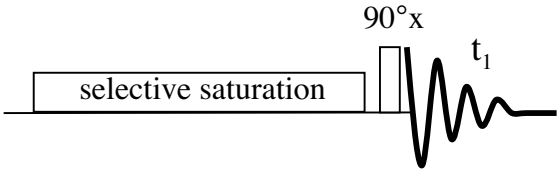
CHEM / BCMB 4190/6190/8189

Introductory NMR

Lecture 19

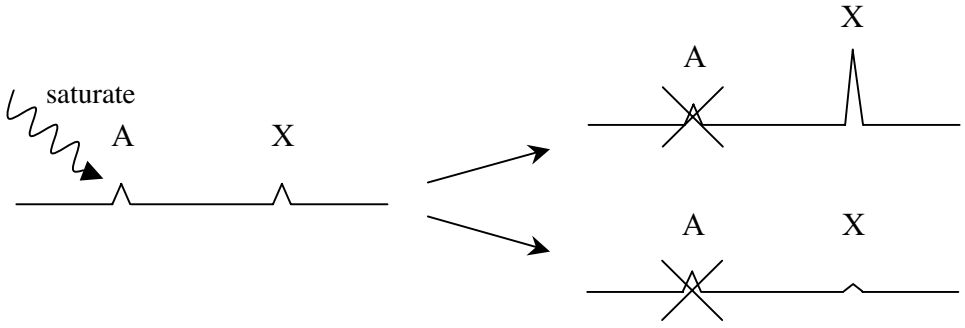
The NOESY Experiment

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

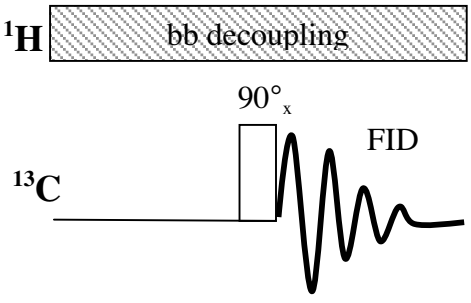


- used in cases of well resolved spectra for assignment purposes or to establish local or global geometry by saturation of selected signals

- 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)



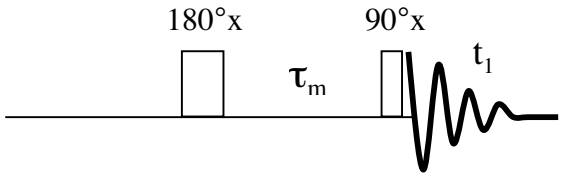
-used most often these days for enhancement of ^{13}C signals by broadband decoupling of ^{1}H



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

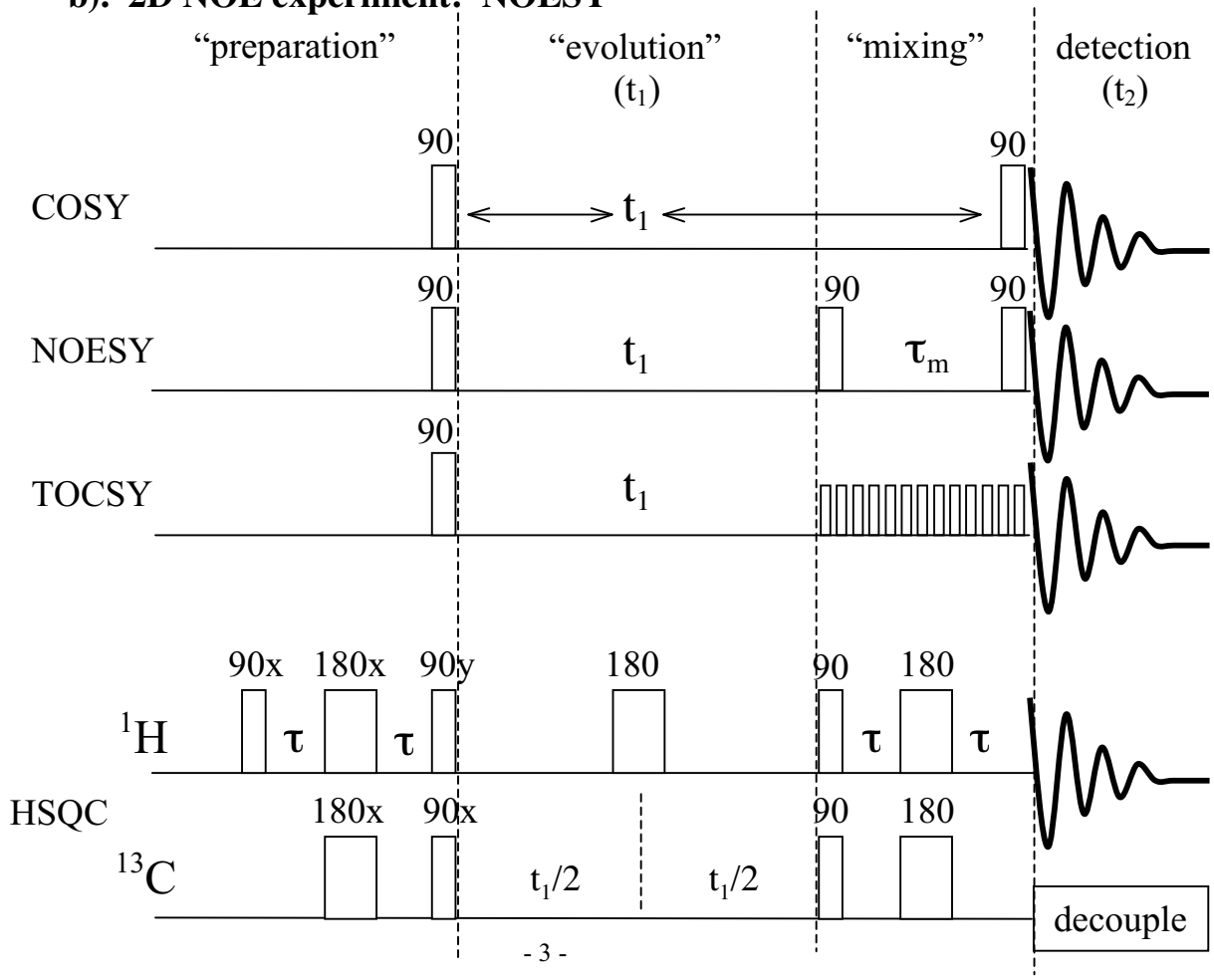
a). 1D selective transient NOE experiment

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

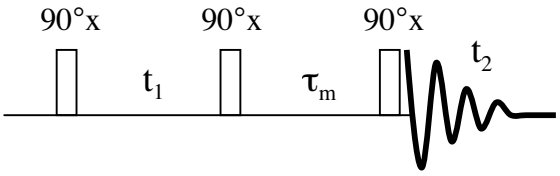


-during the mixing time (τ_m), the NOE starts to build (at the 'X' spin), as the W_0 and W_2 routes redistribute the spin populations
-the NOE is transient, however: it builds to a maximum in a short time (approximately one T_1 period) and then decreases (remember, only a few T_1 periods after a 180° 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 90º pulses (assume, for the moment, that $t_1 = 0$), permitting all transient NOE's to develop during the subsequent mixing time (τ_m)

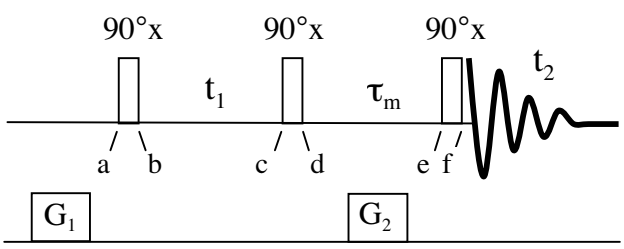
-increasing t_1 provides chemical shift modulation of the signal for the second dimension

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

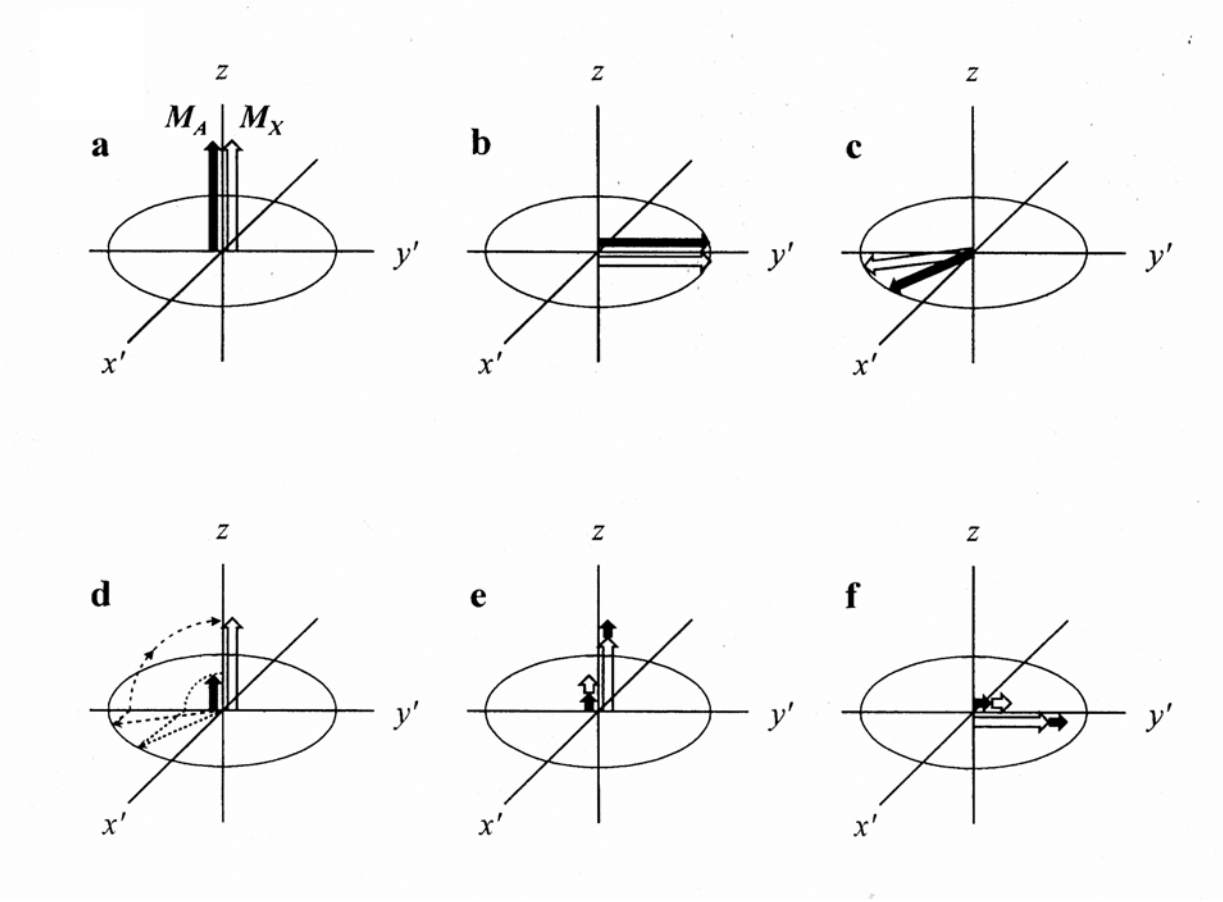
-the crosspeaks appear at the chemical shifts of the spins involved

-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)



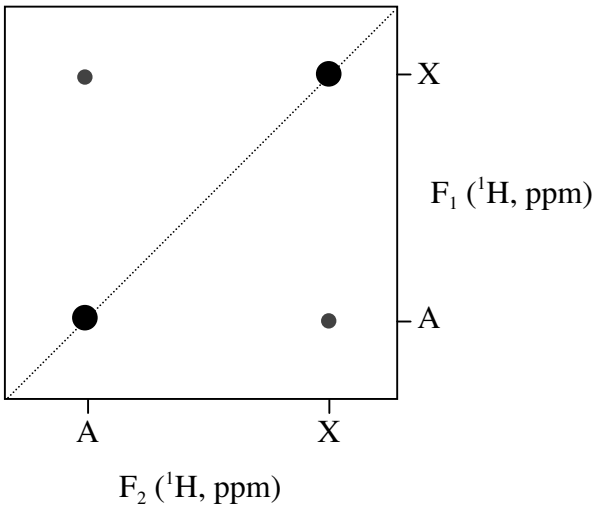
- A and X have different Larmor frequencies, so M_A and M_X precess about z and move apart during t_1

- 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 ^{1}H-^{1}H 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). <u>2D NOESY: role of G_1 and G_2 gradient pulses</u>

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

-ideally, one would wait ~$5T_1$ 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

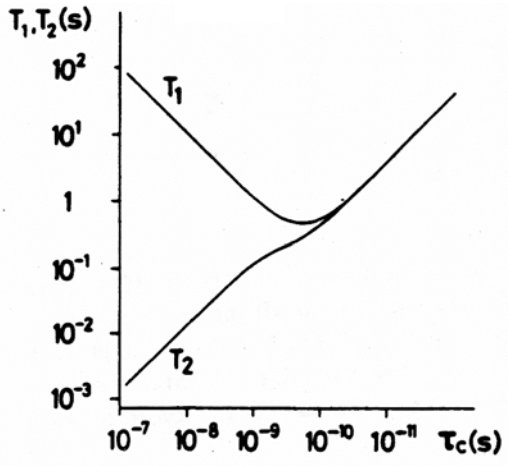
G_2 gradient:

-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. G2 dephases these components

6). <u>2D NOESY: mixing time</u>

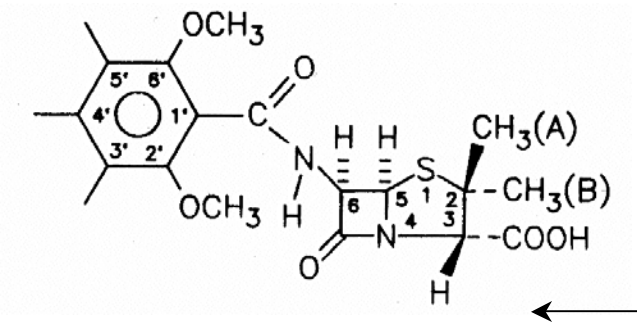
How long should the mixing time, τ_m, be?

- 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

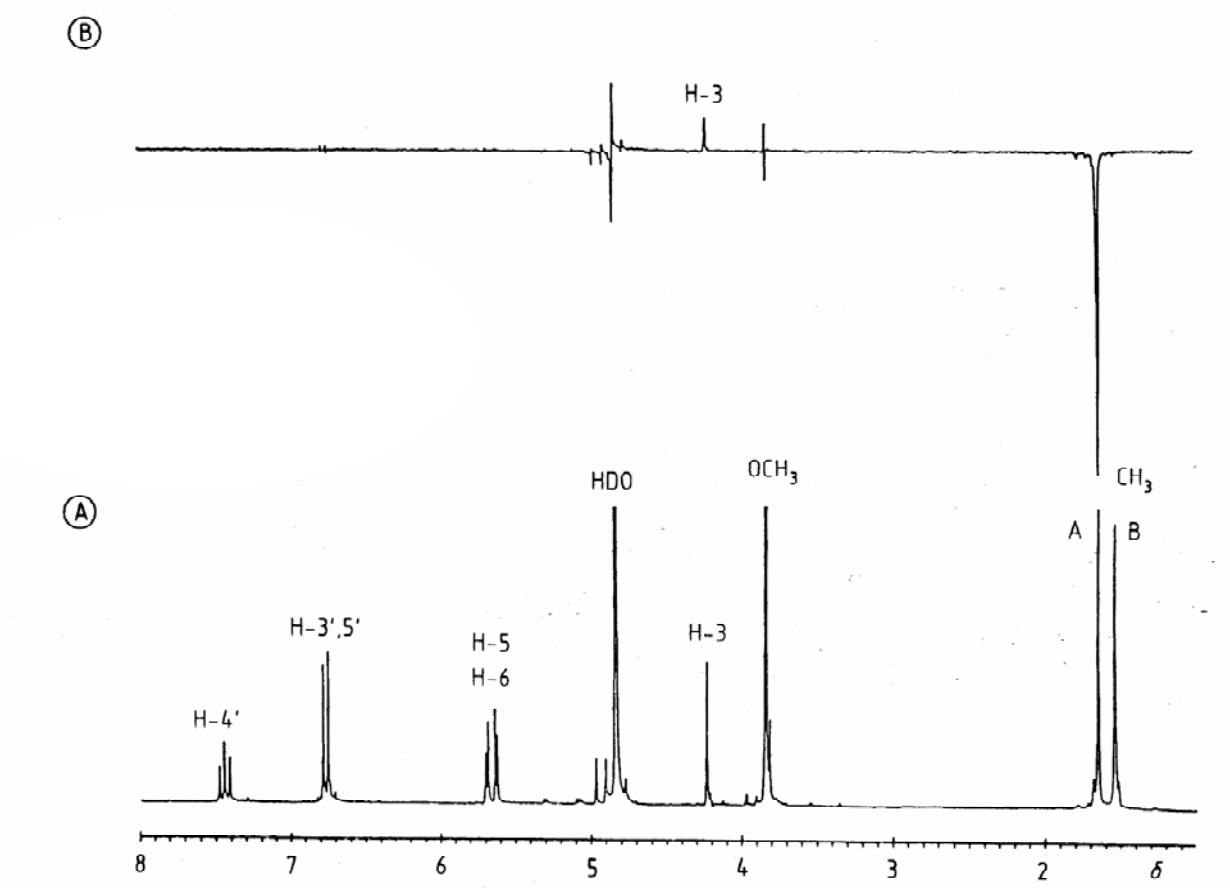


small molecule:	**500 ms – 5 s**
large molecule:	**30 ms – 400 ms**

7). <u>example: 1D steady-state NOE experiment</u>



H-3

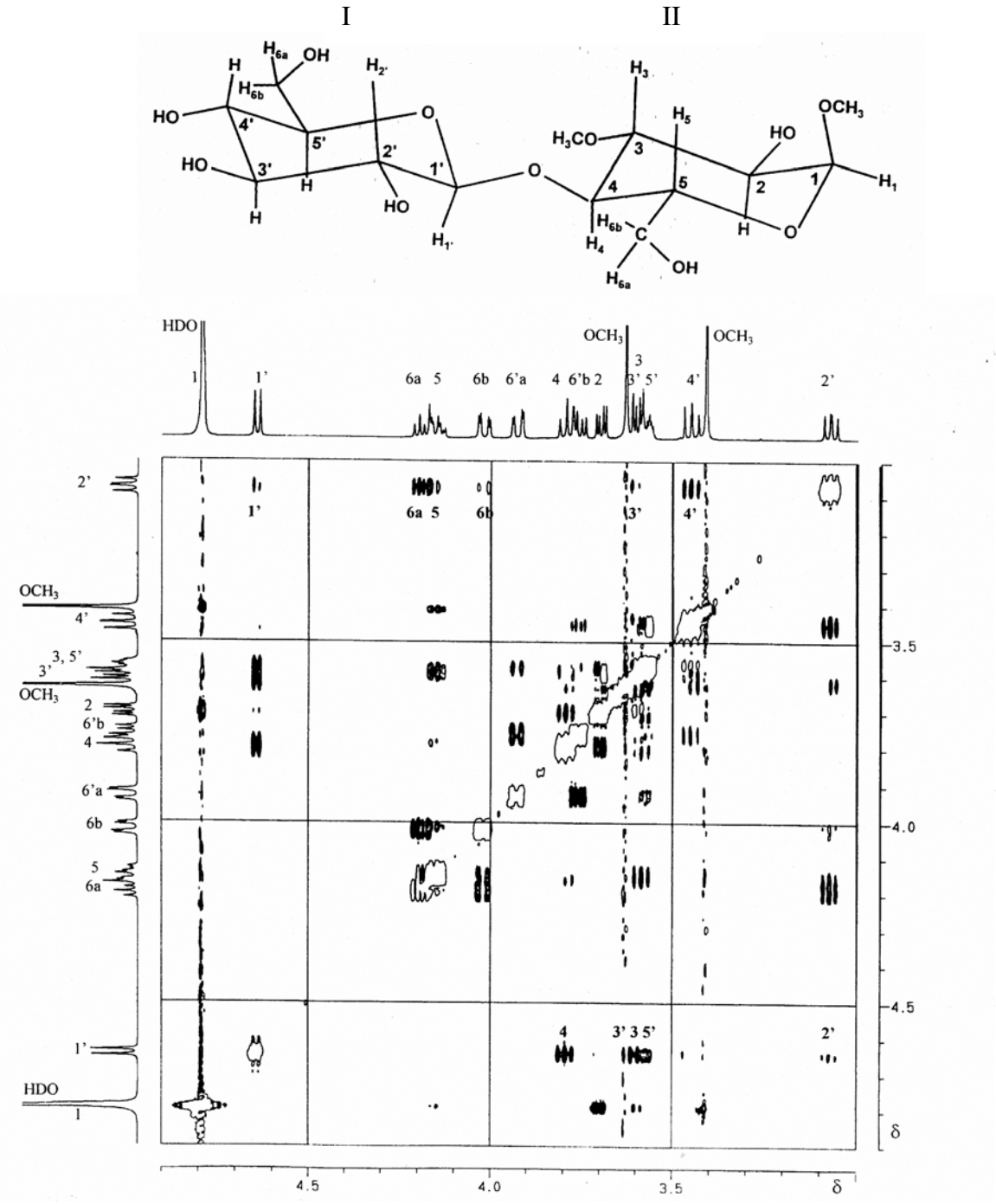


Which methyl signal (δ=1.7 or δ=1.5 ppm) is the methyl group on the same side of the ring as H-3?

-saturate the methyl signals

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

8). <u>example: 2D NOESY experiment</u>



(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)