Compounds **1a-c** bind strongly to DNA, as indicated by changes in their fluorescence quantum yields and by hyperchromism and bathochromic shifts in their absorption spectra (Table I) on DNA addition.

Evidence that the DNA binding of **1a-c** is intercalative comes from consideration of their molar extinction coefficient $\epsilon(\lambda_{max})$ at the maximum of their first absorption band. In the free state, ϵ(412 nm) of **1c** is only about twice as large as ϵ(409 nm) for the monomeric 9-(ethylamino)acridine, due to intramolecular stacking, a phenomenon observed for many polychromophores.[5] However, ϵ(417 nm) for bound **1c** is just 3 times as large as ϵ(417 nm) for bound 9-(ethylamino)acridine, suggesting that all three chromophores of **1c** are in environments identical with that of 9-(ethylamino)acridine (i.e., intercalated).

Definitive evidence concerning the mode of DNA intercalation of **1a-c** comes from their ability to unwind and rewind closed circular supercoiled DNA. The D/P ratios (r) for the compounds to completely relax the supercoils of PML-21 closed circular DNA as determined by viscometry are recorded in Table I,[11] together with the r values for ethidium bromide, 9-aminoacridine, and 9-(ethylamino)acridine measured under the same conditions. Assuming an unwinding angle of 26° for ethidium[12] results in an unwinding angle of 15° for the monomeric 9-aminoacridines, in agreement with other determinations.[6,13] This indicates that the chromophore determines the geometry of the ligand-DNA complex. The diacridines **1a** and **1b** have comparable unwinding angles (29 and 33°, respectively), of about twice that of the monomer, indicating bis-intercalation. A similar value for the triply charged compound **1b** suggests that the point charge in the chain has little effect on ligand-DNA geometry.

The triacridine **1c**, where the point charge of **1b** has been replaced by a third acridine chromophore, has an unwinding angle of 45°, 3 times that of the monomer, indicating strongly that all three chromophores bind by intercalation. The value of 45° is a minimum, for the compound binds avidly to all surfaces, including glass. The binding constant K of **1c** to PML-21 DNA was estimated to be $>10^6$ M^{-1} from a Vinograd plot[14] by using a site exclusion model.[15]

The spacing of 7 Å between the chromophores demands that **1c** forms single base pair sandwiches on intercalation as suggested for the $(CH_2)_6$- and $(CH_2)_7$-linked diacridines.[6] However, the present data do not allow determination of the exact nature of the triacridine-DNA complex. NMR studies of triacridine-oligonucleotide complexes provide more information and are underway. The possibility that **1c** tris-intercalates only in presence of certain DNA sequences is under study using several analogous compounds. This work is facilitated by the synthetic scheme outlined (Scheme I), which permits easy variation of both linker chain and chromophores.

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Registry No. 1a·2HCl, 85185-21-3; **1b**·3HCl, 85185-22-4; **1c**·3HCl, 85185-23-5; **2a**, 110-94-1; **2d**, 17336-01-5; **2e**, 85185-24-6; **3d**, 85185-25-7; **4b**, 85185-26-8; 9-chloroacridine, 1207-69-8; *N*-(benzyloxycarbonyl)propane-1,3-diamine, 46460-73-5.

(10) All compounds analyzed correctly for the assigned structure. The end products **1** had NMR spectra consistent with the assigned structures. The compounds showed one spot on TLC (Merck silica gel 60, developed in the top phase of *n*-BuOH/water/AcOH (5:4:1) and eluted for 20 cm).

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Sequential Resonance Assignments in DNA ^{1}H NMR Spectra by Two-Dimensional NOE Spectroscopy

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The utility of ^{1}H NMR in structural studies of biological macromolecules has grown enormously since the advent of two-dimensional (2-D) techniques.[1-7] Systematic methods have been developed to obtain resonance assignments in ^{1}H NMR spectra of small proteins[8,9] by a combination of 2-D NOE (NOESY)[10,11] and 2-D J-correlated spectroscopy (COSY, SECSY).[6,12] These assignments are a prerequisite for a detailed analysis of 2-D NOE data, which yields a large amount of proton-proton "contacts" (proton-proton distances within about 0.4 nm) from which the solution structure of the protein may be deduced.[13]

In this communication we show that a similar strategy can be used in nucleic acid conformational studies. A sequential assignment procedure is described for most of the base and deoxyribose protons in DNA duplexes employing 2-D NOE spectroscopy. As in the case of proteins the individual resonance assignments thus found may form the basis for a quantitative analysis of 2-D NOE spectra in terms of proton-proton distances and, eventually, the DNA structure in solution.

A 360-MHz 2-D NOE spectrum was recorded of a mixture of two synthetic[14,15] complementary heptamers d(TGAGCGG) and d(CCGCTCA), which form a duplex under the experimental conditions employed (see legends to Figure 1). This DNA fragment is homologous to part of the *lac* operator of *Escherichia coli*, which is the subject of current research on specific protein-nucleic acid interactions in our laboratory. In the 2-D NOE experiment dipole-dipole cross relaxation between a pair of closely spaced protons results in the appearance of an off-diagonal peak linking the two corresponding diagonal resonances, thereby establishing the promixity of the two protons.[10]

Three parts of the 2-D spectrum will be discussed in detail here. Figure 1A shows the region of cross peaks that link the base proton (AH8, GH8, CH6, TH6) resonances with those of the CH5 and H1′ protons. In Figure 1B the contacts are shown between the same base protons and the TCH_3 and sugar H2′, H2″ protons.

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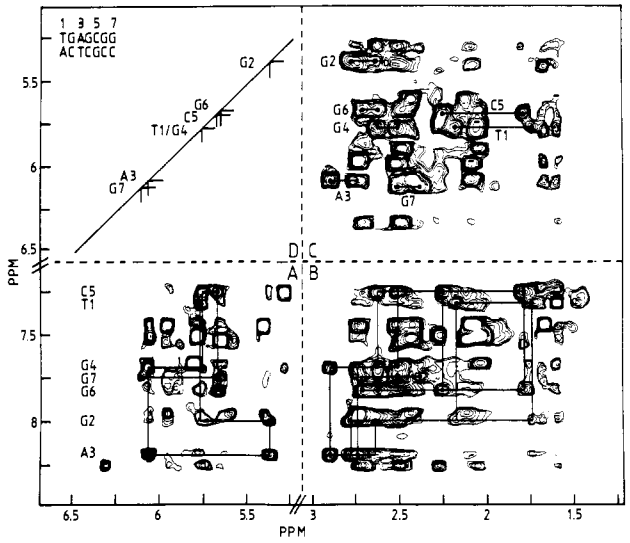

Figure 1. 360-MHz 2-D NOE spectrum (contour plot) of an equimolar mixture of d(TGAGCGG) and d(CCGCTCA), 5 mM in duplex, in 50 mM phosphate, 0.2 M KCl, 0.02% (w/v) NaN_3, T = 28 °C, pH 6.5 (meter reading in ^2H_2O). A $(\pi/2\text{-}t_1\text{-}\pi/2\text{-}\tau_m\text{-}\pi/2\text{-}t_2)_n$ sequence was used,[10] with a 2-s relaxation delay after each acquisition and 0.2-s mixing time τ_m. 128 free induction decays of 2048 points were accumulated at 512 t_1 values, using quadrature-phase detection in t_2 (±5000 Hz) with the carrier frequency at the low-field side of the spectrum. The pulses and receiver phases were cycled through a 32-step phase program that keeps intact the pure amplitude modulation, and only real t_1 data were collected.[16] Gaussian multiplication[17] and zero-filling preceded Fourier transformation in both dimensions and the symmetrized pure absorption phase spectrum is shown. The drawn lines connect intra- (indicated with a dot) and internucleotide contacts between base protons and sugar H1′ protons (1A) or sugar H2′ and H2″ protons (1B) in the d(TGAGCGG) strand. In Figure 1C the corresponding H1′–H2′, H2″ (intranucleotide) cross peaks are indicated. Figure 1D shows schematically the H1′ resonance positions on the diagonal.

Table I. Proton-Proton Distances[a] (nm) in B-DNA

type of contact	B-B	B-H1′	B-H2′	B-H2′	B-TCH_3	B-CH5
intra-nucleotide		0.38	0.36	0.21	0.28	0.24
to 5′-neighbor	0.50	0.28	0.23	0.37	>0.6	>0.6
to 3′-neighbor	0.50	>0.6	>0.6	>0.6	0.32	0.38

[a] The distances between the purine H8 or pyrimidine H6 protons (B) and the adjacent aromatic or ribose protons were calculated from the optimized X-ray coordinates of the carbon atoms of B-DNA[20] and a C-H bond length of 0.109 nm. Distances for all combinations of nucleotides fall within 0.015 nm from the average given in the table.

Many of the cross peaks in these two regions represent intranucleotide contacts. The most intense peaks, corresponding to the shortest proton–proton distances, are readily identified as the five CH6–CH5 contacts and the two TH6–TCH_3 contacts,[19] which also appear in a COSY spectrum (not shown). However from the number of cross peaks found at H1′, H2′, and H2″ frequencies it can be concluded that each of the purine H8 and pyrimidine H6 protons (except those at the 5′ termini) is close enough to the H1′, H2′, and H2″ protons on *two* sugar residues for cross relaxation to occur. For right-handed helices, with nucleotides in the anti conformation, it is clear that one of these sugar residues forms part of the same nucleotide as the base proton while the other must belong to the 5′-neighboring nucleotide. This is illustrated in Table I, where the relevant proton–proton distances are given for B-DNA.[20] These internucleotide cross peaks in the

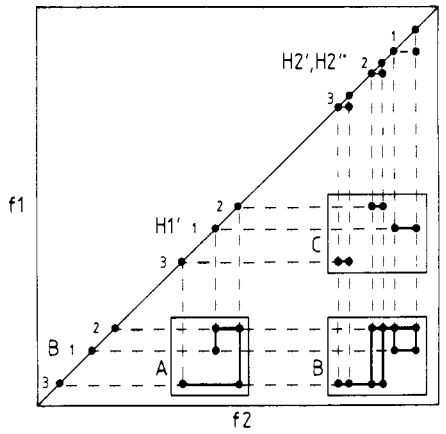

Figure 2. Schematic 2-D NOE spectrum of a DNA fragment. Only resonances of the base protons (B) and the sugar H1′, H2′, and H2″ protons of three nucleotides are shown with their cross peaks in one of the two symmetrical triangles. Drawn lines indicate the cross-relaxation networks in regions A and B that allow sequential assignment of the resonances shown. See text for explanation.

2-D NOE spectrum make it possible to step from one base proton to the next in the sequence via its H1′, H2′, and H2″ contacts. This is illustrated in Figure 2.

Figure 2 shows schematically part of a 2-D NOE spectrum of a single DNA strand, consisting of three nucleotides in a right-handed helix conformation. Two regions of the spectrum (regions A and B, corresponding to the spectral regions of Figure 1, A and B) contain useful sequence information, as discussed above. The sequential assignment procedure, then, may start at the 5′ terminus with base proton B(1). Only intranucleotide cross peaks appear in regions A and B connecting B(1) with H1′(1), H2′(1), and H2″(1). These sugar assignments can be checked immediately in region C, where a pair of cross peaks links the H1′ resonance to both the H2′ and H2″ resonances of a given nucleotide. These H1′ and H2′, H2″ resonances are linked to a common new base proton resonance B(2), which, on this basis, can be assigned to the purine H8 or pyrimidine H6 of the 3′-neighboring nucleotide. Also linked to B(2) are the H1′(2), H2′(2), and H2″(2) resonances, which are again reflected by a pair of cross peaks in region C. This completes the first step, which can be repeated until the complete cross-relaxation networks in regions A and B are found and checked for consistency with region C. All purine H8, pyrimidine H6, sugar H1′, and H2′, H2″ resonances of the DNA strand can be assigned in this way.

The procedure was applied successfully to both the d(TGAGCGG) and d(CCGCTCA) strands in our DNA fragment. In Figure 1, A and B, the three cross-relaxation networks are shown for the d(TGAGCGG) strand, whereas in Figure 1C the seven pairs of H1′–H2′, H2″ cross peaks, consistent with these networks, are indicated.

The strong CH6–CH5 and TH6–TCH_3 contacts can be used to identify the cytosine and thymine H6 resonances, thereby characterizing the DNA strand. Apart from these strong intrabase contacts each of the CH5 and TCH_3 resonances is linked to the purine H8 or pyrimidine H6 resonance of its 5′-neighbor in accordance with the spatial requirements of a right-handed helix (see Table I). Most of these interbase cross peaks are clearly resolved in Figure 1A. Thus, when a pyrimidine occurs in the sequence, an alternative cross-relaxation pathway exists, not involving sugar protons but CH5 and TCH_3 protons as the linkage between purine H8 and/or pyrimidine H6 protons on adjacent nucleotides.

The systematic procedure presented here has yielded the complete assignment of all GH8, AH8, TH6, TCH_3 CH6, CH5, H1′, and H2′, H2″ resonances as well as most of the H3′ resonances in both strands of our DNA duplex. This will be described in more

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detail elsewhere. The complete systematic assignment of the remaining sugar resonances is impeded thus far by strong overlap in the H4′, H5′, and H5″ regions, even in a 2-D spectrum. The remaining aromatic resonances (AH2) are part of another cross-relaxation network in double-stranded DNA, which also includes the exchangeable hydrogen-bridged imino protons. Systematic assignment of these resonances is also possible by using the nuclear Overhauser effect.[21,22]

The procedure presented here for DNA resonance assignments has the usual advantages over analogous one-dimensional approaches in that it avoids the necessity of selective irradiation of many resonances in crowded spectral regions[12] and is experimentally straightforward. Furthermore, it does not rely on the availability of numerous subfragments[23] or analogues[22] of the DNA duplex of interest, nor on assumptions about the exact solution structure as required for chemical-shift calculations,[24] nor on assumptions concerning the temperature dependence of the chemical shift such as are involved in the interpretation of melting behavior.[25]

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Angle-Resolved SIMS Studies of Organic Monolayers on Ag(111)

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Organic molecules are desorbed and ionized directly from the solid state by using secondary ion mass spectrometry (SIMS).[1,2] The mechanism of molecular desorption has been open to discussion particularly with regard to species with molecular weights greater than several hundred daltons.[1-4] Of special concern is to explain how a >1000-eV Ar^+ ion (or Ar atom) induces the desorption of a molecule whose bond strengths may be on the order of several electron volts. To address this question, the energy dissipation for a model system of benzene adsorbed into a $c(4 \times 4)$ overlayer on Ni(001) has been followed by using classical trajectory techniques.[5,6] In this work, we test several predictions of the classical dynamics model by measuring for the first time

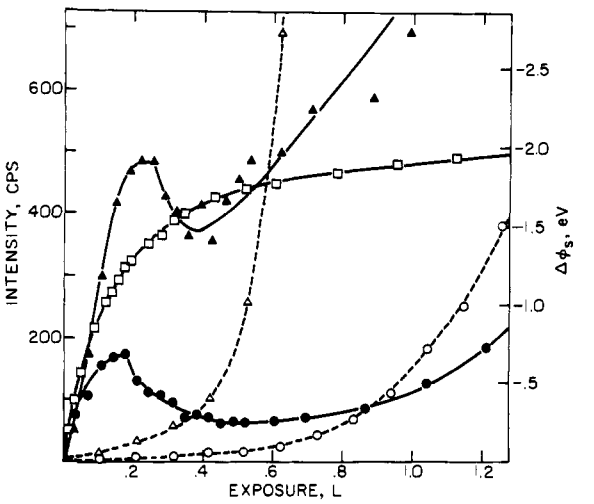

Figure 1. Ion yields of $C_6H_5^+$ (○) and $AgC_6H_6^+$ (△) from benzene on Ag(111) and ion yields of $C_5H_5NH^+$ (●) and $AgC_5H_5N^+$ (▲) from pyridine on Ag(111) as a function of exposure at 153 K. The Ar^+ primary ion was incident perpendicular to the surface, and the polar collection angle θ was 45° relative to the surface normal; work function change (□) for pyridine on Ag(111) as a function of exposure at 153 K. Bombardment conditions: 1 keV, 2 nA, Ar^+.

the ion yield as a function of the polar angle of ejection and coverage for benzene and pyridine adsorbed on Ag(111) at 153 K. These model systems are of interest for a number of reasons. (i) The molecules are similar in size and shape and should behave in a closely related fashion under the influence of ion bombardment. (ii) Classical dynamics calculations have been performed on these molecules adsorbed on Ni(001) where dramatic differences in the molecule yield are predicted to occur with molecular orientation.[6] (iii) Electron energy loss spectroscopy indicates that pyridine on Ag(111) initially adsorbs in a π-bonded configuration but undergoes a compressional phase transition to a σ-bonded configuration as the coverage is increased.[7,8] Benzene, on the other hand, is believed to remain in the π-bonded configuration at all coverages.[9-12]

The details of the angle-resolved SIMS apparatus have been described elsewhere.[13] The Ag(111) surface was cleaned by cycles of heating at 700 K and ion bombardment. After a final annealing at 620 K for 5 min, the crystal exhibited sharp 6-fold symmetric LEED spots. Exposure values in langmuir units were corrected by a guage factor of 5.8. The SIMS spectra of a Ag(111) surface exposed to pyridine or benzene at 153 K are characterized by a series of cluster ions including the molecular ion M^+, $(M + H)^+$, Ag^+, $(Ag + M)^+$, and $(Ag + 2M)^+$. For benzene, all positive ion yields begin to increase after 0.6 langmuir of exposure and reach a plateau at monolayer coverage after 2.5 langmuirs of exposure. Exposure to benzene beyond 2.5 langmuirs results in a decrease in the intensities and an increase in the relative fragment yields as has been reported to occur using the Ni(001) surface.[14] For pyridine on Ag(111), however, the ion yields exhibit a distinct maximum at 0.2 langmuir of exposure before increasing to a second maximum after 4.5 langmuirs of exposure. We do not believe this first structure arises from any anomolous electronic effects since the surface work function change measured simul-

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