ISTANBUL TECHNICAL UNIVERSITY ★ INSTITUTE OF SCIENCE AND TECHNOLOGY

INVESTIGATION OF STRUCTURE AND FUNCTIONAL PROPERTIES OF LNA (LOCKED NUCLEIC ACIDS) BY COMPUTATIONAL TOOLS

M.Sc. Thesis by
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Science Programme:	**Advanced Technologies in Engineering**
Programme:	**Molecular Biology, Genetics and Biotechnology**

Supervisor : Assoc. Prof. Dr. Cenk SELÇUKİ

May 2007

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MAY 2007

İSTANBUL TEKNİK ÜNİVERSİTESİ ★ FEN BİLİMLERİ ENSTİTÜSÜ

HESAPSAL ARAÇLARLA KİLİTLİ NÜKLEİK ASİTİN (LNA) YAPISAL VE FONKSİYONEL ÖZELLİKLERİNİN ARAŞTIRILMASI

YÜKSEK LİSANS TEZİ

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PREFACE

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May, 2007 Neslihan Canbek

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ABBREVIATIONS

A: Adenine
ATP: Adenosine Triphosphate
CLL: Chronic Lymphocytic Leukaemia
C: Cytosine
DFT: Density Functional Theory
DIS: Dimerization Initiation Site
DNA: Deoxyribonucleic Acid
$E_C[P]$: The Correlation Functional.
$E_X[P]$: The Exchange Functional
FAD: Flavin Adenine Dinucleotide
G: Guanine
HF: Hartree-Fock Self-Consistent Field Approach
HIV: Human Immunodeficiency Virus
LNA: Locked Nucleic Acid
MM: Molecular Mechanics
NAD: Nicotinamide Adenine Dinucleotide
N-type: Northern- Type
ON: Oligonucleotide
P: The density matrix.
PCR: Polymerase Chain Reaction
PES: Potential energy surface
QM: Quantum Mechanics
RNA: Ribonucleic Acid
PNA: Peptide Nucleic Acid
SNP: Single Nucleotide Polymorphism
S-type: Southern- Type
T: Thymine
T_m: Melting Temperature
U: Uracil
V: The Nuclear Repulsion Energy
<hP>: The One-Electron Energy
1/2<PJ(P)>: The Classical Coulomb Repulsion of the Electrons.
-1/2<PK(P)>: The Exchange Energy Resulting from the Quantum Nature of Electrons

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ÖZET

Hesapsal Araçlarla Kilitli Nükleik Asitin (LNA) Yapısal Ve Fonksiyonel Özelliklerinin Araştırılması

Kilitli nükleik asit (LNA) bir veya daha fazla LNA nükleotid monomeri içeren sentetik RNA türevidir. Şeker-fosfat ana hattında riboz kısmı yapısal olarak 2'-oksijen ve 4'-karbon atomlarından metilen köprüsü ile sınırlandırılmıştır. Bu bağlantı şeker halkasını DNA ve RNA sekanslarına komplementer hibrid oluşumuna müsade eden 3'-endo (N-tip) konformasyonuna sabitler. Bu sayede LNA bisiklik furanoz birimine sahip RNA taklidi konformasyona kilitlenmiştir. Bu çalışmada kilitli nükleik asitin beş bazı (adenin, timin, urasil, guanin ve sitozin) gaz fazındaki farklı konformasyonları araştırılmıştır. Bu çalışmanın amacı her bir LNA bazının gaz fazındaki konformasyonal evreninden yola çıkarak literatürde deneysel olarak kanıtlanmış tanı ve tedavi yöntemlerinde LNA'ya avantaj getiren nedenleri saptamak ve ileriki çalışmalar için öngörülerde bulunmaktır. Bu yapıların yapısal ve elektronik karakterlerinin hesaplamaları ayrıntılı olarak öncelikle Spartan programı aracılığıyla moleküler mekanik düzeyde ve daha sonra Gaussian 03 sürüm C.02 programı aracılığıyla B3LYP/6-31G** ve HF/6-31G** düzeylerinde incelenmiştir. Çalışmada başlangıç konformasyonu Spartan programında Moleküler Mekanik opsiyonunda seçildi. Konformasyonel araştırma sistematik metotla değiştirildi. Her dönebilen bağ 360° içinde 6 kere döndürüldü. Farklı konformasyonlar seçildi ve mümkün olan tüm yapılar çıkan sonuçlardan elde edildi. Bütün konformasyonlar yoğunluk fonksiyon teorisi düzeyinde B3LYP/6-31G** kullanılarak optimize edildi. Optimize olan yapıların tüm enerjileri, dipol momentleri, frekansları ve geometrileri karşılaştırıldı. Aynı ya da benzer olmayanlar konformasyon listesine eklendi. Optimize olan tüm konformasyonlar için aynı prosedür Hartree-Fock ile HF/6-31G** düzeyinde tekrar yapıldı.

SUMMARY

Investigation of Structural and Functional Properties of LNA (Locked Nucleic Acid) by Computational Tools

Locked nucleic acid (LNA) is a synthetic RNA derivative containing one or more LNA nucleotide monomers. The ribose moiety in sugar-phosphate backbone is structurally constrained by a methylene bridge between the 2'-oxygen and the 4'-carbon atoms. The link 'locks' the sugar ring in the fixed 3'-endo (N-type) conformation preferable for the formation of hybrids with complementary DNA or RNA sequences. Five bases with locked nucleic acid (adenine, thymine, uracil, guanine, and cytosine) have been investigated for their different conformations. The aim of this study is to start with investigating the conformational space of each LNA's completely in the gas phase to determine the advantages of LNA in the diagnostic and therapeutic applications proven in the literature and foresight further studies. These structures have been investigated in detail structural and electronic characteristics firstly at Molecular Mechanics as implemented in Spartan and secondly at B3LYP/6-31G** and HF/6-31G** levels as implemented in Gaussian 03 Version C.02. In the study, an initial structure is chosen by using the Molecular Mechanics (MMFF conformer distribution) option in Spartan program with systematic method. Every flexible bond is rotated 6 times through 360°. Different conformers and all possible structures are found from the resultant output. All the conformers are optimized at the Density Functional Theory (DFT) by using B3LYP/6-31G**. Total energies, dipole moments, frequencies and geometries of the optimized structures are compared. If they are not same or similar, they are added to conformer list. The same procedure is done to optimize all the conformers by using HF/6-31G**.

1. INTRODUCTION

Watson–Crick proposed nucleic acid recognition between two complementary nucleic acid strands by hybridization in 1953; this was a discovery that has proven to be the key to molecular biology and modern biotechnology. Various explorations and examinations of nucleic acid hybridization have been done, and a multitude of nucleic acid analogues have been synthesized. This research effort has been prompted by the promise of therapeutic applications, possible uses within biotechnology and sheer scientific curiosity. Modifications to native nucleic acids can be introduced in the nucleobase, the sugar ring or the phosphodiester backbone [1-4]. In an effort to increase binding affinity towards RNA by conformational restriction, many sugar modified nucleic acids have been prepared [2].

Especially, there is a great interest in synthesized oligonucleotides with a modified furanose sugar moiety. Recent researches have focused on restricting the conformation of the furanose ring into either an Southern- or Northern- type conformation (S-type, N-type) [3, 4]. Double-stranded RNA and DNA are generally favorable to forming A- and B-type helical structures, respectively. In each structure, the furanose moieties in RNA exist in an N-type and those in DNA generally exist in an S-type. When conformation is locked in N-type, it showed strong hybridization ability towards RNA complements, while conformation having a restricted S-type conformation, a linkage exhibited favorable features as an antisense/antigene molecule [4].

In 1998, laboratories in Japan and Denmark first described the synthesis and properties of a novel series of nucleotideanalogues called Locked Nucleic Acids (LNA). LNA is a synthetic RNA derivative containing one or more LNA nucleotide monomers in which the ribose moiety in sugar-phosphate backbone is structurally constrained by a methylene bridge between the 2'-oxygen and the 4'-carbon atoms (Figure 1.1). Initial research focussed on the ß–D-LNA form [5].

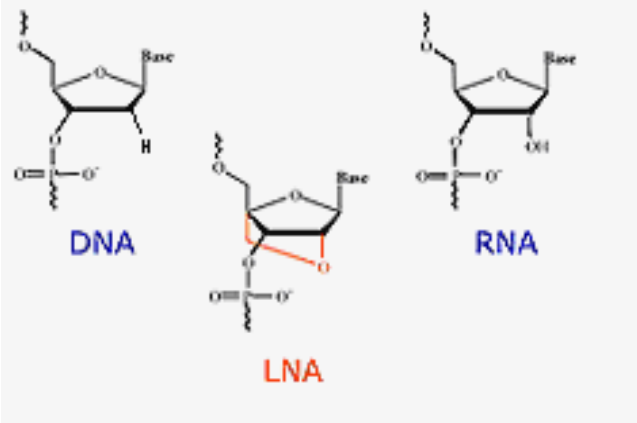


Figure 1.1 : Chemical structures of DNA, RNA and LNA nucleotide.

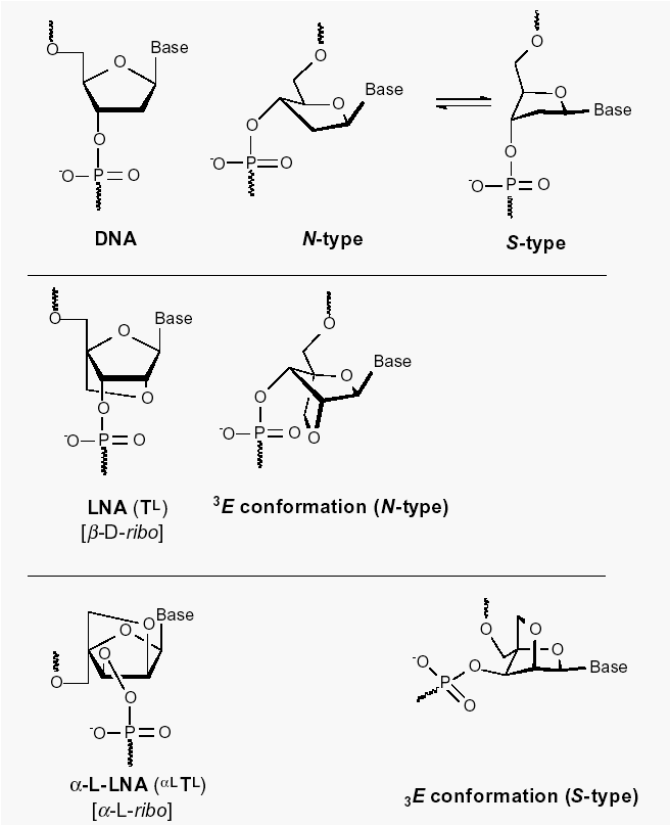


Figure 1.2 : The structures of nucleotide monomers of DNA, LNA (T^L : Base=thymin-1-yl) and α-L-LNA ($\alpha^L T^L$: Base = thymin-1-yl). The conformational equilibrium between N-type and S-type conformers (the C3'-endo/^{3}E north(N)-type and C3'-exo/$_3$E south(S)-type) of an unmodified DNA monomer. Locked N-type (C3'-endo/^{3}E) and S-type (C3'-exo/$_3$E) furanose conformations of an LNA and an α-L-LNA monomer, respectively [21].

The link ‘locks’ the sugar ring in the fixed 3’-endo (N-type) conformation, which is preferable for the formation of hybrids with complementary DNA or RNA sequences [6] (Figure 1.2). Therefore, LNA has a bicyclic furanose unit locked in an RNA-mimicking conformation. This feature gives the LNA probes very high binding affinity but does not compromise their sequence specificity [7-9].

For DNA diagnostics it is important that LNA is stable against cleavage by nucleases, which might contaminate biogenic DNA analytes. Several studies have demonstrated that LNA-modified oligonucleotides exhibit unprecedented thermal stabilities when hybridized with their RNA target molecules [10-12]. Thus, an increase in melting temperature (T_m value) of +2 to +10°C per LNA monomer against complementary RNA compared to unmodified duplexes has been reported [11]. LNA analogues and derivatives have been designed and characterized in the studies. The main LNA analogues are 2'-amino-LNA [13, 14], 2'-thio-LNA [13,16, 75], phosphorothioate-LNA [16], xylo-LNA [49, 50], L-LNA [50] and the diastereoisomeric α-L-LNA [21, 26, 35, 39, 41, 53, 75]. α-L-LNA is the most studied LNA stereoisomer which mimics DNA in contrast to LNA mimics RNA. It is also locked in a C-3'-endo conformation but it induces B-type duplex conformations wheras LNA induces A-type. α-L-LNA shows positive results, it is widely used in antisense studies. Higher affinity LNA hybridization has been tried towards DNA, RNA, LNA and α–L-LNA complementary sequences [13-28].

The pre-organized conformation of the LNA nucleoside was predicted to be an N-type sugar puckering (Figure 1.2), characteristic for A-type double helices, such as RNA-RNA duplexes. This assumption has been confirmed by NMR solution studies and X-ray crystallographic analysis [12, 29-46]. The LNA oligonucleotide conformational structure, examining both sugar puckering and oligonucleotide backbone, has been determined by two-dimensional NMR analysis. The preliminary LNA nucleoside spectra demonstrated the fixed N-type conformation of LNA [7, 8]. Subsequent NMR studies have analyzed the structure of LNA oligonucleotides, either as single stranded oligonucleotides or hybridized to complementary DNA and RNA [12, 31, 33, 35, 40]. The spectra confirmed the locked N-type conformation of the LNA sugar pucker, but also revealed that LNA monomers are able to twist the neighboring, unmodified nucleotides from an S-type towards an N-type

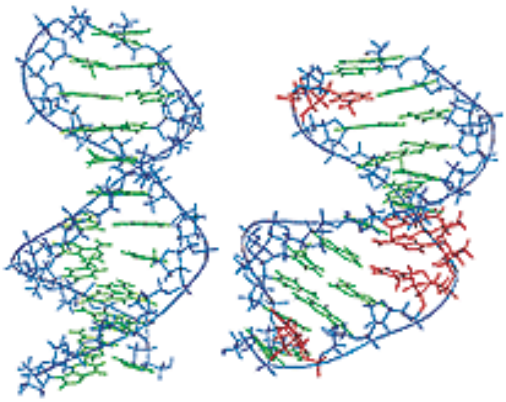
conformation in DNA/LNA mixmer oligos and LNA-containing duplexes. The fixed N-type (3'-endo) conformation of the LNA nucleoside, together with enhanced stacking of the nucleobases results in higher thermal stability of LNA-containing duplexes [38].

By changing the conformation of the helix and by increasing the stability of the duplex, the integration of LNA bases into oligo sequence opens new perspectives to DNA affinity based studies. Affinity determines the ability of the oligo to effectively compete with other molecules for binding to its target messenger RNA (mRNA) and dictates the half-life of the resulting complex. An increase in affinity, therefore, correlates positively with potency and broadens the number of sites in the target that the oligo can address. For instance, LNA may be used to improve techniques requiring high affinity probes as specific as possible like single nucleotide polymorphism (SNP) detection, expression profiling, and in situ hybridization [47-72].

The integration of LNA bases into probes changes the conformation of the duplex when the annealing with DNA bases occurs [38]. The integration of LNA moieties on every third position changes the structure of the double helix from the B to the A type. This conformation allows a much better stacking and then a higher stability. Therefore B-helix DNA has poor stacking and low stability on the other hand A-helix DNA with LNA has good stacking and high stability as seen in Figure 1.3.

By increasing the stability of the duplex, the integration of LNA monomers into the oligonucleotide sequence allows to increase consequently the T_m of the duplex. This characteristic allows reducing the size of the probes and, by the way, to increase the specificity of the probes.

Every corporation of LNA increases the T_m of the duplex. The following table (Table 1.1) shows the average T_m increase for DNA or RNA duplex with oligos containing LNA, RNA or PNA moieties [11].



B-helix DNA A-helix DNA with LNA bases (red-colored)

Figure 1.3 : B-helix DNA and A-helix DNA containing LNA bases

Table 1.1 : A direct advantage of T_m increases of LNA versus RNA and PNA

	LNA	**RNA**	**PNA**
T_m increase / monomer against DNA (°C)	2 - 6	-0.5 - 0.5	0.5 – 2
T_m increase / monomer against RNA (°C)	3 - 8	1 - 1.5	0.5 – 2

1.1. Diagnostic Applications of LNA

Application areas of LNA are wide in molecular biology for analytic and diagnostic purposes. LNA may be used to enhance Real-Time polymerase chain reaction (PCR) probes [60, 66, 67]; in situ hybridization probes [73]; primers for single, multiplex and allele specific PCR [48, 65], capture probes for SNP genotyping [52, 58, 59, 63, 64, 66], for expression analysis [56, 85, 87] and to monitor exon skipping. LNA should be used in any hybridization assay, which requires high specificity and/or reproducibility. The LNA modification suits perfectly to SNP detection. First, the reduction of the size of the probe increases the impact of one mismatch in the stability of the duplex probe/target. Also, by designing probes with an LNA moiety in front of the variable position it becomes possible to discriminate very efficiently the allelic variations [58]. The mismatch would avoid the A helix structure stabilization and then decrease the T_m considerably. This modification increases the specificity of the probe but also its power of discrimination.

There are a lot of advantages of LNA use in vitro or in vivo experiments. First of all the affinity of LNA is higher than the other synthetic probes [11]. The thermal stability of duplexes is increased by LNA due to its RNA-like structure. LNA duplex formation constitutes the most stable Watson-Crick base pairing system [36]. T_m modulation of LNA is higher than the others. Depending on their position along the sequence, LNA bases allow to reach the desired T_m level without losing specificity. Introduction of LNA allows for shorter probes while maintains the same T_m. LNA enhances hybridization performance relative to native DNA, RNA or phosphorothioate. LNA lowers experimental error rates due to better mismatch discrimination. LNA improves signal-to-noise ratio. Also LNA is an enzyme compatible probe in the experiments [71]. It shows increased resistance to certain exo- and endonucleases thus leading to biostability [70]. DNA-LNA chimeras readily activate RNAse H. LNA acts as a substrate for standard molecular biology enzymes: T4 PNK, T4 DNA ligase, DNA polymerases. LNA behaves like DNA, so it is easily transferable to DNA-based assays [48, 50, 70]. LNA is highly soluble in water, complies with oligonucleotide synthesis and analysis methods (QC, purification, etc) and exhibits the same salt dependence as DNA and RNA.

1.2. Therapeutic Applications of LNA

1.2.1 Human immunodeficiency virus (HIV)

LNA antisense oligonucleotides can enhance inhibition of HIV-1 genome dimerization and inhibit virus replication [74, 75, and 77]. Antisense design and efficacy of LNA and DNA oligonucleotide (ON) mixmers have been evaluated targeting the conserved HIV-1 dimerization initiation site (DIS) in these studies. LNA is a high affinity nucleotide analog, nuclease resistant and elicits minimal toxicity. They show that inclusion of LNA bases in antisense ONs augments the interference of HIV-1 genome dimerization. The concomitant RNase H activates by six consecutive DNA bases in an LNA/DNA mixmer. ON uptake via receptor-mediated transfection of a human T-cell line in which the mixmers subsequently inhibit replication of a clinical HIV-1 isolate. Thus, the technique of LNA/DNA

mixmer antisense ONs targeting the conserved HIV-1 DIS region may provide a strategy to prevent HIV-1 assembly in the clinic shown in Figure 1.4.

The HIV genome is a homo-dimer of two sense RNA single strands. The DIS is a stem loop structure with six self-complementary nucleotides at the top. The loop is located between the primer binding site and the splice donor site at the end of the long terminal repeat (LTR) and is involved in the dimerization of the HIV-1 genome, packaging and proviral synthesis the aim is at increasing the potency of the DIS-targeting ONs by exchanging DNA nucleotides for high affinity, nuclease resistance LNA in the DNA sequence creating LNA/DNA mixmers.

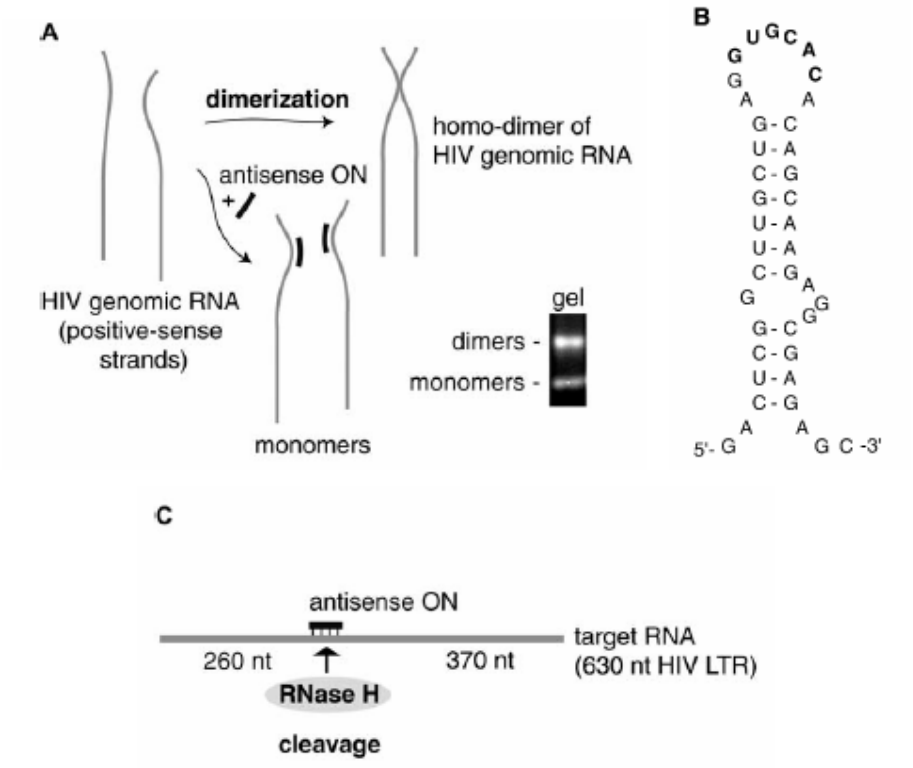


Figure 1.4 : A) Schematic of HIV-1 dimerization and appearance of dimer and monomer on a gel. B) HIV-1 subtype A dimerization initiation site (DIS). The six self-complementary bases are marked in bold. C) Schematic of RNase H activation [77].

1.2.2 Chronic lymphocytic leukaemia (CLL)

Santaris Pharma has a number of innovative new cancer drugs in development each of which targets a molecular mechanism known to be important in the development and progression of certain cancers.

In clinical development of the cancer drugs, Phase III failures have with a number of undesired features that substantially limit their therapeutic use. These include insufficient biostability and low affinity for their target mRNA (both of which negatively affect potency) and a range of dose limiting toxicities that narrows their therapeutic window [76, 92].

The current development programs are based on antisense RNA drugs as seen in Table 1.2. Antagonist technology, termed Locked Nucleic Acid (LNA). To provide high quality manufactured product for these programs, Santaris Pharma has developed extensive and cost competitive manufacturing competencies.

Table 1.2 : Current pipeline of new cancer drugs development in Santaris Pharma. (Retrieved in 04.24.2007)

DRUG ENTITY	MODE OF ACTION	INDICATION	DISCOVERY		DEVELOPMENT		
			In-Vitro	*In-Vivo*	Preclinic	Phase I/II	Phase II/III
SPC2996	Bcl-2 mRNA Antagonist	CLL B-cell Lymphoma					
SPC2968	Hif-1α mRNA Antagonist	Renal & Colerectal Carcinoma Multiple Myeloma					
SPC3042	Survivin mRNA Antagonist	Acute Leukemias Solid tumours + Chemo					
Lead NCE*	Pan-Ras mRNA Antagonist	Cancer					
NCEs	ApoB100 mRNA Antagonist	Hyperlipidemia					
NCEs	MicroRNA Antagonists	Cancer Metabolic Disorders					
NCEs	Soluble TNF-R modulator	Cachexia & Inflam. Dis.					

Improving the potency and safety of antisense oligos are needed to bring antisense therapy into mainstream therapeutics. The term RNA Antagonists signals the strong belief that they will transform antisense therapy into a robust drug platform.

For antisense applications, increased ON stability towards nuclease degradation is important. Other desired properties are high affinity for the target, RNaseH activation, and low toxicity along with good solubility and uptake.

In May, 2005, the company´s most advanced drug candidate (SPC2996) entered a multi-center Phase I/II clinical study in patients with Chronic Lymphocytic Leukaemia (CLL). Further clinical studies will follow with two additional drug candidates, SPC3042 and SPC 2968, which are currently undergoing preclinical development.

There are a lot of review papers from 1999 to date which states LNA as an antisense drug candidate [78-89], optimal oligo design of LNA as a diagnostic tool [90] and making LNAzymes by incorporating LNA-type monomers into DNAzymes to increase RNA cleavage [91].

In present study, locked nucleic acid has been chosen for investigation. Adenine, guanine, cytosine, thymine and uracil bases have been chosen because LNA is a synthetic molecule that can be composed of either DNA or RNA bases. There are some NMR and X-ray representations of LNA in the literature but there is not any conformational data in order to check the reliability and accuracy of the method used. The aim of this study is to investigate the conformational space of each base completely in the gas phase. These structures will be investigated in detail in terms of their structural and electronic characteristics for possible implications in science.

2. THEORY

Modelers study systems ranging from single isolated molecules to proteins containing thousands of atoms immersed in a sea of solvent molecules, yet at the heart of all these calculations is some procedure for calculating the energy of the system. Energies can be calculated using two basic methods known as quantum mechanics and molecular mechanics. Quantum mechanics (QM) offers the most fundamental approach but is restricted to relatively smaller systems. Molecular mechanics (MM) is particularly useful for modeling large molecules and assemblies of molecules [92]. Molecular mechanics (empirical energy calculation) have the great utility in the study of the structure, dynamics and thermodynamics of proteins and other biological macromolecules. For biomolecular systems, computational speed is premium, and the use of more complex terms (higher-order expansions, cross terms, etc.), as employed for accurate modeling of smaller systems, is not practical. Simplicity of the method makes possible simulations of thousand of atoms in a nanosecond range.

One methodological development in recent years has been the density functional theory (DFT) which enables accurate calculations to be performed much more efficiently than traditional quantum mechanical methods, at least for certain types of systems. DFT still uses the Hartree-Fock (HF) self-consistent field approach but does not rely on the wavefunction. Instead, it calculates all the electron interactions in terms of the electron densities within the system. This approach has the potential to give the system's exact energy and is now being used to perform accurate calculations on crystalline solids, molecules on surfaces and large transition-metal complexes [93].

In Hartree-Fock theory the multi-electron wavefunction is expressed as a Slater determinant which is constructed from a set of N single-electron wavefunctions. DFT also considers single-electron functions. However, whereas Hartree-Fock theory does

indeed calculate the full N-electron wavefunction, DFT only attempts to calculate the full electronic energy and the overall electronic density distribution. The central idea underpinning DFT is that there is a relationship between the total electronic energy and the overall electronic density.

In Hartree-Fock theory, the energy has the form:

$E_{HF} = V + \langle hP \rangle + 1/2\langle PJ(P) \rangle - 1/2\langle PK(P) \rangle$ (2.1)

where the terms have the following meanings:

V: The nuclear repulsion energy.

P: The density matrix.

<hP>: The one-electron (kinetic plus potential) energy

1/2<PJ(P)>: The classical coulomb repulsion of the electrons.

-1/2<PK(P)>: The exchange energy resulting from the quantum (fermion) nature of electrons.

In density functional theory, the exact exchange (HF) for a single determinant is replaced by a more general expression, the exchange-correlation functional, which can include terms accounting for both exchange energy and the electron correlation which is omitted from Hartree-Fock theory:

$E_{KS} = V + \langle hP \rangle + 1/2\langle PJ(P) \rangle + E_X[P] + E_C[P]$ (2.2)

where $E_X[P]$ is the exchange functional, and $E_C[P]$ is the correlation functional.

Hartree-Fock theory is really a special case of density functional theory, with $E_X[P]$ given by the exchange integral $-1/2\langle PK(P) \rangle$ and $E_C=0$. The functionals normally used in density functional theory are integrals of some function of the density and possibly the density gradient:

$E_X[P] = \int f(\rho_\alpha(r), \rho_\beta(r), \nabla \rho_\alpha(r), \nabla \rho_\beta(r)) dr$ (2.3)

where the methods differ in which function f is used for E_X and which (if any) f is used for E_C. In addition to pure DFT methods, hybrid methods in which the exchange functional is a linear combination of the Hartree-Fock exchange and a functional integral of the above form have been developed and are widely used. Proposed functionals lead to integrals which cannot be evaluated in closed form and are solved by numerical quadrature.

The true test of any theoretical technique is how well its predictions agree with the values obtained from the experiment. The systems, bioscientists are interested in, are often so complex that experimental data are usually regarded as 'definitive'. Quantum mechanics really scores for problems that involve the making or breaking of bonds and so has been extensively used to investigate the detailed mechanisms of reactions -the nature of the intermediate of the transition state that forms when molecules react. Pople who was awarded the Nobel Prize for chemistry in 1998, aimed to make his empirical calculations produce answers that aspired to the more exact *ab initio* calculations and his "Gaussian" series of programs rapidly became the standard for *ab initio* calculations [93, 94]. Dewar also aimed at direct agreement with the experiment and developed the semi-empirical theories and incorporated them into series of packages which are also widely used around the world [92, 93].

For larger molecules or assemblies of the molecules, quantum mechanics is not usually feasible; consequently such systems are the realm of the molecular mechanics, or *force field methods*. Molecular mechanics approach considers the energy of any arrangement of atoms, for example a particular geometrical shape governed by rotation of bonds, the conformation of a molecule which can be broken into several distinct parts.

First, there is a contribution from the stretching or compressing of bonds. Each of the bonds in the molecule has an 'ideal' value; the equilibrium value of the bond length and energy must be expended to force a bond to deviate from its ideal value. To reasonable first approximation can be modeled is the variation in the energy with the degree of stretching or compression Hooke's law relationship.

The second contribution to the molecular mechanics energy is that due to the 'angle bending'. As with the bond stretching, there is an 'ideal' value for each angle, and deviations from this ideal value for each angle, and deviations from this ideal value require the expenditure of energy. A Hooke's law of relationship is again employed, with ideal values for atoms having a tetrahedral arrangement of bonds.

Most of the variation in the structure of a molecule comes not from bond-stretching or angle-bending, but from rotation about bonds. It is well known that the energy of a molecule varies with its conformation, in other words, the particular 3-D configuration arising from bond rotation. In the example of ethane (C_2H_6), as the carbon- carbon bond rotates, the interactions between the hydrogen atoms change, creating the three-fold periodicity in the energy of the molecule (Figure 2.1). To enable molecular mechanics to model behavior, a torsional potential is employed which enables the locations and relative magnitudes of the potential energy minima and maxima to be reproduced. The Potential energy surface (PES) is conceptual plot of molecular energy for all possible atomic configurations.

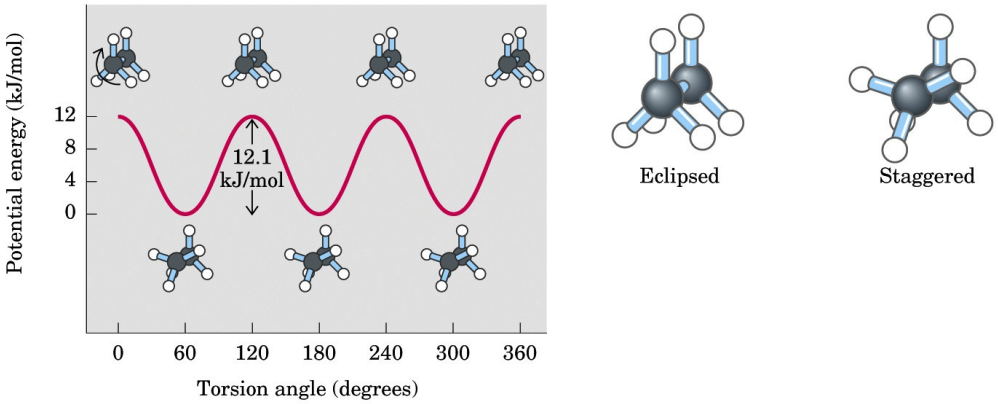


Figure 2.1 : Potential energy surface (PES) of ethane (C_2H_6) [95]

The fourth contribution to the molecular mechanics energy rises from non-bonded forces. The non-bonded forces are usually modeled by a combination of electrostatic interactions and van der Waals interactions. The electrostatic forces arise from the interaction between permanent charges within the molecule due to uneven distributions of electron density in the atoms. Although the electron distribution around an atom is spherically symmetrical on average, the distribution at one instant

is non-uniform, leading to an instantaneous electric dipole. This instantaneous dipole can then induce a dipole in a neighboring atom. The two dipoles now attract together. As the atoms approach closer they attract more and more until energy minimum is reached, whereupon they start to repel each other.

One of the most common applications of the molecular mechanics is the *conformational analysis*. This is the task of exploring all the ways in which the various parts of a molecule rotate in relation to each other, called *conformational space*, in order to identify the conformations with the lowest energy. There are obviously the conformations the molecule is mostly to have. There are more than one minimum which correspond to more than one stable arrangement in the conformational energy surface. Moreover, one of these minima is more stable (lower in energy) than the others.

The variation in the energy of a molecule as it changes its conformation can be linked to the way in which the height above sea-level on the Earth varies with the geographical location (Figure 2.2). The height is a function of just two variables (the longitude and latitude), whereas a molecule's energy can be a function of many variables. In the case of a molecule, the energy, which is equivalent to the altitude, varies with the torsion angle values of the rotatable bonds. There three special types of points in both the geographical and the molecular systems. Points of maximum altitude correspond to the tops of fells; these may be either local or global. Local maximum is the highest peak in its locality but it is lower than the global maximum. The third important point of feature is the saddle points; these correspond to mountain passes and are the easiest path from one minimum to another. On the molecular energy surface conformations corresponding to energy minima are interested in primarily because these are the most stable structures of a molecule. It may also be important to take account of the actual energy value; only those conformations that are within a few kilojoules of the global energy minimum are likely to be accessible to any significant extent [92].

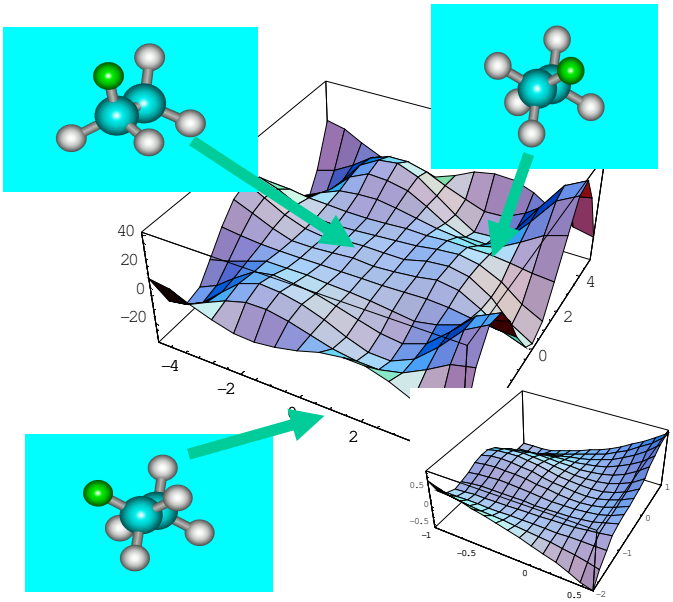


Figure 2.2 : 3-D PES for 1-chloro-ethane (C_2H_5Cl) [42].

There are several different ways to carry out a conformational search. Two of the simplest methods are known as the systematic research and the random search. The purpose of the methods such as those is to locate conformations of molecules in the region of conformational minima. These 3-Dimensional structures then passed to a minimization program which actually locates the true position of the nearest minimum energy structure.

The possible conformers of LNA bases have been studied by the potential energy surface scan using the the Molecular Mechanics (MMFF) option in Spartan Program [96] (Figure 2.3). The rotational analysis has been carried out through C–N, C–C, C–O and O-P bonds to locate all the conformers. The main torsional angles (OCNC, $CCOP_1$, $CCOP_2$, CCCN, CCCO, COPO, CCOC, OCNC, and NCNC) are varied in steps of 30˚ between 0˚ and 360˚ generating different number of initial points in each base. After that these structures have been carefully examined and different structures have been determined for further optimization. The geometries were then optimized at the B3LYP level of theory and HF theory implementing the 6-31G** basis set by using the Gaussian03 series of programs [97].

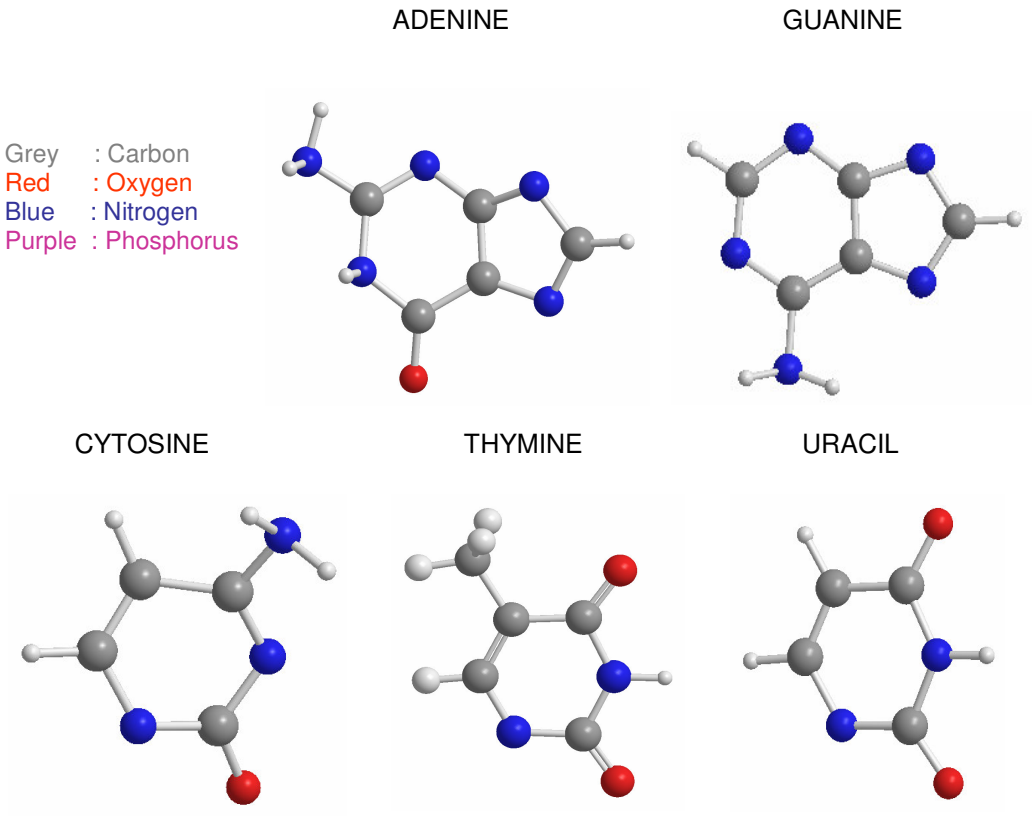


Figure 2.3 : Chemical structures of bases

3. METHODOLOGY

In this study five bases with locked nucleic acid (adenine, thymine, uracil, guanine, and cytosine) have been investigated for their different conformations in the gas phase. These structures have been investigated in detail for structural and electronic characteristics firstly with Molecular Mechanics as implemented in Spartan [96] and secondly at B3LYP/6-31G** and HF/6-31G** levels as implemented in Gaussian 03 Version C.02 [97]. The theoretical procedure is given in the below list and the representations of two side views of LNA adenine base comparing with the literature data is given in the Figure 3.1.

- Choose an initial structure
- Using the Molecular Mechanics (MMFF conformer distribution) option in Spartan program perform an initial conformational search. The net charge is -4 and multiplicity is 1. Conformation is altered with systematic method. Every flexible bond is rotated 6 times through 360°. The torsion increment is 12 in the bicylic sugar oxygen.
- Select different conformers and find all possible structures from the resultant output.
- Optimize all the conformers at the Density Functional level (DFT) by using B3LYP/6-31G**.
- Compare total energies, dipole moments and geometries of the optimized structures.
- If they are not same or similar, add to conformer list.
- Frequency calculations are done for all optimized conformers. When frequency is (+) and forces are lower from the threshold value, the exact conformer is handled. The others are saddle points.
- Repeat same DFT procedure to optimize all the conformers at the Hartree Fock by using HF/6-31G**.

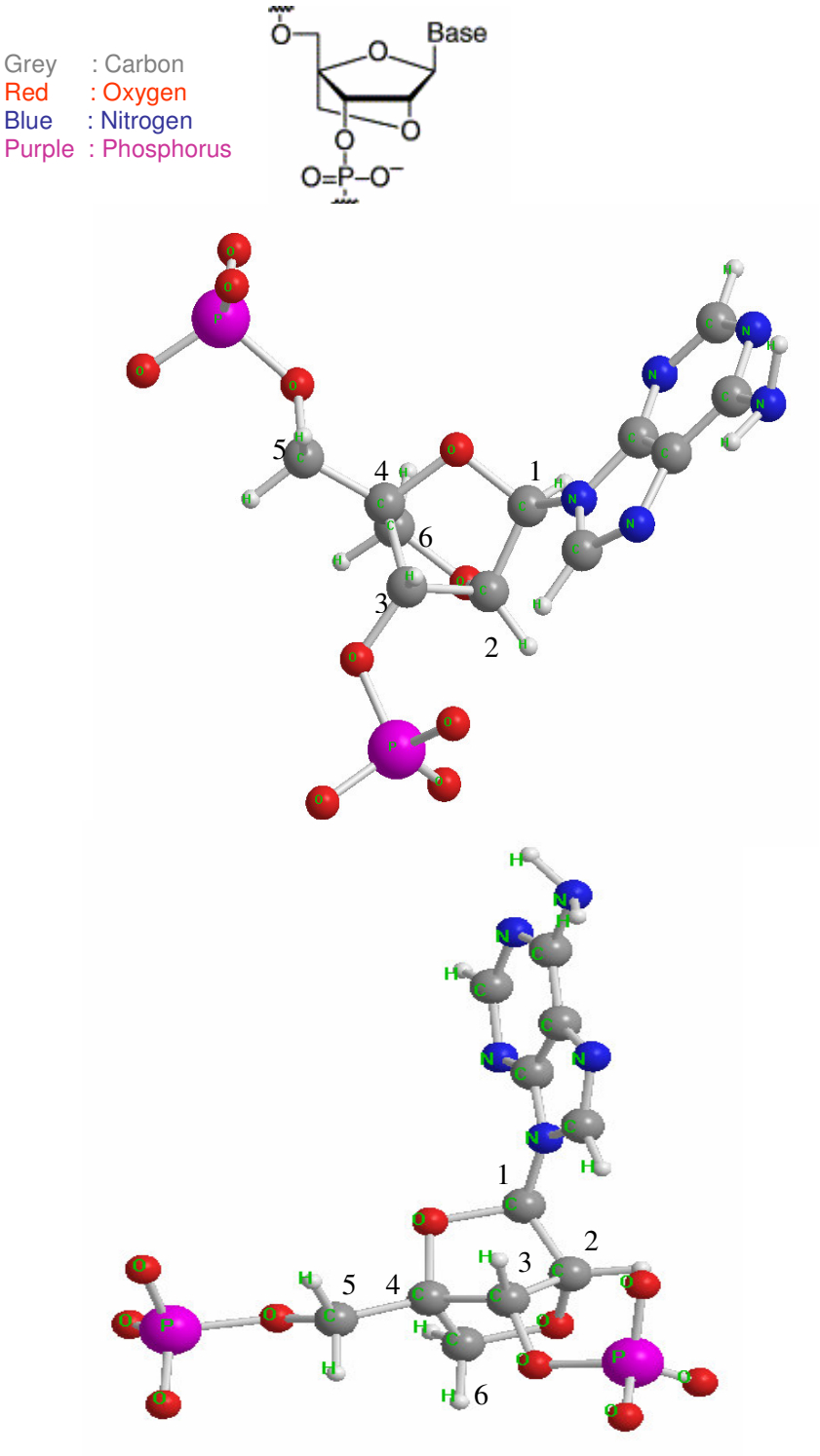


Figure 3.1 Schematic structure of LNA in the literature and two side views of LNA in the study. (Order of numbering is based on the study of Jensen et al.) [38].

4. RESULTS AND DISCUSSION

In this study, five bases with locked nucleic acid (adenine, thymine, uracil, guanine, and cytosine) have been investigated for their different conformations in the gas phase by using Molecular Mechanics as implemented in Spartan and the conformational space for each LNA base has been scanned to determine all possible conformers in the gas phase. All structures are taken which are determined by a Molecular Mechanics (MM) conformational search by using the graphical interface program Spartan [96]. All the structures are then fully optimized with Quantum Mechanics (QM) at B3LYP/6-31G** and HF/6-31G** levels as implemented in Gaussian 03 Version C.02 [97]. Also there are some exceptional structures which are reproduced by comparing the bases conformations taken from Gaussian results. The nature of all optimized structures is determined by the number of positive and negative frequencies in the Hessian matrix. Minima have all positive frequencies, whereas transition states are characterized by one and only one negative frequency. Two or more negative frequencies indicate a saddle point on the potential energy surface. We have shown the negative frequencies of the some structures in Tables 4.2.1, 4.5.1 and Figures 4.2.3, 4.5.3 to emphasize that although similar structures can be obtained with the different methods, their characteristics and nature may be different.

The computed energies, dipole moments, relative energies are given in the Tables 4.1.1, 4.2.1, 4.3.1, 4.4.1 and 4.5.1. The optimized structures are displayed in Figures 4.1.1-4.1.3, 4.2.1-4.2.3., 4.3.1-4.3.3, 4.4.1-4.4.3 and 4.5.1-4.5.3. Some selected interatomic geometrical parameters are also shown on the structures. The following discussion will focus on comparisons of the base structures, their effects on the relative stability. Computed results will be compared to the experimental and computational data in the literature where available.

4.1. Adenine LNA

Adenine is one of the two purine nucleobases used in forming nucleotides of the nucleic acids. It has a variety of roles in biochemistry including cellular respiration, in the form of both the energy-rich adenosine triphosphate (ATP) and the cofactors nicotinamide adenine dinucleotide (NAD) and flavin adenine dinucleotide (FAD), and protein synthesis, as a chemical component of DNA and RNA. In DNA, adenine binds to thymine via two hydrogen bonds to assist in stabilizing the nucleic acid structures. In RNA, which is used in the cytoplasm for protein synthesis, adenine binds to uracil [95].

There are seven conformers in the Molecular Mechanics approach taken from Spartan program. The overall stability order of conformers has been determined to be 1> 2 > 3 > 4 > 5 > 6 > 7 as seen in the Figure 4.1.1 and the Table 4.1.1. 3rd conformer of the MM approach gives the same result in each QM approaches (2nd conformer in DFT and HF) and it is the most stable conformer in DFT and HF. The second stable conformer is also the same in each approach (4th in MM, 2nd in DFT, 2nd in HF). 5th and 7th conformers pairs with 3rd and 4th conformers of DFT. 1st, 2nd and 6th pairs with 5th of DFT and 3rd and 4th of HF conformers. Dipole moments also give the same results because it presents the orientation of lone pairs, thus the electronegative O and N atoms. The alignment in the same direction causes a large value whereas alignment in reverse direction results in a smaller dipole moment.

There are five conformers in DFT taken from Gaussian program. The overall stability order of conformers has been determined to be 1> 2 > 3 > 4 > 5 as seen in the Figure 4.1.2 and the Table 4.1.1. In the most stable conformer, conformer 1, bases nitrogen is much closer to close phosphate than the second stable conformer, conformer 2. First four conformers show 2nd type of structure. When the distant phosphate group comes close to the base, the overall stability decreases (conformer 3 and 4). In the 5th conformer first type of structure can be seen. There is not any proton transfer in the conformers of LNA adenine.

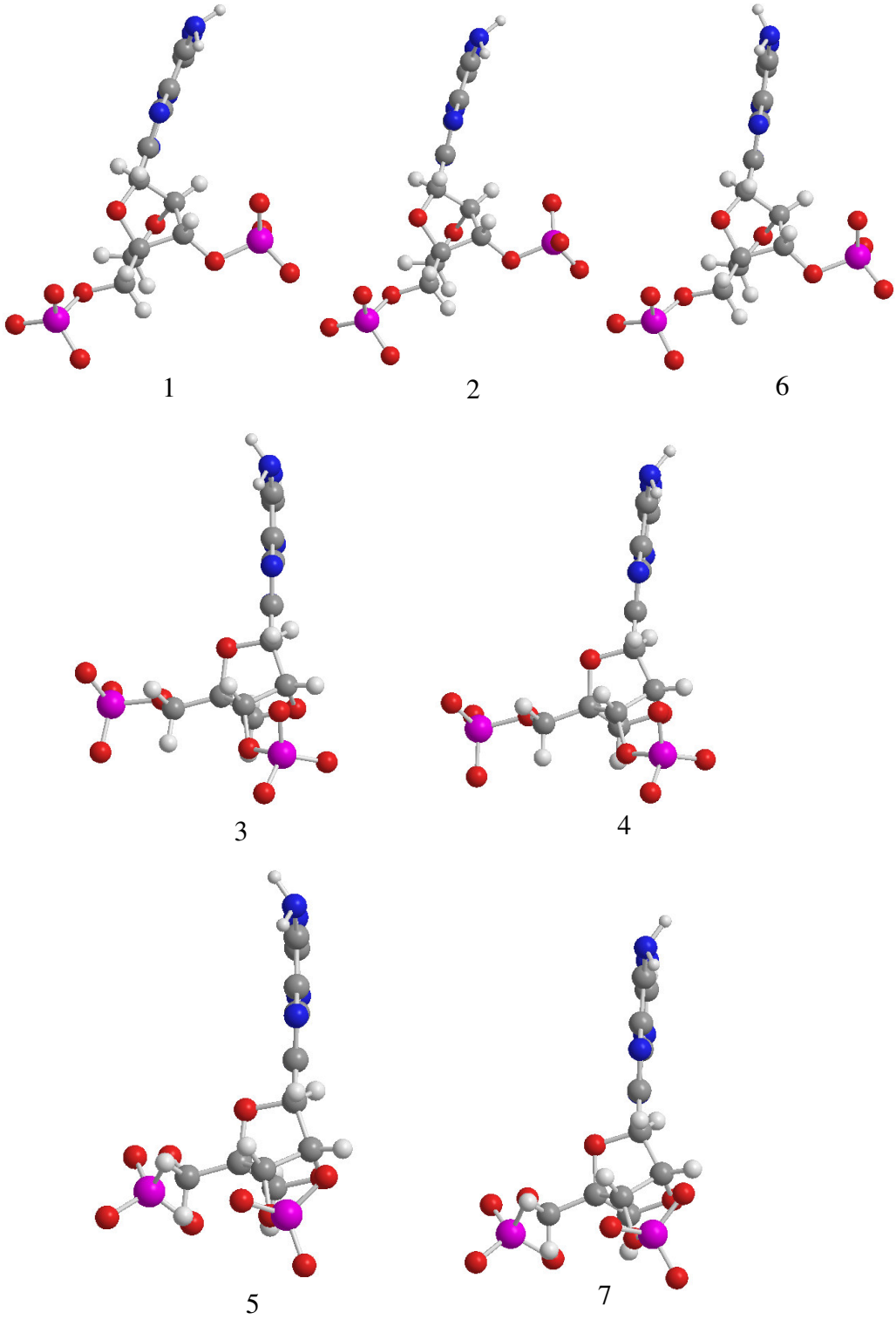


Figure 4.1.1 : MM conformers of adenine LNA monomer

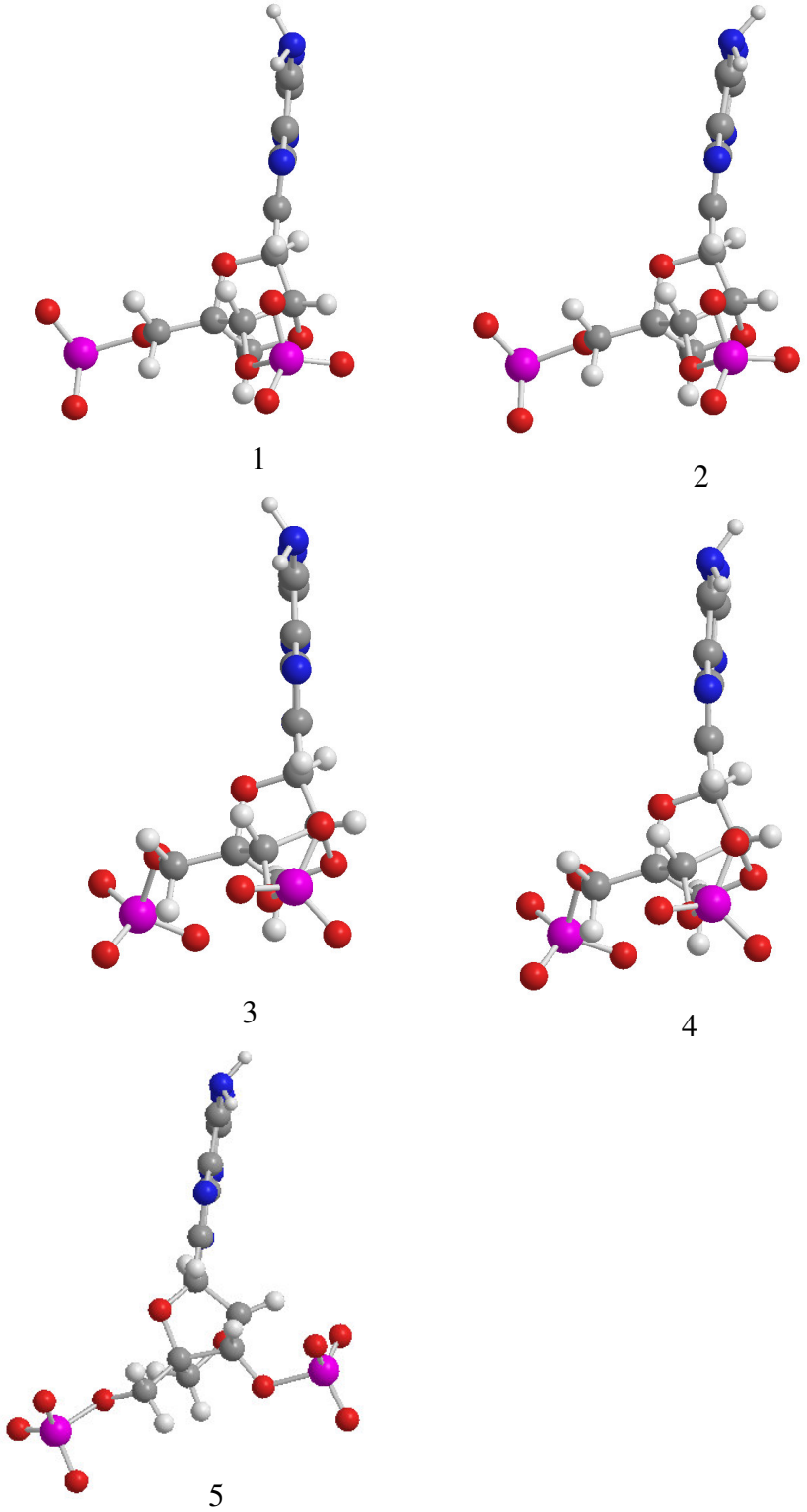


Figure 4.1.2 : DFT conformers of adenine LNA monomer

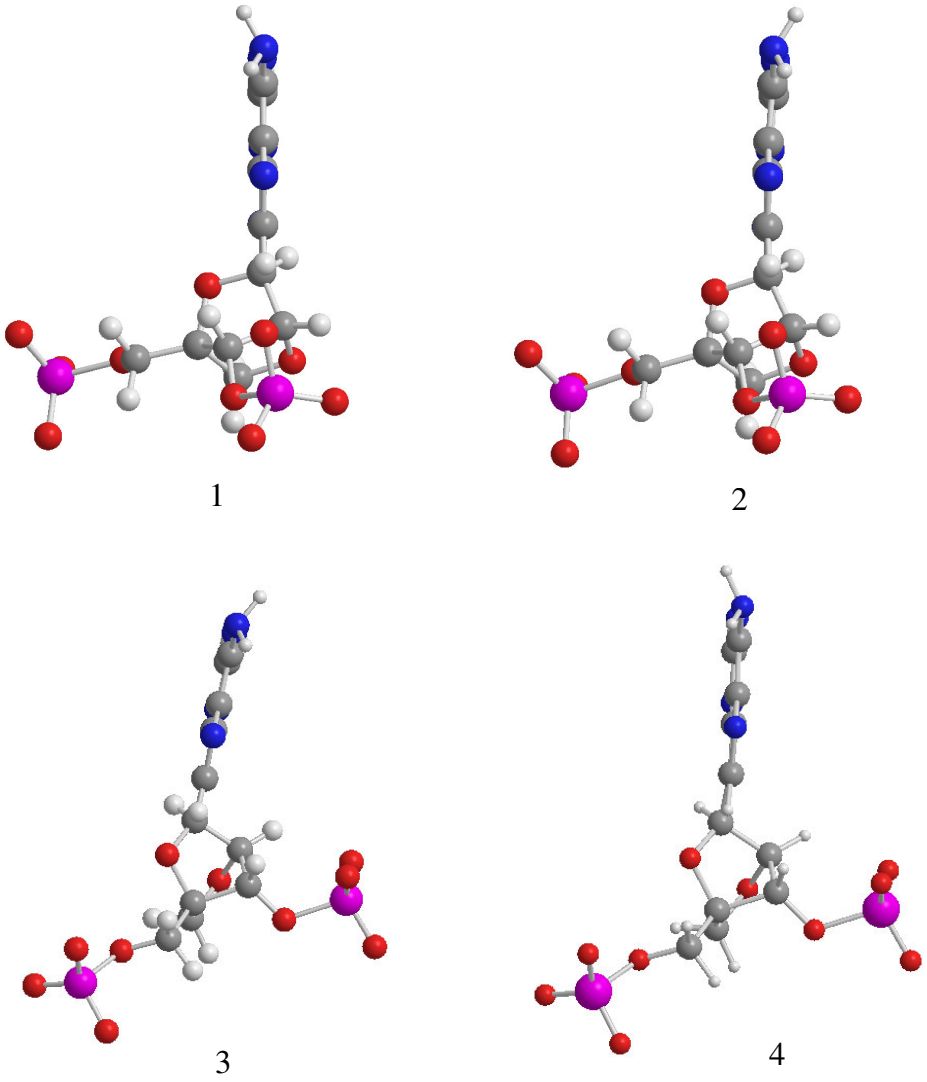


Figure 4.1.3 : HF conformers of adenine LNA monomer

There are four conformers in HF. The overall stability order of conformers has been determined to be 1> 2 > 3 > 4 as seen in the Figure 4.1.3 and the Table 4.1.1. First two conformers seem like first two conformers of DFT. 3rd conformer of DFT seems like 5th conformer of HF. 4th conformer of HF base nitrogen is further away from phosphate group, therefore it is less stable than 3rd conformer of HF. HF calculations can find conformers which cannot be optimized by DFT

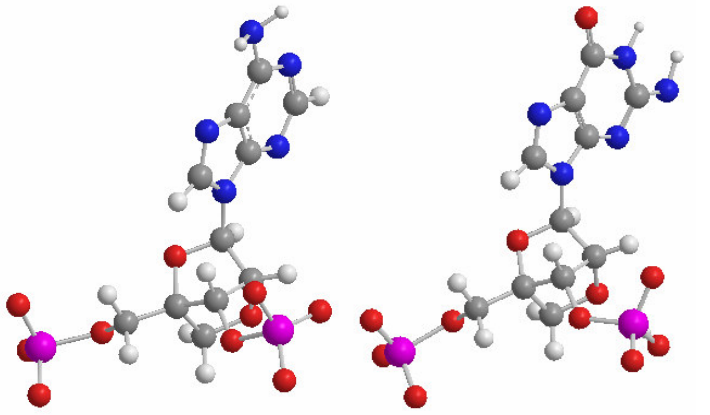
Table 4.1.1 : Conformer Distribution (Conf), Heats of Formation (Energy, kcal/mol for MM), Total Electronic Energies (Energy, hartrees for DFT and HF), Relative

Energies (E_{REL}, kcal/mol), and Dipole Moments (μ, Debye) of adenine LNA conformers

MM			DFT				HF			
Conf	Energy	E_{REL}	Conf	Energy	E_{REL}	μ	Conf	Energy	E_{REL}	μ
1	104.18	0.00					3	-2125.014	6.05	36.20
2	104.88	0.69	5	-2134.275	7.92	30.14				
3	105.16	0.97	1	-2134.288	0.00	29.65	1	-2125.024	0.00	34.71
4	105.75	1.56	3	-2134.283	2.94	30.06				
5	106.25	2.06	2	-2134.287	0.32	30.98	2	-2125.023	0.60	36.39
6	106.34	2.16					4	-2125.013	6.88	36.63
7	106.70	2.51	4	-2134.283	3.25	31.09				

.

Adenine base ring structure does not allow become close to proton transfer from bases nitrogen to either close or distant phosphate group like other LNA bases shown in Figure 4.1.4. On the other hand all LNA guanine conformers have proton transfer shown in Figure 4.5.1 and 4.5.2. We can design our LNA sequence considering this information in the studies.



LNA Adenine LNA Guanine

Figure 4.1.4 : LNA adenine and LNA guanine monomer structures

4.2. Uracil LNA

Found in RNA, it pairs with adenine base and is replaced by thymine in DNA. Methylation of uracil produces thymine. It turns into thymine to protect the DNA and to improve the efficiency of DNA replication. Uracil can base pair with any of the bases depending on how the molecule arranges itself on the helix, but readily pairs with adenine because the methyl group is repelled into a fixed position. As stated, uracil pairs with adenosine through hydrogen bonding. Uracil is the hydrogen bond acceptor and can form two hydrogen bonds [95].

There are seven conformers in the Molecular Mechanics approach taken from Spartan program. The overall stability order of conformers has been determined to be 1> 2 > 3 > 4 > 5 > 6 > 7 as seen in the Figure 4.2.1 and the Table 4.2.1. 2^{nd} and 4^{th} conformers do not have proton transfer but they seem like a transition state. 1^{st} conformer seems like unprotonated structure of 2^{nd} conformer and 3^{rd} conformer seems 4^{th} one. 5^{th} conformer pairs with 3^{rd} conformer in DFT and 1^{st} conformer of HF. 6^{th} conformer cannot be found in DFT, it seems like 3^{rd} conformer of HF. 7^{th} conformer seems like 2^{nd} conformer of DFT and 5^{th} conformer of HF.

There are five conformers in DFT taken from Gaussian program. The overall stability order of conformers has been determined to be 1> 2 > 3 > 4 > 5 as seen in the Figure 4.2.2 and the Table 4.2.1. There are proton transfers in the first two most stable conformers. The stability increases when the hydrogen of bases nitrogen group transferred to the phosphate group oxygen. The most stable conformer in the non transferred conformers, 3^{rd} conformer, shows 2^{nd} numbered structure type like adenine LNA conformer distribution. 4^{th} conformer seems like unprototaned structure of the 1^{st} conformer and 5^{th} conformer seems like unprotonated structure of the 2^{nd} conformer.

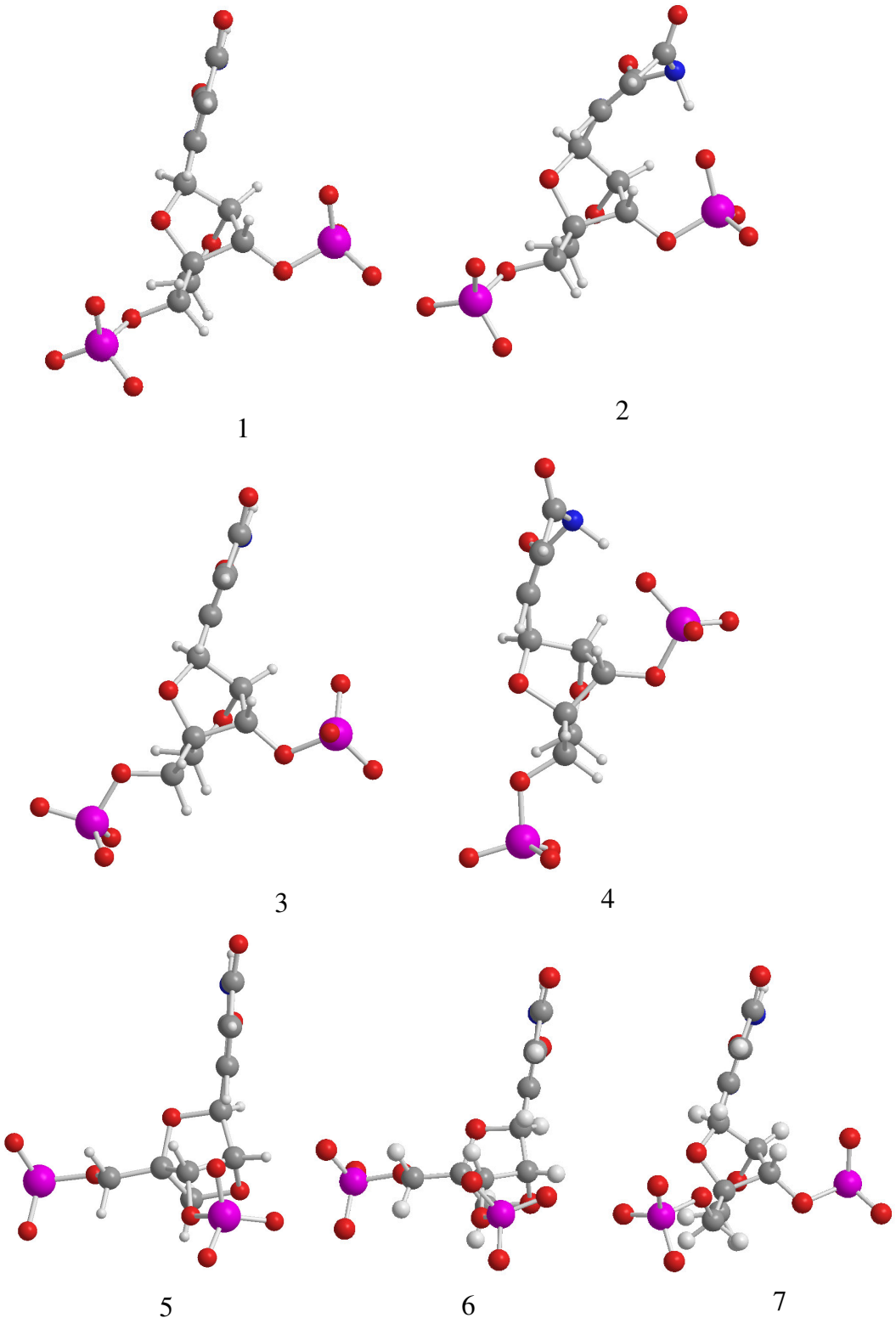


Figure 4.2.1 : MM conformers of uracil LNA monomer

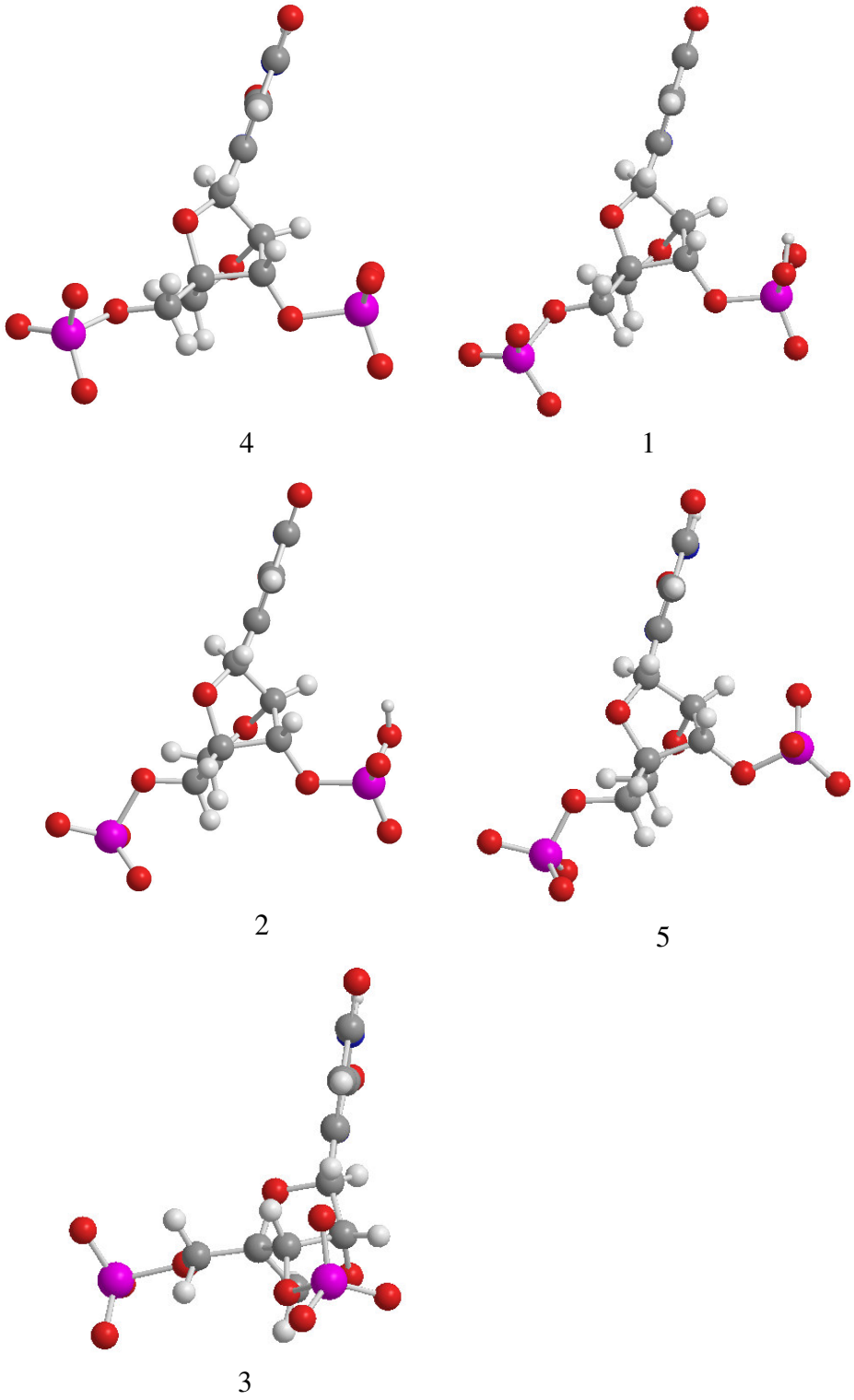
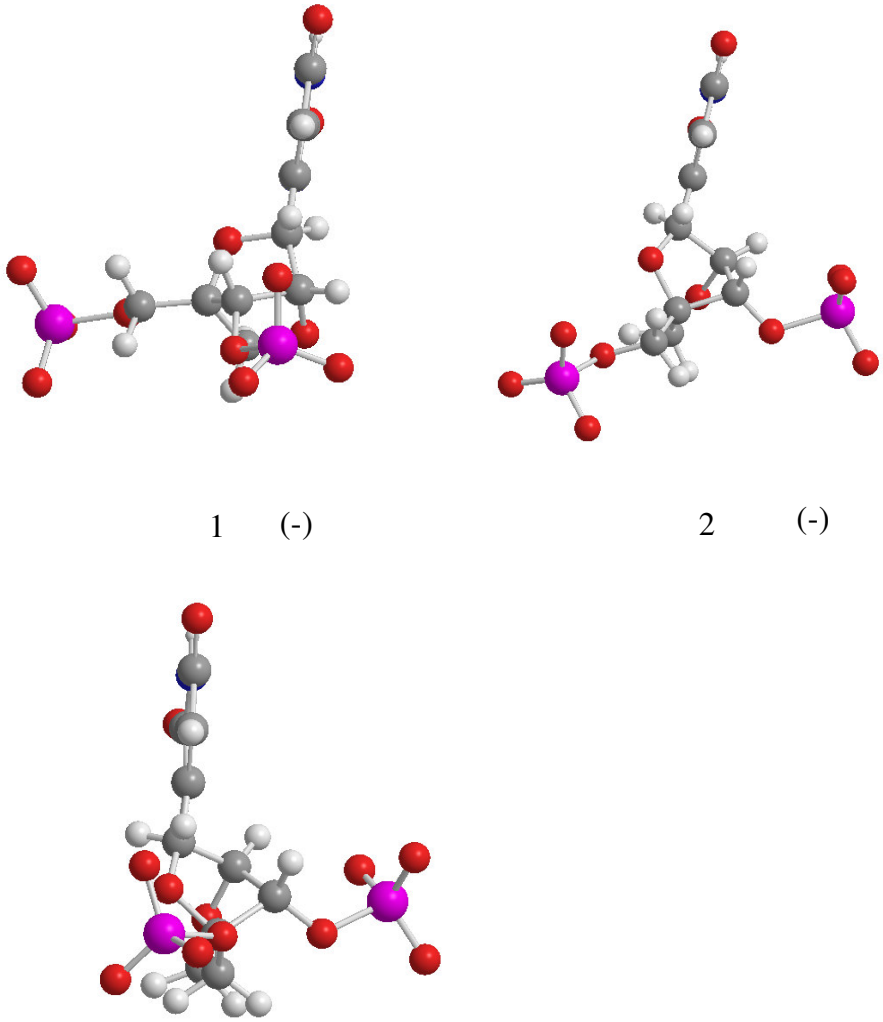


Figure 4.2.2 : DFT conformers of uracil LNA monomer



1 (-) 2 (-)

3

Figure 4.2.3 : HF conformers of uracil LNA monomer

Table 4.2.1 : Conformer Distribution (Conf), Heats of Formation (Energy, kcal/mol for MM), Total Electronic Energies (Energy, hartrees for DFT and HF), Relative Energies (E_{REL}, kcal/mol), and Dipole Moments (μ, Debye) and Frequencies (Freq) of uracil LNA conformers.

MM			DFT				HF				
Conf	Energy	E_{REL}	Conf	Energy	E_{REL}	M	Conf	Energy	E_{REL}	μ	Freq
1	10.45	0.00	4	-2081.776	46.02	23.61	2	-2072.969	5.67	26.69	(-)
2	11.46	1.01	1	-2081.849	0.00	15.70					
3	11.69	1.24	5	-2081.772	48.48	23.55					
4	12.18	1.73	2	-2081.848	0.49	13.97					
5	12.28	1.83	3	-2081.788	38.26	25.41	1	-2072.978	0.00	27.72	(-)
6	12.58	2.14									
7	18.00	7.56					3	-2072.967	7.29	18.83	

There are three conformers in HF taken from Gaussian program. The overall stability order of conformers has been determined to be 1 > 2 > 3 seen in the Figure 4.2.3 and the Table 4.2.1. Conformers with the proton transfer cannot be seen in HF. The most stable conformer pairs with 3rd conformer of DFT. 2nd conformer pairs with 5th conformer of DFT. 3rd conformer does not pair with DFT and it is 3rd numbered type of structure; the distant phosphate group is closest to the base. In HF and 1st and 2nd conformers each have one imaginary (negative) frequency indicating that they correspond to transition states. 3rd conformer of HF cannot be found by DFT.

4.3. Thymine LNA

Thymine, also known as 5-methyluracil, is a pyrimidine nucleobase. As the name implies, thymine may be derived by methylation of uracil at the 5^{th} carbon. Thymine is found in the nucleic acid DNA. In RNA thymine is replaced with uracil in most cases. In DNA, thymine (T) binds to adenine (A) via two hydrogen bonds to assist in stabilizing the nucleic acid structures [95].

There are seven conformers in the Molecular Mechanics approach taken from Spartan program. The overall stability order of conformers has been determined to be 1> 2 > 3 > 4 > 5 > 6 > 7 as seen in the Figure 4.3.1 and the Table 4.3.1. There are six conformers in DFT taken from Gaussian program. The overall stability order of conformers has been determined to be 1> 2 > 3 > 4 > 5 > 6 as seen in the Figure 4.3.2 and the Table 4.3.1. There are four conformers in HF taken from Gaussian program. The overall stability order of conformers has been determined to be 1> 2 > 3 > 4 as seen in the Figure 4.3.3 and the Table 4.3.1.

There are 5 conformers found in MM and all of them can also be located with DFT. HF can find 4^{th} of them. The most stable conformers are not same in DFT and HF. The most stable conformer in HF has a proton transfer from amino terminal to phosphate terminal. This transfer decreases the energy and dipole moment in both DFT and HF. Methyl group hydrogens of the base can interact with distant phosphate group oxygens. All conformers of thymine LNA are similar to uracil LNA. 5^{th} conformer of uracil LNA does not found in thymine LNA with DFT and 4^{th} conformer of thymine LNA cannot be found in uracil LNA with DFT but it can be found in HF. These conformers are designed again and 5^{th} conformer of uracil LNA can be found in thymine LNA as a 6^{th} conformer.

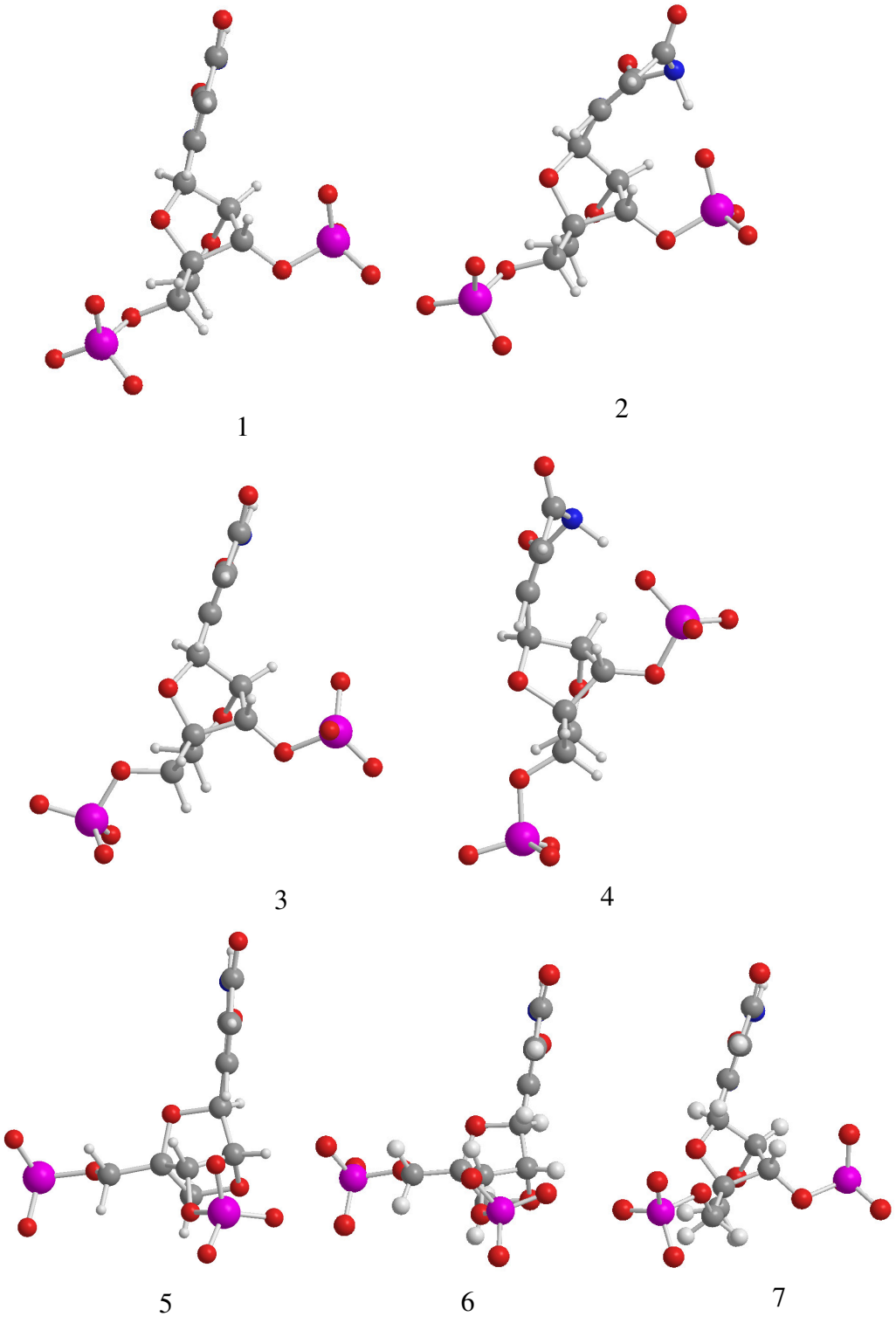


Figure 4.3.1 : MM conformers of thymine LNA monomer

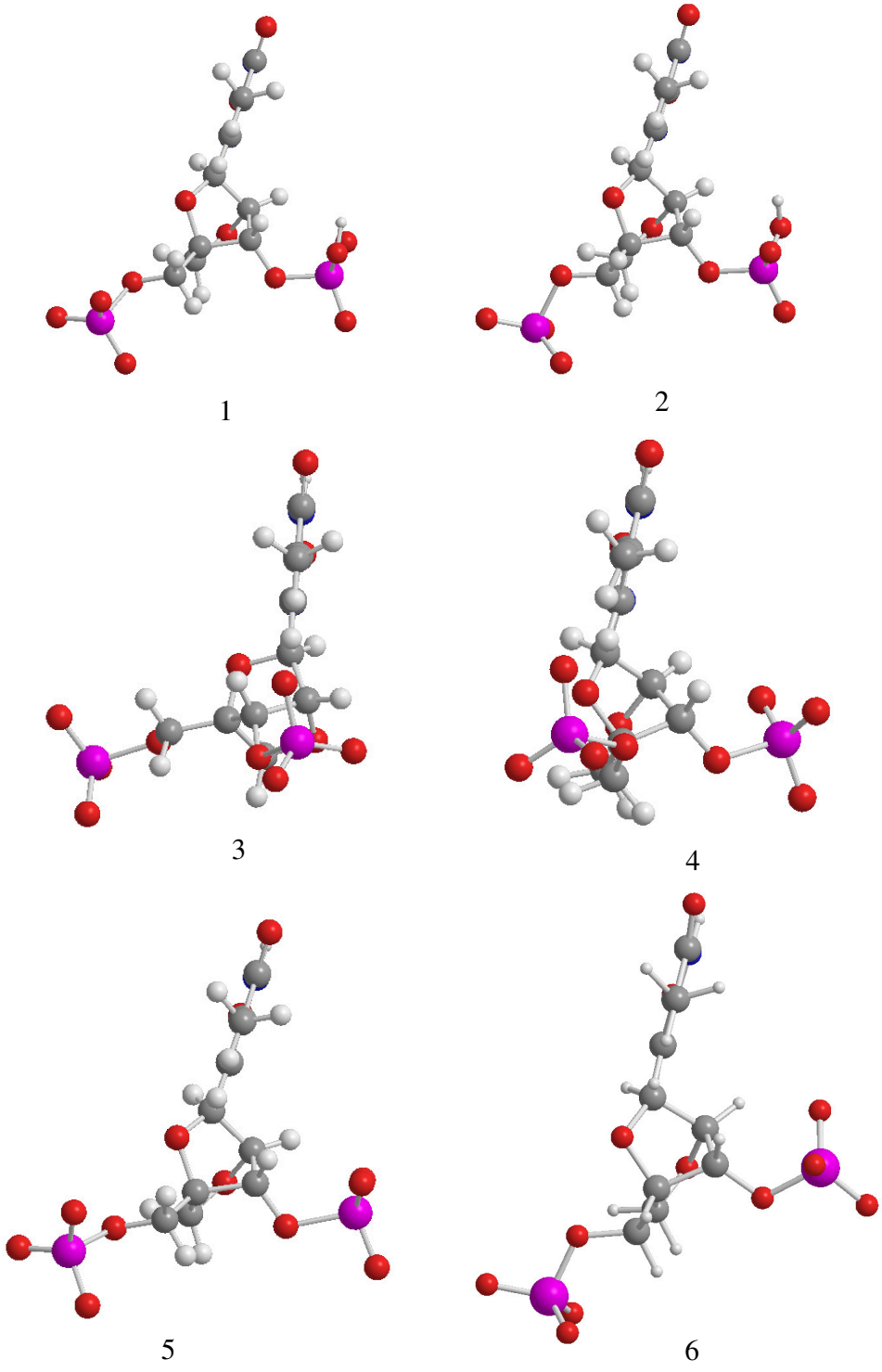


Figure 4.3.2 : DFT conformers of thymine LNA monomer

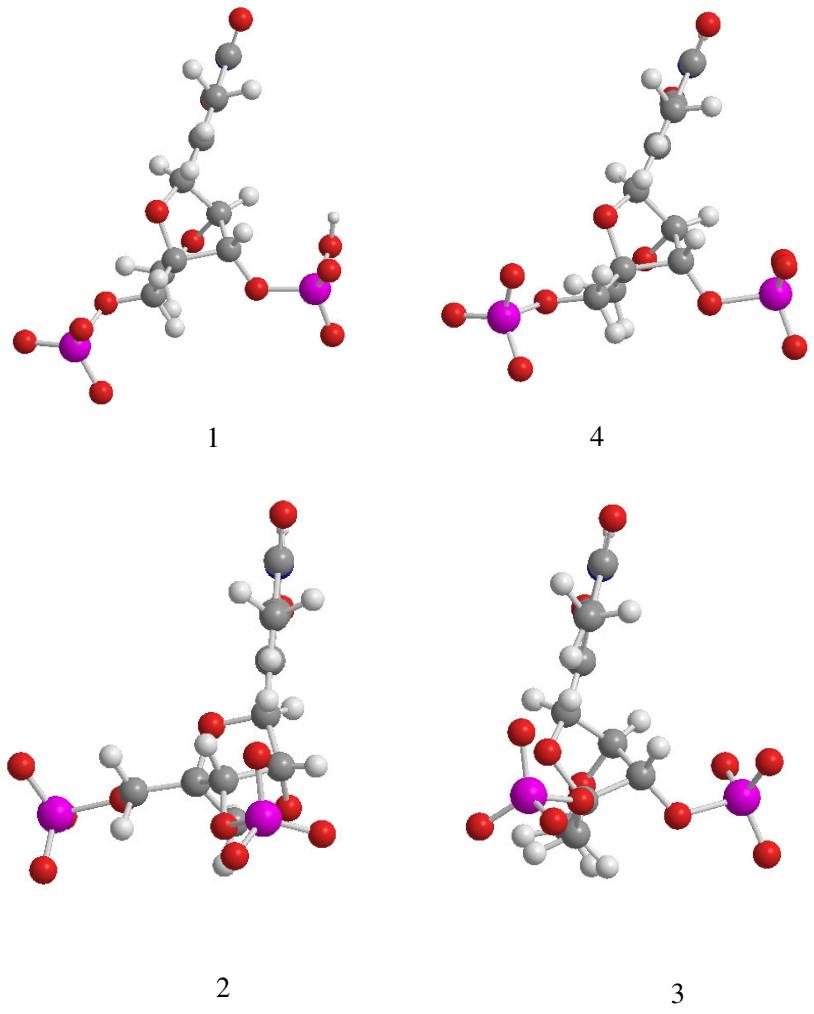


Figure 4.3.3 : HF conformers of thymine LNA monomer

Table 4.3.1 : Conformer Distribution (Conf), Heats of Formation (Energy, kcal/mol for MM), Total Electronic Energies (Energy, hartrees for DFT and HF), Relative Energies (E_{REL}, kcal/mol), and Dipole Moments (μ, Debye) of thymine LNA conformers.

MM			DFT				HF			
Conf	Energy	E_{REL}	Conf	Energy	E_{REL}	μ	Conf	Energy	E_{REL}	μ
1	12.49	0.00	5	-2121.097	47.07	25.45	4	-2112.010	49.61	28.16
2	13.05	0.56	1	-2121.172	0.00	17.33				
3	13.72	1.23	2	-2121.171	0.41	16.05	1	-2112.089	0.00	18.23
4	14.18	1.70	3	-2121.114	36.02	27.79	2	-2112.022	41.88	30.02
5	19.86	19.86	4	-2121.103	43.20	18.13	3	-2112.012	48.14	19.71
			6	-2121.093	49.49	25.94				

4.4. Guanine LNA

Guanine, along with adenine and cytosine, is present in both DNA and RNA, whereas thymine is usually seen only in DNA and uracil only in RNA. It binds to cytosine through three hydrogen bonds. In cytosine, the amino group acts as the hydrogen donor and the C-2 carbonyl and the N-3 amine as the hydrogen-bond acceptors. Guanine has a group at C-6 that acts as the hydrogen acceptor, while the group at N-1 and the amino group at C-2 acts as the hydrogen donors [95].

There are six conformers in the Molecular Mechanics approach taken from Spartan program. The overall stability order of conformers has been determined to be 1> 2 > 3 > 4 > 5 > 6 as seen in the Figure 4.4.1 and the Table 4.4.1. Although MM results show tendency for proton transfer, no proton transfer has been observed.

There are five conformers in DFT taken from Gaussian program. The overall stability order of conformers has been determined to be 1> 2 > 3 > 4 > 5 as seen in the Figure 4.4.2 and the Table 4.4.1. Proton transfer occurs in all conformers like the 4^{th} numbered type of structure. Two oxygen of the close phosphate group can take proton from the bases' nitrogen which can be seen in conformer 1, 2 and 3. 4^{th} and 5^{th} conformers also transfers proton from close phosphate group.

There is one conformer in HF taken from Gaussian program given in the Figure 4.4.3 and the Table 4.4.1. HF can find only the most stable conformer it looks like 3^{rd} conformer of DFT. MMFF conformers are so close to each other therefore DFT conformers shapes, energies and moments are also similar. Different initial conformers can be prepared related to the adenine base and new conformers can be investigated. Control conformers are designed for adenine and guanine from the point of adenine has two rings like guanine. But neither protonated adenine nor unprotonated guanine can be found. This information gives us a real difference between adenine and guanine and it differs in the specifity and affinity when bases make hydrogen bonds in the double helix.

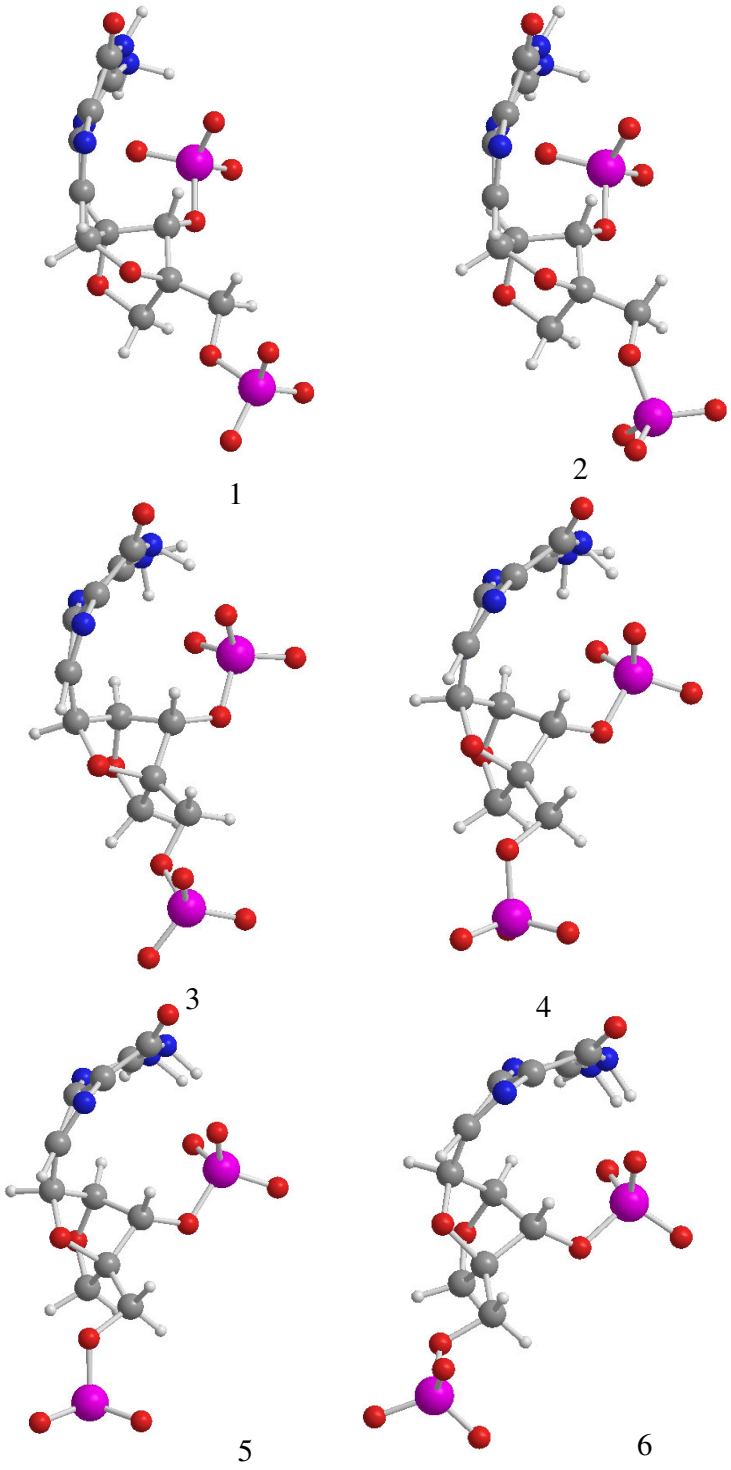


Figure 4.4.1 : MM conformers of guanine LNA monomer

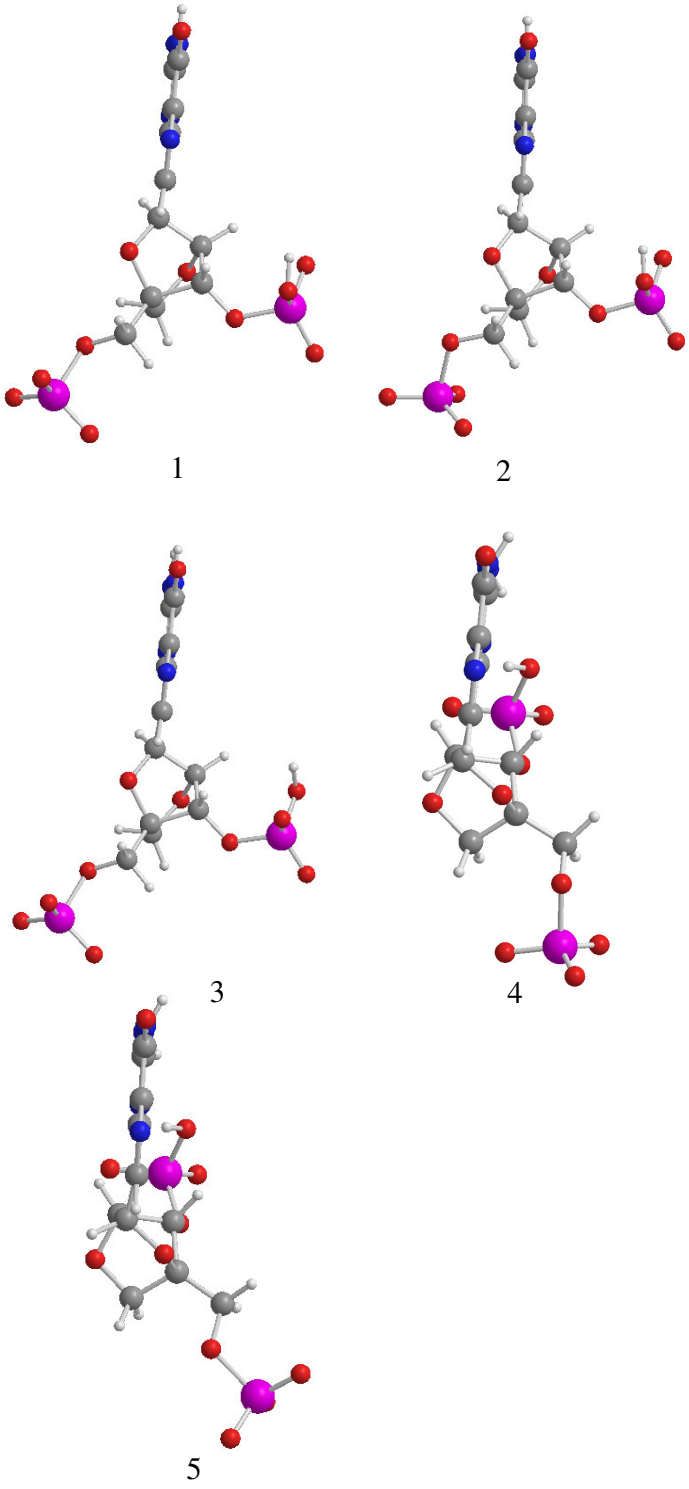
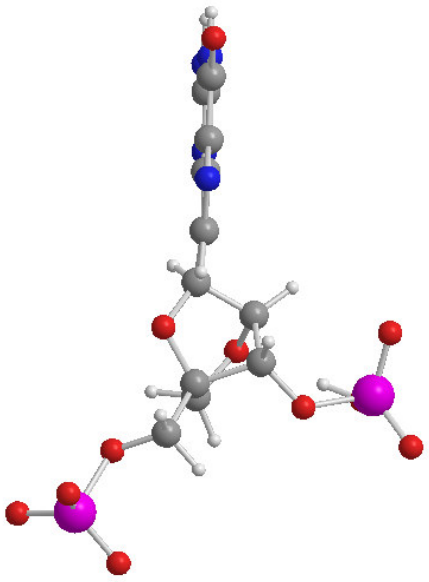


Figure 4.4.2 : DFT conformers of guanine LNA monomer



1

Figure 4.4.3 : HF conformers of guanine LNA monomer

Table 4.4.1 : Conformer Distribution (Conf), Heats of Formation (Energy, kcal/mol for MM), Total Electronic Energies (Energy, hartrees for DFT and HF), Relative Energies (E_{REL}, kcal/mol), and Dipole Moments of guanine LNA conformers.

MM			DFT				HF		
Conf	Energy	E_{REL}	Conf	Energy	E_{REL}	μ	Conf	Energy	μ
1	14.78	0.00	1	-2209.621	0.00	22.13	1	-2200.000	23.22
2	14.83	14.83	2	-2209.620	0.39	19.88			
3	21.72	21.72	5	-2209.608	8.20	24.67			
4	21.74	21.74							
5	22.65	22.65	3	-2209.614	4.29	19.54			
6	22.92	22.92	4	-2209.614	4.31	22.61			

4.5. Cytosine LNA

Cytosine is one of the five main nucleobases used in storing and transporting genetic information within a cell in the nucleic acids DNA and RNA. It is a pyrimidine derivative, with a heterocyclic aromatic ring and two substituents attached. [95].

There are five conformers in the Molecular Mechanics approach taken from Spartan program. The overall stability order of conformers has been determined to be 1> 2 > 3 > 4 > 5 as seen in the Figure 4.5.1 and the Table 4.5.1. The first three conformers resemble a transition state for a proton transfer.

There are five conformers in DFT taken from Gaussian program. The overall stability order of conformers has been determined to be 1> 2 > 3 > 4 > 5 as seen in the Figure 4.5.2 and the Table 4.5.1. First three conformers have proton transfers. 4^{th} conformer is unprotonated structure 2^{nd} numbered type of structure in correlation with the other bases the most stable uprotonated ones. In the 5^{th} conformer phosphate groups look like parallel to the base, 1^{st} type, and the bases nitrogen is close to phosphate group. 4^{th} and 5^{th} conformers of DFT look like 3^{rd} and 4^{th} conformers of HF and 4^{th} and 5^{th} conformers of MM. But the stability order is reverse in DFT.

There are four conformers in HF taken from Gaussian program. The overall stability order of conformers has been determined to be 1 > 2 > 3 > 4 as seen in the Figure 4.5.3 and the Table 4.5.1. There is not any proton transfer in HF results. The second most stable conformer in HF has negative frequency and it looks like a transition state for a proton transfer. DFT calculations have more conformations and they are more reliable than HF.

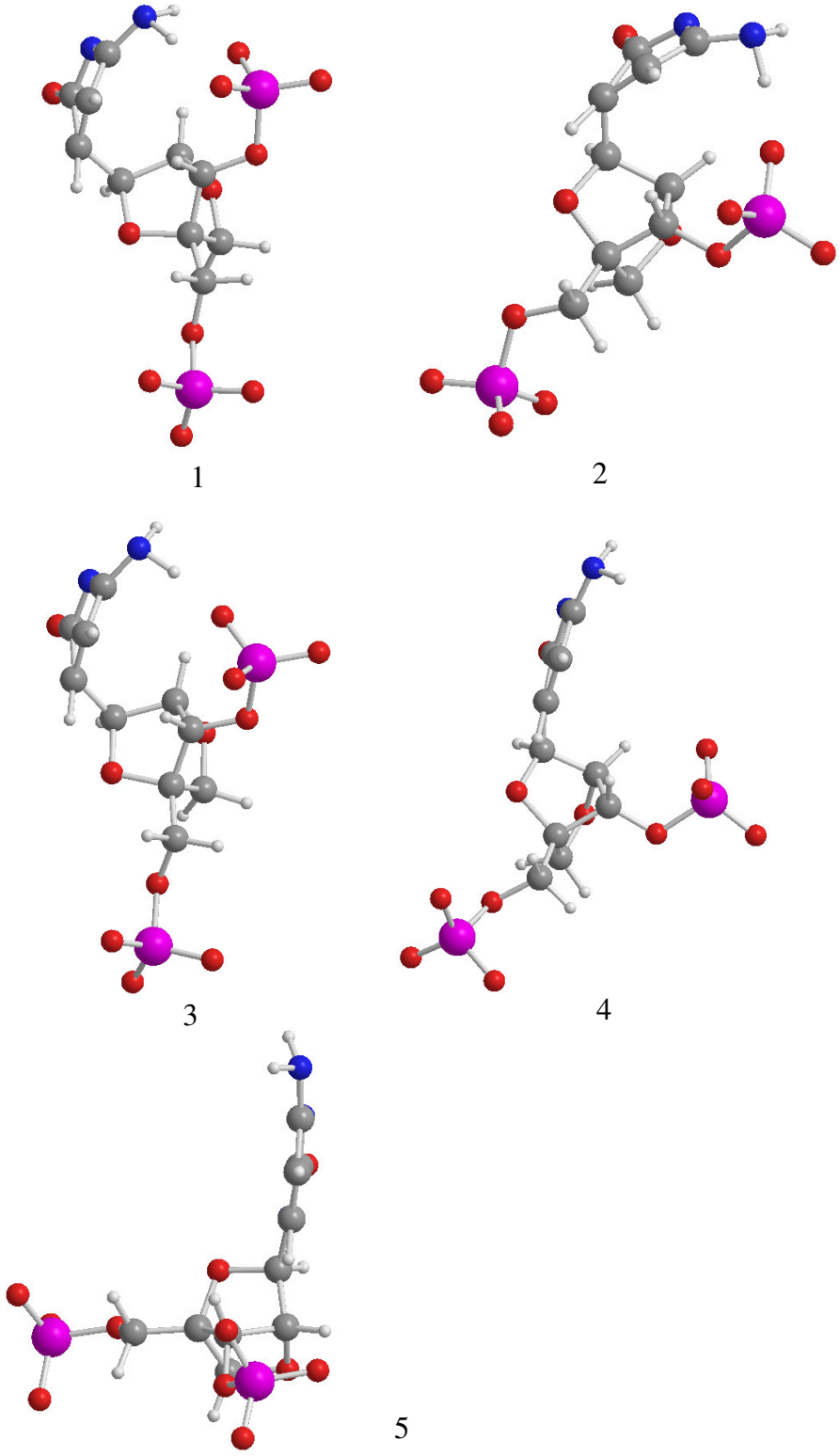


Figure 4.5.1 : MM conformers of cytosine LNA monomer

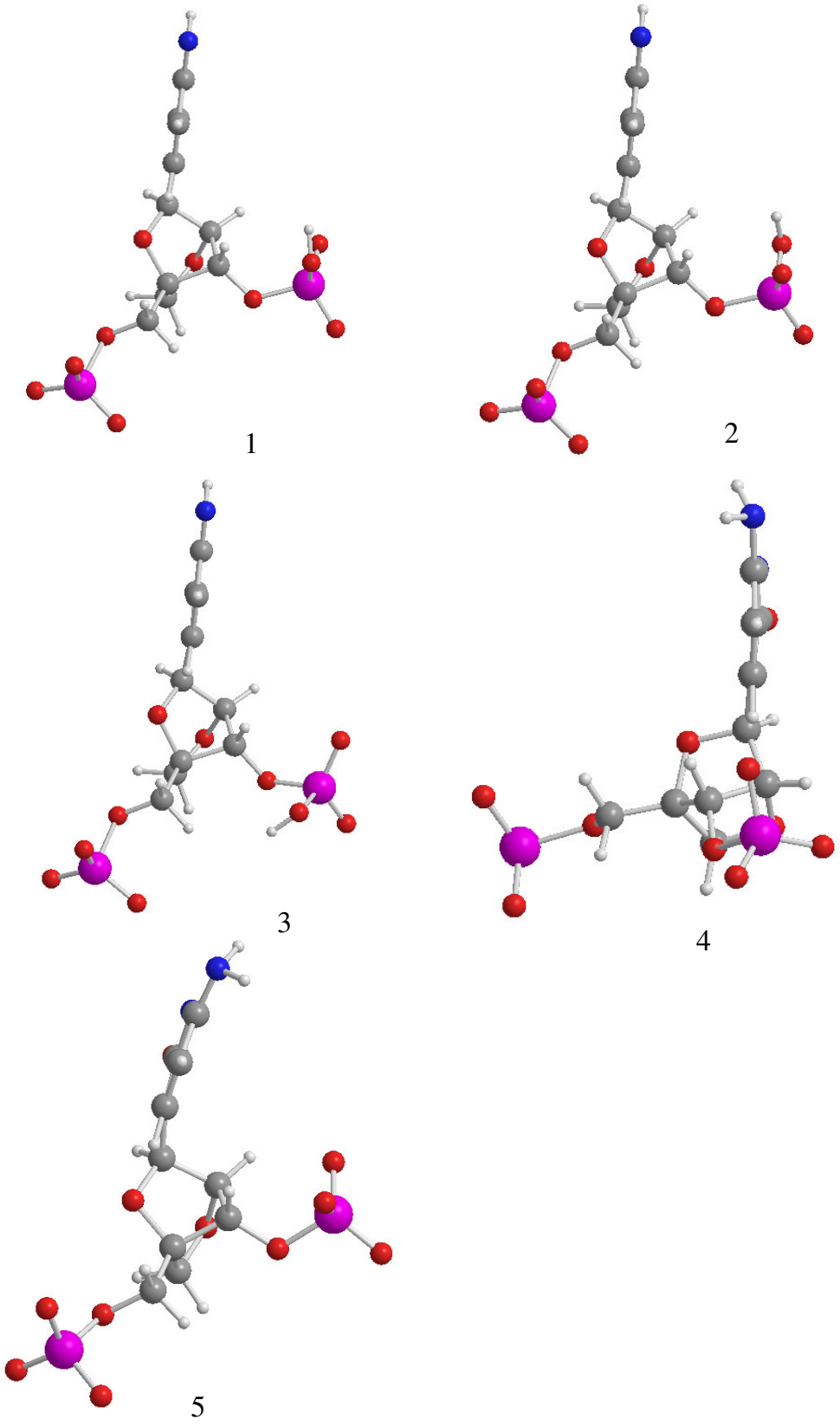


Figure 4.5.2 : DFT conformers of cytosine LNA monomer

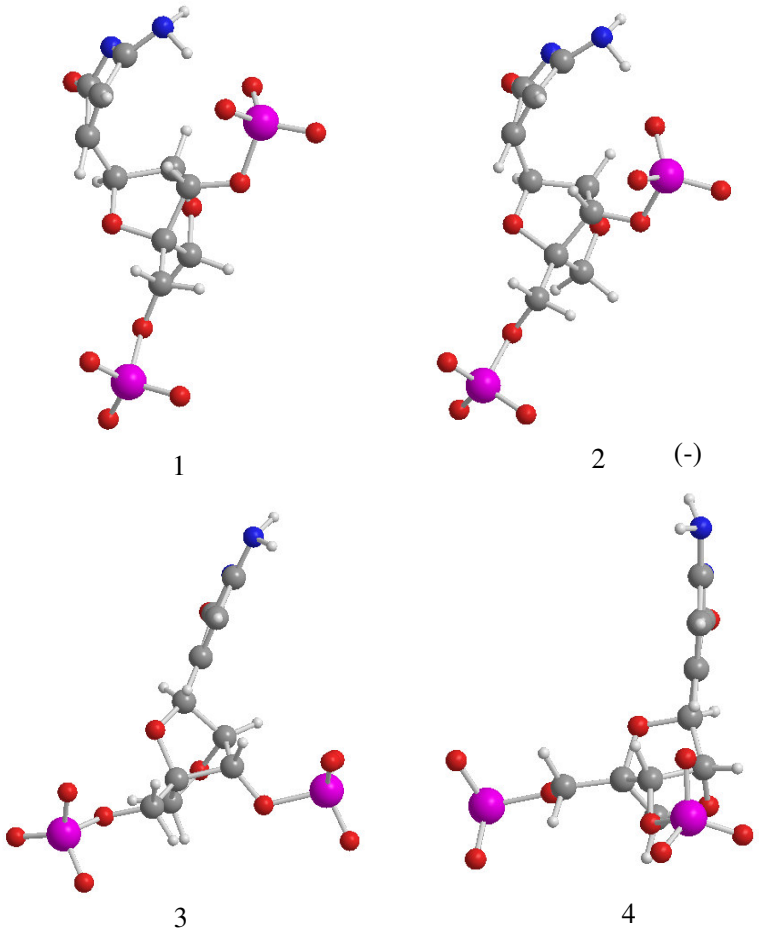


Figure 4.5.3 : HF conformers of cytosine LNA monomer

Table 4.5.1 : Conformer Distribution (Conf), Heats of Formation (Energy, kcal/mol for MM), Total Electronic Energies (Energy, hartrees for DFT and HF), Relative Energies (E_{REL}, kcal/mol), Dipole Moments (μ, Debye) and Frequencies (Freq) of cytosine LNA conformers.

MM			DFT				HF				
Conf	Energy	E_{REL}	Conf	Energy	E_{REL}	μ	Conf	Energy	E_{REL}	μ	Freq
1	14.74	0.00	2	-2061.960	1.06	16.68	2	-2053.107	3.16	22.52	(-)
2	19.17	4.44	3	-2061.960	1.11	17.67	4	-2053.101	7.34	25.14	
3	19.23	4.49	1	-2061.962	0.00	13.65					
4	21.28	6.55	5	-2061.878	52.50	24.75	3	-2053.105	4.46	27.40	
5	24.36	9.63	4	-2061.889	45.54	26.74	1	-2053.112	0.00	29.66	

5. CONCLUSION

The results for the gas phase conformers of five LNA bases; adenine, uracil, thymine, guanine and cytosine have been discussed in term of structure and energetics. It has been found that the bicyclic sugar ring of LNA is a restricted structure therefore two phosphate groups and base affect the conformational changes of LNA. The stability of conformers mainly depends on the type, number and strength of the intramolecular hydrogen bonding and proton transfer. C-H bond in methyl group is more polar and stable than N-H bond, there is no proton transfer from methyl group to phosphate group.

There are four types of interactions in LNA conformers.

1. Base and phosphate groups are parallel
2. Oxygens of close phosphate group interact with hydrogens of base
3. Oxygens of distant phosphate group interact with hydrogens of base
4. Proton transfer from amino group of base to oxygen of close phosphate group

All conformational space have been scanned using MMFF, B3LYP/6-31G** and HF/6-31G** methods. Not only LNA base differences but also method differences are defined for LNA base conformations in the study.

The most stable conformer is 4^{th} type and the second most stable conformer is 2^{nd} type when the base allow proton transfer. When the base does not allow proton transfer, like LNA adenine, the most stable conformer is 2^{nd} type. MM approach cannot find proton transfer and gives a conformer like a transition state for a proton transfer and after this conformer, the most stable conformer is 1^{st} type.

LNA can be used in further experimental studies as a linker to bind nucleic acids and peptides. Not only it has a great specifity and affinity to nucleic acids like RNA anda DNA, but also it can interact with amino acids especially with its highly positively charged phosphate groups.

To describe the electronic nature of the complex components, charge analysis should be carried out. If possible the studies should be extended to calculations in solution which will mimic the true physiological conditions in living systems. Our current results are promising and enforce us to carry out more detailed computations on similar systems for a better quantitative explanation of the structure and functional properties of LNA.

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RESUME

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