

LECTURE NOTES CHEM 781:

PART 8: NOE and through space correlation

November 18, 2014

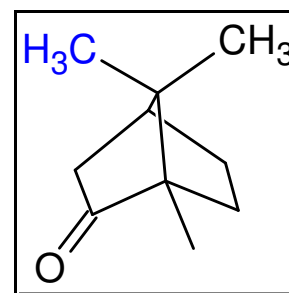
8.1 Introduction

All 2D methods discussed so far utilized scalar couplings to establish through bond connectivities
=> determination of two dimensional structure

The magnitude of scalar coupling could be used to determine dihedral angles and establish configuration and conformational information.

For many stereo chemical problems groups more than three bonds apart have to be related. For example to assign the methyl groups of camphor coupling constants are not useful. *Through space* interaction (direct distance information) is often needed

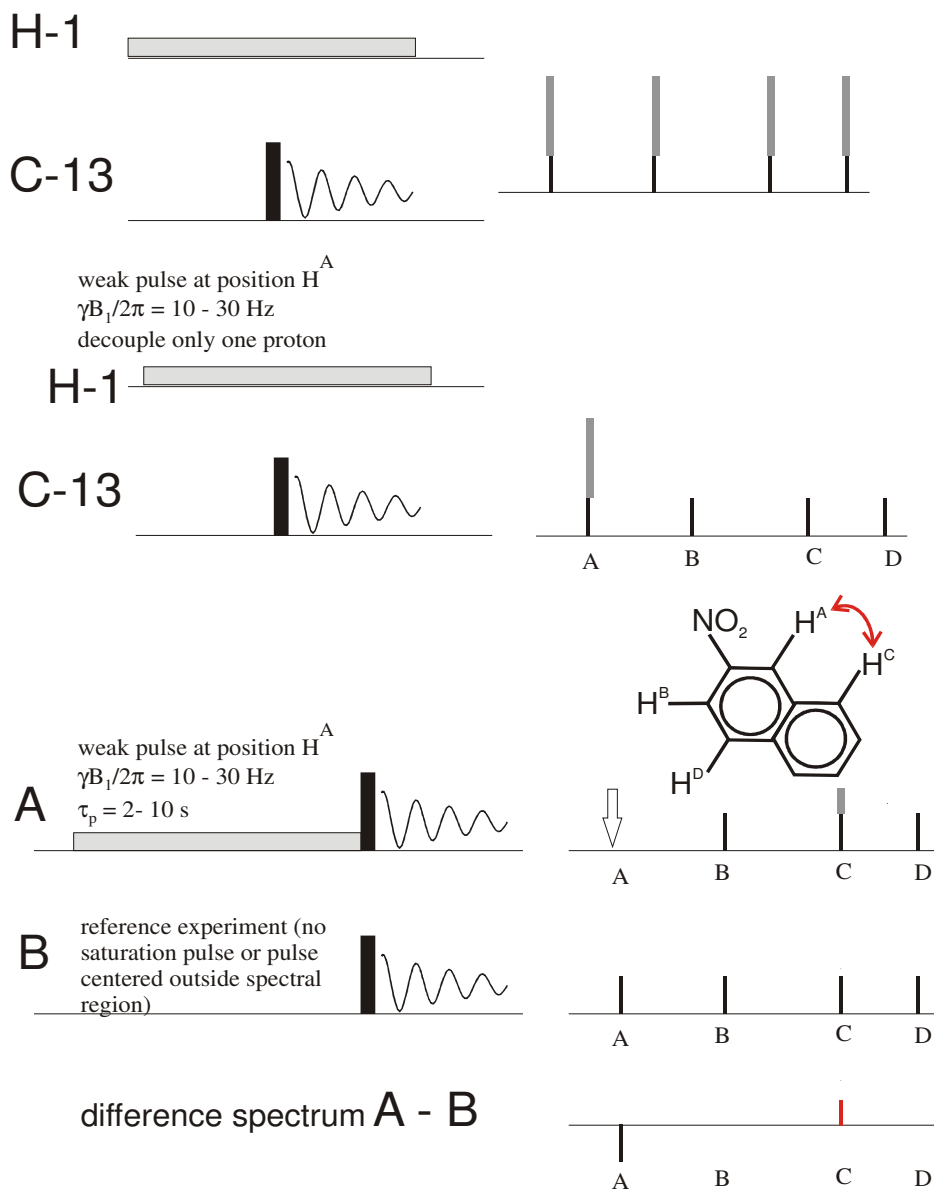
=> Dipolar coupling does not depend on bonds but only on proximity through space ($\sim r^{-3}$)



In solution dipolar coupling can usually not be measured directly as it is averaged out by the fast tumbling of the molecule. It can be measured by its effect on relaxation and creation of Nuclear Overhauser Effect (NOE) as discussed for decoupled ^{13}C spectra.

NOEs in ^{13}C spectra arise from decoupling of protons and are indicative of spatial proximity of the carbon and hydrogen atoms, causing protonated carbons to appear more intense in the spectrum. In the regular decoupled carbon experiment, no information available as to which particular proton(s) caused the NOE. To obtain that information, one has to decouple (saturate) one proton at a time without affecting the other protons. This can be achieved by irradiating with very low power ($\gamma B_1/2\pi \approx 10\text{-}30\text{ Hz}$) at the frequency of one specific hydrogen for several seconds before taking the carbon spectrum. This would result in a spectrum where only carbons close to the irradiated proton show enhancement.

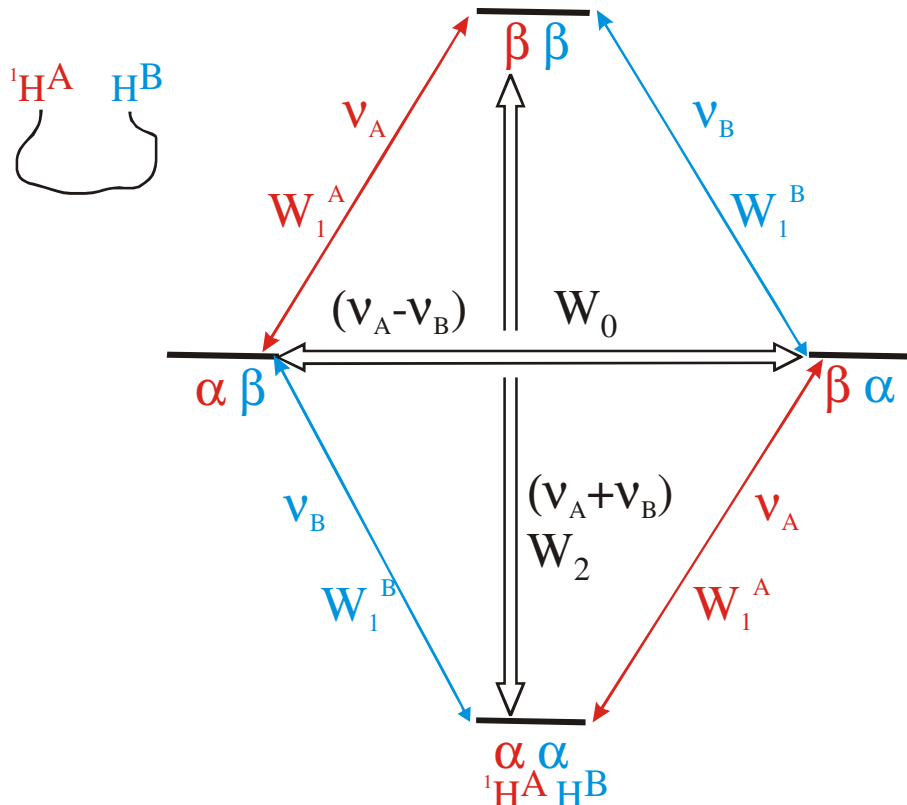
In a similar manner, NOE's can be measured between protons and can be used to assess H-H distances. After selectively irradiating one proton one acquires spectrum with a high power pulse. This results in a spectrum where protons in close proximity to the irradiated proton exhibit increased



intensities due to NOE. As the effects often are small typically the difference to a non irradiated spectrum is typically determined.

8.2 NOE and relaxation

As already mentioned when discussing ¹H decoupling of ¹³C NMR spectra, the NOE arises from a change in population caused by relaxation. Consider a pair of protons close in space but not necessarily coupled:



- *four transitions* can give rise to an NMR signal, corresponding to transitions involving flip of *one spin* (single quantum transitions). If there is no scalar coupling, the frequencies of the two H^{A} (and H^{B}) lines are identical and two singlets are observed.
- relaxation can occur across *six transitions*, the four single quantum transitions and the double- and zero quantum transitions, with the transition rates (probabilities) W_1 , W_0 and W_2 .
- The maximum possible NOE is given by the ratio of cross- to total relaxation:

$$\eta = \frac{\overbrace{W_2 - W_0}^{\sigma}}{\underbrace{W_1^{\text{A}} + W_1^{\text{B}} + W_0 + W_2}_{\frac{1}{T_1}}} = \frac{\text{cross relaxation}}{\text{total spin lattice relaxation}} \quad (8.1)$$

Relaxation requires the presence of randomly oscillating magnetic fields. In solution that occurs if Brownian motion of the molecules modulates the effective field around a nucleus. So we need to

closer look at two things: molecular motion and interactions which depend on the orientation of the molecule (anisotropic interactions):

$$\text{relaxation} \quad \sim \quad \text{molecular motion}(\text{frequency}) \cdot \text{interaction}(\text{changing field})$$

8.2.1 Molecular motion and Relaxation

The frequency of any random fields is directly related to *inter-* and *intra-*molecular motion. Brownian motion of the molecule involves both translational and rotational motion. For most contributions to relaxation, the rotational motion is the important one.

- Rotational Brownian motion is described by the *rotational* diffusion coefficient D_{rot} (not to be mixed up with the translational diffusion) or the correlation time τ_c (the time a molecule on average remains in one orientation). For spherical molecules the Stokes-Einstein equation can be used:

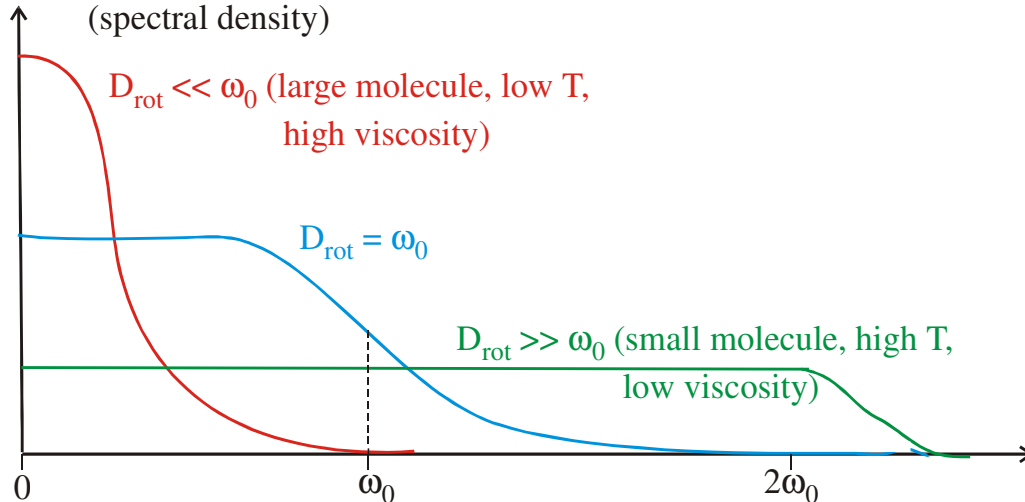
$$D_{rot} = \frac{1}{6\tau_c} = \frac{kT}{8\pi r^3 \eta_{solv}} \quad (8.2)$$

- As one can see the rate of reorientation depends on molecular size (given by the volume r^3), temperature T and viscosity of the solvent η (note that the same symbol is used for the NOE, don't get them mixed up). As the motion is random, D_{rot} represents an average rate of motion, with a certain distribution of frequencies present in the ensemble of molecules determined by the Boltzmann distribution. This distribution is described by the spectral

$$\text{density } J(\omega) = \frac{\tau_c}{1 + \omega^2 \tau_c^2} \quad (8.3)$$

- The relaxation rates W_i will be most efficient when the tumbling at the transition frequency reaches a maximum. One can already see that for very fast or very slow tumbling the single quantum relaxation rate W_1 at frequency ω_0 will become very inefficient
- The double quantum transition W_2 at $\omega = 2 \cdot \omega_0$ will be more important for fast tumbling (small molecules), W_0 at $\omega = \omega^A - \omega^B \approx 0$ will become important for small molecules, so the sign of H-H NOE will be different for different size molecule

$J(\omega)$: number of molecules at a certain frequency
(spectral density)



8.2.2 Interactions leading to relaxation

The second ingredient to relaxation is an interaction which gets modulated by the motion.

Some important interactions are:

- Anisotropy of the chemical shielding anisotropy - see part 3 of lecture notes: important for tertiary carbons
- dipolar (through space) nuclear spin-spin coupling - part 4 of lecture notes: Important for protons and protonated heteronuclei (C-H, N-H)
- dipolar nuclear spin - electron spin coupling : important when paramagnetic molecules are present (like oxygen)
- quadrupolar coupling: orientation of non spherical nuclei in electric field. Only for $I > \frac{1}{2}$ nuclei, but then almost always dominant

All these interactions cause the nucleus to experience a fluctuating field in the presence of motion, even if only a constant average value is observed in solution.

- All interactions will contribute to single quantum transitions at ω_0 (W_1)
- *only dipolar coupling between A and B will cause double- and zero quantum transitions W_0^{AB}*

and $W_2^{AB} \Rightarrow$ **only dipolar coupling will create NOE's**

- as a consequence, maximum NOE between two nuclei will only be observed if the dipolar interaction between the two nuclei is the dominant relaxation process for that nucleus dominant and other processes are negligible (problem I > 1/2 nuclei, paramagnetic impurities like oxygen, other hydrogens coupled by dipolar coupling)

8.3 Dipolar relaxation and NOE

8.3.1 General Theory

The dipolar coupling between two spins depends only on the distance and the gyromagnetic ratio of the spins:

$$D_{AB} = (\gamma_A \gamma_B \hbar^2) / r_{AB}^3 \quad (8.4)$$

From a lengthy and tedious quantum mechanical derivation one can obtain values for the relevant relaxation rates:

$$\begin{aligned} W_I^A &= 3/20 \cdot D_{AB}^2 \cdot J(\omega_A); & W_I^B &= 3/20 \cdot D_{AB}^2 \cdot J(\omega_B) \\ W_0 &= 1/10 \cdot D_{AB}^2 \cdot J(\omega_A - \omega_B) = 1/10 \cdot D_{AB}^2 \cdot J(0) \text{ for A,B both } ^1\text{H} \\ W_2 &= 6/10 \cdot D_{AB}^2 \cdot J(\omega_A + \omega_B) = 6/10 \cdot D_{AB}^2 \cdot J(2\omega) \text{ for A,B both } ^1\text{H} \end{aligned} \quad (8.5)$$

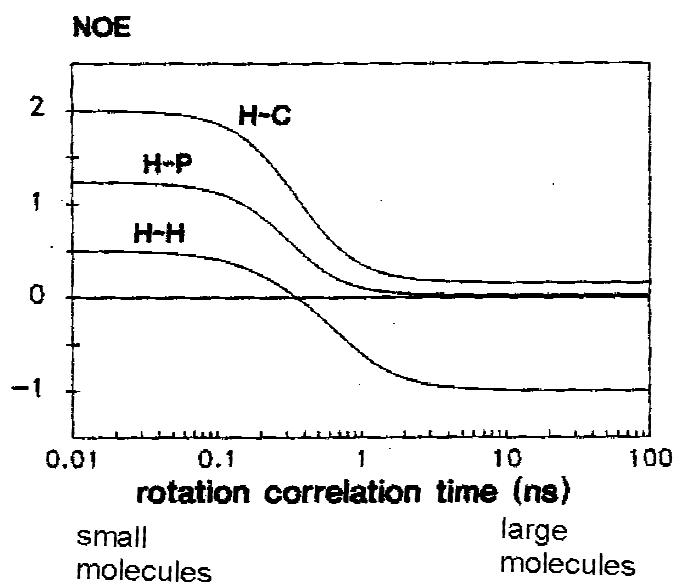
Note that the relaxation rates depend on the square of the dipolar coupling constant, and thus are related to inter nuclear distance by r_{AB}^{-6} .

Combining that with (8.1) and (8.3) and some more algebra for an homonuclear spin system ($I^A = I^B$) leads to

$$\eta_{AB}^{\max} = \frac{5 + \omega_0^2 \tau_c^2 - 4\omega_0^4 \tau_c^4}{10 + 23\omega_0^2 \tau_c^2 + 4\omega_0^4 \tau_c^4} \quad (8.6)$$

Lets consider some extreme cases:

- very small molecules; $D_{rot} \gg \omega_0$ or $\tau_c \omega_0 \ll 1$: $\eta_{AB}^{\max} = + 1/2$
- very large molecules or viscous solutions: $D_{rot} \ll \omega_0$ or $\tau_c \omega_0 \gg 1$: $\eta_{AB}^{\max} = -1$
- somewhere in between there will be a case with $\eta_{AB}^{\max} = 0$!!!

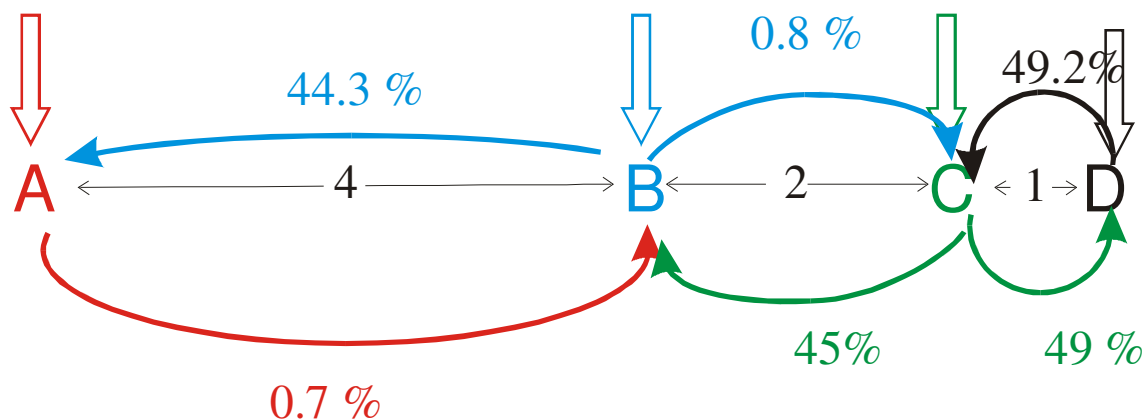


This leads to the apparently ridiculous result that the NOE does not appear on the distance A-B anymore, the measurement of which was the main reason for all the trouble. But note that we were talking of *maximum NOE* after achieving a steady state, considering an isolated pair of protons.

- For a two spin system it is not the NOE itself which depends on the distance, but the rate the NOE is generated, which is $\sigma = W_2 - W_0$. Therefore measurement of NOE buildup will be a more appropriate experiment in most cases.
Meaning: if there were only two protons in the universe, irradiating one would always eventually result in the same NOE no matter what the distance. But if they are 1 ly apart it will take the age of the universe to build up that NOE
- Usually more than two isolated spins are present and it is the competing effect of different dipolar couplings which will lead to different NOE values. *Only cross relaxation between A and B will contribute to the NOE between the two (numerator of 8.1), all other relaxation including dipolar interaction with other protons will contribute to the denominator only.*

8.3.2 Example linear four spin system:

If more than two spins are present, the maximum steady state NOE for a nucleus will be determined



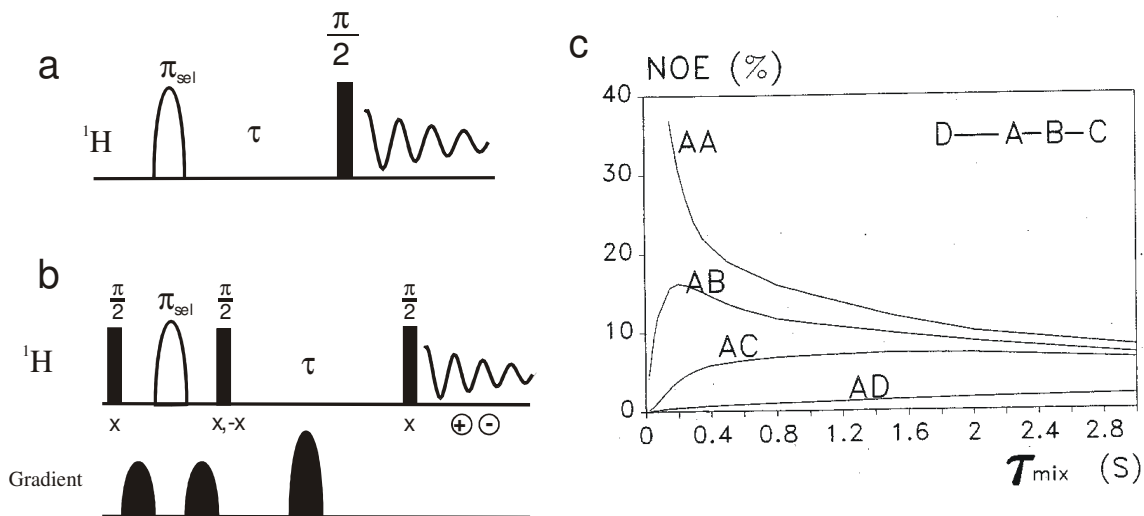
by the competition of relaxation pathways between different pairs of nuclei. We assume that only dipolar coupling between proton spins contributes to relaxation

- steady state NOE can be deceiving for isolated protons as they don't have a competing relaxation mechanism. As B is the only proton near A saturation of B will give almost full NOE on A
- On the other hand saturation of A has barely any effect on B due to the close proximity of C to B which will contribute to the denominator of (8.1). Reversely saturation of C will have a large effect on B.

In practice that means that more than one NOE should be considered before interpreting any result. Also NOE's to CH₃ groups will be weaker than the NOE from the CH₃ group as the methyl protons will relax each other, so saturating the methyl group will cause to larger NOE

8.3.3 Transient vs. steady state NOE

As it is the rate of cross relaxation ($\sigma = W_2 - W_0$) which is directly related to proton-proton distance it is usually more meaningful value to measure the rate of buildup. This can be achieved by the transient NOE experiment. Instead of a saturation one applies a selective 180° pulse on one proton resonance, waits for a time τ_{mix} and then excites all protons with a 90° pulse. As long τ_{mix} is

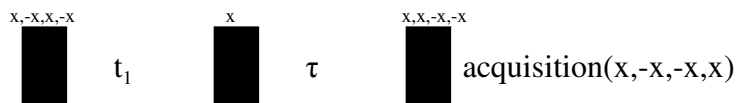


a) Basic transient 1D NOE experiment involving inversion of one proton using a selective 180° pulse. **b)** In the Pused Field gradient Spin echo 1D NOE experiment only signal from protons inverted by the selective 180° pulse are observed. The phase of the first two 90° pulses is cycled such that their total effect alternates between a 180° and a 0° pulse. **c)** NOE buildup curve for a linear four spin system. For short mixing times ($\tau < 200$ ms) the NOE intensity is roughly linear with the cross relaxation rate.

kept short enough ($\tau < 0.2\text{s}$ in the figure above), the NOE intensities between A and B will be proportional to the cross relaxation rate σ_{AB} . As shown in the figure the NOE A-D grows in at a much smaller rate than A-B or A-C. If one is not sure about the proper value of τ , multiple experiment with different length of τ should be acquired to establish linearity, or measure the full buildup curve. A newer gradient enhanced version of the 1D NOE experiment allows to filter only signal arising from the signal selected by the selective 180° pulse, with the reference experiment build into the phase cycle by reverting the phase on the second 90° pulse, thus alternating between inverted and non inverted spectrum and avoiding the need for later subtracting a reference spectrum.

8.4 Two dimensional NOESY

The 1D transient NOE experiment requires irradiating one signal at a time using a selective pulse. In practice often many distances are required. This is often best accomplished by a two dimensional experiment:



The Sequence is identical to DQF COSY with a mixing time τ added. However a different phase cycle is employed selecting the z-magnetization present after the second 90° pulse (term I in the description of the COSY experiment).

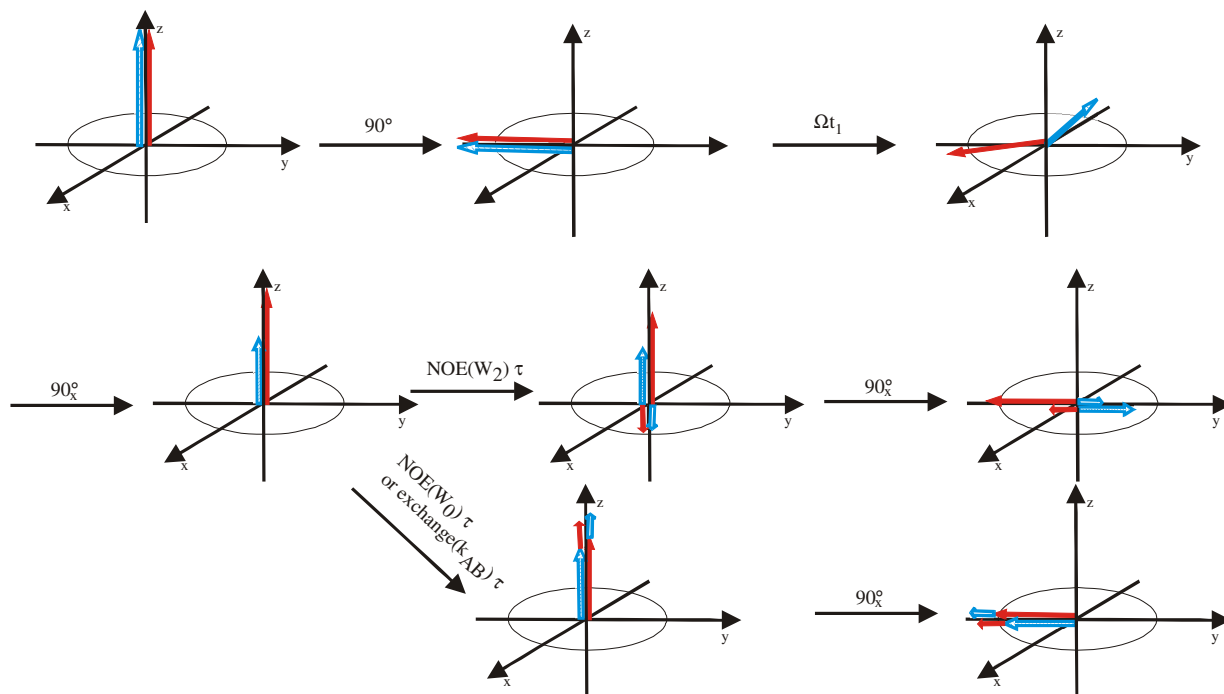
The Incremented delay t_1 will create z-magnetization modulated with chemical shift information (and J coupling if present):

$$I_z^A \xrightarrow{90^\circ_x} \xrightarrow{\Omega^A t_1} \xrightarrow{90^\circ_{x(\text{select } z)}} -I_z^A \cos(\Omega^A t_1)$$

During the mixing time τ cross relaxation and chemical exchange with spin B can take place with the rates σ_{AB} and k_{AB} , respectively. Both processes will create z-magnetization of spin B modulated with the chemical shift of spin A during t_1 :

$$\xrightarrow{\text{NOE}(W_2 - W_0) \tau, \text{ exchange}(k_{AB}) \tau} \underbrace{-I_z^A \cos(\Omega^A t_1) (1 - 1/T_1 \tau_m)}_{\text{Diagonal peak}} + \underbrace{\overbrace{(W_2 - W_0 - k_{AB}) \tau_m}_{\sigma_{AB}} I_z^B \cos(\Omega^A t_1)}_{\text{cross peak}}$$

A further 90° x pulse converts the z magnetization after τ into detectable y magnetization. The



linear relationship towards τ is only valid as approximation for short values of τ , otherwise a bi-exponential function has to be used.

case I: σ_{AB} dominated by W_0 (large molecules) or chemical exchange: negative σ_{AB}
=> Diagonal and cross peak have the same sign (both normally phased positive, negative NOE gives positive cross peaks)

case II: NOE dominated by W_2 (small molecules) positive σ_{AB}
=> Diagonal and cross peak have opposite sign (phase correction typically makes diagonal positive and cross peaks negative, positive NOE gives negative cross peaks)

- The two dimensional NOESY experiment will give cross peaks between protons exhibiting NOE between each other and which are therefore in close spatial proximity
- For short enough mixing times (small molecules 400 -800 ms, large molecules 100-200 ms) the cross peak intensity is proportional to the cross relaxation rate and thus to r_{ab}^{-6} .
- correlation time $\tau_c = 1/6D_{rot}$ is usually not known or may be different for different parts of the molecule in case of internal rotation. Therefore only relative distances can be determined. Usually known fixed distances can be used for calibration (geminal CH_2 , aromatic $CH=CH$, ...) or qualitative interpretation
- For small molecules NOE and chemical exchange will give cross peaks of opposite sign and can be distinguished.
- As sequence is identical to DQF-COSY, coupling can cause artefacts in spectrum. These will be anti-phase in nature

8.5 ROESY experiment

8.5.1 General

If the molecular weight is around 1000 g mol^{-1} D_{rot} is of the order of the lamor frequency and W_0 and

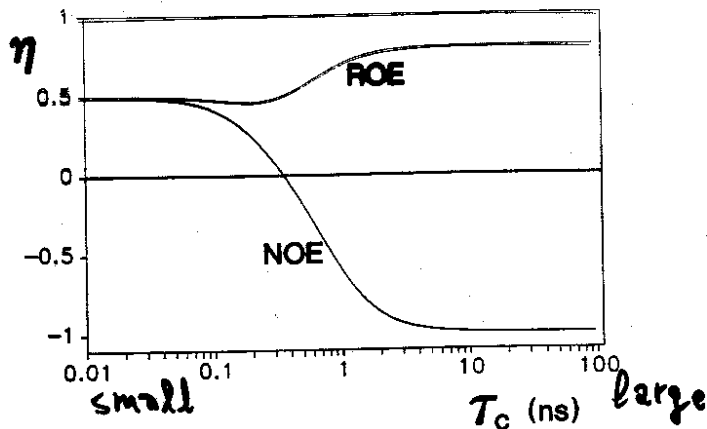


Figure 4.47. Nuclear Overhauser enhancement factor η , plotted as function of the rotation correlation time τ_c for NOESY and ROESY. The curves were calculated assuming a spectrometer frequency of 500 MHz and an interproton distance of 2.0 Å.

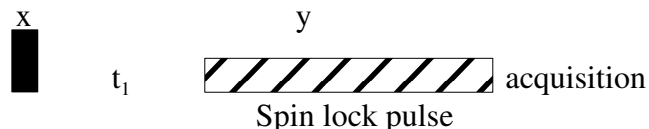
W_2 become very similar or equal, and NOE's will be very weak or zero no matter how close the distance ($5 + \omega_0^2\tau_c^2 - 4\omega_0^4\tau_c^4 = 0$). NOESY experiments will fail in that case.

The location of the zero NOE point is given by $\omega_0\tau_c = \sqrt{\frac{5}{4}}$ and thus depends both on ω_0 and τ_c .

For a given molecule both parameters can be varied to reach a region where NOE $\neq 0$:

- One can vary τ_c by changing temperature or solvent (viscosity). This option is often limited due to sample considerations
- change ω_0 . Using a different magnetic B_0 field. On first sight, this option appears even more limited in practice as extremely weak magnets would give poor sensitivity and resolution.
- However, relaxation taking place during a spin lock depends only on the effective field, which is the applied B_1 field, which is much weaker than the static B_0 field.

This fact is used in the ROESY experiment (Rotating Frame Overhauser Effect):



Instead of generating z-magnetization, y-magnetization will be locked along the y-axis by the spin

lock pulse. During that time the spins only experience the B_1 field of the spin lock pulse, so the effective larmor frequencies for transitions will be $\omega^{\text{eff}} = \omega_1^{\text{SL}} = \gamma_{\text{H}}B_1$ which is typically 3-5 kHz. Therefore the condition $D_{\text{rot}} \gg \omega^{\text{eff}}$ (or $\tau_c \cdot \omega^{\text{eff}} \ll 1$) will be always be fulfilled and NOE's will always be positive.

- ROE's will be observable for molecules where NOESY gives no signal
- also for large molecules (or low temperature) ROE peaks and exchange peaks will have opposite sign and can be distinguished (as ROE will be dominated by W_2)

8.5.2 ROESY versus TOCSY:

The ROESY experiment utilizes the same pulse sequence as the TOCSY experiment. The only difference are the strength and length of the spin lock pulse:

- TOCSY: $\gamma B_1/2\pi \approx 10$ kHz, $\tau_p^{\text{SL}} = 15 - 80$ ms, pulse is too short for effective ROESY
- ROESY: $\gamma B_1/2\pi \approx 1.5$ kHz, $\tau_p^{\text{SL}} = 400$ ms, field is too weak for effective TOCSY
- sign of TOCSY peaks is always same as diagonal (positive), for ROESY peaks it is opposite to diagonal (negative)

Therefore TOCSY spectrum spectra can exhibit weak ROE peaks, an ROESY spectra can exhibit weak TOSY peaks.

8.6 Examples and applications of NOE experiments:

8.6.1 Assignment of methyl groups in camphor

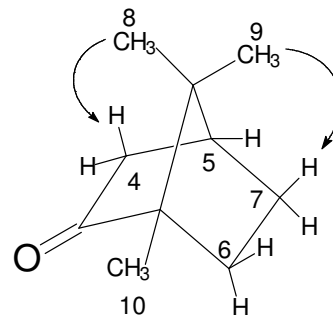
NOE's can be used to assign the methyl groups of Camphor. While C-10 can be identified by the HMBC experiment, that experiment can not distinguish between C-8 and C-9.

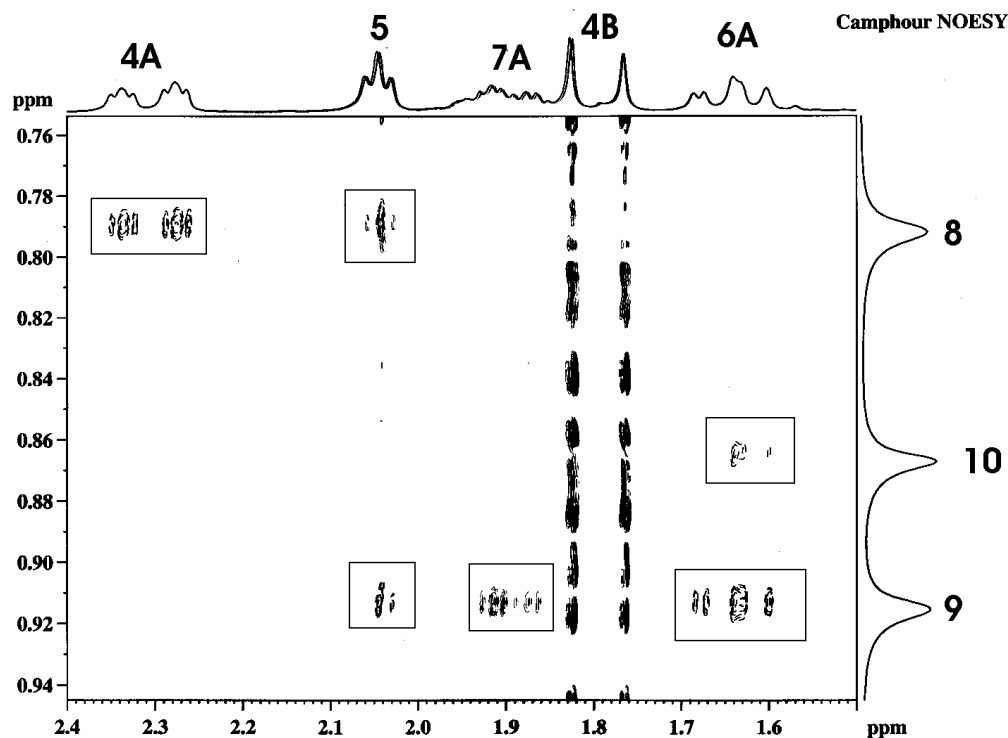
The 2D NOESY experiment shows very distinct NOE correlations for the three methyl groups:

$\text{CH}_3(8)$ correlates with C-H(4A) and C-H(5)

$\text{CH}_3(9)$ correlates with C-H(7A) and C-H(5)

$\text{CH}_3(10)$ correlates with C-H(6A)



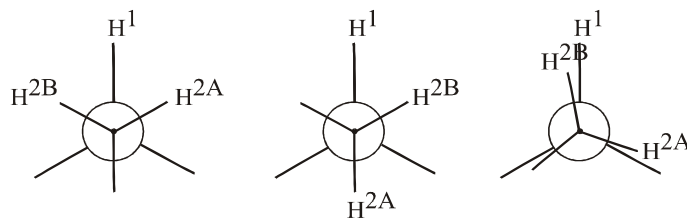


2D NOESY spectrum of Camphor: CH_3 -8 is the one on the side of the CO group as it shows NOE's to H-4A whereas CH_3 -9 exhibits NOE's to H-6A and H-7A. Also H7A appears to be the exo hydrogen and H7B the endo.

These data show that CH_3 (8) is on the side of the C=O group, and CH_3 (9) on the opposite side of the molecule. It also allows the assignment of the diastereotopic protons 4, 6, and 7, with C-H(4A), C-H(6A) and C-H(7A) pointing towards the top (bridged) side of the molecule, and C-H(4B), C-H(6B) and C-H(7B) pointing towards the bottom of the molecule.

8.6.2 NOE and conformational analysis

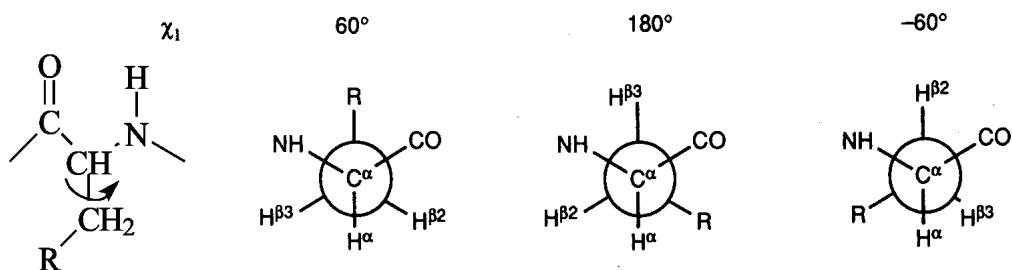
NOE's can be used to supplement coupling constants in the measurement of dihedral angles. For a pair of vicinal protons in a staggered conformation coupling- and NOE information is exactly complementary with large coupling constants accompanied by a relatively weak NOE 's (*trans*-interaction) and vice versa. However a different pattern is observed if an eclipsed conformations is present, and measuring a combination of NOE's and coupling constants will help to identify such conformations.



$J_{1,2A}$	< 5Hz	> 10 Hz	< 5 Hz
$J_{1,2B}$	< 5Hz	< 5 Hz	> 9 Hz
NOE(1,2A)	strong	weak	medium
NOE(1,2B)	strong	strong	strong

Combination of coupling constant and NOE for three different conformations.

In many examples vicinal coupling constants and -NOE's alone do not allow the determination of the conformation. In the example below (amino acid side chain) an additional distance (H^N-H^β NOE) is needed in order to determine the dihedral angle $N-C_\alpha-C_\beta-C_R$ and assign the diastereotopic protons of the prochiral $C^\beta H_2$ group.



${}^3J_{\alpha\beta 2}$ (Hz)	<4	<4	> 10
${}^3J_{\alpha\beta 3}$ (Hz)	<4	>10	<4
NOEs	$\alpha\beta 2 \sim \alpha\beta 3$	$\alpha\beta 2 > \alpha\beta 3$	$\alpha\beta 2 < \alpha\beta 3$
NOEs	$NH\beta 2 < NH\beta 3$	$NH\beta 2 \sim NH\beta 3$	$NH\beta 2 > NH\beta 3$

8.6.3 Structure determination of Macromolecules

NOE's and coupling constants can be used to determine three dimensional structures of macromolecules like proteins, nucleic acids and carbohydrates. Typically the sequence of aminoacids or nuclides is known (connectivity), but the folding (three dimensional structure) needs to be established, and the procedure usually follows the steps listed below:

Some special considerations when working with biological macromolecules:

- molecular weight of such compounds is ~5000 - 30000 g/mol:
- short T_2 => broad lines, experiments generating anti phase cross peaks through small couplings (COSY, HMBC) may fail. Experiments utilizing multiple steps of one-bond correlations work better than those utilizing long range couplings. In-phase transfer preferred over antiphase transfer (TOCSY vs. COSY)
- heavy overlap of lines due to complexity of molecule => highest magnetic field possible should be used, two dimensional experiments reach their limits
- usually computer software needed to keep track of assignment data
- Often more than two dimensions are needed to resolve all signals.
- Proteins can be produced using expression media, and enrichment with ^{13}C and/or ^{15}N which will allow experiments not possible with natural abundance samples

