Models of Bleomycin Interactions with Poly(deoxyadenylylthymidylic acid). Fluorescence and Proton Nuclear Magnetic Resonance Studies of Cationic Thiazole Amides Related to Bleomycin A_2†

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ABSTRACT: The interaction of eight 2-substituted thiazole-4-carboxamides, structurally related to the cationic terminus of bleomycin A_2, with poly(deoxyadenylylthymidylic acid) [poly(dA-dT)] has been studied by using proton nuclear magnetic resonance and fluorescence spectroscopy. These analogues have been used as probes of the complex formed between the parent drug molecule and poly(dA-dT). Aliphatic substituents on the 2′ position of 2,4′-bithiazole derivatives restrict the ability of the aromatic ring system to intercalate in the double-helical form of the polynucleotide. Absence or partial removal of the 2′ substituent enhances intercalation of the bithiazole system. The cationic side chain does not appear to be involved in the stabilization of any of these complexes, although it may be necessary for their formation. A 2,4′:2′,4″-terthiazole derivative shows a substantial degree of intercalation which is accompanied by extensive immobilization of the cationic side chain. This suggests that insertion of the aromatic system into the nucleic acid causes the cationic side chain to be pulled in also. Monothiazole analogues do not appear to bind, indicating that at least two thiazole rings are necessary for binding or that proper spacing between the two side chains on either side of the thiazole system is important for binding. The relation of the interactions of these analogues to the biochemical and biological properties of the parent bleomycins is discussed as is the possible use of these data in the design of synthetic bleomycin derivatives having varying affinities and specificities for DNA.

The bleomycins, a group of related antitumor antibiotics (Figure 1), are believed to exert their biological activity through the degradation of DNA [see articles in Hecht (1979)]. In vitro, this degradation is mediated by iron(II) complexes of the bleomycins in a cyclic process requiring dioxygen and a reducing agent [see reviews by Sausville & Horwitz (1979), Grollman & Takeshita (1980), and Burger et al. (1981)]. Previous studies (Chien et al., 1977; Kasai et al., 1978; Takita et al., 1978; Glickson et al., 1981; Sakai et al., 1981) have shown that the bleomycin molecule consists of two distinct and relatively independent regions: One region is localized around the bithiazole-containing moiety and the cationic terminus (R group in Figure 1) and is responsible for the association with DNA; the other is comprised of the pyrimidine group and associated residues and is responsible for binding the metal ion cofactor. Some of the ligands implicated in the binding of the metal ion are indicated in Figure 1. Proton nuclear magnetic resonance (NMR) studies (Sakai et al., 1981) have shown that the acetyl derivative of the terminal "dipeptide" fragment (acetyl-dipeptide, **1**) contains all of the hydrogen atoms of bleomycin A_2 (the most common congener) which are perturbed upon binding of the intact drug molecule to the model DNA poly(deoxyadenylylthymidylic acid) [poly(dA-dT)]. In an effort to obtain more detailed information about the binding of bleomycin A_2 to DNA, the interaction of synthetic dipeptide analogues of bleomycin A_2 with poly(dA-dT) have been studied by using fluorescence and proton NMR spectroscopy. These derivatives have been used to help determine the geometry of the complex formed between the intact drug and polynucleotide and to learn how changes in the structure of the DNA-binding region of the antibiotic might affect its interaction(s) with its target.

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The NMR methods utilized were initially described by Patel in studies of nucleic acid–binding drugs (Patel, 1978, 1979; Patel & Canuel, 1977, 1978). The temperature dependence of the chemical shifts of the drug–nucleic acid complex, when compared to the spectra of the constituent drug and nucleic acid separately, provides structural information about the complex. Poly(dA-dT) has a relatively short rotational correlation time which allows the attainment of high-resolution NMR spectra of the nucleic acid; in addition, the regular sequence of the polynucleotide gives rise to relatively simple spectra, the resonances of which have been assigned for the most part (Patel, 1978, 1979).

The thiazole derivatives employed in this study are amides containing the (3-aminopropyl)dimethylsulfonium chloride side chain of bleomycin A_2 (Riordan & Sakai, 1981). The structures of these derivatives are summarized in Figure 2. The various modifications shown have been employed to probe the relationship between the structure of the ligand and its ability to bind to the nucleic acid. The pertinence of the results to the mechanism of DNA degradation and to the design of potential active analogues is discussed.

Experimental Procedures

Materials. Poly(dA-dT) was obtained from P-L Biochemicals (Milwaukee, WI). Some samples were dissolved in D_2O (99.8 atom %; Aldrich Chemical Co., Milwaukee, WI) and heated at 70–75 °C for 18–24 h to exchange the A(H-8) hydrogen, the resonance of which overlapped with aromatic resonances of some of the thiazole derivatives studied. By this procedure, >95% exchange of D for H was effected. No differences were detectable in the NMR spectra or in the temperature dependence of the resonances of the exchanged preparation when compared to unexchanged samples. Deuterated and untreated polynucleotide preparations were purified by dialysis against 10 mM sodium phosphate (pH 6.8)–1 M NaCl–1 mM EDTA and then distilled water and finally

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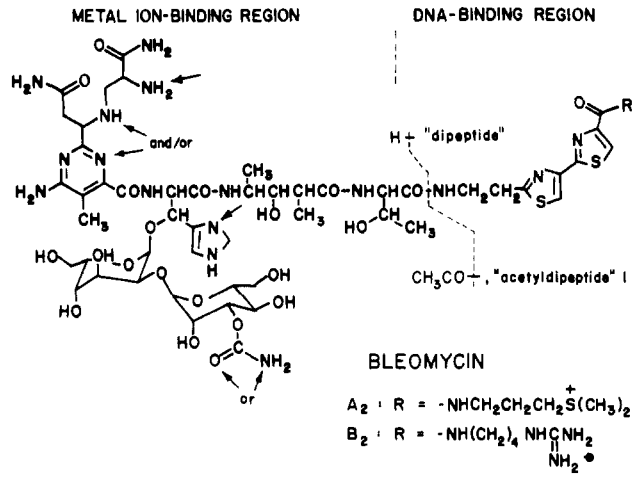

FIGURE 1: Structure of the bleomycins, the terminal dipeptide, and the acetyl-dipeptide. Arrows indicate possible ligands involved in the binding of metal ions.

obtained as a lyophilized solid. Concentrations of poly(dA-dT) were determined by using a molar absorptivity at 262 nm of 6.6×10^3 M^{-1} cm^{-1} per nucleotide residue (Inman & Baldwin, 1962).

The synthesis and proton NMR spectral properties of the cationic thiazole derivatives used in this study have been described elsewhere (Riordan & Sakai, 1981). 2,4′-Bithiazole and 2′-amino-2,4′-bithiazole were prepared by the method of Erlenmeyer et al. (1948). The concentrations of all derivatives were determined by weight on the basis of the molecular weights determined by combustion analysis.

Methods. NMR measurements were made on a Bruker WH-400 spectrometer operating in the Fourier-transform mode as described previously (Chen et al., 1980; Sakai et al., 1981). Temperatures were determined to ±1 °C by using the separation of the resonances of ethylene glycol (Van Geet, 1968). Samples for NMR measurements contained 10 mM poly(dA-dT) and 1.17 mM of the thiazole derivative. At these concentrations, all perturbations of derivative resonances were maximal; i.e., the chemical shift perturbations did not increase with decreasing derivative concentrations at this fixed polynucleotide concentration. This analogue concentration was chosen to allow spectra to be collected in a shorter period of time and to allow ready comparison with previous data (Sakai et al., 1981). The buffer employed was 10 mM sodium phosphate (pH_m 6.8)–0.1 M NaCl in D_2O, where pH_m is the meter reading uncorrected for isotope effects. Normally 128 or 256 transients (8000 data points, 4 KHz spectral width) were averaged for each spectrum, employing a 1-s pulse with a 2-s delay between pulses. Chemical shifts are referenced to internal sodium 4,4-dimethyl-2,2,3,3-tetradeuterio-4-silapentanoate (TSP; Stohler Isotope Chemicals, Waltham, MA). Thermal reversibility of the binding of the derivatives was determined by measuring spectra during both heating and cooling cycles. No hysteresis effects were noted for the temperature dependence of any of the resonances in the experimental spectra. For all of the derivatives studied, the interactions with poly(dA-dT) were at least moderately fast on the chemical shift time scale, as indicated by the observation of only a single resonance for each hydrogen atom of the derivative and the nucleic acid in the spectra of complexes.

Fluorescence measurements were performed at 25 °C on a Perkin-Elmer MP3A fluorometer. Buffer solutions contained 10 mM sodium phosphate (pH 6.8)–0.1 M NaCl and 10 mM sodium phosphate (pH 6.8)–25 mM NaCl. A solution (20 μM) of the thiazole derivative in the desired buffer was titrated

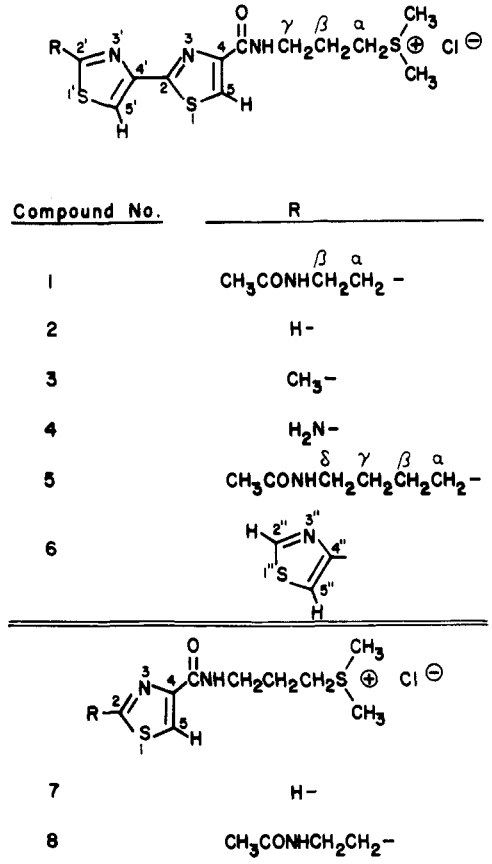

FIGURE 2: Structures of the cationic thiazole derivatives used in this study (Riordan & Sakai, 1981).

with aliquots of a 5 mM solution of poly(dA-dT) containing 20 μM derivative in the same buffer, and the fluorescence intensities were measured after each addition. The titration curves obtained in this manner were analyzed by using Scatchard plots as described previously (Sakai et al., 1981): The fraction f of the thiazole derivative bound at any ratio of poly(dA-dT) to an analogue in a solution having a fluorescence intensity F is given by $f = (F_0 - F)/(F_0 - F_\infty)$, where F_0 and F_∞ are the fluorescence intensities of the analogue at zero and saturating concentrations of poly(dA-dT), respectively. By appropriate choice of the wavelength of the exciting light (260–280 nm, depending on the derivative), F_∞ could be made to approach zero. Values of C_f and C_b, the concentrations of free and bound analogue, respectively, were obtained from the values of f and the known input concentration of the derivative. The data were analyzed by using plots of n vs. n/C_f, where n is C_b divided by the poly(dA-dT) concentration at the point in question. The straight-line portions of the plots were analyzed by least-squares analysis to provide estimates of the binding constants and numbers of binding sites in each class. It has been shown (Klotz & Hunston, 1971) that such a treatment of the data provides good approximations of the binding parameters if the binding constants of the two classes of sites differ by approximately 2 orders of magnitude.

Results

NMR Studies. Role of the 2′ Substituent in 2,4′-Bithiazole Derivatives. Data previously obtained on bleomycin A_2 (Chen et al., 1980) and the acetyl-dipeptide **1** (Sakai et al., 1981) suggested that the substituent at the 2′ position of the bithiazole ring system imposed some constraints on the ability of the respective molecules to bind to poly(dA-dT). So that

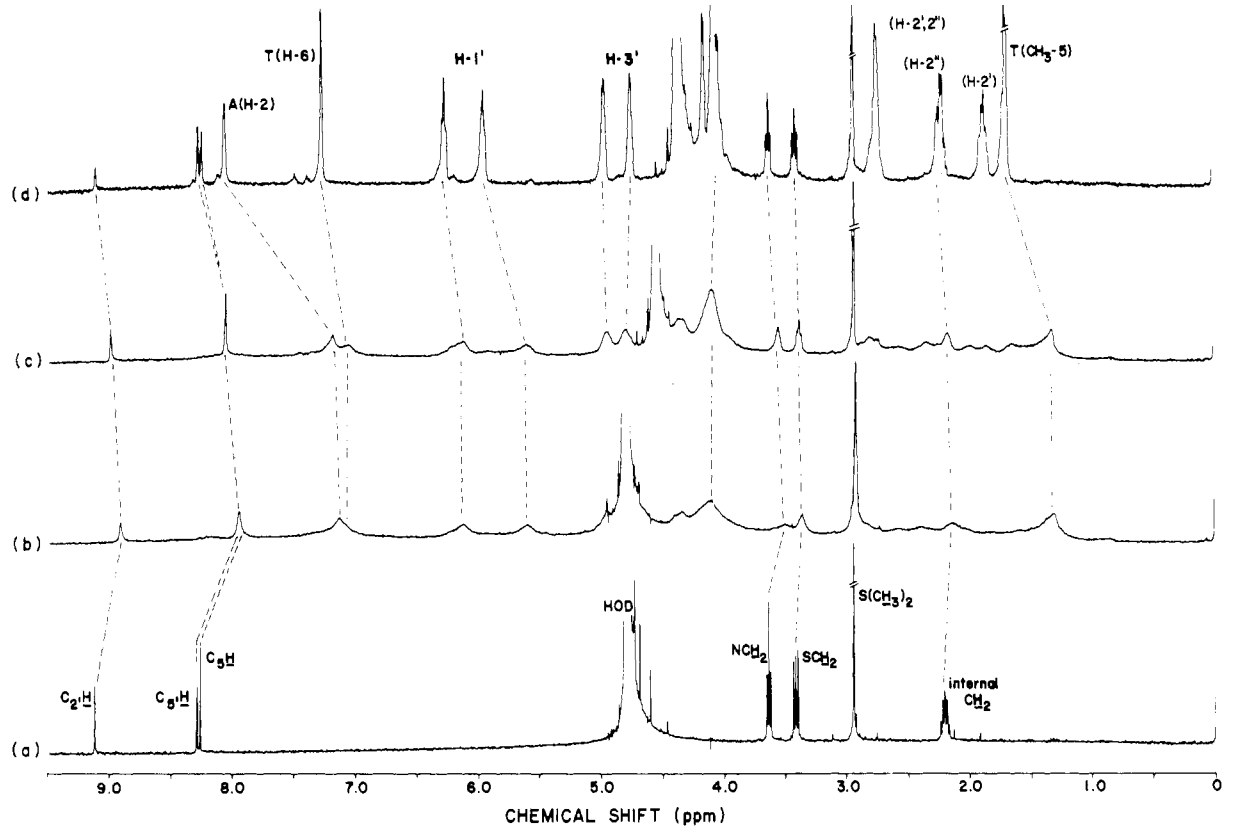

FIGURE 3: Proton NMR spectra of derivative **2** (1.17 mM) (a) free at 25 °C and in the presence of 10 mM poly(dA-dT) at (b) 25, (c) 50, and (d) 75 °C. Buffer is 0.01 M sodium phosphate (pH_m 6.8)–0.10 M sodium chloride in D_2O. Assignments of the poly(dA-dT) resonances are the original ones of Patel (1978).

this idea could be further tested, derivatives modified in this portion of the molecule were studied for their ability to bind to poly(dA-dT).

Typical proton NMR spectra obtained in these experiments are shown in Figure 3 for the unsubstituted analogue **2**. The aromatic resonances, in particular, show considerable broadening consistent with immobilization due to binding to the nucleic acid. In addition, the resonances are shifted upfield, indicating that those hydrogens are experiencing the ring-current effects of the nucleic acid bases. Similar spectra were obtained for all of the derivatives, which interact with poly(dA-dT).

The temperature dependence of the chemical shifts of the aromatic resonances and the resonances of the 2′ substituents of these derivatives is shown in Figures 4 and 5. The acetyl-dipeptide **1** shows a slightly skewed inverted bell-shaped response of its C_5H and C_5H resonances, with maximum shifts of about 0.15–0.17 ppm between 50 and 55 °C (Figure 4A). This is just below the T_m of poly(dA-dT) which is 61 ±1 °C under the conditions of these experiments. The broadness of the shifted resonances makes accurate determination of the chemical shifts difficult in some instances. The values of the chemical shifts in these instances were the average of a minimum of five determinations and are reproducible to within 0.02–0.03 ppm.

All shifted resonances move back downfield as the nucleic acid undergoes the helix–coil transition. The temperature dependence of these shifts parallels the changes seen in the resonances of the nucleic acid (see below). The shifts observed with **1** are slightly larger than those observed with intact bleomycin A_2 (Chen et al., 1980). The acetyl group shows no perturbation while the 2′-α- and 2′-β-CH_2 (NCH_2) resonances show shifts of 0.05 and 0.07 ppm, respectively. (The cationic chain γ-CH_2 (4-NCH_2) resonance overlaps with and shifts with the 2′-α-CH_2 resonance.) The side chain resonances do not show the bell-shaped response of the aromatic resonances. This behavior indicates that the ligand remains bound to the nucleic acid even when the bithiazole resonances experience little or no ring-current shifts resulting from neighboring nucleic acid bases.

For the unsubstituted derivative **2**, the C_5H and $C_{5'}H$ resonances show high field shifts of the order of 0.30 ppm relative to the free derivative (Figure 4B). The shifts are considerably larger than those observed with bleomycin A_2 or **1**. In addition, maximum shifts occur well below the T_m and do not show the bell-shaped response of the intact drug or derivative **1**. These shifts are maintained up to the temperature of the helix–coil transition of the nucleic acid at which point the resonances move back downfield in a highly cooperative transition. At the same time, they sharpen, and the line widths apprach those of the free derivative.

In compound **2**, the $C_{2'}H$ resonance experiences a shift of about 0.15 ppm, which is considerably less than the shifts observed for the C_5H resonance or for the $C_{5'}H$ resonance which is on the same ring. Similarly, the 2′-CH_3 resonance of the methyl derivative **3** shows a maximum shift of 0.15 ppm while the C_5H and $C_{5'}H$ resonances of the same molecule show shifts of the order of 0.30–0.31 ppm (Figure 4C).

The implication of aromatic amino groups of some intercalating agents in the stabilization of their complexes with nucleic acids suggested the use of a similar substituent in the bithiazole series. The 2′-amino derivative **4** shows a temperature dependence for the chemical shift of the C_5H resonance which is very similar to the corresponding resonances of derivatives **2** and **3**, with a maximum shift of about 0.40 ppm (not shown). Unfortunately, the $C_{5'}H$ resonance could not be monitored because it exchanged readily with the deuterium of the solvent.

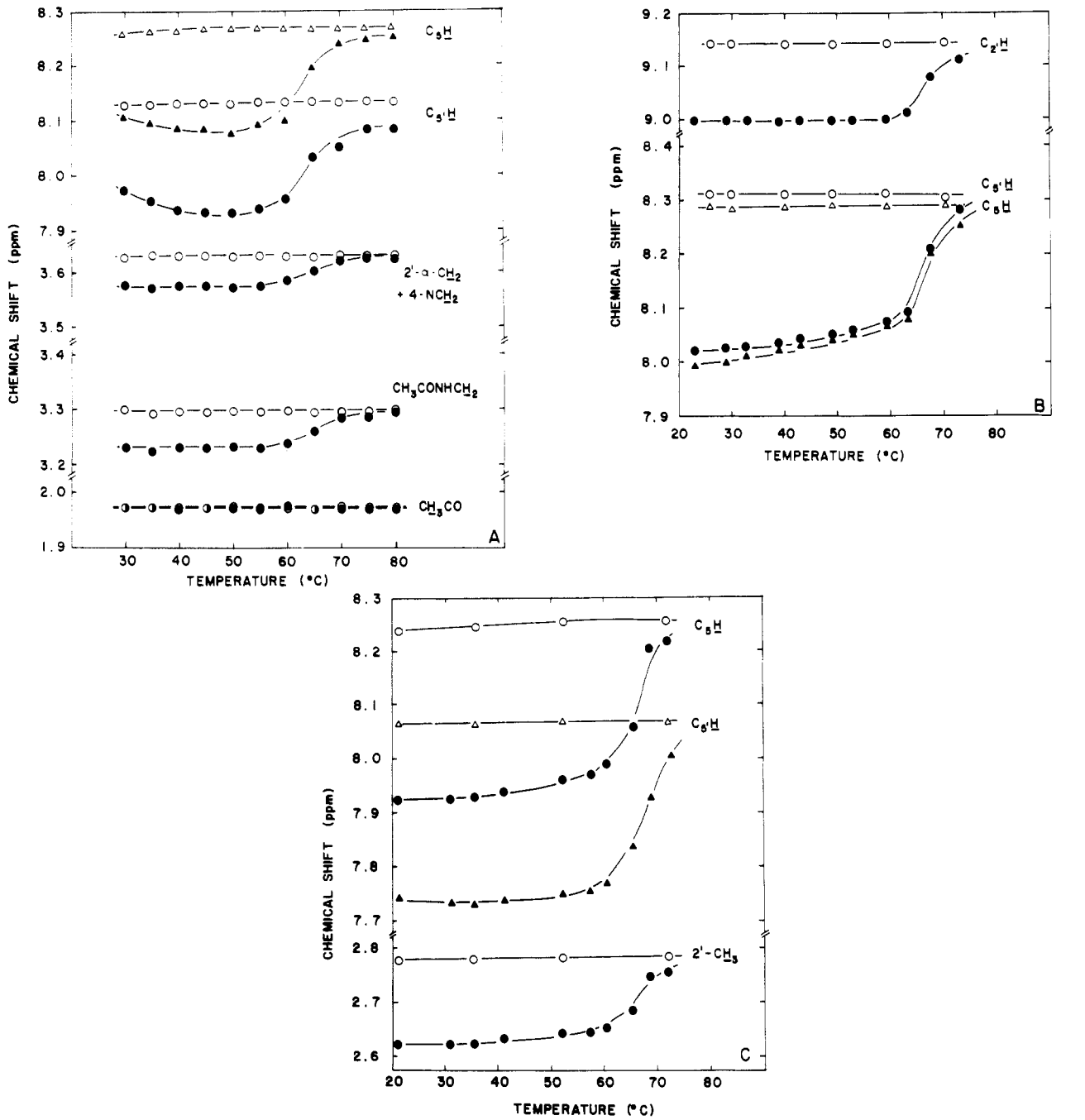

FIGURE 4: Temperature dependence of the chemical shifts of (A) the C_5H and $C_{5'}H$ resonances of **1**, together with the resonances of the 2′ substituent, (B) the $C_{2'}H$, C_5H, and $C_{5'}H$ resonances of **2**, and (C) the 2′-CH_3, C_5H, and $C_{5'}H$ resonances of **3** in the absence (open figures) and the presence (closed figures) of poly(dA-dT). Conditions are described in Figure 3.

The high field shifts observed in compounds **2**–**4** indicate that a steric effect is present in the interactions of bleomycin A_2 and **1** with poly(dA-dT). The two 2′-methylenes of **1** should show high field shifts of the same magnitude as the 2′-CH_3 of **3** in the absence of any other effects, but they show shifts of only 0.05 ppm, about one-third that of the 2′-CH_3 resonance of **3**. The mere presence of a 2′ substituent is not sufficient to impede binding since the 2′-CH_3 of **3** experiences substantial ring-current effects from the nucleic acid; thus, the steric effect must be manifesting itself further down the 2′ side chain than the first (2′-α) atom on the ring. These results suggested the possibility that the amide function might be involved in restricting the accessibility of the 2′ side chain to the nucleic acid interior and that it might serve as an anchoring point in **1** (and bleomycin A_2). It was reasoned that elongation of this side chain might allow sufficient flexibility for the bithiazole ring system to more fully insert itself between base pairs. The acetamidobutyl derivative **5** was used to test this idea.

The C_5H and $C_{5'}H$ resonances of **5** show a temperature dependence similar in shape to those of **1** (Figure 5). The maximum shift is slightly larger (~0.22 ppm); however, as with **1**, the high field shifts are diminished to some extent at temperatures below 50 °C. Interestingly, the four CH_2 resonances of the tetramethylene side chain show a graded response in their high field shifts: the 2′-α-CH_2 exhibits a shift of about 0.12 ppm in the presence of poly(dA-dT), a value which compares favorably with the shifts experienced by the $C_{2'}H$ and 2′-CH_3 of **2** and **3**. The 2′-β-, γ-, and δ-CH_2 (NCH_2) resonances show maximal shifts of 0.08, 0.02, and 0.01 ppm, respectively, and the acetyl CH_3 resonance shows no perturbation at all.

Role of the Thiazole Rings. The involvement of the thiazole rings in the binding of these derivatives to poly(dA-dT) was

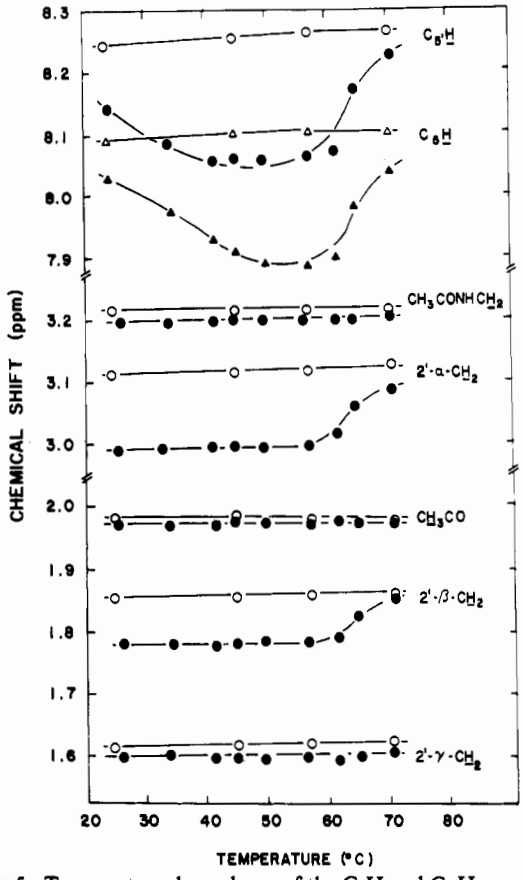

FIGURE 5: Temperature dependence of the C_5H and $C_{5'}H$ resonances and the resonances of the 2′ substituent of **5** in the presence of poly(dA-dT). Conditions are described in Figures 3 and 4.

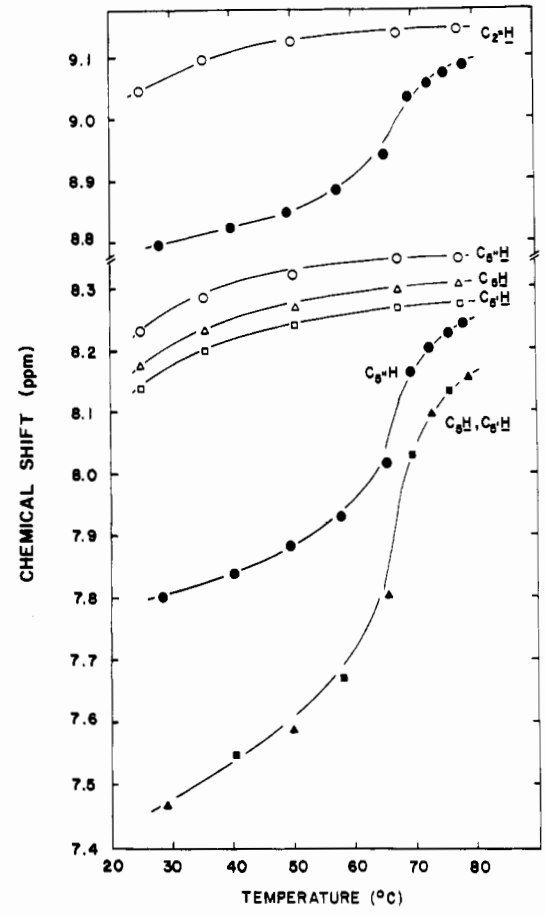

FIGURE 6: Temperature dependence of the aromatic resonances of **6** in the presence and absence of poly(dA-dT). Conditions are given in Figures 3 and 4.

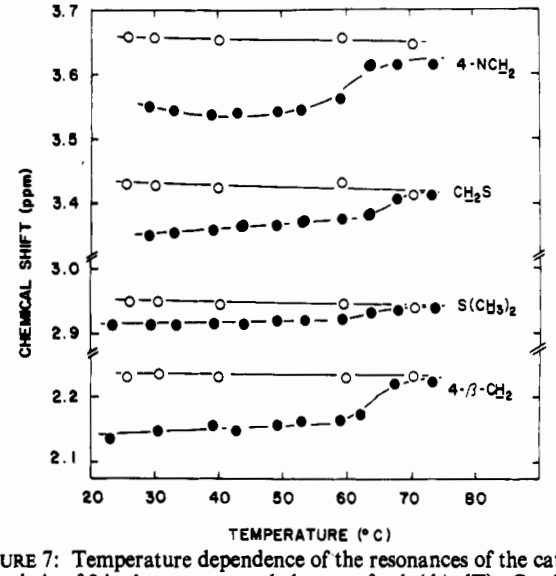

FIGURE 7: Temperature dependence of the resonances of the cationic side chain of **2** in the presence and absence of poly(dA-dT). Conditions are given in Figures 3 and 4.

studied by using three additional derivatives. The terthiazole derivative **6** was used to determine if an aromatic 2′ substituent would serve to block in the manner of the 2′-alkyl chains or enhance the binding of the bithiazole system as a result of extending the aromatic system. The temperature dependence of the aromatic hydrogen resonances (Figure 6) clearly shows that the high field shifts are enhanced in the terthiazole system. The lowest field resonance, arising from $C_{2''}H$, shows a maximum complexation shift of nearly 0.3 ppm. At ambient temperatures, two of the other three aromatic resonances (arising from C_5H, $C_{5'}H$, and/or $C_{5''}H$) are coalesced into a single broadened peak at $\delta \sim 7.45$, while the third occurs at a somewhat lower field at $\delta \sim 7.80$. As the temperature is raised, the resonances move close to one another as they track back to lower field and approach the positions of the free resonances. When the resonances are carefully tracked, the lower field resonance is found to arise from $C_{5''}H$, which thus shows a total shift of about 0.45 ppm. The C_5H and $C_{5'}H$ resonances appear to be degenerate in the complex with respective complexation shifts of about 0.70 and 0.72 ppm. The differential effects show that the aromatic hydrogens are experiencing ring-current effects at different positions relative to the nucleic acid base pairs.

The fact that the C_5H resonances of many of the bithiazole derivatives show a temperature dependence and high field shift virtually identical with the respective $C_{5'}H$ resonances suggested that the two hydrogens occupy very similar regions of the nucleic acid and that both rings are intercalated to similar extents. On this basis, it was thought that the aromatic hydrogens of monothiazole derivatives might show perturbations which could be used to help characterize the binding of each of the rings of bithiazole derivatives.

Somewhat unexpectedly, both the unsubstituted monothiazole derivative **7** and the 2-(2-acetamidoethyl) derivative **8** did not interact at all with poly(dA-dT). No shifts were observed for any of the resonances of **7** or **8**, and no line broadening indicative of immobilization of the ligand was evident; in addition, no perturbations of the nucleic acid resonances could be detected.

Effects on the Cationic Side Chain. In derivatives **1**–**6**, small high field shifts are observed for the trimethylene side chain containing the sulfonium group. Figure 7 shows the temperature dependence for the cationic side-chain resonances

Table I: Cationic Side-Chain High Field Chemical Shifts[a]

compd	Δδ			
	γ-CH_2	β-CH_2	SCH_2	$S(CH_3)_2$
1	0.05	0.05	0.03	0.03
2	0.11	0.07	0.05	0.03
3	0.12	0.08	0.07	0.02
4	0.15	0.07	0.08	0.02
5	0.10	0.05	0.10	0.03
6	0.17	0.08	0.08	0.03

[a] The values are the chemical shifts of the free resonance *minus* the chemical shift of the corresponding resonance in the complex, both measured at 30 °C.

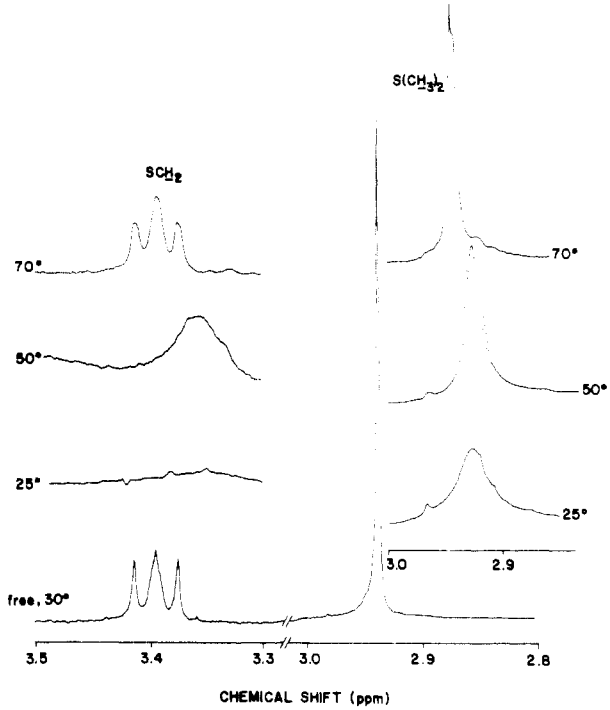

FIGURE 8: Spectra of the SCH_2 and $S(CH_3)_2$ resonances of derivative **6** free and in the presence of poly(dA-dT) at 25, 50, and 75 °C. Conditions are described in Figures 3 and 4.

of derivative **2**. The values for the high field shifts of these methylenes in each of the derivatives are compared in Table I. The similar behavior of the analogous CH_2's of all of these derivatives suggests a similar environment for this side chain in complexes of these derivatives. As expected, the CH_2 closest to the bithiazole system experiences the largest high field shift, consistent with its proximity to the nucleic acid bases. This shift is particularly pronounced in derivative **6** (~0.17 ppm). The sulfonium methyl hydrogens generally show smaller chemical shift responses to the presence of poly(dA-dT), although the line widths of these resonances and the SCH_2 resonances increase substantially in the presence of the nucleic acid for derivatives **2**, **3**, **4**, and **6** (Figure 8). The side chain of the bleomycin-like derivatives **1** and **5** shows smaller chemical shift changes than that of the other derivatives, while showing little or no broadening of the SCH_2 and $S(CH_3)_2$ resonances.

The requirement for an initial ionic interaction for proper placement of the thiazole system is suggested by studies of 2′-amino-2,4′-bithiazole. The resonances of this derivative show high field shifts only of the order of 0.05 ppm in the presence of poly(dA-dT) under the same conditions employed in the study of the cationic derivatives. The simpler 2,4-bithiazole was not sufficiently water soluble for these studies.

Poly(dA-dT) Resonances. The responses of the chemical

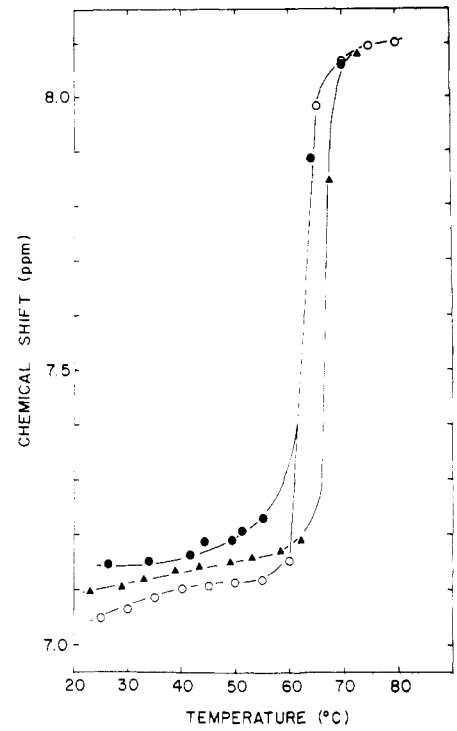

FIGURE 9: Temperature dependence of the A(H-2) resonance of poly(dA-dT) (10 mM) in the presence of no additions (○), 1.17 mM derivative **4** (●), and 1.17 mM derivative **6** (▲). Buffer is 0.01 M sodium phosphate (pH_m 6.8)–0.10 M sodium chloride in D_2O.

shifts of the polynucleotide resonances to structural changes in the thiazole derivatives have allowed us to pinpoint likely sites of interaction between the nucleic acid and the derivatives. At the ratio of poly(dA-dT) to analogue used in these studies, the perturbations of the nucleic acid resonances are not maximal. No attempt was made to maximize these shifts.

Only small perturbations are generally observed on the temperature dependence of the poly(dA-dT) resonances. All of the derivatives affect the A(H-2) resonance which, in the presence of the derivatives, experiences a low field shift of the order of 0.05–0.10 ppm at temperatures below T_m (Figure 9). This perturbation of the premelting region generally indicates that the base pairs of the helix have become slightly separated due to intercalation of the analogues and are feeling smaller ring-current shifts of the other base pairs as a result. Depending on the derivative, the T_m of this resonance is shifted to higher temperatures, by varying amounts, indicating stabilization of the helix by the binding of the derivative.

Compounds **1**, **4**, and **5** increase the T_m only slightly, as monitored by the midpoints of the transitions of this and other poly(dA-dT) resonances. The chemical shift of the A(H-2) resonance in the random coil form of poly(dA-dT) in the presence of these three derivatives is essentially the same as that in free poly(dA-dT), showing the absence of residual stacking interactions between these analogues and the random-coil form of the polynucleotide.

Derivatives **1** and **5** show 0.05- and 0.07-ppm low field shifts in the premelting region of the A(H-2) temperature curve. Compound **1** also causes a 0.17-ppm high field shift in the premelting region of the temperature curve of an H-2″ resonance (Patel, 1978) (Figure 10) and little or no other detectable perturbations. By comparison, compound **5** produces a 0.22-ppm high field shift on the premelting transition of an H-2′ resonance and a small effect on an H-2″ resonance (Figure 10). Both derivatives have no effect on the T_m of poly(dA-dT).

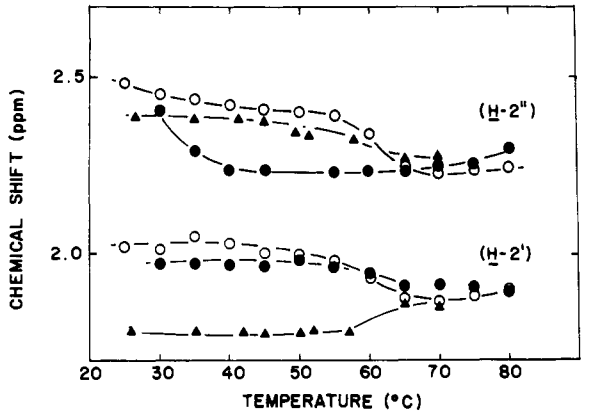

FIGURE 10: Temperature dependence of H-2′ and H-2″ resonances of poly(dA-dT) in the presence of derivative **1** (●) and derivative **5** (▲). Open figures (○) are points obtained in the absence of derivatives. Conditions are described in Figures 3, 4, and 9.

Derivatives **2** and **3**, in addition to small low field shifts of the A(H-2) resonance, cause slight high field shifts (0.05–0.07 ppm) in the premelting region of both sets of H-2′ and H-2″ resonances.

The amino derivative **4** shows only a small (~0.05 ppm) shift on the premelting transition of the A(H-2) resonance (Figure 9). In this case, however, one of the H-2″ resonances appears to split at temperatures below the T_m, with one peak essentially unchanged from the control resonance and one displaced approximately 0.2 ppm to high field in the premelting region (not shown). The two peaks appear to coalesce when the nucleic acid is in the random coil. Lesser perturbations are indicated for the H-2′ resonance. Derivative **4** causes only a small (<2 °C) increase in the T_m of the polynucleotide.

Compound **6**, the terthiazole, produces the most pronounced perturbations of all the derivatives on the poly(dA-dT) resonances. In addition to a 0.05-ppm low field shift for the premelting region of the A(H-2) resonance, this derivative causes a 5 °C increase in the T_m and shows an approximately 0.10-ppm residual high field shift for the A(H-2) resonance for temperatures greater than T_m (Figure 9).

The same H-2″ resonances which are split in the presence of derivative **4** again appear to be split below the T_m; in this case, however, one peak is shifted 0.10 ppm to higher field and one peak 0.10 ppm to low field relative to the control (not shown). Once again the two peaks merge at temperatures beyond the T_m. The T(H-6) (Figure 11) and T(H-1′) resonances show 0.05-ppm high field shifts in the premelt region, while the other (H-2′ and H-2″) resonance shows a slightly smaller effect. The T(H-6) resonance (Figure 11) exhibits a residual high field shift of about 0.10 ppm at temperatures beyond the T_m. One set of H-1′ and H-2′ resonances experiences small high field shifts in the premelting region.

All of the six derivatives which bind to poly(dA-dT) cause no high or low field shifts of the T(CH_3-5) resonance. The only effects seen on this resonance are increases in the T_m as a result of stabilization of the nucleic acid by the analogues (Figure 11). This absence of effects on the thymine methyl resonance suggests that the binding of the analogues is not occurring in the large groove occupied by the methyl group; however, further experiments are necessary before this can be shown conclusively.

The high field shifts experienced by the T(H-6) and T(H-1′) resonances in the presence of **6** indicate that extension of the thiazole aromatic system allows the thymine residues to experience greater ring-current effects than experienced in the double-helical form of poly(dA-dT). It is not known if this

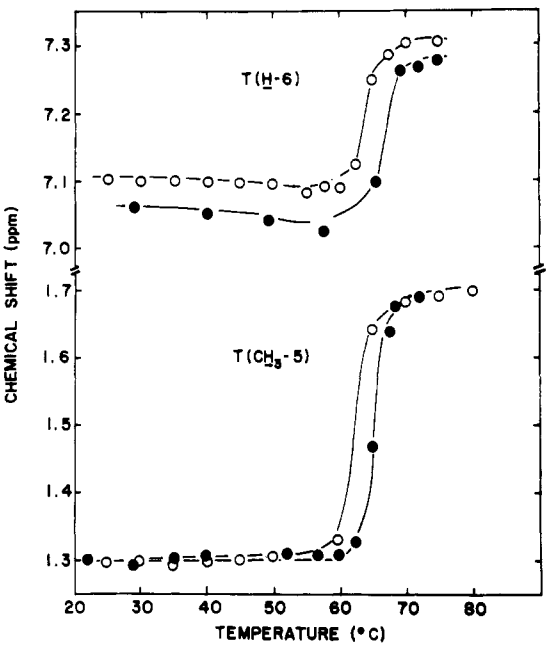

FIGURE 11: Temperature dependence of the T(CH_3-5) and T(H-6) resonances of poly(dA-dT) in the absence (○) and presence (●) of derivative **3**. Conditions are described in Figures 3 and 4.

Table II: Spectral Data for Thiazole Derivatives[a]

compd	λ (nm) UV (max)	excitation (max)	emission (max)	excitation (exptl)
1	292	300	350	260
2	285	296	350	260
3	291	300	350	260
4	310	278	300	278
5	292	300	350	270
6	240 (sh), 265, 300	270 (sh), 310	378	275
bleomycin A_2	292	300	350	260

[a] Determined in 0.01 M sodium phosphate (pH 6.8)–0.10 M NaCl.

effect arises from overlap with the terthiazole system or from an altered overlap of the A rings with the T residues.

Fluorescence Studies. The interactions of analogues **1–6** with poly(dA-dT) could be monitored by the quenching of the fluorescence of these derivatives in the presence of the polynucleotide. The fluorescence spectral properties of these compounds are given in Table II. Derivatives **1**, **2**, **3**, and **5** all have roughly the same quantum yield as bleomycin A_2 (≲0.01) (Chien et al., 1977); derivative **4** has a somewhat lower yield while the terthiazole has a quantum yield of about 0.05–0.07. These values are based on comparative measurements with bleomycin A_2 using solutions having the same absorbancies.

The use of the wavelength maxima for excitation and emission for any of the analogues gave variable results and only small decreases in the fluorescence intensities of the analogues upon the addition of poly(dA-dT). Examination of the excitation spectra of the analogues in the presence and absence of the nucleic acid showed that the region between 260 and 280 nm was particularly sensitive to the presence of poly(dA-dT) (Figure 12). The presence of saturating amounts of the polynucleotide resulted in the loss of this region of the excitation spectra so that by exciting at these wavelengths and monitoring the emission at the normal maxima, much more pronounced decreases in fluorescence intensity could be ob-

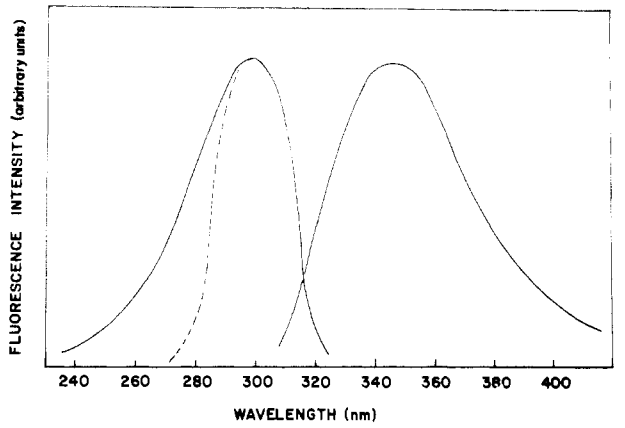

FIGURE 12: Fluorescence excitation and emission spectra of derivative **2** (2×10^{-5} M) in the absence (solid line) and the presence (broken line) of poly(dA-dT) (10^{-3} M) in 0.01 M sodium phosphate (pH 6.8)–0.10 M sodium chloride.

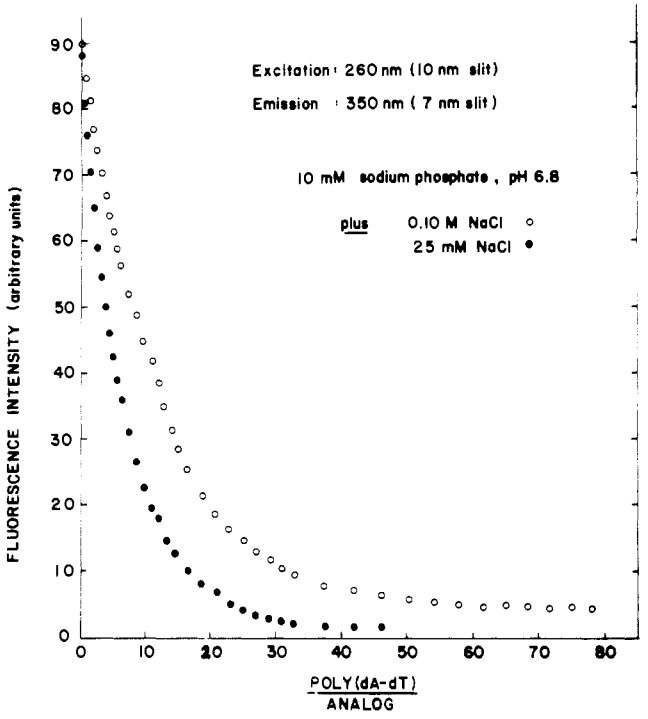

FIGURE 13: Fluorescence quenching of derivative **3** by poly(dA-dT) under conditions shown.

tained. The excitation wavelengths used are also given in Table II.

For each derivative, smooth saturation curves were obtained which could be analyzed by Scatchard plots. Typical plots of fluorescence intensity vs. the molar ratio of poly(dA-dT)–phosphate to analogue are shown in Figure 13 in both 10 mM sodium phosphate–25 mM NaCl and 10 mM sodium phosphate–0.1 M NaCl. Data were obtained for bleomycin A_2 by using the same "off-center" excitation wavelength. The monothiazole derivatives gave no reproducible fluorescence spectra or data which could be correlated with increases in poly(dA-dT) concentrations.

The Scatchard plots obtained for each of the six derivatives which experienced quenching of fluorescence intensity in the presence of poly(dA-dT) were characterized by two apparent classes of binding sites, one of high affinity but low occupancy and the other of considerably lower affinity but of high occupancy (Figure 14). As indicated under Methods, advantage was taken of the large difference in the apparent binding constants of the two classes of binding sites to obtain values

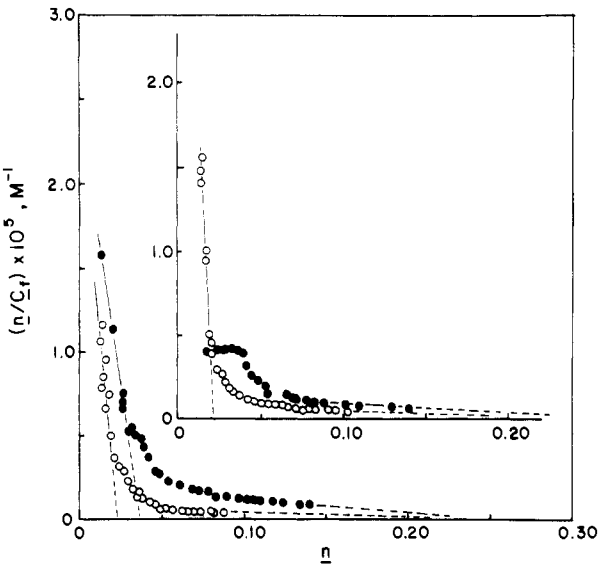

FIGURE 14: Scatchard plots of data obtained in Figure 13 for derivative **3**. Insert shows similar plots for derivative **2**. Open figures are data obtained in 0.01 M sodium phosphate (pH 6.8)–0.10 M sodium chloride, and closed figures are data obtained in 0.01 M sodium phosphate (pH 6.8)–0.025 M sodium chloride.

for both binding constants and numbers of binding sites from the straight-line extremes of the Scatchard plots. The apparent binding constants and the number of binding sites for each class are summarized in Table III for each analogue. Data for bleomycin A_2 obtained in the same manner are shown for comparison. The binding constants are the average of three experiments and are accurate to ±10–15%. For this reason, comparison of the derivatives with one another may be tenuous, although the salt effects on a single derivative may be compared.

The acetyl-dipeptide **1** has apparent binding constants of 2.8×10^4 and 6.5×10^6 M^{-1} for the low and high affinity sites, respectively, in 0.10 M NaCl. The numbers of binding sites correspond to one analogue molecule per 5 and 42 nucleotide residues, respectively. At the lower NaCl concentration, the affinity of the high occupancy site increases more than 2-fold, although the number of binding sites remains essentially unchanged. The higher affinity site, on the other hand, experiences a nearly 3-fold decrease in the apparent binding constant (to 2.7×10^6 M^{-1}) and about a 2-fold decrease in binding sites. The structurally similar acetamidobutyl derivative **5** shows very similar behavior.

Bithiazole derivatives **2–4** all appear to show increased binding relative to **1** for both classes of binding sites, with the high affinity sites affected by the structural changes to a greater degree than the low affinity sites. The occupancy in the low affinity class in 0.10 M NaCl corresponds to about one analogue molecule per 5 or 6 nucleotide residues and that in the higher affinity class to one analogue, bound per 38–45 nucleotides, depending on the particular derivative. Once again, at the lower NaCl concentration, the lower affinity classes of sites show a general increase in association constants and a slight increase in the number of binding sites. The higher affinity classes show the opposite trend, with decreases of approximately 2-fold in binding constants and corresponding increases in numbers of binding sites.

Derivative **2**, the unsubstituted bithiazole, gives a Scatchard plot at the lower NaCl concentration which is generally characteristic of positive cooperativity (Figure 14, inset). The binding constant of the higher affinity class was not determined in this instance. The other derivatives do not show this cooperative behavior.

Table III: Binding Constants and Numbers of Sites for Thiazole Derivatives

	K (M^{-1}) × 10^{-5} (n)[a]			
	buffer A[b]		buffer B[c]	
compd	class 1	class 2	class 1	class 2
1	74.9 (0.024)	0.28 (0.20)	27.0 (0.048)	0.66 (0.21)
2	186.0 (0.024)	0.64 (0.18)		0.66 (0.24)
3	146.0 (0.022)	0.39 (0.20)	51.9 (0.038)	0.66 (0.28)
4	208.0 (0.026)	0.42 (0.17)	11.6 (0.042)	0.84 (0.10)
5	79.2 (0.025)	0.36 (0.20)	29.3 (0.048)	0.46 (0.32)
6	249.0 (0.020)	0.53 (0.15)	130.7 (0.034)	0.45 (0.34)
bleomycin A_2	87.0 (0.049)	0.50 (0.39)	69.5 (0.052)	0.51 (0.36)

[a] Numbers of binding sites are expressed as moles of ligand per mole of poly(dA-dT) nucleotide residue. [b] Buffer A: 0.010 M sodium phosphate (pH 6.8)–0.100 M NaCl. [c] Buffer B: 0.010 M sodium phosphate (pH 6.8)–0.025 M NaCl.

The terthiazole analogue **6** shows the highest apparent association constants of the derivatives studied, with a value for the higher affinity site which is more than 3 times that of **1** and about 1.3 times that of the analogous bithiazole **2**. The apparent binding constant of the lower affinity site is about 5.3×10^4 M^{-1}. The numbers of binding sites correspond to about one analogue molecule per 49–50 and 7 nucleotide residues for the high and low affinity sites, respectively. At the lower NaCl concentration, both classes of binding sites show decreased affinity and increased occupancy. The low salt, low affinity site for this derivative appears not to obey the neighbor exclusion limit; that is, the stoichiometry allows for adjacent base pairs to bind ligand rather than every other base pair as in a neighbor exclusion model. Bleomycin A_2 also does not adhere to the neighbor exclusion limit based on these fluorescence experiments (Table III).

Discussion

The high field shifts observed for many of the hydrogen resonances of the various bithiazole derivatives indicated that these derivatives were experiencing the ring-current effects of the stacked A–T base pairs of the helical regions of poly(dA-dT). Such shifts are generally taken as evidence for intercalation or partial intercalation of the ligand molecule in question. Hydrogens on molecules in the ring-current fields of nucleic acid bases may experience high field shifts anywhere from approximately 0.10 ppm to greater than 1.0 ppm, depending on the degree of overlap of the molecule (and the hydrogen) with the bases (Giessner-Prettre & Pullman, 1976a,b; Krugh & Nuss, 1979; Patel, 1979). Thus, changes in the degree of intercalation may be monitored by changes in high field shifts experienced by hydrogens on the potential intercalant in question. Clearly, other contributions to the chemical shift perturbations such as charge and polarizability effects may play a role; however, it has been assumed that their effects are relatively small compared to the ring-current and diamagnetic anisotropy effects of the adjacent base pairs of the nucleic acid (Krugh & Nuss, 1979). The variation in the magnitudes of the shifts observed as the structures of the thiazole derivatives were modified could then be used to monitor the intercalative process and to determine possible geometric differences in the mode of interaction as a result of these modifications. These data could also be correlated with perturbations of the nucleic acid resonances to obtain an idea of likely points of interaction between the analogues and the polynucleotide and to obtain possible geometries for the complexes.

The initial observations on bleomycin A_2 and on the acetyl-dipeptide **1** had shown some unique features in the temperature dependence of the high field shifts of resonances perturbed by the presence of poly(dA-dT). In particular, the aromatic C_5H and $C_{5'}H$ resonances of the bithiazole system of bleomycin A_2 showed an unusual bell-shaped temperature dependence for the high field shifts, with the maximum shifts observed just below the helix–coil transition temperature of the nucleic acid. The loss of high field shifts with increasing temperature correlated with the melting transition of the nucleic acid to the single-stranded form, indicating that the drug required a duplex structure for optimum binding. At lower temperatures, however, these perturbations of the aromatic resonances were lost. The persistence of small shifts of other drug resonances suggested a residual interaction of the drug with poly(dA-dT) which did not involve intercalation of the bithiazole moiety.

Compound **1** exhibits a similar temperature dependence for the shifts of its aromatic bithiazole protons at temperatures near the T_m of the nucleic acid. However, below this temperature, the high field shifts were retained to a larger extent than in bleomycin A_2, indicating a greater degree of intercalative binding of the analogue at the lower temperatures at which the intact drug has lost most of its intercalative binding. Other resonances, including those of the 2′ side chain, remained perturbated at the lower temperatures, as were the analogous resonances in bleomycin A_2.

The loss of high field shifts above the T_m of the polynucleotide undoubtedly reflects the conversion of the nucleic acid to a random coil which cannot bind bleomycin or the acetyl-dipeptide fragment. The reason(s) for the loss of high field shifts below the T_m is (are) not clear, but this phenomenon may also reflect a change in the structure of the nucleic acid. In this case, the helix may become compact or stable enough to expel the bithiazole from its binding site. The difference in the response to temperature of the aromatic resonances of bleomycin A_2 and **1** may be due to the lack of bulk of **1** which would make it less likely to feel the restrictions of a helical nucleic acid. However, the similar values for the maximum high field shifts (0.15–0.17 ppm) indicate that the complexation must result in a similar geometry for the bithiazole ring system in both bleomycin A_2 and **1** at the optimum temperature.

Removal of the 2′ substitutent of the bithiazole system as in compounds **2**–**4** allows the aromatic hydrogens of the analogues to experience substantially larger high field shifts than are seen by the corresponding hydrogens of bleomycin A_2 or **1**. In addition, these shifts are maintained as long as the nucleic acid is in the helical form. This shows that the 2′ side chain of **1** and bleomycin A_2 or some portion of that chain is responsible for the loss of intercalative binding in these compounds.

The 2′ side chain of analogue **5**, the homologous acetyl-dipeptide, appears to play a similar inhibitory role. However,

compound **5**, because the side chain is longer, can place its bithiazole moiety slightly further into the poly(dA-dT) helix. The fact that the complexation shifts experienced by the 2′-α-CH_2 of **5** and by the $C_{2'}H$ and 2′-CH_3 resonances of **2** and **3** are all about 0.12–0.15 ppm suggests that these atoms may occupy similar regions in the intercalation complex. However, the smaller magnitude of the C_5H and $C_{5'}H$ resonances ($\sim$ 0.20 ppm) of **5** suggests that the geometry of binding of derivative **5** may be intermediate to the structures of the bleomycin complex and the complexes of derivatives **2**–**4** in the degree of intercalation. Since the tetramethylene side chain of **5** experiences relatively large ring-current shifts, it may be argued that the bithiazole in **5** is intercalated to a greater extent than it is in **1** but that the new orientation fortuitously gives rise to shifts which are not substantially different from those seen with **1**. Examination of the isoshielding contours of Giessner-Prettre & Pullman (1976a,b) shows that there are many possible orientations of the bithiazole systems which would give rise to the same chemical shifts for two sets of aromatic resonances. The decrease in the complexation shifts of the 2′ side chain of **5** as the chain is followed from the thiazole rings toward the amide function shows that the part of the chain distal to the bithiazole system is extending away from the nucleic acid helix and does not feel the ring-current effects of the base pairs.

The interdependence of the 2′ side chain amide function, the thiazole aromatic system and the cationic side chain is evident in the monothiazole derivatives. Although compound **7** is not sterically constrained and was expected to bind much as compound **2**, it was found not to interact at all with poly(dA-dT). This suggests that the monothiazole is not sufficiently long to intercalate. The ability of the C_5H region of the bi- and terthiazole derivatives to experience the high field shifts associated with intercalation clearly requires the second (and third) thiazole ring(s). It appears that the initial ionic binding process does not allow the first thiazole ring to get close enough so that the intercalation process can stabilize the interaction of all the rings. That an initial ionic interaction between the cationic group and the phosphate backbone of the nucleic acid may be a necessary prerequisite for intercalation to occur is verified by the small NMR spectral perturbations observed with 2′-amino-2,4′-bithiazole which lacks the cationic side chain. Once complexation has occurred, the cationic group is no longer used to stabilize the complex to any great degree. Bleomycin A_2 and derivatives **1** and **5** exhibit little or no broadening of the SCH_2 and $S(CH_3)_2$ resonances, even though these compounds show the least intercalation and might be expected to show a greater ionic component in the binding. On the other hand, those derivatives which intercalate most strongly, e.g., compounds **2**, **3**, and **6**, show the greatest broadening of those resonances associated with the cationic center. This apparently anomalous behavior may be explained by the ability of the strong intercalators to draw the side chain in close to the nucleic acid. The resultant broadening of the resonances is due to a general immobilization of the group within the narrow groove of the nucleic acid rather than to a more specific immobilization by ion pair interactions.

That the monothiazole **8** also does not bind suggests a minimum distance requirement between the 2′-amide function and the cationic group. This distance is just met in bleomycin A_2 and the acetyl-dipeptide **1** and surpassed by the homologous acetyl-dipeptide **5**, but is too short in derivative **8**. With compound **5**, the excess length is placed into the nucleic acid helix to help stabilize the complex while the contact between the amide function and nucleic acid is maintained. The monothiazole results clearly show that although the cationic group may be *necessary* for binding, it is not sufficient. Thus, even the low affinity class of binding sites (class 2 in Table III) probably involves interactions in addition to the ionic interactions.

The use of fluorescence quenching to estimate binding constants makes assumptions about the interactions of the bithiazole derivatives with poly(dA-dT); in particular, it has been assumed that quenching is a sensitive measure of nonintercalative binding as well as intercalative binding. That this is true remains to be determined for these derivatives, as well as for the bleomycins.

In studies of the intact drug (Chien et al., 1977; Huang et al., 1979, 1980a; Povirk et al., 1979), buffers of low ionic strength have been used to enhance the fluorescence changes observed in the presence of DNA. Such ionic strengths (less than 1 mM in one instance) undoubtedly have a profound effect on the nucleic acid structure, and the values of binding constants obtained in such cases must reflect binding of bleomycins to the structures assumed by the nucleic acids under those conditions. In those instances, only a single class of binding site is apparent for bleomycin binding. In the present study, use of moderate ionic strengths to maintain the double-helical structure of the nucleic acid and the use of a modified fluorescence quenching method have allowed the observation of two classes of binding sites, one of which appears to be primarily intercalative in nature and one of which appears to be governed primarily by ionic interaction(s). The interrelations between the low ionic strength class and the two classes observed at moderate ionic strengths are not clear, but the low ionic strength class may be a combination of the latter two classes which arises as a result of the loss of nucleic acid structure. Data obtained from studies in such low ionic strength media have been cited as evidence for intercalative binding of the bleomycins to DNA (Povirk et al., 1979); however, as mentioned above, such data must be viewed in the context of possible effects on nucleic acid structure and should be taken as evidence "in agreement with but not proving" intercalation. This present study has indicated that bleomycin A_2 and bleomycin-like fragments are sensitive to the nucleic acid structure to such an extent that intercalative binding is favored only when the nucleic acid is slightly unwound and that helical poly(dA-dT) does not bind such derivatives by an intercalative mechanism. Similarly, when the nucleic acid is double helical, only these derivatives will intercalate which have had the bleomycin-like 2′ side chain removed, showing that that substituent determines to a large extent the mode of binding of bleomycins.

The low affinity sites detected in the fluorescence experiments for derivatives **1**–**5** appear to correspond to "outside binding" sites, i.e., binding on the outside of the nucleic acid helix in a process which is sensitive to salt concentration. This process presumably involves primarily an electrostatic interaction between the positively charged sulfonium group of the analogues and the anionic nucleic acid backbone, a process which would be expected to be enhanced as the ionic strength is lowered, although, as indicated above, such a process is not sufficient for binding. The number of sites are seen to be close to the number expected for a neighbor exclusion model of binding, although there is some evidence that such a model may not be operative in some cases (Table III). The high affinity site appears to reflect an intercalative complex and is presumed to be the type of complex monitored in the NMR experiments.

The fluorescence data show that the increase in outside binding brought about by lowered salt concentration does not result in a concomitant increase in intercalative binding, a

result which would not be expected for a coupled process in which ionic binding occurs prior to and/or is a prerequisite for intercalation. It appears that the ionic binding and intercalative binding in these derivatives are not interdependent or are only weakly connected.

This apparent independence of the low and high affinity sites is generally not encountered in intercalating drugs such as ethidium bromide (Sakai et al., 1975) and is difficult to explain. In the simplest case, intercalation and ionic binding are processes completely independent of one another, and the explanation is trivial—the outside sites are destabilized by high salt and the intercalative sites are destabilized by low salt. However, if the ionic binding process is a prerequisite for intercalation and if, as some of the data indicate, the cationic side chain is not a major determinant in the maintenance of the complex, the intercalation of the ring system (and the associated freeing of the cationic group) must be fast relative to the on-rate of the strictly ionic interaction in order that the intercalation process be insensitive to salt concentration. To account for the fact that many more ligand molecules are bound to ionic sites than to intercalated sites, it must be argued that not all ionic interactions lead to intercalation and/or that the off-rate for intercalated species must also be rapid.

An explanation for the effect of low salt on the high affinity sites may lie in the possible destabilization of the nucleic acid helix brought about by the loss of electrostatic shielding. This destabilization might lead to some elongation of the helix with resulting increases in the distance between base pairs. Such "loose" helical regions may not sufficiently stabilize the binding of intercalating groups. The apparently cooperative binding of derivative **2** may reflect the ability of this compound to restore some structure to the nucleic acid, with the result that as more analogue is bound, more structure is given to the polynucleotide, allowing, in turn, more binding. Increasing cooperative behavior with decreasing ionic strength has been observed in the binding of actinomycin D and some aromatic carcinogens to DNA (Winkle & Krugh, 1981). It is not clear, however, why derivative **2** is the only one of the thiazole analogues showing this effect.

The 2′-amino analogue (**4**) shows several interesting properties which bear on the same idea of nucleic acid destabilization in low salt. Of the bithiazole derivatives, it has the highest apparent association constant ($2.1 \times 10^7\ M^{-1}$); however, the high affinity site is very sensitive to lowered salt concentration, with the association constant decreasing nearly 20-fold in going from 0.10 M NaCl to 25 mM NaCl. Other simple derivatives such as **2** and **3** show less exaggerated responses to the salt concentration. It is tempting to suggest that the amino group of **4** is involved in stabilizing its complex with poly(dA-dT), perhaps via hydrogen bonding, as has been suggested for the binding of ethidium bromide to helical nucleic acids (Tsai et al., 1975; Mandal et al., 1980; Wakelin & Waring, 1980). This stabilization is not possible at low salt because of the disruption of the nucleic acid structure which would normally allow this stabilization to occur. Since the binding of the other analogues is not as sensitive to the "correct" structure, their binding is not as strongly influenced by low salt concentrations. Such a stabilization would require that the 2′-amino group be oriented toward the phosphate backbone of the nucleic acid rather than in toward the helix.

The ring-current effects felt by the $C_{2'}H$ resonance of **2** and the 2′-CH_3 resonance of **3** are somewhat lower than those expected on the basis of the shifts experienced by the respective C_5H and $C_{5'}H$ resonances. It is possible that the binding of these analogues places the hydrogens of or near the 2′ position in a region between the two bases of a base pair where ring-current effects would be diminished. This idea is substantiated by the terthiazole derivative in which the resonances of the hydrogens on the third (″) ring show substantial shifts. The third ring would be expected to allow interaction of the three thiazole rings with both bases of the base pair. The ring-current effects experienced by the hydrogens of the third thiazole ring and the appearance of perturbations on the T-(H-6) resonance indicate that the three contiguous rings can span the entire base-pair region of poly(dA-dT).

Scission of DNA by bleomycins occurs predominantly at G(5′→3′)–pyrmidine sequences, and preferential association of bleomycins with G residues may account for this reaction (D'Andrea & Haseltine, 1978; Takeshita et al., 1978). It is tempting to attribute some of the NMR spectral perturbations to sequence-specific interactions of analogues with poly(dA-dT); however, the tentative nature of the assignments of the sugar resonances of the nucleic acid precludes any model for a sequence–specific interaction between the thiazole systems and the polynucleotide. It seems possible that changes in preference for cleavage may result if an appropriately designed thiazole system could be introduced into the bleomycins or into synthetic bleomycin analogues. That the bithiazole region may function as such a "recognition site" is indicated by the observations of Mirabelli et al. (1979). They have found that the phleomycins and the talisomycins which are structurally related to the bleomycins but which contain modifications in and around the bithiazole region appear to have different sequence preferences in their cleavage reactions. In addition, the relative numbers of single- and double-stranded nicks introduced into DNA is affected (Mirabelli et al., 1980).

That the cationic side chain may not be involved in stabilization of complexes suggests that it may not be a requirement for the biological activity of the bleomycins. In this regard, it has been shown that bleomycin amide (which lacks the cationic side chain) can cleave DNA (Asakura et al., 1975), albeit at a reduced rate (Huang et al., 1980b). The effect of this modification on the association with the target DNA is not known, although the reduction in rate of cleavage may reflect a decreased affinity for the DNA resulting from lack of the cationic group.

The results presented here have shown that full intercalation of the bithiazole system can occur for bleomycin derivatives of the proper structure. This suggests that by use of appropriate synthetic and biosynthetic precursors bleomycin derivatives might be prepared which have different affinities and modes of action with DNA and tumors. This manipulation of the affinities and structures in synthetic or semisynthetic derivatives may provide bleomycins of varying specificities in DNA cleavage reactions and, ultimately, in biological activity and toxicity.

Conclusions

It has been suggested that the binding of bleomycin A_2 to DNA occurs with intercalation of the bithiazole rings on the basis of viscometric and dichroic measurements at low ionic strengths (Povirk et al., 1979). However, the data presented here argue against intercalation as a predominant mode of binding of the intact drug molecule under conditions of moderate ionic strength (and physiological temperatures). Only when the bulk of the bleomycin A_2 structure is removed is significant intercalative binding of the bithiazole system evident. This is based on the fact that the synthetic derivatives described here are able to show NMR complexation shifts consistent with a greater degree of intercalation. It seems likely that the threonyl peptide bond with the aminoethyl side chain

of the bithiazole moiety is involved in an interaction with poly(dA-dT) which serves to limit the intercalation process. Extension of this chain allows somewhat more intercalation.

A minimum of two thiazole rings appears to be necessary for any binding to occur. A third thiazole ring greatly enhances the intercalation process. The derivatives do not appear to require the ionic interaction with the nucleic acid backbone to stabilize complexes with the nucleic acid. The binding of all derivatives appears to occur in the narrow or minor groove of the nucleic acid. The ring system of the terthiazole derivative appears to extend across both bases of the nucleic acid base pair, while bithiazole derivatives appear only to span the purine ring.

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References

Asakura, H., Hori, M., & Umezawa, H. (1975) *J. Antibiot. 28*, 537–542.

Burger, R. M., Peisach, J., & Horwitz, S. B. (1981) *Life Sci. 28*, 715–727.

Chen, D. M., Sakai, T. T., Glickson, J. D., & Patel, D. J. (1980) *Biochem. Biophys. Res. Commun. 92*, 197–205.

Chien, M., Grollman, A. P., & Horwitz, S. B. (1977) *Biochemistry 16*, 3641–3647.

D'Andrea, A. D., & Haseltine, W. A. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 3608–3612.

Erlenmeyer, H., Weber, O., Schmidt, P., Küng, G., Zinsstag, C., & Prijs, B. (1948) *Helv. Chim. Acta 31*, 1142–1158.

Giessner-Prettre, C., & Pullman, B. (1976a) *Biochem. Biophys. Res. Commun. 70*, 578–581.

Giessner-Prettre, C., & Pullman, B. (1976b) *C.R. Hebd. Seances Acad. Sci., Ser. D 283*, 675–677.

Glickson, J. D., Pillai, R. P., & Sakai, T. T. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 2967–2971.

Grollman, A. P., & Takeshita, M. (1980) *Adv. Enzyme Regul. 18*, 67–83.

Hecht, S. M., Ed. (1979) *Bleomycin. Chemical Biochemical and Biological Aspects*, Springer-Verlag, New York.

Huang, C.-H., Galvan, L., & Crooke, S. T. (1979) *Biochemistry 18*, 2880–2887.

Huang, C.-H., Galvan, L., & Crooke, S. T. (1980a) *Biochemistry 19*, 1761–1767.

Huang, C.-H., Mirabelli, C. K., & Crooke, S. T. (1980b) *Proc. Am. Assoc. Cancer Res. 21*, 31.

Inman, R. B., & Baldwin, R. L. (1962) *J. Mol. Biol. 5*, 172–184.

Kasai, H., Naganawa, H., Takita, T., & Umezawa, H. (1978) *J. Antibiot. 31*, 1316–1320.

Klotz, I. M., & Hunston, D. L. (1971) *Biochemistry 10*, 3065–3069.

Krugh, T. R., & Nuss, M. E. (1979) in *Biological Applications of Magnetic Resonance* (Shulman, R. G., Ed.) pp 113–175, Academic Press, New York.

Mandal, C., Englander, S. W., & Kallenbach, N. R. (1980) *Biochemistry 19*, 5819–5825.

Mirabelli, C. K., Mong, S., Huang, C.-H., & Crooke, S. T. (1979) *Biochem. Biophys. Res. Commun. 91*, 871–877.

Mirabelli, C. K., Huang, C.-H., & Crooke, S. T. (1980) *Cancer Res. 40*, 4173–4177.

Patel, D. J. (1978) *J. Polym. Sci., Polym. Symp. 62*, 117–141.

Patel, D. J. (1979) *Acc. Chem. Res. 12*, 118–125.

Patel, D. J., & Canuel, L. L. (1977) *Biopolymers 17*, 857–873.

Patel, D. J., & Canuel, L. L. (1978) *Eur. J. Biochem. 90*, 247–254.

Povirk, L. F., Hogan, M., & Dattagupta, N. (1979) *Biochemistry 18*, 96–101.

Riordan, J. M., & Sakai, T. T. (1981) *J. Heterocycl. Chem. 18*, 1213–1221.

Sakai, T. T., Torget, R., I, J., Freda, C., & Cohen, S. S. (1975) *Nucleic Acids Res. 2*, 1005–1022.

Sakai, T. T., Riordan, J. M., Booth, T. E., & Glickson, J. D. (1981) *J. Med. Chem. 24*, 279–285.

Sausville, E. A., & Horwitz, S. B. (1979) in *Effects of Drugs on the Cell Nucleus* (Busch, H., Crooke, S. T., & Daskal, Y., Eds.) pp 181–205, Academic Press, New York.

Takeshita, M., Grollman, A. P., Ohtsubo, E., & Ohtsubo, H. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 5983–5987.

Takita, T., Muraoka, Y., Nakatani, T., Fujii, A., Iitaka, Y., & Umezawa, H. (1978) *J. Antibiot. 31*, 1073–1077.

Tsai, C.-C., Jain, S. C., & Sobell, H. M. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72*, 628–632.

Van Geet, A. L. (1968) *Anal. Chem. 40*, 2227–2229.

Wakelin, L. P. C., & Waring, M. J. (1980) *J. Mol. Biol. 144*, 183–214.

Winkle, S. A., & Krugh, T. R. (1981) *Nucleic Acids Res. 9*, 3173–3186.