by the Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute.[36] Modifications in the selection and number of doses used have been made to increase test efficiency.[37]

Registry No. 4, 96389-69-4; 5, 96404-51-2; 7, 133550-74-0; 8, 1140-79-0; 9, 2143-81-9; 10, 26842-00-2; 11, 7466-50-4; 12, 41498-43-5; 13, 104500-25-6; 14, 28440-63-3; 15, 96403-40-6; 16, 96403-41-7; 17, 104500-30-3; 18, 26947-45-5; 19, 104500-12-1; 20, 96403-57-5; 21, 96403-23-5; 22, 22138-85-8; 23, 96404-79-4; 24, 104500-22-3; 25, 22245-51-8; 26, 24295-83-8; 27, 14214-57-4; 28, 4466-76-6; 29, 133550-75-1; 40, 67064-61-3; 41, 133550-76-2; 42, 104500-21-2; 50, 96403-91-7; 51, 133550-77-3; 52, 133550-78-4; 53, 133550-79-5; 54, 133550-80-8; 55, 133550-81-9; 56, 133550-82-0; 57, 133550-83-1; 58, 96404-62-5; 59, 96403-65-5; 60, 96538-95-3; 61, 133550-84-2; 62, 96404-35-2; 63, 96403-63-3; 64, 96403-62-2; 65, 96404-20-5; 66, 104500-26-7; 67, 133550-85-3; 68, 133550-86-4; 69, 96389-48-9; 70, 96403-44-0; 71, 104500-31-4; 72, 104500-28-9; 73, 96403-30-4; 74, 133550-87-5; 75, 104500-13-2; 76, 96403-59-7; 77, 96403-25-7; 78, 133550-88-6; 79, 133550-89-7; 80, 96422-39-8; 81, 104500-23-4; 82, 104500-07-4; 83, 104500-10-9; 84, 104500-15-4; 85, 104525-00-0; 86, 133550-90-0; 87, 96403-22-4; 88, 96403-20-2; 89, 96403-55-3; 90, 96403-26-8; 91, 133550-91-1; 92, 133550-92-2; 93, 96403-21-3; 94, 96403-56-4; 95, 103395-25-1; 96, 206-44-0; 97, 42050-05-5; 98, 832-69-9; 99, 33543-31-6; 100, 3351-30-2; 101, 2871-91-2; 102, 88746-58-1; 103, 133550-93-3; 104, 133550-94-4; 105, 133550-95-5; $SnCl_4$, 7646-78-8; Cl_2CHOCH_3, 4885-02-3; 2-amino-2-methyl-1,3-propanediol, 115-69-5.

(36) Goldin, A.; et al. *Methods in Cancer Research*; Academic Press: New York, 1979; Vol. XVI, p 165.

(37) After the LD_{20} was determined, antitumor screening was performed under contact by Southern Research Institute, 2000 Ninth Avenue South, P.O. Box 55305, Birmingham, AL 35255-5305. We acknowledge the consultation of the late Frank Schabel Jr. and the assistance of W. Russell Laster Jr., the late Mary Trader, and Daniel P. Griswold in the testing of these compounds.

Computer Simulation of the Binding of Saframycin A to d(GATGCATC)$_2$

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The binding of Saframycin A to the octanucleotide duplex d(GATGCATC)$_2$ was investigated using molecular dynamics. For covalent binding at N2 of the central guanine, only the *R* configuration at the alkylating carbon (C7) was permitted for B DNA and the 3′ direction in the minor groove was preferred by 50.6 kcal/mol. The dihydroquinone form of saframycin A gave stronger binding than the quinone, in agreement with the literature. Addition of solvent and counterions made no significant change in the geometry model. The proposed mechanism of DNA alkylation, involving iminium ion intermediates from the dihydroquinone or quinone, was investigated by modeling these species. They gave models with good net binding enthalpies, and C7 was in close proximity to N2 of guanine. The noncovalent binding of saframycin A and its dihydroquinone in the vicinity of guanine also was favorable in the 3′ direction.

The saframycins were discovered in a thorough investigation of satellite antibiotics produced by *Streptomyces lavendulae* No. 314.[1] Saframycin A (Figure 1, IUPAC numbering) had the best antibacterial and antitumor activity among the earlier compounds isolated, and it has been the one most thoroughly studied. Structures of the saframycins are based mainly on the X-ray diffraction of saframycin C.[2] Saframycin A and otherr saframycins were related to it by ^{1}H and ^{13}C NMR spectrometry.[3-5] Absolute stereochemistry of the saframycins follows from that of a closely related compound, the 15-bromo derivative of safracin A (Figure 1, 4-bromo in IUPAC numbering).[6]

The saframycins have pentacyclic, dimeric structures containing two units of 7-methoxy-6-methyl-1,2,3,4-isoquinoline-5,8-dione joined through the fifth ring and bearing a methylene group substituted with a pyruvamide moiety.[7] Certain saframycins have labile leaving groups at C7, including cyano (saframycin A) and hydroxyl (saframycin S). Thus, saframycin S is converted into saframycin A on treatment with sodium cyanide.[8] Furthermore, acid hydrolysis of saframycin A results in the release of 1 equiv of HCN (presumably with formation of saframycin S).[3] Saframycins have two tertiary nitrogens, but only one of them, N16, is protonated in dilute acid.[9] The two quinone rings of safracins or saframycins are at 75° angles to each other (Figure 2).[6] Solution conformations of saframycins A and C, determined by ^{1}H NMR studies, show that ring B deforms from half-boat to half-chair and the chair conformation of ring C is slightly twisted, when compared with their crystal structures.[4,5] The pyruvamide side chain has a slightly skewed gauche conformation with a dihedral angle of about 90°, and the orientation of this chain with respect to the C9 methylene depends on the solvent. The 7-cyano group of saframycin A has the axial conformation.[9]

A mode of action has been proposed for the antitumor activity of saframycins. It incorporates the chemical evidence described above, plus the following biochemical studies. (1) Treatment of [^{14}C]saframycin A (derived from [^{14}C]tyrosine) with dithiothreitol in the presence of calf thymus DNA resulted in incorporation of radioactivity into the DNA; however, excess sodium cyanide inhibited this reaction.[10] There was no radioactivity incorporated when saframycin A with ^{14}CN at C7 was used. Reduction of the

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(8) Arai, T.; Takahashi, K.; Ishiguro, K.; Yazawa, K. *J. Antibiot.* 1982, *35*, 1708.
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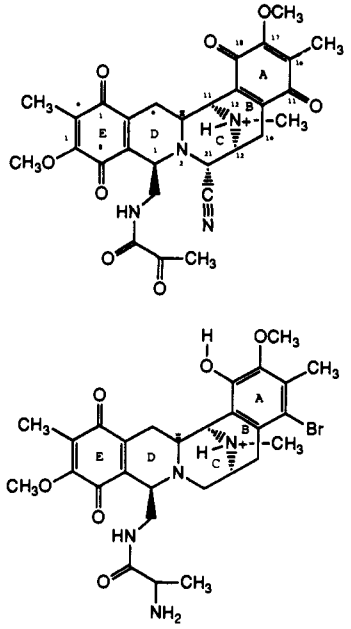
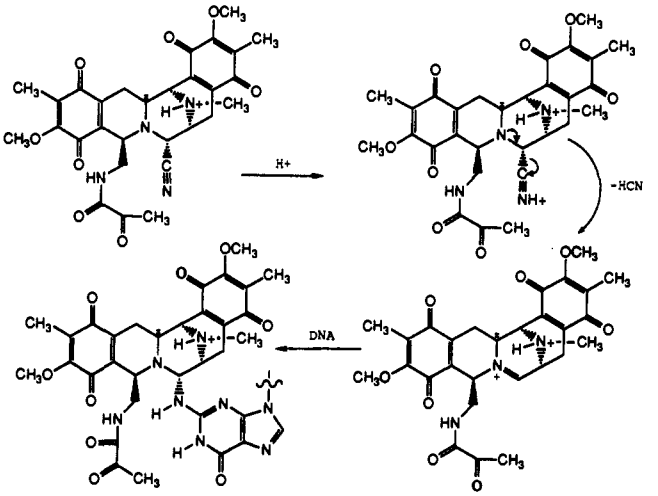


Figure 1. Structures of saframycin A (upper) and 15-bromosafracin A (lower).

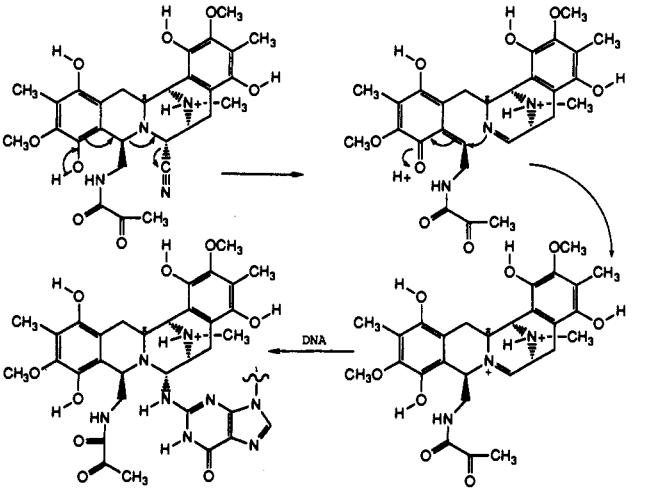
quinone rings of saframycin A substantially increased DNA binding.[10] Saframycin B, which has no 7-cyano group, is 2 orders of magnitude less potent than saframycin A in inhibiting DNA template function.[10] These results suggest that saframycin A is converted into a reactive intermediate such as an iminium ion before it binds covalently to DNA, and that this process is promoted by reduction to a hydroquinone. (2) Saframycin A binding to DNA is slow and reversible to heat and lower pH. DNA sequences containing G + C units are preferred for binding.[9] Strand scission is induced in poly(dG)·poly(dC), but not in poly(dA)·poly(dT).[10] Covalent binding to the 2-amino group of guanine in the minor groove of DNA, involving an aminal linkage, is consistent with these observations. (3) Saframycins A and C are protonated at pH 5 and they bind weakly and reversibly to DNA, partly extruding intercalated ethidium; however, they do not bind above pH 6.0.[9] Decyanosaframycin A (saframycin B) has no 7-substituent, but it can react with DNA in the absence of a reducing agent. Its binding is "equivalent" to that of saframycin A in the presence of a reducing agent, which shows the importance of hydroquinone functionality to DNA binding.[10] These results indicate that there is a noncovalent binding process that may be either independent of the covalent binding process or a precursor to it, for appropriately substituted compounds. The slow covalent bonding that occurs with unreduced saframycin A, but not saframycin C is consistent with this concept.

From the above discussion, a scheme for the activation and covalent bonding of saframycin A to DNA can be

Scheme I



Scheme II



derived (Scheme I).[7,9] It has many of the features associated with the alkylation of DNA by anthramycin and other pyrrolo[1,4]benzodiazepines. In this scheme, the 7-cyano group, protonated at low pH, leaves with formation of an iminium ion. This ion then alkylates N2 of a guanine residue on the DNA. If the quinone is reduced to a hydroquinone or dihydroquinone, the 7-cyano group (not necessarily protonated) can leave with formation of an iminium ion in a process involving participation by both the 10-hydroxyl group and N8.[9] Then, the iminium ion can alkylate the 2-amino group of a guanine residue (Scheme II). Although these schemes are consistent with most of the experimental evidence, there are many important aspects of the DNA binding of saframycins that remain unknown. For example, the conformations of the covalent and noncovalent complexes, the reason for the enhanced binding of hydroquinone species over quinone

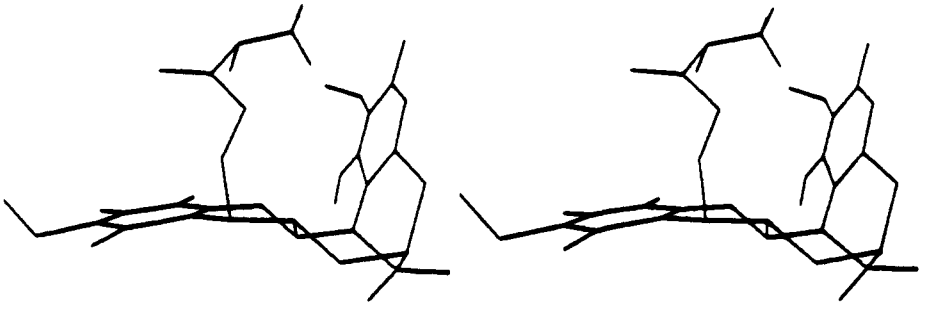


Figure 2. Stereopair for 15-bromosafracin A.

Table I. Enthalpies (kcal/mol) for Interactions between Saframycin A Species and d(GATGCATC)$_2$

model[a]	total	intermolecular			helix[b] dist	drug[c] dist	net[d] binding
		vdw	elstat	total			
HQ, CV, 3′R	-636.0	-30.9	-143.0	-172.8	59.4	6.6	-106.8
HQ, CV, 5′R	-585.13	-18.8	-104.8	-123.6	61.8	5.6	-56.2
HQ, NC, 3′	-631.4	-33.2	-122.0	-155.2	43.5	9.5	-102.2
HQ, I, NC, 3′	-674.6	-31.9	-180.7	-212.6	44.1	14.4	-154.1
Q, CV, 3′R	-588.7	-34.0	-95.8	-129.8	51.1	6.7	-72.0
Q, NC, 3′	-590.3	-28.1	-83.9	-112.0	36.6	1.9	-73.5

[a] Abbreviations: HQ = dihydroquinone, Q = quinone, CV = covalent, NC = noncovalent, I = iminium ion. [b] Helix distortion enthalpy is obtained by subtracting the enthalpy of the unbound helix (-561.4 kcal/mol) from its value in the adduct. [c] Drug distortion enthalpy is obtained by substrating the enthalpy of the drug minimized without DNA present (kcal/mol) from its value in the adduct. [d] Net binding enthalpy is obtained by adding the total intermolecular, helix distortion, and drug distortion enthalpies.

species, and the possibility that noncovalent binding can lead directly to covalent binding are all unexplored. Our continuing interest in the DNA binding of antitumor antibiotics has led us to examine these problems through the use of molecular modeling, with emphasis on molecular dynamics calculations.

The following objectives were set for a molecular modeling study. (1) Find, if possible, the single best representation for the covalent binding of saframycin A to a representative DNA fragment, d(GATGCATC)$_2$, employing molecular dynamics and molecular mechanics. Study the effects of solvation and counterions on this complex. (2) Determine the preferred direction for binding in the minor groove and the configuration at the C7 alkylation center. (3) Calculate the comparative binding energies for hydroquinone and quinone structures in both covalent and noncovalent complexes. Determine if the noncovalent complexes can be direct precursors to covalent complexes. (4) Model the iminium ion intermediate to determine if it binds noncovalently in a conformation such that subsequent covalent binding occurs without substantial distortion in the DNA or the saframycin.

Our previous studies on the binding of drugs at N2 of guanine in the minor groove of DNA involved the hexanucleotide duplex d(ATGCAT)$_2$, which was chosen because it is known to exist in the B form and because extensive NMR studies have been made on its adducts with anthramycin and tomaymycin.[10-13] Unfortunately, this DNA fragment is unsuitable for molecular dynamics because the terminal AT pairs fray so badly that they do not reform Watson–Crick base pairs. (Fraying also is observed in the NMR spectra at room temperature.[14]) Based on NMR studies,[15] GC "caps" were added at both ends of the duplex, affording d(GATGCATC)$_2$, and they maintained its integrity throughout the dynamics.[16]

The structure of saframycin A dihydroquinone (both quinone rings reduced, based on the assumption that they have equal reduction potentials) was constructed with the program AMBER,[17] with geometry derived from the X-ray

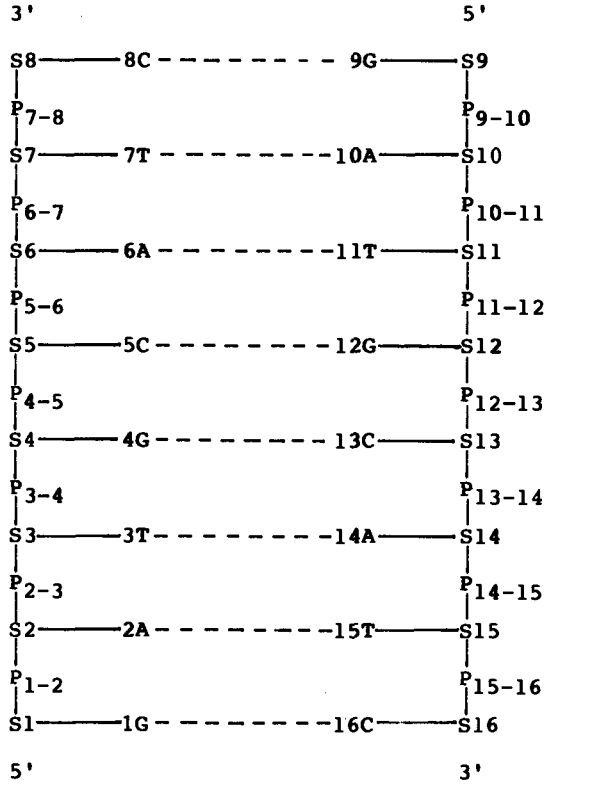


Figure 3. Schematic for d(GATGCATC)$_2$.

structure of 15-bromosafracin A (Experimental Section). It was not necessary to derive any new parameters for the dihydroquinone; however, in order to establish the trans coplanar relationship of the pyruvamide carbonyls, we defined some new atom types N8, C8, and O8 having exactly the same parameters as those in AMBER for N, O, and C atom types and set the appropriate dihedrals at 180° or 0°. A proton was placed on N16, the preferred site of protonation according to Lown's NMR study.[18] Partial atomic charges (supplementary material) were calculated with GAUSSIAN-80 (UCSF).[19] This structure was refined in AMBER and docked with the aid of MIDAS[20] on d(GATGCATC)$_2$ close to N2 of G4 (see Figure 3 for a schematic of this duplex), which was previously built and refined in AMBER. Docking was made in both the 3′ and 5′ directions (specified by the orientation of ring E of saframycin with respect to the strand to which it is cova-

(11) Remers, W. A.; Mabilia, M.; Hopfinger, A. J. *J. Med. Chem.* **1986**, *29*, 2492.
(12) Cheatham, S.; Kook, A.; Hurley, L. H.; Barkley, M. D.; Remers, W. *J. Med. Chem.* **1988**, *31*, 583.
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(15) Hurley, L. H., personal communication.
(16) In the course of this investigation, sequence selectivities for the covalent binding of saframycins A and S to DNA were published (Rao, E. K.; Lown, J. W. *Chem. Res. Toxicol.* **1990**, *3*, 262). The most preferred sequences were GGG and GGC. Although our polynucleotide duplex does not contain this sequence, it is suitable for the present study, which is based on mode of action and not sequence. It is known to be B DNA and has served as the duplex for modeling related compounds such as the pyrrolo[1,4]benzodiazepines.
(17) Sing, U. C.; Weiner, P. K.; Caldwell, J.; Kollman, P. A. *AMBER (UCSF), Version 3.0*; Department of Pharmaceutical Chemistry, University of California: San Francisco, 1986.
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Table II. Interaction Enthalpies (kcal/mol) for Saframycin A Species with Individual Residues of d(GATGCATC)$_2$[a]

	residue												
model[b]	P3–4	G4	P4–5	C5	P5–6	P6–7	G12	P12–13	C13	P13–14	A14	P14–15	P15–16
HQ, CV, 3′*R*[c]	−3.9	−16.6	−3.5	−4.0	−35.9	−3.8	−3.8	−3.8	−6.4	−23.5	−13.5	−29.8	−18.7
HQ, CV, 5′*R*		−11.6	−4.0	−4.0	−25.6	−5.9		−4.6	−10.2	−20.3		−31.0	−4.2
HQ, NC, 3′			−5.5	−3.5	−32.5	−3.0	−5.0	−3.0	−3.0	−29.3	−7.7	−42.4	−5.1
HQ, I, NC, 3′[d]	−8.7	−3.0	−11.9	−7.2	−47.0	−6.7		−7.1	−10.5	−34.0	−17.2	−52.5	−10.0
Q, CV, 3′*R*	−4.1	−19.0	−3.1	−3.7	−21.5		−3.2	−4.0	−7.7	−11.4	−14.8	−22.5	−6.4
Q, NC, 3′			−3.9		−22.4	−7.8	−4.8			−8.3		−45.1	−3.4

[a] Residues are listed only if the enthalpies are >3.0 kcal/mol. [b] Abbreviations: HQ = dihydroquinone, Q = quinone, CV = covalent, NC = noncovalent, I = iminium ion. [c] This species also makes the following interactions: A6, −7.2; S15, +5. [d] This species also makes the following interactions: P2–3, −3.8; S4, +3.0; S5, +3.1; A6, −3.3; P7–8, −3.4; P11–12, −3.5; S14, +6.8; S15, +3.6.

lently bonded); however, only the *R* configuration at C7 gave, in either direction, models that had appropriate fits between drug and DNA as judged by the absence of obvious strong steric hindrance. Coordinates of the docked adducts were captured and the structures were energy minimized until a root mean square value of 0.1 kcal/mol Å was obtained. The resulting structures were subjected to a total of 48 ps of molecular dynamics in AMBER, done in a series of 16-ps runs, using nonclassical dynamics with constant temperature and shake on (Experimental Section). There was no significant change in the conformation after the first 16 ps. It was then reminized using molecular mechanics. Table I shows the binding enthalpies for the final models. The net binding enthalpies, which reflect the intermolecular binding and the helix and drug distortions resulting from the induced fit, are the best measures of relative binding. Thus, the dihydroquinone model with 3′ direction (Figure 4) was greatly favored (50 kcal/mol) over the one with 5′ direction.

The binding of an iminium ion derived from saframycin A dihydroquinone was modeled to show that it could provide a reasonable noncovalent intermediate for subsequent formation of the 3*R* covalent structure. This modeling was carried out as described above, with partial atomic charges for the dication calculated in GAUSSIAN-80 (UCSF) using the STO 3G basis set. The resulting structure showed high net binding enthalpy (Table I) and a significant hydrogen-bond network (Table III). Direct comparison of its net binding enthalpy with those of the other species should not be made because the second positive charge strongly influences the result of simulations in vacuum. A distance of only 3.06 Å from C7 of the drug to N2 of G4 indicated that subsequent covalent bonding should readily occur.

It is conceivable that there is a mechanism, alternative to those in the literature, involving noncovalent binding of the drug followed by S_N2 displacement. The noncovalent binding of protonated saframycin A dihydroquinone was modeled in the 3′ direction, which was clearly preferred for covalent binding. The resulting adduct (Figure 5) had a substantial net binding enthalpy of −102.2 kcal/mol, which is only 4.6 kcal/mol different from that of the corresponding covalent model (Table I). A lower intermolecular binding enthalpy for the noncovalent model, caused mainly by decreased electrostatic attraction, was partly balanced by less helix distortion. Despite the favorable binding enthalpy of the noncovalent hydroquinone, it does not appear to be a viable precursor to covalent bonding. The distance from C7 to N2 of G4, 3.44 Å is favorable, but the N2–C7–(7-cyano) angle is only 58°, rather than the 180° optimal for an S_N2 displacement. Furthermore, the cyano group would be expelled into the DNA, resulting in severe steric hindrance. The last mentioned effect would also prevent a S_N1 process. The cyano group fits neatly into the DNA and there is no room for it to move away from the saframycin A nucleus.

Table III. Hydrogen-Bond Parameters Involving Saframycin A–d(GATGCATC)$_2$ Interactions[a]

model[a]	hydrogen donor	acceptor atom	length, Å
HQ, CV, 3′*R*	HO4 (SM)	OA (P5–6)	1.63
	HO10 (SM)	N3 (A6)	2.06
	HN2B (G12)	O10 (SM)	1.83
	HO13 (SM)	OA (P13–14)	1.66
	HO1 (SM)	OA (P14–15)	1.63
	HN16 (SM)	N3 (A14)	1.81
	HO10 (SM)	O11 (SM)	2.23
HQ, CV, 5′*R*	HO13 (SM)	OA (P5–6)	1.67
	HN16 (SM)	O2 (C4)	2.46
	HO4 (SM)	OA (P14–15)	1.64
	HO10 (SM)	O11 (SM)	2.11
	HO1 (SM)	O2 (SM)	2.10
HQ, CV, NC, 3′	HO4 (SM)	OA (P5–6)	1.63
	HN2B (G12)	O10 (SM)	1.81
	HO13 (SM)	OA (P13–14)	1.65
	HO1 (SM)	OA (P14–15)	1.62
	HN16 (SM)	O1′ (A14)	2.17
	HO10 (SM)	O11 (SM)	2.03
HQ, I, NC, 3′	HO4 (SM)	OA (P5–6)	1.65
	HN2B (G12)	O10 (SM)	1.83
	HO13 (SM)	OA (P13–14)	1.64
	HO1 (SM)	OA (P14–15)	1.61
	HN16 (SM)	N3 (A14)	1.76
	HO10 (SM)	O11 (SM)	2.08
Q, CV, 3′*R*	HN2B (G12)	O10 (SM)	1.90
	HN16 (SM)	N3 (A14)	1.79
NC, 3′	HN2B (G12)	O10 (SM)	2.16
	HN16 (SM)	OA (P14–15)	1.65

[a] Abbreviations: HQ = dihydroquinone, Q = quinone, CV = covalent, NC = noncovalent, I = iminium ion, SM = saframycin species.

However, the S_N1_{CA} process could occur if the helix becomes distorted temporarily.

Covalent binding of protonated saframycin A in the quinone form was modeled as described above for the dihydroquinone. As discussed in the Experimental Section, the only new atom types are for the cyano group, which appears in the noncovalent models. Parameters for the quinone ring were taken from the literature.[21] The CT atom type for C12 was renamed CU in order to make a selective improper dihedral to it so that the quinone ring could be kept flat. It retained the CT parameters. The method described above was used to establish a trans coplanar arrangement for the pyruvamide carbonyls. Parameters for the new cyano group were taken from the literature.[22] These parameters and all partial atomic charges are given in the supplementary material. Only the *R* configuration and 3′ direction were used, based on the superiority of these factors in the dihydroquinone model. The resulting structure (Figure 6) had a net binding enthalpy (Table I) that was significant, although much poorer

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(22) *Handbook of Chemistry and Physics*, 53rd ed.; Weast, R. D., Ed.; CRC Press: Cleveland, OH, 1972–1973; p F-180.

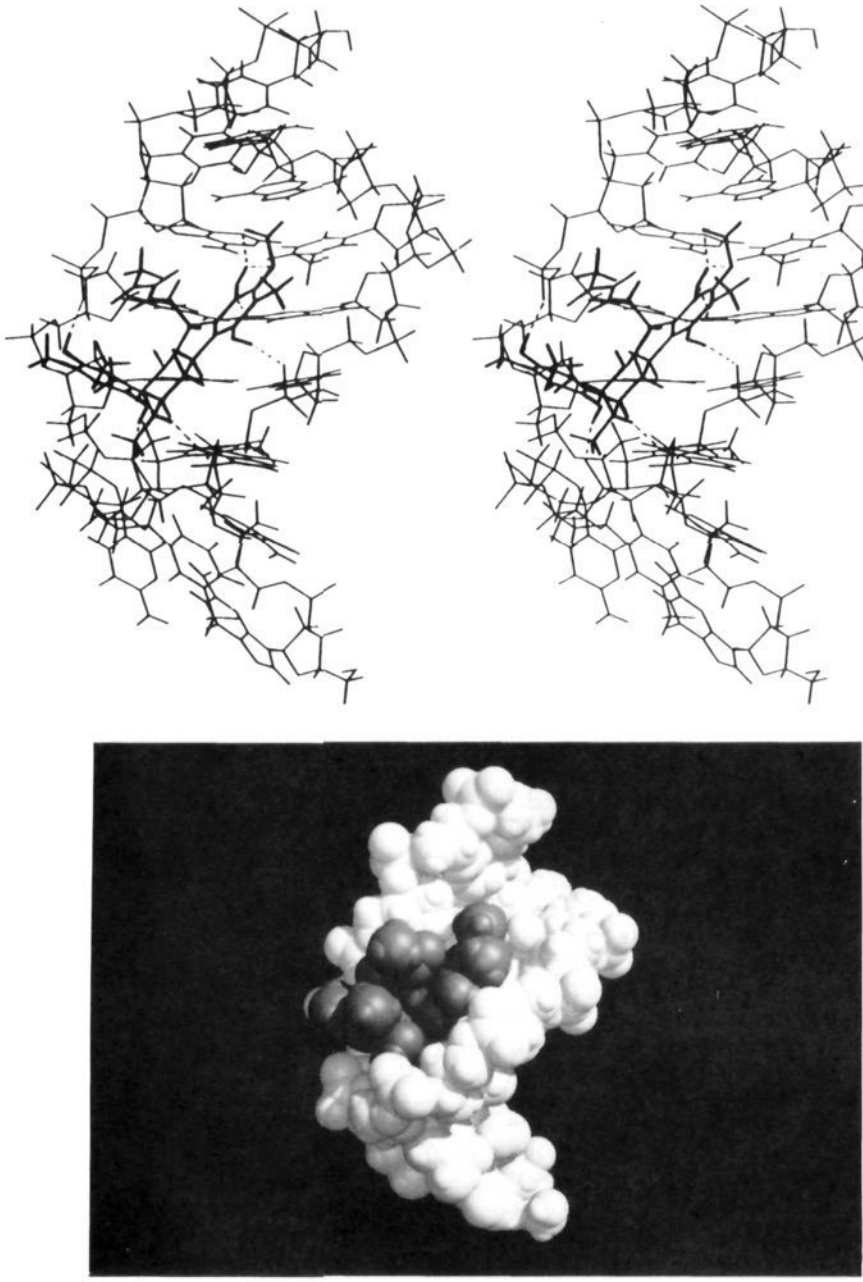

Figure 4. Covalent complex between d(GATGCATC)$_2$ and saframycin A dihydroquinone with *R* configuration at C7 and ring E in the 3′ direction. (a, top) Line drawing with hydrogen bonds indicated by dashes. (b, bottom) Space-filling model illustrating how the A and B rings and the pyruvamide side chain project outward without significantly disturbing the minor groove geometry.

than that of the corresponding dihydroquinone. This difference results mainly from decreased electrostatic interactions, which are related to fewer hydrogen bonds as discussed below.

A model for noncovalent binding of the quinone in the same orientation (Figure 7) had approximately the same net binding enthalpy (Table I). It had less intermolecular binding than the covalent model, but this was overcome by decreased helix and drug distortion. The distance from C7 to N2 of G4 was 6.47 Å, which is not convenient for subsequent covalent binding. Furthermore the N2–C7–(7-cyano) angle of 62.3° rules out a S_N2 process. The need to expel the cyano group into the DNA appears to rule out a S_N1_{CA} process as well, unless the helix is distorted substantially. Noncovalent modeling of an iminium ion was not examined, but it should be favorable because this species is smaller than the quinone retaining the 7-cyano group and it does not need to liberate this group into the DNA.

Group Interactions Including Hydrogen Bonding. The main differences between the 3′*R* and 5′*R* covalent binding models for protonated saframycin A hydroquinone are in interactions between functional groups on this drug and specific residues on d(GATGCATC)$_2$. Analysis by groups (Table II) shows some similar group interaction profiles, as in the interactions with G4, P5–6, P13–14, and P14–15. The 5′*R* model has unfavorable interactions with S13 and S14, whereas the 3′*R* model has only one with S15. Major differences appear where the 3′*R* model interacts favorably with A6, A14, and P15–16. These interactions, which involve hydrogen bonds, are missing or much weaker in the 5′*R* model. The 3′*R* model has six intermolecular plus one intramolecular hydrogen bonds (Table III), but the 5′*R* model has only three intermolecular plus two in-

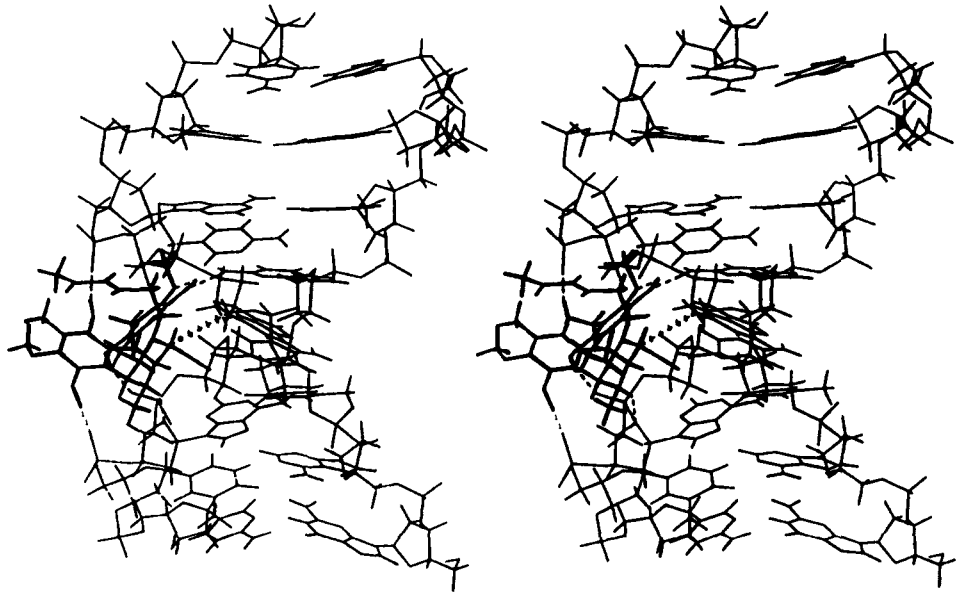

Figure 5. Stereopair for the noncovalent complex between d(GATGCATC)$_2$ and saframaycin A dihydroquinone with ring E in the 3′ direction. Hydrogen bonds are indicated by dashed lines and the distance for potential covalent bonding is indicated by a line of plus signs. Note that the drug will have to move upward as well as inward to form a covalent bond.

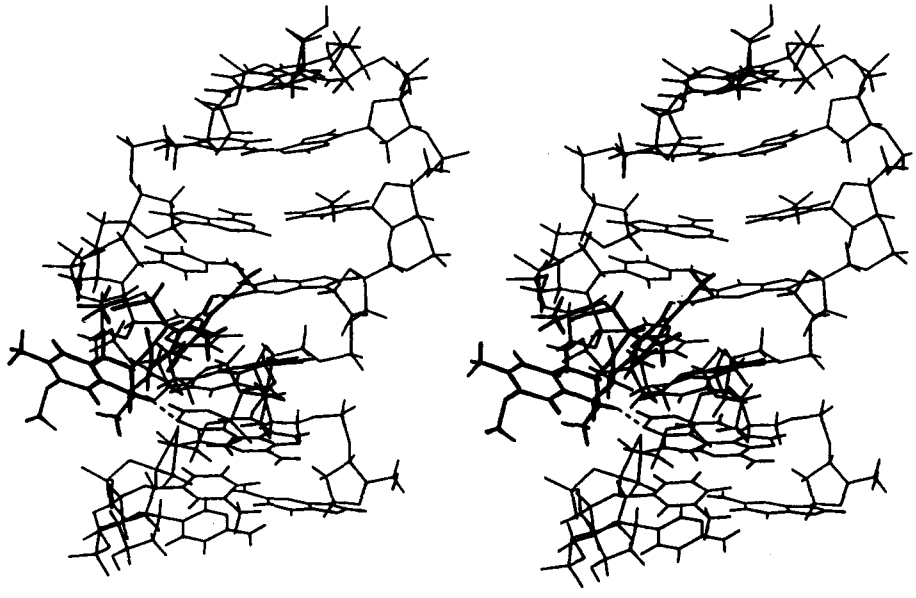

Figure 6. Stereopair for the covalent complex between d(GATGCATC)$_2$ and saframycin A. Hydrogen bonds are indicated by dashed lines.

tramolecular hydrogen bonds. Every hydroquinone hydroxyl in the former (HO1, HO4, HO10, and HO13) is strongly hydrogen bonded (H–O distance <2.0 Å) to an acceptor atom that usually is a phosphate oxygen. These hydrogen bonds include HO10 with N3 and A6 and O10 with HN2B of G4. In the 5′*R* model O10 can only form an intramolecular hydrogen bond because there is no hydrogen donor nearby. There is a short hydrogen bond between HN16 and N3 of A14 (1.81 Å), whereas in the 5′*R* model HN16 only makes a weak hydrogen bond (H–O distance = 2.46 Å) with O2 of C13.

The 3′ iminium ion model also has a strong network of hydrogen bonds. It is the same as the one for the corresponding covalent 3′*R* model, except it lacks the hydrogen bond with N3 of A6. Group interactions are closely parallel to those of the covalent model, although the P3–4, P4–5, and P14–15 interactions are strengthened because of the second positive charge. New unfavorable interactions with S4, S5, S14, and S15 are countered by new favorable interactions with P2–3, P7–8, and P11–12. Absence of the interaction with G4 and decreased interactions with A6 and P15–16 result from the iminium ion not being able to get in closer to the DNA. The C7–N2 distance is 3.28 Å compared with 1.47 Å in the covalent model. In the 3′ noncovalent saframycin A dihydroquinone model, the same hydrogen-bonding network is present as in the iminium ion model. The group interactions are nearly the same as those found in the 3′*R* covalent model, except that the P14–15 interaction is stronger and the P3–4 and G4 interactions are absent.

Covalent binding of protonated saframycin A (quinone) in the 3′*R* orientation results in a set of interactions with individual residues of d(GATGATC)$_2$ that resemble those of the corresponding dihydroquinone (Table II). The interaction with P6–7 is absent and interactions with P5–6, P13–14, and P15–16 are much weaker. This quinone from has four fewer hydrogen bonds than the dihydroquinone. Only those between HN16 and N3 of A14, and O10 and HN2B of G12 are present. The 3′ noncovalent model for the quinone has the HN2B of G12–O10 hydrogen bond and one between HN16 and OA of P14–15. It has fewer group interactions than the covalent model (Table II),

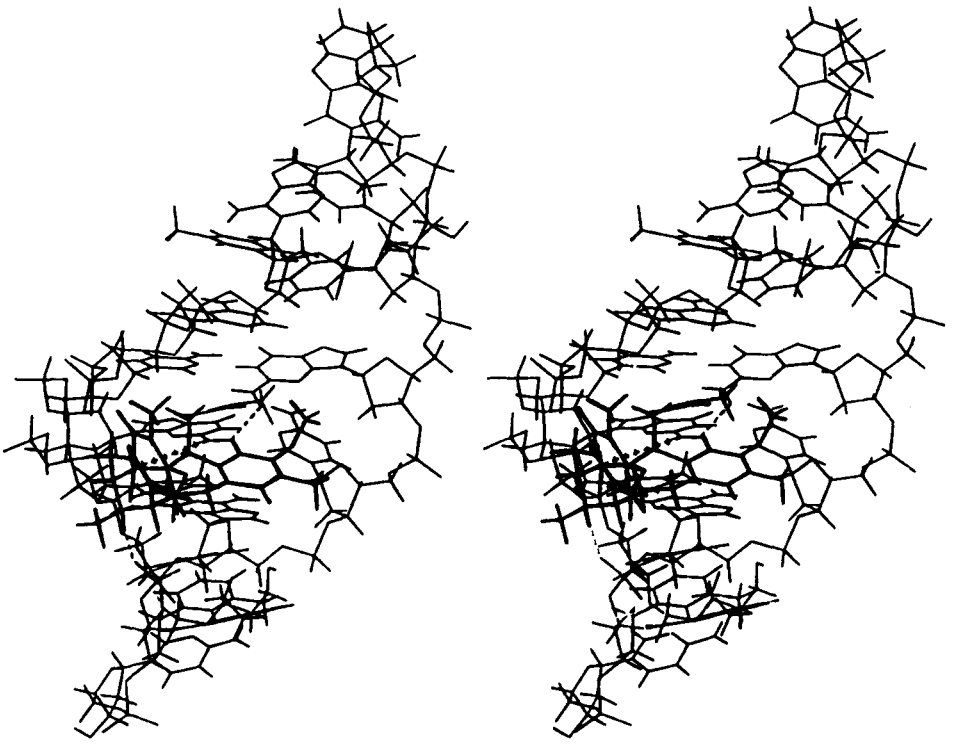

Figure 7. Stereopair for the noncovalent complex between d(GATGCATC)$_2$ and saframycin A. Hydrogen bonds are indicated by dashed lines and the distance for potential covalent bonding is indicated by a line of plus signs.

although the P14–15 interaction is very strong.

The 3′*R* covalent quinone model was solvated in a box of water with counterions present (solvated sodium for each phosphate, 3.0 Å from the phosphorus atom, and chloride for the protonated amino group, see the Experimental Section) to determine the effect on conformation of the adduct and on hydrogen bonding. After molecular mechanics, 15 ps of molecular dynamics and further refinement by molecular mechanics very little change in the overall conformation was apparent when the model was superimposed with the one determined in vacuum. The two hydrogen bonds between the drug and the polynucleotide remained, although a slight change in the conformation lengthened the one between HN16 and N3 of A4 by 0.21 Å. A 180° rotation of the 11-methoxy group allowed its methyl group to interact with hydrophobic groups, rather than water. Water molecules formed hydrogen bonds with O1, O2, O11, O13, and OS3 (side chain) of saframycin A.

Conformational Analysis. In the binding models of saframycin A species, which have helix distortion enthalpies in the range of 36.6–61.8 kcal/mol, it is expected that there will be many distortions in the dihedral angles from their typical values in unbound d(GATGCATC)$_2$. Table IV gives those dihedrals in the adducts differing by a significant amount from the range of their values in the unbound duplex.[23] It arbitrarily includes only those dihedrals differing by more than 40° (30° for χ), although there are many lesser deviations. This table shows that the distortion enthalpies are shared by many dihedrals, rather than being concentrated in a few. In this manner, the approximate form of B DNA is maintained. The Ψ and ω dihedrals are distorted most often, and there is a high frequency of distortion in the Φ and ω' dihedrals. Of course, the actual situation with saframycin A bound DNA would be dynamic, with the DNA "breathing" and rapidly changing dihedral angles. The present models, nevertheless, provide useful "snapshots" of this process and aid our understanding of factors involved in the induced fits of the drug and DNA.

The saframycin pyruvamide side chain maintains planar geometry and projects away from the minor groove to minimize steric interactions with the DNA (Figure 4). Movements of this chain are not possible without significant distortion of the DNA. It makes no significant interactions with d(GATGCATC)$_2$ (Table II), although it does hydrogen bond to added water molecules. The role of this side chain in the mode of action of saframycins is unknown. Molecular modeling provides no insight into this problem, except to suggest that it is in a position to interact with protein molecules that might bind to DNA.

Conclusion

Because of its relatively large size and highly folded geometry, protonated saframycin A has a unique covalent binding mode at N2 of G4 in d(GATGCATC)$_2$. It adopts the 3′ direction in the minor groove and R configuration at its alkylating carbon atom, whether it is in the quinone form or dihydroquinone form. This outcome contrasts the binding of smaller molecules, such as tomaymycin, at the same site, for which there may be two or more reasonable binding models.[12] The dihdyroquinone form has a much higher net binding enthalpy than the quinone, according to molecular dynamics and mechanics, and this difference results from a more extensive hydrogen bond network.

The corresponding noncovalent species also gave good binding models, but their particular geometries precluded subsequent covalent binding by S_N2 or S_N1_{CA} mechanisms. This left alkylation by way of iminium ions, proposed previously in the literature,[9] as the most reasonable pathway. An iminium ion model, based on the dihydroquinone, had a high net binding energy and the two atoms that would form the covalent bond were close to each other. Thus, the proposed mechanism is supported by modeling.

(23) Description of the dihedrals follows the notation of Sundaralingham, M. *Biopolymers* **1969**, *7*, 821. They are defined in Table IV.

Table IV. Backbone Dihedrals in the Adducts Differing Substantially from Those in Unbound d(GATGCATC)$_2$[a]

model[c]	dihedral in adduct (value in degrees)[b]					
	Ψ	Φ′	ω′	ω	Φ	χ
HQ, CV, 3′*R*	A6 (168)	C5 (287)	A2–T3 (206)	C5–A6 (60)	C5 (218)	
	A14 (183)	C13 (279)	C5–A6 (189)	C13–A14 (78)	15 (58)	
	T15 (173)	A14 (276)	C13–A14 (178)			
HQ, CV, 5′*R*	G1 (293)	C5 (282)	C5–A6 (183)	T11–A12 (140)	T15 (121)	G12 (10)
	G12 (178)	A14 (288)	A14–T15 (139)	C13–A14 (127)		
	A14 (184)					
HQ, iminium-ion, NC, 3′	T3 (182)	G4 (278)	G4–C5 (137)	A2–T3 (166)	A14 (75)	G9 (2)
	T5 (165)	C5 (289)	C15–A6 (191)	C5–A6 (56)		
	C8 (175)			A14–T15 (128)		
	A14 (161)					
	T15 (178)					
HQ, NC, 3′	T3 (183)	A2 (179)	A2–T3 (169)	A2–T3 (67)	T15 (235)	G9 (0)
	A6 (168)	G4 (274)	G4–C5 (187)	C5–A6 (60)		
	C8 (177)	C5 (280)	CA–A6 (187)	T7–C8 (158)		
	A10 (184)	A14 (282)	A14–T15 (182)	G9–A10 (177)		
	T11 (186)			A10–T11 (107)		
	A14 (181)			C13–A14 (145)		
	T15 (180)			A14–T15 (61)		
Q, CV, 3′*R*	A6 (170)	C5 (286)	C5–A6 (192)	C5–A6 (59)	C5 (220)	G1 (357)
	A14 (180)			C13–A14 (159)		
	T15 (175)			A14–T15 (136)		
Q, NC, 3′	A2 (181)	C5 (280)	C5–A6 (180)	G1–A2 (93)	A10 (90)	G1 (2)
	T3 (179)	T7 (282)	T7–C8 (149)	A2–T3 (167)		G9 (350)
	A6 (183)	G9 (267)		C5–A6 (81)		A10 (11)
	C8 (188)			T7–C8 (84)		
	A10 (180)			A10–T11 (91)		
	T11 (177)			C13–A14 (153)		
	A14 (173)			A14–T15 (98)		
	T15 (184)					

[a] Definitions of the dihedrals and their ranges over the 14 or 16 residues in which they occur are as follows: Ψ, O5′–C5′–C4′–C3, 57–67°; Ψ′, C5′–C4′–C5′, 103–141°; Φ′, C4′–C3′–O3′–P, 181–185°; ω′, C3′–O3′–P–O5′, 243–272°; ω, O3′–P–O5′–C5′, 289–295°; Φ, P–O5′–C5′–C4, 167–180°; χ, O1′–C1′–N1(9)–C6(8), 40–68°. [b] There were no deviations greater than 30° in the Ψ′ dihedral, which is not included in the table. [c] Abbreviations: HQ = dihydroquinone, A = quinone, CV = covalent, NC = noncovalent.

A frequent criticism of modeling in vacuum is that it ignores the important effects of solvent and counterions. Addition of these species to the covalent quinone model made almost no change in its geometry, except for rotation of a methyl group out of the solvent. There were some hydrogen bonds found between water and saframycin A, but none of the existing ones were broken between the drug and DNA. This result does not mean that solvent and counterions should be ignored in future studies, but it does suggest that earlier in vacuum studies by our group and others may be good approximations.

Experimental Section

Saframycin A was constructed by modifying the X-ray coordinates of the known 15-bromosafracin A (Figure 1).[6] This process involved converting the bromophenol ring into a quinone ring. It was accomplished by superimposing the quinone ring of a second 15-bromosafracin molecule, using the MIDAS interactive graphics program,[20] capturing the coordinates, and substituting those of the quinone ring for those of the bromophenol ring. Saframycin A dihydroquinone was constructed by replacing the bromine by OH to get the appropriate E ring and then transforming the A ring from quinone to hydroquinone by overlaying the E ring. The resulting structures were protonated on N16, which is the singular site of protonation according to Lown et al.[18] They were then minimized in AMBER 3.0, using parameters contained therein, wherever possible. Parameters for the quinone ring of saframycin were taken from those used previously for the quinone ring of mitomycins.[21] The C=C carbons are denoted C5 (CQ in the mitomycin article) and for the carbonyl groups, carbon is CY and oxygen is OY (supplementary material, PREP input file for AMBER). Cyano groups had to be newly defined. We have used C7 and N7 for the atoms, a bond length of 1.158 Å, (taken from the literature[22]) with a force constant of 470.0 kcal/mol Å, and a CT–C7–N7 angle of 180° (supplementary material). Problems arose in maintaining planarity in the quinone ring and the pyruvamide group during molecular mechanics minimizations. They are overcome by using the same parameters given in AMBER but defining new atom types to which improper dihedrals or trans stereochemistry could be made, without interfering with similar atom types that were not involved in problems. Thus, the C12 methyl group on the quinone ring was designated CU, but it retained the CT parameters. It was included in a C9A–C11–C13A–C12 improper dihedral to keep the quinone ring flat. For the pyruvamide chain, the carbonyl groups were defined as C8–O8 and the NH group was N8–H8. Using these designations, it was possible to define a trans-coplanar geometry by using 180° dihedral angles for H8–N8–C8–O8, H8–N8–C8–C8, and N8–C8–C8–O8. It was not necessary to use improper dihedrals to maintain this geometry. The usual AMBER parameters for amide groups were used. Thus, the only really new parameters are those of the cyano group. They are given in the supplementary material.

Coordinates from the minimized structures were used to calculate the partial atomic charges (ESP), which was done with GAUSSIAN 80 (UCSF) using the STO 3G basis set.[18] These charges are listed in the supplementary material. The molecules were then refined in AMBER,[16] using these charges, a distance dependent dielectric constant, a cutoff distance of 99 Å for nonbonded pairs, and updating of the pair list every 100 cycles, until the root mean square gradient was <0.1 kcal/mol Å. A table of input data on protonated saframycin A dihydroquinone for the PREP module of AMBER is given in the supplementary material. The control file for molecular mechanics simulations also is given there. The minimized structure was nearly superimposable with the starting structure, which shows that there were no significant errors in the parameters used. The possibility of nitrogen inversion at N16 on binding to DNA was ruled out because steric hindrance would result with both H14a and the 7-cyano group. Furthermore, if the positions of the proton and methyl group were reversed, the methyl group would point directly into the DNA, causing severe steric hindrance and eliminating important hydrogen bonds.

The dihydroquinone iminium ion was constructed by removing the 7-cyano group and making a C=N bond with a positive charge. The resulting structure was minimized and then partial atomic charges were calculated for the resulting dication (supplementary

material). Reminimization was then done in AMBER.

The octanucleotide duplex d(GATGCATC)$_2$ was constructed and minimized in AMBER using Arnott's B DNA geometry.[24] Figure 3 shows a schematic for this sequence. Minimized saframycin A species were docked onto it near G4 using MIDAS. In many cases, it was obvious that certain orientations would give unsatisfactory models. For protonated saframycin A hydroquinone the covalent models were made in both the 3′ and 5′ direction. Subsequent models were made only in the 3′ direction. Coordinates of the docked models were captured and the structures were refined in AMBER to a root mean square value of <0.1 kcal/mol Å. They were then subjected to 48 ps of molecular dynamics at 300 ± 10 K in AMBER, with a temperature increase from 10 to 300 K in the first 16 ps. The equilibrium conditions were non-classical dynamics with velocity scaling (constant temperature). There was no periodicity and shake was on. A control file for molecular dynamics is given in the supplementary material. The resulting structures were then reminimized using molecular mechanics under the conditions described above.

Helix distortion enthalpies (Table I) were calculated by subtracting the helix enthalpies in the adduct from the enthalpies of the unbound helix, and drug distortion enthalpies were calculated in the same way. Net binding enthalpies were obtained by adding the total intermolecular binding and the distortion enthalpies. Enthalpies for the binding of the drug to individual DNA residues were generated by the analysis module (ANAL) of AMBER 3.0. Hydrogen-bond data were generated in the same way.

Solvation and counterions were added to the completed model of protonated saframycin A with 3′*R* geometry. This was done by placing the complex in a box of water extending ±7 Å from its farthest coordinates. Solvated sodium cations were placed at the bisector of each phosphate group at a distance of 3.0 Å from the phosphorus atom[25] and a solvated chloride ion was placed near the protonated amino group. The system was then minimized in AMBER until the root mean square gradient was <0.2 kcal/mol Å. The model was then submitted for molecular dynamics in AMBER for 15 ps at 300 ± 10 K (starting at 10 K) and then reminimized using molecular mechanics. A control file for molecular mechanics on the solvated adduct is given in the supplementary material.

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Registry No. Saframycin A, 66082-27-7; saframycin A dihydroquinone, 133966-18-4; d(GATGCATC), 133983-37-6.

Supplementary Material Available: Tables of bond, angle, and torsional parameters for the cyano group of saframycin A, atomic charges for saframycin A species, and input and control files for AMBER (7 pages). Ordering information is given on any current masthead page.

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New α-Amino Phosphonic Acid Derivatives of Vinblastine: Chemistry and Antitumor Activity

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A series of new amino phosphonic acid derivatives of vinblastine (1, VLB) has been synthesized and tested in vitro and in vivo for antitumor activity. The compounds were obtained from O^4-deacetyl-VLB azide (5). All of the new products studied were capable of inhibiting tubulin polymerization in vitro. The most potent antitumor compounds bore an alkyl substituent on the phosphonate. In these compounds, the antitumor activity strongly depended on the stereochemistry of the phosphonate. The phosphonate (1*S*)-[1-[[[O^4-deacetyl-3-de(methoxycarbonyl)vincaleukoblastin-3-yl]carbonyl]amino]-2-methylpropyl]phosphonic acid diethyl ester (15) exhibited a remarkable activity against cancer cell lines both in vitro and in vivo.

Bisindole alkaloids (*Vinca* alkaloids) extracted from the Madagascan periwinckle (*Catharantus roseus*) are complex, dimeric structures that occupy a particular place among natural substances. The antitumor activity of this class of compounds was discovered during the 1960s by serendipity, and this instigated many studies concerning the chemical, pharmacological, and clinical aspects of these substances.[1-3]

Vinblastine (1, VLB) and vincristine (2, VCR) have been used in human anticancer chemotherapy for several years (Chart I). It is striking that a minor structural modification (compared with the size and complexity of these compounds) induces very different clinical responses, as VLB and VCR differ only by the transformation, by oxidation, of the indolic methyl of vindoline into a formyl group.

The mechanism of action of *Vinca* alkaloids at the molecular level is not well known, but it is generally considered that they inhibit microtubule formation and subsequently arrest cells in mitosis.[4] Microtubules are involved in many other essential biological processes and inhibiting their formation with drugs can produce important secondary effects. In addition, the antitumor activity of the *Vinca* alkaloids seems to depend critically on their uptake by, and release from, the tumor cells.[5]

Numerous structural analogues have been prepared in order to reduce clinical side effects (particularly neuro-

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