Conformations of Complexes between Mitomycins and Decanucleotides. 3. Sequence Specificity, Binding at C-10, and Mitomycin Analogues

William A. Remers,*,† Shashidhar N. Rao,‡,[1] Timothy P. Wunz,† and Peter A. Kollman‡

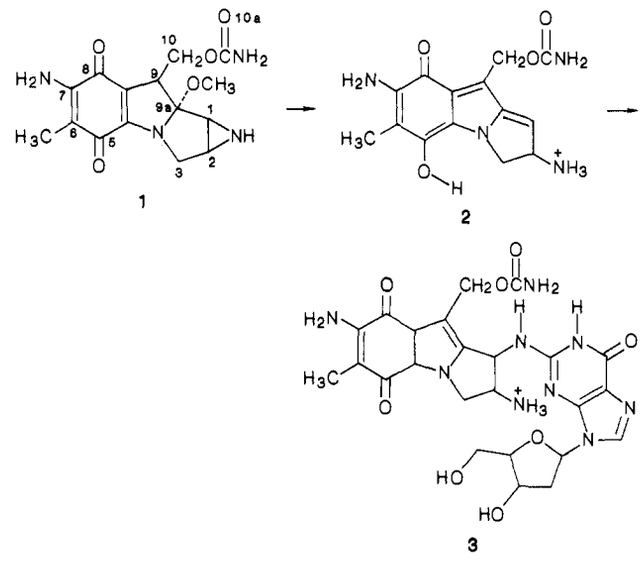
Department of Pharmaceutical Sciences, University of Arizona, Tucson, Arizona 85721, and Department of Pharmaceutical Chemistry, University of California, San Francisco, California 94143. *Received October 2, 1987*

Molecular mechanics simulation of the interactions of mitomycin C and certain analogues with DNA models are presented. The sequence specificity of mitomycin C binding was investigated by using a $d(GCGCGCGCGC)_2$ decanucleotide duplex, abbreviated herein as GC10, in which the base pair was varied on either side of the covalent binding site. A CGT fragment was favored, although its correlation with the diverse findings in the literature is questionable. A model was derived for the monocovalent binding at C10 of 2,7-diaminomitosene with GC10 and for the noncovalently bound hydroquinone intermediate. Revised models were established for three highly active mitomycin C analogues: M-83, BMY-25282, and RR-150. They involved covalent binding at the 2-amino group of a guanine residue, and they accounted for enhanced noncovalent binding afforded by specific interactions of the C7 substituents with residues in GC10.

The two previous articles in this series examined models for various binding modes of mitomycin C (**1**) and certain of its analogues with double helical DNA, represented by the decanucleotide fragment GC10.[2,3] In the first article, molecular mechanics simulations were made for noncovalent, monocovalent, and crosslinked binding in the major and minor grooves and for noncovalent intercalative binding. The intercalative mode did not provide a good model, but useful models were obtained for noncovalent binding in both grooves. They showed a striking feature of stabilization by hydrogen bonds in conformations that could lead readily to monoalkylation by C1 of the mitomycin intermediate with O6 of guanine in the major groove or N2 of guanine in the minor groove. The resulting monocovalent complexes also were stabilized by a network of specific hydrogen bonds, and the molecules were oriented such that crosslinking was possible between O6 of two guanines in the major groove or N2 of two guanines in the minor groove. Only small helix distortion energy occurred in the noncovalent binding models, but it increased as the covalent bond formed, especially in the case of O6 alkylation wherein Watson–Crick hydrogen bonding with the complementary cytosine was changed.[2] In the monocovalent mitomycin binding model, the mitomycin structure used is that of a 1-substituted 2,7-diaminomitosene protonated on its 2-amino group (**3**). This structure results from bioreductive activation of mitomycin C (**1**), which involves elimination of methanol from the 9 and 9a positions, followed by opening of the aziridine ring. An intermediate (**2**) proposed by Moore is thought to be the alkylating species. Following alkylation, reoxidation to the quinone occurs. The final form, **3**, has been identified as covalently bound to DNA hydrolysis fragments (Scheme I).

The second article was concerned with extending these studies to covalent binding with N6 of adenine, with the use of Moore's intermediate (**2**) as the initial noncovalent binding species and with three mitomycin C analogues that showed important antitumor activity. Binding to N6 of adenine in the major groove gave a model that was similar to that of binding to O6, except that the Watson–Crick base pairing was not disturbed. Replacement of the previously used mitomycin intermediate, a protonated 7-aminoaziridinomitosene with **2** made little difference in the calculated binding energy or conformation of the noncovalent complex. The rationale for using **2** was that there was indirect evidence for its existence in solution following reductive activation of mitomycin C. The three mitomycin analogues M-83 (**4**), BMY-25282 (**5**), and RR-150 (**6**) were chosen because they showed promise as improved anticancer agents, and two of them (**4** and **5**) were entered into clinical trial. Their binding was modeled as covalent at O6 of guanine in the major groove.[3] At the time that article was written, O6 appeared to be the best documented alkalation site, based on the isolation from a hydrolyzed mitomycin C–DNA covalent complex of a fragment containing mitomycin and 2-deoxyguanosine linked at positions thought to be C1 and O6, respectively.[4] These mitomycin analogues gave excellent binding models in which their new functional groups were seen to make specific hydrogen bonds or electrostatic interactions that further enhanced the noncovalent binding energies.[3] Unfortunately, since that modeling study was published, the structure of the hydrolysis fragment has been revised to one with covalent binding of the mitomycin C1 to N2

Scheme I. Bioreductive Activation and DNA Binding by Mitomycin C



† University of Arizona.
‡ University of California.

(1) Present address: Searle Research and Development, 4901 Searle Parkway, Skokie, IL 60077.
(2) Rao, S. N.; Singh, U. C.; Kollman, P. A. *J. Am. Chem. Soc.* **1986**, *108*, 2058.
(3) Remers, W. A.; Rao, S. N.; Singh, U. C.; Kollman, P. A. *J. Med. Chem.* **1986**, *29*, 1256.
(4) Tomasz, M.; Lipman, R.; Snyder, J. K.; Nakanishi, K. *J. Am. Chem. Soc.* **1983**, *105*, 2059.

0022-2623/88/1831-1612$01.50/0

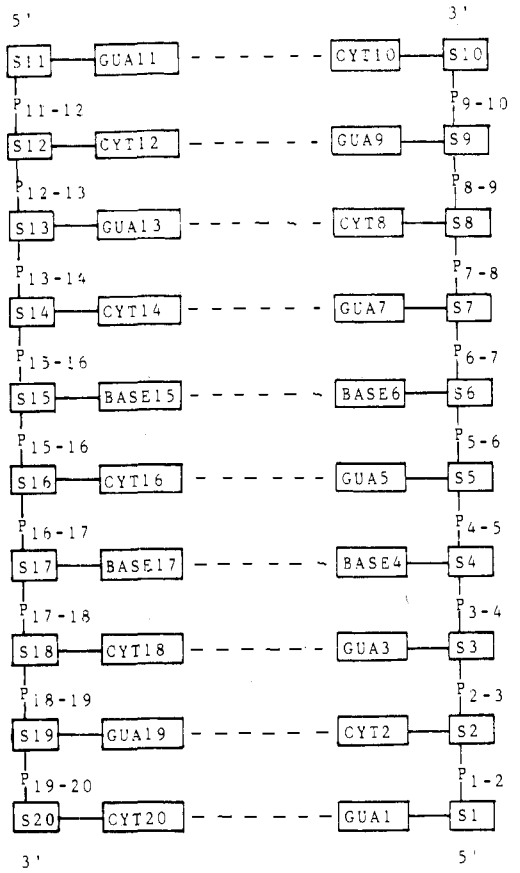
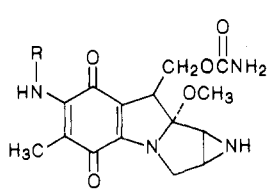


Figure 1. Schematic for decanucleotide duplexes. S stands for sugar.

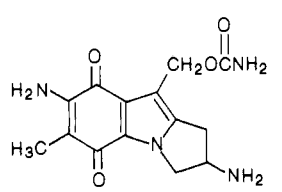
of 2-deoxyguanosine.[5] Revision of our modeling studies on the mitomycin analogues to fit this revised binding site is one goal of the present work.



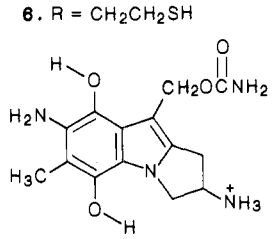
4, R = C_6H_4OH

5. R = $CH{=}N^+(CH_3)_2$ (after protonation on N7)

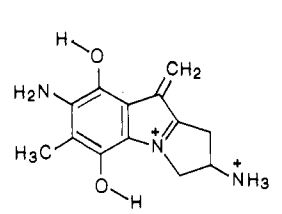
6. R = CH_2CH_2SH



7



8

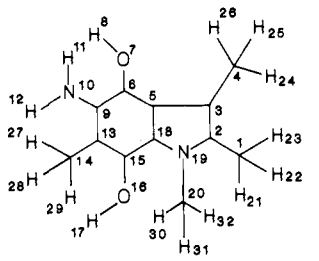


9

Results and Discussion

The first problem investigated was the sequence specificity of mitomycin C binding to DNA. Previous modeling studies had been limited to the decanucleotide duplex $d(GCGCGCGCGC)_2$, abbreviated here as GC10.[2,3] However, it seemed important to extend them to variations in this sequence, because a possible preferred binding site might emerge from the calculations. A recent study on sequence-specific DNA damage induced by mitomycin C and its analogue M-83 (4) showed that reduction of either compound in the presence of calf thymus DNA resulted in damage at deoxyguanosines preferentially in the dinucleotide sequence GT, with emphasis on PuGT sequences.[6,7]

Table I. Partial Atomic Charges on an Indole Related to 2,7-Diaminomitosene Hydroquinone[a]



atom	charge	atom	charge
C1	-0.308	H17	0.353
C2	-0.048	C18	-0.355
C3	0.213	N19	0.331
C4	-0.443	C20	-0.361
C5	-0.303	H21	0.091
C6	0.376	H22	0.102
O7	-0.515	H23	0.091
H8	0.336	H24	0.105
C9	-0.004	H25	0.120
N10	-0.503	H26	0.105
H11	0.251	H27	0.075
H12	0.243	H28	0.036
C13	-0.096	H29	0.077
C14	-0.220	H30	0.114
C15	0.443	H31	0.122
O16	-0.542	H32	0.114

[a] Determined by GAUSSIAN-80 UCSF with a STO-3G basis set.

In the present investigation, monoalkylation between C1 of mitomycin C and the 2-amino group of GUA5 was assumed (see Figure 1). Base pairs immediately above and below the GUA5–CYT16 pair were varied one at a time through each of the three possible new combinations. Thus, models were built for complexes between mitomycin C and the six decanucleotides: d(GCGGGCGCGC)·d(GCGCGCCCGC) (C17), d(GCGAGCGCGC)·d(GCGCGCTCGC) (T17), d(GCGTGCGCGC)·d(GCGCGCACGC) (A17), d(GCGCGTGCGC)·d(GCGCACGCGC) (T6), d(GCGCGGGCGC)·d(GCGCCCGCGC) (G6), and d(GCGCGAGCGC)·d(GCGCTCGCGC) (A6). These complexes are designated MC–C17, MC–T17, MC–A17, MC–T6, MC–G6, and MC–A6, respectively. The original complex between mitomycin C and GC10, designated MC–GC10 (Figure 2), is used for comparisons. Results from AMBER[8] energy refinements of these complexes are given in Tables II–IV and Figures 3–5 give stereo pairs for some of them. The remaining stereo pairs are in the microfilm edition. Table II lists the relative net binding energies for drug–DNA interactions. It is not possible to compare total energies among the seven complexes because they are chemically different. However, the net binding energies, which equal the total intermolecular binding energies minus the energies of distortion in the drug and DNA that occur on binding, may be com-

(5) Tomasz, M.; Lipman, R.; Verdine, G.; Nakanishi, K. *Biochemistry* **1986**, *25*, 4337.

(6) Ueda, K.; Komano, T. *Nucleic Acids Res.* **1984**, *12*, 6673.

(7) Ueda, K.; Morita, J.; Komano, T. *Biochemistry* **1984**, *23*, 1634.

(8) Weiner, P. K.; Kollman, P. A. *J. Comput. Chem.* **1984**, *2*, 287. Version 3.0 of AMBER (UCSF) was developed for the FPS by Singh, U. C.; Weiner, P. K.; Caldwell, J. W.; Kollman, P. A., Deptartment of Pharmaceutical Chemistry, University of California, San Francisco, CA 94143 (1985).

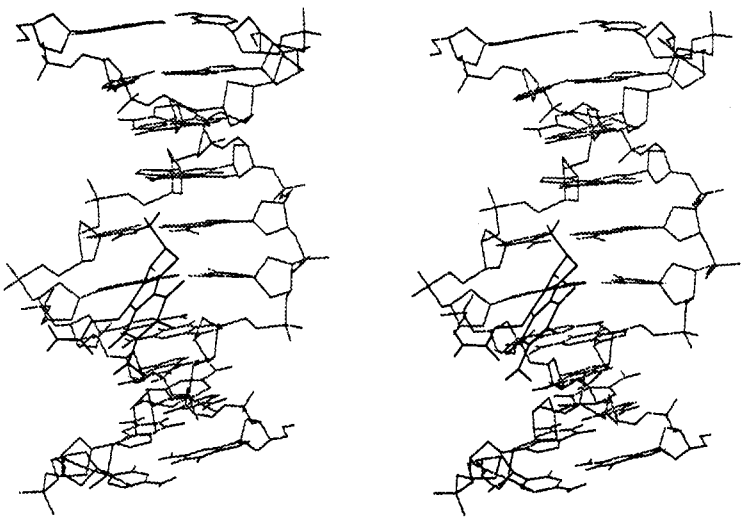


Figure 2. Stereopair for the covalent complex MC-GC10.

Table II. Energies (in kcal/mol) for Interactions between Mitomycins and Decanucleotides[a]

	total distortion energies							intermolecular				intramolecular				
		bond length		bond angle		dihedral				total[c]			helix[d]		drug[e]	net
complex	total	drug	DNA	drug	DNA	drug	DNA	elstat[b]	vdw	distort	total	helix	distort	drug	distort	binding
MC-GC10	-970.5	0.5	5.0	1.9	107.8	12.6	271.3	-117.3	-33.2	4.0	-146.9	-824.1	26.3	0.2	8.5	-120.4
MC-C17	-964.3	0.5	4.9	1.8	107.6	12.2	271.2	-114.7	-32.1	3.9	-140.6	-820.4	29.8	0.0	8.3	-110.4
MC-T17	-981.2	0.5	5.2	1.9	107.0	13.2	271.1	-113.9	-30.5	3.9	-140.8	-840.9	29.8	0.4	8.9	-110.8
MC-A17	-984.0	0.5	5.1	1.8	107.8	13.0	271.0	-112.6	-31.2	3.9	-143.8	-843.4	29.9	0.2	8.5	-113.9
MC-T6	-988.6	0.5	5.2	1.9	107.5	12.7	271.3	-119.9	-32.2	4.2	-148.7	-862.0	21.9	0.4	8.9	-126.4
MC-G6	-963.3	0.5	5.0	1.8	110.3	12.0	271.3	-116.6	-33.2	4.6	-148.8	-840.4	26.3	-0.4	7.9	-122.1
MC-A6	-984.3	0.5	5.1	1.9	108.5	12.0	271.1	-119.4	-33.0	4.1	-145.7	-861.3	22.6	-0.4	7.9	-122.6
7-GC10	-936.0	0.9	5.8	6.7	117.2	4.1	277.0	-106.3	-18.2	1.6	-124.5	-829.1	21.3	16.7	1.0	-102.2
8-GC10	-952.7	1.1	5.3	7.9	108.3	49.3	279.5	-126.5	24.6	0.0	-151.1	-835.6	14.8	34.0	6.9	-129.4
4 GC10	-969.0	1.6	4.9	6.0	110.8	20.7	268.8	-140.0	-31.1	4.0	-171.1	-819.0	31.4	21.2	14.0	-125.7
5 GC10	-1010.2	1.1	5.0	5.5	110.7	27.0	268.5	-183.6	-28.0	4.0	-211.6	-816.0	34.4	27.5	12.5	-164.7
6 GC10	-964.8	1.0	5.0	3.0	109.9	14.0	268.4	-119.0	-31.4	3.9	-150.4	-820.2	36.0	5.8	2.8	-111.6

[a] All the complexes are covalent except for 8-GC10. [b] In the AMBER force field, the electrostatic component of hydrogen bonds is evaluated as a normal Coulombic interaction with distance-dependent dielectric constant, and it is included with the other electrostatic energies. The other component is evaluated as a soft 10–12 van der Waals term, which accounts for only a small part of the energy (±1.5 kcal/mol). [c] The sum of components of bond length, bond angle, and dihedral angle distortion in the drug–DNA interaction energies. These energies should not be compared directly because the structures differ chemically. However, they play a very small role in relative stabilization of one complex over another. Thus, the differences among the C1 covalently bonded compounds are within 0.3 kcal/mol. The 2.7-diamitosenes 7 and 8 have larger energy differences from the C1 covalent compounds, but they differ only 1.6 kcal/mol from each other. [d] The total energies of the energy refined decanucleotides GC10, C17, T17, A17, T6, G6 and A6, starting from B-DNA in the absence of interactions with the mitomycins, are, respectively, -850.4, -850.3, -870.7, -873.2, -866.7, -883.9, and -887.6 kcal/mol. [e] The energies of the drugs in forms for covalent binding (except 8) when minimized by themselves outside the complexes are as follows: mitomycin C, -8.3; 7, 15.7; 8, 27.1; 4, 7.2; 5, 14.9; 6, 3.0 kcal/mol.

pared. These distortion energies are calculated by subtracting the intramolecular energies of the drug and DNA in the complex from their energies obtained by separately minimizing each isolated molecule. Table II clearly shows that any change in the CYT4–GUA17 base pair (models MC–C17, MC–T17, and MC–A17) resulted in a significant decrease in net binding energy (6.5–10.0 kcal/mol) compared with MC–GC10. Thus, there is an apparent preference for a 5′-GC-3′ segment in the DNA strand containing the covalent linkage.

Specific interactions between drug and DNA residues that underlie the differences in net binding energies can be found in Table III. These residues are identified in the schematic of Figure 1. The phosphate groups are referred to as P_{n-m}, where n and m are the sequence numbers for bases at the 5′ and 3′ ends, respectively, and they include both the O3′ and O5′ atoms. For example, P_{3-4} is the phosphate group between the sugars attached to GUA3 and CYT4. Among the mutations of base pairs at positions 4 and 17 in the DNA, the most favorable arrangement is CYT4, GUA17 as found in MC–GC10. This arrangement has the best energy because of a hydrogen bond between the carbonyl oxygen in the carbamate group of the drug and the 2-amino group of GUA17 in MC–GC10 (Figure 2). Table IV lists the various hydrogen bond interactions. A similar hydrogen bond in the GUA4, CYT17 arrangement of MC-C17 (Figure 3) gives the second best net binding energy to MC–C17. Such a hydrogen bond cannot be formed when A–T or T–A base pairs are present at positions 4 and 17. In these cases, the carbonyl group swings away from the floor of the minor groove, as shown for MC–T17 in Figure 4. Consequently MC–T17 and MC–A17 have poorer energies than MC–GC10 and MC–C17. In the three cases in which GUA17 is replaced by another base, one of the hydrogens on the carbamate amino group is oriented toward P_{5-6}. However, the P–O dipole is not oriented suitably to afford strong hydrogen bonding. In all the complexes, the double helix has not undergone any major conformational changes compared with B-DNA. The only conformations affected significantly are the sugar pucker and the P–O3′ bond rotation (ω). For the sake of convenience in describing sugar puckers, we have divided the phase space (W) into three broad regions. In accordance with this classification, W values from 0° to 72°, 72° to 144°, and 144° to 180° correspond to C3′ endo, O1′ endo–C1′ exo, and C2′ endo, respectively.[1] In the case of intermediate puckers (O1′ endo–C1′ exo), phases of individual sugars are mentioned explicitly. In the complexes in which mitomycin C is covalently bound to N2 of GUA5 through its C1 atom, the

Table III. Interaction Energies (in kcal/mol) between Individual DNA Residues and Mitomycins[a]

	mitomycin-DNA system											
	sequence specificity study											
inter-action	MC-GC10	MC-C17	MC-T17	MC-A17	MC-T6	MC-G6	MC-A6	7-GC10	8-GC10	4-GC10	5-GC10	6-GC10
drug with												
P_{4-5}											-6.4	
GUA5	-7.0	-6.1	-6.0	-6.1	-5.8	-5.3	-5.7			-7.01	-7.6	-5.3
P_{5-6}	-13.5	-12.7	-14.0	-13.6	-12.9	-13.4	-13.0	-9.4	-7.1	-13.7	-17.6	-11.5
S6	13.6	12.9	13.5	13.3	13.1	13.3	12.9	13.7		13.1	13.3	10.2
base 6	-23.6	-23.6	-22.6	-22.4	-22.6	-19.2	-13.8	-20.4	-6.3	-23.9	-21.4	-20.4
P_{6-7}	-38.3	-37.0	-36.1	-36.8	-36.3	-37.1	-37.2	-34.3	-48.7	-37.5	-32.6	-26.6
S7	6.8	6.5	6.3	6.5	6.2	5.9	5.9	5.9		6.9	5.8	
GUA7	-10.7	-10.2	-9.9	-10.2	-10.4	-10.4	-10.6	-7.0		-10.3	-8.1	-7.4
P_{7-8}	-9.3	-8.9	-8.8	-8.9	-8.8	-8.8	-8.7	-8.5	-7.3	-9.2	-8.7	-5.6
P_{15-16}	-5.3	-5.0	-5.0	-4.9	-5.0	-5.2	-5.2			-5.1	-6.0	
CYT16	-11.9	-10.9	-10.7	-10.4	-11.3	-12.0	-12.0	-5.1	-6.5	-10.8	-11.5	-10.2
P_{16-17}	-8.0	-7.5	-7.4	-7.5	-7.6	-7.5	-6.1	-6.1	-9.9	-8.1	-11.1	-6.1
S17									6.4			
base 17	-10.4	-7.1	-6.3	-6.5	-10.1	-10.5	-10.4	-9.7	-9.6	-10.0	-11.4	-9.8
P_{17-18}	-20.6	-20.5	-20.3	-20.2	-20.2	-20.2	-20.1	-8.6	-37.2	-19.7	-53.3	-17.9
S18											9.3	
P_{18-19}								-18.0	-11.7	-24.0	-12.4	
poor stacking energies												
CYT6-GUA7										-1.3	-1.2	
CYT16-GUA17								-0.6	-2.5			
GUA5-base 6		-7.1	-6.9	-7.0	-5.6	-4.9	-6.8					
CYT16-base 17		-2.5	-4.1	-4.3	-3.8	-3.3	-3.7					

[a] All complexes are covalent except for 8-GC10, which is a noncovalent complex. Only interactions for which energies are greater in maagnitude than 5.0 kcal/mol are listed, except for base stacking in models for sequence specificity of mitomycin C-DNA interactions and for significantly differing interactions in the remaining complexes.

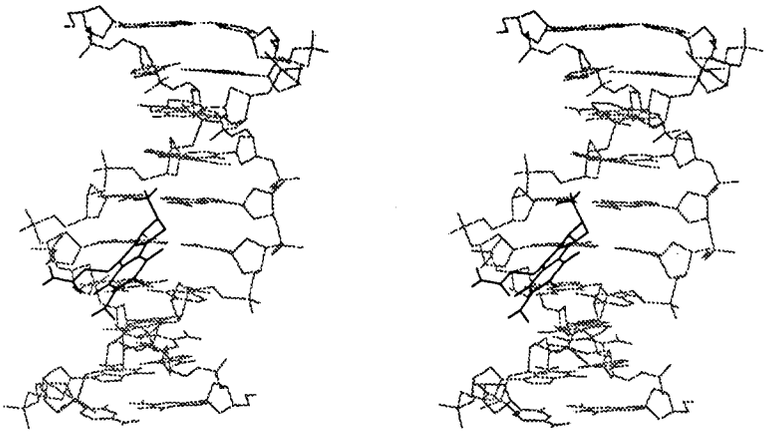


Figure 3. Stereopair for the covalent complex MC-C17.

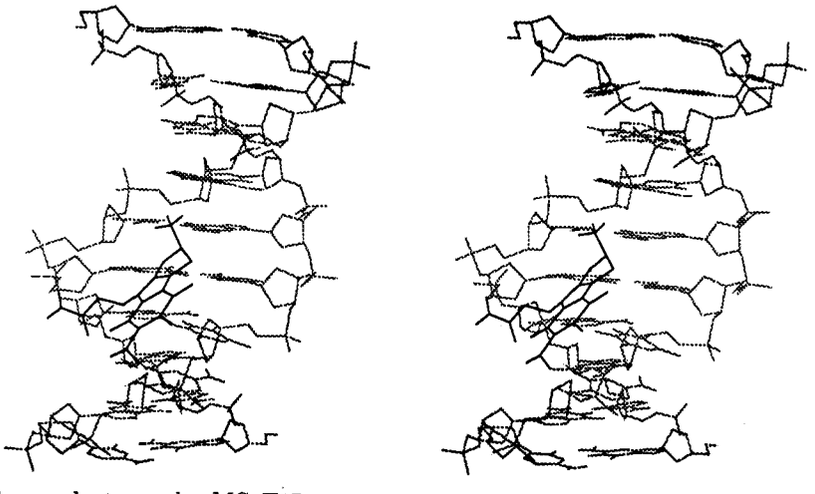


Figure 4. Stereopair for the covalent complex MC-T17.

sugar geometries of CYT2, GUA3, CYT12, GUA13, and CYT18 are in the O1′ endo–C1′ exo regions. The phases of these sugars are typically around 135°, with the exception of GUA3 whose sugar has W values around 105°. Further, the P–O3′ conformations at the 3′ ends of CYT6, CYT8, GUA15, and CYT18 are trans (ω ranging from 200° to 220°) in these complexes. All the sugars in complexes of 4–6 are in the C2′ endo region and GUA5 (in 7) and CYT14 (in 8) have respectively O1′ endo (W = 82°) and C1′ exo (W = 111°) puckers. The P–O3′ conformation is

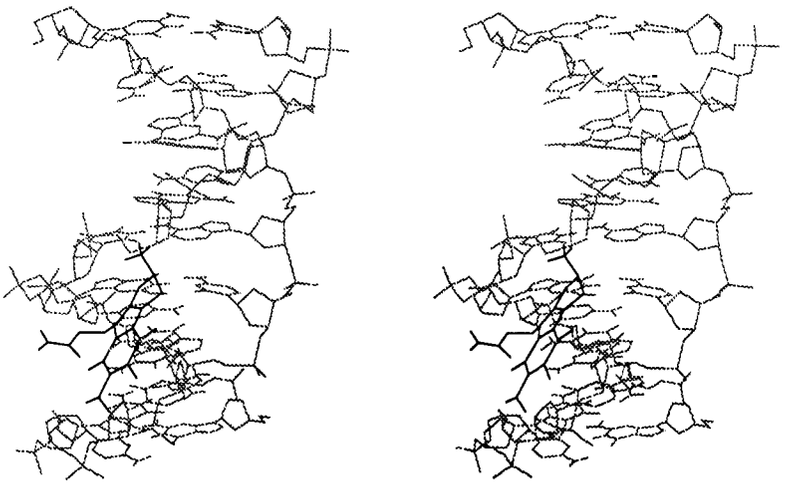


Figure 5. Stereopair for the covalent complex MC-T6.

Table IV. Hydrogen-Bond Parameters Involving Mitomycin-Polynucleotide Interactions[a]

complex	hydrogen donor (X-H)	acceptor atom (Z)	length, Å	angle, deg
MC-GC10	N7-HN7B(MIT)	O2(CYT6)	1.75	142.2
	N2-HN2B(MIT)	$O(P_{17-18})$[b]	1.75	141.3
	N2-HN2B(GUA17)	O10A(MIT)	2.00	158.0
MC-C17	N7-HN7B(MIT)	$O(P_{17-18})$	1.76	140.8
	N2-HN2B(MIT)	O2(CYT6)	1.75	143.2
	N2-HN2B(GUA4)	O10A(MIT)	2.18	150.2
MC-T17	N7-HN7B(MIT)	$O(P_{17-18})$	1.75	141.9
	N2-HN2B(MIT)	O2(CYT6)	1.77	142.1
MC-A17	N7-HN7B(MIT)	$O(P_{17-18})$	1.76	140.9
	N2-HN2B(MIT)	O2(CYT6)	1.73	143.9
MC-T6	N7-HN7B(MIT)	$O(P_{17-18})$	1.75	141.1
	N2-HN2B(MIT)	O2(CYT6)	1.76	143.0
	N2-HN2B(GUA17)	O10A(MIT)	2.0	157.7
MC-G6	N7-HN7B(MIT)	$O(P_{17-18})$	1.75	141.3
	N2-HN2B(GUA17)	O10A(MIT)	2.13	153.0
MC-A6	N7-HN7B(MIT)	$O(P_{17-18})$	1.75	141.4
	N2-HN2B(GUA17)	O10A(MIT)	2.17	151.4
7-GC10	N7-HN7B(MIT)	O2(CYT6)	1.71	171.8
	N2-HN2A(MIT)	$O(P_{18-19})$	1.66	157.8
8-GC10	$N7-HN_7A(MIT)$	$O(P_{6-7})$	1.61	150.4
	O1-HO1(MIT)[c]	$O(P_{17-18})$	1.63	164.8
	N2-HN2B(MIT)	$O(P_{18-19})$	1.77	140.5
bifurcated	N2-HN2A(GUA17)	O10A(MIT)	2.54	
	N2-HN2B(GUA17)	O10A(MIT)	2.49	
4-GC10	N7-HN7B(MIT)	O2(CYT6)	1.73	146.6
	N2-HN2B(MIT)	$O(P_{17-18})$	1.75	151.0
	O6-HO6(MIT)[c]	$O(P_{18-19})$	1.63	164.2
	N2-HN2B(GUA17)	O10A(MIT)	2.13	
5-GC10	N7-HN7B(MIT)	O2(CYT6)	1.75	143.3
	N2-HN2B(MIT)	$O(P_{17-18})$	1.66	142.8
	N2-HN2B(GUA17)	O10A(MIT)	2.08	
6-GC10	N7-HN7B(MIT)	O2(CYT6)	1.75	143.7
	N2-HN2B(MIT)	$O(P_{17-18})$	2.19	
	N2-HN2B(GUA17)	O10A(MIT)	2.12	

[a] In a hydrogen bond X-H···Z, X and Z are respectively donor and acceptor atoms and the hydrogen bond length corresponds to the distance between H and Z, whereas the angle is X-H···Z. [b] Phosphate oxygens are always the one designated B. [c] HO6 is the phenolic one.

trans at the 3′ ends of GUA15 in 4–6 and at 3′ ends of CYT18 in 7 and 8. In addition, ω' is around 215° in the phosphodiester between CYT16 and GUA17 in the complex 7.

Mutations in the bases at positions 6 and 15, as in covalent complexes MC-T6, MC-G6, and MC-A6, produce small changes in the intermolecular binding energies. In MC-T6, wherein thymine replaces cytosine, the drug-DNA interactions are very similar to those in MC-GC10, because the carbonyl oxygen of the pyrimidine is still in the right position to form hydrogen bonds with the protonated amine at C2 of mitomycin (Figure 5). However, when a purine replaces cytosine, as in MC-G6 and MC-A6, this interaction is not feasible. If the purine is adenine (MC-A6), the remaining drug-DNA interactions are more favorable than when it is guanine, possibly because the 2-NH_2 group of guanine projects into the minor groove. As a consequence, MC-G6 has the poorest intermolecular binding energy by about 3 kcal/mol. The helix distortion energies for MC-GC10 and MC-A6 are 4.4 kcal/mol greater than that of MC-T6, and the helix for MC-G6 is 1.6 kcal/mol less stable (Table II). These values, combined with drug distortion energies and total intermolecular binding energies, give MC-T6 a greater net binding energy than those of MC-G6 and MC-A6 by about 4 kcal/mol and by 6 kcal/mol over MC-GC10. These differences might not be large enough to give sequence specificity with certainty, but there is a preference for thymine at base position 6. The combined preferred sequence then becomes 5′-CGT-3′ for the 4–6 positions.

In MC-T6 and MC-A6 the predominant conformational changes are in the sugar puckers of CYT2, GUA5, CYT12, GUA13, and CYT18, as they were in the other four complexes (Table V). The torsion about the covalent bond between N2 of GUA5 and C1 of mitomycin is not significantly different when the base at position 6 is a purine than when it is a pyrimidine. Consequently, a purine can be accommodated with only small changes in the protonated amino group at C2 of mitomycin. In all three complexes, the lengths of the hydrogen bonds between the 2-amino group of GUA17 and the carbonyl oxygen of the carbamate chain in mitomycin are longer than in MC-GC10 by about 0.1–0.2 Å (Table IV).

Table V. Comparison of Net Binding Energies for Mitomycin Analogues with Their Potencies against P388 Leukemia in Mice[a]

compd	net binding energy, kcal/mol	minimum effective dose, mg/kg
1	−119.2	0.2
4	−125.7	0.1
5	−164.7	0.05
6	−111.6	0.2

[a] Antitumor assays conducted by Dr. W. T. Bradner at Bristol-Myers Co., Syracuse, NY. A tumor inoculum of 10^6 ascites cells was implanted in CDF_1 femal mice. Six mice were used at each dose of the compound, given on day 1, and 10 control mice were injected with saline. The minimum effective dose is the lowest dose required to give a 25% increase in lifespan with respect to the controls.

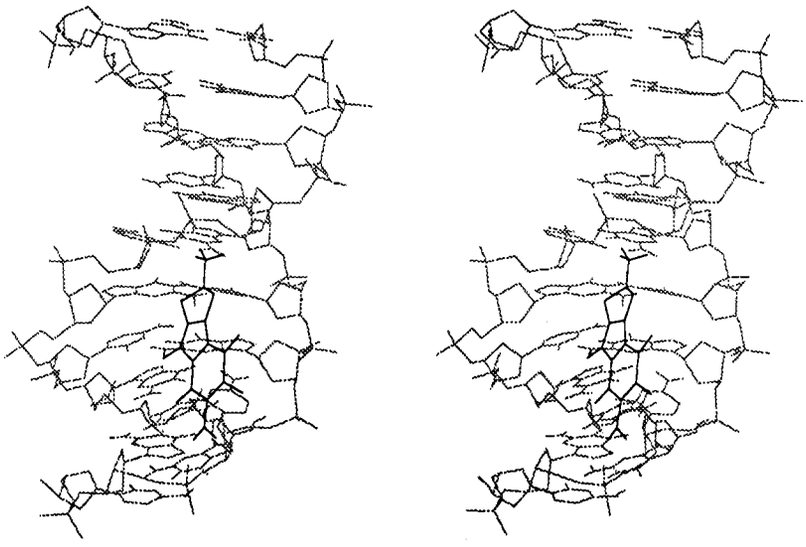


Figure 6. Stereopair for the covalent complex between **7** and GC10.

Our next investigation involved the derivation of a satisfactory model for the covalent binding of 2,7-diaminomitosene (**7**), a mitomycin C reduction product,[9] with a DNA segment. It lacks a substituent on C1, which prevents it from alkylating a guanine residue DNA according to the first step used by mitomycin C. However, it does possess the 10-carbamoyloxy substituent, which is used by mitomycin C in its second, cross-linking, alkylation of a guanine residue in the complementary strand. Recently it has been found that this compound can alkylate DNA, at least when it is reduced catalytically in the presence of isolated calf thymus DNA.[10] The alkylation site on DNA has not been determined, but previous work on mitomycin C suggests that it should be the 2-NH_2 group of a guanine residue.[5] GC10 was chosen as the polynucleotide for our modeling study because it had been used in previous mitomycin modeling studies.[2,3] However, no attempt had been made to model monocovalent binding at C10 in the earlier work.

The starting geometry for 2,7-diaminomitosene, protonated on its 2-amino group, was derived from the mitomycin portion of monoalkylated GC10. After replacement of the C–N bond at C1 by a C–H bond and replacement of the carbamoyloxy group at C10 by a C–H bond, the structure was reminimized in AMBER. Previously used partial atomic charges were retained, except for C1, which was adjusted to reflect its substitution by hydrogen rather than nitrogen, and for C10, which was adjusted to reflect replacement of the carbamoyloxy group (C–O bond) by a C–N bond in the monocovalent complex to be modeled. The resulting structure was docked so that its C10 was within covalent bond distance from the 2-NH_2 group of GUA17, the same base bonded in the second alkylation by mitomycin C, and the complex was minimized in AMBER. As shown in Figure 6, an excellent model was obtained. The orientation of the mitosene molecule was approximately the same as that found in the cross-linked species derived from mitomycin C;[2] however, the former had less net binding energy (noncovalent) than the latter by about 11 kcal/mol (Table II). This difference reflects higher electrostatic binding energy (36 kcal/mol) in the cross-linked complex partly cancelled by its higher helix distortion energy (27.7 kcal/mol). Significant differences in electrostatic energies involve GUA5 and CYT16. One of the key interactions is a hydrogen bond between its O2 of CYT16 and HN3C of mitomycin.

The helix distortion energy for GC10 covalently bound with 2,7-diaminomitosene was 21.3 kcal/mol (Table II). There was very little decrease in the energies of the Watson–Crick hydrogen bonds, but some significant losses in base stacking energies were evident (Table IV). In particular, the CYT6–GUA7 and CYT16–GUA17 interactions were only −1.3 and −0.6 kcal/mol, respectively, compared with about −8 kcal/mol for a typical interaction. It can be seen in Figure 6 that these bases have poor overlap, and CYT16 and GUA17 are tipped away from each other. The main conformational changes were in the pucker of the sugar attached to GUA5, which had a phase angle of 82.2° (C3′ endo) compared with 176.5° in GC10 and dihedral angle C3′–O3′–P–O5′, which was 153.5° compared with 212.3° in GC10.

Once a satisfactory model was obtained for the covalent binding of 2,7-diaminomitosene to GC10, it became interesting to examine the noncovalent binding that precedes this alkylation. Protonated 2,7-diaminosene could not be used for a model because this compound does not alkylate DNA unless it is reduced. The corresponding hydroquinone (**8**) and methylene iminium ion **9** appeared to be better choices. The former was selected because there is no evidence for the latter as there had been for the Moore's intermediate[11] used in noncovalent binding of mitomycin C.[3]

Partial atomic charges for hydroquinone **8** were obtained by combining those for the indoloquinone fragment, which were obtained by calculation with GAUSSIAN-80 UCSF[12] with a STO-3G basis set (Table I), with those of the ammonium ion substituted pyrrole ring fragment from mitomycins used in our previous publications. The hydroquinone structure was built in AMBER, given the partial atomic charges, and refined by energy minimization. It was then docked in several ways in the minor grove of GC10 so that C10 was reasonably close to the 2-NH_2 group of GUA17, and the resulting complex was refined in AMBER. Figure 7 shows a stereo pair for the best refined structure. This model has a surprisingly close resemblance to that of mitomycin C covalently bonded by its C1 (Figure 2), which was derived completely independently. This result suggests that there might be a rather specific binding site for

(9) Tomasz, M.; Lipman, R. *Biochemistry* **1981**, *20*, 5060.
(10) Iyengar, B. S., personal communication.
(11) Moore, H. *Science (Washington, D.C.)* **1964**, *145*, 55.
(12) Singh, U. C.; Kollman, P. A. *J. Comput. Chem.* **1984**, *2*, 129.

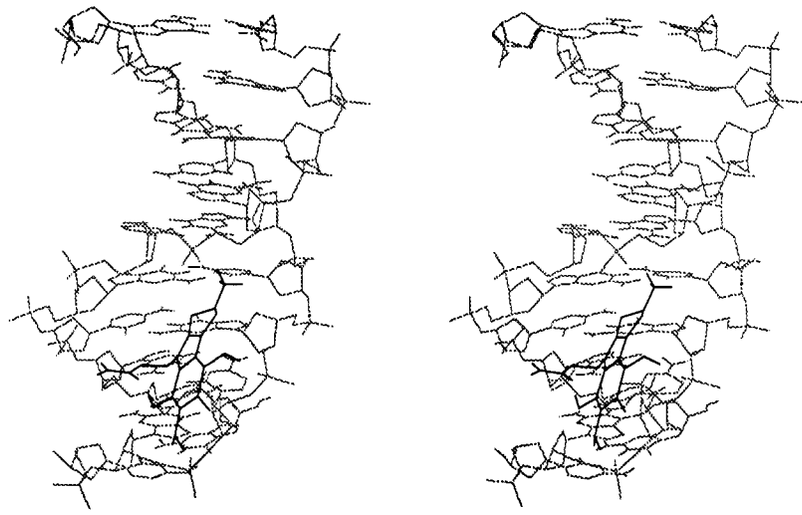


Figure 7. Stereopair for the noncovalent complex between 8 and GC10.

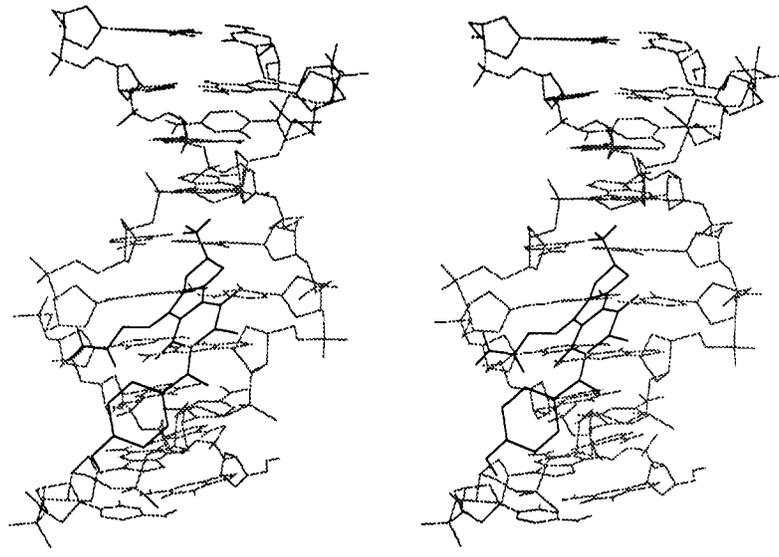


Figure 8. Stereopair for the covalent complex between **4** and GC10.

mitomycins in the minor groove of DNA. The distance between C10 of the hydroquinone and the 2-amino nitrogen of GUA17 is 3.17 Å, approximately the combined van der Waals radii of these two atoms and very favorable for subsequent covalent bond formation. As shown in Table II, the net binding in the complex is strong, with both the electrostatic and van der Waals forces making substantial contributions. Interactions between the drug and phosphate groups P_{6-7} and P_{17-18} are particularly strong as shown in Table III. They reflect hydrogen bonding between both HN1B and HO1 of the hydroquinone ring and P_{17-18}, plus a hydrogen bond between HN3A of the NH_3^+ group and P_{6-7} (Table IV). There also was a weak intramolecular hydrogen bond between HN1B and O2 in the drug.

Helix distortion energy in the noncovalent complex was less than that in the corresponding covalent complex but still significant (14.8 kcal/mol). No significant changes were found in Watson–Crick base pair energies, but decreased base-stacking energies were apparent (Table IV). As in the covalent complex, the binding between the CYT6–GUA7 and CYT16–GUA17 pairs was reduced to −1.2 and −2.5 kcal/mol, respectively. The main conformational changes were in puckers of the sugars attached to CYT2 (168.5° compared with 103.9° in GC10) and GUA5 (118.7° compared with 176.5°), and dihedral angle C3′–O3′–P′O5′, which was 131.6° compared with 212.3°. Two of these changes were also found in the covalent binding model.

As discussed in the introduction, revision of models for the covalent binding of important mitomycin C analogues to GC10 is necessary because recent experimental evidence showed that the binding site was the 2-amino group of guanine, rather than O6 of guanine. Revised models were derived for RR-150, M-83, and BMY-25282, on the basis of the previously described structures and partial atomic charges of these drugs.[3] Table II lists the energies obtained for the noncovalent interactions of each drug with GC10. All of them have substantial net binding energies, which fall in the order BMY-25282 > M-83 > mitomycin C > RR-150. Most of the differences in net binding energies result from differences in the relative electrostatic binding energies, which, in turn, can be assigned to specific interactions between the drugs and individual DNA residues as given in Table III. Thus, M-83 (**4**) makes a strong interaction with P_{18-19} in addition to the interactions that it has in common with mitomycin C. This interaction results from a hydrogen bond between the phenolic hydroxyl group and the phosphate residue (Table III and Figure 8). BMY-25282 (**5**) also interacts with P_{18-19}, but not by hydrogen-bond formation. It has an electrostatic attraction between its positively charged 7-substituent group and this phosphate. The strongest interaction for **5** is with P_{17-18}, probably because it involves a hydrogen

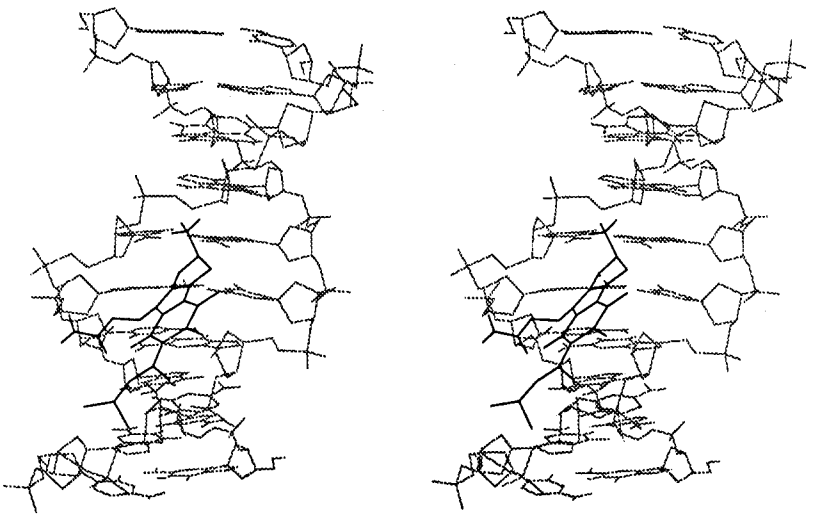


Figure 9. Stereopair for the covalent complex between **5** and GC10.

bond plus electrostatic attraction between the positively charged 7-substituent and the phosphate (Table III and Figure 9). The ethylthio substituent of RR-150 (**6**) confers no additional binding energy with GC10. In the guanine-O6 binding model it did form a hydrogen bond with a phosphate group,[3] but this does not occur in the present model. Even biasing the model by placing the SH group near a phosphate leads to a minimized structure without the expected hydrogen bond.

None of the models for mitomycin C or its analogues showed any significant decrease in the strength of Watson–Crick base pair interactions or any substantial changes in sugar puckers or backbone dihedrals. However, there were two significant decreases in base stacking energies in all cases. The interactions between CTY4 and GUA5 were reduced to −3.5 to −3.8, and the interactions between CYT6 and GUA7 varied from 0.0 to +0.3.

A question naturally arises as to whether or not the calculated net binding energies of mitomycin C and its analogues show any correlation with their observed antitumor activities. A preliminary attempt at such a correlation is given in Table V, in which the net binding energies are compared with the minimum effective doses against P388 lymphocytic leukemia in mice. Rather surprisingly, there is an apparent qualitative correlation. We acknowledge that this correlation might be fortuitous and that there are too few compounds for statistical significance. However, the result suggests that it might be worthwhile to undertake a more extensive correlation, perhaps including other factors such as partition coefficient and quinone reduction potential.

Conclusions

Our previously derived model for covalent binding of mitomycin C in the minor groove of the decanucleotide duplex GC10 was extended to examine the possibility that sequence specificity might exist in mitomycin–DNA binding. The results indicate that the best base to occupy the 5′ side of the guanine to which mitomycin C is covalently bound is cytosine, because of strong hydrogen bonding between the mitomycin carbamate carbonyl and the 2-amino group of the guanine complementary to this cytosine. This conclusion is qualitatively consistent with the experimentally observed preference of mitomycin C binding to poly(dG-dC)·poly(dG-dC) over that to poly(dG)·poly(dC).[13,14] The presence of guanine on the 5′ end of the alkylated guanine results in weaker hydrogen bonding interactions between the carbamate chain of the drug and the exocyclic amino group of the guanine. Specificity for the base at the 3′ side appears less certain, but the preference is for thymine. Thus, the best calculated sequence specificity is 5′-CGT-3′, which agrees partially with a PuGT sequence found by studies on DNA damage induced by the mitomycin C. However, other experimental evidence, obtained from hydrolysis of the covalent mitomycin–calf thymus DNA complex by nuclease P1, suggested no specificity for the base at the 3′ side.[15] The discrepancy between the two experimental methods makes any conclusion about sequence specificity uncertain. However, the calculated preference for CGT is not well supported. An obvious reason for the discrepancy is that there are two components to drug–DNA specificity: desolvation of the DNA and drug and interaction of the drug with DNA. We have only calculated the latter in a rather simple fashion. Thus, the lack of correlation with observed sequence specificity is not too surprising.

A satisfactory model was obtained for the monocovalent binding of 2,7-diaminomitosene, by way of its carbamate side chain, with the decanucleotide duplex GC10. The corresponding hydroquinone, which is a reasonable intermediate in this reaction, also gave a model that had a low energy position with five hydrogen bonds and low distortion in the mitomycin binding site. The fact that independently derived models for C10 alkylation by 2,7-diaminomitosene and C1 alkylation by mitomycin have closely related energy refined structures suggests that there is one best way for mitomycins to bind in the minor groove of double-helical DNA. This binding involves positioning of the mitomycin by specific hydrogen bonds between the mitomycin 7-amino group and P_{17-18} and between the protonated 2-amino group of mitomycin and O2 of CYT6 in the GC10 model.

The presence of a favored binding site for mitomycins on GC10 also was supported by models for three highly active mitomycin C analogues. In these models, the analogues made all of the same hydrogen bonds as mitomycin C, and one of them had an additional specific hydrogen

(13) Mercado, C. M.; Tomasz, M. *Biochemistry* **1977**, *16*, 2040.

(14) Tomasz, M.; Barton, J. K.; Magliozzo, C.; Tucker, D.; Lafer, E. M.; Stollon, B. D. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 2874.

(15) Tomasz, M.; Chowdary, D.; Lipman, R.; Shimotakahara, S.; Veiro, D.; Walker, V.; Verdine, G. L. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 6702.

bond that further stabilized the complex. Although drug–DNA binding is only one of many factors involved in the antitumor activity of mitomycins, it is a necessary condition, and we expect that any analogue with good activity will give a good binding model. This clearly is the case with BMY-25282, RR-150, and M-83. Of course, the converse is not necessarily true; a compound that binds well to DNA might have poor activity because of problems with uptake by tumor cells, bioactivation, and nonspecific interaction with proteins.

It must be emphasized that the molecular mechanical approach used here does not give a quantitative analysis of the free energies of interactions between mitomycins and DNA, or of the energetics for the reaction pathway for covalent binding. The calculated total energies do not include solvent and counterion effects. However, by limiting the investigation to closely related mitomycin analogues and by using net binding energies for comparisons, the results of this investigation should be useful in increasing our understanding of the way in which mitomycins interact with DNA. And this understanding might lead to improved efficiency in the development of newer analogues. Although we do not know at this time that a compound that shows good binding will have good antitumor activity, it seems reasonably certain that one that does not bind well will have poor activity. Thus, fewer compounds will need to be synthesized and screened by rejecting those that give poor calculated binding energies.

Experimental Section

Initial representations for the binding of mitomycin C and related compounds to decanucleotide duplexes were obtained by docking the drugs near the site of covalent binding at the 2-amino group of GUA5 in the minor groove of appropriate decanucleotides by using the MIDAS[16] or CHEM[17] molecular graphics programs. Nomenclature used in describing these decanucleotides is illustrated in Figure 1. Coordinates for the docked structures were captured, and the resulting models were refined by energy minimization using the program AMBER[8] with the covalent bond formed between N2 of GUA5 and either C1 or C10 of mitomycin C and its derivatives and analogues. Force field parameters presented by Weiner et al.,[18] and extended by Rao et al.,[2] were employed in the calculations. The united atom model was chosen for both drugs and DNA residues. Each isolated drug model and each isolated decanucleotide sequence was modeled separately so that the distortion energy induced in each when the drug bound to the decanucleotide could be calculated. As in our earlier studies, the starting structure for each decanucleotide was based on B-DNA geometry proposed by Arnott and co-workers.[19] All structures were refined until the root mean square gradient was less than 0.1 kcal/mol Å. A distance-dependent dielectric constant was used in all calculations. All nonbonded pairs were included in the calculations. Charges on the atoms of 2,7-diaminomitosene hydroquinone were calculated by GAUSSIAN-80 UCSF with an STO-3G basis set (Table 1).[12] Other mitomycins had been calculated previously by using the same method.[2,3]

AMBER has been used in a number of investigations on drug-nucleic acid complexes, protein–substrate complexes, and small molecule conformational analysis in our laboratories.[20-26] Among the drug-nucleic acid complexes, both intercalation (ethidium, actinomycin, and daunomycin) and nonintercalation (anthramycin, CC-1065, and diol epoxides of polycyclic aromatic hydrocarbons) have been studied. The results have been generally qualitatively consistent with the experimental data, implying that the force field parameters are physically meaningful. Although the parameters in the AMBER force fields were developed for peptides and nucleic acids, their extension to a number of other structural types has been successful.[18,20-26] The values of bond lengths and bond angles in energy-minimized structures are quite close to those of equilibrium values obtainable in the force field.[18] Typically the bond length deviations are 0.1–0.3 Å and the bond angle deviations are 1–5° from the ideal equilibrium geometries. These deviations are within the ranges observed through high-resolution crystallographic studies on small molecules (with the probable exception of highly strained molecules, which we do not model). It should be noted that equilibrium bond lengths and angles in the AMBER force field are derived from the statistical averaging of crystallographic and spectroscopic data.

The helix distortion energy, as evaluated in the present study, is the difference in energies of polynucleotide part of the complexes (not corrected for the missing hydrogen in the 2-amino group of the covalently bound guanine) and the polynucleotide without the drug covalently bound to it. The absolute value of this distortion energy cannot be used to compare the distortion in a complexed polynucleotide relative to uncomplexed polynucleotide, because they are chemically different molecules. However, it is meaningful to compare the distortion energies among various complexes based on the same drug and polynucleotide and to draw inferences on their relative stabilities. The dominant components in the relative distortions are van der Waals and electrostatic (including hydrogen bonding) interactions. Bond length and bond angle contributions make little difference, as seen in Table II.

Acknowledgment. This investigation was supported by NIH National Cancer Institute Grants CA-37798 (to W.R.) and CA-25644 (to P.K.). We also gratefully acknowledge the use of facilities of the University of California, San Francisco, Computer Graphics Laboratory (R. Langridge, director, and T. Ferrin, facility manager), supported by NIH RR-1081. The FPS-264 computer used for some of the calculations was purchased through grants from the NSF (DMB-84-13762) and NIH (RR-02441).

Registry No. 1, 50-07-7; 1-GC10, 114883-90-8; 1-C17, 114883-91-9; 1-T17, 114925-29-0; 1-A17, 114883-92-0; 1-T6, 114883-93-1; 1-G6, 114883-94-2; 1-A6, 114923-87-4; 4, 114820-09-6; 4-GC10, 114923-98-7; 5, 114836-85-0; 5-GC10, 114925-30-3; 6, 83586-81-6; 6-GC10, 114883-95-3; 7-GC10, 114820-05-2; 8-GC10, 114820-07-4; 4,7-dihydroxy-1,2,3,6-tetramethyl-5-indolamine, 114820-08-5.

Supplementary Material Available: Figures 10–13 showing the stereopairs for the covalent complexes MC-A17, MC-G6, and MC-A6 and the covalent complex between 6 and GC10 (5 pages). Ordering information is given on any current masthead page.

(16) Langridge, R.; Ferrin, T. E. *J. Mol. Graphics* **1984**, *2*, 5.
(17) CHEM program written at UCSF Computer Graphics Laboratory by A. Dearing (1981).
(18) Weiner, S. J.; Kollman, P. A.; Case, D.; Singh, U. C.; Ghio, C.; Alagona, G.; Profeta, S., Jr.; Weiner, P. K. *J. Am. Chem. Soc.* **1984**, *106*, 765.
(19) Arnott, S.; Campbell-Smith, P.; Chandrasekaron, R. In *CRC Handbook of Biochemistry*; Fasman, G. D., Ed.; CRC: Cleveland, OH, 1976; Vol. 2, pp 411–422.
(20) Rao, S. N.; Singh, U. C.; Kollman, P. A. *J. Med. Chem.* **1986**, *29*, 2484.
(21) Remers, W. A.; Mabilia, M.; Hopfinger, A. J. *J. Med. Chem.* **1986**, *29*, 2942.
(22) Lybrand, T.; Kollman, P. A. *Biopolymers* **1985**, *24*, 1863.
(23) Nuss, M. E.; Marsh, F. J.; Kollman, P. A. *J. Am. Chem. Soc.* **1979**, *101*, 825.
(24) Bash, P. A.; Singh, U. C.; Brown, F. K.; Langridge, R.; Kollman, P. *Science (Washington, D.C.)* **1987**, *235*, 574.
(25) Alagona, G.; Desmeules, P.; Ghio, C.; Kollman, P. A. *J. Am. Chem. Soc.* **1984**, *106*, 3623.
(26) Blaney, J. M.; Weiner, P. K.; Dearing, A.; Kollman, P. A.; Jorgensen, E. C.; Oatley, S. J.; Burridge, J. M.; Blake, C. C. F. *J. Am. Chem. Soc.* **1982**, *104*, 6424.