were formed (1–6 h). The solid products **8a–d** were collected either by filtration (**8b–d**) or centrifugation (**8a**) and then successively washed with water (2 × 1.0 mL) and ethyl ether (1.0 mL) and dried at 40 °C (0.01 torr). Difficulties were experienced in combustion of **8a–c**. As a consequence, the microanalyses are not within 0.4% for **8a–c**. The yields and analytical data are presented in Table II.

Acknowledgment. These studies were conducted pursuant to a contract with the National Foundation for Cancer Research. The cancer lines were supplied by the DCT Tumor Repository, NCI Frederick Cancer Research Facility. We thank Dr. Walter Lürken for his expert advice on nomenclature and Dr. Suzanne Wehrli and Frank Laib for helpful discussions and technical assistance in obtaining the NMR spectra. One of the authors (N.U.M.R.) thanks the Graduate School of the University of Wisconsin—Milwaukee for a research assistantship.

Registry No. 4, 111-90-0; **5a**, 101347-40-4; **5b**, 101347-41-5; **5c**, 101347-42-6; **5d**, 101347-43-7; **6a**, 66943-05-3; **6b**, 33941-15-0; **7a**, 101347-44-8; **7b**, 101347-45-9; **7c**, 101347-46-0; **7d**, 101347-47-1; **8a**, 101248-21-9; **8b**, 101248-23-1; **8c**, 101248-25-3; **8d**, 101315-82-6; aziridine, 151-56-4; 2,2-dimethylaziridine, 2658-24-4.

Conformations of Complexes between Mitomycin and Decanucleotides. 2. Application of the Model to Mitomycin C Derivatives. Extension to Covalent Binding with Adenine[1]

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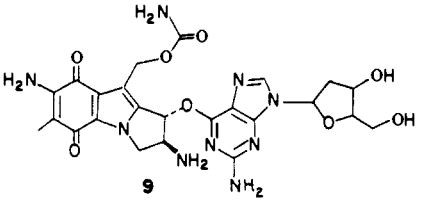
Molecular mechanics simulation of the interactions of important mitomycin C analogues monocovalently bound to DNA models are presented. These analogues included substituents such as *p*-hydroxyphenyl, 2-mercaptoethyl, and dimethylamidinium on N7 of mitomycin C and the DNA models consisted of $d(GCGCGCGCGC)_2$ and $d(GCGCATGCGC)_2$. The excellent fits and strong binding affinities of these highly potent analogues support the usefulness of the model. The binding of a mitomycin-related *N*-phenylpyrrole with a carbamoyloxy substituent to O6 of guanine was studied. Finally, a reactive mitomycin intermediate proposed by Moore was shown to interact with DNA in a way consistent with the formation of a covalent adduct.

The mode of binding of mitomycins to DNA has challenged investigators for over 20 years. An early proposal by Iyer and Szybalski that mitomycin C (**1**) is activated by reduction to a hydroquinone **2** followed by spontaneous loss of methanol (Scheme I) and that the resulting intermediate **3** alkylates DNA probably at O6 of guanine[3] has generally stood the test of time. However, a number of modifications have been proposed. Lown suggested that the initial alkylation probably involves aziridine ring opening with C1 of the mitomycin becoming the electrophilic center (**6** → **8**).[4] The second alkylation, involving DNA cross-linking, is a separate step (**8** → **7**).

More recently, an alternative reactive intermediate derived from the hydroquinone by aziridine ring opening and protropic rearrangement (Scheme I) was proposed by Moore.[5] This intermediate (**5**) has received indirect support from experiments that showed that reduction of mitomycin C in acidic medium gave mostly the 1-unsubstituted mitosene **4**.[6,7] Bachur has demonstrated that in biological systems the first intermediate formed from mitomycin C is a radical anion.[8] It would be surprising if this negatively charged species alkylates DNA directly. Whether it undergoes disproportionation to hydroquinone **3** is unknown.

Although the status of various reactive intermediates remains unclear, progress has been made in establishing the alkylation sites on DNA. By digestion of mitomycin-alkylated DNA with phosphatases, Hashimoto's group isolated mononucleotides covalently bound to the 1-position of the resulting 2-aminomitosene (**8**). The nucleotide bonds were O6 of guanine, *N*6 of adenine, and *N*2 of guanine, in decreasing order of abundance.[9] Tomasz confirmed the fragment **9** involving O6 of guanine.[10] Hashimoto further showed that the N^7-(*p*-hydroxyphenyl)



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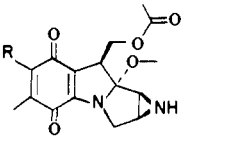
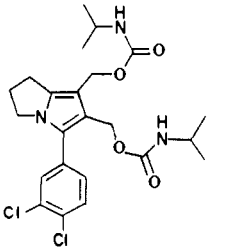


10 R = NHC_6H_5OH-P
11 R = NHC_6H_5I-P
12 R = $NHCH_2CH_2SH$
13 R = $N{=}CHN(CH_3)_2$
14 R = $NHCH{=}\overset{+}{N}(CH_3)_2$



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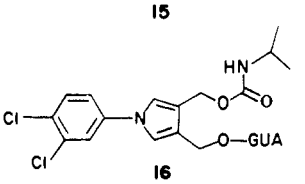

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(1) For the preceding article, see: Rao, S. N.; Singh, U. C.; Kollman, P. A. *J. Am. Chem. Soc.*, in press.
(2) Visiting Professor from the University of Arizona.
(3) Iyer, V. N.; Szybalski, W. *Science (Washington, D.C.)* **1964**, *145*, 55.
(4) Lown, J. W.; Begleiter, A.; Johnson, D.; Morgan, A. R. *Can. J. Biochem.* **1976**, *54*, 110.
(5) Moore, H. W. *Science (Washington, D.C.)* **1977**, *197*, 527.
(6) Tomasz, M.; Lipman, R. *Biochemistry* **1981**, *20*, 5056.
(7) Kohn, H.; Zein, N. *J. Am. Chem. Soc.* **1983**, *105*, 4105.
(8) Pan, S.-S.; Andrews, P. A.; Glover, C. J.; Bachur, N. *J. Biol. Chem.* **1984**, *259*, 959.
(9) Hashimoto, Y.; Shudo, K.; Okamoto, T. *Chem. Pharm. Bull.* **1983**, *31*, 861.
(10) Tomasz, M.; Lipman, R.; Snyder, J. K.; Nakanishi, K. *J. Am. Chem. Soc.* **1983**, *105*, 2059.

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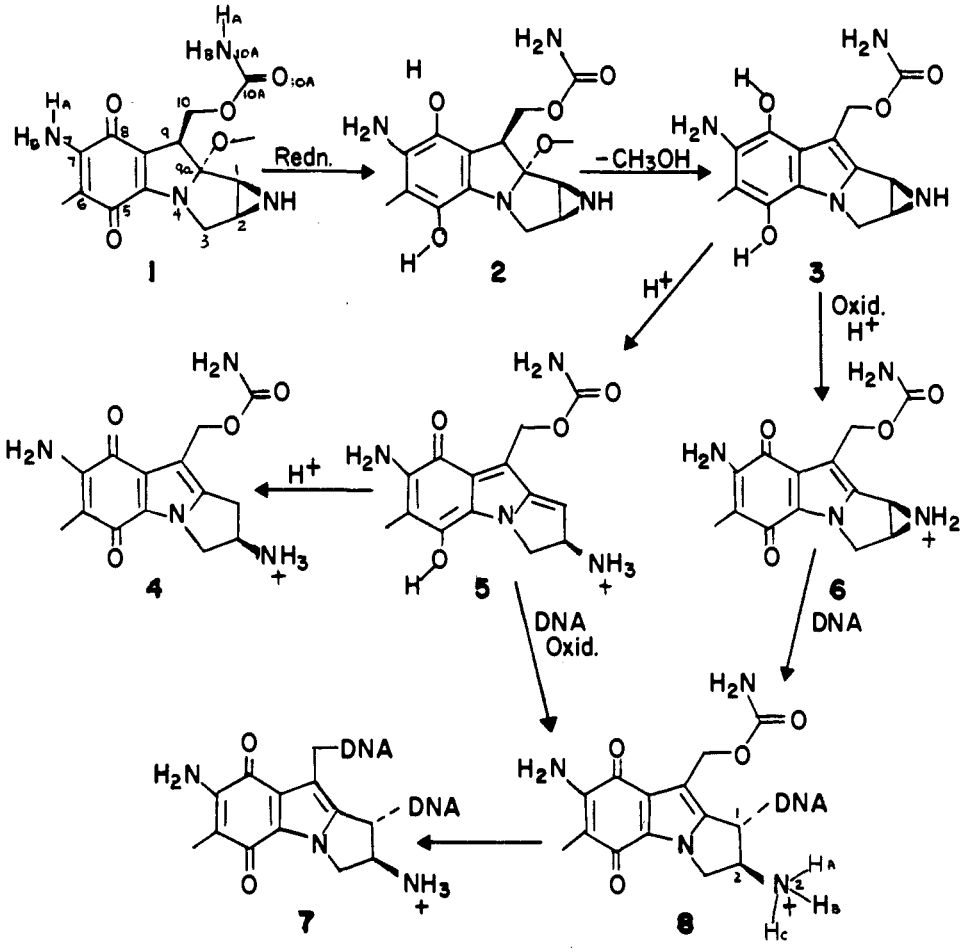
Scheme I



derivative (10) of mitomycin C (a clinical trial agent denoted M-83)[11,12] alkylated DNA with a preference of N6 of adenine over O6 of guanine.[13] Thus far, the isolation of fragments corresponding to cross-linked mitomycin has not been reported.

The results described above provide a basis for constructing models of mitomycin bound to DNA, but they offer little in the way of detailed structure and specific functional group interactions. Experimental evidence on which to further develop the model will be difficult to obtain because of the low frequency of alkylation sites per base pair (about one in 200). Fortunately it is now possible to construct computer models of considerable complexity using a combination of molecular graphics and molecular mechanics. Such models ultimately must be confirmed by physical measurements, but even in the absence of confirmation they can be used in drug development. The test of their value is whether they successfully correlate existing structure–activity relationships and aid the design of new analogues. In the case of mitomycin, a potentially useful model was derived, based on energy calculations performed with the program AMBER.[1] It encompassed noncovalent interaction of the reactive intermediate, protonated 7-aminoziridinomitosene (6, Scheme I), in the major groove, minor groove, and intercalated into d(GCGCGCGCGC)$_2$ with Arnott's BDNA geometry[14] (hereinafter called GC10).

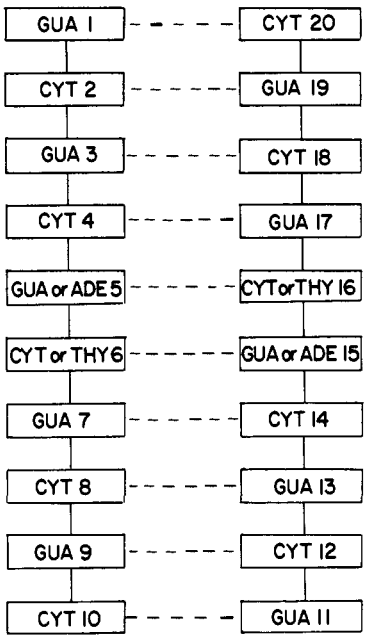


Figure 1. Schematic illustration of the nomenclature used in describing the decanucleotides d(GCGCGCGCGC)$_2$ and d-(GCGCATGCGC)$_2$, referred to as GC10 and GC2ATGC2 in the text.

It also included 2,7-diaminomitosenes, protonated on N2 (8), covalently bound to O6 of guanine (major groove) and

(11) Imai, R.; Morimoto, M.; Marumo, H.; Kobayashi, T.; Tsuruo, T.; Inaba, M.; Tsukagoshi, S.; Sakurai, Y. *Gann* 1981, *72*, 944.
(12) Meguro, S.; Nagata, T.; Yokoyama, K.; Chineu, T.; Yamazaki, H.; Kobayashi, T.; Isogai, Y.; Ogawa, M. *Invest. New Drugs* 1984, *2*, 381.
(13) Hashimoto, Y.; Shudo, K.; Okamoto, T. *Chem. Pharm. Bull.* 1982, *30*, 2644.
(14) Arnott, S.; Campbell-Smith, P. J.; Chandrasekaran, R. In *Handbook of Biochemistry and Molecular Biology*; Fasman, O. D., Ed.; CRC Press: Cleveland, OH, 1976; Vol. 2, p 411.

N2 of guanine (minor groove), and cross-linked structures (7). Among the salient features of structures obtained from this model were that the major groove was preferred for the noncovalently bonded structure. A network of four hydrogen bonds stabilized this structure and little distortion occurred in the structure of the DNA helix. For the monocovalent structures, the one with binding to O6 of GUA5 (see Figure 1 for an illustration of nomenclature of the decanucleotide residues) retained the pattern of hydrogen bonds, but destabilization of the helix resulted from the loss of a Watson–Crick hydrogen bond between GUA5 and CYT 16 and the "pulling" of GUA5 into the major groove. The corresponding monocovalent structure with binding to N2 of GUA5 was stabilized by only three hydrogen bonds, but little distortion occurred in the helix. Structures for cross-linking were based on the assumption that the carbamate was the second alkylating functionality. Two major groove possibilities involved C1 and C10 of mitomycin C binding to either O6 of GUA5 and O6 of GUA15 or to O6 of GUA15 and O6 of GUA5, respectively, whereas the only reasonable possibility for minor groove binding involved C1 and C10 of mitomycin C to N2 of GUA5 and N2 of GUA17, respectively. These structures retained important hydrogen bonds and caused little additional distortion in the structure of the double helix, except that, in the cases of major groove binding, a second change in Watson–Crick base pairing occurred. Thus, the model derived for mitomycin binding is reasonable for a variety of possibilities including noncovalent, monocovalent, and dicovalent (cross-linked) binding, and it accounts for productive hydrogen-bond interactions between DNA and the major functional groups of mitomycin C.

In the present paper, we extend this model to include noncovalent binding by another reactive intermediate, structure **5**, proposed by Moore.[5] Monocovalent linking is considered for N6 of adenine on the basis of a decanucleotide $d(GCGCATGCGC)_2$ with the right-handed B form (hereinafter denoted GC2ATGC2). The main emphasis, however, is on applying the model to certain mitomycin C analogues with significant activity in clinical or preclinical antitumor studies. Although the antitumor activity of mitomycins depends on a number of factors including their partition coefficient, quinone reduction potential, and substituent size,[15] it is anticipated that the best analogues should make optimal or at least very good binding to DNA. Comparisons of the binding energies of these analogues to that of mitomycin C are based on the structure with monocovalent binding to O6 of guanine because this structure is the best documented one.[9,10]

Methods

As in the earlier investigations,[1] conformational analysis has been carried out by molecular mechanics, wherein energy calculations were performed with the program AMBER (Assisted Model Building with Energy Refinement).[16] Force field parameters presented by Weiner et al.,[17] and extended to mitomycins as described earlier,[1] were used. Molecular mechanical energies were evaluated by eq 1, and the structures were refined until the rms gradient was less than 0.1 kcal/mol Å. A distance-dependent dielectric constant $\epsilon = R_{ij}$ was used in all the calculations. The charges on atoms in Moore's intermediate (**5**) were obtained by combining unchanged portions of the previously modeled mitosene with fragment A, whose charge distribution was obtained with use of quantum chemically derived electrostatic potentials from ab initio calculations employing an STO-3G basis set.[18] For mitomycin C analogues with substituents on N7, the 7-amino group was replaced by fragments including *p*-hydroxyaniline and dimethylamidinium ion, whose charges were calculated in the same manner. These charge distributions are available as supplementary material (appendix 1). The charge distribution for the 2-mercaptoethyl group was estimated from cysteine, whereas that of *p*-iodophenyl was estimated from the observed structure and dipole moment of iodobenzene.

$$E_{\text{total}} = \sum_{\text{bonds}} K_r(r - r_{eq})^2 + \sum_{\text{angles}} K_\theta(\theta - \theta_{eq})^2 + \sum_{\text{dihedrals}} \frac{V_n}{2}[1 + \cos(n\phi - \delta)] + \sum_{i<j} \left[\frac{A_{ij}}{R_{ij}^{12}} - \frac{B_{ij}}{R_{ij}^{6}} + \frac{q_i q_j}{\epsilon R_{ij}} \right] + \sum_{\text{H bonds}} \left[\frac{C_{ij}}{R_{ij}^{12}} - \frac{D_{ij}}{R_{ij}^{10}} \right] \quad (1)$$

As in an earlier study[1] the phosphate groups have been referred to as P_{n-m} where n and m are the sequence numbers of the bases at respectively the 5′ and 3′ ends. For example, P_{3-4} is the phosphate group intervening GUA3 and CYT4. As mentioned earlier in this paper, the nomenclature for the bases is illustrated in Figure 1.

Results and Discussion

A preliminary model for the nocovalent complex between Moore's intermediate (**5**) and the major groove of GC10 was constructed by using interactive computer graphics and the program CHEM.[19] The energy of this complex was then minimized by using the molecular mechanics program AMBER. Stereo pairs for the resulting structure are displayed in Figure 3. The distance between C1 of this intermediate and O6 of GUA5 is 3.20 Å, which is near the sum of the van der Waals radii of this atom pair. Thus, it is ready to form a covalent bond. Interestingly, the distance between the C10 atom of **5** and O6 of GUA17 in the opposite strand is virtually identical (3.22 Å) to the C1–O6 distance. This observation alone would suggest an equal probability for C1 and C10 alkylation. The predominance of C1 alkylation presumably reflects greater reactivity of the carbonyl system of **5** over the carbamate.

Hydrogen-bond interactions between **5** and GC10 (Table I) are similar to those obtained earlier for **6**. The main difference between these two structures is a longer distance between O10A of the mitomycin and HN4 of CYT14 in the former (see Scheme I for atom numbering of mitomycins). This results in a slightly reduced drug–helix interaction, but this effect is cancelled by a slightly enhanced internal drug energy, as shown in Table II. On balance, the interaction energies of the two intermediates are nearly equal. Consequently, molecular mechanics does not provide the basis for a choice between them. An analysis of the energies of interaction between **5** and individual residues of DNA is presented in Table III (see Figure 2).

Analysis of the sugar and phosphate units in DNA complexed with **5** revealed significant changes from normal BDNA values in two different structural elements. The

(15) Sami, S. M., Iyengar, B. S.; Tarnow, S. E.; Remers, W. A.; Bradner, W. T.; Schurig, J. E. *J. Med. Chem.* **1984**, *27*, 701.
(16) Weiner, P. K.; Kollman, P. A. *J. Comput. Chem.* **1984**, *2*, 287.
(17) 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.
(18) Singh, U. C.; Kollman, P. A. *J. Comput. Chem.* **1984**, 129.
(19) CHEM program written at the UCSF Computer Graphics Laboratory by A. Dearing (1981).

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

complex	hydrogen atom	acceptor atom	length, Å
5/GC10[c]	HN42(MIT)	O(P13-14)	1.68
	HN7B(MIT)	O(P14-15)	1.69
	HN2C(MIT)	O(P3-4)	1.63
	HN4(CYT14)	O10A(MIT)	2.31
mit C/GC2ATGC2	HN42(MIT)	O(P13-14)	1.66
	HN7B(MIT)	O(P14-15)	1.71
	HN2C(MIT)	O(P14-15)	1.61
	HN4(CYT14)	O10A(MIT)	2.12
10/GC2ATGC2	HO7(MIT)[b]	O(P13-14)	1.64
	HN7B(MIT)	O(P14-15)	1.70
	HN2C(MIT)	O(P3-4)	1.63
	HN4(CYT14)	O14A(MIT)	2.05
10/GC10	HO7(MIT)	O(13-14)	1.64
	HN7B(MIT)	O(P14-15)	1.70
	HN2C(MIT)	O(P3-4)	1.63
	HN4(CYT14)	O10A(MIT)	2.06
11/GC10	HN7B(MIT)	O(P14-15)	1.69
	HN2C(MIT)	O(P3-4)	1.63
	HN4(CYT14)	O10A(MIT)	2.07
12/GC10	HS(MIT)	O(P13-14)	1.73
	HN7(MIT)	O(P14-15)	1.70
	HN2(MIT)	O(P3-4)	1.62
	HN4(CYT14)	O10A(MIT)	2.06
14/GC10	HN7B(MIT)	O(P14-15)	1.63
	HN2C(MIT)	O(P3-4)	1.62
	HN4(CYT14)	O10A(MIT)	2.07
16/GC10	HN4(CYT14)	carbamate O(15)	2.03

[a] See Scheme I for illustration of mitomycin atoms; standard nomenclature for DNA. [b] HO7 is on the phenol ring. [c] 5: Moore's intermediate.

puckers of sugars in CYT2, CYT12, and GUA13 have O1′ endo–C1′ exo (ω = 106.5–119.9°) geometries and the O3′–P conformation between GUA3 and CYT4 has changed to trans (ω' = 208.3°). Values for conformations differing from standard values by more than 30° are listed in the supplementary material (appendix 2), along with those of other mitomycin analogues discussed in this paper. The patterns of helix deformations are similar for all of these compounds, with the addition of an O1′ endo–C1′ exo pucker in the sugar attached to CYT6 or THY6 when the mitomycin is monocovalently bound to DNA. In certain cases, the O3′–P torsion between CYT18 and GUA19 is trans ($\omega' \sim 223°$).

The model for monocovalent binding of mitomycin C at N6 of adenine was constructed by analogy to the previously modeled monocovalent complexes at O6 of guanine.[1] Stereo pairs for the resulting energy refined structure are illustrated in Figure 4. This structure shows that the network of hydrogen bonds stabilizing the binding is similar to that of the corresponding complex at O6 of guanine,[1] and Table I confirms that the hydrogen-bond lengths are as expected. The main difference between the

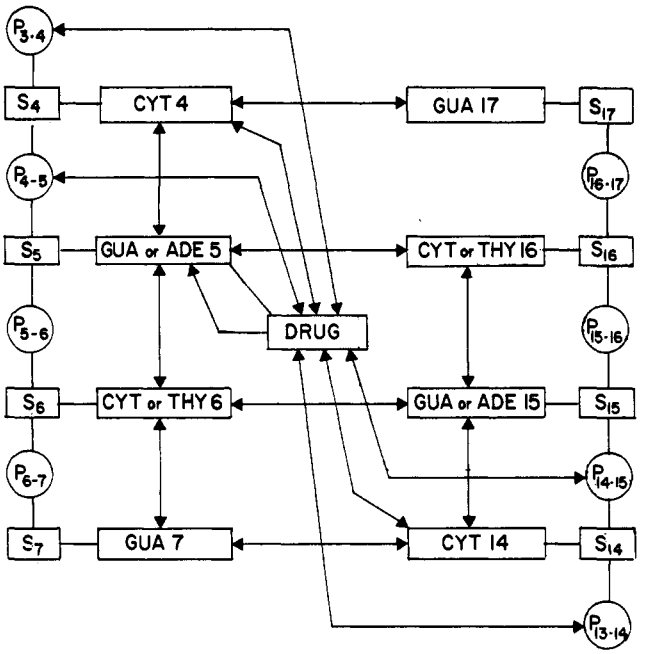


Figure 2. Diagram for the interaction of DNA residues with mitomycins and with each other.

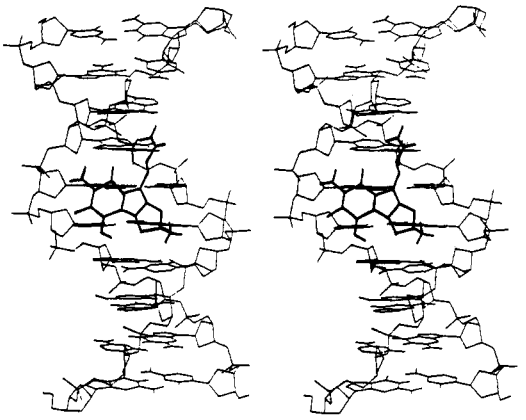


Figure 3. Stereopairs of the noncovalent complexes between GC10 and Moore's intermediate **5**.

two complexes is that the helical distortion resulting from loss of a Watson–Crick hydrogen bond and pulling of covalently attached guanine into the major groove that occurs on O6 alkylation does not occur on N6 alkylation. Tables II and III clearly show that the energy loss from helix distortion is about 11 kcal/mol less for N6 alkylation than for O6 alkylation. A similar difference was observed for N2 vs. O6 alkylation.[1] These results indicate that if alkylation were subject to thermodynamic control, N6 and

Table II. Energies (in kcal/mol) for Drug–Polynucleotide Interactions: Mitomycin Analogues Covalently Bound to Double-Helical Deoxycanucleotides[a,b]

mitomycin analogue	nucleotide	covalent bond	total	drug	drug–helix	helix	helix destab[c]
6[c,e]	GC10	none	-986.4	-5.8	-149.9	-830.7	19.7
5[d]	GC10	none	-984.7	-2.4	-149.3	-833.0	17.4
mit C[e]	GC10	GUA-O6	-962.3	-0.5	-151.1	-810.7	39.7
mit C	GC2AGC2	ADE-N6	-1008.9	0.8	-157.3	-851.6	28.0
10	GC10	GUA-O6	-962.5	6.9	-157.9	-811.5	38.9
10	GC2ATGC2	ADE-N6	-1010.0	8.9	-165.3	-853.5	26.1
11	GC10	GUA-O6	-952.9	5.1	-145.4	-812.6	37.8
12	GC10	GUA-O6	-957.6	3.8	-149.5	-811.9	38.5
14	GC10	GUA-O6	-1021.7	7.0	-210.5	-811.2	39.2
16	GC10	GUA-O6	-857.2	54.3	-35.6	-821.7	28.7

[a] These energies are valid only for relative comparison within this series. [b] The interactions are illustrated in Figure 2. [c] Energies of the uncomplexed helices: all GC, -850.4 kcal/mol; (GC)2AT(GC)2, -879.6 kcal/mol. [d] Moore's intermediate. [e] Taken from ref 1.

Table III. Interaction Energies (in kcal/mol) between DNA Residues and Mitomycin Analogues Bound Noncovalently or with N6 of Adenine

interaction	moore's intermed with GC10	mit C at ADEN6 of GC2ATGC2	**10** at ADE N6 of GC2ATGC2
drug with			
P3-4	-48.0	-48.2	-48.8
CYT4	-4.7	-5.5	-6.3
P4-5	-13.2	-12.1	-12.9
ADE or GUA5	-17.1	-17.9	-17.7
P13-14	-15.7	-20.0	-21.5
CYT14	-4.4	-7.4	-9.2
P14-15	-14.5	-18.1	-17.7
base pairing			
CYT-GUA17	-21.1	-21.7	-21.7
GUA5-CYT16	-11.6		
ADE5-THY16		-11.0	-10.8
CYT6-GUA15	-6.4		
THY6-ADE15		-12.0	
GUA7-CYT14	-11.1	-21.9	-21.7
base stacking			
CYT4-GUA5	2.6		
CYT4-ADE5		2.4	2.3
GUA5-CYT6	-9.1		
ADE5-THY6		-5.3	-4.7
CYT6-GUA7	-5.8		
THY6-ADE7		-5.0	-4.7
CYT16-GUA15	-8.4		
THY16-ADE15		-2.6	-2.3
GYA15-CYT14	-9.7	2.1	1.5

N2 alkylation would predominate greatly over O6. The experimentally observed preference for O6 alkylation suggests that the products are kinetically controlled. If one considers that an approaching electrophile has direct access to 6 of guanine, whereas access to N6 of adenine or N2 of guanine is possible only after abstraction of a proton or substantial helix distortion, the ability of O6 to compete for alkylation is reasonable.

Another interesting aspect of alkylation is that monocovalently linked compounds including mitomycin C and the analogues discussed below have C10 in close proximity to O6 of guanine in the complementary strand (GUA17). For compounds bonded to N6 of ADE5, the model shows that this distance is 3.17–3.2 Å, whereas for those bonded to O6 of GUA5 it is only 3.07–3.09 Å. These values suggest that cross-linking should be highly favored whenever the DNA sequence is appropriate. The reported frequency of about one cross-link per 10 monocovalent links[3] is lower than expected. Possibly this is a consequence of the need to reactivate the mitomycin by a second reduction. Lown showed that this process significantly increased cross-linking.[4]

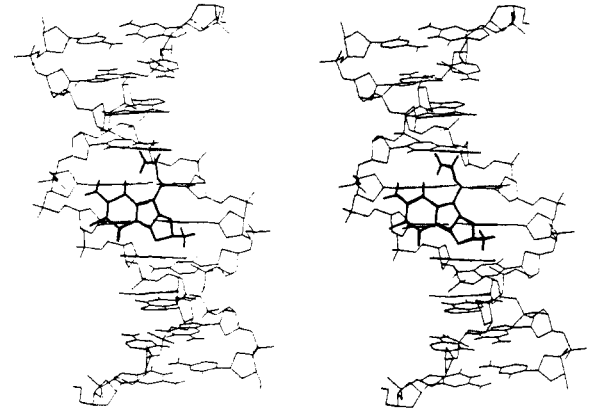


Figure 4. Stereopairs of the monocovalent complex between the product from mitomycin C and ADE N6 of GC2ATGC2.

Hashimoto's report that the N^7-(*p*-hydroxyphenyl) derivative **10** of mitomycin C showed a preference for alkylation at N6 of adenine over 6 of guanine[13] prompted us to model its interaction with DNA when monocovalently bound to each of these sites. The resulting energy minimized structures are shown in stereopairs **5a** and **5b**, respectively. These structures resemble each other closely, as expected. Their main difference from the monocovalent structures derived from mitomycin C is that the phenolic hydroxyl group (O7) of **10** replaces the amino group of the carbamate (N2) as hydrogen-bond donor to the oxygen of P13-14 (Table I). This change causes no significant difference in the total binding or helix destabilization energies (Table II). However, the closeness of total binding energies results from an increase in drug–helix interaction balanced by a decrease in internal drug energy for the *p*-hydroxyphenyl derivatives. Examination of the detailed interactions between residues (Tables III and IV) reveals that there are few differences between mitomycin C and its *p*-hydroxyphenyl derivative in their binding to a specific base; however, for both mitomycins there is a greater helix destabilization in the case of binding to O6 of guanine. Conformational analysis (appendix 1, supplementary material) shows a similar pattern of dihedral angle distortions from normal β-DNA for the polynucleotides in all

Table IV. Interactions of DNA with Mitomycins Bound Covalently to O6 of Guanine in $d(GCGCGCGCGC)_2$ (Energy in kcal/mol)[a]

		mitomycin analogue				
interaction	mit C[b]	**10**	11	12	14	16
drug with						
P3-4	-47.5	-49.0	-49.7	-49.2	-53.7	-3.1
CYT4	-5.5	-6.9	-6.9	-7.0	-7.6	-2.4
P4-5	-10.1	-13.1	-13.5	-13.4	-16.0	-2.5
GUA5	-14.5	-14.5	-12.0	-12.0	-14.0	-3.0
P13-14	-21.4	-21.4	-6.3	-13.9	-21.5	-2.1
CYT14	-9.6	-9.5	-9.8	-9.3	-11.1	-6.7
P14-15	-18.5	-18.1	-18.3	-17.3	-50.6	-1.0
base pairing						
CYT4-GUA17	-21.8	-21.7	-21.7	-21.6	-21.6	-21.9
GUA5-CYT16	-8.6	-8.0	-8.0	-8.0	-8.0	-8.4
CYT6-GUA15	-21.9	-20.3	-18.9	-18.9	-19.1	-18.9
GUA7-CYT14	-22.0	-21.8	-21.9	-21.9	-21.8	-21.9
base stacking						
CYT4-GUA5	-2.8	-1.4	-1.4	-1.4	-1.3	-3.0
GUA5-CYT6	-6.5	-6.6	-2.5	-2.6	-2.5	-1.8
CYT6-GUA7	-5.0	-4.8	-0.6	-0.5	-0.6	0.0
CYT16-GUA15	-8.3	-6.0	-8.3	-8.6	-8.3	-9.0
GUA15-CYT14	-5.0	-3.3	-4.4	-4.5	-4.2	-4.8

[a] The interactions are illustrated in Figure 2. [b] Taken from ref 1.

the four compounds. Within this pattern, the distortions in the sugar pucker and C4′–C3′ torsion in CYT14 are especially pronounced for the *p*-hydroxyphenyl derivative. This is a result of the phenolic hydroxyl replacing the carbamate amine as hydrogen-bond donor to P13-14. Although these observations provide additional insight into the binding of mitomycins to DNA, they provide no guidelines to the reported preference for adenine alkylation by the *p*-hydroxyphenyl derivative. It must be concluded that such preference is a result of kinetic control and subject to small energy differences in the transition states.

The comparison of monoalkylation products from mitomycin C and its N^7-(*p*-hydroxyphenyl) derivative **10** serves as the first example of our series of comparisons between mitomycin C and its analogues with important clinical or preclinical antitumor activity. Before additional examples are considered, it is useful to focus on a series of N^7-phenyl derivatives, including the *p*-hydroxy analogue, whose structure–activity relationships were subjected to a Hansch analysis.[15] This analysis revealed that the only physical property with a good correlation to antitumor activity was the partition coefficient, wherein the most hydrophilic analogues (those with OH and NH_2 substituents) were the most potent in terms of minimum effective dose. Quinone reduction potentials and substituent size were insignificant in accounting for the variance in antitumor activity in this particular family of mitomycin analogues.

In attempting to evaluate the importance of strong hydrogen bonding to DNA as a contributor to antitumor potency, it is desirable to separate this factor from partition coefficient (and any others that influence potency). Unfortunately, in the case of N^7-phenyl derivatives with OH or NH_2 substituents, both of these factors work in the same direction. The Hansch analysis did reveal one compound, bearing a glycinyl substituent, that is highly hydrophilic but unlikely to hydrogen bond to P13-14 because of ionization at pH 7.4. This compound was the main outlier in the direction of reduced activity in the plot of potency vs. partition coefficient.

The effects of hydrogen bonding and substituent size on antitumor activity were examined further by modeling the binding of the N^7-(*p*-iodophenyl) derivative 11 of mitomycin C. This relatively inactive compound has less hydrophilicity and greater steric bulk than the corresponding *p*-hydroxyphenyl derivative. Stereopairs for its energy minimized monocovalent complex with GC10 are shown in Figure 6. This structure closely resembles that of the *p*-hydroxyphenyl derivative, except that the C7–N7 bond is rotated to move the iodine away from P13-14. The bulk of the iodophenyl group still prevents N10A of the carbamate from approaching P13-14 close enough to make a hydrogen bond. Thus only three hydrogen bonds are possible (Table I). Energy analysis (Tables III and IV) reflects this loss of a hydrogen bond, but other changes in the mitomycin–DNA interactions are small. The most interesting result is that there is no increase in helix destabilization over that in the complex with the *p*-hydroxyphenyl derivative. This result indicates that large substituents on N7 can be accommodated by simply rotating them away from the DNA. Such a process is consistent with the result of the Hansch analysis that showed no contribution from substituent size to the variance in antitumor potency.

The next mitomycin C analogue examined was its N^7-(2-mercaptoethyl) derivative (**12**). This compound, known as RR-150 or NSC 329697, shows enhanced antitumor activity in a number of assays in the NC1 mouse

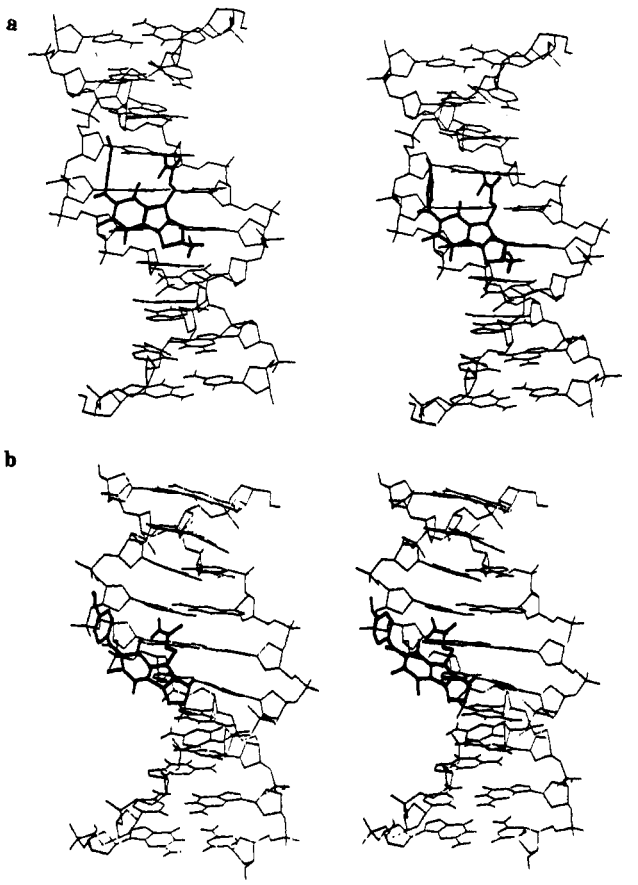


Figure 5. Stereopairs of the monocovalent complexes between the product from N^7-(*p*-hydroxyphenyl) mitomycin C and decanucleotides: (a) covalent bond at ADE N6 of GC2ATGC2; (b) covalent bond at GUA O6 of GC10.

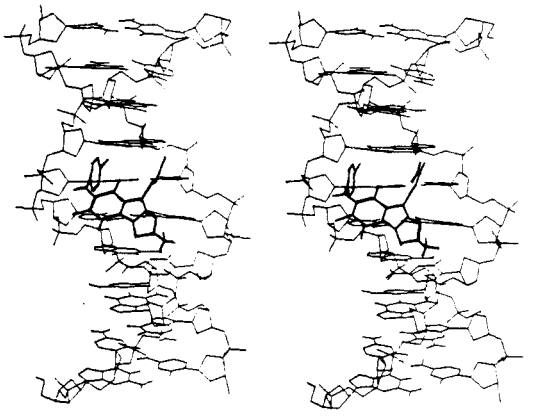


Figure 6. Stereopairs for the monocovalent complex from N^7-(*p*-iodophenyl)mitomycin C at GUA O6 of GC10.

tumor panel and it is possibly less leukopenic than mitomycin C.[20,21] Its reduced hydrophilicity ought to be valuable in focusing on the value of model building in rationalizing biological activity because the partition coefficient and binding energy effects should be opposed. Unfortunately, it is readily oxidized in air to a more hydrophilic disulfide. Its status in biological systems is unknown. Nevertheless, it fits the model nicely (appendix 3, supplementary material). The sulfur atom acts as the hydrogen-bond donor for P13-14, replacing N10A of the

(20) Iyengar, B. S.; Sami, S. M.; Remers, W. A.; Bradner, W. T.; Schurig, J. E. *J. Med. Chem.* **1983**, *26*, 16.
(21) Bradner, W. T.; Rose, W. C.; Schurig, J. E.; Schlein, A.; Huftalen, J. B. *Cancer Res.* **1984**, *44*, 5619.

carbamate (Table I). This hydrogen bond is weaker than others by about 8 kcal/mol, consistent with the weaker SH-bond capabilities of S–H···O than O–H···O discussed elsewhere.[17] Otherwise, the interaction energies and conformational properties are closely related to those of the other analogues.

A highly active new agent is the *N*,*N*-dimethylamidino analogue **13** of mitomycin C, known as BMY-25282. This compound is potent and active against a number of mouse tumors resistant to mitomycin C. At this stage in its development it appears to be substantially better than its parent.[22,23] The chemical status of BMY-25282 in biological systems is uncertain, although it appears to be reduced readily. For our modeling study, we are making the reasonable assumption that the amidino function is protonated. This change should have a significant effect on the partition coefficient. We estimate from Hansch's substituent constants[24] that conversion of mitomycin C to **13** increases log *P* by +0.90, whereas protonation of **13** decreases the resulting log *P* by −3.00. The proton should add to N7[25] (affording structure **14**) and create positive charges on atoms including the dimethylamino group. Protonation of the 2-amino group has been retained. The resulting dication, bonded covalently to O6 of guanine, makes a good fit onto the major groove (appendix 3, supplementary material). The dimethylamino group positions itself about midway between P13-14 and P14-15, with distances of 2.96 and 3.94 Å, respectively, for the upper methyl group and nitrogen to the nearest oxygen of P13-14, and 2.97 and 3.45 Å for the lower methyl group and nitrogen to the nearest oxygen of P14-15. These interactions prevent N10A of the carbamate from hydrogen bonding with P13-14, but HN7B forms a hydrogen bond with P14-15 and the other two hydrogen bonds remain (Table I). The additional positive charge increases the drug–nucleoside interaction by about 60 kcal/mol (Table II) and it is divided into the interactions with P13-14 and P14-15 (Table IV). Conformational changes resemble those produced by the N^7-(*p*-hydroxyphenyl) analogue **10**. Thus, the dimethylamidinium substituent significantly enhances the binding of mitomycin to DNA without destabilizing the helix more than other substituents. This might be an important principle for the design of future mitomycin analogues.

Our concluding example involves application of the mitomycin–DNA binding model to another type of alkylating agent that resembles mitomycin C. Series of biscarbamates of bis(hydroxymethyl)-5-phenylpyrroles[26] and 6,7-bis(hydroxymethyl)-8-phenyl-1*H*-pyrrolizines[27] bearing various substituents in the phenyl rings were designed by Anderson on the basis of structural features of mitomycin and pyrrolizidine alkaloids. One such compound, NSC 278214 (**15**) is considered a "compound of interest" by the National Cancer Institute. For modeling of DNA binding we have chosen a structure (**16**) representative of the *N*-phenylpyrroles.[26] This choice was based on the ease of estimating pyrrole ring charges from mitomycin. It is modeled with a monocovalent link to O6 of guanine in GC10, even though the actual binding site or sites have not been determined. Thus the example is purely hypothetical. Even so, it might be useful to inspect the energy minimized structure and see how it fits into the major groove of DNA. Table I shows that only one hydrogen bond, involving N4 of CYT14 and the carbamate carbonyl of **16**, stabilizes it. The carbamate nitrogen does not interact with P13-14 or P14-15, possibly because of steric hindrance by the isopropyl group (appendix 3, supplementary material). The energy analysis (Table II) shows only a small amount of drug–helix interaction (−35.6 kcal/mol) and about the same helix destabilization as caused by mitomycin. Conformational analysis reveals the pattern of sugar puckers and dihedral distortions usually found for the mitomycins (appendix 1, supplementary material). It is clear from these data that **16** fits the mitomycin–DNA binding model but not with strong interactions. Needless to say, the assumption of binding of O6 of guanine might be invalid, in which case other models would be more relevant. However, the mitomycin model does provide clues for the design of future analogues of **16**. In particular, the addition of functional groups that could increase the hydrogen bonding to P3-4 or P14-15 appears desirable, especially if they carried positive charge at physiological pH.

Conclusions

We have applied molecular mechanics methods to suggest possible binding models for mitomycin C analogue–DNA interactions, considering both noncovalent and monolinked drug–DNA interactions. Elsewhere, we have applied such methods to study cross-linked complexes.[1] One is, of course aware that strong interactions with DNA are likely to be a *necessary* but not *sufficient* condition for useful anticancer activity. Besides the role of partitioning, which can be analyzed with a Hansch-like model, there are many other factors that mask a simple relation between biological activity and DNA binding affinity. Some of the most obvious include: drug metabolism to inactivate the drug, reductive *activation* of the drug, differences in rate of *dissociation* of the drug from the DNA, selective or more rapid absorption of the drug by cancer cells, and other factors that might modulate the drug–DNA interaction, e.g., DNA-binding proteins.[28]

Further, we emphasize that the molecular mechanical approach employed here is not capable of giving a quantitative analysis of the free energies of interactions between mitomycin analogs and DNA, or of the energetics for the reaction pathway of the covalent attack. In addition, the calculated total energies do not explicitly include solvation and counterion atmosphere effects. However, useful qualitative structural insights might be obtained from the models and we hope such models can be tested by 2D NMR/NOE and crystallographic studies on oligonucleotide complexes with the mitomycin analogs.

These caveats notwithstanding, the previously developed model for the interaction between mitomycin C and DNA is able to accommodate a variety of analogues and it accounts for the effectiveness of some of their unique substituents. It also accounts for some of the results obtained from a Hansch analysis of mitomycin C analogues with aryl substituents on the 7-amino group, especially the lack of influence of substituent size on antitumor potency.[15] In

(22) Vyas, D. M.; Doyle, T. W.; Bradner, W. T.; Rose, W. C. *Abstracts of Papers* 187th National Meeting of the American Chemical Society, St. Louis, MO; American Chemical Society: Washington, D.C., 1984; MEDI 30.
(23) Bradner, W. T.; Rose, W. C.; Schurig, J. E.; Huftalen, J. B.; Florczyk, A. P.; Vyas, D. M. *Proc. Am. Soc. Cancer Res.* **1984**, *25*, 228.
(24) Hansch, C.; Leo, A. *Substituent Constants for Correlation Analysis in Chemistry and Biology*; Wiley-Interscience: New York, 1979.
(25) Noller, C. R. *Chemistry of Organic Compounds*, 3rd ed.; W. B. Saunders: Philadelphia, 1965; p 274.
(26) Anderson, W. K.; Corey, P. F. *J. Med. Chem.* **1977**, *20*, 1691.
(27) Anderson, W. K.; Corey, P. F. *J. Med. Chem.* **1977**, *20*, 812.
(28) Altona, C.; Sundaralingam, M. *J. Am. Chem. Soc.* **1972**, *91*, 8505.

addition, the model allows one to go beyond the correlation between activity and *hydrophilicity* and to suggest that some of the reasons for this correlation is that the more hydrophilic substituents include many that are effective proton donors in hydrogen bonds to the DNA. The examination of substituents such as 4-SO_3^- would further validate this suggestion, since such a compound would be very hydrophilic but not a proton donor. The two outliers in the current Hansch analysis are R = $NHCH_2CO_2H$, which is likely to be anionic at physiological pH and, thus, less effective than suggested by its hydrophilicity, and R = $NHCONH_2$, for which our model cannot suggest a reason for inactivity. It is possible that the presence of the CO prevents as effective hydrogen bonding as found in the OH and NH_2 substituents.

An obvious problem in correlating biological activity with models based on molecular mechanics is the separation of binding effects from physicochemical properties such as partition coefficient that can cause parallel changes in activity. It should be possible to include binding energies in the multiple linear regression analysis. Unfortunately, the mitomycins are not a good family of compounds for this purpose because only one type of substituent tried thus far, the N^7-phenyl derivatives, gave any useful regression analysis. At this time, one can at least design structural modifications that fit the model well while making certain that they enhance hydrophilicity and do not increase the difficulty of quinone reduction.

Application of the mitomycin binding model to nonmitomycins such as **16** is the least well-founded aspect of our study, yet it might hold the greatest promise for future drug design. This approach should be tested further by the synthesis and evaluation of simpler molecules that can interact with the same binding sites as mitomycins.

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Registry No. 1, 50-07-7; 1·2GC2ATGC2, 102046-81-1; 5·2GC10, 102046-79-7; 10·2GC2ATGC2, 102046-83-3; 10·2GC10, 102130-36-9; 11·2GC10, 102046-85-5; 12·2GC10, 102046-87-7; 13-2GC10, 102072-56-0; 16·2GC10, 102046-89-9; adenine, 73-24-5.

Supplementary Material Available: Quantum mechanically derived charges for mitomycin fragments, conformational analysis of distorted segments in the sugar–phosphate backbone of DNA, and stereo pairs for the monocovalent adducts of mitomycin C analogues to $d(GC10)_2$ (7 pages). Ordering information is given on any current masthead page.

Folate Analogues. 25. Synthesis and Biological Evaluation of N^{10}-Propargylfolic Acid and Its Reduced Derivatives[1]

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N^{10}-Propargylfolic acid (**2**), which is the closest pteridine analogue of the thymidylate synthase inhibitor N^{10}-propargyl-5,8-dideazafolic acid (PDDF), was synthesized starting from diethyl [*p*-(*N*-propargylamino)benzoyl]-L-glutamate (**5**) and *N*-(3-bromo-2-oxopropyl)phthalimide (**8**). The 7,8-dihydro derivative of propargylfolic acid served as a synthetic substrate of *Lactobacillus casei* dihydrofolate reductase. Propargylfolic acid and its reduced derivatives were weak inhibitors of *L. casei* thymidylate synthase compared to PDDF. All derivatives of propargylfolate were active against the growth of *Streptococcus faecium*, but with the exception of 7,8-dihydropropargylfolic acid, all were inactive against *L. casei*. Although less potent than PDDF, marked inhibition of thymidylate synthase by **2** was observed in permeabilized L1210 cells.

Specific inhibitors of thymidylate synthase[2] (EC 2.1.1.45) are useful chemotherapeutic agents for the treatment of various forms of human cancers.[2-4] Most inhibitors of this enzyme are analogues of the nucleotide substrate deoxyuridine monophosphate (dUMP), and relatively few are coenzyme analogues structurally related to folic acid (**1**). Recently, Jones and co-workers reported[5] that the quinazoline derivative 5,8-dideaza-N^{10}-propargyl folic acid (PDDF) is an excellent inhibitor of L1210 thymidylate synthase and that it exhibited remarkable activity against the L1210 tumor in vivo. We have subsequently determined the antifolate activity of this compound using both methotrexate- (MTX-) sensitive and resistant strains of *Lactobacillus casei* (ATCC 7469) and *Streptococcus faecium* (ATCC 8043).[6] The propargyl derivative was as active as MTX against *S. faecium* and showed good activity against the MTX-resistant strain of this organism. It was a very powerful inhibitor of *L. casei* and *S. faecium* thymidylate synthases. The poly-γ-glutamyl derivatives of PDDF were remarkably more active than the parent compound in inhibiting thymidylate synthase derived from several species,[7] including man.[8] Since the introduction

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(1) Previous paper: Nair, M. G. *J. Org. Chem.* **1985**, *50*, 1879.
(2) Friedkin, M. *Adv. Enzymol.* **1973**, *38*, 235.
(3) Santi, D. V. *J. Med. Chem.* **1980**, *23*, 103.
(4) Heidelberger, C.; Danenberg, P. V.; Moran, R. G. *Adv. Enzymol.* **1983**, *54*, 57.
(5) Jones, T. R.; Calvert, A. H.; Jackman, A. L.; Brown, S. J.; Jones, M.; Harrap, K. R. *Eur. J. Cancer* **1981**, *17*, 11.
(6) Nair, M. G.; Salter, D. C.; Kisliuk, R. L.; Gaumont, Y.; North, G.; Sirotnak, F. M. *J. Med. Chem.* **1983**, *26*, 605.
(7) Kisliuk, R. L.; Gaumont, Y.; Kumar, P.; Coutts, M.; Nair, M. G.; Nanavati, N. T.; Kalman, T. I. In *Proceedings of the Second Workshop on Folyl and Antifolyl Polyglutamates*; Goldman, I. D., Ed.; Praezer Scientific: New York, 1985; pp 319–328.
(8) Cheng, Y. C.; Dutschman, G. E.; Starnes, M. C.; Fisher, M. H.; Nanavati, N. T.; Nair, M. G. *Cancer Res.* **1985**, *45*, 598.

0022-2623/86/1829-1263$01.50/0