Nuclear Magnetic Resonance Studies of Drug–Nucleic Acid Interactions at the Synthetic DNA Level in Solution

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The design of clinically useful pharmacological agents is dependent to a great degree on an understanding of the molecular basis of antibiotic action.[1] Several antitumor agents (adriamycin, bleomycin, *cis*-dichlorodiammineplatinum(II)) currently used in combination chemotherapy form complexes with DNA and exhibit their antibiotic activity at the nucleic acid level. The deleterious side effects associated with these natural drugs have stimulated considerable effort to be directed toward the chemical synthesis of nontoxic analogues. Such research would benefit greatly from an increase in our knowledge of the stereochemical details of the complementary interactions between different classes of antibiotics and the DNA double helix.

Antibiotics can bind to the nucleic acid duplex by several mechanisms.[2,3] Bleomycin probably belongs to the class in which the antibiotic interacts along the grooves of the sugar–phosphate backbone. The resulting complexes are stabilized by electrostatic interactions involving the backbone phosphates and hydrogen-bonding interactions with the base-pair edges. Adriamycin belongs to a second type in which the nucleic acid duplex undergoes local conformational transitions to generate intercalative binding sites. These complexes are stabilized primarily by stacking interactions between aromatic chromophores of the antibiotic and adjacent base pairs. Finally, antibiotics such as *cis*-dichlorodiammineplatinum(II) may form covalent linkages with the nucleic acid and perturb the DNA structure sufficiently to inhibit the transcription and translation processes.

The recognition process is further manifested in the specificity of antibiotic–nucleic acid interactions at the helix-type, base-pair, and sequence level. Thus, the antibiotics actinomycin[4] and netropsin[5] bind to double-stranded DNA but not to RNA or DNA–RNA hybrids. Actinomycin complexes selectively to dG·dC-rich base-pair regions[4] while netropsin binds only at dA·dT base-pair sites in the minor groove of the double helix.[5] Sequence specificity is best demonstrated by the antibiotics actinomycin and ethidium bromide which intercalate at purinyl-(3′–5′)-pyrimidine[6] and pyrimidinyl-(3′–5′)-purine[7] sites, respectively. The elucidation of the stereochemical basis of this remarkable specificity has stimulated a multidisciplinary effort to probe and characterize antibiotic–nucleic acid complexes in the crystalline, fiber, and solution states. The goal of these studies is the elucidation of the available topologies for ligand and receptor at the molecular level and an understanding of the role of symmetry in the recognition process.

The most detailed structural information on antibiotic–nucleic acid complexes has been deduced from single-crystal X-ray investigations of a series of drugs intercalated into miniature self-complementary dinucleotide RNA duplexes.[8] Such investigations at atomic resolution pinpoint the torsion angle changes in the sugar–phosphate backbone and about the glycosidic bonds which result on generation of an intercalation site. Current efforts are directed toward the crystallization of antibiotics complexed to longer oligonucleotide duplexes in order to estimate the extent of structural distortion adjacent to the binding site. Metallointercalators such as terpyridineplatinum(II) have been used to enhance the X-ray diffraction maxima of antibiotics complexed to DNA fibers.[9] The results support a neighbor exclusion model in which the intercalating agent complexes at every other potential binding site on the DNA helix.

High-resolution nuclear magnetic resonance (NMR) spectroscopy is a powerful technique for monitoring antibiotic–nucleic acid complexes since resonances distributed throughout each component can be independently monitored to yield detailed structural, thermodynamic, and kinetic information on the interactions in solution. The initial NMR studies con-

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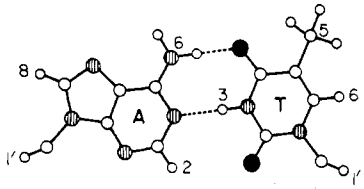
centrated on complexes at the dinucleotide duplex level[10] and the solution results compared favorably with related crystallographic information. The complexity of the spectra increased on studying the complexes at the stable oligonucleotide duplex level,[11] with the published work to date focussing on structural aspects of intercalation complexes. Proceeding to the macromolecular level,[12] NMR has been applied with considerable success to elucidate the structure and function of tRNA,[13] while the resonances of rod-like biopolymers such as DNA are broadened out beyond detection.

We therefore initiated a search for high molecular weight ($\sim 10^6$ daltons) nucleic acid duplexes which would exhibit a minimum number of resolvable NMR resonances with moderate line widths (<100 Hz). Our efforts were guided by the thermodynamic and kinetic measurements of Baldwin and co-workers on synthetic DNAs with an alternating purine–pyrimidine sequence using absorbance and temperature-jump spectroscopy.[14] They found that poly(dA-dT) folds via hairpin loops into smaller branched duplexes which melt independently of each other during the thermal helix–coil transition.[14] We reasoned that the rapid migration of the branched duplexes of poly(dA-dT) should provide sufficient segmental mobility to narrow the line widths of the NMR resonances compared to their values in natural DNA. In addition, the NMR spectrum of poly(dA-dT) should be greatly simplified since symmetry considerations require that every dA·dT base pair (1) exhibit the same distribution of neighboring residues.



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Our laboratory has probed the structure and dynamics of poly(dA-dT) by monitoring the NMR resonances distributed at the Watson–Crick hydrogen bonds, the base pairs, and the sugar–phosphate backbone.[15] The section below characterizes the helix–coil transition of this synthetic DNA by NMR techniques. It is followed by examples of ligands that intercalate between nucleic acid base pairs, that are partially inserted between tilted base pairs at the binding site, and that complex to the sugar–phosphate backbone of the nucleic acid in solution. The NMR parameters are distinct for these three kinds of complexes at the synthetic DNA level and provide structural and dynamic information on different types of ligand–nucleic acid interactions in solution.

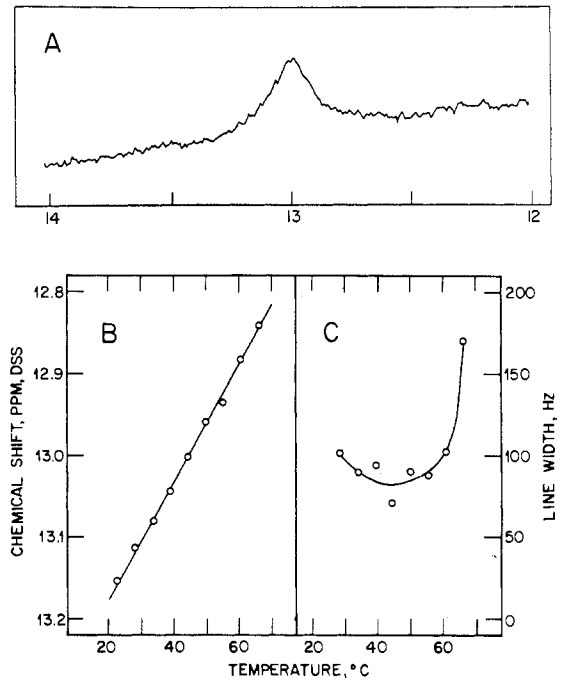

Figure 1. (A) The 360-MHz continuous-wave NMR spectrum (12–14 ppm relative to 4,4-dimethyl-4-silapentane-1-sulfonate, DSS) of 26 mM poly(dA-dT) in 1 M NaCl, 10 mM cacodylate, 0.1 mM ethylenediaminetetracetic acid, EDTA, H_2O, pH 6.53 at 44.5 °C. The temperature dependence of the chemical shifts and line widths of this exchangeable resonance between 20 and 70 °C are plotted in (B) and (C), respectively.

Helix–Coil Transition of Poly(dA-dT). Previous NMR studies have demonstrated that the Watson–Crick hydrogen-bonded ring NH resonances of stable nucleic acid base pairs resonate between 12 and 14 ppm in H_2O solution.[16] Base-pair formation for poly(dA-dT) in high salt (1 M NaCl) is readily demonstrated by the observation of an exchangeable resonance in this region (Figure 1A) with an area comparable to single proton nonexchangeable resonances. This thymidine H-3 resonance (1) shifts linearly downfield from 65 to 20 °C (Figure 1B). The chemical shift change is indicative of either a change in the base-pair overlap or a variation in the fraction of hairpin loops and branch points in the folded structure of poly(dA-dT) with temperature in the premelting region or both. Alternately, it may also reflect a contribution from an increase in the strength of the hydrogen bond at the lower temperature. The line width of the exchangeable resonance is a measure of the lifetime of the hydrogen-bonded state.[17] The thymidine H-3 proton exhibits a line width of ~90 Hz between 30 and 60 °C but broadens out between 60 and 70 °C (Figure 1C). This broadening occurs at tem-

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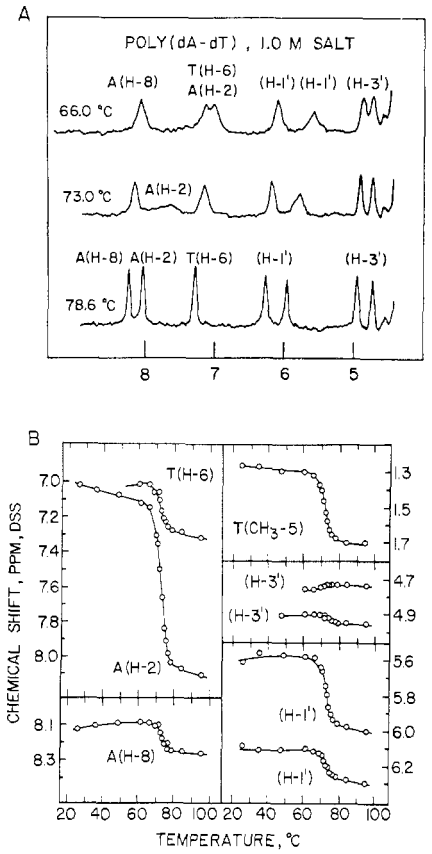

Figure 2. (A) The temperature dependence of the 360-MHz continuous-wave proton NMR spectra (4.5 to 9.0 ppm relative to DSS) of 22.2 mM poly(dA-dT) in 1 M NaCl, 10 mM cacodylate, 1 mM EDTA, D_2O, pH 5.4 at 66.0, 73.0, and 78.6 °C. (B) The temperature dependence of the chemical shifts of the base and sugar resonances between 25 and 95 °C.

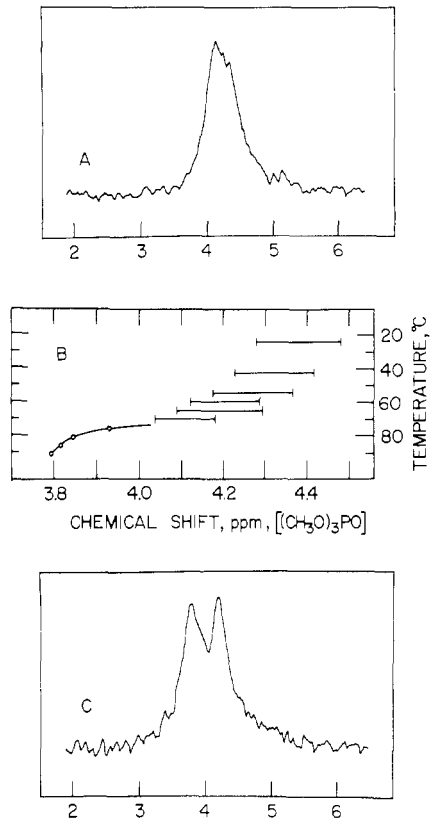

Figure 3. (A) The proton noise-decoupled 145.7-MHz phosphorus FT NMR spectrum (upfield from standard trimethyl phosphate) of poly(dA-dT) in 1 M NaCl, 10 mM cacodylate, 10 mM EDTA, D_2O, pH 6.2 at 65 °C. (B) The temperature dependence of the poly(dA-dT) chemical shifts between 20 and 90 °C. The spectra are an envelope of broad overlapping resonances between 20 and 70 °C. A shift in intensity from the upfield to the downfield components is observed on going from 55 to 70 °C. (C) The proton noise-decoupled ^{31}P spectrum of proflavin–poly(dA-dT) complex (P_i/drug = 10) in 1 M NaCl, 10 mM cacodylate buffer at 65 °C.

peratures below the midpoint of the melting transition (T_m = 72.4 °C), so that exchange with solvent must occur by transient opening of the duplex.

The potential of NMR spectroscopy to characterize the helix–coil transition of poly(dA-dT) in 1 M NaCl is demonstrated by the observation of well-resolved base (adenosine H-8 and H-2, thymidine H-6 and CH_3-5) and sugar (H-1′ and H-3′) resonances in the duplex state (Figure 2A). These nonexchangeable protons between 4.5 and 8.5 ppm shift as average peaks during the duplex to strand transition (Figure 2A) and exhibit a transition midpoint of 72.4 °C and a transition width of 3.7 °C (Figure 2B). The upfield shifts of the base protons on duplex formation (Figure 2B) reflect predominantly the effect of ring currents from nearest- and next-nearest-neighbor base pairs.[18] The estimated contributions increase in magnitude in the order A(H-8) ~ T(H-6) < T(CH_3-5) < A(H-2)[15] for a B-DNA helix.[19] This agreement with experiment suggests that poly(dA-dT) exhibits base-pair overlap geometries of the B-DNA type in solution.

The adenosine H-2 resonance undergoes the largest chemical shift change during the melting transition (Figure 2B). The line width of the adenosine H-2 resonance (>100 Hz) is much broader than that observed for the other resonances at the midpoint of the transition (Figure 2A). This suggests that the poly(dA-dT) duplex dissociation rate constant is on the order of the melting transition shift difference (0.935 ppm) at the adenosine H-2 resonance. The calculated rate constant for the duplex to strand transition of 2.3 × 10^3 s^{-1} (at T_m = 72.4 °C) is several orders of magnitude faster than the corresponding values for DNA of comparable length. This verifies the earlier proposal that the melting of poly(dA-dT) proceeds through the opening of short branched duplex regions which melt independently of each other.[14]

Recent examples have demonstrated the ability of ^{31}P NMR spectroscopy to probe the structural environment about the phosphate groups in nucleic acids.[20] Poly(dA-dT) exhibits broad overlapping ^{31}P resonances in the premelting transition range in 1 M NaCl solution (Figure 3A). The resonances narrow and shift downfield on proceeding from duplex to strands during the melting transition (Figure 3B). Theoretical conformational calculations of nucleic acid structure suggest that the transition from stacked to unstacked states results predominantly from a gauche to trans change in the O3′–P backbone torsion angle.[21] The

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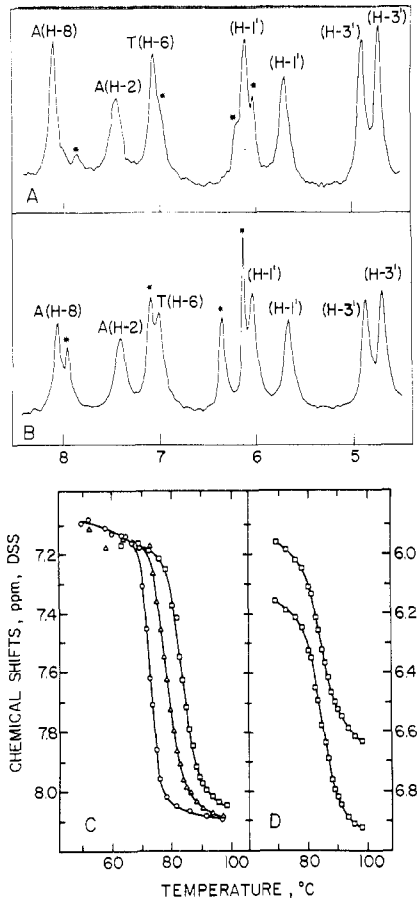

Figure 4. (Top) The 360-MHz Fourier-transform NMR spectra (4.5–8.5 ppm relative to DSS) of the proflavin–poly(dA-dT) complex in 1 M NaCl, 10 mM cacodylate, 10 mM EDTA, D_2O, pH 7. Spectrum A represents the P_i/drug = 24 complex at 78.5 °C (T_m of proflavin resonances in the complex = 80.0 °C), while spectrum B represents the P_i/drug = 8 complex at 81.4 °C (T_m of proflavin resonances in the complex = 84.3 °C). The synthetic DNA concentration was 12.6 mM in phosphates. The proflavin (**2**) resonances H_a, H_b, H_c, and H_d from low to high field are designated by asterisks and have relative areas 1:2:2:2 per mutagen molecule. (Bottom) The temperature dependence of the chemical shifts of the resonances in the proflavin–poly(dA-dT) complex, P_i/drug = 24 (△) and 8 (□) in 1 M NaCl, 10 mM cacodylate, D_2O, pH 7. The synthetic DNA concentration was 12.6 mM in phosphates. (C) The adenosine H-2 resonance. The chemical shifts for poly(dA-dT) alone are represented by (○). (D) The two upfield proflavin resonances.

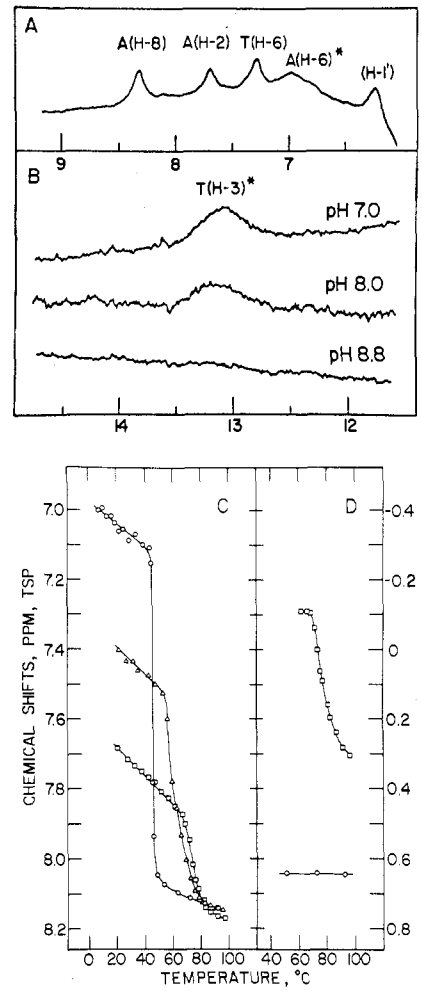

Figure 5. (Top) The continuous-wave 360-MHz proton NMR spectra of the dipyrandium–poly(dA-dT) complex, P_i/drug = 5, in 25 mM cacodylate, 0.25 mM EDTA, H_2O, pH 7. The spectra are calibrated relative to standard sodium 3-(trimethylsilyl)-propionate-2,2,3,3-d_4 (TSP). (A) The spectral region 6–9 ppm at pH 7 and 31.6 °C. The exchangeable adenosine H-6 protons (**1**) are designated by an asterisk. (B) The spectral region 11.5–14.5 ppm at 20.3 °C as a function of pH. (Bottom) The temperature dependence of the chemical shifts of resonances in the dipyrandium–poly(dA-dT) complex, P_i/drug = 11.5 (△) and 5 (□) in 10 mM cacodylate buffer, 0.1 mM EDTA, D_2O solution. The synthetic DNA concentration was fixed at 17 mM. (C) The adenosine H-2 resonance. The chemical shifts for poly(dA-dT) alone are represented by (○). (D) The highest field dipyrandium resonance. The chemical shifts of dipyrandium alone are represented by (○).

downfield shifts for the ^{31}P resonances (Figure 3B) probably monitor this change during the melting transition of poly(dA-dT) in solution.

In summary, the exchangeable protons of the synthetic DNA monitor the Watson–Crick hydrogen bonds, the base proton chemical shifts are a measure of the base-pair overlap geometries, and the torsion angles about the backbone phosphates can be probed by the phosphorus chemical shifts. The coupling constant information (magnitude <10 Hz) is lost for proton resonances with line widths of ≥50 Hz at the synthetic DNA duplex level, so that we are unable to evaluate the pucker of the sugar ring and the remaining backbone torsion angles.

The methodology described above is quite general, and we have applied it to characterize and compare the NMR parameters for synthetic RNAs and DNAs with the same alternating inosine–cytidine and adenosine–urdine sequences[22] through the premelting, melting, and postmelting transitions. These experiments demonstrate that the pyrimidine H-6 resonance is very sensitive to nucleic acid conformational transitions in solution.

Intercalation of Mutagens into DNA. Proflavin (**2**), a cationic acridine dye, is a powerful mutagenic agent against T-even phages of *Escherichia coli*. Frame shift mutation studies with proflavin have played a fundamental role in the establishment of the triplet nature of the genetic code.[23] Proflavin binds to DNA

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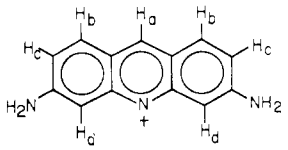
by intercalation between base pairs,[3] and detailed stereochemical models have been proposed based on linked-atom conformational calculations for intercalation of proflavin into B-DNA and A-DNA.[24] Heavily hydrated crystals of 3:2 and 2:2 proflavin·C-G complexes have been grown and analyzed by X-ray diffraction methods.[25] These studies have yielded stereochemical details at atomic resolution for proflavin intercalated into a miniature RNA duplex. The discussion below summarizes the NMR parameters for the proflavin·poly(dA-dT) complex at high phosphate (P_i) to drug ratios in 1 M NaCl solution.[26] The results permit an estimation of several structural aspects of drug–nucleic acid complexes involving intercalating agents.



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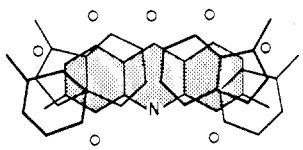
The excellent resolution of the nucleic acid and mutagen proton resonances for the proflavin·poly(dA-dT) complex at P_i/drug ratios of 24 and 8 is demonstrated in Figures 4A and 4B, respectively. The individual nucleic acid resonances shift as average peaks during the dissociation of the complex and hence can be monitored through the helix–coil transition. The transition midpoints of the adenosine H-2 melting curves for the synthetic DNA and its proflavin complexes (Figure 4C) demonstrate stabilization of the nucleic acid duplex by bound mutagen. The variations in the poly(dA-dT) base and sugar proton chemical shifts can be monitored on gradual addition of proflavin to the duplex state. Thus, similar values are observed for the adenosine H-2 nonexchangeable resonance on complex formation (Figure 4C), while the Watson–Crick thymidine H-3 exchangeable resonance shifts to higher field with increasing mutagen concentration. These chemical shift changes reflect the net difference at a given proton between the ring currents of the proflavin ring[27] and that of a base pair[18] displaced to twice its distance as a result of the intercalation.

The mutagen resonances shift as average peaks during the dissociation of the proflavin·poly(dA-dT) complex, indicative of fast exchange of the intercalating agent between potential binding sites. This is demonstrated for two proflavin protons in the P_i/drug = 8 complex; these undergo large upfield shifts on complex formation (Figure 4D). The high-temperature shifts correspond to free proflavin in solution, while the low-temperature values correspond to proflavin intercalated between poly(dA-dT) base pairs.

Alden and Arnott have proposed an overlap geometry for intercalation of proflavin at a pyrimidinyl-(3′–5′)-purine site in B-DNA based on linked atom conformational calculations.[24] We have modified this geometry so that the proflavin ring overlaps to a greater extent with adjacent base pairs at the intercalation site (**3**). The calculated upfield shifts of the four proflavin protons [designated by (O) in **3**] based on ring-current and atomic diamagnetic anisotropy contributions of adjacent base pairs for the overlap geometry depicted in **3** compare favorably with the observed experimental values. This agreement is subject to the assumption that proflavin intercalates at dT-dA sites and that its amino groups are directed toward the major groove.



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The NMR results demonstrate that the long axis of the proflavin molecule is collinear with the direction of the Watson–Crick hydrogen bonds of adjacent base pairs and that there is maximum overlap between the mutagen and base-pair planes down the helix axis (**3**). These solution results at the synthetic DNA level support Lerman's seminal proposal for overlap geometries in acridine–DNA complexes[3] and more recent X-ray crystallographic studies of proflavin complexes at the dinucleotide level.[25]

Phosphorus NMR can be used as a probe for investigating the specificity of drug–nucleic acid interactions. There are two potential intercalation sites in poly(dA-dT) which can be monitored at the chemical shifts of the corresponding dTpdA and dApdT internucleotide phosphates. Experimentally two partially resolved ^{31}P resonances of approximately equal area are observed for the proflavin–poly(dA-dT) complex at various P_i/drug ratios in the duplex state in 1 M NaCl solution (Figure 3C). One of the ^{31}P resonances in the complex exhibits a chemical shift similar to the unresolved ^{31}P envelope of poly(dA-dT) while the other resonates to lower field (Figures 3A,C). This suggests that proflavin exhibits a sequence specificity for one of the two potential binding sites and shifts the ^{31}P resonance of the internucleotide phosphate corresponding to that site on complex formation.

The NMR techniques outlined above have general applicability and have been extended to a study of the intercalation complexes of poly(dA-dT) with the trypanocidal antibiotic ethidium bromide and the anthracycline antitumor agent daunomycin in solution.[28]

Partial Insertion of Steroid Diamines into DNA. Investigations into the structure and dynamics of nucleic acids as a function of salt and solvent suggest that the DNA double helix exhibits considerable flexibility in solution.[29] This flexibility is manifested in the folding of DNA around histones in chromatin and the packaging of nucleic acids into phage heads. It has

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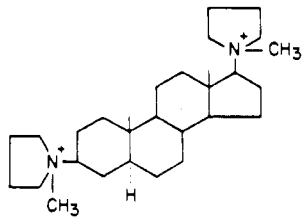
been proposed that DNA may fold either smoothly[30] or that its chain direction may change abruptly by the generation of kinks every few base pairs.[31] Sobell has proposed that kinks are generated by partial unstacking and unwinding of adjacent base pairs and that steroid diamines recognize and bind to such sites.[32] The strong sites for the binding of steroid diamines to DNA are saturated when one drug binds every two base pairs (P_i/drug = 4), corresponding to a neighbor exclusion model for complex formation.[33]

We have applied NMR spectroscopy to evaluate the structure and dynamics of steroid diamine–poly(dA-dT) complexes in solution.[34] The studies with dipyrandium (**4**) were undertaken in the absence of added salt in order to maximize the contributions from electrostatic interactions to the stability of the complex. The nonexchangeable nucleic acid proton line widths are narrower in the dipyrandium–poly(dA-dT) complex (Figure 5A) compared to their values in poly(dA-dT) at the same temperature. This suggests that the synthetic DNA exhibits considerable segmental flexibility in the steroid diamine–nucleic acid complex.



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The thymidine H-3 resonance was utilized as a probe to monitor the integrity of the base pairing in the dipyrandium–poly(dA-dT) complex. This resonance is observed at ~13.0 ppm in the P_i/drug = 5 steroid diamine complex (Figure 5B) with an area somewhat less (by ~25%) than that of the narrower adenosine H-8 nonexchangeable resonance. The line width of the thymidine H-3 Watson–Crick proton in the complex (Figure 5B) was twice its value in poly(dA-dT) under the same conditions. This exchangeable resonance broadened out on raising the pH of the complex (Figure 5B), indicative of base catalysis of the exchange with solvent. These results demonstrate that the base pairs of the synthetic DNA are intact but partially exposed to solvent in the steroid diamine–nucleic acid complex. The similarity in chemical shifts of the thymidine H-3 resonance (~13.0 ppm) in poly(dA-dT) (Figure 1A) and its dipyrandium complex (Figure 5B) suggests a compensation between contributions from a decrease in hydrogen-bond strength (upfield shift) and a reduction in base-pair overlaps (downfield shift) at the complexation site.

The nucleic acid nonexchangeable proton chemical shifts in the duplex state are sensitive markers of perturbations of the base-pair stacking interactions on complex formation. Experimentally, the adenosine and thymidine resonances of the synthetic DNA shift downfield on addition of dipyrandium, indicative of a partial loss of base-pair stacking at the steroid diamine binding site. This is best demonstrated by a comparison of the temperature-dependent chemical shifts of the adenosine H-2 resonance of poly(dA-dT) and its dipyrandium complex (P_i/drug = 11 and 5) in the premelting transition region (Figure 5C). The magnitude and direction of the tilt between unstacked base pairs in the steroid diamine–DNA complex cannot be unambiguously deduced from the NMR parameters. A tilt of 30° from the plane perpendicular to the orientation axis has been evaluated from transient electric dichroism studies of the complex.[35]

The interaction of dipyrandium (**4**) with the nucleic acid can be monitored at the CH_3 groups, NCH_3 groups, and unassigned proton resonances of the steroid diamine. The CH_3 groups and several proton resonances shift dramatically upfield on complex formation, as demonstrated by the temperature dependence of the highest field dipyrandium proton during the thermal transition of the P_i/drug = 5 complex (Figure 5D). By contrast, much smaller shifts are observed for the NCH_3 groups located at either end of the steroid diamine. The observation of selective upfield shifts requires that the corresponding steroid diamine proton and CH_3 groups be located in the shielding region of the tilted base pairs at the complexation site.

The nucleic acid and steroid diamine proton resonances shift as average peaks through the melting transition of the dipyrandium–poly(dA-dT) complexes (Figure 5C,D), indicative of fast exchange of the dipyrandium between potential nucleic acid binding sites on the NMR time scale. The premelting transition observed at the adenosine H-2 resonance of poly(dA-dT) (Figure 5C) probably reflects an increase in branch formation of the synthetic DNA on raising the temperature.[14] The premelting transition persists in the dipyrandium–poly(dA-dT) complexes (Figure 5C), which suggests that the degree of branching of the nucleic acid duplex structure is not perturbed by complex formation with steroid diamines. The similarity of the ^{31}P spectra of poly(dA-dT) and its neighbor exclusion dipyrandium complex suggests that the O–P polynucleotide torsion angles of the synthetic DNA remain unchanged on complex formation.

The NMR data support Sobell's proposal that the steroid diamine is partially inserted between tilted base pairs[32] in the dipyrandium–poly(dA-dT) complex.

Netropsin-Induced Conformational Changes at Adjacent Antibiotic-Free Base-Pair Regions. Netropsin (**5**) is a basic oligopeptide which exhibits antiviral, antifungal, and antibacterial activities and inhibits DNA and RNA tumor viruses in mammalian cells.[5] The antibiotic complexes double-stranded DNA at dA·dT sites in the minor groove of the helix, with the complex stabilized by electrostatic and hydrogen-bonding interactions. There is a single major binding site for netropsin which spans ~3 base pairs of the

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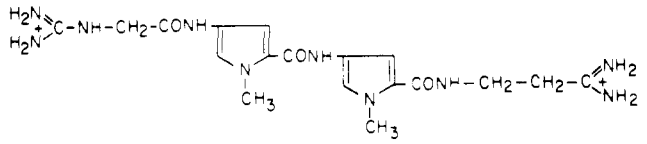
nucleic acid double helix.[5]



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Biphasic absorbance (260 nm) melting curves have been reported for the netropsin–poly(dA-dT) complex in solution.[36,37] The lower temperature cooperative transition was assigned to the opening of antibiotic-free base-pair regions, while the higher temperature cooperative transition was assigned to the opening of base-pair regions centered about bound netropsin. Proton NMR studies were undertaken on the netropsin–poly(dA-dT) complex at low antibiotic concentrations in a buffer of intermediate ionic strength (0.1 M cacodylate solution) in order to monitor and characterize the individual transitions in the biphasic melting curve.[37]

Let us first consider the NMR parameters for the netropsin–poly(dA-dT) complex in the temperature range corresponding to the lower temperature cooperative transition. The nonexchangeable proton spectra of the P_i/drug = 50 complex (Figure 6A) demonstrate that the antibiotic-free base-pair regions have melted out on raising the temperature from 53 to 66 °C. The opening of the antibiotic-freee base-pair regions is slow on the NMR time scale ($<10^2$ s^{-1}) since doubling of resonances is observed at the midpoint (61 °C) of this transition (Figure 6A). Selective upfield shifts are observed at the adenosine and thymidine sugar H-1′ resonances on formation (<60 °C) of the P_i/drug = 50 netropsin complex (Figure 6B). The variations in the sugar H-1′ chemiccal shifts reflect netropsin-induced changes in the glycosidic torsion angles[38] of the synthetic DNA in solution. By contrast, much smaller complexation shifts are observed at the base proton resonances which monitor the base-pair overlap geometries. The sugar H-1′ chemical shift changes have been followed on gradual addition of the antibiotic to the synthetic DNA duplex in solution. These shifts level off when 1 netropsin is bound per 25 base pairs even though the antibiotic covers only 3 base pairs at its binding site. The NMR results demonstrate that the netropsin-induced structural perturbation extends into the antibiotic-free base-pair regions on either side of the netropsin binding site.

We next consider the NMR parameters for the higher temperature cooperative transition in the biphasic netropsin–poly(dA-dT) melting curve. The NMR spectrum of the P_i/drug = 50 complex at 66 °C exhibits resonances from antibiotic-free base-pair regions that have melted out (major resonances, Figure 6A) adjacent to netropsin-bound intact base pair regions (minor resonances, designated by asterisks, Figure 6A). The minor resonances in the complex exhibit constant area between 64 and 71 °C but broaden out and coalesce with the major resonances about 75 °C. This is indicative of the onset of rapid migration of netropsin along the polynucleotide backbone at the higher temperature. The sugar H-1′ chemical shifts of the major resonances exhibit a cooperative transition with a midpoint at 95 °C (Figure 6B) which monitors the release of netropsin from base-pair regions centered about the bound antibiotic.

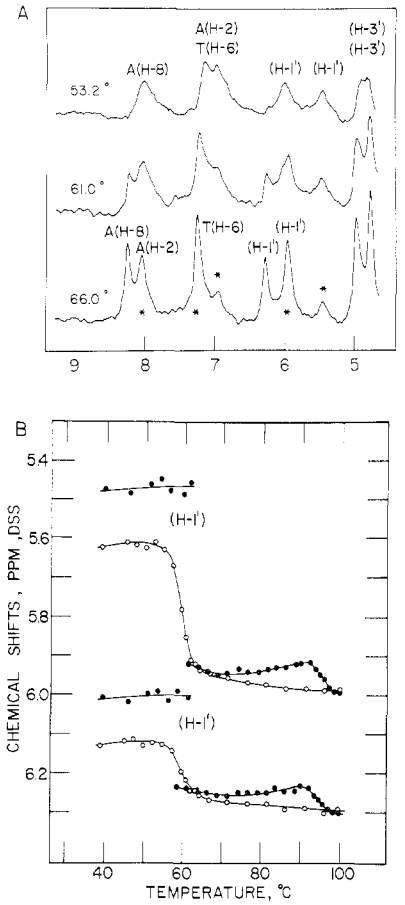

Figure 6. (A) The 360-MHz Fourier transform NMR spectra (4.5–9 ppm relative to DSS) of the first heating cycle of the netropsin–poly(dA-dT) complex, P_i/drug = 50, in 0.1 M cacodylate, 4.4 mM EDTA, D_2O, pH 7.5 at 53.2, 61.0, and 66.0 °C. The spectra were recorded without sample spinning and signal to noise ratio was improved by adding a line width of 10 Hz. The asterisks designate the minor resonances from base-pair regions centered at the binding site. The poly(dA-dT) concentration was 21.6 mM in phosphates. (B) The temperature dependence (40 to 100 °C) of the sugar H-1′ chemical shifts of poly(dA-dT) (○) and the netropsin–poly(dA-dT) complex (●) in 0.1 M cacodylate, 4.4 mM EDTA, D_2O.

The above application of NMR spectroscopy demonstrates that it is possible to separately monitor base-pair regions adjacent to and centered about the antibiotic binding site in drug–nucleic acid complexes in solution. Structural and kinetic information about each melting transition can be estimated from the chemical shift and line-shape variations as a function of temperature.

Synergistic Binding of Antibiotics to DNA. The majority of investigations to date on drug–nucleic acid complexes have concentrated on the structural and kinetic aspects of a single antibiotic complexed to DNA. There is considerable interest to extend such investigations to probe the interaction of two or more drugs to DNA. Thus, Wells and co-workers have reported on the simultaneous binding of actinomycin and netropsin to DNA and have shown that these antibiotics can

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occupy adjacent binding sites.[39]

We demonstrated in the previous section that netropsin alters the nucleic acid conformation at its binding site on poly(dA-dT) and that the structural perturbation (changes in the glycosidic torsion angles) is propagated to adjacent antibiotic-free base-pair regions.[37] Recent examples demonstrate that a second drug may exhibit enhanced binding to such an altered nucleic acid structure,[40] so that an understanding at the molecular level of such synergistic effects could lead to the successful design of classes of antibiotics to be used in combination chemotherapy. We predict that NMR spectroscopy will contribute to our understanding of this challenging problem.

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Zwittazido Cleavage

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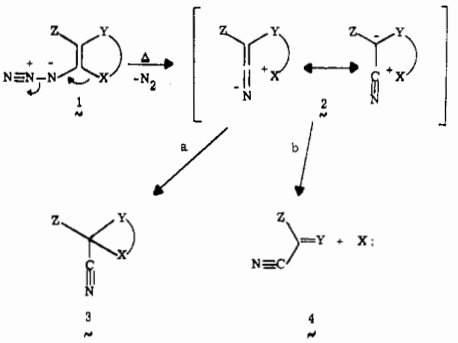
The idea of generating reactive intermediates or inducing concerted molecular rearrangements by the extrusion of a stable gaseous molecule from an organic compound has been the subject of extensive research effort. This is particularly true as it applies to the thermolysis and photolysis of organic azides.[1] Although a number of very interesting transformations have been discovered, the well-known Curtius rearrangement of acyl azides is one of the few that has found its place in the arsenal of the synthetic chemist. The prime objective of this Account is to add to this arsenal by defining and illustrating a new reaction of certain organic azides which has both synthetic and mechanistic significance. This reaction, which we call the *zwittazido cleavage*, constitutes the foundation of a research effort encompassing a number of intimately related topics, several of which will be discussed here.

First of all, let me define the zwittazido cleavage reaction. The definition stems from a generalized mechanistic model which can be used to predict the products from the thermolysis of appropriately substituted vinyl azides.[2] Specifically, we proposed that vinyl azides of general structure **1** (Scheme I) cleave to the zwitterions **2** when X is a substituent capable of cation stabilization and Y and/or Z are anion stabilizing groups. The zwitterionic intermediate **2** can then ring-close to **3** (ring contraction, path a) or cleave to **4** (fragmentation, path b). Henceforth, we wish to refer to reactions of this type as examples of the zwittazido cleavage, a name aptly describing the fact that cyclic vinyl azides of the structural type mentioned are thought to cleave to zwitterionic intermediates which proceed to products by pathways a or b.

This generalized mechanism is the fundamental "take home" message of this Account since it relates to the other objectives to be discussed. For example: (1) it defines the zwittazido cleavage reaction and illustrates the mechanism of this reaction; (2) it predicts a synthetic route to cyanoketenes by pathway b provided Y is a carbonyl and X is an appropriate leaving group; (3) it provides a powerful mechanistic probe for studying dipolar cycloadditions of cyanoketenes since one can independently generate the same zwitterionic intermediates from appropriately substituted vinyl azides, **1**, as are formed when cyanoketenes react with imidates, other ketenes, or aldehydes; (4) it illustrates a strategy for a new β-lactam synthesis from appropriately substituted 4-azido-Δ^3-2-pyrrolinones. Such compounds undergo ring contraction (pathway a) to 3-cyano-2-azetidinones via a zwitterionic intermediate analogous to **2**.

Scheme I



Synthesis of Cyanoketenes

A particularly suitable class of cyclic vinyl azides which are examples of structure **1** (Scheme I) are 2,5-diazido-1,4-benzoquinones. The thermal chemistry of such compounds has now been explored in moderate depth, and we have observed that they are ideal precursors to alkyl- and arylcyanoketenes.[3] A particularly

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