course of these investigations and for his suggestions during the preparation of this paper. We also thank Dr. T. A. Krugh and Dr. L. H. Hurley for making ref 31 and 22, respectively, available to us prior to their publication. We gratefully acknowledge the support of the National Cancer Institute (Grant CA-25644) in this research. The use of the facilities of the UCSF Computer Graphics Laboratory (R. Langridge, director, and T. Ferrin, facility manager), supported by Grant NIH RR-1081, is also gratefully acknowledged.

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Supplementary Material Available: Tables of charges on anthramycin in its covalently bonded form (Appendix 1), additional bond lengths, bond angles and dihedral parameters for anthramycin (Appendix 2), stereodiagrams of structures of the complexes G10C10-AM$_{rev}$ (Figure 2c), GC10-AM$_{rev}$ (Figure 3c), GCA, GCT, G10C10A, and G10C10T (Figures 4a,b and 5a,b, respectively), and energy component analysis diagrams (Figures 7-9) (12 pages). Ordering information is given on any current masthead page.

Conformations of Complexes between Pyrrolo[1,4]benzodiazepines and DNA Segments[1]

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The molecular mechanics program AMBER, assisted by CHEMLAB II, was used to model the covalent and noncovalent binding of anthramycin, tomaymycin, and neothramycin A to the hexanucleotide duplex d(GCATGC)$_2$ in the B-form conformation. Structures covalently bonded at N2 of guanine gave excellent fits when placed in either direction in the minor groove. However, energy analysis showed a preference for the direction wherein the side chain points toward the 5′ end of the covalently bound strand. This preference agrees with published NMR studies. Noncovalent binding of anthramycin in the minor groove near guanine gave good fits with almost no distortion in the helix, and the reactive center of the ligand was close enough to N2 for subsequent covalent bond formation. Anthramycin also gave a good noncovalent complex near adenine in the minor groove, but binding in the major groove had decreased dispersion attractions. Binding of tomaymycin was similar to that of anthramycin, although the smaller size of tomaymycin resulted in less binding energy. Neothramycin noncovalent binding was characterized by strong electrostatic interactions, partly involving the 3-OH group, and by part of the molecule lying outside the minor groove. AMBER was used for the exploratory design of an anthramycin analogue that theoretically would bind as well as anthramycin but not cause cardiotoxicity. A related study involving anthramycin, tomaymycin, and the pentanucleotide duplex d(AAGAA/TTCTT) was undertaken to evaluate further the ability of AMBER to predict sequence specificity. It indicated a preferred direction of binding toward 5′ in the minor groove of the duplex, but rather weak interaction with the noncovalently bound strand. This prediction agreed with experiments on tomaymycin that showed separation of the duplex and alignment of the drug toward the 5′ end of the covalently bound strand.

This paper and the preceding paper in this issue[1] represent independent investigations on modeling the binding of anthramycin and related pyrrolo[1,4]benzodiazepine antibiotics to specific segments of double helical B DNA. The other paper utilizes all atom force field parameters for the drug, emphasizes the appropriate stereochemistry for covalent bond formation at C11 of anthramycin, and explores the effects of sequence specificity when the covalent bond is made with the 2-amino group of guanine. The present paper utilizes united atom force field parameters to investigate both covalent and noncovalent binding at guanine and other locations on DNA. It includes anthramycin, tomaymycin. and neothramycin A. It also explores the use of molecular mechanics in the design of anthramycin analogues.

The pyrrolo[1,4]benzodiazepine antitumor antibiotics are potent agents produced by actinomycetes.[4-7] Included in this family are anthramycin, tomaymycin, sibiromycin, and neothramycins A and B. Their structures and numbering are shown in Figure 1. All of them have the same structural nucleus but differ in the benzene ring substituents and in the degree of saturation and substituents of the pyrrole ring.[8,9] Anthramycin and tomaymycin are observed by X-ray diffraction to possess a right-handed twist conformation along the length of the molecule, controlled mainly by the C11a hydrogen.[10,11] The neothramycins by structural analogy should have a similar twist, but unsaturation in the pyrrole ring of sibiromycin limits twisting. A carbinolamine group or its equivalent at N10–C11 are required for covalent binding to DNA.[9] Although the structure of tomaymycin often is written with a methyl ether group at C11, this is an artifact of crystallization from methanol. The natural product probably has an OH group like that of the anthramycin.[12] The neothramycins are N10–C11 imines, but they are known to undergo hydration in aqueous solution.[12]

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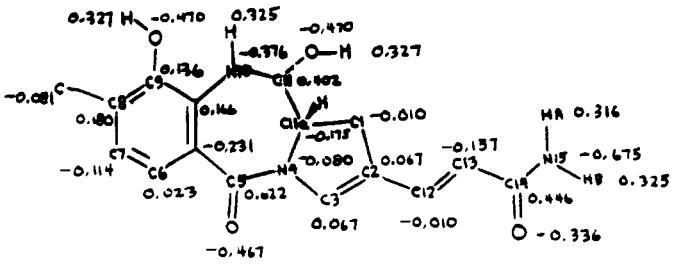
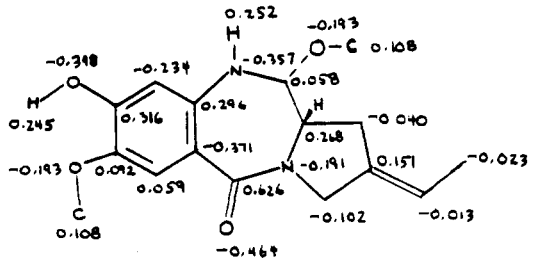
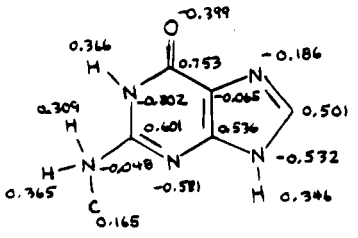


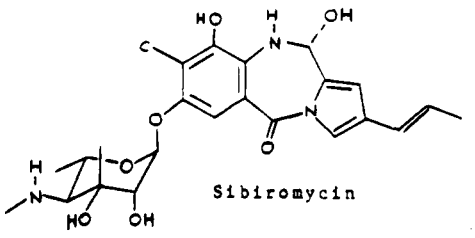
Anthramycin



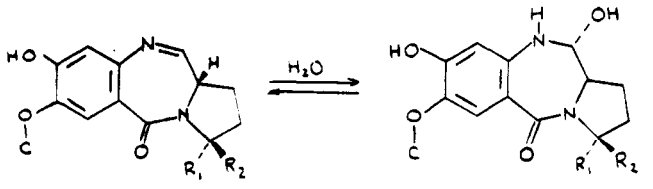
Tomaymycin



2N-methylguaninium ion



Sibiromycin



Neothramycins
A: R_1=OH, R_2=H
B: R_1=H, R_2=OH

Figure 1. Pyrrolo[1,4]benzodiazepine antibiotics and N^2-methylguaninium ion.

Pyrrolo[1,4]benzodiazepine antibiotics cause potent inhibition of nucleic acid synthesis in cells.[13] Persistent single- and double-strand DNA breaks occur, and they are dependent on excision repair.[14] Unscheduled DNA synthesis results in excision-proficient cells.[15] These antibiotics are nonmutagenic, but highly recombinogenic in bacteria.[16] Their cytotoxicity has led to experimental use

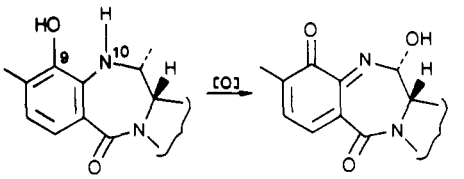


Figure 2. Oxidation of anthramycin to a quinone imine.

in treating human cancer. Anthramycin and sibiromycin possess serious cardiotoxicity, but the neothramycins do not. Hurley has suggested that the cardiotoxicity might be related to the phenolic 9-OH substituent, which in combination with the 10-NH can give quinone imine on oxidation (Figure 2).[12]

The covalent binding reaction of anthramycin with DNA is slow, requiring 60 min for saturation binding.[17] There is strict specificity for guanine in double-stranded DNA.[18] The resultant covalent adduct is unstable below pH 5, with the drug released. However, it remains covalently bound at neutral or alkaline pH.[12,19] The drug also is released unchanged upon treatment with heat or the enzyme DNase I.[20] Digestion by S_1 nuclease of DNA bound to anthramycin showed no regions of distortion or helix unwinding.[21] Attempts to isolate a small fragment of DNA covalently bonded to anthramycin have failed because of the lability of this bond. However, a variety of indirect methods have shown that a covalent bond is established between C11 of anthramycin and the 2-amino group of guanine, with anthramycin positioned within the minor groove of DNA. Thus, the selectivity for guanine in GC pairs was determined by adding [^{3}H]anthramycin to poly(dG)·poly(dC) and denaturing the adduct under alkaline conditions.[12] The 2-NH_2 group of guanine was found to be the alkylation site by eliminating all of the other possibilities: N7 and C8 by using [C8-^{3}H]guanine, O6 by using T-4 DNA, and N3 by the heat stability at neutral pH.[12] Proof of this site was obtained by an experiment in which d(ATGCAT)$_2$ gave an adduct wherein one of the 2-NH_2 protons was replaced by alkyl. Furthermore, a 15.5 ppm upfield shift for C11 in the ^{13}C spectrum of anthramycin on reaction with DNA was consistent with the carbinolamine to animal transformation (see Scheme I).[22]

The sequence specificity for microheterogeneity in DNA was tested by utilizing modified restriction enzyme fragments in combination with a footprinting method using methidiumpropyl-EDTA. The preferred binding sequence was demonstrated to be PuGPu, with PyGPy sequences being the least favored.[23]

A relatively detailed picture of the covalent binding of pyrrolo[1,4]benzodiazepines with double-helical B-form DNA has resulted from Hurley's CPK model building studies, which were made for all members of this family and certain analogues.[12] These studies showed that the drugs fit snugly in the minor groove without distortion of

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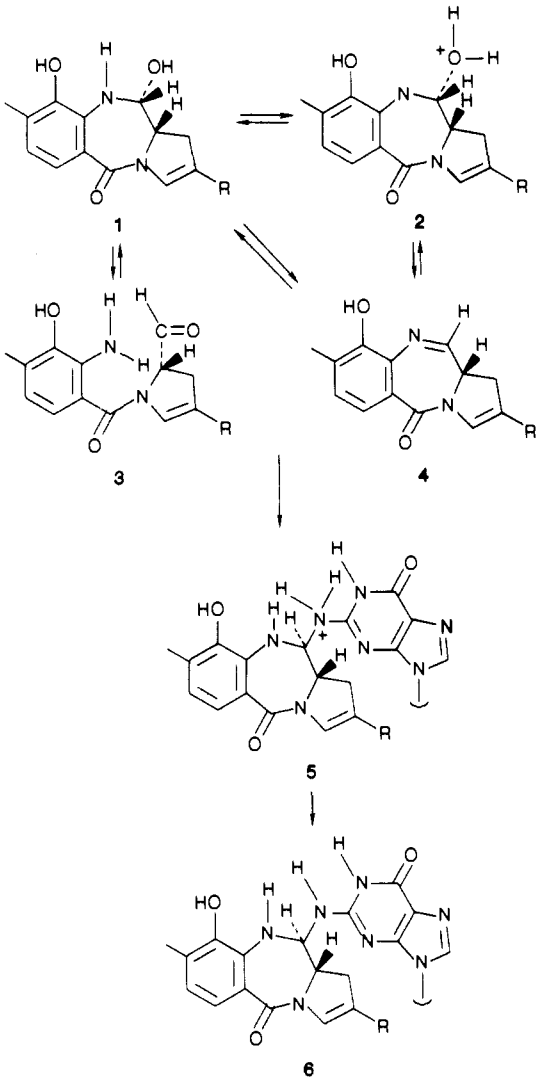
Scheme I. Some Possible Intermediates for the Alkylation of DNA by Anthramycin



the B helix. The only part of any of these drugs lying outside of this groove was the amino sugar moiety of sibiromycin. A 45–55° angle of the chromophore relative to the helix axis was consistent with electric dichroism measurements.[8] Stabilization of the drug–DNA interaction by hydrogen bonding was predicted as follows: hydrogen bonds between the N10 NH (all drugs except sibiromycin) and a CO group of thymine or cytosine or N1 of guanine or adenine in an adjacent base pair on the same strand as that bound to the drug; a hydrogen bond between 9-OH of anthramycin and O2 of cytosine in the same base pair to which the drug is covalently bonded. Arora has modeled the interaction of tomaymycin and noted that its 8-OH group is too far from O2 of cytosine to form a hydrogen bond. He suggested a bifurcated hydrogen bond to sugar and phosphate oxygens.[11]

Our main interest in the pyrrolo[1,4]benzodiazepines was to both extend and evaluate Hurley's model building studies by applying molecular mechanics intermolecular energy calculations.[24,25] This approach would enable us to obtain estimates of the relative interaction energies between DNA and these drugs. It also would provide information on the changes in intramolecular and intermolecular conformational preferences that accompany drug–DNA binding. Although it was anticipated from Hurley that these conformational changes would be small,[12] it seemed likely that some cooperative accommodation of the fit would occur. Another of our goals was to examine the initial noncovalent binding of pyrrolo[1,4]benzodiazepine, not just near the 2-NH_2 group of guanine, but at other sites on DNA. We were also interested in the "direction" in the groove the drug would prefer to take at a given sequence distribution site. Finally, we wished to make a preliminary evaluation of the use of intermolecular modeling using molecular mechanics in the design of anthramycin analogues. The pyrrolo[1,4]benzodiazepines chosen for our study were anthramycin, tomaymycin, and neothramycin A. For all of these compounds we used the hexanucleotide duplex $d(ATGCAT)_2$ in the B form, based on Krugh's and Hurley's experiment that demonstrated a covalent bond between C11 of anthramycin and 2-NH_2 of guanine.[22] This appeared to be the firmest evidence available.

Methods

The crystal structures of anthramycin methyl ether[10] and tomaymycin methyl ether[8,11] were used as initial structures in this investigation. Following the replacement of methyl ethers by hydroxyl groups and the addition of calculated charges, the crystal structures were minimized in the force fields provided by AMBER (UCSF) (assisted model building with energy refinement)[26] and the MMFF option of CHEMLAB II.[27] Initial atomic coordinates of neothramycin A were based on those of tomaymycin, with the 3α-OH substituent added by the CHEMLAB II editor. Minimization by AMBER produced the pucker (envelope conformation) expected for a pyrrolidine ring, and the orientation and distance (1.90 Å) of the 3α-OH group relative to the 4-carbonyl group indicated intramolecular hydrogen bonding. The hexanucleotide duplex was generated by AMBER from Arnott's coordinates for B DNA,[28] and the pyrrolo[1,4]benzodiazepines, or their 11-deoxy or derivatives for covalently bonded structures, were docked by real-time graphics of the PSSHOW program on an Evans and Sutherland terminal. For this docking, the drugs were simply placed in the minor groove near the chosen site (guanine NH_2 for covalent binding). AMBER was then used to minimize the energy of the drug–DNA complex. Force field parameters presented by Weiner et al.[29] were employed in these minimizations.

Charge distributions for deoxyanthramycin (furnished by S. N. Rao),[1] deoxytomaymycin, and N^2-methylguaninium ion were obtained from ab initio calculations employing an STO-3G basis set to derive the partial charges,[30] which are shown in Figure 1.

The structural effects of water and counterions on complexing were neglected in the energy calculations. These interactions certainly should contribute to the absolute values of binding energies of each drug to DNA. However, these contributions should have limited effects on the relative energies of noncovalent binding to DNA for each of the various pyrrolo[1,4]benzodiazepines mod-

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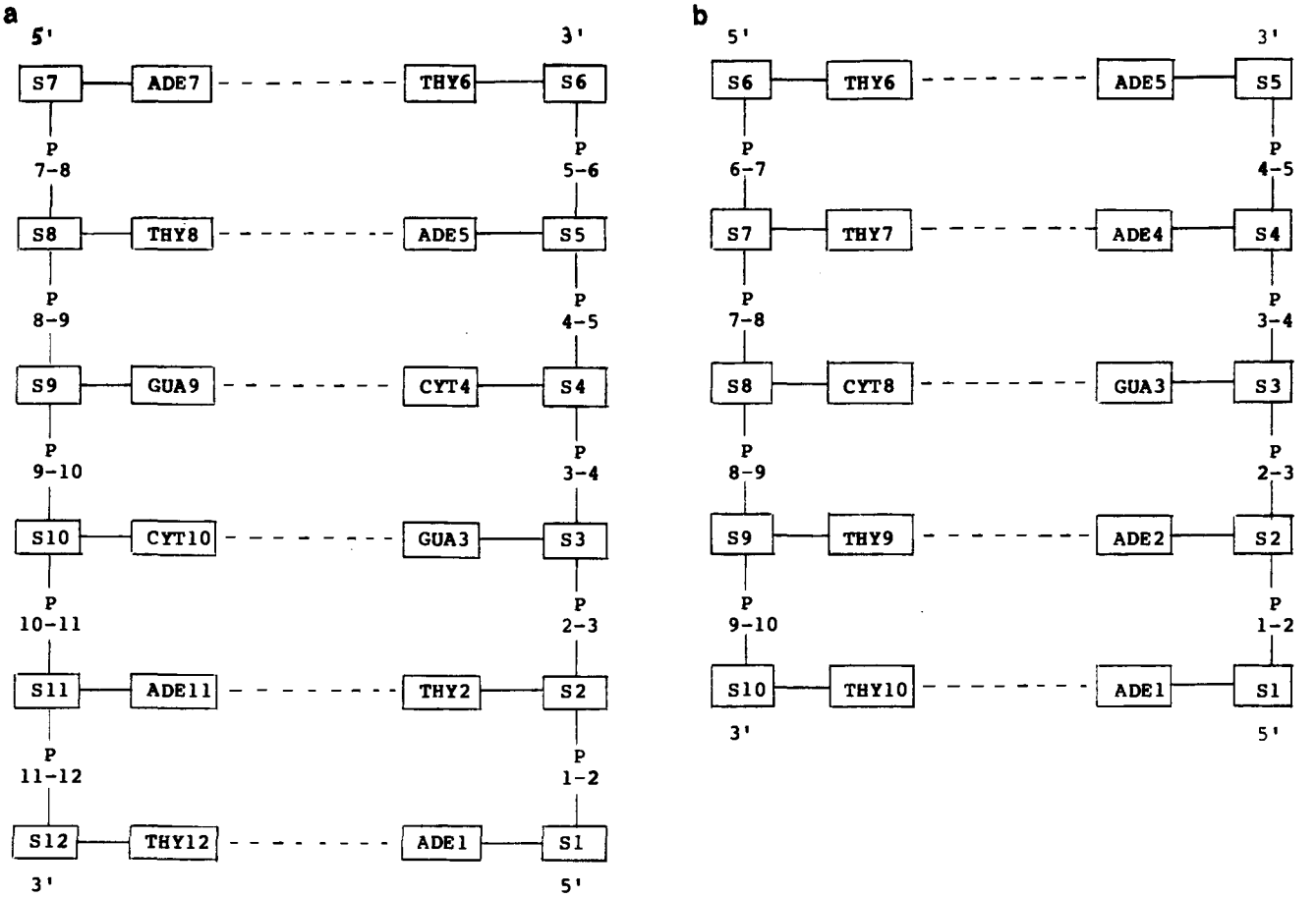
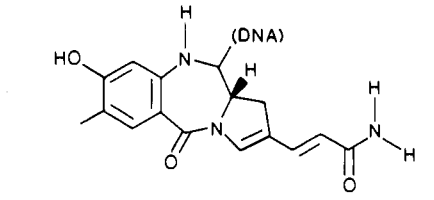


Figure 3. (a) Schematic for d(ATGCAT)$_2$. S stands for sugar. (b) Schematic for d(AAGAA/TTCTT).

eled for its interactions in the minor groove, in different directions, and at different sites, because each drug should displace about the same amount of water irrespective of its location in the minor groove. The counterion energetics might be more susceptible to site variation, although the drug molecules are neutral. In contrast, the desolvation and counterion energy differences should be somewhat greater among the various drugs even though they might be congeneric, because they differ in length and polarity. The contrast between anthramycin and its analogues is important in this respect.

Results and Discussion

Anthramycin. The starting point for developing a model was covalent binding of anthramycin at the 2-NH_2 group of guanine. As an aid in discussing this interaction and subsequent ones, the schematic diagram for d(ATGCAT)$_2$ in Figure 3 will be used. There were two possible directions in which anthramycin, covalently bound to GUA3, could lie in the minor groove: with the acrylamide side chain pointing toward the 3′ end of the hexanucleotide (→3′) or pointing toward the 5′ end (→5′). Modeling was done in both directions. The stereochemistry of binding at C11 of anthramycin was not specified. We simply used the united atom method, in which C11 was considered as an sp^3 carbon with one hydrogen,[26] and let the minimization by AMBER determine the best stereochemistry. As discussed below, this amounted to an inversion of configuration at C11 on going from noncovalent to covalent binding. This result predicted correctly the lower energy structure that would be obtained when anthramycin was bound both ways by the all atom force field parameters of AMBER.[1] It is not always possible to use the united-atom force field for calculations involving small molecule to DNA interactions. For example, Lybrand and



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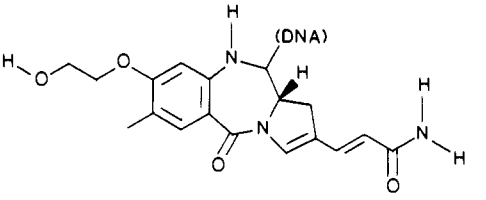

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Figure 4. Anthramycin analogues.

Kollman reported that experimental results for an ethidium–DNA complex were in agreement with the all-atom force field, but not with the united-atom force field.[31] However, in the case of the anthramycin the latter force field produces the same geometry as the former for C11 covalently bound to DNA, and as discussed below, it correctly predicts the preferred direction in the minor groove for the anthramycin–d(ATGCAT)$_2$ complex. Furthermore, our calculations gave nearly the same energy values as those in the related study in which the all-atom model was used for anthramycin.[1]

As shown in the stereopairs of Figure 5, covalently bound anthramycin fits well, lying in either direction of the major

(31) Lybrand, T. P.; Kollman, P. A. *Biopolymers* **1985**, *24*, 1863.

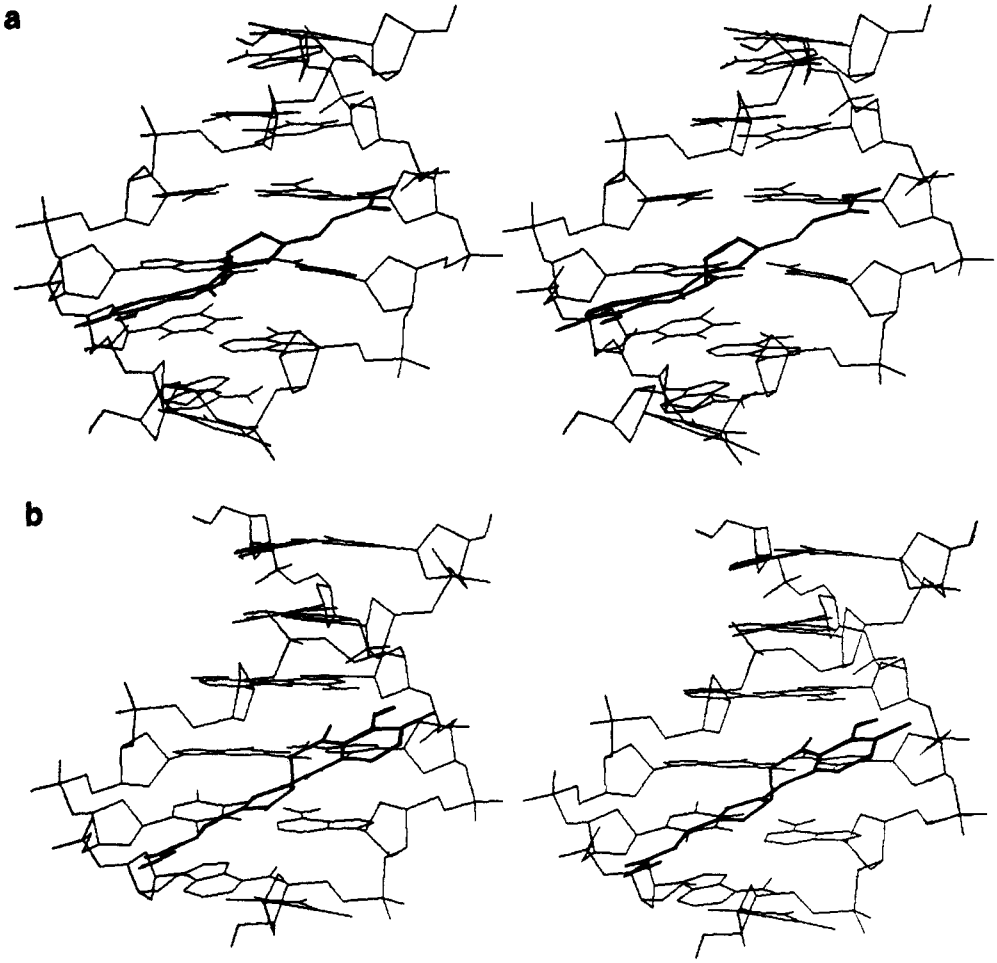

Figure 5. Stereopairs of the covalent complexes between d(ATGCAT)$_2$ and anthramycin: (a) side chain →C3′; (b) side chain →C5′.

groove. There is little apparent distortion in either the drug or the DNA, although as discussed below, the helix is distorted by about 20 kcal/mol. Analysis by AMBER of the energies for interaction between the drug and hexanucleotide (Table I) showed that the total energy for the components of binding in the minor groove was greater by about 10 kcal/mol for the drug in the 5′ direction. This table also sorts the interactions between drug and DNA into steric (largely van der Waals) and electrostatic components. The difference between the internal energy of the DNA and that of an isolated helix of DNA (also minimized by AMBER) is a measure of the distortion introduced by drug binding. This distortion energy, about 20 kcal/mol, is more than compensated for by the DNA–drug interactions of about −65 kcal/mol. The question arises as to whether an anthramycin analogue that causes less distortion in the helix would be a better drug. As discussed below, much of the distortion appears to involve fitting the DNA closer to the drug in order to achieve the substantial binding energy. Thus, the main consideration is to find an analogue with greater *total* binding energy.

Table II lists all interactions greater than −4.0 kcal/mol between the drug and individual residues of DNA, as defined in Figure 3. A number of significant interactions are apparent for both orientations. The interaction with GUA 9 for the 3′ orientation represents a hydrogen bond (1.95 Å) between amide hydrogen H15A of anthramycin and N3 of GUA9, whereas the 5′ orientation has hydrogen bonds between H15A and O2 of THY2 (1.82 Å) and HN10 of anthramycin and O2 of CYT4 (2.45 Å). It should be noted that shorter hydrogen bonds are stronger than longer ones. These hydrogen bonds are listed in Table III. In comparison, the crystal structure of anthramycin methyl ether monohydrate shows a variety of intermolecular hydrogen bonds with the 9-hydroxyl group and the 15-amino group acting as hydrogen-bond donors and the carbonyl oxygen on C14, oxygen of the 9-OH, and oxygen of the methyl ether on C11 acting as hydrogen-bond acceptors. There also was an intramolecular hydrogen bond between HN10 and the oxygen of the 9-OH. The water molecule was both a hydrogen-bond donor and acceptor.[10,11] Thus, most of the hydrogen-bonding patterns seen in the crystal structure are not significant in the structure of anthramycin covalently bound with DNA, except for those involving the 15-amino group and HN10 as hydrogen-bond donors. Analysis of the helix distortion energy does not reveal many significant individual distortions. The Watson–Crick base-pairing energies are normal, and the only changes in base stacking energies are those for the CYT4–ADE5 interaction (3.2 kcal/mol for →3′ and −4.0 kcal/mol for →5′) and the CYT10–ADE11 interaction (−3.9 for →3′). They represent small tilts of the bases. These energies and the conformational parameters, including sugar puckers and the dihedral C3′–O3–P–O5′ are given in Tables IV and V (supplementary material). There were few significant changes in the sugar–phosphate backbone.

A recent paper[32] on the one- and two-dimensional proton NMR spectra of the anthramycin–d (ATGCAT)$_2$ adduct in solution reported that the preferred direction for anthramycin in the minor groove was with the acrylamide side chain toward 5′. This is in agreement with our model that shows the 5′ orientation favored by −9.3 kcal/mol over the 3′ orientation, based on the relative differences of the

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Table I. Energies (kcal/mol) for Interactions between Pyrrolo[1,4]benzodiazepines and DNA Segments (These Energies Valid for Comparisons within This Table)[a]

DNA and Drug	binding mode	Location[b] or groove dir	total	DNA–drug steric	DNA–drug elstat	DNA–drug sum	internal drug	internal DNA	helix distortion[d]
$d(ATGCAT)_2$									
anthramycin	NC	3′	−599.9	−38.2	−10.4	−48.6	2.4	−553.7	0.5
	NC	5′	−596.7	−31.3	−13.3	−44.6	0.8	−552.9	1.3
	NC	at ADE5	−599.3	−35.1	−15.3	−50.4	2.1	−551.0	2.1
	NC	major[c]	−587.0	−22.3	−18.2	−40.5	6.4	−552.9	1.3
	C	3′	−587.2	−35.1	−26.1	−61.2	5.5	−531.5	22.7
	C	5′	−597.5	−40.9	−26.3	−67.2	4.6	−534.9	19.3
tomaymycin	NC	3′	−581.2	−31.4	−8.7	−40.1	9.7	−551.1	3.1
	NC	5′	−584.8	−32.6	−10.8	−43.4	11.9	−553.0	1.1
	NC	at ADE5	−577.3	−27.5	−7.0	−34.5	9.7	−552.5	1.7
	C	3′	−573.8	−29.0	−19.5	−48.9	10.8	−535.7	18.5
	C	5′	−576.2	−33.7	−19.1	−52.8	10.5	−533.9	20.3
neothramycin A	NC	3′	−585.2	−28.3	−20.0	−48.3	13.1	−550.0	4.2
	NC	5′	−588.1	−29.0	−23.1	−53.0	13.9	−549.0	5.2
	C	3′	−567.8	−26.3	−18.3	−44.6	12.1	−535.3	18.9
	C	5′	−570.1	−30.4	−17.7	−48.1	12.1	−534.1	20.1
anthramycin–DNA intermed	C	3′	−656.2	−35.5	−37.0	−72.5	8.4	−592.1	
	C	5′	−659.1	−37.6	−31.6	−69.2	4.2	−594.1	
anthramycin analogue 7	C	5′	−591.6	−35.9	−28.6	−64.1	8.1	−535.6	18.6
anthramycin analogue 8	C	5′	−597.1	−42.7	−29.2	−71.9	9.6	−534.8	19.4
d(AAGAA/TTCTT) anthramycin	C	3′	−524.7	−37.3	−35.4	−72.7	4.2	−456.2	20.4
	C	5′	−532.2	−37.2	−37.7	−79.4	4.8	−457.6	19.0
tomaymycin	C	3′	−498.4	−30.9	−19.0	−49.9	10.6	−459.1	17.5

[a] Abbreviations: NC = noncovalent, C = covalent at 2-NH_2 of GUA3. [b] Location is near GUA3 unless otherwise specified. Direction refers to orientation of the drug pyrrole ring and side chain relative to C11 of the 7-membered ring. For noncovalent binding near ADE5, the side chain lies toward 5′. [c] Noncovalent binding in the major groove is near O6 of GUA3 with the side chain toward 5′. [d] Energies of the uncomplexed helixes determined by minimization in AMBER are −554.2 and −476.6 kcal/mol, respectively for $d(ATGCAT)_2$ and d(AAGAA/TTCTT). Helix distortions are equal to these values minus the internal DNA energy in the complex.

sum of the intermolecular interactions and the helix distortion energies. Evidence for the 5′ orientation included the ADE11 H_2 proton closer to anthramycin than any other ADE H_2, an NOE between the anthramycin methyl group and ADE5 H_2, and significant upfield resonances in the $H_{4'}$, $H_{5'}$, and $H_{5''}$ protons on sugar 11 caused by their close proximity to the π-electron system of the anthramycin benzene ring. These interactions can be discerned in Figure 5b.

The main problem in modeling the noncovalent binding of anthramycin (and other pyrrolo[1,4]benzodiazepines) to DNA is deciding which form of the drug to use. Scheme I illustrates some of the possible species that might be formed from anthramycin. In principle, any of them could have noncovalent interactions near the 2-NH_2 group of GUA3 and subsequently make a covalent bond to it. Hurley suggested originally that the protonated form **2** is the reactive intermediate.[17] However, he later noted the possibility that the open amino aldehyde form **3** reacts.[8] Another structure of interest is the imine **4** resulting from loss of water from **1** of hydronium ion from **2**.[33] In the absence of cogent evidence for any of these species, we have chosen to simply model the drug itself. This choice minimizes problems associated with proper representation of molecular dielectric, which would be especially serious for charged species in a vacuum.

Minimized structures resulting from the noncovalent binding of anthramycin near GUA3 in the minor groove, placed in both directions, are shown in stereopairs in the supplementary material. These structures reveal excellent fits in both directions with almost no distortion of the DNA. The energy analysis is consistent with this picture. Table I shows total noncovalent binding energies comparable with those of the covalently bound species, but the helix distortion energies are negligible. The main difference in interactions between noncovalent and covalent complexes (besides the covalent bond) is that the latter have stronger electrostatic bonding between drug and DNA. This gain is offset by the helix distortion accompanying covalent bonding. Among the interactions between anthramycin and individual DNA residues listed in Table II are those resulting from hydrogen bonds listed in Table III between HN10 and N3 of ADE11 (2.02 Å) and between HO at C9 and O2 of THY12 (2.02 Å) for the 3′ direction and between HN15A and O2 of THY12 (1.95 Å) for the 5′ direction. There were no significant changes in Watson–Crick base-pairing energies or base-stacking energies for either orientation. Furthermore, only one small backbone dihedral angle change occurred for →3′, affording ω = 143.6° for CYT9 (supplementary material). Distances between the potential reactive centers of C11 in anthramycin and the 2-amino nitrogen of GUA3 were 3.22 Å for the →3′ complex and 3.08 Å for the →5′ complex. These distances are about the same as the combined van der Waals radii of the two atoms (3.00 Å), and they are consistent with an ease of subsequent covalent binding.

A further analysis of the changes in geometry of DNA and anthramycin on going from DNA alone to the noncovalent complex (→5′) to the resulting covalent binding is illustrated in the supplementary material through a figure produced by superimposing the two minimized systems with the GUA3 residue held constant. It is apparent that only a small change in the DNA with no distortion occurs on noncovalent binding. A figure prepared in the same way from minimized structures for the noncovalent complex and the covalent one shows how covalent binding brings the drug further into the minor groove, rotating it to give the optimal fit. The overall process corresponds to an inversion of configuration at C11 of the

(33) Barkley, M. D.; Cheatham, S.; Thurston, D. E.; Hurley, L. H. *Biochemistry* **1986**, *25*, 3021.

Table II. Interactions of Pyrrolo[1,4]benzodiazepines with Individual Residues of DNA Segments[a,b]

DNA and drug	binding mode and direction	THY2	S3	GUA3	S4	CYT4	S5	ADE5	S6	GUA9	S10	CYT10	S11	ADE11	S12	THY1
d(ATGCAT)$_2$																
anthramycin	NC 3′				-5.0								-6.8	-7.4	-6.8	-6.5
	NC 5′					-7.8	-5.7						-4.5			-7.1
	NC at ADE5[c]					-7.2		-6.3	-4.1							
	NC major	-10.4		-16.2						-4.4						
	C 3′		-4.0		-7.1					-7.7		-6.1		-5.9	-6.2	
	C 5′	-7.3		-6.5	-5.9	-6.7	-5.3					-5.5	-6.5		-4.8	
tomaymycin	NC 3′			-5.5	-5.9									-5.8	-6.9	
	NC 5′						-5.1					-6.3	-4.7	-4.6		
	NC at ADE5[d]								-4.8	4.8						
	C 3′		-7.3	-4.5	-5.5	-5.4						-5.0		-5.0	-5.3	
	C 5′		-4.5	-4.4	-5.2							-9.4	-5.0	-6.3	-4.0	
neothramycin A	NC 3′					-4.2	-5.0				6.5		-9.8	-7.6		
	NC 5′				-8.8	-5.7							-5.5	-5.0	-6.4	
	C 3′				-7.3	-6.3									-5.6	
	C 5′				-4.5	-4.4	-5.2					-8.0	-5.2	-6.3		
anthramycin–DNA intermed	C 3′			+10.5	-6.7	-6.1		-10.4				-9.7	-6.2	-9.8	-5.4	-8.6
	C 5′	-7.2		+5.7	-9.1		-6.1					-6.5	-7.9	-5.3	-6.0	-4.0
anthramycin																
analogue 7	C 5′			-5.8	-6.0	-8.4	-5.8				-5.1	-5.6	-7.0	-4.2		
analogue 8	C 5′			-7.1	-6.8	-9.1	-5.1					-5.1	-7.1	-4.0	-4.6	-7.2
d(AAGAA/TTCTT)		ADE2	S3	GUA3	S4	ADE4	S5	THY7	CYT8	S9	THY9	$P_{9\to10}$	S10			
anthramycin	C 3′	-8.1			-4.4		-4.4	-7.2	-7.8	-4.5	-6.7	-7.1				
	C 5′	-9.7		-8.4	-8.3	-9.0	-4.6		-6.2	-7.3			-5.7			
tomaymycin	C 3′				-6.8	-5.3			-5.1	-4.3	-4.5		-6.3			
	C 5′				-5.5	-6.1			-7.9	-5.3	-4.9		-5.6			

[a] Only energies > +4.0 kcal/mol are listed. [b] See Figure 3 for definitions and sequence of residues. [c] Also interacts with S8, THY8, and GUA9 with energies of -5.3, -5.2, and -6.0 kcal/mol, respectively. [d] Also interacts with THY6, THY8, and S9 with energies of -4.4, -5.3 and -4.7 kcal/mol, respectively.

drug. Another interesting feature of this model is that the amide group in the side chain of anthramycin changes its hydrogen bonding from one strand of the helix (O2 of THY12) to the other (O2 of THY2). Only a small overall movement occurs in the DNA on covalent binding. It appears to be one of closing in of the two strands to increase interactions with the drug, rather than one of being pushed out because of steric crowding.

The covalent binding process was analyzed in further detail by modeling the initially formed covalent species with a positive charge on the 2-NH_2 of GUA3. This corresponds to structure **5** in Scheme I. Although it is not known which reactive intermediate alkylates DNA, species including **1**–**4** should give **5**. In any event, it seemed important to determine whether structure **5** was compatible with low helical distortion in the process of going from noncovalent binding to covalent binding (**1** → **5** → **6** in Scheme I). Thus, structure **5** was modeled for both directions in the minor groove, using calculated ab initio charges for guaninium ion (Figure 1). The resulting stereopairs (supplementary material) show excellent fits with no distortion of the DNA. Even the Watson–Crick base pairing between GUA3 and CYT10 is preserved. The added proton points toward CYT4 and makes a hydrogen bond with it. Analysis of the interaction energies (Table I) shows the usual amount of dispersion energy with strongly increased electrostatic energies between drug and DNA and (presumably) within the DNA. However, electrostatic energies are high in AMBER minimizations on systems under vacuum. It can be concluded that formation of the tetrahedral ammonium ion at the 2-NH_2 of GUA3 offers no structural impediment to the alkylation process.

Although it appears that noncovalent binding near GUA3 followed by alkylation of its 2-NH_2 is a reasonable process, the question arises as to how feasible noncovalent binding is by anthramycin at other sites on DNA. There is no evidence for covalent binding elsewhere, but this does not rule out the possibility of noncovalent complexes. To explore this possibility, we modeled the binding of anthramycin at two additional sites on d(ATGCAT)$_2$: near ADE5 in the minor groove and near GUA3 in the major groove. The stereopairs for binding near ADE5 (supplementary material) show an excellent fit with no distortion of the helix, and the calculated total binding energy (Table I) compares favorably with those for binding near GUA3 in the minor groove. Furthermore, the detailed energy analysis, hydrogen-bonding, and conformational parameters resemble the others (Tables II and III and supplementary material). The main difference is a somewhat longer distance of 3.50 Å for the potential covalent bond between C11 and N3 of ADE5. Alkylation of N3 of adenine is known; however, it might not be favored for anthramycin because of the distance and possibly because of a relatively high-energy transition state associated with disruption of aromaticity in the pyrimidine ring. The noncovalent modeling at ADE5 raises two new questions. Can the drug move in the groove from ADE to GUA without dissociating from the DNA? What effect does noncovalent binding have on biological activity? There is no definitive experimental evidence relevant to either of these questions, although it has been reported that covalent binding is needed for cytotoxicity.[8] The minimized structure for noncovalent binding near GUA3 in the major groove is given in the supplementary material. This figure shows that the drug is aligned parallel to the groove, although the groove is too wide for a good fit. Significant hydrogen bonding occurs between the OH at C9 and O4 of THY2 (1.79 Å) and between HN10 and O6 of GUA3

Table III. Hydrogen-Bond Parameters Involving Drug–Polynucleotide Interactions[a,b]

DNA and drug	binding mode and direction	hydrogen atom	acceptor atom	length, Å
		d(ATGCAT)$_2$		
anthramycin	NC 3′	HN10	N3 (ADE11)	2.02
		HOC9	O2 (THY12)	2.02
	NC 5′	HN15A	O2 (THY12)	1.95
	NC at ADE5	HN15A	O2 (CYT4)	1.88
		HN10	O2 (THY8)	1.93
	NC major	HN10	O4 (THY2)	1.79
		HOC9	O6 (GUA3)	1.90
	C 3′	HN15A	N3 (GUA9)	1.95
	C 5′	HN15A	O2 (THY12)	1.88
		HN10	O2 (CYT4)	2.45
tomaymycin	NC 3′	HN10	N3 (GUA3)	2.18
		HOC12	O1′ (S12)	2.33
	NC 5′	HN10	O2 (CYT10)	1.84
		HOC12	O1′ (S4)	2.10
		HN2B (GUA3)	OC12	1.76
	NC at ADE5	HN10	O2 (THY8)	1.85
		HOC12	O1′ (S6)	2.12
	C 3′	HN10	N3 (GUA3)	1.84
	C 5′	HN10	O2 (CYT10)	1.84
neothramycin A	NC 3′	HOC3	O3′ (S9)	2.12
		HN10	N3 (ADE5)	2.42
		HN2A (GUA3)	OC11	2.16
	NC 5′	HOC3	O1′ (S4)	2.24
		HN10	O2 (CYT4)	2.42
		HN2B (GUA3)	OC11	1.76
	C3′	HN10	N3 (GUA3)	1.85
	C 5′	HN10	O2 (CYT10)	1.87
anthramycin–DNA intermed	C 3′	HN15A	N3 (ADE5)	1.86
		HN10	N3 (ADE11)	2.02
		HOC9	O2 (THY12)	1.90
	C 5′	HN15A	O2 (THY2)	1.81
		HOC9	O1′ (S5)	2.18
anthramycin				
analogue 7	C 5′	HN15A	O2 (THY12)	1.85
		HN10	O2 (CYT9)	1.94
analogue 8	C 5′	HN15A	O2 (THY12)	1.92
		HN10	O2 (CYT9)	1.91
		HOEthyl	O1′ (S6)	2.41
		d(AAGAA/TTCTT)		
anthramycin	C 3′	HN10	N3 (GUA3)	2.00
		HN15A	O2 (THY7)	1.88
	C 5′	HN10	N3 (ADE4)	2.15
		HN15A	N3 (ADE2)	1.86
tomaymycin	C 3′	HN10	N3 (GUA3)	1.97
		HN10	O2 (CYT8)	2.12

[a] Only bond lengths <2.5 Å are listed. [b] See Figure 3 for definitions and sequence of residues.

(1.90 Å). This bonding is consistent with the substantial energy for the electrostatic interaction between the drug and DNA (Table I). However, the significant decrease in dispersion energy between drug and DNA results in a total binding energy that is unfavorable by about 10 kcal/mol to that of anthramycin at sites in the minor groove (Table I). Thus, the major groove does not appear to be significant for binding in the particular sequence that was modeled. It is interesting to note, however, that C11 of anthramycin would lie close to both O6 and N7 of GUA3 (3.07 and 3.38 Å, respectively) if it did bind in this manner.

Tomaymycin. The binding of tomaymycin with d(ATGCAT)$_2$ was modeled in the same manner as anthramycin, except that this study was more limited. For the case of covalent binding to the 2-NH$_2$ group of GUA3, the stereopairs are shown in Figure 6a for the 3′ direction and Figure 6b for 5′. The total interaction energies (Table I) are somewhat lower than those of the corresponding anthramycin covalent structures, with decreases occurring in both dispersion and electrostatic interactions. This is a consequence of the lack of a side-chain amide and an unfavorable location of the OH group at C8. Tomaymycin does hydrogen bond with its 10-NH (N3 of GUA3 for →3′ and O2 of CYT10 for →5′) as shown in Tables II and III. In the tomaymycin crystal, the 10-NH does not hydrogen bond but the 8-OH and C5 carbonyl group do.[34] The helix distortion energy is about the same as that produced by anthramycin, and the only significant changes in the helix are tilts in the CYT4–ADE and CYT10–ADE11 base stacking (supplementary material). A recent study by Cheatham and Hurley revealed that tomaymycin showed no preference for covalent binding in either direction of the minor groove of d(ATGCAT)$_2$.[35] Table I shows the 5′ direction favored by −2.4 kcal/mol, but this difference might be too small to control binding specificity.

Noncovalent binding of tomaymycin was modeled with the intact antibiotic (hydroxy substituent on C11). Stereopairs for binding near GUA3 with 3′ and 5′ directionality and near ADE5 in the minor groove are given in the supplementary material. The interaction energies (Table I) show a preference (−4 kcal/mol) for binding in the 5′ direction. There was very little helix distortion for any binding direction. Three intermolecular hydrogen bonds occurred in each binding mode at GUA3, and there were two hydrogen bonds at ADE5 (Tables II and III). As in

(34) Arora, S. K. *J. Antibiotics* 1981, *34*, 462.
(35) Cheatham, S.; Hurley, L. H., manuscript in preparation.

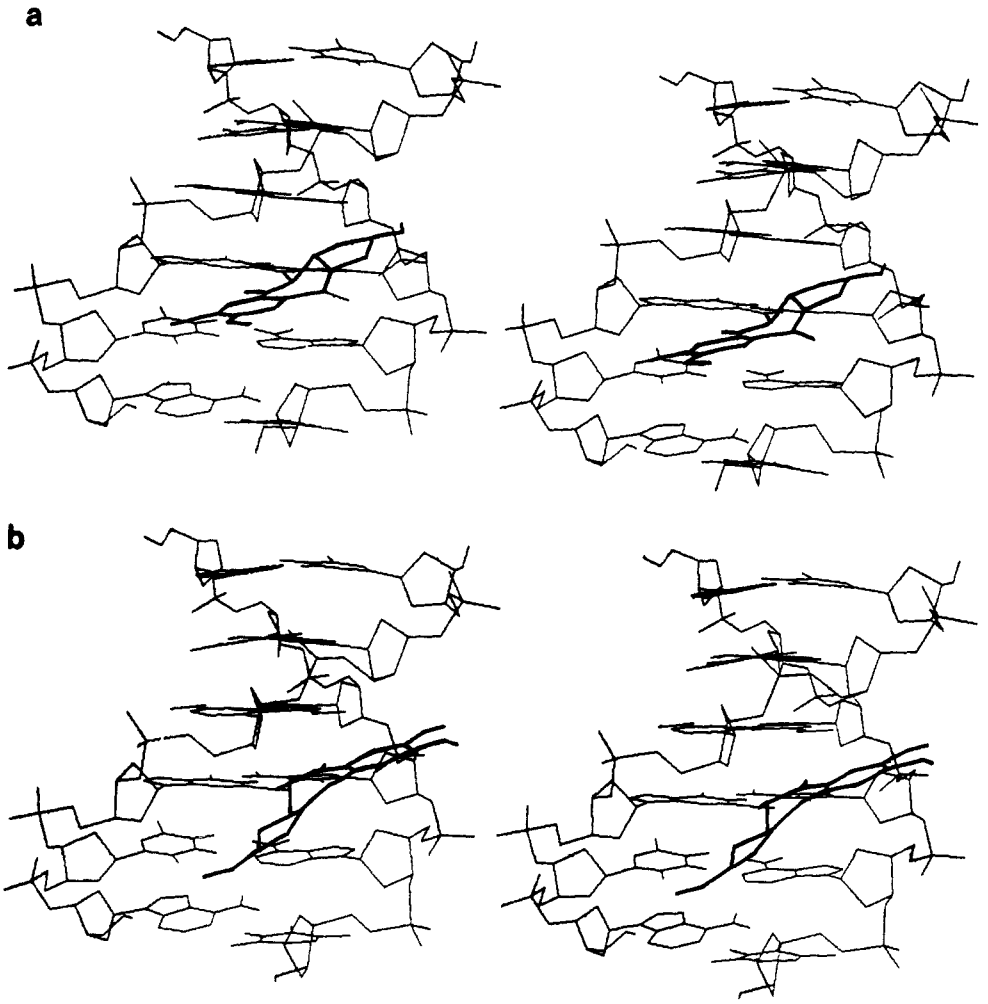

Figure 6. Stereopairs of the covalent complexes between tomaymycin and d(ATGCAT)$_2$: (a) side chain →C3′; (b) side chain →C5′.

the anthramycin examples, the distances between potential covalent binding centers, C11 of tomaymycin and the 2-NH_2 of GUA3, were short, 3.01 Å for →3′ and for →5′. However, a C11–N3 of ADE5 distance of 3.93 Å was found for noncovalent binding in the 5′ direction near ADE5. No significant changes in backbone dihedral and sugar puckers were observed.

Neothramycin A. Covalent binding of neothramycin A with d(ATGCAT)$_2$ was modeled according to the previous examples. Stereopairs for the resulting minimized structures are shown in Figure 7, parts a and b, respectively, for the pyrrolidine ring toward 3′ and 5′. The energies for binding interactions (Table I) are very close to those of tomaymycin, as are the helix deformation energies. Hydrogen bonding involves only HN10 of neothramycin A (Table III), with N3 of ADE11 for →3′ or O2 of CYT10 for →5′ as the acceptors. The hydroxyl group on C3 of neothramycin prefers intramolecular hydrogen bonding with the C5 carbonyl to any intermolecular interaction. No significant sugar puckers or backbone distortions occur, and the only decreases in base stacking energy are in the CYT4–ADE5 and CYT10–ADE11 interactions.

As noted above, neothramycins undergo hydration in water. On this basis, the hydrated form (Figure 1) was used for modeling the noncovalent binding with d(ATGCAT)$_2$. This choice maintains consistency with anthramycin and tomaymycin in that the latter now has a C11 hydroxyl group. Energy-minimized structures for the noncovalent complexes, with the drug placed in both directions of the minor groove, differed from anthramycin and tomaymycin in that part of neothramycin A (benzene ring substituents) lay outside of the minor groove (supplementary material). In contrast to the covalently bound neothramycin structures, which have an intramolecular hydrogen bond between HOC3 and OC5, the noncovalent structures have an intramolecular hydrogen bond between HOC11 and OC3 (1.96 Å) with HOC3 involved in hydrogen bonding to DNA (Table III). These structural changes correspond to intermolecular electrostatic interactions that are stronger than those of anthramycin (Table I). However, the larger size and more complete fit in the minor groove give anthramycin greater dispersion and total intermolecular binding energies. Neothramycin A utilizes HOC3, HN10, and OC11 for intermolecular hydrogen bonding, regardless of which direction it is placed in the minor groove. Of course, the hydrogen-bonding partner on DNA varies with direction (Table III). There were no significant changes in Watson–Crick hydrogen bonding and only the usual tilts resulting in reduced base stacking energies (CYT4–ADE5 and CYT10–ADE11). Only one deviation >30° in the backbone dihedrals was observed (supplementary material). The distances for potential covalent bonding between C11 of neothramycin A and N2 of GUA3 were 3.23 Å for the 3′ direction and 3.19 Å for the 5′ direction, in good agreement with corresponding distances in anthramycin and tomaymycin.

Anthramycin Analogues. Modeling with AMBER should provide a valuable aid in the design of antitumor drugs that bind to DNA. However, little use has been made of it for direct applications in analogue design. The following example is an attempt to explore the use of AMBER in designing a new analogue of anthramycin. Based

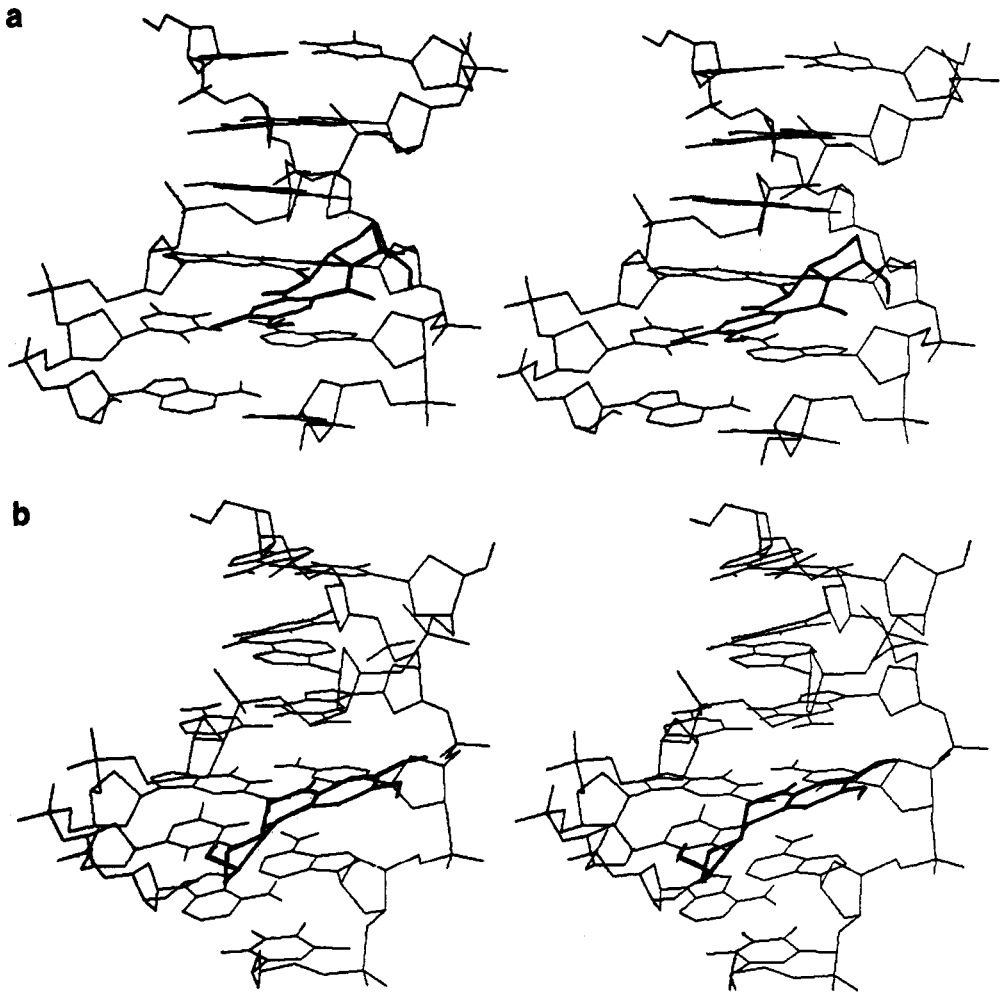

Figure 7. Stereopairs of the covalent complexes between neothramycin A and d(ATGCAT)$_2$: (a) pyrrolidine ring →C3′; (b) pyrrolidine ring →C5′.

on Hurley's analysis of anthramycin cardiotoxicity,[8] the simplest way to avoid this undesirable property would be to move the 9-OH group to some other position on the benzene ring. The resulting structure, for example **7** in Figure 4, might have decreased hydrogen bonding with DNA, but there would be no change in octanol/water partition coefficient. The partition coefficient (log *P*) of a drug often is a critical measure of its biological activity because it describes the ability of a drug to cross cell membranes. In at least one series of antitumor drugs it accounted for nearly all of the variation in potency.[36] Because the optimal log *P* for anthramycins has not been determined, we are basing analogue design on the log *P* of anthramycin itself. It must have a favorable log *P*, if not the optimal one, in view of its high potency. This problem was investigated by modeling the covalent binding of **7** to the 2-NH_2 group of GUA3 with the acrylamide chain →5′. The resulting minimized structure (Figure 8a) shows an excellent fit in the minor groove with the usual modest helix distortion. However, the difference in the total of drug–DNA interaction energy plus helix distortion energy between **7** and anthramycin was −3.4 kcal/mol in favor of anthramycin. In order to improve the DNA binding properties of **7**, a 2-hydroxyethyl group was substituted on the oxygen at C8. The resulting analogue **8** has nearly the same partition coefficient as **7** according to Hansch's π values:[37] π for aromatic OH, −0.67; $\sum\pi$ for aromatic OCH_3 + aliphatic CH_3 + aliphatic OH, −0.02 + 0.50 + (−1.12) = −0.64. It was modeled in the same manner as **7**. The resulting minimized structure (Figure 8b) shows an excellent fit, with the hydroxyethyl group completely in the minor groove and making a weak hydrogen bond to O1′ of sugar **6** (2.44 Å). The difference in the total of drug–DNA interaction energy plus helix distortion energy (Table I) between **8** and anthramycin was −4.6 kcal/mol in favor of **8**. Thus, the hydroxyethyl group has overcome the loss in interaction energy that accompanied relocation of the phenolic OH group.

Studies with d(AAGAA/TTCTT). Cheatham and Hurley recently have studied the directionality of covalent minor-groove binding of anthramycin and tomaymycin to the deoxynucleotide d(AAGAA/TTCTT).[35] At the suggestion of Professor Hurley, we have examined this system of molecular modeling with AMBER in order to evaluate further the ability of AMBER to predict the preferred binding direction. The methodology and the structures and charges for anthramycin and tomaymycin were the same as those described above. Structures resulting from energy minimization (supplementary material) showed excellent fits in the minor groove, and they resembled those depected for the analogous binding to d(ATGCAT)$_2$ in Figures 5 and 6. Energies for the relative stability of the drugs with side chains in the 3′ and 5′ directions can be

(36) Sami, S. M.; Iyengar, B. S.; Tarnow, S. E.; Remers, W. A.; Bradner, W. T.; Schurig, J. E. *J. Med. Chem.* **1984**, *27*, 701.

(37) Hansch, C.; Leo, A. *Substituent Constants for Correlation Analysis in Chemistry and Biology*; Wiley-Interscience: New York, 1979.

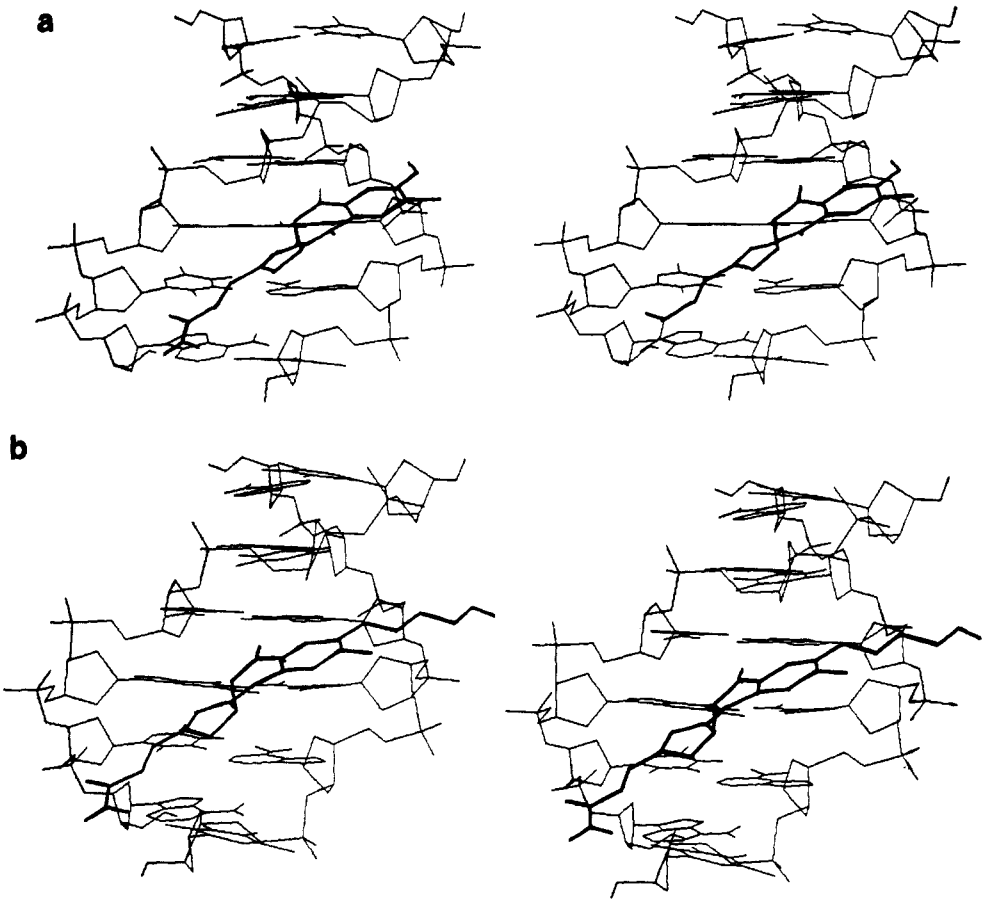

Figure 8. Stereopairs of the covalent complexes between $d(ATGCAT)_2$ and anthramycin analogues: (a) analogue **7**; (b) analogue **8**.

derived by adding the sum of DNA–drug interactions and the helix distortion energies given in Table I. They predict that binding in the 5′ direction is more stable by–8.1 kcal/mol for anthramycin and –4.4 kcal/mol for tomaymycin. Table II gives the energies greater than –4.0 kcal/mol for binding of the individual residues of the DNA with the two drugs, and Table III lists the important hydrogen bonds. None of the Watson–Crick hydrogen bonds are significantly distorted, but there is some loss in base-stacking energies, especially near the end of the duplex at CYT8–THY9 and THY9–THY10 (supplementary material, Table IV). The helix distortions listed in Table I are revealed in all cases by changes in the C3–O3′–P–O5′ dihedral angle between THY9 and THY10 or ADE4 and ADE5 and in the anthramycin cases by changes in the puckers of sugars **7** and **8** (supplementary material, Table V).

The NMR experiments of Cheatham and Hurley indicated that tomaymycin binds only in the 5′ direction of the AAGAA strand. They also found that the double helix separates in solution under the experimental conditions. This result renders our prediction unverifiable; however, the calculations still have some significance. Thus, analysis of the residue data in Table II reveals that binding between tomaymycin and the strand not covalently bound (TTCTT residues CYT8 through S11) is –23.7 kcal/mol, whereas the helix distortion energy is +17.7 kcal/mol. Consequently, the calculations show that the drug has only a small effect on holding the DNA together in solution. However, it must be noted that effects of solvent and counterions were neglected in these calculations. Given its small size and the predominance of A–T pairs, it is not surprising that this duplex separates. The data in Table II indicate no directional preference for tomaymycin if only the covalently bound AAGAA strand is considered. Directional selectivity must derive from covalent binding while the DNA is in a double helix, rather than covalent binding to the dissociated AAGAA strand. Kohn and co-workers have reported that anthramycin reacts preferentially with double-stranded DNA, although single-stranded DNA also is reactive.[18] The question remains as to whether the orientation of covalently bound tomaymycin would change after dissociation of the helix. This change appears unlikely in view of the significant calculated binding of tomaymycin to the adjacent ADE4 and S4 (Table II) and the possibility of bad steric hindrance to the rotation.

Summary and Conclusions

Modeling of the covalent binding of anthramycin with the hexanucleotide duplex $d(ATGCAT)_2$ gave excellent fits in either direction in the minor groove. The intermolecular binding and helix distortion energies were in good agreement with those obtained in the companion study (see the preceding paper). There were differences in the atom-specific binding interactions in the two studies, even allowing for the differences in sequences of residues. In particular, we found no interactions with the phosphate groups, whereas the companion study found interactions between them and HN15B of anthramycin. The ligand-binding geometries found in these two studies, using the same force field (AMBER), are also nearly identical in energy. This would suggest that each binding geometry is about equally likely. Moreover, experience suggests that intermolecular space is rich with energy minima for ligands interacting with macromolecules. Thus, other stable intermolecular assemblies could be expected to be uncovered with additional modeling of this system.

The key strategy in analyses of this type is to reasonably explore the energy of the system as a function of the degree

of freedom, but not get so carried away in the exploration that the study is never completed. Unfortunately, there is no general way to implement such a strategy. From our perspective, the best way to limit the exploration process, and define the problem, is to make maximum use of experimental data. We believe this has been done in our work and that of the related study. Both studies also agreed that there were no gross distortions in the DNA helix, confirming Hurley's CPK modeling results. No changes in Watson–Crick base pairing were observed in either study, although we noted some tilt of the bases. This effect would be less pronounced in a longer DNA sequence, especially in one with G–C pairs near the ends as modeled in the related study.[1]

Covalent binding of tomaymycin and neothramycin A in the minor groove also gave good fits to the hexanucleotide duplex. Their total binding energies were about −10 kcal/mol less stable than that of anthramycin, presumably because of their smaller size. Helix distortion energies were comparable to those of anthramycin.

Noncovalent binding studies showed that anthramycin and tomaymycin made favorable interactions at ADE5 as well as at the ultimate covalent binding site at GUA3. The drugs lay within the minor groove and caused almost no distortion in the DNA helix. Binding in the major groove gave good electrostatic attraction, but the DNA backbone was too distant from the drug to allow effective dispersion interactions. The additional C3 hydroxyl group in neothramycin A was significant in increasing its electrostatic binding with DNA. As a result, the drug did not fit completely in the minor groove, which decreased attractive dispersion interactions.

Any discussion of molecular energetics and relative energies of minimum energy complexes must be viewed with the understanding that the force fields are approximate and incomplete. In the present study functional presentation of electrostatic interactions is probably incomplete, and the contribution of solvent interactions to the molecular energetics are neglected. While these factors probably will not destroy and/or create energy minima, based upon past studies, the relative differences in minimum energy states is likely to be altered.[35,36] In view of this, the importance assigned to differences in energy between stable states should not be too great. The identification of stable states should be taken as the major task.

An exploratory use of AMBER for the design of an anthramycin analogue showed how AMBER might be used to improve ligand-binding energy to DNA. The success of this attempt cannot be determined unless the analogue is actually synthesized and tested, but it certainly is more rational and quantitative than most approaches now in use.

Perhaps the most significant result of our studies is an indication that modeling with AMBER can predict the direction of minor groove binding and, hence, the sequence specificity of anthramycin and tomaymycin. Thus, the calculated preference for covalent binding of anthramycin with the acrylamide side chain toward the 5′ end of the bound strand in $d(ATGCAT)_2$ is supported by the recent 2D NMR studies by Krugh.[32] Whether the calculated binding difference of −2.1 kcal/mol for tomaymycin with $d(ATGCAT)_2$ can be considered predictive of no directional preference (the observed result)[35] or small preference for the 5′ direction is debatable at this time. We simply have too few examples to establish a cutoff point in energy for directional preference. Further studies on the ability of AMBER to predict sequence specificity for pyrrolo[1,4]-benzodiazepines and other antitumor drugs clearly are desirable.

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Supplementary Material Available: Tables IV and V of data on energies for base-stacking interactions and for nonstandard sugar puckers and backbone dihedrals and stereopairs for energy-mimized complexes of pyrrolo[1,4]benzodiazepines and DNA segments (Figures 9–16) (19 pages). Ordering information is given on any current masthead page.