NMR Experiments for Structure Determination

Dr Michael Thrippleton

Introduction

Nuclear Overhauser Effect (NOE)

•through **space**

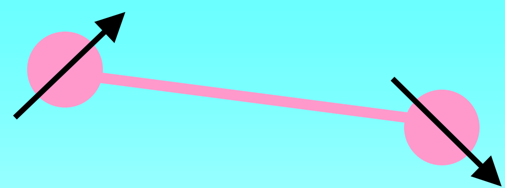
•1D NOE

•2D NOESY

•ROESY

J-coupling

•through **bond**



•*J*-spectroscopy

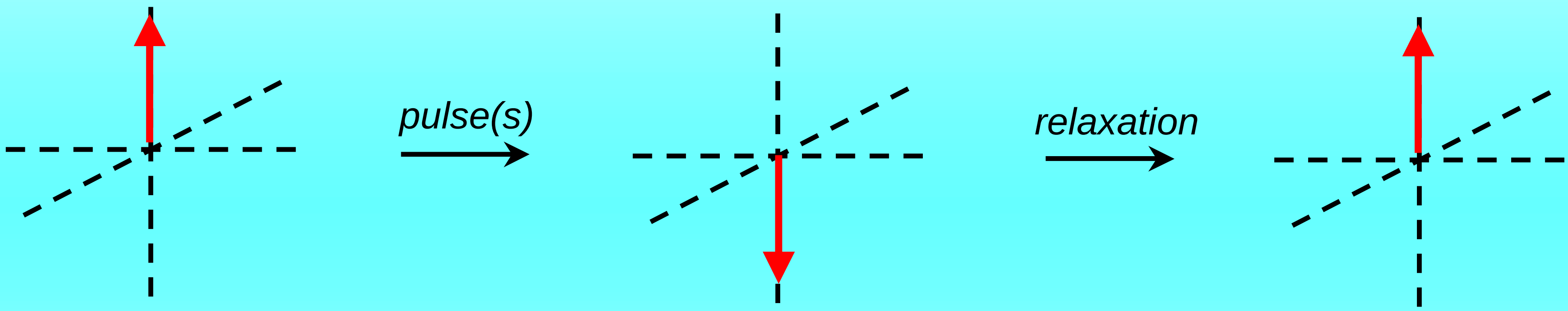
•DQF COSY

•*z*-COSY

•HMBC

The NOE

Self-Relaxation

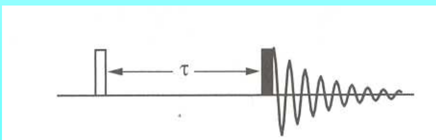


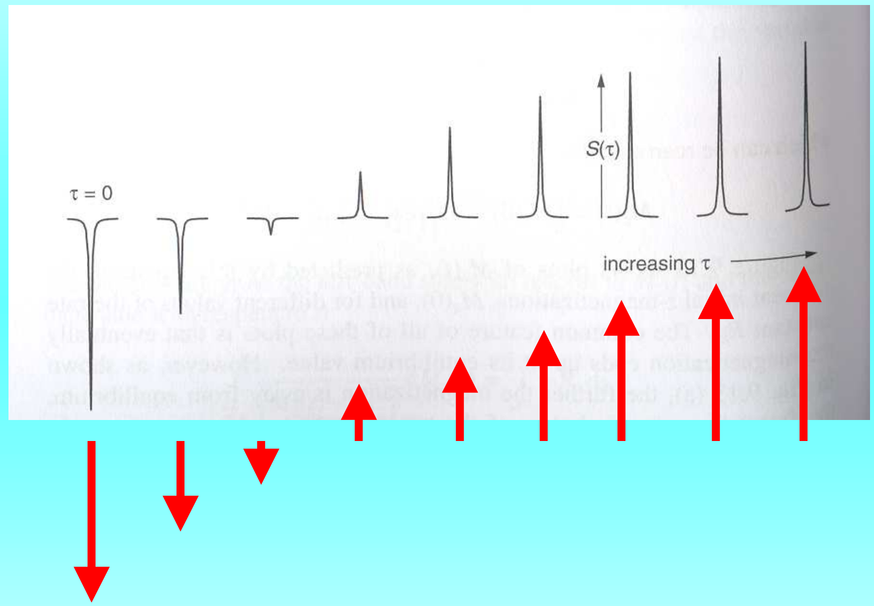
Relaxation is the process by which magnetisation returns to equilibrium

- longitudinal relaxation rate R_1
- Transverse relaxation rate R_2

The NOE

Inversion recovery



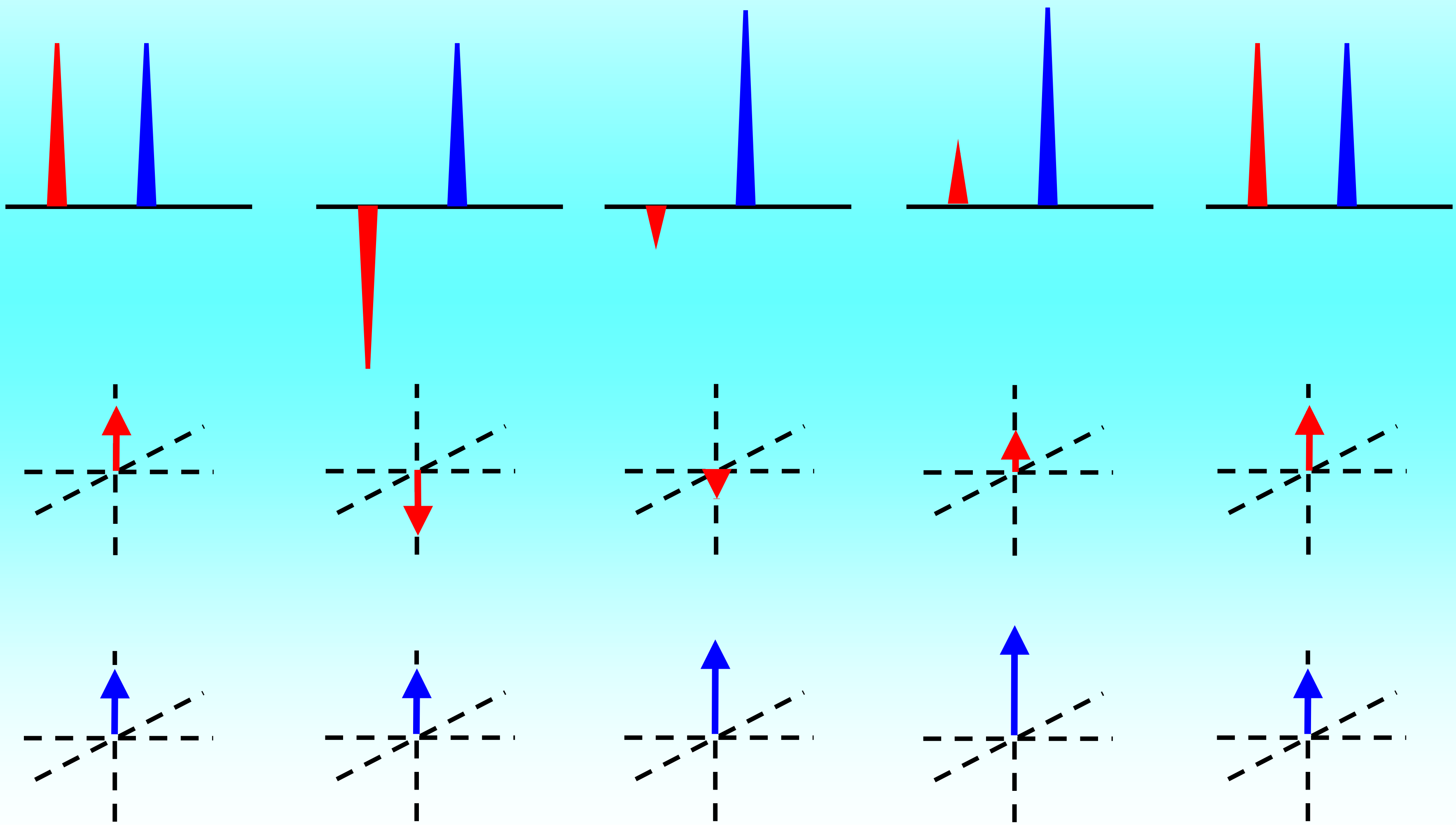


Magnetisation returns to +z axis during τ at rate R_1

•figures reproduced from *Understanding NMR spectroscopy*, by James Keeler

The NOE

Cross-relaxation



The NOE

Cross-relaxation

Perturbation of spin I from equilibrium causes spin S to grow/shrink

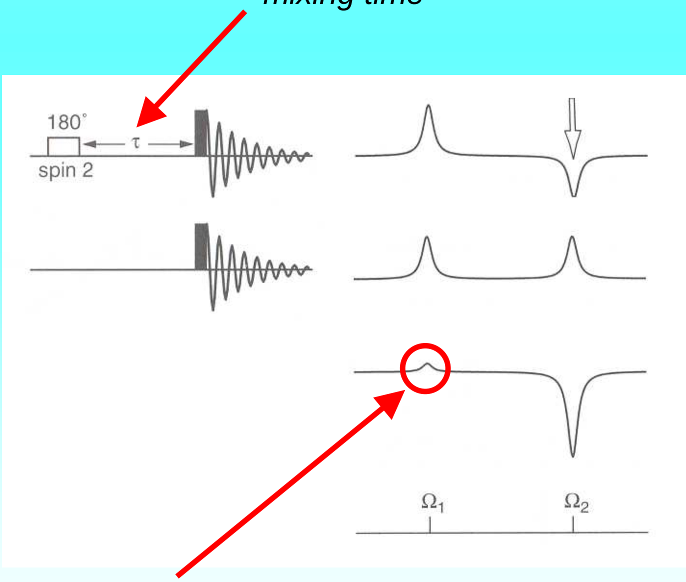
- cross-relaxation rate constant σ_{12}
- spins must be close in space

The NOE

Transient NOE

•Target spin inverted and allowed to relax to equilibrium
•Cross-relaxation generates NOE on neighbouring spin

"mixing time"



NOE enhancement

irradiated spectrum

reference spectrum

difference spectrum

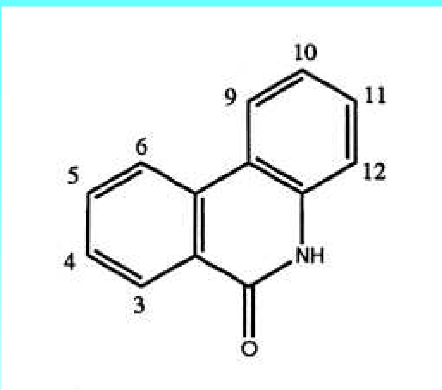
$$\eta = \frac{S_{irr} - S_{ref}}{S_{ref}}$$

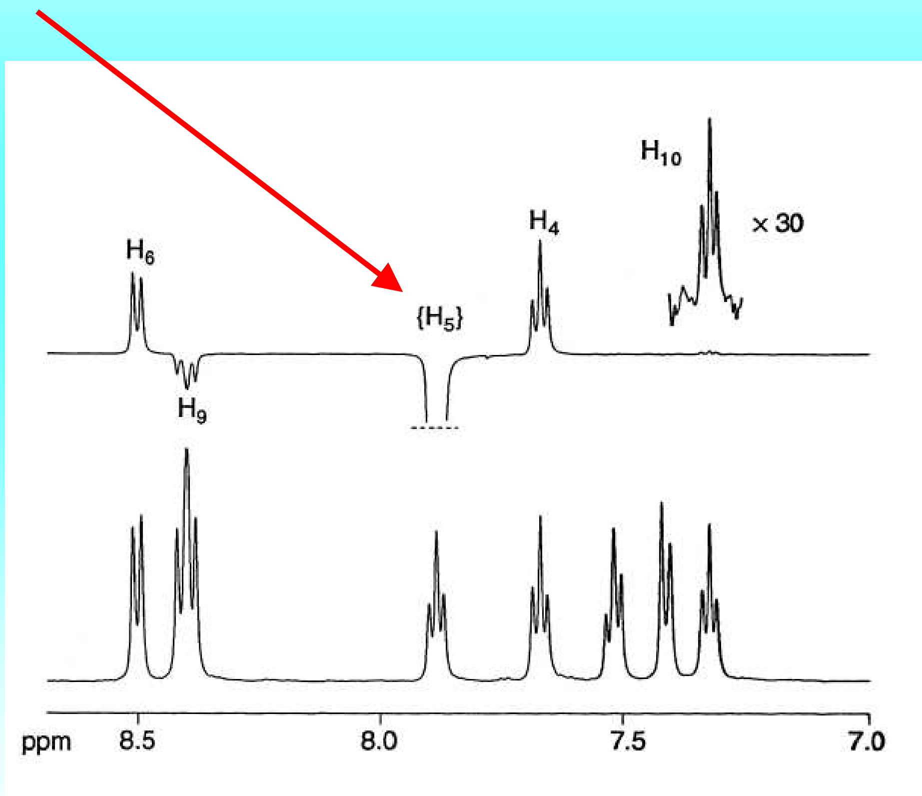
•figures reproduced from *Understanding NMR spectroscopy*, by James Keeler

The NOE

Transient NOE

irradiated spin





difference spectrum

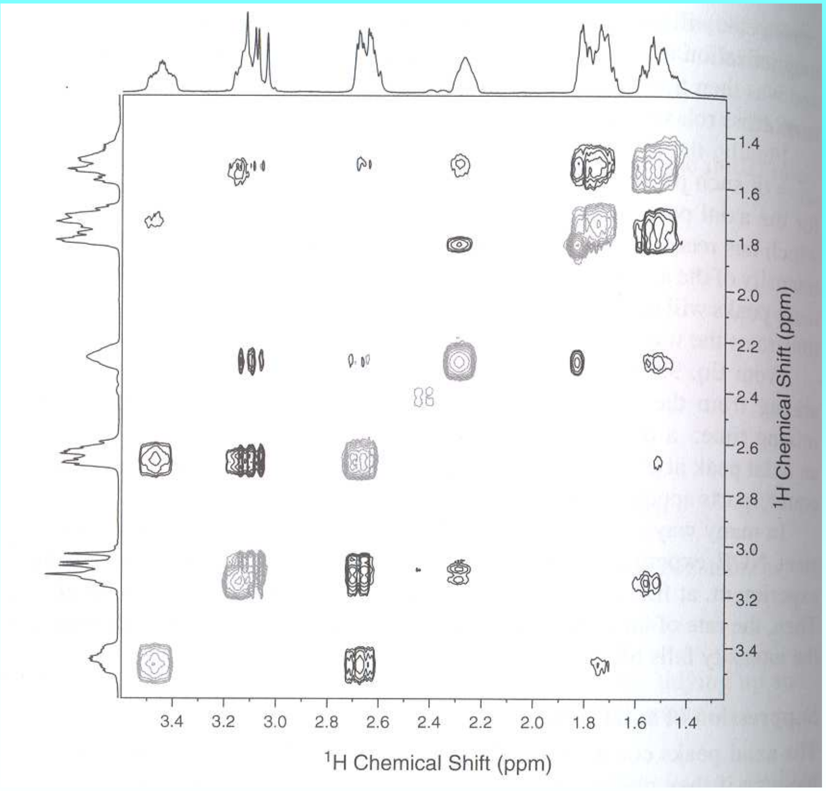
reference spectrum

•figures reproduced from Stott et al., *J. Magn. Reson.* **125**, 302-324

The NOE

NOESY

- 2D version of 1D transient NOE
- takes longer, but contains more information
- row from NOESY looks like 1D experiment



•spectrum reproduced from *Understanding NMR spectroscopy*, by James Keeler

The NOE

Relaxation Mechanisms

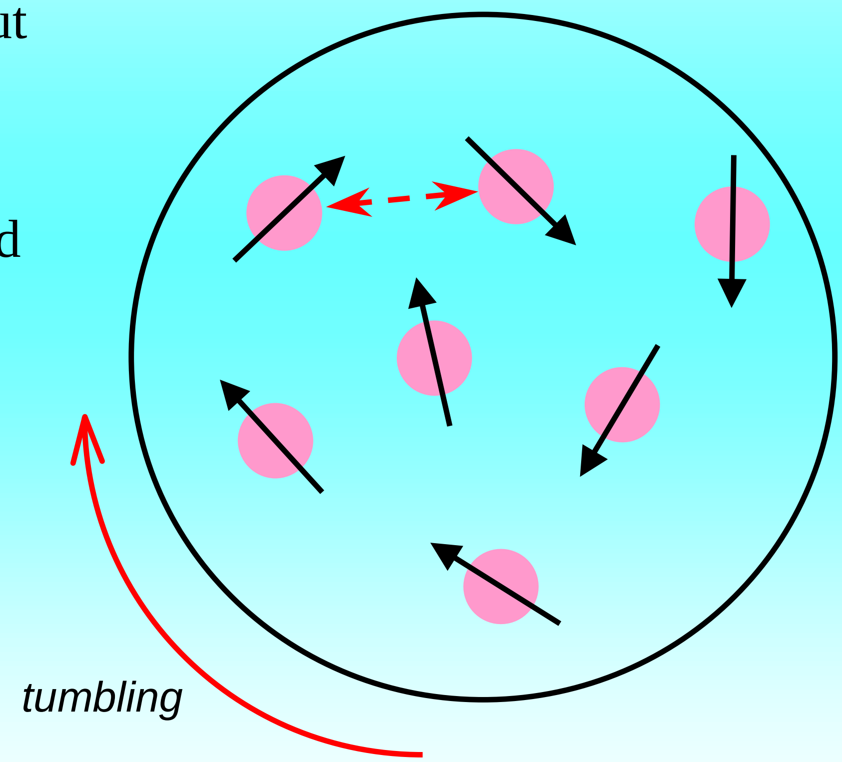
- Dipole-dipole coupling *nearly* averaged out by molecular tumbling
- Remainder responsible for relaxation
- Rate depends on *timescale* of motion τ_c and *distance* between spins (r^{-6})

Small molecules, e.g. quinine

- rapid motion / short τ_c

Large molecules, e.g. proteins

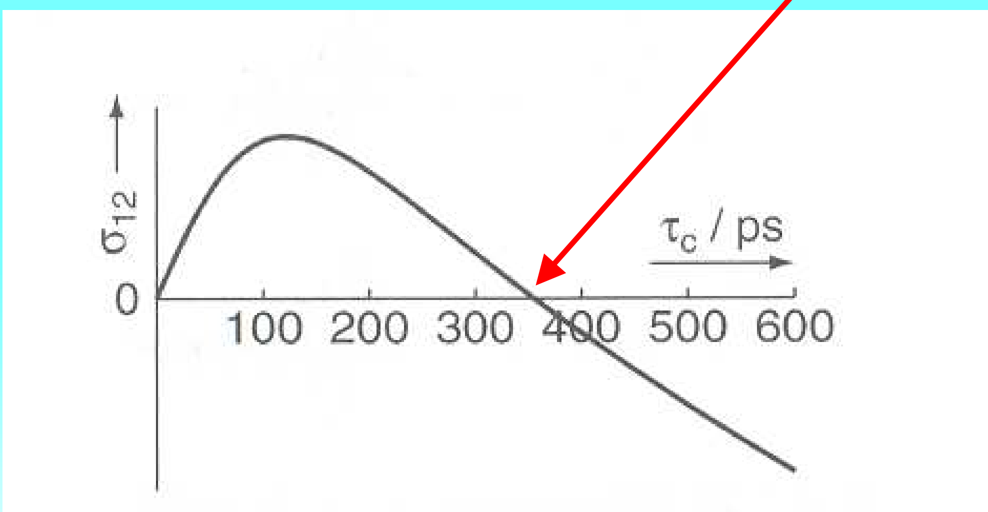
- slow motion / long τ_c



The NOE

How big, how fast?

"zero crossing" $\omega_0 \tau_c = \sqrt{\frac{5}{4}}$



small molecules *large molecules*

- slow / absent for v. small or intermediate-size molecules
- NOE positive for small molecules
- NOE negative for large molecules

•figure reproduced from *Understanding NMR spectroscopy*, by James Keeler

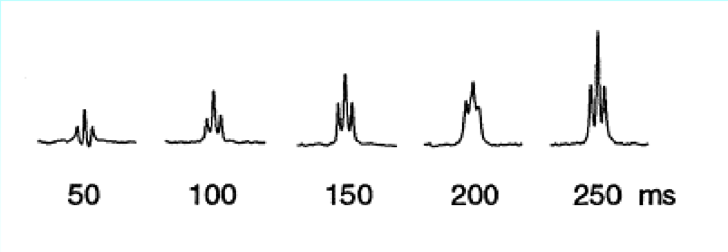
The NOE

How big, how fast?

•NOE experiments are in general **not** quantitative
•presence of NOE implies r < 5Å

However:

•Transient NOE usually recorded under "initial rate" conditions
•Distances can sometimes be estimated from rate of NOE buildup using a known reference distance



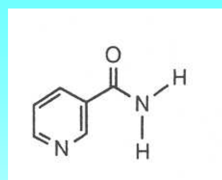
$$\eta = 2\sigma_{12}\tau$$

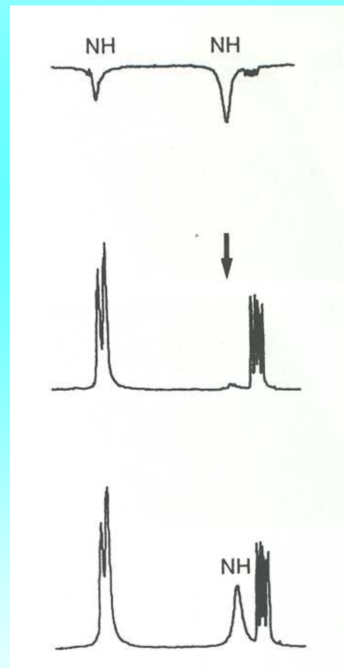
•figure reproduced from *Understanding NMR spectroscopy*, by James Keeler

NOE pitfalls

Exchange Peaks

•"NOEs" between exchanging sites
- •appear as *negative* "NOEs"
- •easy to distinguish for small molecules





difference spectrum

irradiated spectrum

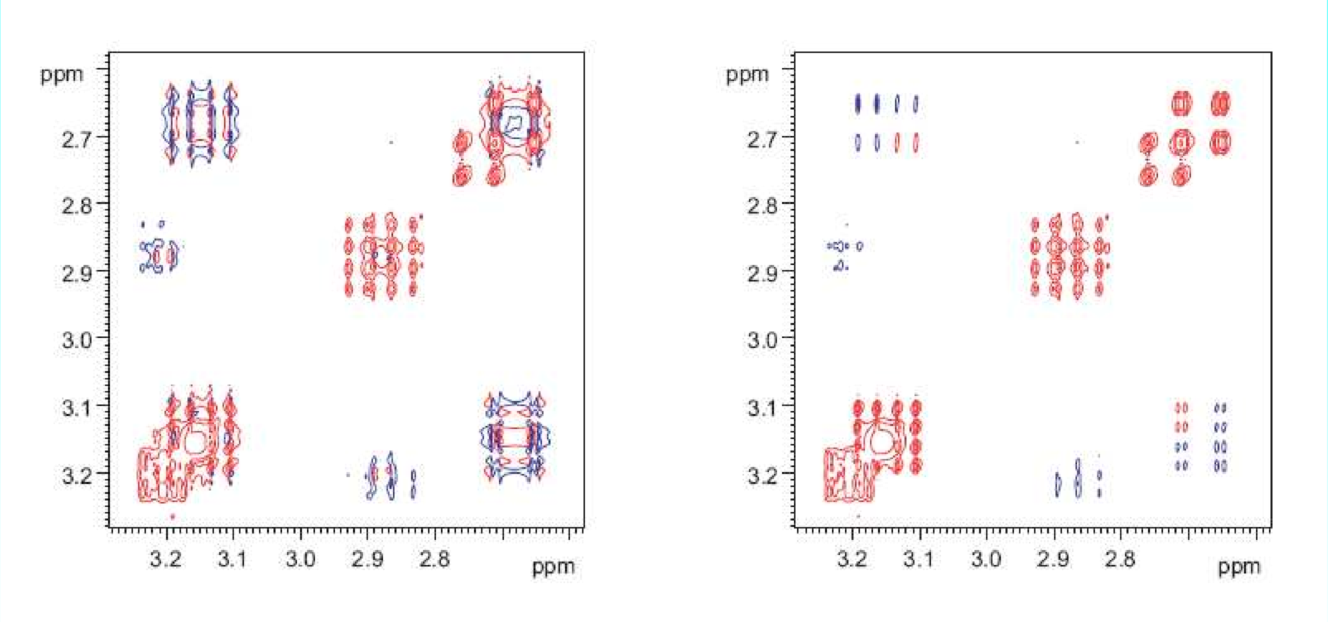
reference spectrum

•spectrum reproduced from *Modern NMR Spectroscopy*, by Sanders and Hunter

NOE pitfalls

Zero-quantum interference

- Appearance of "NOEs" or NOESY cross-peaks between *J*-coupled spins
  - easy to spot due to broad, dispersive lineshape
  - should be suppressed using modern NMR techniques



without zero-quantum suppression *with zero-quantum suppression*

- spectra reproduced from MJT Phd thesis

NOE pitfalls

Subtraction Artefacts

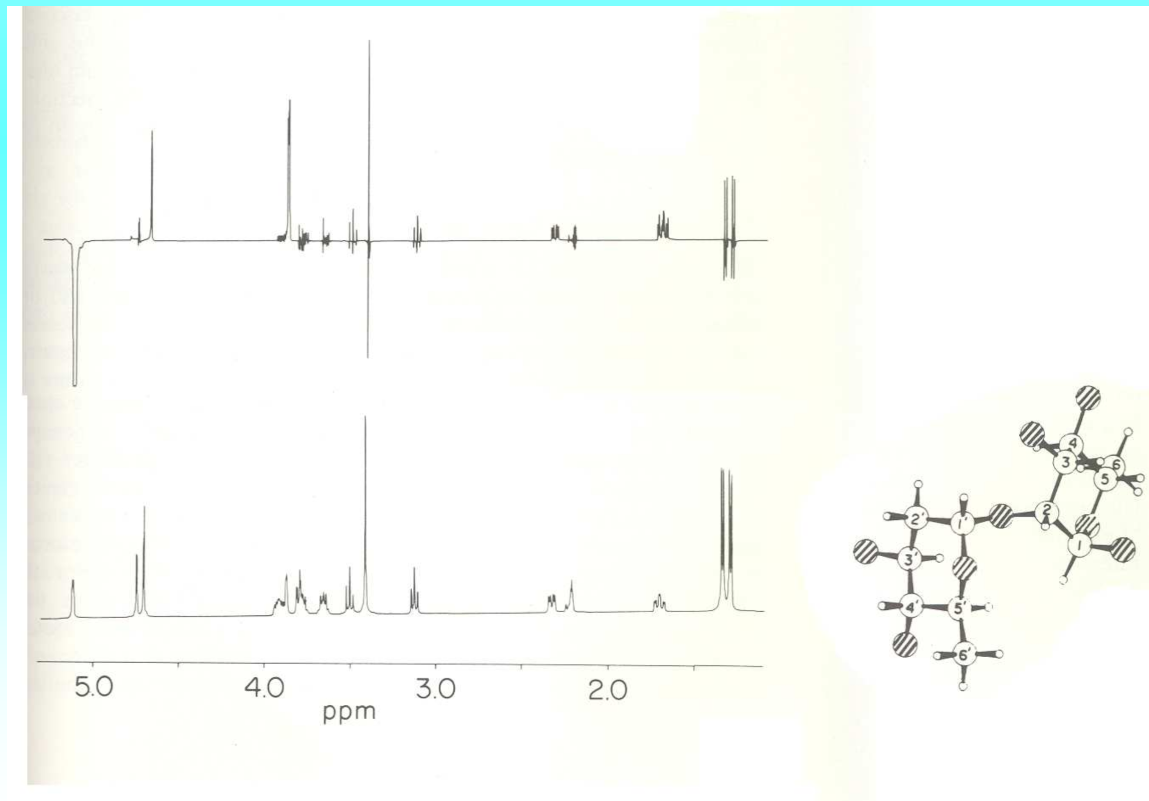
•Poor subtraction of spectra leads to imperfect cancellation

•Due to spectrometer instability

•Subtraction artefacts can obscure small NOEs

difference spectrum

reference spectrum



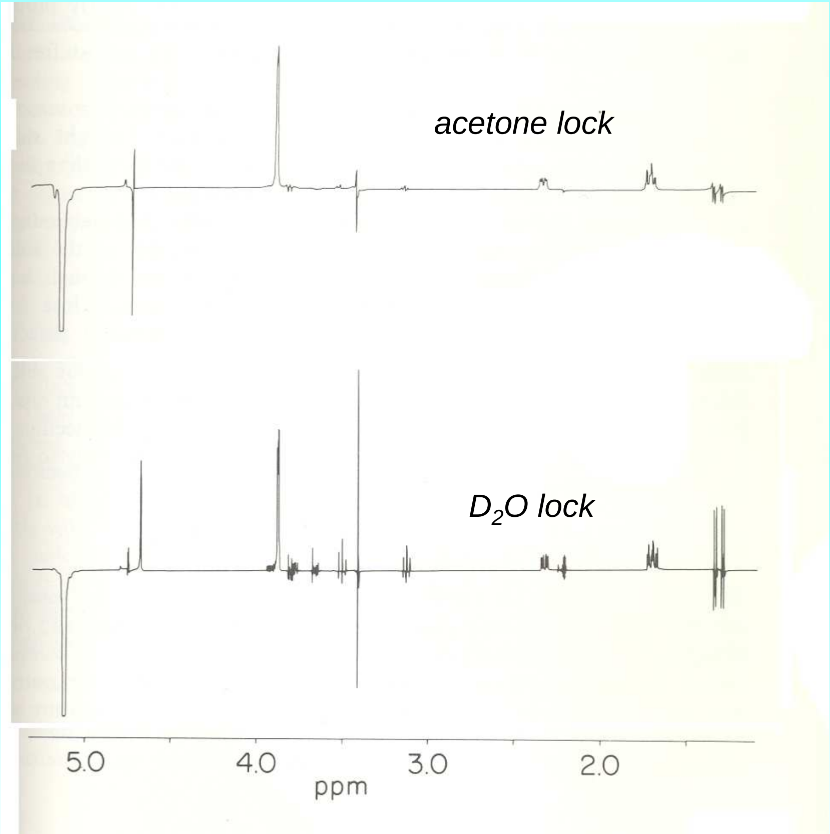
•spectra reproduced from *The Nuclear Overhauser Effect*, by D. Neuhaus and M. Williamson

NOE pitfalls

Subtraction Artefacts

•Remedy:

- •modern spectrometer
- •modern gradient-based NOE experiments, e.g. DPFGSE-NOE, GOESY
- •acquire more scans
- •temperature stability
- •solvent with strong, sharp lock signal, e.g.d6-acetone, not D_2O

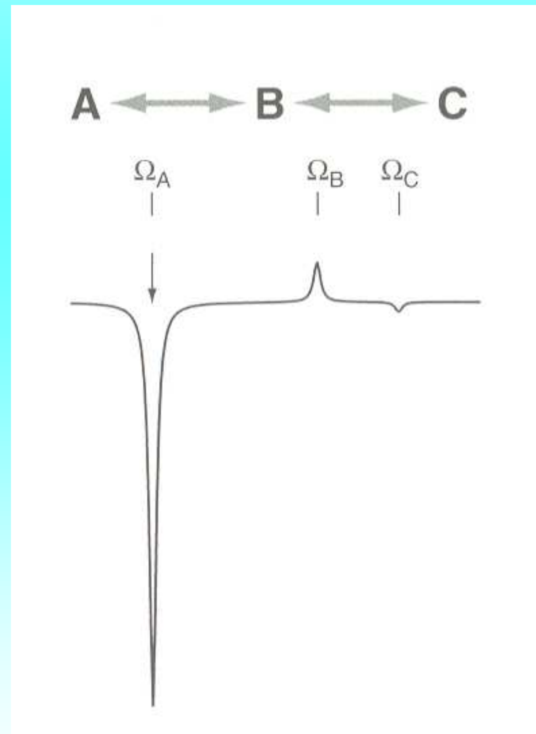


•spectra reproduced from *The Nuclear Overhauser Effect*, by D. Neuhaus and M. Williamson

NOE pitfalls

Indirect transfer

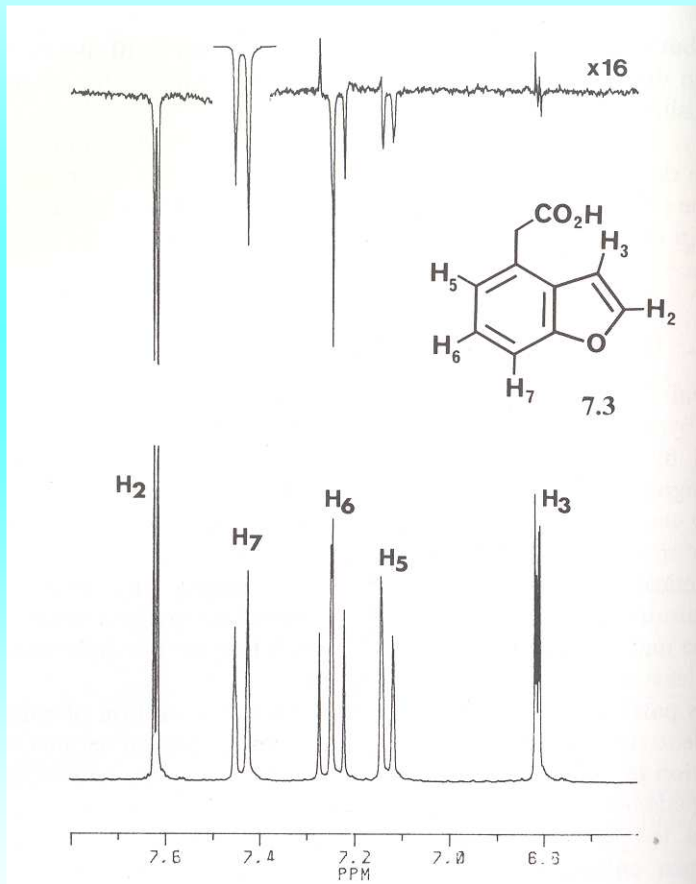
- multi-step transfer can generate unexpected "NOEs"
- Easily distinguished by alternating sign for small molecules
- Minimised by using short mixing times
- Can be a serious problem in very large molecules ("spin diffusion")



•figure reproduced from *Understanding NMR spectroscopy*, by James Keeler

NOE pitfalls

Irradiation Power too high



difference spectrum

reference spectrum

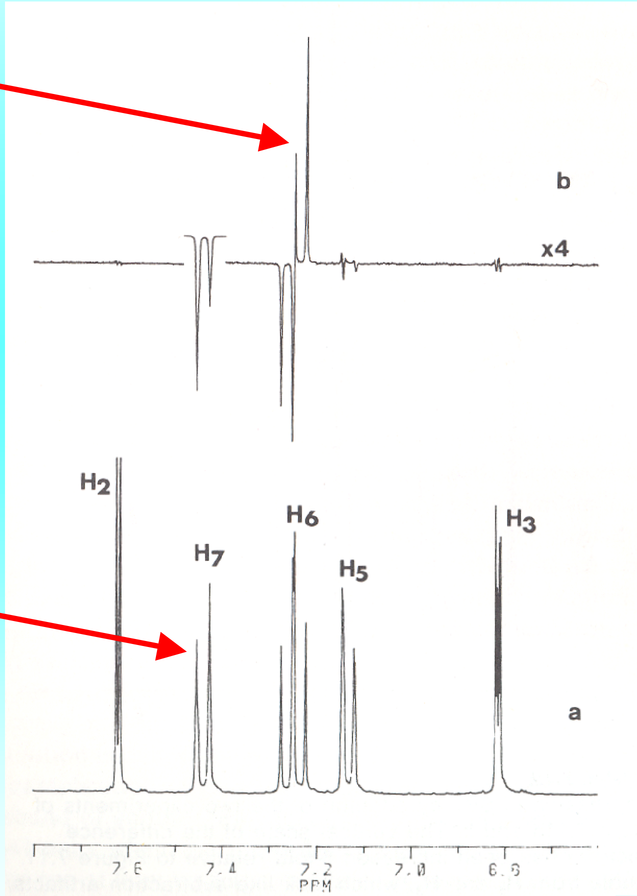
•spectra reproduced from *The Nuclear Overhauser Effect*, by D. Neuhaus and M. Williamson

NOE pitfalls

Irradiation Power too low

"selective polarisation transfer (SPT)"

only 1/2 of doublet irradiated



difference spectrum

reference spectrum

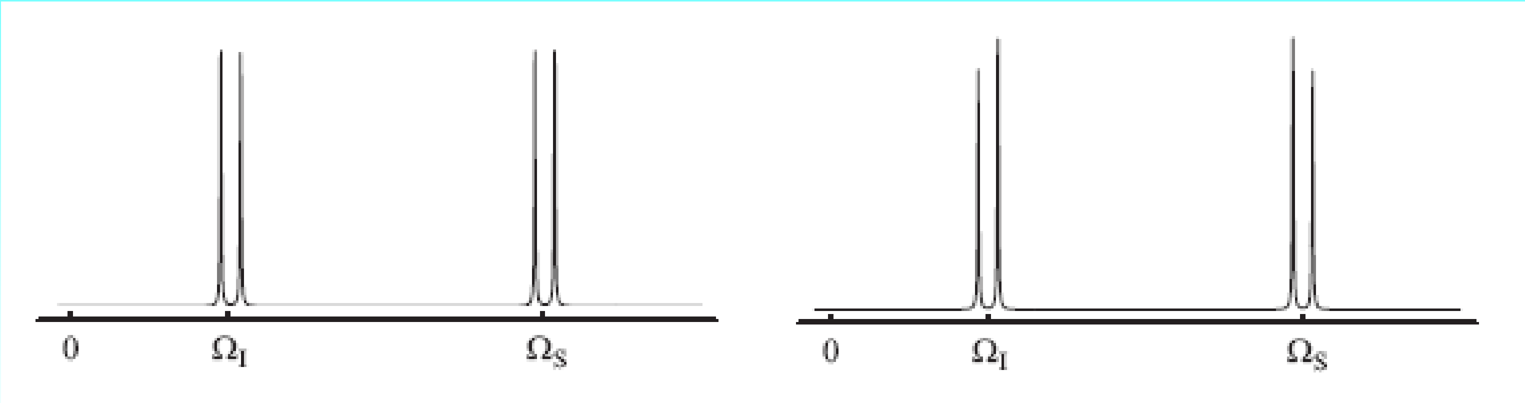
•spectra reproduced from *The Nuclear Overhauser Effect*, by D. Neuhaus and M. Williamson

NOE pitfalls

Strong Coupling

•Apparent "NOEs" between *strongly J-coupled* spins

- •look for multiplet distortions
- •still present at zero mixing time
- •difficult to suppress
- •minimised at high fields



"weak coupling" *"strong coupling"*

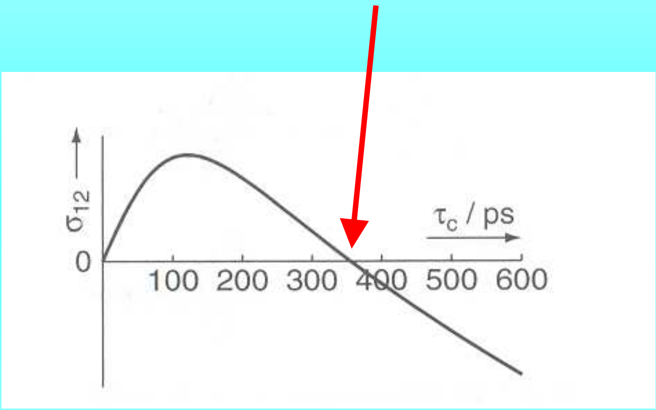
•spectra reproduced from MJT Phd thesis

NOE pitfalls

Absent NOEs

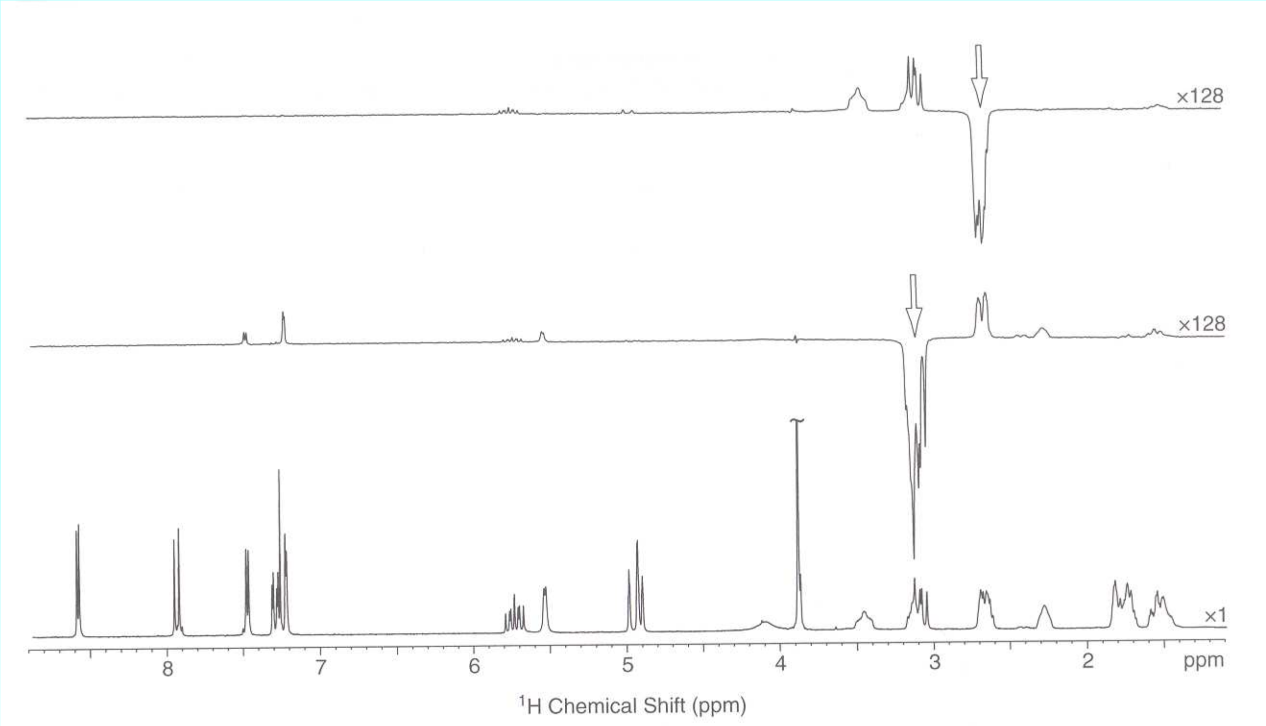
- zero-crossing of σ_{12}
    - change solvent
    - change B_0
    - use ROE / ROESY experiment
- quenching by paramagnetic ions or dissolved O_2
    - degas sample
- mixing time too short (or too long)

"zero crossing" $\omega_0 \tau_c = \sqrt{\frac{5}{4}}$



The NOE

Inspite of the pitfalls it's possible to generate very high quality NOE spectra using modern spectrometers and gradient techniques…



difference spectrum

difference spectrum

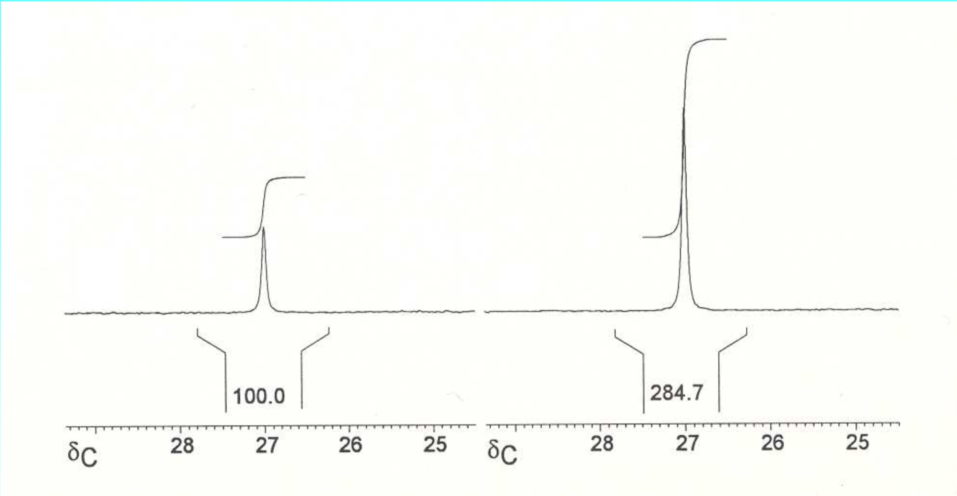
reference spectrum

•spectrum reproduced from *Understanding NMR spectroscopy*, by James Keeler

The NOE

Heteronuclear NOE

- ^{1}H—^{13}C steady-state enhancement of 200% (for small molecules)
- enhancement may be negative or positive, depending on signs of γ_I and γ_S

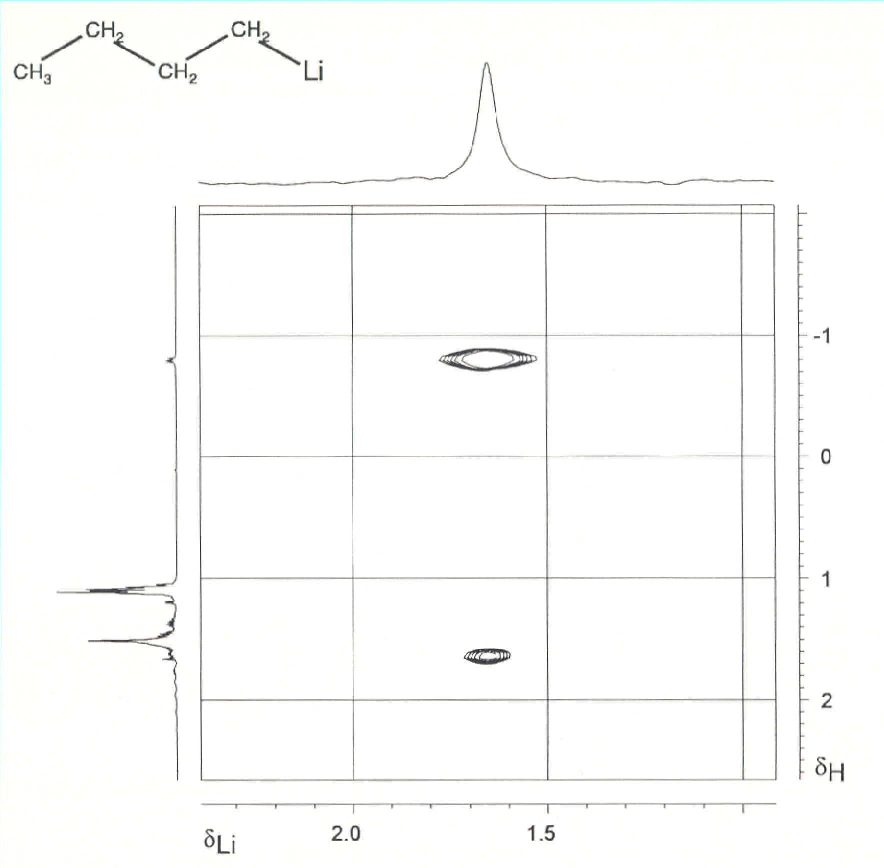


$$\eta_{SS} = \frac{\gamma_I}{2\gamma_S}$$

•spectrum reproduced from *100 and more Basic NMR Experiments*, by Braun, Kalinowski and Berger

The NOE

HOESY

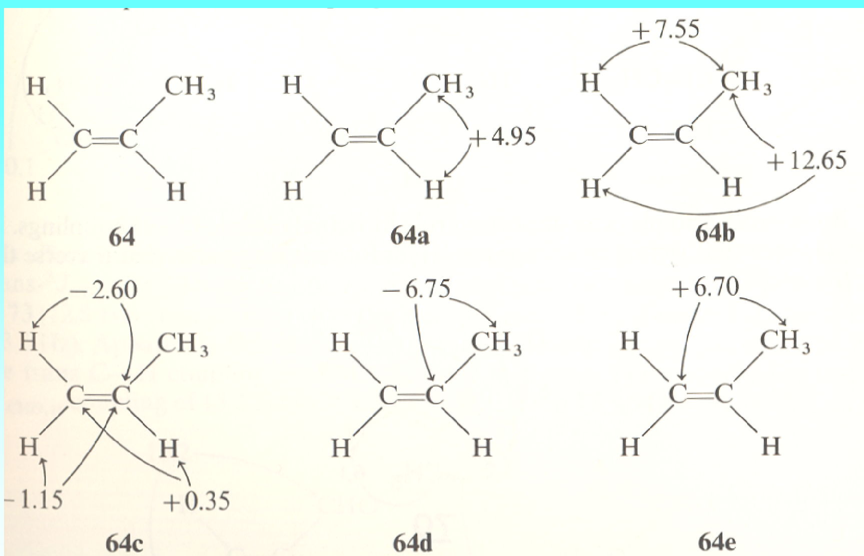


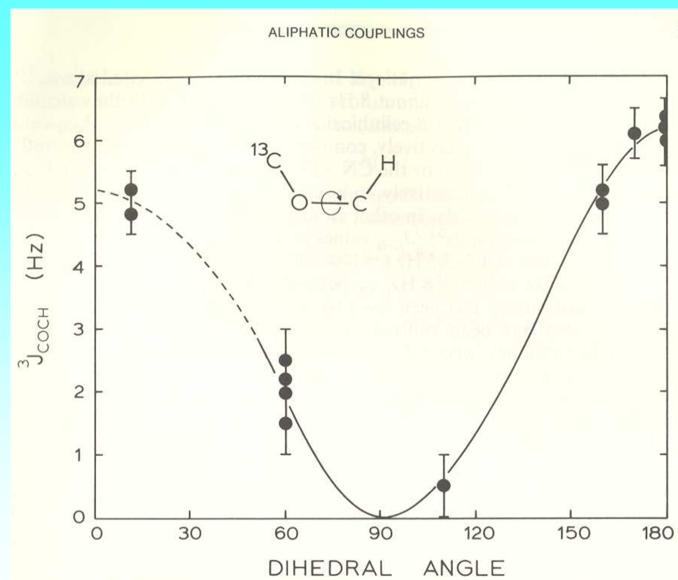
•spectrum reproduced from *100 and more Basic NMR Experiments*, by Braun, Kalinowski and Berger

J-coupling

J-coupling is a through bond interaction and therefore depends strongly on geomtry

- •Karplus relationships
- •empirical formulae
- •tables of coupling constants
  - •*Spectroscopic Methods in Organic Chemistry,* by Williams and Fleming
  - •*Carbon-carbon and C—H NMR couplings,* by James L. Marshall



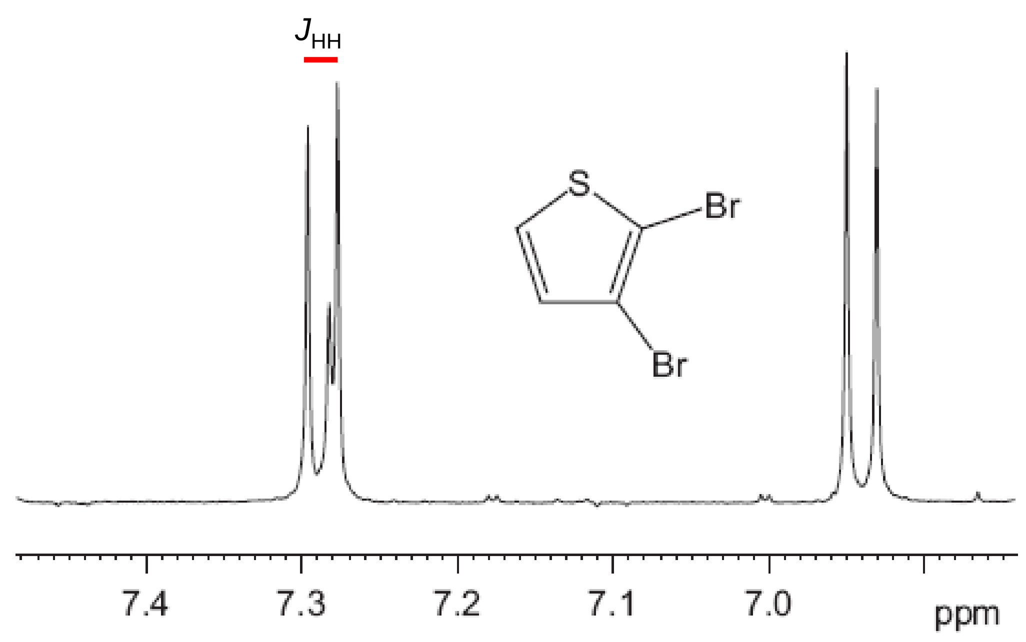


•figures reproduced from *Carbon-carbon and C—H NMR couplings,* by James L. Marshall

^1H—^1H *J*-coupling

1D spectrum

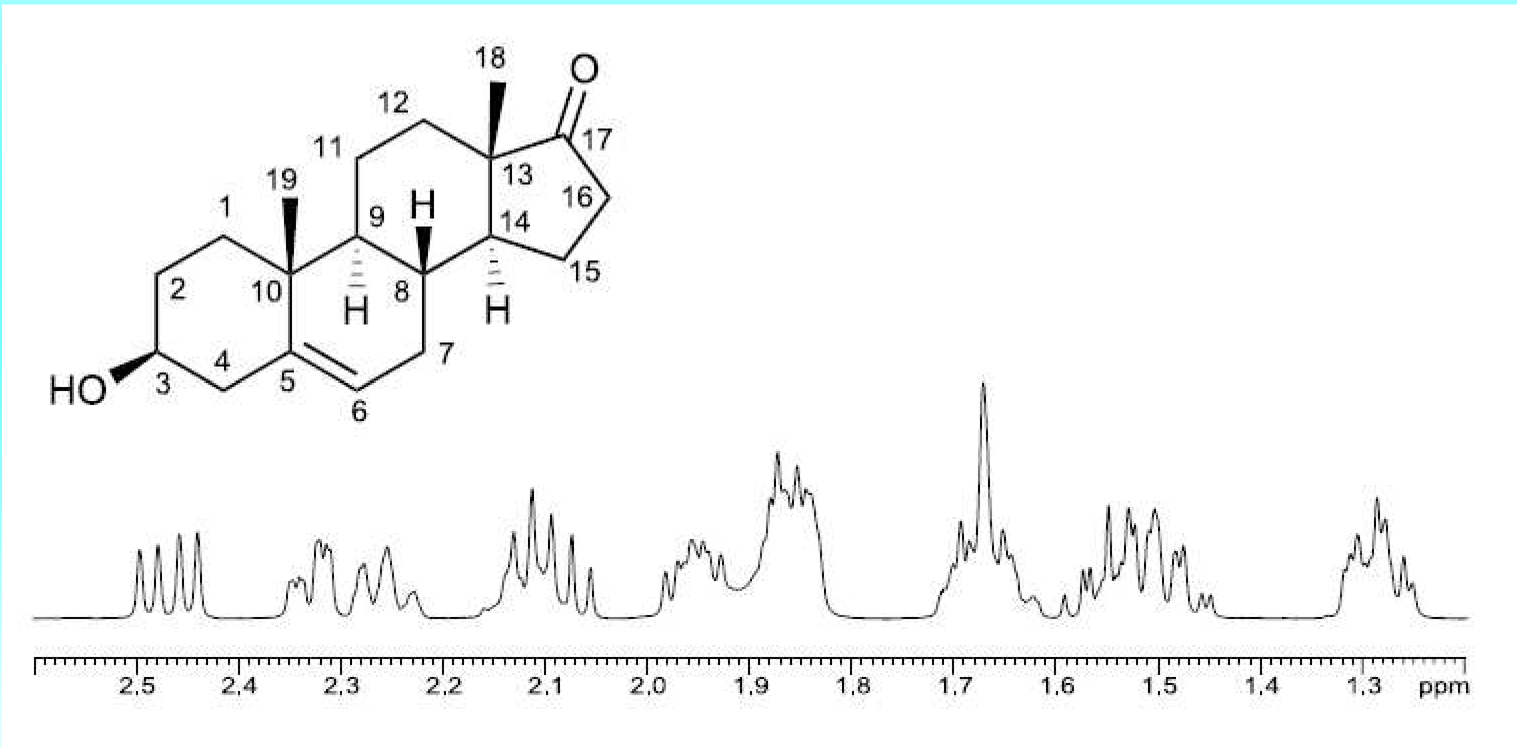
•Measure J_{HH} from splitting in 1D spectrum



•spectrum reproduced from MJT Phd thesis

^1H—^1H *J*-coupling

1D spectrum

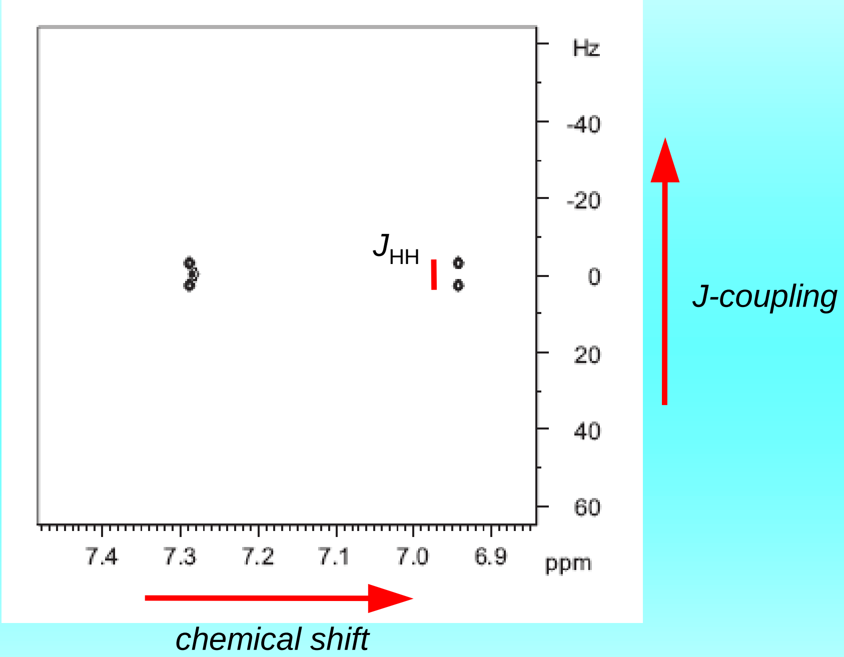


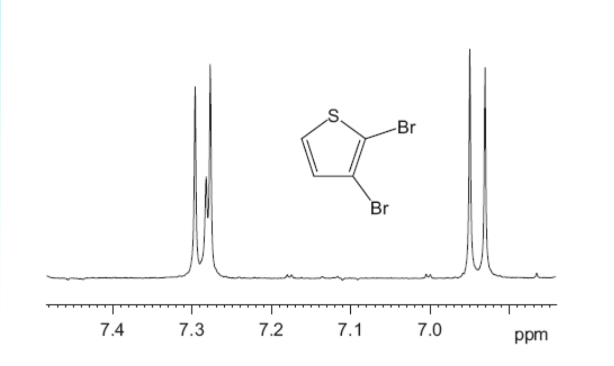
•spectrum reproduced from MJT Phd thesis

^{1}H—^{1}H *J*-coupling

2D *J-resolved spectroscopy*

•Separation of chemical shift and *J*-coupling

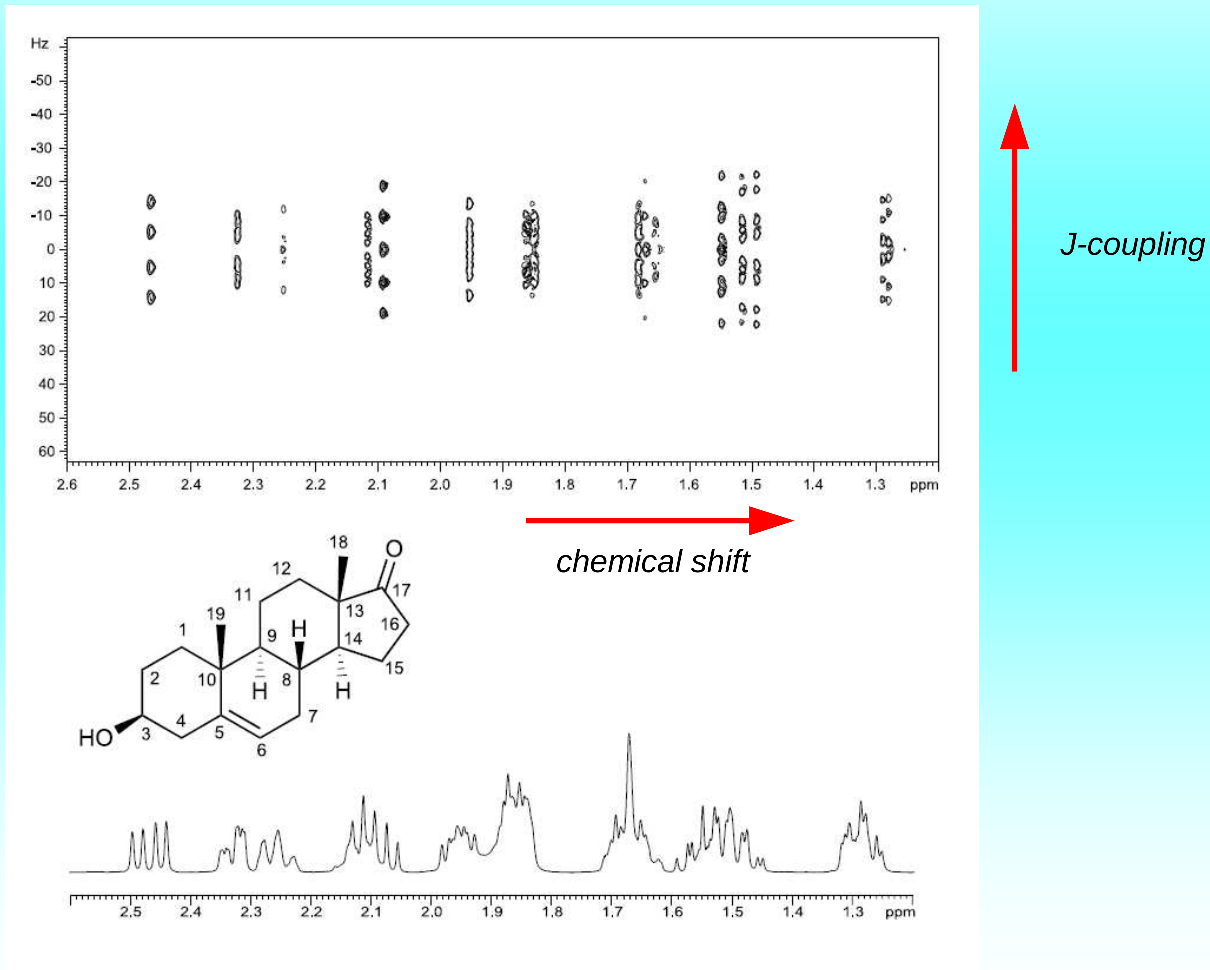




•spectrum reproduced from MJT Phd thesis

^1H—^1H *J*-coupling

2D *J-resolved spectroscopy*

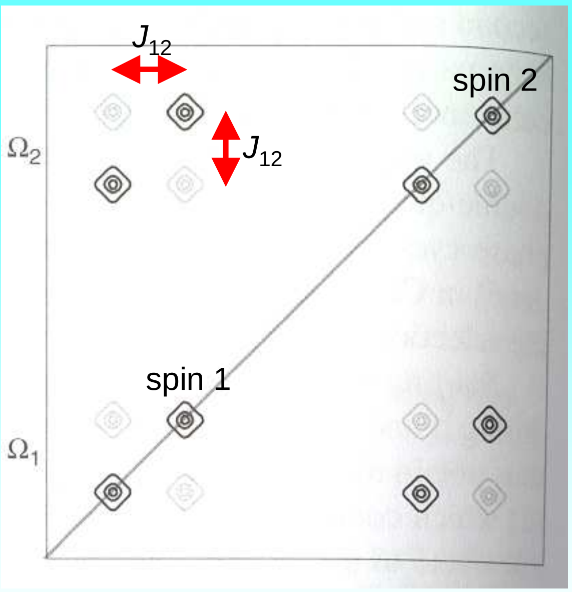


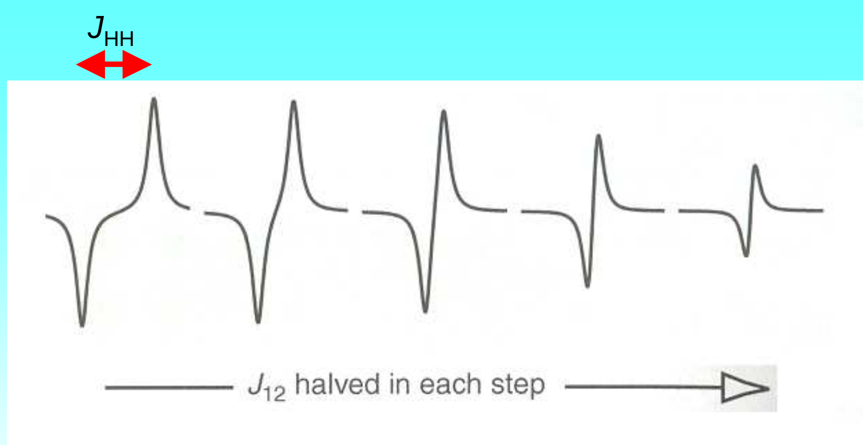
•spectra reproduced from Thrippleton et al., *J. Magn. Reson.* **174**, 97-109

^{1}H—^{1}H *J*-coupling

DQF-COSY

- J_{HH} can be measured from cross-peaks in a phase-sensitive DQF COSY spectrum
- valid when linewidth << J



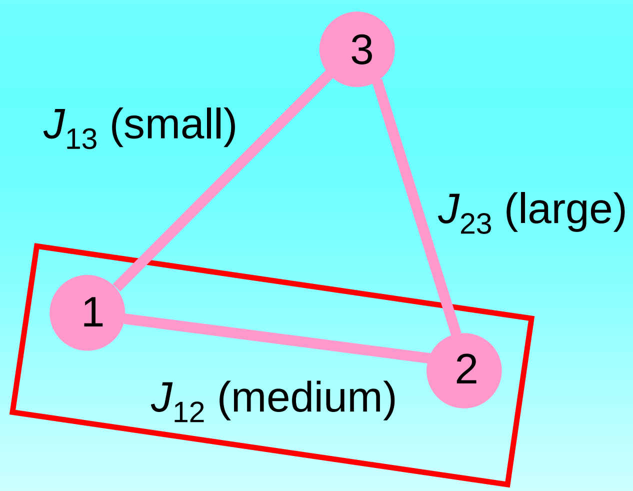


- figures reproduced from *Understanding NMR spectroscopy*, by James Keeler

^1H—^1H *J*-coupling

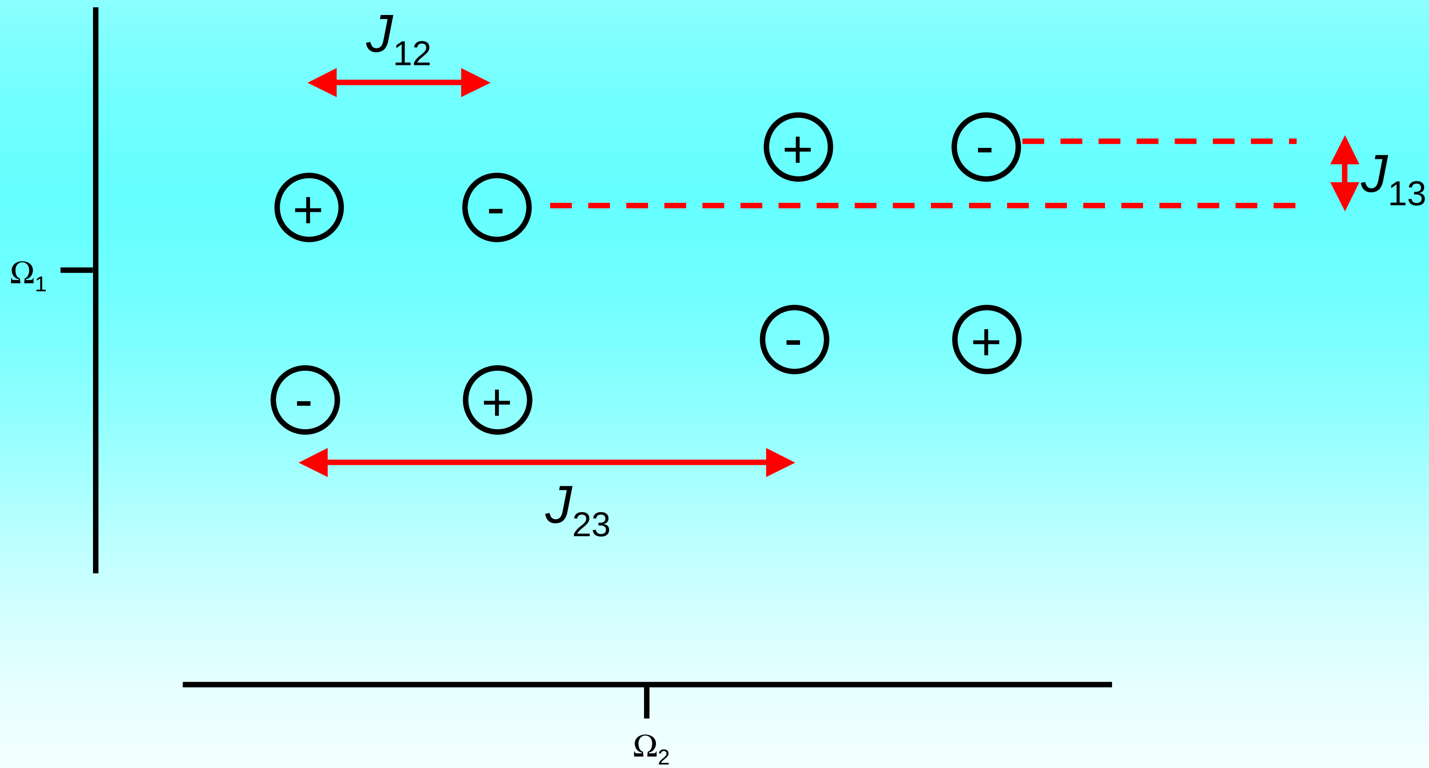
measuring small J values

- J_{HH} can be measured from cross-peaks in a phase-sensitive DQF COSY spectrum
- valid when linewidth << J



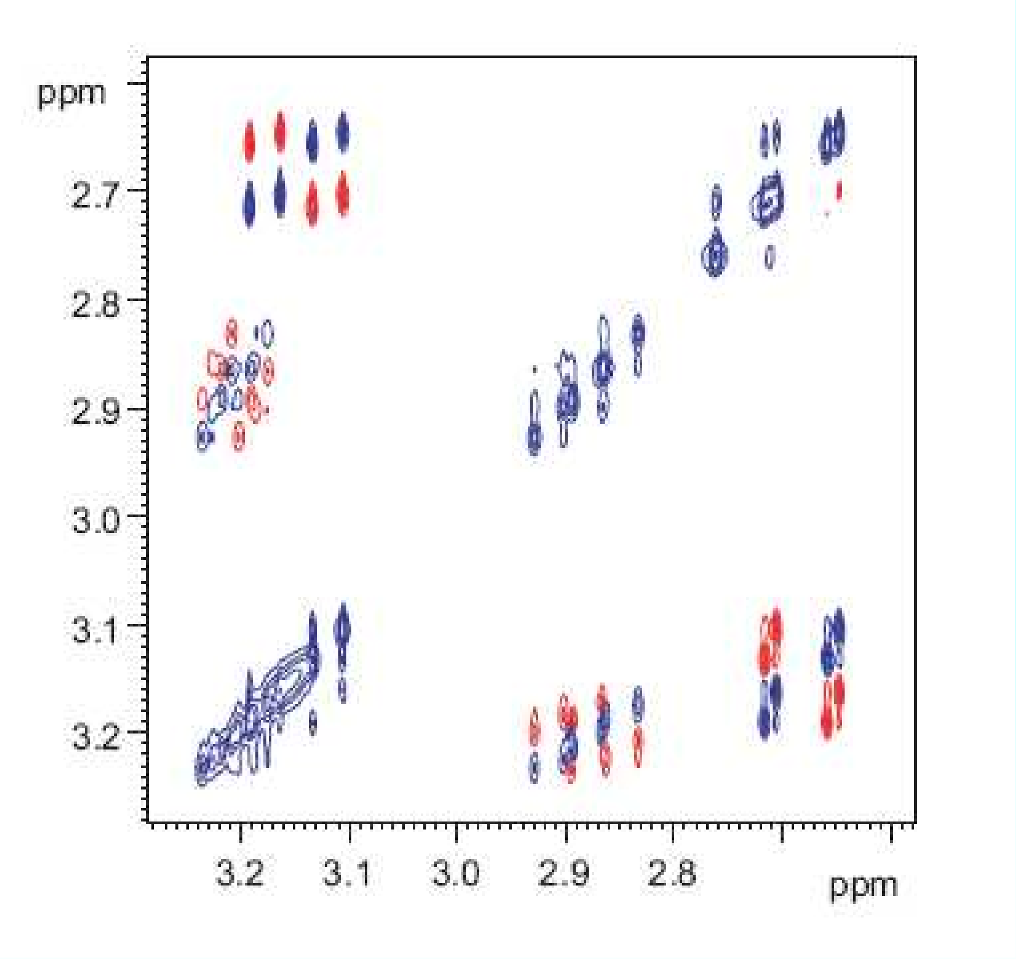
^1H—^1H *J*-coupling

z-COSY



$^1H—^1H$ *J*-coupling

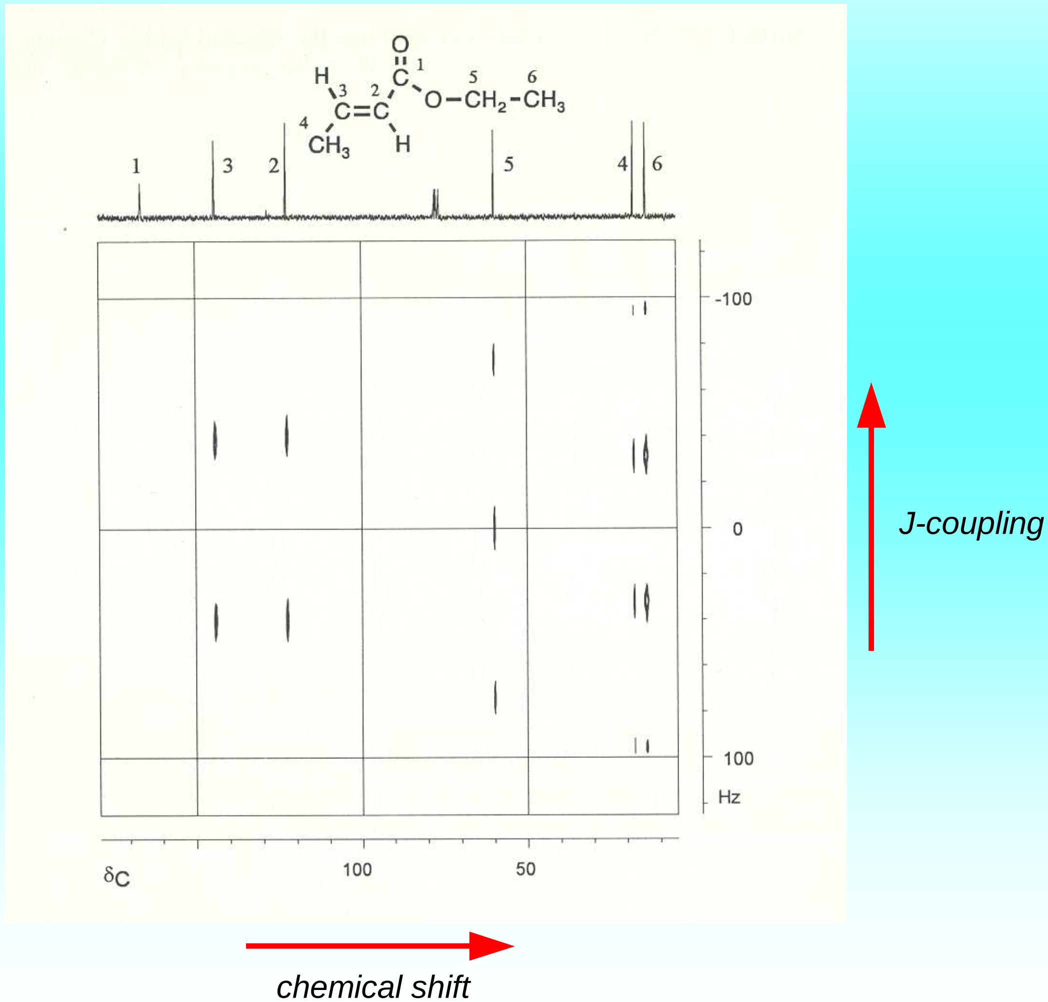
z-COSY



•spectrum reproduced from MJT Phd thesis

^{1}H—^{13}C *J*-coupling

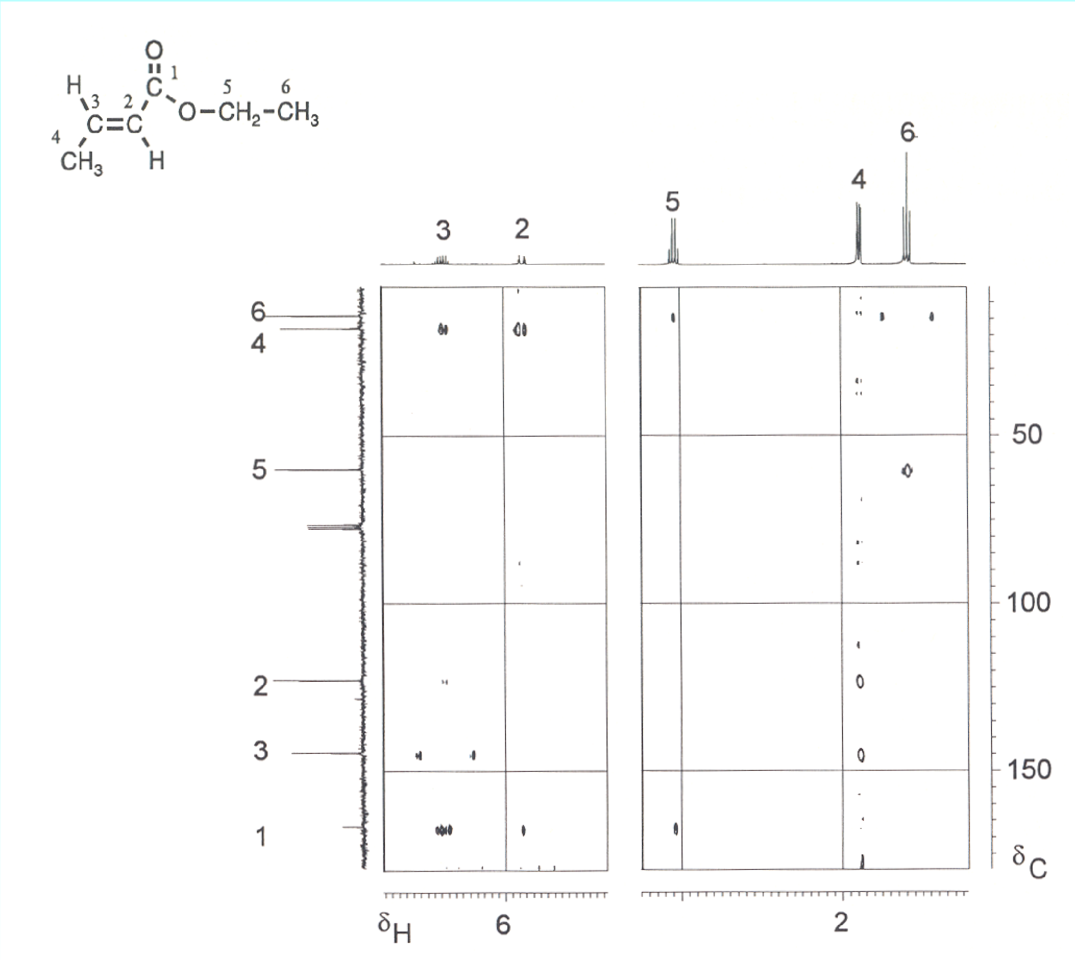
heteronuclear 2D *J-resolved spectroscopy*

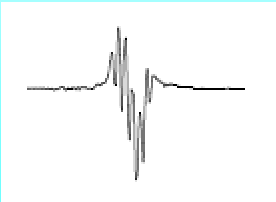


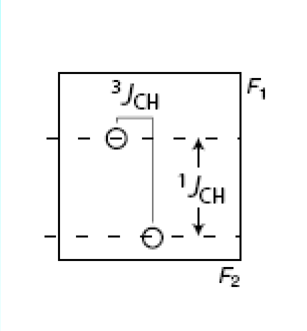
•spectrum reproduced from *100 and more Basic NMR Experiments*, by Braun, Kalinowski and Berger

^1H—^{13}C *J*-coupling

HMBC







•spectrum reproduced from *100 and more Basic NMR Experiments*, by Braun, Kalinowski and Berger

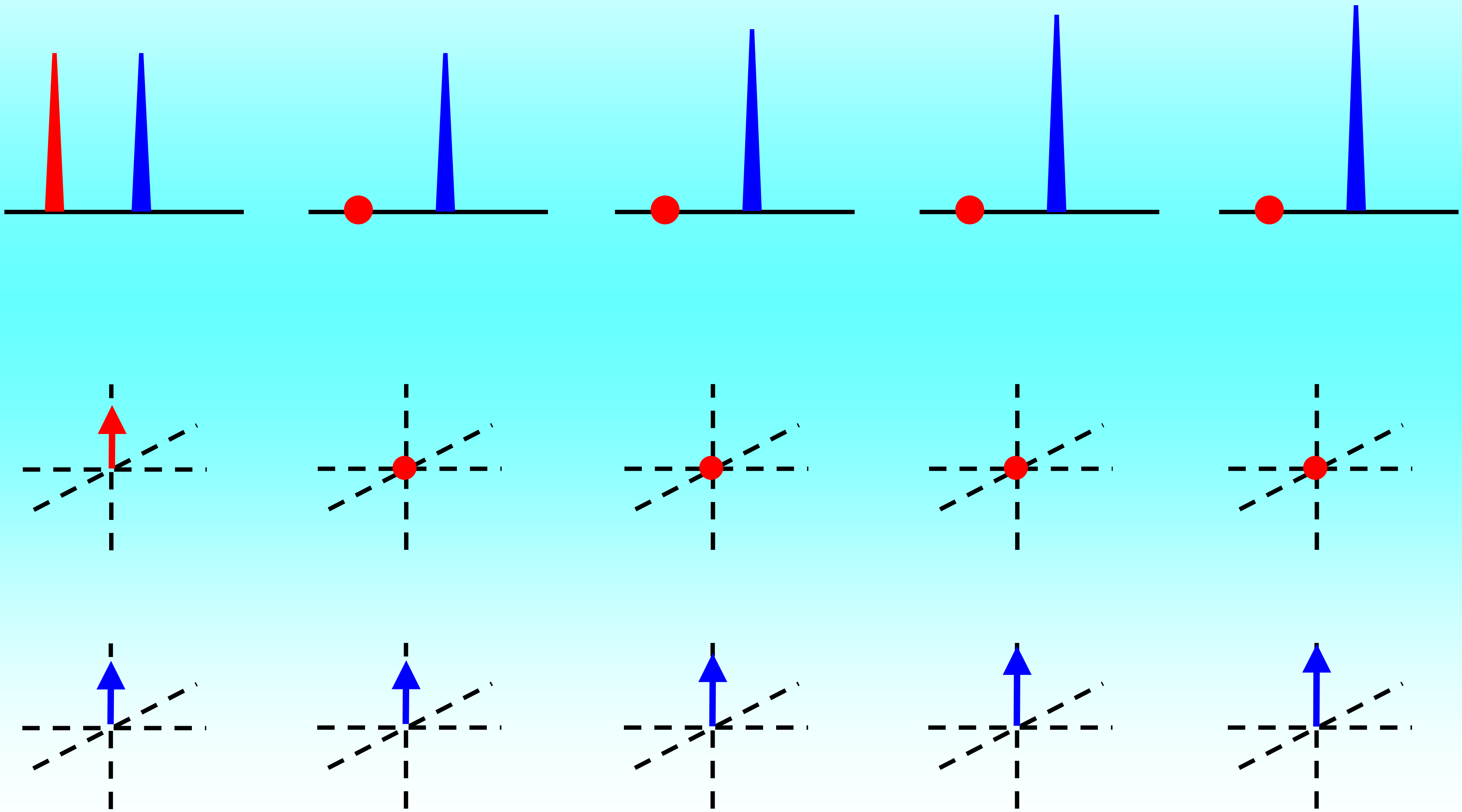
Useful Reading

- *The Nuclear Overhauser Effect* (look for 2nd edition), by D. Neuhaus and M. Williamson

- *Spectroscopic Methods in Organic Chemistry,* by Williams and Fleming
- *Carbon-carbon and carbon-proton NMR couplings,* by James L. Marshall
- *Understanding NMR spectroscopy,* by James Keeler
- *Modern NMR Spectroscopy,* by J.K.M. Sanders and B.K. Hunter
- the literature, e.g. *Magnetic Resonance in Chemistry*

The NOE

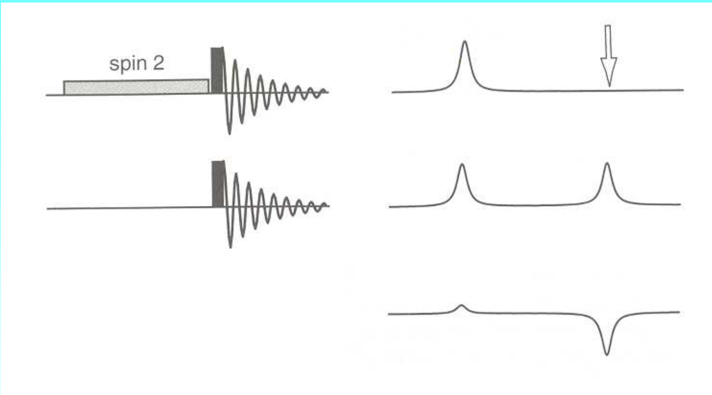
Steady-state NOE



The NOE

Steady-state NOE

- Target spin saturated
- Cross-relaxation generates steady-state NOE on neighbouring spin
- size of steady-state NOE determined by σ_{12} and R_1



irradiated spectrum

reference spectrum

difference spectrum

$$\eta = \frac{S_{irr} - S_{ref}}{S_{ref}}$$

•figure reproduced from *Understanding NMR spectroscopy*, by James Keeler

The NOE

Steady-state NOE

- Maximum steady-state enhancement:
    - *fast* tumbling +50%
    - *slow* tumbling –100%
- Enhancement independent of distance (in theory)
- Indirect effects

The NOE

ROE and ROESY

•NOE in the *x*—*y* plane

•no zero-crossing for the ROE

•exchange peaks and indirect ROEs have opposite sign to direct ROEs

•watch out for TOCSY-like cross-peaks between coupled spins