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An experimental and theoretical study of drug/nucleic-acid interactions

Author
Nuss, Merrill Emery

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AN EXPERIMENTAL AND THEORETICAL

STUDY OF DRUG/NUCLEIC-ACID INTERACTIONS

by

MERRILL EMERY NUSS, JR.

B.S., Emory University 1967

DISSERTATION

Submitted in partial satisfaction of the requirements for the degree of

DOCTOR OF PHILOSOPHY

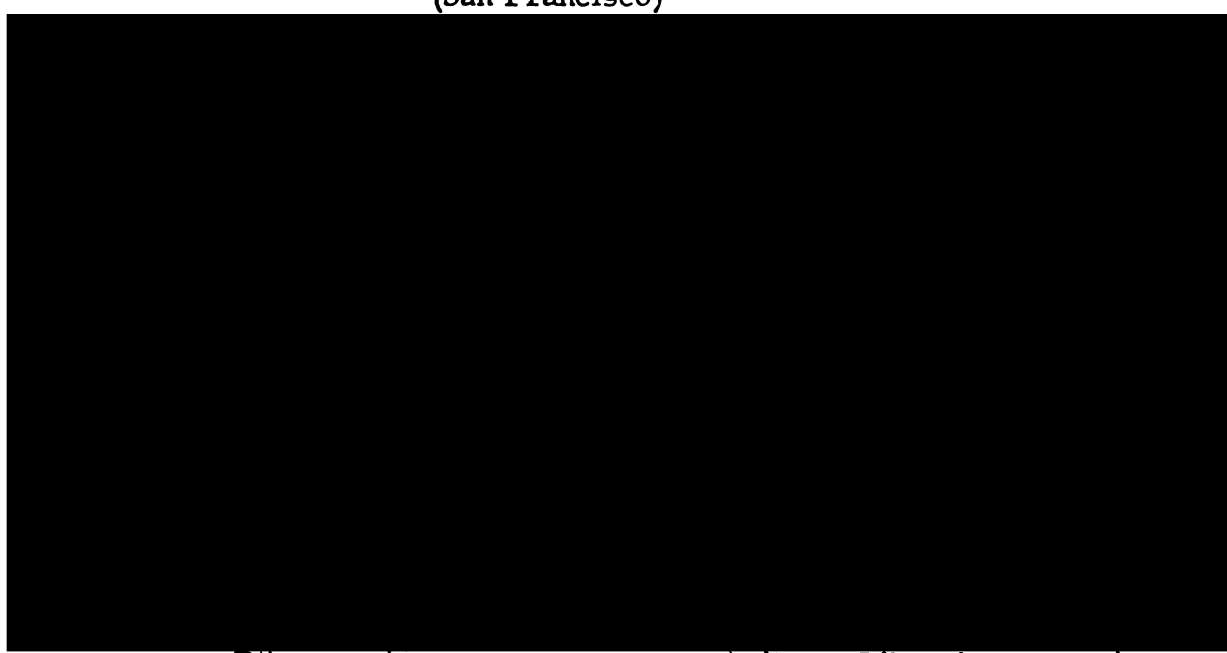
in

Pharmaceutical Chemistry

in the

GRADUATE DIVISION

(San Francisco)



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DEDICATION

This thesis is dedicated to three very special people, my wife Virginia and two children, Kellie and Kirk. To start and bring an undertaking such as this to a conclusion requires a great deal of support and understanding from those around you. I have been fortunate because my wife and children have given of themselves to allow me to finish and complete my doctoral degree. I hope that in some way I can repay my family for their love and understanding and what it has meant to me.

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Dallas Tuck for letting me clutter up his lab with paper and NMR tubes.

My parents for realizing the value of an education.

My wife's parents for their help and encouragement.

ABSTRACT

AN EXPERIMENTAL AND THEORETICAL

STUDY OF DRUG/NUCLEIC-ACID INTERACTIONS

MERRILL EMERY NUSS, JR.

Ph. D. DISSERTATION

Drugs such as daunomycin, actinomycin D, ethidium bromide, and the aminoacridines bind to nucleic acids by intercalating between the base pairs of the nucleic acids. Many of these drugs are potent chemotherapeutic agents. This thesis investigates the interaction of these drugs with nucleic acids (intercalation) using experimental and theoretical techniques in an effort to understand the mechanism of action of the intercalators on a molecular level.

High resolution FT NMR (360 MHz) has been used to study the interaction of daunomycin, an anticancer drug, with five deoxydinucleotides. The chemical shifts of the daunomycin protons are plotted as a function of the dinucleotide/drug ratio. The results indicate that daunomycin binds to all of the deoxydinucleotides, complementary and non-complementary, in a 1:1 complex. A molecular model is presented for the interaction of the drug with the dinucleotides based on the induced chemical shifts of the drug protons in the daunomycin/dinucleotide complex. The model is based on two assumptions. First, the daunomycin chromophore "intercalates" between the bases of the dinucleotide. The induced upfield chemical shift of the aromatic protons of daunomycin suggest that the chromophore is stacked between the nucleic acid bases. Second, the terminal phosphate group of the deoxydinucleotide interacts strongly with the positively charged 3'-amino group of the daunomycin sugar ring. The biological

implications of the formation of such a complex (daunomycin with a single-stranded nucleic acid) are discussed in the second chapter.

A complete potential energy function is used to study the interaction of several drugs with the deoxydinucleoside monophosphate, deoxyguanidylyl(3'-5')cytosine. The complex consists of the drug intercalated between two dinucleosides that are hydrogen-bonded by guanine-cytosine Watson-Crick base pairing. The energy of the complex is minimized with respect to seven torsional variables for each dinucleoside and six variables to position each small nucleic acid fragment with respect to the other one and to the drug (a total of 26 variables for the complex). The pucker of the sugar ring was not varied during the optimization procedure. The calculations indicate that there is a stronger interaction between proflavine and ethidium bromide with deoxyguanine(3'-5')deoxycytosine than for 10-aminoacridine. In addition, the results suggest that there is no large energy barrier (excluding dispersion attraction of the bases of the dinucleoside) for destacking the bases in the intercalation complex.

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Figure Captions (Continued)

numbers are used to represent the interaction energy contours (kcal): (1) = -2.5; (2) = -3.5; (3) = -4.5; (4) = -5.5; (5) = -6.5

Figure 18: See Figure Caption 17. Only the electrostatic contribution to the interaction energy is plotted in this figure. The following numbers are used to represent the interaction energy contours (kcal): (1) = -.6; (2) = -1.2; (3) = -1.8; (4) = -2.4; (5) = -2.8.

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Figure

Figure

Figure 2

Figure 23

Figure Captions (Continued)

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CHAPTER I: INTRODUCTION

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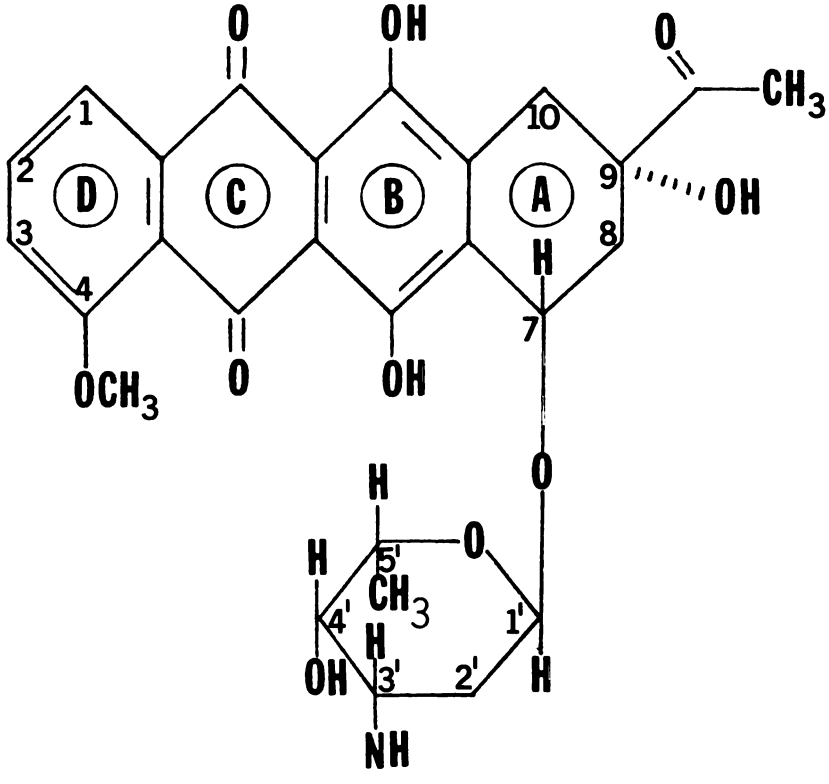
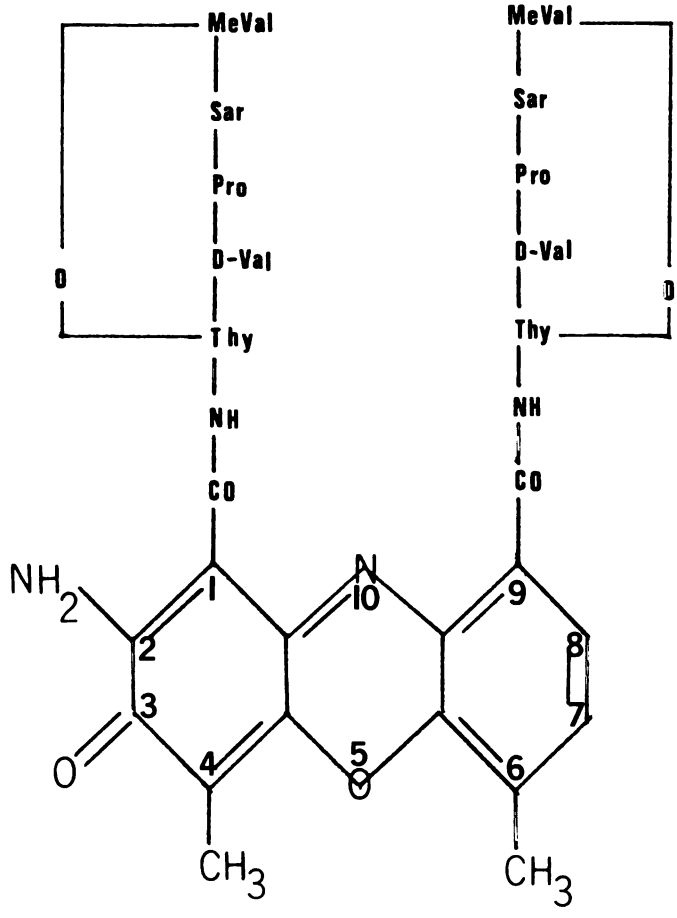
Many drugs such as the aminoacridines, ethidium bromide, actinomycin D, and daunomycin apparently bind to nucleic acids by intercalating between the base pairs of nucleic acids. The structure of these molecules and other drugs that are classified as intercalators is given in Figure 1. Many of the intercalators are very active chemotherapeutic agents. The anthracycline adriamycin is one of the most effective anticancer drugs available. Actinomycin D and are also used in the treatment of certain types of cancer. The aminoacridines are powerful antibacterial agents while quinacrine and quinine are very useful in the treatment of malaria. In each case, although the drugs are used in a wide variety of diseases, the activity of the drug has generally been associated with its ability to form complexes with nucleic acids (intercalation). The overall objective of this research was to investigate the interaction of drugs with nucleic acids (intercalation) using theoretical and experimental techniques in an effort to understand the mechanism of action of intercalators on a molecular level.

In 1961 Lerman[1] proposed a model for the binding of aminoacridine molecules to DNA. This model was based on several experimental results. The X-ray diffraction pattern of oriented fibers of a lithium DNA-proflavine complex was unlike the pattern observed for the B form of DNA. The altered patterns revealed a loss of long-range regularity of the helical structure but retention of the 3.4 Å stacking of base pairs perpendicular to the helical axis. The reflections also indicated

Figure 1a

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Pro
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NH
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MeVal
Sar
Pro
D-Val
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Figure 1c

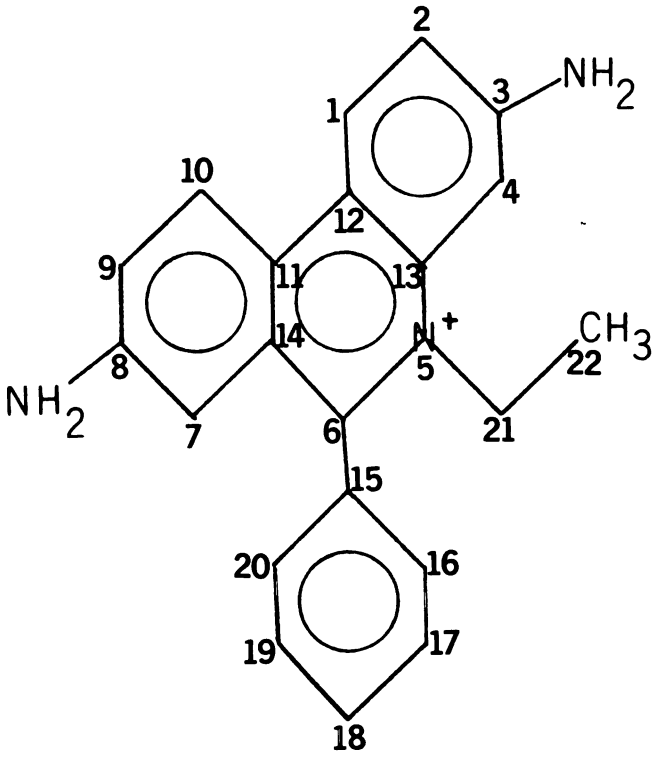
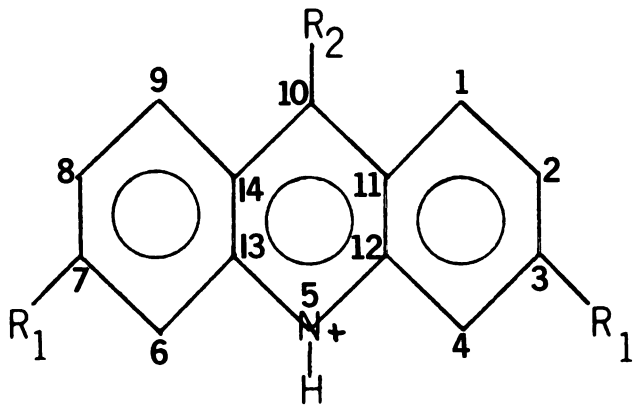


Figure 1d



R_1=H; R_2=H; Acridine

R_1=NH_2; R_2=H; Proflavine

R_1=H; R_2=NH_2; 10-Aminoacridine

that the drug was not bound to the outside of the helix. In addition, Lerman noted that the viscosity of proflavine/DNA complexes increased as compared to DNA solutions while the sedimentation coefficient decreased. From this information Lerman proposed that the aminoacridine molecule was inserted between adjacent base pairs of DNA. To accommodate the acridine molecule he suggested that the distance between the base pairs of DNA must increase from 3.4 Å to 6.8 Å at the site of intercalation. The increased distance between the base pairs forces the helix to unwind 45 degrees from its characteristic 36 degree right-hand twist to a left-handed twist of 9 degrees. Lerman subsequently revised his estimate of the unwinding of DNA necessary to accommodate the intercalator. He suggested that it would be possible for a drug to intercalate between the base pairs of DNA and only unwind the helix 36 degrees. The base pairs adjacent to the intercalation site still remain perpendicular to the helix axis.

More recently Fuller and Waring[2] proposed a refined intercalation model based on the interaction of ethidium bromide with DNA. The model is basically the same as Lerman's except for the unwinding angle at the site of intercalation. They propose that an unwinding angle of only 12 degrees is sufficient for the DNA to accommodate the ethidium bromide molecule. One attractive feature of this model as compared to Lerman's is that the smaller unwinding angle allows a maximum separation of the negatively charged phosphate groups of DNA.

A wide variety of experimental techniques have been used to investigate the nature of the drug/nucleic-acid complex. Perhaps the easiest method of detecting a drug/nucleic complex is the change in the visible

absorbance of the drug when DNA is present. Generally drugs that intercalate have their absorption maxima at longer wavelengths than DNA.[3] When the drug binds to DNA by intercalating between the base pairs the absorption maxima is generally shifted to longer wavelengths and decreased in intensity. If the extinction coefficient is known for both the free and bound drug,an apparent binding constant for the drug/nucleic-acid complex can be calculated using a Scatchard plot. The main source of error in calculating the binding constant for the drug/nucleotide complex is accurately determining the extinction coefficient of the bound drug. Small errors in the extinction coefficient of the bound drug can lead to dramatically different binding constants for the drug/nucleic-acid complex. The number of binding sites per mole of base pairs of the nucleic acid can also be estimated from a Scatchard plot.

The fluorescence of a molecule complexed to nucleic acid is also used to monitor the drug/nucleic-acid complex. Normally the fluorescence of a drug is altered (quenched or enhanced) when it intercalates between the base pairs of nucleic acids.[4] With proflavine the fluorescence is quenched, while with ethidium bromide it is enhanced. The fluorescence of the drug can also be affected by a change in the flexibility of the nucleic acid or the chromophore itself.

The optical rotary dispersion (ORD) and circular dichroism (CD) curves of optically active drugs shift when they bind to DNA.[5] Also drugs that are not optically active have an induced CD spectra when they form a complex with nucleic acids. The electronic transition of the symmetrical chromophore will be distorted by the asymmetric electric field of DNA producing an induced ORD and CD spectrum. Unfortu-

nately the ORD and CD spectra normally contain components from dye-dye interactions which may not be separable from the drug/nucleic-acid complex making a detailed analysis almost impossible.

The absorbance of polarized light by the drug in a drug/nucleic-acid complex can be measured when the complex has been oriented by a flowing liquid. This technique is known as flow dichroism.[6] The change in absorbance of the polarized light by the drug gives some indication of the orientation of the drug with respect to the base pairs of nucleic acid. With intercalators the absorbance is normally a maximum when the polarized light is perpendicular to the helical axis of the nucleic acid indicating that the drug is perpendicular to the helical axis. This does not, however, verify that a drug has intercalated between the base pairs of the nucleic acid. It would be possible for a drug to bind to the outside of the DNA helix parallel to the base pairs and produce the same type of results.

The melting point of DNA (T_m) is often used to detect the formation of complexes between drugs and nucleic acids.[7] Drugs that intercalate bind more tightly to double-stranded DNA than to DNA in a random coil. This shifts the equilibrium between the helical and coil forms of DNA toward the helix and thereby stabilizing the helix. Therefore drugs that intercalate or link any two parts of the helix together increase the T_m of DNA by stabilizing the helical form of DNA. It might be noted that if a drug bound more tightly to single-stranded DNA than to double-stranded DNA, the melting point of DNA would actually be decreased.

The unwinding of the supercoiled circular DNA is also used as a diagnostic test for drugs that intercalate.[8] The sedimentation velocity of circular DNA is measured as the ratio of drug to nucleic acid is

increased. If a drug intercalates between the base pairs of the circular DNA, the sedimentation velocity will decrease initially as the superhelix unwinds. The unwinding of the helix makes the circular DNA less compact and hence sediment more slowly. After reaching a minimum value, the sedimentation velocity of the complex will begin to increase as more drug is added. This is because the helix begins to rewind in the opposite sence from the initial unwinding of the circular DNA. The helix becomes more compact and thus begins to sediment at a faster rate. All of the intercalators tested with this method have demonstrated similar changes in the sedimentation coefficient for the drug/circular-DNA complex. There is at least one example (irehdiamine A) of a nonintercalator which can produce similar results with the supercoiled DNA.

Microcalorimetric studies of the binding of several drugs that intercalate have been used to study the thermodynamic properties of the drug-nucleic-acid interaction. All of the drugs tested except actinomycin D, which is one of the best documented cases of intercalation, demonstrated that the binding of drugs to nucleic acids by intercalation is an exothermic process.[9] Typical enthalpy values for the intercalation of the drug between the base pairs of the nucleic acid range from -5 to -7 kcal/mole. When a derivative of actinomycin D in which the pentapeptide side chains were replaced by smaller side chains, N,N-diethylethylenediamine, the thermodynamic properties of the drug/nucleic-acid complex were consistent with other intercalators. This suggests that the anomalous thermodynamic behavior of actinomycin D is related to the pentapeptide side chains rather than the aromatic chromophore which is presumed to fit between the successive base pairs of DNA.

X-ray crystallography has also been used to investigate the complexes of drugs with small nucleic components.[10,11,12] Ethidium bromide and 10-aminoacridine have been co-crystallized with a few self-complementary ribodinucleoside monophosphates. The drug/nucleotide interaction represents the minimum components in the intercalation model and, as such, provides an excellent opportunity to evaluate the intermolecular interactions of the intercalation process. These complexes will be discussed in more detail in Chapter III.

Nuclear Magnetic Resonance (NMR) techniques, although of great importance in studying the binding of drugs to small nucleic acids, have had little success in probing the nature of drug binding to high molecular weight DNA. An unresolved continuum of signals is observed for the drug protons when actinomycin D,[13] acridine,[14] and daunomycin[15] bind to high molecular weight DNA making any kind of structural interpretation impossible. The broadened spectral lines of the drugs is due to the long correlation time of the drug/nucleic-acid complex in solution, i.e. the complex does not rotate fast enough for the protons to "see" an averaged magnetic field. The study of the binding of drugs (actinomycin D and ethidium bromide) to small nucleic acid components using NMR has demonstrated that a drug/small-nucleic-acid complex is a reasonable model for the drug/DNA complex.[16,17]

The binding of drugs to DNA by intercalation has received considerable attention over the past few years. One reason is that the intercalation model for drugs that complex with nucleic acids has offered the medicinal chemist a rare opportunity--the chance to study a reasonable three-dimensional model of a drug/receptor complex. Structure-activity

relationships for the antimalarial quinine[18] and the antibacterial aminoacridines[19] were postulated in the 1940s. These rules were not rationalized using a drug/receptor complex until the intercalation model was presented in 1961 by Lerman. Since then the biological activity of a large number of drugs has been associated with their ability to intercalate between the bases of DNA. The activity of ethidium bromide, an antitrypanocidal drug, has also been related to its ability to form complexes with nucleic acids.[20] The anticancer activity of the anthracyclines,[21] adriamycin and daunomycin, and actinomycin D[22] is also thought to be related to their ability to bind to nucleic acids.

Intercalators have also been used as a probe for the structure of biological macromolecules. In 1947 Michaelis[23] attempted to elucidate the structure of DNA based on the results of binding of aminoacridines to DNA. Although his model was not correct, his comments are worth repeating:

> Nucleic acids, whether of high or low molecular weight, may be imagined to consist of strings of bundles of nucleotides arranged in such a way that the pyrimidine, or purine rings lie parallel to each other, connected by phosphate groups; the dye molecules attached to the negatively charged end of the phosphate group. Each dye cation combined with one phosphate group must lie in the space between the planes of the pyrimidine or purine rings, and so they are prevented from approaching each other in such a way as to interfere optically with each other and from exhibiting the spectrum of a higher dyestuff aggregate.

Ethidium bromide has been used to investigate the nature of the interaction of DNA, histones, and nonhistone proteins in chromatin.[24] A Scatchard plot of the binding of ethidium bromide to chromatin indicates that there are two strong binding sites, one similar in binding affinity to free DNA and another an order of magnitude weaker. The

number of binding sites of ethidium bromide in chromatin is substantially less than that for free DNA. These data suggest that the DNA in chromatin exists in at least two different states. The binding of actinomycin D to chromosomes has also been investigated. The amount of the drug bound in chromosomes is less than that found for free DNA.[25] It has also been shown that quinacrine, an antimalarial drug, binds in a region of chromosomes different from that of actinomycin D. One can conclude that chromosomes, like chromatin, consist of DNA in more than one state.

Many intercalators, especially the aminoacridines, have been noted for their mutagenic properties. The mutagenic effect of the aminoacridines actually led to the discovery of frameshift mutations.[26] Most theories of mutagenicity, in particular frameshift mutations, assume that intercalation is the primary event. Although intercalation certainly plays an important role, it is clear that other processes must be involved. Although both 10-aminocridine and 5-methyl-10-aminoacridine bind very tightly to DNA by intercalation, only the 10-aminoacridine compound is mutagenic.[27] The difference in metabolism of the two aminoacridines might be responsible for their different mutagenic properties. Some intercalators can even act as antimutagens. Actinomycin D, for example, reduces the frequency of X-ray induced mutations in Drosophila melanogaster by approximately one-half.[28] In control experiments the normal frequency of mutations was not significantly changed when actinomycin D was added to the control cultures. Quinacrine also acts as an antimutagen by decreasing the frequency with which bacteria mutate to resist the action of drugs.[29]

In a few instances the actual biological event responsible for an intercalator's activity has been determined. The antibacterial activity of the aminoacridines is related to its ability to block the DNA-starter required by the enzymes which synthesize DNA and RNA and repair damaged DNA.[30] The molecular event responsible for the antiviral activity of ethidium bromide has also been investigated.[31] When cells were infected by an RNA tumor virus (avian sarcoma virus) ethidium bromide blocked the integration of the viral DNA into the host DNA while allowing the synthesis of normal amounts of viral DNA. Presumably the integration of the viral DNA into the host genome is blocked because the drug interferes with the formation of the covalently closed circular DNA.

Of the different techniques and methods used to study drug/nucleic-acid complexes, I have chosen NMR spectroscopy and theoretical techniques to investigate the origin and nature of these complexes. Both of these methods, although impractical for the study of drug binding to high molecular weight DNA, are potentially useful in determining the nature of the drug/small-nucleic-acid complex. The only other technique which permits a detailed molecular picture of the drug/receptor complex is X-ray crystallography. Chapter II describes the results of using NMR to study the daunomycin/nucleic-acid complex.

A few attempts have been made to study the interaction of drugs with nucleic acids using theoretical calculations. In Chapter III the most complete model to date is presented for studying the drug/nucleic-acid interaction. The model consists of a drug intercalated between the base pairs of two self-complementary deoxydinucleoside monophosphates. The minimum energy conformation is determined varying the torsional

and intermolecular degrees of freedom for the complex. A model is also presented which evaluates the binding energy of different actinomycin D chromophores to the ten different base pair combinations using only an electrostatic contribution.

CHAPTER II: NMR STUDIES

Daunomycin is one of the glycosidic anthracyclines antibiotics that is currently being used in the treatment of cancer in man.[32] The activity of the anthracyclines in general is thought to be related to their ability to form complexes with DNA. In vivo, daunomycin inhibits both RNA and DNA synthesis, while in vitro, DNA and RNA-dependent polymerases are inhibited. The inhibition of the in vitro nucleic acid synthesis has been shown to be due to drug binding to the template rather than to the direct interaction with the enzyme.[33] The inhibition of a nucleic acid synthesis by the drug can be reversed by adding more drug. However, if more enzyme is added there is little, if any effect.

The objective of this experimental work is to study a daunomycin/nucleic-acid complex to determine (1) if there is any base specificities for daunomycin/nucleotide interactions, (2) possible structure(s) of the daunomycin/receptor complex in solution and (3) the stoichiometry of the drug/nucleotide complex.

A model for the interaction of daunomycin with double-stranded DNA has been proposed by Pigram.[32] He suggested that there are three important components of binding for the daunomycin/nucleic-acid complex--

(1) hydrophobic interaction due to the intercalated aglycone

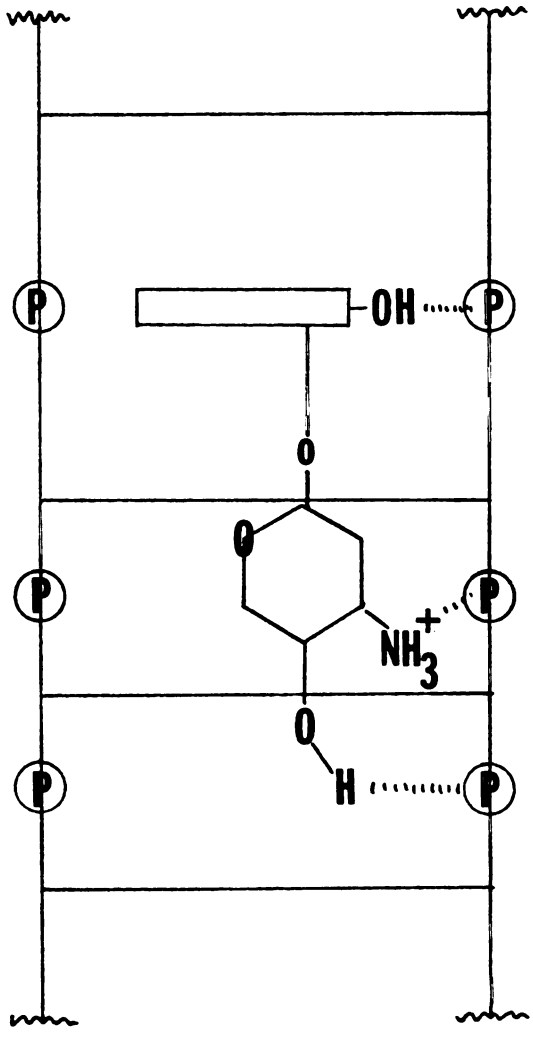
(2) electrostatic attraction between the protonated 3'-amino group of the daunosamine sugar and the phosphate groups of the helix

(3) hydrogen bonds of unspecified character

Recently Henry[32] has refined the model proposed by Pigram by suggesting that (1) the 9-hydroxyl group can form a hydrogen bond with the phosphate group at the intercalation site, (2) the 3'-amino group forms a hydrogen bond with the phosphate group adjacent to the intercalation site and (3) the 4'-hydroxyl group forms a hydrogen bond with the phosphate group which is two away from the phosphate group at the intercalation site. A schematic diagram (Figure 2) illustrates the position of the chromophore of daunomycin and the position of the three hydrogen bonds as proposed by Henry. The work that is proposed should provide information to evaluate the validity of the binding model of daunomycin and nucleic acids. An understanding of the daunomycin/nucleotide interaction is essential if one hopes to understand the mechanism of action of the anthracyclines on the molecular level.

NMR is well suited to study the structure of intermolecular complexes (drug/nucleic-acids) is solution since the induced chemical shifts of the drug protons are a function of the molecular environment. Other techniques which are frequently used to study drug/nucleic-acid interactions (UV-Visible, CD, Fluorescence, unwinding of circular DNA and others) can easily demonstrate the formation of drug/nucleic-acid complexes.[34] They can even distinguish, although not on an individual basis, between drugs that intercalate and those that bind to the outside of the DNA helix. They can not, however, provide specific information about the molecular details of the drug/nucleic-acid complex. NMR, however, is also limited because it can only be used to study the interaction of drugs with small nucleic acid components (mononucleotides, dinucleotides and others). The interaction of drugs with small nucleic

Figure 2



acids using NMR has proven quite useful. Recently, the binding of ethidium bromide to self-complementary dinucleoside monophosphates has demonstrated that the drug binds to certain sequence isomers more strongly than others.[35] Ethidium bromide shows a preference for binding to dinucleoside monophosphates that have a pyrimidine (3'-5') purine sequence when compared to their isomeric purine (3'-5') pyrimidine sequence. The large induced upfield chemical shifts of the ethidium aromatic protons suggests that the complex results from stacking of the nucleic acids with the drug forming a miniature double helix (two small self-complementary nucleosides with one drug molecule). In addition, NMR has been used to study the interaction of actinomycin D with a variety of small nucleic acids. It has been demonstrated that actinomycin D will form strong complexes with (1)d-pG,[36] (2) d-pGpC,[37] and (3) d-ApTpGpCpApT.[38] In each case the stoichiometry of the drug/nucleic-acid complex is 1:2. Also, the induced chemical shifts of the actinomycin protons are consistent with a stacking model for the drug/nucleic-acid interaction. These studies indicate a preference for the binding of actinomycin D to guanine.

EXPERIMENTAL

The deoxydinucleotides and deoxydinucleoside monophosphates were purchased from Collaborative Research, Inc., and used without further purification. Daunomycin and calf-thymus DNA were obtained from Sigma Chemical Co. An extinction coefficient of 9870 at λ = 480 nm for daunomycin and 6600 at λ = 260 for DNA was used to

calculate the concentration of the drug and nucleic acid. The concentrations of the small nucleic acids were determined using extinction coefficients from the P-L Biochemical catalog No. 104. All of the samples were dissolved in a 5 mm sodium phosphate/D_2O buffer with a pH meter reading of 6.8. The titrations were performed by keeping the drug concentration constant (1 mm) and adding different amounts of the nucleic acid components. Typically the ratio of nucleic acid to drug would vary from a maximum of 6:1 to a minimum value of 0.5:1. A small amount of EDTA (ethylenediaminetetraacetic acid) was added to eliminate broadening due to paramagnetic impurities. The chemical shifts of the drug (free and complexed) are reported relative to the sodium salt of 2,2-dimethyl-2-silapentane-5-sulfonic acid (DSS). The drug/nucleotide titrations were actually run using tetramethylammonium chloride (TMA) as an internal standard. The change in standards (TMA to DSS) was done to facilitate the interpretation of the data.

The proton NMR spectra were recorded on the Bruker HXS-360 spectrometer at the Stanford Magnetic Resonance Laboratory and the Varian XL-100 instrument at the UCSF Magnetic Resonance Laboratory. All of the titrations that are reported were done at a temperature of 313 degrees Kelvin to sharpen the resonance during the titrations.

METHODS

In studying drug/nucleic-acid interactions with NMR it is necessary to distinguish the type of chemical exchange for a nucleus between the free and complexed state. In general, if

the rate of chemical exchange between the two states is slower than their chemical shift difference

$$\tau_A, \tau_B >> 2\pi/(|\nu_A - \nu_B|)$$

where τ_A and τ_B are the first order lifetimes of the nuclei in their environments and ν_A and ν_B are the resonance frequencies, then the rate of exchange between the two sites is defined as slow.[39] In this case there will be two resonances (ν_A and ν_B) corresponding to the free and bound nucleus. If the chemical shift differences are much greater than the lifetime of the nuclei at each site, there will be a single resonance peak which will be a weighted average of the chemical shifts for the free and complexed nuclei. It is also possible to have an intermediate exchange rate where the chemical shift difference is approximately equal to the lifetime of the nuclei at each site. The lineshape for intermediate exchange will depend on the exact rate of chemical exchange and the lifetimes for the nucleus in each site.

Nuclear magnetic relaxation, although not of primary importance in this work, has important implications in the study of intermolecular complexes such as the interaction between drugs and nucleic acid molecules. In general, two conditions must be fulfilled for magnetic relaxation to occur. First, there must be a magnetic field which interacts directly on the spin of the nuclei. Secondly, this interaction must be time dependent, i.e. fluctuate with time. Although relaxation can take place by a wide variety of mechanisms, the nuclear magnetic

dipole-dipole interaction is normally the dominant component of relaxation for a proton in a liquid.[40] Specifically there are two types of relaxation, spin-lattice (T_1) and spin-spin (T_2). The spin-spin relaxation mechanism is of importance because it determines the resonance lineshape in the absence of exchange contributions. With very viscous solutions or with large molecules (high molecular weight DNA), T_2 relaxation is very fast. This results in broad lines for the nuclei in question. With small molecules, however, the spin-spin relaxation is sufficiently long to permit relatively sharp resonance lines.

An understanding of the chemical shifts for protons is important in the study of drug/nucleic-acid interactions. The chemical shifts of the drug are monitored as the ratio of nucleic acid to drug is changed by adding nucleic acid while keeping the concentration of the drug (daunomycin) constant. The factors responsible for the chemical shifts of nuclei can be arbitrarily divided into three contributions, local paramagnetic effects, local diamagnetic currents, and interatomic current effects. For protons, the first contribution can normally be neglected because there are no low-lying excited states for protons.[41]

When an atom is placed in a magnetic field, the electrons circulate around the nucleus and produce a magnetic field that opposes the applied filed. This has the net effect of shielding the nucleus. It therefore, takes a larger magnetic field to produce the resonance condition because of the diamagnetic effect. The shielding factor (diamagnetic effect) is roughly proportional to the electron density around the nucleus.

The third contribution to the chemical shifts of a proton is of primary importance in this work because it can be used to determine the molecular geometry of the drug/nucleic-acid complex. The ring current effects are produced in planar aromatic molecules by the circulation of the π electrons in such a direction that an induced magnetic field opposes the applied magnetic field. The induced magnetic field will reenforce the applied magnetic field in the plane of the molecule. It will, however, oppose the applied field above and below the plane of the molecule. This has the net result of shifting the resonance of a proton downfield if it lies in the plane of the molecule or upfield if it is above or below the plane of the molecule.

Since the induced chemical shifts of the drug protons resulting from ring-current effects are monitored in the daunomycin/nucleic-acid titrations and then used to determine the structure of the complex, it is necessary to consider in some detail the magnitude and spatial dependence of these effects. One of the earliest attempts to quantitate the ring-current effect was the work of Johnson and Bovey.[42] Their approach was based on the assumption that the π electrons of the aromatic molecule (benzene in this case) circulate in the π-clouds giving rise to a magnetic field that opposes the applied field. The qualitative features of the shielding can be described by the relation

$$(3 \cos^2 \theta - 1)/r^3$$

where θ is the angle between the axis of a planar molecule and a vector from the center of the molecule to the nuclei of interest and r is the distance from the observed nucleus to the center of the molecule. This expression indicates that a proton situated

above the planar molecule (θ will be small) will experience an upfield chemical shift while a proton in the plane of the molecule (θ will be close to 90 degrees in this case) will experience a downfield shift. The relationship also indicates that when a proton is at an angle (θ) of 54.7 degrees, it will not be shifted upfield or downfield. Although the qualitative features of their work is correct, the exact magnitude of the induced chemical shifts has been the subject of recent papers.[43,44,45]

The most complete description of the ring-current effect is the paper of Giessner-Prettre and Pullman. They have calculated the spatial dependence of the ring-current magnetic anisotropies of the nucleic acid bases as a function of radial distances from the center of the planar molecule. It is thus possible to calculate the ring-current shift at any point around the molecule to a radius of 10 Å as well as to a distance of 8 Å above or below the molecule. They have made some assumptions in their work which lead to some uncertainty in the results. First, they assumed that the ring-current intensities are not modified by intermolecular interactions. Secondly, atomic contributions to the diamagnetic effect are neglected. Finally, an averaged distance of the electrons of the $2p_z$ orbital is used regardless of whether it is a nitrogen or carbon atomic orbital. All of these factors could lead to a difference in the calculated magnitude of the induced chemical shift when it is compared to experimental data.

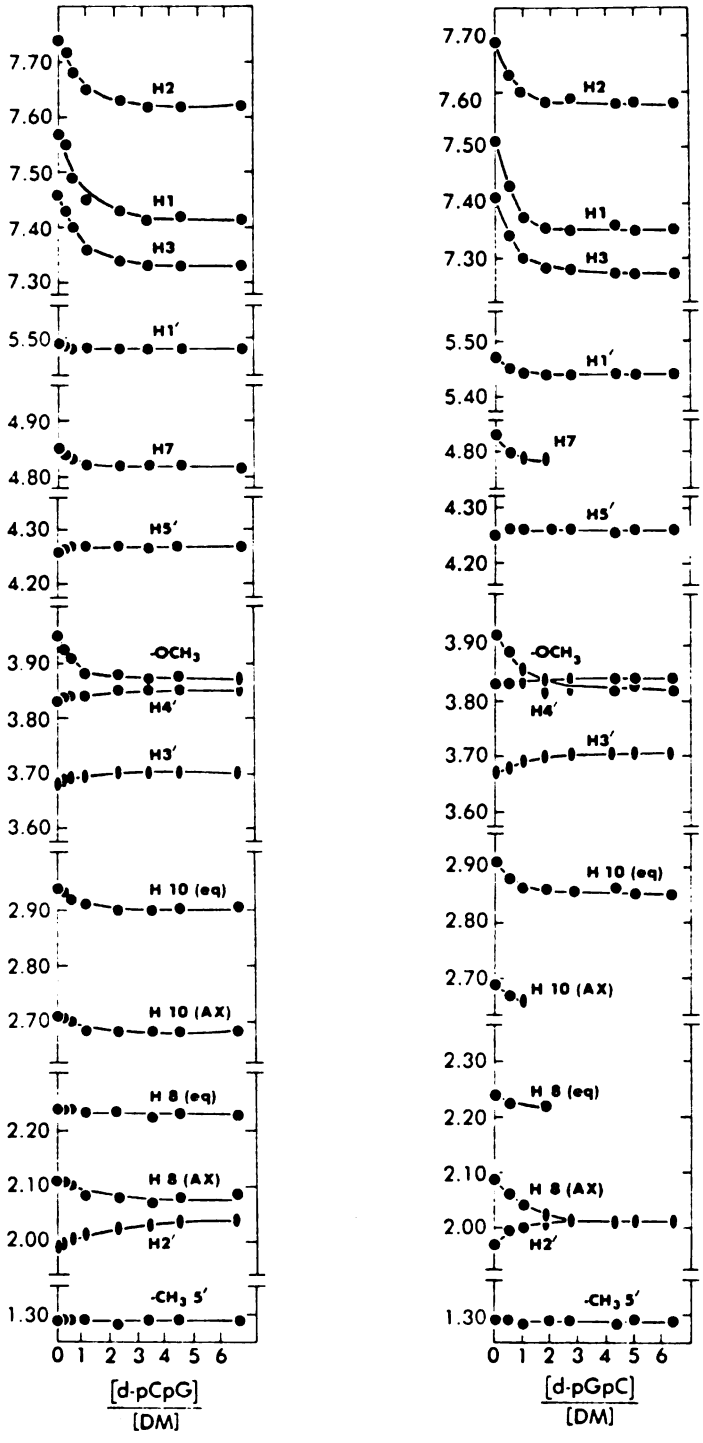
RESULTS

All of the deoxydinucleotides used in this study (d-pApT, d-pTpA, d-pGpC, d-pCpG, and d-pCpC) form strong complexes with daunomycin.

The chemical shifts of all of the drug protons except the C-14 methyl protons are plotted as a function of the nucleotide to daunomycin ratio (Figure 3a-3e). In Appendix A the 360 MHz proton FT NMR spectra is shown for daunomycin and three drug/d-pCpG comples of different ratios. The C-14 methyl protons are not observed in the titrations because they exchange with the solvent (D_2O). The complete spectrum of daunomycin at 220 MHz in pyridine-d_5 has been reported.[46]

We believe there is one error in the previous assignment of the spectrum, the H-8(eq) and H-8(ax) resonances being misassigned. The previous assignment was based on the H-7 proton being in a "pseudoequatorial" position. Looking down the C-8 to C-7 bond, it is possible to have two conformation of the A ring of daunomycin as indicated in Figure 4a and 4b. Using the Karplus relationship[47] it should be possible to distinguish between the two conformations. The Karplus theory suggests that there is a relationship between the coupling constants and the dihedral bond angle associated with an ethane-like fragment, $H_a-C_a-C_b-H_b$. Large coupling constants, between the H_a and H_b protons, are predicted for cis (0°) and trans (180°) conformations but small values for gauche (60° and 120°) conformations. In 4b, both of the H-8 protons would be split by the same amount, if split at all. However, in Figure 4a the H-7 proton would split the H-8(eq) proton by a larger amount than it would the H-8(ax) proton. The spectrum in pyridine demonstrates that the splitting is much greater for one of the two protons (5.0 to 1.5 Hz). In D_2O the splitting of one of the H-8 protons is approximately 6 Hz while there is no observed splitting on the other proton. These observations indicate that the conformation as indicated in Figure 4a is correct. The H-8 proton that is split is the equatorial one and not the axial proton as

Figure 3*



(a) (b)

* The equatorial and axial designations are reversed for the H8 protons in all of the titration curves.

Figure 3 (cont.)

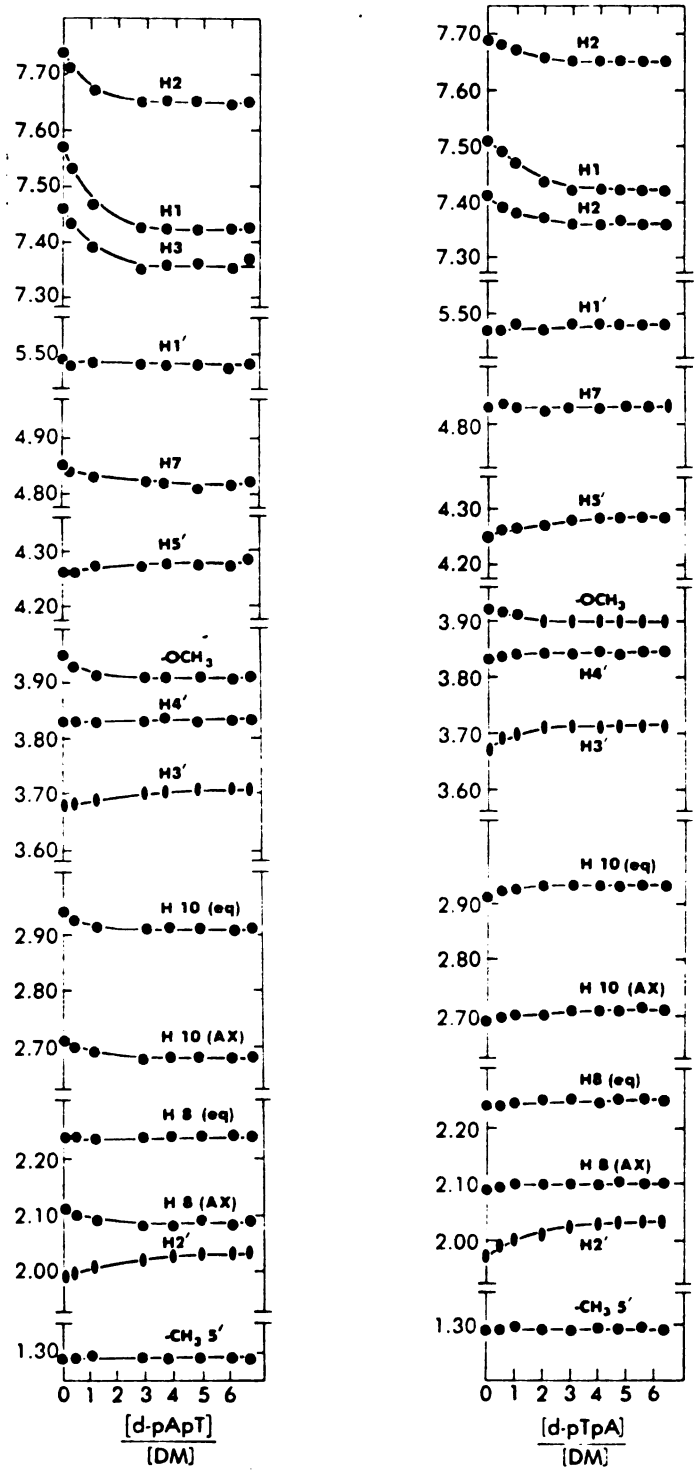
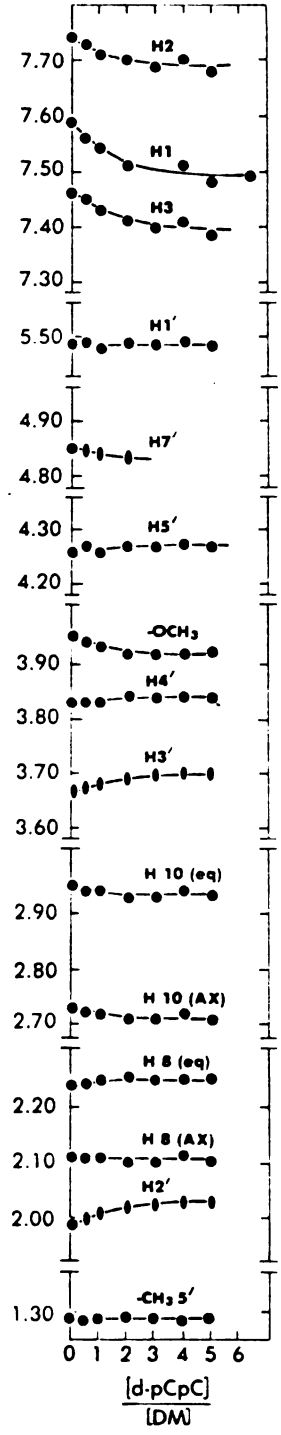
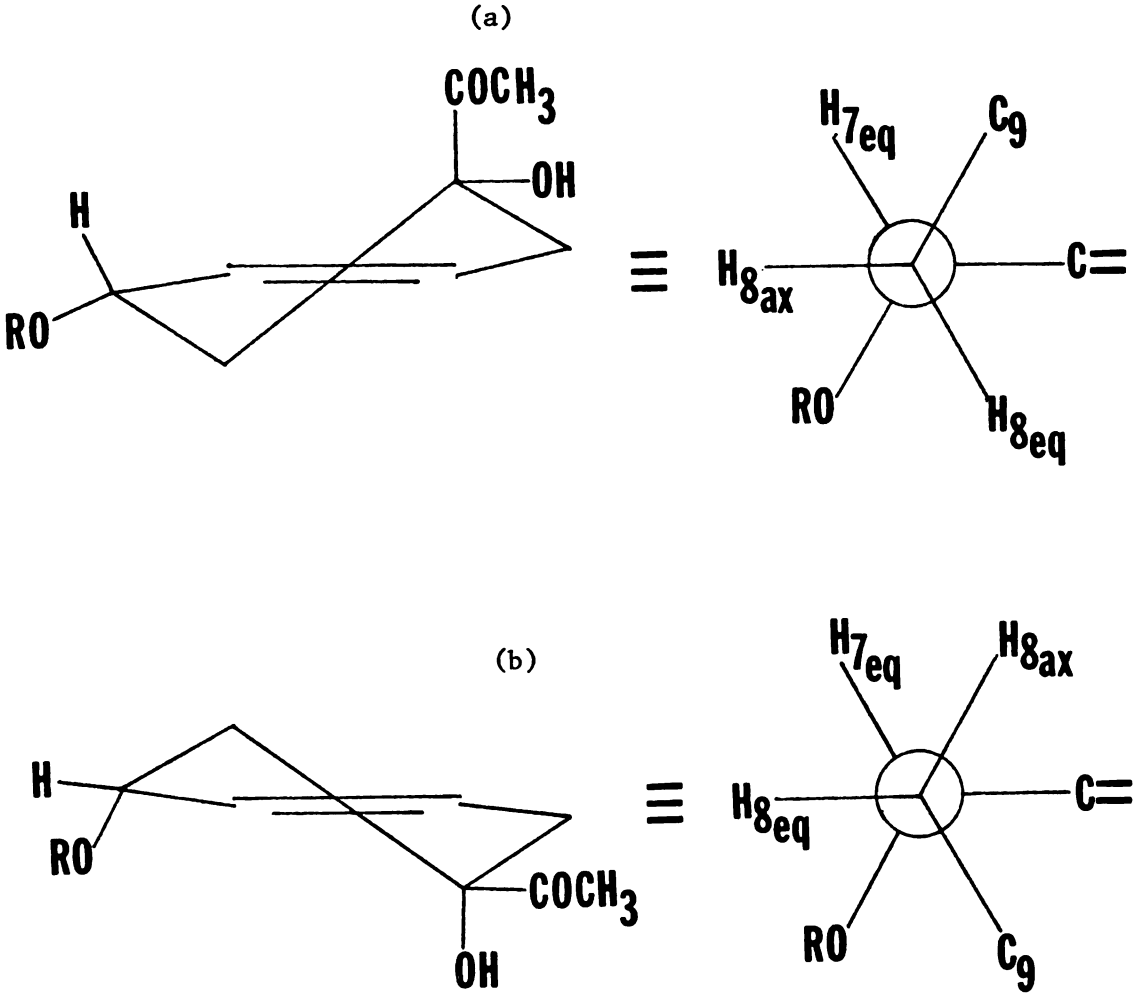


Figure 3 (cont.)



(e)

Figure 4


(a)
COCH3
OH
H
RO
≡
H7eq
C9
H8ax
C=
RO
H8eq
(b)
H
RO
COCH3
OH
≡
H7eq
H8ax
H8eq
C=
RO
C9

was originally assigned. Also, if the A ring is in the conformation as shown in Figure 4a, the H-8(ax) proton would be closer to the plane of the aromatic chromophore of daunomycin than the H-8(eq) proton. This would result in the H-8(eq) proton being shifted upfield as compared to the H-8(ax) proton due to ring current effects of the aromatic chromophore and this is indeed experimentally observed.

When the spectrum of daunomycin is compared for the two solvents (pyridine and D_2O), most of the daunomycin proton resonances in D_2O can be unambiguously assigned by comparing them to the drug spectra in pyridine. It is not, however, possible to make a clear distinction between the two C-2' and two C-8 protons by comparing the drug spectra in the different solvents. The chemical shifts of these protons are fairly close, less than one ppm, and the splitting pattern is not the same in each solvent. By spin decoupling it was demonstrated conclusively that both of the H-8 protons were downfield in D_2O as compared to the H-2' protons, supporting the original assignment.

Before a detailed analysis of the data can be made, one must consider the effect of the self-association of daunomycin on the drug proton chemical shifts. At the concentrations of daunomycin used in these experiments ($\sim$ 1 mm), the proton chemical shifts of the drug are significantly changed from their values in a monomeric state. Table 1 lists the chemical shifts of the different drug protons at infinite dilution (monomer) and in the presence of an excess of each of the different deoxydinucleotides. The chemical shifts for the daunomycin monomer were determined from the concentration dependence of the drug proton chemical shifts extrapolated to zero

Table 1. Chemical Shift (ppm) of daunomycin protons (relative to DSS) in the presence of an excess of each of the five different deoxydinucleotides.

DM Protons	d-pTpA	d-pApT	d-pGpC	d-pCpG	d-pCpC	Infinite Dilution
H2	7.65	7.65	7.58	7.62	7.67	7.83
H1	7.42	7.43	7.35	7.42	7.48	7.87
H3	7.36	7.37	7.27	7.33	7.38	7.61
H1'	5.48	5.48	5.43	5.48	5.48	5.57
H7	4.84	4.84	4.87	4.83	4.86	5.01
H5'	4.28	4.28	4.26	4.27	4.28	4.29
OCH_3	3.91	3.91	3.81	3.87	3.92	4.07
H4'	3.84	3.83	3.84	3.87	3.85	3.81
H3'	3.71	3.71	3.70	3.71	3.70	3.66
H10(e)	2.94	2.92	2.86	2.92	2.94	3.09
H10(a)	2.72	2.69	2.67	2.69	2.72	2.89
H8(a)	2.24	2.24	2.22	2.23	2.25	2.27
H8(e)	2.06	2.09	2.04	2.09	2.10	2.27
H2'	2.02	2.02	2.02	2.04	2.01	1.97
CH_3	1.29	1.29	1.28	1.28	1.29	1.28

concentration. The aromatic protons and the methoxy protons are shifted by the largest amount (downfield) as the concentration of the drug is decreased. The direction and magnitude of these chemical shifts indicates that the self-association of daunomycin is a result of stacking of the planar chromophore of the drug.

In titrations of the drug with the different dinucleotides, the induced chemical shifts reach a limiting value in the presence of an excess of nucleic acid. The limiting value of the chemical shift is assumed to be the chemical shift of daunomycin/deoxydinucleotide complex since the addition of more nucleic acid no longer influences the chemical shifts of the drug. At intermediate nucleic-acid/drug concentrations, a ratio greater than the monomer but less than that needed to produce the limiting value of the chemical shift, the induced chemical shift of the drug arises from two sources, (1) disruption of the self-association complex of daunomycin and (2) a change in the chemical shifts that is a direct result of the complex formation of the drug with the nucleotides. To facilitate interpretation of the spectra, the chemical shifts will be measured at their limiting value (excess nucleic acid) since the drug chemical shifts will only be a function of the drug/nucleotide complex.

The induced chemical shifts of the daunomycin protons in the presence of an excess of nucleic acid are useful in determining the geometry of the drug/nucleic-acid complex. There are, however, several reasons which make an exact structural determination of the complex difficult. The ring-current magnetic anisotropy effects, which are used to determine the geometry of stacking complexes (drug/nucleotide), have not been unequivocally determined. Although there have been several papers

recently,[43,45] which have attempted to calculate the spatial dependence of the ring-current shifts, it is doubtful that these effects have been totally and accurately determined. In addition, there is a problem of accurately determining the chemical shift of the daunomycin monomer. When one compares the induced chemical shifts from the same proton, with the different dinucleotides, an error in the monomer shifts is not that important. It is much more critical when one compares the induced shifts of the different protons of the drug with the same nucleic acid. Inaccuracies in the induced chemical shifts will lead to errors in the structural interpretation of the drug/nucleotide complex.

There are, also, two problems which are specific to the daunomycin/nucleotide complex. As has been noted previously, the induced chemical shift for the complex is obtained by subtracting the proton chemical shift of the complex (daunomycin in the presence of an excess of nucleic acid) from the chemical shift of the drug monomer. For the aromatic protons of daunomycin, H-1, H-2, and H-3, the induced shifts can be calculated directly. The induced chemical shifts for the protons of the A ring and the daunosamine moiety, can be determined in a similar fashion. Using this method, however, it is implied that the conformation of the two rings (A ring and daunosamine) is the same in the monomer and complexed state. If the conformation of the A ring, for example, was not the same in both cases, then the induced chemical shift would be the result of the stacking complex and the difference in chemical shifts for the different conformations. However, since the splitting pattern of the H-8 protons does not change, it appears that the A ring does not change its conformation. It is not known whether the conformation of the sugar ring changes when it binds to nucleic acids. Although the splitting pattern for the two

2'-protons has not been resolved (ABMX), it is evident from the spectra that the splitting pattern, and hence the conformation, has been altered when the drug interacts with the dinucleotides. Even if the induced chemical shifts have been corrected for the aforementioned effects, there is still one unresolved question--What are the relative positions of the base with respect to each other and to the chromophore? The induced shifts of the drug protons is a combination of the ring current effect from the base above and below the chromophore in an intercalated complex. In double-stranded DNA the position of one base relative to another is accurately known. The entire macromolecule (DNA) restricts the conformational freedom of the bases. In a 1:1 complex between deoxydinucleotide and drug, the nucleic acid bases have a great deal of conformational flexibility which would allow a large number of possible orientations of one base with respect to another. The induced chemical shift of the protons on the drug can be one of several possible combinations of orientations of the two bases. An exact molecular geometry for the drug/nucleic-acid complex becomes difficult to specify. However, a qualitative interpretation (base stacking vs. outside binding) is certainly feasible using the information obtained from the titrations. The magnitude of the induced upfield chemical shift for the aromatic protons can only be interpreted as a stacking complex of the drug chromophore and the bases of the dinucleotide.

The titration curves for the interaction of the different deoxydinucleotides with daunomycin are shown in Figure 3a-3e. Also, the limiting value of the induced chemical shifts of the drug protons (chemical shift of the drug protons in the presence of an excess of nucleic acid minus the chemical shift for the drug monomer) is listed in Table 2. In comparing the relative magnitude and direction of the chemical shifts of the daunomycin protons when the drug interacts with the different dinucleotides, it is apparent that the drug/nucleotide complexes are very similar. In each complex the aromatic protons shift substantially upfield. Of the aromatic protons, the H-1 proton of daunomycin shifts by the largest amount. It was tentatively assigned as being the downfield doublet in the aromatic region by comparing the daunomycin spectrum with that of a substituted anthraquinone (Sadtler No. 182). The methoxy protons also shift upfield but generally not as much as the aromatic protons. All of the protons on the A ring H-7, H-8, and H-10, shift upfield approximately the same amount except for the H-8(ax) proton. The H-4', H-3' and the H-2' protons of the sugar ring (daunosamine) are shifted downfield by small amounts in the presence of the nucleotides. The 5'-methyl protons and the 5'-proton are not shifted by a significant amount. The H-1' proton is shifted upfield approximately 0.01 ppm.

The shape of the titration curves of daunomycin and the different deoxydinucleotides are informative about the stoichiometry and the relative binding constants of these complexes. The stoichiometry is determined by drawing a line tangent to the binding curve at small values of the nucleic-acid/drug ratio and letting it intersect with

Table 2. Induced chemical shifts (ppm) of the daunomycin protons relative to the chemical shift of the drug monomer for each of the five dinucleotide drug complexes. Positive values indicate that the resonances move upfield.

DM Protons	d-pTpA	d-pApT	d-pGpC	d-pCpG	d-pCpC
H2	0.18	0.18	0.25	0.21	0.16
H1	0.45	0.44	0.52	0.45	0.39
H3	0.25	0.24	0.34	0.28	0.23
H1'	0.09	0.09	0.14	0.09	0.09
H7	0.17	0.17	0.14	0.18	0.15
H5'	0.01	0.01	0.03	0.02	0.01
OCH_3	0.16	0.16	0.26	0.20	0.15
H4'	-0.03	-0.02	-0.03	-0.06	-0.04
H3'	-0.05	-0.05	-0.04	-0.05	-0.04
H10(e)	0.15	0.17	0.23	0.17	0.15
H10(a)	0.17	0.20	0.22	0.20	0.17
H8(a)	0.03	0.03	0.05	0.04	0.02
H8(e)	0.21	0.18	0.23	0.18	0.17
H2'	-0.05	-0.05	-0.05	-0.07	-0.04
CH_3	0.01	0.01	0.00	0.00	0.01

the line which represents the limiting value of the induced chemical shift. At this point the approximate stoichiometry can be deduced. In addition, by comparing the slope of the tangent line, relative values of the binding constants can be determined. It should be noted that, at best, the stoichiometry and relative magnitude of the binding constants for these complexes are only approximate.

The analysis of the curves is not straightforward since the chemical shifts at intermediate values of nucleic-acid/drug ratios are influenced by the self-association of the drug and the complex formed. If one focuses attention on the aromatic proton H-1, it is evident that either the binding constants of the drug with the five dinucleotides are different or that the stoichiometry of the complexes varies with the small nucleic acid components. With three of the dinucleotides, d-pApT, d-pGpC, and d-pCpG, the binding curves suggest that the drug/nucleic-acid complex ratio is 1:1. With d-pTpA and d-pCpC the shape of the curves could either be interpreted in one of two ways: first, the complex has a stoichiometry of two dinucleotides to one drug molecule or secondly, the binding constant is weaker for these two small nucleic acid components when compared to the other three. The second explanation seems the more reasonable of the two. If a 2:1 nucleic acid to drug complex was formed, one would expect a larger upfield chemical shift at the limiting values than for a 1:1 complex. However, this is not experimentally observed. The shifts for these two dinucleotides are as small or smaller than the other three. In addition, the deoxydinucleotide d-pCpC, is not self-complementary and would not be expected to form a hydrogen-bonded complex under

these conditions. The evidence, in total, suggests very strongly that the complexes that are being formed in solution are the result of one drug molecule interacting with one nucleic acid component. It is also apparent that daunomycin does not bind to all sequences of the dinucleotides with equal strength.

A qualitative binding constant for the interaction of daunomycin with the dinucleotides can be calculated using the limiting chemical shifts of the drug in the monomer and dimer states and in the drug/nucleotide complex. In addition, the dimerization constant for daunomycin ($\sim 10^3$)[48] is taken into account to determine the magnitude of the binding constant for the daunomycin/small-nucleic acid complex. For example, our NMR results indicate that the binding constant of daunomycin with d-pGpC is approximately 10^3. It must be noted, however, that this is only a rough estimate of the binding constant because of the uncertainty of the different chemical shift values.

It is interesting to compare our values ($\sim 10^3$) for the binding constant of daunomycin with a dinucleotide to the value obtained for the drug binding to double-stranded DNA ($\sim 10^6$)[49] and single-stranded DNA ($\sim 10^5$).[49] The small difference in the binding constant of daunomycin with single- and double-stranded DNA most likely results from the "extra" base stacking of the second nucleic acid strand with the aromatic chromophore of the drug. The large difference in binding constants for the drug with small nucleic acids ($\sim 10^3$) as compared to the nucleic acid macromolecules ($\sim 10^6$ and $\sim 10^5$) is mainly due to the loss of translational and rotational entropy of brining two small molecules (drug+dinucleotide) together as opposed to the binding of one small

molecules (drug) with one macromolecule (high molecular weight DNA).

The loss of translational and rotational entropy is a major factor why daunomycin does not form a 2:1 small-nucleic-acid/drug complex as does ethidium bromide and actinomycin D. With daunomycin the three important components of binding to nucleic acids are located on a single dinucleotide. First, the chromophore can "intercalate" between the two bases of the dinucleotide. Second, the 3'-amino group can bind to the terminal phosphate group and finally, a hydrogen bond can be formed between the 9-hydroxyl group of the A ring of daunomycin with the phosphate group at the intercalation site. A 2:1 dinucleotide/drug complex is not formed because the loss of the entropy (translational and rotational) is energetically more important than any increase in base stacking for the second dinucleotide with the 1:1 daunomycin/dinucleotide complex. For ethidium bromide and actinomycin D, a 2:1 nucleic-acid/drug complex is formed because both small nucleic acid fragments contribute significantly to the drug/dinucleotide binding energy. With daunomycin most of the interaction energy results from the interaction of the drug with a single dinucleotide.

Binding of Daunomycin to Deoxydinucleotide Monophosphates

The chemical shift of the daunomycin protons in the presence of four self-complementary deoxydinucleoside monophosphates (d-GpC, d-CpG, d-Apt, and d-TpA) is given in Table 3. The lack of any significant changes in the chemical shifts, especially the aromatic protons, indicate that a strong complex is not formed between the drug and these nucleic acids. These dinucleosides were used to evaluate the importance of the 3'-amino/phosphate group interaction. Our model suggests that

Table 3. Chemical Shifts (ppm) of daunomycin protons (relative to DSS) for different deoxydinucleoside-monophosphates/drug ratios

DM Protons		d-TpA/DM			d-ApT/DM		
		1.0	2.5	5.0	1.0	2.5	5.0
7.74	H2	7.74	7.75	7.73	7.74	7.73	7.73
7.59	H1	7.57	7.58	7.56	7.57	7.55	7.54
7.45	H3	7.46	7.46	7.45	7.45	7.44	7.44
5.48	H1'	5.49	5.50	5.51	5.50	5.50	5.51
4.86	H7	---	---	---	4.86	---	---
4.29	H5'	---	---	---	---	---	---
4.95	OCH_3	3.95	3.96	3.95	3.95	3.95	3.96
3.82	H4'	3.83	3.83	3.83	3.84	3.83	---
3.67	H3'	---	---	---	3.68	3.69	3.71
2.96	H(10e)	2.96	2.95	2.94	2.94	2.95	2.96
2.74	H(10a)	2.73	2.72	2.74	2.71	2.71	2.72
2.24	H8(a)	2.24	--	---	2.24	2.24	---
2.11	H8(e)	2.13	2.12	2.12	2.10	2.10	2.12
1.97	H2'	1.99	1.99	2.01	1.99	2.00	2.02
1.28	CH_3	1.28	1.28	1.28	1.28	1.28	1.29

Table 3 Continued

DM Protons		d-GpC/DM		d-CpG/DM		
		1.0	5.0	1.0	2.5	4.0
7.74	H2	7.70	7.70	7.70	7.68	7.70
7.59	H1	7.53	7.53	7.55	7.51	7.54
7.45	H3	7.41	7.41	7.42	7.39	7.42
5.48	H1'	5.48	5.49	5.47	5.47	5.48
4.86	H7	---	---	4.84	---	---
4.29	H5'	4.26	---	4.26	4.26	4.26
3.95	OCH_3	3.93	3.94	3.93	3.93	3.93
3.82	H4'	3.83	3.84	3.82	3.82	3.83
3.67	H3'	3.67	---	3.65	3.63	3.64
2.96	H10(e)	2.95	2.95	2.95	2.95	2.95
2.74	H10(a)	2.72	---	2.73	2.72	2.71
2.24	H8(a)	---	---	2.25	2.24	2.23
2.11	H8(e)	2.11	2.09	2.12	2.11	2.10
1.97	H2'	2.00	2.01	1.99	2.00	2.00
1.28	CH_3	1.30	1.28	1.30	1.29	1.28

this interaction would be a key component for stabilizing the daunomycin/deoxydinucleotide complex.

Binding of Daunomycin to High Molecular Weight DNA

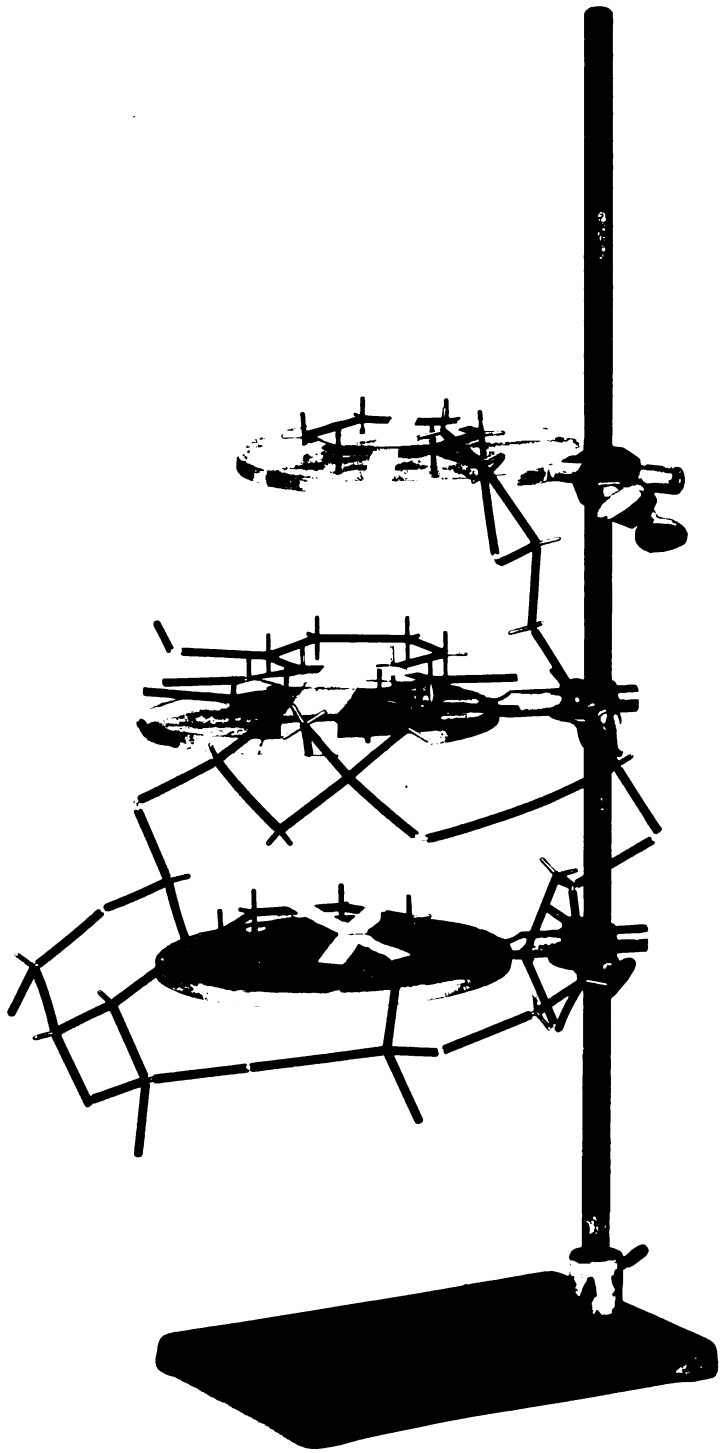
The binding of daunomycin to high molecular weight DNA has been studied using NMR. The results are not very descriptive because of (1) the slow exchange rate of daunomycin between the free and complexed state and (2) the fast relaxation (T_2) with consequent line broadening of the daunomycin protons when the drug is bound to high molecular weight DNA. As DNA is added to a daunomycin solution, the proton signals of the drug begin to disappear without a concomitant broadening of the resonances. At an approximate ratio of six moles of phosphate (DNA) to one mole of daunomycin, the signals of the drug protons have totally disappeared. This is consistent with the drug intercalating between the base pairs of DNA.

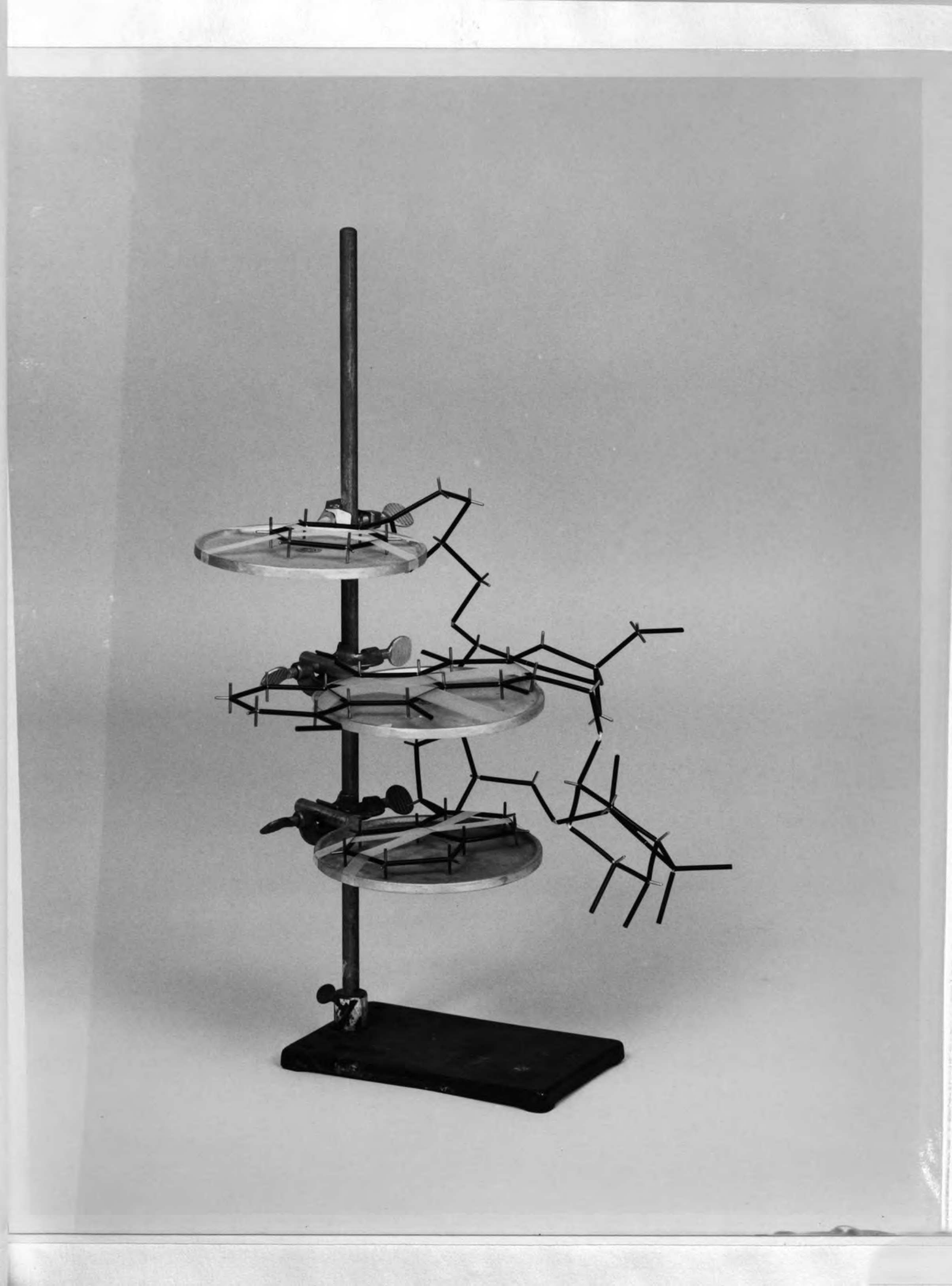
DISCUSSION

The interpretation of the chemical shifts of the daunomycin/dinucleotide complexes and the shape of the titration curves indicate that the drug forms a 1:1 complex with the nucleic acid components. Ethidium bromide and actinomycin D, on the other hand, form 2:1 complexes with deoxydinucleotides under similar conditions.[50,51] The limiting value of the upfield chemical shifts for daunomycin is too small for a 2:1 complex. In addition, the shape of the titration curves indicate the formation of a 1:1 complex. However, the most convincing argument for a 1:1 complex is the induced upfield chemical shift of daunomycin in the presence of an excess of d-pCpC. Although the magnitude of

the shift is not as great as for the other four dinucleotides, this is as expected because the ring current effects for cytosine are much smaller than those of adenine and guanine. The fact that daunomycin can form strong complexes with a single deoxydinucleotide has important biological implications that will be discussed later.

Using the results (chemical shifts) obtained from the daunomycin/nucleic-acid titrations a molecular model is proposed for the drug/receptor complex (Figure 5). The model is consistent with the observed chemical shifts of the (1) aromatic methoxy protons being shifted upfield, (2) small but significant upfield chemical shifts of the protons on the A ring and (3) downfield shifts of some of the daunosamine protons. The aromatic protons and the methoxy protons are shifted upfield by an amount consistent with a stacking complex with the D ring of the chromophore extended away from the bases. The A ring of daunomycin is in a conformation as shown in Figure 4a. This conformation allows the 9-hydroxyl group to hydrogen bond to the phosphate group at the intercalation site. The A ring is not situated under the bases but out to the side next to the sugar-phosphate-sugar backbone that connects the two bases of the deoxydinucleotides. In this region, the protons of the A ring would be expected to be shifted upfield but not by an amount larger than the aromatic protons. The chemical shifts of several of the protons of the sugar ring (H-2', H-3', and H-4') are shifted downfield by small amounts. In the model we propose for the 1:1 complex, a necessary component for the complex is the interaction of the 3'-amino group with the phosphate group adjacent to the intercalation site. This would necessitate that the daunosamine ring be situated close to the plane of the first base



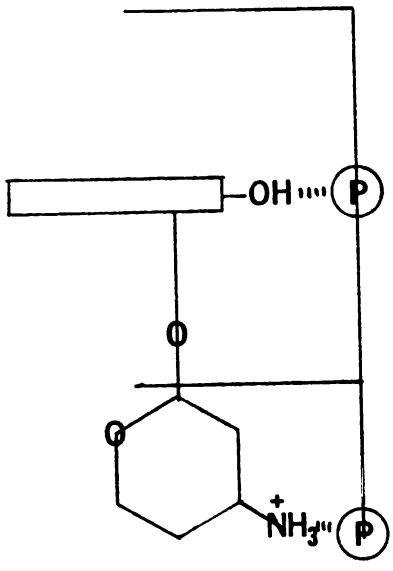


of the dinucleotide. The protons would thus be shifted downfield to some small amount. As was mentioned previously, it is impossible to exactly determine the molecular geometry of the daunomycin/deoxydinucleotide complex. It is possible, however, to present a general model which is consistent with the interaction of daunomycin with all of the different deoxydinucleotides.

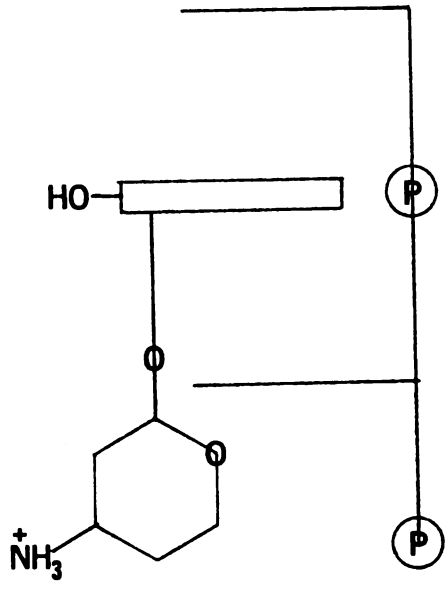
A schematic diagram of the model we propose for the binding of daunomycin to deoxydinucleotides is shown in Figure 6a. Our model is based on two major points (1) a large induced upfield shift of the aromatic protons of daunomycin that is consistent with a stacking complex of the drug and the nucleic acid bases and (2) the interaction of the 3'-amino group with the terminal phosphate group of the dinucleotide. The importance of the second component (3'-amino and phosphate interaction) is demonstrated clearly because daunomycin binds very strongly to dinucleotides and not at all to dinucleoside monophosphates. It will be necessary to include these two facts (base stacking and binding of the 3'-amino group to the terminal phosphate) in any model for the interaction of daunomycin with dinucleotides. If daunomycin approached from the side opposite the sugar-phosphate backbone and formed a complex as indicated in Figure 6b, the upfield chemical shifts of the aromatic protons would be comparable to the ones we observed. However, it is sterically impossible for the 3'-amino group to interact with the terminal phosphate group of the nucleotide. Therefore, this model can be discounted. If a complex such as the one indicated in Figure 6c was formed, it would be likely that daunomycin would also form a complex with dinucleoside monophosphates. However, as indicated earlier, daunomycin does not form a strong complex with dinucleoside monophosphates and

Figure 6

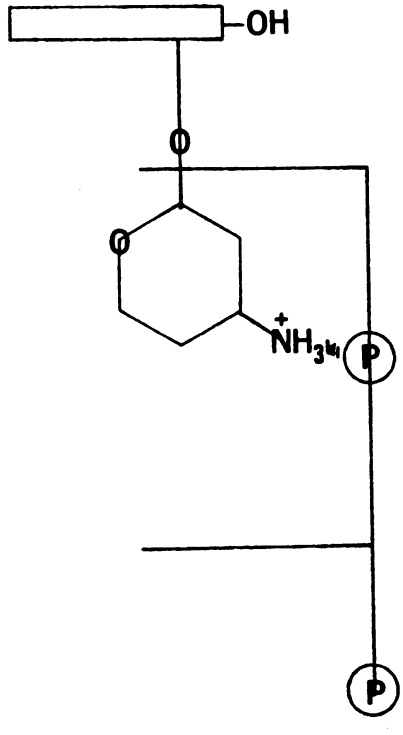
(a)



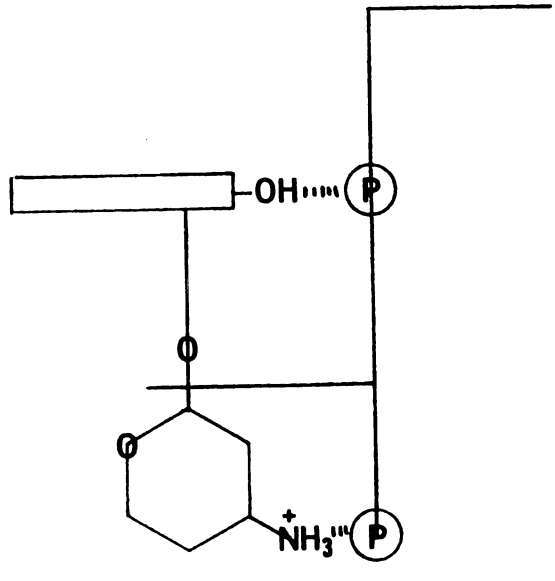
(b)



(c)



(d)



hence this model (6c) can be excluded. The representation in Figure 6d is actually an equilibrium conformation of our proposed model. It can be excluded because of the magnitude of the induced chemical shifts of the aromatic protons of daunomycin. In d-pCpC, for example, the H-1 proton is shifted upfield approximately 0.4 ppm. In our model, each cytosine base would contribute approximately 0.2 ppm to the chemical shift. In Figure 6d, the cytosine base on the bottom would have to contribute all of the upfield chemical shift (0.4 ppm). This would require the H-1 proton be directly above the first cytosine base according to ring current calculations of Giessner-Prettre and Pullman.[45] Sterically, this complex would not be allowed.

The conformation of the A ring of daunomycin, when it binds to nucleic acids, has been the subject of some speculation.[32] Two possible conformations of the A ring are given in Figure 4. Figure 4b shows the A ring conformation which is similar to that found in the crystal structure of N-bromoacetyldaunomycin. The conformation in Figure 4a has been suggested as a possible alternative. This conformation (Figure 4a) has one attractive feature that is not present in the other conformation--it is possible to form a hydrogen bond between the 9-hydroxyl group in the A ring with the phosphate group at the intercalation site. It might be noted that the 9-hydroxyl group of the drug is necessary for activity.[52] The NMR spectra supports the conformation shown in Figure 4a when the drug is complexed to nucleic acids and when it is free in solutions. The benzylic proton (H-7) splits the H-8(eq) proton but not the H-8(ax) proton. In the conformation as shown in Figure 4b, one would expect both of the H-8 protons to be split by a comparable amount. This is

not observed when daunomycin is bound or free. In addition, the fact that the H-8(ax) proton is shifted downfield, when compared to the H-8(eq) proton, supports the conclusion that the conformation in Figure 4a is the one of importance.

In the model proposed by Henry, there are three specific hydrogen bonds formed between the 9-hydroxyl, 3'-amino group, and the 4'-hydroxyl group with the phosphate groups of the DNA helix when daunomycin binds to DNA. In our model of the daunomycin/deoxydinucleotide complex, two of the hydrogen bonds can still be formed involving the 9-hydroxyl and the 3'-amino groups with the phosphates of the dinucleotides. The 4'-hydroxyl group, in our model, points away from the dinucleotides so it should provide little, if any, stabilization for the drug/dinucleotide complex. It is interesting to note that the activity (in vitro) of daunomycin and a derivative in which the position of the 4'-hydroxyl group has changed (equatorial to axial), is very similar.[32]

In the model we propose the hydrogen bond between the 3'-amino group of daunomycin and the terminal phosphate is of primary importance. In recent work by DiMarco,[49] the binding constant of daunomycin to single-stranded DNA was at least ten times smaller when compared to double-stranded DNA. In another report,[53] the concentration of daunomycin needed to inhibit the polymerase (DNA) action of denatured DNA was seven times the amount needed to inhibit DNA polymerase on double-stranded DNA. This suggested that single-stranded DNA, which has phosphate groups that can interact with both the 9-hydroxyl and the 3'-amino group, is not a potential receptor for daunomycin because of the weak complex that is formed. In addition, the hydrophobic effect of which is a key component of the intercalation complex, would be smaller for daunomycin binding

to single-stranded than double-stranded DNA. Our results, however, strongly imply that single-stranded DNA can form strong complexes with daunomycin. A key factor in our experiments is the importance of the terminal phosphate of the dinucleotide. The second pK_a of a terminal phosphate is approximately 7.2, which means at physiological pH (7.4), more than half of the terminal phosphate groups will be doubly ionized (-2). Since the electrostatic interaction is a key component of the total interaction energy, the "extra" binding energy stabilizes the drug/single-stranded complex. Each single-stranded nucleic acid, with a terminal phosphate on the 5'-hydroxyl group, will be a potential binding site for daunomycin. The other binding sites of single-stranded nucleic acids, the internal region, do not have this "extra" electrostatic component to stabilize the interaction between daunomycin and the nucleic acid.

Biological Implications and Further Studies

The proton NMR results suggest that daunomycin can bind very tightly, in a "pseudo-intercalated" complex, to a single deoxydinucleotide. One implication of this is that the receptor for daunomycin in a cell might be the terminal region of a single-stranded nucleic acid which has a phosphate group on the 5'-hydroxyl group of the nucleic acid chain. There are two potential targets in the cell with which daunomycin might bind, and, therefore, exert its action. First, the synthesis of DNA requires a primer (RNA) to initiate the DNA polymerase action.[54] If the primer has a 5'-phosphate group daunomycin could possible bind to the primer and, thus, prevent the polymerase from initiating the synthesis of DNA properly or at all. Since the primer is a small single-stranded RNA chain one of the first experiments would be to study the binding of daunomycin to small fragments such as ribodinucleotides. A second potential receptor for daunomycin is a section of DNA where one of the nucleic acid chains is not continuous. In a cell there are at least two situations where this might occur: (1) discontinuous replication of DNA polymerase and (2) breakage of one of the strands of DNA. In both cases there is probably a 5'-phosphate group on one of the single-stranded chains to which daunomycin can bind. Under normal conditions the DNA enzyme ligase will join the two ends of the nucleic acids and make the strand continuous. The interaction of daunomycin with discontinuous strands of DNA that are based paired to the other strand of DNA can be investigated by the following procedure. First, monitor the interaction of daunomycin with a non-complementary hexanucleoside, e.g. GGGTTT, and then with a trinucleotide, e.g., CCC. Then add the two nucleic acid fragments together with

the drug and determine the strength of interaction using spectroscopic techniques. A possible model for the interaction is given in Figure 7.

The data also indicates that daunomycin can bind to certain base sequences at the terminal end of the nucleic acid chain more strongly than others. In addition, there does not seem to be any influence of Watson-Crick hydrogen bond formation on the drug/receptor complex. More work needs to be done using non-complementary base sequences to determine which of the sixteen two-base combinations of the dinucleotides is the "best" receptor for daunomycin. Techniques, other than NMR, e.g. UV-Visible, would actually be more suitable for these studies.

Experiments should also investigate the binding of daunomycin to nucleic acid chains longer than the dinucleotides. Our experiments have suggested that the terminal phosphate group, with a (-2) charge, is essential for the binding of the drug to the ends of nucleic acids. To check this hypothesis one should study the binding of daunomycin with a trinucleoside diphosphate. A possible complex between the drug and the trinucleoside is shown in Figure 8. If the -2 charge on the terminal phosphate is crucial to the binding of the drug to terminal regions of nucleic acids, this complex should be very weak. If, on the other hand, a strong complex is formed, then our hypothesis regarding the importance of the terminal phosphate will be in doubt.

In our model of the drug binding to a dinucleotide, the 4'-hydroxyl group of daunomycin can not form a hydrogen bond with the nucleic acid. To help elucidate the importance of the 4'-OH group it is necessary to monitor the interaction of the drug with a tetranucleoside

Figure 7

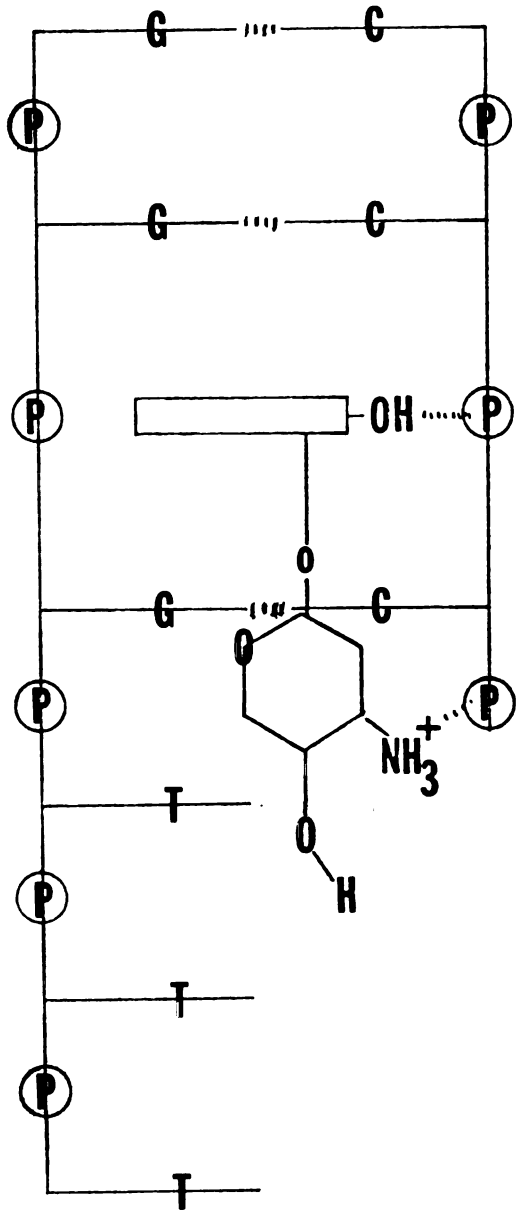
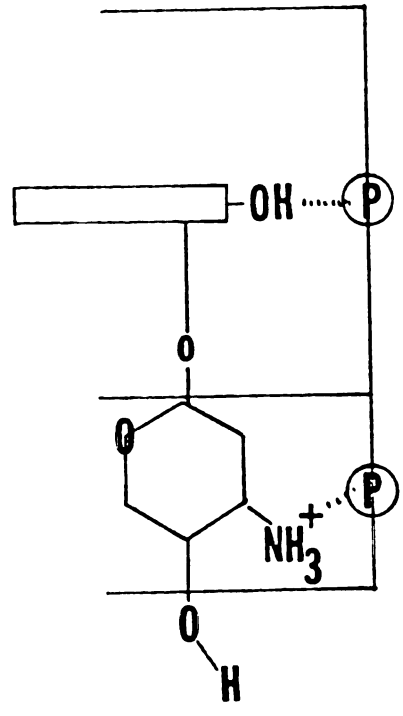


Figure 8

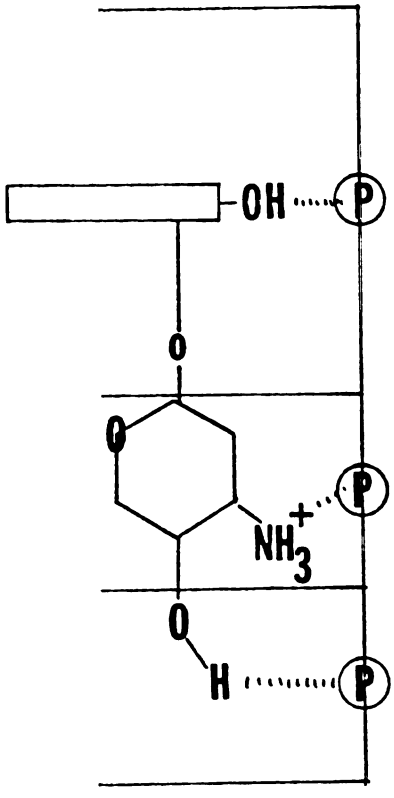


triphosphate. This complex, daunomycin/tetranucleoside, has the components to form all of the hydrogen bonds that Henry[32] has suggested are important when daunomycin binds to DNA. A possible structure for this interaction is shown in Figure 9.

The binding of daunomycin to mononucleotides (5'-phosphate) should also be investigated to determine the importance of the second base of the dinucleotide and the hydrogen bond formed between the 9-OH group of the A ring of daunomycin and phosphate group at the intercalation site. By monitoring the chemical shifts of the aromatic protons of the drug, the interaction of daunomycin and the mononucleotides should help determine the importance of these two components of binding.

Finally, NMR could be used to look "directly" at the hydrogen-bonded protons in the daunomycin/dinucleotide complex. The techniques, e.g., correlation spectroscopy, are now available to monitor hydrogen-bonded resonances in water if the lifetime of the hydrogen-bonded is sufficiently long. It is not clear that one could actually "see" this proton in the drug/receptor complex; however, if the temperature was lowered (even below 0°C using methanol) it might be possible to slow the exchange rate enough to distinguish these proton resonances.

Figure 9



CHAPTER III: THEORETICAL STUDIES

INTRODUCTION

The nature and magnitude of the forces which determine the energy and conformation of small-molecule/macromolecule complexes has been the subject of a great deal of interest in the last few years.[55] Quantum mechanical calculation (ab initio) have been successful for predicting the structure and properties of small non-covalent complexes, e.g. $(H_2O)_2$. For larger non-covalent complexes, ab initio calculations are too costly and semi-empirical molecular orbital methods (CNDO/2 and others) have been unsuccessful.[56] Empirical energy calculations, on the other hand, have been successful in studying intermolecular complexes of reasonable size.[57,58]

In general, empirical potential energy functions contain four terms (assuming fixed bond lengths and angles), (1) electrostatic, (2) dispersion attraction, (3) exchange repulsion and (4) torsional. The electrostatic, dispersion attraction, and exchange repulsion are evaluated on an atom-atom basis. The torsional contributions is a function of the dihedral angle associated with rotation around a specific bond. These terms will be discussed in more detail in the following section. Other interaction components, such as polarization energy, are occasionally included in the energy calculation but they rarely have any large effect on the total energy or conformation of intermolecular complexes.

In a small-molecule/macromolecule complex the four components used to calculate the interaction energy can be separated into intermolecular and intramolecular contributions. The intermolecular terms represent the interaction of the small molecule with the macromolecule. The conformational change of the small molecule itself or the macromolecule is represented by the intramolecular contribution.

Electrostatic. The electrostatic contribution to the total energy results from the interaction of charges on different atoms of the same or different molecules (intra- or intermolecular effects). The form of the electrostatic terms is

$$V = \sum_{i \neq j}\sum \frac{332.0 * q_i * q_j}{\varepsilon r_{ij}}$$

where q_i and q_j are the charges on atoms i and j, ε is the dielectric constant (for vacuum $\varepsilon = 1$; for water $\varepsilon = 80$), and r_{ij} is the distance between atoms i and j. Compared to the exchange repulsion and dispersion attraction components in the potential energy function, the electrostatic dies off much more slowly with increasing distance between two atoms. Therefore at large distances, the electrostatic term will dominate the total energy. It should be noted that the interaction between charges on different atoms can either be attractive or repulsive depending on the sign of the charges.

Exchange Repulsion. This repulsive component in the potential energy function dies off very rapidly as the distance between atoms increase. This interaction is generally represented by either (1) A_{ij}/r_{ij}^{12} or (2) $B_{ij} * e^{-C_{ij}r_{ij}}$. In the first and second terms A_{ij}, B_{ij}, and C_{ij} are parameters that represent the magnitude of the repulsive interaction between the different atom-atom pairs. In our optimization calculations we use the second term because studies on small molecules demonstrate that the repulsive component roughly decreases with an exponential dependence of the atom-atom separation.[59] The repulsive term is based on the functional form of the exchange repulsion between the electron clouds of two atoms as they are brought together. The repulsive

contribution is classified as a short-range interaction.

Dispersion Attraction. This attractive component of the potential energy function arises as an induced dipole-induced dipole attraction between two atoms. This attractive component of the interaction energy is a function of the inverse sixth power of the distance between two atoms and is often referred to as the van der Waals attraction.

Torsional Contribution. If potential energy functions were only to be used in studying interactions between two rigid molecules, the three components of the energy of interaction that we have discussed would be sufficient to calculate reasonable structures and energies. These functions can also be used to study intramolecular effects, i.e., the conformation of a molecule. However, another term must be added to the potential energy function when studying intramolecular effects, i.e., a torsional term to represent the energy for rotation around a chemical bond. When one studies the conformation of a molecule such as ethane, one neglects the change in bond distance (C-H and C-C) and internal bond angles (H-C-C and H-C-H) because changes in their equilibrium value are generally very small. These interactions (bond distance and bond angle) can be classified as 1-2 and 1-3 contributions. If one excluded all 1-2 and 1-3 interactions of ethane and calculated the intramolecular energy of ethane as a function of its dihedral angle using only electrostatic, dispersion and exchange terms, the energy difference between the staggered and eclipsed conformations would be smaller than the experimental value. To reproduce the experimental rotational barrier of ethane, a torsional term is added to the potential energy function. The analytical

form of the torsional potential can be expressed as

$$V(\phi) = \sum_{n=1,3} V(n) \text{ Cos } n\phi$$

where k(n) refers to the weighting factor for each Fourier component of the potential function, n refers to which Fourier component and ϕ is the dihedral angle. In ethane the dihedral angle is defined in terms of four consecutive atoms, ABCD; the sense of rotation is counterclockwise from A to D while looking down the BC bond. The torsional angles are designated as 1-4 interactions.

In our calculations the electrostatic exchange and dispersion contributions are determined for atom-atom interactions that are not defined as 1-2, 1-3 or 1-4 interactions. In ethane, for example, the potential function would only include a torsional term to determine the conformation of the molecule. For larger molecules all terms in the potential energy function, electrostatic, exchange dispersion and torsional, are used in the calculation.

HISTORICAL

Compared to the use of potential functions to calculate the energy and conformation of small peptides, there have been relatively few theoretical studies on the interactions of drugs with nucleic acids. In the previous studies that have been done, there are serious problems with oversimplification of the potential energy functions or complete neglect of some of the interaction components. Jordan[60] in one of the earliest attempts to study the interaction of drugs with nucleic acids, calculated the interaction energy using electrostatic,polarization and dispersion terms. These contributions, however, were on a molecular basis and not on an atom-atom level. The electrostatic and polarization

terms included the dipole-dipole and dipole-induced dipole interaction between the drug and the different bases. The dispersion attraction was calculated using molecular polarizabilities of the drug molecules and the bases. The approximation of using molecular terms is probably not adequate to evaluate the structure and energy of the drug/nucleic acid complexes at the short distances involved.

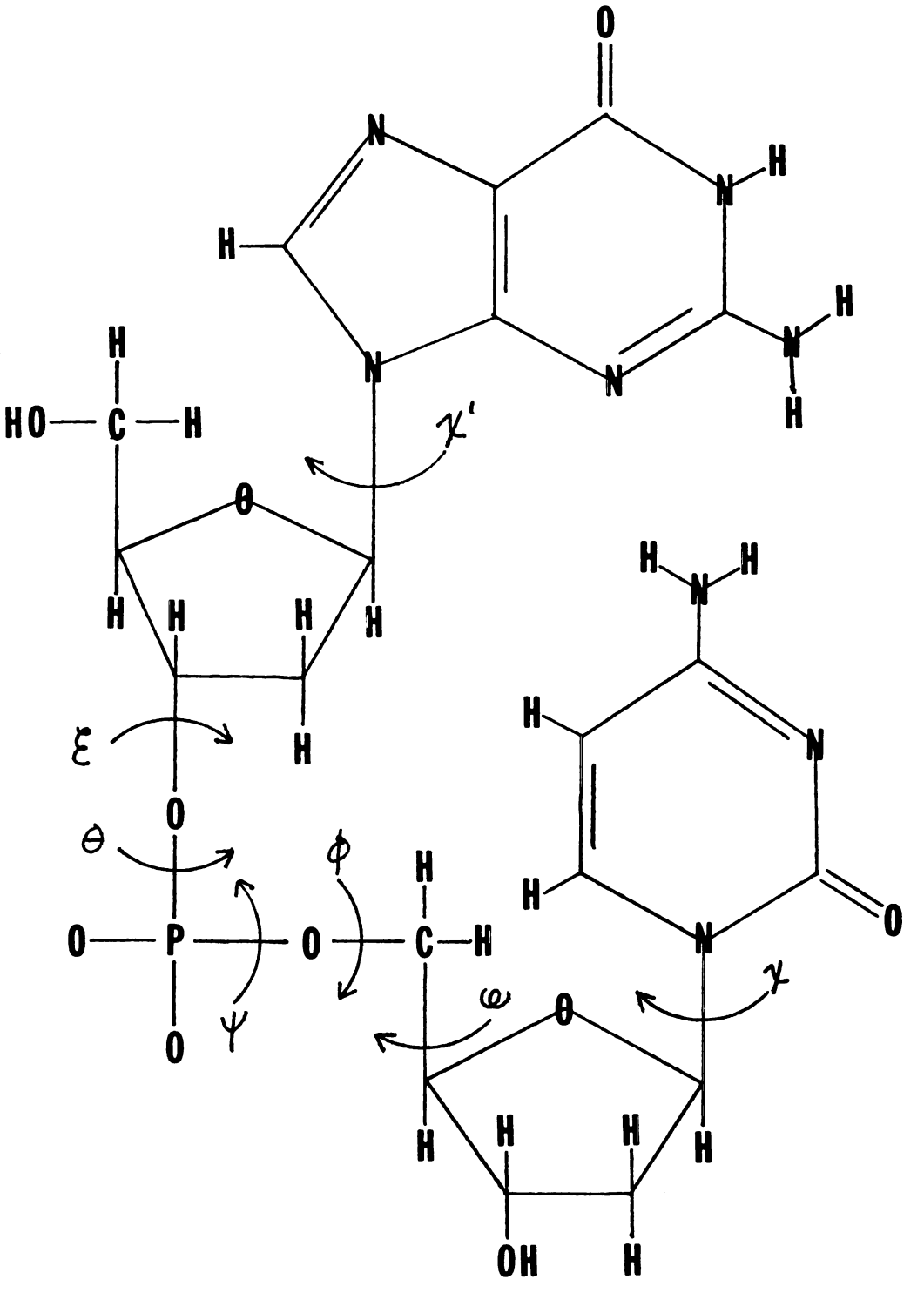
More recently the interaction energy of the ten different base pair combinations of DNA has been determined using atom-atom interactions to calculate both the electrostatic and dispersion terms of the total energy.[61] The pairing interaction (hydrogen bonds) were also included in these calculations. The results indicate that guanosine-cytosine base pairs are generally more stable than are adenine-thymine base pairs. There are, however, two serious omissions in these calculations of the interaction energies. First, no repulsive terms are used in the calculations and secondly, there are no base-backbone interaction terms included in the energy calculation. On a crude level of approximation these terms can be neglected. A more realistic model, however, would include all of the terms in the potential energy function.

Arnott[62] has proposed a model to examine the stereochemical requirements for intercalation of planar drugs into DNA. His model consists of a drug intercalated between a base-paired dinucleotide, tetranucleotide or hexanucleotide. The interaction energy is evaluated using exchange and dispersion atom-atom interactions. He allows the drug/nucleic-acid structure to minimize with respect to (1) stacking interactions between the drug and nucleic acid, (2) nonbonded contacts of the base-backbone and backbone-backbone fragments and (3) the steric strain of the sugar-phosphate chain. In addition, various sugar puckers (C3'-endo and C2'-endo) are tried to determine their contribution to the drug/nucleotide

model. This work is realistic except that (1) Arnott neglects an important component of intermolecular interaction (the electrostatic contribution) and (2) he forces the hydrogen bonds to remain in their Watson-Crick form with pseudo-potential to keep them fixed at the appropriate distance.

In our calculation we propose to evaluate the interaction energy and determine the minimum energy conformation of several drug/dinucleoside-monophosphate complexes using a complete potential energy function. The energy will be calculated using all atom-atom interactions to determine the dispersion attraction, exchange repulsion, electrostatic and torsional contributions to the total interaction energy. The energy of the drug/dinucleoside-monophosphate complex is minimized with respect to 13 variables on each nucleic acid fragment, six of which are used to position each dinucleoside with respect to the intercalator and to the other nucleic acid chain. The other seven variables are torsional angles which determine the conformation of the sugar-phosphate backbone. Figure 10 shows the deoxydinucleoside monophosphate, guanosine (3'-5') cytosine, that was used in these calculations and indicates the seven torsional variables on each nucleic acid chain. Six drug/nucleic-acid structures were optimized with respect to the 26 variables of the complex. Figure 1 in chapter 1 shows the structure of the different drugs that were used for these calculations. Ethidium Bromide, with and without the phenyl and ethyl side chains, was optimized with the N5 atom of the chromophore pointing towards the minor groove (this is the same side of the DNA helix as the N3 atom of guanine). Drug/dinucleoside-monophosphate structures, using proflavine and 10-aminocridine, were also minimized

Figure 10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with the drugs pointing towards the minor groove in one case and the major groove in the other. Finally, the nucleic acid complex of one dinucleoside monophosphate, guanosine(3'-5')cytosine(GpC), base paired with a second dinucleoside (GpC), forms a miniature double helix. This structure is minimized with respect to 20 degrees of freedom (6 translational and rotational and 14 torsional).

Before going further, it would be appropriate to ask the following question--What evidence supports the use of potential energy functions such as the one we employ to determine the structure and properties of intermolecular complexes? There are two papers which focus on the interaction energy in non-covalent complexes.

Caillet and Claverie,[63] using a potential energy function similar to ours, calculated the interaction energy between two molecules to simulate their crystal structure and properties. Their function differed from ours in two ways. First, they used a variable repulsive parameter for a hydrogen atom that could participate in a hydrogen bond. The choice of the repulsive parameter depended on the distance of this hydrogen atom from other atoms. Secondly, they included polarization components in their calculation. In both of these instances there should not be any significant difference between the final structure and energy of a non-covalent complex using their function as compared to ours. Caillet and Claverie used crystal structures of several molecules including methane, carbon dioxide, benzene and nitrobenzene, to evaluate the validity of their potential energy function. They found good agreement between the intermolecular energy of these molecules in a crystal and in gas phase. Also, the minimum energy position for carbon dioxide and nitrobenzene was very similar for the calculated position as compared to the experimentally observed structure.

The work of Hagler and Lifson[64] also supports the concept that potential energy functions of the form we employ can accurately predict the energy and structure of intermolecular complexes. They derived a potential energy function and tested it by minimizing the interaction energy of ten amide crystals. The minimum energy was very close to the experimental sublimation energy of a number of the crystal structures. In addition, the deviation between the experimental and calculated position of the amide molecules in the crystal are very small. Their results suggest that it is not unreasonable to use such potential energy functions to describe the properties and structures of small amides in a crystal complex. Considering the work of Caillet and Claverie and Hagler and Lifson, we feel confident that potential energy functions can be used to study the structure and properties of drug/nucleic-acid complexes.

There are, however, several problems in using empirical potential energy functions to study the structure and properties of intermolecular complexes. The most obvious problem is that "empirical" parameters are being used. The total form of the potential function used in our calculations in given below

$$V = \sum_{i\neq j}\sum \frac{332.0\, q_i q_j}{\varepsilon r_{ij}} - \sum_{i\neq j}\sum \frac{A_{ij}}{r_{ij}^{6}} + \sum_{i\neq j}\sum \frac{B_{ij}}{e^{\alpha_{ij} r_{ij}}}$$

$$+ \sum_{n=1,3} V(n)\mathrm{Cos}\, n\, \phi$$

In this equation the only terms that are known explicitly are the distance between the atoms (r_{ij}) and the dihedral angle (ϕ) used in the calculation of the torsional energy. The other terms, used to

calculate the energy, are normally derived from calculations of crystal structures. In the electrostatic term the charges, q_i and q_j, can be obtained from CNDO/2 (semi-empirical) or ab initio calculations. There is some uncertainty in determining the atomic charges on the individual atoms in a molecule, but the dielectric constant (ε) introduces the greatest source of error in these calculations. For calculations in a gas phase (vacuum), ε is one, while in water the dielectric constant has a value of 80. A reasonable assumption for most calculations is that the dielectric constant is between one and eighty. The problem is extremely complicated because of the inhomogeniety of the medium. In the drug/nucleic-acid complex, for example, part of the molecule will be exposed directly to the solvent (sugar-phosphate backbone), while other parts (the bases and intercalator) will be excluded from water except around the edges. Using a single value for the dielectric constant,is at best, a crude representation of reality.

The A_{ij} and B_{ij} terms that are used for the non-bonded interactions are derived using various algorithms.[65] It is doubtful that any one set of terms is, in general, more accurate than the others. A set of parameters derived using crystal structures of small amides is obviously better (more accurate) for reproducing properties of amides than nucleic acids. It is safe to say, however, that any error introduced in these calculations by nonbonded terms is not of the magnitude of the dielectric constant.

There is also a small error associated with the torsional contribution of the total energy. In general, the torsional parameter is used as one component of a Fourier series to describe the rotational barrier around a chemical bond. Usually only small molecules with the

appropriate symmetry (ethane has three-fold rotational symmetry) can be described by a single term for the torsional contribution. A better representation is a combination of three terms representing a one-, two-, and three-fold rotational barrier. The rotational barrier around the phosphate-oxygen bond in dimethyl phosphate has been determined using a Fourier component analysis.[66] It is evident, even in this small molecule, that there are contributions from a V_1, V_2, and V_3 term which represent the one-, two-, and three-fold torsional barriers.

One very important interaction that is neglected in most theoretical calculations is the effect of the solvent in determining the interaction energy. Solvation of drug, dinucleoside phosphate and the complex is obviously a very important factor in determining the relative binding affinities of drugs interacting with nucleic acids as well as the conformational aspects. A major difference between theoretical calculations and experimental work is that theoretical calculations can investigate a large number or orientations individually while, in solution, the properties of the system under investigation are averages of many possible conformations. Therefore, to determine the structure and properties of water(s) interacting with intermolecular complexes, it is necessary to carry out calculations on several different orientations of the water molecules surrounding the structure. The best approximation would include an infinite number of water molecules around the complex; however, computationally this is not feasible. As modeling of the solvation effect becomes more successful, the properties of the drug/nucleic-acid complex can be calculated more accurately.

Besides the inherent limitations of theoretical calculations in

general, it is important to note what assumptions have been used in modeling the drug/nucleic-acid interaction. It is appropriate, therefore, to point out the remainder of the chapter will be divided into two parts depending on what constraints have been imposed on our drug/nucleic-acid model. The initial work evaluated the interaction energy of the chromophore of actinomycin D and several derivatives with the ten different base pair combinations of DNA. The energy was calculated using only an electrostatic term and neglected the dispersion attraction and exchange repulsion contributions. Neither the drug nor the nucleic acid was given any freedom of movement to minimize the interaction energy. The position of the atoms of the drug and the nucleic acid were taken from Sobell.[67] Clearly, this approach is a crude representation of the drug/nucleic-acid complex.

In the second part the interaction energy of the drug/deoxydinucleoside-monophosphate is minimized with respect to 26 variables, 12 variables which allows each small nucleic acid component to move with respect to each other and to the drug, and 14 internal (torsional) variables, 7 for each small nucleic acid. The seven torsional variables were shown in Figure 10. These are considered to be the "most important" torsional variables of the nucleic acid.

Two important considerations were made regarding these calculations. First, the sugar pucker in the rings was not allowed to change and secondly, the "strain" of intercalation was centered in the sugar-phosphate backbone at the site of intercalation and the base pairs above and below the intercalator. By using these constraints, the energy and structure of the drug/small-nucleic-acid complex can not

be compared directly to drug binding to high molecular weight DNA. It is unrealistic to think that all of the distortion of DNA, as a result of intercalation, is confined to such a small part of the nucleic acid chain. Another problem is incurred by not letting the sugar rings of the nucleic acid fragment "repucker" as the torsional angles change. Recent calculations[68] have demonstrated that there is a great deal of flexibility (conformational freedom) in the sugar rings. This suggests that one should consider "repuckering" the sugar rings as the torsional angles are optimized.

COMPUTATIONAL DETAILS

Energy Calculations and Parameters

Most of the calculations were carried out on the CDC 7600 computer at the Lawrence Berkley Laboratory. A few computations were done on the IBM 370/145 at the UCSF computer center.

All of the atomic charges of the different drug molecules were calculated using the CNDO/2[69] method. On the fragments of DNA (bases, sugar ring and phosphate group) STO-3G calculations[70] were carried out to determine the Mulliken charges. Because such charges are relatively insensitive to hydrogen bonding and conformational changes, the same set of charges were used for different base pair combinations. Since the calculations were carried out on particular fragments and then hooked together, there were small edge effects which were "smoothed out" so the net charge of a deoxydinucleoside monophosphate was (-1). Most of the electrostatic potential calculations included a (+1) charge to represent a sodium ion bifurcating the  group at R (Na-O) = 1.98 Å.

In the drug/nucleic-acid interaction, the interaction energy is calculated as the sum of five components (1) drug/nucleic-acid, (2) nucleic-acid/nucleic-acid, (3) torsional components, (4) 1-5 interactions of each dinucleoside monophosphate and (5) sodium/nucleic-acid and sodium/drug interaction. Preliminary calculations have shown that the interaction of the sodium ion with the complex does not have a large effect in the total energy or gradients, so it has been calculated by evaluating all of the atom-atom interactions (non-bonded contributions) as a sum of dispersion, exchange repulsion and electrostatic terms. In calculating the drug/nucleic-acid contribution, for example, the interaction of the first atom of the drug with each atom of both nucleic acid components is calculated; then, the interaction of the second atom of the drug with the nucleosides is calculated and so on, until, finally, the energy is calculated for all the atoms of the drug interacting with all of the atoms of the nucleic acid components. The energy of each individual atom-atom interaction is then summed as a total of three (dispersion attraction, exchange repulsion and electrostatic) terms. The energy is calculated in basically the same way for interactions (2) and (4).

The torsional term (3), as has been mentioned earlier, is not a nonbonded interaction and, therefore, is calculated by a different method than the other four components. The energy is a function of the dihedral angle. With each of the seven torsional angles (Figure 10) that are variables (in each dinucleoside monophosphate), the torsional

energy is evaluated for each angle and then summed for the total contribution. The torsional potential is represented by the equation

$$V = \sum_{n=1,3} V(n) \, \mathrm{Cos}(n) \, \phi$$

where V(n) is a weighting factor, n represents a one-, two-, or three-fold rotational barrier and ϕ represents the dihedral angle. In the dinucleoside monophosphates, there are four types of torsional barriers (1) C-N, (2) C-O, (3) C-C, and (4) O-P. The parameters V(n) for each of the rotational barriers is given in Table 4.

With empirical potential energy calculations, as one might expect, the energy is dependent on the values of the parameters. The form of the equation that we used to calculate the non-bonded terms (excluding the electrostatic term) is

$$V = -\sum_{i \neq j}\sum \frac{A_{ij}}{r_{ij}} + \sum_{i \neq j}\sum \frac{B_{ij}}{e^{C_{ij} r_{ij}}}$$

In our calcultions the values of A_{ij}, B_{ij}, and C_{ij} are taken from the Levit-Warshel program.[71]

Most of the values of the parameters used in the calculations are consistent with previous values.[65] There are, however, two significant differences which should be noted. First, the rotational barrier around the P-O bond is a combination of a two- and three-fold rotational barrier. This type of potential gives a better representation of the torsional freedom around the P-O bond in dimethyl phosphate than a single three-fold barrier as has been suggested by several sources.[72,73] Secondly, the hydrogen bond interaction, which is important in nucleic

Table 4. Different weighting factors (kcal) for the different torsional barriers.

Bond	V(1)	V(2)	V(3)
C-N	0.0	0.9	0.0
C-O	0.0	0.0	1.21
C-C	0.0	0.0	1.16
O-P	0.0	1.5	1.5

acid interactions, is treated in a somewhat unusual way.[57] All of the hydrogen atoms, that can participate in a hydrogen bond (OH and NH), are assumed to have a negligible radius. This, in effect, minimizes the importance of the attractive and repulsive contributions for the hydrogen atom with any other atom in the calculation. Therefore, the attractive (A_{ij}) and repulsive (B_{ij}) parameters for a hydrogen atom that can participate in a hydrogen bond, are set to zero. The electrostatic term is still included in the calculation and, as such, represents the contribution to the hydrogen bond interaction.

Choice of Geometry

All of the calculations, either the electrostatic or potential energy, use the same drug/nucleic-acid model: a drug is placed between two base-paired deoxydinucleoside monophosphates to form a miniature intercalation complex. A schematic diagram of this structure is shown below

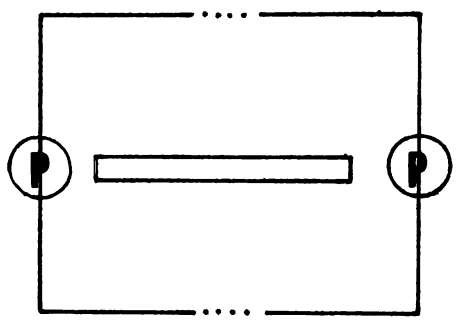
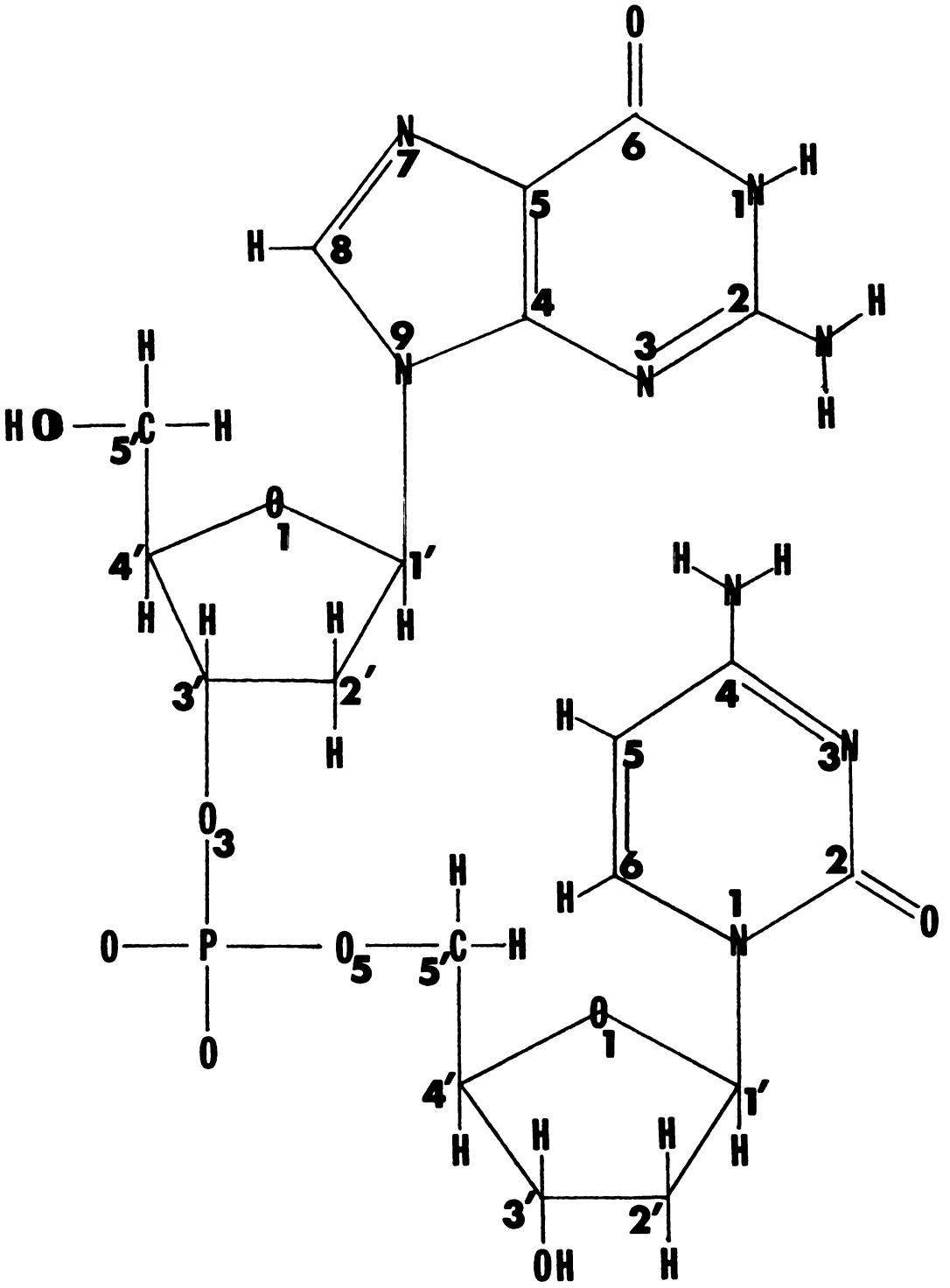


Figure 11 gives the standard numbering system for the nucleic acid bases and sugar-phosphate backbone of the deoxydinucleoside monophosphate that was used in all of the calculations, guanosine(3'-5')cytosine (GpC). In the potential energy calculations the atoms of the nucleic acid fragment (GpC) are numbered continuously beginning with atom N1 of

Figure 11


O
N
7
6
H
N
1
H
5
H
8
4
2
H
9
3
N
N
H
H
HO
C
H
5′
O
1
4′
1′
H
H
H
H
H
H
3′
2′
H
H
O
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H
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4
H
5
3
N
6
2
H
O
1
O
P
O
5
C
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5′
N
O
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4′
1′
H
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H
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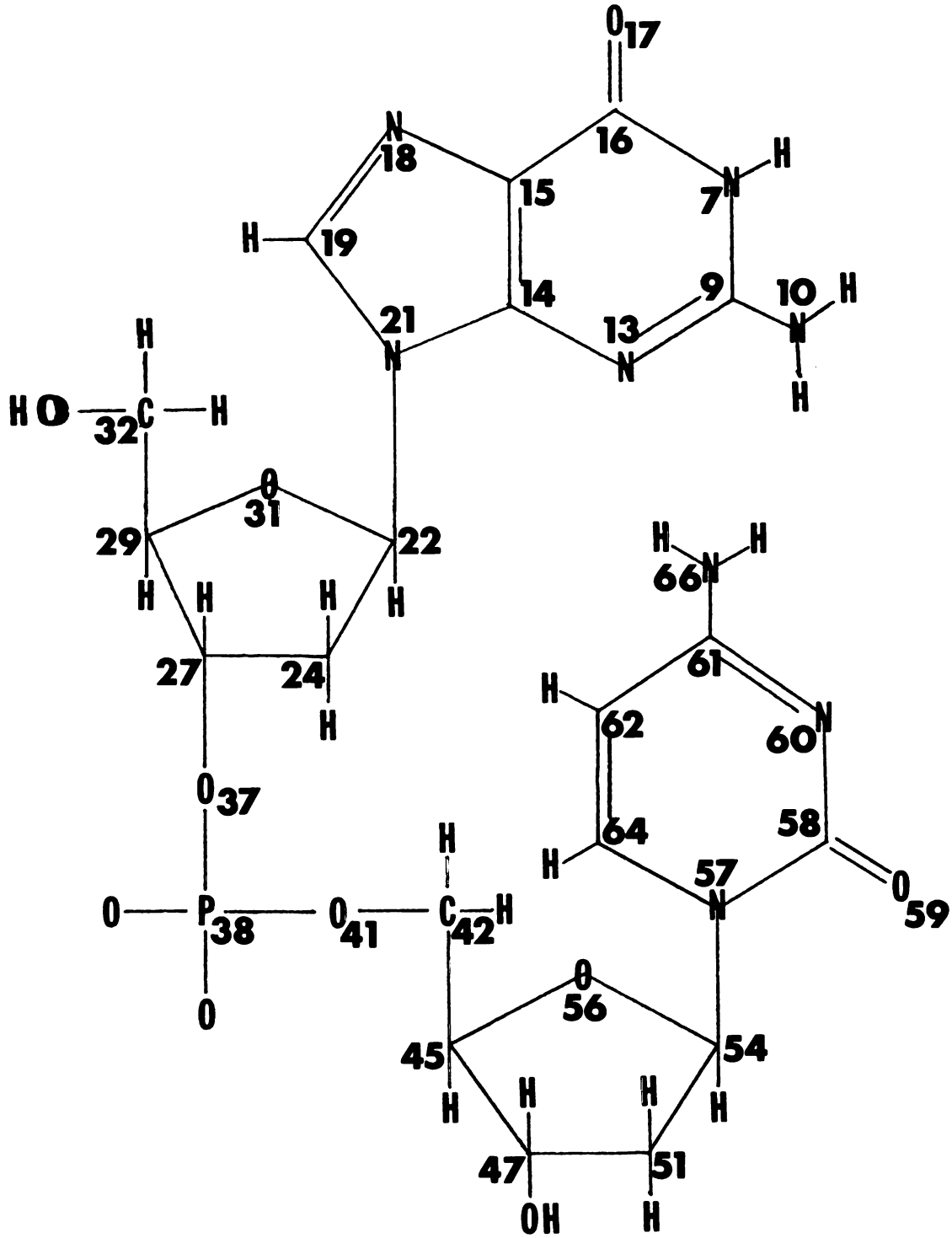
3′
2′
OH
H

Figure 12


O 17
N 18
16
H
N 7
H
15
H 19
14
9
10 H
21
13
N
N
N
H
H
HO
C
H
32
O 31
29
22
H
H
H
H
27
24
H
H
66 N
H
H
61
H
62
60 N
O 37
64
58
H
H
57
N
O 59
H
O
P 38
O 41
C 42
H
O
O 56
45
54
H
H
H
H
47
51
OH
H

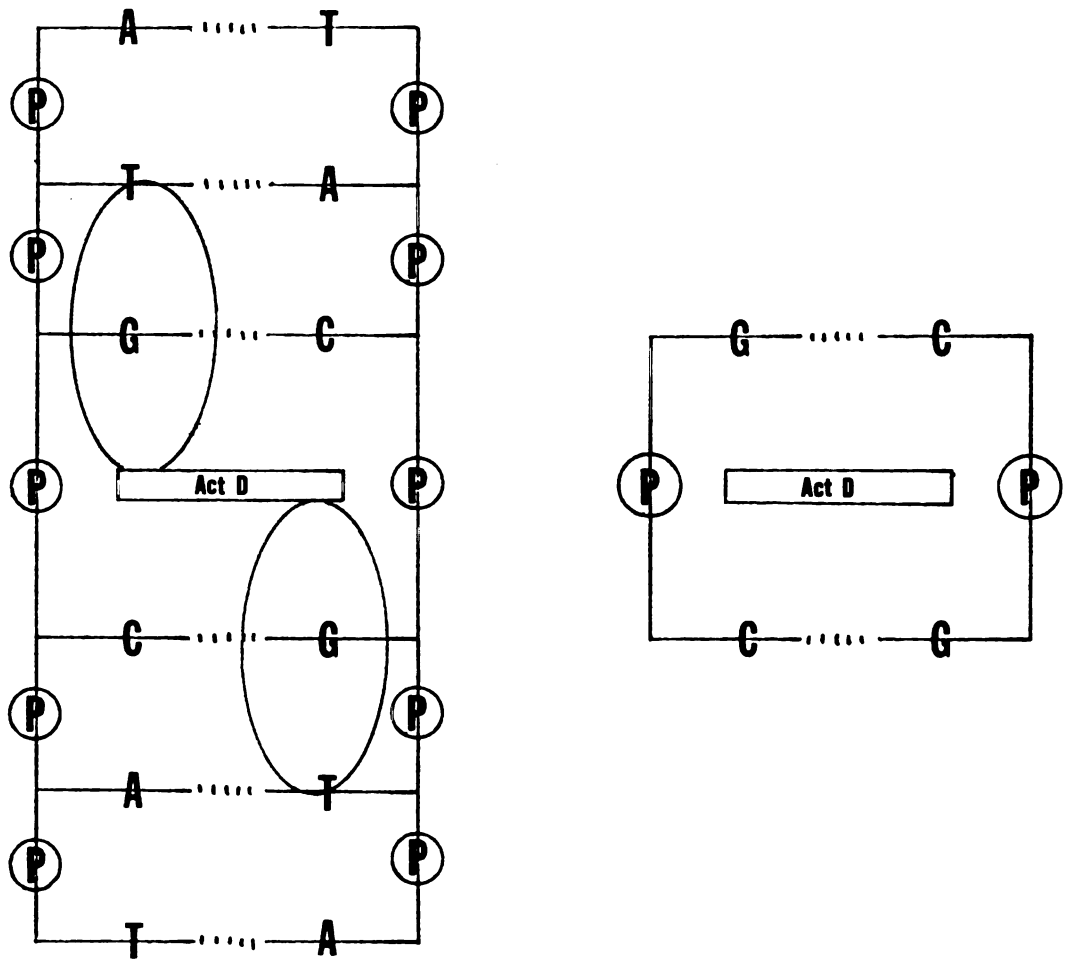
guanine, which is labelled as number 7, and ending with the hydrogens on the N4 atoms of cytosine (numbers 67 + 68). Figure 12 shows the dinucleoside (GpC) with the numbering scheme we employ in these calculations. Since the two dinucleoside monophosphates used in the potential energy calculations are identical (GpC), the numbering sequence of both fragments is identical except that the corresponding atom numbers of the second dinucleoside are 62 larger than the atoms of the first nucleic acid. For example, the N1 atom of guanine of the first fragment is number 7. The corresponding atom of the other dinucleoside, also the N1 atom of guanine, is designated as atom number 69 (7+62).

(A) Actinomycin D-Deoxydinucleoside Monophosphate

The relative positions (atomic coordinates) of the actinomycin chromophore and the nucleic acids were based on a model presented by Sobell.[67] He proposed a detailed molecular model for the interaction of actinomycin D with a self-complementary hexanucleotide (ApTpGpCpApT). In our model we are only interested with the two dinucleoside monophosphates that "surround" the intercalator. The pentapeptide side chains of actinomycin have not been included in the calculations. A schematic diagram (Figure 13) illustrates the model as proposed by Sobell and the model we have used. In Figure 13 the viewer is looking at the DNA chains from the major groove.

When an actinomycin derivative is used the coordinates of the drug are the same except for the atoms to be changed. For example, if the derivative has a hydrogen atom in the 2 position on the actinomycin chromophore instead of the naturally occurring amine, standard bond

Figure 13



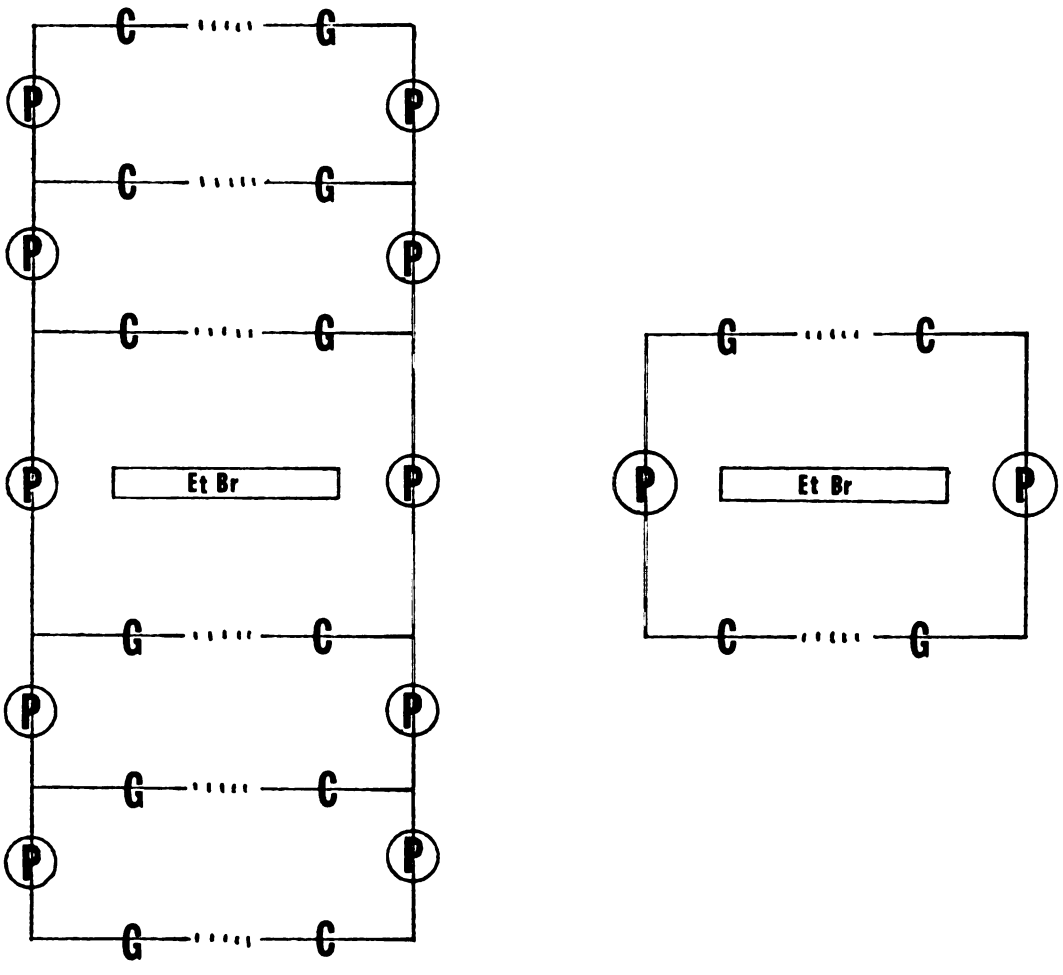
lengths and bond angles (internal and dihedral) are used to specify the coordinates of the hydrogen atoms. Defining the position of one of the ten different base pair combinations depends on the specific base pair sequence. Substituting adenine for guanine or thymine for cytosine was relatively straightforward. The basic ring structure for both the purines and pyrimidines does not change. In changing guanine to adenine, the 2'-amino group was replaced with a hydrogen, the H-1 proton was removed, and the C-6 oxygen was replaced with an amino group. Changes from cytosine to uracil were done in a similar fashion.

To change from a pyrimidine-purine base pair combination to a purine-pyrimidine base sequence required building the base structure starting with one of the sugar rings on the nucleic acid backbone. The internal structure of the Watson-Crick base pairs was not altered. The internal angles (C1'-N9-C4 for purine and C1'-N1-C2 for pyrimidine) were varied within acceptable limits so that the base sequences would fit properly between the sugar-phosphate backbones. The sugar-phosphate backbone coordinates were not changed from the Sobell model.

(B) Ethidium Bromide-Guanidyl(3'-5')Cytosine

The starting geometry (atomic coordinates) for the calculations in which the energy of the drug/nucleic-acid complex is minimized, is based on a model presented by Sobell[74] in which ethidium bromide is intercalated between the two middle base pairs of a self-complementary hexanucleotide. A schematic diagram of Sobell's model of this interaction is given in Figure 14. In our model we are only interested in the base pairs directly above and below the intercalator and the sugar-phosphate backbone of DNA which connects the bases of each strand. Our model of

Figure 14



the drug interacting with the small nucleic acids is shown in Figure 14. It might be noted that there are two differences between the model of Sobell's and the one we use in our calculations. First, the most obvious difference is the length of the nucleic acid. His model is based on the drug interacting with two hexanucleotides and ours focuses on the interaction of the drug with two dinucleosides. The smaller size was chosen because (1) the number of variables associated with a drug interacting with two hexanucleotides makes the calculations too costly and (2) drugs do form complexes with small nucleic acids components in crystals and in solution. Secondly, in our model the dinucleoside sequence is deoxyguanosine(3'-5')cytosine while the Sobell model has the sequence of the bases reversed, deoxycytosine (3'-5')guanine.

In the minimization calculations, the x, y, and z coordinates of the nucleic acid are not read into the program. The coordinates of each atom are generated using a distance (r), an internal angle (θ), and a dihedral angle (ϕ) which relates the position of one atom with the x, y, and z coordinates of three previously defined atoms. For instance, the N-1 atom of guanine, the first atom of the dinucleoside monophosphate that is defined, is specified by three terms, a distance (1.0), an internal angle (120.0) and a dihedral angle (0.0) which relate it to the x, y, and z coordinates of three reference atoms. The reference atoms are only used to determine the position of the nucleosides with respect to each other and to the drug. They are not included in the energy calculations. The x, y and z coordinates of the N-1 atom of guanine can now be defined with relation to the three "reference" atoms using the appropriate r, θ , and ϕ values. The second atom of the nucleic acid (H-1) is then

defined in relation to any three previous atoms using the three terms r, θ, and ϕ that are specific for the H-1 atom. The rest of the atoms of the dinucleoside are defined in a similar manner.

In the Sobell model the position of the hydrogen atoms have not been specified. Their atomic coordinates were defined using normal hydrogen bond lengths, and internal angles, and appropriate dihedral angle. For example, to position a hydrogen atom at the end of a bond where two dihedral angles have already been defined by non-hydrogen atoms, the hydrogen atom is placed 1.09 Å away from the atom to which it is bonded (assuming it is an aliphatic carbon) at an angle of 109.5° with a dihedral angle that bisects the other two dihedral angles. For placement of other hydrogen atoms the same type of procedure was used.

The atomic coordinates of ethidium bromide, when it is intercalated between the base-paired small nucleic acid components, were also obtained from Sobell.[74] The coordinates for ethidium bromide, without the ethyl and phenyl side chain, are the same as for the complete ethidium molecule except that hydrogen atoms have replaced the side chains. Proflavine and 10-aminoacridine were positioned by placing them parallel and on top of the ethidium chromophore. In one case the amines of proflavine point towards the minor groove and in the other model they point towards the major groove. An analagous situations occurs with the direction of the amine group of 10-aminoacridine. In the nomenclature that we use regarding the position of the drug in relation to the minor and major groove, the designation is based on the C-10 atom of the acridine chromophore. For example, if the C-10 atom of proflavine points toward the major groove, proflavine is designated as pointing towards the major groove. All of the x, y, and z coordinates of the different

atoms of the different drugs and their charges are listed in Appendix B.

(C) BDNA

The starting geometry (atomic coordinates) of two self-complementary deoxydinucleoside monophosphates that are base-paired in the B-DNA conformation were obtained from the values given by Arnott.[75] The hydrogen atoms of the nucleic acid were not specified by Arnott. They were placed in the appropriate position by an algorithm that has been previously described.

Minimization Procedure

The Fletcher-Powell algorithm[76] was used to minimize the energy of the drug/small-nucleic-acid complex. This program is very efficient when accurate gradients of the variables can easily be obtained. The gradients that the program used are calculated directly in the program in the following way. After calculating the energy of the complex (drug/nucleic-acid) with a set of values of the 26 variables used in the program, each of the variables is changed one at a time and the energy is then calculated. The change in energy of the structure, for each of the variables, is then divided by the small increment used to change the value of the variable. This determines the value of the gradient, amplitude and direction, for each of the 26 variables. Using the gradients, the values of the variables are changed (using the Fletcher-Powell program) in such a way as to minimize the energy of the function. The energy and gradients of the new calculation are then compared to the previous calculation. This procedure is repeated until a change in the variables does not result in any large "deviation" in energy or structure of

the complex. Specifically, the structure is assumed to be optimized when the change in energy from one iteration to the next is 0.1 kcal or less. We have found the gradients are generally very small, < 1 kcal/Å or < 0.1 kcal/°, and the values of the variables are not changing significantly.

RESULTS AND DISCUSSION

(A) Electrostatic Calculations

We first studied the interaction of uracil and cytosine with the center of the molecules 3.4 Å apart and the planes of the molecules parallel (figure 15). We then rotated the uracil with respect to the cytosine and evaluated the energy with the CNDO/2 method, with the partial charges derived from CNDO/2 and STO-3G *ab initio* and with the *ab initio* partial charges plus exchange and repulsion terms. The results of these calculations are presented in figure 16 and clearly demonstrate that: (1) the relative energies are dominated by the electrostatic term and (2) the CNDO/2 electrostatic charges do a good job mimicking the minimal basis *ab initio* charges. Dispersion attraction does not change the directionality of the interaction. In all of the calculations, the minimum energy occurred at θ approximately 135°.

The magnitude of the dispersion attraction for these planar "stacking" interactions is substantial, however. If one relaxes the constraint of forcing the center of the two molecules to be on top of each other, we now have three degrees of freedom to vary: the position of the center of the uracil molecule (2 variables X and Y) and the angle it makes (1 variable - θ). The minimum energy in such a surface (CNDO/2 electrostatic plus Lennard-Jones) is at X = 1.0, Y = 0.0, θ = 0°, and is illustrated in figure 17. Both the electrostatic energy and dispersion attraction are substantially attractive at this point. The minimum energy predicted from varying the electrostatic energy alone is X = 2.50, Y = 0.50, and θ = 90° (see figure 18).

Figure 15

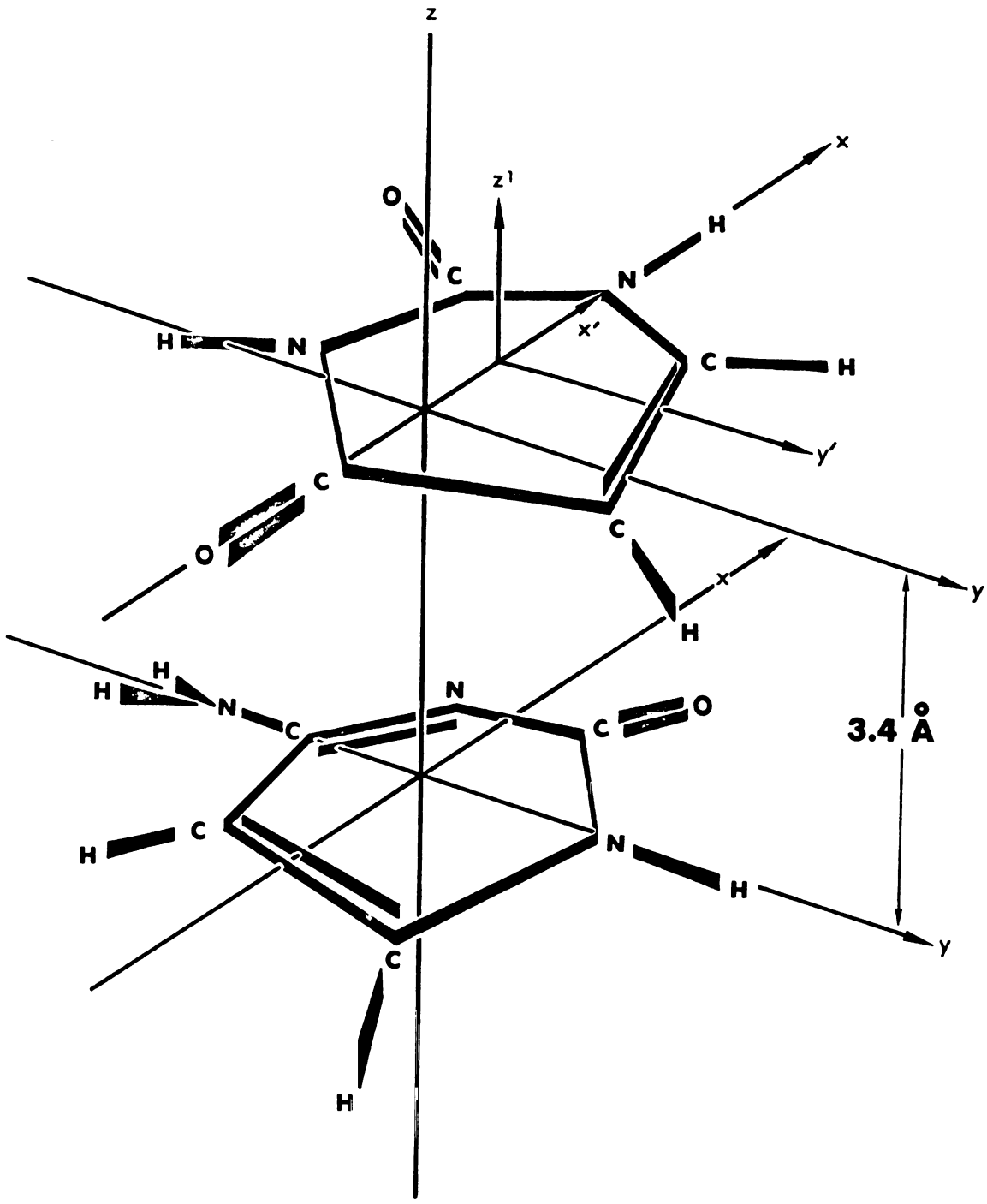


Figure 16

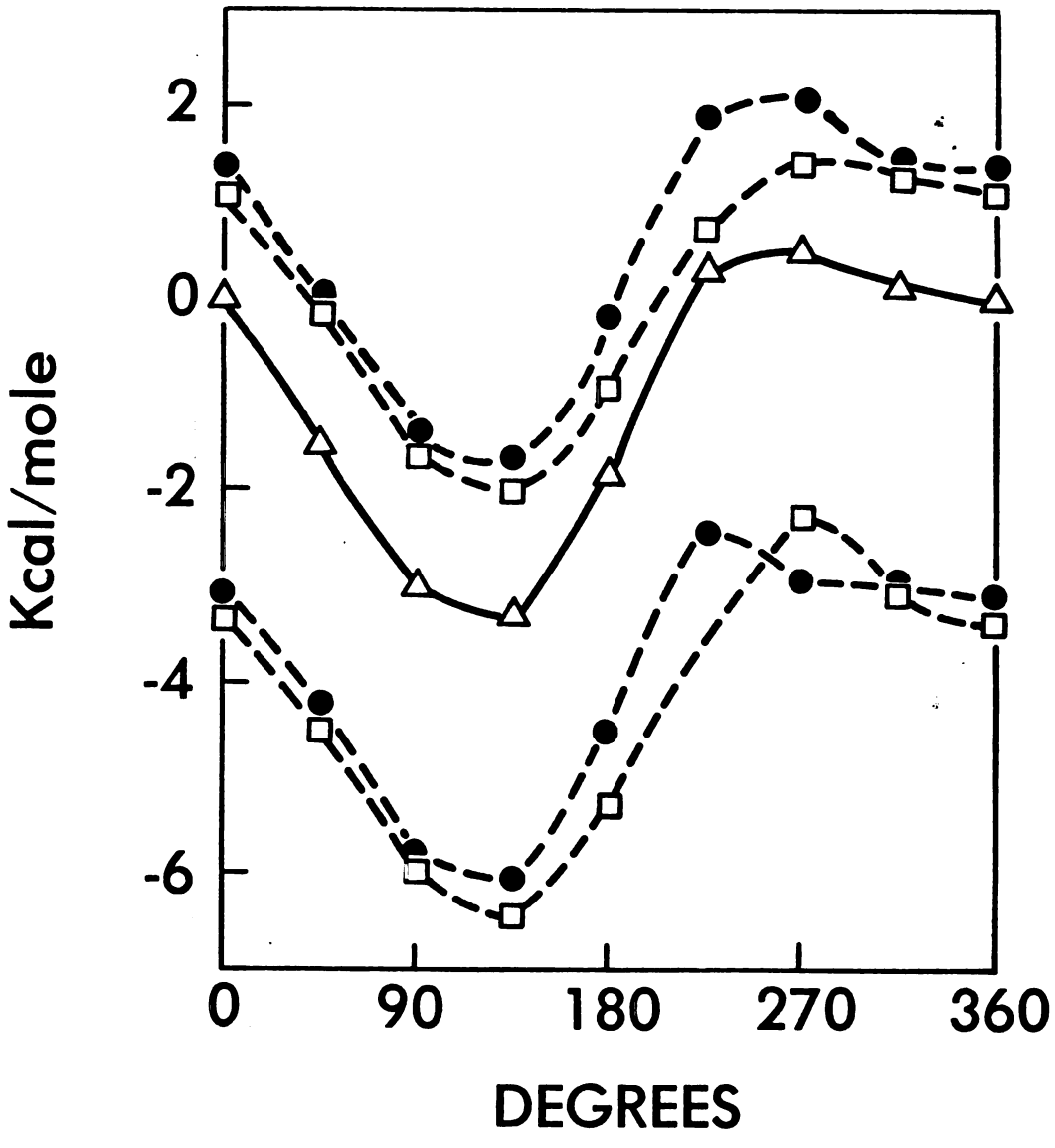


Figure 17

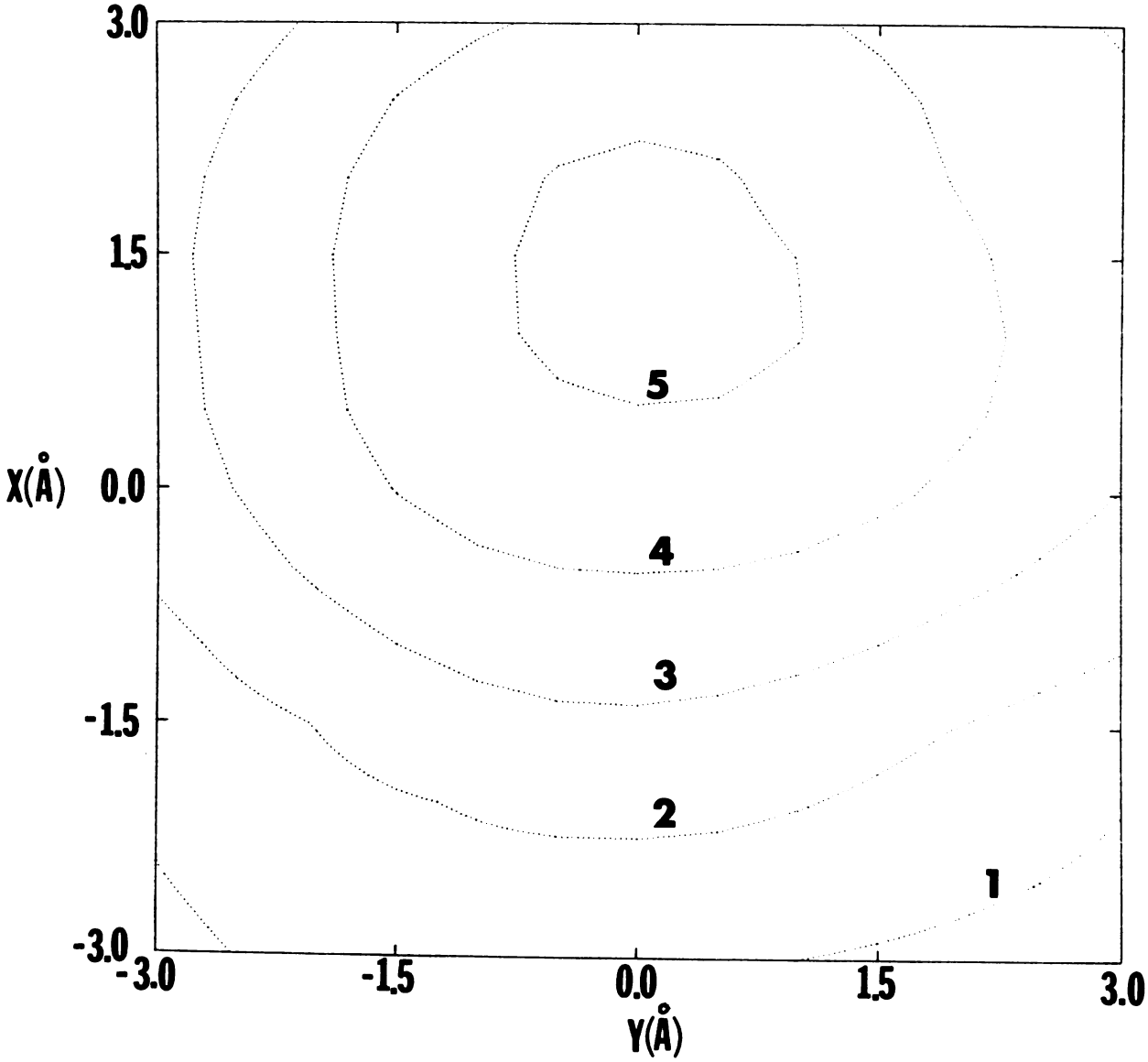


Figure 18

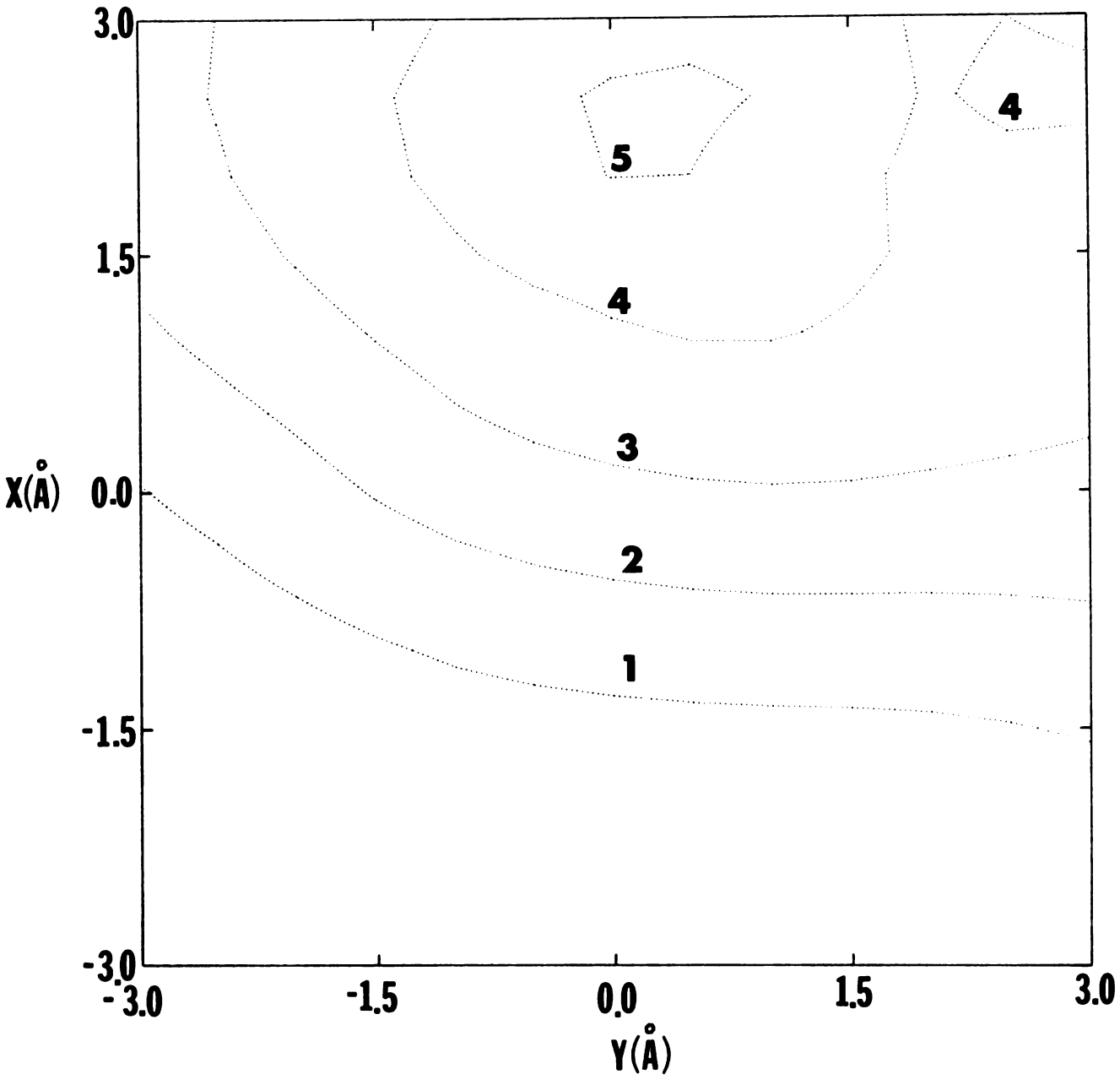


Figure 19a

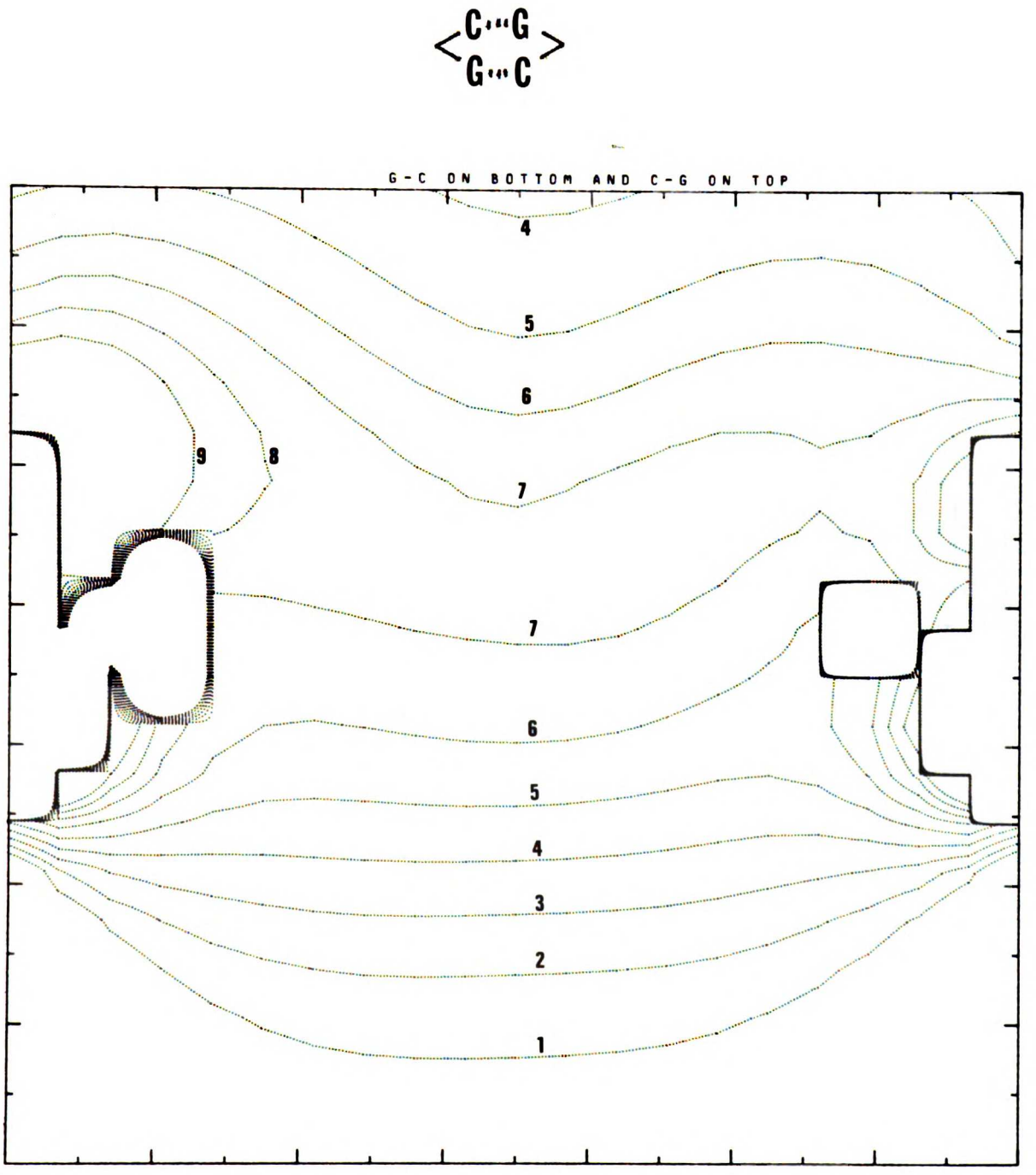


Figure 19b

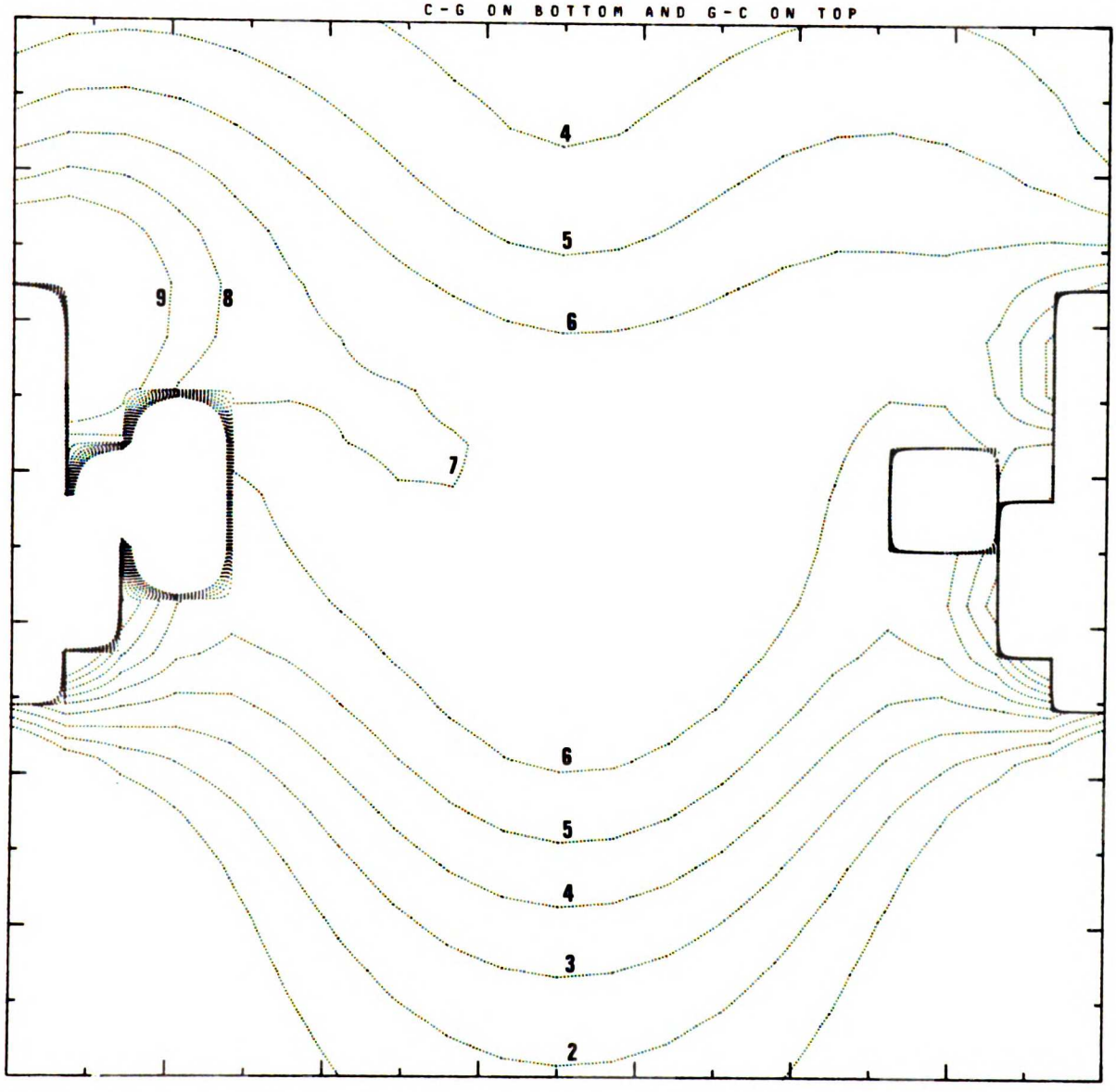
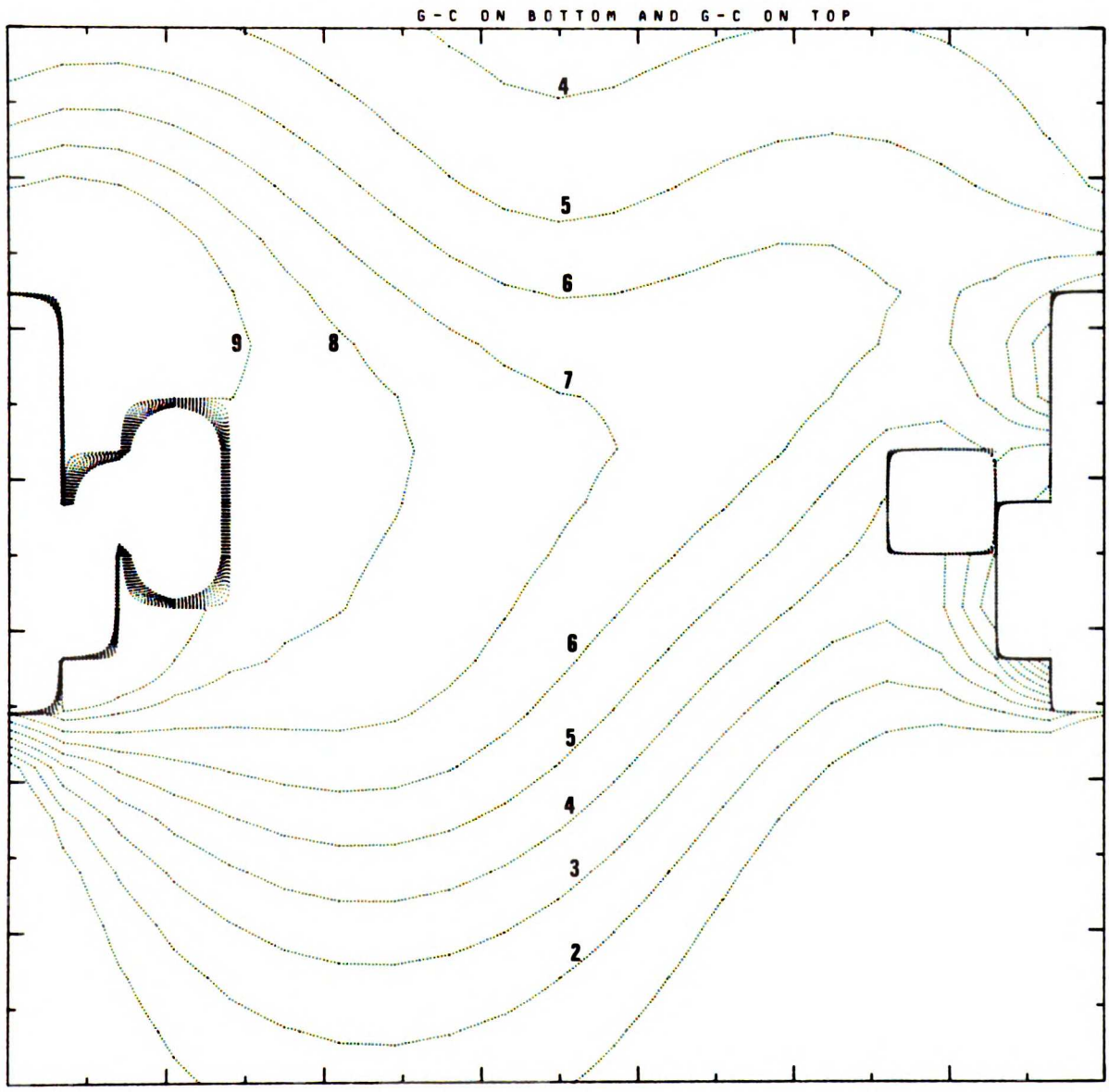


Figure 19c




T···A
A···T

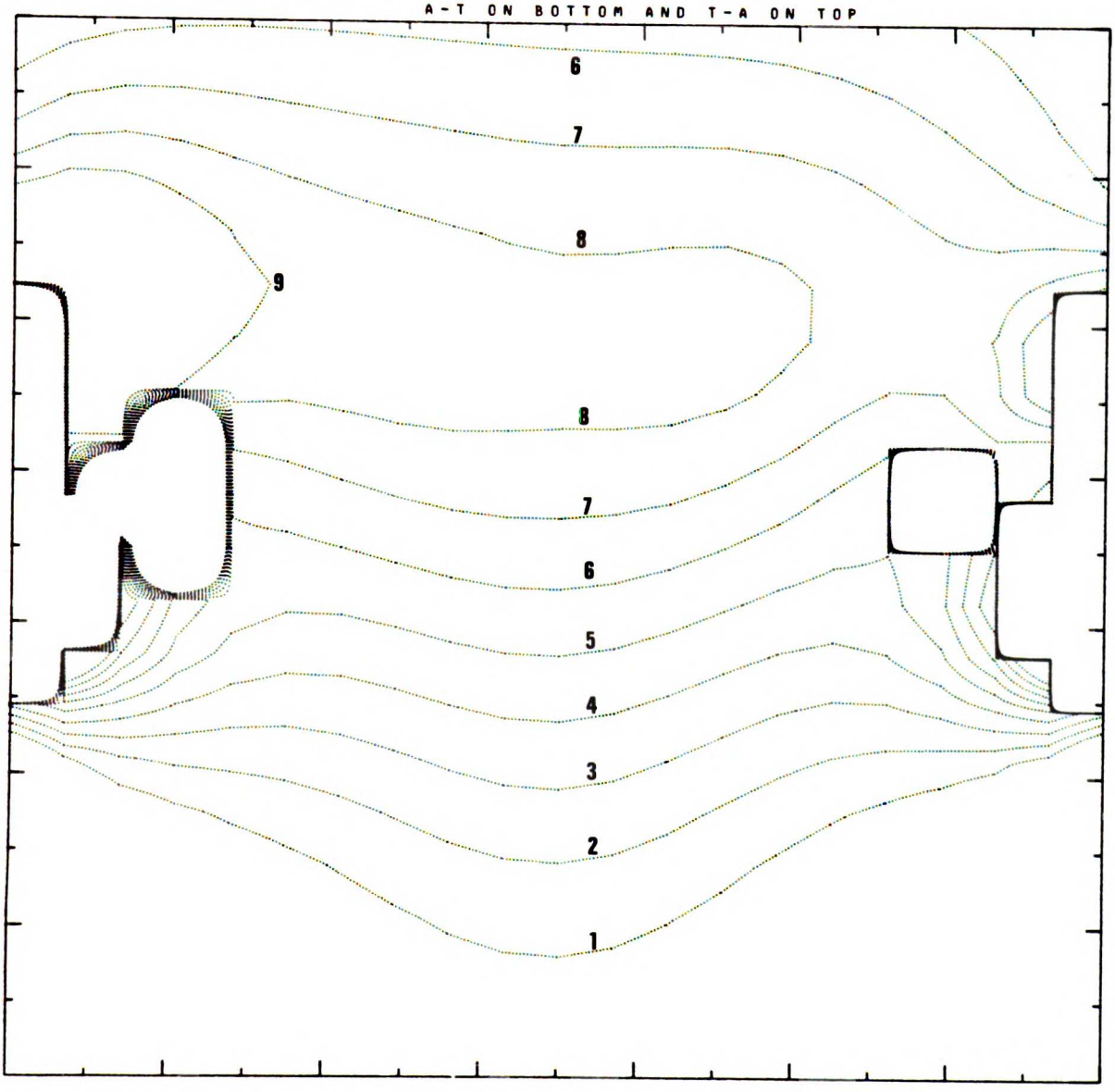
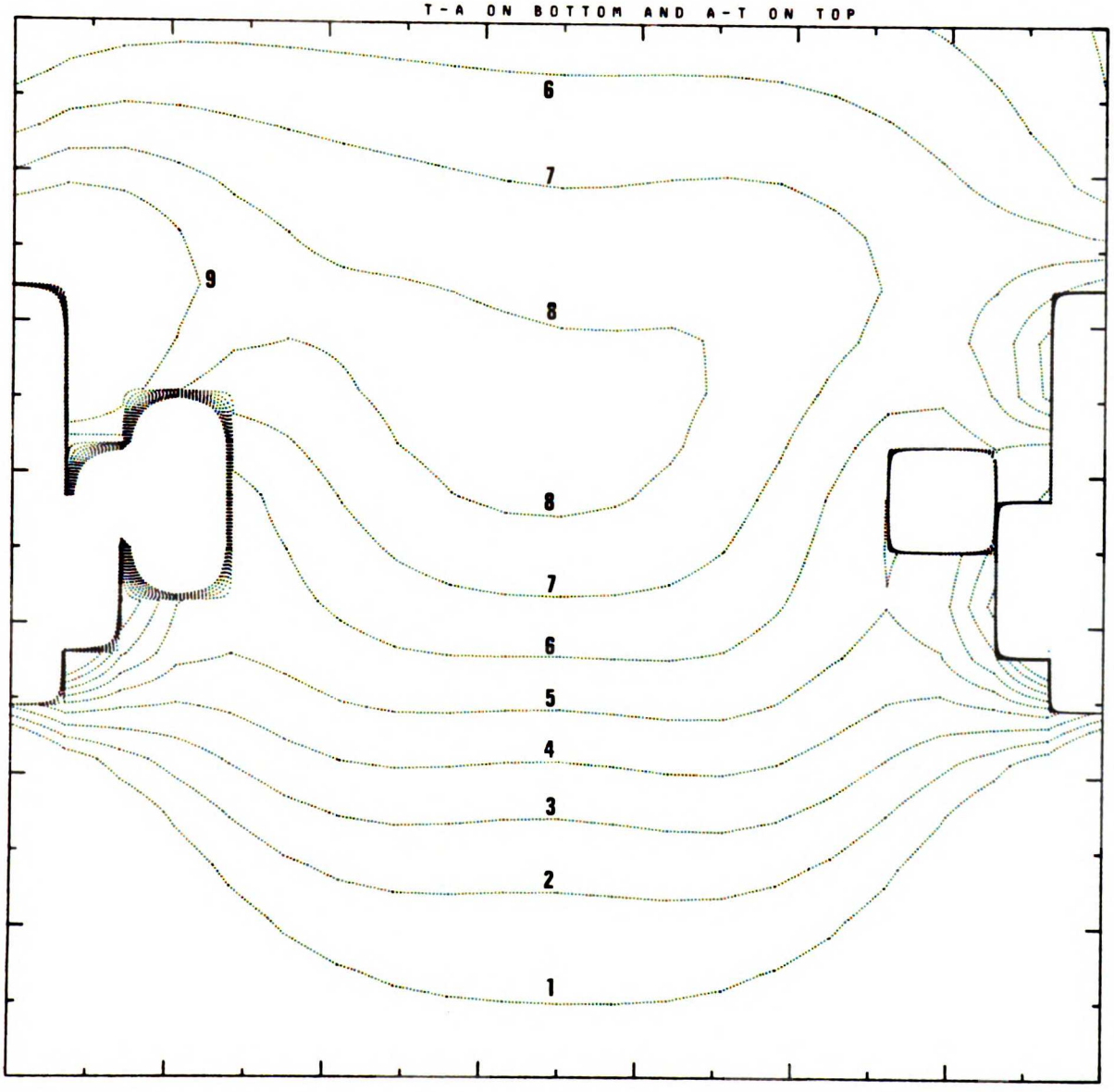

A-T ON BOTTOM AND T-A ON TOP
6
7
8
9
8
7
6
5
4
3
2
1

Figure 19e



$$\left\langle \begin{matrix} T \cdots A \\ T \cdots A \end{matrix} \right\rangle$$

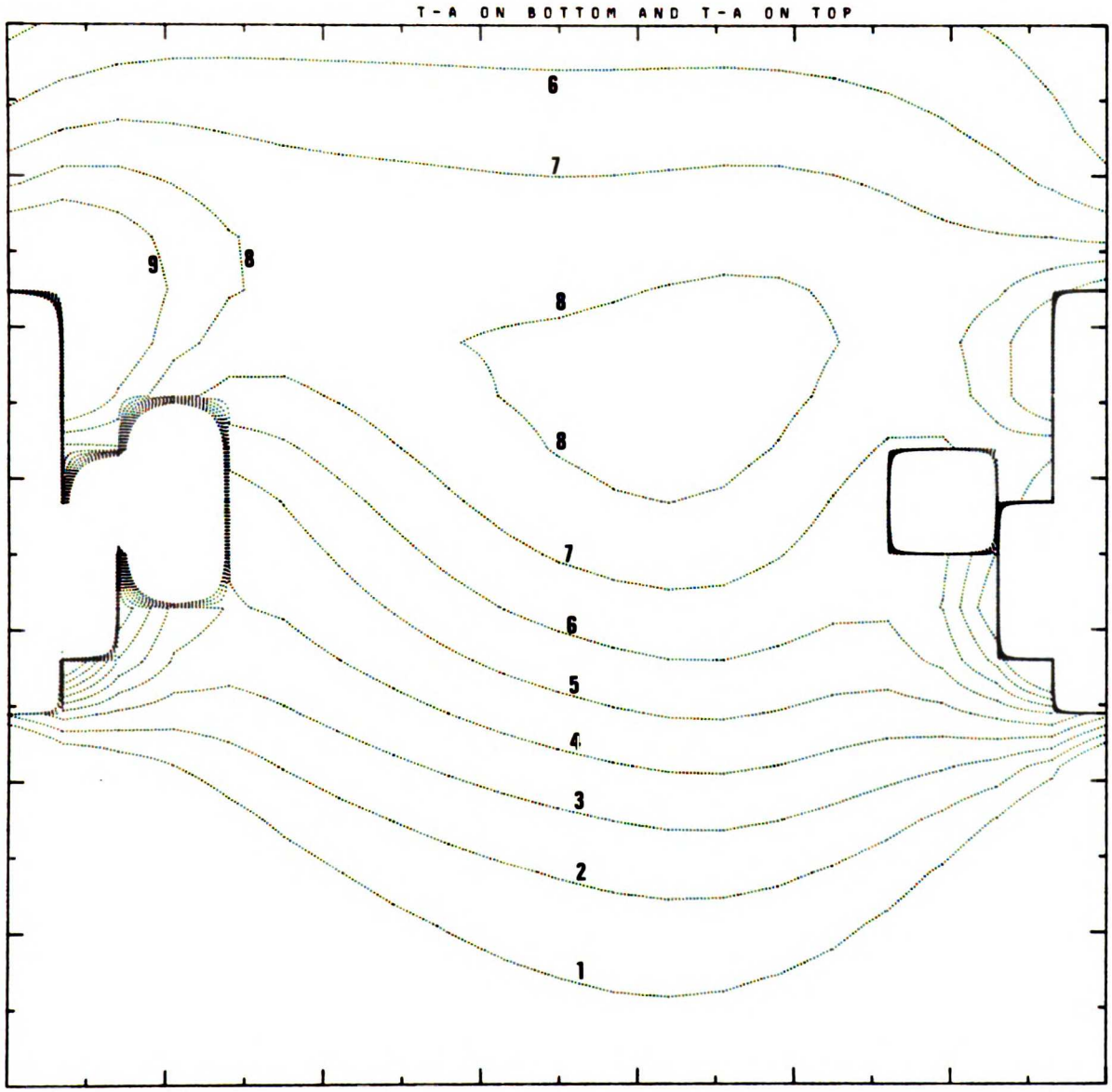
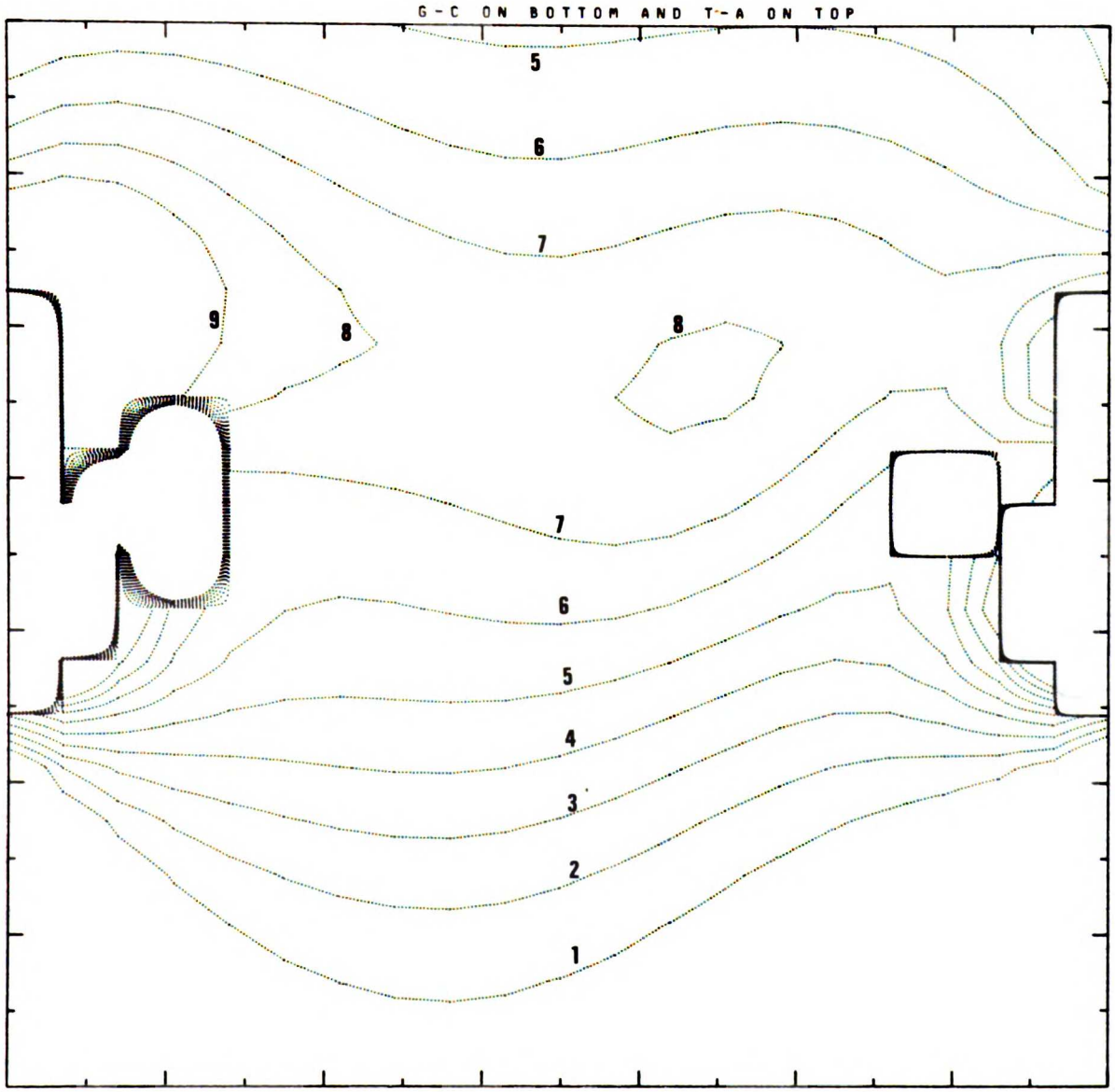
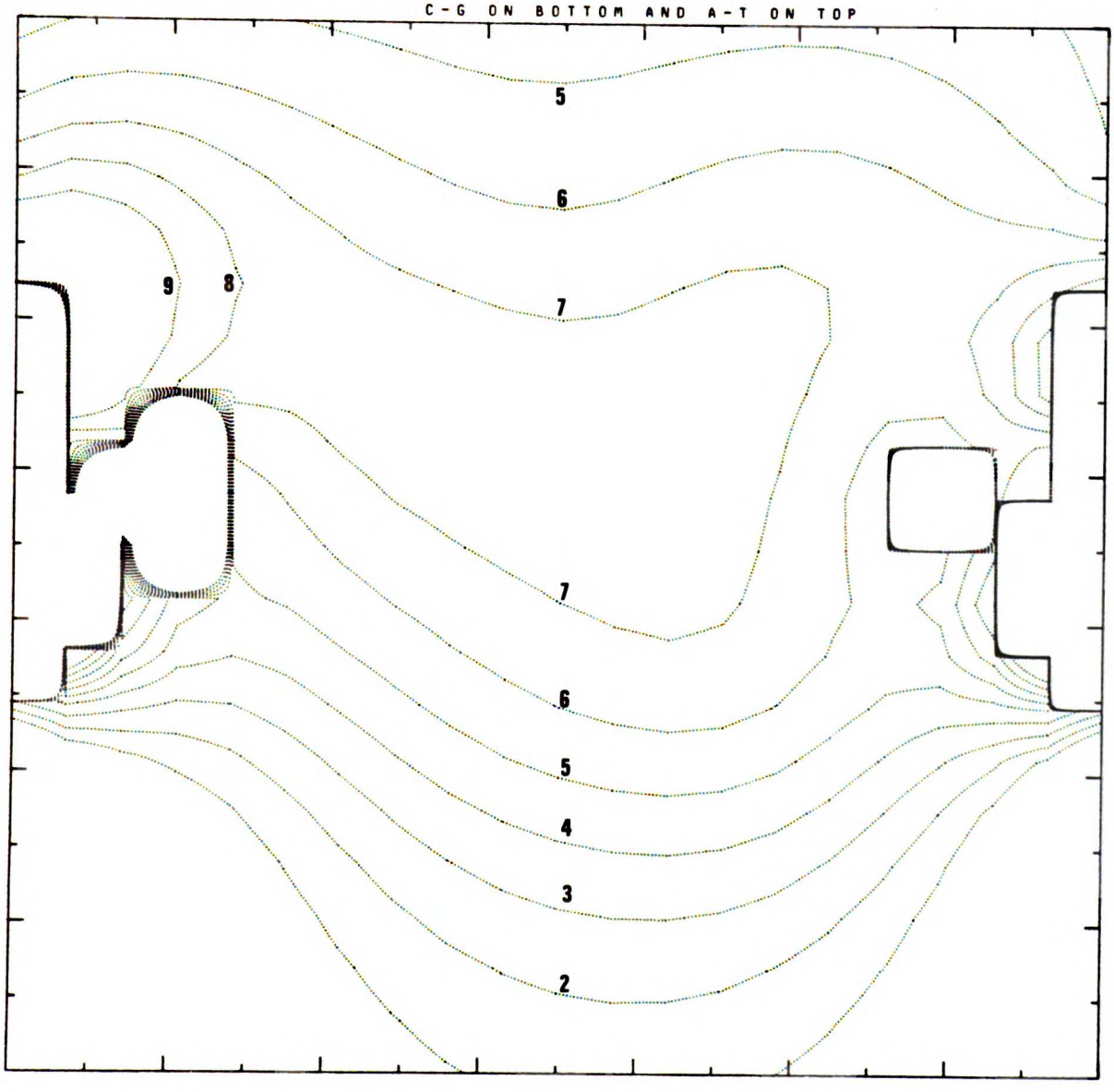


Figure 19g




A···T
C···G


C-G ON BOTTOM AND A-T ON TOP
5
6
9
8
7
7
6
5
4
3
2

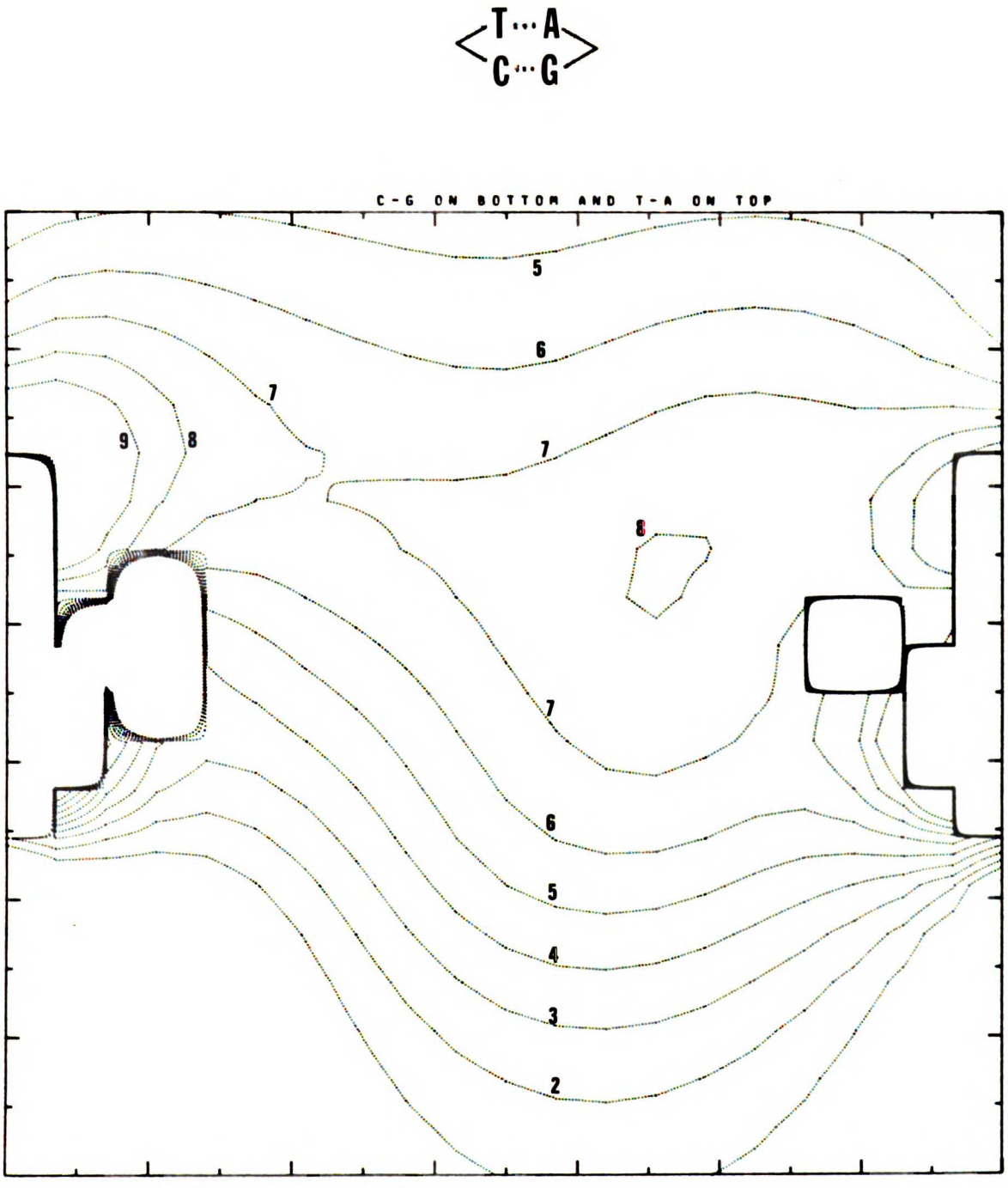

C-G ON BOTTOM AND T-A ON TOP
5
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Figure 19j

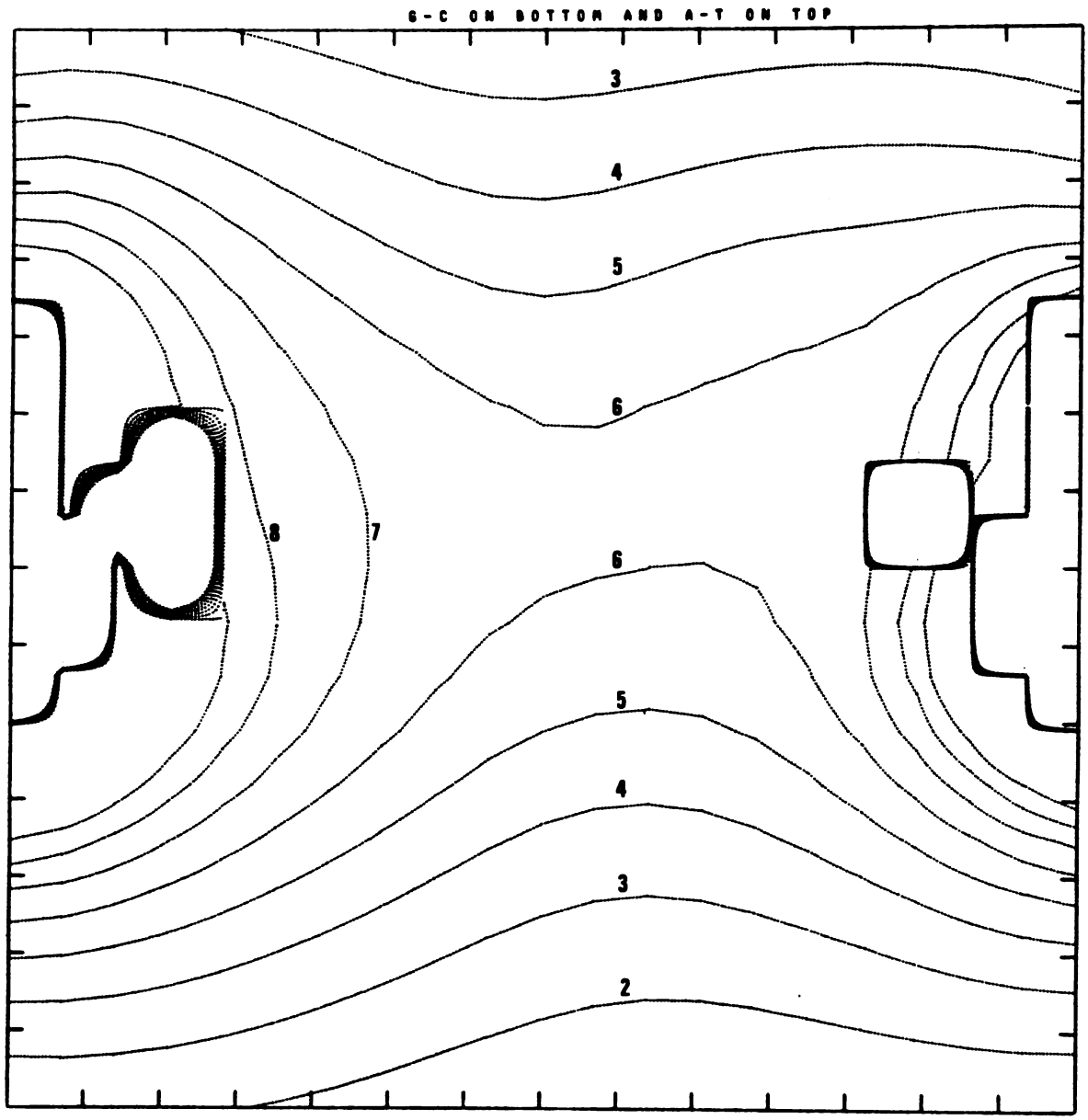
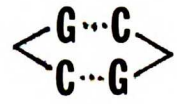
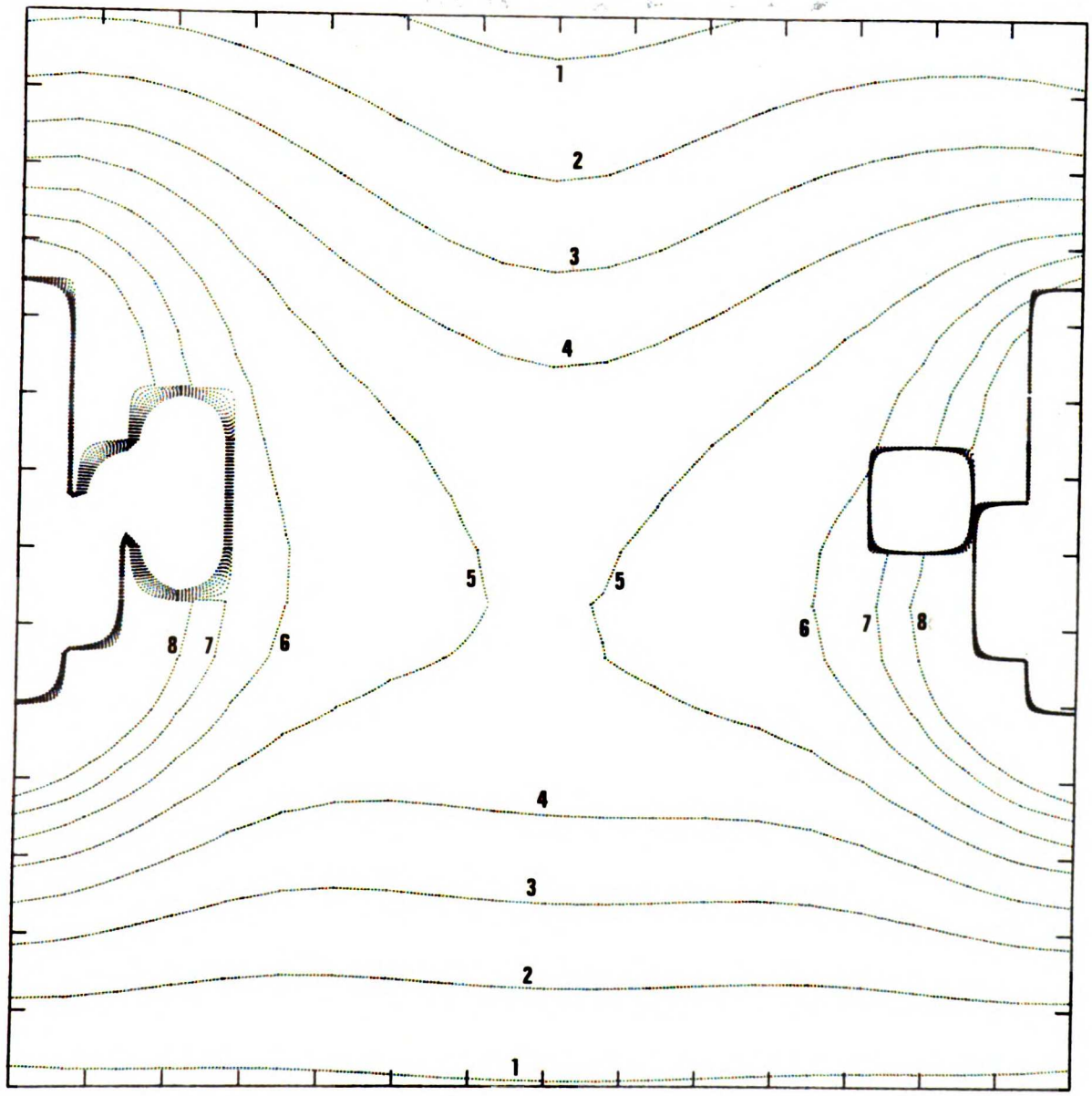
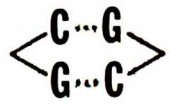
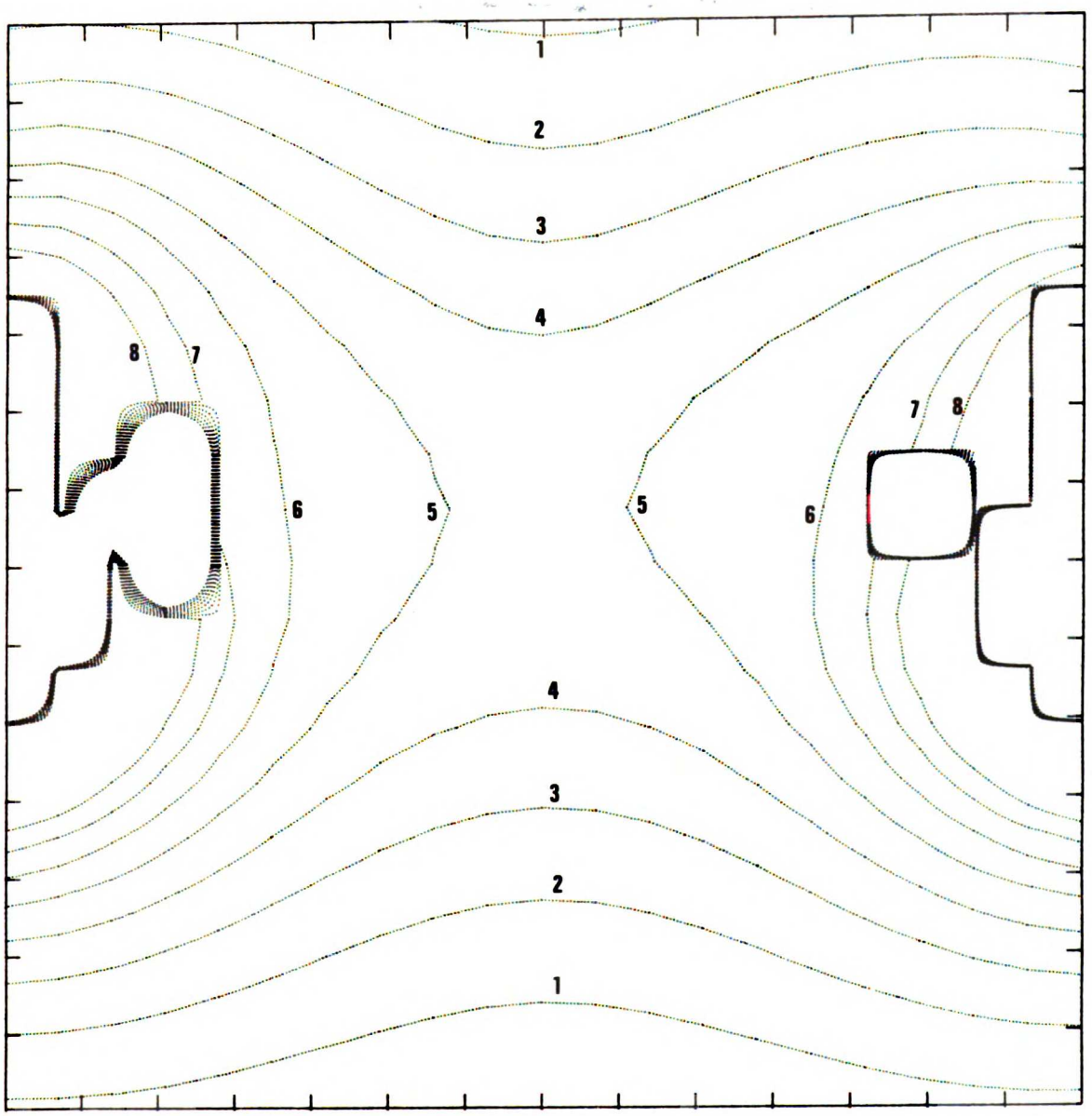


Figure 20a






C···G
G···C


1
2
3
4
8
7
6
5
5
6
7
8
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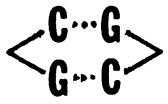
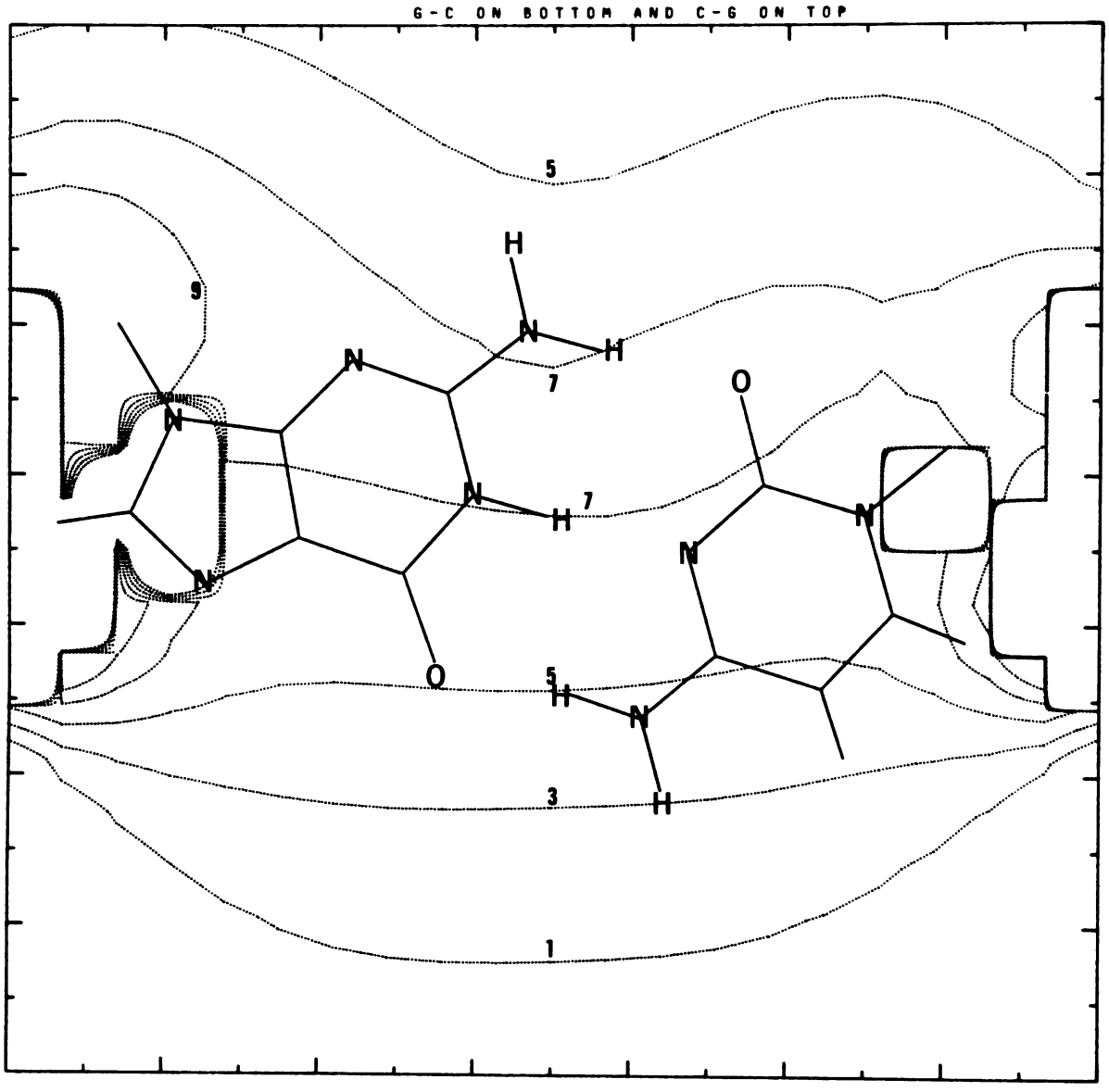
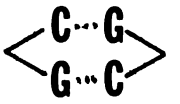
3
2
1

Figure 21a






C···G
G···C

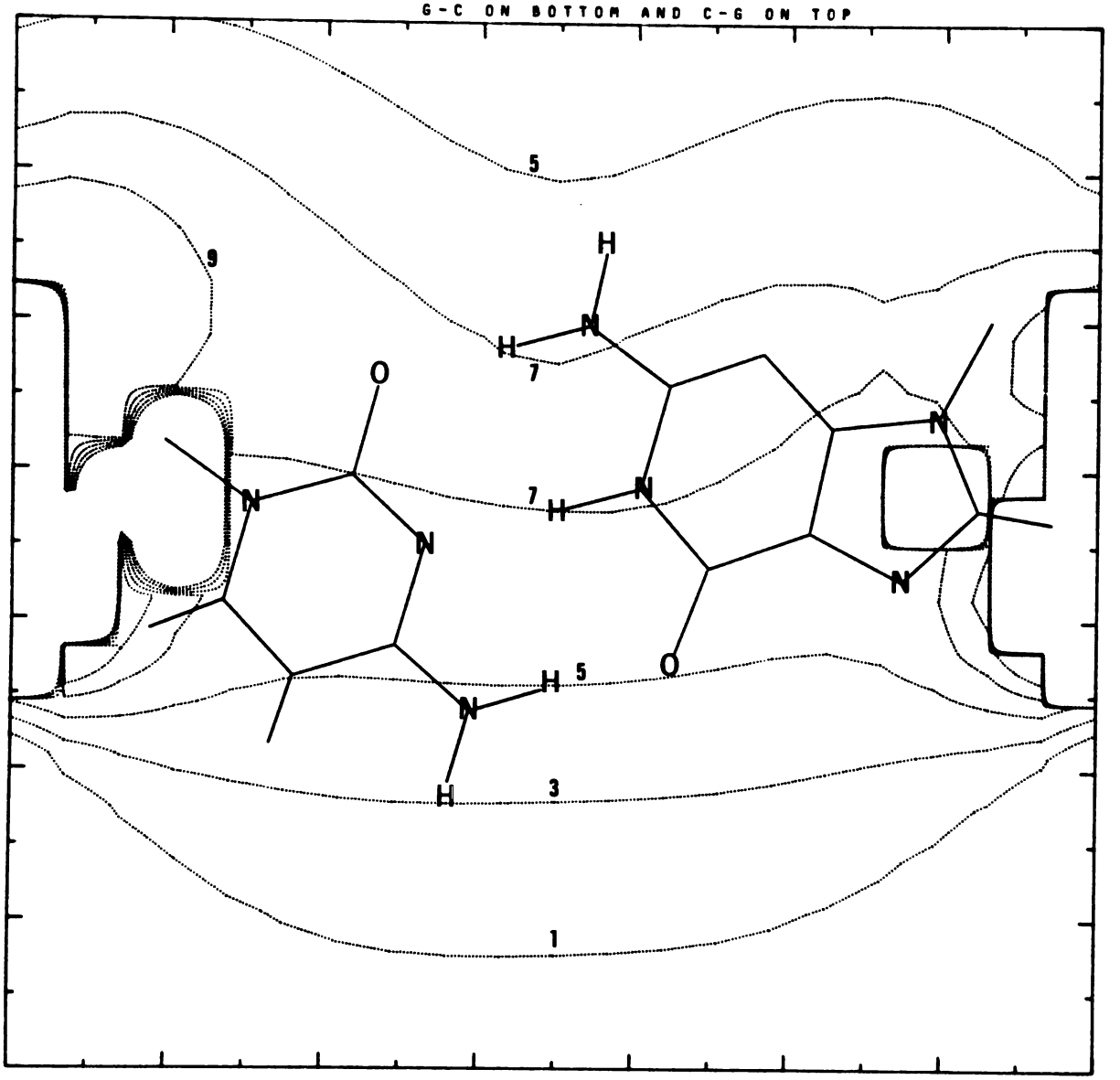
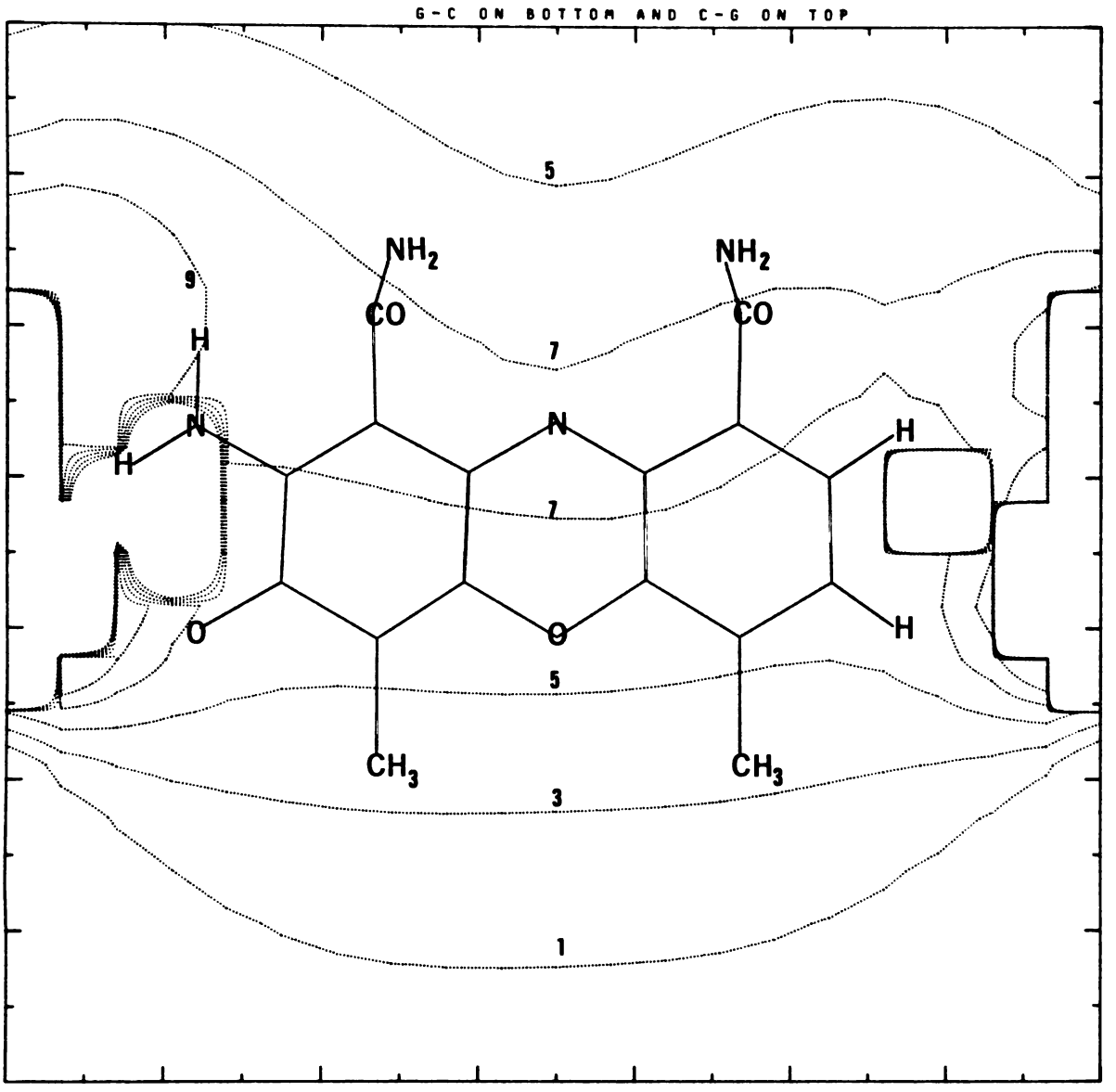

G-C ON BOTTOM AND C-G ON TOP
5
9
7
7
5
3
1
H
N
O
N
H
N
N
H
N
O
N
H
H

Figure 22


C···G
G···C


G-C ON BOTTOM AND C-G ON TOP
5
9
7
7
5
3
1
NH2
CO
NH2
CO
H
N
H
N
H
O
O
H
CH3
CH3

Next we examine the electrostatic potential in the plane of the actinomycin chromophore due to the presence of the dinucleotide base pair. As noted earlier, we used the Sobell geometry for the chromophore plane and the dinucleoside base pair. We examined the electrostatic potential for all 10 base pair combinations and these are illustrated in figure 19a-j (with sodium atoms). In figure 20a-b the electrostatic potential maps are presented for two base pair combinations without the sodium atoms. The projection of a G-C base pairs, above and below the intercalator, is illustrated in figure 21a and 21b; the location of the actinomycin D chromophore is presented in figure 22. The differences in electrostatic potential for different base pair combinations are quite noticeable and of potential utility in aiding understanding of base specificity for different intercalators. For example, if the ethidium bromide chromophore was located at same position as the actinomycin chromophore, its $N5^{+}$ atom would prefer the orientation of the actinomycin oxygen (O5) over that of the nitrogen (N10). Similarly, there is a preference for the minor groove (upper part of the electrostatic potential maps) over the major groove (lower part of figures). These electrostatic potential maps clearly show: (1) that one should expect significant base specifity in intercalators to design molecules with increased base pair specificity and stronger binding. For example, electronegative substituents at the 6 position might (generally) be expected to increase binding and those at the 8 position to decrease binding (relative to the unsubstituted molecule).

As a more precist test of our electrostatic model for analyzing substituent effects, we studied the interaction of 2 and 7 substituted actinomycin chromophores with the 10 base pair combinations. We kept the actinomycin chromophore at the geometry proposed by Sobell and added substituents at standard geometries (checking to see that they fit without van der Waals repulsion). We used the CNDO/2 Mulliken populations for the substituted actinomycins and evaluated the electrostatic interactions energies between the chromophore and the dinucleoside base pair combinations. Table 5 contains the results and the large base dependence and substituent dependence of the interaction energies. It is interesting that the actinomycin chromophore has the largest interaction energy with the $\left\langle \begin{matrix} C\text{-}G \\ G\text{-}C \end{matrix} \right\rangle$ dinucleoside, in qualitative agreement with the experimental results. Table 6 contains the comparison between calculated interaction energies, experimental binding affinities and <u>in vivo</u> anti-cancer activities of some of the substituted chromophores.

There is qualitative agreement between the calculated affinities and experimental affinities, which is as good as would be expected in view of the simplicity of our model. As we have pointed out before the Sobell and Alden and Arnott models differ in the intercalation structural parameters for the sugar-phosphate backbone, but we feel these differences will not effect our calculated <u>base specificities</u>.

The Mulliken populations are relatively geometry independent and we used standard geometries to derive the populations. We have also further broken down the intercalation energies into atom-atom contributions. The amide group on the actinomycin chromophore does play

Table 5. Interaction Energies for Actinomycin Chromophore with Dinucleotides[a]

Substitution		Di Nucleotide[b]									
2	7	$\langle^{G-C}_{G-C}\rangle$	$\langle^{G-C}_{C-G}\rangle$	$\langle^{C-G}_{G-C}\rangle$	$\langle^{T-A}_{T-A}\rangle$	$\langle^{T-A}_{A-T}\rangle$	$\langle^{A-T}_{T-A}\rangle$	$\langle^{T-A}_{C-G}\rangle$	$\langle^{A-T}_{C-G}\rangle$	$\langle^{A-T}_{G-C}\rangle$	$\langle^{T-A}_{G-C}\rangle$
H	H	-1.87	-3.35	-3.29	-2.22	-1.52	-1.35	-3.43	-2.56	-1.08	1.95
OH	H	-2.15	-3.18	-3.25	-1.84	-1.33	-1.17	-2.97	-2.30	-1.27	-1.94
NH_2	H	-3.30	-4.06	-4.41	-2.76	-2.51	-2.06	-3.89	-3.19	-2.42	-3.12
NO_2	H	+2.31	-0.79	+0.54	+0.10	+1.91	+1.05	-1.24	-0.29	+2.80	+1.85
F	H	-0.73	-2.48	-1.96	-1.23	-0.15	-0.51	-2.32	-1.60	+0.15	-0.57
Cl	H	-1.18	-2.70	-2.41	-1.39	-0.61	-1.39	-2.57	-1.83	-0.32	-1.06
Br	H	-1.47	-2.90	-2.72	-1.58	-0.89	-0.82	-2.79	-2.03	-0.60	-1.36
H	OH	-1.02	-2.78	-2.36	-1.48	-0.58	-0.91	-2.76	-2.19	-0.43	-1.01
H	NH_2	-0.98	-3.15	-3.60	-2.71	-1.61	-1.17	-4.12	-2.57	-0.41	-1.95
H	NO_2	-3.33	-3.97	-2.48	-1.87	-1.54	-2.04	-2.70	-2.87	-2.22	-2.06
H	F	-2.47	-3.74	-2.83	-2.05	-1.40	-1.73	-3.16	-2.83	-1.56	-1.89
H	Cl	-2.40	-3.69	-2.54	-1.82	-1.15	-1.62	-2.93	-2.74	-1.45	-1.65
H	Br	-2.00	-3.55	-3.23	-2.51	-1.74	-1.72	-3.64	-2.85	-1.29	-2.10
NH_2	OH	-2.31	-3.28	-3.26	-1.61	-1.19	-1.25	-2.85	-2.49	-1.53	-1.89
NH_2	NH_2	-2.22	-3.60	-4.40	-2.75	-2.11	-1.45	-4.11	-2.81	-1.43	-2.73
NH_2	NO_2	-4.78	-4.42	-3.41	-1.63	-1.94	-2.08	-2.51	-2.96	-3.32	-2.51
NH_2	F	-3.89	-4.46	-3.93	-2.60	-2.39	-2.44	-3.62	-3.47	-2.90	-3.62
NH_2	Cl	-3.37	-3.98	-3.86	-2.14	-1.93	-1.77	-3.29	-2.92	-2.31	3.29
NH_2	Br	-3.23	-3.91	-3.94	-2.18	-1.94	-1.69	-3.36	-2.87	-2.19	-3.36

[a]Units are kcal/mole.

[b] $\langle^{G-C}_{G-C}\rangle$ has the left hand chain 3' on top; right hand chain 5' on top.

Table 6. Comparison between Calculated Interaction Energies DNA Binding and In Vivo Activities.

DNA Binding[a]			
Compound	Buffer	K_{ap} X 10^{-6}	Interaction Energy (Max)[c]
Actinomycin C3	BPES	2.4	-4.41
7-NO_2 Actinomycin C3	BPES	3.05	-4.78
7-NH_2 Actinomycin C3	BPES	3.2	-4.40
7-Br Actinomycin C3	BPES	7.0	-3.94
Actinomycin C3	0.01 PO_4	12.0	-4.41
7-NO_2 Actinomycin C3	0.01 PO_4	38.0	-4.78
7-NH_2 Actinomycin C3	0.01 PO_4	38.0	-4.40
Actinomycin C1	BPES	2.3	-4.41
7-OH Actinomycin C1	BPES	4.2	-3.28
2-Cl Actinomycin C1	BPES	0.025	-2.70

In Vivo Activity[b]		
Substituent	Activity	Interaction Energy (Max)[c]
$2NH_2$, 7H (Actinomycin)	+	-4.41
2OH, 7H	-	-3.25
2Cl, 7H	-	-2.70
$2NH_2$ $7NH_2$	-	-4.40
$2NH_2$, 7OH	-	-3.28
$2NH_2$, 7Cl	+	-3.98
$2NH_2$, 7Br	+	-3.94
$2NH_2$, $7NO_2$	+	-4.78

[a] See reference 6 for experimental results.

[b] See reference 14.

[c] Maximum interaction energy of the ten base-pair combinations, see Table II.

an important role in the base specificity, as has been suggested by Sobell.[67] Table 7 compares the interaction energies for the different base pairs with and without the amide side chains.

It should be emphasized here that our interaction energy calculations are very crude; they leave out many important factors. We envisage the intercalation process to proceed as follows (Figure 23) where (1) involves conformational changes and unstacking energies of the base pairs, (2) the desolvation of the intercalator and (3) the interaction of the separated base pairs (6.8 Å) and intercalator.

We have focused our attention mainly on the energy of step (3) and are currently carring our analyses of steps (1) and (2) by classical conformational analysis (1) and simple empirical solvation energy estimates (2). However, we should point out that the use of only step (3) in the comparison of the relative interaction energies for isomers, e.g., 2-H, 7-NH_2 compared to 2-NH_2, 7-H should be close to correct.

The ΔS of intercalation is another important consideration and appears to the dominant[77] thermodynamic variable for actinomycin-D/DNA interactions (the interaction of charged intercalators with DNA appears, on the other hand, to be enthalpy dominated). In fact, without any side chain, intercalation of the actinomycin chromophore is undetectable. Our results (Table 5) are consistent with very little net energetic attraction between the actinomycin chromophore and dinucleosides. However, considering the pentapeptide as a constant, it is still meaningful to compare the relative interaction energies of different "chromophoric" isomers of actinomycin D.

Table 7. Role of amide side chains in Actinomycin Dinucleotide Interactions[c]

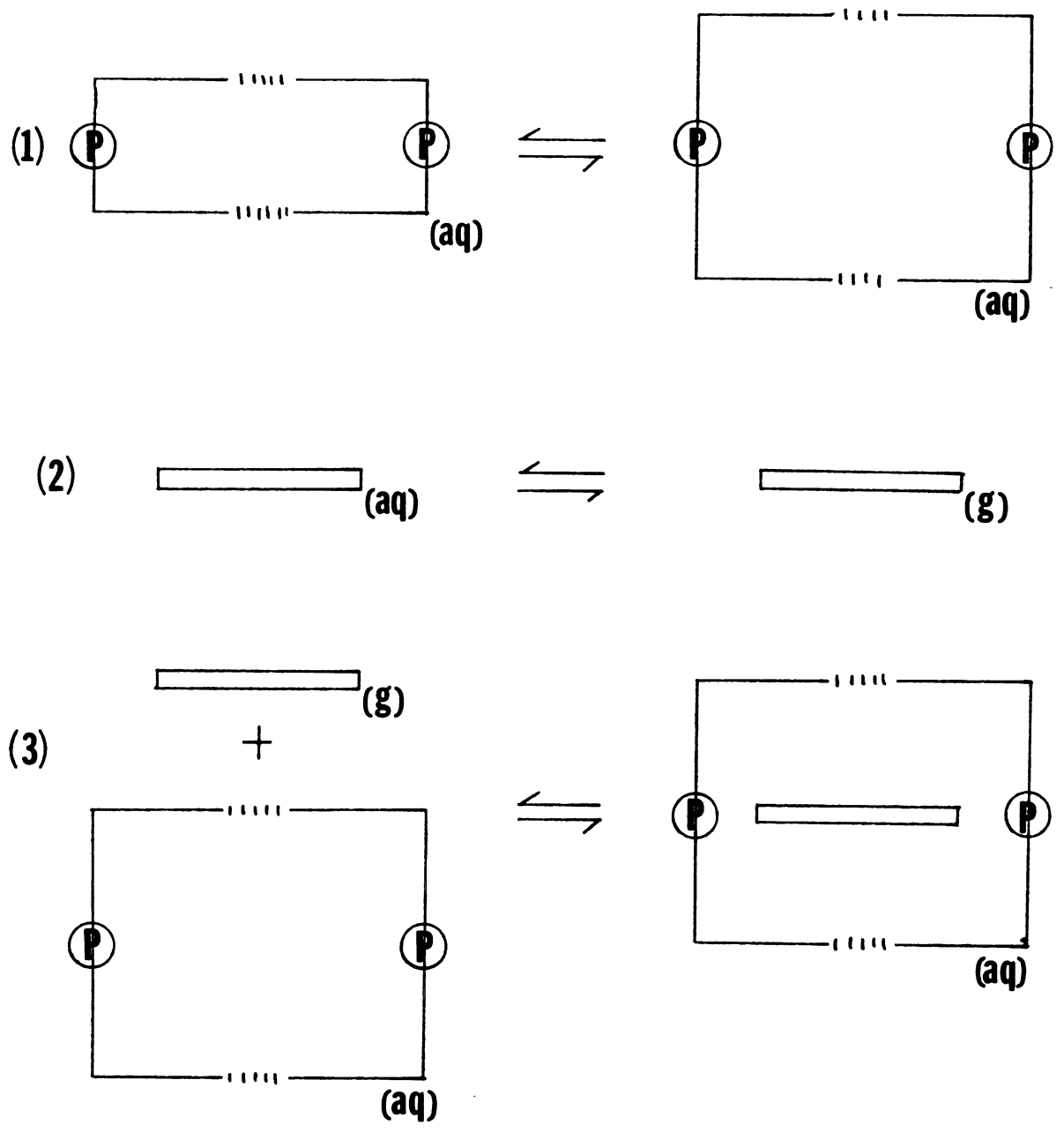
Interaction	$\langle \frac{G-C}{G-C} \rangle$	$\langle \frac{G-C}{C-G} \rangle$	$\langle \frac{C-G}{G-C} \rangle$	$\langle \frac{T-A}{T-A} \rangle$	$\langle \frac{T-A}{A-T} \rangle$	$\langle \frac{A-T}{T-A} \rangle$	$\langle \frac{T-A}{C-G} \rangle$	$\langle \frac{A-T}{C-G} \rangle$	$\langle \frac{A-T}{G-C} \rangle$	$\langle \frac{T-A}{G-C} \rangle$
sidechain[a]	-3.81	-3.12	-4.08	-1.43	-2.08	-0.86	-2.35	-1.80	-2.50	-3.04
chromophore[b]	0.51	-0.94	-0.33	-1.33	-0.43	-1.20	-1.54	-1.39	+0.08	-0.08

[a]Electrostatic interaction of phenoxazone chromophore with dinucleotide.

[b]Electrostatic interaction of 2 amide groups with dinucleotide.

[c]The sum of side chain and chromophore interactions is equal to the interactions in Table III (2 NH_2, 7H).

Figure 23



(B) Empirical Potential Calculations

A potential energy function as described above (including electrostatic, dispersion, exchange and torsional terms) was used to calculate the conformation and energy of six different drug/nucleic-acid complexes. In these complexes the drug was intercalated between two base-paired deoxydinucleoside monophosphates (GpC). Four different drugs were used in the calculations (1) ethidium bromide, (2) ethidium bromide without the phenyl and ethyl side chains, (3) proflavine and (4) 10-aminoacridine. In the proflavine and 10-aminoacridine/dinucleoside complexes the chromophore was either pointed towards the major or minor groove. With ethidium bromide, with and without the side chains, the N5 atom of the polyaromatic ring points in the direction of the minor groove. For the purpose of comparing the binding energy of these drugs to nucleic acids, the structure of two GpC fragments that are base paired in the Watson-Crick form was optimized. In each case the energy was minimized with respect to the torsional (7 for each nucleic acid fragment) and intermolecular (6 for the dinucleoside monophosphate that was base paired in the BDNA structure and 12 for the base-pair/intercalator complex) degrees of freedom.

Geometry

Torsional Angles. Table 8 summarizes the values of the torsional angles that define the sugar-phosphate backbone and sugar-base orientation. It should be noted that in the drug/nucleic-acid complex the normal C2'-endo deoxyribose sugar ring puckering of BDNA is altered to a mixed sugar puckering of the type C3'-endo(3'-5')C2'-endo. The conformation of the sugar rings, however, are not varied during the minimization calculations.

Table 8 Comparison of Model Calculations with Crystal Structure

	Sobell Model*	Calculated Model**	Deviation
Torsional Angles*** (°)			
(A) First Dinucleoside			
χ	199	235	36
ω	288	272	16
ϕ	150	149	1
ψ	74	71	3
θ	79	60	19
ε	134	155	21
χ'	91	83	12
(B) Second Dinucleoside			
χ	191	240	49
ω	305	275	30
ϕ	136	144	8
ψ	69	70	1
θ	69	57	12
ε	135	154	19
χ'	96	87	9
Hydrogen Bond Lengths (Å)			
A,D	2.88,2.91	2.98,2.85	0.01
B,E	2.89,3.01	2.95,2.99	0.02
C,F	2.81,3.02	3.29,3.45	0.46

*H. M. Sobell, private communication

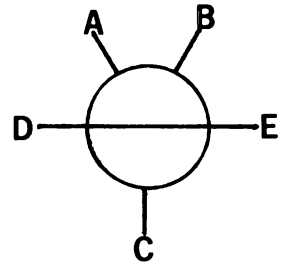
**These results.

***The torsional angles is defined in terms of four consecutive atoms, ABDC; the positive sense of rotation is counterclockwise from A to D while looking down BC bond.

As one might expect, the corresponding torsional angles of the two dinucleoside are almost identical for each complex since the drug/nucleic-acid or BDNA complexes have a dyad or "pseudo" dyad axis. In addition, the dihedral angles for most of the drug/nucleic-acid complexes are similar indicating that the different drug/dinucleoside structures are closely related. The one exception is proflavine with the C10 atom of the chromophore pointing towards the major groove. The starting conformation for this structure has the chromophore stacked between the base pairs of the dinucleosides with the amine groups pointing directly at the phosphate backbone. In this orientation the repulsive contribution for the interaction of the amine groups of the drug with the sugar-phosphate backbone of the nucleic acid dominates the total energy of the complex. To relieve the steric repulsion the dinucleosides are forced to move away from each other. This complex is the least stable of all the drug/nucleic-acid structures because of this steric repulsion of the amine groups with the backbone.

Most of the conformational changes that occur when a drug intercalates between the base pairs of the nucleic acids results from (1) repuckering of the sugar rings and (2) a change in the dihedral angles of the sugar-phosphate backbone. The distance between the bases must increase from 3.4 Å to 6.8 Å when a drug intercalates between the base pairs of DNA. Our results indicate that by changing the pucker of the sugar rings of DNA, the backbone of DNA can be extended approximately 3.4 Å to accommodate the intercalator without a "large" change in the torsional angles. For example, a comparison of the differences in the torsional angles (excluding χ and χ') of the optimized BDNA structure with the optimized drug/nucleic-acid structures, excluding proflavine

(major), shows that the largest change in the dihedral angle is approximately 40° for ω and θ. For the other torsional angles the changes are generally less than 20°. This is very interesting because previous work by Arnott[62] suggests that the angle ψ must increase by 120° (approximately) when a drug molecule binds to DNA by intercalation. The same angle, in our calculations, only increases by approximately 40°. The values of χ and χ' do not vary by much with the different drug/nucleic-acid complexes. Their angle is essentially determined by the base stacking interactions which are very similar for all of the intercalators. There is a change, however, in the value of χ' between the optimized BDNA structure and the BDNA angles taken from Arnott,[75] the value changing from 35.0° to 71.3°. The angular dependence of the glycosidic bond (χ and χ') suggests, as shown below, that there are several minima



depending on which four atoms are used to designate the dihedral angle. In the Newman projection the nucleic acid base (D and E) is situated at a minimum with respect to three atoms of the sugar ring (A, B, and C). If atoms C and D are used to define the torsional angle, the minimum is at 90° (counterclockwise). However, if A and E are used the minimum will occur at 215°. There are several other minima for this structure depending on which atoms are designated as end atoms. In our optimized drug/nucleic-acid structures the angle χ' is generally within 10° of one of the minimum. The other glycosidic angle, χ, is more

than 20° away from the nearest minimum which means it is almost eclipsing one of the sugar bonds. There is no obvious reason why this angle is so far away from a minimum.

Hydrogen Bond Distances

One important test of the validity of our potential energy function is the length of the hydrogen bond between the bases of the two dinucleoside monophosphates (GpC) that are base paired in the BDNA and drug/nucleic-acid structures. Table 9 gives the length of the six hydrogen bonds that are formed between the two self-complementary dinucleosides (GpC). The first three hydrogen bonds (A, B, and C) refer to the bonds formed between the guanine base of the first dinucleoside with the cytosine base of the other (second) dinucleoside. The "first dinucleoside" refers to the nucleic fragment that is numbered from 7 to 68. Bonds D, E, and F designate the other three hydrogen bonds that are formed in these complexes. All of the hydrogen bonds, with the exception of the proflavine (major) structure, have "acceptable" distances.

In each complex there is a small variation in the length of the different hydrogen bonds. This effect is accentuated if ethidium bromide is the intercalator. It is likely that the side chains of ethidium, especially the phenyl ring, interact with the base in such a way as to shorten one hydrogen bond and lengthen the other. When the side chains are removed from ethidium, the hydrogen bond lengths for the drug/nucleic-acid complex are very similar to the other intercalator structures which also do not have side chains. In addition,

Table 9. Hydrogen Bond Lengths (Å)

Atom-Atom**	BDNA†	EB†	BDNA	EB	EB*	PF (minor)	PF (major)	10AA (minor)	10AA (major)
(A) 10-121	2.85	2.98	2.82	2.98	2.82	2.86	4.42	2.84	2.75
(B) 7-122	2.91	2.91	2.96	2.95	2.88	2.91	3.99	2.90	2.96
(C) 17-128	2.84	2.72	3.02	3.29	3.15	3.05	3.43	3.11	3.30
(D) 59-62	2.85	2.98	2.81	2.85	2.85	2.85	4.46	2.83	2.77
(E) 60-69	2.91	2.91	2.94	2.99	2.89	2.92	4.04	2.91	2.91
(F) 66-79	2.83	2.72	2.99	3.45	3.12	3.10	3.49	3.12	2.97

*Ethidium Bromide without the ethyl and phenyl side chains.

**The number refer to the end atoms of the hydrogen bonds.

†Starting values

the difference in the bond lengths is more pronounced for the first set (A, B and C) of three hydrogen bonds than it is for the second set (D, E and F). It is possible that the positive side chain (ethyl group) of ethidium bromide which has the methyl group pointing up, interacts with the base in such a way as to distort the first set of hydrogen bonds (A, B and C) more than the second set. The first set of hydrogen bonds represent the hydrogen bonds on "top".

Comparison of Crystal Structure With Optimized Complex

The starting values for the r, θ, and ϕ (internal and intermolecular coordinates) terms in our minimization calculations were based on a set of atomic coordinates that Sobell[74] has proposed for a general intercalation model with ethidium bromide. As noted earlier, we change from x, y, and z coordinates of the nucleic acids to r, θ, and ϕ terms in our computer program. Sobell has based his ethidium-bromide/DNA intercalation model on the atomic coordinates of some drug/dinucleoside crystal complexes.[74] It is therefore appropriate to compare the results of our calculations (hydrogen bond lengths and torsional angles) with the crystal complex of ethidium bromide with cytosine (3'-5')guanosine. Table 10 compares bond lengths and torsional angles for the two complexes.

There are several reasons for the differences between our calculated model and the crystal structure of Sobell. First, the dinucleoside base sequence in the Sobell model is pyrimidine(3'-5')purine (CpG) while we use the isomeric nucleic acid, purine(3'-5')pyrimidine (GpC). In addition, the dinucleoside in the crystal complex (ethidium/CpG) has an iodine atom on the cytosine base. The puckering of the

sugar rings in the calculated and crystal structures is also not identical. Finally, the obvious, and perhaps most important difference, is that theoretical calculations simulate gas phase conditions while crystal structures have intermolecular interactions involving water and other molecules. These considerations will contribute to the deviation between the calculated and crystal structures. However, these differences should not lead to any "large" changes between the structures.

A comparison between the crystal and calculated structures of ethidium bromide with dinucleosides is important to test the reliability of the potential energy functions for these types of calculations. Table 10 compares the values of the 14 torsional angles that are variables and the six hydrogen bond lengths for a crystalline complex of ethidium bromide with 5-iodocytidyl(3'-5')guanosine (CpG) and our optimized structure of the drug with guanidyl(3'-5')cytidine monophosphate(GpC). The deviations of the angles and bond lengths are relatively small indicating that our potential energy functions can (1) reproduce the characteristic changes (torsional angles) necessary for the sugar-phosphate backbone to extend from 3.4 Å to 6.8 Å and (2) maintain the hydrogen-bonds that help stabilize the DNA and drug/nucleic-acid structures.

It is important to compare both the hydrogen bonds and the torsional angles in the drug/nucleic-acid complex. Without knowing that our hydrogen-bonds were "acceptable" lengths, it would be difficult to determine whether the changes in the torsional angles would give a reasonable nucleic-acid/intercalator structure. Our model, however,

Table 10 Torsional Angles*

Greek symbol†	BDNA***	EB***	BDNA	EB	EB**	PF (Minor)	PF (major)	10 AA (minor)	10AA (Major)
χ	215.0	214.7	205.0	234.6	231.7	233.7	214.5	234.0	232.5
ω	329.1	318.4	310.9	272.1	270.6	273.0	318.9	273.9	274.3
ϕ	151.4	167.3	155.0	148.5	161.8	160.9	167.6	163.4	162.9
ψ	39.2	33.1	65.5	71.4	72.6	69.7	34.7	69.6	69.8
θ	98.9	80.8	97.8	59.6	59.6	63.8	81.8	62.6	62.5
ε	200.9	136.8	176.4	155.4	157.9	153.6	137.7	151.1	155.0
χ'	35.0	90.5	71.3	83.1	97.6	99.6	90.7	101.4	99.6
χ	215.0	214.7	205.1	240.5	232.0	233.5	214.6	235.1	238.4
ω	329.1	318.4	310.9	274.9	270.6	273.0	318.9	273.7	277.1
ϕ	151.4	167.3	155.0	144.5	160.8	162.1	167.6	164.5	168.7
ψ	39.2	33.1	65.5	69.9	73.7	70.6	34.7	69.5	71.5
θ	98.9	80.8	97.8	56.9	58.5	61.8	81.8	61.5	64.7
ε	200.9	136.8	176.4	154.1	160.5	153.4	137.6	151.7	153.3
χ'	35.0	90.5	71.3	86.8	96.7	98.5	90.6	100.6	89.3

*The torsional angle is defined in terms of four consecutive atoms, ABDC; the positive sense of rotation is counterclockwise from A to D while looking down the BC bond.

**Ethidium Bromide without the ethyl and phenyl side chains.

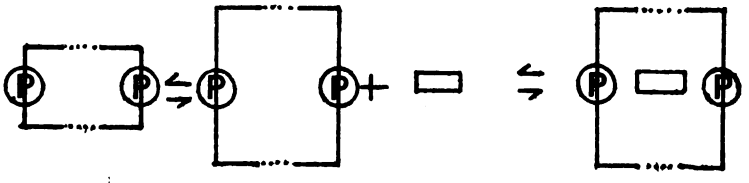
***Starting values.

†These symbols are also used to designate the torsional angles of the dinucleoside monophosphate.

is constrained (in a figurative sense) by the hydrogen bonds between the bases. We feel confident, therefore, that our potential energy function has produced a reasonable model for the ethidium-bromide/small-nucleic-acid structure which can be extended to other drugs, proflavine and 10 aminoacridine, and different base pair combinations.

Interaction Energies

The interaction of a drug with two base-paired dinucleoside monophosphates (GpC) can be envisaged as a two step process in the gas phase. A schematic diagram of the sequence of events for the formation of a drug/nucleic-acid complex is shown below--



The first step is the unstacking of the base-paired nucleic acid followed by the insertion of a drug chromophore between the unstacked base pairs. The first step is an endothermic process, i.e. energy is required to separate the bases. The second step, the insertion of the drug between the base pairs, must be an exothermic process that is larger in magnitude than the initial process if intercalation is to occur.

Table 11 gives a breakdown (component analysis) of the different contributions to the interaction energy of a drug interacting with two base-paired self complementary dinucleoside monophosphates (GpC). The components are divided into two sections (1) intramolecular effects, base-base, base-backbone (bk), and backbone-backbone, and torsional terms, and (2) intermolecular contributions resulting from base-base, base-backbone, backbone-backbone, drug-base, and drug-backbone interactions. A more detailed listing of the component analysis, from which the numbers in Table 11 were tabulated is given in Appendix C.

The designation for base-base contributions refers to individual atom-atom interactions where both atoms are on one of the four bases of the two base paired dinucleosides. An atom-atom interaction such as drug-bk (backbone) gives the contribution to the energy based on the sugar-phosphate backbone. The atoms of the drug refer to the different intercalators.

In evaluating the component analysis data two points become apparent regarding the interaction energy of the drug/nucleic-acid complexes we have studied. First, all of the drugs that from "reasonable" drug/dinucleoside complexes have essentially the same magnitude for step one (the unstacking of the bases of the dinucleoside) for the intercalation process. To form an intercalation complex the distances between the bases of the dinucleoside must be increased from 3.4 to 6.8 Å. This requires a conformational change in the sugar-phosphate backbone (it must be extended). This results in an increase in energy as the bases of the dinucleoside monophosphate become "unstacked". The different components of this increase in energy for the different

Table 11. Energy Component Analysis (kcal/mol)

	BDNA**	EB**	BDNA	EB	EB*	10AA (minor)	10AA (major)	PF (minor)	PF (major)
(A) Intramolecular Components									
Base-Base***	88.36	108.24	94.30	109.35	108.45	108.36	108.03	108.49	108.01
Base-Bk	56.79	68.70	58.99	59.07	61.09	60.77	60.04	60.90	73.20
Bk-Bk	-45.01	-58.67	-65.09	-63.90	-62.23	-62.47	-62.37	-62.49	-58.93
Torsional	-7.30	- 8.77	-13.51	-12.57	-14.81	-14.66	-15.48	-14.71	- 6.71
(B) Intermolecular Components									
Base-Base	-21.38	-17.17	-22.33	-17.99	-18.25	-20.11	-19.15	-18.71	- 7.94
Base-Bk	8.40	6.18	8.58	9.26	8.84	14.88	8.25	8.64	4.91
Bk-Bk	10.82	10.06	11.25	12.23	10.95	6.65	10.62	11.04	9.53
Drug-Base	---	-68.42	---	-66.81	-71.84	-72.17	-71.89	-67.55	-55.54
Drug-Bk	---	-59.99	---	-94.03	-79.35	-72.80	-69.97	-82.05	-49.56
(C) Total Components									
E	90.68	-19.84	72.19	-65.39	-57.15	-51.55	-51.92	-56.44	-16.97
ΔE	---	---	0	-137.58	-126.34	-123.74	-124.11	-128.63	-89.16

*Ethidium Bromide without the phenyl or ethyl side chains.

**Starting values.

***Base refers to atoms of guanine and cytosine of dinucleoside; Bk refers to all other atoms of dinucleoside monophosphate (sugar and phosphate group); Drug refers to atoms of the intercalator.

complexes are very similar. For example, there is less than a 2 kcal difference in the base-base interactions (intramolecular) for the different complexes when the bases are unstacked (most of this results from a loss in the dispersion attraction term). In addition, there is less than a 3 kcal difference in the "total" intramolecular interaction of the sugar-phosphate backbone for the different complexes in the "extended" form. There is also a small variation in the torsional ($\sim$ 3 kcal) and intermolecular ($\sim$ 4 kcal) energy components for the unstacked nucleic acid structure. The increase in the intermolecular contributions is a result of the "distorted" hydrogen bonds in the intercalation complex as compared to the hydrogen bonds in the BDNA structure. The net destabilization for step (1) is 21 ± 4 kcal/mole for all 6 dinucleotide drug interactions in Table 11. The second point is that the intercalation complex is formed because of the strong interaction between the drug and the two dinucleosides. For example, there is a gain of $\sim$ 161 kcal when ethidium bromide interacts with both dinucleosides in their "unstacked" conformation (step two). The total difference, therefore, is a net stabilization in gas phase of approximately 138 kcal for the ethidium-bromide/nucleic-acid structure. There is a large variation in the interaction energy of step two for the different intercalation complexes. This will be discussed in more detail in a following section.

Several factors will change the binding energy of the complex in solution compared to the gas phase calculations. First, a dielectric constant of one was used for the electrostatic contributions. This

represents the maximum electrostatic effect between two charges in a vacuum. Picking the "correct" dielectric constant is almost impossible. Any single value of the dielectric constant infers that the complex and the solvent around it can be represented as a homogenous medium. This obviously is incorrect. The correct value of the dielectric constant must take into account the medium through which the charges are calculated. At the present time this presents a very serious complication for all potential energy calculations. A change in the dielectric constant can either decrease or increase the interaction energy. In addition, we have neglected solvation effects in the formation of the drug/nucleic-acid complex. In the first step when the distance between the bases increases, water molecules will more completely solvate the bases. In the second step, the drug must be "desolvated" so it can intercalate between the base pairs. Both of these processes will tend to decrease the energy difference between the initial components and final complex making the structure less stable than the gas phase calculations have predicted. Thirdly, translational and rotational entropy effects due to bringing two components together to form a single complex will decrease the net attractive energy for complex formation.

In comparing the interaction energy of different intercalators with GpC, it is clear that the different binding energies result mainly from the interaction of the drug with the sugar-phosphate backbone and the bases of the dinucleosides. In some cases this can be explained very nicely by considering the position of the positively charged amine group(s) of the intercalator. The difference

in binding energy for ethidium bromide, with and without the phenyl and ethyl side chains, and proflavine (minor), as compared to 10-aminoacridine (major and minor) can be rationalized by examining the position of the amine groups of the different intercalators with respect to the negatively charged (-1) phosphate group of the backbone. For ethidium and proflavine the amine groups are very close to the phosphate group while in 10-aminoacridine (major and minor) the amine group is situated between the two phosphate groups on the different monophosphates. This favorable electrostatic energy for ethidium and proflavine enhances their ability to bind to nucleic acids by intercalating between the base pairs as compared to 10-aminoacridine. The data in Table 11 is consistent with this observation. With this type of analysis one would also not expect there to be a great difference in the binding energy between proflavine and ethidium without the side chains. The difference in the interaction energy for these two molecules is indeed very small (less than one kcal). The difference in binding energy for the two ethidium molecules, with and without side chains, is a result of the positive side chains of the "complete" ethidium bromide drug molecule interacting with the negatively charged side chains, since there is essentially no difference of the charges on the amine groups of the two intercalators. The relatively high energy of the proflavine (major) orientation is a result of the amines actually being too close to the phosphate backbone and distorting the nucleic acid structure.

Sobell has recently suggested that intercalators may be divided into three classes depending on the direction of entrance into DNA. Actinomycin D and ethidium bromide enter from the minor groove while drugs such as daunomycin and proflavine enter from the wide groove. Some drugs (10-aminoacridine) can enter from either side. Our work has provided a convenient check on some of Sobell's assumptions. Proflavine, for steric considerations, has to bind in the major groove. However, there are two orientations of the drug when it approaches from this direction (1) the amine groups point towards the phosphate group of the helix or (2) the amine groups point away from the phosphates. Our work suggests very strongly that the amines are pointing away from the helix. This allows the chromophore to slide between the base pairs of DNA to maximize the base stacking interaction. If the amines are pointing toward the helix the bases will not be able to stack effectively with the chromophore which will result in a higher energy for the complex. Our results show that the drug/base and drug/backbone energy is about 50 kcal higher for the amines pointing towards the helix than for the opposite orientation. In addition, our results on the preferential orientation of 10-aminoacridine is consistent with Sobell's assumption that the chromophore can enter and bind from either groove. There is no steric consideration with the drug entering from either side of the helix because it does not have amine groups on the side of the chromophore. Also, there is apparently no preference in terms of the interaction energy for the chromophore to point towards the major or minor groove.

There is some direct experimental evidence which suggests that ethidium bromide binds more tightly to DNA than doses proflavine.[78] It is premature, however, to compare directly our results with the experimental work. Besides the difficulty, as discussed previously of comparing experimental and theoretical work, DNA has ten different base pair combinations with which the two chromophores can bind. It is expected that each of the ten drug/base-pair combinations will have a different equilibrium constant because of the different drug/base interactions.

CONCLUSION AND FURTHER CONSIDERATIONS

Our calculations are the first attempt to use a complete potential energy function to study the interaction of drugs with small nucleic acid components to determine the origin, nature, and magnitude of the forces that dictate the conformation of such a complex. Comparing hydrogen bond lengths and torsional angles of the sugar-phosphate backbone in the calculated and crystal structure of ethidium-bromide/dinucleoside-monophosphate gives us confidence that our program is capable of producing reasonable geometries for this type of complex. We also feel that the difference in interaction energies between drug/nucleic-acid structures is qualitatively correct.

There are three areas where our program should be used to study drug/nucleic-acid interactions. First the complexes of small nucleic acids with different intercalators than the ones we have used can be studied to elucidate the important components of binding. Our results have suggested that the difference is binding energy of different

chromophores binding to DNA by intercalation is a function of the interaction of positive groups such as amines interacting with the sugar-phosphate backbone. In addition, our NMR results have suggested that daunomycin could bind to certain base sequences more tightly than others. Using potential energy functions it would be possible to determine the reason for the drug/base specificity as well as the most likely conformation of the drug in the complex. Other intercalators, such as ellipticine, quinacrine, and actinomycin D, could also be studied to determine what forces stabilize the drug/nucleic acid interaction.

Secondly, our calculations should be extended to base pair combinations other than guanosine(3'-5')cytosine. Our results indicate that there is not much difference in drug-base interactions for different base-pair combinations. Does this apply to the same chromophore with different base-pair combinations? This is very important question because of the recent interest is drug/base specificity.[35,79]

Finally, these studies should be extended to drugs binding to larger fragments of nucleic acids such as tetra- and hexanucleotides. If one attempts to model drug/high-molecular DNA interactions it is crucial that fragments larger than dinucleosides be used. It is very doubtful when a drug binds to DNA that all of the conformational strain of the nucleic acid helix is centered at the site of intercalation. It is more likely that the distortion is "spread" over several base pairs of the nucleic acid. Therefore, a drug/base-paired hexanucleotide complex would be a more reasonable model for drug binding to DNA than the dinucleoside structure.

Calculating the energy and structure of different intercalators with the same small nucleic acid fragments are projects that are feasible at the present time with our program. There are a few modifications that must be made to look at different dinucleoside components. The non-bonded terms of the intramolecular calculations for the untried base-pair combinations must be verified as being correct. Also, the specification of the six variables to translate and rotate the nucleic acid fragments must be determined for adenine and thymine.

To investigate the binding of drugs to tetranucleotide (or larger) fragments will require extensive modifications in the current program. If all atom-atom interactions in a drug/tetranucleotide complex were calculated, the cost of the calculation would be prohibitive. It will be necessary to generate arrays for the inter- and intramolecular interactions so the individual interactions will only be counted for atoms less than 10 Å apart. If these arrays are generated in the beginning of the program and recalculated every 10 iterations it would be possible to look at larger fragments.

One very important consideration in future work in this area involves the pucker of the sugar rings of the helix. In our calculation, their conformation, which was based on the Sobell model, remained fixed as the structure and energy were optimized. Recent work has shown that the energy changes by only 0.5 kcal as the sugar pucker changes from C3'-endo to C2'-endo. This suggests that as the torsional angle of the sugar-phosphate backbone and glycosidic bond are optimized, the ring pucker should also be varied. The conformation of the helix depends to a great extent on the puckering on the sugar ring.

Our calculations, which uses the Fletcher-Powell minimizer, can not easily change the sugar pucker as a variable. An alternative procedure would be to take the starting guess of Sobell and optimize the sugar-phosphate-sugar fragment of the dinucleoside monophosphate using the Levitt-Warshel Consistent Force Field program.[71] This would allow this part of the helix to "relax" (the sugars could pucker along with the torsional changes of the sugar-phosphate backbone). The coordinates from this program could then be used as a starting guess in the Fletcher-Powell program. After several iterations of the minimizer, the sugar-phosphate backbone could be reoptimized with the Levitt-Warshel program. After several calculations with the two programs, Fletcher-Powell and Levitt-Warshel, one might obtain a structure which is consistent with both programs. This procedure would not only allow variations in the torsional angles, but also in the conformation of the sugar rings of the sugar-phosphate backbone.

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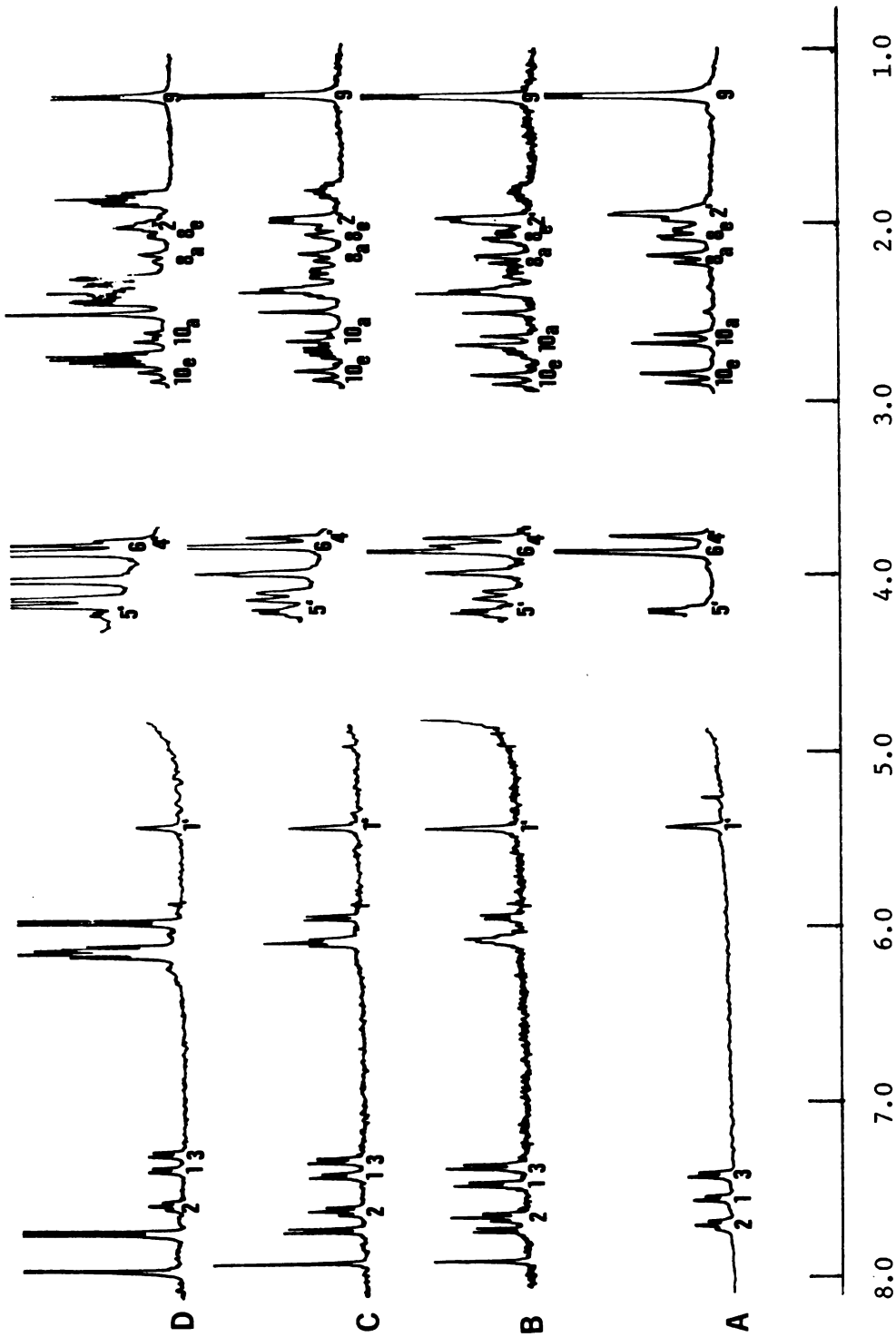
Appendix A

360 MHz spectrum of (a) daunomycin; (b) d-pCpG and daunomycin in a 0:5:1 ratio; (c) d-pCpG and daunomycin in a 1:1 ratio; (d) d-pCpG and daunomycin in a 4:1 ratio. The numbers below each labeled resonance corresponds to the carbon atom to which the proton is bonded except for the methoxy and methyl protons. These protons (methoxy and methyl) are denoted as 6 and 9 respectively.


D
C
B
A
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
PPM (Relative to DSS)

Appendix B

Section 1: This section contains the atomic coordinates and charges for the deoxydinucleoside monophosphates (optimized structures except where otherwise indicated) in the following complexes (the 3rd, 4th and 5th columns are the x, y, and z coordinates respectively while the 6th column contains the charges for each of the atoms):

I (a) BDNA (starting guess)

I (b) Ethidium Bromide (starting guess)

I (c) BDNA

I (d) Ethidium Bromide

I (e) Ethidium Bromide without the phenyl and ethyl side chains

I (f) Proflavine (minor)

I (g) Proflavine (major)

I (h) 10-Aminoacridine (minor)

I (i) 10-Aminoacridine (major)

Section II: This section contains the x, y, and z coordinates (3rd, 4th, and 5th columns respectively) and the charges (6th column) for the atoms of the different intercalators:

II (a) Ethidium

II (b) Ethidium Bromide without phenyl and ethyl side chains

II (c) Proflavine (minor)

II (d) Proflavine (major)

II (e) 10-Aminoacridine (minor)

II (f) 10-Aminoacridine (major)

(a)

7	N	1	.190945	-.806213	3.432022	-.360000	
8	H	1	.681342	-.072924	3.350283	.218000	
9	C	2	.952897	1.456295	3.484902	.388000	
10	N	2	2.275484	1.785986	3.421794	-.420000	
11	HB	2	2.772901	.909770	3.351632	.240000	
12	HN	2	2.840747	2.621678	3.468664	.218000	
13	N	3	.432011	3.174309	3.603307	-.353000	
14	C	4	-.926895	3.149555	3.651914	.201000	
15	C	5	-1.763867	2.060146	3.594542	-.022000	
16	C	6	-1.203180	.761668	3.468115	.299000	
17	O	6	-1.778838	-.323257	3.401374	-.284000	
18	N	7	-3.092736	2.459508	3.676503	-.252000	
19	C	8	-3.021340	3.763473	3.779831	.126000	
20	H	8	-3.893075	4.395492	3.863674	.084000	
21	N	9	-1.732799	4.257437	3.771984	-.180000	
22	C	1	-1.264913	5.667628	3.869784	.180000	
23	C	1	-.283132	5.758299	3.405040	.056000	
24	C	2	-2.186415	6.668861	3.181314	-.132000	
25	HA	2	-1.819521	6.868423	2.174505	.085000	
26	HB	2	-3.194088	6.257089	3.125282	.059000	
27	C	3	-2.085045	7.880659	4.106545	.008000	
28	O	3	-2.986102	8.486733	4.012367	.028000	
29	C	4	-1.946475	7.240799	5.487183	.060000	
30	H	4	-1.413777	7.921077	6.151680	.072000	
31	O	4	-1.198218	6.014504	5.243952	-.265000	
32	C	5	-3.245977	6.891730	6.185540	.007000	
33	HA	5	-3.195568	5.823433	6.396020	.068000	
34	HB	5	-3.470543	7.400487	7.123003	.054000	
35	O	5	-4.411791	7.034816	5.345576	-.338000	
36	HO	5	-5.284649	7.114510	5.737199	.194000	
37	P	3	-.938035	8.643442	3.757587	-.477000	
38	O		-.658612	8.964553	2.215248	1.343000	
39	OA		-.053979	10.309549	2.089531	-.738000	
40	OB		-1.892747	8.738869	1.430151	-.738000	
41	O	5	.426712	7.847024	1.850281	-.477000	
42	C	5	1.444451	7.520733	2.815341	-.032000	
43	HA	5	1.684417	8.405865	3.404456	.033000	
44	HB	5	1.080873	6.733191	3.475407	.033000	
45	C	4	2.696324	7.040859	2.107674	.060000	
46	H	4	2.641864	5.961796	1.963598	.072000	
47	C	3	2.951245	7.643485	.726863	.067000	
48	O	3	2.594584	8.673305	.707826	.053000	
49	O	3	4.317006	7.694608	.292769	-.309000	
50	HO	3	4.444182	7.613217	-.655283	.187000	
51	C	2	2.151126	6.724682	-.195305	-.132000	
52	HA	2	2.580382	6.751018	-1.196877	.085000	
53	HB	2	1.115375	7.061884	-.235494	.059000	
54	C	1	2.312771	5.371486	.489060	.180000	
55	O	1	3.138383	4.827815	.029835	.056000	
56	N	1	2.579608	5.610178	1.863187	-.265000	
57	H	1	1.103856	4.505362	.397107	-.217000	
58	C	2	1.296771	3.132193	.275236	.366000	
59	C	2	2.453598	2.692072	.248968	-.314000	
60	O	3	.207699	2.325457	.190007	-.357000	
61	C	4	-1.027568	2.841147	.223199	.272000	
62	C	5	-1.248435	4.251463	.348562	-.138000	
63	C	6	-2.229521	4.700185	.398735	.065000	
64	O	6	-.147146	5.039948	.431642	.100000	
65	N	7	-.436844	6.076466	.521681	.094000	
66	H	7	-2.057271	2.011769	.136774	-.415000	
67	HN	4	-2.984901	2.409253	.176872	.233000	
68	HB	4	-1.904078	1.017866	.043003	.213000	
69	N	1	.628049	-.540082	-.053026	-.360000	
70	H	1	.508245	.459458	.028622	.218000	
71	C	2	1.920405	-1.022863	-.105902	.388000	
72	C	2	2.890441	-.107829	-.042911	-.420000	
73	HB	2	2.778438	.869477	.026882	.240000	
74	HN	2	3.833266	-.449775	-.089500	.218000	
75	N	3	2.214723	-2.314483	-.224193	-.353000	
76	C	4	1.100671	-3.093031	-.272691	.201000	
77	C	5	-.216729	-2.703428	-.215314	-.022000	
78	C	6	-.526129	-1.323313	-.089008	.299000	
79	O	6	-1.629462	-.783773	-.022282	-.284000	
80	N	7	-1.057244	-3.807484	-.297143	-.252000	
81	C	8	-.233194	-4.820588	-.400402	.126000	
82	H	8	-.567115	-5.844250	-.484137	.084000	
83	N	9	1.099659	-4.463036	-.392630	-.180000	
84	C	1	2.306937	-5.329087	-.490387	.180000	
85	C	1	3.154602	-4.825455	-.025719	.056000	
86	O	2	2.149744	-6.680657	.198216	-.132000	
87	HA	2	2.563908	-6.626420	1.205006	.085000	
88	HB	2	1.092448	-6.939651	.254306	.059000	
89	C	3	2.943858	-7.601651	-.726953	.008000	
90	O	3	2.574392	-8.612179	-.633531	.028000	
91	C	4	2.679913	-7.002634	-2.107640	.060000	
92	H	4	3.510673	-7.240072	-2.772140	.072000	
93	O	4	2.564700	-5.570685	-1.864541	-.265000	
94	C	5	1.423318	-7.483927	-2.805911	.007000	
95	HA	5	.836305	-6.589955	-3.016457	.068000	
96	HB	5	1.540565	-8.027625	-3.743326	.054000	
97	O	5	.564159	-8.284720	-1.965844	-.338000	
98	HO	5	-.095258	-8.862180	-2.357391	.194000	
99	O	3	4.320180	-7.544743	-.378044	-.477000	
100	P		4.735017	-7.640205	1.164291	1.343000	
101	OA		6.014633	-8.373125	1.290036	-.738000	
102	OB		3.603867	-8.182778	1.949475	-.738000	
103	O	5	4.956450	-6.098168	1.529105	-.477000	
104	C	5	5.588134	-5.236172	.563942	-.032000	
105	HA	5	6.302429	-5.811379	-.025141	.033000	
106	HB	5	4.831132	-4.812687	-.096140	.033000	
107	C	4	6.319056	-4.112161	1.271479	.060000	
108	H	4	5.640877	-3.271072	1.415497	.072000	
109	C	3	6.879497	-4.449815	2.652305	.067000	
110	O	3	7.196103	-5.492645	2.671430	.053000	
111	O	3	8.014597	-3.688528	3.086296	-.309000	
112	HO	3	8.069692	-3.547851	4.034333	.187000	
113	C	2	5.692201	-4.176511	3.574485	-.132000	
114	HA	2	6.055025	-3.945470	4.576023	.085000	
115	HB	2	5.052326	-5.058008	3.614777	.059000	
116	C	1	5.027752	-2.986700	2.840027	.180000	
117	O	1	5.376284	-2.061591	3.349154	.056000	
118	N	1	5.383876	-3.023149	1.515892	-.265000	
119	H	1	3.540626	-2.996332	2.982029	-.217000	
120	C	2	2.889765	-1.771907	3.103804	.366000	
121	C	2	3.567124	-.735980	3.129953	-.314000	
122	O	3	1.534504	-1.759164	3.189076	-.357000	
123	C	4	.838086	-2.902331	3.156014	.272000	
124	C	5	1.488160	-4.173237	3.030751	.138000	
125	H	5	.956048	-5.112851	2.980685	.065000	
126	C	6	2.842580	-4.164035	2.947627	.100000	
127	O	6	3.217298	-5.172943	2.857672	.094000	
128	N	7	-.482444	-2.835379	3.242475	-.415000	
129	HB	4	-.999415	-3.[illegible]160	3.202476	.233000	
130	HN	4	-.942564	-1.[illegible]170	3.336176	.213000	
131	NA	L	-1.585864	10.[illegible]177	.872847	1.000000	
132	NA	R	4.952936	-9.520373	2.507377	1.000000	

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7	N	1	3.190932	1.680740	3.155037	-.360000	
8	H	1	3.051103	.691595	3.303636	.218000	
9	C	2	4.489245	2.148184	3.171736	.388000	
10	N		5.441421	1.230065	3.386256	-.420000	
11	HA	2	5.187834	.262116	3.523621	.240000	
12	HB	2	6.412693	1.505999	3.410581	.218000	
13	C	3	4.809712	3.426052	2.989378	-.353000	
14	C	4	3.714586	4.206812	2.786829	.201000	
15	C	5	2.392310	3.830960	2.753713	.022000	
16	C	6	2.054958	2.465498	2.948930	.299000	
17	O	6	.943106	1.939524	2.954788	.284000	
18	N	7	1.576823	4.931972	2.519507	-.252000	
19	CA	8	2.419929	5.929556	2.419018	.126000	
20	CB	8	2.108121	6.946844	2.233811	.084000	
21	N	9	3.742076	5.564529	2.570941	-.180000	
22	C		4.975471	6.399184	2.524149	.180000	
23	HC	1	5.733255	5.965498	3.176666	.056000	
24	C	2	5.337499	6.334496	1.100896	-.132000	
25	HA	2	6.415962	6.208597	1.005143	.077000	
26	HB	2	4.830766	5.489575	.634602	.077000	
27	C	3	4.833975	7.695061	.614867	.008000	
28	C	3	3.851202	7.577428	.158336	.028000	
29	C	4	4.889560	8.524082	1.911491	.060000	
30	O	4	5.889306	8.940596	2.034471	.072000	
31	N	5	4.600812	7.616303	2.937436	-.265000	
32	C	5	3.927167	9.713144	1.940295	.007000	
33	HA	5	4.496996	10.641674	1.975344	.061000	
34	HB	5	3.337788	9.686099	2.868681	.061000	
35	O	5	3.071604	9.662617	.795586	-.338000	
36	N	5	2.608991	10.498812	.704116	.194000	
37	H	5	2.704096	8.386050	-.301116	-.477000	
38	CA		5.280263	8.614388	-1.826969	.343000	
39	CB		5.936911	9.759449	-2.496334	-.738000	
40	C		3.847375	8.983495	-1.858442	-.738000	
41	O		5.388536	7.256312	-2.614749	-.477000	
42	N		6.721690	8.422233	-2.225037	-.032000	
43	HA	5	6.497560	5.920731	-1.283569	.033000	
44	HB	5	7.607208	7.045810	-2.102116	.033000	
45	C	4	7.063695	5.326849	-3.266989	.060000	
46	O	4	8.077436	4.965047	-3.095148	.072000	
47	C	3	7.027507	5.692283	-4.741963	.067000	
48	C	3	7.020421	7.777092	-4.647978	.053000	
49	O	3	8.182159	5.157776	-5.394636	-.309000	
50	HC	3	8.145972	5.365748	-6.331128	.187000	
51	C	2	5.736580	5.037600	-5.172075	-.132000	
52	HA	2	5.806050	4.750771	-6.221362	.077000	
53	HB	2	4.912023	5.738395	-5.041339	.077000	
54	C		5.765368	3.836974	-4.145397	.180000	
55	O		6.405346	3.043500	-4.531308	.056000	
56	N		6.350575	4.236624	-2.914864	-.265000	
57	H		4.384393	3.284071	-4.059913	-.217000	
58	CA	2	4.246134	1.923458	-3.875482	.366000	
59	CB	2	5.256727	1.227574	-3.789756	-.314000	
60	N	3	2.990151	1.407908	-3.796024	-.357000	
61	C	4	1.910935	2.196173	-3.893651	.272000	
62	O	4	2.038398	3.597640	-4.083501	-.138000	
63	H	5	1.196205	4.270739	-4.147133	.065000	
64	C	6	3.304163	4.089188	-4.159802	.100000	
65	H	6	3.280336	5.159181	-4.304535	.094000	
66	N	4	.713188	1.647683	-3.810219	-.415000	
67	HN		-.088439	2.258985	-3.871894	.233000	
68	HB		.617651	.650610	-3.680534	.213000	
69	N		3.191690	-1.680106	-3.155192	-.360000	
70	H		3.051861	-.690961	-3.303991	.218000	
71	C	2	4.490003	-2.147550	-3.171891	.388000	
72	N	2	5.442179	-1.229431	-3.386411	-.420000	
73	HA	2	5.188592	-.261483	-3.523777	.240000	
74	HB	2	6.413451	-1.505365	-3.410736	.218000	
75	C	3	4.810469	-3.425418	-2.989533	-.353000	
76	C	4	3.715344	-4.205978	-2.786984	.201000	
77	C	5	2.393068	-3.830326	-2.753869	-.022000	
78	C	6	2.055716	-2.464865	-2.949086	-.299000	
79	O	6	.943863	-1.938890	-2.954943	-.284000	
80	N	7	1.577581	-4.931338	-2.519662	-.252000	
81	CA	8	2.420686	-5.928922	-2.419173	.126000	
82	CB	8	2.108879	-6.946210	-2.233966	.084000	
83	N	9	3.742834	-5.563895	-2.571096	-.180000	
84	C		4.976228	-6.398550	-2.524304	.180000	
85	HC	1	5.727061	-5.968842	-3.170835	.056000	
86	C	2	5.338257	-7.333862	-1.101051	-.132000	
87	HA	2	6.416719	-6.207984	-1.005298	.077000	
88	HB	2	4.831523	-5.488941	-.634757	.077000	
89	C	3	4.834733	-7.694427	-.615022	.008000	
90	C	3	3.851960	-7.576795	-.158491	.028000	
91	C	4	4.890318	-8.523448	-1.911646	.060000	
92	O	4	5.890064	-8.939962	-2.034626	.072000	
93	N	5	4.601570	-7.615669	-2.937591	-.265000	
94	C	5	3.927965	-9.712510	-1.940450	.007000	
95	HA	5	4.497754	-10.641040	-1.975499	.061000	
96	HB	5	3.343904	-9.685711	-2.860396	.061000	
97	O	5	3.072363	-9.661984	-.795740	-.338000	
98	N	5	2.609751	-10.498160	-.704271	.194000	
99	H	3	5.704852	-8.385417	.300962	-.477000	
100	C		5.281017	-8.613755	1.826814	1.343000	
101	CA		5.937662	-9.758817	2.496179	-.738000	
102	CB		3.848128	-8.982859	1.858285	-.738000	
103	O	5	5.589291	-7.255679	2.614594	-.477000	
104	C	5	6.722446	-6.421602	2.224883	-.032000	
105	HA	5	6.498318	-5.920100	1.283415	.033000	
106	HB	5	7.607964	-7.045180	2.101963	.033000	
107	C	4	7.064453	-5.326219	3.266836	.060000	
108	O	4	8.078195	-4.964420	3.094997	.072000	
109	N	3	7.028263	-5.691653	4.741810	.067000	
110	C	3	7.021175	-6.776462	4.847825	.053000	
111	O	3	8.182915	-5.157147	5.394484	-.309000	
112	HC	3	8.146727	-5.365169	6.330976	.187000	
113	C	2	5.737336	-5.036968	5.171921	-.132000	
114	HA	2	5.806805	-4.750139	6.221208	.077000	
115	HB	2	4.912778	-5.737762	5.041164	.077000	
116	C		5.766127	-3.836342	4.145243	.180000	
117	O		6.406106	-3.042869	4.531155	.056000	
118	N		6.351333	-4.235994	2.914710	-.265000	
119	H		4.385153	-3.283437	4.059757	-.217000	
120	CA	2	4.246896	-1.922823	3.875326	.366000	
121	CB	2	5.257490	-1.226941	3.789602	-.314000	
122	N	2	2.990914	-1.407272	3.795868	-.357000	
123	C	4	1.911696	-2.195535	3.893494	.272000	
124	O	5	2.039158	-3.597003	4.083343	.138000	
125	C	5	1.196963	-4.270100	4.146975	.065000	
126	C	6	3.304921	-4.088552	4.159640	.100000	
127	H	6	3.281093	-5.158545	4.304378	.094000	
128	N	4	.713951	-1.647043	3.810060	-.415000	
129	H		-.087677	-2.258344	3.871735	.233000	
130	H		.618415	-.649970	3.680375	.213000	
131	NA	L	4.078249	10.427555	-2.635662	1.000000	
132	NA	R	4.536823	-10.596934	2.775271	1.000000	

7	N	1	.188121	-.890797	3.113236	-.360000
8	HC	1	.744782	.064345	3.278178	.218000
9	C	2	.860613	2.070107	2.862166	.388000
10	N	2	2.193546	1.995849	2.863250	-.420000
11	HN	2	2.756554	1.175671	3.038631	.240000
12	HN	2	2.693486	2.855980	2.689082	.218000
13	N	3	.247295	3.229247	2.640501	-.353000
14	C	3	-1.106906	3.109457	2.677782	.201000
15	C	3	-1.858518	1.981156	2.907224	-.022000
16	C	3	-1.199303	.745820	3.143403	.299000
17	O	3	-1.690462	-.359717	3.365729	-.284000
18	N	3	-3.215304	2.279837	2.862260	-.252000
19	C	3	-3.244160	3.564976	2.609699	.126000
20	H	3	-4.162617	4.124182	2.509273	.084000
21	N	3	-1.996200	4.140451	2.484109	-.180000
22	C	3	-1.637560	5.557897	2.202422	.180000
23	C	3	-.570017	5.702914	2.368017	.056000
24	C	2	-1.937684	5.994049	.772275	-.132000
25	HA	2	-1.037550	5.895889	.165460	.085000
26	HB	2	-2.725160	5.364745	.357635	.059000
27	C	3	-2.371977	7.445828	.968516	.008000
28	C	3	-3.021322	7.745712	.146007	.028000
29	H	3	-3.102022	7.401570	2.310104	.060000
30	H	3	-3.050372	8.381348	2.784939	.072000
31	O	1	-2.392502	6.384144	3.074482	-.265000
32	C	3	-4.571333	7.033208	2.249352	.007000
33	HA	3	-4.687595	6.157982	2.888542	.068000
34	HB	3	-5.295142	7.778559	2.578976	.054000
35	O	5	-5.002468	6.583017	.946821	-.338000
36	H	5	-5.934028	6.590837	.715014	.194000
37	O	3	-1.221080	8.277842	1.017795	-.477000
38	P	3	-.376992	8.515965	-.320414	1.343000
39	OA		.179950	9.887194	-.318985	-.738000
40	OB		-1.200830	8.156250	-1.496729	-.738000
41	O	5	.804590	7.451747	-.143610	-.477000
42	C	5	1.748260	7.628197	.929681	-.032000
43	HA	5	1.925900	8.692091	1.086755	.033000
44	HB	5	1.347041	7.188646	1.842872	.033000
45	C	4	3.060085	6.951427	.584205	.060000
46	N	4	2.931484	5.869585	.618534	.072000
47	C	3	3.628157	7.270057	-.798023	.067000
48	C	3	3.381200	8.297631	-1.064864	.053000
49	O	3	5.049978	7.156238	-.947277	-.309000
50	H	3	5.346133	6.894256	-1.821466	.187000
51	C	2	5.933510	6.244702	-1.692951	-.132000
52	HA	2	3.545269	6.058463	-2.575655	.085000
53	HB	2	1.961004	6.629405	-2.000094	.059000
54	C	1	2.833063	5.035517	-.769152	.180000
55	O	1	3.675178	4.367772	-.950976	.056000
56	N	1	2.856209	5.509742	-.569121	-.269000
57	H	1	1.586637	4.240756	-.955907	-.217000
58	C	2	1.614023	2.896230	-.596512	.366000
59	C	2	2.662641	2.421556	-.140740	-.314000
60	N	3	.488153	2.154485	-.758980	-.357000
61	C	3	-.626653	2.706190	-1.254711	.272000
62	C	3	-.676492	4.088146	-1.630482	-.138000
63	O	3	-1.562947	4.571070	-2.014388	.065000
64	C	3	.459081	4.811427	-1.460817	.100000
65	C	3	.301661	5.831969	-1.777159	.094000
66	N	4	-1.698755	1.940062	-1.393635	-.415000
67	HN	4	-2.538618	2.369003	-1.755236	.233000
68	HN	4	-1.664508	.964501	-1.134465	.213000
69	N	1	.676925	-.606140	.212824	-.360000
70	C	1	.640921	.390376	.052330	.218000
71	C	2	1.914810	-1.165733	.458900	.388000
72	C	2	2.949321	-.321925	.459399	-.420000
73	HB	2	2.922344	.669192	.289119	.240000
74	HN	2	3.854396	-.722072	.628920	.218000
75	N	3	2.100715	-2.464911	.674480	-.353000
76	C	4	-.934825	-3.164096	.636520	.201000
77	C	5	-.337020	-2.692380	.411757	-.022000
78	C	6	-.530632	-1.304519	.182067	.299000
79	O	6	-1.578422	-.698116	-.035449	.284000
80	N	7	-1.258780	-3.731933	.454046	-.252000
81	H	7	-.525972	-4.789510	.700262	.126000
82	C	8	-.939881	-5.782307	.797379	.084000
83	C	9	.822096	-4.521708	.824464	-.180000
84	N	1	1.946194	-5.458652	1.999733	.180000
85	H	1	2.894631	-4.947529	.934436	.056000
86	C	2	1.962814	-5.994167	2.527511	-.132000
87	HA	2	2.634439	-5.388193	3.135616	.085000
88	HB	2	.956662	-5.950019	2.944414	.059000
89	C	3	2.464735	-7.422972	2.323963	.008000
90	O	3	2.120694	-8.045190	3.136908	.028000
91	N	4	1.845445	-7.810533	.981928	.060000
92	H	4	2.462336	-8.370585	.502484	.072000
93	O	5	1.819506	-6.567033	.223055	-.265000
94	C	5	.440492	-8.376771	1.043076	.007000
95	HA	5	-.169473	-7.734385	.407952	.068000
96	HB	5	-.292595	-9.403796	.709241	.054000
97	O	5	-.170272	-8.271846	2.347336	-.338000
98	H	5	-.918714	-8.826929	2.578244	.194000
99	O	3	3.884771	-7.419037	2.271788	-.477000
100	P	3	4.710301	-7.121232	3.609627	1.343000
101	OA		5.967034	-7.902876	3.602186	-.738000
102	OB		3.835991	-7.321439	4.786869	-.738000
103	O	5	5.039943	-5.564885	3.438979	-.477000
104	C	5	5.904799	-5.148037	2.365732	-.032000
105	HA	5	6.673712	-5.903443	2.203761	.033000
106	HB	5	5.319944	-5.024398	1.454273	.033000
107	C	4	6.568686	-3.830813	2.715635	.060000
108	O	4	5.828495	-3.031214	2.686336	.072000
109	C	3	7.218368	-3.760596	4.096854	.067000
110	C	3	7.623348	-4.738141	4.358574	.053000
111	O	3	8.302994	-2.834794	4.247955	-.309000
112	H	3	8.389121	-2.450977	5.123665	.187000
113	C	2	6.055436	-3.343593	4.996011	.132000
114	HA	2	6.442579	-2.837165	5.880155	.085000
115	HB	2	5.449627	-4.227928	5.300421	.059000
116	C	1	5.261318	-2.420530	4.077884	.180000
117	O	1	5.550239	-1.386068	4.263660	.056000
118	N	1	5.556169	-2.784628	2.737391	-.265000
119	H	1	5.786198	-2.511371	4.267268	-.217000
120	C	2	3.016854	-1.406935	3.912310	.366000
121	O	2	3.584848	-.405077	3.458099	-.314000
122	N	3	1.670367	-1.469680	4.077232	-.357000
123	C	4	1.094228	-2.572872	4.571149	.272000
124	C	5	1.867458	-3.720829	4.942360	-.138000
125	O	5	1.435326	-4.633789	5.324677	.065000
126	C	6	3.210920	-3.037425	4.770301	.100000
127	H	6	3.684473	-4.556225	5.083277	.094000
128	N	4	-.223150	-2.584227	4.712607	-.415000
129	HN	4	-.649409	-3.426050	5.072797	.233000
130	HN	4	-.769752	-1.774402	4.456656	.213000
131	NA	L	-.769894	10.010650	-2.051888	1.000000
132	NA	X	5.273213	-8.568381	5.334529	1.000000

7		N	1	5.425070	.138730	3.800203	-.360000	
8			1	5.641861	-.843201	3.894626	.218000	
9			2	6.420394	1.041828	4.113502	.388000	
10				7.585507	.515923	4.515418	-.420000	
11		NA	2	7.693633	-.466568	4.573905	.240000	
12		NH	2	8.355564	1.122024	4.759868	.218000	
13		N	3	6.264896	2.360197	4.032131	-.353000	
14		C	4	5.019905	2.705540	3.607472	.201000	
15			5	3.971561	1.880404	3.274681	-.022000	
16		C	6	4.142245	.473475	3.363106	.299000	
17		CO	6	3.333238	-.418339	3.111958	-.284000	
18		N	7	2.865104	2.627360	2.887500	-.252000	
19		C	8	3.271938	3.867831	2.996202	.126000	
20		N	8	2.643090	4.715359	2.766752	.084000	
21			9	4.575777	3.994952	3.430073	-.180000	
22			1	5.386536	5.220954	3.674384	.180000	
23		C	1	6.149647	5.009256	4.423346	.056000	
24		C	2	5.947569	5.489838	2.342527	-.132000	
25		HA	2	6.993177	5.781792	2.440332	.077000	
26		HB	2	5.877635	4.590936	1.730003	.077000	
27		C	3	5.036100	6.624759	1.871274	.008000	
28			3	4.251270	6.219709	1.232465	.028000	
29		C	4	4.580549	7.229619	3.212250	.060000	
30			4	5.314323	7.961892	3.549077	.072000	
31		O	4	4.517470	6.147191	4.097896	-.265000	
32		C	5	3.242016	7.967893	3.147585	.007000	
33		HA	5	3.399800	9.026208	3.355370	.061000	
34		HB	5	2.580572	7.599512	3.945575	.061000	
35		O	5	2.647039	7.765902	1.863023	-.338000	
36		H	5	1.917203	8.378842	1.747946	.194000	
37		O	3	5.696659	7.705260	1.185869	-.477000	
38				5.781941	7.738074	-.411519	1.343000	
39		O	A	6.485685	8.910011	-.978714	-.738000	
40		O	B	4.408748	7.880273	-.944923	-.738000	
41		O	5	6.491405	6.381983	-.878078	-.477000	
42		C	5	7.910373	6.165888	-.610809	-.032000	
43		HA	5	8.024139	5.592519	.309194	.033000	
44		HB	5	8.410953	7.128111	-.502886	.033000	
45		C	4	8.634956	5.387150	-1.738216	.060000	
46		H	4	9.507588	4.879643	-1.327059	.072000	
47		C	3	9.165517	6.164863	-2.931560	.067000	
48			3	9.191341	7.227649	-2.690898	.053000	
49		O	3	10.486407	5.715588	-3.245095	-.309000	
50		H	3	10.811261	6.188041	-4.015068	.187000	
51		C	2	8.144081	5.811740	-3.986108	-.132000	
52		HA	2	8.613640	5.834450	-4.969520	.077000	
53		HB	2	7.327214	6.532850	-3.957352	.077000	
54		C	1	7.785401	4.370364	-3.447422	.180000	
55			1	8.530640	3.655613	-3.796494	.056000	
56		C	1	7.849715	4.329000	-2.029483	-.265000	
57			1	6.481920	3.975132	-4.051433	-.217000	
58		C	2	9.455535	2.844658	-4.842466	.366000	
59			2	7.497200	2.214120	-5.016408	-.314000	
60		O	3	5.271043	2.474593	-5.398911	-.357000	
61			4	4.155045	3.185664	-5.187898	.272000	
62		C	5	4.167638	4.350805	-4.376302	-.138000	
63		H	5	3.288071	4.940638	-4.164504	.065000	
64		C	6	5.363568	4.700337	-3.831205	.100000	
65		H	6	5.255138	5.596262	-3.237946	.094000	
66		C	4	3.030010	2.785247	-5.750359	-.415000	
67		HN	4	2.196159	3.325480	-5.568859	.233000	
68		H	B	3.017393	1.960141	-6.332716	.213000	
69			1	5.351862	-.109065	-3.902570	-.360000	
70			1	5.629227	.857871	-3.993137	.218000	
71		N	2	6.270843	-1.070512	-4.270668	.388000	
72		NA	2	7.450295	-.615981	-4.715521	-.420000	
73			2	7.622224	.378037	-4.765214	.240000	
74		HN	2	8.167356	-1.267404	-5.001126	.218000	
75			3	6.031667	-2.376935	-4.200289	-.353000	
76		C	4	4.785543	-2.646431	-3.726848	.201000	
77			4	3.809346	-1.759593	-3.338029	-.022000	
78		C	6	4.069246	-.365699	-3.414925	.299000	
79			6	3.332622	.573236	-3.117123	-.284000	
80		O	7	2.673057	-2.438288	-2.913388	-.252000	
81		N	8	2.991611	-3.700920	-3.056163	.126000	
82		C	8	2.318099	-4.508832	-2.811092	.084000	
83		N	8	4.264617	-3.906524	-3.547672	-.180000	
84			1	4.981071	-5.179105	-3.843190	.180000	
85		C	1	5.760936	-4.994058	-4.567046	.056000	
86		C	2	5.501869	-5.553817	-2.520594	-.132000	
87		HA	2	6.522905	-5.921533	-2.622426	.077000	
88		HB	2	5.493710	-4.682259	-1.866055	.077000	
89		C	3	4.499004	-6.635191	-2.113420	.008000	
90			3	3.739533	-6.200862	-1.463294	.028000	
91		C	4	4.015754	-7.138373	-3.486328	.060000	
92			4	4.694960	-7.908891	-3.851133	.072000	
93		O	4	4.048515	-6.013548	-4.319211	-.265000	
94		C	5	2.623283	-7.772141	-3.470157	.007000	
95		HA	5	2.701299	-8.828495	-3.727312	.061000	
96		HB	5	2.008659	-7.319974	-4.248542	.061000	
97		O	5	2.028778	-7.585466	-2.183061	-.338000	
98		H	5	1.252165	-8.144331	-2.104686	.194000	
99		O	3	5.064675	-7.795242	-1.474751	-.477000	
100				5.154029	-7.896870	.119516	1.343000	
101		O	A	5.696331	-9.172652	.637868	-.738000	
102		O	B	3.777336	-7.871869	.662186	-.738000	
103		O	5	6.042843	-6.666487	.625644	-.477000	
104		C	5	7.481862	-6.649768	.379587	-.032000	
105		HA	5	7.684017	-6.140056	-.562446	.033000	
106		HB	5	7.853664	-7.672971	.325547	.033000	
107		C	4	8.284671	-5.919752	1.486418	.060000	
108		H	4	9.199216	-5.507121	1.060458	.072000	
109		C	3	8.745965	-6.718675	2.694445	.067000	
110			3	8.668545	-7.784011	2.477267	.053000	
111		O	3	10.105707	-6.391221	2.992397	-.309000	
112		H	3	10.388754	-6.875828	3.771268	.187000	
113		C	2	7.769638	-6.246669	3.745205	-.132000	
114		HA	2	8.241066	-6.292937	4.726895	.077000	
115		HB	2	6.887405	-6.886730	3.735858	.077000	
116		C	1	7.547012	-4.789573	3.176251	.180000	
117			1	8.359357	-4.142053	3.506273	.056000	
118		C	1	7.606040	-4.785245	1.757485	-.265000	
119			1	6.291123	-4.258411	3.776841	-.217000	
120		C	2	6.355230	-3.048645	4.437716	.366000	
121			2	7.434348	-2.462200	4.504710	-.314000	
122		O	3	5.214856	-2.554313	4.989788	-.357000	
123		C	4	4.055899	-3.220569	4.897474	.272000	
124			5	3.975403	-4.466213	4.220502	-.138000	
125		C	6	3.058624	-5.025469	4.105835	.065000	
126			6	5.128733	-4.939568	3.677026	.100000	
127		C	4	4.950197	-5.887419	3.191121	.094000	
128		N	4	2.976669	-2.698788	5.450111	-.415000	
129			4	2.109298	-3.208069	5.358456	.233000	
130			4	3.030288	-1.815633	5.937207	.213000	
131		NA	+	4.715182	9.277675	-1.787536	1.000000	
132		NA	+	4.318259	-9.607362	1.446959	1.000000	

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7		N	1	4.800822	1.040300	3.266917	-.360000
8		H	1	4.914454	.038919	3.333427	.218000
9		C	2	5.935687	1.817177	3.380676	.388000
10	N		2	7.084782	1.154321	3.569895	-.420000
11	HA		2	7.083363	.145627	3.621223	.240000
12	HB		2	7.952310	1.663400	3.661187	.218000
13		N	3	5.923754	3.145309	3.311227	-.353000
14		C	4	4.671335	3.638471	3.116706	.201000
15		C	5	3.490295	2.945418	2.992422	-.022000
16		C	6	3.507947	1.527500	3.067230	.299000
17		O	6	2.567639	.739355	2.980351	.284000
18		N	7	2.427096	3.820742	2.803965	-.252000
19		C	8	2.989697	5.003677	2.819612	.126000
20		H	8	2.433363	5.921276	2.697526	.084000
21		N	9	4.356697	4.972657	3.006021	-.180000
22			1	5.336689	6.092499	3.081323	.180000
23		C	1	6.104508	5.855170	3.817685	.056000
24		C	2	5.854690	6.141630	1.706492	-.132000
25	HA		2	6.933780	6.293740	1.729465	.077000
26	HB		2	5.631333	5.202788	1.199750	.077000
27		C	3	5.079107	7.337545	1.150471	.008000
28		O	3	4.213164	6.981864	.592140	.028000
29		C	4	4.783117	8.135193	2.434117	.060000
30		N	4	5.625865	8.789056	2.658643	.072000
31		C	1	4.824236	7.169427	3.435142	-.265000
32		C	5	3.554134	9.041911	2.342659	.007000
33	HA		5	3.863553	10.083711	2.426392	.061000
34	HB		5	2.894116	8.653606	3.202263	.061000
35	O	5		2.867792	8.793076	1.113060	-.338000
36	HP		5	2.221510	9.486430	.960806	.194000
37	O	3		5.839907	8.238942	.324476	-.477000
38		P		5.964078	8.004701	-1.253407	1.343000
39		OA		6.741570	9.032295	-1.981387	-.738000
40		OB		4.613102	8.123188	-1.846047	-.738000
41		C	5	6.611901	6.557853	-1.470106	-.477000
42		C	5	8.031207	6.341471	-1.204872	-.032000
43	HA		5	8.188372	6.232733	-.131758	.033000
44	HB		5	8.603815	7.193668	-1.570907	.033000
45		C	4	8.596239	5.071102	-1.890001	.060000
46	H		4	9.418069	4.672059	-1.295476	.072000
47		C	3	9.159836	5.195225	-3.296185	.067000
48		O	3	9.329890	6.246508	-3.528531	.053000
49		O	3	10.397209	4.483230	-3.379075	-.309000
50	HP		3	10.739877	4.539332	-4.274078	.187000
51		C	2	8.051331	4.571233	-4.109769	-.132000
52	HA		2	8.470728	4.114623	-5.006269	.077000
53	HB		2	7.333203	5.339660	-4.395993	.077000
54		C	1	7.542848	3.553070	-3.013801	.180000
55		O	1	8.174910	2.665068	-3.020970	.056000
56		O	1	7.671706	4.104397	-1.711560	-.265000
57		N	2	6.171930	3.118791	-3.403783	-.217000
58		C	2	5.919606	1.763510	-3.466603	.366000
59		O	2	6.825818	.975302	-3.201262	-.314000
60		N	3	4.671650	1.355765	-3.821519	-.357000
61		C	4	3.707880	2.242871	-4.104055	.272000
62		C	5	3.953471	3.040159	-4.043460	.138000
63	H		5	3.200528	4.388107	-4.243584	.065000
64		C	6	5.208675	4.022902	-3.686291	.100000
65	H	H	6	5.280414	5.100448	-3.674066	.094000
66		N	4	2.512777	1.796920	-4.443550	-.415000
67	HN		4	1.794942	2.479956	-4.639372	.233000
68	HB		4	2.334990	.803928	-4.493136	.213000
69		N	1	4.666085	-1.468088	-3.249462	-.360000
70		H	1	4.889589	-.485858	-3.322734	.218000
71		C	2	5.704817	-2.367210	-3.379904	.388000
72	N		2	6.917074	-1.837414	-3.592854	-.420000
73	HA		2	7.027361	-.835020	-3.648916	.240000
74	HB		2	7.720503	-2.440483	-3.697298	.218000
75		N	3	5.545924	-3.685500	-3.303974	-.353000
76		C	4	4.250054	-4.035107	-3.084669	.201000
77		C	5	3.156276	-3.214096	-2.942479	-.022000
78		C	6	3.330779	-1.807236	-3.024295	.299000
79		O	6	2.486119	-.918729	-2.924275	-.284000
80		N	7	2.005623	-3.984630	-2.730828	-.252000
81		C	8	2.432225	-5.203058	-2.750981	.126000
82		H	8	1.779240	-6.052415	-2.614590	.084000
83		N	9	3.790483	-5.325479	-2.962047	-.180000
84			1	4.637743	-6.548008	-3.049636	.180000
85		C	1	5.396651	-6.407293	-3.805016	.056000
86		C	2	5.189834	-6.638338	-1.690248	-.132000
87	HA		2	6.244225	-6.909579	-1.743038	.077000
88	HB		2	5.087651	-5.674664	-1.191257	.077000
89		C	3	4.304469	-7.734188	-1.093470	.008000
90		O	3	3.501059	-7.678081	-.515065	.028000
91		C	4	3.882092	-8.508932	-2.355599	.060000
92		N	4	4.639866	-9.254885	-2.595251	.072000
93		C	1	3.799863	-7.543218	-3.365861	-.265000
94		C	5	2.563809	-9.272459	-2.213981	.007000
95	HA		5	2.753312	-10.343077	-2.291211	.061000
96	HB		5	1.908152	-9.024336	-3.046636	.061000
97	O	5		1.947965	-8.934657	-.968378	-.338000
98	HP		5	1.234024	-9.550145	-.786549	.194000
99		O	3	4.986316	-8.704808	-.277064	-.477000
100		P		5.130973	-8.496869	1.302798	1.343000
101		OA		5.794916	-9.605806	2.023796	-.738000
102		OB		3.773752	-8.477587	1.892695	-.738000
103		O	5	5.926410	-7.127591	1.531616	-.477000
104		C	5	7.356460	-7.052633	1.247118	-.032000
105	HA		5	7.509156	-6.959964	.171850	.033000
106	HB		5	7.846864	-7.957274	1.606600	.033000
107		C	4	8.053507	-5.844360	1.922914	.060000
108	H		4	8.902697	-5.528555	1.316908	.072000
109		C	3	8.620933	-6.023590	3.321594	.067000
110		O	3	8.689309	-7.086534	3.553056	.053000
111		O	3	9.923649	-5.437452	3.386957	-.309000
112	HP		3	10.271098	-5.527155	4.277368	.187000
113		C	2	7.590609	-5.293023	4.149123	-.132000
114	HA		2	8.065124	-4.880115	5.039315	.077000
115	HB		2	6.803951	-5.986662	4.445946	.077000
116		C	1	7.170601	-4.229580	3.058673	.180000
117		O	1	7.887413	-3.408438	3.056187	.056000
118		O	1	7.226778	-4.790966	1.755565	-.265000
119		N	2	5.854716	-3.661851	3.466366	-.217000
120		C	2	5.736666	-2.288144	3.524638	.366000
121		O	2	6.711170	-1.593323	3.240974	-.314000
122		N	3	4.540013	-1.758997	3.895618	-.357000
123		C	4	3.498863	-2.546529	4.197941	.272000
124		C	5	3.606153	-3.961375	4.142273	-.138000
125			5	2.787067	-4.631360	4.358195	.065000
126		C	6	4.812418	-4.466377	3.768778	.100000
127		O	6	4.778611	-5.545827	3.762101	.094000
128		N	4	2.358293	-1.984566	4.552479	-.415000
129			4	1.580472	-2.593418	4.763178	.233000
130			4	2.278907	-.978748	4.596612	.213000
131	NA			5.011977	9.341845	-2.894121	1.000000
132	NA			4.483295	-9.986468	2.960509	1.000000

7		N	1	4.489058	1.265949	3.246424	-.360000
8				4.655992	.272588	3.320372	.218000
9		C	2	5.581967	2.102697	3.345439	.388000
10				6.765812	1.503183	3.531700	-.420000
11		NA	2	6.818141	.496306	3.591370	.240000
12		HB	2	7.605785	2.058188	3.612421	.218000
13			3	5.499271	3.427640	3.265107	-.353000
14		C	4	4.221194	3.852244	3.075703	.201000
15			5	3.077667	3.096669	2.965811	-.022000
16		C	6	3.170864	1.682366	3.052201	.299000
17			6	2.273017	.844886	2.978733	-.284000
18		O	7	1.968354	3.912893	2.777926	-.252000
19			8	2.467645	5.124011	2.779673	.126000
20		N	8	1.862693	6.009822	2.654091	.084000
21			9	3.035621	5.166923	2.956307	-.180000
22			C	4.755457	6.337622	3.015168	.180000
23			1	5.521219	6.161961	3.770506	.056000
24		C	2	5.294613	6.374915	1.648120	-.132000
25		HA	2	6.363914	6.583216	1.684278	.077000
26		HB	2	5.128693	5.411939	1.165162	.077000
27			3	4.468782	7.513056	1.045181	.008000
28		C	3	3.632259	7.097551	.483323	.028000
29			4	4.109546	8.330003	2.300230	.060000
30		C	4	4.913523	9.032608	2.519469	.072000
31			5	3.982588	7.385756	3.326037	-.265000
32		C	5	2.837541	9.169022	2.162568	.007000
33		HA	5	3.091641	10.227369	2.221177	.061000
34		HB	5	2.172864	8.971009	3.016380	.061000
35		O	5	2.186811	8.850647	.929649	-.338000
36		HP	5	1.508663	9.505106	.746894	.194000
37			5	5.196959	8.429084	.205984	-.477000
38			P	5.220059	8.249737	-1.383765	-.343000
39			OA	5.908146	9.328637	-2.127346	-.738000
40			OB	3.827979	8.335483	-1.878903	-.738000
41		C	5	5.902651	6.836127	-1.693239	-.477000
42			5	7.332608	6.646074	-1.468069	-.032000
43		HA	5	7.517772	6.505871	-.403101	.033000
44		HB	5	7.875902	7.523710	-1.818334	.033000
45		C	4	7.911291	5.413290	-2.208253	.060000
46			4	8.782334	5.040945	-1.669046	.072000
47		C	3	8.387203	5.585347	-3.641537	.067000
48			3	8.498329	6.646794	-3.863081	.053000
49		HP	3	9.647175	4.929883	-3.808080	-.309000
50			3	9.935059	5.015815	-4.719058	.187000
51		C	2	7.261531	4.926821	-4.402657	-.132000
52		HA	2	7.647866	4.504002	-5.330057	.077000
53		HB	2	6.495807	5.867589	-4.632942	.077000
54		C	1	6.861117	3.869272	-3.299181	.180000
55			1	7.529250	3.010143	-3.359137	.056000
56		O	1	7.040901	4.404069	-1.996081	-.265000
57			1	5.489938	3.381697	-3.618929	-.217000
58		N	2	5.299710	2.018941	-3.724334	.366000
59			2	6.258492	1.267931	-3.552222	-.314000
60		N	3	4.052121	1.562722	-4.015855	-.357000
61		C	4	3.030066	2.410158	-4.197115	.272000
62			5	3.211407	3.814578	-4.091670	-.138000
63		H		2.411643	4.530604	-4.210345	.065000
64		C	6	4.467575	4.246147	-3.799454	.100000
65		H		4.487877	5.324582	-3.744989	.094000
66		N	4	1.838018	1.917869	-4.478316	-.415000
67		H		1.076711	2.570739	-4.597783	.233000
68		H		1.705469	.919905	-4.559547	.213000
69			1	4.234007	-1.263533	-3.288634	-.360000
70		H	1	4.428831	-.275421	-3.364637	.218000
71		C	2	5.299479	-2.132099	-3.410141	.388000
72		N	2	6.496693	-1.567571	-3.618913	-.420000
73		HA	2	6.577666	-.562580	-3.678372	.240000
74		HB	2	7.318099	-2.147061	-3.716812	.218000
75			3	5.179134	-3.454205	-3.329815	-.353000
76		C	3	3.892996	-3.841066	-3.115835	.201000
77			4	2.774746	-3.052157	-2.982556	-.022000
78		C	5	2.908113	-1.641081	-3.069036	.299000
79		O	6	2.037100	-.777514	-2.976905	-.284000
80			7	1.645624	-3.835511	-2.773890	-.252000
81		CH	8	2.108664	-5.060877	-2.786933	.126000
82			8	1.480300	-5.928611	-2.650560	.084000
83		N	9	3.471038	-5.143963	-2.990484	-.180000
84			9	4.354435	-6.341280	-3.068850	.180000
85			C	5.091171	-6.195545	-3.844982	.056000
86			1	4.939871	-6.381016	-1.721042	-.132000
87		C	2	6.000862	-6.620486	-1.792119	.077000
88		HA	2	4.818545	-5.409052	-1.242851	.077000
89		HB	2	4.103502	-7.488961	-1.077735	.008000
90			3	3.299386	-7.044066	-.491589	.028000
91		C	3	3.677328	-8.307194	-2.310809	.060000
92			4	4.452735	-9.034825	-2.550385	.072000
93		C	4	3.541528	-7.369566	-3.341543	-.265000
94			5	2.387483	-9.107723	-2.120141	.007000
95		HA	5	2.609041	-10.173488	-2.176315	.061000
96		HB	5	1.705484	-8.900682	-2.944830	.061000
97		O	5	1.789709	-8.758842	-.868802	-.338000
98		HP	5	1.099965	-9.391634	-.655664	.194000
99			5	4.834172	-8.417560	-.254675	-.477000
100			P	4.922027	-8.221059	1.330780	1.343000
101			OA	5.562849	-9.333756	2.066751	-.738000
102			OB	3.544647	-8.209405	1.872157	-.738000
103		C	5	5.705203	-6.851635	1.597821	-.477000
104			5	7.132113	-6.752534	1.305060	-.032000
105		HA	5	7.274618	-6.607565	.234183	.033000
106		HB	5	7.632039	-7.669612	1.616734	.033000
107		C	4	7.826599	-5.572709	2.031836	.060000
108			4	8.693475	-5.249834	1.455313	.072000
109		C	3	8.358575	-5.798971	3.437612	.067000
110			3	8.409353	-6.868887	3.639606	.053000
111		HP	3	9.665948	-5.230693	3.550468	-.309000
112			3	9.991113	-5.350182	4.445784	.187000
113		C	2	7.317258	-5.080183	4.261688	-.132000
114		HA	2	7.775161	-4.698830	5.174374	.077000
115		HB	2	6.515922	-5.772556	4.519720	.077000
116		C	1	6.935376	-3.980793	3.193088	.180000
117			1	7.661330	-3.168614	3.231260	.056000
118		O	1	7.016101	-4.505051	1.875880	-.265000
119			1	5.616716	-3.409232	3.586220	-.217000
120		N	2	5.524013	-2.039127	3.722668	.366000
121			2	6.522153	-1.350470	3.516832	-.314000
122		N	3	4.325086	-1.506533	4.081162	-.357000
123		C	4	3.257809	-2.287345	4.297617	.272000
124			5	3.338843	-3.698422	4.160882	-.138000
125		C	5	2.499038	-4.361738	4.306272	.065000
126			6	4.547950	-4.206982	3.801680	.100000
127		C	6	4.492673	-5.283116	3.728872	.094000
128		H	4	2.116185	-1.722269	4.643717	-.415000
129		N	4	1.319005	-2.325160	4.789070	.233000
130		H	4	2.055221	-.719452	4.747351	.213000
131		NA	+	4.109642	9.600945	-2.909415	1.000000
132		NA	+	4.219769	-9.724509	2.953393	1.000000

7	N	1	2.540175	2.229703	3.019740	-.360000
8	H	1	2.462922	1.435528	3.180197	.218000
9	C	2	3.808326	2.773894	3.003327	.388000
10	N		4.818063	1.916030	3.204431	-.420000
11	HA	2	4.625766	.936130	3.355819	.240000
12	HB	2	5.771431	2.249481	3.204621	.218000
13	C	3	4.047887	4.066478	2.802777	.353000
14	C	4	2.903961	4.778451	2.618032	.201000
15	C	4	1.606060	4.324495	2.617774	-.022000
16	C	4	1.355157	2.943401	2.832317	.299000
17	C	4	.477077	2.352304	2.867614	.284000
18	N	4	.721388	5.372655	2.392346	.252000
19	C	1	1.501048	6.417572	2.264373	.126000
20	C	1	1.125106	7.412611	2.077383	.084000
21	N	1	2.845628	6.133375	2.389825	-.180000
22			4.025685	7.039413	2.308210	.180000
23	C	1	4.821423	6.659068	2.948709	.056000
24	C	2	4.361305	6.980224	.878261	-.132000
25	HA	2	5.443144	6.917692	.760725	.077000
26	HB	2	3.896413	6.101380	.431484	.077000
27	C	3	3.767368	8.302777	.389392	.008000
28	C	3	2.784118	8.121646	-.044786	.028000
29	C	4	3.800206	9.148321	1.676084	.060000
30	C	4	4.775603	9.625003	1.773421	.072000
31	C	1	3.587602	8.236679	2.717097	-.265000
32	C	5	2.769260	10.278213	1.713459	.007000
33	HA	5	3.283170	11.239366	1.726916	.061000
34	HB	5	2.201954	10.226672	2.654473	.061000
35	O	5	1.894665	10.163816	.587894	-.338000
36	HP	5	1.381095	10.969885	.497899	.194000
37	O	3	4.575422	9.033898	-.551942	-.477000
38	P		4.116273	9.203082	-2.075280	1.343000
39	OA		4.665151	10.390033	-2.768303	-.738000
40	OB		2.656852	9.448339	-2.093677	-.738000
41	O	5	4.530286	7.864865	-2.848436	-.477000
42	C	5	5.753310	7.155157	-2.484944	-.032000
43	HA	5	5.614412	6.659343	-1.524227	.033000
44	HB	5	6.577483	7.864671	-2.411256	.033000
45	C	4	6.162023	6.070939	-3.514470	.060000
46	H	4	7.214290	5.820192	-3.380454	.072000
47	C	3	6.024684	6.387869	-4.994704	.067000
48	O	3	5.900485	7.462698	-5.126675	.053000
49	H	3	7.199427	5.955985	-5.686336	-.309000
50	N	3	7.101593	6.131993	-6.624978	.187000
51	C	2	4.791381	5.592005	-5.349195	-.132000
52	HA	2	4.845045	5.283556	-6.393264	.077000
53	HB	2	3.904931	6.207986	-5.197958	.077000
54	C	1	4.988770	4.430903	-4.295971	.180000
55	O	1	5.690453	3.696444	-4.691333	.056000
56	H	1	5.581725	4.923952	-3.103618	-.265000
57	N	1	3.677605	3.741929	-4.133916	-.217000
58	C	2	3.689457	2.380571	-3.908162	.366000
59	C	2	4.769633	1.795123	-3.850094	-.314000
60	C	3	2.498357	1.741391	-3.758738	-.357000
61	C	4	1.339885	2.411277	-3.827994	.272000
62	C	5	1.312830	3.811979	-4.059733	-.138000
63	C		.403292	4.392746	-4.102747	.065000
64	C	6	2.516278	4.428396	-4.205824	.100000
65	C	6	2.375254	5.485367	-4.377071	.094000
66	N	4	.210246	1.745450	-3.676372	-.415000
67	HN		-.652475	2.269003	-3.717659	.233000
68	HE		.224444	.748259	-3.516659	.213000
69	N	1	2.436325	-2.312155	-3.000437	-.360000
70	H	1	2.359046	-1.318011	-3.161080	.218000
71	C	2	3.704365	-2.856457	-2.988002	.388000
72	N		4.713973	-1.999110	-3.189094	-.420000
73	HA	2	4.521664	-1.019210	-3.340469	.240000
74	HB	2	5.667256	-2.332799	-3.190796	.218000
75	N	4	3.943943	-4.149237	-2.785445	-.353000
76	C	4	2.800155	-4.860865	-2.598532	.201000
77	C	5	1.502369	-4.406584	-2.596216	-.022000
78	C	5	1.251448	-3.025497	-2.810774	.299000
79	O	5	.173456	-2.434141	-2.844430	-.284000
80	H	5	.617820	-5.454451	-2.368951	-.252000
81	C	1	1.397436	-6.499522	-2.241967	.126000
82	N	1	1.021563	-7.494407	-2.054021	.084000
83	N	2	2.741871	-6.215701	-2.369792	-.180000
84	C	1	3.921839	-7.122008	-2.289691	.180000
85	H	1	4.708652	-6.746506	-2.927327	.056000
86	C	2	4.261424	-7.060502	-.860976	-.132000
87	HA	2	5.343604	-6.998146	-.746531	.077000
88	HB	2	3.798067	-6.180739	-.414415	.077000
89	C	3	3.668386	-8.382016	-.368220	.008000
90	C	3	2.686399	-8.199806	.068360	.028000
91	C	4	3.697384	-9.229760	-1.653556	.060000
92	H	4	4.672346	-9.706944	-1.752772	.072000
93	O	1	3.482220	-8.319816	-2.695530	-.265000
94	C	5	2.665953	-10.359356	-1.686164	.007000
95	HA	5	3.179494	-11.320709	-1.699402	.061000
96	HB	5	2.101251	-10.309675	-2.617155	.061000
97	O	5	1.794508	-10.242743	-.558385	-.338000
98	HP	5	1.280912	-11.048479	-.465602	.194000
99	O	3	4.478782	-9.111816	.572125	-.477000
100	P		4.024940	-9.276232	2.097574	.343000
101	OA		4.574581	-10.462139	2.791778	-.738000
102	OB		2.565233	-9.519262	2.121944	-.738000
103	O	5	4.443749	-7.936567	2.863621	-.477000
104	C	5	5.666490	-7.229660	2.495773	-.032000
105	HA	5	5.524826	-6.736215	1.534241	.033000
106	HB	5	6.489337	-7.940596	2.420991	.033000
107	C	4	6.080559	-6.143297	3.520889	.060000
108	H	4	7.132701	-5.894480	3.382371	.072000
109	C	3	5.948148	-6.456054	5.002459	.067000
110	O	3	5.822839	-7.530340	5.137767	.053000
111	H	3	7.126043	-6.024071	5.688647	-.309000
112	N	3	7.031370	-6.197418	6.628108	.187000
113	C	2	4.717326	-5.657405	5.359300	-.132000
114	HA	2	4.775252	-5.346240	6.402335	.077000
115	HB	2	3.829418	-6.272466	5.212947	.077000
116	C	1	4.912594	-4.499425	4.302251	.180000
117	O	1	5.616801	-3.764956	4.693081	.056000
118	H	1	5.500465	-4.996552	3.109073	-.265000
119	N	1	3.601869	-3.808934	4.143122	-.217000
120	C	2	3.614981	-2.448562	3.911572	.366000
121	C	2	4.695860	-1.865183	3.846228	-.314000
122	C	3	2.424293	-1.808013	3.764749	-.357000
123	C	4	1.265027	-2.475649	3.841985	.272000
124	C	5	1.236673	-3.875314	4.079753	-.138000
125	C		.326370	-4.454363	4.129264	.065000
126	C	6	2.439732	-4.493136	4.223091	.100000
127	C	6	2.297713	-5.549136	4.399425	.094000
128	N	4	.135834	-1.808566	3.692580	-.415000
129			-.727565	-2.330488	3.739913	.233000
130	H	4	.150981	-.812062	3.528596	.213000
131	NA	+	2.754796	10.895346	-2.893216	1.000000
132	NA	+	3.104202	-11.170853	3.071766	1.000000

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7		N	1	4.587233	1.554562	3.241782	-.360000
8		H	1	4.749428	.564698	3.359948	.218000
9		HC	2	5.680941	2.391856	3.326292	.388000
10		N	2	6.859122	1.797519	3.559210	-.420000
11		HA	2	6.906950	.793855	3.661546	.240000
12		HB	2	7.699327	2.353211	3.632432	.218000
13		C	3	5.604204	3.712581	3.189560	-.353000
14		C	4	4.331276	4.132225	2.959007	.201000
15		C	5	3.187433	3.375712	2.859324	-.022000
16		C	6	3.274256	1.965980	3.006007	.299000
17		O	6	2.375101	1.128508	2.950670	-.284000
18		N	7	2.084530	4.186312	2.617180	-.252000
19		C	7	1.587780	5.395155	2.577974	.126000
20		C	8	1.988235	6.276557	2.404550	.084000
21		N	9	1.952401	5.441809	2.778053	-.180000
22		C	1	4.874933	6.611568	2.805388	.180000
23		C	2	5.603252	6.481909	3.605916	.056000
24		C	2	5.478118	6.559645	1.465845	-.132000
25		HA	2	6.545667	6.767139	1.539229	.077000
26		HB	2	5.329795	5.568073	1.038192	.077000
27		C	3	4.687958	7.658813	.752839	.008000
28		C	3	3.876549	7.210208	.179717	.028000
29		C	4	4.274516	8.555546	1.934590	.060000
30		O	4	5.071160	9.268746	2.146262	.072000
31		C	4	4.094108	7.679423	3.011558	-.265000
32		N	5	3.015095	9.387204	1.883384	.007000
33		HA	5	3.272028	10.446486	1.886110	.061000
34		HB	5	2.309827	9.246088	2.515660	.061000
35		O	5	2.421437	8.991981	.443921	-.338000
36		P	5	1.756303	9.635066	-.187722	.194000
37		O	5	5.459957	8.517238	-.107815	-.477000
38		O	P	5.524324	8.259772	-1.685651	1.343000
39		O	A	6.263105	9.282723	-2.459068	-.738000
40		O	B	4.150105	8.357379	-2.226388	-.738000
41		O	5	6.176543	6.815430	-1.909845	-.477000
42		C	5	7.588008	6.593483	-1.605670	-.032000
43		HA	5	7.725481	6.534518	-.525983	.033000
44		HB	5	8.178547	7.419474	-2.002033	.033000
45		C	4	8.146774	5.284283	-2.219082	.060000
46		O	4	8.988607	4.933189	-1.622300	.072000
47		C	3	8.670242	5.317451	-3.645715	.067000
48		C	3	8.823248	6.351933	-3.953184	.053000
49		C	3	9.911981	4.611639	-3.715063	-.309000
50		H	3	10.229346	4.809773	-4.621105	.187000
51		C	2	7.546144	4.630703	-4.383876	-.132000
52		HA	2	7.945331	4.117847	-5.258935	.077000
53		HB	2	6.812719	5.372060	-4.701011	.077000
54		C	1	7.078252	3.685010	-3.207805	.180000
55		C	1	7.718793	2.603773	-3.172766	.056000
56		C	1	7.237279	4.324347	-1.949886	-.265000
57		O	1	5.701581	3.214087	-3.528911	-.217000
58		C	2	5.477221	1.852737	-3.557009	.366000
59		O	2	6.411707	1.089321	-3.118610	-.314000
60		O	3	4.224311	1.411659	-3.869013	-.357000
61		C	3	3.229362	2.272352	-4.103740	.272000
62		C	5	3.445956	3.675505	-4.078292	-.138000
63		H	5	2.668329	4.403188	-4.257663	.065000
64		C	6	4.706765	4.091809	-3.783928	.100000
65		H	6	4.754767	5.170699	-3.793539	.094000
66		O	4	2.030778	1.794490	-4.382028	-.415000
67		N		1.289913	2.458127	-4.557543	.233000
68		HB		1.873315	.797140	-4.406485	.513000
69		N	1	4.468383	-1.433455	-3.292617	-.360000
70		H	1	4.641214	-.445332	-3.410265	.218000
71		C	2	5.550061	-2.284123	-3.396231	.388000
72		N	2	6.731968	-1.703866	-3.645226	-.420000
73		HA	2	6.790868	-.700560	-3.745267	.240000
74		HB	2	7.563907	-2.269814	-3.732859	.218000
75		C	3	5.458788	-3.604212	-3.262308	-.353000
76		C	4	4.184317	-4.008595	-3.013717	.201000
77		C	5	3.051725	-3.238121	-2.894413	-.022000
78		C	6	3.154053	-1.829128	-3.038172	.299000
79		O	6	2.266450	-.980629	-2.966681	-.284000
80		N	7	1.942501	-4.035596	-2.637986	-.252000
81		C	7	2.431033	-5.250773	-2.610047	.126000
82		C	8	1.823137	-6.125137	-2.430184	.084000
83		N	9	3.791774	-5.313896	-2.830957	-.180000
84		C	1	4.698991	-6.495016	-2.875800	.180000
85		C	2	5.401100	-6.379670	-3.688289	.056000
86		C	2	5.337660	-6.443406	-1.552797	-.132000
87		HA	2	6.400319	-6.664397	-1.652890	.077000
88		HB	2	5.212655	-5.447825	-1.127013	.077000
89		C	3	4.553161	-7.528968	-.813191	.008000
90		C	3	3.762561	-7.067379	-.221594	.028000
91		C	4	4.098025	-8.426826	-1.978654	.060000
92		O	4	4.880123	-9.150882	-2.207052	.072000
93		C	4	3.900113	-7.554293	-3.055463	-.265000
94		N	5	2.835608	-9.241569	-1.669925	.007000
95		HA	5	3.079491	-10.303949	-1.693392	.061000
96		HB	5	2.117129	-9.097565	-2.496860	.061000
97		O	5	2.279484	-8.832510	-.437615	-.338000
98		P	5	1.613528	-9.465998	-.160494	.194000
99		O	5	5.336960	-8.392229	.031818	-.477000
100		O	P	5.434286	-8.135527	1.608089	1.343000
101		O	A	6.160247	-9.176140	2.369999	-.738000
102		O	B	4.067361	-8.198500	2.171959	-.738000
103		C	5	6.125936	-6.707779	1.815832	-.477000
104		C	5	7.537788	-6.521858	1.493797	-.032000
105		HA	5	7.657812	-6.448253	.412928	.033000
106		HB	5	8.110692	-7.371422	1.865443	.033000
107		C	4	8.145589	-5.240284	2.118814	.060000
108		O	4	8.988916	-4.905591	1.514762	.072000
109		C	3	8.688821	-5.312177	3.536605	.067000
110		C	3	8.814444	-6.355622	3.825641	.053000
111		C	3	9.952585	-4.645814	3.597935	-.309000
112		H	3	10.283216	-4.668094	4.498927	.187000
113		C	2	7.597427	-4.603280	4.302389	-.132000
114		HA	2	8.025101	-4.116879	5.179092	.077000
115		HB	2	6.846300	-5.326856	4.619161	.077000
116		C	1	7.141527	-3.825099	3.148387	.180000
117		C	1	7.808299	-2.763389	3.117230	.056000
118		C	1	7.262187	-4.248849	1.870436	-.265000
119		O	1	5.784900	-3.117532	3.497728	-.217000
120		C	2	5.597102	-1.750698	3.527647	.366000
121		O	2	6.546054	-1.012566	3.267739	-.314000
122		O	3	4.362757	-1.276276	3.845319	-.357000
123		C	3	3.351165	-2.110030	4.123051	.272000
124		C	5	3.530157	-3.518444	4.096037	-.138000
125		C	5	2.737653	-4.225085	4.293512	.065000
126		C	6	4.772998	-3.968313	3.775783	.100000
127		O	6	4.792779	-5.048077	3.786709	.094000
128		N	4	2.171789	-1.600291	4.425774	-.415000
129		H	4	1.417630	-2.243864	4.618470	.233000
130		H	4	2.041191	-.599099	4.451416	.213000
131		NA	L	4.496252	9.563139	-3.307595	1.000000
132		NA	R	4.860046	-9.628093	3.291793	1.000000

7	C 1	2.370	1.416	-.221	.048
7	C 2	2.385	2.766	-.437	-.05
7	C 3	3.64	3.472	-.553	.208
8	N3	3.686	4.835	-.771	-.231
7	C 4	4.806	2.754	-.44	-.112
8	N 5	5.985	.665	-.108	-.05
7	C 6	5.98	-.668	.104	.21
7	C 7	4.818	-2.785	.445	-.051
7	C 8	3.643	-3.501	.562	.169
8	N8	3.636	-4.862	.779	-.237
7	C 9	2.428	-2.842	.458	.006
7	C 10	2.374	-1.507	.246	-.002
7	C 11	3.559	-.734	.12	-.03
7	C 12	3.565	.667	-.104	.113
7	C 13	4.79	1.363	-.218	-.02
7	C 14	4.775	-1.401	.224	.067
7	C15	7.255	-1.39	.217	.001
7	C16	7.75	-2.13	-.85	.006
7	C17	8.98	-2.79	-.73	.026
7	C18	9.62	-2.86	.45	.019
7	C19	9.16	-2.17	1.54	.026
7	C20	7.94	-1.46	1.43	.007
7	C21	7.3	1.38	-.23	.108
7	C22	7.93	1.24	-1.61	-.036
3	H1	1.429	.893	-.135	.017
3	H2	1.453	3.305	-.522	.024
2	HA3	2.837	5.357	-.853	.132
2	HB3	4.568	5.30	-.847	.125
3	H4	5.759	3.255	-.523	.021
3	H7	5.769	-3.29	.525	.014
2	HA8	2.767	-5.351	.858	.127
2	HB8	4.499	-5.36	.858	.124
3	H9	1.507	-3.39	.548	.026
3	H10	1.418	-1.011	.168	.021
3	H16	7.24	-2.10	-1.81	.006
3	H17	9.35	-3.34	-1.58	.005
3	H18	10.55	-3.41	.51	.021
3	H19	9.68	-2.21	2.49	.022
3	H20	7.59	-.9	2.28	.021
3	H21	7.15	2.44	-.04	.008
3	H21	8.00	.98	.50	.008
3	H22	8.11	.19	-1.82	.027
3	H22	7.26	1.66	-2.36	.028
3	H22	8.888	1.79	-1.63	.028

7	C 1	2.370	1.416	-.221	.044
7	C 2	2.385	2.766	-.437	-.04
7	C 3	3.64	3.472	-.553	.206
8	N3	3.686	4.835	-.771	-.232
7	C 4	4.806	2.754	-.44	-.110
8	N 5	5.985	.665	-.108	-.029
7	C 6	5.98	-.668	.104	.183
7	C 7	4.818	-2.785	.445	-.055
7	C 8	3.643	-3.501	.562	.173
8	N8	3.636	-4.862	.779	-.24
7	C 9	2.428	-2.842	.458	.014
7	C 10	2.374	-1.507	.246	.003
7	C 11	3.559	-.734	.12	.073
7	C 12	3.565	.667	-.104	-.019
7	C 13	4.79	1.363	-.218	.13
7	C 14	4.775	-1.401	.224	.009
3	H1	1.429	.893	-.135	.019
3	H2	1.453	3.305	-.522	.031
2	HA3	2.837	5.357	-.853	.136
2	HB3	4.568	5.30	-.847	.135
3	H4	5.759	3.255	-.523	.024
3	H5	6.856	1.15	-.186	.169
3	H6	6.913	-1.205	.189	.048
3	H7	5.769	-3.29	.525	.02
2	HA8	2.767	-5.351	.858	.132
2	HB8	4.499	-5.36	.858	.129
3	H9	1.507	-3.39	.548	.028
3	H10	1.418	-1.011	.168	.019

7 C 1	5.05	-2.41	.38	.076
7 C 2	4.34	-3.60	.58	-.075
7 C 3	2.94	-3.59	.58	.238
8 N 3	2.23	-4.78	.78	-.223
7 C 4	2.25	-2.39	.39	-.14
8 N 5	2.26	0.0	0.0	-.127
7 C6	2.27	2.40	-.38	-.14
7 C 7	2.98	3.59	-.57	.238
8 N 7	2.29	4.79	-.76	-.223
7 C 8	4.38	3.58	-.57	-.075
7 C 9	5.07	2.38	-.38	.076
7 C10	5.06	-.01	0.0	.147
7 C11	4.35	-1.21	.19	-.051
7 C12	2.95	-1.20	.19	.2047
7 C13	2.97	1.19	-.19	.204
7 C14	4.37	1.19	-.19	-.051
3 H 1	6.13	-2.41	.38	.024
3 H 2	4.88	-4.53	.72	.034
2 HA 3	1.22	-4.77	.78	.14
2 HB 3	2.73	-5.65	.92	.14
3 H 4	1.17	-2.39	.39	.032
3 H 5	1.18	.01	0.0	.151
3 H 6	1.19	2.40	-.38	.032
2 HA 7	1.28	4.8	-.76	.14
2 HB 7	2.80	5.65	-.9	.14
3 H 8	4.92	4.50	-.72	.034
3 H 9	6.15	2.38	-.38	.024
2 H 10	6.14	-.02	.00	.031

7	C 1	2.25	-2.39	.39	.076
7	C 2	2.94	-3.59	.58	-.075
7	C 3	4.34	-3.60	.58	.238
8	N 3	5.03	-4.80	.77	-.223
7	C 4	5.05	-2.41	.38	-.14
8	N 5	5.06	-.01	0.0	-.127
7	C 6	5.07	2.38	-.38	-.14
7	C 7	4.38	3.58	-.57	.238
8	N 7	5.09	4.77	-.76	-.223
7	C 8	2.98	3.59	-.57	-.075
7	C 9	2.27	2.40	-.38	.026
7	C10	2.26	0.0	0.0	.147
7	C11	2.95	-1.20	.19	-.051
7	C12	4.35	-1.21	.19	.204
7	C13	4.37	1.19	-.19	.204
7	C14	2.97	1.19	-.19	-.051
3	H 1	1.17	-2.39	.39	.024
3	H 2	2.40	-4.52	.73	.034
2	HA 3	6.04	-4.81	.77	.14
2	HB 3	4.52	-5.66	.91	.14
3	H 4	6.13	-2.41	.38	.032
3	H5	6.14	-.02	.00	.151
3	H 6	6.15	2.38	-.38	.032
2	HA 7	6.10	4.77	-.76	.14
2	HB 7	4.59	5.64	-.9	.14
3	H 8	2.44	4.52	-.72	.034
3	H 9	1.19	2.40	-.38	.024
2	H 10	1.18	.01	.01	.031

7	C 1	5.05	-2.41	.38	.06
7	C 2	4.34	-3.60	.58	-.012
7	C 3	2.94	-3.59	.58	.085
7	C 4	2.25	-2.39	.39	-.055
8	N 5	2.26	0.0	0.0	-.148
7	C6	2.27	2.40	-.38	-.055
7	C 7	2.98	3.59	-.57	.085
7	C 8	4.38	3.58	-.57	-.012
7	C 9	5.07	2.38	-.38	.06
7	C10	5.06	-.01	0.0	.278
7	C11	4.35	-1.21	.19	-.065
7	C12	2.95	-1.20	.19	.179
7	C13	2.97	1.19	-.19	.179
7	C14	4.37	1.19	-.19	-.065
3	H 1	6.13	-2.41	.38	.012
3	H 2	4.88	-4.53	.72	.032
3	H 3	2.40	-4.52	.73	.032
3	H 4	1.17	-2.39	.39	.033
3	H 6	1.19	2.40	-.38	.033
3	H 7	2.44	4.52	-.72	.032
3	H 8	4.92	4.50	-.72	.032
3	H 9	6.15	2.38	-.38	.012
3	H 5	1.18	.01	0.0	.155
8	N10	6.46	-.02	0.0	-.211
2	HA	6.96	-.89	.14	.162
2	HB	6.97	.84	-.14	.162

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7	C 1	2.25	-2.39	.39	.06
7	C 2	2.94	-3.59	.58	-.012
7	C 3	4.34	-3.60	.58	.085
7	C 4	5.05	-2.41	.38	-.055
8	N 5	5.06	-.01	0.0	-.148
7	C 6	5.07	2.38	-.38	-.055
7	C 7	4.38	3.58	-.57	.085
7	C 8	2.98	3.59	-.57	-.012
7	C 9	2.27	2.40	-.38	.06
7	C10	2.26	0.0	0.0	.278
7	C11	2.95	-1.20	.19	-.065
7	C12	4.35	-1.21	.19	.179
7	C13	4.37	1.19	-.19	.179
7	C14	2.97	1.19	-.19	-.065
3	H 1	1.17	-2.39	.39	.012
3	H 2	2.40	-4.52	.73	.032
3	H 3	4.88	-4.53	.72	.032
3	H 4	6.13	-2.41	.38	.033
3	H 6	6.15	2.38	-.38	.033
3	H 7	4.92	4.50	-.72	.032
3	H 8	2.44	4.52	-.72	.032
3	H 9	1.19	2.40	-.38	.012
8	N10	.86	.01	.00	-.211
2	HA10	.35	-.85	.14	.162
2	HB10	.36	.87	-.13	.162
3	H5	6.14	-.02	.00	.155

Appendix C

Detailed Energy Component Analysis for the different optimized structures (except where otherwise indicated)

(1) BDNA (starting guess)

(2) Ethidium Bromide (starting guess)

(3) BDNA

(4) Ethidium Bromide

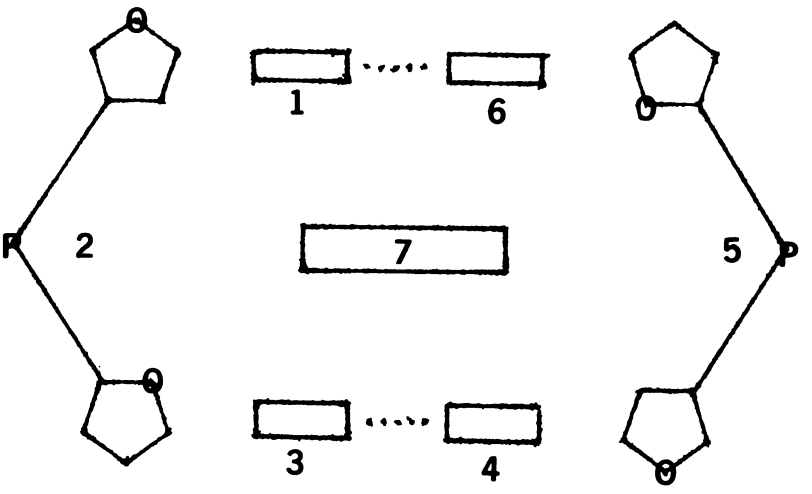
(5) Ethidium Bromide without phenyl and ethyl side chains

(6) Proflavine (minor)

(7) Proflavine (major)

(8) 10-Aminoacridine (minor)

(9) 10-Aminoacridine (major)



		Dispersion Attraction	Exchange Repulsion	Electrostatic
(A) Drug/Nucleic Acid				
Drug-Base	7-1			
	7-3			
	7-4			
	7-6			
Drug-Bk	7-2			
	7-5			
(B) Nucleic-Acid/Nucleic-Acid				
Base-Base	1-4	-4.19	0.63	2.38
	1-6	-9.11	7.80	-10.04
	3-4	-9.10	7.79	-10.02
	3-6	0.62	0.00	3.10
		-23.02	16.22	-14.58
Base-Bk	1-5	-0.32	0.00	1.39
	2-4	-0.32	0.00	1.39
	2-6	-0.16	0.00	3.29
	3-5	-0.16	0.00	3.29
		-0.96	0.00	9.36
Bk-Bk	2-5	-0.07	0.00	10.89
(C) Nucleic-Acid (Intramolecular)				
Base-Base	1-1	-2.66	0.41	71.65
	1-3	-29.68	13.62	-4.06
	3-3	-1.09	0.19	39.98
		-33.43	14.22	107.57
Base-Bk	1-2	-16.98	6.54	38.11
	2-3	-26.93	19.34	36.71
		-43.91	25.88	74.82
Bk-Bk	2-2	-54.32	61.00	-51.69
(D) Torsional		-7.30		

Ethidium Bromide (start)

		Dispersion Attraction	Exchange Repulsion	Electrostatic
(A) Drug/Nucleic Acid				
Drug-Base	7-1	-19.98	8.11	-2.08
	7-3	-13.84	4.86	-10.15
	7-4	-22.12	9.86	-3.01
	7-6	-13.75	4.38	-10.70
		-69.69	27.21	-25.94
Drug-Bk	7-2	-15.86	16.88	-28.70
	7-5	-17.59	15.79	-30.51
		-33.45	32.67	-59.21
(B) Nucleic-Acid/Nucleic-Acid				
Base-Base	1-4	- .21	0.00	0.21
	1-6	- 8.24	8.54	-9.41
	3-4	- 8.24	8.54	-9.41
	3-6	- 0.08	0.00	1.13
		-16.77	17.08	-17.48
Base-Bk	1-5	- 0.32	0.00	0.80
	2-4	- 0.32	0.00	0.80
	2-6	- 0.09	0.00	0.80
	3-5	- 0.09	0.00	2.70
		- 0.82	0.00	7.00
Bk-Bk	2-5	0.05	0.00	10.11
(C) Nucleic-Acid (Intramolecular)				
Base-Base	1-1	-2.65	0.41	72.44
	1-3	-0.90	0.00	- .11
	3-3	-1.11	0.19	39.97
		-4.66	0.60	112.30
Base-Bk	1-2	-14.65	6.86	36.42
	2-3	-13.61	11.64	42.04
		-28.26	18.50	78.46
Bk-Bk	2-2	-31.54	18.34	-45.47
(D) Torsional		- 8.77		

			Dispersion Attraction	Exchange Repulsion	Electrostatic
(A)	Drug/Nucleic Acid				
	Drug-Base	7-1			
		7-3			
		7-4			
		7-6			
	Drug-Bk	7-2			
		7-5			
(B)	Nucleic-Acid/Nucleic-Acid				
	Base-Base	1-4	-8.01	2.68	2.80
		1-6	-8.39	6.48	-9.32
		3-4	-8.62	6.86	9.42
		3-6	-0.36	0.00	2.97
			-25.38	16.02	-12.97
	Base-Bk	1-5	-0.41	0.00	1.42
		2-4	-0.42	0.00	1.42
		2-6	-0.14	0.00	3.42
		3-5	-0.14	0.00	3.43
			-1.11	0.00	9.69
	Bk-Bk	2-5	-0.07	0.00	11.32
(C)	Nucleic-Acid (Intramolecular)				
	Base-Base	1-1	-2.66	0.41	71.65
		1-3	-16.70	5.44	-2.92
		3-3	-1.09	0.19	39.98
			-20.45	6.04	108.71
	Base-Bk	1-2	-15.12	6.09	36.56
		2-3	-34.26	29.09	36.63
			-49.38	35.18	73.19
	Bk-Bk	2-2	-38.60	27.77	-54.26
(D)	Torsional		-13.51		

			Dispersion Attraction	Exchange Repulsion	Electrostatic
(A)	Drug/Nucleic Acid				
	Drug-Base	7-1	-22.10	8.54	-7.09
		7-3	- 6.82	2.23	-5.72
		7-4	-23.22	8.85	-8.05
		7-6	- 9.54	3.39	-7.28
			-61.68	23.01	-28.14
	Drug-Bk	7-2	-15.16	6.92	-36.16
		7-5	-21.51	10.29	-38.41
			-36.67	17.21	-74.57
(B)	Nucleic Acid/Nucleic Acid				
	Base-Base	1-4	- 0.34	0.00	1.14
		1-6	- 8.91	5.83	-6.60
		3-4	- 9.44	6.34	-6.60
		3-6	- 0.02	0.00	0.61
			-18.71	12.17	-11.45
	Base-Bk	1-5	- 0.49	0.00	2.77
		2-4	- 0.62	0.00	2.83
		2-6	- 0.09	0.00	2.53
		3-5	- 0.09	0.00	2.42
			-1.29	0.00	10.55
(C)	Nucleic Acid (Intramolecular)				
	Base-Base	1-1	- 2.65	0.41	72.44
		1-3	- 0.35	0.00	0.44
		3-3	- 1.10	0.19	39.97
			- 4.10	0.60	112.85
	Base-Bk	1-2	-14.58	6.86	36.56
		2-3	-15.14	9.33	36.04
			-29.72	16.19	72.60
(D)	Torsional	2-2	-12.57		

Ethidium Bromide (without side chains)

		Dispersion Attraction	Exchange Repulsion	Electrostatic
(A) Drug/Nucleic Acid				
Drug-Base	7-1	-19.78	7.70	-5.74
	7-3	-14.10	6.40	-11.23
	7-4	-18.67	7.14	-5.48
	7-6	-13.39	6.26	-10.95
		-65.94	27.50	-33.40
Drug-Bk	7-2	-10.15	4.57	-35.41
	7-5	-9.70	4.64	-33.30
		-19.85	9.21	-68.71
(B) Nucleic Acid-Nucleic Acid				
Base-Base	1-4	-0.27	0.00	0.82
	1-6	-9.43	7.78	-8.44
	3-4	-9.34	7.53	-8.25
	3-6	-0.09	0.00	1.44
		-19.13	15.31	-14.43
Base-Bk	1-5	-0.34	0.00	1.81
	2-4	-0.33	0.00	1.85
	2-6	-0.11	0.00	3.04
	3-5	-0.11	0.00	3.03
		-0.89	0.00	9.73
Bk-Bk	2-5	-0.07	0.00	11.02
(C) Nucleic Acid (Intramolecular)				
Base-Base	1-1	-2.65	.41	72.44
	1-3	-0.88	0.00	0.08
	3-3	-1.11	0.19	39.97
		-4.64	0.60	112.49
Base-Bk	1-2	-15.27	7.32	37.10
	2-3	-14.93	9.56	37.31
		-30.20	16.88	74.41
Bk-Bk	2-2	-26.95	12.59	-47.87

		Dispersion Attraction	Exchange Repulsion	Electrostatic
(A) Drug/Nucleic-Acid				
Drug-Base	7-1	-19.13	7.71	-7.44
	7-3	-12.42	5.30	-8.23
	7-4	-18.48	7.09	-7.26
	7-6	-11.54	4.71	-7.86
		-61.57	24.81	-30.79
Drug-Bk	7-2	-11.10	5.48	-34.88
	7-5	-12.02	6.27	-35.80
		-23.12	11.75	-70.68
(B) Nucleic-Acid/Nucleic-Acid				
Base-Base	1-4	- 0.27	0.00	0.83
	1-6	- 9.21	7.18	-8.14
	3-4	- 9.19	7.11	-8.17
	3-6	- 0.07	0.00	1.22
		-18.74	14.29	-14.26
Base-Bk	1-5	- 0.33	0.00	1.87
	2-4	- 0.34	0.00	1.82
	2-6	- 0.11	0.00	2.88
	3-5	- 0.11	0.00	2.96
		- 0.89	0.00	9.53
Bk-Bk	2-5	- 0.06	0.00	11.10
(C) Nucleic Acid (Intramolecular)				
Base-Base	1-1	- 2.65	0.41	72.44
	1-3	- 0.78	0.00	0.02
	3-3	- 1.11	0.19	39.97
		- 4.54	0.60	112.43
Base-Bk	1-2	-15.23	7.56	37.07
	2-3	-14.89	9.44	36.95
		-30.12	17.00	74.02
Bk-Bk	3-3	-26.85	12.19	-47.83
(D) Torsional		-14.71		

		Dispersion Attraction	Exchange Repulsion	Electrostatic
(A) Drug/Nucleic Acid				
Drug-Base	7-1	-15.87	6.59	-2.41
	7-3	-12.82	4.75	-7.88
	7-4	-15.41	6.32	-2.66
	7-6	-12.73	4.77	-8.19
		-56.83	22.43	-21.14
Drug-Bk	7-2	-13.14	15.34	-26.40
	7-5	-12.52	13.98	-26.82
		-25.66	29.32	-53.22
(B) Nucleic-Acid/Nucleic-Acid				
Base-Base	1-4	- 0.14	0.00	0.31
	1-6	- 2.98	0.85	-2.66
	3-4	- 2.97	0.90	-2.14
	3-6	- 0.08	0.00	0.94
		- 6.17	1.75	-3.55
Base-Bk	1-5	- 0.11	0.00	0.97
	2-4	- 0.10	0.00	0.95
	2-6	- 0.06	0.00	1.69
	3-5	- 0.06	0.00	1.67
		- 0.33	0.00	5.28
Bk-Bk	2-5	- 0.02	0.00	9.55
(C) Nucleic-Acid (Intramolecular)				
Base-Base	1-1	- 2.65	0.41	72.44
	1-3	- 0.98	0.00	- .26
	3-3	- 1.11	0.19	39.97
		- 4.47	.60	112.15
Base-Bk	1-2	-16.10	9.08	36.00
	2-3	-15.35	15.16	44.41
		-31.45	24.24	80.41
Bk-Bk	2-2	-31.01	17.59	-45.51
(D) Torsional		- 6.71		

		Dispersion Attraction	Exchange Repulsion	Electrostatic
(A) Drug/Nucleic Acid				
Drug-Base	7-1	-18.69	7.75	-6.78
	7-3	-13.63	6.32	-10.95
	7-4	-18.57	7.47	-6.87
	7-6	-13.64	6.28	-10.86
		-64.53	27.82	-35.46
Drug-Bk	7-2	- 8.62	3.86	-31.10
	7-5	- 9.55	4.59	-31.98
		-18.17	8.45	-63.08
(B) Nucleic Acid/Nucleic Acid				
Base-Base	1-4	- 0.24	0.00	0.78
	1-6	- 9.30	7.37	-8.18
	3-4	- 9.41	7.51	-8.13
	3-6	- 0.10	0.00	1.42
		-19.05	14.88	-14.11
Base-Bk	1-5	- 0.33	0.00	1.81
	2-4	- 0.34	0.00	1.82
	2-6	- 0.12	0.00	3.06
	3-5	- 0.10	0.00	3.11
		- 0.89	0.00	9.80
Bk-Bk	2-5	- 0.07	0.00	10.96
(C) Nucleic Acid (Intramolecular)				
Base-Base	1-1	- 2.65	0.41	72.44
	1-3	- 0.90	0.00	0.01
	3-3	- 1.11	0.19	39.97
		- 4.66	0.60	112.42
Base-Bk	1-2	-15.43	7.87	37.10
	2-3	-14.90	9.37	36.76
		-30.33	17.24	73.86
Bk-Bk	2-2	-27.08	12.32	-47.71
(D) Torsional		-14.66		

		Dispersion Attraction	Exchange Repulsion	Electrostatic
(A) Drug/Nucleic-Acid				
Drug-Base	7-1	-16.53	7.16	-7.30
	7-3	-12.52	5.73	-9.67
	7-4	-20.36	8.18	-7.14
	7-6	-15.94	7.23	-10.73
		-65.35	28.30	-34.84
Drug-Bk	7-2	- 6.38	2.93	-27.11
	7-5	-10.35	4.73	-33.79
		-16.73	7.66	-60.90
(B) Nucleic-Acid/Nucleic-Acid				
Base-Base	1-4	- 0.25	0.00	0.68
	1-6	- 9.19	7.37	-7.96
	3-4	- 9.48	7.89	-9.45
	3-6	- 0.11	0.00	1.35
		-19.03	15.26	-15.38
Base-Bk	1-5	- 0.36	0.00	1.73
	2-4	- 0.32	0.00	1.56
	2-6	- 0.10	0.00	2.95
	3-5	- 0.12	0.00	2.91
		- 0.90	0.00	9.15
Bk-Bk	2-5	- 0.06	0.00	10.68
(C) Nucleic Acid (Intramolecular)				
Base-Base	1-1	- 2.65	0.41	72.44
	1-3	- 1.13	0.00	- .09
	3-3	- 1.11	0.19	39.97
		- 4.89	0.60	112.32
Base-Bk	1-2	-15.08	7.24	36.94
	2-3	-14.99	9.33	36.60
		-30.07	16.57	73.54
Bk-Bk	2-2	-26.80	12.07	-47.64
(D) Torsional		-15.48		