DFT Study of NMR Chemical Shifts and Spin-Spin Scalar Interactions in Nucleic Acids

Diploma thesis

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I declare that I wrote this diploma thesis on my own using only the referenced literature.
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Thus, the task is, not so much to see what no one has yet seen; but to think what nobody has yet thought, about that which everybody sees.

Erwin Schrödinger
To my mum
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Chapter 1

Introduction

The knowledge of 3D structures of proteins and nucleic acids is important for understanding the biological functions of biomacromolecules. Nuclear Magnetic Resonance (NMR) spectroscopy is one of the most powerful techniques used for the structure determination. The recent advances in NMR instrumentation and methodology provide new possibilities for application of theoretical approaches.

NMR parameters, i.e., chemical shifts, indirect spin-spin coupling constants, and direct dipol-dipol coupling constants, are quantities determined by the electronic structure. This electronic structure is, in turn, intimately related to both local and global geometry. Thus, NMR parameters are influenced by internal flexibility and intramolecular interactions. Chemical shifts as well as spin-spin coupling constants found experimentally are an average over the values belonging to all geometrical arrangements arising during the course of NMR experiment. Unfortunately, in the case of chemical shifts, the dependence on neither the internal dynamics nor the intermolecular interactions is generally described. Therefore, most experimental NMR techniques employ coupling constants or the nuclear Overhauser effect, rather than chemical shifts, for obtaining structural information. However, the insight into structure-chemical shift/spin-spin coupling constants relationships may also be provided by \textit{ab initio} calculations which can make the interpretation of the experimental data easier in this way. That is why the capability of reliable computational predictions is in high demand.

As generally known, results of sufficient quality are achievable only when electron correlation is included in the calculation. This is fulfilled by the application of various wave function based strategies. Unfortunately, these methods are limited to small and medium sized systems. However, with the advent of density functional methods, where the electron correlation effects are implicitly accounted for via the exchange-correlation functional, it is pos-
sible to obtain relevant results even for larger molecules, such as fragments of proteins and nucleic acids.

The link between geometrical and NMR parameters has already been successfully investigated \[1\] [2] [3] [4] [5] [6] [7] [8] [9] [10] [11] [12] [13] for the case of (deoxy)nucleosides. Various models have been employed, exceeding the range of structural parameters attainable from experiment. The results obtained emerged to provide valuable information. The goal of my diploma thesis was to extend the theoretical knowledge about the structure-NMR relationships in two directions: The first one was focused on understanding the influence of the sugar pucker, the exocyclic dihedral angle, and the glycosidic torsion angle on $^{13}\text{C}$ and $^{15}\text{N}$ chemical shifts in deoxyribonucleosides. The other one was aimed to comprehend the influence of the sugar-phosphate backbone conformation in nucleic acids on $^{31}\text{P}$ chemical shielding and heteronuclear $J_{\text{PX}}$ spin-spin coupling constants.
Chapter 2

Theory of NMR Parameters

2.1 The NMR Spin Hamiltonian Parameters

2.1.1 The Spin Hamiltonian

According to the postulates of quantum mechanics, the quantum state of the system is fully described by a wave function $|\psi_{\text{full}}(t)\rangle$, which contains information on the positions, velocities, and spin states of all electrons and nuclei. The wavefunction obeys the time-dependent Schrödinger equation:

$$\frac{d}{dt}|\psi_{\text{full}}(t)\rangle = -i\hat{H}_{\text{full}}|\psi_{\text{full}}(t)\rangle.$$ (2.1)

This equation is complete but it can be solved exactly only for a couple of simple systems [14]. For purposes of NMR, a much simpler equation is used [15], in which only the nuclear spin states appear:

$$\frac{d}{dt}|\psi_{\text{spin}}(t)\rangle = -i\hat{H}_{\text{spin}}|\psi_{\text{spin}}(t)\rangle.$$ (2.2)

Here, $|\psi_{\text{spin}}(t)\rangle$ denotes the spin state of the nuclei, and $\hat{H}_{\text{spin}}$ is the nuclear spin Hamiltonian [16]. From now on, the operator $\hat{H}$ is taken to imply the nuclear spin Hamiltonian, and the quantum states $|\psi\rangle$ are taken to imply the nuclear spin states.

The effective spin Hamiltonian may be written as

$$\hat{H} = -\sum_{M} \gamma_{M} h B^{T}(1 - \sigma_{M}) I_{M} + \frac{1}{2} \sum_{M \neq N} \gamma_{M} \gamma_{N} h^{2} I_{M}^{T}(D_{MN} + K_{MN}) I_{N}.$$ (2.3)

\(^{1}\)The superscript $T$ indicates that the corresponding vector and operator, respectively, is written as a transponated matrix.
where $\gamma_M$, $\gamma_N$ are the nuclear magnetogyric ratios, $I_M$ the nuclear spin operator, $\sigma_M$ the nuclear magnetic shielding tensor, $D_{MN}$ the tensor describing the classical dipolar interaction of the nuclear spins $M$ and $N$, and $K_{MN}$ the reduced indirect nuclear spin-spin coupling tensor. $\hat{H}$ reproduces the transition energies between the Zeeman states \[15\] of nuclear magnetic dipole moments

$$\mu_M = \gamma_M \hbar I_M$$ (2.4)

placed in the magnetic field $B$.

If rotational averaging of the spin Hamiltonian is taken into account, then the direct spin-spin coupling constants $D_{MN}$ vanish but the indirect couplings $K_{MN}$ do not, and the equation (2.3) may be rewritten in the form

$$\hat{H}_{iso} = -\sum_M \gamma_M \hbar (1 - \sigma_M) B I_M \gamma_x + \frac{1}{2} \sum_{M \neq N} \gamma_M \gamma_N \hbar^2 K_{MN} I_M \cdot I_N$$ (2.5)

where the nuclear shielding constants $\sigma_M$ and the reduced indirect spin-spin coupling constants $K_{MN}$ are associated with the corresponding tensors

$$\sigma_M = \frac{1}{3} \text{Tr}\{\sigma_M\}$$ (2.6)

$$K_{MN} = \frac{1}{3} \text{Tr}\{K_{MN}\}.$$ (2.7)

$K_{MN}$ is often discussed using the related indirect spin-spin coupling tensors

$$J_{MN} = \frac{\hbar}{2\pi} \gamma_M \gamma_N K_{MN}.$$ (2.8)

Despite this fact, there is one advantage of using $K_{MN}$ instead of $J_{MN}$. Since the parametric dependence on the magnetogyric ratios is removed from $K_{MN}$ (see eq (2.8)), it enables studies of trends in indirect spin-spin coupling between various elements and/or isotopes without having to consider the nuclear factors.

### 2.1.2 Spin-Hamiltonian Parameters as Energy Derivatives

Spin-Hamiltonian parameters, namely the nuclear shielding and spin-spin coupling constants, can be determined from a knowledge of the electronic wave function of the system.

Generally, the total energy of a molecular electronic system varies under the influence of a perturbation $x$ according to the equation:

$$E(x) = E^{(0)} + E^{(1)} x + \frac{1}{2} x^T E^{(2)} x + \ldots$$ (2.9)
The coefficients of this expansion are characteristic of the molecular system in a given quantum state and are known as molecular properties. If the perturbation is static (i.e., time-independent, as in our case of NMR properties studied for a homogeneous magnetic field), the molecular properties may be calculated by differentiation

\[ E^{(1)} = \frac{\partial E}{\partial x} \bigg|_{x=0} \quad (2.10) \]

\[ E^{(2)} = \frac{\partial^2 E}{\partial x^2} \bigg|_{x=0} \quad (2.11) \]

and are called first-order \((2.10)\) and second-order \((2.11)\) molecular properties, respectively.

The electronic energy in the presence of an external magnetic field and nuclear magnetic moments is expanded in terms of the small perturbations caused by the magnetic induction \(B\) and the nuclear moments \(\sigma_M\) around \(B = \sigma_M = 0\), which gives for closed-shell systems \(18\)

\[ E(B, \mu) = E_0 + \frac{1}{2} B^T E^{(20)} B + \sum_M B^T E^{(11)}_{M} \mu_M + \frac{1}{2} \sum_{M \neq N} \mu_M^T E^{(02)}_{MN} \mu_N \quad (2.12) \]

using the following notation for energy derivatives

\[ E^{(20)} = \frac{\partial^2 E(B, \mu)}{\partial B^2} \bigg|_{B=0,\mu=0} \quad (2.13) \]

\[ E^{(11)}_M = \frac{\partial^2 E(B, \mu)}{\partial B \partial \mu_M} \bigg|_{B=0,\mu=0} \quad (2.14) \]

\[ E^{(02)}_{MN} = \frac{\partial^2 E(B, \mu)}{\partial \mu_M \partial \mu_N} \bigg|_{B=0,\mu=0} \quad (2.15) \]

where

\[ \mu \in \{\mu_M, \mu_N\} \quad (2.16) \]

Comparing the Eqs. (2.3) and (2.12), we identify the \(E^{(11)}_M\) with the nuclear shielding tensors and \(E^{(02)}_{MN}\) with the spin-spin coupling tensors:

\[ \sigma_M = E^{(11)}_M + 1 \quad (2.17) \]

\[ K_{MN} = E^{(02)}_{MN} - D_{MN}. \quad (2.18) \]

The \(E^{(20)}\) tensor represents the molecular magnetizability and it does not enter the conventional spin-Hamiltonian.
2.2 NMR Parameters for Exact States

In section 2.1.2, we have identified the spin-Hamiltonian parameters as the rovibrationally averaged derivatives of the electronic energy. Thus, $\mu_M$ and $J_{MN}$ might seem straightforward to calculate using the relations (2.14) and (2.15), respectively. Nevertheless, this would necessitate the determination of the eigenvalues of the Schrödinger equation. Unfortunately, even the regular time-independent Schrödinger equation, where no magnetic operators appear, can be solved exactly only for some one-electron systems. However, the fact that magnetic effects are generally very small compared to the total molecular energy enables us to approach the problem in a different way, that is by employing the perturbation treatment.

Within the non-degenerate time-independent perturbation theory \[19\], the first- and second-order energy derivatives with respect to a perturbation $x$ are given by

$$\frac{\partial E(x)}{\partial x_i} = \langle 0 | \frac{\partial H}{\partial x_i} | 0 \rangle$$  \hspace{1cm} (2.19)

$$\frac{\partial^2 E(x)}{\partial x_i \partial x_j} = \langle 0 | \frac{\partial^2 H}{\partial x_i \partial x_j} | 0 \rangle + 2 \sum_{n \neq 0} \langle 0 | \frac{\partial H}{\partial x_i} | n \rangle \langle n | \frac{\partial H}{\partial x_j} | 0 \rangle \frac{E_n - E_0}{E_n - E_0}. \hspace{1cm} (2.20)$$

The first derivative or the first order property eq (2.19) is simply the expectation value of the first-order Hamiltonian (the Hellmann-Feynman theorem \[20\]) and requires only a knowledge of the unperturbed reference state $| 0 \rangle$. The second derivative or the second-order property eq (2.20) contains an expectation-value term analogous to the first-order properties but also a sum-over-states contribution from each excited state $| n \rangle$ of energy $E_n$. For magnetic properties, the expectation-value contribution to the second-order property is known as the diamagnetic part, the sum-over-states contribution is referred to as the paramagnetic part \[18\].

In order to obtain explicit expressions for the nuclear shielding and spin-spin coupling constants using the equations above (2.19, 2.20), we must consider the form of the electronic Hamiltonian in the presence of the magnetic field. That is, we must determine its dependence on the magnetic induction $B$ and on the nuclear magnetic moments $\mu_M$.

2.2.1 The Molecular Electronic Hamiltonian

In the presence of magnetic perturbations $B$ and $\mu_M$, the non-relativistic Hamiltonian in atomic units has the form

$$\hat{H}(B, \mu) = \frac{1}{2} \sum_i \pi_i^2 - \sum_i m_i \cdot B^{\text{tot}}(r_i) - \sum_{iM} \frac{Z_M}{r_{iM}} + \frac{1}{2} \sum_{i \neq j} \frac{1}{r_{ij}} +$$
\[ + \frac{1}{2} \sum_{M \neq N} \frac{Z_M Z_N}{R_{MN}} - \sum_M \mu_M \cdot B^{\text{tot}}(R_M) + \]
\[ + \sum_{N > M} \mu_M^T D_{MN} \mu_N \quad (2.21) \]

where \( r_{ij}, R_{MN} \) and \( r_{iM} \) is the distance between the electrons \( i \) and \( j \), the distance between the nuclei \( M \) and \( N \), and the distance between the electron \( i \) and nucleus \( M \), respectively. \( Z_A (Z_B) \) represents the atomic number of the nucleus \( A (B) \), \( \mu_i \) is the permanent magnetic moment

\[ \mu_i = -g \mu_B s_i = -s_i, \quad (2.22) \]

where \( s_i \) denotes the vector of the electron spin, \( \mu_B = \frac{e}{2m_e} \) is the Bohr magneton (\( \mu_B \) is equal to 1/2 in atomic units, \( g \) factor in eq (2.22) has been set equal to 2), and \( \pi_i \) stands for the operator of the kinetic momentum

\[ \pi_i = -i \nabla_i + A^{\text{tot}}(r_i). \quad (2.23) \]

The vector potential \( A^{\text{tot}}(r_i) \) at the position of an electron \( i \) is connected to the magnetic induction \( B^{\text{tot}}(r_i) \) according to the equation

\[ B^{\text{tot}}(r_i) = \nabla_i \times A^{\text{tot}}(r_i). \quad (2.24) \]

2.2.2 The First-Order (Paramag.) Interaction Terms

The first-order interaction terms contribute to the paramagnetic parts of the magnetic properties eq (2.20) and can be obtained by differentiating the Hamiltonian equation (2.21) firstly with respect to the magnetic induction \( B \) at zero field and zero magnetic moments, and secondly with respect to the magnetic moments at zero field and zero magnetic moments.

- The First-Order Interaction of the Electrons with the Magnetic Field

\[ \frac{\partial \hat{H}}{\partial B} = h_B^{\text{orb}} + h_B^{\text{spin}} \quad (2.25) \]

where

\[ h_B^{\text{orb}} = \frac{1}{2} \sum_i l_i \quad (2.26) \]

\[ h_B^{\text{spin}} = -\sum_i \mu_i = \sum_i s_i \quad (2.27) \]
The term $h_{orb}$ couples the external field to the orbital motion of the electron by means of the orbital angular-momentum operator

$$l_{iO} = -i \mathbf{r}_{iO} \times \nabla_i$$  \hspace{1cm} (2.28)

whereas the second term couples the field to the spin-angular momentum operator expressed by eq (2.22). Hence, the first-order coupling of the electrons with $\mathbf{B}$ depends on the orbital and spin-angular momentum of the electron and is known as the *electron Zeeman interaction*.

**The First-Order Interaction of the Electrons with the Nuclear Magnetic Moments**

$$\frac{\partial \hat{H}}{\partial \mu_M} = h_{M}^{\text{pso}} + h_{M}^{\text{sd}} + h_{M}^{\text{fc}}$$  \hspace{1cm} (2.29)

Hyperfine operators in eq (2.29) describe three distinct interactions: one involving the orbital motion of the electron and two involving the electron spin. The paramagnetic spin-orbit (PSO) operator or the orbital hyperfine operator

$$h_{M}^{\text{pso}} = \alpha^2 \sum_i \frac{l_{iM}}{r_{iM}^3}$$  \hspace{1cm} (2.30)

couples the nuclear magnetic moments to the orbital motion of the electrons whereas the spin-dipole (SD) operator

$$h_{M}^{\text{sd}} = \alpha^2 \sum_i \frac{r_{iM}^2 \mathbf{m}_i - 3(\mathbf{m}_i \cdot \mathbf{r}_{iM}) \mathbf{r}_{iM}}{r_{iM}^5}$$  \hspace{1cm} (2.31)

and the Fermi-contact (FC) operator

$$h_{M}^{\text{fc}} = -\frac{8\pi\alpha^2}{3} \sum_i \delta(\mathbf{r}_{iM}) \mathbf{m}_i$$  \hspace{1cm} (2.32)

couple the nuclear magnetic moments to the spin of the electron. The spin-dipole operator, multiplied by the nuclear magnetic moment, represents the direct interaction of the dipole moment of the electron with the source of the magnetic field. $\alpha$ is a dimensionless constant ($\alpha = \frac{e^2}{\hbar c} \approx 1/137$) and $\delta(\mathbf{r}_{iM})$ is the Dirac’s delta function whose presence in eq (2.32) causes that $h_{M}^{\text{fc}}$ makes a contribution only when the electron is at the nucleus. It must be emphasized that the FC interaction is usually the dominant mechanism for the coupling of nuclear spins.
CHAPTER 2. THEORY OF NMR PARAMETERS

2.2.3 The Second-Order (Diamag.) Interaction Terms

If we differentiate eq (2.21) twice with respect to the perturbations at zero field and zero magnetic moments, we arrive at the second order interaction terms contributing to the diamagnetic parts of the magnetic properties eq (2.20):

\[ \frac{\partial^2 H}{\partial B \partial \mu_M} = -1 + h^\text{dia}_{BM} \]

(2.33)

\[ \frac{\partial^2 H}{\partial \mu_M \partial \mu_N} = D_{MN} + h^\text{dso}_{MN}. \]

(2.34)

The diamagnetic electronic operators in eqs (2.33) and (2.34) are given by

\[ h^\text{dia}_{BM} = \frac{\alpha^2}{2} \sum_i \left( \frac{\mathbf{r}_i \cdot \mathbf{r}_M}{r^3_i M} \right) \]

(2.35)

\[ h^\text{dso}_{MN} = \frac{\alpha^4}{2} \sum_i \left( \frac{\mathbf{r}_M \cdot \mathbf{r}_N}{r^3_i M T^3_i N} \right). \]

(2.36)

The operator \( h^\text{dso}_{MN} \) is known as the diamagnetic spin-orbit (DSO) operator.

2.2.4 Ramsey’s Expressions for the Nuclear Shielding and Spin-spin Coupling Tensor

The final expressions for the nuclear shielding and spin-spin coupling tensors (Ramsey’s expressions [21, 22]) can be obtained by substituting the terms for the interaction operators considered in the preceding two sections into the general equation (2.20) for second order properties:

\[ \sigma_M = \frac{\partial^2 E}{\partial B \partial \mu_M} = \langle 0 | h^\text{dia}_{BM} | 0 \rangle - 2 \sum_{nS \neq 0} \frac{\langle 0 | h^\text{orb}_{BM} | nS \rangle \langle nS | (h^\text{pso}_{BM})^T | 0 \rangle}{E_{nS} - E_0} \]

(2.37)

\[ K_{MN} = \frac{\partial^2 E}{\partial \mu_M \partial \mu_N} = \langle 0 | h^\text{dso}_{MN} | 0 \rangle - 2 \sum_{nS \neq 0} \frac{\langle 0 | h^\text{pso}_{MN} | nS \rangle \langle nS | (h^\text{pso}_{MN})^T | 0 \rangle}{E_{nS} - E_0} - 2 \sum_{nS \neq 0} \frac{\langle 0 | h^\text{sd}_{M} + h^\text{fc}_{M} | n_T \rangle \langle n_T | (h^\text{sd}_{N})^T + (h^\text{fc}_{N})^T | 0 \rangle}{E_{n_T} - E_0}. \]

(2.38)

\( |n_S\) and \( |n_T\) denotes a singlet and a triplet excited state, respectively. Both expressions comprise a diamagnetic part corresponding to an expectation value of the unperturbed state, and a paramagnetic part, which represents the relaxation of the wave function in response to the external perturbations.
Chapter 3

Computational Methods

3.1 Methods for Solving the Gauge Problem

3.1.1 The Gauge-Origin Problem

In shielding tensor calculations there is a problem associated with the freedom in the choice of a gauge origin for the vector potential $A$ of eq (2.24). Theoretically, we may choose the gauge origin $O$ freely without results being influenced since the vector potential in the form

$$A_O(r) = \frac{1}{2} B \times r$$

as well as

$$A_O(r) = \frac{1}{2} B \times (r - O)$$

satisfies the requirement (Maxwell’s equation)

$$B = \nabla \times A_O(r).$$

An exact wave function will of course give origin-independent results, as will a HF wave function if a complete, i.e. infinite, basis set is used. However, in practice, a finite basis set must be employed. Therefore, for an approximate wave function the results do depend on the choice of the gauge origin. Whereas in the case of an exact wave function the sum of the diamagnetic and paramagnetic contributions remains constant for different gauge origins \[23\], the error due to the use of a finite basis set is larger for the paramagnetic part than for the diamagnetic part. The reason is that while $\sigma_{\text{dia}}$ depends on the ground state only, $\sigma_{\text{para}}$ depends on the excited state MO’s as well. This in turn leads to a dependence of the results on the choice of the gauge origin for the vector potential $A$, which is called the gauge-origin problem. For solving this problem the GIAO and IGLO methods are the ones most commonly used.
3.1.2 GIAO and IGLO

The GIAO method

In the GIAO method one evaluates matrix elements of the Hamiltonian in terms of a basis of field-dependent atomic orbitals (AO). The basis functions are made explicitly dependent on the magnetic field by inclusion of a complex phase factor referring to the position of the basis function (usually the nucleus). Such orbitals are known as the London Atomic Orbitals (LAO) or the Gauge Including Atomic Orbitals (GIAO). The gist is that matrix elements involving GIAOs only contain a difference in the vector potentials, thereby completely removing the reference to an absolute gauge origin.

The IGLO method

The Individual Gauge for Localized Orbitals method was the first to enable the systematic study of nuclear shieldings in larger systems using distributed gauge origins. The idea of the approach consists in evaluating the shielding tensor in terms of localized molecular orbitals (MO) whose individual gauge origins are chosen so that they minimize the absolute value of the paramagnetic contribution. In contrast to the London orbital approach where a phase factor is connected to each AO, in the IGLO method local phase factors are attached to the MO’s. The IGLO approach is computationally less demanding compared to the GIAO method since it makes possible the use of special approximations of derivative twoelectron integrals. These approximations are enabled by employing localized orbitals and should be kept in mind when choosing a proper basis set in an IGLO calculation.

3.2 Calculation of NMR Parameters within Kohn-Sham Density Functional Theory

3.2.1 Density Functional Theory

The Hohenberg-Kohn Theorems

The essence of the Density Functional Theory (DFT) consists in using electron density $\rho(r)$, instead of the number of electrons $N$ and the external potential $v(r)$, as a variable for determining all electronic properties of the system. This idea is a consequence of the first Hohenberg-Kohn
**Theorem:** The external potential $v(r)$ is determined, within a trivial additive constant, by the electron density $\rho(r)$. Since $\rho$ also determines the number of electrons, it in turn determines the ground-state wave function $\Psi$ and all other electronic properties of the system. Accordingly, the total electronic energy can be written as

$$E[\rho] = T[\rho] + V_{ne}[\rho] + V_{ee}[\rho] = \int \rho(r)v(r)dr + F_{HK}[\rho]. \quad (3.4)$$

Here, $T[\rho]$ denotes the kinetic energy, $V_{ne}$ and $V_{ee}$ the potential energy due to the nuclei-electron and electron-electron interaction, respectively, and $F_{HK}$ is given by

$$F_{HK}[\rho] = T[\rho] + V_{ee}[\rho]. \quad (3.5)$$

As seen from eq (3.5), $F_{HK}$ is defined independently of the external potential $v(r)$, which implies that $F_{HK}$ is a universal functional of $\rho(r)$. We may write $V_{ee}$ as a sum of two contributions

$$V_{ee}[\rho] = J[\rho] + \text{nonclassical term}, \quad (3.6)$$

where

$$J[\rho] = \frac{1}{2} \int \int \frac{1}{|r - r'|} \rho(r)\rho(r')drdr'. \quad (3.7)$$

is the classical self-repulsion energy of an electron distribution $\rho(r)$, i.e. the Coulomb energy.

The second Hohenberg-Kohn theorem states: For a trial density $\tilde{\rho}(r)$, such that $\tilde{\rho}(r) \geq 0$ and $\int \tilde{\rho}(r)dr = N$, the energy $E[\tilde{\rho}(r)]$ is always an upper bound to the exact energy $E_0$. This is of course nothing else than the variational principle that in the present context can be expressed as

$$E_0 \leq E[\tilde{\rho}(r)] = T[\tilde{\rho}] + V_{ne}[\tilde{\rho}] + V_{ee}[\tilde{\rho}]. \quad (3.8)$$

The variational principle requires that the ground state density satisfies the stationary principle

$$\delta \left\{ E[\rho] - \mu \left[ \int \rho(r)dr - N \right] \right\} = 0, \quad (3.9)$$

which leads to the Euler-Lagrange equation, the basic working equation of the density functional theory:

$$\mu = \frac{\delta E[\rho]}{\delta \rho(r)} = v(r) + \frac{\delta F_{HK}[\rho]}{\delta \rho(r)}. \quad (3.10)$$

The Lagrange multiplier $\mu$ represents a quantity denoted as the chemical potential. If we knew the exact $F_{HK}$, eq (3.10) could be used as an exact
equation for the ground state electron density. However, the explicit form of \( F_{HK} \) is hard to achieve and its approximate forms are needed. A direct approach, whereby one constructs explicit forms for \( T[\rho] \) and \( V_{ee}[\rho] \), is complicated since it is difficult to get beyond a crude level of approximation of \( T[\rho] \).

An ingenious indirect approach has been suggested within the *Kohn-Sham* (KS) method.

### The Kohn-Sham Method

The Kohn-Sham method introduces a (hypothetical) noninteracting reference system with exactly the same electron density as that of the real system. The exact wave function of the noninteracting system is given by a Slater determinant constituted from \( N \) spin-orbitals (Kohn-Sham orbitals) \( \psi_i(x) \). For such a system, the exact kinetic energy becomes

\[
T_s = \sum_i \langle \psi_i | -\frac{1}{2} \nabla^2 | \psi_i \rangle. \tag{3.11}
\]

Employing the first Hohenberg-Kohn theorem, this exact kinetic energy can be viewed as a functional of the charge density

\[
\rho(r) = \sum |\psi_i(x)|^2. \tag{3.12}
\]

Naturally, \( T_s[\rho] \) is not equal to the true kinetic energy of the interacting system \( T[\rho] \). Kohn and Sham faced this problem by considering \( T_s[\rho] \) as a kinetic energy component of \( T[\rho] \). In this case, eq (3.5) has a form

\[
F_{HK}[\rho] = T_s[\rho] + J[\rho] + E_{xc}[\rho] \tag{3.13}
\]

where

\[
E_{xc}[\rho] \equiv T[\rho] - T_s[\rho] + V_{ee}[\rho] - J[\rho]. \tag{3.14}
\]

The quantity \( E_{xc}[\rho] \) is called the *exchange-correlation energy*. Now, the Euler-Lagrange equation (3.10) associated with the stationarity of \( E[\rho] \) can be rewritten as

\[
\mu = v_{\text{eff}}(r) + \frac{\delta T_s[\rho]}{\delta \rho(r)} \tag{3.15}
\]

where the Kohn-Sham (KS) effective potential is given by

\[
v_{\text{eff}}(r) = v(r) + \frac{\delta J[\rho]}{\delta \rho(r)} + \frac{\delta E_{xc}[\rho]}{\delta \rho(r)} = v(r) + \int \frac{\rho(r')}{|r - r'|} dr' + v_{xc}(r) \tag{3.16}
\]

with the *exchange-correlation potential*

\[
v_{xc}(r) = \frac{\delta E_{xc}[\rho]}{\delta \rho(r)}. \tag{3.17}
\]
The Kohn-Sham treatment runs as follows. In conventional DFT, eq (3.15) is exactly valid for a system of noninteracting electrons moving in the external potential $v_s(r) = v_{\text{eff}}(r)$. Therefore, for a given $v_{\text{eff}}(r)$, one obtains $\rho(r)$ that satisfies (3.15) simply by solving the $N$ one-electron equations

$$\left[-\frac{1}{2}\nabla^2 + v_{\text{eff}}(r)\right] \psi_i = \epsilon_i \psi_i$$  (3.18)

and setting

$$\rho(r) = \sum |\psi_i(x)|^2 .$$  (3.19)

Equations (3.16)–(3.19) are the so called Kohn-Sham equations. Here, $v_{\text{eff}}$ depends on $\rho(r)$ through eq (3.17). Thus, (3.16), (3.18) and (3.19) must be solved self-consistently, like the Hartree-Fock equations. The calculation begins with a guessed $\rho(r)$, follows with constructing $v_{\text{eff}}(r)$ according to (3.16), and then a new $\rho(r)$ is found using (3.18) and (3.19). The total Kohn-Sham energy can be computed as

$$E = \sum_{i}^{N} \epsilon_i - \frac{1}{2} \int \frac{\rho(r)\rho(r')}{|r-r'|} d\mathbf{r} d\mathbf{r'} + E_{\text{xc}}[\rho] - \int v_{\text{xc}}(r) \rho(r) d\mathbf{r}$$  (3.20)

with

$$\sum_{i}^{N} \epsilon_i = \sum_{i}^{N} \langle \psi_i | -\frac{1}{2}\nabla^2 + v_{\text{eff}}(r) | \psi_i \rangle = T_s[\rho] + \int v_{\text{eff}}(r) \rho(r) d\mathbf{r} .$$  (3.21)

If $E_{\text{xc}}$ is ignored, the physical content of the theory becomes identical to that of the Hartree-Fock approximation. The Kohn-Sham equations differ from the Hartree-Fock equations only by the inclusion of the exchange-correlation potential $v_{\text{xc}}$. Whereas in HF theory, electron correlation effects are by definition neglected, the more general local potential $v_{\text{xc}}$ in the KS equations assures that the Kohn-Sham theory is in principle exact. This is owing to the fact that KS theory fully incorporates the exchange-correlation effects, provided that the exact $E_{\text{xc}}$ is used in (3.17). In other words, the only error in the theory is due to approximations of $E_{\text{xc}}$.

**Exchange-Correlation Functionals**

As mentioned above, the explicit form of the exchange-correlation functional $E_{\text{xc}}$ is not known and therefore the quality of the description hinges solely on the accuracy of the approximation to $E_{\text{xc}}$. Hence, the quest for better and better functionals is at the very heart of DFT but unfortunately, there is no systematic way of improving exchange-correlation functionals. Various approximations used for $E_{\text{xc}}$ are given below.
1. The Local Density Approximation (LDA)

This model approximates \( E_{xc} \) on the basis of the idea of a uniform electron gas. \( E_{xc} \) is then written as

\[
E_{xc}^{LDA}[\rho] = \int \rho(\mathbf{r}) \epsilon_{xc}[\rho] d\mathbf{r}. \tag{3.22}
\]

Here, \( \epsilon_{xc}[\rho] \) is the exchange-correlation energy per particle of a uniform electron gas of density \( \rho(\mathbf{r}) \). The \( E_{xc}^{LDA}[\rho] \) can be formally split into exchange and correlation contributions

\[
E_{xc}^{LDA} = E_x^{LDA} + E_c^{LDA}. \tag{3.23}
\]

The exchange part of LDA functional is given by \( E_x^{LDA} \)

\[
E_x^{LDA} = -\frac{3}{4} \left( \frac{3}{\pi} \right)^{1/3} \int \rho^{4/3}(\mathbf{r}) d\mathbf{r}. \tag{3.24}
\]

\( E_x^{LDA} \) frequently called the Slater exchange and is abbreviated by S. No such explicit form is known for the correlation part \( E_c^{LDA} \). However, various analytical expressions were derived on the basis of quantum Monte-Carlo simulations of the homogeneous electron gas \( \text{[34]} \), the most widely used one being due to Vosko, Wilk and Nusair (VWN) \( \text{[35]} \). We usually use acronym SVWN to identify the corresponding exchange-correlation functional.

If we extend LDA to the unrestricted case, we arrive at the Local Spin Density Approximation (LSD). Here, not the electron density \( \rho(\mathbf{r}) \), but the two spin densities, \( \rho_\alpha(\mathbf{r}) \) and \( \rho_\beta(\mathbf{r}) \), with \( \rho_\alpha + \rho_\beta = \rho \), are employed

\[
E_{xc}^{LSD}[\rho_\alpha, \rho_\beta] = \int \rho(\mathbf{r}) \epsilon_{xc}[\rho_\alpha(\mathbf{r}), \rho_\beta(\mathbf{r})] d\mathbf{r}. \tag{3.25}
\]

2. The Generalized Gradient Approximations (GGA)

In contrast to LDA, the generalized gradient approximations for \( E_{xc} \) are not only functions of the local density \( \rho(\mathbf{r}) \) but also functions of the gradient of the charge density \( \nabla \rho(\mathbf{r}) \). They can be generically written as

\[
E_{xc}^{GGA}[\rho_\alpha, \rho_\beta] = \int f(\rho_\alpha, \rho_\beta, \nabla \rho_\alpha, \nabla \rho_\beta) d\mathbf{r}. \tag{3.26}
\]

Here, \( \alpha \) and \( \beta \) refers to "up" and "down" spin, respectively. Again, in practice, \( E_{xc}^{GGA} \) is usually split into its exchange and correlation contributions

\[
E_{xc}^{GGA} = E_x^{GGA} + E_c^{GGA} \tag{3.27}
\]

and approximations for these two terms are sought individually.
The best known representatives of GGA exchange functionals are functionals by Becke, 1988 (B or B88) [36], and by Perdew and Wang, 1986 (P or PW86) [37]. Among the most widely used choices of GGA correlation functional belongs the one by Perdew, 1986 (P or P86) [38]. It is the correlation counterpart of the Perdew and Wang’s exchange functional mentioned above, and in combination with the latter it creates $E_{xc}$ abbreviated as PP[^1]. Perdew and Wang also developed the PW91 exchange-correlation functional [39] consisting of an exchange and correlation component, both denoted PW91 if used separately. The exchange part of PW91 is similar to B88 and the correlation part is a modified version of P86. Nowadays, probably the most popular correlation functional is due to Lee, Yang and Parr, 1988 (LYP) [40]. It differs from the other GGA functionals in that it contains some local components.

3. The Hybrid Methods
The so-called hybrid functionals are exchange-correlation functionals where the exchange part is composed of exact Hartree-Fock exchange and pure density functionals for exchange. In other words, these functionals include a mixture of Hartree-Fock exchange with DFT exchange and correlation:

$$E_{xc}^{\text{hybrid}} = c_{HF}E_{x}^{HF} + c_{DFT}E_{x}^{DFT}$$

Becke’s three-parameter hybrid functional (B3) [41] is an example of such hybrid models. Using B3, the exchange energy is calculated [26] as a suitable combination of local $E_{x}^{LSD}$, exact Hartree-Fock $E_{x}^{HF}$ and gradient correction $E_{x}^{B88}$ term for exchange energy. The correlation energy may similarly be taken as the local correlation energy $E_{c}^{LSD}$ plus gradient correction term for the correlation energy $E_{c}^{GGA}$:

$$E_{xc}^{B3} = (1 - a)E_{x}^{LSD} + aE_{x}^{HF} + bE_{x}^{B88} + E_{c}^{LSD} + cE_{c}^{GGA}$$

The $a$, $b$ and $c$ parameters are determined by fitting to experimental data and depend on the form chosen for $E_{c}^{GGA}$. If we adopt correlation functional LYP or PW91 for $E_{c}^{GGA}$, we obtain B3LYP or B3PW91, respectively. The former is the exchange-correlation functional most widely used nowadays and it was also intensively employed throughout this thesis.

[^1]this exchange-correlation functional has been used in this thesis for the calculation of $^{31}$P chemical shieldings and heteronuclear $J_{PX}$ spin-spin coupling constants in the deMon program.
3.2.2 FPT and Coupled DFT approaches

We have outlined in section (2.1.2) that second-order properties, and consequently NMR parameters, can be expressed as a mixed second derivative of the total electronic energy with respect to two perturbations

\[
\sigma_{M,uv} = \left. \frac{\partial^2 E(\mu_M, B)}{\partial \mu_M, u \partial B_v} \right|_{\mu_M=0, B=0} \tag{3.30}
\]

\[
J_{MN,uv} = \left. \frac{\partial^2 E(\mu_M, \mu_N)}{\partial \mu_M, u \partial \mu_N, v} \right|_{\mu_M=0, \mu_N=0} \tag{3.31}
\]

where \( \sigma_{M,uv} \) and \( J_{MN,uv} \) are elements of the nuclear shielding and spin-spin coupling tensor, respectively. However, in section (2.2) we have explained why it is advantageous to calculate NMR parameters by employing the perturbation theory rather than using equations (3.30), (3.31). Diamagnetic contributions to a second-order property are straightforward to compute using DFT because they depend only on the ground state wave function. However, paramagnetic contributions are the ones most important to compute accurately (they are much more sensitive to the electronic and geometrical structure). These contributions depend on the linear response of the many-electron wave function to an external perturbation. To evaluate this response the coupled Hartree-Fock (CHF), the coupled DFT (CDFT) (the so called response theory), the Finite Perturbation Theory, or the Sum-Over-States Density Functional Perturbation Theory (SOS-DFPT) are usually used.

In DFT-based approaches the paramagnetic term is calculated by solving the Kohn-Sham (KS) orbital equations for a system in the presence of a perturbation \( \lambda \) (cf. eq (3.18))

\[
\left[ -\frac{1}{2} \nabla^2 + \lambda \hat{H}_u^1 + v_{\text{eff}}(\lambda) \right] \varphi_k(\lambda) = \epsilon_k(\lambda) \varphi_k(\lambda) \tag{3.32}
\]

where \( \hat{H}_u^1 \) is the operator connected with the particular type of perturbation, and \( v_{\text{eff}} \) is the effective KS potential.

In Finite Perturbation Theory, eq (3.32) is solved in the same self-consistent way as the unperturbed KS equation. The additional matrix elements corresponding to the perturbation operator \( \lambda \hat{H}_u^1 \) are added to the Fock matrix. An appropriate value of the parameter \( \lambda \) must be chosen in order to correctly estimate the linear response of the KS density matrix.

Alternatively, we can solve eq (3.32) by expanding all terms in powers of the parameter \( \lambda \) up to first-order, and exclude the zero-order terms since these
satisfy the unperturbed equations. Then, the parameter $\lambda$ can be eliminated from the resulting equations, owing to the linear dependence on $\lambda$

$$\left[ -\frac{1}{2}\nabla^2 + v_{\text{eff}}^0 \right] \varphi_k^1 + \left[ \tilde{H}^1 + v_{\text{eff}}^1 \right] \varphi_k^0 = \epsilon_k^0 \varphi_k^1. \quad (3.33)$$

These so called Coupled Perturbed Kohn-Sham Equations (CPKS) are the basis of the response theory or coupled DFT (CDFT) \[42, 43\]. An analogous method based on the Hartree-Fock approximation is called the coupled Hartree-Fock (CHF) approach \[19\]. The perturbed molecular orbitals are coupled with each other by $v_{\text{eff}}^1$, which implies the equations must be solved self-consistently as in the FPT approach.

For a case of a purely imaginary perturbation, i.e. the magnetic field, the situation is slightly more complicated. As the electron density $\rho$ has no linear term in the expansion in powers of a purely imaginary perturbation, the exchange-correlation potentials $v_{\text{xc}}(\rho, \nabla \rho)$ commonly used in DFT also have no linear response. Consequently, $v_{\text{eff}}^1$ disappears from eq (3.33) and one ends up with the so called uncoupled DFT (UDFT) equations \[44, 45\]. The loss of $v_{\text{eff}}^1$ is a consequence of the fact that the commonly used exchange-correlation functionals depend only on $\rho$ and $\nabla \rho$ and therefore they are proper approximations solely for a system in the absence of the magnetic field. However, in the presence of the magnetic field, the usual Hohenberg-Kohn theorems \[30\] do not hold any more. The corresponding exchange-correlation functional not only has to depend on the electron density $\rho(\mathbf{r})$ but also on the current density $j(\mathbf{r})$ induced by the magnetic field. This is a basis of the Current Density Functional Theory \[46\] which is, however, still in the early development phase. An approximation that for most purposes the dependence on the current density can be neglected is generally accepted.

Within FPT as well as CDFT eqs (3.32) and (3.33) must be solved self-consistently four times (for the unperturbed system and for the three components of the magnetic field). In order to simplify practical calculations, an alternative approach, the Sum-Over-States Density Functional Perturbation Theory (SOS-DFPT), has been developed \[47\]. SOS is not a self-consistent variational perturbation theory. It is based on a simple approximation of the sum-over-states contribution from the equation (2.20) and it is aimed at an implicit introduction of the current dependence.

### 3.2.3 Sum-Over States Density Functional Perturbation Theory (SOS-DFPT)

Within the SOS perturbation theory, the perturbed many-electron wave function of the ground state can be expanded with respect to the unperturbed
wave function

\[
\Psi_0(B_u) = \Psi^0_0 + i B_u \sum_{k \rightarrow a} \frac{\langle \Psi^0_0 | \hat{H}^1(B_u) | \Psi^0_{k \rightarrow a} \rangle}{E^0_0 - E^0_{k \rightarrow a}} \Psi^0_{k \rightarrow a} + \cdots \tag{3.34}
\]

where \( \hat{H}^1(B_u) \) is a perturbation operator, \( u = \{x,y,z\} \) and the superscripts denote the order of perturbation. \( \Psi^0_0 \) is the wave function of the ground state, \( \Psi^0_{k \rightarrow a} \) represents the wave function of the excited state corresponding to the transition of an electron from the occupied MO ”\( k \)” into the virtual MO ”\( a \)”. \( E^0_0 \) and \( E^0_{k \rightarrow a} \) are the energies of the ground state and excited state, respectively. After approximating the many-electron wave functions by appropriate Slater determinants built from occupied KS MO’s and keeping in eq (3.34) only terms that are linear with respect to the external magnetic field, eq (3.34) can be rewritten in the form

\[
\Psi_0(B_u) = \Psi^0_0 + i B_u \sum_{k \rightarrow a} \beta_{ak}(u) \Psi^0_{k \rightarrow a} + \cdots \tag{3.35}
\]

The coefficients \( \beta_{ak} \) are given by the expression

\[
\beta_{ak}(u) = -\frac{(1/2c)\langle a | l_{ku} | k \rangle}{e_k - e_a - \Delta E^{xc}_{k \rightarrow a}} \tag{3.36}
\]

with

\[
l_{ku} = \{(r - R_k) \times \nabla\}_u \tag{3.37}
\]

where \( R_k \) is the gauge origin for MO ”\( k \)”, \( c \) is the velocity of light, \( e_a \) and \( e_k \) are the energies of MO’s ”\( a \)” and ”\( k \)”, respectively. \( \Delta E^{xc}_{k \rightarrow a} \) is a correction term that describes the difference in the exchange-correlation energy between the ground state \( E_0 \) and a singlet excited state corresponding to a ”\( k \rightarrow a \)” transition. Several different approximations for \( \Delta E^{xc}_{k \rightarrow a} \) were derived \[47\] and of them the Loc.1 approximation

\[
\Delta E^{xc}_{k \rightarrow a} = \frac{1}{3} C_x \int \rho^\uparrow(r)^{-2/3} \rho_k(r) \rho_a(r) d\mathbf{r} \tag{3.38}
\]

appears to be most suitable. In the equation \(3.38\), \( \rho^\uparrow \) is the overall electron density in occupied up-spin molecular orbitals, \( \rho_k \) and \( \rho_a \) stands for the electron density in molecular orbitals ”\( k \)” and ”\( a \)”, respectively, when \( \psi_k \) is chosen to be an up-spin orbital. The value of \( C_x \) is given by

\[
C_x = \left( \frac{3}{2} \right) ^{1/3} \left( \frac{3}{4\pi} \right) ^{1/3} \tag{3.39}
\]

The ”Malkin correction” \( \Delta E^{xc}_{k \rightarrow a} \) is viewed as an a posteriori attempt to model the current dependence of \( E^{xc} \) or a correction for the deficiencies in the orbital energy denominators.
Chapter 4

Carbon and Nitrogen Chemical Shifts in B-DNA

4.1 Studied Systems

This part of the thesis focuses on studying $^{13}$C and $^{15}$N chemical shifts in deoxyribonucleosides (2’-deoxyadenosine, 2’-deoxyguanosine, 2’-deoxycytidine, 2’-deoxythymidine; see Fig. 4.1) as a function of the following structural parameters:

- the orientation of the base about the glycosidic bond
- the sugar ring conformation, the so called sugar pucker
- the CH$_2$OH group conformation
- the hydrogen bonding

Changes in chemical shifts caused by the rotation about the glycosidic bond have already been studied [2] but we wished to involve the relaxation of the geometry after changing $\chi$, which has not been considered in the previous work. The results obtained have been compared to available experimental data from the BMRB database [48] and data for the [d(G$_4$T$_4$G$_4$)]$_2$ quadruplex [49, 50]. This comparison showed the necessity of considering other geometrical features which could be responsible for the observed experimental trends. Whereas the effect of the rotation about the glycosidic bond has been explored for all of the four deoxyribonucleosides, the influence of the other three structural parameters has been investigated only for deoxyguanosine. The impact of the hydrogen bonding has been studied on models simulating the presence of hydrogen bonds as found in the guanine-quartet (Fig. 4.10) of the [d(G$_4$T$_4$G$_4$)]$_2$ quadruplex (Fig. 4.11).
CHAPTER 4. $^{13}$C AND $^{15}$N CHEMICAL SHIFTS IN B-DNA

4.2 Computational Details

4.2.1 Geometry Optimizations

All geometries of deoxyribonucleosides have been optimized using the Gaussian 98 suite of programs [51] at the DFT level of theory. The B3LYP functional [36, 52] along with the 6-31G(d) basis set [53, 54] has been employed. For purposes of studying chemical shifts as a function of the structural features mentioned in the preceding section, geometry optimizations have been approached in a different way.

In the case of exploring the influence of the base orientation for a model close to real B-DNA structure, all backbone torsion angles have been constrained to their average experimental values [55] as found in B$_1$-DNA: $\beta = 176^\circ$, $\gamma = 48^\circ$, $\delta = 128^\circ$, $\epsilon = 184^\circ$. The glycosidic torsion angle $\chi$ has been varied within the range ($-180^\circ$...$0^\circ$...$180^\circ$) (Fig. 4.2), freezing its value in $15^\circ$ increments.

$^1\beta, \gamma, \delta$ and $\epsilon$ refer to the P–O5'–C5'–C4', O5'–C5'–C4'–C3', C5'–C4'–C3'–O3', and C4'–C3'–O3'–P torsion angles, respectively [56].

Figure 4.1: Studied nucleosides.
Besides $\beta$, $\gamma$, $\delta$, $\epsilon$ and $\chi$, all other structural parameters have been relaxed after changing $\chi$. The *south* (S) conformation of the sugar uniformly adopted for all starting geometries has not changed significantly after the optimization. The use of the constraints for the sugar-phosphate backbone torsion angles can be justified as follows:

- these constraints allowed to keep the number and type of interactions while varying $\chi$ as constant as possible [6]
- changes in backbone torsion angles, especially $\delta$, cause changes in the sugar pucker [56], and we wished to model the influence of $\chi$ separately from the influence of the sugar ring conformation
- freezing the torsion angle $\beta$ to 176° excluded the possibility of creating an artificial hydrogen bond between H(O5’) of the sugar and O2 or N3 of the base for conformers with $\chi \sim 60^\circ$
- the constant value of $\gamma$ enabled us to separate the effect of $\chi$ and of the hydroxymethyl conformation on calculated chemical shifts

Chemical shifts as influenced by the *sugar pucker* have been investigated using structures optimized with the $\delta$ and $\epsilon$ torsions relaxed but $\beta$ and $\gamma$ torsions constrained. This strategy has been chosen since the values of $\delta$ and $\epsilon$ in S and N conformers are significantly different. Accordingly, after changing the sugar conformation from S to N, the $\delta$ and $\epsilon$ dihedral angles had to be relaxed so that they could correspond with the modified sugar pucker.
Upon the optimization procedure, sugar ring conformations remained within the north and south range of the pseudorotation cycle \[56\], respectively. The N vs. S comparison has been performed for both the syn (\(\chi = 60^\circ\)) and anti (\(\chi = -120^\circ\)) orientation of the base (with \(\chi\) frozen to the accordant values).

For the studies of chemical shifts under the influence of hydroxymethyl-group conformation, geometry optimizations have been conducted with all backbone torsion angles except for \(\beta\) relaxed and with the sugar conformation assigned to the south region. The torsion angle \(\gamma\) (see Fig. 4.3), determining the CH\(_2\)OH group conformation, has been adjusted to its approximate values corresponding to the \(gg\) (\(\gamma = 48^\circ\)), \(gt\) (\(\gamma = 180^\circ\)) and \(tg\) (\(\gamma = -60^\circ\)) rotamers \[56\]. The comparison of chemical shifts for the three conformers has been done for the syn as well as anti base position (\(\chi\) fixed to 60° and to -120°, respectively).

Models for studying the effect of the hydrogen bonding were constructed so that they simulated the molecular arrangement of the G-quartet (Fig. 4.10) in the \([\text{d(G}4\text{T}4\text{G}4])_2\) quadruplex. That is, guanine and/or its various fragments were added to the optimized structures of the deoxyguanosine (S, syn, \(gg\)) and deoxyguanosine (S, anti, \(gg\)). All backbone torsion angles and \(\chi\) were constrained during the optimization. The distance between the particular fragment and deoxyguanosine was adjusted to the lengths of the hydrogen bonds found in the experimental structure of the quadruplex. Guanine as well as all the fragments were placed to the plane of the base in deoxyguanosine. The geometries obtained in this way were used without
further optimization. Either two or all of the four hydrogen bonds found in the G-quartet have been involved in the models studied, which allowed to investigate the influence of hydrogen bonding at various levels of complexity. All of the models are summarized in Fig. 4.11.

4.2.2 Calculations of Chemical Shifts

Isotropic $^{13}$C and $^{15}$N chemical shifts have been calculated using the DFT-GIAO $ab$ initio method as implemented in Gaussian 98. Becke’s three-parameter hybrid functional employing the Lee, Yang, and Parr GGA for correlation (B3LYP), along with the 6-311+G(2d,p) basis set, has been uniformly adopted in all cases. The standard relationship $\delta_{\text{iso}} = \sigma_{\text{standard}} - \sigma_{\text{iso}}$ has been used for referencing. Carbon chemical shifts have been obtained relative to the calculated shielding constant of TMS ($\sigma = 182.59$ ppm, B3LYP/6-311+G(2d,p)//B3LYP/6-31G(d); TMS optimized in $T_d$ symmetry) whereas nitrogen chemical shifts have been referenced to the absolute shielding constant of NH$_3$ ($\sigma = 244.6$ ppm) [57].

4.3 Results and discussion

4.3.1 Base orientation

In agreement with the results of Xu and Au-Yeung [2], C1’, C2’ and N1/N9 atoms appeared to be most sensitive to the position of the base relative to the sugar ring. This feature is understandable considering the fact that the atoms are involved in the glycosidic bond or they are very close (Fig. 4.2). Besides C1’, C2’ and N1/N9 chemical shifts, the results obtained for N7 (Fig 4.7) will be discussed below in order to show the difference between $\delta_{\text{N9}}$ and $\delta_{\text{N7}}$ in deoxyguanosine in regard to the correlation with experimental data (cf. section 4.3.5). In addition, observed features of N3 chemical shifts as modulated by $\chi$ will be demonstrated for purine (Fig. 4.8) as well as for pyrimidine (Fig. 4.9) nucleosides. Possible explanations for the presented trends will be outlined.

The trends uncovered in chemical shifts of the particular atoms are mostly significantly different from those reported previously [2], which is naturally caused by the different approach to the geometry optimizations (cf. section 4.1). Solely C1’ chemical shift revealed more or less the same tendencies no matter whether the relaxation of the geometry has or has not been performed.

In the case of all the three atoms (C1’, C2’, N1/N9), the behaviour of the
chemical shifts as a function of $\chi$ appeared to be similar for the two purine and the two pyrimidine nucleosides (cf. Fig. 4.4–4.6). Moreover, the trends for the C2’ atom have been found to be close for all studied models in the syn region but different in the anti region (cf. Fig. 4.5). In contrast, the most pronounced difference in C1’ chemical shift between A and G on one hand, and C and T on the other hand, has been observed within the syn range of $\chi$ (cf. Fig. 4.4). Finally, the most striking fact about absolute values of $\delta_{N9}$ is the 10 ppm upfield shift for C compared to T (cf. Fig. 4.6). This phenomenon can be ascribed to the different substitution at C4 of the two bases, i.e., to electron-withdrawing effect of O4 in T which is naturally stronger than the effect of NH$_2$ in C. The structural distinction between C and T has also recently appeared to give rise to higher values of $J_{C2-H1'}$ in deoxycytidine in contrast to those in deoxythymidine and deoxyuridine (U) [12]. The methyl group present in T but missing in C seems to have a negligible influence on the chemical shielding of the studied atoms as is obvious, e.g., from the comparison of N1 chemical shift in deoxycytidine with that in deoxyuridine. If we simply replace the CH3 group in the optimized structure of T by a hydrogen (i.e. we construct U), we arrive at a value of $\delta_{N1}$ larger by not more than 1 ppm.
Generally, chemical shifts are a result of a complex interplay of various geometrical features, which implies serious difficulties in seeking a detailed insight into structure-chemical shift relationships. Therefore, an exact analysis of the particular contributions to the chemical shielding was outside the scope of this thesis. However, some possible explanations for the results obtained are still suggested. Fig. 4.4–4.9 demonstrate that maximal/minimal values of the chemical shift are usually found for $\chi$ close to $0^\circ$, $60^\circ$ and $-120^\circ$. For $\chi \sim 60^\circ$, especially the through-space polarization interaction between the base and the sugar ring is at work. Extremes of the chemical shift in the syn as well as anti region also arise from the fact that for such base positions, the lone pair at N1/9 is orientated perpendicular to the plane defined by H1’, C1’ and N1/9. As a result, this lone pair is as close as possible to the lone pair at O4’.

Now, let’s consider the situation of (approximately) zero glycosidic torsion angle. Obviously, in such a base position, the interaction of the O4’ lone pairs with those at N3(pu) or O2(py) is maximized, which necessarily has an impact on calculated chemical shifts. The effect of the electron clouds overlap is most explicitly revealed in results for N3 in purine nucleosides (cf. Fig. 4.8). For $\chi \sim 0^\circ$, N3 and O4’ are as close as possible and the N3 chemical shift
reaches its largest values. However, with the increasing $r_{O4',N3}$ distance, i.e., with $\chi$ departing from zero, calculated chemical shifts continuously diminish. Similar behaviour has been disclosed for N3 in pyrimidine nucleosides (cf. Fig. 4.9) in the range of $\chi$ between $-60^\circ$ and $60^\circ$. In this case, however, the modulations in N3 chemical shift result from the interaction with O4’ mediated by O2.
Figure 4.7: N7 chemical shift plotted vs. $\chi$. 
Figure 4.8: N3 chemical shift plotted vs. $\chi$ for A and G.
Figure 4.9: N3 chemical shift plotted vs. $\chi$ for C and T.
CHAPTER 4. $^{13}$C AND $^{15}$N CHEMICAL SHIFTS IN B-DNA

4.3.2 Sugar Pucker

In order to assess the effect of the sugar pucker, chemical shifts for deoxyguanosine (S, syn, $gg$) vs. deoxyguanosine (N, syn, $gg$) and deoxyguanosine (S, anti, $gg$) vs. deoxyguanosine (N, anti, $gg$) have been compared. The highest sensitivity of the base atoms to S→N repuckering has been encountered in the case of N9 (cf. Table 4.1). For this atom, the change of the sugar conformation from S to N resulted in 10.5 and 7.8 ppm upfield shift in the syn and anti regions, respectively. C1’ and C2’ chemical shifts experienced much smaller changes, the S vs. N difference of the former reaching approximately the same value (a bit larger than 3 ppm) for the both base orientations. Interestingly, the effect of opposite sign has been found for C2’ in the syn, as distinct from anti, position of the base (-2 ppm compared to 1.3 ppm). Except for N9, base atoms appeared to be more or less independent of the sugar pucker, especially in the case of $\chi = -120^\circ$. In the other region of $\chi$, the S-N difference of the most affected atoms reaches values from ca. 1 to 2 ppm, the largest one being that of N3, namely 1.8 ppm.

<table>
<thead>
<tr>
<th>atom</th>
<th>Chemical shift/ppm</th>
<th>Chemical shift/ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>syn</td>
<td>N</td>
</tr>
<tr>
<td>C1’</td>
<td>92.08</td>
<td>88.63</td>
</tr>
<tr>
<td>C2’</td>
<td>44.80</td>
<td>46.80</td>
</tr>
<tr>
<td>N9</td>
<td>181.56</td>
<td>192.10</td>
</tr>
<tr>
<td>C8</td>
<td>142.67</td>
<td>141.05</td>
</tr>
<tr>
<td>N7</td>
<td>276.31</td>
<td>275.19</td>
</tr>
<tr>
<td>C4</td>
<td>157.11</td>
<td>155.70</td>
</tr>
<tr>
<td>N3</td>
<td>195.21</td>
<td>193.41</td>
</tr>
</tbody>
</table>

Table 4.1: The comparison of $^{13}$C and $^{15}$N chemical shifts for the S (south) and N (north) conformation of the sugar ring in deoxyguanosine ($\Delta_{S-N} = \delta_S - \delta_N$).

4.3.3 Hydroxymethyl Group Conformation

The influence of the CH$_2$OH group conformation has been examined by comparing chemical shifts for the $gg$ ($\gamma \sim 60^\circ$), $gt$ ($\gamma \sim 180^\circ$) and $tg$ ($\gamma \sim -60^\circ$) rotamers (Fig. 4.3) of deoxyguanosine with the syn as well as anti base orientation, and with the S type of the pucker. All sugar ring and base atoms revealed small sensitivity to the hydroxymethyl group rotation (cf. Table 4.2).
except for two cases. Firstly, N9 chemical shift in deoxyadenosine (S, anti, gg), which shows significantly larger value (by ca. 7 ppm) than all the other rotamers with either of the two base positions. Secondly, the rotation about the C4′-C5′ bond causes no change in N3 chemical shift in the anti region, contrary to syn, where δN3 of the gg conformer is shifted upfield by 5 ppm relative to gt and tg. Strikingly distinct values exclusively obtained for the gg-syn combination are indicative of a direct, through-space, interaction between the base and the CH2OH group. Since γ, δ and ϵ torsion angles have been relaxed during the optimization (cf. section 4.2.1), the change of the hydroxymethyl group conformation is naturally associated with the change of the sugar pucker. Therefore, the modifications in chemical shifts are also due to a mediated, indirect, interaction.

### 4.3.4 Hydrogen Bonding

As mentioned in section 4.3.2, a significant difference in N9 chemical shift has been found between the syn and anti orientations of the base in deoxyadenosine (S, syn). Such a difference has not been observed in experimental data for the [d(G4T4G4)]2 quadruplex. Hence, the identification of the factor that gives rise to this discrepancy was in high demand. Since the geometries used for calculations up to this stage of investigation did not include hydrogen bonds, the consideration of hydrogen bonding seemed to be a reasonable step to approach the reality of the experimental structure even more.

Therefore, several models simulating the presence of two or all of the four hydrogen bonds appertaining to each deoxyguanosine in the guanine-quartet...
The two simplest models (A, B) comprising deoxyguanosine and urea or guanidine contain only fragments of the bases and exclude the so called ring current effect\(^2\). Despite this fact, A and B provide very similar results as the other models (C, D, E). This finding suggests negligible importance of the ring current effect in regard to its influence on N9 chemical shifts.

Hydrogen bonds created through the mediation of N7 and O1 (A, B, C) in deoxyguanosine showed to have a slightly stronger influence on \(\delta_{N9}\) than those created by H2 and H3 (D, E). Moreover, comparing the impact of the two and four hydrogen bonds, respectively, we see that it is nonadditive. Finally, the hydrogen bonding resulted in the systematic upfield shift of N9 (3 ppm for A, B, C and 1 ppm for D, E) for both the syn and anti glycosidic torsion angles.

\(^2\)changes in chemical shifts arising from the electron currents induced for example by the presence of an aromatic ring
Figure 4.11: Models used for studying the influence of hydrogen bonds.

<table>
<thead>
<tr>
<th>base position</th>
<th>G</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>syn</td>
<td>181.55</td>
<td>184.18</td>
<td>184.19</td>
<td>184.74</td>
<td>182.45</td>
<td>182.89</td>
</tr>
<tr>
<td>anti</td>
<td>189.06</td>
<td>191.94</td>
<td>191.95</td>
<td>192.74</td>
<td>189.89</td>
<td>–</td>
</tr>
</tbody>
</table>

Table 4.3: The comparison of N9 chemical shifts in the syn and anti regions for models A–E (cf. Fig. 4.11) and for deoxyguanosine (G).
Figure 4.12: The schematic illustration of the quadruplex \([d(G_4T_4G_4)]_2\). The strand directions are indicated by arrows. Nucleotides of one strand are numbered 1–12; the symmetry related strand is numbered 1∗–12∗. The grey and white boxes are indicative of the syn and anti conformation of the glycosidic torsion angles, respectively.

### 4.3.5 Comparison with Experimental Data

**The source of experimental data**

In order to compare the theoretical results with experimental chemical shifts, the data from the BMRB (BioMagResBank) database \(^{[48]}\) and data for the \([d(G_4T_4G_4)]_2\) quadruplex \(^{[3]}\) have been used. The database served as a source of C1’ and C2’ chemical shifts whereas the quadruplex (K\(^+\) form; see below) provided data for N9, whose chemical shifts were not available in BMRB. Moreover, C1’ chemical shifts of the Na\(^+\) form of \([d(G_4T_4G_4)]_2\) have been employed.

**The structure of the quadruplex \([d(G_4T_4G_4)]_2\)**

The quadruplex \([d(G_4T_4G_4)]_2\) is a symmetrical bimolecular DNA structure with four guanine-quartets and two thymine loops which span the diagonal of the end G-quartets (Fig. 4.12). Guanines are alternately syn and anti along each strand and syn-syn-anti-anti around each quartet. The structure of the quadruplex is stabilized by the presence of sodium, potassium or ammonium counterions \(^{[50]}\).

\(^{[3]}\)the \([d(G_4T_4G_4)]_2\) quadruplex has been intensively studied at Molecular Biology Institute at the University of California Los Angeles, USA in cooperation with NMR Laboratory at National Centre for Biomolecular Research.
CHAPTER 4. \(^{13}C\) AND \(^{15}N\) CHEMICAL SHIFTS IN B-DNA

<table>
<thead>
<tr>
<th>atom</th>
<th>(\delta_{\text{theor}} - \delta_{\text{exp}}/\text{ppm})</th>
<th>I</th>
<th>II</th>
<th>([d(G_4T_4G_4)]_2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1’</td>
<td>3.9</td>
<td>4.4</td>
<td>5.7 (G)</td>
<td>6.6 (T)</td>
</tr>
<tr>
<td>C2’</td>
<td>7.1</td>
<td>8.7</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Table 4.4: The difference between theoretical and experimental C1’, C2’ chemical shifts in selected structures. I and II represents \([d(TTGGCCCAA)]_2\) and 14mer DNA duplex (with the sequences of GAAAGCCATTAGAG and CTCTAATGGCTTTC for the two strands), respectively. The values given in the table are averages over all of the four deoxynucleosides unless otherwise noted in parentheses.

<table>
<thead>
<tr>
<th>Residue</th>
<th>G1</th>
<th>G2</th>
<th>G3</th>
<th>G4</th>
<th>G9</th>
<th>G10</th>
<th>G11</th>
<th>G12</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\delta_{N9}/\text{ppm})</td>
<td>166.2</td>
<td>164.9</td>
<td>165.6</td>
<td>165.7</td>
<td>164.7</td>
<td>165.0</td>
<td>165.3</td>
<td>–</td>
</tr>
</tbody>
</table>

Table 4.5: The values of N9 chemical shifts in the quadruplex \([d(G_4T_4G_4)]_2\). The odd residues correspond with the syn, and the even residues with the anti, orientation of the base.

The results of the comparison

Basically, for the purposes of the comparison, two B-DNA structures have been selected from the BMRB database, i.e., \([d(TTGGCCCAA)]_2\) (I) [58], and 14mer DNA duplex (II) [59] with the sequences of GAAAGCCATTAGAG and CTCTAATGGCTTTC for the plus and minus strand, respectively. Using the data for these structures, the comparison has revealed a systematic overestimation of the theoretical results by ca. 4 ppm and 8 ppm for C1’ and C2’, respectively (cf. Table 4.4). A substantially larger difference has been encountered in the case of the quadruplex, which is understandable considering the structural distinctions of \([d(G_4T_4G_4)]_2\) from usual double-stranded B-DNA such as I and II.

However, we were especially interested in the comparison of the theoretical and experimental syn-anti difference rather than in the extent of correlation of absolute values. For this purpose, the quadruplex is a unique structure as it contains bases orientated alternately syn and anti along each strand, while bases in classical B-DNAs almost exclusively adopt anti position. Whereas a promising agreement has been obtained for C1’, this is not

\(^4\)for all other structures in the database only \(^1H\) chemical shifts were available
Table 4.6: The difference of C1’, N7 and N9 chemical shifts between the syn and anti regions ($\Delta \delta = \delta_{\text{syn}} - \delta_{\text{anti}}$). Experimental values of the chemical shift for the syn and anti region belong to G1 and G10 residue in the quadruplex $[d(G_TG_TG_T)_2]$, respectively.

The case for N9 (cf. Table 4.6). Contrary to theoretical results which provided ca. 7 ppm difference in N9 chemical shift between the syn and anti regions (cf. sections 4.3.3, 4.3.4), experimental values of $\delta_{\text{N9}}$ in the quadruplex are approximately the same for both orientations of the base (cf. Table 4.5). In contrast, if we make the same comparison for N7 (Table 4.6), we ascertain that the theory does correlate with the experimental observations – in the $^1$H-$^15$N 2D-correlation spectrum (see Appendix A) are found two groups of peaks separated by several ppm.

In section 4.3.4 we have shown that the discrepancy in N9 chemical shielding described above is not brought about by the presence of hydrogen bonds. Besides, we know that the 7 ppm difference between the syn and anti region can be obtained only when the orientation about the C4’-C5’ bond in the anti region corresponds with the gg rotamer (cf. section 4.3.3). Thus, the observed discrepancy would be accounted for if the orientation about the C4’-C5’ bond for anti-residues in the quadruplex was tg or gt (i.e., if $\gamma$ was ca. 180° or ca. 300°). Unfortunately such values of $\gamma$ have been found solely
for the _syn_-residues whereas deoxyguanosines with \( \chi \sim -120^\circ \) always adopt _gg_ conformation. Another possible reason of the disagreement could be the type of the sugar conformation in the quadruplex. In section 4.3.2 the S→N repuckering has been shown to give rise to 10.5 or 7.8 ppm upfield shift for N9. Therefore, it was necessary to make sure that the sugar ring conformations in \([d(G_4T_4G_4)]_2\) are the same as that in structures used for the calculations. Indeed, the values of the pseudorotation phase angle (P)\(^{[56]}\) in computed geometries have been found to fall within the range of P in the quadruplex.

Considering the arguments given above, the discussed disagreement seems to be caused by a specific geometrical feature of \([d(G_4T_4G_4)]_2\), possibly by the presence of metal counterions. That is why there is a future need of providing some additional experimental data suitable for comparison. This, naturally, is not an easy task with regard to the rarity of the occurrence of DNAs with alternating _syn_ and _anti_ glycosidic torsion angles.

\(^{5}\)the value of the pseudorotation phase angle determines the type of the sugar pucker
Chapter 5

$^{31}$P NMR Parameters in Nucleic Acids

5.1 Background and Motivation

The present chapter deals with the calculation of $^{31}$P NMR parameters in models of nucleic acids. The study has been motivated by the fact that phosphorus chemical shifts and heteronuclear $J_{PX}$ spin-spin coupling constants are significantly influenced by the phosphodiester conformation in the DNA backbone \[60, 61\]. Reproducing, predicting and interpreting of $^{31}$P NMR parameters can thus offer a valuable help in the structure determination of nucleic acids.

The internucleotide linkage is generally described by six torsion angles $(\alpha, \beta, \gamma, \delta, \epsilon, \zeta)$ from one phosphorus atom to the next along the DNA backbone. The two P–O ester torsion angles, i.e., $\alpha$ (O3’–P–O5’–C5’) and $\zeta$ (C3’–O3’–P–O5’), have appeared to significantly influence $^{31}$P chemical shifts and $J_{PX}$ spin-spin coupling constants. Besides $\alpha$ and $\zeta$, changes in phosphorus NMR parameters are also mediated by the modifications in $\epsilon$, since a strong correlation between $\zeta$ and $\epsilon$ has been ascertained. Two very similar relations expressing the correlation have been derived: $\zeta = -317 - 1.23\epsilon$ \[62, 63\] and $\zeta = -348.11 - 1.42\epsilon$ \[64\].

Phosphorus chemical shifts and $J_{H3'–P}$ coupling constants for a phosphate diester in a gauge, gauge $(g,g)$ conformation have been shown to be notably distinct from those for a phosphate diester in a conformation denoted as gauche, trans $(g,t)$ \[60, 61\]. Gauche and trans refer to the values of the P–O ester torsion angles $\alpha$ and $\zeta$, gauge being either $+60^\circ$ or $-60^\circ$ and trans

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$^{1}$\(\alpha, \beta, \gamma, \delta, \epsilon\) and $\zeta$ refer to the O3’–P–O5’–C5’, P–O5’–C5’–C4’, O5’–C5’–C4’–C3’, C5’–C4’–C3’–O3’, C4’–C3’–O3’–P and C3’–O3’–P–O5’ torsion angles, respectively.
**Figure 5.1**: B$_I$ (gg) and B$_{II}$ (gt) conformation.

being 180°. The gg and gt conformation is found, e.g., in the B$_I$ and B$_{II}$ type of B-DNA, respectively. Similarly, the gg and gt patterns alternate along the sequence of poly(dC-dG) in Z-DNA resulting in different phosphate-diester conformations for the CG step on the one hand (gg), and the GC step on the other hand (gt) [56].

Semiempirical and *ab initio* GIAO chemical shift calculations as well as later empirical studies suggested that a phosphate diester in the B$_I$ conformation should have a $^{31}$P chemical shift 1.6 ppm upfield from a phosphate diester in the B$_{II}$ conformation ($\sigma_{B_I} = -4.6$ ppm and $\sigma_{B_{II}} = -3.0$ ppm) [60, 65]. Likewise, $J_{\text{HX-P}}$ for B$_I$ has been estimated to be 1.3 Hz as opposed to 10.0 Hz for B$_{II}$ [60]. A slightly larger difference of ca. 2.0 ppm has been found in $^{31}$P chemical shifts between the gg and gt conformation in Z-DNA ($\delta_{\text{CG}} = -4.68$ ppm and $\delta_{\text{GC}} = -2.62$ ppm) [66].

Pure B$_I$ and B$_{II}$ conformations are not present in experimental structures for purposes of conveniently describing the dependence of $^{31}$P chemical shifts and $J_{\text{PX}}$ spin-spin coupling constants on P–O ester torsion angles, no distinction is usually made between R–O–P–O(R’) torsion angles +60°(+g) or −60°(−g). Moreover, $\alpha$ and $\zeta$ torsion angles of $g$, $t$; $-g$, $t$; $t$, $g$; and $t$, $-g$ are often grouped as $g$, $t$. Similarly, $g$, $g$ includes conformers $-g$, $-g$; $g$, $-g$; and $-g$, $g$. 

\[ \begin{align*}
\text{B}_I & \quad \epsilon = t \\
\zeta &= -g \\
\alpha &= -g \\
\text{B}_{II} & \quad \epsilon = -g \\
\zeta &= t \\
\alpha &= -g 
\end{align*} \]
and both types are encountered along the DNA backbone. This fact implies that the measured values are weighted averages of chemical shifts and coupling constants in the two states. Thus, the dispersion in the $^{31}$P chemical shifts and $J_{PX}$ spin-spin coupling constants is likely attributable to different populations of the $B_I$ and $B_{II}$ states in the sequence. Therefore, the knowledge of $^{31}$P NMR parameters can be very helpful in the proper interpretation of the phosphate ester conformation.

### 5.2 Studied Systems

This study is aimed at investigating the impact of sugar-phosphate backbone conformation on $^{31}$P chemical shielding and $J_{PX}$ spin-spin coupling constants. Particularly, $^{31}$P NMR parameters for the model system of the $B_I$ conformation are examined under the influence of phosphate-diester torsion angles $\alpha$ and $\zeta$. In addition, differences in chemical shielding and coupling constants between the $gg$ and $gt$ conformations are studied for the case of B- as well as Z-DNA (namely $Z_{II}$). The correlation of the results obtained with experimental observations is outlined.
CHAPTER 5.  $^{31}$P NMR PARAMETERS IN NUCLEIC ACIDS

5.3 Computational Details

5.3.1 Geometry Optimizations

Molecular geometries have been optimized in DFT-KS calculations employing the hybrid B3LYP [36, 52] functional and the 6-31G(d) basis set [53, 54] as implemented in Gaussian 98 [51]. Phosphorus NMR parameters of both B- and Z-DNA have been studied on a model comprising two sugar rings connected by a phosphate group (Fig. 5.3). Bases normally present in DNA have been replaced by hydrogens in order to obtain systems of a reasonable size. In initial calculations geometries with hydroxyl groups bound to C1’ have been tested. At first, these systems seemed to be the most suitable approximations to real structures. However, during the optimization several difficulties associated with creating artificial hydrogen bonds between the two sugars have been met. The hydrogen bonds were driving structures into inappropriate geometries and therefore, the approach simply employing hydrogens had to be chosen.

For the purposes of the optimization of $B_I$ and $B_{II}$ conformations, those torsion angles which are included twice in the backbone of the model (Fig. 5.3)
\section*{CHAPTER 5. \textsuperscript{31}P NMR PARAMETERS IN NUCLEIC ACIDS}

<table>
<thead>
<tr>
<th>Torsion Angle</th>
<th>Degree</th>
<th>Mean</th>
<th>Conformation Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\alpha$ (O\textsuperscript{3'}-P-O\textsuperscript{5'}-C\textsuperscript{5'})</td>
<td>270\textdegree–330\textdegree</td>
<td>298\textdegree</td>
<td>–</td>
</tr>
<tr>
<td>$\beta$ (P-O\textsuperscript{5'}-C\textsuperscript{5'}-C\textsuperscript{4'})</td>
<td>130\textdegree–200\textdegree</td>
<td>176\textdegree</td>
<td>B\textsubscript{I}</td>
</tr>
<tr>
<td>$\gamma$ (O\textsuperscript{5'}-C\textsuperscript{5'}-C\textsuperscript{4'}-C\textsuperscript{3'})</td>
<td>20\textdegree–80\textdegree</td>
<td>48\textdegree</td>
<td>–</td>
</tr>
<tr>
<td>$\delta$ (C\textsuperscript{5'}-C\textsuperscript{4'}-C\textsuperscript{3'}-O\textsuperscript{3'})</td>
<td>70\textdegree–180\textdegree</td>
<td>128\textdegree</td>
<td>B\textsubscript{I}</td>
</tr>
<tr>
<td>$\epsilon$ (C\textsuperscript{4'}-C\textsuperscript{3'}-O\textsuperscript{3'}-P)</td>
<td>160\textdegree–270\textdegree</td>
<td>184\textdegree</td>
<td>B\textsubscript{I}</td>
</tr>
<tr>
<td>$\zeta$ (C\textsuperscript{3'}-O\textsuperscript{3'}-P-O\textsuperscript{5'})</td>
<td>230\textdegree–300\textdegree</td>
<td>265\textdegree</td>
<td>B\textsubscript{I}</td>
</tr>
</tbody>
</table>

Table 5.1: Experimental values of backbone torsion angles in B-DNA.

have been labeled by the same variable. Particularly, the notation $\beta_1, \beta_2 \rightarrow$ BET, $\gamma_1, \gamma_2 \rightarrow$ GAM, $\delta_1, \delta_2 \rightarrow$ DEL and $\epsilon_1, \epsilon_2 \rightarrow$ EPS has been used. Such a way of defining the variables assured that both torsion angles within each couple were changing by the same amount during the optimization. This strategy appeared to be necessary to adopt in order to obtain relevant geometries. It enabled to provide equivalent electronic surroundings for phosphorus atom from both sides of the backbone. It also restrained the motion of atoms reasonably so that the known relationship between the torsion angles $\zeta$ and $\epsilon$ (cf. section 5.1) could be fulfilled, at least to a certain extent.

For all optimizations of the B\textsubscript{I} as well as the B\textsubscript{II} conformations, the starting values of backbone torsion angles have been set to average experimental ones (Table 5.1) \cite{55}: $\alpha = 298\textdegree$, BET = 176\textdegree, GAM = 48\textdegree, DEL = 128\textdegree, EPS = 184\textdegree, and $\zeta = 265\textdegree$ for BI; $\alpha = 298\textdegree$, BET = 146\textdegree, GAM = 48\textdegree, DEL = 144\textdegree, EPS = 246\textdegree, and $\zeta = 174\textdegree$ for BII. Structural parameters have been relaxed except for the constraints mentioned below. The torsion angle $\beta$ has always been frozen because the repulsion of lone pairs at O\textsuperscript{5'} and O\textsuperscript{4'} caused artificial rotation about the C\textsuperscript{5'}-O\textsuperscript{5'} bond, pushing the (O\textsuperscript{5'})H proton towards H3'. In real structures, such rotation is prevented by the sugar-phosphate backbone. In order to study \textsuperscript{31}P chemical shifts and $J_{PX}$ spin-spin coupling constants in B\textsubscript{I} conformation under the influence of the $\zeta$ torsion angle, its value has been varied within the experimental range of 230\textdegree–300\textdegree and constrained, namely to 240\textdegree, 265\textdegree, 270\textdegree, 285\textdegree, and 300\textdegree. By analogy, \textsuperscript{31}P NMR parameters have been investigated as a function of the $\alpha$ torsion...
Table 5.2: Experimental values of backbone torsion angles in Z_{II}-DNA.

<table>
<thead>
<tr>
<th>Torsion Angle</th>
<th>Degree</th>
<th>Mean</th>
<th>Residue Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>α (O3'–P–O5'–C5')</td>
<td>40°–100°</td>
<td>71°</td>
<td>G</td>
</tr>
<tr>
<td></td>
<td>150°–250°</td>
<td>168°</td>
<td>C</td>
</tr>
<tr>
<td>β (P–O5'–C5'–C4')</td>
<td>150°–250°</td>
<td>183°</td>
<td>G</td>
</tr>
<tr>
<td></td>
<td>160°</td>
<td>166°</td>
<td>C</td>
</tr>
<tr>
<td>γ (O5'–C5'–C4'–C3')</td>
<td>160°–210°</td>
<td>179°</td>
<td>G</td>
</tr>
<tr>
<td></td>
<td>20°–90°</td>
<td>54°</td>
<td>C</td>
</tr>
<tr>
<td>δ (C5'–C4'–C3'–O3')</td>
<td>80°–160°</td>
<td>95°</td>
<td>G</td>
</tr>
<tr>
<td></td>
<td>20°–90°</td>
<td>141°</td>
<td>C</td>
</tr>
<tr>
<td>ε (C4'–C3'–O3'–P)</td>
<td>180°–300°</td>
<td>189°</td>
<td>G</td>
</tr>
<tr>
<td></td>
<td>20°–90°</td>
<td>267°</td>
<td>C</td>
</tr>
<tr>
<td>ζ (C3'–O3'–P–O5')</td>
<td>40°–100°</td>
<td>52°</td>
<td>G</td>
</tr>
<tr>
<td></td>
<td>20°–90°</td>
<td>75°</td>
<td>C</td>
</tr>
</tbody>
</table>

angle for which a constraint to 270°, 298°, and 330° has been used. In the latter case, two sets of optimizations have been performed – either with or without freezing ζ to its average value of 265°. For the purpose of comparing $^{31}$P chemical shifts and $J_{PX}$ spin-spin coupling constants in B_{I} and B_{II} conformation, two approaches to geometry optimizations have been adopted. Firstly, all backbone torsion angles have been fixed to average experimental values (Table 5.1) and secondly, all of them have been relaxed, except for ζ and β.

Table B.1 in Appendix B gives the values of the backbone torsion angles before and after the optimization of the B_{I} conformation. For the set of geometries optimized while varying the torsion angle ζ, one can observe the notable correlation of the ζ and ε torsion angles previously uncovered in the empirical studies [62, 63, 64]. It is possible to see that for ζ = 240°–300°, we obtain values of ε = 187°–211°. However, for this range of ζ, the relation $\zeta = -317 - 1.23\epsilon$ [62, 63] predicts values of $\epsilon = 200°–151°$. That is, the direction of the correlation in the optimized structures is opposite compared to the one derived from the empirical relationship. Nevertheless, the range of values of the ε torsion angle provided by the geometry optimizations falls within the dispersion of experimental data [60].

In the case of Z-DNA the scheme employing the same label for the two corresponding variables could not be used since geometrical parameters in poly(dC-dG) appertaining to deoxycytidine are different from those appertaining to deoxyguanosine [56]. Therefore, an individual value has been as-
CHAPTER 5. $^{31}$P NMR PARAMETERS IN NUCLEIC ACIDS

31

P NMR PARAMETERS IN NUCLEIC ACIDS

54

Fixed torsion angles

<table>
<thead>
<tr>
<th>The dependence of $\sigma_{31P}$ and $J_{PX}$ on $\zeta$ for B_1 conformation</th>
<th>$\zeta = 240^\circ, 265^\circ, 270^\circ, 285^\circ, 300^\circ$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\beta = 176^\circ$</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>The dependence of $\sigma_{31P}$ and $J_{PX}$ on $\alpha$ for B_1 conformation</th>
<th>$\alpha = 270^\circ, 298^\circ, 330^\circ$</th>
</tr>
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<tbody>
<tr>
<td>$\beta = 176^\circ$</td>
<td></td>
</tr>
<tr>
<td>$\zeta = 265^\circ$</td>
<td></td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>The comparison of $\sigma_{31P}$ and $J_{PX}$ for B_1 and B_II conformation</th>
<th>$\zeta = 265^\circ(B_1), 174^\circ(B_\text{II})$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\beta = 176^\circ(B_1), 146^\circ(B_\text{II})$</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>The comparison of $\sigma_{31P}$ and $J_{PX}$ for CG and GC step in Z_II-DNA</th>
<th>$\zeta = 265^\circ(B_1), 174^\circ(B_\text{II})$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\beta = 176^\circ(B_1), 146^\circ(B_\text{II})$</td>
<td></td>
</tr>
</tbody>
</table>

Table 5.3: The summary of geometry optimizations.

Table 5.3: The summary of geometry optimizations.

signed to every backbone torsion angle. Models representing the CG and GC steps have been optimized two times. Firstly (A), all backbone torsion angles have been constrained to average experimental values as found in Z_II-DNA (see Table 5.2) and secondly (B), all backbone torsion angles have been fixed to original values of one specific Z_II-DNA selected from the NDB database [67]. Besides backbone torsion angles, all other parameters have been optimized. The sugar pucker of deoxyguanosine always experienced large changes upon the optimization procedure ($^3T\rightarrow^O E$ for A and $^3T\rightarrow^3E$ for B). This feature correlates with the broad range of pseudorotation phase angle $P$ (from C2'-exo to C2'-endo, i.e., $P \in (-18^\circ, +162^\circ)$) found for deoxyguanosine in experimental structures of Z-DNA [56]. On the contrary, the sugar pucker of deoxycytidine remained essentially unchanged after the optimization, which is consistent with the well defined C2'-endo (S) experimental conformation of the sugar ring. The summary of all geometry optimizations is given in Table 5.3.

5.3.2 NMR parameter calculations

All computed NMR parameters have been obtained with the modified version of the deMon-KS program [68, 69] along with the deMon-NMR code...
Calculations of chemical shielding tensors employed sum-over-states density functional perturbation theory with the IGLO choice of the gauge origin (SOS-DFPT-IGLO) \[47\]. On the contrary, spin-spin coupling constants have been obtained with a combined SOS-DFPT (DSO and PSO terms) \[47\] and DFT/FPT (FC term) \[73, 74, 75\] approach. The SD term has been neglected (cf. below). All parameters have been calculated using the Perdew and Wang exchange \[37\] and the Perdew correlation functional (PP) \[38, 76\]. The basis set IGLO-III \[23\] of Kutzelnigg et al. has been uniformly adopted for all atoms. The perturbation defined by the perturbation parameter \(\lambda = 0.001\) has been imposed on P atom (see below for the discussion on the choice of the center of the finite perturbation).

In order to obtain chemical shifts, chemical shieldings are usually referenced to the shielding constant of a standard (trimethylphosphate (TMP) in the case of the phosphorus chemical shifts in nucleic acids). In this part of the thesis, only the values of the chemical shielding are presented for two reasons. Firstly, we have especially been interested in relative differences of phosphorus chemical shielding between particular conformations rather than in absolute values of chemical shifts. Secondly, using the currently available exchange-correlation functionals it is difficult to achieve an absolute agreement of theoretical and experimental chemical shifts of heavier nuclei such as phosphorus \[77, 78, 79, 80\].

**Additional details about the calculation of \(J_{MN}\) in \textit{deMon}**

There are four main contributions to the nuclear spin-spin coupling constants: the Fermi contact (FC), the paramagnetic spin-orbit (PSO), the diamagnetic spin-orbit (DSO), and the spin-dipolar (SD) contributions \[73, 74, 75, 81\]. As mentioned in section \(2.2.2\), the Fermi contact term is usually the most important one and also the one most sensitive to the geometry changes. Its origin consists in the interaction between the two nuclei through spin polarization of the electronic system (even if a closed-shell system is formally treated). The calculation of the FC term using FPT requires basis sets of high quality, enlarged grid for the numerical integration (approximately twice as large as for the PSO and DSO contributions) and the ”spin-unrestricted” approach as normally used for open shell systems \[70, 71\]. Due to all of these reasons, the calculation of the FC term is much more time demanding than the calculation of the PSO and DSO contributions (as the SD term is neglected).

The second crucial contribution is the PSO term. Its calculation using \[3\]IGLO-III is roughly of ”quadruple-\(\zeta\)” quality.
the SOS-DFPT method differs from the SOS-DFPT procedure for the NMR chemical shift calculation only in the choice of the gauge origin – it is common for all molecular orbitals and placed at the position of the nucleus selected as the centre of perturbation. To obtain the PSO term, one does not have to make a great effort. A “spin-restricted” calculation for the ground state employing SOS-DFPT is performed only once and it is directly followed by a calculation of the PSO term with the same approach (which is faster than coupled perturbed DFT as well as coupled perturbed Hartree-Fock calculations). Moreover, the PSO contribution is not as sensitive to the quality of the grid (therefore a smaller one is used) and basis set as the FC contribution.

The last term is the SD (spin-dipolar) contribution. In the deMon implementation, it is neglected due to the following reasons. In the first place, the SD term is known to be relatively small, at least for long range couplings. In the second place, this contribution is usually smaller than the error in the DFT calculations of the FC term. And finally, the quest for the value of this term is the most time consuming step of nuclear spin-spin coupling calculations at the DFT level [82]. It is a consequence of the fact that in the case of SD contribution, the perturbation comprises both real and imaginary components.

Besides the four contributions mentioned above, there is also the so called FC-SD (Fermi contact-spin dipolar) cross term. This term contributes especially to the anisotropy and can be calculated together with the FC term employing the FPT approach. The FC-SD cross term, as distinct from SD term, requires only very small computational effort.

The choice of the centre of perturbation

If we wish to obtain the spin-spin coupling constant of two nuclei $M$ and $N$, we have to impose a FPT perturbation on one of them. The chosen nucleus produces the perturbed spin-density in the system, and this density then interacts with the other nucleus. The extent of the perturbation is defined by the perturbation parameter $\lambda$. Having a look at eq (3.31), we see that it is symmetrical with respect to the interchange of the indices $M$ and $N$. Moreover, FPT is, in principle, an exact perturbation theory. Therefore, the results should be independent of which of the two nuclei $M$ and $N$ is selected as the centre of perturbation. In reality, it is not true [70]. This contradiction arises from the fact that the used exchange-correlation functional is never exact and that the grid used for the numerical integration is always finite. For distinct choices of the perturbation origin, the difference in calculated spin-spin coupling constants decreases if a larger grid is used. On the other hand, the choice of the lighter nucleus (usually hydrogen) as the centre of
Table 5.4: Calculated $^{31}$P chemical shielding in B$_{II}$ conformation as a function of torsion angles $\zeta$ and $\alpha$. $\Delta \sigma$ is the chemical shielding anisotropy calculated according to the relation: $\Delta \sigma = \sigma_{33} - \frac{1}{2}(\sigma_{11} + \sigma_{22})$.

<table>
<thead>
<tr>
<th>$\zeta$/degs</th>
<th>$\sigma_{iso}$/ppm</th>
<th>$\Delta \sigma$/ppm</th>
<th>$\sigma_{11}$/ppm</th>
<th>$\sigma_{22}$/ppm</th>
<th>$\sigma_{33}$/ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>240</td>
<td>303.2</td>
<td>286.9</td>
<td>178.427</td>
<td>236.761</td>
<td>494.482</td>
</tr>
<tr>
<td>265</td>
<td>303.6</td>
<td>288.4</td>
<td>175.900</td>
<td>239.066</td>
<td>495.922</td>
</tr>
<tr>
<td>270</td>
<td>303.9</td>
<td>289.2</td>
<td>175.973</td>
<td>239.037</td>
<td>496.724</td>
</tr>
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<td>285</td>
<td>303.3</td>
<td>289.3</td>
<td>174.390</td>
<td>239.335</td>
<td>496.124</td>
</tr>
<tr>
<td>300</td>
<td>302.1</td>
<td>293.9</td>
<td>171.764</td>
<td>236.616</td>
<td>498.064</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>$\alpha$/degs</th>
<th>$\sigma_{iso}$/ppm</th>
<th>$\Delta \sigma$/ppm</th>
<th>$\sigma_{11}$/ppm</th>
<th>$\sigma_{22}$/ppm</th>
<th>$\sigma_{33}$/ppm</th>
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<td>303.1</td>
<td>286.9</td>
<td>174.401</td>
<td>240.633</td>
<td>494.395</td>
</tr>
<tr>
<td>298</td>
<td>304.1</td>
<td>293.2</td>
<td>175.781</td>
<td>236.959</td>
<td>499.544</td>
</tr>
<tr>
<td>330</td>
<td>304.9</td>
<td>308.0</td>
<td>176.458</td>
<td>227.913</td>
<td>510.212</td>
</tr>
</tbody>
</table>

perturbation appeared to generally provide much better results, very close to those obtained with an enlarged grid.

In this study the heavier nucleus, i.e., phosphorus, has been chosen as the centre of perturbation. The adequacy of this strategy has been verified on the basis of grid tests. Calculations employing an enlarged grid provided essentially the same results as those employing a smaller grid. Imposing the perturbation on phosphorus has been motivated by the computational convenience. Within one calculation in deMon, one obtains coupling constants of the nucleus selected as the centre of perturbation with all other nuclei in the system. That is, besides $J_{H3'-P}$ spin-spin coupling constant, which was primarily the focus of investigation, it was possible to arrive at some other, potentially interesting, coupling constants without additional computational effort.

### 5.4 Results and Discussion

#### 5.4.1 The Influence of Torsion Angles $\alpha$ and $\zeta$

The phosphodiester conformation in B$_I$ and B$_{II}$-DNA differ in the value of the $\zeta$ torsion angle whereas the experimental range of values of $\alpha$ is the same for both the types of B-DNA (cf. Table 5.1). Therefore, it was logical to explore at first what would happen if the $\zeta$ torsion angle was varied within the range of 230°–300° observed experimentally (cf. Table 5.1). Not very large changes have been expected because of two reasons. Firstly, the exper-
Chapter 5. $^{31}$P NMR Parameters in Nucleic Acids

Figure 5.4: Calculated $\sigma^{(31)P}$ and $\Delta\sigma^{(31)P}$ plotted vs. $\zeta$. $\Delta\sigma$ is the chemical shielding anisotropy.

Experimental difference in the phosphorus chemical shielding between the $\text{B}_1$ and $\text{B}_\text{II}$ conformation is quite small (only 1.6 ppm). Secondly, measured values of the phosphorus chemical shielding fall within a tight range from ca. -3 ppm to ca. 1 ppm.

Despite this fact, it was quite surprising that the calculated $^{31}$P chemical shielding was essentially the same for all values of $\zeta$, except for the case of $\zeta = 300^\circ$ (cf. Table 5.4, Figure 5.4). This finding raised a question of the geometrical parameters corresponding to such a value of $\zeta$. The comparison of backbone torsion angles before and after the optimization showed that
Figure 5.5: The plot of phosphorus chemical shielding anisotropy vs. bond angle O–P–O ($\varphi$) in geometries with the corresponding value of $\zeta$ and $\alpha$, respectively.

<table>
<thead>
<tr>
<th>$\zeta$/degs</th>
<th>$\varphi$/degs</th>
<th>$\triangle\sigma$/ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>240</td>
<td>96.83</td>
<td>286.9</td>
</tr>
<tr>
<td>265</td>
<td>98.39</td>
<td>288.4</td>
</tr>
<tr>
<td>270</td>
<td>98.91</td>
<td>289.2</td>
</tr>
<tr>
<td>285</td>
<td>100.42</td>
<td>289.3</td>
</tr>
<tr>
<td>300</td>
<td>100.93</td>
<td>293.9</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>$\alpha$/degs</th>
<th>$\varphi$/degs</th>
<th>$\triangle\sigma$/ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>270</td>
<td>100.04</td>
<td>286.9</td>
</tr>
<tr>
<td>298</td>
<td>99.53</td>
<td>293.2</td>
</tr>
<tr>
<td>330</td>
<td>99.98</td>
<td>308.0</td>
</tr>
</tbody>
</table>

Table 5.5: Values of the O–P–O bond angle ($\varphi$) and values of the phosphorus chemical shielding anisotropy in geometries with the corresponding value of $\zeta$ and $\alpha$, respectively. The plot of $\triangle\sigma$ vs. $\varphi$ is shown in Fig. 5.5.

in the geometry with $\zeta = 300^\circ$, the $\alpha$ torsion angle experienced a significant change. In particular, the optimization procedure shifted the starting value from 289° to 271°. Such a large change has not been observed for other structures (see Appendix B). This fact suggests that the notable distinction of phosphorus chemical shielding found for the geometry with $\zeta = 300^\circ$ is a consequence of the change in the value of $\alpha$ but not $\zeta$ torsion angle.
Considering what has been said above, it was natural to explore the effect introduced by varying the torsion angle $\alpha$. As mentioned in section 5.3.1 for purposes of exploring $^{31}$P NMR parameters as modulated by $\alpha$, two sets of optimized geometries have been used. The one obtained by the optimization with the fixed value of $\zeta$(B) appeared to provide essentially the same results as the other set obtained by the optimization with the relaxed value of $\zeta$(A). Therefore, from now on, only the results provided using the set A are presented. The phosphorus chemical shielding appeared to be quite strongly dependent on the $\alpha$ torsion angle. The change of $\alpha$ from 270° to 298° resulted in a 1 ppm difference, and the change from 298° to 300° resulted in the difference of 0.8 ppm (cf. Table 5.4). That is, the transition from the lowest to the largest experimental value of $\alpha$ gave rise to a 1.8 ppm upfield shift.

Besides the dependence of phosphorus chemical shielding on backbone torsion angles, Gorenstein noted that a correlation between the phosphorus chemical shielding anisotropy $\Delta\sigma$ and the bond angle O–P–O ($\phi$) may exist [60]. Table 5.5 gives the values of $\phi$ and $\Delta\sigma(^{31}P)$ for the two sets of geometries optimized with either $\zeta$ (set I) or $\alpha$ (set II) constrained. The plot of these values is shown in Fig. 5.5. If we take set I into account, we see that the chemical shielding anisotropy of phosphorus slightly increases with the increasing value of the O–P–O bond angle. No such correlation has been observed for the case of set II.

Heteronuclear $J_{PX}$ Spin-Spin Coupling Constants

Whereas the phosphorus chemical shielding did not appear to be influenced by the torsion angle $\zeta$, calculated spin-spin coupling constants showed a strong dependence on this torsion angle. Namely, the $^3J_{H3'-P}$ spin-spin coupling constant has been influenced most of all calculated coupling constants by the rotation about the O3'-P bond (cf. Table 5.6). The variation of $\zeta$ within the range of 240°–300° gave rise to values of $^3J_{H3'-P}$ from 4 to 10 Hz (see Fig. 11). The changes in $^3J_{H3'-P}$ observed upon varying $\zeta$ are due to the related changes in the $\epsilon$ torsion angle which is directly associated with the $^3J_{H3'-P}$ coupling constant. The sensitivity of $^3J_{H3'-P}$ to the value of the $\zeta$ torsion angle is not very surprising as $^3J_{H3'-P}$ has already been reported to serve as a useful probe of phosphodiester backbone conformation [60, 83]. Besides $^3J_{H3'-P}$, the $^3J_{C4'-P}$ coupling constant $^4$ appeared to be significantly affected by $\zeta$ (Table 5.6, Figure 11). The most encouraging fact about the

$^4$ $^3J_{C4'-P}$ and $^3J_{C4'+1}$ denote the coupling constant of the phosphorus with the carbon C4' in the sugar ring above and below the phosphorus atom, respectively, when the orientation of the model is as that in Fig. 5.3.
Table 5.6: Calculated heteronuclear $J_{PX}$ spin-spin coupling constants in the B1 conformation as a function of the $\zeta$ torsion angle.

<table>
<thead>
<tr>
<th>$\zeta$/degs</th>
<th>$^3J_{H3'\rightarrow P}$</th>
<th>$^3J_{iC4'\rightarrow P}$</th>
<th>$^3J_{iC4'+1\rightarrow P}$</th>
<th>$^3J_{H3'\rightarrow P}+^3J_{iC4'\rightarrow P}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>240</td>
<td>4.04</td>
<td>14.25</td>
<td>11.98</td>
<td>18.29</td>
</tr>
<tr>
<td>265</td>
<td>5.36</td>
<td>13.51</td>
<td>11.72</td>
<td>18.87</td>
</tr>
<tr>
<td>270</td>
<td>6.85</td>
<td>13.02</td>
<td>11.71</td>
<td>19.87</td>
</tr>
<tr>
<td>285</td>
<td>8.75</td>
<td>12.48</td>
<td>11.51</td>
<td>21.23</td>
</tr>
<tr>
<td>300</td>
<td>9.97</td>
<td>11.61</td>
<td>11.22</td>
<td>21.58</td>
</tr>
</tbody>
</table>

Table 5.7: Calculated heteronuclear $J_{PX}$ spin-spin coupling constants in the B1 conformation as a function of the $\alpha$ torsion angle.

<table>
<thead>
<tr>
<th>$\alpha$/degs</th>
<th>$^3J_{H3'/5'\rightarrow P}$</th>
<th>$^3J_{iC2'\rightarrow P}$</th>
<th>$^3J_{iC5'\rightarrow P}$</th>
<th>$^3J_{C3'\rightarrow P}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>270</td>
<td>9.21</td>
<td>12.30</td>
<td>11.54</td>
<td>21.51</td>
</tr>
<tr>
<td>298</td>
<td>6.97</td>
<td>12.94</td>
<td>11.67</td>
<td>19.91</td>
</tr>
<tr>
<td>330</td>
<td>6.91</td>
<td>12.05</td>
<td>11.74</td>
<td>18.96</td>
</tr>
</tbody>
</table>

results for $^3J_{H3'\rightarrow P}$ and $^3J_{iC4'\rightarrow P}$ is a very good agreement with experimental data. That is, the sum of experimentally measured values of $^3J_{H3'\rightarrow P}$ and $^3J_{iC4'\rightarrow P}$ has been found to fall within the range from 18.5 to 21.7 Hz [60]. The same range has been obtained by theoretical calculations (cf. Table 5.6). Other results also reproduce experimental data quite well. Calculated values of $^3J_{iC5'\rightarrow P}$ coupling constant are close to those usually found in a regular helix (10–11 Hz) [84], and theoretical results for $^3J_{C5'\rightarrow P}$ (3.6–3.8 Hz) nicely
fits the experimentally observed range of 2.8–4.0 Hz [60].

The same good agreement with experimental data has also been encountered for coupling constants calculated for geometries with different values of the α torsion angle. However, very similar values of $^3J_{\text{H3'}-\text{P}}$ have been obtained for α = 298° and α = 330° but different for α = 270° (cf. Table 5.7). This finding reveals that $^3J_{\text{H3'}-\text{P}}$ strongly depends on ζ but very slightly on α. This fact is understandable since based on the geometry consideration, the H3'-P coupling pathway is not related to the α torsion angle (α does not influence the positions of H3’ and P relative to each other). The difference obtained between $^3J_{\text{H3'}-\text{P}}$ for α = 270° on the one hand and $^3J_{\text{H3'}-\text{P}}$ for α = 298° and α = 330° on the other hand results from the value of ζ and ϵ. The ϵ torsion angle is essentially the same in optimized geometries with α = 298° and α = 330° (ca. 198°), whereas ϵ = 206° for the case of α = 270° (cf. Appendix B).
Figure 5.6: $^3J_{\text{H}^\prime - \text{P}}, ^3J_{\text{C}^\prime - \text{P}}$ coupling constants in B\textsubscript{1} conformation and their sum plotted vs. $\zeta$. 
5.4.2 The Comparison of the $gg$ and $gt$ Conformations

$^{31}$P Chemical Shielding

Since the experimental difference in phosphorus chemical shielding between the $B_I$ and $B_{II}$ conformations is quite small (1.6 ppm) \[60\], its reproduction by the theory is a challenging task. In the very first approach, chemical shielding has been calculated for the geometries of $B_I$ and $B_{II}$ optimized with all backbone torsion angles fixed to average experimental values (A). In this way, the difference of 6.6 ppm has been obtained, indicating that theoretical calculations are able to provide distinct values of $\sigma(^{31}\text{P})$ for the two conformation types. A more realistic approach has been adopted afterwards with the $\beta$ and $\zeta$ torsions angles constrained and the other torsions relaxed during the optimization (B). In this case, we have arrived at the difference of 3.6 ppm. Such a difference is certainly still quite large compared to the one found experimentally. The overestimation may be due to the fact that the value of the $\epsilon$ torsion angle in the optimized model of $B_{II}$ conformation (283°) does not fall within the experimental range (160°–270°). Despite the overestimation discussed, it is promising that the direction of the calculated chemical shieldings is in an agreement with experimental phosphorus chemical shifts in the $B_I$ and $B_{II}$ conformation (the value of $\delta(^{31}\text{P})$ smaller for $B_I$ than for $B_{II}$ correlates with the theoretically found value of $\sigma(^{31}\text{P})$ larger for $B_I$ than for $B_{II}$, cf. Table 5.8).

$B_I$ and $B_{II}$ are the representatives of the $gg$ and $gt$ phosphodiester backbone conformations, respectively. These two conformation types are also found in Z-DNA. They alternate along the sequence of the poly(dC·dG), giving rise to the $gg$ pattern for the CG step and the $gt$ pattern for the GC step. Since the experimental difference in phosphorus chemical shielding between $gg$ and $gt$ in Z-DNA (2.1 ppm) \[66\] is larger than the one in B-DNA (1.6 ppm), Z-DNA seemed to be a suitable system for testing the capability of theoretical calculations \[85\]. For the case of Z-DNA, the geometries of the $gg$ and $gt$ conformations have been optimized two times (cf. section 5.3.1). In the second approach (B), theoretical results provided the $gg$-$gt$ difference of 1.5 ppm. This result is comparable with the experimental observation of 2.1 ppm (cf. Table 5.9). Like in the case of B-DNA, the direction of the calculated chemical shieldings fits the experimental data.

Heteronuclear $J_{PX}$ Spin-Spin Coupling Constants

Theoretical calculations show that besides the $^3J_{H3'-P}$ coupling constant, $^3J_{C4'-P}$ and $^3J_{C2'-P}$ may serve as a probe of the phosphodiester conformation. As seen from Table 5.10 for the $B_I$ conformation $^3J_{C4'-P} > ^3J_{C2'-P}$ whereas
### Table 5.10: Calculated heteronuclear $J_{PX}$ spin-spin coupling constants in the $B_I$ and $B_{II}$ conformations.

<table>
<thead>
<tr>
<th>conf. type</th>
<th>$J_{H3'}-P$ /Hz</th>
<th>$J_{C4'}-P$ /Hz</th>
<th>$J_{C2'}-P$ /Hz</th>
<th>$J_{H3'}-P + J_{C4'}-P$ /Hz</th>
</tr>
</thead>
<tbody>
<tr>
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<td>3.13</td>
<td>14.36</td>
<td>0.26</td>
<td>17.49</td>
</tr>
<tr>
<td>B</td>
<td>5.36</td>
<td>13.51</td>
<td>-0.05</td>
<td>18.87</td>
</tr>
</tbody>
</table>

Table 5.11: Calculated heteronuclear $J_{PX}$ spin-spin coupling constants in the $Z_{II}$-CG and $Z_{II}$-GC conformations.

<table>
<thead>
<tr>
<th>conf. type</th>
<th>$J_{H3'}/5''-P$ /Hz</th>
<th>$J_{C4'/5''}-P$ /Hz</th>
<th>$J_{C5'-P}$ /Hz</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>3.70/1.94</td>
<td>11.76</td>
<td>-6.50</td>
</tr>
<tr>
<td>B</td>
<td>3.63/1.69</td>
<td>11.72</td>
<td>-6.39</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>conf. type</th>
<th>$J_{H3'}/5''-P$ /Hz</th>
<th>$J_{C5'-P}$ /Hz</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2.74/3.47</td>
<td>-5.81</td>
</tr>
<tr>
<td>B</td>
<td>1.11/5.57</td>
<td>-5.95</td>
</tr>
</tbody>
</table>

Table 5.12: Calculated heteronuclear $J_{PX}$ spin-spin coupling constants in the $Z_{II}$-CG and $Z_{II}$-GC conformations.

<table>
<thead>
<tr>
<th>conf. type</th>
<th>$J_{H3'}/5''-P$ /Hz</th>
<th>$J_{C5'-P}$ /Hz</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>8.01/0.15</td>
<td>-3.71</td>
</tr>
<tr>
<td>B</td>
<td>2.74/3.47</td>
<td>-5.81</td>
</tr>
</tbody>
</table>

Table 5.13: Calculated heteronuclear $J_{PX}$ spin-spin coupling constants in the $Z_{II}$-CG and $Z_{II}$-GC conformations.

<table>
<thead>
<tr>
<th>conf. type</th>
<th>$J_{H3'}/5''-P$ /Hz</th>
<th>$J_{C5'-P}$ /Hz</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>8.01/0.15</td>
<td>-3.71</td>
</tr>
<tr>
<td>B</td>
<td>1.11/5.57</td>
<td>-5.95</td>
</tr>
</tbody>
</table>

Table 5.14: Calculated heteronuclear $J_{PX}$ spin-spin coupling constants in the $Z_{II}$-CG and $Z_{II}$-GC conformations.

<table>
<thead>
<tr>
<th>conf. type</th>
<th>$J_{H3'}/5''-P$ /Hz</th>
<th>$J_{C5'-P}$ /Hz</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2.74/3.47</td>
<td>-5.81</td>
</tr>
<tr>
<td>B</td>
<td>1.11/5.57</td>
<td>-5.95</td>
</tr>
</tbody>
</table>

Table 5.15: Calculated heteronuclear $J_{PX}$ spin-spin coupling constants in the $Z_{II}$-CG and $Z_{II}$-GC conformations.

<table>
<thead>
<tr>
<th>conf. type</th>
<th>$J_{H3'}/5''-P$ /Hz</th>
<th>$J_{C5'-P}$ /Hz</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>8.01/0.15</td>
<td>-3.71</td>
</tr>
<tr>
<td>B</td>
<td>2.74/3.47</td>
<td>-5.81</td>
</tr>
</tbody>
</table>
$^{3}J_{C4'-P}^{i} < ^{3}J_{C2'-P}$ for $B_{II}$. Similarly, $^{3}J_{C4'-P}^{i} < ^{3}J_{C2'-P}$ for $Z_{II}$-CG but the trend is opposite for $Z_{II}$-GC: $^{3}J_{C4'-P}^{i} > ^{3}J_{C2'-P}$ (cf. Table 5.11). This behaviour is due to the position of $P$ relative to $C2'$ and $C4'$, respectively. When $P$ and $C2'$ are placed anti to each other, then $^{3}J_{C2'-P} > ^{3}J_{C4'-P}$ and vice versa for the case of $C4'$ in the anti position relative to $P$.

Experimental values of the $^{3}J_{H3'-P}$ coupling constant in B-DNA have been reported to be 1.3 Hz for $B_{I}$ with $\epsilon = 170^\circ$ and 10 Hz for $B_{II}$ with $\epsilon = 255^\circ$ [60]. The fact that the published values of the $^{3}J_{H3'-P}$ coupling constant correspond to certain values of $\epsilon$ explains why a good agreement with these experimental data has been obtained using the approach $A$ but not the approach $B$ (cf. Table 5.10). The values of $\epsilon$ for $B_{I}$ and $B_{II}$ were 189$^\circ$ and 267$^\circ$, respectively, in the case of $A$ but 191$^\circ$ and 283$^\circ$, respectively, in the case of $B$.

For Z-DNA, no experimental NMR data suitable for comparison were available, as most Z-DNA structures have been determined by X-Ray crystallography due to difficulties in inducing the Z-form of DNA in a solution.
Chapter 6

Conclusions

The central objective of this thesis was to gain insight into relationships between the geometric features of the nucleic acids (NA) and their NMR parameters. Theoretical calculations at the DFT level have been performed on models representing structural constituents of B- and Z-DNA. These models employed various geometric constraints whose choice appeared to be crucial for attaining the relevant description of the systems studied. The behaviour of chemical shieldings and spin-spin coupling constants as influenced by structural parameters has been interpreted in terms of torsion angle effects, through-bond and through-space interactions. Moreover, several correlations of the theoretical results and experimental observations have been established.

In the first part of the study, an attention has been paid to carbon and nitrogen chemical shifts in B-DNA. The models of deoxyribonucleosides have been employed in the investigation. For these systems, the influence of the base orientation, the sugar ring conformation, the hydroxymethyl group conformation and the hydrogen bonding has been explored. Changes in the base position have been found to introduce the largest effects on the chemical shift of C1’, C2’, N1/N9 and N3. In the case of all the four atoms the behaviour of the chemical shifts as a function of $\chi$ has been ascertained to be similar for the two purine and two pyrimidine nucleosides. Varying the torsion angle $\chi$ gave rise to larger changes in C1’ chemical shifts for deoxyadenosine and deoxyguanosine than for deoxycytidine and deoxythymidine. On the contrary, the trends found for C2’ were close for all bases. In the case of N9, the calculations revealed a significant downfield shift for T relative to A, G and C. In addition, the N9 chemical shift appeared to provide direct information on the sugar pucker. In contrast, the hydroxymethyl group conformation has emerged to cause only slight changes. An exception to this finding is the N9 chemical shift in the gg-conformer of deoxyguanosine ($S$, anti) compared
to other hydroxymethyl group conformers with either syn or anti base position. This exception results in 7 ppm difference in N9 chemical shift between the syn and anti orientations of the base.

Such a difference has not been observed in experimental data for the $[d(G_4T_4G_4)]_2$ quadruplex. Since the comparison of the theory and experiment provided a promising agreement in the case of C1’ and N7, the discrepancy found for N9 seems to be due to a specific geometric feature of the quadruplex, other than the hydrogen bonding, influencing the N9 chemical shift. This conclusion has been highly supported by the results of the study of the hydrogen bonding influence.

The latter study has been performed on models representing the arrangement of hydrogen bonds in the G-quartet of the $[d(G_4T_4G_4)]_2$ quadruplex. It revealed that the hydrogen bonding gives rise to a systematic increase of the chemical shift in both the syn and anti regions of the glycosidic torsion angle.

The second part of the thesis focused on studying $^{31}$P NMR parameters in B- as well as Z-DNA. The aim was to explore the impact of the phosphodiester conformation on $^{31}$P chemical shielding and heteronuclear $J_{PX}$ spin-spin coupling constants. Namely, the influence of P–O ester torsion angles $\zeta$ and $\alpha$ has been investigated on the model of B$\Gamma$-DNA. Using the chosen approach, the phosphorus chemical shielding has appeared to be independent of the value of the $\zeta$ torsion angle. On the contrary, the variation of the $\alpha$ torsion angle resulted in changes which were quite significant with regard to experimental observations. Despite several suggestions that the phosphorus chemical shielding anisotropy may be related to the O–P–O bond angle, no simple correlation has been provided by theoretical calculations. The systematic study of spin-spin coupling constants confirmed that $^3J_{H3'P}$ may serve as a useful probe of the phospho-diester conformation in nucleic acids. An encouraging agreement of calculated and experimental coupling constants has been ascertained, especially in the case of the sum of $^3J_{H3'P}$ and $^3J_{C4'P}$. Besides studying $^{31}$P NMR parameters as a function the $\zeta$ and $\alpha$ torsion angle, differences in the phosphorus chemical shielding and $J_{PX}$ coupling constants between two distinct phosphodiester backbone conformations, i.e., $gg$ and $gt$, have been assessed. The comparison of the NMR parameters for these two conformation types has been performed for both B-DNA (B$\Gamma$ vs. B$\Pi$) and Z-DNA (Z$\Pi$-CG vs. Z$\Pi$-GC). A promising agreement of the direction of the calculated phosphorus chemical shielding and the direction of experimental chemical shifts in the two conformations has been found. Moreover, for the case of Z-DNA the absolute theoretical $gg$-$gt$ difference obtained is close to the experimental one. Using the different approaches to the geometry optimizations, the calculations of chemical shieldings as well as spin-spin
couplings provided different extent of the correlation between the theoretical results and the experimental data. This fact indicates the high importance of a deliberate choice of geometric constraints for the optimizations. The study of $^{31}$P NMR parameters in the sugar-phosphate backbone of nucleic acids is still in progress.

**Presentations**

The part of this thesis concerning calculations of $^{13}$C and $^{15}$N chemical shifts in B-DNA was presented at the following national and international meetings:


2. J. Přečchtělová, M. L. Munzarová & V. Sklenár: *$^{13}$C and $^{15}$N Chemical Shifts in Deoxyribonucleosides as Studied by DFT* (lecture) – 18th NMR Valtice, Valtice, Czech Republic, April 28–30, 2003
DFT studium NMR chemických posunů a spin-spinových interakčních konstant v nukleových kyselinách

Tato práce se na teoretické úrovni zabývala studiem chemického stínění a spin-spinových interakčních konstant v nukleových kyselinách v závislosti na jejich geometrických charakteristikách. Studium bylo zaměřeno do dvou oblastí. První se týkala $^{13}$C a $^{15}$N chemických posunů v B-DNA, zatímco v oblasti druhé šlo o výpočet $^{31}$P NMR parametrů, a to nejen v B-, ale i v Z-DNA.

Uhlíkové a dusíkové chemické posuny byly studovány na modelech desoxyribonukleosidů. Ze strukturních rysů byl sledován vliv orientace báze okolo glykosidické vazby, vliv konformace cukru a hydroxymethyllové skupiny a v neposlední řadě také vliv tvorby vodíkových vazeb. Ukázalo se, že nejcitlivější na pozici báze jsou chemické posuny atomů C1', C2', N1/N9 a N3. Ze srovnání *south* a *north* konformeru desoxyguanosinu (G) pak plyne, že N9 chemický posun ($\delta_{N9}$) může poskytovat užitečné informace o konformaci cukru v nukleových kyselinách. Na základě studia vlivu orientace hydroxymethyllové skupiny bylo zjištěno, že $gg$-rotamer desoxyguanosinu s umístěním báze do *anti* polohy má oproti ostatním rotamerům mnohem větší N9 chemický posun, což vede k rozdílu 7 ppm mezi *syn* a *anti* oblasti glykosidického torzního úhlu. Tento rozdíl nebyl pozorován v experimentálních datech pro [d(G₄T₄G₄)]₂ quadruplex. S ohledem na objasnění zmíněné nesrovnalosti bylo potřeba zjistit, jaký efekt má na $\delta_{N9}$ tvorba vodíkových vazeb. Proto byly provedeny výpočty s použitím modelů reprezentujících geometrické uspořádání v gauaninovém quartetu quadruplexu. Výsledky ukázaly, že přítomnost vodíkových vazeb systematicky zvyšuje hodnotu $\delta_{N9}$ v *syn* i *anti* oblasti.

Cílem druhé části práce věnované $^{31}$P NMR parametrům bylo studovat vliv konformace cukr-fosfátové páteře v DNA na velikost chemického stínění...
SOUHRN

fosforu a na velikost heteronukleárních $J_{PX}$ spin-spinových interakčních konstant. Pro model B1-DNA byla sledována závislost NMR parametrů na torzních úhlech $\zeta$ a $\alpha$ určujících rotaci okolo P–O esterových vazeb. Nalezené hodnoty chemického stínění vykázaly pouze závislost na úhlu $\alpha$, nikoliv na úhlu $\zeta$. Na základě systematické studie interakčních konstant se potvrdilo, že $J_{H3'P}$ může sloužit jako užitečné vodítko při určování fosfodiesterové konformace. Tento závěr byl navíc silně podpořen dosažením mimořádně dobré shody výpočtu s dostupnými experimentálními daty. Kromě závislosti NMR parametrů na konkrétních torzních úhlech byla pozornost věnována srovnání chemického stínění a interakčních konstant pro $gg$ a $gt$ konformaci fosfodiesteru. Toto srovnání bylo provedeno pro B-DNA (B1 vs. B1H) i Z-DNA (krok deoxycytidin-deoxyguanosin vs. krok deoxyguanosin-deoxycytidin v $Z_{II}$ konformaci poly(dCdG)). Směr vypočteného stínění fosforu koreluje s experimentálními hodnotami chemických posunů, a to u obou typů DNA. U Z konformace bylo navíc dosaženo slibné shody i co se týče absolutní korelace teoretického a experimentálního rozdílu chemického stínění mezi $gg$ a $gt$ konformací.
Bibliography and references


[58] BMRB accession number 4753; related PDB entries: 1EKH, 1EKI.

[59] BMRB accession number 4104; related PDB entry: 1AHD.


Appendix A

Experimental data
Figure A.1: $^1$H-$^{15}$N 2D correlation spectrum of H6/H8 and N1/N9 in the K$^+$ quadruplex [d(G$_4$T$_4$G$_4$)]$_2$. Peaks are denoted by the residue number.
Appendix B

Geometry Optimizations
### Torsion Angle Optimization

<table>
<thead>
<tr>
<th>Torsion Angle</th>
<th>Before the Optimization [degs]</th>
<th>After the Optimization [degs]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \zeta = 240 )</td>
<td>( \zeta = 265 )</td>
</tr>
<tr>
<td>( \beta_1 )</td>
<td>176</td>
<td>176.0</td>
</tr>
<tr>
<td>( \gamma_1 )</td>
<td>48</td>
<td>47.4</td>
</tr>
<tr>
<td>( \delta_1 )</td>
<td>128</td>
<td>140.6</td>
</tr>
<tr>
<td>( \epsilon_1 )</td>
<td>184</td>
<td>187.0</td>
</tr>
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<td>( \zeta )</td>
<td>240–300</td>
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<tr>
<td>( \alpha )</td>
<td>298</td>
<td>293.0</td>
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<tr>
<td>( \beta_2 )</td>
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<td>176.0</td>
</tr>
<tr>
<td>( \gamma_2 )</td>
<td>48</td>
<td>47.4</td>
</tr>
<tr>
<td>( \delta_2 )</td>
<td>128</td>
<td>140.6</td>
</tr>
<tr>
<td>( \epsilon_2 )</td>
<td>184</td>
<td>187.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Torsion Angle</th>
<th>Before the Optimization [degs]</th>
<th>After the Optimization [degs]</th>
</tr>
</thead>
<tbody>
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<td>( \alpha = 298 )</td>
</tr>
<tr>
<td>( \beta_1 )</td>
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<td>176.0</td>
</tr>
<tr>
<td>( \gamma_1 )</td>
<td>48</td>
<td>50.3</td>
</tr>
<tr>
<td>( \delta_1 )</td>
<td>128</td>
<td>135.9</td>
</tr>
<tr>
<td>( \epsilon_1 )</td>
<td>184</td>
<td>206.2</td>
</tr>
<tr>
<td>( \zeta )</td>
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<td>285.1</td>
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<tr>
<td>( \alpha )</td>
<td>270, 298, 330</td>
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<tr>
<td>( \beta_2 )</td>
<td>176</td>
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<td>( \gamma_2 )</td>
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<td>50.3</td>
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<tr>
<td>( \delta_2 )</td>
<td>128</td>
<td>135.9</td>
</tr>
<tr>
<td>( \epsilon_2 )</td>
<td>184</td>
<td>206.2</td>
</tr>
</tbody>
</table>

Table B.1: The optimization of geometries representing the models of B1 conformation with different values of the \( \zeta \) and \( \alpha \) torsion angle, respectively – the comparison of backbone torsion angles before and after the optimization.