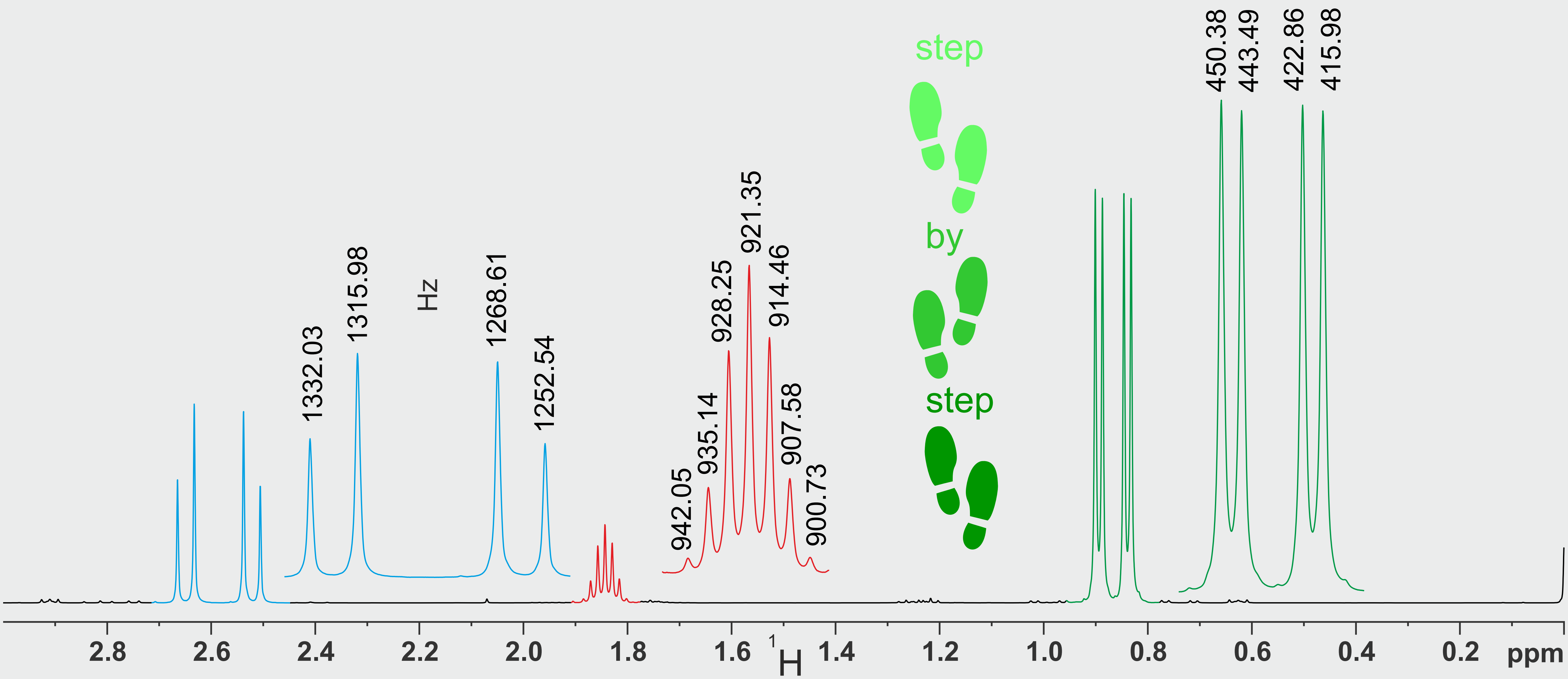
Exercise plus Solution – Quick PDF overview

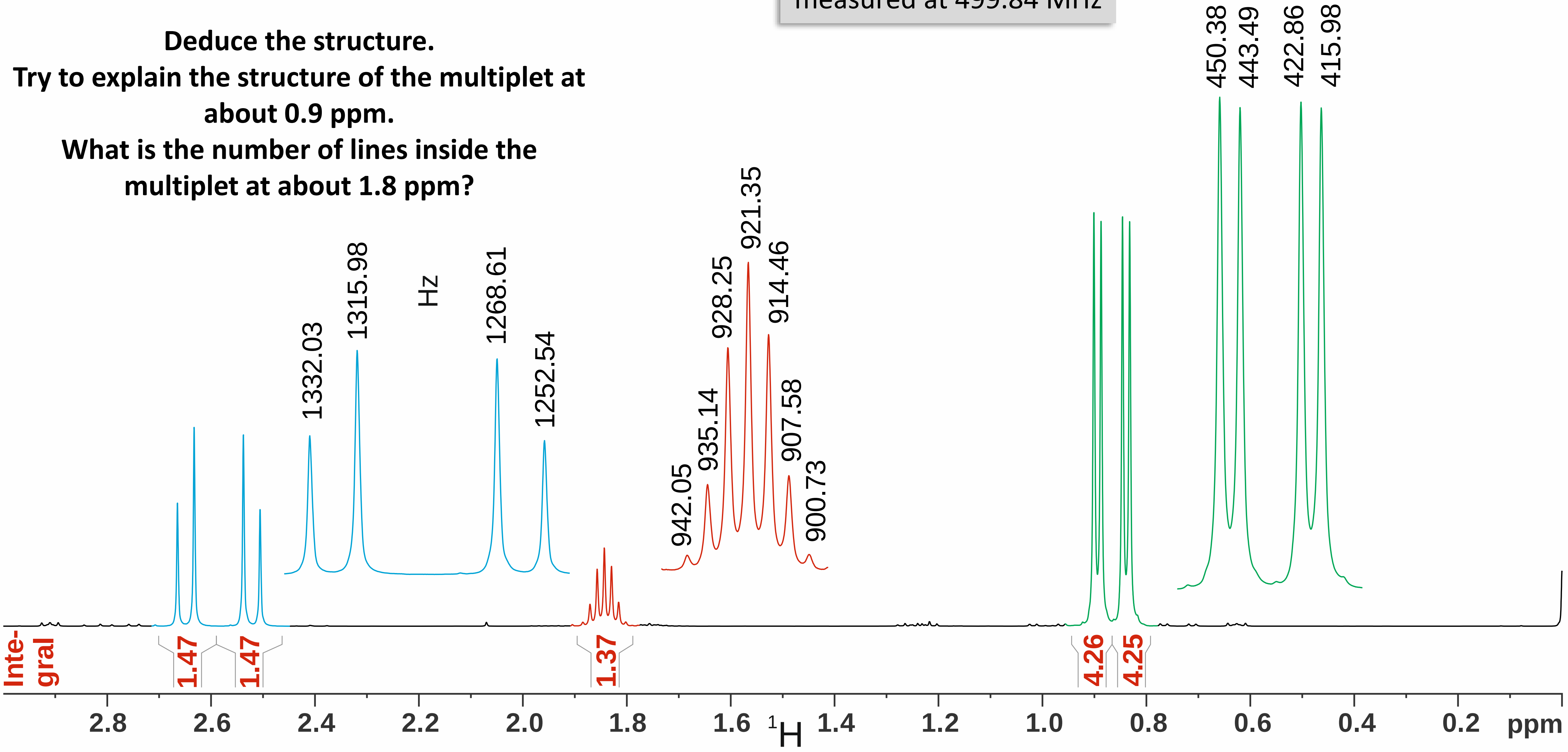
It is recommended to use this PDF version only for a quick overview of the NMR challenge. All animations of the PowerPoint version are missing, under certain circumstances quality deficiencies may also occur.
The higher quality PowerPoint files are freely available for download at any time.

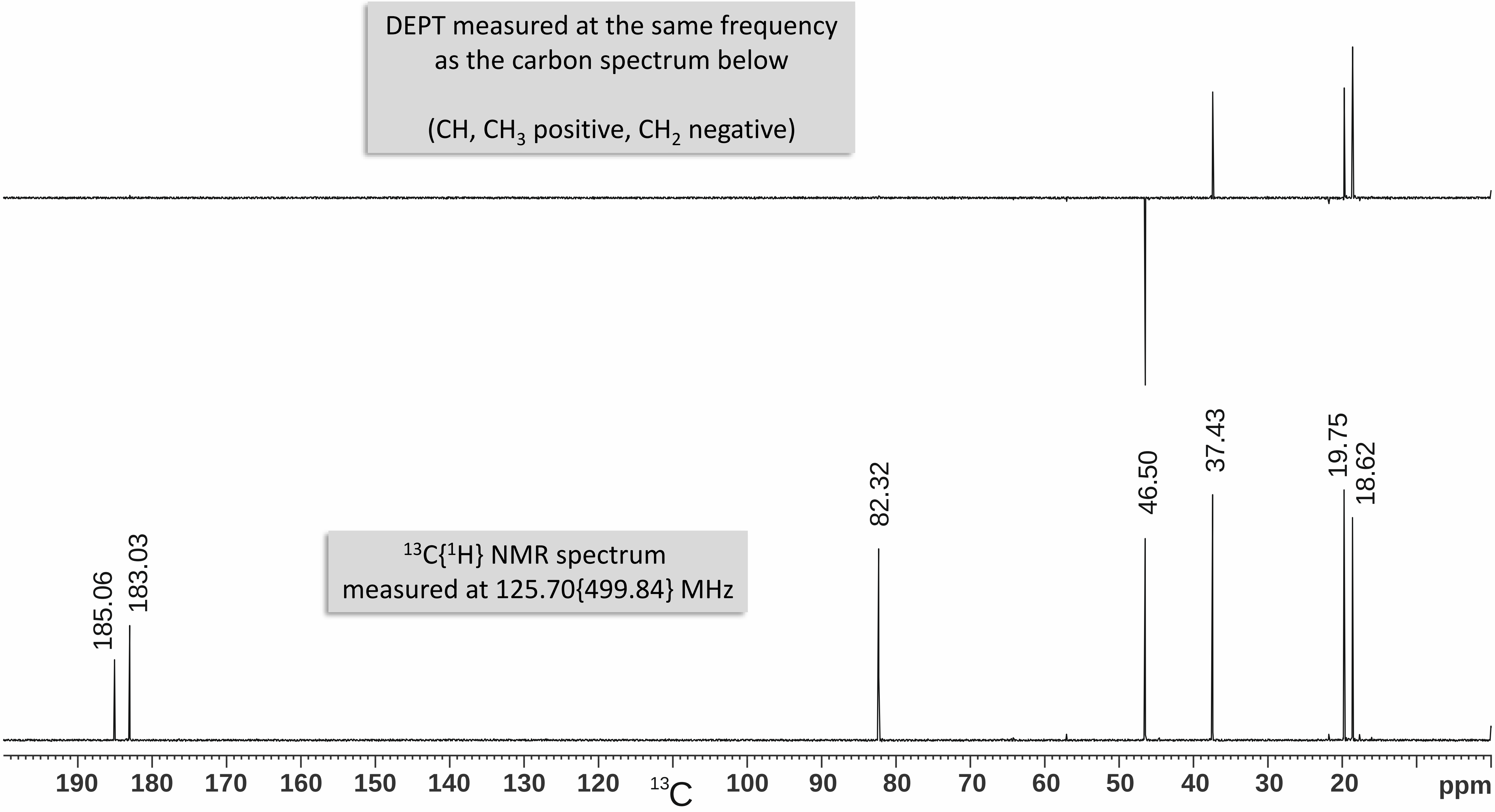


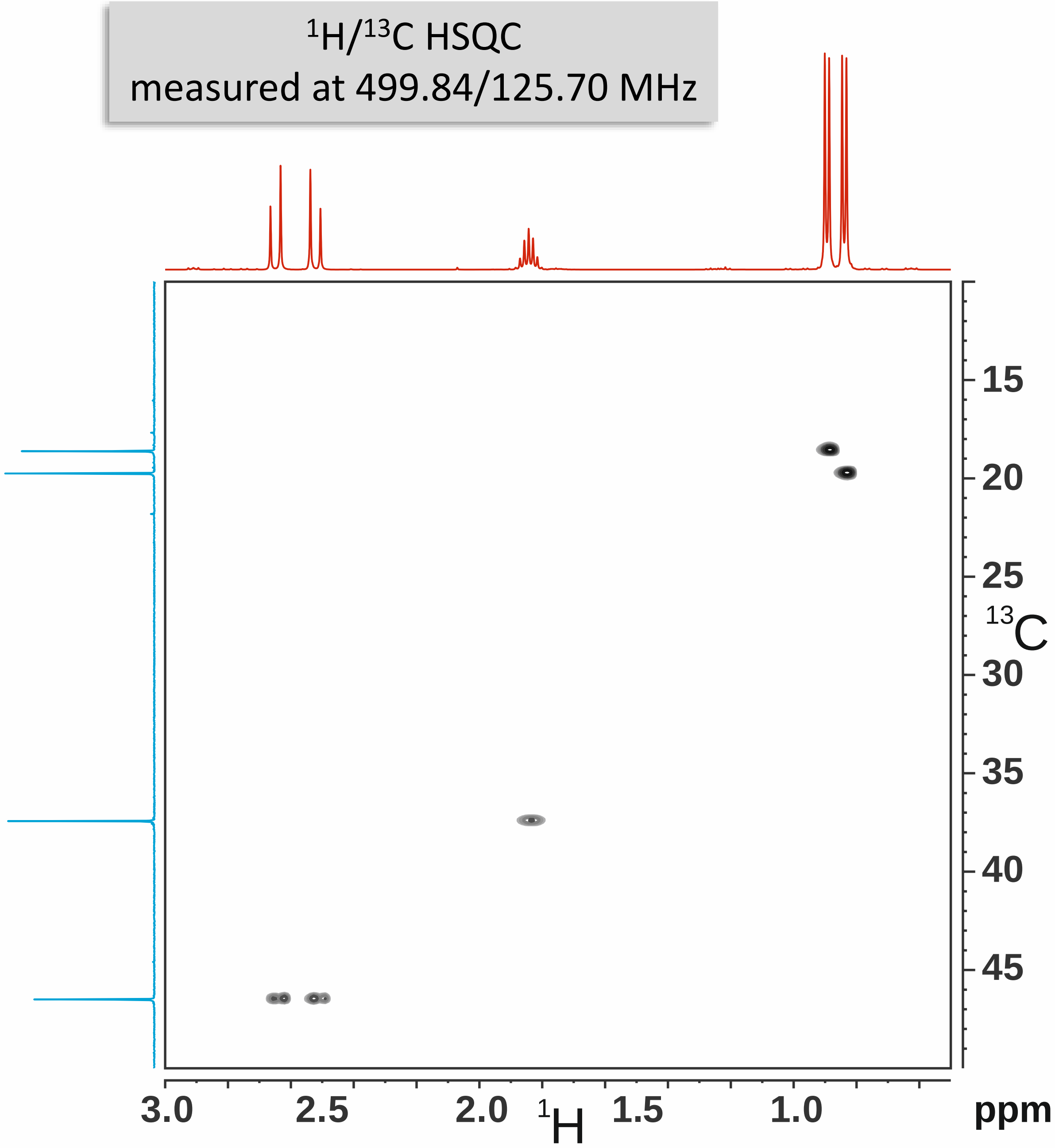
$C_7H_{12}O_5$ measured in D_2O

^{1}H NMR spectrum
measured at 499.84 MHz

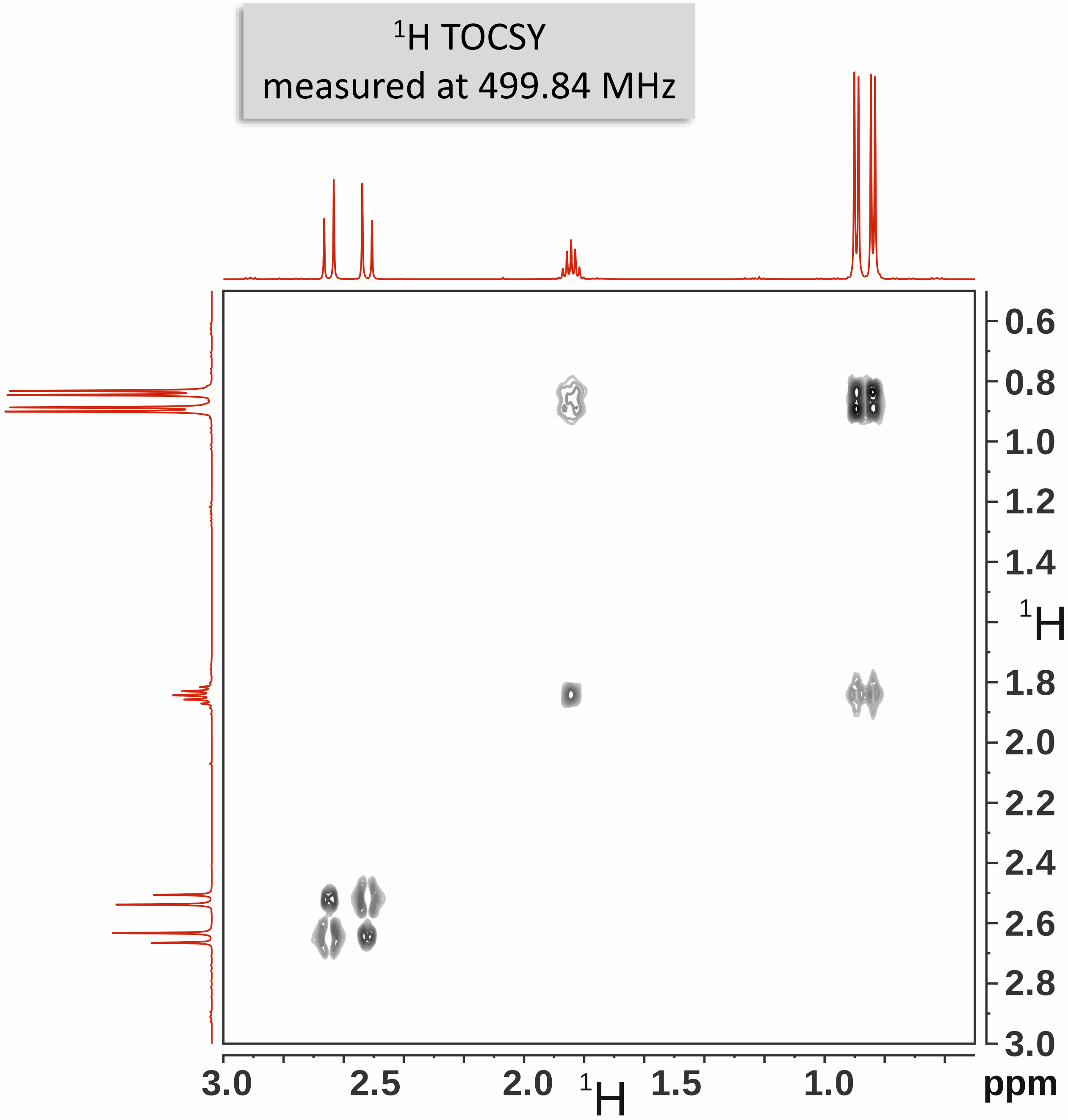
Deduce the structure.
Try to explain the structure of the multiplet at about 0.9 ppm.
What is the number of lines inside the multiplet at about 1.8 ppm?




DEPT measured at the same frequency
as the carbon spectrum below
(CH, CH_3 positive, CH_2 negative)
185.06
183.03
$^{13}C\{^1H\}$ NMR spectrum
measured at 125.70{499.84} MHz
82.32
46.50
37.43
19.75
18.62
190
180
170
160
150
140
130
120
^{13}C
100
90
80
70
60
50
40
30
20
ppm



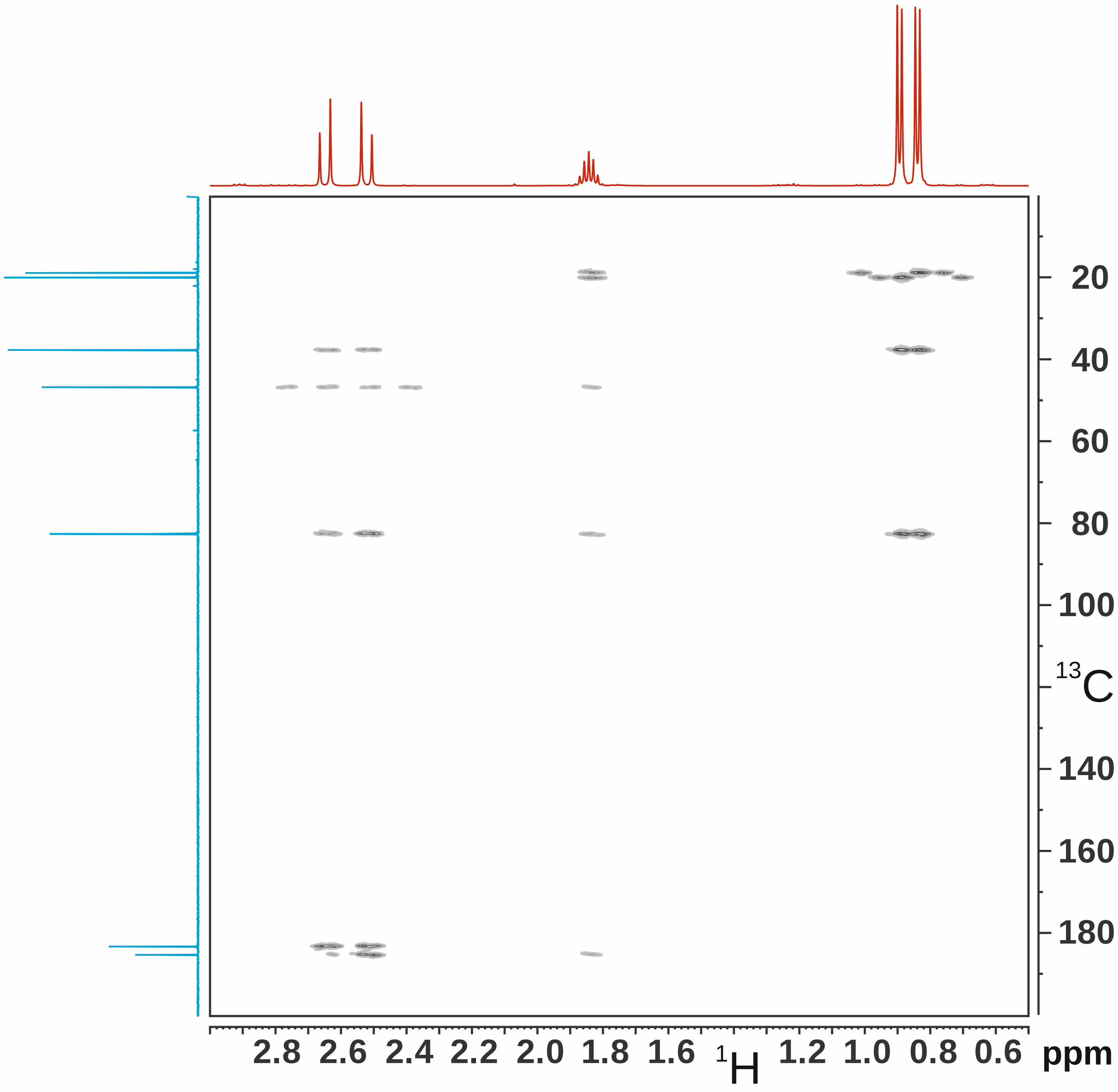
$^1H/^{13}C$ HSQC
measured at 499.84/125.70 MHz



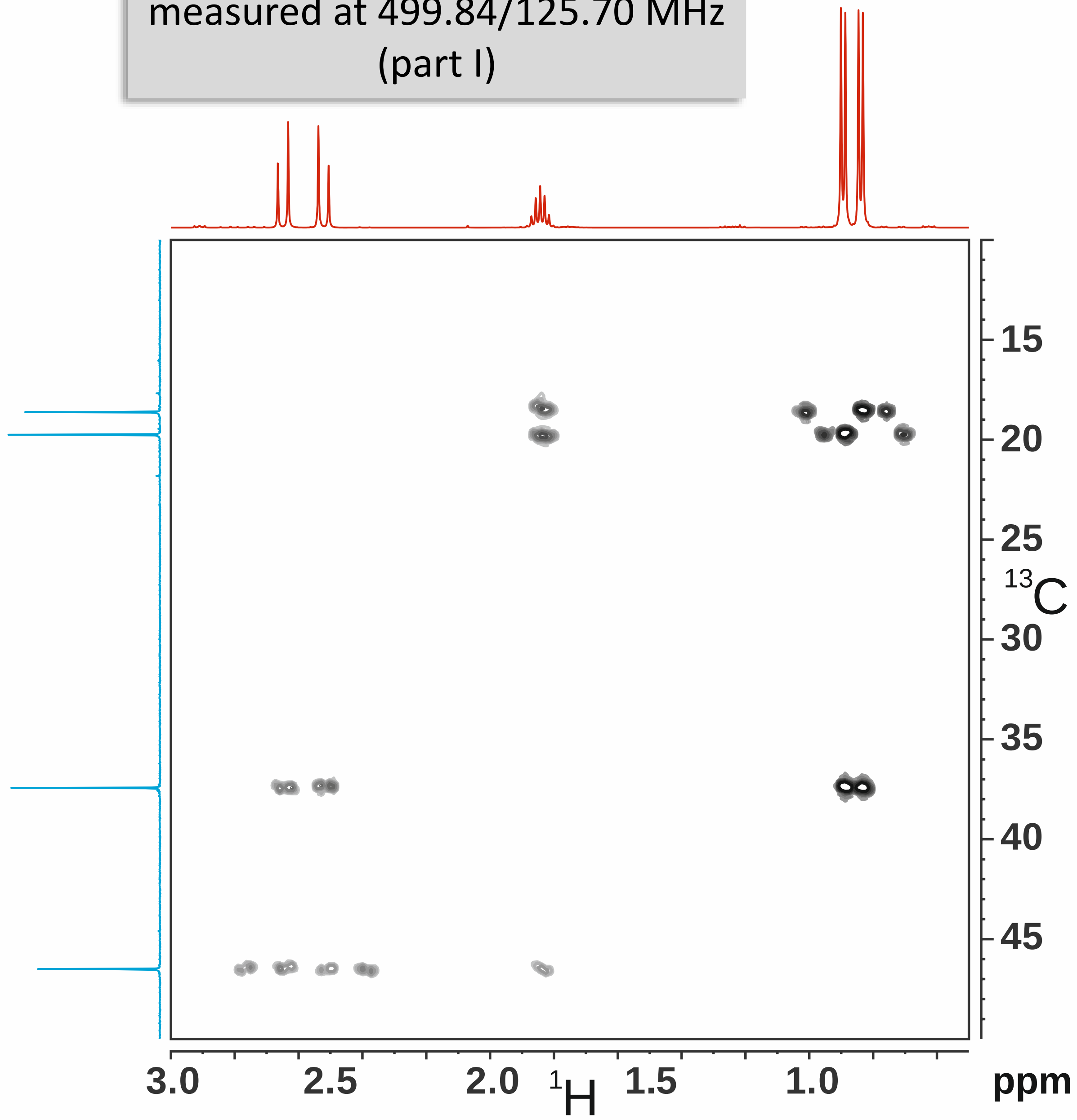
^1H TOCSY
measured at 499.84 MHz

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

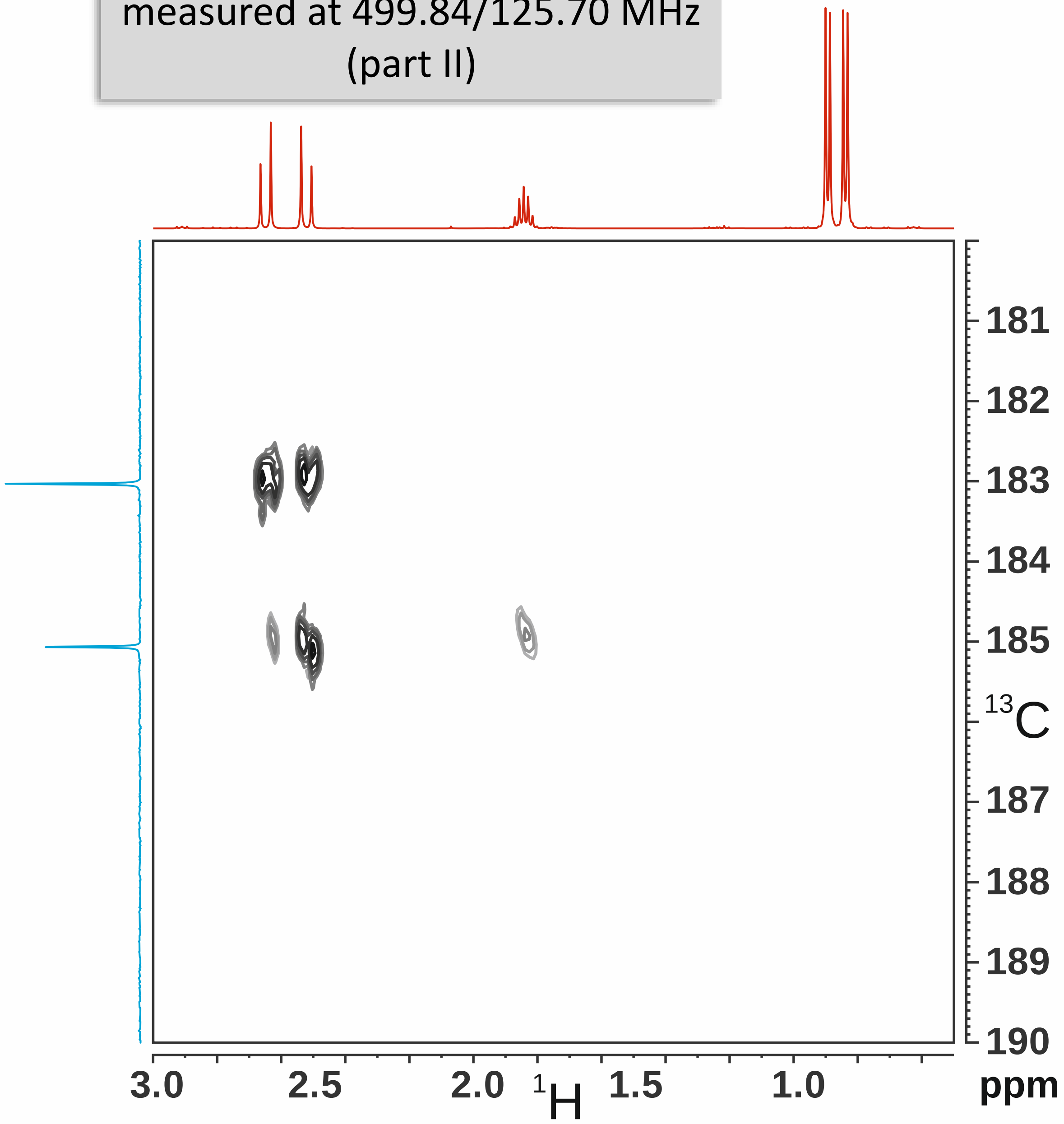
Please also use the enlarged parts of this HMBC on the next page.



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



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



Integration

The sum of the five integrals presented in the proton spectrum gives a value of **12.82**.
This value seems to agree quite well with the twelve protons of the molecular formula.

On the one hand, this is entirely a coincidence. The integral in NMR spectra always contains an unknown proportionality coefficient, which, of course, could be accidentally close to **1** here.

If we assume this factor of **1** here, then the well-separated signal at about 1.8 ppm would consist of about **1.35** protons. That is completely impossible on the other hand. We have to end in **1,0** or **2,0** protons.

We can tentatively normalise the integral of the signal at approx. 1.8 ppm one time to **1.0** and another time to **2.0** and have a look to the changes of the other integrals.

Values taken from spectrum:	1.47	1.47	1.37	4.26	4.25	
$I_{1.8\ \mathrm{ppm}}$ normalised to $I = 1,0$	**1.07**	**1.07**	**1.00**	**3.11**	**3.10**	$\Sigma I = 9.35$
$I_{1.8\ \mathrm{ppm}}$ normalised to $I = 2.0$	2.15	2.15	2.00	6.22	6.20	$\Sigma I = 18.72$

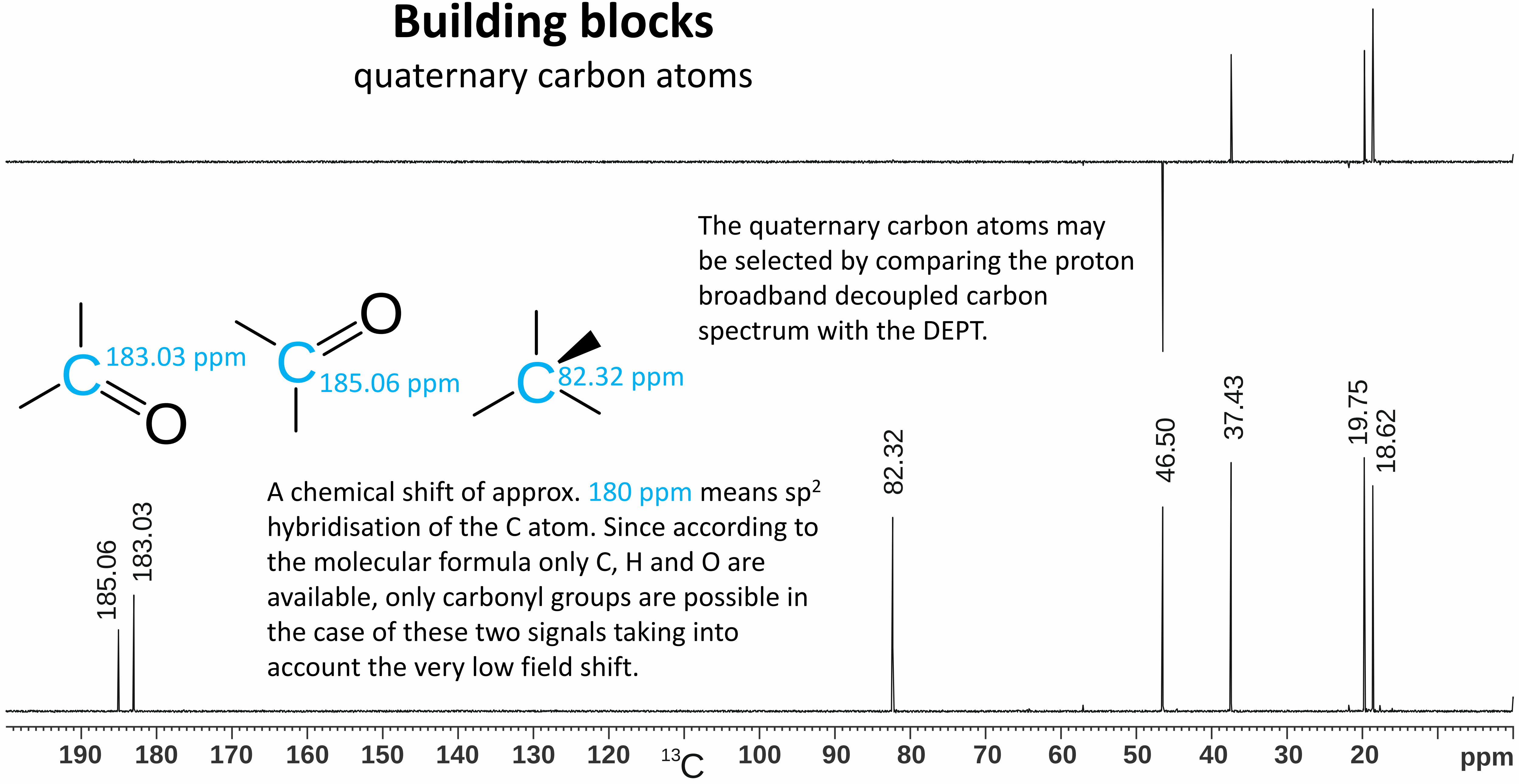
The normalisation to **2.0** is impossible because the compound contains only twelve protons. With slight rounding, the five proton signal groups are distributed in the ratio of

1 : 1 : 1 : 3 : 3

The three missing protons can only be OH groups, which are not visible due to the exchange with D_2O.

Building blocks

quaternary carbon atoms



The quaternary carbon atoms may be selected by comparing the proton broadband decoupled carbon spectrum with the DEPT.

A chemical shift of approx. 180 ppm means sp^2 hybridisation of the C atom. Since according to the molecular formula only C, H and O are available, only carbonyl groups are possible in the case of these two signals taking into account the very low field shift.

Building blocks

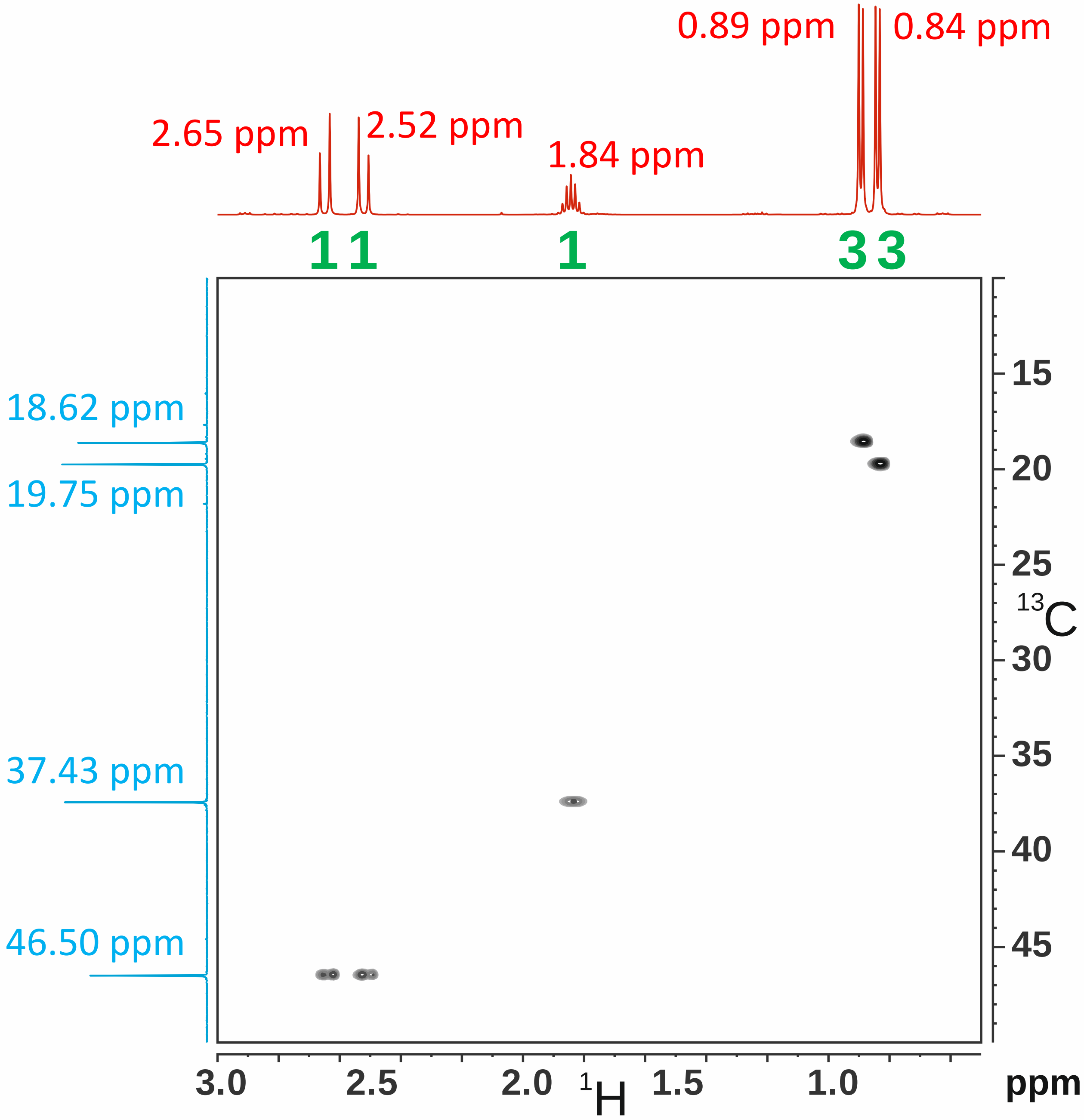
CH_n fragments

The best source to find protonated carbons is the HSQC.

The integrals from the proton spectrum have just been determined, the chemical shifts of the carbon signals can be taken from the one-dimensional carbon spectrum.

To get the chemical shifts of the proton multiplets, some simple arithmetics is required. An example for the high-field doublet :

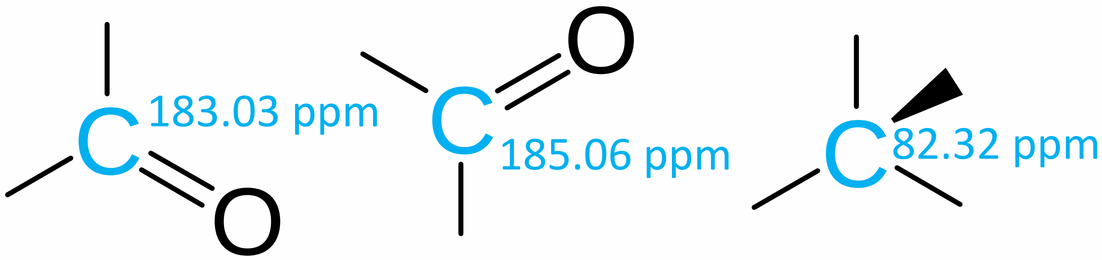
(422.86 Hz + 415.98 Hz/ (2 * 499.84 MHz)



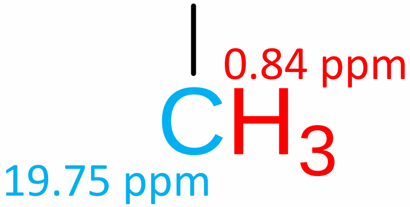
Building blocks

CH_n fragments

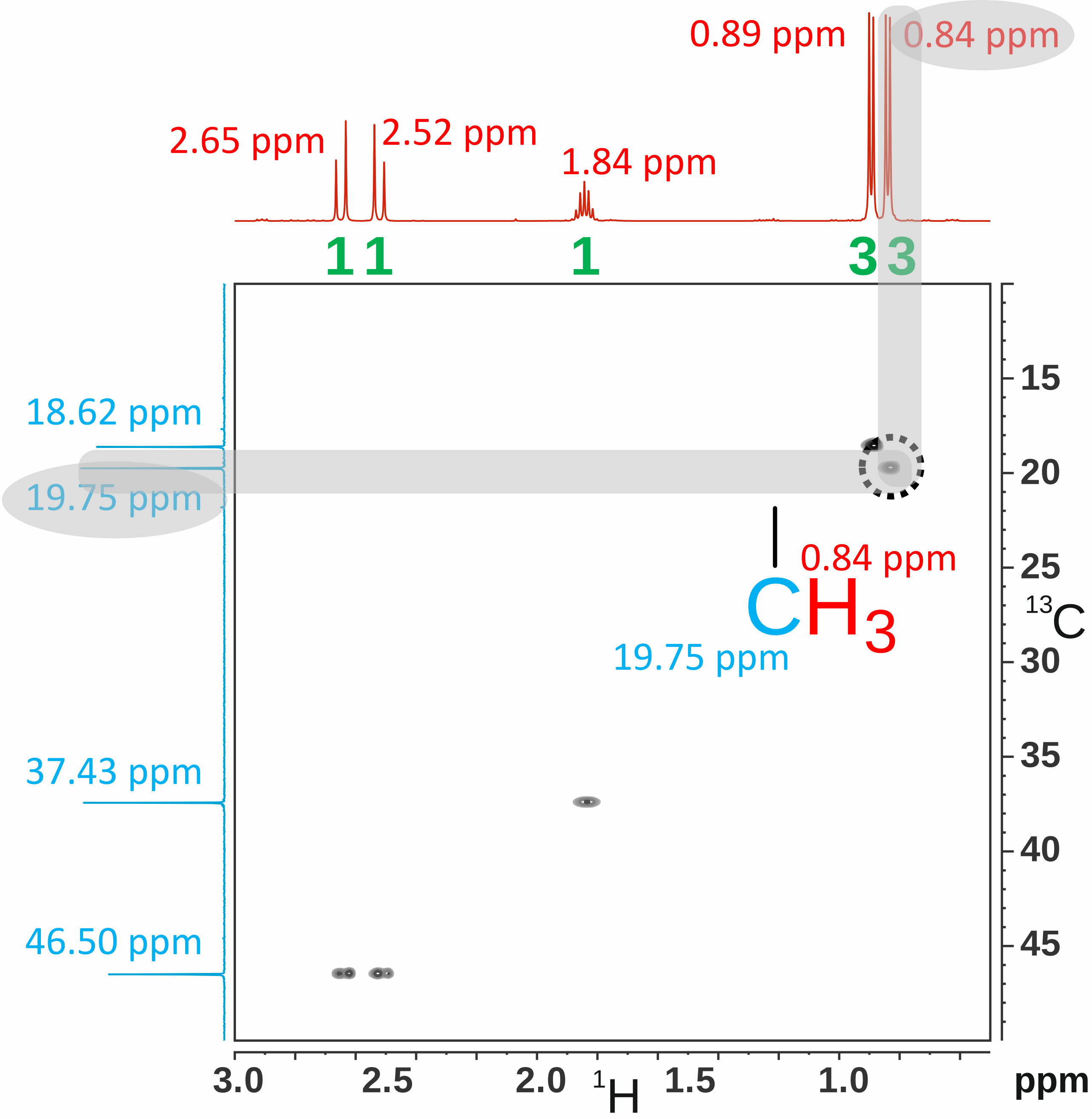
An Integral of **3** for a proton multiplet at high field almost certainly indicates a methyl group.



(taken from the one dimensional carbon spectrum)



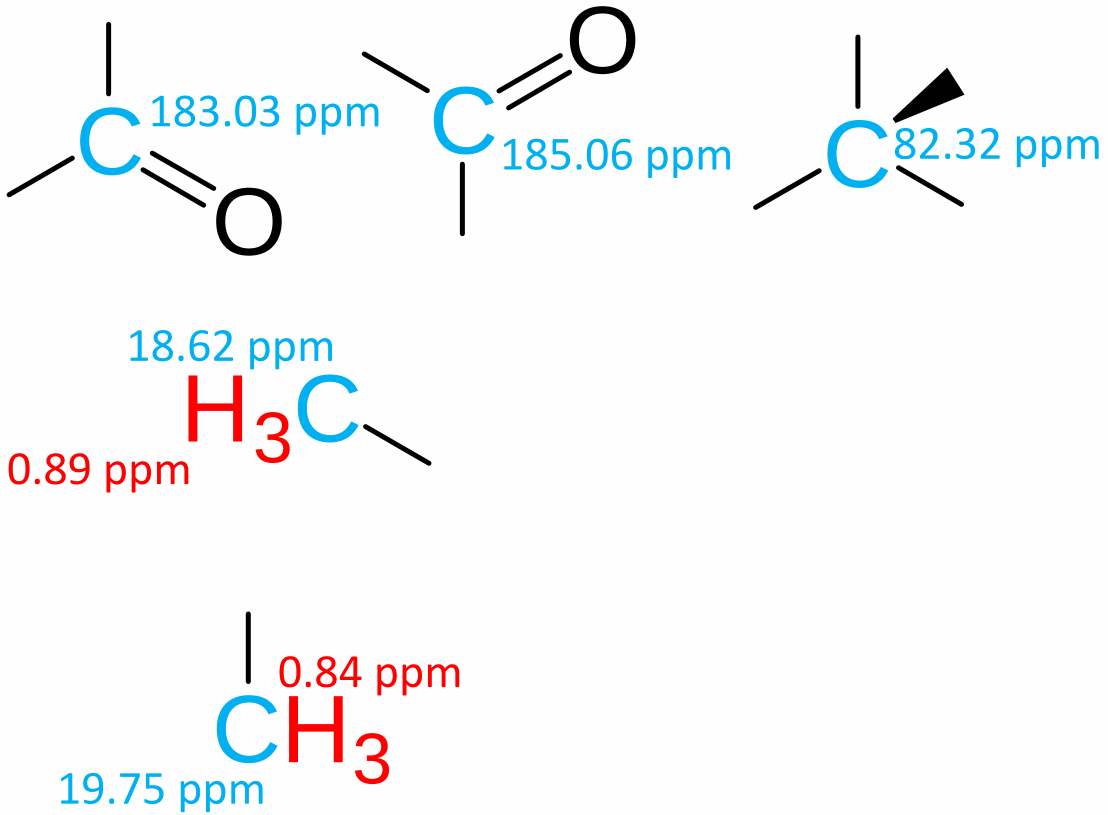
CH_3

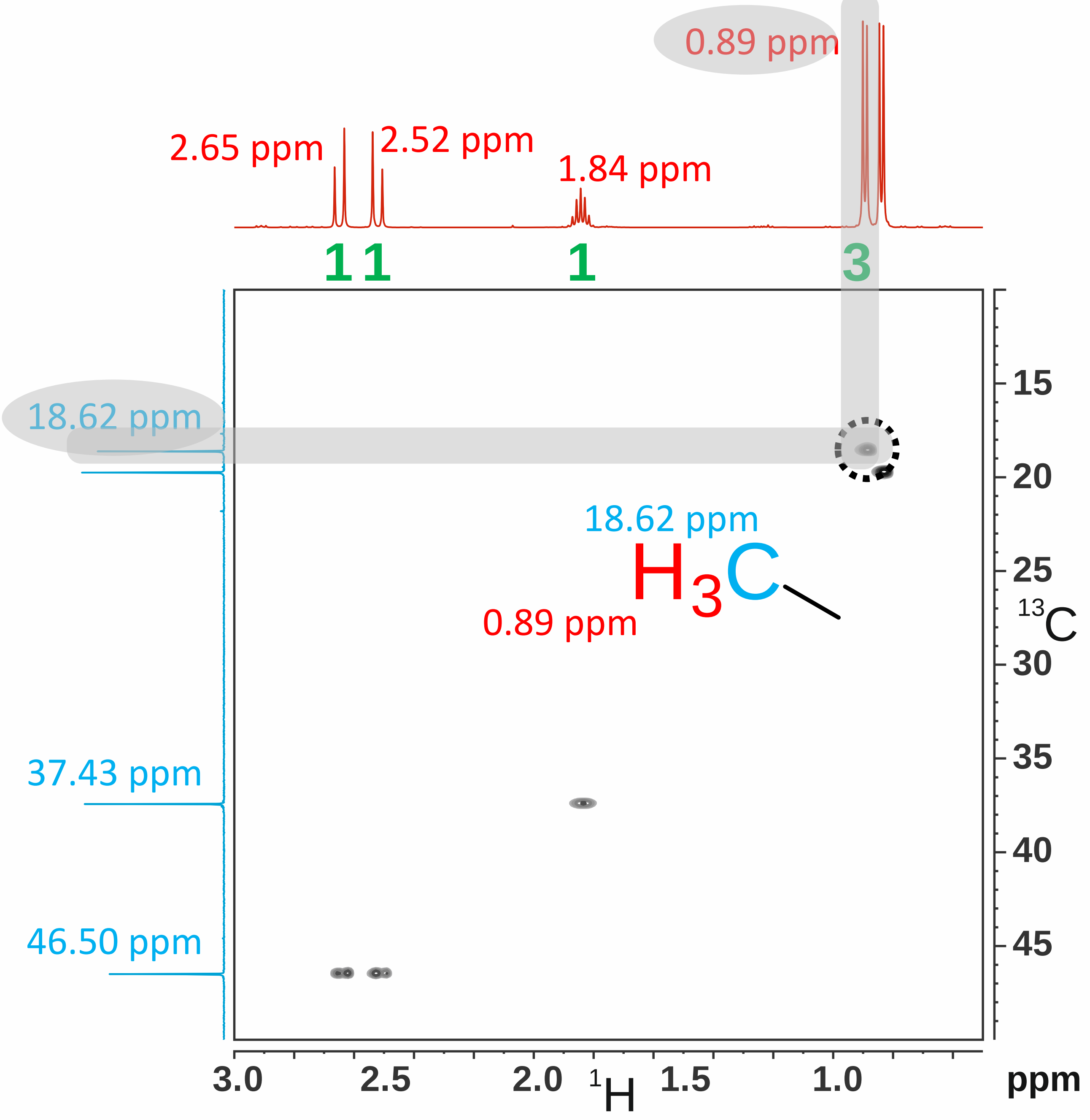


Building blocks

CH_n fragments

The cross peak of the next methyl group is close by.

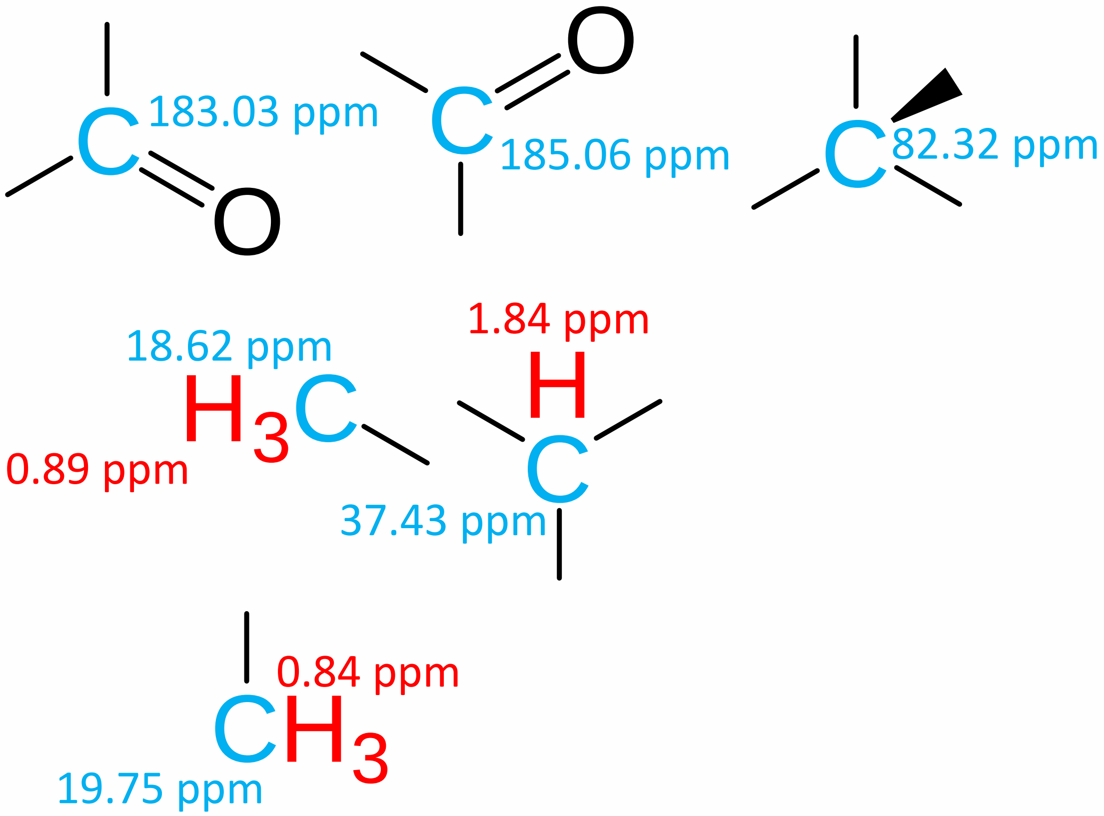


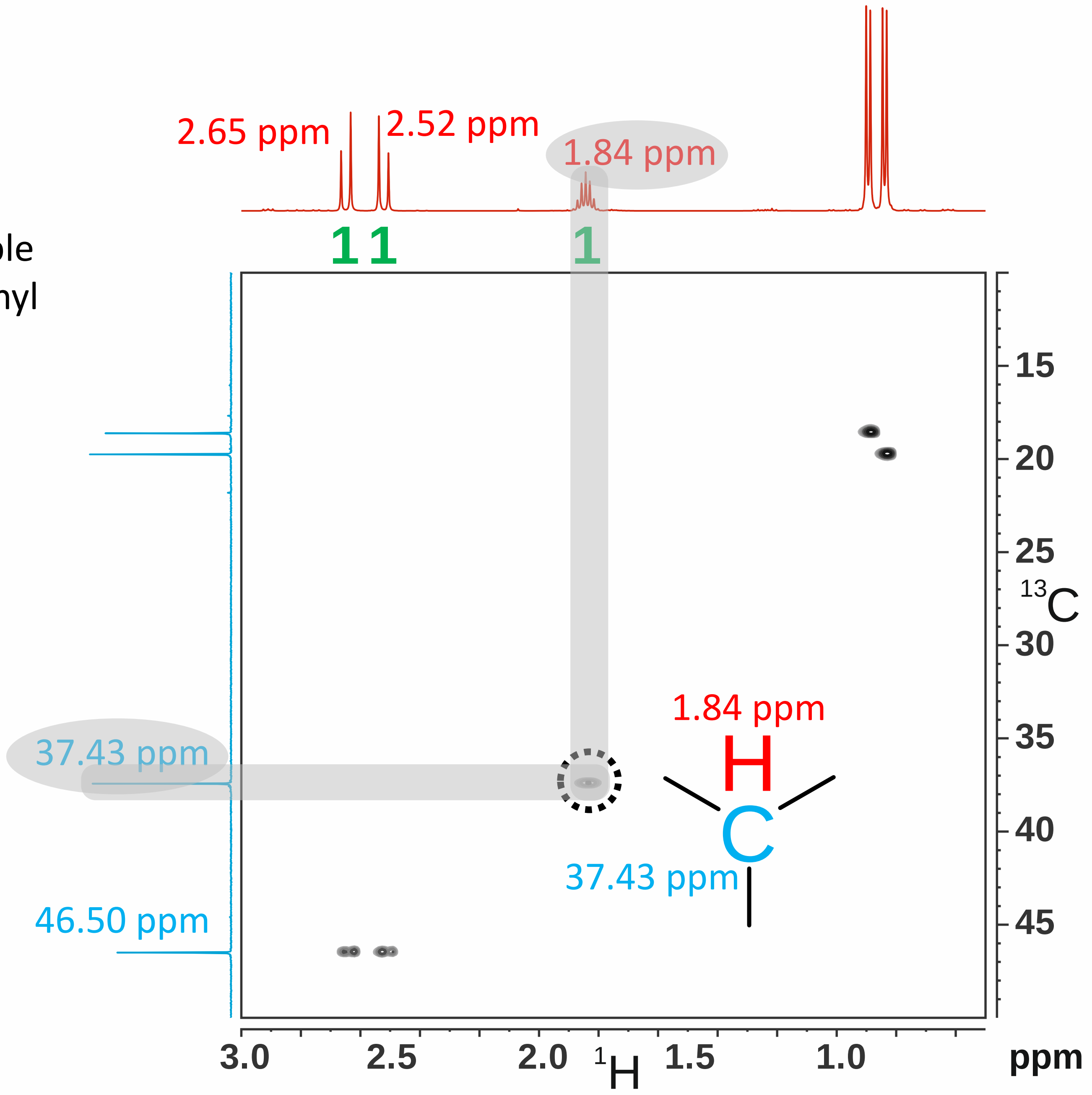


Building blocks

CH_n fragments

The next cross peak reveals a methine group. A CH with carbon in sp hybridisation can be excluded, since both double bond equivalents have already been assigned for the carbonyl groups.

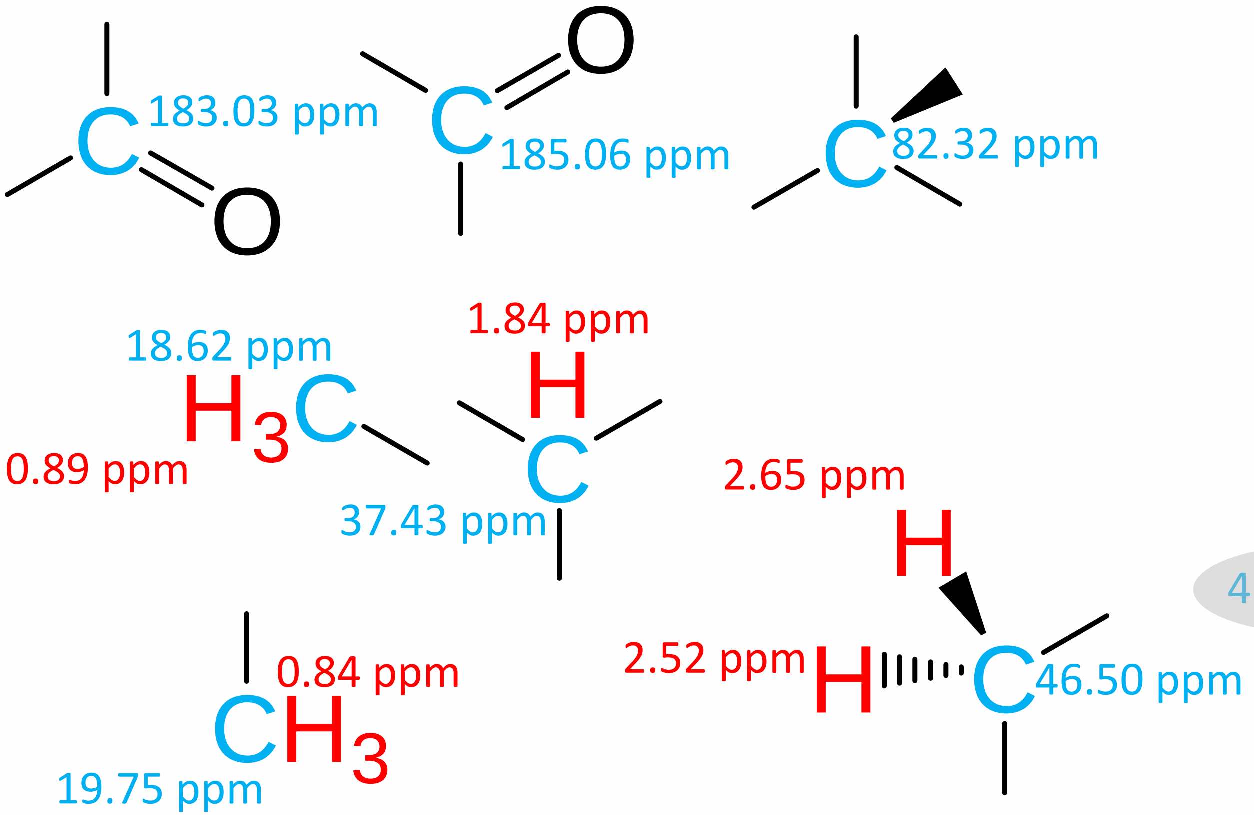


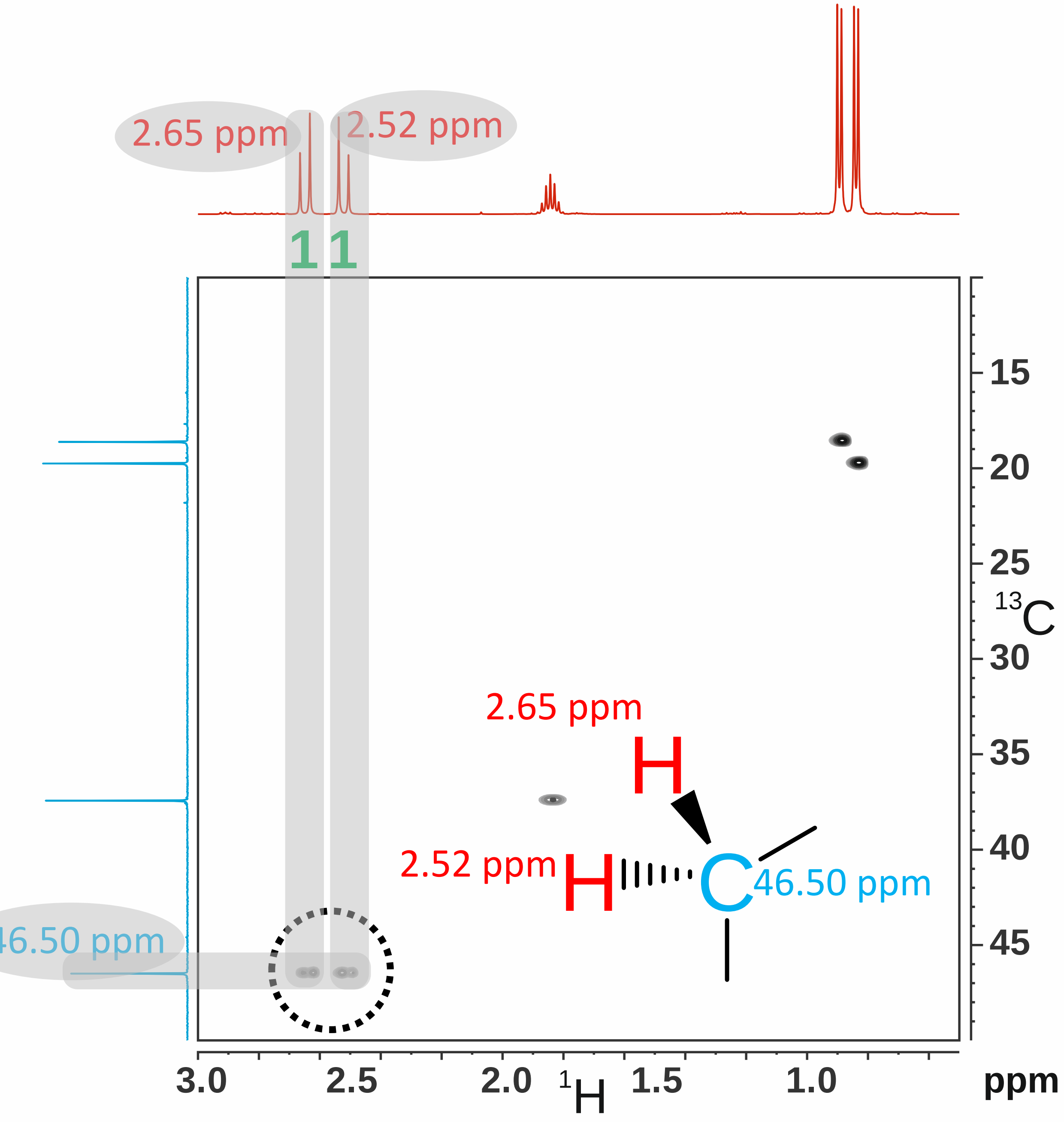


Building blocks

CH_n fragments

A CH_2 group with diastereotopic protons remains as the last CH_n fragment, which indicates that there is probably a center of chirality somewhere in the final molecule.

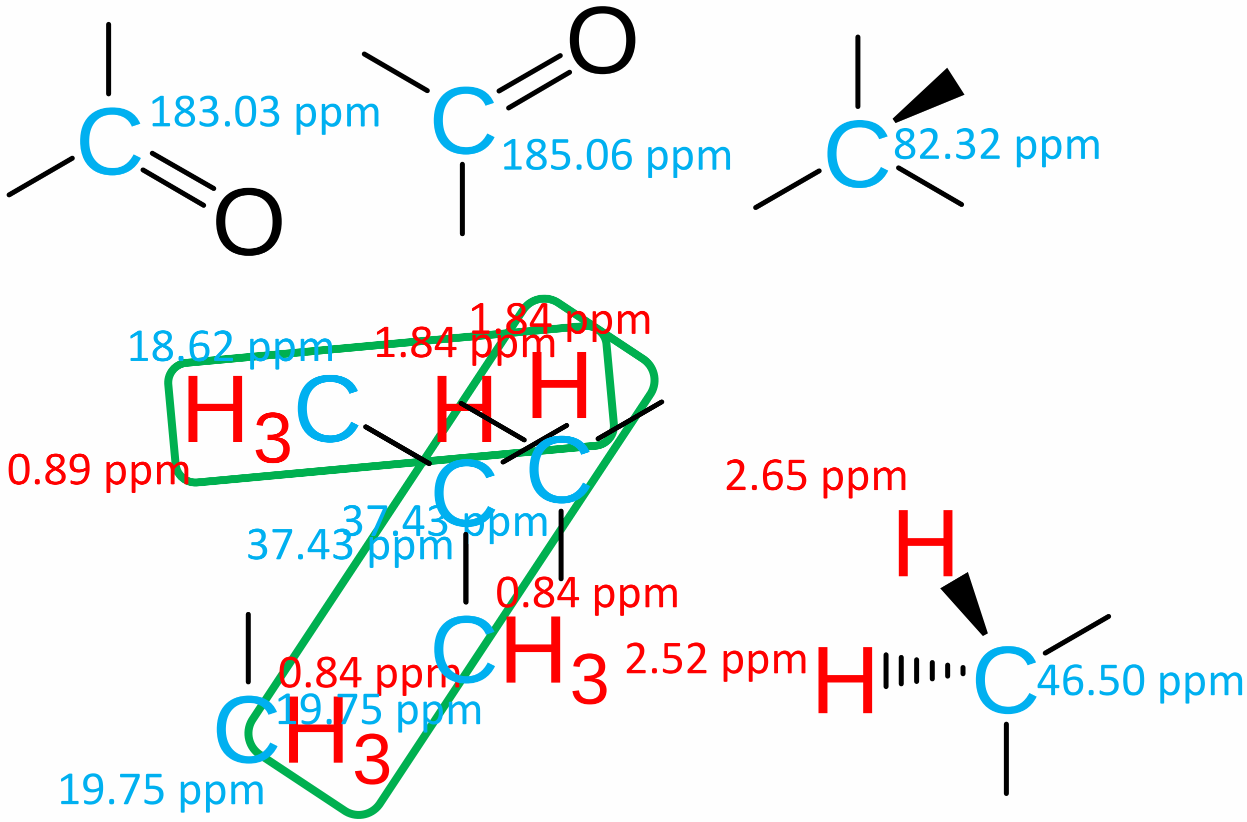


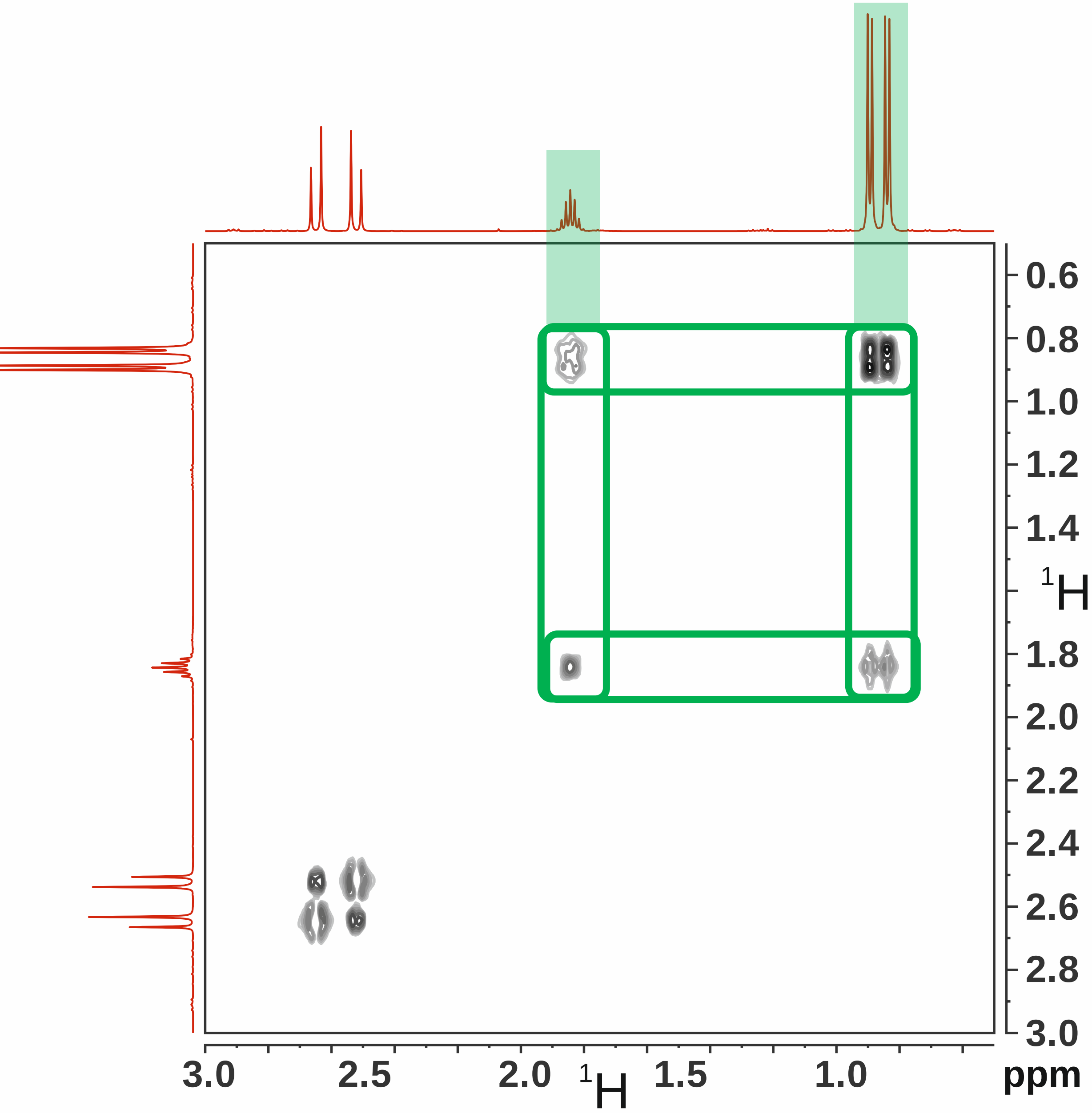


Fragment linking

Isopropyl group

The only helpful piece of information presented in the TOCSY is the link between the methine group and the two methyl groups.



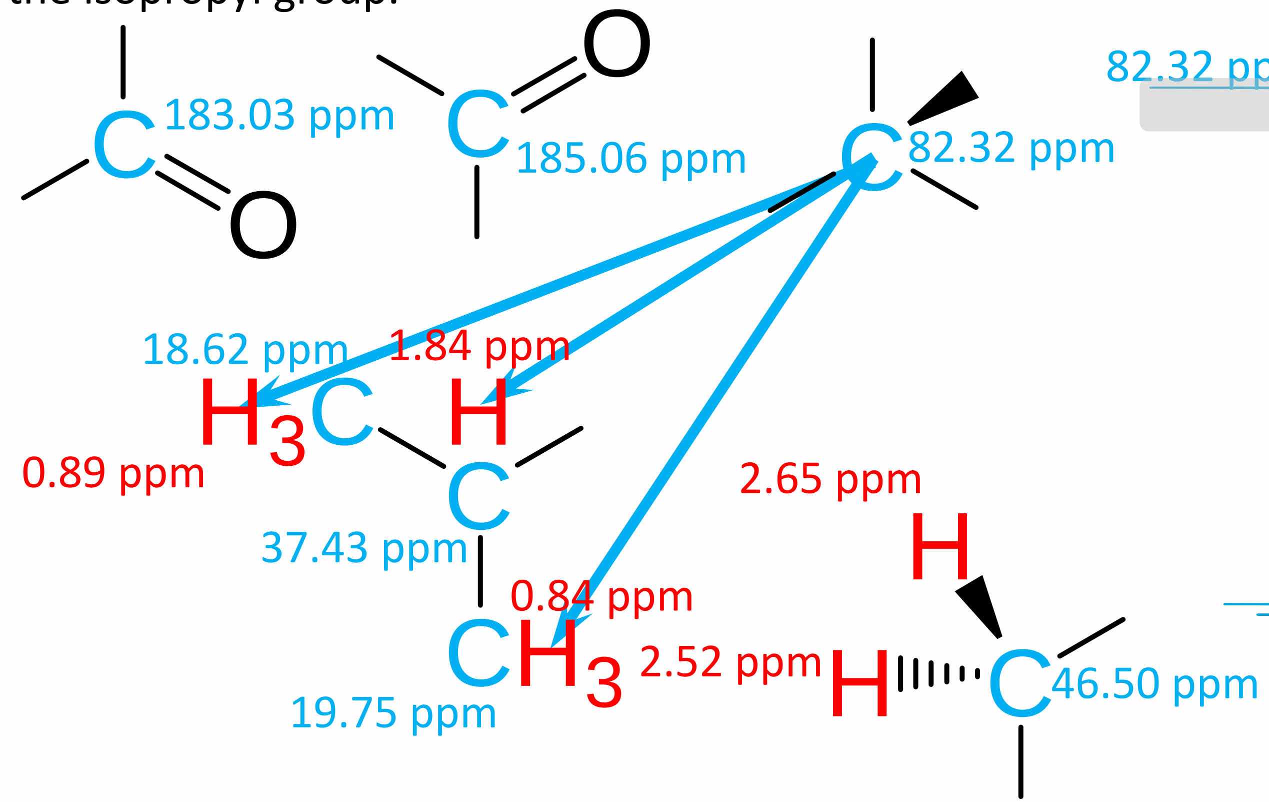


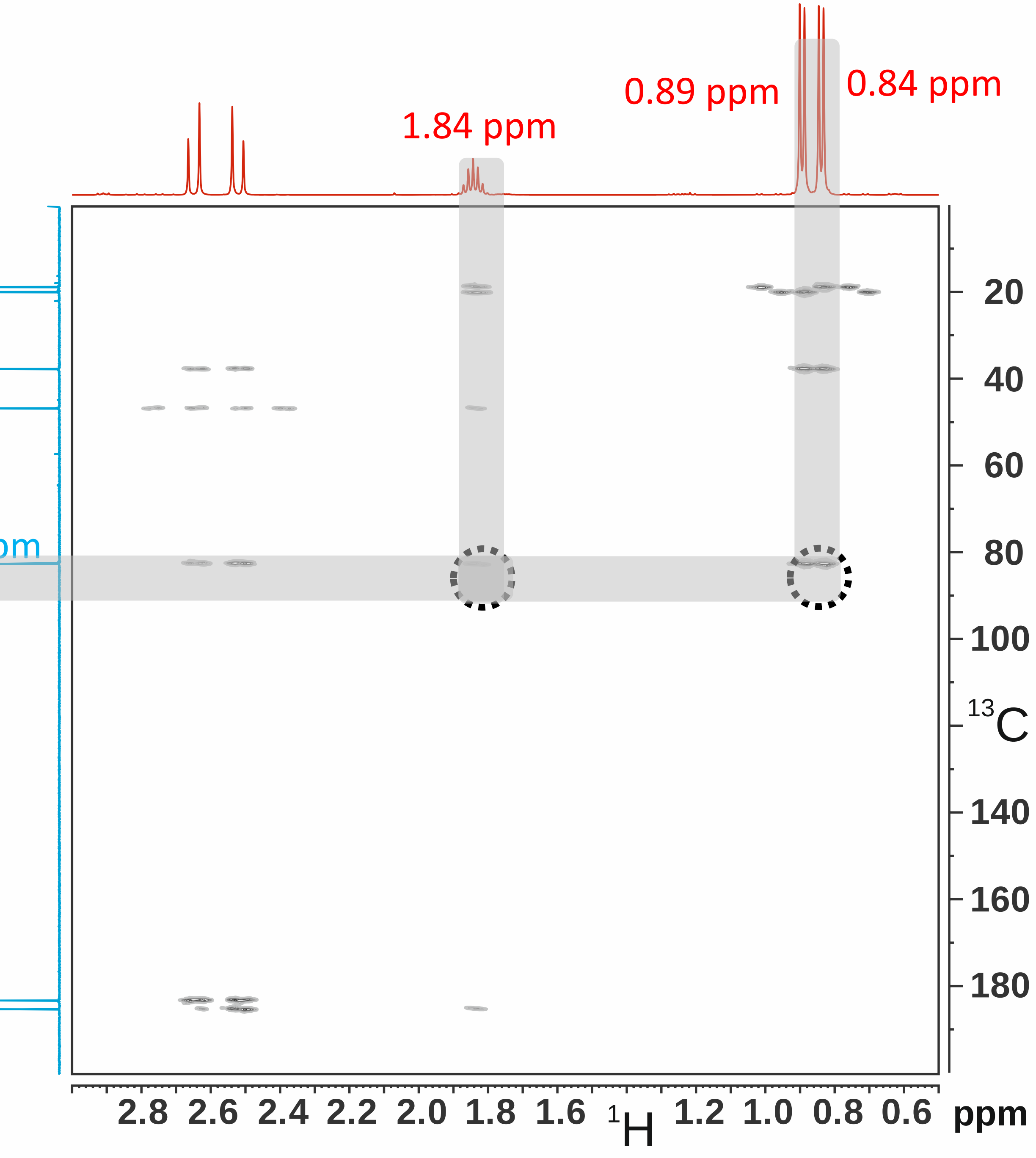
Fragment linking

Quaternary carbon atoms

Most of the remaining structural fragments have no directly attached proton (no HSQC correlation). This is where HMBC helps.

Starting from the carbon with the chemical shift of 82.32 ppm, there are two correlations to the protons of the isopropyl group.

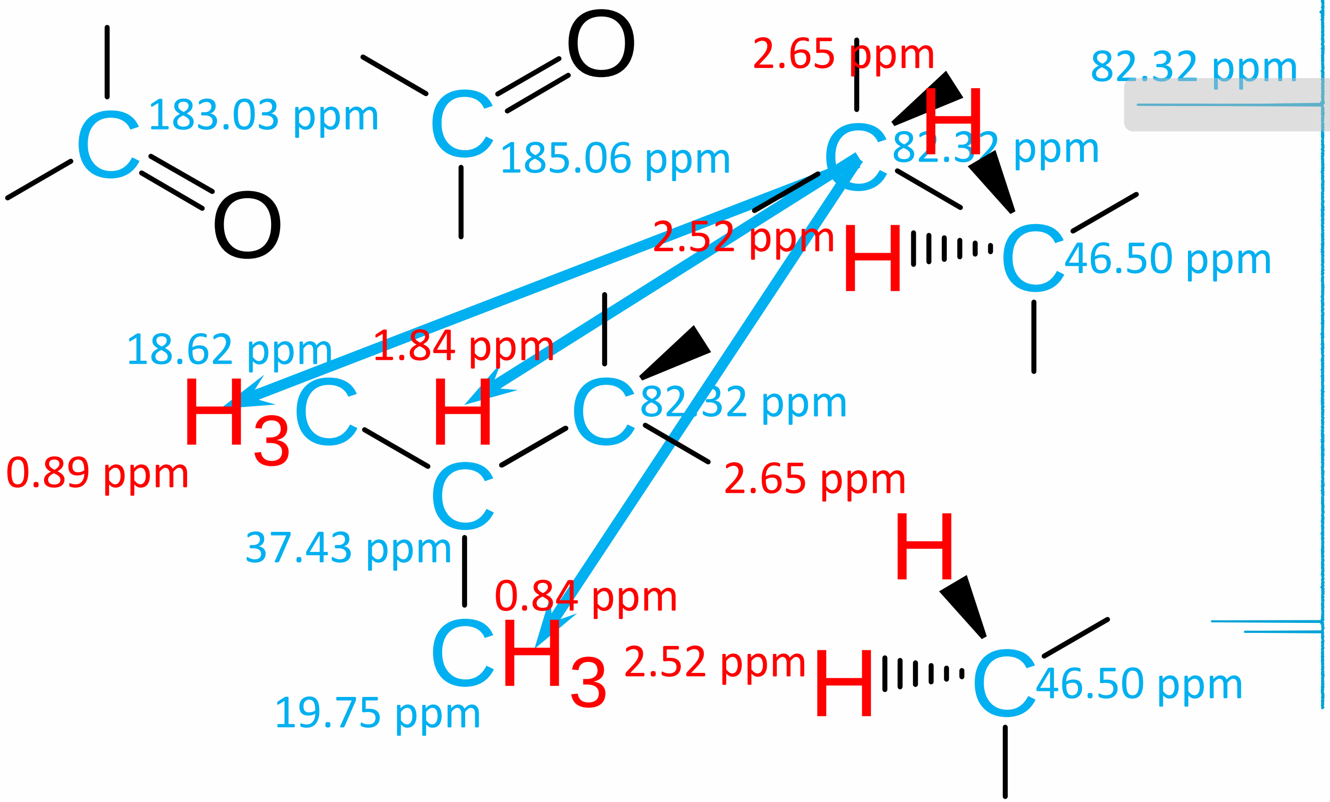


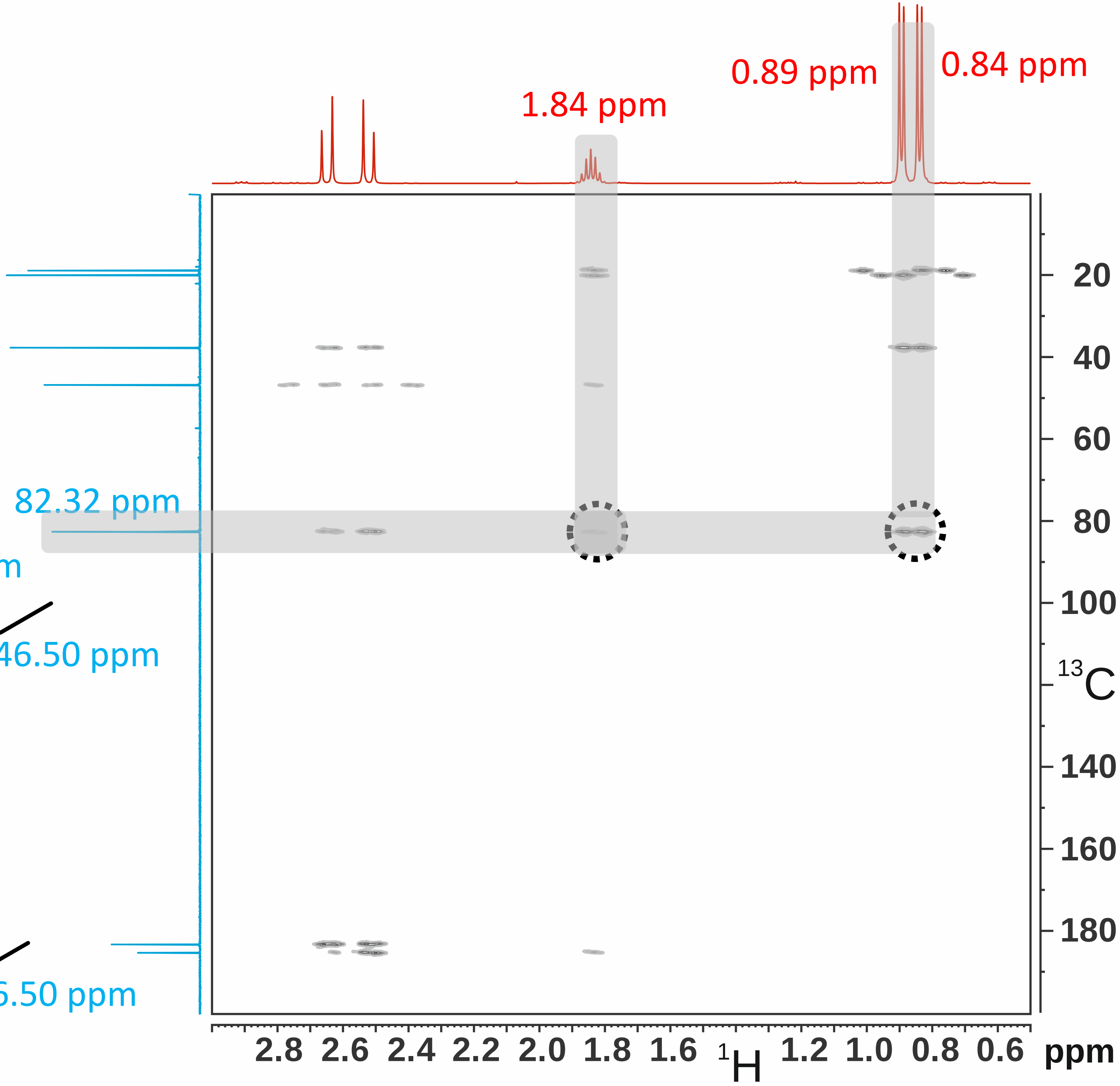


Fragment linking

Quaternary carbon atoms

Assuming we only see coupling pathways over a maximum of three bonds, there is only one arrangement possibility for the quaternary carbon atom.

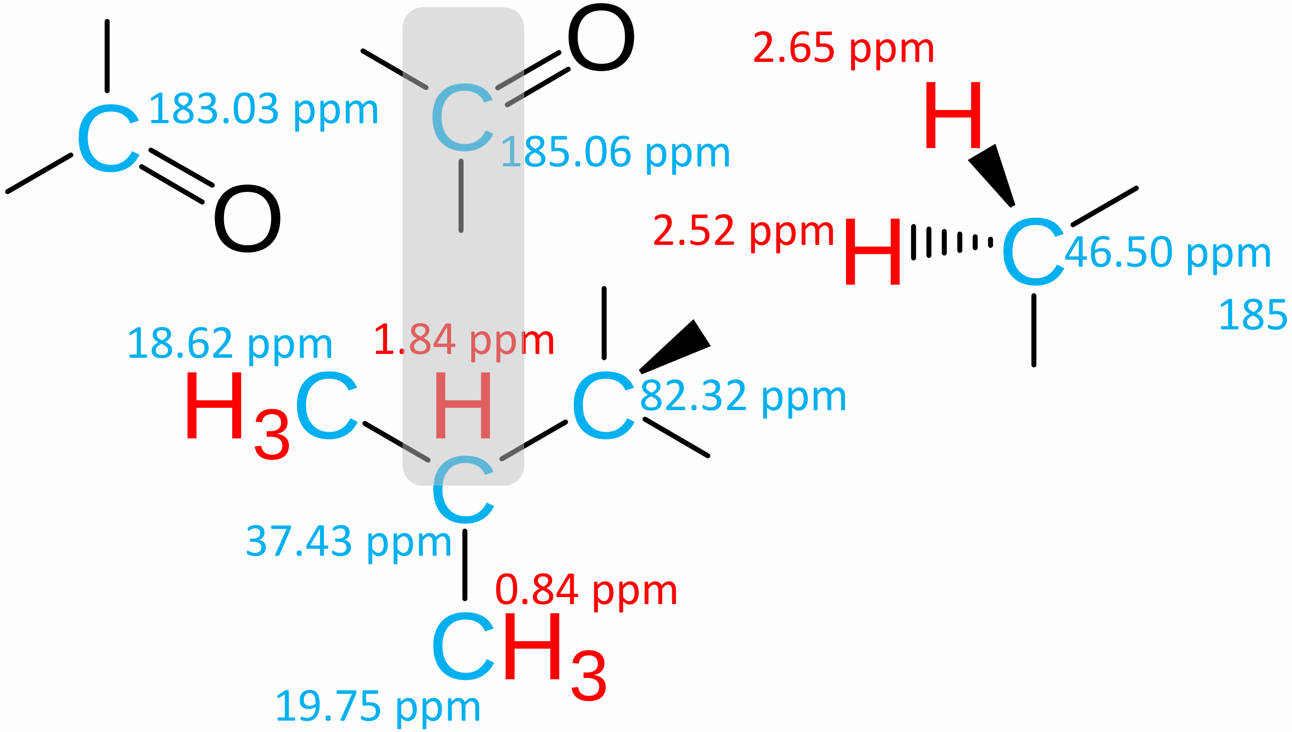


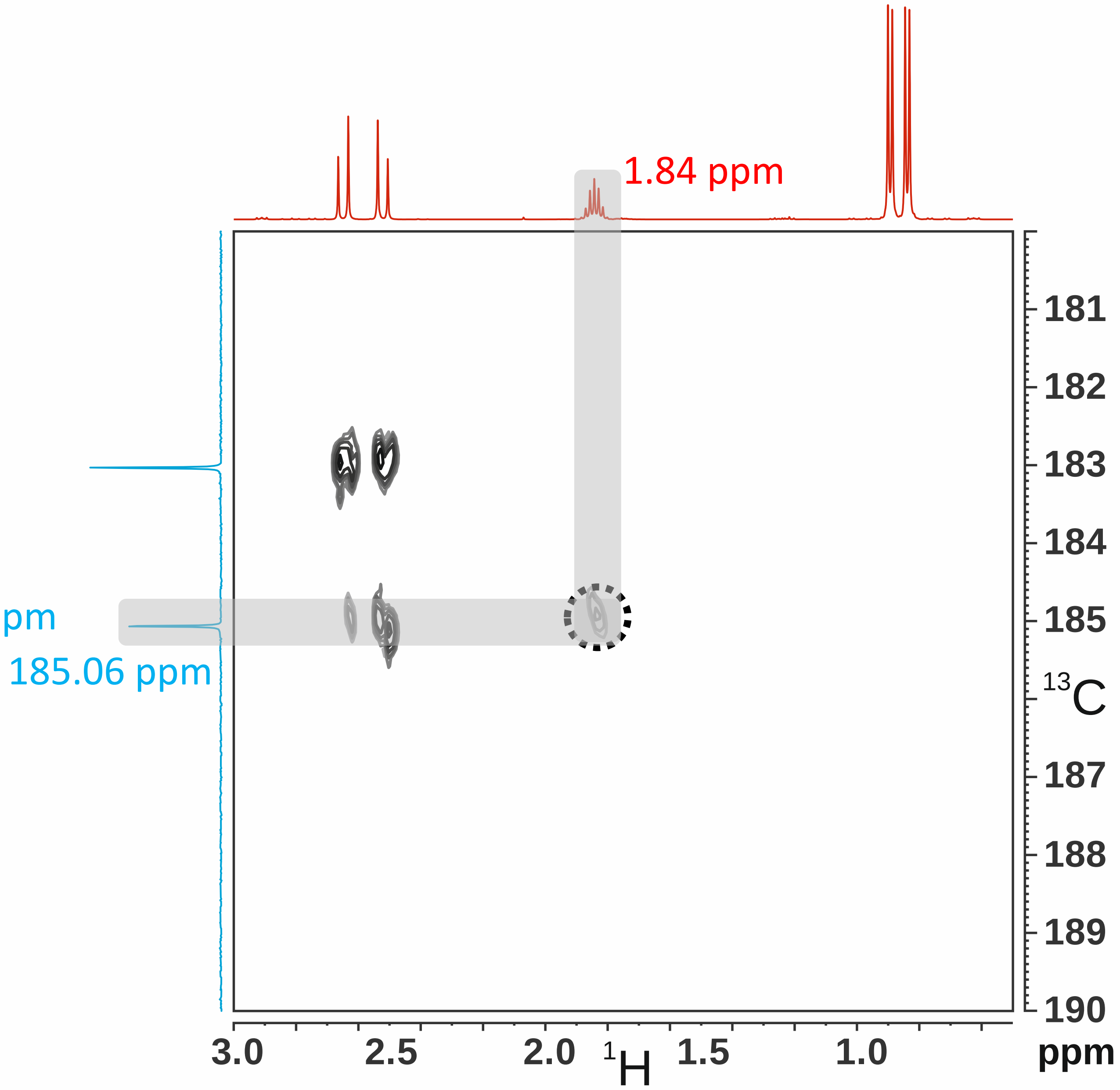


Fragment linking

First carbonyl group

Starting from the carbonyl carbon atom with the chemical shift of 185.06 ppm, there is one correlation to the methine proton at 1.84 ppm visible.

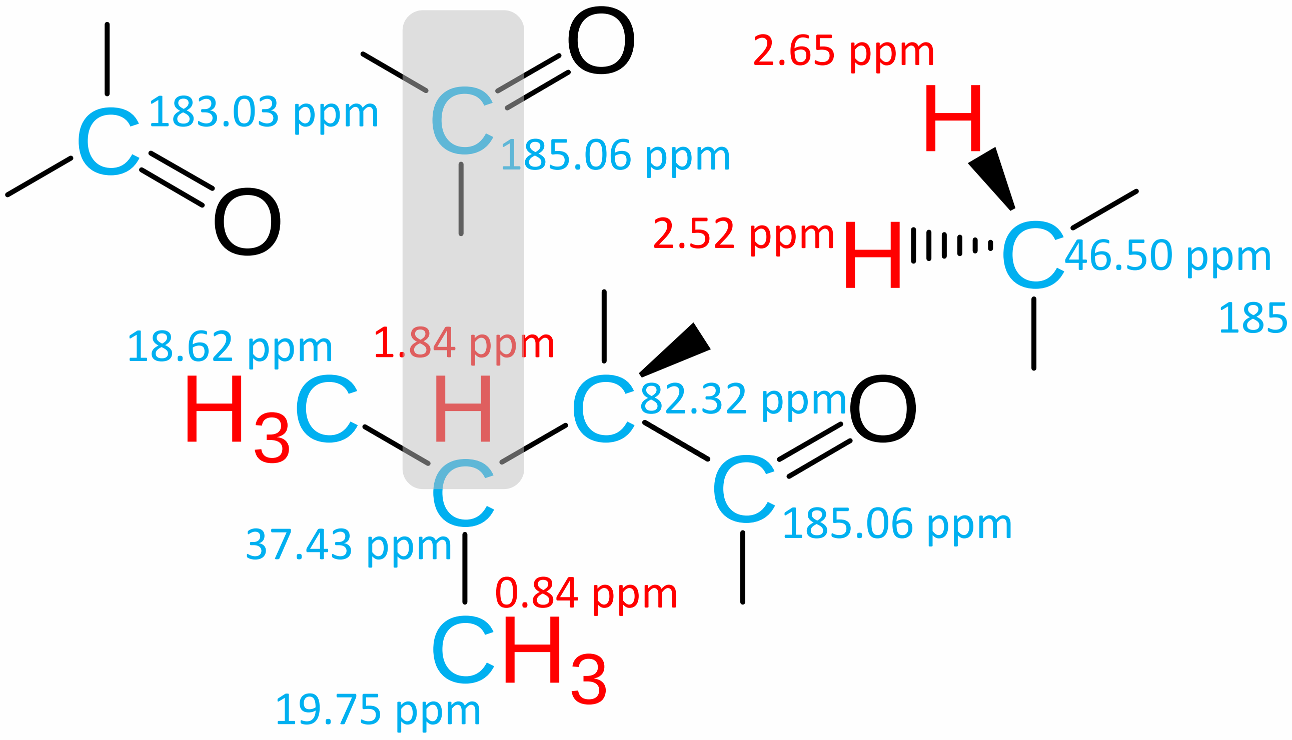


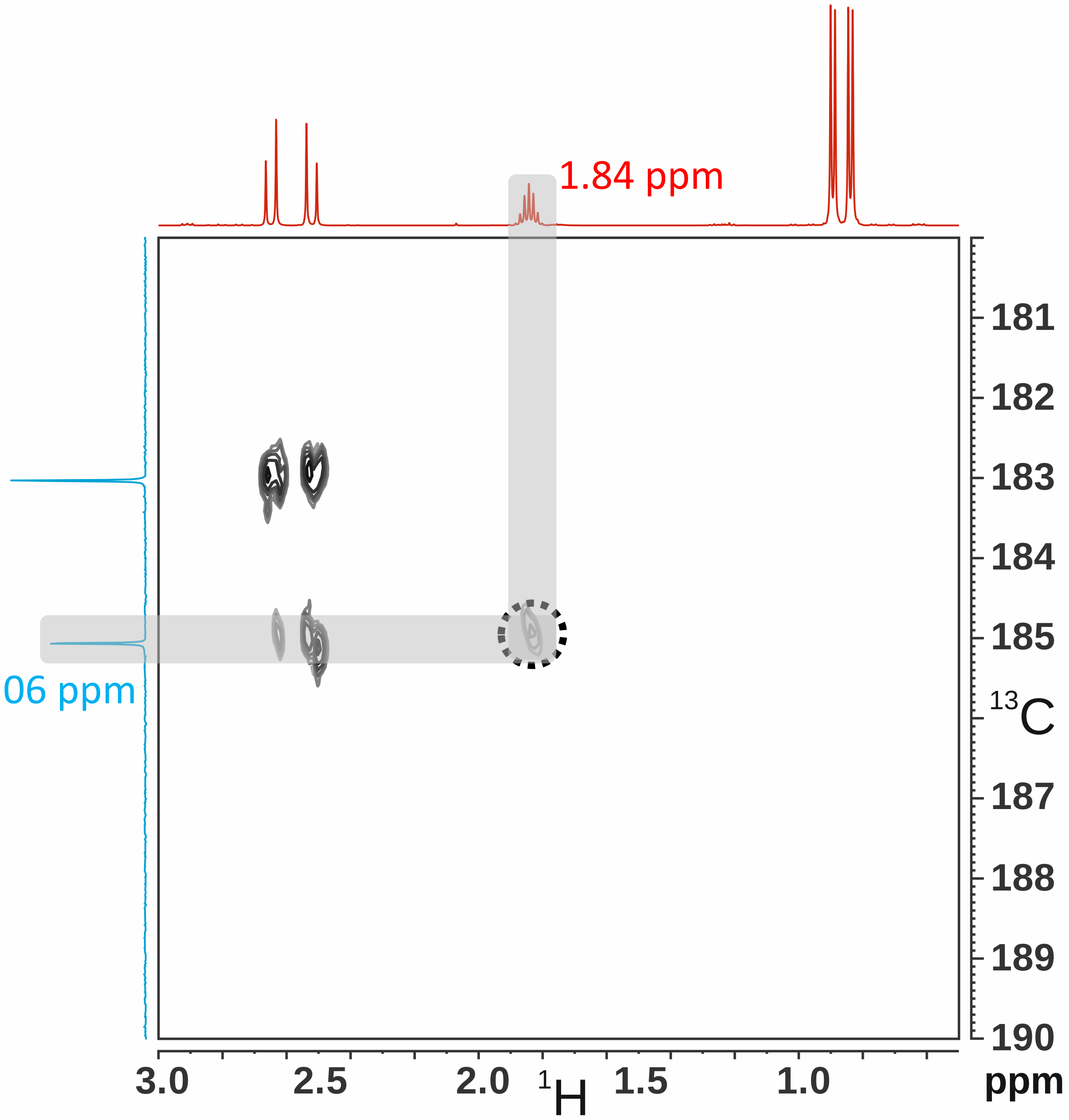


Fragment linking

First carbonyl group

A coupling pathway via a maximum of three bonds is only possible if the carbonyl group is directly bound to the quaternary C at 82.32 ppm.

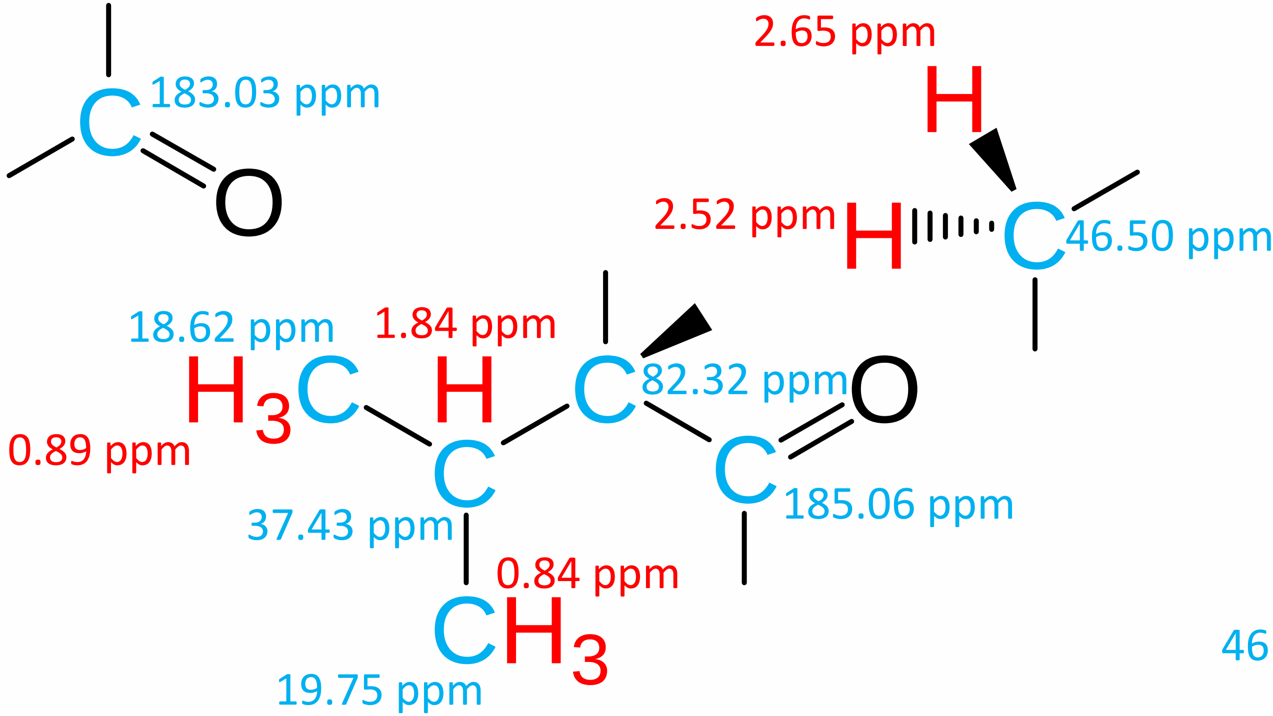


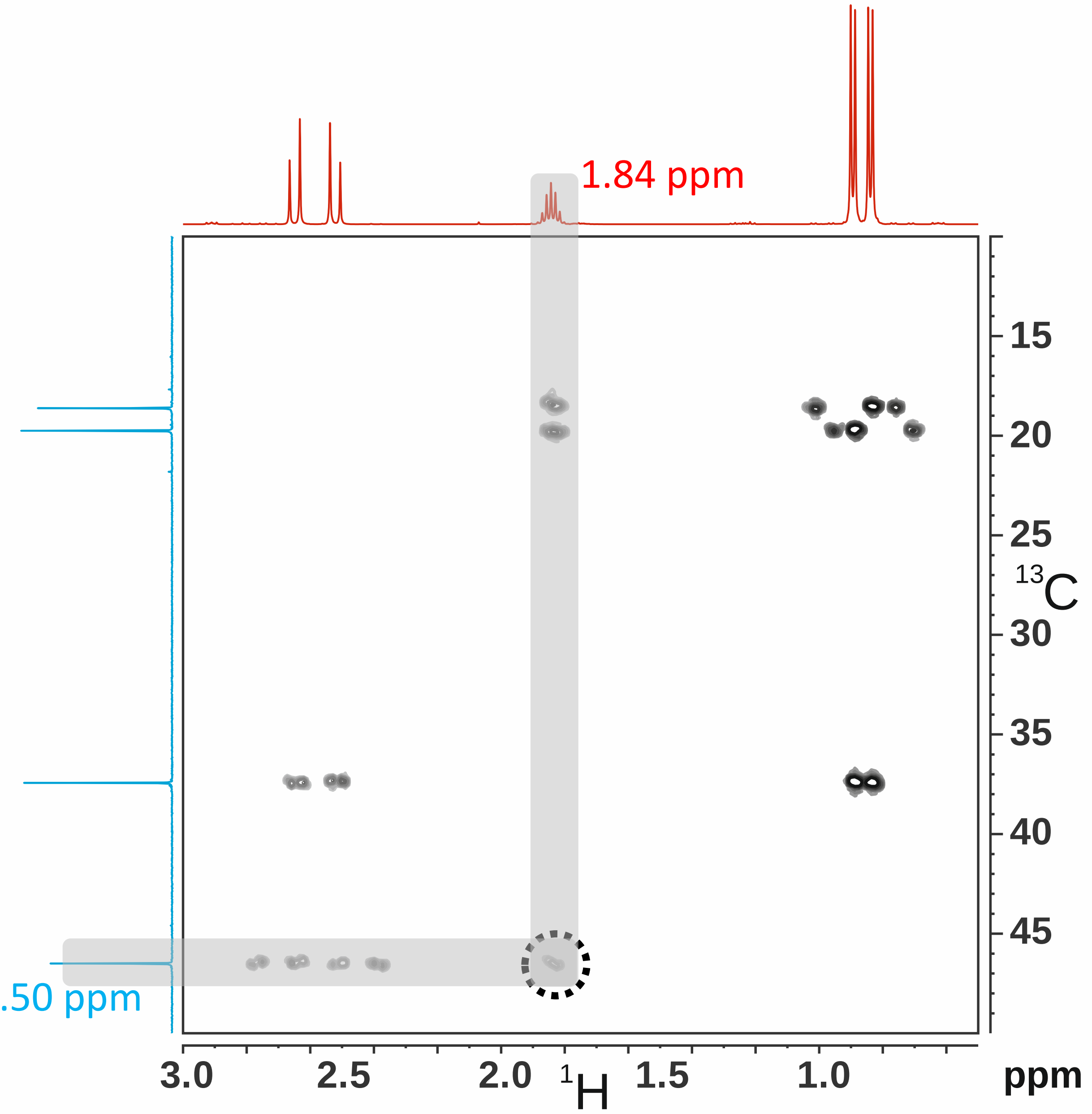


Fragment linking

Methylene group

Starting from the carbon of the methylene group with the chemical shift of 46.50 ppm, there is also a correlation to the methine proton at 1.84 ppm.

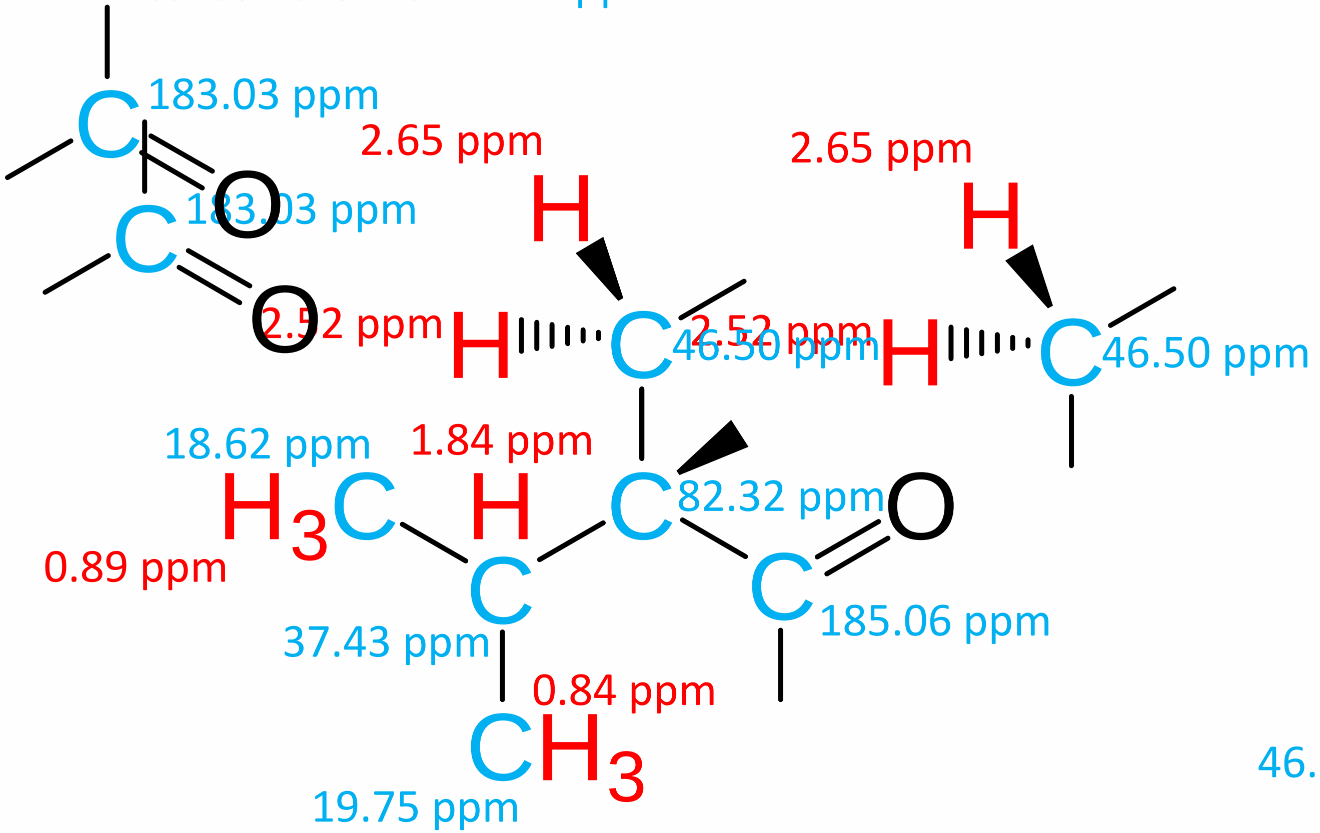


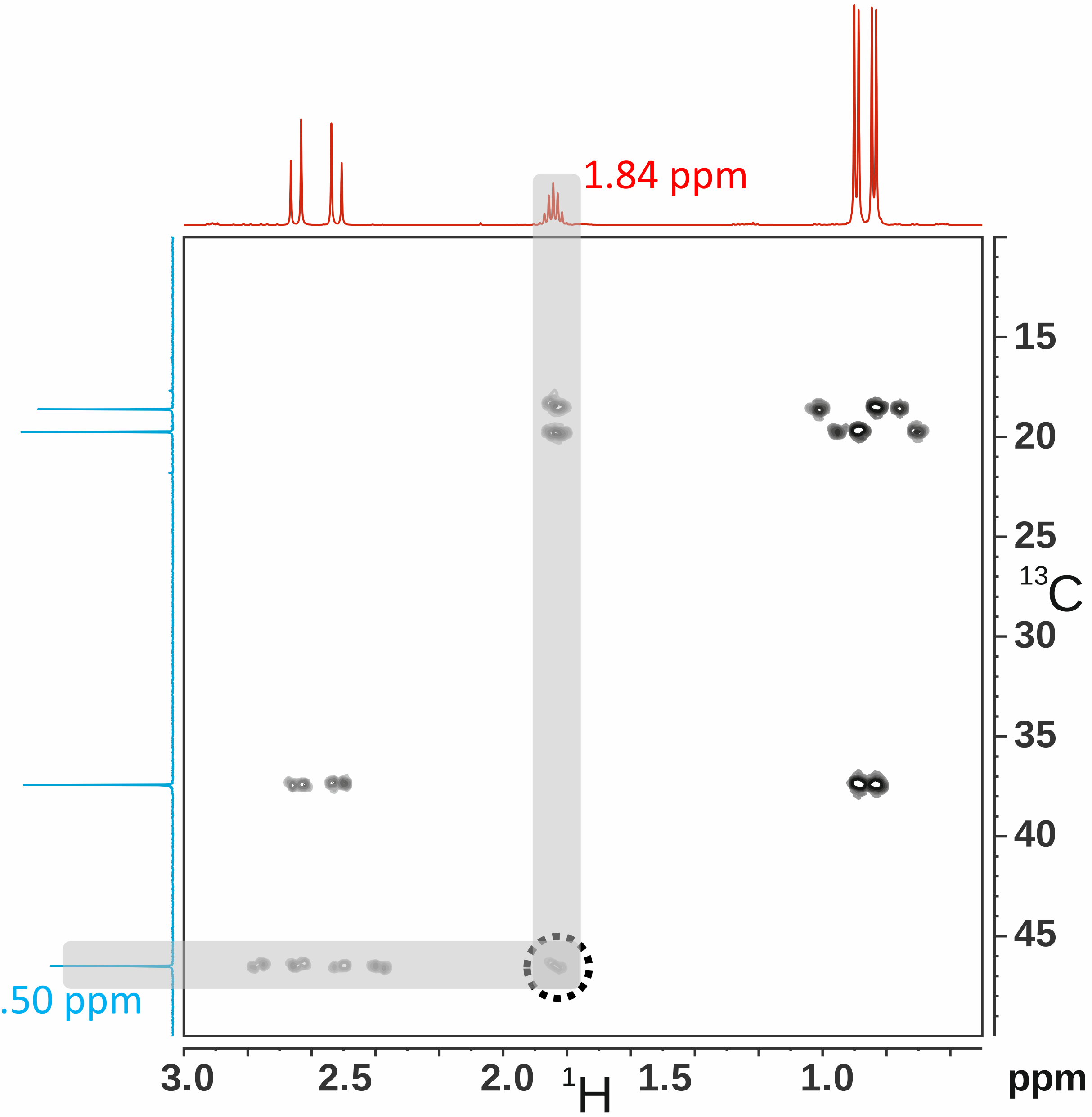


Fragment linking

Methylene group

Assuming again that the visibility of a coupling pathway is over a maximum of three bonds, the methylene group must also be directly bound to the quaternary carbon atom at 82.32 ppm.

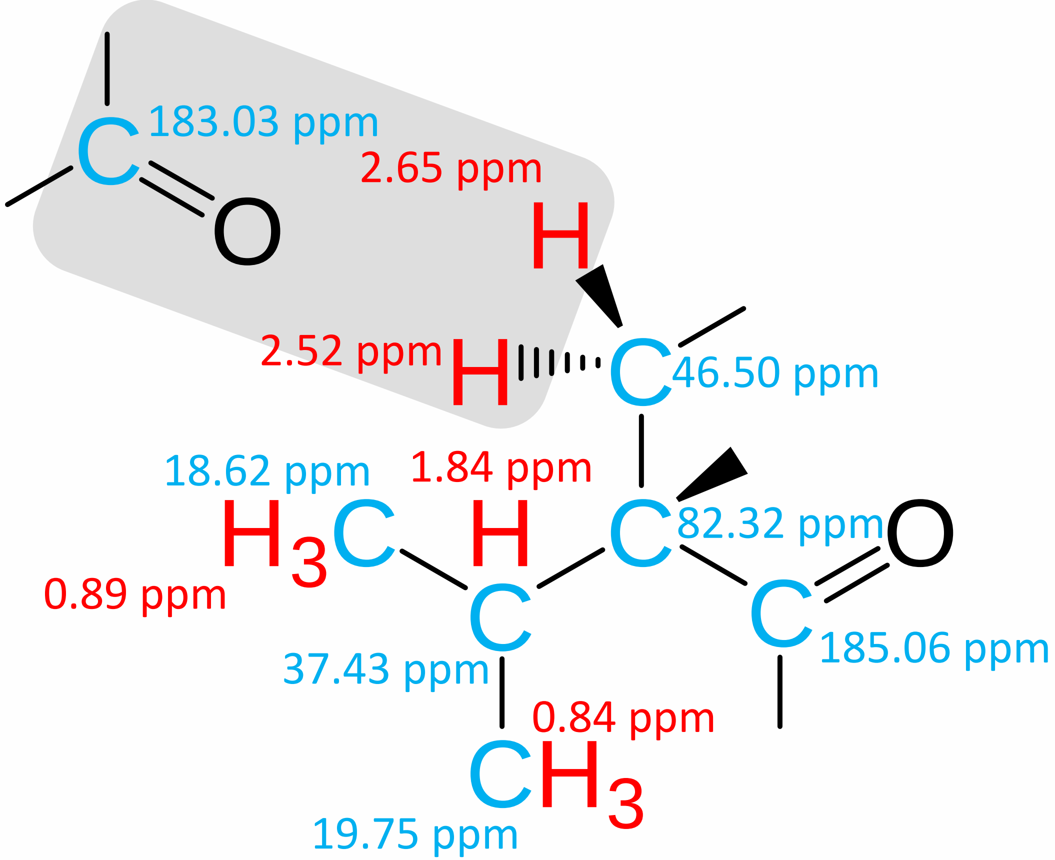


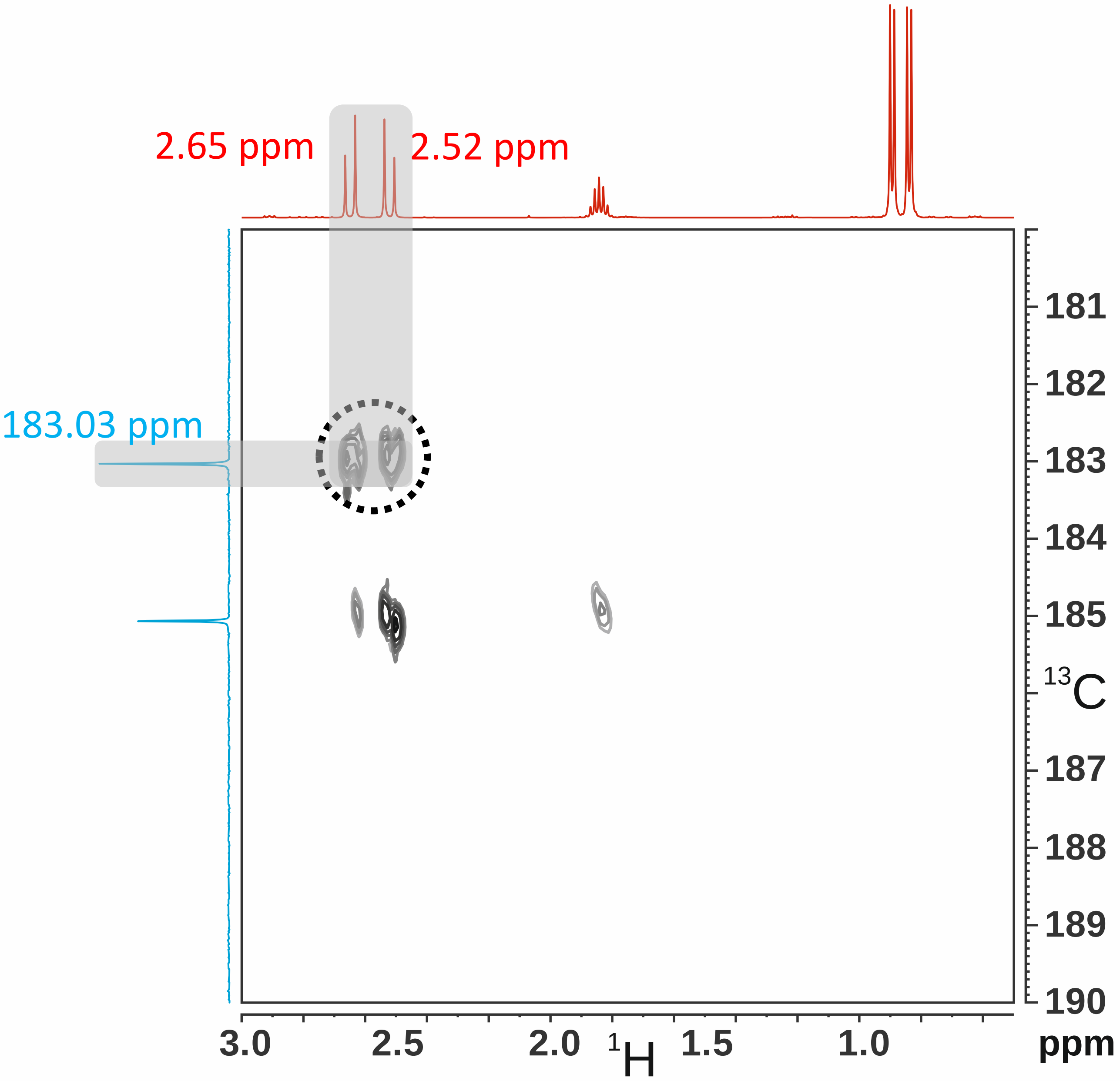


Fragment linking

Second carbonyl group

Starting from the carbonyl carbon at 183.03 ppm, only correlations to the protons at 2.52/2.65 ppm are visible.

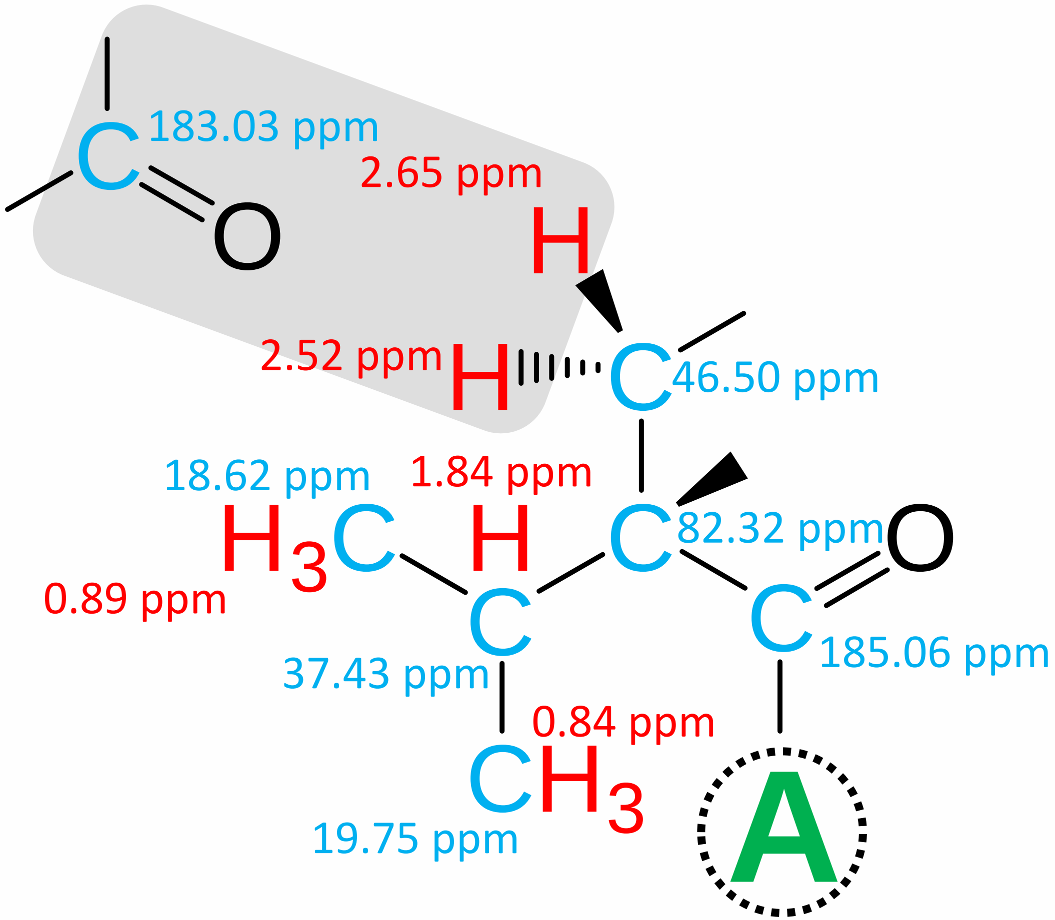


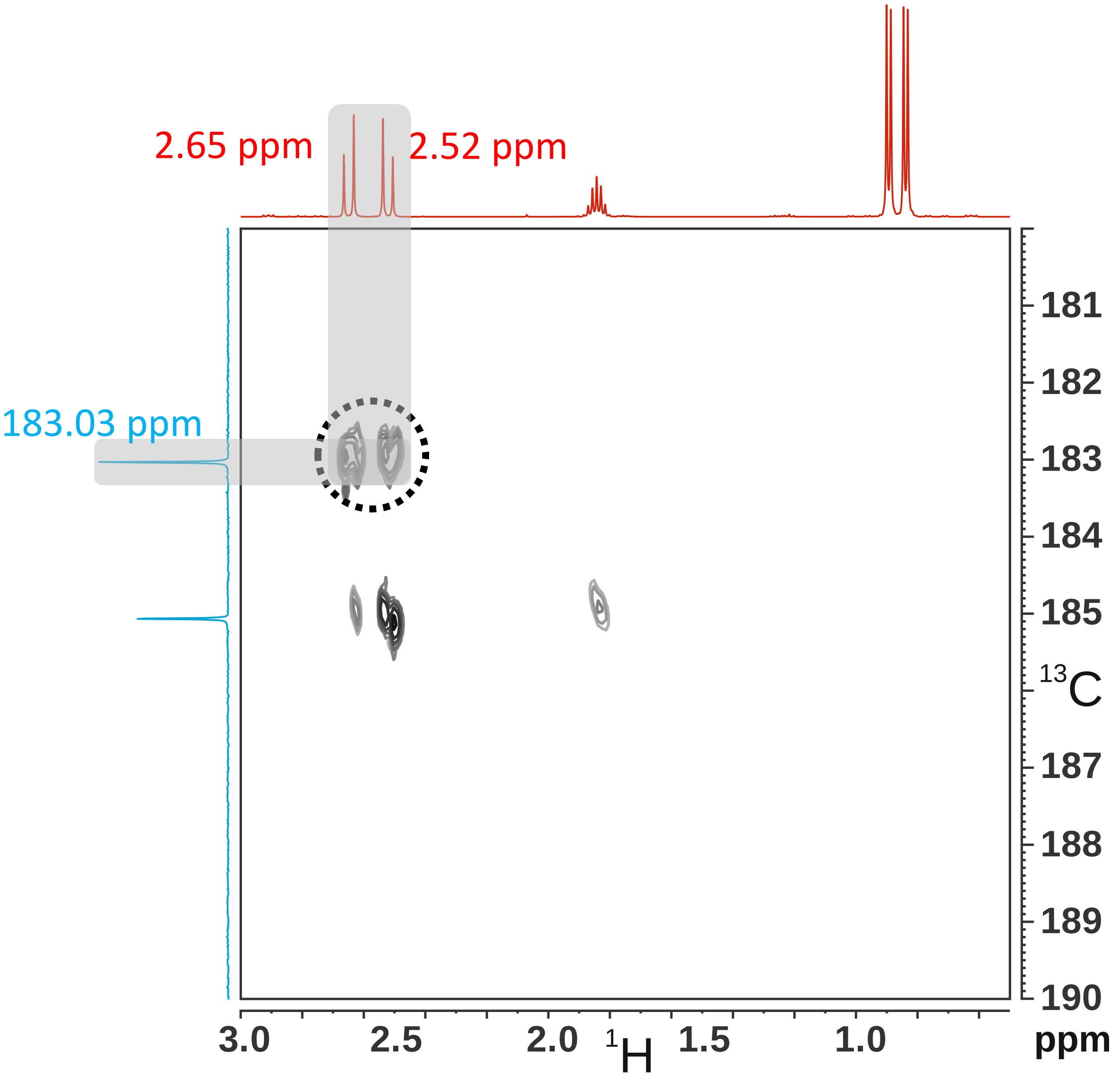


Fragment linking

Second carbonyl group

A carbonyl group in position **A** would require a coupling pathway across four bonds. This is unlikely.

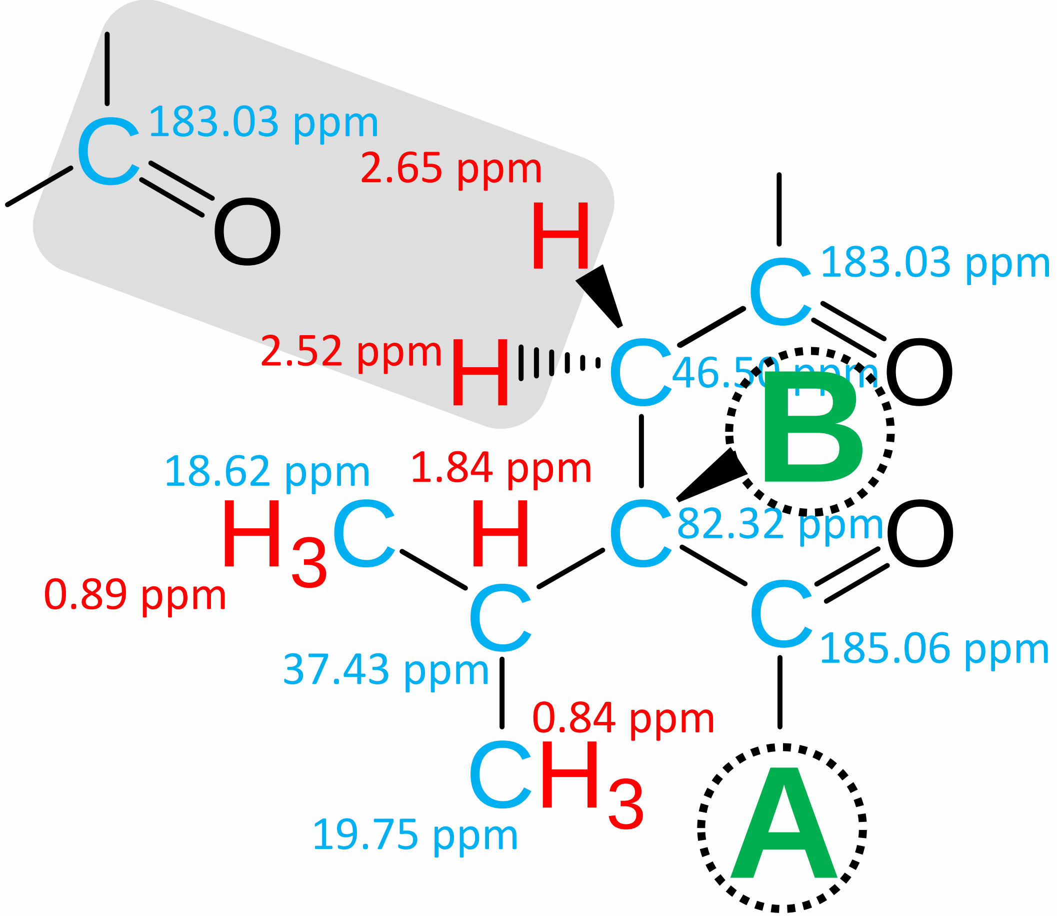


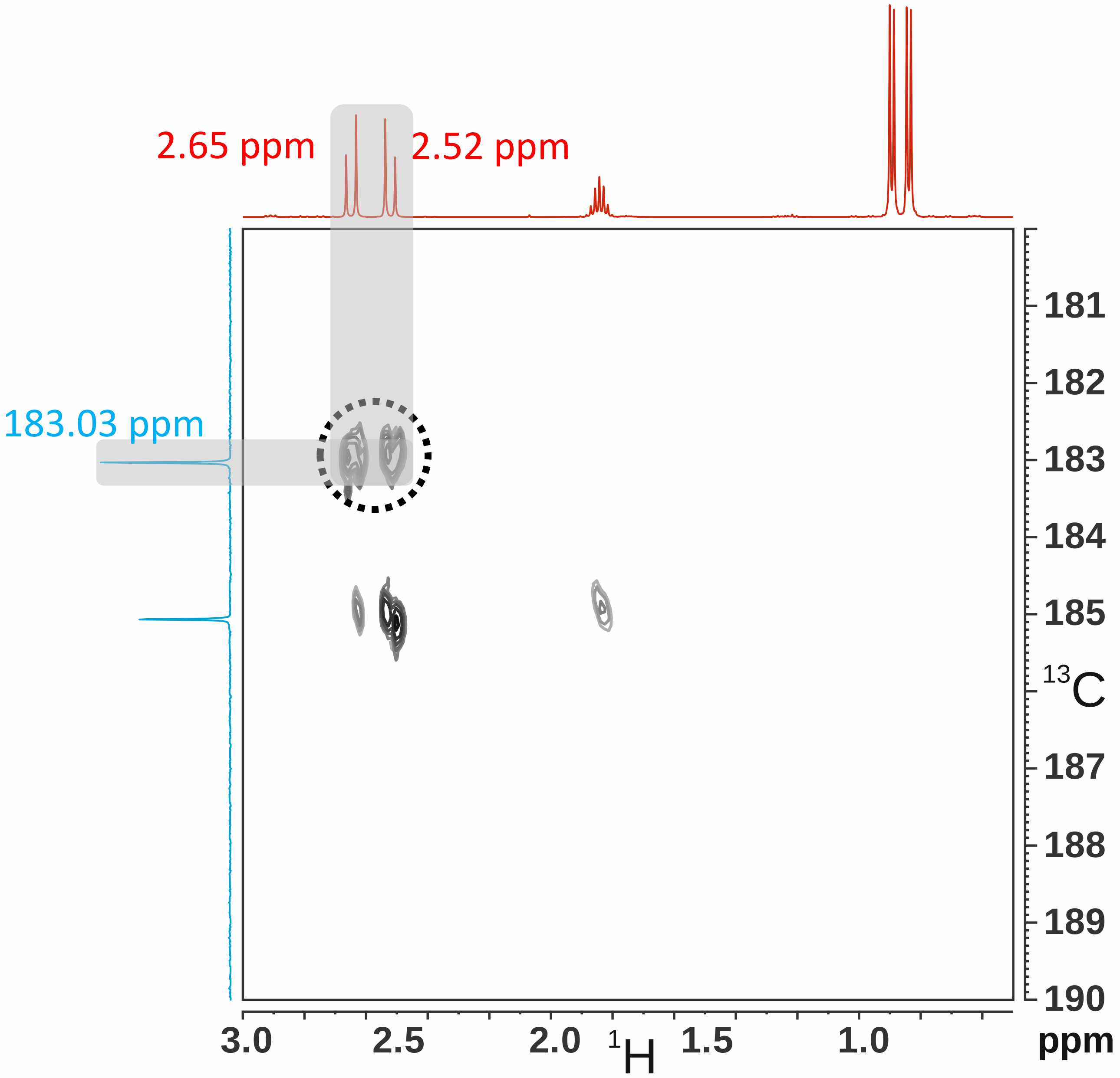


Fragment linking

Second carbonyl group

In position **B**, one would additionally expect a cross peak to the proton at 1.84 ppm. Which means, there is only one remaining possibility.

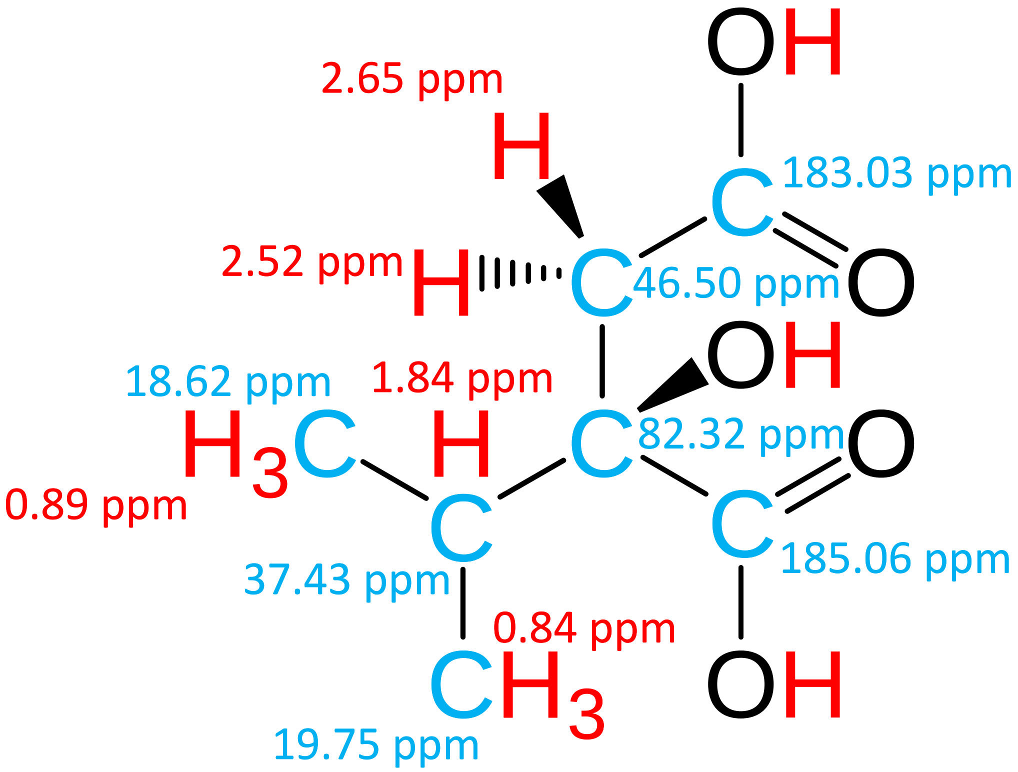




Fragment linking

Hydroxyl groups

The structural fragment has three open bonds, and at the same time the three OH groups found during the integration still have to be positioned. Finally we get:



Some additional remarks concerning HMBC

The previous slide contains an assumption that is hard to justify. The absence of an expected cross peak in the HMBC is actually **no evidence at all**. The transfer function, which describes the intensity of the cross peaks as a function of time, has zero crossings.

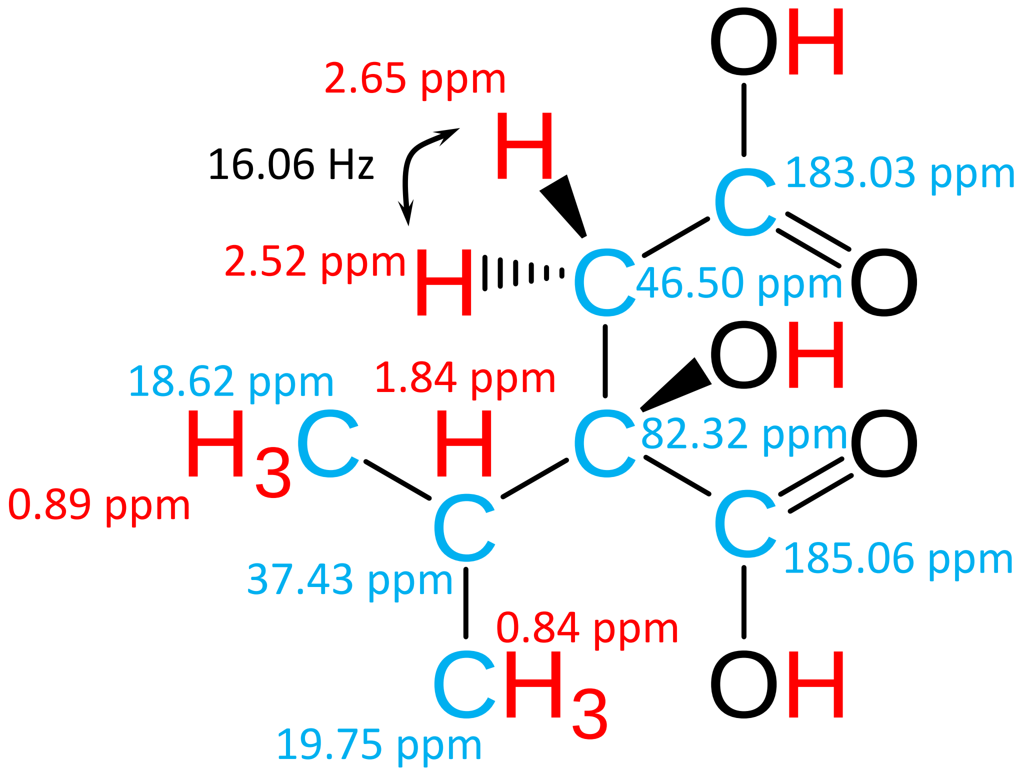
The adjustable experimental parameter "evolution time" could coincidentally hit such a zero crossing.

With these thoughts in mind, there would definitely be a few more ways to combine the fragments, but they will not all be derived here.

The solution presented here is the simplest available.

Multiplets

Methylene group



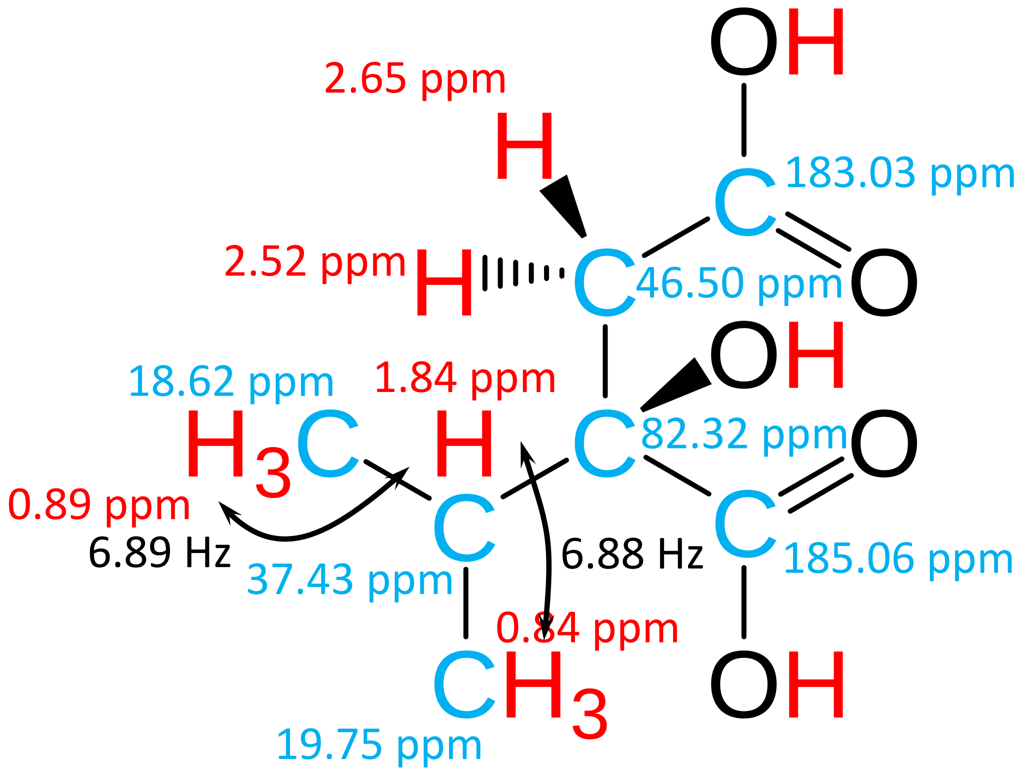
Because of the center of chirality at the carbon with the chemical shift of 82.32 ppm, the two protons of the methylene group are diastereotopic.
A geminal coupling constant of 16.06 Hz is measurable between these two protons with the chemical shifts of 2.52 ppm and 2.65 ppm.

The distance to all other protons is larger than three bonds. The coupling constants to them are smaller than the spectroscopic resolution.

Maybe you read somewhere: "There is no coupling over more than three bonds“. That’s not quite correct. The coupling constant is merely usually too small to be measurable.

Multiplets

Methyl groups



Because of the center of chirality, the two methyl groups with the signals at 0.84 ppm and 0.89 ppm are diastereotopic as well.

The protons of each of the two chemically different methyl groups are adjacent to the methine proton via three bonds. We expect a doublet for the methyl group protons. The two coupling constants should be different.
Note: The difference of 0.01 Hz measured here is within the margin of error and does not proof the claim just made.

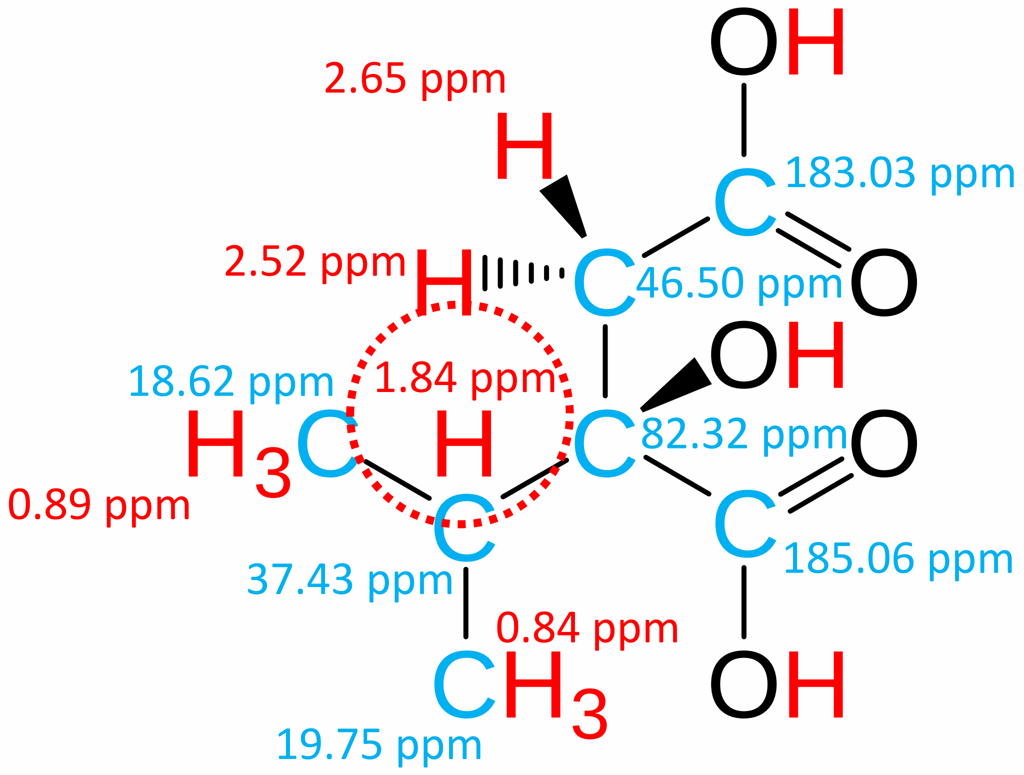
But what kind of multiplet does the methine proton actually show?

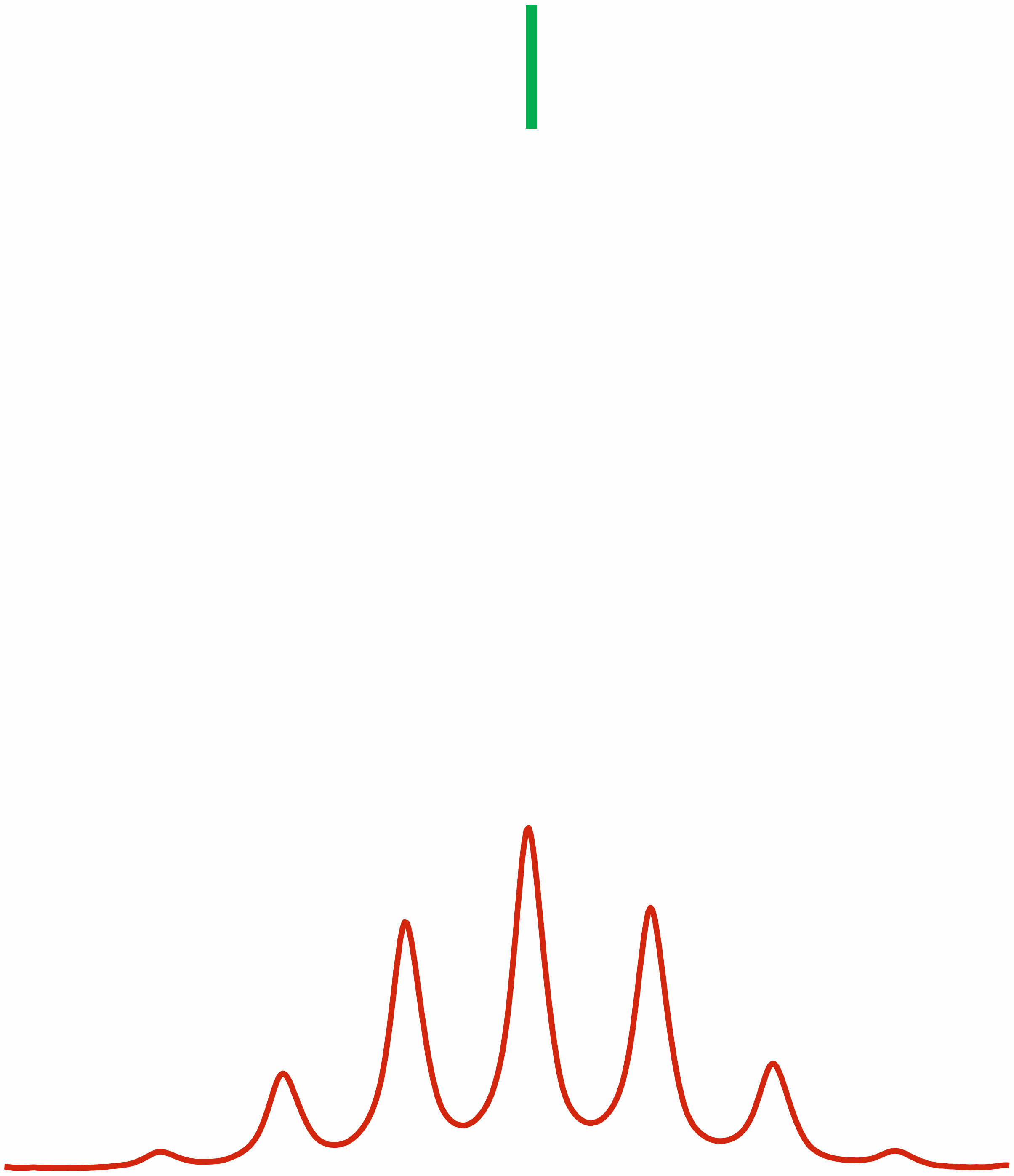
‚Septet' would be the wrong answer.

Multiplets

Methine group

Let us start with the methine proton and neglect all adjacent protons for the moment.





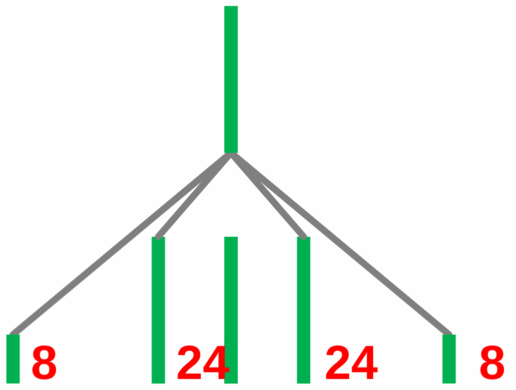
Multiplets

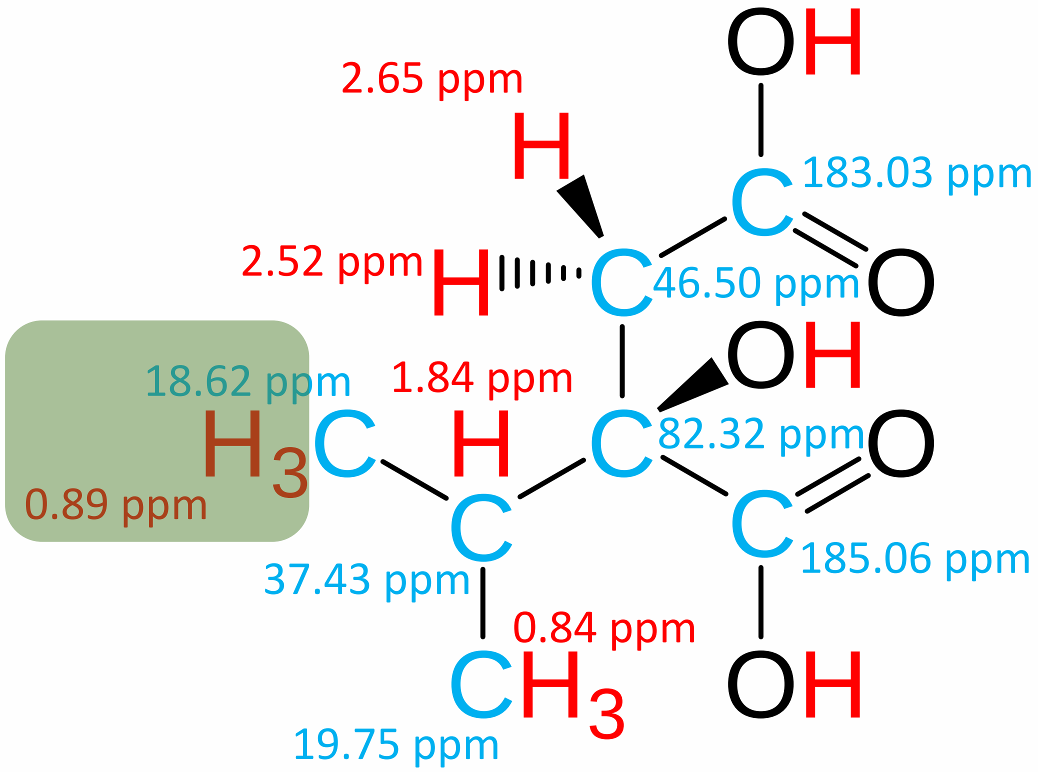
Methine group

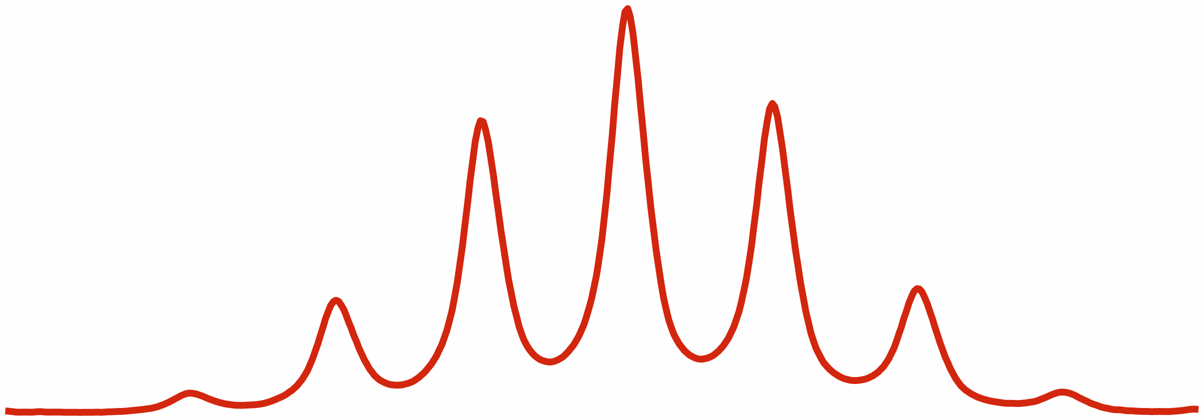
By coupling with the three protons of the methyl group at 0.89 ppm, this becomes a quartet showing an intensity ratio for the individual lines of

1 : 3 : 3 : 1

(the proportionality coefficient here is 8)



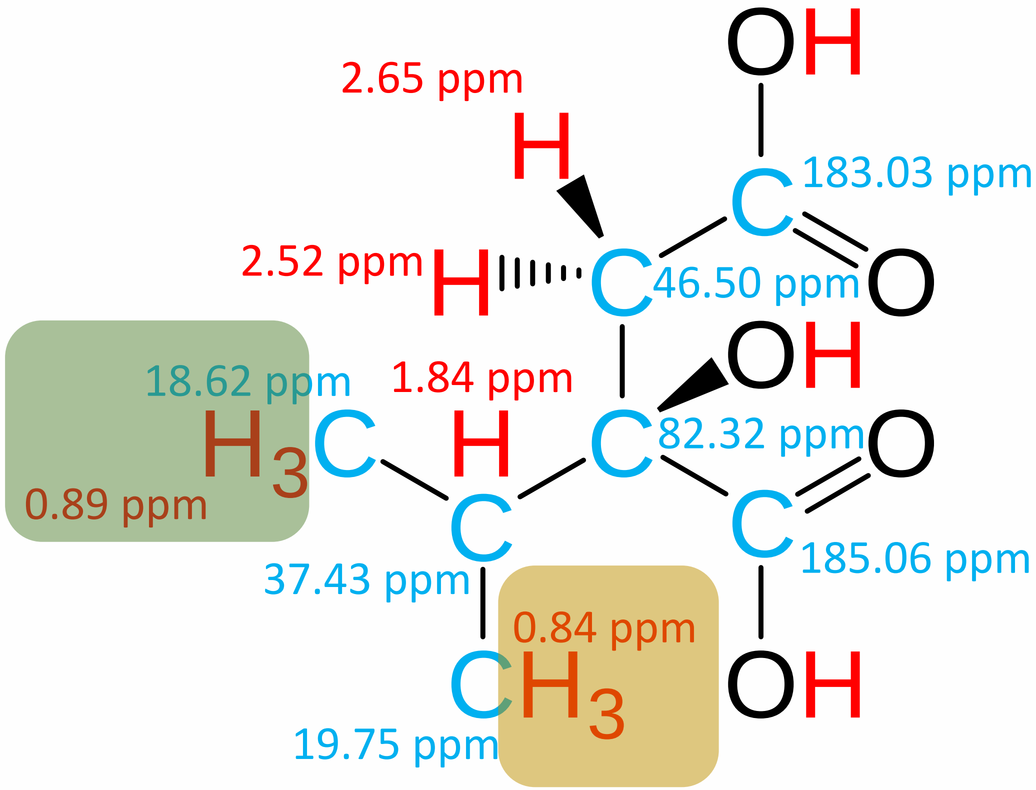


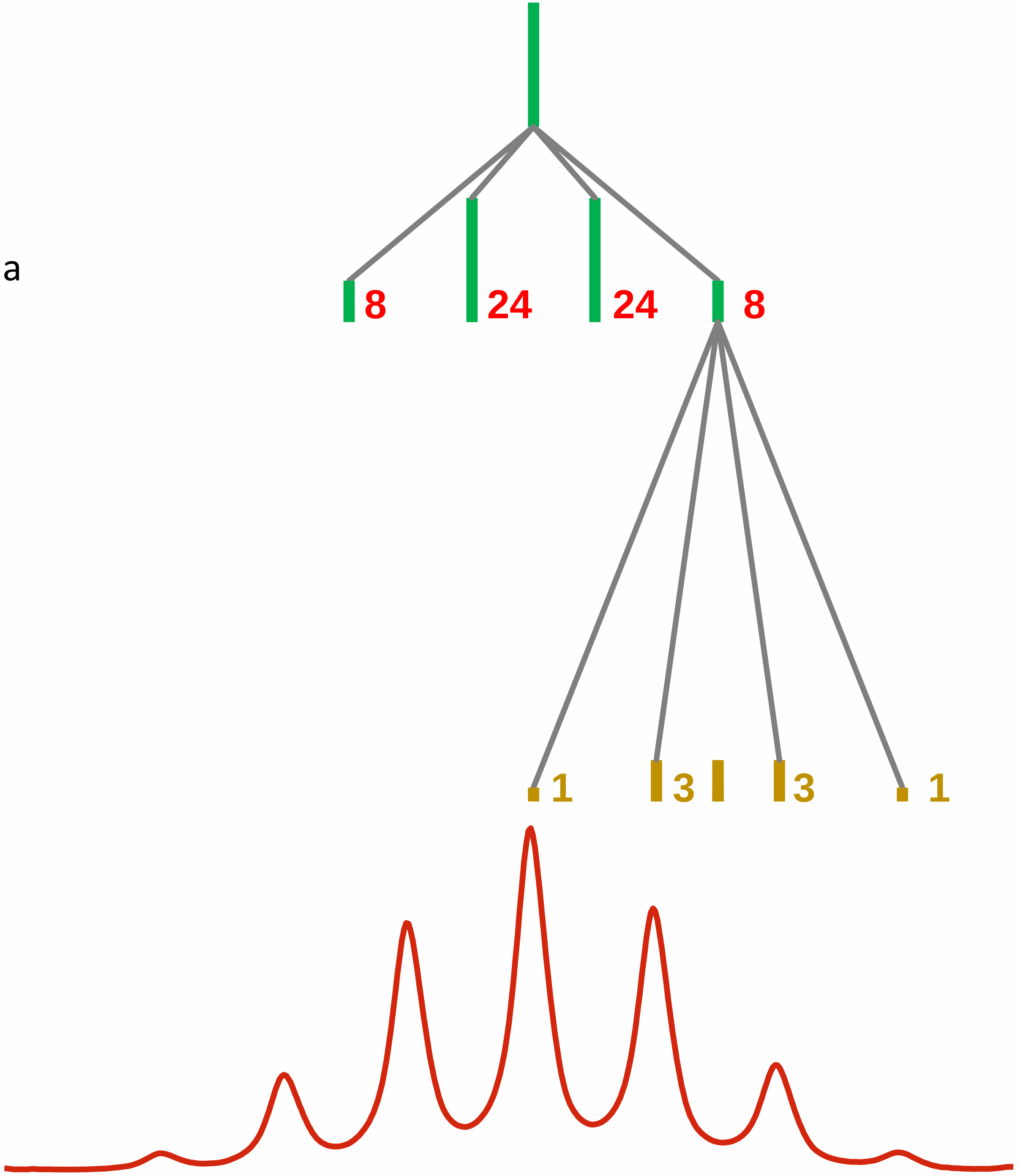


Multiplets

Methine group

The three protons of the methyl group at 0.84 ppm now create a quartet from **each** of the four lines.
Let's look at the splitting of the four lines line by line.

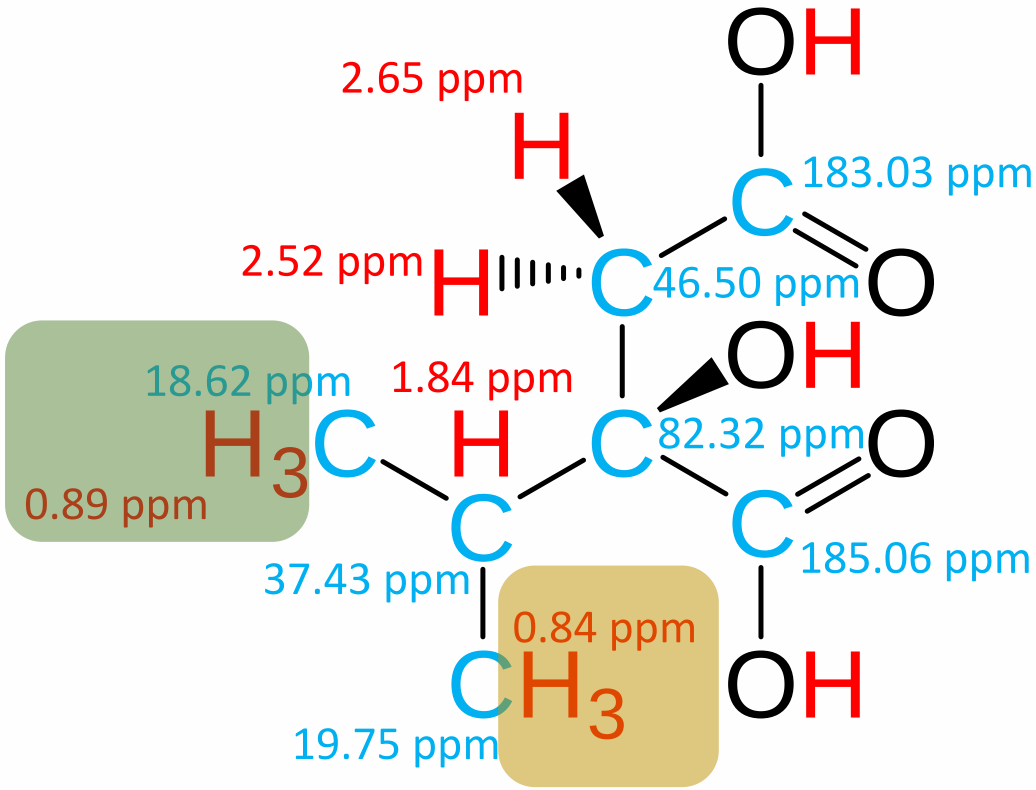


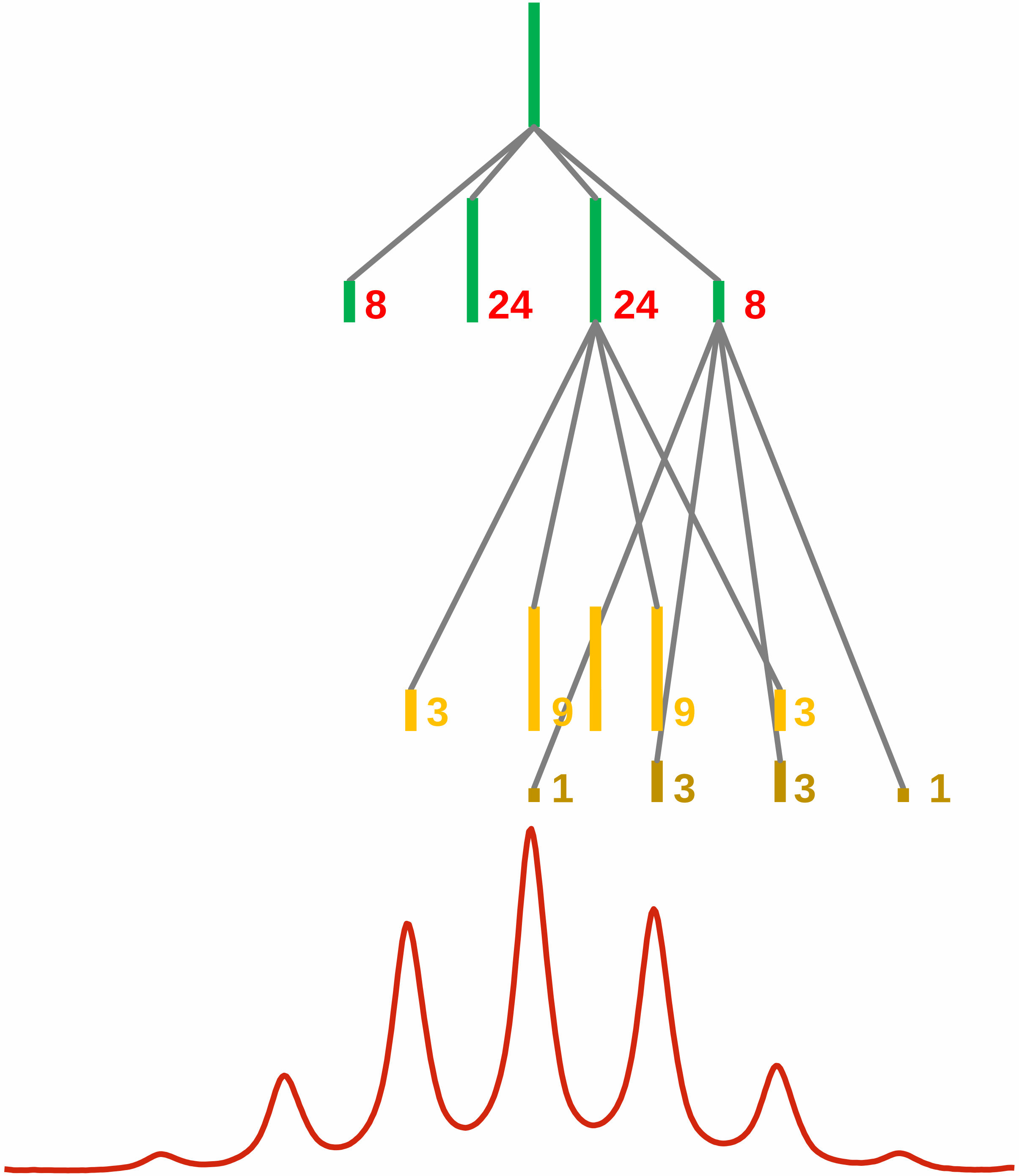


Multiplets

Methine group

For sake of clarity the four new multipletts are displayed vertically staggered.

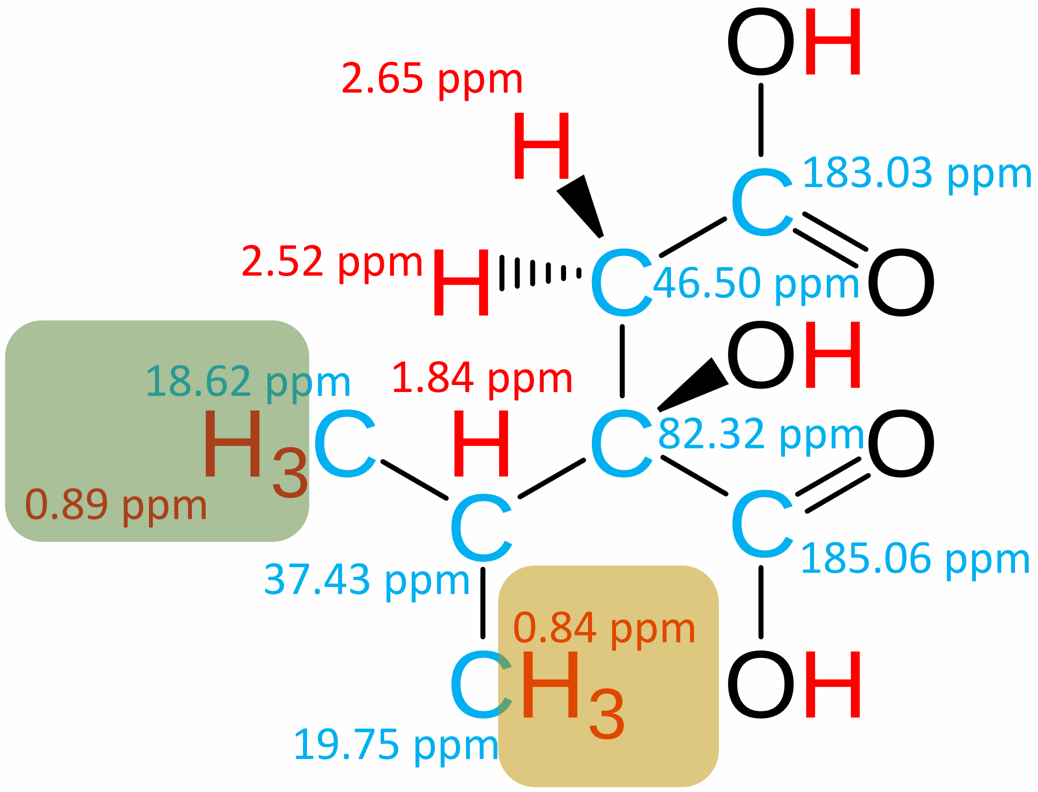


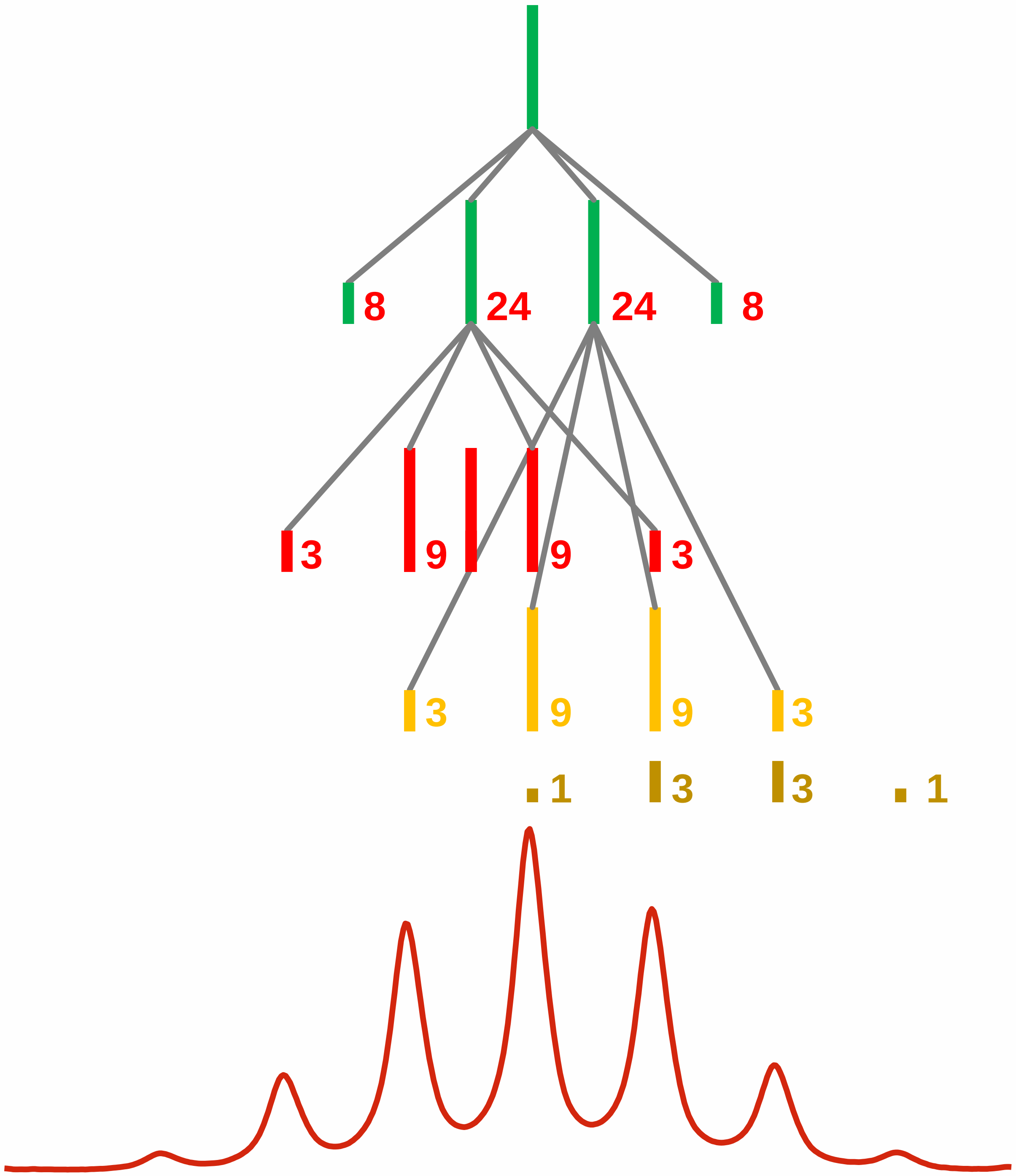


Multiplets

Methine group

Next splitting …

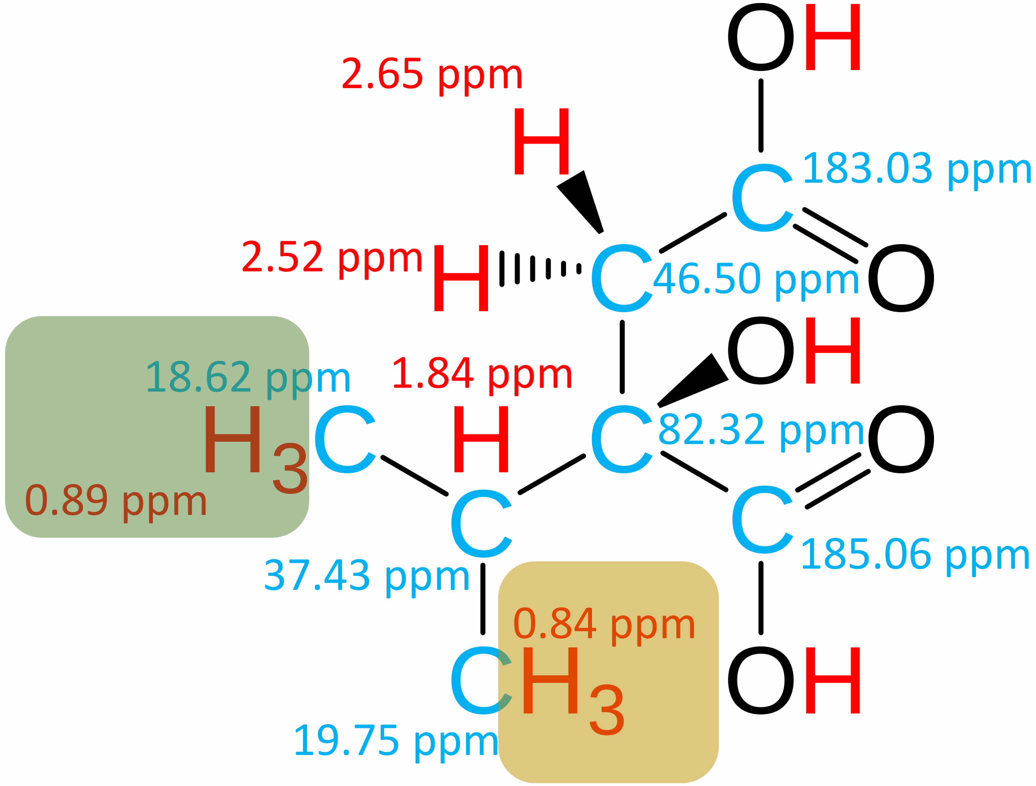


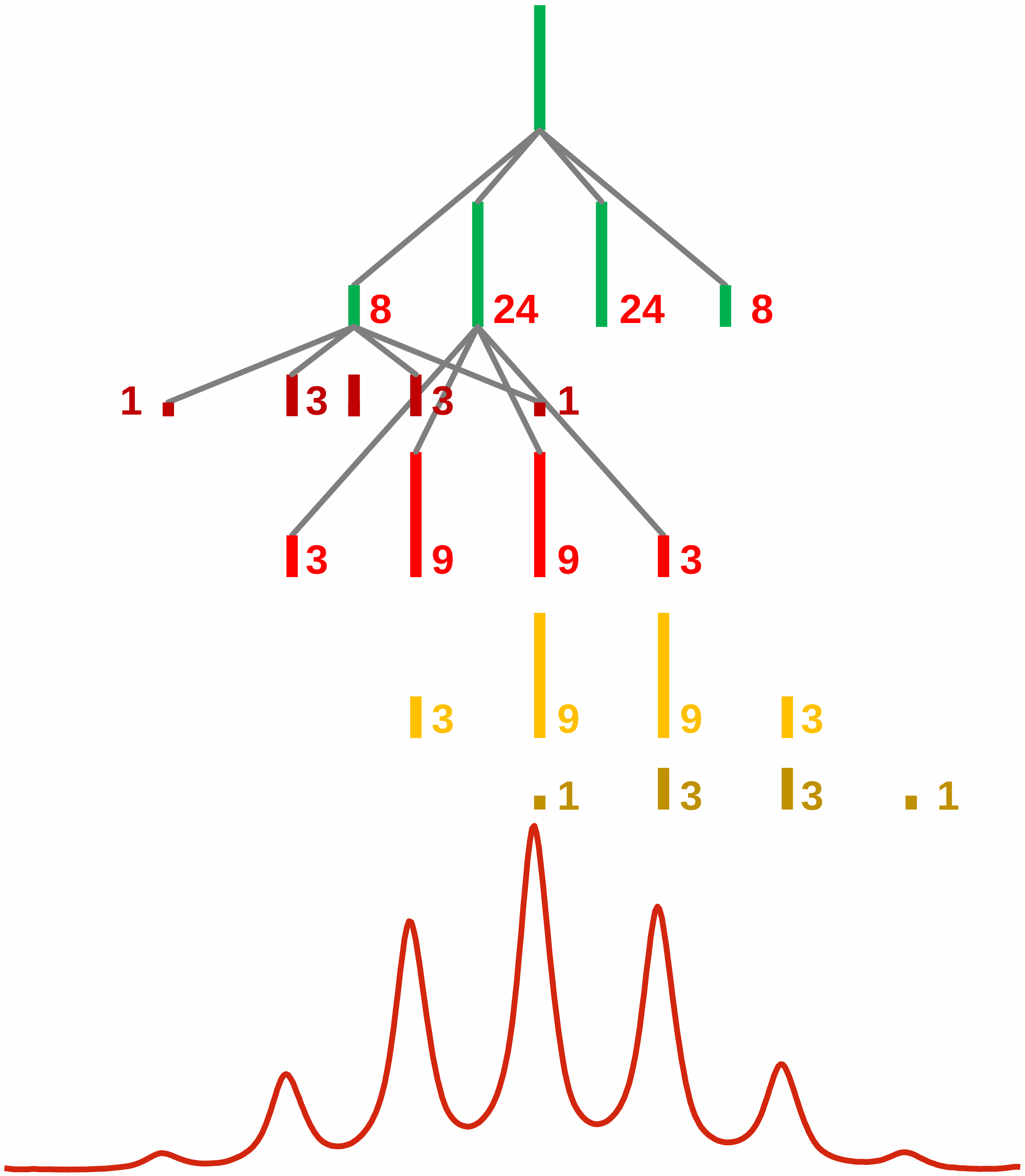


Multiplets

Methine group

And the final one …

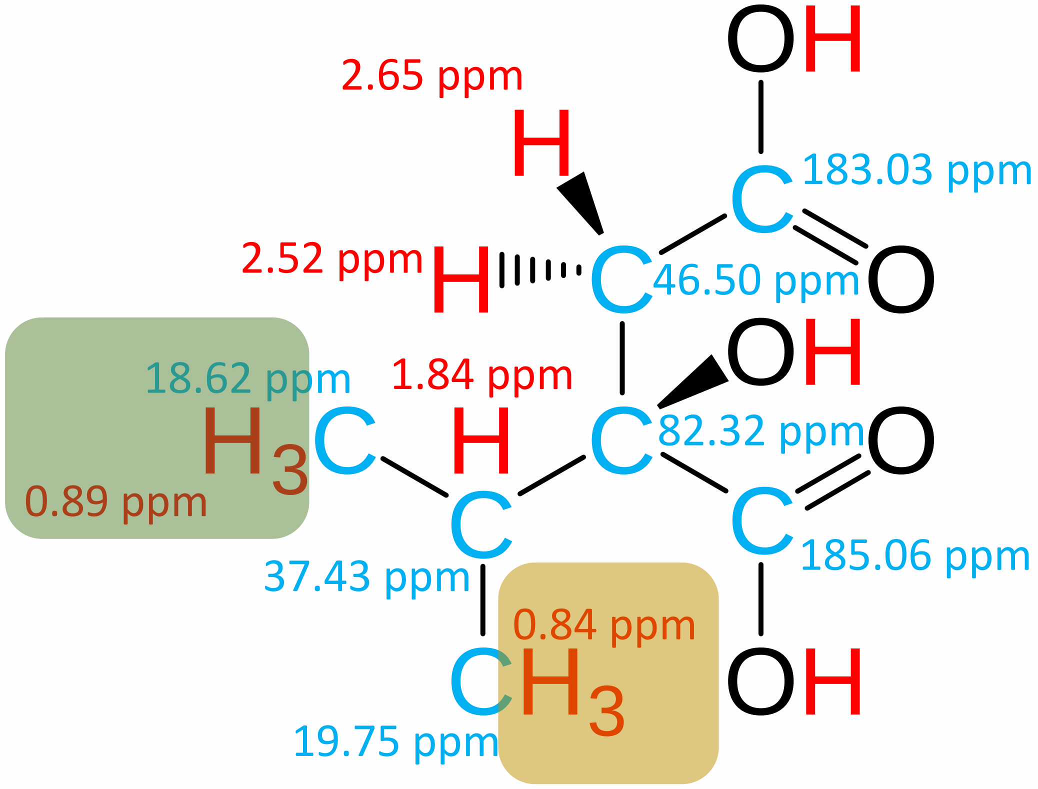


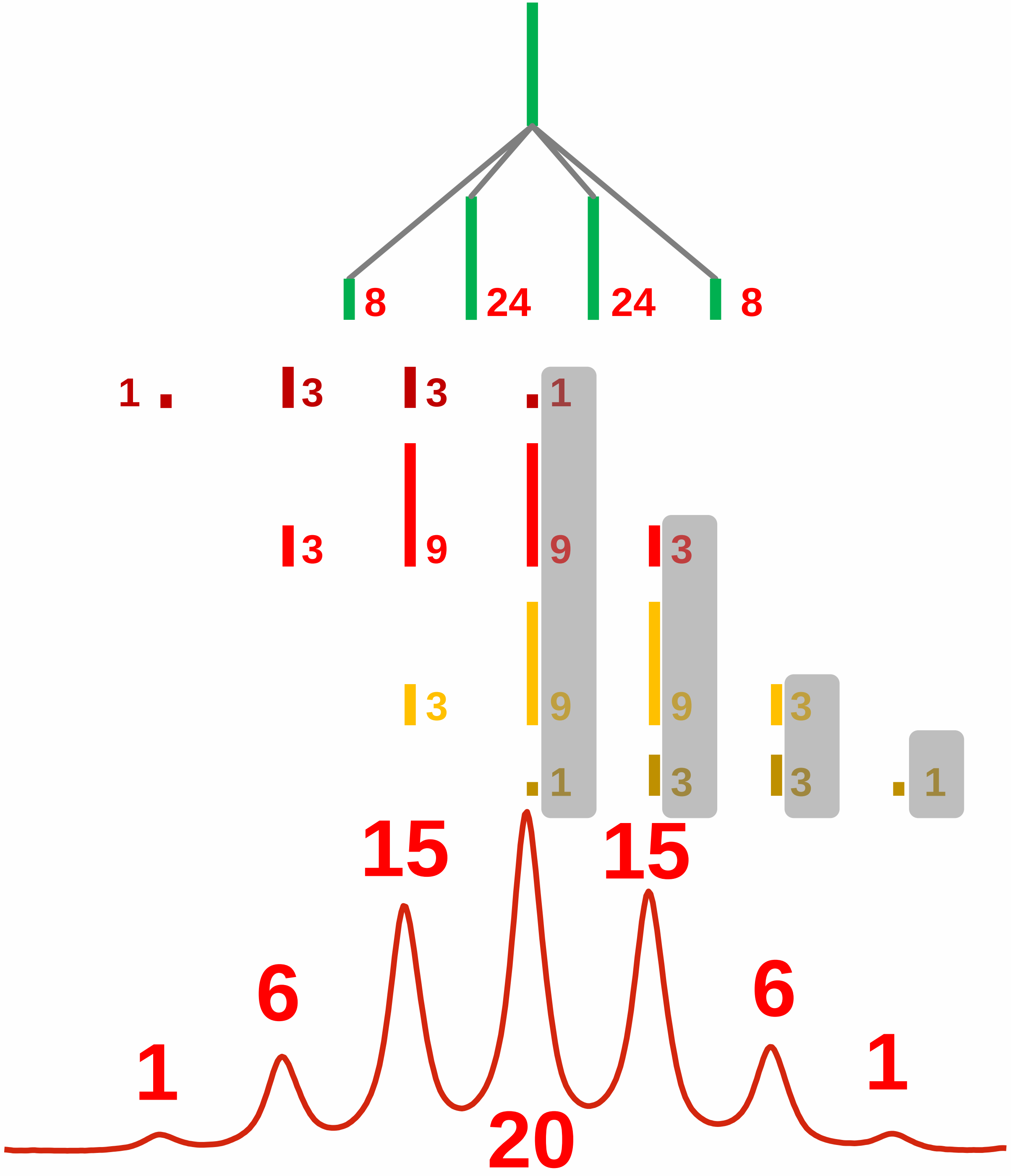


Multiplets

Methine group

Now we simply have to add everything.

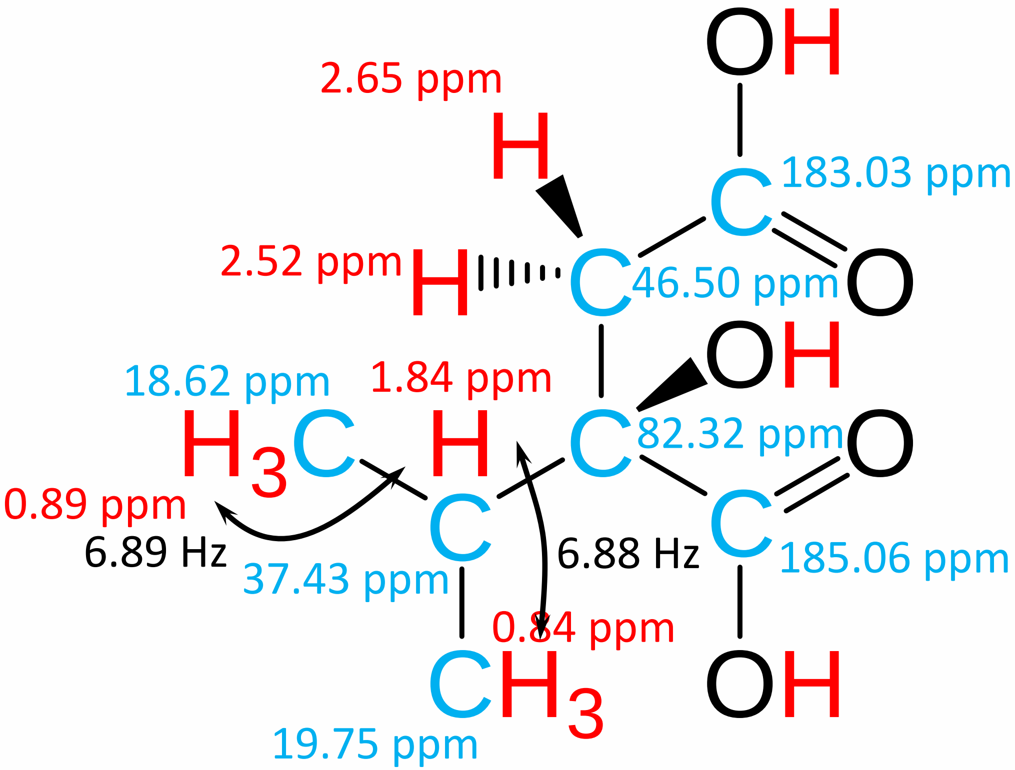




Multiplets

Methine group

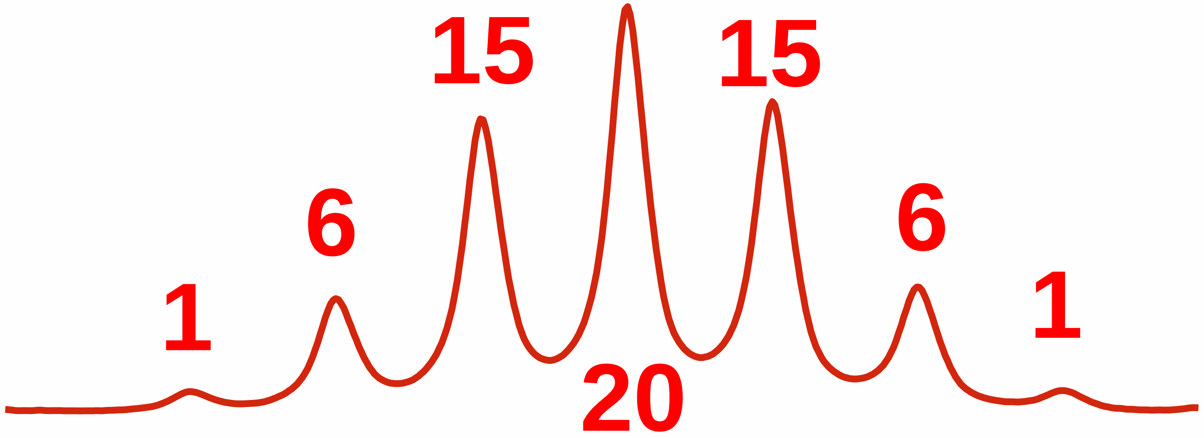
But **this is** a septet?!



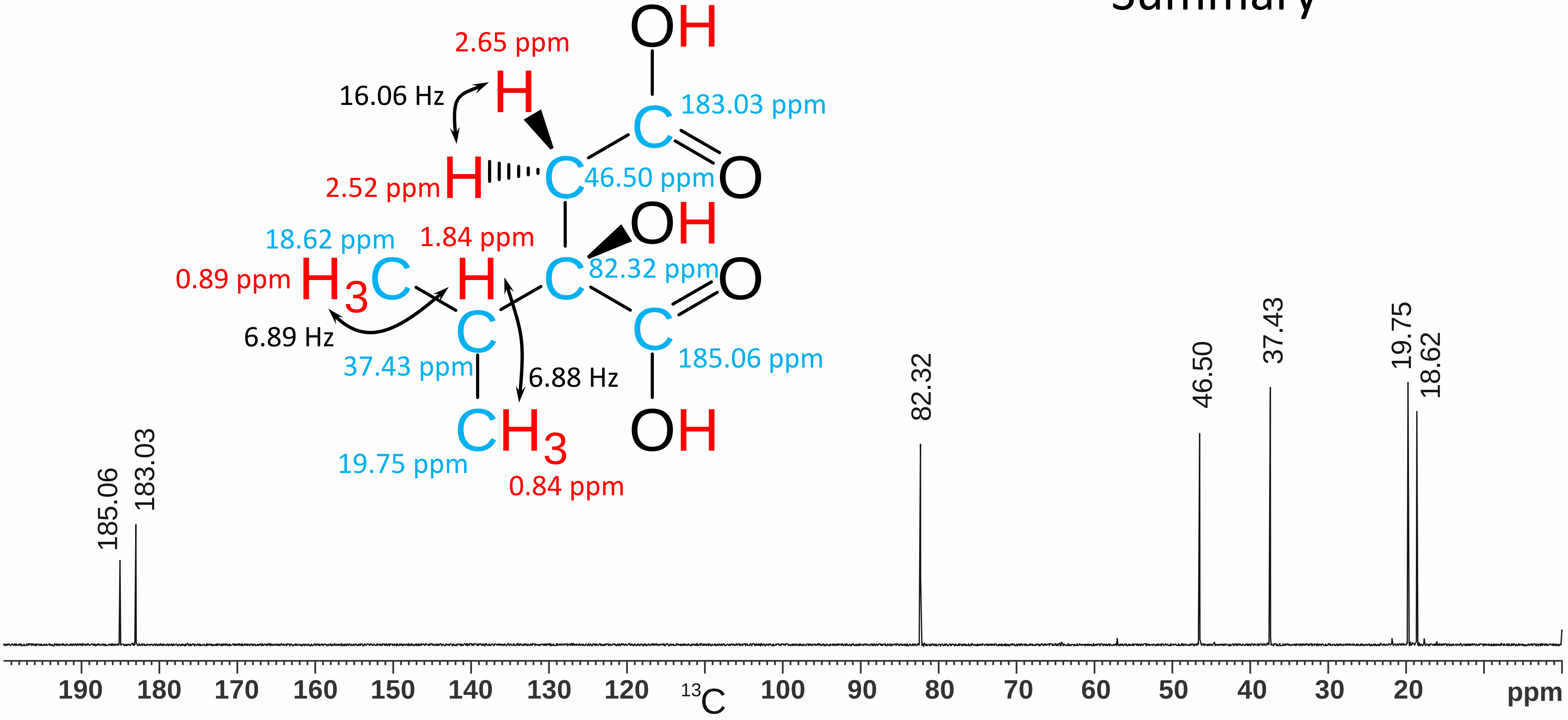
No!

It would be a septet if the couplings from the methine proton to the two sets of methyl protons were identical, but because the two methyl groups are diastereotopic that isn't necessarily the case. In principle they should be different but in practice they may be very (very!) similar. Because of the almost identical vicinal coupling constants between the methine proton and the protons of each individual methyl group, the quartet of quartets **looks like** a septet.

The correct name of the multiplet is, therefore „pseudo septet".

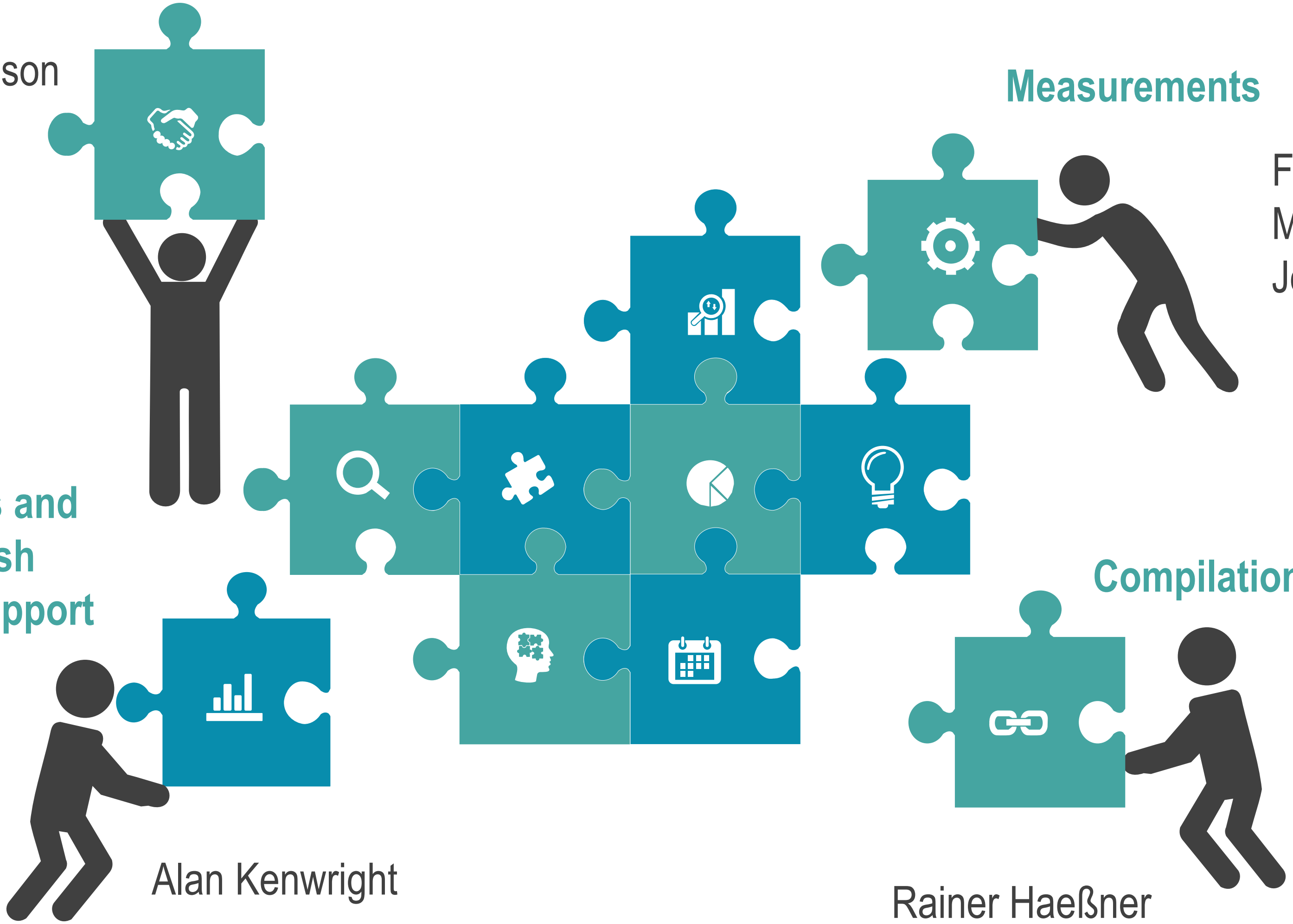


Summary



2.65 ppm
16.06 Hz
2.52 ppm
183.03 ppm
46.50 ppm
18.62 ppm
1.84 ppm
0.89 ppm
82.32 ppm
6.89 Hz
37.43 ppm
6.88 Hz
185.06 ppm
19.75 ppm
0.84 ppm
OH
O
OH
O
OH
H
H
H
H_3C
CH_3
C
185.06
183.03
82.32
46.50
37.43
19.75
18.62
190
180
170
160
150
140
130
120
^{13}C
100
90
80
70
60
50
40
30
20
ppm

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 …