# NMR course at the FMP:

# Basic concepts

# organics

26.01.2009

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AG Solution NMR

# The program

General aspects

Basic principles

Parameters in NMR spectroscopy

Multidimensional NMR-spectroscopy

Two examples

# General aspects of NMR-spectroscopy

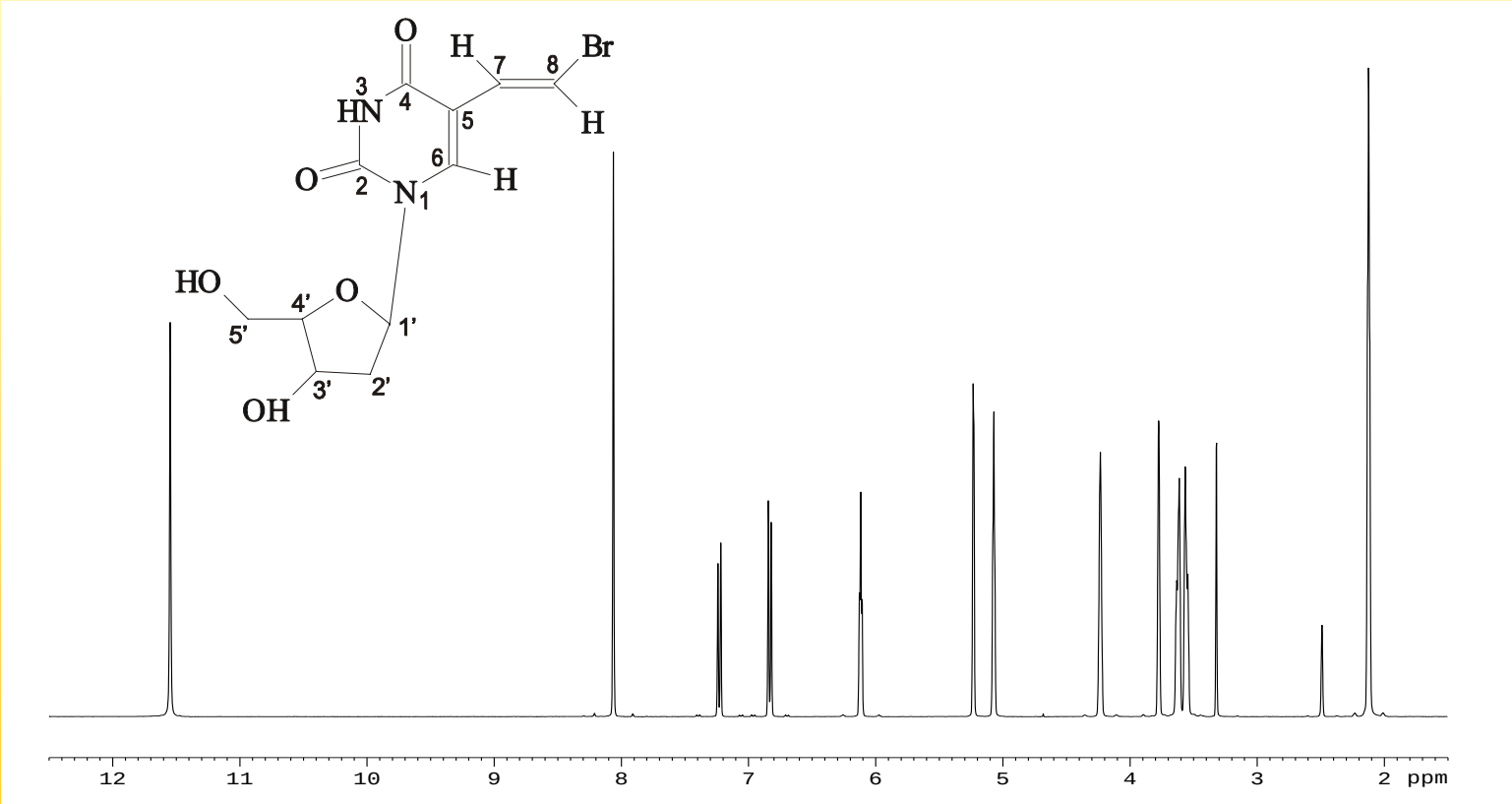
## General aspects of NMR spectroscopy

# Nuclear Magnetic Resonance

NMR-spectroscopy observes the resonance interaction of atomic nuclei with electromagnetic waves. The effect is only detectable in a strong magnetic field. Every atomic nucleus is observed separately and in addition interactions between nuclei can be visualized. NMR therefore corresponds well to the chemists view of a molecule as atoms connected by bonds.

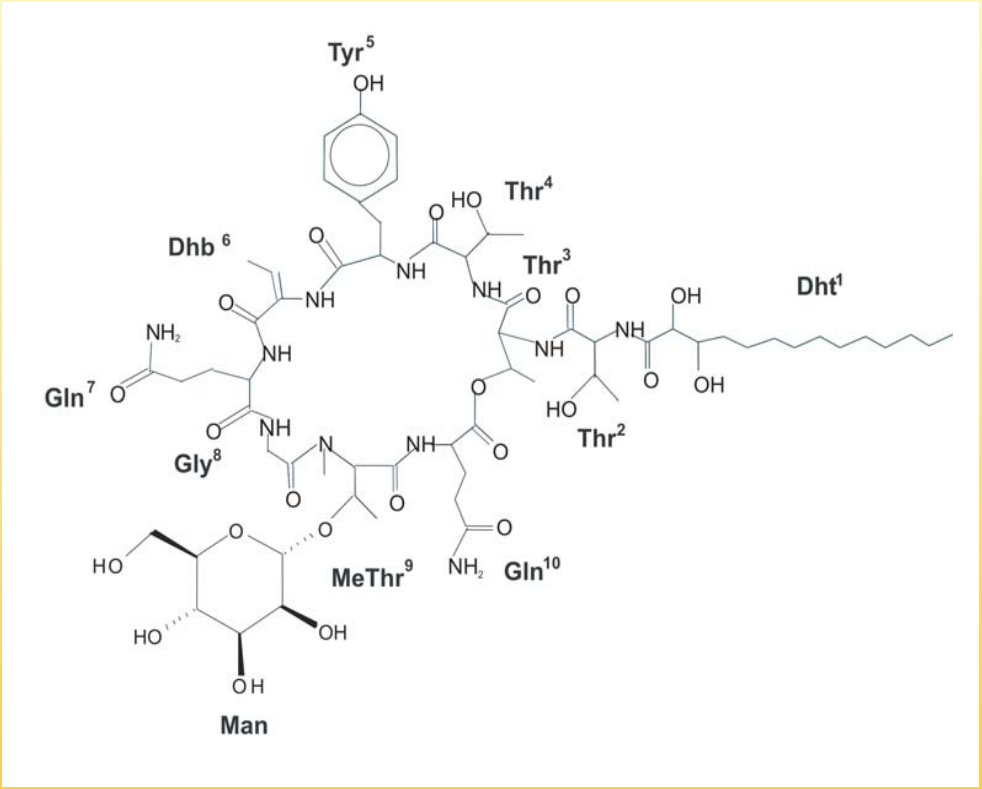
# General aspects of NMR spectroscopy

## Analytical method accompanying synthetic work



# General aspects of NMR spectroscopy

## Structure elucidation of natural compounds

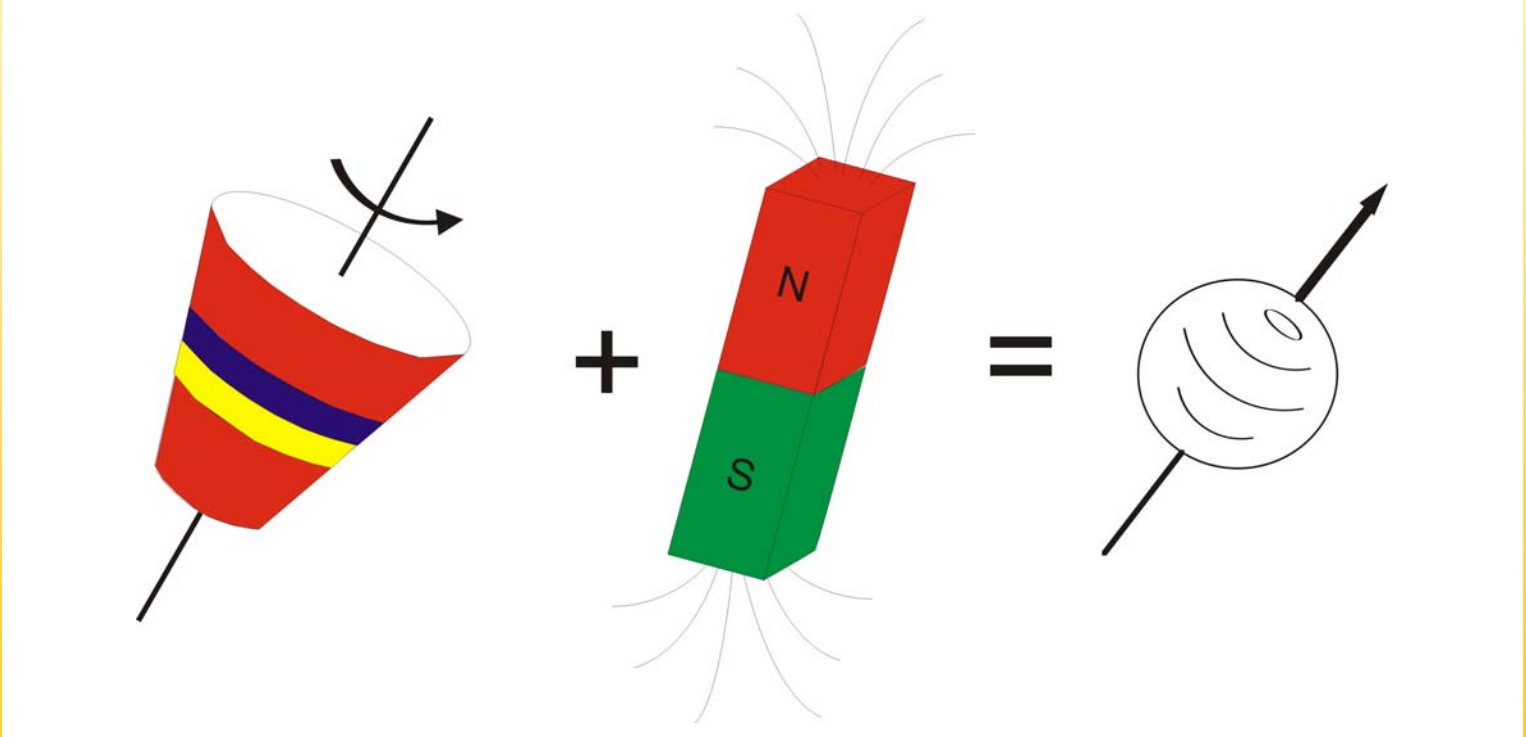



NMR is very powerful in the determination of the constitution of natural products

# Basic principles of NMR-spectroscopy

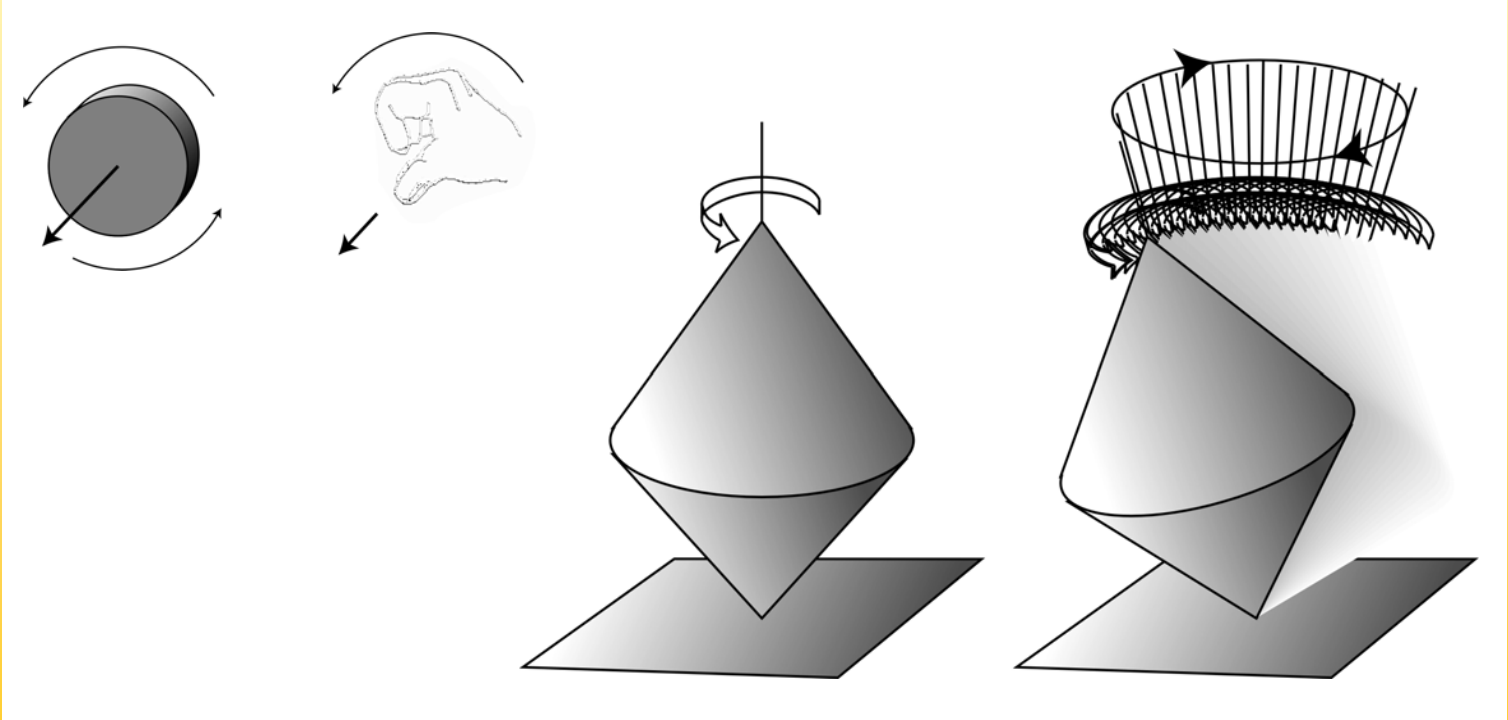
# Basic principles of NMR-spectroscopy

Basis of the effect of nuclear magnetic resonance is the nuclear spin, that can be imagined as a mixture of gyroscope and magnetic needle



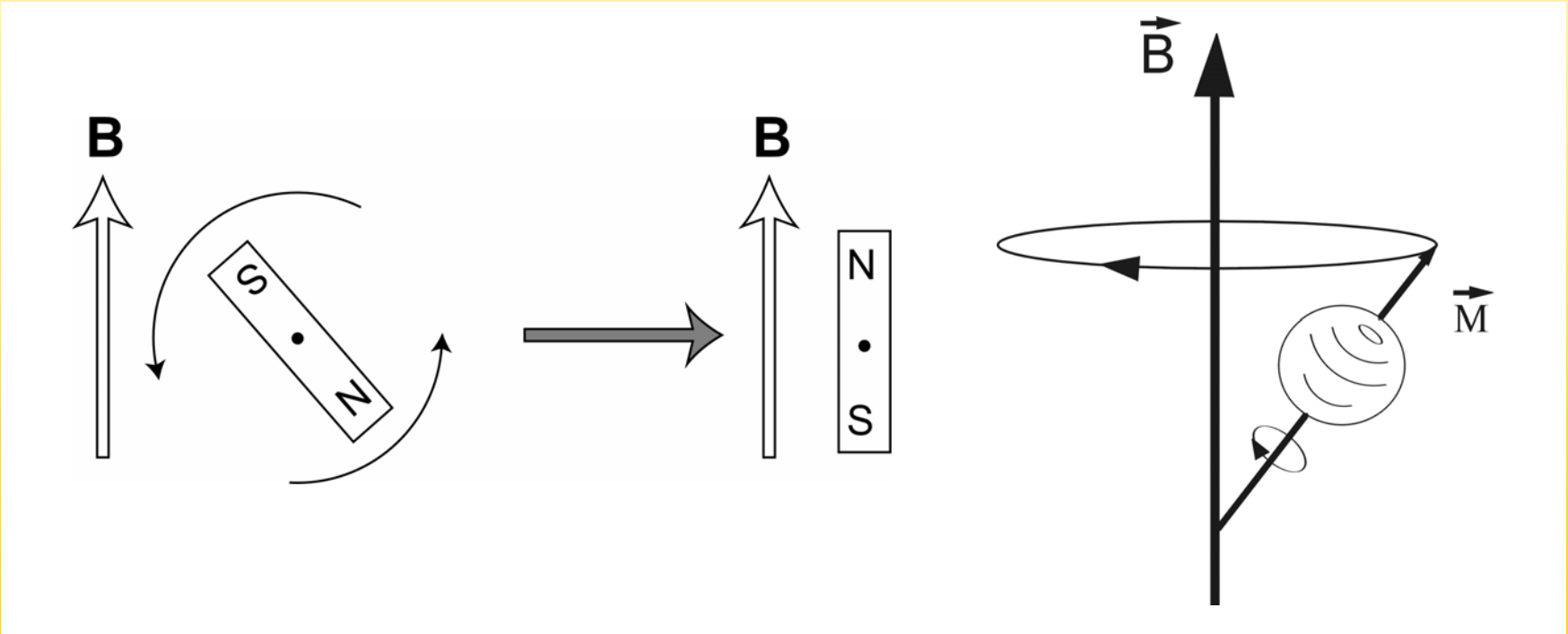
# Basic principles of NMR-spectroscopy

A gyroscope has an angular momentum whose axis is stable in three-dimensional space



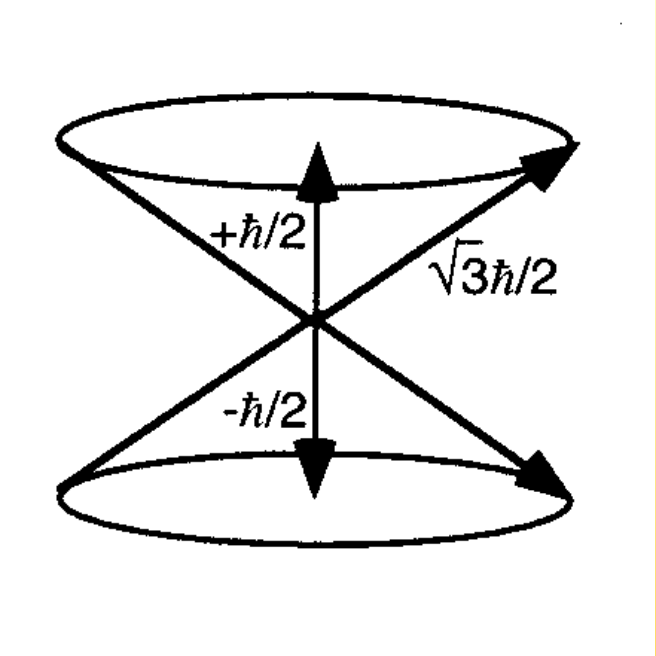
# Basic principles of NMR-spectroscopy

An alignment of the "magnetic needle" with an external magnetic field is prevented by the properties of a gyroscope, a precession begins



# Basic principles of NMR-spectroscopy

In case of the nuclear spin we have a „quantum mechanic gyroscope" and not all orientations of the angular momentum are allowed, in case of high resolution NMR there are only two, named $\alpha$ and $\beta$ state.

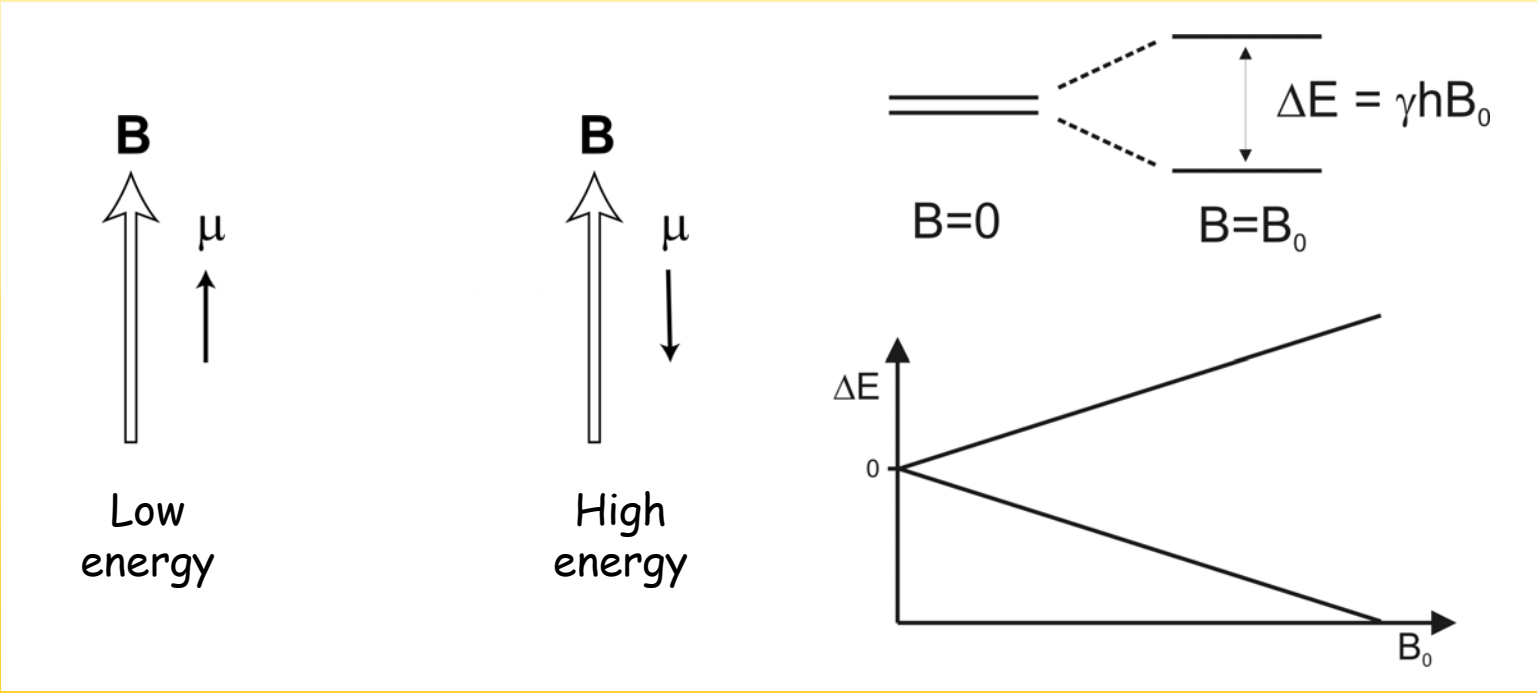



Symbolic representation of the two states of the spin

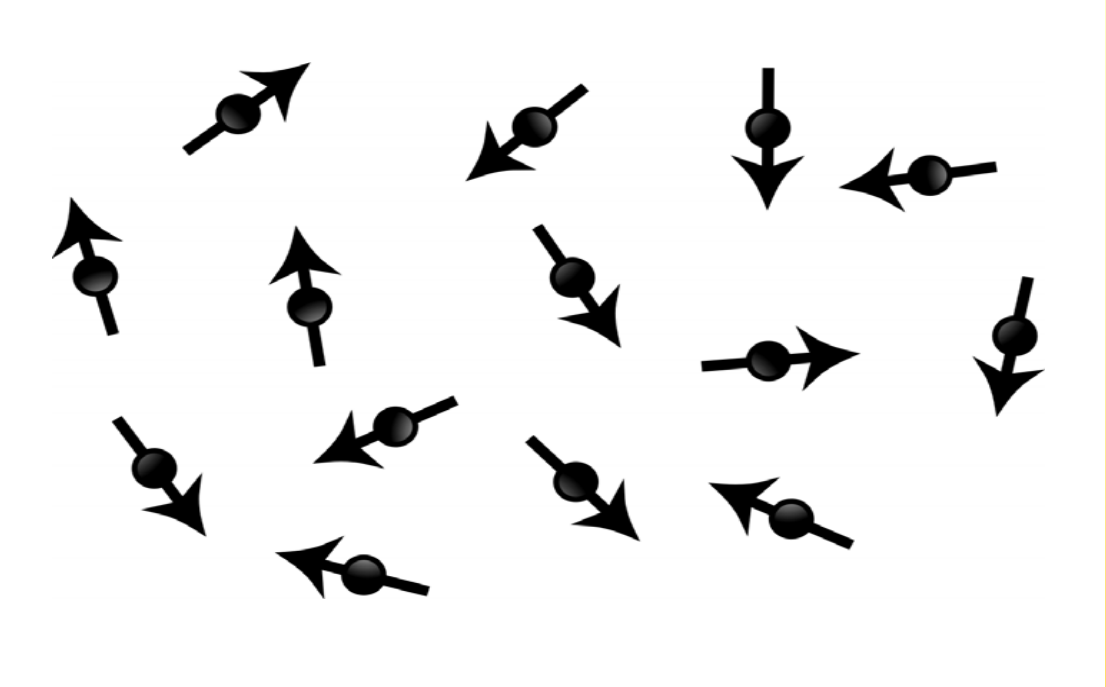
# Basic principles of NMR-spectroscopy

The energy of the two possible states is not equal:

$$E = -\mu \mathbf{B} \qquad \Delta E = \hbar \gamma B_0$$

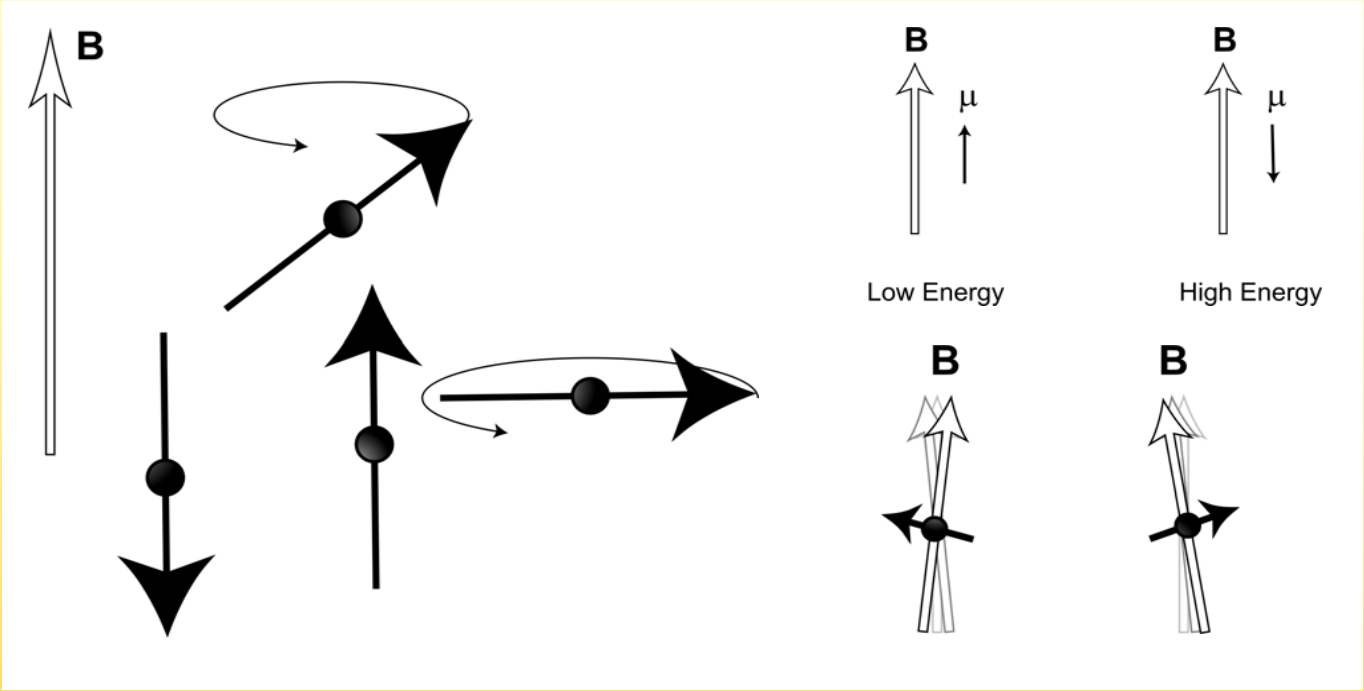


# Basic principles of NMR-spectroscopy



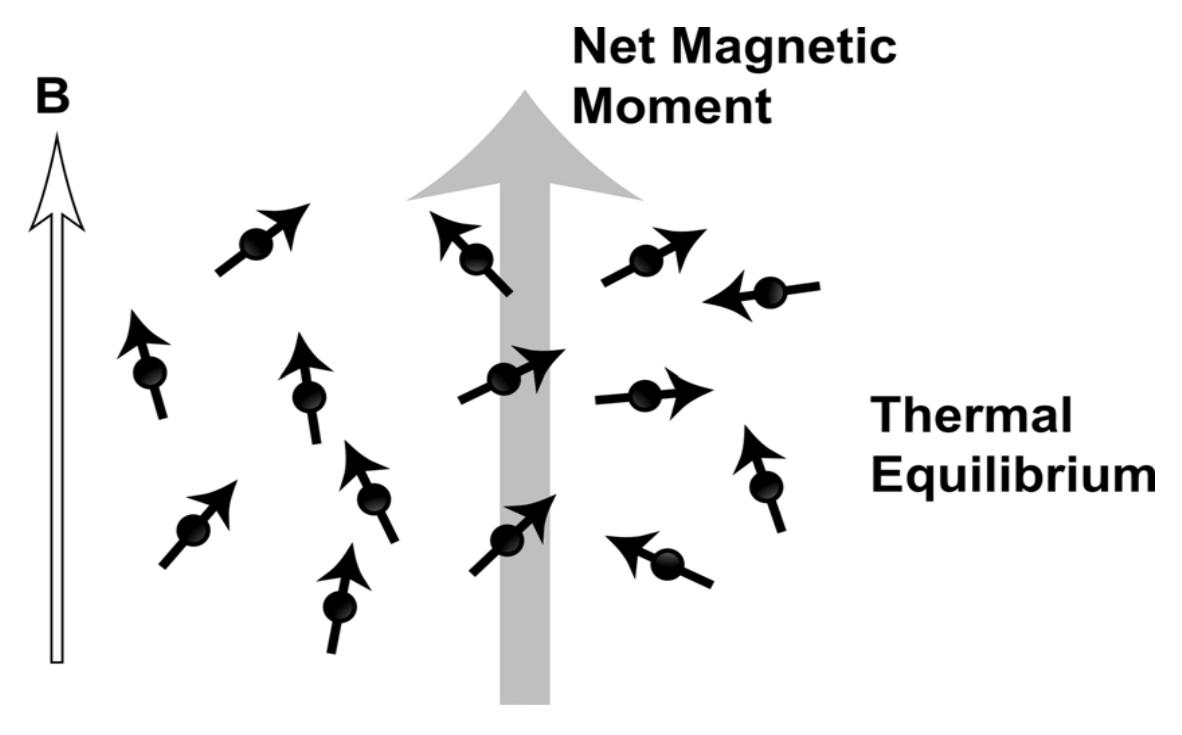
Without an external magnetic field the spins are dustributed in all directions

# Basic principles of NMR-spectroscopy




When a magnetic field is present this leads to the build-up of a magnetic moment due to an orientation of the spin with a preferred direction

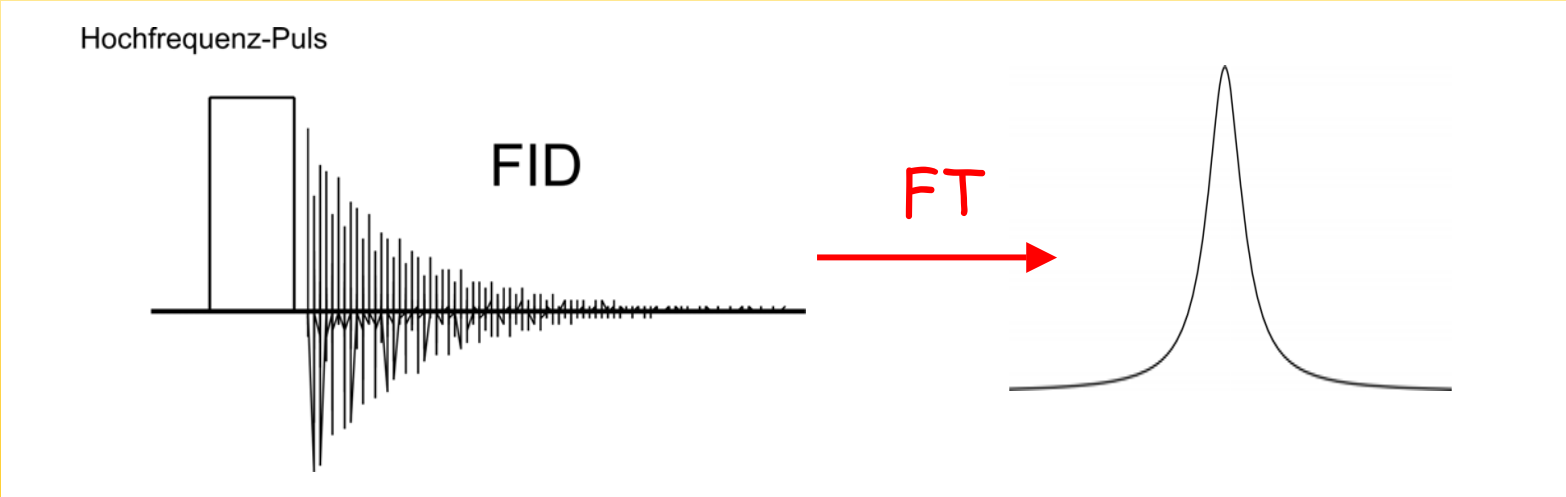
# Basic principles of NMR-spectroscopy



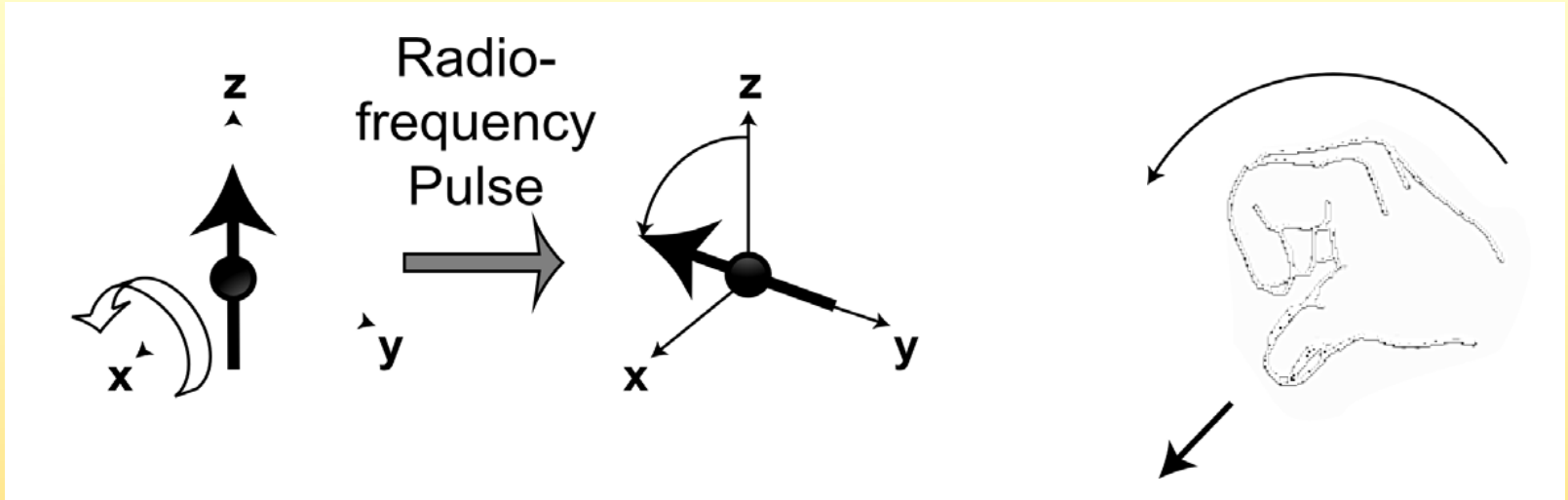

The result is a Bolzman distribution and a net magentic moment at thermal equilibrium, that, however, does not give rise to a detectable signal

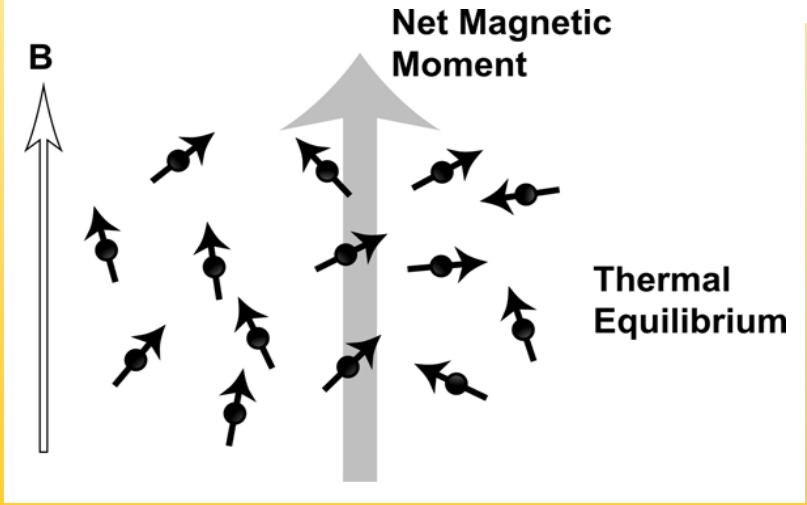
# Basic principles of NMR-spectroscopy

Modern NMR experiments all use the „FT-principle" and are based on the concept of „pulsed NMR". The reason is the ease with that signal-to-noise can be improved and the possibility of multidimensional NMR.



# Basic principles of NMR-spectroscopy

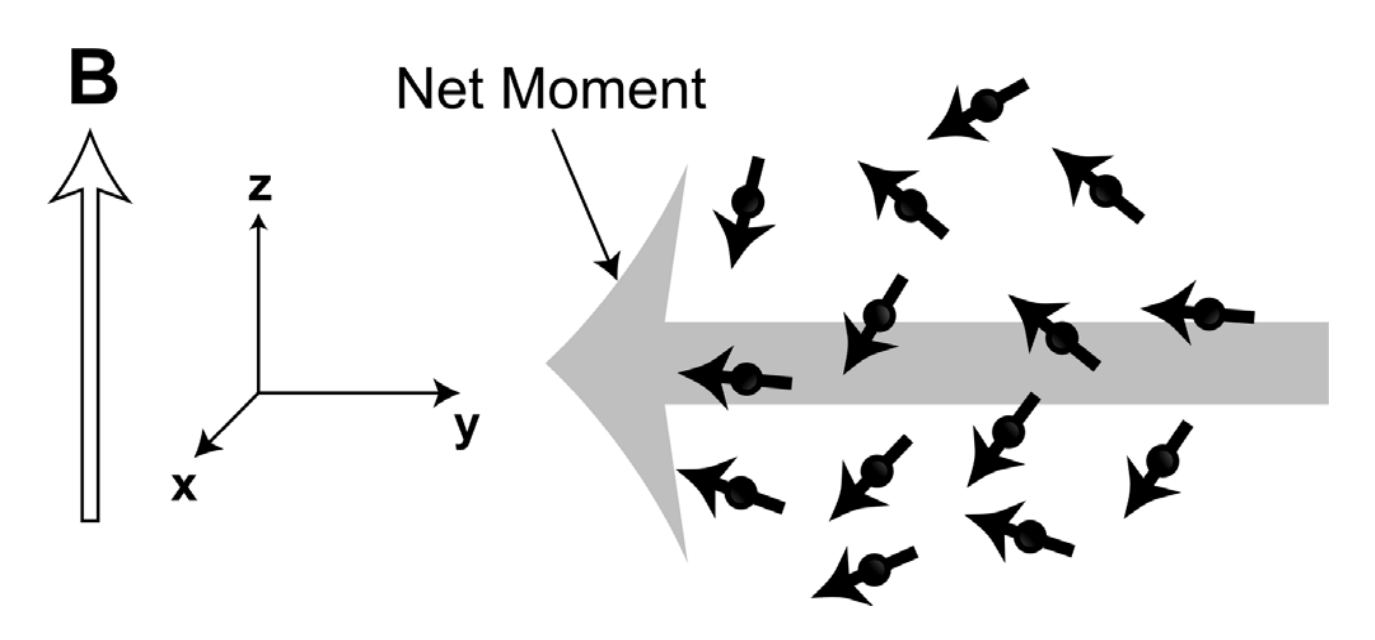


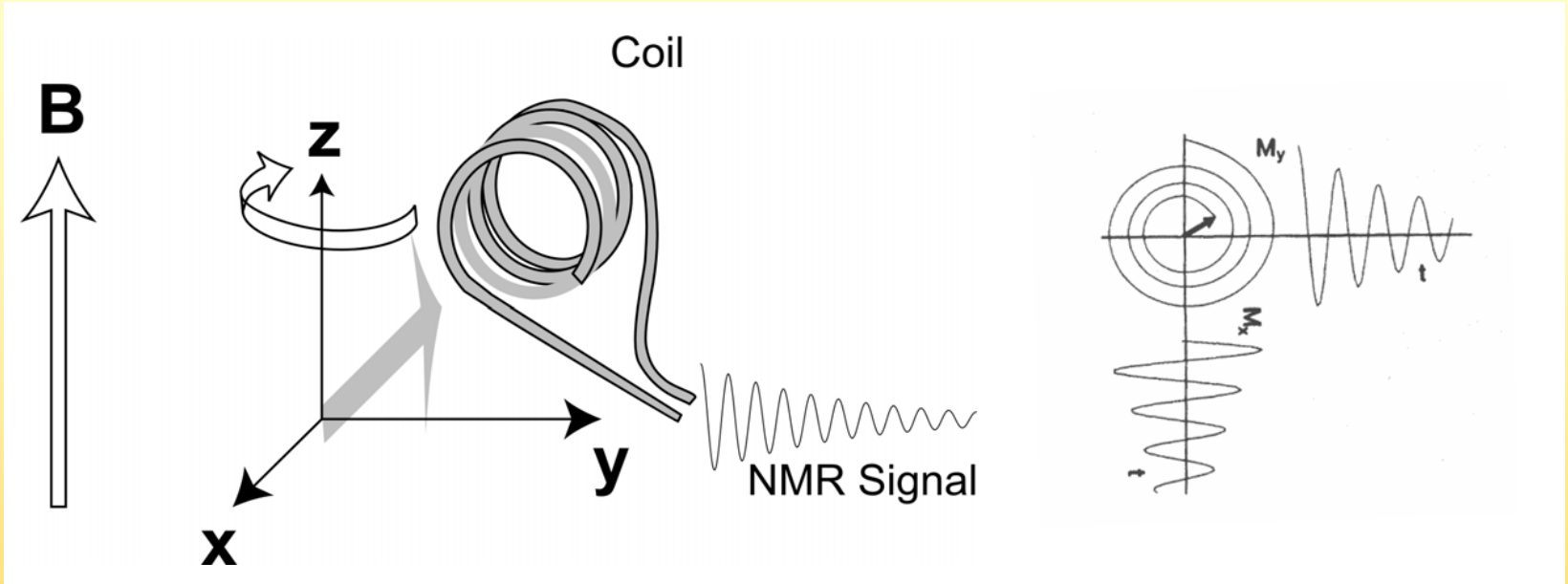

The puls rotatestes every spin and thus the net magnetic moment into the x,y-plane

# Basic principles of NMR-spectroscopy

The resulting magnetic moment points into the x,y direction, no z-magnetisation is present.



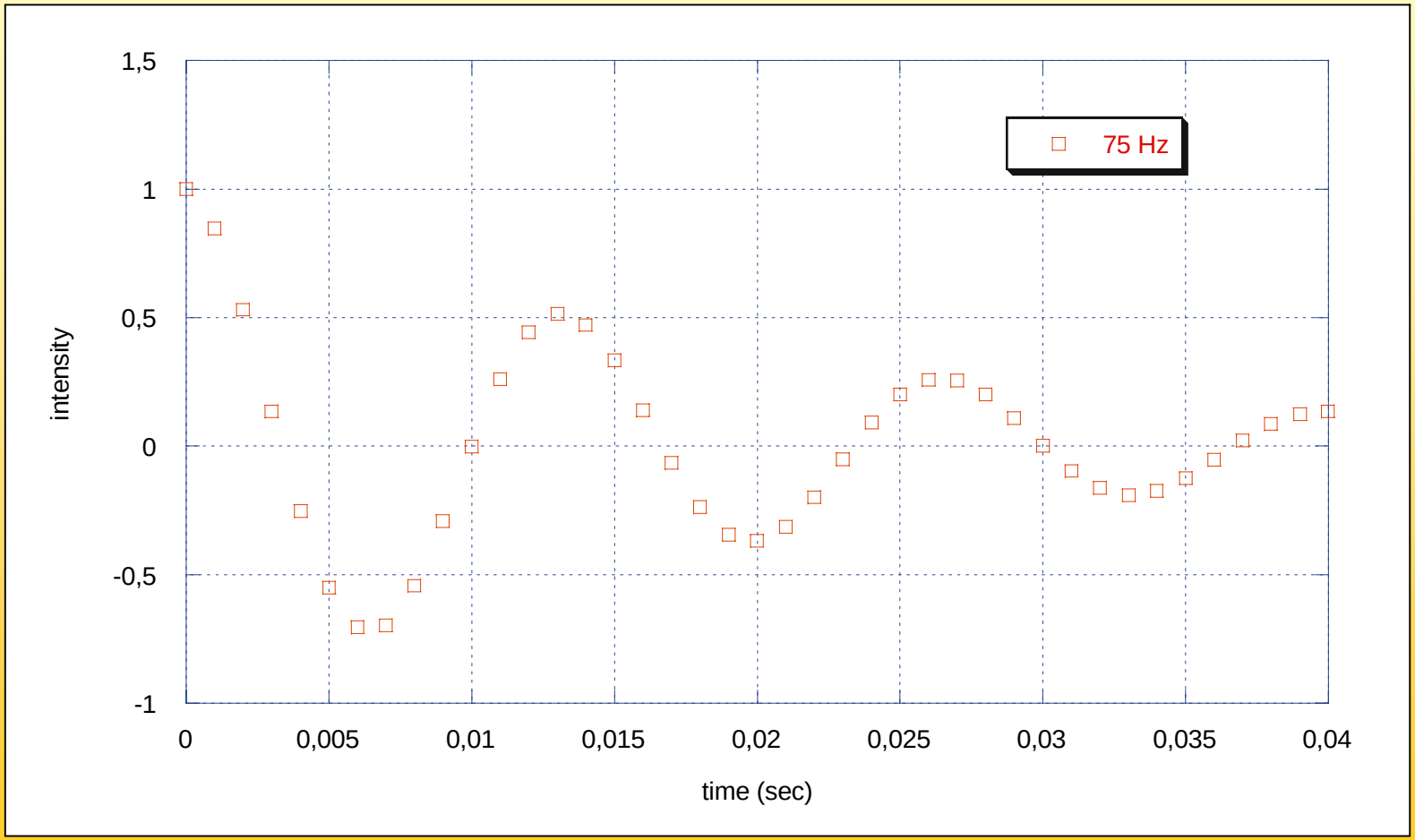
# Basic principles of NMR-spectroscopy




After the pulse has stopped the magnetic moment takes up its former precessional motion and induces a current in the detection coil: the NMR signal !

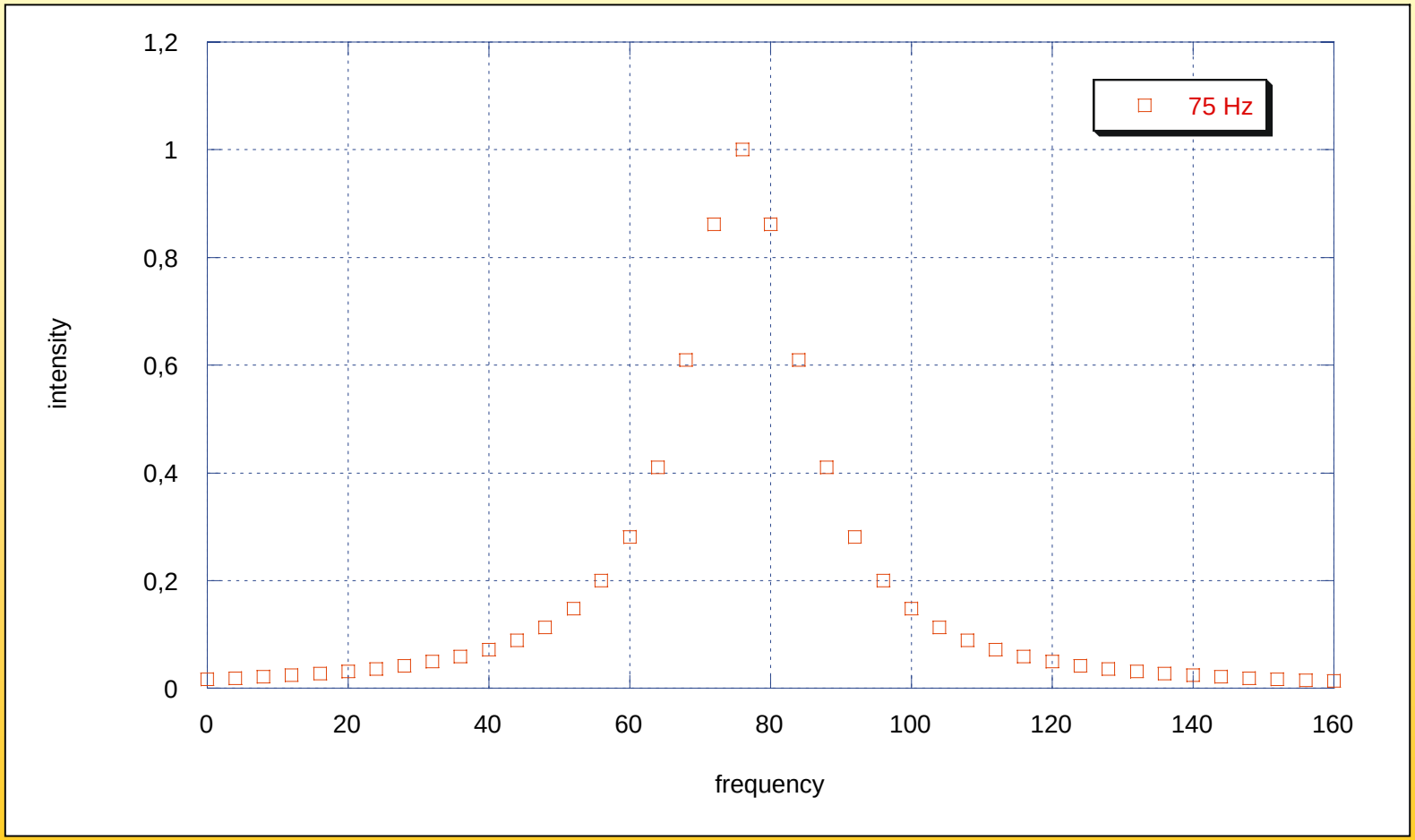
# Basic principles of NMR-spectroscopy

## After digitisation an „FID" is obtained…



# Basic principles of NMR-spectroscopy

## ....that is converted into a spectrum by an FT

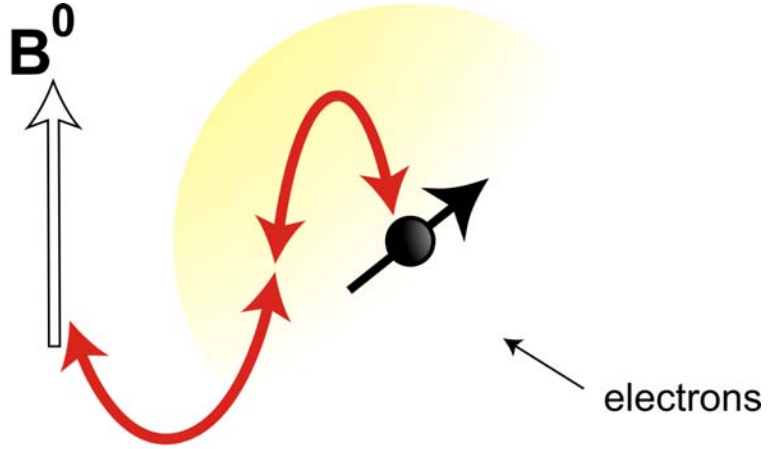


# Parameters in NMR-spectroscopy

# Parameters in NMR-spectroscopy

## Chemical shift

Electrons around the nucleus shield it from the external magnetic field, the more electrons the weaker the field




$$B_{eff} = (1 - \sigma)\, B_0$$

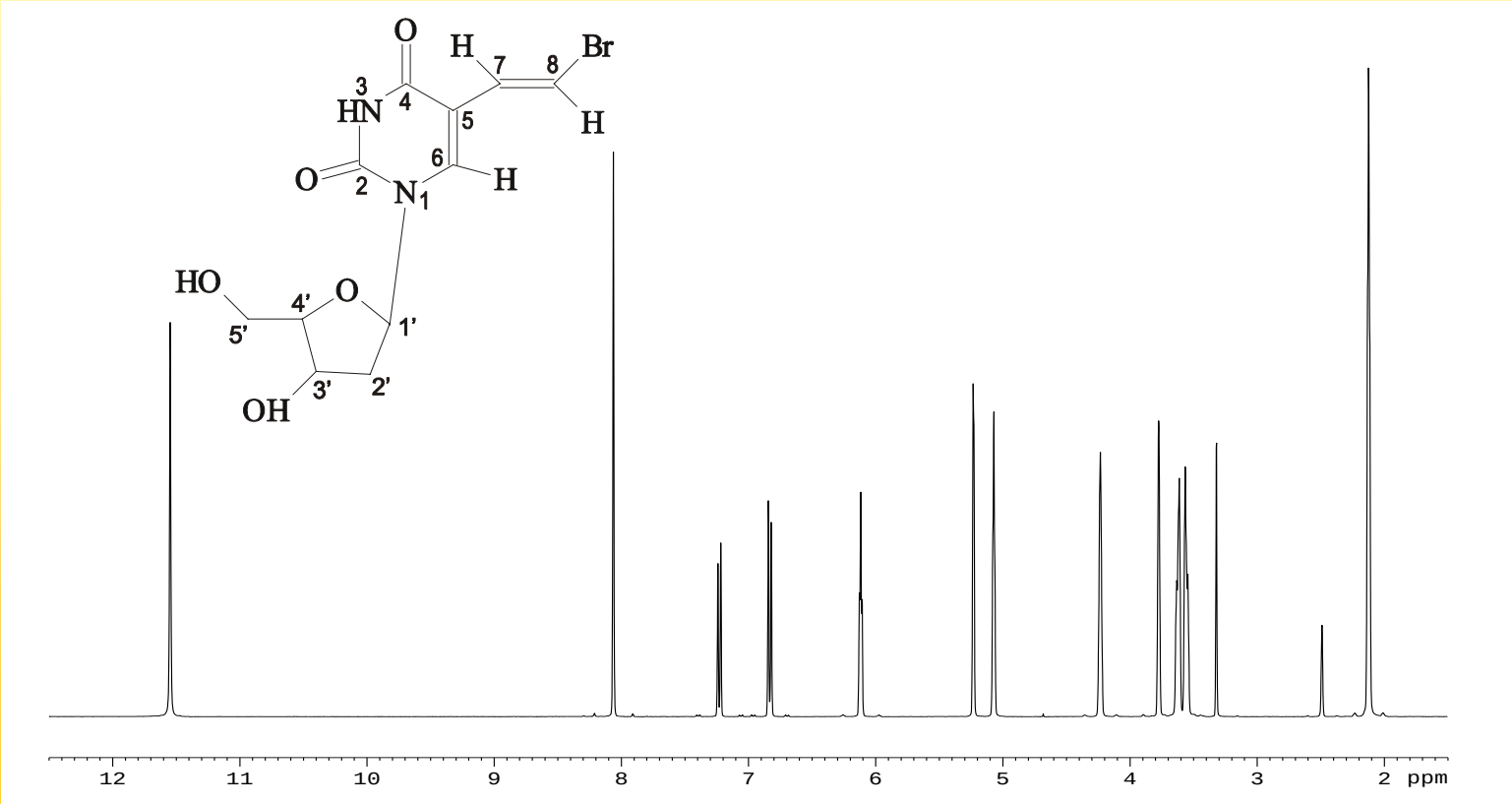
$$\omega = \gamma\, (1 - \sigma)\, B_0$$

$$\delta = (\omega - \omega_{ref}) / \omega_0 \times 10^6$$

$$= (\sigma_{ref} - \sigma) \times 10^6$$

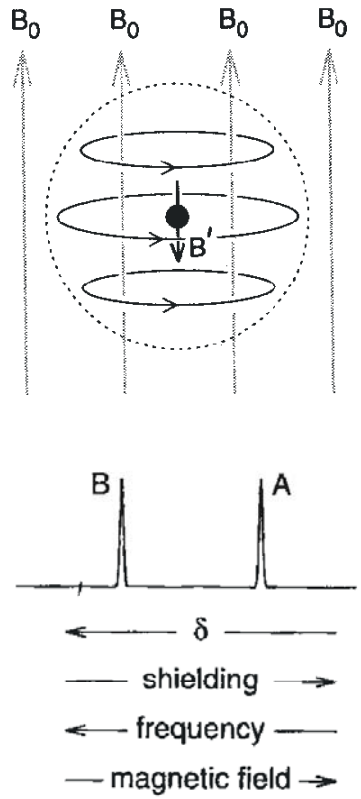
# Parameters in NMR-spectroscopy

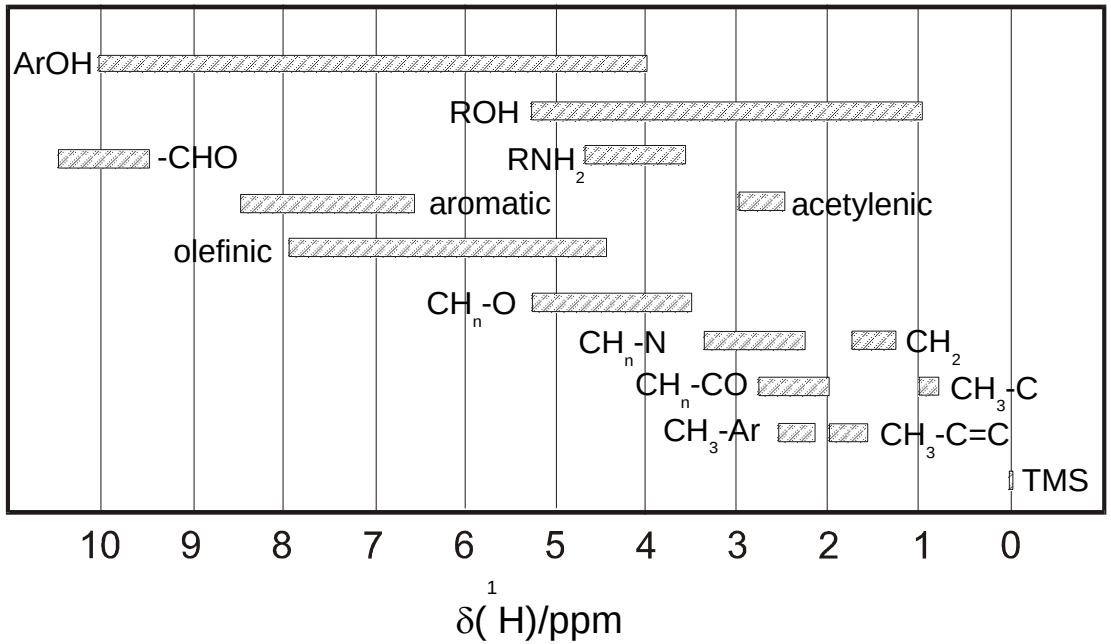
Each atom in the molecule gives rise to a resonance line



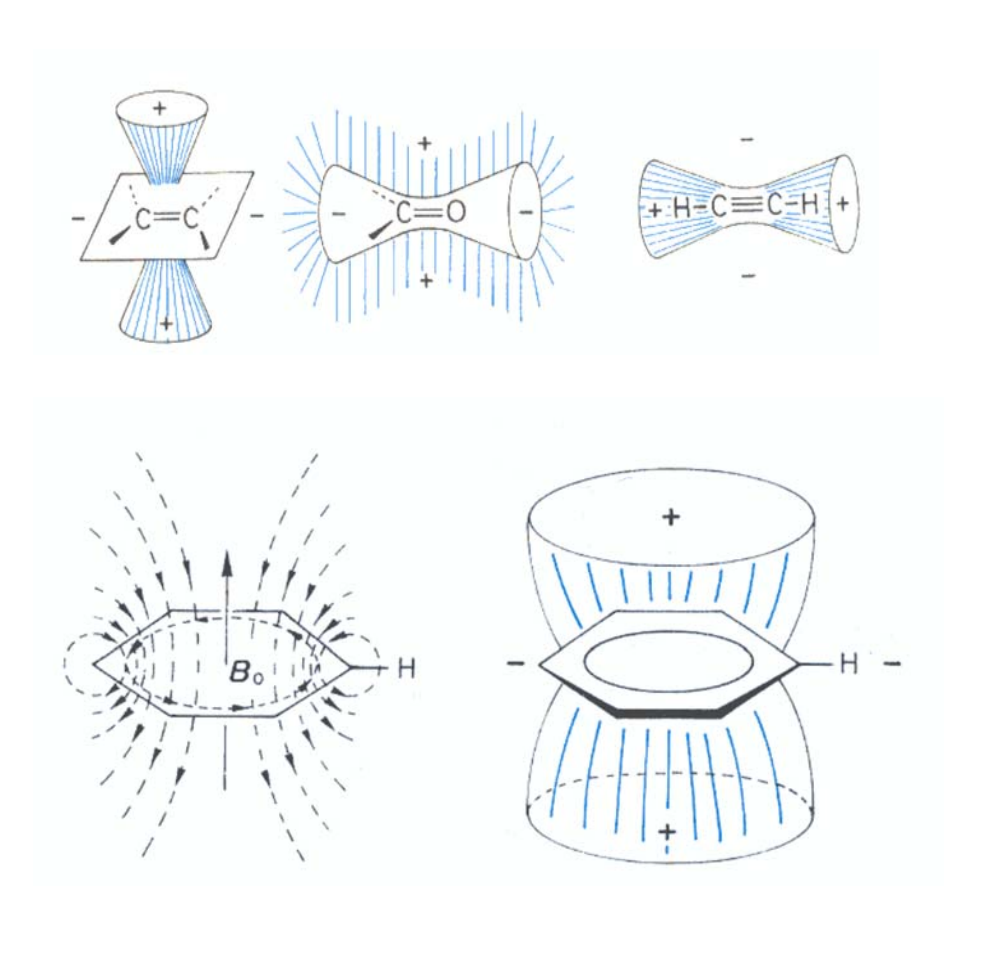
# Parameters in NMR-spectroscopy

## The chemical shift depends on the chemical environment



# Parameters in NMR-spectroscopy

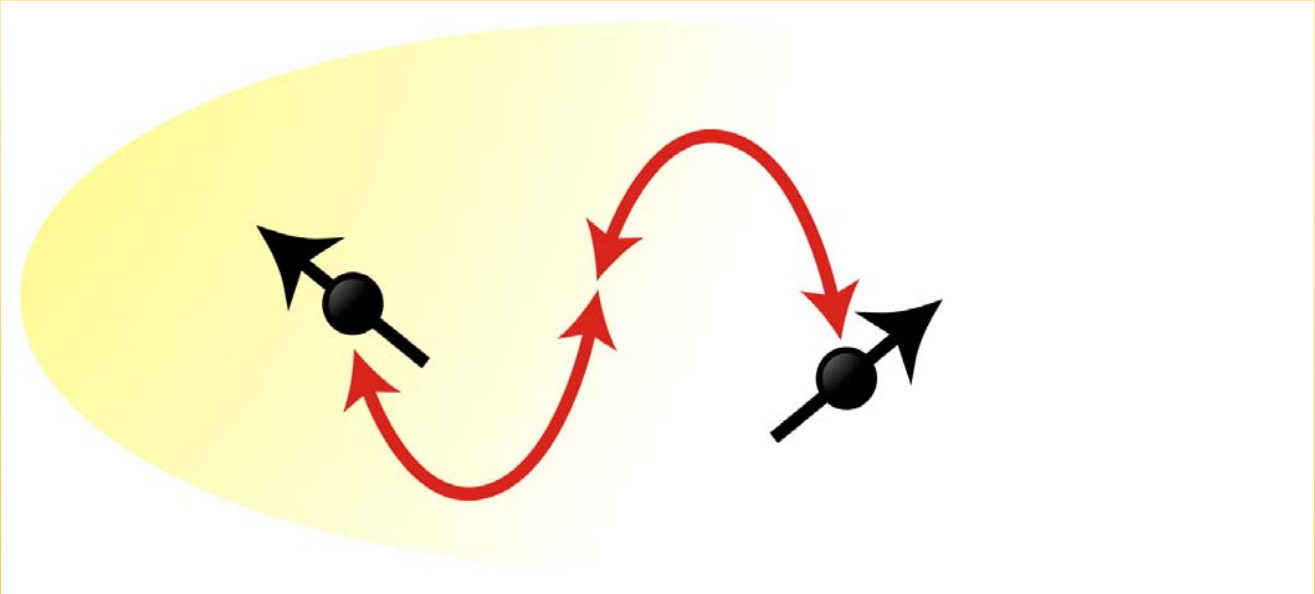



An important factor influencing the chemical shift are anisotropy effects, that are created by small additional fields

# Parameters in NMR-spectroscopy

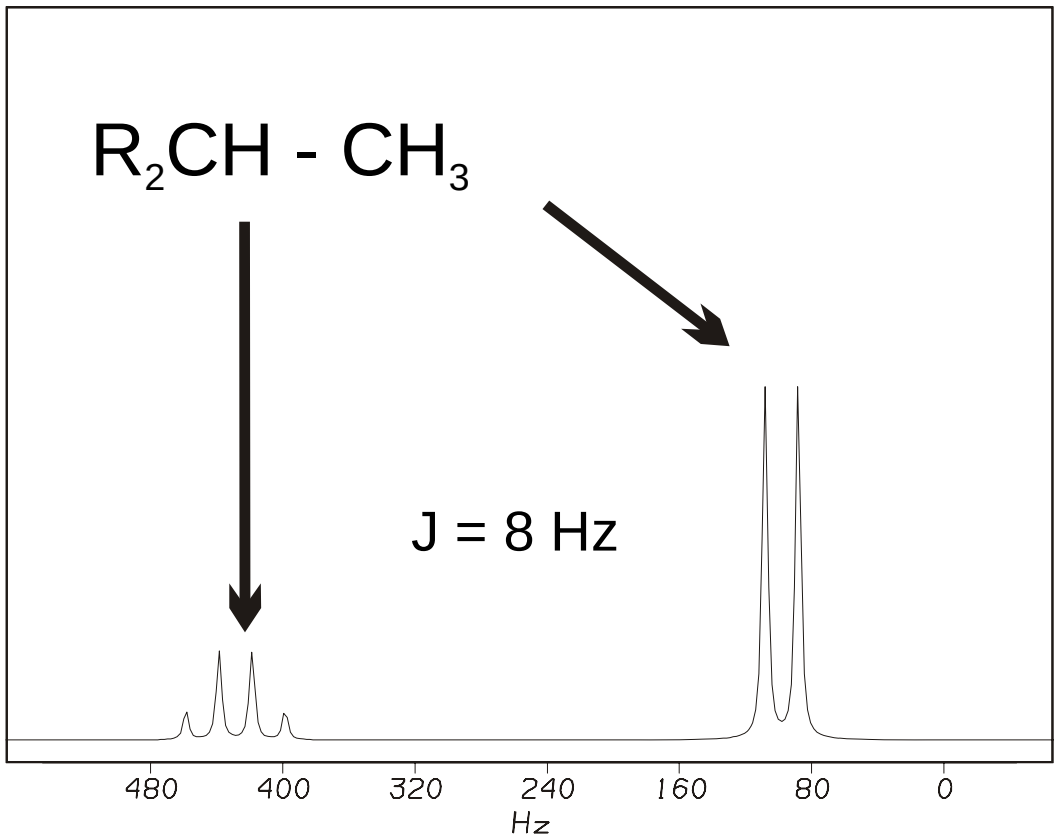
## Scalar or J-coupling

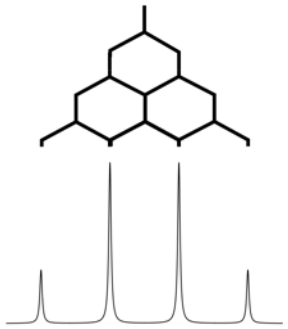
Electrons in the bonds between the nuclei mediate an interaction, the scalar coupling

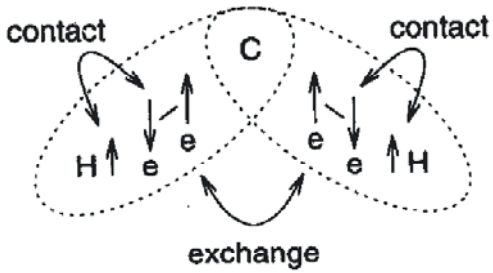


# Parameters in NMR-spectroscopy

## Scalar coupling splits the signals according to the number of neighboring nuclei

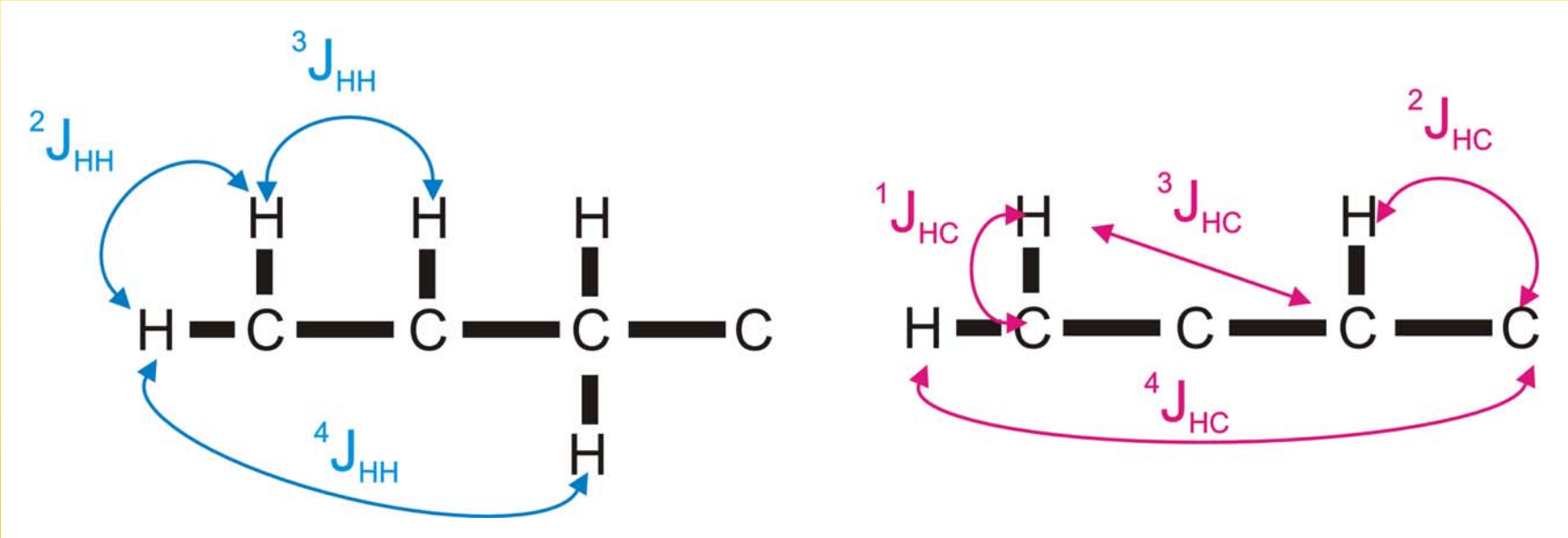




# Parameters in NMR-spectroscopy

Coupling constants can either be homonuclear (between like nuclei) or heteronuclear (between different nuclei) and can either be direct (one-bond) or long-range (multiple bonds)



# Parameters in NMR-spectroscopy

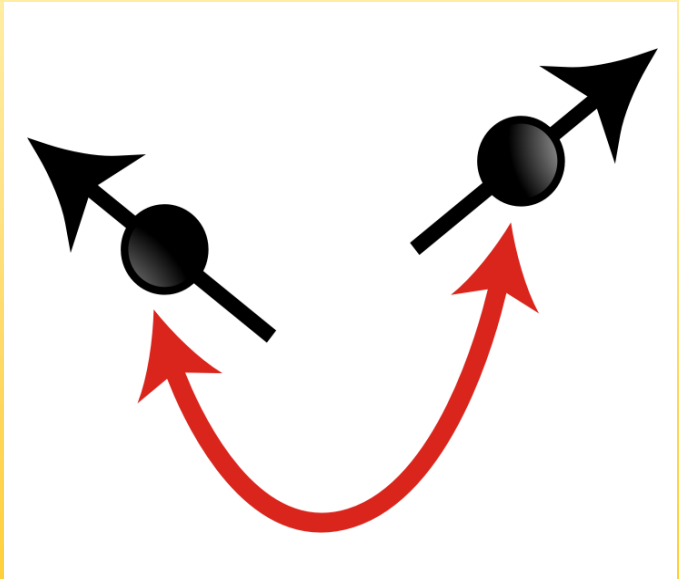
Direct couplings are usually one order of magnitude larger than the so-called long-range couplings

| | | |
|---|---|---|
| $^{1}J_{HH}$ = 276 Hz | $^{1}J_{HC}$ = 125 .. 200 Hz | $^{1}J_{HN}$ = 60 .. 100 Hz |
| $^{2}J_{HH}$ = 0 .. 30 Hz<br>$^{3}J_{HH}$ = 0 .. 20 Hz<br>$^{4}J_{HH}$ = 0 .. 3 Hz | $^{2}J_{HC}$ = 0 .. 20 Hz<br>$^{3}J_{HC}$ = 0 .. 15 Hz<br>$^{4}J_{HC}$ = 0 .. 2 Hz | $^{2}J_{HN}$ = 0 .. 15 Hz<br>$^{3}J_{HN}$ = 0 .. 8 Hz<br>$^{4}J_{HN}$ = 0 .. 1 Hz |

# Parameters in NMR-spectroscopy

## Dipolar coupling

The nuclei interact directly through space via a dipol-dipol interaction



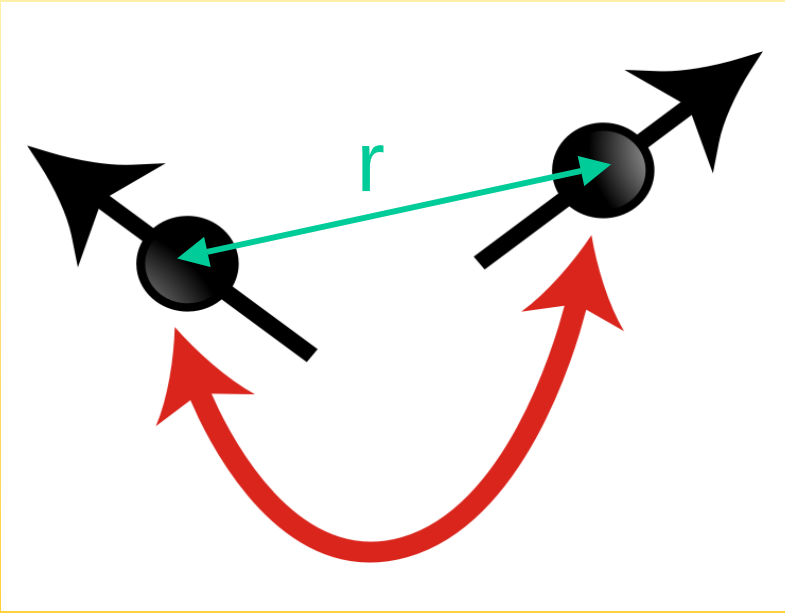
In solution NMR this interaction is averaged to zero due to the fast isotropic movement of the molecules

# Parameters in NMR-spectroscopy

But it does show up as NOE-Effect, that depends on the distance between two nuclei

$$I_{NOE} \sim 1/r^6$$

Since the intensity drops quickly with increasing distance the effect can only be observed up to 500 pm

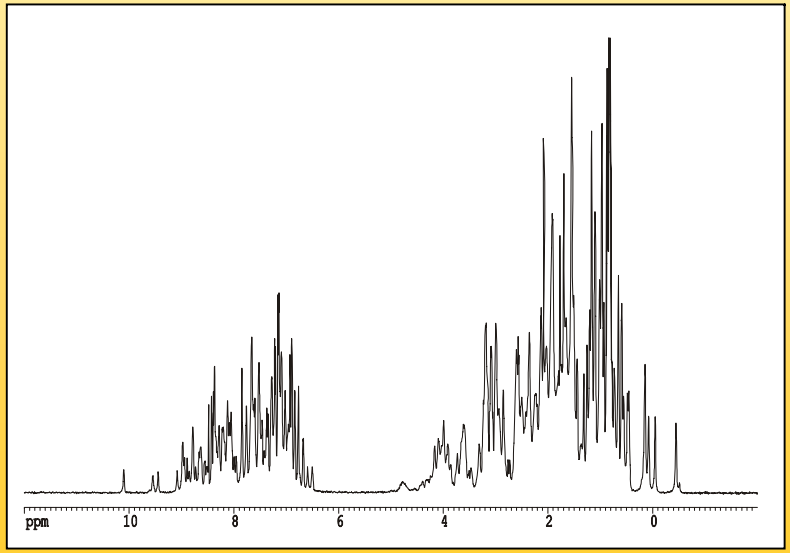


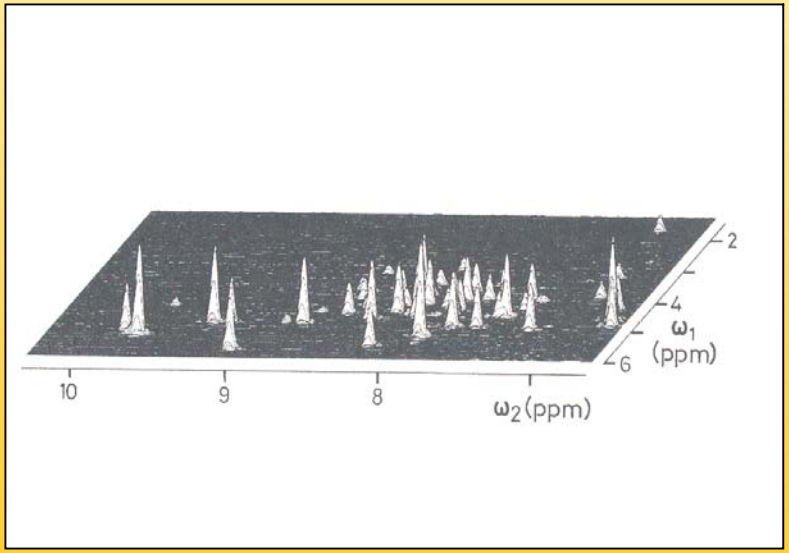
# Multidimensional NMR-spectroscopy

# Multidimensional NMR-spectroscopy

1D-NMR:
2 axis
intensity vs. frequency

2D-NMR:
3 axis
intensity vs. frequency (1)
vs. frequency (2)

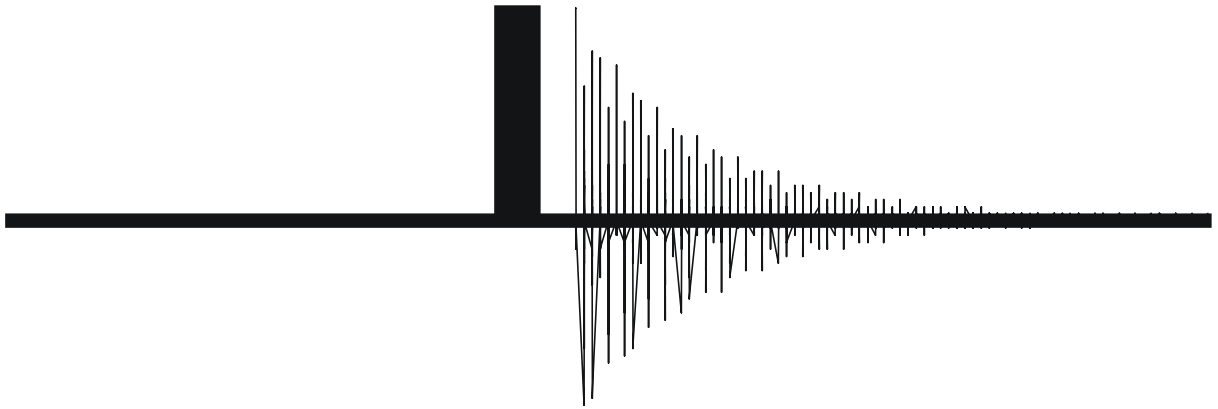


# Multidimensional NMR-spectroscopy

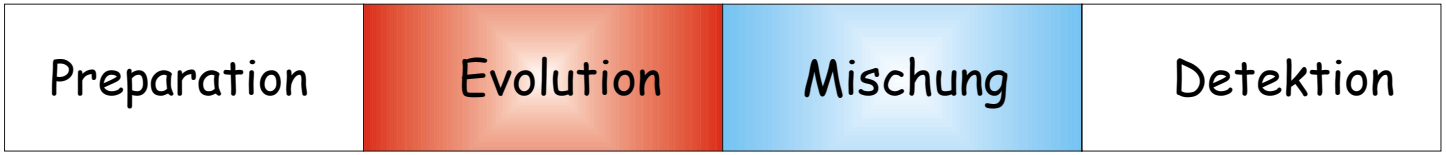
## 1D-NMR schematisch

| Preparation | Detektion |
|---|---|



# Multidimensional NMR-spectroscopy

2D-NMR sequences contain two novel types of time periods:
evolution time and mixing time




($t_1$) ($t_2$)

evolution time:
a second frequency dimension is created by indirect detection

mixing time:
magnetisation is transferred from spin to spin via interactions between spins

# Multidimensional NMR-spectroscopy

The two major advantages of multidimensional NMR are:

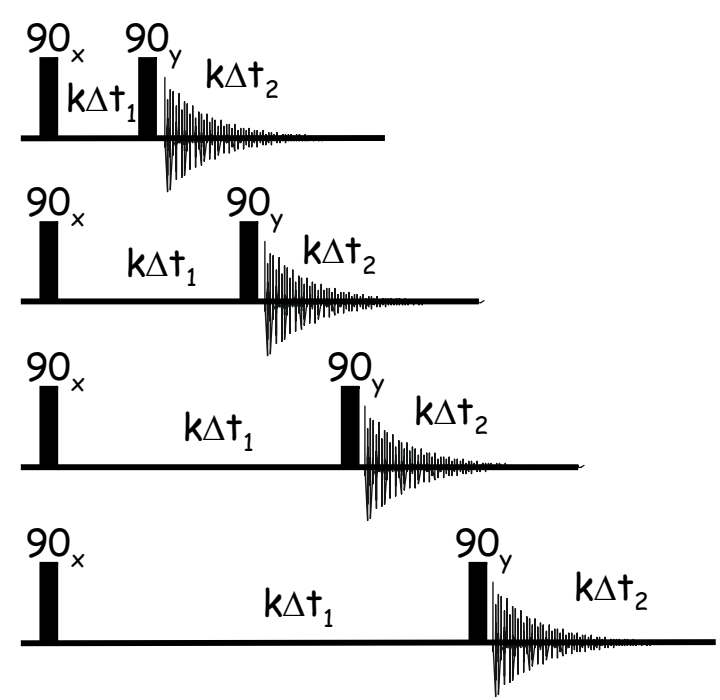
Improved resolution: Signals are spread over a surface (2D) or in a three-dimensional space (3D, 4D)

Magnetization transfer: Signals result from the interaction between nuclei. That can be interactions through bond (via J-coupling) or through space (via NOE).

Taken together this eases the interpretation and the assignment of the spectra considerably

# Multidimensional NMR-spectroscopy

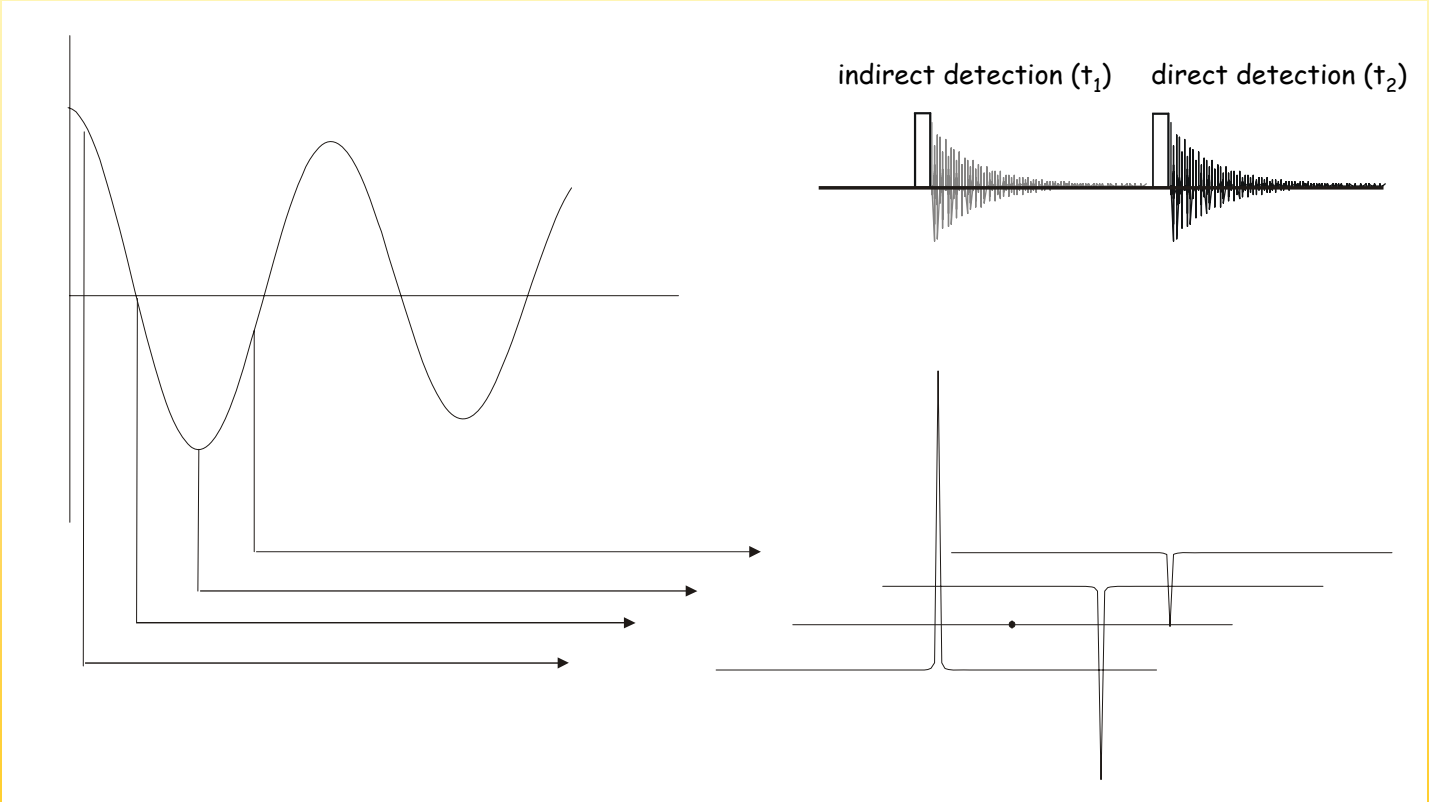
The indirect dimension is created by implementing an evolution time in the pulse sequence that is systematically increased during the NMR experiment




A series of one-dimensional experiments is thus created

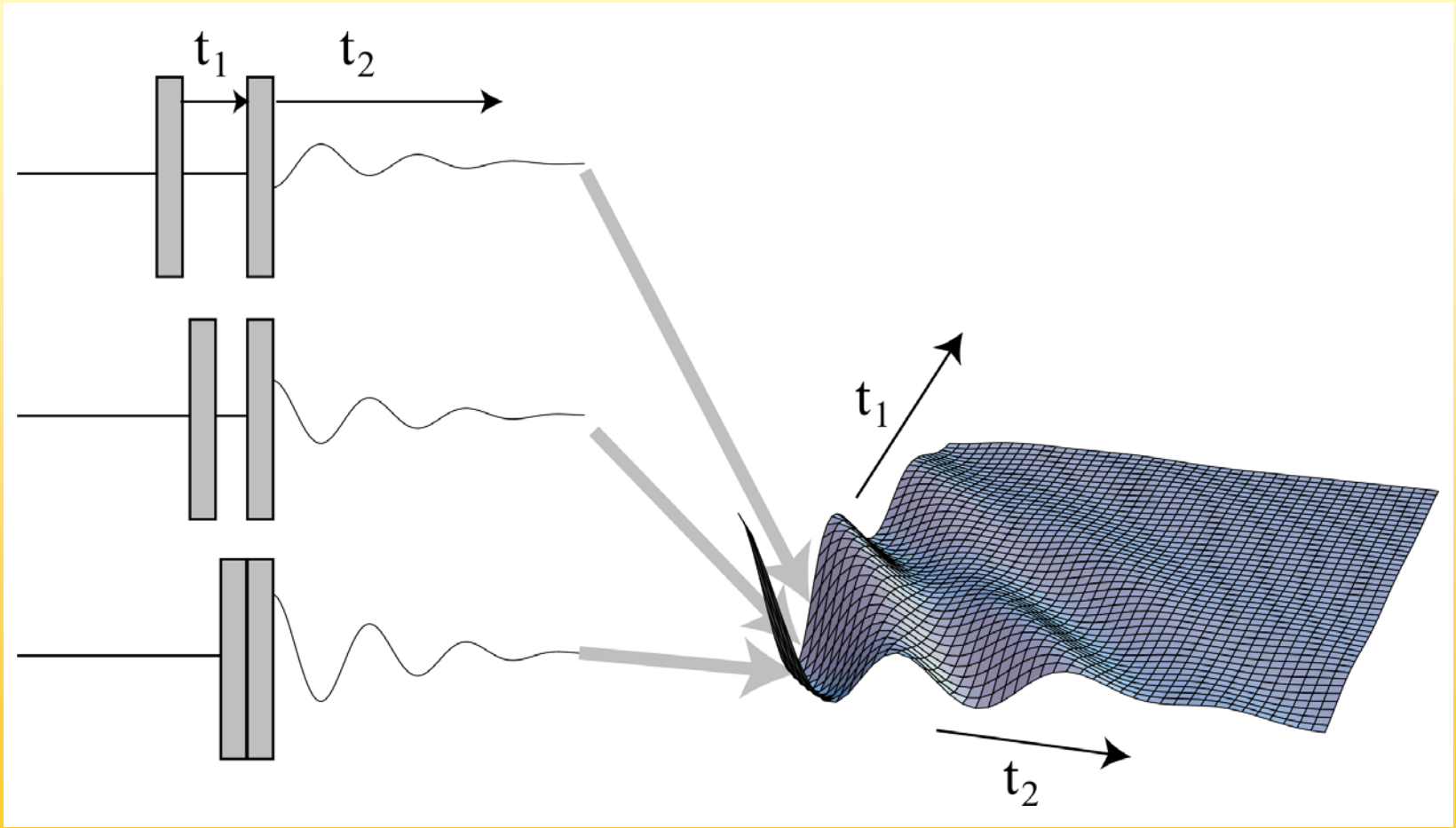
# Multidimensional NMR-spectroscopy

Each 1D differs from the previous one depending on the evolution time



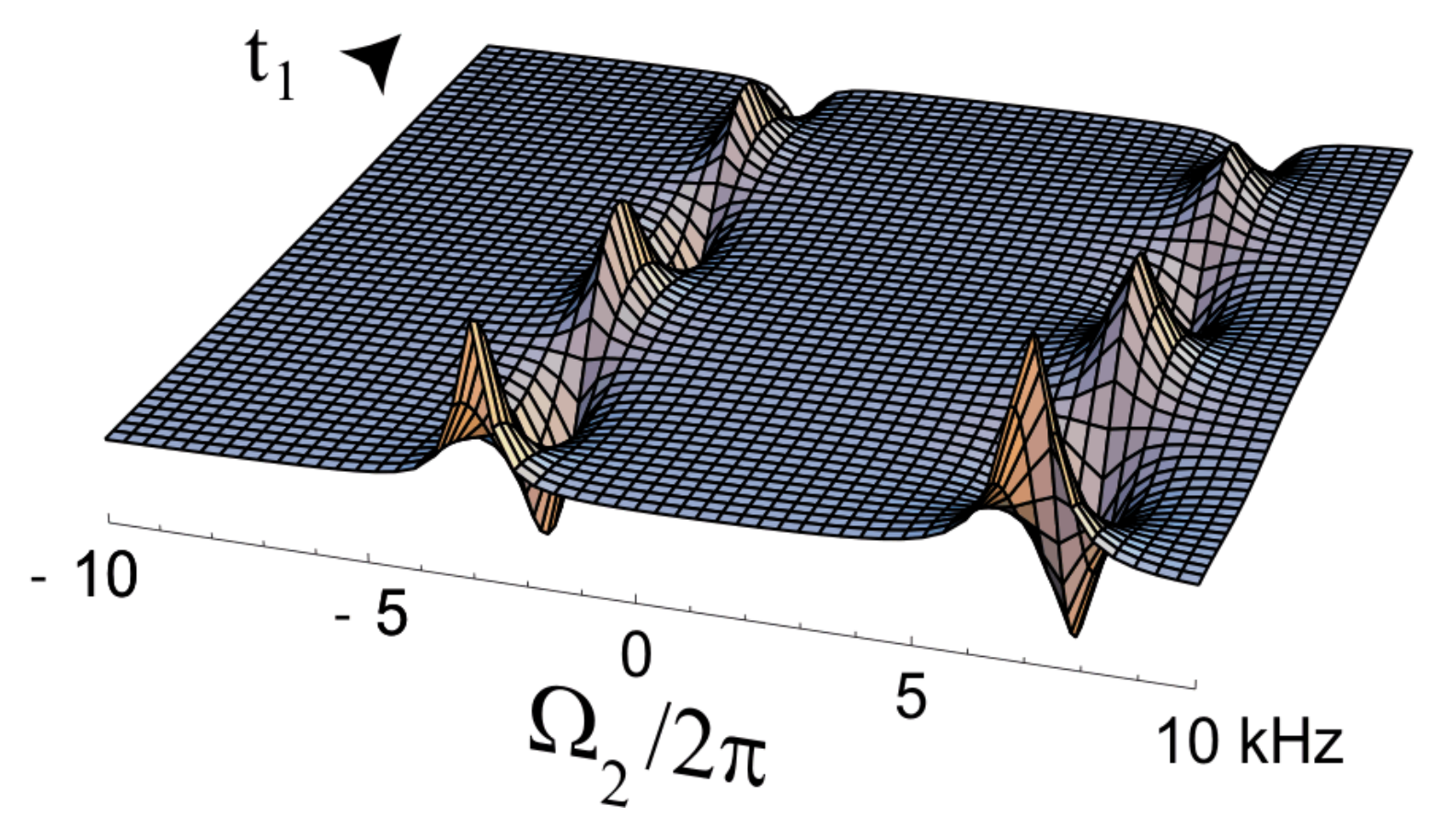
# Multidimensional NMR-spectroscopy

A two-dimensional FID is thus created



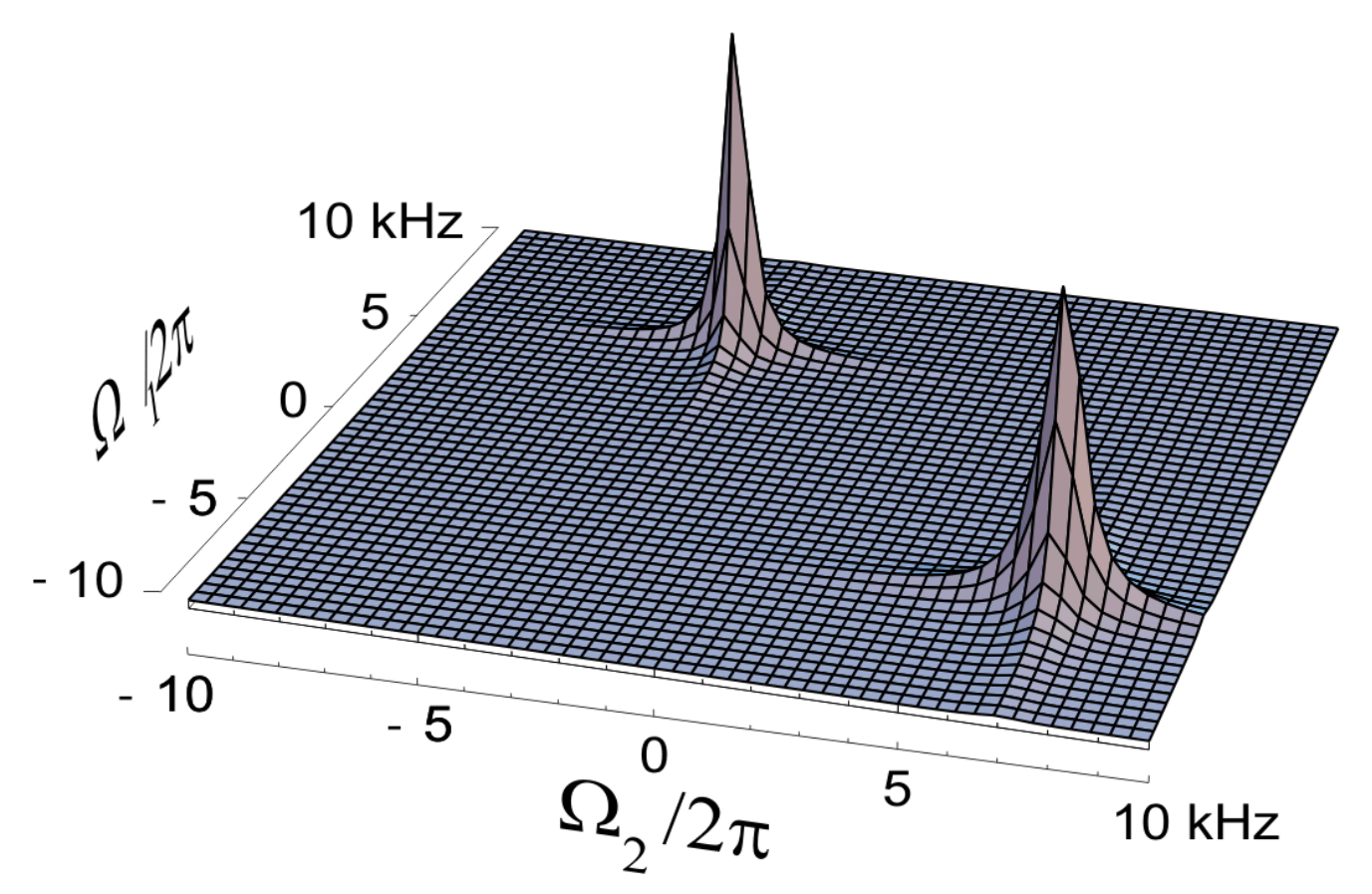
# Multidimensional NMR-spectroscopy

The first FT yields an „interferogram“

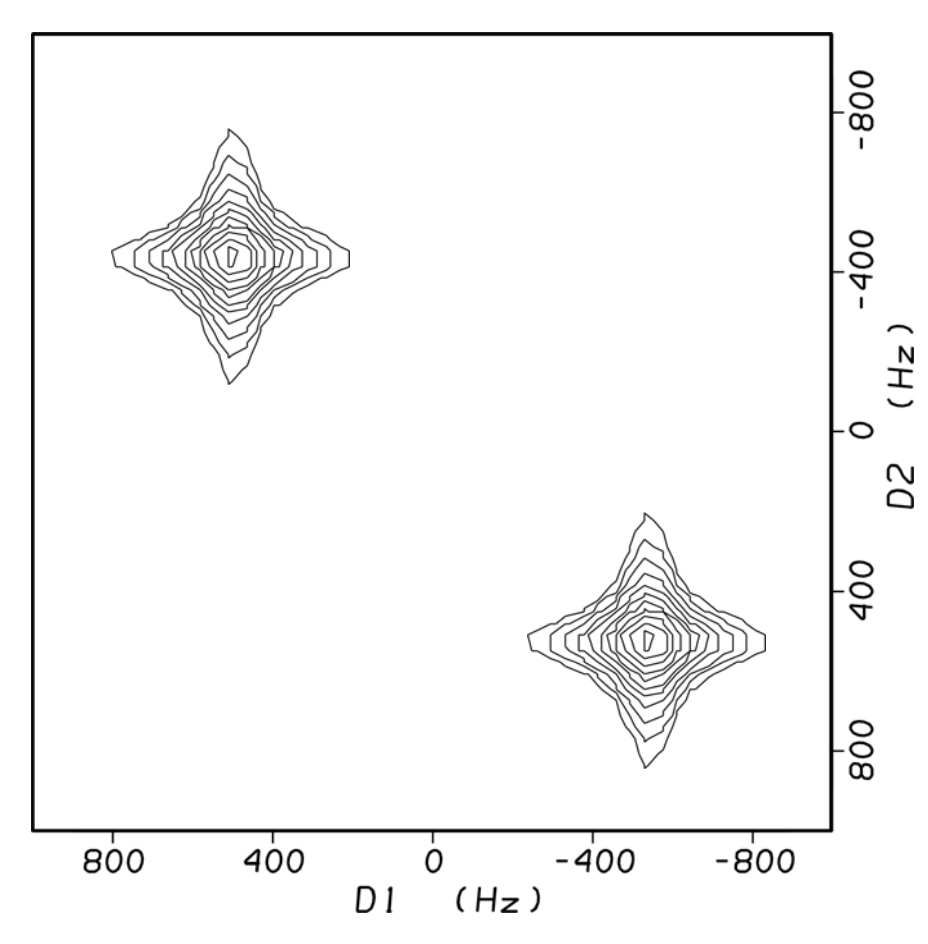


# Multidimensional NMR-spectroscopy

## The second FT yields a 2D Spektrum



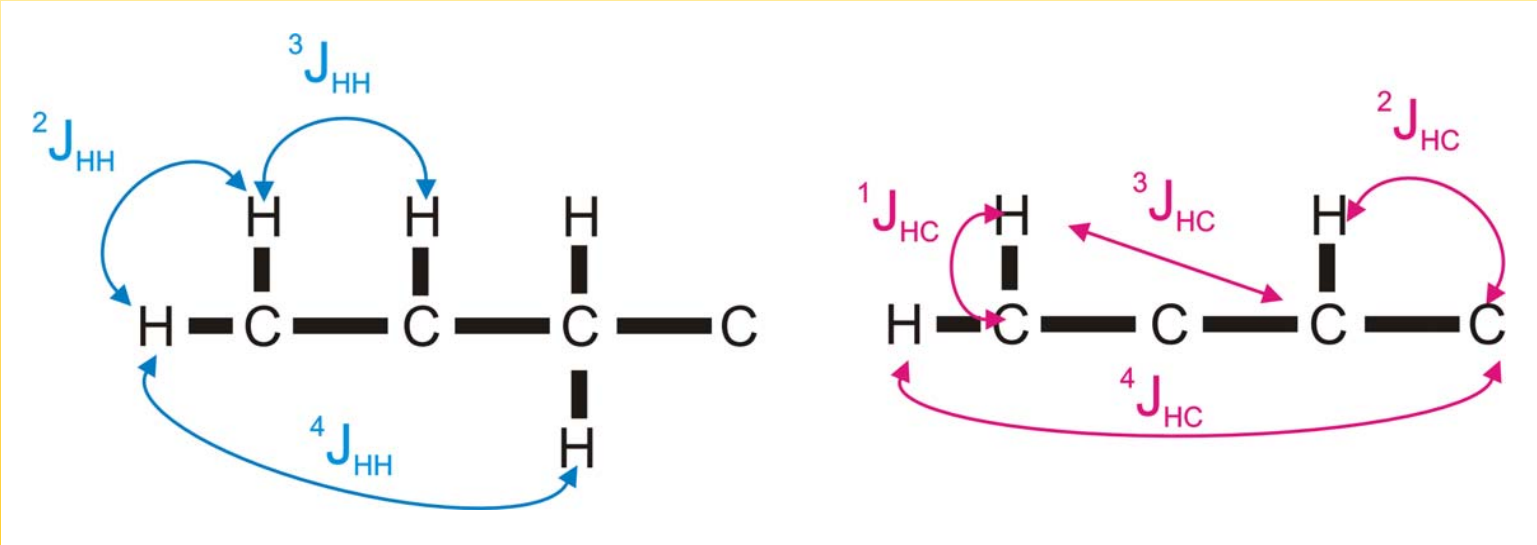
# Multidimensional NMR-spectroscopy




For analysis of the data the spectra are converted in contour plots

# Multidimensional NMR-spectroscopy

More importantly the mixing time results in a transfer of magnetization from one nucleus to another, in most cases scalar coupling is used, sometimes also the NOE-effect



# Multidimensional NMR-spectroscopy

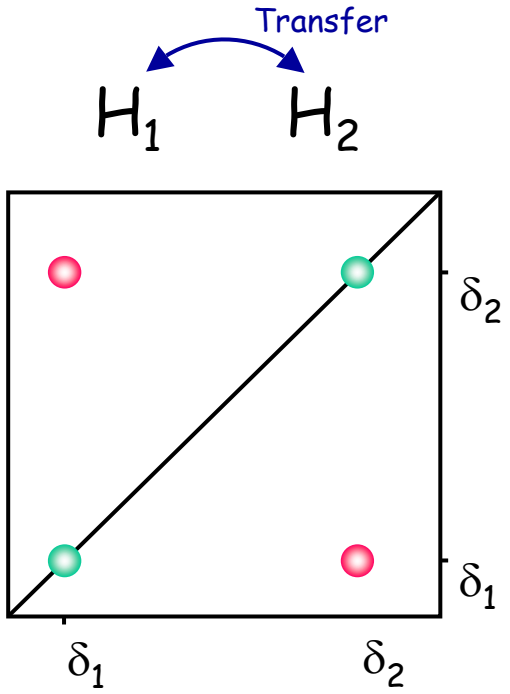
## homonuclear spectra

Transfer of magnetization takes place between like nuclei. Both axis exhibit the chemical shift of the same type of nucleus. If a transfer has taken place, the signal has different frequencies in the two dimensions:

cross peak

If no transfer has taken place, the shifts are the same in both dimensions:

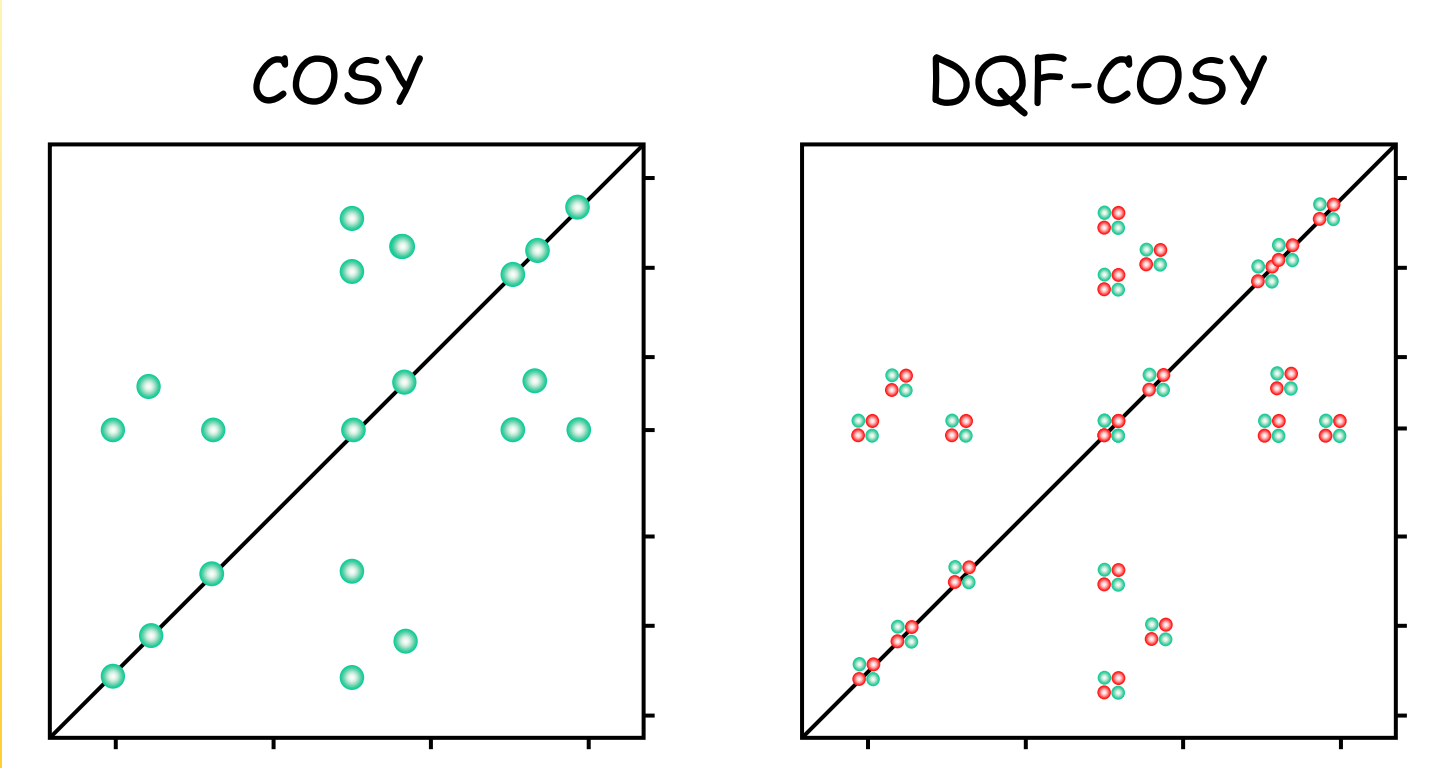
diagonal signal




cross peak
diagonal signal

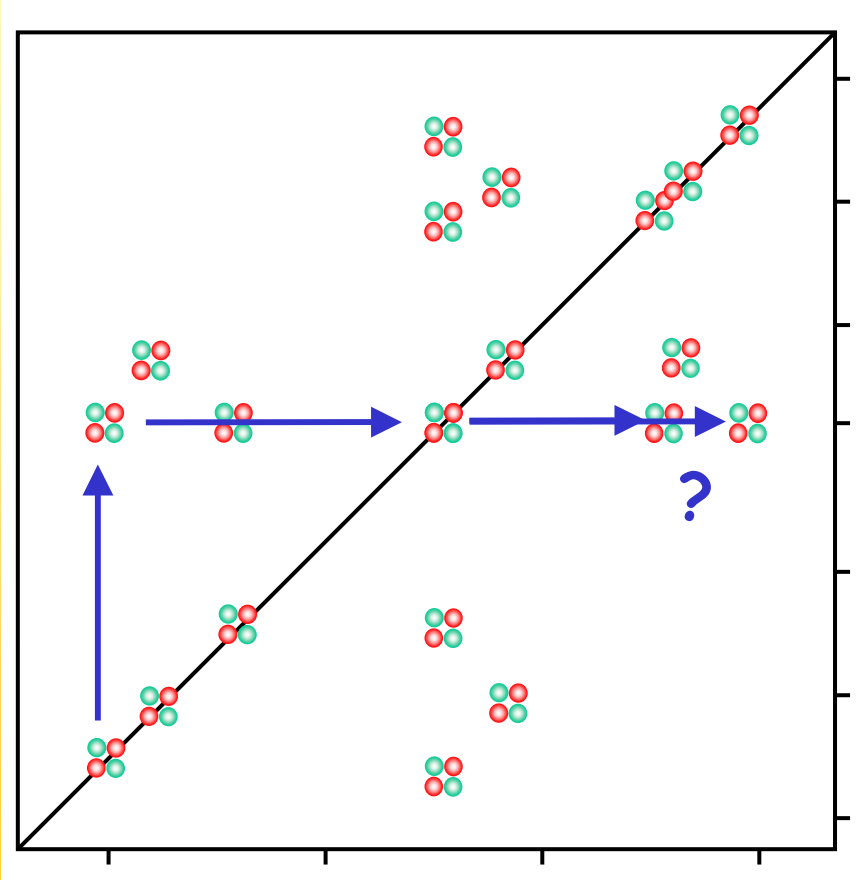
# Multidimensional NMR-spectroscopy

COSY and DQF-COSY accomplish a transfer via scalar coupling, usually via not more then three bonds



# Multidimensional NMR-spectroscopy

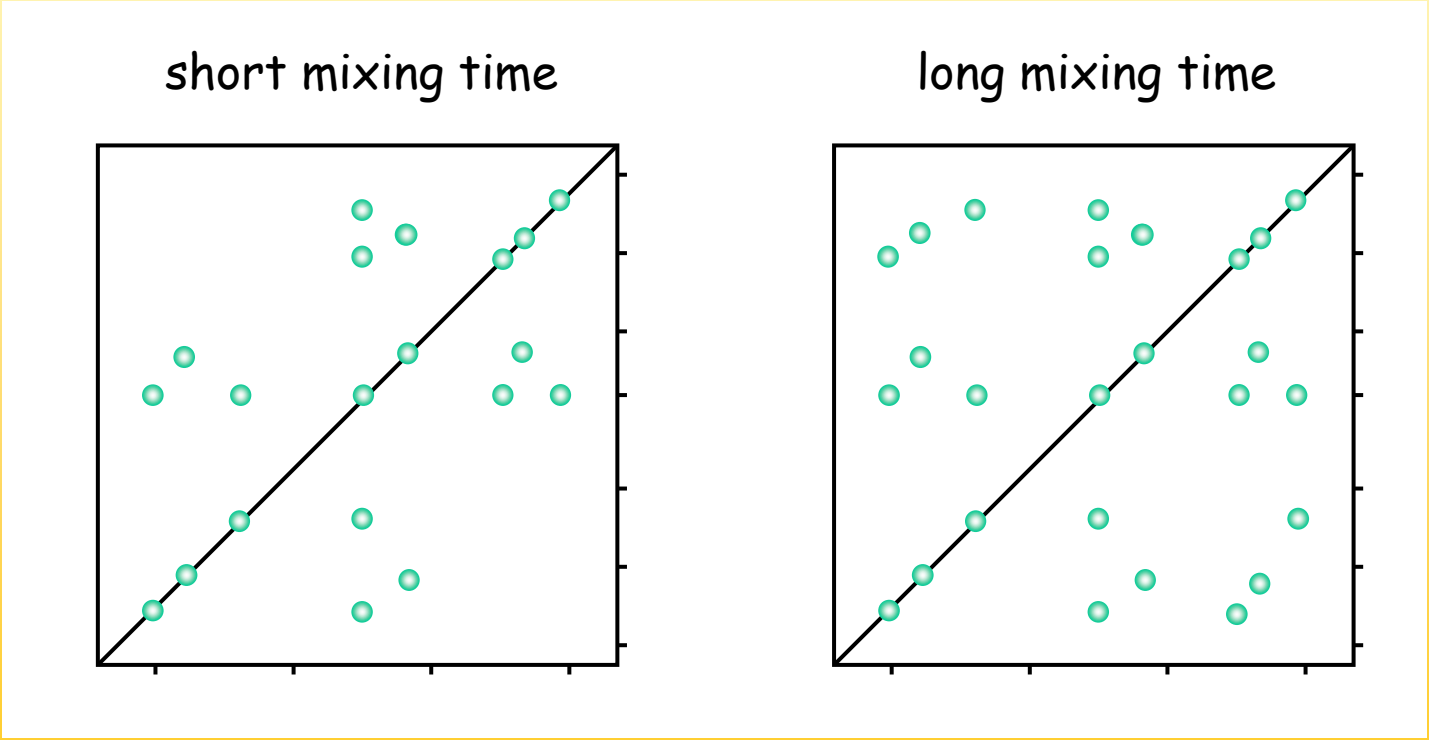
A problem can be created by overlap….




…but can be resolved by another 2D

# Multidimensional NMR-spectroscopy

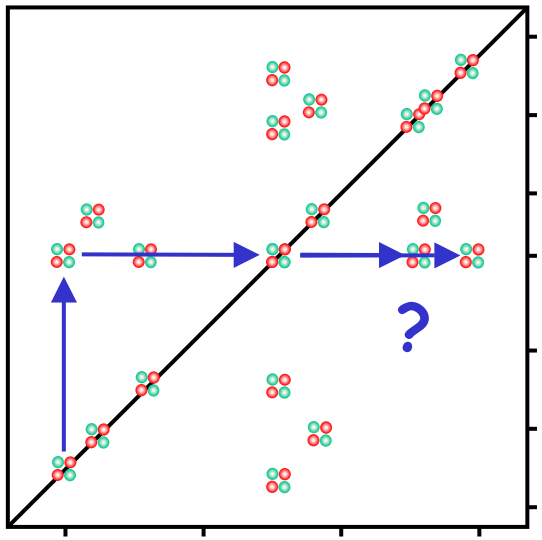
A TOCSY also accomplishes a transfer via scalar coupling, but via more then three bonds. But if there is no coupling there is no transfer (e.g. via quarternary carbons)



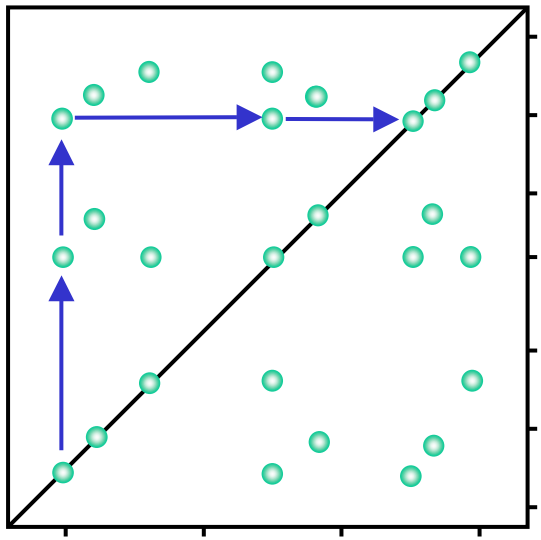
# Multidimensional NMR-spectroscopy

A TOCSY can resolve overlap and does still work when molecules get bigger

COSY



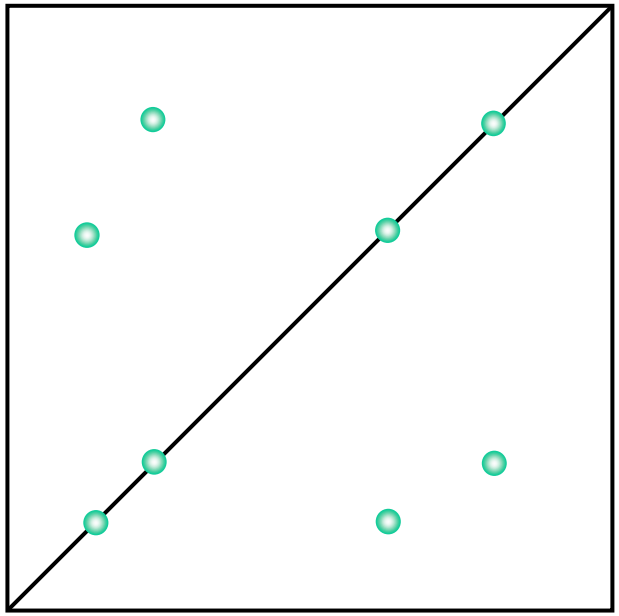

TOCSY



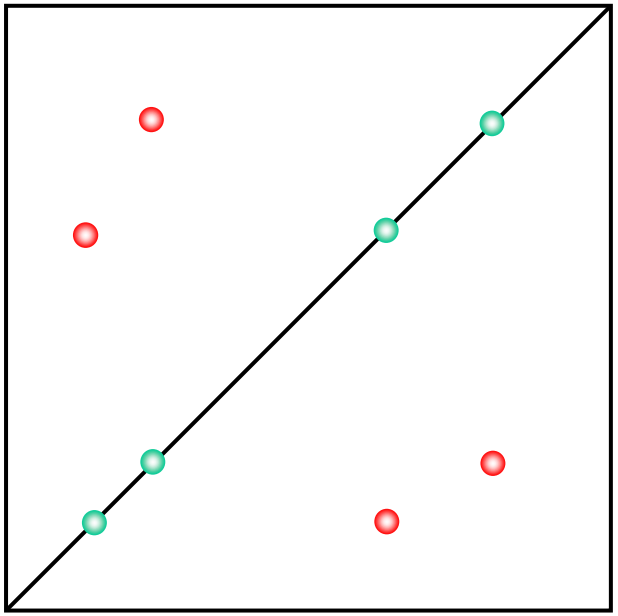
# Multidimensional NMR-spectroscopy

Besides the transfer via scalar coupling there is the possibility to transfer via the NOE-effect, i.e. via a distance dependent interaction through space

NOESY ($\omega\tau_c > 1$)

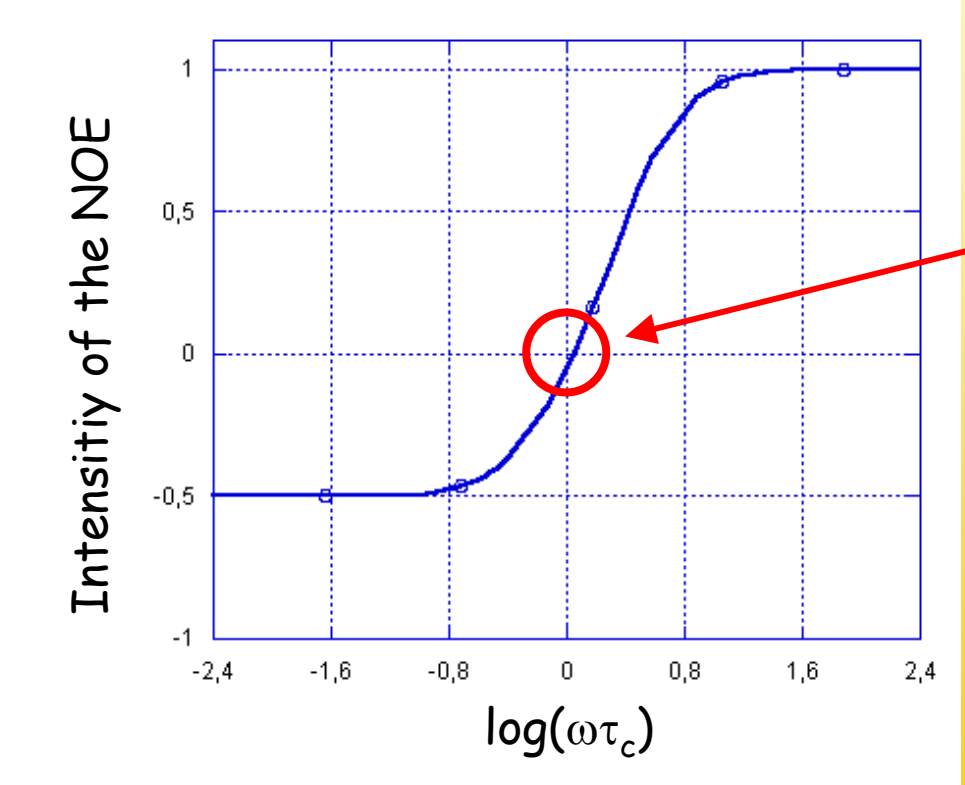


NOESY ($\omega\tau_c < 1$)



# Multidimensional NMR-spectroscopy

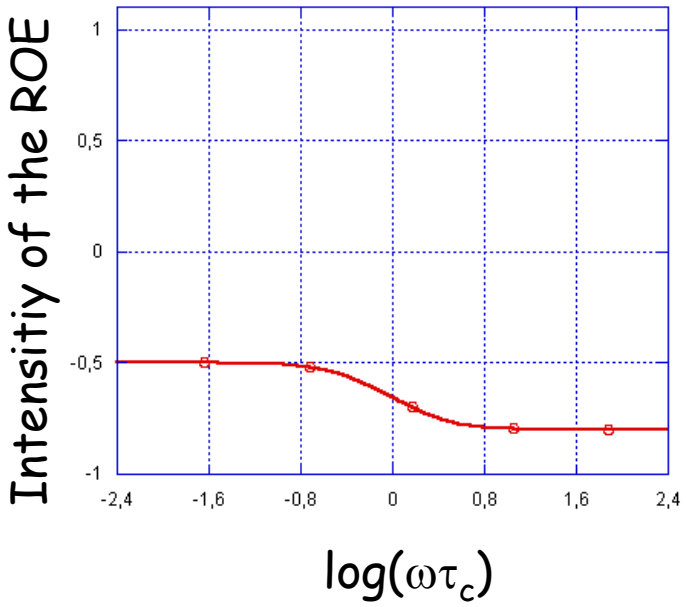
There is a problem with the NOE for a certain combination of size and solvent viscosity

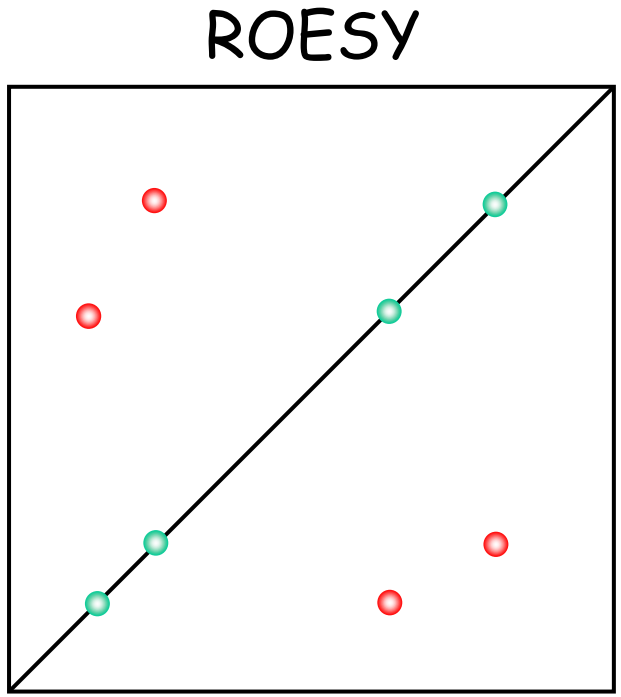



The theoretical intensity of the NOE is zero at $\omega\tau_c = 0$

# Multidimensional NMR-spectroscopy

This problem can be solved by using the ROE, i.e. the rotating-frame NOE in a slightly modified experiment called the ROESY

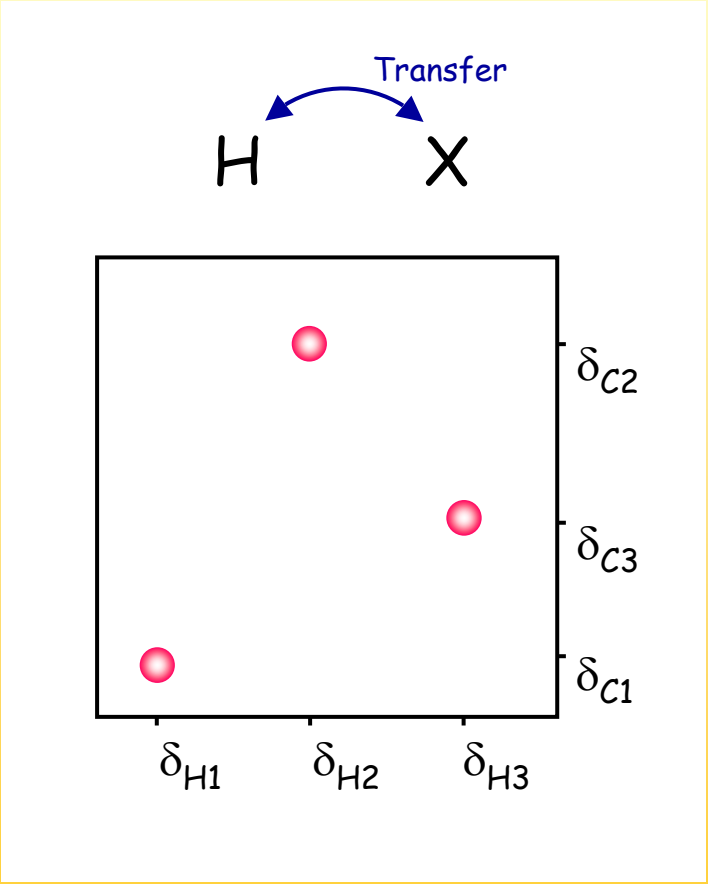


# Multidimensional NMR-spectroscopy

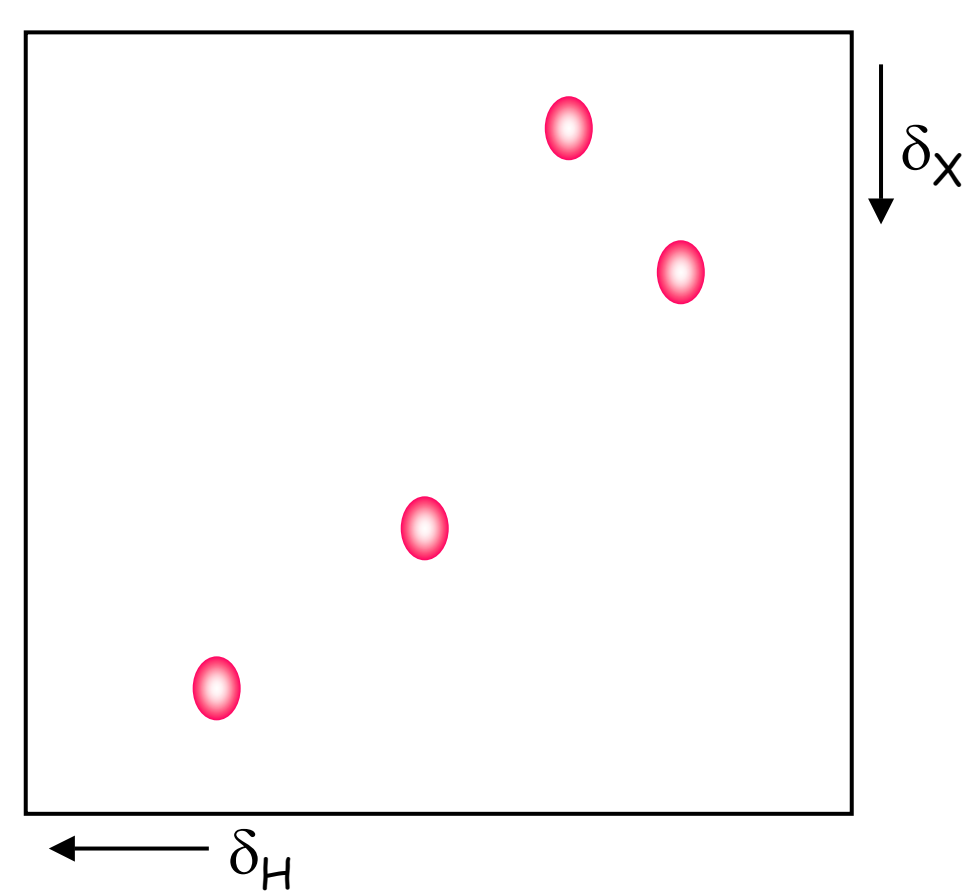
## heteronuclear spectra

Transfer of magnetization takes place between nuclei of different types. The two axis show the chemical shift of the respective type of nucleus. If a transfer has taken place, a signal appears at the intersection of the two frequencies, without a transfer there is no signal.



# Multidimensional NMR-spectroscopy

HMQC = Heteronuclear MultipleQuantum Correlation

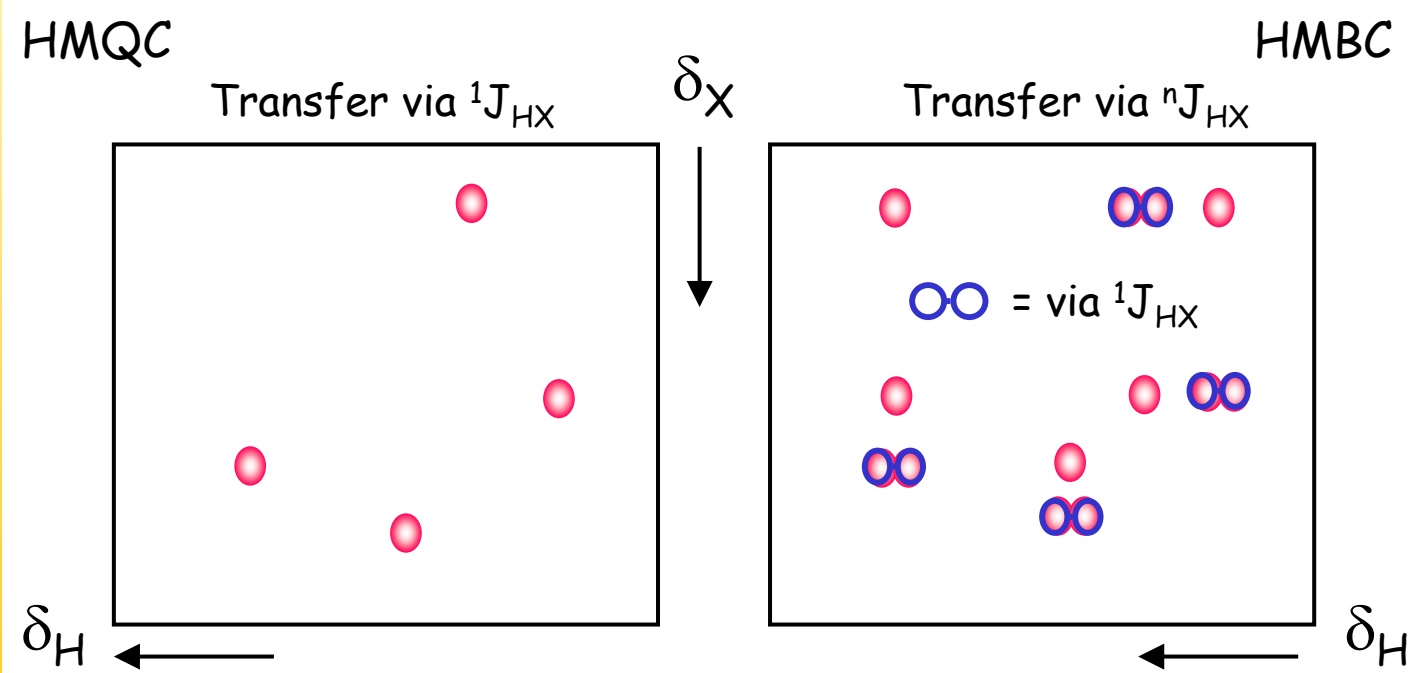



A signal indicates a direct bond between the proton and the heteronucleus

# Multidimensional NMR-spectroscopy

## HMBC = Heteronuclear MultipleBond Correlation

A signal indicates a correlation via two, three or four bonds between the proton and the heteronucleus.

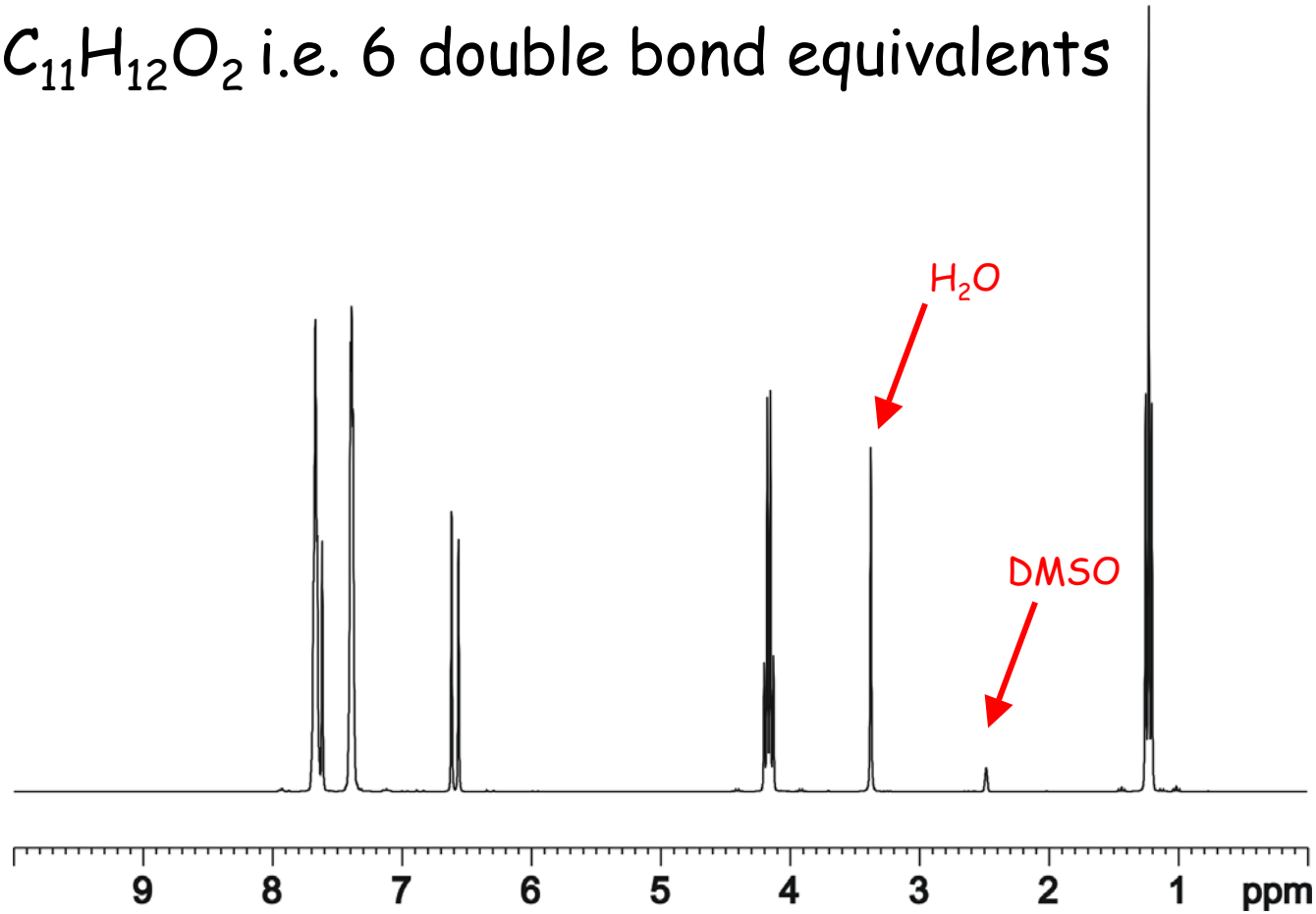


# A first example:

# An „unknown" compound

# An „unknown" compound

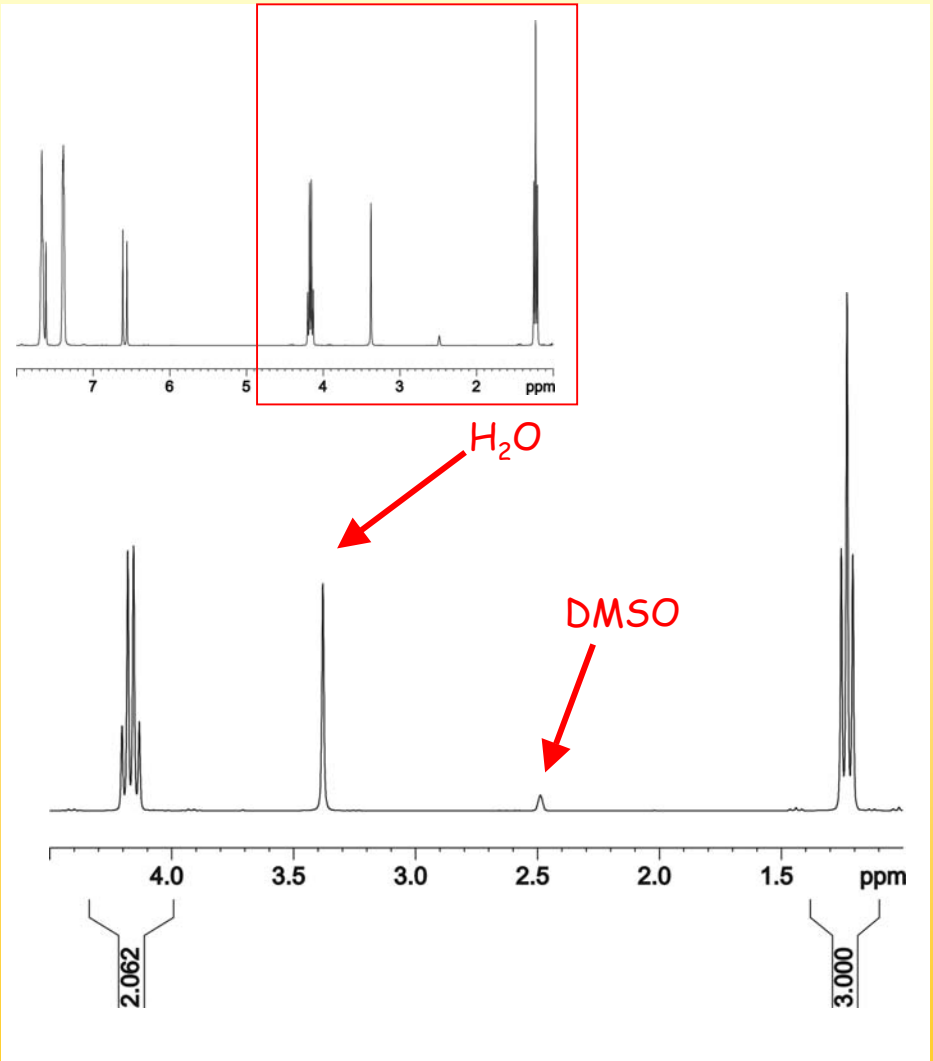
$C_{11}H_{12}O_2$ i.e. 6 double bond equivalents



# An „unknown" compound

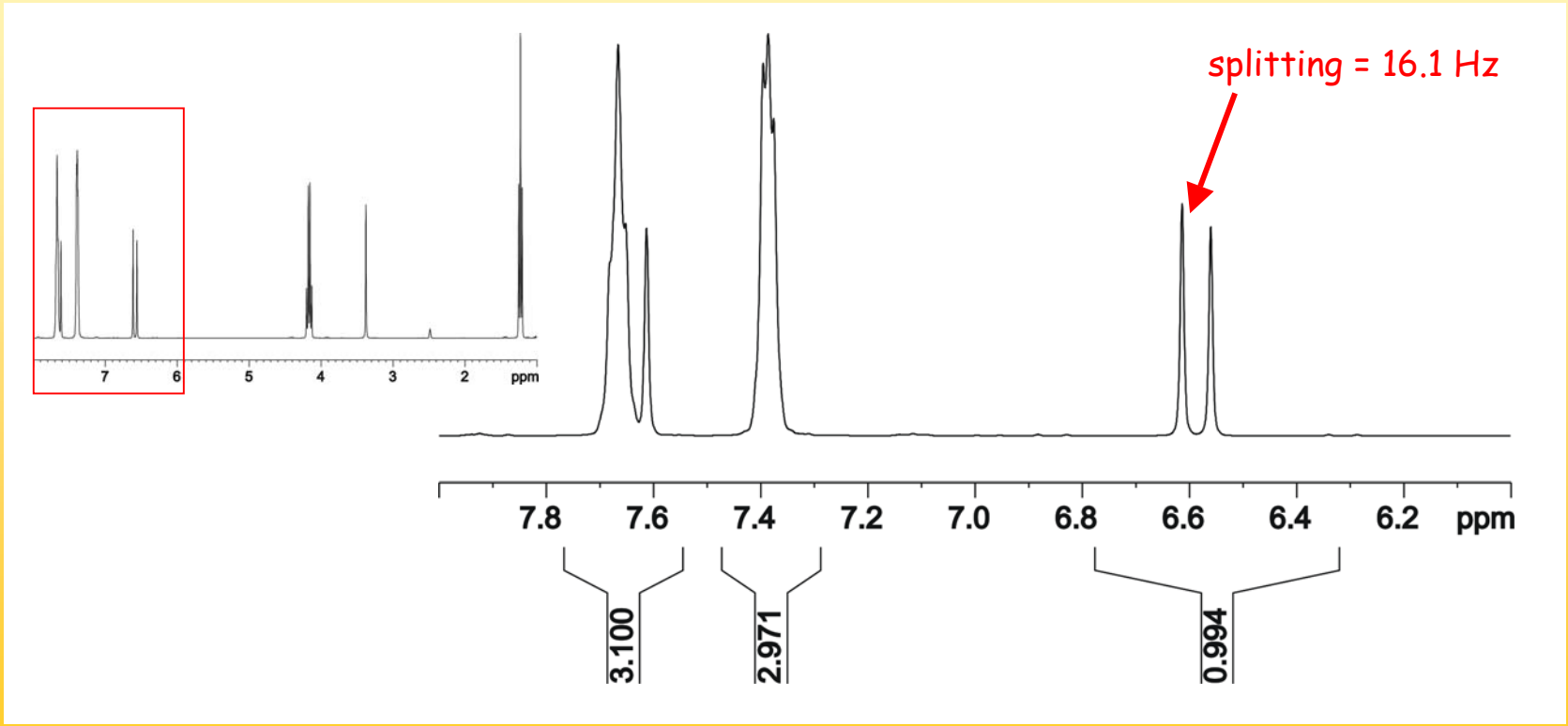
First we use all information we can get from the 1Ds

A quartet and a triplet with an intensity ratio of 2:3, that sounds familiar ?



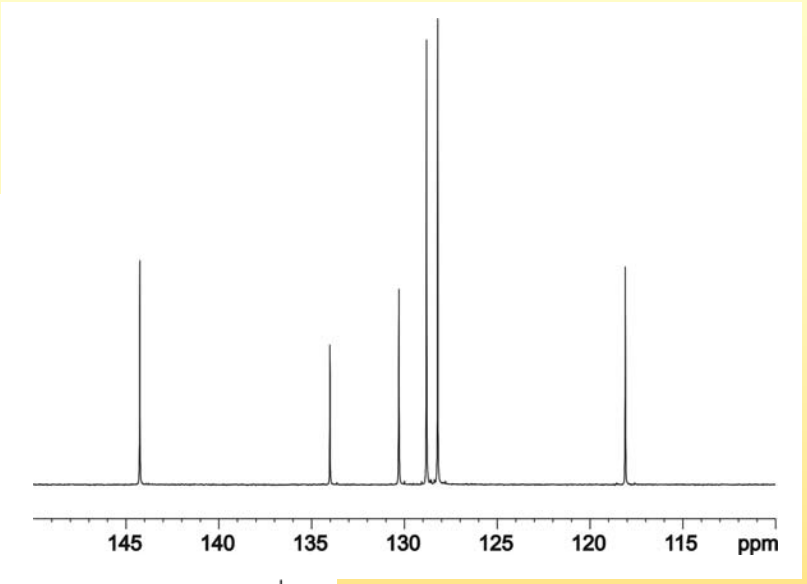
# An „unknown" compound

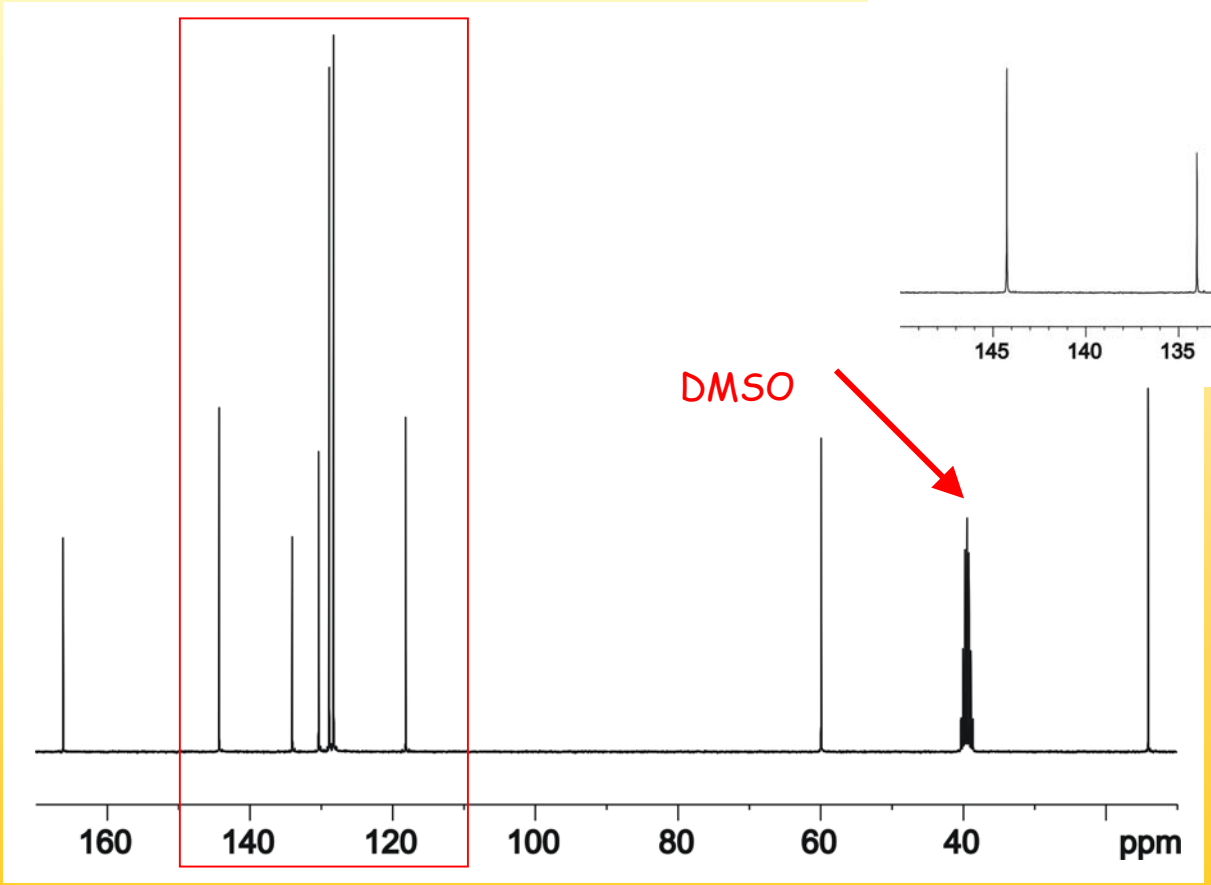
That leaves us 7 protons which we find in the other region of the spectrum



# An „unknown" compound

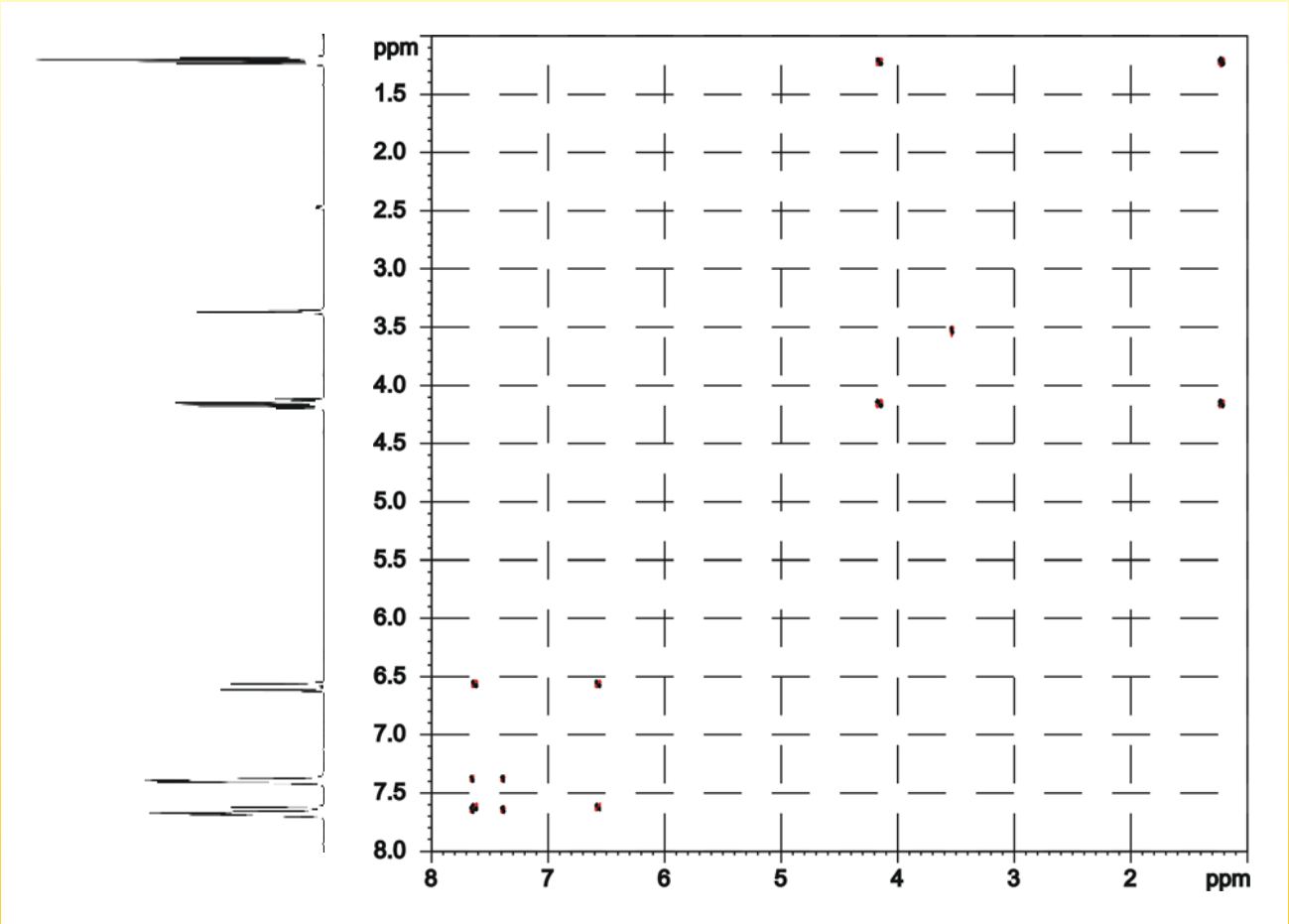
In the carbon 1D we find 9 signals instead of 11



# An „unknown" compound

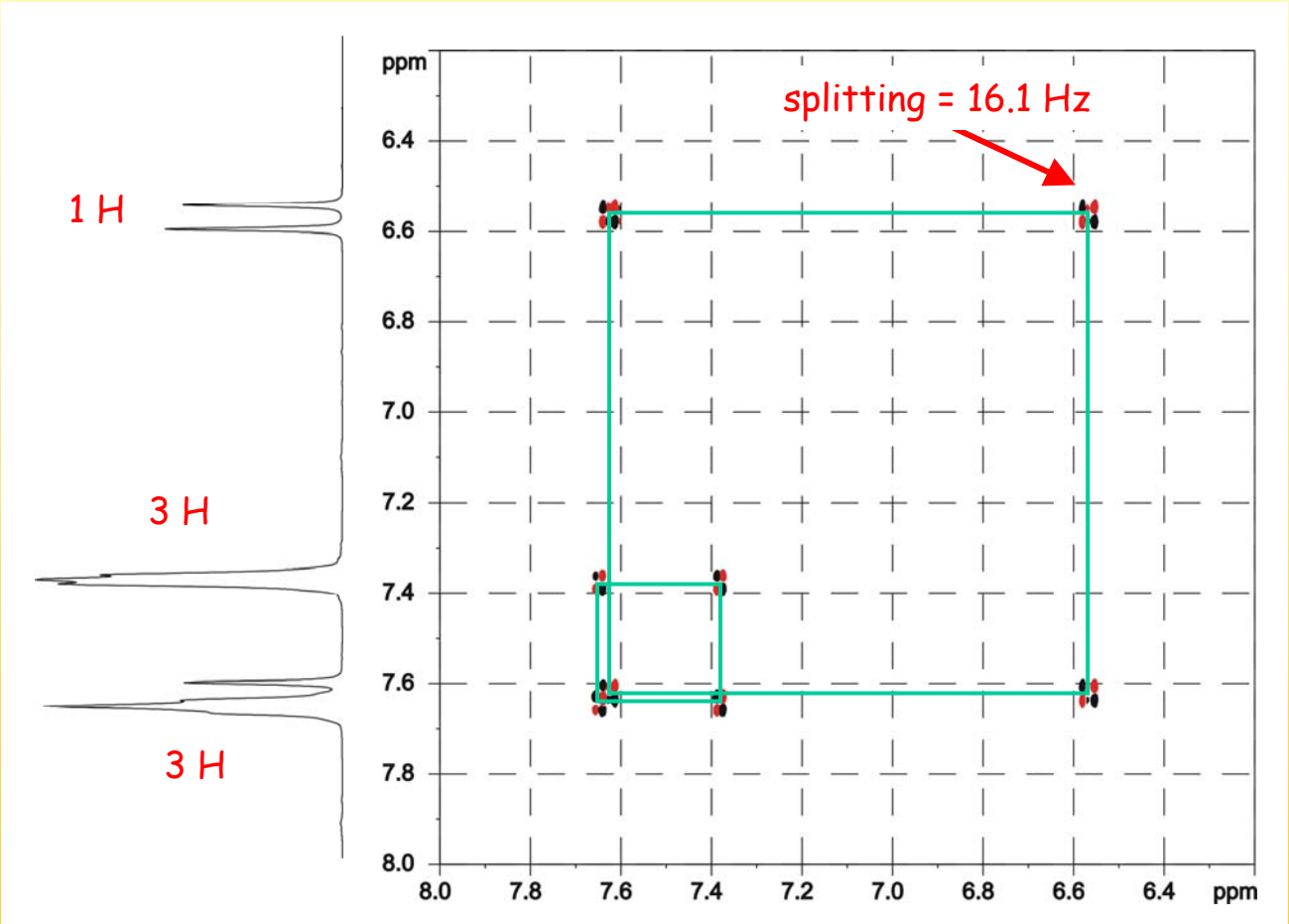
The first 2D we look at is the DQF-COSY




Only a few "pairs" are visible

# An „unknown" compound

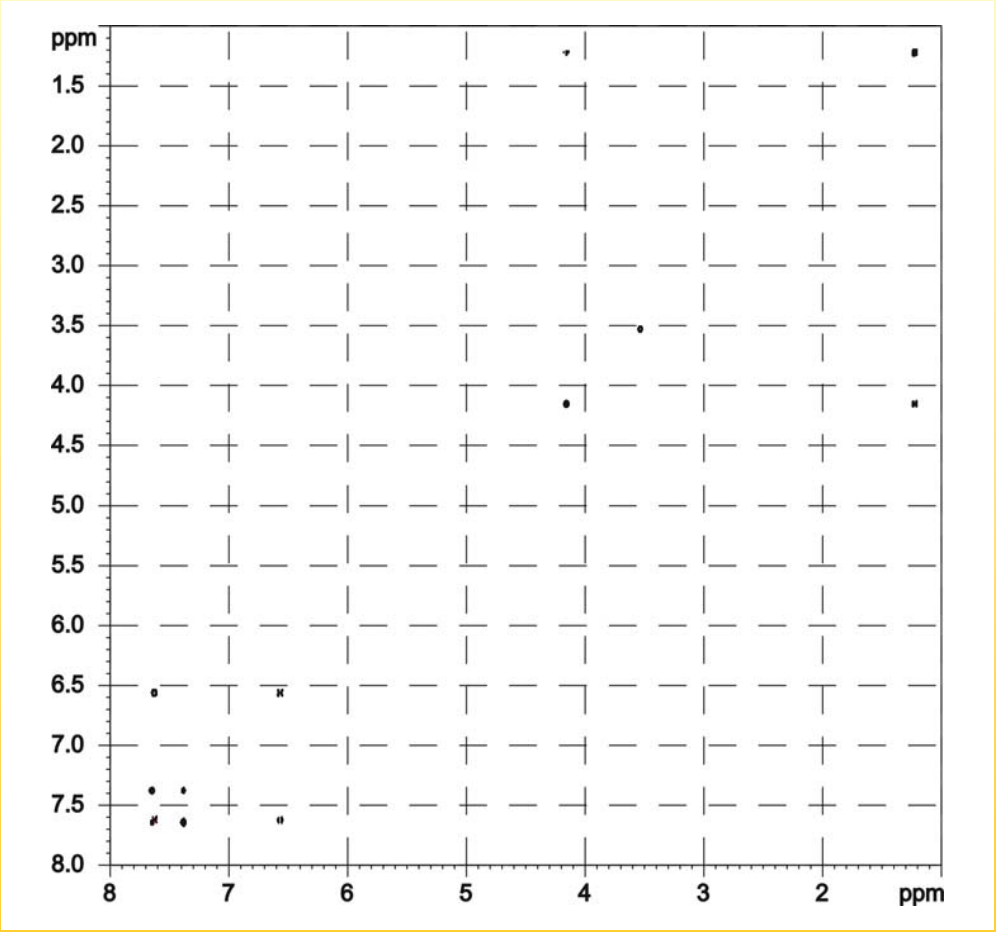
## The region of the aromatic/olefinic protons is more interesting




There is a discrepancy between crosspeaks and intergrals !

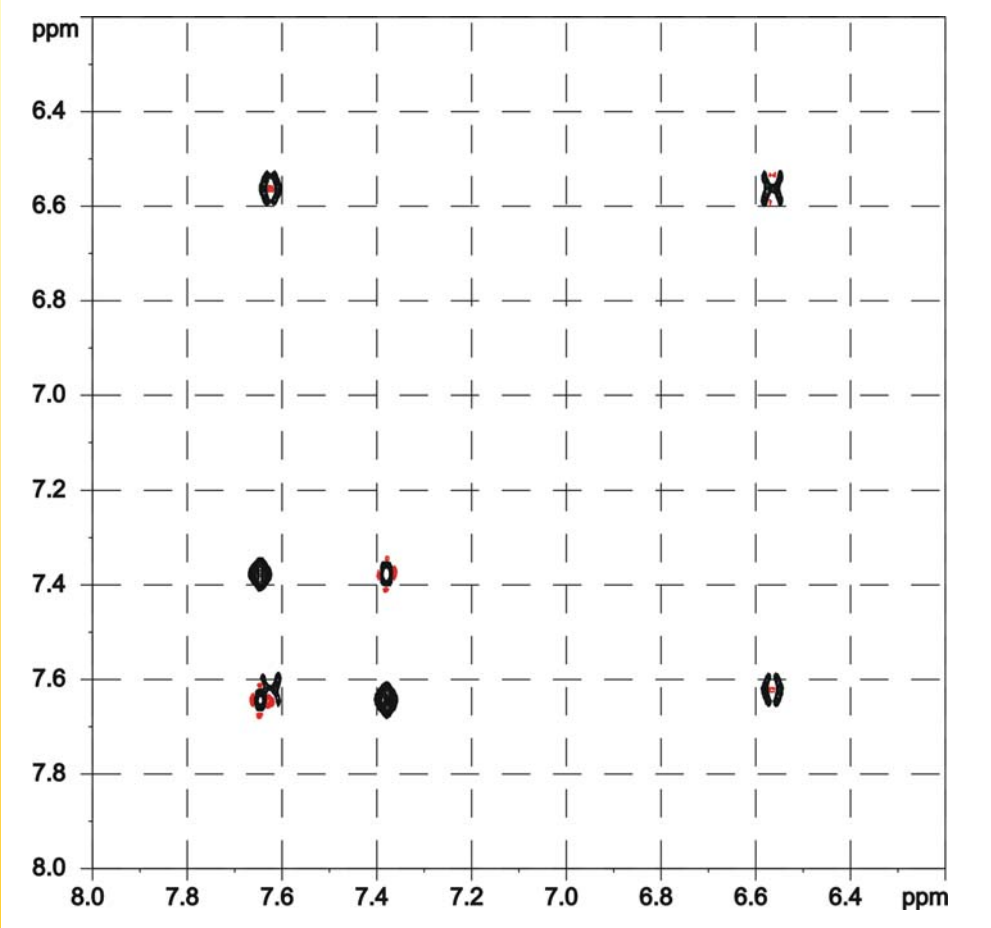
# An „unknown" compound

## Maybe the TOCSY helps ?



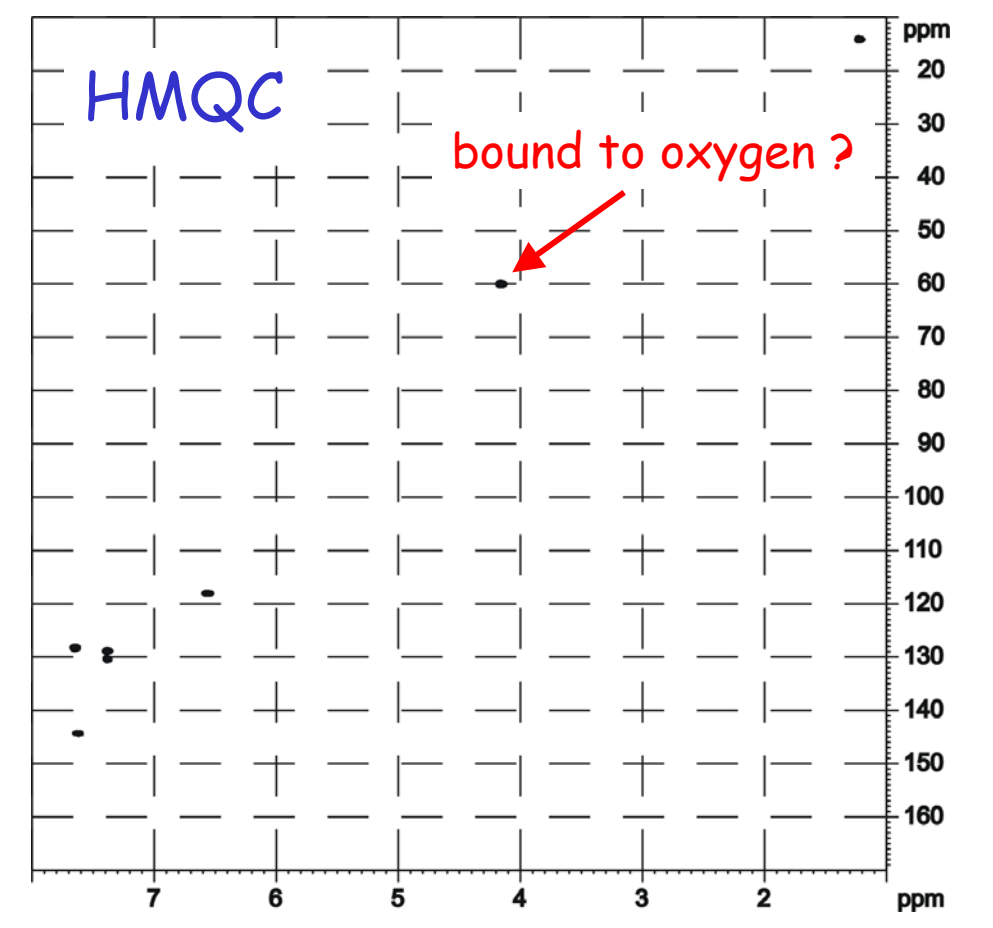
# An „unknown" compound

But that looks identical to the DQF-COSY



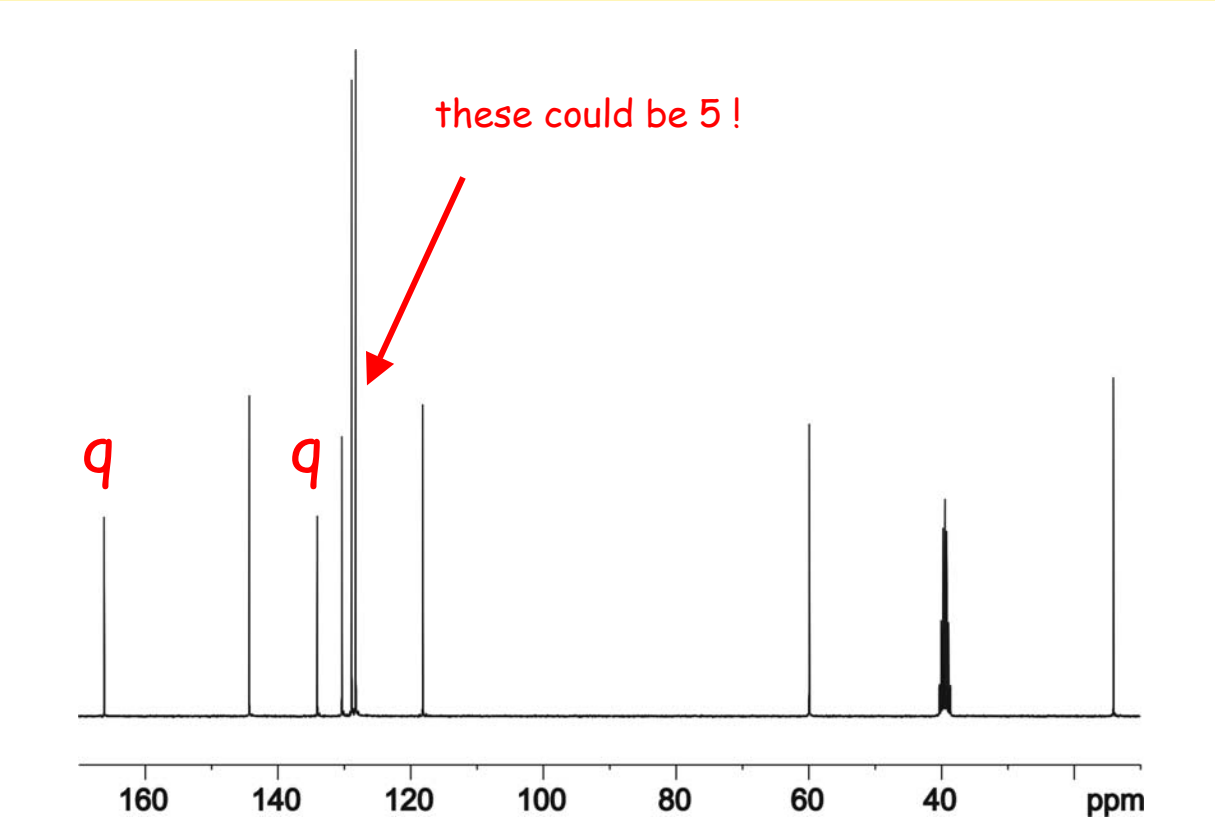
# An „unknown" compound

The resolve the discrepancies we have to look into heteronuclear data.



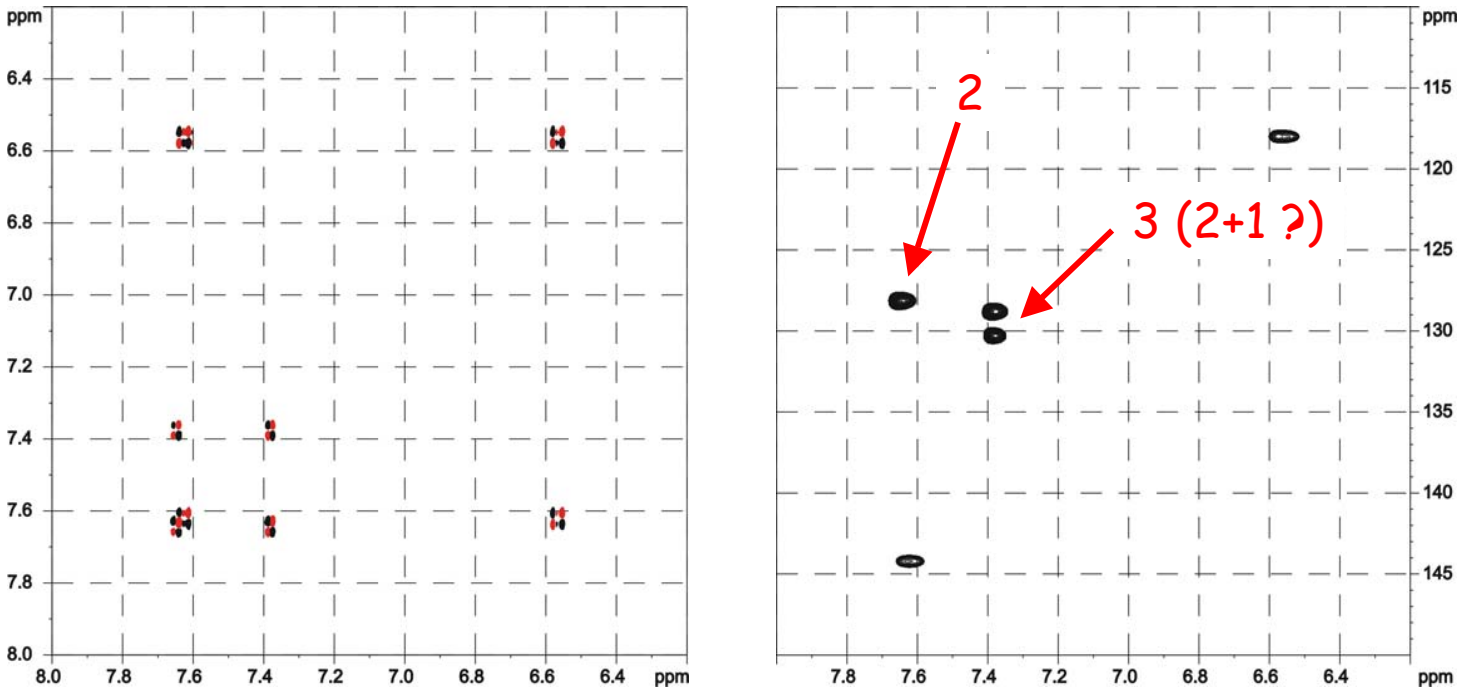
# An „unknown" compound

Comparing 1D and HMQC shows the quartenary carbons. The upfield part of the HMQC looks o.k., what about the aromatic/olefinic region ?



# An „unknown" compound

The pattern resembles an aromatic ring with 5 proton-attached carbons



# An „unknown" compound

So what do we have up to now ?

We have three spin-systems isolated from each other.

One is an ethyl group attached to oxygen, one seems to be a trans-double bond and the last an aromatic ring with 6 carbons.

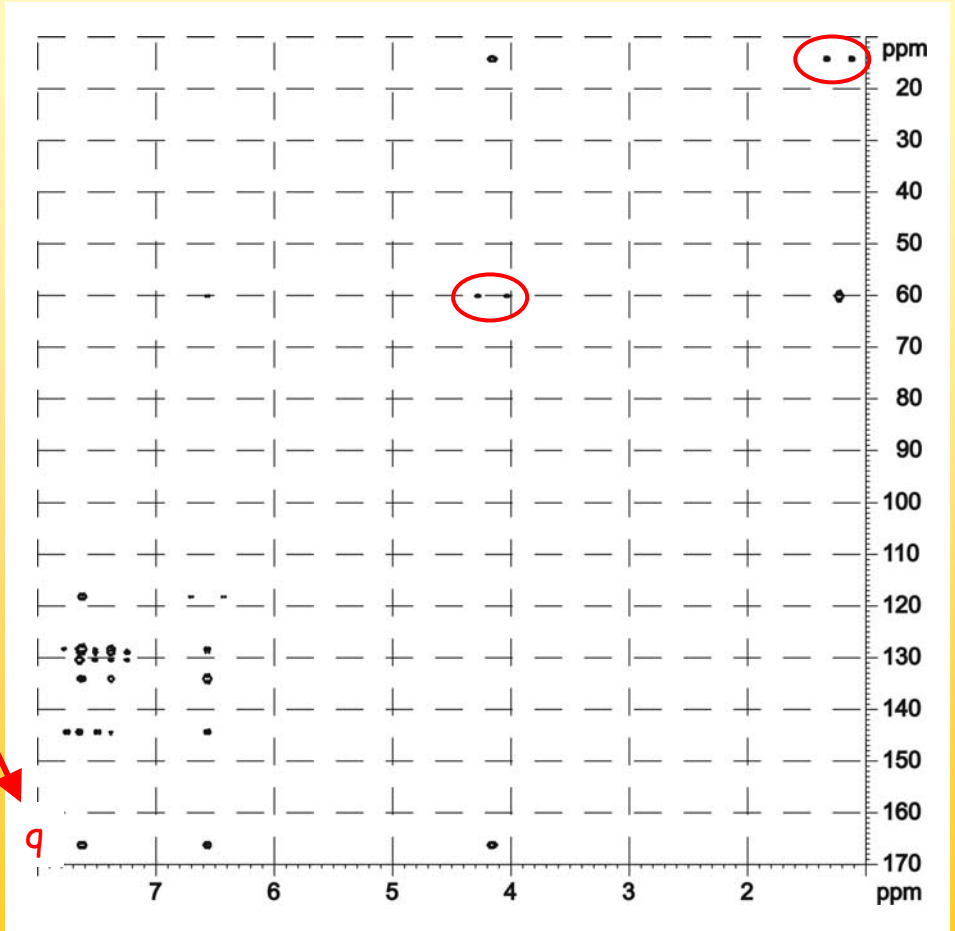
Since we have 6 double bond equivalents the oxygen most likely belongs to an carboxyl group.

# An „unknown" compound

## The final answer is in the HMBC

It helps with our ethyl group that shows a correlation to a -COO- (chemical shift)

And both of our olefinic protons show correlations as well

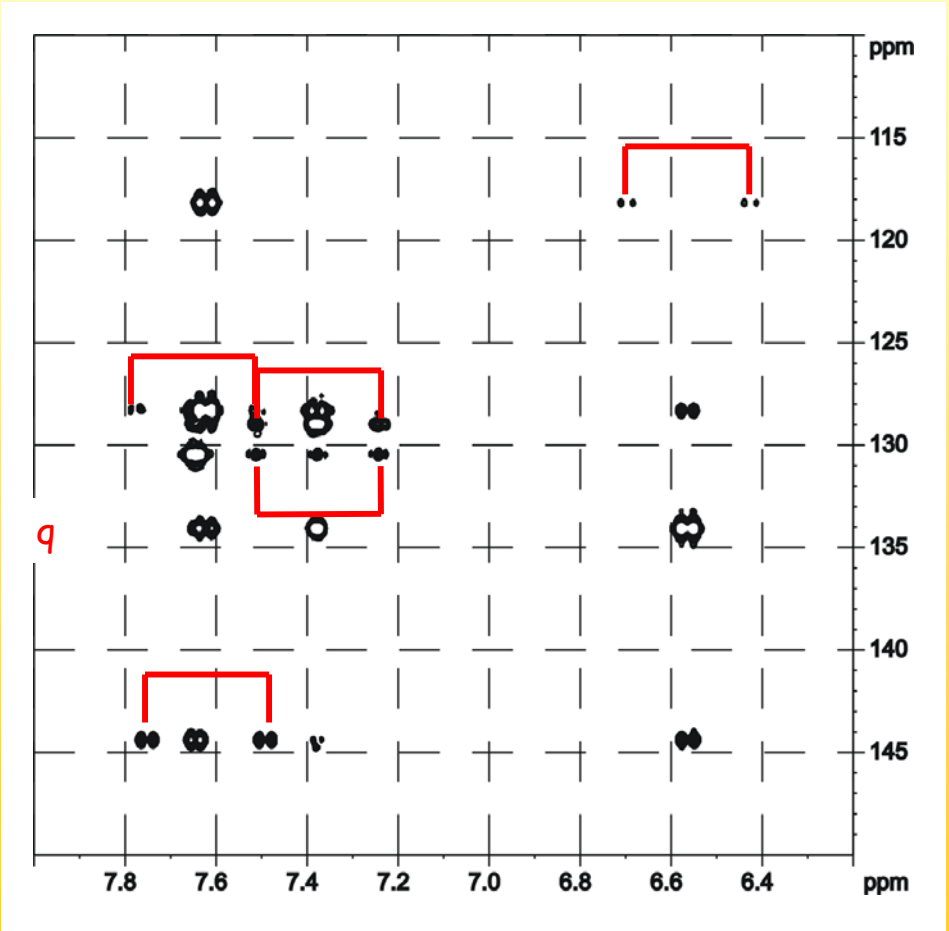


# An „unknown" compound

Now we look at the "crowded" region

Here it helps to assume that $^{3}J$ correlations are more intense than $^{2}J$ and $^{4}J$

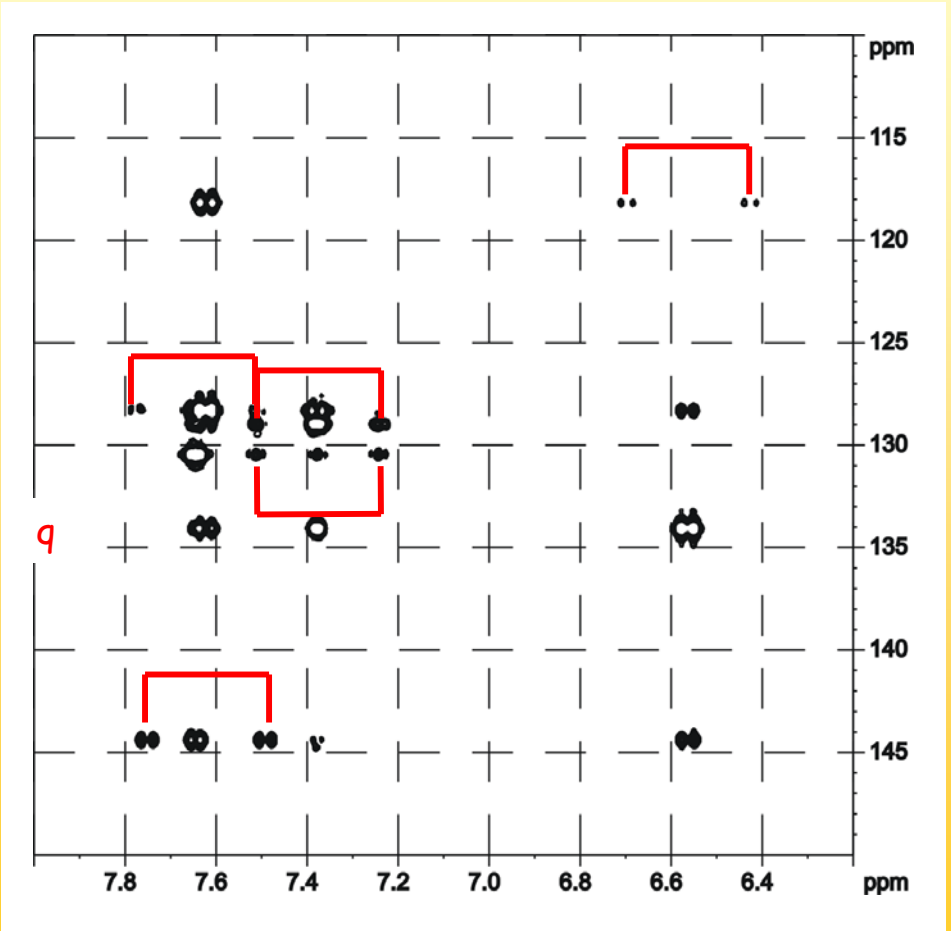
The olefinic protons show correlations to the other olefinic carbon but different pattern for the other correlations



# An „unknown" compound

Now we look at the "crowded" region

In the aromatic rings things are more complicated due to crowding but everything fits with a phenyl-ring



# An „unknown" compound

In the end we put the pieces together

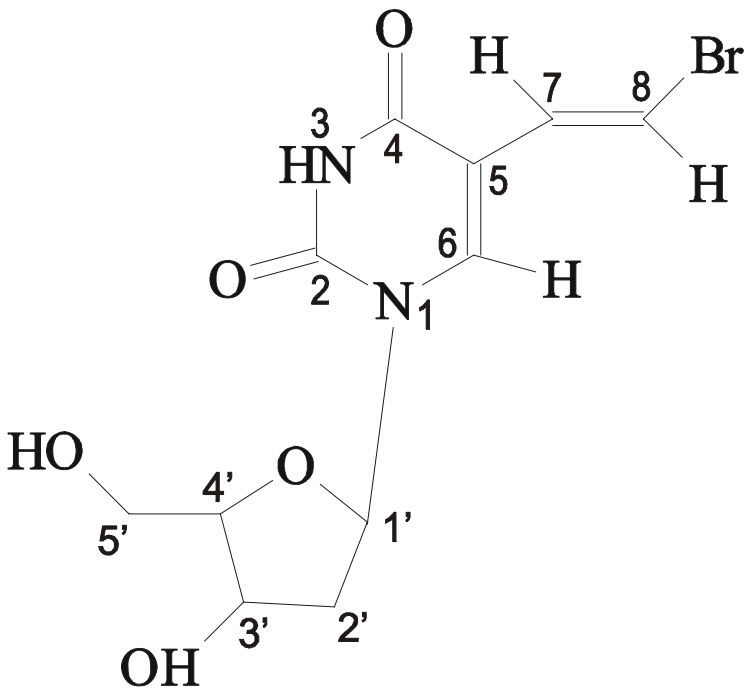
to be revealed in the lecture.....

# A second example:

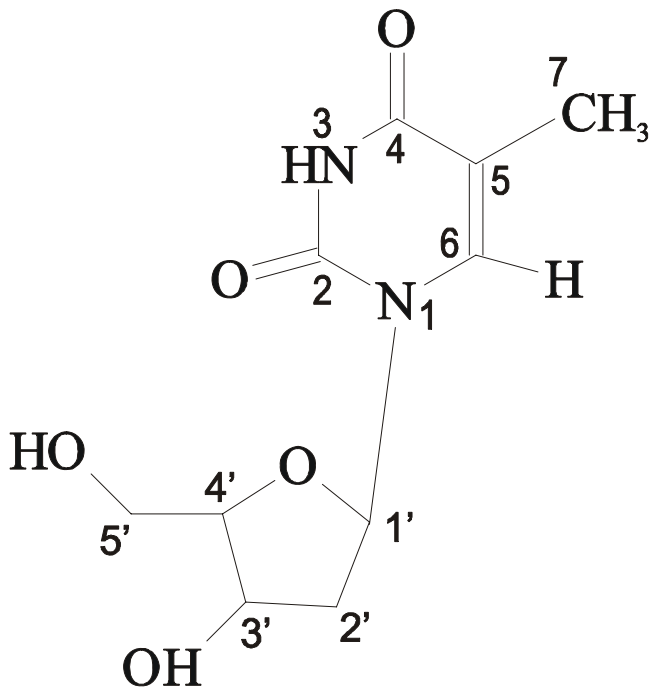
# Privudin

# Application to privudin

## the molecule




$C_{11}H_{13}N_2O_3Br$




Desoxythymidin

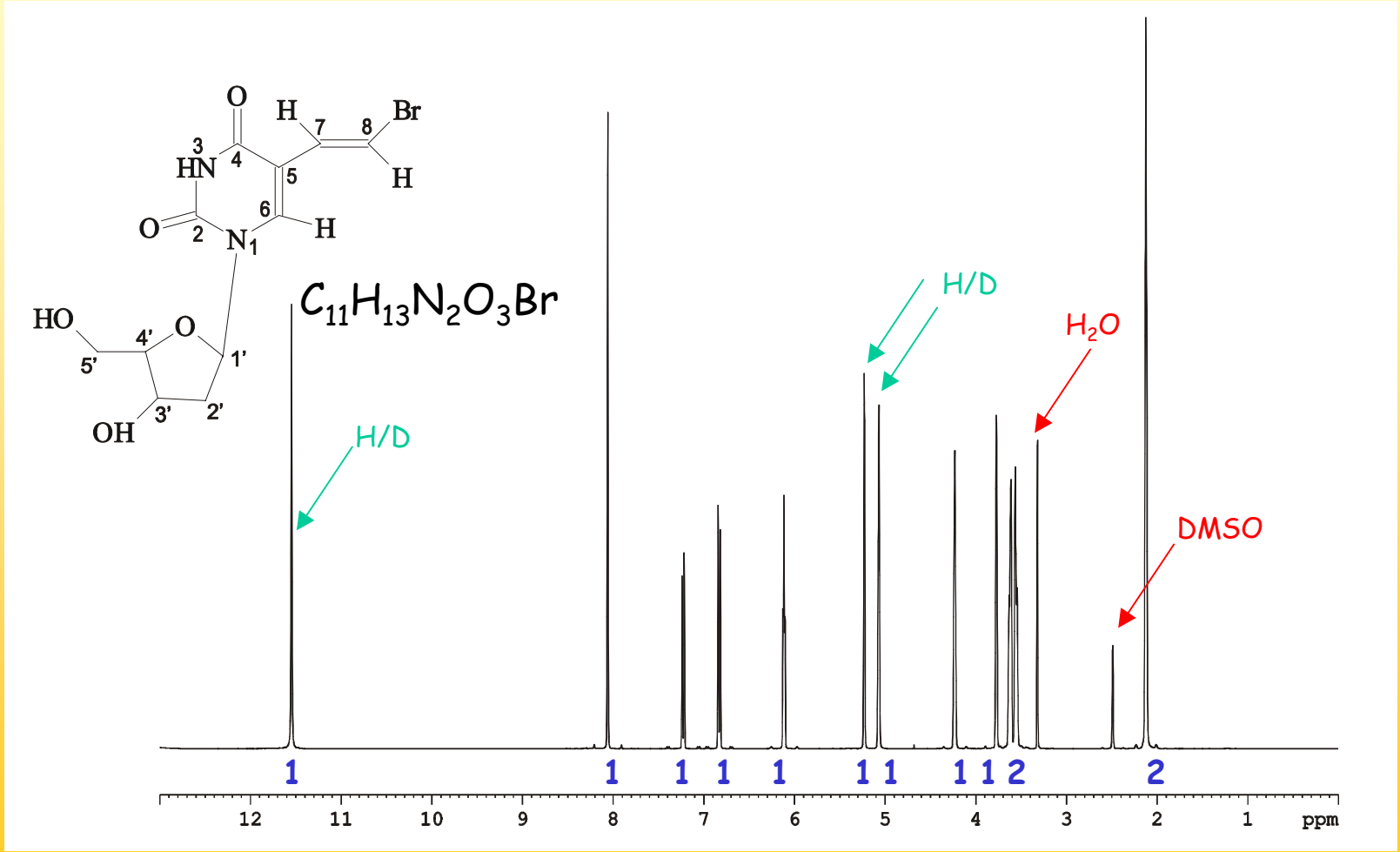
# Application to privudin

## the NMR experiments

| | |
|---|---|
| 1D-$^{1}$H | 1 min |
| 1D-$^{13}$C | 1 h |
| DEPT | 10 min |
| DQF-COSY | 90 min |
| TOCSY | 20 min |
| $^{13}$C-HMQC | 10 min |
| $^{13}$C-HMBC | 50 min |
| $^{15}$N-HMQC | 3 h |
| $^{15}$N-HMBC | 35 h |

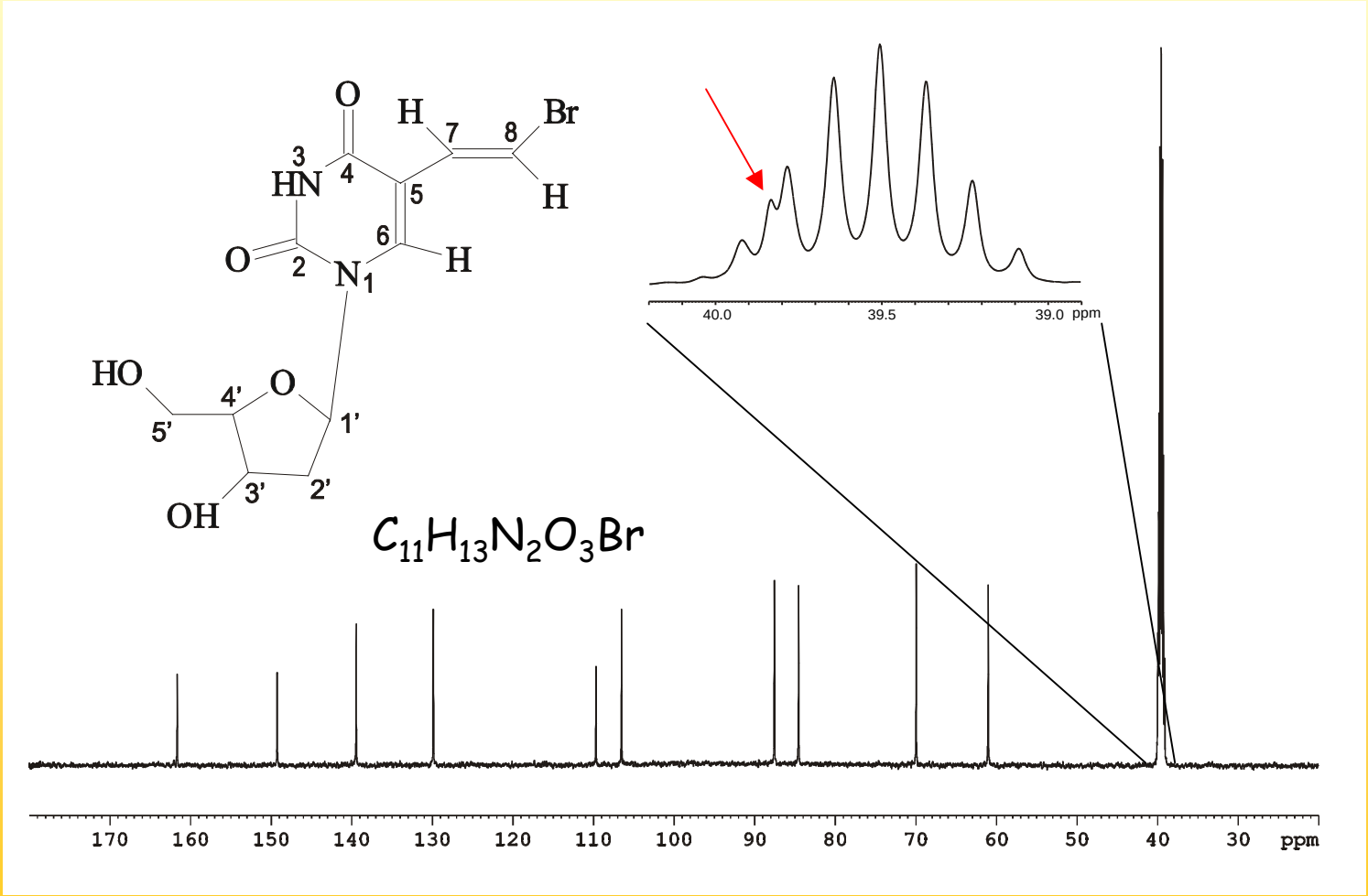
# Application to privudin

## 1D-$^1$H-NMR-spectrum



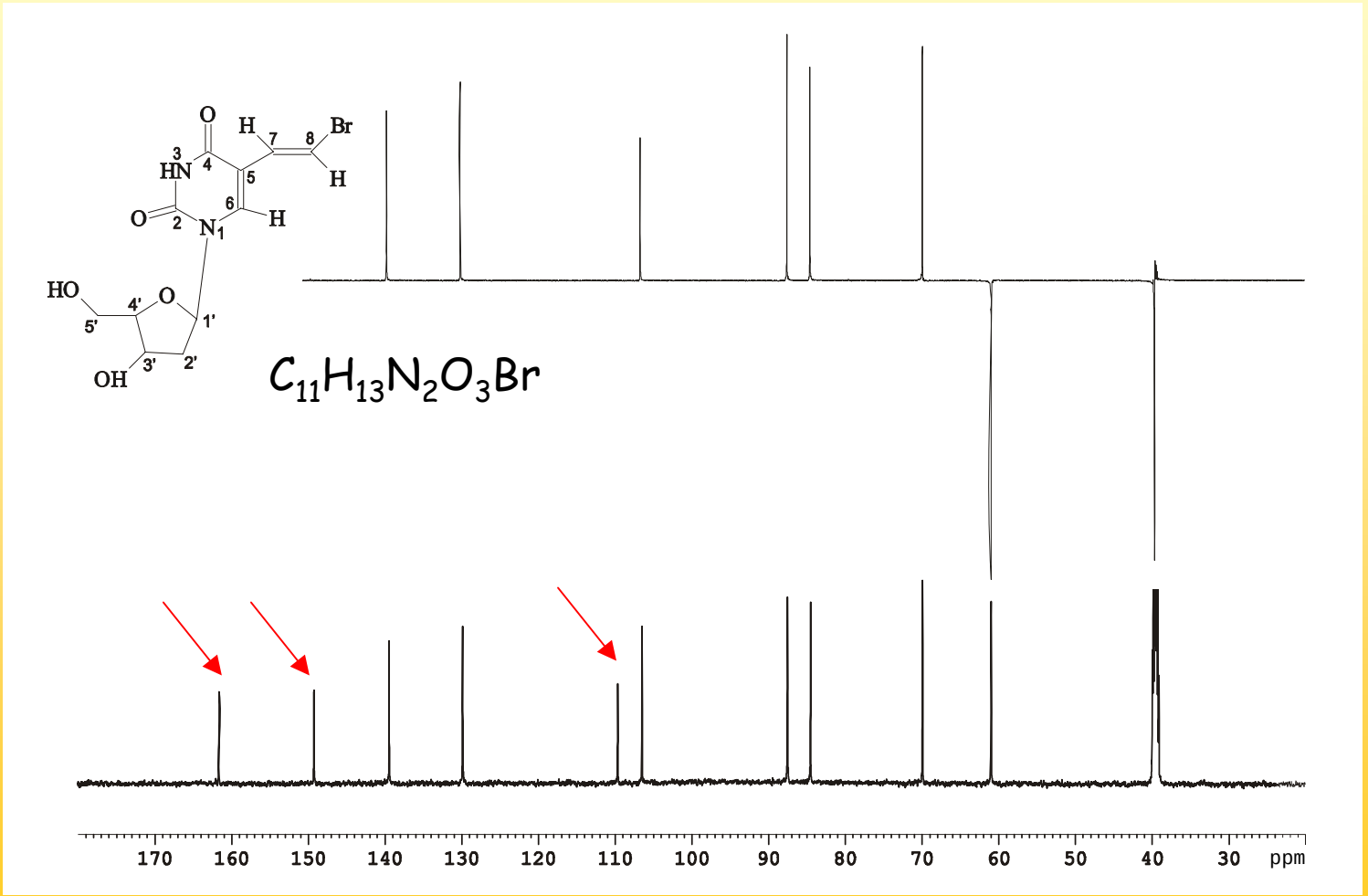
# Application to privudin

## 1D-$^{13}$C-NMR-spectrum

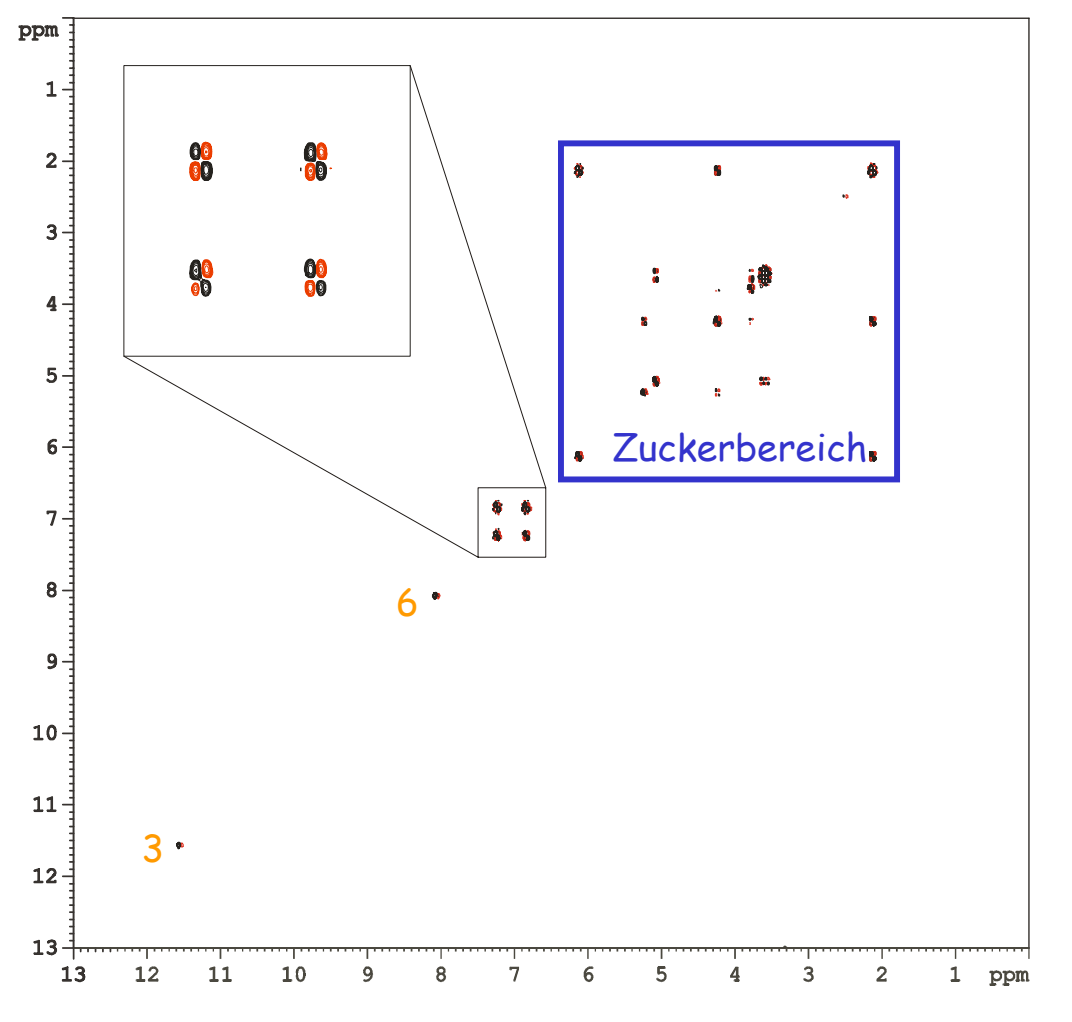


# Application to privudin

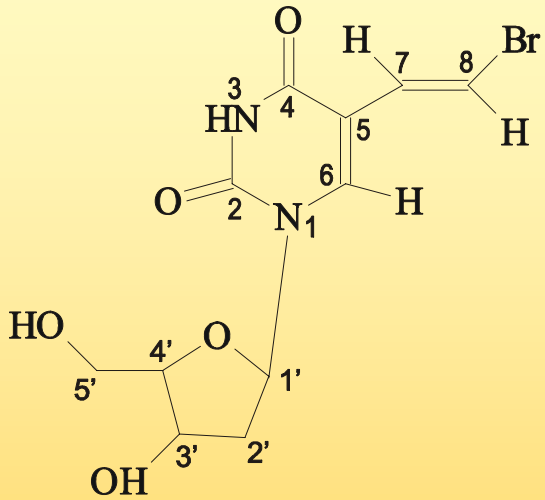
## DEPT-spectrum



# Application to privudin

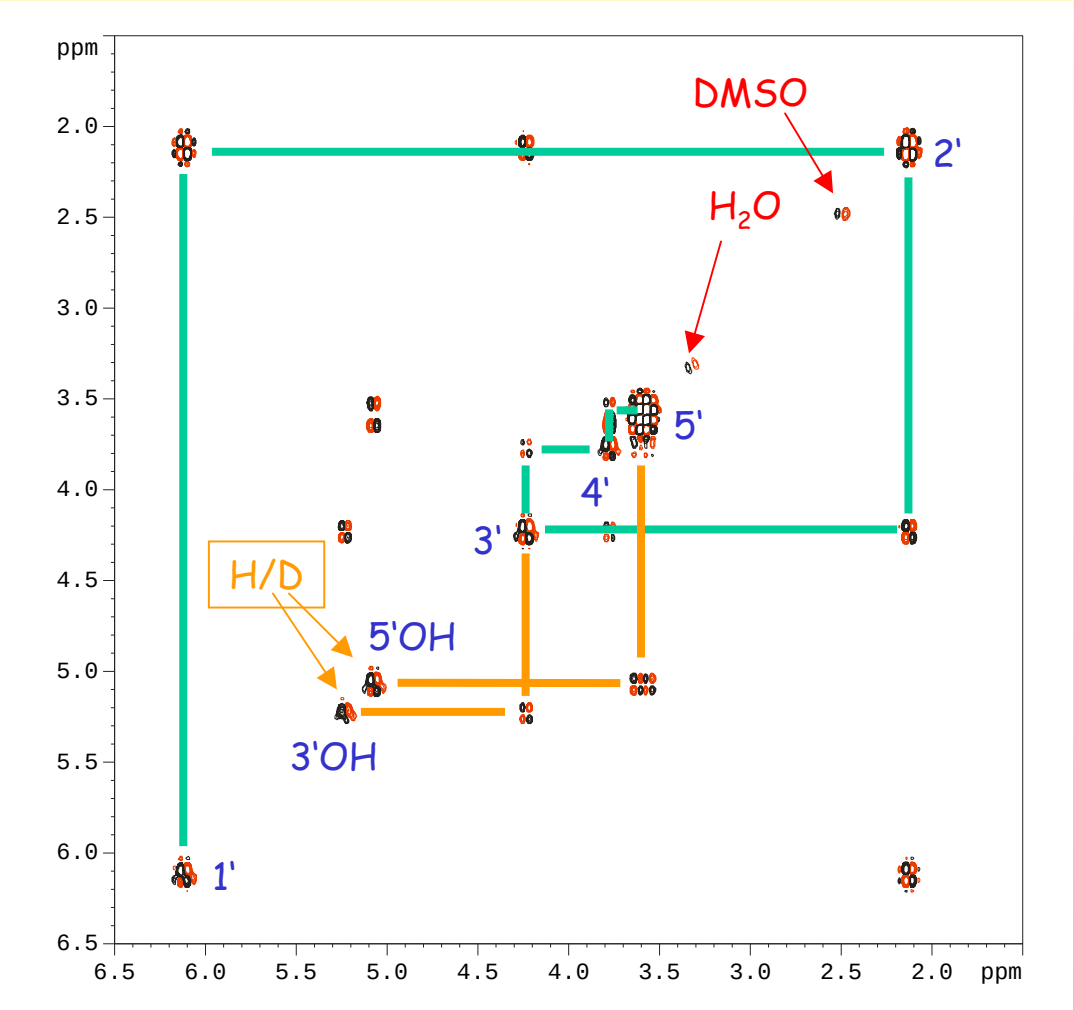



## DQF-COSY

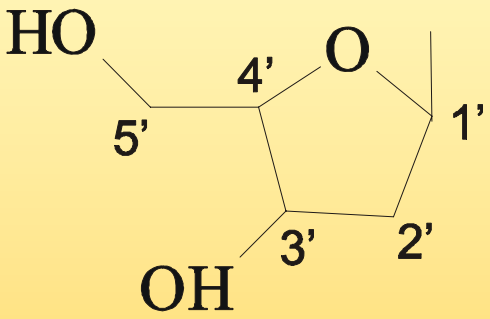



Correlation of frequencies via $^{1}H$-$^{1}H$ scalar couplings, only two or three-bond couplings give crosspeaks

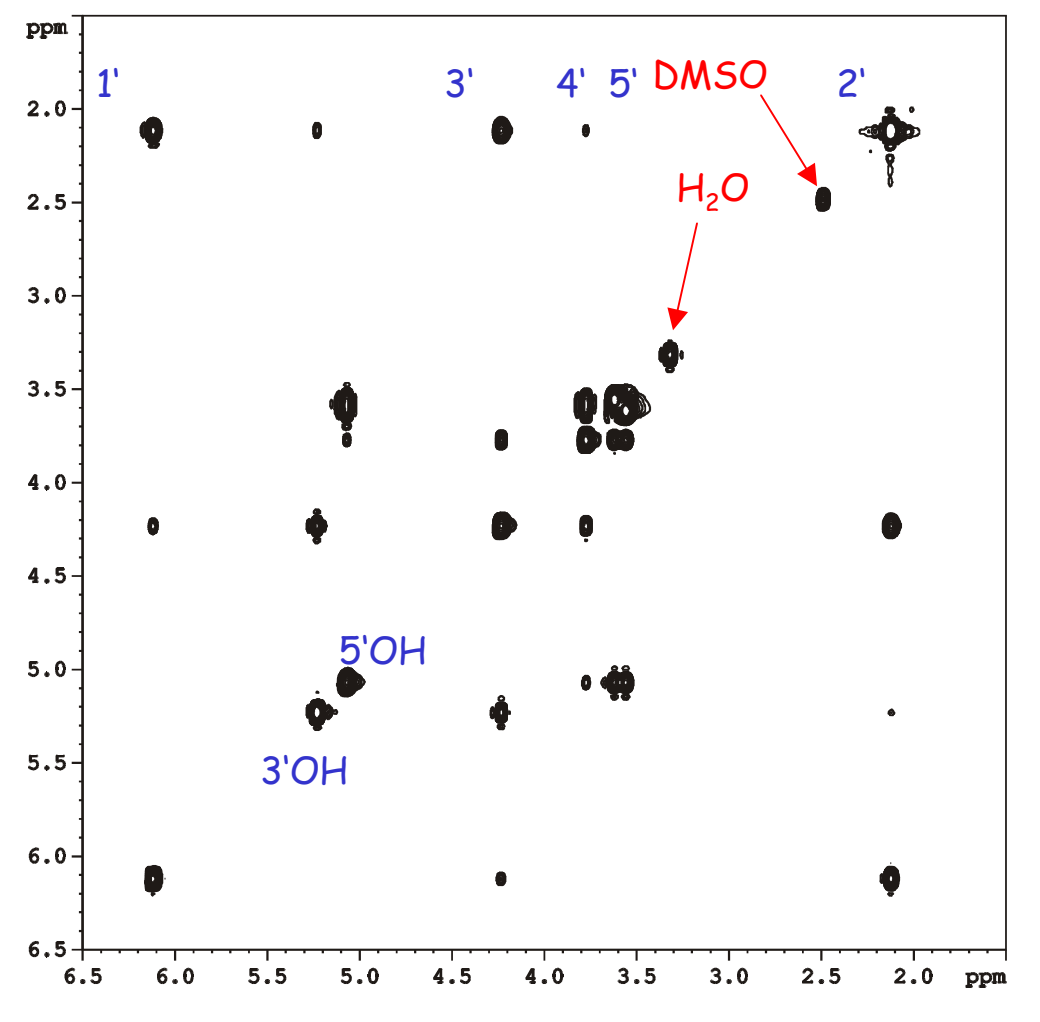
# Application to privudin



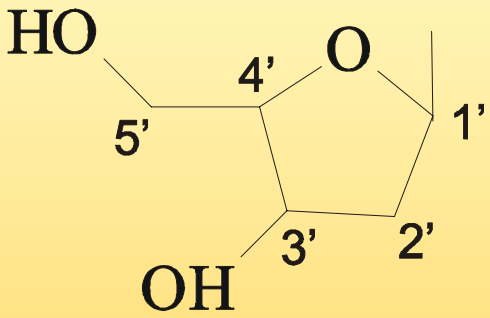

DQF-COSY



# Application to privudin

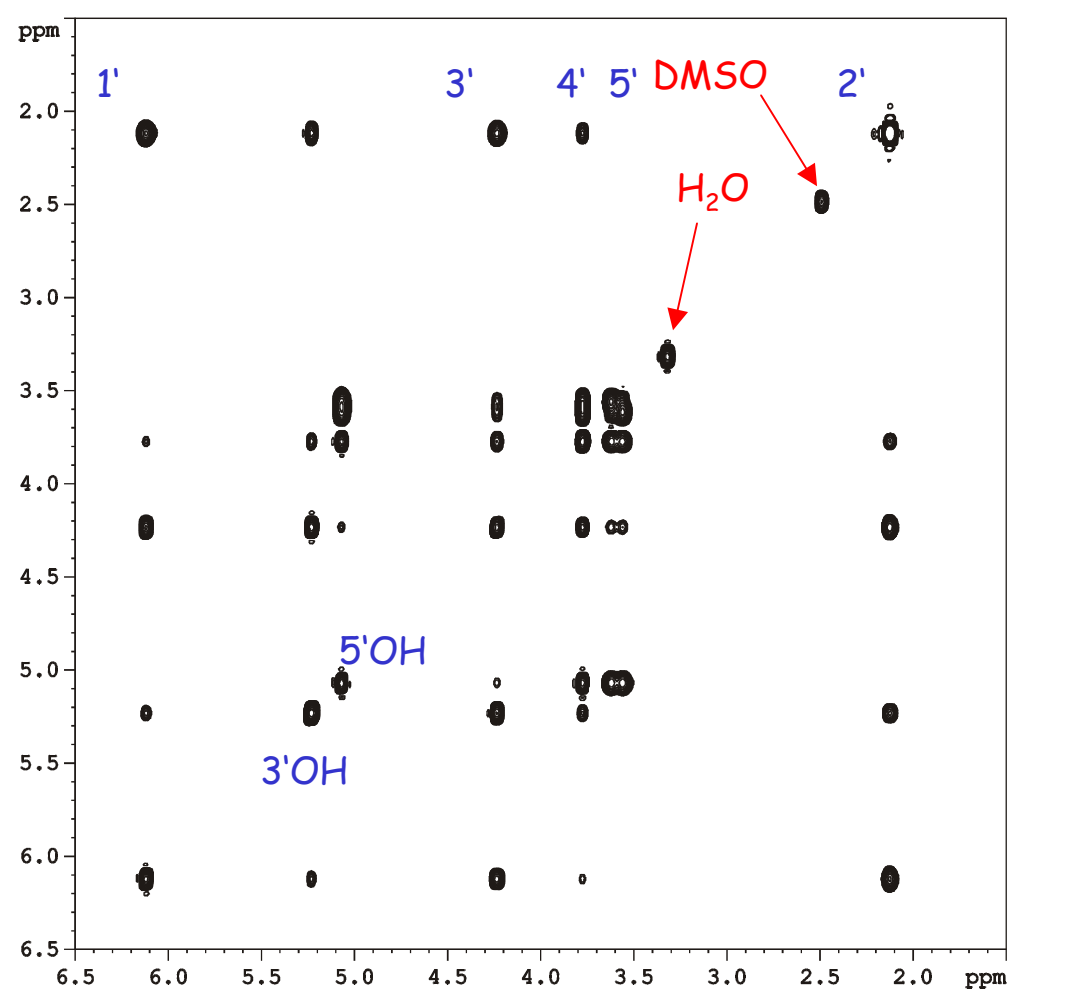



## TOCSY (short mixing time)

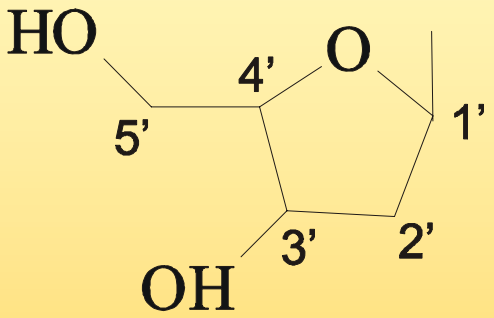



Correlation of frequencies via $^1H$-$^1H$ scalar couplings, several transfersteps are possible

# Application to privudin

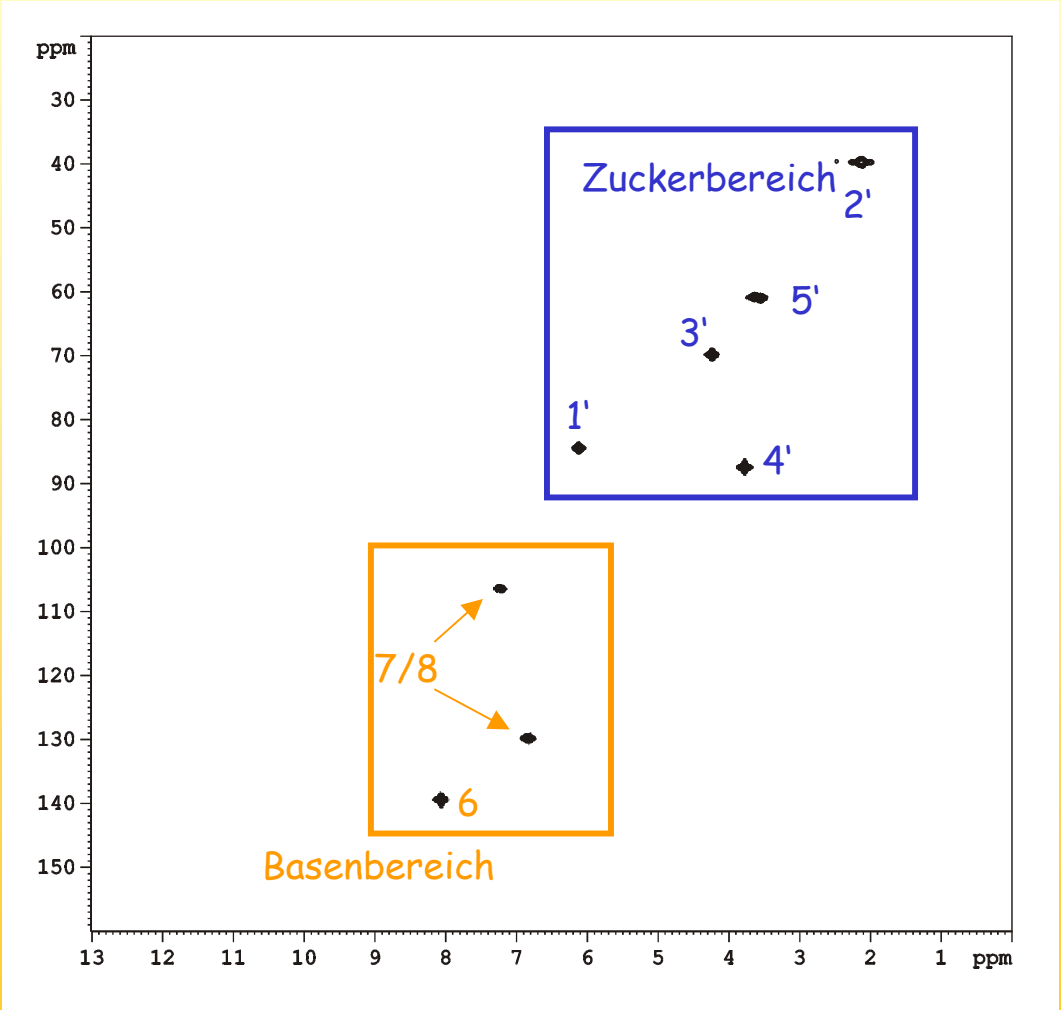



## TOCSY (long mixing time)

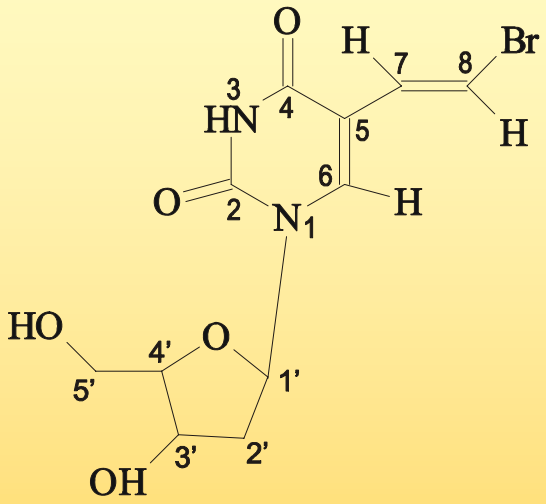



Correlation of frequencies via $^{1}H$-$^{1}H$ scalar couplings, several transfersteps are possible

# Application to privudin

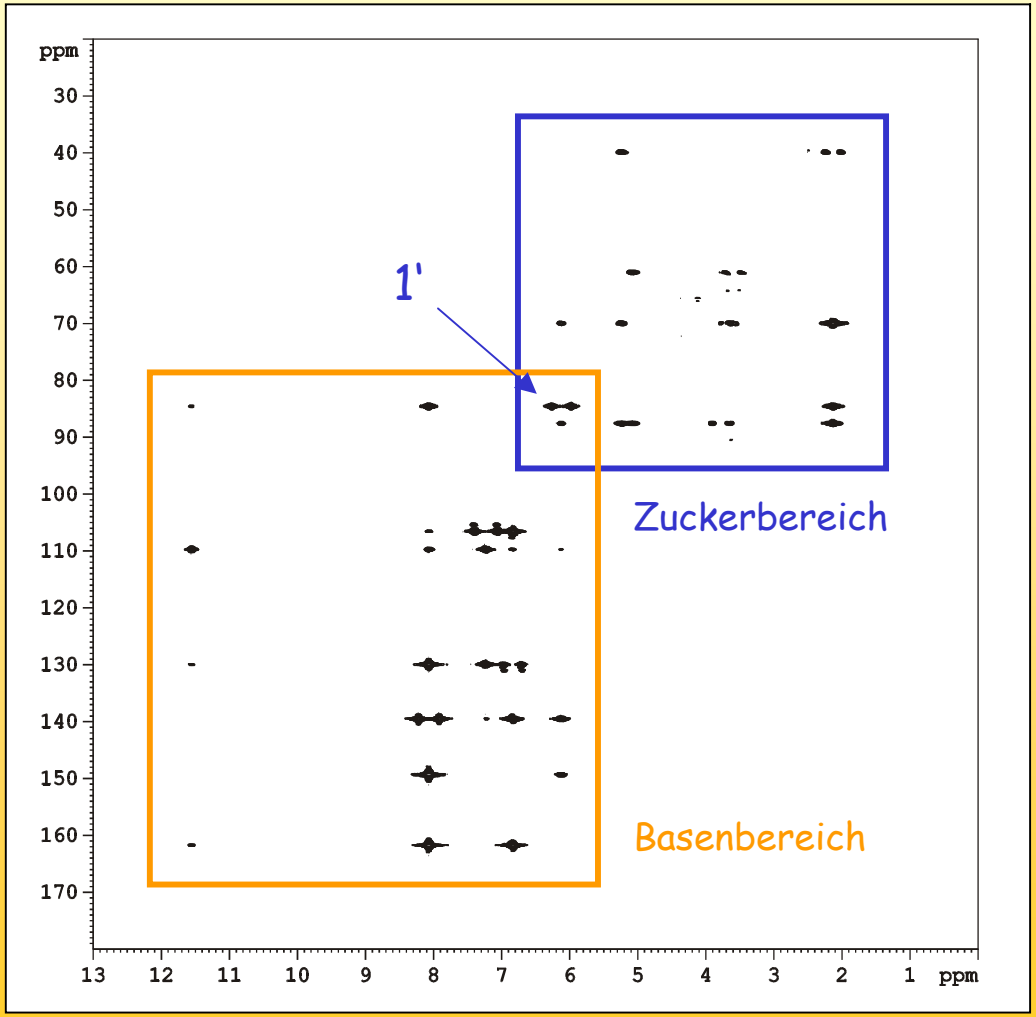



## $^{13}C$-HMQC

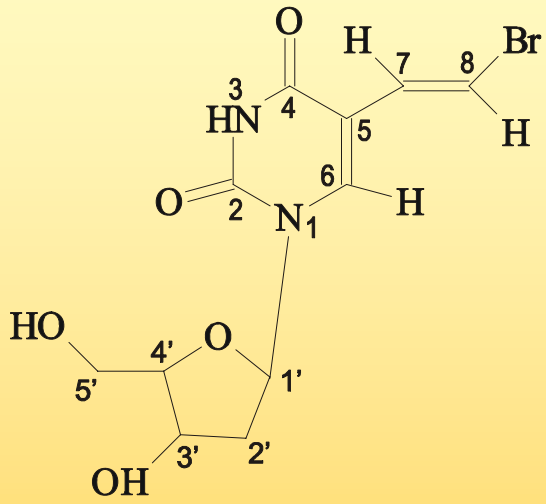



Correlation of frequencies via $^{1}H$-$^{13}C$ scalar couplings, only directly bound nuclei give correlations

# Application to privudin

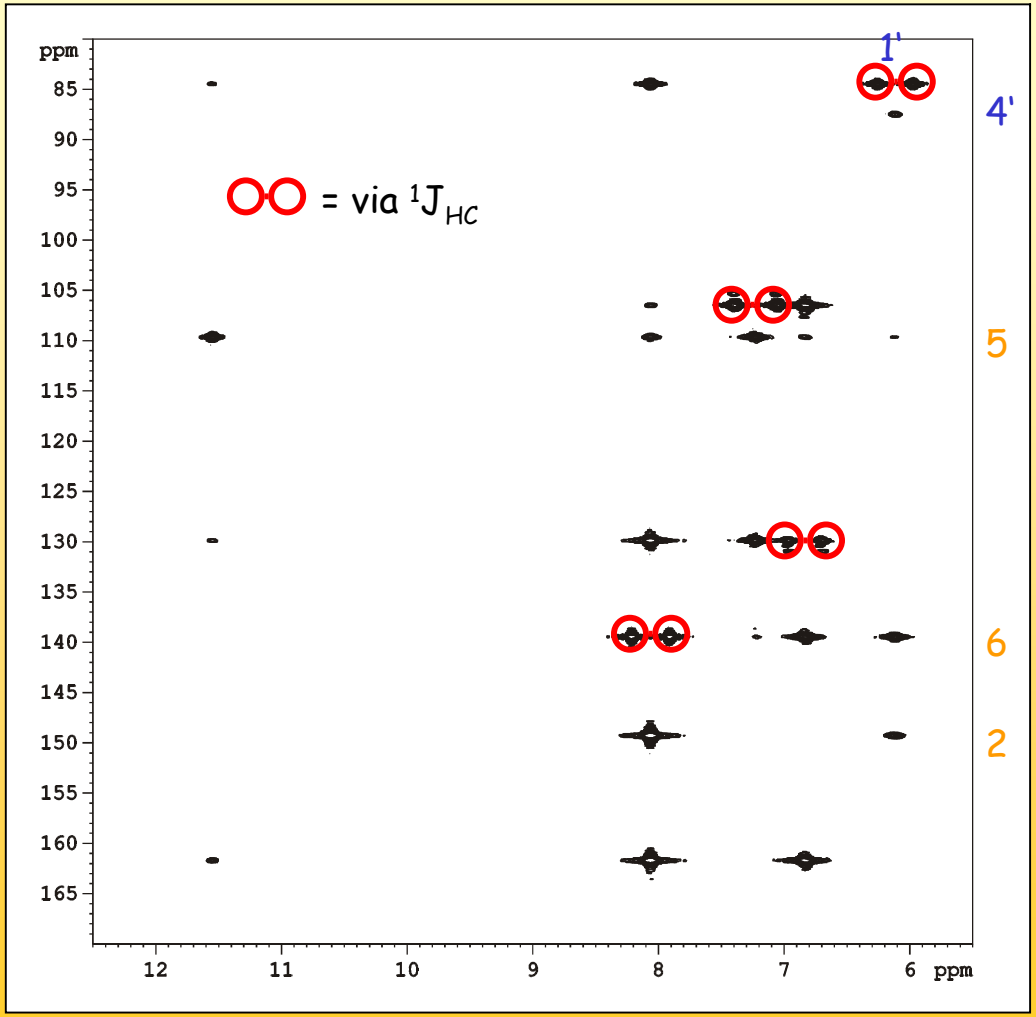



## $^{13}C$-HMBC

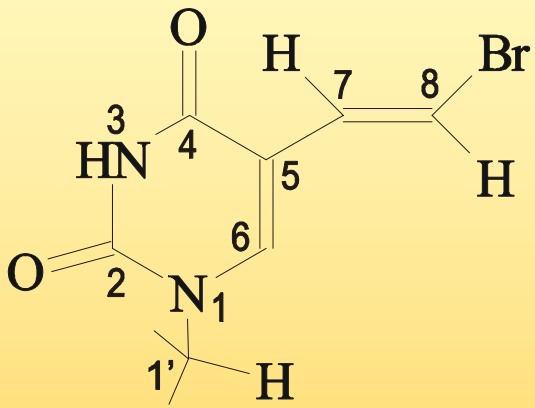



Correlation of frequencies via $^{1}H$-$^{13}C$ scalar couplings, correlations via up to four bonds ($^{2}J_{HC}$ to $^{4}J_{HC}$), $^{1}J_{HC}$ gives a doublett

# Application to privudin

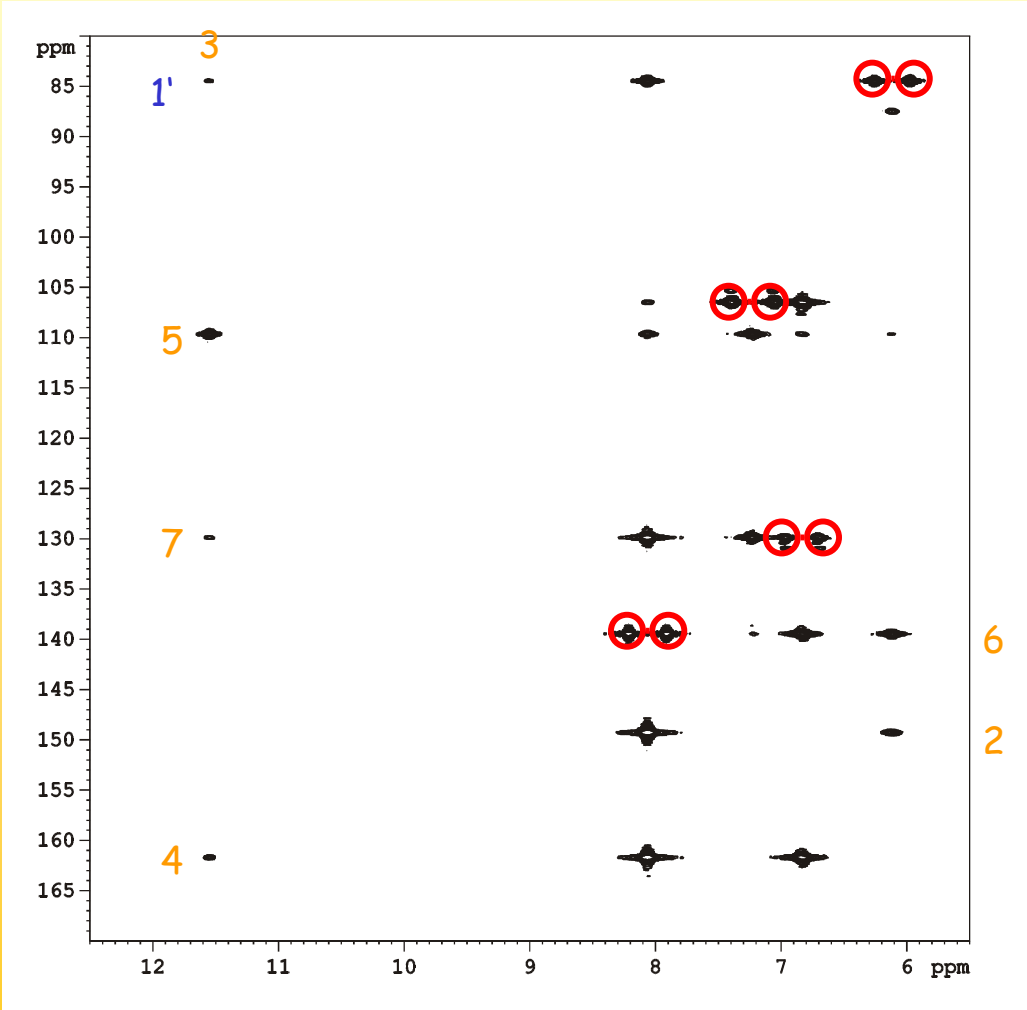



## $^{13}$C-HMBC (region of the base signals)

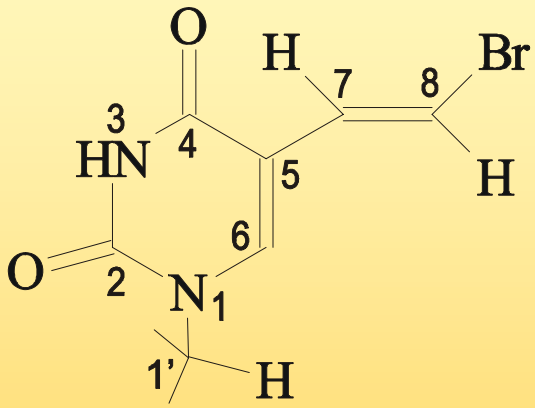



Correlations from H1': correlation to 5 is weak (4 bond) the other two are 3 bond, 6 and 2 can be distinguished by the proton attached

# Application to privudin

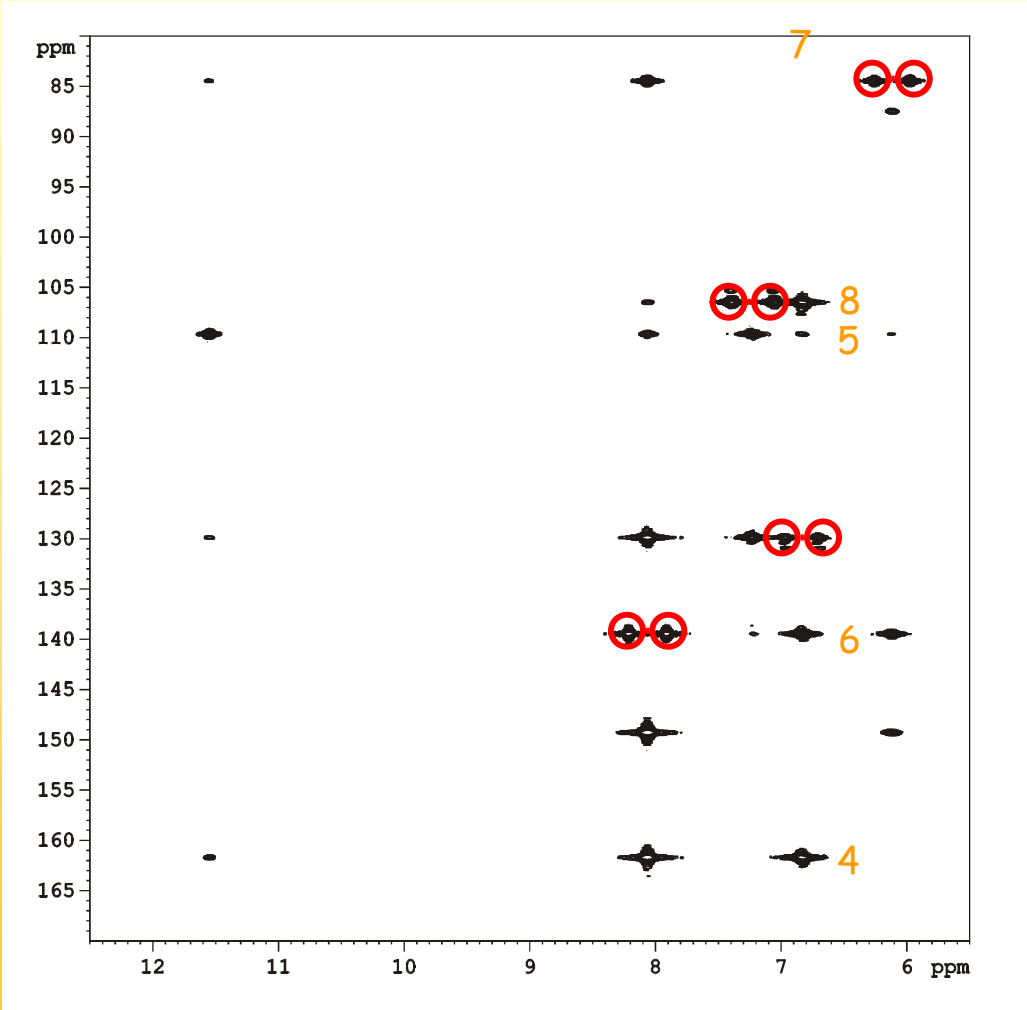



## $^{13}$C-HMBC (region of the base signals)

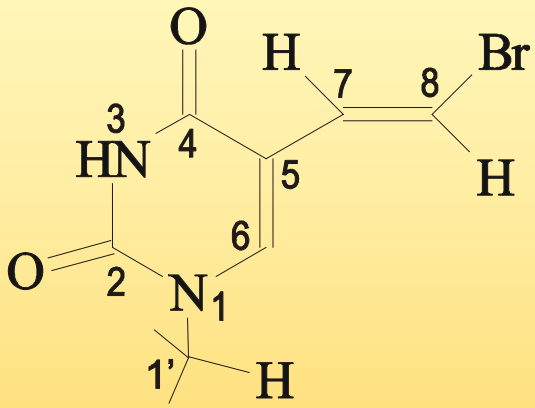



Correlations from H3: correlation to 7 is weak (4 bond), to 5 is 3 bond, the one to 2 is missing (why ?) thus the other one must be 4

# Application to privudin

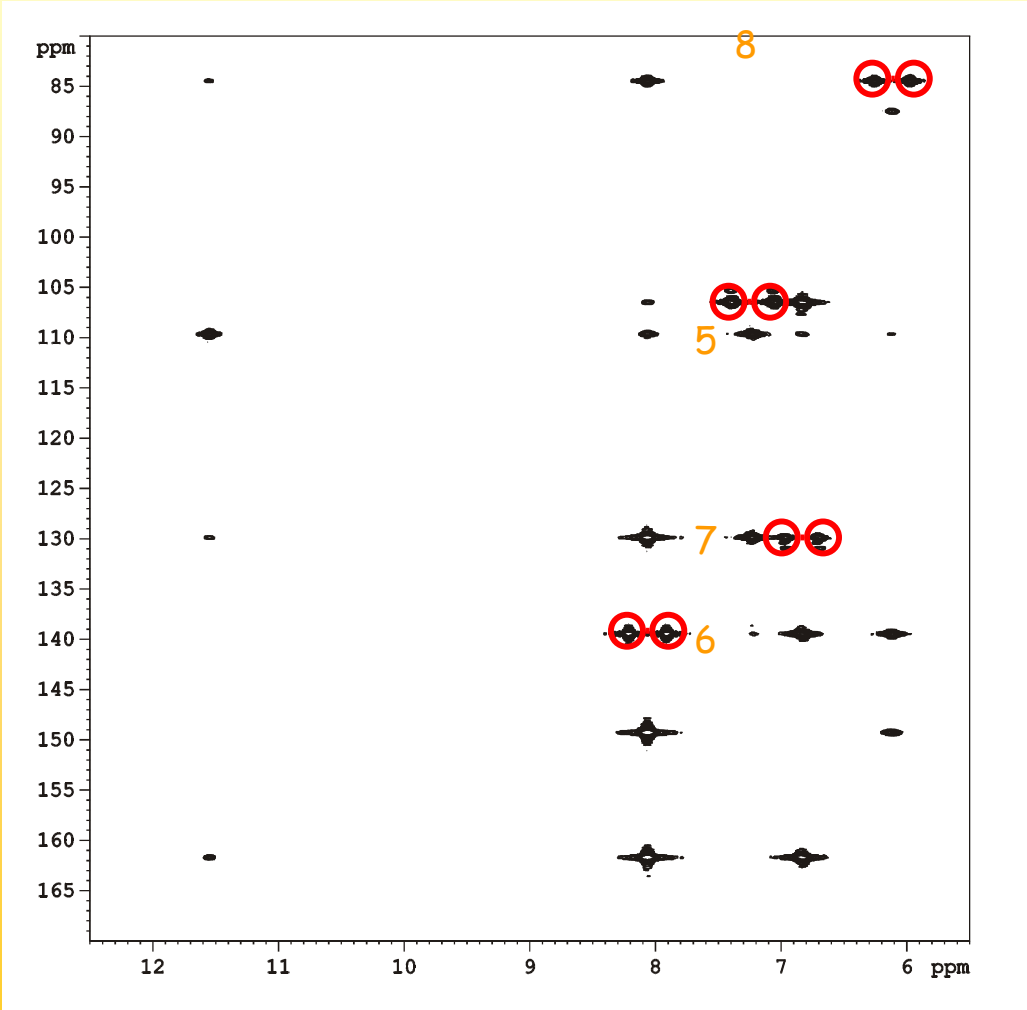



## $^{13}$C-HMBC (region of the base signals)

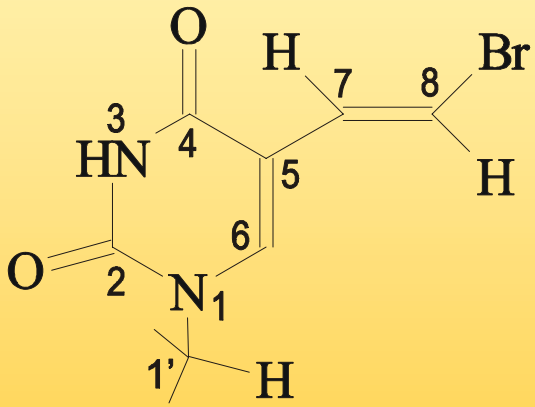



Correlations from H7: correlation to 6 and 4 are 3 bond, the one to 5 is 2 bond, those are often weaker than 3 bond

# Application to privudin

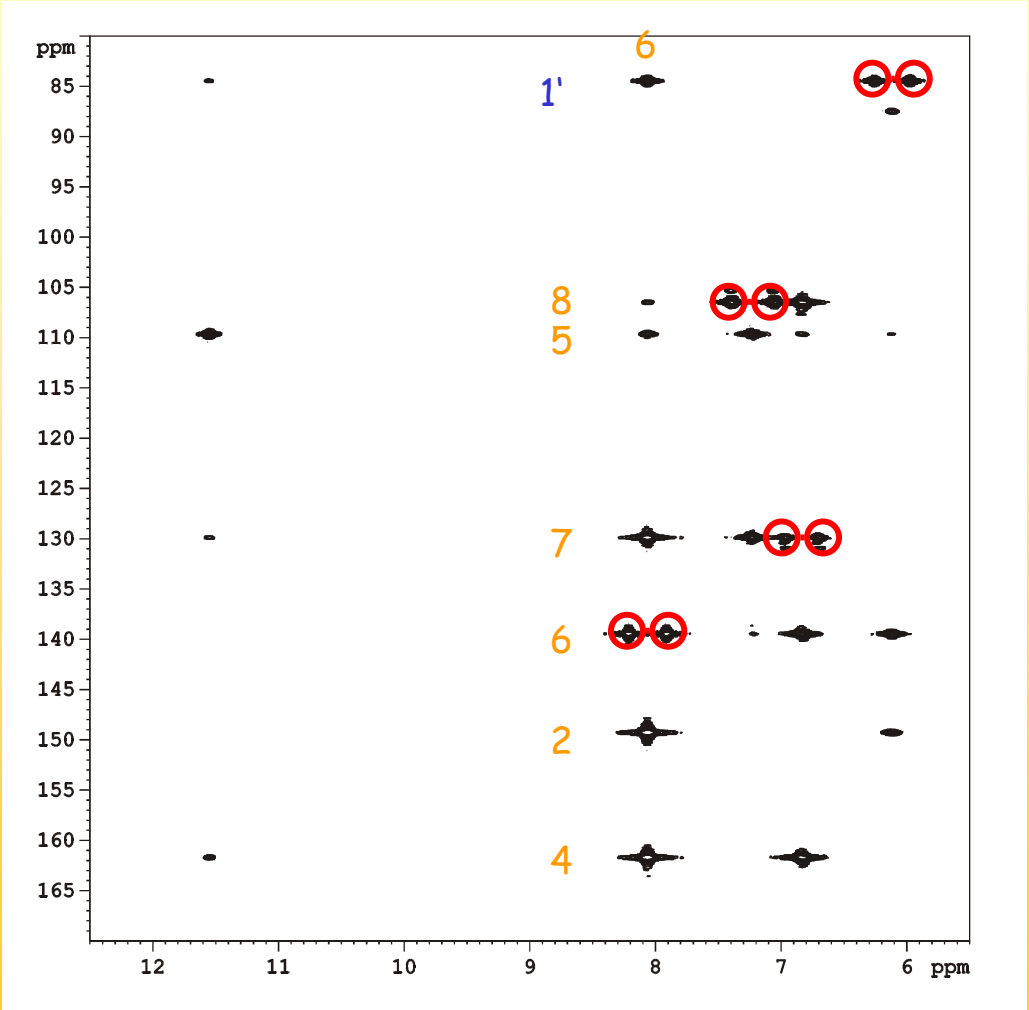



$^{13}$C-HMBC (region of the base signals)

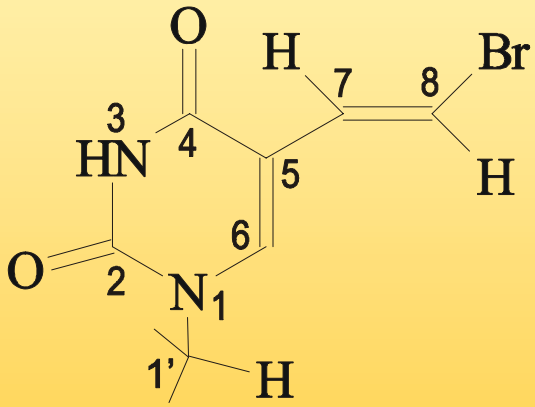



Correlations from H8

# Application to privudin

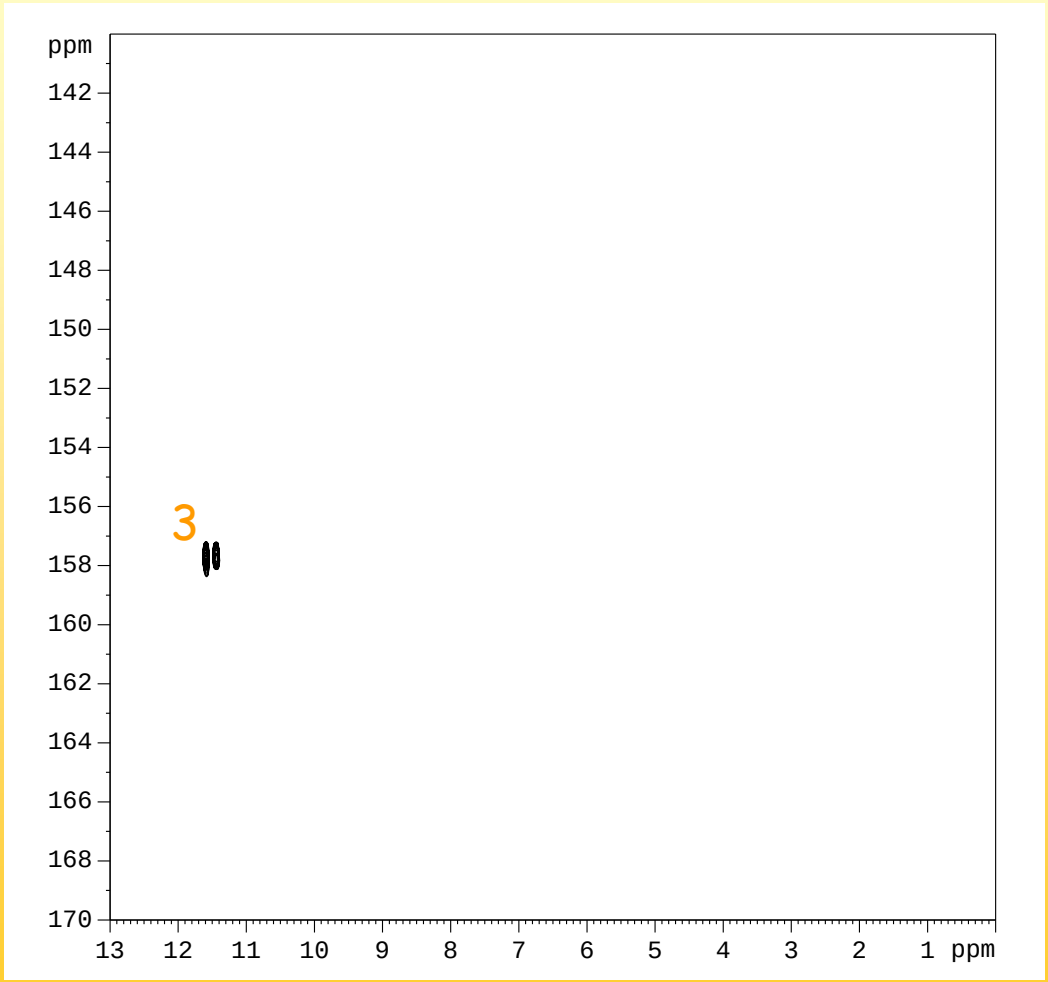



## $^{13}$C-HMBC (region of the base signals)

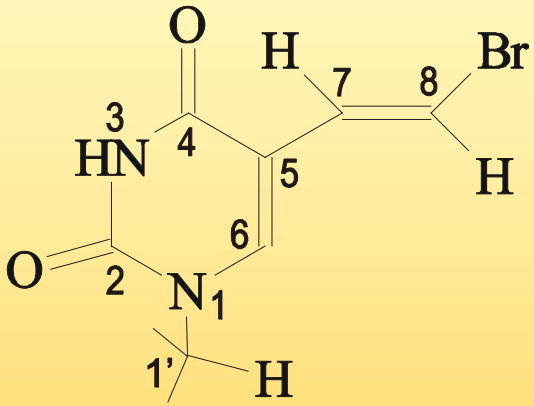



Correlations from H6

# Application to privudin

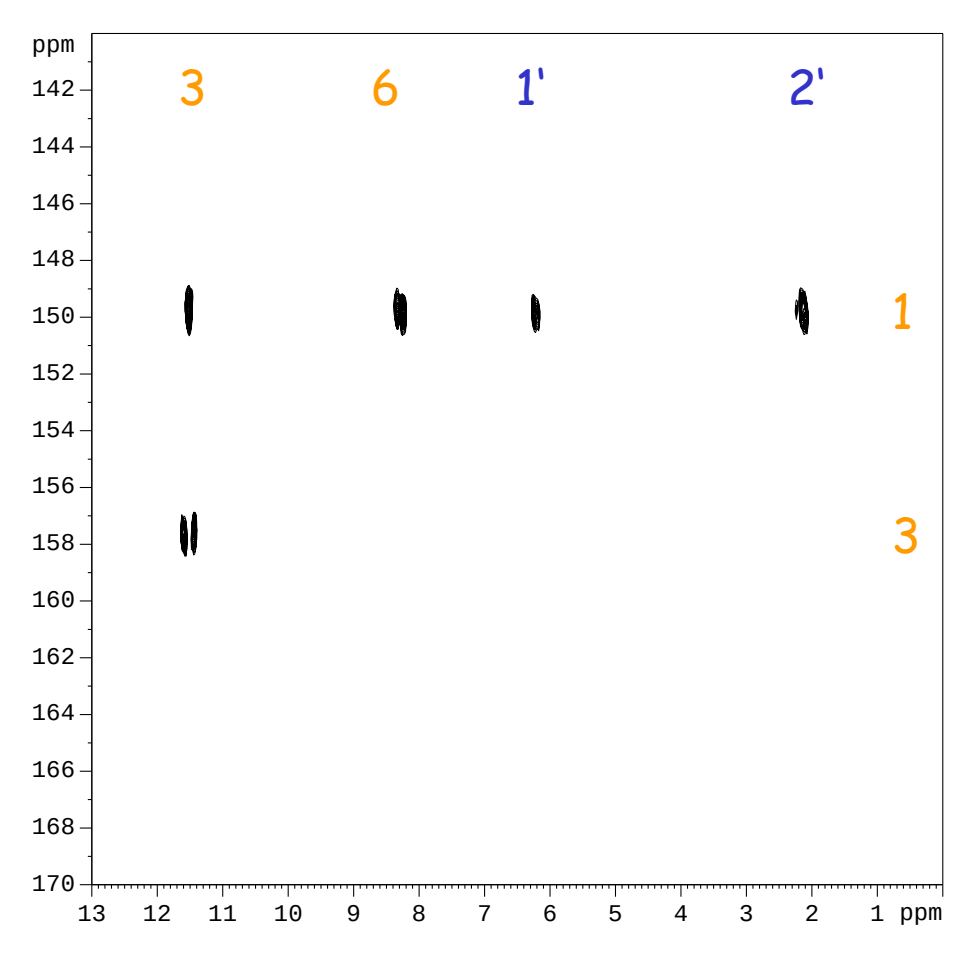



## $^{15}$N-HMQC

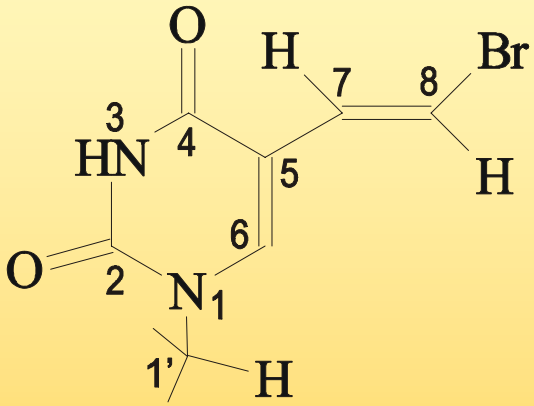



Correlation of frequencies via $^{1}$H-$^{15}$N scalar couplings, only directly bound nuclei give correlations

# Application to privudin




## $^{15}$N-HMBC




Correlation of frequencies via $^{1}$H-$^{15}$N scalar couplings, correlations via up to four bonds ($^{2}J_{HN}$ to $^{4}J_{HN}$), $^{1}J_{HN}$ gives a doublett

# Application to privudin

## Assignment

| | $^{1}H$ [ppm] | $^{13}C$ [ppm] | $^{15}N$ [ppm] |
|---|---|---|---|
| | | | |
| 1' | 6,16 | 84,5 | - |
| 2' | 2,17 | 39,8 | - |
| 3' | 4,27 | 69,9 | - |
| 4' | 3,79 | 87,5 | - |
| 5' | 3,62 | 61 | - |
| 1 | - | - | 149,7 |
| 2 | - | 149,2 | - |
| 3 | 11,92 | - | 157,7 |
| 4 | - | 161,6 | - |
| 5 | - | 109,7 | - |
| 6 | 8,09 | 139,4 | - |
| 7 | 6,87 | 129,9 | - |
| 8 | 7,25 | 106,5 | - |

| | $^{13}C$ [ppm] | $^{13}C$ [ppm] | $^{15}N$ [ppm] |
|---|---|---|---|
| | predicted | dT | dT |
| 1' | 77,8 ± 8,2 | 85,1 | - |
| 2' | 39,9 ± 5,1 | 40,4 | - |
| 3' | 68,7 ± 1,6 | 71,7 | - |
| 4' | 74,8 ± 9,2 | 88,4 | - |
| 5' | 63,8 ± 0,3 | 62,4 | - |
| 1 | - | - | 142,7 |
| 2 | 156,8 ± 10,6 | 151,6 | - |
| 3 | - | - | 153,5 |
| 4 | 135,7 ± 6,9 | 137,3 | - |
| 5 | 117,5 ± 3,1 | 110,5 | - |
| 6 | 135,7 ± 11,4 | 164,9 | - |
| 7 | 132,6 ± 6,9 | - | - |
| 8 | 89,4 ± 15,8 | - | - |

# That's it

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