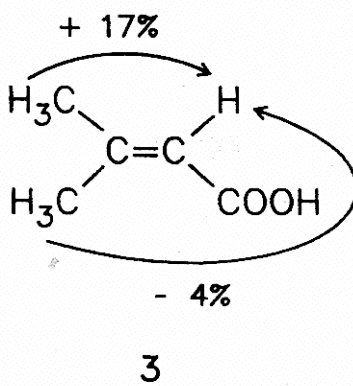
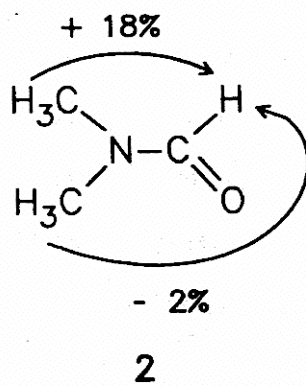
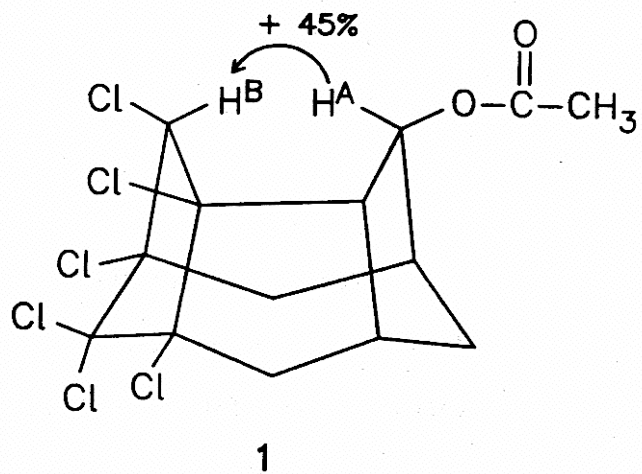


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Introductory NMR

Lecture 18



The Nuclear Overhauser Effect

NOE: Nuclear Overhauser Effect or Nuclear Overhauser Enhancement

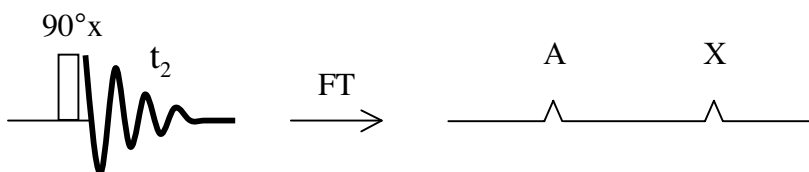
- Depends on dipole-dipole relaxation (an important mechanism of T_1 relaxation)
- Is based on through-space interactions that change M_z
- The NOE changes the intensities of particular signals
- The magnitude of the NOE is related to nuclear distances and molecular motions

1. 1D steady-state NOE experiment:

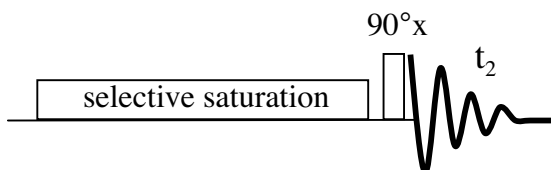
Consider a two-spin system AX:

- Assume the homonuclear case (A and X are both ^1H , although they need not be)
- Assume no scalar coupling between A and X ($J = 0$)
- Assume the internuclear A-X distance is $\leq \sim 5\text{\AA}$ (NOE enhancement depends on $1/r^6$ where r is internuclear distance)

So, the simple 1D spectrum gives 2 signals with no splitting ($J=0$):



To establish the NOE, we will selectively saturate the 'A' transition with an RF field to equilibrate the spin populations:



What happens to the population levels when we saturate one of the signals, and what happens afterwards?

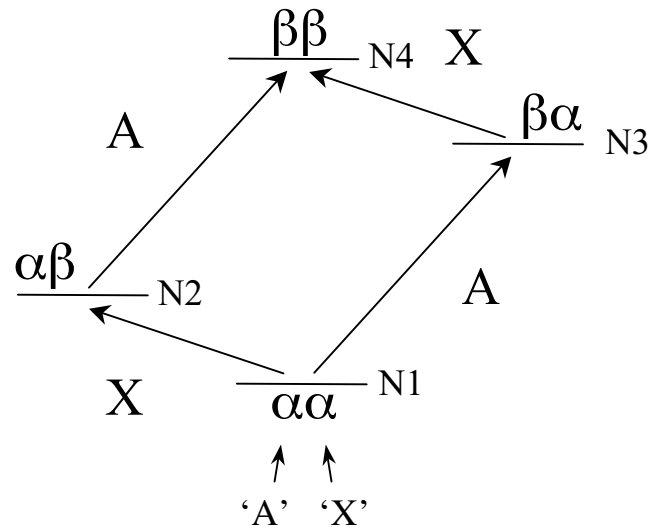
-Let's look at the energy level diagrams:

A). At equilibrium:

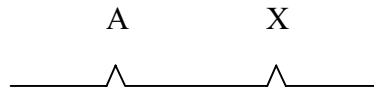
$$\begin{aligned} N_4 &= N \\ N_3 &= N + \Delta H \\ N_2 &= N + \Delta H \\ N_1 &= N + 2\Delta H \end{aligned}$$

'A' transitions: $N_1 - N_3 = N_2 - N_4 = \Delta H$

'X' transitions: $N_1 - N_2 = N_3 - N_4 = \Delta H$



- 'A' and 'X' transitions are allowed and observable
- 'A' and 'X' transitions give singlets in 1D spectrum

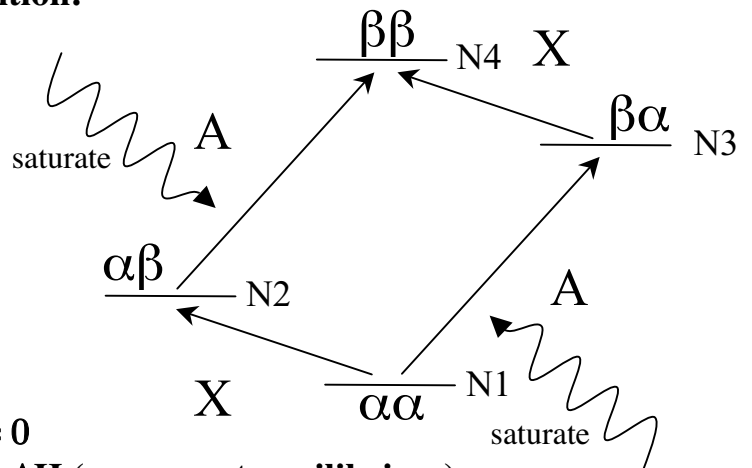


B). After saturation of the 'A' transition:

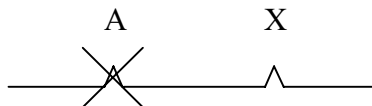
$$\begin{aligned} N_4 &= N + \Delta H/2 \\ N_3 &= N + 3\Delta H/2 \\ N_2 &= N + \Delta H/2 \\ N_1 &= N + 3\Delta H/2 \end{aligned}$$

'A' transitions: $N_1 - N_3 = N_2 - N_4 = 0$

'X' transitions: $N_1 - N_2 = N_3 - N_4 = \Delta H$ (same as at equilibrium)

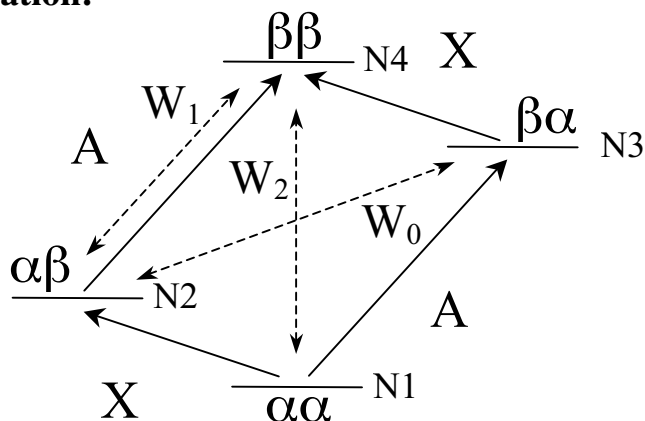


-populations of N_1 , N_2 , N_3 , and N_4 are now different than they were at equilibrium



C). Now, allow relaxation during saturation:

	at equilibrium	after saturation
N4 =	N	$N + \Delta H/2$
N3 =	$N + \Delta H$	$N + 3\Delta H/2$
N2 =	$N + \Delta H$	$N + \Delta H/2$
N1 =	$N + 2\Delta H$	$N + 3\Delta H/2$



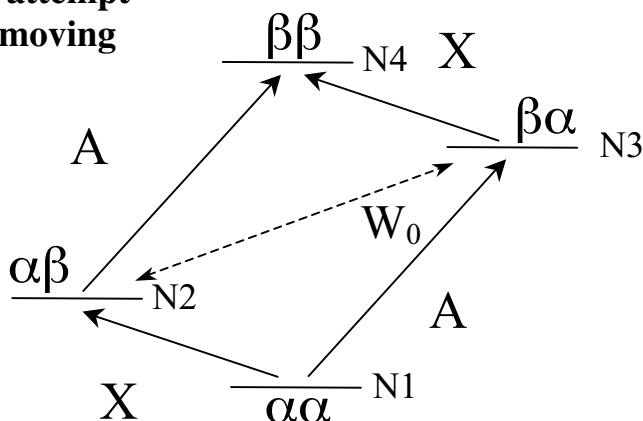
- W_0 , W_1 , and W_2 are *rate constants or transition probabilities*
- W_1 is a one-spin transition or single quantum transition
- W_0 and W_2 are two-spin transitions
- W_0 is a zero quantum transition
- W_2 is a double quantum transition
- W_0 and W_2 are spectroscopically **FORBIDDEN** and cannot be observed
- **HOWEVER**, they are **ALLOWED** for relaxation

Let's suppose W_0 is more efficient than W_2 (large molecule case):

-in this case, the system will attempt to return to equilibrium by moving spins from N3 to N2

after saturation
and W_0 relaxation

N4 = $N + \Delta H/2$
 N3 = $N + 3\Delta H/2 - \delta$
 N2 = $N + \Delta H/2 + \delta$
 N1 = $N + 3\Delta H/2$



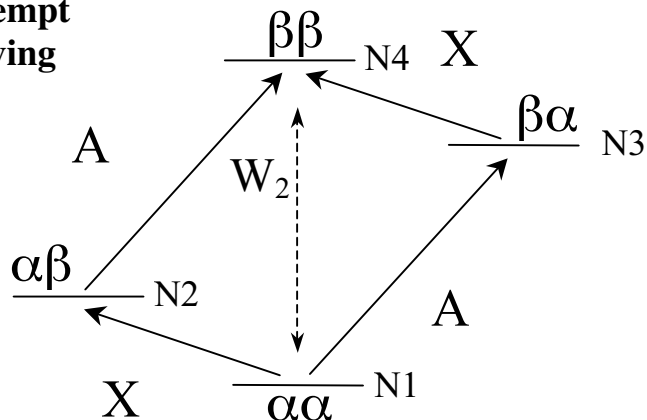
'X' transitions: $N1 - N2 = N3 - N4 = \Delta H - \delta$: intensity of 'X' reduced !!



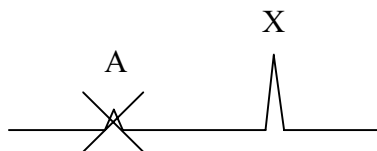
Let's suppose W_2 is more efficient than W_0 (small molecule case):

-in this case, the system will attempt to return to equilibrium by moving spins from N4 to N1

after saturation
and W_0 relaxation
 $N4 = N + \Delta H/2 - \delta$
 $N3 = N + 3\Delta H/2$
 $N2 = N + \Delta H/2$
 $N1 = N + 3\Delta H/2 + \delta$



'X' transitions: $N1 - N2 = N3 - N4 = \Delta H + \delta$: intensity of 'X' increased !!



Summary:

(reminder: τ_c is the correlation time of the molecule (sec))

- for small molecules (short τ_c) W_2 dominates
- for large molecules (large τ_c) W_0 dominates
- because W_0 and W_2 depend on B_0 , the NOE also depends on B_0

2. Enhancement Factor for Small Molecules:

Maximal enhancement for small molecules (η):

$$\eta = \gamma_a / 2\gamma_x \quad \text{A: saturated} \quad \text{X: observed}$$

(γ is gyromagnetic ratio, of course)

Total Intensity (I):

$$I = (1 + \eta) I_0 \quad I_0 \equiv \text{original intensity}$$

Examples:

- a. Fractional increase in ^{13}C NMR signal intensity with BB ^1H decoupling:

$$\eta = \gamma_a / 2\gamma_x$$
$$\eta = 4 / (2 \times 1) = 2 \quad \text{i.e. signal increased by a factor of 2 (200\%)}$$

- b. Fractional increase in ^1H NMR signal intensity with BB ^{13}C decoupling:

$$\eta = 1 / 8 = 0.125 \quad \text{i.e. signal increased by a maximum of 12.5\%}$$
$$I = (1 + 0.125) I_0 = 1.125 \times I_0 \quad (\text{signal is slightly more intense})$$

3. Multi-Spin Systems:

Consider a homonuclear 3-spin system A-B-C

- assume no J coupling
- assume the molecule is a small molecule

- If A is saturated, the intensity of the signal from B is increased
- The saturation of A affects the populations of the B-C system, which causes an indirect NOE to C.
- Thus, this indirect NOE is negative

4. Dependence of Maximum ^1H - ^1H Homonuclear NOE on B_0 and molecular mass:

The magnitude and sign of the ^1H - ^1H NOE are dependent on:

- the Larmor frequency, ν (sometimes ω), which depends on B_0
- the correlation time, τ_c , which depends on molecular size (mass)
 - VERY crudely, $\tau_c \approx 10^{-12} \text{ MW}$ (where MW is molecular mass in Daltons)
 - example: $\tau_c \approx 10 \text{ ns}$ for 10 kDa molecule

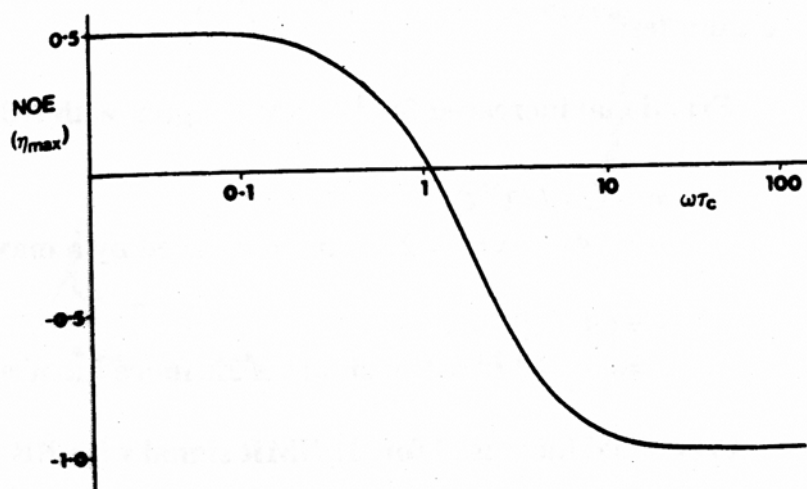


FIGURE 2.6
Dependence of maximum homonuclear NOE enhancement on $\omega\tau_c$. Note the log scale of $\omega\tau_c$.

Note:

- NOE is *positive* for *small* molecules
- NOE is *negative* for *large* molecules
- NOE is zero at $\omega\tau_c \approx 1$
 - examples: -at 200 MHz, NOE is zero for molecules with $\tau_c \approx 5 \text{ ns}$ (approximately 5 kDa)
 - at 800 MHz, NOE is zero for molecules with $\tau_c \approx 1.25 \text{ ns}$ (approximately 1.25 kDa)

5. Dependence of the magnitude of the NOE on Internuclear Distance:

In the absence of external relaxation:

- the maximal enhancement is achieved
- the NOE is distance independent

In the presence of external relaxation:

- two-spin steady-state NOE depends on internuclear distance

$$f = \text{NOE enhancement} \propto 1/r^6$$

(r = internuclear distance)

- this effect is usually observed only for ^1H - ^1H distances $\leq 5 \text{ \AA}$

Examples of external relaxation:

a). intermolecular relaxation caused by paramagnetic agents

- field at a nucleus depends on the presence of unpaired electrons
- examples: dissolved O_2 , manganese and other metal impurities

b). quadrupolar relaxation

- energy of quadrupole nuclei varies with the fluctuating electric field gradient
- dominant mechanism for nuclei with $I > 1/2$ (^2H and ^{14}N)

c). chemical shift anisotropy (CSA)

- anisotropy produces a different field at the nucleus depending on the orientation of the bond relative to the applied field
- significant for nuclei with large CSA (^{19}F , ^{31}P)

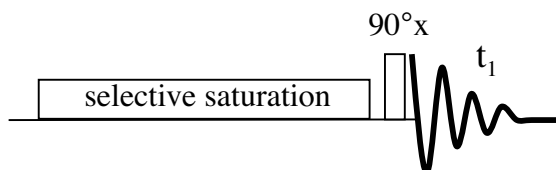
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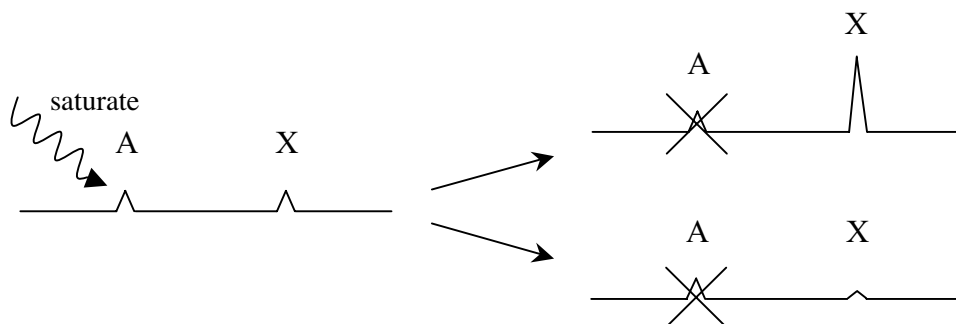
Lecture 19

The NOESY Experiment

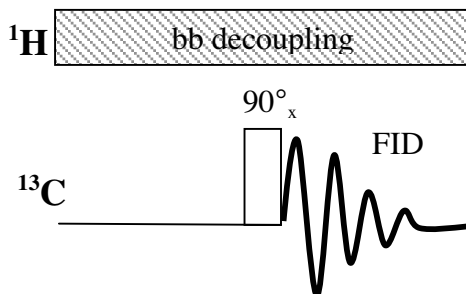
1). Review: 1D steady-state NOE experiment



- used in cases of well resolved spectra for assignment purposes or to establish local or global geometry by saturation of selected signals
- the *steady-state NOE* develops during saturation of one spin, and results in a change in the intensity of the signal from another spin
-this is in contrast to the *transient NOE* (see below)



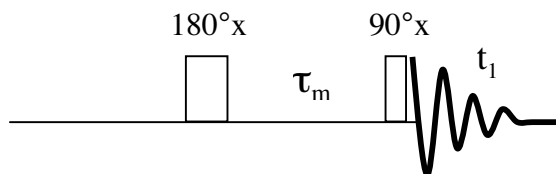
- used most often these days for enhancement of ^{13}C signals by broadband decoupling of ^1H



2). 1D and 2D transient NOE experiments: NOESY

a). 1D selective transient NOE experiment

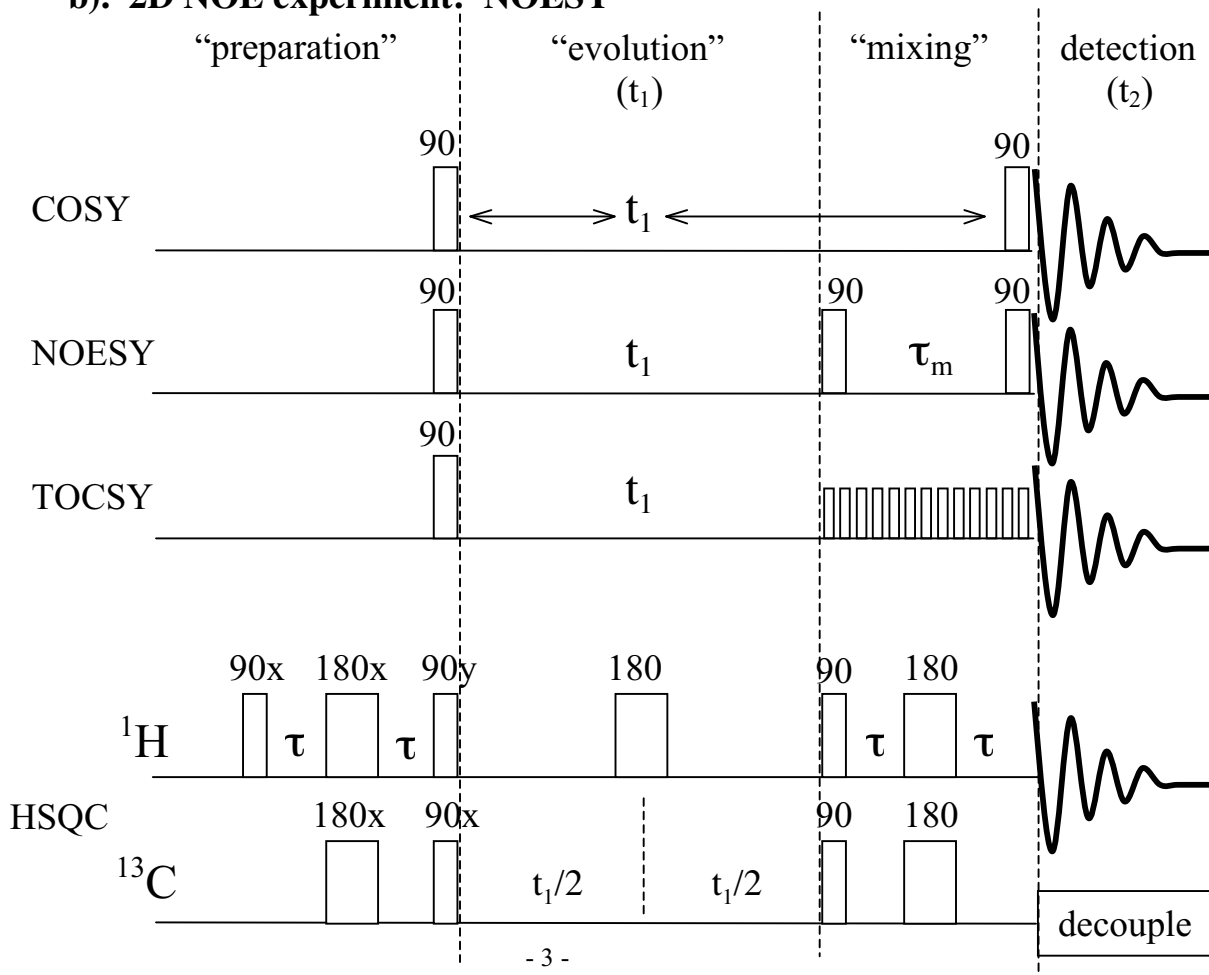
-an NOE can also be induced by application of a selective 180° pulse to one spin (the 'A' spin)



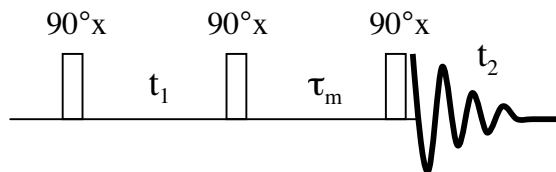
-during the mixing time (τ_m), the NOE starts to build (at the 'X' spin), as the W_0 and W_2 routes redistribute the spin populations

-the NOE is transient, however: it builds to a maximum in a short time (approximately one T_1 period) and then decreases (remember, only a few T_1 periods after a 180° pulse, equilibrium populations are established)

b). 2D NOE experiment: NOESY



- the NOESY (Nuclear Overhauser Effect Spectroscopy) experiment plays a central role in the structural elucidation of molecules ranging from small organic molecules to large biological molecules (nucleic acids and proteins)



-in the 2D NOESY experiment, *all* spins are inverted by the first two 90° pulses (assume, for the moment, that $t_1 = 0$), permitting all transient NOE's to develop during the subsequent mixing time (τ_m)

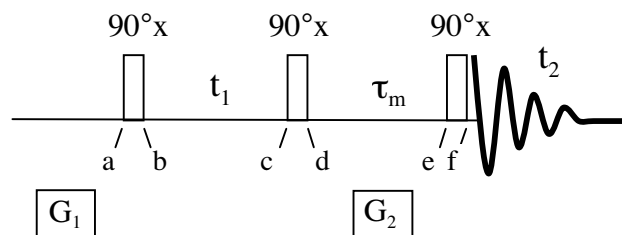
-increasing t_1 provides chemical shift modulation of the signal for the second dimension

-in the 2D NOESY spectrum, the peaks representing the transferred magnetization (the “NOEs”), appear as crosspeaks (off-diagonal peaks)

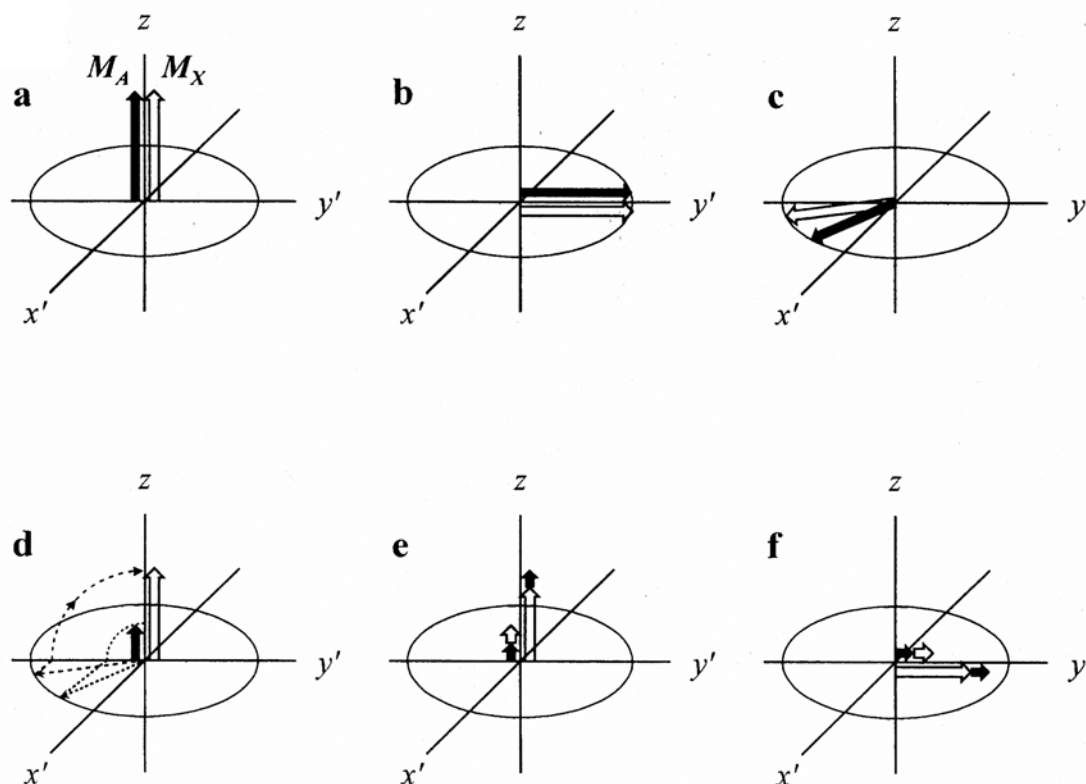
-the crosspeaks appear at the chemical shifts of the spins involved

-the intensities of the crosspeaks are much less than the intensities of the diagonal peaks

3). 2D NOESY: vector diagrams

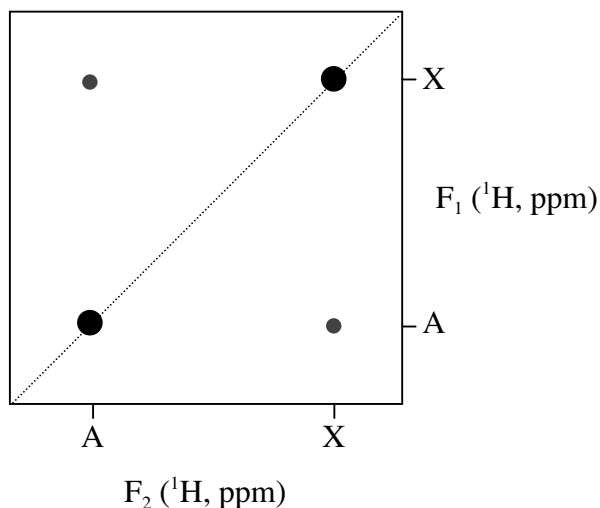


-we again will assume a simple AX system with no coupling ($J_{AX}=0$)



- A and X have different Larmor frequencies, so M_A and M_X precess about z and move apart during t_1
- the second 90° pulse converts the 'y' components of M_A and M_X to 'z'
- during τ_m , polarization is transferred from A to X and from X to A via cross-relaxation (W_0 and W_2)
 - the transferred components are represented by the small arrows with opposite coloring

4). 2D NOESY: spectrum



Cross-relaxation / cross-polarization between nuclei results in cross peaks in the 2D NOESY spectrum, usually referred to as ‘NOE cross-peaks’ or simply ‘NOEs’

- the *intensity* of the cross peaks is substantially less than the intensity of the diagonal peaks (the diagonal peaks can be problematic in these experiments because they are very intense)

- the *intensity* of the cross peaks is proportional to $1/r^6$ where r is the internuclear distance

$$I \propto 1 / r^6$$

- so, for ^1H - ^1H distances, if the distance is \leq approximately 5 Å, crosspeaks can be observed

- the *sign* or *phase* of crosspeaks (relative to the diagonal peaks) depends on the size of the molecule

- for small molecules, the crosspeaks and diagonal peaks are of opposite phase

- for large molecules, the crosspeaks are the same phase as the diagonal peaks

5). 2D NOESY: role of G_1 and G_2 gradient pulses

Normally, the data from many scans at each t_1 are added together during collection of NOESY spectra

- ideally, one would wait $\sim 5T_1$ between scans to allow the system to equilibrate
- this of course require far too much time to acquire a complete 2D data set
- one can wait for $<5T_1$ between scans, but this causes a loss in signal intensity and an increase in the appearance of artifacts in the 2D spectra (some of which can be alleviated by phase cycling and other techniques)
- linear field gradient pulses can be used to

G_1 gradient:

- in the gradient NOESY experiment, the G_1 gradient pulse will dephase any transverse magnetization during the recycle delay, preventing artifacts in the spectra resulting from these components

G_2 gradient:

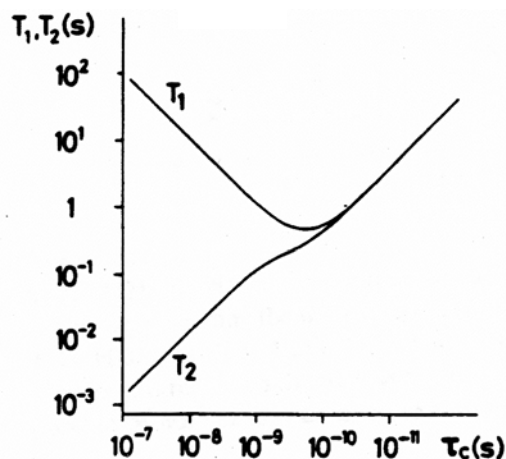
- performs a function similar to the G_1 gradient, only during the mixing time, τ_m . During τ_m , transverse magnetization components often can cause artifacts in NOESY spectra. G_2 dephases these components

6). 2D NOESY: mixing time

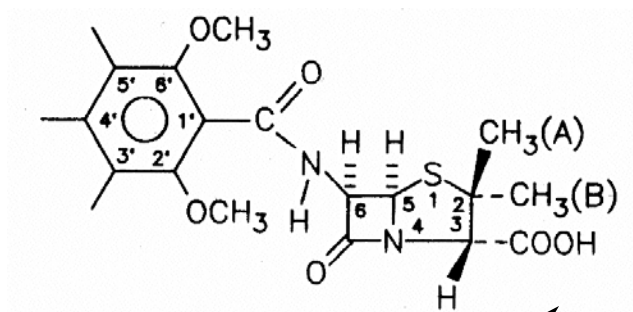
How long should the mixing time, τ_m , be?

- in order to maximize the polarization transfer that can be accomplished by the transient NOE, and therefore to maximize the crosspeak intensity in the NOESY spectrum, τ_m should be set to approximately T_1 for the ^1H spins involved

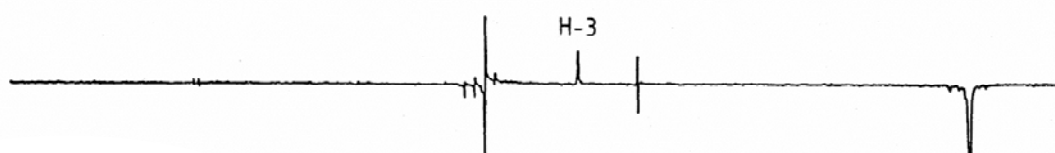
small molecule: 500 ms – 5 s
large molecule: 30 ms – 400 ms



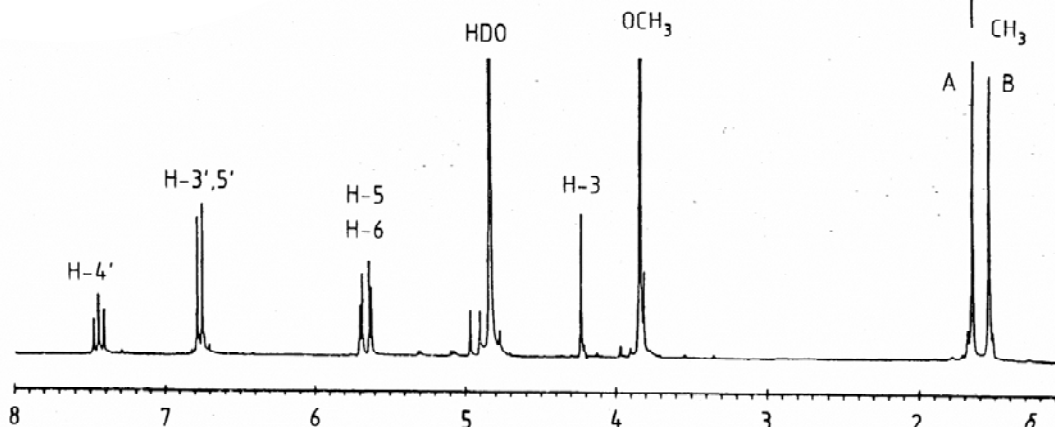
7). example: 1D steady-state NOE experiment



(B)



(A)

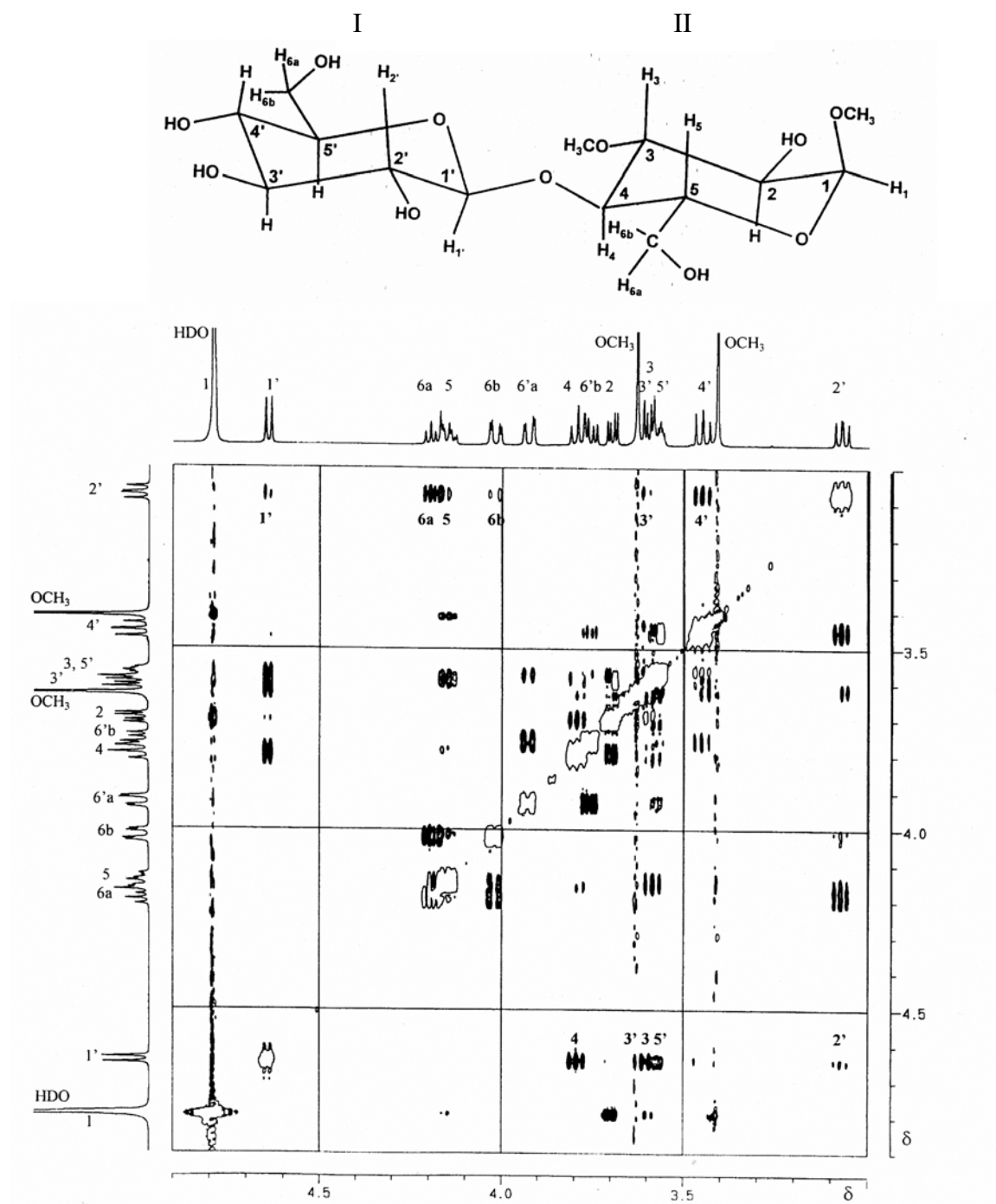


Which methyl signal ($\delta=1.7$ or $\delta=1.5$ ppm) is the methyl group on the same side of the ring as H-3?

-saturate the methyl signals

-when the signal at $\delta=1.7$ ppm is saturated, an NOE enhancement is observed for H-3, indicating the signal at $\delta=1.7$ ppm corresponds to the methyl group on the same side of the ring as H-3

8). example: 2D NOESY experiment



(20 mg in 0.5 ml D₂O, 128 points in t₁, mixing time = 2 seconds, 16 scans/FID: 2 hours)

- crosspeaks observed between H-1' (I) and H-3 and H-4 (II)
- crosspeaks observed between H-2' (I) and H-5, H-6a and H-6b (II)
- no crosspeaks between H-2' (I) and H-4 (II)