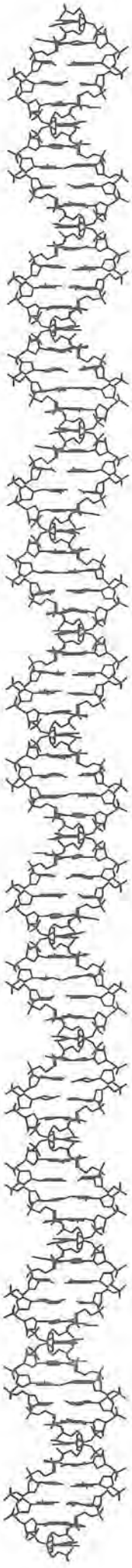

THE CIRCULAR DICHROISM OF DNA-LIGAND SYSTEMS

A computational study.

Reidar Lyng

Department of Physical Chemistry
Chalmers University of Technology
Sweden

MCMXCII

THE CIRCULAR DICHROISM OF DNA-LIGAND SYSTEMS
A computational study.

Reidar Lyng

Department of Physical Chemistry
Chalmers University of Technology
S-412 96 Göteborg
Sweden
MCMXCII

Errata & Amendments

Page	Line	Original text	Change to
vii	8	Manuscript submitted to Biopolymers	Accepted for publication in Biopolymers
3	10	relate	examine
6	4-5	magnetic transition dipole moment	magnetic dipole transition moment
7	15	gives	give
10	6b	The results of the	The
13	17	decorrelation	random phase
23	8b	$\left\lvert \underline{r}_{BA}^{3} \right\rvert$	$\left\lvert \underline{r}_{BA} \right\rvert^{3}$
23	7b	$\left\lvert \underline{r}_{BA}^{2} \right\rvert$	$\left\lvert \underline{r}_{BA} \right\rvert^{2}$
23	6b	& Davis	& Davis [1974b]
28	foot-note	(1 + kK + kK (K-1) / 2)	(1 + kK + k^2K (K-1) / 2)
28	foot-note	190000	19000
29	5	densities.	densities
32	4b	planes	plane
32	3b	base pair surrounding	base pairs surrounding
35	last	are the numbering convention for the atoms in these molecules and	is

continued...

Page	Line	Original text	Change to
37	6	aussian	Gaussian
40	8	electric transition dipole moment	electric dipole transition moment
41	5b	$\sqrt{f \cdot \lambda}$	$\sqrt{f \cdot \lambda}$
42	6	is a comparison of	compares
46	last	oscillators	oscillators.
47	5b	One admittedly attractive feature of this explanation is that there is no mechanism by which an eda transition in a DNA adduct can pick up circularly dichroic intensity from an mda transition in the DNA molecule. For all intents and purposes of these induced CD calculations the model adduct transition is effectively blind to the suggested presence of a magnetic dipole transition.	The presence of an mda transition in the DNA molecule is expected to give only a small contribution to the induced CD of the model adduct transition.
52	7b	were	was
55	9	intercalation between	intercalation, on the helix axis, between
56	18	resonably	reasonably
58	last	666 nm	664 nm
59	19	Paper III,	Paper III
60	last	666 nm	664 nm
61	16	Popov, 1979	Platonova & Popov, 1979
66	2	rotation in the plane	rotation of the intercalator in the plane
74		(Missing reference)	Petke, J.D., Maggiora, G.M. & Christoffersen, R.E. (1990) ***J. Am. Chem. Soc.*** **112**, 5452-5460.
75	8	Redman	Redmann

Paper II

15	8	have no influence	have little influence

Abstract

Circular dichroism (CD) is an important spectroscopic tool for the study of nucleic acids, often used to monitor changes in their secondary structure. CD is often easily measured, but difficult to interpret in terms of detailed molecular geometry. When an achiral substance binds to DNA it will show an induced CD (ICD). The ICD, which is the main subject of this thesis, contains valuable information on the geometry of the DNA-ligand complex.

This thesis presents calculations of the CD of $[poly(dA\text{-}dT)]_2$ and $[poly(dG\text{-}dC)]_2$, and their complexes with achiral small molecules. The matrix formalism of Schellman and co-workers [Bayley, Nielsen and Schellman (1969) J. Phys. Chem. 73, 228-243] is applied to the calculation of the induced rotatory strength (IRS) of a DNA-ligand, as a function of the position and orientation of the ligand relative DNA. Both intercalation and groove-binding is considered. The ligand is described by a single electric dipole transition moment. The intrinsic CD spectra of the two alternating purine-pyrimidine polymers is also calculated.

The calculated intrinsic CD spectrum of $[poly(dG\text{-}dC)]_2$ agrees well both with experiment and previous calculations. The CD spectrum of $[poly(dA\text{-}dT)]_2$ is calculated using recently assigned transition moments parameters for adenine; for this case the result agrees less well, and the reasons for this is discussed.

The results of the calculations of IRS show that (1) the magnitude of the IRS for an intercalator is less than one order of magnitude weaker than that for a groove bound ligand, and, (2) the IRS of an intercalated ligand depends both on the orientation of the ligand in the intercalation site, and on its displacement from the helix axis. Both for intercalators and groove-binders, the IRS is significantly different if the ligand is bound to 5'pyrimidine-3'purine or to 5'purine-3'pyrimidine sites. The signs and magnitudes of the calculated IRS agree well with the experimental observations.

The calculated results are compared to experimentally observed induced rotatory strengths for a number of adducts bound to DNA, among them methylene blue, 4',6-diamidino-2-phenylindole, and Ru(1,10-phenanthroline)$_3^{2+}$. The binding geometries of these adducts bound to DNA are discussed in view of the calculated results.

Keywords

circular dichroism, optical activity, induced circular dichroism, calculation of circular dichroism, transition moments, DNA, DNA ligand, methylene blue, 4',6-diamidino-2-phenylindole, Ru(1,10-phenanthroline)$_3^{2+}$.

List of papers

The thesis is based on the work presented in the following papers, referred to by Roman numerals in the text.

I. **Reidar Lyng, Alison Rodger & Bengt Nordén.**
The CD of Ligand-DNA Systems. I. Poly(dG-dC) B-DNA.
Biopolymers (1991), **31**, p 1709-1720.

II. **Reidar Lyng, Alison Rodger & Bengt Nordén.**
The CD of Ligand-DNA Systems. II. Poly(dA-dT) B-DNA.
Manuscript submitted to Biopolymers.

III. **Reidar Lyng, Torleif Härd & Bengt Nordén.**
Induced CD of DNA Intercalators: Electric Dipole Allowed Transitions.
Biopolymers (1987), **26**, 1327-1345.

IV. **Daniel Fornasiero, Tomas Kurucsev, Reidar Lyng & Bengt Nordén.**
Circular Dichroism and Absorption Spectra of Mono and Di-Aminoacridines Complexed to DNA.
Croatica Chimica Acta (1989), **62**, 339-350.

CONTENTS

1. Introduction.

The subject of this thesis is the relation of circular dichroism (CD) to the binding geometry of a DNA-ligand complex.

Throughout the work presented here I have found it consistently problematic to explain in plain language to friends outside the Department what the meaning of this simple first statement is. I have often begun by asking if she or he knows what plane-polarized light is. The answer is usually no, so I simply state that I am examining a method for studying the geometry of how small molecules are bound to DNA. This has the advantages both of being true and of giving room for a motivation of why such a study is interesting, and quite often the next question is what good it is to know about such things. My answer is that a number of substances are known to bind to the DNA molecule and the manner of the interaction is intimately related to the binding geometry of the two molecules. Examples of substances that interact with DNA are: proteins in the cells of living organisms; carcinogenic substances that interfere harmfully with the functions of DNA; cell poisons used in cancer therapy, deliberately designed to sabotage the DNA functions of cancer cells; and, molecules with known structures that are used as laboratory models for any of the previous three categories. I round off the explanation by saying that CD is easily measured, but difficult to interpret and that there is a need for relating the measured CD to the binding geometry. However satisfying people may claim this explanation to be, I invariably feel slightly guilty of not being able to give a full explanation covering all aspects of my work.

To fully understand CD it is necessary to know something about the electromagnetic nature of light, something about quantum mechanics and something

about symmetry. This introduction aside I shall assume that the reader already has more than an inkling of these concepts.

Circular dichroism is intimately related to optical activity which in turn is defined as the observed rotation of plane-polarized light when it is passed through a substance. The term optical activity harks back to the last century when substances able to rotate plane-polarized light were said to be active in an optical sense, as opposed to substances which left the nature of the light intact when it shone through them. The criterion for optical activity can be stated in terms of the symmetry of the substance. A chiral molecule is one that can be distinguished from its mirror image, in the same way that we know our left hand from our right. A pair of chiral molecules that are each others mirror images are called enantiomers. There is a corresponding chirality of light: plane-polarized light can be decomposed into a left and a right circularly polarized component. The simplest way of understanding optical activity is to think of two kinds of light of different handedness interacting differently with a molecule of a specific handedness. Optically active media are circularly birefringent, i.e., have different refractive indices for left (lcpl) and right (rcpl) circularly polarized light. Since refraction and absorption are closely related, an optically active medium also absorbs lcpl and rcpl differently. Circular dichroism is defined as the difference between the absorption of lcpl and of rcpl for a substance.

The particular attraction of using CD for studies of biomolecules is that CD is the only direct probe of the asymmetry of a system. NMR techniques require either assumptions or additional information about the structure of a system to resolve the absolute structure of enantiomeric substrates. X-ray diffraction methods are able to determine the absolute structure of any compound that can be grown as a crystal. The procedure is quite time consuming and the three-dimensional structure of the crystal need not be the same as the structure in the environment of interest. CD contains in principle all information about the asymmetry of a system, and it is the problem of extracting the resident information that has been the main reason for all attempts to calculate the CD of

different systems. Identifying one enantiomer from another may be very important as is amply illustrated by the case of thalidomide. Thalidomide was synthesized in 1954 by the Chemie Grünenthal G.m.b.H. in West Germany. The drug was found to have sedative effects and was soon marketed in a number of countries under a number of names, among them Neurosedyn [Mellin & Katzenstein, 1962]. It soon became apparent that use of the drug during pregnancy led to teratogenicity, i.e., fetal deaths and malformations [ibid.]. Thalidomide exists as two enantiomers and it is only the S-(-)-enantiomer that is teratogenic [Blaschke et al., 1979].

The aim of this work is to relate the CD induced by the DNA base transitions into an electric dipole allowed transition of a ligand bound to DNA. The motivation is to determine the induced CD as a function of the geometric arrangement of an adduct transition and DNA. To do so a number of assumptions have been made, both within the theory underlying the program used, and in the particular choice of input parameters. Most importantly, only $\pi \rightarrow \pi^*$ transitions in the nucleic acid bases have been considered. The intrinsic CD spectra of some polynucleotides have also been calculated as a simple check of the quality of the spectroscopic parameters for DNA.

Though this is not the proper forum for a lengthy consideration of CD I wish to name some relevant references. There are a number of books on the subjects of optical activity and circular dichroism. I have found "The molecular basis of optical activity" by Elliot Charney [1979] quite informative. I have also found a manuscript of John Schellman's for a book on CD very useful indeed, especially in providing a thorough presentation of the matrix formulation of the CD calculations; I am indebted to many of the limpid explanations given in this unpublished textbook. A copy of the manuscript was very kindly given to me by John Schellman during his six months stay at our department in 1986. Two sources for the quantum mechanics that I have often consulted are "Molecular Quantum Mechanics" by P.W. Atkins [2nd ed., 1986] and "Quantum Chemistry" by Eyring, Walter & Kimball [1944]. The necessary descriptions of the nature

of polarized light and electromagnetic radiation are often covered in the same text books as optical activity. A thorough exposition of polarized light is found in the monograph by Shurcliff [1962]. The symmetry aspects mentioned will not be dwelt on here, but are very useful for understanding and discussing the CD of many systems. Symmetry can be treated very powerfully by group theory; this has been extensively discussed in papers by Schellman [1966; 1968] and Schipper [1978].

This thesis is divided into chapters dealing with the background, the theory, and the results. Chapter 2 is a brief review of previous calculations both of the intrinsic CD of DNA molecules, and of the induced CD of ligands bound to DNA. Chapter 3 deals with the theory. Between the extreme alternatives of merely citing the relevant references or writing a text book on CD theory, I have opted for a chapter of the kind I myself would have liked to read when I started the work. In chapter 4 the input parameters to the program are discussed. Chapters 5 and 6, finally, constitute the presentation of the results and the conclusions.

2. Background.

Circular dichroism is the method of choice for monitoring the secondary structure of nucleic acids in solution, and has been so ever since the first CD measurement on nucleic acids nearly thirty years ago [Brahms & Mommaerts, 1964]. It is singularly sensitive to the conformation of nucleic acids, and measurements can be made on relatively small amounts of material. Unfortunately, its usefulness in actually determining or defining structural details of nucleic acids is still limited, though progress has been made over the years. Consequently the CD of nucleic acids and nucleic acid systems is most often used in an empirical manner [Johnson, 1985], and catalogues of CD spectra of DNA have been compiled [Johnson, 1990]. A number of approximate methods for calculating the CD of DNA have been formulated. Many of these works have increased the understanding of the CD of the nucleic acids, and they are briefly reviewed in section 2.2 and 2.3.

2.1 Optical activity and the rotatory strength.

Throughout my undergraduate years I lived under the delusion that all optical activity was "caused" by asymmetric carbon atoms. Instead, optical activity results from the chiral currents of electrons which are generated in molecules by radiation fields. In 1928 Rosenfeld [Rosenfeld, 1928] derived a quantum mechanical formula for optical activity in non-absorbing regions. He did it by treating the radiation field classically, deriving the electric moments induced in a quantized system by the time derivative of the magnetic field, and the magnetic moments induced by the time derivative of the electric field. The kernel of Rosenfeld's work is the rotatory strength

$$\mathbf{R} = \Im m\ (\underline{\mu}^{if} \cdot \underline{m}^{fi}), \tag{2.1}$$

where $\Im m()$ means the imaginary part of the expression contained within the parentheses. This fundamental equation, which holds also in absorbing regions, relates the rotatory strength, $\mathbf{R}$, to the matrix elements for the electric dipole transition moment (edtm), $\underline{\mu}^{if} = \langle i|\underline{\mu}|f\rangle$, and for the magnetic transition dipole moment (mdtm), $\underline{m}^{fi} = \langle f|\underline{m}|i\rangle$, belonging to the $i \rightarrow f$ transition of a molecule.†

For a transition to be optically active it is necessary that the transition is accompanied both by a linear displacement of charge, otherwise the electric dipole moment, $\underline{\mu}^{if}$, is zero; and by a net circular flow of charge, otherwise the magnetic dipole moment, $\underline{m}^{fi}$, will be zero. The presence of the dot product in the formula guarantees that a component of the circular flow is around the direction of the linear displacement of charge. The result can be seen as a helical displacement of charge.

We shall not delve into the mysteries of electromagnetic radiation here. Suffice to say that circularly polarized light is defined as radiation in which the field vectors have constant amplitude, and change orientation in a uniform circular motion. The simplest way to distinguish between left and right circularly polarized light is to follow the tip of the field vector in space; chirality is a spatial, not a temporal property. For right circularly polarized light the tip describes a right handed helix in space. It is then not so hard to imagine that the interaction of the helical rotation of photons with the helical rotation of electrons will depend on their relative chirality. Rigorous expositions of polarized light are found in Shurcliff [1962] and in Schellman & Jensen [1987].

In principle, Equation 2.1 contains all the necessary information for calculating the optical activity of any system. Calculations of optical activity can be made in two fundamentally different ways, depending on whether the molecule

† Throughout the rest of the thesis the following abbreviations are used: edtm = electric dipole transition moment; mdtm = magnetic dipole transition moment; eda/mda = electric/magnetic dipole allowed; edf/mdf = electric/magnetic dipole forbidden.

can be divided into specific groups which have little or no electronic exchange. If this division into groups is not possible the Schrödinger equation must be solved for the entire molecule; the solution gives the electric and magnetic dipole moments for all transitions of interest and the corresponding rotatory strengths can be calculated using Eq. 2.1. If, on the other hand, the molecule can be divided into electronically independent groups another approach to the calculations is possible.

The second method, sometimes called the independent systems approach, is less exact but has proven useful for understanding the CD of many systems. The basic premise is that the molecule is optically active and can be divided into chromophores. An ideal chromophore is a group of atoms which remains structurally intact when combined with other groups, and which interacts with radiation as a separate unit independent of the nature of the other groups. There is no interchromophoric exchange of electrons, and the perturbing interactions of the constituent chromophores gives rise to optical activity. An example of such a system is DNA where the chiral arrangement of the DNA bases in the helix is the major source of circular dichroism for the molecule, at least for wavelengths longer than 200 nm [Moore & Wagner, 1974; Johnson, 1990]. The planar DNA bases themselves are optically inactive. Clearly, the first method is inappropriate for systems the size of a DNA-molecule for the simple reason that even a short fragment of DNA is far too large to be handled effectively by direct quantum mechanical calculations. An added advantage of the independent systems approach, in contradistinction to direct calculations, is that the results can be transferred to other systems.

When circular dichroism is interpreted it is often thought to arise from one or more of three basic models for optical activity. The division into different models for optical activity is heuristically motivated, as a perturbation expression to the first order for the rotatory strength of an eda transition leads to three different terms which can be understood to arise from different interactions in the system.

The three mechanisms are often called 'static coupling', 'dynamic coupling' and the coupled oscillator mechanism.

Optical activity due to static coupling is caused by the presence of a chiral static field surrounding the transition of interest on the (otherwise) achiral chromophore. The perturbation mixes the states on the achiral chromophore so that the chromophore behaves as though it were intrinsically chiral. Static coupling is also known as the 'one-electron-mechanism', from an analogy with an electron moving in a chiral field. The model was originally derived by Condon, Altar & Eyring [1937].

Dynamic coupling is caused by the circular motion of electrons in mda transitions in the surroundings of the achiral chromophore. The circular motion of the electrons induces some helical character into the eda transitions on the achiral chromophore. This mechanism is also known by the name μm-coupling. It was originally considered by Kauzmann et al. [1940].

The coupled oscillator mechanism corresponds to the coupling of eda transitions that are chirally arranged in space. It is often referred to as Kirkwood-Kuhn coupling, after J.G. Kirkwood [Kirkwood, 1937] and W.A. Kuhn [Kuhn, 1930].

None of these three mechanisms provides a general description of optical activity by itself; in any real system all mechanisms are operative simultaneuosly, together with effects arising from higher order interactions.

2.2. Calculations of intrinsic CD of DNA.

The early years of the decade 1950-59 saw the discovery of the ordered structures of both proteins (the α-helix and the β-sheet) and the Watson-Crick helix for DNA [Pauling et al., 1951; Watson & Crick, 1953]. In 1956 Moffitt presented a theoretical study on the optical rotatory dispersion of helical polymers [Moffitt, 1956]. The study extended Kirkwood's treatment [1937] to allow for the nondegenerate interactions of eda transitions. Moffitt conceived of the α-helix as a one-dimensional crystal with a screw axis and treated the helical polymer

as a degenerate exciton system. The treatment leads to a qualitatively correct description for the origin of CD arising from strong transitions in helices similar to the α-helix, but, unfortunately, transition moments that are perpendicular to the helix axis do not contribute at all to the rotatory strength of the polymer. The exciton approach to the optical activity of helices was further developed by Moffitt, Fitts & Kirkwood [1957], and by Tinoco [Tinoco et al., 1963; Bradley et al., 1963; Tinoco, 1964]. Bradley et al. calculated the CD of poly A with only interactions between nearest neighbours [1963].

Another approach was taken by Tinoco in a paper from 1960, and in the systematic investigation of the optical activity of polymers published in 1962 [Tinoco, 1960;1962]. The aim was to formulate a method for the calculation of a polymer's optical properties from the properties of the constituent groups and the polymer's geometry. Relations between polymer wave functions and group wave functions were derived, assuming that no exchange of electrons between groups occur. In the course of the derivation expressions for the rotatory strength, including all major mechanisms (Cf next chapter), were presented. For computational convenience in the derivation first order perturbation theory was used. Also, the potential energy from the Coulombic interactions was expanded in terms of $1/r$, and only pairwise interactions were considered. The resulting expressions are instructive but unwieldy to implement and have not been used in their full form to calculate the CD of DNA.

DeVoe presented a classical model for the frequency dependence of absorption, refraction and optical rotation of polymers in terms of complex polarizabilities [DeVoe, 1964; 1965]. The incident light induces electronic polarizations of the constituent monomer units, and the polymer polarization is modified by Coulombic interactions between the monomers. Each monomer transition is described by a complex frequency-dependent polarizability, which is derived by Krönig-Kramer transforms from the absorption properties of the monomer.

Bush & Brahms made partial use of Tinoco's derivations [1962] to discuss the contribution to the CD of single stranded oligonucleotides by $\pi \rightarrow \pi^*$ transitions. They also considered the effect of tilting the base planes with respect to the helix axis [Bush & Brahms, 1967]. Bush also investigated the optical activity of $n \rightarrow \pi^*$ transitions in polynucleotides [Bush, 1970], and concluded that the contribution of $n \rightarrow \pi^*$ transitions to the polynucleotide CD was small, but noticeable.

A simple theory of polynucleotide CD was presented by Tinoco in 1968 [Tinoco, 1968]. It was applied by Johnson & Tinoco in 1969 for the calculation of the CD of RNA and DNA [Johnson & Tinoco, 1969a]. The CD of a polynucleotide is considered as the sum of two fundamental contributions: one related to interactions among the electronic transitions in the bases in the wavelength region of interest, and one related to the coupling of these transitions with transitions outside the region, such as all unknown far-UV transitions. The CD, as a function of frequency, was expanded in a Taylor series about the average frequency in the region of interest (260 nm), and the CD was calculated for the wavelength region 220-300 nm. The interactions between the base transitions were calculated using first order perturbation theory and the monopole approximation of London [1942] and Haugh & Hirschfelder [1955]. The interactions with transitions outside the range of interest were calculated using group polarizabilities. A problem with the method, shared with other methods mentioned in this section, is that it requires transition moments for the transitions of interest. The most common source for the transition moments is experiment, although theoretically calculated values have occasionally been used. The results of the calculations were surprisingly successfull, considering the simplicity of the theory. The low CD intensity of natural DNA and RNA (in the region 220-300 nm) was found to be explained by cancellation of a great number of CD bands of opposite signs. The method fails to predict the maxima of the CD bands, and it fails to reproduce the CD spectra of polynucleotides of repeating sequences. CD

spectra with more than one peak and one trough seemingly cannot be accounted for by this approximation.

This method of Johnson & Tinoco [1969a] was subsequently used by Moore & Wagner in two papers [Moore & Wagner, 1973; 1974], and by Studdert & Davis in a series of three papers [Studdert & Davis, 1974a-c]. The studies are applications of the method and present no new theory. In the first study Moore & Wagner calculated the CD of DNA and RNA considering only $\pi \rightarrow \pi^*$ transitions of the DNA bases [1973]. The polymer CD spectrum was found to depend on the distance between the transition moments and the helix axis. In their second study they investigated the contribution of the sugar-phosphate transitions to the CD spectrum of DNA, and found them to be negligible [1974]. Studdert & Davis examined the effects on the DNA CD of $\pi \rightarrow \pi^*$ transitions alone [1974a] and including $n \rightarrow \pi^*$ transitions [1974b], and of the choice of wave functions to describe the monomer properties [1974c]. They found the overall agreement with experiment to be moderately good. The calculations show a strong dependence on the geometrical parameters that define the helicity of the polynucleotide, i.e., the distance of the base pairs from the helix axis and the tilt of the base pairs [1974a]. Inclusion of the $n \rightarrow \pi^*$ transitions improves some spectra [1974b]. Studdert & Davis added terms for the $n \rightarrow \pi^*$ transitions from Tinoco's treatment [1962] to their rotatory strength expressions. They concluded that the largest contributions of $n \rightarrow \pi^*$ transitions to the CD of double helical DNA are comparable in magnitude to those of $\pi \rightarrow \pi^*$ transitions only where the CD is weak. They found the DNA CD due to the μm-coupling mechanism to be one order of magnitude weaker than the CD due to the coupling of the $\pi \rightarrow \pi^*$ transitions, and one order of magnitude stronger than the CD due to the 'one-electron-mechanism.'

A general method for calculating the CD of any molecular system that can be divided into chromophores, i.e., groups of atoms with no electronic exchange, was presented by Schellman and co-workers in 1967 [Schellman & Nielsen, 1967], and developed in a paper from 1969 by Bayley, Nielsen & Schellman. The method

is used throughout the thesis work presented here and it is described in some detail in the next chapter. The method has mainly been used for calculations of polypeptide CD [Bayley et al., 1969; Hooker & Schellman, 1970; Madison & Schellman, 1972; Goux & Hooker, 1980]. With the exception of the work of Cech [1975, *vide infra*] it was not applied to calculations of nucleic acid CD until 1984 [Rizzo & Schellman, 1984].

Johnson & Tinoco published a second method for calculation of nucleotide CD in 1969 [Johnson & Tinoco, 1969b]. The division of the monomer properties of DNA into transitions above and below 220 nm is retained, but the interactions between the dimers are considered explicitly using expansions of the polymer wave functions in a restricted basis set. The result is a method reminiscent of the method of Bayley et al. [1969]. The method was used on dinucleoside phosphates [Johnson & Tinoco, 1969b]. Johnson & Switkes used the method to calculate the CD of a number of dinucleotides [1978].

In the thesis of Cech [1975], and in two following papers [Cech et al., 1976; Cech & Tinoco, 1977] the all-order, coupled-oscillator polarizability theory developed by DeVoe [1964; 1965] was applied to calculations of polynucleotide CD. Cech compared the results of the calculations with sample results calculated by the matrix method of Bayley et al. [1969]. Cech disregarded the absorption properties of the sugar-phosphate backbone and the $n \rightarrow \pi^*$ transitions, but included a set of "background oscillators" intended to mimic the host of unknown transitions in the far-UV. The use of such background oscillators was originally suggested by Fitts & Kirkwood [1957]. These consisted of three mutually perpendicular polarizabilities, arbitrarily placed at 119 nm, and their magnitudes were estimated on the basis of model compounds. In the study Cech found an effective dielectric constant, twice that of vacuum, to give the best results. The problem of inconsistent representations of transition moments was also discussed. In the papers by Johnson & Tinoco [1969a-b], and in the work of Cech, the transition moments used to calculate the rotatory strength of the polymer were represented by polarizabilities, but the Coulombic interactions of the transitions were

most often calculated using transition density monopoles [Tinoco, 1962; London, 1942]. These transition monopoles were taken from semi-empirical calculations by Hug & Tinoco [1973] on the nucleic acid bases. Cech thought the choice of monopoles to be one of the most critical factors in predicting correct CD patterns [Cech et al., 1976]. The results of the calculations of polynucleotide spectra were uneven: agreement with experiment was remarkably good in some cases and disappointing in others [Cech & Tinoco, 1977]. Cech also discussed the differences between the polarizability approach of DeVoe and the matrix method of Schellman. The matrix method does not take bandshapes of monomers or polymers into account; these have to be assigned more or less arbitrarily and can only be thought of as representations of the bands. The DeVoe method, on the other hand, requires integrated polarizabilities from absorption properties, and consumes more computer time as a result of the explicit calculation of point-by-point spectra [Cech et al., 1976]. An extension of the DeVoe model for the calculation of CD in helical polymers was presented by Levin & Tinoco in 1977 [Levin & Tinoco, 1977].

Redmann & Rhodes [1978] made use of linear response theory in the decorrelation approximation to calculate the CD of single stranded poly A in a helical conformation. The method has the advantage that spectral band shapes of the polymer arise naturally from those of the monomer, and from the geometry dependent interactions in the helix. The polymer CD spectrum was found to depend on the monomer bandshape. Rabenold has used linear response theory together with time dependent Hartree theory for the CD of unordered [1974] and helical [1990] polymers. Rabenold and Rhodes have presented joint papers on the CD, both of short and long, helical polymers [Rabenold & Rhodes, 1976; 1977]. In a paper from 1987 they reach the conclusion that the CD bands for infinitely long helical polymers are composed of two parts: a derivative shaped band, plus an ordinary Gaussian band. The paper can be seen as a more rigorous theoretical justification of the method of Johnson & Tinoco [1969a], mentioned above.

In a series of papers Moore & Williams treated the problem of obtaining electronic transition moment parameters for the calculation of polynucleotide CD spectra [Moore, 1980; Williams & Moore, 1983; Moore & Williams, 1986]. They combined DeVoe's and Kirkwood's polarizability concepts, and calculated the CD of cyclic nucleotides in energy minimized conformations. They also optimized the transition moment parameters from CD calculations of cyclic nucleotides. These optimized parameters were found to result in better agreement with experimental CD spectra than did transition moments obtained from polarized spectra of the nucleic acid bases in crystals or stretched films. In order to achieve better results, the polarizations of the first two transitions of guanine were switched, a procedure that has been criticized by Callis [1983].

In 1984 Rizzo & Schellman presented matrix method calculations of absorption, LD and CD spectra of DNA. Their input consisted largely of the same parameters as were used by Cech, and wave-functions and transition moments parameters were extensively borrowed from the works of Johnson and Tinoco. In order to avoid the inconsistent representations of the transition moments in the calculations of Coulombic interaction and rotatory strength, Rizzo & Schellman optimized the transition density monopoles so that their directions agreed with the directions of the transition moments in the expressions for rotatory strength. Rather than calculating the CD of the polynucleotide as a function of structure, they chose to calculate the CD of polynucleotides for a great number of experimentally determined or proposed structures. The results of the calculations were an improvement over most earlier calculations, but failed to identify the CD spectrum of Z-DNA with the high salt CD spectrum of $[poly(dG\text{-}dC)]_2$. Using the program of Cech [Cech, 1975], Vasmel & Greve [1981] found the Z-DNA structure to result in a CD spectrum that corresponded to the high salt CD spectrum of alternating GC. Rizzo & Schellman concluded that the problems could arise from not using the right coordinates or from a need for improved transition parameters [1984]. Williams et al. [1986] used the matrix method with improved transition moment parameters, still excluding sugar-phosphate and

$n \rightarrow \pi^*$ transitions. Their calculations included a number of far UV monomer transitions, making it possible to calculate the CD down to 160 nm. The results agree well with experiment, especially for alternating GC, and are consistent with the observations of Sutherland and co-workers that the vacuum-UV CD spectra of DNA are sensitive to helical handedness [Sutherland et al., 1981 ; Sutherland & Griffin, 1983].

Callahan & Hooker [1987] have also performed matrix method calculations of DNA CD. For their calculation of the CD of B- and Z-form DNA, they used crystal structures taken from the Protein Data Bank [Bernstein et al., 1977], and an effective dielectric constant that was a function of the distance between the interacting units. They found that the agreement between experiment and calculation was improved by the use of such a function.

The influence of basic optical parameters was investigated by Richterich & Pohl who calculated the CD for a tetramer of alternating GC in the A, B and Z forms of DNA [Richterich & Pohl, 1987]. They used the classical polarizability theory of DeVoe and the point dipole approximation. The spectra show only moderate agreement with experiment, and they concluded that the transition moment directions of the nucleic acid bases are the most important parameters in their calculations.

A method for estimating the nearest neighbour base pair content of RNAs using CD and absorption spectroscopy has been presented by Johnson & Gray [1991a; 1991b]. The nearest neighbour content is estimated by linear combinations of a large number of known CD and absorption spectra. The method is reminiscent of the methods for determining the secondary structures of proteins. By fitting the spectra of a basis set containing 58 CD and 58 absorption spectra to the CD of an RNA molecule of known sequence, Johnson & Gray were able to determine the fractions of each of the nearest neighbour base pairs [1991a]. They have also applied the method to RNA molecules of unknown sequence [1991b].

2.3. Calculations of DNA induced CD.

In his review of induced CD in biopolymer-dye systems Hatano [1986] mentions only one theoretical or computational study of DNA induced CD: that of Schipper, Nordén & Tjerneld [1980]. Comparatively few such studies of the DNA induced CD have been made, and these are almost exclusively concerned with intercalators. That intercalators have been the subject of interest to a much higher degree than groove binders is not surprising. Since Lerman's proposal in 1961 of intercalation as a possible binding mode, intercalation has attracted widespread interest as a mechanism of potential biological significance. It is not until the last decade that groove binding of drugs to DNA has been recognized. With improved NMR and foot-printing techniques more time and interest have been spent on elucidating the binding geometries of groove binders to DNA.

The aforementioned study of Bradley et al. [1963] is an early example of the calculation of the CD of a DNA-dye system. Their calculations could not, however, reproduce the induced CD of Acridine Orange bound to DNA. The same system of Acridine Orange and DNA has been considered by Imae & Ikeda [1976] and Imae et al. [1987], who used the exciton model of Moffitt, Fitts & Kirkwood [1957]. They considered the degenerate interaction of dye molecules bound to the polynucleotide which was seen merely as a helical framework.

Ito & I'Haya have also been interested in the DNA-Acridine Orange system. They have made use of linear response polarizability theory to calculate the induced CD of Acridine Orange bound to DNA in varying phosphate/dye ratios [1979]. In a later paper they investigated the dependence of the CD bandshapes on the interaction between the dye and DNA [1989].

The induced CD of proflavine bound to DNA has been treated by Kamiya who used a Frenkel exciton model for the dye-polymer interaction [1979]. Kamiya also used linear response theory to investigate the optical activity for DNA-dye systems [Kamiya, 1980; 1988a-b]. Some of this work is based on the more general formalism of Philpott, who has formulated an exciton theory for the electronic

states of polymer-dye systems [Philpott, 1970; 1972]. A linear response theory for the CD of polymer-dye complexes has been derived by Rabenold [Rabenold, 1983].

In the two papers by Schipper et al. [1980] and Nordén & Tjerneld [1982] the induced CD of an in-plane transition of an intercalated adduct was related to its orientation. Schipper et al. [1980] assigned a single electronic transition moment to each base, and considered the pairwise interactions between an intercalator and the base pairs of the DNA molecule. The results were compared to the experimentally observed CD for a number of intercalators bound to DNA, and were found to be consistent. From the results presented by Schipper et al. [1980], Nordén & Tjerneld [1982] derived a simple formula which can be written

$$R = f \cdot |\underline{\mu}|^2 \; cos2\gamma, \tag{2.2}$$

where f is a positive constant, $|\underline{\mu}|^2$ is the square of the length of the edtm of the intercalated adduct, and γ is the angle between the adduct's edtm and the pseudo dyad axis of the surrounding base pairs. The paper also presents an experimental observation, for the dye methylene blue bound to DNA, of an induced CD band which changes sign as a function of ionic strength. The observation can be explained by a rotation of the dye in the intercalation pocket as the ionic strength increases [Nordén & Tjerneld, 1982].

The approach of Schipper et al. [1980] was developed further by Schipper & Rodger, who derived symmetry rules for the use of CD to determine the intercalation geometry of host/guest systems [1983]. In the derivation the guest and the host were assumed to have no electronic interchange, and the generalized selection rules of Schipper [1978] were used. The results were applied to aromatic guests intercalated into DNA and cyclodextrin hosts, and the agreement with experimental results was found to be good.

The paper of Kubista, Åkerman & Nordén [1988] presents a simple nondegenerate coupled oscillator approach where an eda transition of a groove bound adduct interacts with the in-plane $\pi \rightarrow \pi^*$ transitions of the DNA bases. The

DNA was modelled as an helical array of transitions placed on the helix axis, and the ligand was defined by a single eda transition. All transitions were described by point dipoles, and the CD was calculated from pairwise interactions. Fair agreement with experimental magnitudes was noted. In order to avoid end effects for the induced CD of the ligand it was necessary to include three full turns of DNA in the calculations.

The studies on DNA induced CD by Schipper & Nordén have in common that they aim to find general results which can be applied to any system of DNA and ligand. It is in that spirit this thesis work has been carried out. The Papers I-IV comprise an extensive study of the DNA induced CD of achiral DNA ligands, where the induced CD of an adduct bound to DNA has been systematically investigated as a function of the binding geometry. The adduct was represented by a single eda transition, and only interactions between eda transitions are considered. The DNA was represented by the known $\pi \rightarrow \pi^*$ transitions of the bases, and the transitions were placed in the center of each base. The DNA transitions were taken from experiment, primarily from the studies of polarized reflection by Clark and co-workers [Clark, 1977; Clark, 1989; Clark, 1990; Novros & Clark, 1986; Zaloudek et al., 1985]. For the calculations the matrix method [Bayley et al., 1969] was used. The matrix method has, so far, produced the best agreement between experiment and calculation for the intrinsic CD of DNA [Williams et al., 1986]. In the work presented here only the interactions of electric dipole allowed transitions are considered.

3. Theory.

In section 2.1 it was said that calculations of optical activity can be characterized by whether the molecule of interest was divided into chromophoric groups or not. A number of methods for such calculations were mentioned in the previous chapter and most of them assume that there is no electronic exchange between chromophores. Obviously there are a number of ways to implement calculations of optical activity under this assumption. A straightforward method is to make use of first order perturbation theory to calculate the perturbed electric (edtm) and magnetic (mdtm) dipole transition moments of the molecule. Another method, the one used in this work and which will be presented in this chapter, is the matrix method derived by Schellman and co-workers [Schellman & Nielsen, 1967; Bayley et al., 1969]. Before presenting this method, an expression for the rotatory strength due to coupled edtms on different chromophores will be derived by first order perturbation theory. The result serves to illustrate how the CD can be seen to arise, and provides a formula that can be used in discussions of the induced CD.

3.1 Coupling of electric dipole transition moments.

The use of perturbation theory demands that the transition of interest is located in an achiral chromophore, whilst the perturbing surroundings may or may not consist of intrinsically chiral chromophores. For a system of achiral chromophores in the absence of coupling the rotatory strength is zero, since by symmetry the transitions of such chromophores are either electric dipole forbidden (edf), magnetic dipole forbidden (mdf), or, have perpendicular electric and

magnetic transition moments. Consequently, any rotatory strength of a transition in such a system must be the result of the perturbation of that transition by the interactions with surrounding chromophores.

There are two basic possibilities: the transition in question is electric dipole-allowed (eda), but mdf; or, it is magnetic dipole-allowed (mda), but edf. In the former case the transition becomes optically active by virtue of an induced magnetic moment, $\delta\underline{m}^{fi}$: $\mathbf{R} = \Im m\ (\underline{\mu}^{if} \cdot \delta\underline{m}^{fi})$. In this work we are only concerned with the coupling of eda-mdf transitions.

Consider a system consisting of two chromophores. For the sake of simplicity we shall assume that the transitions on these chromophores are non-degenerate. As the chromophore concept presupposes that there is no electronic exchange between the two units we can write the zeroth order wavefunction as a product of the electronic wavefunctions for A and B. Let $\underline{r}_{BA}$ be the vector from A to B. We shall examine the rotatory strength of an eda transition on chromophore A. As this transition is mdf by assumption we need to evaluate the first non-zero contribution to the magnetic transition. The perturbation is assumed sufficiently weak that only terms to the first order need be considered.

The magnetic dipole transition moment operator can be written [Bayley, Nielsen & Schellman, 1969]

$$\underline{m} = \underline{m}_A + \underline{m}_B + \frac{e}{2m_e}\,(\underline{r}_{BA} \times \underline{p}_A), \tag{3.1}$$

where m_e is the mass of the electron, the first two terms represent the intrinsic magnetic moment operators for the two chromophores, and where $\underline{p}$ represents the electronic momentum operator. The matrix elements of the electronic momentum and electric dipole moment operators are related by the so-called dipole-velocity relation

$$\underline{p}^{if} = \frac{im_e}{e\hbar}\,(E_i - E_f)\,\underline{\mu}^{if}, \tag{3.2}$$

where E_i and E_f are the respective energies of states i and f. First order non-degenerate perturbation theory gives the wavefunctions for the ground state and the state with chromophore A in its first excited state as

$$\mid 0_A 0_B > = \mid 00) - \sum_{a,b} \frac{(ab \mid V \mid 00)}{E_a + E_b} \mid ab) \tag{3.3a}$$

and

$$\mid 1_A 0_B > = \mid 10) - \sum_{c,d} \frac{(cd \mid V \mid 10)}{E_c + E_d - E_1} \mid cd), \tag{3.3b}$$

where the ground state energies of A and B are taken to be zero. Here sharp bras and kets are used for the perturbed states and round ones for the zeroth order states. Also, the position in the bras and kets indicates whether the wavefunction belongs to chromophore A or B, with A henceforth to the left of B. The sums are understood to be taken so that the denominators are non-zero. We wish to find the non-zero terms to the first order for the mdtm associated with the transition $0 \rightarrow 1$ on A. This is given by

$$< 1_A 0_B \mid \underline{m} \mid 0_A 0_B > = < 10 \mid \underline{m} \mid 00 > =$$

$$(10 \mid \underline{m} \mid 00) - \sum_{a,b} \frac{(ab \mid V \mid 00)}{E_a + E_b} (10 \mid \underline{m} \mid ab) -$$

$$\sum_{c,d} \frac{(cd \mid V \mid 10)}{E_c + E_d - E_1} (cd \mid \underline{m} \mid 00) \; + \; (Higher\ order\ term), \tag{3.4}$$

where the higher order term is of second order in V and therefore discarded. It can be shown that the sought magnetic transition moment can be written as

$$< 10 \mid \underline{m} \mid 00 > = \; \underline{m}_A^{10} + \tag{3.5a}$$

$$- \sum_{n \neq 0} \underline{m}_A^{1n} \frac{(n0 \mid V \mid 00)}{E_n} - \sum_{n \neq 1} \underline{m}_A^{n0} \frac{(n0 \mid V \mid 10)}{E_n - E_1} \tag{3.5b}$$

$$-\sum_{n\neq 1} \underline{m}_B^{0n} \frac{2E_1}{E_n^2 - E_1^2} (0n \mid V \mid 10) \tag{3.5c}$$

$$-\frac{i}{\hbar} \sum_{n\neq 1} \frac{(E_1 E_n)}{E_n^2 - E_1^2} (0n \mid V \mid 10)\, \underline{r}_{BA} \times \underline{\mu}_B^{0n} \tag{3.5d}$$

The first term, $\underline{m}_A^{10}$, is the zeroth order magnetic dipole transition on chromophore A which is zero by assumption. Before the rotatory strength for the eda transition on chromophore A can be calculated we need an expression for the perturbation V. V is described by Coulombic interaction and expanded in terms of $\frac{1}{r}$. Only the leading dipole-dipole term of the multipole expansion [Hinchliffe & Munn, 1985] is retained

$$(0n \mid V \mid 10) = \frac{1}{4\pi\epsilon} \left(\frac{\underline{\mu}_A^{10} \cdot \underline{\mu}_B^{n0}}{|\underline{r}_{BA}|^3} - \frac{3\,(\underline{\mu}_A^{10} \cdot \underline{r}_{BA})\,(\underline{\mu}_B^{n0} \cdot \underline{r}_{BA})}{|\underline{r}_{BA}|^5} \right), \tag{3.6}$$

where ϵ is the dielectric constant for the medium, and $|\underline{r}_{BA}|$ is the (absolute) distance between chromophores A and B. With this choice of interaction the rotatory strength of the $0 \rightarrow 1$ transition on A can be written

$$\mathbf{R} = \Im m\, (\underline{\mu}_A^{10} \cdot \underline{m}) =$$

$$-\frac{1}{4\pi\epsilon\hbar} \sum_{n\neq 1} \frac{E_1 E_n}{E_n^2 - E_1^2} V_{BA}\, O_{BA} \tag{3.7}$$

$$+(\textit{Terms corresponding to } 3.5b \textit{ and } 3.5c),$$

where V_{BA} is the corresponding dipole-dipole interaction energy already given within the large parentheses in Equation 3.6, and where the factor O_{BA} depends on the geometrical arrangement of the interacting electric dipole transition moments, $\underline{\mu}_A^{10}$ and $\underline{\mu}_B^{n0}$.

$$O_{BA} = \underline{r}_{BA} \cdot (\underline{\mu}_B^{n0} \times \underline{\mu}_A^{10}). \tag{3.8}$$

The summation in Equation 3.7 is over all transitions from ground to excited state on chromophore B. In a system where magnetic transitions can not be

disregarded, the terms corresponding to 3.5b and 3.5c in Equation 3.5 must be included. Inclusion of the term 3.5b leads to a CD due to the mixing of states on chromophore A by perturbation from B. This is the one-electron mechanism of Condon, Altar and Eyring [1937]. Inclusion of the term 3.5c leads to the coupling of a mdtm on chromophore B with the edtm on A. This is the so called μm-mechanism [Schellman, 1968]. The term 3.5d leads to a CD due to chirally arranged eda transitions; this is the coupled oscillator mechanism. Equation 3.7 is often called the Kirkwood-Kuhn formula for optical activity [Kirkwood, 1937; Kuhn, 1930]. Note that it is not applicable to the coupling of degenerate transitions. To handle degenerate transitions it is necessary to derive yet another formula [Moffitt, 1956].

The main value of the Kirkwood-Kuhn formula (Eq. 3.7) lies in its simple description of the CD arising from the non-degenerate coupling of eda transition moments. In all Papers contained in this thesis the DNA-adduct is represented by a single eda transition, well removed in energy from the DNA transitions, and magnetic moments have been disregarded altogether. For these systems Equation 3.7 is helpful in discussing the results. The reason for disregarding mda transitions in the DNA is mainly that no mda transitions have been assigned with any degree of confidence in the nucleic acid bases, and calculations with eda transitions alone have proven quite successfull [Williams et al., 1986]. Also, the terms 3.5a-3.5c give rise to rotatory strength expressions inversely proportional to $|\underline{r}_{BA}^3|$, whereas the rotatory strength in Eq. 3.7 depends on the inverse of $|\underline{r}_{BA}^2|$. The exclusion of $n \rightarrow \pi^*$ transitions can be questioned, though. Studdert & Davis found the contribution of $n \rightarrow \pi^*$ transitions to the DNA CD to be one order of magnitude weaker than the CD due to the coupling of the $\pi \rightarrow \pi^*$ transitions of the bases.

2.3 The matrix method.

To use first order perturbation methods for calculations on DNA or DNA-adduct systems is questionable because of the multiple near degeneracies of the nucleic

acid transitions. Also, if the perturbation approach is to be used with systems where more than one mechanism is operative each mechanism must be treated separately. Perturbation formulas capable of dealing with all mechanisms, as well as both degenerate and non-degenerate transitions have been developed but are quite unwieldy to apply [Tinoco, 1962].

In 1969 Bayley, Nielsen and Schellman presented a procedure that incorporates all the considered mechanisms in a systematic way. The method operates entirely by matrix transformations spanning a chosen basis of wavefunctions. With this procedure there is no need to distinguish between degenerate and non-degenerate interactions and the result is equivalent to "all-order" perturbation calculations. The procedure was named the matrix method not because of its extensive use of matrix algebra, but because of its likeness to the original Heisenberg matrix formulation of quantum mechanics.† Most of the remainder of this chapter is based upon the paper by Bayley, Nielsen and Schellman [1969] where the matrix method is presented in its entirety. Further details of the matrix method are given by Madison & Schellman [1972], Goux & Hooker [1980], and Rizzo & Schellman [1984].

Consider a molecule which can be subdivided into chromophores. The chromophores have independent electronic eigenstates which are perturbed in the molecular framework. These interactions can include all CD mechanisms briefly outlined at the end of the previous section. The products of the chromophore's wave functions are taken to be the initial basis functions for the non-interacting

† With the matrix formulation of quantum mechanics direct consideration of the wave functions is suppressed and emphasis is placed on the coefficients which define the state of a system in terms of a set of eigenstates, and on the transformation equations which are required to go from one set of eigenstates to another. The original references are: Heisenberg, W., [1925], Born, M. and Jordan, P., [1925], and, Born, M. et al., [1925]. A more modern reference is Merzbacher [1970].

system. In the general case the set of basis functions would be complete. In forming the Hamiltonian it is expedient to label each state of the system with an ordinal number. State 1 is the ground state, with all chromophores in their ground states. States 2 to N+1 are singly excited states numbered in order through all states on chromophore A, those of B, etc.. In this context, a singly excited state corresponds to a state where one electron in one of the chromophores is in an excited state. N is the total number of possible single excitations in the basis set. Next come all double, triple, etc. excitations. Each state is then referred to by its ordinal number, I^0, J^0, etc.. Superscript ^{0} designates the unperturbed state in the initial basis.

The Hamiltonian for the molecule is given by

$$H = H^0 + V = (\sum_i H_i^0) + V , \tag{3.9}$$

where H_i^0 is the local Hamiltonian for the ith group, and V represents the potential energy of interactions amongst the chromophores. The diagonal elements of the Hamiltonian are

$$H^0_{I^0,I^0} + V_{I^0,I^0} = E^0_{I^0} + V_{I^0,I^0}, \tag{3.10}$$

where $E^0_{I^0}$ is the energy of state I^o of the isolated chromophore, and V_{I^0,I^0} is the shift in energy produced by the intramolecular interactions. The diagonal elements can often be estimated directly from the spectroscopy of the groups in the appropriate solvent. The off-diagonal elements are of the form V_{I^0,J^0}. The Hamiltonian matrix is diagonalizable by a unitary matrix C.

$$C^{-1}HC = H_{diagonal} \tag{3.11}$$

C contains the eigenvectors and Eq. 3.11 corresponds to a rotation of the basis set, resulting in a diagonal Hamiltonian; the diagonal elements (the eigenvalues λ_i) are the energies of the polymer transitions. All the important electronic spectroscopy of the system is contained in the matrices of the electric and magnetic dipole operators. In accordance with the theorem of matrix mechanics, the

matrix elements for the electric dipole and magnetic dipole operators in the interacting system are obtained from the same transformation which diagonalizes H.

$$\underline{\mu} = C^{-1}\underline{\mu}^0 C; \quad \underline{M} = C^{-1}\underline{M}^0 C, \tag{3.12}$$

where $\underline{\mu}^0$ and $\underline{M}^0$ are the electric and magnetic transition moment matrices in the original representation. In other words, the new (polymer) transition moments are obtained as linear combinations of the original (monomer) transition moments. Both $\underline{\mu}^0$ and $\underline{M}^0$ are assumed to be known experimentally or to be pre-calculated. The rotatory strength matrix for the molecule is given by

$$R = \Im m\{\underline{\mu} : \underline{M}'\} \tag{3.13}$$

$$R_{I,J} = \Im m\{\underline{\mu}_{I,J} \cdot \underline{M}_{I,J}\},$$

where the symbol ":" means that scalar products are formed element by element and the prime indicates transposition. Capital indices without superscripts designate states in the final (diagonal) energy representation. Equation 3.13 contains implicitly all the mechanisms discussed briefly at the end of section 3.1 and in section 2.1. Equations 3.9-3.13 constitute the entire theory and if the original basis were a complete set for the Hamiltonian, it would be an exact theory for the model. For practical reasons these equations are limited basis approximations to the complete formalism.

3.2.1 Advantages of the matrix method. The advantages of the matrix method are: 1) It treats degenerate and non-degenerate states, as well as all the mechanisms mentioned in sections 2.1 and 3.1, simultaneuously with a single formalism. 2) It is an "all-order" calculation, whereas first order perturbation theory is restricted to terms that are first order in the off-diagonal elements of the Hamiltonian. 3) Digital computers are especially well suited to handle matrix manipulations.

3.2.2 Restriction of the basis. The electronic states of individual chromophores are limited to a finite set. Usually, only states involved in well-characterized

	$\|oo)$	$\|lo)$	$\|mo)$	$\|on)$	$\|op)$	$\|ln)$	$\|lp)$	$\|mn)$	$\|mp)$
$\|oo)$	$\mathbf{V_{11}}$	V_{12}	V_{13}	V_{14}	V_{15}	V_{16}	V_{17}	V_{18}	V_{19}
$\|lo)$	V_{21}	$\mathbf{V_{22}}$	$\mathbf{V_{23}}$	$\mathbf{V_{24}}$	$\mathbf{V_{25}}$	V_{26}	V_{27}	V_{28}	V_{29}
$\|mo)$	V_{31}	$\mathbf{V_{32}}$	$\mathbf{V_{33}}$	$\mathbf{V_{34}}$	$\mathbf{V_{35}}$	V_{36}	V_{37}	V_{38}	V_{39}
$\|on)$	V_{41}	$\mathbf{V_{42}}$	$\mathbf{V_{43}}$	$\mathbf{V_{44}}$	$\mathbf{V_{45}}$	V_{46}	V_{47}	V_{48}	V_{49}
$\|op)$	V_{51}	$\mathbf{V_{52}}$	$\mathbf{V_{53}}$	$\mathbf{V_{54}}$	$\mathbf{V_{55}}$	V_{56}	V_{57}	V_{58}	V_{59}
$\|ln)$	V_{61}	V_{62}	V_{63}	V_{64}	V_{65}	V_{66}	V_{67}	V_{68}	V_{69}
$\|lp)$	V_{71}	V_{72}	V_{73}	V_{74}	V_{75}	V_{76}	V_{77}	V_{78}	V_{79}
$\|mn)$	V_{81}	V_{82}	V_{83}	V_{84}	V_{85}	V_{86}	V_{87}	V_{88}	V_{89}
$\|mp)$	V_{91}	V_{92}	V_{93}	V_{94}	V_{95}	V_{96}	V_{97}	V_{98}	V_{99}

TABLE 3.1

transitions from the ground state are chosen. Clearly, the success of the method depends on finding values for all pertinent matrix elements in the zeroth order basis and also on the validity of cutting off the, in principle, infinite dimensions of the problem to a finite number. An example illustrates the restriction of the basis set.

Consider two chromophores, each of which can exist in the ground state or in one of two excited states. The reasoning can easily be extended to a larger number of states and chromophores, but the notation quickly gets cumbersome and little is gained in clarity. The states are labelled $|o)$ for the ground state, $|l)$ and $|m)$ for the excited states of the first chromophore, and $|n)$ and $|p)$ for the other chromophore. In the absence of interaction there are nine product states indicated by the row and column headings of Table 3.1. The table is a schematic representation of the interaction matrix V before diagonalization, indicating the retained elements after the restriction of the basis set. As a result of Coulombic interaction between groups, nine new eigenstates are formed that, in general, are linear combinations of the unperturbed states. The different basis functions result in 81 integrals of the perturbation energy V. The diagonal elements are of the form $(jk|V|jk)$, where $j = o, l, m$ and $k = o, n, p$ [Table 3.1]. These are the first order corrections to the energy of the states.

Only the ground state and states with a single excitation are considered, and the mixing of the ground state with the excited states is assumed sufficiently small to be ignored. As a result only the boldfaced integrals of Table 3.1 are retained, and the dimensions of the matrix C may be reduced from N+1 to N dimensions by removing the ground state from consideration, where N is the number of single excitations in the basis.‡ In addition the matrices for $\underline{\mu}^0$ and $\underline{M}^0$ are contracted to N dimensional row vectors. Consequently, when the Hamiltonian is to be diagonalized only the matrix

$$H = H^0 + V = \begin{pmatrix} H^0_{22} + V_{22} & (lo|V|mo) & (lo|V|on) & (lo|V|op) \\ (mo|V|lo) & H^0_{33} + V_{33} & (mo|V|on) & (mo|V|op) \\ (on|V|lo) & (on|V|mo) & H^0_{44} + V_{44} & (on|V|op) \\ (op|V|lo) & (op|V|mo) & (op|V|on) & H^0_{55} + V_{55} \end{pmatrix} \tag{3.14}$$

need be considered, where the diagonal energies are of the form given in Eq. 3.10. The remaining elements in the interaction matrix 3.14 represent implicitly the specialized CD mechanisms mentioned at the end of section 2.1. An instructive illustration for the case of two chromophores, with one eda transition and one mda transition each, is found in the paper of Bayley, Nielsen & Schellman [1969]. The outcome is that at the level of dipole-dipole coupling all one needs to know is the array of transition dipoles in the molecule. As in previous studies we incorporated only eda transitions [Johnson & Tinoco, 1969a-b; Cech, 1975; Rizzo & Schellman, 1984; Williams et al., 1986].

‡ This reduces the size of the problem to tractable dimensions: K chromophores with k excited states each have Kk functions in a single excitation basis and (1 + kK + kK(K-1)/2) functions in a double excitation basis. For the AT polynucleotides in Paper II K=20 and k=10, which gives 200 single excitation functions but ca 190000 double excitation functions!

3.2.3 Input. The input consists of: (1) the three dimensional structure of the molecule, determining position and orientation of all chromophores; (2) position vectors for the transitions; (3) energies for each transition; (4) transition moments for each transition, obtained from experiment or calculation; (5) and, estimates of the transition monopole densities. for the calculation of the off-diagonal elements of the Hamiltonian. The input is described in detail in the next chapter.

3.2.4 Off-diagonal interaction terms. Because of the multiple near-degenerate transitions in the DNA bases where the stacking interactions are strong it is questionable whether the multipole expansion converges properly. The distance between the bases in DNA is smaller than the extension of the bases themselves, and if a multipole expansion shall be used it is probably necessary to include higher order terms. A more practical method for calculating these short range interactions is the London method of monopoles [London, 1942; Haugh & Hirschfelder, 1955; Tinoco, 1962]. Instead of using a multipole expansion, monopoles are used to represent the charge interactions. A number of ways of modelling such charge distributions have been used [Hooker & Schellman, 1970; Stigter & Schellman, 1973; Cech et al., 1976]. In the manner of Rizzo & Schellman [1984] we have used transition monopoles centered on the atoms. These are available from the transition density matrix, which can be calculated from LCAO wave functions.

Unfortunately, theory and experiment seldom agree on the energies and directions of the transition moments [Callis, 1983; Matos & Roos, 1988; Petke et al., 1990]. Calculated transition monopoles correspond to transition moment directions that often disagree with experimentally determined directions. Particular heed must be paid both to the phase and to the direction of transition moments. Phases are arbitrary and are selected at the beginning of a problem; once phase conventions are established they must be unconditionally adhered to. In particular, it is desirable that the transition monopole representations in the Hamiltonian have the same phase as the transition moments in Eq. 3.13. In

some cases calculations have been carried out where transition monopole representations and transition moments have had different polarizations. For this reason Rizzo & Schellman [1984] used Lagrangian multipliers to bring about a minimum variation in the LCAO coefficients, subject to the constraints that intensities and transition moment directions must agree with the experimental assignments. No doubt more sophisticated methods than Lagrangian multipliers can be used to optimize the monopoles.

3.2.5 Bandwidths. The matrix method calculates integrated intensities, and if spectra are to be calculated, band shapes and bandwidths must be assigned to each transition. We have used Gaussian shapes and have assumed the bandwidths to be the same as for the isolated chromophore. The assumption of Gaussian bandshapes is merely a practical representation of the bandshape [Schellman, 1975]. The assumption that the isotropic absorption spectrum and the CD spectrum of the same transition have the same band shape ultimately relies on the assumptions that the motions of the nuclei and the electrons can be separated, and that the transition is sufficiently strongly allowed. This assumption has been discussed in the literature [Simpson & Peterson, 1957; Weigang, 1965; Schellman, 1975].

3.2.6 The dielectric constant. Coulomb's law requires a dielectric constant in condensed media and this generates an inescapable difficulty in all such calculations. A dielectric constant is not really defined for distances of the order of atomic radii, and should depend on position in the molecule as well as on conformation and solvent. The problem is intractable, and is bypassed by assuming an effective dielectric constant and using it indiscriminately for all interactions. We have used a value of twice the vacuum dielectric constant. This value was found by Cech et al. [1976] to give acceptable results, and the value has been used by Schellman and co-workers for a number of years [Rizzo & Schellman, 1984].

4. Input parameters.

4.1. Structure.

In Papers I and II, for the calculations of the intrinsic CD of the polynucleotides, we used different DNA structures taken from fiber diffraction data. Only B-DNA geometries were used in the calculations of induced circular dichroism.

4.1.1. DNA Structure. For [poly(dG-dC)]$_2$ in Paper I we used the B-form structure of Arnott & Hukins [1972].

For [poly(dA-dT)]$_2$ in Paper II we calculated the CD spectrum for two different structures. The B-DNA coordinates used for this polynucleotide were taken from Chandrasekaran & Arnott [1989, Structure No 4.]. This B-form geometry differs in the refinement of the coordinates from the one presented by Arnott & Hukins [1972]. CD spectra calculated for the two structures are practically identical (results not presented). We also used a D-DNA geometry in the calculations for this polymer [Arnott et al., 1974]. The D-structure is very compact with an eight-fold helical repeat and an axial rise per residue of 3.03 Å. The D-DNA structure belongs to the larger genus of B-DNA structures [*ibid.*].

For poly(dA):poly(dT) in Paper II we calculated the intrinsic CD spectrum for three different fiber structures, one B-form and two H-form structures. The B-DNA structure was the same as for [poly(dA-dT)]$_2$ [Chandrasekaran & Arnott, 1989, Structure No. 4]. The two slightly different H-DNA structures are heteromerous and specific for poly(dA):poly(dT), with different features for each strand. They, too, belong to the B-DNA family. The fiber data for the

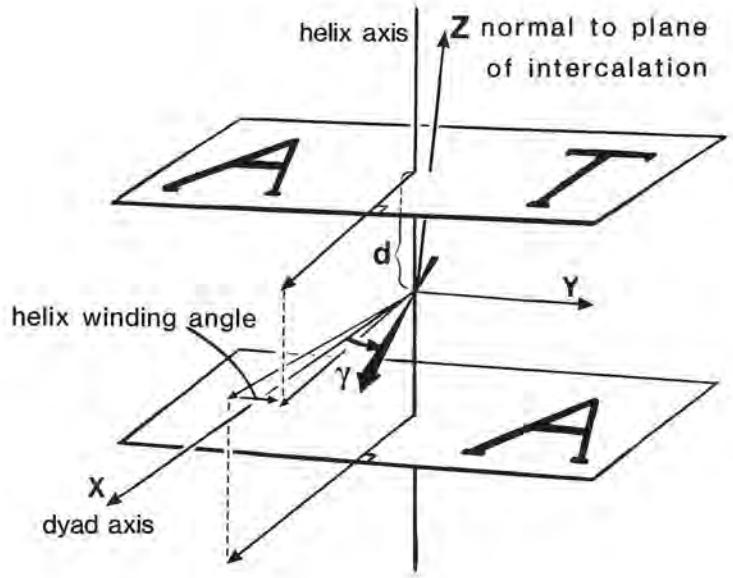

Figure 4.1. Schematic view of intercalation site and the angle γ.

H-DNA structures were taken from Chandrasekaran & Arnott [1989, Structures No 18-19. †].

4.1.2. Structure of the DNA-ligand complex. In all the calculations we assume that a conformation change of the ligand is not a source of induced circular dichroism. We also assume that DNA retains its B-form structure. The intercalation structure used in all three papers is based on crystal data for the acridine type of intercalators in (dG-dC)-intercalation sites [Reddy et al. 1979; Sakore et al., 1979; Wang et al., 1979; Sobell, 1985]. The distance between the planes of the intercalator and the base planes is 3.4 Å and the decrease in helix winding angle for the base pair surrounding the intercalation site is 26°, from 36° to 10°. Figure 4.1 is a schematic view of an intercalation site and shows the coordinate system used in the calculations in Papers I-IV for the induced CD of

† The original references for the two H-DNA structures are Arnott et al. [1983] and Park et al. [1987].

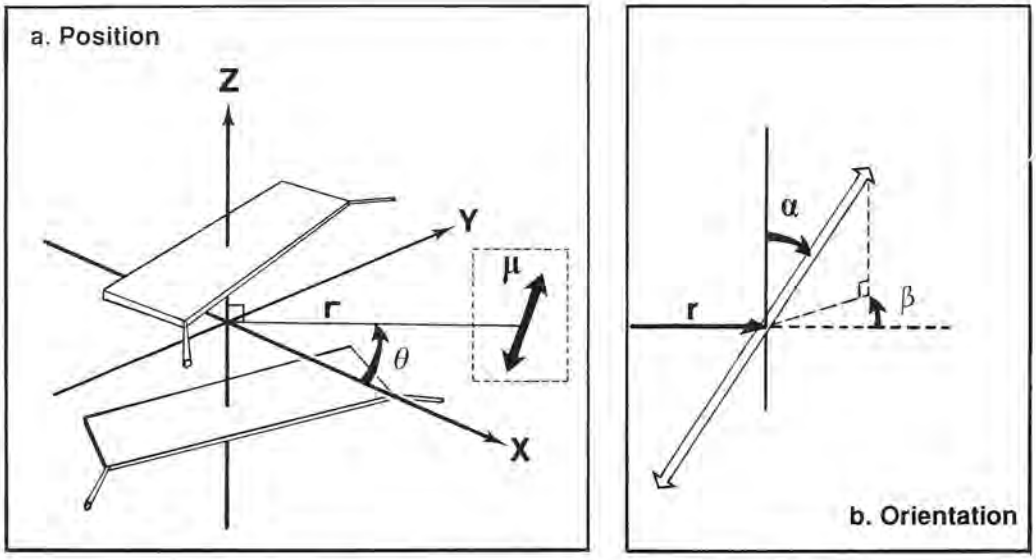

Figure 4.2. The Parameters defining the DNA binding geometry of a groove binder. (Cf Papers I and II.)

intercalators. The DNA structure is assumed to be of B-form on both sides of the intercalation site.

No specific structure of the binding site was assumed for the calculations of the induced circular dichroism of a groove binder. From general considerations of the structure of B-DNA we decided on a reference geometry for the ligand relative to DNA. This standard geometry corresponds to the most likely binding orientation and position for a planar aromatic molecule bound to the minor groove of an AT polymer. Figure 4.2 shows the coordinate system used in the calculations on groove binders in Papers I-II. The details of the standard geometry are explained in these papers.

There are two more aspects of the structure of the DNA-ligand complex that deserve illustration: the difference between 5'pyrimidine (pyr)-3'purine (pur) and 5'pur-3'pyr base pair sequences, and the physical appearance of the major and the minor grooves. Figure 4.3 shows projections onto a plane perpendicular to the helix axis of 5'pyr-3'pur and 5'pur-3'pyr sequences for the bases A and T. Figure 4.4 is a view of a DNA molecule seen along the grooves. The different

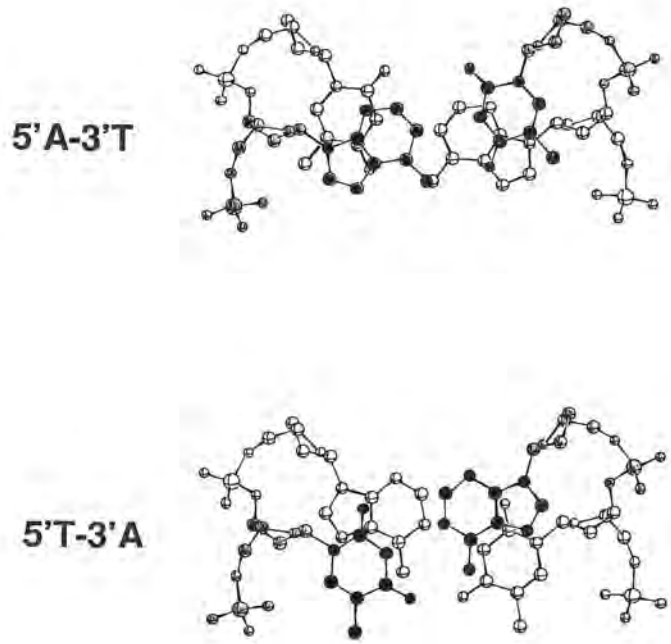

Figure 4.3. Illustration of the different local geometries of 5'pyr-3'pur and 5'pur-3'pyr sequences. Projection onto a plane perpendicular to the helix axis.

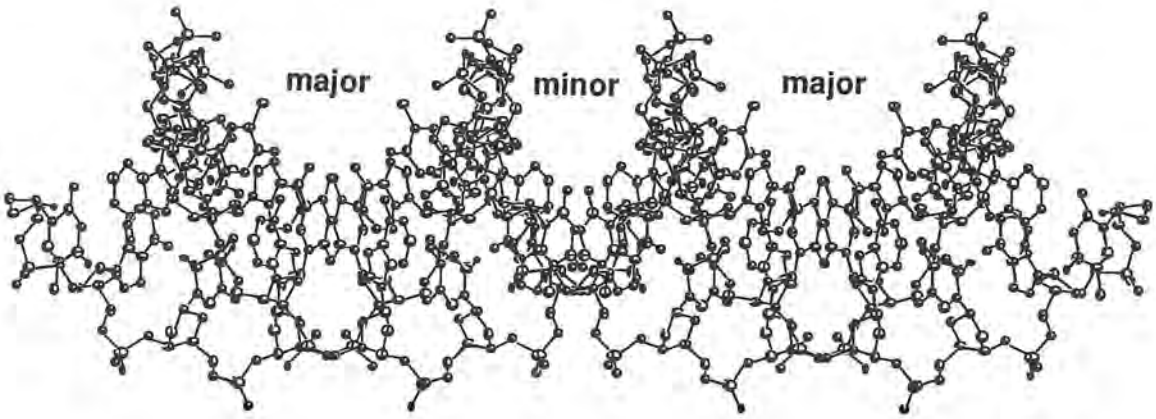

Figure 4.4. Illustration of groove features. A 20-mer of $[poly(dA\text{-}dT)]_2$ viewed along the grooves. The helix axis is tilted 40° away from the viewer.

widths of the grooves can clearly be seen. For the B-DNA structure in Paper II the major groove is 9 Å deep and 12 Å wide, and the minor groove 8 Å deep and 6 Å wide [Chandrasekaran & Arnott, 1989, p 116]. The width of the minor groove is a good match for the thickness of planar aromatic molecules. The deep negative electrostatic potential of the minor groove is considered especially attractive to cationic ligands [Pullman & Pullman, 1981]. For a full description of the features of DNA see, e.g., Mathews & van Holde [1990] or Chandrasekaran & Arnott [1989].

4.2. Spectroscopic parameters.

As stated in Chapter 3 the spectroscopic parameters for the calculations were taken from experiment when possible. Following previous studies we disregarded the absorption properties of the sugar-phosphate backbone [Cech et al., 1976; Rizzo & Schellman, 1984]. Moore & Wagner [1974] estimated the contribution of the base-backbone interactions to the CD of DNA and found them to be negligible compared to the base-base interactions. Also, the absorption spectra of nucleic acid bases and their corresponding nucleotides and nucleosides are almost superimposable [Voet et al., 1963; Sprecher & Johnson, 1977]. This indicates a weak perturbing effect of the sugar-phosphate backbone on the monomer base. No $n \rightarrow \pi^*$ transitions, or magnetic dipole allowed transitions, were included in the optical parameter sets as there still is no direct evidence of their character or location in the DNA bases [Callis, 1983; 1986].

In Papers III-IV the parameters for DNA were the same as those used by Rizzo & Schellman [1984]. In Paper I the parameters, excepting bandwidths, were those used by Williams et al. [1986]. In Paper II we present the first calculations of the CD of AT polynucleotides using Clark's new transition moment assignments for adenine [1989; 1990].

Figure 4.5 shows the structures of purine, pyrimidine and their nucleic acid derivatives. Also shown are the numbering convention for the atoms in these

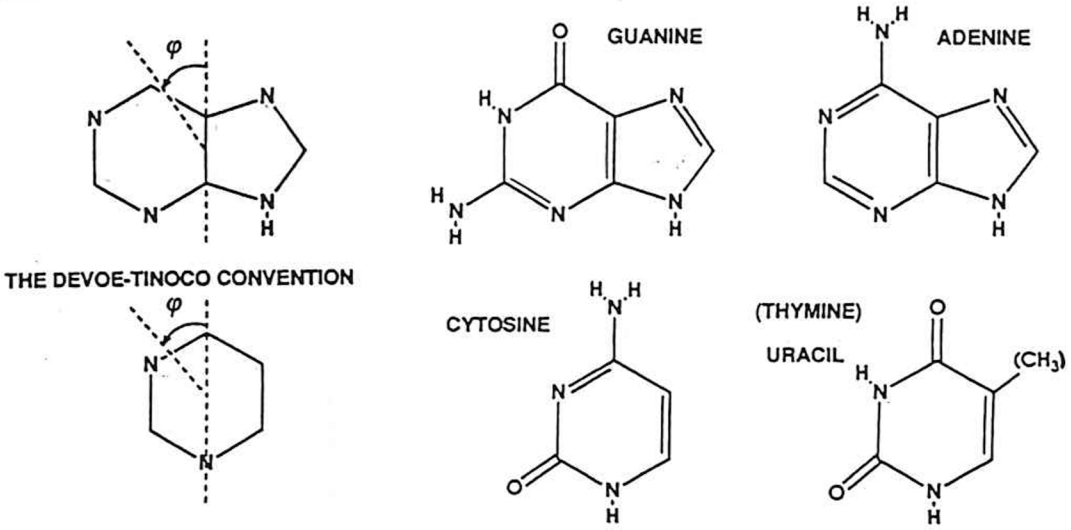

Figure 4.5. Nucleic acid bases.

molecules and the so called Devoe-Tinoco convention used to define the in-plane transition moment polarizations of the bases [Devoe & Tinoco, 1962].

4.2.1. Transition moments of guanine and cytosine. The transition moments and other parameters for guanine and cytosine used in Papers III-IV are presented in Paper III and in the paper of Rizzo & Schellman from 1984. The parameters used in Paper I for guanine and cytosine are presented in Figure 4.6 and Table 4.1. We used the data presented by Williams et al. [1986], which in turn are based upon crystal studies of guanine and cytosine [Clark, 1977; Zaloudek et al., 1985]. The bandwidths necessary for calculation of the CD spectra were estimated by fitting Gaussian bands to the experimental spectra of the monomeric DNA units dGMP and dCMP [Sprecher & Johnson, 1977]. The intensities and energies of the transitions as given by Williams et al. [1986] were strictly adhered to. The results of the fitting are presented in Figure 4.6 and the bandwidths used to generate the fitted bands (dotted spectrum) are listed in Table 4.1.

4.2.2. Transition moments of adenine and thymine. The transition moments for adenine and cytosine used in Papers III-IV are presented in Paper III

and in the paper of Rizzo & Schellman [1984]. The parameters used in Paper II are presented in Figure 4.6 and in the Tables 4.1 and 4.2. The transition assignments for adenine are based upon the recent work of Clark on adenine derivatives [1989; 1990]. Because the matrix method requires the transition moments of the monomer in the proper solvent, we have modified the published transition parameters of Clark [1989; 1990] so as to be able to fit aussian bands to the experimental absorption spectrum of dAMP [Sprecher & Johnson, 1977]. The crystal spectra of the adenine derivatives are all markedly redshifted [Stewart & Davidson, 1963; Clark, 1990]. As an example, the maximum of the first absorption band of adenine from the crystal data lies at approximately 270 nm [Clark, 1989; 1990], while for dAMP and adenine alike the maximum in water lies at ca 260 nm [Sprecher & Johnson, 1977].

When fitting, the oscillator strengths were held as close as possible to the published values. The resulting set of transition moment parameters for adenine are presented together with those for thymine in Table 4.2. The differences between the used parameters and the literature data are presented in full in Paper II. A theoretical treatment of the effects of local electrostatic fields in crystals on the excited states and transition properties for guanine has been published recently by Theiste et al. [1991].

The parameters used for thymine in Paper II have a more composite origin than the parameters for adenine. We follow the procedure of Williams et al. [1986] in assigning four $\pi \rightarrow \pi^*$ transitions to the three broad absorption bands of thymine. The two first transitions were assigned on the basis of crystal data of 1-methyluracil [Novros & Clark, 1986]. The third and fourth transitions were assigned the energies and intensities used by Williams et al. [1986]. The polarizations of these two transitions were chosen as those resulting in the "best" agreement between the calculated and experimental CD spectra for [poly(dA-dT)]$_2$. The details of this choice are given in Paper II. As for adenine the transition parameters were slightly modified to allow fitting Gaussian bands to

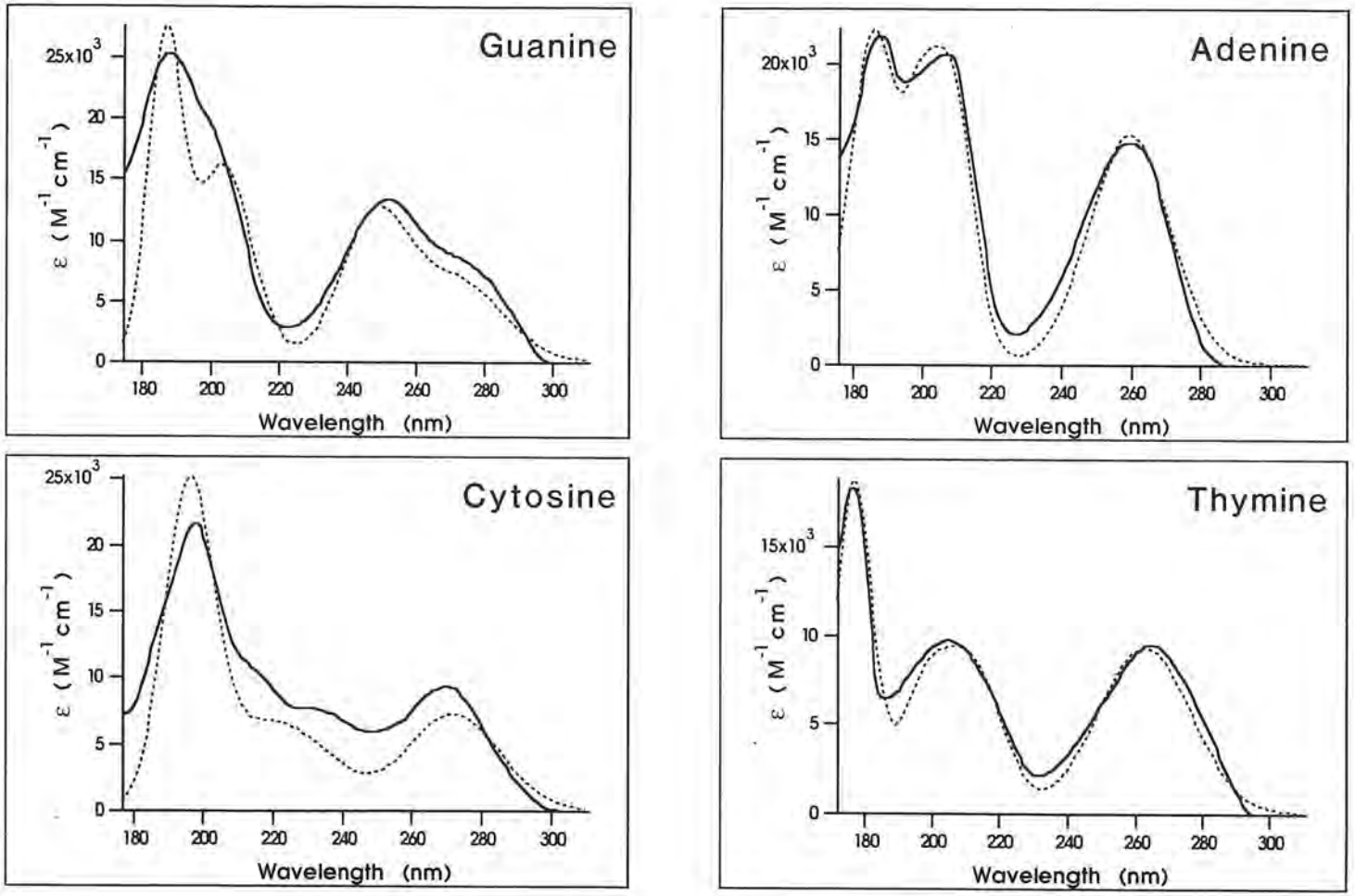

Figure 4.6. Experimental absorption spectra of dGMP, dCMP, dAMP and dTMP in aqueous solution (———), compared to absorption bands (.....) constructed from the parameters presented in Table 4.1. The experimental spectra are redrawn from Sprecher & Johnson [1977].

TABLE 4.1. PARAMETERS FOR FITTING GAUSSIAN BANDS TO EXPERIMENTAL SPECTRA.

GUANINE		
λ nm	$\lvert \underline{\mu} \rvert$ Debye	Δ_e nm
272	2.76	19.1
248	3.18	13.7
204	3.92	12.0
187	3.92	7.0
159	2.76	-
154	1.84	-
145	2.30	-

CYTOSINE		
λ nm	$\lvert \underline{\mu} \rvert$ Debye	Δ_e nm
272	2.86	19.0
231	2.07	16.0
214	2.44	16.0
196	4.43	10.0
163	2.17	-
151	2.44	-

ADENINE		
λ nm	$\lvert \underline{\mu} \rvert$ Debye	Δ_e nm
261	2.20	16.4
259	3.30	16.4
208	3.70	9.2
198	2.45	6.8
187	3.49	7.5
160	2.80	-

THYMINE		
λ nm	$\lvert \underline{\mu} \rvert$ Debye	Δ_e nm
263	3.35	19.5
213	2.65	12.0
198	2.45	11.0
177	3.58	11.0

The parameters used to create the fitted spectra presented in Figure 4.6. λ is the wavelength at the maximum of the band. $\lvert \underline{\mu} \rvert$ is the length of the transition moment. Δ_e is the halfwidth at 1/e of the maximum.

the experimental spectrum of dTMP (Cf Fig. 4.6 and Table 4.1). The set of transition moment parameters used are presented in Table 4.2.

In Paper I the calculated spectrum of [poly(dG-dC)]$_2$ was compared to the experimental spectrum of Riazance et al. [1985]. In Paper II the calculated spectra of [poly(dA-dT)]$_2$ and poly(dA):poly(dT) were compared to the corresponding experimental spectra of Gray et al. [1990].

4.2.3. The ligand. In all calculations throughout Papers I-IV the ligand chromophore was represented by a single electric transition dipole moment. In Paper I-II the energy and intensity of the ligand was chosen to correspond to the 335 nm long-axis polarized transition of 4',6-diamidino-2-phenylindole (DAPI). The dipole strength of that transition is 37.2 Debye^2. Other values corresponding to transitions in other compounds were used for some calculations in both Papers I and II.

In Paper III a transition at 664 nm with a dipole strength of 93.7 Debye^2 was used, corresponding to the first, isolated, long-axis polarized absorption band of the cationic dye methylene blue.

4.3. The choice of transition monopoles.

In Papers III and IV we used the same transition monopoles as Rizzo & Schellman [1984]. These monopoles were taken from the work of Tinoco and co-workers [Hug & Tinoco, 1973; Cech et al., 1976] and optimized by Lagrangian multipliers to yield transition monopole representations of the transitions that agree with the experimentally determined transition moment directions [Rizzo & Schellman, 1984]. Their assignment to the transitions used in the calculations is explicated in Rizzo & Schellman [1984].

In Paper I the same transition monopoles as in Paper III were used for guanine and cytosine. We followed the procedure of Williams et al. [1986] in assigning the transition monopoles to the transitions used. The transition monopoles of the first two LCAO [Cech, 1975] transitions of both guanine and

SET 1

ADENINE

	Wavelength (nm)	Oscillator strength[a] f	$\|\mu\|$[a] (Debye)	Polarization DeVoe-Tinoco angle[b] °
I	261	(0.084)	2.20	76
II	259	(0.191)	3.30	20
III	208	(0.298)	3.70	-50
IV	198	(0.137)	2.45	10
V	187	(0.295)	3.49	48
VI	160	(0.222)	2.80	9

THYMINE

I	263	(0.193)	3.35	-8
II	213		2.65	62
III	198		2.45	-55
IV	177		3.58	-50

TABLE 4.2. TRANSITION MOMENTS OF ADENINE AND THYMINE.

The adenine and thymine transition moments used for calculation of the induced rotatory strengths in Paper II.

(a) The oscillator strength values within parentheses are calculated by the interrelating formula $|\mu| = 14584.1 \cdot \sqrt{f} \cdot \lambda$, where λ is the wavelength (in m), f is the oscillator strength, and $|\mu|$ is the length of the transition moment vector (in Debye).

(b) Polarization according to the DeVoe-Tinoco convention. [DeVoe & Tinoco, 1962]

cytosine were recalculated using Lagrangian multipliers to agree with the experimental assignments. The remaining transition monopoles were calculated from an initial set of transition monopoles all equal to zero.

For the study presented in Paper II we used transition monopoles very kindly provided by Professor Patrik Callis, originally presented in 1986 [Callis, 1986]. That paper is a comparison of three different LCAO programs applied to the calculation of electronic states and transition moments of the DNA bases. The three programs in that paper are designated RZ-INDO/SCI, RZ-INDO/SDCI and HT-CNDO/S, and correspond to different parametrizations. These designations are used here without further motivation, merely as practical labels to distinguish between the different sets of transition charge densities. The HT-CNDO/S program used the same parametrization as was used by Hug & Tinoco [1973, 1974] [Callis, 1986]. We found the monopoles corresponding to the HT-CNDO/S program to result in slightly better polynucleotide CD spectra (cf Fig 5.2), and on that account these monopoles were used in our calculations in Paper II. The calculated transitions were assigned in order of increasing energy to the experimental transitions, with one exception. The third calculated transition of adenine in the HT-CNDO/S calculation was omitted from the transition monopole set, and the transition monopoles IV-VII were assigned to the experimental adenine transitions III-VI, in that order. The motivation for this is twofold. The calculated spectrum is significantly improved by the omission, and there may be a weak in-plane polarized eda transition at ca 235 nm which has been disregarded in our set of transition moments for adenine. There is only scant evidence of the transition [Matsuoka & Nordén, 1982], and Clark finds no evidence for it [1990].

5. Results and Discussion.

The main objective of this work is the systematic investigation of the dependence of the induced CD of a DNA adduct transition on its position and orientation relative to DNA. Both the intrinsic CD spectra of several polynucleotides of different structure, and the rotatory strength induced into an edtm of an adduct bound to B-form DNA were calculated. The results of the Papers I-IV are presented according to the subject, not in the chronological order of the papers.

5.1 Intrinsic CD of DNA

Strictly speaking the subheading should be "The intrinsic CD spectrum of alternating purine-pyrimidine polynucleotides". The calculated CD spectra of the three polynucleotides $[poly(dG\text{-}dC)]_2$, $[poly(dA\text{-}dT)]_2$ and poly(dA):poly(dT) are presented in the Papers I and II for some different geometries. The spectra were calculated to check the credibility of the DNA parameters used. The rationale is that it is desirable that the intrinsic CD spectra of the DNA be reasonably well-reproduced if the DNA induced CD is to be calculated.

5.1.1. $[poly(dG\text{-}dC)]_2$:

The CD spectrum of alternating GC presented in Paper I was calculated using a set of optical DNA parameters used by Williams et al. [1986], assuming the geometry of the polynucleotide to be of the B-form. No alternative DNA structures were used. We found the agreement between the calculated and the measured spectra to be good for wavelengths longer than 225 nm. This region corresponds to the wavelength region where the transition moments of guanine and cytosine are considered to be assigned with confidence. For wavelengths shorter than

225 nm the agreement between experiment and calculation becomes increasingly worse, albeit acceptable, down to 180 nm. Two contributing reasons for this are that the experimental uncertainty of the assignment of transition moments increases with decreasing wavelength, and, also, that the closer to the far UV the transitions are, the more important will be the (unknown) high energy transitions, both of the bases themselves and of the sugar-phosphate backbone.

5.1.2. $[poly(dA\text{-}dT)]_2$:

The calculated intrinsic CD spectra of the AT polynucleotides (Paper II) do not agree as well with the corresponding measured spectra as do the results for $[poly(dG\text{-}dC)]_2$.

With the exception of the region 205-245 nm, the differences between the experimental and measured spectra of B-form $[poly(dA\text{-}dT)]_2$ can be characterized using the very same words as for B-form $[poly(dG\text{-}dC)]_2$. The wavelength region closest to the visible UV is the region where experiment and calculation agree best. This is also where the first two transitions of adenine and the first transition of thymine may be considered to be assigned with confidence. Also (still excepting the region 205-245 nm) the calculated CD spectra agree qualitatively with the experimental spectrum down to 175 nm.

To investigate the importance of the assumed DNA geometry, we did calculations for two B-DNA geometries [Arnott & Hukins, 1972; Chandrasekaran & Arnott, 1989] differing only in the refinement of the coordinates, and for a D-DNA geometry [Arnott et al., 1974]. The resulting spectra for both the B-form and the D-form geometries are very similar, and differ from the experimental CD spectrum in much the same way.

It seems that the structure of $[poly(dA\text{-}dT)]_2$ would have to be distinctly different from the standard B-form geometry, if the discrepancies between experimental and calculated spectra are to be explained by the choice of DNA structure alone.

As the transition moment directions taken from the literature are subject to uncertainties, it is conceivable that a concerted change, within experimental error, of input polarizations might result in significant changes in the calculated CD spectrum. To test this hypothesis, as well as the sensitivity of the calculations to the assignment of the transition moment polarizations, we adopted a procedure for generating input sets of transition moments, with properties randomly distributed within the experimental uncertainties. The procedure is described in Paper II. This random variation did not result in an improved CD spectrum for [poly(dA-dT)]$_2$. The extensive random generation of sets of transition moments results in spectra which differ mainly in intensities, while bandshapes are not much affected. Also, we find that the calculations are not overly sensitive to the transition moment directions of the bases. Variations of as much as 20 degrees for any single transition has little discernible effect on the calulated CD spectrum of the polynucleotide.

5.1.3. Poly(dA):poly(dT):

The calculated results for this polynucleotide are worse than the results for the alternating AT polynucleotide (Paper II). Spectra were calculated for different possible geometries. One B-form geometry [Chandrasekaran & Arnott, 1989] and two H-form geometries [Chandrasekaran & Arnott, 1989] were used. The CD spectra calculated for the two H-form geometries agree better with experiment [Gray et al., 1990] than do the spectrum calculated for the standard B-form geometry. The agreement for the calculated H-DNA spectra is in both cases semi-qualitative, and agree best in the wavelength region 205-245 nm. In this region the B-DNA spectrum for poly(dA):poly(dT) is similar to the calculated spectrum for poly(dA-dT) (Paper II).

For poly(dA):poly(dT) the discrepancy between calculation and experiment can not be explained simply by the addition of a single CD band around 230 nm, as is possible for the alternating AT polynucleotide. The solution structure of poly(dA):poly(dT) may be distinctly different from the standard B-geometry. In their experimental study on the CD spectra of polynucleotides of repeating

sequence Gray et al. [1990] found that the CD spectrum of poly(dA):poly(dT) could not be explained by the linear combination of the CD spectra of poly d(A-T):d(A-T), poly d(A-A-T):poly d(A-T-T), and poly d(A-A-T-T):poly d(A-A-T-T).

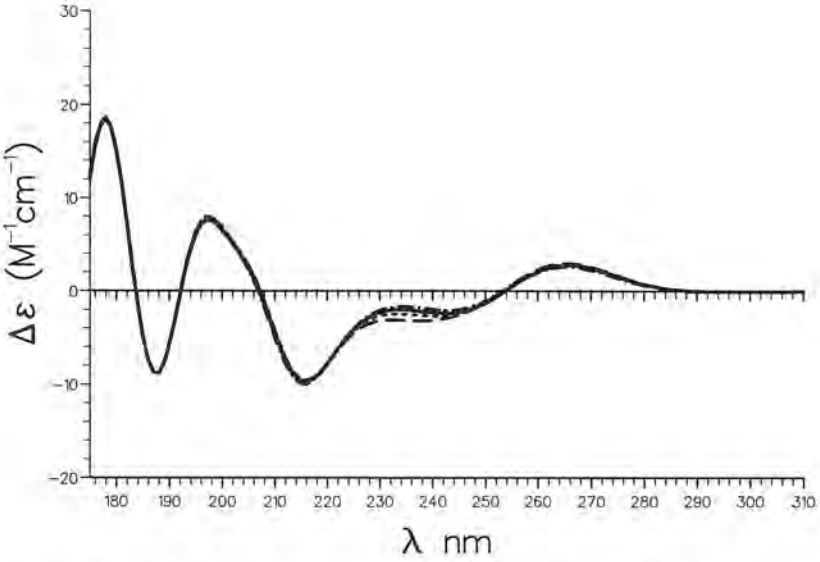

Figure 5.1. Calculated CD spectra for $[poly(dA\text{-}dT)]_2$. The figure shows six spectra calculated with the standard set of transition moments (Table 4.2) with an added eda transition at 235 nm in adenine ($|\underline{\mu}|$= 1 Debye). The spectra are calculated for six different polarizations of this transition: Θ = 0°; 30°; 60°; 90°; 120°; 150°. Θ is the DeVoe-Tinoco angle (cf Fig 4.2).

The calculations presented in Papers I-IV depend on a number of factors, and it is desirable that some idea of their sensitivity to the input parameters be attained. As stated at the end of section 5.1.2 the calculations do not seem overly sensitive to the directions of the transition moments in the DNA bases. This observation agrees with Cech's for DeVoe polarizability calculations [Cech, 1976]. Cech found that variation of the transition moment polarization of a single oscillator did little more than change magnitudes arising from other oscillators

A source of inaccuracy is the outright omission of some transition. For this possibility, Figure 5.1 indicates that such a transition is unlikely to be a weak in-plane polarized $\pi \rightarrow \pi^*$ transition, and experimental evidence for additional strong $\pi \rightarrow \pi^*$ transitions in any of the DNA bases is lacking [Callis, 1983; Callis, 1986; Clark, 1990]. On the other hand both Bush [1970] and Studdert & Davis [1974b] found that $n \rightarrow \pi^*$ transitions may be expected to contribute to give a small but noticeable contribution to the CD spectra of polynucleotides. There is some experimental evidence of base transitions that are out-of-plane polarized [Callis, 1983; Novros & Clark, 1986], and also of $n \rightarrow \pi^*$ transitions [Callis, 1983]; both of which may be magnetically dipole allowed.

In Paper II we suggest that an mda transition, not accounted for in our input, may be the reason for the discrepancy between experiment and calculation for [poly(dA-dT)]$_2$. The arguments are the following: there is a conspicuous absence of absorption intensity in the wavelength region 220-240 nm of both adenine and thymine, and there is no strong eda transition in the region [Callis, 1983; Novros & Clark, 1986; Clark, 1991]; the CD spectrum of [poly(dA-dT)]$_2$ has a maximum at 230 nm [Gray et al., 1990; Johnson, 1990]; a weak eda in-plane polarized transition as has been suggested in adenine around 235 nm [Bush & Scheraga, 1969; Matsuoka & Nordén, 1982] can not account for the CD intensity (cf Fig. 5.1); and mda transitions have anomalously high dichroic ratios $\Delta\epsilon/\epsilon$.† One admittedly attractive feature of this explanation is that there is no mechanism by which an eda transition in a DNA adduct can pick up circularly dichroic intensity from an mda transition in the DNA molecule. For all intents and purposes of these induced CD calculations the model adduct transition is effectively blind to the suggested presence of a magnetic dipole transition.

---

† The relation between the dichroic ratio and the dipole (D) and rotatory (R) strengths of a transition is approximately given by $\Delta\epsilon/\epsilon = 4R/cD$, where c is the speed of light and where all entities should be given in SI- units. (1 Debye = $3.3356 \cdot 10^{-30}$ Asm. 1 Bohr magneton = $9.2731 \cdot 10^{-24}$ Am^2.

It should be noted that the observed CD band in [poly(dA-dT)]$_2$ at ca 230 nm has no corresponding absorption band. This band is found in a number of AT oligomers [Bush & Scheraga, 1969; Johnson, 1990]. It has been suggested to arise from an $n \rightarrow \pi^*$ transition at 235 nm in adenine [Bush, 1970; Sprecher & Johnson, 1977]. In addition, Novros & Clark have reported evidence for an out-of-plane polarized $n \rightarrow \pi^*$ transition at 217 nm in 1-methyluracil from polarized reflection spectra [Novros & Clark, 1986]. Further research into the existence and character of $n \rightarrow \pi^*$ transitions in adenine and thymine is desirable if the CD spectrum of the AT polynucleotides shall be understood.

Another problem is the representation of the transitions in the Hamiltonian matrix. Cech et al. (1976) thought the transition monopoles likely to be one of the most important determinants of the calculated CD pattern. As was explained in the theory chapter, it may be necessary to optimize the transition monopoles in some manner before using them in the calculations, as it is of paramount importance that the transition moments be represented consistently. Figure 5.2 shows calculated CD spectra of [poly(dA-dT)]$_2$ for five different sets of monopoles, all optimized to agree with the same transition moment polarizations (Cf Table 4.2). The experimental spectrum of [poly(dA-dT)]$_2$ is also shown for comparison. (See the figure caption for details.) The overall impression is one of very similar spectra, at least for wavelengths below 250 nm. The chosen set (the dotted line) results in the best agreement for wavelengths longer than 250 nm. The spectra corresponding to the other monopole sets can be improved by changing the assignments of the calculated transitions and the experimental transitions. The CD spectrum of [poly(dA-dT)]$_2$ around 260 nm arises from three near degenerate transitions and the exact assignment of energies and intensities for these transitions have a noticeable effect on the shape of the CD spectrum in the region (not shown). The assigned energies and intensities for the transitions reproduce the monomeric absorption spectra with fair agreement (Fig 4.6).

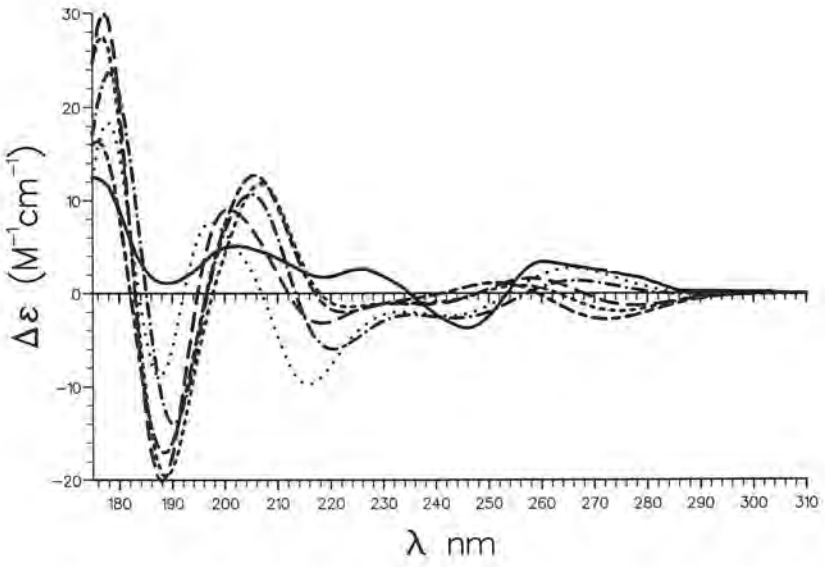

Figure 5.2. Calculated CD spectra for [poly(dA-dT)]$_2$ for five different sets of transition monopoles optimized to agree with the polarizations in Table 4.2. (———) For comparison the experimental spectrum is shown; (- — -) Spectrum calculated using zero monopoles only; Monopoles for the remaining spectra are taken from a study comparing three different LCAO programs for calculation of nucleic acid transition moments [Callis, 1986]. The abbreviations below refer to the LCAO programs in that work. (· · · · · ·) the set of monopoles used in Paper II [HT-CNDO/S, Callis 1986] Cf Chapter 4.3; The monopoles for the last three calculations were assigned to the transitions in increasing order of energy. (— · — ·) HT-CNDO/S; (— —) RZ-INDO/S-SCI; (- - - - - -) RZ-INDO/S-SDCI.

Figure 5.3 illustrates the importance of consistent representations of the transition moment polarizations in the Hamiltonian matrix and in the set of transition dipole moments used to calculate the rotatory strengths. The full line is the "best" spectrum for [poly(dA-dT)]$_2$, described in Paper II, calculated with consistent representations. The other spectra are 45°, 90° and 180° out of phase for the third transition in adenine (208 nm in the unperturbed monomer). Which

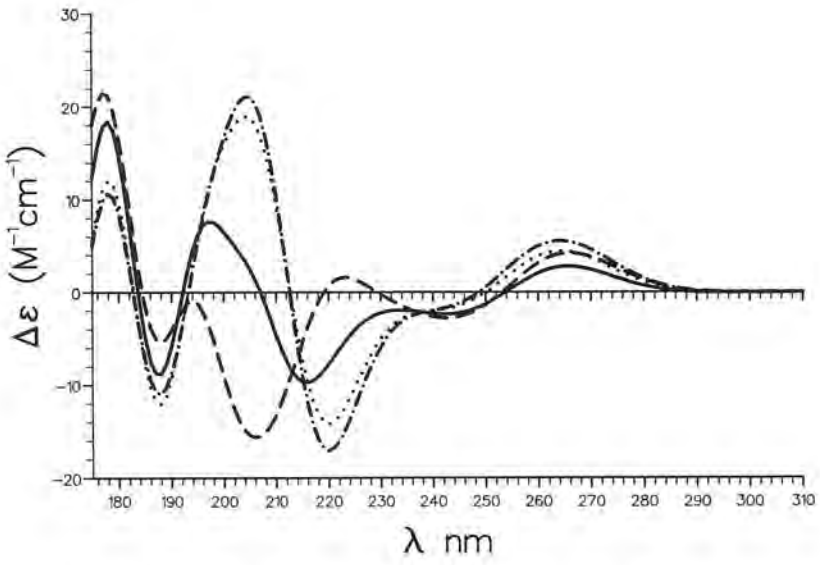

Figure 5.3. Calculated CD spectra for $[poly(dA\text{-}dT)]_2$. (———) the spectrum calculated with consistent representations. The remaining spectra were calculated with deliberately inconsistent representations of transition monopoles and transition dipole moments for the third transition of adenine (208 nm). The representations differ by: (· · · · · · ·) 45°; (— · — · —) 90°; and (— — —) 180°.

set of transition monopoles to use for a starting set is clearly less a problem than the need for a consistent representation of the transition moments.

How important are the far-UV transitions below 200 nm for the calculation of the CD spectrum of the polynucleotides? The spectra of $[poly(dA\text{-}dT)]_2$ and poly(dA):poly(dT) are non-conservative in the wavelength region 175-300 nm. The reason for the non-conservative character has been attributed to the body of (unknown) higher energy transitions in the far-UV [Johnson & Tinoco, 1969a]. One way of dealing with these transitions is to adopt the method of Cech et al.[1976] who incorporated background oscillators to represent the host of all such transitions. In their calculations on the intrinsic CD of a number of DNA polymers Rizzo & Schellman [1984] found that inclusion of these background oscillators worsened the agreement between experiment and calculation. In Paper III we tried background oscillators for the calculations of induced CD.

They were found to affect only the intensities of the induced CD: the intensities were scaled by a factor of two, but the nodal lines were unaffected. No background oscillators were used in Papers I and II. As for the influence of the transitions in the sugar-phoshate backbone, Moore & Wagner found these to be of negligible importance for the DNA CD [1974]. It may be added that from experimental studies on the mononucleosides Sprecher & Johnson found the CD for B-form DNA to result almost exclusively from base-base interactions, at least for wavelengths above 200 nm [Sprecher & Johnson, 1977].

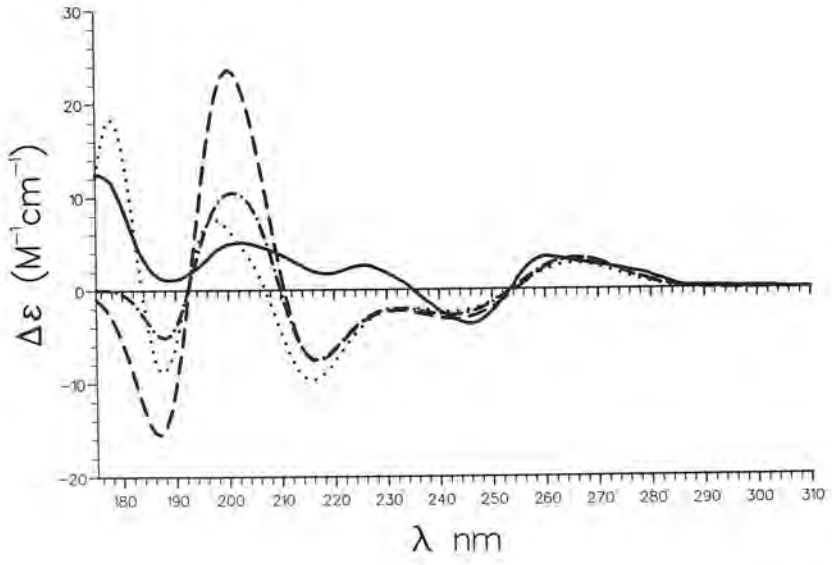

Figure 5.4. Calculated CD spectra for [poly(dA-dT)]$_2$. (———) Experimental CD spectrum [Gray et al., 1990]; (· · · · · · ·) The standard calculated spectrum; (·—·—·–) Transitions at 160 nm in adenine and 177 nm in thymine removed from the input file; (— — –) Transitions at 160 and 187 nm in adenine, and at 177 nm in thymine removed from input file.

The final query concerns the effect of truncating the basis set, excluding transitions below a certain wave length. Figure 5.4 shows calculated CD spectra for [poly(dA-dT)]$_2$ that are the results of calculations where some of the higher energy transitions have been omitted from the basis set. From the Figure it can

be seen that the spectrum above 220 nm is virtually unaffected by the inclusion or exclusion of the far-UV transitions in the basis set. Calculations for [poly(dG-dC)]$_2$ lead to the same observation [not shown]. It would thus seem that only the best characterized DNA base transitions need be included in the basis set, for the CD above 220 nm to be represented with acceptable accuracy. Also, including the far-UV transitions improves the agreement between the calculated CD spectrum and the experimental spectrum.

5.2 Induced Circular Dichroism

The induced circular dichroism of an eda transition in an adduct bound to DNA was calculated as a function of the position and orientation of the transition. The results of the calculations are presented in contour maps throughout Papers I-III. These results can be seen as "sector rules" for the DNA-induced CD.

The application of these "sector rules" demands consideration. The transition of the DNA adduct should have a well known polarization, and also be well separated in energy from the DNA transitions. By the same token the adduct transition must not have any magnetic character, or the adduct be chirally deformed upon binding. For obvious reasons such adducts have to be fairly small systems; if larger molecules are to be considered only specific chromophores can come into question.

In Paper III and IV, where comparison with experiment were made, we chose to calculate the circular dichroism of the adduct transition assuming Gaussian bandshapes. In the later Papers (I-II) we chose instead to present the results in terms of rotatory strengths. For the record, the headings of the subsections below will be "Induced CD...", but the terms IRS (induced rotatory strength) and ICD (induced circular dichroism) will be used in the text to specify which of the properties is presented.

5.2.1 Induced CD of a groove bound molecule:

The IRS was calculated for an edtm in an adduct bound to DNA. In Paper I the DNA was [poly(dG-dC)]$_2$; in Paper II the DNA was [poly(dA-dT)]$_2$. In both papers the adduct transition was chosen to have the intensity and energy of a transition in the DNA binding molecule 4',6-diamidino-2- phenylindole (DAPI).

The results in Papers I and II applicable to both polynucleotides are: there are pronounced differences between the major and the minor grooves, as well as between 5'pur-3'pyr and 5'pyr-3'pur binding sites; and, it is sufficient to include ca 20 basepairs in the inducing polynucleotide to ensure that end effects are taken care of.

In Papers I and II contour maps of the DNA induced rotatory strength (IRS) of a groove binder as a function of position and orientation are presented. The contour maps show the IRS of the adduct edtm for pairwise variations of the parameters describing position and orientation. Three kinds of contour maps of the rotatory strength, R, are presented: R as a function of position along the groove with a fixed orientation; R as a function of orientation relative the polynucleotide in a fixed position in either groove, for both the 5'pyr-3'pur and the 5'pur-3'pyr sites; and, R calculated in a cross-section of the groove perpendicular to the helix axis. The results presented in these plots constitute the "sector rules" mentioned above.

A feature common to the two alternating polynucleotides is that the IRS for an edtm oriented along the groove is largest in magnitude halfway between two base pairs, and is smallest between the two neighbouring basepairs. In [poly(dG-dC)]$_2$ the maximum was found for the 5'pur-3'pyr site, while for [poly(dA-dT)]$_2$ the maximum was found for the 5'pyr-3'pur site. Two points can be made. First, as expected from the differing local geometries of the two basepair sequences (cf Fig 4.3), the IRS is significantly different for the two sites. Second, the non-degenerate Kirkwood-Kuhn coupling is sensitive only to the relative orientations

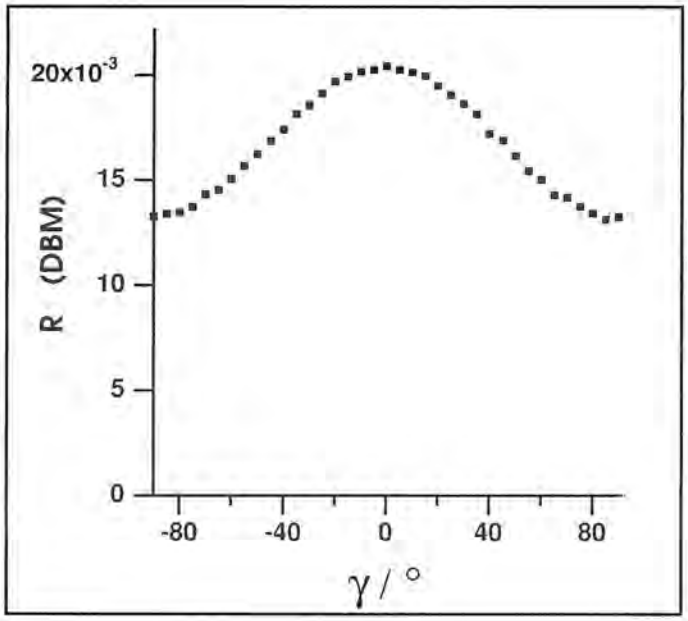

Figure 5.5. Rotatory strength as a function of the angle (γ) for an intercalator centered on the helix axis in the plane of intercalation between base pairs in a 5'A-3'T sequence. The intercalator has the characteristics of the 335 nm transition in 4',6-diamidino-2-phenylindole.

of transition moments in the DNA bases and in the adduct; the mechanism obviously cannot distinguish between pyrimidines and purines as such.

5.2.2. Induced CD of an intercalator:

There are three major results presented in Papers I-III. 1) The magnitude of the largest IRS for an intercalator is approximately 1/20 of that for a groove bound edtm (Papers I-II). For the case of the adduct transition in a groove bound position the dichroic ratio $\Delta\epsilon/\epsilon$ is of the order $5 \cdot 10^{-4}$, and it is of the order $1 \cdot 10^{-5}$ in an intercalated position. Thus, if the dichroic ratio $\Delta\epsilon/\epsilon$ is larger than 10^{-4} the results in Papers I and II provide argument that the chromophore in question is not intercalated. A smaller ratio, on the other hand, may be the result of an orientation or position close to a nodal region. In section 5.3.2 we shall see that the dye methylene blue bound to [poly(dA-dT)]$_2$ is seemingly intercalated at high ionic strengths, yet shows a dichroic ratio of $2 \cdot 10^{-4}$. It is quite possible that, due to uncertainties in the calculations, the dichroic ratios

for groove binders and intercalators should be stated in relative terms, not in absolute magnitudes. 2) The IRS of an intercalated edtm depends not only on its orientation relative to the surrounding base pairs, but also on the lateral displacement of the adduct relative to the helix axis. 3) The angular dependence of the IRS predicted by Schipper et al. [1980] and Nordén & Tjerneld [1982; cf Eq. 2.2], is supported by the results in Papers I-III. However, the IRS for an intercalator edtm centered on the helix axis need not change sign upon rotation in the intercalation pocket. This can be seen in Paper II where the results for intercalation between the basepairs in a 5'A-3'T sequence show the IRS to be positive for both perpendicular ($\gamma = 0°$) and parallel ($\gamma = 90°$) orientations of the edtm (cf Fig 5.5).

I wish to add a few comments on Paper III. The main conclusions of this study agree with the results in the later studies (cf results 2 and 3 above). Nevertheless, there are some particular limitations in the study presented in Paper III. One is that the calculations were performed only for 5'pur-3'pyr intercalation sites, the other that only the immediately surrounding basepairs were included. The reason for performing calculations for only one of the two possible site configurations is simply the embarrassing fact that, at the time, I did not realize the difference between 5'pur-3'pyr and 5'pyr-3'pur sites in the alternating AT and GC polynucleotides. The size of the system considered was partially motivated by the limited access to CPU.‡ In addition, the DNA parameters used result in intrinsic CD spectra that agree less well with experiment than do the parameters used in Papers I and II. This can be seen if the results of Rizzo & Schellman [1984], are compared to those of Williams et al. [1986]. For these reasons calculations representing parallel and perpendicular orientations of methylene blue, intercalated in both 5'pur-3'pyr and 5'pyr-3'pur sites of 20-mers of alternating AT and GC, have been performed using the DNA parameters of Paper I and II.

‡ As an example, each of the Figures 5.6 a-d and 5.7 a-d presented in this chapter would have required ca 30 hours of CPU-time on the IBM 3081 used for the calculations presented in Paper III.

These new results are presented in Figures 5.6 and 5.7, and are discussed in the next section.

5.3. Comparison with experiment.

As there is no unequivocally determined DNA-adduct binding geometry to check the calculated results with, we have to resort to complexes whose binding geometries are conjectures to varying degrees. The results from the calculations have been compared to some DNA-adduct systems. In Paper I comparisons were made with experimental data on DAPI and Ru(1,10-phenanthroline)$_3^{2+}$; in Paper II with DAPI and a number of AT minor groove binders; in Paper III with methylene blue; and, in Paper IV, with a pair of mono- and diaminoacridines. In general the magnitudes of the calculated rotatory strengths are in good agreement with experiment, and the signs are consistent with the experimentally determined binding geometries. The difference in magnitude for the induced CD of groove binders and intercalators is apparently in accord with experimental findings (Papers I-IV).

5.3.1 Groove binders:

Comparison of the calculated "sector rules" to experimentally determined IRS for DNA-ligand complexes of resonably known structures, serves the dual purposes of assessing the plausibility of the calculations and of illustrating how the results can be applied in discussions of possible binding geometries.

The discussions of the possible groove binding modes of Ru(1,10-phen)$_3^{2+}$ (Paper I) and DAPI (Paper II) both arrive at binding geometries for the ligands that are consistent with suggested models for their binding to DNA. In the case of Ru(1,10-phen)$_3^{2+}$ the results agree well with the binding characteristics concluded by Hiort et al. [1990] and by Haworth et al. [1991]. It should be pointed out, though, that the binding of Ru(1,10-phen)$_3^{2+}$ to DNA is still subject to investigation, and there is evidence of other binding modes than the two mentioned [Rehmann & Barton, 1990; Nordén et al., 1991]. The data presented

by Nordén et al. [1990] is consistent with binding of the metal complex in an AT minor groove site. Upon this binding the DNA structure may be considerably disturbed, which disallows the use of our "sector rules" since these are calculated under the assumption of intact DNA structure. The structures discussed for the DAPI-polynucleotide complexes seem less controversial [Nordén et al., 1990; Wilson et al., 1990]. The "sector rules" can not be used to determine the binding geometry, only to test whether the calculated and the experimental CD can be reconciled with a suggested geometry.

5.3.2. Intercalators:

In Paper I the calculated induced rotatory strength of the DAPI transition is compared to the experimentally observed CD of DAPI bound to $[poly(dG\text{-}dC)]_2$. The results are consistent with intercalation of DAPI in $[poly(dG\text{-}dC)]_2$, though this is not the only binding geometry the results agree on.

In Paper III the calculated induced circular dichroism for methylene blue (MB) bound to the dinucleotides $[(dA\text{-}dT)]_2$ and $[(dG\text{-}dC)]_2$ is presented and compared to experimental data measured for methylene blue bound to DNA. While the induced CD for MB in the presence of alternating GC remains weak and of the same bandshape, as the NaCl concentration is raised, the signal increases by one order of magnitude for MB under the same circumstances.

The following is a discussion of the experimentally observed CD of MB bound to DNA, in view of the new results presented in the contour maps of Figures 5.6 and 5.7. The figures show the calculated IRS induced of an intercalated edtm. The edtm in these calculations correspond to the intensity and energy of the intense, long-axis polarized transition at 664 nm in MB. These contour maps differ both from the maps presented in Paper III, and from the maps presented for an intercalated edtm corresponding to the transition at 335 nm in DAPI (Papers I-II). The features of the maps are consistent with the results reported in section 5.2.2.

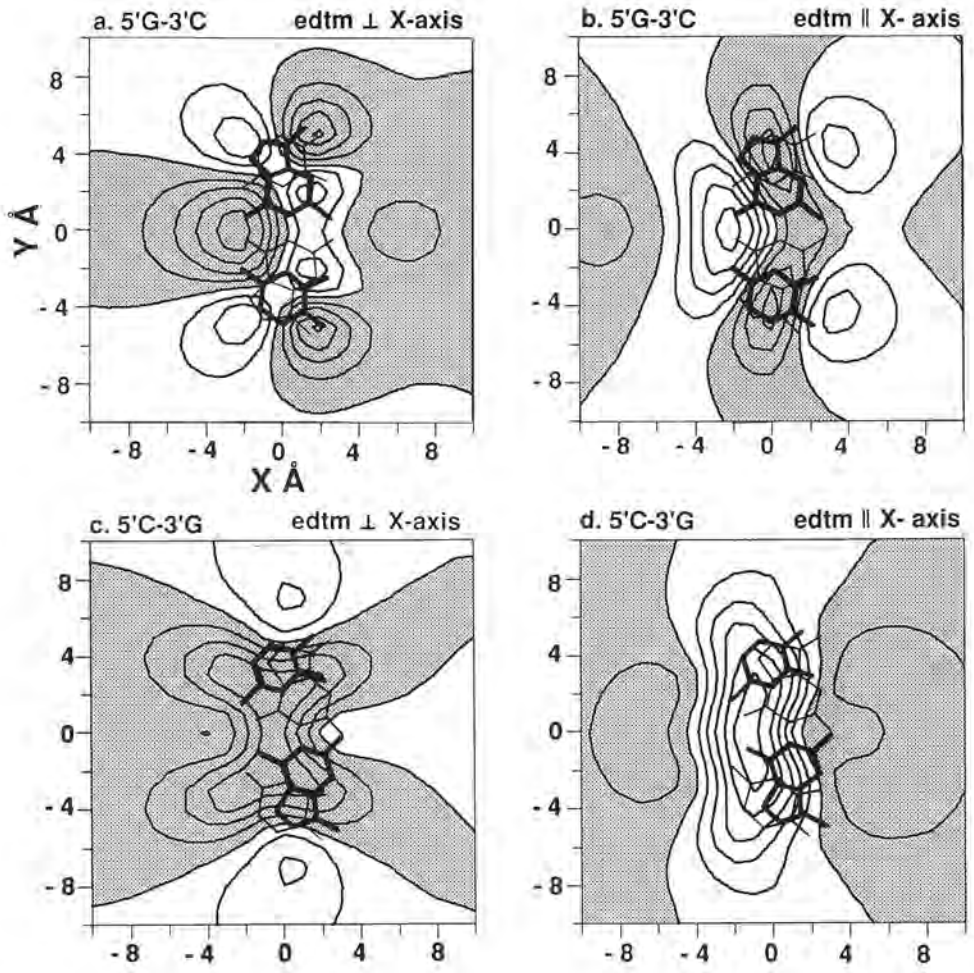

Figure 5.6. Contour maps of the IRS of an intercalator as a function of lateral displacement between base pairs in a 5'G-3'C sequence (a and b), and a 5'C-3'G sequence (c and d). (a and c): $\gamma = 90°$, edtm perpendicular to the X axis. (b and d): $\gamma = 0°$, edtm parallel to the X axis. The map is 10x10 Å with the helix axis in the center. The distance between the contour levels is 0.01 DBM. The shaded areas represent negative IRS. Also shown are the base pairs surrounding the intercalator. The base pair above the intercalation site is indicated by thick lines. The intercalator has the characteristics of the 666 nm transition in methylene blue (Paper III).

Figures 5.6 and 5.7 show the IRS of an intercalated ligand edtm as a function of lateral displacement in the plane of intercalation for the orientations $\gamma = 90°$ (edtm parallel to the surrounding base pairs) and $\gamma = 0°$ (edtm perpendicular to the base pairs). Figure 5.6 shows the results for intercalation of an edtm in the two possible GC sites, while Figure 5.7 shows the corresponding results for AT sites. The edtm corresponds to the intense transition at 664 nm in methylene blue (cf Paper III). The geometric and spectroscopic parameters for DNA are those used in Papers I and II; the inducing DNA molecule contains 20 basepairs. The results in Figures 5.6 and 5.7 illustrate the effect of changing the ligand's transition energy and intensity; if these contour maps are compared to the maps presented for the intercalation of a DAPI transition in Papers I and II, it can be seen that the differences are slight. At the same time the observation (Paper II) that the IRS for an edtm intercalated in an 5'A-3'T site, and placed on the helix axis, does not change sign if the edtm is rotated is found also here (cf Figs. 5.7a and 5.7b). The three general results listed in section 5.2.2 are not contradicted by these maps, and the result that the energy and intensity of the interacting adduct has little effect on the IRS can be added to the list.

On the basis of the results presented in Papers I and II, the discussion in Paper III, of the experimental results for MB bound to alternating double-stranded AT and GC, shall be commented. 1) $[poly(dA\text{-}dT)]_2$: The intensity of the ICD signal for MB bound to $[poly(dA\text{-}dT)]_2$ increases one order of magnitude when the ionic strength increases from 2 mM NaCl to 200 mM NaCl. As a result the dichroic ratio $\Delta\epsilon/\epsilon$ is ca $2{\cdot}10^{-4}$ at 200 mM NaCl. Considering the observed differences in the calculated magnitudes for the IRS of groove binders and intercalators, it is tempting to conclude that MB is intercalated at low ionic strength, but groove bound at high ionic strength. Linear dichroism measurements on MB bound to $[poly(dA\text{-}dT)]_2$, however, clearly show that MB has the same LD^r as the DNA bases throughout a salt titration from 1-200 mM NaCl [K. Jansen, unpublished results]. Hence MB must be oriented with its plane parallel to the DNA base planes, which is consistent with intercalation. A groove bound

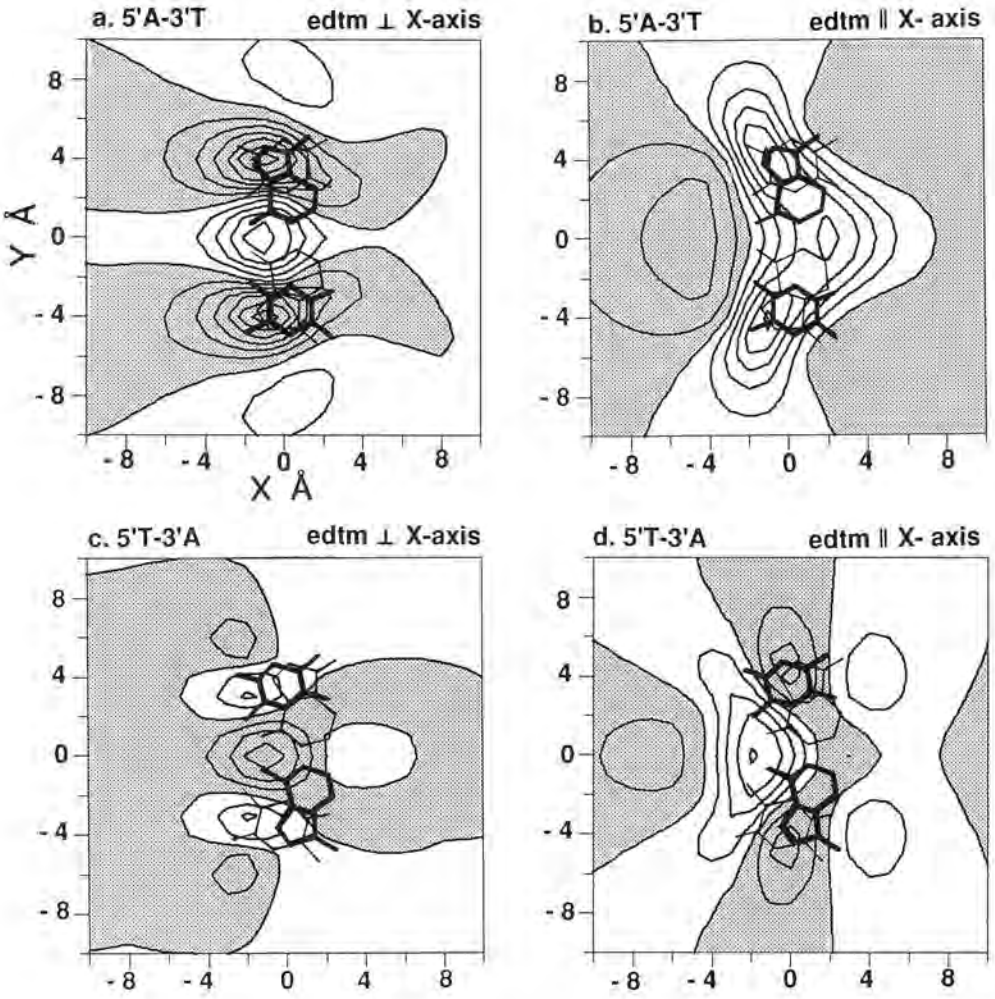

Figure 5.7. Contour maps of the IRS of an intercalator as a function of lateral displacement between base pairs in a 5'A-3'T sequence (a and b), and a 5'T-3'A sequence (c and d). (a and c): $\gamma = 90°$, edtm perpendicular to the X axis. (b and d): $\gamma = 0°$, edtm parallel to the X axis. The map is 10x10 Å with the helix axis in the center. The distance between the contour levels is 0.01 DBM. The shaded areas represent negative IRS. Also shown are the base pairs surrounding the intercalator. The base pair above the intercalation site is indicated by thick lines. The intercalator has the characteristics of the 666 nm transition in methylene blue (Paper III).

binding geometry for a planar compound oriented with its plane parallel to the base pairs has been discussed for DAPI bound to $[poly(dG\text{-}dC)]_2$ [Nordén et al., 1990]. Thus MB bound to AT may be groove bound with its plane parallel to the base pairs.

2) $[poly(dG\text{-}dC)]_2$: The ICD band for MB bound to $[poly(dG\text{-}dC)]_2$ does not change with ionic strength (Paper III). In contrast to the case of MB bound to AT, the ICD for MB bound to GC is bisignate. The shape of this spectrum was discussed in Paper III. One possibility that was not discussed was the presence of more than one transition in MB in this band. The absorption of MB between 400-700 nm shows one band with a maximum at 664 nm and a pronounced shoulder at 620 nm [Bergmann & O'Konski, 1963]. Lewis and coworkers demonstrated that the shoulder was due to the vibrational resolution of a single electronic band [Lewis et al., 1943]. The absorption band corresponds to a single, intense long-axis polarized transition, with possibly a weak perpendicularly polarized transition hidden in the red edge of the absorption envelope [Nordén, 1977; Popov, 1979]. There is also some argument for a hidden, perpendicularly polarized, transition in this band [Hogan et al., 1986], and a weak indication that such a transition is out-of-plane polarized [Kubista et al., 1988]. Altogether, the evidence for a hidden transition in the red edge of the absorption band of MB is inconclusive. Nevertheless, the bisignate shape of the ICD band for MB bound to DNA may, conceivably, be the result of the interaction of two transitions in MB with DNA.

In Paper IV a straightforward discussion of the possible intercalation geometries of a pair of aminoacridines is carried out. With the results from Papers I-II in mind (Paper IV, Page 348 in that paper), the conclusions on the intercalation geometry of the mono- and the diaminoacridines can be reviewed. Of the four conclusions presented it is only the first that is unaffected by the results of Papers I-II: the geometries of the intercalated 9-aminoacridine moiety in the mono- and bisfunctional forms differ. The reasoning that leads to the conclusions remains both sound and illustrative in the use of two transitions of known

(perpendicular) polarization in the ligand molecule. It must be remembered that a structure can not be concluded from the CD data alone; by itself CD may possibly exclude geometries from consideration, but it is in combination with other methods that the potential for using CD can be realized.

It is often difficult to decide whether the CD induced in an achiral chromophore upon binding to DNA is caused by chiral deformation, or by one or more of the perturbation mechanisms discussed at the end of section 3.1. This problem is more pronounced for groove binders than for intercalators, since it is in the nature of an intercalator to be planar and hence achiral. Two methods for addressing this question is that of Krueger & Prairie [1991], and that of Manning & Woody [1986]. Krueger & Prairie investigated the binding properties of a natural antitumor antibiotic known as CC-1065 and concluded that the induced CD of the drug is the result of a delocalized chiral electronic transition, i.e., the molecule is chirally deformed upon binding to DNA [Krueger & Prairie, 1991]. They reached their conclusion by comparing the ICD spectra of CC-1065 to the measured CD spectra of a number of analog compounds bound to DNA. Manning & Woody did molecular orbital calculations for netropsin bound to DNA and found that a chiral deformation of the ligand could not explain the features of the experimentally observed CD. They concluded that a change of conformation was not the dominant source of ICD for netropsin bound to DNA [Manning & Woody, 1986]. In both cases it is clear that in addition to the information of how the ligand is bound to DNA, circular dichroism may provide information of whether the ligand is conformationally changed upon binding. The results presented in this work should be helpful in such discussions.

Finally, the question of further work shall be considered. The understanding of the CD of nucleic acids, and of the relation of CD to the structure of the polymer in aqueous solutions, has reached a stage where specific questions both about the assumed DNA structures and about the spectroscopic properties of the monomeric nucleic acid bases can be discussed. The most obvious extension is to investigate the effects of $n \rightarrow \pi^*$ transitions, the sugar-phosphate backbone, and

the choice of dielectric constant. Of these three, the last two can be approached more easily. Since the work of Cech [1975] the sugar-phosphate backbone has either been described by background oscillators or been disregarded completely. The question of how to derive a reliable set of background oscillators has not been addressed. The possibility of using a dielectric constant, other than just an effective constant, has been discussed [Callahan & Hooker, 1987; Mazur & Jernigan, 1991].

The inclusion of additional transitions in the DNA bases, whatever character they have, hinges on the results of spectroscopic investigations of the bases. Over the years a number of absorption and CD bands of the bases have been suggested as candidates for $n \rightarrow \pi^*$ transitions, but there is still no unequivocal evidence for an $n \rightarrow \pi^*$ transition. (Some candidates have been eliminated, though (cf, e.g., Williams et al., 1991).) The matrix method, as well as many other methods, depends on well characterized transitions to be used for input in the calculations. As the matrix method makes use of transition densities which can be calculated from LCAO programs, it is desirable that the discrepancies between experimental and computational descriptions of the transition properties be resolved [Callis, 1983]. Also, characterization of the far UV $\pi \rightarrow \pi^*$ transitions is of interest if the DNA CD in this region is to be calculated, and it seems as though the far-UV CD must be included if the CD is to be used as an indicator of helical handedness [Johnson, 1990]. An immediately feasible approach may be to place a magnetic dipole allowed transition at a carbonyl group or an azine nitogen in A or T, and calculate the DNA CD spectrum. The effects of placement (A or T), energy and intensity, as well as polarization of the transition could then be evaluated.

The calculations of the CD induced into a DNA-adduct's transition presented here have been limited to interactions of electric dipole allowed transitions. The calculations correspond to the case where the adduct retains its conformation upon binding, so that it is achiral. If a magnetic dipole allowed transition is to be considered, then it seems simplest to add it to the adduct.

This would result in an additional CD mechanism (cf section 2.1) contributing to the ICD of the adduct: the so-called one-electron-mechanism of Condon, Altar & Eyring [1937]. For the μm-mechanism to contribute to the ICD of the adduct there would have to be magnetic transitions in the DNA. The formalism of the matrix method is especially well-suited to comparisons of the contributions of different mechanisms.

6. Conclusions.

The results presented in this thesis show how the rotatory strength induced into an electric dipole allowed transition in a DNA binding adduct depends upon position and orientation relative the DNA. The only interaction considered is that between the adduct's transition and the $\pi \rightarrow \pi^*$ transitions in the helically arranged DNA bases. The CD spectra of the polynucleotides [poly(dA-dT)]$_2$, [poly(dG-dC)]$_2$ and poly(dA):poly(dT) have been calculated, and compared to the experimental spectra. The differences and agreements have been discussed in terms both of DNA structure and of the spectroscopic parameters of the DNA bases.

These are the conclusions from the calculations on induced rotatory strength:

The induced rotatory strength for an intercalator is one order of magnitude weaker than that for a groove bound ligand.

The dependence upon orientation for the induced rotatory strength is significantly different if the ligand is bound to 5'pyrimidine-3'purine, or to 5'purine-3'pyrimidine sites. This holds for groove binders and intercalators alike.

The induced rotatory strength of an intercalated ligand depends both on the orientation of the ligand in the intercalation site, and on its displacement from the helix axis.

The angular dependence of the induced rotatory strength of an intercalator proposed by Norden & Tjerneld [1982] is partially confirmed, as the induced

rotatory strength of an intercalator varies as cos 2γ, but need not change sign upon rotation in the plane of the intercalation site.

Both signs and magnitudes of the calculated IRS agree well with the experimental observations. The results of the intrinsic DNA CD calculations are harder to itemize. The calculations using the recently assigned transition moments parameters of Clark [1989; 1990] result in AT polynucleotide CD spectra that agree less well with experiment than do the calculations of Williams and co-workers [1986]. As often is the case with calculations of DNA CD, it is difficult to determine whether the discrepancy between calculation and experiment arises from wrongly assumed DNA structures, or from deficient transition moment parameters. In line with previous studies we suggest that in the case of [poly(dA-dT)]$_2$, both the structure and the $\pi \rightarrow \pi^*$ transition moment parameters are correctly described, and that there is a magnetic dipole allowed transition in either adenine or thymine, that we have not considered in our input, and which contributes significantly to the experimental CD spectrum of [poly(dA-dT)]$_2$. It is not unlikely that magnetic dipole transitions must be considered if the DNA CD shall be fully understood.

The results presented here can indubitably be improved upon - a number of the assumptions made are crude approximations of the properties both of DNA and of DNA adducts. If CD is to be useful in the study of molecules relevant to biophysical systems, and it is a very attractive technique in its sensitivity to chirality, then it is necessary to perform studies of the kind presented here. The measurement of CD is straightforward, the interpretation complicated and any investigation that leads to simple empirical relations between experiment and structure is of help. Having said so it is fitting to end with a final caveat. The allure of simple interpretation of CD measurements must not lead to applications where the possible contributions of, e.g., magnetic dipole allowed transitions have not been considered. Even though the objective of this work is a systematic study

of how to make use of DNA induced CD for determining the geometry of a DNA-adduct complex, it must be remembered that it clearly is impossible to determine the conformation of such a complex from the sign and magnitude of a rotatory band alone. I firmly contend, though, that results such as those presented here are of help in discussing the structure of a DNA-adduct complex - at the very least by making it possible to eliminate some structure under consideration.

7. Acknowledgment.

Whether in the end the work presented here could have been done faster, better, badder or worse is of little importance in the long run; on the whole I have enjoyed these seven long years.

I wish to thank:

Alison Rodger, who never made me work harder than she herself would;

John Schellman, who explained and encouraged at the outset when I understood so little;

And, Bengt Nordén, who instigated my collaboration with Alison and without whom – as the saying goes – this wouldn't have been possible.

Daniel Fornasiero, Torleif Härd, Tom Kuruscev, and Eddie Schipper are all gratefully acknowledged for co-authorship and discussion on CD.

I wish to name Kjell Sandros, Svante Eriksson, Bo Albinsson, Per Hagmar, and Catharina Hiort, all of whom I over the years have come to count as my friends and with whom I share more than one esoteric interest.

This sentence, finally, is dedicated to all those whom I have unforgivably forsaken to mention by name.

March 1992.
Reidar L

8. References.

Arnott, S. & Hukins, D.W.L. (1972) ***Biochem. Biophys. Res. Commun.*** **47**, 1504-1509.

Arnott, S., Chandrasekaran, R., Hukins, D.W.L., Smith, P.J.C. & Watts, L. (1974) ***J. Mol. Biol.*** **88**, 523-533.

Arnott, S., Chandrasekaran, R., Hall, I.H. & Puigjaner, L.C. (1983) ***Nucleic Acids Res.*** **11**, 4141-4155.

Atkins, P.W.A. (1983) ***"Molecular Quantum Mechanics"***, 2nd ed., Oxford University Press, Oxford.

Bayley, P.M., Nielsen, E.B. & Schellman, J.A. (1969) ***J. Phys. Chem.*** **73**, 228-243.

Bergman, K. & O'Konski, C.T. (1963) ***J. Phys. Chem.*** **67**, 2169-2177.

Bernstein, F.C, Koetzle, T.F., Williams, G.J.B., Meyer, E.F.Jr., Bruce, M.D., Rogers, J.R., Kennard, O., Shimanouchi, T. & Tasumi, M. (1977) ***J. Mol. Biol.*** **112**, 535-542.

Blaschke, V.G., Kraft, H.P., Fickentscher, K. & Köhler, F. (1979) ***Arzneimittel-Forschung/Drug Res.*** **29**, 1640-1642.

Born, M. & Jordan, P. (1925) ***Z. Physik.*** **34**, 858-888.

Born, M., Heisenberg, W. & Jordan, P. (1925) ***Z. Physik*** **35**, 557-615.

Bradley, D.F., Tinoco, I.Jr. & Woody, R.W. (1963) ***Biopolymers*** **1**, 239-267.

Brahms, J. & Mommaerts, W.F.H.M. (1964) ***J. Mol. Biol.*** **10**, 73-88.

Bush, C.A. & Brahms, J. (1967) ***J. Chem. Phys.*** **46**, 79-88.

Bush, C.A. (1970) ***J. Chem. Phys.*** **53**, 3522-3530.

Bush , C.A. & Scheraga, H.A. (1969) ***Biopolymers*** **7**, 395-409.

Callahan, D.E. & Hooker, T.M.Jr. (1987) ***Biopolymers*** **26**, 457-461.

Callis, P.R. (1983) ***Ann. Rev. Phys. Chem.*** **34**, 329-357.

Callis, P.R. (1986) ***Photochem. Photobiol.*** **44**, 315-332.

Cech, C.M. (1975) ***Ph.D. Thesis***, University of California, Berkeley.

Cech, C.M., Hug, W. & Tinoco, I.Jr. (1976) ***Biopolymers*** **15**, 131-152.

Cech, C.M. & Tinoco, I.Jr. (1977) ***Biopolymers*** **16**, 43-65.

Chandrasekaran, R. & Arnott, S. (1989) in ***Landolt-Börnstein New Series VII/1b***, Saenger, W., Ed., Springer Verlag, pp 31-170.

Charney, E. (1979) ***"The Molecular Basis of Optical Activity"***, John Wiley & Sons, New York, NY.

Clark, L.B. (1977) ***J. Am. Chem. Soc.*** **99**, 3934-3938.

Clark. L.B. (1989) ***J. Phys. Chem.*** **93**, 5345-5347.

Clark, L.B. (1990) ***J. Phys. Chem.*** **94**, 2873-2879.

Condon, E.U., Altar, W. & Eyring, H. (1937) ***J. Chem. Phys.*** **5**, 753-775.

DeVoe, H. & Tinoco, I.Jr. (1962) ***J. Mol. Biol.*** **4**, 500-517.

DeVoe, H. (1964) ***J. Chem. Phys.*** **41**, 393-400.

DeVoe, H. (1965) ***J. Chem. Phys.*** **43**, 3199-3208.

Eyring, H., Walter, J. & Kimball, G.E. (1944) ***"Quantum Chemistry"***, John Wiley & Sons, New York, NY.

Fitts, D.D. & Kirkwood, J.G. (1957) ***Proc. Natl. Acad. Sci.*** **42**, 1046-1052.

Goux, W.J. & Hooker, T.M.Jr. (1980) ***J. Am. Chem. Soc.*** **102**, 7080-7087.

Gray, D.M., Johnson, K.H., Vaughan, M.R., Sutherland, J.C. & Ratliff, R.L. (1990) ***Biopolymers*** **29**, 317-323.

Hatano, M. (1986) ***Adv. Polymer Sci.*** **77**, 1-121.

Haugh, E.F. & Hirschfelder, J.O. (1955) ***J. Chem. Phys.*** **23**, 1778-1796.

Haworth, I.S., Elcock, A.H., Freeman, J., Rodger, A. & Richards, W.G. (1991) ***J. Biomol. Struct. & Dyn.*** **9**, 23-44.

Heisenberg, W. (1925) ***Z. Physik*** **33**, 879-893.

Hinchliffe, A. & Munn, R.W. (1985) ***"Molecular Electromagnetism"*** John Wiley & Sons, New York, NY.

Hiort, C., Nordèn, B. & Rodger, A. (1990) ***J. Am. Chem. Soc.*** **112**, 1971-1982.

Hogan, M.E., Hayes, B., Wang, N.C. & Austin, R.H. (1986) ***Biochemistry*** **25**, 5070-5082.

Hooker, T.M.Jr. & Schellman, J.A. (1970) ***Biopolymers*** **9**, 1319-1348.

Hug, W. & Tinoco, I.Jr. (1973) ***J. Am. Chem. Soc.*** **95**, 2803-2813.

Hug, W. & Tinoco, I.Jr. (1974) ***J. Am. Chem. Soc.*** **96**, 665-673.

Imae, T. & Ikeda, S. (1976) ***Polymer Journal*** **8**, 531-539.

Imae, T. Hayashi, S. & Ikeda, S. (1987) ***Macromolecules*** **20**, 589-597.

Ito, H. & I'Haya, Y.J. (1979) ***Bull. Chem. Soc. Japan*** **52**, 2823-2831.

Ito, H., Uchiyama, S., Tabe, N. & I'Haya, Y.J. (1989) ***Chem. Phys. Lett.*** **162**, 103-109.

Johnson, K.H. & Gray, D.M. (1991) ***Biopolymers*** **31**, 373-384.

Johnson, K.H. & Gray, D.M. (1991) ***Biopolymers*** **31**, 385-395.

Johnson, N.P. & Switkes, E. (1978) ***Biopolymers*** **17**, 857-872.

Johnson, W.C.Jr. & Tinoco, I.Jr. (1969a) ***Biopolymers*** **7**, 727-749.

Johnson, W.C.Jr. & Tinoco, I.Jr. (1969b) ***Biopolymers*** **8**, 715-731.

Johnson, W.C.Jr. (1985) ***Methods of Biochem. Anal.*** **31**, Glick, D., Ed., 61-163.

Johnson, W.C.Jr. (1990) in ***Landolt-Börnstein New Series VII/1c***, Saenger, W., Ed., Springer Verlag, pp 1-21.

Kamiya, M. (1979) ***Biochim. Biophys. Acta*** **562**, 70-78.

Kamiya, M. (1980) ***Chem. Phys.*** **52**, 337-351.

Kamiya, M. (1988a) ***Chem. Phys.*** **120**, 79-89.

Kamiya, M. (1988b) ***Chem. Phys. Lett.*** **144**, 339-345.

Kauzmann, W.J., Walter, J.E. & Eyring, H. (1940) ***Chem. Rev.*** **26**, 339-407.

Kirkwood, J.G. (1937) ***J. Chem. Phys.*** **5**, 479-491.

Krueger, W.C. & Prairie, M.D. (1991) ***Chem. -Biol. Interactions*** **79**, 137-149.

Kubista, M., Albinsson, B., Åkerman, B., Lyng, R. & Nordén, B. (1988) ***Finn. Chem. Lett.*** **15**, 38-39.

Kubista, M. Åkerman, B. & Nordèn B. (1988) ***J. Phys. Chem.*** **92**, 2352-2356.

Kuhn, W.A. (1930) ***Trans. Faraday Soc.*** **26**, 295-308.

Lerman, L.S. (1961) ***Proc. Natl. Acad. Sci.*** **3**, 18-30.

Levin, A.I. & Tinoco, I.Jr. (1977) ***J. Chem. Phys.*** **66**, 3491-3497.

Lewis, G.N., Goldschmid, O., Magel, T.T. & Bigeleisen, J. (1943) ***J. Am. Chem. Soc.*** **65**, 1150-1154.

London, F. (1941) ***J. Phys. Chem.*** **46**, 305-316.

Madison, V. & Schellman, J.A. (1972) ***Biopolymers*** **11**, 1041-1076.

Manning, V. & Woody, R.W. (1986) ***Biopolymers*** **25**, 2065-2082.

Mathews, C.K. & van Holde, K.E. (1990) ***"Biochemistry"***, The Benjamin/Cummings Pub. Co., Redwood, CA.

Matos, J.M.O. & Roos, B.O. (1988) ***J. Am. Chem. Soc.*** **110**, 7664-7671.

Matsuoka, Y. & Nordén, B. (1982) ***J. Phys. Chem.*** **86**, 1378-1386.

Mazur, J. & Jernigan, R.L. (1991) ***Biopolymers*** **31**, 1615-1629.

Mellin, G.W. & Katzenstein, M. (1962) ***New Engl. J. Med.*** **267**, 1184-1193.

Merzbacher, E. (1970) ***"Quantum Mechanics"***, 2nd Ed., John Wiley & Sons, New York, NY.

Moffitt, W. (1956) ***J. Chem. Phys.*** **25**, 467-478.

Moffitt, W., Fitts, D.D. & Kirkwood, J.G. (1957) ***Proc. Natl. Acad. Sci.*** **43**, 723-xxx.

Moffitt, W. & Moscowitz, A. (1959) ***J. Chem. Phys.*** **30**, 648-660.

Moore, D.S. & Wagner, T.E. (1973) ***Biopolymers*** **12**, 201-221.

Moore, D.S. & Wagner, T.E. (1974) ***Biopolymers*** **13**, 977-986.

Moore, D.S. (1980) ***Biopolymers*** **19**, 1017-1038.

Moore, D.S. & Williams, A.L.Jr. (1986) ***Biopolymers*** **25**, 1461-1491.

Nordén, B., Eriksson, S., Kim, S.K., Kubista, M., Lyng, R. & Åkerman, B. (1990) in ***Molecular Basis of Specificity in Nucleic Acid-Drug Interactions***, Pullman, B. & Jortner, J., Eds., Kluwer Academic Publishers, pp 23-41.

Nordén, B. (1977) in ***Linear Dichroism Spectroscopy: Proceedings of a Nobel Workshop in Lund, October 25-27, 1976.*** Lund University Press, Lund, Sweden.

Nordén, B., Patel, N., Hiort, C., Gräslund, A. & Kim, S.K. (1991) ***Nucleosides and Nucleotides*** **10**, 195-205.

Nordén, B. & Tjerneld, F. (1982) ***Biopolymers*** **21**, 1713-1734.

Novros, J.S. & Clark, L.B. (1986) ***J. Phys. Chem.*** **90**, 5666-5668.

Park, H.-S., Arnott, S., Chandrasekaran, R., Millane, R.P. & Campagnari, F. (1987) ***J. Mol. Biol.*** **197**, 513-524.

Pauling, L., Corey, R.B. & Branson, H.R. (1951) ***Proc. Natl. Acad. Sci.*** **37**, 205-211.

Philpott, M.R. (1970) ***J. Chem. Phys.*** **53**, 968-981.

Philpott, M.R. (1972) ***J. Chem. Phys.*** **56**, 683-688.

Platonova, N.V. & Popov, K.R. (1979) ***Zh. Prikl. Spektrosk.*** **30**, 464-469.

Pullman, A. & Pullman, B. (1981) ***Quart. Revs. Biophys.*** **14**, 289-380.

Rabenold, D.A. (1974) ***J. Chem. Phys.*** **62**, 376-387.

Rabenold, D.A. (1983) ***J. Chem. Phys.*** **80**, 1326-1333.

Rabenold, D.A. (1990) ***J. Phys. Chem.*** **94**, 6171-6175.

Rabenold, D.A. & Rhodes, W. (1986) ***J. Phys. Chem.*** **90**, 2560-2566.

Rabenold, D.A. & Rhodes, W. (1987) ***Biopolymers*** **26**, 109-119.

Riazance, J.H. Baase, W.A., Johnson, W.C.Jr., Hall, K., Cruz, P. & Tinoco, I.Jr. (1985) ***Nucleic Acdis Res.*** **13**, 4983-4989.

Reddy, B.S., Seshadiri, T.P., Sakore, T.D. & Sobell, H.M. (1979) ***J. Mol. Biol.*** **135**, 787-812.

Redman, S.M. & Rhodes, W. (1979) ***Biopolymers*** **18**, 393-409.

Rehmann, J.P. & Barton, J.K. (1990) ***Biochemistry*** **29**, 1710-1717.

Richterich, P. & Pohl, F.M. (1987) ***Biopolymers*** **26**, 231-250.

Rizzo, V. & Schellman, J.A. (1984) ***Biopolymers*** **23**, 435-470.

Rosenfeld, L. (1928) ***Z. Physik*** **52**, 161-174.

Sakore, T.D., Reddy, B.S. & Sobell, H.M. (1979) ***J. Mol. Biol.*** **135**, 763-785.

Schellman, J.A. (1966) ***J. Chem. Phys.*** **44**, 55-63

Schellman, J.A. & Nielsen, E.B. (1967) in ***Conformation of Biopolymers***, Ramachandran, G.N., Ed., Academic Press, New York, NY, pp 109-122.

Schellman, J.A. (1968) ***Accounts. Chem. Res.*** **1**, 144-151.

Schellman, J.A. (1975) ***Chem. Revs.*** **75**, 323-331.

Schellman, J.A. & Jensen, H.P. (1987) ***Chem. Rev.*** **87**, 1359-1399.

Schipper, P.E. (1978) ***J. Am. Chem. Soc.*** **100**, 3658-3668.

Schipper, P.E., Nordén, B. & Tjerneld, F. (1980) ***Chem. Phys. Lett.*** **70**, 17-21.

Schipper, P.E. & Rodger, A. (1983) ***J. Am. Chem. Soc.*** **105**, 4541-4550.

Shurcliff, W.A. (1962) ***"Polarized Light"***, Harvard University, Cambridge, MA.

Simpson, W.T. & Peterson, D.L. (1957) ***J. Chem. Phys.*** **26**, 588-593.

Sobell, H.M. (1985) in ***Biological Macromolecules & Assemblies, Volume 2: Nucleic Acids and Interactive Proteins.*** Jurnak, F.A. & MacPherson, A., Eds., John Wiley & Sons, New York, NY, pp 171-232.

Sprecher, C. & Johnson, W.C.Jr. (1977) ***Biopolymers*** **16**, 2243-2264.

Stewart, R.F. & Davidson, N. (1963) ***J. Chem. Phys.*** **39**, 255-266.

Stigter & Schellman, J.A. (1973) ***Int. J. of Quant. Chem.*** **7**, 1-13.

Studdert, D.S. & Davis, R.S. (1974a) ***Biopolymers*** **13**, 1377-1389.

Studdert, D.S. & Davis, R.S. (1974b) ***Biopolymers*** **13**, 1391-1403.

Studdert, D.S. & Davis, R.S. (1974c) ***Biopolymers*** **13**, 1405-1416.

Sutherland, J.C., Griffin, K.P., Keck, P.C. & Takas, P.Z. (1981) ***Proc. Natl. Acad. Sci.*** **78**, 4801-4804.

Sutherland, J.C. & Griffin, K.P. (1983) ***Biopolymers*** **22**, 1445-1448.

Theiste, D., Callis, P.R. & Woody, R.W. (1991) ***J. Am. Chem. Soc.*** **113**, 3260-3267.

Tinoco, I.Jr. (1960) ***J. Chem. Phys.*** **33**, 1332-1338; **34**, 1067.

Tinoco, I.Jr. (1962) ***Adv. Chem. Phys.*** **4**, 113-160.

Tinoco, I.Jr., Woody, R.W. & Bradley, D.F. (1963) ***J. Chem. Phys.*** **38**, 1317-1325.

Tinoco, I.Jr. (1964) ***J. Am. Chem. Soc.*** **86**, 297-298.

Tinoco, I.Jr. (1968) ***J. Chim. Phys.*** **65**, 91-97.

Vasmel, H. & Greve, J. (1981) ***Biopolymers*** **20**, 1329-1332.

Voet, D., Gratzer, W.B., Cox, R.A. & Doty, P. (1963) ***Biopolymers*** **1**, 193-206.

Wang, A.H.-J., Quigley, G.J. & Rich, A. (1979) ***Nucleic Acids Res.*** **6**, 3879-3890.

Watson, J.D. & Crick, F.H.C. (1953) ***Nature*** **171**, 737-738.

Weigang, O.E.Jr. (1965) ***J. Chem. Phys.*** **43**, 71-72.

Williams, A.L.Jr. & Moore, D.S. (1983) ***Biopolymers*** **22**, 755-786.

Williams, A.L.Jr., Cheong, C., Tinoco, I.Jr. & Clark, L.B. (1986) ***Nucleic Acids Res.*** **14**, 6649-6659.

Williams, S.A., Renn, C.N. & Callis, P.R. (1987) ***J. Phys. Chem.*** **91**, 2730-2734.

Wilson, W.D., Tanious, F.A., Barton, H.J., Jones, R.L., Fox. K., Wydra, R.L. & Strekowski, L. (1990) ***Biochemistry*** **29**, 8452-8461.

Zaloudek, F., Novros, J.S. & Clark, L.B. (1985) ***J. Am. Chem. Soc.*** **107**, 7344-7351.

PAPER I

Reidar Lyng, Alison Rodger & Bengt Nordén

The CD of Ligand–DNA Systems. I. Poly(dG-dC) B-DNA

Biopolymers (1991), **31**, p 1709-1720
https://doi.org/10.1002/bip.360311405

PAPER II

Reidar Lyng, Alison Rodger & Bengt Nordén

The CD of Ligand-DNA Systems. 2. Poly(dA-dT) B-DNA

Biopolymers (1992), **32**, p 1201-1214
https://doi.org/10.1002/bip.360320910

PAPER III

Reidar Lyng, Torleif Härd & Bengt Norden

Induced CD of DNA Intercalators: Electric Dipole Allowed transitions

Bipolymers (1987), **26**, p 1327-1345
https://doi.org/10.1002/bip.360260809

PAPER IV

Daniel Fornasiero, Tomas Kuruscev, Reidar Lyng & Bengt Nordén

Circular Dichroism and Absorption Spectra of Mono and Di-Aminoacridines Complexed to DNA

Croatica Chimica Acta (1989), 62, p 339-350