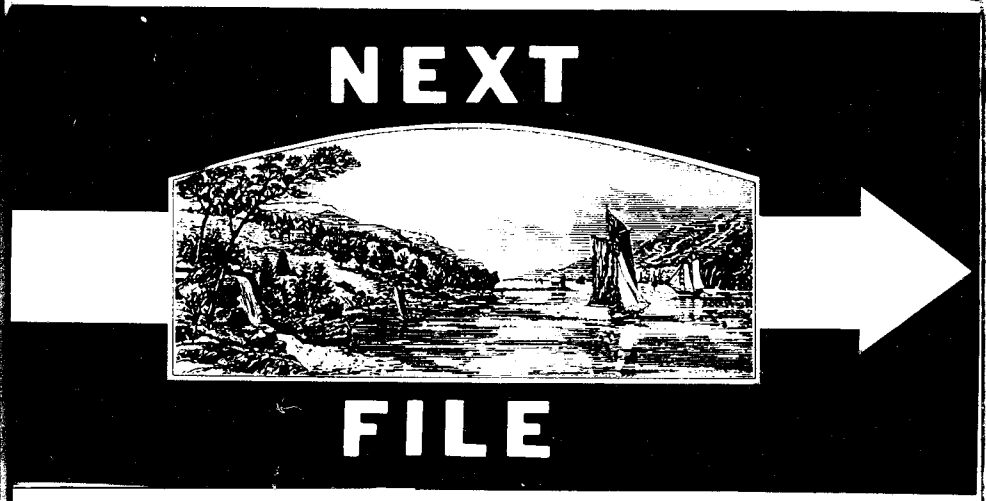

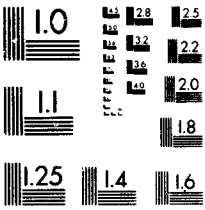

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CONFORMATIONS OF DNA OLIGOMERS

BY

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ABSTRACT:

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This work examines the conformations of various oligonucleotides as a function of two environmental conditions, neighboring base combinations and surrounding media. The conformations were determined by molecular mechanical energy refinement using the internal minimization function of a program written by Ken Miller.

The first condition examined was the effect of various neighboring base combinations on the conformation of the interior base. Our results indicate that the NH2 amino groups are the primary factors in determining differences in conformations of various base pairs sequences. One way in which the NH2 amino groups are able to affect the conformation of the molecule is by repulsive forces which separate the two groups when **C** and **A** groups are stacked upon each other. The second way is that the distance between the amino group of a nucleotide base and the C1' carbon directly influences the value of the dihedral angle Xi, which is between the C4 and C5 carbon atoms.

The second condition examined was the effect of including rough approximations of solvent and cations conditions that exist in the cellular environment into the energy refinement calculations. Our results indicate that the mere inclusion of hydrated sodium cations, in the proximity of the phosphate groups along the oligonucleotide backbone, insulates the phosphate groups from the substantial repulsive forces they were observed to exert upon each other in the gaseous medium calculations.

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I would like to express my sincere appreciation to a number of people who have helped to make this thesis what it is now. First and foremost is my patient and understanding advisor, **Dr. Janet Anderson**. She has given me the support, guidance and above all friendship to make this one of the most enjoyable parts of my education. I would also like to thank my advisor, **Dr. T. C. Werner**, for his support and guidance through my four years at Union. Finally, I would like to thank all of the proofreaders who volunteered their time, in particular **Mr. Eric Turer**.

TABLE OF CONTENTS

INTRODUCTION:

Some oligopeptide [small polypeptide] drugs have been reported to undergo highly specific interactions with DNA, such as daunomycin (1), triostin (1), netropsin (2). However, in order for research in this field to advance, a better understanding of how and when these oligopeptide drugs will interact with and/or alter DNA is required. Information on which characteristics of these oligopeptides are important for these interactions is similarly helpful. (2) Towards this end we are investigating how the conformations of DNA oligomers vary with different conditions, such as the size and composition of the oligamer and characteristics of the surrounding medium, ie. the presence of water and cations.

Our data are acquired through calculations of theoretical minimum energy conformations. This approach has been taken by a number of investigators. (3, 4, & 5) The primary advantage of these calculations is that variables can be tested individually, without the possibility of other variables having been coincidentally altered. However this method also has its drawbacks. First among these is the problem with the parameters which we use. These include bond lengths, angles, and strengths as well as dihedral angles, and charges. Often we used approximations for these parameters because the true values are not known. Therefore there is substantial variation in the approximations that different investigators use. This results in a small side benefit however, because we are able to use this variation to test the relative importance of a number of variables. In addition to discovering the magnitude of the effect of these variables on the final conformation, it is possible, given enough data, to determine the kind of effect that these

variations in parameters have on conformational energy.

Our conformations were determined by energy minimization calculations. A VAX computer was used with a program written by K. J. Miller (5) and adapted to the VAX system by Janet Anderson. The program allows many means of minimizing the total potential energy of the system. We used two of these, Internal Rotation and Cartesian Coordinate. Both methods minimize the total potential energy by varying some function which represents a dependency of the total energy on the shape of the molecule.

Internal Rotational minimization varies the dihedral angles of the molecule and the ribose sugar pucker, a composite of the dihedral angles of the ribose group. A dihedral angle is the rotation of one side of a single bond relative to the next; for example, in propane there would be two dihedral angles, between the H-C-H group in the middle and each of the terminal methyl groups. The energy function in this type of minimization combines terms for Van der Waals (which includes terms for both steric repulsion and dispersion attraction), and electrostatic forces (including H-bond attractions). (6)

Cartesian Coordinate minimization works by varying the location of each atom in a theoretical three-dimensional grid. This allows for more movement than Internal Rotational minimization, because both the bond lengths and bond angles of the molecule are allowed to vary in addition to its dihedral angles. However this also greatly increases the number of variables and the complexity of the calculations. In Cartesian Coordinate minimization the energy function includes not only the terms used in the Internal Rotational formula, hydrogen bonding, Van der Waals attraction, and electrostatic attraction, but also two new terms to account for the bond stretching and bond bending.

Unfortunately, what is found by these methods is not the global minimum energy conformation, but instead the nearest local minimum to the starting conformation. Because we are working with a tremendous number of variables, approximately one thousand for a DNA trimer (a chain of three base pairs with two backbone phosphate-ribose chains) minimized with the Cartesian Coordinate method, there are an extraordinary number of local minima. For this reason the program is only effective at resolving the precise shape of a structure whose gross structure is already known. We are using the Watson and Crick B-DNA as our starting model.

Among the variables that we are interested in are the size of the DNA chain, the combination of bases, the effect of water, and the effect of cations. The chain length needs to be long enough so "end effects" will be negligible; however the longer the chain, the more complicated and lengthy the minimization process becomes. "End effects" is a term used to describe any change in the shape of a chain structure in the vicinity of the ends of that structure, which is primarily due to the lack of base-base-stacking attractions. [When discussing we are refering to the anomalous behavior of the terminal base pairs.] These effects are the result of a greater freedom of movement in the ends because they are not constrained as the internal links are. The goal is to find an optimum length of DNA oligomer, which will be the minimum length necessary to obtain results representative of the conformations of internal base pairs.

The base combinations are probably also important. Does a **G-C** base pair act differently when surrounded by **A-T** pairs than it would when surrounded by **G-C** pairs? If the neighboring base pairs affect the conformation of a given base pair, it would be helpful to find a pattern to

these variations in conformation.

We know that water has a tremendous effect on the entropy of the total system. This results from its very stable structure, as shown by its high boiling point. Thus the water-water hydrogen bonds that are affected by the DNA molecule could play a significant role in the final conformation of that molecule. If some way can be devised to account for this factor in our energy calculations, a better picture of the final conformations would be obtained.

A clearer model would also be obtained if the effect of the negatively charged phosphate groups were modified to represent the cellular environment with its abundance of charged ions. In the cell many cations are available to serve as bridges between the phosphate groups and replace their repulsive forces with a negative-positive-negative attractive force. This is an important force and some method must be devised to include it in the calculations.

With each of these relationships better defined, the next step would be to include the oligopeptides directly. Some investigators have performed energy minimizations on these oligopeptides and obtained minimum energy conformations for them. If these conformations prove compatible with our program, and some method is derived to introduce them into the DNA minimization calculations, then conformations can be calculated to represent the interactions of the oligopeptides with DNA oligomers. This would be a significant benefit to the research into oligopeptide anti-cancer drug research.

EXPERIMENTAL:

The first point to explore was the amount of leeway that the minimization procedure would leave us in the original gross structure approximations which are being fine tuned by the program. Table 1 shows the original dihedral angles that four investigators used to model Watson and Crick B-DNA. Figure 1 defines the dihedral angles. The large variation in starting dihedral angle values shows evidence of tremendous flexibility in the original conformations which can minimize to the desired conformation. However, because of the extremely complex potential energy surface, care must be taken to assure that the minimization is toward the conformation of interest when the initial conformation is on a ridge. This sometimes can be accomplished by initially placing constraints on the acceptable conformations, and then, when the structure has minimized toward the conformation of interest, these constraints can be removed. For example if the molecule tends to denature, that is, the two strands separate, a maximum distance between the two strands can be introduced. After the conformation has moved into the desired energy well, the strands can be freed to find the local minimum energy conformation.

The next point was to determine at what point the minimization could be considered finished. Figure 2 shows the total energy as a function of number of iterations minimized. It clearly points out the rate of descent of the total energy is very uneven and that it would be unreasonable to use some energy difference to determine what the end point of the minimization should be. From Figure 3 we see that after 75 iterations this structure has reached its approximate energy minimum. I arbitrarily assigned 200 iterations as the duration for minimization as a compromise

between time efficiency and optimum precision.

The final preliminary point was to determine the dependence of the final structure on the method of minimization used. To do this both the Internal Rotational and Cartesian Coordinate methods of minimization were used. As Table 2 shows the only deviation is in the **C**-side Ξ (xi) angle, and even this is only one degree, the minimum possible increment. This demonstrates that both methods are equally accurate. Because the Internal Rotational method is much simpler and faster we used it to do all of the following calculations.

The first area of concern was to determine how far into the DNA oligomer end effects were felt. We minimized a monomer, dimer, and trimer of **G-C** chains. As is shown in Table 3, only the angles from the terminal sugars out (Ψ (psi), Θ (theta), and Ξ of that group), were affected. This means that on one side of the chain the Ψ (psi), Θ (theta), and Ξ angles are affected, while on the other side the Ω (omega) and Φ (phi) angles are affected. Therefore, the internal base of any trimer is free from end effects in all of its dihedral angles.

The next area to be explored was the effect of the nature of the surrounding bases on an internal base. All symmetrical normal trimers, that is trimers where a nucleotide is surrounded on each side by the same nucleotide, and has on the opposite chain, complimentary nucleotides, **A** for **T**, **G** for **C**, and vice versa. Each of these molecules were then minimized, and the resultant dihedral angles are listed in Table 4.

One interesting pattern that these results show is that the Φ and Ξ angles are significantly different when the adjoining groups are **C** and **A** (italicized). Because these are the two bases which have relatively large

NH2 groups diagonally across from the C1' carbon, it is probable that repulsive forces between NH2 groups deform the normal structure to increase the distance between these groups.

A second interesting trend is with the Ξ angles. For **C** bases the Ξ angle values (bold) are more consistent and lower than all of the other Ξ angles (underlined). This is true to *a* progressively lesser extent for **A** than **G** and finally **T** bases. The NH2 group can explain this pattern also. When the NH2 group is directly across from the C1' carbon, as in **C** bases (Figure 4), the deviation in Ξ is zero (0), that is all the molecules with C as the interior base have the same Ξ value. As the NH2 group becomes farther from this position (figures 5-7), the deviation (or variation) in Ξ values increases consistantly.

The importance of an individual NH2 amino group was demonstrated by Lavery and Pullman (7). They studied both the electrostatic potential and the steric accessibility of two oligonucleotides. The two oligomers, d**I**.d**C** and d**G**.d**C**, differed only by the 2-amino group of the **G** base which is absent in the **I** base. They found that the major differences between **G**-**C** and **A**-**T** base pairs were a result of the 2-amino group of the **G** base because the **I_C** oligomer behaved like the **A_T** oligomer more and not like the **G_C** oligomer. These differences included the accessibility of the N3 atom of the purine and the O2 atom of the base-paired pyrimidine to the **G** or **C** base. The same amino group renders the electrostatic surface potential of the minor groove shallower than both the **A-T** and **I-C** base pairs. (7) These findings were supported by Kollman et. al. in their work on Netropsin. (8)

The final area to explore is the effect of adding water and sodium

cations to the system. As is graphically represented in Figure 8, one investigator (9) has found that approximately 20 water molecules are located in the first hydration sphere of a two-nucleotide molecule. There is evidence that the motion of water molecules in this hydration sphere is reduced relative to liquid water, specificaly from NMR and IR spectroscopic data as well as comparisons of dielectric constants. Of these twenty waters, approximately one half, or ten, are in the first hydration sphere of each of the two sodium ions. In Figure 9, a second investigator (10) has found that ten of sixteen water molecules, including ten of the twelve most tightly associated water molecules that are in direct contact with the atoms of a two nucleotide molecule, are associated with the two phosphate groups in the molecule, even without a cation present.

Our approach then to adding the water and cations to the system, is to place a model representing a sodium molecule with six water molecules in its first hydration sphere in proximity to each phosphate group. Oligomers of six, three, and one base pair lengths were built in this way, and the dihedral angles of each molecule's minimum energy conformation are then compared in Table 5 with those values obtained before the sodium and water molecules had been added.

The most important result from this procedure is that the hexamer, which denatured when minimized in a gaseous medium, did not change appreciably from its original B-DNA form when limited solvent characteristics were included in the energy minimixation, Figures 10 &11. Unfortunately the energy minimization of these hexamers took approximately 20-24 hours of CPU time on the VAX system which hinders extensive study of these larger oligomers. It is, however, rewarding to

learn that this limited representation of solvent characteristics adequately damps the phosphate-phosphate repulsive forces so that these longer oligomers can be energy refined without denaturation.

From Figures 10 and 11, we see that the only substantial change in base pair pairings occur at the two terminal base pairs, and that these "end effects" are common to both energy refined structures. For the energy refined hexamer with solvent properties included in the procedure, no constraints were placed on the acceptable conformations to produce this desired structure. This is not true of the oligomer minimized in a gaseous medium (Figure 12); here the base pairs were forced to maintain their starting distances from one another. This was done by "tying" the C1'-C1' distance between conjugate bases at the original value, 10.85.

DISCUSSION:

The basic purpose of this research has been to lay a foundation upon which future researchers can build, allowing them to build models to examine DNA oligomer-oligopeptide drug interactions. Similar research has recently (1986) been carried out by Kollman et al. on the interactions between the oligopeptide drug netropsin and DNA oligomers. There were three goals to their examination. First, they hoped to rationalize and order the DNA base sequence preference of the drug. Second, they attempted to propose detailed three-dimensional structures of the drug-DNA complex. And third, they wished to compare their results with results obtained experimentally. I will now analyze their procedure in order to determine its value, and assess the applicability of their method to Union's facilities, and therefore appraise the value of future work in this field at Union. I will attempt to point out possible shortcomings and suggest alternative approaches to both our current procedure and that of Kollman et. al.

Kollman's group employed a three-part procedure in analyzing the netropsin-DNA system. The first step was to provide models for the two constituent parts, netropsin and the DNA oligomer. The DNA oligomer's conformation was obtained by molecular mechanical energy refinement of a model-built structure whose parameters were obtained from NMR data. The drug's conformation was model-built using X-ray structures obtained from the literature. The second step was to combine the two component molecules in one complex. This was done by "docking" the drug to the DNA oligomer using an interactive graphics package. The final step is to energy-refine the complex. This was done with molecular mechanical energy refinements which included, when necessary, temporary

constraints being placed on the conformations of the complex. One admitted shortcoming of the procedure is that they did not include the solvent in their calculations. This resulted in an overemphasis of the electrostatic forces of the backbone atoms, especially the negatively charged oxygen atoms of the phosphate groups.(8)

A second shortcoming results from the use of molecular mechanics as the sole means of energy refinement. This is a disadvantage because minimum energy conformations are "correct" only at absolute zero, 0°K. In the cell, there is substantial energy which can allow the molecule, or complex, a large amount of freedom of movement, and there can be many "stable conformations". Molecular mechanics gives no regard to kinetic factors, and can therefore misleadingly produce a single conformation, when, in fact, there are many conformations which are all close enough in energy to allow the molecule to spend considerable time in each conformation. When this is the case it is difficult and time consuming to even approximate the energy barriers between these conformations. Similarly, it is possible that the structure given by the energy refinement can not be reached in the cell because the energy barrier to reach that conformation is prohibitive. (11)

However, even with these limitations, the data that Kollman's group obtained was valuable, because it was useful in each of the group's three goals. Comparisons could be made between NMR data and the energy-refined data to prove that the energy-refined conformation was not prohibited by energy barriers. This energy refined data was then useful in proposing more detailed conformational structures than were available from the NMR data, and it also provided both rationale and support for the experimentally observed DNA base sequence binding

tendencies of the drug.

The accuracy of these data was primarily limited by approximations made to adjust for the non-inclusion of solvent characteristics. In an aqueous medium, such that of the cells where DNA is naturally found, the ability of water to form hydrogen-bonds is extremely important. In their work with netropsin, Kollman's group found H-bonding to be the primary mode of binding in DNA complexes. Quigley has found specific examples of the importance of including water in these conformational calculations, (1). Pictured in Figure 13 is the complex of the oligopeptide drug Triostin A and a DNA oligomer. Here we see three H-bonds; the first is between the lower carbonyl group of the drug and the N2 amino group of the lower **G** base of the oligomer. The other two H-bonds are between two backbone amino groups of the drug, and the two **G** bases. It is unlikely that these bonds would have been observed to be as important in the complex if Kollman's group's procedure had been used. Because Kollman et al. did not include solvent characteristics directly into their calculations, they reweighted the importance of various forces in order to artificially overcome the denaturation problem. This reweighting lessens the importance of electrostatic interactions, and therefore lessens the importance of the bonds which hold the drug in place.

In Figure 14, we see an even clearer example. In this complex between the oligopeptide drug, daunomycin, and a DNA oligomer, a water molecule actually forms H-bonds to each of the larger molecules. Thus a water molecule is able to serve as a link, binding the drug and the oligomer together by binding both to itself. The water molecule bonds to both the O2 group of the **C** base and the carbonyl group of the drug at the point labeled 13. Another H-bond is seen between the drug's O9 hydroxyl

group and the N3 amino group of the G base. And finally, an electrostatic attraction of a magnitude approaching that of an H-bond is noted between the same O9 hydroxyl group and the N2 amino group of the same base. It is also interesting to note that because all three of these major binding forces are with atoms on the oligomer which are common to all bases, the interaction should not be strongly specific to any particular sequence, and this is what is observed experimentally. (1) By not including the solvent directly in these calculations, an important element in the binding of an oligopeptide drug to the DNA oligomer can be lost.

Although we made some attempts to include solvent characteristics in our work as described in the previous section, many of the same problems will be present in our calculations of drug-DNA complexes. Clearly, because we have not included any individual water molecules, we would not be able to observe a water molecule acting as an H-bond bridge between the drug and the DNA oligomer as in Quigley's work. However the inclusion of the cation-water structure should reduce the over-emphasis of the electrostatic forces of the backbone atoms that was observed by Kollman et al.

A further shortcoming of our simplified method of including water in our calculations is seen in another researcher's findings. In Kennard's work (12), there is substantial evidence of a "spine of hydration" along the minor grove. This "spine of hydration" is apparently much more a factor in regions where there are many A-T base pairs, because the minor grove is both narrower and deeper in these regions. Kennard also speculates that long-chain H-bonding molecules, such as netropsin, replace this spine. Therefore, the energy of hydration of this spine is important in the calculation of binding of a drug in its place in the minor groove of the

oligomer. (12)

A similar effect was noted by Berman (13), in her examination of the binding of two oligopeptides, actinomycin D and proflavin, with DNA oligomers. She found a positive entropy change in the binding of actinomycin and a negative entropy change in the binding of proflavin to duplex DNA. In the case of the nonintercalating binding of actinomycin, the drug displaces the highly ordered waters which are present along the minor grove of the oligonucleotide. However proflavin attaches to the oligonucleotide by an intercalating bond and is able to retain the highly ordered water pattern along the minor grove as well as remove the drug from the surrounding medium where it had disrupted the normal structure of liquid water. Thus, here we see that the highly ordered solvent has a substantial effect on the change in entropy for the binding of oligopeptide drugs to oligonucleotides.

In both cases we see that our current method of including water into our calculations is a gross over-simplification. We have no way to account for changes in entropy that result from disruptions in the highly ordered patterns of hydration about these molecules. Also, we are not allowing individual water molecules to serve as links in the binding of the DNA oligomer and an oligopeptide drug. The major advantage gained by our method over not including any solvent, is that we are able to more realistically depict the magnitude of the electrostatic forces that the backbone atoms would exert on the rest of the system. The primary reason that we are unable to include more explicit solvent interaction is the limited capacity of the computer system with which we are working; that is, minimizations that include individual water molecules would take days of CPU time if they are possible at all.

The second problem with the procedure of Kollman's group, which is the not accounting for energy barriers in the calculations, will be even more of a problem at Union. Kollman's group was able to run an interactive graphics program to dock them and therefore demonstrate that it is plausible for the drug to approach the oligonucleotide in a manner which avoids prohibitive energy barriers. This procedure, while not completely accounting for kinetic factors, does address some of the concerns about how realistic these computer calculations are in predicting the conformation of physical structures. They were further able to justify their conformations by making comparisons with NMR data.

The biggest problem at Union, however, is how to "dock" the two molecules. We do not have the computer facilities to run a graphics "docking" program as Kollman's group does. Therefore, it would require considerable luck to "guess" a conformation which would fall within the desired energy well. Further, to test these "guesses" to see if they did indeed fall within the energy well, would require considerable computer time which would have to be repeated with each guess.

The most likely solution to this problem, is to use the NMR data to provide starting conformations from which to carry out the energy refinements. Therefore, it seems worthwhile to explore the possibility of doing this NMR work in order to maintain some association between the data that is being calculated and environmental realities. One can use Nuclear Overhauser Effects to provide data on the conformations of complex biomolecules in various solvents. (14 and 15) This data could also demonstrate the existence of specific conformations, from which no energy barriers would be crossed, and therefore bypass the objectionable practice of not taking into account energy barriers.

It seems that for research to continue much further in this field three problems have to be overcome. First, we must find some method which will enable us to foresee when it is necessary to include individual water molecules in our energy refinements, ie. when they are suspected of forming links between the DNA oligomer and the drug. Second, it is necessary to find some method of testing whether the arrived-upon conformation is plausible. This could be either making comparisons with experimental data or somehow accounting for energy barriers in the calculations; possibly by including molecular dynamics/quantum mechanics, as Kollman has. And finally, some procedure for "docking" the two molecules must be arrived at, which will minimize the computer time needed to run the energy minimizations.

Hopefully, the recent investment in Union's new NMR equipment will allow future researchers to find experimental data which would partially answer all of these questions. It is likely that the NMR procedure could identify when a water molecule is locked into a single position, which is an indication that a water molecule should be included in the energy refinement at that position. It would clearly provide data which could support the plausibility of calculated conformations. And as described above, it could provide data on the "docked" conformation of the DNA oligomer-oligopeptide drug complex.

CONCLUSION:

This research has produced two results. First through our examination of various base pair combinations, we have shown the importance of amino groups in determining the conformation of DNA oligomers. This supports other experimentor's findings which were determined by different methods. Further research to support these findings could be obtained by substituting various substituent groups, ie. OH, CH3, and H, in place of the various amino groups using a subroutine in Miller's program which allows for such substitutions, and then carrying out the energy refinements on these oligomers. This would allow a more specific analysis of which constituent parts are most critical in determining an oligomer's conformation.

The second result is that, by our inclusion of limited solvent characteristics, we have shown that hydrated cations interact with the phosphate groups to prevent denaturing of the oligomer as its length increases and phosphate groups begin stacking upon each other. Further research to determine which is the more important component, ie. whether the cation, water, or both are responsible for this phenomenon, could be done by minimizing the hexamer with an unhydrated cation and with an uncharged hydration shell. If one of these systems did not denature, that would be evidence that system included the essential element for nullifying the phosphate-phosphate repulsive forces.

TABLE 1:

STARTING CONFORMATIONS OF INVESTIGATORS

	OMEGA	PHI	PSI	THETA	XI
MILLER (5)	-161.99	-131.52	- 33.16	159.66	32.89
--IN COMMON UNITS*	198.01	258.48	326.84	159.66	32.89
ARNOTT (16)	159	261	321	209	35
LEVITT (16)	187	269	299	180	57
KOLLMAN (16)	159.2	261.0	320.8	208.6	30.9

Note that Kollman uses refined Arnott structures.

* Common units are merely the conversion of the angles into positive numbers.

TABLE 2:

CONFORMATIONS BY ENERGY FUNCTION

	Theta	Psi	Omega	Xi	Chi
DG-DC					
Internal Rotational:					
G-side	142.66	-61.16	-231.99	47.89	137.90
C-side	165.66	-67.16	-210.99	*54.89*	119.90
Cartesian Coordinate:					
G-side	142.66	-61.16	-231.99	47.89	137.90
C-side	165.66	-67.16	-210.99	*53.89*	119.90

TABLE 3:

CONFORMATIONS BY SIZE OF DNA CHAIN

	THETA	PSI	PHI	OMEGA	XI	CHI
DGDC (1)						
G-SIDE	142.66	-61.16		-231.99	47.89	137.90
C-SIDE	165.66	-67.16		-210.99	54.89	119.90
DGDC (2)						
G-SIDE	159.66	-34.16	-132.52	-231.99	32.89	140.90
	137.66	-76.13		-161.99	50.89	129.90
C-SIDE	117.66	-64.16	-131.52	-161.99	56.89	150.90
	158.66	-35.16		-166.99	34.89	133.90
DGDC (3)						
G-SIDE	121.66	-76.16	-131.52	-164.99	59.89	132.90
	159.66	-31.16	-132.52	-164.99	33.89	133.90
	160.66	-35.16		-165.99	32.89	136.90
C-SIDE	158.66	-34.16	-130.52	-183.99	32.89	145.90
	159.66	-34.16	-131.52	-161.99	29.89	133.90
	131.66	-61.16		-162.99	47.89	133.90

TABLE 4:

CONFORMATION BY BASES

	THETA	PSI	PHI	OMEGA	XI	CHI
AAA (MIDDLE)	158.66	-34.16	-131.52	-163.99	31.89	136.90
CAC (MIDDLE)	158.66	-34.16	*-133.52*	-164.99	*31.89*	132.90
GAG (MIDDLE)	158.66	-34.16	-131.52	-164.99	32.89	135.90
TAT (MIDDLE)	158.66	-33.16	-131.52	-165.99	31.89	134.90
ACA (MIDDLE)	158.66	-33.16	*-128.52*	-165.99	***29.89***	139.90
CCC (MIDDLE)	159.66	-34.16	-131.52	-161.99	**29.89**	133.90
GCG (MIDDLE)	158.66	-33.16	-131.52	-162.99	**29.89**	137.90
TCT (MIDDLE)	159.66	-33.16	-130.52	-168.99	**29.89**	133.90
AGA (MIDDLE)	158.66	-34.16	-129.52	-164.99	31.89	137.90
CGC (MIDDLE)	158.66	-35.16	-129.52	-163.99	32.89	137.90
GGG (MIDDLE)	159.66	-31.16	-132.52	-164.99	33.89	133.90
TGT (MIDLE)	159.66	-35.16	-130.52	-161.99	33.89	132.90
ATA (MIDDLE)	158.66	-37.16	-131.52	-161.99	34.89	133.90
CTC (MIDDLE)	159.66	-33.16	-131.52	-162.99	32.89	133.90
GTG (MIDDLE)	158.66	-34.16	-133.52	-164.99	31.89	132.90
TTT (MIDDLE)	159.66	-35.16	-131.52	-162.99	32.89	134.90

TABLE 5:

COMPARISONS BETWEEN GASEOUS AND SOLVENT MEDIUMS

	NANG	THETA	PSI	OMEGA	XI	CHI
6 BASES:						
C-SIDE						
-SOLVENT	33	159.7	-33.2	-162.	32.9	130.9
-GASEOUS*		160.7	-34.2	-158.	28.9	135.9
G-SIDE						
-SOLVENT	35	159.7	-34.2	-162.	32.9	140.9
-GASEOUS*		160.7	-32.2	-165.	32.9	133.9
3 BASES:						
C-SIDE						
-SOLVENT	62	158.7	-34.2	-165.	32.9	137.9
-GASEOUS		159.7	-34.2	-162.	29.9	133.9
G-SIDE						
-SOLVENT	62	159.7	-34.2	-163.	33.9	131.9
GASEOUS		159.7	-31.2	-165.	33.9	133.9
1 BASE:						
C-SIDE						
-SOLVENT		156.7	-30.2	-182.		95.9
-GASEOUS		165.7	-67.2	-211.	54.9	119.9
G-SIDE						
-SOLVENT		183.7	-43.2	-181.		135.9
-GASEOUS		142.7	-61.2	-232.	47.9	137.9

*NOTE THAT THE GASEOUS HEXAMER DENATURED UNDER FREE MINIMIZATION AND THAT THE VALUES LISTED ARE FOR A MINIMIZATION WITH THE DISTANCE BETWEEN BASES FIXED AT THE NORMAL B-DNA DISTANCE

FIGURE 1:

DIHEDRAL ANGLES IN DNA

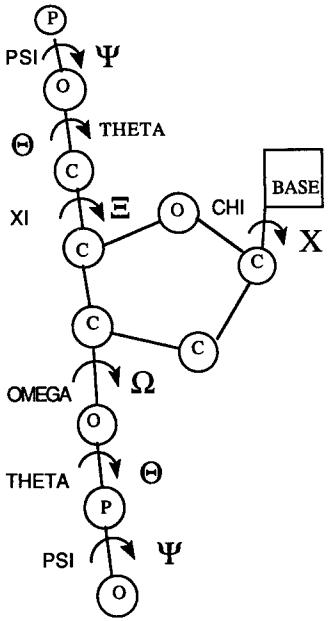


FIGURE 2:

DG DC minimization for 100 iterations

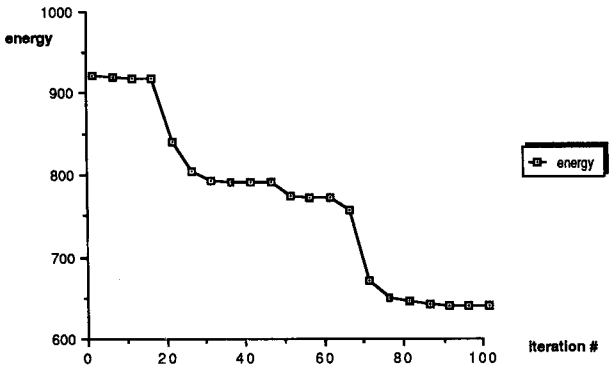


FIGURE 3:

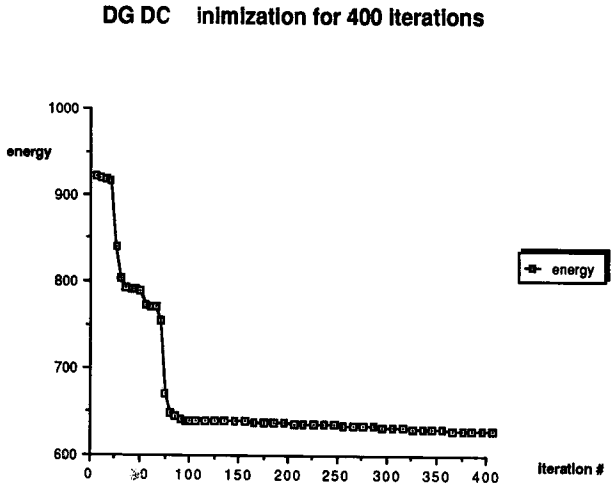
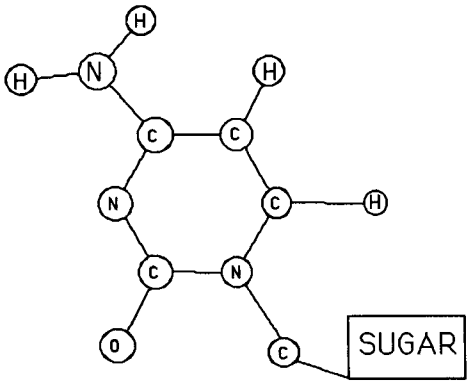

DG DC inimization for 400 iterations
energy
1000
900
800
700
600
0
50
100
150
200
250
300
350
400
iteration #
energy

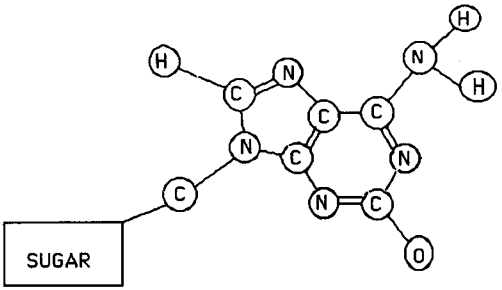
FIGURE 4:



CYTOSINE

Notice that the angle between the only multiple atom group attatched to the benzene ring, the amino group, and the **C1'** group connecting to the sugar is 180 degrees.

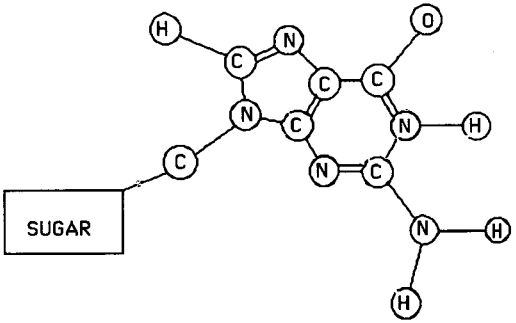
FIGURE 5:



ADENINE

Notice that the angle between the only multiple atom group attatched to the benzene ring, the amino group, and the **C1'** group connected to the sugar is approximately 150 degrees.

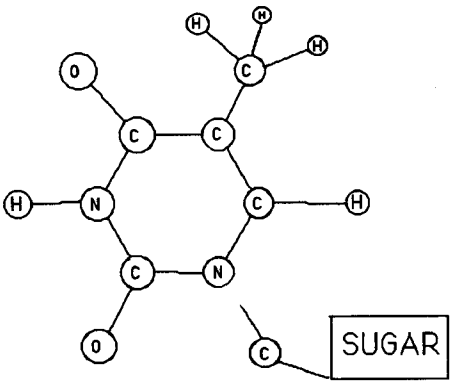
FIGURE 6:



GUANINE

Notice that the angle between the only multiple atom group attatched to the benzene ring, the amino group, and the **C1'** group connecting to the sugar is approximately 90 degrees.

FIGURE 7:



THYMINE

Notice that the only multiple atom group attatched to the benzene ring is a methyl group.

FIGURE 8:

PREFERRED HYDRATION SITES

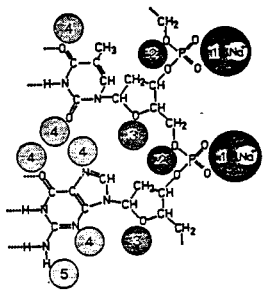
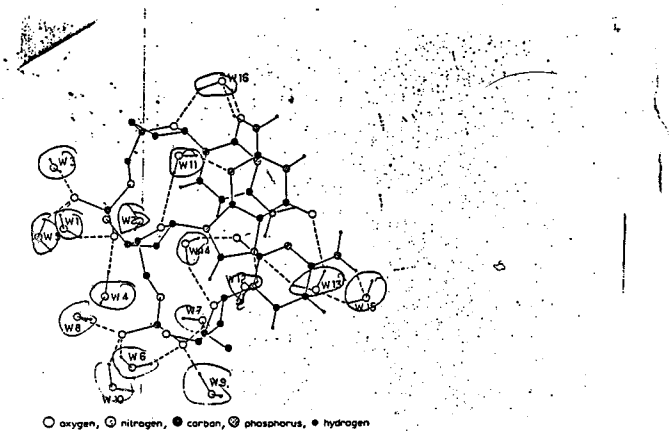


Figure 17-1. Preferred hydration sites in B-DNA. Numbers 1 to 5 indicate strength of binding, in decreasing order. Around phosphate groups, about five water molecules are found. Adapted from (1158).

FIGURE 9:

WATER MOLECULES OF THE FIRST HYDRATION SHELL

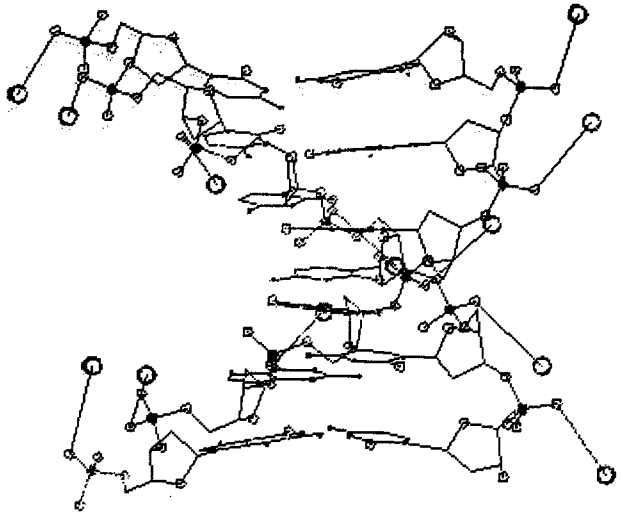


○ oxygen, ⊙ nitrogen, ● carbon, ⊘ phosphorus, • hydrogen

Fig. 4. Projection along the helixal axis of the model compound indicating the locations of the water molecules of the first hydration shell.

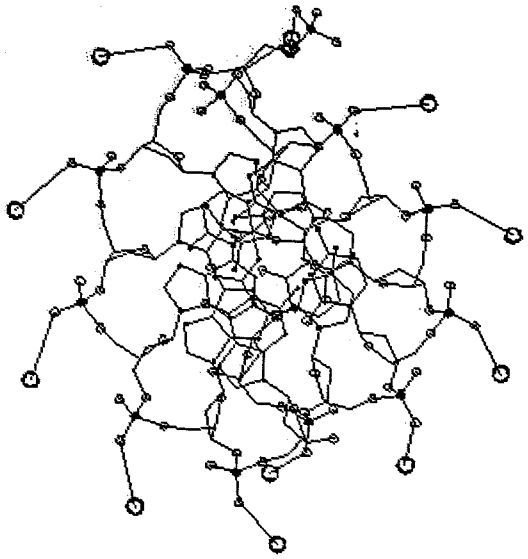
FIGURE 10:

HEXAMER IN SOLVENT MEDIUM



SIDE VIEW

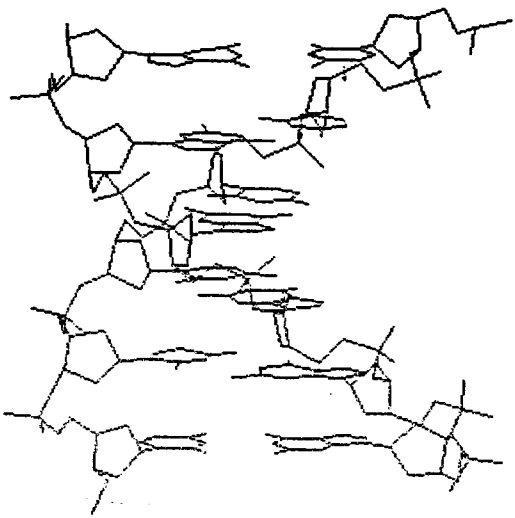
FIGURE 10B:



TOP VIEW

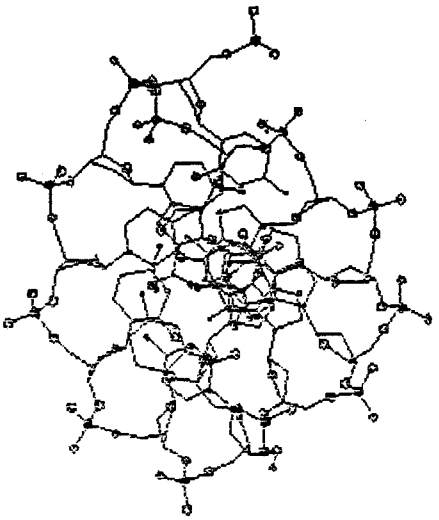
FIGURE 11:

UNREFINED HEXAMER CONFORMATION



SIDE VIEW

FIGURE 11B:



TOP VIEW

FIGURE 12:

HEXAMER IN GASEOUS MEDIUM

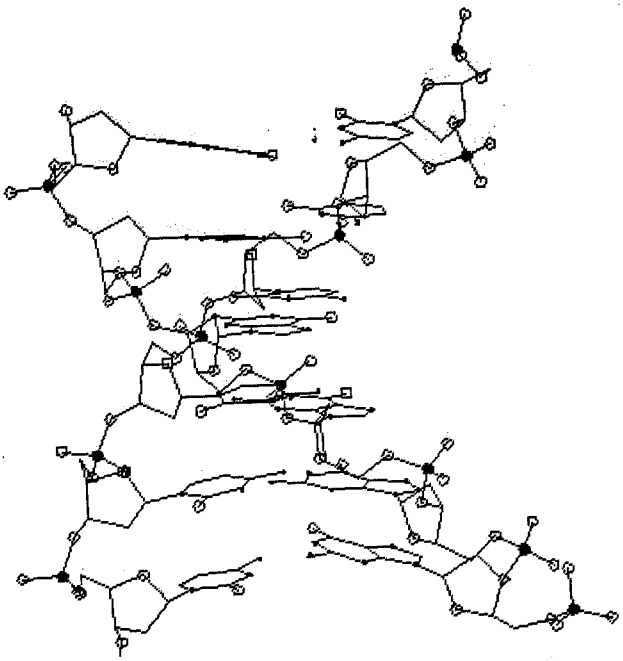


FIGURE 13:

BINDING OF TRIOSTIN A

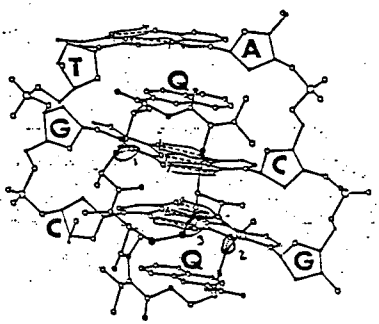


Figure 7. A diagram of the drug triostin A interacting with a fragment of DNA. In addition to the base pairing hydrogen bonds, three more hydrogen bonds are seen stabilizing the drug-DNA interaction. There are two pseudo-symmetrically disposed hydrogen bonds from the two backbone amino hydrogens to the two N3 atoms of the guanines. In addition, there is a hydrogen bond from the N2 of the lower G to a peptide carbonyl. The two pseudo-symmetrically related atoms are substantially farther apart with no indication of hydrogen bonding (refs 17,18).

FIGURE 14:

THE BINDING OF DAUNOMYCIN

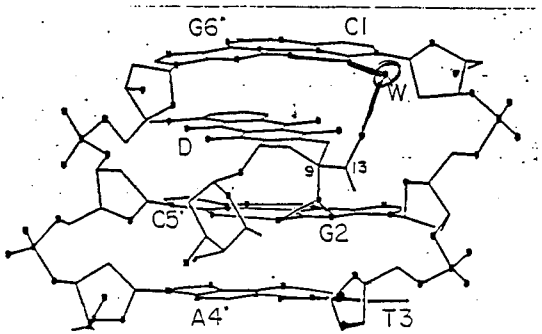


Figure 6. A diagram of the drug daunomycin binding to the minor groove of a fragment of right-handed DNA. The 09 hydroxyl of the daunomycin is donating a hydrogen bond to the N3 of the central G of the right strand. The N2 amino group of the same G may be contributing a weak non-linear hydrogen bond to the same hydroxyl. A very well-defined water molecule forms a hydrogen bonding bridge between the drug and DNA by donating hydrogen bonds to both the carbonyl O2 of the top right C and the carbonyl O13 of the drug (ref 15).

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