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Ultrahigh-Quality NOE Spectra

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The observation of nuclear Overhauser effect (NOE) enhancements is a crucial part of the process of structure determination by NMR.[1] Such effects are generally observed using difference spectroscopy in which a control spectrum is subtracted from a spectrum in which the resonances due to one spin have been irradiated selectively.[2] The resulting *NOE difference spectrum* contains, in principle, just those resonances from the irradiated spin and from those spins which are cross-relaxing with the irradiated spin. In practice as a result of imperfect subtraction, such spectra often contain "subtraction artifacts" whose presence makes it difficult to visualize the NOE enhancements. In this communication we propose a new method of measuring transient NOE enhancements which, by the use of pulsed field gradients,[3,4] avoids the need to compute difference spectra and thus gives spectra which contain *no* subtraction artifacts. As a result, very small NOE enhancements can be detected readily and with complete confidence.

The basic experiment utilizes the following pulse sequence

$$90°(x,\text{sel})–G_1–90°(x)–\tau_m–G_m–90°(x)–\tau_2–180°(x)–G_2–\text{acq}$$

where the first pulse excites selectively only those resonances from the spin of interest. The gradient G_1 gives the magnetization excited by this pulse a phase (in the xy-plane) which depends on position in the sample; the magnetization is dephased. The second pulse turns the y-components of this magnetization onto the z-axis. During the mixing time, τ_m, cross-relaxation transfers this magnetization, together with its phase encoding from the first gradient, to other spins. The final 90° pulse turns this magnetization into the xy-plane, where it is refocused by the gradient G_2. A 180° pulse refocuses the evolution of chemical shifts (offsets) during the final gradient;[5] the delay τ_2 is equal in length to the gradient G_2. The length and strength of G_1 and G_2 are set so that the magnetization defocused by the first is refocused by the second. A third gradient, G_m, is applied during the mixing time in order to purge all but the z-magnetization. This sequence has similarities to experiments used for water suppression in *in vivo* NMR[6,7] and NOESY-type experiments used to measure chemical exchange.[8]

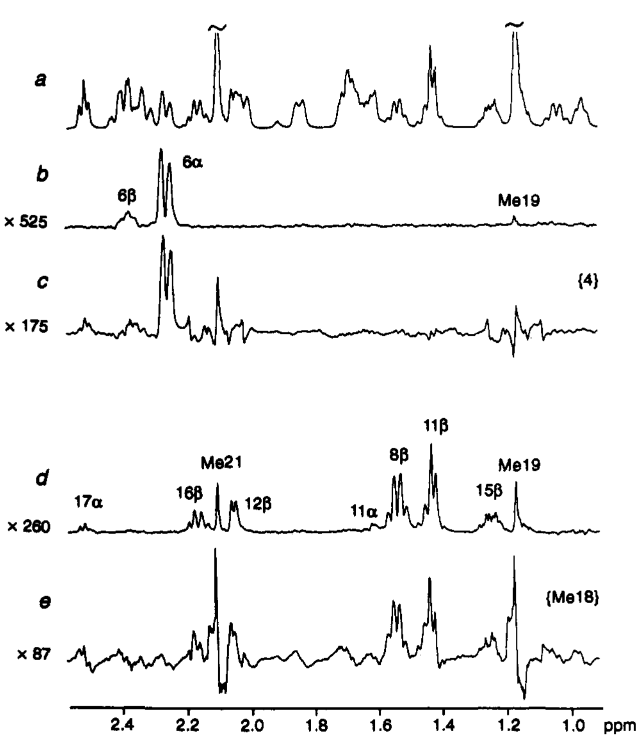

Figure 1. Proton spectra of progesterone, **1**. Spectrum a is the conventional spectrum, while spectra b and c are NOE spectra recorded using the gradient experiment proposed in the text and the conventional transient NOE difference experiment, respectively; the target is H_4, and the mixing time is 150 ms. Note the complete absence of subtraction artifacts from spectrum b, thus enabling the very small NOE enhancements of $H_{6\beta}$ and Me_{19} to be observed; the latter is just 0.03%. Spectra d and e are similar to b and c except that Me_{18} is the target. Parameters used for the gradient experiments: the selective 90° and 180° pulses were of duration 20 and 40 ms, respectively, and shaped to a 1% truncated Gaussian; the gradients G_3 and G_4 were of duration 2 and 4 ms respectively, shaped to a 5% truncated Gaussian and of peak strength 20 G cm^{-1}; the gradient G_m was similarly shaped and of duration 5 ms. Each spectrum was the result of time averaging for 14 min, with a recycle delay of 6.25 s. EXORCYCLE phase cycling of both 180° pulses was used. Spectra were recorded on a Bruker AMX600 spectrometer equipped with a triple-resonance probe head incorporating a single shielded gradient coil. The sample concentration was approximately 50 mM.

The crucial advantage of this sequence is that the only magnetization which is refocused by the final gradient is that which was defocused by the first gradient. Thus the only resonances observed in the spectrum are those which arise from the spin which is excited by the selective 90° pulse and from spins to which magnetization has been transferred by cross-relaxation. NOE enhancements thus appear directly in the resulting spectrum, without the need to take a difference; artifacts associated with poor subtraction are thus avoided, and excellent quality spectra result.

In coupled spin systems there is a potential difficulty that, as a result of the evolution of couplings during the selective pulse and G_1, the second 90° pulses may generate zero-quantum coherence which cannot be separated from the wanted z-magnetization and appears in the spectrum as antiphase dispersive contributions.[9] There are a number of modifications to the

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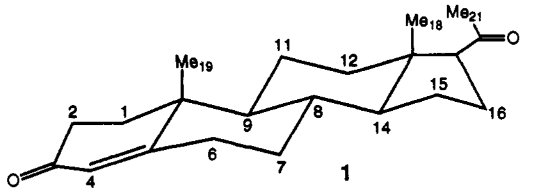
sequence which result in the suppression of these contributions; one such results in the sequence

$$90°(x)\text{–}G_3\text{–}180°(x,\text{sel})\text{–}\bar{G}_3\text{–}90°(x,\text{sel})\text{–}\tau_m\text{–}G_m\text{–}90°(x)\text{–}\tau_4\text{–}180°(x)\text{–}G_4\text{–acq}$$

The selective 180° pulse, applied to the resonances of interest, refocuses any evolution of the coupling which takes place during the gradients G_3 and $\bar{G}_3$. As a result no antiphase states are created and hence zero-quantum coherence is not generated. As an added precaution, the second 90° pulse is also made selective so that any antiphase states which are generated, perhaps due to incomplete refocusing, are turned into *zz* terms which are not rendered observable by the final 90° nonselective pulse.[10] The second gradient $\bar{G}_3$ is applied in the opposite sense to the gradient G_3 so that the magnetization from the selected spin continues to dephase. As before, G_4 is chosen to rephase the effects of G_3 and $\bar{G}_3$.

We demonstrate the excellent results obtained using this sequence by observing transient NOE enhancements in progesterone, **1**. Figure 1 shows NOE spectra recorded using the above sequence and with the conventional transient NOE experiment. In Figure 1b H_4 is the target and enhancements of $H_{6\beta}$, $H_{6\alpha}$, and Me_{19} are clearly visible, whereas in the conventional NOE spectrum, Figure 1c, subtraction artifacts obscure all but the strongest enhancement of $H_{6\alpha}$. The exceptional quality of the spectrum enables us to detect, with complete confidence, the tiny enhancement (~0.03%) of Me_{19}; the distance involved is ~4.3 Å. This result is all the more remarkable when it is noted that the spectrum was recorded in just 14 min. Figure 2 shows the buildup of the enhancements of $H_{6\alpha}$ and $H_{6\beta}$ as a function of mixing time.



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The use of gradients results in the refocusing of just one out of two possible coherence transfer pathways,[11] leading to a reduction in the signal intensity by a factor of 2. In addition, compared to a transient NOE experiment the initial buildup rate of the NOE in the gradient experiment is reduced by a factor of 2.[1] However, the gradient experiment records the NOE spectrum directly so that no reference spectrum is needed. Taking all these factors into account, the signal-to-noise ratio of the gradient experiment is predicted to be one-half of that of the transient NOE experiment. In practice, due to the effects of diffusion during the mixing time,[12,13] relaxation during the selective pulses, gradients and associated delays, this ideal is not achieved. However, as is seen in Figure 1, the detectability of enhancements in NOE difference spectra is limited by the presence of subtraction artifacts and the complete absence of these from the gradient experiment more than compensates for a reduction in signal-to-noise ratio.

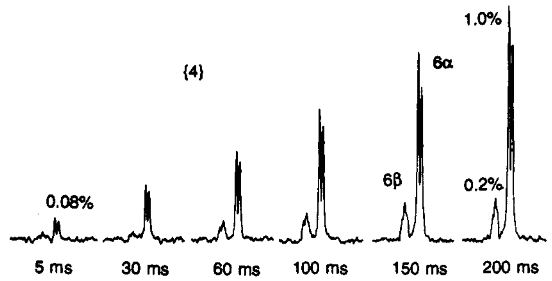

Figure 2. NOE spectra, recorded using the gradient experiment described in the text, showing the buildup of the enhancements on $H_{6\alpha}$ and $H_{6\beta}$ as a function of mixing time. The maximum enhancements are shown, as is the minimum enhancement for $H_{6\alpha}$; H_4 is the target.

It is clear from these spectra that by avoiding difference spectroscopy it is possible to measure, with complete confidence, NOE enhancements of less than one-tenth of 1%. There are three implications of this result. Firstly, the fact that much smaller NOE enhancements can be observed extends the range of the NOE as a structural probe. Secondly, measurement of the buildup of NOE enhancements at short mixing times becomes much easier and much more reliable. Finally, the high quality of the spectra obtained in this way implies that it may well be preferable to the measurement of steady-state enhancements. For example, a single transient experiment recorded with a relatively long mixing time will reveal all the NOE enhancements that might be seen in a steady-state experiment; the absolute size of the enhancements seen will be smaller, but this is of no consequence given the absence of subtraction artifacts. We suggest that the acronym GOESY (gradient enhanced nuclear Overhauser effect spectroscopy) might be suitable for this experiment.

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