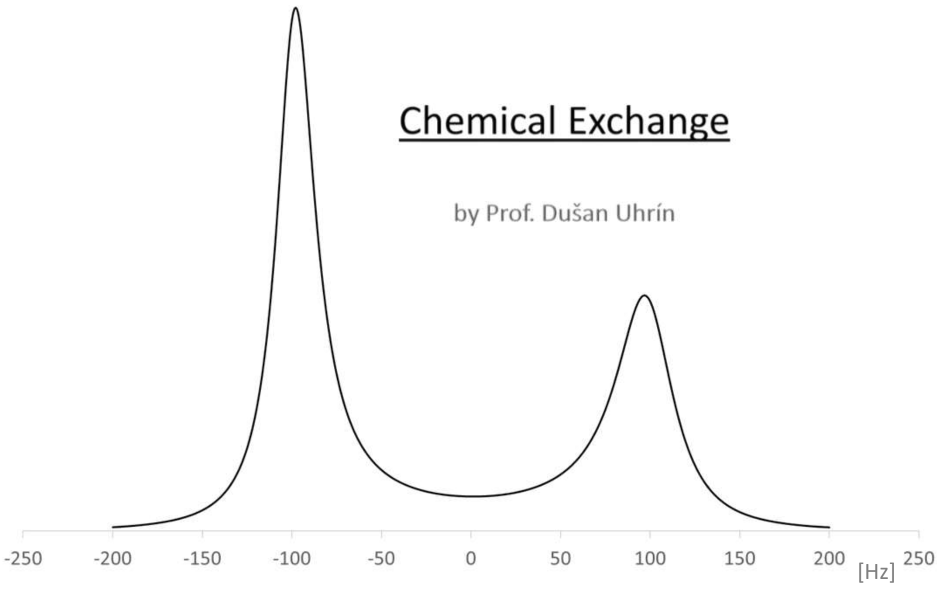
Chemical Exchange

by Prof. Dušan Uhrín



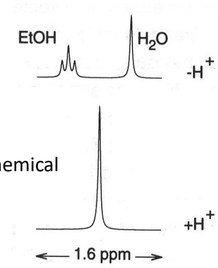
2024 SNUG Postgraduate Course in NMR Spectroscopy
University of Edinburgh, 3rd of December, 2024

What come first to mind when hearing "chemical exchange" ?

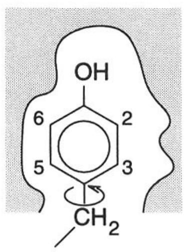
Example I
Intermolecular exchange
Exchange of labile protons

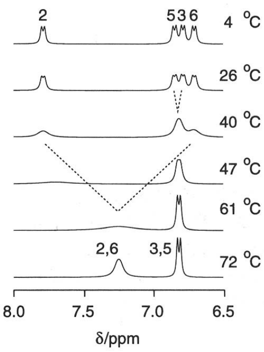
OH in ethanol/water without a catalyst (H^+)

With a catalyst (H^+)
- averaging of chemical shifts
- quenching of *J* coupling by chemical exchange



Example II. Hindered rotation of a tyrosine side chain in the core of a protein.

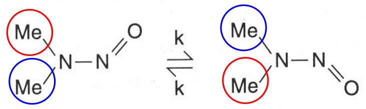




Ch.E. - change of the environment while NMR signal is measured

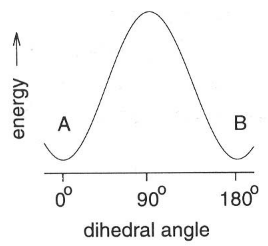
Dynamic equilibrium between two conformations with equal energy, or a **symmetrical two-site exchange**:

Dimethylnitrosamine



$$A \underset{k_{BA}}{\overset{k_{AB}}{\rightleftharpoons}} B$$

Temperature affects the rate constant of the exchange



$k_{ex} = k_{AB} + k_{BA}$ [s^{-1}] (in many equations describing exchange)

Average lifetime: $\tau_A = 1/k_{AB}$ [s]
$\tau_B = 1/k_{BA}$ [s]

Effects of the N=O group is different on the two Me groups – in a rigid structure they have different chemical shifts

Rotation around the N-N bond is slowed down because of the conjugation of the free electron pairs of N and N=O electrons

The effect of chemical exchange on NMR spectra

NMR spectra for two nuclei exchanging between two sites with equal population

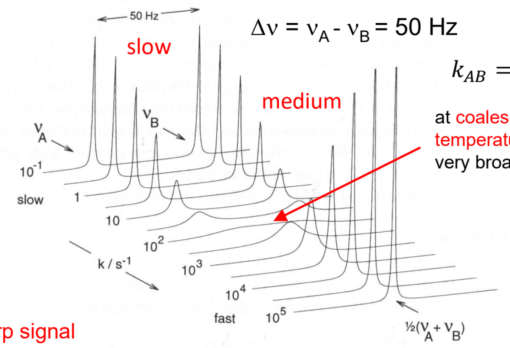
$$A \underset{k_{BA}}{\overset{k_{AB}}{\rightleftharpoons}} B$$

| $\nu_A - \nu_B$ | >> k, two sharp signals

k ~ | $\nu_A - \nu_B$ |, signals broaden, eventually merge

k >> | $\nu_A - \nu_B$ |, signals sharpen eventually one sharp signal

$\Delta\nu = \nu_A - \nu_B = 50$ Hz



$$k_{AB} = \frac{\pi\Delta\nu}{\sqrt{2}}$$

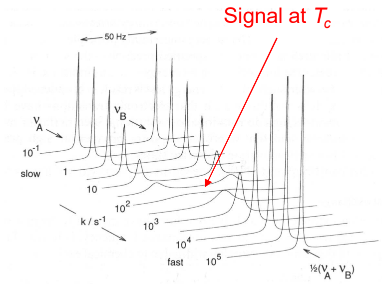
at coalescence temperature – very broad signal

The process is slow (two signals), intermediate (broad signals) or fast (one signal) on the chemical shift time scale depending on the relation between $\Delta\nu$ and k.

Change of temperature or B_o can affect the chemical exchange regime.
1. Temperature affects k. Increase of T increases k = move towards fast exchange.
2. B_o affects $\Delta\nu$ (in Hz). Increase of B_o increases $\Delta\nu$ = move towards slow exchange.

How can we analyse exchange spectra?

The appearance of the exchange spectra depends on:



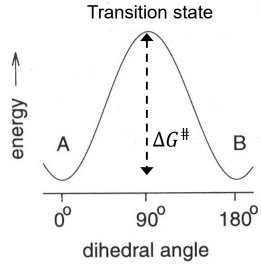
- Chemical shift of individual species
- Rate constants of the exchange
- Population of exchanging species

Full line shape analysis

- general and most powerful
- suitable for k_{AB} ~ 10^1 to 10^4 s^{-1}

Approximate solutions

- valid only in certain regimes, be careful!
- (e.g. Abergel D, and Palmer, A.G. *ChemPhysChem* 2004, **5**, 787-793)



At coalescence temperature, T_c, (when signals merge into one):

$$k_{cA} = \frac{\pi \Delta \upsilon}{\sqrt{2}}$$

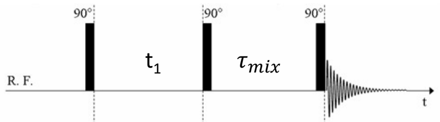
$$\Delta G^{\#} = RT_c \left[23.76 + ln\left(\frac{T_c}{\Delta \upsilon}\right)\right] kJmol^{-1}$$

T_c – coalescence temperature

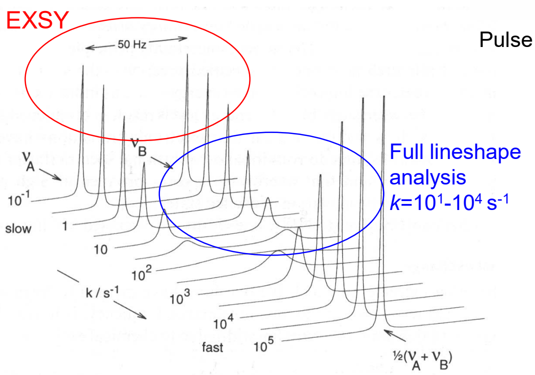
$\Delta\nu$ - difference between individual chemical shifts

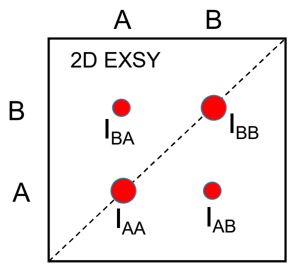
2D Exchange spectroscopy (EXSY)

k_{AB} 10^{-2} to 10^2 s^{-1} EXSY can investigate much slower processes than the line shape analysis.



Pulse sequence identical to NOESY





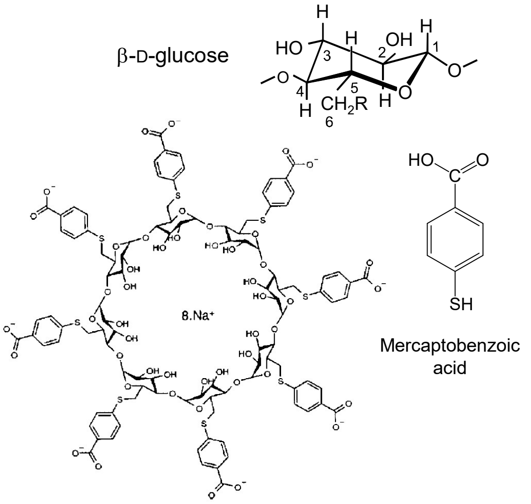
Rate constant from peak intensities

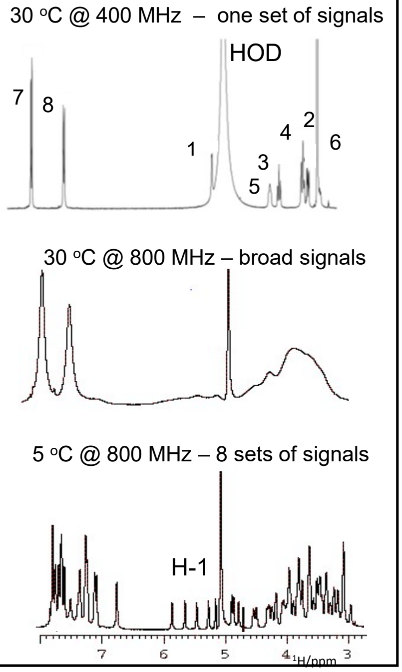
$$k_{AB} = \frac{1}{\tau_{mix}} ln \frac{r+1}{r-1}$$

$$r = \frac{(I_{AA} + I_{BB})}{(I_{AB} + I_{BA})}$$

Perrin, C.L. and Dwyer T.J. *Chem. Rev.* 1990, 90, 6, 935–967

Derivative of γ cyclodextrin





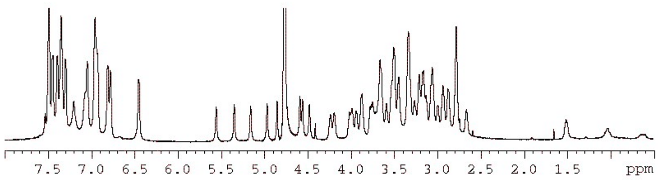
Inclusion of mercaptobenzoic acid at any time into the cyclodextrin cavity breaks the symmetry.

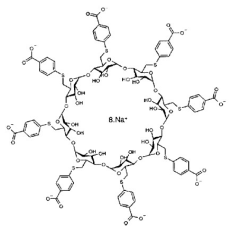
Instead of one set of signals, eight sets per each of the proton type are observed.

EXSY (Chemical EXchange SpectroscopY) – NOESY pulse sequence

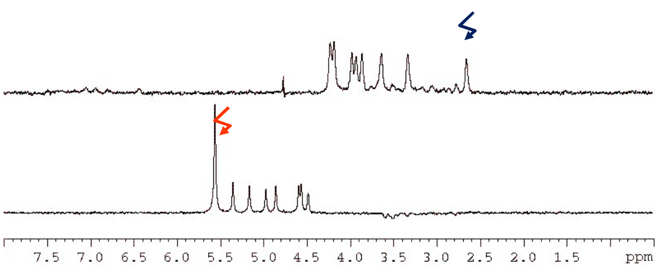
Correlates spins that exchange between different chemical environments

1D ^{1}H NMR spectrum





1D EXSY spectra of **I** at 800 MHz and 5 °C

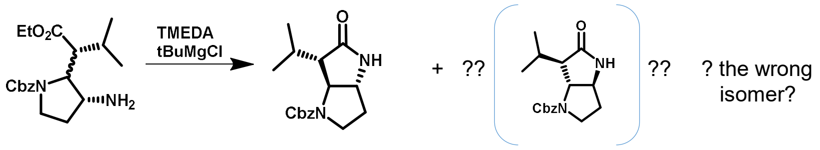


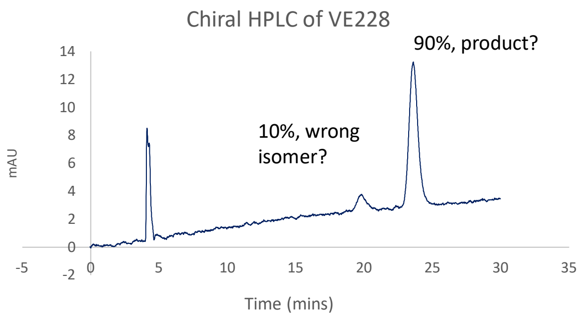
Eight-site exchange
H-4 protons

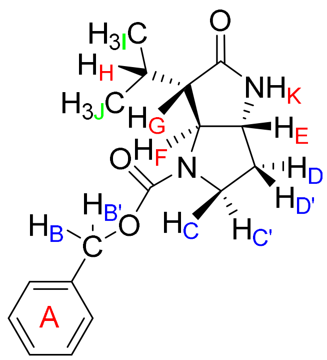
Eight-site exchange
H-1 protons

Cameron, K.S., Fielding, L., Palin, V., Uhrín, D, *Magn. Reson. Chem.* **43,** 647-653 (2005)

Troubles with cyclisation

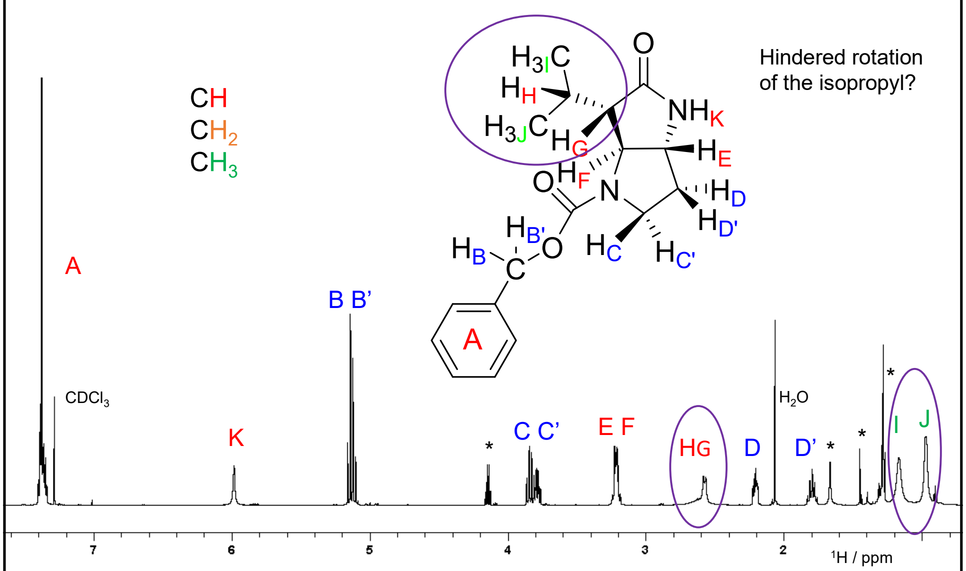




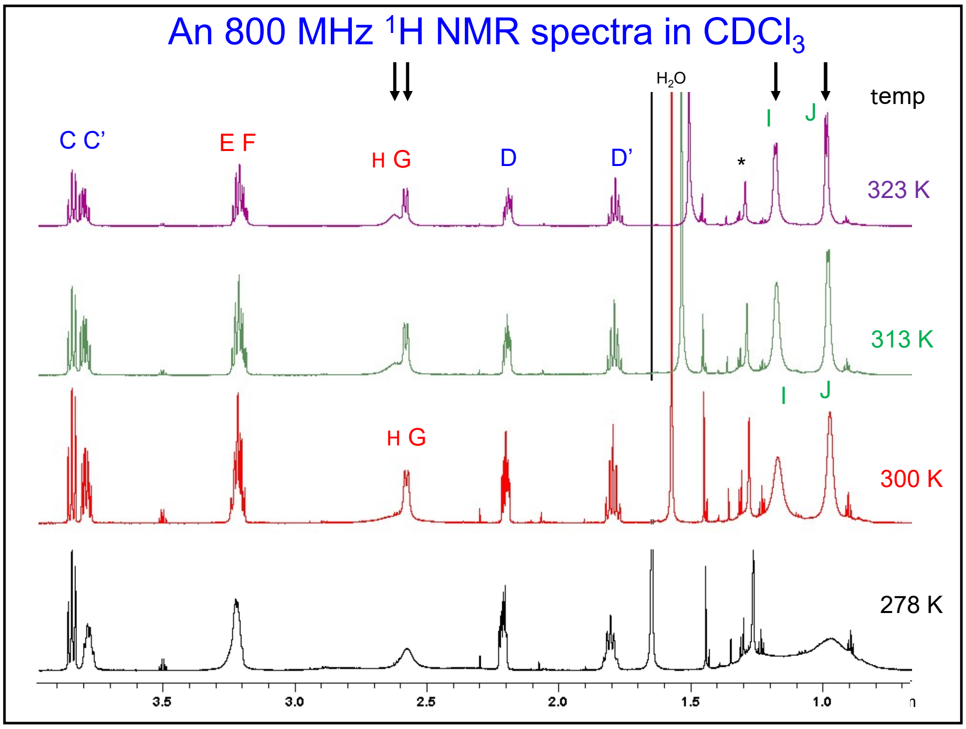


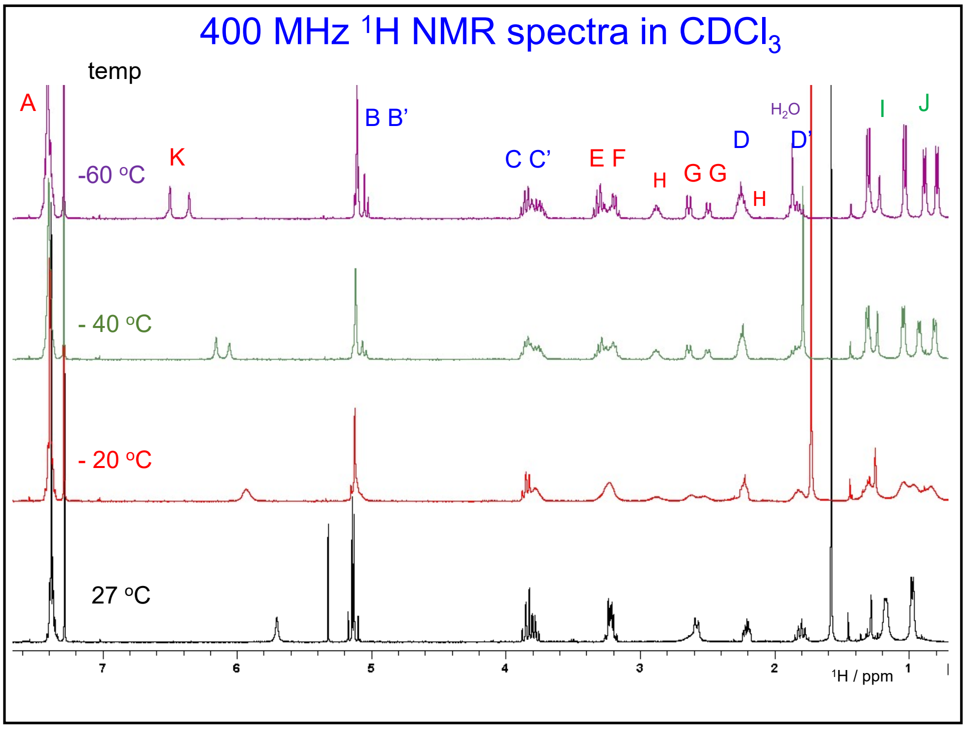
Courtesy of Vikki Evans

A 600 MHz ^{1}H NMR spectrum at 300 K in $CDCl_3$

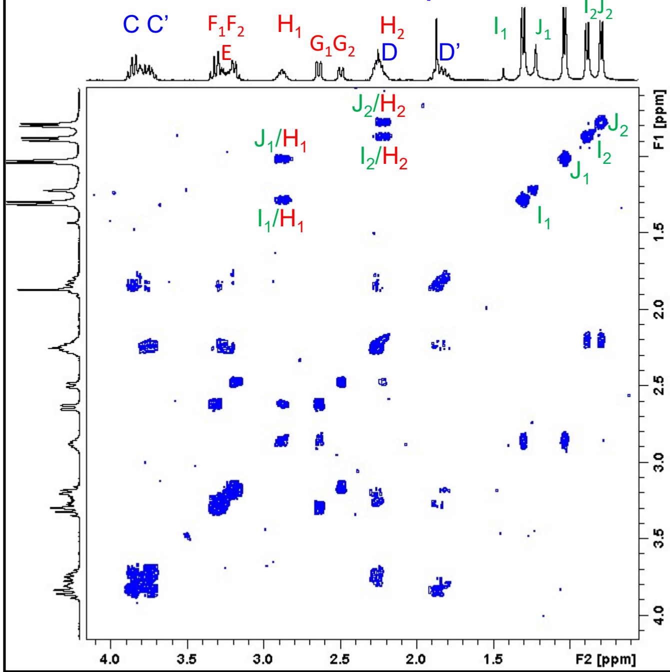


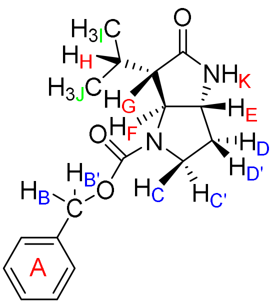
* impurities


An 800 MHz 1H NMR spectra in CDCl3
temp
H2O
C C'
E F
H G
D
D'
*
I
J
323 K
313 K
H G
I
J
300 K
278 K
3.5
3.0
2.5
2.0
1.5
1.0


400 MHz 1H NMR spectra in CDCl3
temp
A
K
B B'
C C'
E F
H
G G
D
H
H2O
D'
I
J
-60 oC
- 40 oC
- 20 oC
27 oC
7
6
5
4
3
2
1H / ppm
1

A 400 MHz COSY spectrum at -60 °C in $CDCl_3$

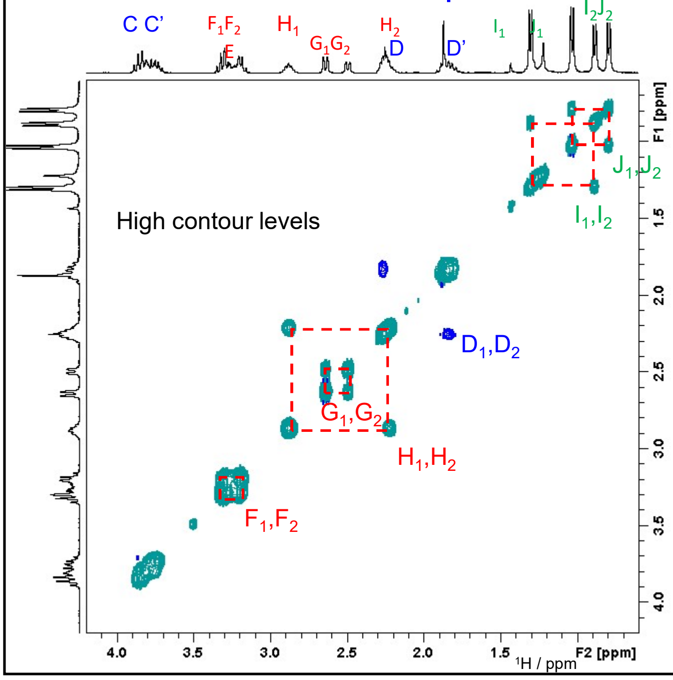


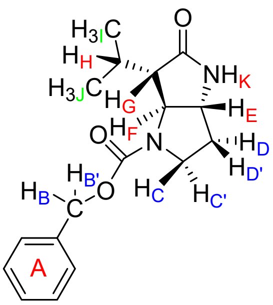


No X_1,X_2 cross peaks (X = F, G, H, I, J)

No COSY cross peaks between different rotamers!

A 400 MHz NOESY spectrum at -60 °C in $CDCl_3$

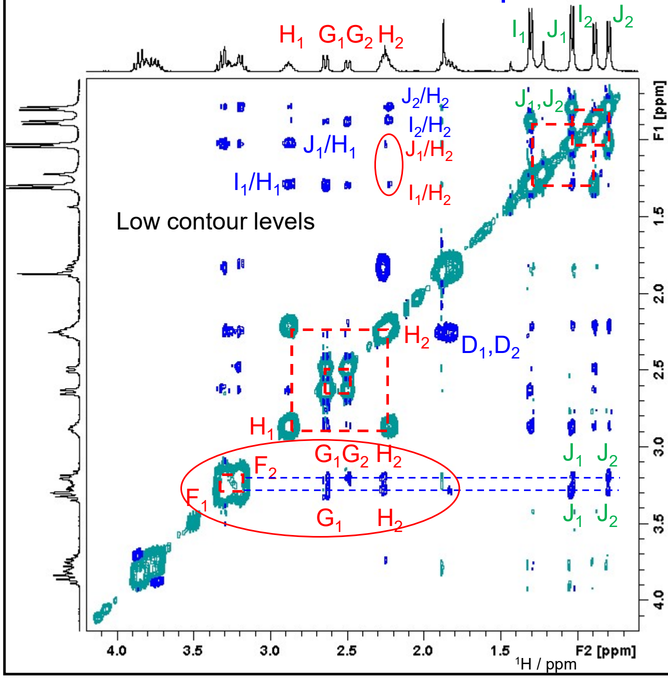


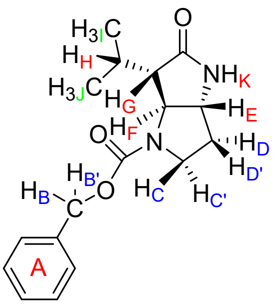


Intense chemical exchange negative cross peaks – turquoise, e.g. I_1I_2 or H_1H_2.

Positive through space NOESY cross peaks – blue, e.g. D_1,D_2.

A 400 MHz EXSY/NOESY spectrum at -60 ^{o}C in $CDCl_3$



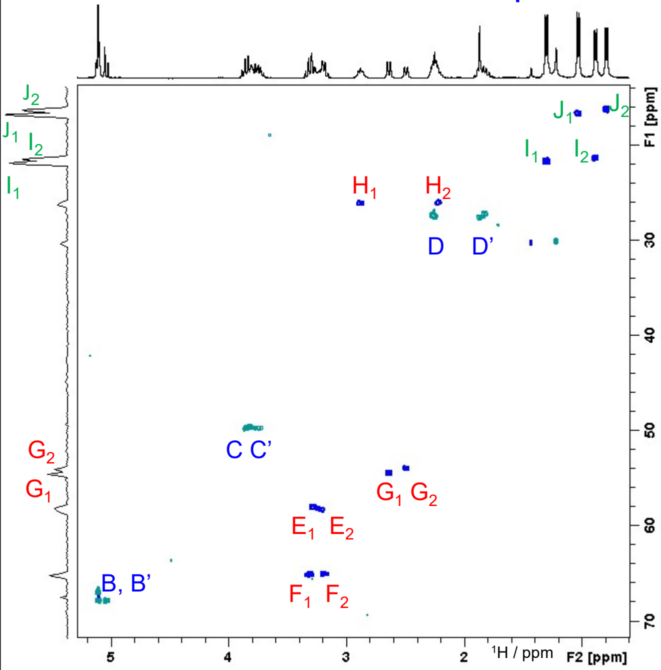


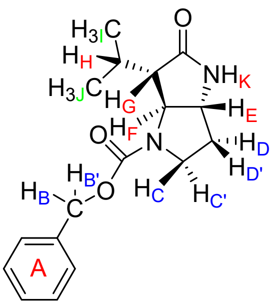
Negative (chemical exchange) cross peaks - turquoise

Positive (through space) cross peaks – blue

Positive cross peaks also appear between different rotamers, e.g. FGH cross peaks. This would also be the case for 2D TOCSY

A 400 MHz 2D ^{1}H, ^{13}C HSQC spectrum at -60 ^{o}C in $CDCl_3$





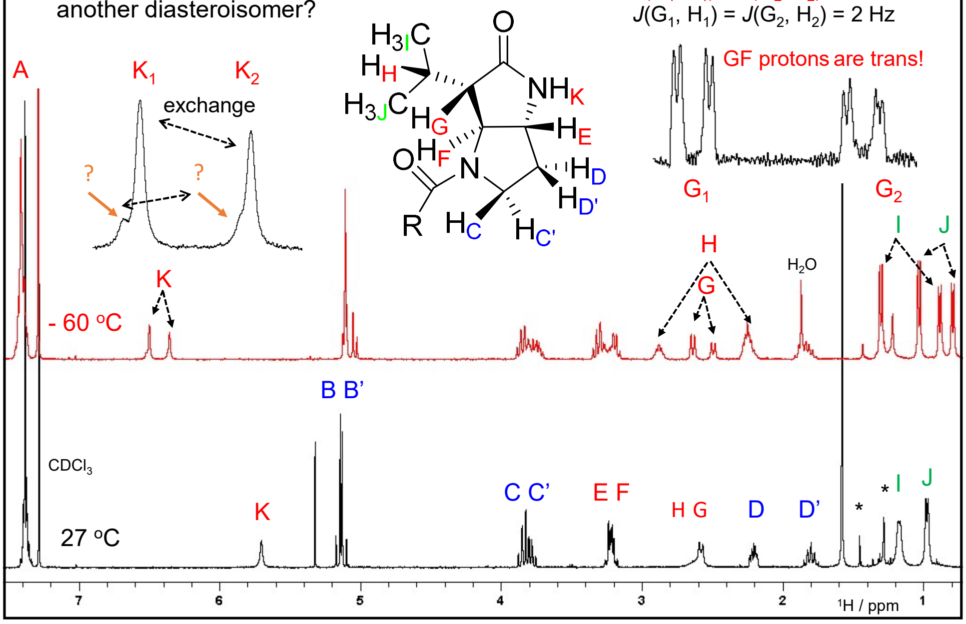
^{13}C chemical shift also differ between different rotamers (typically less)

Similar to COSY, no HSQC cross peaks between different rotamers!

Source of ChE: hindered rotation of the isopropyl

Minor signals of K. Hint of another diasteroisomer?

$J(G_1, F_1) = J(G_2, F_2) = 11$ Hz
$J(G_1, H_1) = J(G_2, H_2) = 2$ Hz



Acknowledgements

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Dynamic Nuclear Magnetic Resonance spectroscopy,
Lloyd Jackman, Ed ISBN: 978-0-12-378850-4, Academic Press 1975
http://www.sciencedirect.com/science/book/9780123788504