Computer Simulation of the Binding of Naphthyridinomycin and Cyanocycline A to DNA

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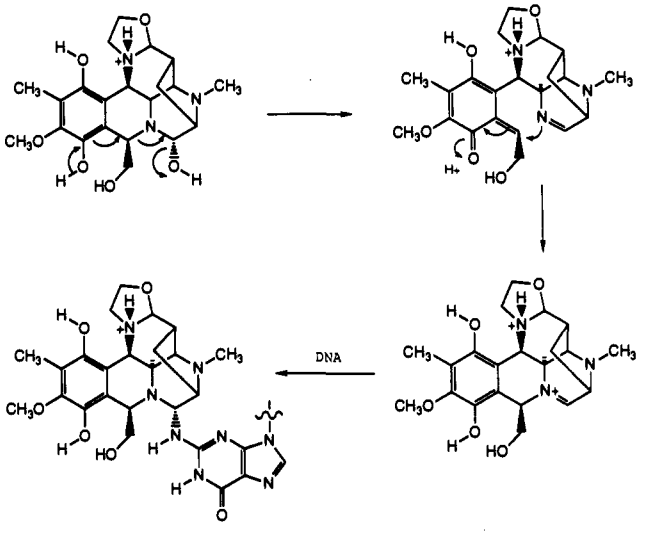
Cyanocycline A was found to have a pK_a of 6.6. Protonation of N14 was established by ^{1}H NMR spectroscopy. In strongly acidic solution the oxazolidine ring opened irreversibly. A model was derived for the binding of naphthyridinomycin and cyanocycline A to the hexanucleotide duplex d(ATGCAT)$_2$, by using the molecular mechanics and dynamics modules of AMBER 3.0. It involved protonation on the oxazolidine-ring nitrogen, reduction of the quinone ring to a hydroquinone, formation of an iminium ion with loss of the C7 substituent, noncovalent binding in the minor groove with the hydroquinone ring in the 3′-direction from guanine, and covalent binding to the 2-amino group of this guanine with C7 adopting the *R* configuration. This model is consistent with the experimental evidence on the DNA binding of these drugs. An alternative binding mode based on opening of the oxazolidine ring and alkylation at C3a also was feasible according to molecular mechanics calculations. The geometry of naphthyridinomycin does not permit interstrand cross-linking involving both C3a and C7, but formation of a cross-link to protein appears possible. When the covalent naphthyridinomycin–d(ATGCAT)$_2$ models were refined in the presence of water and counterions, the models with the most favorable net binding enthalpies were the same as those produced by simulation in vacuum. Qualitative estimates of the relative entropy changes resulting from adduct formation were based on the number of ordered (hydrogen bonded) water molecules released from d(ATGCAT)$_2$ and from the drug. In all cases but one, d(ATGCAT)$_2$ loses five water molecules. It loses six in the C3a covalent model with 5′,*S* geometry. Naphthyridinomycin hydroquinone loses up to two water molecules, depending on the particular adduct. The 3′,*R* model was again favored for the C7 covalent adduct. Among the C3a covalent models, the one with 5′,*R* geometry lost the second most water molecules, but it had the best binding enthalpy.

Naphthyridinomycin and cyanocycline A are complex hexacyclic antibiotics that contain three tertiary nitrogen atoms and a quinone ring.[1-6] The only difference between these two antibiotics is at C7, where the former has a hydroxyl group and the latter has a cyano group (Figure 1). This 7-substitutent is labile, as shown by the facile conversion of naphthyridinomycin into cyanocycline A by cyanide ion.[2] Another antibiotic, differing from naphthyridinomycin only by a hydroxyl group rather than a methoxyl group on the quinone ring (Figure 1), is SF-1739 HP.[7] It was obtained by treating an unstable antibiotic, SF-1739, with HCl. The corresponding 7-cyano derivative is known as cyanocycline F (Figure 1).[7] Structures of both naphthyridinomycin and cyanocycline A have been determined by X-ray crystallography.[5,6] They have slightly puckered quinone rings and a hydrogen bond between the 9′-hydroxyl group and the oxazolidine nitrogen. Rings B and D have distorted chair conformations, ring E is a distorted sofa, ring A has a twist conformation and ring C is a distorted envelope. Some of these conformations are evident in the stereo pair of Figure 2. Treatment of cyanocycline A with HCl or HBr resulted in opening of the oxazolidine ring, with its nitrogen converted into an iminium ion and its oxygen protonated.[6] The X-ray crystallographic structures of these products showed that ring B changed to a distorted sofa conformation. An arbitrarily assigned absolute configuration for naphthyridinomycin, cyanocycline A, and related compounds was reversed in 1986,[8] which means that the literature prior to this time has incorrect absolute stereochemistry.

Scheme I



The mode of antitumor action of these compounds has been studied by a number of investigators.[9-13] Although much work remains to be done in this area, the following findings appear to be significant: (1) Cytotoxicity results mainly from inhibition of DNA synthesis, which produces unbalanced cell growth.[9] RNA synthesis and protein synthesis are inhibited at concentrations higher than cytotoxic ones.[9,10] (2) Binding selectivity is highest in DNAs rich in GC pairs and there is no binding to polynucleotides that lack GC pairs.[12] (3) T_4 DNA, which is 100% glycosylated in the major groove, is readily bound by na-

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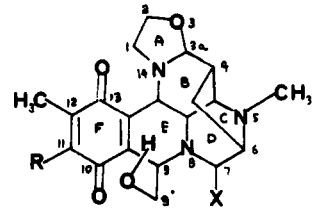
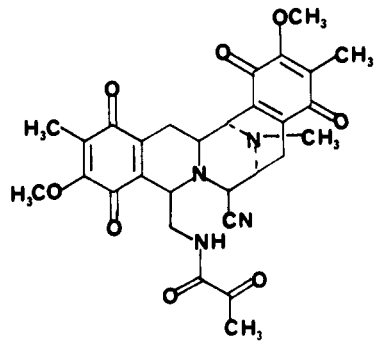


Napthyridinomycin: R=CH_3O, X = OH

Cyanocycline A: R=CH_3O, X = CN

Cyanocycline F: R=HO, X = CN

SF-1739 HP: R=HO, X = OH



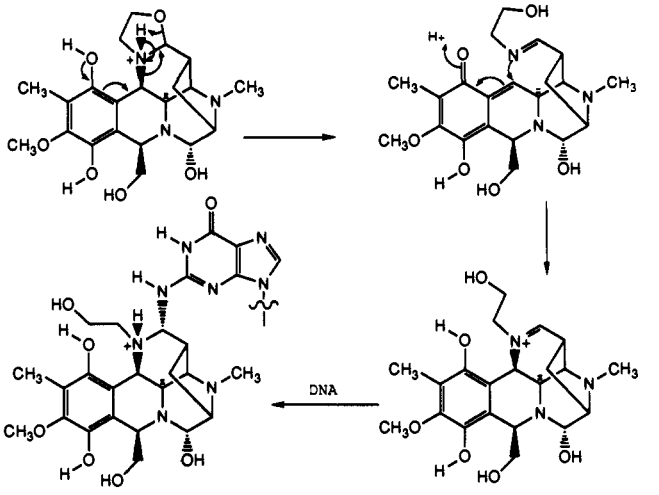
Saframycin A

Figure 1. Structures of naphthyridinomycin, cyanocyclines, and saframycin A.

phthyridinomycin. This result suggests that binding occurs in the minor groove.[12] (4) Unreduced naphthyridinomycin binds slowly to DNA, with the maximum rate occurring at pH 5. Reduction to the hydroquinone by dithiothreitol or penicillamine resulted in a rapid burst of binding followed by a slower phase. Very little binding occurred below pH 5 or above pH 7.09 in the presence of these reducing agents.[12] (5) The relative stability of the complex to dialysis indicated covalent or other strong binding; however, the gradual release of the drug over a number of days suggested slow reversibility of the binding.[12] This effect can be imagined to be the formation and gradual hydrolysis of aminal linkages derived from alkylation of the 2-amino groups of guanine residues.

On the basis of the foregoing evidence, Zmijewski and co-workers proposed two different mechanisms for the interaction of naphthyridinomycin with DNA.[12] Their first mechanism was based on that of saframycin A. It involved the conversion of either the antibiotic or its hydroquinone to iminium ions, which bind in the minor groove of DNA and then form covalent bonds with the 2-amino group of a guanine residue. In this mechanism, the hydroquinone would be expected to be more reactive than the quinone because of participation of hydroxyl groups of the hydroquinone in iminium ion formation, as shown in Scheme

Scheme II



I. These groups might also help initial DNA binding by forming specific hydrogen bonds. Enhanced reactivity of the iminium ion of the quinone form at pH 5 could be explained by protonation of one or more tertiary nitrogen atoms. The second mechanism involved noncovalent binding of the hydroquinone, followed by alkylation of guanine through a S_N1_{CA} process.

Although the mechanisms described are reasonable and they do not contradict the experimental evidence, it should be possible to gain much more insight into their feasibility by computer modeling of the DNA-binding process. In particular, this approach could determine if covalent binding in the minor groove to a guanine residue can take place without serious distortion of the DNA or the drug. It could also predict the preferred direction in the groove and configuration at C7 of the drug. Relative enthalpy differences in the initial binding of quinone and hydroquinone forms could be calculated and specific hydrogen bonds involving the latter could be identified. Finally, the possibility of DNA alkylation by the oxazolidine ring of the drug molecules could be evaluated. This possibility has been neglected in considerations of mode of action because of the demonstrated ability of C7 substituents and the absence of cross-linking. The biphasic release of bound drug from DNA, however, suggested there was more than one mode of binding.[12] Consideration of the structures of naphthyridinomycin and cyanocycline A (Figure 1) shows that the oxazolidine oxygen has the same relationship as the C7 substituent to the quinone ring and a similar mechanism for DNA alkylation can be written (Scheme II). Furthermore, opening of the oxazolidine ring by HBr or HCl to give a stable iminium ion suggests that this process might be enhanced in cells that have lower pH, as is the case with certain tumor cells.

For the reasons cited above, and an interest in the comparative binding of DNA by various antitumor antibiotics, we undertook a study on computer modeling of

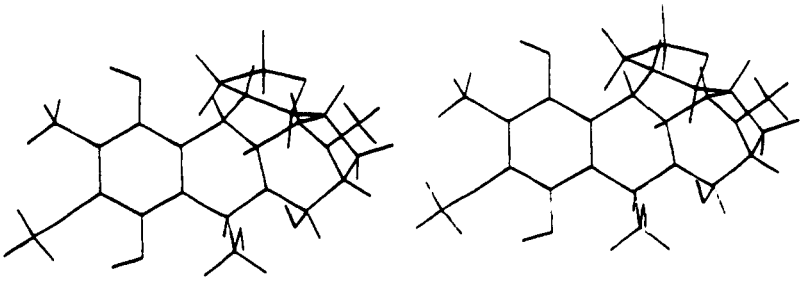


Figure 2. Stereo pair for naphthyridinomycin hydroquinone protonated on N14.

Table I. ^{1}H Chemical Shifts of Cyanocycline A and the Protonated Derivative

position number[a]	cyanocycline A[b]	protonated cyanocycline A	chemical shift different ($\Delta\delta$)[c]
1	4.00, 3.70	4.05, 4.21	0.05, 0.51
2	4.20	3.61, 3.28	-0.59, -0.92
3a	4.72	4.13	-0.60
4	3.17	3.28	0.11
4′	1.70, 2.37	1.81, 2.45	0.11, 0.08
6	3.39	3.43	0.04
7	3.94	3.98	0.04
9	2.97	4.25	1.38
9′	3.64, 2.97	3.75, 3.95	0.11, 0.98
13b	3.78	4.23	0.45
13c	2.83	2.95	0.12
5′	1.97	1.96	-0.01
11′	4.05	4.07	0.02
12′	2.42	2.42	0

[a] See Figure 1 for numbering system. [b] Data from S. Gould, Oregon State University. [c] δ protonated cyanocycline A - δ cyanocycline A.

naphthyridinomycin and cyanocycline A binding. Hydroquinone forms were chosen on the basis of their faster reactions with DNA. The following goals were set for this study: (1) determine the preferred site of protonation by NMR techniques and the pK_as by titration; (2) model the covalent binding of the hydroquinone form at the 2-amino group of a guanine residue of DNA using both directions in the minor groove and both *R* and *S* configurations at C7 and calculate the relative net binding enthalpies, hydrogen-bond formation, changes in conformations of the drugs and DNA, and interaction enthalpies between the drugs and individual DNA residues (both drugs give the same species when the C7 substituent leaves); (3) model in a similar way the noncovalent binding of the hydroquinone form of naphthyridinomycin (arbitrarily chosen over cyanocycline A) and determine if the distance and alignment of functional groups is suitable for subsequent covalent binding; and (4) investigate the covalent binding of naphthyridinomycin hydroquinone to DNA by way of C3a in its oxazolidine ring, using both directions in the minor groove and both configurations at C3a. If any model with good binding enthalpy and relatively low distortion of the DNA is found, investigate initial noncovalent binding by the hydroquinone.

Results and Discussion

Before modeling the binding of naphthyridinomycin and cyanocycline to DNA, it was necessary to determine the position and extent of protonation that they would undergo. Titration of cyanocycline A gave a pK_a of 6.6. This result indicates that there would be 25% protonation at pH 7 and considerably higher protonation in the acidic environment of some tumor cells. Below pH 6.6 the drug underwent a structural change that prevented determination of pK_as for the other two nitrogens. A monocation was selected as the species to be modeled, because even if it does not predominate over the neutral molecule, it would be more attracted to DNA because of its charge. The position of protonation was unknown. On the basis of protonation of the related structure of saframycin A, it appeared that N5 might be preferentially protonated; however the site of protonation was established to be N14 by the following NMR experiments.

Analysis of the TOCSY and DQF-COSY spectra of the protonated form of cyanocycline A gave the assignments of the proton resonances shown in Table I. The chemical shifts can be compared with those assigned to the proton resonances for cyanocycline A (Gould and He, private communication) and it can be seen that significant changes in chemical shift (>0.15 ppm) only occur at positions 1, 2, 3a, 9, 9′, and 13b (Table I). Positions 1, 2, 3a, and 13b are situated either α or β with respect to N14 and their observed chemical shifts in protonated cyanocycline A are consistent with protonation occurring at this nitrogen. The explanation for the chemical shift changes occurring at positions 9 and 9′ is that protonation at N14 breaks the original hydrogen bond between the hydrogen of the 9′-hydroxyl group and N14, as seen in the crystal structure,[5,6] and is replaced by a hydrogen bond between the oxygen of the 9′-hydroxyl and the protonated N14. Computer modeling using the minimized structure for the protonated form of naphthyridomycin shows this bond is relatively strong (1.91 Å) and the resulting juxtaposition of the 9′-hydroxyl with the now protonated N14 causes the observed chemical shift changes of the protons at 9′ and, similarly, that of 9. The 9′-hydroxyl hydrogen resonance is observed at 5.21 ppm in the ^{1}H NMR spectrum of protonated cyanocycline A and a NOESY spectrum shows intense nuclear Overhauser enhancements (NOEs) from this resonance to positions 4′ and 9′ and medium NOEs to positions 1 and 2. These interactions are consistent with our model of cyanocycline A protonated at N14. Further corroboration that the site of protonation is indeed N14 can be found by default. That is, if N5 was the preferred site of protonation, it could be expected that the protons at 4a, 5′, and 6 would display a significant change in chemical shift. These changes are, however, only 0.11, -0.01, and 0.04 ppm, respectively. Similarly, if N8 is protonated, significant chemical shift changes would be expected at positions 7 and 13c. Again, the changes of 0.04 and 0.12 ppm, respectively, are not significant.

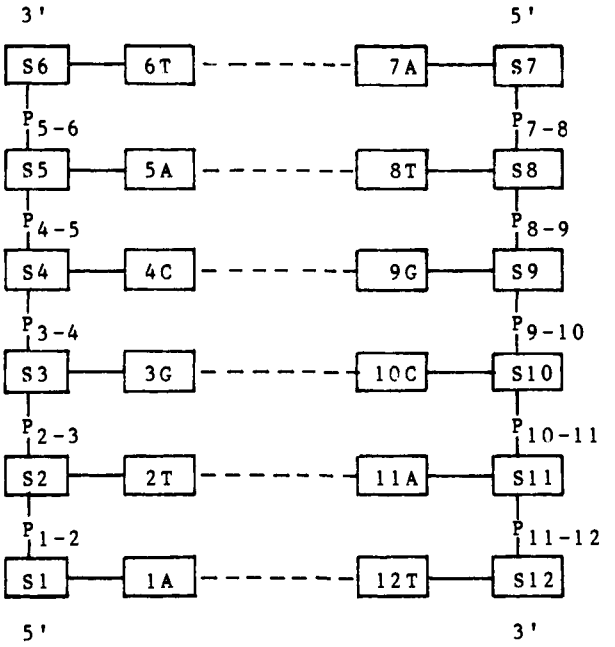


Figure 3. Schematic for d(ATGCAT)$_2$. S stands for sugar.

C7 Alkylation Models

As noted above, there are four different ways in which covalent binding between C7 of naphthyridinomycin or cyanocycline A and the 2-amino group of GUA3 can occur (See Figure 3 for schematic of the d(ATGCAT)$_2$ sequence). The configuration at C7 can be *R* or *S* and the drug molecule can be aligned in the minor groove with its hydroquinone ring pointing toward either the 3′-end or the 5′-end of the strand to which the covalent bond is formed. As shown in Table II, there is a clear preference (by 15.0

Table II. Interaction Enthalpies (in kcal/mol) for Naphthridinomycin Hydroquinone with d(ATGCAT)$_2$

binding mode[a]	direction and config[b]	intermolecular[e] total	vdW	elstat	total	helix disort[c]	drug distort	net binding[d]
CV at C7	3′,*R*	−365.2	−23.7	−76.8	−100.5	25.3	6.2	−69.0
	3′,*S*	−349.4	−17.5	−87.9	−105.4	40.8	11.4	−53.2
	5′,*R*	−335.8	−22.8	−52.3	−75.1	28.0	7.4	−39.7
	5′,*S*	−335.7	−15.2	−60.3	−75.5	30.1	5.9	−39.5
NC at C7	3′	−368.5	−27.2	−73.0	−100.2	19.1	8.7	−72.4
	5′	−375.3	−19.6	−99.4	−119.0	22.0	17.9	−79.1
CV at C3a	3′,*R*	−322.4	−16.2	−61.8	−78.0	33.0	8.8	−36.2
	3′,*S*	−322.4	−9.8	−63.2	−73.0	32.6	14.2	−26.2
	5′,*R*	−349.3	−7.0	−102.7	−109.7	37.9	19.8	−52.0
	5′,*S*	−344.2	−11.7	−80.3	−92.0	30.1	14.0	−47.9
NC at C3a	3′	−356.3	−10.2	−71.5	−81.7	16.3	5.3	−60.1
	5′	−349.5	−15.5	−76.8	−92.3	20.6	18.4	−53.3

[a] CV indicates covalently bound and NC indicates noncovalently bound. [b] Direction refers to the direction in the minor groove that the hydroquinone ring takes with respect to the point of covalent or potential covalent binding. [c] The enthalpy of d(ATGCAT)$_2$ minimized in the absence of drug is −424.3 kcal/mol. [d] Net binding is total intermolecular binding plus drug distortion and helix distortion. [e] van der Waals and electrostatic.

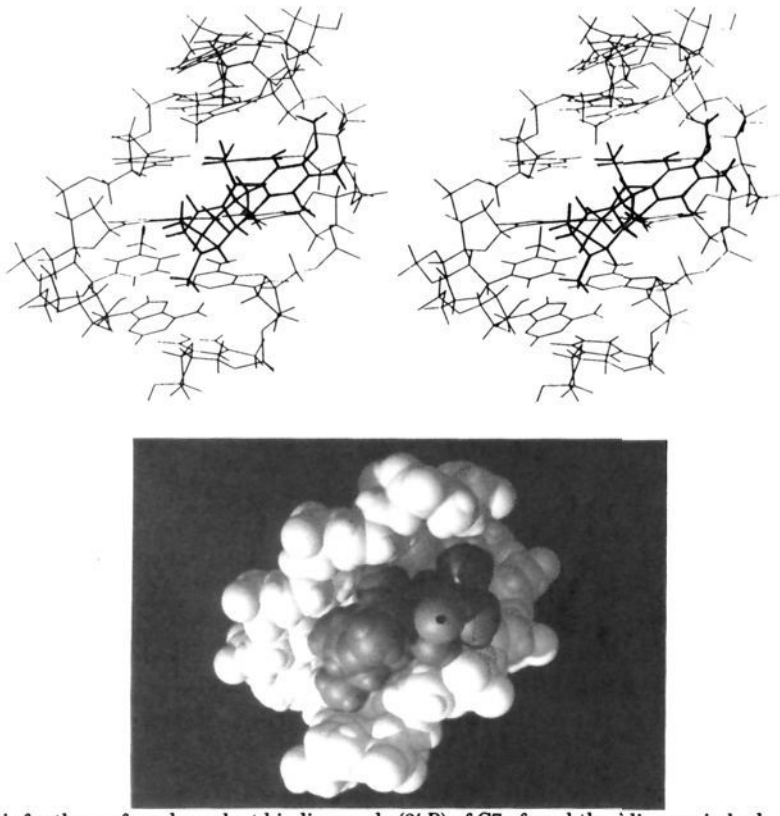


Figure 4. (top) Stereo pair for the preferred covalent binding mode (3′,*R*) of C7 of naphthyridinomycin hydroquinone with d(ATGCAT)$_2$. (bottom) Space-filling model showing waters (dot) that solvate the drug.

kcal/mol or more) for the *R* configuration and 3′-orientation of the hydroquinone over the other three possibilities, based on their net binding enthalpies, which equal the total intermolecular binding enthalpies minus the helix- and drug-distortion enthalpies. These enthalpy values were obtained by "belly" molecular dynamics (DNA fixed and drug free to move) followed by molecular mechanics with no constraints. The stereo pair for this preferred model is shown in Figure 4. A significant part of the intermolecular binding enthalpy involves interactions between the drug and P_{10-11} (Table III). It is derived from a strong hydrogen bond with HO13 of the drug. The covalent model with the second highest binding enthalpy has *S* configuration and 3′-direction of the aromatic ring (Table II). Its strongest electrostatic interaction is with P_{11-12} and it participates in three hydrogen bonds, one involving the proton on N14, another the proton on HO9, and the third involving the proton from HO13, one of the hydroquinone hydroxyls. The corresponding 5′-direction models have poorer net binding enthalpies, mainly as a result of decreased electrostatic forces.

One of the mechanisms advanced by Zmijewski et al. for covalent binding of naphthyridinomycin at C7 is based on initial binding of an iminium ion.[12] The formation of an *N8–C7* iminium from naphthyridinomycin or cyanocycline A should be facile, because a trans–coplanar arrangement exists among the electron pair on N8, C7, and the leaving group on C7. This concept is supported by the ease of transforming naphthyridinomycin into cyanocycline A.[2] The alternative mode of binding at C7 featured initial noncovalent binding by the hydroquinone followed by alkylation.[12]

Both the 3′- and 5′-directions were modeled for noncovalent complexes of the hydroquinone. As shown in Table

Table III. Interaction Enthalpies (in kcal/mol) for Naphthridinomycin Hydroquinone with Individual Residues of d(ATGCAT)$_2$[a]

binding mode[b]	direction and configuration[c]	P_{2-3}	G3	P_{3-4}	C4	P_{4-5}	P_{5-6}	P_{9-10}	C10	P_{10-11}	A11	P_{11-12}	S12
CV at C7	3′,*R*[d]	-11.4	-3.7	-3.7	-5.4	-14.7	-8.4	-3.9	-4.8	-28.2		-35.5	
	3′,*S*	-3.6	-9.0	-3.4	-11.1	-23.1	-6.5	-4.0	-5.1	-9.1		-33.0	
	5′,*R*[e]	-4.3	-6.4	-3.8		-7.0	-3.2		-5.2	-7.0	-5.3	-13.0	-4.9
	5′,*S*	-3.7	-7.0	-5.4	-3.9	-6.1	-3.2	-3.4	-5.8	-7.0	-8.9	-22.8	
NC at C7	3′[f]		-6.3	-5.6	+4.2	-13.6	-6.0	-3.0		-22.1	-6.0	-4.2	-6.2
	5′[g]	-4.1	-7.0	-24.6		-4.1					-9.7	-39.6	-18.0
CV at C3a	3′,*R*	-3.3	-5.8		-12.9	-8.4	-6.0	-5.6	-6.9	-10.5	-3.6	-10.4	
	3′,*S*	-3.6	-3.9		-6.5	-7.0		-5.3	-7.9	-11.0	4.0	-11.7	
	5′,*R*[h]	-5.6	-10.9	-4.9		-27.5	-4.1	-3.0	-4.0	-6.0	-4.4	-46.4	
	5′,*S*[i]	-4.8	-16.6	-5.0	-4.2	-34.5	-5.5	-3.3	-5.0	-8.4	-3.4	-5.2	-5.1
NC at C3a	3′[j]						-22.3	-7.7	-3.9	-25.5	-5.2	-5.2	
	5′[k]	+5.4	-8.4	-23.0		-21.6					-7.1	-9.3	-17.8

[a] Residues are listed only if the energies are <3.0 kcal/mol. See Figure 3 for location of these residues within the DNA. [b] CV indicates covalently bound and NC indicates noncovalently bound. [c] Direction refers to the direction that the drug lies in the minor groove and configuration refers to the stereochemistry at C7 or C3a in the covalent adducts. [d] Also G9, -3.8. [e] Also T2, -6.7. [f] Also S4, -9.0; A5, -13.6; S6, -6.6; G9, -3.9; S10, -5.9. [g] Also S4, -12.1; S11, +4.0; T12, +3.6. [h] Also S3, +3.4; S11, +3.3. [i] Also S3, +3.4. [j] Also S4, -3.4; A5, -4.8; S6, -7.7; G9, -3.1; S10, +3.5. [k] Also T2, -5.0; S3, +5.4; S4, -7.4; S10, -3.0; S11, +3.4; T12, +5.3.

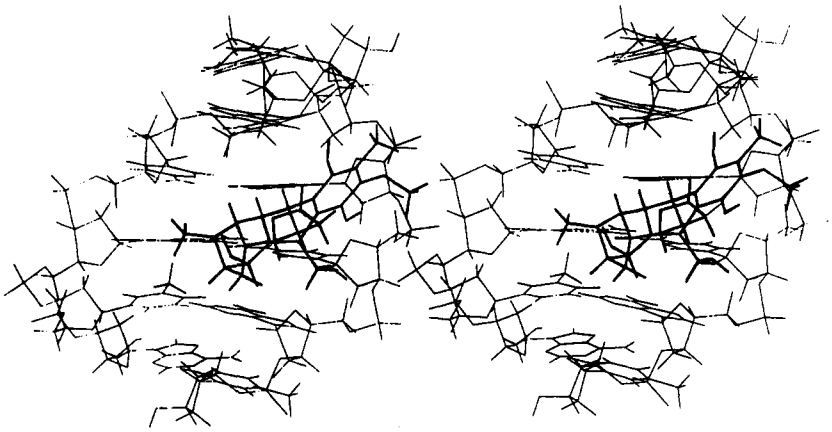


Figure 5. Stereo pair for the noncovalent binding in the 3′-direction of naphthridinomycin hydroquinone (near C7) with d(ATGCAT)$_2$.

II, both of these complexes have relatively high net binding enthalpies, but the 5′-direction is preferred by 6.7 kcal/mol. It has much stronger electrostatic binding interactions than the model with 3′-direction. Figure 5 is a stereo pair for the hydroquinone 3′-direction model which could lead to the preferred covalent model, which is favored by 29.3 kcal/mol. Interaction enthalpies by residue are given in Table III and the hydrogen bonds are listed in Table IV. Both models have substantial hydrogen-bond networks and each network involves the 7-substituent that will leave in the next step of the reaction sequence. For both directions, O7 binds with HN2 of GUA3. The proximities of the NH_2 group of GUA3 to C7 in the 3′- and 5′-direction models, 3.53 and 3.62 Å, respectively, suggest that they are close enough for subsequent covalent bond formation. However, the atoms N2 of GUA3 and C7 and O7 of naphthyridinomycin do not have the desired 180° angle. Their angles are 43.1° and 38.4° for the 3′- and 5′-models. Consequently, a concerted alkylation process is ruled out. The proposed S_N1_{CA} process seems favorable because loss of the 7-OH group with formation of water and an iminium ion would be promoted by the HN2B–O7 hydrogen bonds in both models. Molecular modeling, therefore, supports both mechanisms of Zmijewski et al.[12]

C3a Alkylation Models

Models for the covalent binding of naphthyridinomycin hydroquinone at C3a also were explored. The 5′,*R* model (Figure 6) was preferred by 4.1 kcal/mol over the 5′,*S* model, but the other models were much poorer (Table II). High total intermolecular binding enthalphies were found for the 5′,*R* and 5′,*S* models, with the main contributions coming from electrostatic interactions. The strongest interactions between drug and individual residues of d(ATGCAT)$_2$ involved P_{4-5} (H bond with HO7) for the 5′,*R* model and P_{4-5} (H bond with HO9) for the 5′,*S* model (Tables III and IV). In addition to adequate net binding enthalpy, the covalent binding at C3a is favored by geometry in the oxazolidine ring that should permit facile ring opening to an iminium ion. The trans–coplanar relationship between the electron pair on N14, N14, C3a, and O3 (Figure 2) suggests that this transformation could take place without a conformational change in the molecule. (In a related study it was found that quinocarcin had to undergo such a change, requiring about 7 kcal/mol of enthalpy, before its oxazolidine ring could open in a concerted manner.)[14]

Satisfactory models in both the 5′- and 3′-directions were obtained for the noncovalent binding of naphthyridinomycin in the vicinity of C3a. The 3′-direction was strongly preferred (Table II). Both models made two strong hydrogen bonds with phosphate groups (Tables III and IV). In the 5′-direction model (Figure 7), the distance between C3a and N2 of GUA3 was 3.66 Å, which is close enough for facile covalent bond formation. However, the possibility of a concerted process involving attack of N2 on C3a with opening of the oxazolidine ring is ruled out by the geometry. The N2–C3a–O3 angle is only 65.0°, rather than

(14) Hill, G. C.; Wunz, T. P.; Remers, W. A. J. *Comput.-Aided Mol. Des.* **1988**, *2*, 91.

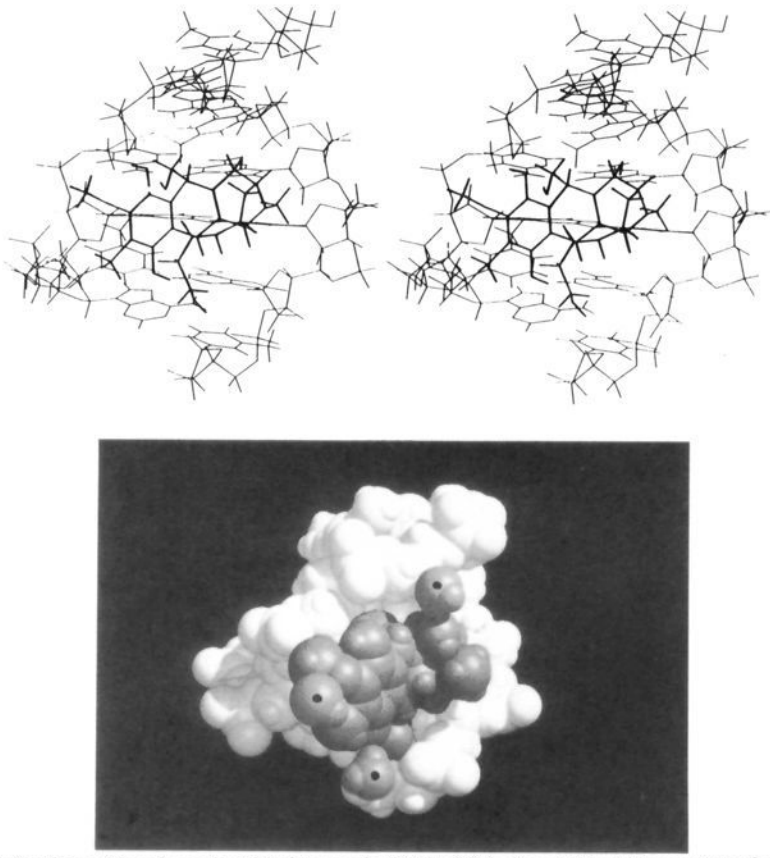


Figure 6. (top) Stereo pair for the preferred covalent binding mode (5′,*R*) of C3a of naphthyridinomycin hydroquinone with d(ATGCAT)$_2$. (bottom) Space-filling model showing waters (dots) that solvate the drug.

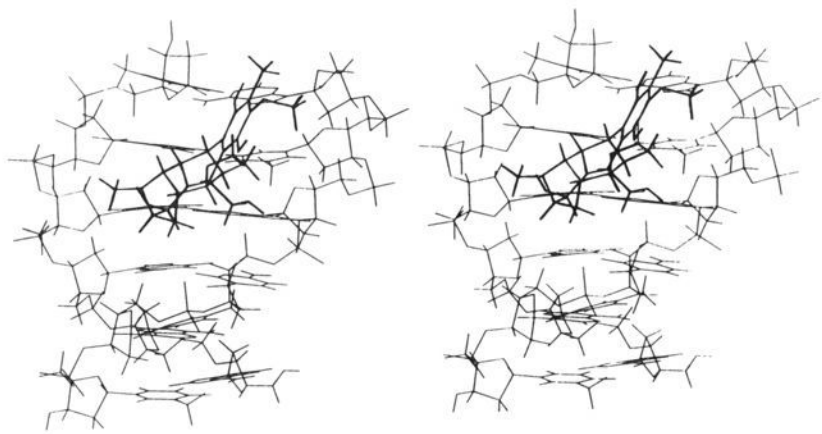


Figure 7. Stereo pair for the noncovalent binding in the 5′-direction of naphthyridinomycin hydroquinone (near C3a) with d(ATGCAT)$_2$.

the optimal 180°. An S_N1_{CA} process involving protonation of O3, followed by opening of the oxazolidine ring to give an iminium ion and alkylation, does appear possible, although enhancement by hydrogen-bond formation with HN2B (GUA3) would not be pronounced as it was for the reaction at C7, because the O3–HN2B distance is 2.59 Å. The 3′-direction model shows the same characteristics, with a 3.66 Å separation of C3a and N2 (GUA3), an N2–C3a–O3 angle of 55.4 Å, and a HN2B (GUA3)–O3 hydrogen bond length of 2.80 Å. Thus, the two mechanisms proposed by Zmijewski for alkylation at C7 also are feasible for alkylation at C3a.

Solvation Models

Each of the above eight models for the covalent binding of naphthridinomycin hydroquinone to d(ATGCAT)$_2$ was examined further for the effects of solvent and counterions on the relative enthalpies of the minimized structures and for qualitative estimation of entropy changes that might occur on solvation. The energy-refined structures were solvated in a box of TIP3P water and positive ions were placed on the bisectors of the phosphate groups. They were then refined by molecular mechanics in AMBER. The hexanucleotide duplex similarly solvated and with counterions, but without the drug, was minimized independently. Figure 8 depicts the first-shell waters in the minor groove of d(ATGCAT)$_2$. There are 10 water molecules (Table V) and they form a regular water structure that is similar to the "strings" running down the minor groove of CCAAGATTGG.[15] Table VI lists the enthalpies for

(15) Prive, G. G.; Heinmann, U.; Chandrasegaran, S.; Kan, L. S.; Kopka, M. L.; Dickerson, R. E. *Science* **1987**, *238*, 498.

Table IV. Hydrogen-Bond Parameters Involving Naphthyridinomycin–Polynucleotide Interactions

binding mode[a]	direction and config[b]	hydrogen atom	acceptor atom	length, Å
CV at C7	3′,*R*	HN2B(G9)	O10(NHQ)	1.82
		HO9(NHQ)[d]	O1′(A5)	1.82
		HO13(NHQ)	OA(P_{11-12})	1.64
		HO10(NHQ)	O11(NHQ)	2.14
		NH14(NHQ)	O9(NHQ)	1.71
	3′,*S*	HO9(NHQ)	O2(C4)	1.87
		HO10(NHQ)	O11(NHQ)	2.31
		HO13(NHQ)	OA(P_{12-13})	1.64
		HN14(NHQ)	O9(NHQ)	1.71
	5′,*S*	HO9(NHQ)	O1′(T2)	1.83
		NH14(NHQ)	O9(NHQ)	1.70
	5′,*S*	NH14(NHQ)	O9(NHQ)	1.77
		HO9(NHQ)	N3(A11)	1.97
		HO10(NHQ)	O11(NHQ)	2.14
NC at C7	3′	HN2B(G3)	O7(NHQ)	1.81
		HN2B(G9)	O10(NHQ)	1.90
		HO13(HNQ)	OA(P_{11-12})	1.64
		HN14(NHQ)	O9(NHQ)	1.75
		HO9(NHQ)	O3′(C4)	1.90
		HO10(NHQ)	N3(A5)	2.04
		HO10(NHQ)	O11(NHQ)	2.33
	5′	HN2B(G3)	O7(NHQ)	1.78
		HO9(NHQ)	OA(P_{11-12})	1.65
		HN14(NHQ)	O9(NHQ)	1.82
		HO8(NHQ)	O1′(C4)	1.95
		HN14(NHQ)	OA(P_{11-12})	2.11
		HO10(NHQ)	O11(NHQ)	2.22
CV at C3a	3′,*R*	HO3(NHQ)	O2(C4)	1.81
		HN14(NHQ)	O9(NHQ)	1.74
		HO13(NHQ)	O1′(A5)	2.41
		HO9(NHQ)	O1′(A11)	1.82
		HO10(NHQ)	O11(NHQ)	2.15
		HO13(NHQ)	O3(NHQ)	1.83
	3′,*S*	HN2B(G9)	O3(NHQ)	2.20
		HN14(NHQ)	O9(NHQ)	1.69
		HO3(NHQ)	O2(C4)	2.34
		HO9(NHQ)	O1′(A11)	1.81
		HO10(NHQ)	O11(NHQ)	2.16
		HO13(NHQ)	O3(NHQ)	1.82
	5′,*R*	HN14(NHQ)	O9(NHQ)	1.76
		HO7(NHQ)	OA(P_{4-5})	1.66
		HO9(NHQ)	O1′(C4)	2.33
		HO3(NHQ)	OA(P_{11-12})	1.65
		HO10(NHQ)	O11(NHQ)	2.16
	5′,*S*	HO9(NHQ)	OA(P_{4-5})	1.63
		HO13(NHQ)	O3(NQH)	1.82
		HN14(NHQ)	O9(NHQ)	1.73
		HO3(NHQ)	N3(G3)	2.46
		HO10(NHQ)	O11(NHQ)	2.16
NC at 3a	3′	HN14(NHQ)	O9(NHQ)	1.73
		HO13(NHQ)	OA(P_{5-6})	1.63
		HO9(NHQ)	OA(NHQ)	1.65
		HO10(NHQ)	O9(NNQ)	2.17
	5′	HO9(NHQ)	OA(P_{3-4})	1.63
		HO7(NHQ)	OA(P_{4-5})	1.68
		HN2B(G3)	O3(NHQ)	1.94
		HO10(NHQ)	O11(NHQ)	2.15

[a] All hydrogen bonds less than 2.5 Å long are listed. Most of the enthalpies for hydrogen bonds are included in the electrostatic fields, which are given in Table II. [b] CV indicates covalently bound and NC indicates noncovalently bound. [c] Direction refers to the direction that the drug lies in in the minor groove and configuration refers to the stereochemistry at C7 or C3a in the covalent adducts. [d] NHQ = Naphthridinomycin hydroquinone.

drug–DNA binding in the eight solvation models. Total enthalpies are not included because they reflect the total number of water molecules in the models and these numbers vary substantially as a result of constructing the box of water around the drug–DNA adduct. The net binding enthalpies, however, are appropriate for comparing the various models. Among the models with covalent bonding at C7, 3′,*R* is clearly favored, as it was in the model in vacuum. The main difference in rank order of the models occurs with the 3′,*S* model, which had the second best net binding enthalpy in vacuum by 13.5 kcal/mol, but is no better than the 5′,*S* model in solvent. For the models with covalent bonding at C3a, the 5′,*R* model has the best net binding enthalpy solvated and in vacuum and the rank order of the other models is unchanged between the two media. Such maintenance of relative binding enthalpy order is consistent with the observation that the drug–DNA parts of the models are nearly superimposible when the solvated and unsolvated adducts are compared by the ANAL module of AMBER. The root mean square (rms) differences are in the range 0.02–0.04 Å.

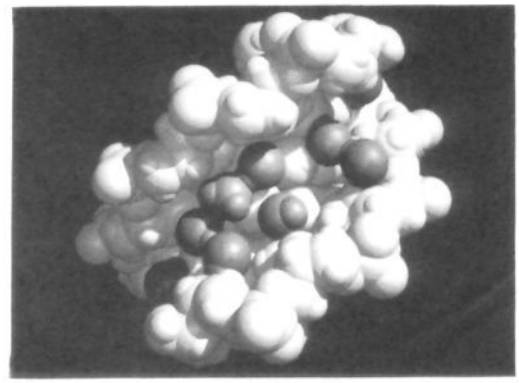


Figure 8. Space-filling model of d(ATGCAT)2 duplex showing first-shell waters in the minor groove. Waters are listed from the top and left to right in the following order: 426, 389, 356, 423, 394, 568, 691, 362, 645, 668.

Table V. Hydrogen Bonds in First Hydration Shell in the Minor Groove of $d(ATGCAT)_2$

hydrogen donor[a] (H–X)	acceptor atom	length, Å	angle, deg
H1 (W668)	N3 (ADE1)	2.10	136.8
H2 (W691)	N3 (GUA3)	2.27	152.0
N1 (W389)	N3 (GUA9)	1.97	156.7
H1 (W362)	N3 (ADE11)	1.85	170.0
H1 (W645)	O2 (THY2)	1.83	149.4
H2 (W356)	O2 (CYT4)	1.86	158.6
H2 (W426)	O2 (THY6)	1.82	163.1
H1 (W426)	O2 (THY8)	1.87	151.7
H2 (W568)	O2 (CYT10)	1.86	156.0
HN2A (GUA3)	O (W568)	2.44	106.9
HN2B (GUA3)	O (W394)	1.91	163.3
HN2B (GUA9)	O (W389)	2.07	133.8
H1 (W668)	O1′ (THY2)	2.45	131.8
H2 (W423)	O1′ (CTY10)	1.90	163.3
H1 (W362)	O1′ (THY12)	1.85	153.3

[a] W = water.

Hydrogen bonds formed between the drug and DNA, and between the drug and water in the solvated adducts are listed in Table VII. Most of the drug–DNA interactions, including intramolecular hydrogen bonds, are the same as they were in the models made in vacuum. The number of hydrogen bonds between drug and solvent is not large. It ranges from one to three. The drug in models with covalent bonding at C3a generally solvates better than in models with covalent bonding at C7 and this correlates with the higher drug–water enthalpies shown in Table VI. In one model, the 3′,*S* adduct covalent at C7, a water molecule formed a bridge between O11 of the drug and OA of P_{11-12}.

The free energy change accompanying adduct formation determines the relative stability of the different models for the covalent binding of naphthyridinomycin hydroquinone to $d(ATGCAT)_2$. Enthalpic contributions to the

Table VI. Binding Enthalpies (kcal/mol) for Naphthyridinomycin Hydroquinone with d(ATGCAT)$_2$ Covalent Adducts in the Presence of Water and Counterions[a]

binding site	direction and config[b]	helix distort[c]	drug distort	total intermol	net binding[d]	drug–water (in adduct)
C7	3′,*R*	3.4	6.3	−94.4	−84.7	−22.9
	3′,*S*	24.9	16.7	−99.2	−57.6	−20.7
	5′,*R*	11.1	14.1	−72.7	−47.5	−15.2
	5′,*S*	1.1	13.8	−71.8	−58.0	−32.4
C3a	3′,*R*	18.6	2.7	−72.6	−51.3	−33.4
	3′,*S*	11.7	7.0	−66.0	−47.3	−25.8
	5′,*R*	12.6	13.0	−85.3	−59.7	−22.4
	5′,*S*	17.7	9.2	−81.3	−54.5	−31.6

[a] Total binding energies are not included in this table because they reflect different numbers of water molecules in the various solvated adducts. These differences reflect the construction of boxes of water around adducts that vary in shape. [b] Direction refers to the direction in the minor groove that the hydroquinone ring takes with respect to the point of covalent bonding. [c] The enthalpy of d(ATGCAT)$_2$ minimized in the presence of water and counterions, but the absence of drug, is −358.7 kcal/mol. [d] Net binding is total intermolecular binding plus drug distortion and helix distortion. This quantity is valid for comparing relative binding enthalpies among the various adducts even though total enthalpies are not.

free energy change are predicted by molecular mechanics calculations as described above; however, the differences in entropy change are not afforded by these calculations. The entropy changes are controlled by the factors of configurational entropy, counterions, and hydration. Configurational entropy and counterions are probably about the same for the four bonding modes at C7 and for the four bonding modes at C3a. Naphthyridinomycin hydroquinone fills the minor groove in the region where it binds and it is anchored to the floor of the groove by the covalent bond and by hydrogen bonds. In each of the C7 covalent models, there is a hydrogen bond between HO9 of the drug and a hydrogen-bond acceptor on the DNA. Each of the C3a covalent models has hydrogen bonds between HO9 of the drug and HO3 of the drug with acceptors on the DNA. Furthermore, there is not substantial distortion in the double helix. Consequently, we do not expect much difference among the binding modes at each alkylation site in terms of configurational entropy. The number of counterions is always the same and they remain at the bisector of the phosphate groups after solvation and minimization. This leaves the entropy change on hydration as the factor that must be estimated.

A qualitative picture of the changes in ordered (hydrogen bonded) water accompanying adduct formation is afforded by the modeling studies. Naphthyridinomycin hydroquinone (independently solvated and minimized) loses two H bonds with water, both involving the 9′-OH group. A water H bond with O13 is retained in the C7 covalent models, but lost in all but one (5′,*R*) of the C3a covalent models. New hydrogen bonds to naphthyridinomycin hydroquinone vary among the models, as listed in Table VII. The resulting net losses of water molecules H bonded to drug upon adduct formation is as follows for the C7 models: 3′,*R*, 2; 3′,*S*, 0; 5′,*R*, 2; 5′,*S*, 1. These numbers are obtained by subtracting waters H bonded to the drug plus waters H bonded to bases in the minor groove from waters H bonded to the drug and minor groove in the adducts. Corresponding losses for the C3a models are 3′,*R*, 0; 3′,*S*, 1; 5′,*R*, 0; 5′,*S* −1. When the drug binds in the minor groove of d(ATGCAT)$_2$, it displaces a number of water molecules. In every case, except for the 3′,*R* model covalent at C7 and the 5′,*S* model covalent at C3a, the same water molecules are displaced (numbers 356, 362, 394, 568, and 691 in Figure 8). In the 3′,*R* model covalent at C7, water 691 is retained, but water 389 is lost. Both waters 389 and 691 are lost from the 5′*S* model covalent at C3a. Thus, in all cases but one, five ordered water molecules are displaced. When the losses of water from both drug and DNA that occur on adduct formations are taken together, it is apparent that the greatest losses among the C7 covalent models are for 3′,*R* and 5′,*R*, both of which lose seven water molecules on adduct formation. This result, combined with the greater binding enthalpy of the 3′,*R* model, suggests that it is the preferred one. For covalent bonding at C3a, the 3′,*S* model loses six water molecules and each of the other models loses five waters. A more favorable entropy change is indicated for the 3′,*S* model; however, this model has the weakest binding enthalpy. It is not preferred to the 5′,*R* model.

Conclusions

Models were derived for the covalent binding of C7 of protonated naphthyridinomycin and cyanocycline A to the 2-amino group of the guanine residue in d(ATGCAT)$_2$. The best of them had the antibiotic at the hydroquinone oxidation state and lying in the minor groove so that the aromatic ring was toward the 3′-side of the guanine residue in the strand to which the covalent bond was formed. It also had the *R* configuration at C7 of the drug. Mechanisms for DNA alkylation proposed by Zmijewski et al.,[12] involving an intermediate N8–C7 iminium ion or noncovalent binding followed by a S_N1_{CA} process, were completely consistent with this model.

Covalent binding at C3a had not been considered in previous publications; however, it was possible to derive a satisfactory model with high net binding enthalpy. Futhermore, models for processes involving either noncovalent binding of an iminium ion obtained by opening of the oxazolidine ring or a two-step process based on initial noncovalent binding of naphthyridinomycin followed by formation of the iminium ion and then covalent binding appeared resonable. No conformational change was required for this ring opening. Although the enthalpy values in Table II favor C7 alkylation over C3a alkylation, we do not suggest that they can be used to predict or confirm that C7 is the preferred site of binding. The relative transition state energies should determine this factor. Increased acidity could lower the transition state for oxazolidine ring opening, thus increasing the possibility of C3a alkylation. In any event, the reasonableness of models for covalent binding at C3a suggests that this position should be carefully considered in future studies on naphthyridinomycin, cyanocycline A, and their analogues. It is clear from the models (for example, Figures 4 and 6) that cross-linking of the duplex by alkylation at C7 and C3a is not possible. In either model, one of these centers projects out from the minor groove. On the other hand, cross-linking between the DNA and protein might be favorable. This possibility could be evaluated by appropriate experiments.

The inclusion of solvent and counterions into the molecular mechanics did not change the models preferred by

Table VII. Hydrogen-Bond Parameters of Naphthyridinomycin Hydroquinone-d(ATGCAT)$_2$ Covalent Adducts in the Presence of Water and Counterions[a]

binding site	direction and config[b]	hydrogen donor (X-H)	acceptor atom (Z)	length, Å
C7	3′,*R*	HO9(NHQ)	O1′ (A5)	1.85
		HN2B (G9)	O10 (NHQ)	1.85
		HO13 (NHQ)	OA (P_{10-11})	1.80
		HO10 (NHQ)	O11 (NHQ)	2.22
		HN14 (NHQ)	O9 (NHQ)	1.76
		H1 (W499)	O13 (NHQ)	1.96
	3′,*S*	HO9 (NHQ)	O2 (C8)	1.94
		HO13 (NHQ)	OA (P_{10-11})	1.72
		NH14 (NHQ)	O9 (NHQ)	1.74
		H2 (W496)	O13 (NHQ)	1.86
		H1 (W476)[c]	O11 (NHQ)	2.46
		HN14 (NHQ)	O9 (NHQ)	1.76
		H1 (W499)	O13 (NHQ)	1.96
	5′,*R*	HO10 (NHQ)	O2 (T2)	2.00
		HO9 (NHQ)	O1′ (T12)	1.86
		HN14 (NHQ)	O9 (NHQ)	1.74
		H1 (W704)	O13 (NHQ)	1.78
	5′,*S*	HO9 (NHQ)	N13 (A11)	2.02
		H1 (W362)	O10 (NHQ)	2.25
		HO10 (NHQ)	O11 (NHQ)	2.15
		HN14 (NHQ)	O9 (NHQ)	1.76
		H2 (W434)	O13 (NHQ)	1.85
		HO13 (NHQ)	O (W366)	1.86
C3a	3′,*R*	HO3 (NHQ)	O2 (C4)	1.80
		HO9 (NHQ)	O1′ (A11)	1.91
		H1 (W334)	O10 (NHQ)	1.87
		HO10 (NHQ)	O11 (NHQ)	2.12
		NH14 (NHQ)	O9 (NHQ)	1.83
		HO7 (NHQ)	O (W497)	1.86
		HO13 (NHQ)	O (W435)	2.04
	3′,*S*	HO3 (NHQ)	O2 (C4)	2.29
		HN2B (G9)	O3 (NHQ)	2.12
		HO9 (NHQ)	O1′ (A11)	1.82
		H2 (W336)	O10 (NHQ)	1.95
		HO10 (NHQ)	O11 (NHQ)	2.14
		HN14 (NHQ)	O9 (NHQ)	1.71
		H2 (W446)	O7 (NHQ)	1.92
	5′,*R*	HO9 (NHQ)	N3 (G3)	2.13
		HN14 (NHQ)	N3 (G3)	2.43
		HO3 (NHQ)	OA (P_{11-12})	1.77
		HO10 (NHQ)	O11 (NHQ)	2.19
		HN14 (NHQ)	O9 (NHQ)	1.81
		H1 (W375)	O7 (NHQ)	1.92
		HO13 (NHQ)	O3 (NHQ)	1.79
		H1 (W113)	O13 (NHQ)	2.47
		H2 (W118)	O11 (NHQ)	2.42
	5′,*S*	HO9 (NHQ)	OA (P_{4-5})	1.74
		H1 (W487)	O10 (NHQ)	1.93
		HO10 (NHQ)	OW (790)	1.90
		HN14 (NHQ)	O9 (NHQ)	1.86
		H2 (W667)	O7 (NHQ)	1.98
		H1 (W790)	O11 (NHQ)	2.29

[a] Only hydrogen bonds made directly to the drug are included. All of the hydrogen bonds less than 2.5 Å long are listed. Most of the enthalpies for hydrogen bonds are included in the electrostatic fields, which are given in Table VI. [b] Direction refers to the direction in the minor groove that the hydroquinone ring takes with respect to the point of covalent bonding.

enthalpy for covalent binding at C7 or C3a. Qualitative estimates of entropy changes, based on the release of ordered water molecules from the drug and the DNA fragment, also indicated no change in which models are preferred. This result is not unexpected in view of the condition that in all models the drug binds at the same site and occupies approximately the same amount of space. The compact structure of naphthyridinomycin hydroquinone probably ensures this result. Drug molecules with structures that are highly unsymmetrical with respect to the covalent binding site might show significant solvent effects on enthalpy and entropy in different orientations.

Experimental Section

Protonation of Cyanocycline A. A solution of 0.3 mg of cyanocycline A in 3.0 mL of water was brought to pH 8.0 by addition of 12.0 mL of 0.00242 N NaOH solution and then titrated with 0.001 N HCl by using a Beckman Model 502 pH meter. The first pK_a found was 6.6, but as more acid was added the pH rose, probably indicating opening of the oxazolidine ring. This reaction prevented measurement of the other two pK_as.

NMR Spectroscopy. All NMR spectra were acquired on a Bruker AM-500 equipped with an ASPECT 3000 computer. Temperature was controlled by a combination of a Haake Thermostat and a Bruker variable-temperature controller and maintained at 25 °C. All two-dimensional spectra were recorded in pure absorption mode with time proportional phase incrementation (TPPI).[16]

Two-dimensional ^{1}H–^{1}H spectra were usually acquired with a spectral width of 5000 Hz, 512 t_1 points, 2K data points in t_2, and 64 transients for each t_1 point. Conventional pulse sequences were used for double quantum filtered correlated spectroscopy (DQF-COSY),[17] 2D total correlated spectroscopy (TOCSY),[18] and 2D NOE correlated spectroscopy (NOESY).[19] All 2D spectra were processed on a VAX 8600 using FTNMR (Hare Research).

Sample Preparation. Cyanocycline A (ca. 8 mg) was dissolved in $CDCl_3$ (0.5 mL) to which deuterated trifluoroacetic acid (2 μL) was added. Chemical shifts (δ) are given in ppm relative to residual $CHCl_3$ at 7.00 ppm.

Molecular Modeling. The structure of protonated naphthyridinomycin hydroquinone was derived from the X-ray crystallographic structure of naphthyridinomycin[5] by changing the F ring geometry from quinone to hydroquinone and adding a proton to N14. It was then minimized in AMBER 3.0,[20] using a distance-dependent dielectric constant, until the rms gradient was less than 0.1 kcal/mol Å. Steepest descent only was used for the first 10 cycles and conjugate gradient for the next 90 cycles at the start and after each update of the nonbonded pair list, which occurred every 100 cycles. The cutoff distance for nonbonded pairs was 99 Å and the nonbonded pair list was updated every 100 cycles. The parameters contained in AMBER 3.0[21] were used wherever possible. They included all of the atom types and bond types. It was necessary, however, to define six new bond angle types: C–CA–CT, C–CA–OS, CA–CT–NT, N3–CT–OS, and NT–CT–OH. These angles were taken from the X-ray structure of naphthyridinomycin and the bending constants were taken from nearly identical angles in AMBER. Their values are given in the supplementary material.

Once the minimized structure was obtained for protonated naphthyridinomycin hydroquinone, the coordinates were used to calculate partial atomic charges (ESP) with the program QUEST 1.0 with a STO-3G basis set.[23] They are listed in the supplementary material. Starting from the previously generated minimized structure and utilizing the calculated partial atomic charges, protonated naphthyridinomycin hydroquinone was reminimized by using the conditions outlined above for the first minimization. The resulting structure (Figure 2) was compared with the X-ray structure of naphthyridinomycin originally input in order to verify that the parameters were appropriate. A calculation by the ANAL module in AMBER for the total atom set in both structures, except the hydroquinone–quinone difference and the N14 proton, showed a rms deviation of only 0.05 Å, which supports the validity of the parameters.

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For experiments in which covalent bonding at C3a was investigated, the geometry was taken from the X-ray crystallographic structure of the HBr derivative of cyanocycline A, which exists as the iminium ion derived from oxazolidine ring opening.[6] Partial atomic charges for this structure, calculated as described above, are given in the supplementary material. With the interactive graphics program MIDAS,[23,24] minimized structures to be used for covalent bonding were docked near the 2-amino group of GUA3, in the hexanucleotide duplex $d(ATGCAT)_2$, and in orientations appropriate to their covalent bonding at C7 or C3a. Structures for noncovalent binding were docked in the same vicinity, but farther from the 2-amino group. The DNA fragment was constructed in AMBER using Arnott's B-DNA geometry[25] and converted into the all-atom model in the EDIT module. This fragment was chosen because it has been intensively studied by 2D-NMR spectroscopy and we anticipate using it in the future to attempt verification of this modeling study. We have used it previously for studies on the binding of anthramycin and tomaymycin, which were done in conjunction with 2D-NMR experiments.[26,27]

Coordinates of the docked structures were captured and the resulting models were subjected to 40 ps of "belly" dynamics in AMBER 3.0, run in a sequence of 10-ps simulations. In this mode, the DNA is constrained and the drug is free to move. Unrestrained molecular dynamics cannot be performed on $d(ATGCAT)_2$ because the AT base pairs at the ends fray so badly that they do not reform Watson–Crick hydrogen bonding. During the dynamics, the temperature was increased from 10 K to 300 ± 10 K during the first 10 ps. The equilibration conditions were nonclassical dynamics with velocity scaling (constant temperature) at the latter temperature. There was no periodicity and shake was on. After each 10-ps period the last frame structure was compared with the proceeding ones. There was essentially no change after the first 10 ps. The final structure obtained after 40 ps was used as the starting one in a molecular mechanics refinement. All of the atoms (DNA as well as drug) were allowed to move and the simulation was continued until the rms gradient was less than 0.1 kcal/mol Å.

In the enthalpy calculations for Table I, the helix distortion was determined by subtracting the enthalpy of the helix minimized separately from the drug, from its value in the complex. The starting structure for the separately minimized helix was obtained by removing the drug from the minimized complex. Distortion enthalpies induced in the drugs were determined in the same way.

For calculations with counterions and solvent, the final structure from molecular dynamics was used. Sodium ions were placed on the phosphate bisectors 3.0 Å from the phosphorus atoms.[28] A sphere of 1.65 Å was used for the sodium ions. The resulting complex was solvated in a box of TIP3P water[29] to a minimum solvent shell thickness of 4 Å from the solute. Any water with an oxygen distance <2.0 Å or a hydrogen distance <1.0 Å from the solute was discarded. Complete solvation of the minor groove could not be effected by molecular mechanics. Consequently, "belly" dynamics was carried out with the complex held constant and the water molecules allowed to move. This procedure did fill the minor groove with solvent molecules. The simulation was run in two consecutive 10-ps runs. The last frame from each run was nearly identical as determined by superposition of these structures using MIDAS. Conditions for the simulation included increase in the temperature from 10 K to 300 ± 10 K over the first 10 ps. Equilibration was run at the latter temperature using nonclassical dynamics with velocity scaling (constant temperature), no periodicity, and shake. The last frame structure was then refined in molecular mechanics with all atoms free to move. A uniform dielectric constant of 1.0 was used and the minimization was continued with steepest descent until the rms gradient was <0.2 kcal/mol Å.

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Supplementary Material Available: Tables of the protonated hydroquinone form of naphthyridinomycin for the PREP module of AMBER, the control files for molecular mechanics minimization, "belly" dynamics on a solvated DNA adduct, and molecular mechanics minimization of a solvated adduct (9 pages). Ordering information is given on any current masthead page.

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