# The Antitumor Drug Nogalamycin Forms Two Different Intercalation Complexes with d(GCGT)·d(ACGC)

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ABSTRACT: The structures of the physical complex of d(GCGT)·d(ACGC) with the anthracycline antitumor drug nogalamycin were studied in order to determine the sequence specifity and the drug orientation at the symmetric d(C2G3)·d(C6G7) binding site of this oligonucleotide. For this purpose, one- and two-dimensional NMR techniques were used in combination with molecular mechanics and molecular dynamics computations. Analysis of the NMR spectra reveals that nogalamycin forms two different intercalation complexes with d(GCGT)·d(ACGC). These complexes are called complex I and complex II and are present in a ratio of 0.45:0.55. In both complexes the nogalamycin is intercalated at the d(C2G3)·d(C6G7) sequence with the bicyclic and nogalose sugars residing in the major and minor groove, respectively. This results in a buckling of the flanking base pairs and a doubling of the inter-base-pair distances at the intercalation site. In complex I, the aglycon ring of the drug stacks with the C6–G7 bases, and the sugars are directed to the G1·C8 end; while in the case of complex II the anthraquinone ring system is stacked with C2–G3 bases, and the sugars are pointed to the T4·A5 base pair end. The two nogalamycin–d(GCGT)·d(ACGC) structures are stabilized by intra- and intermolecular hydrogen bonds, electrostatic interactions, and van der Waals contacts. Comparison of different nogalamycin–oligonucleotide structures reveals a nogalamycin binding specificity to the 3′-side of the cytosine base in cytosine–purine sequences in double-stranded DNA.

The anthracycline antibiotic nogalamycin (Figure 1) isolated from *Streptomyces nogalator* binds to double-stranded DNA via intercalation (Kersten et al., 1966; Das et al., 1974; Sinha et al., 1977; Crooke & Reich, 1980; Gale et al., 1981). In vivo nogalamycin selectively inhibits RNA synthesis (Fox & Waring, 1972; Ennis, 1981) and exhibits an antitumor activity against a number of tumor cell lines (Bhuyan & Reussen, 1970).

Footprinting experiments established a preferential binding of nogalamycin at d(CA)·d(TG) pyrimidine–purine steps in DNA (Fox & Waring, 1986; White & Phillips, 1989). NMR studies on the structure of the complex of nogalamycin with the $d(GCATGC)_2$ (Searle et al., 1988) and $d(AGCATGCT)_2$ (Zhang & Patel), 1990) duplexes, in which the two binding sites are separated by two base pairs, clearly showed that the aglycon rings of nogalamycin intercalate at the d(CA)·d(TG) sequence. In these structures the nogalose and bicyclic amino sugars are positioned in the minor and major groove, respectively, and point in the direction of the ends of the duplex (Figure 2).

The complex of nogalamycin with the $d(*CGTsA*CG)_2$ (*C is either C or $me^5C$) and $d(CGTACG)_2$ duplexes was investigated with crystallographic methods (Liaw et al., 1989; Williams et al., 1990; Egli et al., 1991) and NMR (Robinson et al., 1990). Here, the aglycon rings of nogalamycin intercalate at the two d(*CG)·d(*CG) steps, which are separated by four base pairs and located at the end of the oligomers. The nogalose and bicyclic amino sugars are also found in the minor and major groove, respectively. In contrast to the nogalamycin–duplex structures containing the d(CA)·d(TG) steps, the nogalose and bicyclic amino sugars are directed to the central base pairs of the complex (Figure 2).

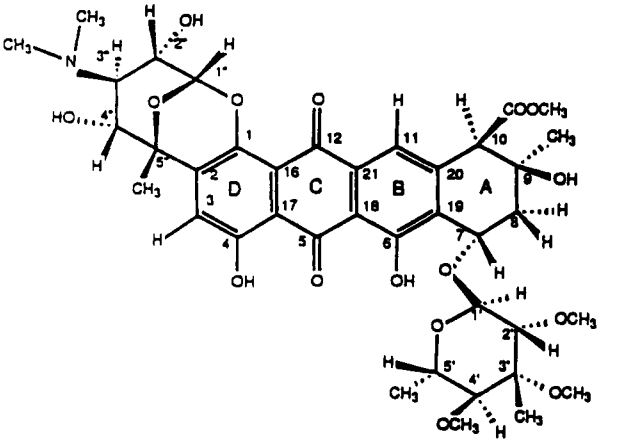

FIGURE 1: Chemical structure of the anthracycline antibiotic nogalamycin.

Steric hindrance of the sugars in the complexes of nogalamycin with $d(GCATGC)_2$ or $d(AGCATGCT)_2$ duplexes might affect the binding specificity and the positioning of the sugars, while in the case of the nogalamycin–$d(*CGTsA*CG)_2$ and –$d(CGTACG)_2$ complexes the drug might lack stabilizing interactions at the end of the duplex to form a structure with the nogalamycin in a reversed position.

In order to investigate the preference in drug orientation and the sequence specificity, the 1:1 complexes of nogalamycin with d(GCAT)·d(ATGC) and d(GCGT)·d(ACGC) were studied with 2D NMR techniques. The data on the d(GCAT)·d(ATGC)–nogalamycin complex (unpublished results) show that the nogalamycin tetramer forms a structure comparable to that found in the $d(GCATGC)_2$ and $d(AGCATGCT)_2$ drug complexes, in which the sugars point to the end of the duplex.

In this paper we report the structural results of the nogalamycin–d(GCGT)·d(ACGC) complex. In contrast to

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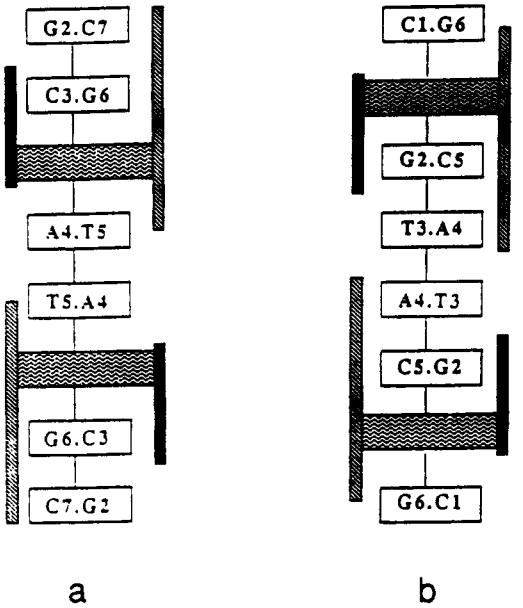


FIGURE 2: Schematic representation of (A) the structures of the complexes of nogalamycin with $d(GCATGC)_2$ (Searle et al., 1988) and $d(AGCATGCT)_2$ (Zhang & Patel, 1990) and (B) the structures of the nogalamycin–d(*CGTsA*CG) (*C is either C or $me^5C$) (Liaw et al., 1989; Williams et al., 1990; Egli et al., 1991) and $d(CGTACG)_2$ (Robinson et al., 1990).

the former data on $d(*CGTsA*CG)_2$– and $d(CGTACG)_2$–drug complexes, our measurements show the formation of two types of complexes: one with the sugars positioned in a similar mode as in the nogalamycin–$d(*CGTsA*CG)_2$ and –$d(CGTACG)_2$ complexes, i.e., toward the center of the duplex, while in the other structure the sugars point in the opposite direction as in the structures with the d(CA)·d(TG) binding step. Comparison of the DNA–drug interactions within the different complexes reveals a nogalamycin binding specificity to the 3′-side of C in cytosine–purine sequences in double-stranded DNA.

## MATERIAL AND METHODS

*Oligonucleotide Synthesis.* The oligonucleotides were synthesized on a Pharmacia LKB Gene Assembler Plus following the solid-phase cyanoethyl phosphoamidite method.

The protected oligomers were removed from the solid phase and deprotected, with the exception of the 5′-dimethoxytrityl group, by incubation with ammonium hydroxide for 48 h at room temperature. The oligonucleotides were purified with preparative reversed-phase HPLC using a Dynamax-300A C4 column (Rainin Instruments Company, Inc.) and a triethylammonium acetate buffer (pH 6.8)/acetonitrile gradient. The protecting 5′-dimethoxytrityl groups were removed by treatment with 80% acetic acid for 30 min. The fully deprotected single-stranded tetramers were again purified with preparative reversed-phase HPLC as described above and desalted on a Sephadex G25 column. Finally, the various tetramers were converted to the sodium form on a Dowex 50 × 8 cation exchange column.

An equimolar amount of complementary oligomer strands were mixed to obtain the tetramer duplexes.

*Nogalamycin–Oligonucleotide Complexes.* Nogalamycin was provided by Upjohn Co. About 2 equiv of solid nogalamycin was added to 500 μL of each duplex in aqueous buffer solution (10 mM sodium phosphate, 100 mM NaCl, 0.1 mM EDTA, pH 7.0). Then 5 mL water was added, and the mixture was shaken for 24 h at 4 °C. The excess of nogalamycin, which is insoluble at neutral pH, was removed by centrifugation. The solution of each nogalamycin–oligonucleotide complex was three times lyophilized and redissolved in 500 μL of 99.8% $D_2O$. Finally, the nogalamycin–oligomers were dissolved in 500 μL of 99.96% $D_2O$ and degassed under vacuum.

*NMR Experiments.* $^1H$ NMR spectra were recorded on a Bruker AM-500 spectrometer at 20 °C. Quadrature detection was used in both dimensions with the carrier frequency placed on the HDO resonance. The spectral width was 5000 Hz (10 ppm). Selective irradiation during the relaxation delay was used to suppress the residual HDO signal.

Magnitude COSY spectra were obtained with 2048 points in the $t_2$ dimension and 400 free induction decays (FIDs) in $t_1$ dimension with 128 transients for each FID. A relaxation delay of 1.5 s was used. Both time domain data sets were multiplied by a 0° phase-shifted sine bell window function.

Phase-sensitive NOESY spectra with mixing times of 100 and 250 ms were recorded. The data sets consisted of 2048 complex points in the $t_2$ dimension and 512 complex FIDs in the $t_1$ dimension with 128 transients for each FID. The relaxation delay was 1.5 s. The time domain sets were multiplied by a 25° phase-shifted sine bell window function. The chemical shifts are reported relative to the resonance of sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS).

*Model Building, Energy Minimization, and Molecular Dynamics.* Model building, energy minimization, and molecular dynamics were done with the programs InsightII 2.0 (Biosym Technologies, Inc.) and Discover 2.5 (Biosym Technologies Inc.). In the energy minimization and molecular dynamics calculations the Amber force field (Weiner et al., 1980) was used. All computations were executed on a Personal Iris computer system (Silicon Graphics, Inc.) interfaced to a CONVEX C2 computer.

The initial DNA–drug structures were subjected to 2000 steps of energy minimization to remove bad contacts within the complex. During this energy minimization, the hydrogen bonds between the base pairs were restrained in order to retain Watson–Crick base pairing in the DNA–drug structure. Next, all the NOE distance constraints were added, and the structures were again energy-minimized for 2000 steps. These structures were subjected to a 10-ps restrained molecular dynamics refinement at 400 K. The resulting structures were further energy-minimized in 2000 steps. Here, the NOE constraints were still present. Finally, all NOE restraints were removed, and the structures were further energy-minimized in 2000 steps.

## RESULTS

*Stoichiometry, Symmetry, and Stability of the Complex.* The nonsymmetric oligonucleotide duplex d(GCGT)·d(CGCA) contains a d(CG)·d(CG) pyrimidine–purine intercalation site for one nogalamycin molecule. However, two different nonsymmetric DNA–drug complexes are formed as can be easily recognized from the 2D NMR spectra (see Figures 4 and 5 below). The formed nogalamycin–DNA complexes appeared to be very stable at pH 7.0 and 20 °C; no time-dependent changes in the NMR spectra were observed over a period of several months.

*Characteristics of 1D NMR Spectra.* The 1D $^1H$ NMR spectrum of the nogalamycin–d(GCGT)·d(ACGC) complex together with that of the free drug and the uncomplexed DNA is shown in Figure 3. The resonances in these spectra are narrow, well-resolved, and amenable to assignment with 2D NMR techniques.

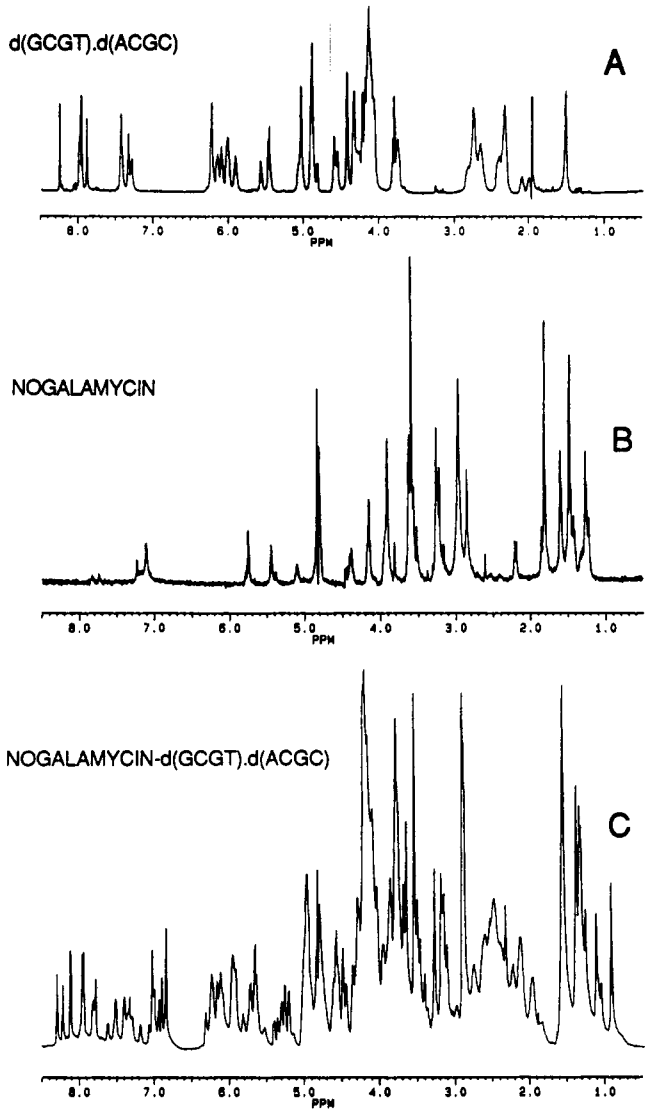


FIGURE 3: 1D NMR spectra of (A) d(GCGT)·d(ACGC), (B) uncomplexed nogalamycin, and (C) the nogalamycin–d(GCGT)·d(ACGC) complex.

*Chemical Shifts and Assignment of the Resonances.* The assignment of the resonances was carried out by established procedures [Hare et al., 1983; reviewed by Patel et al. (1987), Reid (1987), and Van de Ven and Hilbers (1988)] using a combination of COSY and NOESY spectra recorded in $D_2O$ buffer, pH 7.0, at 20 °C.

Figure 4 shows an overview of a 500-MHz NOESY spectrum (mixing time, 250 ms) of the nogalamycin–d(GCGT)·d(ACGC) complex. The spectrum shows intramolecular NOE contacts *within* the DNA and the nogalamycin and intermolecular NOE contacts *between* the nogalamycin and the DNA protons as cross peaks.

An expanded contour plot of a NOESY spectrum (mixing time, 250 ms) containing the cross peaks between the base protons (6.8–8.3 ppm) and the H1′ protons (5.5–6.5 ppm) is shown in Figure 5 (see also block A in Figure 4). Sequential analysis of the cross peaks going from G1 to T4 and from A5 to C8 revealed the presence of two separate sets of sequential traces (Figure 5, panels A and B). These distance connectivities are in accordance with the existence of two different complexes (complexes I and II). Note that for complex I (Figure 5A) the cross peak of the sequential step from H1′ of C6 to H8 of G7 is missing, while for complex II the cross

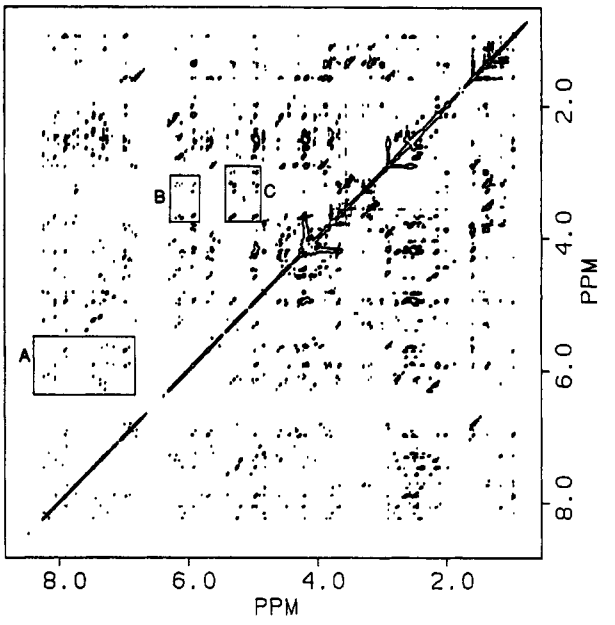


FIGURE 4: 500-MHz NOESY spectrum (mixing time, 250 ms) of nogalamycin–d(GCGT)·d(ACGC) complex.

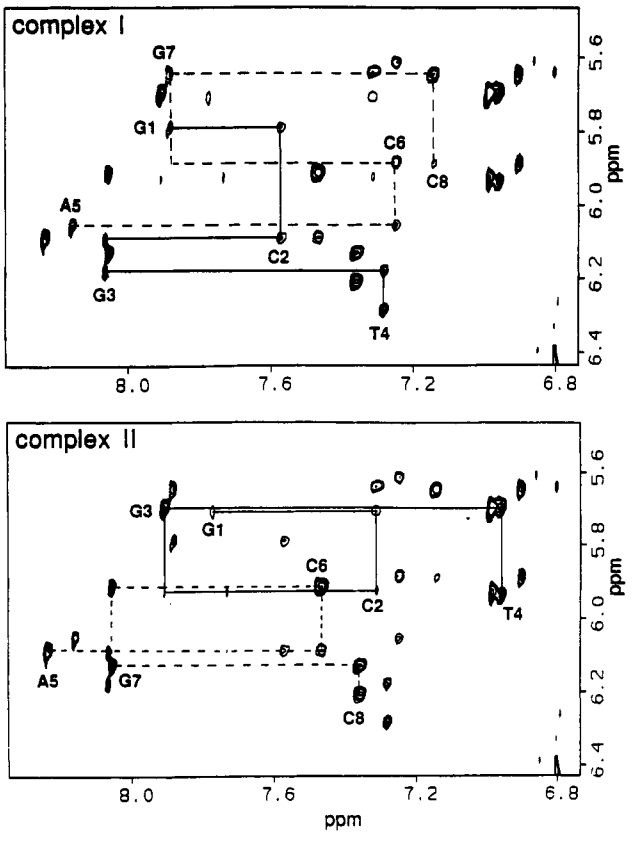


FIGURE 5: Base–H1′ region (block A in Figure 4) from the 500-MHz NOESY spectrum (mixing time, 250 ms) of complexes I (A, top) and II (B, bottom) of nogalamycin–d(GCGT)·d(ACGC). The sequential traces of each strand are indicated.

peak between H1′ of C2 and H8 of G3 is very weak. In addition to the separate sequential paths, all the other cross peaks were found twice. Thus, two sets of DNA cross peaks were found, each belonging to its own complex. Also, two sets of drug resonances were observed (Figure 6A; see also block C in Figure 4). As expected for the presence of two different complexes, each set of oligonucleotide resonances shows only intermolecular contacts represented by cross peaks from the oligonucleotide to its own set of drug resonances. In

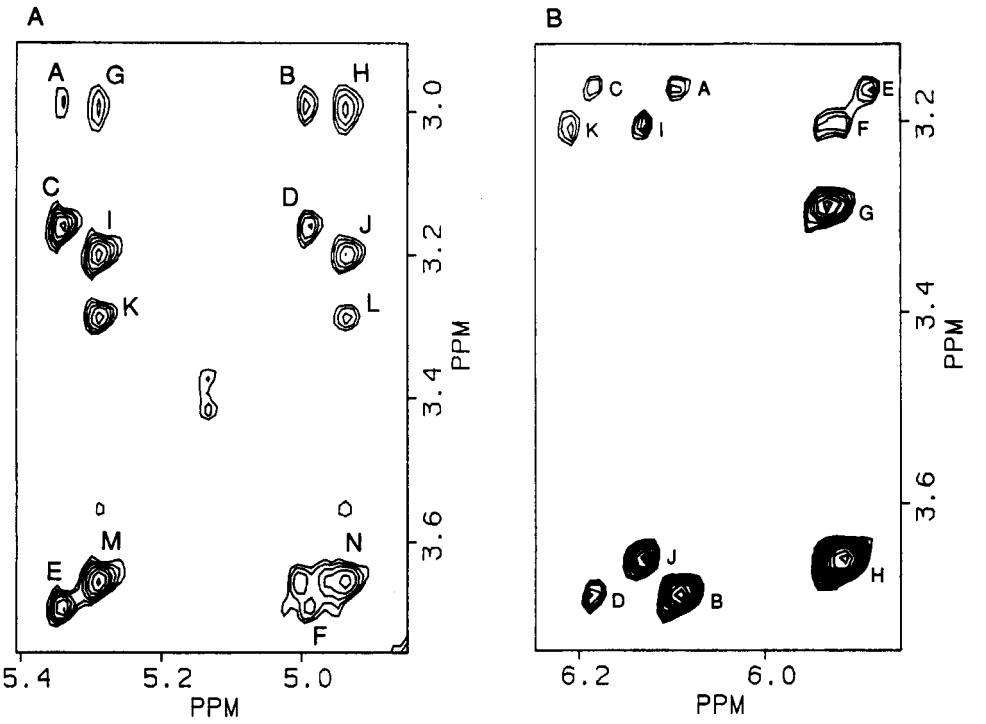


FIGURE 6: Expanded region of the 500-MHz NOESY spectrum (mixing time, 250 ms) of nogalamycin–d(GCGT)·d(ACGC). (A) Drug–drug cross peaks in complex I and II (block C in Figure 4). (Complex I) A. NOG(H1′)–NOG(H8); B. NOG(H7)–NOG(H8); C. NOG(H1′)–NOG(H2′); D. NOG(H7)–NOG(H2′); E. NOG(H1′)–NOG($OCH_3$-2′); F. NOG(H7)–NOG($OCH_3$-2′). (Complex II) G. NOG(H1′)–NOG(H8); H. NOG(H7)–NOG(H8); I. NOG(H1′)–NOG(H2′); J. NOG(H7)–NOG(H2′); K. NOG(H1′)–NOG($OCH_3$-3′); L. NOG(H7)–NOG($OCH_3$-3′); M. NOG(H1′)–NOG($OCH_3$-2′); N. NOG(H7)–NOG($OCH_3$-2′). (B) Selection of intermolecular cross peaks between the drug and the oligonucleotide of complexes I and II (block B in Figure 4). (Complex I) A. C2(H1′)–NOG(H2′); B. C2(H1′)–NOG($OCH_3$-2′); C. G3(H1′)–NOG(H2′); D. G3(H1′)–NOG($OCH_3$-2′); E. C6(H1′)–NOG(H2′). (Complex II) H. C6(H1′)–NOG($OCH_3$-2′); I. G7(H1′)–NOG(H2′); J. G7(H1′)–NOG($OCH_3$-2′); K. C8(H1′)–NOG(H2′).

this way each oligonucleotide–drug combination could be identified. Figure 6B shows a selection of the intermolecular NOEs between the oligonucleotide and nogalamycin for each complex (see also block B in Figure 4). A listing of the resonance assignments of the nogalamycin–d(GCGT)·d(ACGC) is given in Table I. The chemical shifts of the free drug and the uncomplexed DNA are given in the Supplementary Material.

*Ratio of the Complexes.* Integration and comparison of the volumes of the strong H5–H6 magnitude COSY cross peaks of the cytosines of both complexes reveals a ratio of complex I to complex II equal to 0.45:0.55.

*NOE Contacts.* The cross peaks in the NOESY spectrum recorded with a mixing time of 100 ms were used to obtain the intra- and intermolecular proton–proton distances ($r$) within the oligonucleotide–nogalamycin complexes.

The volume integrals of the intensities of these NOE cross peaks provide information about the internuclear distances via the following relation:

$$\mathrm{NOE} \sim f(\tau_c)(1/r^6) \qquad (1)$$

In this relation $f(\tau_c)$ is a function of the rotational correlation time, which represents the influence of the motion of the complex on the NOE intensity. Using the above NOE-distance relationship and the reference H2′–H2″ distance of 1.8 Å for DNA sugar–sugar and DNA sugar–base (with exception of the H1′–base distance) and the cytidine H5–H6 distance of 2.45 Å for H1′–base, base–base, drug–drug, and DNA–drug contacts, the interproton distances were estimated. The lower and upper bounds of the distances were set to $d \pm 1$ Å in most cases. The position of the methyl protons was replaced by pseudoatoms located in the center of mass of the methyl protons. This distance information was applied in the structure computation of the various complexes.

In the 100-ms NOESY spectrum, 77 DNA–DNA and 42 drug–drug NOE contacts were found for complex I, while for complex II 108 and 50 intramolecular DNA and drug cross peaks were observed, respectively. A listing of these data is tabulated in the Supplementary Material. Table II summarizes the intermolecular DNA–drug NOE contacts together with their lower and upper bounds for the interproton distances.

In complex I the NOE contacts are mainly found between the drug and the central d(C2G3)·d(C6G7) base pairs of the nogalamycin–oligonucleotide complex. This indicates that the intercalation site of nogalamycin is at the central d(C2G3)·d(C6G7) base pair step.

The NOG(H11), NOG(H1″), and NOG($CH_3$-9), which are on one side of the drug, interact with the C6 and/or G7 protons, while the NOG(H1′), NOG($OCH_3$-2′), and NOG-(H7) on the other side of the drug interact with the C2 and G3 protons. These specific interactions show that the drug is complexed with the DNA in a mode with the sugars pointing to the G1·C8 base pair. In this context, the internuclear C8 (H1′)–NOG($CH_3$-3′) and G7(H1′/H4′)–NOG($CH_3$-9) contacts are very important. In addition, the C6(H5/H6)–NOG(H1″) contacts show that the bicyclic sugar is placed in the major groove, while the NOEs between NOG($OCH_3$-2′) and C2(H1′/H4′) and G3(H4′) together with those between G3(H1′) and NOG(H7/H1′) show that the nogalose sugar is situated in the minor groove.

In complex II, the NOEs between the oligonucleotide and NOG(H11), NOG($CH_3$-9), NOG(H1″), NOG($OCH_3$-2′), NOG(H3), NOG(H1′), and NOG(H7) reveal that the drug is also intercalated at the d(C2G3)·d(C6G7) site. However,

Table I: Chemical Shifts (ppm) of d(GCGT)·d(ACGC) Complexed with Nogalamycin at 293 K

| | Complex I | | | | | | |
|---|---|---|---|---|---|---|---|
| | H8 | H6 | H2,H5,$CH_3$ | H1′ | H2′,H2″ | H3′ | H4′ |
| G1 | 7.88 | | | 5.79 | 2.49, 2.63 | 4.83 | 4.19 |
| C2 | | 7.57 | 5.39 | 6.09 | 2.25, 2.46 | 5.02 | 4.29 |
| G3 | 8.06 | | | 6.19 | 2.46, 2.78 | 4.95 | 4.49 |
| T4 | | 7.28 | 1.16 | 6.28 | 2.27 | 4.63 | 4.10 |
| A5 | 8.16 | | 7.72 | 5.97 | 2.51, 2.81 | 4.83 | 4.22 |
| C6 | | 7.25 | 4.83 | 5.88 | 2.54 | 4.93 | 4.16 |
| G7 | 7.88 | | | 5.65 | 2.51, 2.59 | 4.90 | 4.58 |
| C8 | | 7.14 | 4.89 | 5.89 | 1.88, 2.14 | 4.35 | 3.88 |

| aglycon ring | | bicyclic amino sugar | | nogalose sugar | |
|---|---|---|---|---|---|
| H3 | 6.86 | H1″ | 5.61 | H1′ | 5.34 |
| H7 | 4.99 | H2″ | 4.20 | H2′ | 3.17 |
| H8 | 2.00, 3.00 | H3″ | 4.35 | $OCH_3$-2′ | 3.70 |
| $CH_3$-9 | 1.58 | $N(CH_3)_2$-3″ | 2.90 | $CH_3$-3′ | 1.36 |
| H10 | 4.33 | H4″ | 3.92 | $OCH_3$-3′ | |
| $COOCH_3$-10 | 3.88 | $CH_3$-5″ | 1.62 | H4′ | 3.19 |
| H11 | 6.90 | | | $OCH_3$-4′ | |
| | | | | H5′ | 3.88 |
| | | | | $CH_3$-5′ | 1.42 |

| | Complex II | | | | | | |
|---|---|---|---|---|---|---|---|
| | H8 | H6 | H2,H5,$CH_3$ | H1′ | H2′,H2″ | H3′ | H4′ |
| G1 | 7.77 | | | 5.71 | 2.41, 2.62 | 4.81 | 4.21 |
| C2 | | 7.31 | 4.82 | 5.93 | 2.52, 2.64 | 4.94 | 4.15 |
| G3 | 7.90 | | | 5.70 | 2.52, 2.65 | 4.97 | 4.60 |
| T4 | | 6.96 | 0.97 | 5.94 | 2.01, 2.14 | 4.44 | 3.86 |
| A5 | 8.23 | | 7.73 | 5.09 | 2.41, 2.74 | 4.82 | 4.20 |
| C6 | | 7.46 | 4.83 | 5.92 | 2.16, 2.39 | 4.96 | 4.22 |
| G7 | 8.05 | | | 6.13 | 2.56, 2.77 | 5.00 | 4.48 |
| C8 | | 7.35 | 5.19 | 6.21 | 2.18 | 4.58 | 4.04 |

| aglycon ring | | bicyclic amino sugar | | nogalose sugar | |
|---|---|---|---|---|---|
| H3 | 6.80 | H1″ | 5.64 | H1′ | 5.29 |
| H7 | 4.94 | H2″ | 4.17 | H2′ | 3.21 |
| H8 | 2.00, 3.00 | H3″ | 4.54 | $OCH_3$-2′ | 3.66 |
| $CH_3$-9 | 1.60 | $N(CH_3)_2$-3″ | 2.92 | $CH_3$-3′ | 1.38 |
| H10 | 4.30 | H4″ | 3.96 | $OCH_3$-3′ | 3.29 |
| $COOCH_3$-10 | 3.87 | $CH_3$-5″ | 1.60 | H4′ | 3.19 |
| H11 | 6.99 | | | $OCH_3$-4′ | 3.56 |
| | | | | H5′ | 3.88 |
| | | | | $CH_3$-5′ | 1.42 |

in this case the NOG(H1″), NOG(H11), and NOG($CH_3$-9) are exposed to the sequential C2–G3 step, while the NOG (H1′), NOG(H7), and NOG($OCH_3$-2′) are facing the C6–G7 bases on the other strand. This indicates that in this DNA–drug complex the sugars point to the T4·A5 base pair end. This is also in agreement with the following NOE contacts: NOG($CH_3$-9)–T4(H1′), NOG($CH_3$-3′)–T4(H1′), and those between A5(H2) and NOG(H2′), NOG($OCH_3$-2′), NOG ($OCH_3$-3′), and NOG($CH_3$-3′).

The protons of the nogalose sugar NOG(H1′), NOG(H2′), NOG($OCH_3$-2′), and NOG($OCH_3$-3′) as well as the NOG-($CH_3$-9) and NOG(H7) have NOE contacts with the protons positioned in the minor groove of the oligonucleotide. The NOG(H1″) shows an NOE contact with C2(H6) in the major groove. An NOE contact between the NOG(H3) of the drug and C6(H5) in the major groove was also observed. These observations show that also in complex II the bicyclic sugar rings are positioned in the major groove.

*Structure Refinement.* In the starting structures of the nogalamycin–d(GCGT)·d(ACGC) complexes, the drug was placed between the d(C2G3)·d(C6G7) base pairs with the bicyclic and nogalose sugar rings in an orientation consistent with the observed NOE contacts. The oligonucleotide part had a right-handed B-type conformation, and for the nogalamycin the coordinates of the crystal structure were used (Arora, 1983). The van der Waals energy of the initial structures of complex I and II were very high; $5.3 \times 10^5$ kcal/mol for complex I and $5.1 \times 10^7$ kcal/mol for complex II. The structure refinement occured in a number of steps. First, the starting structures were energy-minimized to release bad contacts within the complexes. Second, all distance contraints calculated from the NOE cross peak intensities (Table II) were incorporated. These structures were then subjected to a distance-restrained energy minimization followed by a 10-ps molecular dynamics run at 400 K. The resulting structures were energy-minimized under restrained conditions. Finally, the structures were released from all constraints and subsequently energy-minimized. In this final step no additional conformational changes of the complexes were observed, which suggests that the structures reached the bottom of the energy well. In this final stage the van der Waals energy is −32 kcal/mol for complex I and −46 kcal/mol for complex II. The intermolecular proton–proton distances in these final nogalamycin d(GCGT)·d(ACGC) structures are in good agreement with the distance bounds used in the computational structure refinement of the complexes (Table II).

Table II: Distance Bounds between Nogalamycin and d(GCGT)·d(ACGC) in Both Complexes and the Observed Distances after Molecular Refinement[a]

| proton pairs | distance bounds | MD distance |
|---|---|---|
| | Complex I | |
| C2(H1′)–NOG($OCH_3$-2′) | 2.1–4.1 | 3.2 |
| C2(H4′)–NOG($OCH_3$-2′) | 2.3–4.3 | 3.8 |
| G3(H1′)–NOG(H7) | 2.2–4.2 | 2.4 |
| G3(H1′)–NOG(H1′) | 2.2–4.2 | 2.8 |
| G3(H4′)–NOG($OCH_3$-2′) | 2.9–4.9 | 3.5 |
| C6(H1′)–NOG(H11) | 2.2–4.2 | 3.7 |
| C6(H5)–NOG(H1″) | 2.3–4.3 | 3.9 |
| C6(H6)–NOG(H1″) | 2.6–4.6 | 3.3 |
| G7(H1′)–NOG(H11) | 2.0–4.0 | 3.8 |
| G7(H1′)–NOG($CH_3$-9) | 2.8–4.8 | 3.8 |
| G7(H4′)–NOG(H11) | 2.5–4.5 | 4.3 |
| G7(H4′)–NOG($CH_3$-9) | 2.8–4.8 | 5.0 |
| C8(H1′)–NOG($CH_3$-3′) | 2.0–4.0 | 3.5 |
| | Complex II | |
| C2(H1′)–NOG(H11) | 2.7–4.7 | 3.2 |
| C2(H6)–NOG(H1″) | 2.7–4.7 | 3.2 |
| G3(H1′)–NOG(H11) | 2.1–4.1 | 3.7 |
| G3(H1′)–NOG($CH_3$-9) | 2.3–4.3 | 3.4 |
| G3(H4′)–NOG(H11) | 2.2–4.2 | 3.8 |
| G3(H4′)–NOG($CH_3$-9) | 2.7–4.7 | 4.1 |
| T4(H1′)–NOG($CH_3$-9) | 3.7–5.7 | 4.7 |
| T4(H1′)–NOG($CH_3$-3′) | 2.1–4.1 | 2.8 |
| A5(H2)–NOG(H2′) | 3.2–5.2 | 3.7 |
| A5(H2)–NOG($OCH_3$-2′) | 3.9–5.9 | 4.1 |
| A5(H2)–NOG($CH_3$-3′) | 3.6–5.6 | 5.5 |
| A5(H2)–NOG($OCH_3$-3′) | 1.8–3.6 | 3.7 |
| C6(H1′)–NOG($OCH_3$-2′) | 2.0–4.0 | 2.4 |
| C6(H2″)–NOG($OCH_3$-2′) | 3.6–5.6 | 3.2 |
| C6(H5)–NOG(H3) | 3.3–5.3 | 4.7 |
| G7(H1′)–NOG(H1′) | 2.3–4.3 | 3.1 |
| G7(H1′)–NOG($OCH_3$-2′) | 3.4–5.4 | 5.3 |
| G7(H1′)–NOG(H7) | 2.3–4.3 | 2.5 |
| G7(H4′)–NOG($OCH_3$-2′) | 3.0–5.0 | 3.3 |

[a] The distances are given in angstroms.

*Conformation of Complexes I and II.* A ball-and-stick representation of the structure of both complexes is given in Figure 7. The various views show that the aglycon ring system has an orthogonal position with respect to the helix axis and the bicyclic and nogalose sugar rings are positioned in the major and minor groove, respectively. Also, the opposite

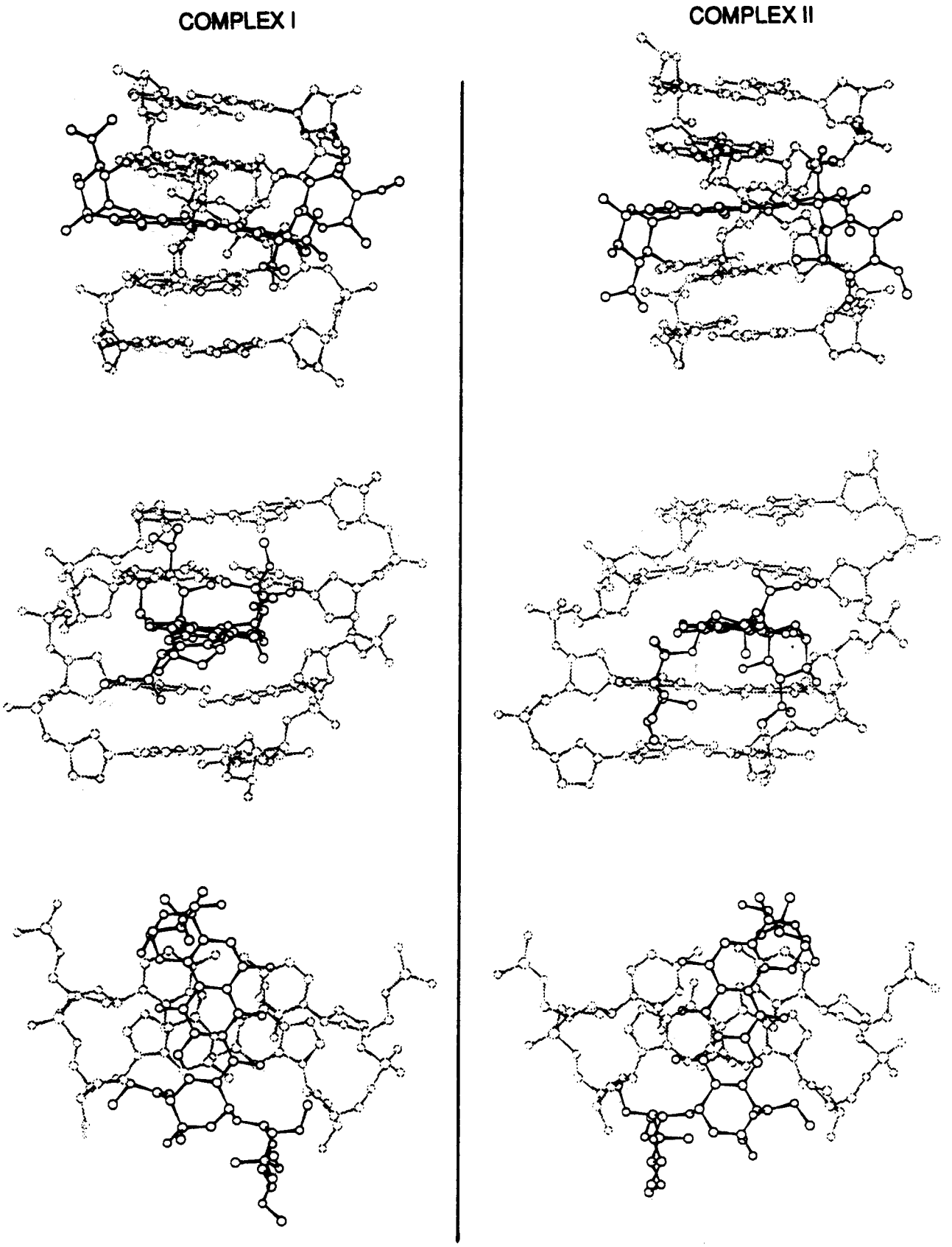


FIGURE 7: Structures of complex I (left) and complex II (right) of the nogalamycin–d(GCGT)·d(ACGC) obtained after molecular mechanics and dynamics calculations. (A) Side view (top), minor groove view (middle), and top view (bottom) of both complexes are shown. For reasons of clarity, only the two central base pairs are shown from the G1·C8 end of the duplex in the top view (bottom).

orientation of the sugars in complex I and II can be recognized immediately in Figure 7. In the major groove of complex I, the $N(CH_3)_2$-3″ group is positioned between G1·C8 and C2·G7 base pairs, while the $OCH_3$-3′ group of the nogalose resides near the G1·C8 plane in the minor groove. In complex II, the $N(CH_3)_2$-3″ atoms are also placed in the major groove. However, here the $N(CH_3)_2$-3″ is near the G3·C6 and T4·A5 base pairs, while the $OCH_3$-3′ group of nogalose is near the

T4·A5 base pair in the minor groove.

Figure 7 (middle) clearly shows the conformational changes of the oligonucleotide at the intercalation site. The distance between the C2·G7 and G3·C6 base pairs is roughly doubled. The inter-base-pair rise at the intercalation site is 7.1 Å for complex I and 6.7 Å for complex II. These base pairs flanking the intercalation site are clearly buckled, especially those base pairs on the $COOCH_3$-10 side of the drug. In addition, the C2·G7/G3·C6 base pairs of both complexes show a large negative roll of about 15° resulting in a slight local bend of the helix axis.

The top views of both nogalamycin–d(GCGT)·d(ACGC) complexes (Figure 7, bottom), as seen from the G1·C8 end of the duplexes, emphasize the stacking of the aglycon chromophore with the bases. The anthraquinone rings of the drug stack with C6 and G7 in complex I and with C2 and G3 in complex II. In addition, Figure 7 (bottom) shows that the nogalose sugar in the minor groove is positioned near the G1–C2 bases in complex I and near the A5–C6 bases in complex II.

*Stabilization of the Nogalamycin d(GCGT)·d(ACGC) Structure.* Detailed inspection of the refined structures of complex I as given in Figure 7 revealed the possible existence of a hydrogen bond in the major groove between O<u>H</u>4″ of nogalamycin and 4-N<u>H</u>$_2$ of C2 (2.46 Å). In the minor groove of complex I, hydrogen bonds might be present between O<u>H</u>-6 and O2 of C2 (2.08 Å) and between C<u>O</u>OCH$_3$-10 and N<u>H</u>$_2$ of G3 (2.17 Å).

In the case of complex II, no hydrogen bonds were found in the major groove. The short distances between O<u>H</u>-6 and O2 of C6 (1.99 Å) and between C<u>O</u>OCH$_3$-10 and N<u>H</u>$_2$ of G7 (2.05 Å) of this nogalamycin oligonucleotide complex suggest the existence of hydrogen bonds in the minor groove.

In addition to these intermolecular hydrogen bonds, an intramolecular hydrogen bond might exist between O<u>6</u> of G3 and N<u>H</u>$_2$ of A5 within the oligonucleotide part of complex II; a distance of 2.49 Å was observed between these protons.

The protonated N(CH$_3$)$_2$-3″ group is positioned in the major groove between the planes of the G1·C8 and C2·G7 base pairs in complex I and between the G3·C6 and T4·A5 base pairs in complex II. Electrostatic interactions between the positively charged N(CH$_3$)$_2$-3″ and the negatively charged functional groups of the bases in the major groove might considerably contribute to the stabilization of the complex. With respect to the structure of the d(GCGT)·d(ACGC)–nogalamycin complex, the O6 of G7 (4.14 Å) and/or N4 of C2 (3.82 Å) in complex I and the O6 of G3 (3.92 Å) in complex II might be involved in these electrostatic interactions. Furthermore, the nogalamycin shows many van der Waals interactions with the oligonucleotide. In particular, the van der Waals contacts between the aglycon rings and the stacking bases and those between the nogalose sugar and the atoms of the oligonucleotide exposed to the minor groove will increase the stability of the complexes.

## DISCUSSION

The structures of the physical complex of d(GCGT)·d(ACGC) with the anthracycline antitumor drug nogalamycin were studied in order to determine the sequence specifity and the drug orientation at the symmetric d(C2G3)·d(C6G7) binding site of this oligonucleotide. For this purpose one- and two-dimensional NMR techniques were used in combination with molecular mechanics and molecular dynamics computations.

Analysis of the NMR reveals that nogalamycin forms two different intercalation complexes with d(GCGT)·d(ACGC). These complexes are called complex I and complex II and are present in a ratio of 0.45 : 0.55. In both complexes the nogalamycin is intercalated at the d(C2G3)·d(C6G7) sequence with the bicyclic and nogalose sugars residing in the major and minor groove, respectively. This results in a buckling of the flanking base pairs and a doubling of the inter-base-pair distances at the intercalation site.

In complex I the aglycon ring of the drug stacks with the C6–G7 bases, and the sugars are directed to the G1·C8 end, while in the case of complex II the anthraquinone ring system is stacked with C2–G3 bases and the sugars are pointed to the T4·A5 base pair end. The two nogalamycin–d(GCGT)·d(ACGC) structures are stabilized by intra- and intermolecular hydrogen bonds, electrostatic interactions, and van der Waals contacts. In this context, the alignment of the nogalose sugar within the walls of the minor groove is of particular importance. The importance of these interactions is stressed by the finding that 7-deoxynogarol, a nogalamycin derivative in which the nogalose sugar is absent, shows a decreased binding affinity to DNA (Plumbridge & Brown, 1979).

The conformation of complex I is more or less similar to the structures in which the nogalamycin is intercalated at the d(CA)·d(TG) sequences (Searle et al., 1988; Zhang & Patel, 1990; Van Houte et al., unpublished results). However, in the d(*CGTsA*CG)$_2$– and d(CGTACG)$_2$–nogalamycin complexes, which contain the d(CG)·d(CG) intercalation site, the complex I conformation was not observed with NMR (Robinson et al., 1990) and X-ray crystallography (Liaw et al., 1989; Williams et al., 1990; Egli et al., 1991). Here, the d(CG)·d(CG) intercalation sites are positioned at the end of the duplexes, so that, most probably, stabilizing intermolecular interactions between nogalamycin and the oligonucleotide are not present to form a complex I conformation as was observed for the d(GCGT)·d(ACGC)–drug complex.

The complex II conformation shows striking similarity with complexes of nogalamycin with d(CGTACG)$_2$ (Robinson et al., 1990) and d(*CGTsA*CG)$_2$ (Liaw et al., 1989; Williams et al., 1990; Egli et al., 1991). This structure might serve as a good example of agreement in structure obtained by NMR in solution and by X-ray crystallography.

However, the complex II structure as found for d(CG)·d(CG) binding sites was not formed within the d(GCAT)·d(ATGC)–drug duplex. Most probably steric hindrance by the adenine N$H_2$ group in the major groove prevents the formation of the complex II structure in d(GCAT)·d(ATGC)–nogalamycin.

Comparison of these structures clearly reveals a sequence specificity of nogalamycin for binding at the 3′-side of C in cytosine–purine sequences in duplex DNA. In these d(CG)·d(CG) and d(CA)·d(TG) sequences, the nogalamycin wraps itself around the C·G base pair, and the aglycon rings stack with the guanine of this base pair and the pyrimidine at the 5′-side of this guanine. In this way the existence of two nogalamycin–d(GCGT)·d(ACGC) structures and one nogalamycin–d(GCAT)·d(ATGC) conformation can be explained. However, footprinting studies (Fox & Waring, 1986; White & Phillips, 1989) suggest a major preference of nogalamycin for d(CA)·d(TG) binding sites. This might be caused by the difference in the kinetics of nogalamycin intercalation at the d(CA)·d(TG) and d(CG)·d(CG) binding sites.

It is speculated that the d(CA)·d(TG) sites are energetically more accessible, because of the presence of two hydrogen bonds in the base pairing of A and T instead of three hydrogen bonds

in the d(CG)·d(CG) sequence. The unravelling of the intercalation mechanism of nogalamycin in DNA is a challenging subject for future investigation.

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## SUPPLEMENTARY MATERIAL AVAILABLE

Five tables listing chemical shifts of d(GCGT)·(ACGC) and free nogalamycin at 293 K, drug–drug and DNA–DNA contacts in complexes I and II of d(GCGT)·(ACGC)–nogalamycin, and coordinates in PDB format of complexes I and II of d(GCGT)·(ACGC)–nogalamycin (19 pages). Ordering information is given on any current masthead page.

## REFERENCES

Arora, S. K. (1983) *J. Am. Chem. Soc. 150*, 1328–1332.

Bhuyan, B. K., & Reuser, F. (1970) *Cancer Res. 30*, 984–989.

Crooke, S. T., & Reich, S. D., Eds. (1980) *Anthracyclines*, Academic Press, New York.

Das, G. C., DasGupta, S., & DasGupta, N. N. (1974) *Biochim. Biophys. Acta 353*, 274–282.

Egli, M., Williams, L. D., Frederick, C. A., & Rich, A. (1991) *Biochemistry 30*, 1364–1372.

Fox, K. R., & Waring, M. J. (1972) *Mol. Pharmacol. 8*, 65–74.

Fox, K. R., & Waring, M. J. (1986) *Biochemistry 25*, 4349–4356.

Gale, E. F., Cundliffe, E., Reynolds, P. E., Richmond, M. H., & Waring, M. J. (1981) *Molecular Basis of Antibiotic Action*, Chapter 5, Wiley, London.

Hare, D. R., Wemmer, D. E., Chou, S. H., Drobny, G., & Reid, B. R. (1983) *J. Mol. Biol. 171*, 319–336.

Kersten, W., Kersten, H., & Szybalski, W. (1966) *Biochemistry 5*, 236–244.

Liaw, Y.-C., Gao, Y.-G., Robinson, H., Van der Marel, G. A., Van Boom, J. H., & Wang, A. H.-J. (1989) *Biochemistry 28*, 9913–9926.

Patel, D. J., Shapiro, L., & Hare, D. (1987) *Q. Rev. Biophys. 20*, 1–34.

Plumbridge, T. W., & Brown, J. R. (1979) *Biochem. Pharmacol. 28*, 3231–3234.

Reid, B. R. (1987) *Q. Rev. Biophys. 20*, 1–34.

Robinson, H., Liaw, Y.-C., Van der Marel, G. A., Van Boom, J. H., & Wang, A. H.-J. (1990) *Nucleic Acids Res. 18*, 4851–4858.

Searle, M. S. Hall, J. G., Denny, W. A., & Wakelin, L. P. G. (1988) *Biochemistry 27*, 4340–4349.

Sinha, R. K., Talapatra, P., Mitra, A., & Mazunder, S. (1977) *Biochim. Biophys. Acta 474*, 199–209.

Van de Ven, F. J., & Hilbers, C. W. (1988) *Eur. J. Biochem. 178*, 1–38.

Weiner, S. J., Kollman, P. A., Nguyen, D. T., & Case, D. A. (1986) *J. Comput. Chem. 7*, 230–252.

White, R. J., & Phillips, D. R. (1989) *Biochemistry 28*, 6259–6269.

Williams, L. D., Egli, M., Gao, Q., Bash, P., Van der Marel, G. A., Van Boom, J. H., Rich, A., & Frederick, C. A. (1990) *Proc. Natl. Acad. Sci. U.S.A. 87*, 2225–2229.

Zhang, X., & Patel, D. J. (1990) *Biochemistry 29*, 9451–9466.