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Heteronuclear Edited Gradient Selected 1D and 2D NOE Spectra: Determination of the NOE Effect between Chemically Equivalent Protons

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With gradient selected HSQC-NOESY spectra it is possible to detect the NOE effect between protons which have the same chemical shift either due to symmetry or due to accidental overlap. The discrimination of the equivalent sites is achieved by allowing one site to couple with ^{13}C in natural abundance. The suppression of unwanted signals is performed with pulse field gradient selection. Both 2D and selective 1D methods are shown. © **1997 by John Wiley & Sons, Ltd.**

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INTRODUCTION

In structural organic chemistry, symmetrical molecules often pose difficult analytical problems. Normal ^{13}C and ^{1}H NMR spectra show only half of the signals expected from the molecular weight and do not reveal the position of the symmetry axis with respect to the molecular frame. Often, however, by the use of proton coupled ^{13}C NMR spectra, the situation can be clarified because the observation of long-range C,H spin coupling allows one to look across the symmetry barrier.[1] Even if there is no chemical symmetry, one often encounters severe overlap of proton signals for medium sized molecules in organic chemistry.

Analysis of the distance between such groups is difficult, since standard NOESY or NOE difference spectra[2] cannot be used. The common solution to the problem of signal overlap in biological NMR is to use 3D heteronuclear resolved NOE spectroscopy.[3] In organic chemistry it proved advantageous to use relayed 2D techniques e.g. as demonstrated in 2D HMQC-TOCSY spectroscopy.[4,5] A remedy to the inherent symmetry problem was originally proposed by Kawabata *et al.*,[6] who used ^{13}C edited techniques, applying in principle such a 2D approach. In their method, an HMQC correlation[7] is followed by a ROESY transfer,[8] hence they can observe ROE effects between chemically equivalent sites. This is made possible by the fact that one starts from one proton site coupled to ^{13}C and the ROE is transferred to the equivalent proton, which is attached to ^{12}C. The proton spectra are acquired without ^{13}C GARP decoupling. This elegant approach, however, has practical problems. NOE and ROE effects are usually small and must be distinguished clearly from signal breakthrough stemming from protons bound to ^{12}C. To achieve this suppression of unwanted signals, a BIRD sandwich at the beginning and extensive phase cycling has been used.[6]

We show here how this problem can be solved more effectively with the use of pulsed field gradients and, furthermore, demonstrate a selective 1D technique, using a modification of our earlier SELINCOR method[9–11] by which, in favorable cases, the structural problem can be solved in a reasonable time. A similar approach, but differing in several details, has been published recently by Parella *et al.*[12,13]

EXPERIMENTAL

The spectra shown were measured in a 25% solution of the compounds in $CDCl_3$ using a Bruker AMX-500 spectrometer equipped with a multinuclear inverse probehead with additional coils for *z*-gradients. The gradient length was set to 1 ms with a strength in the order of 0.2 T m^{-1} using a 10 A BGU gradient unit. The 2D spectra were obtained typically with 128 scans for each of 128 FIDs and a mixing time of 3 s, requiring a total measurement time of *ca.* 6.5 h. Investigation of the signal strength as a function of the mixing time showed the usual build-up curve and gave 2–3 s as the optimum for the molecules investigated. The 1D spectra employed a 180° Gaussian-shaped pulse of 78 ms duration and were typically performed in *ca.* 105 min with 1024 transients. Instead of the gradient selection procedure shown, an echo–anti-echo scheme could be used which should improve the signal-to-noise ratio by a factor of $\sqrt{2}$. This, however, is due to software reasons currently not possible on the instrument used.

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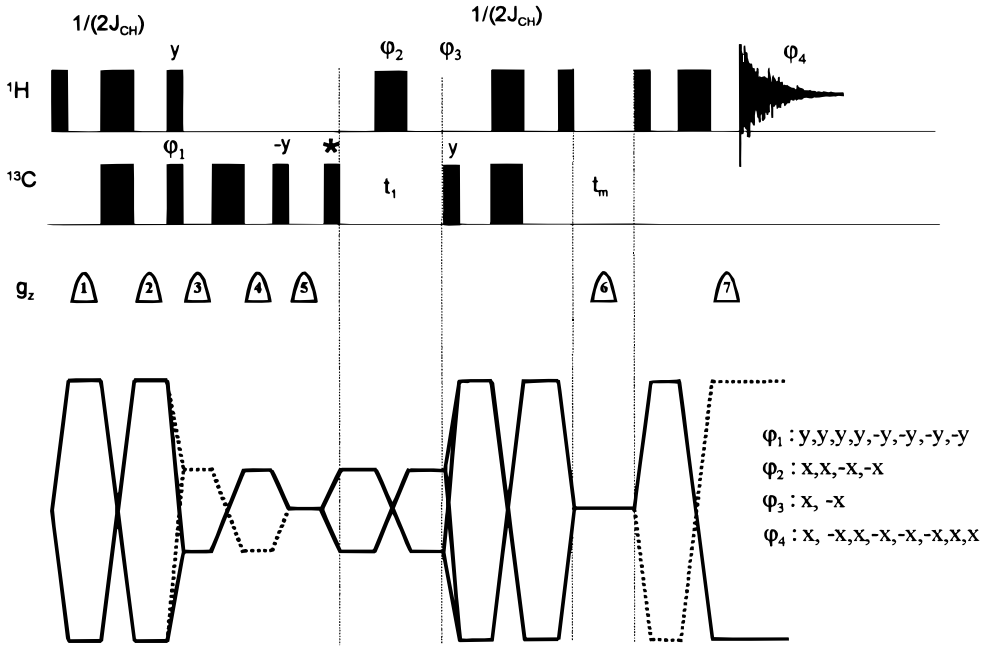


Figure 1. Pulse sequence for the gs-HSQC-NOESY technique. Small bars 90° and thick bars 180° pulses. The 90° pulse marked with an asterisk was phase cycled according to TPPI. The selected coherence transfer pathway is given as a thick line. Gradient length 1 ms in the ratios $g_1 : g_2 : g_3 : g_4 : g_5 : g_6 : g_7 = 5 : 5 : -40 : 40 : 15 : 25 : -20.1153$.

RESULTS AND DISCUSSION

As seen from the pulse sequence given in Fig. 1, we start with an HSQC scheme,[14] which often gives more satisfactory results than the HMQC technique. The HSQC part of the sequence is carried out in the phase sensitive mode,[5] applying the pulsed field gradients outside the t_1 period and using a gradient *zz*-filter.[15] Discrimination of the signs of the frequencies during t_1 is achieved with TPPI phase cycling of the third 90° ^{13}C pulse marked by an asterisk in Fig. 1. The small gradients g_1 and g_2 correct for imperfect 180° pulses and g_5 acts as the gradient *zz*-filter. After back-transfer to protons an NOE transfer part without evolution time follows where another gradient pulse g_6 during the mixing time spoils undesired magnetization. This feature has now been successfully applied in normal homonuclear NOESY spectroscopy.[16] The final rephasing gradient pulse g_7 selects the desired magnetization, which has been dephased by the gradient pulses g_3 and g_4. The coherence pathway diagram below the pulse sequence shows the selection of the desired signal pathway and demonstrates the action of the *zz*-filter as well of the gradient during the NOE mixing time, leading to very clean spectra.

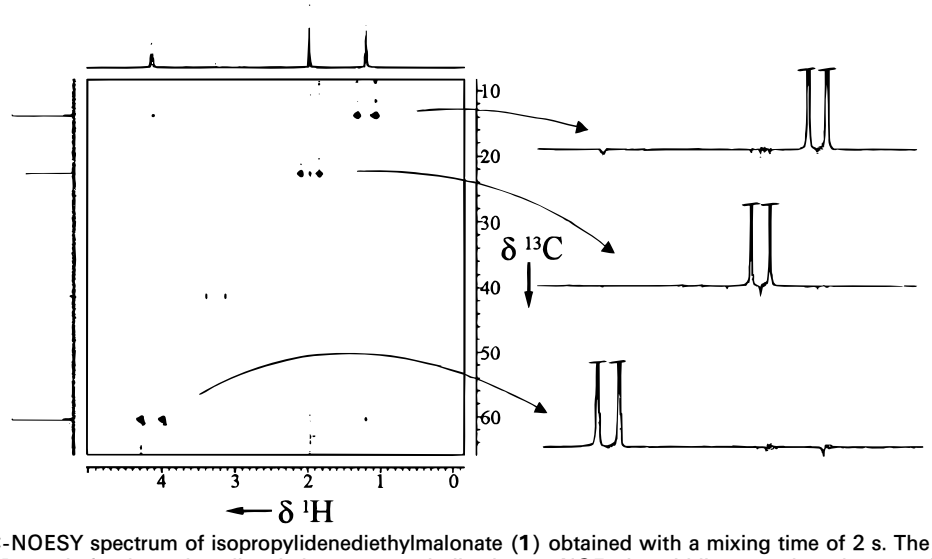


Figure 2. gs-HSQC-NOESY spectrum of isopropylidenediethylmalonate (**1**) obtained with a mixing time of 2 s. The top and bottom trace are the rows of the 2D matrix for the carboxylic ethyl ester group indicating no NOE; the middle trace gives the row of the 2D matrix for the methyl groups attached to the double bond with the negative NOE signal at the center of the doublet. The doublet at $\delta_H = 3.2$ and $\delta_C = 41$ stems from an impurity in the sample.

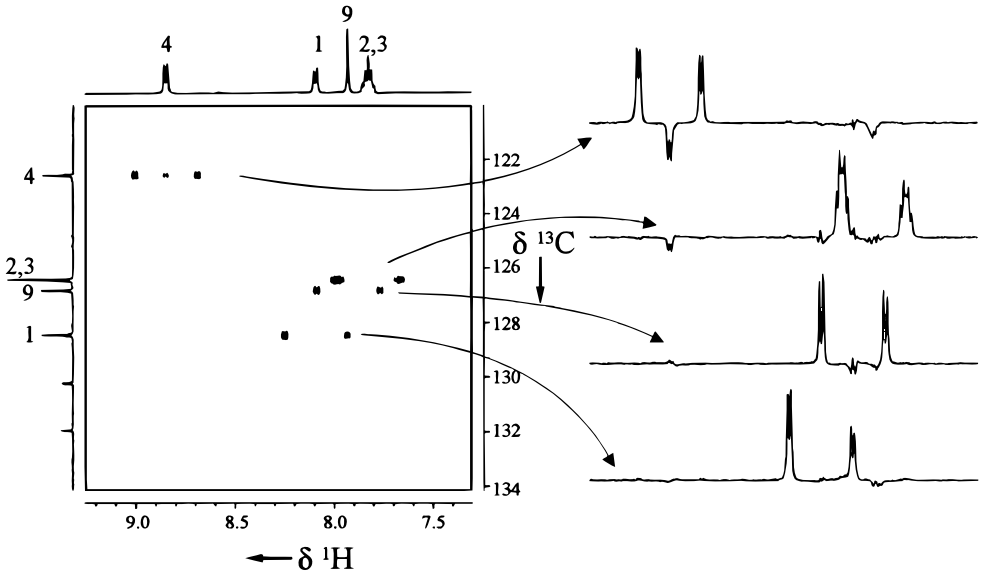
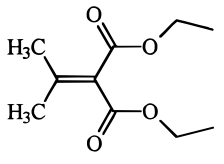


Figure 3. gs-HSQC-NOESY spectrum of phenanthrene (**2**) obtained with a mixing time of 3 s. The top trace is the row of the 2D matrix at the position of H-4 indicating a strong NOE effect between the protons H-4 and an NOE effect between H4 and H3. The other traces are the rows of the 2D matrix at the position of protons H-1, H-9 and H-2/3.

The result of the sequence is demonstrated for two examples in Figs 2 and 3. Isopropylidenediethylmalonate (**1**) served as the molecule with which we developed the method. Here a significant NOE effect is expected between the two methyl groups attached to the olefinic double bond. As can be seen from Fig. 2, one observes a C,H correlation with three C,H doublets. These three signals form the 'diagonal' of this kind of carbon edited NOESY spectrum. Furthermore, 'normal' NOE effects are present between the signals of the ethyl ester group. In the center of the C,H doublet of the olefinic methyl group, however, a negative signal is detected which stems from an NOE between the two equivalent methyl groups. A signal breakthrough of those methyl protons bound to ^{12}C would probably be out of phase, as seen in some other traces; furthermore, the intensity of the negative signal is dependent on the mixing time. This provides experimental proof for identifying a true NOE signal.[6]



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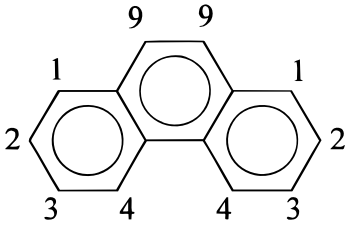

2

An interesting application is provided by phenanthrene (**2**), where one would expect a strong NOE effect between the protons H-4. This is revealed clearly, as seen in the upper trace of Fig. 3, whereas the expected NOE between the protons H-9 is very small and on the detection limit. Again, 'normal' NOEs are observed between H-4 and H-3.

Earlier we introduced the SELINCOR method[9–11] for detecting a particular proton by selectively exciting the attached ^{13}C atom, which is, in principle, the one-dimensional equivalent to the HMQC or HSQC method. With a slightly modified and improved gs-SELINCOR sandwich, as shown in Fig. 4, we succeeded in measuring NOE effects in symmetrical molecules giving the typical time and resolution advantage of a 1D sequence. Compared with earlier versions of gs-SELINCOR,[10] we use here a 180° selective pulse enclosed in a gradient sandwich,[11] which is far easier to calibrate than the previously used 90° selective pulse. Furthermore, the relative phase of the 180° selective pulse does not have to be known. During the mixing time we apply several 180°C pulses embedded between two gradients, as has been demonstrated for the homonuclear DPFGSE method.[17]

In Fig. 5 the result of the 1D measurement on **2** is given. The spectrum was obtained in only 105 min, demonstrating the usefulness of the 1D approach.

In conclusion, the use of pulsed field gradients provides a large advantage in suppressing unwanted coherences, rendering even the observation of NOE effects between equivalent sites easily possible. Our two-dimensional method is a significant technical advance over earlier approaches, and the 1D version will make the measurements of NOEs in symmetrical molecules even simpler.

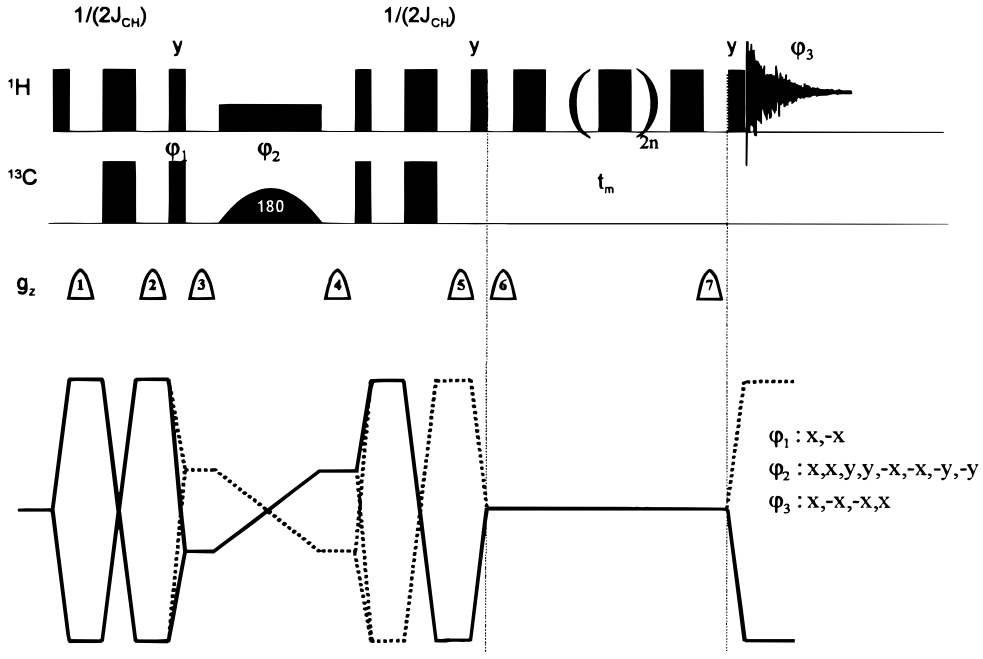

Figure 4. Pulse scheme of the 1D gs-HSQC-NOESY technique. Small bars 90° and thick bars 180° pulses. The selected coherence transfer pathway is given as a thick line. For the decoupling step during the selective 180° carbon pulse cw irridiation was used. Gradient length 1 ms in the ratios $g_1 : g_2 : g_3 : g_4 : g_5 : g_6 : g_7 = 5 : 5 : -40 : 40 : -20.1153 : 10 : -10$.

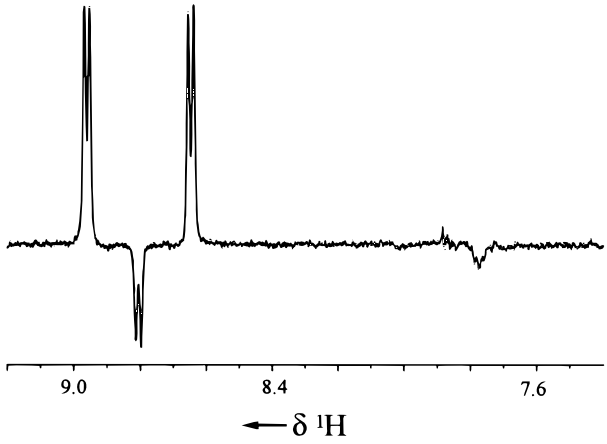

Figure 5. 1D gs-HSQC-NOESY spectrum of phenanthrene (**2**) obtained by adjusting the selective pulse on C-4 and using a mixing time of 3 s.

Acknowledgements

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