Calorimetric and spectroscopic investigation of drug-DNA interactions: II. Dipyrandium binding to poly d(AT)

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ABSTRACT: We report the first calorimetric investigation of steroid diamine binding to a DNA duplex. Absorption spectroscopy, batch calorimetry, and differential scanning calorimetry (DSC) have been used to detect, monitor, and thermodynamically characterize the binding of the steroid diamine, dipyrandium, to poly d(AT). The following thermodynamic data for the binding in 16 mM Na⁺ at 25°C have been obtained: $\Delta G^\circ = -6.5$ kcal/mol, $\Delta H^\circ = +4.2$ kcal/mol, and $\Delta S = +36$ e.u. We interpret the endothermic binding enthalpy in terms of steroid-induced conformational changes in the duplex (e.g. "kinking"). The large positive entropy is interpreted in terms of binding-induced release of bound water and condensed sodium ions. The salt-dependence of the binding constant is interpreted in terms of dipyrandium site-binding involving only one of the two charged ends of the steroid. The optical and DSC curves for the unsaturated steroid-poly d(AT) complexes exhibit biphasic behavior. A comparison of the van't Hoff and the calorimetric transition enthalpies reveals that steroid binding reduces the cooperativity of the transition.

INTRODUCTION

Over the past several years, the binding of steroid-diamines to synthetic and natural nucleic acids has received considerable attention. Most of the early work in this area was part of larger efforts involving broad-based studies of drug-DNA interactions.¹⁻⁶ More recent theoretical and experimental studies⁷⁻¹³ have been motivated by the suggestion of Sobell et al.⁷ that steroid diamine binding may "kink" DNA. Considering the potential importance of such a molecular distortion to drug binding and DNA packaging, interest in and investigations of steroid diamine-DNA complexation has greatly increased. Despite this increased attention, the nature of the molecular forces that control steroid-DNA complexation are not fully defined. To help alleviate this situation, we have begun a program in which calorimetric and spectroscopic techniques are being used to thermodynamically characterize the binding process. In this paper we report the results that we have obtained for the binding of the steroid diamine, dipyrandium, to the poly d(AT) double helix.

EXPERIMENTAL SECTION

Materials

Poly d(AT) was purchased from P.L. Biochemicals, Inc. and used without further purification. The iodide salt of the steroid diamine, dipyrandium, was a gift of Professor Suggs of Brown University and Dr. Dinshaw Patel of Bell Laboratories. All solutions were prepared in a buffer consisting of 0.01 M sodium phosphate, 10^{-4} M sodium EDTA, adjusted to the desired sodium concentration and pH 7.0. The concentration of each poly d(AT) solution was determined optically using a 260 nm molar extinction coefficient of $6650 \text{ M}^{-1} \text{ cm}^{-1}$. Stock solutions of the steroid were prepared by weight using a molecular weight of 678 daltons.

METHODS

Optical Titrations. Optical titrations were performed using a Perkin Elmer 575 spectrophotometer. Small aliquots of a concentrated steroid stock solution were added incrementally to a 1 cm cell containing a solution of poly d(AT) of known volume and concentration. Absorbance changes were monitored at 260 nm.

Temperature-Dependent Ultraviolet Spectrophotometry. Absorbance versus temperature profiles of poly d(AT) and poly d(AT)-dipyrandium complexes were measured using a thermoelectrically-controlled Perkin Elmer 575 programmable spectrophotometer interfaced with a Tektronix 4051 computer. Samples were heated at a rate of $0.5^\circ\text{C}/\text{minute}$ while the absorbance at 260 nm and the temperature were recorded every 30 seconds.

Batch Calorimetry. The batch calorimeter used in this study is based on the design of Prosen and Berger, and has been previously described in detail.¹⁴

Differential Scanning Calorimetry (DSC). DSC was performed on samples of poly d(AT) and poly d(AT)-dipyrandium complexes using a Microcal-1 instrument as previously described.^{15,16}

RESULTS

Figure 1 shows the structure of the steroid diamine, dipyrandium, used in the binding studies reported here. This steroid has been used rather than the structurally similar irehdiamine also shown in Figure 1 due to the instability of irehdiamine at high temperatures.

Temperature-Dependent UV Absorbance

Dipyrandium binding to poly d(AT) can be monitored by taking advantage of the effect of steroid binding on the thermal stability of the duplex. The

Figure 1. Structures of the two steroid diamines.

thermal stability of the duplex is characterized by the temperature midpoint observed in a uv melting curve at 260 nm. Figure 2 shows the family of differentiated melting curves we obtain for poly d(AT) in the presence of increasing concentrations of dipyrandium. The significant features to note are:

- 1) Increasing the concentration of bound steroid increases the thermal stability of the duplex. At saturation, the duplex melts with a t_m some 20°C above that of the free duplex.
- 2) The steroid-saturated duplex melts over a broader temperature range than the steroid-free duplex.
- 3) Below saturation, steroid binding causes the duplex to melt in an apparent biphasic manner.
- 4) Below saturation, increasing the extent of steroid binding increases the t_m 's of both the low and high temperature transitions.

Figure 2. Differentiated 260 nm UV melting curves of the poly d(AT) duplex ($c = 1.65 \times 10^{-4}$ in phosphate) at the indicated phosphate to drug (P/D) ratios in 10 mM sodium phosphate buffer, 1mM EDTA, pH 7.

Figure 3. Optical titration curves. The change in 260 nm absorbance of a poly d(AT) solution upon addition of aliquots of dipyrandium. The inset shows that salt dependence of the steroid binding constant derived from the optical titration curves. Each titration was performed in a pH 7 sodium phosphate buffer containing the following overall sodium ion concentrations: (O) 16 mM; () 33 mM; (Δ) 66 mM.

Possible structural interpretations of these features will be presented in the Discussion Section.

Binding Curves, Binding Constants, and Salt Dependence

In Figure 3 we have plotted the change in nucleic acid absorbance as a function of added steroid at different salt concentrations. The observed hyperchromicity with increasing steroid concentration suggests a mode of binding which results in some unstacking of the bases. The low-salt curves approach saturation at a nucleotide to steroid ratio of 5 to 1 which is consistent with a neighbor exclusion model in which a maximum of one steroid molecule binds per five nucleotides.¹⁷ Both of these observations are consistent with previous studies of steroid diamine binding to both natural and synthetic DNA

TABLE 1
SALT DEPENDENCE OF THE BINDING CONSTANT FOR THE COMPLEXATION OF
DIPYRANDIUM TO THE POLY d(AT) DUPLEX AT 25°C

$[Na^+]$	$K \times 10^{-4}$
16 mM	5.38 M ⁻¹
33 mM	3.20 M ⁻¹
66 mM	1.43 M ⁻¹

TABLE 2
BATCH CALORIMETRY ON THE BINDING OF DIPYRANDIUM TO THE
POLY d(AT) DUPLEX AT 25°C

Phosphate/Drug	r value	ΔH_b (kcal/mol)
15/1	0.133	+ 4.2*

*This value is the average of 8 independent determinations and has a standard deviation of ± 0.6 kcal/mol.

duplexes.^{9,10} Analysis of these curves by previously described methods,⁹ results in the equilibrium constants listed in Table 1 for the binding of the steroid to the poly d(AT) duplex at 25°C. These values compare favorably with previously reported determinations.^{9,10} Using the standard thermodynamic relationship $\Delta G^\circ = -RT \ln K_{eq}$, we calculate a binding free energy of -6.5 kcal/mol for complexation of dipyrandium with poly d(AT) in 10 mM phosphate buffer at 25°C.

The observed decrease in the binding constant with increasing salt concentration (see Table 1) reflects the importance of electrostatic interactions to the binding process. This salt dependence is shown in the inset of Figure 3 as a log-log plot of the equilibrium binding constant, K, versus the molar cation concentration, (Na⁺). The implications of both the slope and intercept of this line will be presented in the Discussion Section.

Batch Calorimetry

An endothermic heat burst results when poly d(AT) is mixed with dipyrandium at 25°C in 10 mM phosphate buffer and at a nucleotide (or phosphate) to steroid ratio (P/D) of 15 to 1. This ratio is equivalent to a steroid to base pair ratio (r) of 0.133. Following small corrections for dilution effects, this heat corresponds to the association reaction shown below:

Integration of the area under the calorimetric curve along with knowledge of the nucleotide to bound steroid ratio allows calculation of the steroid binding enthalpy, ΔH_b , which is listed in Table 2. This value is the average of eight independent determinations. It should be noted that despite the favorable equilibrium constant, the binding enthalpy is endothermic.

Differential Scanning Calorimetry

Figure 4a shows the calorimetric transition curve for the duplex to single strand transition of poly d(AT) in the absence of added steroid. As

Figure 4. DSC curves for the helix-to-coil transition of the poly d(AT) duplex in the presence of the indicated phosphate to steroid ratios. Note: The y-axis in panel A is compressed by a factor of two.

expected, a cooperative, monophasic transition profile is observed.^{18,19} Figures 4b and 4c show the transition in the presence of the steroid at nucleotide to steroid ratios of 17 to 1 and 10 to 1, respectively. Under these conditions, we observe biphasic melting behavior with both peaks having melting temperatures higher than poly d(AT) in the absence of bound steroid. Figure 4d shows the poly d(AT) transition when the duplex is saturated with steroid (a nucleotide to steroid ratio of 5 to 1). Under these conditions, we observe monophasic melting behavior similar to poly d(AT) in the absence of steroid but at a much higher temperature.

Straightforward analysis of the calorimetric curves in Figures 4a-d yields the transition enthalpies per base pair (ΔH_t) and the melting temperatures (t_m) listed in Table 3. No attempt was made to deconvolute the biphasic melting curves. Consequently, only total transition enthalpies are listed although where possible each sub-transition is assigned a separate melting temperature. The significant observations to note are:

TABLE 3
DIFFERENTIAL SCANNING CALORIMETRY ON STERIOD-FREE AND
STERIOD-BOUND POLY d(AT) DUPLEXES

Phosphate/Drug (P/D)	r	t _m (°C)		ΔH _t	ΔH _d
		1st	2nd	(kcal/Mol)	(kcal/mol drug)
∞	0	45	—	+7.60	—
17/1	0.118	50	60	+6.61	-8.39
10/1	0.174	58	66	+6.43	-6.70
5/1	0.256	—	73	+5.09	-9.80
				Average =	-8.30

*This r value corresponds to the actual amount of bound steroid per base pair calculated from the P/D ratio using a K value of $5.38 \times 10^4 \text{ M}^{-1}$ at 25°C.

1) The t_m of the duplex increases with increasing concentration of bound steroid. At saturation, the duplex melts with a t_m some 28°C above that of the free duplex. This drug-induced increase in the t_m compares favorably with the trend seen in the uv melting curves.

2) The steroid-induced increase in the thermal stability of the duplex is paralleled by a decrease in the overall transition enthalpy, ΔH_t, of the duplex.

3) At bound steroid concentrations below saturation, the duplex manifests biphasic behavior. Increasing the concentration of bound steroid causes a decrease in the magnitude of the low-temperature peak and an increase in the magnitude of the high-temperature peak. This behavior is consistent with the melting profiles observed optically (see Figure 2).

4) When saturated with steroid, the duplex melts monophasically. However, the monophasic transition of the saturated complex is considerably broader than the monophasic transition of the free duplex. This observation is consistent with the corresponding comparison in the optical melting curves.

Calculating a Binding Enthalpy from the DSC Data

Inspection of the enthalpy data in Table 3 reveals that the endothermic transition enthalpies of the complex decrease as the concentration of bound steroid increases. This result tells us that the thermally-induced dissociation of the steroid makes an exothermic contribution to the overall endothermic transition enthalpy. Consequently, the binding enthalpy of the steroid must be endothermic. This conclusion deduced from the DSC data is consistent with the directly measured binding enthalpy determined by batch calorimetry

(see Table 2). A specific value for the steroid binding enthalpy can be calculated from the DSC data in the manner described below.

The DSC curves in Figures 4b-d correspond to the thermally-induced transition shown below:

In the absence of steroid-single strand interactions, the observed transition enthalpy for the steroid-bound complex corresponds to the combined enthalpy effects of duplex disruption and steroid dissociation. Thus, as shown below, the steroid dissociation enthalpy (ΔH_d) can be calculated by subtracting the transition enthalpy of the free duplex (ΔH_t^f) from the transition enthalpy of the steroid-bound duplex (ΔH_t^b).

Dividing this enthalpy value by r , the ratio of bound steroid to base pairs, (column two in Table 3), yields the dissociation enthalpy per mole of drug released, ΔH_d .

Dissociation enthalpies, ΔH_d , calculated in this manner are listed in the last column of Table 3. It should be emphasized that these indirectly-obtained dissociation enthalpies have large uncertainties since they are calculated from small differences between two large numbers (the transition enthalpies). For this reason, rather than suggesting a dependence of the molar dissociation enthalpy on the concentration of bound steroid, we simply have averaged the three determinations listed in Table 3 and obtain a value of -8.3 kcal/mol for ΔH_d .

If heat capacity effects are small, this dissociation enthalpy (at 73°C for the saturated complex) should be equal in magnitude but opposite in sign to the steroid binding enthalpy obtained directly by isothermal batch calorimetry at 25°C . However, a comparison of the data in Tables 2 and 3 reveals that the steroid binding enthalpy obtained directly by batch calorimetry at 25°C is in fact lower in magnitude than the corresponding average value calcu-

TABLE 4
COMPARISON OF THE CALORIMETRIC AND THE VAN'T HOFF TRANSITION
ENTHALPIES FOR THE STEROID-FREE ($r=0$) AND THE STEROID-SATURATED
($r=0.256$) POLY d(AT) DUPLEX

r'	t_m (°C)	$\Delta H_{v.H.}$ (kcal)	ΔH_{cal} (kcal/mol of base pair)	$\Delta H_{v.H.}/\Delta H_{cal}$ (size of cooperative unit)
0.000	45	400	7.6	53
0.256	73	125	5.1	25

lated from the DSC data at higher temperatures. For the saturated complex, this difference corresponds to a heat capacity change of +85 cal/K-mol. This heat capacity change may reflect solvation differences at 25 and 73°C. The significant point is that both techniques independently show that dipyrandium binding to poly d(AT) is accompanied by a large endotherm. A possible molecular interpretation for this unfavorable binding enthalpy will be presented in the Discussion Section.

Implicit in the treatment of the DSC data described above is the assumption that the steroid does not energetically interact with the high-temperature, final single-stranded state. To test this assumption, we mixed the steroid in the batch calorimeter at 40°C with the oligomer d(AT)₅ which exists in the single-stranded state at this temperature. We observed no energy absorption or release. Consequently, we conclude that the steroid does not energetically interact with the final, single-stranded state.

Van't Hoff Transition Enthalpies

As previously described²⁰⁻²², a van't Hoff transition enthalpy can be calculated from the shape of a calorimetric transition curve by application of the equation

$$\Delta H_{v.H.} = \frac{-A}{\frac{1}{T_{1/2}} - \frac{1}{T_{3/4}}}$$

where $T_{1/2}$ and $T_{3/4}$ are the half and three quarters temperatures at the half height, respectively and where A is 4.37 for the bimolecular double helix to single strand transition (Figure 4a) and 5.09 for the transition of the steroid-saturated ternary complex (Figure 4d). In a previous study on netropsin binding to poly d(AT),²⁷ this distinction in the value of the numerator was not noted. Table 4 lists the van't Hoff transition enthalpies obtained by application of the appropriate equation to the transition of the free duplex

(Figure 4a) and the steroid-saturated duplex (Figure 4d). The significant observation is that the steroid-bound duplex has a lower van't Hoff transition enthalpy than the steroid-free duplex. The implications of this result will be described in the Discussion Section.

DISCUSSION

Our calorimetric data demonstrate that the binding of dipyrandium to poly d(AT) is an endothermic process and that this binding increases the melting temperature of the duplex. In the sections that follow, we suggest molecular interpretations for our calorimetric and spectroscopic results.

Salt-Dependence of the Binding Constant

The salt-dependence of dipyrandium binding to the poly d(AT) duplex is illustrated in the inset of Figure 3 by a log-log plot of the binding constant, K, versus the sodium ion concentration. The observed linear dependence can be described by the equation

$$\log K = 3.07 - 0.94 \log [\text{Na}^+] \quad (1)$$

Manning and Record,^{23,24} independently have shown that the slope of such a log-log plot is approximately equal to the charge on the binding ligand. Specifically, Manning has shown that for delocalized binding, the salt-dependence of the binding constant can be described by the general equation

$$\log K = -0.62 + 0.51N - N \log C$$

where N is the charge on the ligand and C is the cation concentration. For mono- and divalent cationic ligands, this equation reduces to

$$\log K = -0.11 - \log C \quad [N=1] \quad (2)$$

and

$$\log K = +0.40 - 2 \log C \quad [N=2] \quad (3)$$

In this study, the charge on the binding ligand, dipyrandium, is +2 (see Figure 1). However, the experimentally-determined slope for our log K versus log [Na⁺] plot is very close to one (see inset of Figure 3 and equation 1). In other words, despite the dicationic nature of dipyrandium, its salt-dependent DNA-binding properties follow behavior predicted for a monocationic ligand. As one possible explanation for this observed behavior, we suggest that dipyrandium binds to the poly d(AT) duplex in a manner that involves interaction with the duplex environment of only one of the two charged ends. Such a picture is consistent with the observation that the binding constant of similar steroid diamines is sensitive to the stereochemistry at only one of the two charged ends.⁵

A further comparison between equation (1) and equations (2) or (3) re-

veals a large difference between the predicted and the experimentally-obtained intercept for a plot of $\log K$ versus $\log[\text{Na}^+]$. Specifically, equation 2 or 3 predicts intercepts of only -0.11 or $+0.40$. By contrast, our experimentally-observed intercept is $+3.07$ (equation 1). Since the predicted intercept values are derived for the case of non-specific ionic binding, we interpret our intercept of $+3.07$ as suggesting site-specific binding of dipyrandium.

Thus, based on a comparison between the predicted and observed slopes and intercepts of $\log K$ versus $\log[\text{Na}^+]$ plots for dipyrandium binding to poly d(AT), we propose a site-specific binding mode involving only one of the two charged ends of the steroid molecule.

The Enthalpy Data and Steroid-Induced Conformational Changes

Patel and Canuel have used NMR to investigate the binding of dipyrandium to the poly d(AT) duplex.¹¹ Consequently, we can interpret our calorimetric results in the context of the specific structural features of the complex as deduced from their NMR data.

The proton NMR results of Patel and Canuel reveal the major steroid-induced conformational change upon dipyrandium binding to poly d(AT) to be partial unstacking of the bases at the binding site due to partial insertion of the steroid diamine.¹¹ As noted by Patel and Canuel, this steroid-induced structural alteration is consistent with formation of a "kinked" duplex structure in which base stacking is partially disrupted. Our calorimetrically-observed endothermic binding enthalpy is consistent with such an energetically-unfavorable, steroid-induced conformational change. However, endothermic binding enthalpies also have been observed for steroid diamines that complex with poly d(AT) in a manner that does not disrupt base stacking.²⁵ Consequently, an endothermic binding enthalpy does not necessarily reflect steroid-induced unstacking of the bases and may in part reflect changes in solvation. Current studies using selectively modified steroid diamines should provide insight into the correlation between binding enthalpies, binding modes, solvation effects, and steroid-induced conformational changes.

Comparison with Published Van't Hoff Enthalpy Data

The literature contains two steroid diamine binding studies which report enthalpy data. From the differences between forward and reverse activation energies, Dattagupta et al.¹⁰ calculate endothermic enthalpies of 12 and 22 kcal mol⁻¹ for the complexation of irehdiamine to CT DNA and M. Luteus DNA, respectively. By contrast, based upon temperature-dependent equilibrium data, Saucier⁹ calculates a zero enthalpy for complexation between irehdiamine and CT DNA. Our calorimetric experiments demonstrate that the enthalpy for

complexation of dipyrandium to poly d(AT) is in fact endothermic.

A quantitative comparison of the results of our study with those of Dattagupta et al. and Saucier may not be appropriate. Dattagupta et al. and Saucier studied the binding of their steroid diamine to duplexes formed by natural DNA polymers while we have studied steroid diamine binding to the duplex formed by the self-complementary, synthetic polymer poly d(AT). Furthermore, Saucier and Dattagupta et al. used the steroid diamine, irehdiamine, for their binding studies while we have used dipyrandium. Despite the similarity in the structures of these two steroid diamines (see Figure 1), they may in fact bind differently to duplex DNA. Evidence in support of this possibility comes from the comparison described below of Saucier's salt-dependent binding data for irehdiamine and our corresponding data for dipyrandium.

Manning¹³ has noted that Saucier's salt-dependent data for irehdiamine binding to CT DNA can be described by the equation

$$\log K = 1.1 - 1.5 \log C$$

As shown by Manning,¹⁶ this equation yields excellent agreement between experimental binding constants and binding constants predicted for divalent cation binding to DNA. By contrast, we find that the corresponding relationship for dipyrandium binding to the poly d(AT) duplex is described by equation 1 which deviates substantially from the form predicted for delocalized binding of a dicationic ligand. This difference in salt-dependent binding properties of irehdiamine and dipyrandium may reflect a real difference in the binding modes of these two structurally-similar steroid diamines. Alternatively, the observed differences may result from differences in the nature of the two duplex systems studied. Nevertheless, it seems quite reasonable to suggest that the buried charges of the tetrasubstituted nitrogens in dipyrandium manifest different ionic influences on binding than do the exposed, mono-substituted charges of irehdiamine.

The Entropy Change

Our calorimetrically-determined endothermic binding enthalpy allows us to conclude that the binding of dipyrandium to poly d(AT) is a completely entropy-driven process. Large positive entropy changes for association reactions in aqueous solution usually reflect solvent effects in which bound water is released. One possible molecular interpretation envisions a steroid-induced disruption of the Dickerson water spine that lines the minor groove around AT base pairs.²⁶ Steroid binding would release to bulk solvent water molecules that previously had been structured in the minor groove. This interpretation is consistent with the established picture that steroid diamine

binding to duplex structures involves interaction with the minor groove.¹¹

A second potential source of positive entropy relates to the fact that in free solution the nonpolar steroid ring system is probably surrounded by hydrophobically restricted water. Upon partial insertion into the helix, some of this steroid-bound water would be disrupted and released to bulk solvent thereby resulting in a gain in entropy.

A third source of positive entropy is the steroid binding-induced release of bound sodium counterions. As shown by Manning²³ and Record,²⁴ counterion release is driven by a positive entropy change. Thus, the combined effects of binding-induced release of restricted water around the steroid, disruption of the water spine in the minor groove, and release of sodium counterions provide possible molecular interpretations for the entropy-driven character of the binding process.

Unsaturated Complex

Under non-saturating conditions we observed biphasic melting behavior both optically and calorimetrically. This result is consistent with two separate populations along the poly d(AT) chain. Based upon the melting temperatures, we suggest that the higher-temperature transition has a greater steroid binding density than the lower-temperature transition. Nevertheless, the lower-temperature transition is still stabilized relative to free poly d(AT) (Figure 4a). This stabilization may reflect a small amount of thermally-induced steroid redistribution or could result from the influence of steroid-dense regions on the stability of adjacent steroid-free regions. This observation contrasts with the behavior we have observed for the interaction of drug-bound and drug-free regions in the poly d(AT)-netropsin complex.²⁷

Patel and Canuel observe that the steroid and nucleic acid protons shift as average resonances during the melting process of the unsaturated complex.¹¹ They interpret this to mean that the steroid is exchanging rapidly between potential binding sites. If this were the case then the unsaturated complex should behave in a homogeneous manner and melt with a single peak. This is contrary to the optically-and calorimetrically-observed biphasic melting behavior reported here. At this time we can offer no explanation for these two different observations.

Nature of the Transition

Inspection of Figures 4a and 4d reveals that the transition of the steroid-saturated duplex is considerably broader (has a greater transition width) than the transition of the steroid-free duplex. Thus, in addition to

effecting the transition enthalpy and the melting temperature, steroid binding also alters the nature of the transition as reflected by the increase in transition width.

This steroid-induced effect on the transition width of the steroid-saturated versus steroid-free duplex can be quantified by taking the ratio of the corresponding van't Hoff and calorimetric transition enthalpies. For a given transition this ratio provides a measure of the size of the cooperative unit.^{28,29} The calculated ratios are listed in Table 4.

The significant observation is that the size of the cooperative unit is larger for the free duplex than for the steroid bound duplex. Thus, we conclude that in addition to decreasing the transition enthalpy and increasing the t_m , steroid binding also reduces the cooperativity of the transition. Since we have observed similar behavior when netropsin binds to poly d(AT),²⁷ it is tempting to suggest a general picture in which drug binding sites form barriers to long-range, cooperative melting in DNA polymers.

Our calorimetric and spectroscopic studies of steroid diamine binding are being extended to other synthetic and natural DNA polymers. The result of these investigations will allow us to assess the influence of base composition and sequence on the overall thermodynamic profile of the binding process. In addition, we are studying the binding of selectively modified dipyrandium analogues to define the role of specific structural components in the binding process.

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