# Correlation of DNA Sequence Specificity of Anthramycin and Tomaymycin with Reaction Kinetics and Bending of DNA†

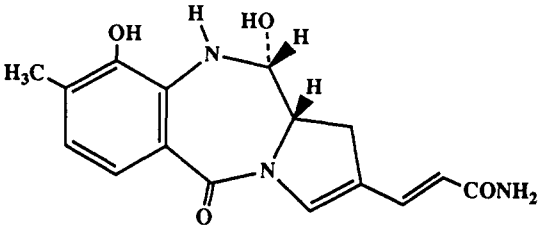
Ryoichi Kizu,‡ Patricia H. Draves, and Laurence H. Hurley*

*Drug Dynamics Institute, College of Pharmacy, The University of Texas at Austin, Austin, Texas 78712*

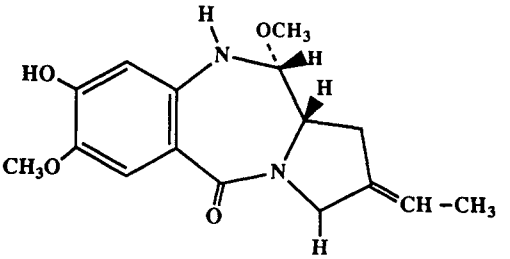
*Received March 9, 1993; Revised Manuscript Received June 9, 1993*

ABSTRACT: Anthramycin and tomaymycin are potent antitumor antibiotics belonging to the pyrrolo[1,4]-benzodiazepine [P[1,4]B] group. Their potent biological effects are thought to be due to their ability to react with DNA within the minor groove, forming covalent adducts through the N2 of guanine with the drug molecules overlapping with a 3–4-bp region. In spite of their small molecular weights, the P[1,4]B's show a surprising degree of sequence selectivity, with 5′-PuGPu sequences being the most reactive and 5′-PyGPy sequences being the least reactive [Hertzberg, R. P., Hecht, S. M., Reynolds, V. L., Molineux, I. J., & Hurley, L. H. (1986) *Biochemistry 25*, 1249–1258]. It has been proposed that inherent DNA flexibility may be one important component of the sequence recognition process for P[1,4]B bonding to DNA, and in this regard, molecular modeling studies are reflective of the experimentally determined hierarchy of bonding sequences [Zakrzewska, K., & Pullman, B. (1986) *Biomol. Struct. Dyn. 4*, 127–136]. In this study, we have used chemical and enzymatic probes (hydroxyl radical, DNase I) to evaluate drug- and sequence-dependent changes in DNA–adduct conformation, gel electrophoresis to measure drug-induced bending in DNA, and HPLC to measure the reaction kinetics of anthramycin bonding to different sequences. The results show that tomaymycin bonding to DNA induces greater conformational changes in the DNA (i.e., bending and associated narrowing of the minor groove) than anthramycin. In addition, we find that within each drug species (i.e., tomaymycin or anthramycin), sequence specificity correlates with the degree of bending and reaction kinetics such that those sequences with the *highest* sequence selectivity produce *more* bending of DNA and react *faster* with DNA and vice versa. On the basis of these results, we propose that sequence-dependent conformational flexibility may be an important factor in determining the hierarchy of bonding sequences for the P[1,4]B's.

Anthramycin and tomaymycin (Figure 1) are antitumor antibiotics belonging to the pyrrolo[1,4]benzodiazepine [P[1,4]B][1] group. Reviews on the chemistry (Remers, 1988), mechanism of action (Kohn, 1975; Hurley, 1977; Hurley & Needham-VanDevanter, 1986), mechanisms for sequence recognition (Mountzouris & Hurley, 1992), structure–activity relationships (Thurston & Hurley, 1983), and multiplicity of drug species bound to DNA (Remers et al., 1992) have appeared. Like mitomycin C, the P[1,4]B's form covalent adducts through the exocyclic amino group of guanine (N2) and lie within the minor groove covering a 2–3-bp region (Hurley & Petrusek, 1979; Petrusek et al., 1981). The imine (Figure 2) is proposed to be the DNA-reactive species (Barkley et al., 1986). In spite of the relatively small size of this DNA ligand, the P[1,4]B's show a surprising degree of sequence specificity (Hertzberg et al., 1986; Hurley et al., 1988), but it is still not clear how this selectivity is achieved (Mountzouris & Hurley, 1992). On the basis of molecular modeling studies (Zakrzewski & Pullman, 1986), it has been proposed that inherent sequence flexibility is the origin of this sequence selectivity. In this study, we have used five different 21-bp oligomers, each containing a unique P[1,4]B bonding site contained in sequences covering the range of hierarchy of bonding specificity in order to compare drug-induced bending and reaction kinetics. Overall, we find that within each drug species (i.e., tomaymycin or anthramycin) sequence specificity correlates with the degree of bending and reaction kinetics such that those sequences with the *highest* sequence selectivity produce *more* bending of DNA and react *faster* with DNA and vice versa. Although this correlation of experimental




ANTHRAMYCIN




TOMAYMYCIN

FIGURE 1: Structures of anthramycin and tomaymycin.

† This research was supported by the Public Health Service (CA-49751), The Welch Foundation, and the Burroughs Wellcome Fund.

* Author to whom correspondence should be addressed. Telephone: (512) 471–4842.

‡ On leave from the Faculty of Pharmaceutical Sciences, Kanazawa University, Takara-Machi, Kanazawa 920, Japan.

[1] Abbreviations: bp, base pair; P[1,4]B, pyrrolo[1,4]benzodiazepine; Tris, tris(hydroxymethyl)aminomethane; Op-Cu, 1,10-phenanthroline–copper complex; EDTA, ethylenediaminetetraacetic acid; HPLC, high-pressure liquid chromatography; MPE, methidium propyl EDTA; TBE, tris–borate–EDTA; ATP, adenosine triphosphate.

0006-2960/93/0432-8712$04.00/0 

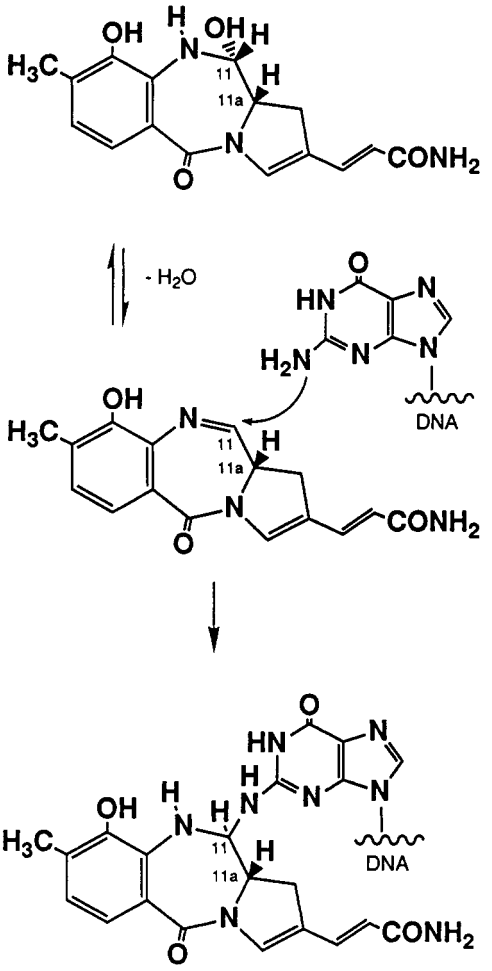



FIGURE 2: Proposed reaction of anthramycin with the N2 of guanine via the imine to form the anthramycin–DNA adduct.

data supports the theoretically based Pullman postulate (Zakrzewski & Pullman, 1986), there are certain experimental findings that do not correspond to the theoretical results.

## MATERIALS AND METHODS

*Chemicals and Enzymes.* Anthramycin 11-methyl ether and tomaymycin were obtained from Hoffmann-La Roche and Fujisawa Pharmaceuticals, respectively. The series of oligonucleotides shown in Figure 2 was synthesized on an automated DNA synthesizer (Applied Biosystems 381A) by the phosphoramidite method (Gait, 1984). The oligomers were deprotected separately with saturated ammonium hydroxide at 55 °C overnight, and the solvent was evaporated at room temperature. HPLC water and methanol were purchased from Fisher Scientific Co. Electrophoretic reagents, acrylamide, bis(acrylamide), ammonium persulfate, and *N,N,N′,N′*-tetramethylethylenediamine were purchased from Bio-Rad and ICN. T4 polynucleotide kinase and T4 DNA ligase were from United States Biochemical Corporation. DNase I from bovine pancreas and bovine serum albumin (Cohn's Fraction V) were from Sigma. [$\gamma$-$^{32}$P]ATP was from ICN. X-ray film (x-OMATAR), intensifying screens, and developing chemicals were from Kodak. Other chemicals were reagent grade or better and were used as received.

Stock solutions of the antibiotics were prepared by suspending anthramycin 11-methyl ether or tomaymycin in 1 mL of annealing buffer (100 mM NaCl and 10 mM Tris Cl, pH 7.5) and stirring for 2 h at room temperature. The antibiotic stock solutions were then stored at −70 °C until use.

*Kinase Reaction and Purification of Oligonucleotides.* Approximately 10 $\mu$g of individual oligonucleotides were labeled at the 5′ end with 30 $\mu$Ci of [$\gamma$-$^{32}$P]ATP and 5 units of T4 polynucleotide kinase in 25 $\mu$L of a solution containing 50 mM Tris-HCl (pH 7.6), 10 mM $MgCl_2$, and 10 mM 2-mercaptoethanol at 37 °C for 30 min. The solution was heated to 70 °C for 5 min to deactivate the kinase enzyme. The [$\gamma$-$^{32}$P]ATP-labeled oligonucleotides were mixed with their complementary oligonucleotides. The combined oligonucleotide solutions were heated to 55 °C and then cooled slowly to 4 °C to form hybridized duplexes.

For purification, the duplexes were electrophoresed [29:1 (w/w) monoacrylamide/bis(acrylamide)] on an 8% nondenaturing polyacrylamide gel until the bromophenol blue marker had migrated 23 cm in a 30 cm × 36 cm × 0.8 mm gel. The electrophoresis buffer was 50 mM Tris–borate (pH 8.3) and 1 mM $Na_2EDTA$ (TBE buffer). The gels were electrophoresed at room temperature at 7 V/cm. The gels were exposed to X-ray film, and the duplexes were excised from the gel, minced with a blade, and extracted with 400 $\mu$L of 10 mM Tris Cl (pH 7.5), 100 mM NaCl, and 1 mM $Na_2$-EDTA.

*Drug Bonding to Oligomers and Ligation.* For the preparation of antibiotic-modified oligomers, 30 $\mu$L of a concentrated suspended stock solution of anthramycin or tomaymycin was added to 100 $\mu$L (~10 $\mu$g) of [$\gamma$-$^{32}$P]ATP-labeled purified DNA duplex. The mixtures were incubated at 4 °C for 7 days, followed by an ethanol precipitation to remove unbound drug molecules.

Antibiotic-modified and unmodified oligomers were self-ligated into multimeters in 15 $\mu$L of ligation buffer with 1 unit of T4 ligase at 4 °C overnight. The ligation buffer contained 50 mM Tris-HCl (pH 7.6), 10 mM $MgCl_2$, 1 mM dithiothreitol, 5% (w/v) poly(ethylene glycol) 8000, and 0.5 mM ATP. These samples were used directly in the bending experiments.

*Bending Experiments.* For bending experiments, equal amounts of radioactivity per self-ligated sample were loaded onto a 6% nondenaturing polyacrylamide gel [30 cm × 36 cm × 0.8 mm; 29:1 (w/w) acrylamide/bis(acrylamide)] and electrophoresed with TBE buffer at 350 V at room temperature until the bromophenol blue marker had migrated 25 cm. The ligated multimers were located by autoradiography, and the ratio of apparent size to actual size ($R_L$) for each product was calculated.

*DNA Sequencing and Sequencing Gel Electrophoresis.* Purine- and pyrimidine-specific sequencing reactions were carried out according to the methods of Maxam and Gilbert (1980). Oligomers digested by the sequencing reaction of Maxam and Gilbert, by DNase I, or by the chemical reagents $Fe^{II}$EDTA and Op-Cu were resuspended in sequencing gel loading buffer. The resulting solutions were heated to 90 °C for 3 min to denature the oligomers and then plunged quickly into ice water. Equal amounts of radioactivity per sample were loaded onto a 20% denaturing gel [30 cm × 35 cm × 0.35 cm; 29:1 (w/w) acrylamide/bis(acrylamide) and 8.3 M urea] and electrophoresed with TBE buffer at 1800 V at room temperature until the bromophenol blue marker had migrated 25 cm. Autoradiograms were made with the aid of intensifying screens and exposed at −70 °C. Films were scanned with an LKB Model 2202 laser densitometer coupled with an LKB Model 2220 recording integrator.

*Footprinting Experiments. (A) DNase I Digestion.* Antibiotic-modified and unmodified oligomers were suspended in 10 $\mu$L of 10 mM Tris-HCl (pH 7.6), 2 mM $MgCl_2$, 0.2 mM

Table I: Sequences of Synthetic Oligonucleotides Used in This Study[a]

| | | |
|---|---|---|
| AGA | 5' | ATATIATCIAGAACTCICITT 3' |
| | 3' | ACTAGCTCTTGAGCGCAATAT 5' |
| CGA | 5' | ATATIATCICGAACTCICITT 3' |
| | 3' | ACTAGCGCTTGAGCGCAATAT 5' |
| AGC | 5' | ATATIATCIAGCACTCICITT 3' |
| | 3' | ACTAGCTCGTGAGCGCAATAT 5' |
| CGC | 5' | ATATIATCICGCACTCICITT 3' |
| | 3' | ACTAGCGCGTGAGCGCAATAT 5' |
| TGC | 5' | ATATIATCITGCACTCICITT 3' |
| | 3' | ACTAGCACGTGAGCGCAATAT 5' |

[a] Duplexes were designed to contain a unique P[1, 4]B binding site covering the range of hierarchy of drug-bonding specificities.

dithiothreitol, and 50 μg/mL bovine serum albumin. DNase I was dissolved in HPLC water at a concentration of 100 μg/mL. DNase I solution (1 μL) was added to each oligomer sample, and digestion was carried out for 1 min at room temperature. The digestion was quenched by the addition of an equal volume of stop dye solution [90% (v/v) deionized formamide, 10% (w/v) sodium lauryl sulfate, 10 mM EDTA, 0.25% (w/v) xylene cyanol FF, and 0.25% (w/v) bromophenol blue]. Samples were then subjected to sequencing gel electrophoresis.

(*B*) *Hydroxyl Radical Cleavage.* Antibiotic-modified and unmodified oligomers were suspended in 20 μL of 10 mM Tris-HCl (pH 7.6). An $Fe^{II}EDTA$ complex was prepared by mixing equal volumes of 2 mM $(NH_4)_2FeSO_4$ and 4 mM EDTA just prior to use. The Fe(II) solution (2 μL), 2 μL of 3% (w/v) $H_2O_2$, and 2 μL of 10 mM sodium ascorbate were mixed on the inside wall of the reaction tube as one drop. The drop was mixed with the oligomer solution, and the cleavage reaction was carried out at room temperature for 10 min. The reaction was quenched by the addition of 2 μL of 0.1 M thiourea. The oligonucleotides were then isolated by ethanol precipitation (2×). The DNA pellet was washed with ethanol and dried in vacuo. The pellet was resuspended in sequencing gel loading buffer and subjected to sequencing gel electrophoresis.

(*C*) *1,10-Phenanthroline–Copper Cleavage.* Antibiotic-modified and unmodified oligomers were suspended in 20 μL of 10 mM Tris-HCl (pH 7.6). The cleavage reaction was carried out at room temperature for 2 min in the presence of 50 μM $CuSO_4$, 200 μL of 1,10-phenanthroline, and 5 mM 2-mercaptopropionic acid. The cleavage reaction was quenched by the addition of neocuproin to give a final concentration of 6 mM. Digested oligomers were isolated by ethanol precipitation and dried in vacuo. The oligomers were resuspended in 100 μL of freshly prepared 1 M piperidine solution and heated to 90 °C for 30 min to transform metastable intermediary products to oligonucleotides with 3′-phospho monoester termini. The oligonucleotides were then isolated by ethanol precipitation and dried in vacuo. The DNA pellets were resuspended in sequencing gel loading buffer and subjected to sequencing gel electrophoresis.

## RESULTS

The purpose of this study was to determine whether any correlation existed between the DNA sequence selectivity of

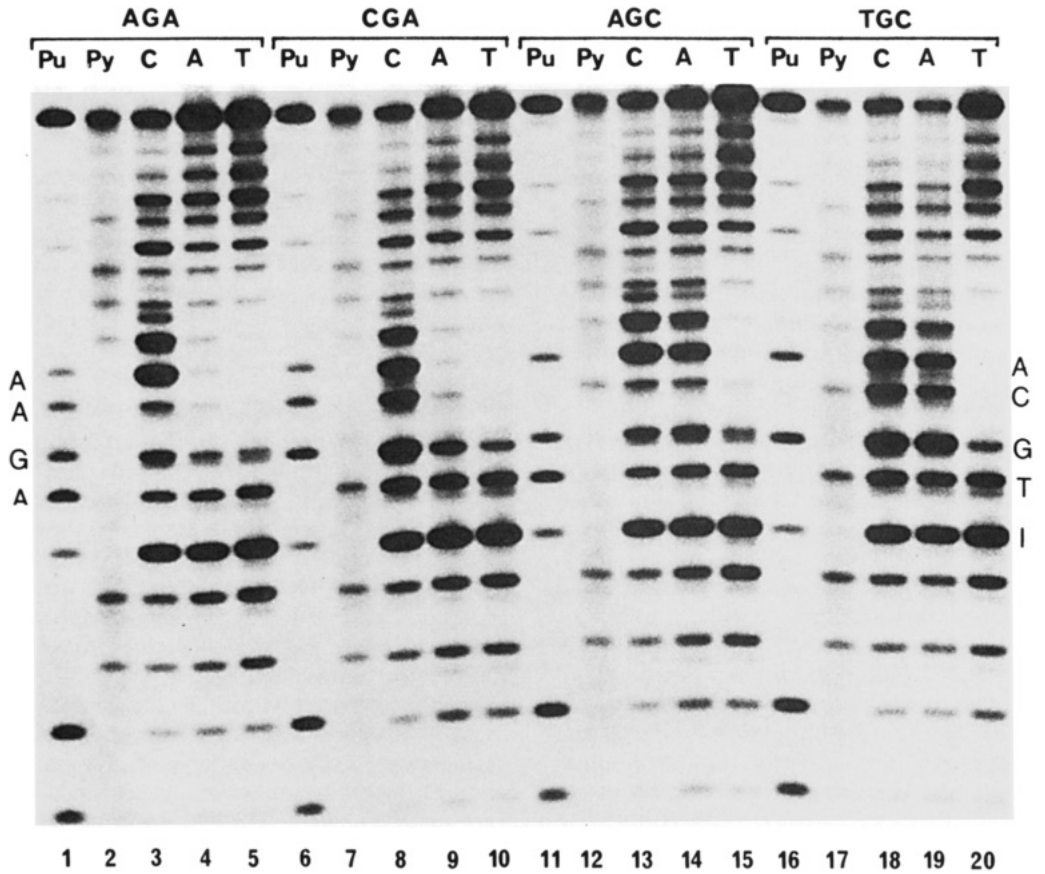



FIGURE 3: Denaturing gel analysis of anthramycin (lanes 4, 9, 14, and 19) and tomaymycin (lanes 5, 10, 15, and 20) modified oligomers and their corresponding unmodified DNA (lanes 3, 8, 13, and 18) following 1,10-phenanthroline–copper(I) footprinting. The sequences of AGA, CGA, AGC, and TGC at the top of the gel correspond to those listed in Table I. DNA was 5′-end-labeled with $^{32}P$ on the top strand only. Pu (lanes 1, 6, 11, and 16) and Py (lanes 2, 7, 12, and 17) represent the Maxam–Gilbert purine- and pyrimidine-specific cleavage reactions.

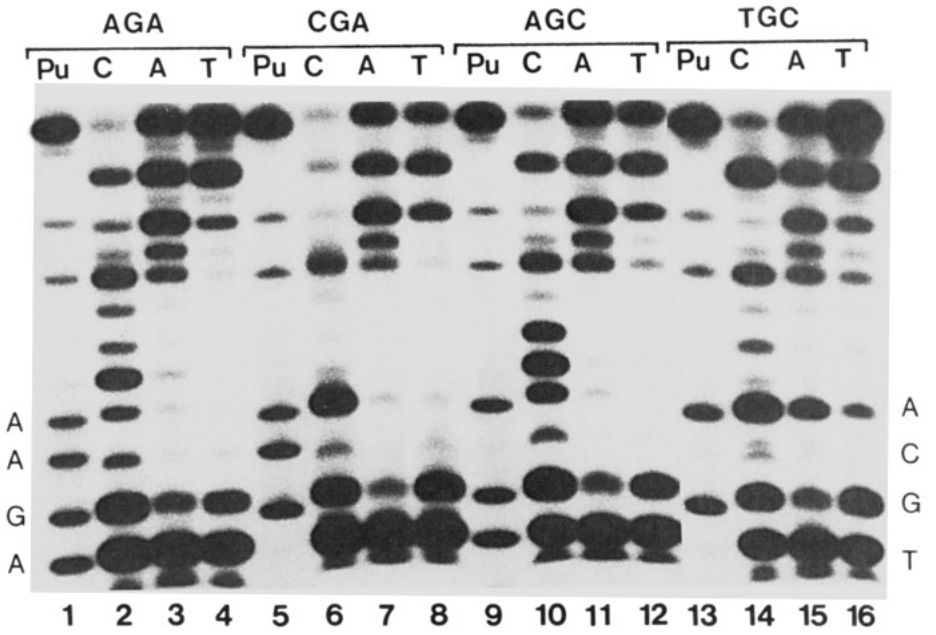



FIGURE 4: Denaturing gel analysis of anthramycin (lanes 3, 7, 11, and 15) and tomaymycin (lanes 4, 8, 12, and 16) and their corresponding unmodified DNA (lanes 2, 6, 10, and 14) following DNase I footprinting. DNA was 5′-end-labeled with $^{32}P$ on the top strand only. Pu represents the Maxam–Gilbert purine-specific cleavage reaction. The sequences AGA, CGA, AGC, and TGC at the top of the gel correspond to those listed in Table I.

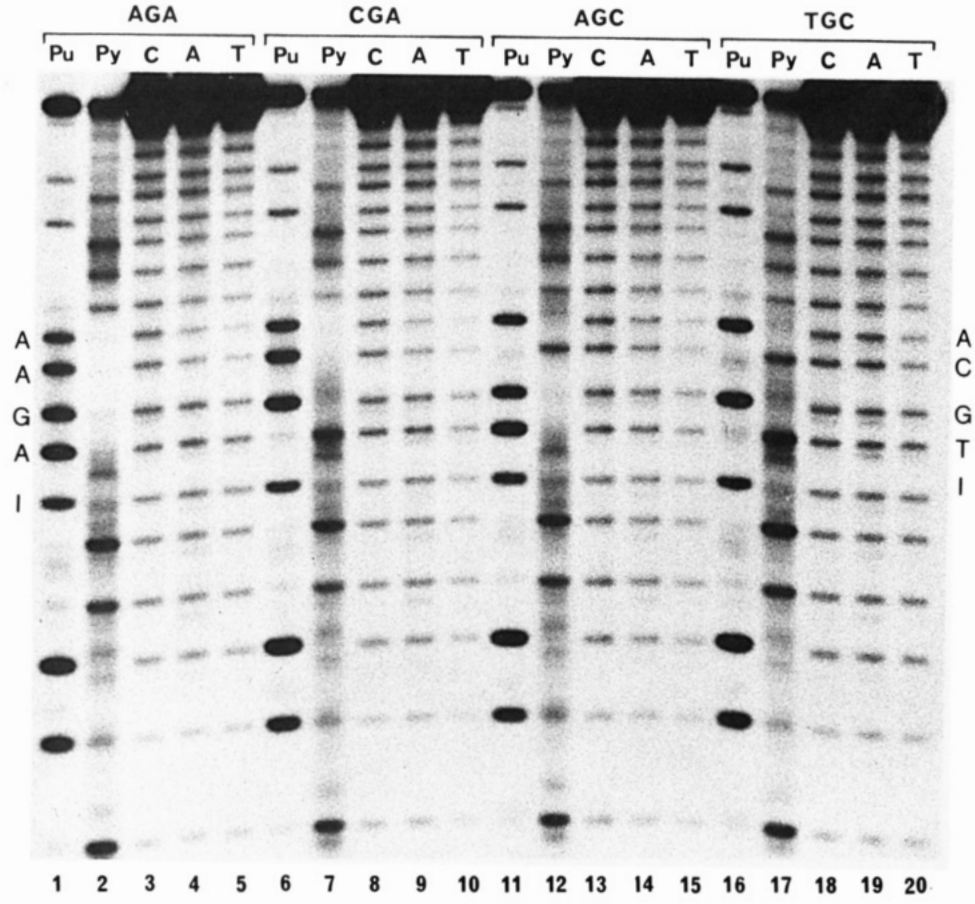
anthraymcin and tomaymycin, the bending of DNA, and the kinetics of anthramycin–DNA adduct formation. The five DNA bonding sequences (Table I) used in this study were chosen in order to cover the range of hierarchy of bonding specificities. Previous chemical and enzymatic footprinting studies on long DNA restriction fragments revealed that the most preferred sequence for P[1,4]B bonding is 5′-PuGPu, while 5′-PyGPy sites are the least preferred sequences (Hertzberg et al., 1986; Hurley et al., 1988). Sequences containing 5′-PyGPu and 5′-PuGPy show intermediate bonding preferences. The first step in our study consisted of an analysis, by three different DNA footprinting methods, of drug–DNA adducts prepared with tomaymycin or anthramycin. First, Op-Cu footprinting was used to determine the *location* and *site size* of the drug on the DNA (Sigman, 1985, 1986, 1990). Second, DNase I footprinting was used to probe the drug *bonding efficiency* and to address questions of *conformation* and *flexibility* of the drug-modified DNA (Lane et al., 1983; Fox & Howarth, 1985). Third, hydroxyl radical footprinting was carried out to examine the minor groove *structure* and *accessibility* (Shafer & Tullius, 1989). Our next step consisted of experiments to examine the electrophoretic mobility of ligated drug-modified 21-bp oligomers to determine whether anthramycin and tomaymycin produced bending of DNA. Finally, an analytical HPLC time course experiment was developed to follow the kinetics of anthramycin–DNA adduct formation. Overall, the results imply that there is a correlation between the sequence selectivity, the drug-induced DNA bending, and the reaction kinetics; that is, for each drug species, the DNA sequences that reacted more rapidly with the P[1,4]B drugs were found to have greater DNA bending and are known to have greater bonding specificity.

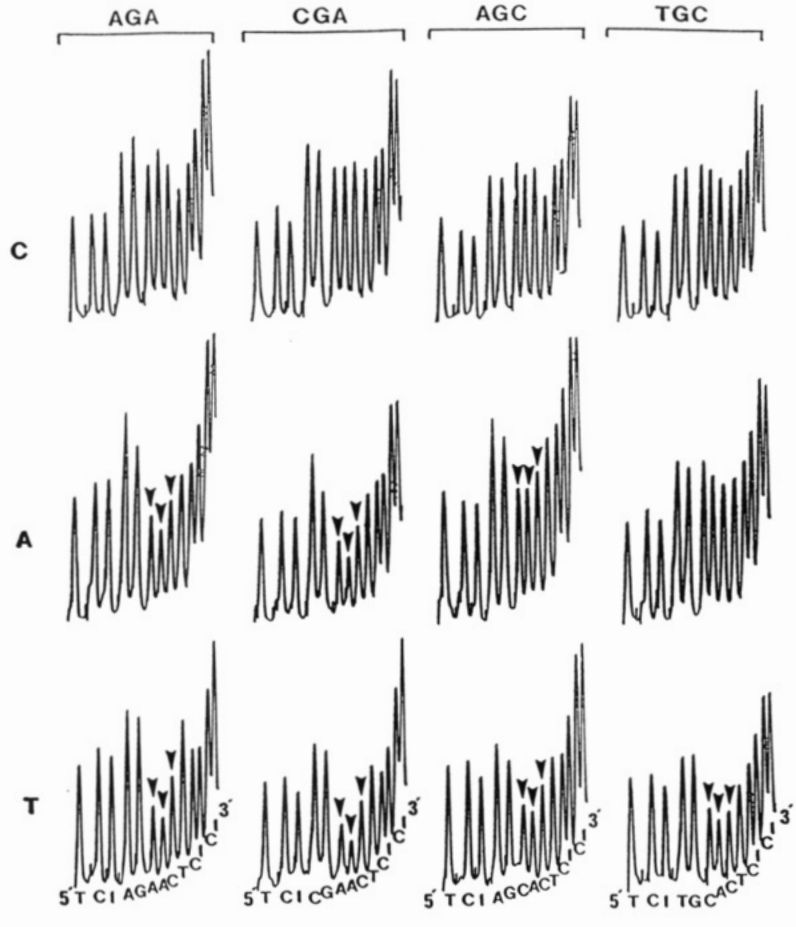
*1,10-Phenanthroline–Copper Footprinting of Drug–DNA Adducts.* The bonding location and size of the anthramycin and tomaymycin footprints on different oligomer sequences (Table I) were analyzed on a 20% sequencing gel by an Op-Cu footprinting assay. The Op-Cu complex binds within the minor groove of DNA and cleaves at its binding site (Sigman, 1986; Yoon et al., 1990). Figure 3 shows the Op-Cu cleavage pattern for four of the 21-bp oligomers modified with anthramycin or tomaymycin. The figure shows a denaturing electrophoresis gel of the footprinting pattern of the DNA strand that is covalently modified with either tomaymycin or anthramycin. The complementary DNA strands that are not involved in covalent interactions with the drugs were also analyzed (data not shown). The analysis of the results of the tomaymycin–DNA adducts for all four oligomer sequences reveals a strong four-nucleotide footprint on the covalently modified strand staggered to the 3′ side of the covalently modified guanine, presumably due to drug occupancy of the minor groove in a right-handed DNA molecule (Sigman, 1986). On the opposite strand of DNA, a similarly positioned 3′ offset footprint of five nucleotides is generated with tomaymycin using Op-Cu footprinting (data not shown). A summary of these results is shown in Figure 6.

The results are more complex for anthramycin than tomaymycin. While a strong four-nucleotide footprint is generated on the covalently modified strand of the 5′-AGA and 5′-CGA sequences (Figure 3) that appears similar to the tomaymycin–DNA adduct, footprinting is not observed on the 5′-AGC or 5′-TGC sequence oligomers. The footprinting reactions on the noncovalently modified strand produced a weak five-nucleotide footprint on the 5′-AGA and 5′-CGA sequence oligomers, while footprints with 5′-AGC and 5′-TGC sequence oligomers were absent. We suspect that the absence of a footprint or the presence of only a weak footprint on the covalent and noncovalent DNA strands is probably due to the Op-Cu destabilization of the duplex, leading to loss of anthramycin from the DNA in these generally poorer bonding sequences.[2]

*DNase I Footprinting of the Anthramycin and Tomaymycin Oligomer Adducts.* The degree of anthramycin and tomaymycin bonding on different 21-bp oligomer sequences (Table I) was analyzed on a 20% sequencing gel by a DNase I

[2] Denaturation of duplex DNA leads to loss of the secondary stabilizing interactions between P[1,4]B and DNA and exposure of the readily reversible aminal linkage and subsequent reversal of the covalent adduct formation (Hurley et al., 1977).


AGA
CGA
AGC
TGC
Pu Py C A T Pu Py C A T Pu Py C A T Pu Py C A T
A A G A I
A C G T I
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20


AGA
CGA
AGC
TGC
C
A
T
5' T C I AGAACTCCI 3'
5' T C I CGAACTCCI 3'
5' T C I AGCACTCCI 3'
5' T C I TGCACTCCI 3'

footprinting assay. The results of the DNase I digestion of the drug–DNA adducts and unmodified DNA controls are shown in Figure 4. While the DNase I digestion of unmodified DNA (lanes 2, 6, 10, and 14) produced an uneven cleavage ladder due to sequence-dependent conformational variability, the DNA samples covalently modified with drug produced the same pattern, except within and around the expected drug-bonding sites. The anthramycin and tomaymycin footprints shown for the 5′-AGA, 5′-CGA, and 5′-AGC sequence oligomers showed an almost total inhibition of DNase I cleavage, demonstrating virtually complete drug bonding. Although the 5′-TGC sequence oligomer shows a footprint at the drug-bonding site, there is only partial inhibition of DNase I cleavage at this site, possibly due to some instability of this adduct toward DNase I cleavage. As also shown in the Cu-Op experiments, both anthramycin and tomaymycin bond to the sole guanine in the 21-bp oligomer sequences. A five-nucleotide footprint produced by anthramycin begins at the adjacent base on the 3′ side of the modified guanine. There is an enhancement of cleavage by DNase I at the two bases to the 3′ side of this footprint. A seven-nucleotide footprint is generated with tomaymycin, which also exhibits an enhancement of cleavage to the 3′ side. For tomaymycin, this seven-nucleotide footprint, rather than the five-nucleotide footprint found for anthramycin, may suggest that tomaymycin has a longer range effect on DNA conformation than anthramycin. These results are also summarized in Figure 6.

*Hydroxyl Radical Footprinting of the Anthramycin and Tomaymycin Oligomer Adducts.* Hydroxyl radical footprinting was used in the study of adducts of anthramycin or tomaymycin with the same 21-bp oligomers. This method was chosen in addition to the Op-Cu and DNase I footprinting because it has produced high-resolution structural data on a number of different DNA molecules, DNA–protein complexes, and DNA–drug adducts (Tullius et al., 1987; Tullius, 1987; Portugal & Waring, 1987, Cons & Fox, 1989a,b; Fox, 1988). Hydroxyl radical, generated by the reduction of hydrogen peroxide by $Fe^{II}EDTA$ is an attractive conformational probe due to small size and high reactivity. The hydroxyl radical abstracts a hydrogen atom, most likely from C4′ of the deoxyribose in the minor groove, leading to DNA strand scission at the point of the attack. With complexes of drugs with DNA, this small, nonspecific, reactive hydroxyl radical probe can yield information on the physical contacts made between drug and DNA molecules and also on variations in the minor groove width.

The sequencing electrophoresis gels of the hydroxyl radical footprinting of the 5′-AGA, 5′-CGA, 5′-AGC, and 5′-TGC 21-bp oligomer sequences modified with tomaymycin or anthramycin are shown in Figure 5A. Both the anthramycin–DNA and tomaymycin–DNA adducts showed diminished cleavage to the 3′ side of the drug-bonding region. Densitometer scans of the footprinting gel lanes provide a more obvious representation of the change in the cleavage pattern of DNA following drug modification of DNA (Figure 5B). In all of the scans of tomaymycin and anthramycin oligomer DNA adducts except one (anthramycin with 5′-TGC), the three bases to the 3′ side of the covalently modified guanine were less reactive to hydroxyl radical (arrows in Figure 5B), while at the covalently modified guanine and to the 5′ side, dramatic changes were not observed. On the noncovalently modified strand, inhibition of hydroxyl radical cleavage was also found, which most often included the cytosine opposite the covalently modified guanine and the two bases to its 3′ side (results not shown, but summarized in Figure 6). The extent of inhibition of hydroxyl radical was quite variable, dependent upon both the drug and the covalently modified sequence. In general, tomaymycin produced a greater inhibition than anthramycin, and for anthramycin, strong inhibition was only found for 5′-AGA and 5′-CGA sequences. Since drug occupancy is almost complete for all of the drug-modified sequences, and cleavage results from attack at the 4′ and 5′ positions of the deoxyriboses, we believe that this uneven pattern of inhibition of hydroxyl radical is largely due to changes in minor groove geometry (i.e., narrowing of groove width). The hydroxyl radical footprinting results for both the covalently and noncovalently modified strands are summarized in Figure 6.

*Determination of Anthramycin- and Tomaymycin-Induced Bending of DNA.* The same four 21-bp DNA sequences used in the footprinting experiments and a fifth, containing the least preferred bonding sequence, 5′-CGC (Table I), were designed in part so that they would be useful for studies to determine whether anthramycin and tomaymycin were capable of bending DNA. The purpose of this aspect of the study was to investigate whether or not any correlation existed between the drug bonding preference and drug-induced conformational changes, such as bending of DNA. In order to study the relationship between DNA sequence preference and DNA bending, the electrophoretic mobility of DNA was monitored (Koo et al., 1986; Koo & Crothers, 1988). The five different 21-bp oligomers were fully modified with anthramycin or tomaymycin and ligated with T4 ligase into multimers, and the electrophoretic mobility was analyzed on 6% nondenaturing gel.

The autoradiogram of the ligation products of unmodified (C) and drug-modified (A and T) oligomers is shown in Figure 7A. Within the set of controls (lanes 1, 4, 7, and 10) there was some variability in electrophoretic mobility, such that 5′-AGA and 5′-CGA sequences show retardation relative to the 5′-AGC, 5′-TGC, and 5′-CGC sequences, suggesting that the former two sequences show some intrinsic bending. While some drug-modified ligation products show considerable retardation in electrophoretic mobility (see lanes 3, 6, 9, and 12) when compared to the unmodified controls, other ligation products of drug–DNA adducts show little or no retardation (see lanes 2, 5, 8, and 11).

The sharp, sometimes more intense band or bands that appear as doublets at the higher molecular weight end of the gel in lanes 3, 6, 9, and 12 (tomaymycin-modified) correspond to circular DNA molecules. This was confirmed by two-dimensional gel electrophoresis (data not shown). For tomaymycin-modified sequences (lanes 3, 6, 9, 12, and 15), a band occurs corresponding to *circular* DNA (C* in Figure 7A) that has a lower molecular weight than other circular

FIGURE 5: (A, top) Denaturing gel analysis of anthramycin (lanes 4, 9, 14, and 19) and tomaymycin (lanes 5, 10, 15, and 20) modified oligomers and their corresponding unmodified DNA (lanes 3, 8, 13, and 18). DNA was 5′-$^{32}$P-labeled on the top strand only and subjected to hydroxyl radical footprinting. Pu and Py represent the Maxam–Gilbert purine (lanes 1, 6, 11, and 16) and pyrimidine (lanes 2, 7, 12, and 17) specific cleavage reactions, respectively. The sequences AGA, CGA, AGC, and TGC at the top of the gel correspond to those listed in Table I. (B, bottom) Densitometer scans of hydroxyl radical footprinting of the AGA, CGA, AGC, and TGC oligomers shown in A. Rows C, A, and T represent unmodified oligomers, oligomers modified with anthramycin, and oligomers modified with tomaymycin, respectively. Arrowheads indicate inhibition of the hydroxyl radical cleavage reaction.

molecules (C in Figure 7A) occurring in either control or anthramycin-modified lanes. This is entirely consistent with the more pronounced bending induced by tomaymycin relative to anthramycin. In addition, this band of circular DNA (C*) and the band corresponding to the next higher molecular weight circular DNA in the AGA sequence lane are in large excess over bands of higher molecular weight circular DNA, which is also consistent with the more pronounced bending in this sequence relative to the other sequences.

For anthramycin, the evidence for bending based upon circularization efficiency is much weaker, which is also in accord with the lower, or absence of, bending calculated from $R_L$ values (see below). The ratio of apparent size to true size ($R_L$) for each of the linear ligation products was calculated and plotted against oligomer length in base pairs (Figure 7B).[3] Unmodified DNA by definition has an $R_L$ value of 1. Increasing changes in the $R_L$ value are an indication of drug-induced bending of DNA. The 5′-AGC, 5′-CGC, and 5′-TGC oligomers modified with anthramycin have $R_L$ values similar to those for the same sequence control DNA. Retardation in electrophoretic mobility was observed for 5′-AGA and 5′-CGA oligomers modified with anthramycin and for all oligomers modified with tomaymycin, indicating drug-induced DNA bending. These oligomers showed an increasing change in the $R_L$ value with the total length of ligation products. The increases in the $R_L$ values are much more pronounced in the tomaymycin–DNA than in the anthramycin–DNA adducts. As with DNase I and hydroxyl radical footprinting, the overall magnitude of the effects, in this case on $R_L$ values, is more pronounced with tomaymycin-modified DNA than with anthramycin-modified DNA. The estimated bending angles for tomaymycin- and anthramycin-modified oligomers are shown in Table II. The bending angles were calculated for each of the five sequences modified with anthramycin or tomaymycin by using a calibration equation for gel mobility anomalies derived by Koo and Crothers (1988). The values varied from a maximum of ~14° for tomaymycin in 5′-AGA and 5′-CGC to a minimum of 0° for anthramycin in 5′-AGC, 5′-CGC, and 5′-TGC.

*Kinetic Analysis of DNA–Drug Adduct Formation.* The five 21-mer oligomer sequences containing the bonding sequences, 5′-AGA, 5′-CGA, 5′-AGC, 5′-CGC, and 5′-TCG, used in this study were incubated with anthramycin, and the time course of the reaction mixture was studied by HPLC. An HPLC system was developed to separate unreacted anthramycin from free DNA and drug–DNA adducts (see Materials and Methods). The relative quantity of unbound drug was then measured by UV absorption at 333 nm. Figure 8A is the HPLC trace of anthramycin alone, showing a retention time of ~5 min. Figure 8B shows DNA (in this case, the 5′-TGC oligomer) alone with a retention time of ~2 min. The small peak at 1.2 min is probably a small amount of single-stranded DNA or an unknown contaminant. Examination of these two plots (Figures 8A,B) shows that anthramycin is well-separated from DNA on the HPLC column. Clean separation of each species is crucial, since the peak height is used to measure the absorbance and to calculate the concentration of the species involved. Figure 8C shows the entire reaction mixture. Briefly, DNA was mixed with an equal volume of anthramycin and injected onto the HPLC, and the retention time was monitored. The chromatograms show peaks at ~5 and ~2 min, representing free drug and unbound DNA, respectively. The large peak that eluted off the column (1.3 min) just prior to the unmodified DNA peak (~2 min) represents the drug–DNA adduct.

Table II: Calculation of DNA Bending Angles Produced by Anthramycin and Tomaymycin in the 21-bp Oligomers Listed in Table I

| oligomer sequence | drug[a] | $R_L$ value[b] | bending angle[c] (deg) |
|---|---|---|---|
| 5′-AGA | A | 1.10 | 6.3–8.4 |
| 5′-CGA | A | 1.08 | 5.6–7.4 |
| 5′-AGC | A | 1.00 | 0 |
| 5′-CGC | A | 1.00 | 0 |
| 5′-TGC | A | 1.00 | 0 |
| 5′-AGA | T | 1.28 | 10.5–14.0 |
| 5′-CGA | T | 1.30 | 10.9–14.4 |
| 5′-AGC | T | 1.27 | 10.3–13.6 |
| 5′-CGC | T | 1.25 | 10.0–13.23 |
| 5′-TGC | T | 1.17 | 8.25–10.9 |

[a] A and T represent anthramycin and tomaymycin, respectively. [b] $R_L$ values were calculated from 189-bp ligation products (nonamers of 21-bp oligomers). [c] The estimate of bending angles of each sequence was calculated using a calibration equation for gel mobility anomalies derived by Koo and Crothers (1988).

The HPLC analysis of drug–DNA adduct formation was extended to all five oligomer sequences (Table I) used in this study. A time course study was conducted to determine the rate constants for these reactions. The plot shown in Figure 9 shows the ln of the ratio of unbound anthramycin to total anthramycin vs time. This plot shows that the 5′-AGA sequence reacts faster with anthramycin relative to the other sequences. The 5′-CGA and 5′-AGC oligomer sequences have intermediate reaction rates, and the 5′-CGC and 5′-TGC sequences react more slowly with the drug. The rate constants for anthramycin monitored here correlate well with the relative sequence selectivity of different sequences (i.e., 5′-PuGPu > 5′-PuGpy = 5′-PyGPu > 5′-PyGPy) monitored in previous studies (Hertzberg et al., 1986; Hurley et al., 1988).

The reaction involved DNA and drug to form drug–DNA adducts, where the concentration of DNA was in vast excess and assumed to remain unchanged over the course of the reaction. The initial concentrations for DNA and anthramycin were ~50 and ~5 μM, respectively, for each DNA sequence investigated. The DNA and drug react at equal stoichiometric amounts, i.e, one drug-bonding site per DNA oligomer molecule. Due to the excess of DNA, a first-order reaction was presumed, and the slopes of the lines in Figure 9 were used to calculate the rate constants. The resulting rate constants for each DNA sequence reacting with anthramycin are shown in Table III. The rate constants varied from $1.12 \times 10^{-1}$ min$^{-1}$ for 5′-AGA to $5.05 \times 10^{-3}$ min$^{-1}$ for 5′-CGC.

## DISCUSSION

The P[1,4]B class of drugs consists of highly potent cytotoxic agents that have significant efficacy as antitumor agents in animal model studies [reviewed in Remers (1988), Thurston and Hurley (1983), and Hurley (1977)], although their clinical use has been limited by the various toxicities they produce (Hurley, 1977). On the basis of a fairly detailed knowledge of the structures of the P[1,4]B–DNA adducts, a template-directed approach to the design of novel interstrand cross-linking molecules has been successfully executed (Wang et al., 1992; Bose et al., 1992). The P[1,4]B antitumor antibiotics not only show a selectivity for covalent bonding through the N2 of guanine in DNA (Petrusek et al., 1981) but also exhibit a modest sequence selectivity despite their small molecular weights. Upon bonding to DNA, the drugs lie within the minor groove overlapping with 2–3 bp (Petrusek et al., 1981; Boyd et al., 1990a,b). DNA footprinting studies using MPE (Hertzberg et al., 1986) and an exonuclease III stop assay (Hurley et al., 1988) have shown that anthramycin and

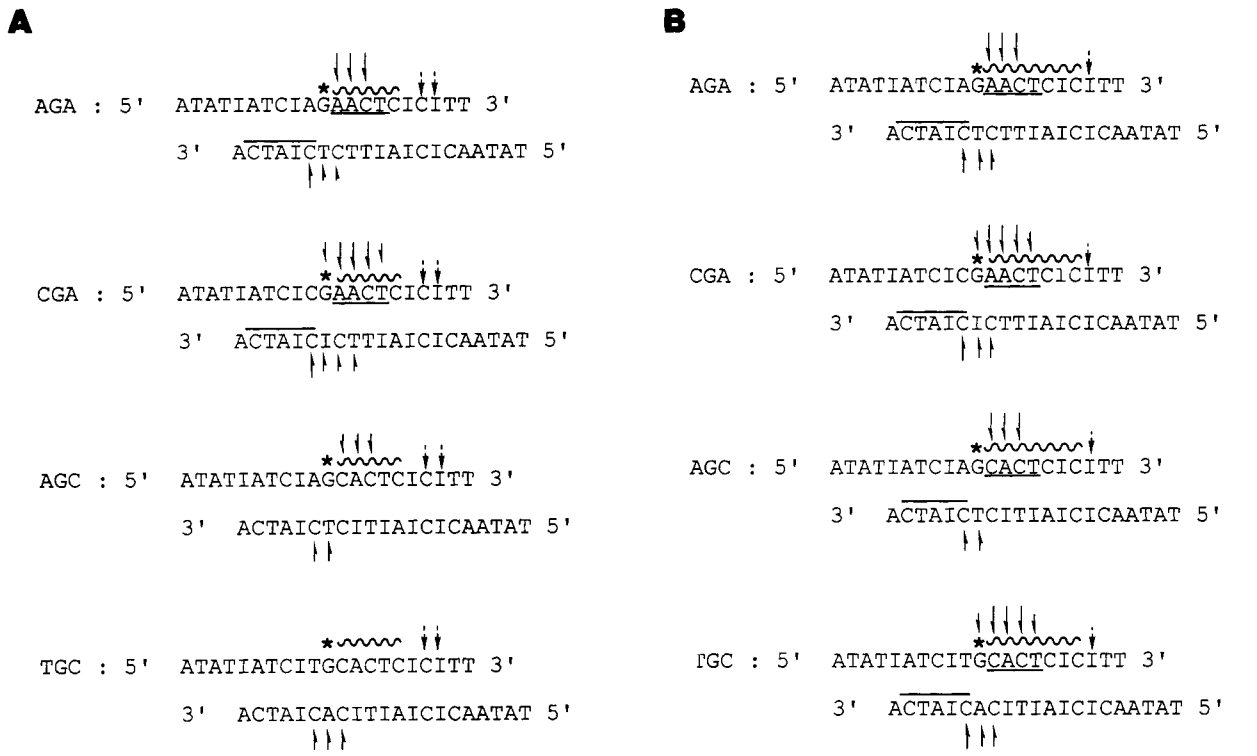



FIGURE 6: Summary of the results of 1,10-phenanthroline–copper footprinting, DNase I footprinting, and hydroxyl radical cleavage pattern of 21-bp oligomers (Table I) modified with anthramycin (A) and tomaymycin (B): asterisk, covalently modified guanine; straight line, inhibition of Op-Cu; wavy line, inhibition of DNase I; arrow, enhancement of DNase I; half-arrow, inhibition of hydroxyl radical. The length of the arrow represents the relative amount of cleavage inhibition.

tomaymycin prefer to bond to 5′-PuGPu sequences, while 5′-PyGPu and 5′-PuGPy sequences are of intermediate preference, and the 5′-PyGPy sequences are the least preferred. The molecular basis for this sequence selectivity is still unclear, and while a variety of molecular modeling studies have been carried out (Rao & Remers, 1990; Rao et al., 1986; Zakrzewska & Pullman, 1986), no firm conclusions have been made, although the Pullman study suggested that DNA flexibility was important. The present study was initiated to address the question of the molecular basis for DNA sequence selectivity of the P[1,4]B's and, in particular, to probe the importance of conformational flexibility. In addition to conformational flexibility, a variety of mechanisms for sequence recognition by covalently modifying drugs, such as catalytic activation and precovalent binding interactions, have been identified [for a review, see Warpehoski and Hurley (1988)].

An additional degree of complexity in the analysis of the P[1,4]B–DNA adduct structure, in comparison to most covalently modifying DNA-reactive compounds, is the possibility of the formation of four different species based upon the stereochemistry at the covalent linkage site (11*R* or 11*S*) and the orientation within the minor groove (aromatic ring to the 3′ or 5′ side of the covalently modified guanine; Barkley et al., 1986). Generally, footprinting experiments are blind to these structural variations unless the drug overlap in the minor groove is biased to one side of the covalent adduct site (Hurley et al., 1984) or there is a distortion of DNA associated with a particular species of adduct that is detectable by chemical or enzymatic cleavage patterns.

On the other hand, high-field NMR studies on defined P[1,4]B–DNA adducts provide definitive information on both the multiplicity (one or more species) and types of species (e.g., 11*S*,3′) bound to DNA [Boyd et al., 1990a,b; Wang et al., 1992; Cheatham et al., 1988; Barkley et al., 1986; reviewed in Remers et al., (1992)], and in the case of tomaymycin, fluorescence is also a useful tool (Barkley et al., 1986; Cheatham et al., 1988; Boyd et al., 1990b). For tomaymycin on calf thymus DNA, fluorescence experiments have shown that there is a 65:35 mixture of 11*S* and 11*R* species and on a defined sequence oligomer either a 50:50 mixture (5′-TGC) of two species or 100% of one species (5′-CGA or 5′-AGC). For anthramycin, while just a *single* species has been detected by high-field NMR on all defined adducts so far examined, the stereochemistry at C-11 is variable and is dependent upon the bonding sequence (J. A. Mountzouris and L. H. Hurley, unpublished results). While this complexity, particularly in regard to tomaymycin, makes direct correlation with structurally defined lesions more elusive, it does not prevent correlations being made between sequence selectivity and bending/distortion of DNA. What is potentially problematic is using molecular modeling to make predictions of sequence preference on specific sequences with the P[1,4]B's where the stereochemistry and orientation of the adducts are as yet undefined. In the case of molecular modeling, where conformational flexibility was predicted to be important (Zakrzewska & Pullman, 1986), a single 11*S*,3′ species was assumed for all species of anthramycin–DNA adduct, irrespective of bonding sequence. While this assumption is apparently true for 5′-CGT, it does not appear to be true for other bonding sequences, such as 5′-CGA or 5′-AGA (J. A. Mountzouris and L. H. Hurley, unpublished results).

Nevertheless, while bearing in mind the potential pitfalls of theoretically based molecular modeling carried out in the absence of definitive experimental data on the identity of the

[3] Since the control lanes for 5′-AGA and 5′-CGA indicated an intrinsic bent DNA structure, the $R_L$ values for all drug-modified sequences were calculated relative to the control lanes for 5′-TGC. If this correction is not carried out, the overall bending magnitude in the final drug-modified duplex is underestimated.

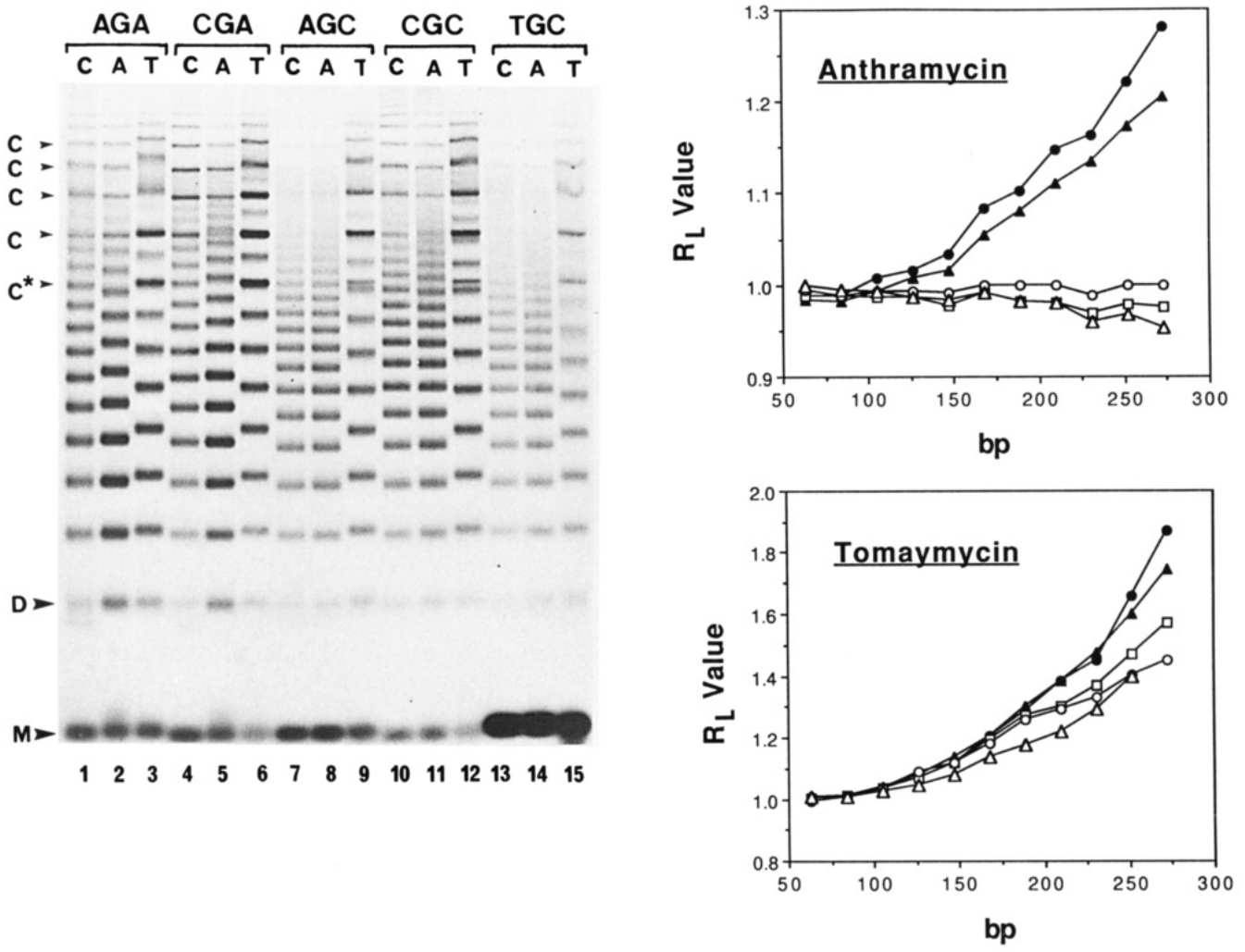



FIGURE 7: (A, left) Electrophoretic behavior of the ligation products of AGA, CGA, AGC, CGC, and TGC oligomers (Table I) modified with anthramycin and tomaymycin on a 6% nondenaturing polyacrylamide gel. Lanes labeled C represent ligated multimers of control oligomers (lanes 1, 4, 7, 10, and 13). Lanes labeled A and T represent oligomers modified with anthramycin (lanes 2, 5, 8, 11, and 14) and tomaymycin (lanes 3, 6, 9, 12, and 15), respectively. M and D represent the positions of monomers (21-bp oligomer) and ligated dimers (42 bp), respectively, on the gel. C* and C refer to circular DNA (see text). (B, right) Plot of $R_L$ values as a function of ligated oligomer length in base pairs for the ligation products of AGA (●), CGA (▲), AGC (□), CGC (○), and TGC (△) (Table I) modified with anthramycin and tomaymycin. $R_L$ = apparent vs actual length.

drug species bound to different sequences, the conclusion that DNA flexibility may be an important determinant in sequence selectivity appears to hold up in the present experimental study. The experimental results presented here suggest that the correlation between the sequence selectivity of anthramycin and tomaymycin with DNA and nucleic acid flexibility may, at least in part, be related to drug-induced DNA bending. The results also demonstrate that the hierarchy of sequence selectivity for anthramycin is correlated to the kinetics of the reaction. The information we have gained from these experiments extends our knowledge on the relationship between the effects of covalent bonding of the P[1,4]B's on DNA structures of different sequences, the reaction kinetics, and the sequence selectivity.

*Comparison of the Effects on DNA Structure of Covalent Modification of DNA by Tomaymycin and Anthramycin.* While the overall trends in the effects of anthramycin and tomaymycin on DNA structure are similar, i.e., 5′-PuGPu sequences are more bent and show greater inhibition of hydroxyl radical cleavage than 5′-PyGPu and 5′-PuGPy sequences, which themselves are subjected to more pronounced effects than 5′-PyGPy sequences, the magnitudes of the drug-induced effects on DNA structure are quite different for the two drugs. Tomaymycin produces much more bending of DNA (about 8.2–14.5°) than anthramycin (5.6–8.9°), the inhibition of hydroxyl radical cleavage is greater for tomaymycin, and the DNase I inhibition/enhancement zone is larger for tomaymycin (see Figure 6). This more pronounced effect of tomaymycin on DNA structure also correlates with a unique effect of tomaymycin–DNA adducts on the normally guanine-specific methylene blue reaction (Hertzberg et al., 1986) and perhaps the slower kinetics of bonding of tomaymycin to DNA (Hurley et al., 1977).

*Correlation of Sequence Specificity of Anthramycin and Tomaymycin with Drug-Induced Conformational Changes and Kinetics of Reaction of Anthramycin with DNA.* The most significant finding from this study is that the hierarchy of bonding sites for sequence specificity parallels the hierarchy for drug-induced bending magnitudes and the rates of reactivity of anthramycin with individual sequences. For anthramycin, theoretical calculations predicted that the preferred bonding sites would also be the most easily distorted (Zakrzewska & Pullman, 1986). If distortion is measured by the bending of DNA and inhibition of hydroxyl radical cleavage, then increased bending correlates with a greater sequence preference.[4] If we assume that the right-handed twist of the P[1,4]B's does not exactly match the twist of the minor groove and, as a consequence, the DNA has to distort

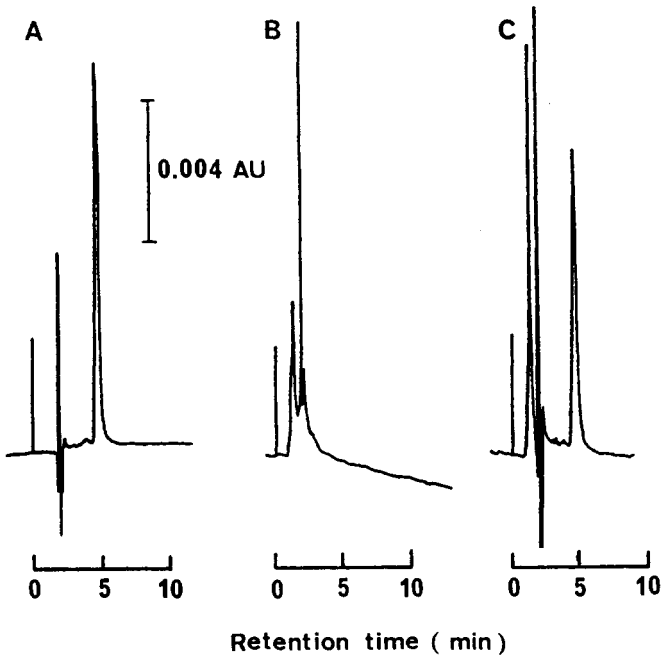



FIGURE 8: HPLC analysis of anthramycin–oligomer DNA complex formation. Chromatograms: (A) 5 μM anthramycin; (B) TGC oligomer solution (diluted 2-fold with annealing buffer); (C) reaction mixture of anthramycin and TGC oligomer. The reaction mixture was prepared by mixing equal volumes of 10 μM anthramycin solution with TGC oligomer solution. HPLC conditions: column, Supelco LC-18-DB (4.6 mm i.d. × 15 cm); column temperature, ambient; mobile phase, $H_2O$/MeOH (55:45); flow rate, 1 mL/min; detection, absorbency at 333 nm; injection volume, 20 μL.

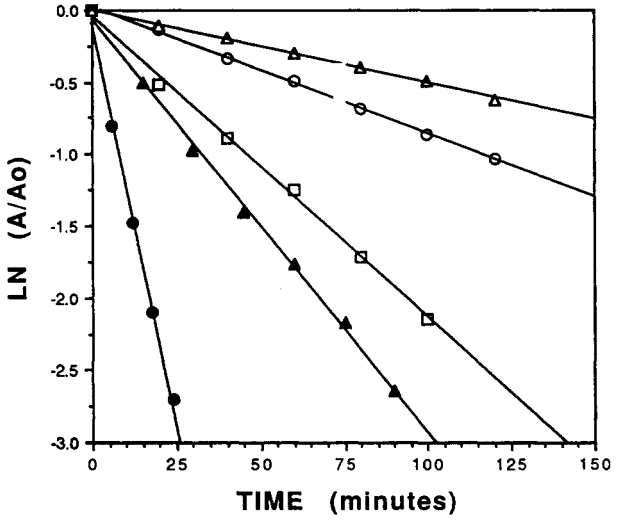



FIGURE 9: Semilogarithmic plot of free anthramycin vs time in the reaction mixture of anthramycin AGA (●), CGA (▲), AGC (□), CGC (○), and TGC (△) oligomer DNA (Table I). The reaction mixture was prepared by mixing equal volumes of 10 μM anthramycin solution and oligomer solution with an absorbency of 30 at 260 nm and was maintained at 23 °C.

slightly around the noncovalently bound drug to produce a stabilized transition state for the covalent reaction, then those sequences that are inherently more flexible and inherently bent (e.g., 5′-AGA and 5′-CGA) will react more rapidly and will probably result in a more distorted DNA structure. Conversely, less pliable sequences (e.g., 5′-TGC) will react slower, be more resistant to distortion, and result in a less distorted drug–DNA adduct structure. Whether the rate of covalent reaction at a particular sequence is affected by creating a favorable noncovalent binding site for the drug,

Table III: Rate Constants for the Binding of Anthramycin to 21-bp Oligomers

| sequence | $k$ (min$^{-1}$) | sequence | $k$ (min$^{-1}$) |
|---|---|---|---|
| AGA | $1.12 \times 10^{-1}$ | CGC | $8.77 \times 10^{-3}$ |
| CGA | $2.89 \times 10^{-2}$ | TGC | $5.05 \times 10^{-3}$ |
| AGC | $2.10 \times 10^{-2}$ | | |

thereby increasing its effective local concentration, or whether the deformation is necessary to bring the reactive atoms of the drug and the target nucleotide into bonding distance to stabilize the transition state is not clear from these studies. In many respects, this mechanism for sequence recognition has analogies to the proposed importance of conformational flexibility in the reaction of (+)-CC-1065 with DNA, where the sequences that can easily adopt a bent DNA structure similar to A-tracts are those that are favored for reaction with this drug (Hurley et al., 1988; Sun et al., 1993).

The variability in species of tomaymycin or anthramycin (11*R* vs 11*S*, 3′ or 5′ orientation) covalently bound to DNA, which is dependent upon both the drug and the bonding sequence, is a unique feature of these drug molecules. In solution, a particular enantiomeric form of the tomaymycin is associated with a specific conformational form (Barkley et al., 1986). However, on DNA the enantiomeric form is not the same one that is associated with the conformational form found in solution (Barkley et al., 1986; Boyd et al., 1990a,b). It is likely that the DNA itself is able to play a decisive role in determining the optimum conformational form for a particular enantiomer form of drug bound to DNA. The implication is that the difference in final binding energies between the different species of anthramycin and tomaymycin bound to DNA may not be very large, and thus drug and DNA distortion energies that are sequence-dependent may play an important role in the selection of the species found on DNA. What remains to be determined is the role, if any, of catalytic activation on DNA of a particular species and whether the kinetically favored forms are those finally observed on DNA. In the latter case, the facile reversibility of the aminal linkage (N–CH–N) makes this a potentially important factor.

In summary, what has emerged from this study is the importance of the flexibility of both the bonding sequence and the P[1,4]B and their relative shape complementarity in determining sequence selectivity. The suggestion by Zakrzewska and Pullman (1986) that inherent sequence flexibility is a determinant of sequence recognition initially inspired us to compare drug-induced distortion of DNA across sequences of different reactivities and between drugs with different reactivities. It is important to recognize that both the conformational flexibility of the bonding sequence *and* the drug are involved in an "induced fit" to stabilize the transition state for the covalent reaction. In this respect, the analogy of an enzyme–substrate interaction is appropriate. For anthramycin, in an optimum bonding sequence (5′-AGA), which has intrinsic bending and high inherent flexibility, an 11*R* stereochemistry and 3′ orientation are favored, but with

[4] It is important to note that, in the theoretical paper by Zakrzewska and Pullman (1984), in the final minimized structures the anthramycin–DNA adduct assumed a distortion globally corresponding to a B→A-like transition. Neither the high-field data (Boyd et al., 1990a; J. M. Mountzouris and L. H. Hurley, unpublished results) nor the bending mode reported here corresponds to this transition. In addition to the previously noted problem with assuming an 11*S*,3′ orientation for all of the adducts, this theoretical study fixed the conformation of the phosphodiester backbone and sugar residues. Both of these factors may have contributed to the discrepancy between theoretical and experimental results.

a less favored sequence (5′-CGT), the flexibility is less and, consequently, the 11*S*,3′ species is found. Likewise, with tomaymycin, in more preferred sequences, the 11*S*,3′ species is favored, while in less preferred sequences, a mixture of species is produced.

## CONCLUSIONS

We present here an experimental study of the interaction of DNA with the covalently bound ligands anthramycin and tomaymycin. The principal results are as follows: (1) Irrespective of bonding sequence, tomaymycin bends DNA upon adduct formation. Although not as dramatic, the anthramycin-induced DNA bending magnitudes mirror the sequence selectivity (PuGPu > PuGPy = PyGPu > PyGPy). (2) Time course analytical HPLC shows that, for anthramycin, the more preferred the bonding site, the more rapid the reaction rate. Thus, there is a correlation between the DNA sequence selectivity of anthramycin with the reaction kinetics and induced bending of DNA, and the conformational flexibility of the drug and DNA may appear to play an important role in sequence recognition.

## ACKNOWLEDGMENT

We thank Mr. Michael Davies for technical assistance and Mr. David M. Bishop for editorial assistance.

## REFERENCES

Barkley, M. D., Cheatham, S., Thurston, D. E., & Hurley L.H. (1986) *Biochemistry 25*, 3021–3031.
Bose, D. S., Thompson, A. S., Ching, J., Hartley, J. A., Berardini, M. D., Jenkins, T. C., Neidle, S., Hurley, L. H., & Thurston, D. E. (1992) *J. Am. Chem. Soc. 114*, 4939–4941.
Boyd, F. L., Cheatham, S. F., Remers, W. A., Hill, G. C., & Hurley, L. H. (1990a) *J. Am. Chem. Soc. 112*, 3279–3289.
Boyd, F. L., Stewart, D., Remers, W. A., Barkley, M. D., & Hurley, L. H. (1990b) *Biochemistry 29*, 2387–2403.
Chetham, S., Kook, A., Hurley, L. H., Barkley, M. D., & Remers, W. (1988) *J. Med. Chem. 31*, 583–589.
Cons, B. M. G., & Fox, K. R. (1989a) *Biochem. Biophys. Res. Commun. 160*, 517–524.
Cons, B. M. G., & Fox, K. R. (1989b) *Nucleic Acids Res. 17*, 5447–5459.
Dougherty, G., & Pilbrow, J. R. (1984) *Int. J. Biochem. 16* (12), 1171–1192.
Fox, K. R. (1988) *Anti-Cancer Drug Des. 3*, 157–158.
Fox, K. R., & Waring, M. J. (1984) *Nucleic Acids Res. 12*, 9271–9285.
Fox, K. R., & Howarth, N. R. (1985) *Nucleic Acids Res. 13*, 8695–8714.
Gait, M. J., Ed. (1984) *Oligonucleotide Synthesis–A Practical Approach*, IRL, Oxford, England.
Hertzberg, R. P., Hecht, S. M., Reynolds, V. L., Molineux, I. J., & Hurley, L. H. (1986) *Biochemistry 25*, 1249–1258.
Hurley, L. H. (1977) *J. Antibiot. 30*, 349–370.
Hurley, L. H., & Petrusek, R. (1979) *Nature 282*, 529–531.
Hurley, L. H., & Needham-VanDevanter, D. R. (1986) *Acc. Chem. Res. 19*, 239–237.
Hurley, L. H., Reynolds, V. L., Swenson, D. H., & Scahill, T. (1984) *Science 226*, 843–844.
Hurley, L. H., Reck, T., Thurston, D. E., & Langley, D. R. (1988) *Chem. Res. Toxicol. 1*, 258–268.
Kohn, K. W. (1975) *Antibiotic III. Mechanism of Action of Antimicrobial and Antitumor Agents*, pp 3–11, Springer-Verlag, New York.
Koo, H.-S., & Crothers, D. M. (1988) *Proc. Natl. Acad. Sci. U.S.A. 85*, 1763–1767.
Koo, H.-S., Wu, H. M., & Crothers, D. M. (1986) *Nature (London) 320*, 501–506.
Lane, M. J., Dabrowiak, J. C., & Vournakis, J. N. (1983) *Proc. Natl. Acad. Sci. U.S.A. 80*, 3260–3264.
Lee, C.-S., Sun, D., Kizu, R., & Hurley, L. H. (1991) *Chem. Res. Toxicol. 4*, 203–213.
Maxam, A. M., & Gilbert, W. (1980) *Methods Enzymol. 65*, 499–560.
Mountzouris, J. A., & Hurley, L. H. (1992) in *Advances in DNA Sequence Specific Agents* (Hurley, L. H., Ed.) pp 263–292, JAI Press, Greenwich, CT.
Petrusek, R. L., Anderson, G. L., Garner, R. F., Fannin, Q. L., Kaplan, D. J., Zimmer, S. G., & Hurley, L. H. (1981) *Biochemistry 20*, 1111–1119.
Portugal, J., & Waring, M. J. (1987) *FEBS Lett. 225*, 195–200.
Rao, S. N., & Remers, W. A. (1990) *J. Med. Chem. 33*, 1701–1707.
Rao, S. N., Singh, U. C., & Kollman, P. A. (1986) *J. Med. Chem. 29*, 2484–2492.
Remers, W. A. (1988) *The Chemistry of Antitumor Antibiotics*, pp 28–29, Wiley, New York.
Remers, W. A., Barkley, M. D., & Hurley, L. H. (1992) in *Nucleic Acid Targeted Drug Design* (Propost, C. L., & Perun, T. J., Eds.) pp 375–421, Marcel Dekker, Inc., New York.
Shafer, G. E., & Tullius, T. D. (1989) *Electrophoresis 10*, 397–404.
Sigman, D. S. (1985) *Biopolymers 24*, 183–195.
Sigman, D. S. (1986) *Acc. Chem. Res. 19*, 180–186.
Sigman, D. S. (1990) *Biochemistry 29*, 9097–9105.
Sun, D., Lin, C. H., & Hurley, L. H. (1993) *Biochemistry 32*, 4487–4495.
Thurston, D. E., & Hurley, L. H. (1983) *Drugs Future 8*, 957–971.
Tullius, T. D. (1987) *Trends Biochem. Sci. 12*, 297–300.
Tullius, T. D., Dombroski, B. A., Churchill, M. E. A., & Kam, L. (1987) *Methods Enzymol. 155*, 537–558.
Wang, J.-J., Hill, G. C., & Hurley, L. H. (1992) *J. Med. Chem. 35*, 2995–3002.
Warpehoski, M. A., & Hurley L. H. (1988) *Chem. Res. Toxicol. 1*, 315–333.
Yoon, C., Kuwabara, M. D., Spassky, A., & Sigman, D. S. (1990) *Biochemistry 29*, 2116–2121.
Zakrzewska, K., & Pullman, B. (1986) *Biomol. Struct. Dyn. 4*, 127–136.