NMR course at the FMP:

NMR of organic compounds and small biomolecules

- I -

09.03.2009

Peter Schmieder

AG Solution NMR

The program

Peptides

Sequence specific assignment (1)

Homonuclear experiments

Sequence specific assignment (2)

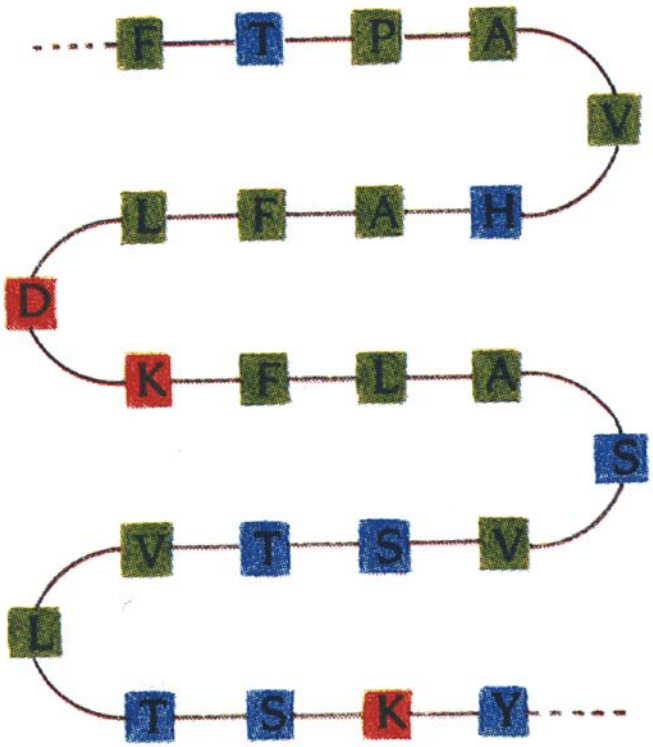
Heteronuclear experiments

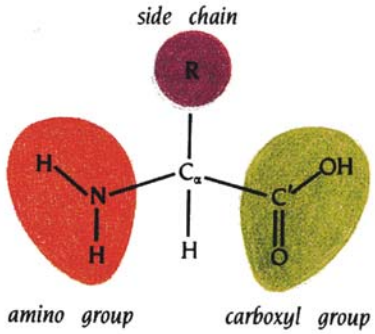
Sequence specific assignment (3)

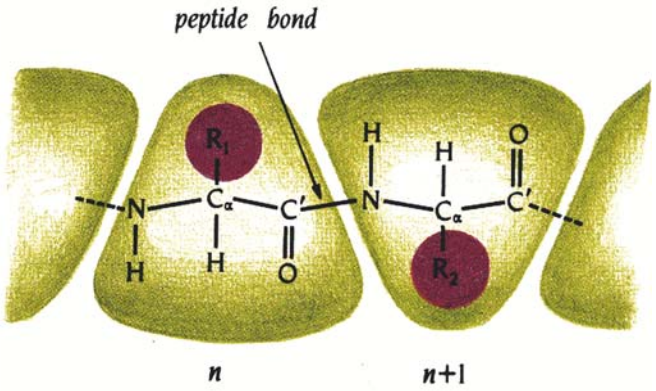
Peptides

Peptides

Primary structure

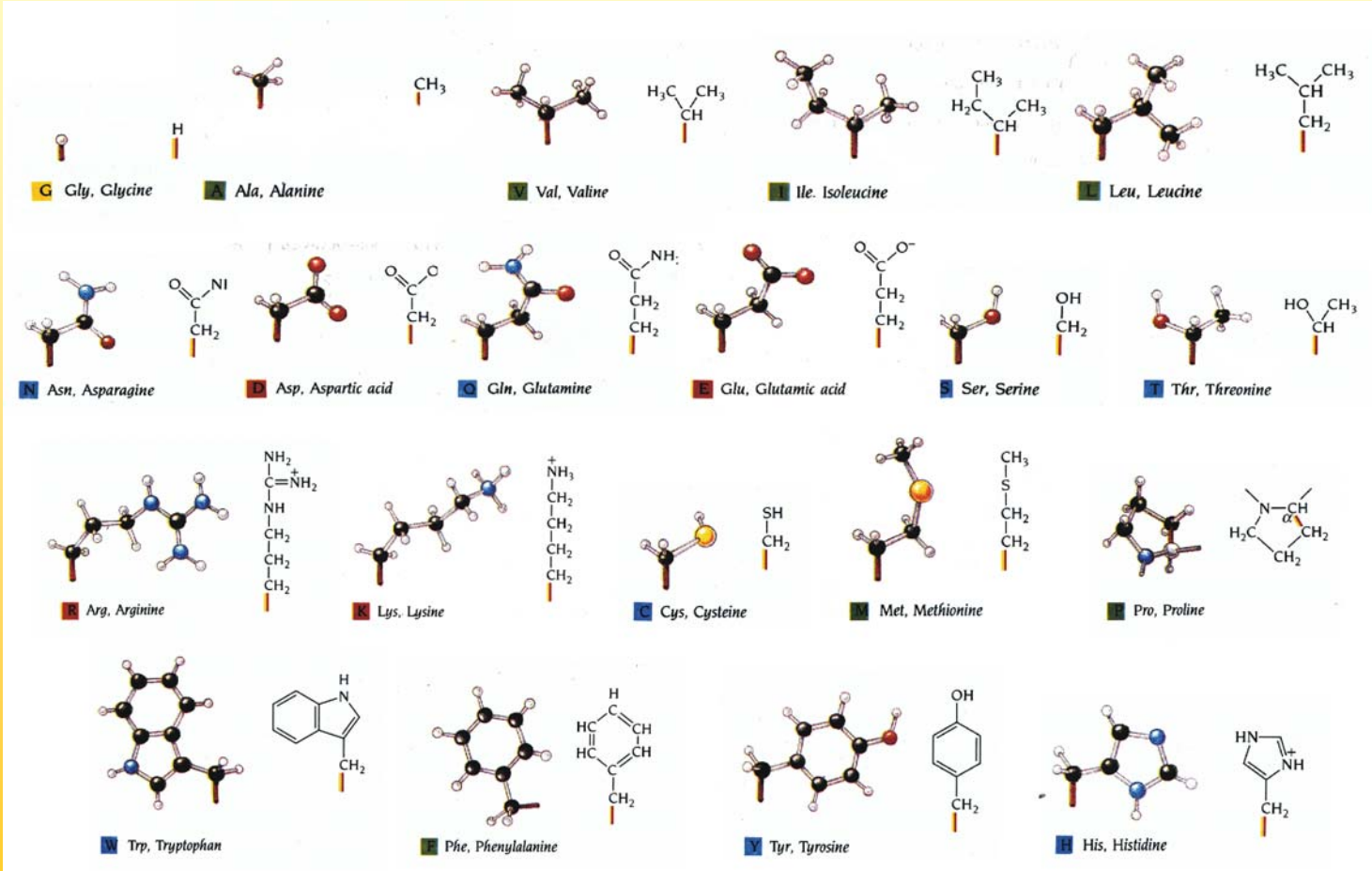






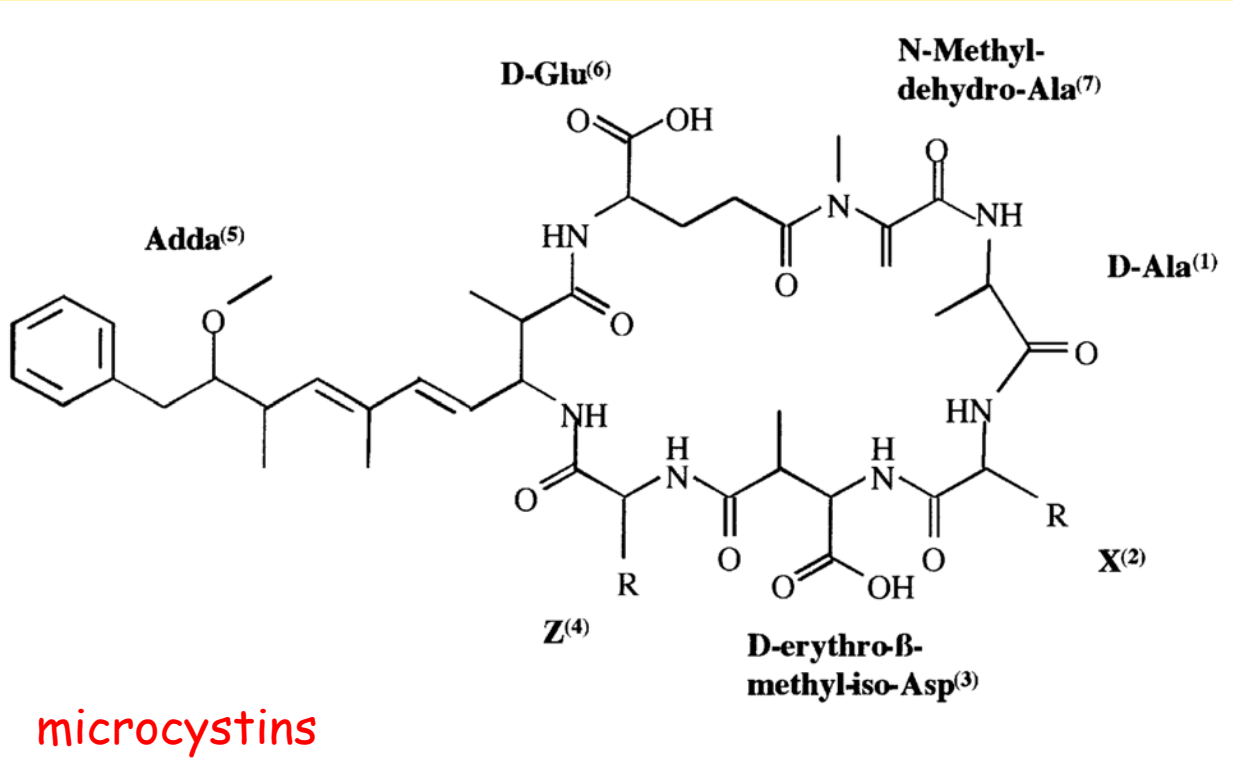
Peptides

20 proteinogenic amino acids



Peptides

In natural products many other amino acids are possible

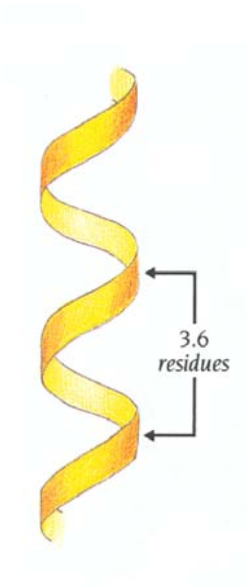


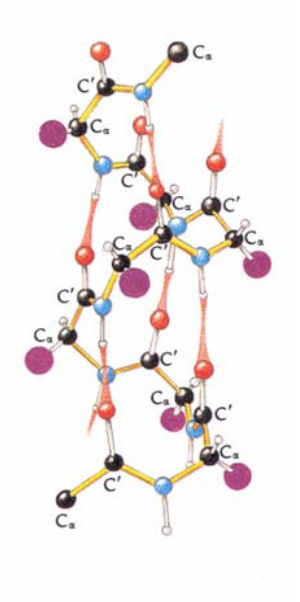
microcystins

Peptides

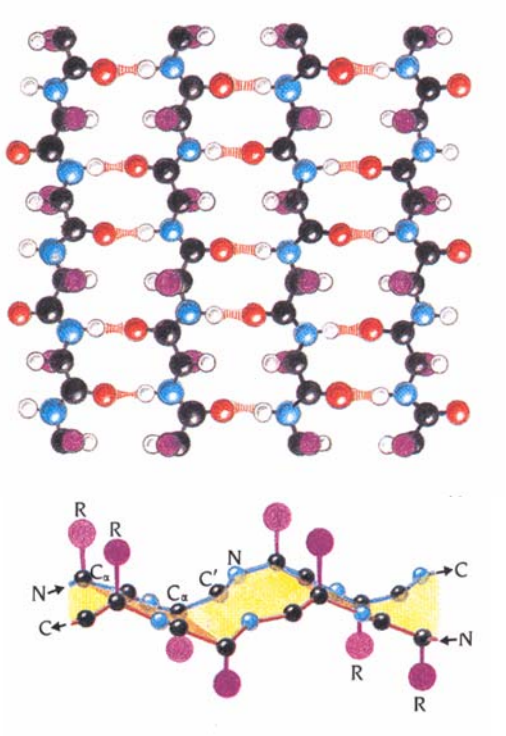
secondary structure

α-helix



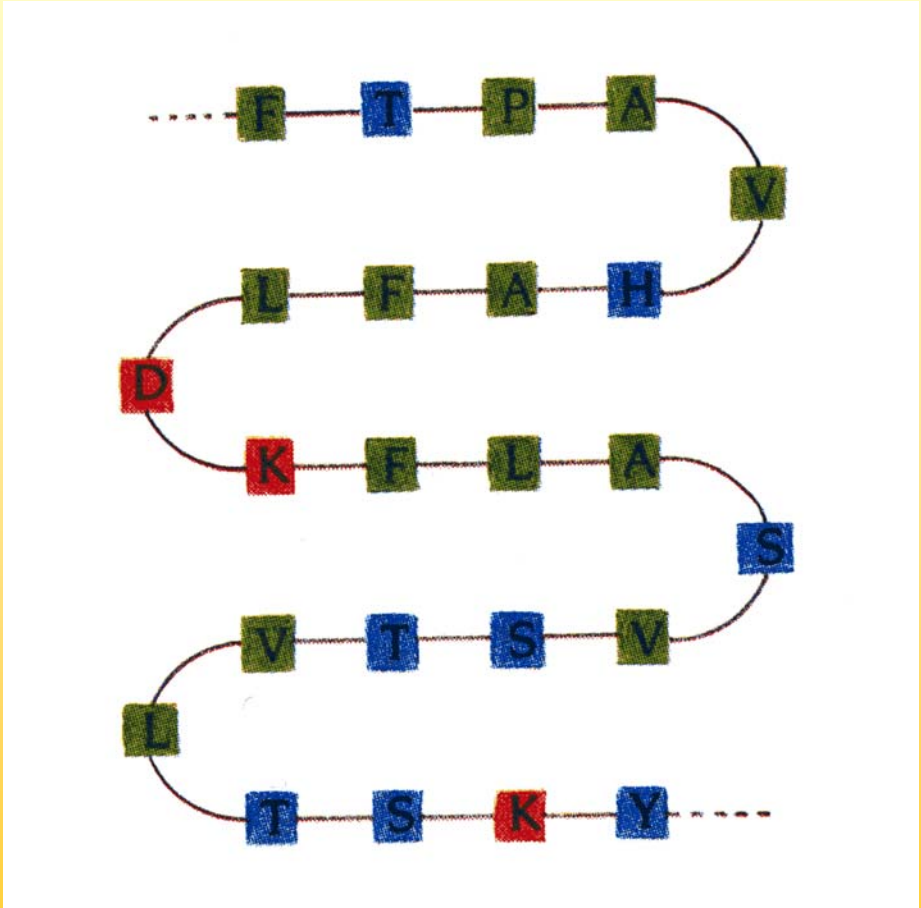


β-sheet



NMR-spectroscopy of peptides

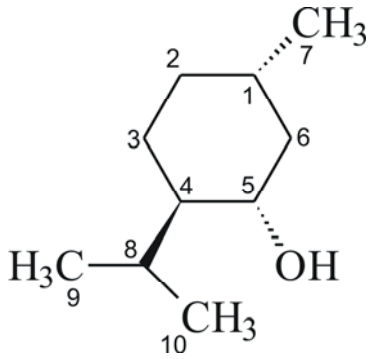
NMR-spectroscopy of peptides



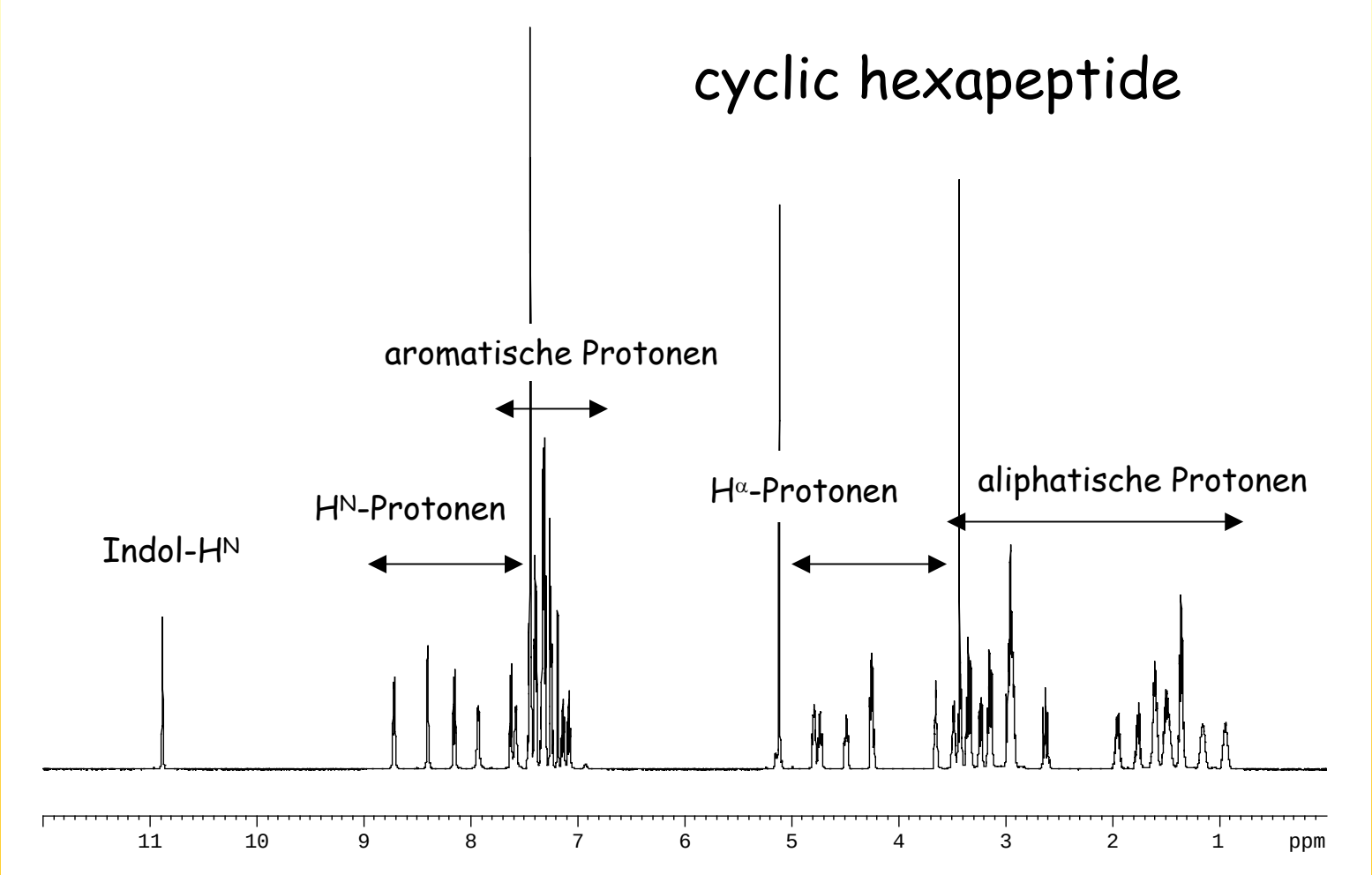
The major problem of protein NMR results from the fact that proteins are polymers, i.e. the repetition of almost identical subunits

NMR-spectroscopy of peptides

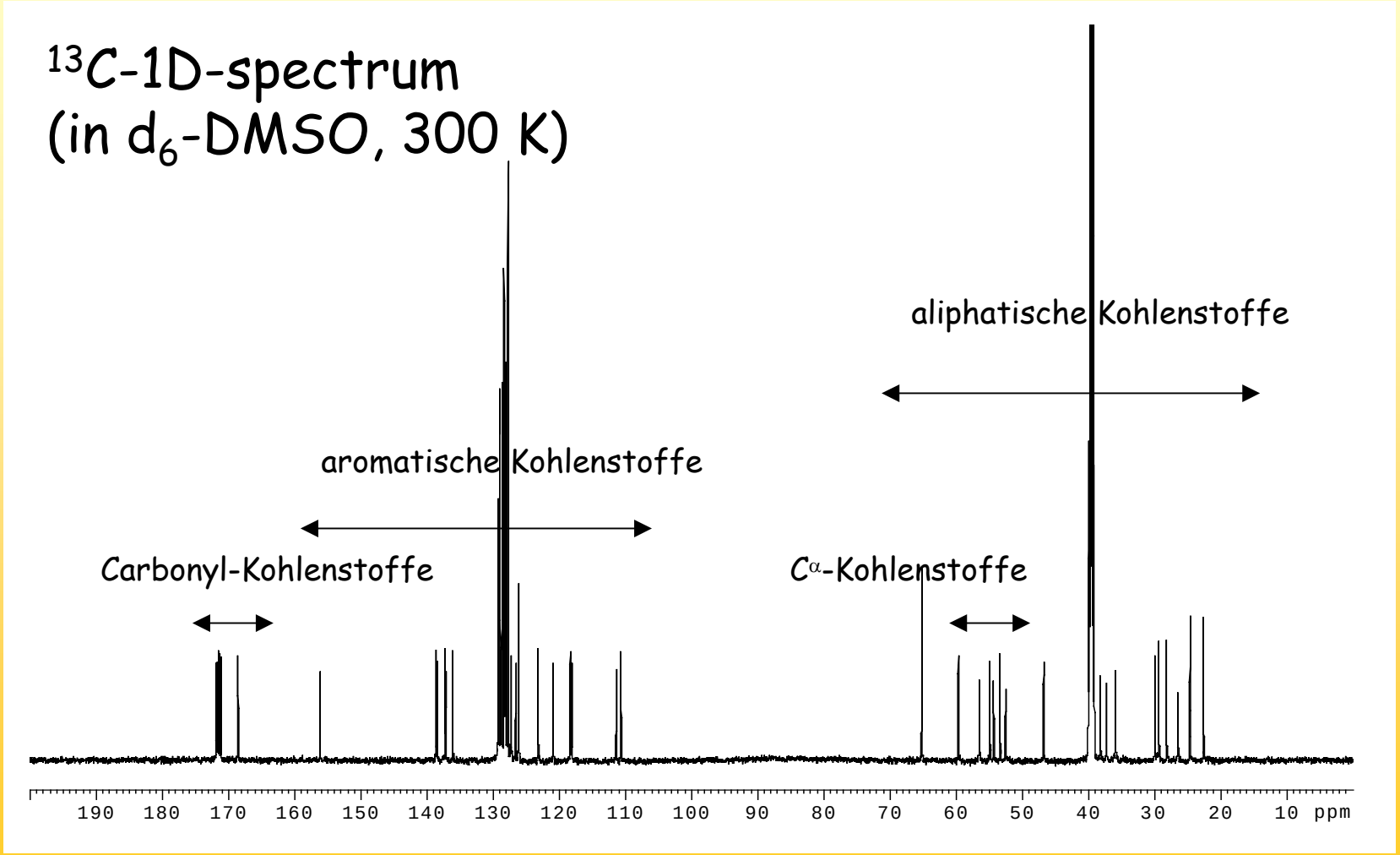
(-)-Menthol



NMR-spectroscopy of peptides

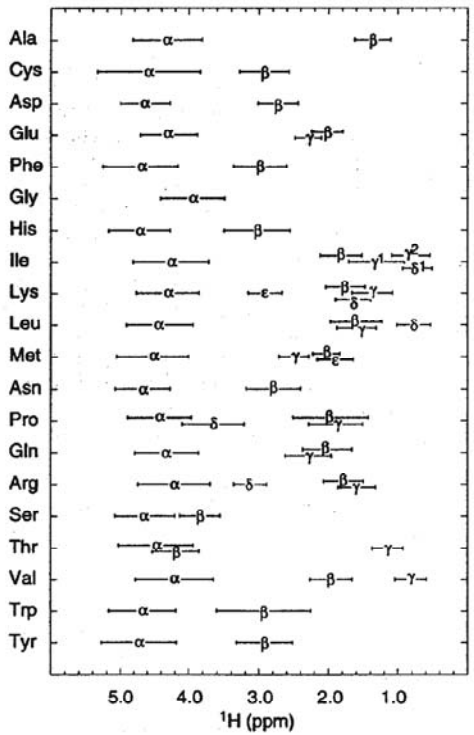


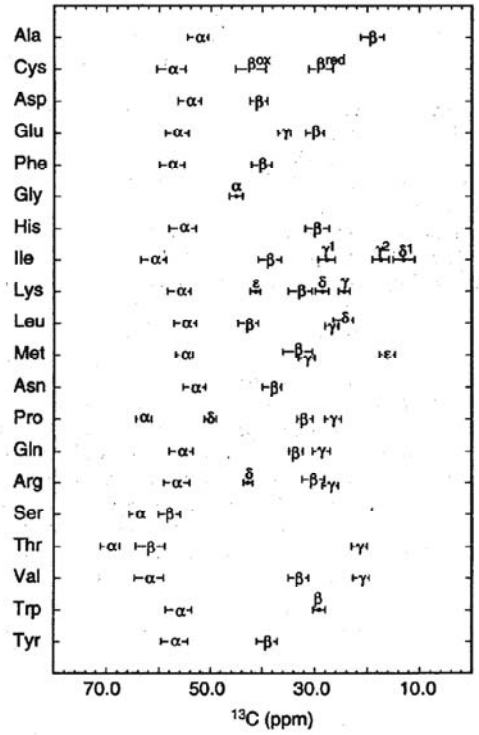
NMR-spectroscopy of peptides

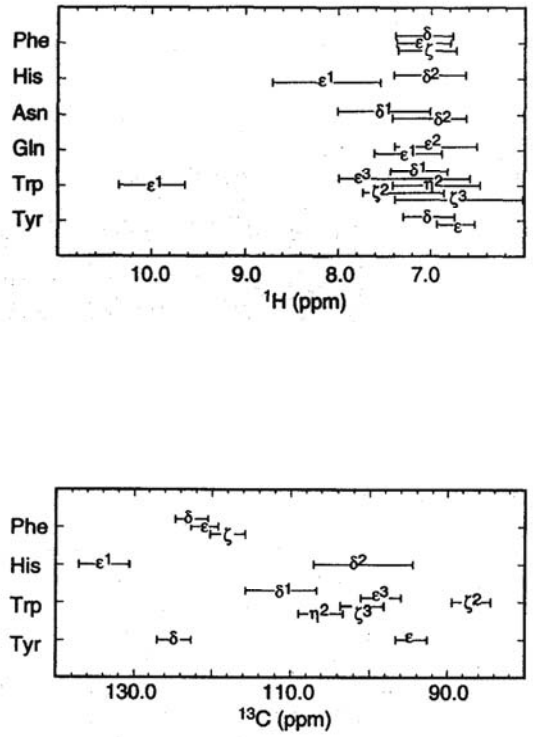


NMR-spectroscopy of peptides

For proton and carbon there are „random coil" chemical shifts

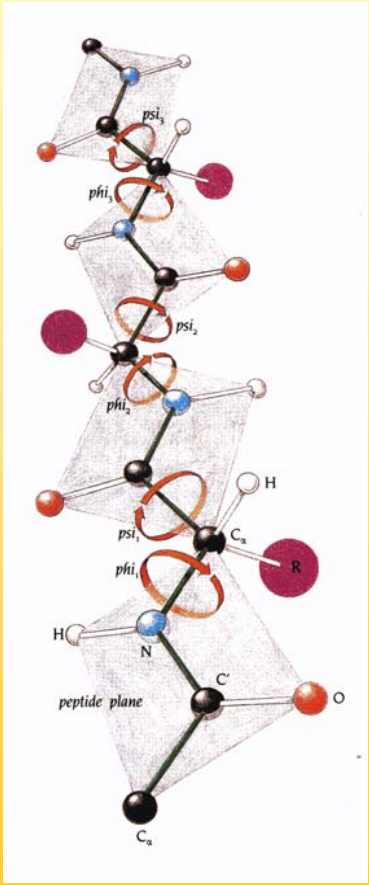


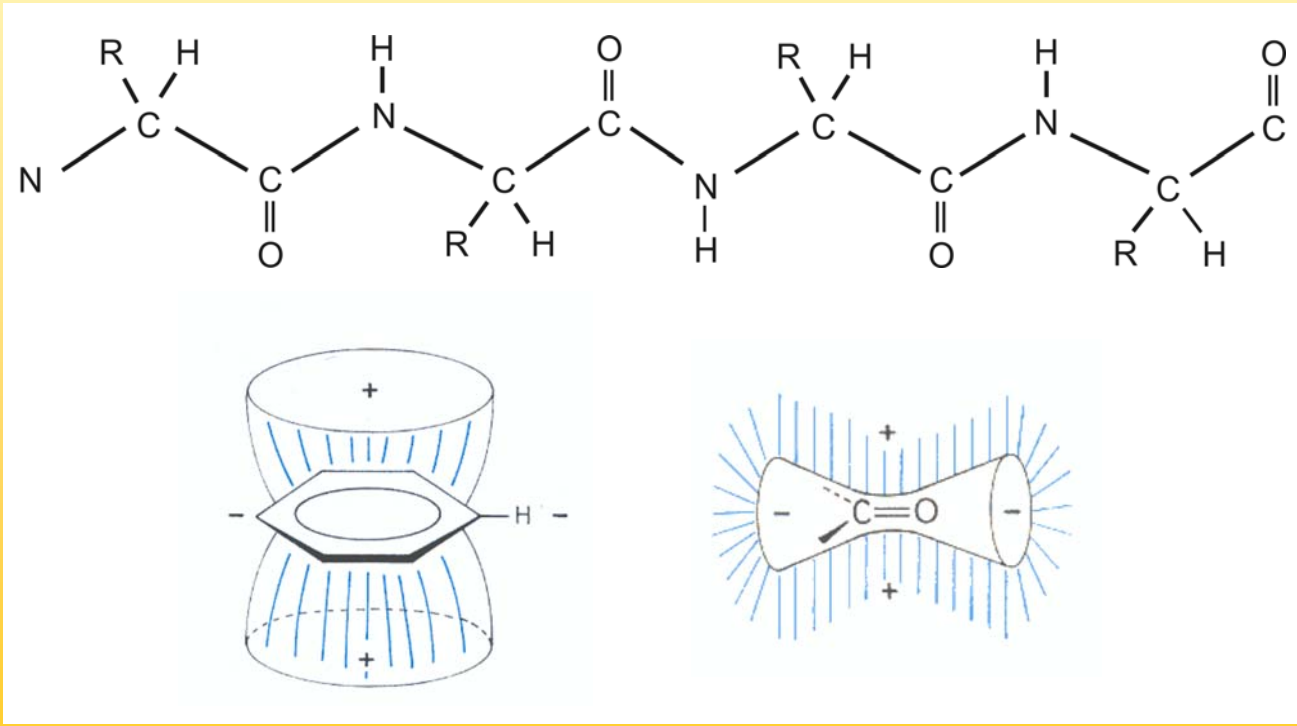




NMR-spectroscopy of peptides

Differences in chemical shifts can be produced by structure and the accompanying anisotropy effect





Sequence specific assignment

Sequence specific assignment

The solution of the assignment problem is the

sequence-specific assignment

The following strategies exist:

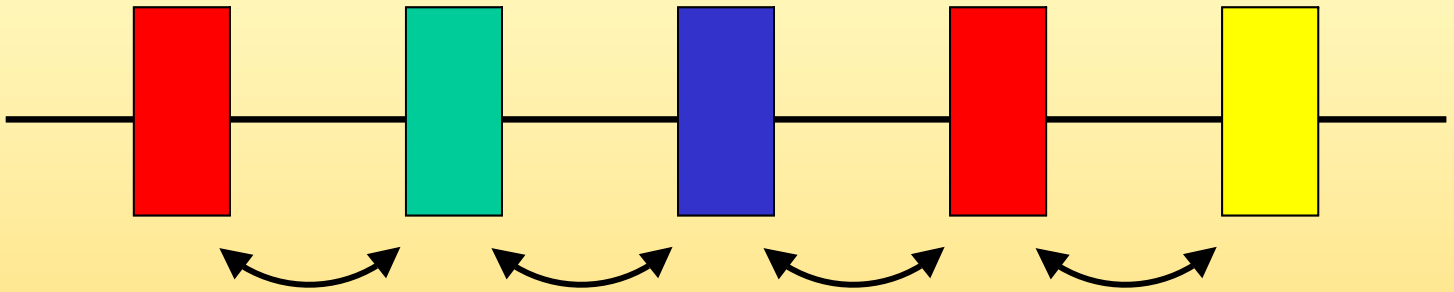
Based on homonuclear spectra (COSY,TOCSY, NOESY)

Based on heteronuclear spectra in natural abundance (HMBC)

In case of larger proteins labeling with ^{13}C and ^{15}N is necessary and heteronuclear triple resonance experiments (CBCA(CO)NNH,CBCANNH) are recorded

Sequence specific assignment

Sequence-specific assignment



1. Which amino acid type is present (which color)
2. Which amino acid is next to which (neighborhood)
3. Comparison of subsequences with that of the peptide

Sequence specific assignment

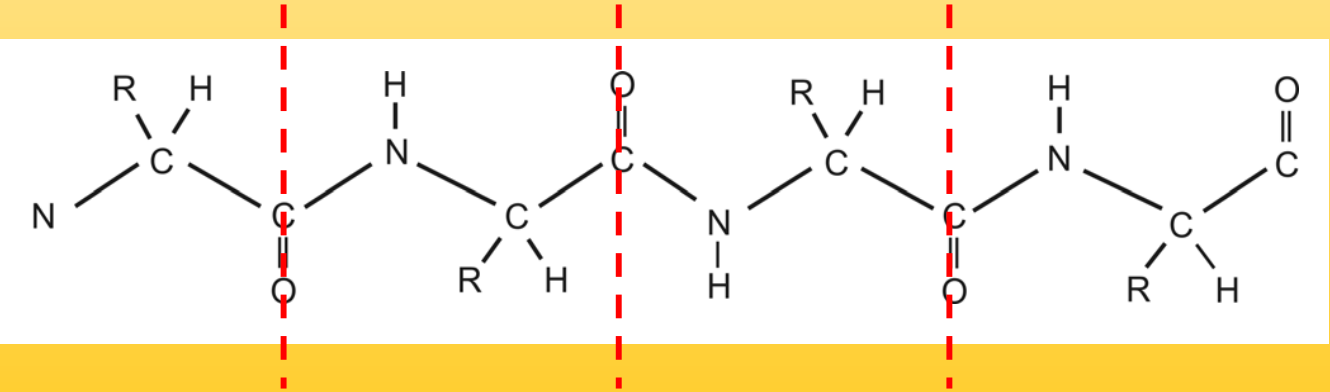
So we need two types of experiments, those that can identify the amino acids type und those that can provide the sequential (neighborhood) inforation.

In case of small, unlabeled peptides the information on the amino acid type will result from spectra based on homonuclear scalar coupling (COSY, TOCSY)

The information on neighborhood will result from NOEs or heteronuclear scalar coupling

Sequence specific assignment

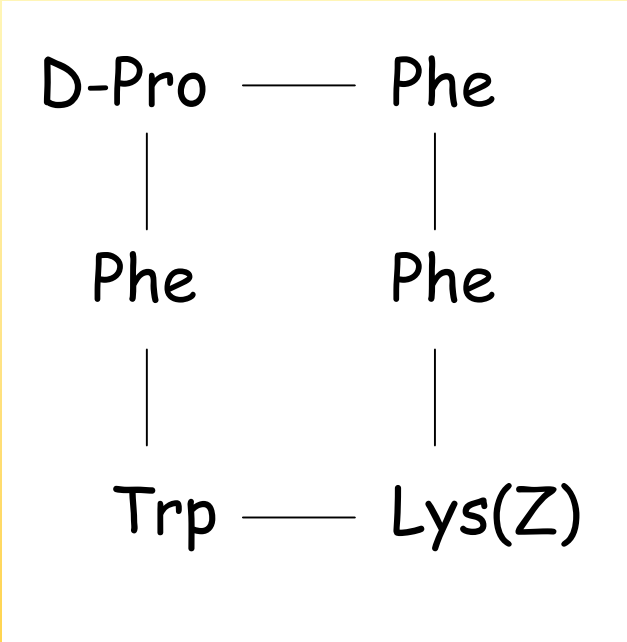
With respect to homonuclear scalar couplings each amino acid represents a separate set of signals, a spin system, since amino acids are separated by the carbonyl carbon that does not have a proton attached.

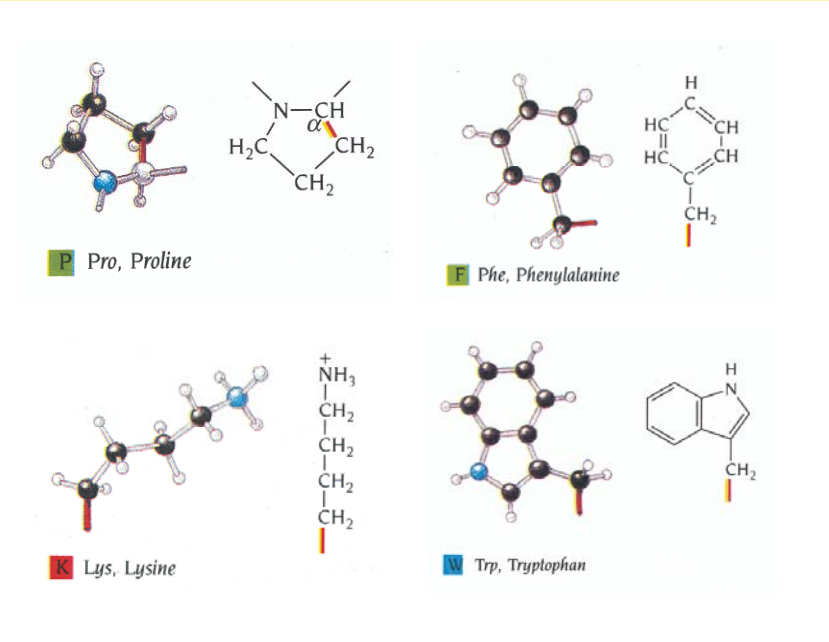


Homonuclear experiments

Homonuclear experiments

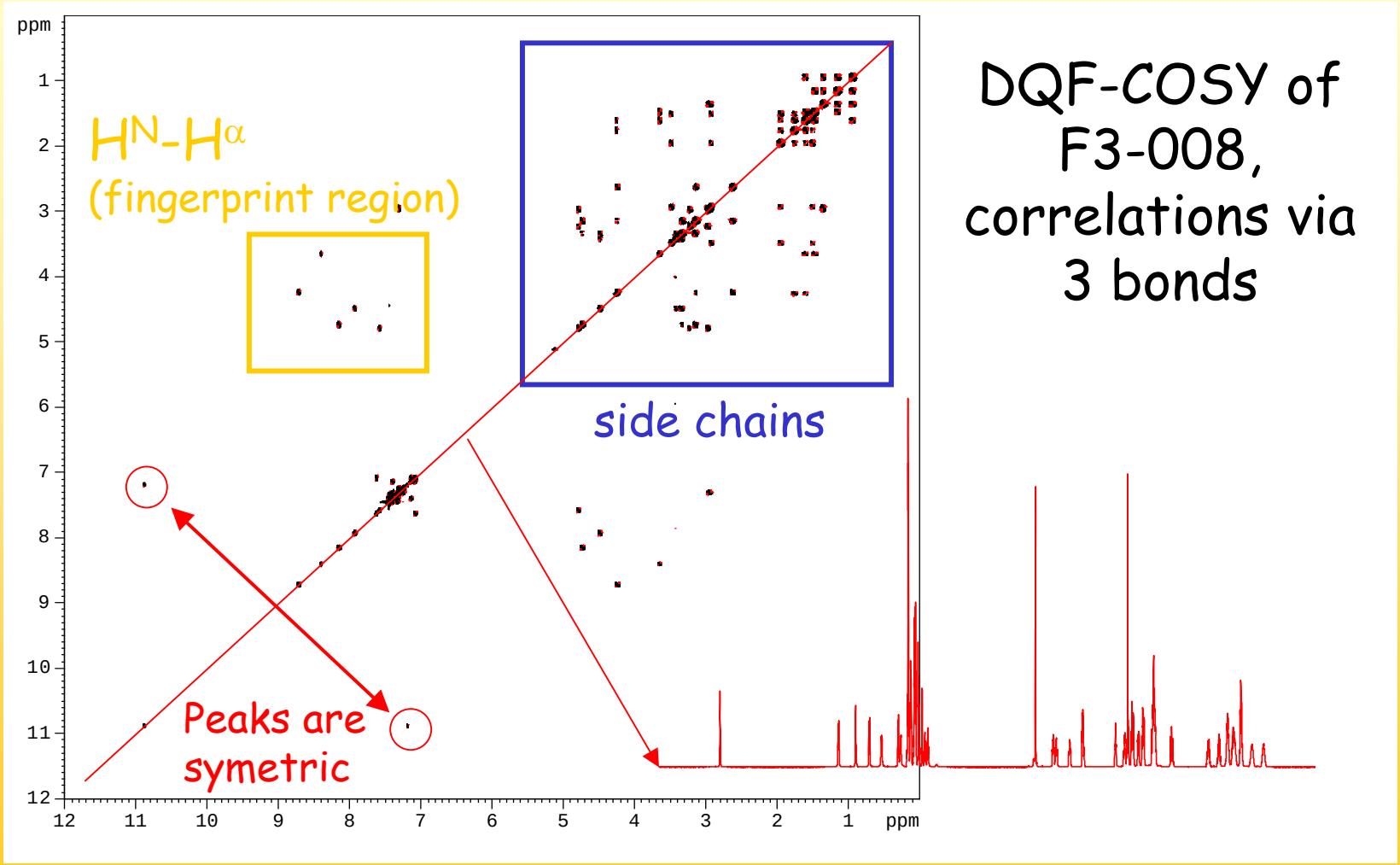
Our example peptide





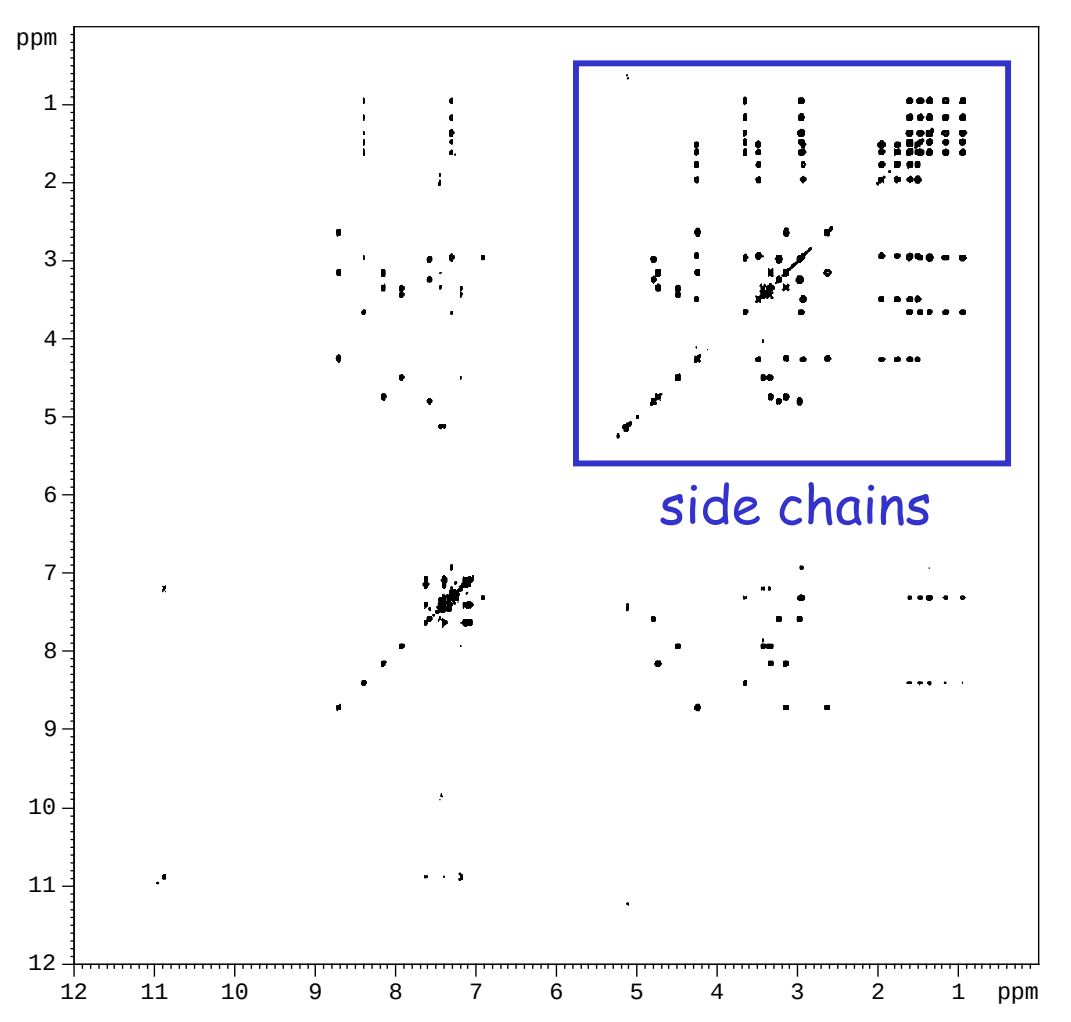
F3-008: *cyc*-(dP-F-F-K(Z)-W-F)

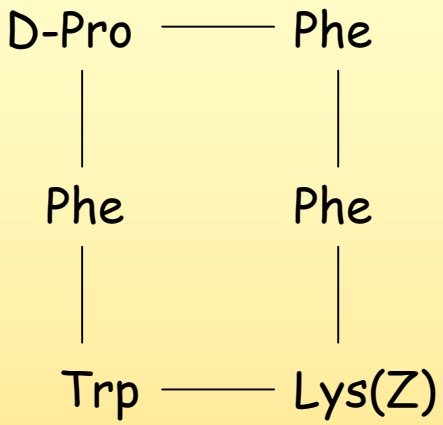
Homonuclear experiments



DQF-COSY of F3-008, correlations via 3 bonds

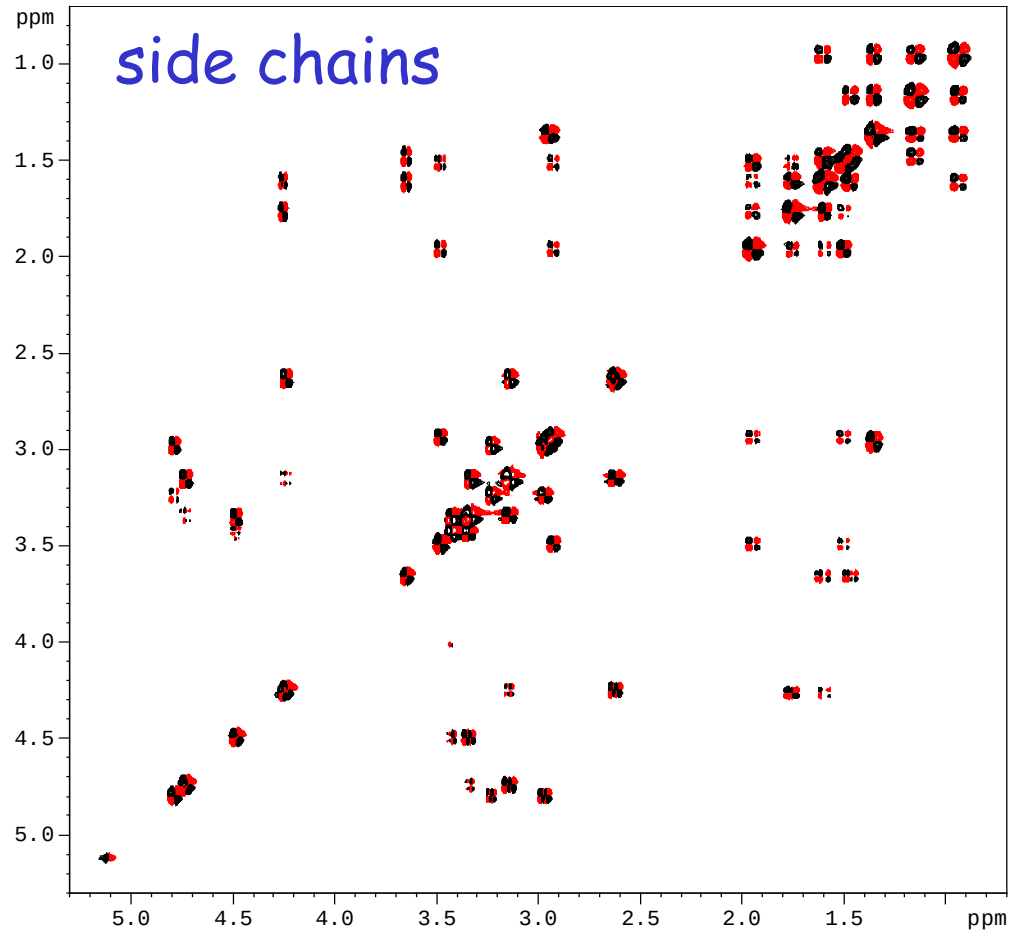
Homonuclear experiments

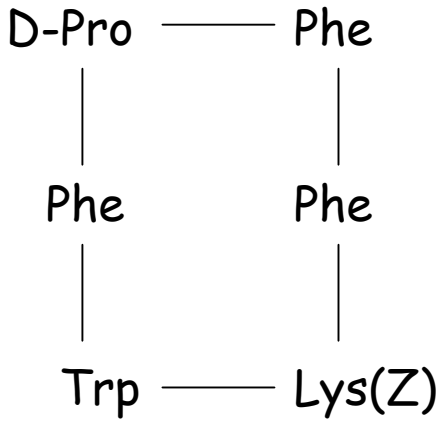




In einem TOCSY mit langer Mischzeit sind ist bei jedem Signal das ganze Spinsystem zu sehen

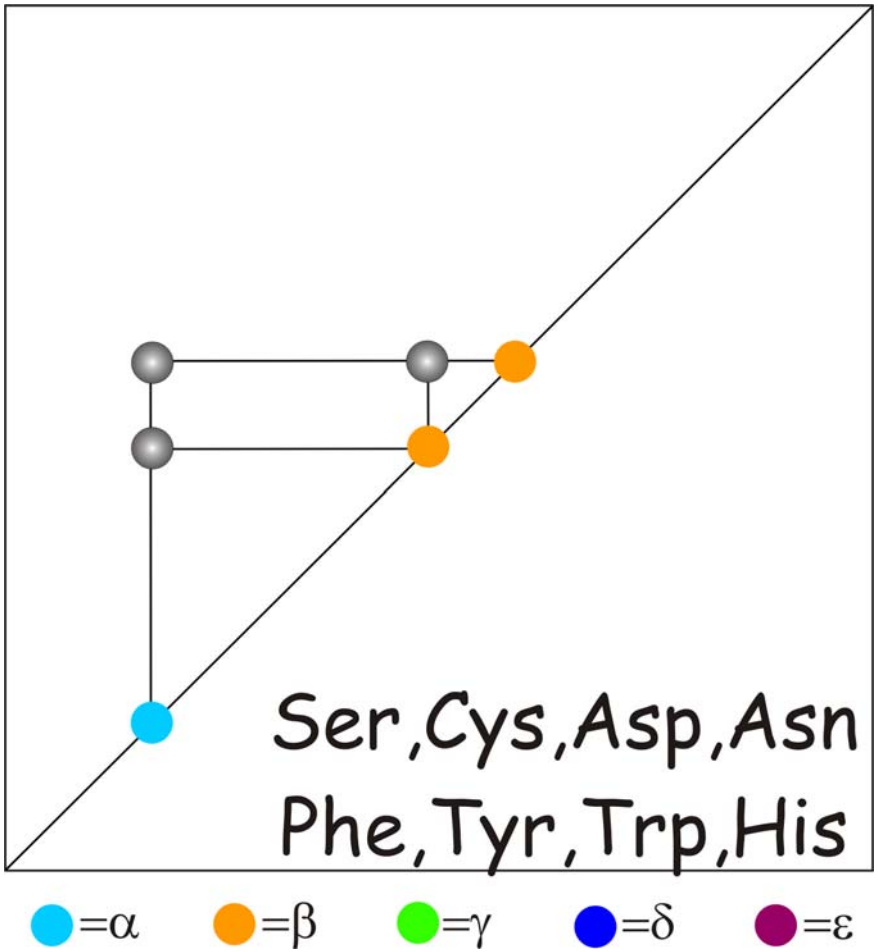
Homonuclear experiments

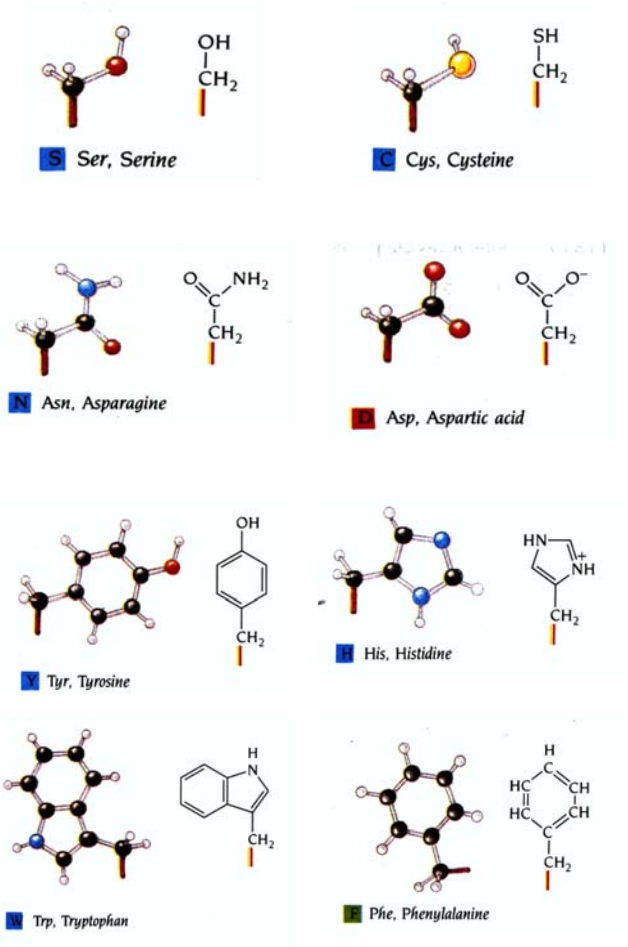




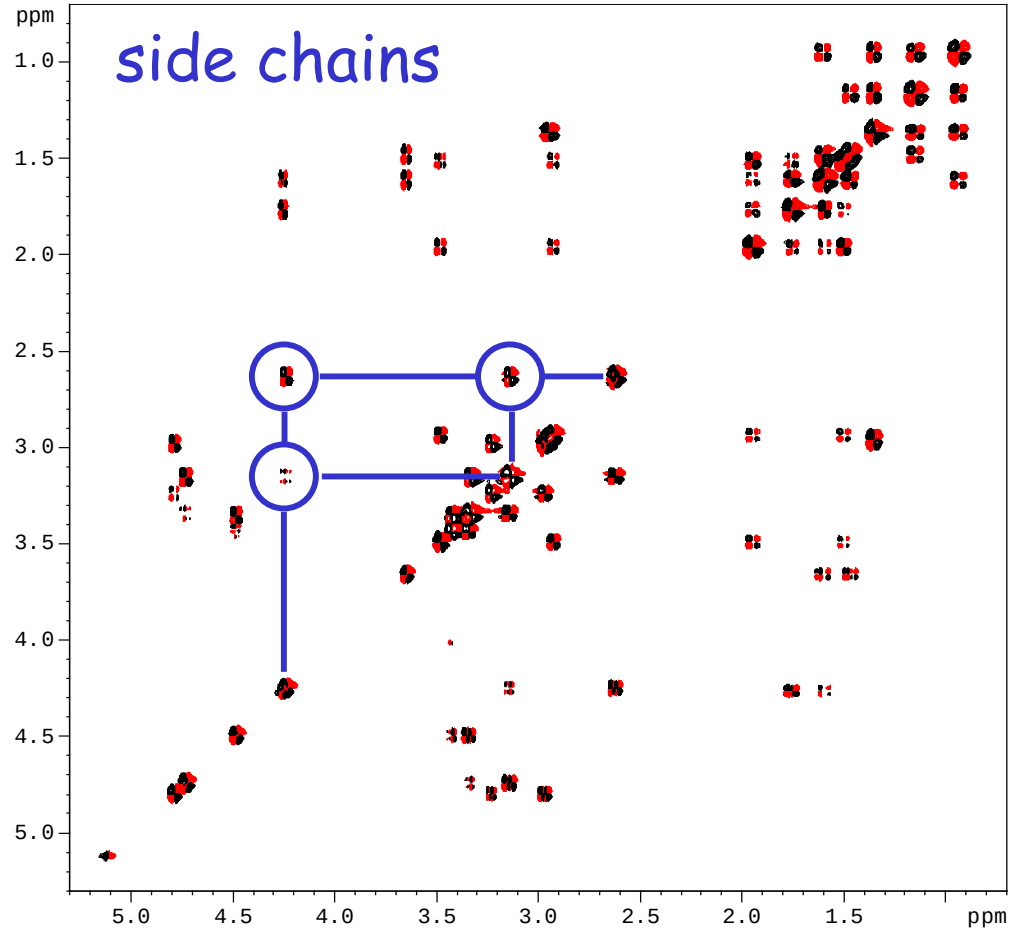
here we can see the different spin systems as different pattern

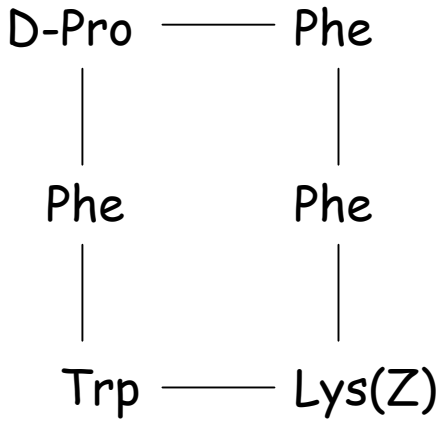
Homonuclear experiments





Homonuclear experiments





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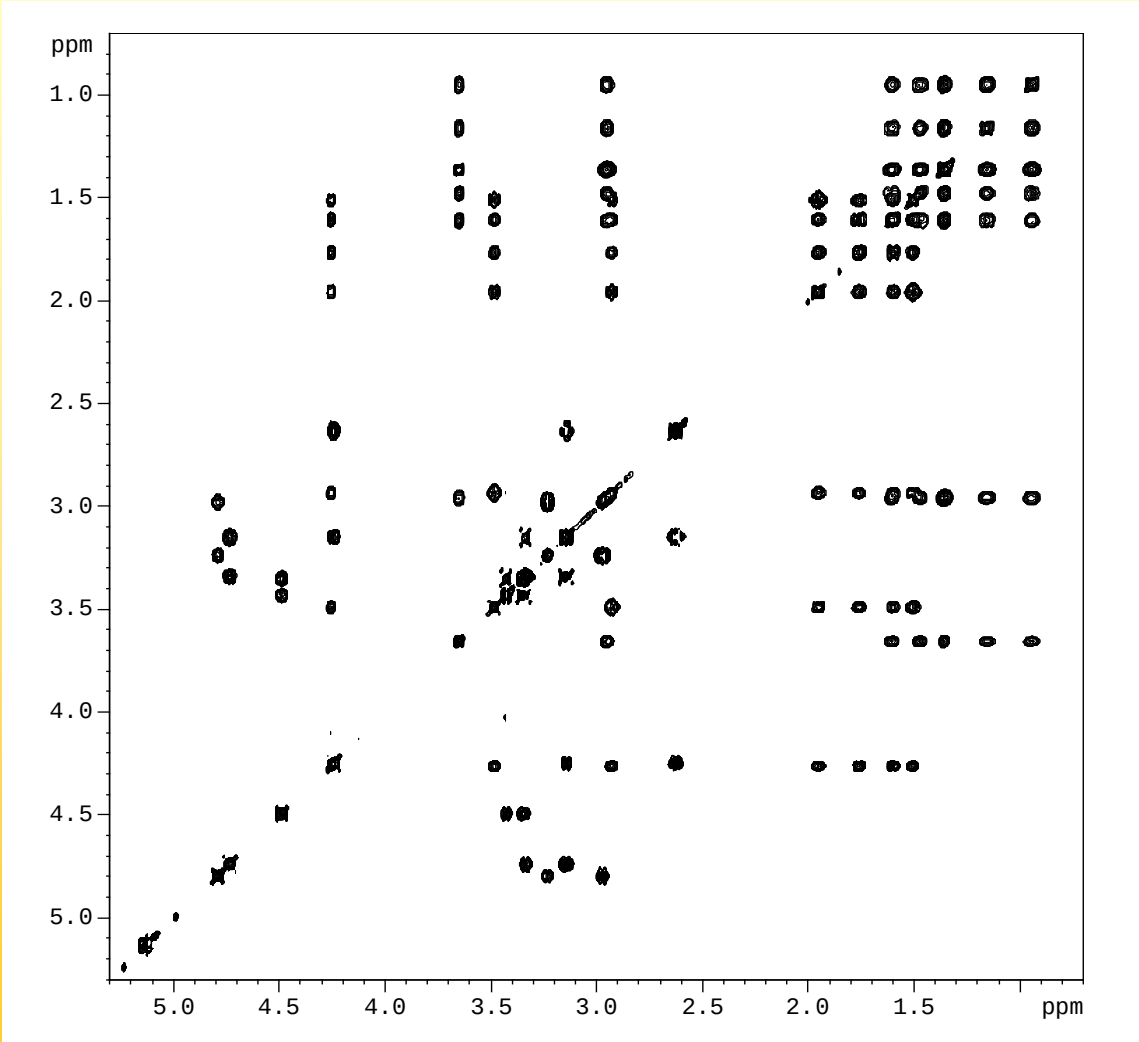
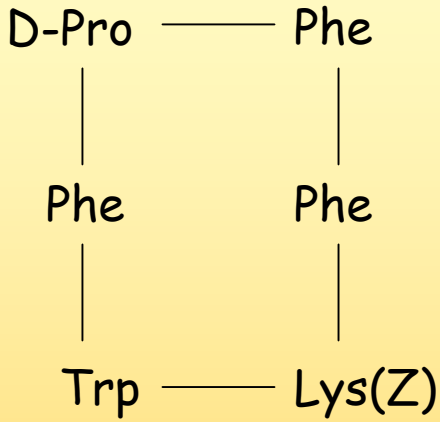
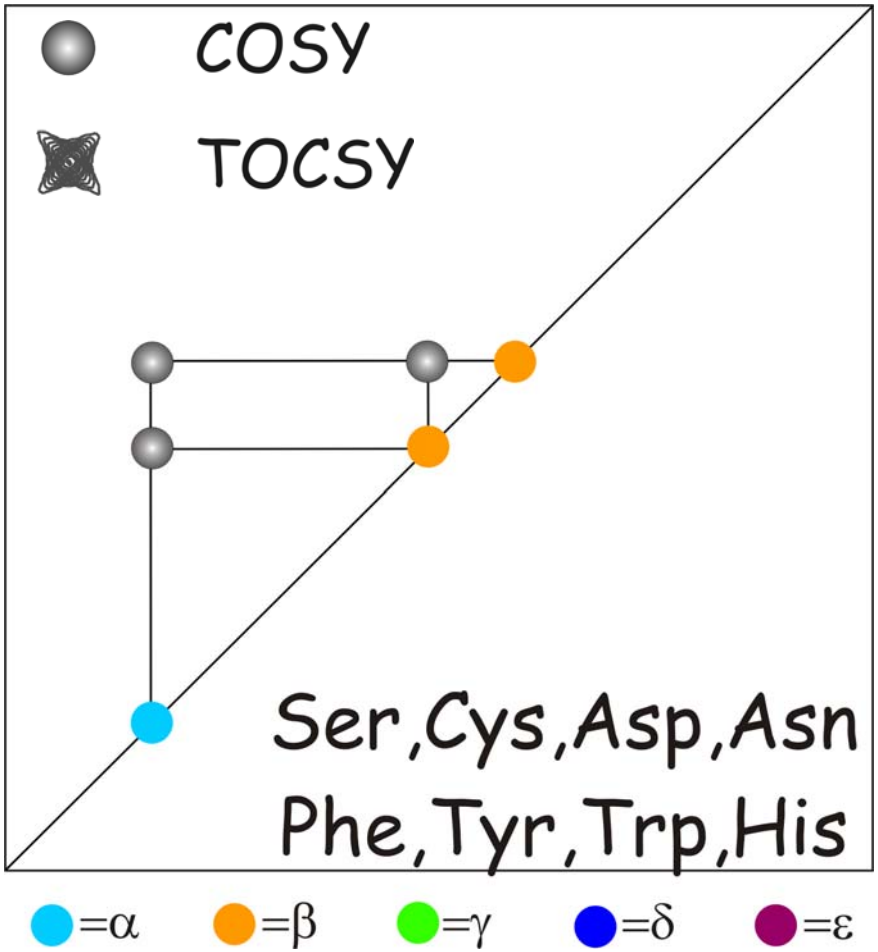
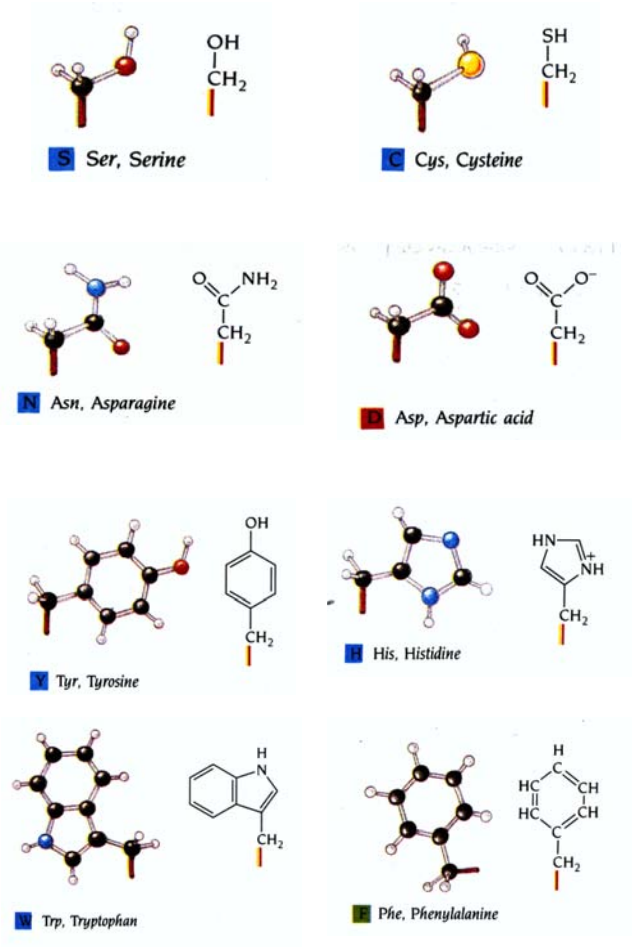




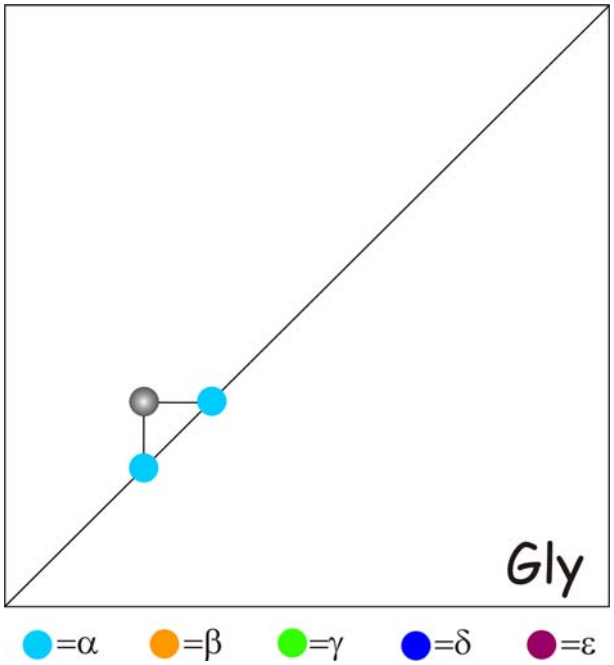
In the TOCSY the pattern is slightly different

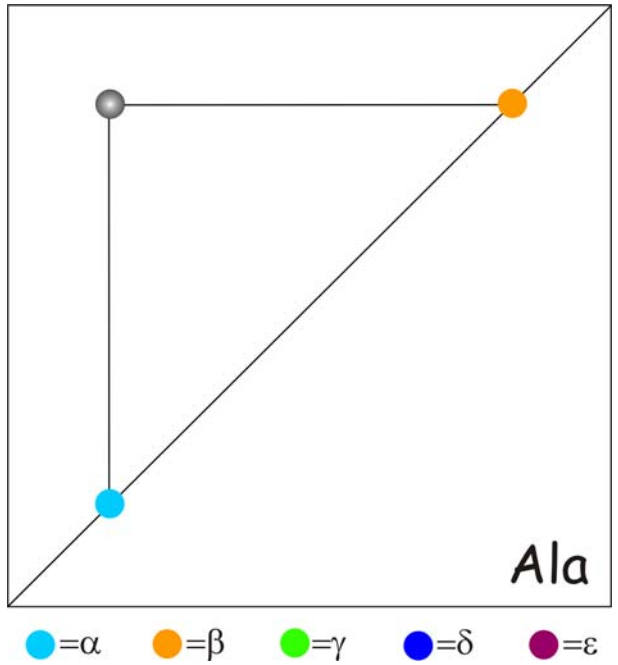
Homonuclear experiments





Homonuclear experiments





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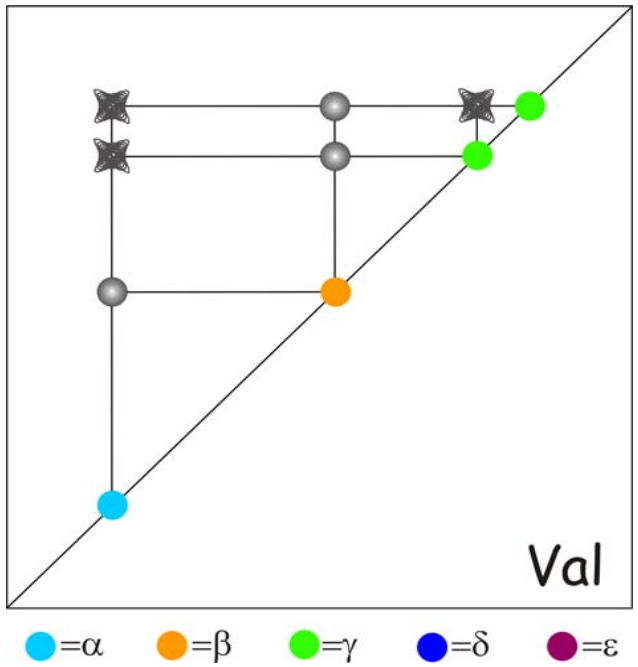
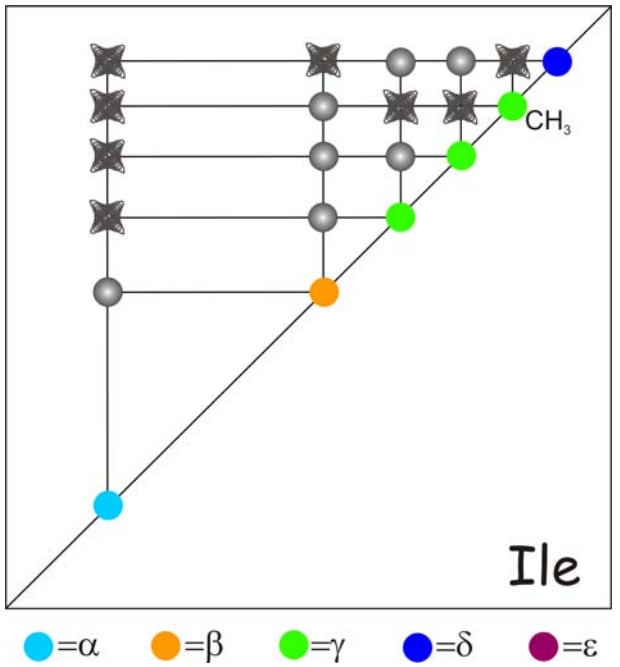
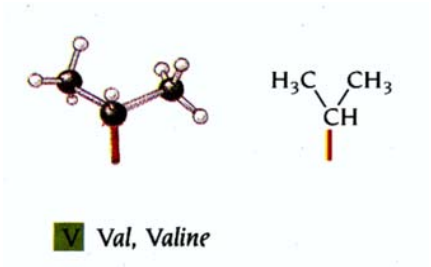
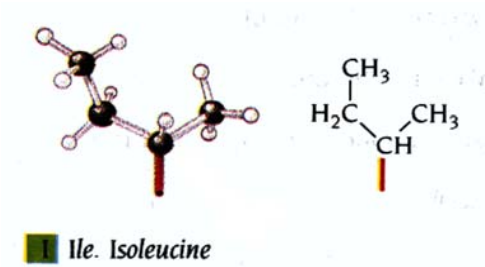




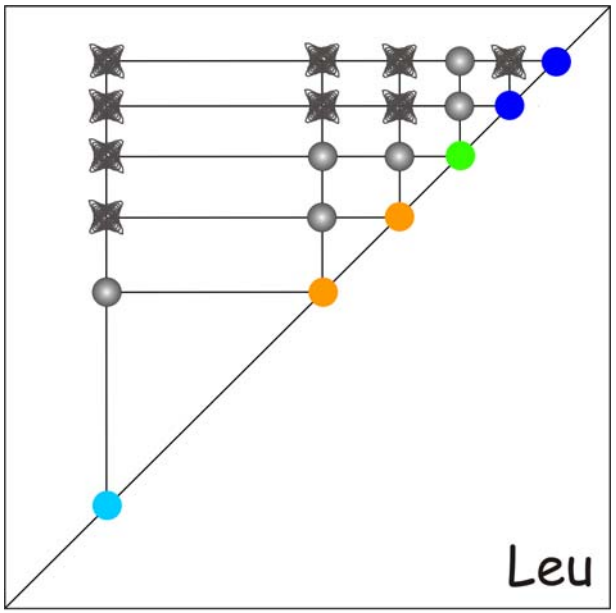


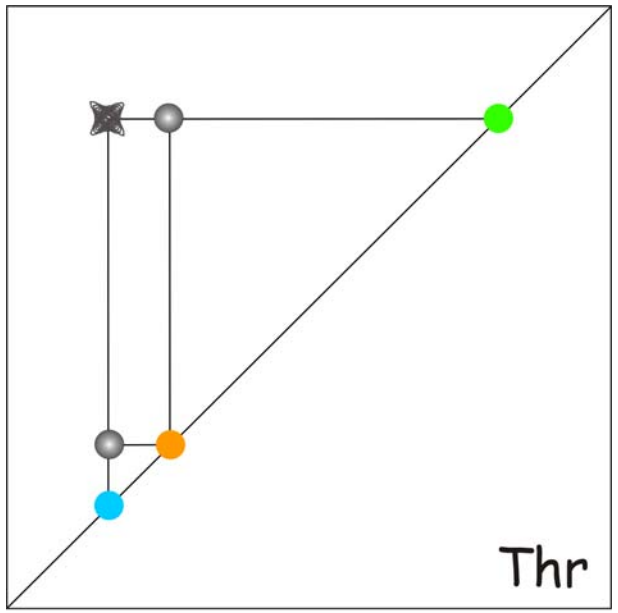
COSY

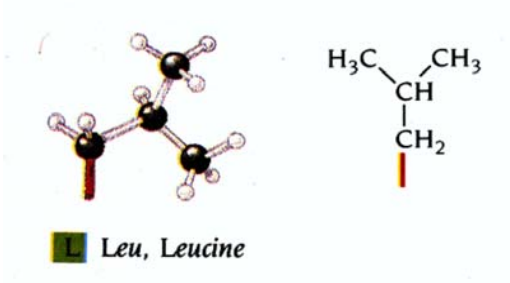
TOCSY



Homonuclear experiments

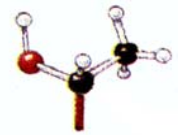


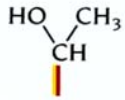




COSY

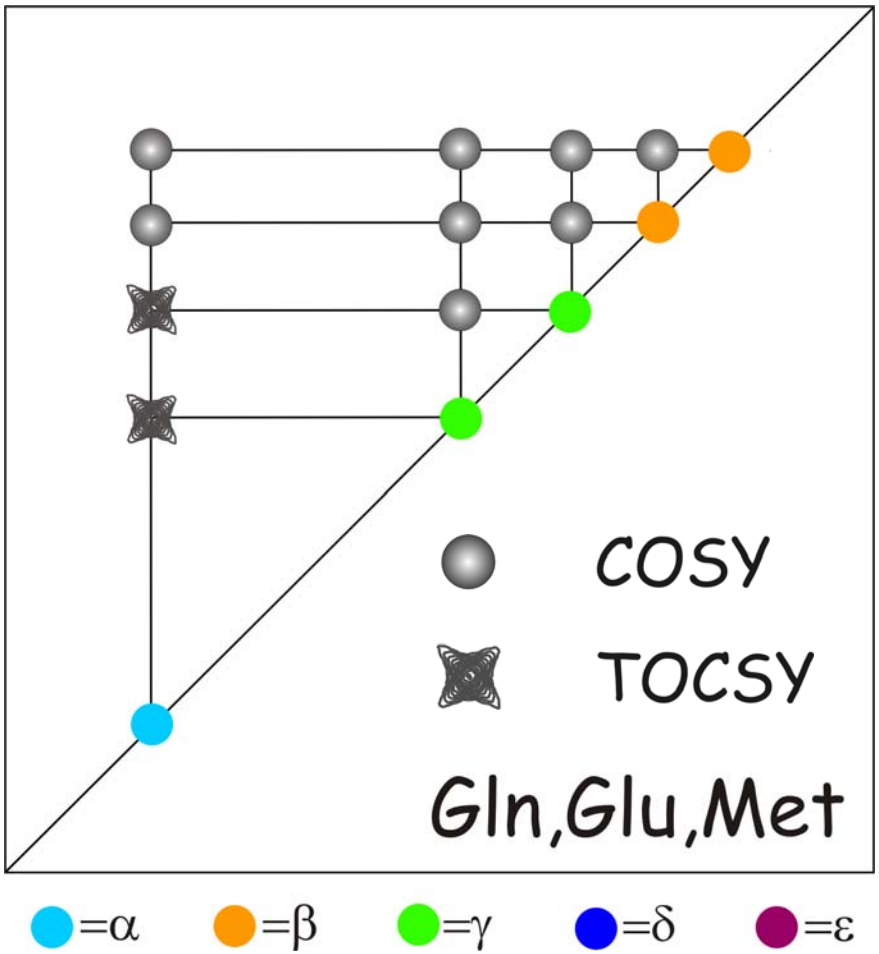
TOCSY

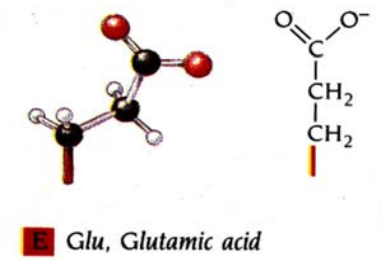


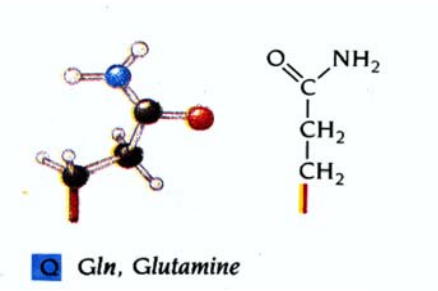


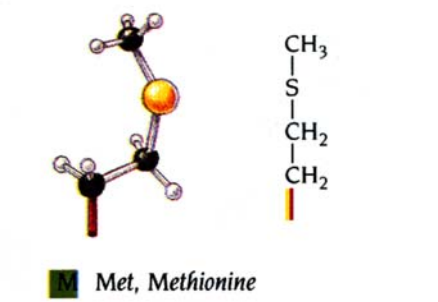
T Thr, Threonine

Homonuclear experiments

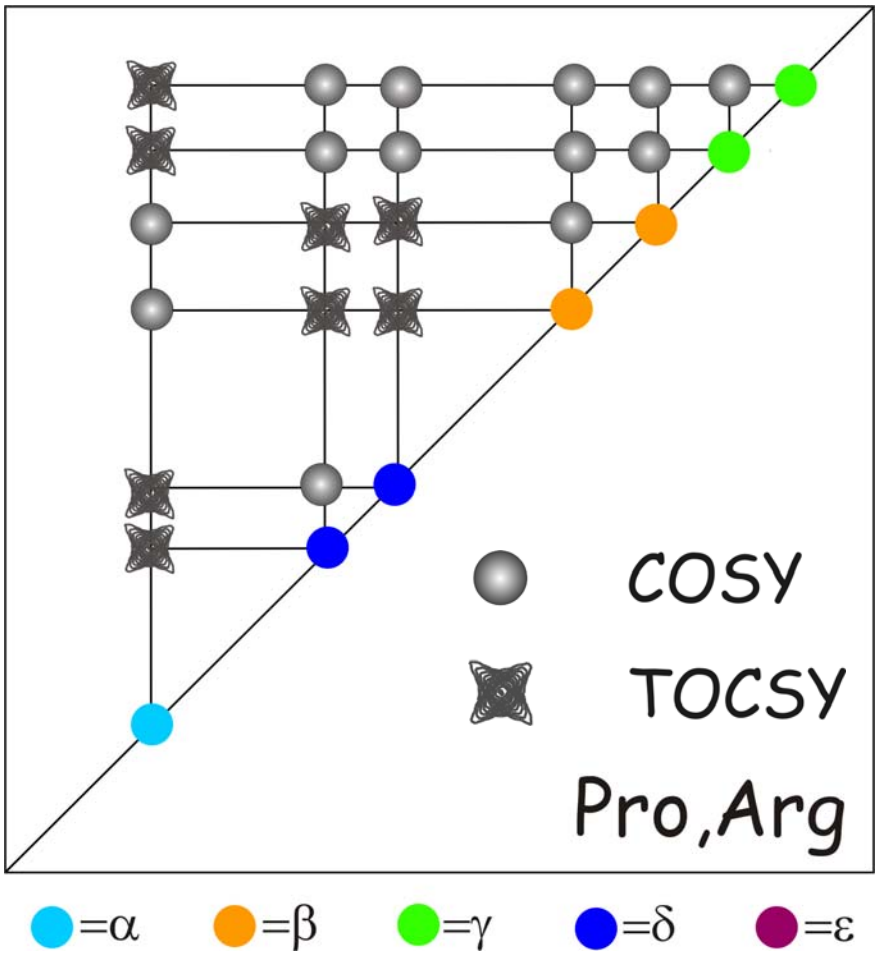


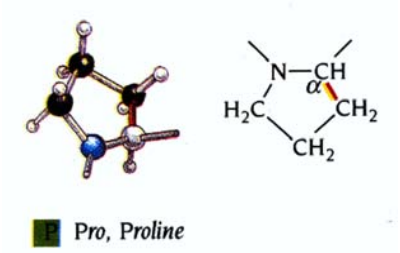


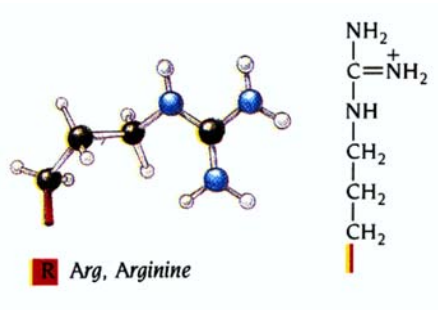




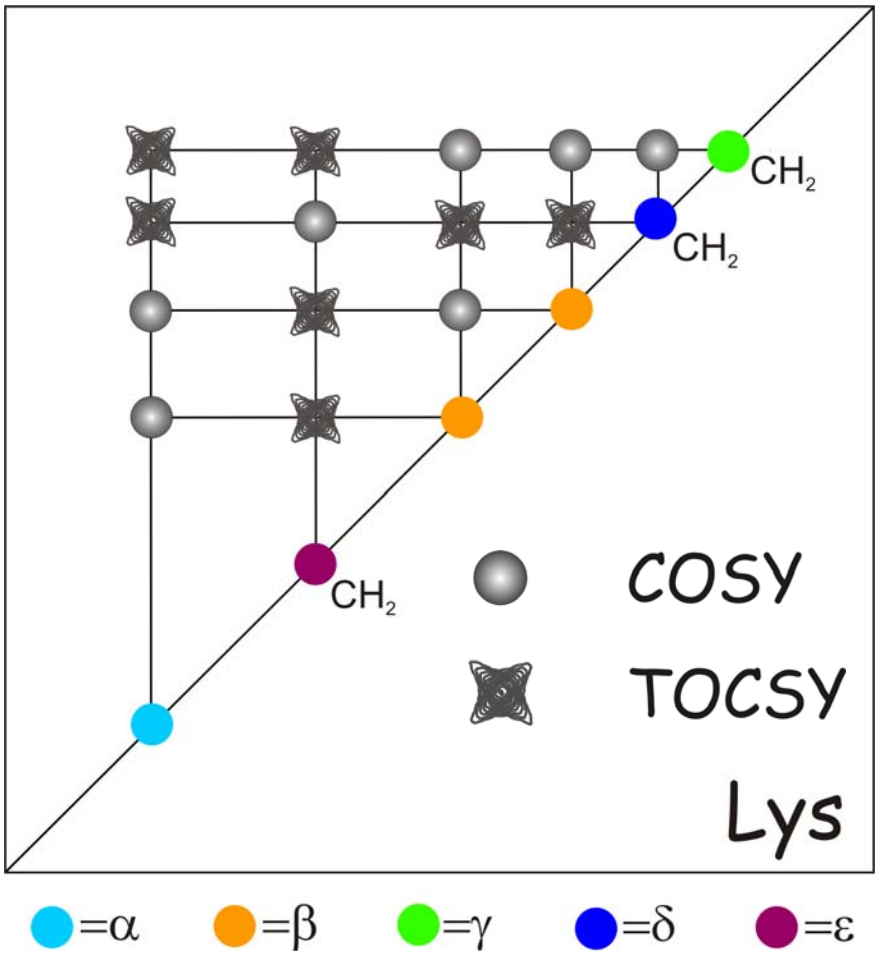
Homonuclear experiments

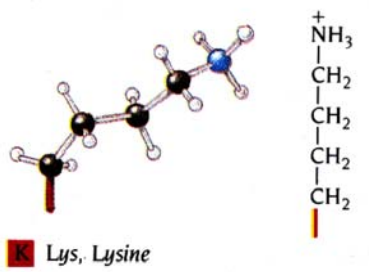






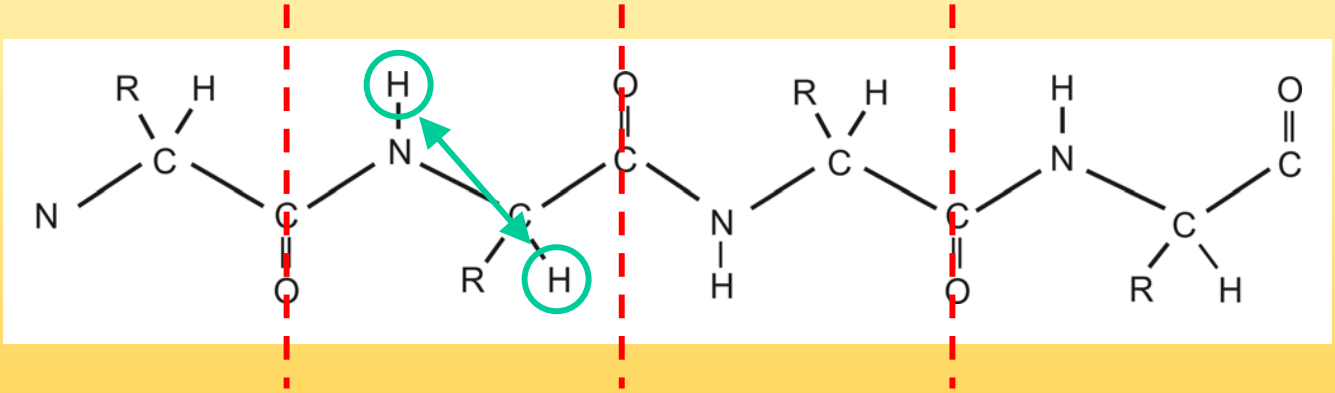
Homonuclear experiments



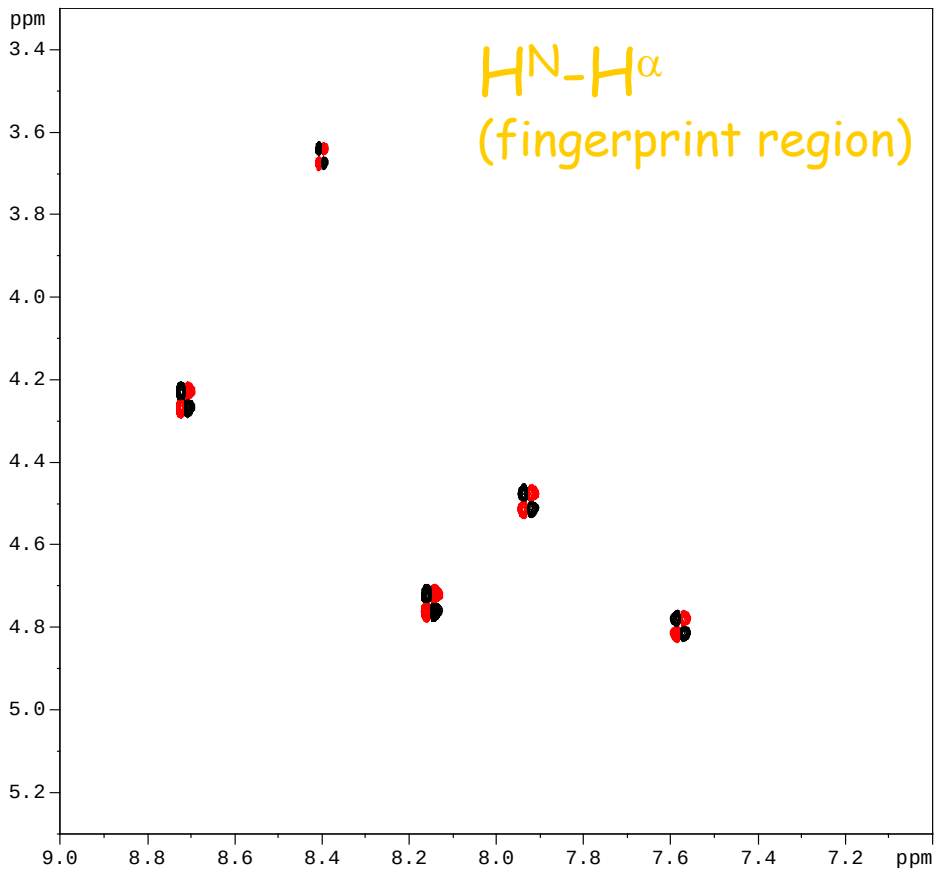


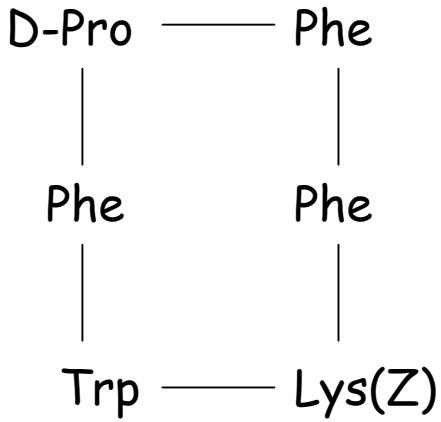
Homonuclear experiments

The amide resonances are connected to the side chain via the H^N-H^α correlation



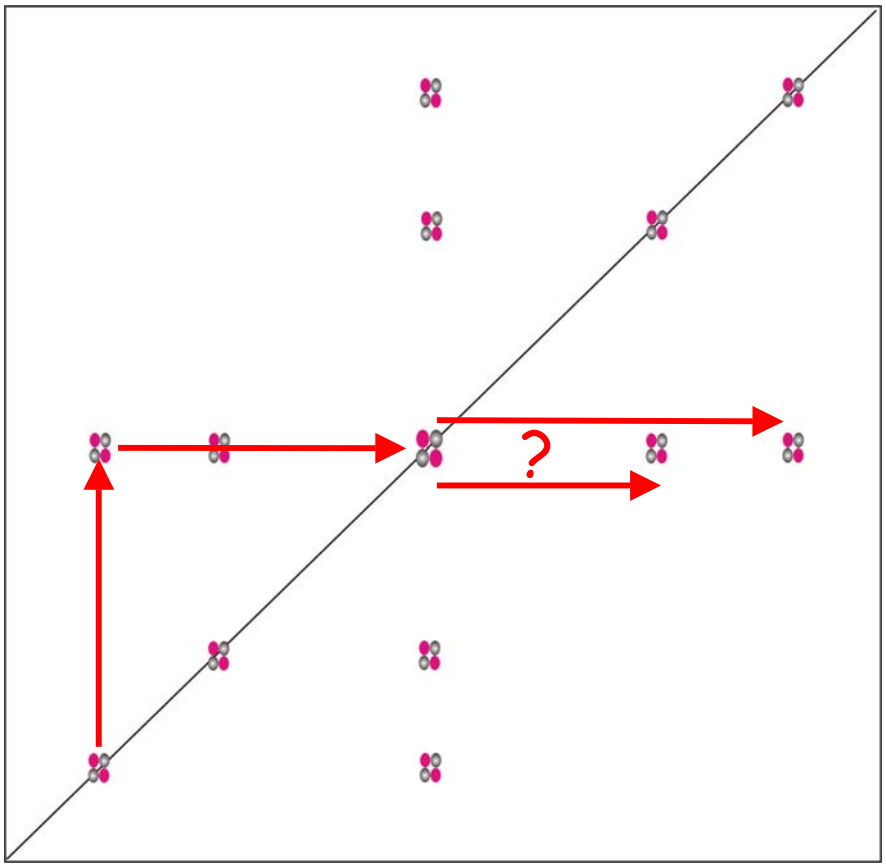
Homonuclear experiments





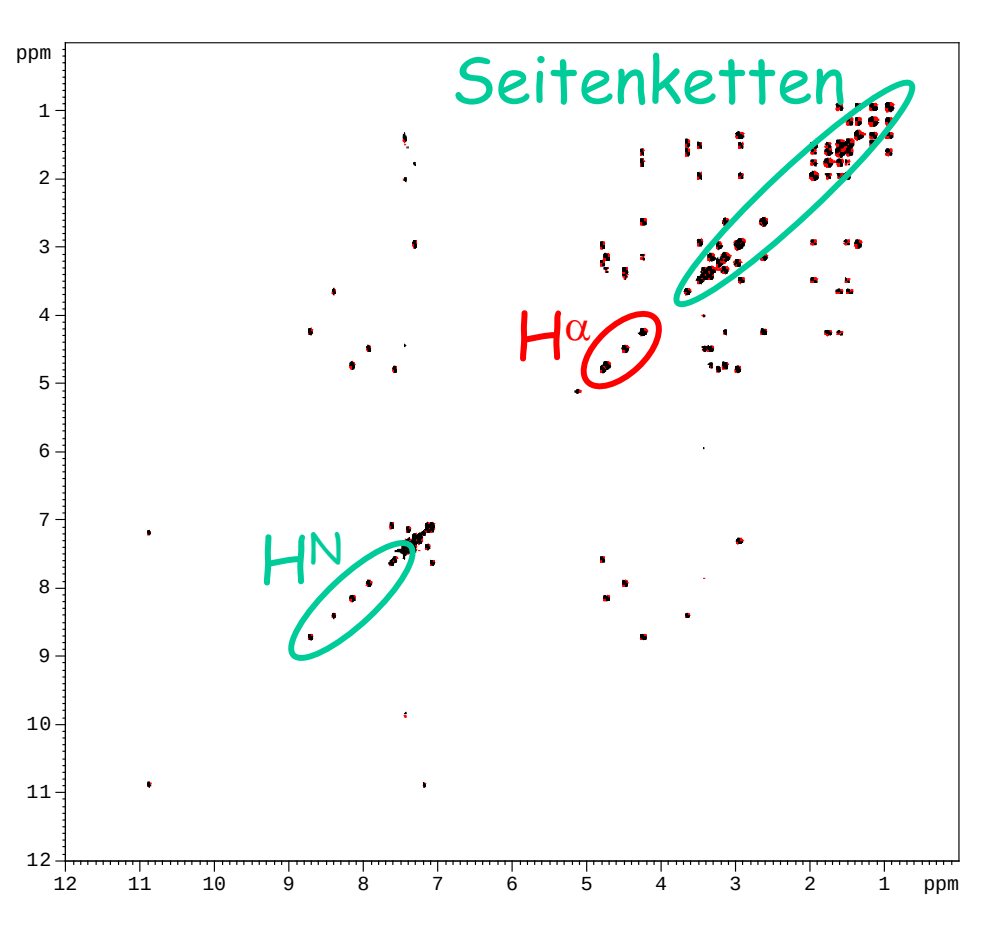
5 H^N-H^α peaks can be expected sind Pro does not have an amide proton

Homonuclear experiments



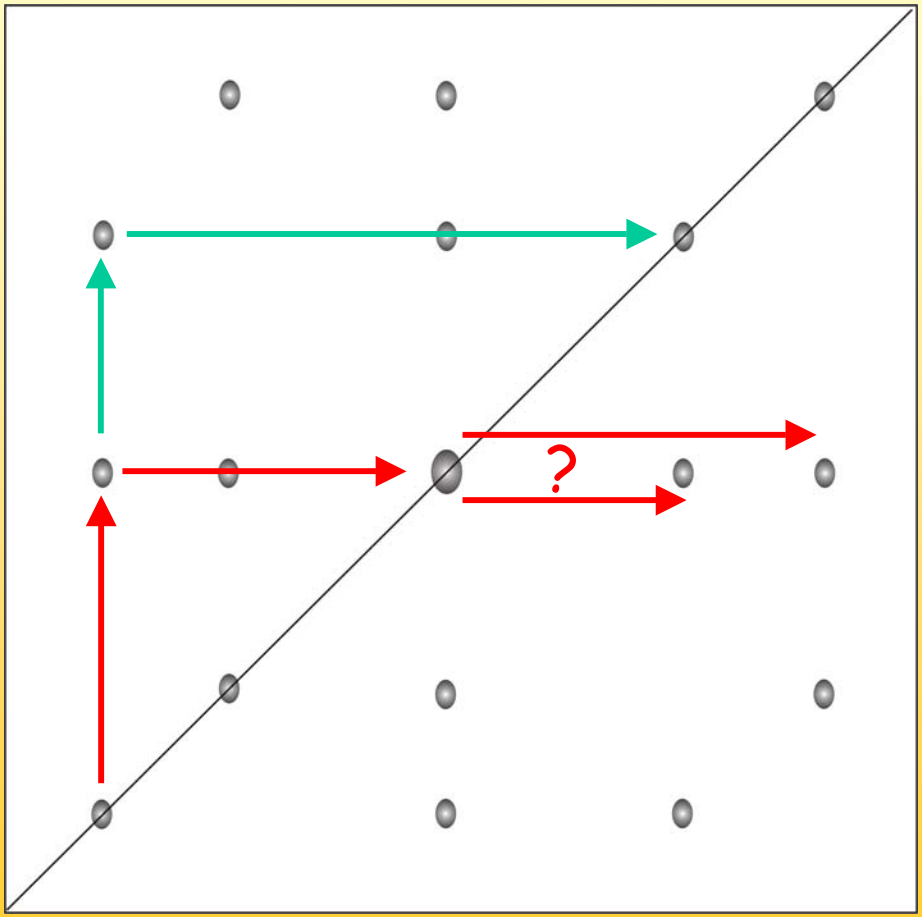
In a DQF-COSY only correlations via 3 bonds are visible, this leads to ambiguity in case of overlap

Homonuclear experiments



Since peptides exhibit very similar H^{α} shifts, this problem does indeed arise

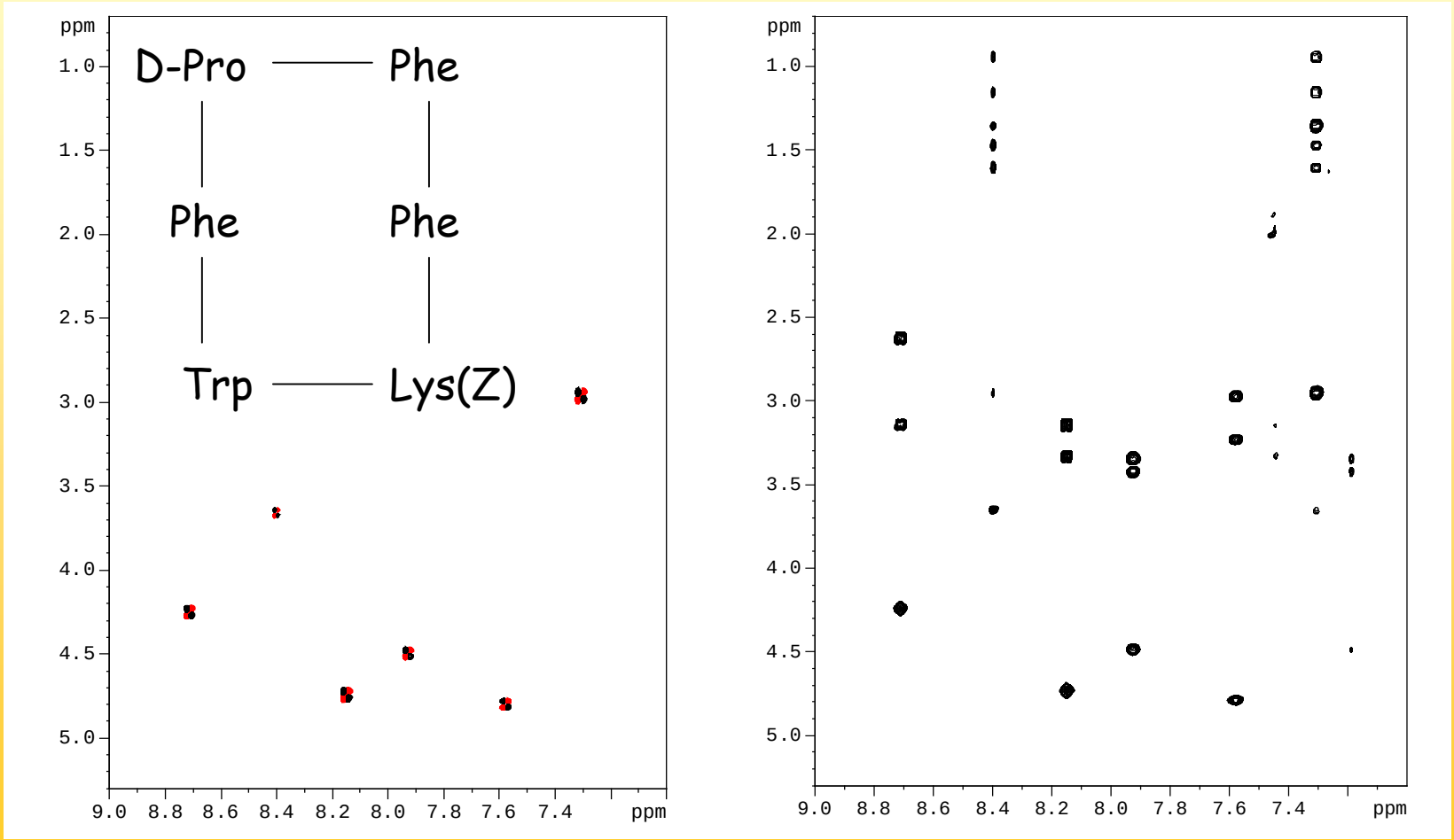
Homonuclear experiments



This ambiguity can be resolved in the TOCSY experiment, Either in the side chain region….

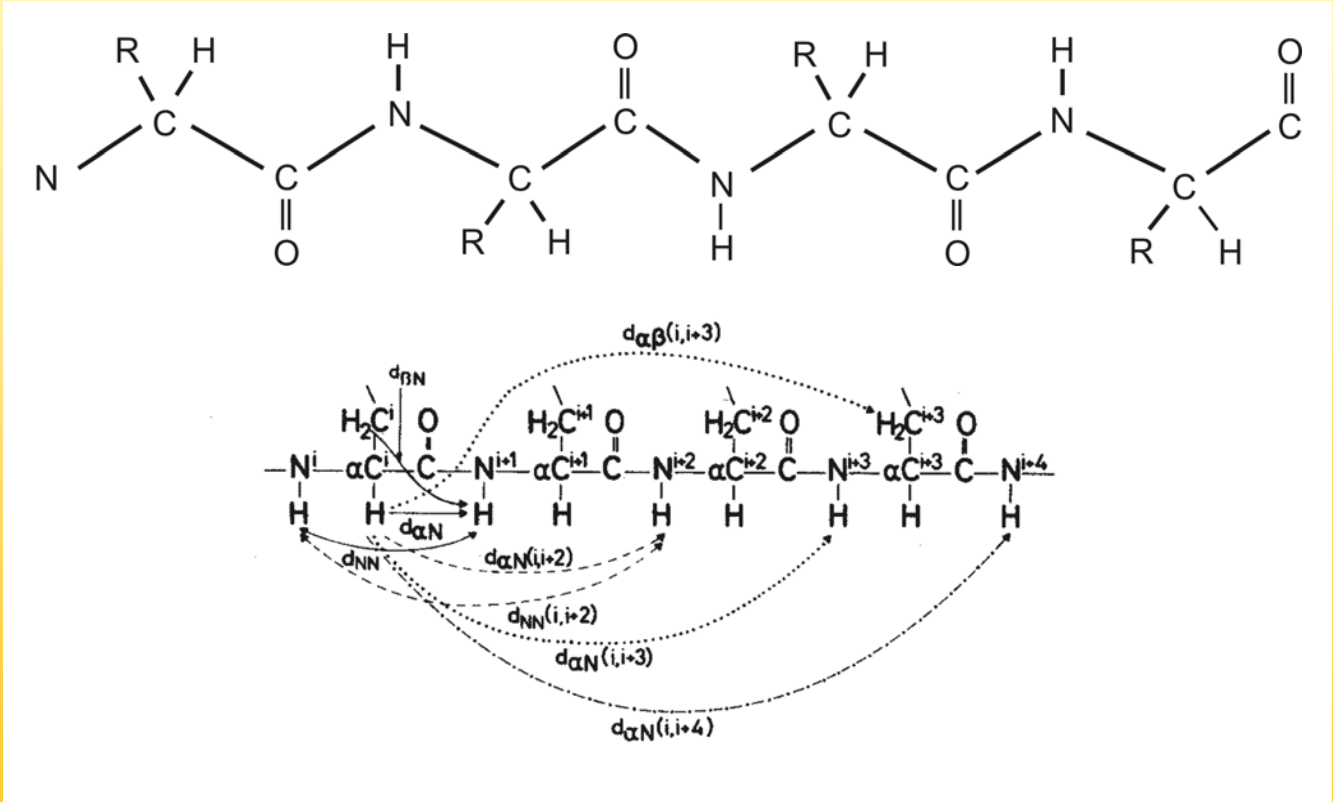
Homonuclear experiments

.....or in the H^N-region

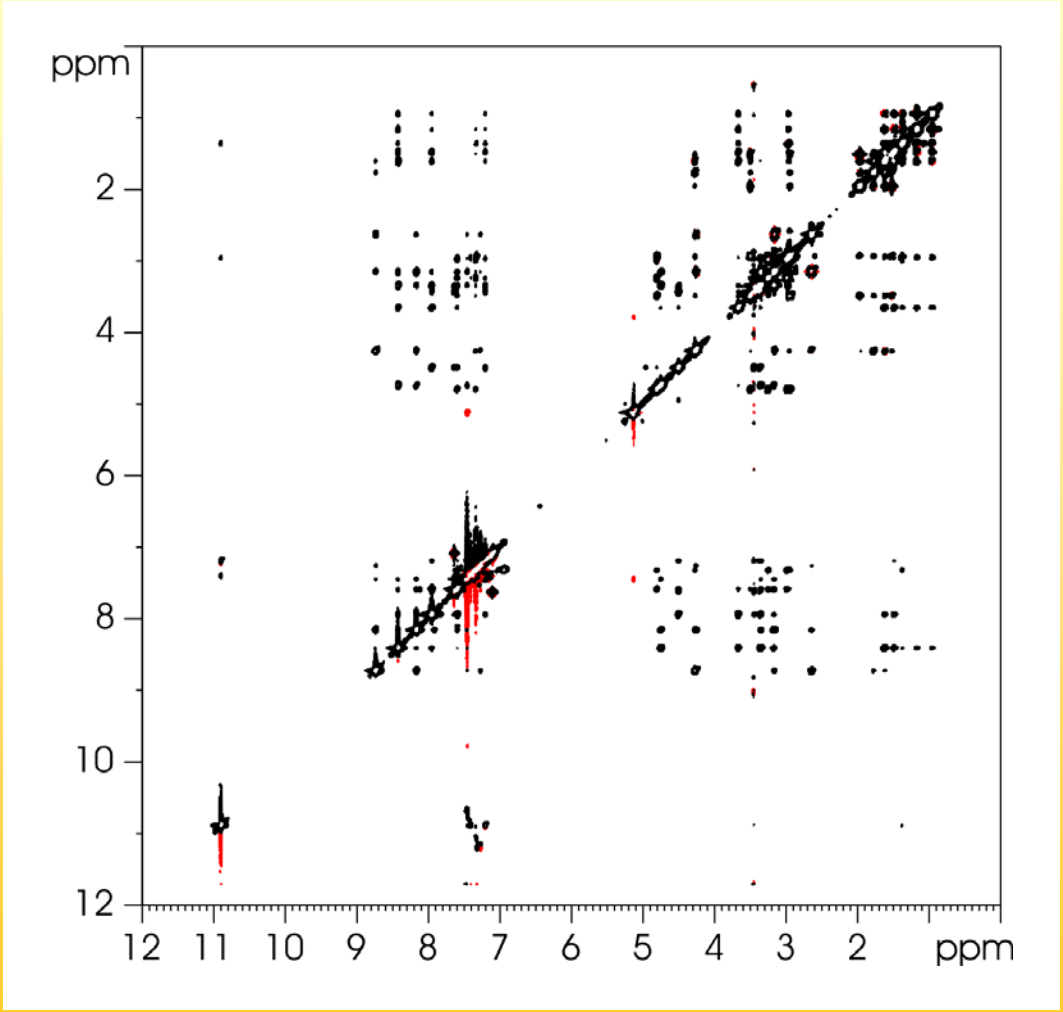


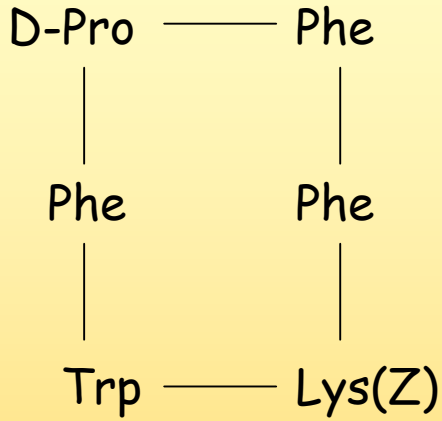
Homonuclear experiments

For the sequential connection the distances along the peptide backbone are important



Homonuclear experiments

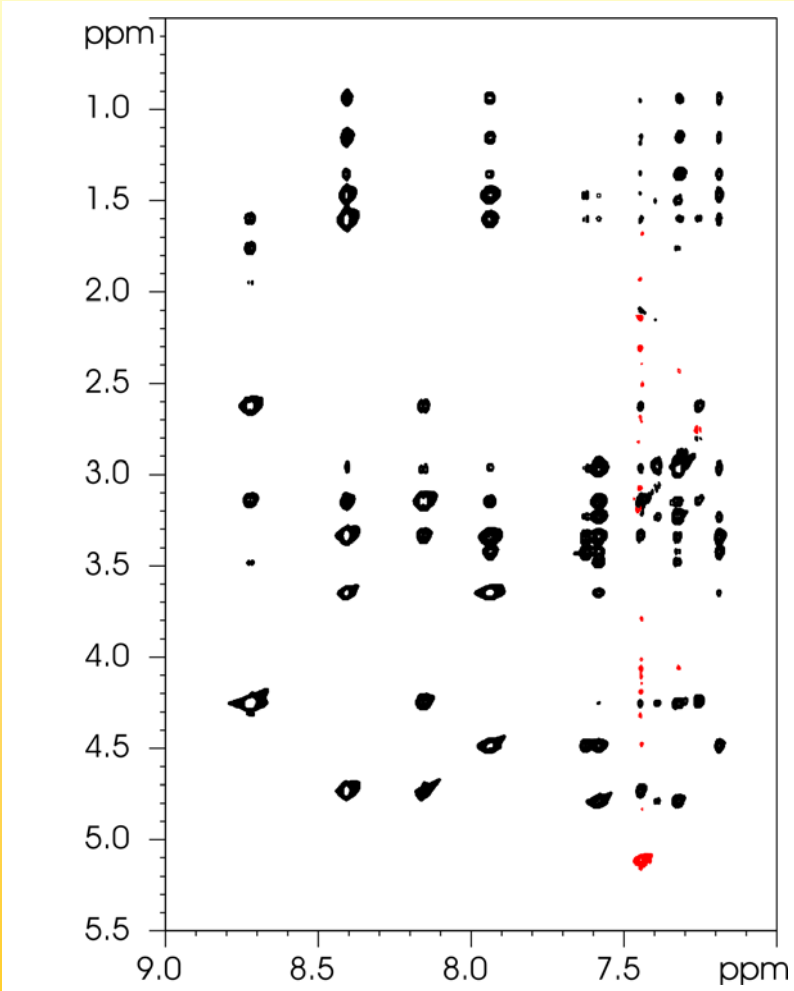




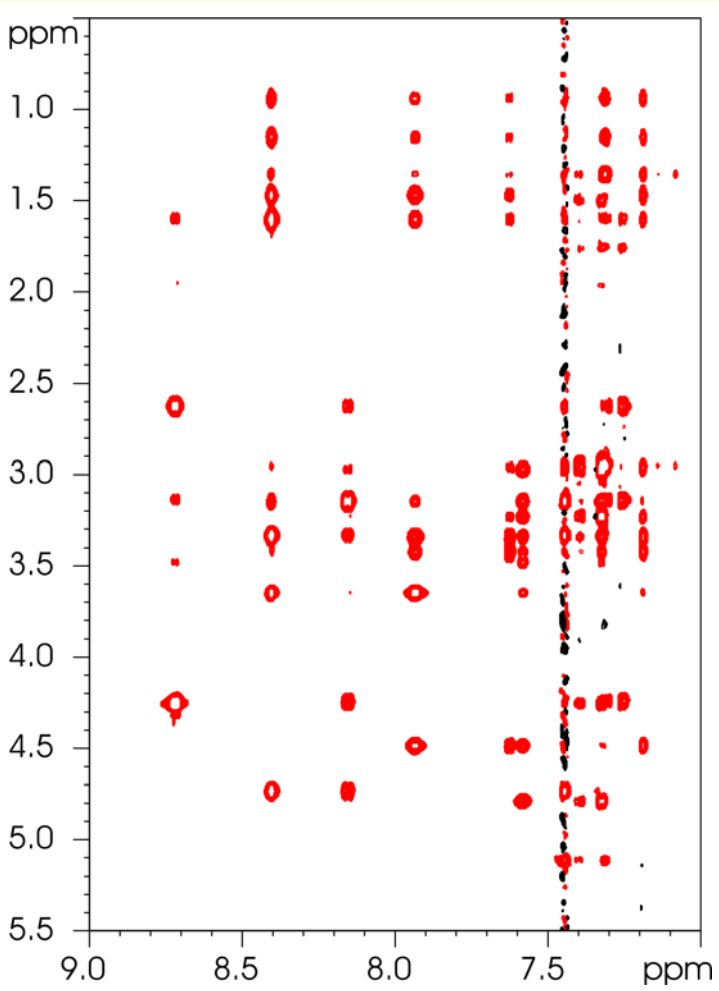
We can finde those distances in the NOESY (or ROESY)

Homonuclear experiments

NOESY

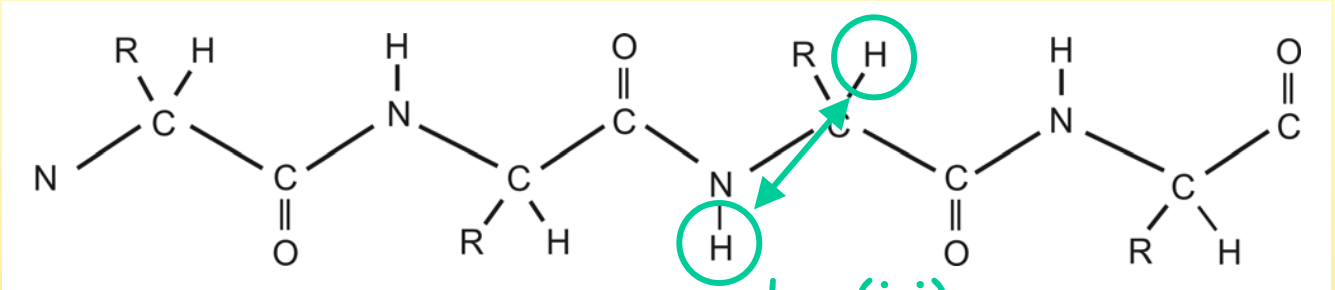


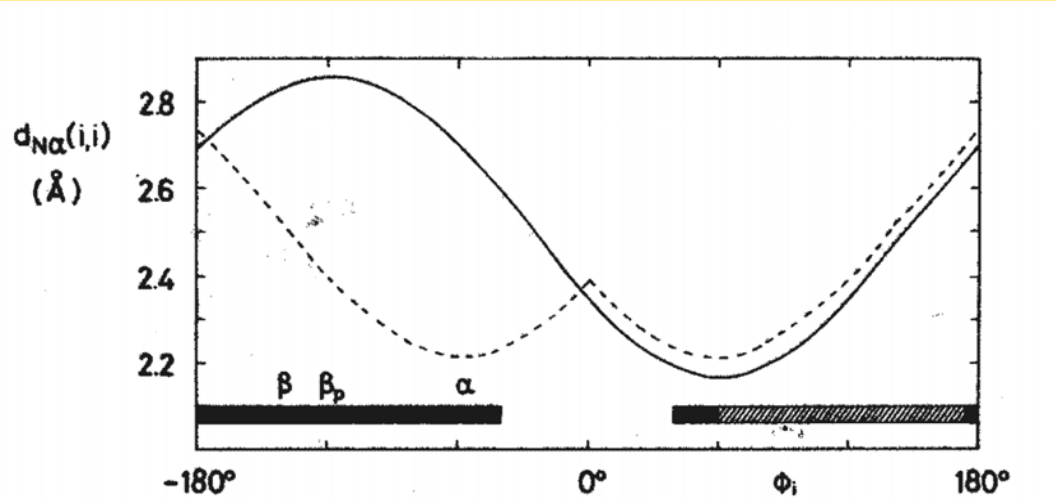
ROESY



Sequence specific assignment (2)

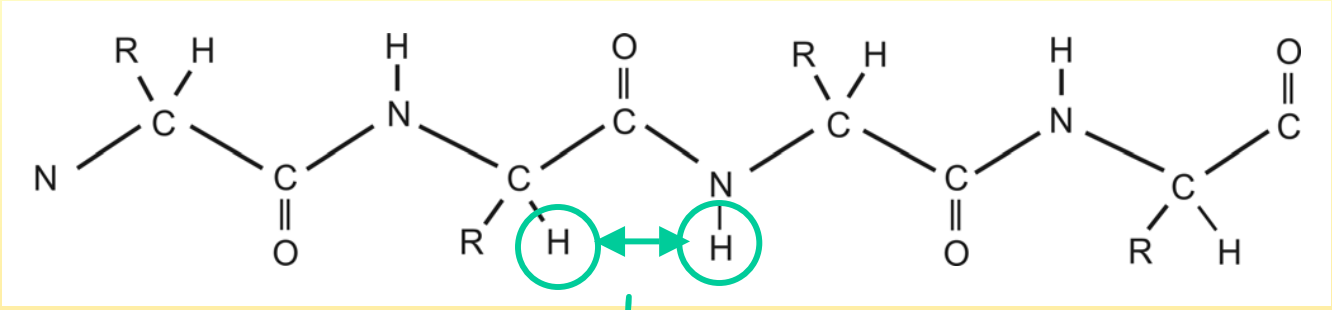
Sequence specific assignment (2)



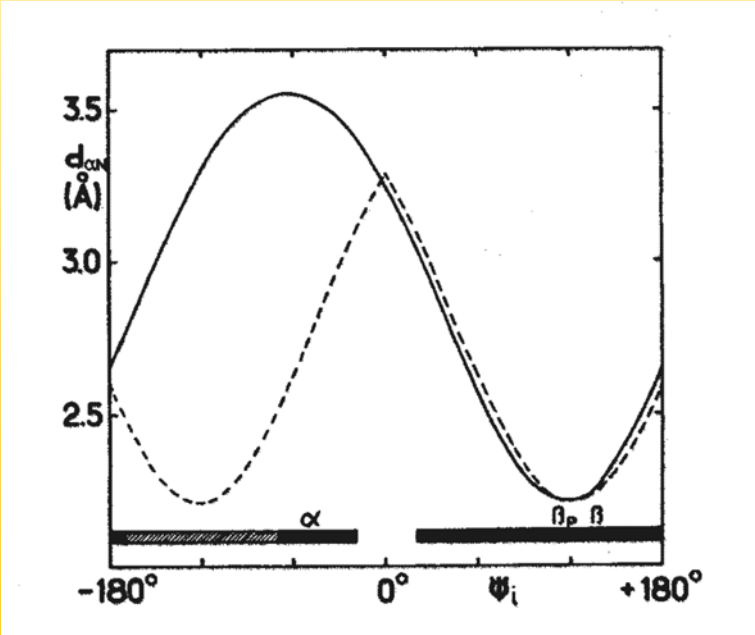


The distance from a H^N to an H^α, $d_{\alpha N}(i,i)$ within one amino acids is always short enough to give rise to an NOE effect

Sequence specific assignment (2)

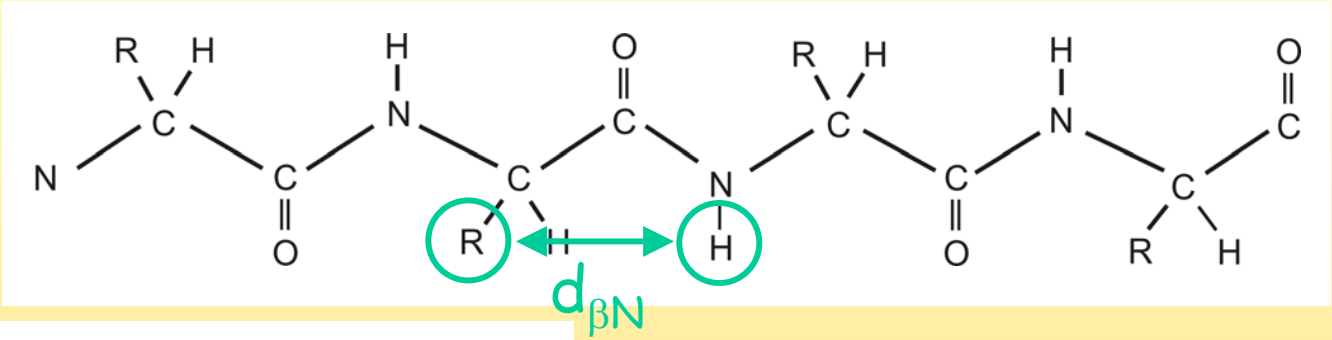


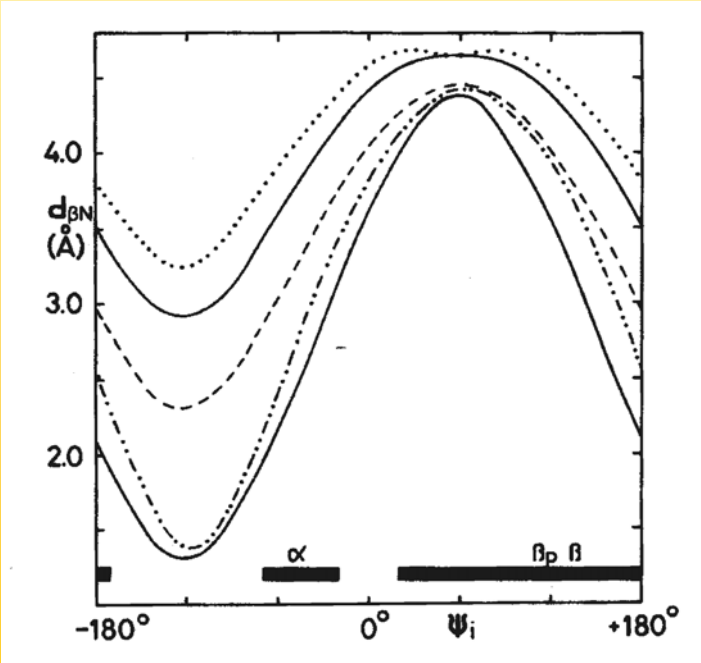
$d_{\alpha N}$



The same is true for the distance from the H^N to the H^α of the amino acids (i-1), $d_{\alpha N}$

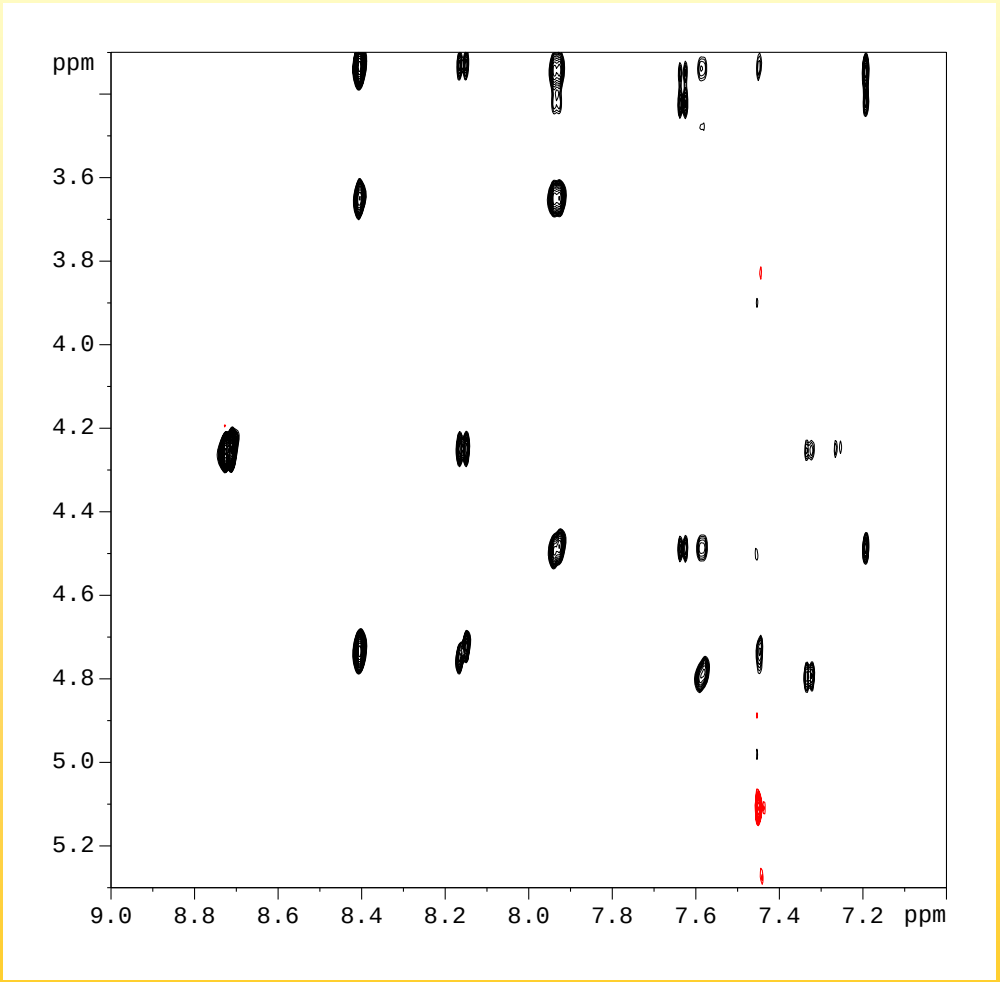
Sequence specific assignment (2)





and usually also for the H^N to H^β of aminoacids (i-1), $d_{\beta N}$

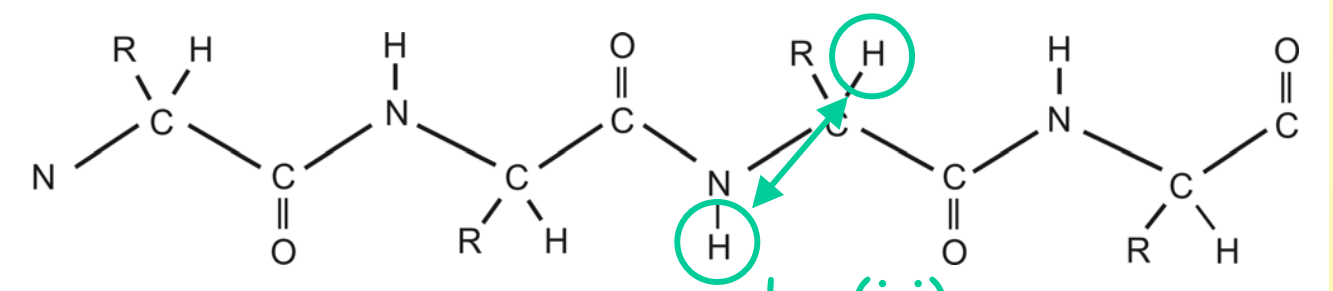
Sequence specific assignment (2)



In the fingerprint region of the NOESY there should be at least two peaks for every amide proton, one for $d_{\alpha N}(i,i)$ and one for $d_{\alpha N}$

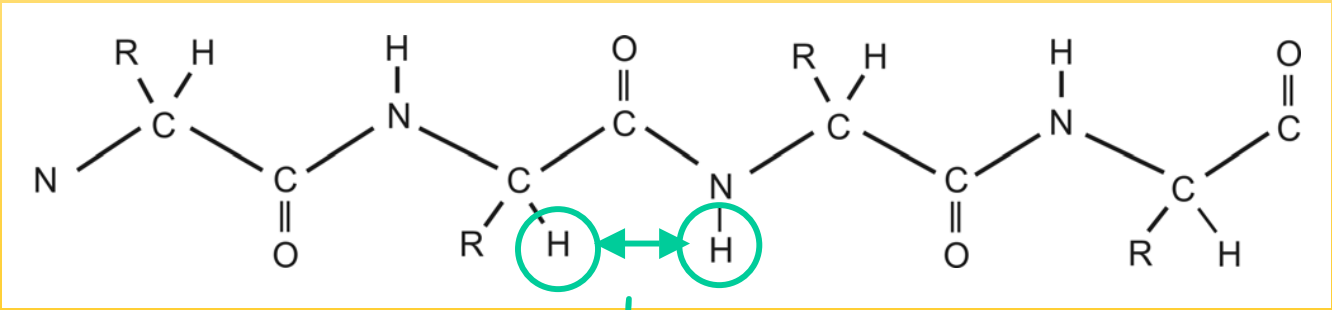
In addition there are peaks to the side chains and all other protons close in space

Sequence specific assignment (2)



$d_{\alpha N}(i,i)$

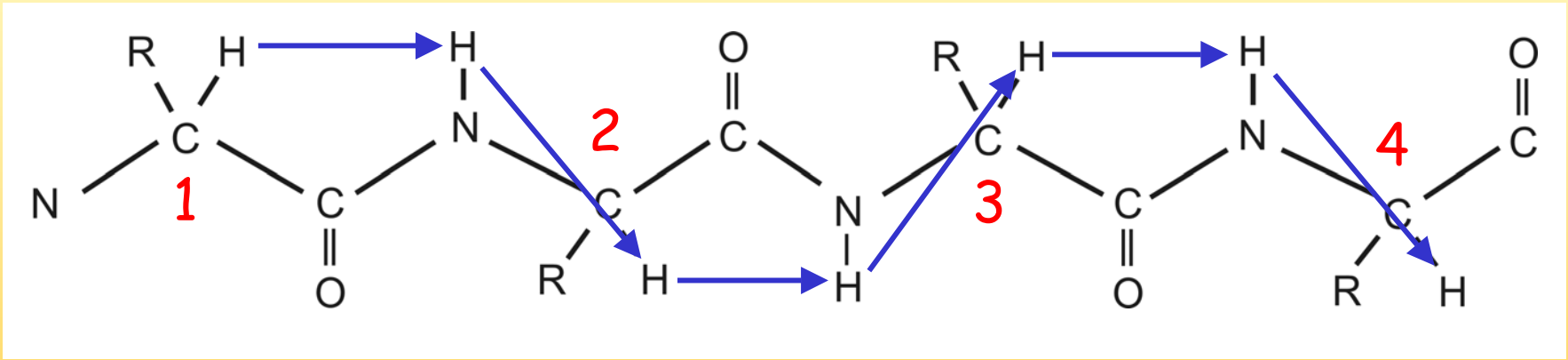
The distance from the H^N to the H^α of the same amino acids, $d_{\alpha N}(i,i)$, yields a peak that can also be found in the COSY.The distance from the H^N to the H^α of the amino acid (i-1), $d_{\alpha N}$, yields a peak that can only be found in the NOESY



$d_{\alpha N}$

Sequence specific assignment (2)

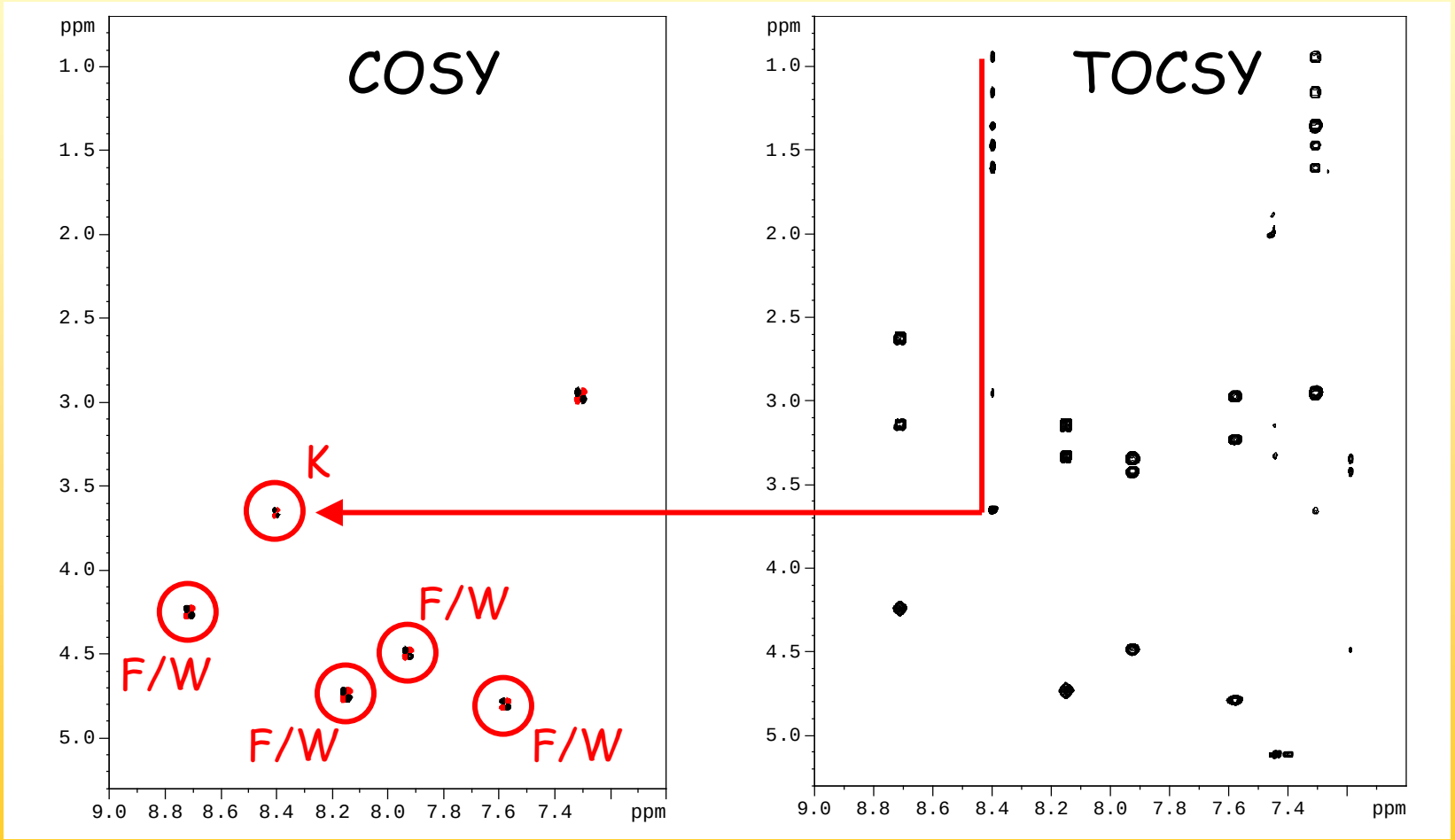
After distinguishing the peaks we can do a "sequential walk"



$1 \xrightarrow{d_{\alpha N}} 2 \xrightarrow{d_{\alpha N}(i,i)} 2 \xrightarrow{d_{\alpha N}} 3 \xrightarrow{d_{\alpha N}(i,i)} 3 \xrightarrow{d_{\alpha N}} 4 \xrightarrow{d_{\alpha N}(i,i)} 4$

Sequence specific assignment (2)

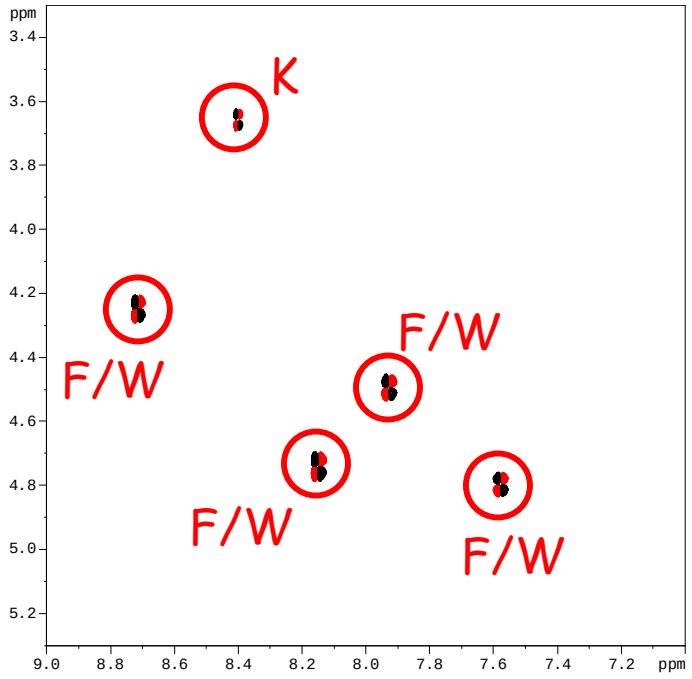
Identification of the amino acids type



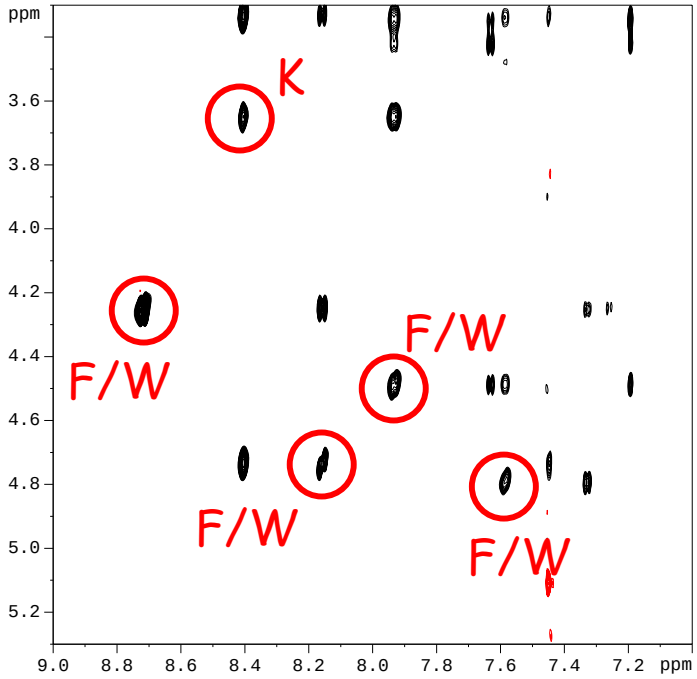
Sequence specific assignment (2)

Transfer of the COSY-Info into a NOESY

COSY

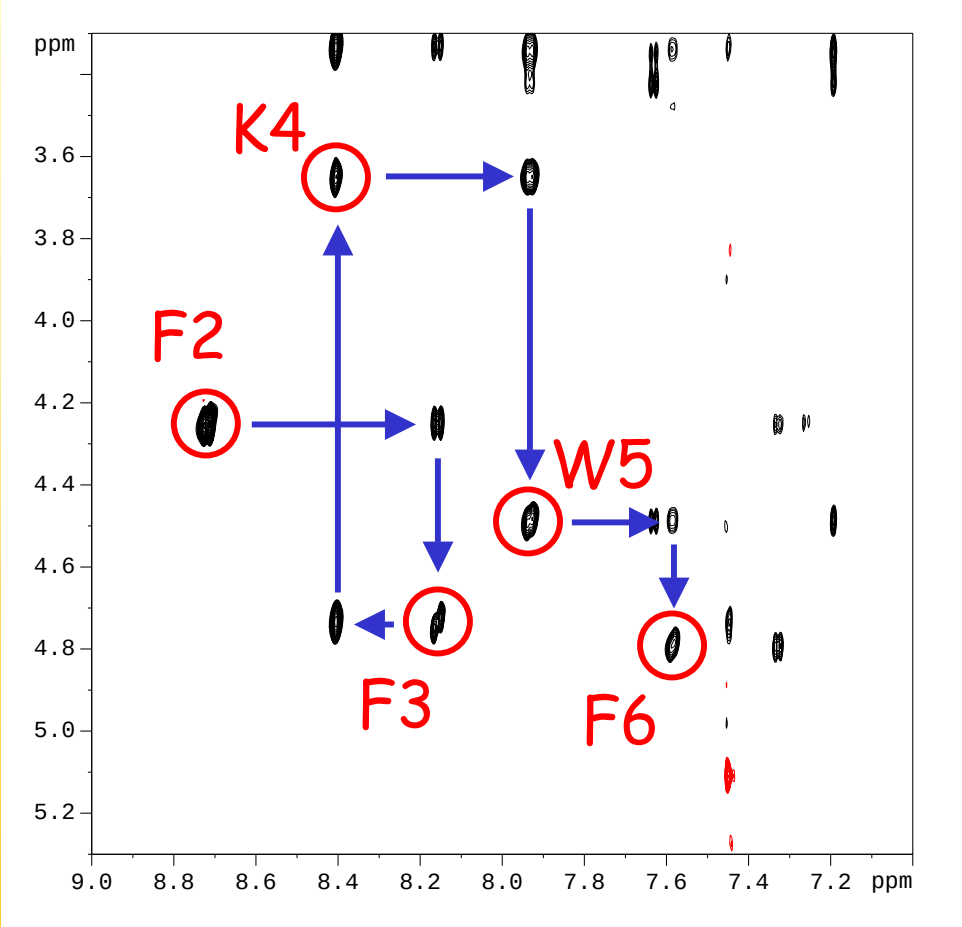


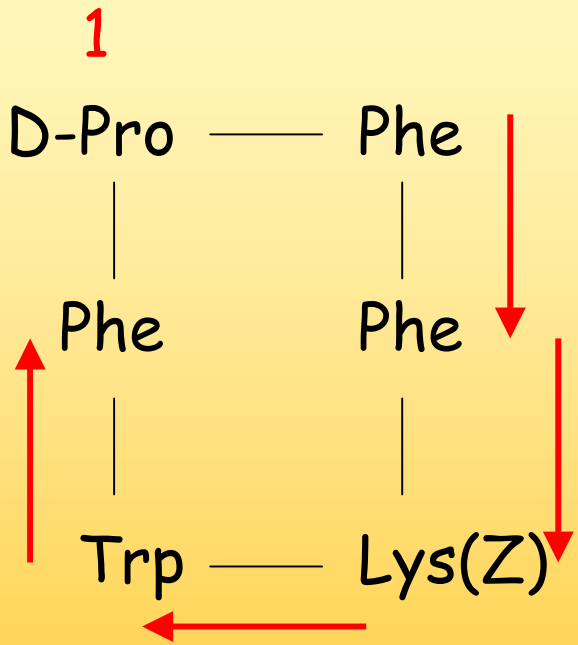
NOESY



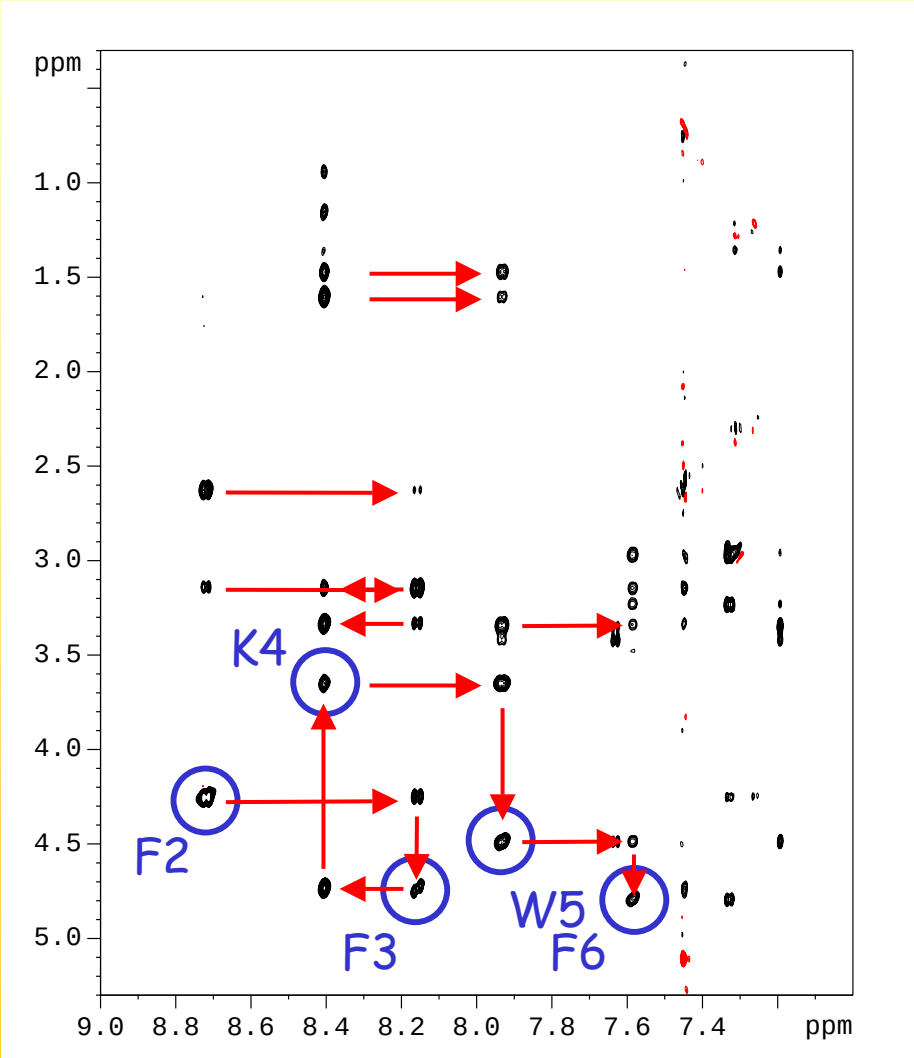
Sequence specific assignment (2)

In the spectrum

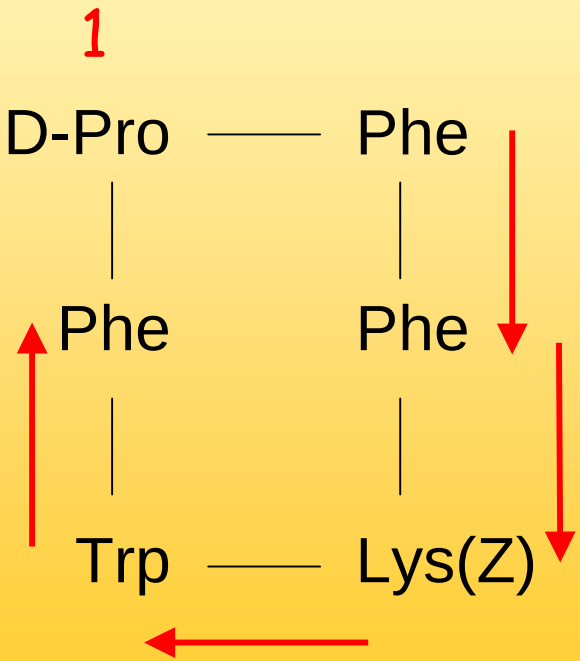




Sequence specific assignment (2)

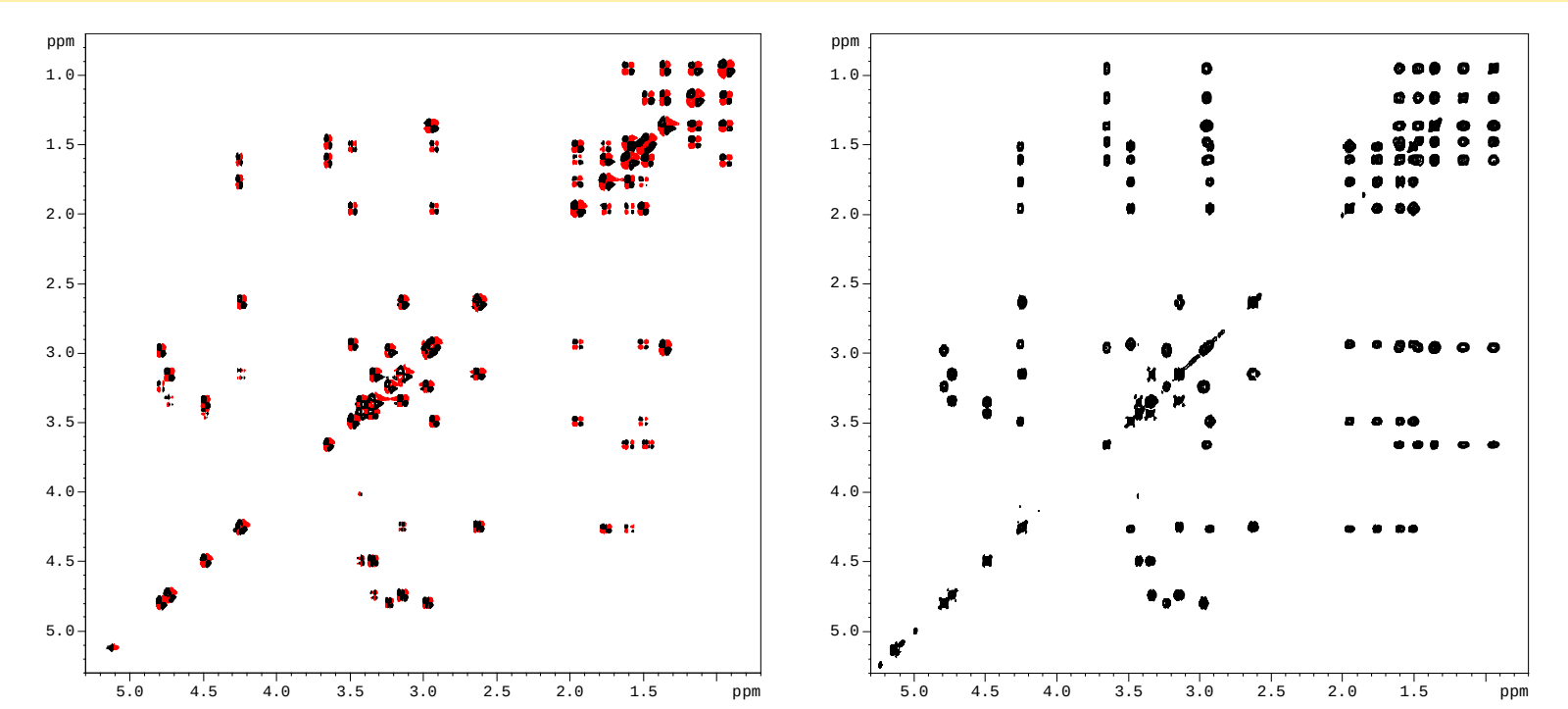


with H^{β} as additional information assignment is more reliable



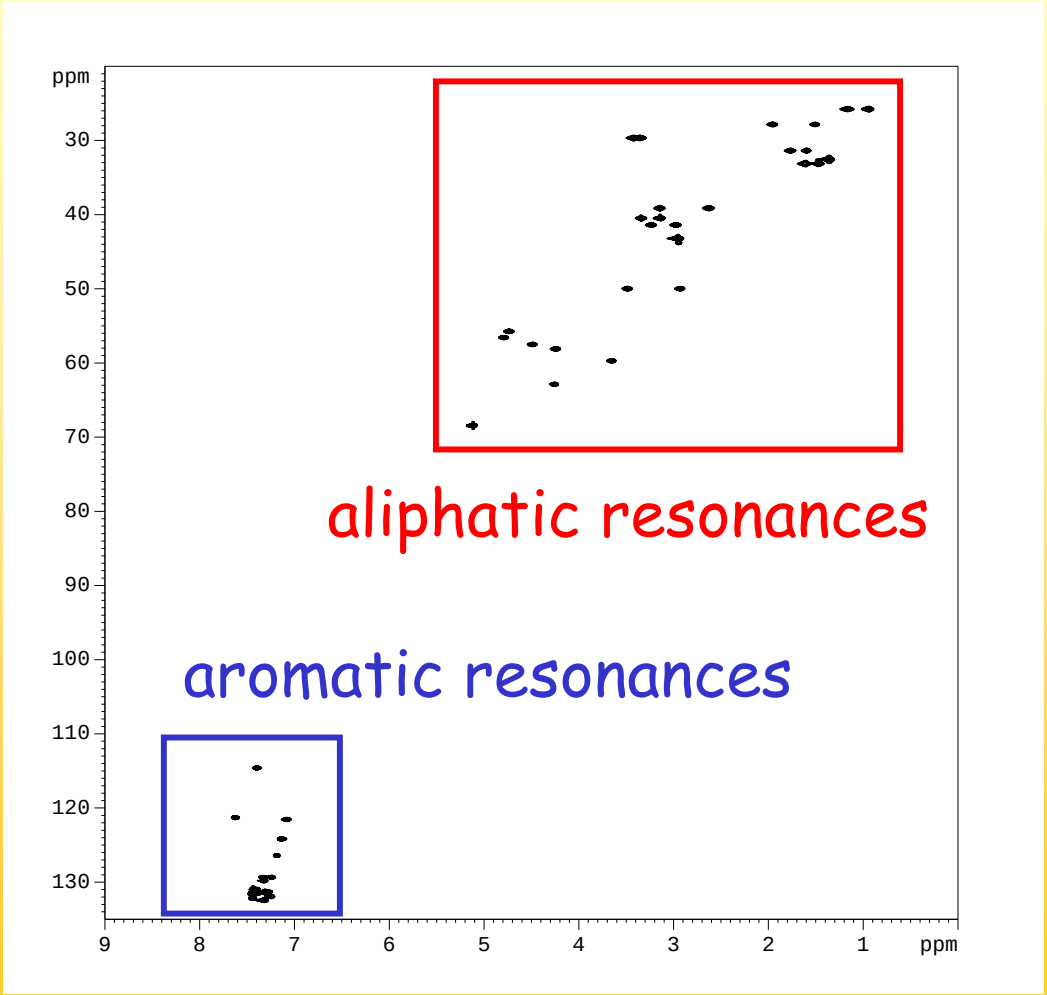
Sequence specific assignment (2)

After having established the assignment of the main chain the assignment can be transferred to the side chain using COSY and TOCSY

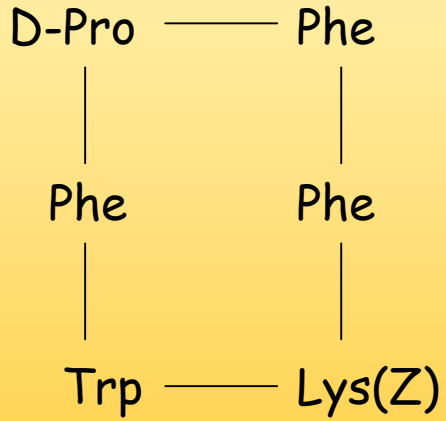


Heteronuclear experiments

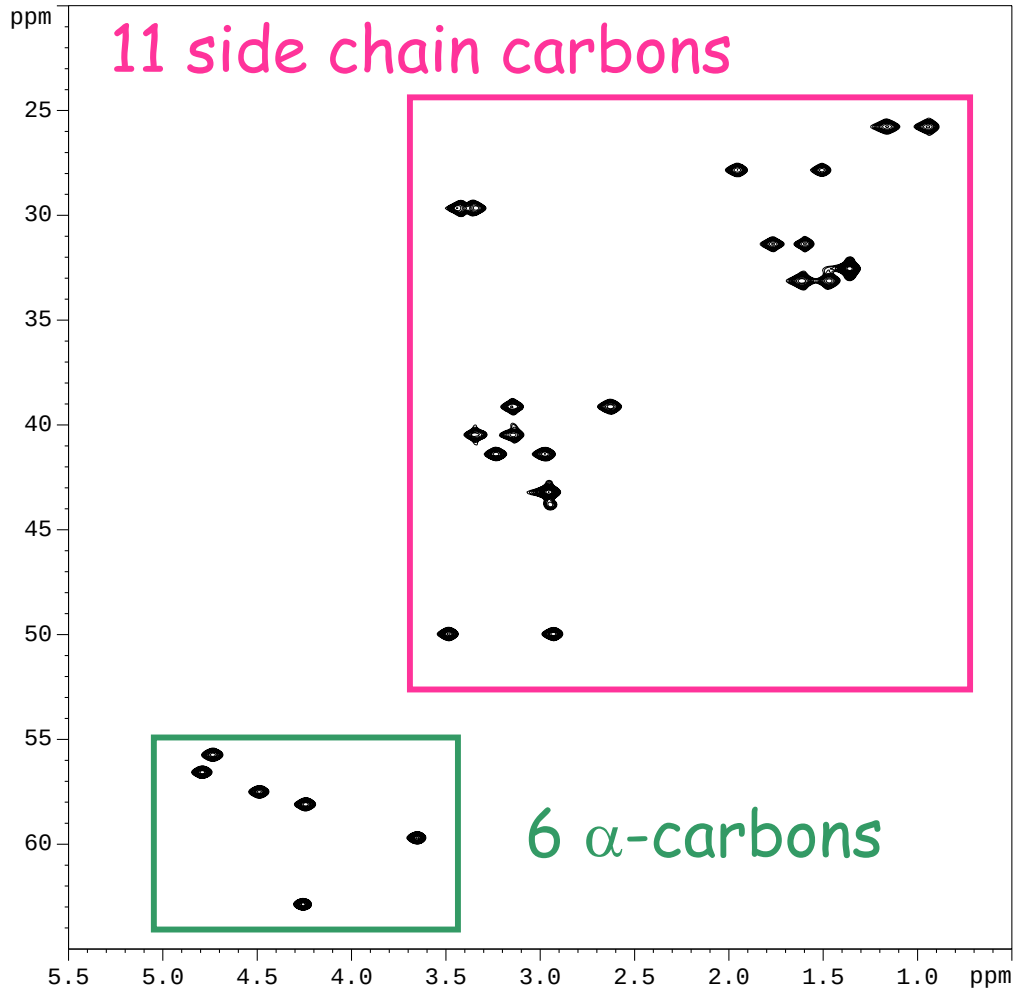
Heteronuclear experiments



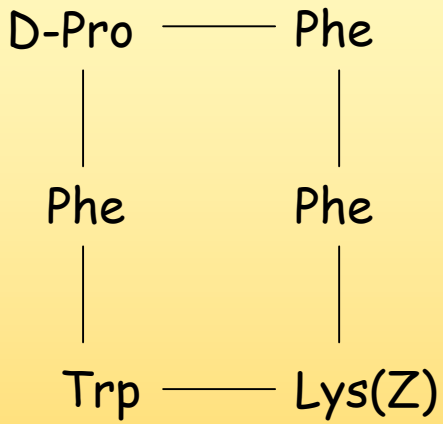
^{13}C-HMQC of F3-008



Heteronuclear experiments



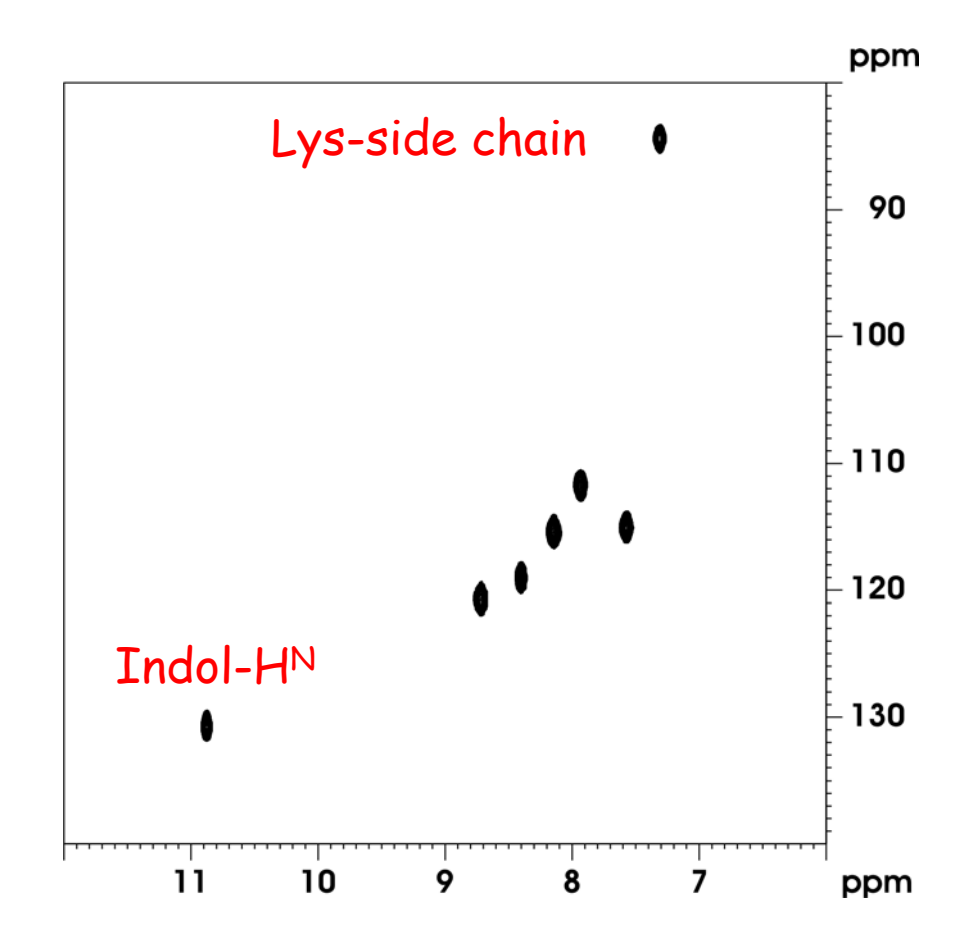
F3-008



overlap is resolved almost completely

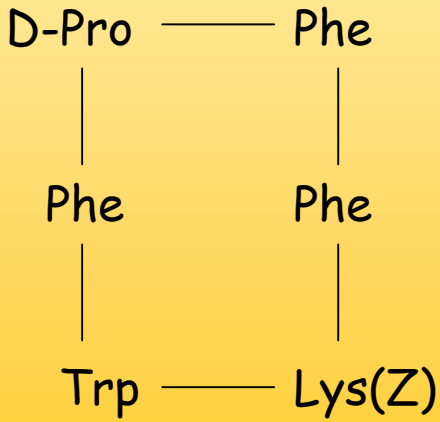
Heteronuclear experiments

^{15}N-HMQC von F3-008

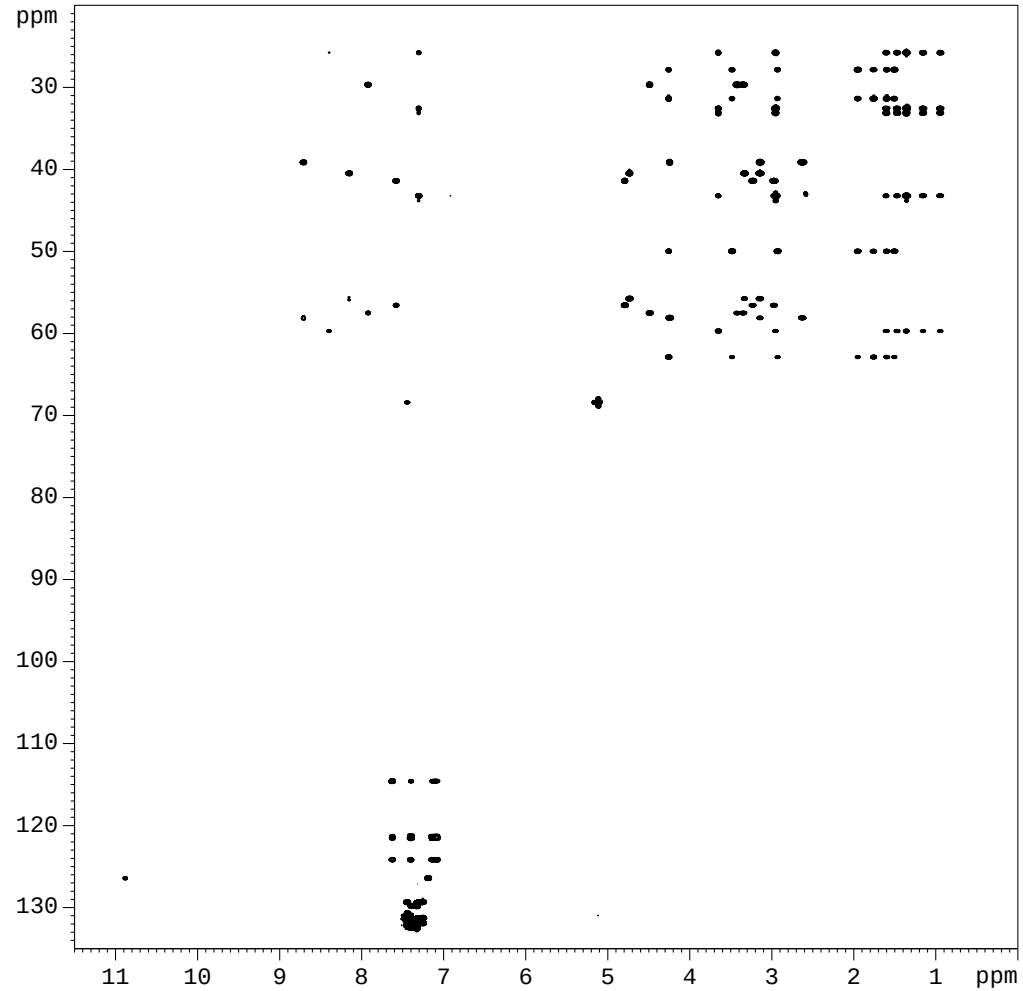


It works with

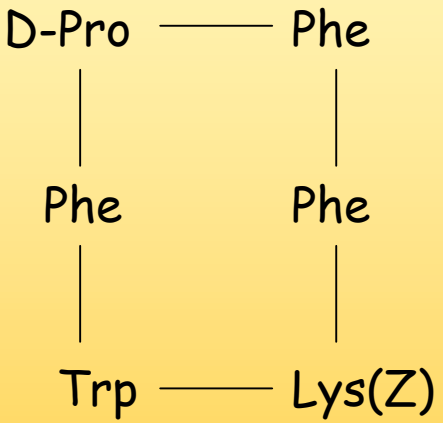
X = ^{15}N as well



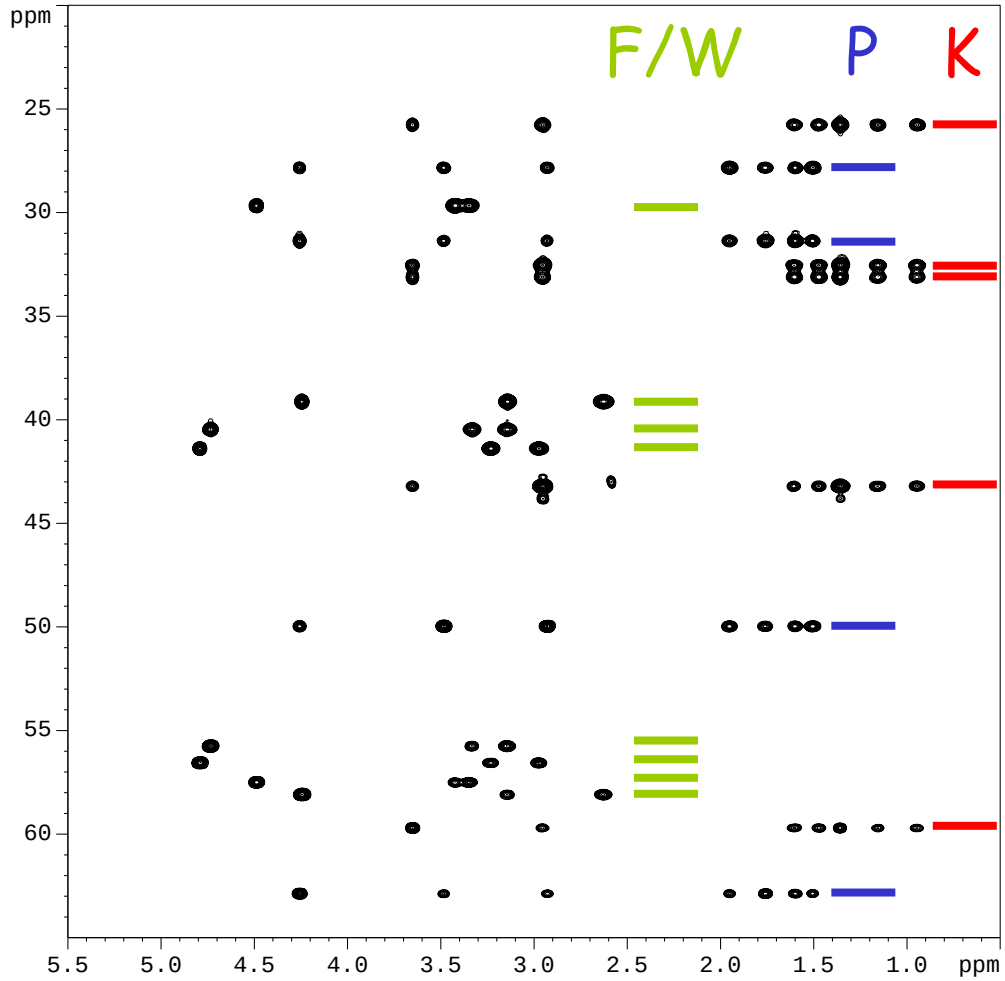
Heteronuclear experiments



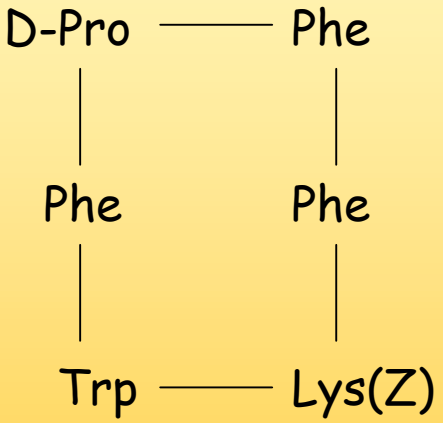
HMQC-TOCSY of F3-008



Heteronuclear experiments

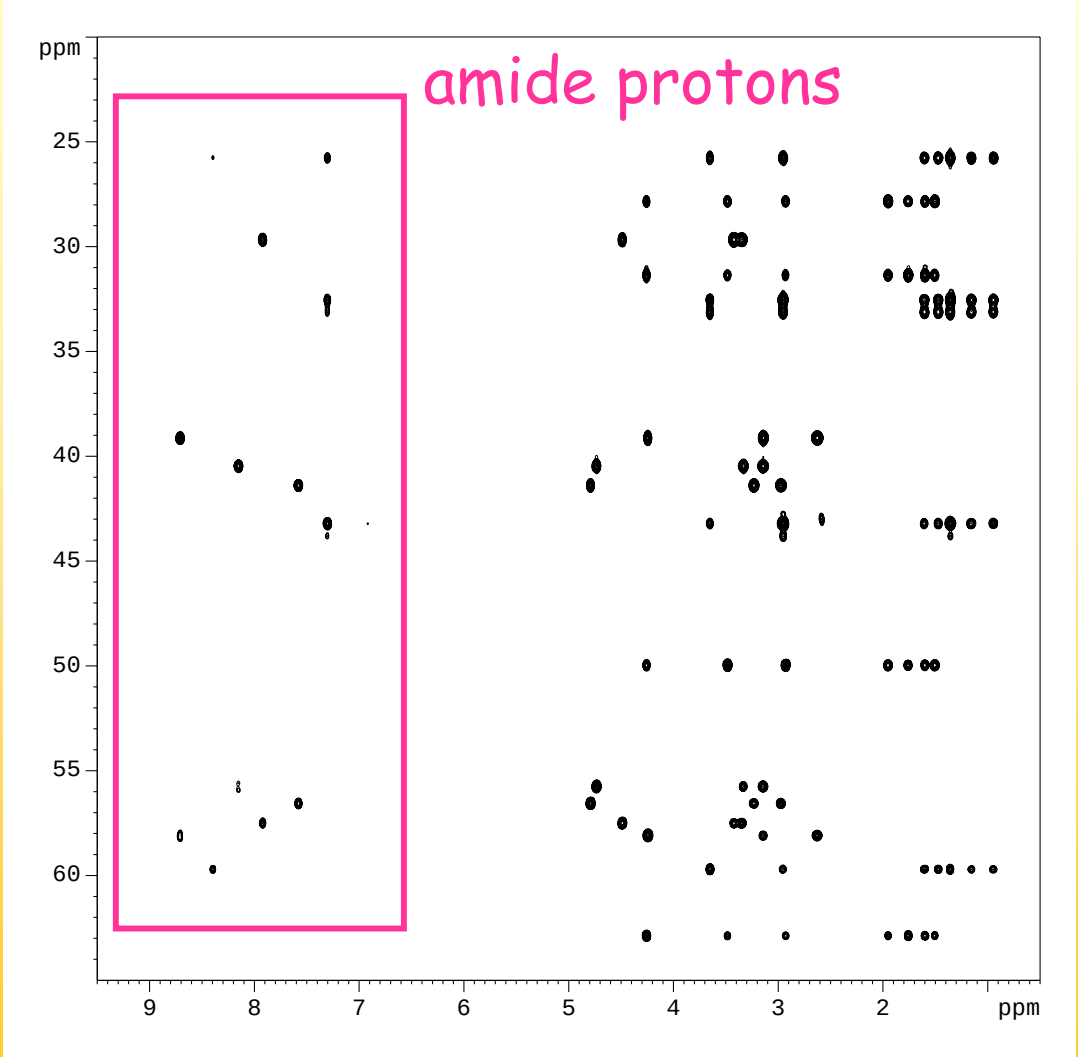


HMQC-TOCSY of F3-008

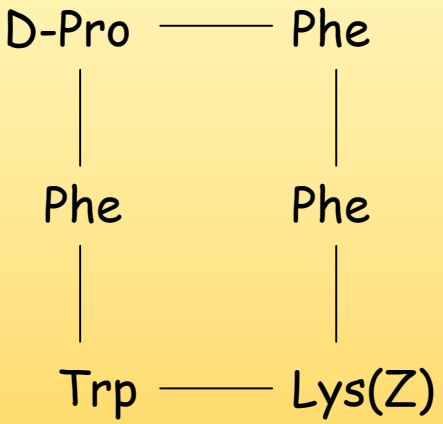


aliphatic region

Heteronuclear experiments



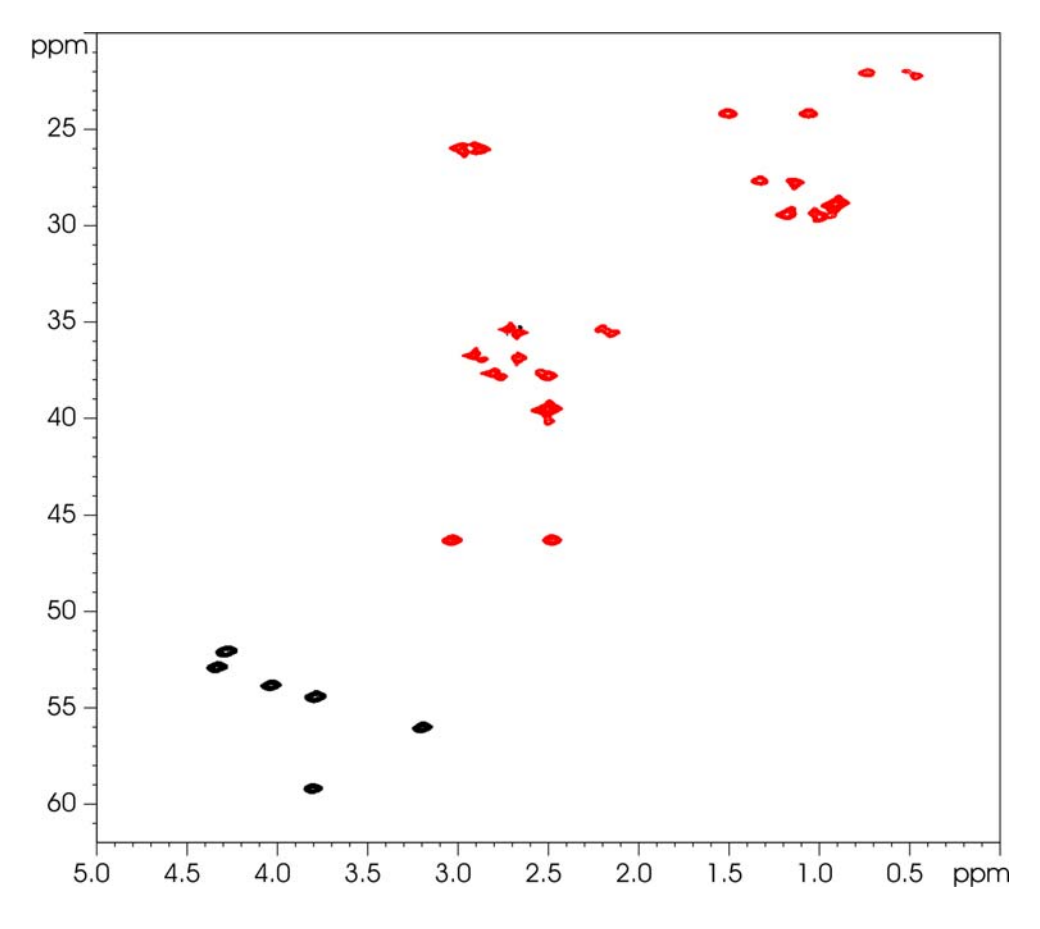
HMQC-TOCSY of F3-008



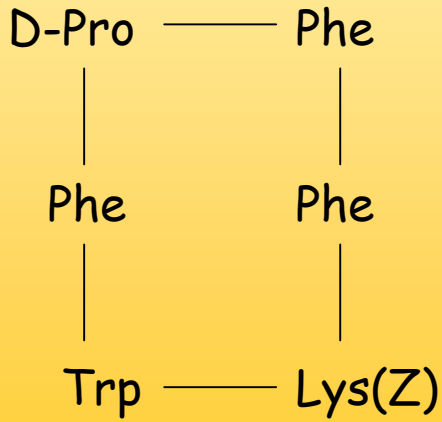
The H^N can thus be reached as well

Heteronuclear experiments

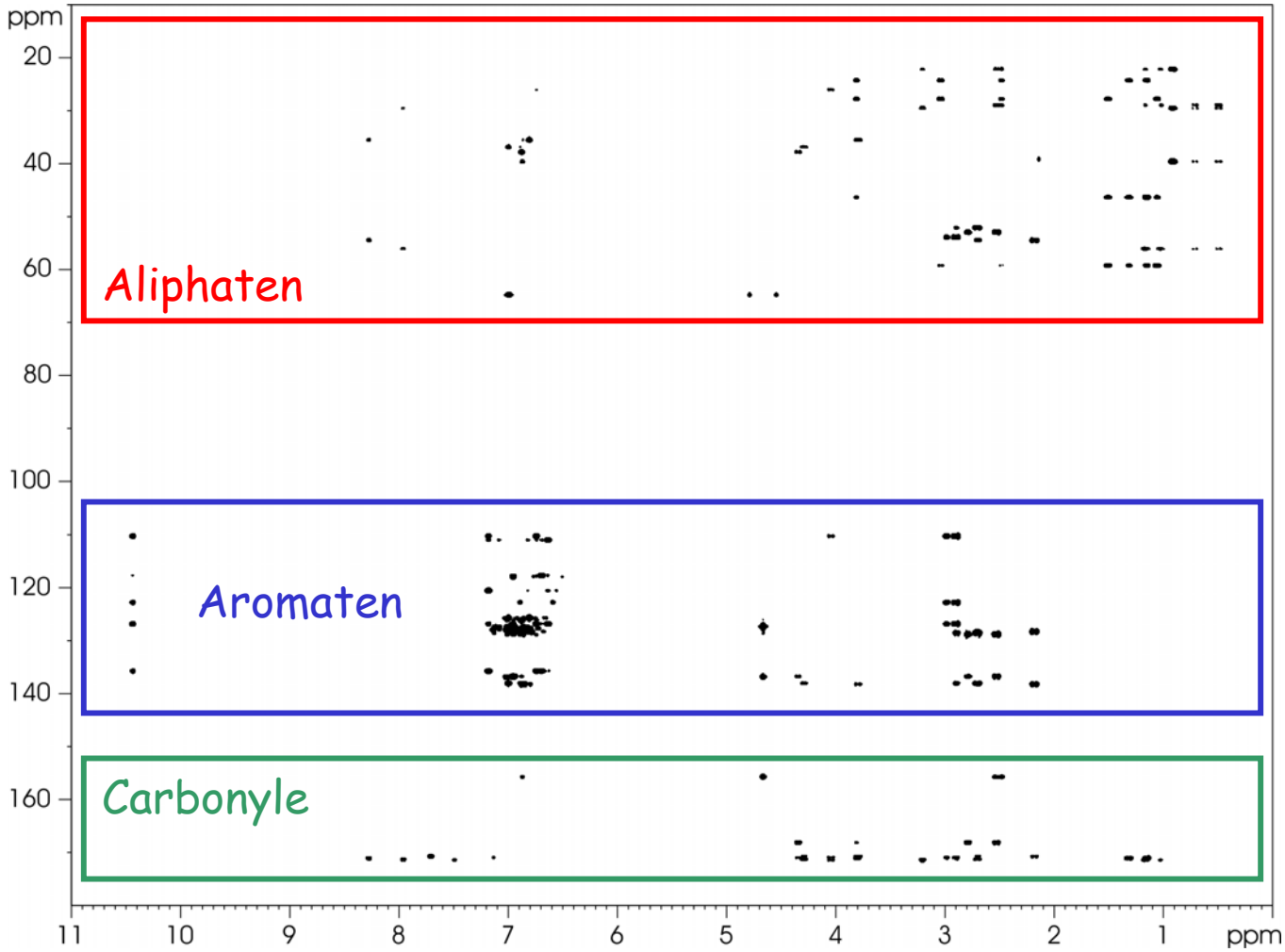
^{13}C-DEPT-HMQC (180) of F3-008



CH_2 can be distiguished from CH and CH_3



Heteronuclear experiments

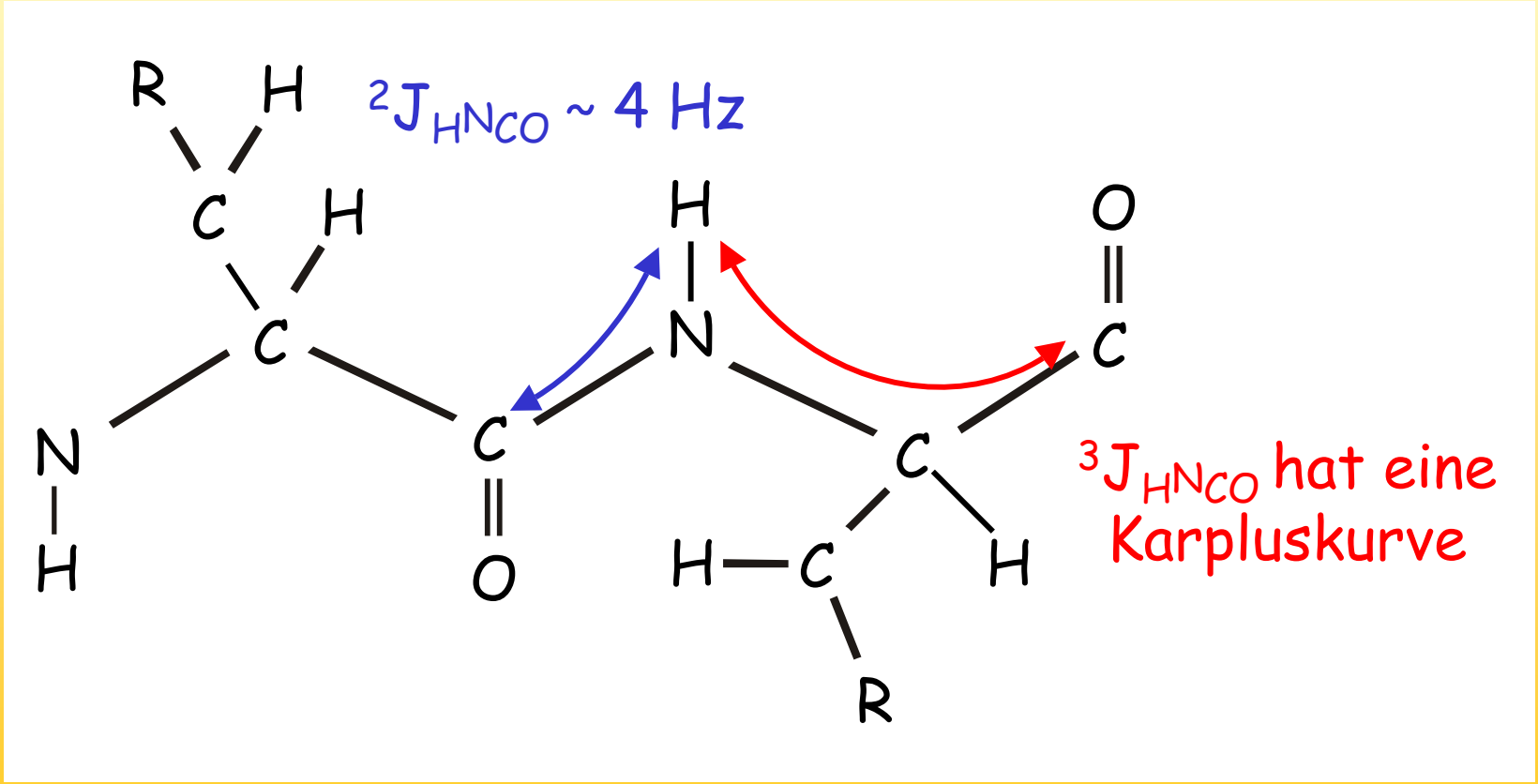


^{13}C- HMBC of F3-008

Sequence specific assignment (3)

Sequence specific assignment (3)

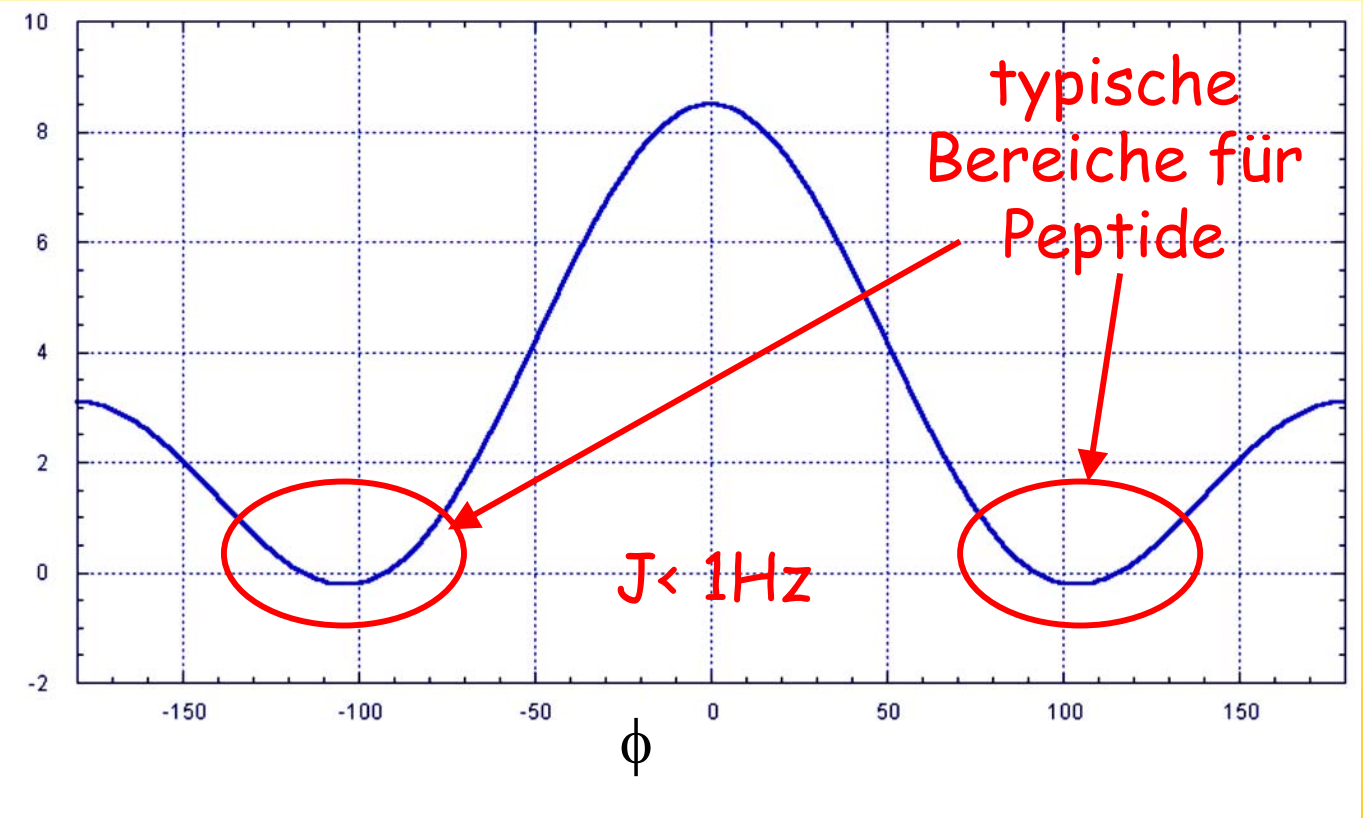
Coupling constants from amino protons to carbonyl carbons



Sequence specific assignment (3)

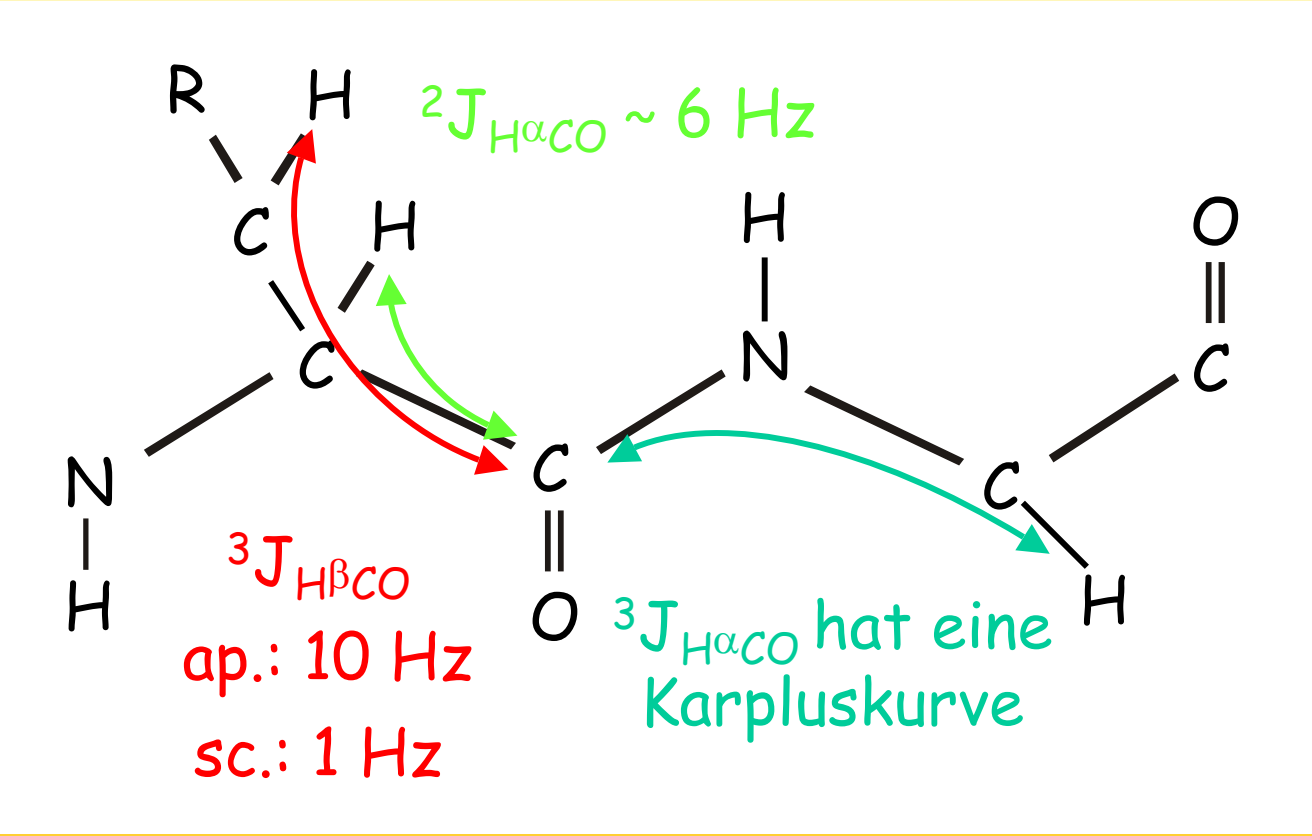
The Karlus-relation for $^3J_{HNCO}$ is

$$^3J_{HNCO} = 5.7 \cos^2(\phi-180) - 2.7 \cos(\phi-180) + 0.1$$



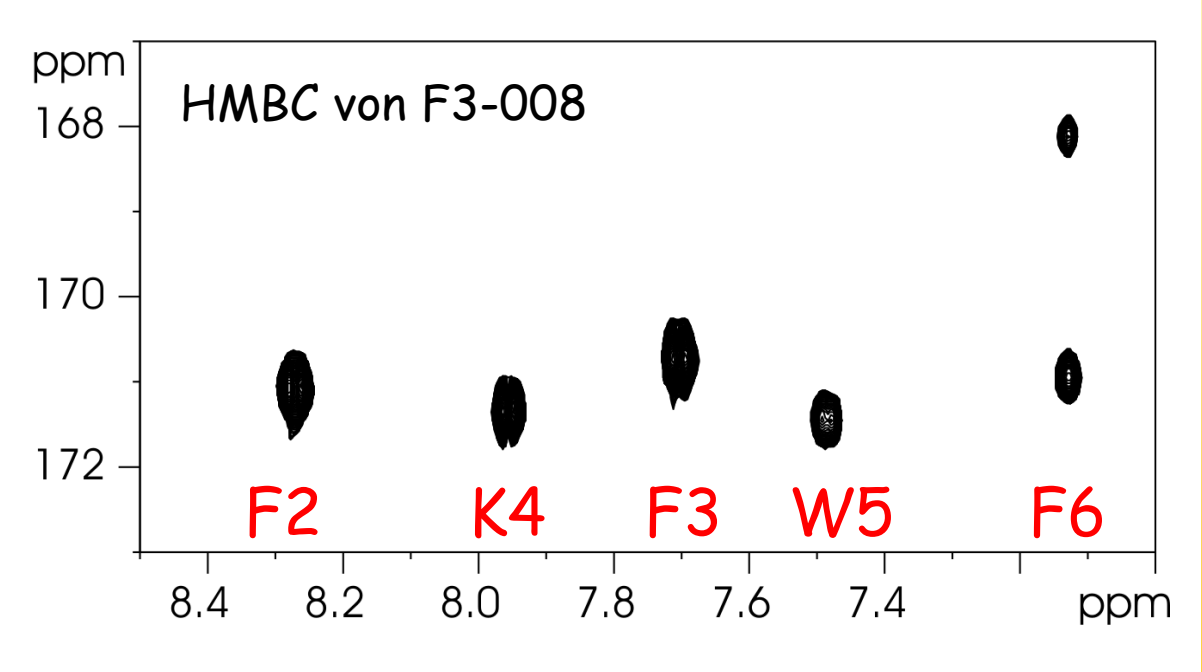
Sequence specific assignment (3)

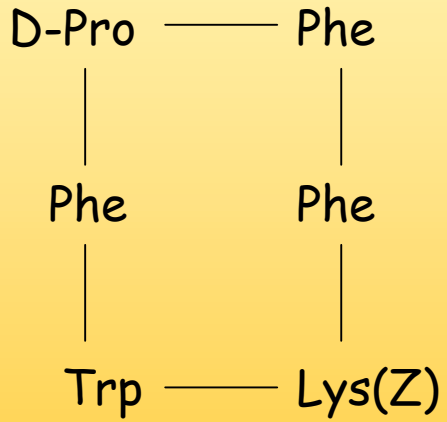
Coupling constants from aliphatic protons to carbonyl carbons



Sequence specific assignment (3)

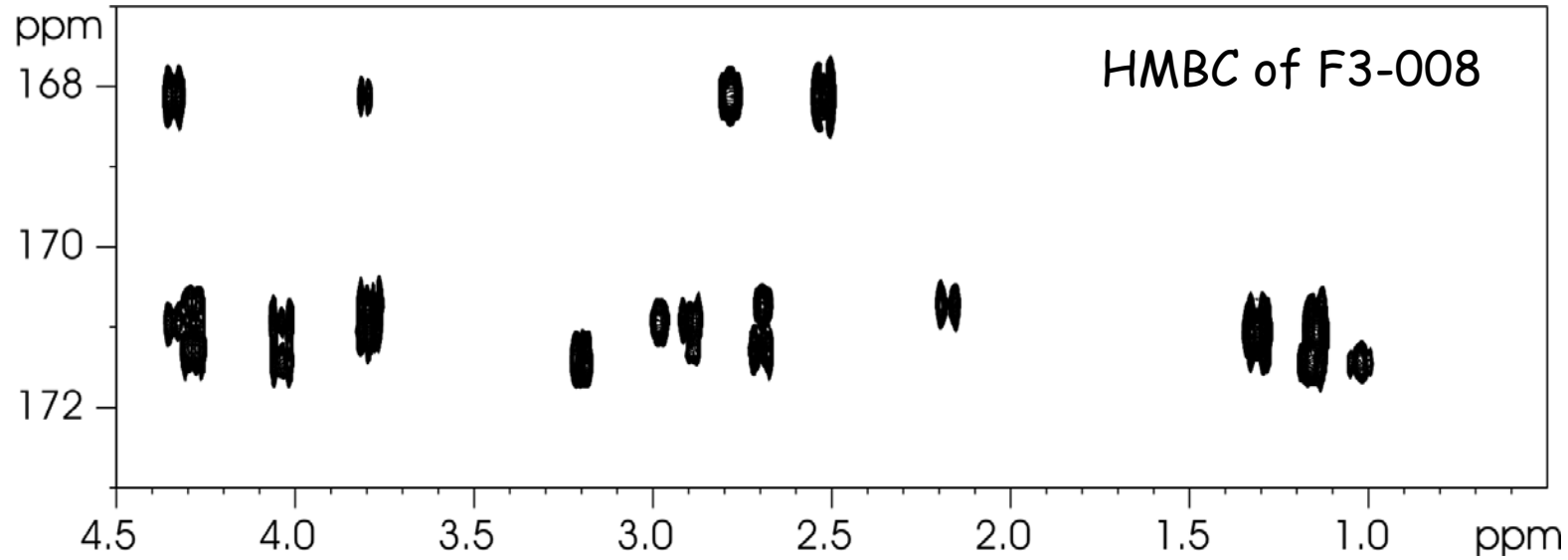
In the area of the amide protons all correlations via ^{2}J are visible, those via ^{3}J only rarely





Sequence specific assignment (3)

In the area of the aliphatic protons most H^{α} show 2 correlations, there are also correlations for the H^{β} protons, all carbonyl carbons can thus be assigned



Sequence specific assignment (3)

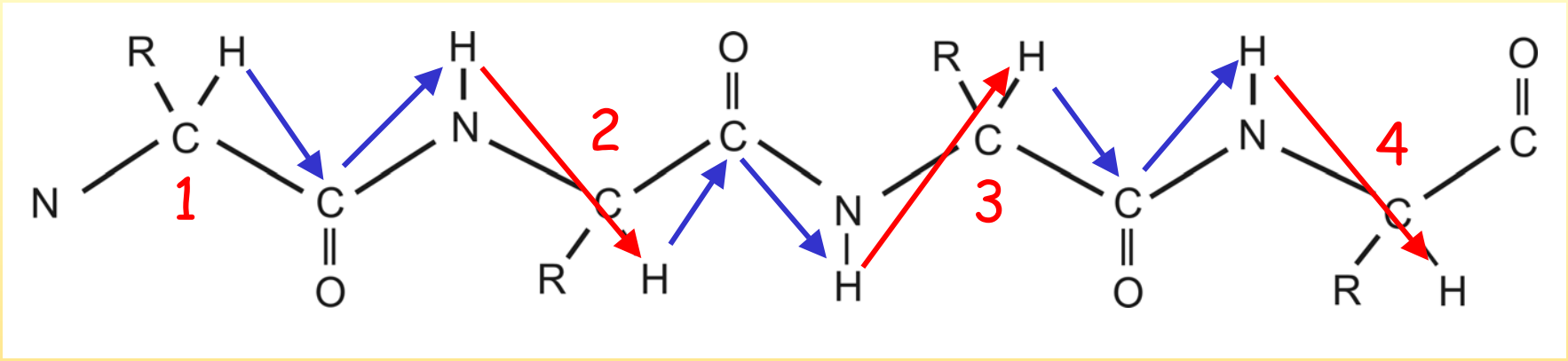
Based on these spectra not only an assignment of the carbonyl resonances is possible but also a sequential assignment.

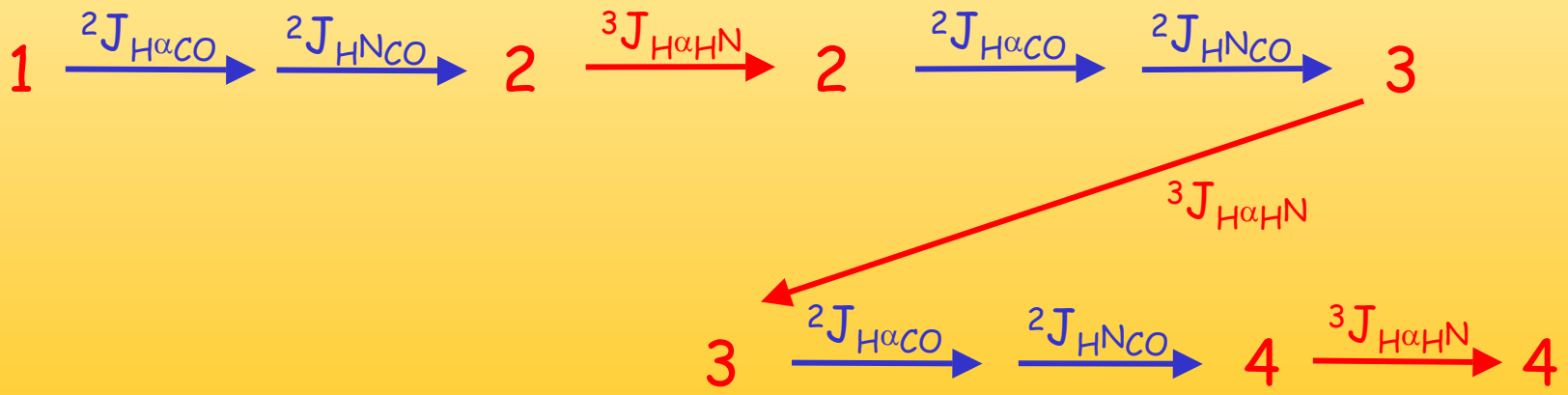
Because of the small coupling between the H^N and the carbonyl of the same amino acid, the DQF-COSY is used instead to get a correlation from the H^N to the H^α.

A sequential assignment would also be possible via the H^α to carbonyl correlation, this is difficult because of overlap in the H^α region

Sequence specific assignment (3)

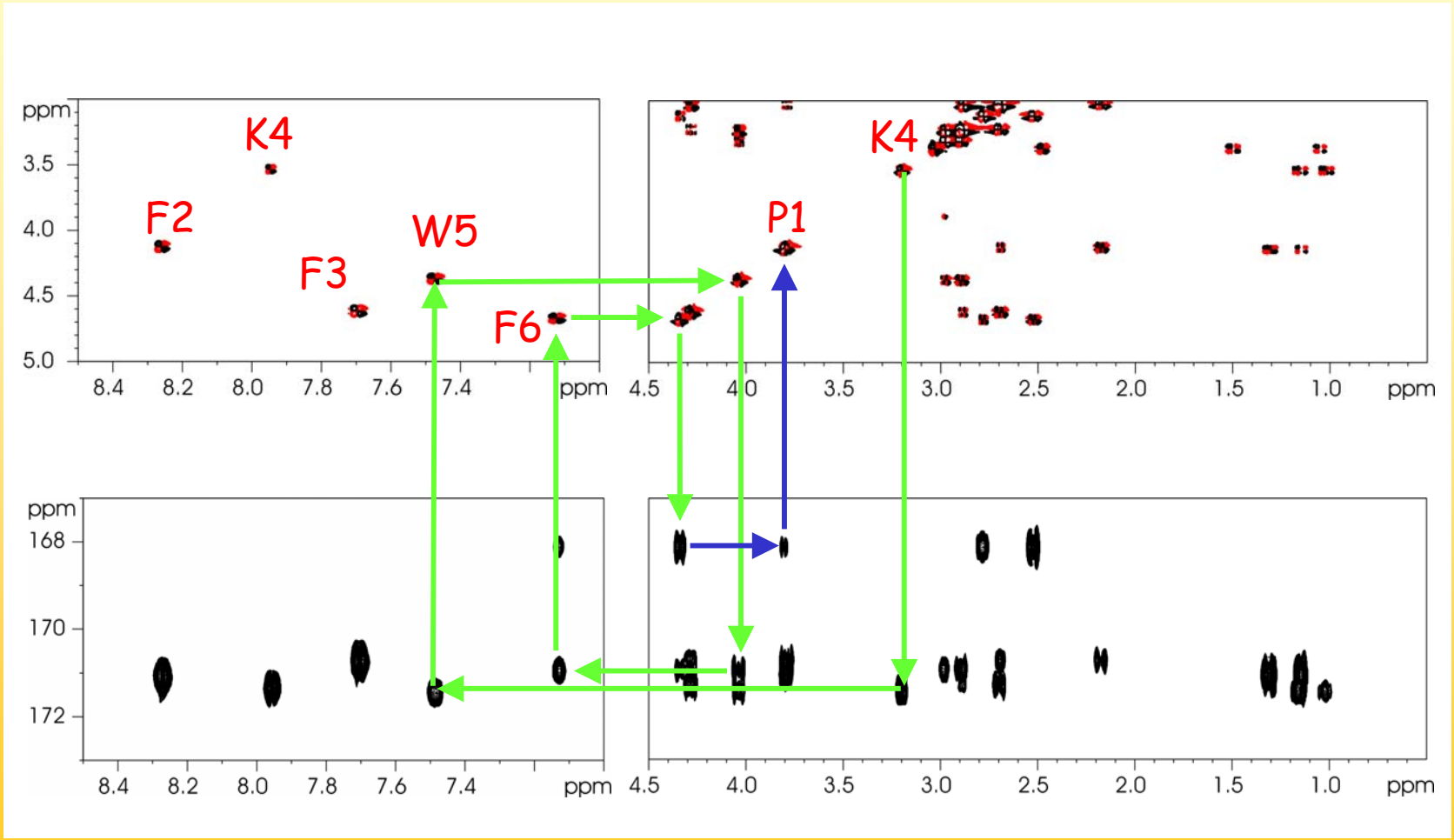
We then have an different „sequential walk"





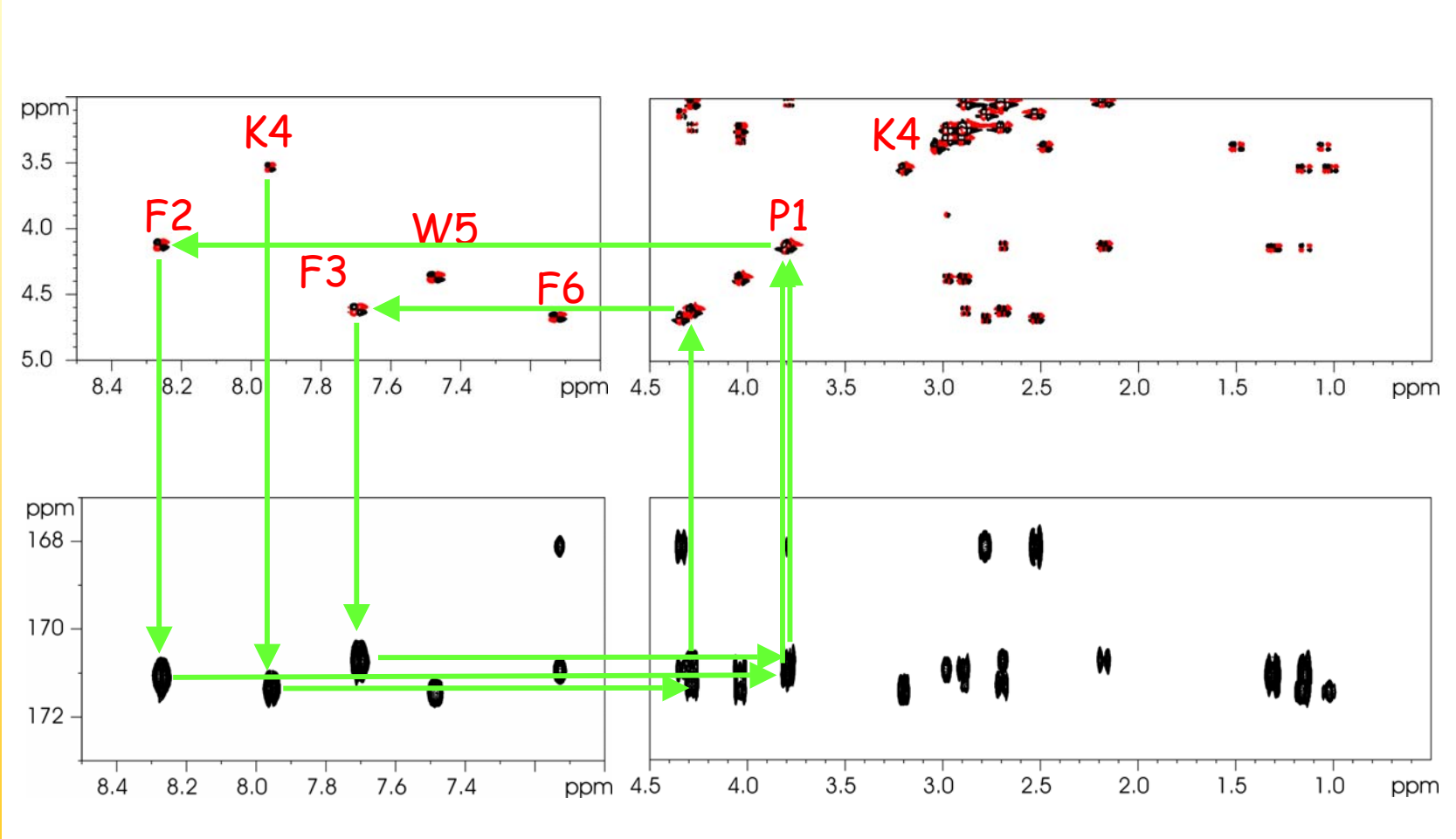
Sequence specific assignment (3)

With real spectra it works like that:



Sequence specific assignment (3)

starting at K4 we go the other way



That's it

www.fmp-berlin.de/schmieder/teaching/selenko_seminars.htm