of ketenes from chromium carbene complexes at 80 °C and 150 atm CO pressure has been claimed.[15,16]) Which, if either, of these mechanisms is in operation is currently under study, as is the application of this method to the synthesis of biologically active β-lactams.

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Registry No. 1a, 82918-98-7; **1b**, 82918-99-8; **1c**, 82919-00-4; **2**, 82919-01-5; **3a**, 82919-02-6; **3b**, 82919-03-7; $(CO)_5Cr{=}C(Me)OMe$, 20540-69-6; $(CO)_5Cr{=}C(Ph)OMe$, 27436-93-7; PhCH=NMe, 622-29-7; PhCH=NPh, 538-51-2; 3,4-dihydro-6,7-dimethoxyisoquinoline, 3382-18-1; 2-thiazoline, 504-79-0; 2-phenyl-2-thiazoline, 2722-34-1.

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Use of Two-Dimensional NMR in the Study of a Double-Stranded DNA Decamer

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The recent development of two-dimensional (2-D) NMR techniques represents a major advance in the use of NMR for the study of biological molecules.[1-5] The usefulness of these techniques in studies of small proteins has already been demonstrated,[6,7] but applications to oligonucleotides have been limited.[8,9] In this communication we present the first application[10] of 2-D NOE (NOESY)[11] and 2-D homonuclear *J*-correlated (COSY)[12] spectroscopy to a double-stranded DNA, the synthetic DNA decamer $d(A_1T_2A_3T_4C_5G_6A_7T_8A_9T_{10})_2$.[13] The results obtained provide information on the conformation of the helix, dynamic properties, and assignments of the resonances, which would have been difficult or impossible to obtain by conventional NMR methods.

The 500-MHz one-dimensional proton NMR spectrum of $d(ATATCGATAT)_2$ at 27 °C is shown in Figure 1A, along with assignments to proton type. The majority of the aromatic resonances were assigned on the basis of various one-dimensional NMR techniques described elsewhere.[10] A stacked plot of a 2-D NOE experiment on $d(ATATCGATAT)_2$ at 27 °C with mixing time τ_m = 350 ms is shown in Figure 1B. The intense diagonal spectrum arises from protons that did not cross relax with other protons during τ_m.[5] The main features of interest, however, are the relatively small off-diagonal cross peaks that arise from dipole–dipole cross relaxation during τ_m.[11,12,14] The long mixing time (τ_m = 350 ms) coupled with the phase-cycling scheme used in these experiments suppresses any *J*-coupling contribution to

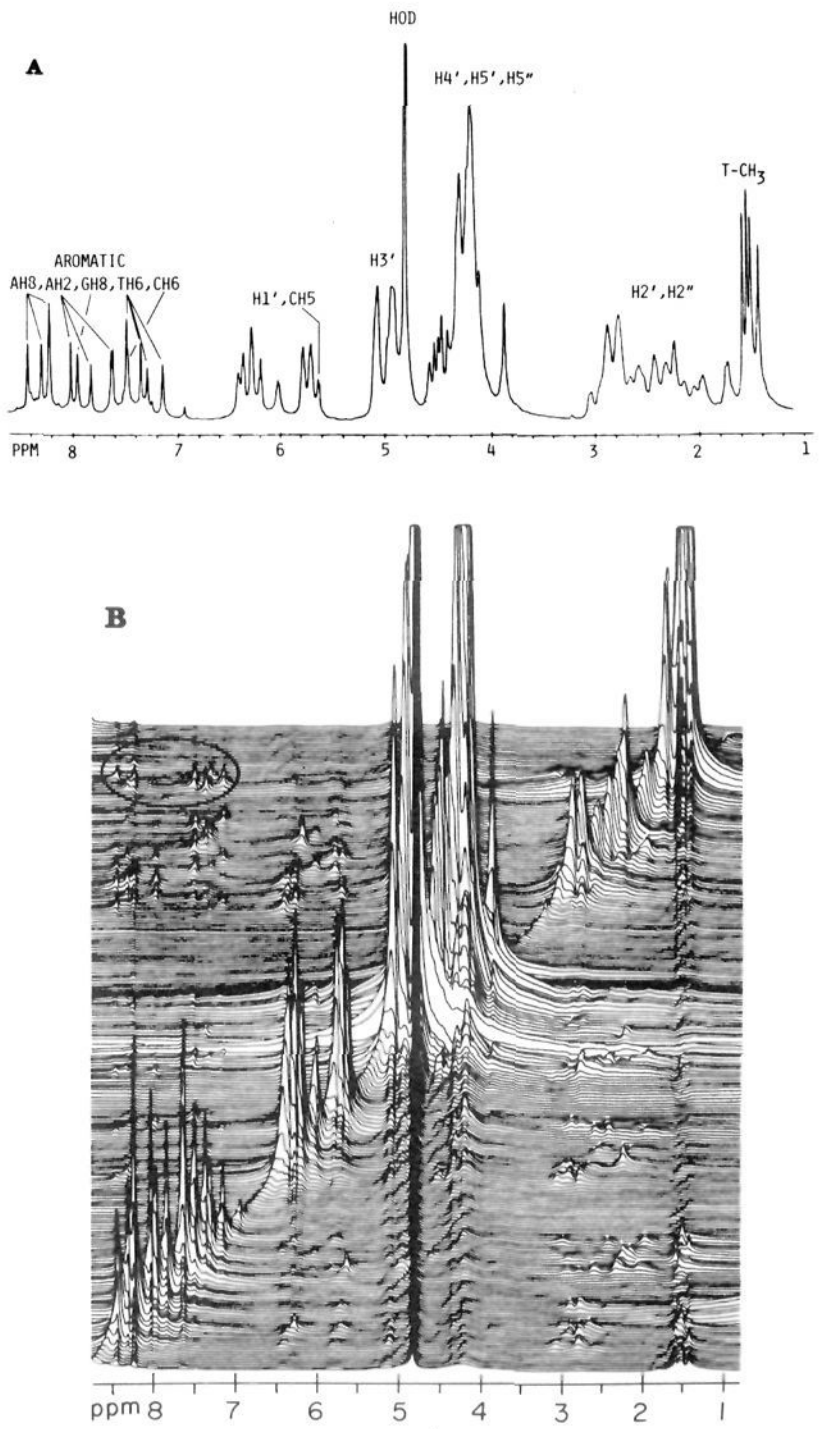

Figure 1. (A) 500-MHz ^{1}H NMR spectrum of the nonexchangeable proton resonances in $d(A_1T_2A_3T_4C_5G_6A_7T_8A_9T_{10})_2$ at 27 °C. Approximately 120 μL of a sample of 3 mM in duplex in 10 mM sodium phosphate, pH 7.0, 0.1 M NaCl, was contained in a Wilmad 508-cp microcell. Samples were repeatedly evaporated to dryness in the NMR tube and redissolved in 99.996% D_2O. Assignments to general proton type are given above the appropriate spectral regions. (B) 500-MHz NOESY spectrum (stacked plot) of $d(ATATCGATAT)_2$ at 27 °C with $\tau_m \approx 350$ ms. The spectral width was ±2000 Hz. The data set consisted of 1024 points in the t_2 dimension and 128 points in the t_1 dimension. 32 FIDs were accumulated for each value of t_1, with a 4-s delay between acquisitions, and the total accumulation time was 5.3 h. Spectra were collected by using quadrature-phase detection with the carrier at the center of the spectrum. A 32-step phase-cycling routine was used to suppress axial ridges and cancel out components of transverse magnetization after the second 90° pulse (details of the phase cycling will be published elsewhere). The resulting data matrix was processed with an exponential broadening of 6 Hz in both dimensions and was zero-filled in the f_1 dimension. The absolute value mode is used. The nonsymmetrical appearance of the cross peaks is a result of the method of data collection and processing. Cross peaks between the thymine methyl and the AH8 and TH6 resonances are circled at the top left.

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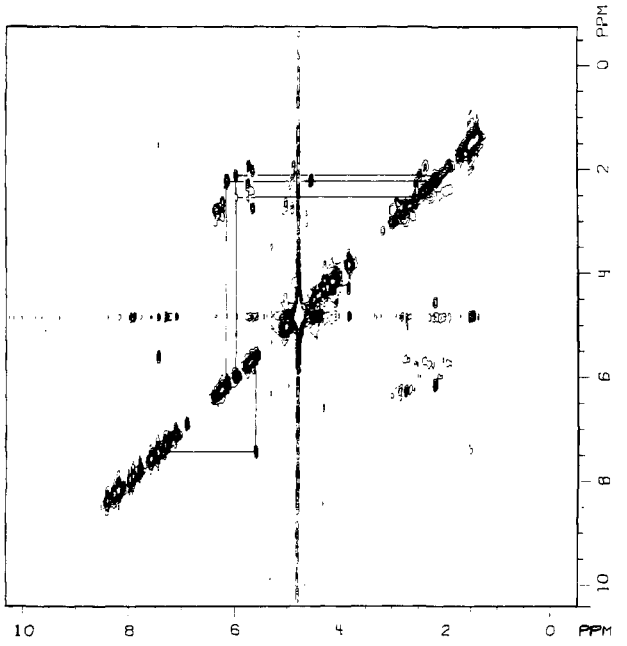

Figure 2. 360-MHz COSY spectrum (contour plot) of d(ATATCGATAT)$_2$ at 29 °C. Sample was the same as in Figure 1. The spectral width was ±2000 Hz. The data set consisted of 1024 points in the t_2 dimension and 128 points in the t_1 dimension. 96 FIDs were accumulated for each value of t_1, with a 4-s delay between acquisitions, and the total accumulation time was ~15 h. Axial peaks were suppressed by a 16-step phase-cycling routine. A 90° pulse (13.5 μs) was used for P_1, but 9.5 μs for P_2. The resulting data matrix was processed with a phase-shifted sine-bell in both dimensions and was zero-filled in the f_1 dimension. The absolute value mode is used. Connectivities manifested as cross peaks between the CH6 and CH5 resonances are indicated by the solid lines (bottom, left). Connectivities between some H1′ resonances (T_{10}, T_4) and the corresponding H2′,H2″ resonances are shown (top). The terminal T_{10} nucleoside shows only one cross peak between H1′ and H2′,H2″ since these latter resonances have the same chemical shift.

the cross peaks.[14] In the absence of second-order NOEs, the appearance of a pair of cross peaks located at (f_1, f_2) and (f_2, f_1) implies a short internuclear separation (probably less than 3.5 Å) between the two protons at (f_1, f_1) and (f_2, f_2).[6] A detailed analysis of these cross peaks in the NOESY spectrum is beyond the scope of this communication, but certain features merit discussion.

Some of the largest cross peaks in the NOESY spectra are between the H2′,H2″ proton resonances and the H1′ as well as the aromatic AH8, GH8, TH6, and CH6 resonances. There are strong cross peaks between each of the four methyl resonances and the four TH6 resonances resulting from intrabase interactions between TH6 protons and rotating methyl protons. Significantly, there are also three or four (two of the AH8 resonances overlap) interbase cross peaks between AH8 and TCH_3 protons. The existence of these interbase cross peaks indicates that the AH8 protons must be located in space near to the thymine methyl protons, and the fact that the intensities for at least two of the TCH_3–AH8 cross peaks are comparable to the intensities of the TCH_3–TH6 cross peaks implies that these interproton distances are similar.[14] Analysis of the assignments reveals that the weaker interactions are between the base pairs at the ends of the helix, and this is attributed to transient opening of the ends.

Examination of a model of B–DNA indicates that for an alternating AT sequence, the closest protons on the methyl group of a T base should be about 2.5 Å, on the average, from the H8 on the A base on its 5′ side (i.e., ApT), but quite distant (>5 Å) from the A base on its 3′ side (i.e., TpA). The observation of strong cross peaks between the AH8 and the methyl protons on a neighboring T base indicates that the base pairs are in the correct spatial configuration for a right-handed B–DNA helix. Furthermore, comparison of the relative magnitudes of the base (purine-H8 or pyrimidine-H6) cross peaks with H1′ and H2′,H2″ at shorter (60–100 ms) mixing times establishes that the bases are in the anti nucleotide conformation. This information, combined with measured coupling constants for the H1′ protons ($J_{1'2'} + J_{1'2''} \simeq 14$ Hz)[10] which indicate a predominantly S-type sugar pucker for the molecule,[15] is consistent with the expectation that d(ATATCGATAT)$_2$ is in the B conformation. The NOESY experiments also provide information that makes it possible to complete the assignments of the base proton resonances and to assign the H1′ sugar resonances. These assignments are discussed in detail elsewhere.[10]

A contour plot of a 2-D *J*-correlated (COSY) spectrum of d(ATATCGATAT)$_2$ is given in Figure 2. The diagonal spectrum is similar to the regular one-dimensional spectrum and off diagonal cross peaks connect homonuclear *J*-coupled proton resonances.[12,16] Interactions between all the *J*-coupled protons in DNA (i.e., sugar protons and CH5–CH6) are seen in the contour plot, although for the coupled sugar protons only the H1′–H2′,H2″ cross peaks are resolvable. The large cross peak connecting resonances at 7.45 and 6.23 ppm is due to coupling between C_5H6 and C_5H5. Analogous cross peaks should be valuable in assigning these resonances in other DNAs.

The preliminary results presented here, and elsewhere,[10,17] indicate that through the use of one- and two-dimensional NMR techniques it should be possible to completely assign the spectra of short DNA molecules and to investigate their solution state structural and dynamic properties at a level of detail heretofore impossible.

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Lifetime of $O_2(^1\Delta_g)$ in Liquid Water As Determined by Time-Resolved Infrared Luminescence Measurements

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The chemical and kinetic properties of singlet molecular oxygen in liquid phase have occupied the attention of chemists for almost 20 years as indicated by numerous reviews on the subject.[1-9] This transient entity, however, has evaded direct observation until very recently when the 1.27-μm luminescence from the forbidden transition $O_2(^3\Sigma_g^-) \leftarrow O_2(^1\Delta_g)$ was detected via photosensitization

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