3.4 ± 0.3 kcal/mol and ln ($A_{ins}/A_{Me}$) = 6.8 ± 0.5, with $r$ = 0.979, 99.9% confidence level, for nine points. From the ratio of the preexponential factors, $\Delta S^{\ddagger}_{ins} - \Delta S^{\ddagger}_{Me}$ = 13.5 eu. If we correct for a 3-fold statistical advantage of insertion (nine CH) over methyl migration (three Me's) in **1**, $\Delta\Delta S^{\ddagger} \sim 11$ eu.[11]

The GC-determined product ratio of **4**/**5** at 293 K (which we take as $k_{ins}/k_{Me}$) is 2.92. Recalling that $k_d$ ($=k_{ins} + k_{Me}$) = 9.3 × $10^5$ $s^{-1}$ at 293 K (see above), we obtain $k_{ins}$ = 6.9 × $10^5$ $s^{-1}$ and $k_{Me}$ = 2.4 × $10^5$ $s^{-1}$ for the competitive 1,3-CH insertion and 1,2-Me shift reactions of *tert*-butylchlorocarbene at 20 °C. We estimate errors of 10–15% in these values.

The 1,3-CH insertion and 1,2-Me migration of **1** are "slow" intramolecular carbene reactions. Thus, reported rate constants at ambient temperatures for 1,2-H shifts range from ≥$10^8$ $s^{-1}$ for $Me_2CHCCl$[2f] to (1–3) × $10^6$ $s^{-1}$ for MeCCl,[2c,e] and the rate constant for the 1,2-C shift of cyclopropylchlorocarbene to chlorocyclobutene is variously reported as 3.8 × $10^5$ $s^{-1}$ [2f,3b] or (8–9) × $10^5$ $s^{-1}$.[3a,c] More importantly, the excess of 1,3-CH insertion (to **4**) over 1,2-Me migration (to **5**), which increasingly obtains above −15 °C, is entropically controlled. Although Me migration is favored over 1,3-CH insertion by $\Delta\Delta H^{\ddagger} \sim 2.8$ kcal/mol, this is more than compensated by the favorable differential entropy of activation attending the insertion (13.5 eu ~ 4 kcal/mol at 293 K).

Our extrapolative methodology is not sufficiently precise to produce absolute activation parameters for these reactions, but the observed entropic disadvantage of 1,2-Me migration is consonant with the very unfavorable entropy of activation (~−20 eu) that attends the 1,2-C shift of cyclopropylchlorocarbene.[12] We are continuing our studies of intramolecular carbene reactions.

**Acknowledgment**. We are grateful to Professor H. D. Roth for a helpful discussion and to the National Science Foundation for financial support.

(11) Although there are nine equivalent C–H bonds and three equivalent C–Me bonds in **1**, conformations of the carbene where insertion or methyl migration are likely to occur present a 1:1 weighting of pathways.

(12) See ref 3c for a discussion of unfavorable activation entropy in a carbene 1,2-rearrangement.

## Simplification of DNA Proton Nuclear Magnetic Resonance Spectra by Homonuclear Hartmann–Hahn Edited Two-Dimensional Nuclear Overhauser Enhancement Spectroscopy

Vladimír Sklenář*,† and Juli Feigon*

*Department of Chemistry and Biochemistry and Molecular Biology Institute, University of California Los Angeles, California 90024*

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Two-dimensional $^1$H NMR spectra of DNA fragments in solution supply a wealth of structural information. The first step in establishing the molecular structure using NMR spectroscopy is the assignment of protons to a particular nucleotide residue. Sequential assignment procedures using a combination of NOESY[1] and COSY[2] (as well as other coherence transfer experiments) have been developed which allow straightforward assignment of the base and most of the sugar protons in short DNA oligonucleotides which adopt a right-handed A- or B-DNA type structure.[3] However, the sequential assignment technique can fail for unusual DNA structures where some of the bases are syn rather than anti such as Z-DNA[4] and some drug–DNA complexes,[5] and in more complicated structures containing non-Watson–Crick base pairs such as DNA triplexes.[6] Spectral overlap in the base–H1′ and base–H2′,H2″ region of the NOESY spectra may also limit assignments in these and in longer B-DNA duplexes.

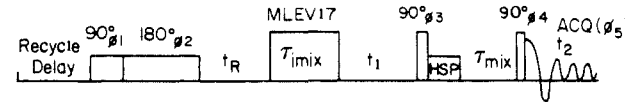
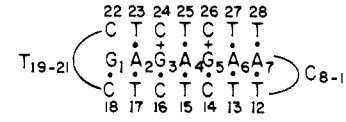



**Figure 1.** Pulse scheme of the HOENOE experiment. The selective 90° and 180° pulses, MLEV17[12b] isotropic mixing, and the nonselective 90° mixing pulses are applied with the carrier positioned in the center of the CH5 region. The nonselective 90° $t_2$ acquisition pulse can be positioned anywhere in the spectrum. The refocusing delay is $t_r = (2/\pi)t_{90}$, where $t_{90}$ is the length of the selective 90° pulse. In order to minimize the required phase cycle extended by the necessity to apply the EXORCYCLE[8] for the selective excitation in the preparation period, a homospoil pulse is applied during the NOE mixing time. The phase cycle used is as follows, with all the elements phase cycled with CYCLOPS after eight scans: $\phi_1 = x$; $\Phi_2 = x, y, -x, -y$; $\phi_3 = -x, x, -x, x, x, -x, x, -x$; $\phi_4 = x$; $\phi_5 = x, x, x, x, -x, -x, -x, -x$. To obtain hypercomplex pure-phase 2D spectra,[16] the phase of $\phi_3$ is changed by 90° for odd and even scans and the data are stored in two separate memory locations, resulting in a 32 × 2 step phase cycle.

**Scheme I**




In order to simplify the NOESY spectra of DNA oligonucleotides for assignment purposes, we present an experiment that enables one to selectively trace the NOE connectivities of cytidine H6 resonances. This approach substantially simplifies the analysis of a 2D NOE spectrum in the base to sugar proton regions and allows straightforward identification of corresponding resonances. The HOENOE (two-dimensional *HO*HAHA[7] *e*dited *NOE* spectroscopy) experiment is based on selective excitation of cytidine H6 protons via in-phase coherence transfer from the scalar-coupled cytidine H5 protons prior to the $t_1$ evolution period of a regular 2D NOE experiment.[8]

The pulse scheme of the HOENOE experiment is shown in Figure 1. During the preparation period a selective excitation pulse sequence is applied in the region of interest, which for our application is between 5.3 and 6.5 ppm where the CH5 as well as the sugar H1′ resonances appear. For simplicity, we used a selective spin echo excitation in which a selective 90° pulse is applied, followed by a selective 180° pulse and a short refocusing period $t_r$. This pulse scheme, with the 180° pulse phase cycled through EXORCYCLE,[9] gives a pure-phase, highly selective excitation of frequencies $\pm 1/3t_{90}$ with an excitation profile that is approximately $(\sin \pi x)/\pi x$ for $-1 < x < 1$. For frequencies outside this range the excitation is negligible. The

† On leave from Institute of Scientific Instruments, Czechoslovak Academy of Sciences, Cs-612 64 Brno, Czechoslovakia.

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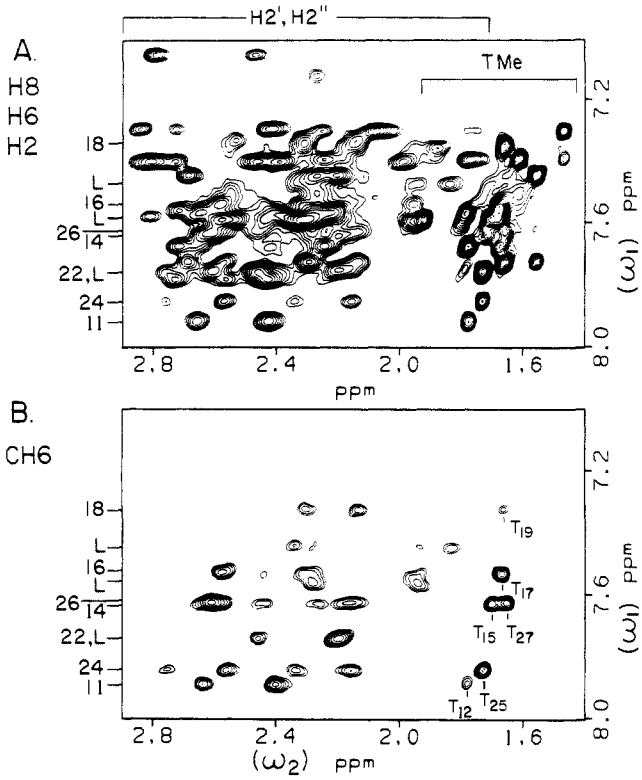


**Figure 2.** Portion of the 500-MHz (GN500) $^1$H NMR spectra of d-($G_1A_2G_3A_4G_5A_6A_7C_8C_9C_{10}C_{11}T_{12}T_{13}C_{14}T_{15}C_{16}T_{17}C_{18}T_{19}T_{20}T_{21}C_{22}T_{23}C_{24}T_{25}C_{26}T_{27}T_{28}$) containing the base-H2',H2", TMe cross peaks of (A) the NOESY experiment (5 °C, $\tau_m$ = 100 ms) and (B) the HOENOE experiment (10 °C). The sample contained a 2.3-mm DNA strand in 100 mM NaCl, 5 mM $MgCl_2$, pH 5.75, 10 °C. For the HOENOE, both the selective and nonselective excitation were centered at 5.76 ppm with $t_{90(sel)}$ = 0.8 ms, $t_r$ = 0.49 ms, $\tau_{imix}$ = 44 ms ($\gamma B_1/2\pi$ = 7.5 kHz), $\tau_m$ = 200 ms, and $t_{90(nonsel)}$ = 16 μs. Data result from 2 × 300 × 2048 matrices which were acquired with the acquisition times $t_1$ = 77 ms and $t_2$ = 205 ms, respectively. Data were processed with a squared sine-bell phase shifted by 50° in both dimensions and zero-filled to give 2048 × 2048 real points after processing. The software program FTNMR (D. Hare) was used for the data processing. Assignments of the CH6 resonances are indicated on the sides of the spectra. Resonances for $C_8$–$C_{10}$ in the C-loop region are labeled L. Interbase cross peaks between the TMe and CH6 resonances are labeled in B. Details of the assignment procedures will be presented elsewhere.

experiment does not require any special hardware on commercial NMR spectrometers. Alternative selective excitation schemes such as shaped pulses[10] could replace the selective spin echo. In the next step of the HOENOE experiment, the magnetization of the cytidine H5 protons is selectively transferred to the *J*-coupled H6 ($J_{CH5-CH6} \sim 7$ Hz) protons via isotropic mixing.[11] The in-phase coherence transfer is accomplished with an MLEV[12] (or WALTZ[13]) broadband decoupling cycle. Although complete coherence transfer is obtained for $\tau_{imix} = 1/2J$, for large molecules (and/or low temperatures) where $T_{1\rho}$ is short, a shorter mixing time should be empirically determined and used in order to obtain maximum sensitivity. After the isotropic mixing, the $t_1$ evolution period of a regular 2D NOE experiment starts. The resulting two-dimensional spectrum obtained is nonsymmetrical, with selective excitation along $\omega_1$ and nonselective excitation along $\omega_2$.

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Application of the HOENOE experiment to the 28-base DNA oligonucleotide d(GAGAGAACCCCTTCTCTCTTTCTCT-CTT), which folds over to form a triple-stranded structure of the form shown in Scheme I (+ indicates protonated C at $N_3$), is illustrated in Figure 2. This molecule provides a model for in vivo triplexes,[14] and its detailed study is presented elsewhere.[15] The base-H2',-2", TMe cross-peak region of a standard NOESY experiment is shown in Figure 2A. Figure 2B shows the same region of a HOENOE experiment. Cross peaks in this region of the HOENOE spectrum arise exclusively from NOEs involving the CH6 resonances. These are CH6-TMe cross peaks and intra- and interbase CH6-H2',H2" cross peaks. Although the CH6 resonances can be readily identified in a COSY spectrum from their scalar coupling to CH5, extension of those assignments to the sugar protons is very difficult in this molecule due to spectral overlap with other base-H2',H2" NOEs. The HOENOE experiment provides a way to unambiguously identify H6-sugar cross peaks. This was essential in assigning this molecule, since standard sequential connectivities are not observed for some resonances and many CH6-sugar cross peaks overlap with other cross peaks in the spectrum. This experiment can also be used to observe selective NOEs from the CH5 resonances via selective excitation and coherence transfer of the CH6.

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**Supplementary Material Available:** The complete HOENOE and NOESY spectra given in Figure 2 as well as the base-H1',CH5 regions of the NOESY and HOENOE spectra (3 pages). Ordering information is given on any current masthead page.

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## Preparation of a Macrocyclic Polynuclear Complex, $[(en)Pd(4,4'\text{-}bpy)]_4(NO_3)_8$,[1] Which Recognizes an Organic Molecule in Aqueous Media

Makoto Fujita,* Jun Yazaki, and Katsuyuki Ogura*

*Department of Synthetic Chemistry*
*Faculty of Engineering, Chiba University*
*1-33 Yayoicho, Chiba 260, Japan*

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Recognition of molecular shapes and functions is becoming an urgent problem in both organic and inorganic chemistry.[2] In contrast to such flexible organic hosts as crown ethers or cyclophanes, some inorganic metal complexes are known to bind organic substances in their latticed or layered infinite framework.[3]

(1) en = ethylenediamine; bpy = bipyridine.

(2) For recent reviews, see: *Molecular Inclusion and Molecular Recognition—Clathrates I (Topics in Current Chemistry, Vol. 140)*; Weber, E., Ed; Springer-Verlag: New York, 1987. *Molecular Inclusion and Molecular Recognition—Clathrates II (Topics in Current Chemistry, Vol. 149)*; Weber, E., Ed; Springer-Verlag: New York, 1987. Iwamoto, T. *Inclusion Compounds*; Atwood, J. L., Davies, J. E. D., MacNicol, D. D., Eds; Academic Press: New York, 1984; Vol. 1, p 29.

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