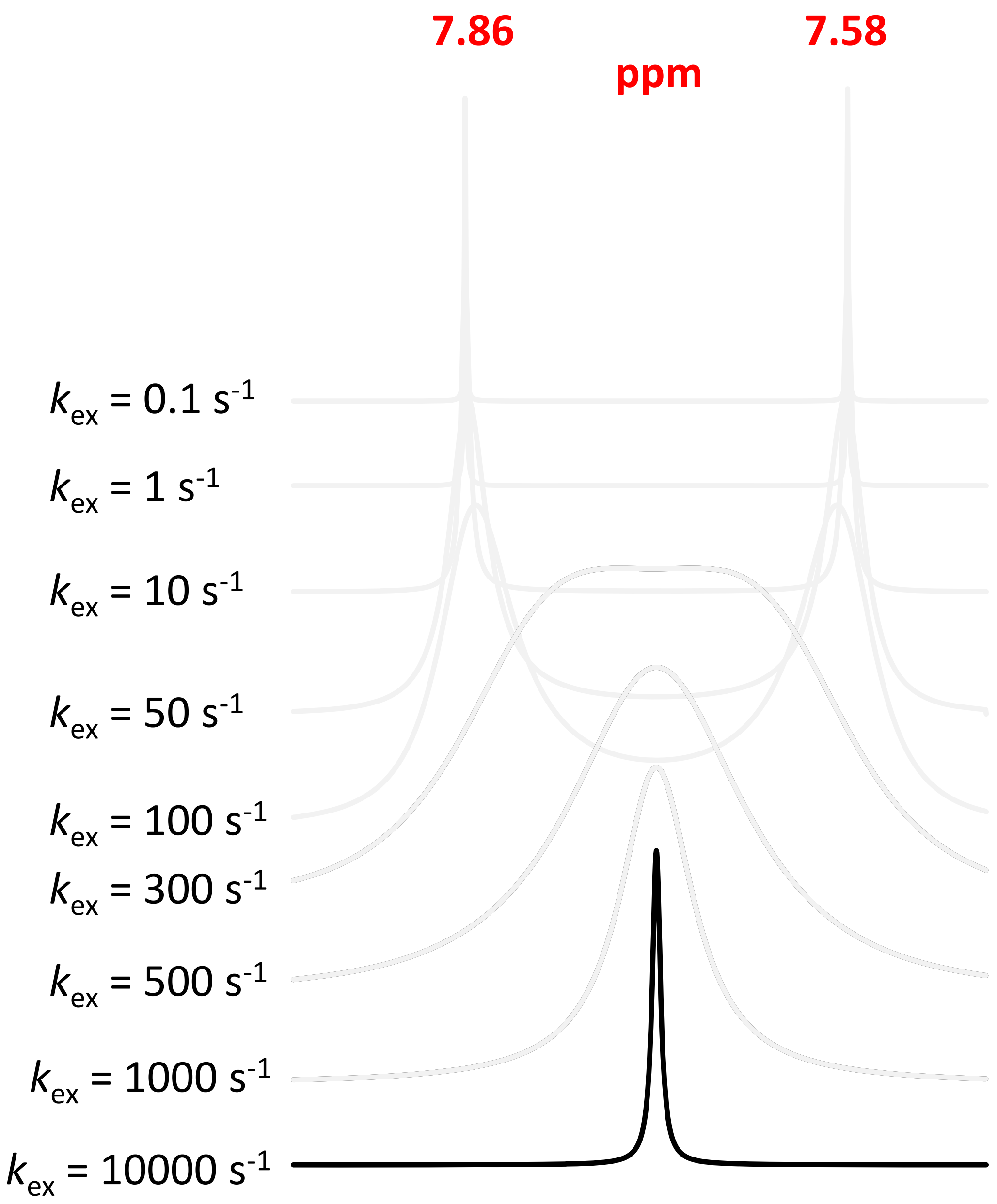
Final structure

Two different NH_2 protons

Finally we end in a sharp single line.

No exact measurement of k_{ex} was done here, but let us assume a value of k_{ex} = 10 s^{-1}. Are we able to explain both **two well separated lines** and two NOESY cross peaks of **identical intensity**?



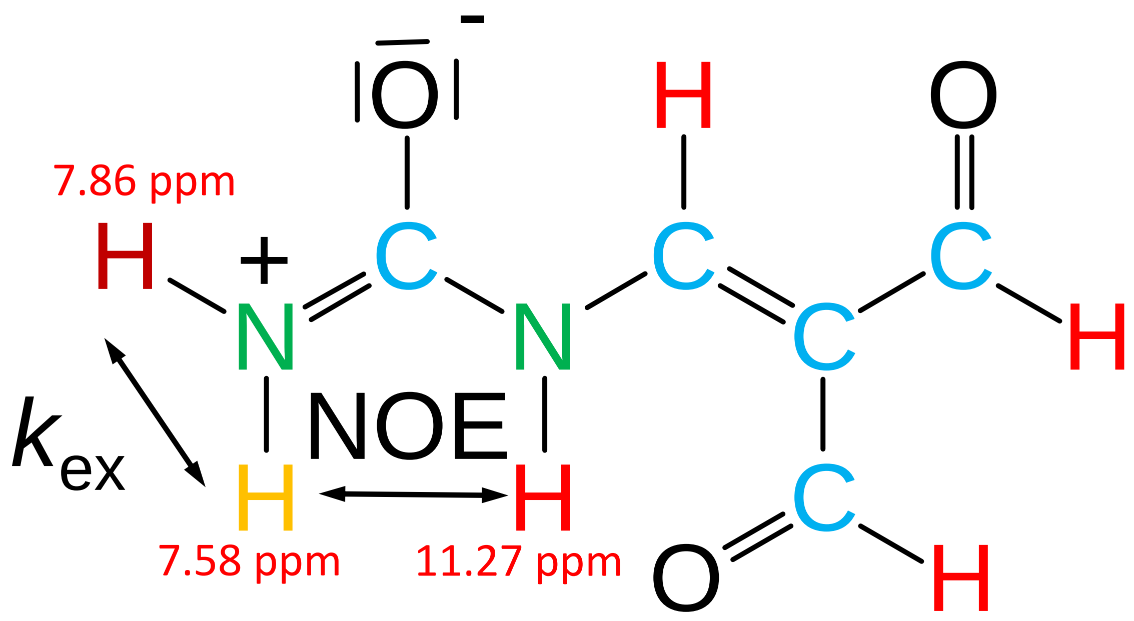


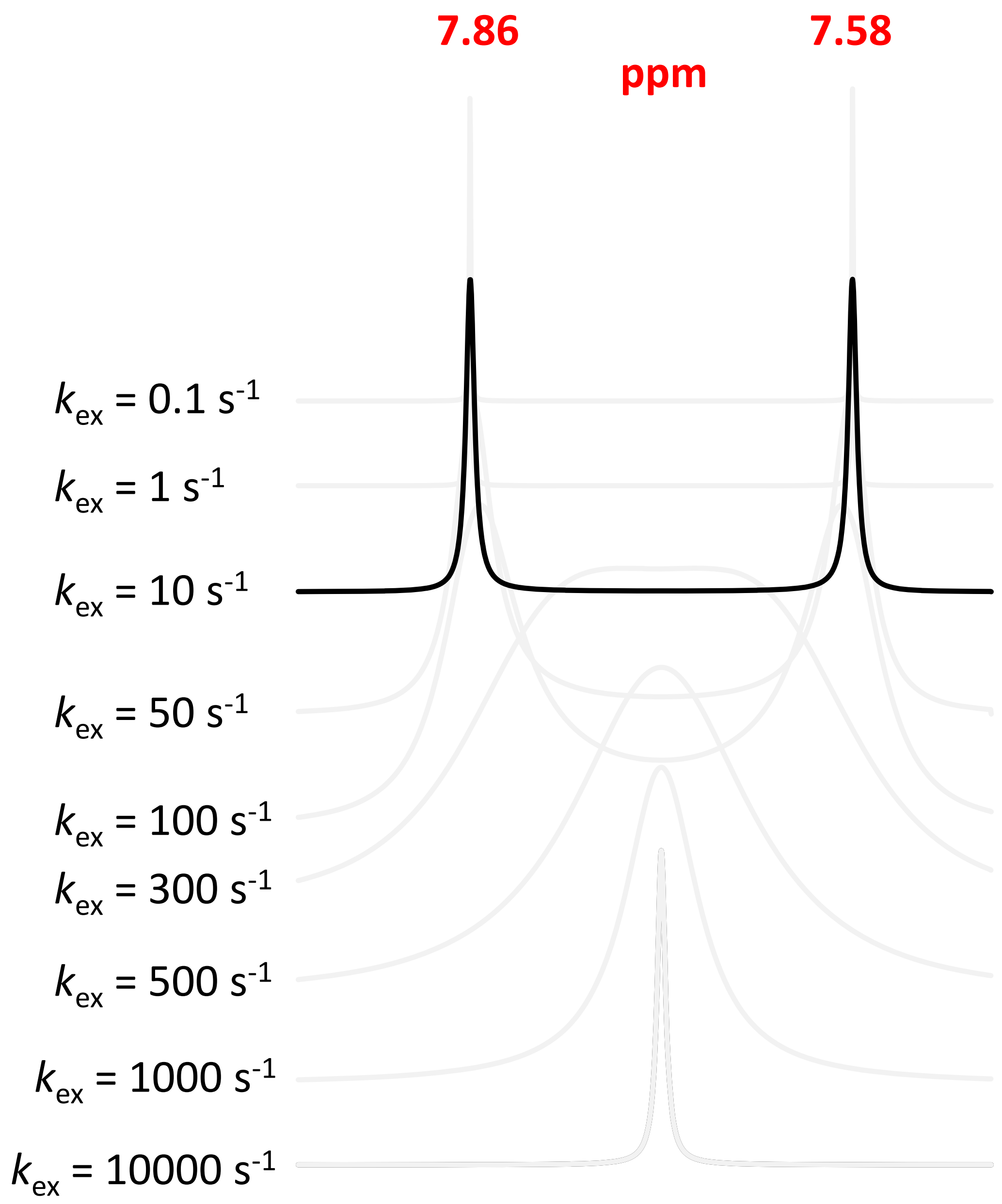
Final structure

Two different NH_2 protons

NOESY mixing time is 1000ms.

This means both NH_2 protons change their positions during the mixing time about ten times. That's enough to average the NOE transfer from the proton with the chemical shift of 11.27 ppm to the protons with the chemical shifts of 7.58 ppm/7.75 ppm.



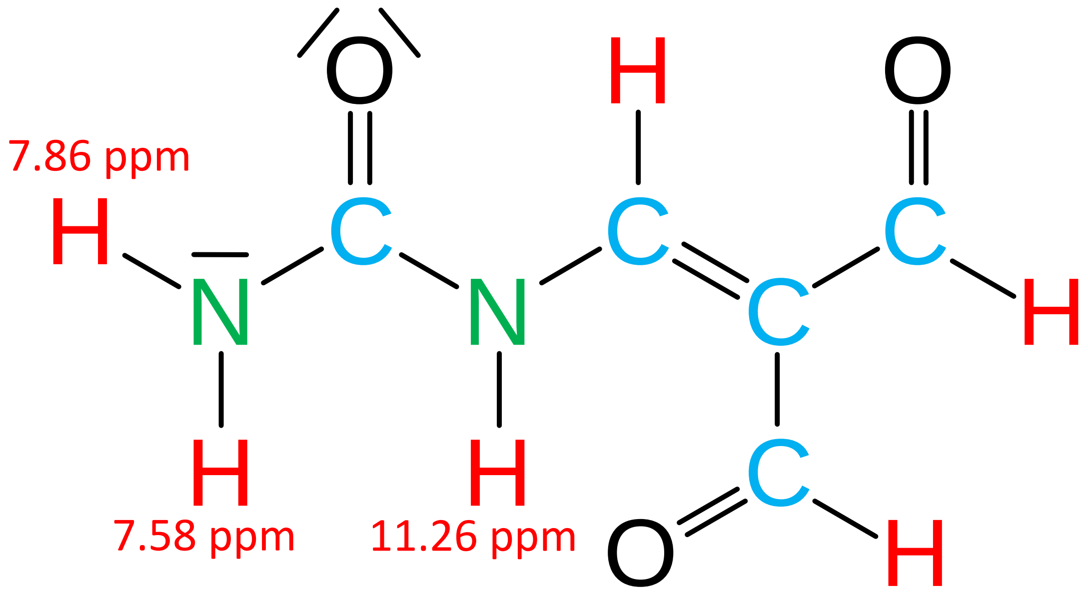


A last challenge

Assign both NH_2 protons

As seen, the restricted rotation around the C-N bond is too fast to do an unambiguouse assignment using the NOE effect.

But due to the excellent signal to noise ratio of the proton spectrum we get a second chance.



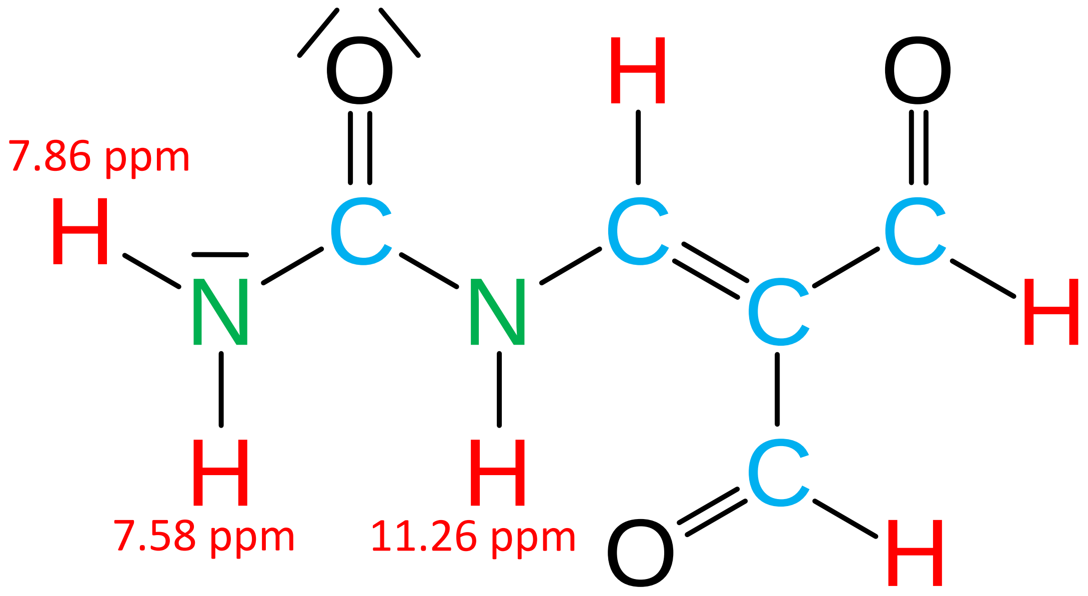
A last challenge

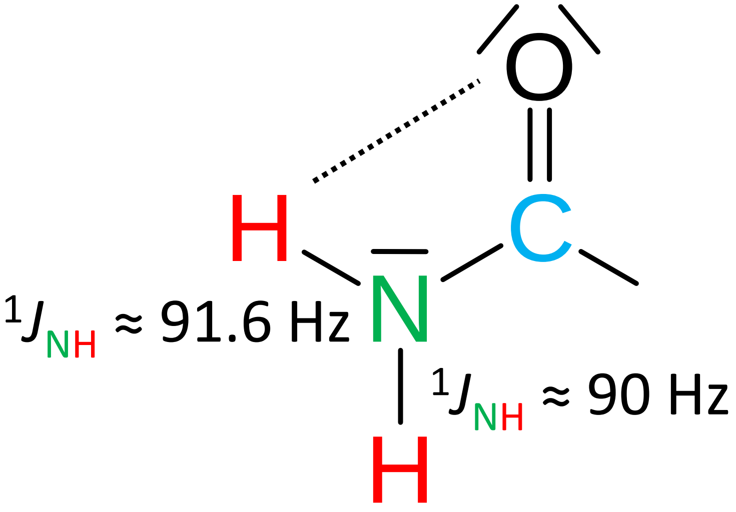
Assign both NH_2 protons

A typical value for a one bond coupling constant between nitrogen and proton in amides is about 90 Hz ($^1J_{NH} \approx 90$ Hz).

This coupling constant is up to 1.6 Hz higher, if there is a hydrogen bond present (*Angew. Chem. Int. Ed.* **2013**, 52, 3525 - 3528).

For the NH_2 part of our molecule this would mean:



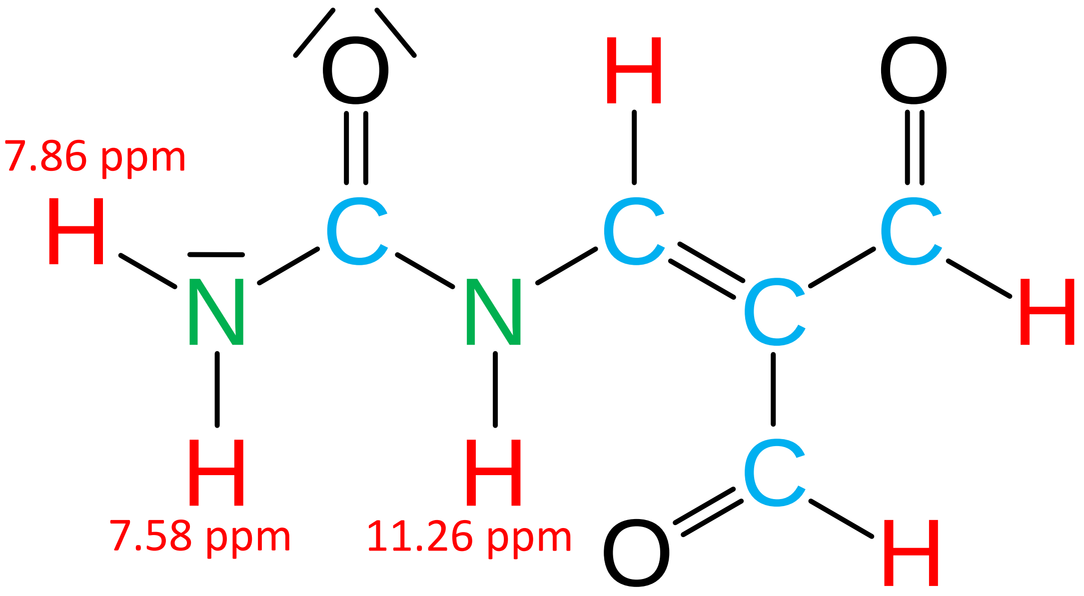


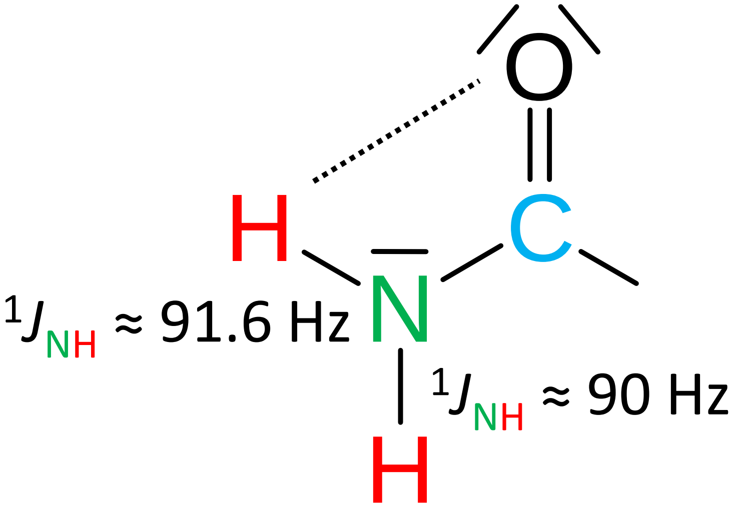
A last challenge

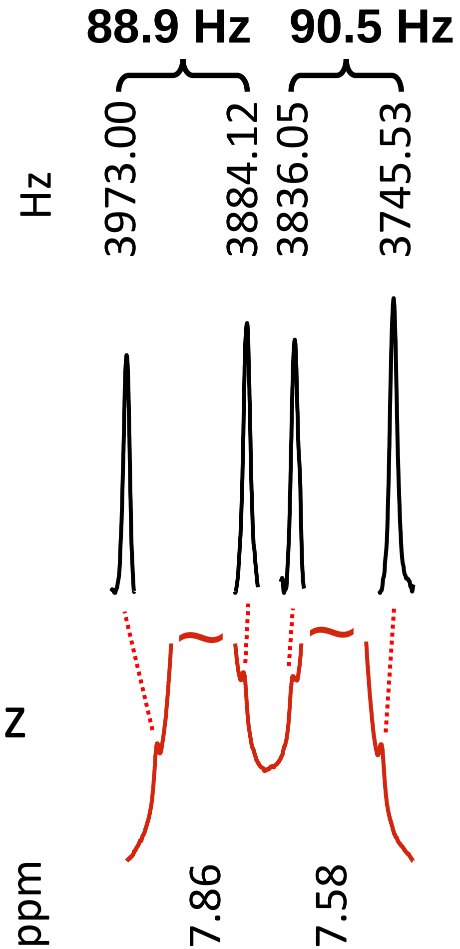
Assign both NH_2 protons

If we have a very carefully look at the amide proton signals at 7.58 ppm and 7.86 ppm, we are able to see the very small ^{15}N satellite signals.

From these satellite signals we get the two one bond coupling constants.





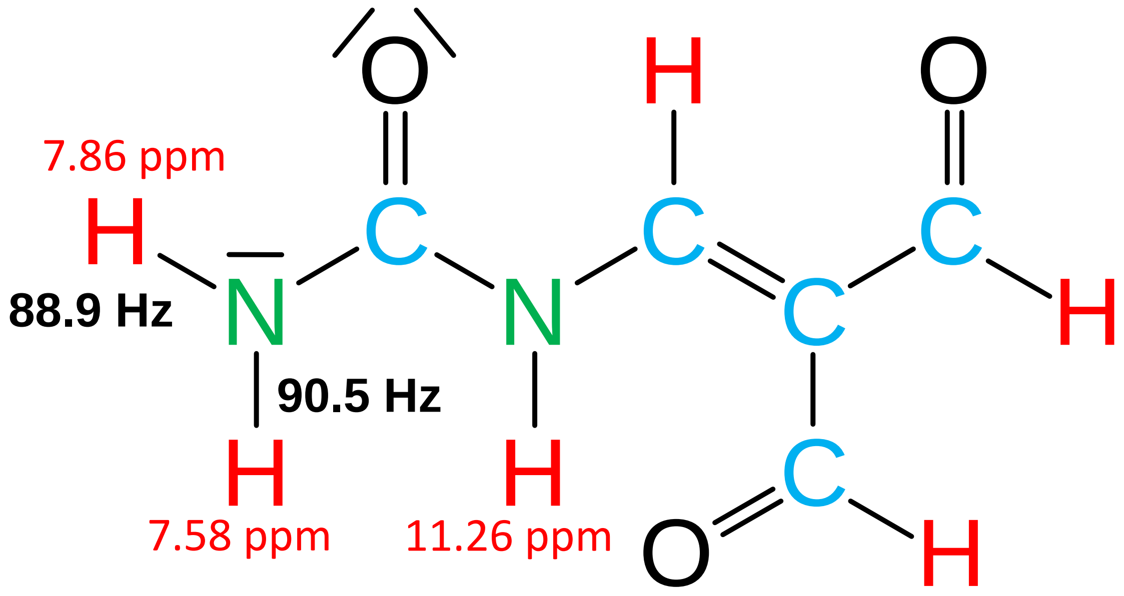


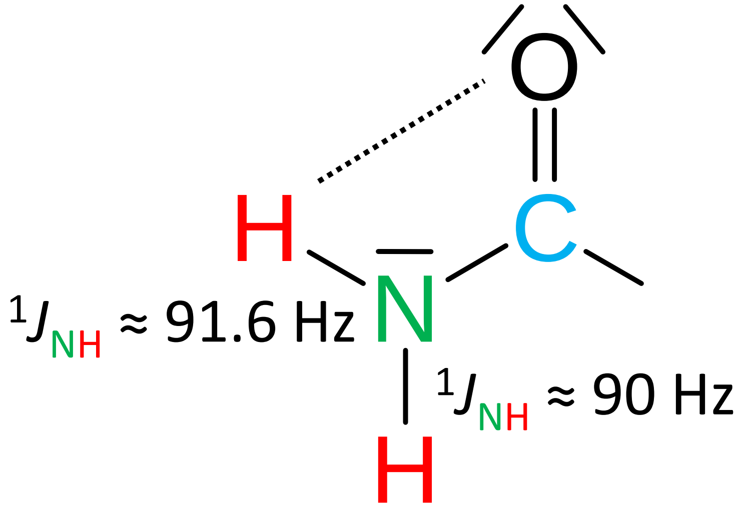
A last challenge

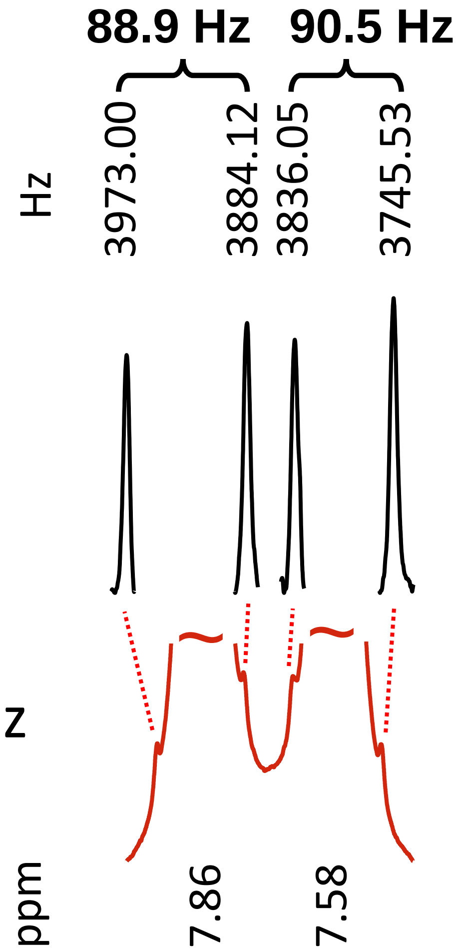
Assign both NH_2 protons

Let us compare the measured values of ${}^{1}J_{NH}$ with our prediction.

Thats the opposite of our expectation. Let us change the proton assignment.





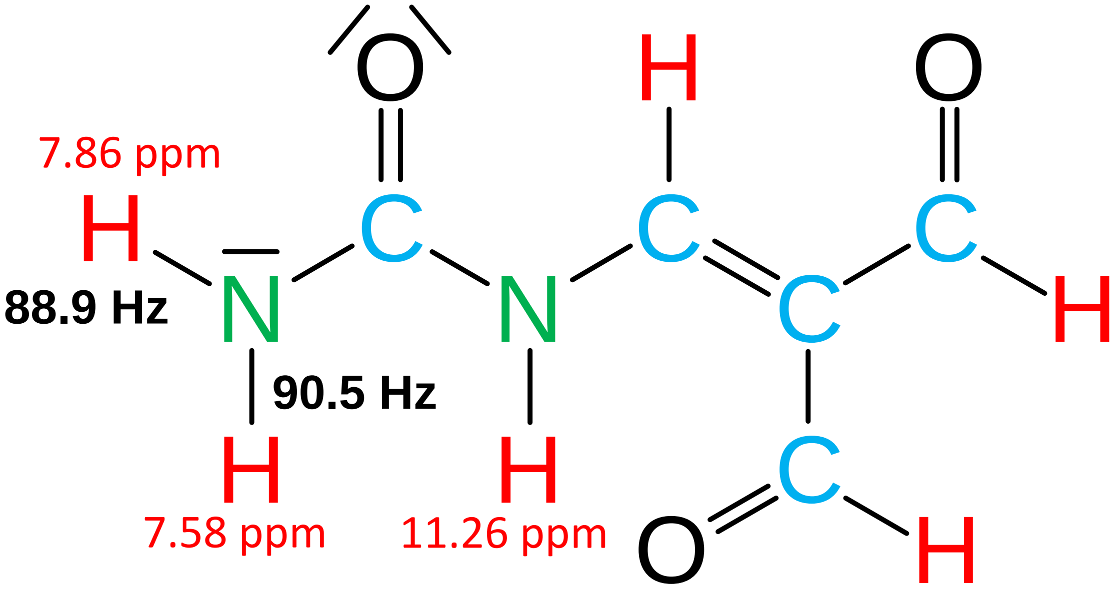


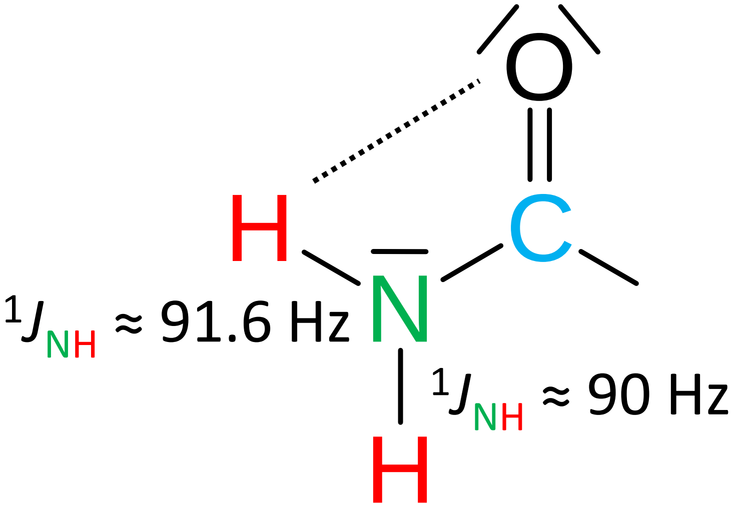
A last challenge

Assign both NH_2 protons

There is a second – although rather weak – proof of our proton assignment. It should be presented here for curious people.

But first let us return to our mesomeric structure with the double bond between carbon and nitrogen.





A last challenge

Assign both NH_2 protons

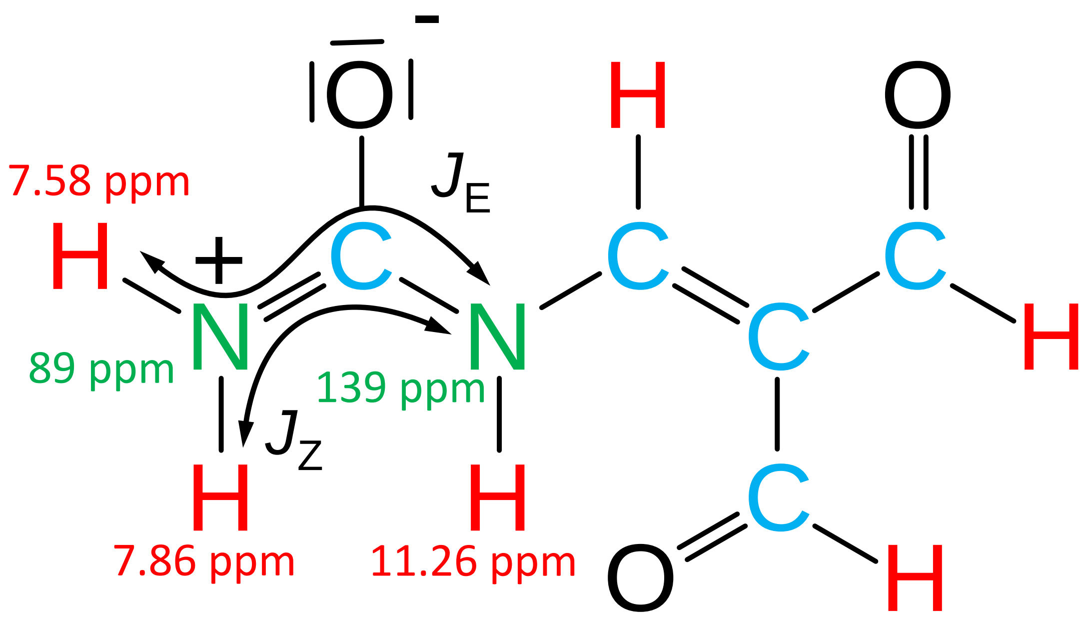
In fragments of the type *A* – X = Y – *B* the atoms *A* and *B* might be in **E** or **Z** position to each other.

Let us introduce the coupling constants

J_Z (*A* and *B* are in **Z** position to each other)

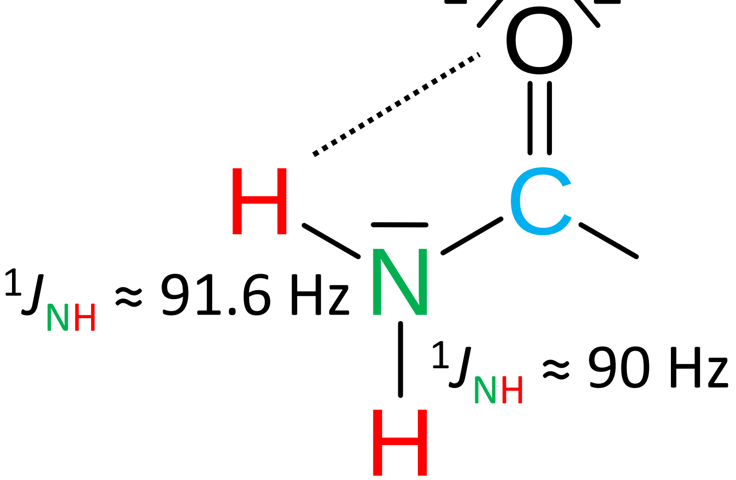
and

J_E (*A* and *B* are in **E** position to each other).



As a general rule – not only in ethylene fragments – we have

$J_E > J_Z$

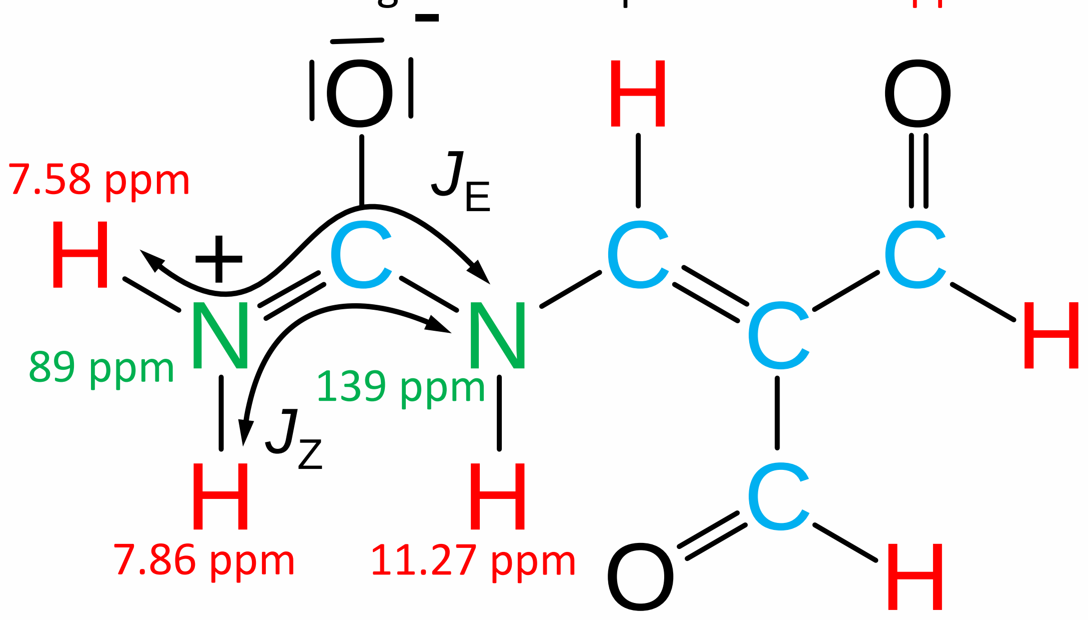


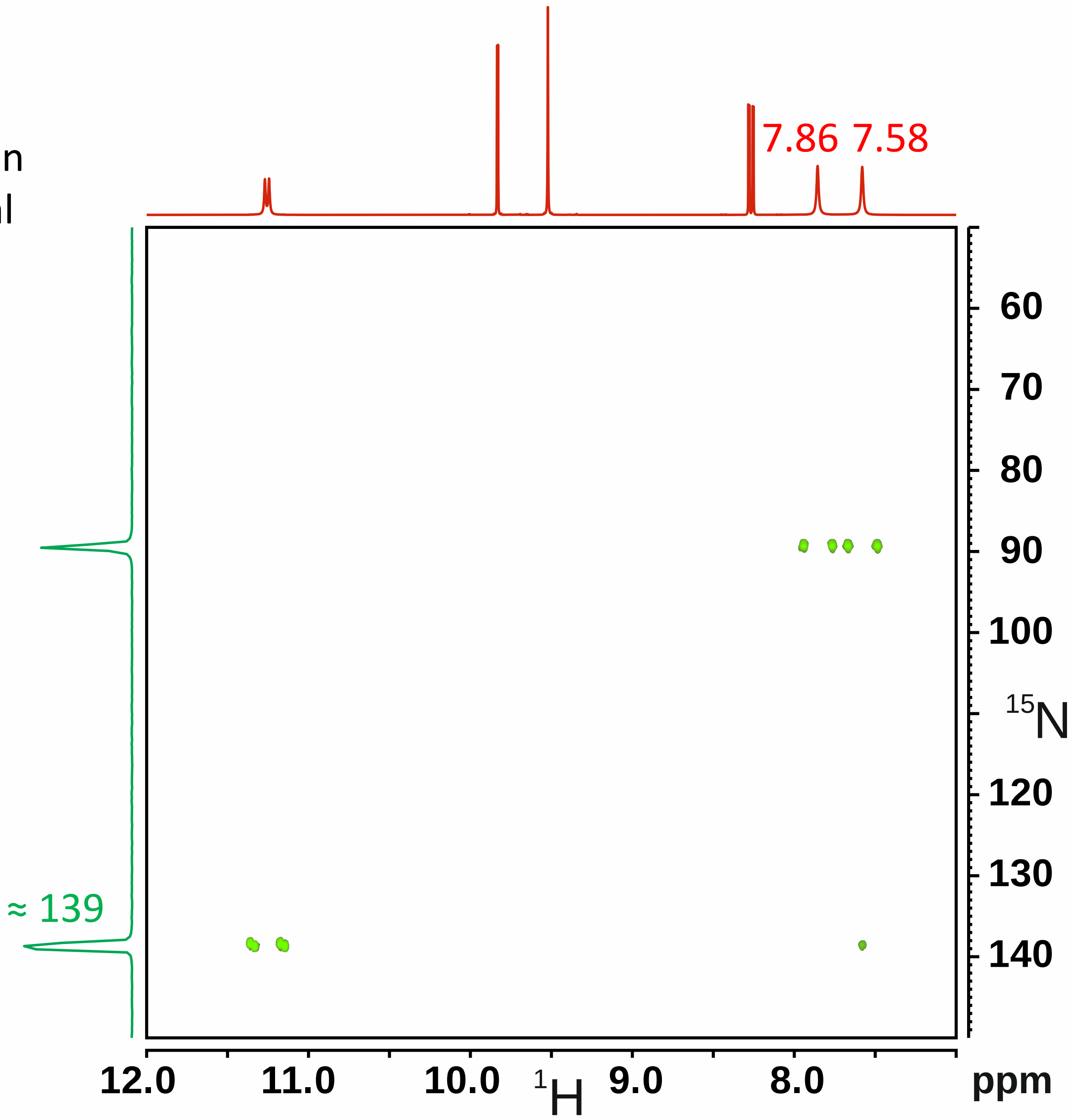
A last challenge

Assign both NH_2 protons

Assuming some boundary conditions concerning HMBC evolution time, J_E and J_Z the intensity of HMBC peaks is nearly proportional to the coupling constant between the nuclei, which are responsible for a given cross peak.

The cross peak between the nitrogen atom at 139 ppm and the proton at 7.58 ppm should be stronger than the cross peak between the same nitrogen and the proton at 7.86 ppm.



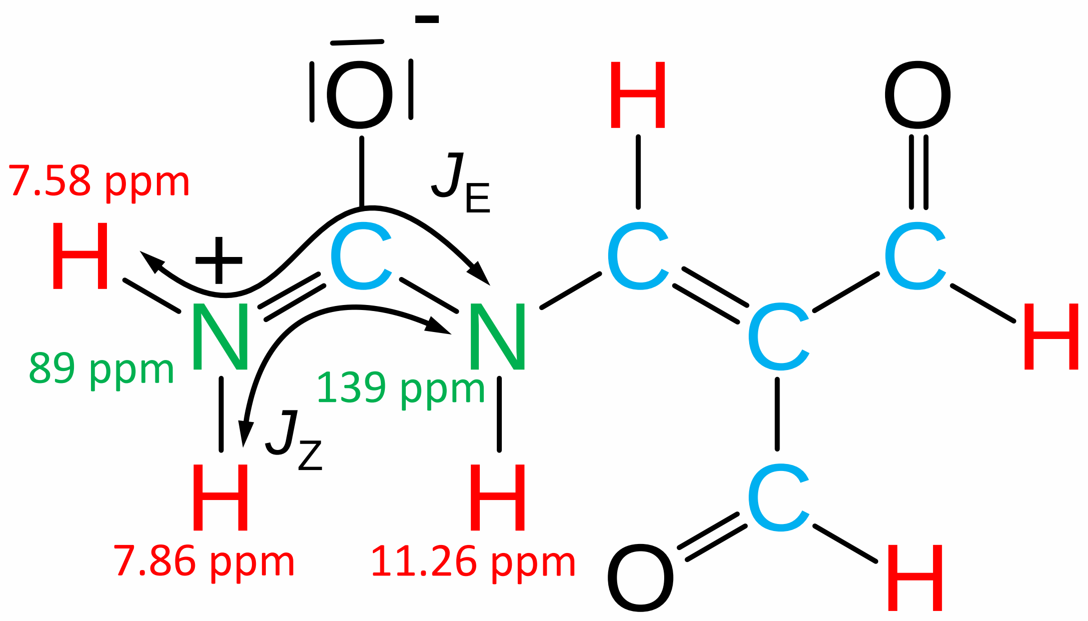


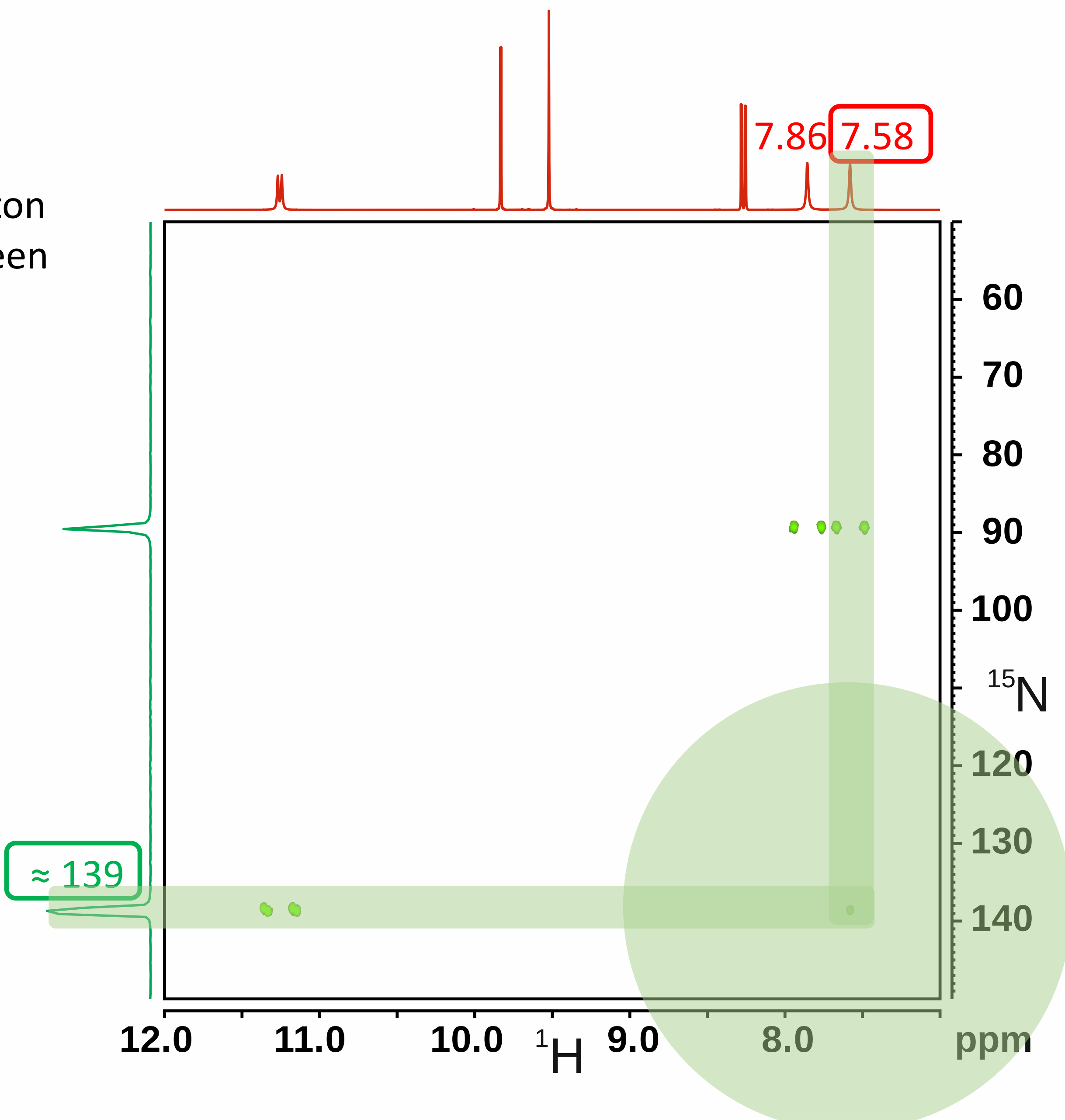
A last challenge

Assign both NH_2 protons

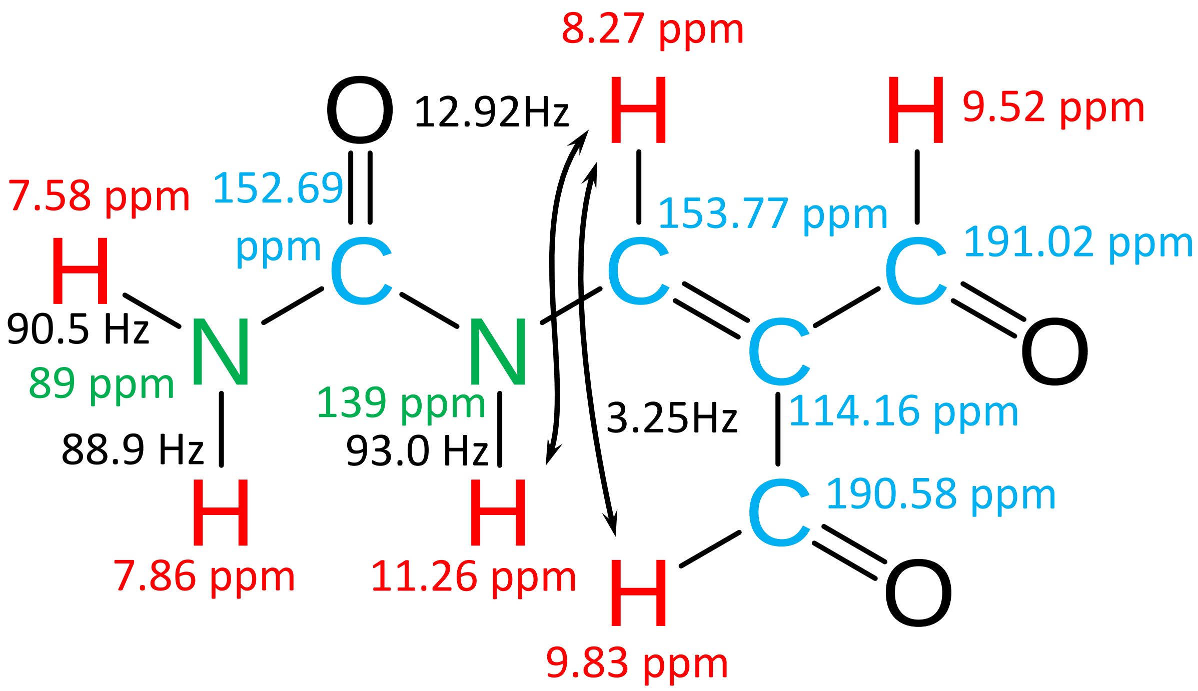
Indeed, the $^1H/^{15}N$-HMBC shows a cross peak between the nitrogen atom with the chemical shift of 139 ppm and the proton at 7.58 ppm and a weaker (in fact not visible) cross peak between the same nitrogen atom and the proton at 7.86 ppm.

But be careful. To really understand the intensity of HMBC cross peaks you have to deal with the HMBC transfer function.





Summary



You get the one bond coupling constant of 93.0 Hz if you analyze the nitrogen satellite signals at 11.27 ppm.

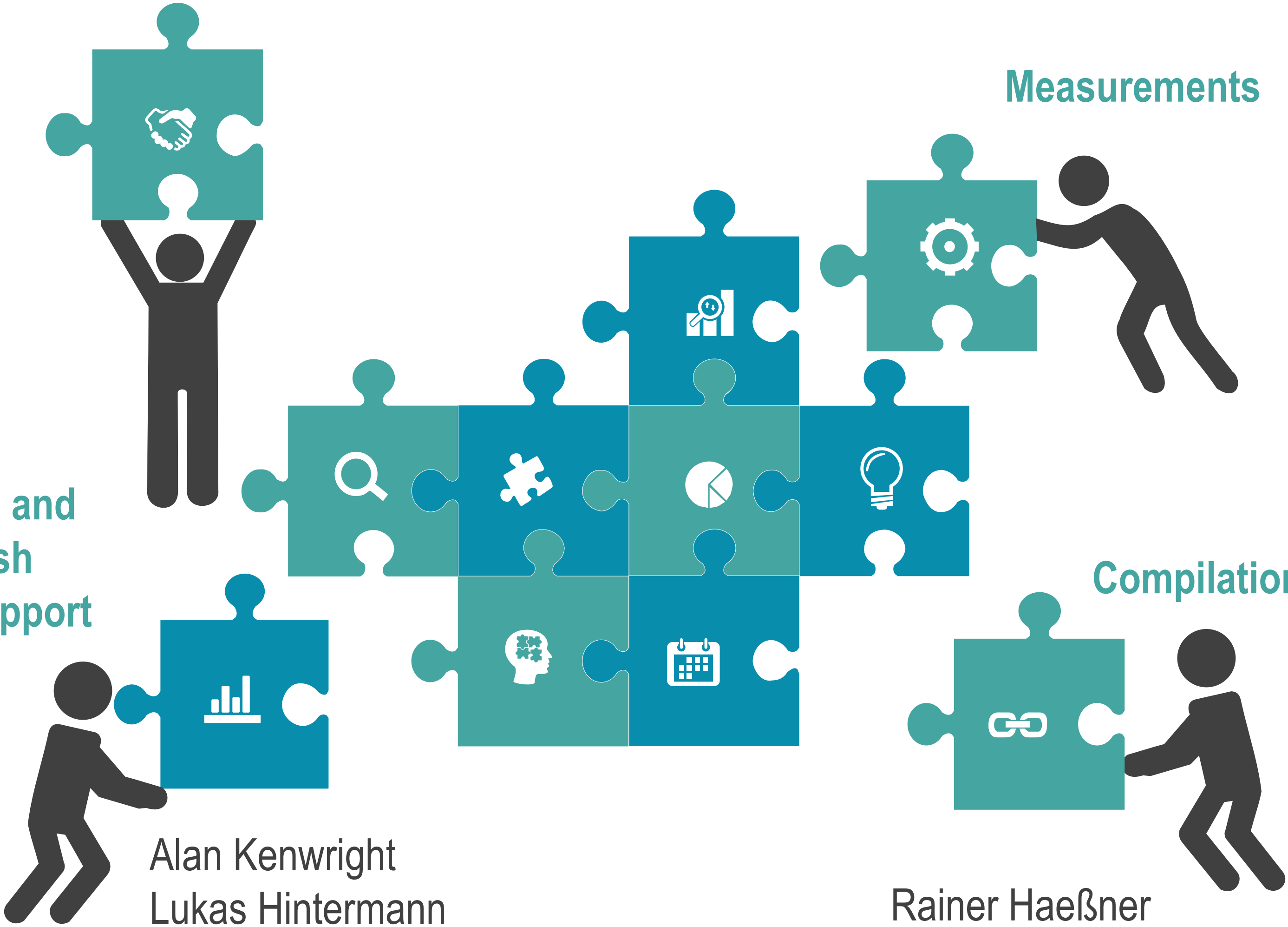
Contributions

Spectrometer time

TU Munich

Measurements

Wolfgang Eisenreich
Lukas Hintermann



Discussions and native English language support

Alan Kenwright
Lukas Hintermann

Compilation

Rainer Haeßner

More exercises …