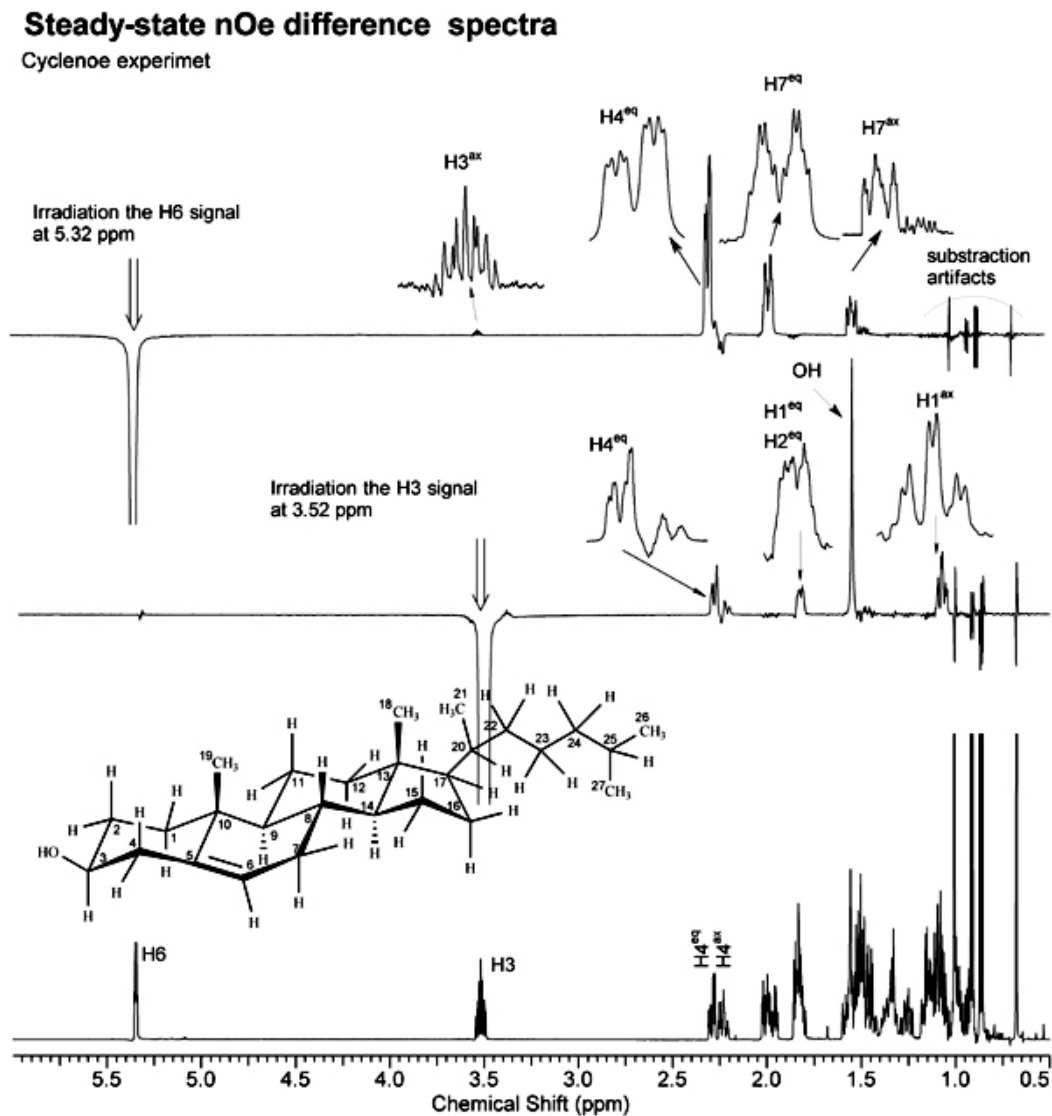


Dipole-dipole coupling as a through space effect in NMR

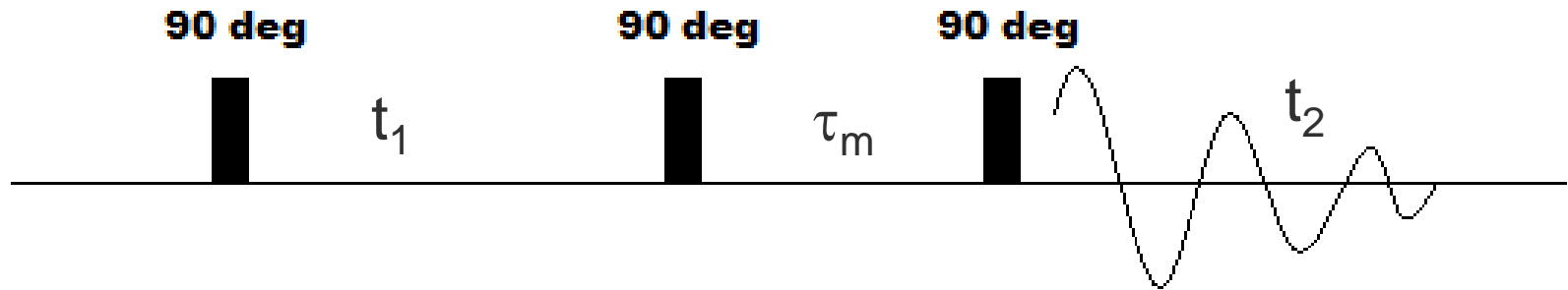
The methods discussed up to this point involve through-bond coupling, also known as scalar coupling. There is a separate type of interactions, which are known as through-space interactions that involve dipolar coupling. These are known collectively under the name of the Nuclear Overhauser Effect (NOE). There is a 1-D version of the NOE experiment that involves observing effects on a spin while exciting a neighboring spin. The intensity of the peak may be increased or decreased depending on the relaxation regime (i.e. the size of the molecule and the tumbling rate). The effect is only observed if the two spins are sufficiently close in space. The cutoff distance is considered to be 5 Å for the NOE.

The 1-D NOE experiment: method

In the 1-D NOE a spin is irradiated until it is saturated. This means that the population in the ground and excited states is equalized. Then one applies a 90° pulse and observes the difference in intensity on other peaks, i.e. relative to the FID obtained without the selective irradiation.



The basic NOE pulse sequence



The initial preparation consists of a 90° pulse followed by a variable delay or evolution time. Thus far the sequence is similar to COSY. However, the remaining pulse sequence differs in that there are two 90° pulses separated by a delay known as the mixing time. The first of these pulses rotates the magnetization from the transverse plane to the z-axis, which eliminates J-coupling. During τ_m , there is cross relaxation closely spaced nuclei. The final 90° pulse converts transferred magnetization into an observable transverse magnetization.

The steady state NOE

If the S spin is selective irradiated so that the S transition is saturated. Since the spin populations in the S transition are equalized the I spin will evolve to a new equilibrium quantity given the expression:

$$\frac{d\langle I_Z^{SS} \rangle}{dt} = -\rho_I (\langle I_Z^{SS} \rangle - \langle I_Z^0 \rangle) + \sigma_{IS}^{NOE} \langle S_Z^0 \rangle = 0$$

$$\frac{\langle I_Z^{SS} \rangle}{\langle I_Z^0 \rangle} = 1 + \frac{\sigma_{IS}^{NOE} \langle I_Z^0 \rangle}{\rho_I \langle S_Z^0 \rangle}$$

$$\frac{\langle I_Z^{SS} \rangle}{\langle I_Z^0 \rangle} = 1 + \eta_{IS}$$

The enhancement factor is

$$\eta_{IS} = \frac{\sigma_{IS}^{NOE} \gamma_S}{\rho_I \gamma_I}$$

The NOE enhancement in terms of the spectral density

Using the isotropic rotor spectral density function the cross-relaxation rate constants for the homonuclear case ($\gamma_S = \gamma_I = \gamma$) is:

$$\sigma_{IS}^{NOE} = \frac{\hbar^2 \mu_0^2 \gamma^4 \tau_c}{160 \pi^2 r_{IS}^6} \left\{ -1 + \frac{6}{1 + 4\omega_0^2 \tau_c^2} \right\}$$
$$\sigma_{IS}^{ROE} = \frac{\hbar^2 \mu_0^2 \gamma^4 \tau_c}{160 \pi^2 r_{IS}^6} \left\{ 2 + \frac{3}{1 + \omega_0^2 \tau_c^2} \right\}$$

The NOE enhancement is given by

$$\eta_{IS} = \frac{-1 + \frac{6}{1 + 4\omega_0^2 \tau_c^2}}{1 + \frac{3}{1 + \omega_0^2 \tau_c^2} + \frac{6}{1 + 4\omega_0^2 \tau_c^2}}$$

Narrowing and spin diffusion limits

The cross relaxation rate constants depend on the inverse sixth power of distance. However, the enhancement factor does not depend on distance. In the extreme narrowing limit $\omega_0\tau_c \ll 1$, the rate constants have the form

$$\sigma_{IS}^{NOE} = \sigma_{IS}^{ROE} = \frac{\hbar^2 \mu_0^2 \gamma^4 \tau_c}{32\pi^2 r_{IS}^6} \quad \text{and} \quad \eta_{IS} = \frac{1}{2}$$

In the spin diffusion limit $\omega_0\tau_c \gg 1$, these values are

$$\sigma_{IS}^{NOE} = \sigma_{IS}^{ROE} = \frac{\hbar^2 \mu_0^2 \gamma^4 \tau_c}{80\pi^2 r_{IS}^6} \quad \text{and} \quad \eta_{IS} = -1$$

Slow tumbling regime

In the slow tumbling regime we have the approximate relaxation

$$\sigma_{ROE} = -2\sigma_{NOE}$$

A special condition is observed when $\omega_0\tau_c = 1.12$. In that case $\sigma_{IS}^{NOE} = 0$ and $\eta_{IS} = 0$.

This regime is applicable to short peptides from 6-10 amino acids. In this regime the NOESY will not work and the ROESY must be implemented.

The 2-D NOE experiment: NOESY

The 2-D NOE is a powerful experiment that is widely used in the determination of molecule structure. This is the through-space complement to the COSY (a through-bond coupling experiment in 2-D). The most common NOESY is a ^1H - ^1H experiment. But others are possible.

The data in the NOESY will be presented in a 2-D format in which the cross-peaks represent dipolar interactions between peaks. Although the NOE effect is distance dependent, it is most common to use the existence of a NOE peak as a constraint on structure. One simply assumes that an observed NOE means that two ^1H nuclei are within 5 Å of each other.

Basic NOESY Pulse Sequence

The NOE pulse sequence shown below includes both preparation and mixing phases.

$$\left(\frac{\pi}{2}\right)_x - t_1 - \left(\frac{\pi}{2}\right)_x - \tau_m - \left(\frac{\pi}{2}\right)_x - t_2 -$$

The preparation of the NOESY is the same as for COSY. But after an evolution time t_1 a second $\pi/2$ pulse rotates the magnetization back to the z axis for mixing.

$$I_{1z} \quad \left(\frac{\pi}{2}\right)_x - t_1 - \left(\frac{\pi}{2}\right)_x$$

----->

$$-I_{1z} \cos(\Omega_I t_1) \cos(\pi J_{I2} t_1) - 2I_{1x} I_{2y} \cos(\Omega_I t_1) \sin(\pi J_{I2} t_1) \\ + I_{1x} \sin(\Omega_I t_1) \cos(\pi J_{I2} t_1) - 2I_{1z} I_{2y} \sin(\Omega_I t_1) \sin(\pi J_{I2} t_1)$$

The significance of the mixing time

The magnetization that is along the z-axis cannot mix by J-coupling. Instead there is mixing by dipolar coupling. This through space effect can occur even when the spins are aligned with the B_0 magnetic field.

$$-I_{1z} \cos(\Omega_I t_1) \cos(\pi J_{I2} t_1) \xrightarrow{\tau_m} - \sum_{k=1} I_{kz} a_{1k}(\tau_m) \cos(\Omega_I t_1) \cos(\pi J_{I2} t_1)$$

The $a_{1k}(\tau_m)$ are the mixing coefficients for transfer of magnetization through the spin system from spin I_1 to spin I_k . Following the final 90°_x pulse the density operator prior to t_2 is:

$$\sum_{k=1} I_{ky} a_{1k}(\tau_m) \cos(\Omega_I t_1) \cos(\pi J_{I2} t_1)$$

The final 90° pulse leads to acquisition

The final spectrum contains diagonal peaks and NOE cross peaks. The term produced by the mixing is a combination of zero-quantum and double-quantum terms. However, the double quantum operator is suppressed by phase cycling. The zero-quantum terms will precess according to the difference in chemical shift.

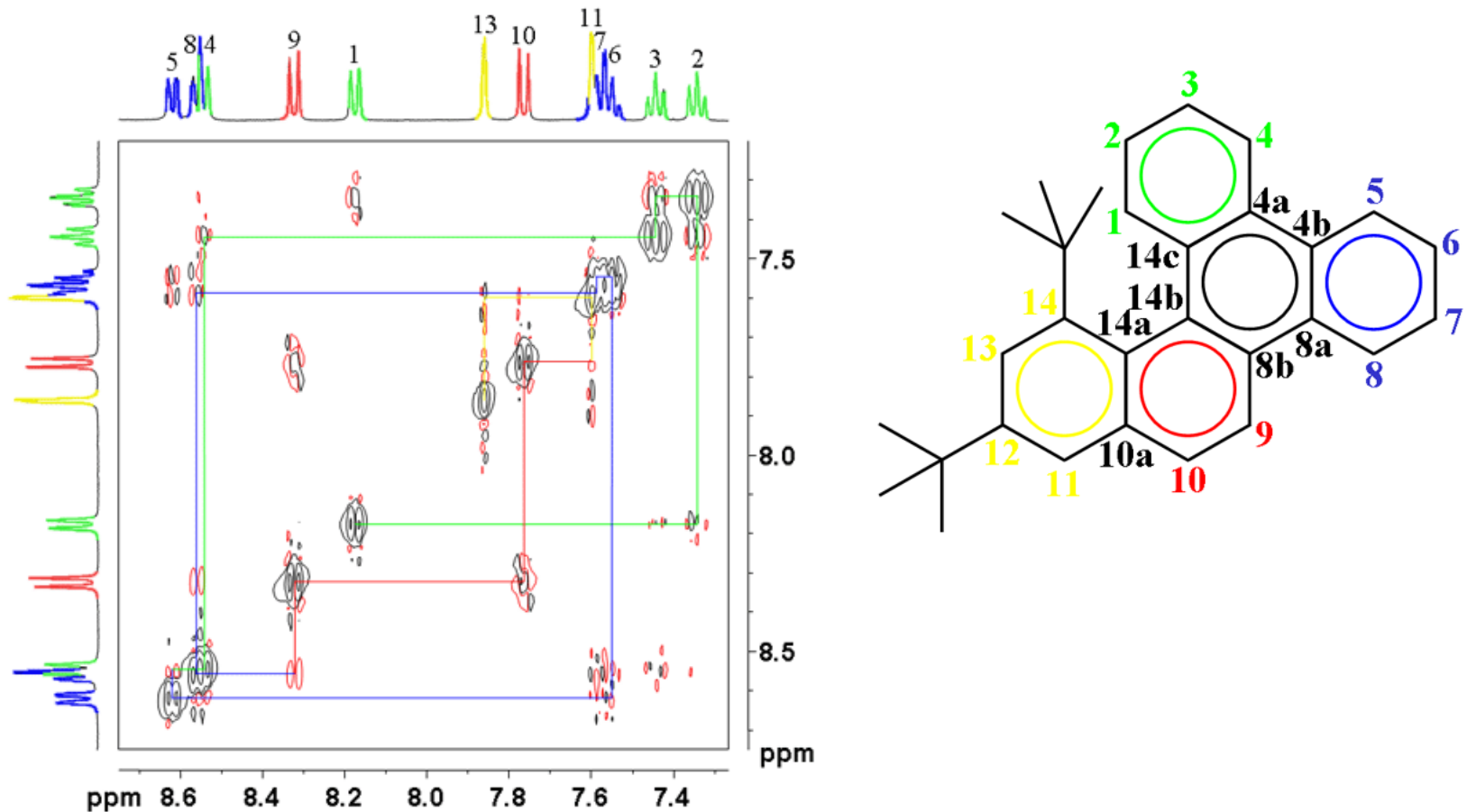
$$-ZQ_y^{12} \cos(\Omega_I t_1) \sin(\pi J_{I2} t_1) \xrightarrow{\tau_m - \left(\frac{\pi}{2}\right)_x}$$

$$\frac{1}{2} [I_{1x} I_{2x} + I_{1z} I_{2z}] \cos(\Omega_I t_1) \sin(\pi J_{I2} t_1) \sin[(\Omega_I - \Omega_2) \tau_m]$$

$$- \frac{1}{2} [I_{1z} I_{2x} - I_{1x} I_{2z}] \cos(\Omega_I t_1) \sin(\pi J_{I2} t_1) \cos[(\Omega_I - \Omega_2) \tau_m]$$

The mixing time should be of the order $1/R_1$. A longer mixing time will minimize zero-quantum peaks, but will permit spin diffusion, which can complicate the analysis.

NOESY Spectrum



Using a COSY experiment only a partial analysis of the molecule 12,14-ditertbutylbenzochrysenes is possible. NOESY provides extra information about the connectivity allowing a full assignment.