Introducing NOE

Previously we have looked at experiments that exploit 'scalar' coupling. However, NOE interactions occur through dipolar coupling. NOE is normally used after the majority of the structure of a molecule has been characterised. NOE is also used routinely for sensitivity enhancement.

TABLE 9.1 Principal Applications of the Main Techniques Described in This Chapter

Technique	Principal Applications
NOESY[a]	Establishing NOEs and hence spatial proximity between protons. Suitable for small ($M_r \ll 1000$) and large ($M_r > 2000$) molecules for which NOEs are positive and negative, respectively, but may fail for mid-sized molecules (zero NOE). Observes *transient* NOEs generated from *inversion* of a target resonance. Estimates of internuclear separations can be obtained in favourable cases.
ROESY[a]	Establishing NOEs and hence spatial proximity between protons. Suitable for any molecule but often essential for mid-sized molecules; NOEs are positive for all molecular sizes. Observes *transient* NOEs in the rotating frame, but is prone to interference from other mechanisms, so requires cautious interpretation. Estimates of internuclear separations can be obtained in favourable cases.
NOE difference	Establishing NOEs and hence spatial proximity between protons. Suitable only for small molecules ($M_r \ll 1000$), for which NOEs are positive. Observes *steady-state* or *equilibrium* NOEs generated from *saturation* of a target resonance. Above methods favoured nowadays.
HOESY[a]	Establishing heteronuclear NOEs and hence spatial proximity between different nuclides (eg ^{1}H–^{13}C or ^{1}H–^{19}F). Can provide useful stereochemical information when homonuclear NOEs prove inadequate. Often suffers from low sensitivity, but ^{1}H-detected variants can help.
EXSY[a]	Qualitative mapping of exchange pathways in dynamic systems when exchange rates are slow on the NMR chemical shift timescale, meaning separate resonances are observed for each exchanging species. Quantitative data on exchange kinetics can be obtained in favourable cases.
RDCs	RDCs provide information on relative bond vector orientations within a molecule and may be used to define the relative configurations and conformations of molecules. These methods require that the sample be weakly aligned in an appropriate medium for RDCs to be apparent.

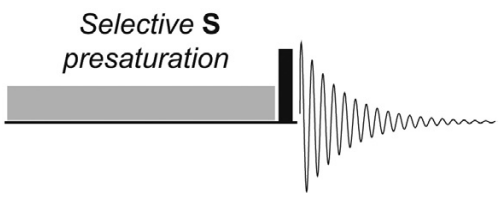
The molecular masses mentioned provide only approximate ranges over which the experiments are applicable (see main text).

[a]*These methods may be executed as 1D or 2D experiments.*

The NOE may be defined as the change in intensity of one resonance when the spin transitions of another are perturbed from their equilibrium populations. We can perturb a spin to get an NOE by *Saturating* or *Inverting* it. *Saturate* a resonance, that is equalising the spin population differences across the corresponding transitions. *Inverting* a resonance (inverting the population differences across the transitions).

Steady state NOE

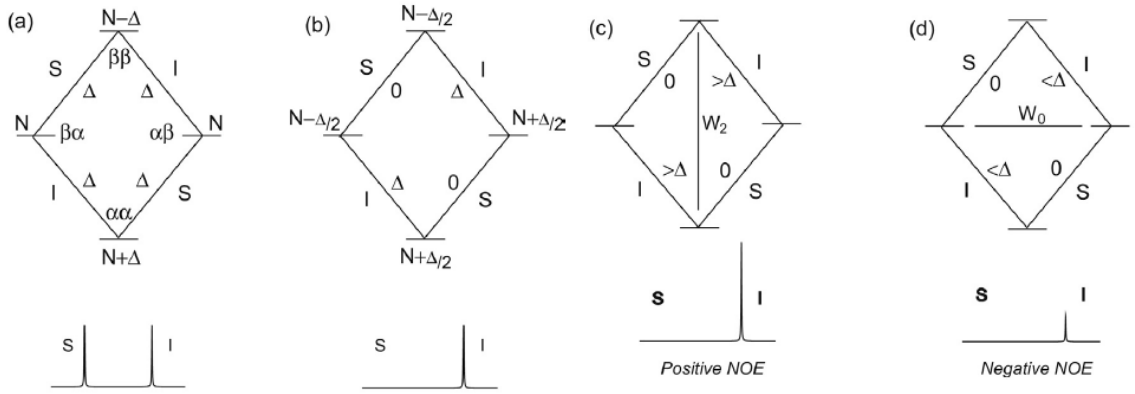
Steady-state NOE signals are brought about by *saturating* specific spin transitions by selective application of weak radiofrequency (rf) irradiation to a resonance. This form of the NOE is observed with the largely deprecated NOE difference method.



Origin of the NOE

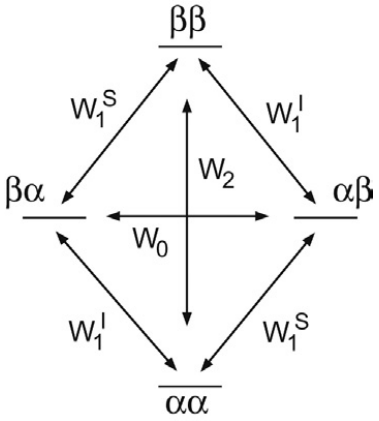
Consider two homonuclear spins-½ that exist in a rigid molecule which tumbles isotropically (has no preferred axis about which it rotates) in solution. The two nuclei do not share a scalar coupling, but do share a dipolar coupling. Dipolar coupling is the direct, through-space magnetic interaction between the two spins such that one spin is able to sense the presence of its dipolar-coupled partner. This coupling may be viewed as being analogous to the interaction one witnesses when two bar magnets are brought close together.

Population differences and cross relaxation

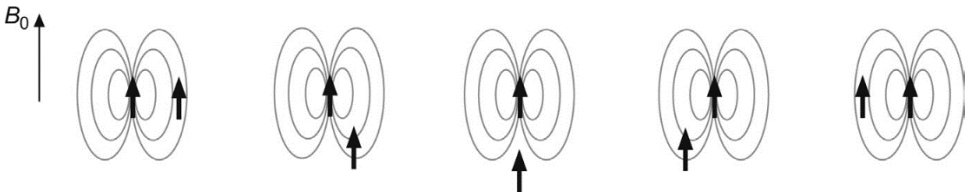


(a) At equilibrium, (b) after instantaneous saturation of the S spins, (c) after relaxation via 'W_2' processes and (d) after relaxation via 'W_0' processes. Below each are the corresponding schematic spectra. The W labels represent transition probabilities – the rates at which the corresponding spin-flips occur.

W_2 and W_0 cross-relaxation processes compete with one another, with the dominant pathway dictating the sign of the observed NOE. W_1 pathways quench NOE.



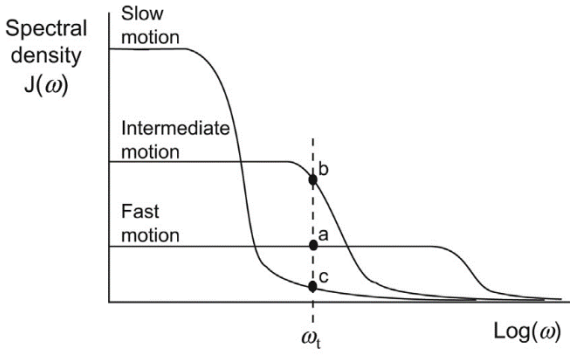
The NOE arises as a result of the redistribution of spin populations and hence flips between spin states. Redistributions occur via longitudinal (T_1) relaxation, which is not spontaneous and requires a magnetic field fluctuating at the field of the corresponding transition. The dipolar coupling between spins is acutely sensitive to the internuclear separation *r*, being proportional to r^{-3}



Correlation Time

Rotational *correlation time* τ_c is the rate at which a molecule tumbles in solution (the average time required for the molecule to rotate through an angle of one radian about any axis. Rapidly tumbling molecules possess small correlation times while slowly tumbling molecules have large correlation times. A *very rough* estimate of this time for a molecule of mass *M*r may be obtained from the relationship: $\tau_c = M_r \times 10^{-12}$ s

Spectral Density

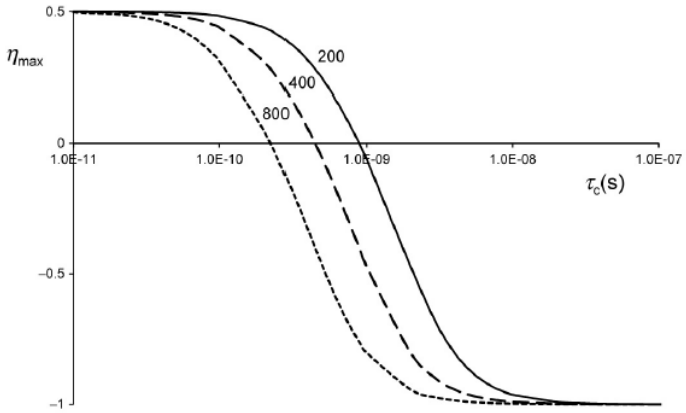


The power available within a molecular system to induce transitions by virtue of its molecular tumbling is referred to as the *spectral density* J(*w*).

J(*w*) provides a measure of how the relaxation rates W_0, W_1 and W_2 vary as a function of tumbling rates.

Molecules which tumble rapidly in solution are likely to favour the higher energy W_2 process and hence *exhibit positive NOEs.*
Molecules that tumble slowly will favour the W_0 process and thus *display negative NOEs.*

NOE and Magnetic Field



τ_c depends on the size and shape of the molecule, solution conditions (viscosity, temperature, possibly pH, etc.) and spectrometer field strength. The use of a higher field strength increases the likelihood of a relatively 'small' molecule falling within the intermediate regime. As a rule of thumb, molecules with a mass of 1000–2000 Da are likely to fall within this intermediate regime

Heteronuclear NOEs

TABLE 9.2 Theoretical Maximum Steady-State Heteronuclear NOE Enhancements in the Presence of Proton Saturation

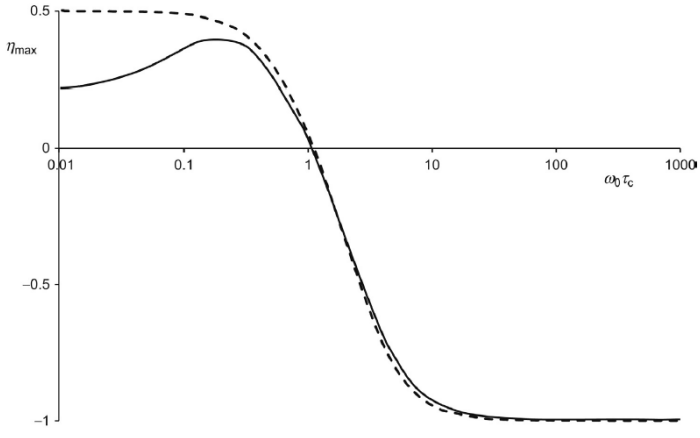
X	^{6}Li	^{7}Li	^{13}C	^{15}N	^{19}F	^{29}Si	^{31}P	^{57}Fe	^{103}Rh	^{109}Ag	^{119}Sn	^{183}W	^{195}Pt	^{207}Pb
$\eta_X\{^1H\}$ (%)	339	129	199	–494	53	–252	124	1548	–1589	–1075	–141	1202	233	239

These numbers assume relaxation exclusively via dipole–dipole interactions, although for the metals, in particular, chemical shift anisotropy may also be a significant mechanism. The lithium isotopes are somewhat anomalous in that they are quadrupolar yet can still demonstrate NOEs. ^{6}Li in particular has the smallest quadrupole of all such nuclei, so the dipole mechanism still makes a significant contribution to relaxation.

For ^{13}C observation in the presence of proton saturation (broadband decoupling) NOE enhancements can be as much as 200% or a threefold intensity increase.

$$\eta_I\{S\} = \frac{\gamma_S}{2\gamma_I}$$

Effect of other relaxation



Maximum homonuclear steady-state NOE in the presence (solid line) and absence (dotted line) of external relaxation sources that compete with cross-relaxation.

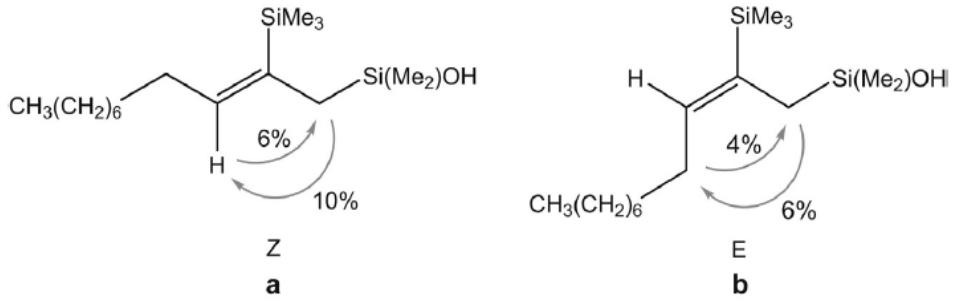
Tips for maximising the size of the NOE

- Remove **paramagnetic oxygen** dissolved in solvents. Degassing of solutions prior to NOE studies may become necessary when seeking longer range interactions in small molecules, but is otherwise unnecessary for the majority of routine studies.
- Other **paramagnetic impurities** will quench the NOE and must be avoided. Samples to which relaxation agents have been deliberately added to promote relaxation are not suitable candidates for NOE studies.
- Solvent molecules are deuterated in most cases, so the relaxation arising from these nuclei is rather inefficient when compared with protons and interactions with other (protonated) solute molecules are generally only likely to be a problem when **very concentrated solutions** are used, so these are thus best avoided for NOE studies.
- Avoid molecules which show relaxation associated with nuclei with spin >½. This is usually the dominant mechanism for such nuclei, so NOEs on **quadrupolar nuclei** are very rarely observed (the exception being ^{6}Li)

Applications – E vs Z

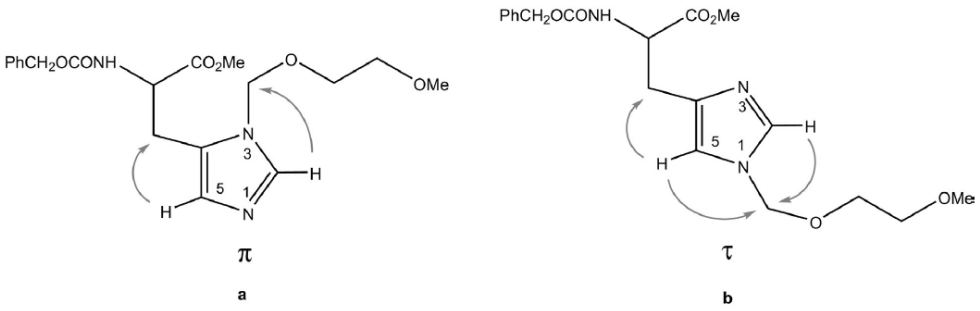
The differentiation of *E* and *Z* alkene isomers is often possible by direct measurement of vicinal proton–proton couplings across the unsaturation, whereby *cis* and *trans* couplings are usually sufficiently different to allow a distinction to be made (typically J_{cis} 7–11 Hz, J_{trans} 12–18 Hz).

When only a single alkene proton exists this method can no longer be used and the NOE then offers an alternative approach provided a protonated group exists across the double bond.



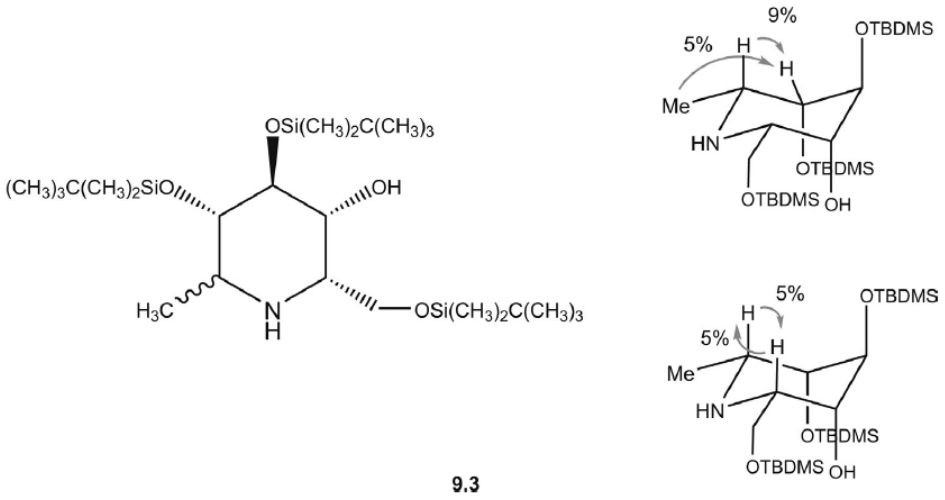
Applications – Aromatic Substitution Position

Determining the position of substitution within a molecule can also be problematic when no direct proton–proton couplings exist. Particularly when dealing with aromatic systems, the NOE can often provide unambiguous solutions. These systems are particularly favourable because they are restricted to being planar and, hence, it is usually safe to assume that nearest neighbours will be those on adjacent positions in the ring. Prior to the use of the NOE, differentiation was possible only through chemical degradation or through empirical rules based on differences in the small (<1.5 Hz) H_2–H_5 coupling constant. An alternative approach to consider nowadays in such cases would be to establish connectivity by identifying long-range proton–carbon correlations across the heteroatom via the heteronuclear multiple bond correlation (HMBC) experiment.



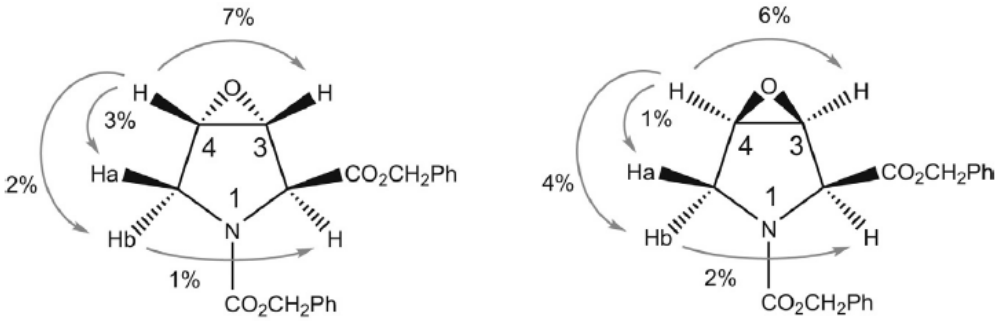
Applications – Substituent Configuration

Arguably the most common problem the NOE is used to address in synthetic chemistry, is the relative configuration of substituents on ring systems. For six-membered rings in particular, direct analysis of proton-coupling constants within the ring can in itself be informative because of the generally distinct differences in *axial–axial versus axial–equitorial/equitorial–equitorial* coupling constants in chair conformations (eg *ax–ax* ≈ 10–12 Hz, *ax–eq*/*eq–eq* ≈ 2–5 Hz). However, in determination of the orientation of the methyl group in this case these would not have been informative, because of the similarity between *ax–eq* and *eq–eq* coupling constants.



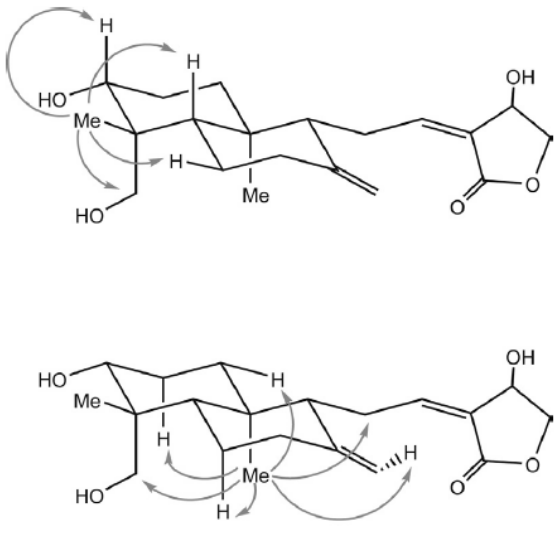
9.3

Vicinal couplings in five-membered rings generally offer greater ambiguity in defining relative configurations and here careful NOE measurements are also required since *cis* and *trans* NOEs between adjacent protons are often of similar magnitude. In these cases it is wise to collect as many NOE enhancements as is possible for the sample and to ensure self-consistency over all of these within the proposed stereochemistry.



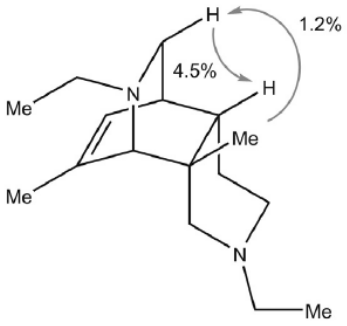
Applications – Resonance Assignment

Most NOE studies first require assignment of the proton spectrum. In some cases the NOE can be used for resonance assignment. It is most likely to be of use for assigning resonances of isolated groups that share no proton scalar couplings, although it is by no means limited to this. The use of long-range heteronuclear correlation experiments should also be considered when addressing such problems, and may be needed in determination of the gross structure in the first place.

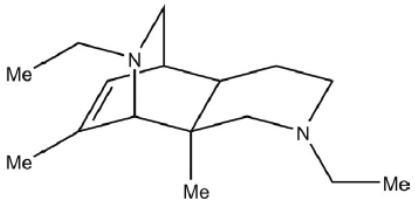


Applications – Endo versus Exo Adducts

NOE can be used to distinguish *exo* and *endo* adducts in fused ring systems. In such cases the NOE patterns observed between the proton(s) at the junction and those on the adjacent rings can often provide an unambiguous stereochemical assignment. Here the bridgehead proton was in fact too heavily overlapped with an adjacent resonance to be selectively saturated, although NOEs on to this were quite distinct. When bridgehead protons cannot be used at all, or if non-existent, the assignment must then rely on the observation of NOEs between ring protons on either side of the junction.



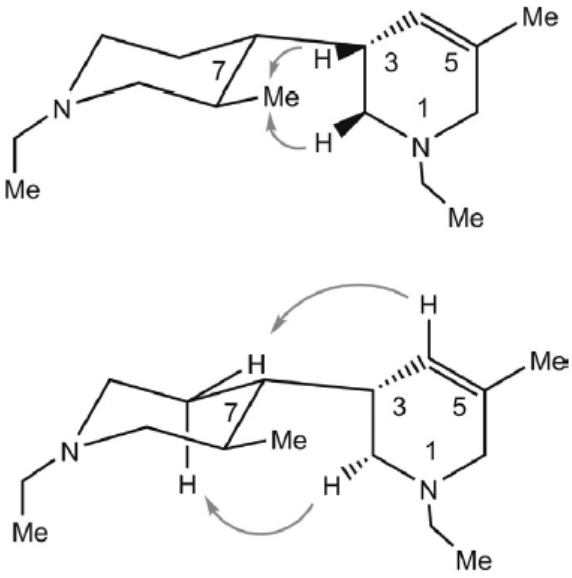
endo



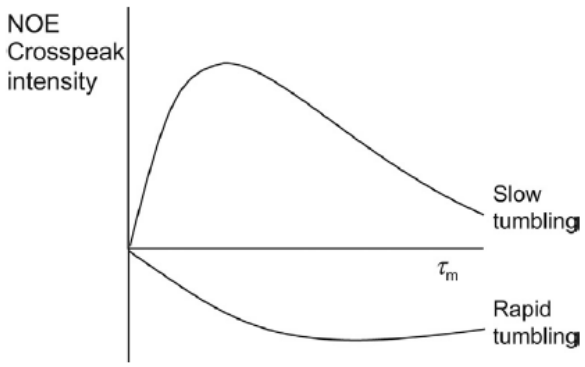
exo

Applications – Conformational Preference

The definition of a favoured conformation in small, flexible molecules by use of the NOE is very difficult. Rapid interchange between many possible conformations means the NOE will represent a weighted average of internuclear separations present within the conformers. When restricted conformational processes lead to one conformation being strongly favoured, sufficient NOE data may be available which allow this to be defined. Examples include the differentiation of chair and boat conformers of cyclohexanes or of slowly interconverting rotamers. Specific conformations may also be favoured in the presence of steric hindrances or strong hydrogen-bonding interactions.



Mixing time

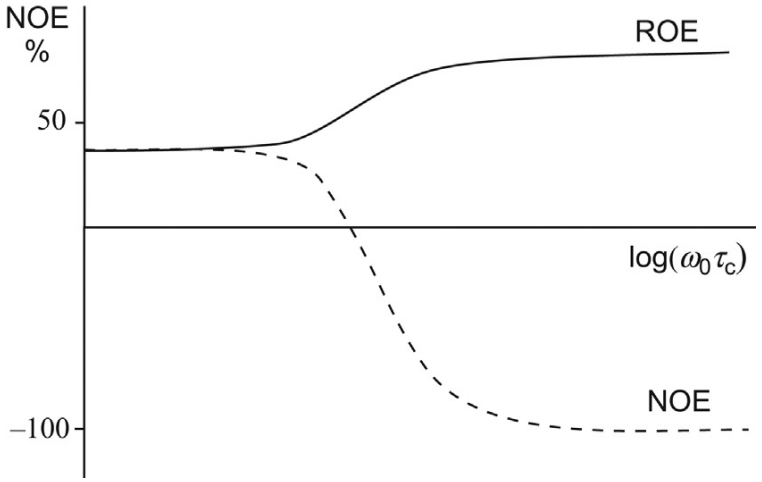


Some tips for setting up NOE experiments

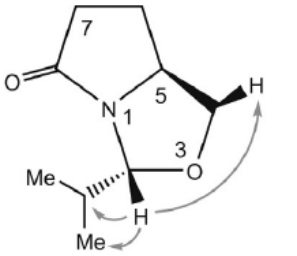
For quantitative measurements, mixing times must be significantly shorter than the T_1 relaxation time of spin *I* (observed, not saturated/irradiated) must be used. If the goal is to qualitatively identify through-space correlations, as is more often the case in routine work, mixing periods comparable with T_1 provide maximum enhancements.

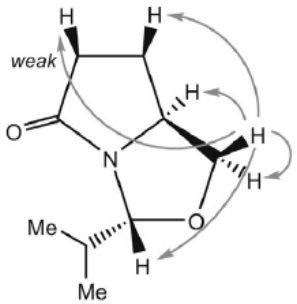
Rotating Frame NOEs (ROESY)

An NOE exploiting experiment (ROESY) is available that avoids the zero crossing point.



1D NOESY





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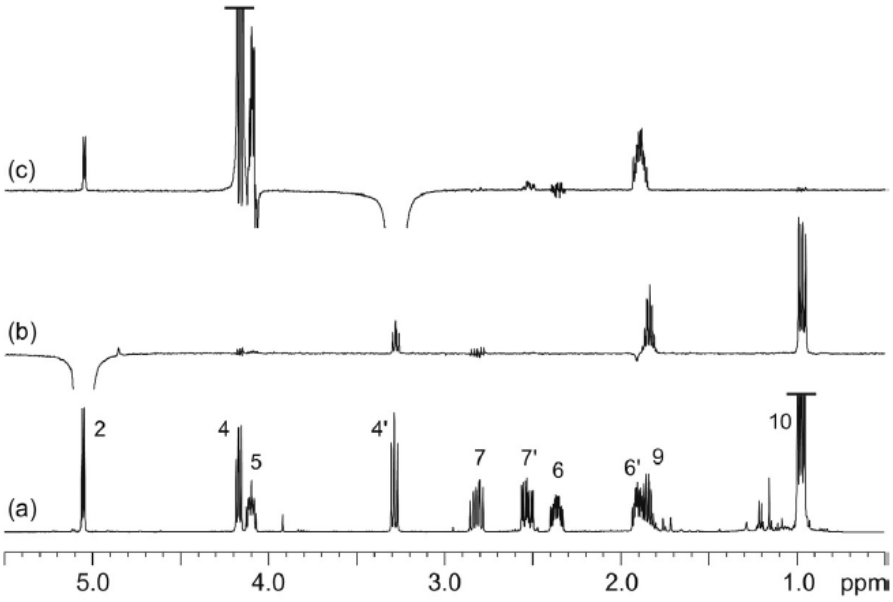
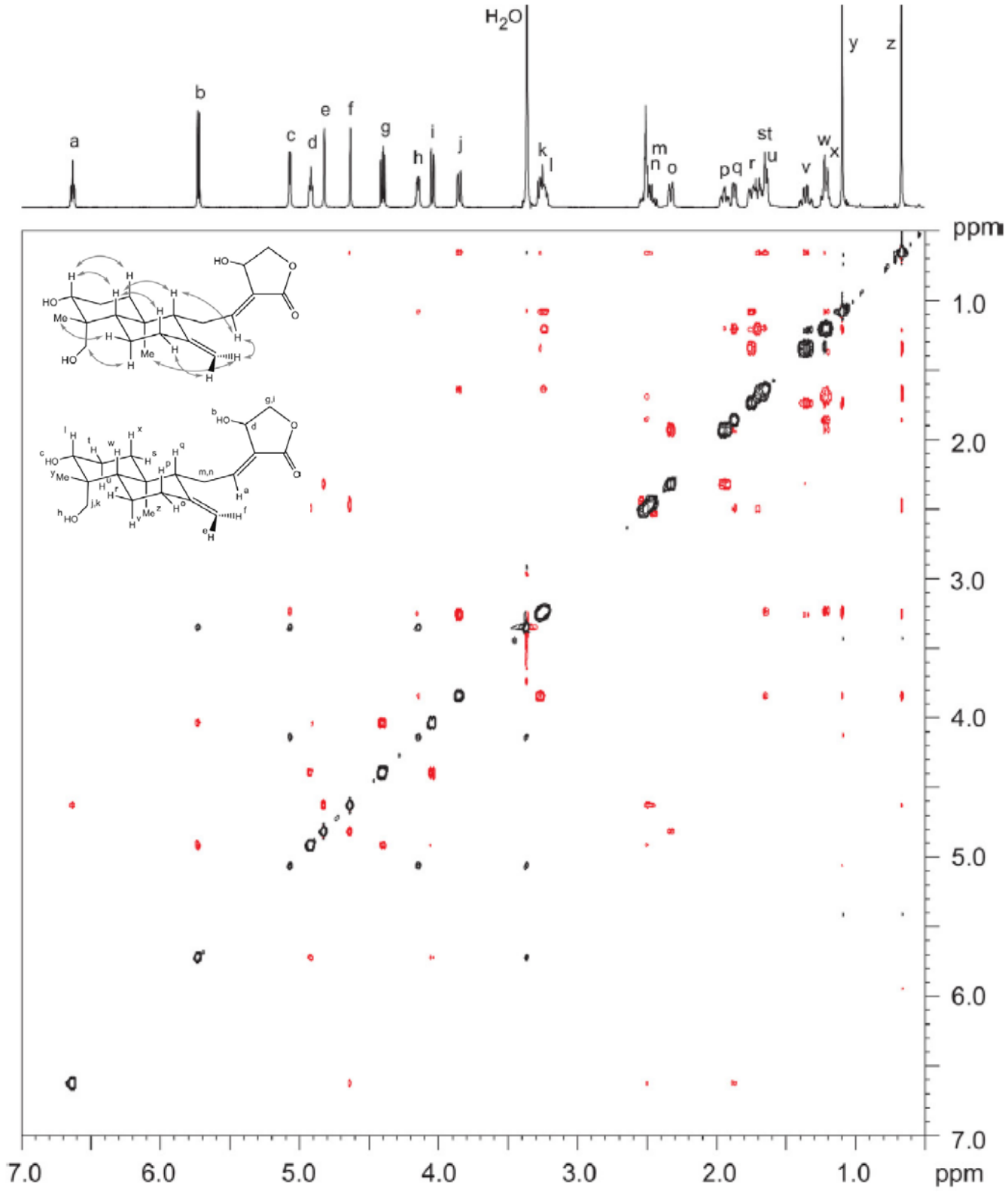


2D NOESY


H2O
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7.0
7.0
6.0
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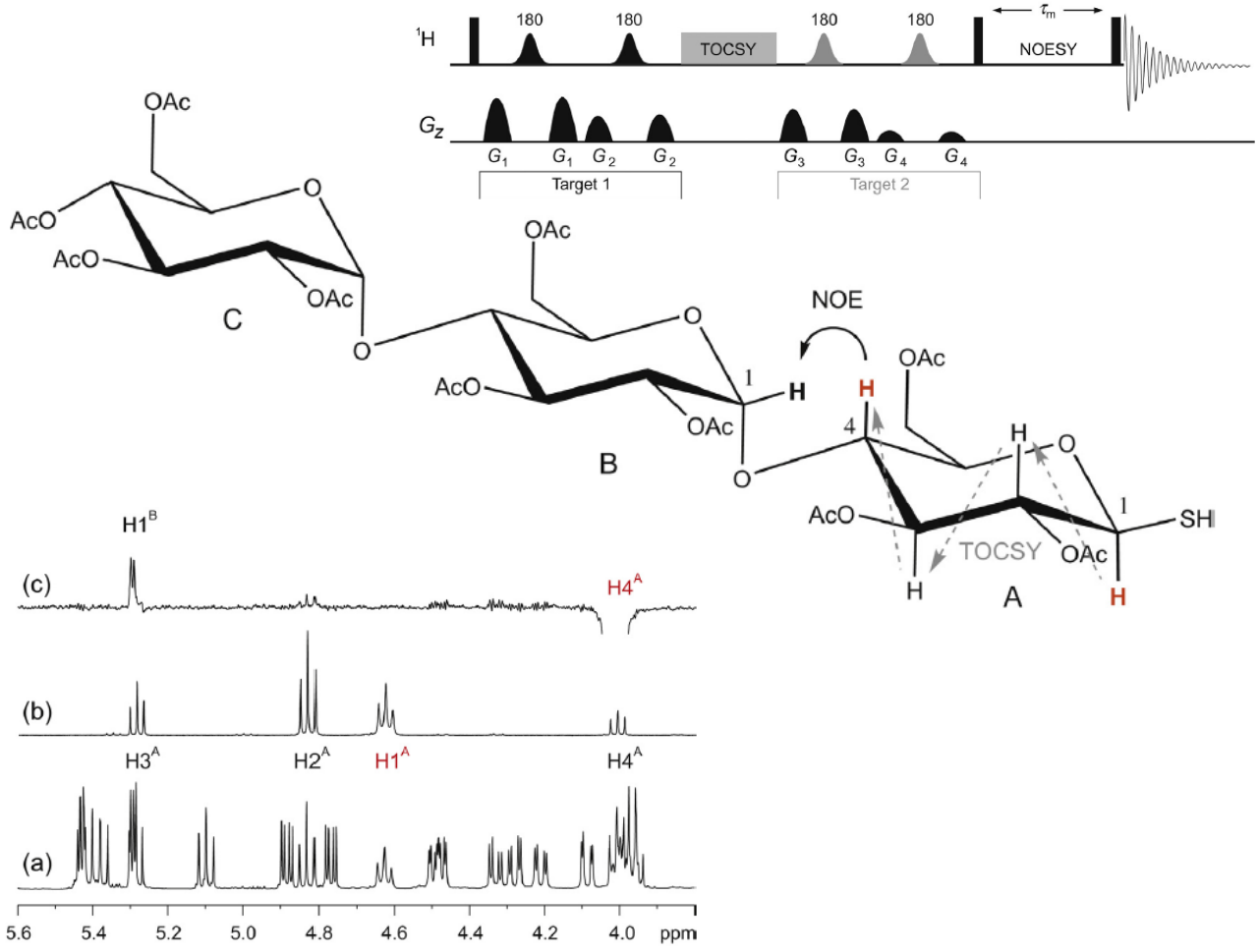
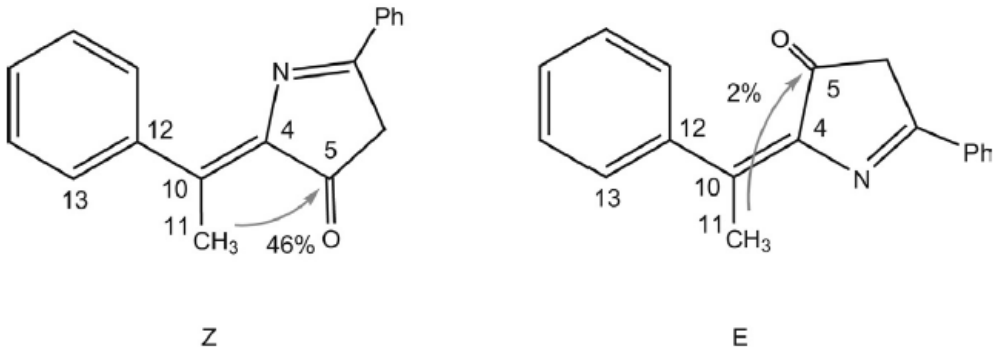
CSSF, Doubly Selective TOCSY-NOESY, HSQC-NOESY, and 2D-pure shift-NOESY

1D selective NOESY experiments need a well-resolved peak for selective inversion. One approach is to incorporate a chemical shift selective filter (CSSF) in the 1D NOESY sequence. The CSSF enables selection of a single resonance at a specified chemical shift even when the resonance is overlapped with others. An alternative approach is to employ a *selective* transfer preparation step from a well-resolved resonance *onto* the overlapped target resonance so as to reveal this. This leads to a concatenated or doubly selective experiment for which a selective TOCSY preparation element is well suited to generating the initial transfer. HSQC can also be used, with a selective pulse used at the chemical shift of the ^{13}C connected to the ^{1}H of choice. If resonances overlap in a high resolution 2D NOESY, pure shift methods can often be used to resolve the signals.



^{13}C HOESY (^{19}F is more sensitive)



Experimental considerations for NOE measurements

You need a well-prepared sample. Sovent (viscosity) and sample temperature has greater influence on the NOE than for most experiments (useful for mid-sized molecules close to the zero NOE condition. It is worth avoiding very high solute concentrations. Paramagnetic impurities should be removed, notably certain metal ions and molecular oxygen. Metal ions can be removed by filtration through suitable chelating resins whereas oxygen may be removed with the freeze–pump–thaw method. In general, the smaller the molecule and the further the internuclear separations being investigated, the greater the gain from solvent degassing. The use of lock solvents with temperature-dependent shifts, notably D_2O, calls for particular attention regarding temperature stability and will likely benefit from active temperature regulation if experiments are to be acquired over some hours. Sample spinning is usually detrimental to NOE experiments.

Some other points of note about NOE

The NOE is the change in intensity of the resonance of a nuclear spin, I ('interesting'), when the population differences across the transitions of a near neighbour, S ('source'), are perturbed from their equilibrium values, usually by *saturation* or by *population inversion*. It arises as the perturbed spin system alters its spin populations in an attempt to regain the equilibrium condition.

Steady-state NOEs are those measured after a period of continuous S-spin saturation during which a new 'steady-state' equilibrium condition has developed for the I-spin populations. The NOE enhancement is usually denoted ηI{S} and may be quantified as a percentage.

Steady-state NOEs can provide information on relative internuclear distances only—not on absolute measurements of internuclear separation.

The NOE only arises between nuclei that share a mutual *dipolar coupling* (a direct, magnetic through-space interaction) and thus relax each other via the dipole–dipole relaxation mechanism. Only this mechanism contributes to the nuclear spin population changes that produce the NOE, which is intimately related to longitudinal spin relaxation. The dependence on internuclear separation that makes the NOE so useful has its origins in the strength of dipolar coupling between two spins, this being inversely proportional to their separation rIS (as rIS–3).

Longitudinal spin relaxation requires a stimulus in the form of a magnetic field fluctuating at a frequency equal to the frequency (energy) of the transition. In the case of dipolar relaxation, this arises from the time-dependent field a spin experiences from its dipolar-coupled neighbour as the molecule rotates or tumbles in solution. The NOE in turn is dependent upon the rates of molecular tumbling.

Rapid molecular tumbling, corresponding to a short correlation time τc, favours the higher energy W_2 process, so small molecules in low-viscosity solvents display positive homonuclear NOEs (the *extreme narrowing limit*). In contrast, slow molecular tumbling (long correlation times) favours the lower energy W0 process, meaning large molecules or smaller molecules in high-viscosity solvents display negative homonuclear NOEs (the *spin diffusion limit*).

The maximum possible positive NOE is $\gamma_S/2\gamma_I$; in other words, 50% in a *homonuclear* system. Enhancements in *heteronuclear* systems can be far larger, for example 199 % from ^{1}H to ^{13}C, and can serve as a useful source of sensitivity enhancement in the observation of low-γ I spin. They also become negative if one of the γ_s is negative, for example −494% from 1H to 15N, and may in some cases lead to severe signal reduction or even disappearance.

The maximum possible negative NOE in a homonuclear system is –100%.

Between the extreme narrowing and spin diffusion limits lies the difficult region in which NOEs can become zero (when $w_0\tau_c \approx 1$) or at least rather weak, often demanding a change in experimental conditions or the use of rotating frame NOE measurements.

In an isolated homonuclear two-spin system in the extreme narrowing limit relaxing exclusively via the dipole–dipole mechanism, the steady-state NOE is predicted to be +50% and *independent of internuclear separation* r_{IS}. However, the initial *rate* at which the NOE grows is proportional to r_{IS}^{-6}.

In a more realistic multi-spin system, neighbouring nuclei N that are close to I can also contribute to its W_1 relaxation pathway. The magnitude of the NOE then becomes dependent on the I–S internuclear separation (inversely as r_{IS}^{-6}), but also has a dependence on the distance(s) between I and its near neighbour(s) (inversely as r_{IN}^{-6}), amongst other factors.

The direct consequence of this is that to correctly interpret steady-state NOE data, it becomes essential to consider not only the I–S internuclear separation, but also the proximity of all other nuclei (relaxation sources) surrounding I.

This also means that steady-state NOEs are rarely symmetrical. That is, the NOE observed at spin A on saturating spin B, ηA{B}, is unlikely to equal the reverse measurement from saturating A and observing B, ηB{A}. This is a consequence of the fact that the neighbours surrounding A are unlikely to match those surrounding B in number and in distance.

The relaxation of a spin with a very near neighbour will be dominated by this neighbour and, as a consequence, NOEs on this spin from a more distant source spin will tend to be small. Conversely, nuclei that have no nearby neighbours will experience relaxation only from distant neighbours and, as a result, NOEs from these neighbours will be large despite the relatively large internuclear distance involved. Thus, it is to be expected that NOEs over similar distances on a methylene and on a methine proton will generally be somewhat smaller for the methylene proton since this will always have at least one near neighbour, its geminal partner.

Longer range NOEs build up only slowly, due to the r^{-6} rate dependence, so require long saturation periods before becoming appreciable.

NOE enhancements may also be *relayed* on to neighbouring spins. For example, an NOE from A to B may be further passed onto a spin C that also cross-relaxes with B, such events being referred to as *indirect effects*. The properties of indirect enhancements differ markedly in the positive and negative NOE regimes.

When direct (A–B) NOEs are positive, indirect effects at C are weak and negative but are favoured when the three spins have an approximately linear relationship (so may provide useful geometrical information), develop only slowly with a characteristic lag time and are thus also favoured by long presaturation periods. These are referred to as *three-spin effects*. Although further relays are theoretically possible, they are generally too weak to be observed.

It is possible that positive direct effects and negative indirect effects can cancel or act to reduce the magnitude of the NOE. Thus, despite two spins being close, NOEs between them may be rather small or even negligible (particularly when longer saturation periods are employed).

When direct (A–B) NOEs are negative, indirect effects are also negative so cannot be distinguished, they spread rapidly and have high intensities. This process is referred to as *spin diffusion* and is fatal for the steady-state NOE since it causes a loss of specificity and hence provides no information on molecular geometry. In this regime, it usually becomes necessary to use kinetic (transient) methods based on the measurement of NOE growth rates.

Taking into account all the subtleties associated with the steady-state NOE presented above, it should be clear that it is unwise to place too much significance on the absolute magnitudes of steady-state NOE enhancements. In reality, differences of a few percent mean little when taken on their own, and it is generally necessary to consider a collection of enhancements when undertaking structural or conformational analysis to be certain of an unambiguous conclusion. A qualitative interpretation of many measurements is the most appropriate approach to interpreting most NOE data.

Source and recommended reading:
High-Resolution NMR Techniques in Organic Chemistry. 3rd Edition, T. D. W. Claridge, Elsevier Science, 2016.