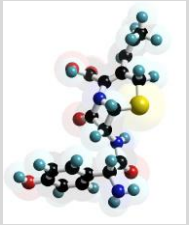
University of Oxford
Doctoral Training Centre

NMR Spectroscopy

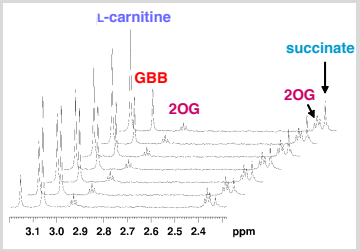
An introduction to NMR methods for Synthetic Chemistry

NMR Spectroscopy

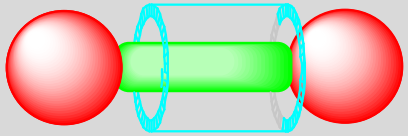
Structure elucidation



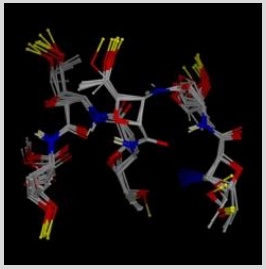
Reaction kinetics



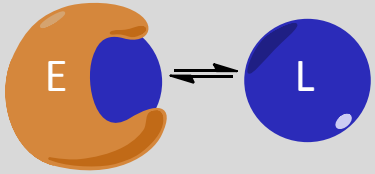
Molecular associations



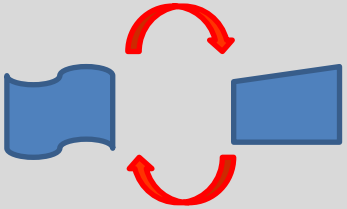
Molecular conformation



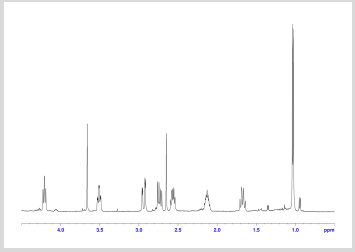
Protein-ligand interactions



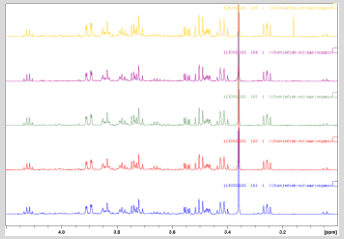
Conformational dynamics



Sample purity



Metabolomics



Using NMR spectroscopy to support synthetic chemistry

- *Elucidation* vs *verification*
  - Determining the structure of a complete unknown is referred to as *structure elucidation* (e.g. a novel natural product).
  - Confirming a structure that is proposed based on prior knowledge is known as *structure verification* (e.g. a synthetic product)
- Identifying a structure involves correlating physical data with features of the structure.
  - we typically speak of *spectrum assignment:* matching spectral features to a structure
  - *or sample characterisation:* collating data for a molecule that provides evidence for the structure.
  - These should ultimately lead to the same overall conclusion.
- Full characterisation is usually required for publication and demands high quality samples to yield good analytical data.

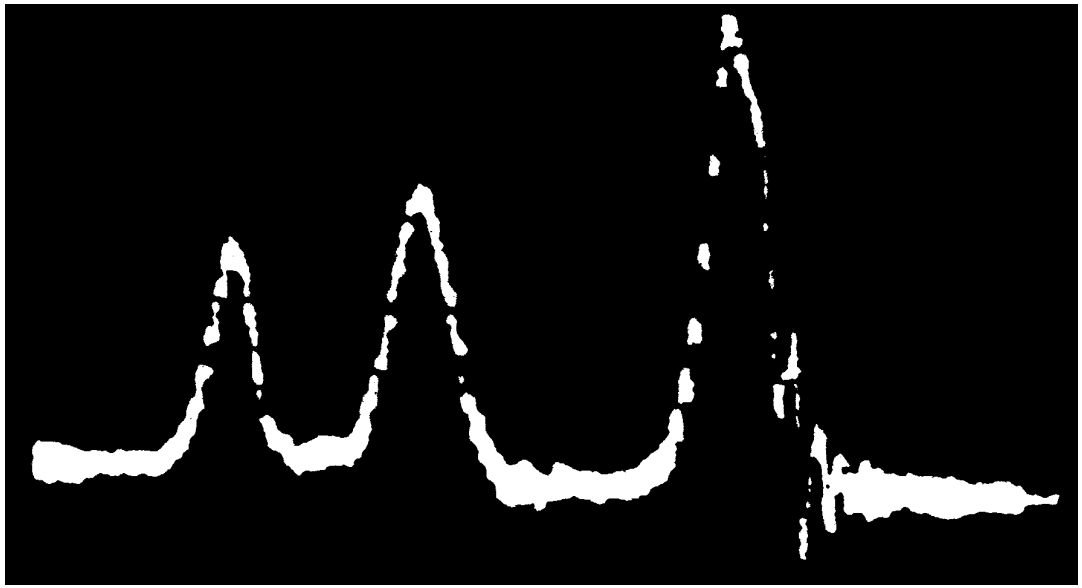
Using NMR spectroscopy to support synthetic chemistry

- Everything starts with ^{1}H NMR…
- In verification, if the ^{1}H NMR doesn't look right, stop and think…
  - Presence of starting materials?
  - Presence of solvents?
  - What features do match what was expected?
  - What other features are present and what do they tell you?
  - What are the most likely other reactions to have occurred- evidence for these?
  - Might the spectrum be easier to interpret in another solvent?
  - Do you need more data to work out what has happened?
- If the ^{1}H spectrum looks consistent, stop and think….
  - Do I need further data, or is this evidence enough to continue?
  - Is the sample clean enough, concentrated enough, important enough to need more?
  - What other experiments would help me to verify the structure?
  - Do I need to obtain full characterisation data (for publication)?

Chemical NMR spectroscopy

1951: First published “high-resolution” NMR spectrum:

Hydrogen- Neat ethanol @ 30 MHz

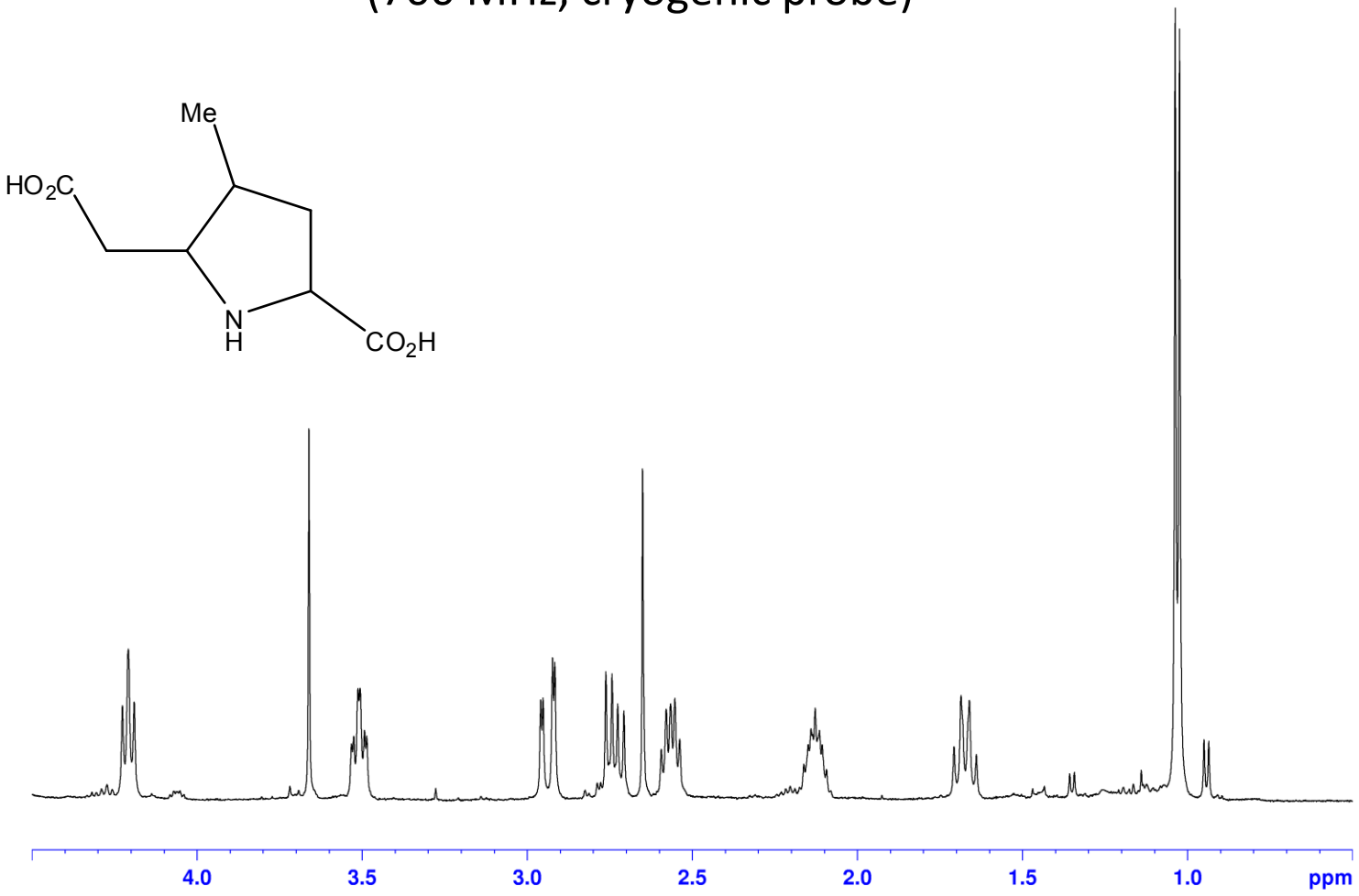


$HO\text{-}CH_2\text{-}CH_3$

Arnold, Dharmatti & Packard
J. Chem. Phys., 1951, 19, 507.

and now…

10 mg incubation product from antibiotic biosynthesis pathway
(700 MHz, cryogenic probe)



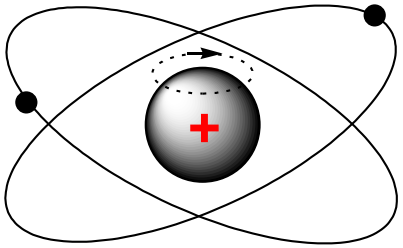
What is “NMR spectroscopy”?

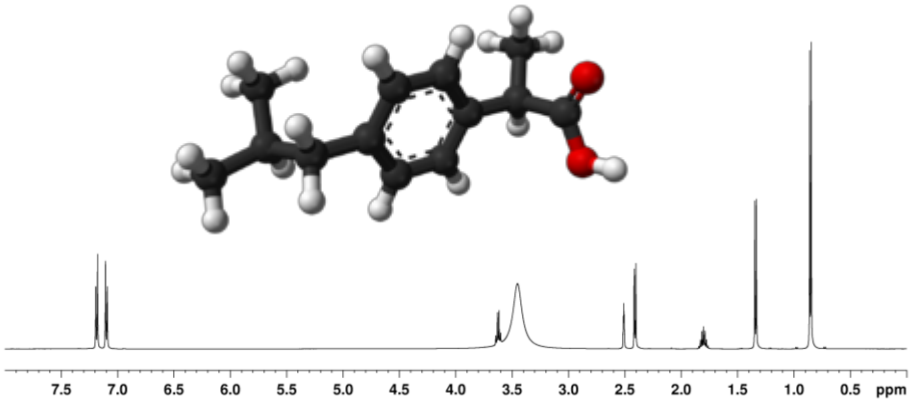
Nuclear- dealing with the properties of nuclei (“spin”)

Magnetic- interaction of nuclear spins with applied magnetic fields

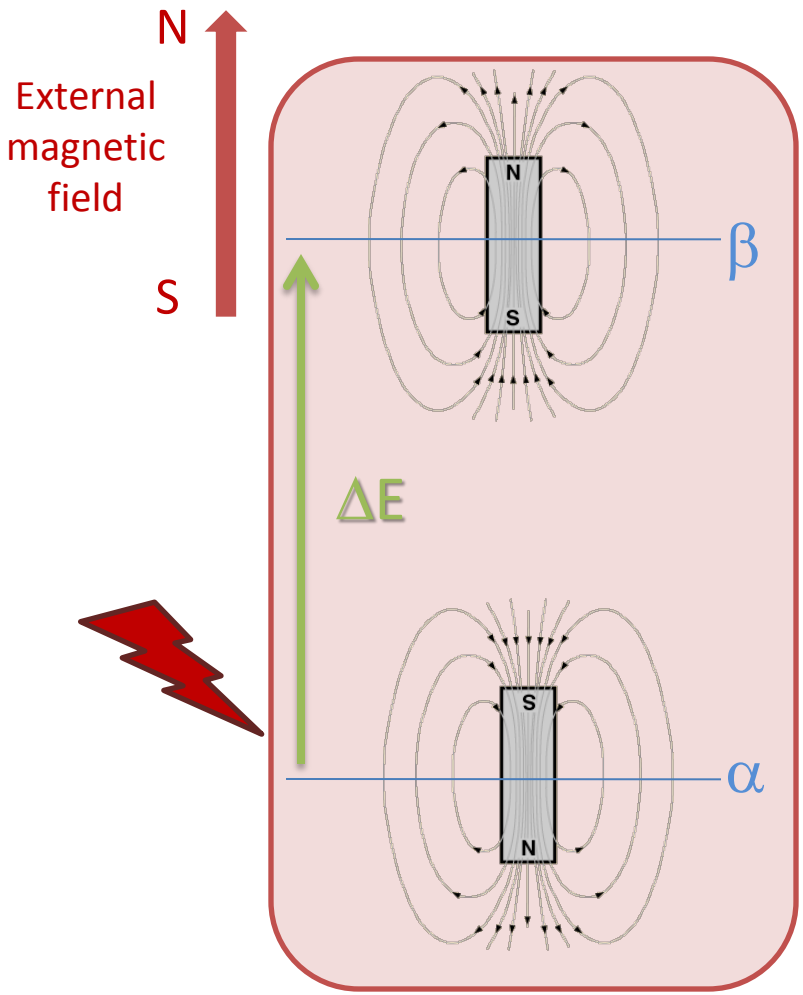
Resonance- excitation of these nuclear spin states

Spectroscopy- through interaction of electro*magnetic* irradiation

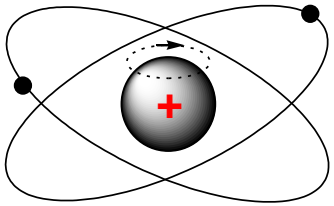




Nuclear Spin and Resonance



spin



"magnetic moment"

Pulse of electromagnetic energy ΔE

$\Delta E = h\nu$

ν = frequency

h = Planck's constant

Only nuclei that posses "spin" can give rise to NMR spectra…

Hydrogen-1 = Yes

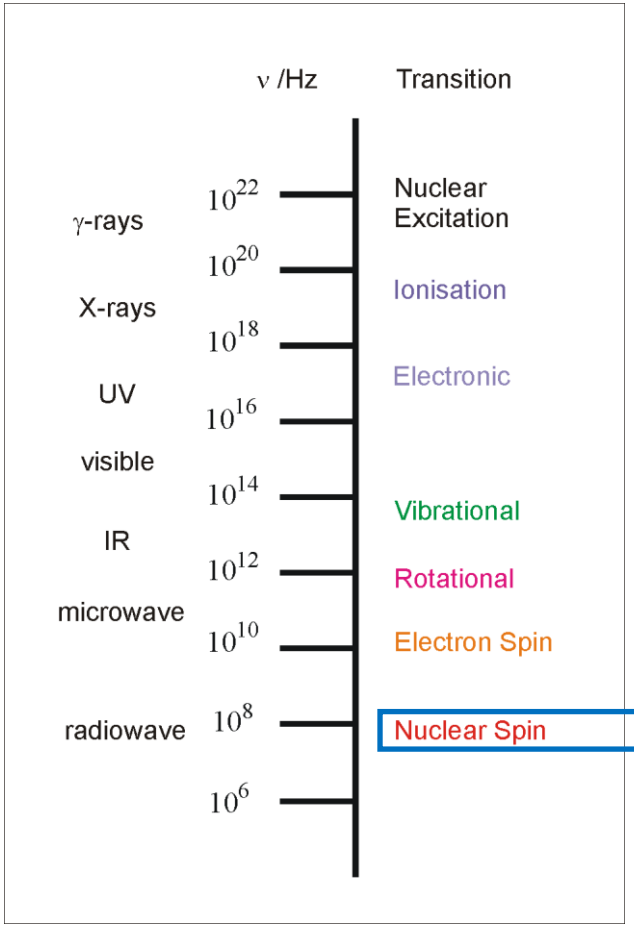
Carbon-12 = No!

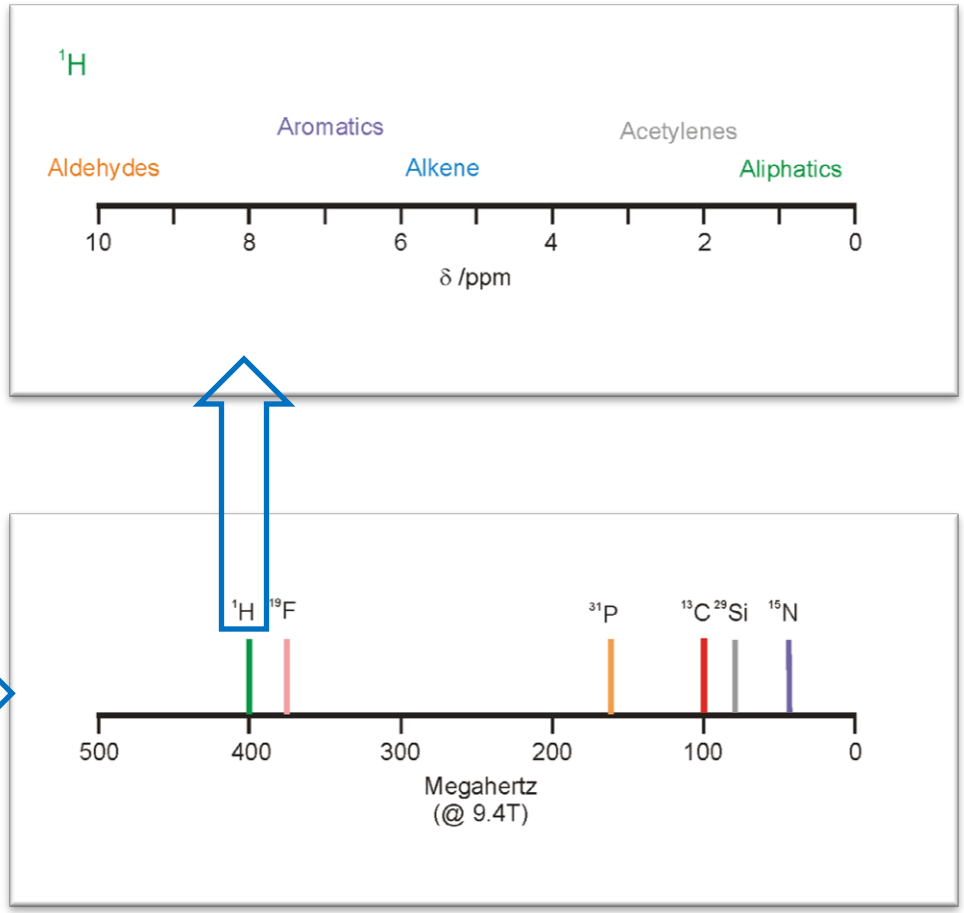
Carbon-13 = Yes (1% abundant)

Oxygen-16 = No!

Introducing NMR Spectroscopy

The electromagnetic spectrum

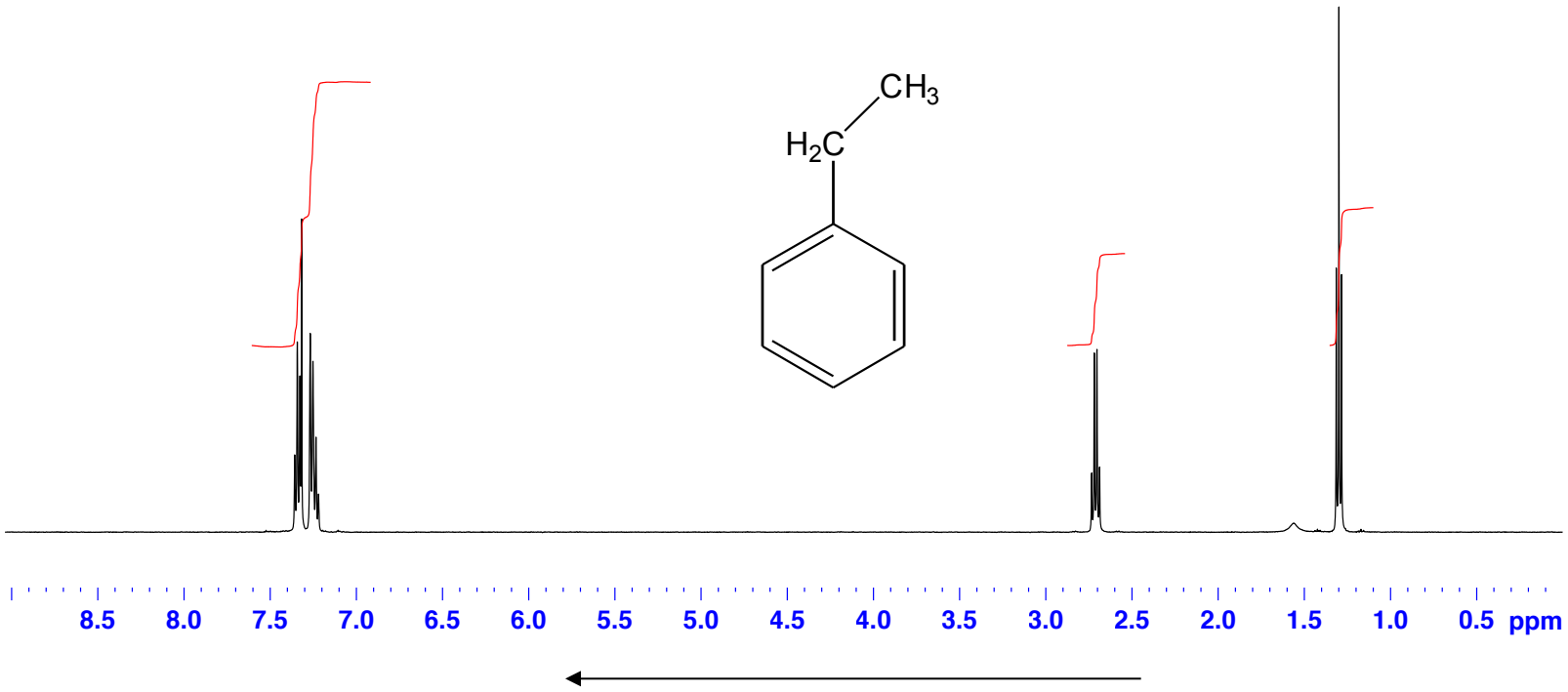




Features of an NMR spectrum (^{1}H)

3) Absorption intensities:
Peak integration

2) Spin-coupling
fine structure

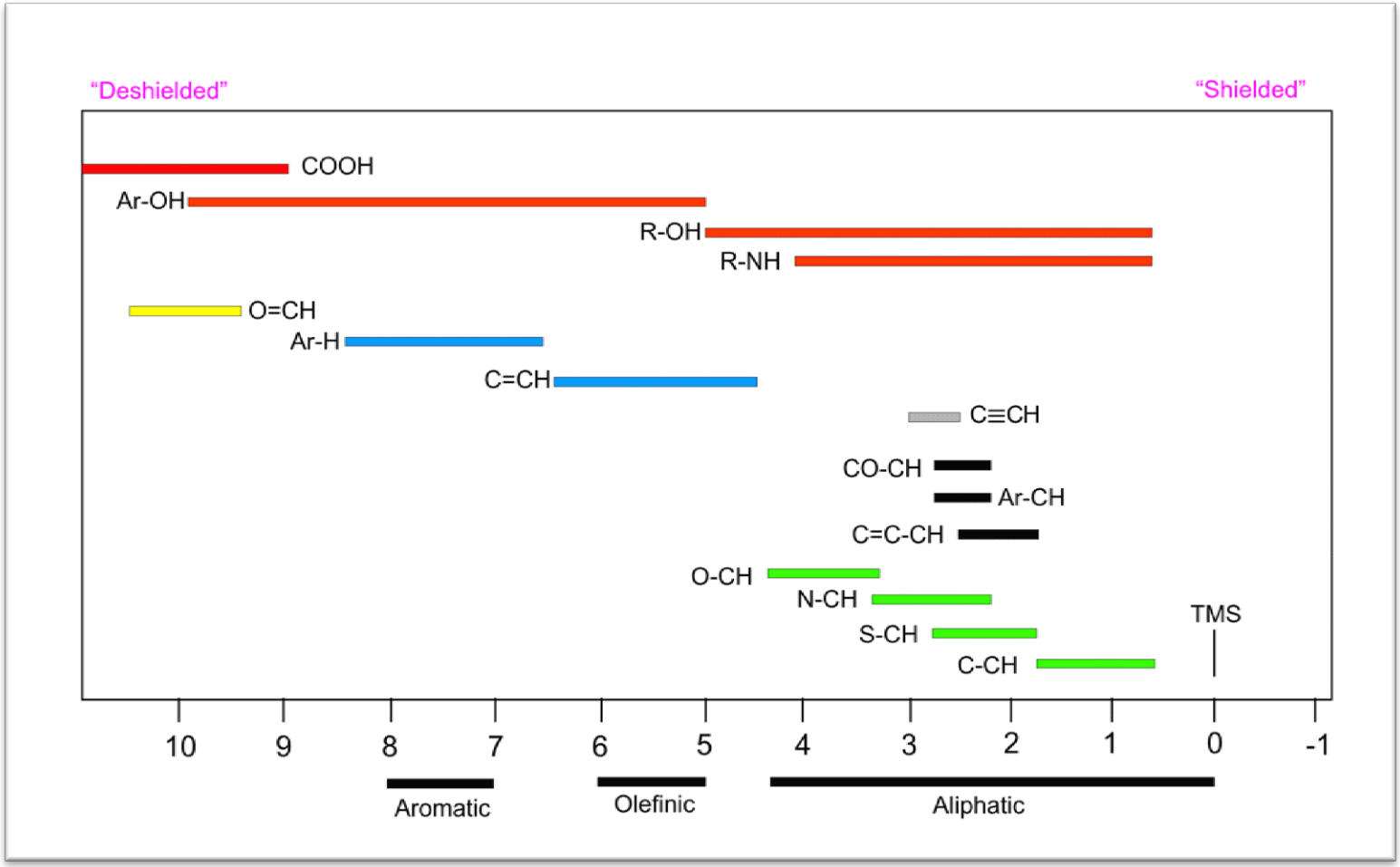


1) Chemical shift

Factors influencing chemical shifts

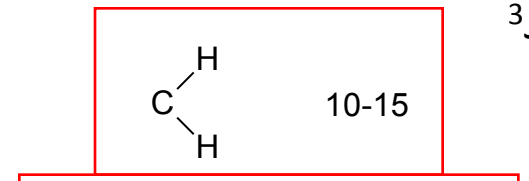
Inductive, anisotropic (aromatic ring currents), mesomeric, hydrogen bonds, solvent

Proton chemical shifts and chemical environments



Typical ^{1}H-^{1}H coupling constants

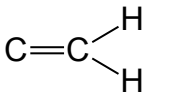
(magnitudes only shown)

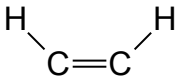


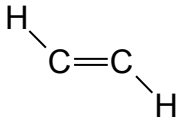
H—C—C—H ≈7 (free rotation)

HC—CH=C 5-7

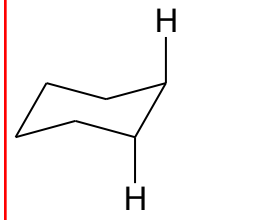
C=CH-CH=C 10-13



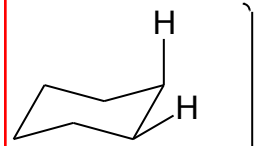


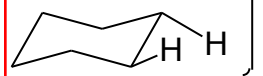


$^{3}J_{HH}$ Karplus relationship

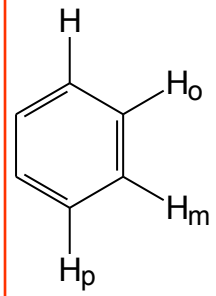


ax-ax
10-12





ax-eq
or *eq-eq*
2-5



ortho 7-8
meta 1-2
para 0-1

HC═C—CH 1-2

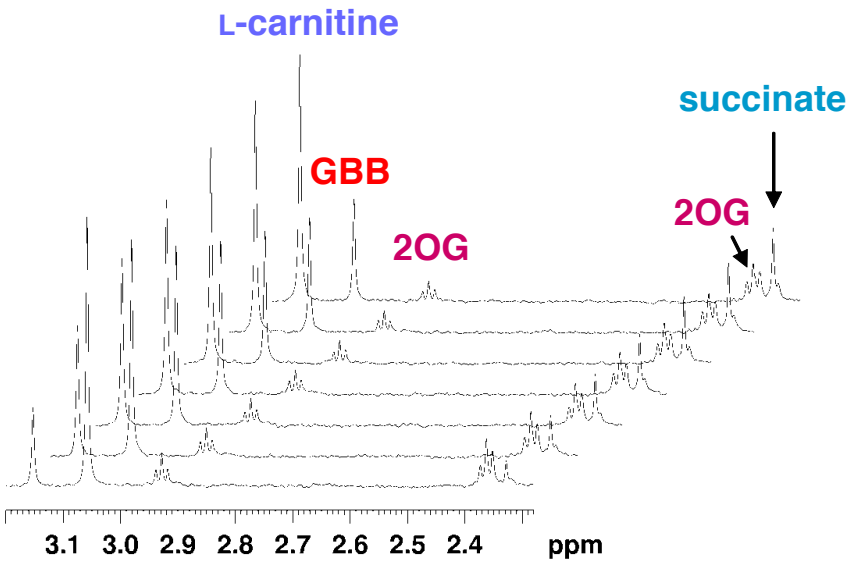
HC—C═C—CH 0-1

HC—C≡C 2-3

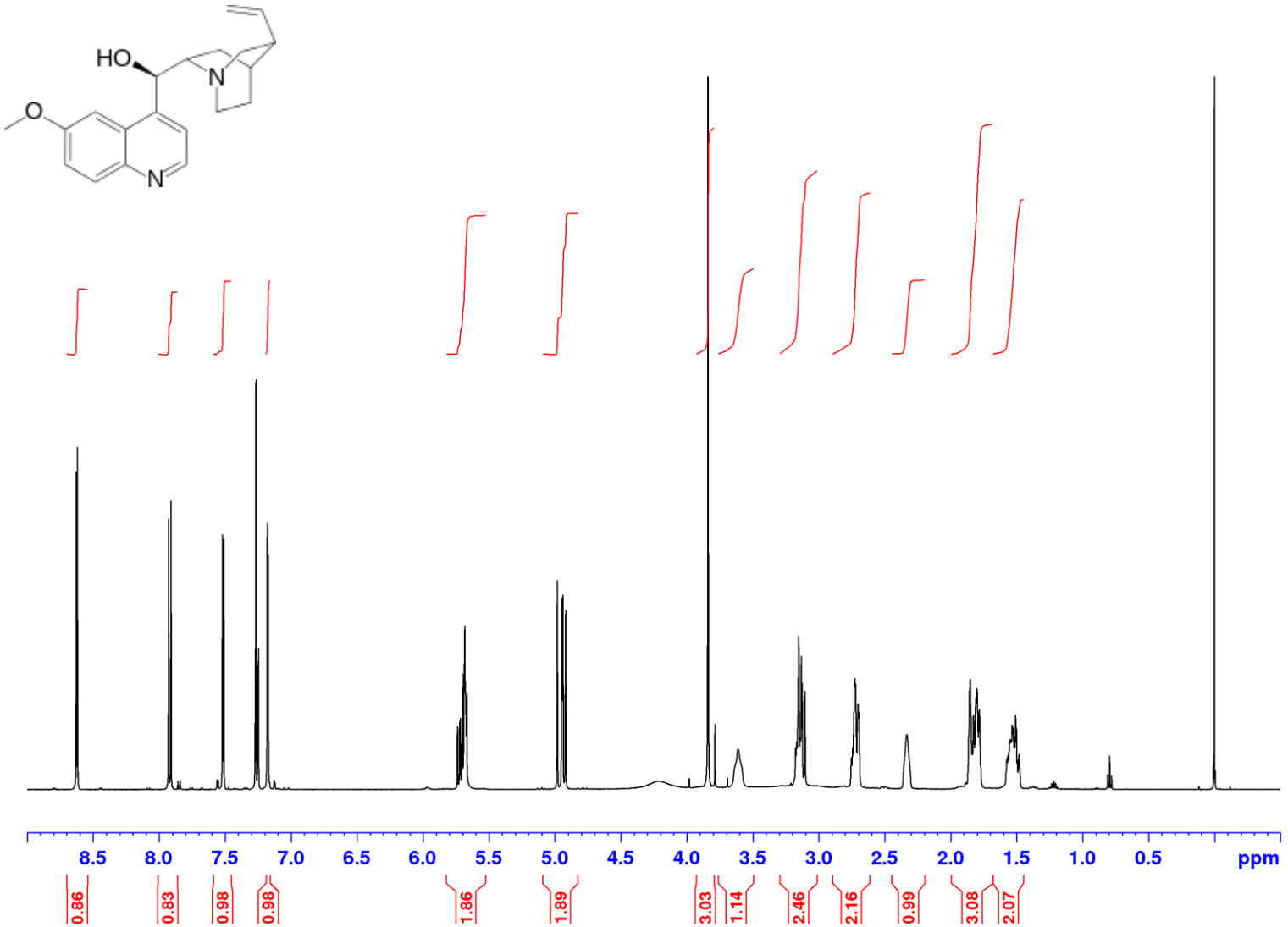
Peak Area Integration

Useful for more than just structure identification

- Peak areas reflect relative concentrations
  - Relative proton count
  - Purity measurement
  - Isomer ratios
    - Regio, diastereo, enantio
  - Kinetic profiles
    - time-dependent concentrations
  - Quantify binding
    - complexed *vs* non-complexed forms
  - Absolute concentration measurement
    - calibration required

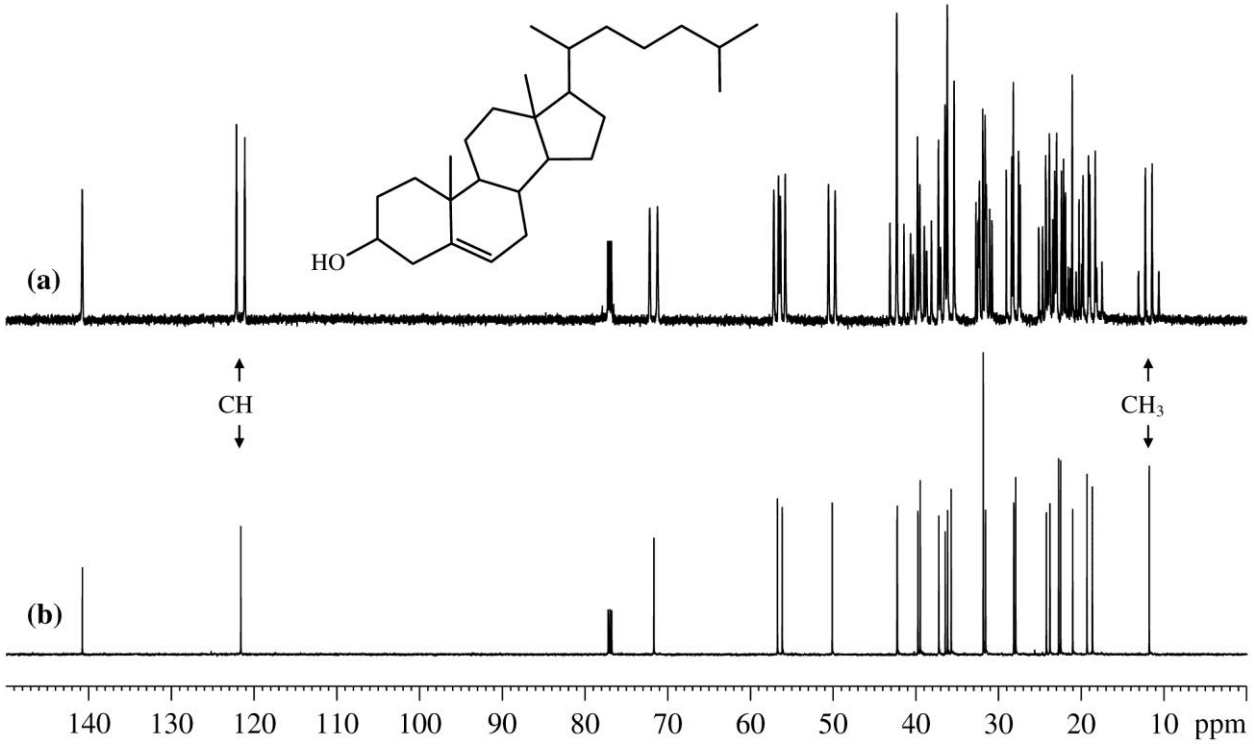


Kinetic reaction profile


HO
N
O
N
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
0.86
0.83
0.98
0.98
1.86
1.89
3.03
1.14
2.46
2.16
0.99
3.08
2.07

Carbon-13 NMR

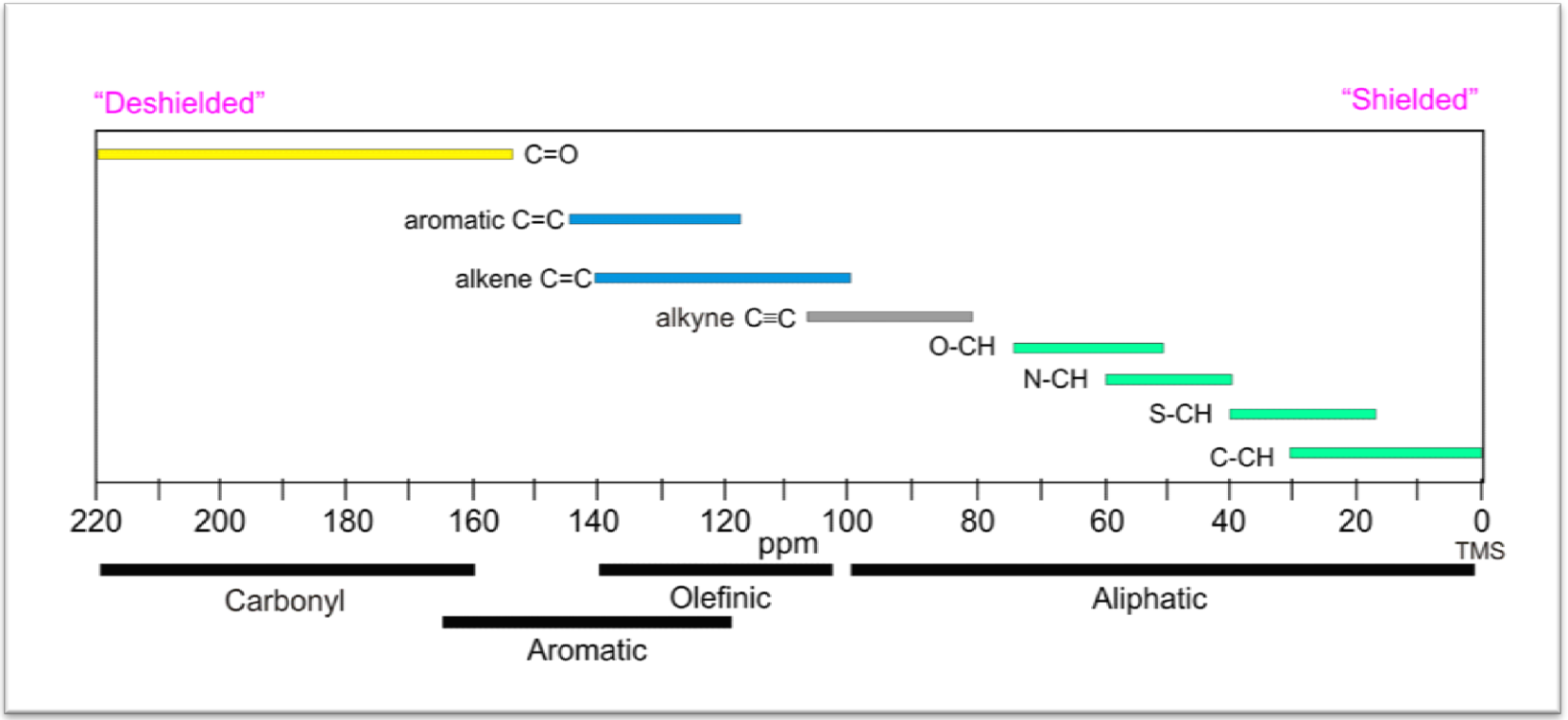
- Chemical shift reference: TMS @ 0 ppm
- Proton decoupled ^{13}C ("heteronuclear decoupling": $^{13}C\{^{1}H\}$)
  - No ^{1}H multiplet structures: peak overlap uncommon
  - All signal intensity into single peak: signal-to-noise enhanced
  - How? Irradiate sample at ^{1}H frequency (e.g. 400 MHz) whilst detecting ^{13}C frequency (e.g. 100 MHz)

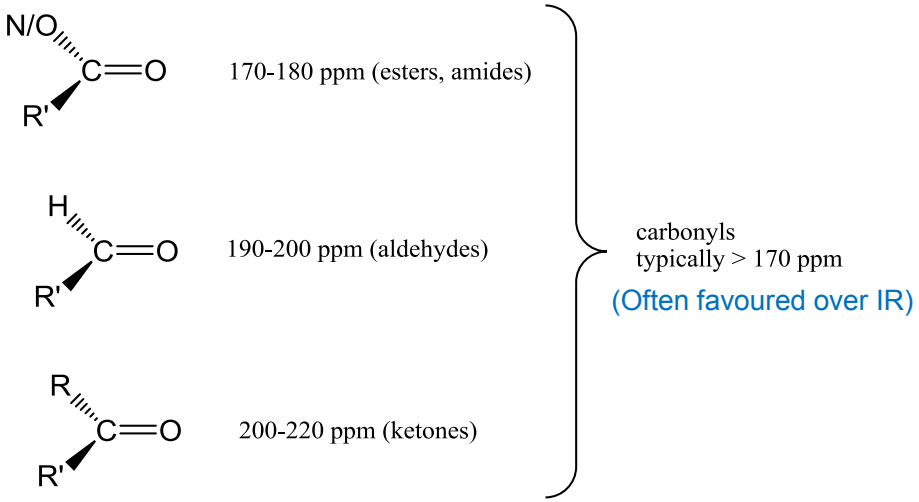


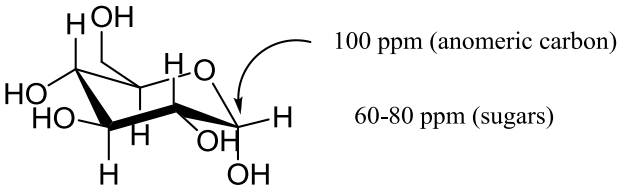
Carbon-13 NMR

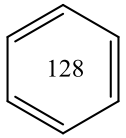
Carbon-13 chemical shifts reflect local chemical environments

As “rule of thumb” ^{13}C shifts are *very roughly* 20x those of the attached proton

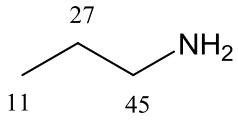


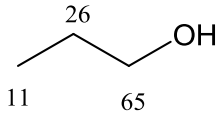


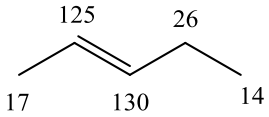




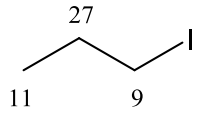
120-140 ppm
(aromatics)

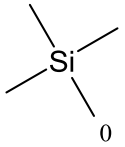






Deshielded environments



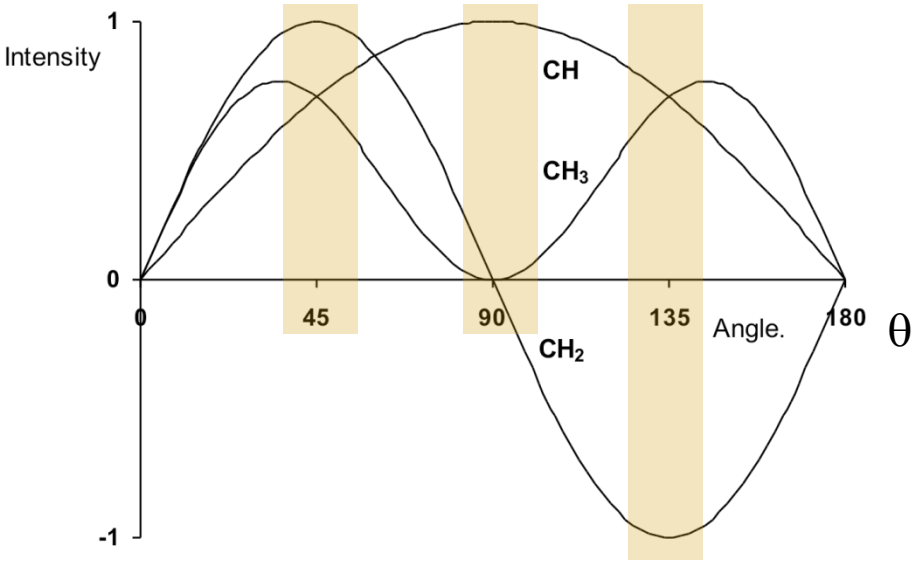


Shielded environments

Carbon-13 shift estimation tables well established (documented in many text books).

Computer programs provide good predictions in many cases (generally more accurate than ^{1}H predictions).

Spectrum editing: DEPT and DEPTQ



DEPT: *Distortionless Enhancement by Polarisation Transfer*

Intensity variation as function of proton pulse tip angle θ

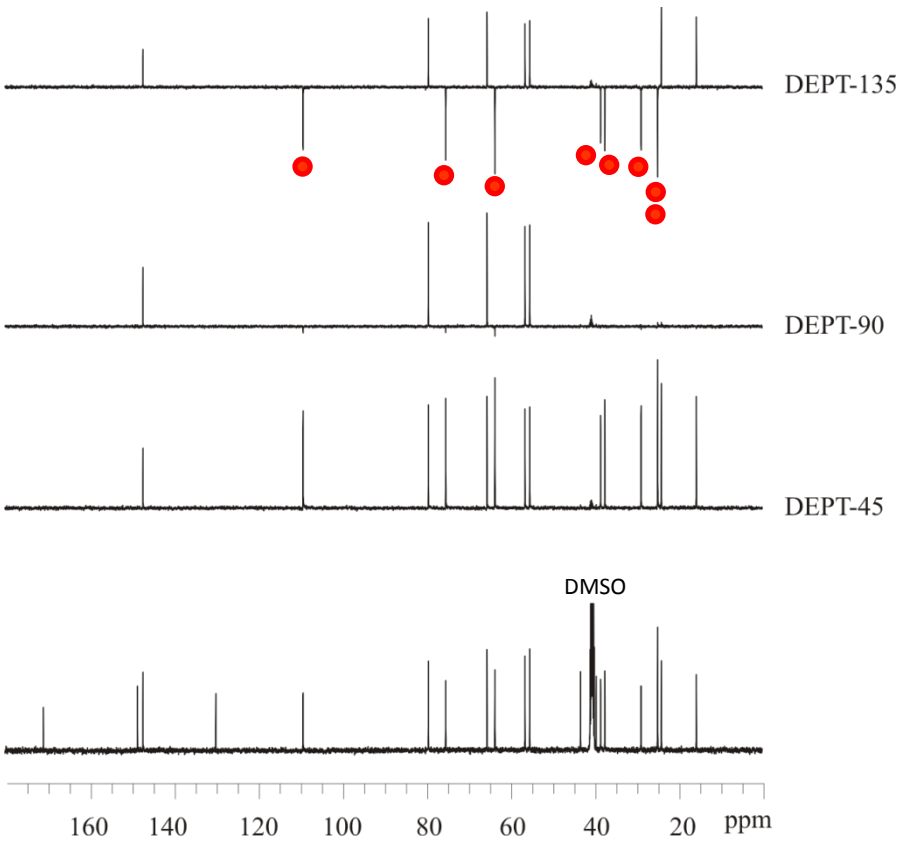
As carbon intensity relies upon magnetisation transfer from protons, non-protonated centres give no response in DEPT

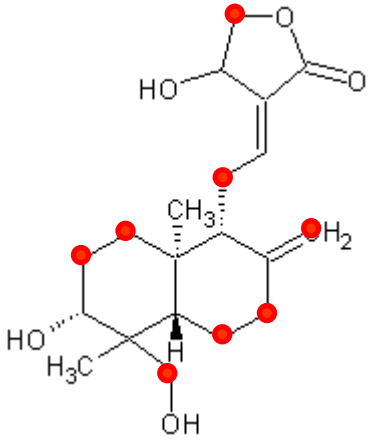
Classic DEPT experiments:

- DEPT-135 is standard
- DEPTQ is modern variant that also retains Quaternary centres

	DEPT-45	DEPT-90	DEPT-135	DEPTQ
C	0	0	0	-
CH	+	+	+	+
CH_2	+	0	-	-
CH_3	+	0	+	+

Spectrum editing: DEPT

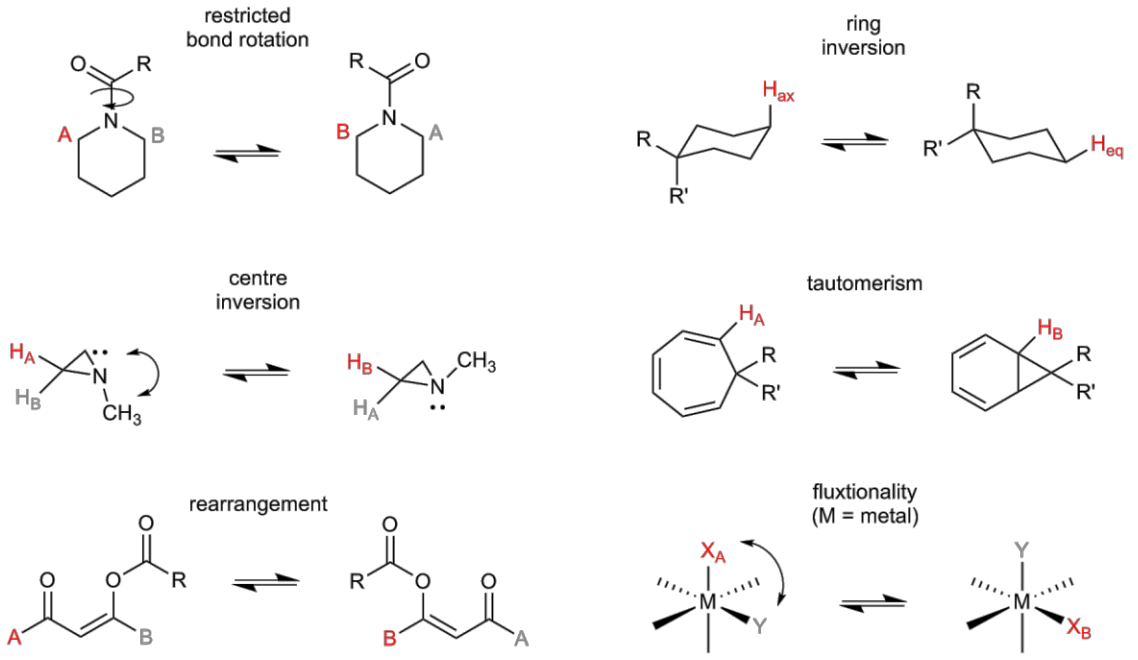




Despite its obvious utility, DEPT still suffers from poor sensitivity relative to ^{1}H. Nowadays editing of proton-detected 2D experiments is more time efficient.

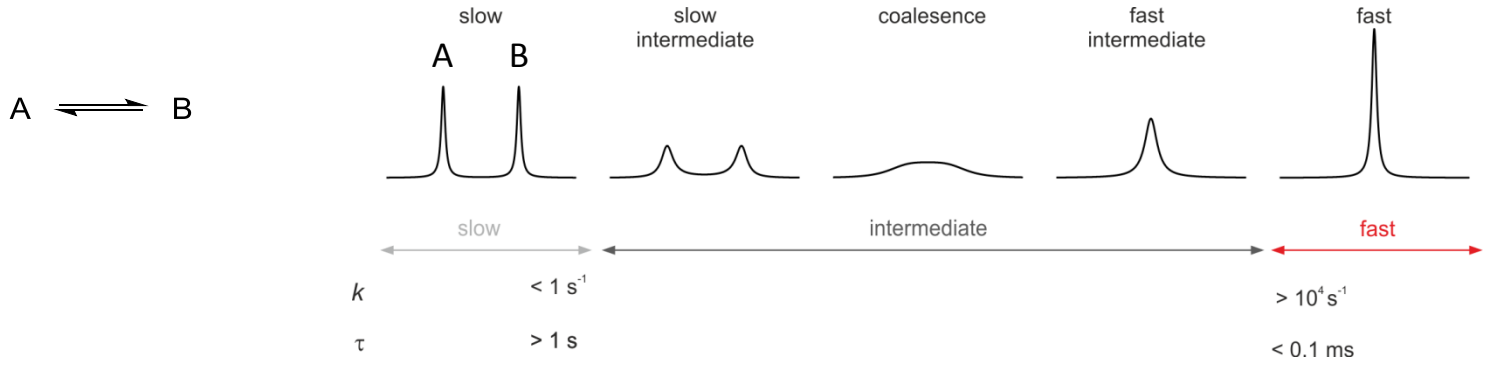
Dynamic effects on spectra

- Line broadening of a subset of peaks is often seen in spectra. Most often this results from relatively slow conformational dynamics (or exchange) occurring within the molecule.



Dynamic effects

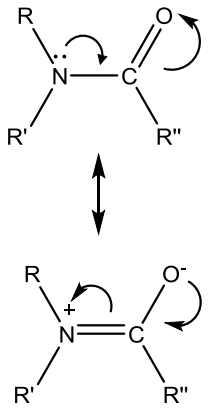
- We talk of motion on the “NMR timescale”- most often this means the chemical shift timescale.
  - Slow: interconverting species show discrete resonances.
  - Intermediate: averaging of resonances produces observable line broadening. Exchanging peaks merge at the ***coalescence point***”.
  - Fast: A single time-averaged peak is observed representing the population-weighted average parameter of each species present.

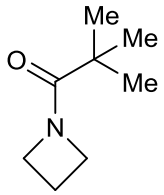


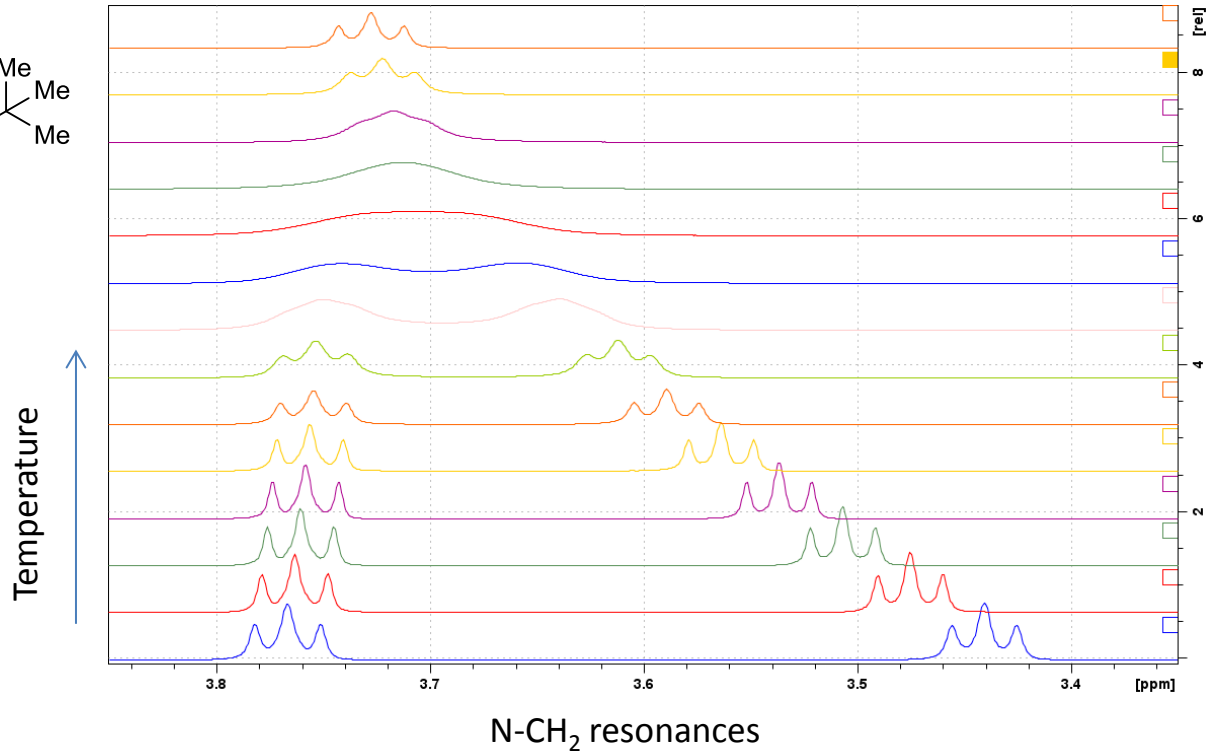
Dynamic effects

Amide bond rotation

- The most common example of restricted dynamics in synthetic chemistry is in bond rotation of tertiary amides:







University of Oxford
Doctoral Training Centre

NMR Spectroscopy

2D NMR and NOE methods for spectrum assignment and structure verification

2D NMR spectroscopy

These methods have been developed to directly map various interactions or correlations between spins. Three basic classes:

1. Through bond: spin-spin (J) couplings (scalar coupling)
2. Through-space: nuclear Overhauser effect (dipolar coupling)
3. Through chemical exchange: dynamic processes

Mapping spin-spin coupling interactions is important as it implies the presence of chemical bonds…

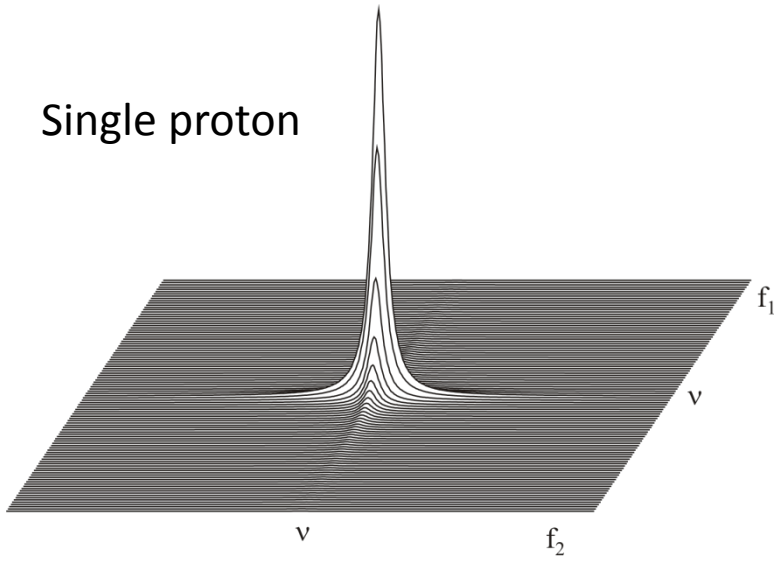
Two sub-classes:

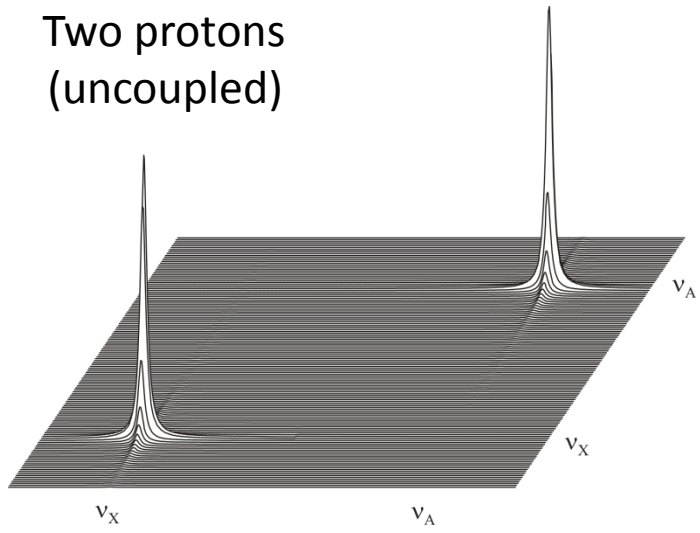
1. Homonuclear- mapping coupling between *similar* spins e.g. ^{1}H-^{1}H
2. Heteronuclear- mapping coupling between *dissimilar* spins e.g. ^{1}H-^{13}C

2D NMR spectroscopy

Why called *two-dimensional* NMR?

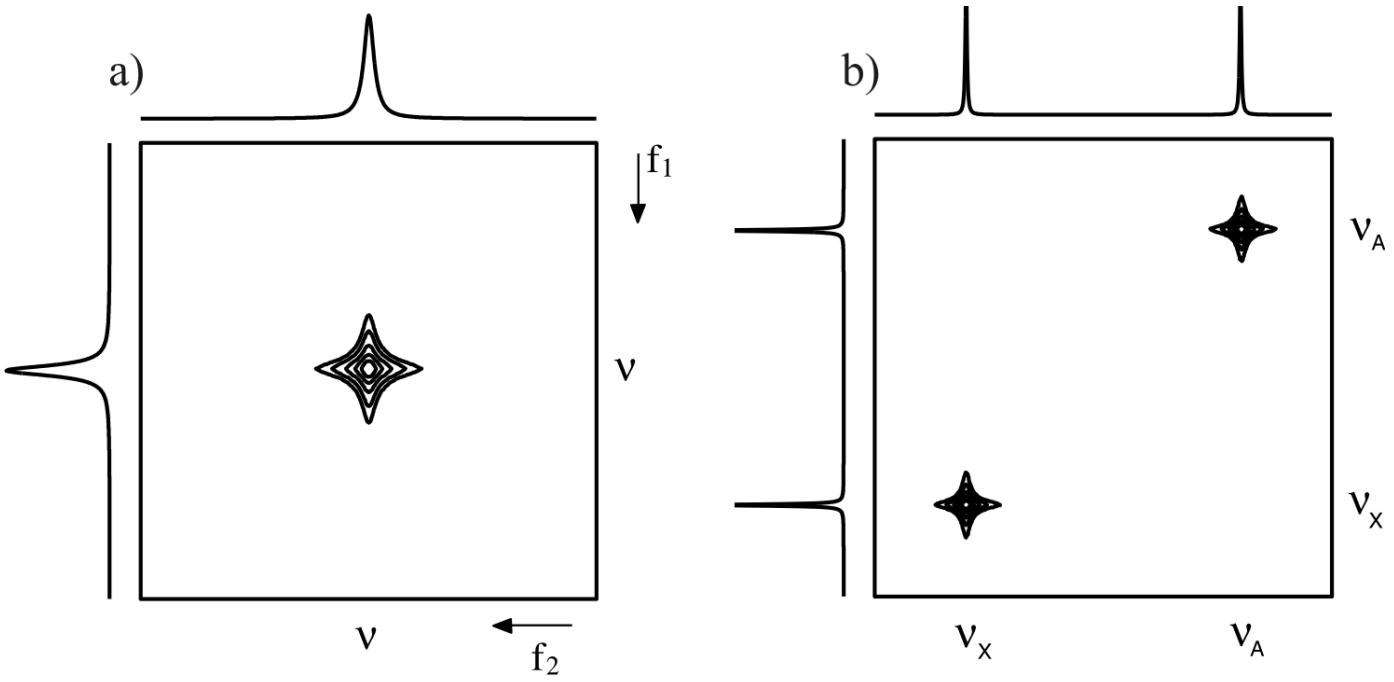
The dimensions refer to the number of chemical shift axes in the experiment (a third dimension corresponds to peak intensity)…





2D NMR spectroscopy

- Contour presentation



2D Homonuclear Correlations

Correlating similar nuclides

COSY

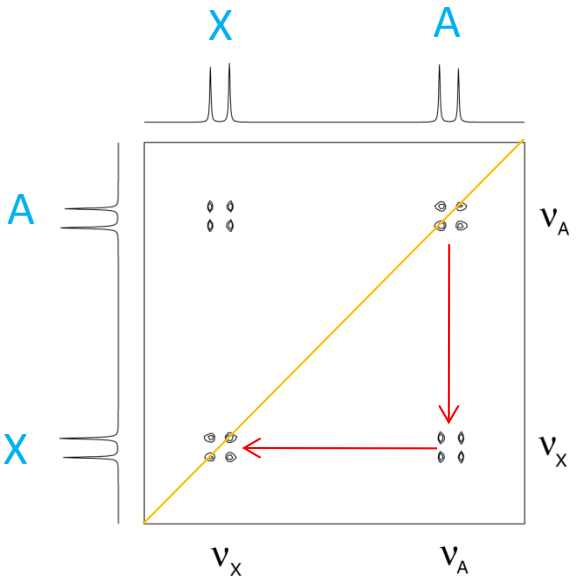
Correlation Spectroscopy

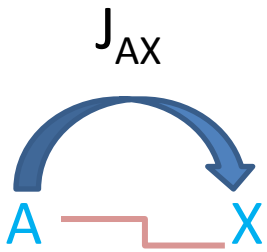
TOCSY

Total Correlation Spectroscopy

2D **CO**rrelation **S**pectroscop**Y**: COSY

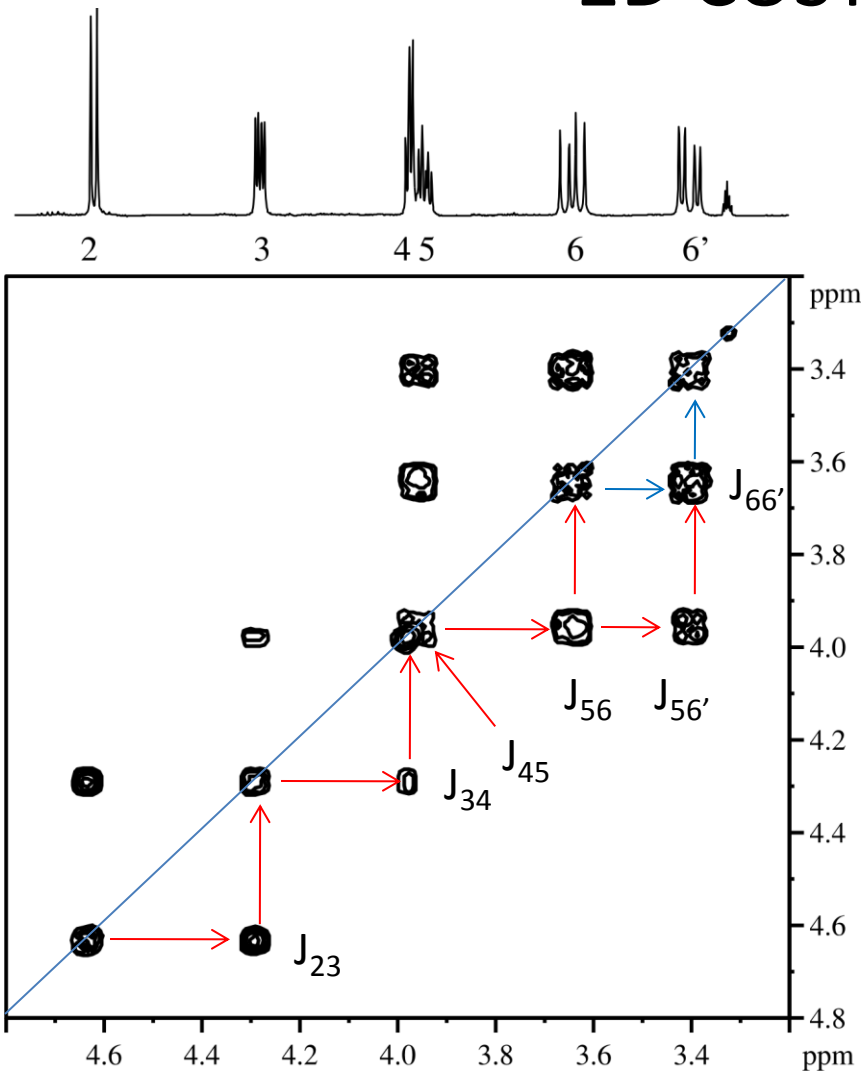
- 2nd COSY pulse also causes "*coherence transfer*" between J-coupled spins
- Crosspeaks from this process map J-coupling interactions between spins
- Typically ^{1}H-^{1}H, but can be used for other **high-abundance** nuclides
  - ^{19}F-^{19}F, ^{11}B-^{11}B, ^{31}P-^{31}P etc

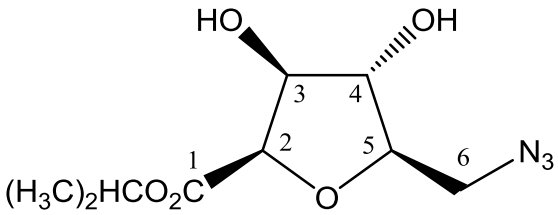




Fine structure within 2D peaks correlates with that of the 1D multiplet, but is often not resolved.

2D COSY



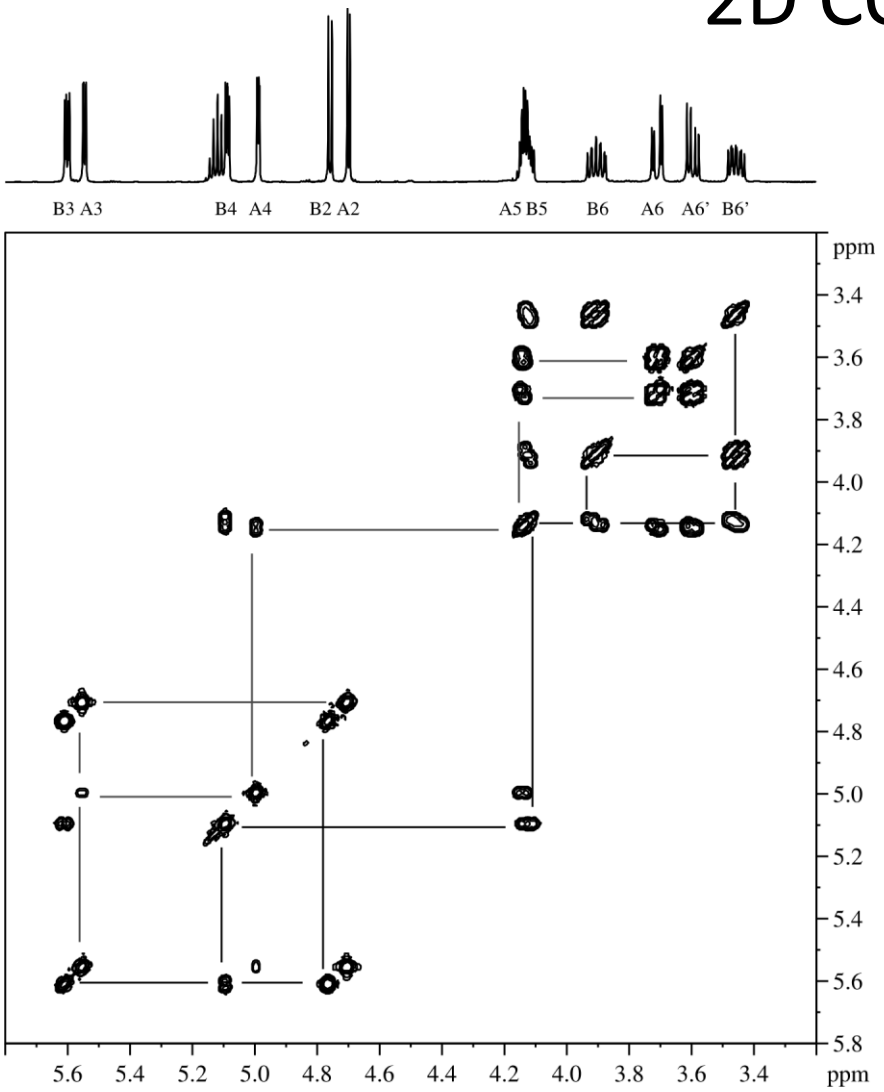


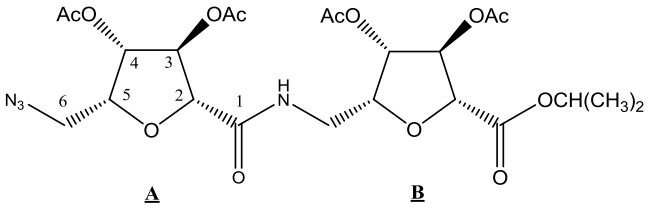
$^2J_{HH}$- geminal

$^3J_{HH}$- vicinal

COSY cannot directly differentiate ^2J from ^3J (or longer range) couplings!

2D COSY



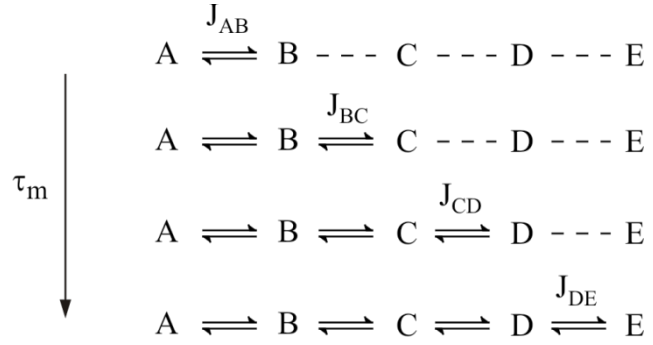


Both ring system couplings can be traced directly:

Upper traces: Ring A
Lower traces: Ring B

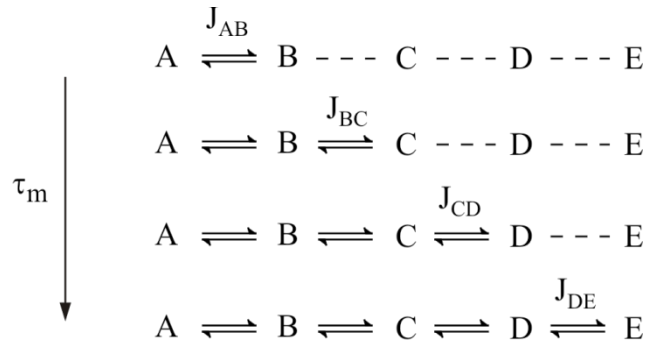
2D **TO**tal **C**orrelation **S**pectroscop**Y** (TOCSY)

- TOCSY sequence is able to relay magnetisation along a chain of coupled spins
  - Provides remote correlations to distant spins within coupled spin-system: *Total* correlations
  - Very powerful tool in the analysis of more complex or heavily overlapped spectra

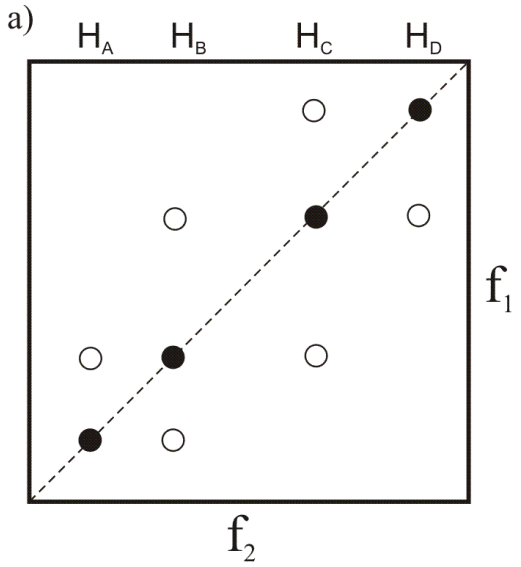


TOCSY

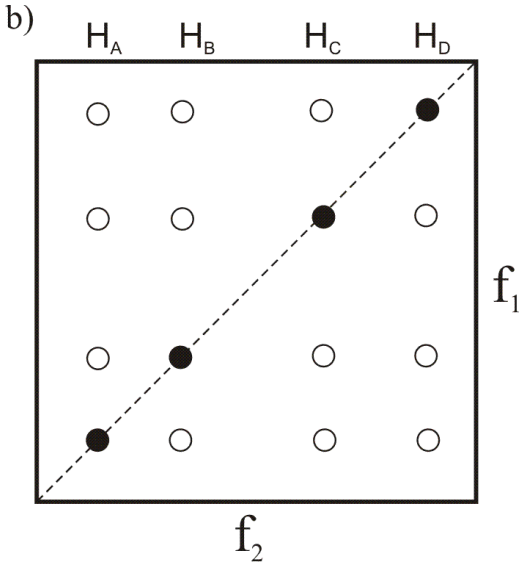
- System with J_{AB}, J_{BC}, J_{CD}



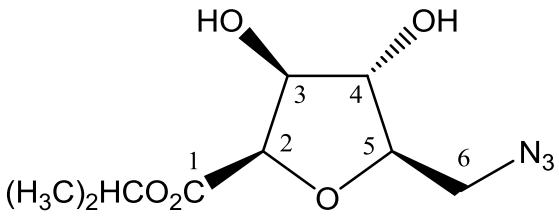
COSY



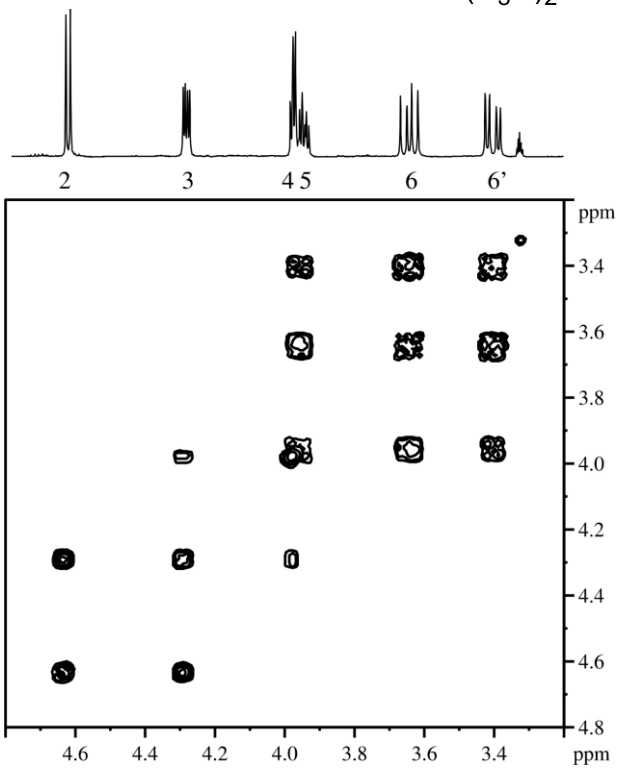
TOCSY



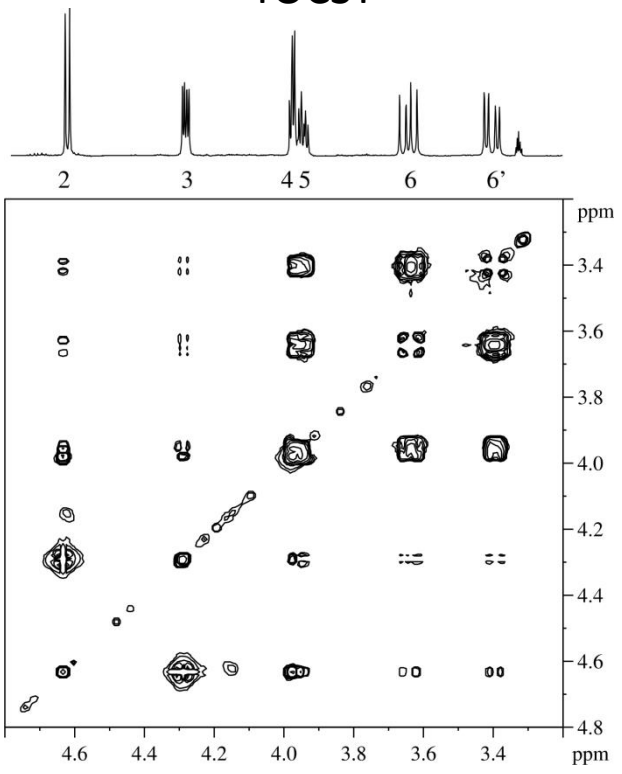
TOCSY



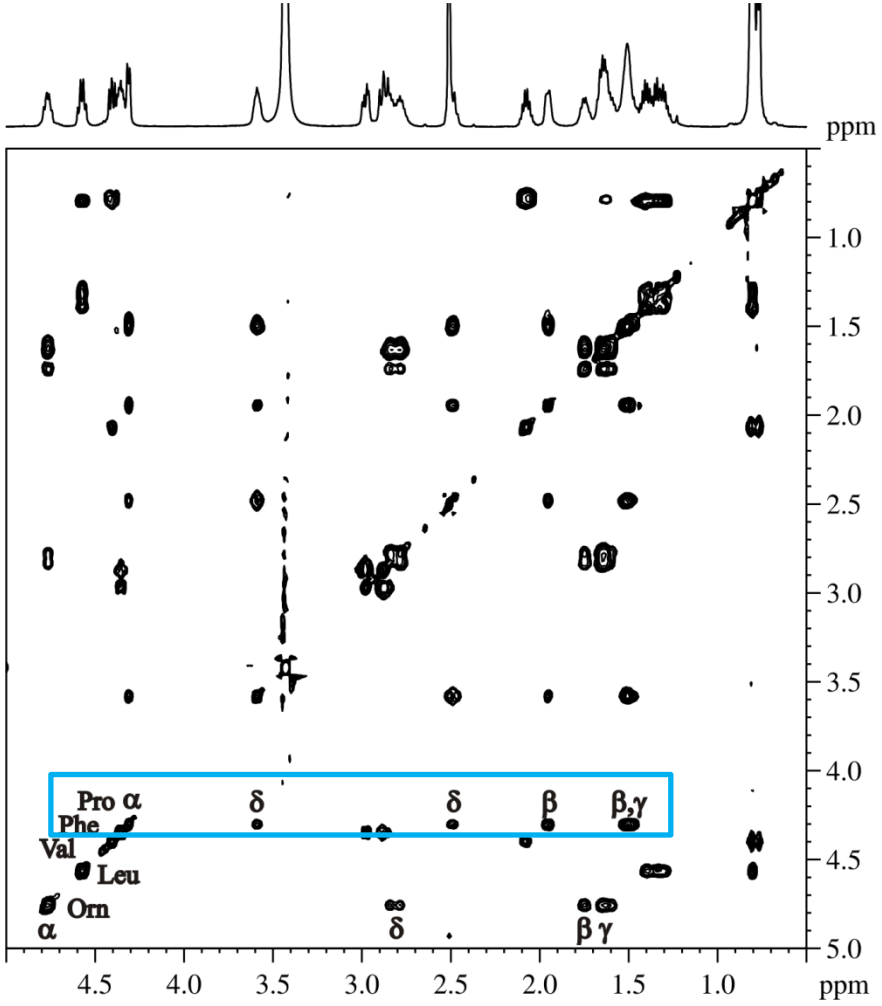
COSY

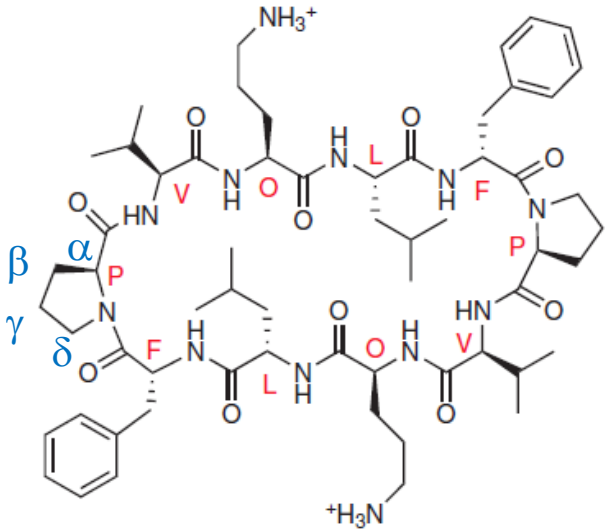


TOCSY



Application of TOCSY





Pro — Val — Orn — Leu — DPhe
DPhe — Leu — Orn — Val — Pro

2D Heteronuclear Correlations

Correlating differing nuclides

HSQC

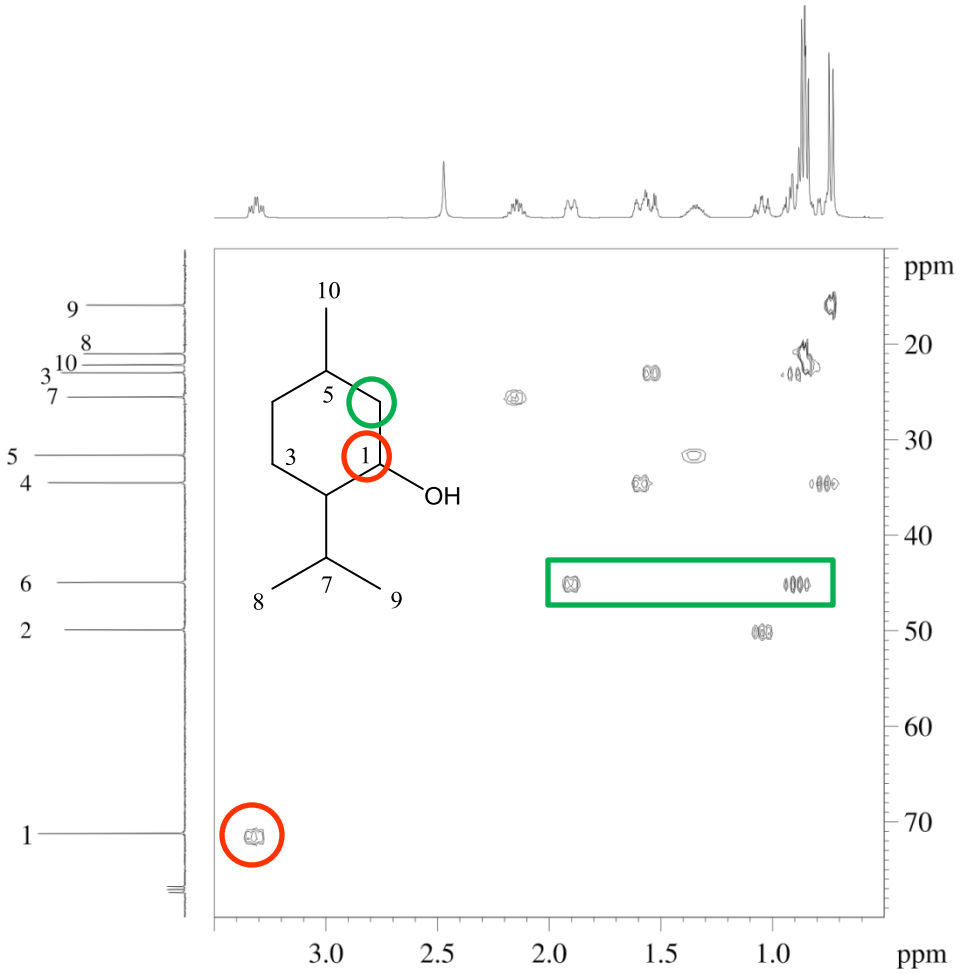
Heteronuclear single-quantum correlation

HMBC

Heteronuclear multiple-bond correlation

(HMQC similar to HSQC)

2D one-bond ^{1}H-^{13}C heteronuclear correlations

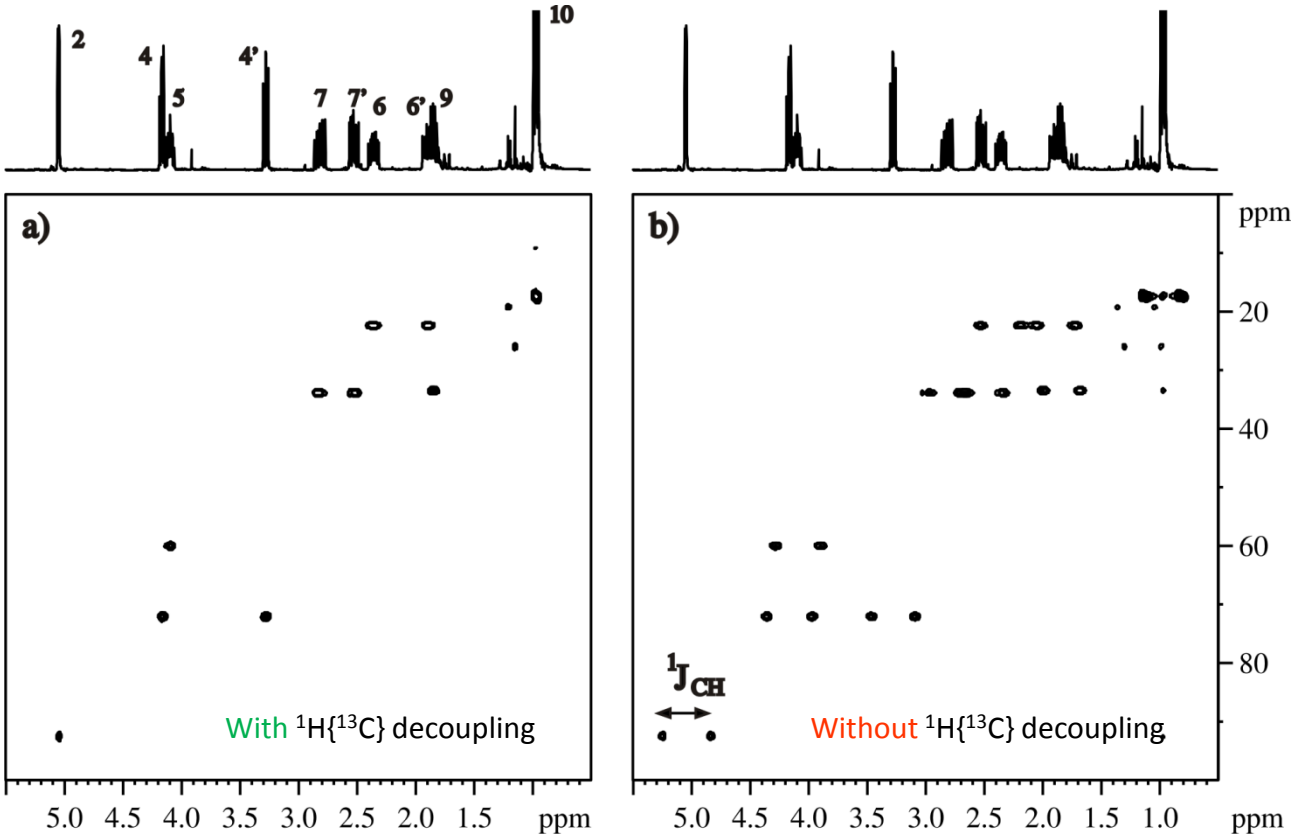


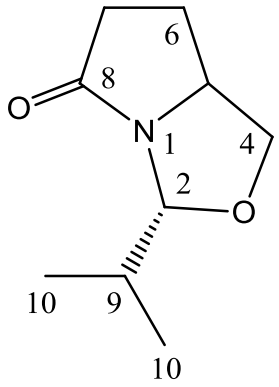
Features:

- Directly correlates ^{1}H-^{13}C pairs
- Spreads ^{1}H multiplets apart according to ^{13}C shifts
- Indirectly provides ^{13}C chemical shifts
- Identifies and correlates diastereotopic CH_2 groups
- *Has greater sensitivity than direct ^{13}C observe experiments due to ^{1}H detection*

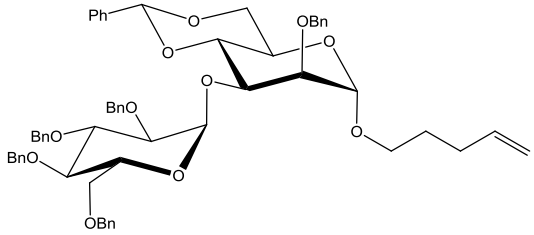
2D one-bond ^{1}H-^{13}C heteronuclear correlation

^{13}C satellites of ^{1}H spectrum are decoupled in 2D experiment: $^{1}J_{CH}$ hidden





Multiplicity edited HSQC

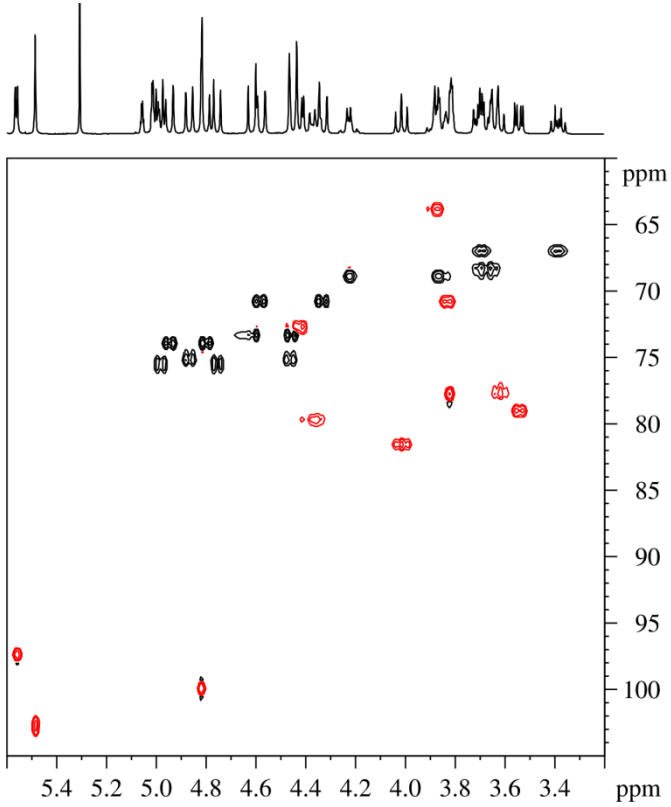


Sign of 2D cross peaks can be modified according to multiplicity of correlated CH groups:

Group	Cross peak sign (relative)
CH	+
CH_2	-
CH_3	+

Editing is equivalent to that seen in DEPT-135…

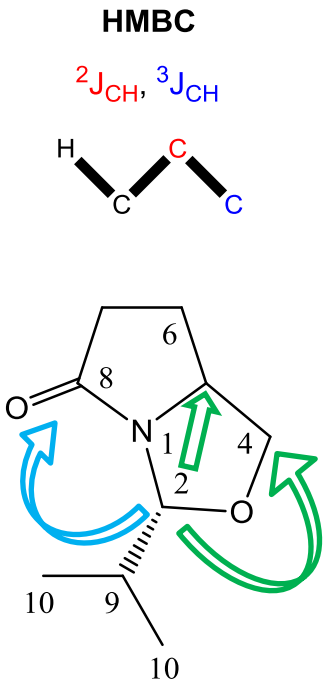
…hence, with edited HSQC DEPT is often unnecessary!



Red= +ve
Black = -ve

2D **H**eteronuclear **M**ultiple-**B**ond **C**orrelation: **HMBC**

- 2D heteronuclear correlation "tuned" to detect J_{CH} couplings over typically 2-3 bonds ($1/2^{n}J_{CH}$).



Correlations across heteroatoms

Correlations to quaternary (non-protonated) centres

Useful for:

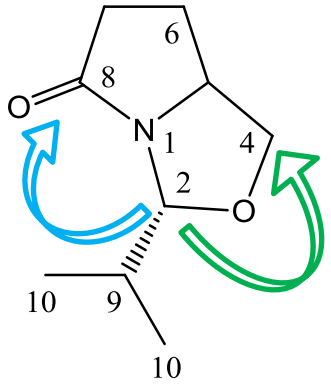
- ✓ connecting structural fragments
- ✓ proving cyclisation
- ✓ identifying substitution positions
- ✓ obtaining ^{13}C shifts for quaternary centres

2D **H**eteronuclear **M**ultiple-**B**ond **C**orrelation: **HMBC features**

- 2D heteronuclear correlation tuned to detect J_{CH} couplings over typically 2-3 bonds.
- Longer-range couplings (4, even 5 bonds) can be observed where couplings exist (e.g. unsaturation)
- Long-range couplings much smaller than one-bond couplings, so sequence optimised for small couplings...

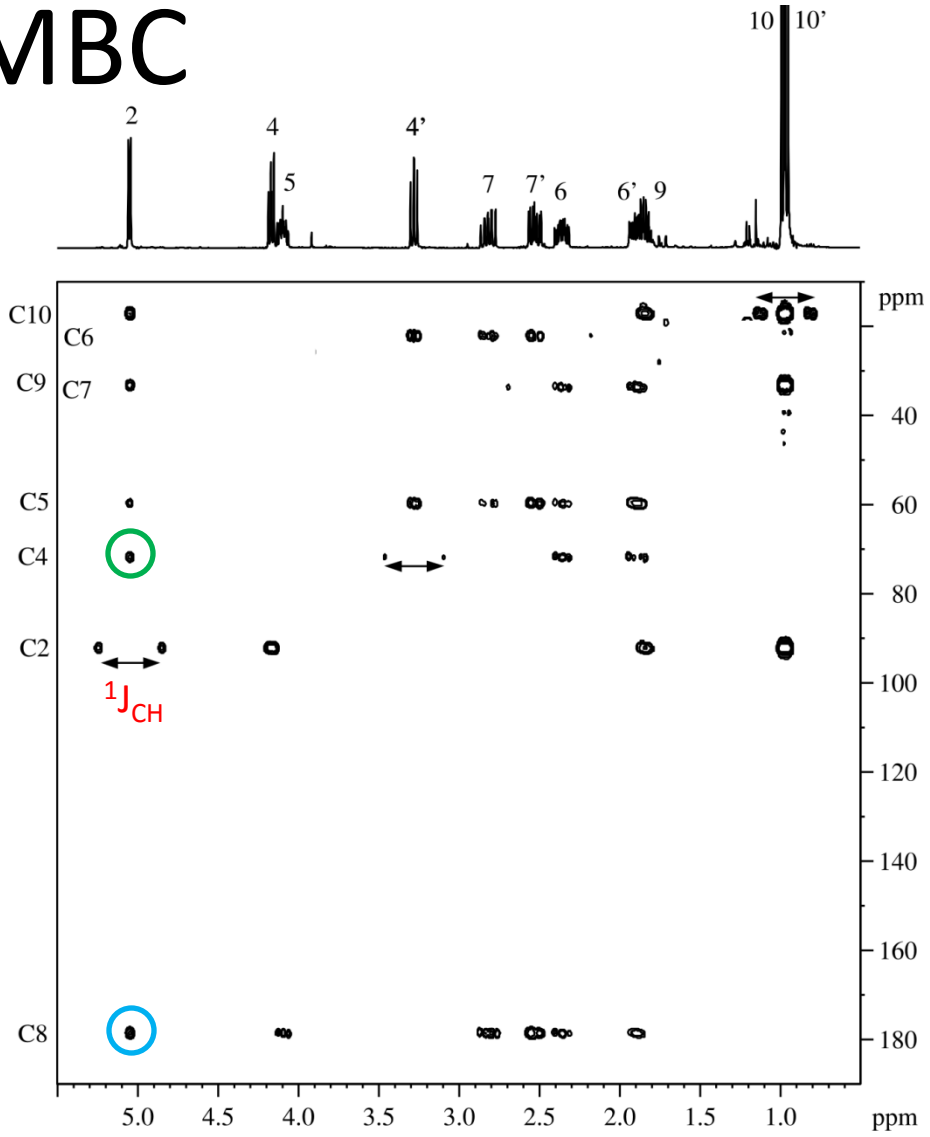
Coupling pathway	$^2J_{CH}$	Coupling pathway	$^3J_{CH}$	Coupling pathway	$^4J_{CH}$
H—C—**C**	(±) ≤ 5	**H**—C—C—**C**	≤ 5	**H**—C=C—C=**C**	(±) ≤ 1
H—C=**C**	≤ 10	**H**—C=C—**C**	≤ 15	**H**—C—C—C—**C**	≤ 1
H—C≡**C**	40-60	**H**—C≡C—**C**	≤ 5		
H—C(=O)—**C**	20-25				

- …however, residual one-bond couplings can sometimes be seen also.
- *3-bond correlations can be stronger than 2-bond correlations.*



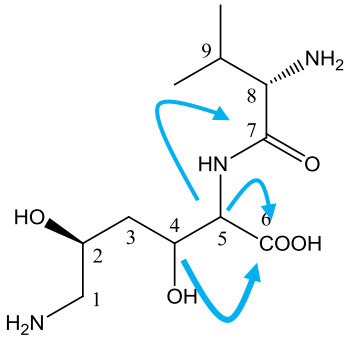
HMBC

- Many long-range correlations
- Cannot distinguish ^{2}J from ^{3}J correlations
- One-bond couplings are filtered out but can breakthrough-appear as $^{1}J_{CH}$ doublets as no ^{13}C decoupling employed.
- Due to small $^{n}J_{CH}$ couplings, broad lines often shown no correlations.



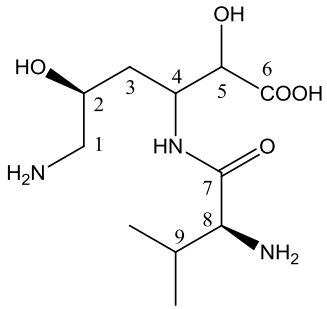
HMBC applications

Which regioisomer?



a

✓



b

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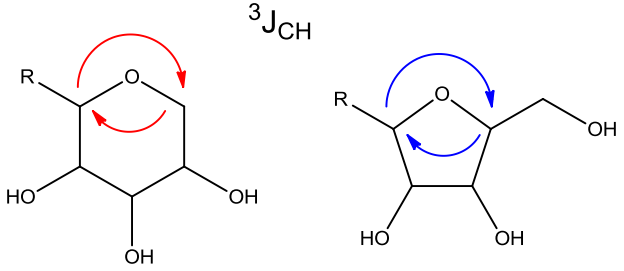
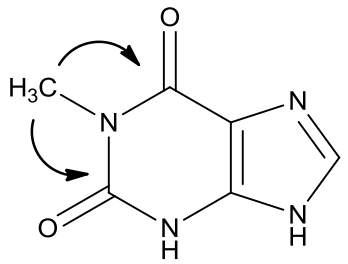
Ring cyclisation



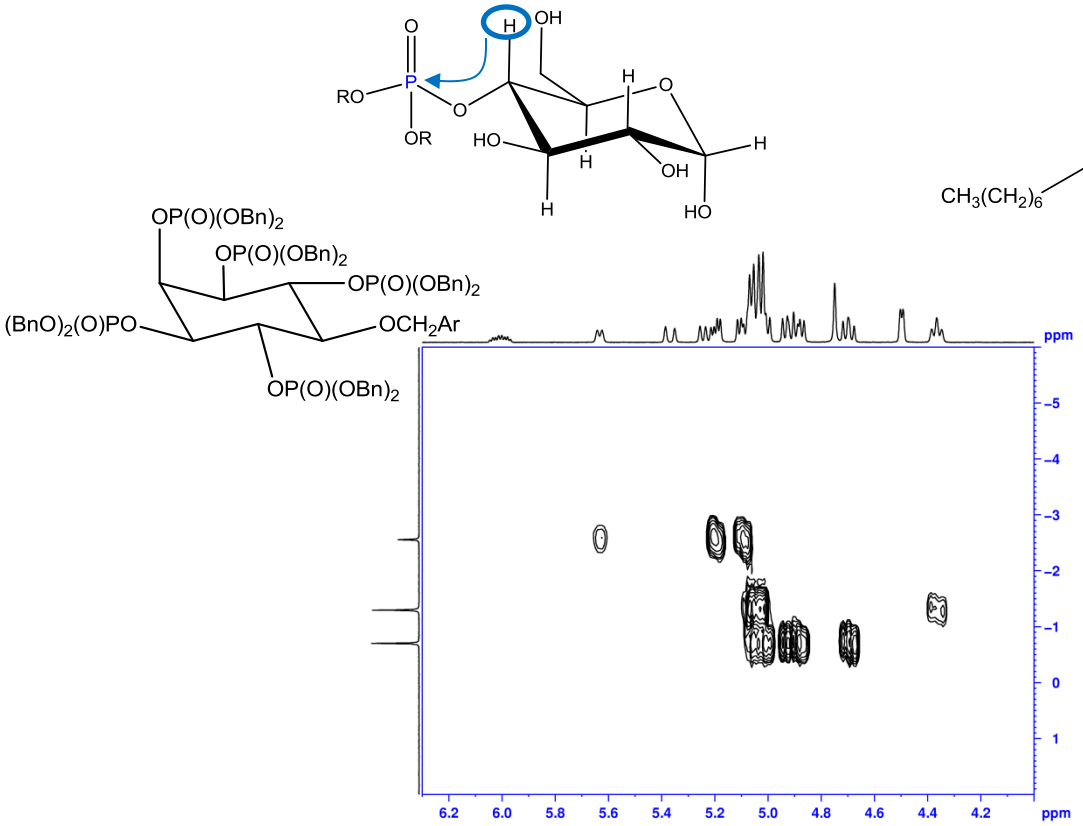
N-Me Substitution position



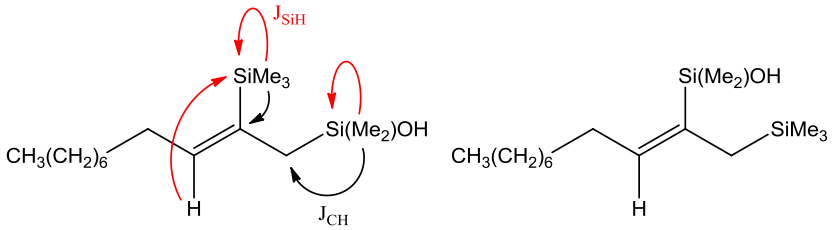
HMBC applications: other nuclei

Long-range correlations to other nuclei can be especially useful when 2 & 3 bond couplings have significant magnitude

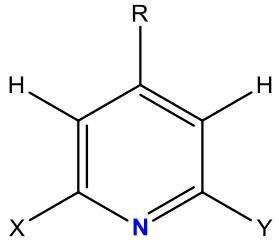
^{1}H-^{31}P (100 % abundant)



^{1}H-^{29}Si (5 % abundant)



^{1}H-^{15}N (0.4 % abundant)



The Nuclear Overhauser Effect

Identifying protons that are close in space

What is the nOe?

- Quantitative definition
  - Fractional enhancement of a resonance intensity,

$$\eta = \frac{I - I_0}{I_0} \quad (\times 100\ \%)$$

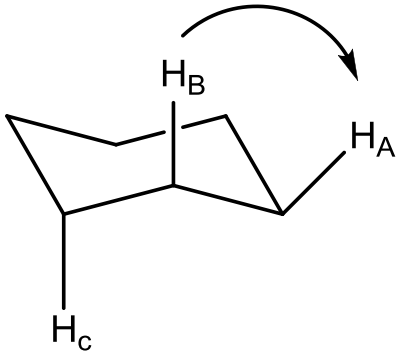
Typically ^1H-^1H nOes are weak (<< 10%) and thus challenging to observe!

I_0= peak intensity of resonance in absence of nOe

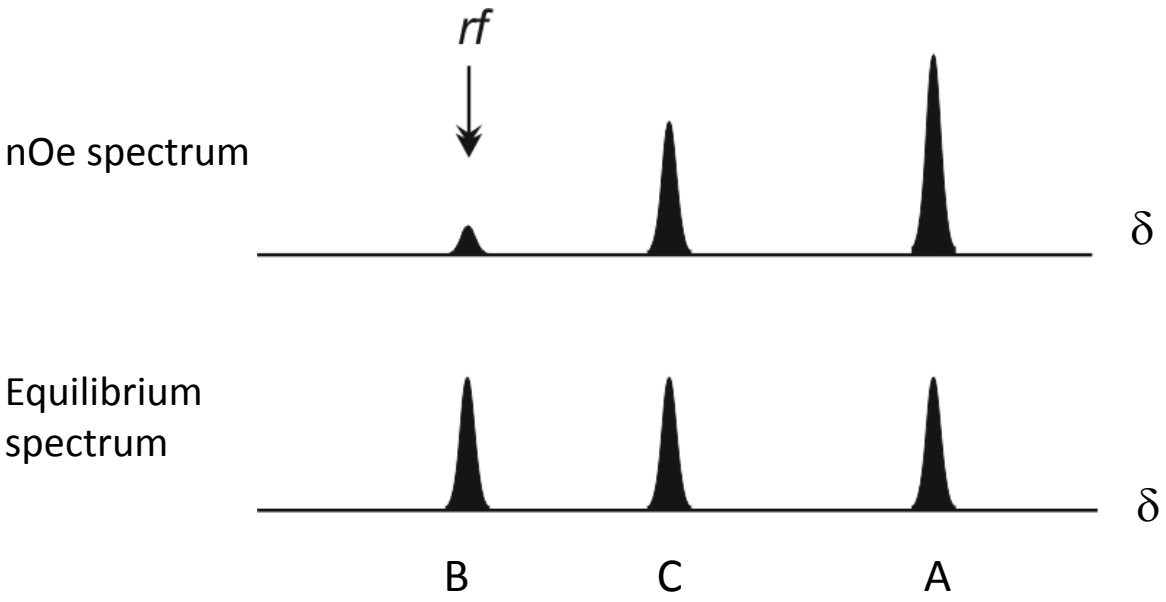
I= peak intensity of resonance in presence of nOe

Schematic ^{1}H-^{1}H nOe experiment:

Three protons A, B & C
-consider the nOe *from* B



rf irradiation acts to either **saturate** or **invert** the resonance of H_B



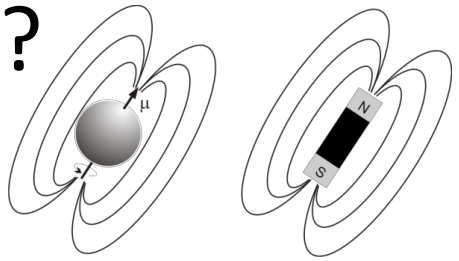
A is "close" to B
C is "far" from B

Signal intensity of A is enhanced by nOe

We say A receives an nOe from B: indicated by arrow

Note the similarity with *spin decoupling* which employs irradiation during spectrum recording.
Here for the nOe we irradiate before spectrum recording.
-both experiments are sometimes described in text books as "*double resonance*" experiments.

What is the nOe?
-some key points



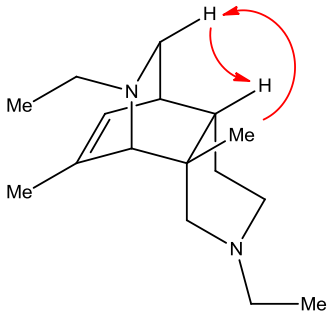
- The nOe reflects changes in spin population differences across resonance transitions
  - Positive nOe: population differences larger than at equilibrium (intensity gain)
  - Negative nOe: population differences smaller that at equilibrium (intensity loss)

- Origin of the nOe lies in *dipolar coupling* between nuclei (direct, through-space magnetic interactions) and is generated through *dipole-dipole spin relaxation* processes

- Scalar (J) couplings play no part!
- Strength of dipolar fields between two nuclei scale as $1/r^3$ (r is the internuclear separation)
  - ^{1}H-^{1}H nOes typically appear when $r \leq 0.4$ nm (4Å); hence indicate close nuclear proximity

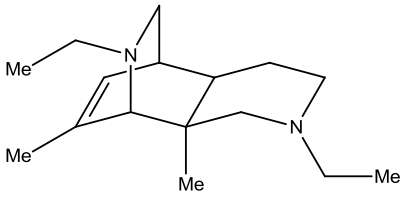
Applications of ^{1}H-^{1}H nOes

Stereochemical assignments



endo

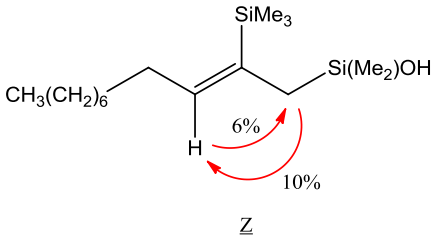
✓



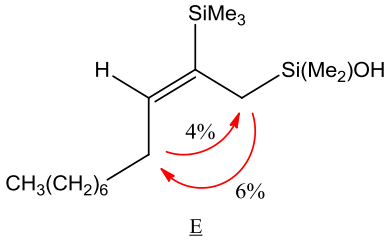
exo

✗

E *vs* Z alkenes

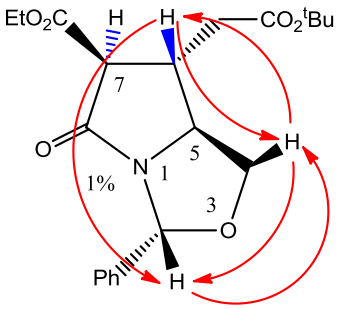


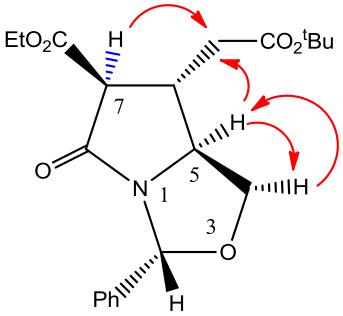
Z

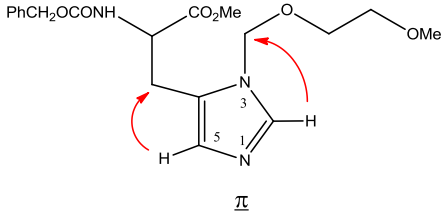


E

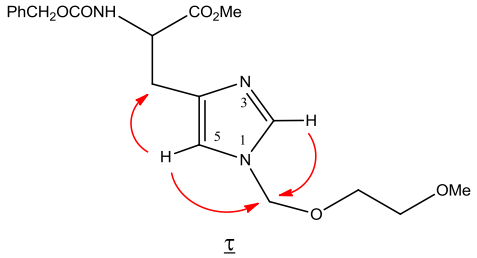
Substitution position







π

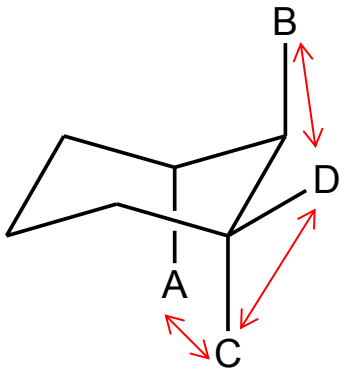


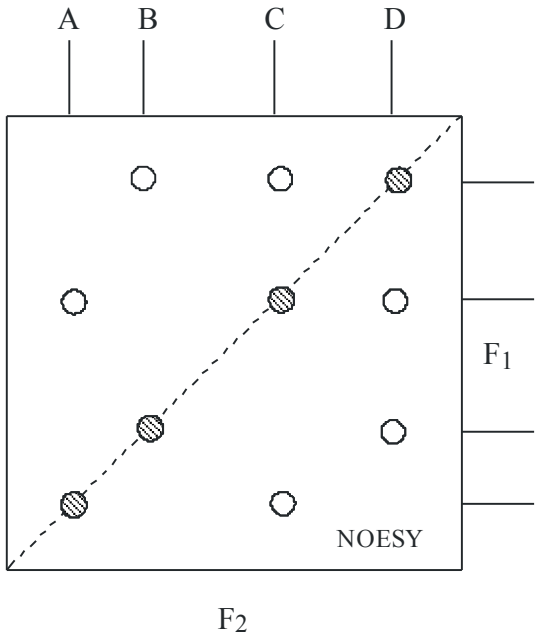
τ

(HMBC possible!)

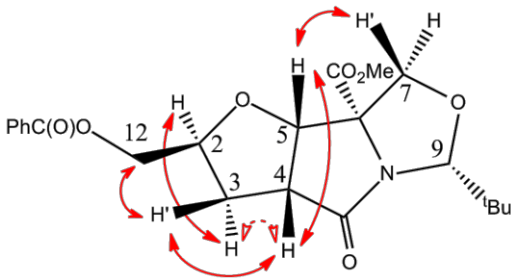
2D NOESY

- Homonuclear ^{1}H-^{1}H 2D correlation
- Maps all ^{1}H nOes within molecule in single experiment
- "Through-space" analogue of 2D ^{1}H-^{1}H COSY for J-couplings
- Often time-consuming as nOes rather weak!

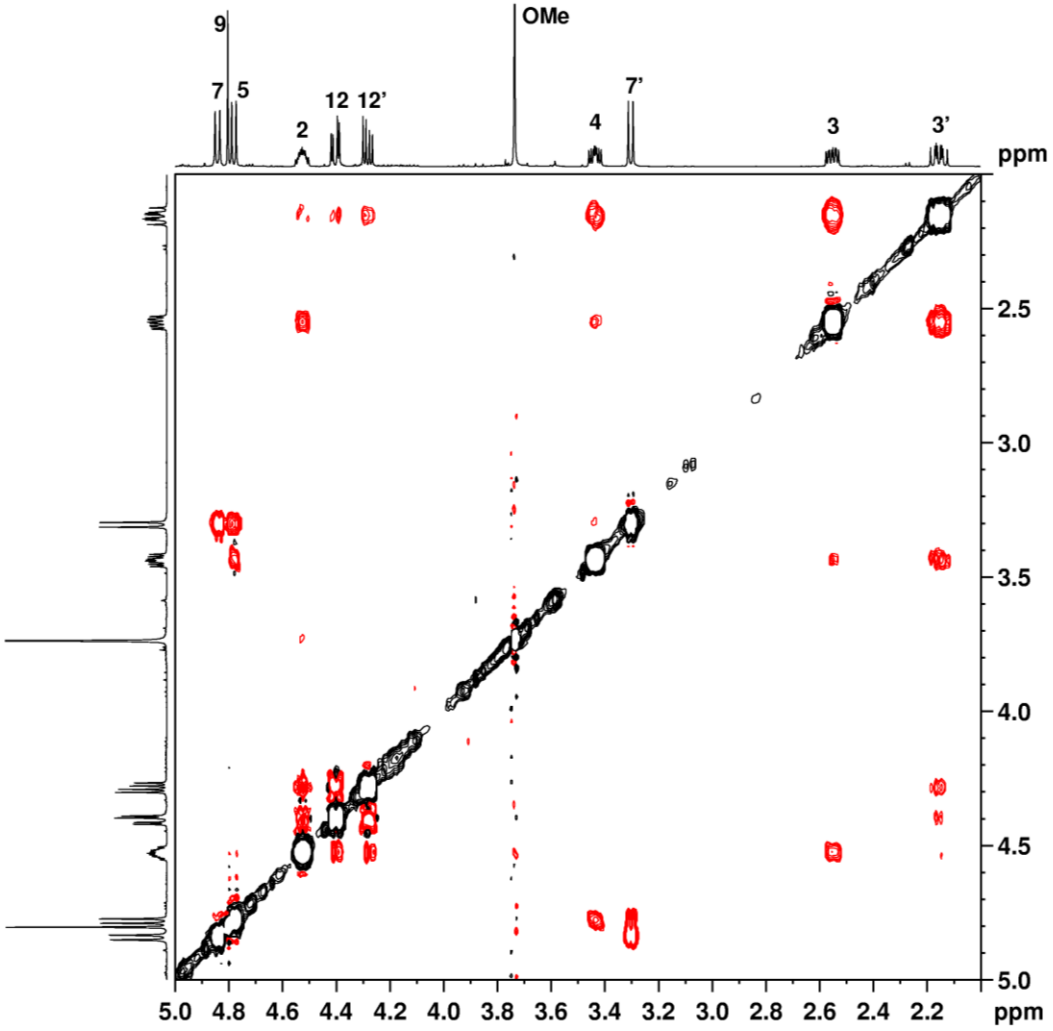




2D NOESY

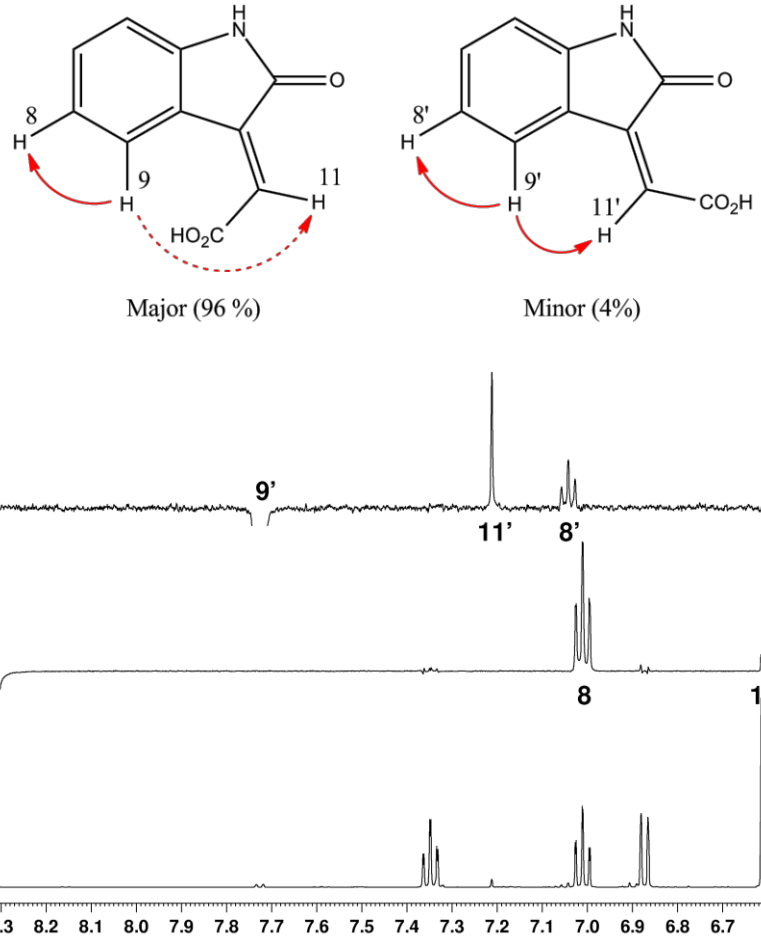


For typical small molecules in low viscosity solvents, nOes are **positive** and have **opposite sign** to the NOESY diagonal peaks

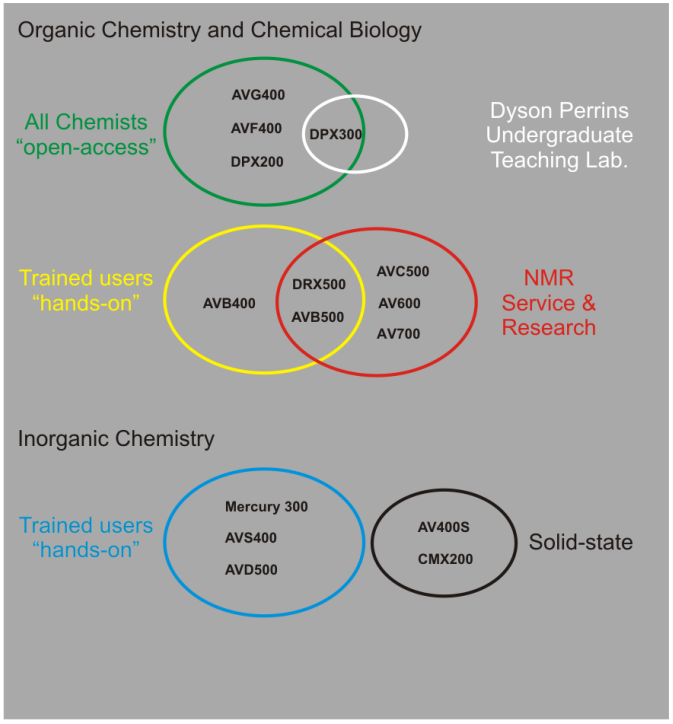


1D NOESY

- NOes observed from a selectively inverted target proton resonance
- Maps nOes for protons from a single resonance at a time
- Quicker than 2D when only a few key nOes required
- Modern “gradient selected” experiments provide clean cancellation of unwanted signals



Chemistry NMR Facility



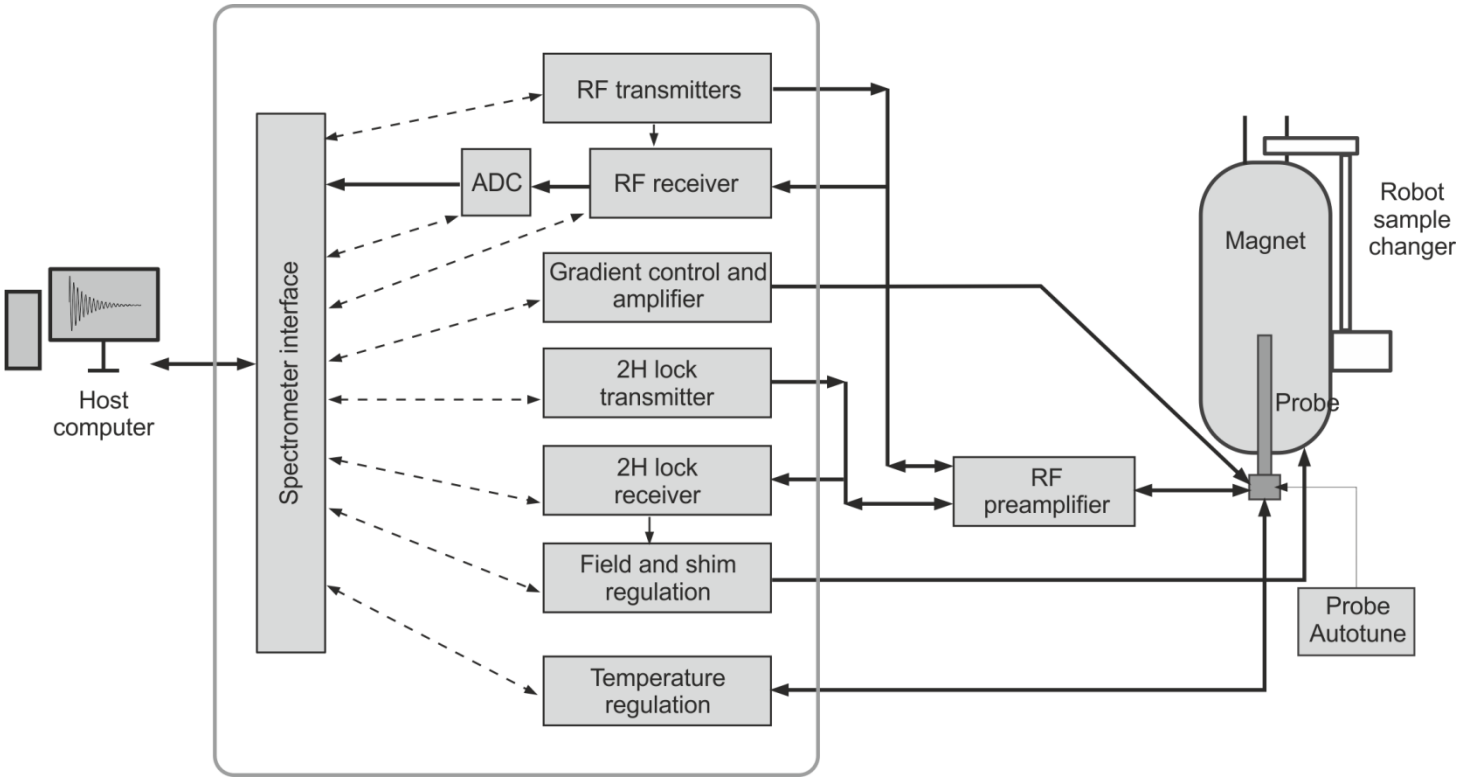
University of Oxford
Doctoral Training Centre

NMR Spectroscopy

Some technical and practical aspects

Inside the NMR spectrometer:

what is the instrument doing??



Probe tuning & matching

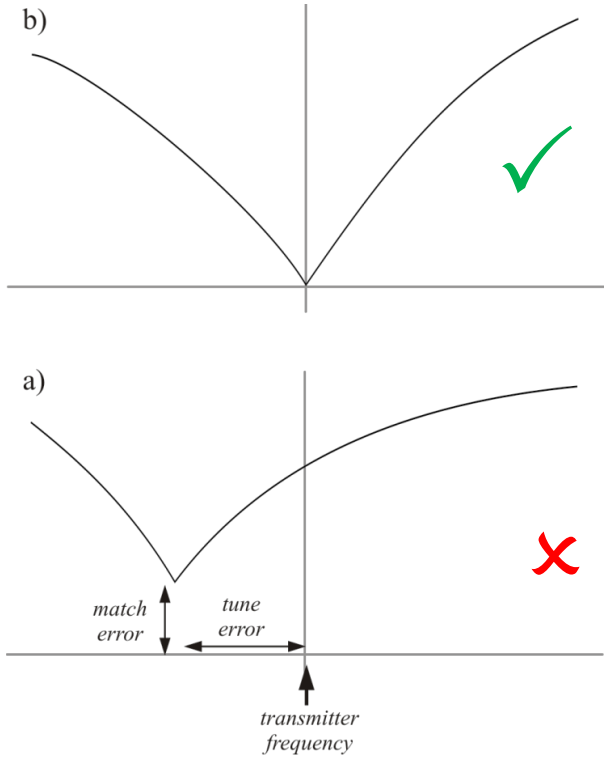
Probehead radiofrequency circuit must be *tuned* to the correct frequency and *matched* to a 50 Ohm load to give the optimum response and correct pulse calibrations

Tuning is analogous to tuning a radio to the desired station

Matching equalises the impedence (AC resistance) of the coil/sample combination to that of the transmitter/receiver

Both are sample dependent

Probehead capacitors adjusted

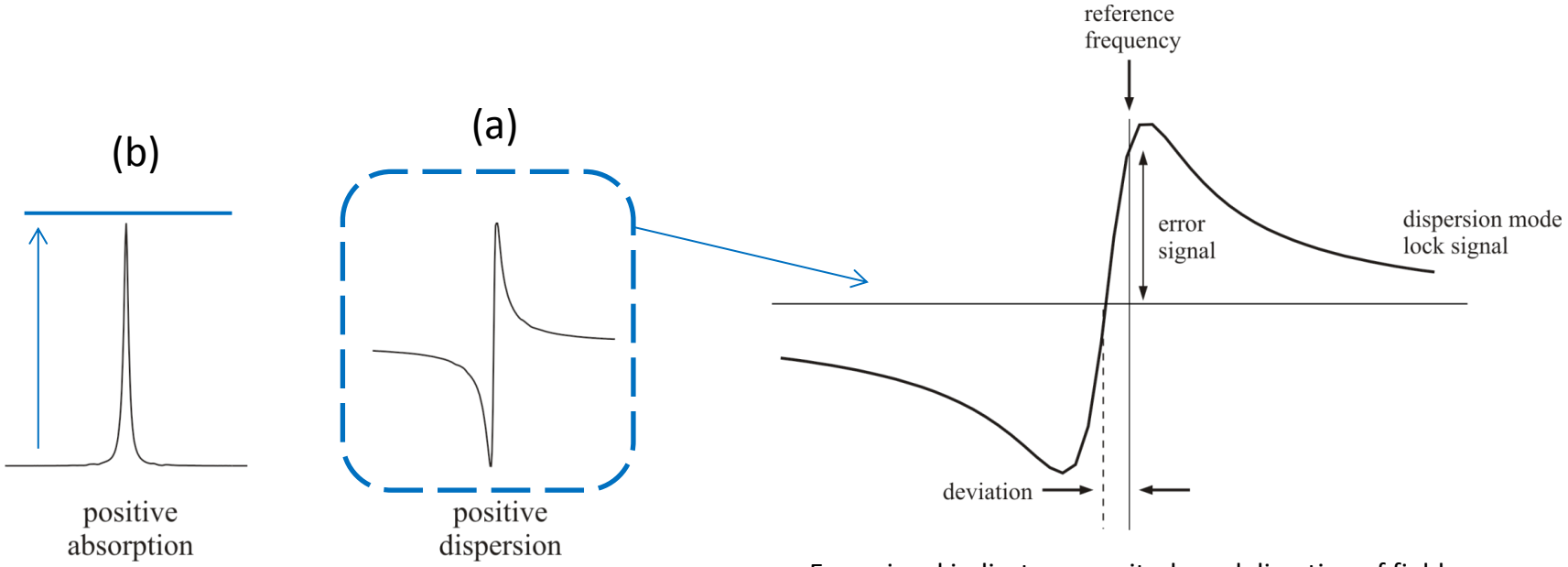


Field-frequency locking

Spectrometer frequencies are “locked” to that of the solvent ^{2}H resonant frequency:

a) Compensates drift in magnetic field (and hence frequencies)

b) Provides a means to measure field homogeneity (for shimming)



Solvent ^{2}H resonance

Error signal indicates magnitude and direction of field drift to compensate

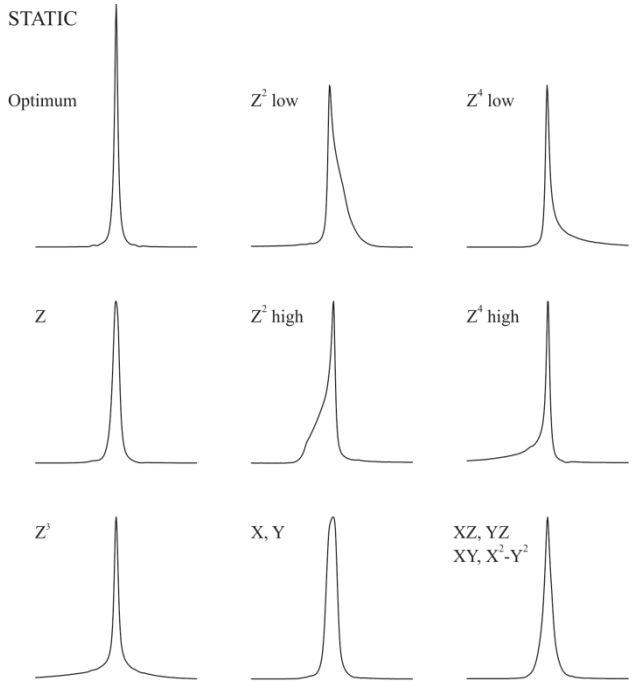
Field shimming

Magnetic field in which sample sits must be homogeneous to ~ 1 part in 10^9 (0.5 Hz at 500 MHz) across whole sample!

Samples distort local magnetic fields (they have *magnetic susceptibilities*) so each requires local field optimisation: *shimming*

Errors in local field leads to lineshape distortions

Shimming involves applying currents to coils surrounding sample to generate small, corrective magnetic fields (term originates from optimising permanent steel magnets)



Common shim errors

Receiver gain adjustment

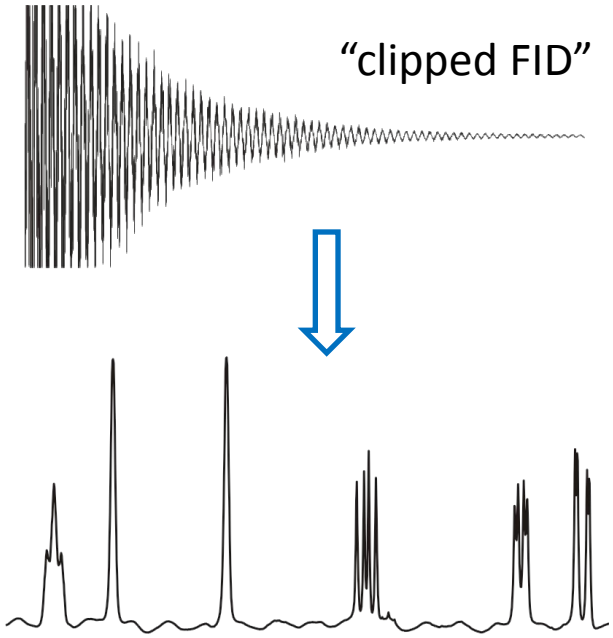
Amplification of weak NMR signals aids their detection

Extent of amplification is dictated by the receiver gain adjustment:

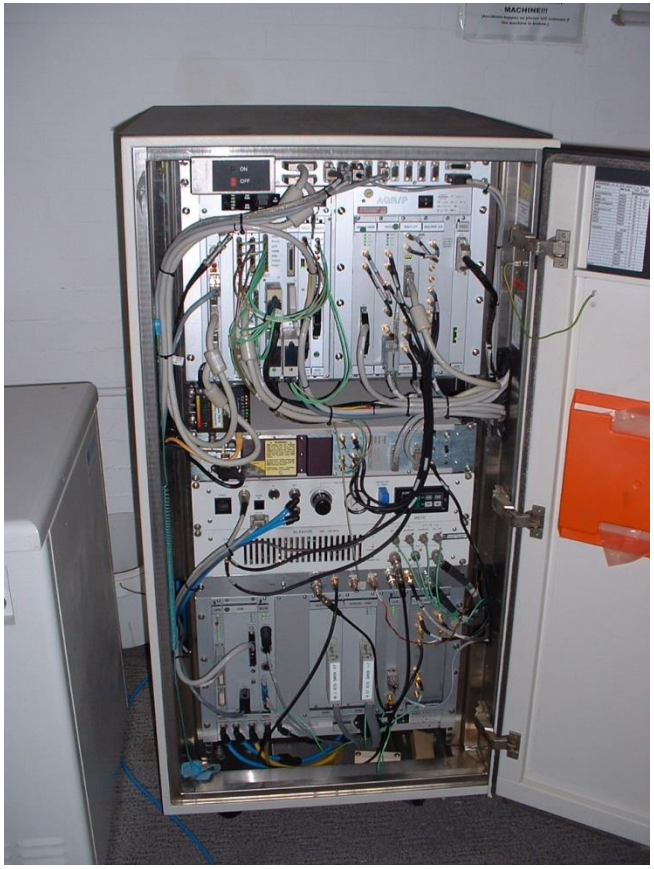
Too low: poor sensitivity

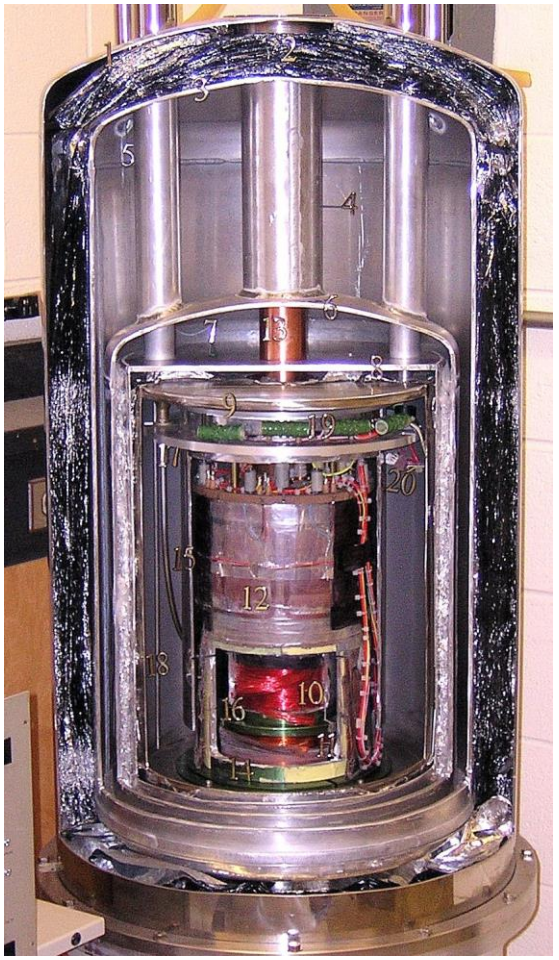
Too high: signal distortion

Analogous to volume button!



Receiver gain overload





http://www.youtube.com/watch?feature=player_detailpage&v=nY3bgZY_nF4

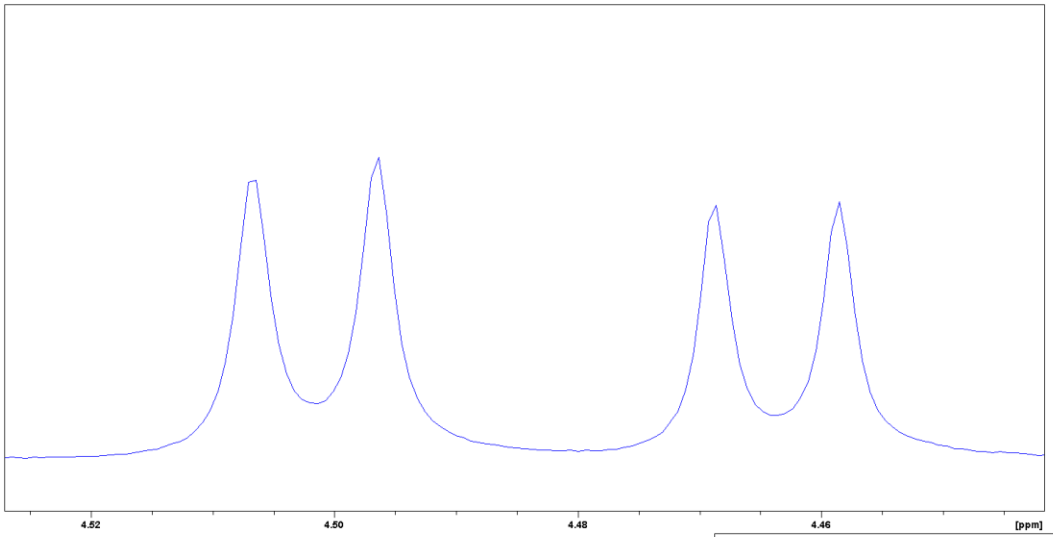
http://www.youtube.com/watch?feature=player_detailpage&v=nBVHnZ8tru0

Optimising 1D spectra

- How accurately can I measure J-coupling values?
- How can I improve spectrum sensitivity?
- How can I improve peak resolution?
- How do I measure accurate integrals?

- All the necessary processing procedures are found in all standard NMR processing packages:
  - TOPSPIN, Mnova, etc

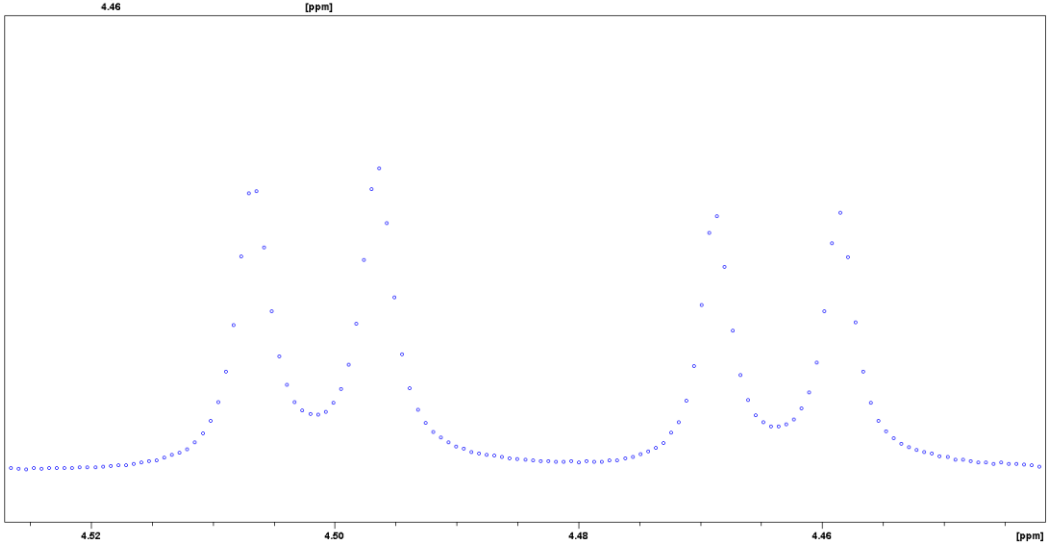
NMR data are digitised…



Frequency spacing between data points is known as the:

digital resolution

Hz per point (Hz/pt)
(smaller value, higher resolution!)

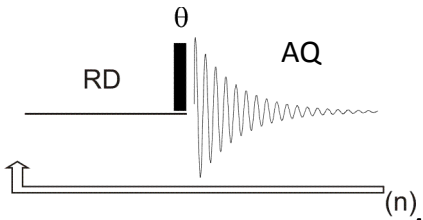


Measuring J-couplings

Accuracy of coupling constant measurement is limited by the ***digital resolution DR*** of the spectrum, that is, the spacing between individual data points

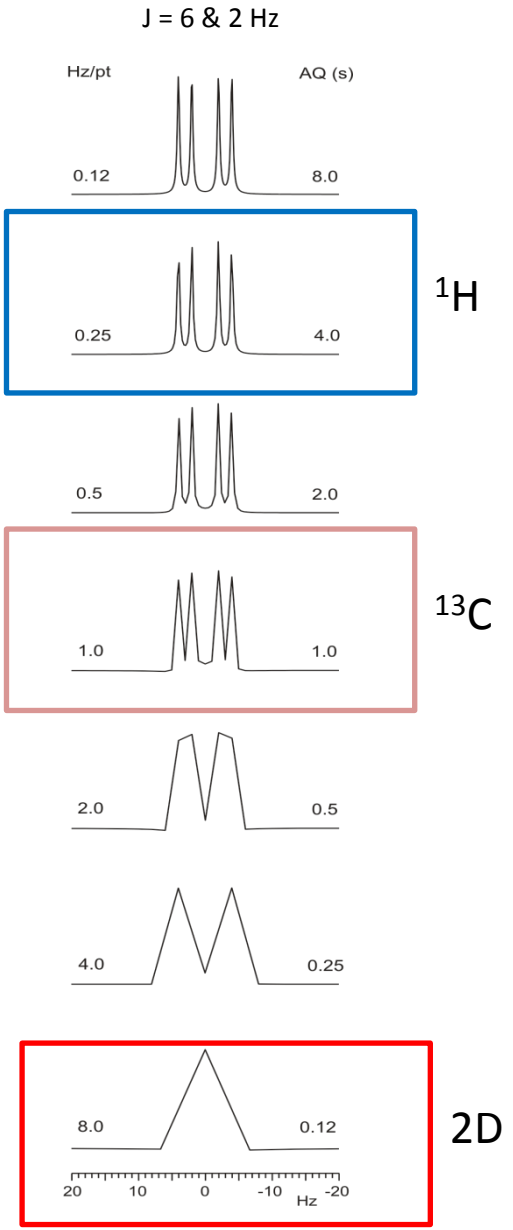
This in turn is dictated by the ***acquisition time AQ*** of the FID

DR (Hz/pt) = 1/AQ



For most proton spectra AQ = ~ 3s and DR ~ 0.3 Hz/pt

Assuming a measurement error of +/- DR, J couplings may be quoted to ~ 0.5 Hz!



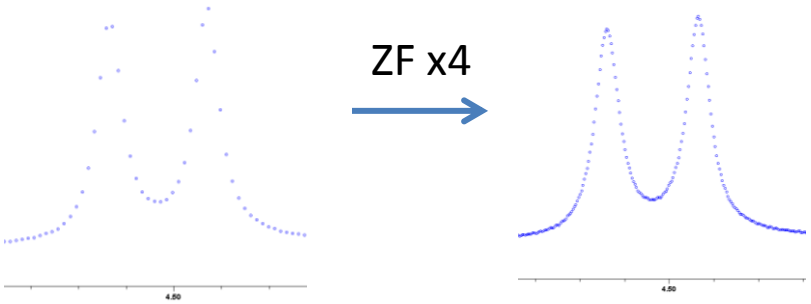
Data processing “tricks”

Zero filling- *improving digital resolution*

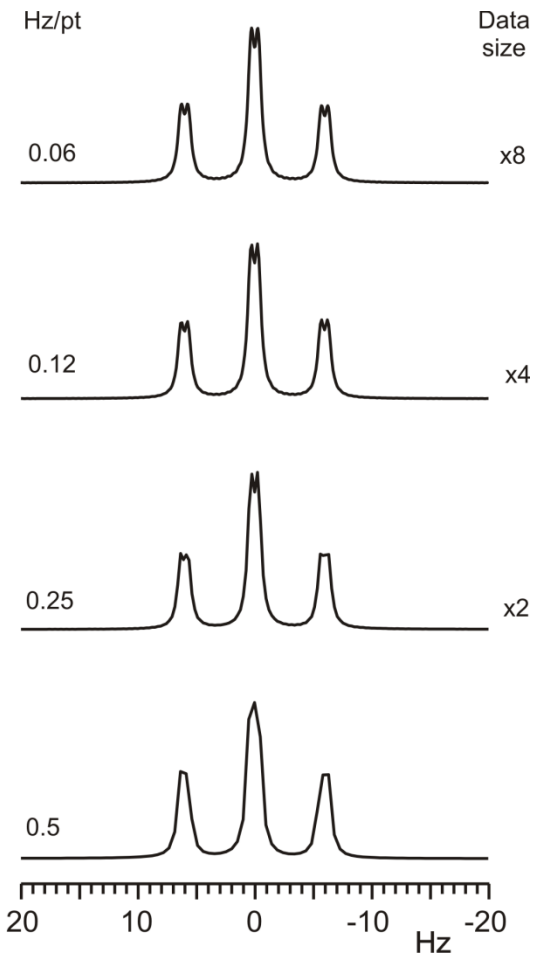
Artificially extend acquired FID by appending “zeros” to end

After FT these points interpolate between “acquired” data points and so enhance digital resolution

Zero filling by factor of 2 (double data size) or 4 improves peak definition (and appearance) and reveals fine structure



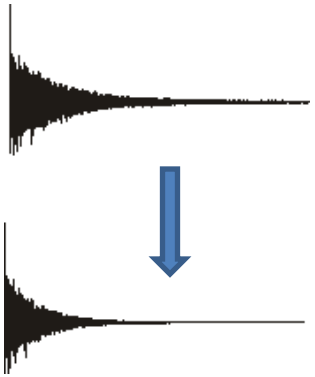
32 k to 128 k points



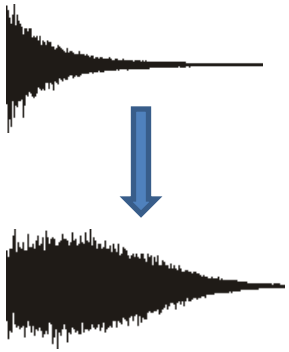
Data processing “tricks”

Window functions (apodisation)

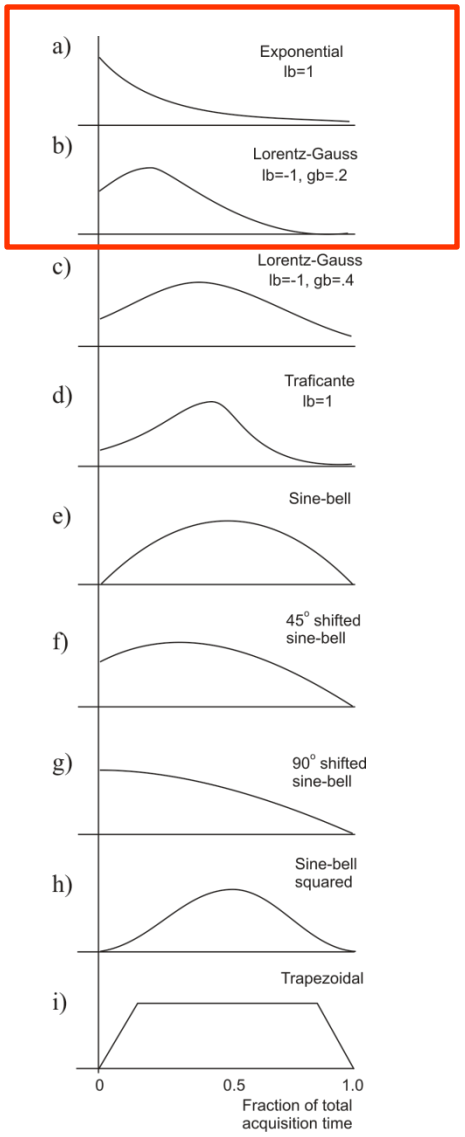
Add numerical shaping to the raw FID to alter its profile.
After Fourier transformation this will alter the appearance of the resulting spectrum



sensitivity

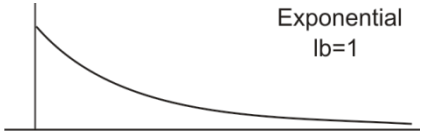


resolution



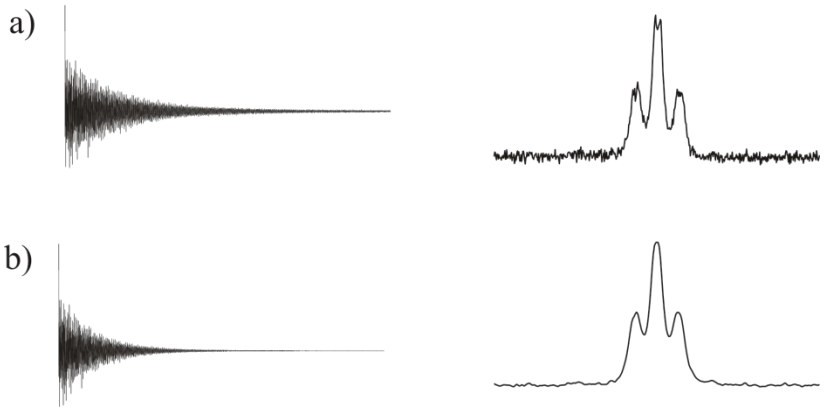
Sensitivity enhancement

Exponential multiplication:



- A function that smoothes the latter part of the FID
- Reduces ***noise*** but increases ***line width***
- Hence, improves sensitivity but decreases resolution

- Parameters: extent of “line broadening” (Hz); “lb”



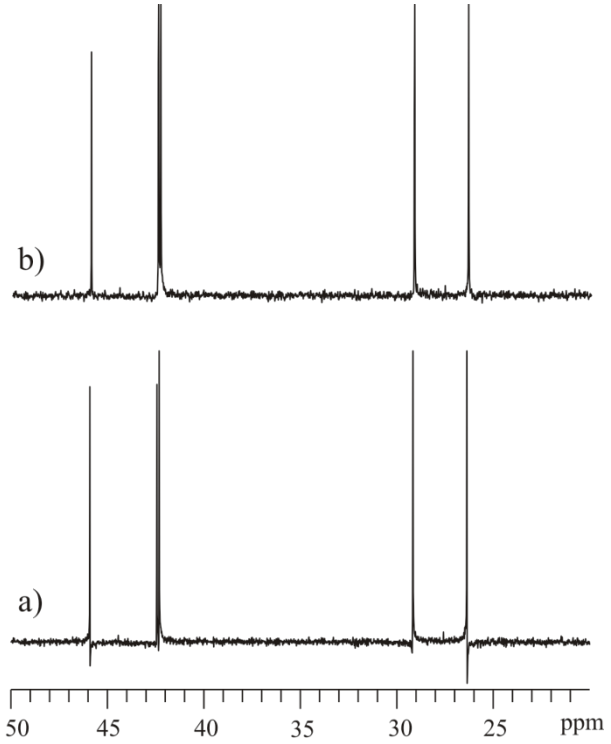
lb = 1 Hz

Some slight sensitivity enhancement is applied to all routine spectra, lb= 0.3 Hz for proton and is **essential** for all heteronuclear spectra, ^{13}C typically at least 1 Hz.

Sensitivity enhancement

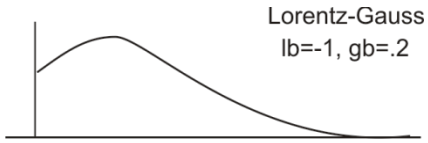
Heteronuclear spectra often display distortions if no line broadening is applied!

This is a consequence of the short acquisition times (low digital resolution) used.



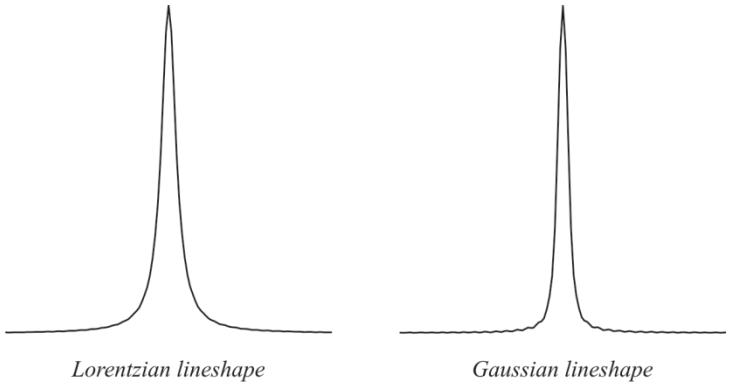
Resolution enhancement

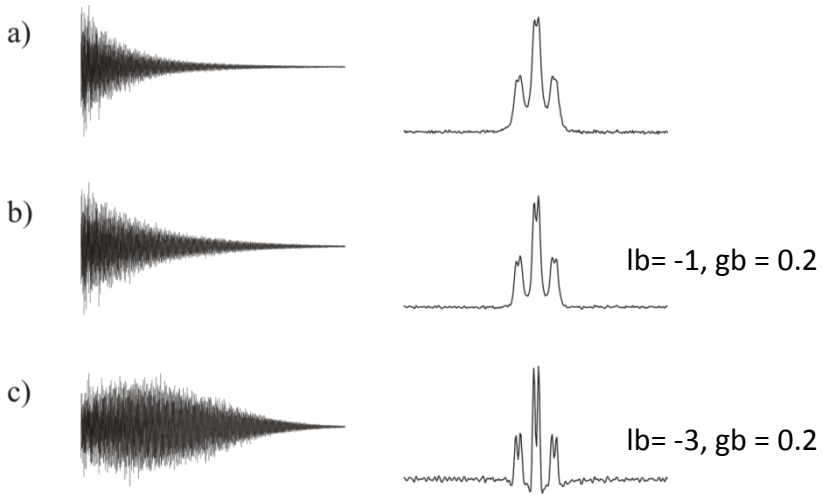
Gaussian multiplication:



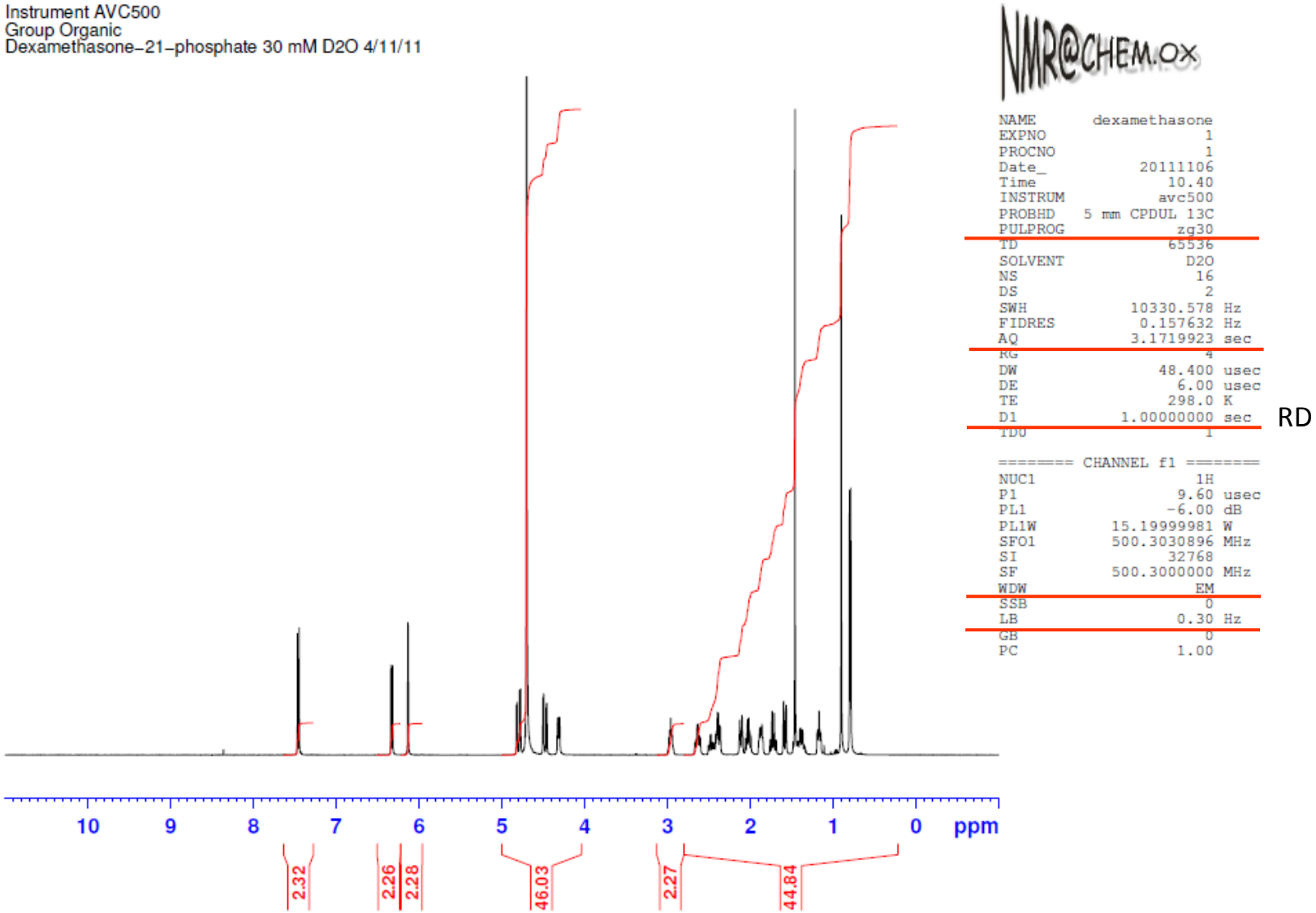
- A function that enhances the latter part of the FID
- Reduces ***line width*** but increases ***noise***
- Hence, improves resolution but decreases sensitivity

- Parameters: position of maximum & extent of line narrowing (Hz); “gb”, “-lb”



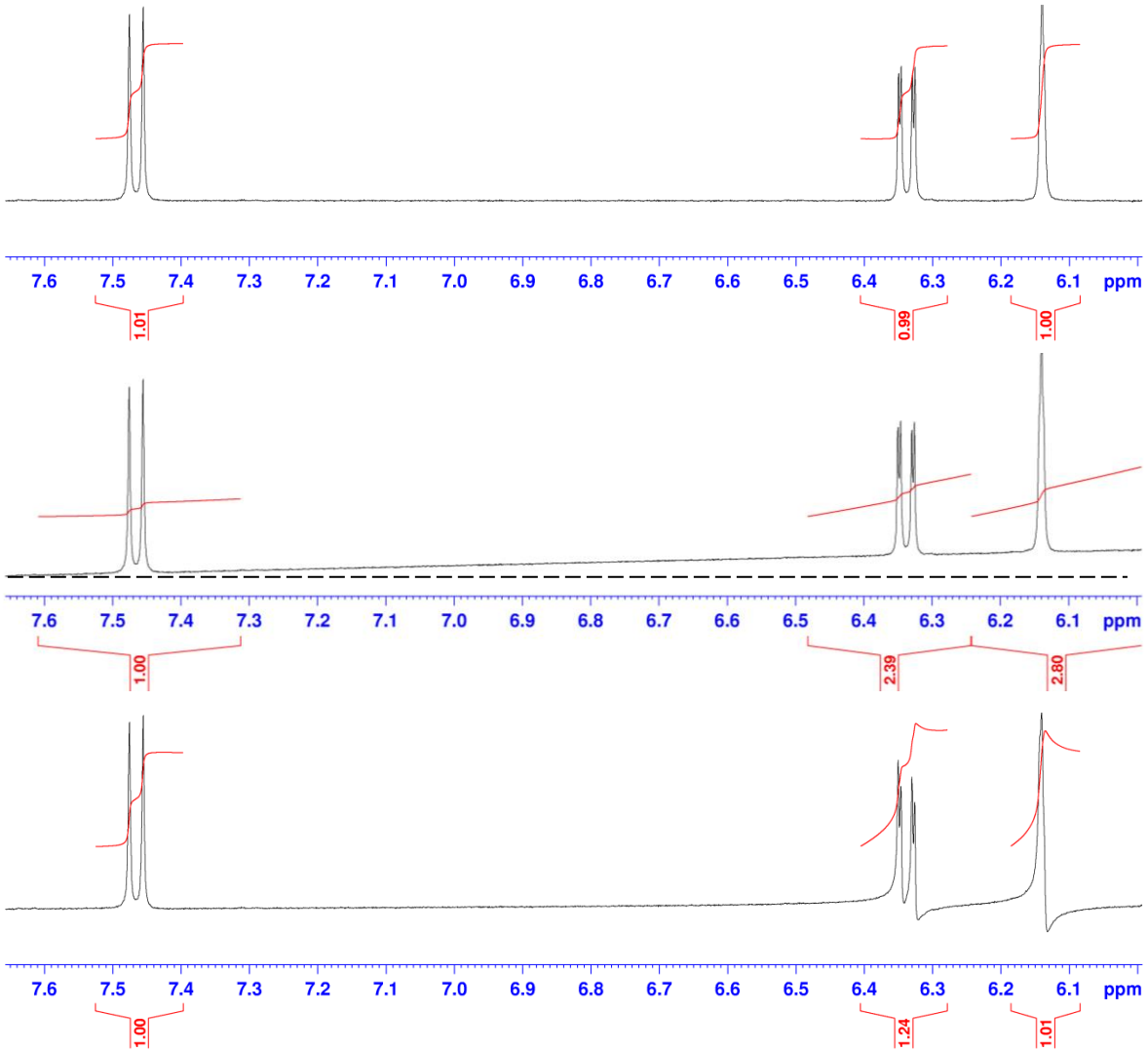


A standard ^{1}H spectrum



Integration

Correct phase and baseline



Incorrect baseline (sloping)

Incorrect phasing