2D-NMR Techniques for the Research Organic Chemist

Andy Thomas

Group Presentation

10-30-12

Outline

- Brief History of NMR (with some general theory)
- Homonuclear Correlations: (^{1}H-^{1}H or ^{13}C-^{13}C etc…)
- Heteronuclear Correlations: (^{1}H-^{13}C or ^{1}H-^{19}F etc…)
- Nuclear Overhauser: through space interactions
- Exchange Correlations: equilibrium

1. Through-Bond interactions

2. Through-Space interactions

3. Chemical Exchange

Key developments

- 1950s-Coupling constants used as structural tools
- 1960s-Signal averaging used to improve sensitivity
- 1970s-Computer controlled instrumentation
- 1980s-2D NMR was developed
- 1990s-Pulse field gradients used
- 2000s-Flow NMR, high sensitivity probes

3 Nobel prizes in physics, 2 Nobel prizes in chemistry and 1 Nobel prize in medicine **(6 total)**

High-Resolution NMR Techniques in Organic Chemistry, Timothy D.W. Claridge, Elsevier Science Ltd, UK, 1999; Chapter 1

Physics Prize 1952
for the development of new methods
of nuclear magnetic precision (NMR)

Felix Boch
Stanford
1905-1983

Edward M. Purcell
Harvard
1912-1997

Richard R. Ernst Physics Prize 1991

for the development of new high resolution
methods of nuclear magnetic precision (NMR)

2002 Chemistry Prize

determination of proteins

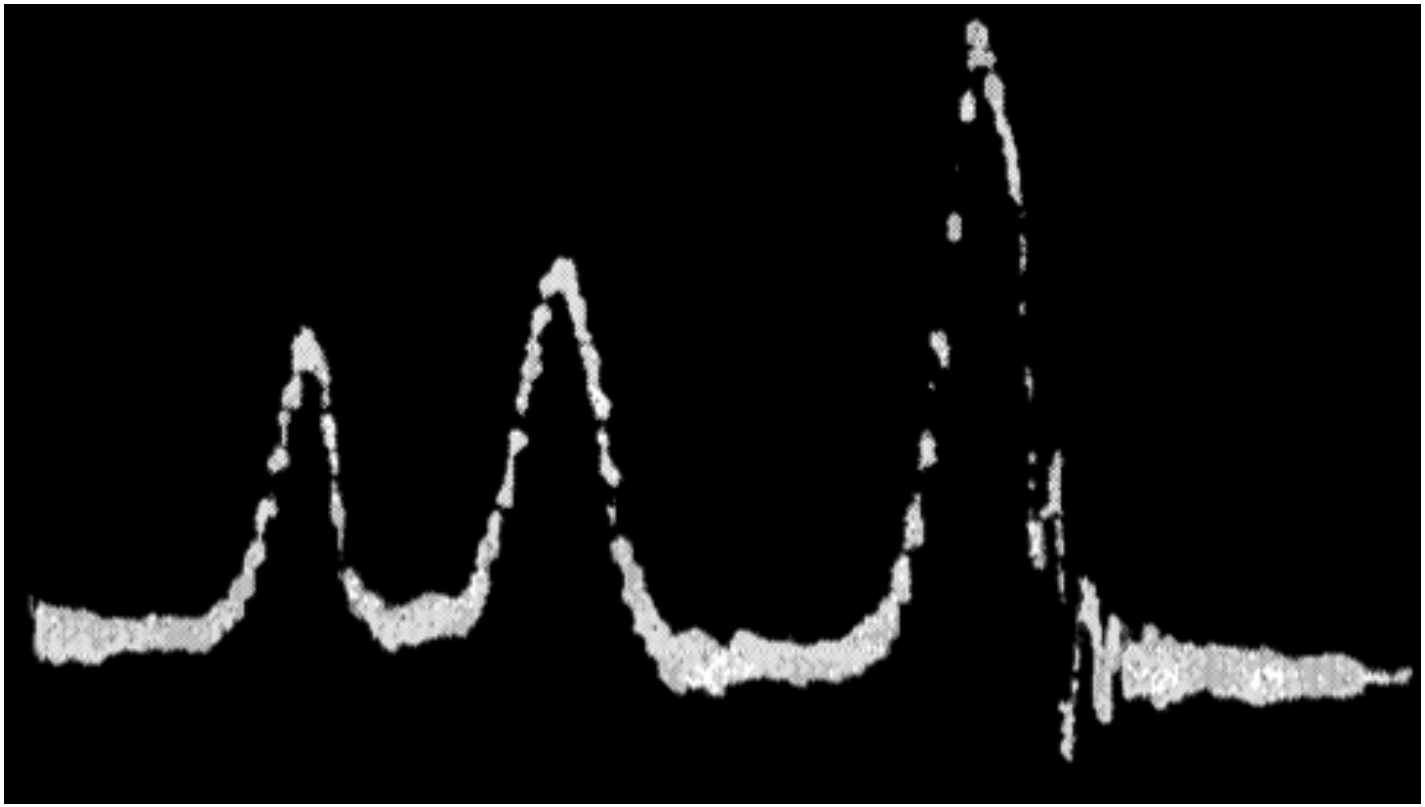
Prize in Medicine 2003
for the discovery of MRI

Paul C. Lauterbur

Peter Mansfield

The beginning of NMR (First Spectrum 1951)

Spectrum of Ethanol at 30 MHz



High Resolution

Arnold, J.T. Dharmatti, S.S., Packard, M.E., *J. Chem. Phys.* **1951**, *19*, 507

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- **Heteronuclear Correlations: (^{1}H-^{13}C or ^{1}H-^{19}F etc…)**

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- Nuclear Overhauser: through space interactions

2. Through-Space interactions

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Homonuclear Correlations (main techniques)

COSY: **CO**rrelation **S**pectroscop**Y**
TOCSY: TOtal **C**orrelation **S**pectroscop**Y**
1D selective TOCSY

^{1}H-^{1}H, ^{19}F-^{19}F etc.

INADEQUATE: (C–C correlations) ^{13}C-^{13}C

- COSY-90-Correlating coupled homonuclear spins. (2-3 bonds)
- DQF-COSY-Correlating coupled homonuclear spins. Coupling Constant information can be obtained. (2-3 bonds)
- TOCSY- Correlates Spin systems

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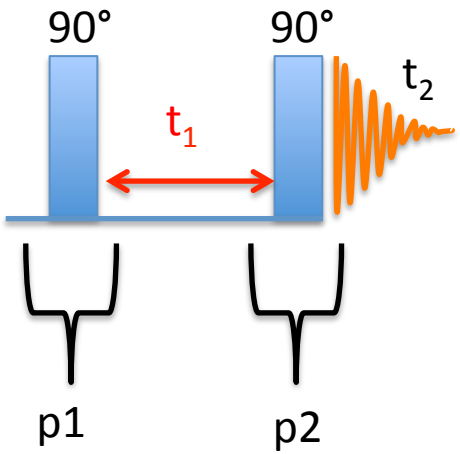
Introducing Two-Dimensional Methods

- Exp. consist of RF pulses with delay periods.
- The dimensions refer to two frequency dimensions. (normally)

p1 = preparation
p2 = mixing period
t1 = time between pulse
t2 = detection period

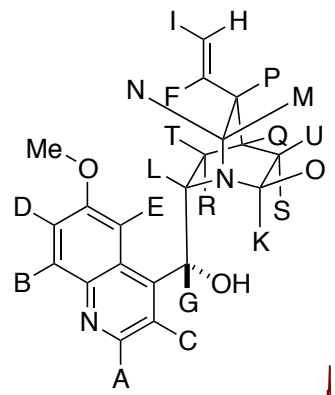
Typical Pulse sequence

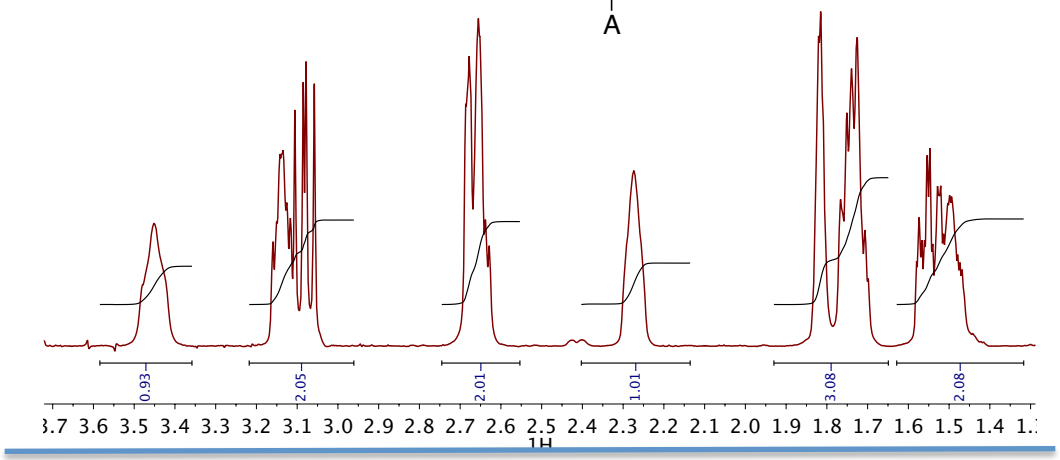


Is COSY Necessary? (quinine)

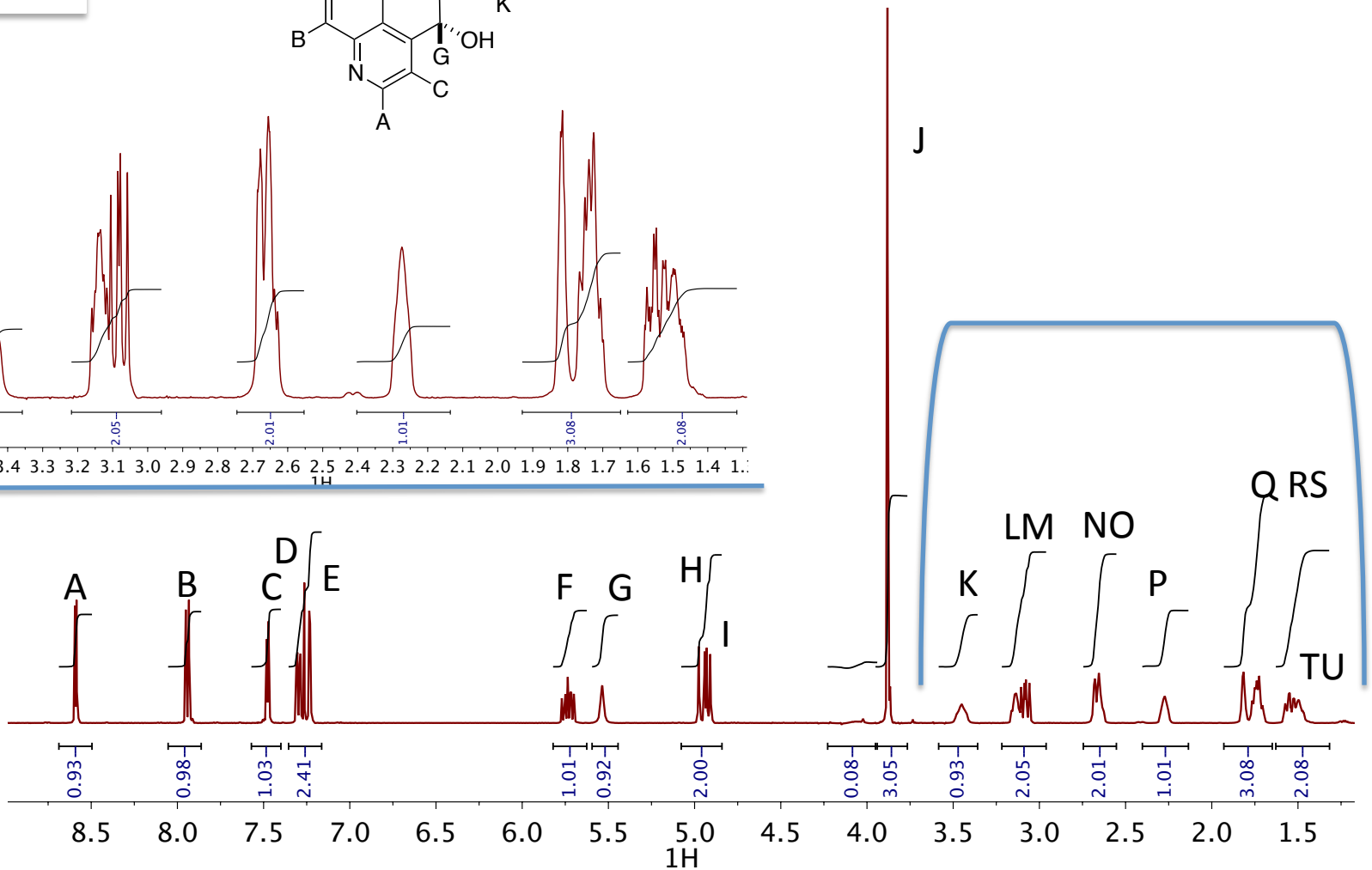
Spectrum from Ian Fleming

- Complex multiplets
- Similar J values



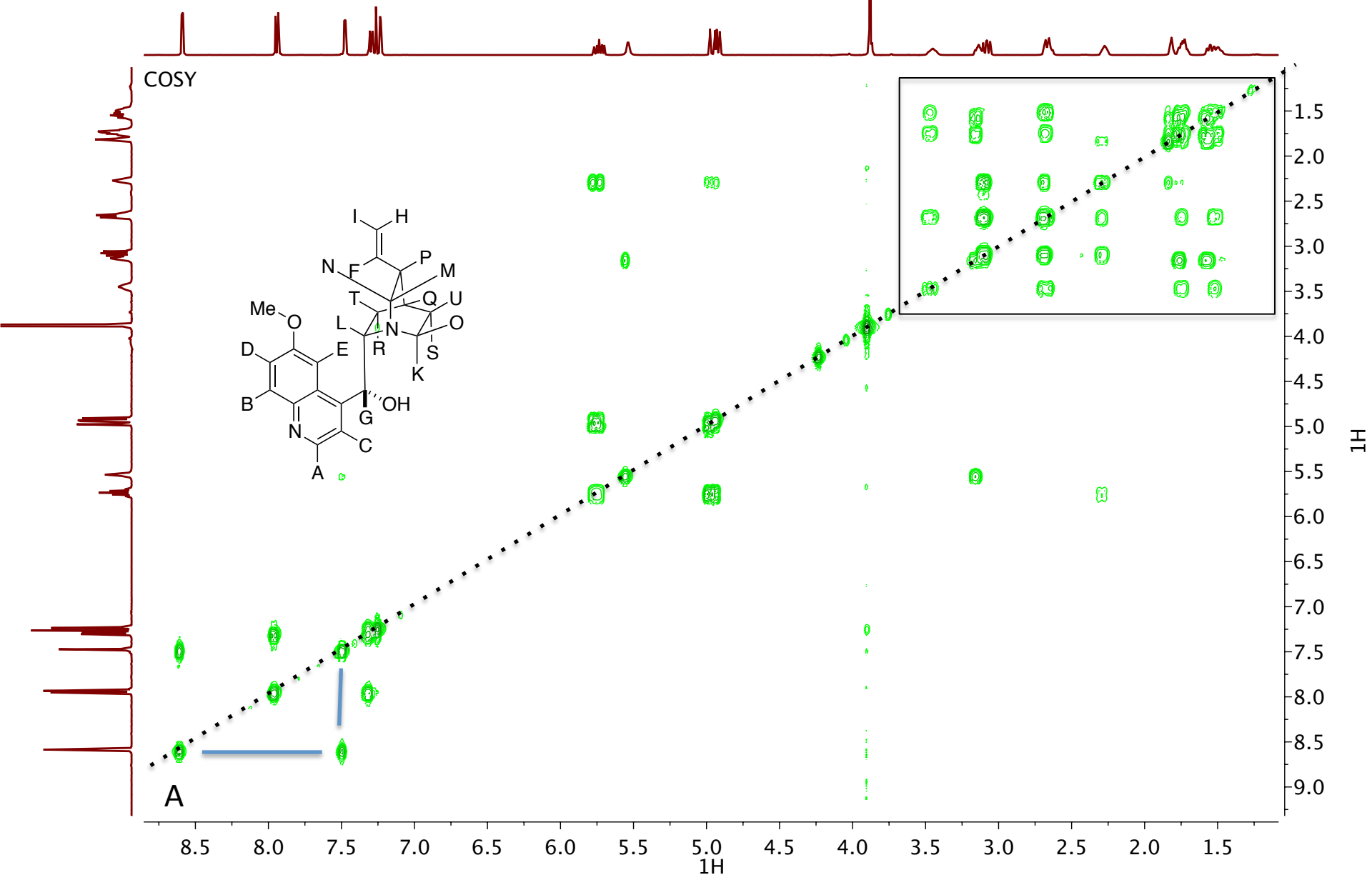


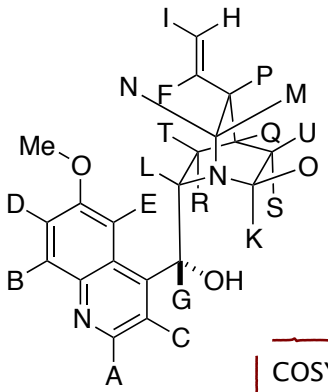
Can we use homonuclear decoupling?
Very difficult in this case:
Overlapping peaks
Also time consuming



COSY of quinine

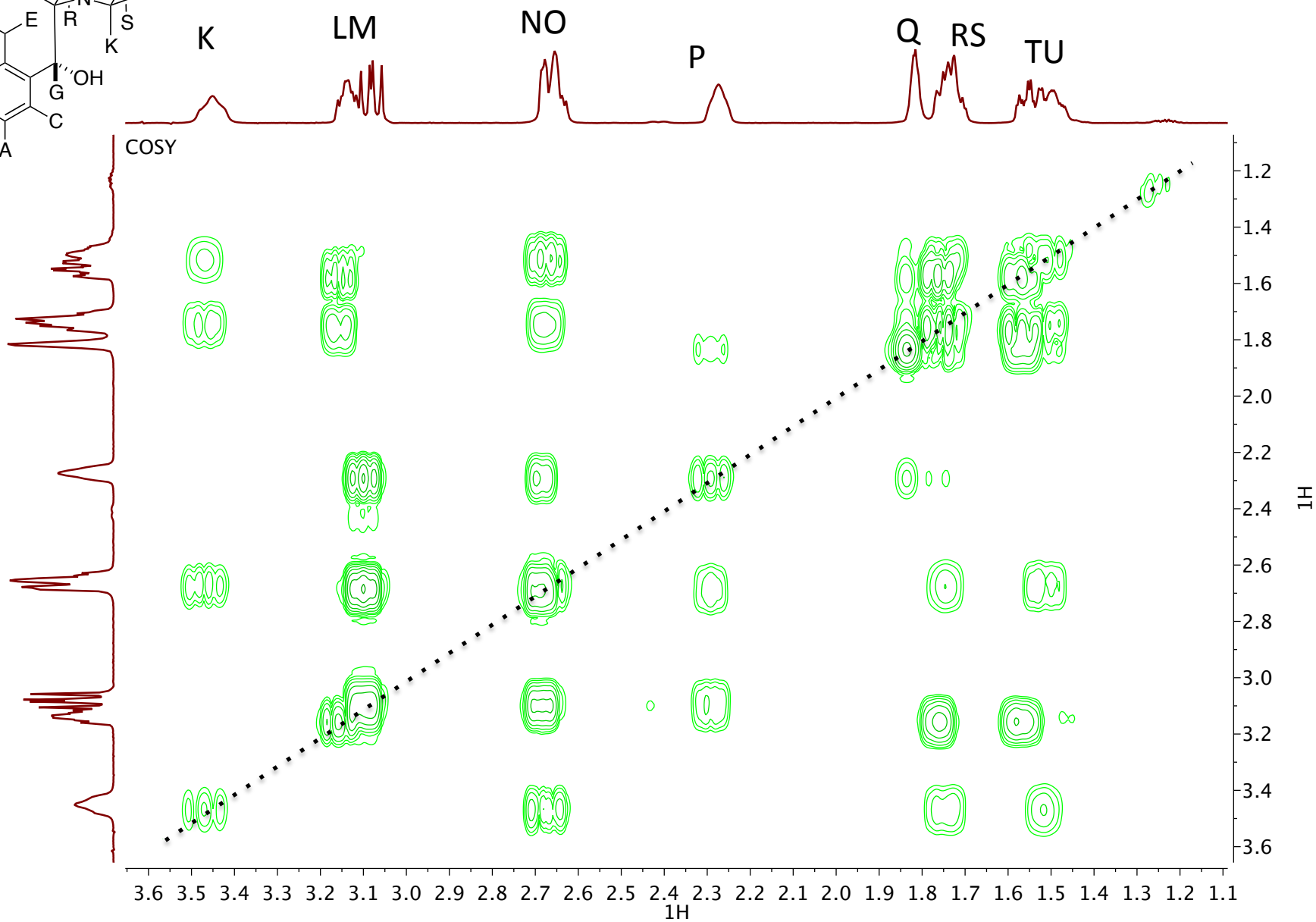
Spectrum from Ian Fleming



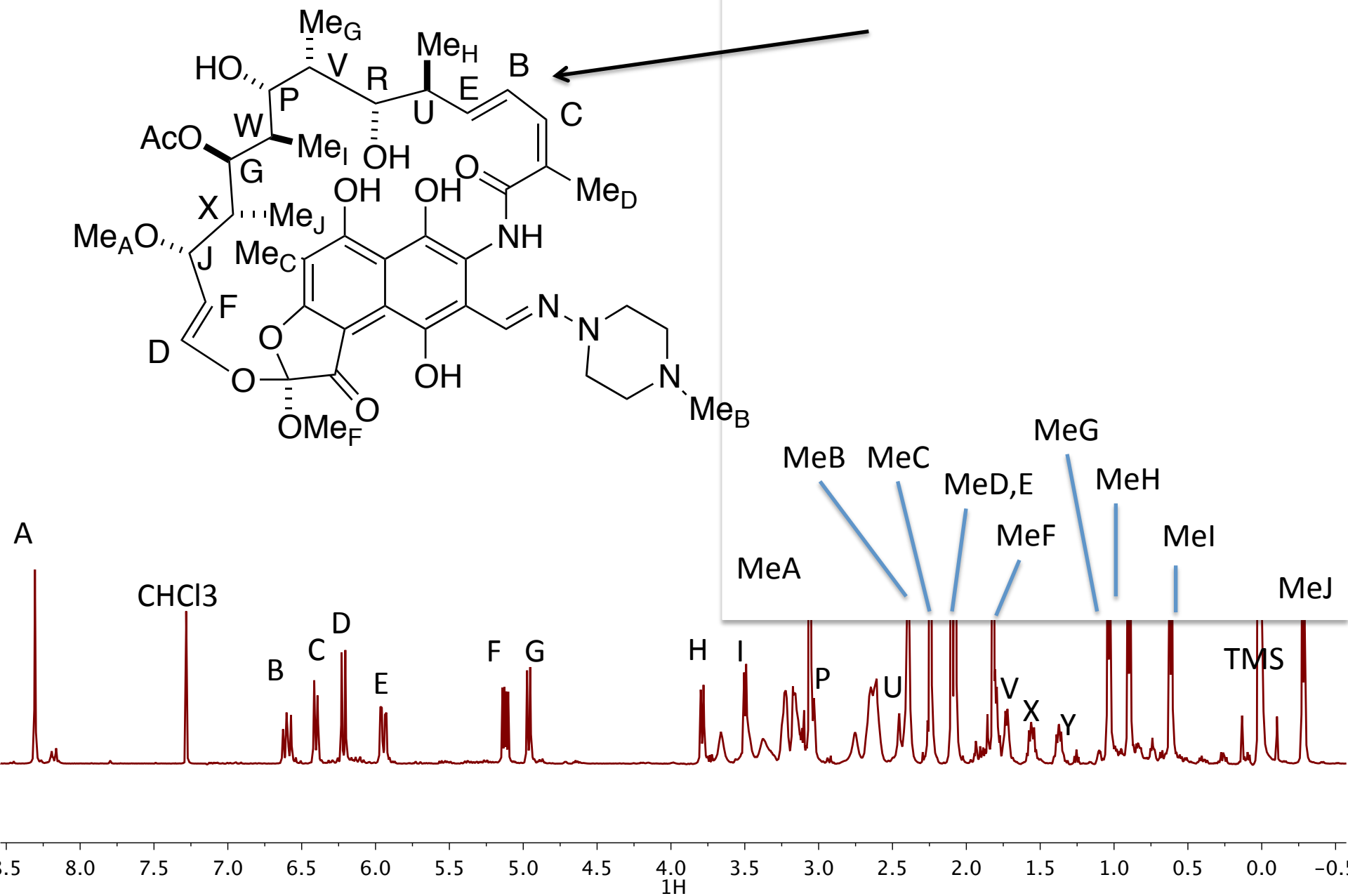


Spectrum from Ian Fleming

The most useful 2D technique for structure determination



Rifampicin (data from Ian Fleming)



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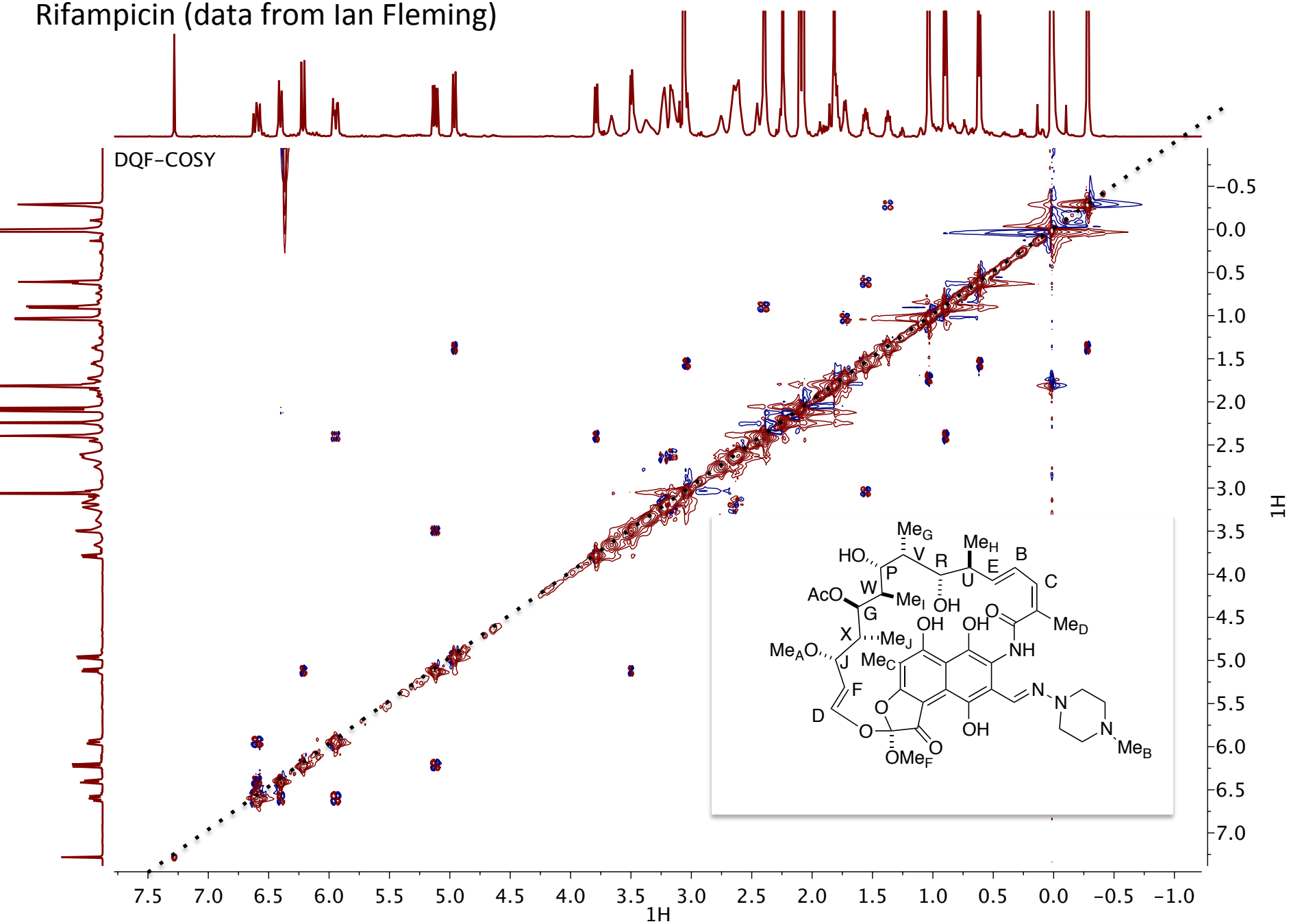

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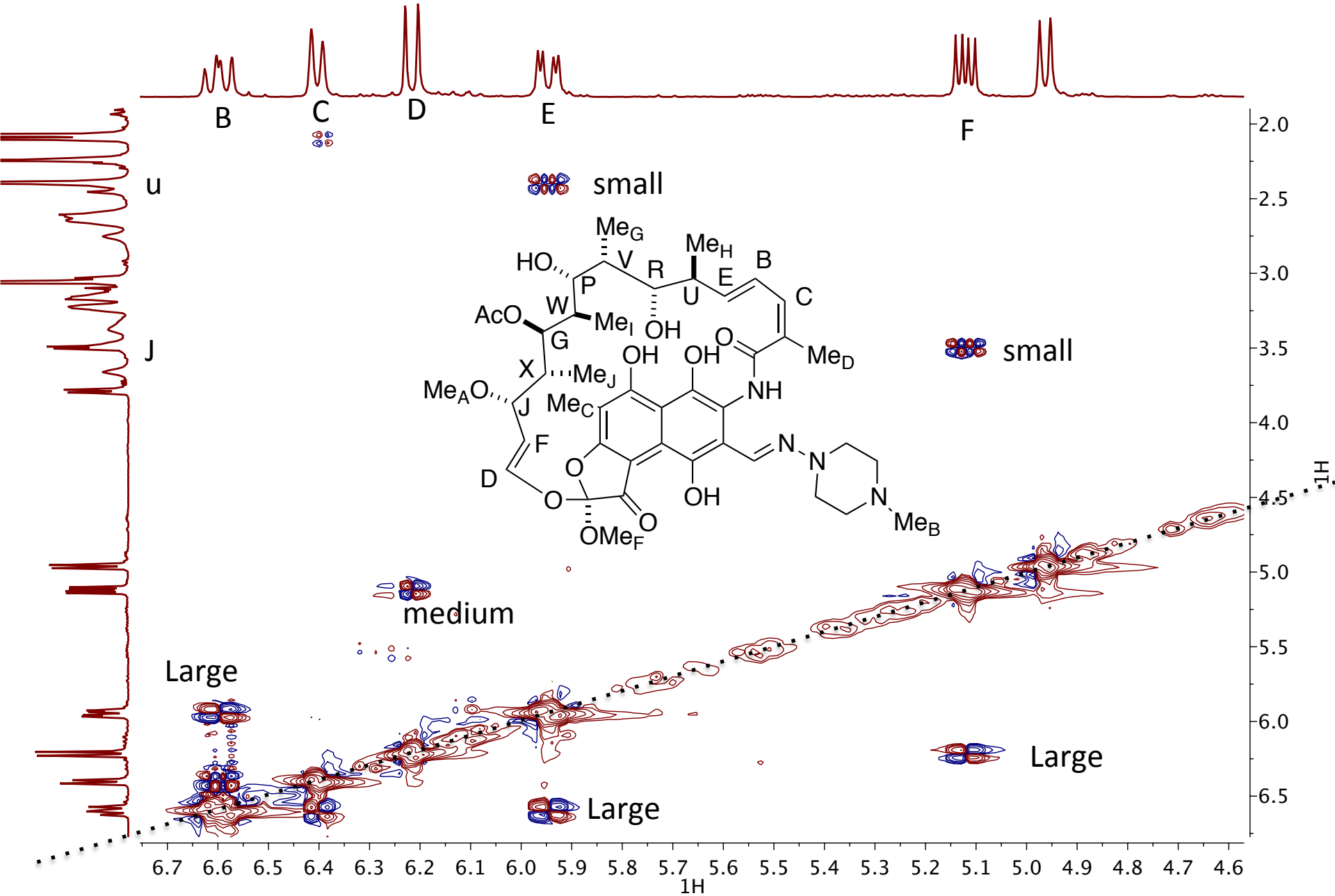

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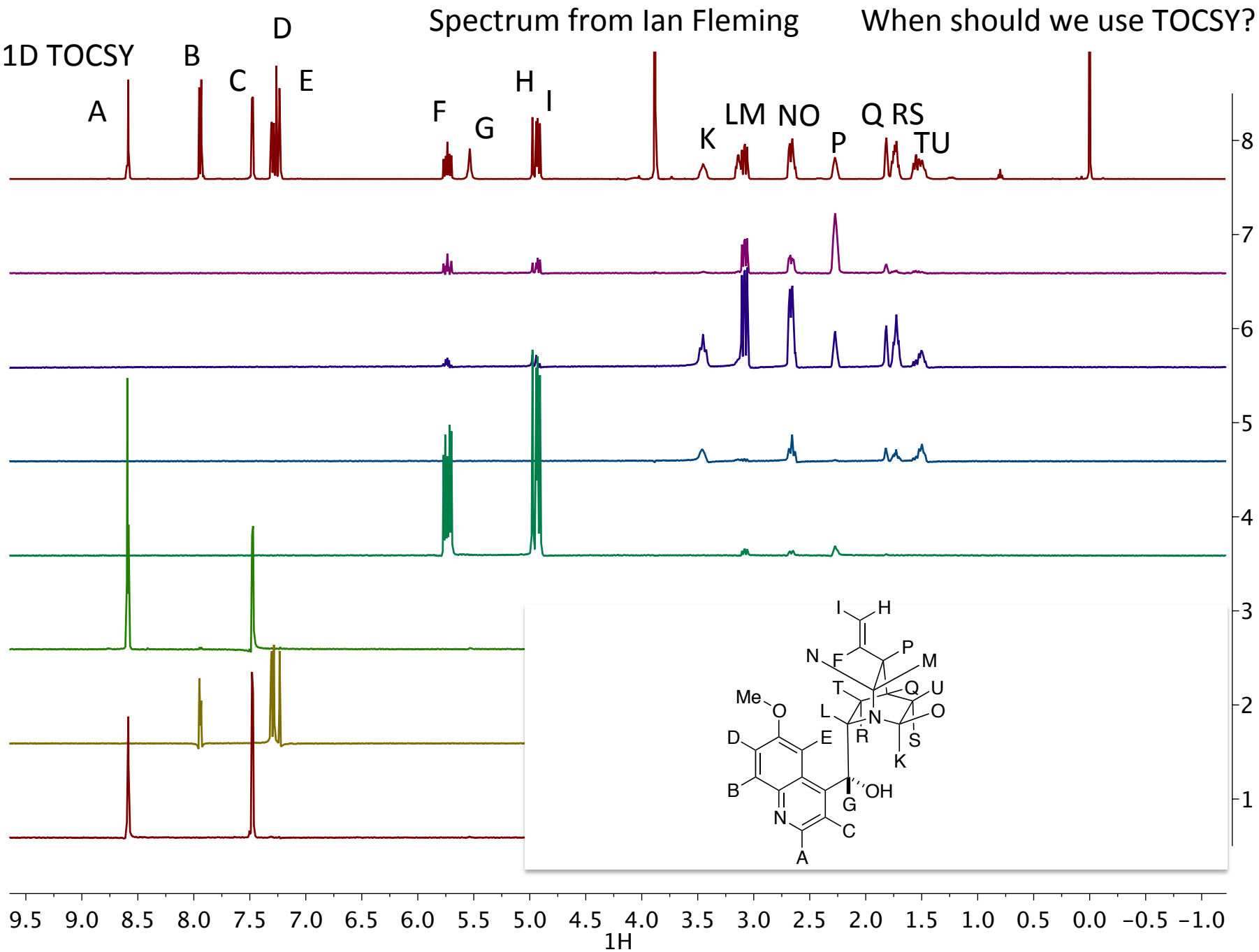
1D TOCSY
Spectrum from Ian Fleming
When should we use TOCSY?
A
B
C
D
E
F
G
H
I
K
LM
NO
P
Q
RS
TU
8
7
6
5
4
3
2
1
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 −0.5 −1.0
1H
Me
O
OH
N

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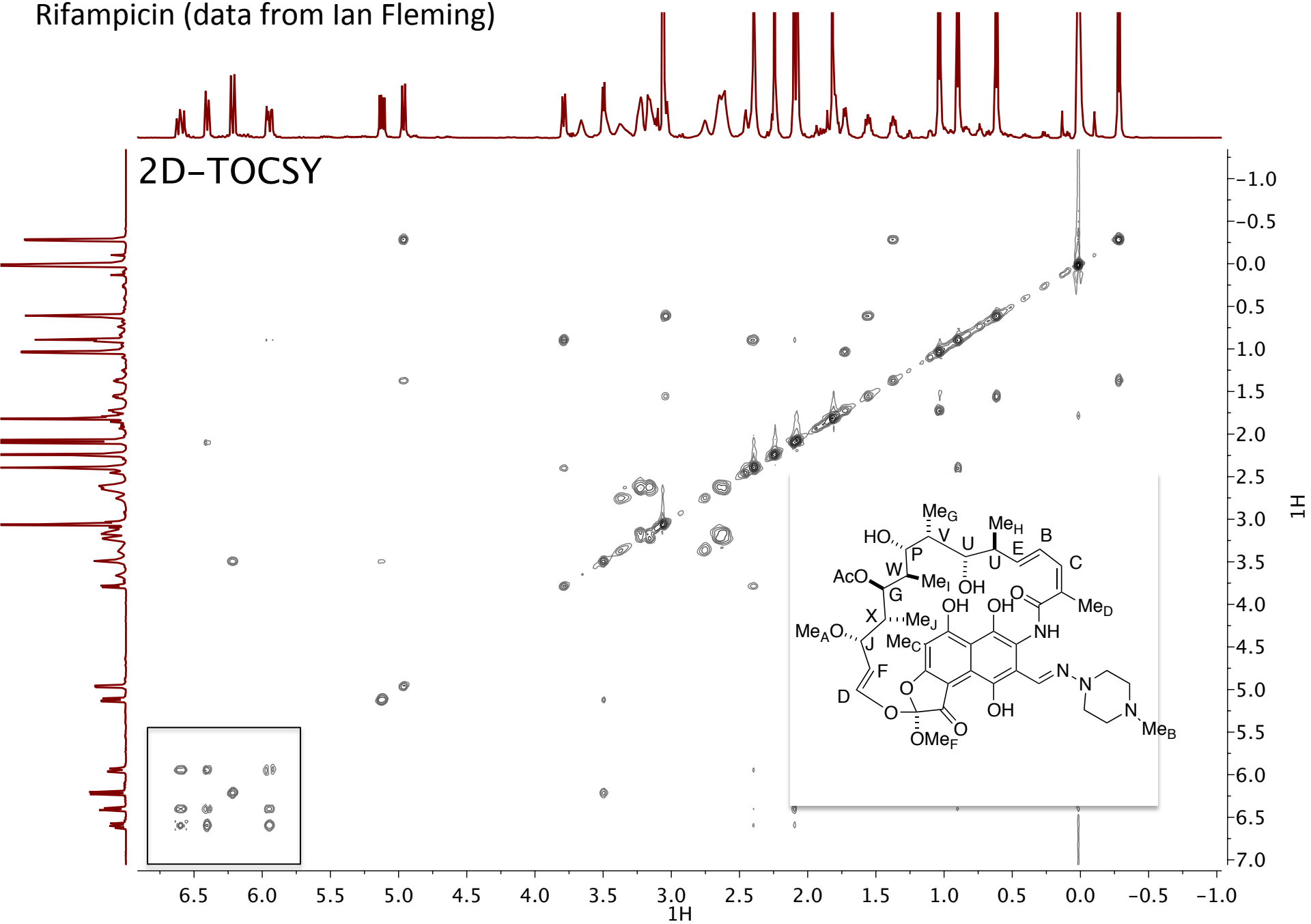

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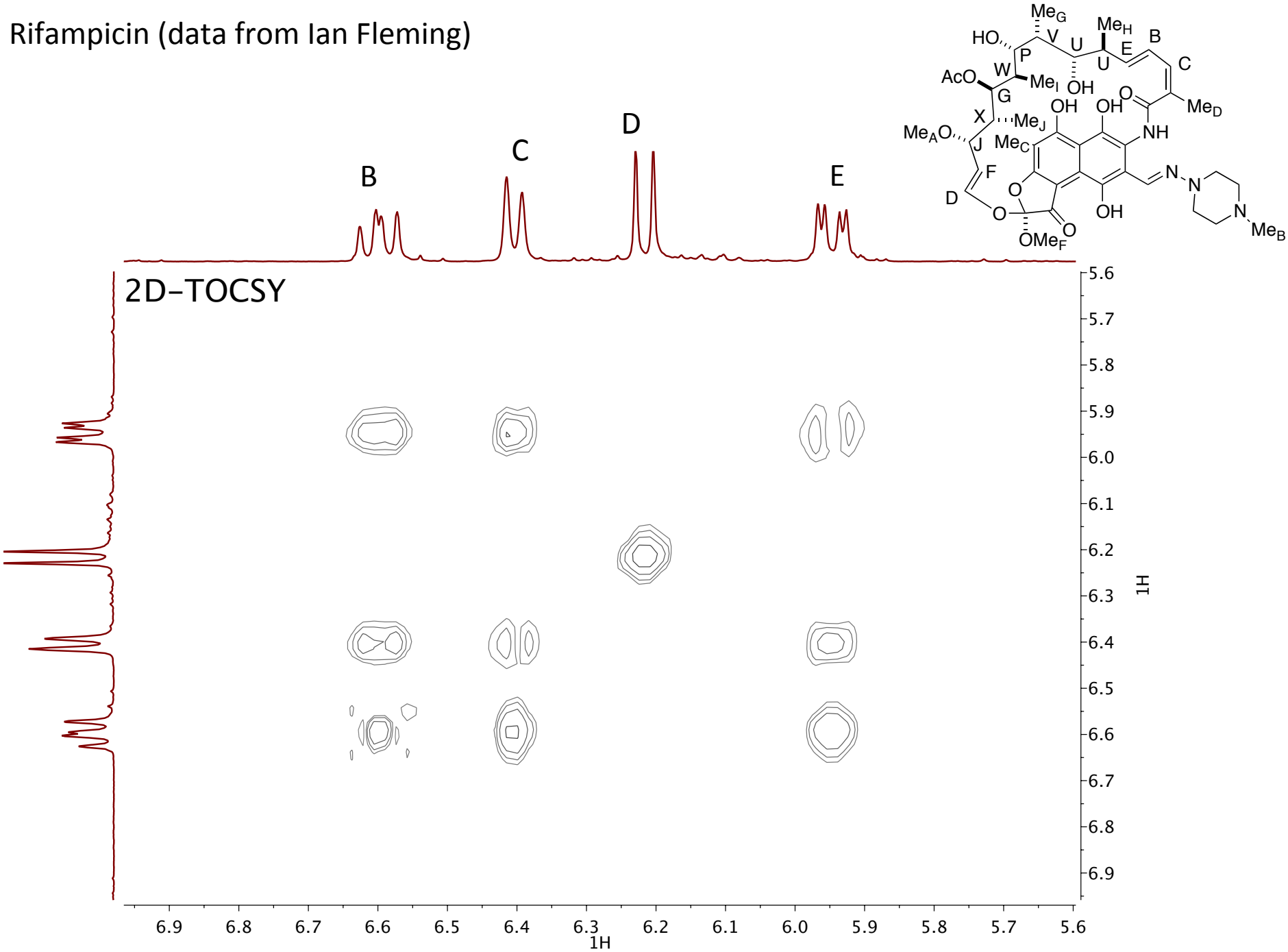

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Heteronuclear Correlations (main techniques)

HMQC: **H**eteronuclear **M**ultiple-**Q**uantum **C**orrelation
HSQC: **H**eteronuclear **S**ingle-**Q**uantum **C**orrelation
HMBC: **H**eteronuclear **M**ultiple-**B**ond **C**orrelation
HETCOR: HETeronuclear **COR**relation spectroscopy

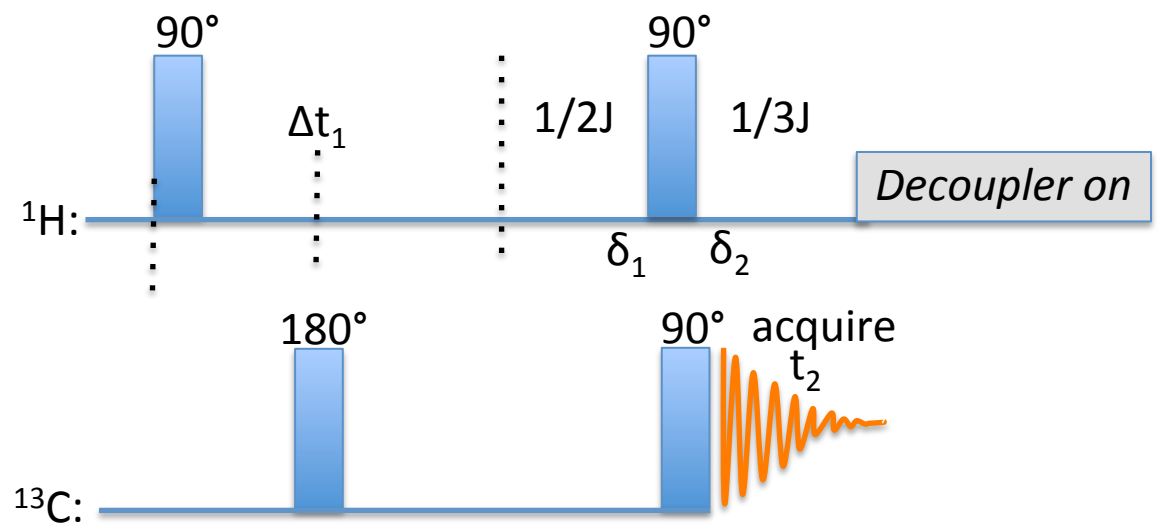
- HMQC-Correlating coupled heteronuclear spins. (used to identify directly connected nuclei)
- HSQC-Same as HMQC except resolution is better (takes longer)
- HMBC-Correlates coupled spins across multiple bonds
- HETCOR-Same as HMQC except is detected through low field channel.

High-Resolution NMR Techniques in Organic Chemistry, Timothy D.W. Claridge, Elsevier Science Ltd, UK, 1999; Chapter 5

HETCOR vs. HMQC

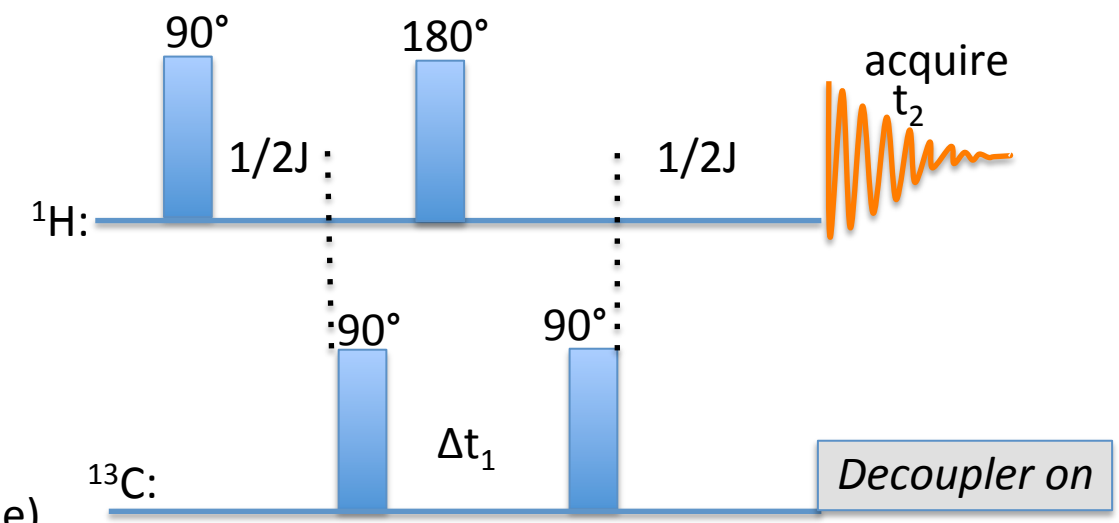
HETCOR: (^{13}C–^{1}H COSY)

- collected in ^{13}C Channel
- increase resolution in ^{13}C
- CH_3Br the carbon is split
- (been replaced by HMQC)



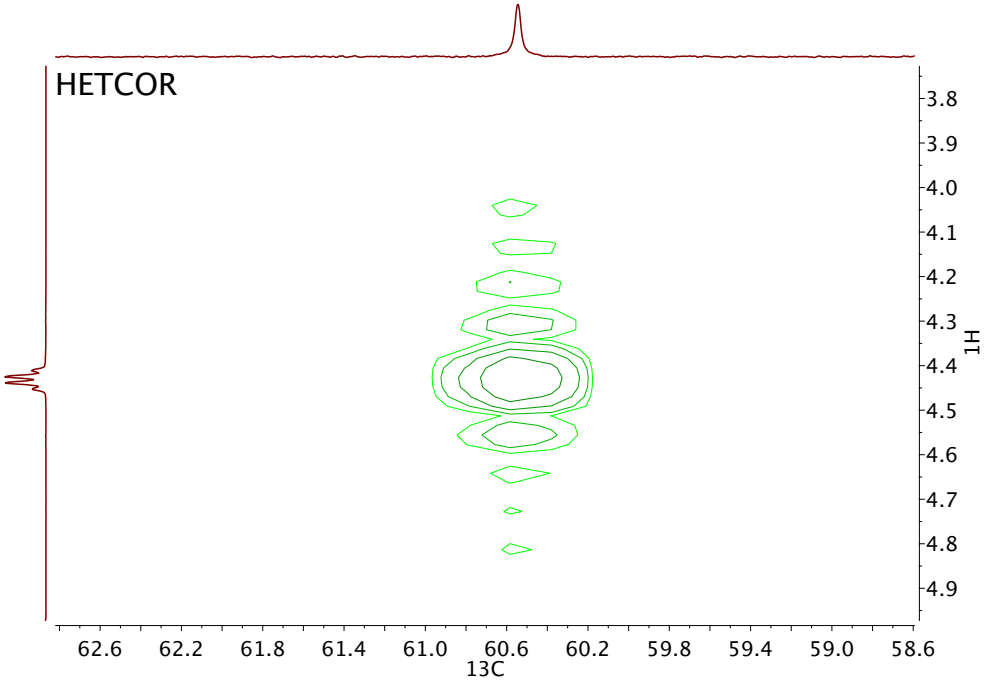
HMQC: (^{1}H–^{13}C COSY)

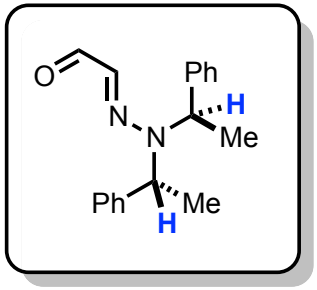
- collected in ^{1}H Channel
- increase resolution in ^{1}H
- CH_3CH_2Br (^{1}H–^{1}H coupling)
- Usually experiment of choice (Time)

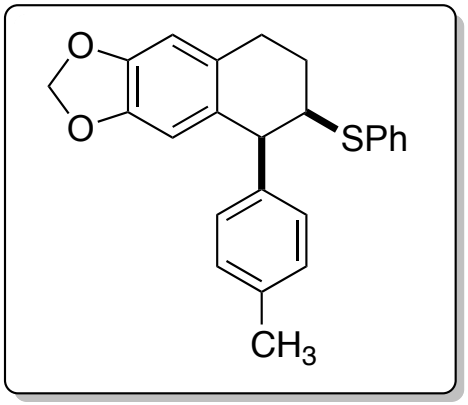


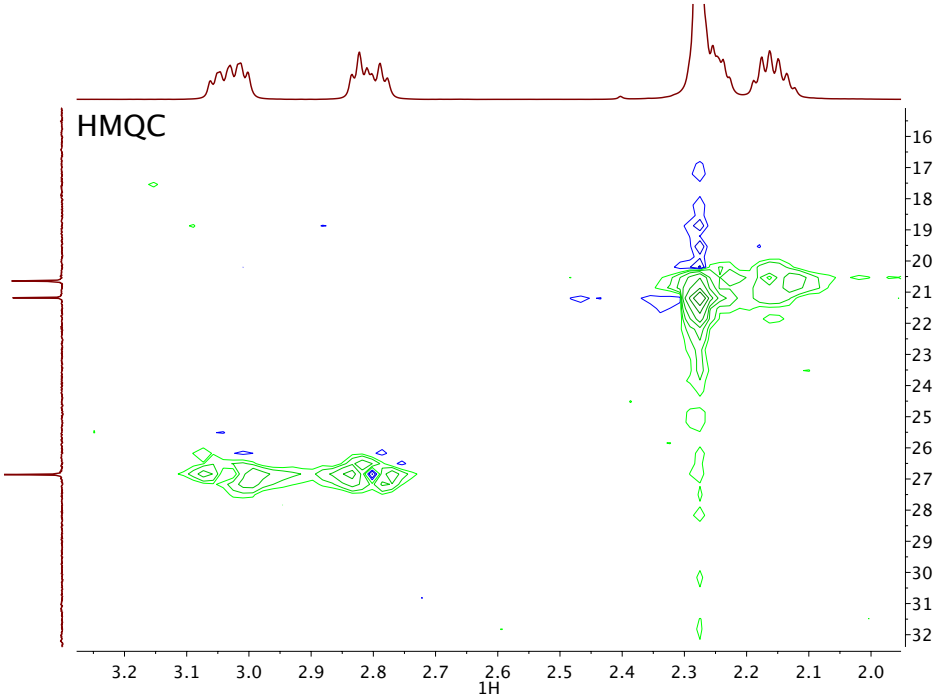
Spectra from Tim and Alex

HETCOR vs. HMQC



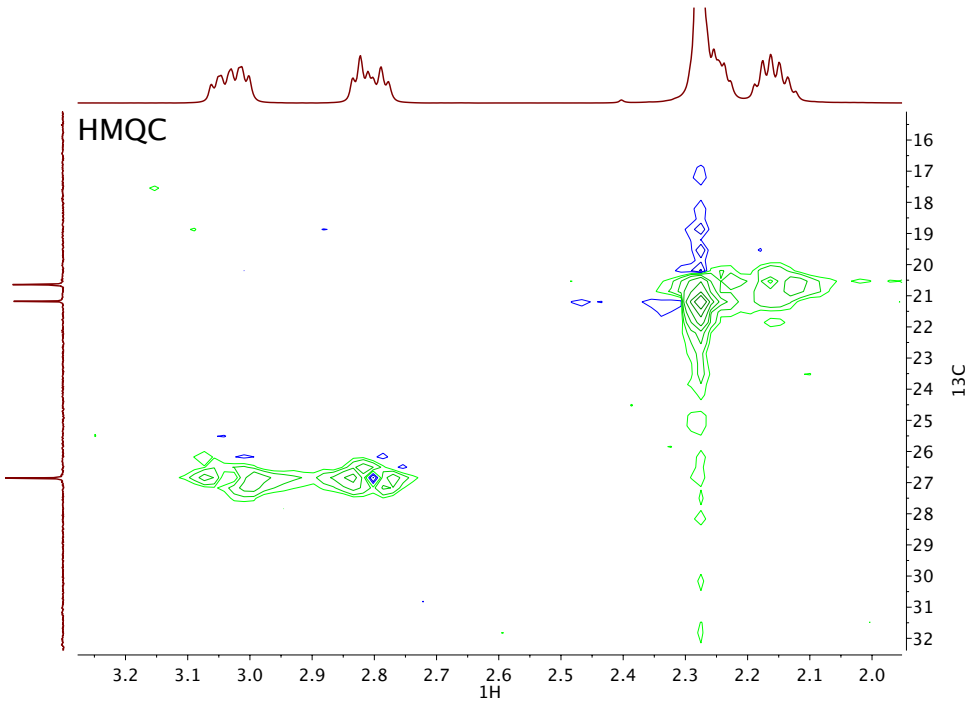


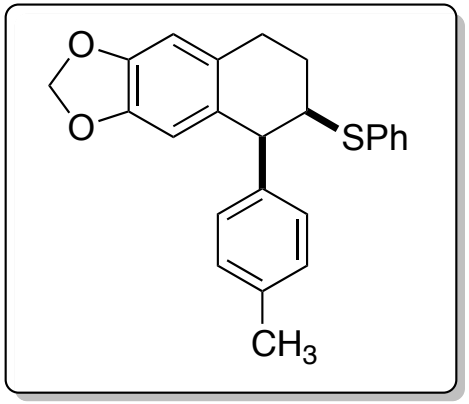


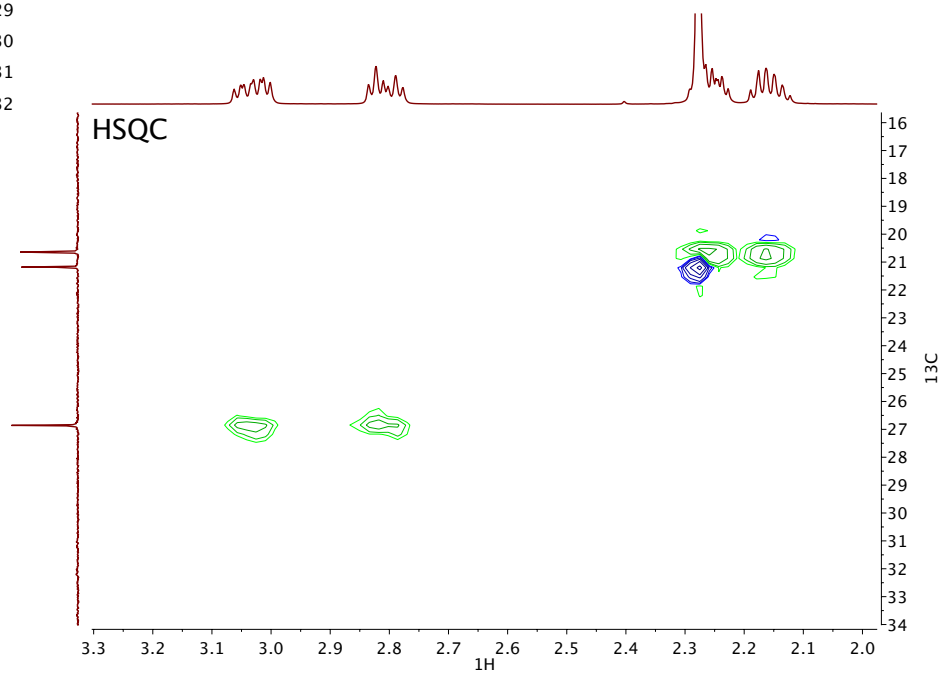


Spectra from Alex

HMQC vs. HSQC





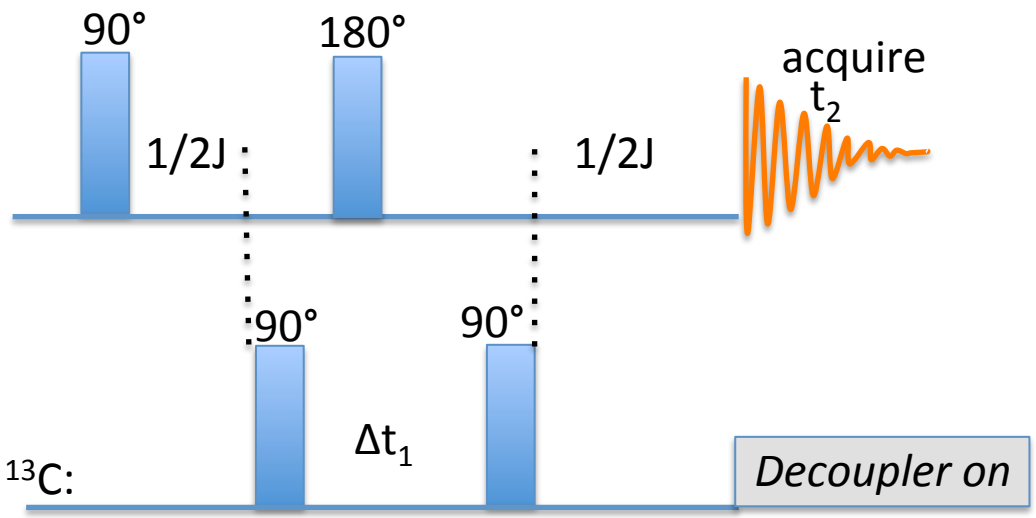


HMQC: Resolution not as good (time is less)

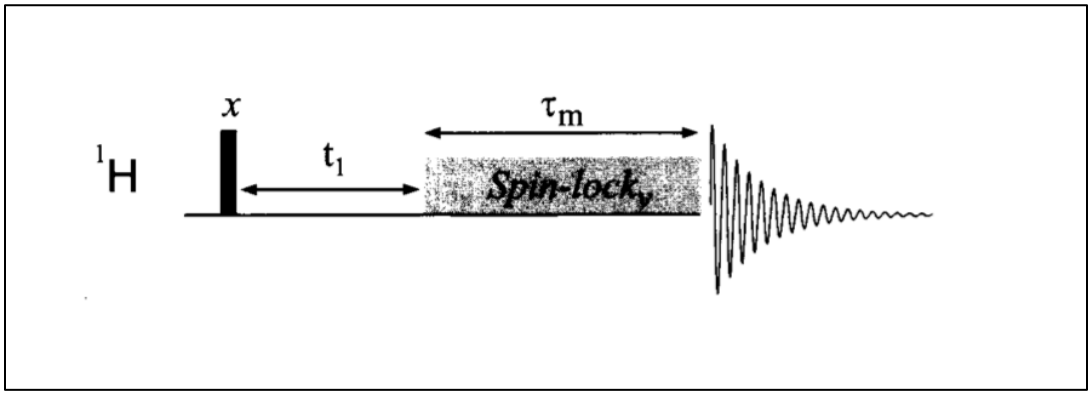
HSQC:
Phase sensitive CH, CH_3 vs. CH_2

Hybrid Experiments (HSQC-TOCSY)?

Sometimes spectra are to crowded to analyze
By adding TOCSY sequence after (HSQC) we can combine the experiments.



TOCSY



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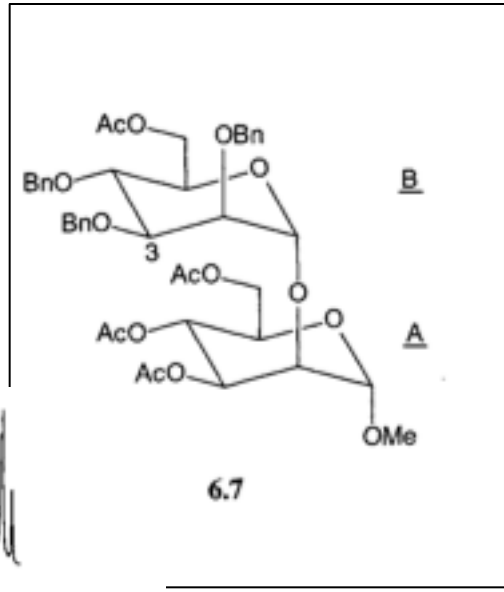
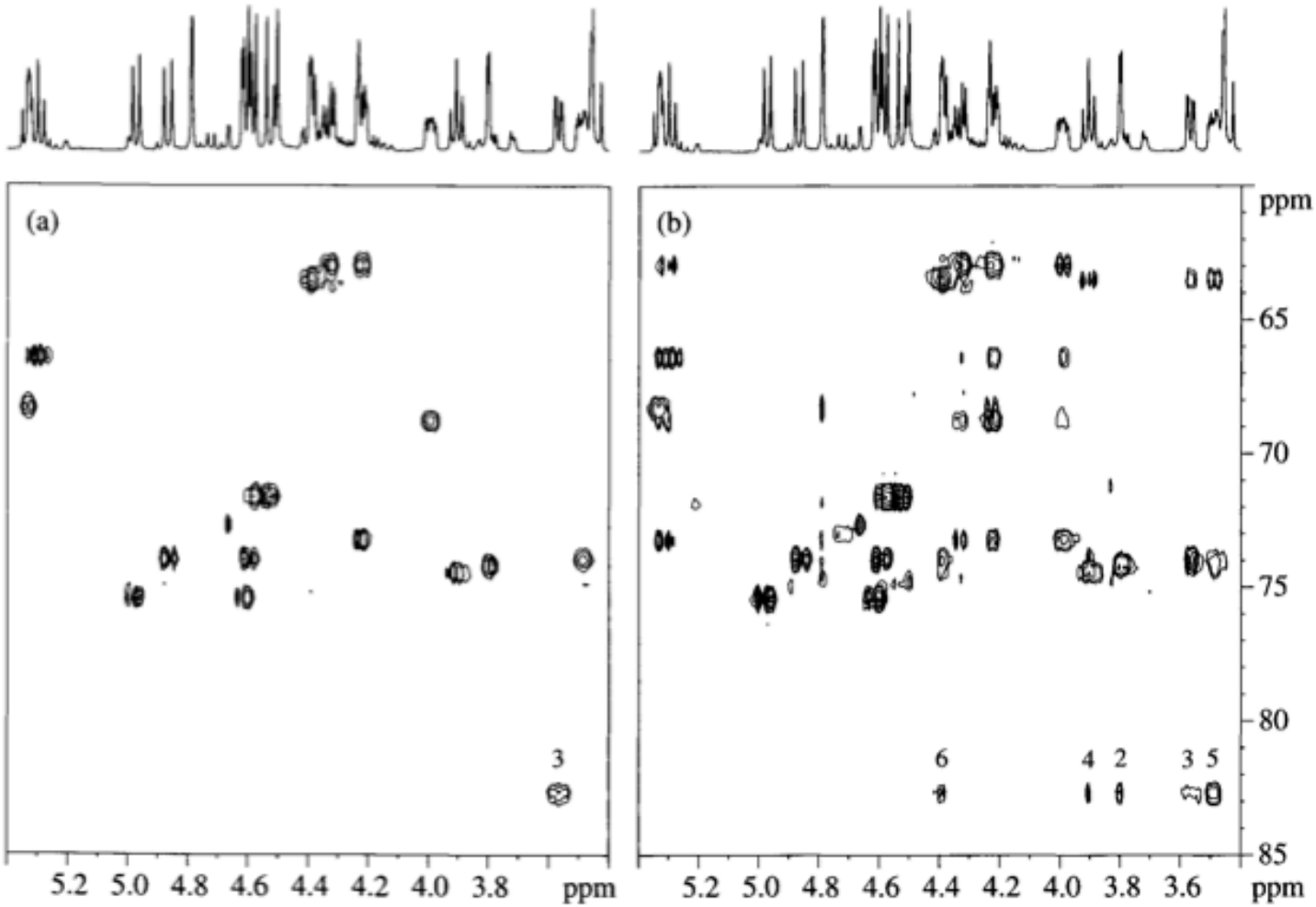
HSQC-TOCSY





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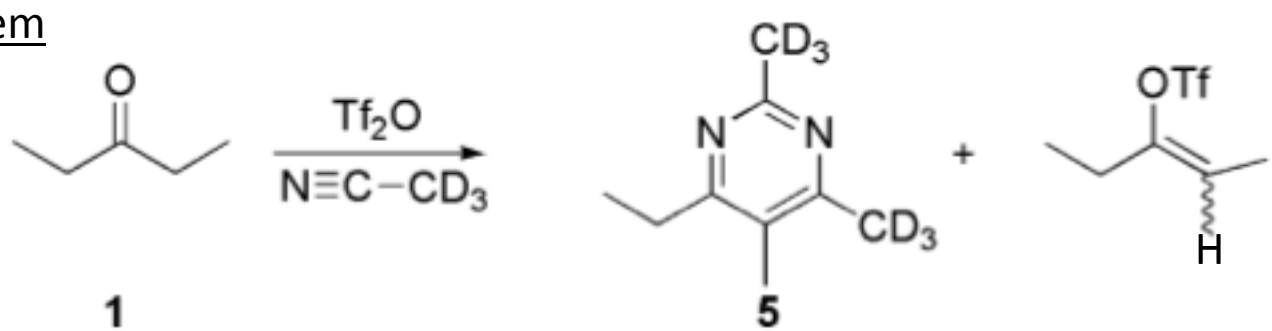
Can we Study mechanism with these methods?

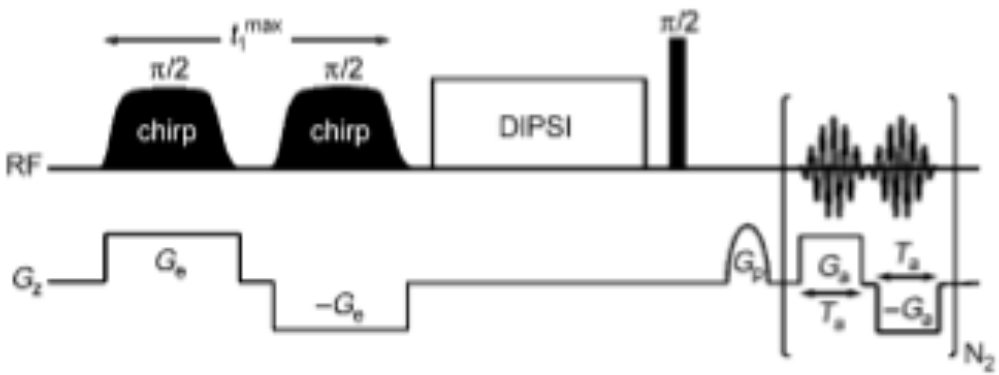
Traditional 2D experiments take on average 7-45 min and up to hrs!!! (needs to be ultrafast)

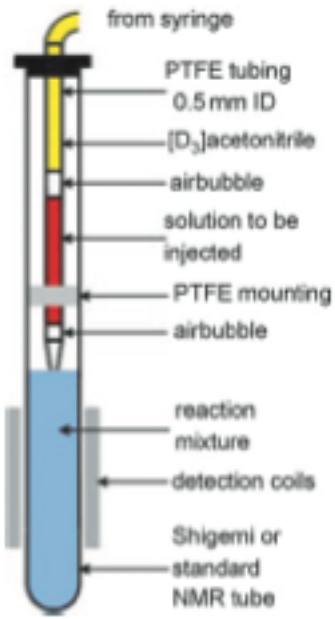
How can we monitor reactions in the NMR by HSQC, HMBC or TOCSY?

Group Problem



Int. Involved in reaction identified by (UF) TOCSY and HSQC





Herra, A., Fernandez-Valle, Martinez-Alvarez, Molero, D., Pardo, Z., Saez, E., Gal, M., *Angew. Chem.* **2009,** *48*, 6274

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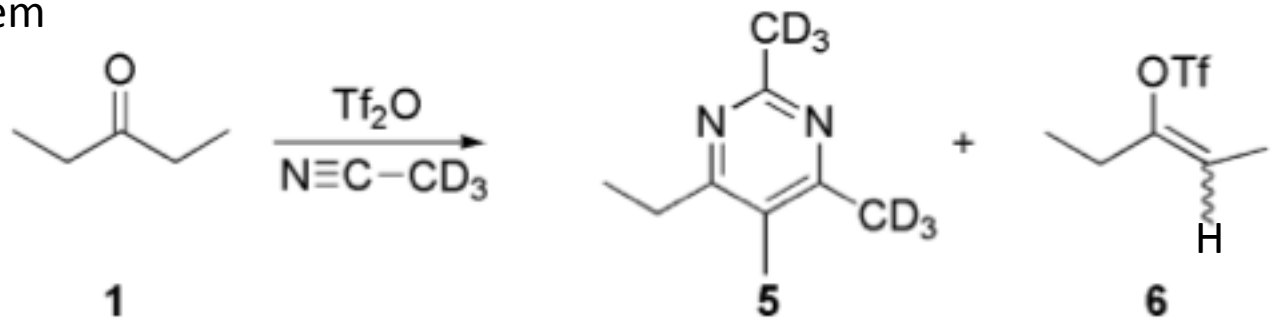

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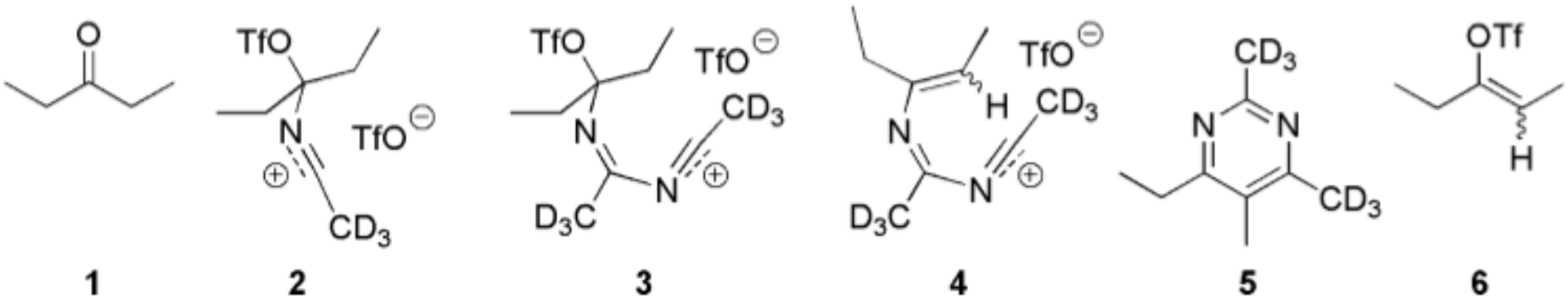

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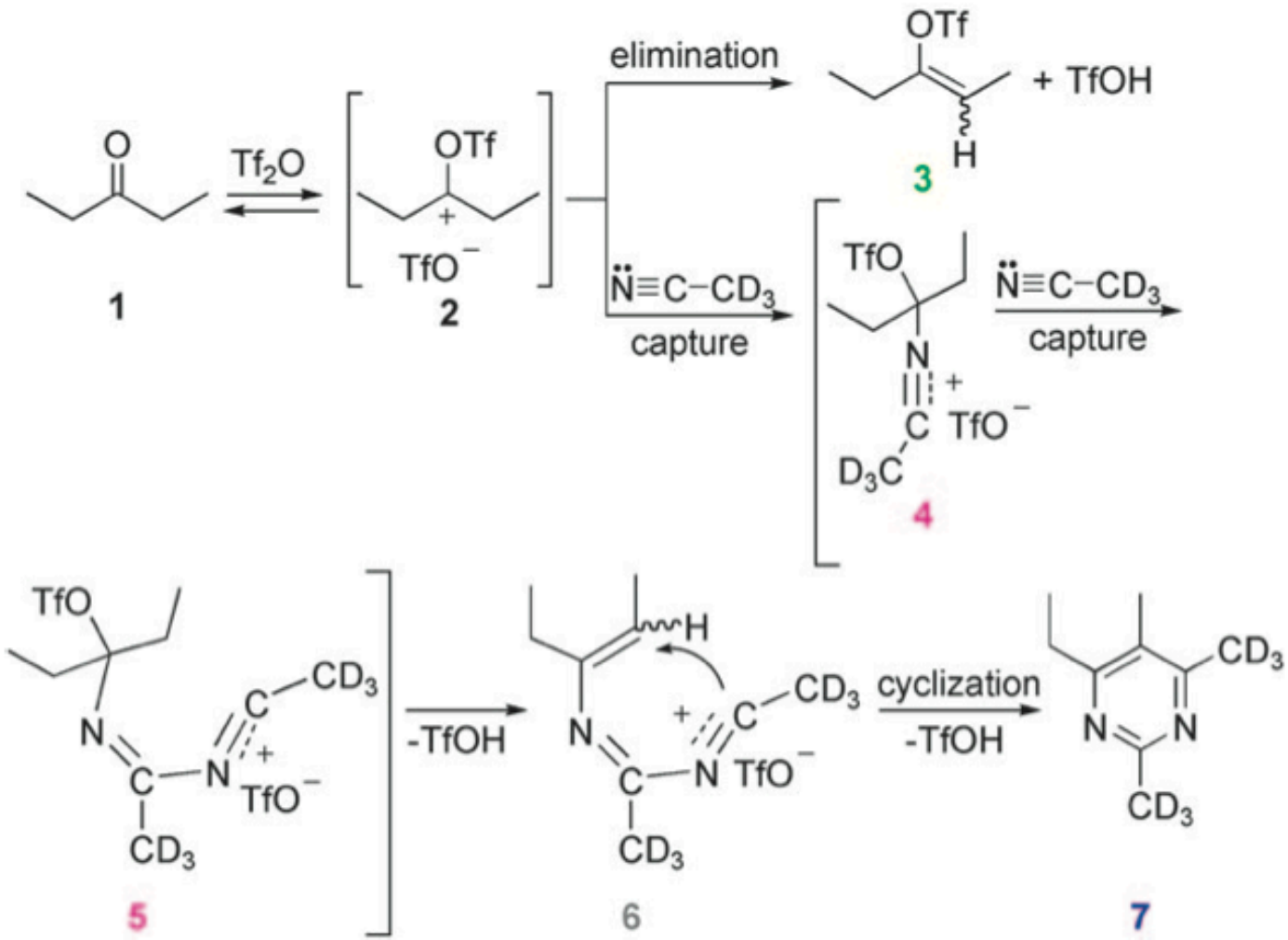
Proposed Mechanism



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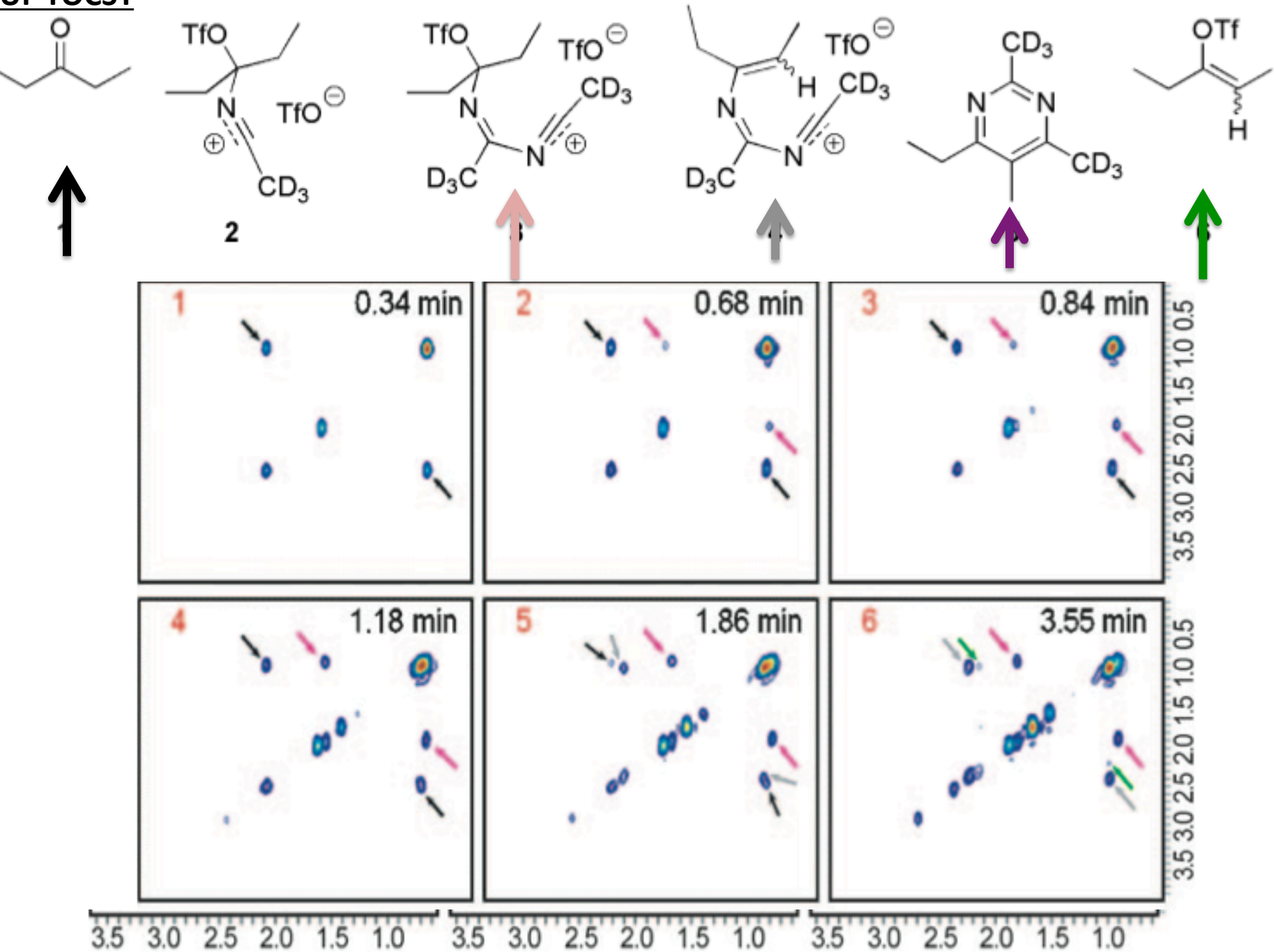
UF TOCSY



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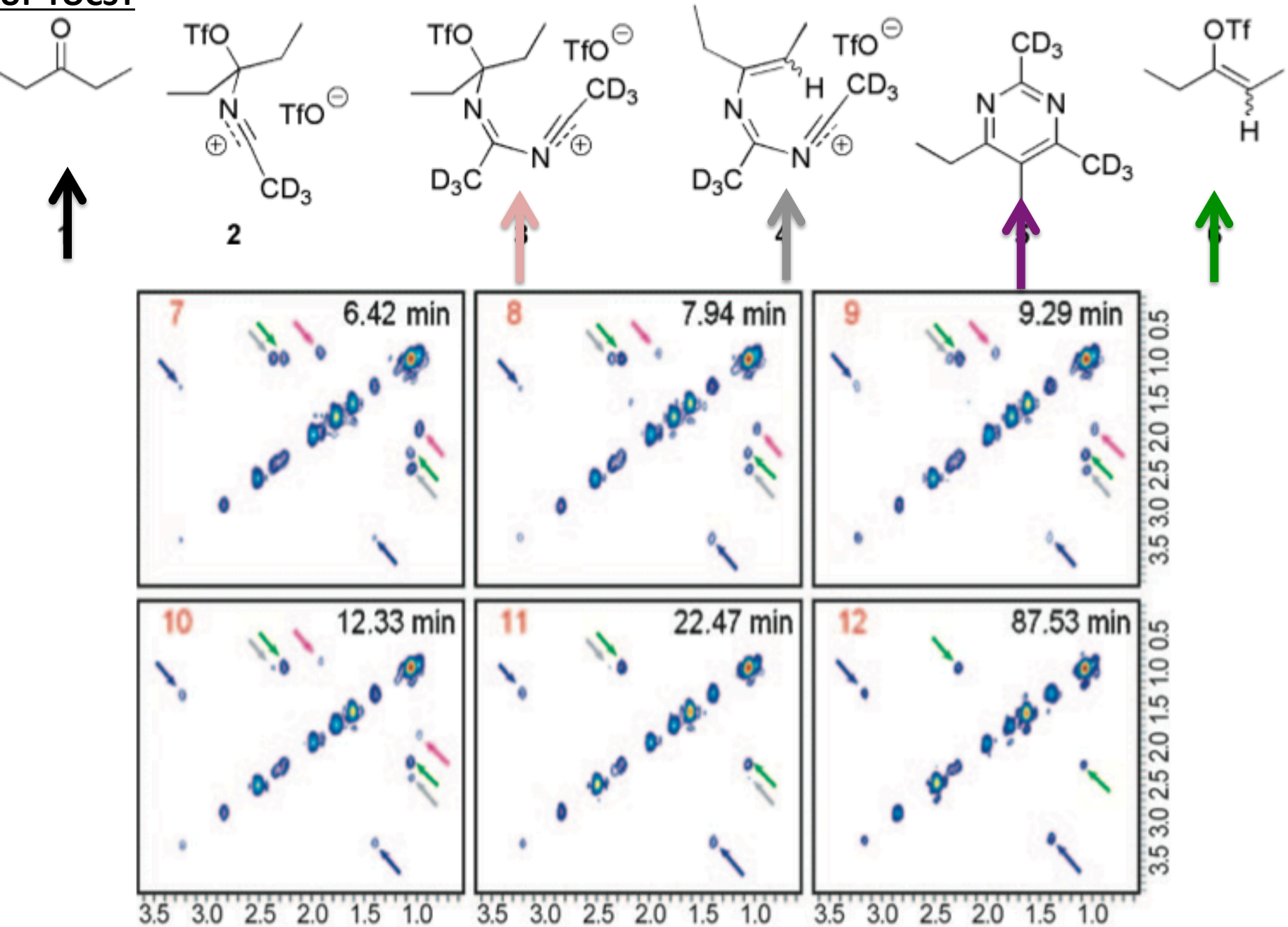

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Data from study

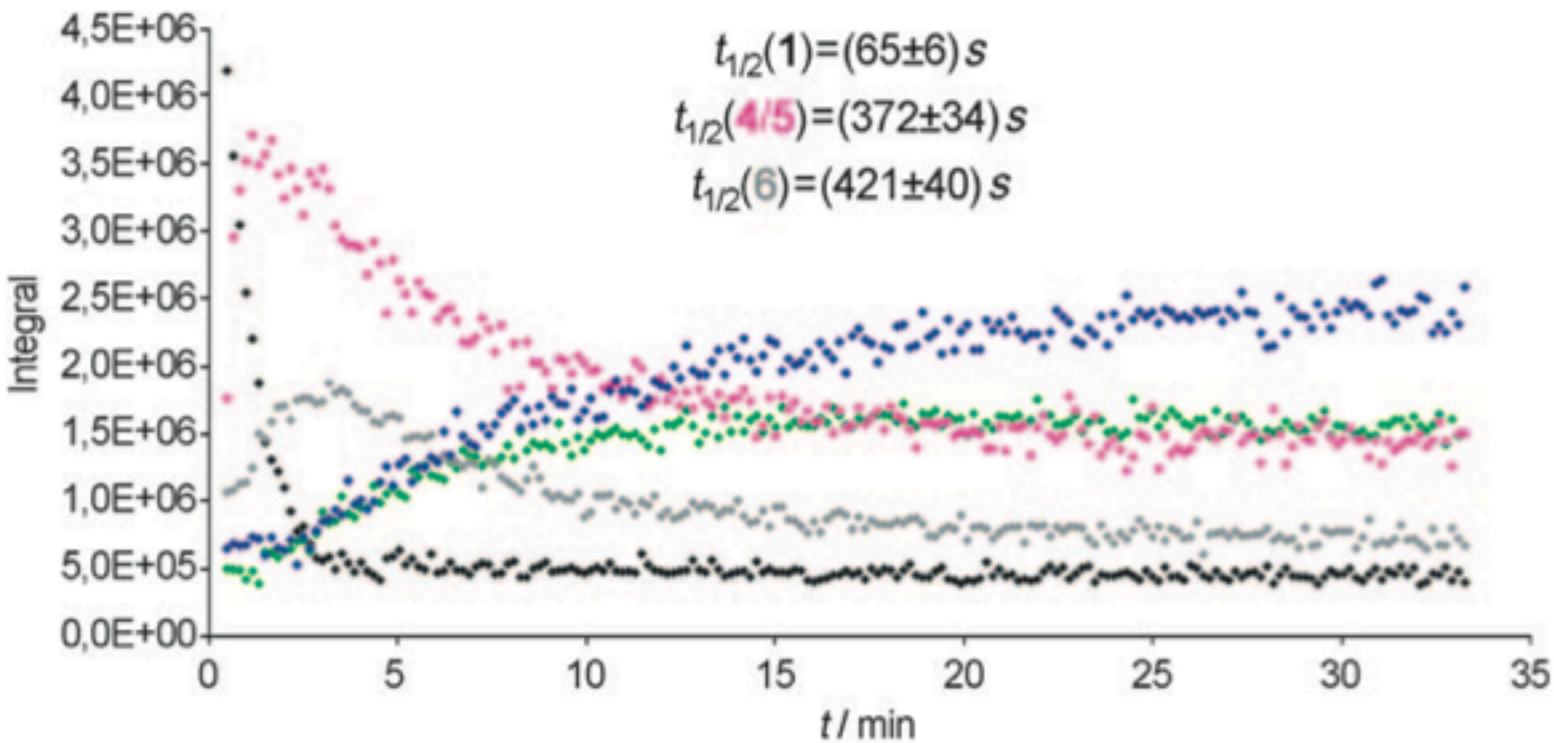

Figure 3. The averaged integrated peak intensity as a function of time for reactant **1** (◆), intermediates **4**, **5** (◆), and **6** (◆), and final products **3** (◆) and **7** (◆).

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NOESY: Nuclear Overhauser Effect

- NOESY-Detects through space interactions
- EXSY-Same as NOESY experiment except chemical exchange is observed
- ROESY-Is used to distinguish exchange from NOE

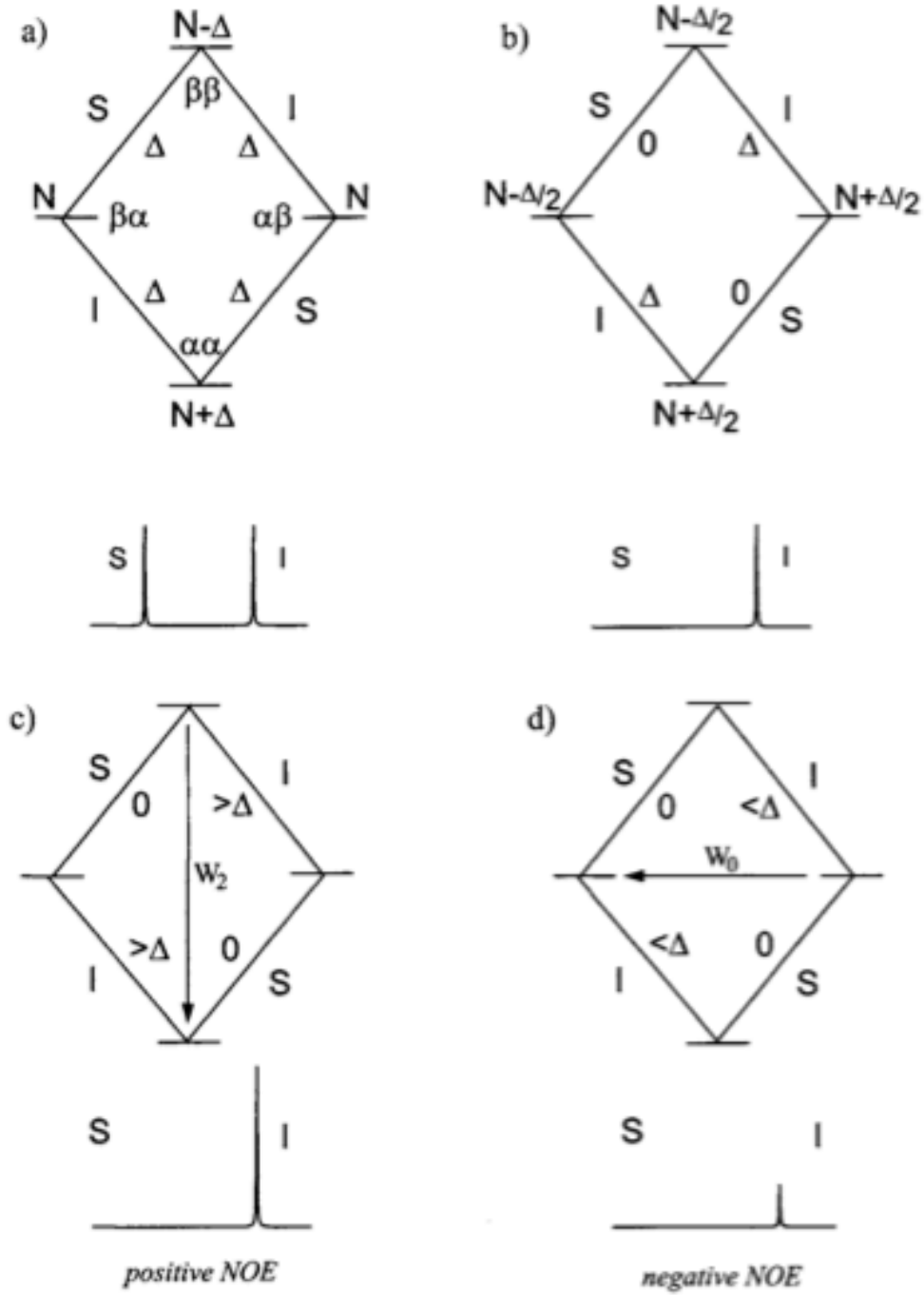
Where does NOE come from? (originate from dipolar coupling)

No coupling observed in solution due to rapid molecular movement

High-Resolution NMR Techniques in Organic Chemistry, Timothy D.W. Claridge, Elsevier Science Ltd, UK, 1999; Chapter 8

Where does the NOE come from?

Boltzmann distribution of spin states



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NOE: Nuclear Overhauser Effect

When a proton is saturated or inverted, spatially-close protons may experience an intensity enhancement which is termed Nuclear Overhauser Effect.

Small molecules NOE can be observed from about 4 angstroms apart
Large molecules NOE can be observed from about 5 angstroms apart

Molecular Weight and Maximum NOE

Maximum NOE depends on the molecular correlation time which (inverse rate of molecular tumbling) depends on the molecular weight and solvent viscosity.

1D vs 2D experiments

	1D	2D
Time	≅ (30 min) good	≅ 1.5 hr (good)
Information	irradiate one proton	all interactions observed
Spectra Crowding	<30 Hz apart is problem	no problem

Parameters needed for 2D NOESY:

1. Tune at desired temp. (Important!!)
2. PW 90°
3. Find T_1
4. Select mixing time (MOST Important)

Normally one is only interested in interactions.

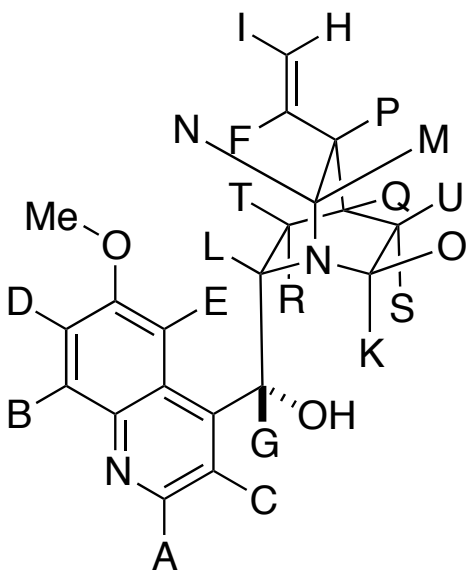
If one is interested in measuring bond distances mixing time is varied from experiment

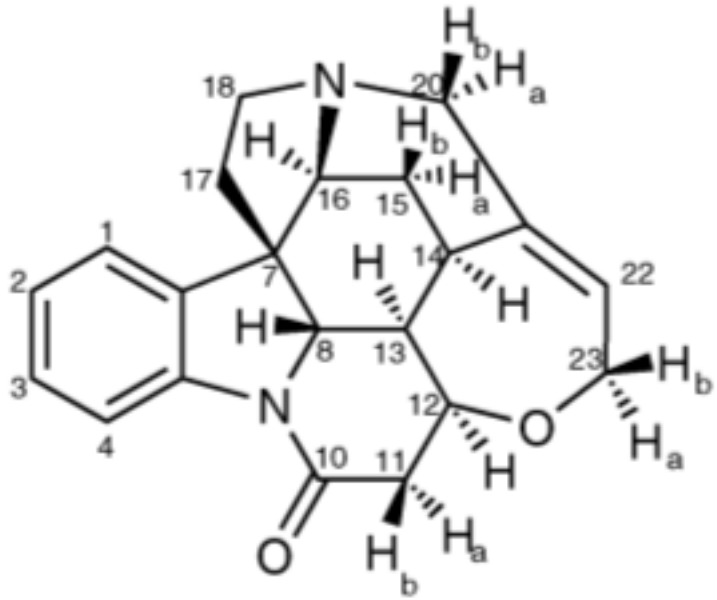
Suggested Mixing time

small molecules 0.5–1 sec
medium molecules 0.1–0.5 sec
large molecules 0.05–0.3 sec

Applications

Relative configuration of compounds
can measure bond distances



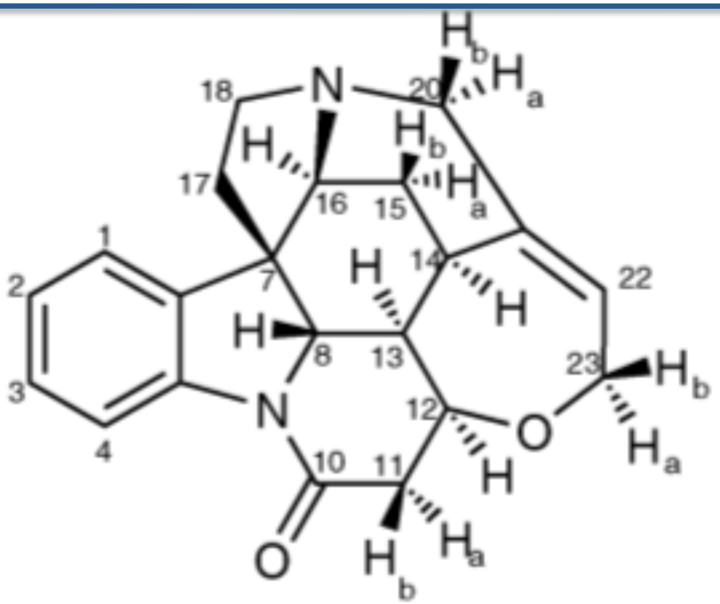


Butts, C., Jones, C., Harvey, J., *Chem. Comm.* **2011**, *47*, -1193

Applications

Relative configuration of compounds
can measure bond distances

Can identify conformations in solution
(also typically need calculation to help support)



Butts, C., Jones, C., Harvey, J., *Chem. Comm.* **2011**, *47*, -1193

2D EXSY: EXchange SpectroscopY and NOE and ROE

- Experiment is homonuclear
- pulse sequence is equivalent to NOESY
- Integration of cross peaks is related to the equilibrium constant
- When finding equilibrium constants multiple experiments are performed varying mixing time
- Technique has gained in popularity over the past few years in rate studies

<u>If it is the same how do we distinguish between chemical exchange and NOE?</u>

- You know you have interconverting species (EXSY)
- USE ROESY experiment

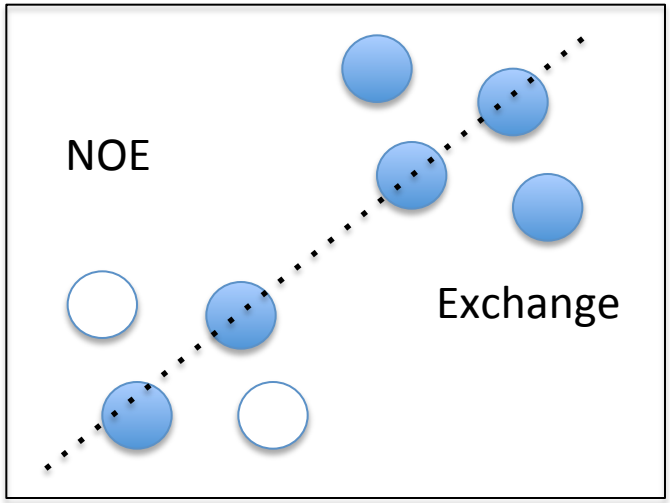
2D ROESY also (called CAMEL SPIN) the spinlock causes the spins to always be positive unlike in the NOE experiment. **This is used to distinguish NOE from exchange**.

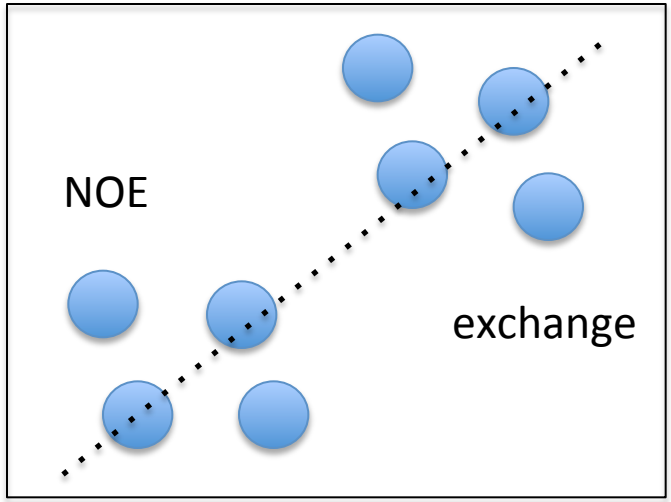
CAMEL SPIN: **C**ross-relaxation **A**ppropriate for **M**inimolecules **E**mulated by **L**ock **SPIN**s

Problem!
TOCSY cross peaks
can be observed

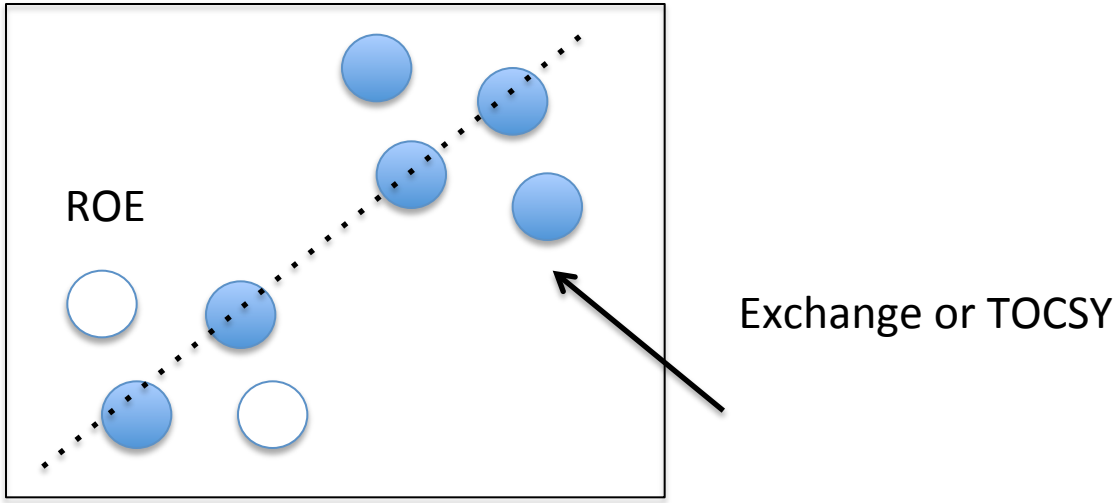
EXSY, NOESY and ROESY

NOESY
or
EXSY



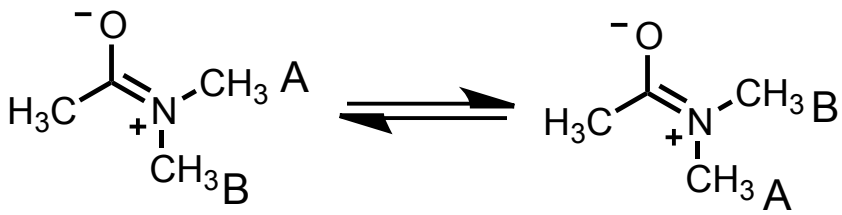


ROESY

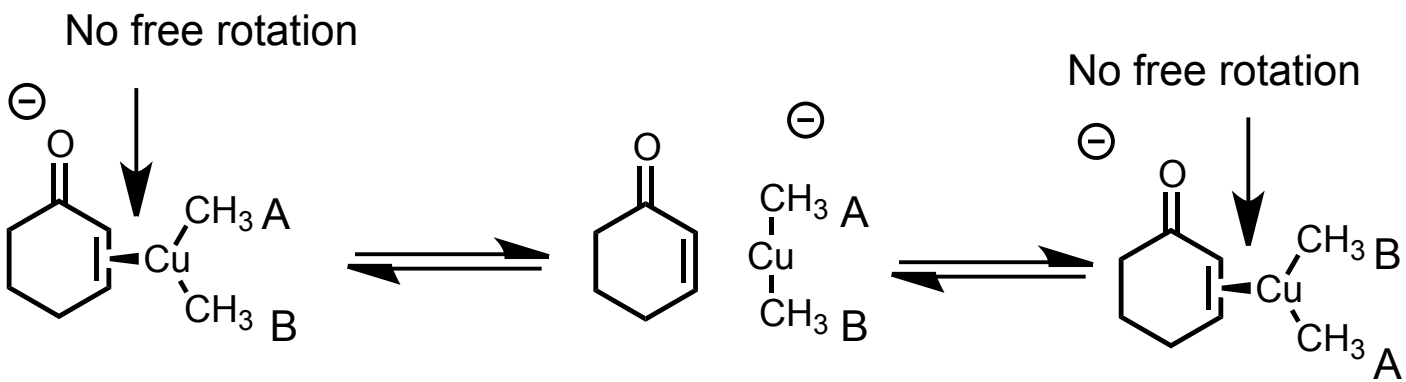


Chemical Exchange (examples)

Hindered rotation about a bond



Equilibrium



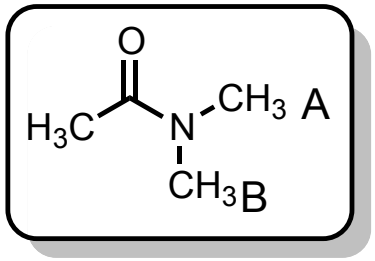
Rate of exchange can be measured by spin population inversion

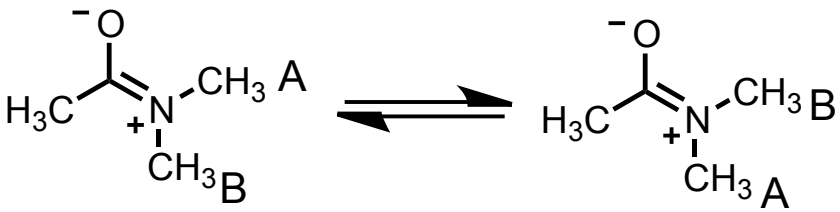
What types of experiments can be done?

1D–SPI: Spin Population Inversion ← Good for simple cases

2D–Exsy Exchange Spectroscopy

SPI: Spin Population Inversion





What does this experiment accomplish?

One can measure rate of exchange and can calculate activation barriers (ΔH and ΔS)

How is the experiment conducted?

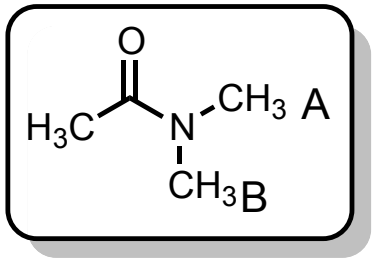
What information does one Need?

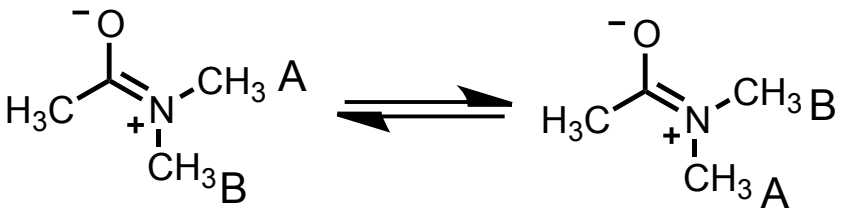
Probe tune

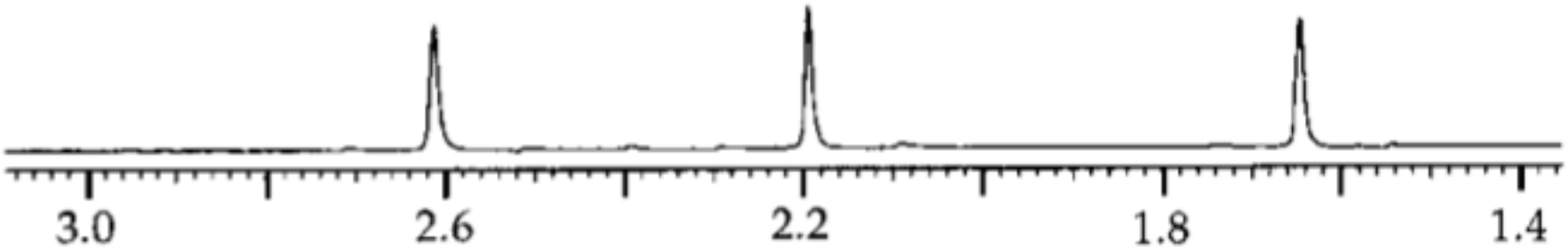
Need to obtain Pw 90 and T_1 values at the desired temperature

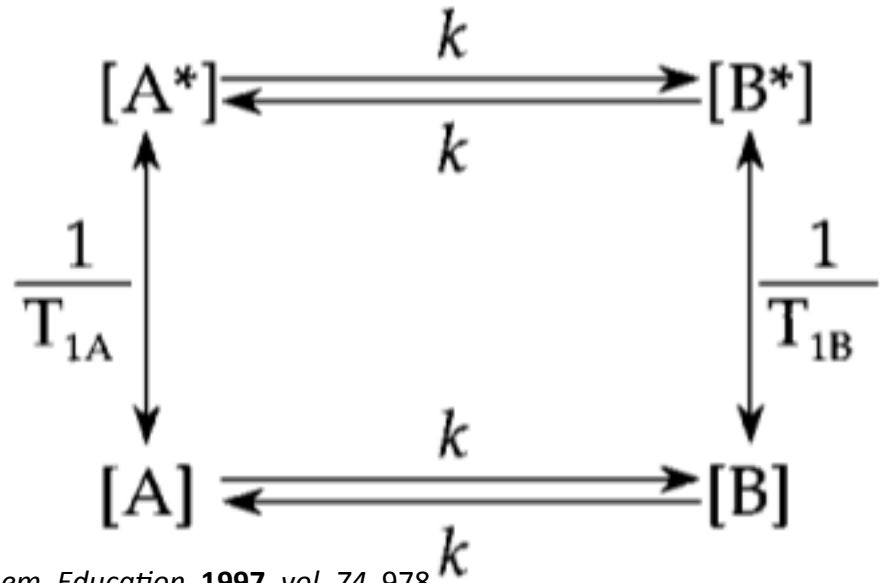
Jarek, R.L., Flesher, R.J., Shin, S.K., *J. Chem. Education.* **1997**, *vol. 74*, 978

SPI: Spin Population Inversion



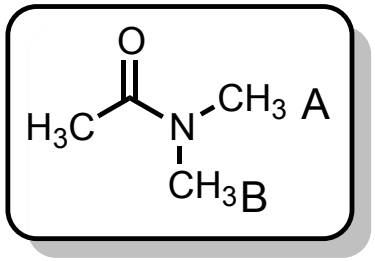


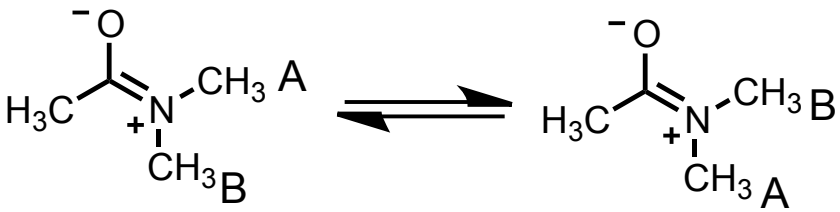


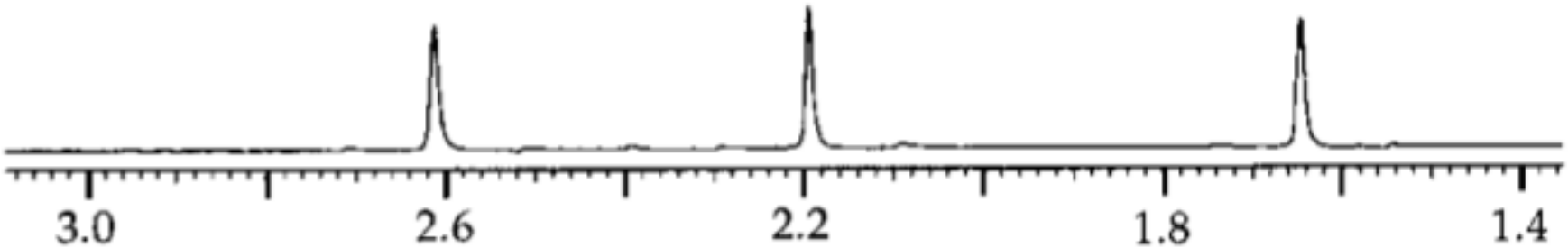


Jarek, R.L., Flesher, R.J., Shin, S.K., *J. Chem. Education.* **1997**, *vol. 74*, 978

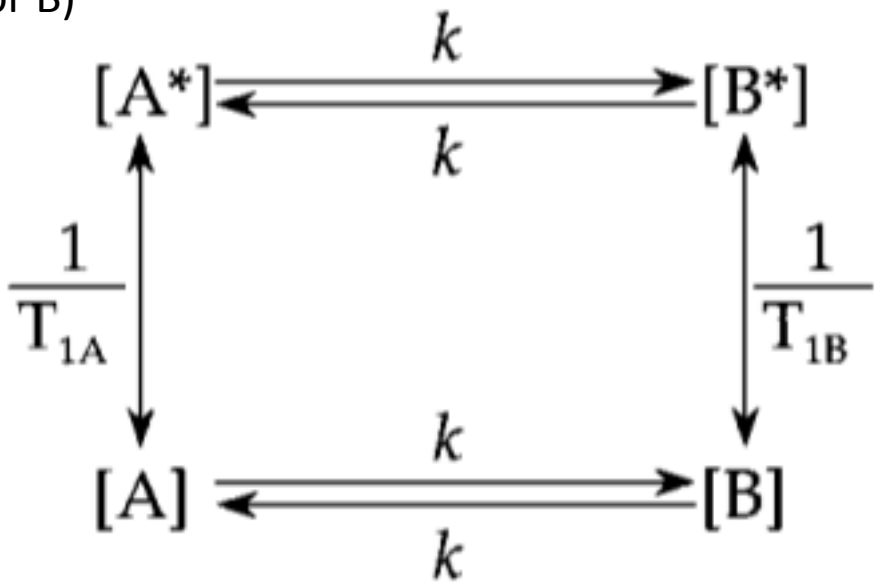
SPI: Spin Population Inversion







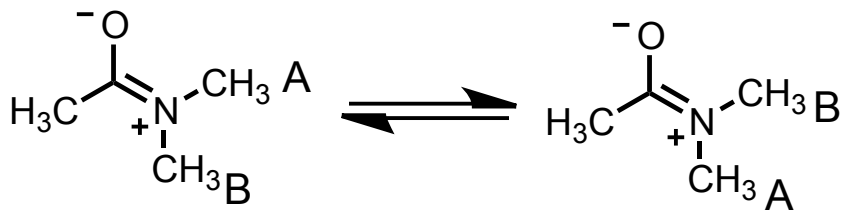
- [A] and [A*] lower and upper spin state (same for B)
- T_{1A} and T_{1B} are spin lattice reaction times
- M_A = [A]−[A*] is the net magnetization
- *k* is the mutual-site exchange site rate constant

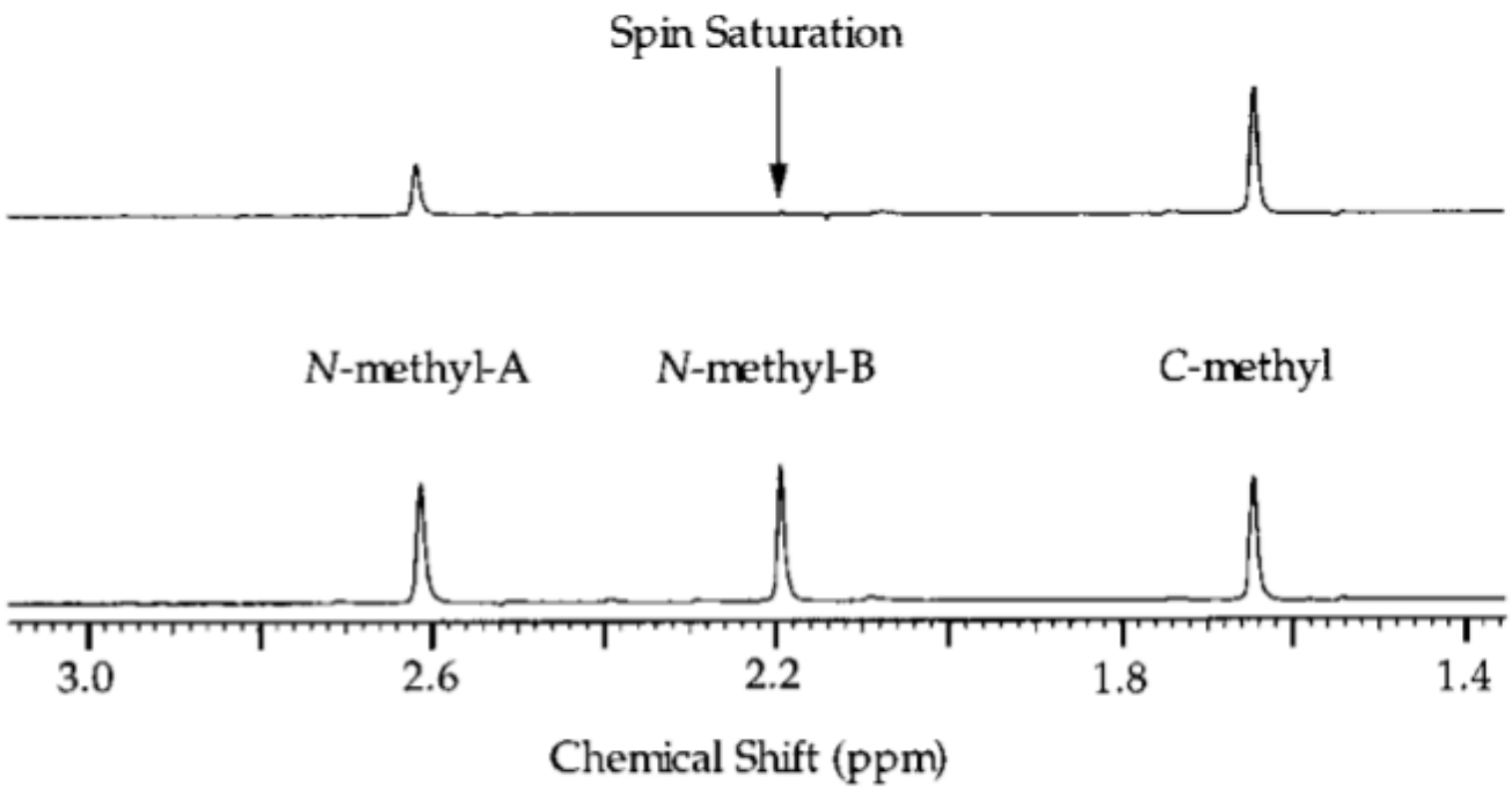


$$k = \frac{1}{T_{1A}}\left(\frac{M_{0A}}{M_A} - 1\right)$$

Jarek, R.L., Flesher, R.J., Shin, S.K., *J. Chem. Education.* **1997**, *vol. 74*, 978

SPI: Spin Population Inversion





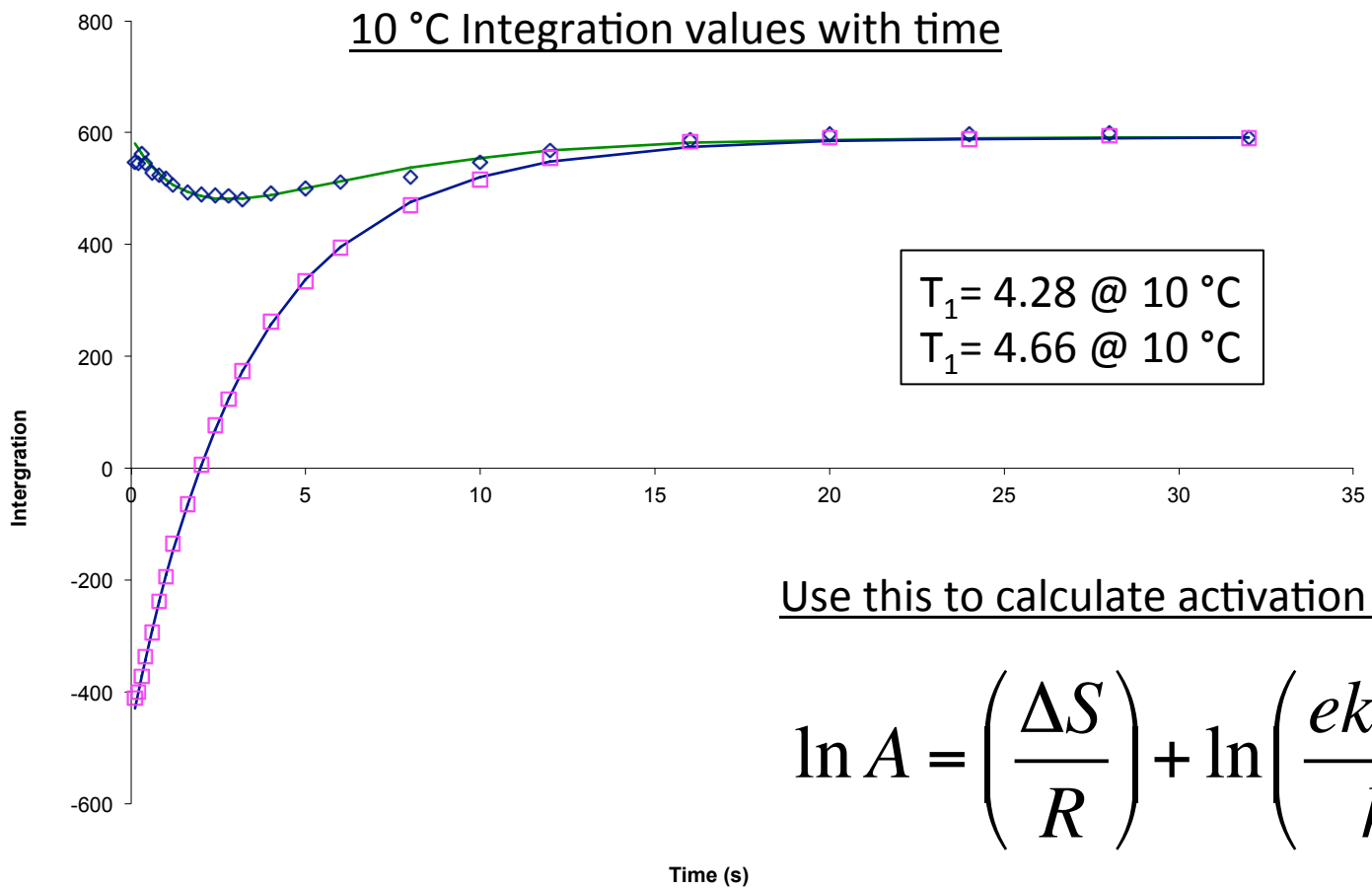
Jarek, R.L., Flesher, R.J., Shin, S.K., *J. Chem. Education.* **1997**, *vol. 74*, 978

SPI: Spin Population Inversion

1. Measure 90° Pulse at 10 °C
2. Measure T_1 for Me_A and Me_B
3. Invert the spin of Me_B
4. Repeat at different Temp

Use this to calculate k

$$M_A(\tau) = M_{0,A}\left\{1 - \left(\frac{100+X}{200}\right)(1-e^{-2k\tau})e^{-\tau/T_1}\right\}$$



Use this to calculate activation energy

$$\ln A = \left(\frac{\Delta S}{R}\right) + \ln\left(\frac{ek_B T}{h}\right)$$

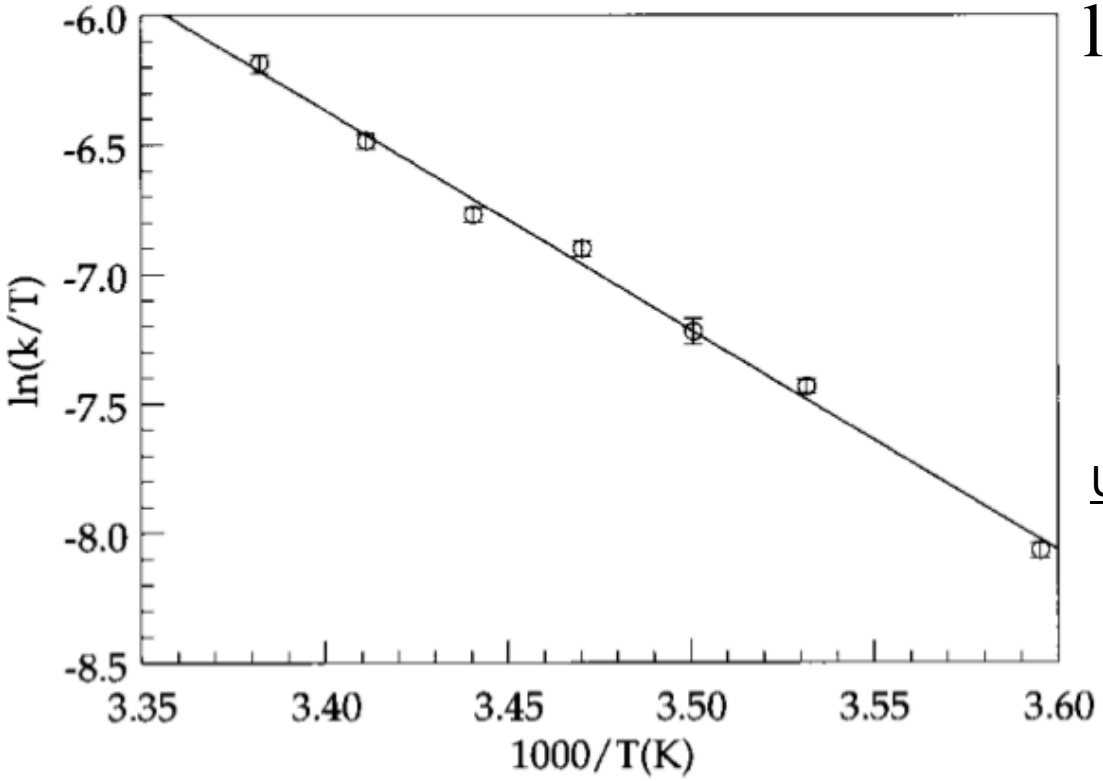
Jarek, R.L., Flesher, R.J., Shin, S.K., *J. Chem. Education.* **1997**, *vol. 74*, 978

SPI: Spin Population Inversion

T_1= 4.28 @ 10 °C
T_1= 4.66 @ 10 °C

Key Points

1. Simple technique
2. Exchange is on NMR time scale
3. How can this be used to study mechanism?
   (equilibrium constants, interconverting species)



$$\ln A = \left(\frac{\Delta S}{R}\right) + \ln\left(\frac{ek_B T}{h}\right)$$

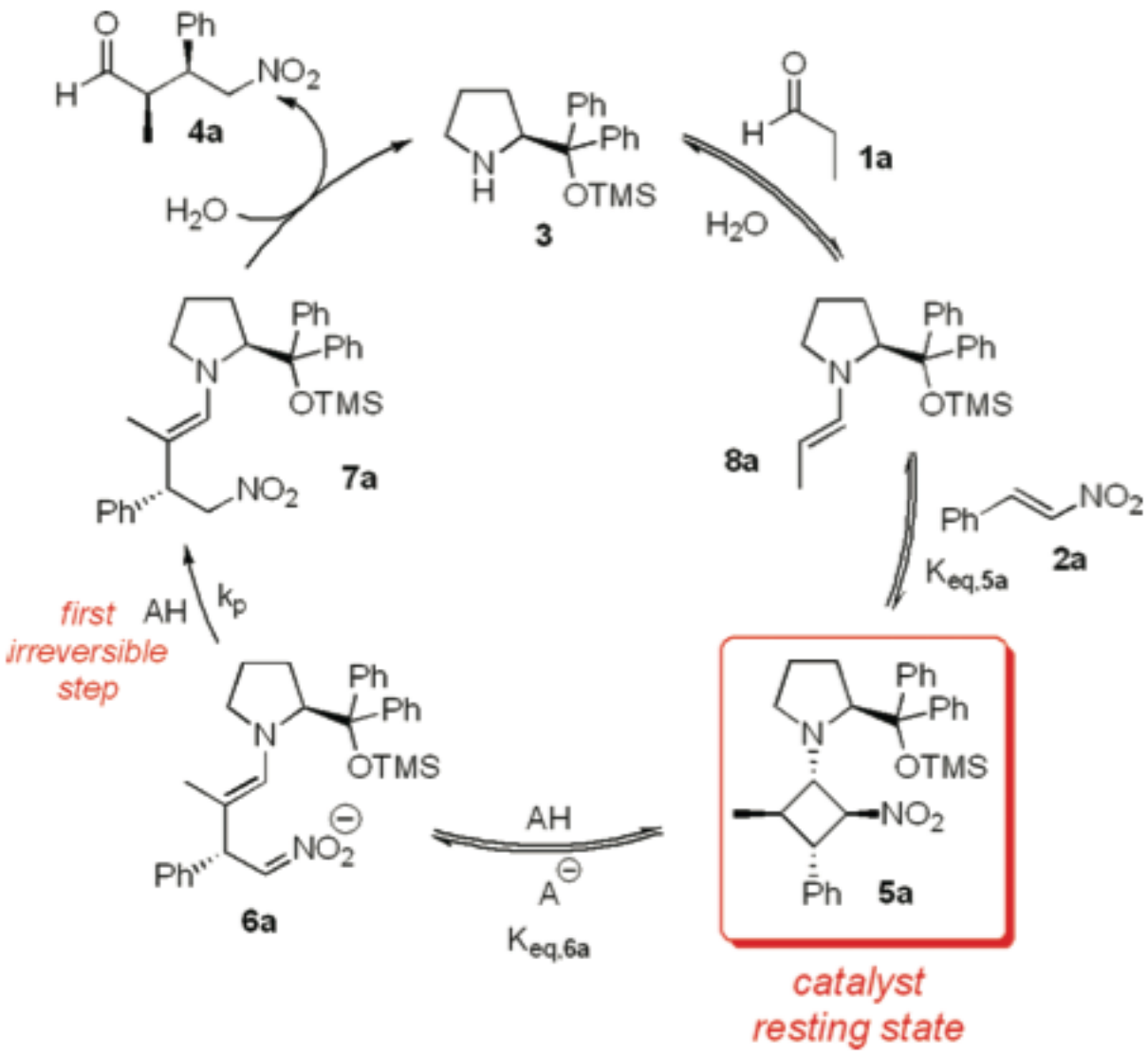
Plot of ln(k/t) vs 1000/T(K)

Use this to calculate activation energy

$$k = Ae^{-E_a/\ RT}$$

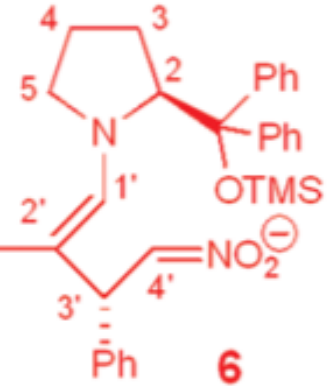
Jarek, R.L., Flesher, R.J., Shin, S.K., *J. Chem. Education.* **1997**, *vol. 74*, 978

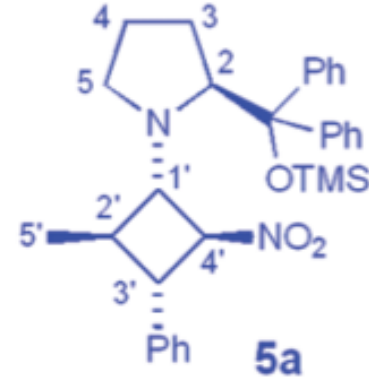
Donna Blackmond Chemistry

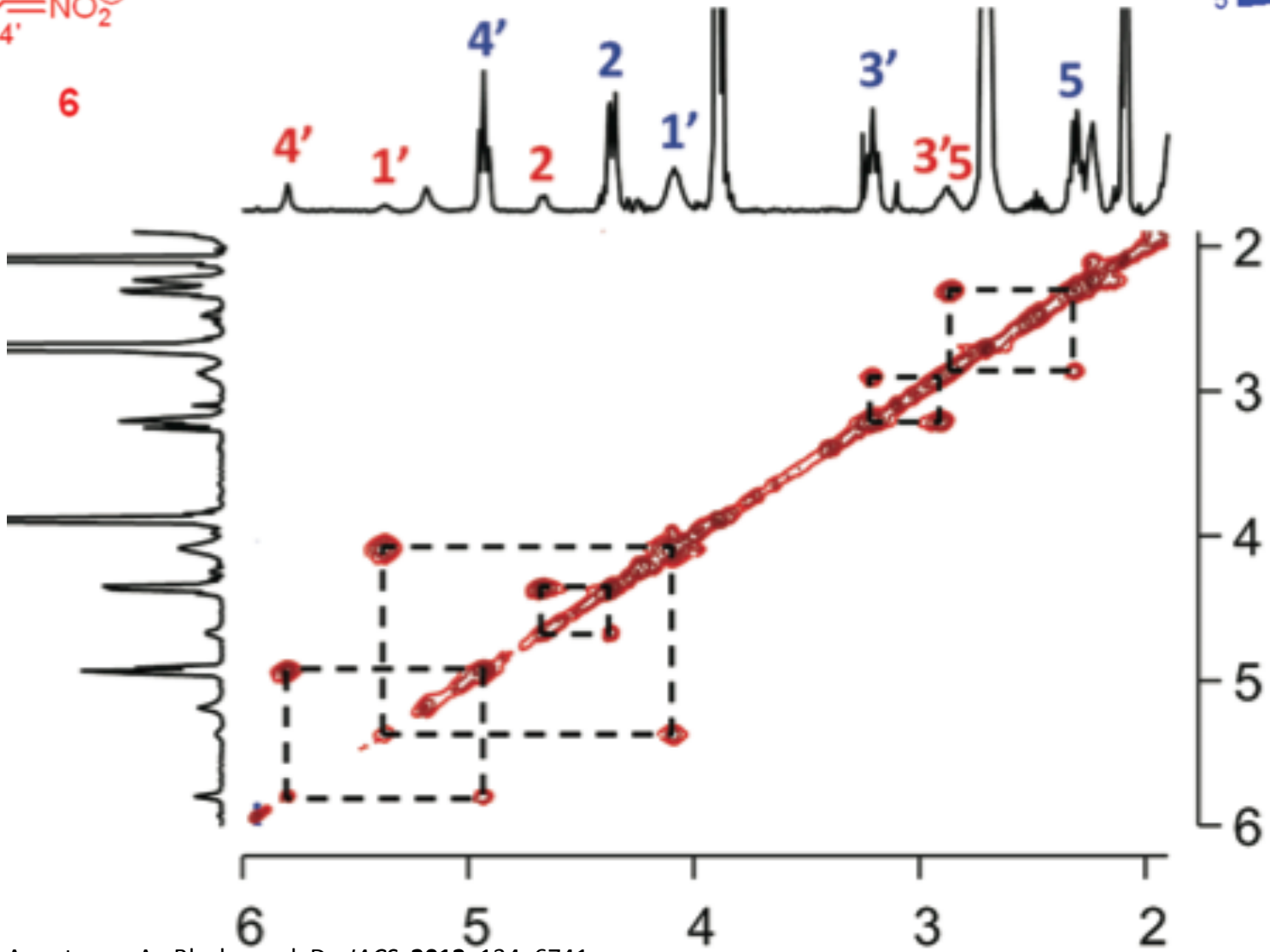


Bures, J., Armstrong, A., Blackmond, D., *JACS*, **2012**, 134, 6741

NOESY(EXSY) from Blackmond Work

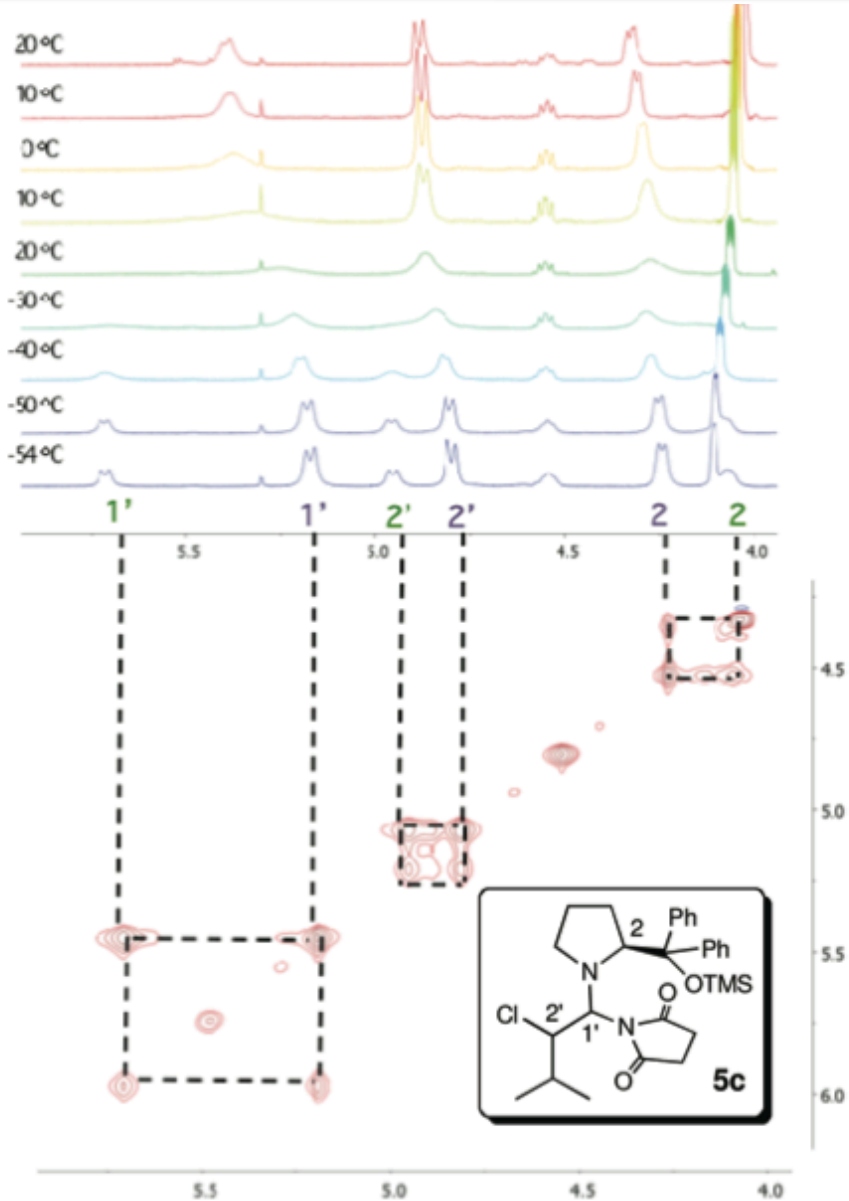






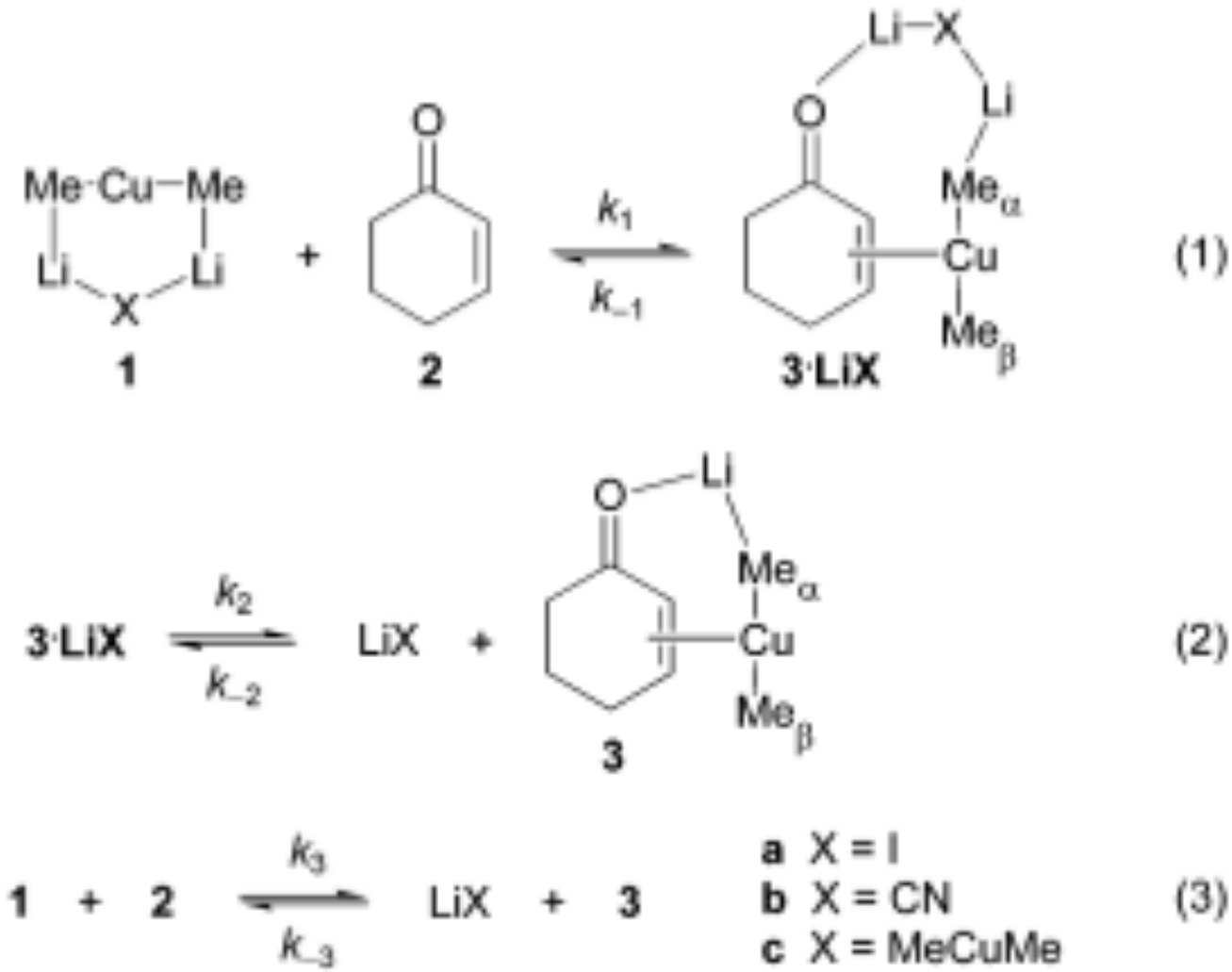
Bures, J., Armstrong, A., Blackmond, D., *JACS*, **2012**, 134, 6741

EXSY experiment (and different temp)



Bures, J., Armstrong, A., Blackmond, D., *JACS*, **2012**, 134, 6741

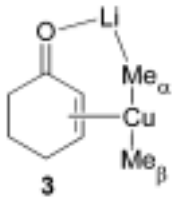
EXSY Impact on kinetics

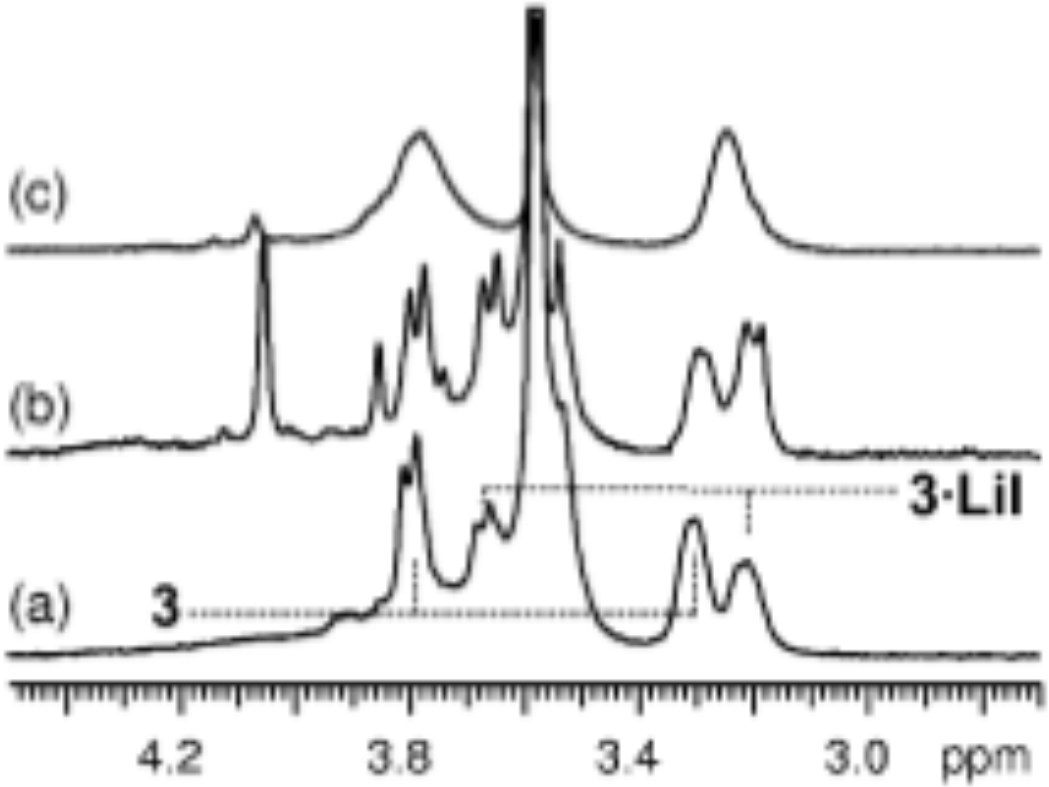


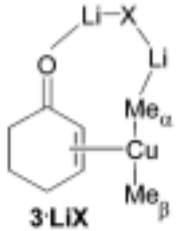
k_1=2.0 $M^{-1}s^{-1}$, k_{-1}=0.085s^{-1}; k_2=0.49 s^{-1}, k_{-2}=28 $M^{-1}s^{-1}$; k_3=k_{-3}=0

Bertz, Carlin, Deadwyler, Murphy, Ogle and Seagle J. Am. Chem. Soc. (2002), 124(46), 13650-1.

Proposed Structure

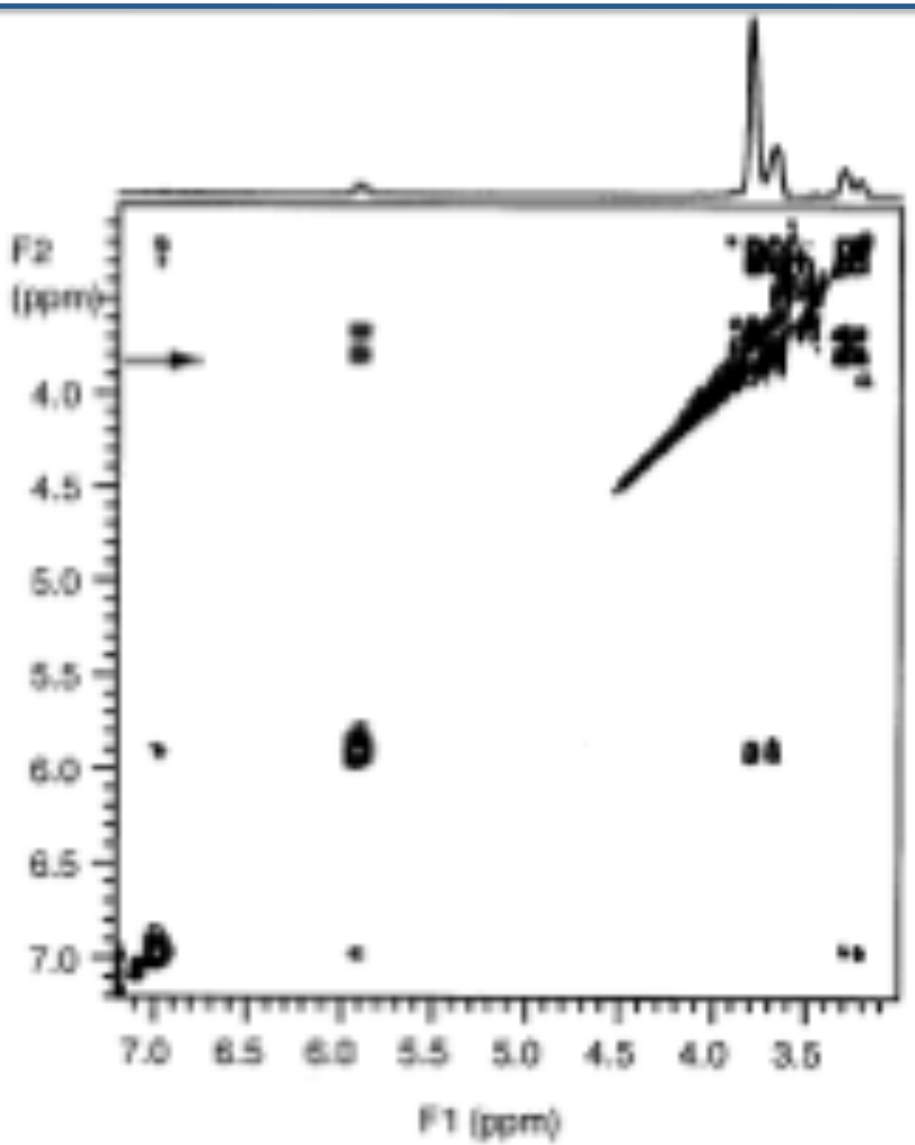


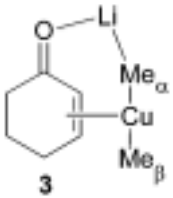


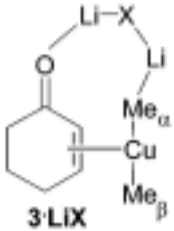


Bertz, Carlin, Deadwyler, Murphy, Ogle and Seagle J. Am. Chem. Soc. (2002), 124(46), 13650-1.

EXSY Data

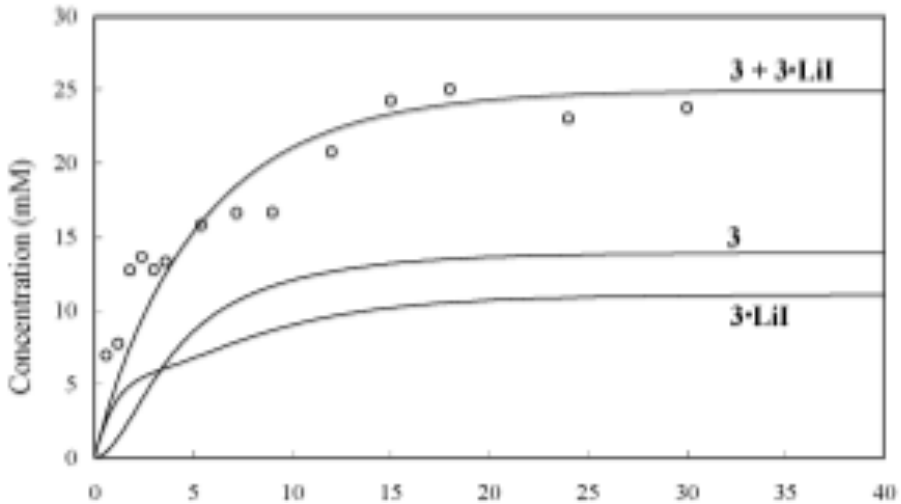


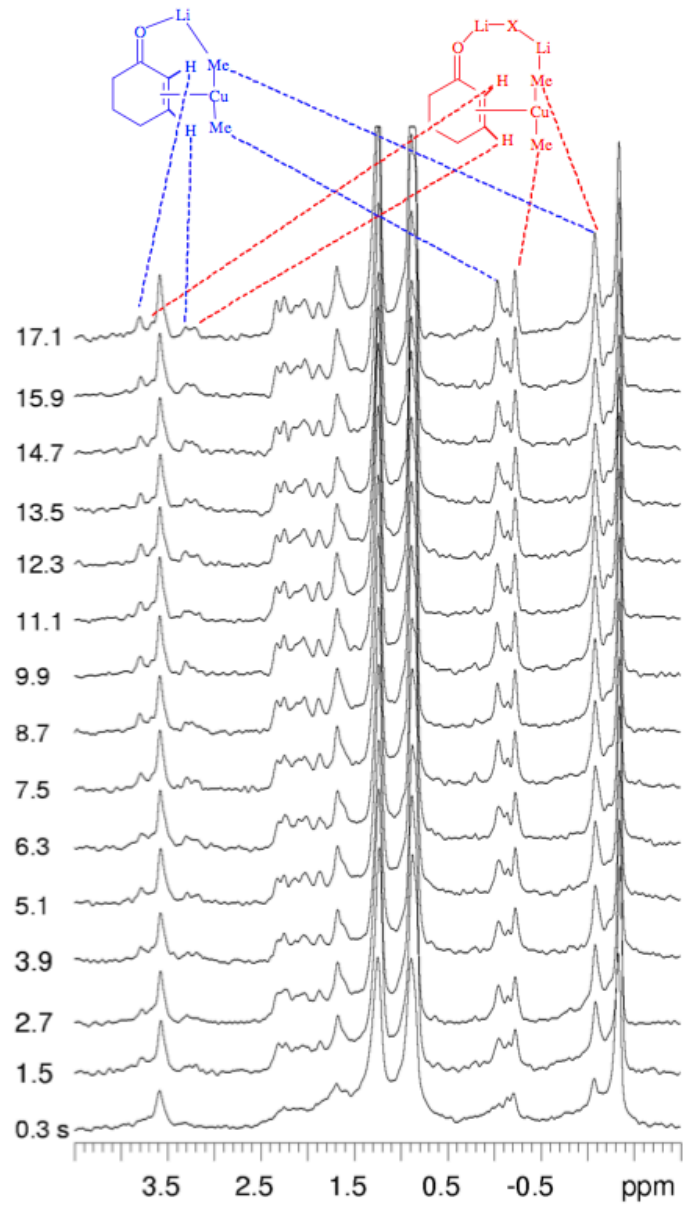




Bertz, Carlin, Deadwyler, Murphy, Ogle and Seagle J. Am. Chem. Soc. (2002), 124(46), 13650-1.

Reaction of cyclohexenone with $Me_2CuLi{\cdot}LiI$ at –100°C: RI vs. EXSY





The rapid-injection ^{1}H NMR data points for the reaction of cyclohexenone with lithiumdimethylcuprate at –100 °C, where the curves for the predicted rates of appearance of π-complexes are calculated from the EXSY rate constants.

Bertz, Carlin, Deadwyler, Murphy, Ogle and Seagle J. Am. Chem. Soc. (2002), 124(46), 13650-1.

Conclusions

- COSY, TOCSY, HMQC, HSQC, HMBC (great structure determination methods)
  - New Ultra fast methods can be used to gain insight into mechanism
- EXSY, NOESY, HOESY, equilibrium constants, aggregation and bond distances can be measured
- ROESY can distinguish between exchange and NOE