48. Assignment of Quaternary Carbon Atoms in ^{13}C-NMR Spectroscopy: Application of an Improved Heteronuclear 2D-NOE Experiment

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Nuclear *Overhauser* effects (NOE) between protons and heteronuclei can be of great value for the assignment of quaternary nuclei, and they can be very informative and unique in view of stereochemistry and molecular dynamics. The application of a modified 2D-NMR experiment for the assignment of quaternary carbon atoms of a medium-sized molecule is demonstrated. Compared to NMR experiments designed to detect scalar instead of dipolar interactions, measuring times are considerably longer, but data analysis is in many cases more straightforward.

1. Introduction. – The nuclear *Overhauser* effect (NOE) has widely been used in the past to detect dipolar interactions among protons and to establish more or less rough estimates of internuclear distances in molecules. *Ernst* and coworkers [1–4] developed a homonuclear 2D experiment (NOESY) to observe NOE phenomena, which is able to resolve many individual spin NOE effects with a single instrument setting. *Rinaldi* [5], and *Yu* and *Levy* [6] independently proposed the corresponding 2D pulse sequence to measure NOEs between protons and heteronuclei (HOESY) a few years ago. A growing number of applications [7–9] and improvements of the basic sequence [10] have meanwhile demonstrated the usefulness of this kind of information. Especially in the case of quaternary centers, the heteronuclear *Overhauser* experiment may yield assignments or stereochemical relationships of an unusual kind. Together with the information from experiments designed to detect scalar interactions between quaternary heteronuclei and protons, as COLOC [11] and related sequences [12], or to selectively measure long-range couplings [13], resonances of these heteronuclei can unambiguously be assigned.

The major problem with experiments of this type is sensitivity, since normally insensitive and less abundant nuclei are measured. Furthermore, only quaternary nuclei with relatively long T1s and reduced NOEs are of interest, since, for proton bearing heteronuclei, better and more efficient methods exist for chemical-shift correlation. The sensitivity problem is even more pronounced in 2D pulse sequences, where only transient instead of steady state NOEs, as for corresponding 1D experiments, are generated. Therefore, all efforts have to be made to improve the basic sequence with respect to sensitivity. We have proposed a modified 2D-HOESY pulse sequence [14] as shown schematically in *Fig. 1*, which considerably improves the sensitivity but by no means removes the inherent problem of this kind of experiment at all.

In contrast to the basic version, data sampling in the superior sequence is initialized after looping not only once but N-times through the evolution (t_1) and mixing (D2)

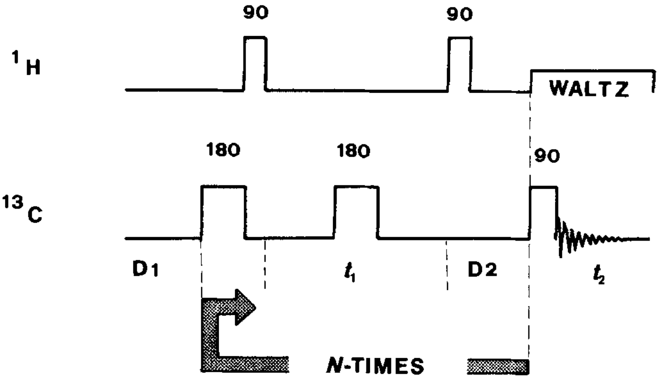
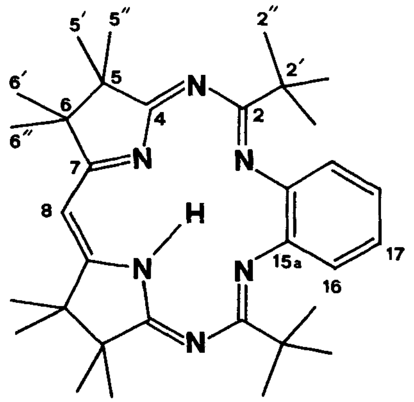


Fig. 1. *Modified 2D-HOESY pulse sequence to detect NOEs between protons and C-atoms.* Compared to the conventional sequence, loops through the evolution (t_1) and mixing (D2) periods are introduced. An additional 180°-^{13}C pulse prevents from partial cancellation of the NOE difference signal for $N > 0$.

periods. In addition to the 180° pulse centered in the evolution period to decouple protons from the C-atoms, a second 180° pulse has to be applied in order to prevent partial cancellation of NOEs for $N > O$. Appropriate shifting of the phases for the first 90°-^{1}H- and the 90°-^{13}C-detection pulse with the subsequent addition of the corresponding C signals generates 2D-NOE difference spectra.

2. Results and Discussion. – Starting with a two-spin system at thermal equilibrium, the first passage through the evolution and mixing periods generates – similar to the basic pulse sequence – transient NOE on the C-atom, the amount of which is dependent on t_1 and D2. Subsequent loops through these periods generate additional, but decreasing amounts of NOE. Due to the two – instead of only one as in the basic version –180°-^{13}C pulses, these partial NOEs are all co-added before the first data acquisition. Compared to the basic version, larger amounts of NOE can, therefore, be measured for the same number of acquisitions, but longer measuring times have to be taken into account. Extensive simulations for a two-spin system [14] based on the master equations for relaxation [15] show that, for a given measuring time and with the experimental parameters optimized for both the basic and the modified version, sensitivity can be gained with the looping procedure. The simulations, furthermore, prove an increase of this gain with T1(C)/T1(H), which means that especially for quaternary C-atoms large improvements have to be expected. Gains in sensitivity in the range of 100–200% for quaternary C-atoms, compared to the basic version of the 2D experiment have been calculated and experimentally confirmed. The simulations show that the sensitivity of steady-state – instead of transient – NOEs can theoretically be achieved or at least approximated, if the number of loops is increased, D2 is optimized (see *Experimental*) accordingly and D1, whose influence on sensitivity rapidly decreases, is set near zero. Some minor deviation from the simulated values have to be taken into account and are due to non-idealities or slight missets of pulses, phases, and delays in the pulse sequence, especially if large numbers of loops N should be applied. Improvements in this respect are in progress. Nevertheless, the gains are respectable and the modification is easy to implement on a modern FT-NMR spectrometer.

The modified 2D-HOESY sequence was applied to a sample of compound **1** *(Fig. 2)* From a 2D contour plot, all of the quaternary C-atoms can unambiguously be assigned. Furthermore, the chemical shifts of non-quaternary C-atoms are correlated with the chemical shifts of their directly bound protons.



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Carbon C(8) shows a positive, intense cross peak with its directly bound proton and small, (due to the indirect magnetization transfer) negative cross peaks with proton signals in the high-field region, attributed to the $CH_3(6')$ and $CH_3(6'')$ groups. The proton signals of these CH_3 groups on the other hand give rise to cross peaks with only one non-olefinic quaternary C-atom, which must, therefore, be assigned to C(6). Carbons C(5) and C(2') are distinguished by their cross peaks with two non-equivalent $CH_3(5')$, $CH_3(5'')$ groups and the intense proton signal of the *t*-Bu group, respectively. Carbons C(7) and C(4) can unambiguously be assigned due to their cross peaks with the signals of the methine proton H–C(8) and $CH_3(5')$, $CH_3(5'')$ groups, respectively. Carbon C(15a) shows a cross peak with only one proton which must, therefore, be assigned to H–C(16). No cross peaks can be detected for C(7) with the proton signals of $CH_3(6')$, $CH_3(6'')$, and for C(2) with the signals of $CH_3(2'')$, probably due to the dominance of non-dipolar relaxation mechanisms or the presence of negative 'three-spin effects' [15] (*i.e.* H–C(6')→H–C(8)→C(7)), which partially cancel direkt positive effects.

As an example and to show the value of NOE data in general, cross sections through carbons C(2'), C(5), and C(6) are shown in *Fig. 2a*. They are compared with the corresponding cross sections of a COLOC [11] experiment *(Fig. 2b)*, which correlates chemical shifts of quaternary C-atoms and protons *via* indirect (scalar) long-range couplings.

From the cross sections of C(5) and C(6), it is obvious that assignments with NOE data are more straightforward due to the strong r^{-6} dependence of the effects, whereas for COLOC data – due to the sometimes similar $^2J(C,H)$ and $^3J(C,H)$ coupling constants – ambiguities may occur and careful set-up of experimental parameters and analysis of spectra is necessary.

^{1}H and ^{13}C chemical-shift data (see *Experimental*) prove the non-equivalence of the methyl groups $CH_3(5')$, $CH_3(5'')$, and $CH_3(6')$, $CH_3(6'')$. This points to a preferred non-planar conformation of the cyclic molecule, whose lifetime at room temperature must be long with respect to the NMR time-scale.

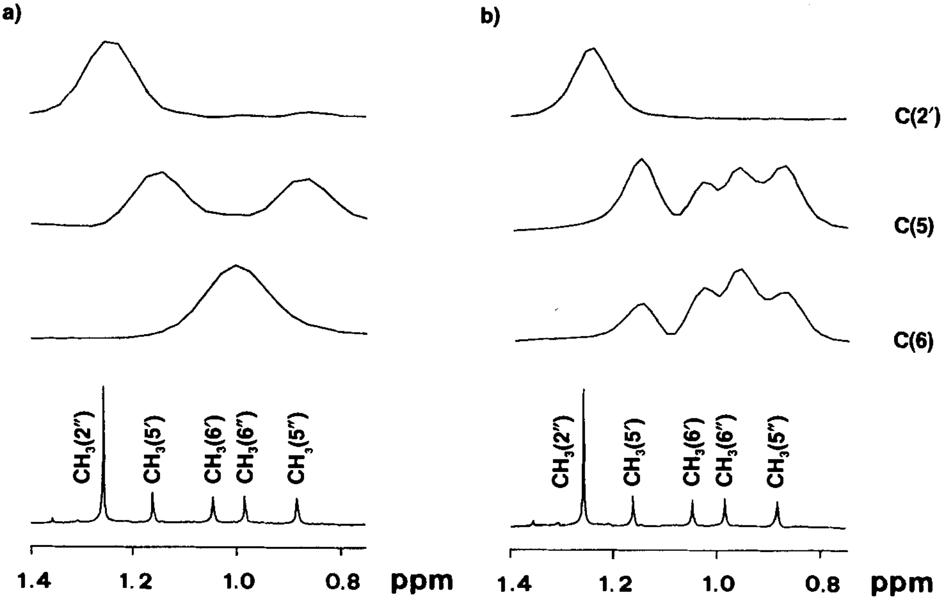

Fig. 2. a) *Columns obtained from a modified 2D-HOESY experiment for C(2'), C(5), and C(6).* b) *Corresponding columns from a COLOC experiment* (fixed evolution delay set to 0.075 s). In *a* and *b*, the corresponding expansion of a 1D-^{1}H spectrum is given below. Due to the more increments used in the COLOC experiment (256 instead of 128 as used for the HOESY sequence), the signals of H–C(6') and H–C(6'') could be resolved in the corresponding 2D spectrum.

3. Conclusion. – Although the sensitivity of the 2D-HOESY experiment could be considerably enhanced and approximates now the values of corresponding 1D steady-state experiments, the concomitant problem of long measuring times or large sample amounts still exists. Therefore, the HOESY pulsesequence can not be considered as an experiment for routine use, but may certainly be a valuable alternative, if other, rather more sensitive methods fail, leave ambiguities, or if data – complementary to data on heteronuclear scalar spin-spin couplings – about dipolar heteronuclear interactions are of interest.

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4. Experimental. – Compound **1** (= *4,11-di(*tert*-butyl)-1,1,2,2,13,13,14,14-octamethyl-1,2,13,14-tetrahydro-12b*H*-benzo[*f*]dicyclopenta[*kl,op*][1,3,5,8,10,12]hexaazapentadecin*; 280 mg) was dissolved in 300 µl of CD_2Cl_2. The soln. was carefully degassed with dry N_2 for a few min.

Spectra were measured at 20° on a *Bruker AM 400* spectrometer equipped with a process controller. Calculations were carried out on a separate data station equipped with an *Aspect 1000* with an array processor and a *NEC 160 MB* disk. A standard 5-mm $^{13}C/^1H$ dual probehead was used.

NMR Data of **1**. ^{1}H-NMR (CD_2Cl_2, TMS, 20°): 6.75 (*m*, H–C(16)); 6.63 (*m*, H–C(17)); 5.09 (*s*, H–C(8)); 1.26 (*s*, H–C(2'')); 1.16 (*s*, H–C(5')); 1.05 (*s*, H–C(6'')); 0.99 (*s*, H–C(6')); 0.88 (*s*, H–C(5'')). ^{13}C-NMR (CD_2Cl_2, TMS, 20°): 180.69 (C(7)); 167.38 (C(2)); 164.15 (C(4)); 140.28 (C(15a)); 121.81 (C(17)); 120.22 (C(16)); 86.29 (C(8)); 48.84 (C(6)); 46.79 (C(5)); 39.39 (C(2')); 27.77 (C(2'')); 25.80 (C(6')); 23.72 (C(5'')); 18.74 (C(5',6'')).

The Experimental Parameters Used. Modified HOESY (see *Fig. 1*). Relaxation delay D1 5 ms, mixing time D2 2 s, number of loops *N* 6, 90° pulse length ^{1}H 11.5 µs and ^{13}C 7.5 µs. Spectral range in f_2 20000 Hz and in f_1 2880 Hz. Quadrature and phase-sensitive detection in both dimensions using TPPI [16]. Zero dummy scans, 32 acquisitions, acquisition time 0.2 s. Number of increments 128, size in f_2 8 K in f_1 512 W (2 × zero filling), application of a gaussian filter in both dimensions. Measuring time 14 h. Experimental set-up (optimizing of D2): A series of experiments with different mixing times D2 for one single t_1 value (which should be as short as possible) and D1 set near zero is run. The corresponding spectra are analyzed with respect to signal-to-noise, taking into account the different measuring times.

COLOC [11]. Sequence: D1 – 90° (^{1}H)-t_1-180° (^{1}H, ^{13}C) – (D2-t_1)-90° (^{1}H, ^{13}C) – D3-t_2. Relaxation delay D1 2s, fixed evolution period D2 75 ms (optimized for ^nJ(C,H) = 6.7 Hz), D3 42.4 ms, 90° pulse lengths see modified HOESY. Zero dummy scans, 32 acquisitions, acquisition time 0.2 s. Number of increments 256, size in f_2 8 k in f_1 512 W (1 × zero filling), application of a gaussian filter in both dimensions. Measuring time 5.3 h.

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