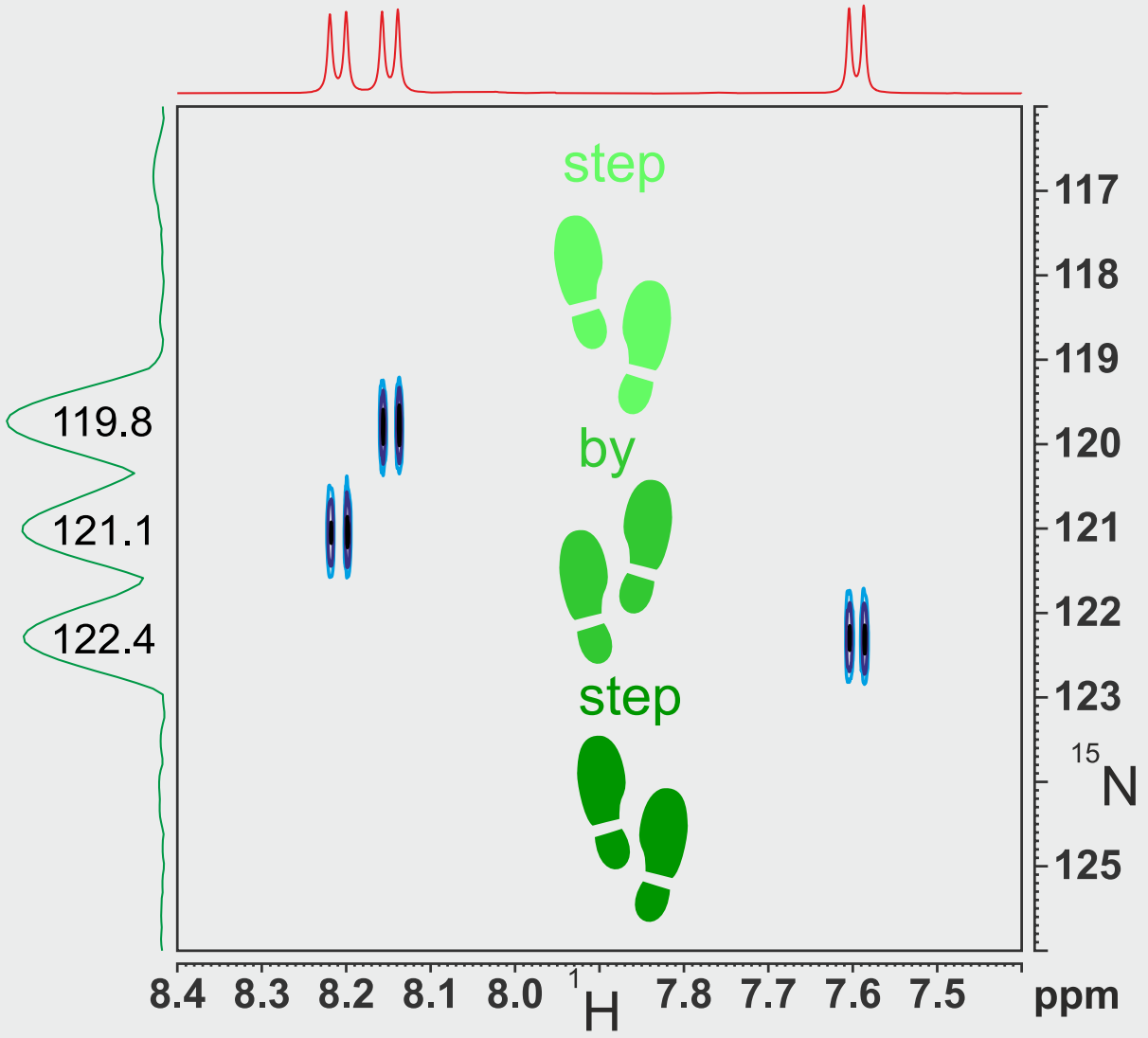
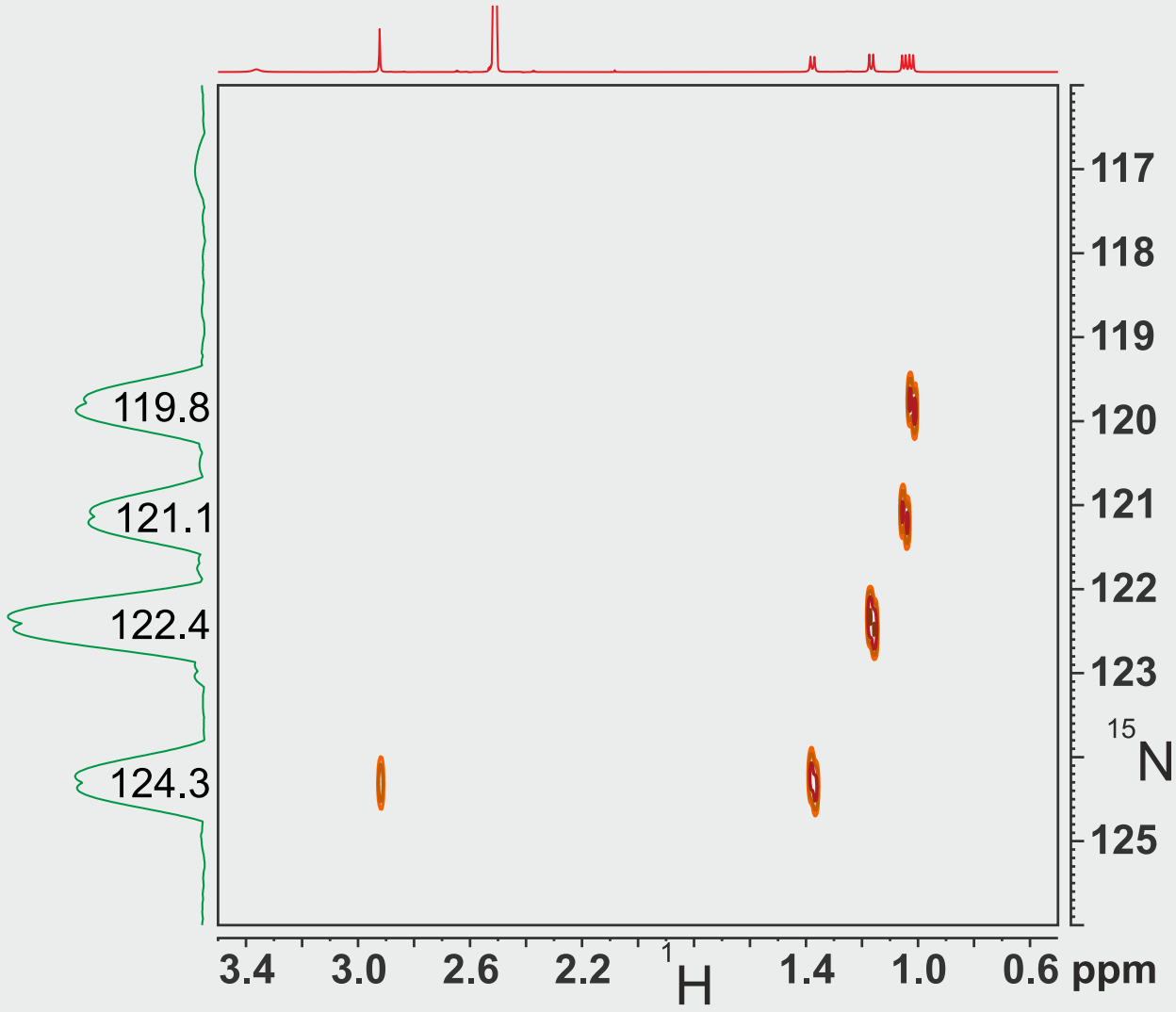
Exercise plus Solution – Quick overview

It is recommended to use this 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.





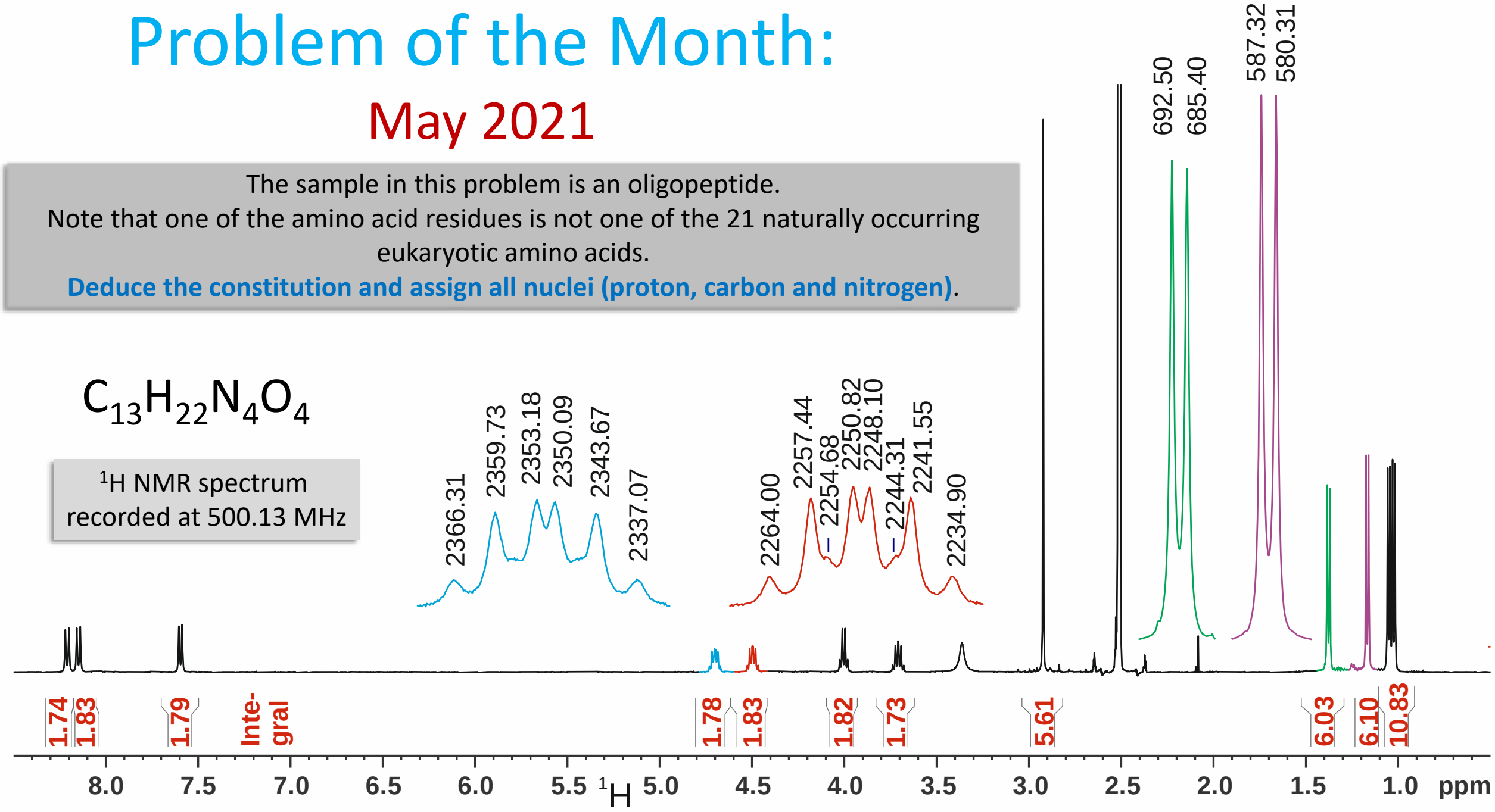
Problem of the Month:

May 2021

The sample in this problem is an oligopeptide.
Note that one of the amino acid residues is not one of the 21 naturally occurring eukaryotic amino acids.
Deduce the constitution and assign all nuclei (proton, carbon and nitrogen).

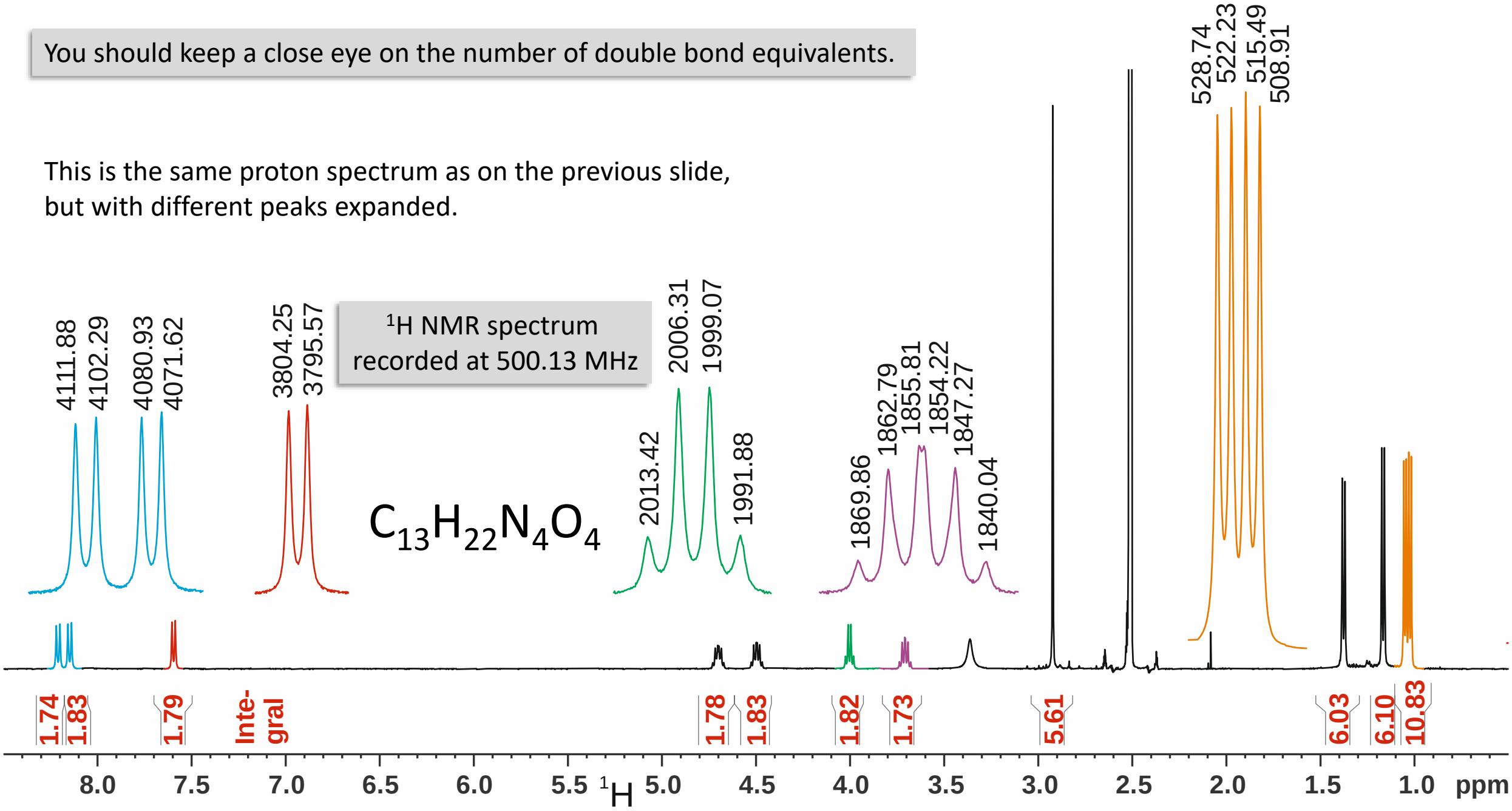
$C_{13}H_{22}N_4O_4$

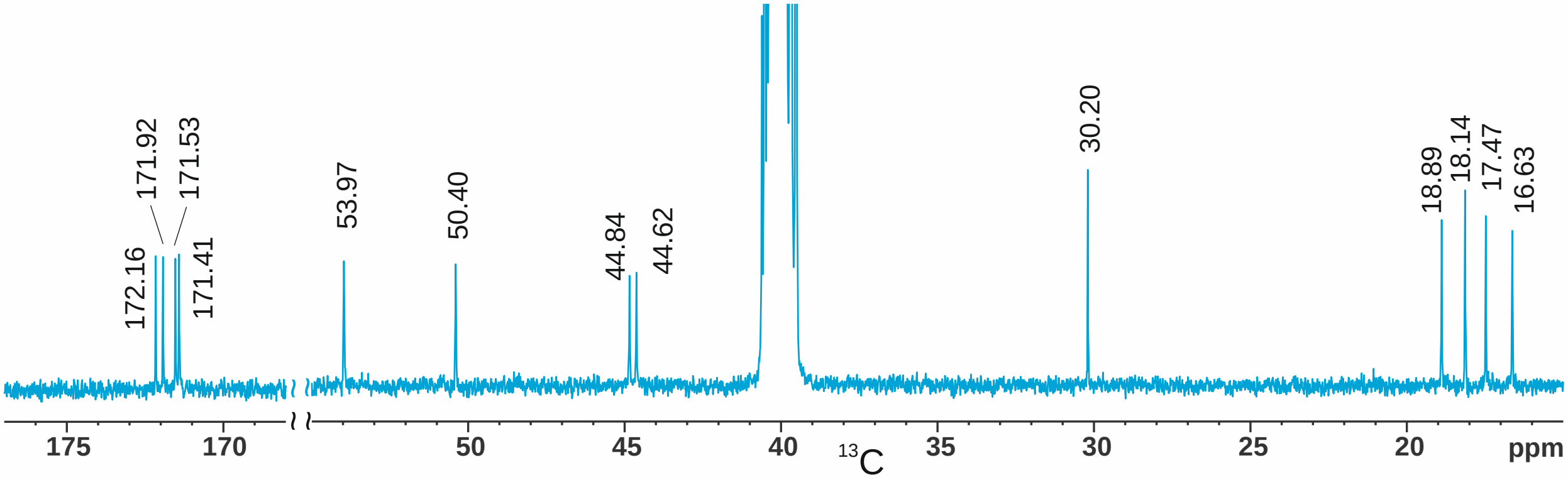
^{1}H NMR spectrum recorded at 500.13 MHz



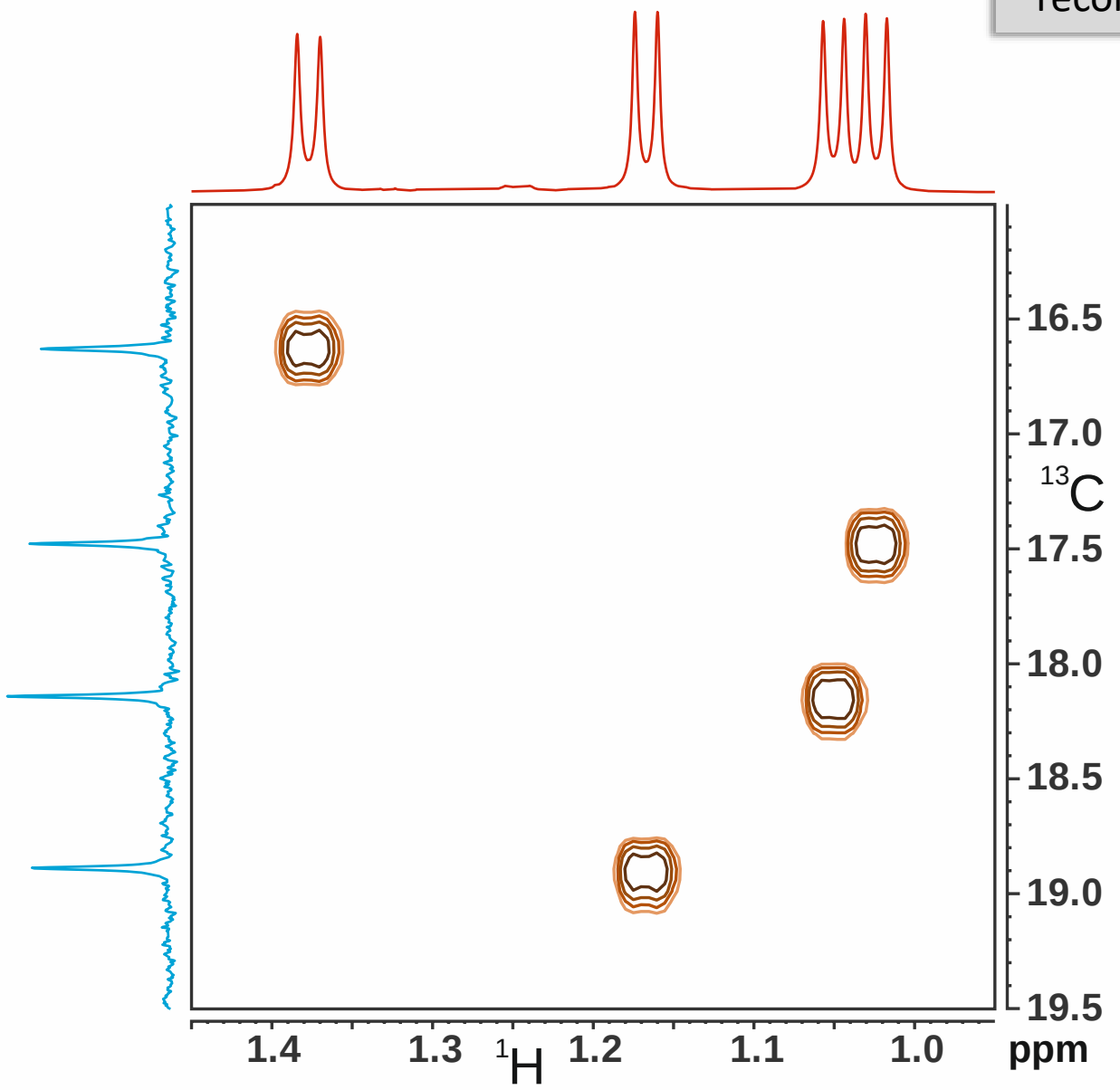
You should keep a close eye on the number of double bond equivalents.

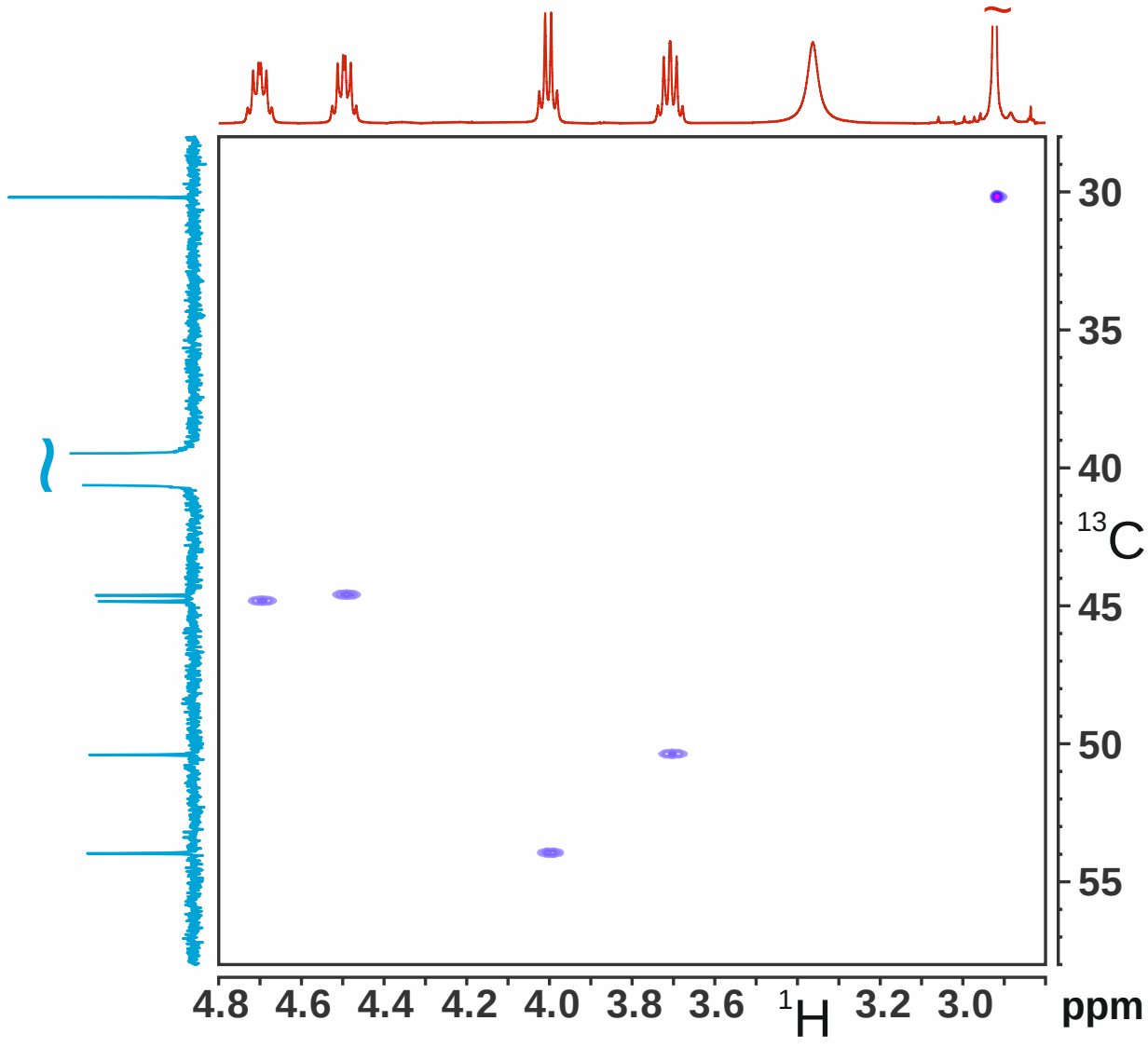
This is the same proton spectrum as on the previous slide,
but with different peaks expanded.



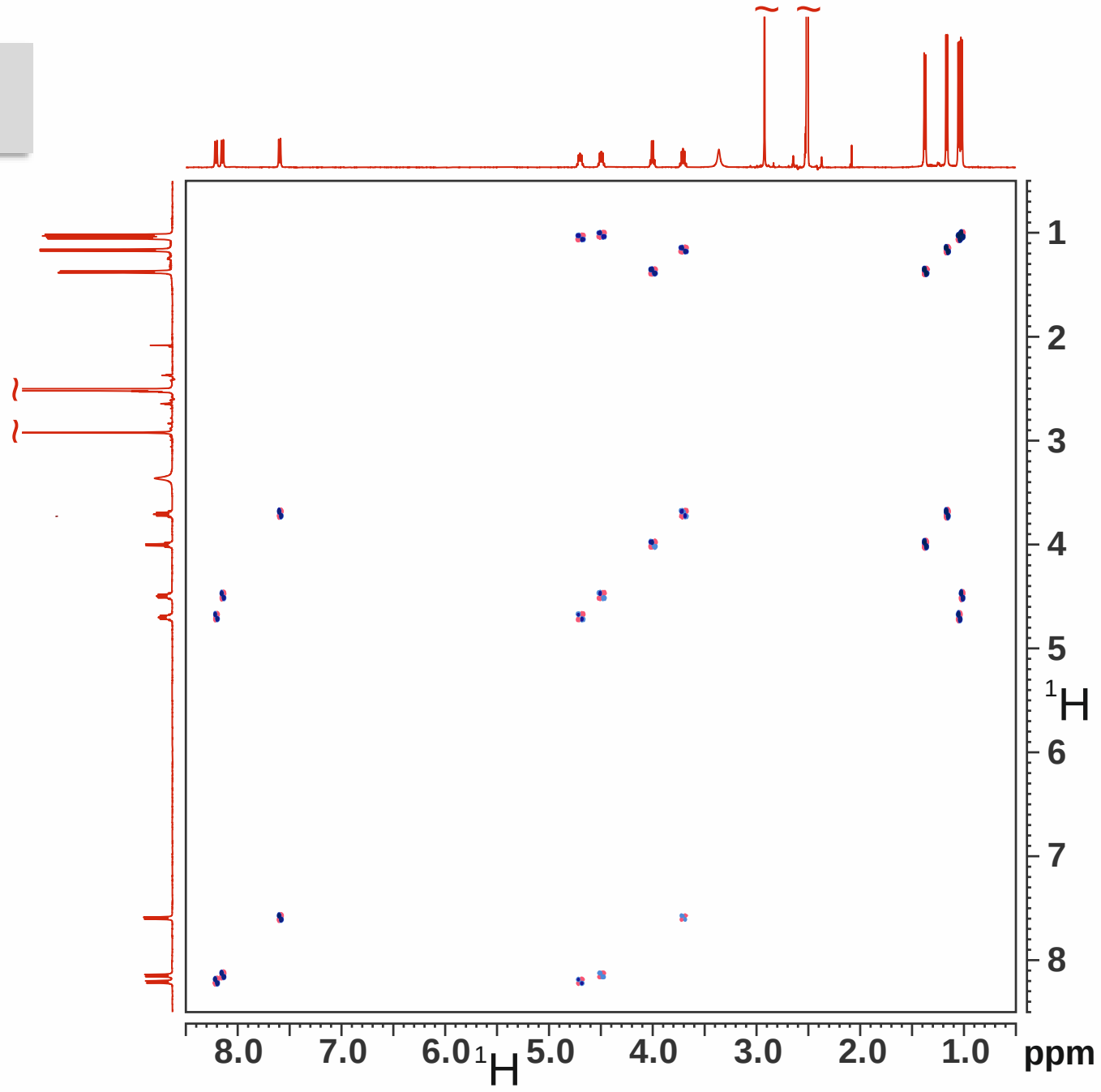

^{13}C{^{1}H} NMR spectrum
recorded at 125.80{500.13} MHz
172.16
171.92
171.53
171.41
53.97
50.40
44.84
44.62
30.20
18.89
18.14
17.47
16.63
175
170
50
45
40
35
30
25
20
ppm
^{13}C

$^{1}H/^{13}C$-HSQC
recorded at 500.13/125.80 MHz

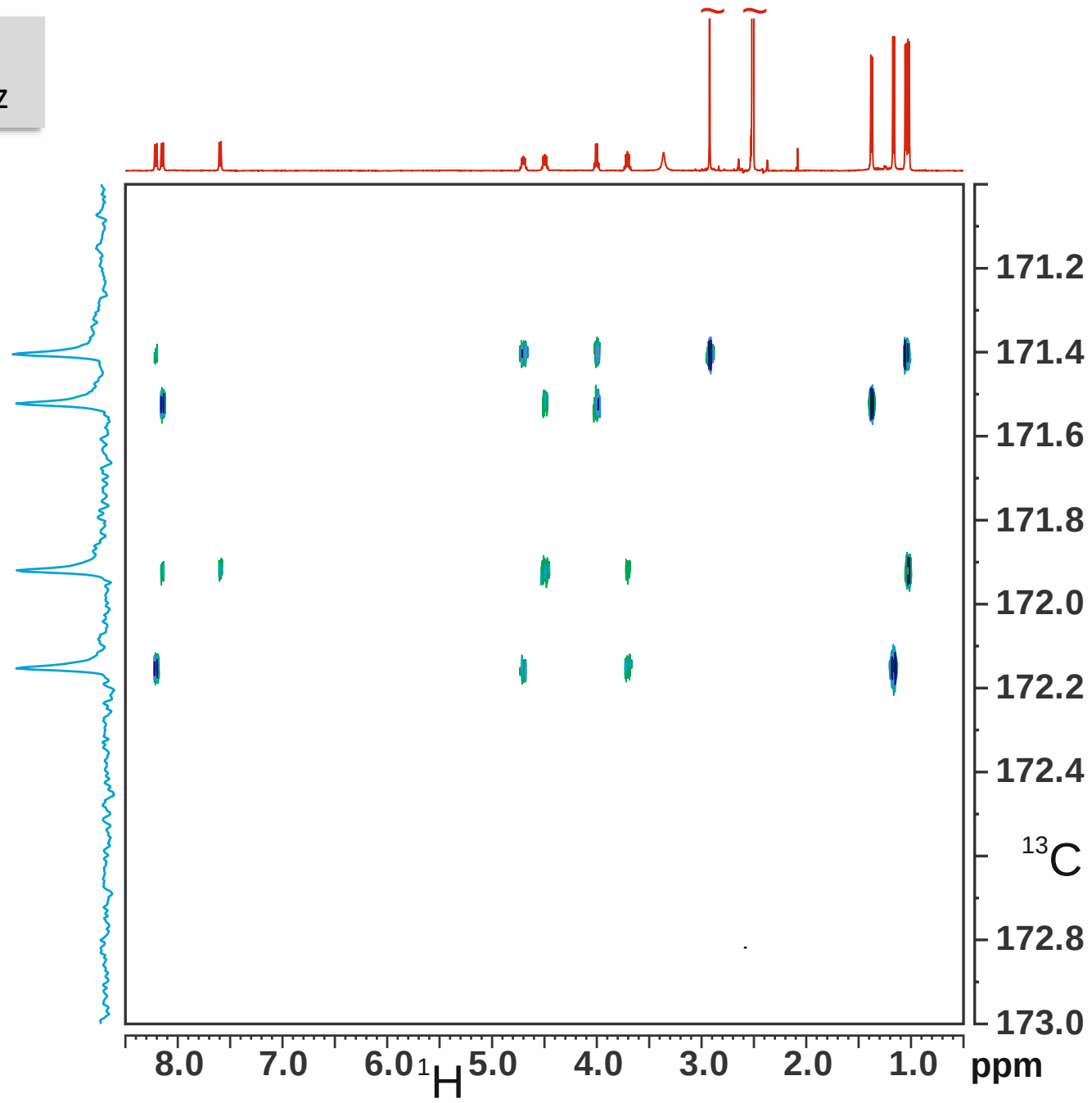


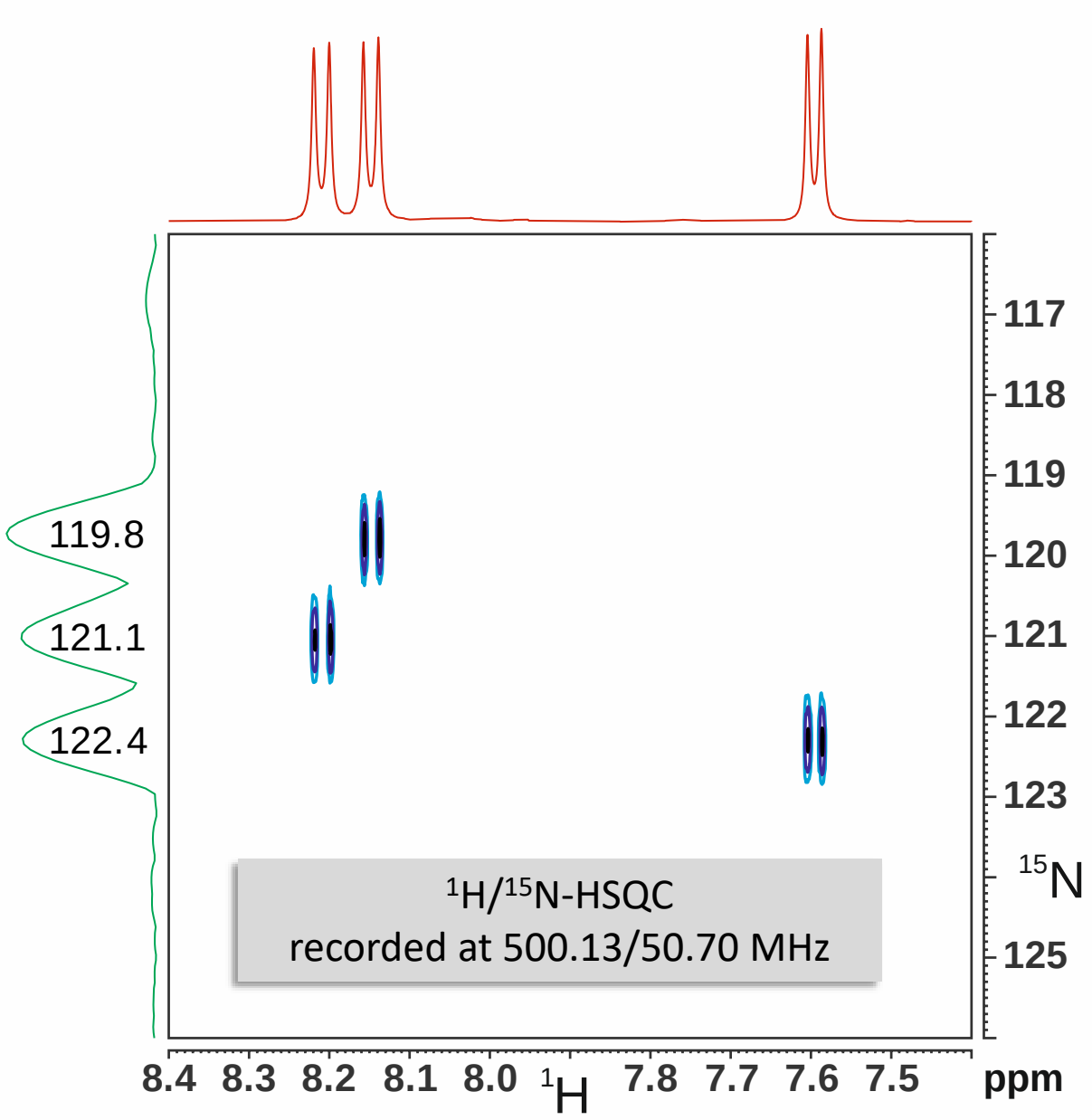


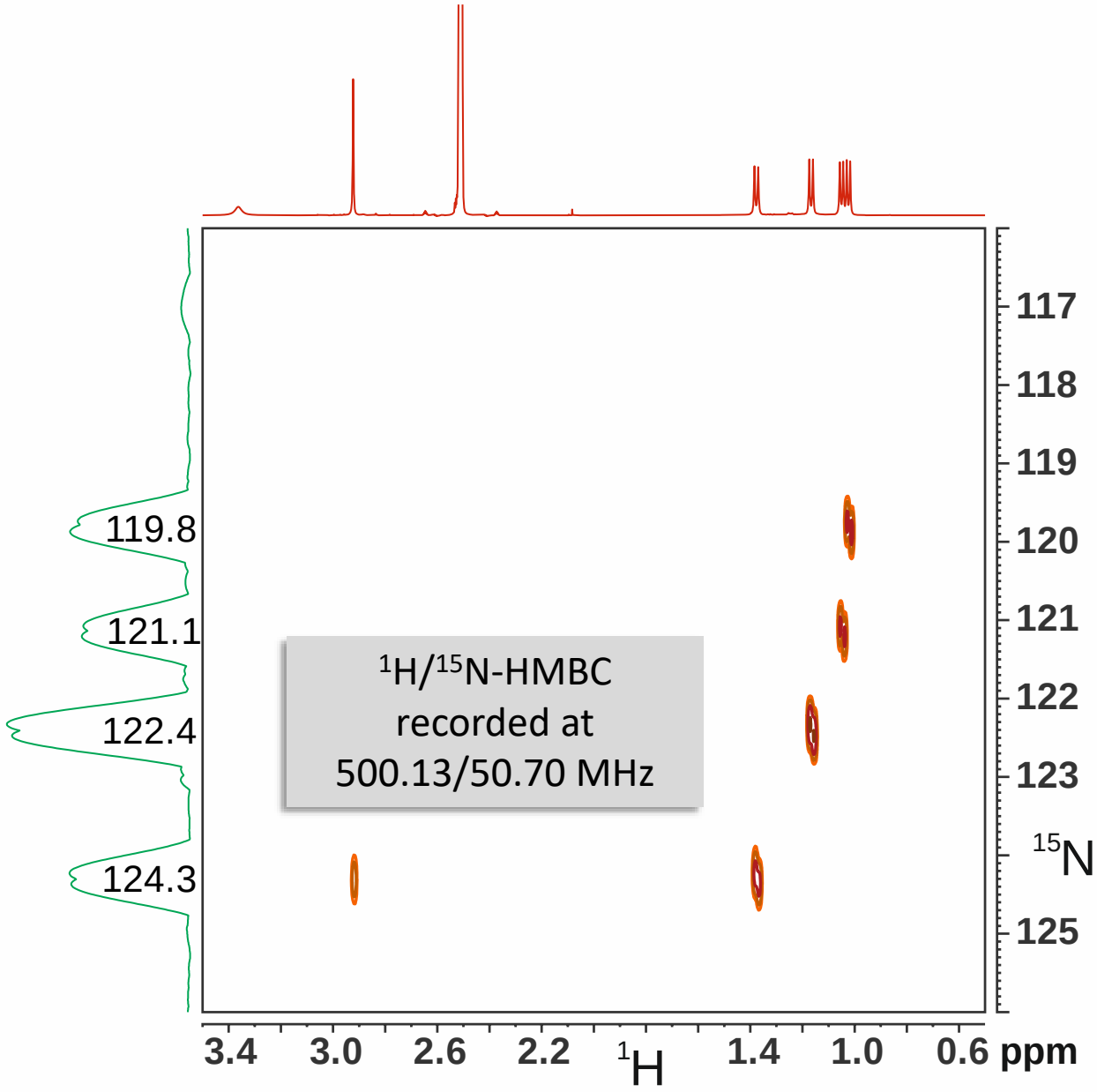
$^{1}H/^{1}H$-COSY
recorded at 500.13 MHz



part of $^{1}H/^{13}C$-HMBC
recorded at 500.13/125.80 MHz

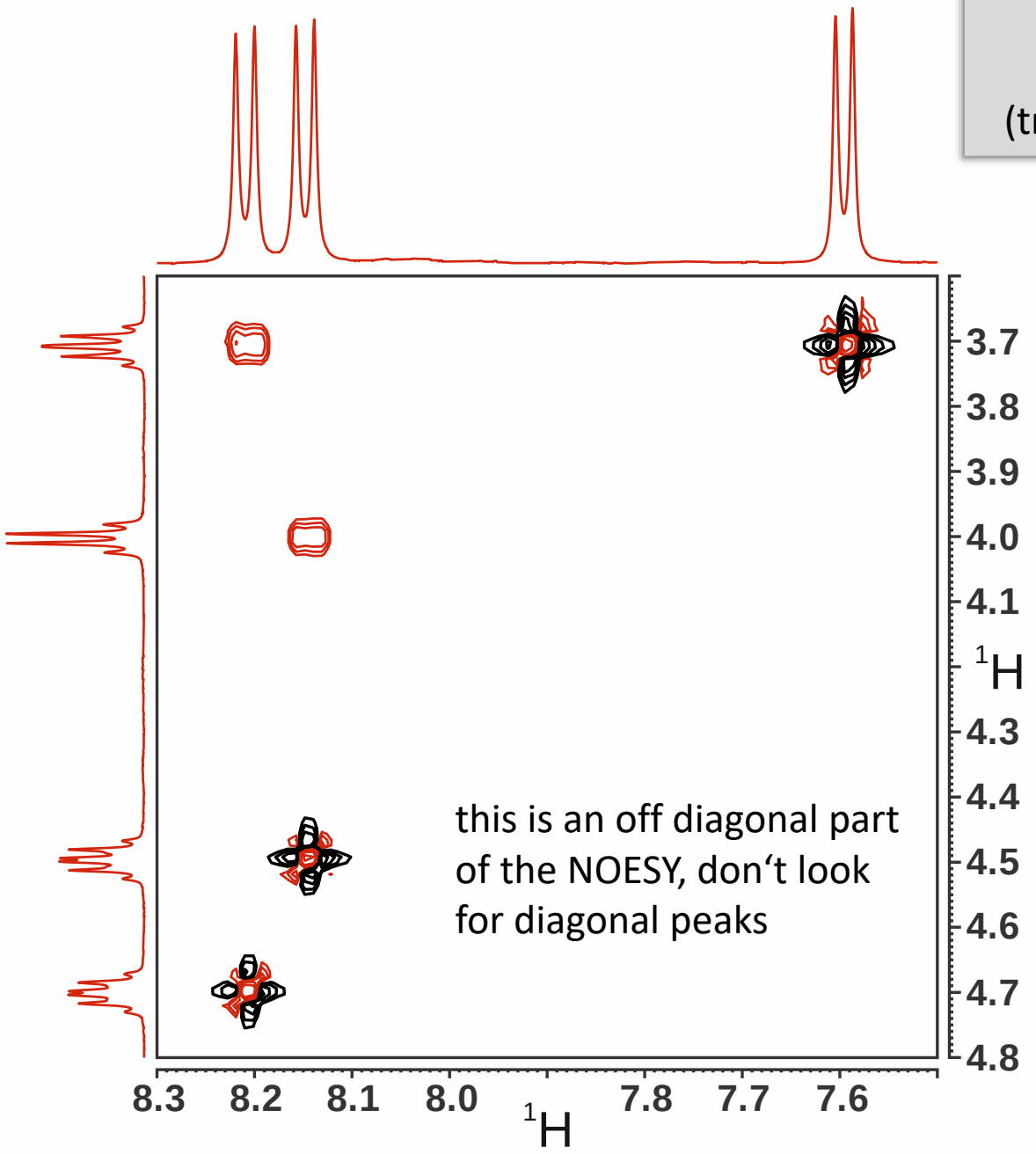


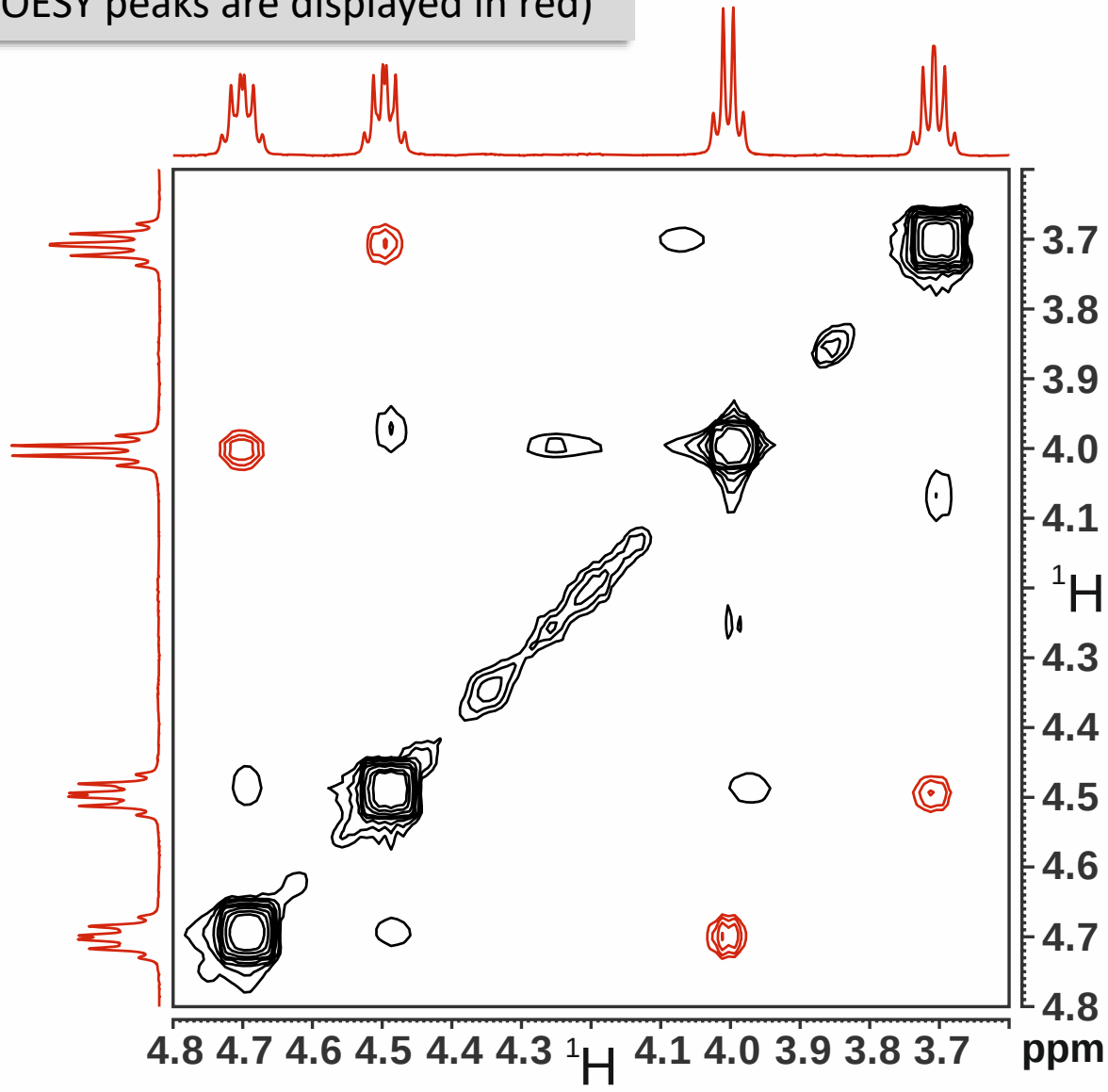

119.8
121.1
122.4
117
118
119
120
121
122
123
125
^{15}N
^{1}H/^{15}N-HSQC
recorded at 500.13/50.70 MHz
8.4 8.3 8.2 8.1 8.0 ^{1}H 7.8 7.7 7.6 7.5
ppm


119.8
121.1
122.4
124.3
117
118
119
120
121
122
123
125
^{15}N
^{1}H/^{15}N-HMBC
recorded at
500.13/50.70 MHz
3.4 3.0 2.6 2.2 ^{1}H 1.4 1.0 0.6
ppm

parts of $^{1}H/^{1}H$-NOESY
recorded at 500.13 MHz

(true NOESY peaks are displayed in red)



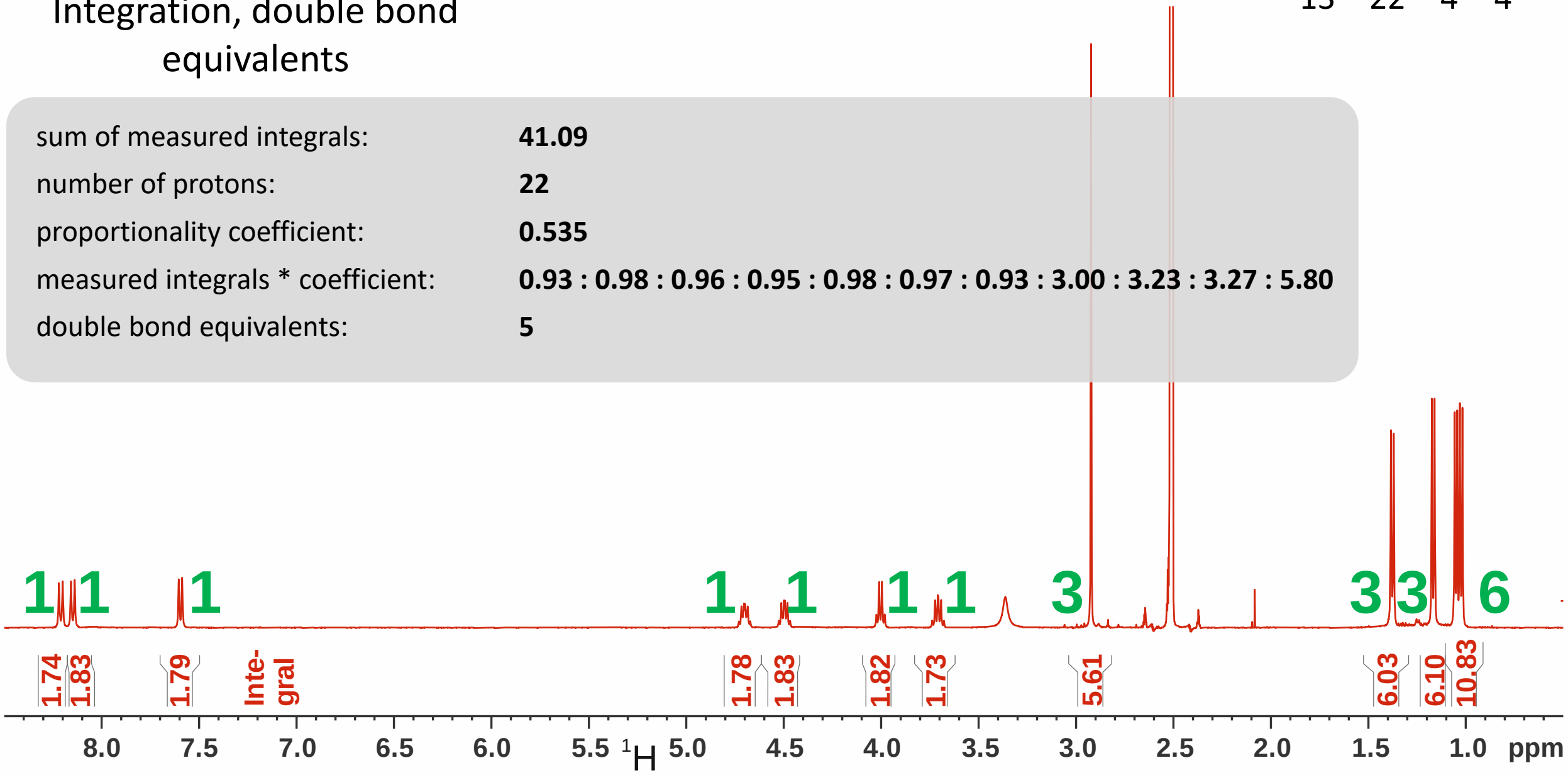


Basics

Integration, double bond equivalents

$C_{13}H_{22}N_4O_4$

sum of measured integrals:	**41.09**
number of protons:	**22**
proportionality coefficient:	**0.535**
measured integrals * coefficient:	**0.93 : 0.98 : 0.96 : 0.95 : 0.98 : 0.97 : 0.93 : 3.00 : 3.23 : 3.27 : 5.80**
double bond equivalents:	**5**



Basics

Number of amino acids

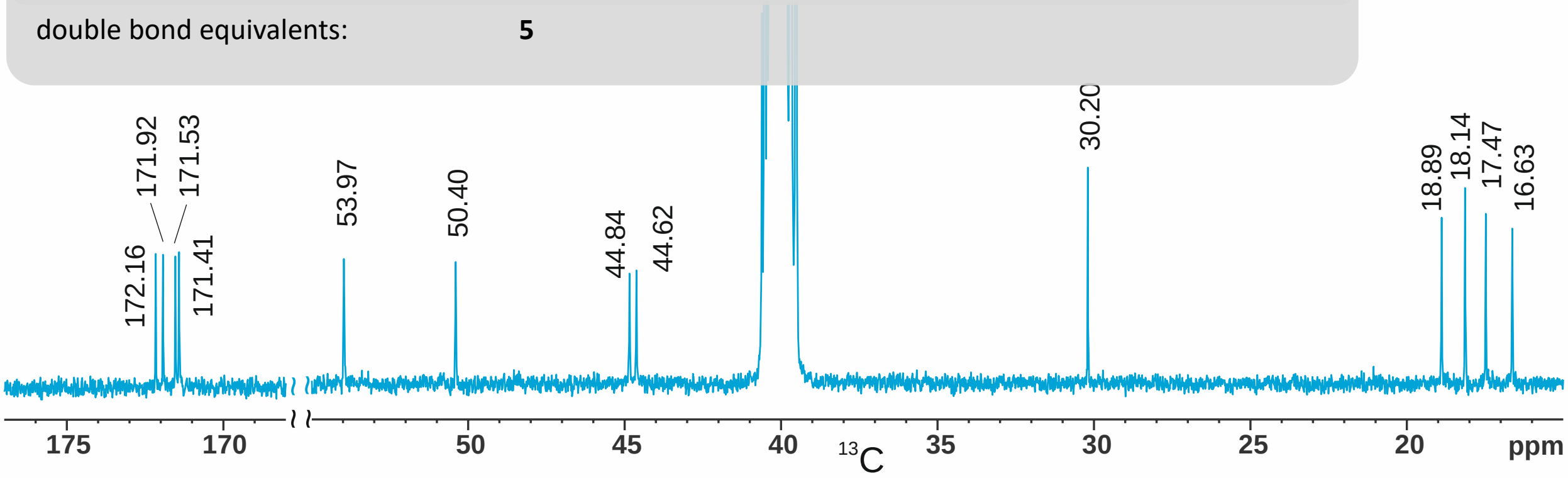
$C_{13}H_{22}N_4O_4$

double bond equivalents: **5**

number of >C=O groups: **4** (171.41, 171.53, 171.92, 172.16 ppm)

Please note:
all oxygen atoms are used now, no –OH group is possible.

double bond equivalents: **5**



Basics

Number of amino acids

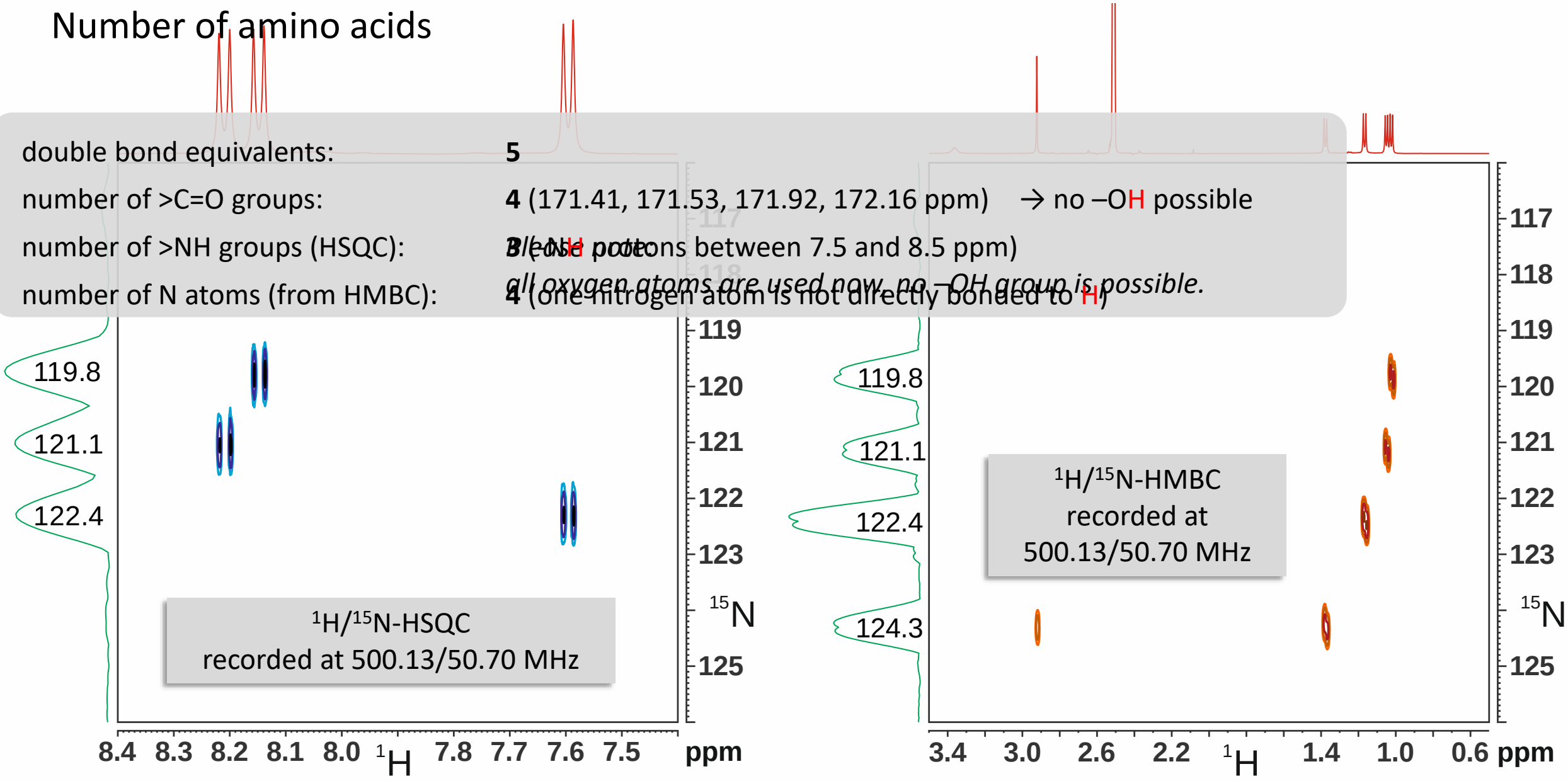
double bond equivalents: **5**

number of >C=O groups: **4** (171.41, 171.53, 171.92, 172.16 ppm) → no –OH possible

number of >NH groups (HSQC): **3** (one NH protons between 7.5 and 8.5 ppm)

number of N atoms (from HMBC): **4** (one nitrogen atom is not directly bonded to H)

all oxygen atoms are used now, no –OH group is possible.



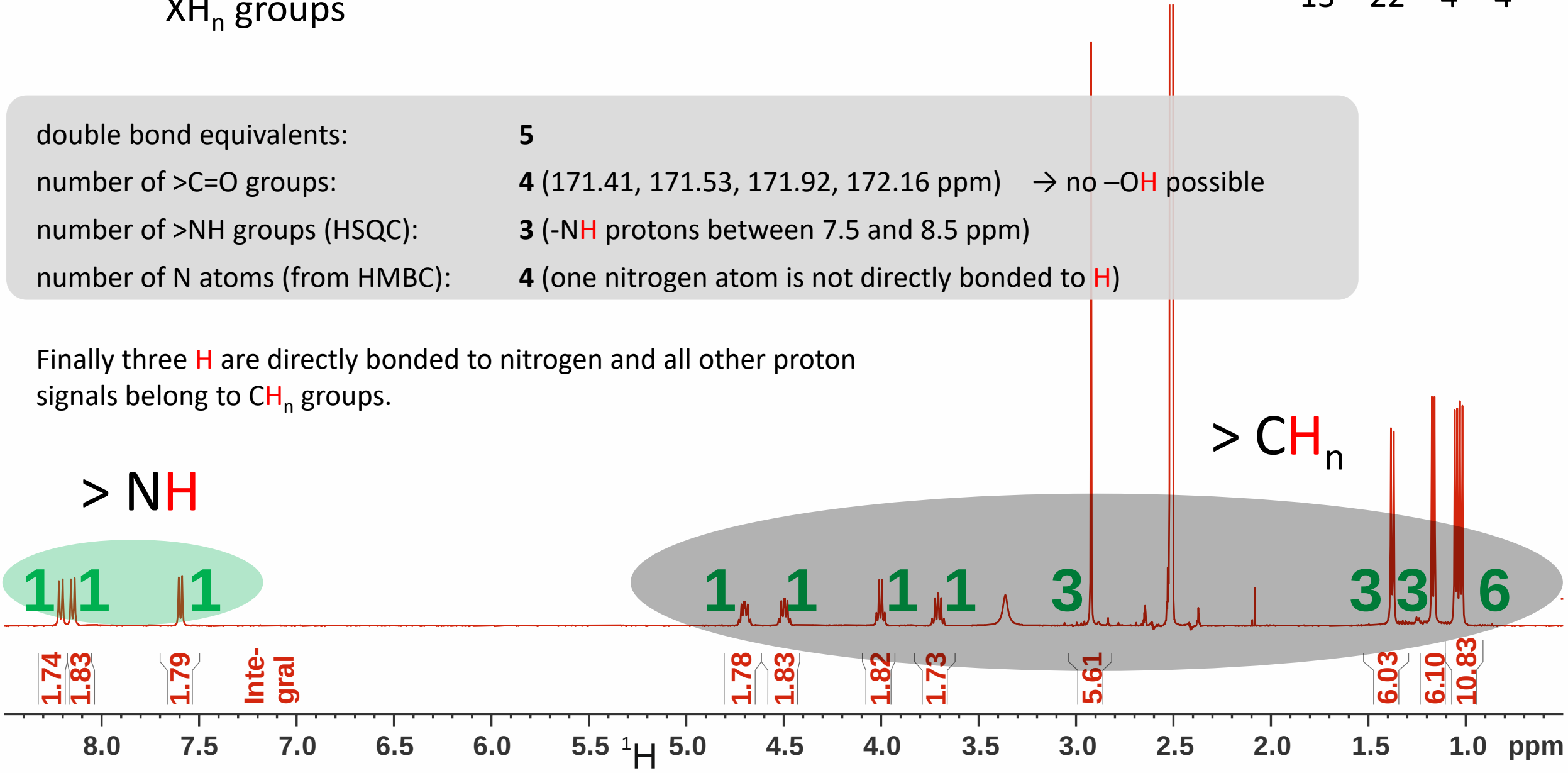
Basics

XH_n groups

$C_{13}H_{22}N_4O_4$

double bond equivalents:	**5**
number of >C=O groups:	**4** (171.41, 171.53, 171.92, 172.16 ppm) → no –OH possible
number of >NH groups (HSQC):	**3** (-NH protons between 7.5 and 8.5 ppm)
number of N atoms (from HMBC):	**4** (one nitrogen atom is not directly bonded to H)

Finally three H are directly bonded to nitrogen and all other proton signals belong to CH_n groups.



Basics

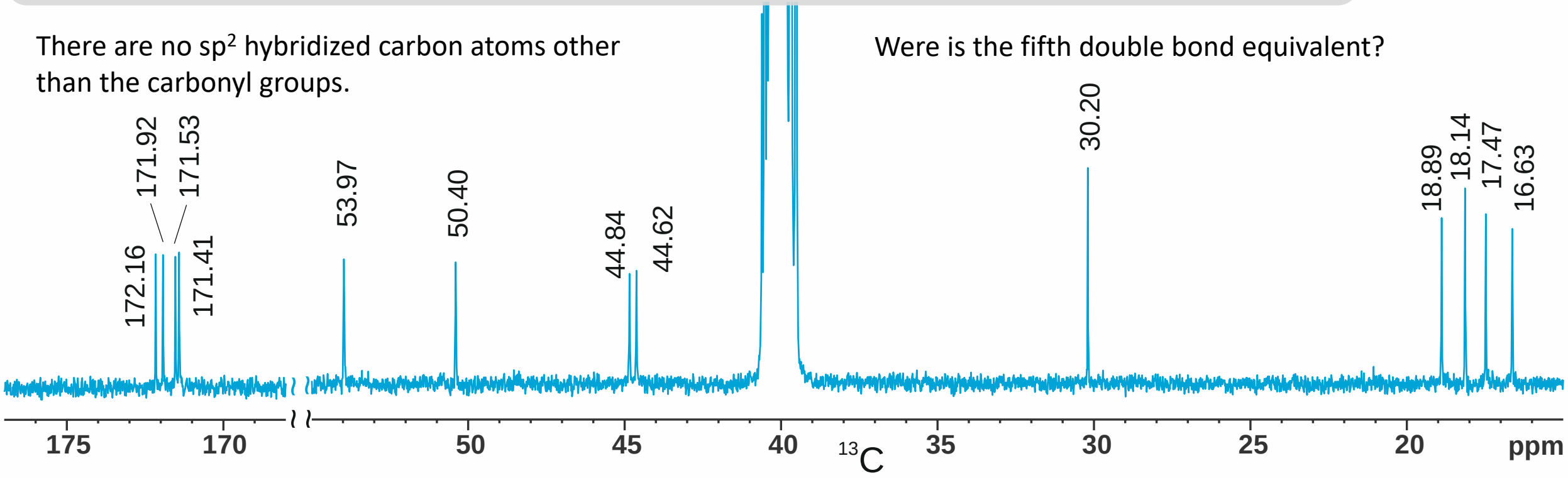
Double bond equivalents revisited

$C_{13}H_{22}N_4O_4$

double bond equivalents:	**5**
number of >C=O groups:	**4** (171.41, 171.53, 171.92, 172.16 ppm) → no –OH possible
number of >NH groups (HSQC):	**3** (-NH protons between 7.5 and 8.5 ppm)
number of N atoms (from HMBC):	**4** (one nitrogen atom is not directly bonded to H)

There are no sp^2 hybridized carbon atoms other than the carbonyl groups.

Were is the fifth double bond equivalent?



Amino acids

We figured out:

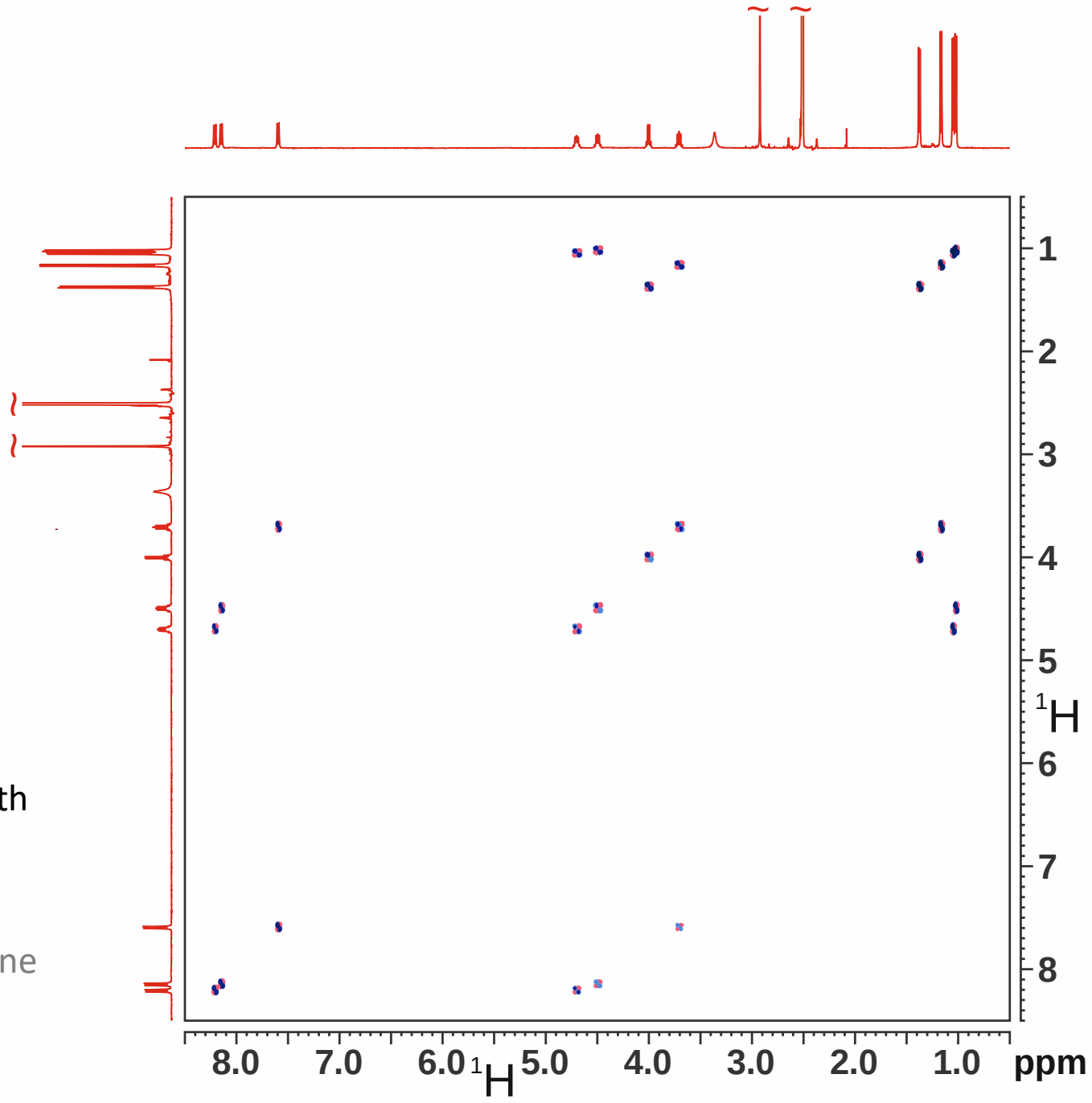
- the three doublets with chemical shifts larger than 7 ppm belong to NH protons.
- all other protons belong to CH_n groups

If we start in a COSY from a NH proton and follow the coupling path as long as possible we will find chains of the kind

– NH – CH_n – CH_n – CH_n –

Because there are three NH protons we should end with three of such chains.

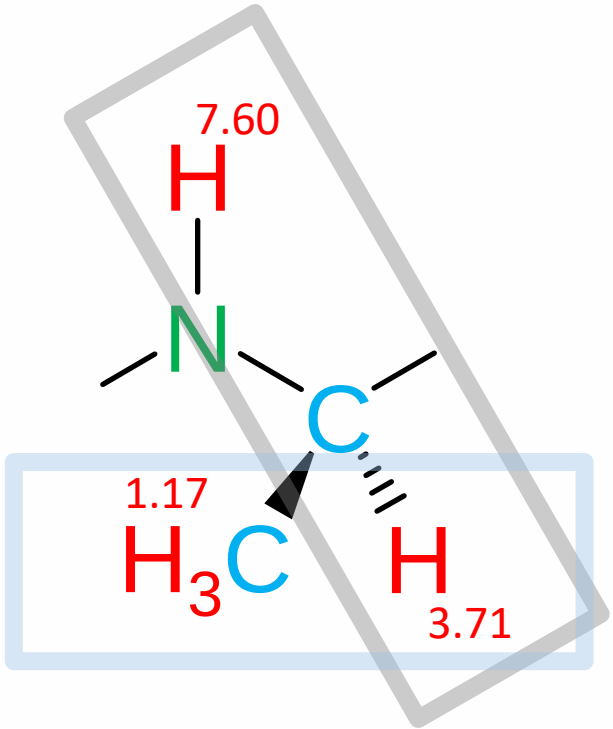
To help us keep an overview, let‘s label the signals of one spin system after the other and remove all signals already assigned.

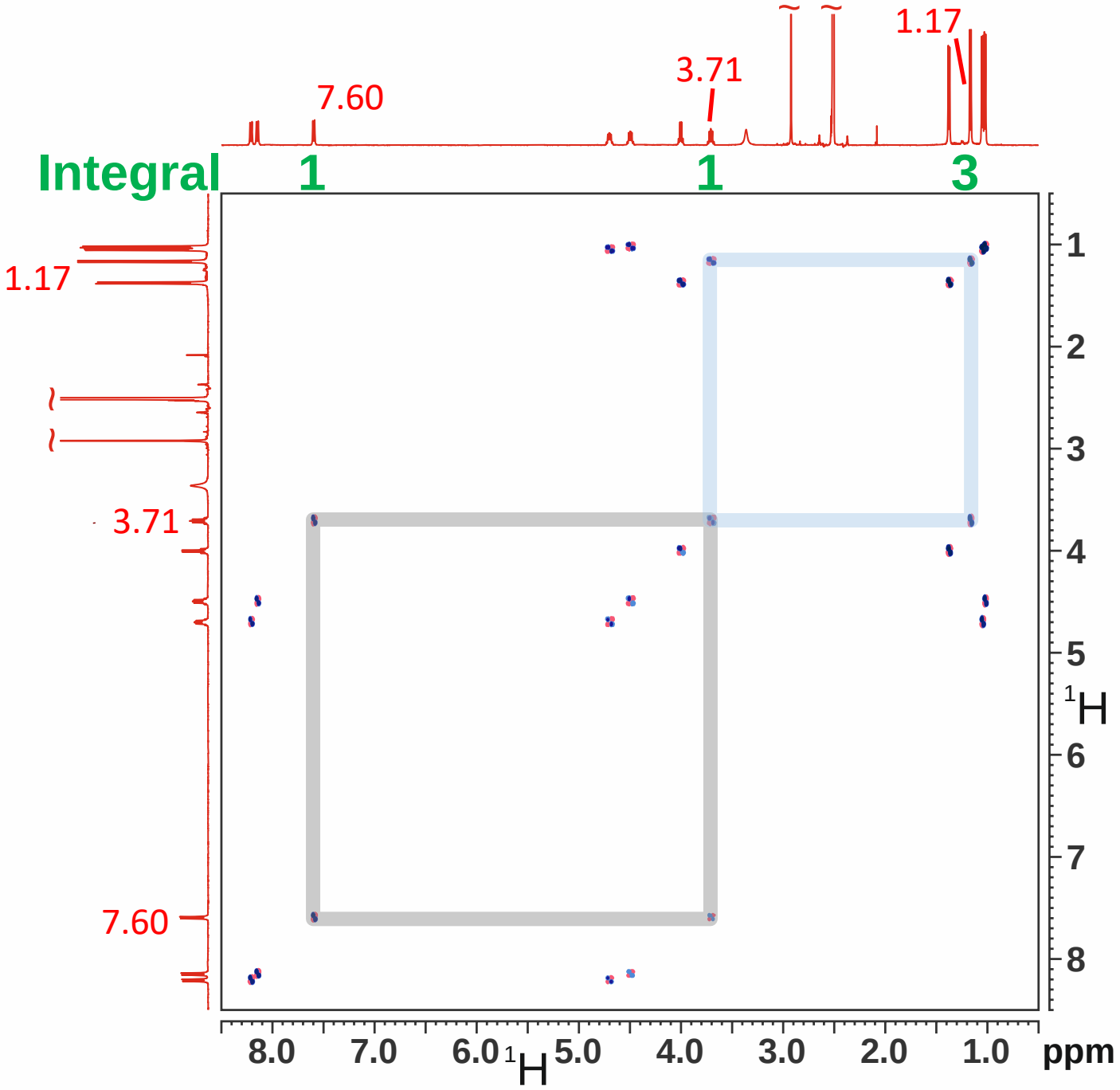


Amino acids

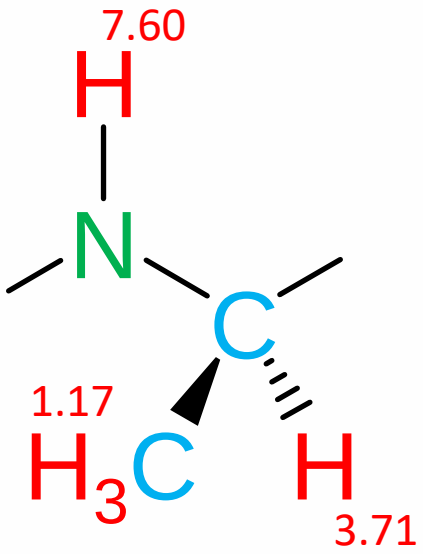
The protons with the chemical shifts of 7.60 ppm and 3.71 pm are neighbours. Please remember, in this case chemical shifts large than 7 ppm mean NH and everything else CH_n. In our case according to the integral n is **1**.

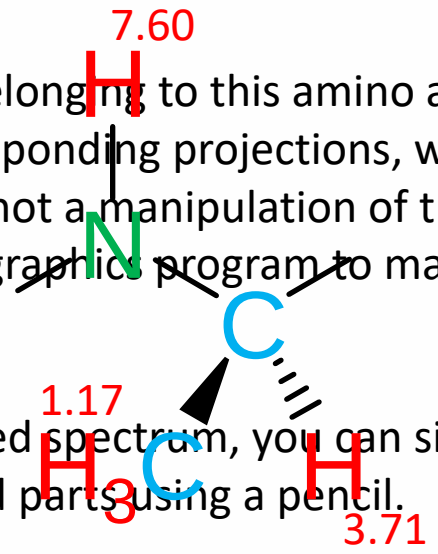
The next pair of neighbours are the protons with the chemical shifts of 3.71 ppm and 1.17 ppm. Please don‘t forget the integral of **3** for the signal at 1.17 ppm.





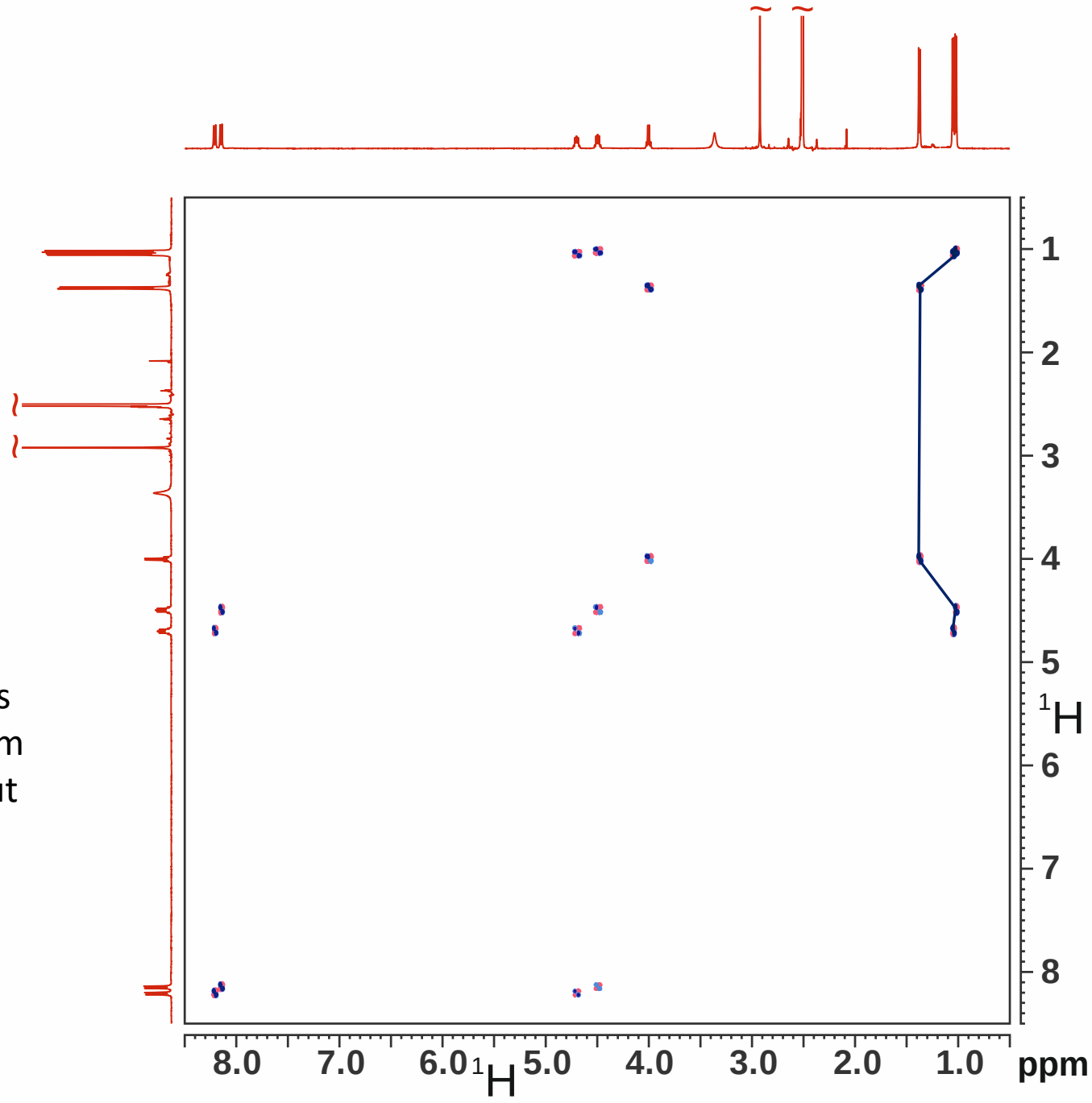
Amino acids



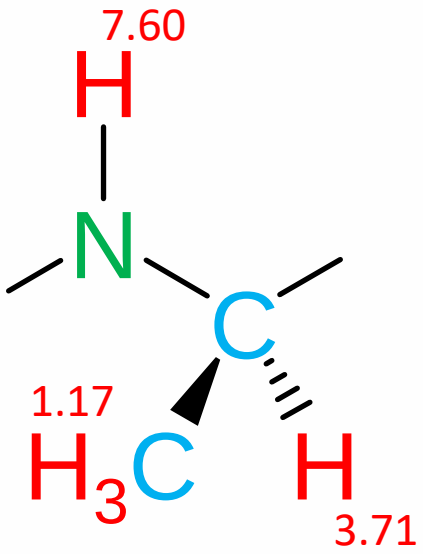


All cross peaks belonging to this amino acid fragment, as well as the corresponding projections, were deleted from the COSY. This is not a manipulation of the spectrum, but was done in the graphics program to make the further evaluation easier.

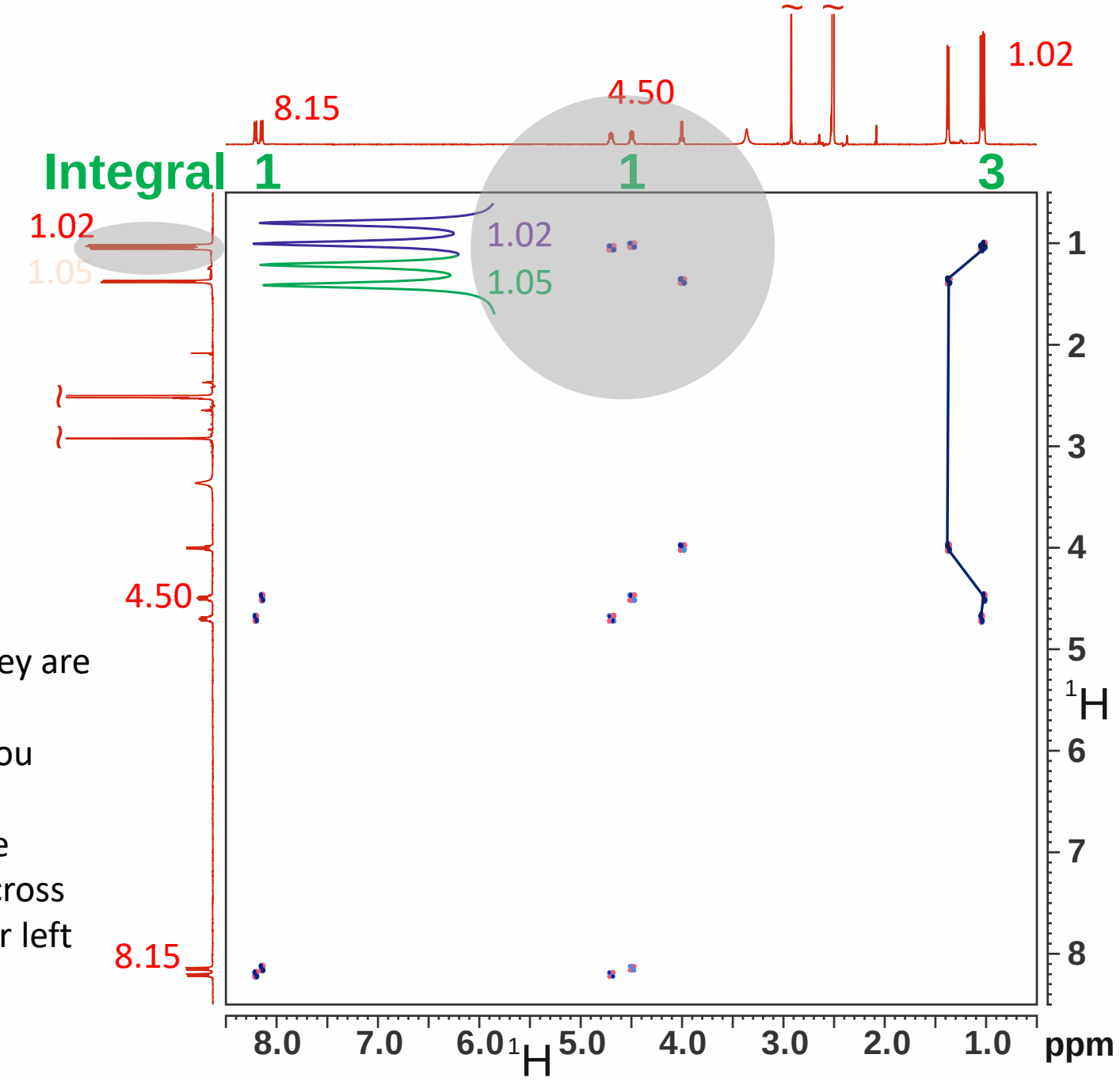
In case of a printed spectrum, you can simply cross out already evaluated parts using a pencil.



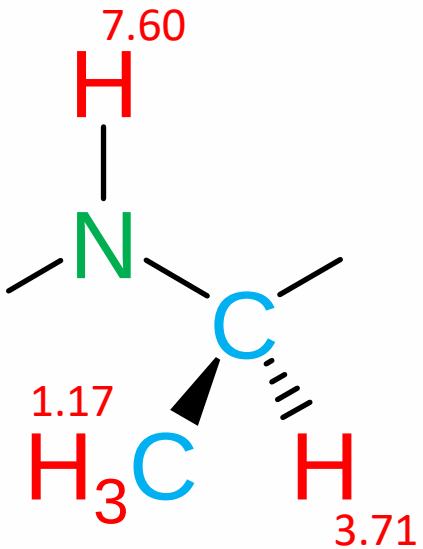
Amino acids



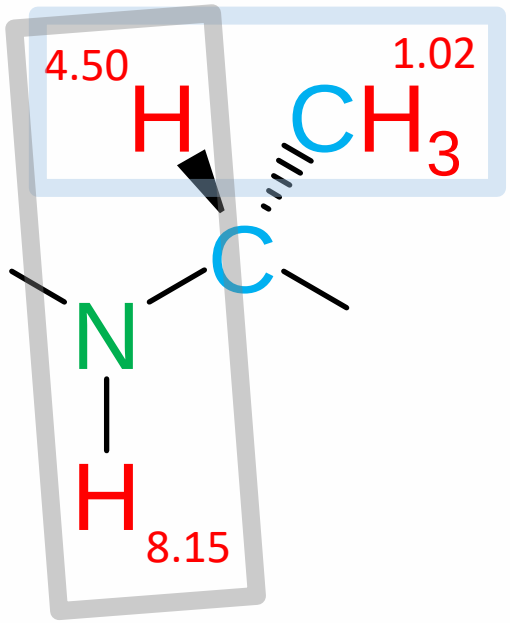
Two signals at 1.02 and 1.05 ppm are very similar. They are easily distinguishable in the one-dimensional proton spectrum, but not in the projections of the COSY. If you take an expansion of the one-dimensional proton spectrum to better recognize the crowded part of the projections, you see that the upper right of the two cross peaks leads to the doublet at 1.02 ppm and the lower left cross peak leads to the doublet at 1.05 ppm.



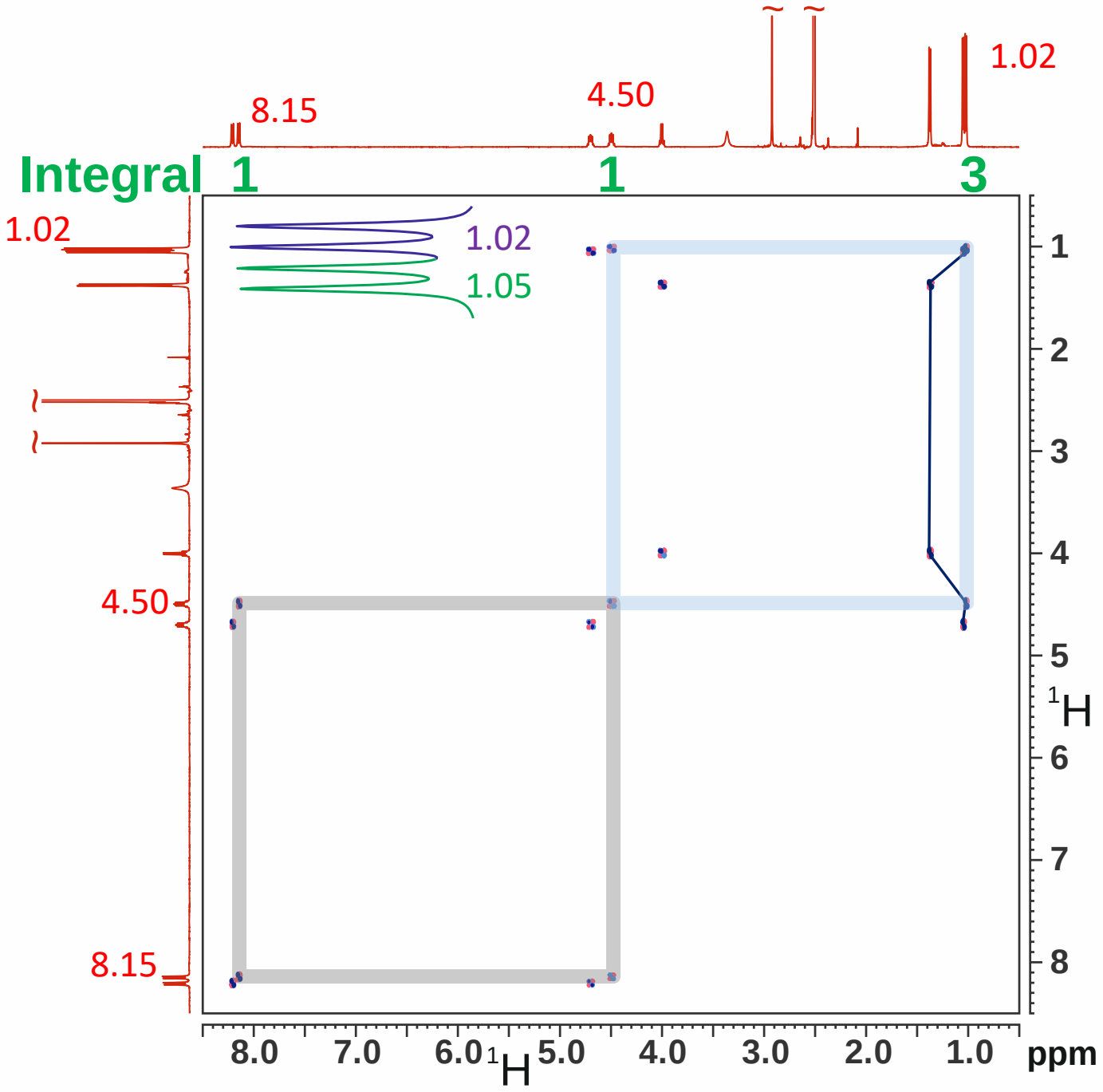
Amino acids



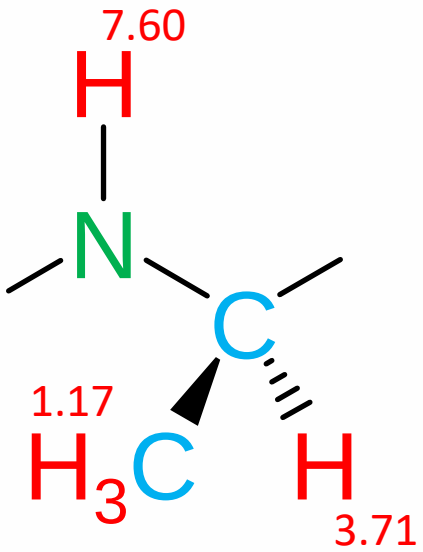
The further procedure is the same as for the first amino acid residue. There is a connection between the protons with the chemical shifts of 8.15 ppm and 4.50 ppm.



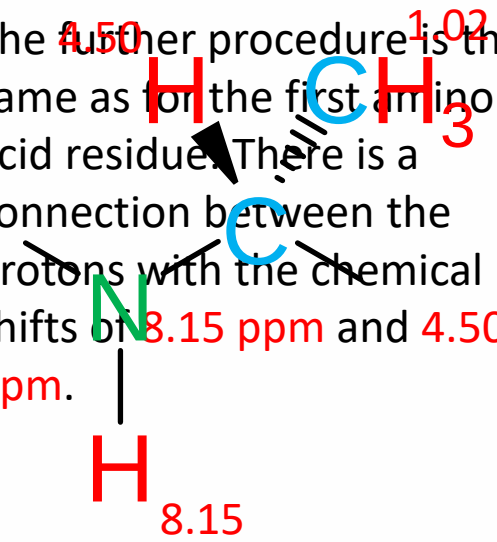
The next connection is between the atoms with the chemical shifts of 4.50 ppm and 1.02 ppm.

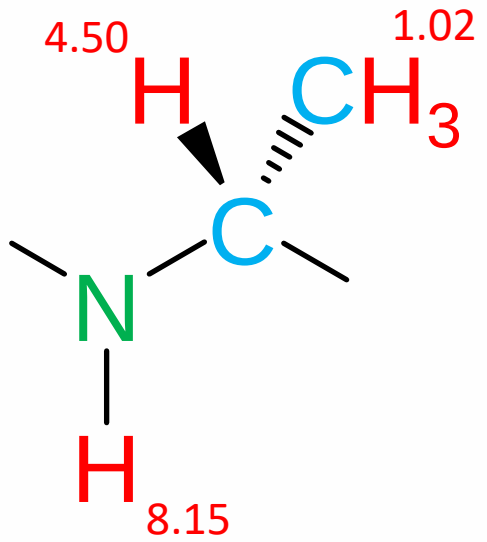


Amino acids

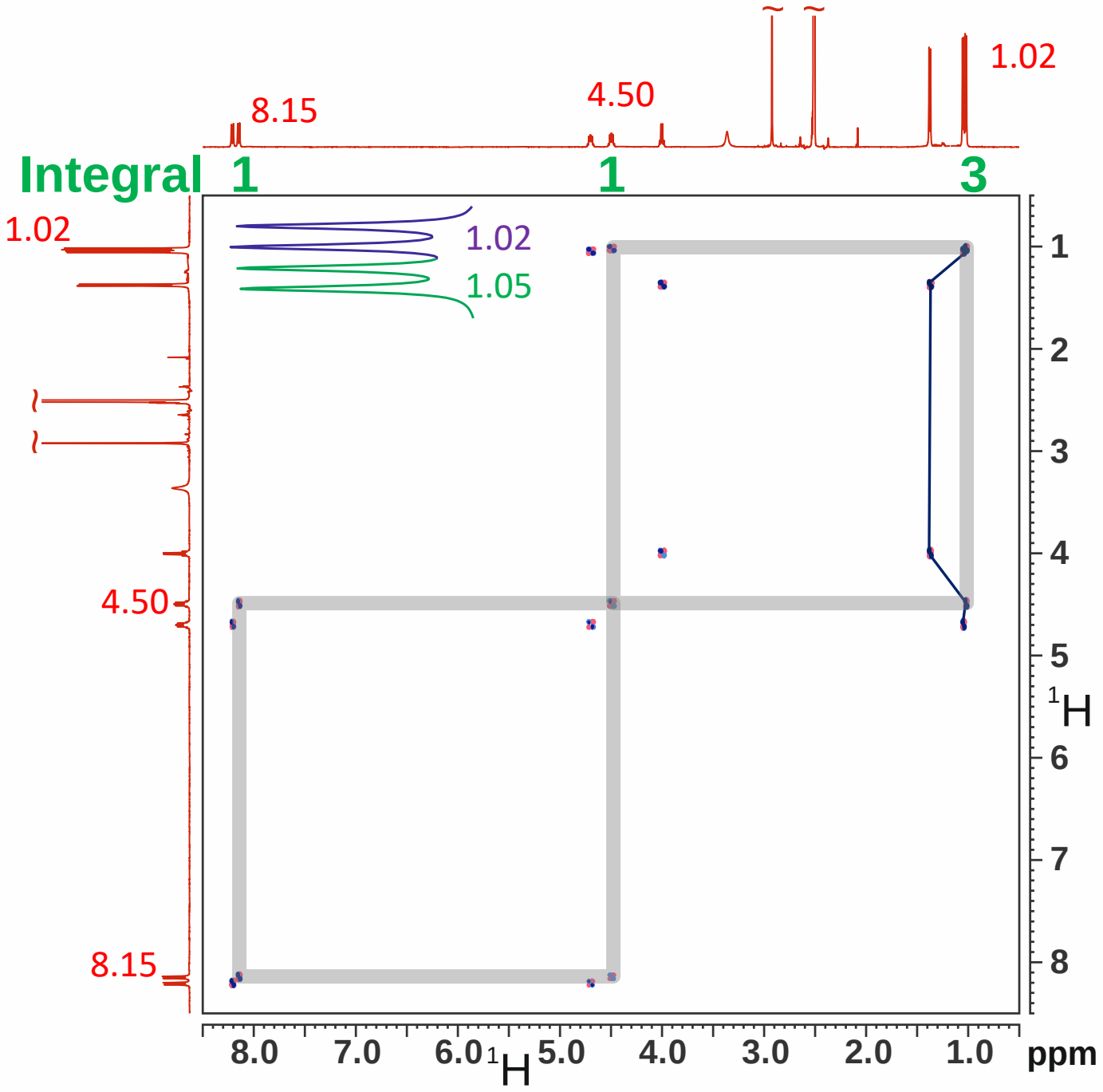


The further procedure is the same as for the first amino acid residue. There is a connection between the protons with the chemical shifts of 8.15 ppm and 4.50 ppm.

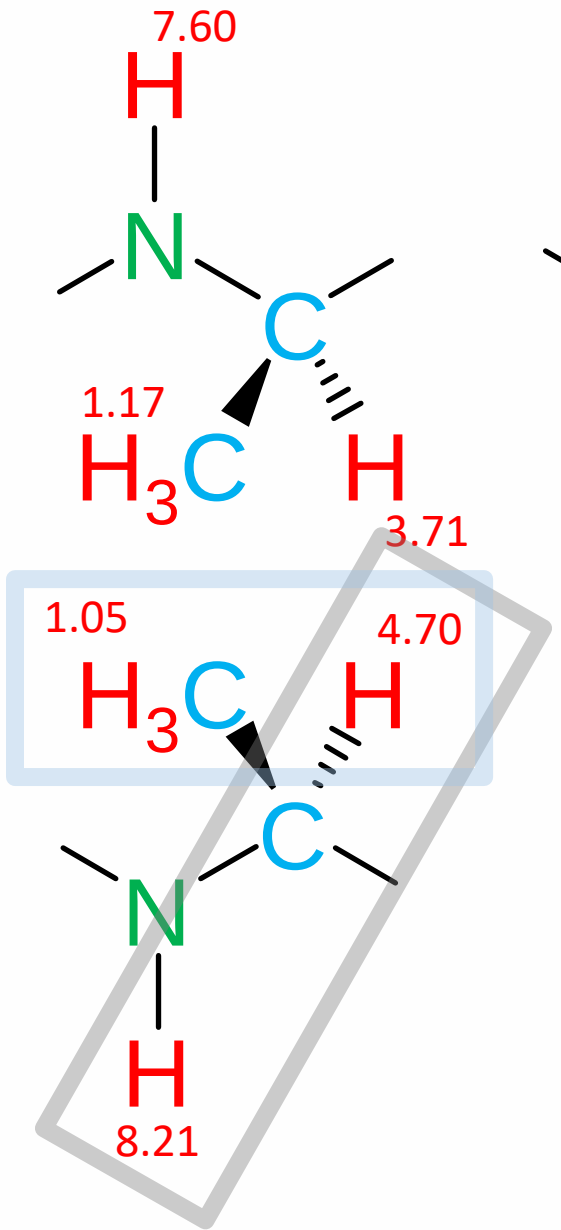


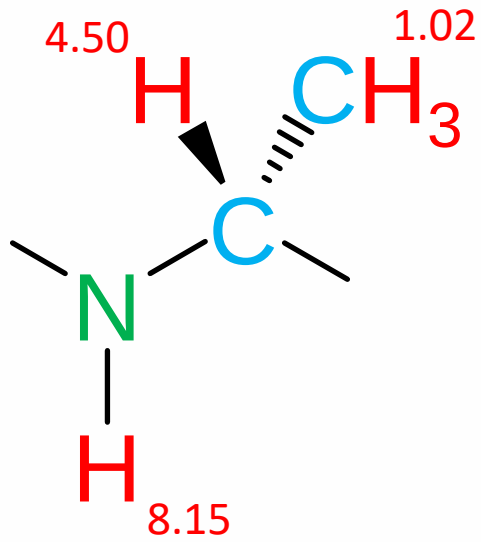


The next connection is between the atoms with the chmical shifts of 4.50 ppm and 1.02 ppm.



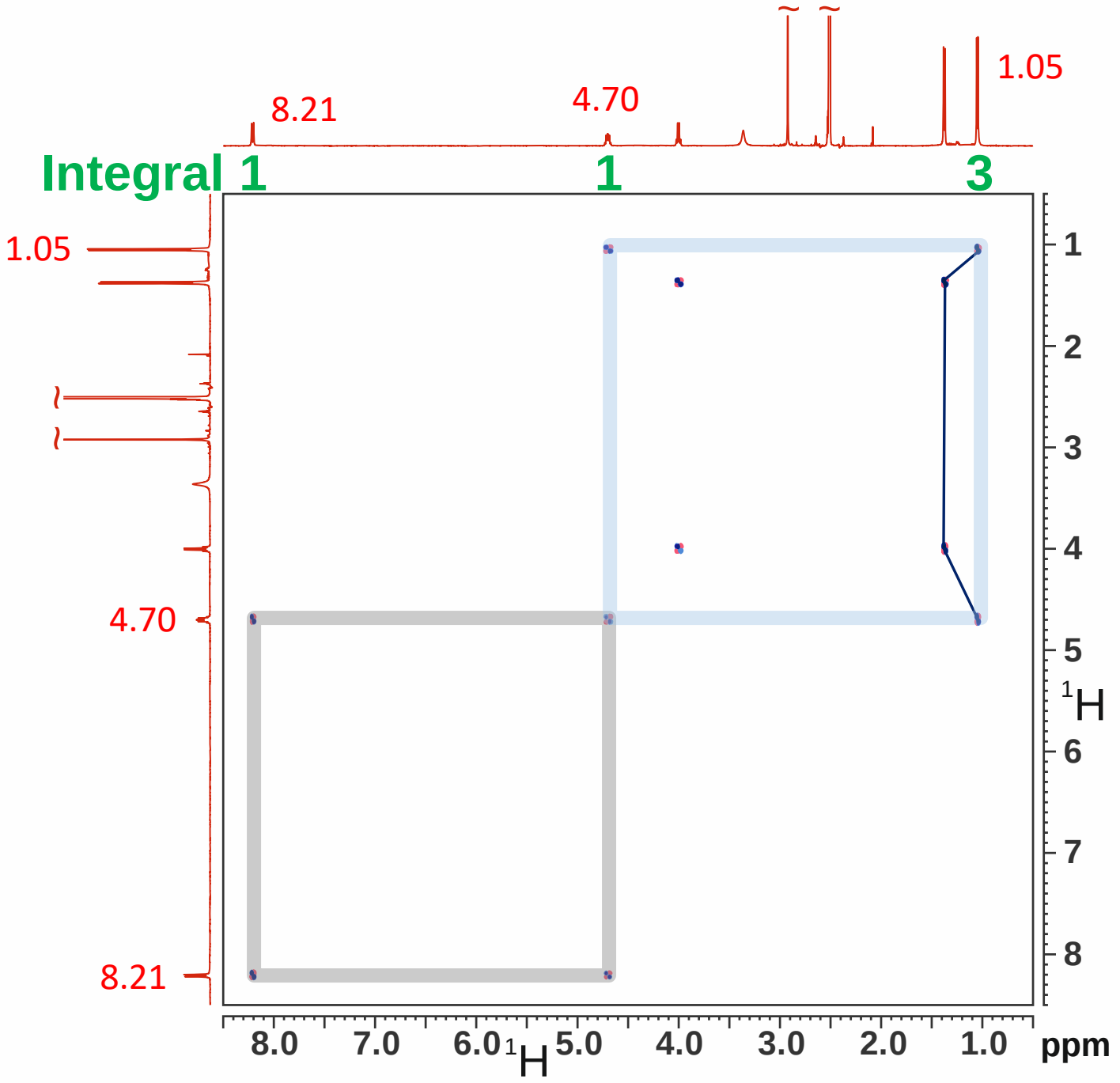
Amino acids



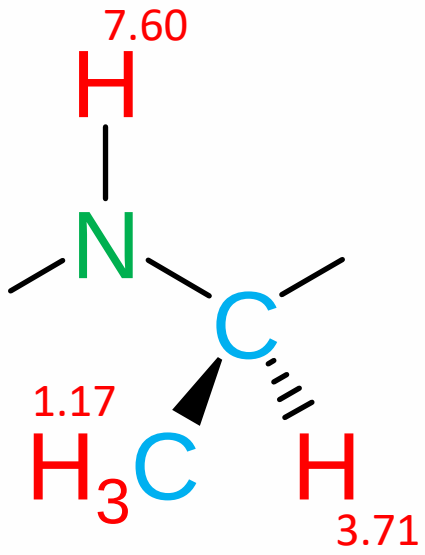


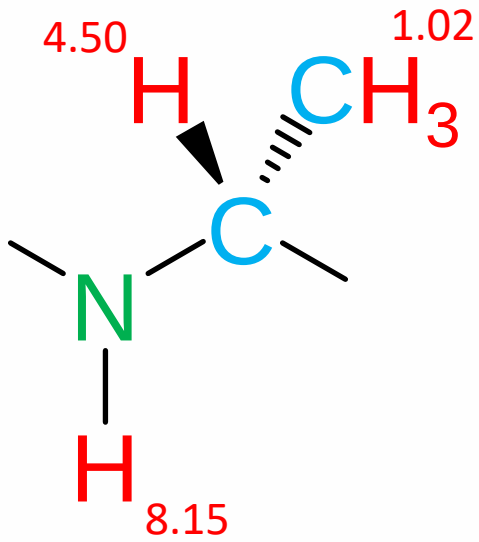
8.21 ppm → 4.70 ppm

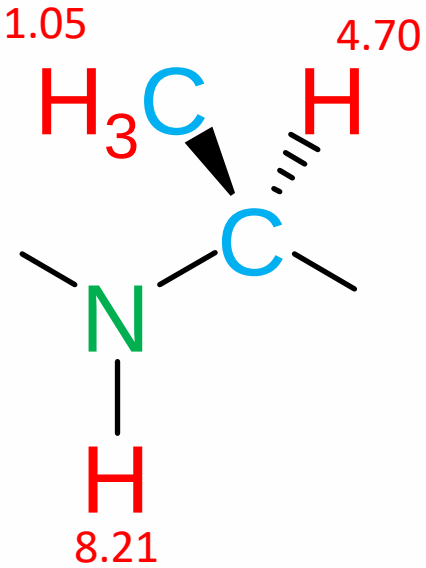
4.70 ppm → 1.05ppm



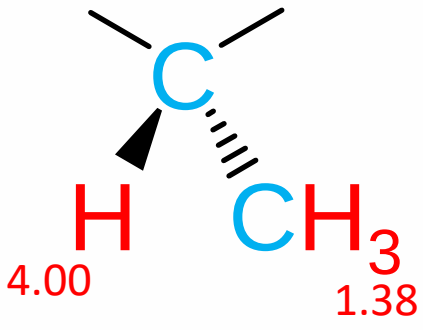
Amino acids

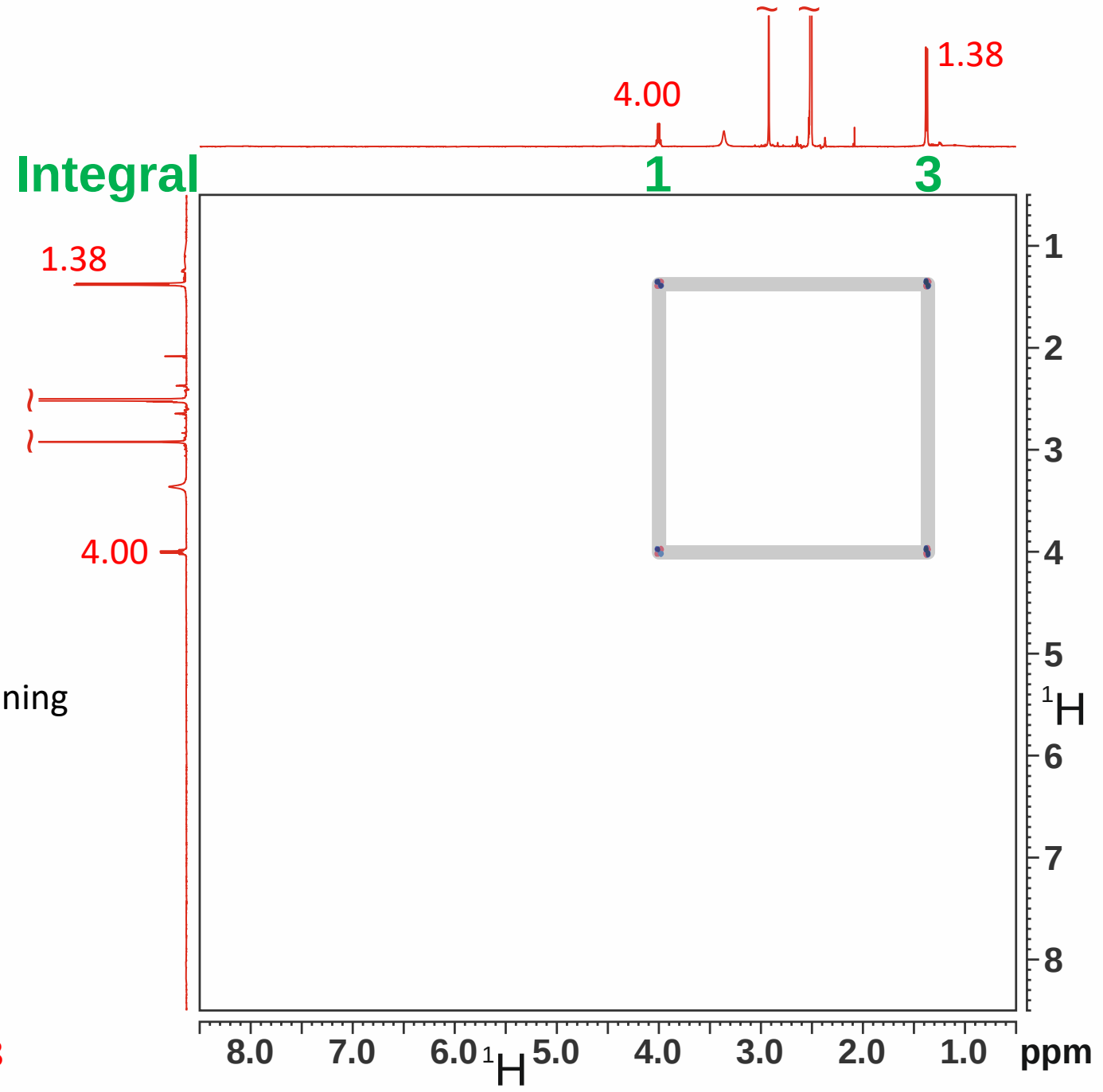




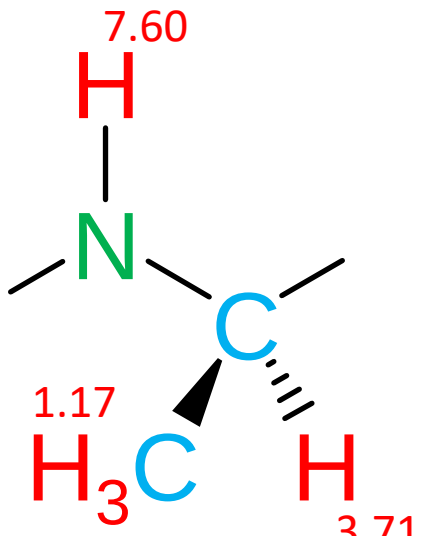


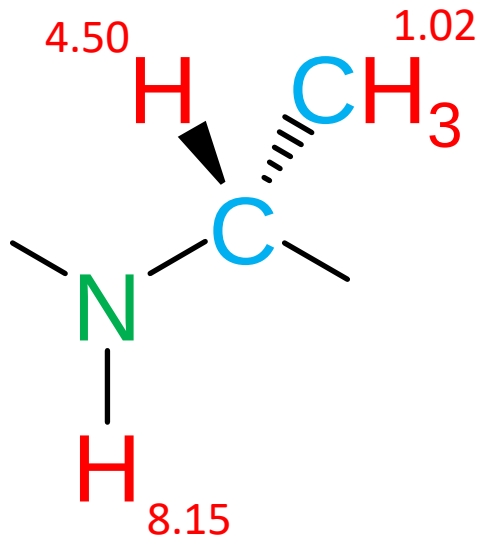
There is only one remaining correlation in the COSY.

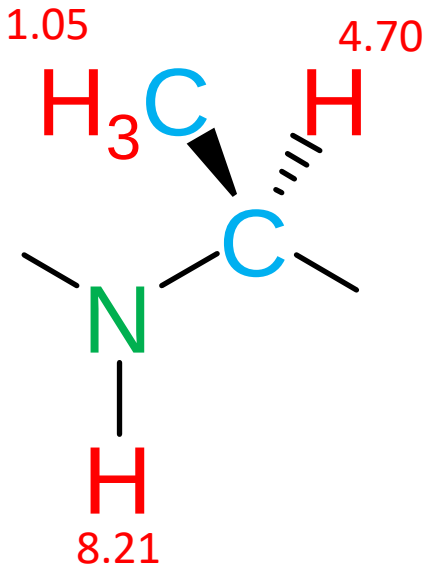


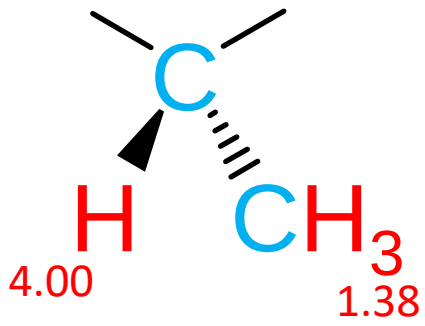


Intermediate inventory









we have

- four double bond equivalents
- four carbonyl groups (C_4O_4)
- for CHN fragments ($C_8H_{19}N_3$)
- all fragments together ($C_{12}H_{19}N_3O_4$)

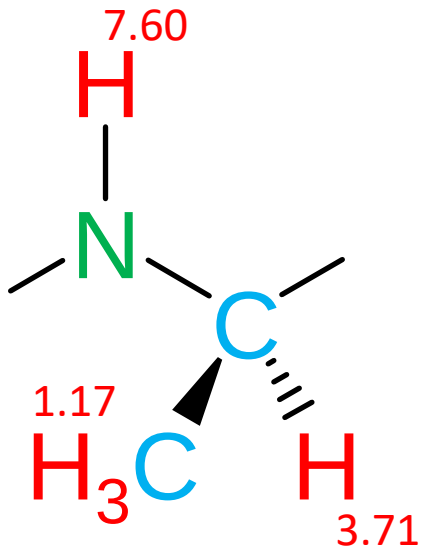
we need

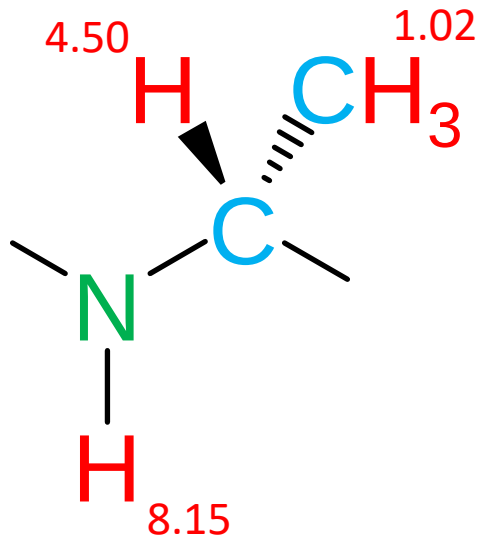
- five double bond equivalents
- molecular formula $C_{13}H_{22}N_4O_4$

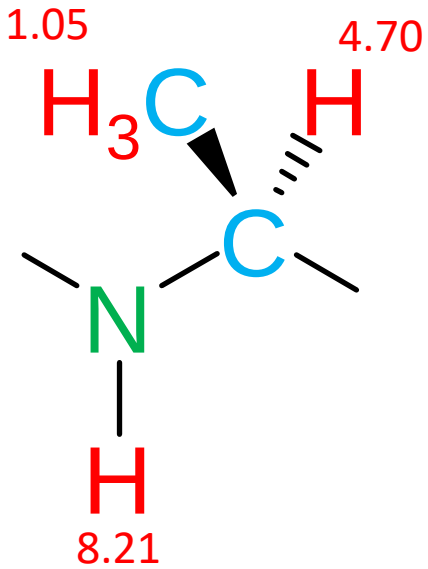
missing

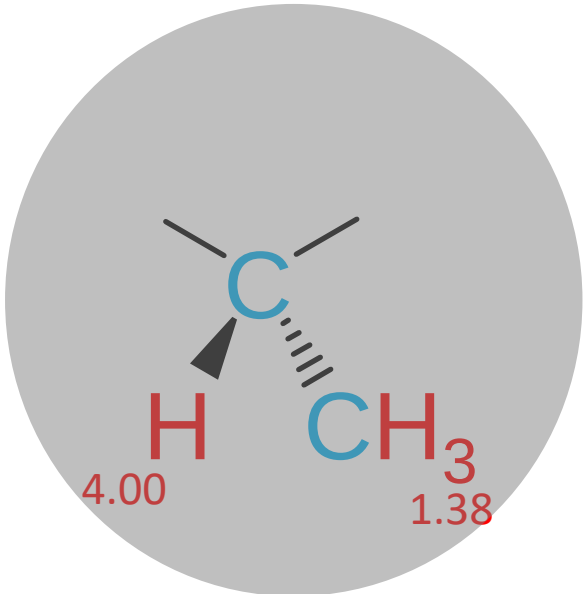
- **one double bond equivalent**
- **CH_3N**

Intermediate inventory









missing

- **one double bond equivalent**
- **CH_3N**

Even though there is no spectroscopic evidence yet, we can speculate a bit about these missing pieces of the puzzle at this point.

The last fragment extracted will probably also be an amino acid. An additional >NH group is clearly not present, but what about an $>NCH_3$ group?

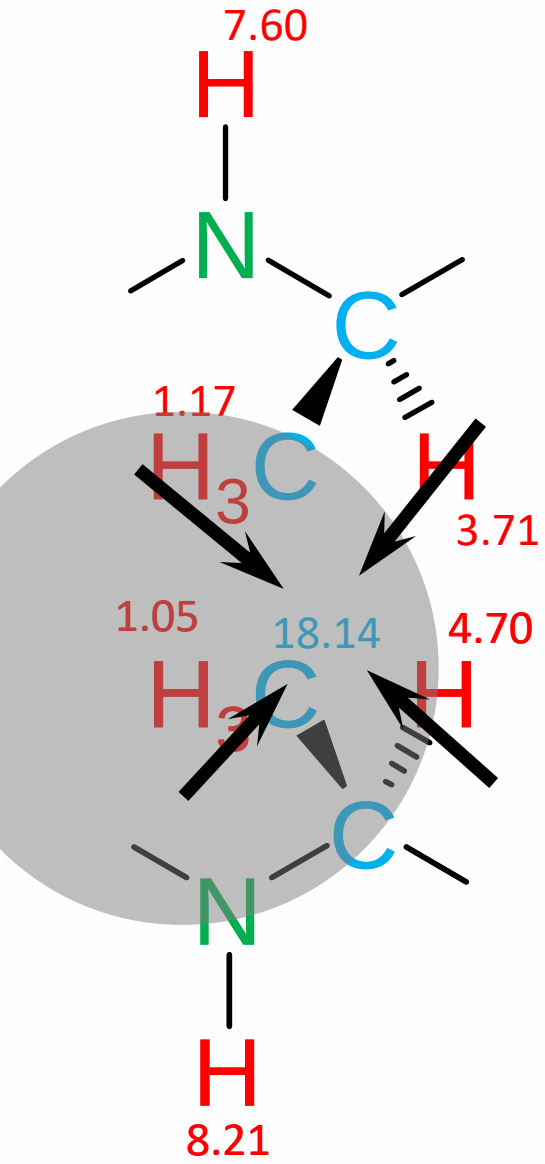
We would then have assigned all atoms, but only accounted for four double bond equivalents. The fifth double bond equivalent should be a ring closure.

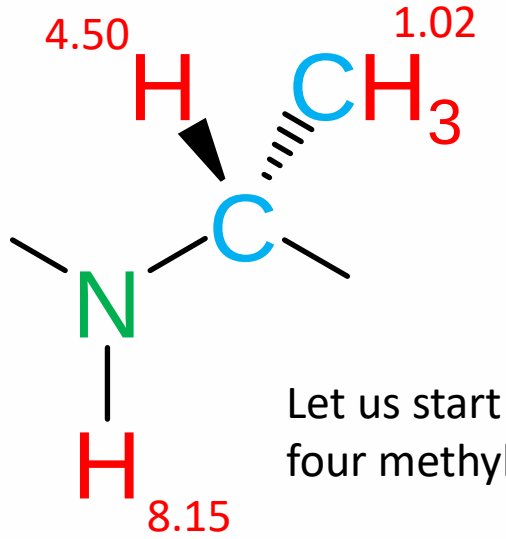
missing

After these speculations, let us return to serious NMR and assign all carbon and nitrogen signals.

- **one double bond equivalent**
- **CH_3N**

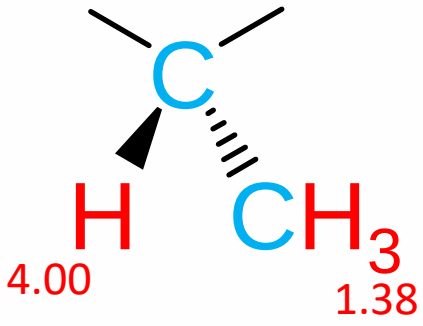
Carbon atom assignment (I)

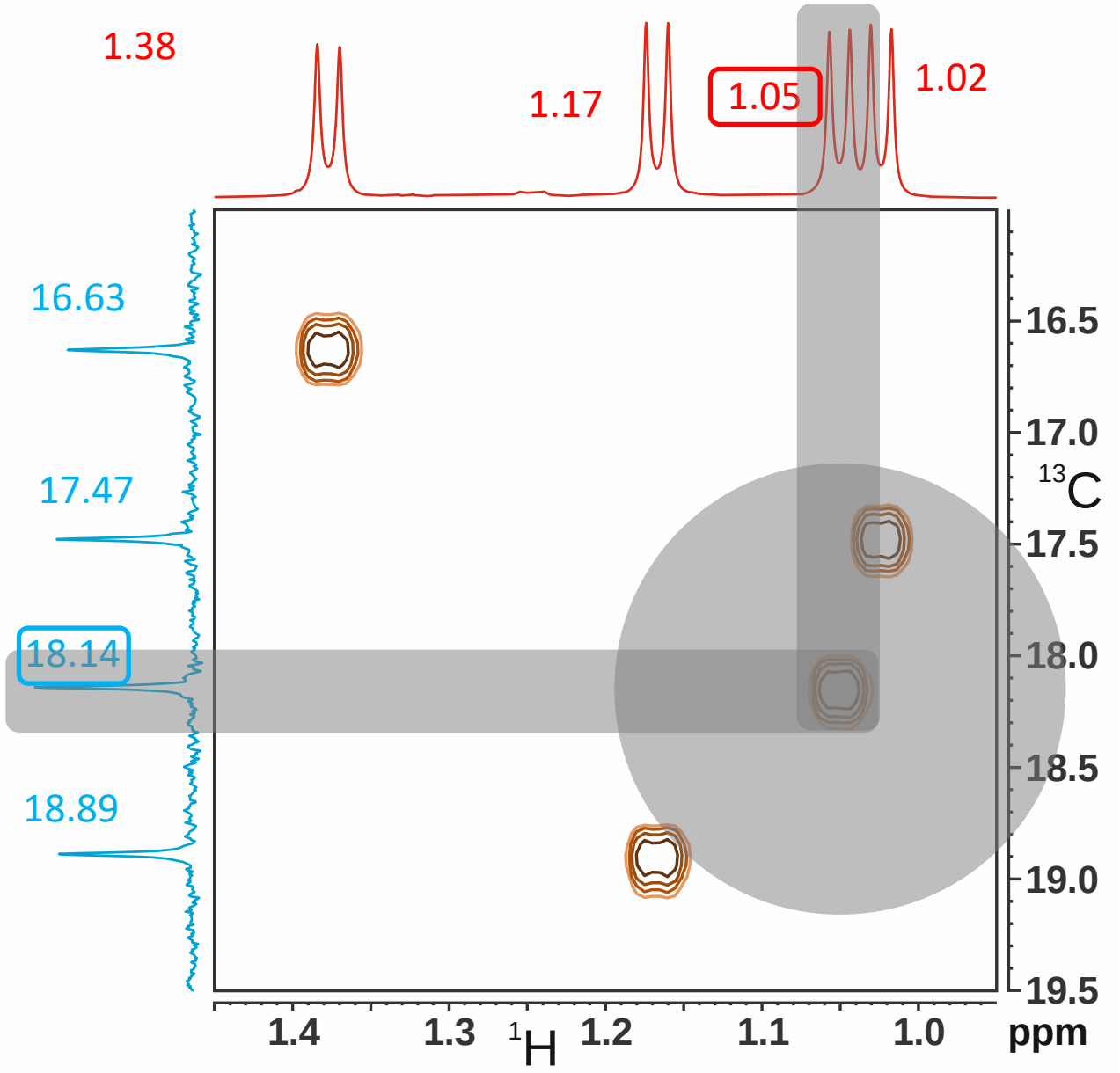




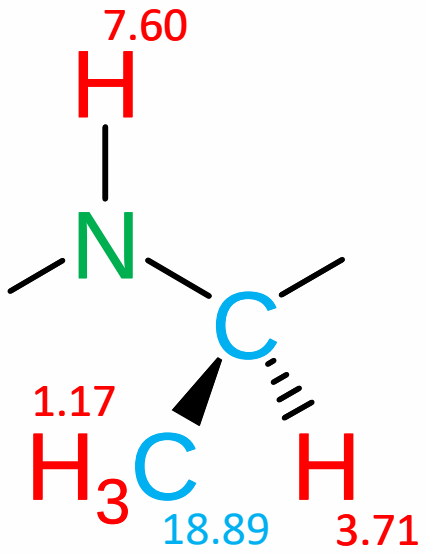
Let us start with the four methyl groups.

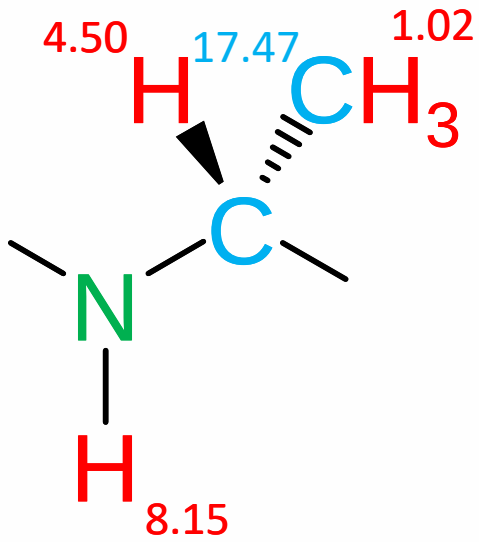
Here is one exemple, which may be used as a template for all four methyl groups.

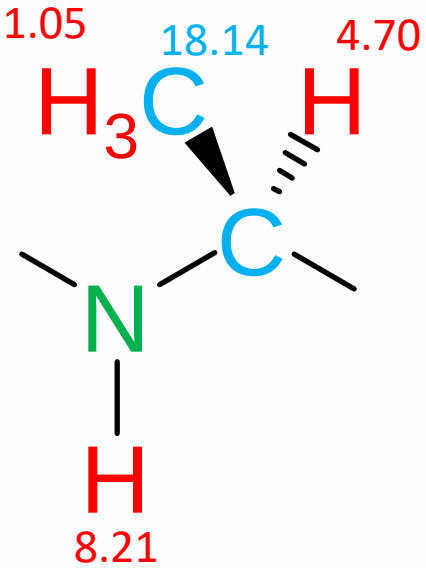




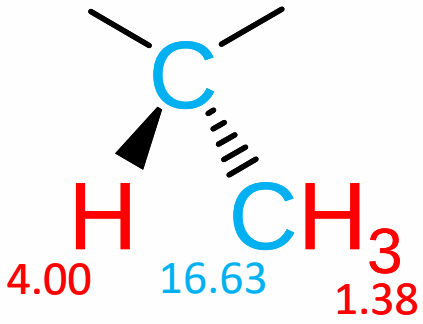
Carbon atom assignment (I)

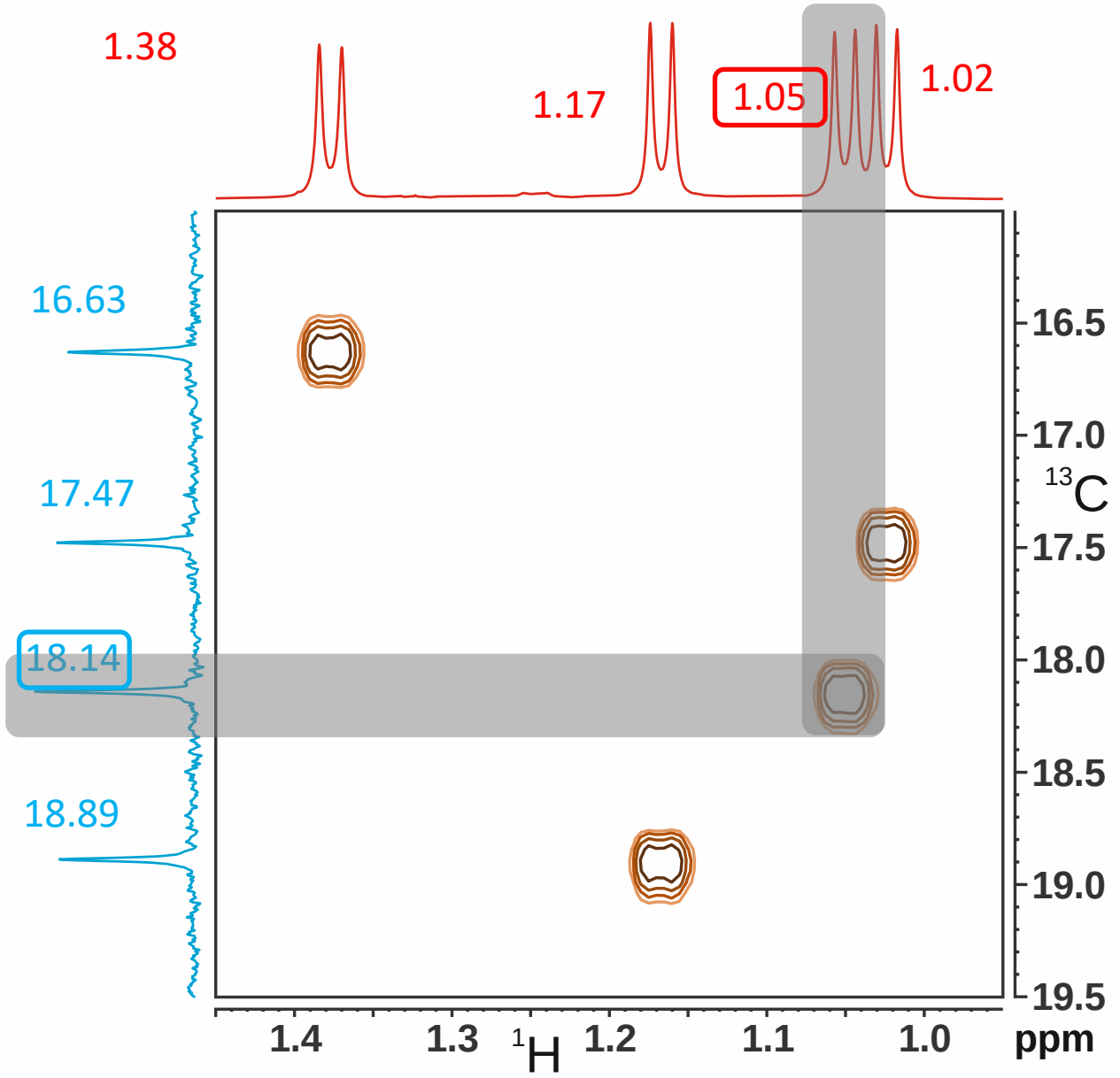




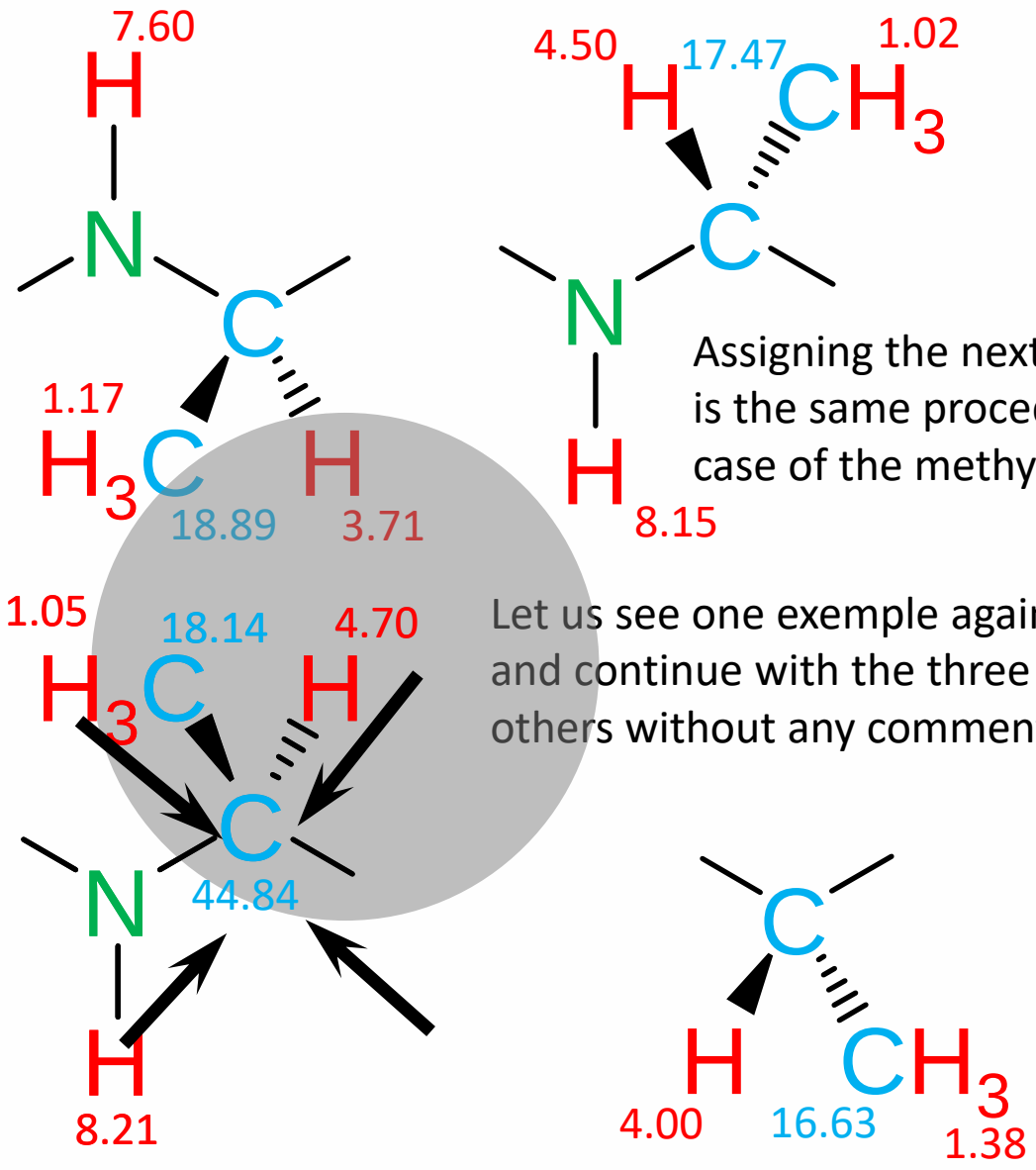


The procedure for the three other methyl groups is the same.



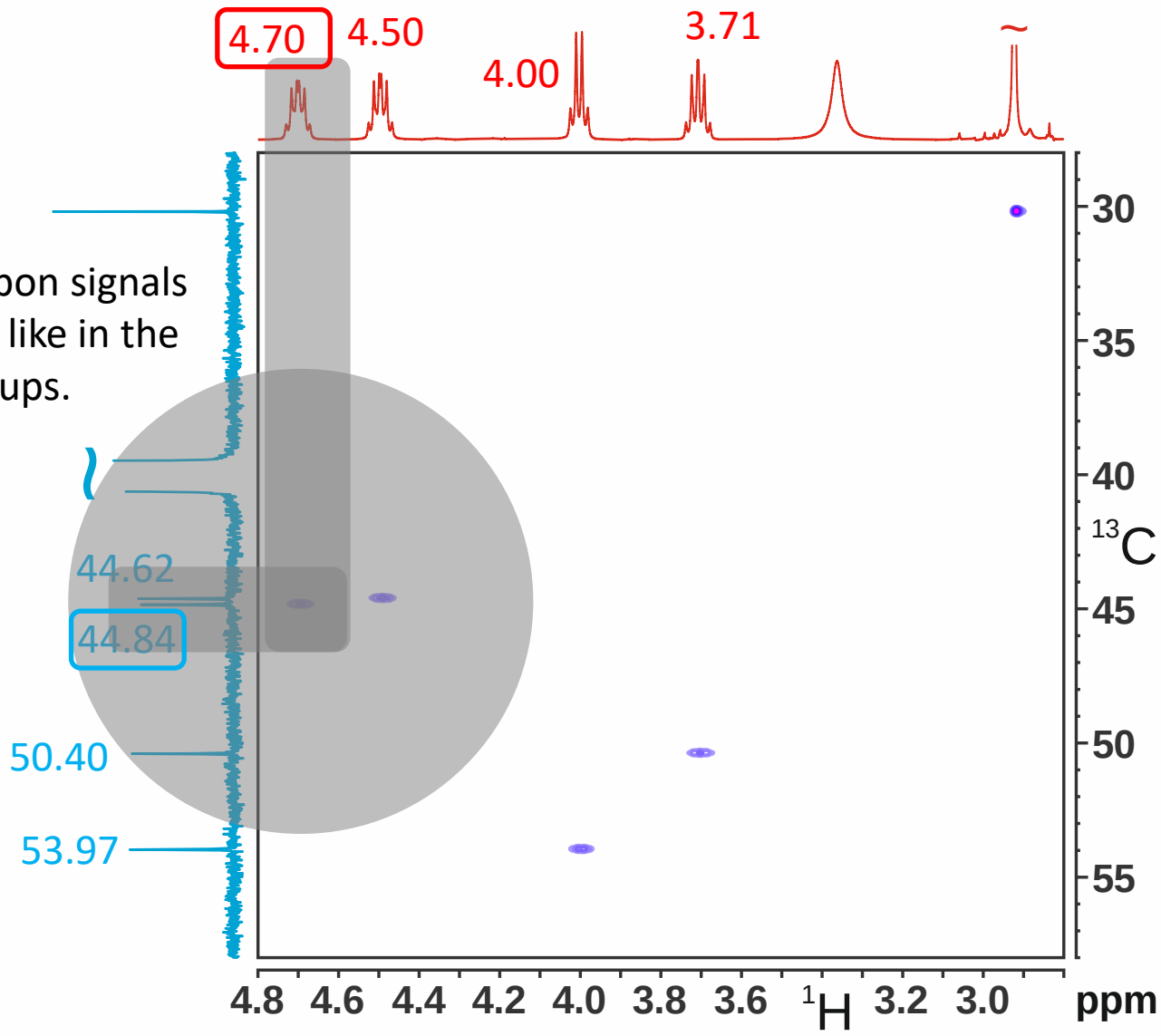


Carbon atom assignment (II)

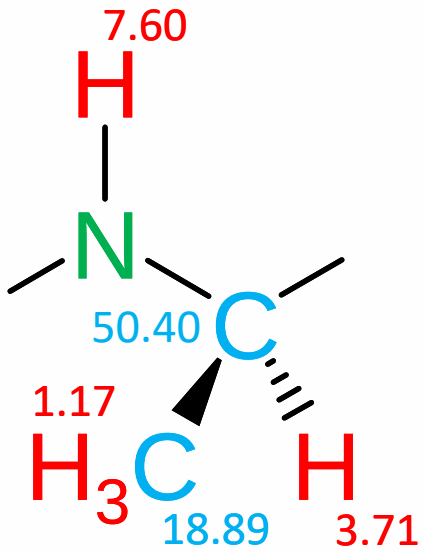


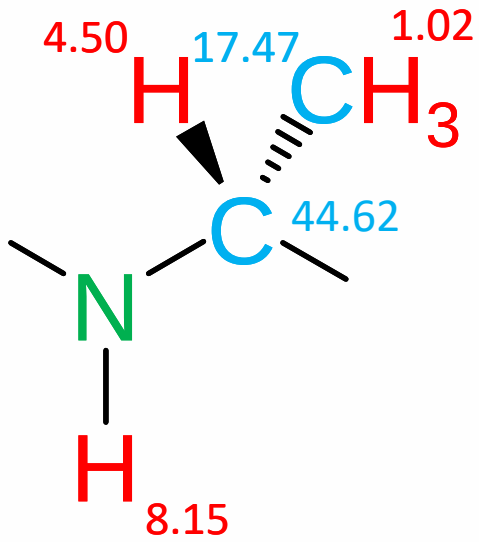
Assigning the next carbon signals is the same procedure like in the case of the methyl groups.

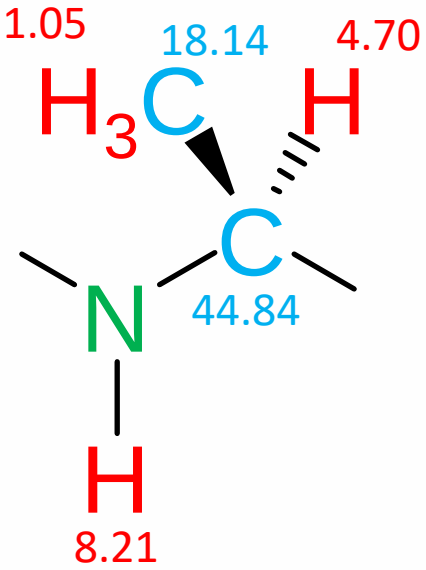
Let us see one exemple again and continue with the three others without any comment.



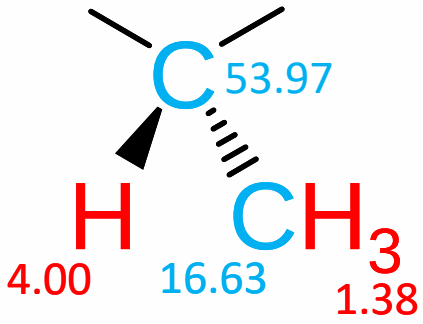
Carbon atom assignment (II)

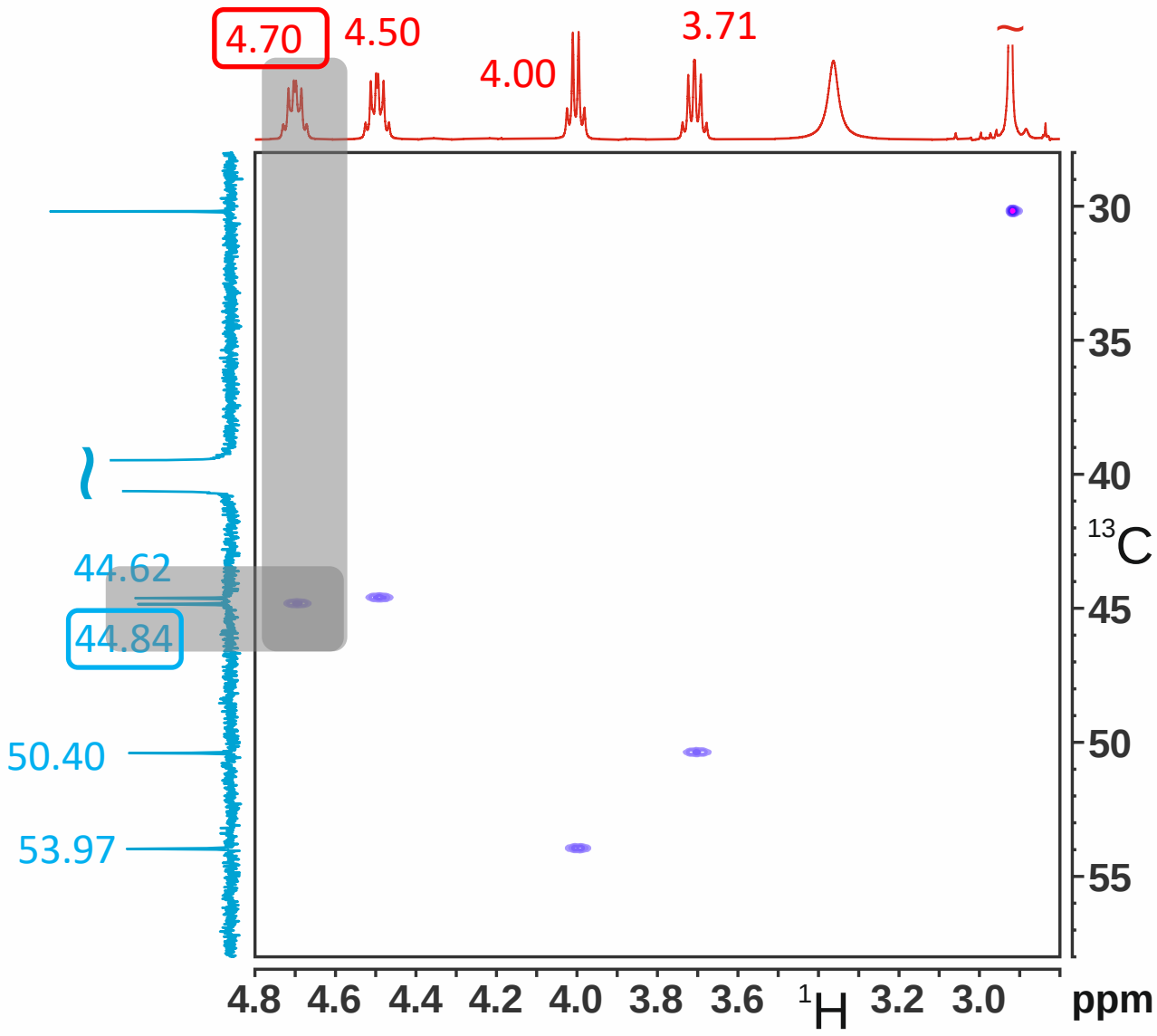




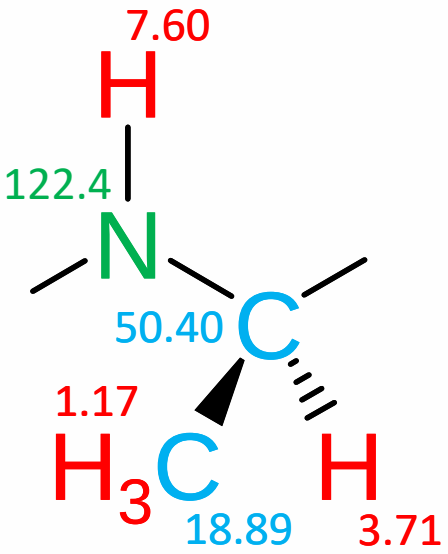


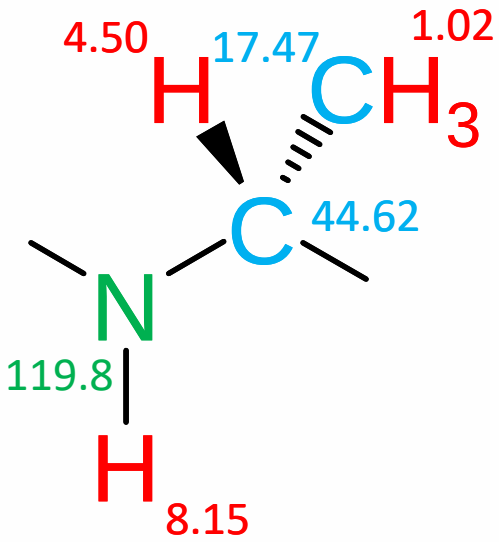
Now let‘s assign the three remaining carbon atoms using the same strategy.

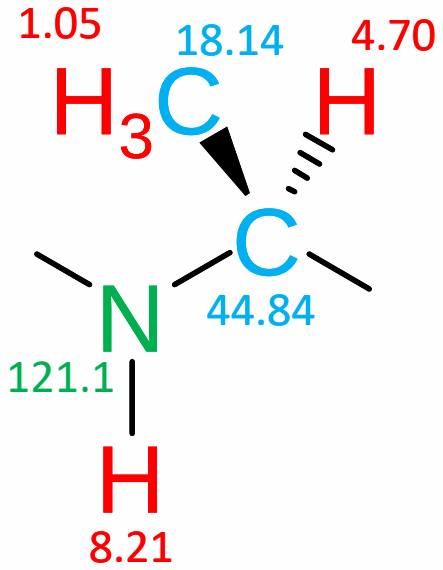




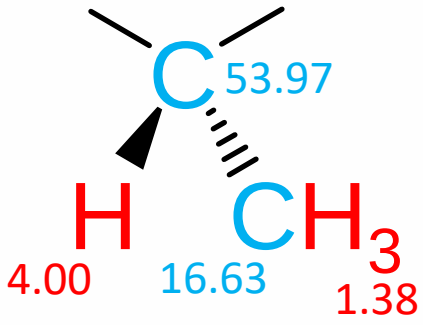
Nitrogen atom assignment

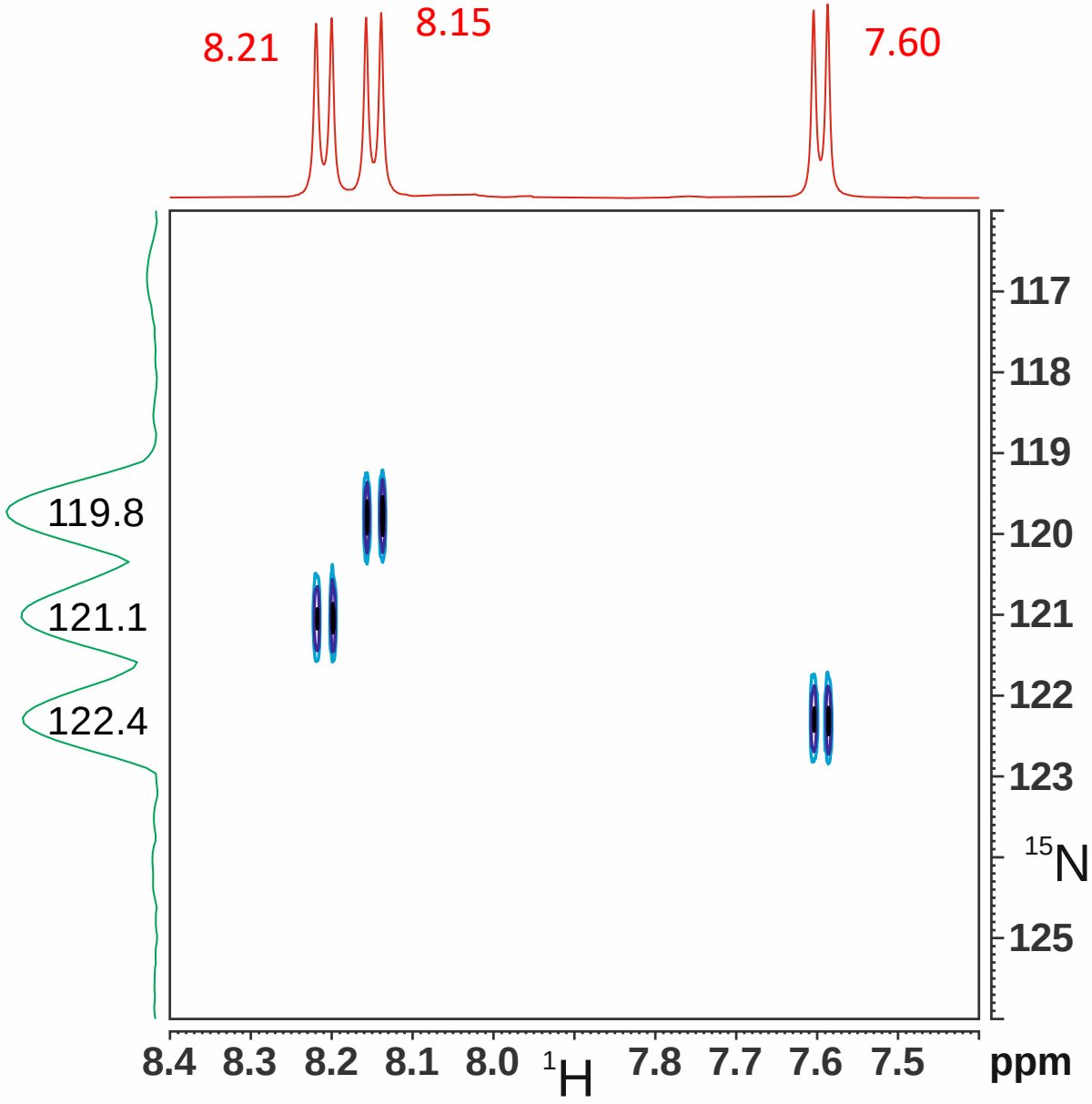




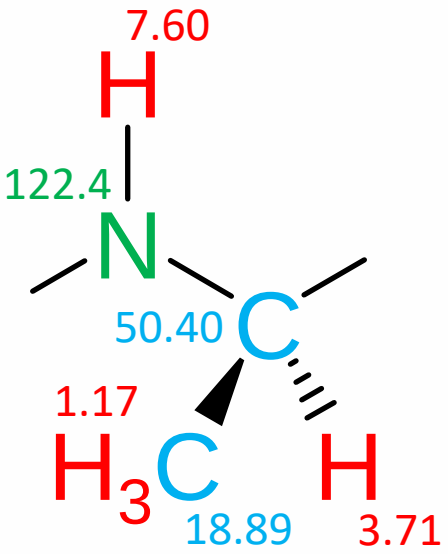


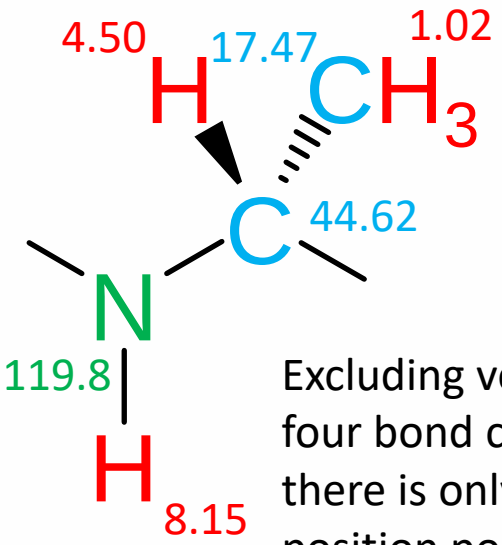
If we repeat the procedure a last time, we get the correct assignment of all protonated nitrogen atoms.

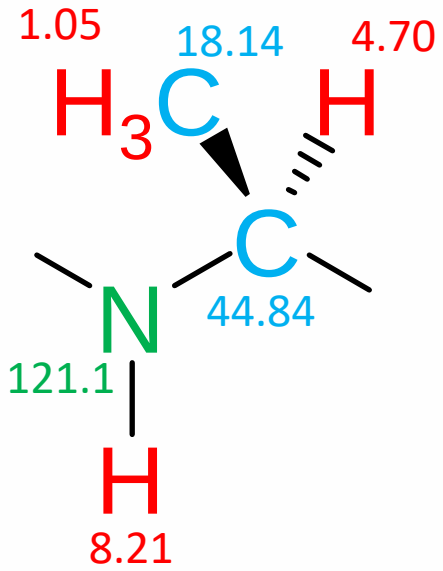




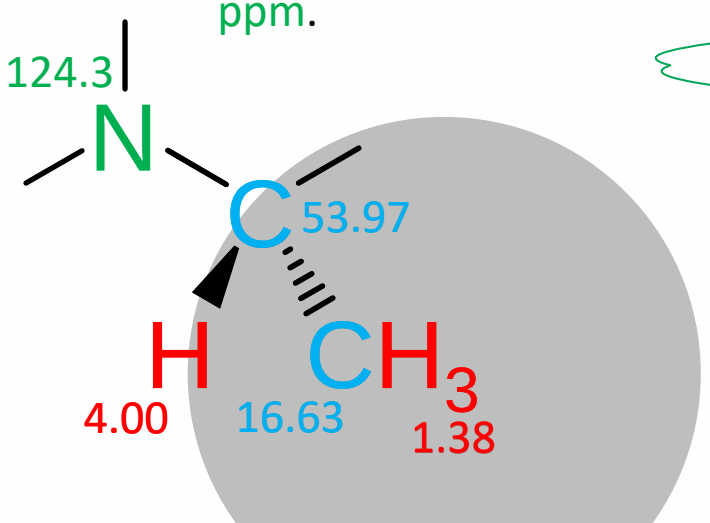
Nitrogen atom assignment



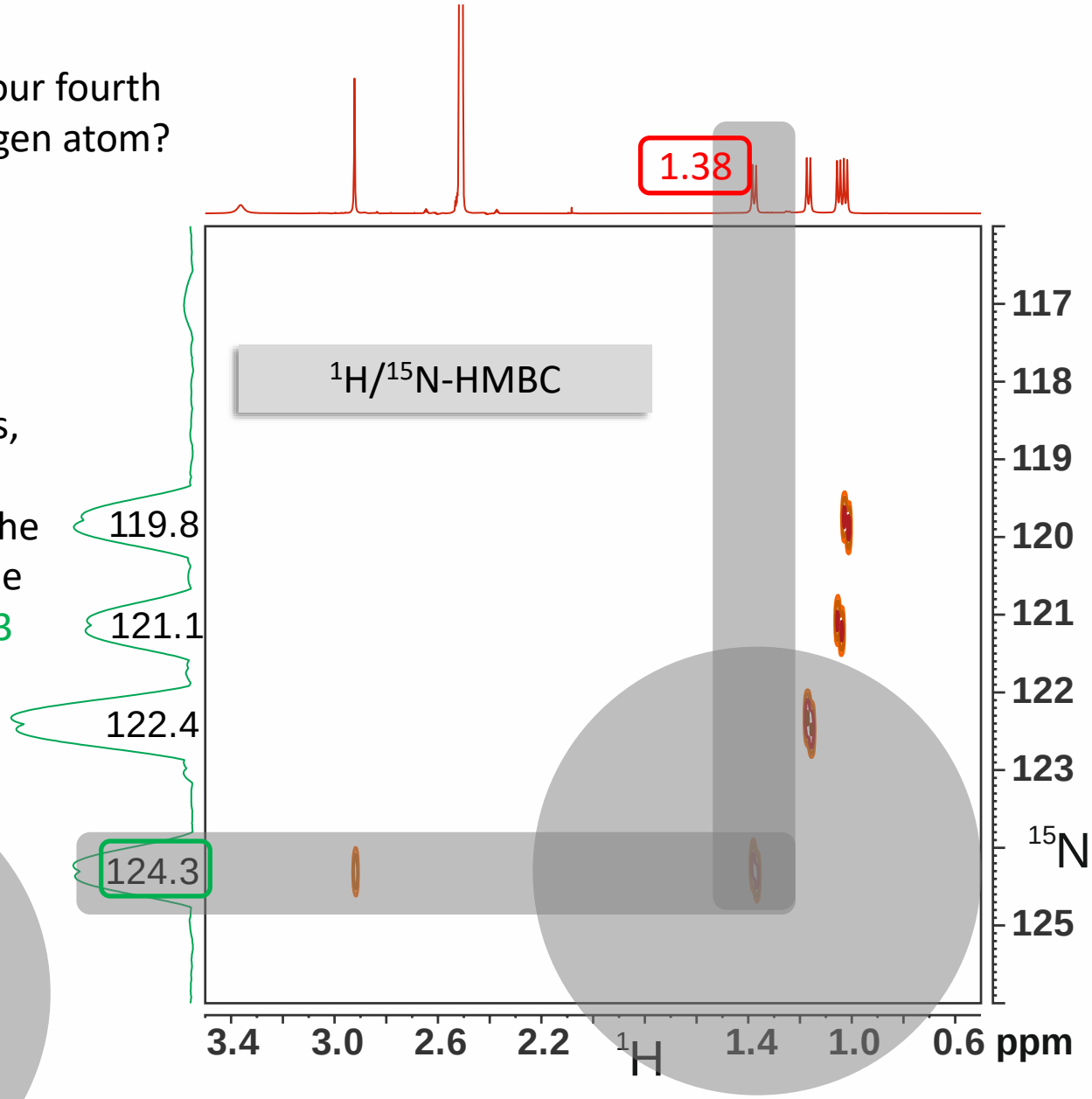




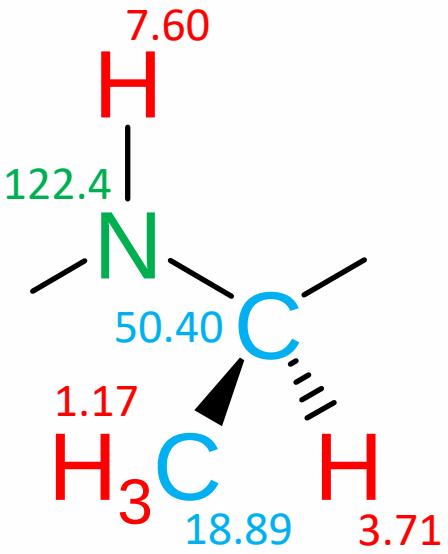
Excluding very unlike four bond correlations, there is only one position possible for the nitrogen atom with the chemical shift of 124.3 ppm.

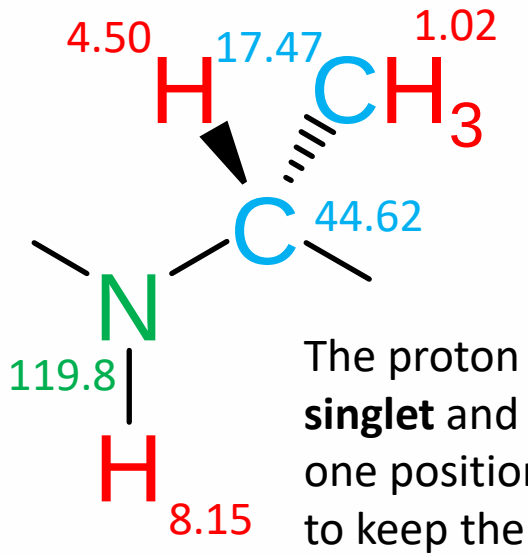


And our fourth nitrogen atom?

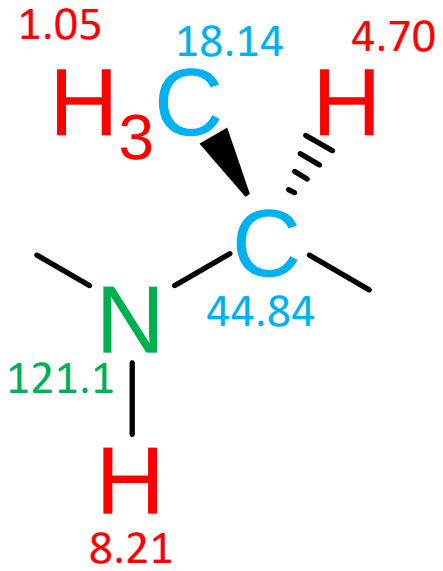


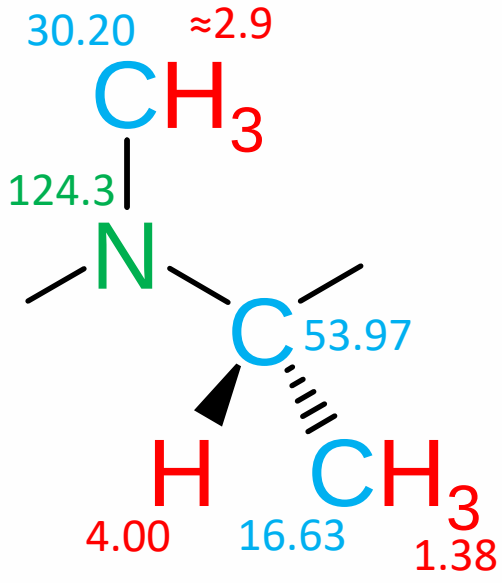
A „forgotten“ methyl group



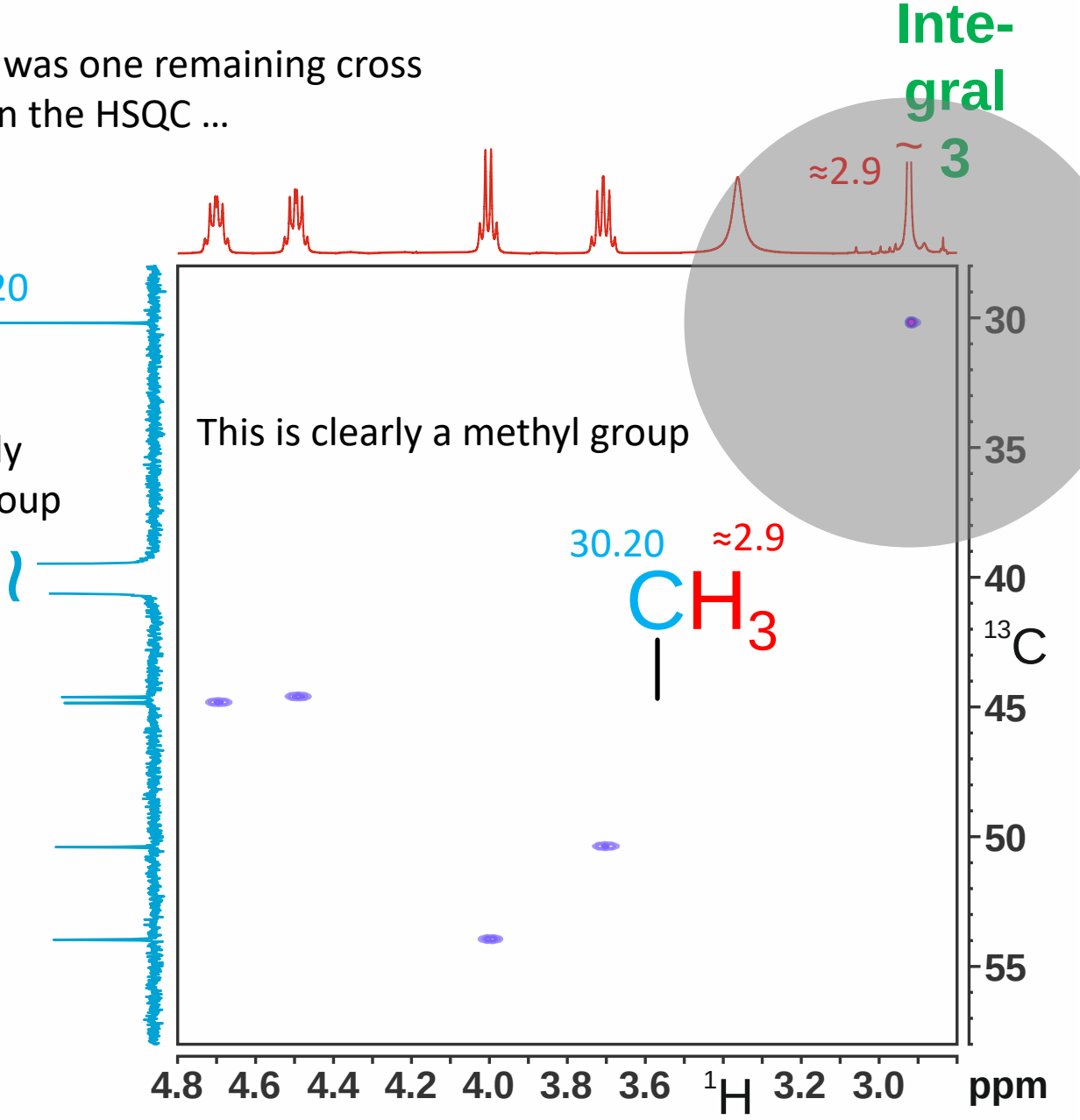


The proton signal is a **singlet** and there is only one position for the group to keep the singlet.

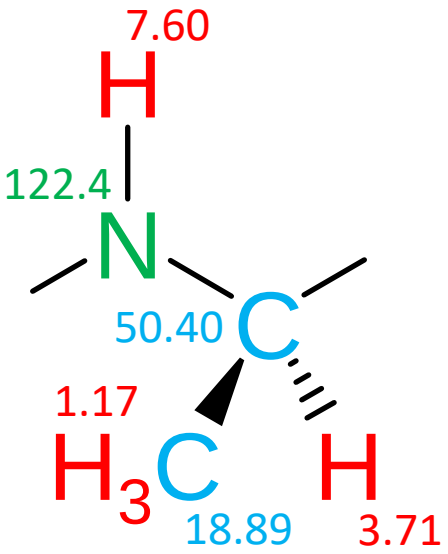


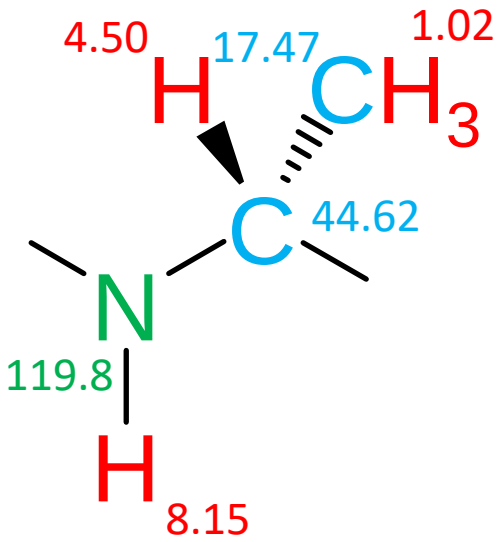


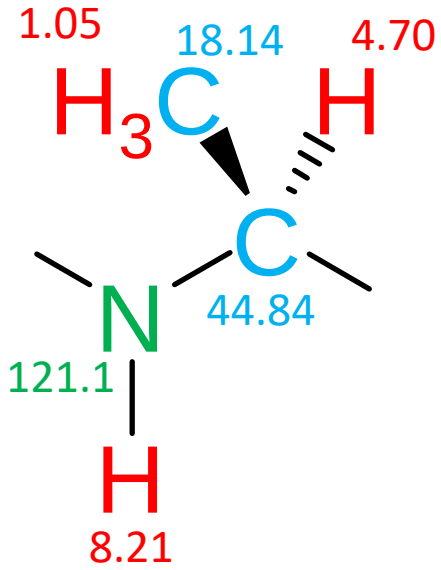
There was one remaining cross peak in the HSQC …

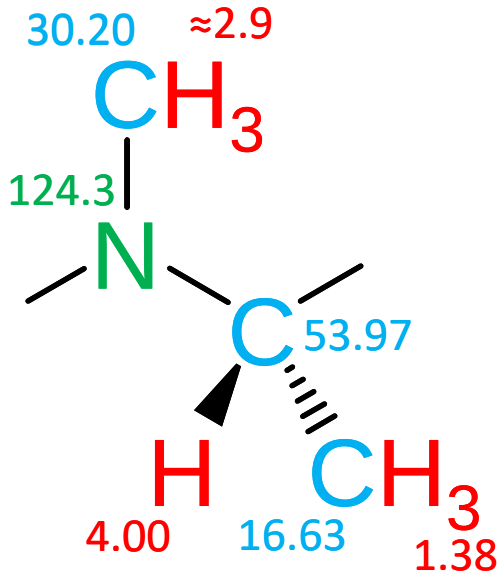


Inventory again









we have

- **four double bond equivalents**
- four carbonyl groups (C_4O_4)
- for amino acid fragments ($C_9H_{22}N_{34}$)
- all fragments together ($C_{13}H_{22}N_4O_4$)

we need

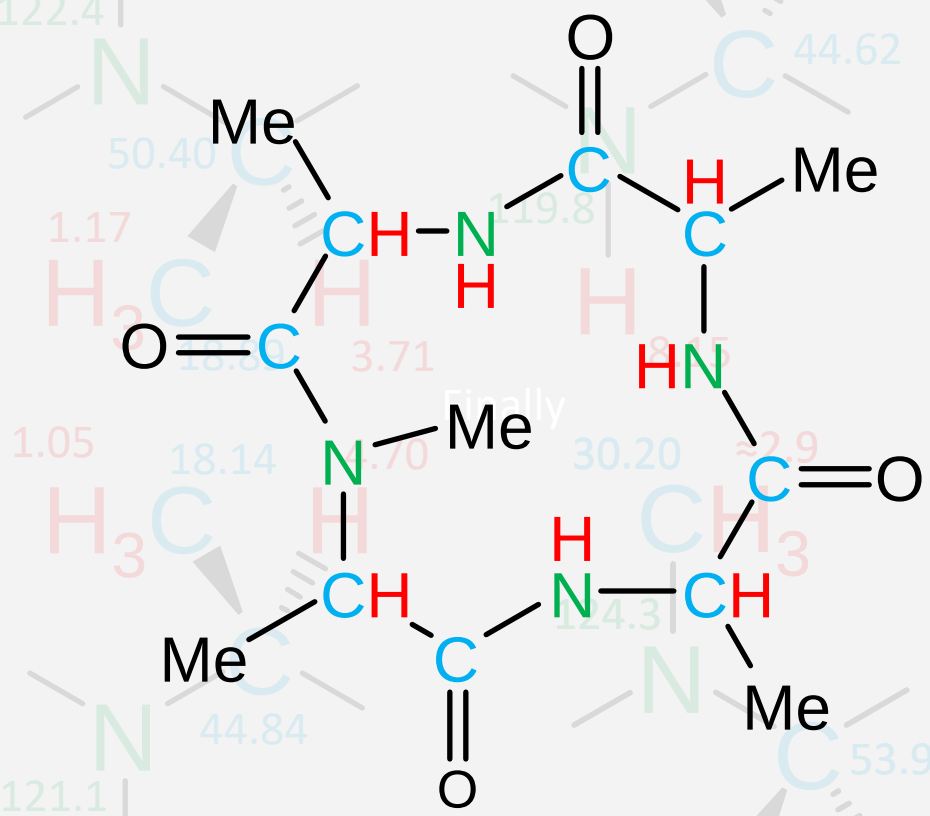
- five double bond equivalents
- molecular formula $C_{13}H_{22}N_4O_4$

No atom is missing anymore. We have eight open bonds coming from the carbonyl groups, 8 open bonds at the amino acid fragments and we still need one double bond equivalent.

The solution is easy: We have to place the carbonyl groups between the amino acid fragments resulting in a ring. The ring is the **last missing double bond equivalent**.

Inventory again

Finally we have to end with this molecule.



But what is the order of the amino acids?

we have

- **four double bond equivalents**
- four carbonyl groups (C_4O_4)
- for amino acid fragments ($C_9H_{22}N_{34}$)
- all fragments together ($C_{13}H_{22}N_4O_4$)

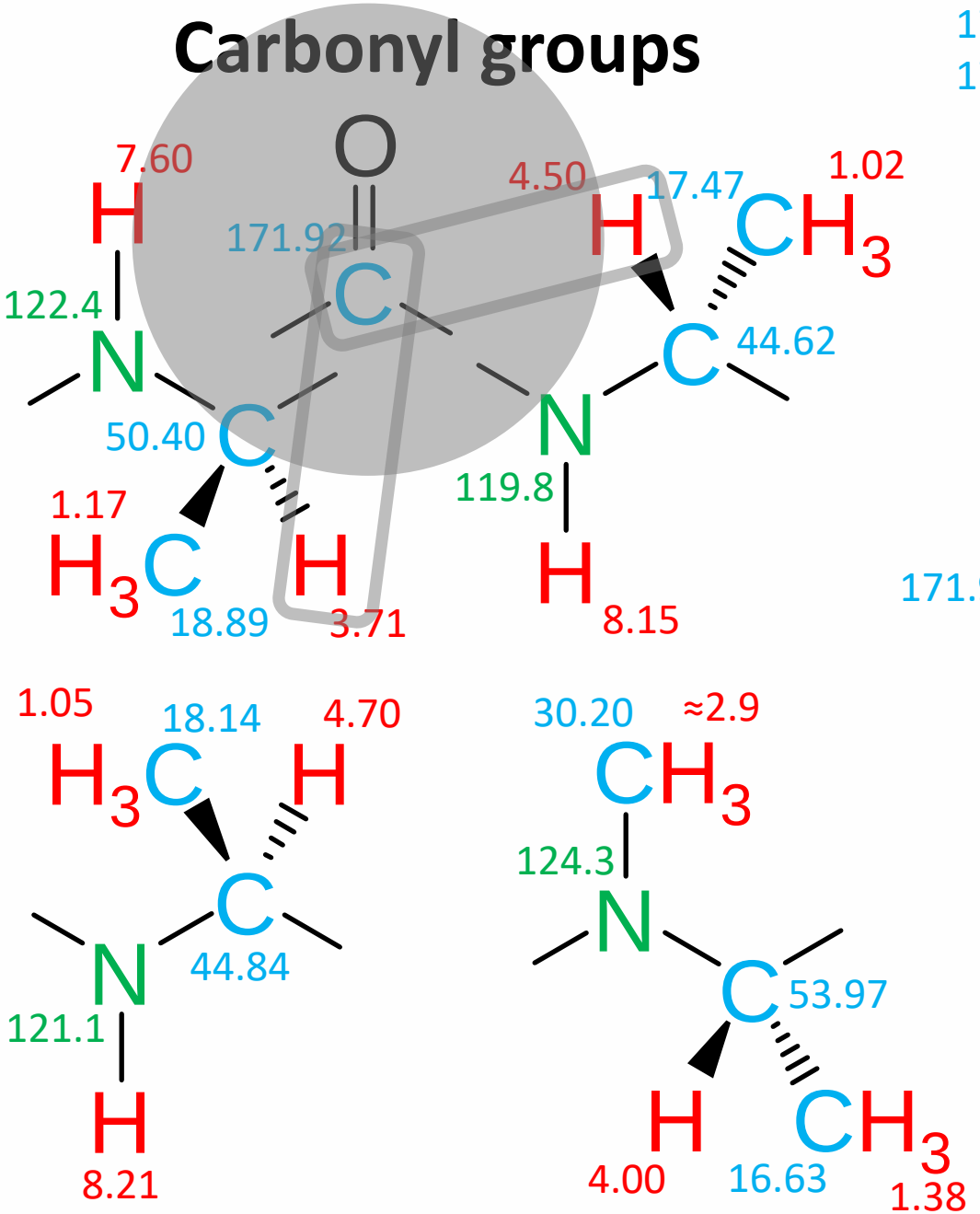
we need

- five double bond equivalents
- molecular formula $C_{13}H_{22}N_4O_4$

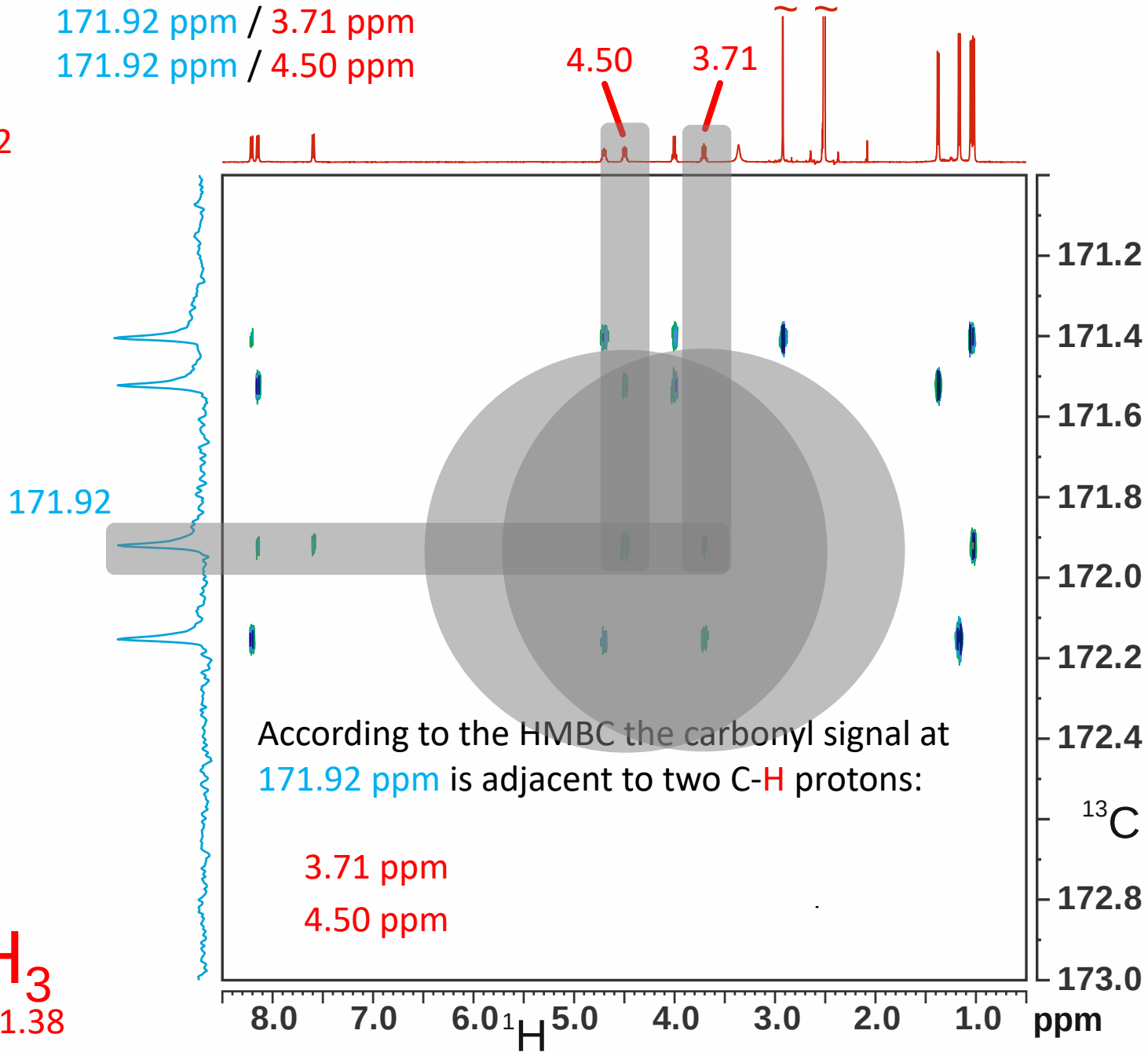
No atom is missing anymore. We have eight open bonds coming from the carbonyl groups, 8 open bonds at the amino acid fragments and we still need one double bond equivalent.

The solution is easy: We have to place the carbonyl groups between the amino acid fragments resulting in a ring. The ring is the **last missing double bond equivalent**.

Carbonyl groups



171.92 ppm / 3.71 ppm
171.92 ppm / 4.50 ppm

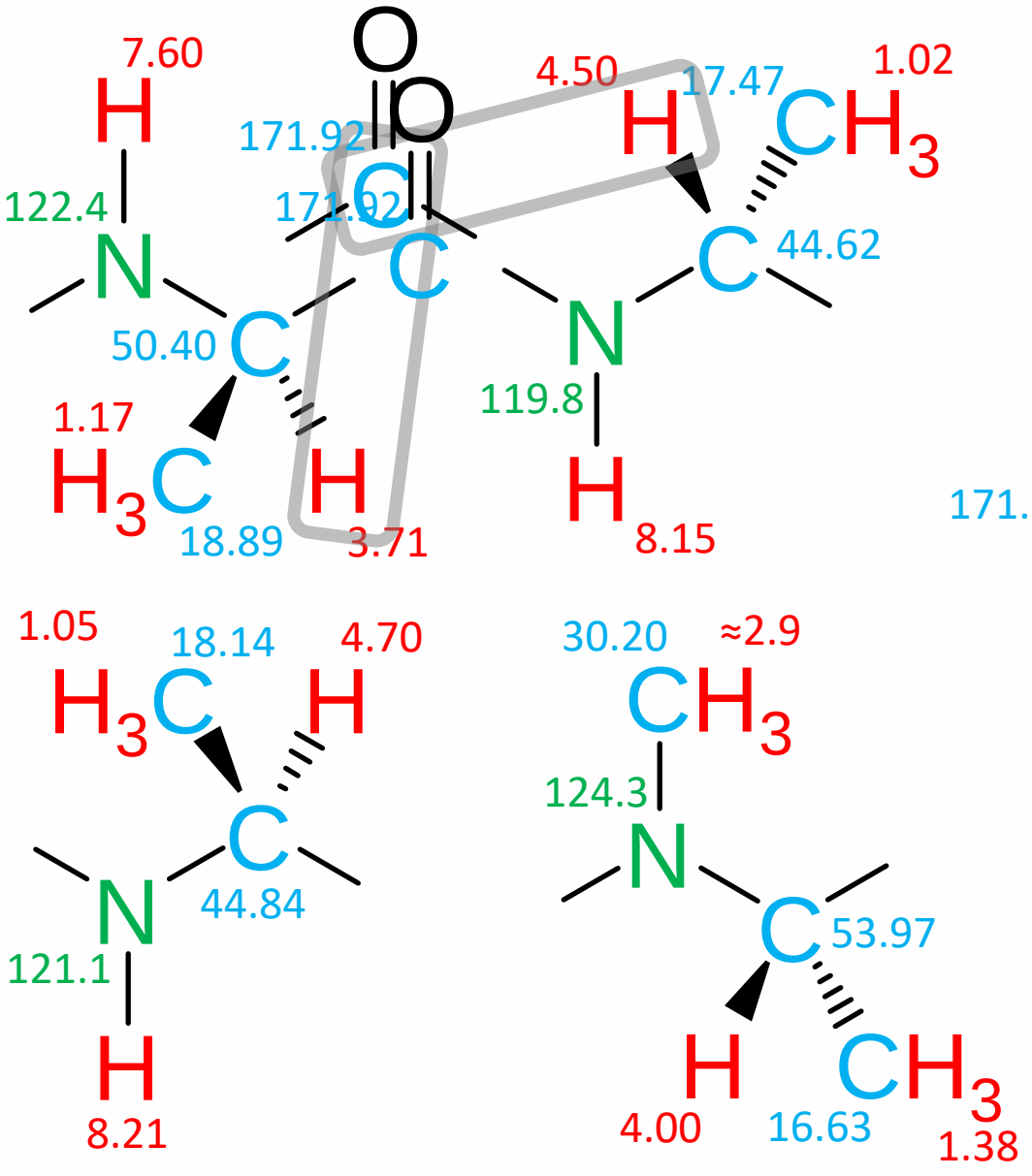


According to the HMBC the carbonyl signal at 171.92 ppm is adjacent to two C-H protons:

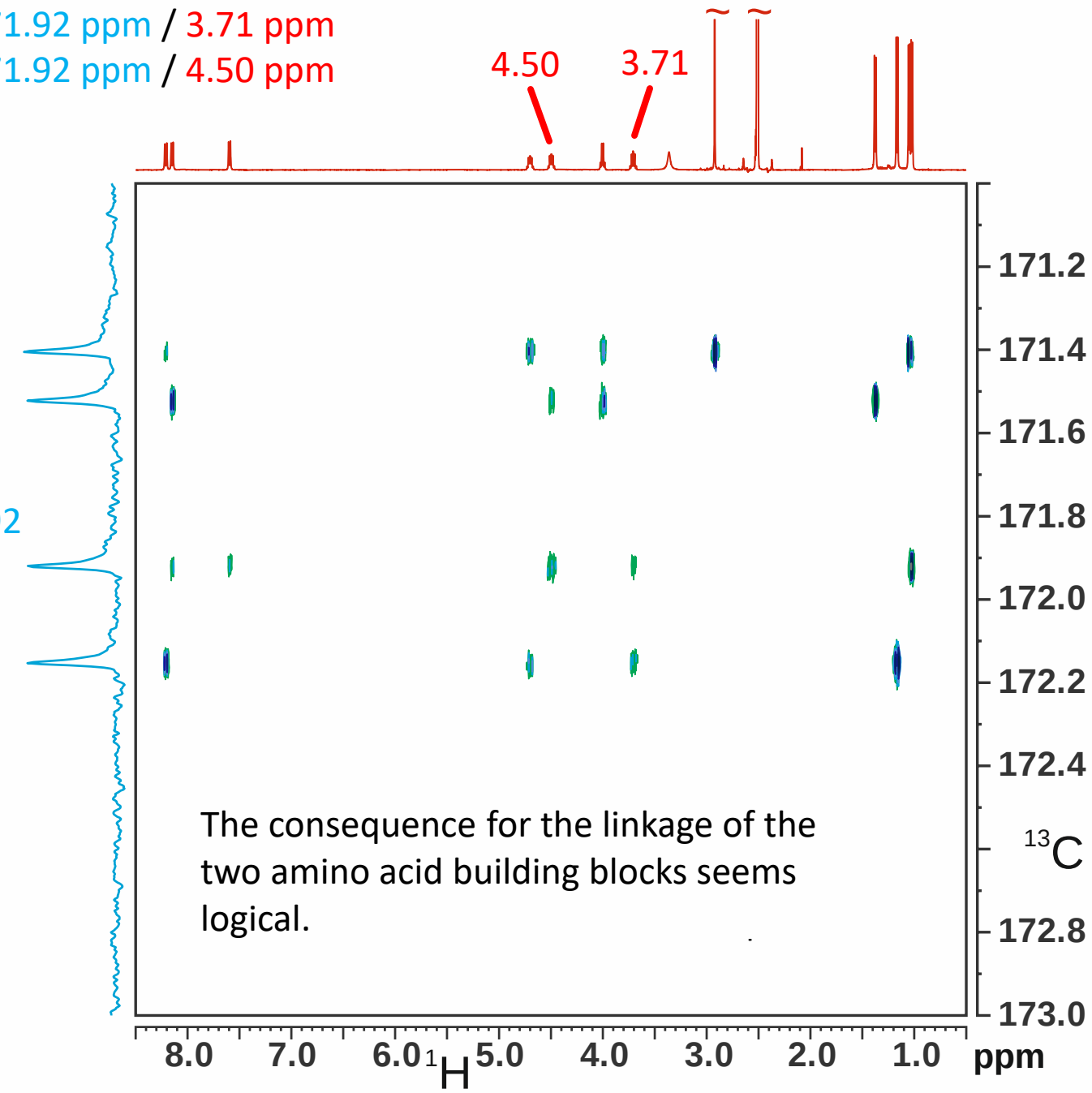
3.71 ppm

4.50 ppm

Carbonyl groups

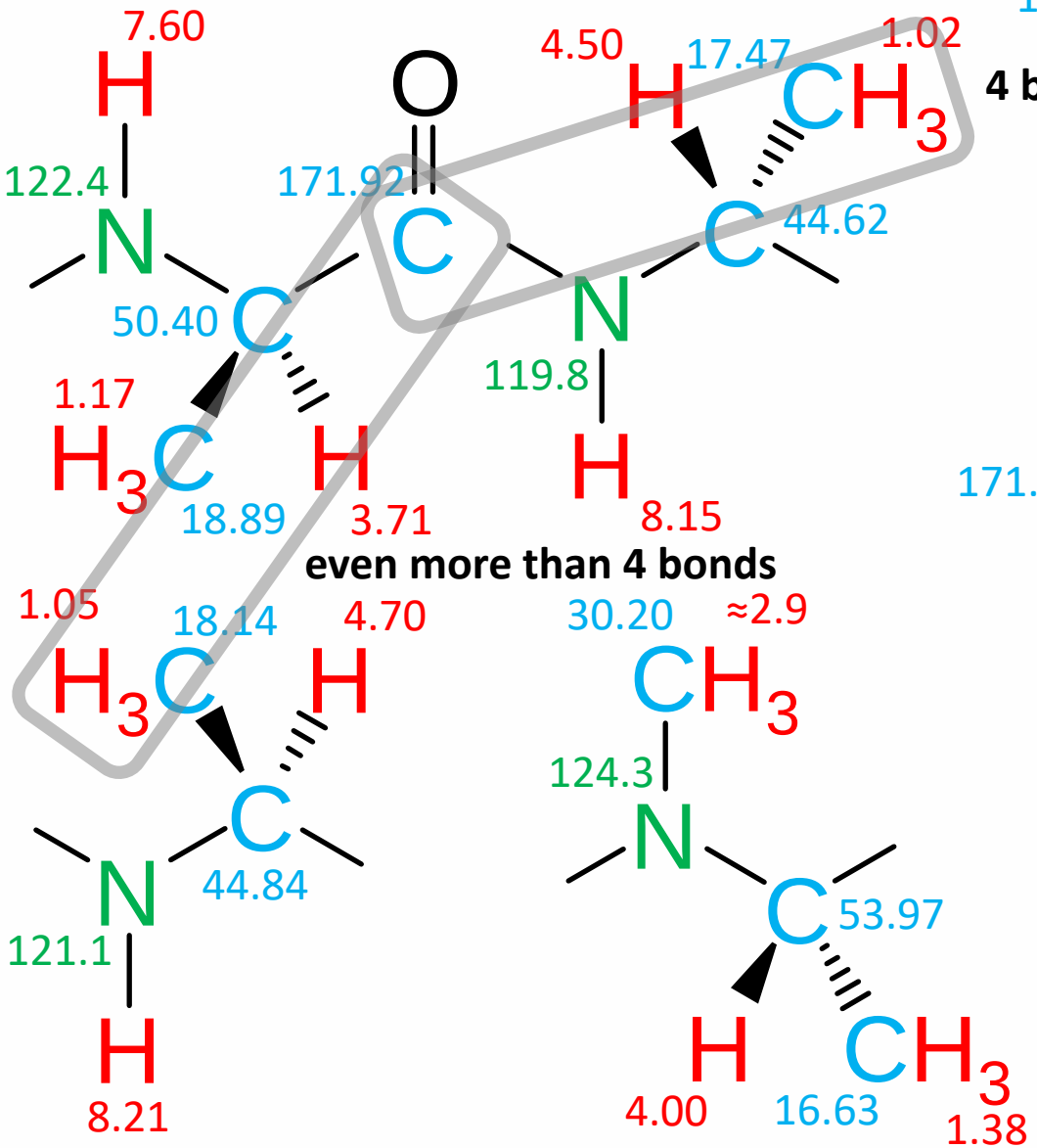


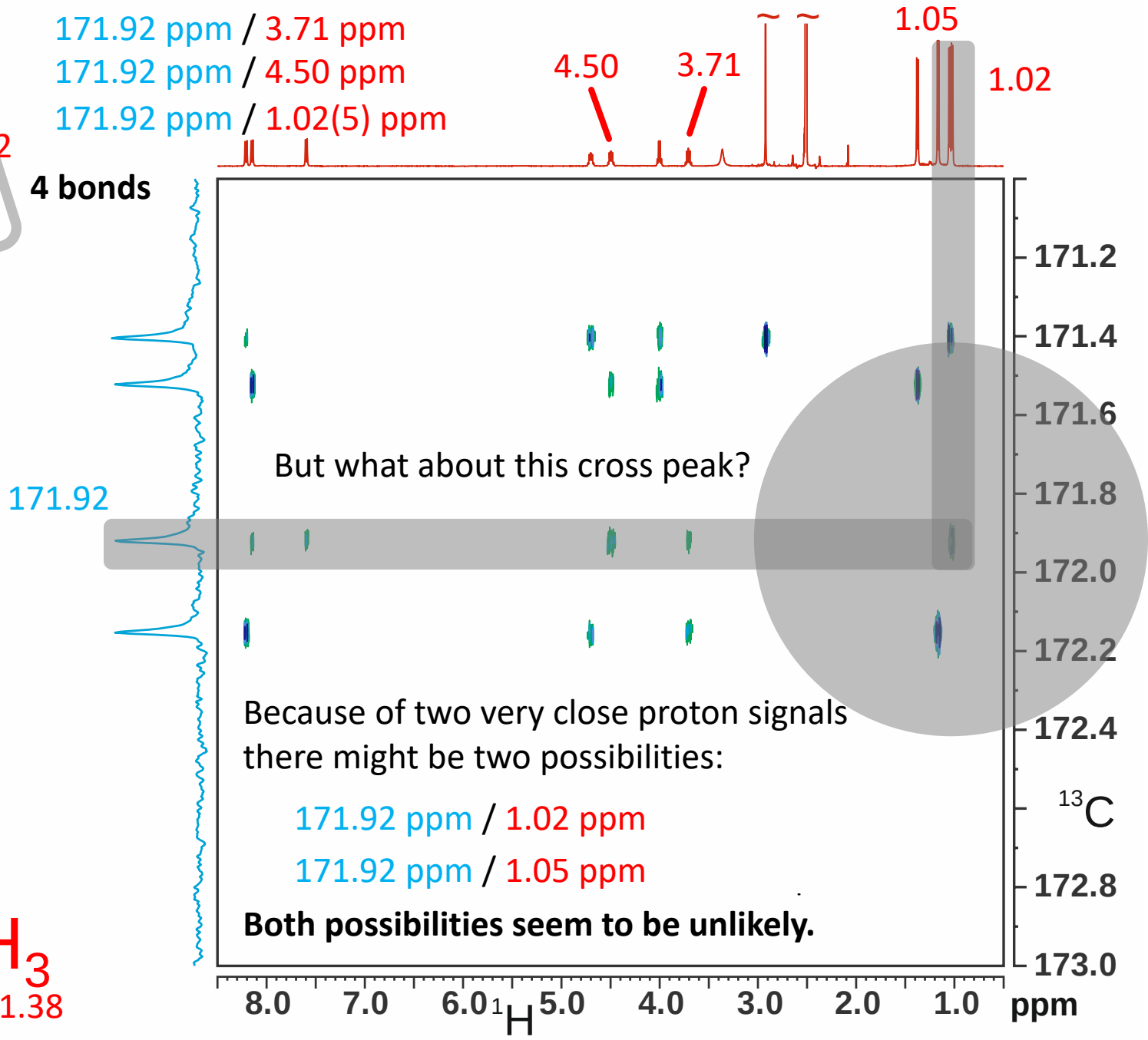
171.92 ppm / 3.71 ppm
171.92 ppm / 4.50 ppm



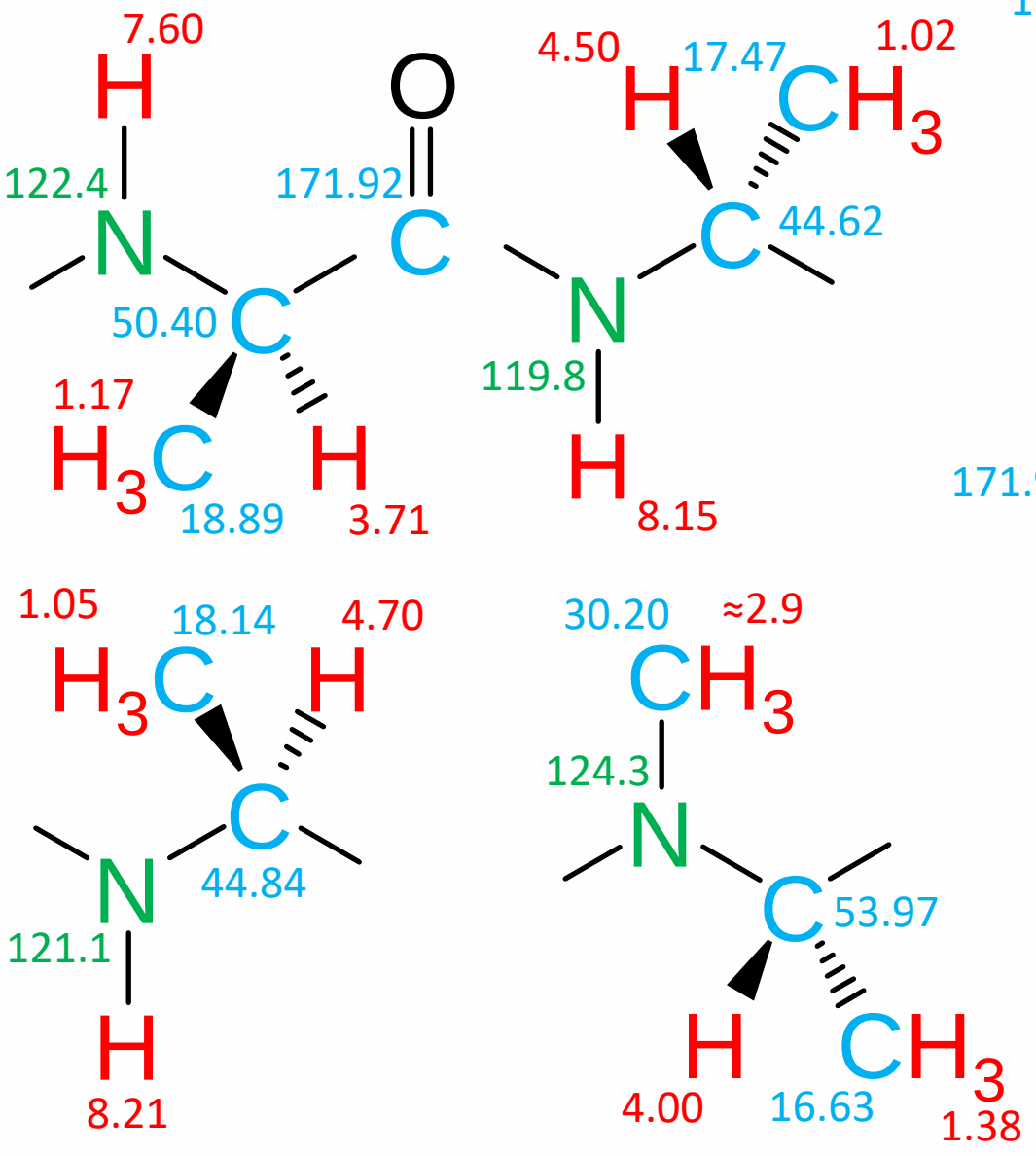
The consequence for the linkage of the two amino acid building blocks seems logical.

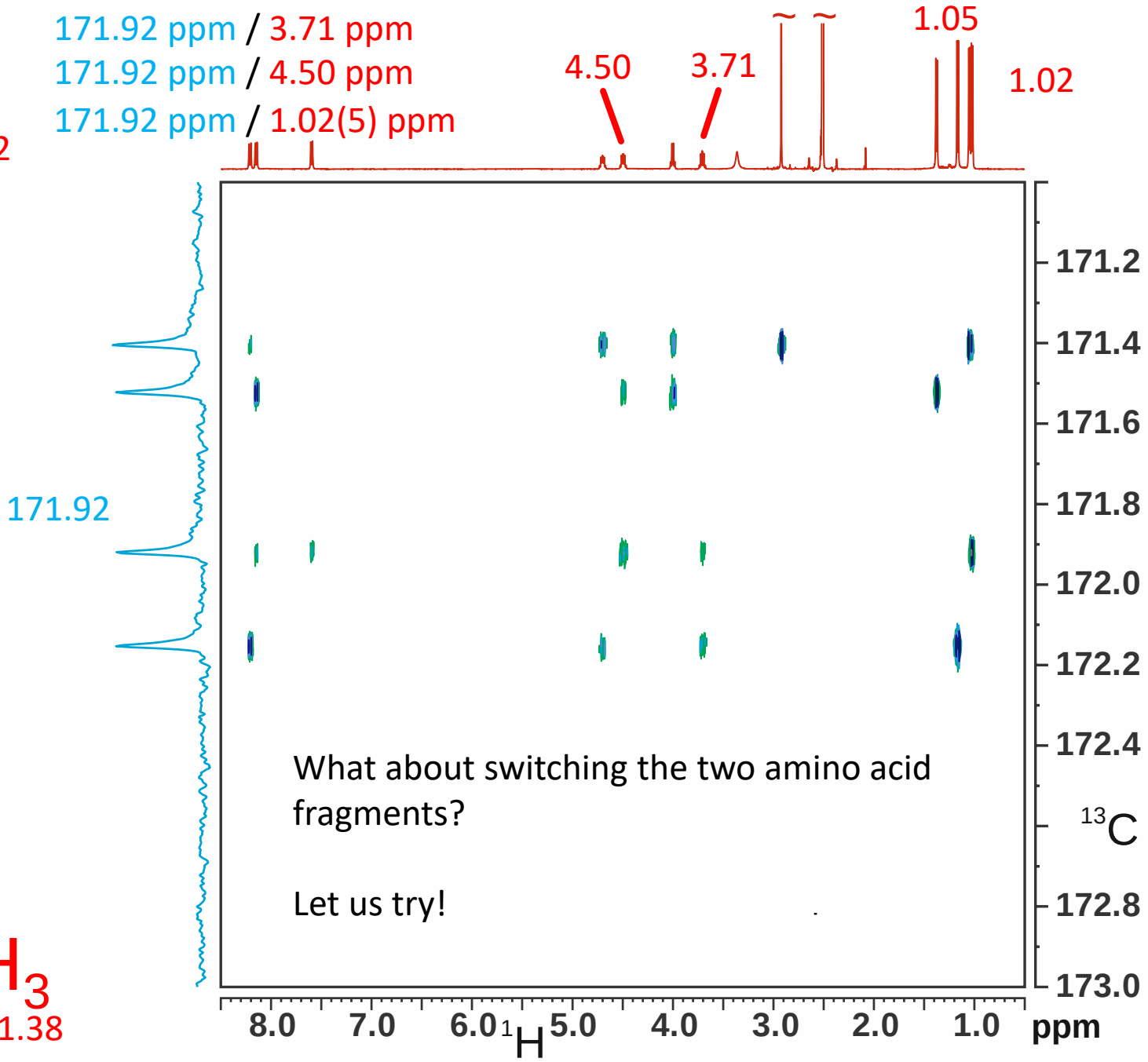
Carbonyl groups



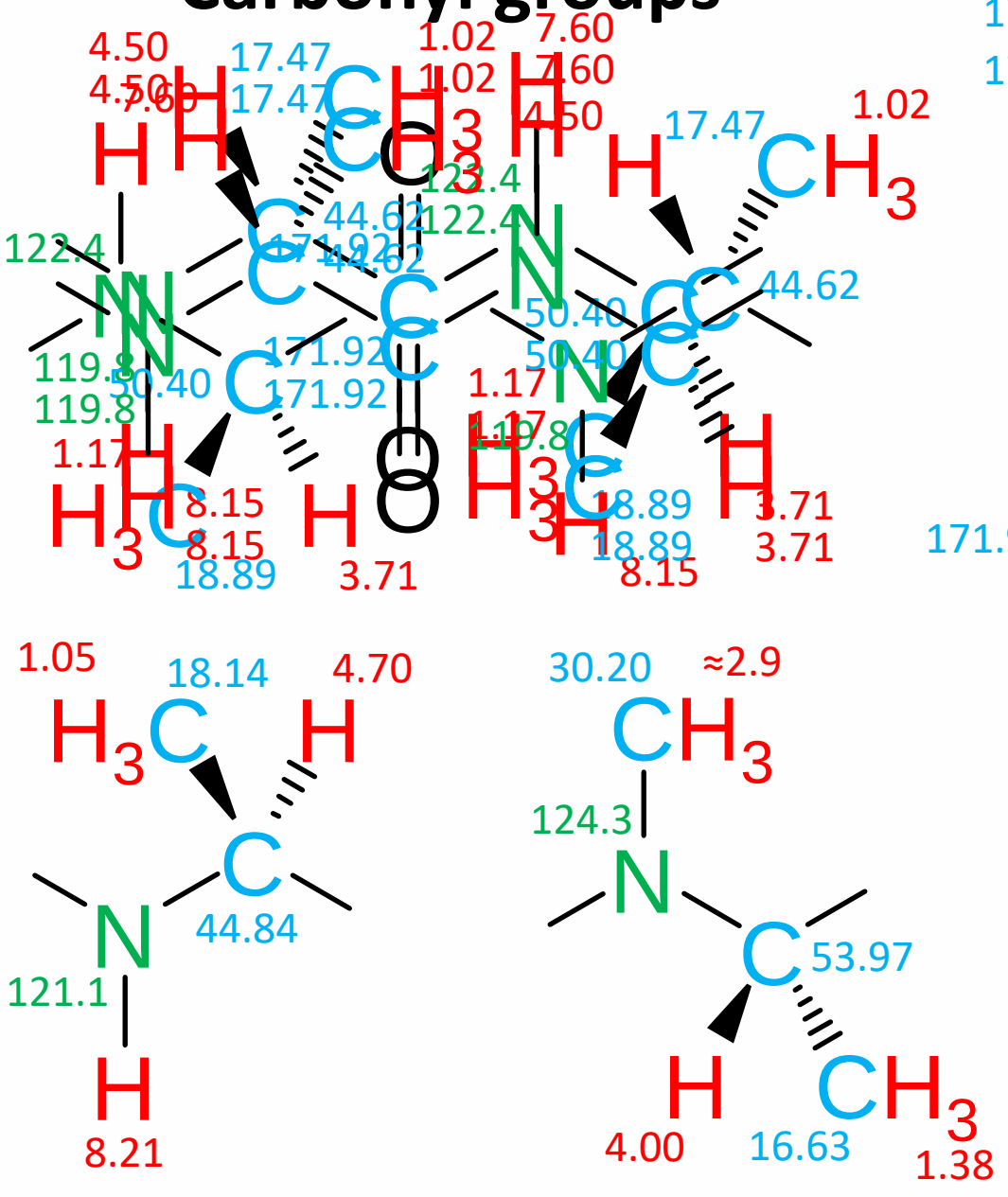


Carbonyl groups

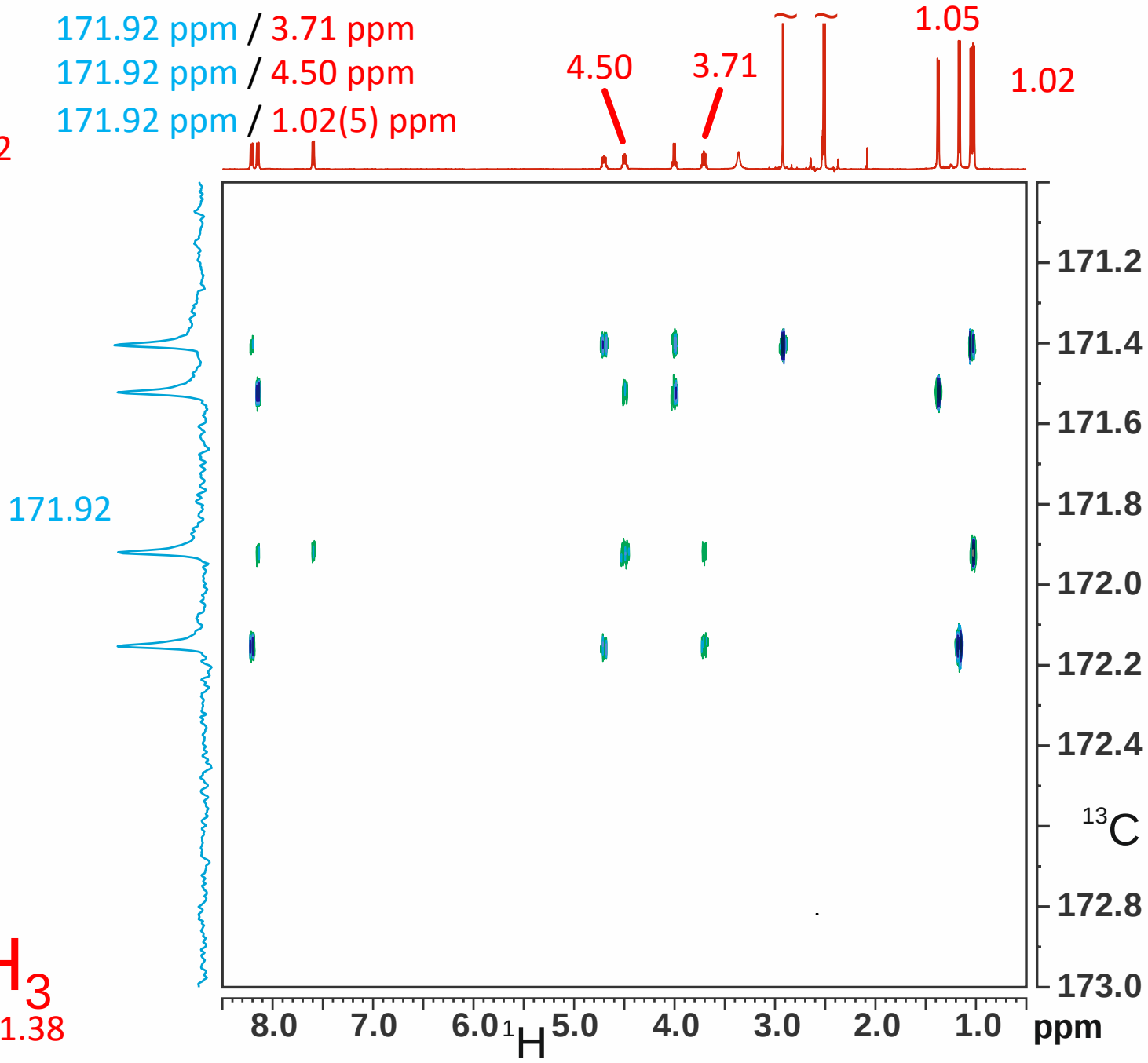




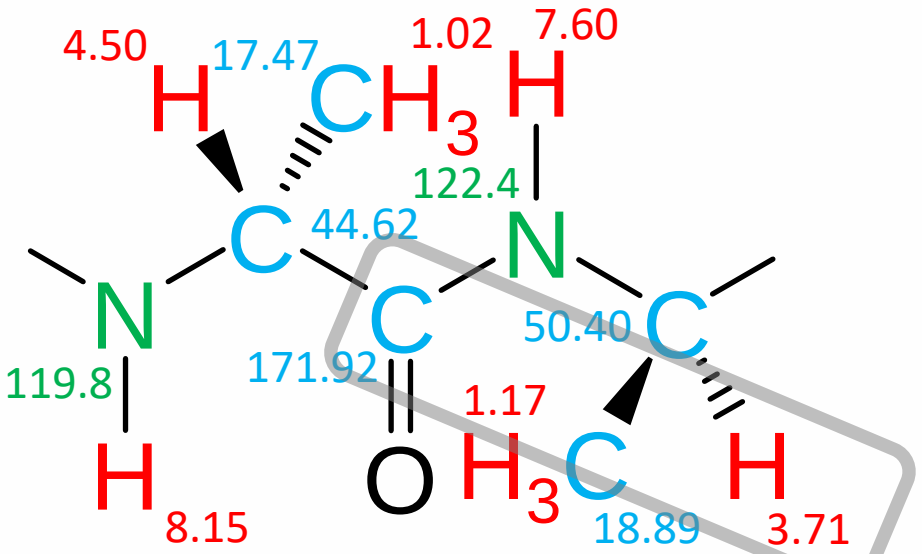
Carbonyl groups

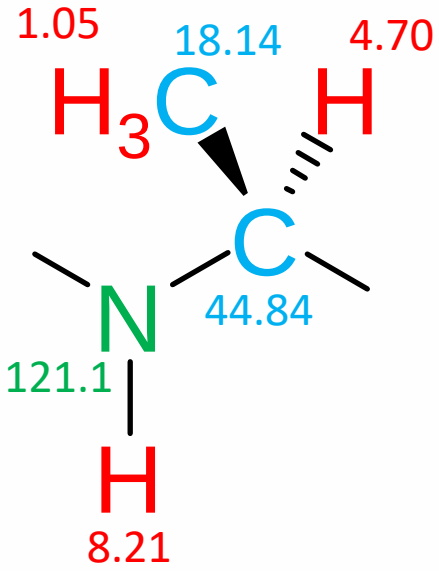


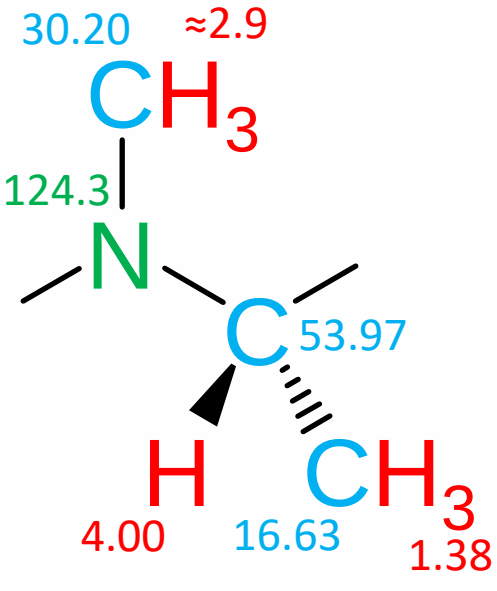
171.92 ppm / 3.71 ppm
171.92 ppm / 4.50 ppm
171.92 ppm / 1.02(5) ppm



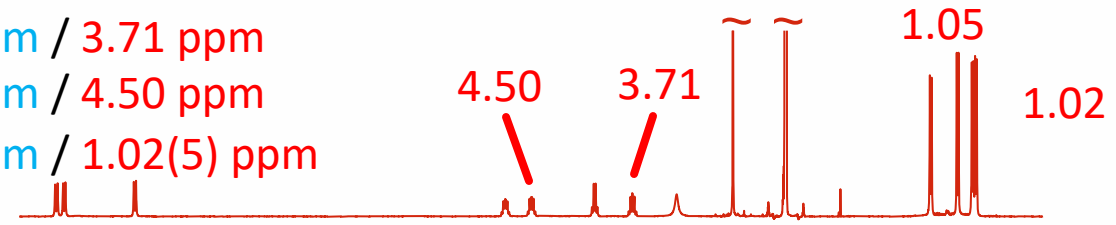
Carbonyl groups

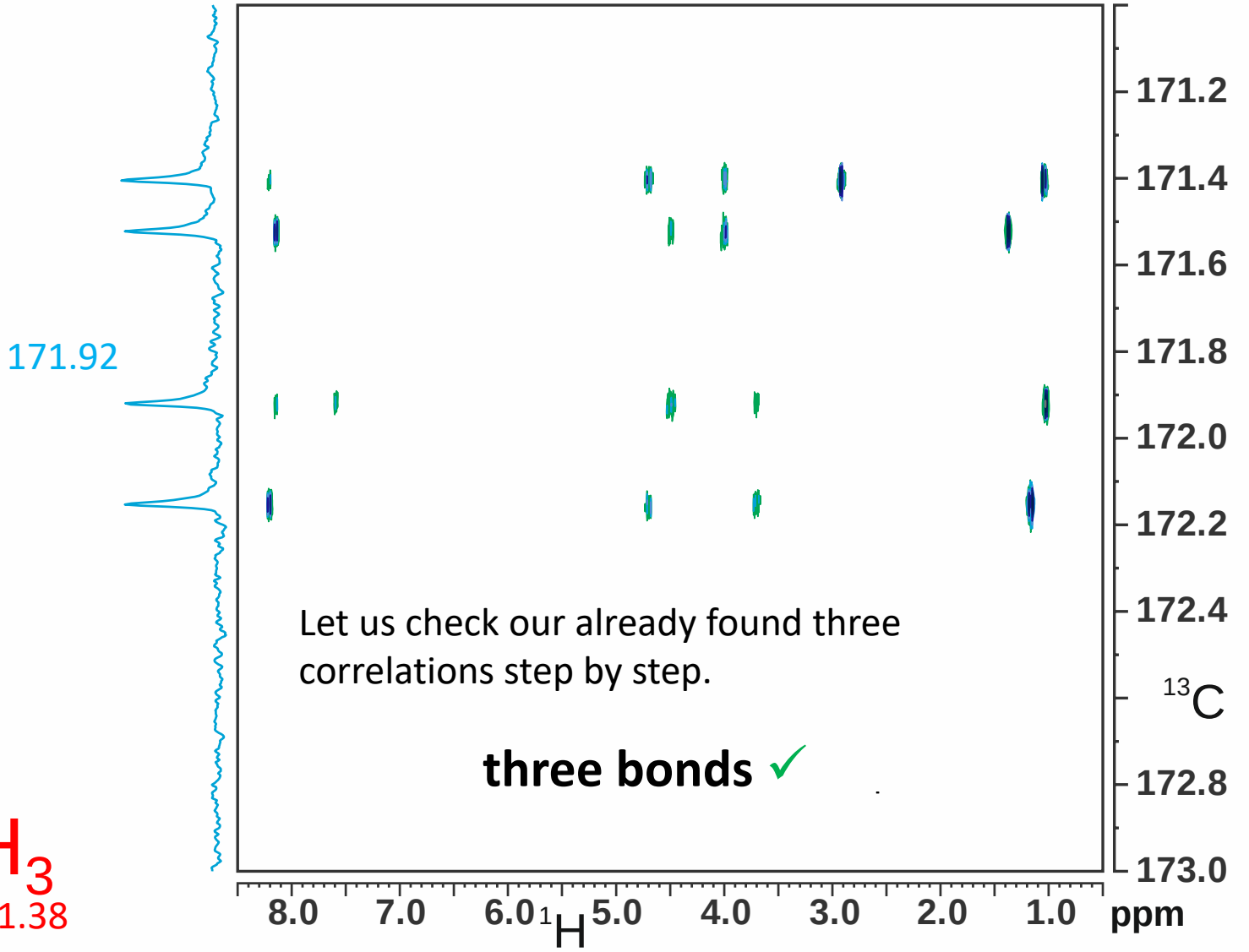






171.92 ppm / 3.71 ppm
171.92 ppm / 4.50 ppm
171.92 ppm / 1.02(5) ppm

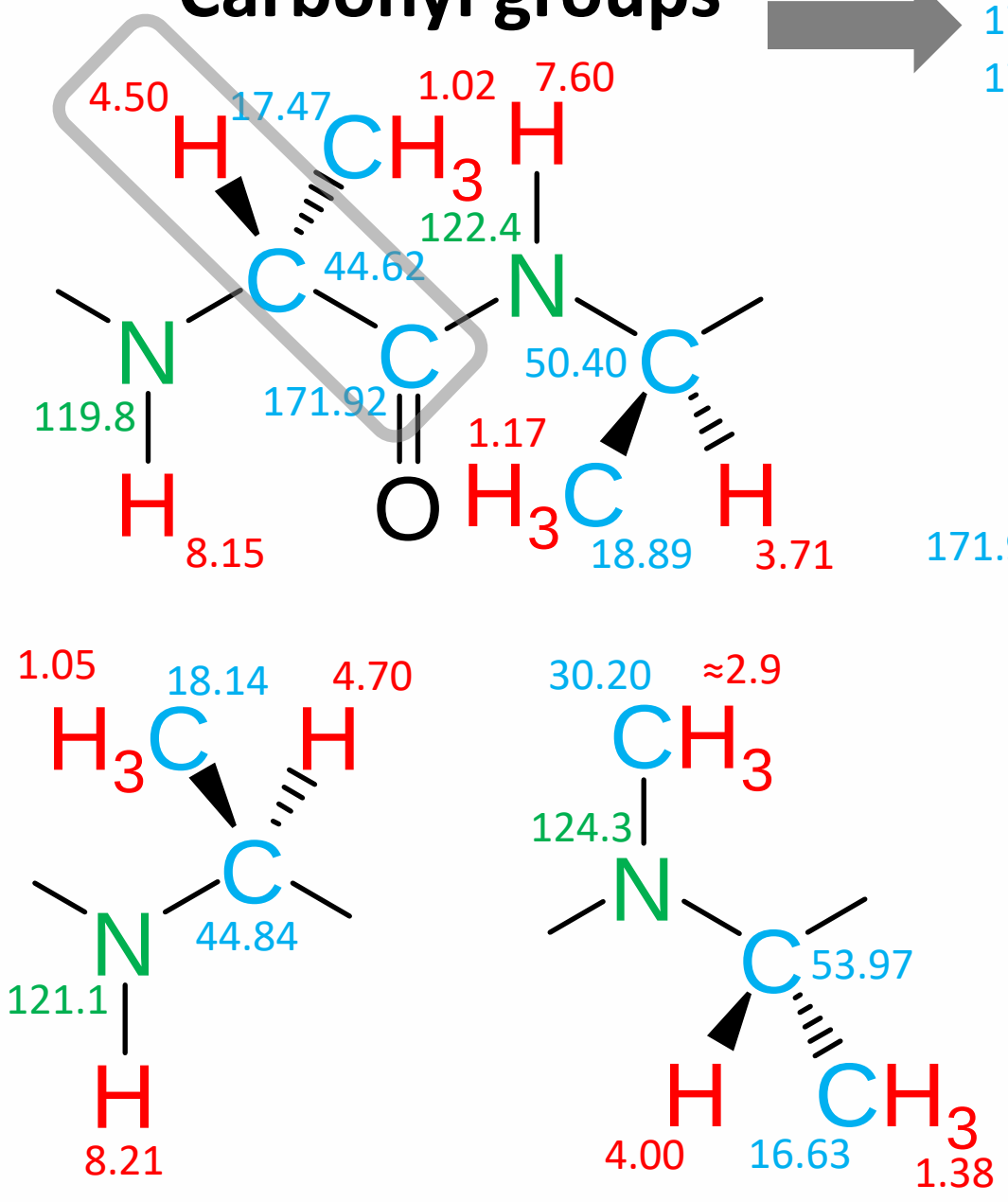




Let us check our already found three correlations step by step.

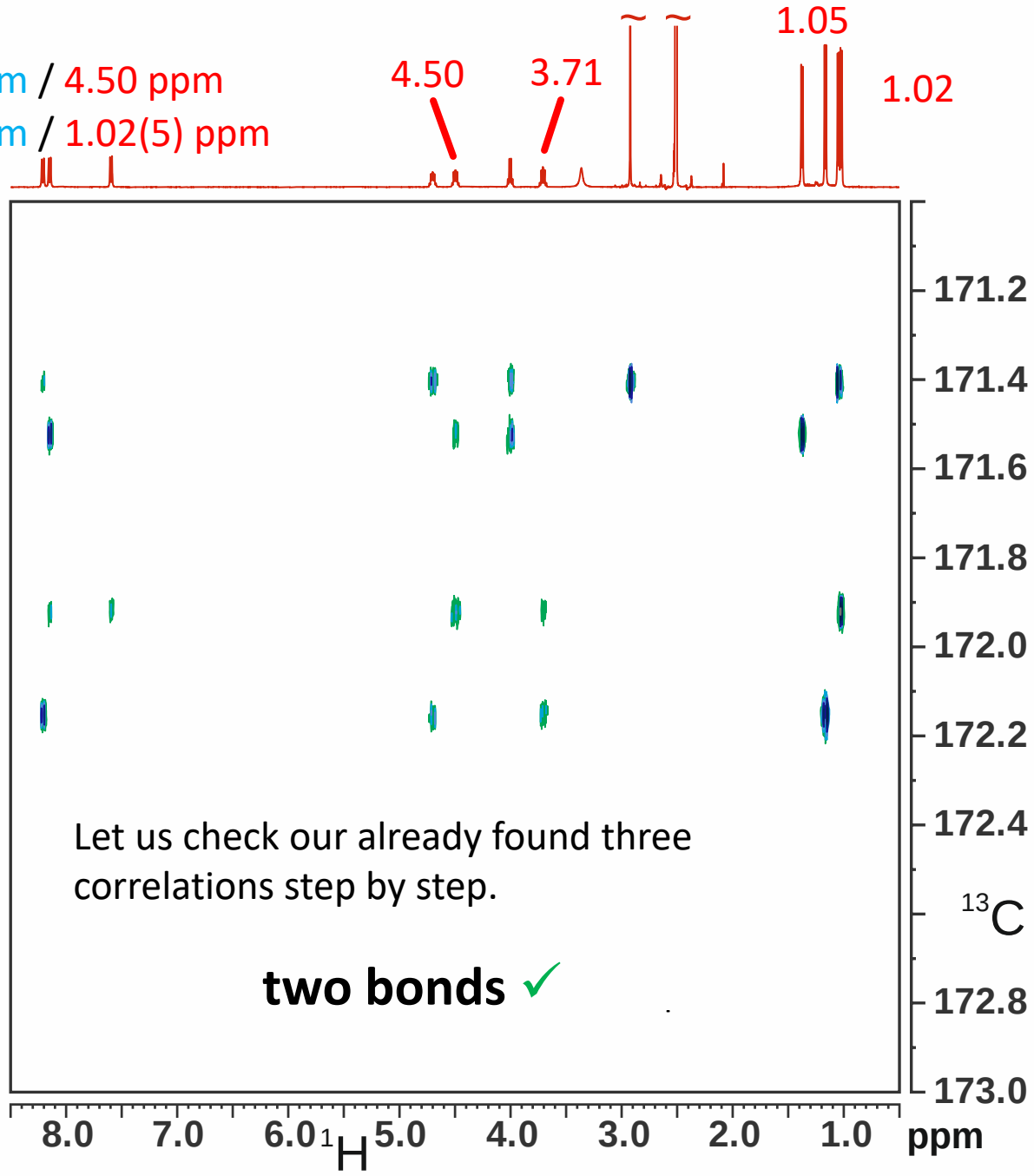
three bonds ✓

Carbonyl groups

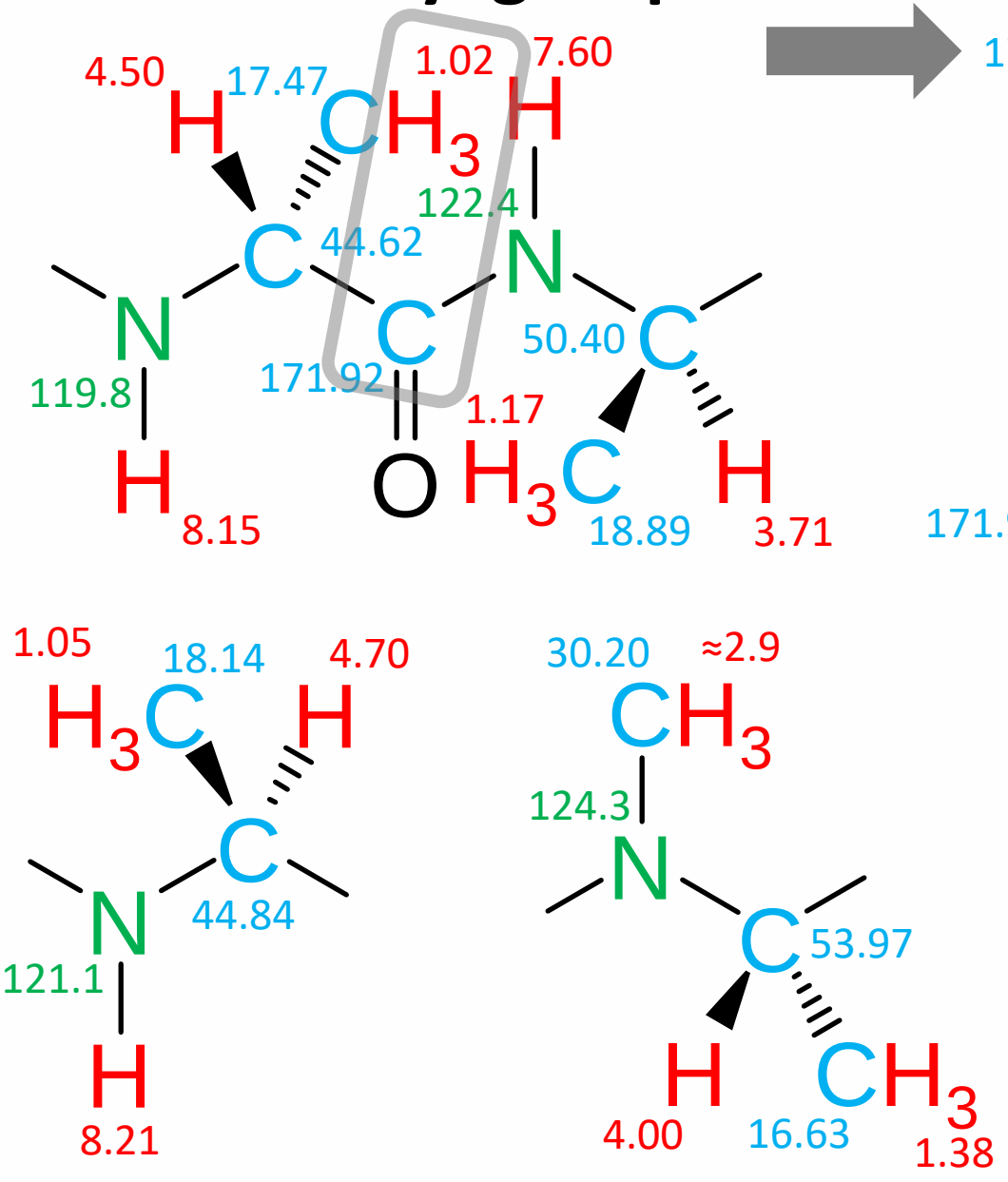


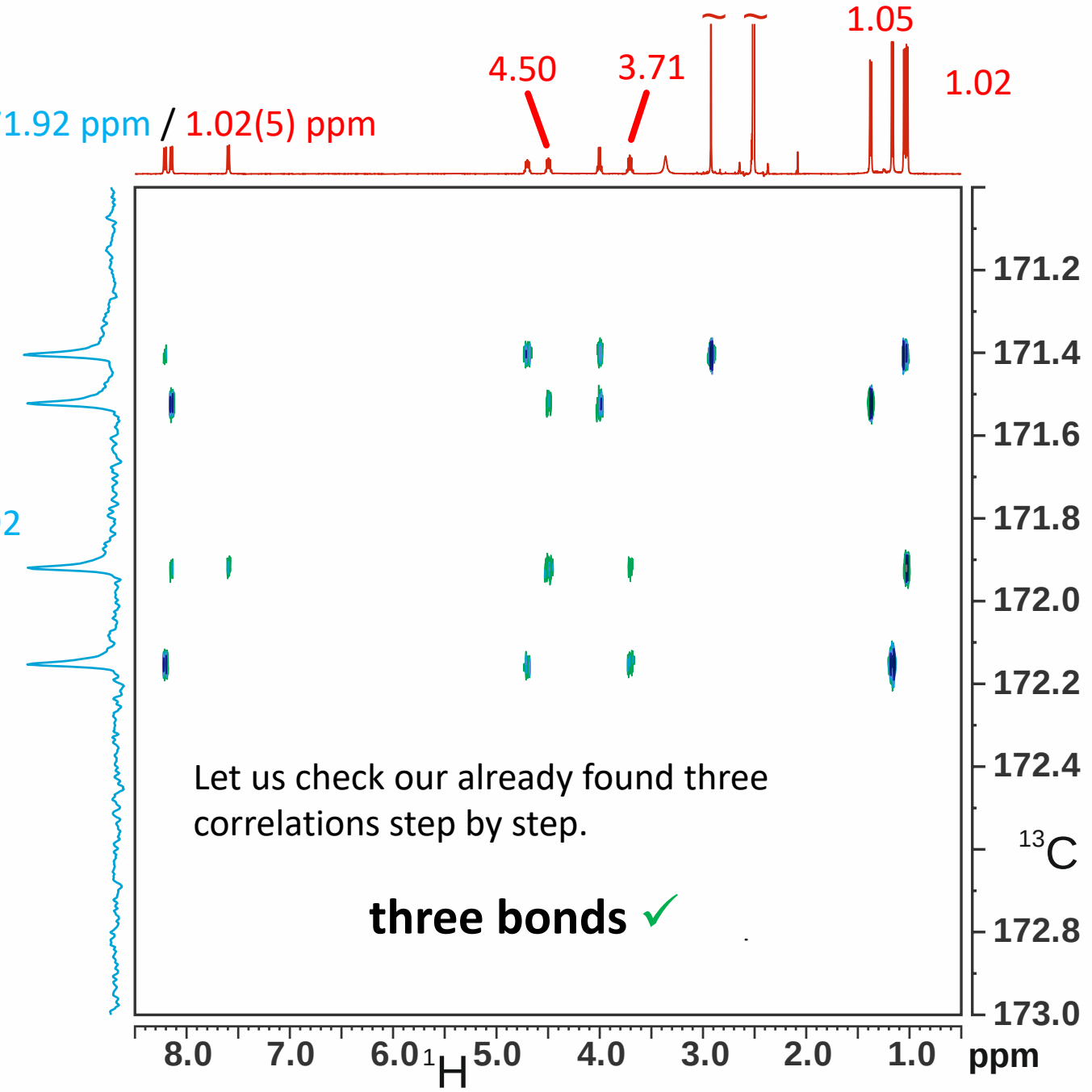
171.92 ppm / 4.50 ppm

171.92 ppm / 1.02(5) ppm

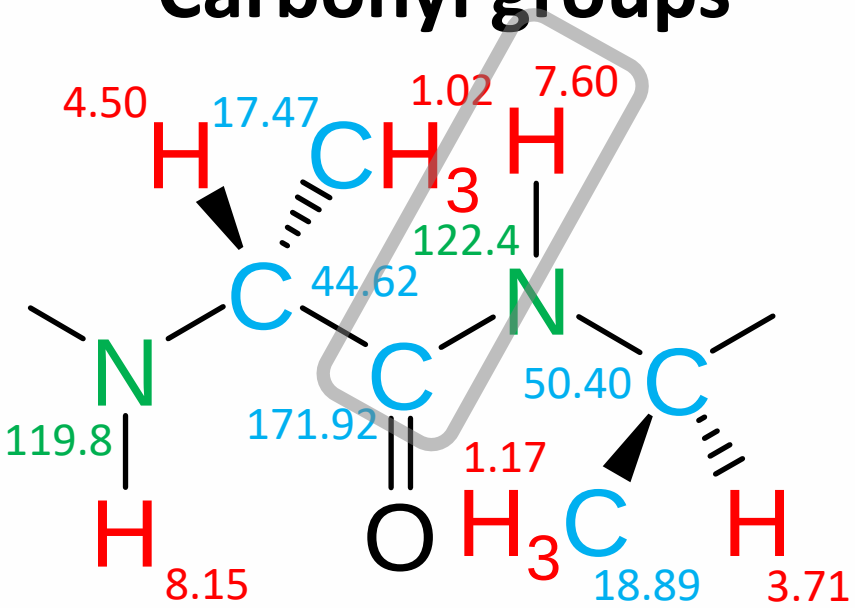


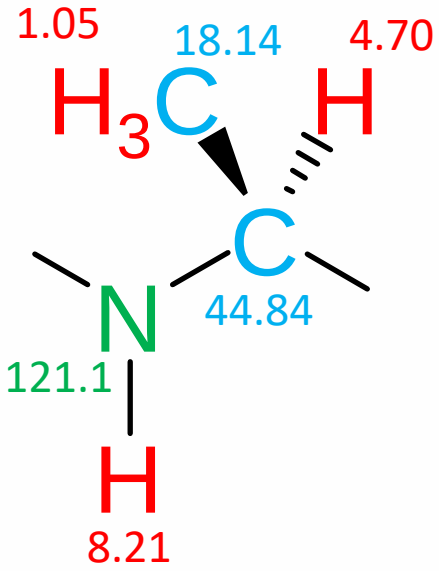
Carbonyl groups

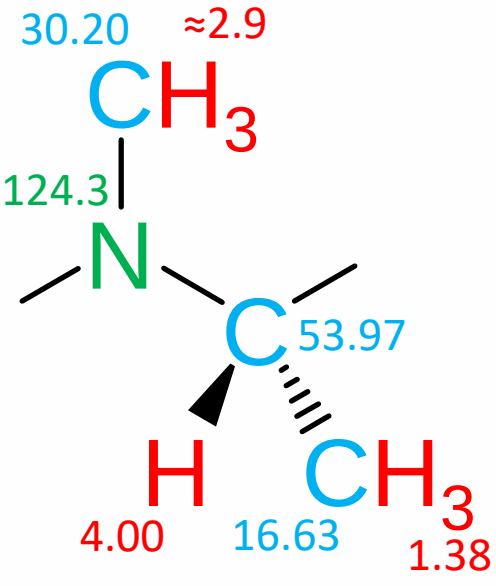


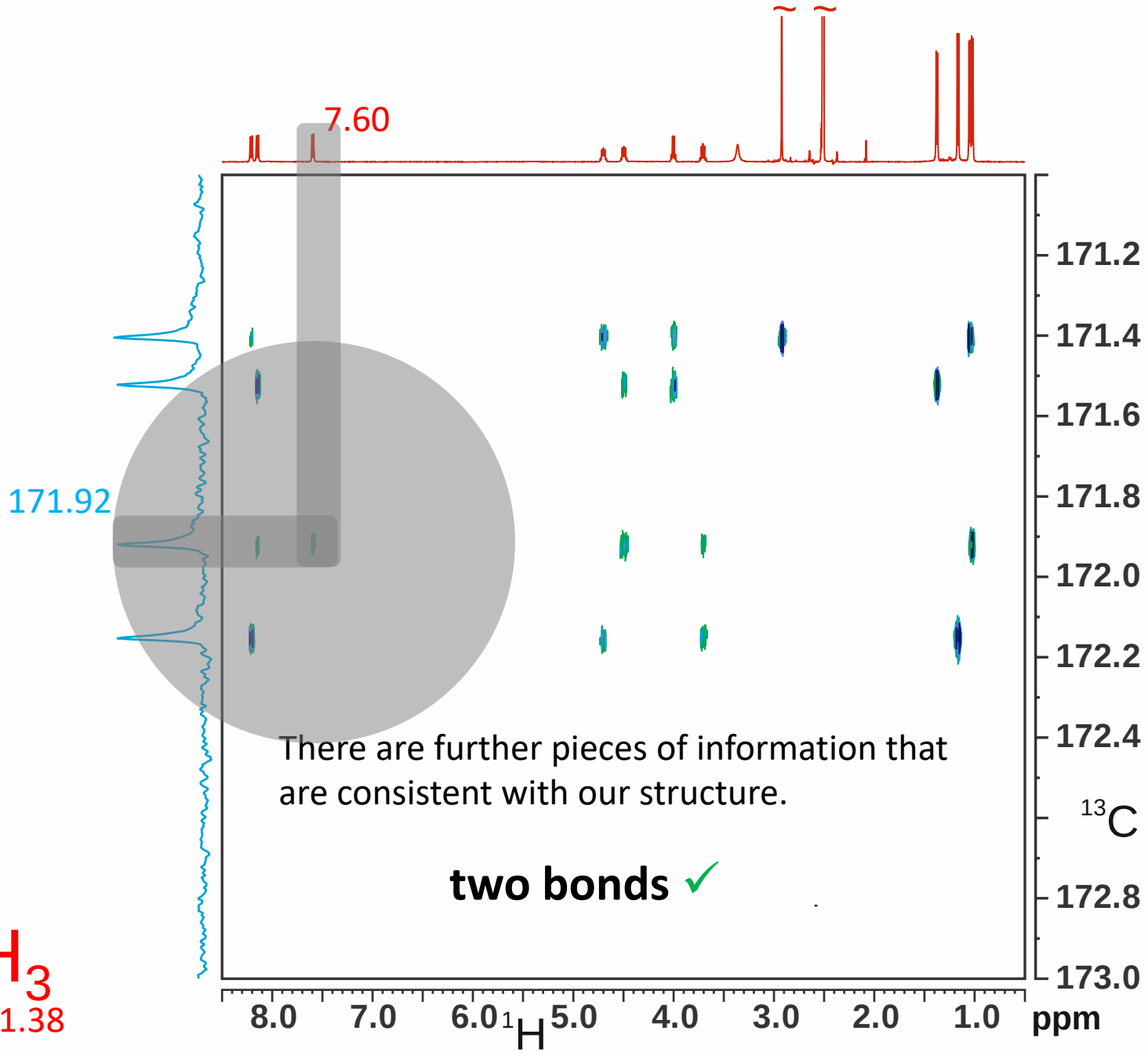


Carbonyl groups

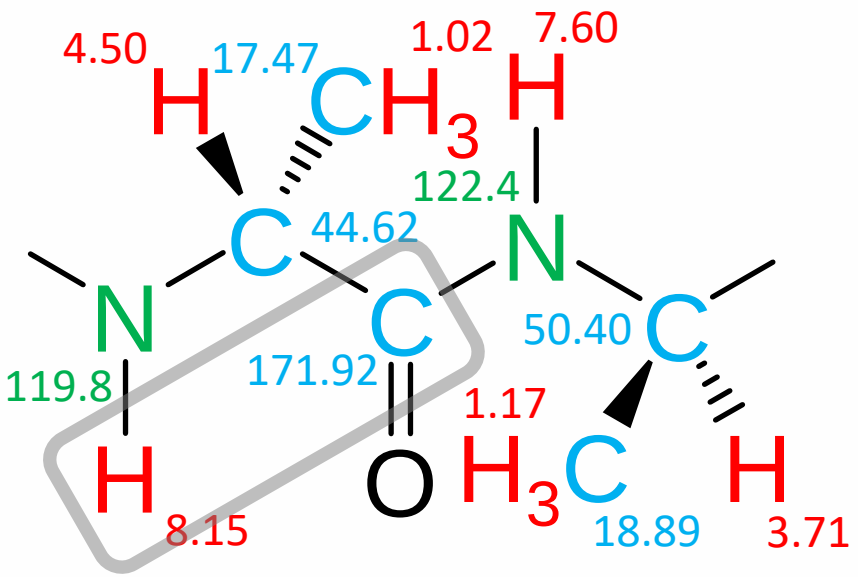


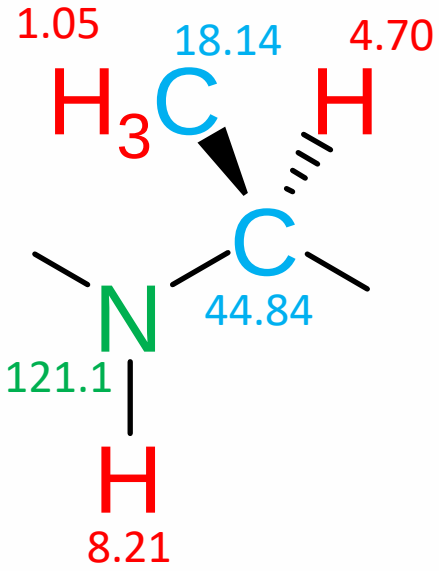


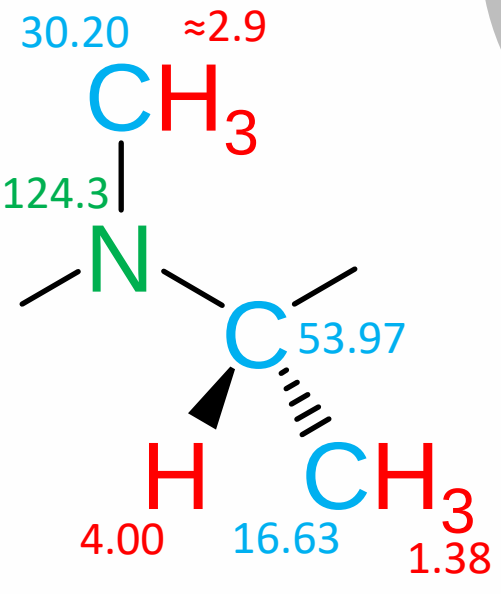


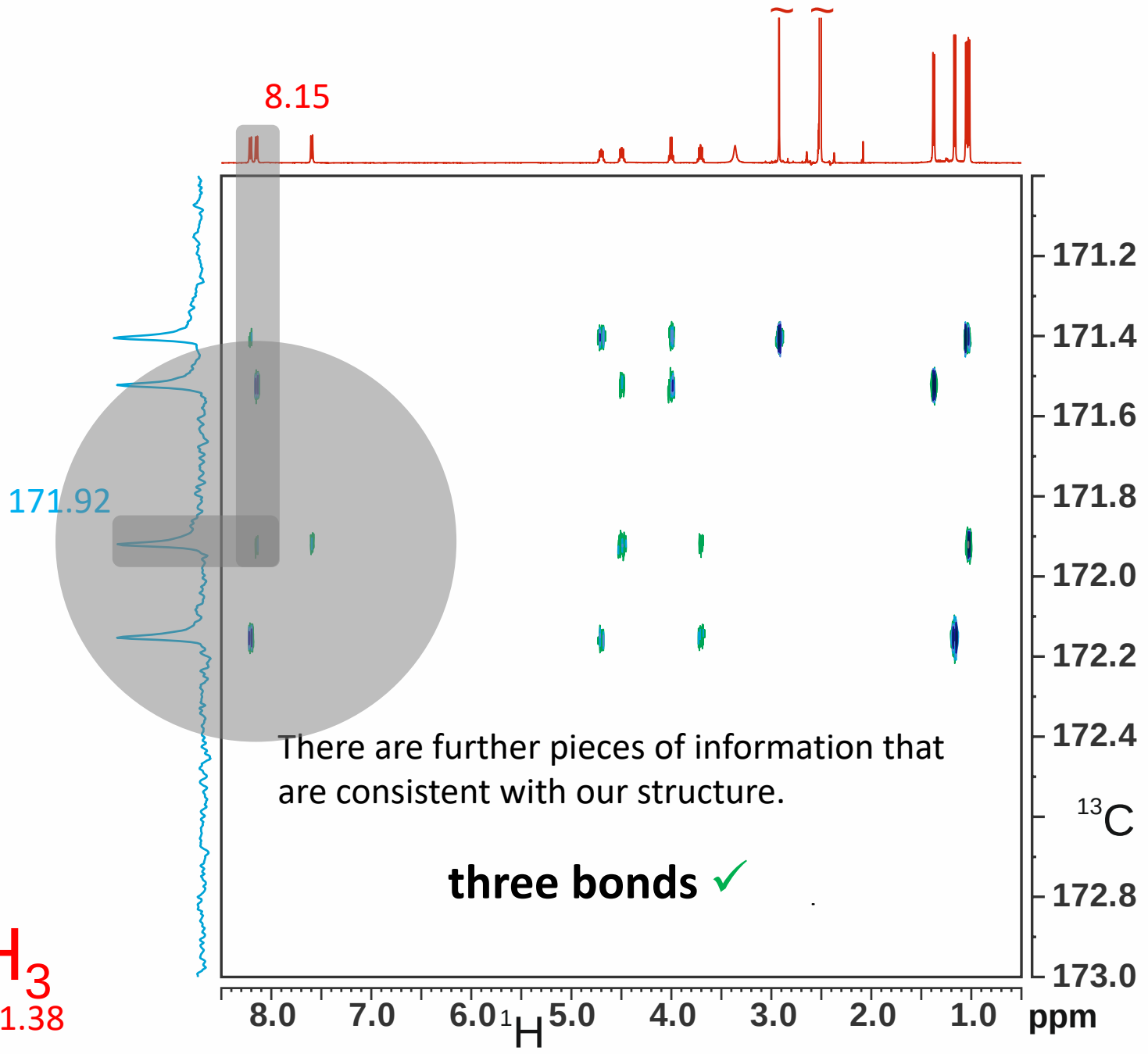


Carbonyl groups

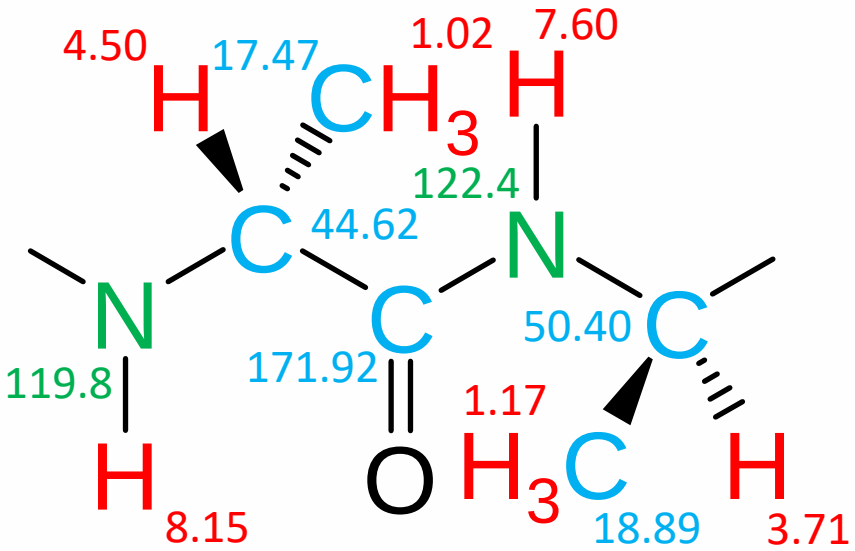


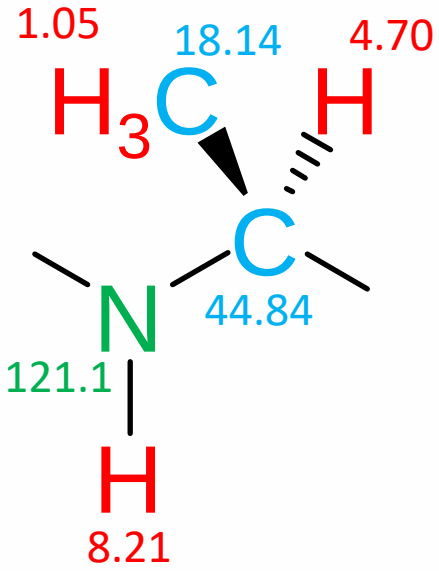


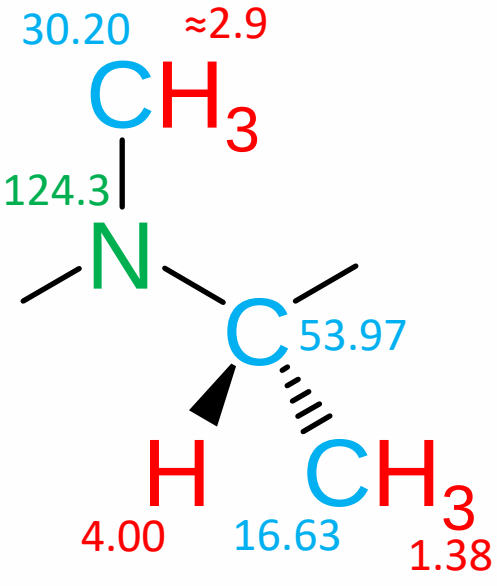


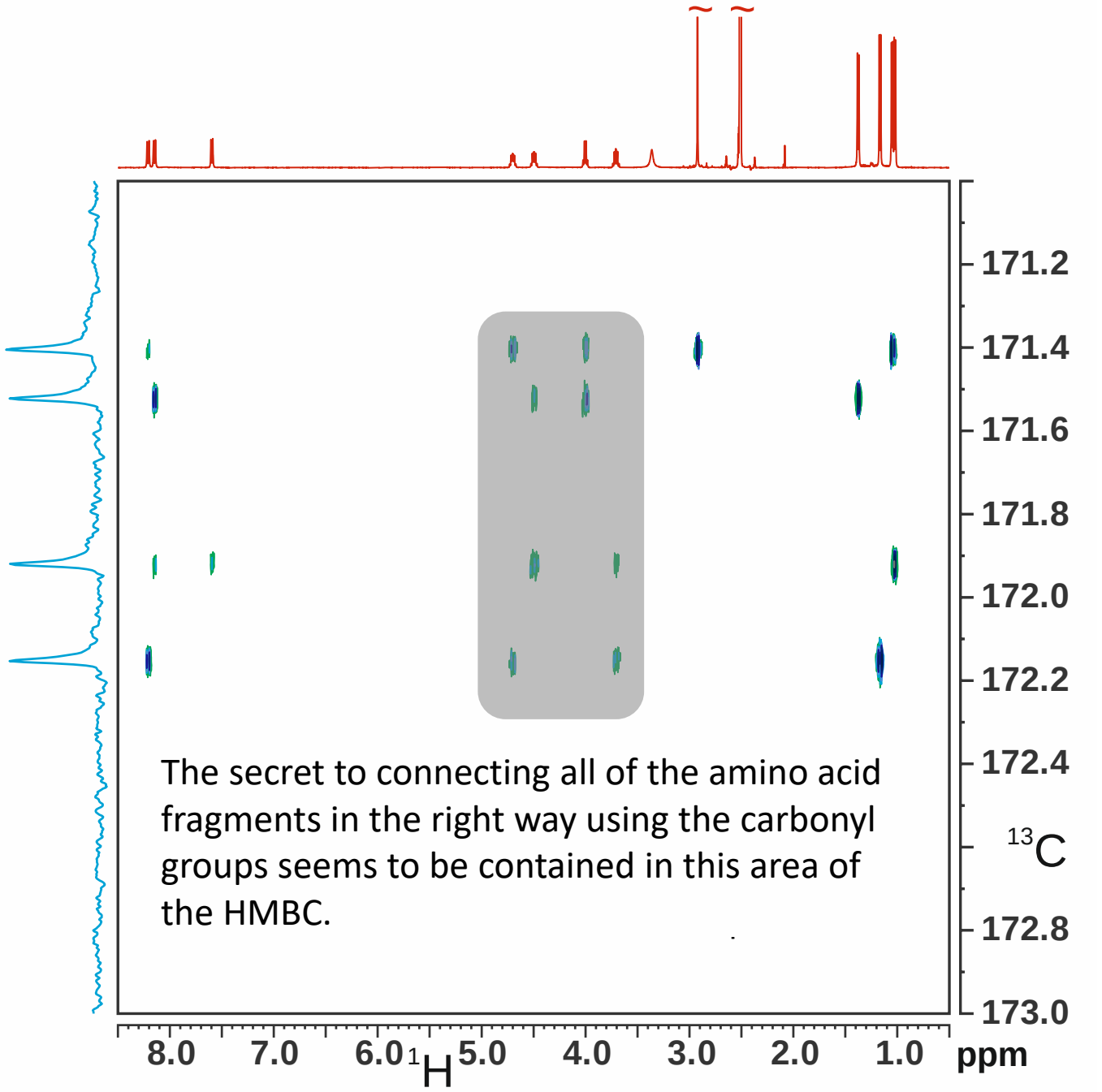


Carbonyl groups

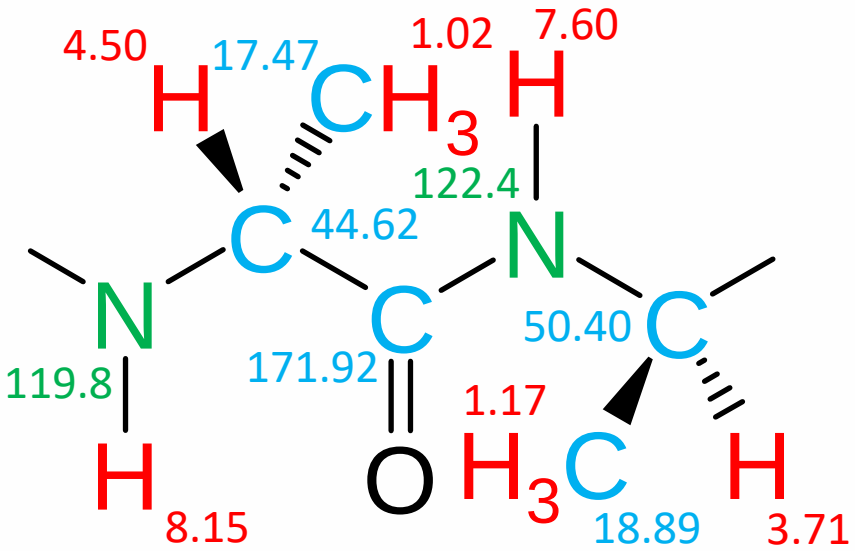


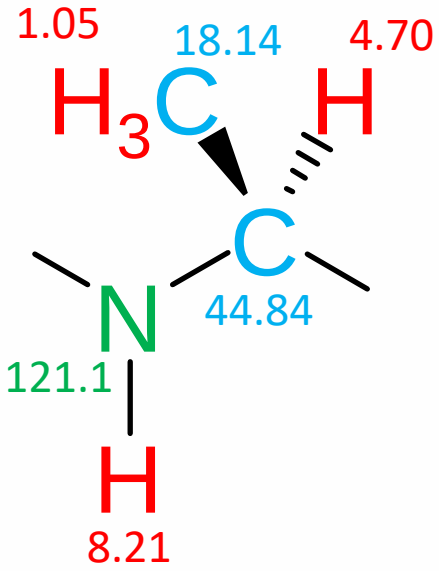


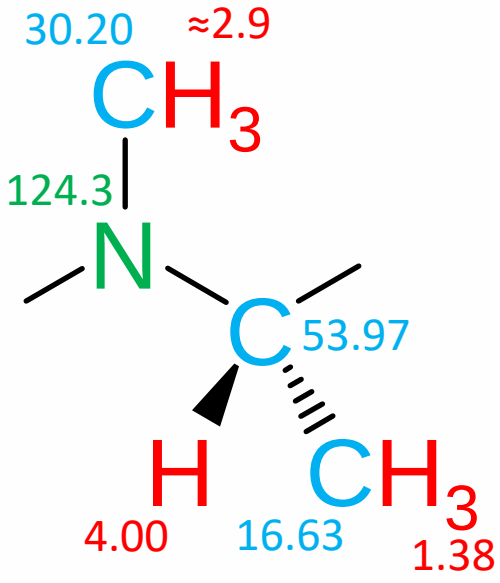


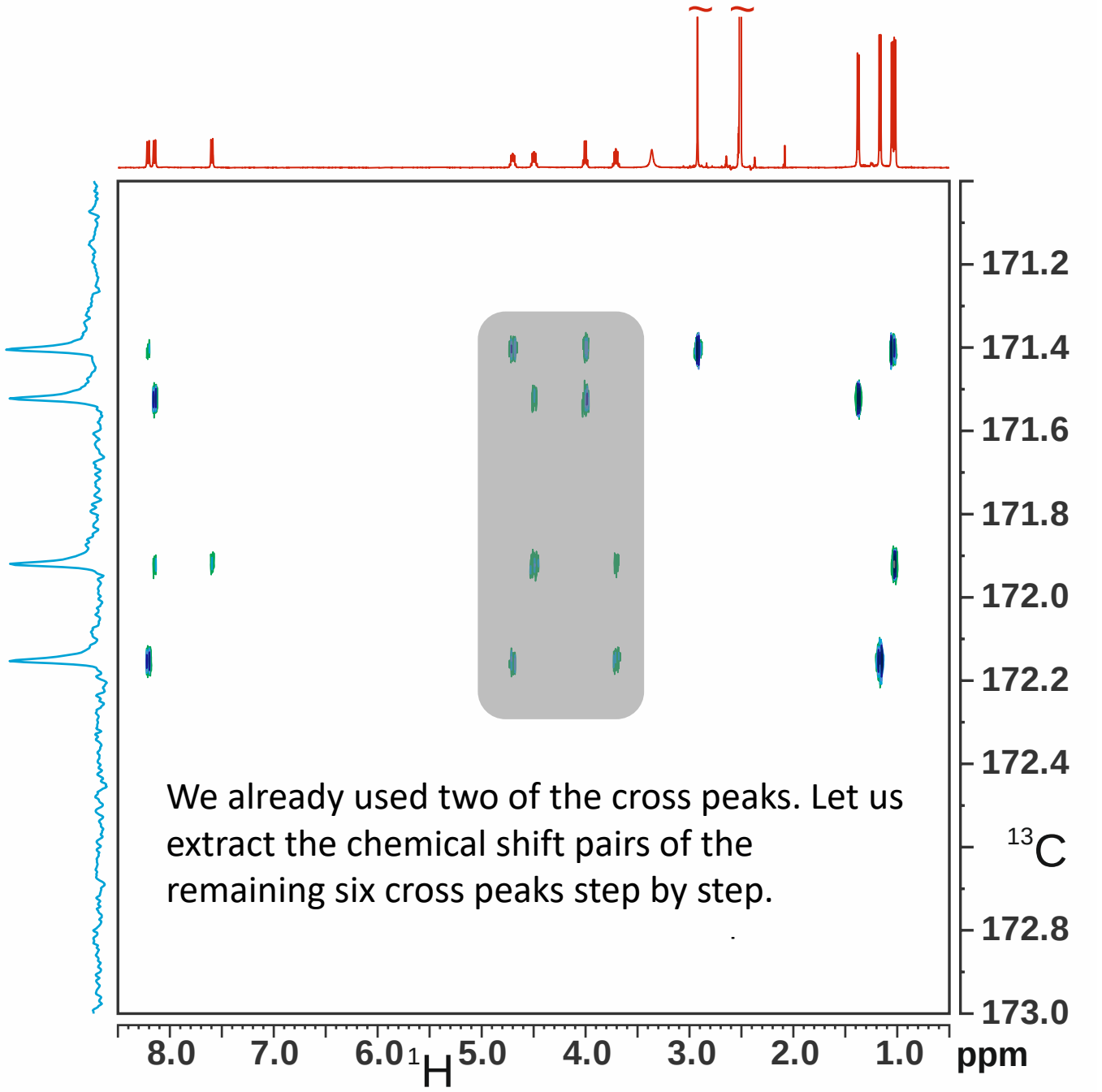


Carbonyl groups

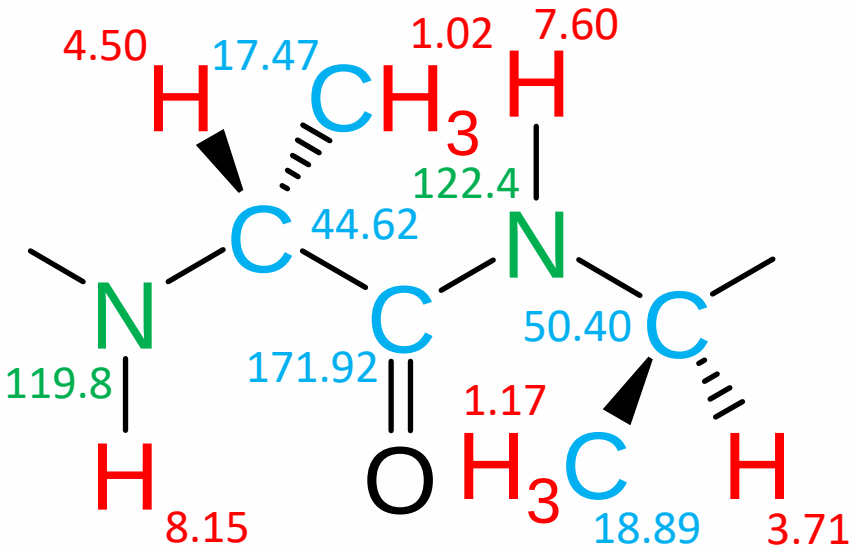


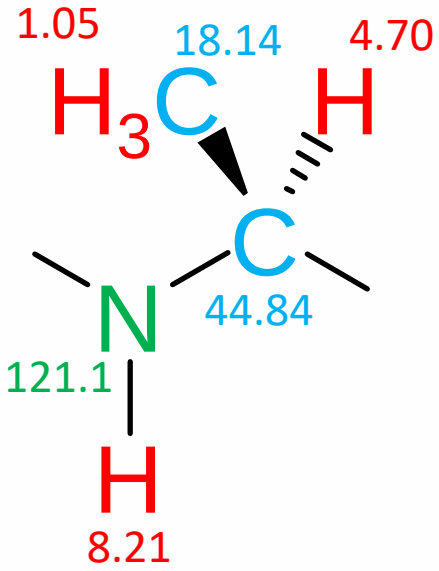


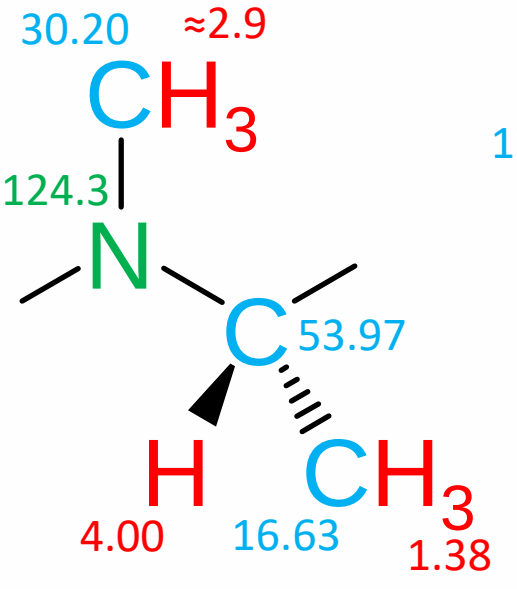


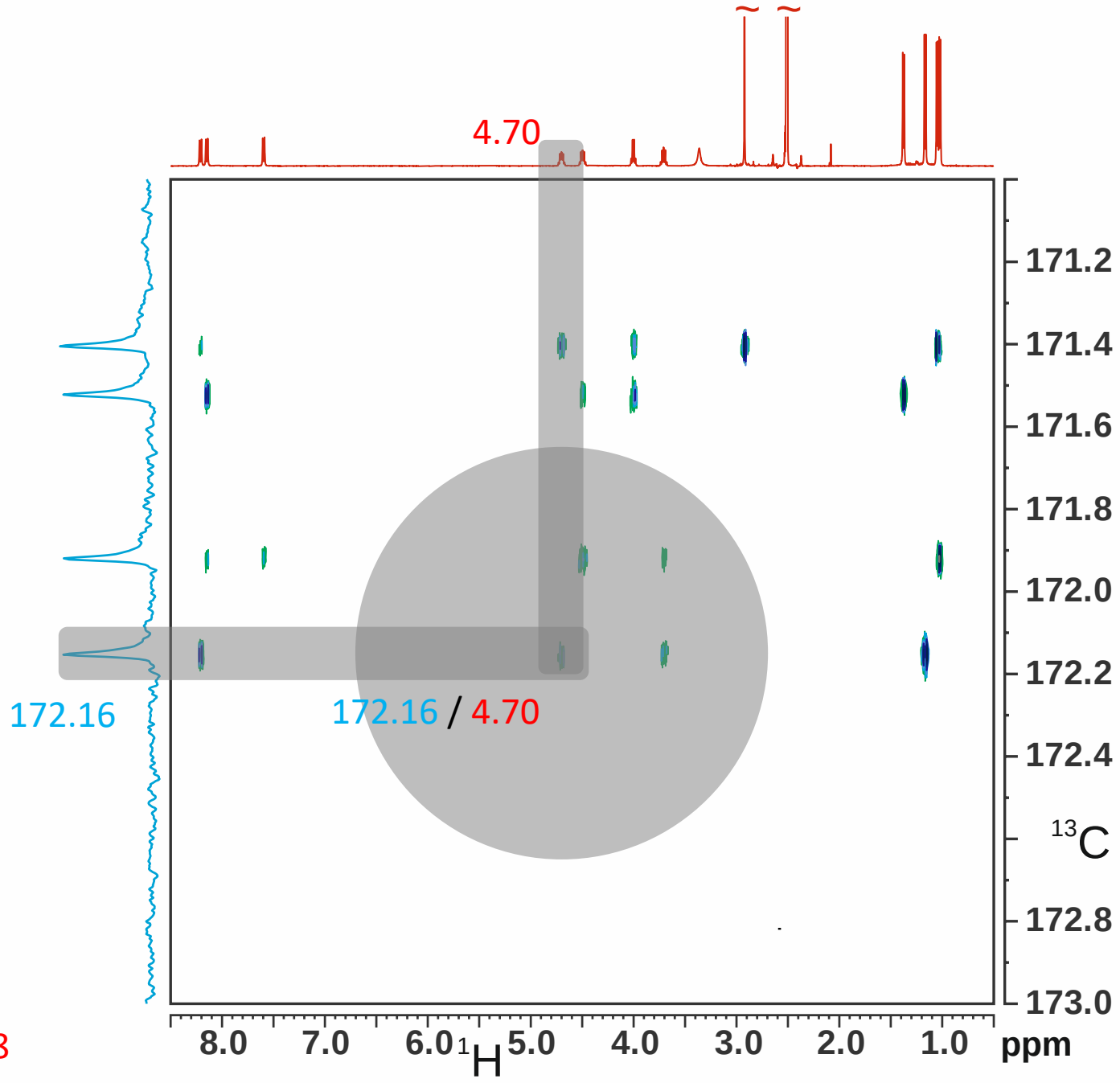


Carbonyl groups

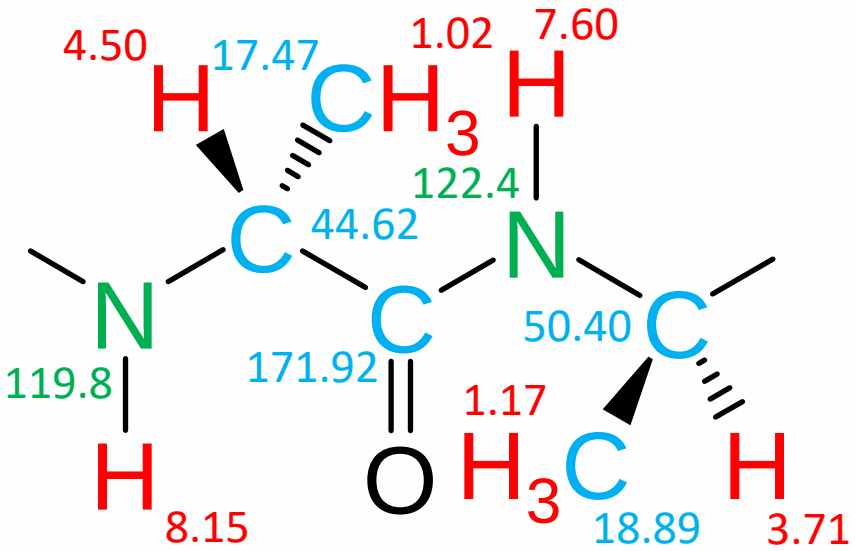


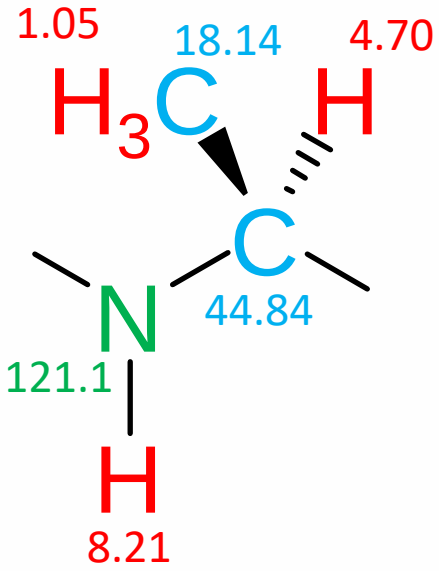


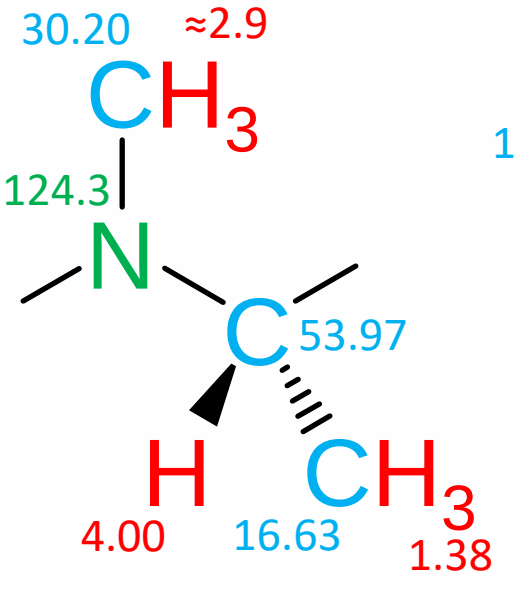


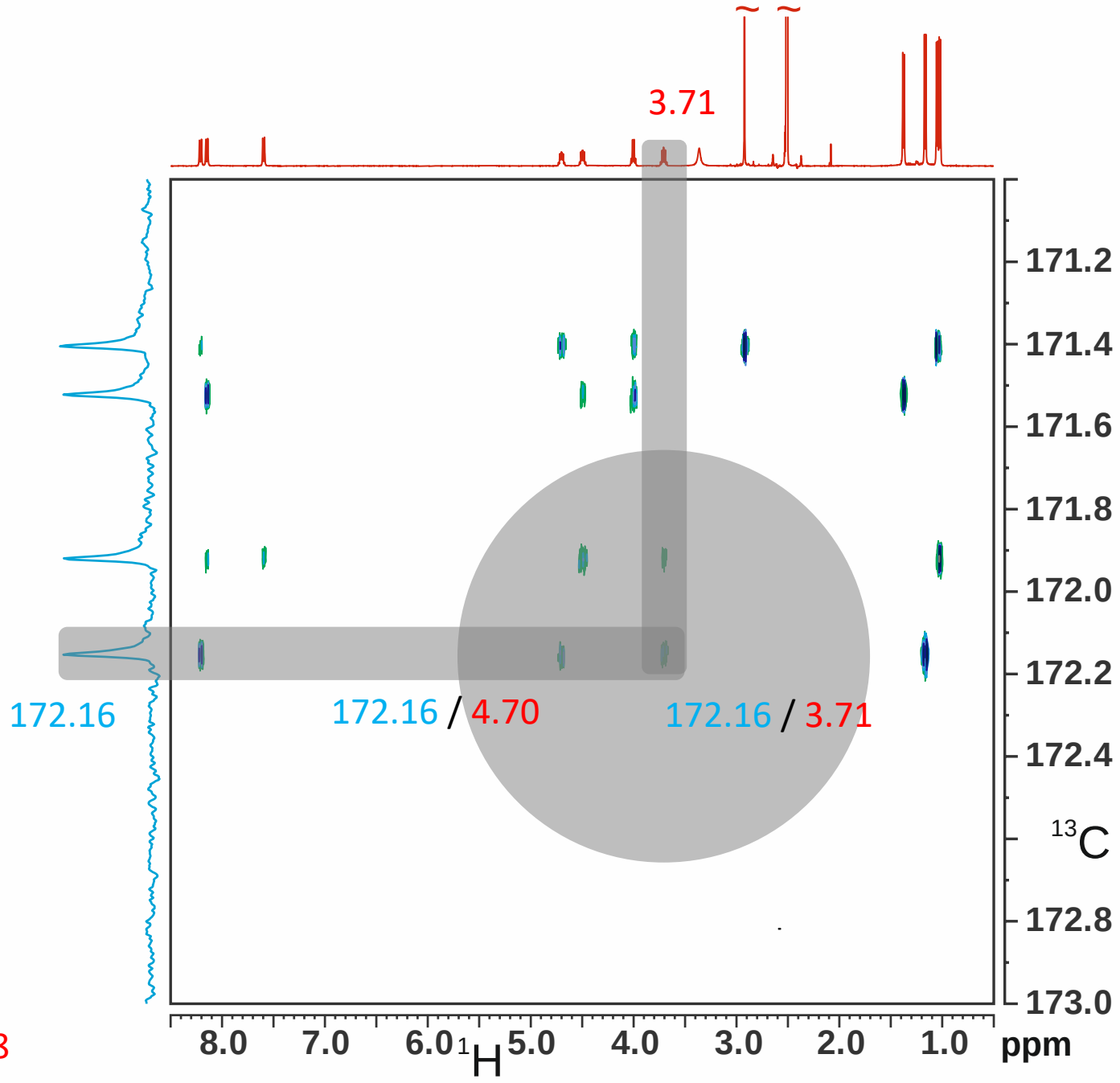


Carbonyl groups

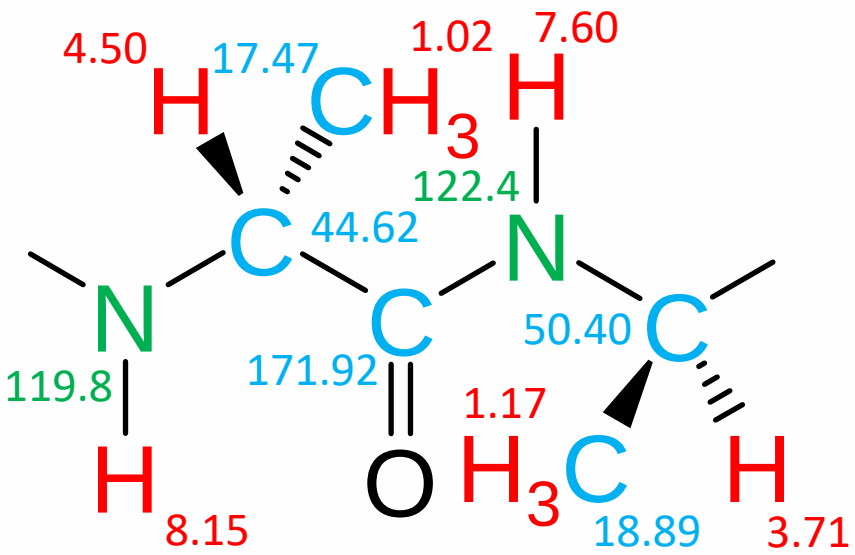


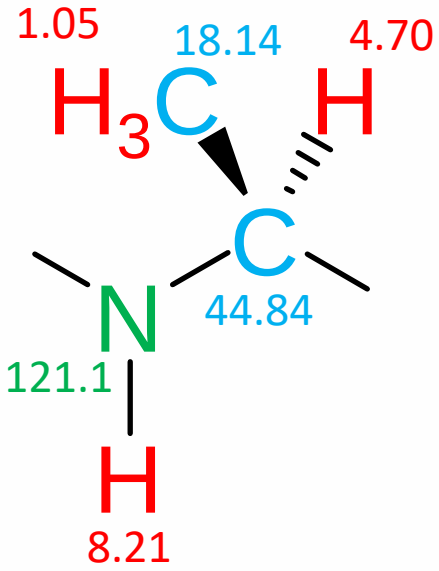


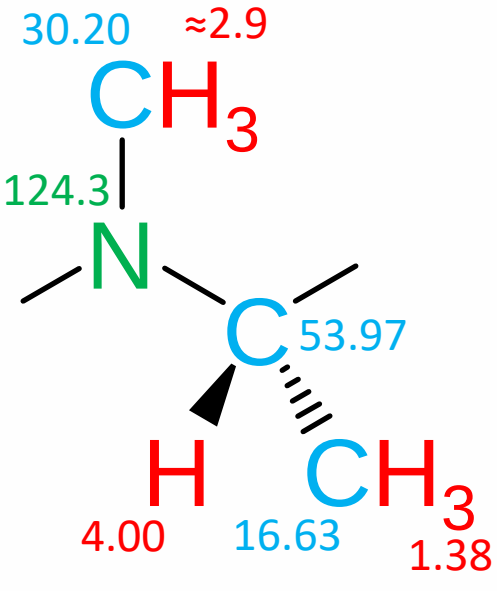


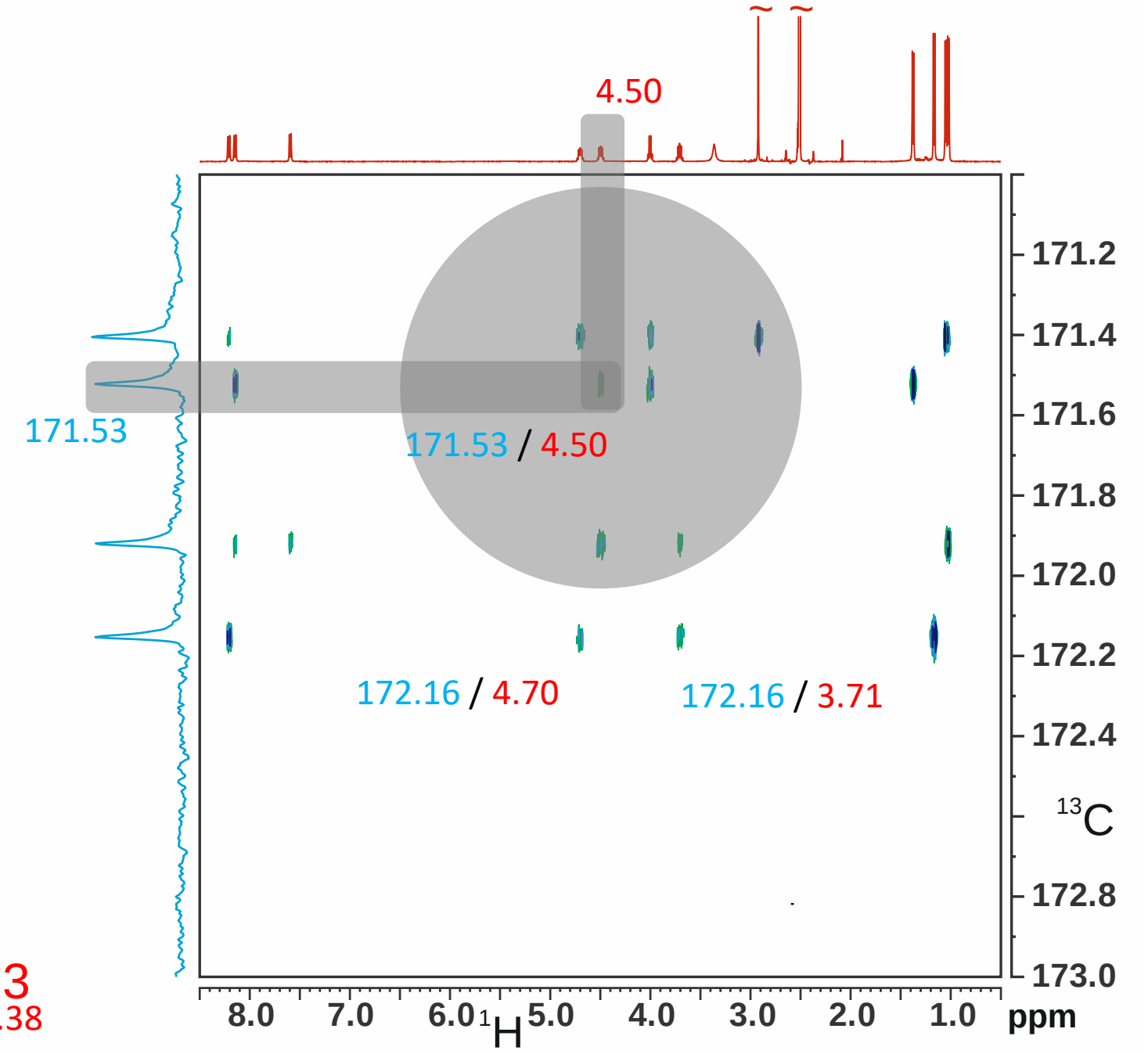


Carbonyl groups

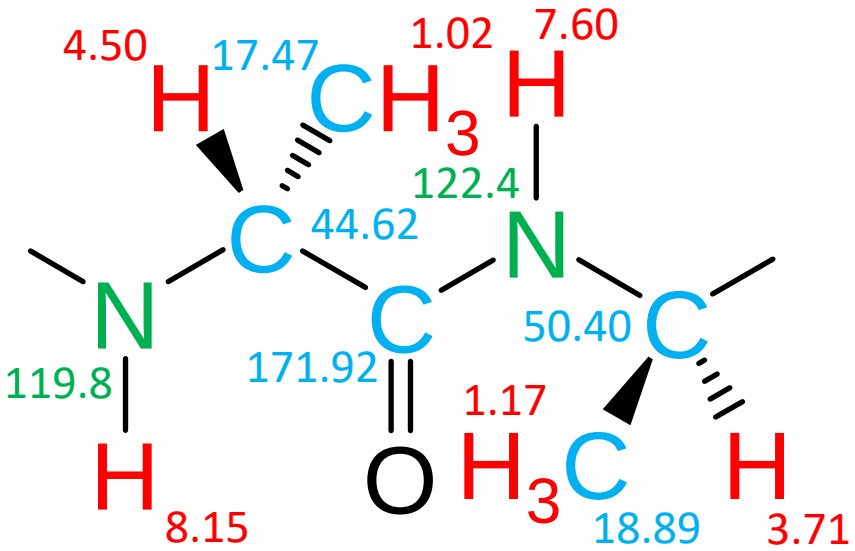


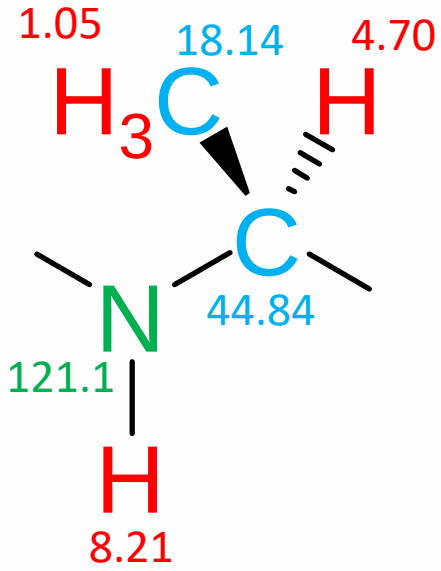


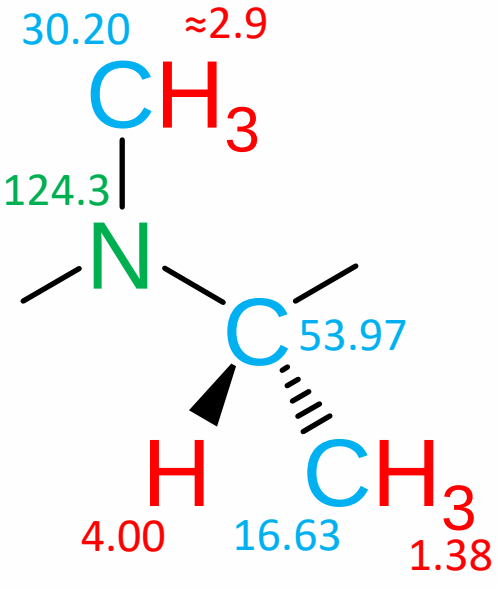


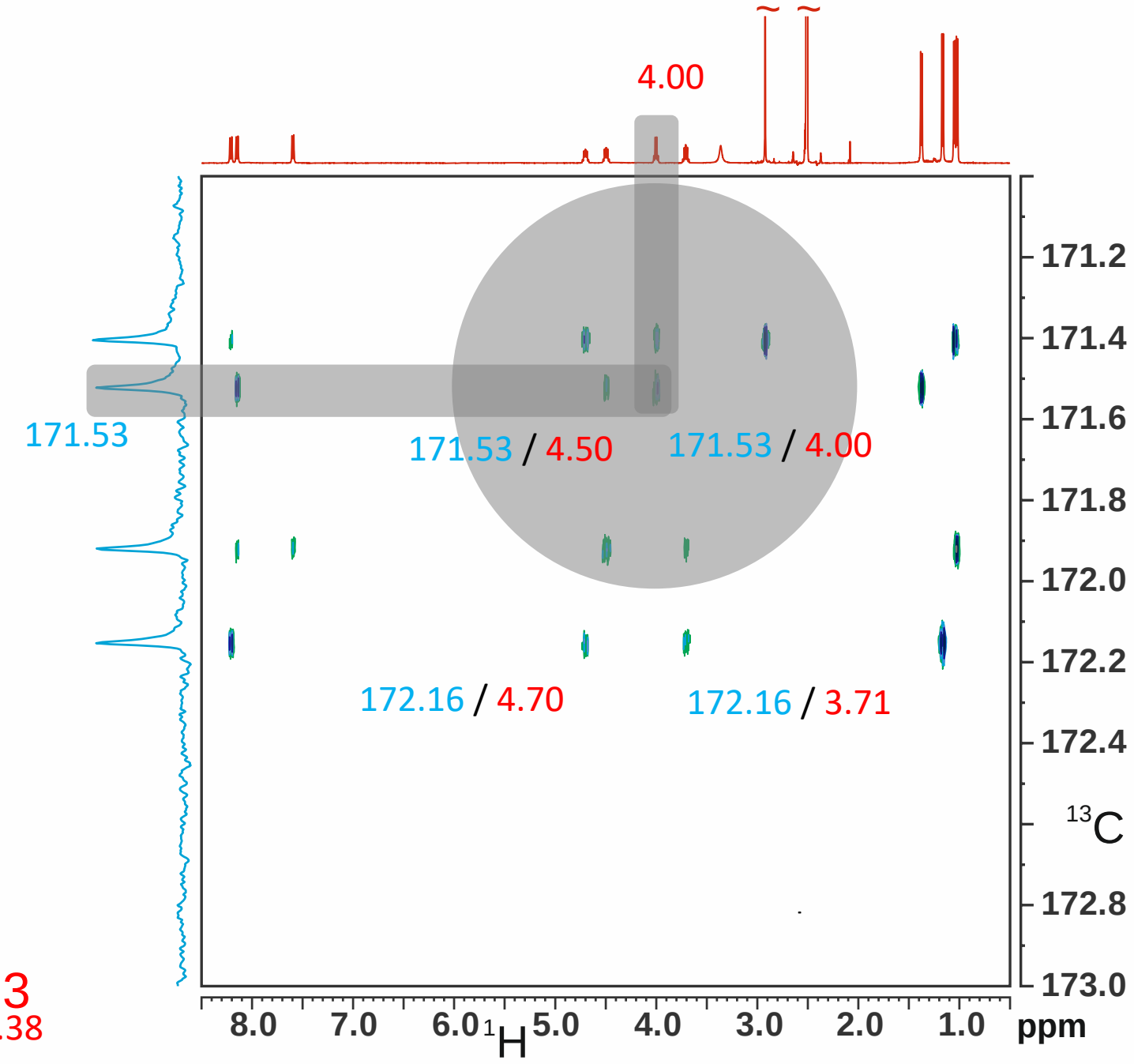


Carbonyl groups

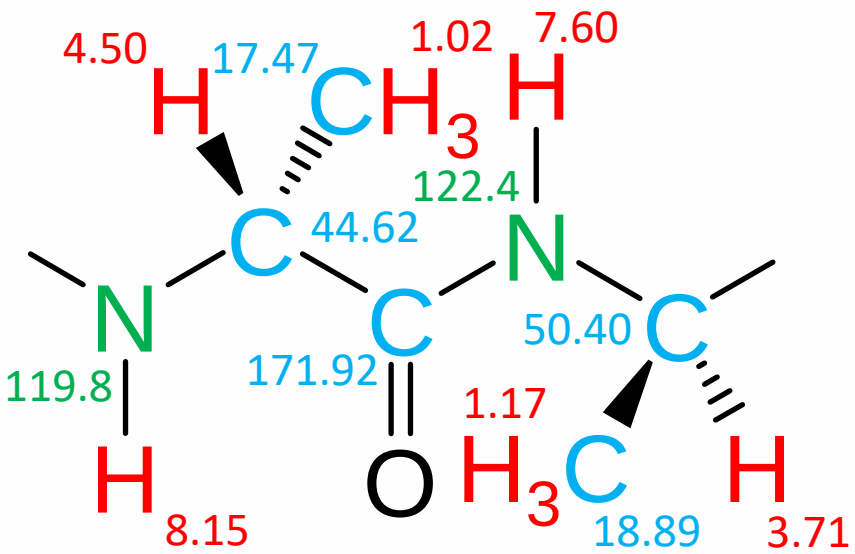


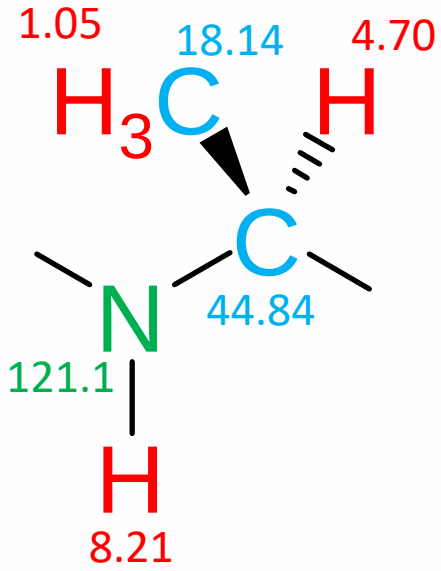


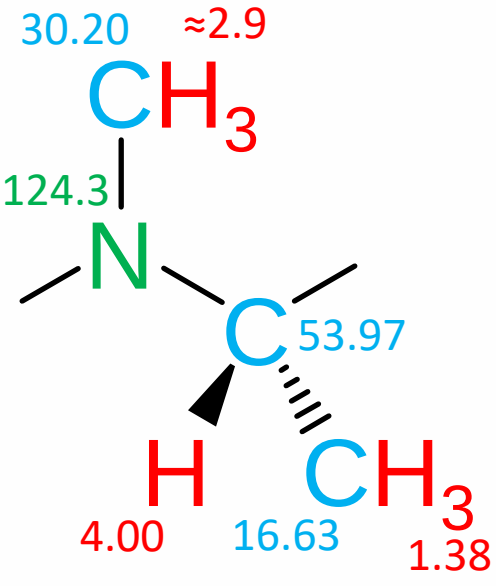


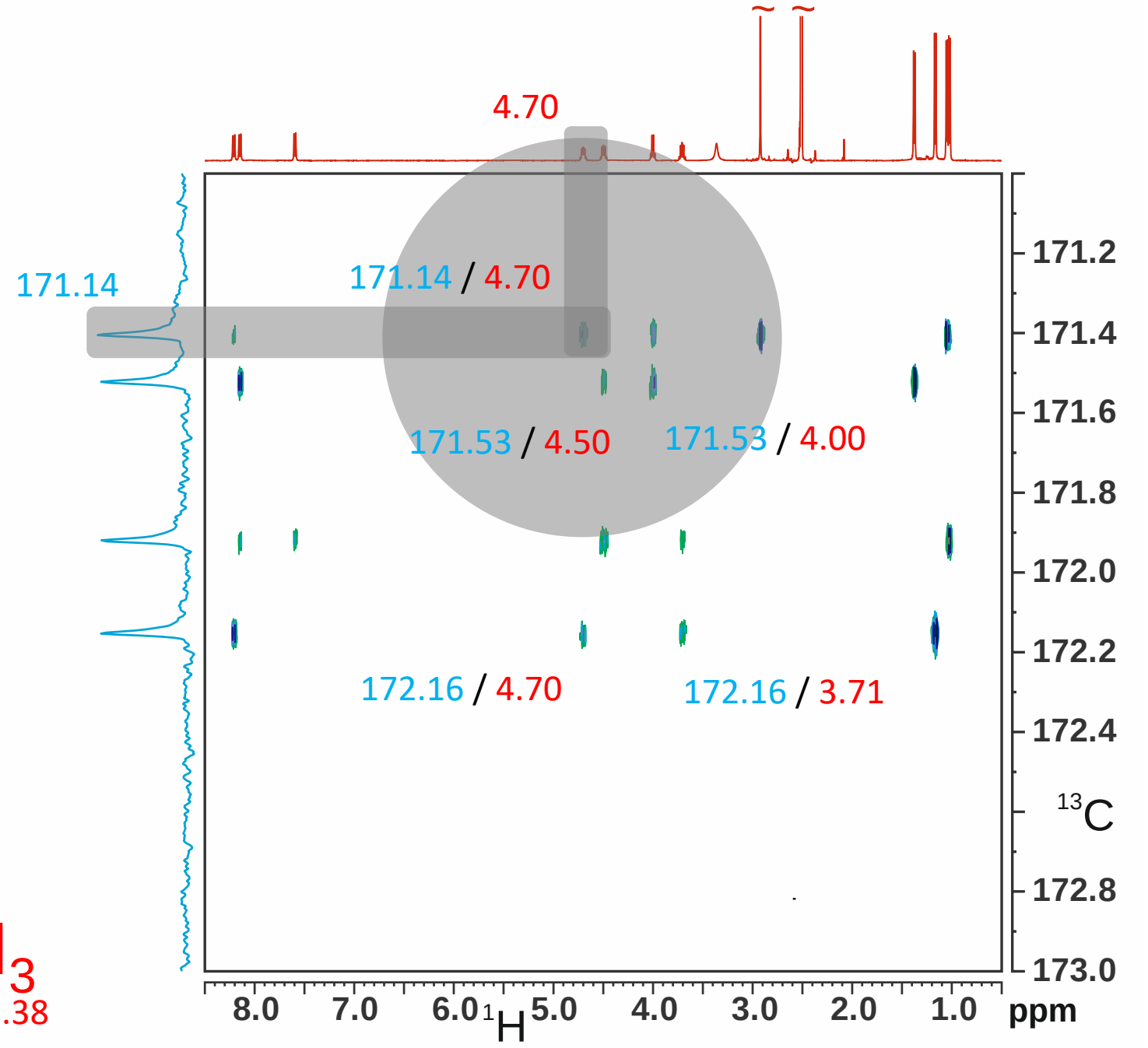


Carbonyl groups

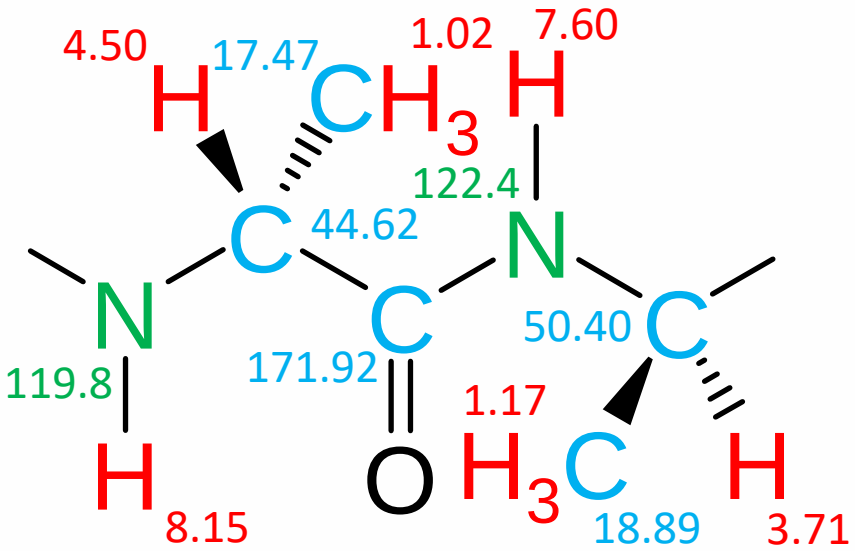


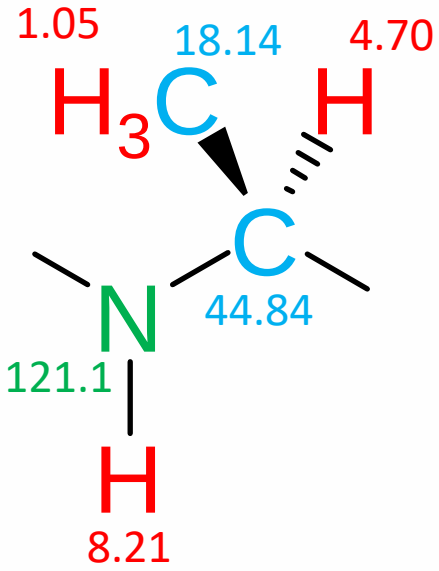


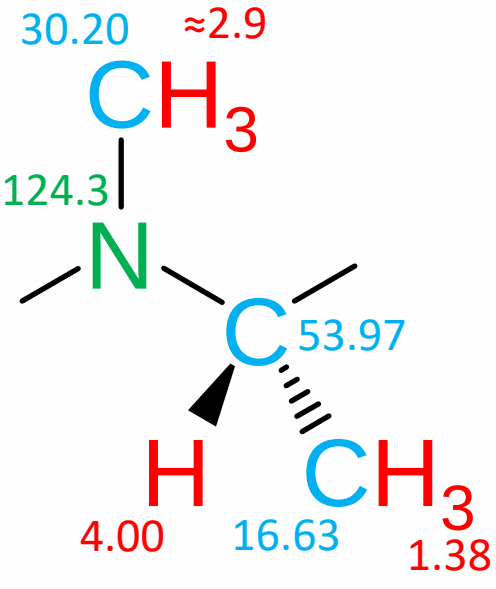


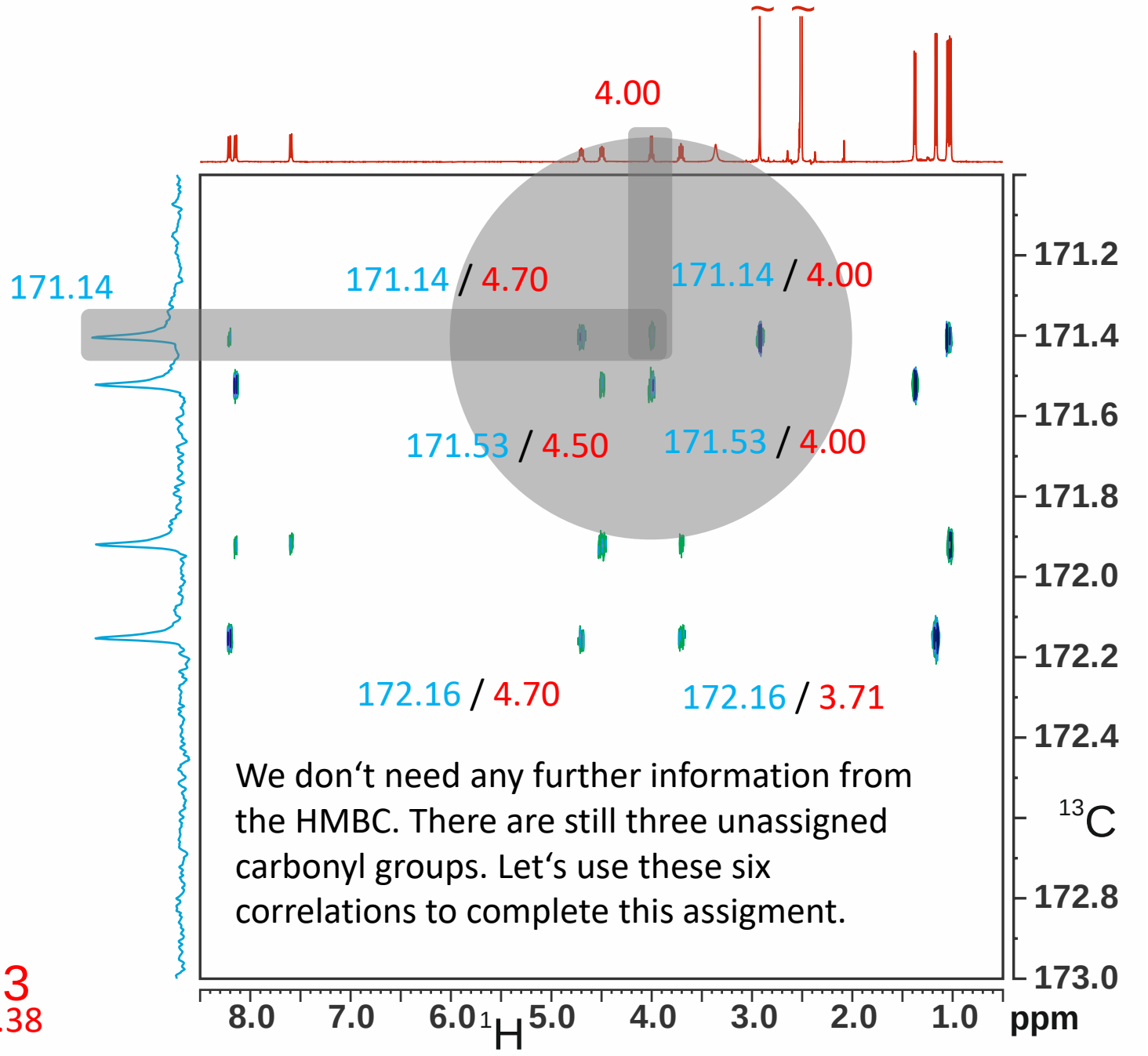


Carbonyl groups

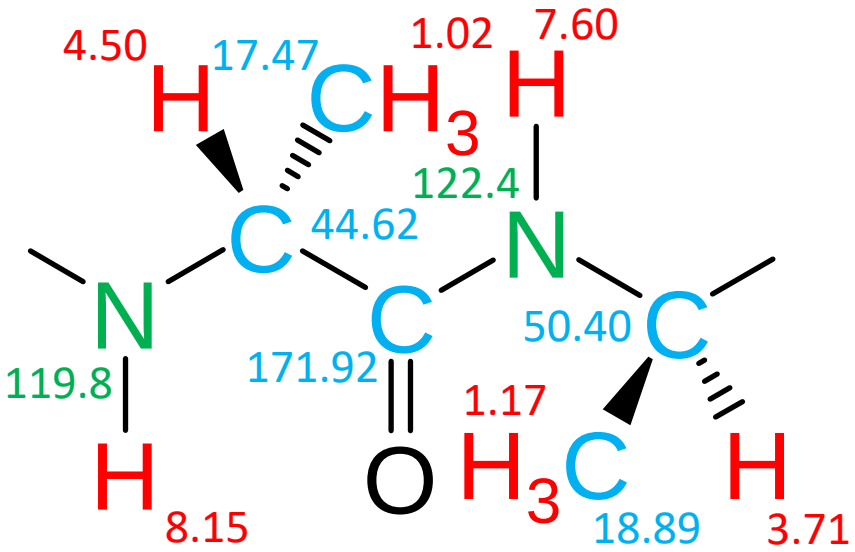


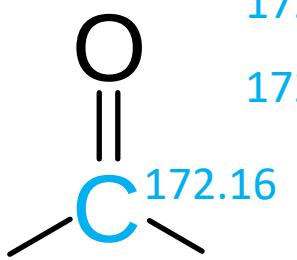






Carbonyl groups





172.16 / 4.70

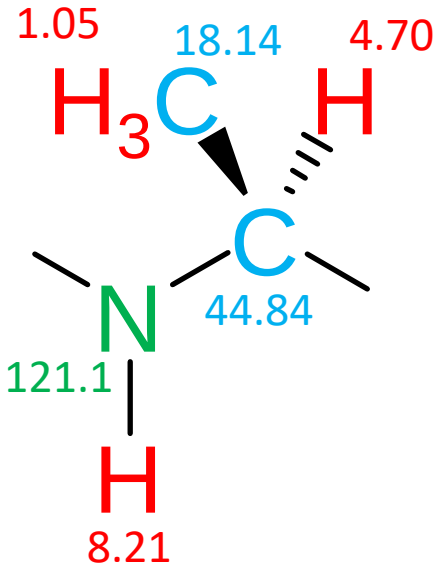
172.16 / 3.71

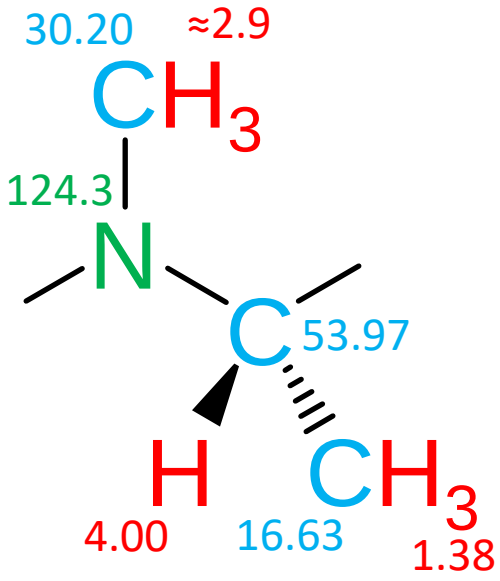
171.14 / 4.70

171.14 / 4.00

171.53 / 4.50

171.53 / 4.00

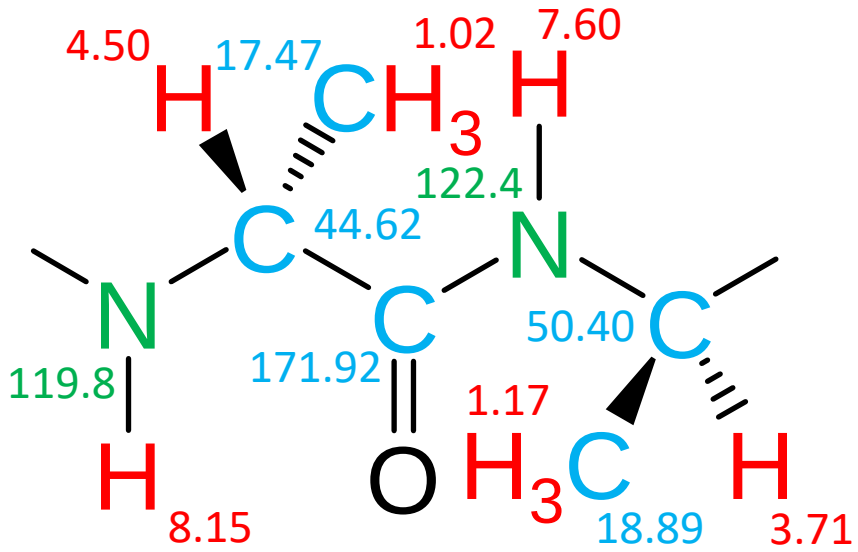


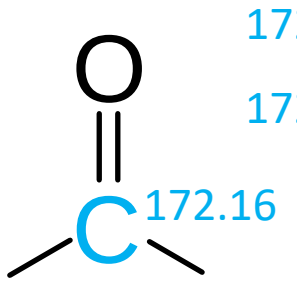


172.16 / 4.70

172.16 / 3.71

Carbonyl groups





172.16 / 4.70

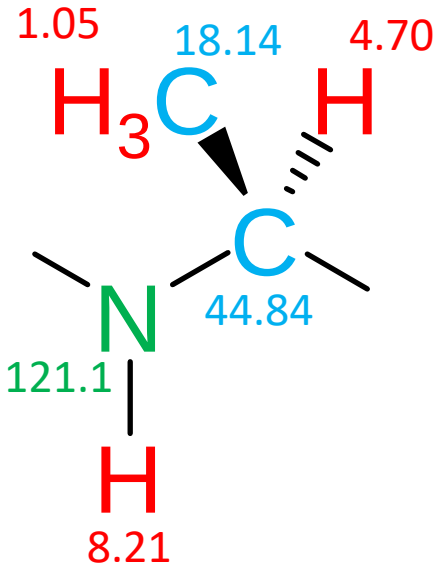
172.16 / 3.71

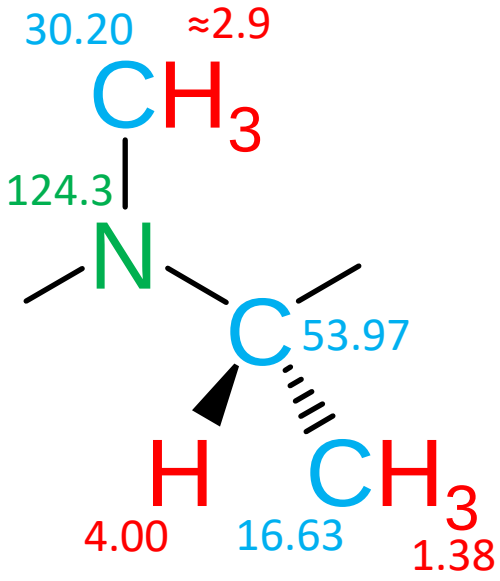
171.14 / 4.70

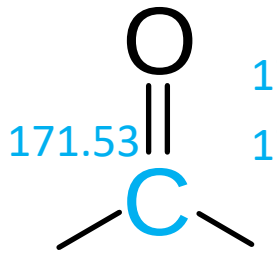
171.14 / 4.00

171.53 / 4.50

171.53 / 4.00



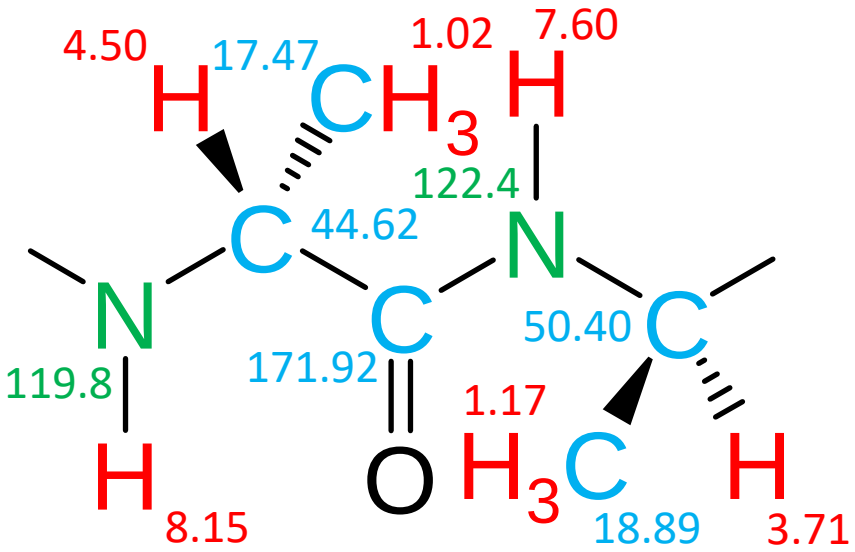


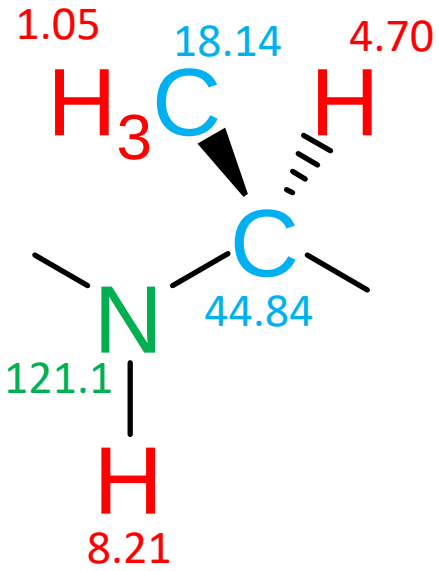


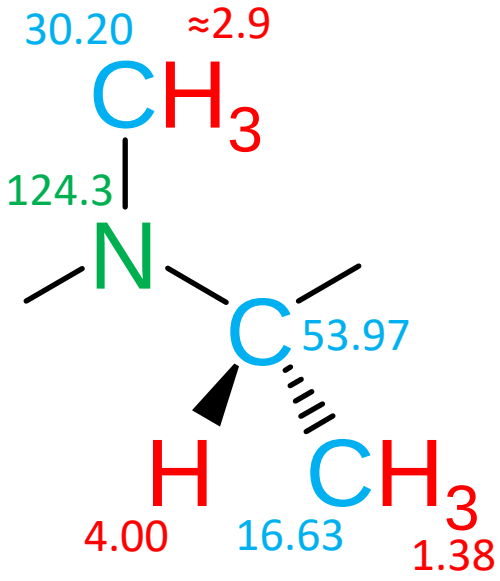
171.53 / 4.00

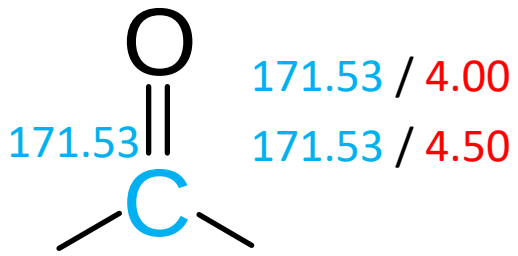
171.53 / 4.50

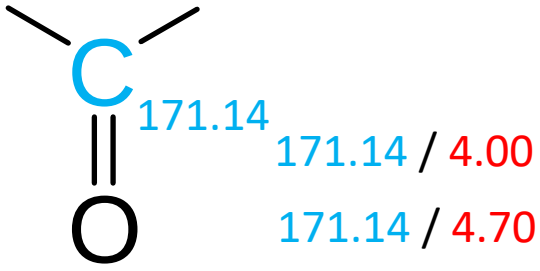
Carbonyl groups



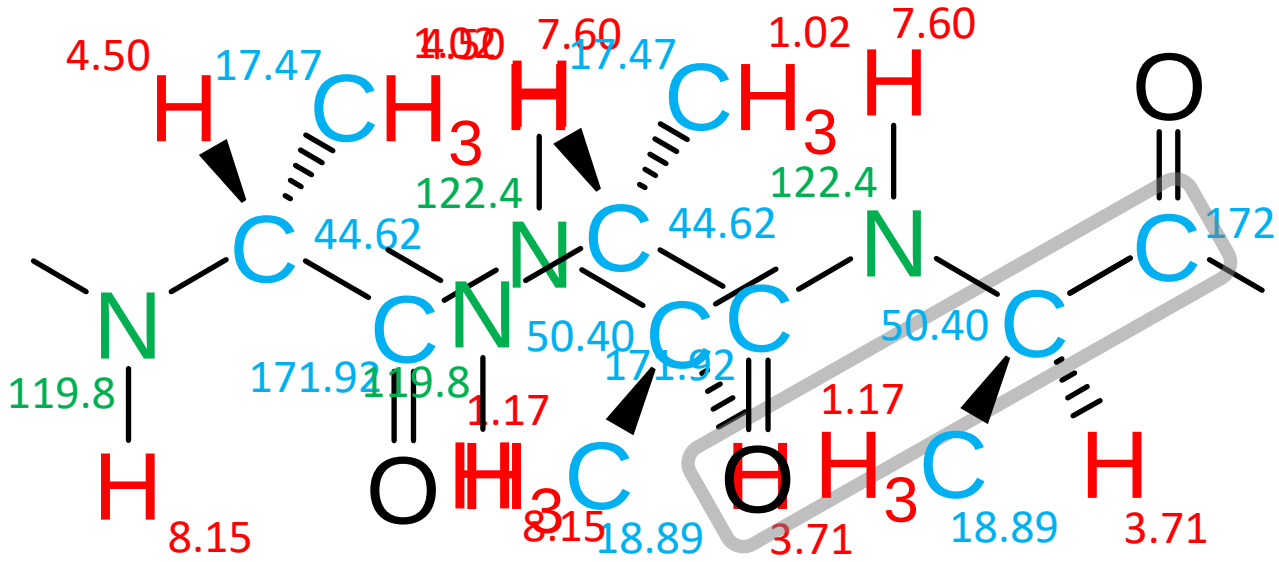








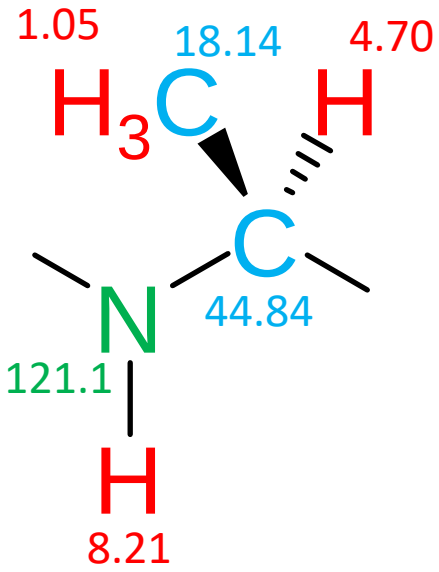
Carbonyl groups

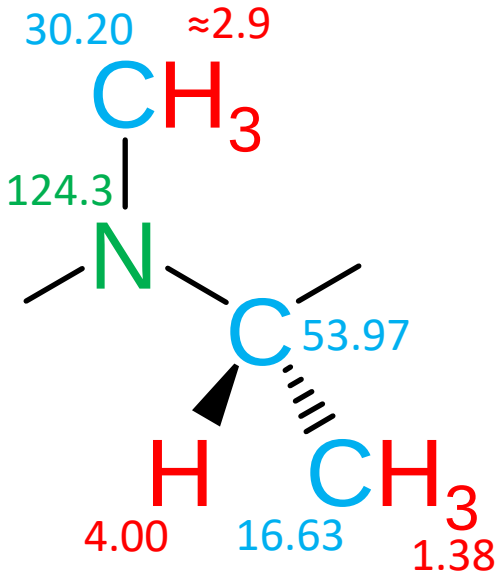


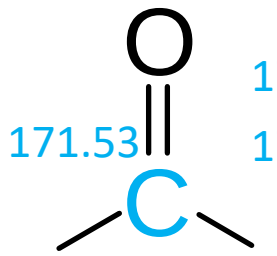
172.16 / 4.70

172.16 / 3.71

Apparently one carbonyl group is next to the already known dipeptide fragment.

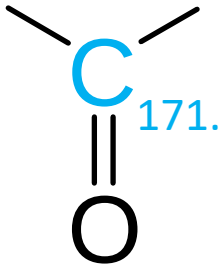






171.53 / 4.00

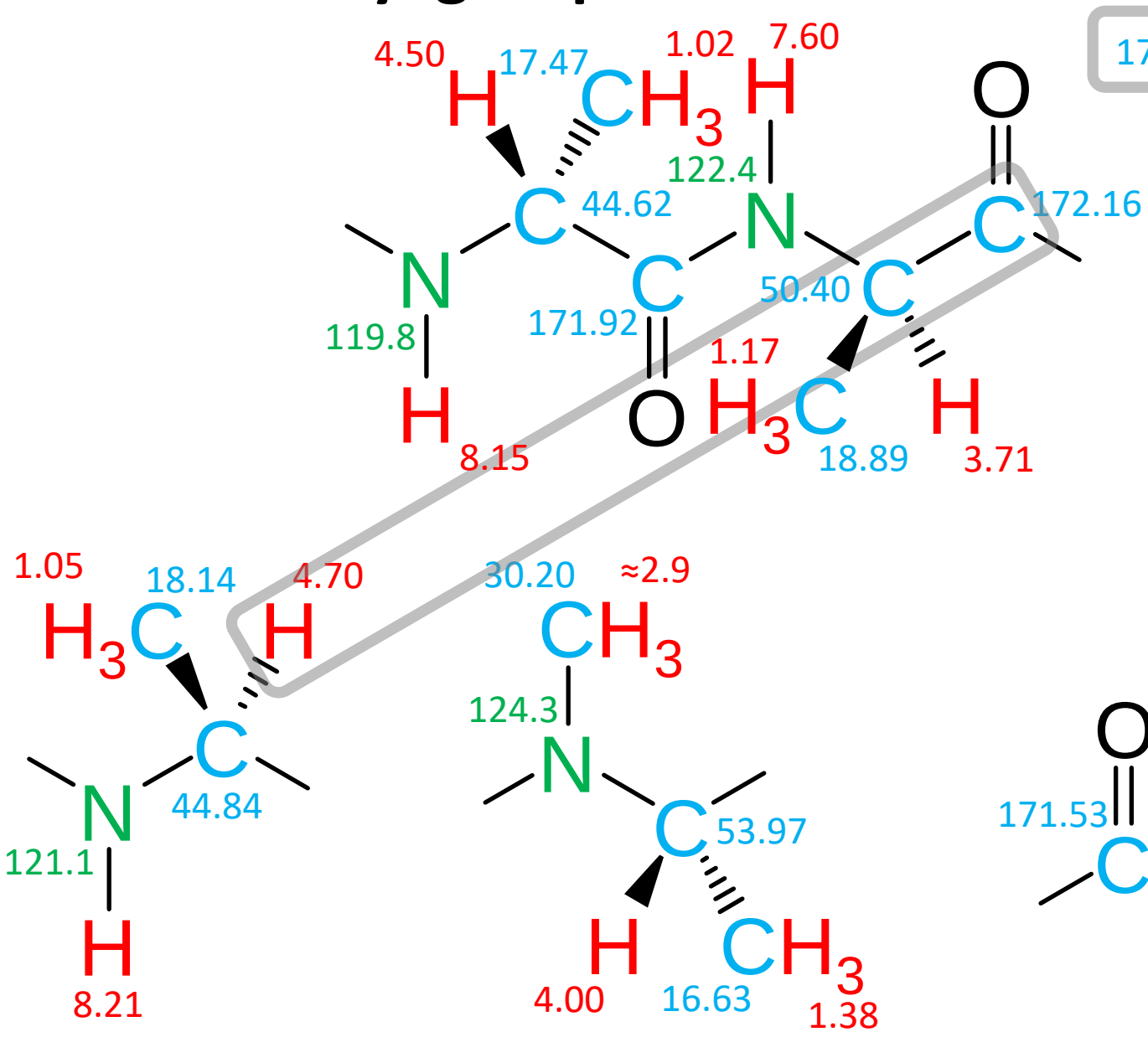
171.53 / 4.50



171.14 / 4.00

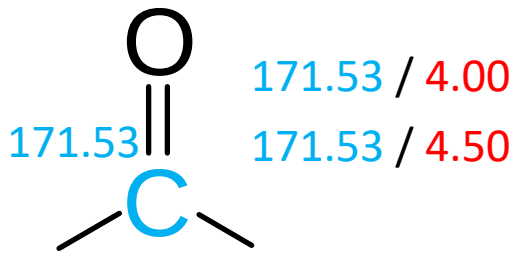
171.14 / 4.70

Carbonyl groups



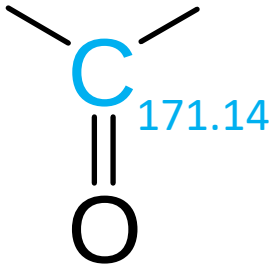
172.16 / 4.70

The next correlation is over three bonds, as soon as we attach the carbonyl atom with the chemical shift of 172.16 ppm and the nitrogen with the chemcial shift of 121.1 ppm together.



171.53 / 4.00

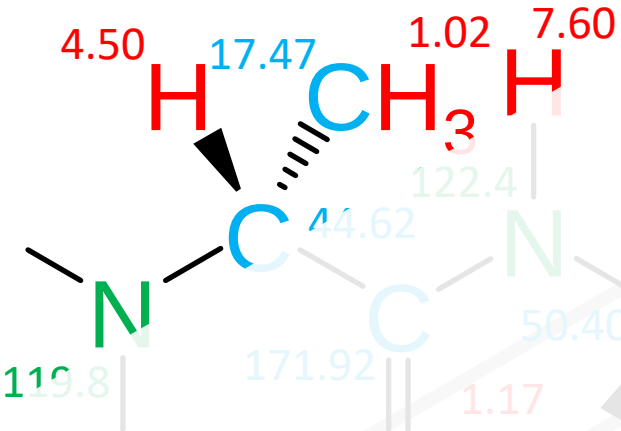
171.53 / 4.50

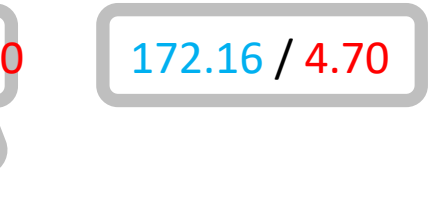


171.14 / 4.00

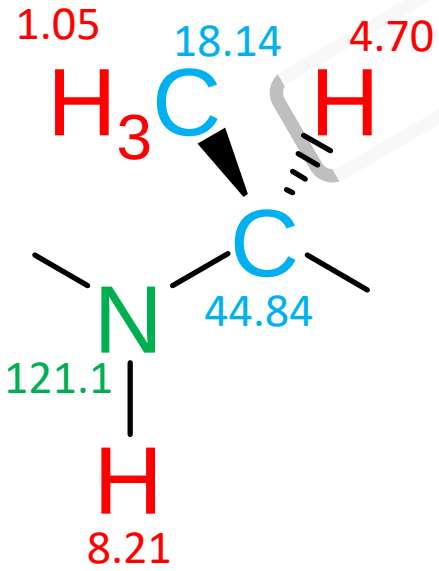
171.14 / 4.70

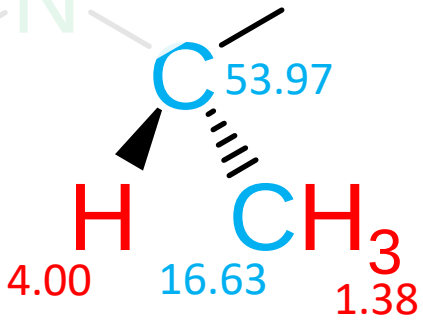
Carbonyl groups

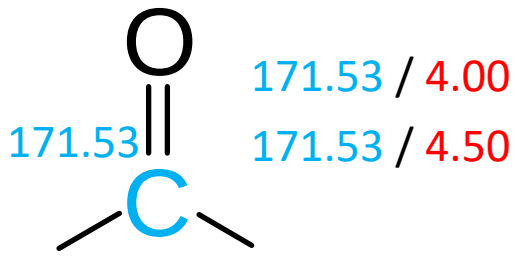


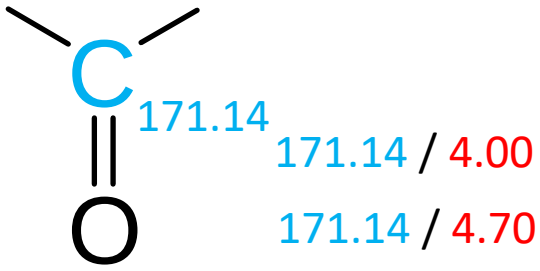


The next coorelation is over three bonds, as soon as we attach the carbonyl atom with the chemical shift of 172.16 ppm and the nitrogen with the chemcial shift of 121.1 ppm together.

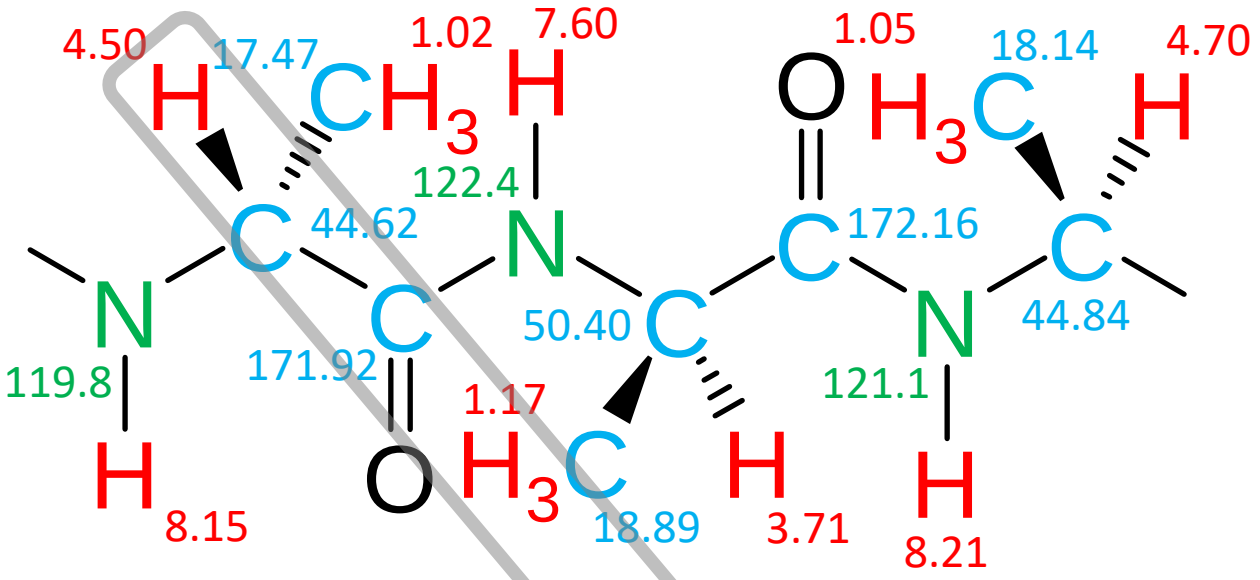




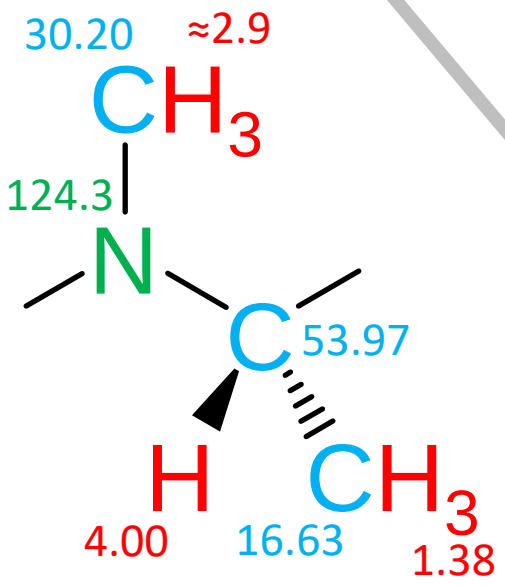


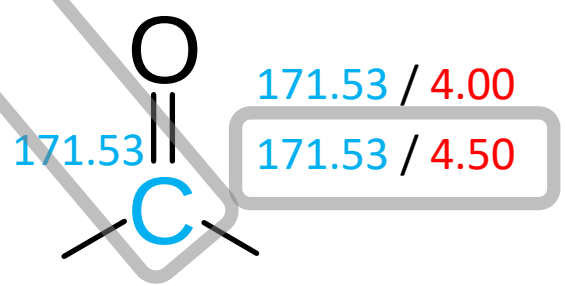


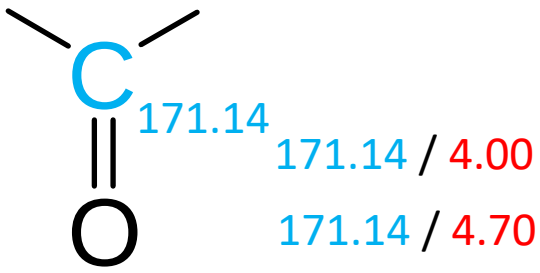
Carbonyl groups



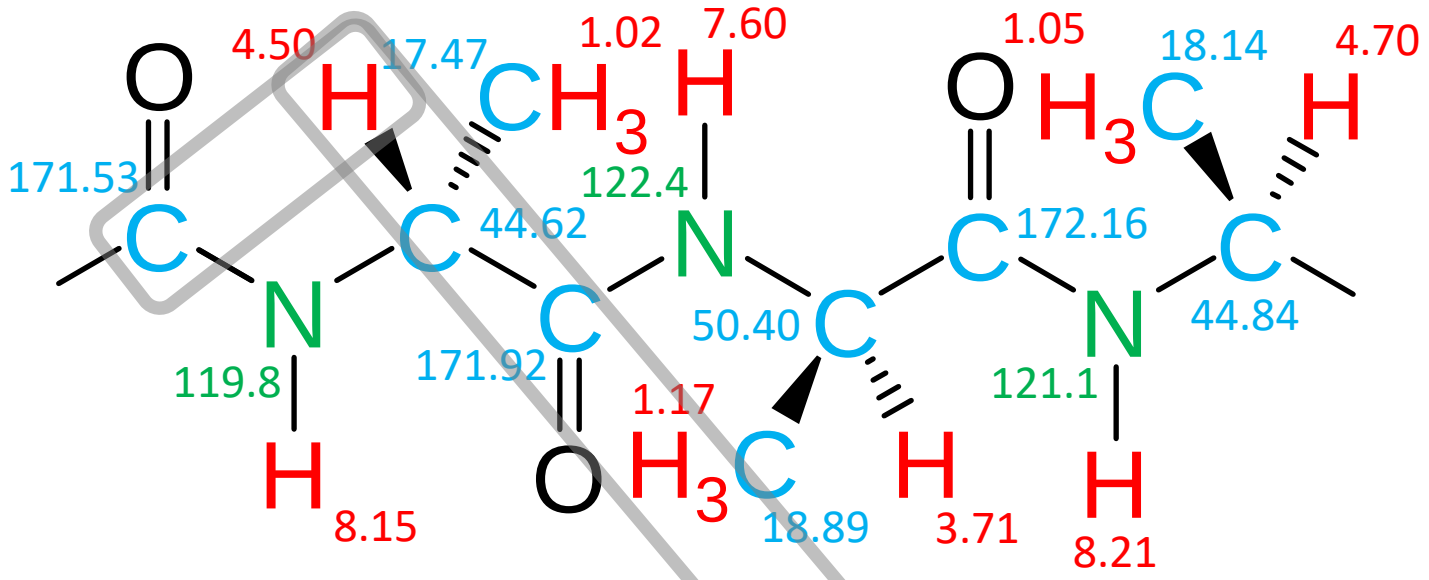
Let us continue.



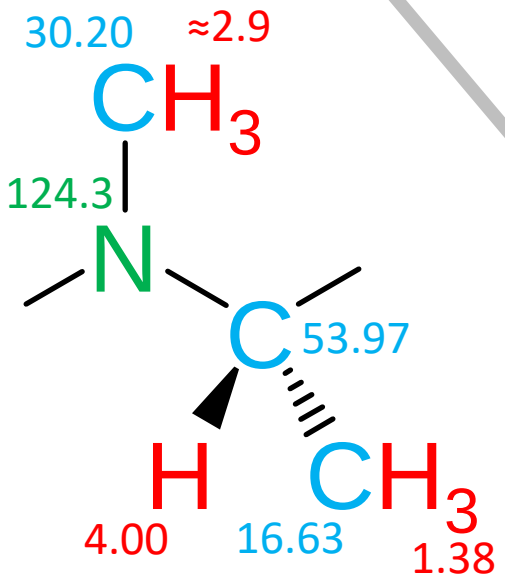


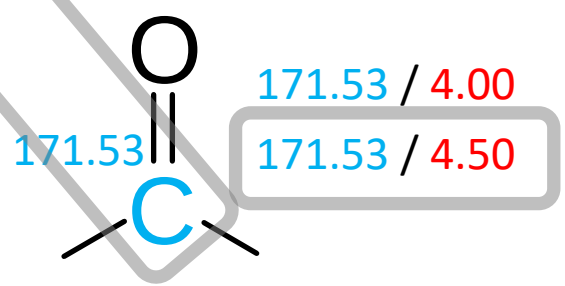


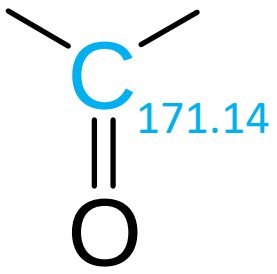
Carbonyl groups



Let us continue.



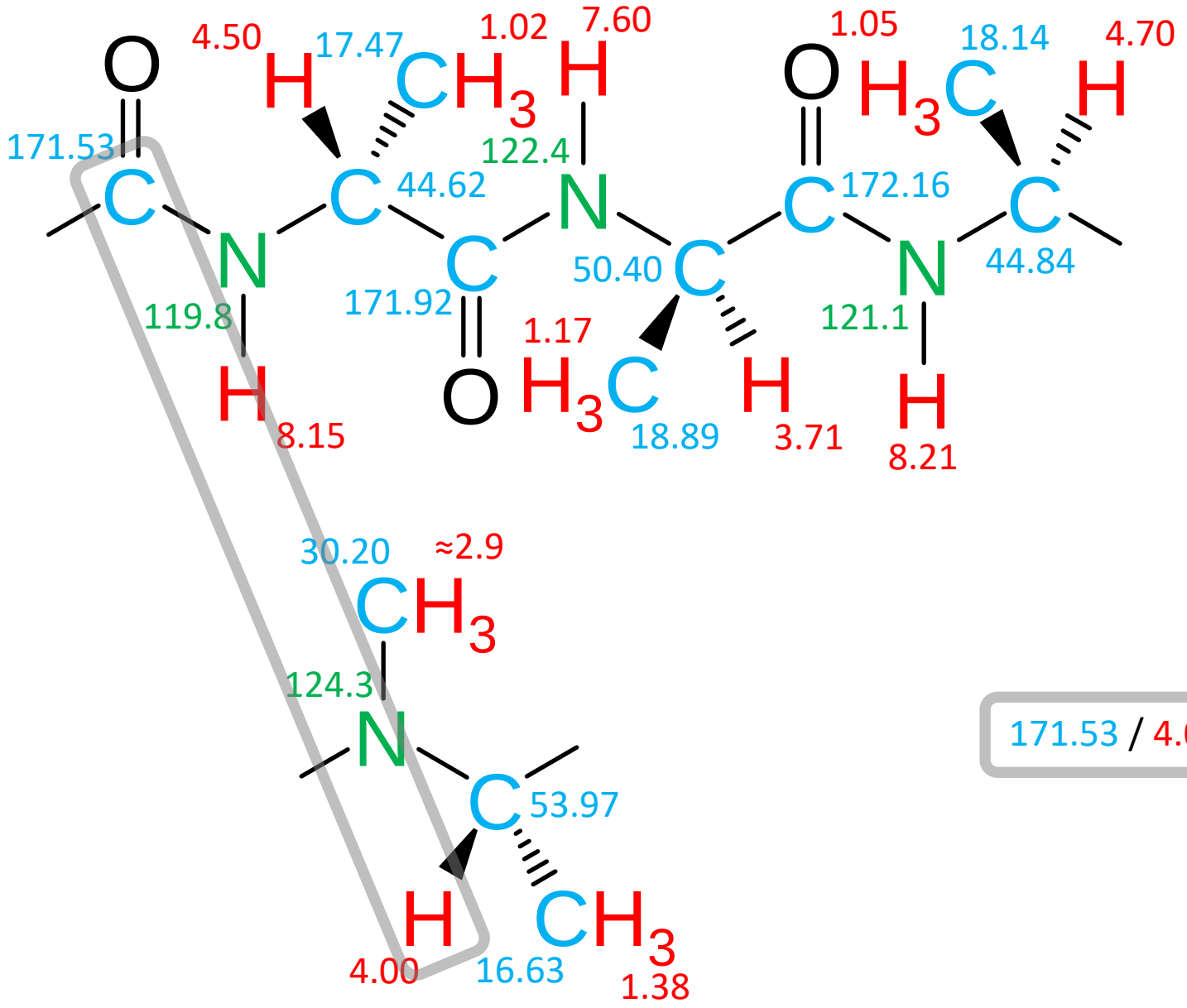




171.14 / 4.00

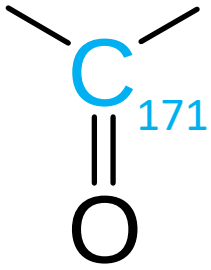
171.14 / 4.70

Carbonyl groups



There is one more correlation.

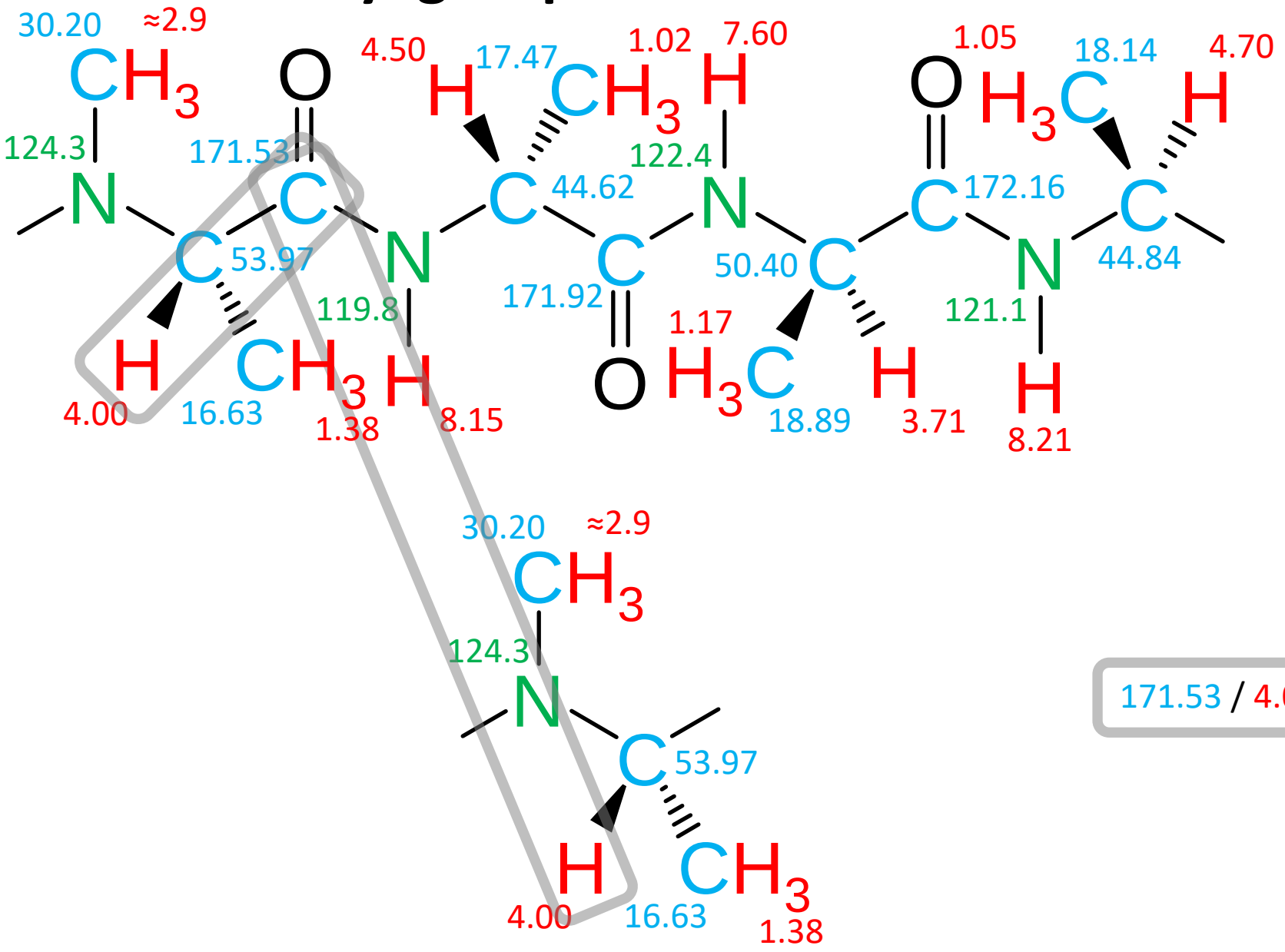
171.53 / 4.00



171.14 / 4.00

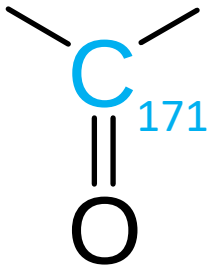
171.14 / 4.70

Carbonyl groups



There is one more correlation.

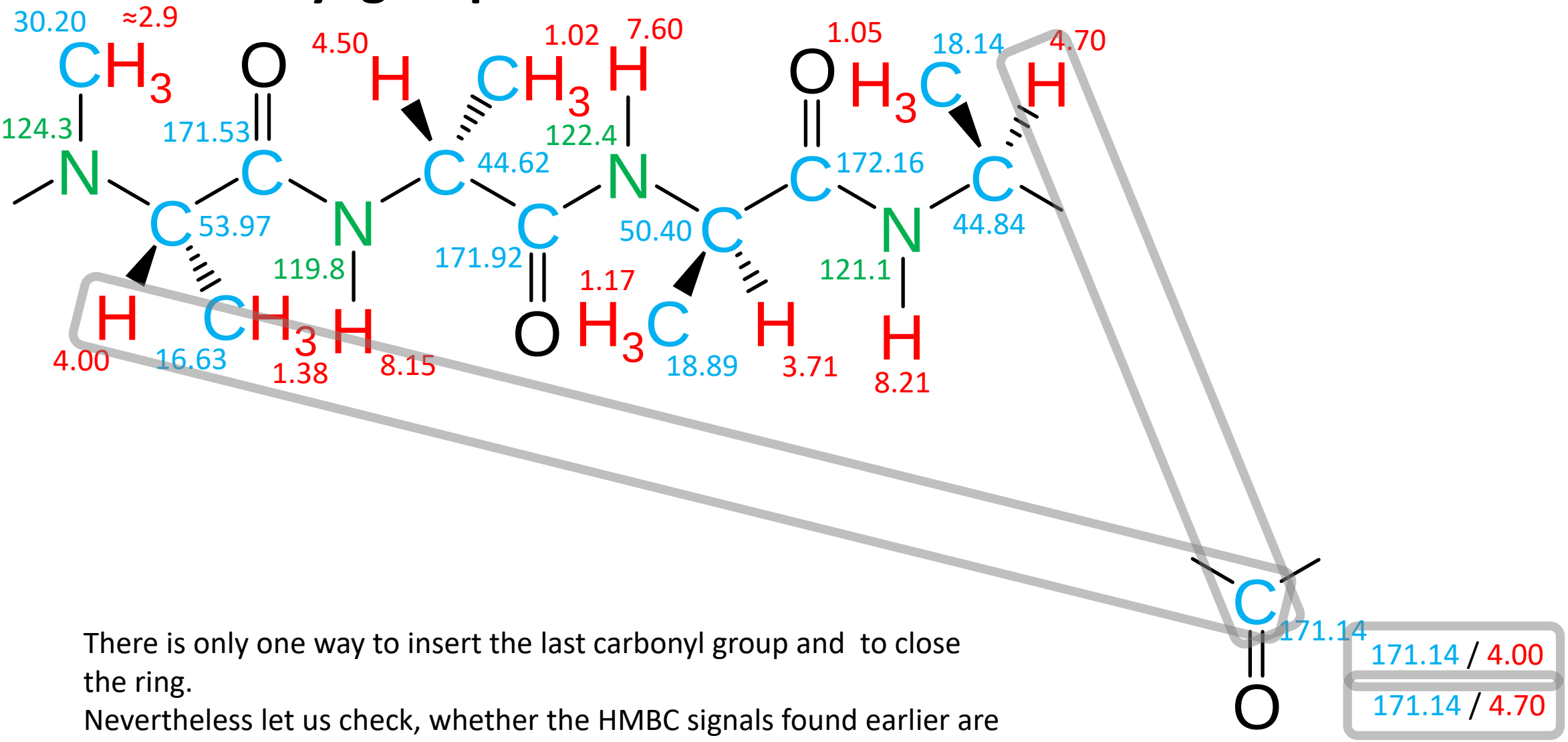
171.53 / 4.00



171.14 / 4.00

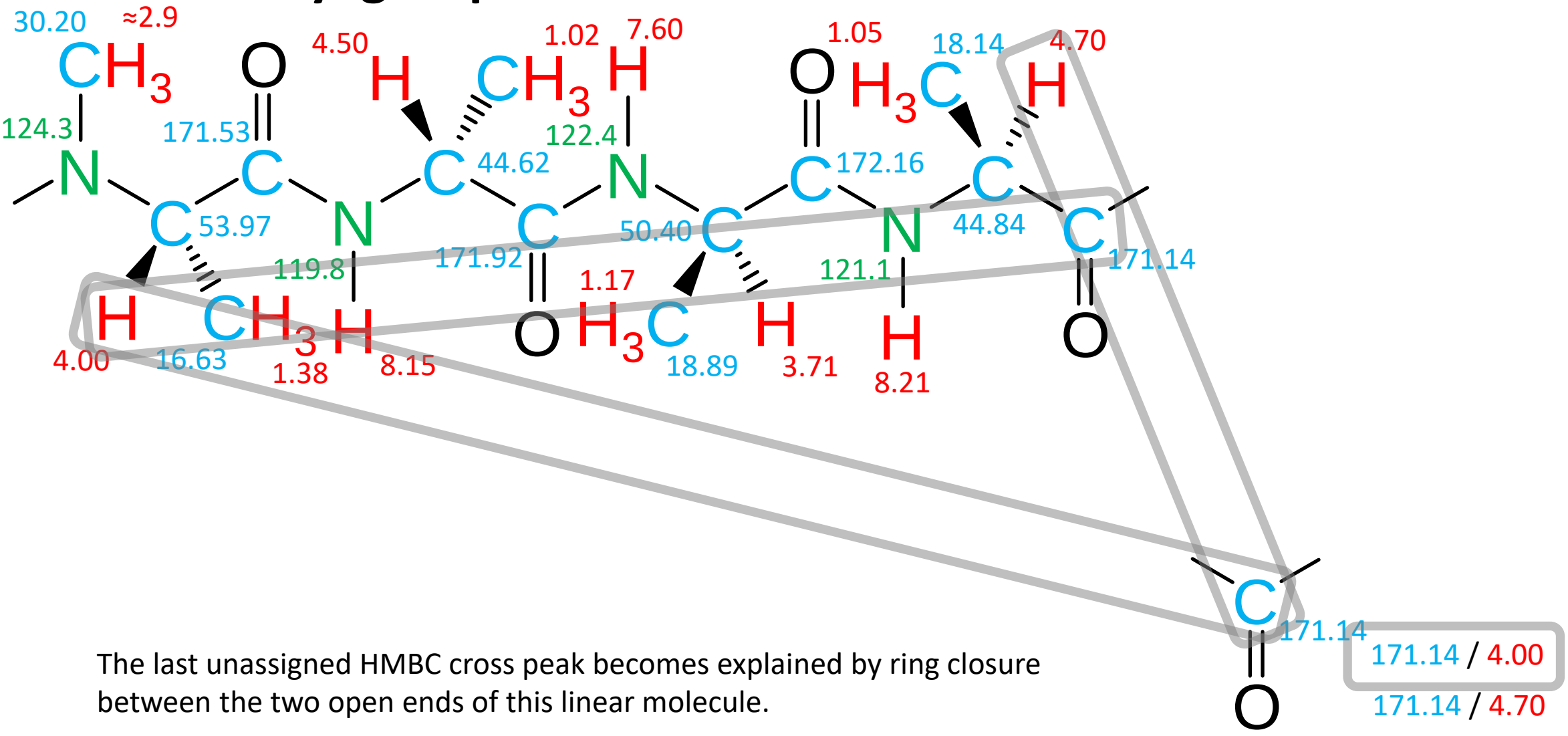
171.14 / 4.70

Carbonyl groups



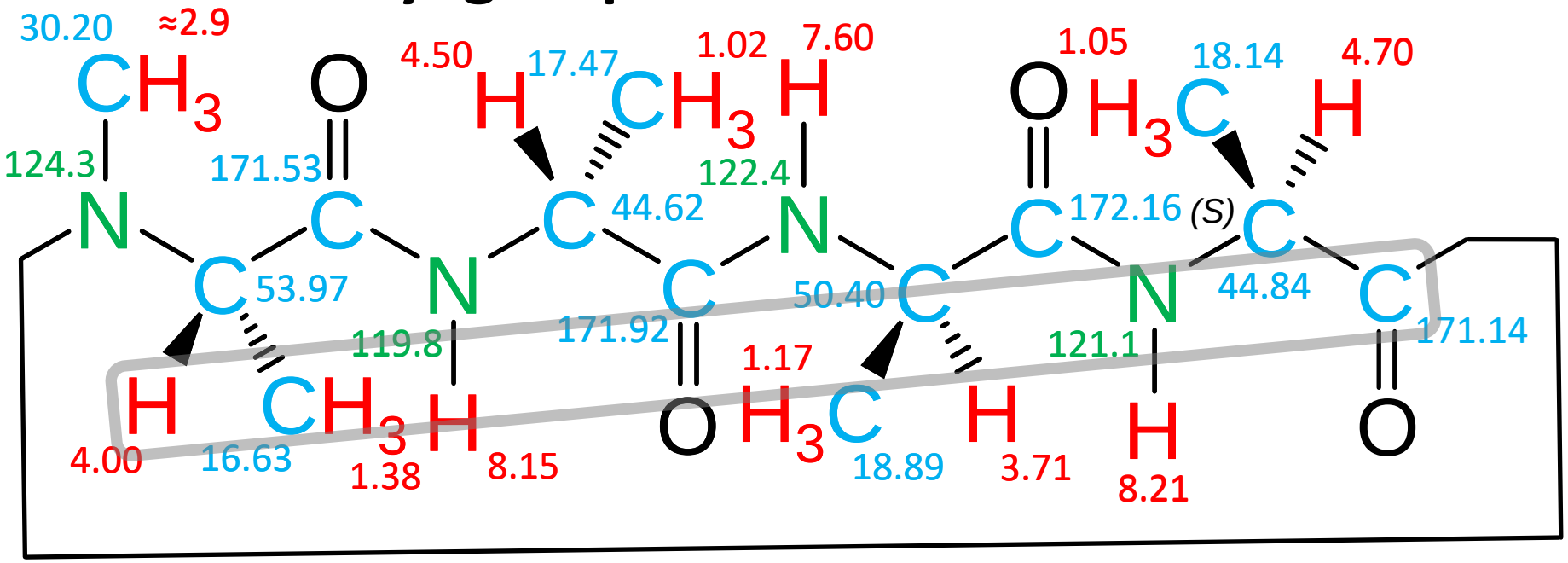
There is only one way to insert the last carbonyl group and to close the ring.
Nevertheless let us check, whether the HMBC signals found earlier are in coincidence with this ring closure.

Carbonyl groups



The last unassigned HMBC cross peak becomes explained by ring closure between the two open ends of this linear molecule.

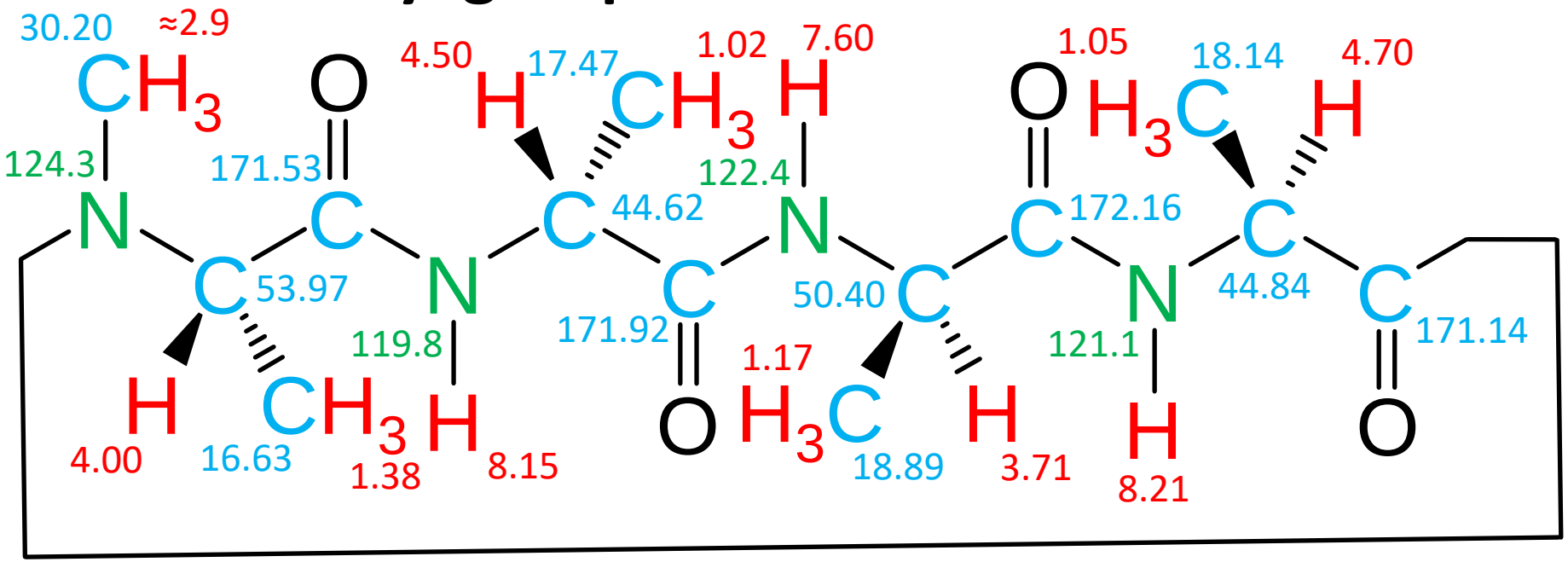
Carbonyl groups



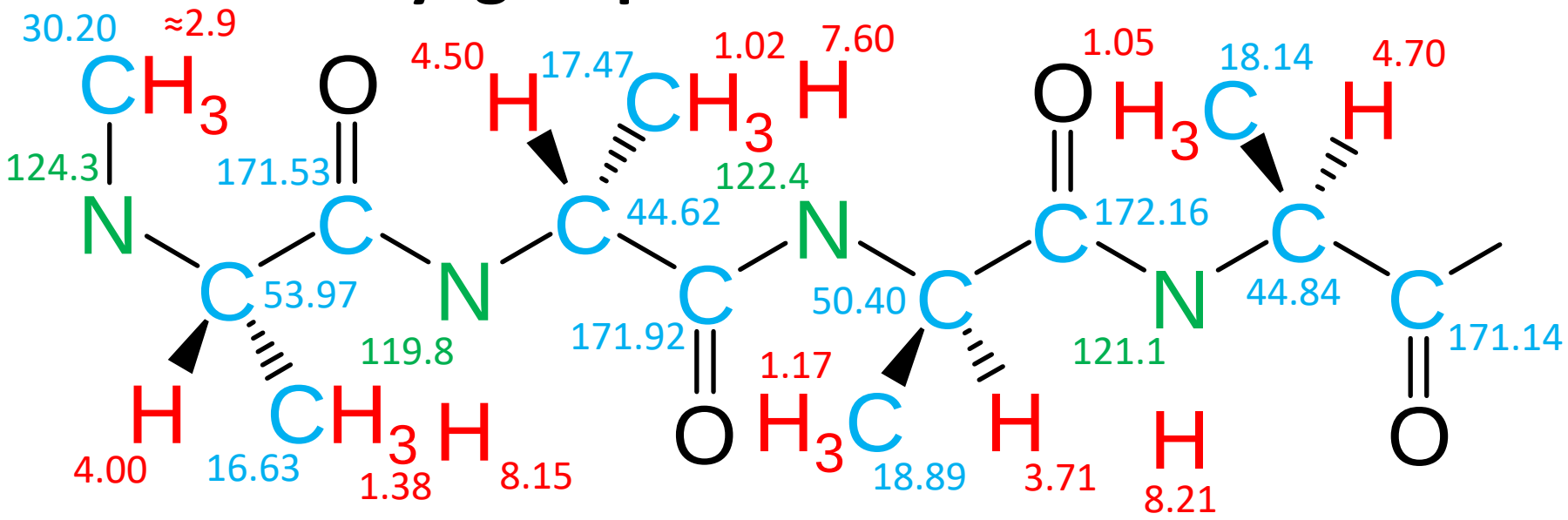
And now let us beautify everything a little bit.

171.14 / 4.00

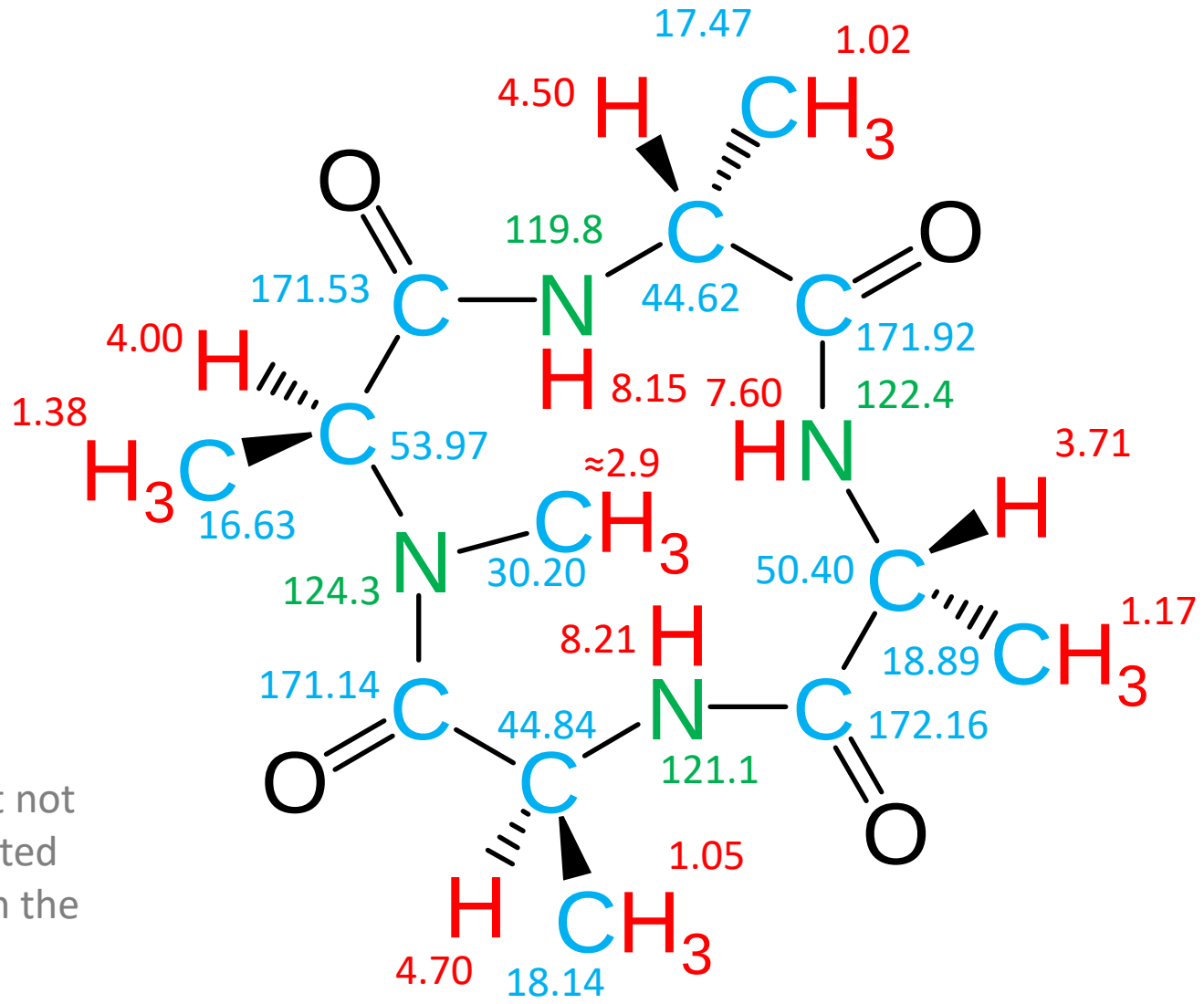
Carbonyl groups



Carbonyl groups



Final structure



Only the constitution, but not the chirality (correctly stated here) can be derived from the HMBC/HSQC data.

Contributions

Spectrometer time

TU Munich

Measurements

Rainer Haeßner

Discussions and native English language support

Alan M. Kenwright
Nils Schlörer

Compilation

Rainer Haeßner