NMR Experiments for Assignment

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Introduction

Selecting experiments for assignment:

1. what type of information do you want?
   - chemical shifts
   - coupling constants
   - correlations

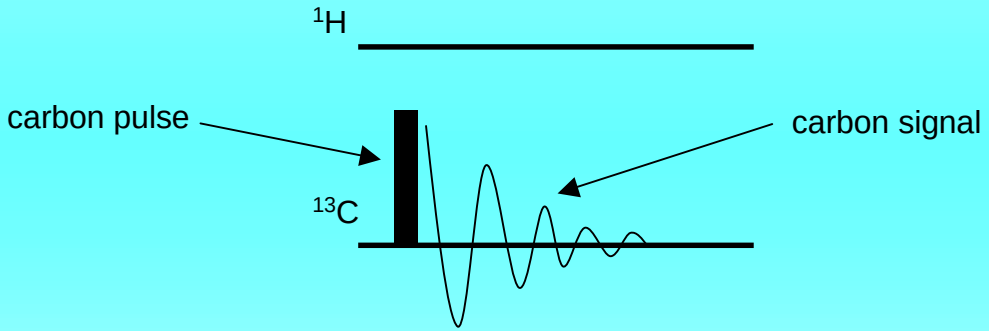
2. what type of spectrum do you want?
   - quick
   - sensitive
   - high-resolution
   - information-rich

3. choose/modify/invent an experiment

1. 1D Carbon NMR
   - 1D carbon spectrum
   - 1D carbon spectrum (proton decoupled)*****
   - APT*****
   - DEPT*****
2. 2D proton NMR
   - COSY
   - DQF COSY*****
   - long-range COSY
   - TOCSY
3. 2D carbon NMR
   - INADEQUATE
4. 2D proton-carbon NMR
   - HMQC
   - HMQC (proton-decoupled)*****
   - multiplicity-edited HSQC*****
   - HMBC*****
   - HSQC
   - HETCOR
5. The rest
   - 1D analogues
   - macromolecules, solids, multinuclear

1D Carbon NMR

1D spectrum



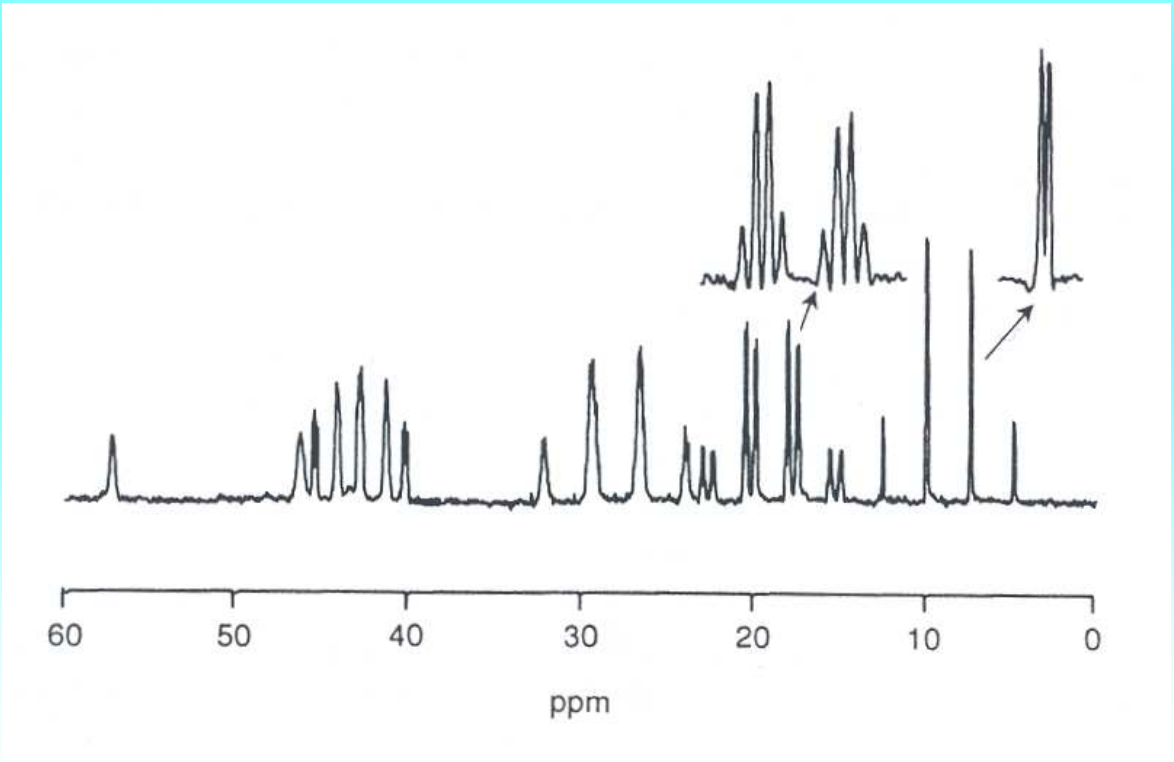
•reveals ^{13}C **chemical shift** interaction

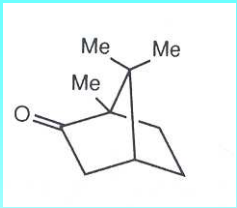
AND $\boldsymbol{J_{CH}}$ interactions (1-bond and multiple-bond)

1D Carbon NMR

1D spectrum

•high information content but low resolution and sensitivity

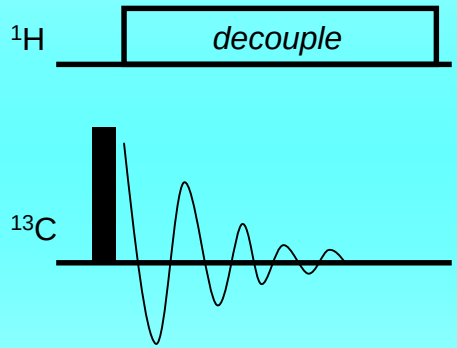




•spectrum reproduced from *Modern NMR Spectroscopy*, by Sanders and Hunter

1D Carbon NMR

proton-decoupled 1D spectrum

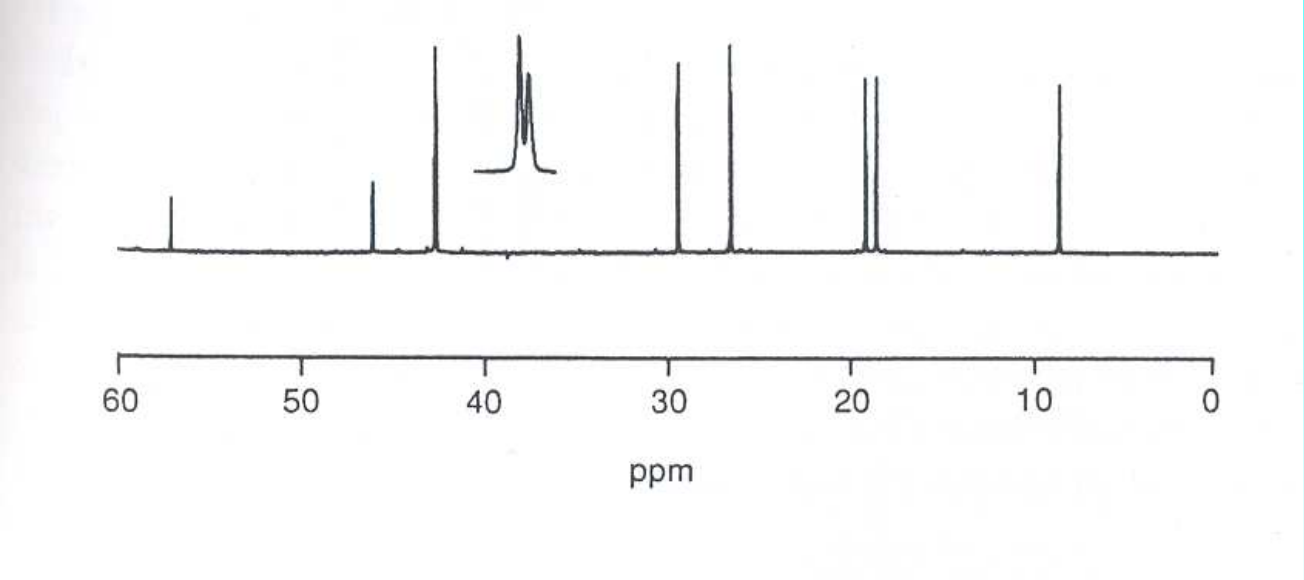


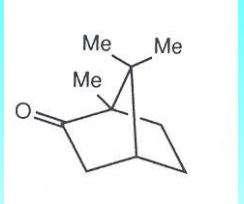
- reveals ^{13}C **chemical shift** interaction
- $\boldsymbol{J_{CH}}$ interactions are removed (decoupled)

1D Carbon NMR

proton-decoupled 1D spectrum

•high resolution and sensitivity but lower information content



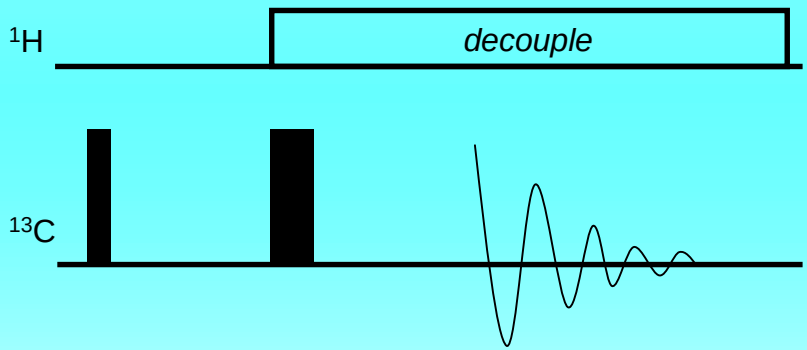


•spectrum reproduced from *Modern NMR Spectroscopy*, by Sanders and Hunter

1D Carbon NMR

APT

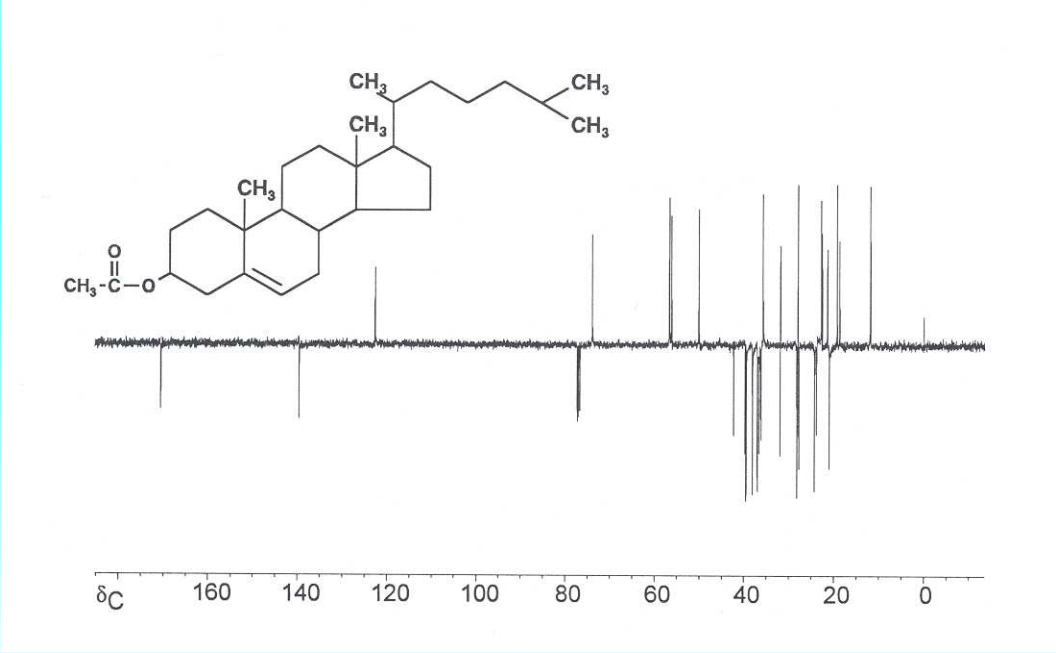
- **Use** $^{1}J_{CH}$ interaction to change sign of peaks and provide multiplicity information.
- **Decouple** $^{1}J_{CH}$ interaction during acquisition for high resolution and sensitivity.



	C	CH	CH_2	CH_3
APT	*negative*	*positive*	*negative*	*positive*

1D Carbon NMR

APT



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

1D Carbon NMR

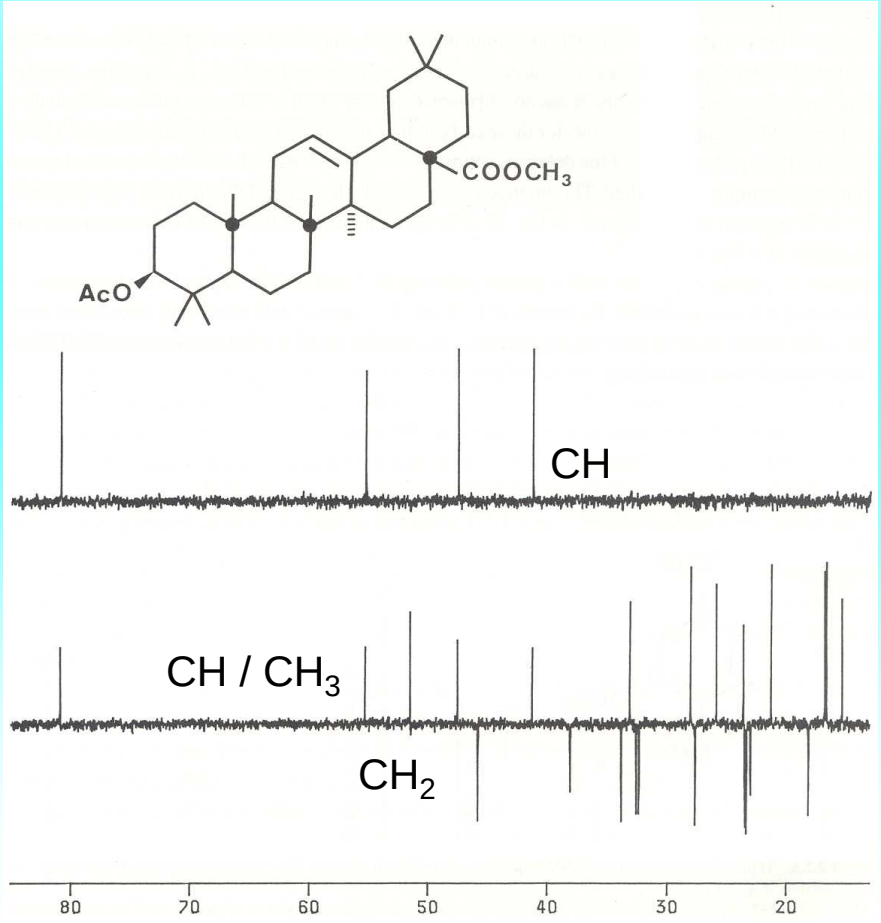
DEPT

- alternative to APT
- better sensitivity than APT
- Quaternaries **not** visible

	C	CH	CH_2	CH_3
DEPT-90	*absent*	+100%	*absent*	*absent*
DEPT-135	*absent*	+70%	–50%	+35%

1D Carbon NMR

DEPT



DEPT-90

DEPT-135

•spectra reproduced from *Structure Elucidation by Modern NMR (workbook)*, by Duddeck and Dietrich

1D Carbon NMR

Summary

1D spectrum	**1D spectrum (proton decoupled)**	**APT**	**DEPT**
•low sensitivity •information-rich •crowded spectra	•high sensitivity and resolution •low information content	•low sensitivity •multiplicity information •quaternaries visible	•high sensitivity •multiplicity information •no quaternaries

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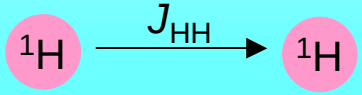
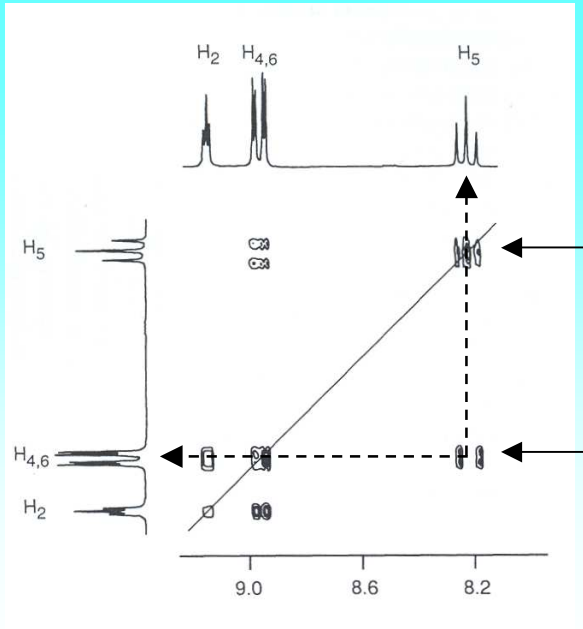
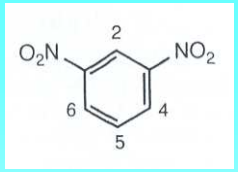
2D Proton NMR

COSY

- Use J_{HH} interaction to correlate protons connected by 2–3 bonds
- Correlations represented by *cross peaks* in 2D spectrum







diagonal peak

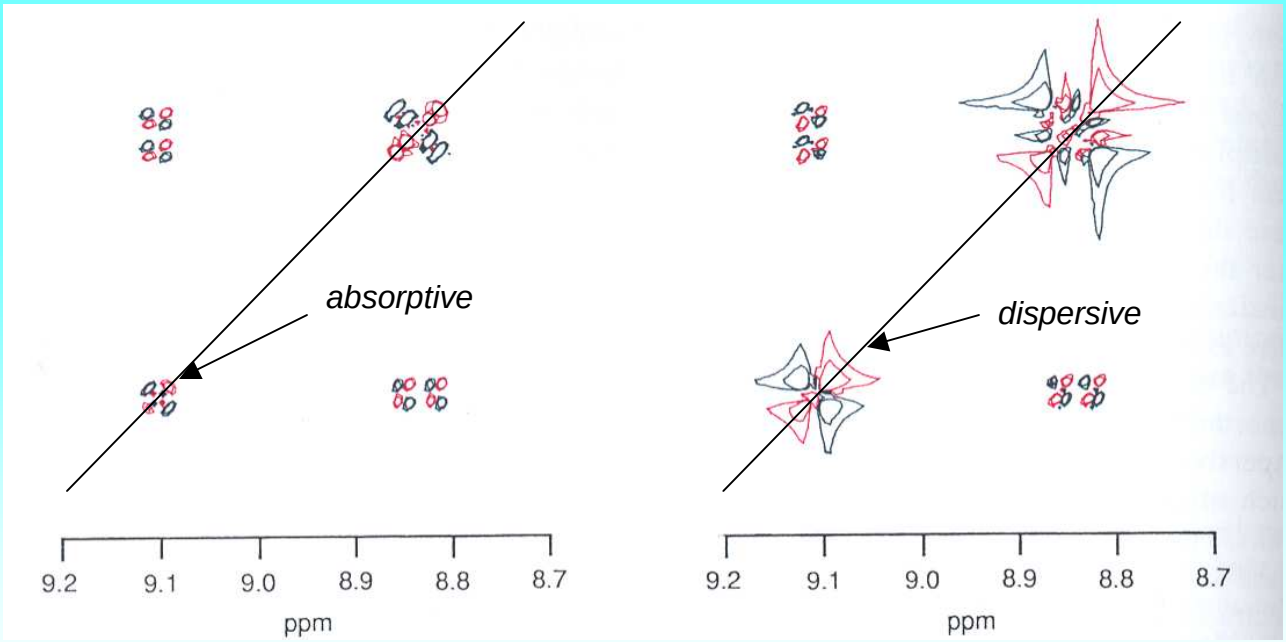
cross peak

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

2D Proton NMR

DQF COSY

- Improved lineshapes and resolution
- Singlet (uncoupled) peaks suppressed



with DQF

without DQF

- spectra reproduced from *Modern NMR Spectroscopy*, by Sanders and Hunter

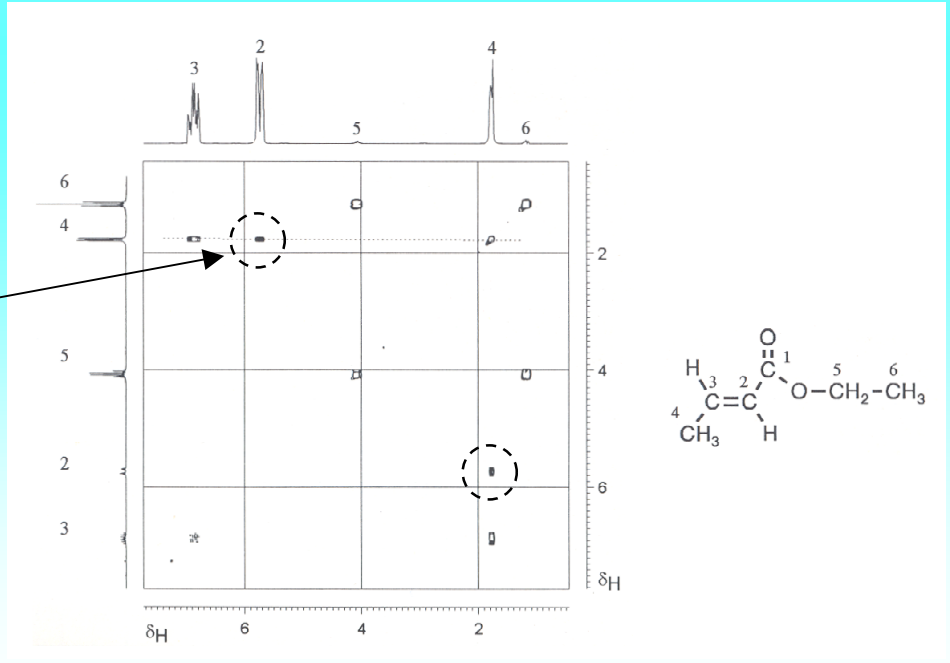
2D Proton NMR

long-range COSY

- correlations via small J_{HH} enhanced
  - e.g. allylic coupling, *W* and *para* coupling in aromatic rings
- short-range correlations may be weakened

H_2–H_4 allylic 4-bond correlation

$^4J_{HH}$ ~ 2 Hz



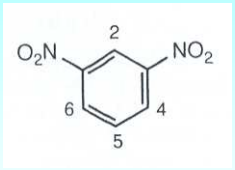
- spectrum reproduced from *100 and more Basic NMR Experiments*, by Braun, Kalinowski and Berger

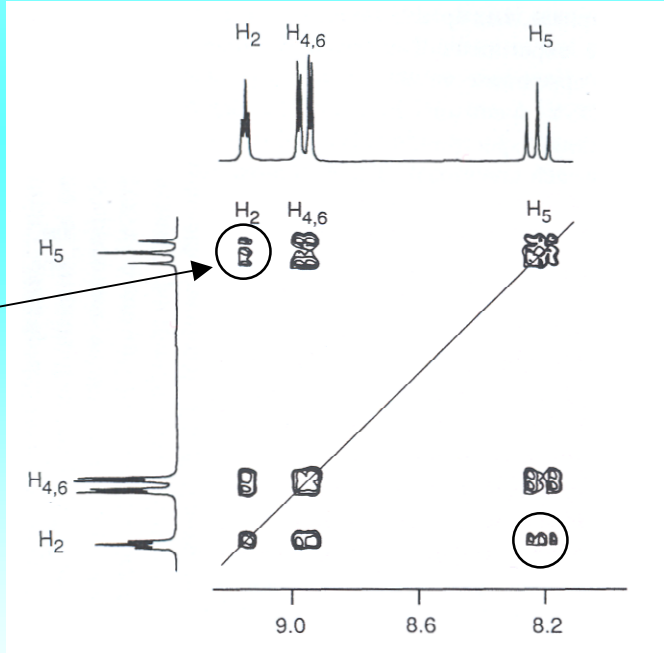
2D Proton NMR

long-range COSY

H_2–H_5 *para* 5-bond correlation

$^5J_{HH}$ < 0.5 Hz



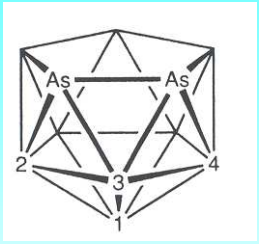


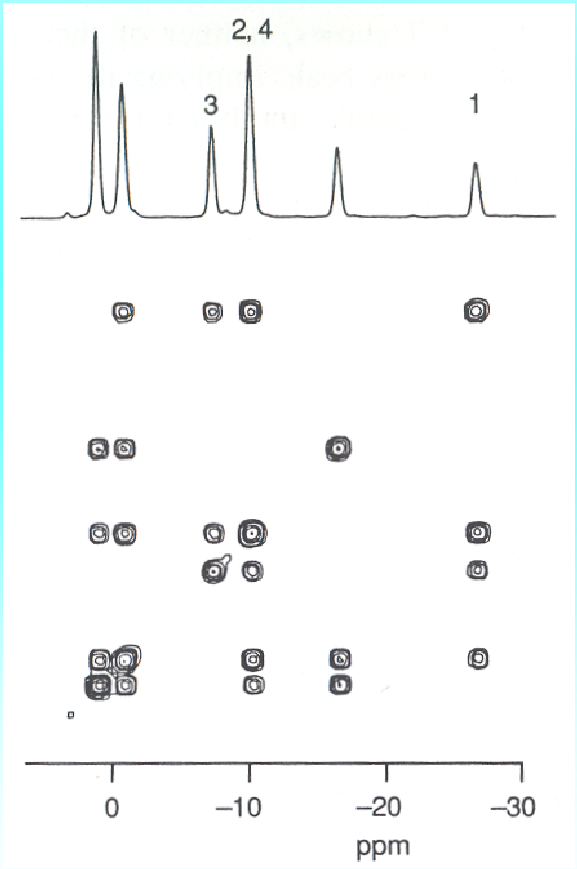
•spectrum reproduced from *Modern NMR Spectroscopy*, by Sanders and Hunter

2D Proton NMR

COSY – inorganic example

- ^{11}B—^{11}B COSY spectrum of $[As_2B_9H_{10}]^-$ ion
- ^{1}H decoupled





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

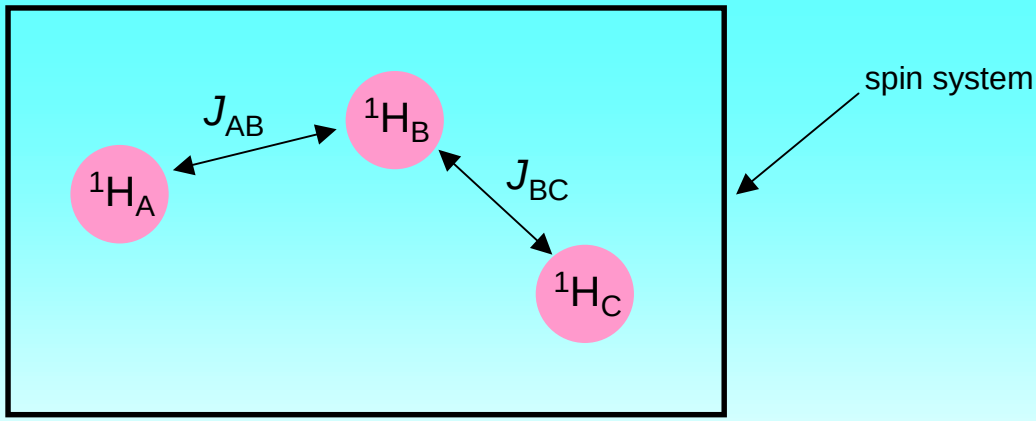
2D Proton NMR

TOCSY

•correlations between **all** protons within spin system (not just those directly coupled)

•useful when key COSY cross-peaks are obscured

•useful for assigning resonances in side-chains of proteins



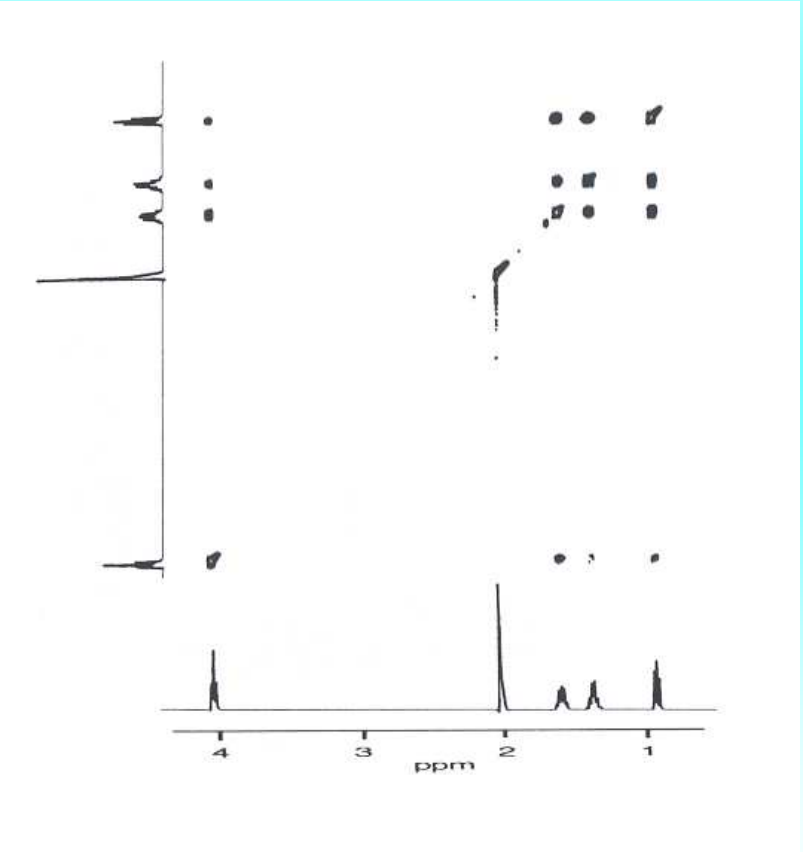
•COSY cross peaks: A—B , B—C

•TOCSY cross peaks: A—B , B—C **and** A—C

2D Proton NMR

TOCSY

- TOCSY spectrum of *n*-butyl acetate
- 2 spin systems



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

2D Proton NMR

Summary

COSY	**DQF COSY**	**long-range COSY**	**TOCSY**
•^{1}H–^{1}H through-bond correlations •mostly 2-bond / 3-bond correlations	•the **best** standard COSY experiment •high resolution and narrow lineshapes •singlet peaks suppressed	•good for obtaining correlations when J_{HH} is small •useful for seeing **long range correlations**, e.g. 4-bond allylic, and "W" and "para" correlations in aromatics	•shows correlations between **all** protons in same spin system •narrow lineshapes

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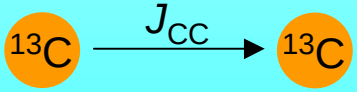
2D Carbon NMR

- Use the J_{CC} interaction to correlate different carbon nuclei.



- A nice way to trace out the carbon skeleton of an organic molecule

- … but to do this you need pairs of ^{13}C nuclei

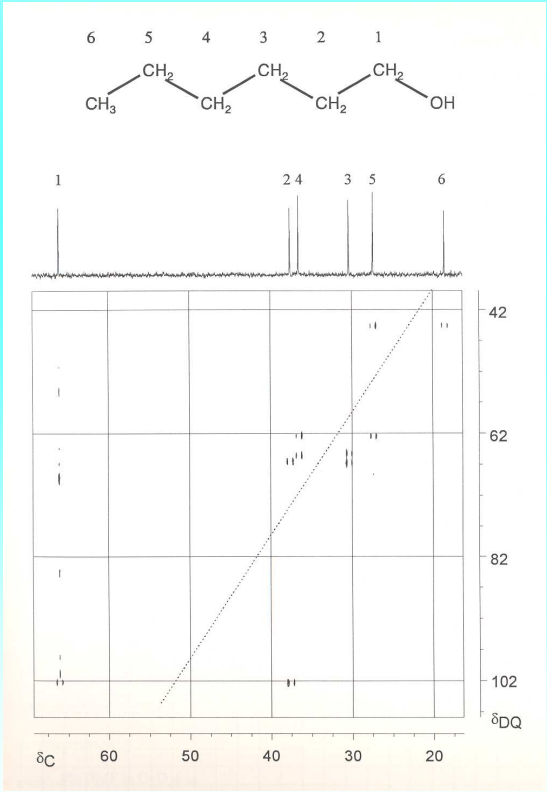
  …the chances of this are $1.1\% \times 1.1\% = 0.0121\ \%$

  …so **sensitivity is low!**

2D Carbon NMR

INADEQUATE

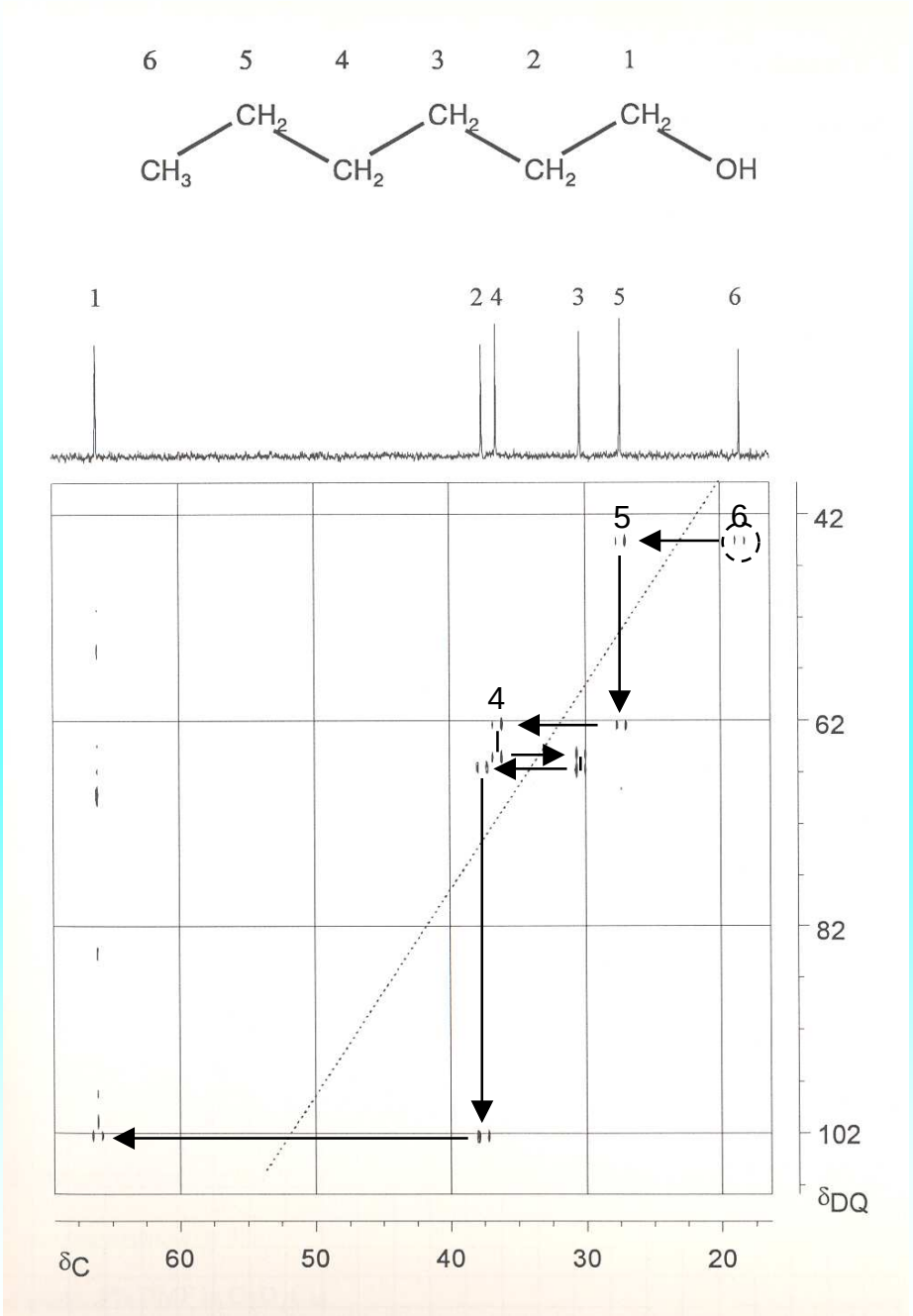
- Use $^{1}J_{CC}$ interaction to correlate neighbouring carbons
- Useful when sensitivity is not an issue



"double quantum" shift

carbon shift

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



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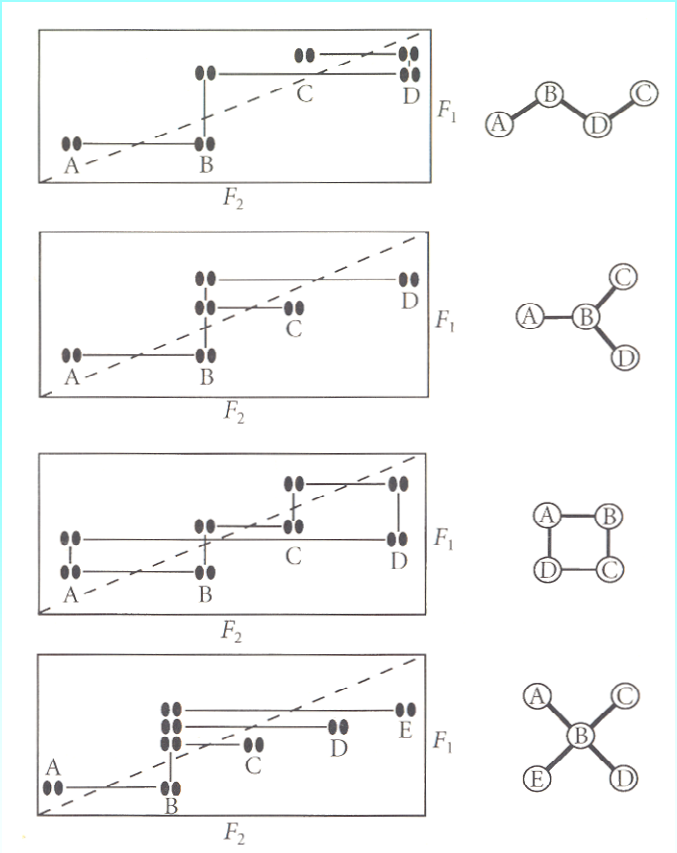
2D Carbon NMR

INADEQUATE

•Useful for distinguishing a variety of “topologies”, e.g. linear, cyclic etc.



•figure reproduced from *Spin Choreography*, by Ray Freeman

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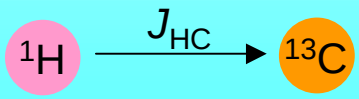
2D Proton-Carbon NMR

options

- Use the J_{HC} interaction to correlate proton and carbon shifts.

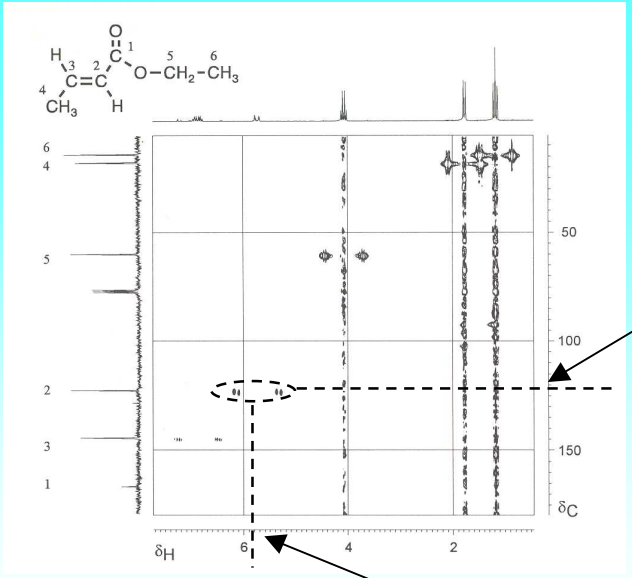


- Great variety of experiments
  - Do we want 1-bond (J_{HC} ~ 130 Hz) correlations or just multiple-bond correlations (J_{HC} ~ 0-20 Hz) ?
  - Detect on proton or carbon?
  - Switch J_{HC} interaction off during acquisition for higher resolution?
  - Leave J_{HC} interaction on during acquisition to retain information?
  - Do we need to *measure* J_{HC} values or just see the correlations?
  - Remove J_{HH} interactions in spectrum?

2D Proton-Carbon NMR

HMQC

- Use $^{1}J_{HC}$ interaction to correlate protons with neighbouring carbons
- Proton detection for high sensitivity



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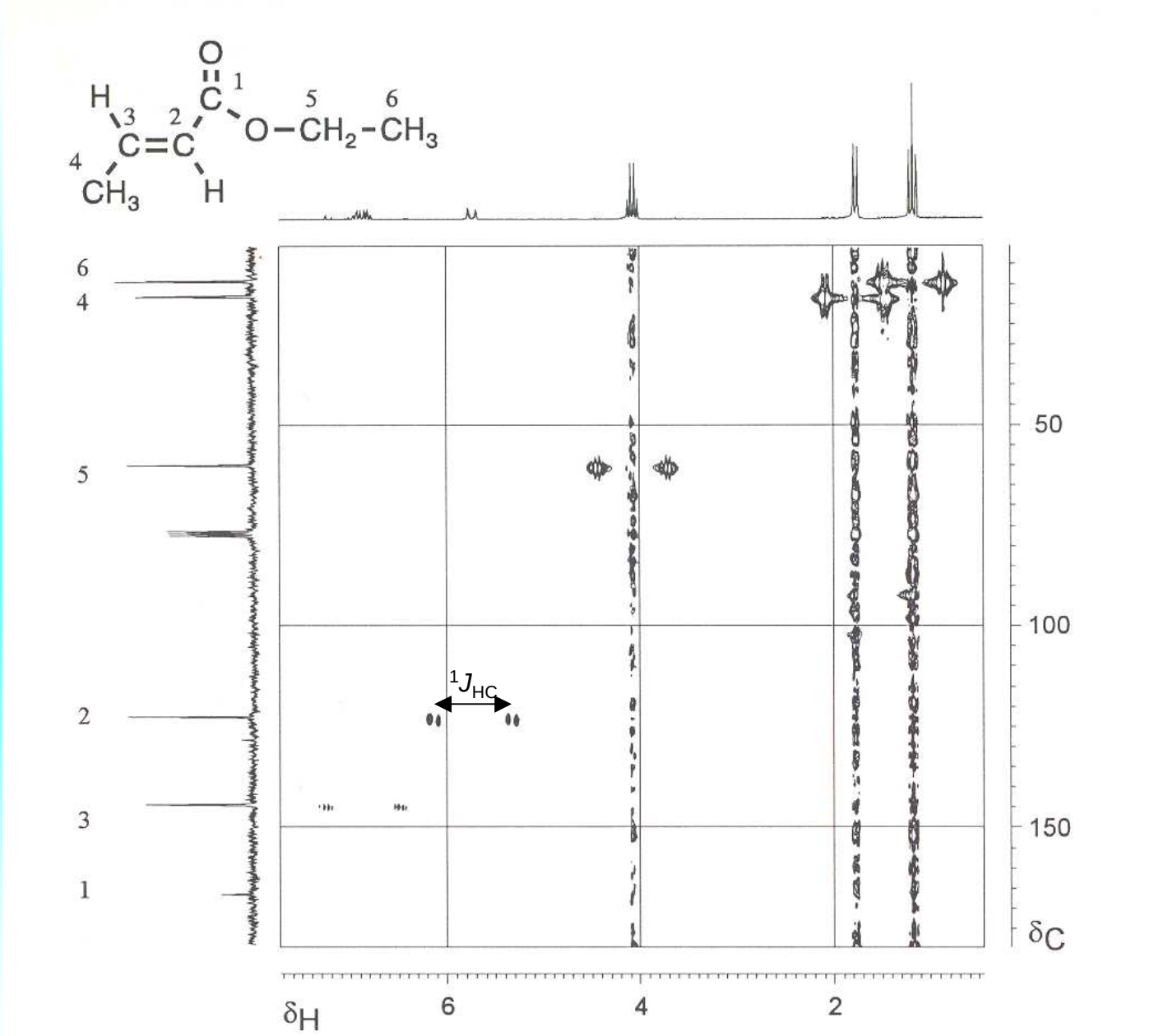
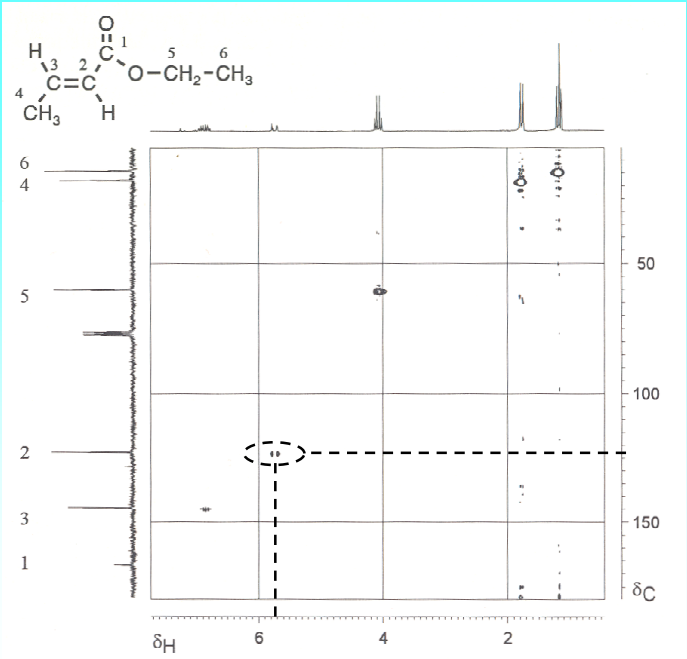


•spectrum reproduced from *100 and more Basic NMR Experiments*, by Braun, Kalinowski and Berger

2D Proton-Carbon NMR

HMQC with decoupling

- Use $^{1}J_{HC}$ interaction to correlate protons with neighbouring carbons…
- …**but** decouple $^{1}J_{HC}$ interaction during acquisition for simpler spectrum and enhanced sensitivity



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

2D Proton-Carbon NMR

multiplicity-edited HSQC

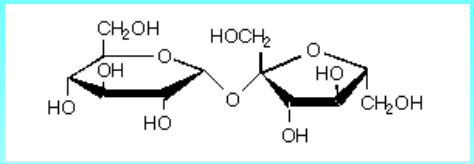
- HMQC-like spectrum
- contains multiplicity information
- 20 minutes

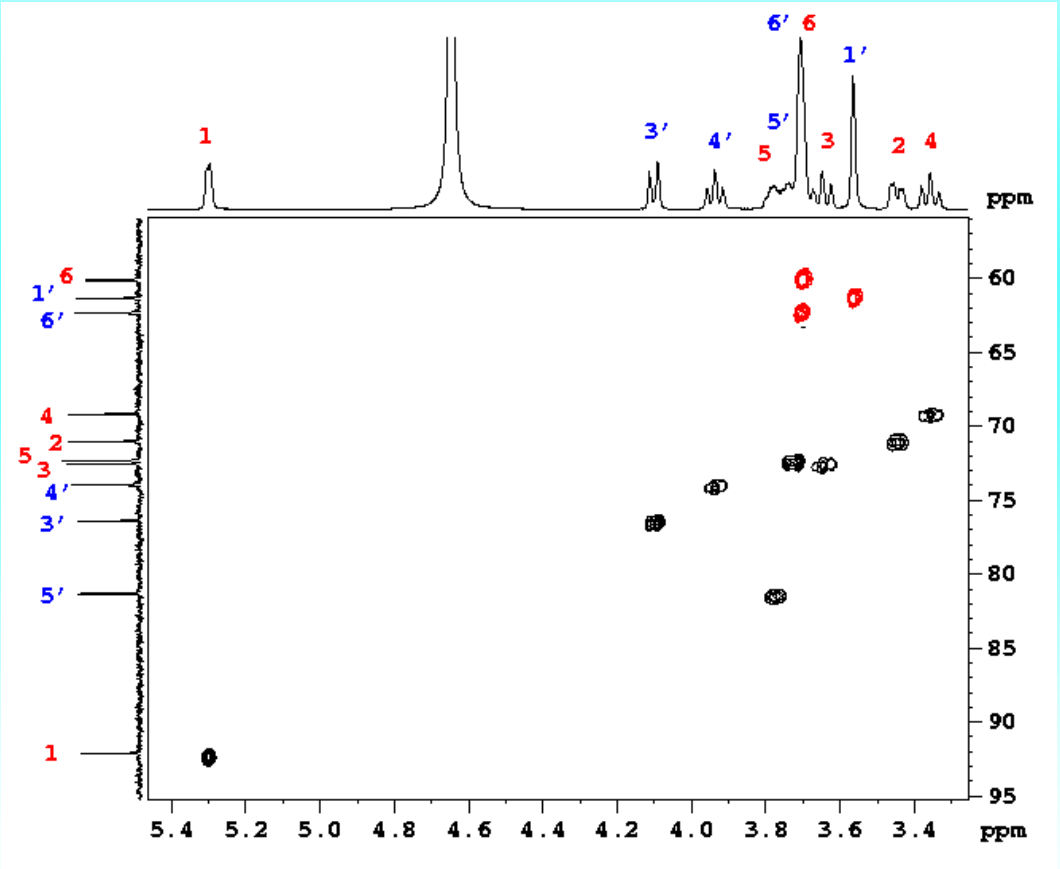
	C	CH	CH_2	CH_3
me-HSQC	*absent*	*positive*	*negative*	*positive*

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

2D proton-carbon NMR

multiplicity-edited HSQC



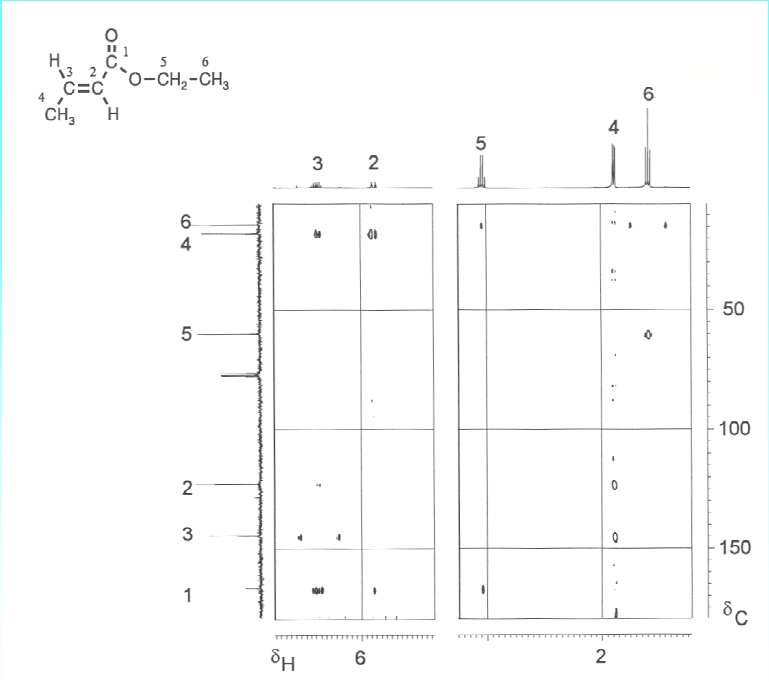


•spectra reproduced from *http://rmn.iqfr.csic.es/guide/tutorials/specdata/spectra/suc_hsqced.html*, by Teodor Parella

2D proton-carbon NMR

HMBC

- Use $^{2}J_{HC}$, $^{3}J_{HC}$ etc. interaction to correlate protons with more distant carbons.
- Usually acquired without decoupling
- Low-pass filter to suppress one-bond correlations



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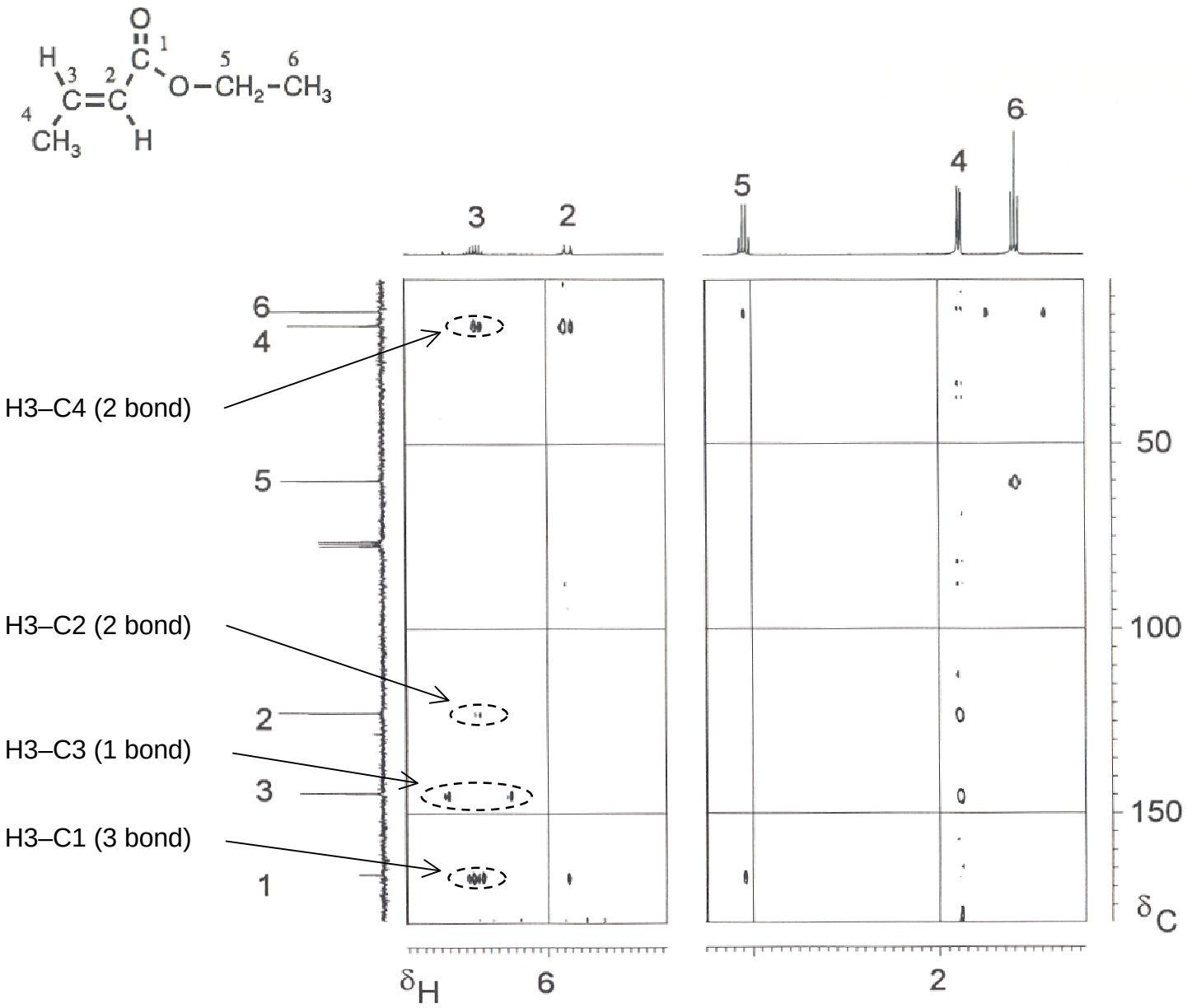
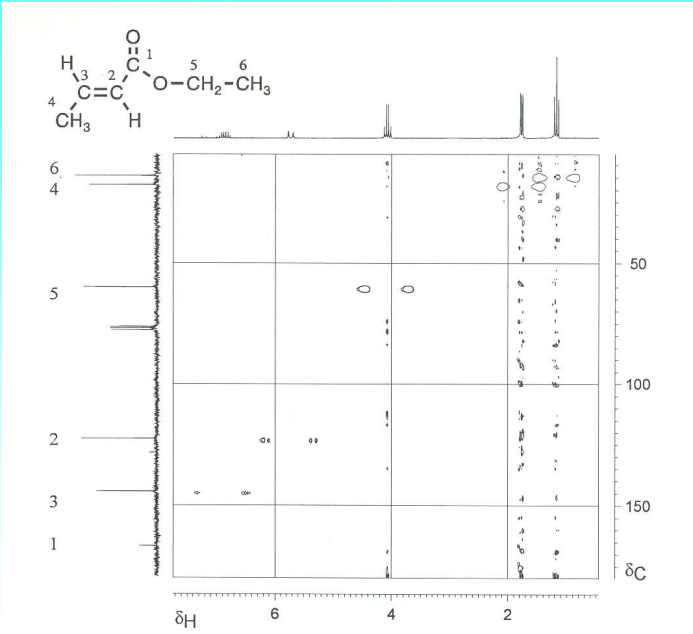


•spectrum reproduced from *100 and more Basic NMR Experiments*, by Braun, Kalinowski and Berger

2D Proton-Carbon NMR

HSQC

- Similar to HMQC
- J_{HH} splitting absent in ^{13}C dimension (useful if carbon spectrum is crowded)
- preferred technique in protein NMR



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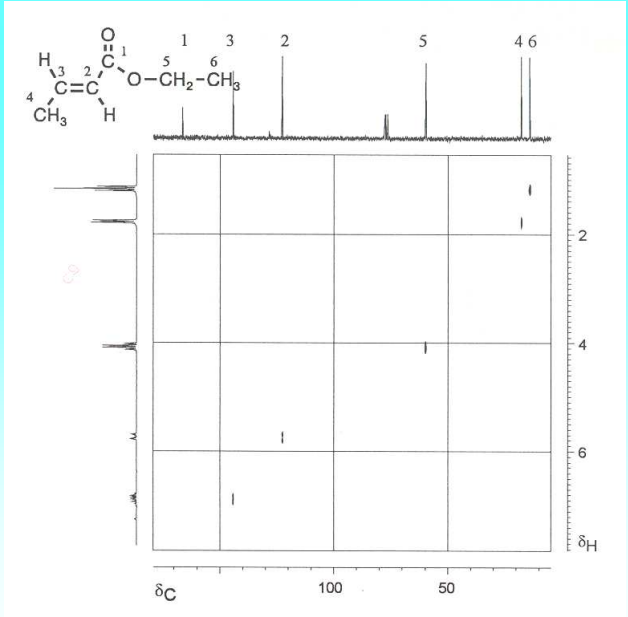
2D Proton-Carbon NMR

HETCOR

- Use $^{1}J_{HC}$ interaction to correlate protons with neighbouring carbons
- Less popular alternative to HMQC
- Carbon signal detected, which means sensitivity is much lower…
- …but useful when high resolution is required in ^{13}C dimension



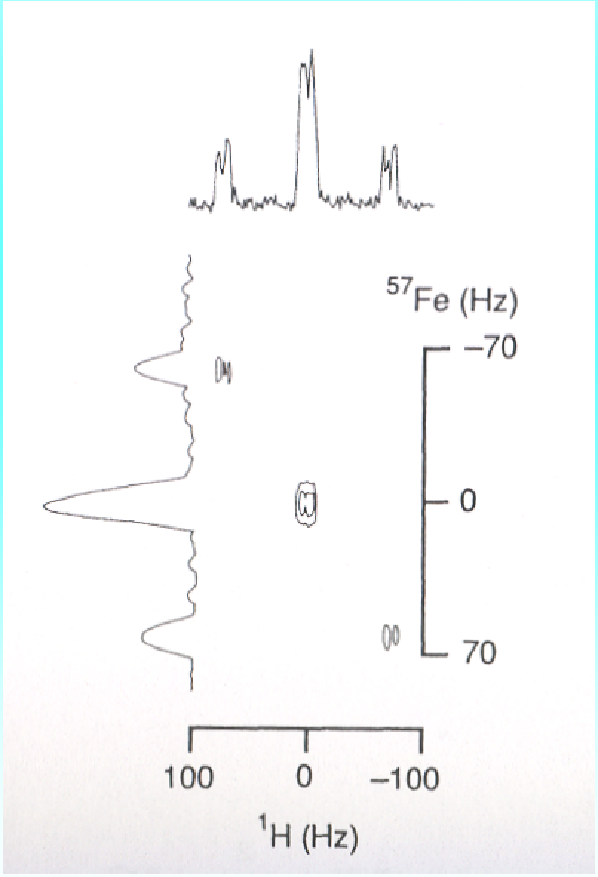
proton shift

carbon shift

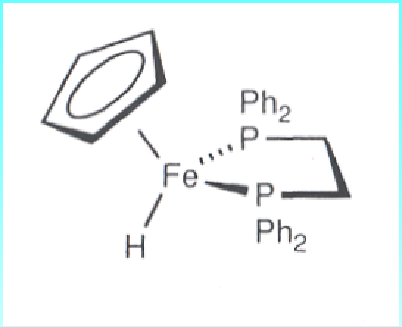
- spectrum reproduced from *100 and more Basic NMR Experiments*, by Braun, Kalinowski and Berger

2D Proton-Carbon NMR

HMQC – inorganic example



- ^{1}H—^{57}Fe correlation spectrum
- $\nu_0(^{1}H) \sim 300$ MHz
- $\nu_0(^{57}Fe) \sim 9.7$ MHz



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2D Proton-Carbon NMR

Summary

HMQC	**HMBC**	**me-HSQC**	**HSQC**	**HETCOR**
•^{1}H–^{13}C one-bond correlations •standard method for small–medium size molecules	•^{1}H–^{13}C 2/3-bond correlations	•^{1}H–^{13}C one-bond correlations •with multiplicity information	•^{1}H–^{13}C one-bond correlations •mainly used for **biomolecules**	•^{1}H–^{13}C one-bond correlations •typically lower sensitivity than HMQC •high resolution in ^{13}C dimension

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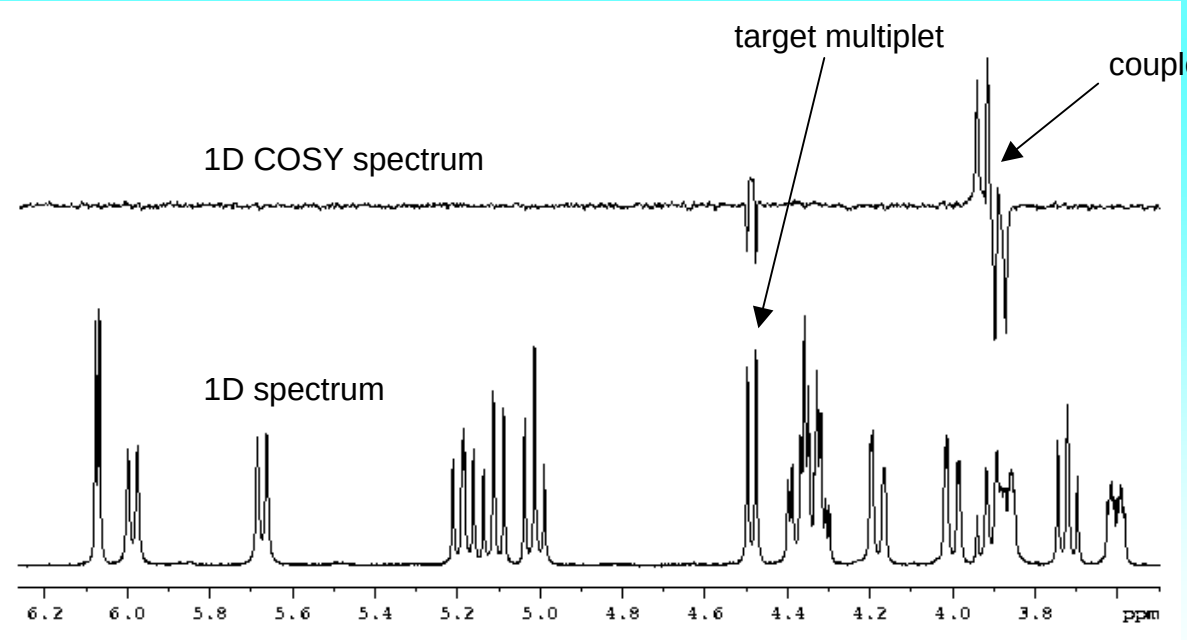
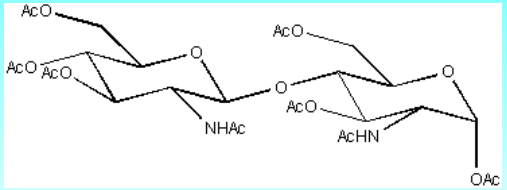
   - multiplicity-edited HSQC*****
   - HMBC*****
   - HSQC
   - HETCOR
5. The rest
   - 1D analogues
   - macromolecules, solids, multinuclear

Further experiments

1D Analogues

- Almost every 2D experiment has a “selective” 1D analogue, e.g. 1D COSY
- experiment time can be much shorter than for 2D
- “target” multiplet must be resolved





- spectra reproduced from *http://rmn.iqfr.csic.es/guide/tutorials/specdata/spectra/dis_selco.html*, by Teodor Parella

Further experiments

Macromolecules

•100s of experiments designed for assigning macromolecules, e.g. proteins, carbohydrates

Solids

•plenty of equivalent experiments for use on solid samples, see *lecture 4*

Multinuclear NMR

•experiments described can be used throughout the periodic table, not just for carbon and proton

Conclusion

- 1000's of NMR experiments have been developed
- about a dozen are really useful
- the rest are occasionally useful…
- …and ***nearly all of them are available in the department!***

Useful Reading

General and Organic:

- *Modern NMR Spectroscopy* (the workbook is also useful), by J.K.M. Sanders and B.K. Hunter
- *100 and more Basic NMR Experiments*, by Braun, Kalinowski and Berger (a practical guide for Bruker users)
- *Structure Elucidation by Modern NMR (workbook)*, by Duddeck and Dietrich
- *Spectroscopic Methods in Organic Chemistry*, by Williams and Fleming
- *Carbon-carbon and C—H NMR couplings*, by James L. Marshall (coupling constants)
- the literature, e.g. *Magnetic Resonance in Chemistry*

Inorganic:

- *NMR Spectroscopy in Inorganic Chemistry (Oxford Chemistry Primer)*, by Jonathan A. Iggo
- several books in department library (look for “multinuclear” in the title)

Proteins:

- *Protein NMR Spectroscopy: Principles and Practice*, by Cavanagh, Fairbrother, Palmer and Skelton

Introductory NMR theory:

- *Understanding NMR spectroscopy*, by James Keeler
- *Nuclear Magnetic Resonance (Oxford Chemistry Primer)*, by P.J. Hore
- *NMR: The Toolkit (Oxford Chemistry Primer)*, by P.J. Hore, Jonathan Jones and Stephen Wimperis