Department Chemie
der Technische Universität München

NMR-Spectroscopic Study of 5-(Pyren-1-yl)-2'-Deoxyuridine Induced Structural Changes in B-Form DNA

Massimiliano Tittarelli

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"Unsinn. Ich halte mich an ein Beispiel Gottes: was hat Gott nicht alles geschaffen – wieviel Mist ist darunter – und was hat Gott für einen Name"

Alexander Roda Roda (a.k.a. Sandór Friedrich Rosenfeld)

Abstract:

Nucleic acids play a central role in biology and life in general: deoxyribonucleic acid (DNA) contains the genetic information used in the development and functioning of living organisms. The most important functions of DNA is transfer information through error-free replication, but at the same time allow evolutionary processes. Damage in the DNA sequence can lead to mutagenesis and carcinogenesis. Often, damage in DNA is caused by radical reactions as a consequence of oxidative stress. Reductive as well as oxidative hole transfer chemistry through DNA is subject of wide interest because of its biological relevance and biotechnical application. One of the most used methods to study charge transfer through DNA is fluorescence spectroscopy which is employed to measure excited state lifetimes of redox probes within the DNA π-stack and to monitor charge transfer quenching of these photo excited reagents. The intrinsic fluorescence of the naturally occurring nucleotide bases is almost absent. This drawback can be overcome by the use of non-natural nucleotide base analogs with optimized fluorescence properties that can be incorporated using automated synthetic methods. Although modified bases are frequently used in modern biochemical approaches to study properties of nucleic acids and their complexes, very little is known about the structural changes of the DNA duplex caused by artificial nucleosides. Structural determination by NMR spectroscopy of a DNA duplex with PydU incorporated and of a control DNA hairpin at natural abundance is accomplished and the two different stuctures are compared in order to elucidate the perturbation induced by the chromophore on the structure of the modified oligomere.

Zusammenfassung:

Nukleinsäuren spielen eine zentrale Rolle in der Biologie und im Leben generell: Deoxy-Nukleinsäuren (DNA) beinhalten die genetische Information zur Entwicklung und Funktionsweise von lebenden Organismen. Die vielleicht wichtigste Funktion der DNA ist der Transfer von Information durch fehlerfreie Replikation, wobei gleichzeitig die Möglichkeit zu evolutionären Prozessen gegeben sein muss. Die Beschädigung von DNA-Sequenzen kann zu Mutagenese und Kanzerogenese führen. Oft werden Schäden in der DNA durch Radikale als Folge oxidativen Stresses verursacht. Reduktiver wie oxidativer Loch-Transfer in DNA-Strängen ist wegen seiner biologischen Relevanz und biotechnischen Anwendungen daher von großem Interesse. Eine der meistgenutzten Methoden zur Untersuchung von Ladungstransfer in DNA ist die Fluoreszenzspektroskopie, mit der die Lebensdauer von angeregten Zuständen von Redox-Sonden innerhalb gestaffelter DNA-Basen und deren Auslöschung durch Ladungstransfer gemessen werden kann. Die intrinsische Fluoreszenz der natürlich vorkommenden Nukleotide ist vernachlässigbar. Dieser Nachteil wird durch den Einbau artifizieller Nukleotid-Basenanaloga mit optimierten Fluoreszenzeigenschaften mehr als ausgeglichen, der heute mit automatisierten synthetischen Methoden passieren kann. Obwohl die modifizierten Basen oft in modernen biochemischen Essays zur Untersuchung von Eigenschaften von Nukleinsäuren und deren Komplexe genutzt werden, ist nur wenig über die strukturellen Änderungen der DNA-Duplexstrukturen durch den Einbau der artifiziellen Nukleosiden bekannt. In dieser Arbeit wurde die Strukturbestimmung einer DNA-Haarnadelstruktur mit und ohne Einbau der unnatürlichen Base PydU mit Hilfe der NMR-Spektroskopie in natürlicher Isotopenhäufigkeit unternommen. Aus den beiden unterschiedlichen Sequenzen wurden die Einflüsse des Chromophors auf die Konformation des modifizierten Oligomers aufgezeigt.

Introduction

Nucleic acids play a central role in biology and life in general: deoxyribonucleic acid (DNA) contains the genetic information used in the development and functioning of living organisms. Moreover, DNA has the role to long-term preserve the genetic information. DNA is a linear, unbranched polymer of four different deoxynucleosides: deoxyadenosine (dA), thymidine (dT), deoxyguanoside (dG) and deoxycytidine (dC); they are covalently linked through phosphodiester bonds. The most important functions of DNA is transfer information through error-free replication, but at the same time allow evolutionary processes. Damage in the DNA sequence can lead to mutagenesis and carcinogenesis. Often, damage in DNA is caused by radical reactions as a consequence of oxidative stress. If and how charge can migrate through DNA has been discussed extensively over the past decades. The fundamental question whether DNA serves as a medium for long-range charge transfer (CT) has been the subject of much controversy. The dispute has been solved by different interpretations of the applied DNA systems and the description of alternative mechanisms. Reductive as well as oxidative hole transfer chemistry through DNA are now widely accepted and the discussion has moved to the decisive question of its mechanism and most recently, to its biological relevance and biotechnical application. CT in DNA is extremely sensitive to the π–stacking of the intervening DNA structure; perturbations of the base stacking caused by base mismatches, DNA lesions or DNA-protein interactions are effectively reported by altered CT. One of the most used methods to study charge transfer through DNA is fluorescence spectroscopy which is finding widespread application in biochemical and biophysical studies of macromolecules, as well as in medicinal, environmental and commercial research efforts. Fluorescence spectroscopy is employed to measure excited state lifetimes of redox probes within the DNA π-stack and to monitor charge transfer quenching of these photo excited reagents. The intrinsic fluorescence of the naturally occurring nucleotide bases in nucleic acids is extremely weak, though. This drawback can be overcome by the use of non-natural nucleotide base analogs with optimized fluorescence properties (i.e. higher quantum yields and longer lifetimes) that can be incorporated using automated synthetic methods. Since no background fluorescence of the natural bases is present, site-specific biophysical information can be obtained. Although modified bases are frequently used in modern biochemical approaches to study properties of nucleic acids and their complexes, very little is known about the structural changes of the DNA/RNA duplex formation caused by artificial nucleosides. The present thesis consists in the structural determination by NMR spectroscopy of a DNA duplex with PydU incorporated and of a control DNA hairpin at natural abundance; The stem is capped by a GAA-motif which forms a triloop useful for stability reasons. The goal is to investigate the possible structural perturbations induced by the incorporated non-natural nucleotide. Nuclear magnetic resonance (NMR) spectroscopy is the most important method to explain molecular structures of either small molecules or large biomacromolecules in solution. It's a general assumption that the conformation in aqueous solution is to a certain degree a significative representation of the bioactive one.

In cap. 2, it is explained how to achieve the fully assignment of oligomere proton resonances which is the first essential step in an NMR study of biomolecules aimed at determining their three-dimensional structure. Subsequently the interproton distance restraints are extracted from 2D-NOESY spectra and these values used to perform a series of simulated annealing calculations using XPLOR NIH 2.9.4a: one of the most popular programs for this purpose. Finally, the structures of the control and modified oligomere are compared in order to highlight the essential structural modifications produced by the aromatic chromophore.

1. DNA

1.1 Structural properties of DNA

1.1.1 Nucleotides

Nucleotides consist of ribose (RNA – ribonucleic acid) or deoxyribose ring (DNA – deoxyribonucleic acid. Latin prefix *de – without*) in furanose β-glycosidic form (fig. 1.1) and specific heterocyclic base (Adenine, Cytosine, Guanine and Thymine in DNA or Uracil instead of Thymine in RNA; see tab. 1.1) connected by a glycosidic bond. This links the sugar's C1' with the N9 of purines or N1 of pyrimidines.

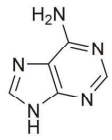
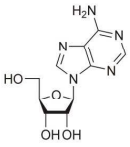
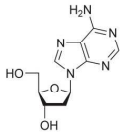
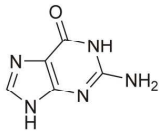
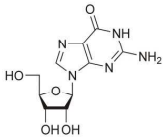
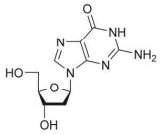
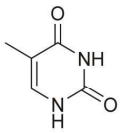
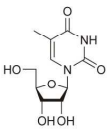
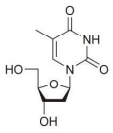
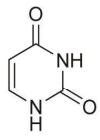
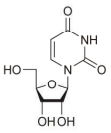
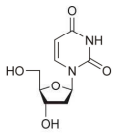
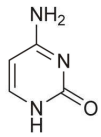
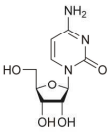
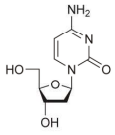
	Nitrogenous base	Nucleoside	Deoxynucleoside
Purines			
	Adenine	Adenine (A)	Deoxyadenosine (dA)
			
	Guanine	Guanosine (G)	Deoxyguanosine (dG)
Pyrimidines			
	Thymine	5'-Methyluridine (m^5U)	Thymidine (dT)
			
	Uracil	Uridine (U)	Deoxyuridine (dU)
			
	Cytosine	Cytidine (C)	Deoxycytidine (dC)

TABELLE 1.1 *Nitrogenous bases, nucleosides and deoxynucleosides in nucleic acids.*

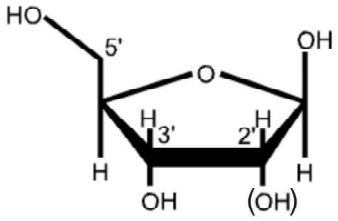


FIGURE 1.1 *β-D-(2' deoxy) ribose in furanose form.*

1.1.2 Sugar pucker

The five-membered deoxyribose sugar ring in DNA is inherently nonplanar. The nonplanarity is termed puckering. The precise conformation of a deoxyribose ring can be completely specified by the five endocyclic torsion angles (ν_0–ν_4) within it (see fig. 1.2). The ring puckering arises from the effect of nonbonded interactions between substituents at the four ring carbon atoms, thus different substituent atoms would be expected to produce differing types of puckering.
It is usual for one of the two atoms to have a larger deviation from the plane than the other, resulting in a twist conformation. If the major deviation is on the same side as the base and C4′–C5′ bond, then the atom involved is termed *endo* (from ancient Greek *ενδω*; within, same side). If it is on the opposite side, it is called *exo* (from ancient Greek *εσω*; external, opposite side). The most commonly observed puckers in crystal structures of isolated nucleosides and nucleotides are either close to C2′-endo or C3′-endo types (fig. 1.3). In practice, these pure forms are rarely observed in solution.
The puckers are described in terms of twist conformations. When the major out-of-plane deviation is on the endo side, there is a minor deviation on the opposite, exo side. The convention used for describing a twist deoxyribose conformation is that the major out-of-plane deviation is followed by the minor one, for example C2′-endo, C3′-exo.
There are energy barriers between major forms. For interconversion of C2′-endo to C3′-endo the preferred pathway is via the O4′-endo state, with a barrier of 2–5 kcal/mole found from an analysis of a large body of experimental data [165], and a somewhat smaller value of 1.5 kcal/mole from molecular dynamic studies [166]; a modest energy barrier anyway which allows conformational interconversion in solution and at ambient temperature (T_{amb}). For a rigorous monitoring of the relative populations of puckers the NMR measurements of coupling constants between H1′, H2′, H2″ and H3′, H4′ protons has to be performed. Since the size of ^3J – couplings is related to dihedral angles via the empirically derived Karlplus relation [147], ν_0– ν_4 can be fitted accordingly from the couplings. Unfortunately, ^3J – couplings not always can be measured. In such cases, distances derived from NOESY spectra allow a rough determination of the preferred puckering. In general, in solution and at T_{amb} there is rapid conformational interconversion but deoxyribose nucleosides are primarily (>60%) in the C2′-endo form.

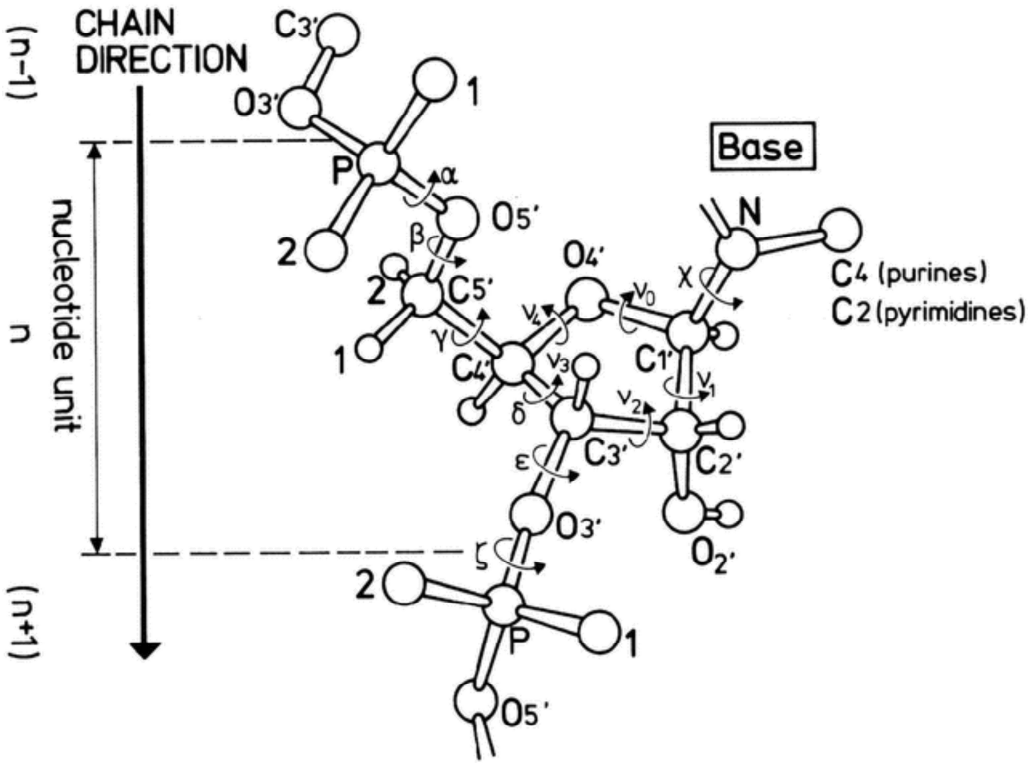
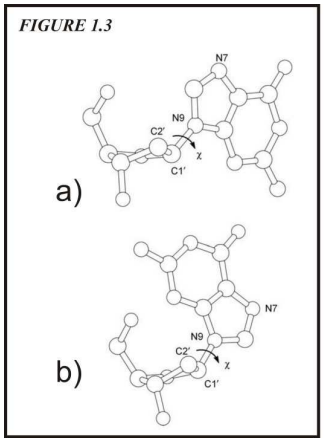


FIGURE 1.2 *5' to 3' chain direction and unit numbering in polynucleotide chain; also shown are the torsion angles in the sugar–phosphate backbone (α, β, γ, δ, ζ) and the glycosidic torsion angle χ. The torsion angles are defined as follow:* (α) O3'–P–O5'–C5'; (β) P–O5'–C5'–C4'; (γ) O5'–C5'– 4'–C3'; (δ) C5'–C4'–C3'–O3'; (ε) C4'–C3'–O3'–P; (ζ) C3'–O3'–P–O5'; (χ) O4'– C1'–N1–C2 (Py); (χ) O4'–C1'–N9–C4 (Pu). *The Endocyclic tosion angles are defined as follow:* (ν_0) C4'-O4'-C1'-C2; (ν_1) O4'-C1'-C2'-C3'; (ν_2) C1'-C2'-C3'-C4'; (ν_3) C2'-C3'-C4'-04'; (ν_4) C3'-C4'-O4'-C1'.



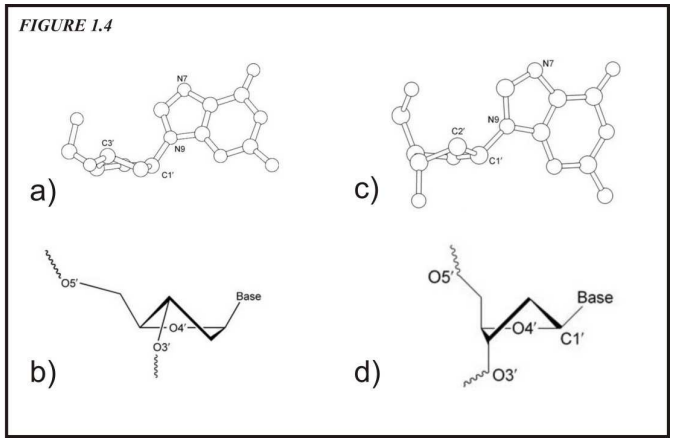


FIGURE 1.3 *two of the most common nucleoside conformation; "anti" (a) and "syn" (b) – they can interconvert rotating along the torsion angle χ.* ***FIGURE 1.4*** *Puckering of the two most common β-D-(deoxy)ribose sugar ring conformations; in a) and b) the C3'-endo (or N-type) and in c) and d) the C2'-endo (also referred to as S-type).*

1.1.3 Conformation about the glycosidic bond

The torsion angle χ around this glucosidic bond can in principle adopt a wide range of values. Theory has predicted two principal low-energy domains for the glycosidic angle, in accord with experimental findings for a large number of nucleosides and nucleotides. The *anti* conformation has the N1, C2 face of purines and the C2, N3 face of pyrimidines directed away from the sugar ring (fig. 1.3.a) so that the hydrogen atoms attached to C8 of purines and C6 of pyrimidines, are lying over the sugar ring. Thus, the Watson–Crick hydrogen-bonding groups of the bases are directed away from the sugar ring. The reverse is true for the *syn* conformation; the hydrogen-bonding groups are now oriented towards the sugar and especially its O5′ atom (fig. 1.3.b).

1.1.4 Base pairing

The –NH– (imino) and $–NH_2$ (amino) groups of the bases are strong hydrogen bond donors, while the sp^2 hybridized electron pairs of the oxygens of the keto groups and the ring nitrogens are strong hydrogen bond acceptors. They are better acceptors than the oxygens of both the phosphate groups and the pentose. The acceptor-donor hydrogen bonds which can be formed are largely electrostatic in character, with a charge of about +0.2e localized on hydrogens and about –0.2e on oxygens and nitrogens. The average strength of the bond is of 6-10 kJ mol^{-1}. Many are the possible combinations for the basis to be linked by multi H-bonds (see fig. 1.5) although the dominant pattern in biology is the Watson-Crick base pairing (see fig. 1.6), named after the two scientists who first proposed the now iconic double helix structure of DNA in the early 1950s.

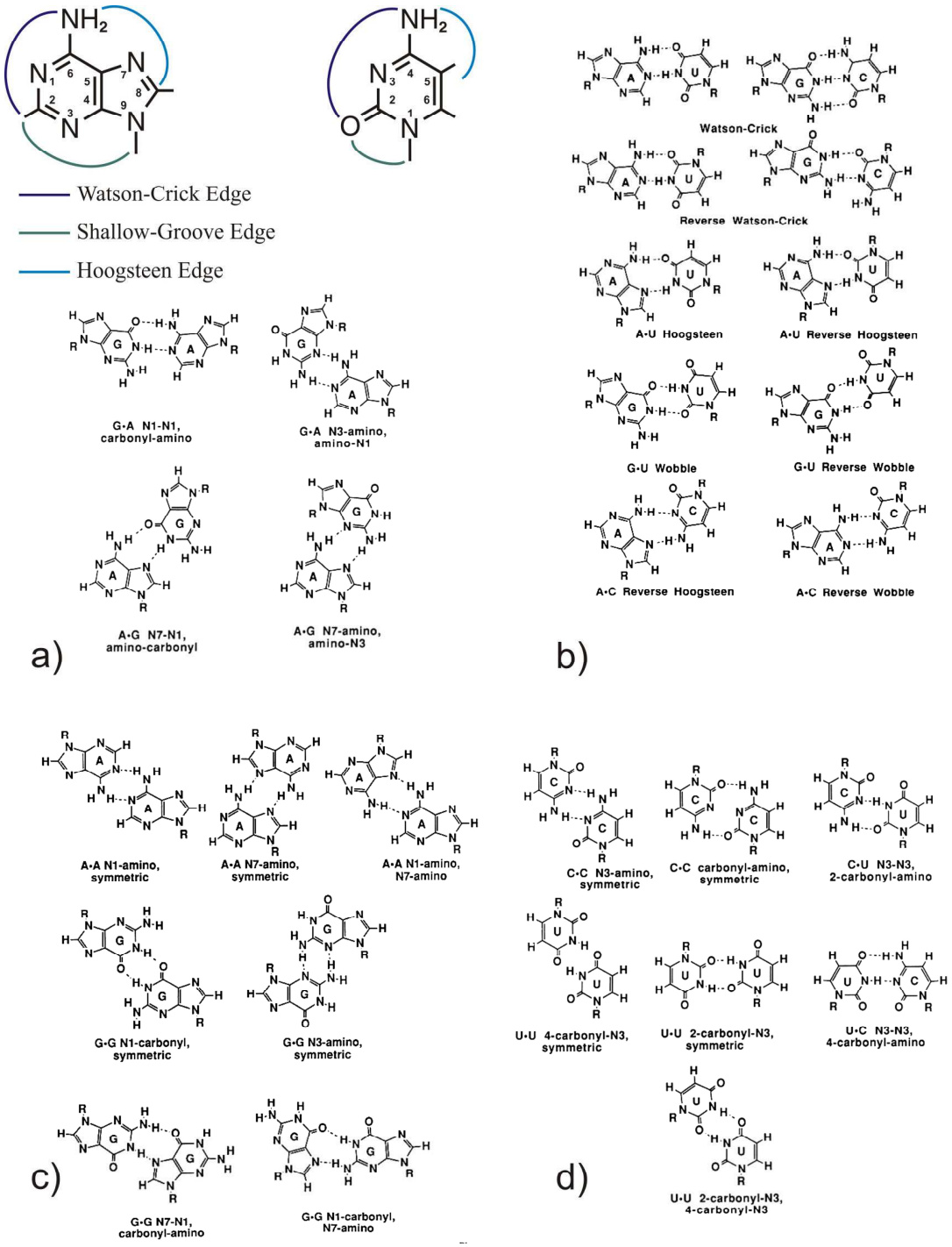


FIGURE 1.5 *Some non canonical base pairs: 28 other distinct and experimentally detected possibilities of forming at least two hydrogen bonds between two bases: a) hetero-purine bp; b) purine-pyrimidine bp; c) homo purine-purine bp; d) pyrimidine-pyrimidine bp* [157].

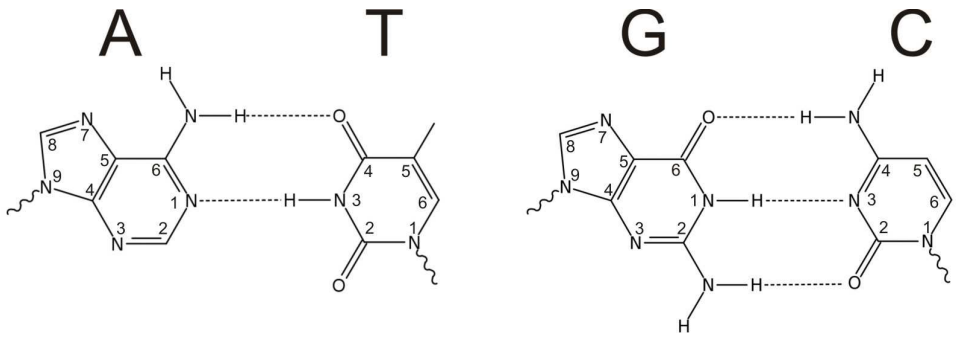


FIGURE 1.6 *A•T base pair with two hydrogen bonds (left) and G•C base pairs with three hydrogen bonds (dashed lines)*

In the canonical Watson-Crick base pairing there are two hydrogen bonds in an A•T pair and three in G•C pairs. The geometry of the pair has been fully analyzed in many structures; in planar base pairs the hydrogen bonds join nitrogen and oxygen atoms that are 2.85–2.95 Å apart (tab. 1.2). This geometry gives a C1'··· C1' distance of 10.60 Å with an angle of 68° between the two glycosidic bonds.

T•A (U•A) C•G	N3-H - - N1	2.84
	O4 - - H-N6 O2 - - H-N2	2.94 2.86
	O2 - - H-N2	2.95
	N4-H - - O6	2.91

TABELLE 1.2 *hydrogen-Bond distances (in Å) in Watson–Crick Base pairs in the crystalline state* [154].

1.1.5 Primary structure of DNA

The primary structure of DNA has each nucleoside joined by a phosphodiester bond from its 5'-hydroxyl group to the 3'- hydroxyl group of the neighbor and by a second similar bond from its 3'-hydroxyl group to the 5'- hydroxyl group of the other neighbor. There are no 5'-5' as well as no 3'-3' linkages in the regular DNA primary structure. By Convention, the direction of nucleic acid single strand always runs from 5' toward 3' side (fig. 1.7).

1.1.6 Secondary structure of DNA

DNA doesn't usually exist as a single molecule but two nucleotide polymers are oriented in an anti-parallel fashion to form a double helix. The two antisense single strands are held together along the Watson-Crick edge by hydrogen bonds of two complementary couple of bases (G•C and A•T; see fig. 1.6); the whole structure is stabilized by π-orbital interaction of the stacked bases. Double helix DNA shows structural polymorphism depending on the environmental conditions; at conditions similar to the biological ones (high humidity, low salt concentration) the dominant structure is B-DNA.

1.1.6.1 B-DNA

The backbone conformation of the DNA B-form has torsion angles of distinct *anti* glycosidic bonds (see tab. 1.3) and C2'-endo sugar puckers (fig. 1.8). The right-handed double helix takes 10.5 base pairs per turn and the paired bases are almost exactly perpendicular to the helix axis. The separation between contiguous stacked planes is the same as the helical rise (3.4 Å, see tab. 1.4). The asymmetry in the base pairs results in two parallel types of grooves, whose dimensions (especially their depths) are related to the distances of base pairs from the axis of the helix and their orientation with respect to the axis. In general, the major groove is richer in base substituents (O6, N6 of purines and N4, O4 of pyrimidines) compared to the minor one. This, together with the steric differences between the two, has important consequences for interaction with other molecules.

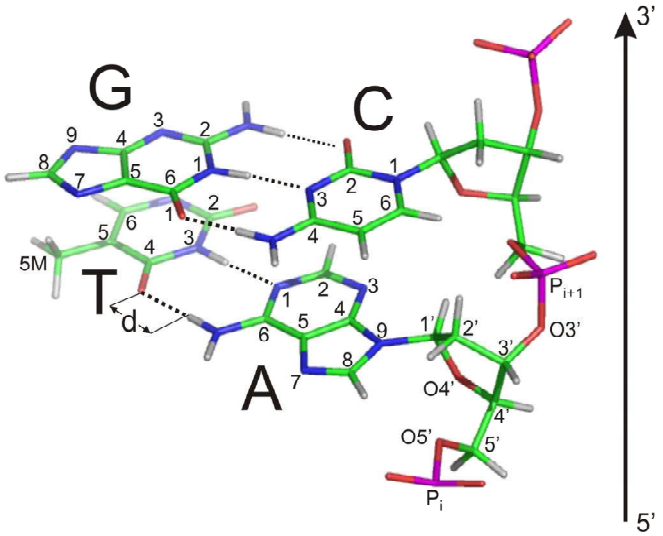


FIGURE 1.7 *conventional Watson-Crick base pairing and atom numbering in nucleic acids [150] of the four common bases (pyrimidines C and T; purines G and A); hydrogen bond distance* $d \approx 2.8/3.0$ *Å*

1.1.6.2 A-DNA

At low humidity and high salt concentration the favored form changes to A-DNA duplex. It has C3′-endo sugar puckers (see tab. 1.3), which causes the base pairs to be twisted and tilted with respect to the helix axis and are displaced nearly 5 Å from it, in striking contrast to the B helix. The helical rise is as a consequence much reduced, to 2.55 Å , compared to 3.4 Å for canonical B-DNA (see tab. 1.4). The helix is wider than in the B form and has an 11 base-pair helical repeat. The combination of base-pair tilt with respect to the helix axis and base-pair displacement from the axis results in very different groove characteristics for the A compared to the B double helix (see fig. 1.9).

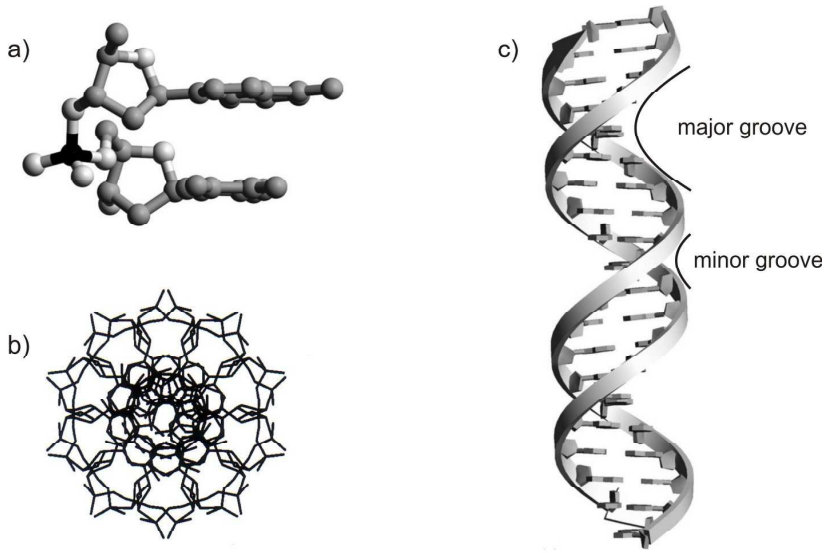


FIGURE 1.8 *conformation of two successive nucleotides in one strand (a) and the structure of canonical B-DNA, from fiber diffraction analysis (b. top view, c. vertical view).*

1.1.6.3 Z-DNA

Rather astonishingly, the earliest (late 70s) ever solved oligodeoxyribonucleotide crystalline structure [152] provided a DNA conformer very different than the expected one grounding on the Watson-Crick's assumptions. The bases show alternated anti- and syn-conformations along the chain (see $Z_{I,II}$ in tab. 1.3). Also, there is a zigzag arrangement of the backbone of the molecule; hence the name Z-DNA. The Z-DNA is a left-handed double helix and can be generally formed by the alternating sequence poly(dC-dG)•poly(dC-dG) and has a dinucleotide repeat with quite distinct conformations for the guanosine compared to the cytosine residues. Each individual repeat has a helical rise of 7.25 Å, so that the rise between successive base pairs is half of this (3.7 Å; fig. 1.10). It is formed in solution in high salt conditions ([NaCl] > 2.5 M) because, being Z-DNA a higher-energy polymorph and showing in its linear form less

stability than either A- or B-forms, it requires high-salt or high-alcohol concentrations for maintenance of its structure. These can reduce the electrostatic interaction between interstrand phosphate groups, which in Z-DNA are much closer than in B-DNA (see section 1.1.7.2). Deoxyribonucleic acid double helix's inherent flexibility defines the polymorphism in terms of A, B and Z duplexes. The majority of polynucleotide structures can readily interconvert under appropriate environmental conditions; under the physiological ones DNA duplex predominantly assumes a B-type conformation while the biological role for both A- and Z-DNA is still not well understood.

1.1.6.4 Other DNA duplexes

In addition to A, B and Z, several other forms of DNA based on the canonical Watson-Crick base pairing have been observed and it seems now clear that the DNA molecule can assume different structures depending on the base sequence and environment. A detailed inspection of the literature reveals that further forms identified as F, Q, U, V and Y are possible. Moreover, several recent constructions show mixtures of various different geometries; therefore a careful conformational analysis is of essential importance, especially in the elucidation of relevant-sized nucleic acids arrangement [156].

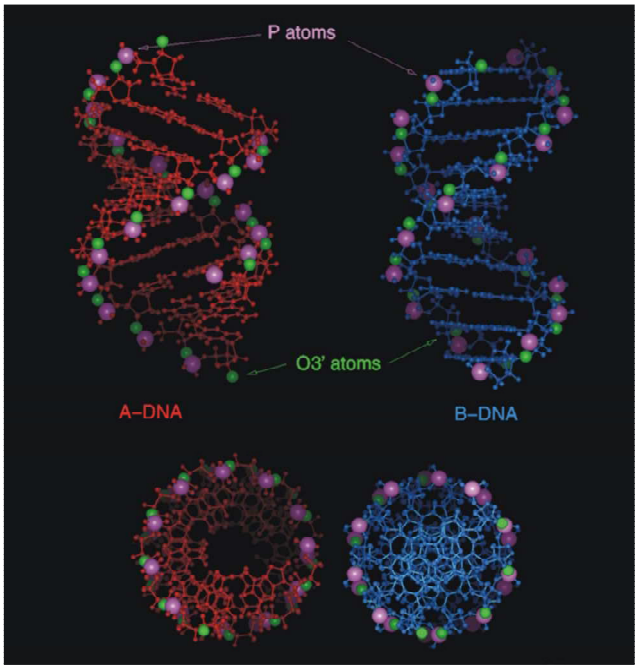


FIGURE 1.9 *Lateral and axial views of A- and B-DNA from which it is possible to appreciate the differences in the structural arrays.*

	α	β	γ	δ	ε	ζ	χ
A	−52	175	42	79	−148	−104	−157
B	**−30**	**136**	**31**	**143**	**−141**	**−161**	**−98**
Z_I	−140	−137	51	138	−97	82	−154
Z_{II}	52	179	−174	95	−104	−65	59

TABELLE 1.3 *Backbone conformational angles for various polymorphs of DNA polynucleotides.* (Taken from S. Arnott, *Oxford Handbook of Nucleic Acid Structure* 1999, Oxford University Press, Oxford).

Parameter	A-DNA	B-DNA	Z-DNA
Helix sense	right-handed	right-handed	left-handed
Residues per turn	11	10.5	12
Axial rise [Å]	2.55	3.4	3.7
Helix pitch(°)	28	34	45
Base pair tilt(°)	20	−6	7
Rotation per residue (°)	33	36	-30
Diameter of helix [Å]	23	20	18
Glycosidic bond configuration dA,dT,dC dG	 anti anti	 anti anti	 anti syn
Sugar pucker dA,dT,dC dG	 C3'-endo C3'-endo	 C2'-endo C2'-endo	 C2'-endo C3'-endo
Intrastrand phosphate-phosphate distance [Å] dA,dT,dC dG	 5.9 5.9	 7.0 7.0	 7.0 5.9

TABELLE 1.4 *Selected helical parameters for various polymorphs of DNA polynucleotides* [151-153]

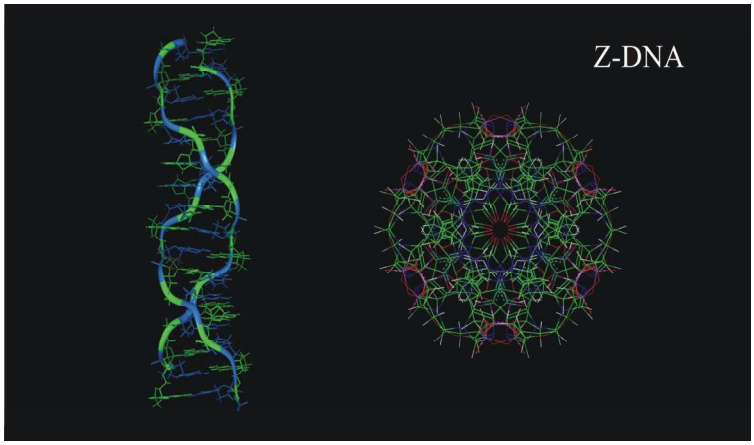


FIGURE 1.10 *Lateral and axial views of poly(dG•dC) in Z-DNA (G in blue, C in green). The phosphate groups are closer in Z-DNA than in B-DNA. Hence, under standard physiological conditions, the electrostatic repulsion of these charged phosphate groups pushes the molecule into the B-DNA conformation. In the presence of a high-salt solution, the electrostatic repulsion of the phosphate residues is vastly decreased, and Z-DNA conformation is stable. In addition, the electrostatic repulsion of phosphate groups is more efficiently tackled by G•C than T•A base pairing (three hydrogen bonds vs. two, respectively); this is consistent with the fact that DNA in Z-form has been mainly observed for alternate poly(dG•dC) sequences.*

1.1.7 Base-pairing stability and melting temperature

DNA melting (also referred to as DNA denaturation), is the process by which double-stranded deoxyribonucleic acid unwinds and separates into single strands through the breakage of hydrogen-bonds which occurs when a mixture is heated. The spectrophotometrically determined T_m (melting temperature) gives a rough estimation of the stability of a double-strand and it depends on base composition, sequence and chain length as well as on salt concentration and pH of the solvent.

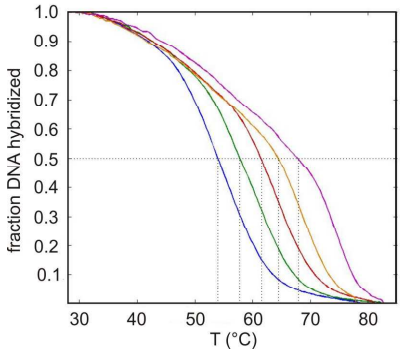


FIGURE 1.10 *Example of melting curves and identification of the respective T_m for different DNAs.*

1.1.7.1 Base composition and sequence:

In conventional base pairing, the interconnection between complementary bases G•C is stronger than A•T (three hydrogen-bonds vs. two, respectively) and that is reverberated into a direct relationship between the G•C content and T_m. There are a number of different ways to approximately calculate the melting temperature of an oligomere. All of these methods will give just a rough to good estimation of the experimental value. These calculations have to be considered as nothing else as a starting point for determining empirically the optimal annealing temperature. Among the several known methods of calculating hybridization temperatures for oligonucleotide probes a commonly used one is that where T_d calculation is based on the number of AT and GC base pairs [199]. The correlation of helix stability with GC content is well-known. An important factor to consider in the definition of the stability is the influence of the sequence. Several experimenters have accumulated evidence on synthetic high polymeric nucleic acids with regular sequences that clearly demonstrate the dependence of stability on factors other than GC content. A more precise method for determination of duplex-melting temperature is based on nearest-neighbor thermodynamic parameters [200], The major drawbacks of using these parameters are first, that they apply to solution hybridization, and second, that calculation of melting temperature is too laborious to be done by hand for long sequences [201].

1.1.7.2 Counter-ion concentration:

Nucleic acids are highly charged polyanions. A nucleoside monophosphate has two ionizable hydroxyl group of $pKa_1 < 1$ and $pKa_2 \approx 6.8$; at biological conditions (pH 6.5 ÷ 7.5) the DNA olygomer has one negative, if not two negative charges each nucleotide. To be stable, DNA needs to associate with counter-ions from solution. The cations bind the helix through electrostatic interactions with the sugar-phosphate backbone; they form a cloud around the charge density of the nucleic acid but are not firmly bound to any specific site (counter-ion condensation) [169-171].
The effect of the hydrated counter-ion condensation is to reduce the effective charge on the nucleic acid; this has considerable consequences on solution properties, binding interactions, stability on nucleic acids and contributes to determine the secondary and tertiary structure assumed by polynucleotide in solution. Therefore nucleic acid structures are generally dependent on salt content, with special impact of divalent cation.

1.1.8 Non-canonical nucleic acid secondary structure.

Each nucleoside is rich of functional groups which can interact with other molecules (nucleotides, proteins, etc.) along edges different than the canonical one. Hence, further base pairs in addition to the canonical Watson-Crick ones can be originated. In fig. 1.5, 28 supplementary experimentally detected distinct possibilities of forming at least two

hydrogen bonds between two bases are reported. That in addition to the inherent conformational flexibility of the polynucleotide backbone – including the puckering of the five-member sugar ring – and rotation about the glycosidic bond produce a wide range of structural variability. Some significative examples of such structures based on non-canonical base pairing are reported in the following:

1.1.8.1 Bulges

Bulges are formed in a duplex when there is an excess of residues on one strand but the rest of the nucleotides on both the sides are Watson-Crick complementary and can base pair. The extra bases can either stack into or be looped out of the duplex. In general the presence of bulges on a duplex has the effect to:

a) distort e stacking of the bases in the duplex
b) induce a bend in the duplex
c) reduce the stability of the helix
d) increase the major groove accessibility at base pairs flanking the bulge

Bulges are motifs very frequently met in nucleic acid structures since they can modulate the stability of the duplex and provide an effective high-affinity site for interaction with other molecules.

1.1.8.2 Telomeres

The ends of the linear chromosomes of eukaryotes are capped by telomeres – protective structures composed of repetitive noncoding sequences of guanine-rich DNA bound to proteins. Telomeric DNA of vertebrates consists of tandem repeats of the sequence d(TTAGGG), and in human somatic cells is typically 5–8 kilo bases (kb) long with a single-stranded 3' overhang of 100–200 bases. These G-rich ends can fold up into four-stranded G-quadruplex structures whose central units are planar arrays of four guanine bases (G-quartets), hydrogen-bonded along Hoogsteen/Watson-Crick edges and with the several Gquartets held together by π–π stacking interactions and connected by TTA edgewise loops. Monovalent countercation (Na^+ or K^+) is sandwiched between the G•G•G•G tetrad planes. The overall structure shows a different geometrical arrangement depending on the cation present in solution (See Pic.) [158, 159].

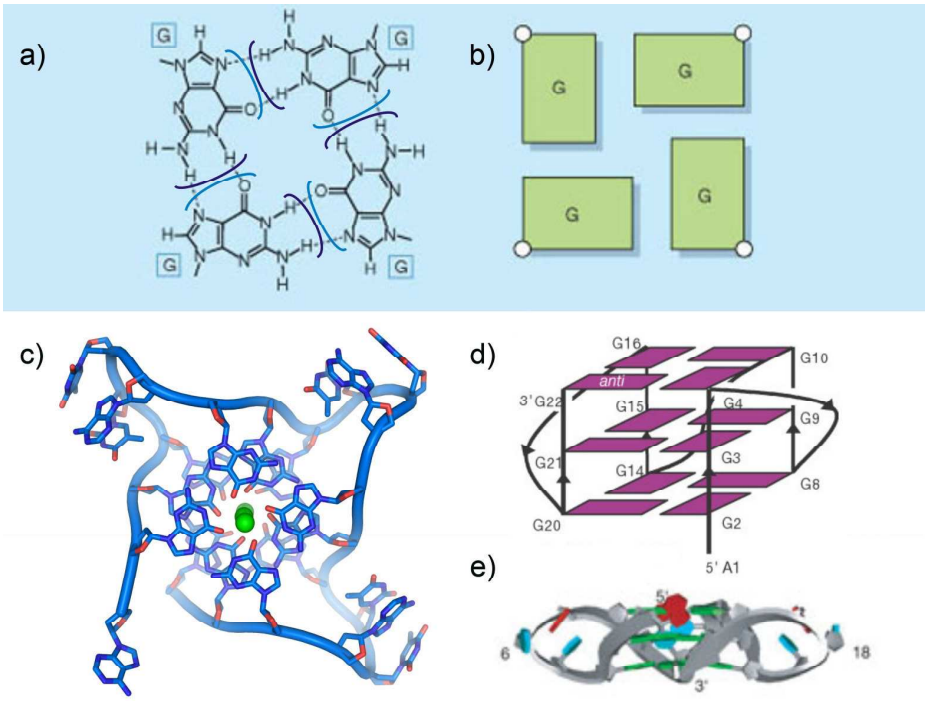


FIGURE 1.11 *the planar G•G•G•G tetrad alignment, viewed from above – each G undergoes two hydrogen-bonds along a Watson-Crick (violet) as well as Hoogsteen (cyan) edges – in chemical (a) and schematic (b) form. Overall folding topology of the d[AGGG(TTAGGG)$_3$] strand: c) axial view from the 3' end of the quadruplex looking down the helical axis – in green is cation K^+ and e) side view; in this latter the edgewise loops are evident (T in cyn and A in red). in d) the schematic diagram of the same 22-mer: all the glycosidic groups show anti conformation.*

1.1.8.3 Hairpin loops

Hairpins are a basic unit of nucleic acid structure and have been shown to play an important role in many biological processes. They consist of a single-stranded loop region closed by a base-paired stem. Normally the stability of a hairpin structure increases with the number of the base pairs in the stem region but some DNA or RNA hairpins having loops with certain specific sequences are unusually stable. DNA fragments such as d(GCGAAAGC) or d(GCGAAGC) occur frequently in biologically important regions, such as replication origins and promoter regions [160]. In this sense hairpin loops together with the bulges present in the stem and originating from base pairing mismatch, play a very important role in the regulatory process because they can modulate the stability of the stem in relation to inhibitor, promoter, regulatory agent, etc. (See Pic.) [165]. The example of two interacting hairpins in a so called loop-loop kissing motif is shown in fig. 1.12. In this thesis especially stable hairpin loops are used to stabilize canonical B-form DNA (see section 3.1).

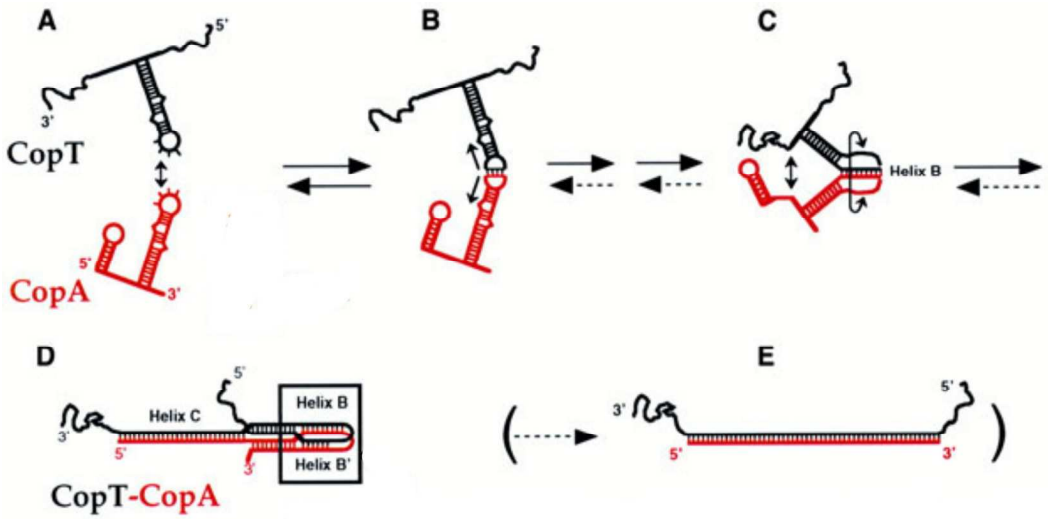


FIGURE 1.12 *Replication of plasmid R1 is regulated at the translational level by rapid and specific binding of the antisense RNA (CopA) to its fully complementary target site (CopT). To initiate, the process requires a rapid and selective interaction between the hairpin loops producing an extended kissing complex (A and B); the interaction is fully reversible and very unstable. Required for rapid binding are the bulged residues located in both the stems which destabilize the upper double helix to facilitate intramolecular base pairs breakage. Inter-strand helix propagation can now occur unidirectionally into the upper stem regions (C). Dissociation rarely occurs once the RNAs have encountered each other in a compatible orientation, and consequently, binding proceeds quasi-irreversibly to the formation of a four-helix junction. This bulky structure is further stabilized by the formation of a third long intermolecular helix involving the 5' tail of CopA and the complementary region of CopT (D). Picture from F. A. Kolb et al., The EMBO Journal 2000, Vol. 19, 21, 5905.*

1.1.8.4 Triple helix

Triple-stranded nucleic acid helices (triplexes) were first described in 1957 [161,162]. It has been mostly considered as laboratory curiosity, until it was found that stretches of triple helix could be formed by oligonucleotides of appropriate sequence binding to much longer duplex DNA molecules, demonstrating the exceptionally high specificity for a target duplex sequence that triplex formation can achieve [163]. This specific nucleic acid geometric arrangement is formed when solutions of poly(A) and poly(T) were mixed in appropriate proportions, forming a 1:1:1 three-stranded polynucleotide complex, poly (T•AT). A molecular model for this novel helix was proposed on the basis of fiber diffraction data [164] on both ribo- and deoxy-polynucleotide. The core structural interaction provides a purine strand Watson–Crick hydrogen-bonded to a pyrimidine one,

and a third pyrimidine strand Hoogsteen hydrogen-bonding the purine strand and parallel to this strand (fig. 1.13). The three oligonucleotides wind around each other and form a right-handed triple helix. Noteworthy, the third-strand cytosine of C^+•GC triplex requires the N3 position being protonated in order for two hydrogen bonds to be formed and so enabling effective Hoogsteen hydrogen-bonding to take place (fig. 1.13.b). The C^+•GC triplex is isosteric with the T•AT one. Protonation of a cytosine base at N3 can only take place at about pH 5.0. This places a potentially severe practical limitation of such triplex formation in biological systems.

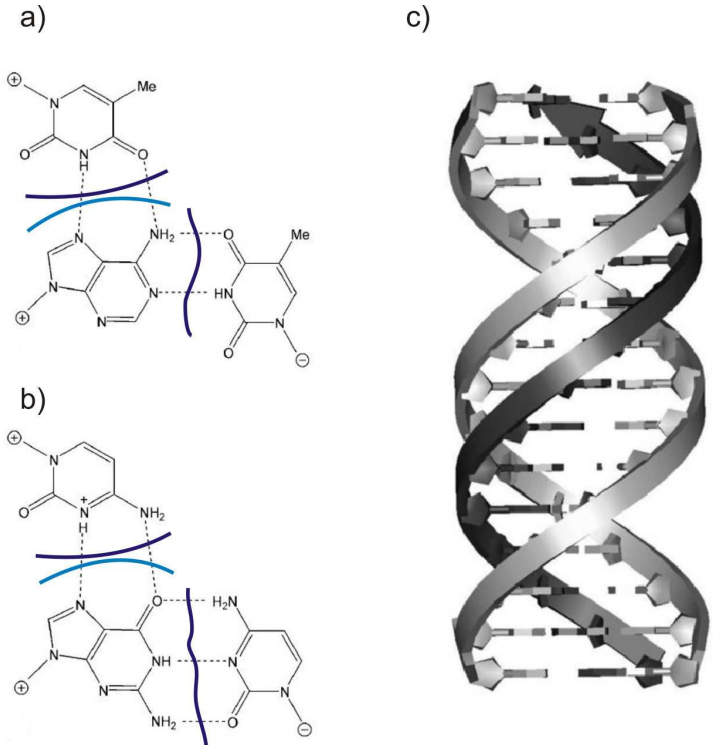


FIGURE 1.13 *a) T•AT and b) C^+•GC triplet: base pairing take place along the Watson-Crick (violet) and Hoogsteen edge (light blue) of the Purine base. c) schematic view of a DNA parallel triple helix, with the third (pyrimidine) strand shown shaded.*

1.2 Charge transfer in DNA

The possibility that the one-dimensional array of π–stacked base pairs in B-form DNA might serve as a pathway for charge migration was suggested over 40 years ago [1]. It did not take long after Watson and Crick described the now iconic double helix structure for a question to arise about the ability of DNA to transport electrical charge. The resemblance of the highly organized array of aromatic bases in the DNA double helix to conductive, one-dimensional aromatic crystals prompted the suggestion that the DNA π-stack might facilitate charge transfer (CT). It seemed apparent that the systematically arranged assembly of orderly stacked aromatic bases might make possible the movement of an electron (or *hole*, i.e. a positive charge in CT jargon) along the length of the

polymer. At the same time, it could actually sound bizarre that DNA, the molecule responsible for the safekeeping of the genetic information, was anything but inert when it comes to the transport charge. Since then, the fundamental question whether DNA serves as a medium for long-range charge transfer (CT) has been the subject of much controversy. The dispute has been solved by different interpretations of the applied DNA systems and the description of alternative mechanisms. Barton and coworkers pioneered this research through remarkable contributions about photo activated CT chemistry in DNA [25] and relevant was the work of Fink and Schönenberger, whose experimental approach to measure the electrical conducting properties of DNA was proposed using a *low energy electron point source* (LEEPS) microscope [58]. Under ultrahigh vacuum conditions, the electrical current of the DNA fibers was directly measured as a function of applied potential. Resistivity values calculated from these measurements are comparable to those of conducting polymers. These measurements were made over large distances (600 nm at least) and unambiguously indicated that DNA transports electrical current as efficiently as a good semiconductor (resistance ~ 10^6 Ω, see fig. 1.14). CT chemistry through DNA is now widely accepted and the discussion has moved to the crucial question of its mechanism [2, 3] and most recently, to its biological relevance and biotechnical application. DNA-mediated charge transfer occurs on an ultrafast time scale and can result in reactions over long range distances [30]; this property along with the fact that DNA molecules of specific composition and different length (from few nucleotides to chains of several tens of micrometers long) can be routinely prepared, make DNA ideally suited for the construction of mesoscopic electronic devices; hence the growing role played by CT in the recent development of microarrays detecting single-base mismatches, various DNA lesions by electrochemical readout methods [32, 33] or DNA chips – segmented, planar arrangement of immobilized DNA fragments used in a wide field of applications, from expression analysis to diagnostic chips [34-37]. In the latter case, a reliable detection of genomic sequence variations, mainly point mutations (*single-nucleotide polymorphism*), is critical for the study of population genetics, for the clinical diagnostic of cancer, for the diagnosis and treatment of genetic or viral diseases such as AIDS and, most recently, for the concept of pharmacogenetics [38]. Furthermore, DNA-mediated charge transfer has been the subject of considerable interest, having biological relevance in the formation of oxidative damage of DNA that can result in severe consequences such as mutagenesis, apoptosis or cancer [21, 31, 59]. Radiation, carcinogens, and metabolic waste products can damage DNA [68] and, if left unrepaired by the normal cellular machinery, these can lead to mutations and carcinogenesis with serious health consequences.[10–18]. Many of the aforementioned damages arise from selective radical reactions with guanine bases. This is consistent with the relative ease with which guanine is oxidized compared to other bases.

Generally the electrochemical oxidation potentials of nucleosides vs. NHE (*normal* – or *standard – hydrogen electrode*) can be described in the series: (+1.3 V) dG < dA < dC ≈ dT < dU (≥1.8 V) and the electrochemical reduction potentials of nucleosides vs. NHE in the series: (<–2.76 V) dG << dA < dC ≈ dT < dU (–2.07 V)]. [19–24]

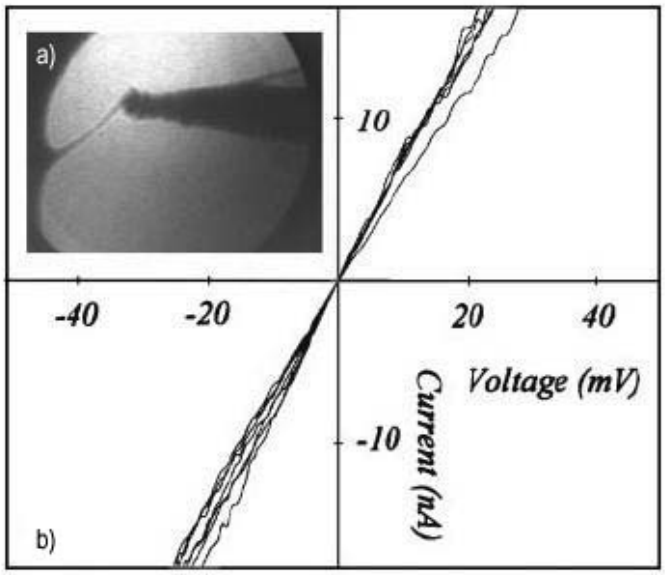


FIGURE 1.14 *a) LEEPS image taken with 70-eV electrons, showing the mechanical and electrical manipulation of DNA ropes. b) I vs. V curve when the manipulation-tip is attached to both DNA ropes. The measured resistance drops to 1.4MΩ. The DNA rope is 900nm long. Picture from H. W. Fink, C. Schonenberger, Nature 1999, 398, 407.*

CT in DNA is extremely sensitive to the π–stacking of the intervening DNA structure; perturbations of the base stacking caused by base mismatches, DNA lesions or DNA-protein interactions are effectively reported by altered CT. While protein interactions can modulate the CT in DNA, mismatches and DNA lesions significantly interrupt charge transfer in DNA and, as a result, the electrochemical response is missing. Despite its obviously fundamental, technological and physiological significance, the mechanism of DNA CT remains mostly unclear [4–9]. Understanding how CT proceeds through DNA would be important, both for a complete description of the reactivity of DNA and because, being such a system with a highly defined yet conformationally dynamic molecular π-stack, it represents a paradigm for studies of CT in donor-bridge-acceptor systems.

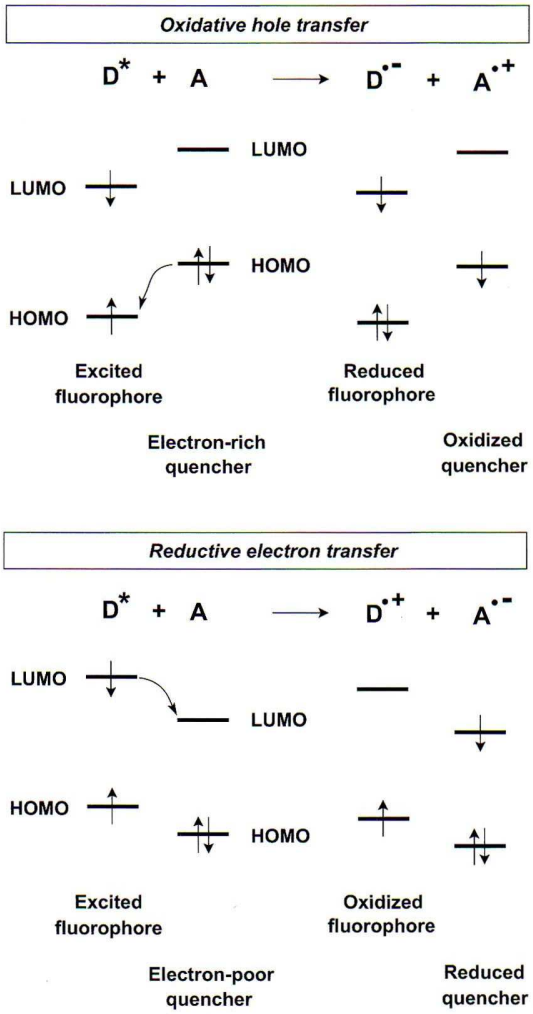


FIGURE 1.15 *Oxidative hole transfer (HT) and reductive electron transfer (ET) schematical mechanisms. In both cases a photo excited pluorophore is used to initiate the CT process. In HT an electron is removed from the highest occupied molecular orbital (HOMO) of the acceptor DNA (A) into the HOMO of donor (D) – DNA is oxidized. In reductive ET the photo excited electron is shifted from the lowest unoccupied molecular orbital (LUMO) of D into the LUMO of the acceptor DNA (A) – DNA is reduced. Pic. from H. A. Wagenknecht, Highlights in Bioorganic Chemistry: Methods and Applications 2004, Wiley VHC; 369.*

1.2.1 Characterization of charge transfer dynamics

1.2.1.1 Spectroscopic approach

Using a variety of spectroscopic techniques, the time-resolved dynamics of charge transfer and transport has been observed on time-scales from 10^{-15} to 10^{-3} seconds. Time-resolved studies are coupled to steady state spectroscopy in order to evaluate both reaction rate constants and yields. Luminescence is the emission of light that can occur when a molecule exited to a higher electronic state relaxes back to the singlet ground state (S_0) (See Transition moment section). Depending on the electronic nature of the excited state – singlet (S_1) or triplet (T_1) – luminescence is divided into two categories: fluorescence and phosphorescence, respectively. The fluorescent emission is spin-permitted ($\Delta S = 0$) and faster (lifetime typically on the order of 10 ns corresponding to emission rate $k_{EM} \approx 10^8\ s^{-1}$) in comparison to phosphorescence (lifetime between 10^{-3} s and s or longer). The emission rate is important to consider since it governs the time window during which a fluorophore can monitor its environment. Fluorescence spectroscopy is employed to measure excited state lifetimes of redox probes within the DNA π-stack and to monitor charge transfer quenching of these photo excited reagents. This defines the time-scale and efficiency of the reaction. An incident photon is exploited to excite a fluorophore and initiate the charge transfer between electron donor and acceptor differently positioned in the DNA helix. The intrinsic fluorescence of the naturally occurring nucleotide bases is extremely weak though [72–73]. This lack has spurred the development of non-natural nucleotide base analogs with optimized fluorescence properties such as higher quantum yield and longer lifetime. In studies of nucleic acids, fluorescence spectroscopy provides a very effective and sensitive instrument to inquire and characterize structural properties and conformational dynamics resulting from interaction with other molecules, ligands or ions. Transient absorption spectroscopy is employed to directly observe the transient intermediates along the CT pathway, and to monitor back electron transfer (BET) [202-204].

1.2.1.2 Biochemical approach

Biochemical assays truly probe *chemistry at distance* by examining DNA damage and repair products generated by long-range DNA-mediated CT. The experiments typically involve steady-state irradiation of an intercalated photo oxidant tethered to DNA, leading to the injection and migration of a hole through the duplex. The aforementioned positive charge is ultimately trapped at the sites of lowest oxidation potential, which are guanine sites in native DNA [19]. The resulting G radical cation ($dG^{\bullet+}$) can react with H_2O and/or O_2 yielding oxidized G products of G^{ox} (see fig. 1.17) [69]. The most common photo oxidants for DNA are metal complexes or organic intercalators such as Rh(III) complexes, Ru(II) complexes, ethidium derivatives, anthraquinone derivatives, uridine modified with cyanobenzoquinones and modified 2'-deoxyribosides bearing a photo reactive group (see fig. 1.16). Despite significantly differences in their structural properties, redox potentials and absorbing wavelengths, in all these systems the positive charge can be transported with high efficiency over very long distances; up to 200 Å. The

ability of the oxidant to initiate damage at a distance is fundamentally related to its association with the DNA base stack. Furthermore, strongly coupled π-stack – including the redox participants and the intervening DNA bases – is essential to CT efficiency. DNA assemblies possessing base bulges between the oxydative-target sites distal and proximal to the intercalated photo oxidant display a dramatic reduction in the distal/proximal ratio of oxidative damage [107]. This oxidative chemistry shows how long range hole transfer (HT) is characterized by a pronounced sensitivity to stacking with minimal sensitivity to distance.

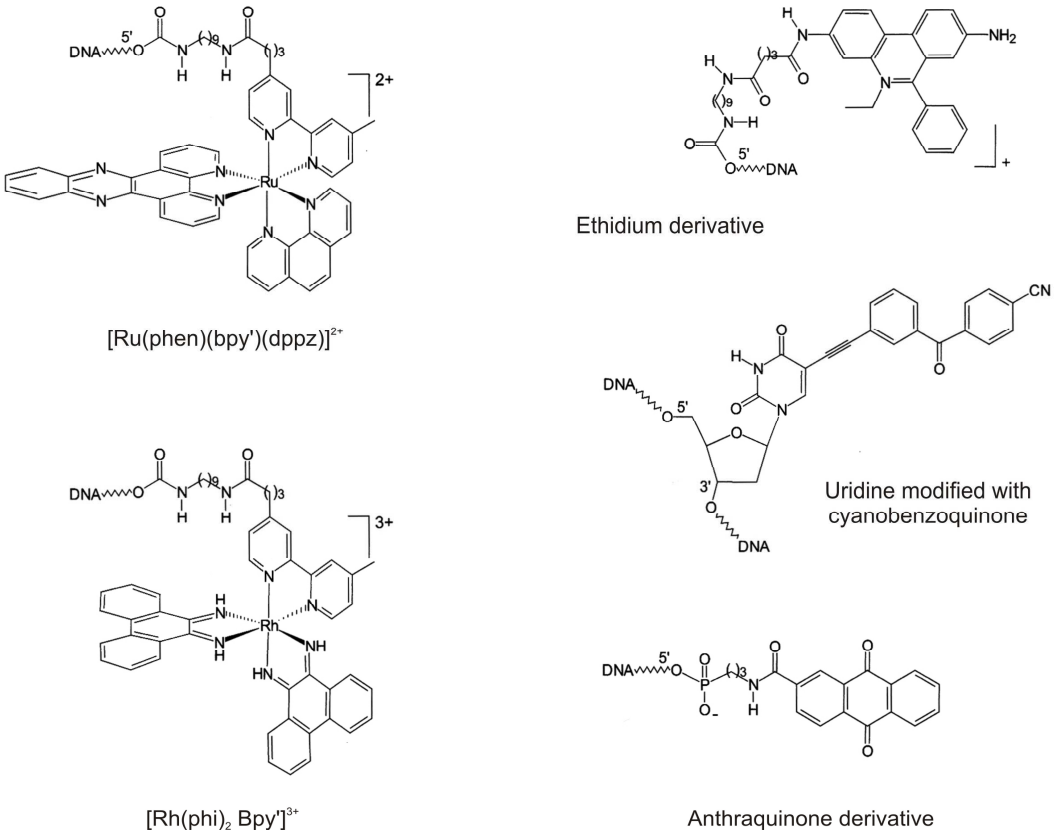


FIGURE 1.16 *Example of photooxidants commonly used in biochemical studies of HT in DNA.*

Interestingly, the 5'-G of 5'-GG-3' doublets has been found to be particularly sensitive to oxidation [24]. *Ab initio* molecular orbital calculations predict that the oxidation potential of 5'-GG-3' doublets is reduced relative to single guanines and that the HOMO (*Highest Occupied Molecular Orbital,* see fig. 1.15) lies predominantly on the 5'-G [23]. Preferential oxidative damage at the 5'-G of 5'-GG-3' doublets has therefore become a signature of HT chemistry. G-rich regions are typically found in CpG islands, introns and telomeres (see section 1.1.8.2), such areas have to be considered as hot spots for G

damage and as a result, efficacious arrays in preventing the genome from oxidative radical damage [46, 47].

The generality of long-range guanine oxidation via DNA-mediated HT has been established by the use of a variety of distinct intercalating oxidants [92, 116–122].

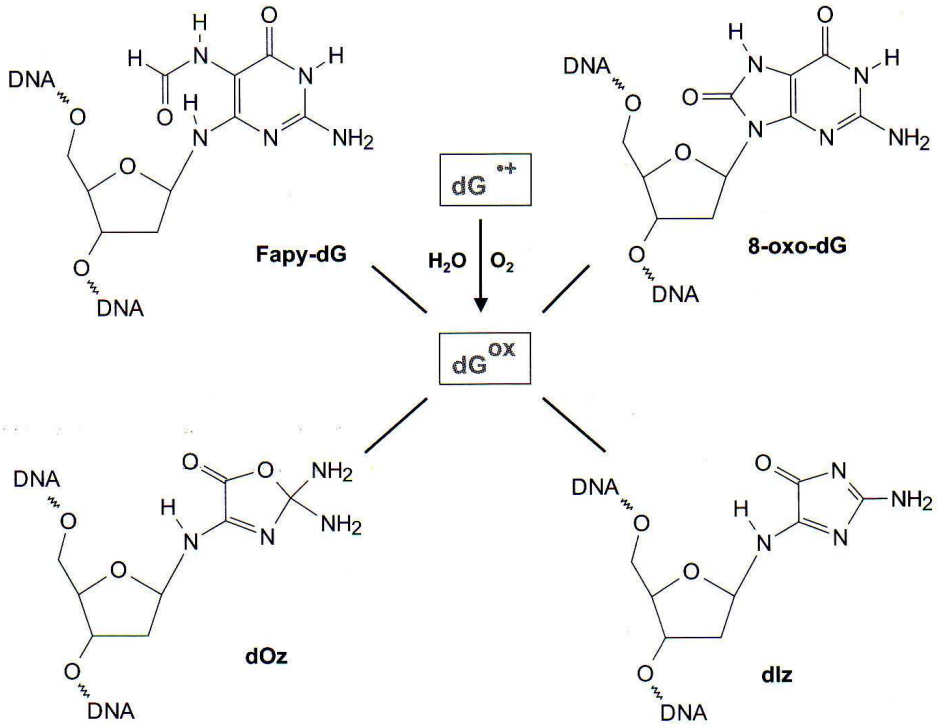


FIGURE 1.17 *Examples of oxidative G damage (G^{ox}): Fapy-dG – Formamidopyrimidine; 8-oxoguanine – 8-oxo-dG; Oxazolone – dOz; Imidazolone (dIz) Pic. from H. A. Wagenknecht, Highlights in Bioorganic Chemistry: Methods and Applications 2004, Wiley VHC; 369.*

1.2.2 Modified bases

Artificial DNA bases with specific photophysics and redox properties are largely used in order to characterize the CT dynamics in DNA. Oligonucleotides containing base analogues at defined positions can be readily prepared by standard solid phase synthesis. These probes are selectively excited to generate powerful photooxidants (or photoreducents) which initiate CT chemistry within the base stack. The base analogues employed are neutral molecules and their CT reactions with natural DNA bases involve charge separation. The hydrogen bonding and stacking interactions of base analogs within DNA duplexes are often comparable to naturally occurring bases. The general

utility of a nucleotide base analog depends on whether the probe exhibits reasonable fluorescence properties under physiological conditions (e.g. aqueous buffer, salinity and pH range between 6.5 and 8) which may also be sensitive to changes in local physical or chemical environment. Variation in pH, ion binding, interaction with other molecules, alteration in secondary or tertiary structure may cause changes in the fluorescence properties like emission intensity, lifetime or anisotropy.

1.2.3 Oxidative electron transfer in DNA: hole transfer and hole hopping in DNA

1.2.3.1 Superexchange mechanism

Photochemically- or photophysically-induced oxidation of DNA generates an electron vacancy in the duplex (*hole*). The so created positive charge can be transferred along the neatly organized DNA π-stack (*hole transfer; HT*). CT processes were initially described in terms of a superexchange mechanism and the results of the pioneering investigations interpreted on the base of the Marcus theory [60]. The charge tunnels in one coherent step from charge donor (D) to charge acceptor (A) and never resides on the intermediate DNA bridge (B) (see fig. 1.18). In this case, the charge transfer kinetic rate k_{CT} depends on the distance R between A and D and the exponential attenuation parameter β, which is itself dependent on the nature of the bridge B and its coupling with D and A.

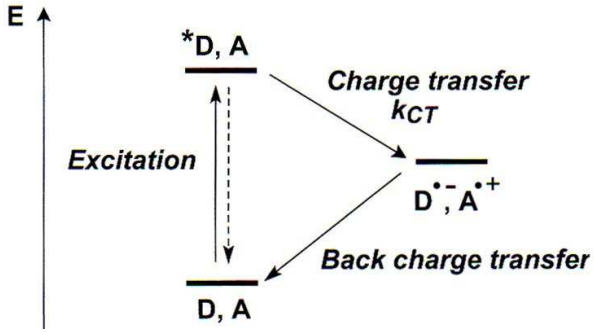


FIGURE 1.18 *Charge transfer scheme.*

$$k_{CT} = k_0^{-\beta R} \quad \text{(eq. 1.2.1)}$$

Values of β determined for CT reactions in DNA can be found in a wide range from β < 0.1 $Å^{-1}$ to β > 1.5 $Å^{-1}$. Differently, β values for CT through proteins lie in the range of 1.0 – 1.4 $Å^{-1}$ [205].

1.2.3.2 The hopping model

The occurrence of β values smaller than the typical (≤ 0.1 $Å^{-1}$) with very shallow distance dependence led to the description of an alternative mechanism – the hopping model. As a

result of the ease with which G is oxidized, the G radical cation plays the role of the intermediate charge carrier during the hopping process. In contrast with the super exchange mechanism, when the positive charge is created into the DNA base stack it hops from G to G and can finally be trapped at a suitable charge acceptor (A in the fig. 1.19). Each hopping step is itself a tunneling process through the intervening adenine – thymine base pairs. In contrast to the super exchange mechanism the rate of HT by hopping does not depend on the overall distance between D and A but is only dependent on the number of hopping steps (N):

$$k_{CT} \propto N^{-\eta} \text{ (eq. 1.2.2)}$$

The power parameter η is experimentally determined by charge hopping random walk [123] Lewis et al. were able to measure the rate for a single hopping step from G to GG, as $k_{HOP}=10^7\ s^{-1}$.[124]

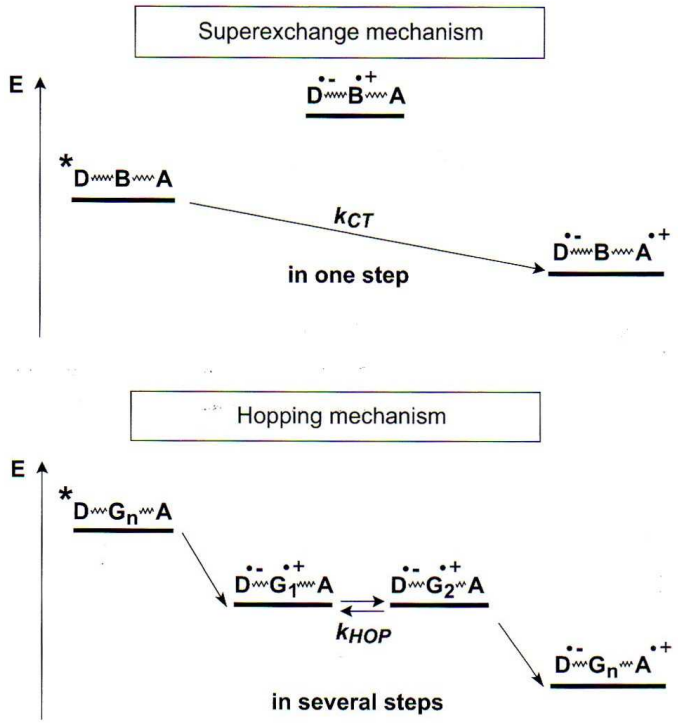


FIGURE 1.19 *Schematic representation of super exchange and hopping mechanism of DNA-mediated hole transfer. Pic. from H. A. Wagenknecht, Highlights in Bioorganic Chemistry: Methods and Applications 2004, Wiley VHC; 369.*

1.2.3.3 2-aminopurine

A fairly wide number of base analog probes are available and used for incorporation into

oligonucleotides for biochemical and biophysical studies. Among others, 2-aminopurine (2AP) is extensively employed as a nucleoside model for HT in DNA. **F**luorescence spectroscopy and calorimetry investigations of the structure stability of DNA duplexes containing the base analogue 2-aminopurine point into the direction that 2AP undergoes pairing with T along the Watson-Crick edge and it is well-stacked within the helix in a similar manner to adenine [88,89]. Differently from the natural DNA bases which are essentially non-fluorescent [90-95], it is possible to selectively excite 2AP in DNA duplexes to generate powerful photo oxidants (2AP excitation wavelength λ_{ex} = 310 nm; $E^{0(*/-)}$ ~ 1.5 V vs. NHE) [40]; 2AP* can initiate CT chemistry within the base stack; ultrafast base-base CT can be directly observed and evaluated as well as the dependence of CT on distance, driving force, and intra- versus interstrand pathways [70,71]. The photoexcited 2AP emits strongly in solution and in DNA and the fluorescence is remarkably sensitive to its environment [77, 78]. While the vast majority of spectroscopic and biochemical investigations of DNA-mediated CT employ pendant redox reagents, survey of DNA-mediated CT benefits from the ability of 2AP* to probe base-base CT in structurally well-defined DNA assemblies unperturbed by auxiliary particiants in the redox reaction [70, 71, 82-87]. Although many hints point out that 2AP is incorporated in the same way into oligomeres as a corresponding A, a structure at atomic resolution in a canonical DNA in solution has not been shown so far.

1.2.4 Reductive electron transfer in DNA

With regard to important biological consequences such as DNA damages, mutagenesis and carcinogenesis, mostly only oxidative experiments have been observed. On the other hand, reductive electron transfer (ET) processes (see fig. 1.15) are currently used in DNA chip technology [48 – 52] and DNA nanotechnology [53, 54, 58]. The DNA bases most easily reduced are the pyrimidines; the reduction potentials of 5-(1-pyrenyl)-2'-deoxyuridine (Py–dU) and 5-(1-pyrenyl)-2'-deoxycytidine (Py–dC) are very similar [22, 55, 56]. The excess electron migration through DNA occurs via hopping mechanism involving all base pairs and the radical anions $C^{\bullet-}$ and $T^{\bullet-}$ as stepping stones.

1.2.4.1 5-(1-pyrenyl)-2'-deoxyuridine (Py–dU) and 5-(1-pyrenyl)-2'-deoxycytidine (Py–dC)

Photoexitation of the pyrenyl group results in intramolecular ET yielding the corresponding pyrimidine radical cation ($Py^{\bullet+}$– $dX^{\bullet-}$ with X = C,U); the pyrenyl group can be selectively excited (λ_{ex} = 340 nm) and the redox potential values is estimated to be in the range of –1.1V to –1.2V for the dU/dU^- and dC/dC^- couples [168]. The properties of the ET in Py–dU and Py–dC have been characterized by steady-state fluorescence spectroscopy and femtosecond transient absorption spectroscopy [167]. The pyridine radical anions generated are of different basicity. The pK_a of the protonated $Py^{\bullet+}$– $dU(H)^{\bullet}$ biradical has been determined by steady state fluorescence to be ~ 5.5. Neither water as the surrounding molecule nor H-bonding donors such as the complementary DNA base A

can protonate the radical anions $U^{\bullet-}$. In contrast, the pK_a of $Py^{\bullet+}$– $dC(H)^{\bullet}$ is estimated to be larger than 12; hence the non-protonated radical anion of dC ($Py^{\bullet+}$– $dC^{\bullet-}$) could not be observed in aqueous solution after excitation (fig. 1.20).

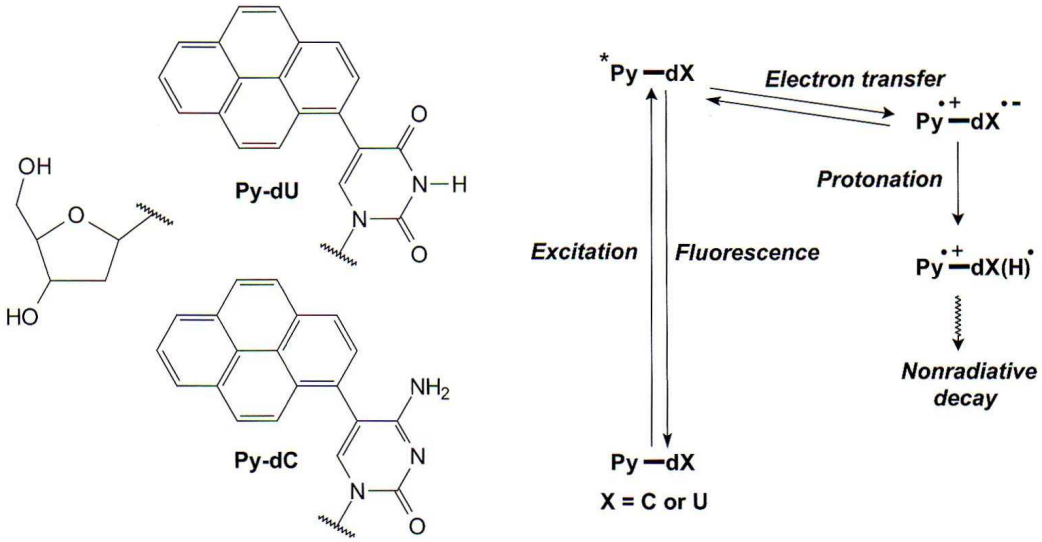


FIGURE 1.20 *Py-dU and Py-dC as nucleoside models for ET in DNA. When Py–dX (X = U or C) is excited at 340 nm intramolecular ET occurs. Subsequently, protonation can take place, yielding the biradical* $Py^{\bullet+}$*–* $dX(H)^{\bullet}$*. Pic. from H. A. Wagenknecht, Highlights in Bioorganic Chemistry: Methods and Applications 2004, Wiley VHC; 369.*

Although the situation in water cannot be directly compared with DNA, the results provide evidence that protonation of $C^{\bullet-}$ by the complementary DNA base G or the surrounding water molecules will occur rapidly. Therefore, $C^{\bullet-}$ is not suitable to act as intermediate charge carrier, whereas $U^{\bullet-}$ can act as efficacious stepping stone for electron injection into the DNA base stack [57]. The properties of PydX with respect to structural changes in DNA have not yet been studied. Specifically upon incorporation of PydU, femtosecond fluorescence data (T. Fiebig, Boston University, unpublished results) suggest a significant change in the dynamic behavior of canonical DNA. High resolution structural analysis is highly desiderable.

2. DNA structure determination: NMR-Spectroscopical aspects

Along with X-ray diffraction, nuclear magnetic resonance (NMR) spectroscopy is the most important method to explain molecular structures of either small molecules or large biomacromolecules. In particular these latter can exhibit a certain structural flexility and their seconday, tertiary and quaternary structure are strongly influenced by the specific environment. Based on observation, it's a general assumption that the conformation in aqueous solution is to a certain degree a significative representation of the bioactive one. It must therefore be considered being more reliable in comparison to the X-ray structure of the isolated molecule in crystalline state. Widely used in NMR spectroscopy are distance restraints derived from nuclear Overhauser effect (NOE) experiments [146]; the short proton–proton distances (< 5–6 Å) which can be derived from NOE data, are essential parameters for the three-dimensional structure determination of biomolecules.
For structural elucidation purpose, in recent years, NOE restraints have been increasingly combined with the use of anisotropic parameters, the most relevant of which are residual dipolar couplings (RDCs). Residual quadrupolar couplings (RQCs), residual chemical shift anisotropy (RCSA) and pseudo - contact shifts (PCSs) are also used in suitable cases. The estimation of anisotropic parameters has been made possible thanks to the recent development of suitable and properly tunable orienting media. Being proportional to the averaged orientation of the solute relative to the static magnetic field as an external reference, anisotropic parameters can provide information regarding the relative orientation of a specific bond vector to the others. This long-range angular information is complementary to traditional short-range interproton distance obtained from NOEs. Another important source of structural informations are angular restraints. Among several empirical relationships between scalar couplings and dihedral angles, the most important one is certainly the Karplus relation for ^{3}J - couplings.

2.1 Chemical shift

Chemical shifts are the most easily measured NMR parameters and carry important structural information. Their values depend on the electron densities around the nuclei, which is influenced by the surroundings in a variety of ways [149]. In principle, chemical shift (δ) could afford detailed structural information, which however cannot be extracted so easily because they either rely on large databases or quantum chemical calculations on MP2 level (Møller–Plesset second-order perturbation theory), which are usually not accessible for DNA oligomeres. In practice, chemical shifts are mainly used for rough assignment purposes. Tab. 2.1 gives an overview of the approximate ranges for the ^{1}H, ^{13}C and ^{15}N shifts in nucleic acids. Resonances of H3' to H5" in DNA are very similar (tab. 2.1). This is a potential source of overlap, which can be avoided or reduced by the use of ^{13}C correlated experiments when accessible.

Name	Thymidine	Uridine	Cytosine	Adenine	Guanine
H1′	5–6	5–6	5–6	5–6	5–6
H2′ (DNA)	1.7–2.3		1.7–2.3	2.3–2.9	2.3–2.9
H2′ (RNA)		4.4–5.0	4.4–5.0	4.4–5.0	4.4–5.0
H2″	2.1–2.7	2.1–2.7	2.1–2.70	2.4–3.1	2.4–3.1
H3′	4.4–5.0	4.4–5.0	4.4–5.0	4.4–5.2	4.4–5.2
H4′	3.8–4.3	3.8–4.3	3.8–4.3	3.8–4.3	3.8–4.3
H5′	3.8–4.3	3.8–4.3	3.8–4.3	3.8–4.3	3.8–4.3
H5″	3.8–4.3	3.8–4.3	3.8–4.3	3.8–4.3	3.8–4.3
H6/8	6.9–7.9	6.9–7.9	6.9–7.9	7.7–8.5	7.5–8.3
H5	1.0–1.9	5.0–6.0	5.0–6.0		
NH (G,T,U)	13–14	13–14			12–13.6
NH2			6.7–7.0	5–6	5–6
(C,G,A)			8.1–8.8	7–8	8–9
C1′ (RNA)	87–94	87–94	87–94	87–94	87–94
C1′ (DNA)	83–89	83–89	83–89	83–89	83–89
C2′ (DNA)	35–38		35–38	35–38	35–38
C2′ (RNA)		70–78	70–78	70–78	70–78
C3′	70–78	70–78	70–78	70–78	70–78
C4′	82–86	82–86	82–86	82–86	82–86
C5′	63–68	63–68	63–68	63–68	63–68
C2	154	154	159	152–156	156
C4	169	169	166–168	149–151	152–154
C5	15–20	102–107	94–99	119–121	117–119
C6	137–142	137–144	136–144	157–158	161
C8				137–142	131–138
N1/N3 (imino)	156	156–162			146–149
N2/6/4 (NH2)			94–98	82–84	72–76
N1(Py)/N9(Pu)	144	142–146	150–156	166–172	166–172
N3			210	220–226	167
N1(Pu)				214–216	
N7(Pu)				224–232	228–238

TABELLE 2.1 *Chemical shift for proton, carbon and nitrogen in nucleic acids; the values are applicable for both DNA and RNA. Data taken from Dieckman et al. [127], Hilbers et al. [128], Ippel et al. [129], Nikonowicz et al. [130], Pardi [131], Van de Ven and Hilbers [132], van Dongen et al. [133], Varani et al. [134] and Wijmenga et al. [135 – 137].*

2.2 Nuclear Overhauser enhancement (NOE)

A dipolar coupling is the interaction of the magnetic moments of two spins through space. The reorientation of molecules in solution produces alternated magnetic fields at the sites of the nuclei. It generates also the mutual relaxation between spatially neighboring nuclei, the so-called cross relaxation. This leads to exchange of spin polarization and induces relaxation of a spin system back to its equilibrium state. The intramolecular cross relaxation gives rise to the effect termed nuclear Overhauser enhancement (NOE). The intensity of the cross relaxation signals (the integral) is inversely proportional to the sixth power of the distances between the nuclei, hence it is possible to accurately extract valuable information about internuclear distance from two-dimensional NOESY experiments.

2.2.1 Determination of distance restraints from 2D-NOESY spectra

The intensities of the cross peaks in a NOESY spectrum are related to distances between spins via the relaxation matrix **R**:

$$\mathbf{A}(\tau_m) = \exp(-\mathbf{R}\tau_m)\mathbf{A}(0)$$

(eq. 2.1)

A(0) is the diagonal matrix of the signal intensity when the mixing time (τ_m) equals zero; **R** is the relaxation matrix whose elements are given by the equations:

$$R_{ij} = \sigma_{ij} = \frac{q}{r_{ij}^6}\left[6J(2\omega) - J(0)\right]$$

(eq. 2.2)

$$R_{ii} = \rho_{ii} = \frac{q}{r_{ij}^6}\left[J(0) + 3J(\omega) + 6J(2\omega)\right]$$

(eq. 2.3)

Each element of **R** depends on the element $q = \gamma^4 (\hbar/2)^2$ and is inversely proportional to the sixth power of the distances between the nuclei *i* and *j* (r_{ij}). For a rigid isotropically tumbling molecule, the spectral density function, J(ω), can be written as:

$$J(\omega) = \frac{2\tau_c}{5(1+\omega^2\tau_c^2)}$$

(eq. 2.4)

With τ_c the characteristic correlation time of the tumbling of the molecule and ω the Larmor frequency.

2.2.1.1 Isolated spin pair approximation (ISPA)

Several approaches exist to quantify distances from NOE-signal intensities; among them maybe the more straightforward is the *isolated spin pair approximation* (ISPA).
Although the relationship between NOE-peak intensity and distance is nonlinear, the time course can be linearized if the mixing time is short enough. In this event the effect of spin diffusion (see 2.1.1.2) can be consider negligible (isolated spin pair approximation).
The argument of eq. 2.1 can be expressed in a Taylor series. For short mixing time the expansion can be truncated after the second term:

$$\exp(-\mathbf{R}\tau_m) = \sum_{n=0}^{\infty} \frac{(-\mathbf{R}\tau_m)^n}{n!} \approx 1 - \mathbf{R}\tau_m$$

(eq. 2.5)

The cross-peak intensities depend only on the corresponding off-diagonal element in **R**. The calibration of the unknown interproton distances is possible using the peak intensity of a proton pair with known distance r_{ref}:

$$r_{ij} = r_{ref}\left(\frac{A_{ref}}{A_{ij}}\right)^{1/6} \quad \text{(eq. 2.6)}$$

Suitable reference proton pairs in nucleic acids are H5 and H6 in Cytosine (r_{HH} = 2.45 Å) or geminal protons in the sugar ring (r_{HH} = 1.80 Å).

2.2.1.2 Possible source of errors in the determination of the distance restraints

For a trustworthy calculation of interproton distances, the dipolar relaxation must be largely predominant in comparison to other relaxation processes, like e.g. paramagnetic relaxation. Hence, it is important in the preparatioon of the sample to avoid the contamination with paramagnetic ions, transition metals or oxygen.

Other possible source of errors are:

a) integration errors in the NOE intensity:
   a common integration mistake is due to overlap of two (or even more) cross – peaks. This specific source of error can be considered of little relevance. Inaccuracy of the NOE intensity lead to strongly damped errors in the derived distances, because of the inverse sixth power relationship; e.g. if a cross-peak intensity is double-estimated, the resulting distance restraint is only affected by the factor $2^{1/6} \approx 1.12$, which is usually included within the error range of distance restraints used in structure calculations [147] . Further remarkable note: for the quality of NMR derived structures, the number of structurally relevant distance constraints is more important than the precision of constraints [148].

b) short relaxation delays (RD) inbetween FID recordings:
   RD doesn't generally mislead structure calculations for medium sized biomacromolecules as in this case T1/T2 < 1. Choose RD ≥ 5 T1 prevent the reliability of measurement from such systematic errors.

c) spin diffusion:
The NOE/ROE vs. τ_m (mixing time) build-up curve loses linearity for long mixing times even in the case of two isolated spins (fig. 2.1A). That trend is more emphasized for short interproton distances. Magnetization is exchanged forth and back to the original spin due to auto-relaxation rate and higher-order transfers (fig. 2.2A). The effect is the underestimation of cross-relaxation rate and, consequently of short distance restraints. In larger spin systems magnetization can be transferred from one spin to its neighboring and further to a third spin. This effect, known as spin diffusion, overestimates cross-relaxation rates for distant spins, provided a shortcut via intermediate spins is present (fig. 2.2B). The calculation of distance restraints is more reliable at short τ_m, where the effects above described don't affect appreciably the linearity of the curve and therefore the accuracy of cross-relaxation rate calibration. In the specific case of nucleic acids, spin diffusion acts just marginally on the base protons because of the paucity of surrounding hydrogens. In the ribose ring conversely, spin diffusion can represent a possible source of errors at long mixing times because of the relatively high proton density. Hence, it is very important to choose an appropriate mixing time: short enough to prevent errors from non-linearity and adequately long to afford sufficient cross-peak intensities. Acceptable calibration results are generally extracted from a single, appropriately choosen mixing time. If more accuracy is required, a sequence of NOESY/ROESY experiments for each single cross-peak has to be acquired at different τ_m and then the results fitted in a build-up curve. For medium-sized biomacromolecules, 200 ms for ROESY and 300 ms for NOESY experiments are reasonable τ_m values [125,126].

d) internal dynamics:
NMR spectroscopy can on one side provide an extremely powerful method for monitoring the exchange of a nucleus due to chemical reaction or conformational transition, but on the other side represent a severe source of error in structure elucidation if the exchange effect is ignored (see fig. 2.2C and section 2.5.1). Compared to proteins, nucleic acids are significantly more flexible and their intrinsic conformational dynamics must be taken into account in stucture calculations; hence, relatively large error bars for derived distances have to be chosen accordingly.

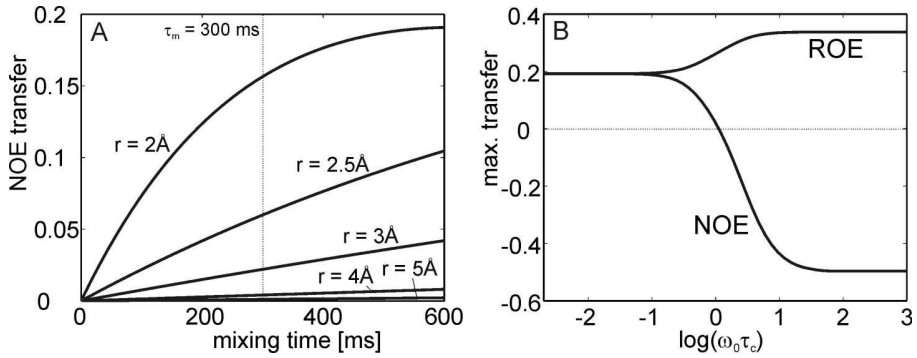


FIGURE 2.1 *The internuclear transfer of magnetization via NOE cross-relaxation in an isolated spin-pair. (A) Build-up curves for the cross peak intensity in a 2D-NOESY experiment at different internuclear distances r. The dashed line stands for* τ_m *= 300 ms tipically used for drug-like molecules. (B) Maximum transfer efficiency for an isolated proton spin pair calculated using only dipolar relaxation processes. The sign-change for the NOE cross-relaxation takes place at* $\omega_0\tau_c$*=1.12.* [138]

From B. Luy, A. Frank, H. Kessler, "Conformational analysis of drugs by nuclear magnetic resonance spectroscopy", in Molecular Drug Properties: Measurement and Prediction, Ed. R. Mannhold, volume 37 of Methods and Principles in Medicinal Chemistry (Eds. R. Mannhold, H. Kubinyi, G. Volkers), pp. 207-254 (2008).

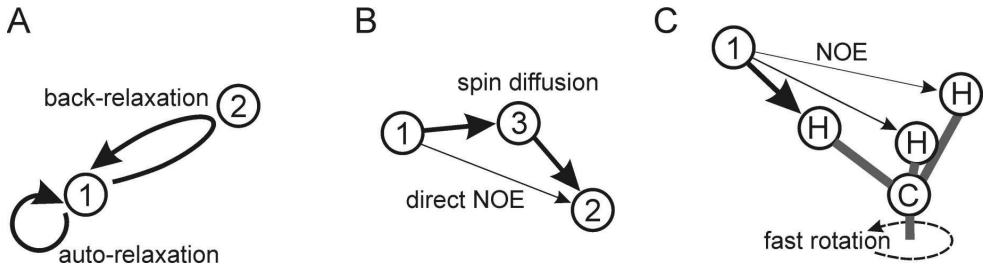


FIGURE 2.2 *Special NOE-transfer pathways that can lead to a wrong interpretation of cross-relaxation rates. (A)* Auto-relaxation and back-relaxation *(B) M*agnetization can transfer from one spin to its neighboring and further to a third spin; *this can lead to a significant contribution to the cross peak intensity for the direct NOE between spins 1 and 2. (C) Example of conformationally averaged distances: for fast rotating methyl groups the intensity of NOE-transfer from spin 1 is mainly determined by the closest distance to spin 1* [138]. From B. Luy, A. Frank, H. Kessler, "Conformational analysis of drugs by nuclear magnetic resonance spectroscopy", in Molecular Drug Properties: Measurement and Prediction, Ed. R. Mannhold, volume 37 of Methods and Principles in Medicinal Chemistry (Eds. R. Mannhold, H. Kubinyi, G. Volkers), pp. 207-254 (2008).

2.3 Assignment strategy for DNA at natural abundance

Assignment of resonances is the first essential step in an NMR study of biomolecules aimed at determining their three-dimensional structure.
For unlabeled compounds the ^{1}H sequential resonance assignment is naturally based on a combination of through-bond ^{1}H-^{1}H and NOE correlations augmented with ^{1}H-^{13}C and ^{1}H-^{31}P connectivities. The assignment for unlabeled nucleic acids consists of essentially four steps:

a) Assignment of the exchangeable protons (imino and amino protons) at low temperature in H_2O

- imino-imino NOE's
- imino-amino NOE's
- imino-aromatics NOE's

b) Assignment of the non-exchangeable protons at higher temperature in D_2O

- Identification of ribose spin systems via TOCSY
- Identification of H5,H5M/H6 connectivities for Pyrimidines in TOCSY experiment (in D_2O)

c) Correlation of the aromatic protons H6,8 to ribose protons via HMBC and/or NOESY (in D_2O)

d) Sequential assignment via (^{1}H-^{31}P) CPMG-correlated experiment and/or H2',H2''/H6,8 NOESY experiment (in D_2O)

solvent: H_2O	
	assignment of imino (and amino) resonances to establish base pairing
	NOESY imino-imino, amino-imino, imino-aromatics

solvent: H_2O	
	partial resonance assignment of non-exchangeable protons via NOE connectivities to amino and/or imino protons
	NOESY imino/amino – H2/H6/H8/H5/H2'/2''

solvent: D_2O	
	identification of sugar proton spin systems (mainly H1'/H2'/H2''/H3')
	(^1H,^1H) TOCSY
	identification of aromatic spin systems (Cytosine/Thymine H5/H6)
	(^1H,^1H) TOCSY
	Sequential resonance assignment
	NOESY H6/H8-H8-H1', H6/H8-H2'H2''

solvent: D_2O	
	unambiguous intranucleotide connection of sugar proton spin systems to the proton(s) of the base
	HMBC
	assignment of ^{31}P resonances and confirm/extend H3', H4', H5', H5'' assignments
	(^1H,^{31}P) CPMG-HSQC-NOESY

TABELLE 2.2 *Flowcharts for resonance assignment in unlabeled nucleic acids.*

2.4 Effects of the temperature

The temperature affect the conformational dynamics and therefore the line sharpe of resulting spectra. As long as one is free to choose the temperature for a given molecule, it can be adjusted to obtain best possible NMR conditions.

2.4.1 Effect on the linewidth

The line width is a factor of primary importance and it must be optimized in order to extract favorable resolution and signal- to- noise ratio. Line width is defined as the full-width at half-height (FWHH) and is affected by intrinsic molecular properties (homogeneous line width) and by instrumental imperfections, such as static magnetic field inhomogeneity, thermal gradients within the sample or imperfection of the NMR tube geometry (inhomogeneous line width). Especially in flexible molecule like nucleic acids, it is also strongly affected by conformational exchange effects (see next section). Considering just the homogeneous, absorptive Lorentzian line shape, the detecting signal $S(\omega)$ depends on the Larmor frequency (ω_0) and on the inverse of the transverse relaxation time constant ($R_2 = 1 / T_2$):

$$S(\omega) = \frac{R_2^2}{R_2^2 + (\omega - \omega_0)^2} \quad \text{(eq. 2.7)}$$

From eq. 2.7 it is easy to extract mathematically the full-width at half-height (FWHH):

$$FWHH = \frac{2}{T_2}$$

(eq. 2.8)

In the case of intra molecular dipole-dipole relaxation as the most important relaxation mechanism for nuclei of spin 1/2 and considering negligible internal molecular motions, R_2 is:

$$R_2 = \frac{3}{20} b^2 \{3J(0) + 5J(\omega_0) + 2J(2\omega_0)\}$$

(eq. 2.9)

Where $J(\omega)$ is the normalized spectral density of the dipole-dipole couplings at frequency ω [$J(\omega) = \tau_c / (1 + \omega^2\tau_c^2)$] and b is the Boltzmann factor ($b = -\mu_0\hbar\gamma^2 / 4\pi r^3$).

In the slow tumbling limit ($\omega\tau_c >> 1$) typical for medium to large molecules, it follows that T_2 is inversely proportional to the characteristic correlation time for the tumbling of the molecule (τ_c):

$$R_2 \propto \tau_c \quad \text{(eq. 2.10)}$$

According to Stoke's law:

$$\tau_c = \frac{4\pi\eta r_m^3}{3k_B T} \quad \text{(eq. 2.11)}$$

The correlation time τ_c is proportional to the effective hydrodynamic radius (r_m) including a potential solvent shell and viscosity of the solvent (η) while it is inversely proportional to the temperature (T); k_B is the Boltzmann's constant.

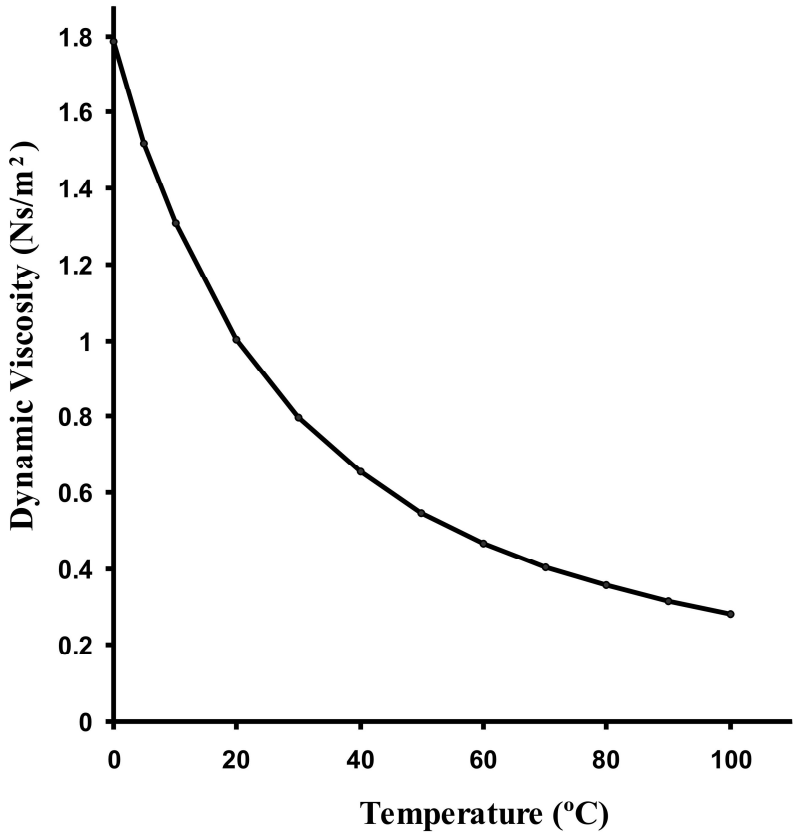


FIGURE 2.3 *Dynamic viscosity of water vs. T. Experimental data and picture taken from www.engineeringtoolbox.com*

Hence, temperature doesn't just affect τ_c but it also acts on the viscosity of water, as well as D_2O. In particular if T rises, η decreases dramatically (see fig. 2.3). For the combination of the aforementioned reasons, in case of slow tumbling molecules, the transverse relaxation time constant T_2 depends on temperature T so that warming the sample increases T_2 and consequently sharpens the absorptive Lorentzian line shape.

2.4.2 Effects of T on conformational dynamics

Consider the simplest, symmetrical two-site exchange system where:

$$A \underset{k}{\overset{k}{\rightleftharpoons}} B$$

The effect of the exchange process on the NMR spectrum depends on the difference in chemical shift frequencies between Ω^0_A and Ω^0_B associated to the state A and B respectively, as compared to the exchange rate (k, also known as the transition probability per unit time). The five following main cases are distinguishable:

e) $k < |\Omega^0_A - \Omega^0_B|$ (slow exchange rate)

f) $k \leq |\Omega^0_A - \Omega^0_B|$ (slow-intermediate exchange rate)

g) $k \approx |\Omega^0_A - \Omega^0_B|$ (intermediate exchange rate)

h) $k \geq |\Omega^0_A - \Omega^0_B|$ (fast-intermediate exchange rate)

i) $k > |\Omega^0_A - \Omega^0_B|$ (fast exchange rate)

According to Arrhenius' equation, k depends on temperature T as follows:

$$k(T) = A(T)e^{\{-E_{act}/RT\}}$$

(eq. 2.12)

with both the pre-exponential factor A(T) and the activation energy (E_{act}) shallowly depending on T; R is the gas constant. Warming the system has the effect of increasing the conformational dynamics. The system moves from slow or intermediate to a fast exchange rate; the final result is that the peaks proper of different states coalesce originating one single peak in the intermediate position of the originals. Further increase of the temperature produces the reduction of the NMR peak width by rapid molecular interconversion; this phenomenon is called motional narrowing, noticeable especially in molecules of remarkable flexibility, e.g. oligomers.

2.4.3 Effects of T on the imino protons

The imino-protons are very good indicators of duplex stability. The 1D NMR spectrum measured in H_2O at 273 K shows a collection of well-resolved imino-protons between 10.5 and 14 ppm. Raising the temperature doesn' t leave the exchangeable imino-protons indifferent though; the peaks coalesce or disappear at higher T because the exchange process with water speeds up according to Arrhenius equation (See Pic. in experimental section). The assignment of the imino-protons has to be made at the lowest temperature reachable, basically just above the freezing point; this condition is different than the optimal one for non-exchangeable protons (see section 3.4.2.1).

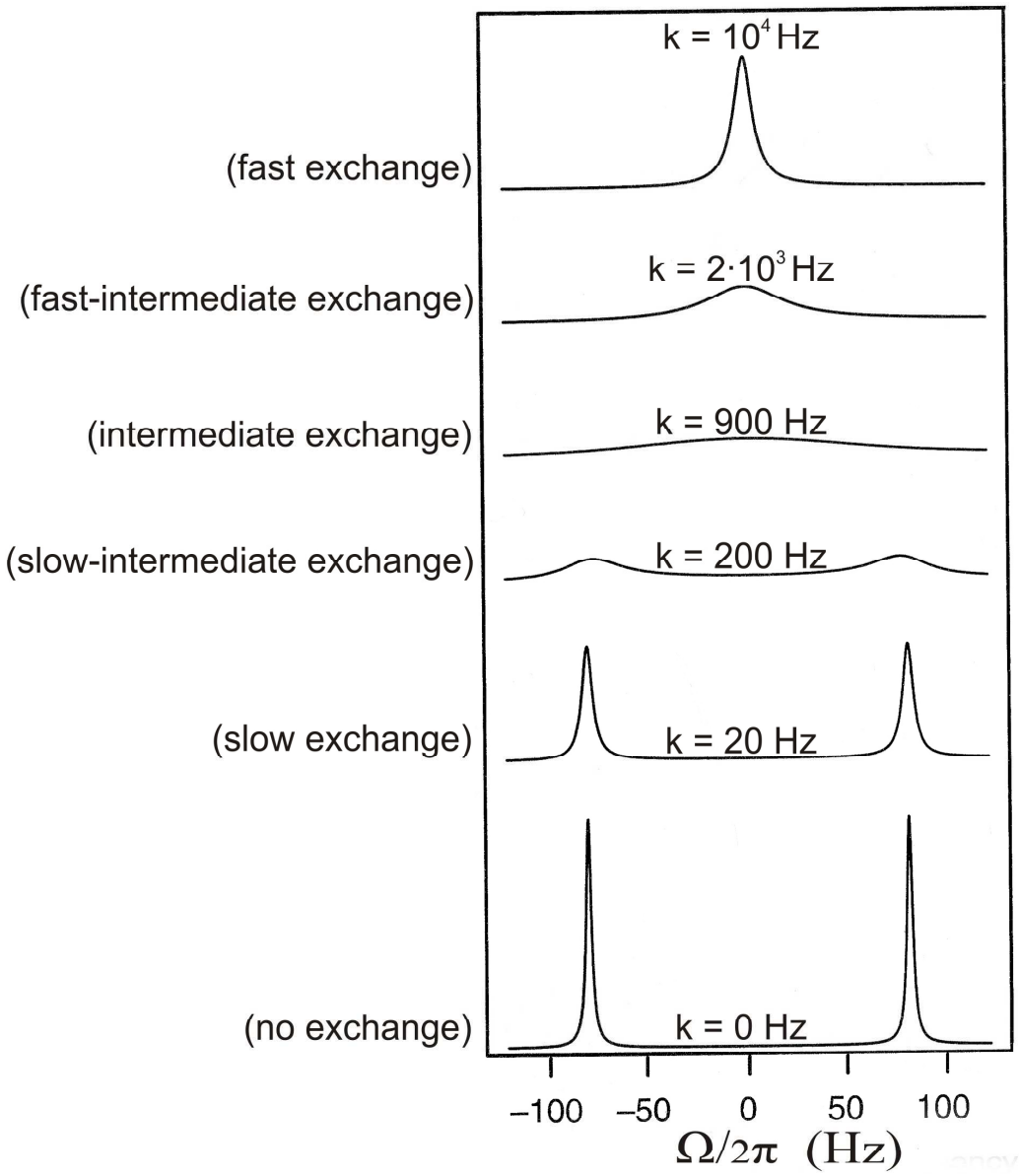


FIGURE 2.4 *Two-site exchange simulated spectra for different k; $\Delta\Omega = |\Omega^0_A - \Omega^0_B| = 10^3$ Hz. Picture adapted from J. Cavanagh, W. J. Fairbrother, A. G. Palmer, M. Rance, N. J. Skelton – protein NMR spectroscopy: principles and practice second edition – Academic Press 2007.*

3. Design and NMR-structure of a canonical B-form helix

The stability of a DNA duplex constituted by two short (5-10 nt) complementary single strands is limited. Typically a stem of six complementary base pairs is characterized by a T_m in the range of 20-25 ºC. In solution such a duplex is rather unstable even at room temperature and it is present in greater extent as denaturated, unstructured form (see section 1.1.7). An appropriate and well-studied solution to overcome the problem of the limited stability of a short duplex is the use of a tri-loop mini hairpin connecting the 3' end of one strand to the 5' end of the complementary one. At the cost of just three additive nucleotides, the T_m of a six base pairs stem is enhanced from 20-25 ºC up to the much more suitable range of 75-80 ºC. The augmented stability of the oligonucleotide enables the appropriate modulation of the measuring temperature in order to reduce the peak overlap in the non-exchangeable proton region where the problem of dispersion can be limiting (see sections 2.4 and 3.4.2.1).

3.1 Triloop mini-hairpin

Hairpins are nucleic acid sequences consisting of a single-stranded loop region closed by a base-paired stem which have been shown to play a significant role in a number of biological processes (see section 1.1.8.3). Short DNA minihairpin sequences, in particular d(GCGAAGC) and d(GCGAAAGC), occur frequently in biologically important regions [160] and are characterized by an extraordinary stability represented by high melting temperature (T_m = 76 °C for the first of the aforementioned sequences), polyacrylamide gel mobility, and resistance against nucleases [172,173]. Melting temperature and thermodynamic studies show how corresponding RNA sequences are much less stable, indicating that the stability of these loops depends on the presence of deoxyribose sugar on the backbone [172]. The 3-D structure of the d(GCGAAGC) fragment determined by NMR spectroscopy revealed that the extraordinary stability is caused by the additional double hydrogen bonded pair formed along the Shallow edge of G3 and the Hoogsteen edge of A5 (see section 1.1.4) and the extensive base-stacking interaction within G1C2G3A4 and A5G6C7 [173]. The extensive base stacking is possible because all of the residues in the fragment adopt a B-form DNA conformation except for the three bonds of A4 O3'-P, A5 O5'-P, and A5 C5'-C4', where the sharp turn occurs. The sheared G-A base pair in the loop is required for the formation of stable trinucleotide-loop hairpins [179]; this enables the two phosphates to be linked with only one nucleoside where for the Watson-Crick base pairs four to five residues are usually needed to link the base pairs [179]. The G-A base pair also contributes to the stacking of bases in the structure because it can connect both strands without destroying the base-base stacking. The observed thermodynamic stability [177], however, does not imply structural rigidity of the hairpin. Sugar pucker wobbling in C7, line broadening in A5, and unrestrained MD simulations showing correlated conformational changes of backbone torsions γ5, ζ4 and α5 suggest certain flexibility of the molecule. Flexible folded form may increase the stability as this is more favorable entropically [178]. The extraordinary stability of the mini-hairpins is widely exploited for stabilizing other oligonucleotides [174], e.g., for stabilization of mRNA in *in vitro* protein synthesizing

systems or for the isolation of full-length RNAs from cells [175,176].

3.2 Design of a stable B-form helix

The imino-protons are a very good indicator of the stability of the duplex. The line-width, the intensity and the persistence of the imino proton peaks as the temperature raises enable to evaluate the stability of the hairpin structure. The iminos are easily distinguishable compared to the rest of the aromatic protons. Because of the exchange, they are visible in water solution where they show rather weak, broad and characteristically downfielded peaks (10-14 ppm, see tab. 2.1). Raising the temperature, the conformational dynamics is speeded up. The solvent can access more easily the segregated imino protons and the exchange of the imino protons with the solvent protons becomes faster as well (see section 2.5.1). As a consequence the intensity of the peaks decreases. The imino proton peaks of a collection of eight different DNA hairpins are measured in water at different temperatures (fig. 3.1). In all the examined cases, the stems are constituted by six bp capped by tri- and tetra-loop (–GAA– and –GAAA– respectively). Formation of the Watson-Crick pairs is confirmed by the six signals in the imino proton region; the number and the chemical shifts of the peaks in the spectra are consistent with the formation of the expected duplexes. In each case, a broad signal at around 10.0-11.0 ppm is observable; it is characteristic of the imino protons of the guanine residue in the sheared G•A base pair. The closing C•G base pairing at the opposite end to the loop is not visible because the bottom side is freely accessible to the solvent and the exchange of G15H1 with H_2O can easily take place [180]. Considering the behavior of the imino proton spectra for all the examined cases, it emerges that the oligomeres *em39* and *em43* show the most stable structures. For both the duplexes six well resolved peaks are distinguishable at the lowest T (273 K). In the case of *em43* the relative full-width at half-height (FWHH, see section 2.5.1) of each peak falls in the range between 30 and 60 Hz for the conventional Watson-Crick base pair while it is 40 Hz for the sheared G•A bp (see fig. 3.2.d). Moreover, in comparison to the rest of the examined hairpins, the intensity of the peaks is the most persistent raising the temperature.

3.3 Effect of the counter-ion on the duplex stability

Positive cations have the effect of stabilizing the structure of DNA helix. The sugar-phosphate backbone is negatively charged and the cations bind to the external of the helix through electrostatic interactions. The hydrated counter-ion condensate along the backbone reduces the effective charge (see section 1.1.7.2). The effect of Na^+ as counter-ion is visible in the comparison of the imino spectra of the duplex em43 without and with [NaCl] = 50 mM (see fig. 3.2.a). In the presence of Na^+, the intensity of imino signals is considerably enhanced and the FWHH is reduced by of 2-3 Hz on average all peak (see detail in fig. 3.2.a).
Notoriously the presence of magnesium cation in solution influences the stability of a loop region [128]. In our case though, the addition of Mg^{2+} does not seem to have any

detectable effect on the imino protons spectra (see fig. 3.2.b).
The action of EDTA is advised in case of presence in solution of contaminating paramagnetic ions mainly represented by heavy-metals cations (see section 2.2.1.2). EDTA (sodium ethylenediamidetetraacetate) is an effective chelating agent which can sequestrate the paramagnetic ions averting such undesirable source of relaxation. Neither in this case any appreciable variation in the imino proton spectrum can be observed after the addition of EDTA (see fig. 3.2.c).

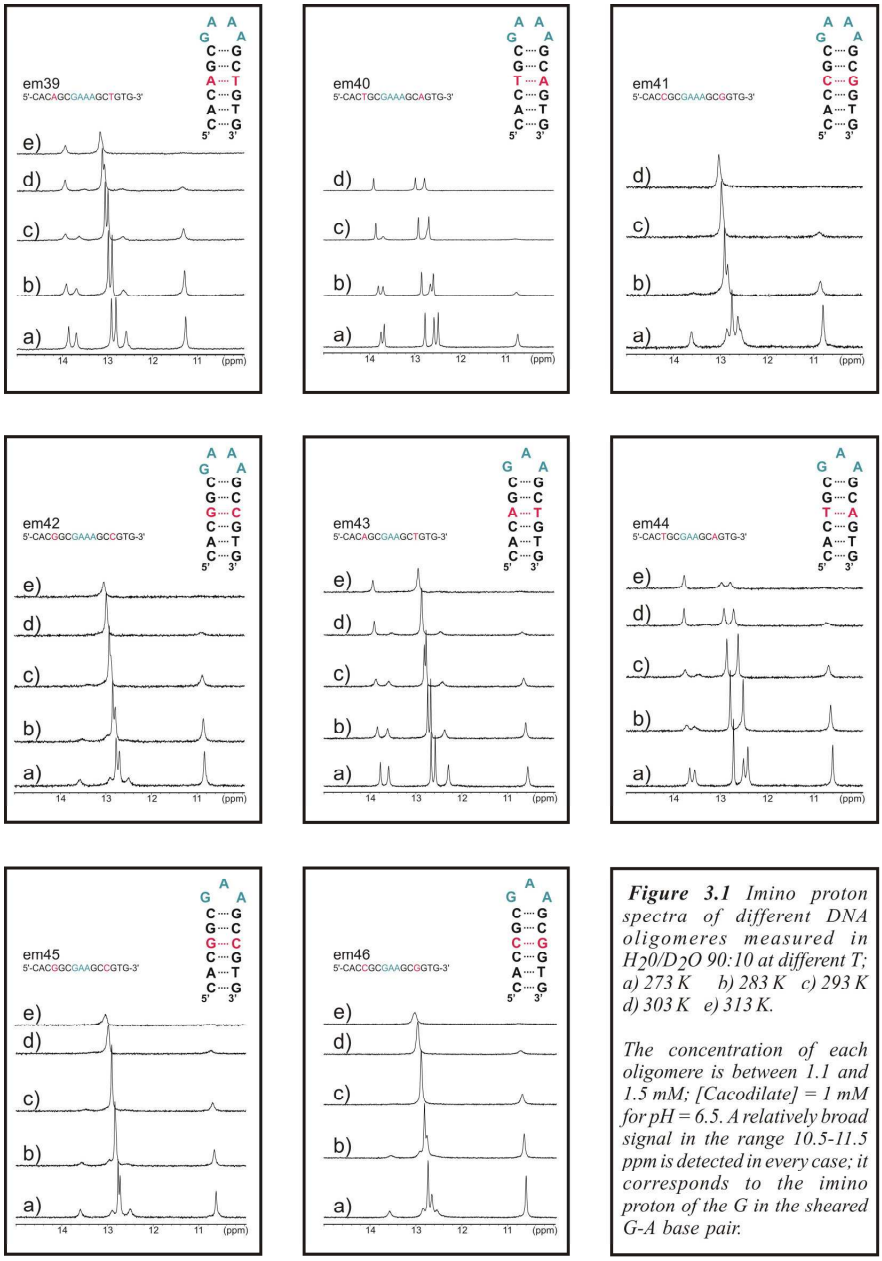


Figure 3.1 *Imino proton spectra of different DNA oligomeres measured in H_2O/D_2O 90:10 at different T; a) 273 K b) 283 K c) 293 K d) 303 K e) 313 K.*

The concentration of each oligomere is between 1.1 and 1.5 mM; [Cacodilate] = 1 mM for pH = 6.5. A relatively broad signal in the range 10.5-11.5 ppm is detected in every case; it corresponds to the imino proton of the G in the sheared G-A base pair.

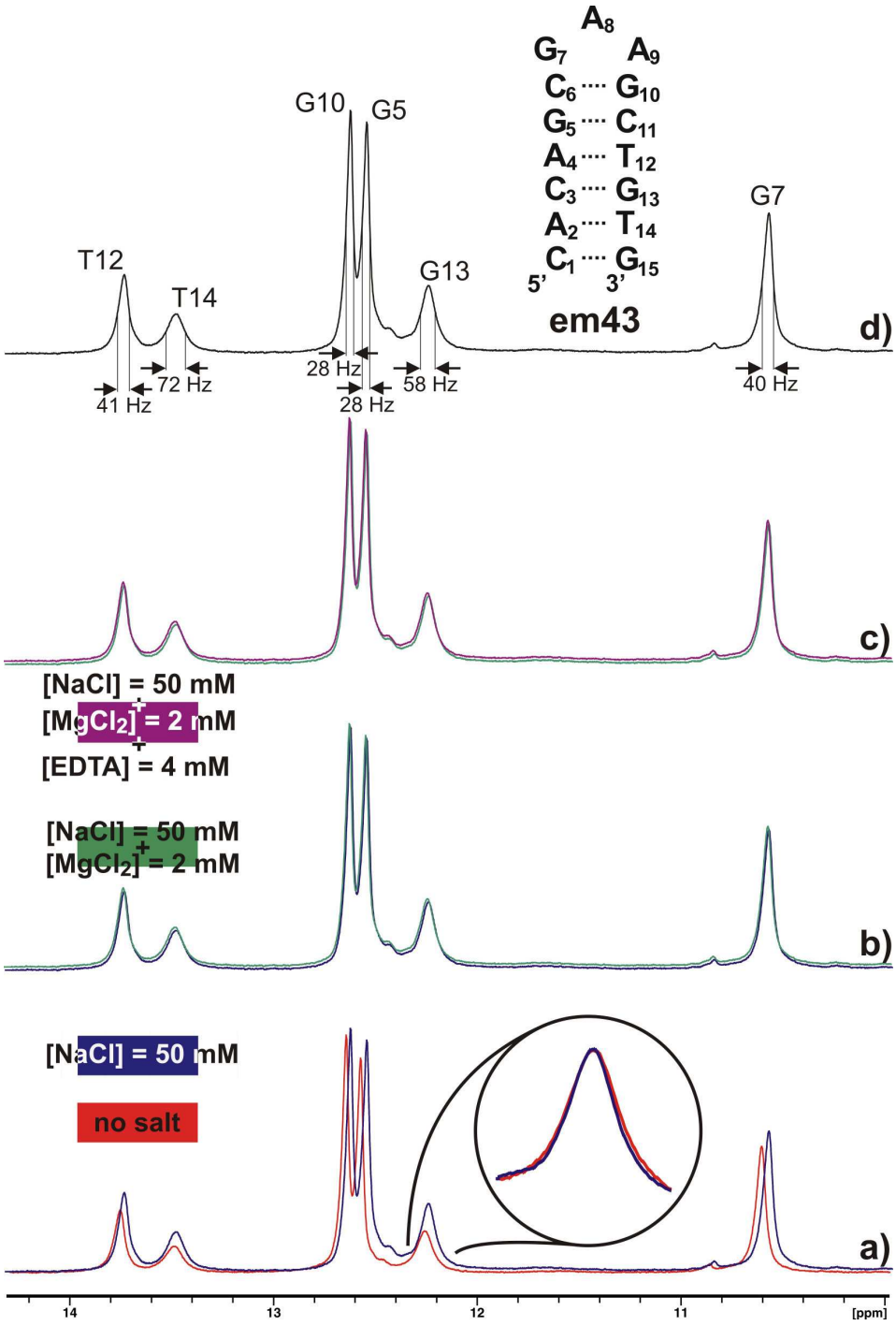


FIGURE 3.2 *Effect of the counterion on the imino proton peaks of em43. a) comparison of em43 with no salt (red) and with NaCl (blue); in detail overlap of same peak obtained in the two different cases: here the intensity of the peak in red is magnified in order to show more easily the slightly different line widths b) comparison after the addition of* Mg^{2+} *(green). c) comparison after the addition of EDTA (purple) d) imino proton peaks with assignment and FWHH.*

3.4 Assignment

Assignment of resonances is the first critical step in the process of three-dimensional structure determination of biomolecules. For unlabeled compounds the ^{1}H sequential resonance assignment is naturally based on a combination of through-bond ^{1}H-^{1}H and NOE correlations enhanced with ^{1}H-^{13}C and ^{1}H-^{31}P connectivities (see section 2.3). The fifteen-residue oligodeoxyribonucleotides d(CATCACGAAGCTGTG) was synthesized on a 1 μmol scale by standard phosphoramidite chemistry [189] and subsequently HPLC purified in the laboratory of Prof. H. A. Wagenknecht at TU München.

3.4.1 Assignment of exchangeable protons

Assignment of the exchangeable amino and imino protons is accomplished with NOESY spectrum recorded at 273 K in H_2O/D_2O 90:10 of the aromatic and imino protons region and and HSQC of the aromatic protons (see fig. 3.4). The through-space connectivities between imino protons are shown in fig. 3.3.a, the connectivities between imino (10.5-14 ppm), amino and aromatic protons (5.0-8.5 ppm) are shown in fig. 3.3.b. The connectivities between imino and H5M of thymidines are shown in fig. 3.3.c. The amino protons of cytidine residues were assigned from intraresidue NOESY cross-peaks between the hydrogen bonded (H41), non-hydrogen bonded (H42), and H5 protons of the four cytidine bases in the oligomere (fig. 3.5.b). Watson-Crick base pairing of all G•C base pairs except C1•G15 were established by the observation of NOESY cross-peaks between the guanosine imino proton and the amino protons of the cytidine (see fig. 3.6). Similarly, the imino protons of the thymidines are assigned from intraresidual NOESY cross-peak with H2 of the relative base paired adenosines (fig. 3.3.b). At the same time, this enables the definition of the A•T Watson-Crick base pairing (fig. 3.5.a). Amino protons of the guanosine residues are not detected in the acquired spectra; this is the typical behavior for DNA duplexes [181]. The imino proton of G7 resonates at 10.58 ppm, which is about 2.5 ppm upfield from the other guanosine imino resonances. The FWHH as well is broader than for the other guanosine imino peaks (see fig. 3.2.d). The direct assignment of this imino proton is not possible because it does not give any kind of NOESY cross-peaks. Nevertheless, previous structural studies of the GAA triloop have shown that the broad, upfielded peak at about 10.0-11.0 ppm is typical of imino protons of the guanine residue in the sheared G-A base [173,177,178,180, 182]. These results established that the oligomer em43 forms a stable hairpin and the A•Ts and G•Cs form canonical Watson-Crick base pairs with the exceptions of C1•G15, which is at the *terminus* of the duplex, A8 which is located at the apical position of the triloop and doesn't take part to any base pairing and G7-A9; here the base pairing is non canonical but along the Shallow edge of G7 and the Hoogsteen edge of A9. T12H3 and T14H3 are the most downfielded imino protons, they resonate at 13.74 and 13.59 ppm respectively and both have a wider line width than those of the guanines G5 and G10 positioned upstream. This results to the fact that in the canonical Watson-Crick base paired A•T two hydrogen bonds take place vs. the hydrogen bonds in C•G. Hence, the imino proton of T is more accessible for exchange with the solvent and/or experiencing line broadening by conformational exchange dynamics. T14H1 shows a broader line than T12H1; this is

consistent with the fact that T12 is proximal to the closing base pair of the stem where the structure is locally less stable. The imino proton of G13 shows a rather broad line width, comparable to the imino protons of thymidines and to the imino proton of G7 in the sheared G-A base pair. A reasonable explanation is that G13 is flanked upstream and downstream by A•Ts which can confer limited local stability to the structure. Consequently the G5H1 is more accessible than the imino protons of the upstream guanosine residues closer to the triloop. This is further evidence of the degree of stabilization supplied by the triloop.

3.4.2 Assignment of nonexchangeable protons

3.4.2.1 Tuning of the temperature

Temperature is an important variable. Since T affects the line width and the conformational dynamics of the oligomere, the appropriate temperature can be chosen in order to achieve the peak sharpness favorable for the assignment. The scarce degree of dispersion can be limiting for the assignment process, especially in the case of nonexchangeable protons. A raise of temperature has the effect of increasing the conformational dynamics. The system moves from slow or intermediate to fast exchange rate; the final result is the reduction of the NMR peak width by rapid molecular interconversion (motional narrowing; see section 2.4) This variation on the peak width is evident in the examined oligomer attesting its flexibility (see fig. 3.7).

3.4.2.2 Base and sugar protons

The assignment of the nonexchangeable base and sugar protons is accomplished at 313 K temperature in D_2O. Natural abundance ($^1H,^{13}C$) HSQC spectra of the oligomere in the aromatic and aliphatic regions are acquired. The different spectral regions for different carbon proton correlations are indicated in boxed regions (see fig. 3.8.a, b for aliphatic and aromatic regions respectively). The increase of T moves the chemical shift down-field in comparison to the HSQC measured at 273 K. By means of HSQC spectra acquired at different intermediate temperatures it is possible to trace back each single δ (see fig. 3.9). The sugar proton spin systems and the pyrimidine proton spin systems (H6 to H5/5M) are identified in ($^1H,^1H$) TOCSY spectrum (via through-bond coherence transfer). In TOCSY spectra of DNA, the cross peaks are between cytosine H6 and H5 resonances, a direct and easy way to identify these residues; the cross peaks between H6 and methyl resonances H5M provide the identification of thymine residues. The difference of chemical shift between methyl protons H5M and aromatic protons H5 enables the discrimination from cytosine residues and thymidines. Moreover, the TOCSY cross peaks of pyrimidines allow them to be distinguished from the guanine and adenine bases (see fig. 3.10). Depending on the sugar puckering, it may be possible to identify the sugar spin system more or less completely from ($^1H,^1H$) TOCSY spectra. In the acquired TOCSY spectrum with a mixing time of 80 ms it is possible the partial identification of the sugar proton spin systems i.e., H1', H2', H2'' and H3'. The H4's are hardly identified

and no cross peak for H5' and H5" has been detected. In DNA the TOCSY transfer path from H1' up to H3' is always open independently of the sugar puckering, because of the presence of the H2" proton [148]. The identification of these spin systems aids in the subsequent sequential assignment of the non-exchangeable base and sugar proton resonances via sequential NOE contacts, i.e. the set of contacts involving H6/H8 and H1' resonances and especially the set involving the H6/8 and H2'/2" resonances (see section 3.4.2.3).

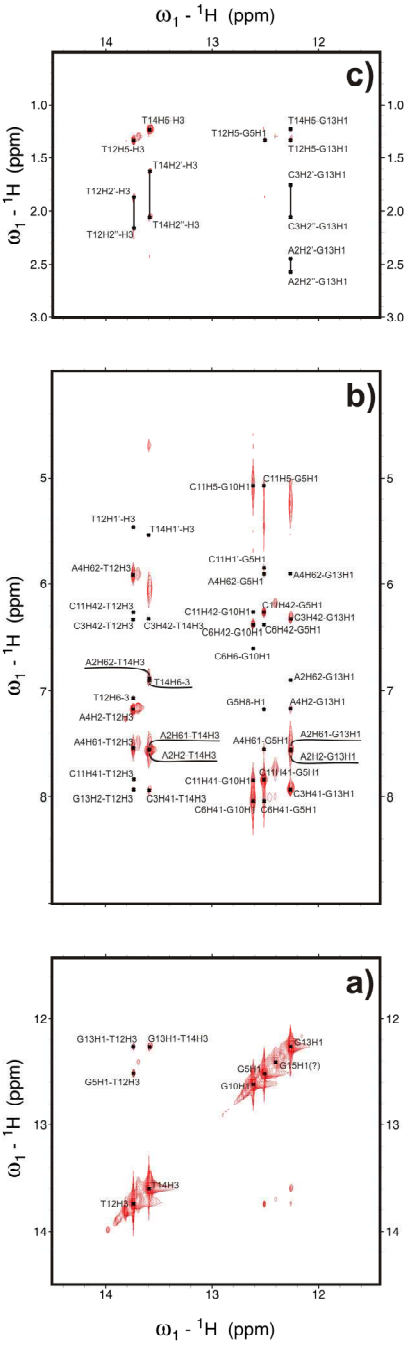


FIGURE 3.3 *Expanded regions of contour plots with the imino proton NOESY contacts between imino protons (a) between imino and aromatic and amino protons (b) and imino and methyl protons (c). NOESY spectrum measured in H2O/D2O 90:10 at 273 K and with 80 ms mixing time.*

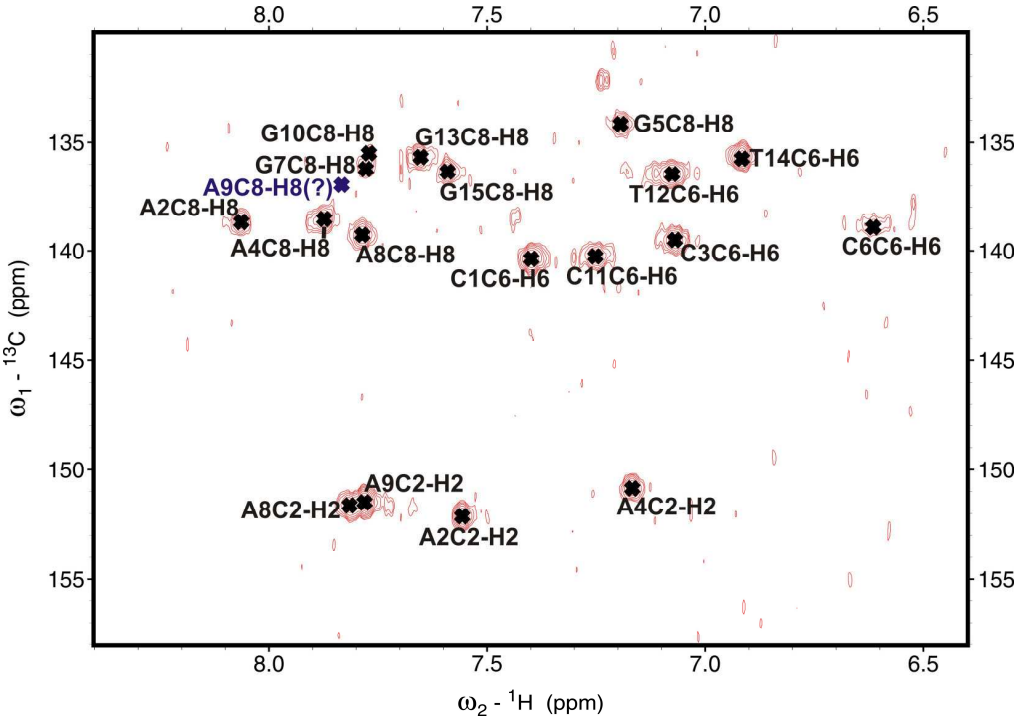


FIGURE 3.4 *HSQC of the aromatic region measured at 273 K in H_2O/D_2O 90:10 .*

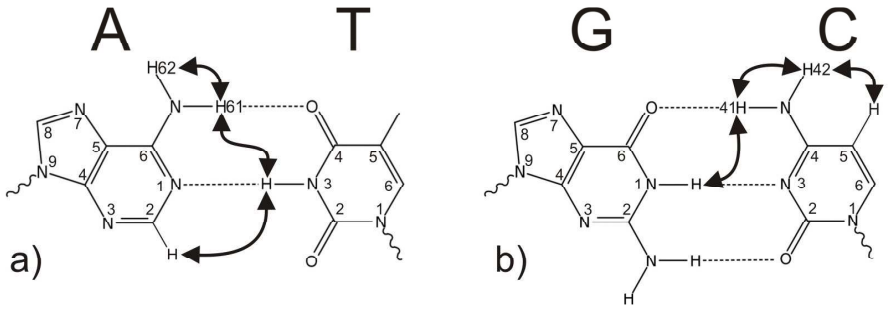


FIGURE 3.5 *The imino proton of thymidine is assigned from interresidual NOESY cross-peak with H2 of the relative base paired adenosine (a). The assignment of the imino protons of guanine residues can be estabilished from the interresidual NOESY cross-peaks with the hydrogen bonded (H41), non-hydrogen bonded (H42), and H5 protons of the cytidine base (b).*

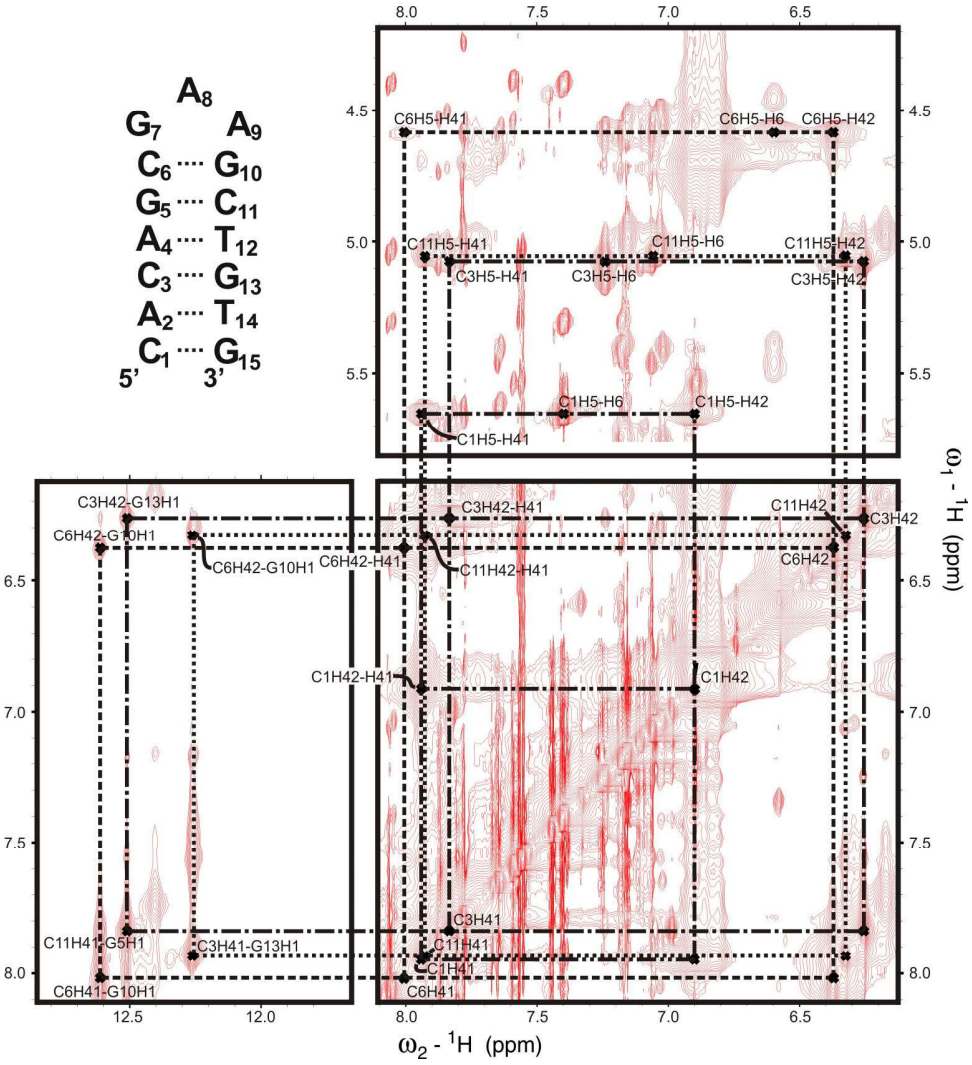


FIGURE 3.6 *The NOESY cross-peaks between guanosine imino protons and the cytidine amino protons establish the G•C Watson-Crick base pairs.*

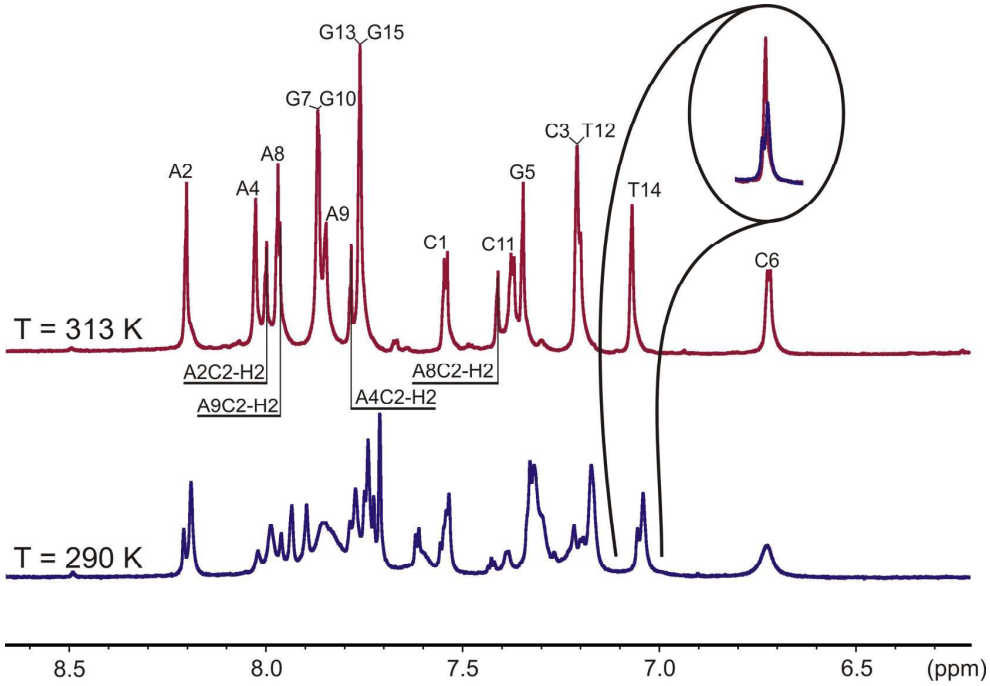


FIGURE 3.7 *Aromatic proton region of DNA in solution (H_2O/D_2O 90:10, [NaCl] = 50 mM, [Cacodilate] = 1 mM, pH = 6.5) at different temperatures. In detail, overlap of the T14H6 peak at different T exemplifies how increasing the temperature changes the dynamic behavior of the DNA and improves the resonance resolution.*

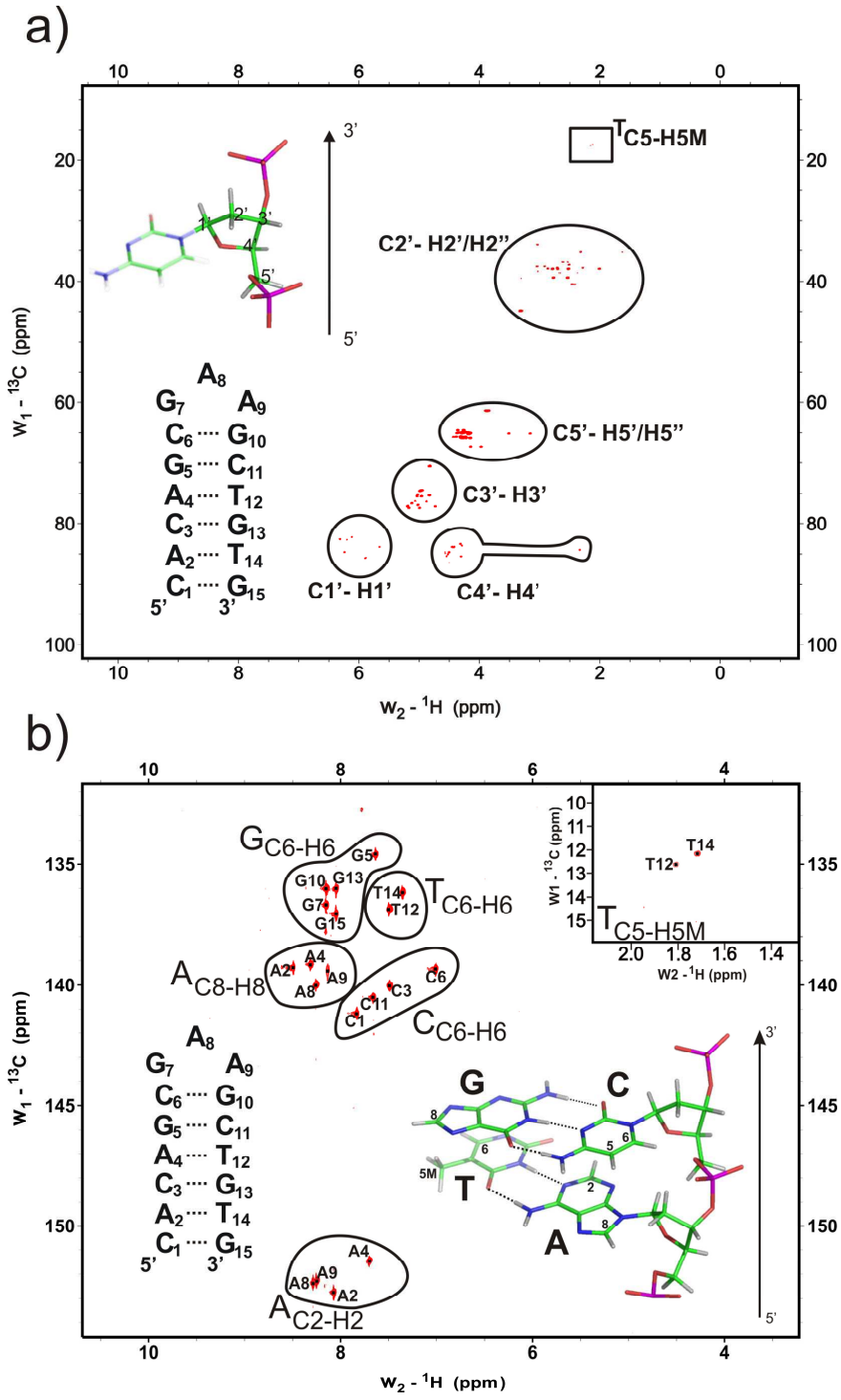


FIGURE 3.8 *Aliphatic (a) and aromatic (b)regions of natural abundance HSQC spectra of DNA; the different spectral regions for different carbon-proton correlations are circled in black. In the right upper corner the insert shows the region of 5M protons of thymidines. Remarkable in spectrum a) is the 2.5 ppm upfielded chemical shift of one H4' in the triloop.*

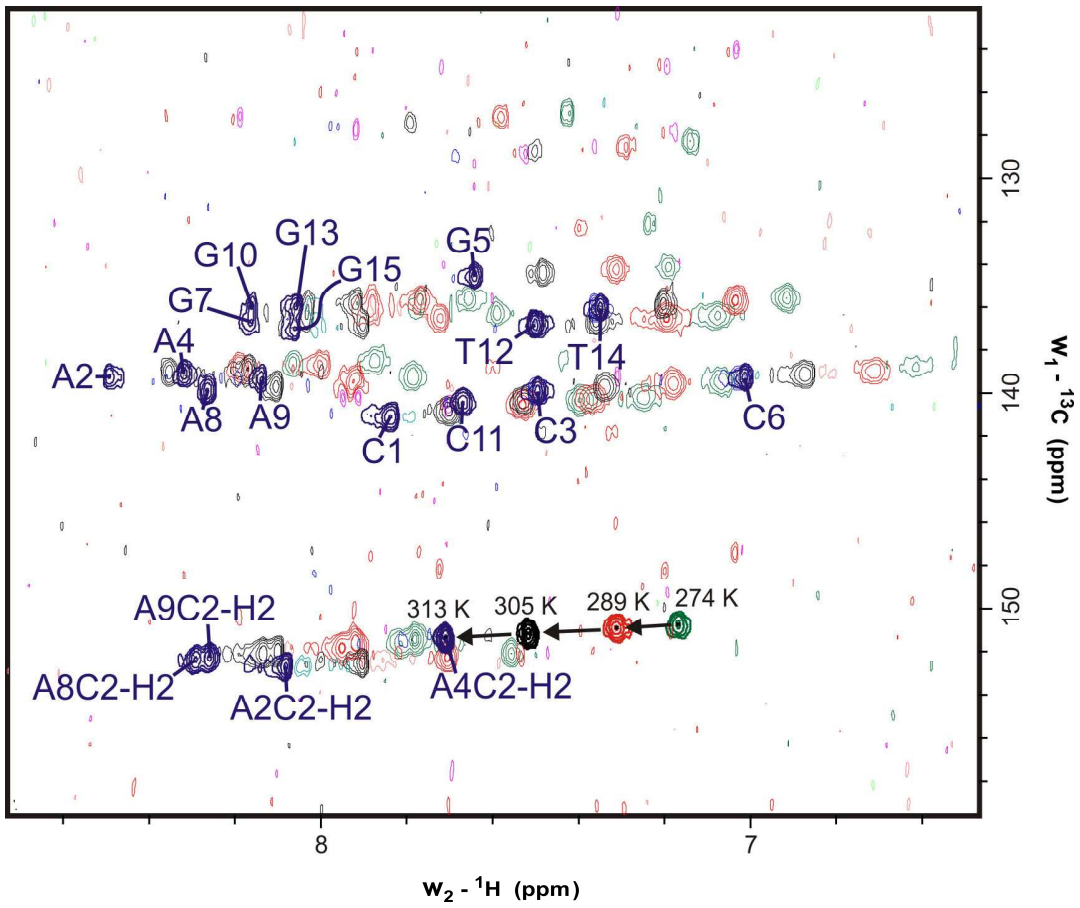


FIGURE 3.9 *Overlap of HSQC spectra of aromatic protons measured at different temperatures; from the picture the downfield shift of proton resonances with increasing T can clearly be followed.*

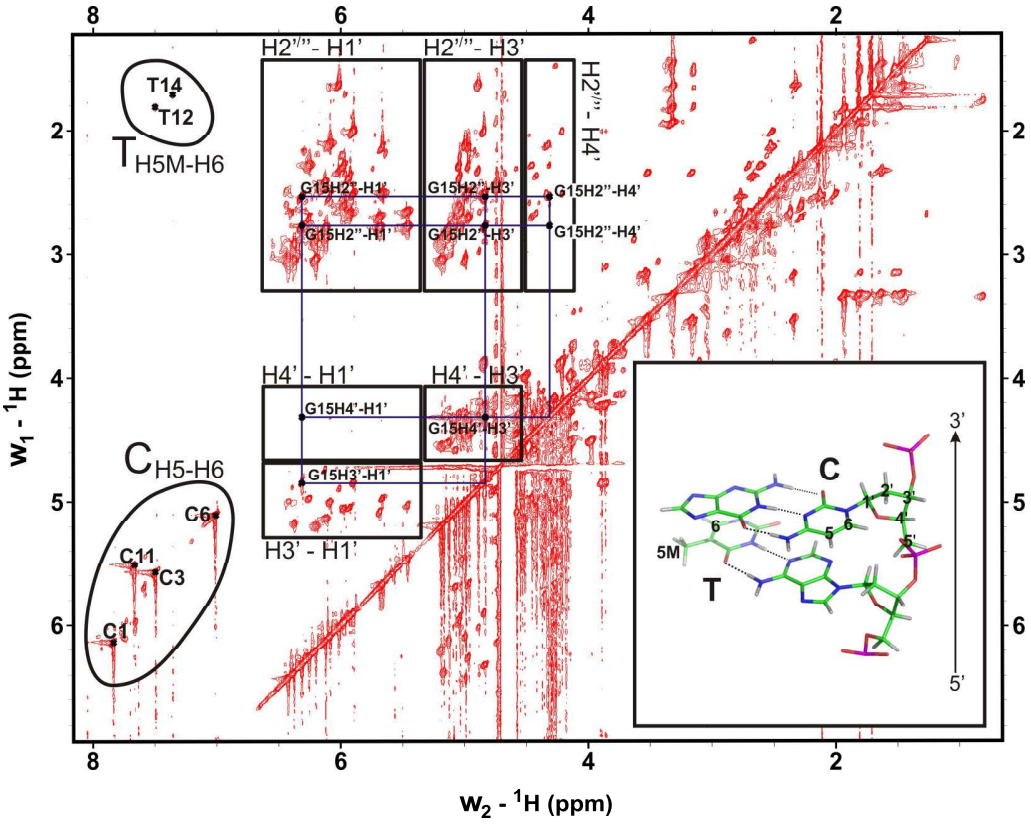


FIGURE 3.10 *TOCSY spectrum with a mixing time of 80 ms measured for the DNA stem-loop em43; the cross peaks between cytosine H6 and H5 and between thymidine H6 and H5M resonances are circled in black. The different spectral regions for different proton-protons correlations of sugar hydrogens are put in rectangular boxes. In the spectrum the partial identification of the sugar proton spin systems is possible. H1', H2', H2'' and H3' can be determined for each of the fifteen spin systems while the H4's are hardly identified and no cross peak for H5' and H5'' has been detected. The violet lines identify the spin system of G15; the H5' and H5'' are not detectable.*

3.4.2.3 Sequential assignment

Given the absence of a sufficiently large J-coupling between the sugar protons and the ring protons of the base, the signals of a particular sugar and those of its corresponding base (and similarly sugar protons of nucleotide *i* and base protons of the contiguous nucleotide *i+1*) need to be connected by NOEs (see excerpt of fig. 3.11.a).
The non-exchangeable protons have proven to be the most useful for this purpose. The connectivity pathway used is that formed by the cross-peaks between H6/8 and H2'/H2'' (see fig. 3.11.a). Once the connectivity patterns of individual nucleotides have been identified, the following step is to connect these patterns to obtain sequential assignments. From the TOCSY experiment the resonances of the aromatic protons of

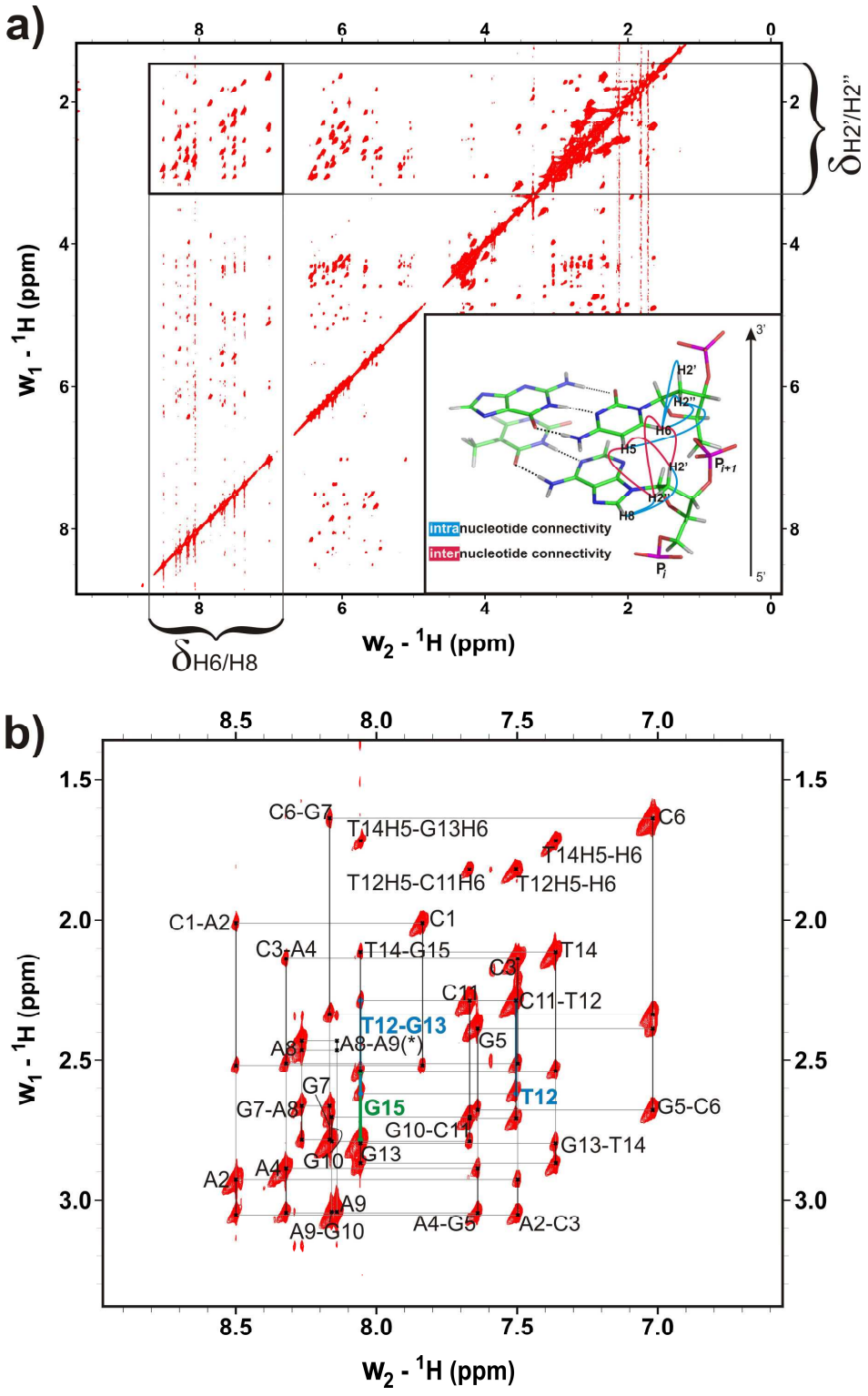


FIGURE 3.11 *a) black boxed the NOESY cross-peaks between H6/8 and H2'/H2'' b) full sequential assignment of the oligomere em43. The only one lacking peak is star marked and not surprisingly is relative to the loop region.*

cytosine residues as well as the aromatic and methyl protons of thymidines are known. Moreover, it is known from the DNA sequence examined that the last nucleotide G15 will give just intranucleotide NOE connectivities between H8 and H2'/H2" but not connectivities between G15H8 and H2'/H2" of the following nucleotide. Similarly, the first nucleotide (C1) will give just intranucleotide NOE connectivities between H8 and H2'/H2". These prerogatives are useful in the identification of the resonances of C1 and G15 and they act as helpful stepping-stones in the process of sequential assignment. The correctness of the ultimate assignment must be based on internal consistency of the NOE contacts. The NOE network for the H8, H6, H5, H5M and sugar ring protons is quite dense and contains a relatively large number of NOE contacts related to conformation independent distances, providing a large number of internal checks of the internal consistency of the assignment.

3.4.3 Validation of the assignment strategy

The main problems associated with sequential assignment of proton resonances in unlabeled nucleic acids is related to the fact that the above described strategy is based on NOE contacts, which are inherently ambiguous with regard to intra- and internucleotide contacts. In addition, NOE-based sequential assignment requires assumptions with regard to the conformation of structural elements of the sequence studied. In contrast, sequential assignment based on through-bond connectivities (via ^{31}P, for example) and connecting intra-residually the ribose spin system to the proton(s) (via HMBC spectra) does not suffer from these two drawbacks.

3.4.3.1 Correlation of aliphatic H1' protons to base C6/C8 carbons via HMBC

Heteronuclear Multiple Bond Correlation (HMBC) spectroscopy is an experiment suitable for determining long-range ^{1}H-^{13}C connectivity [184,185]. Protons are correlated to remote ^{13}C nuclei two or three bonds away in a 2D experiment via their long-range heteronuclear J couplings ($^{2}J_{CH}$ and $^{3}J_{CH}$). This enables the determination beyond reasonable doubts of the connection between each sugar spin system and the aromatic protons of the respective base (see fig. 3.12). In an HMBC, the J value selected for coherence transfer has to be small so that the two- and three-bond correlations can be detected ($^{2,3}J_{CH} \sim$ 6-10 Hz) and cross peaks originating from one-bond $^{1}J_{CH}$ couplings are rejected by setting the delays according to the corresponding coupling constants ($^{1}J_{CH} \sim$ 150 Hz for aliphatics and $^{1}J_{CH} \sim$ 210 Hz for aromatics) [183]. The experiment generally has low sensitivity; the delay Δ for the evolution of the long range coupling is rather long ($\Delta = 1/|2J_{CH}| = 50$ ms) and during this time there is considerable loss of magnetization. Moreover, during Δ not just the desired H-C long-range coupling is evolving but also proton-proton couplings. For that reason, the originated proton signals have complex splitting patterns that further reduce their peak height.

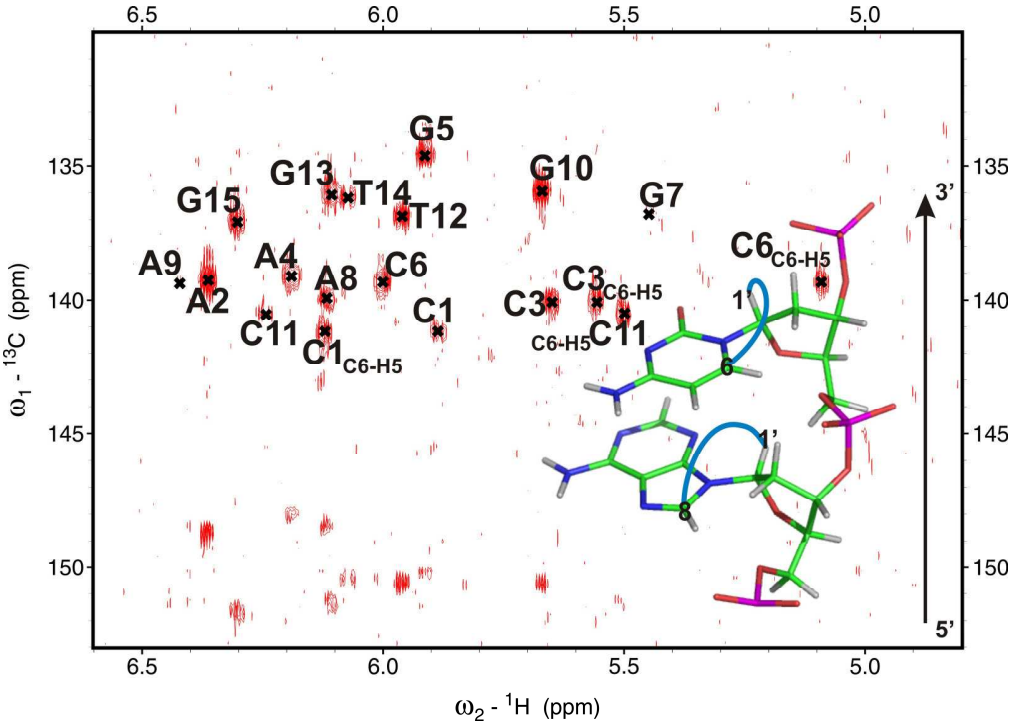


FIGURE 3.12 *HMBC spectrum of em43 measured in D_2O at 313 K. The picture in the inset shows the long-range connectivities of 1' protons of the sugars with the C6 of pyrimidinic bases or C8 of purinic bases. In the case of cytidines also correlations between H5 and C6 within the same base are visible.*

3.4.3.2 Sequential assignment via (^{1}H-^{31}P) CPMG-correlated experiment

The effective application of HSQC correlation of phosphorus and protons is achieved by the application of a train of closely spaced 180° pulses during the periods of magnetization transfer between phosphorus and scalar coupled proton nuclei; such a train of pulses, known as a CPMG pulse sequence building block (Carr-Purcell-Meiboom-Gill) has the characteristic to preserve spin coherence in the presence of conformational exchange and cross-correlated relaxation, providing an optimized refocusing .
Furthermore, the HP-CPMG-HSQC is applied in combination with a nuclear Overhauser effect (NOE) mixing period (HP-CPMG-HSQC-NOESY) to correlate the phosphorus nuclei with additional ribose and aromatic base protons that are better resolved than the H5´ and H3´ protons directly coupled to phosphorus [138]. Last but not least, the described pulse sequence provides an enhanced sensitivity of approximately 2-fold with respect to phosphorus-excited experiments (See fig. 3.13, 3.14).

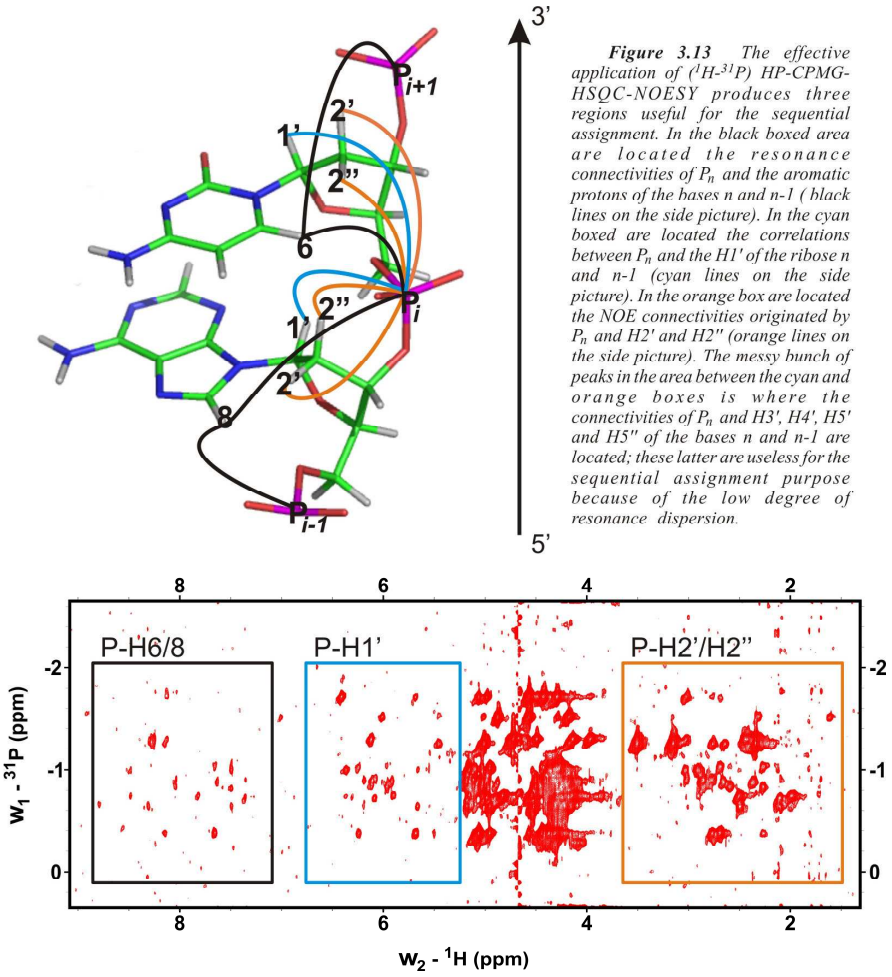


Figure 3.13 *The effective application of (^{1}H-^{31}P) HP-CPMG-HSQC-NOESY produces three regions useful for the sequential assignment. In the black boxed area are located the resonance connectivities of P_n and the aromatic protons of the bases n and n-1 (black lines on the side picture). In the cyan boxed are located the correlations between P_n and the H1' of the ribose n and n-1 (cyan lines on the side picture). In the orange box are located the NOE connectivities originated by P_n and H2' and H2'' (orange lines on the side picture). The messy bunch of peaks in the area between the cyan and orange boxes is where the connectivities of P_n and H3', H4', H5' and H5'' of the bases n and n-1 are located; these latter are useless for the sequential assignment purpose because of the low degree of resonance dispersion.*

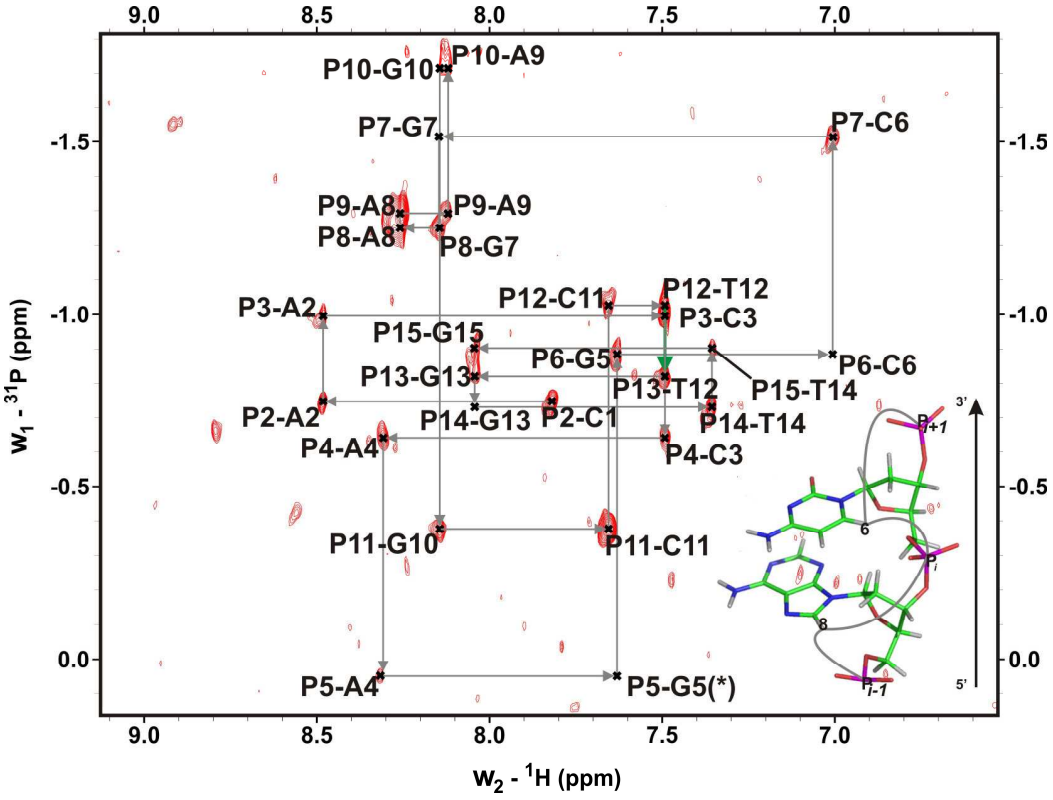


FIGURE 3.14 *sequential assignment of the resonance connectivities of P_n and the aromatic protons of the bases n and n-1 (corresponding to the black box in Figure 3.13). All the connectivities for the full sequential assignment have been found with the exception of the star-marked one.*

^{1}H Chemical Shift Assignments (ppm) of the DNA Stem-Loop											
Residue	H1'	H2'	H2''	H3'	H4'	H5'	H5''	H6/8	H2/5/Me	NH^	NH2^
dC_1	5.90	2.01	2.52	4.85	4.22	3.86	3.90	7.83	6.14	–	6.91/7.95
dA_2	6.34	2.93	3.05	5.17	4.56	4.16	4.28	8.49	8.08	–	n.d.
dC_3	5.67	2.14	2.51	4.97	4.34	4.30	4.39	7.50	5.57	–	6.33/7.93
dA_4	6.20	2.89	3.05	5.20	4.54	4.23	4.32	8.32	7.70	–	5.91/7.54
dG_5	5.93	2.67	2.37	5.04	4.42	4.33	4.36	7.64	–	12.51	n.d.
dC_6	6.02	1.64	2.33	4.90	4.23	4.19$^{(*)}$	4.19$^{(*)}$	7.02	5.10	–	6.38/8.03
dG_7	5.46	2.78	2.66	5.02	4.582	4.19$^{(*)}$	4.19$^{(*)}$	8.16	–	10.58	n.d.
dA_8	6.13	2.47	2.44	4.75	2.34	3.16	6.13	8.26	8.29	–	n.d.
dA_9	6.43	3.04$^{(*)}$	3.04$^{(*)}$	5.00	4.51	4.15	3.98	8.14	8.25	–	n.d.
dG_{10}	5.69	2.79	2.70	5.08	4.58	4.44	4.31	8.16	–	12.61	n.d.
dC_{11}	6.26	2.29	2.71	4.97	4.46	4.29$^{(*)}$	4.29$^{(*)}$	7.67	5.51	–	6.26/7.84
dT_{12}	5.97	2.29	2.61	5.05	4.31	4.28$^{(*)}$	4.28$^{(*)}$	7.51	1.820	13.74	–
dG_{13}	6.12	2.80	2.87	5.13	4.52	4.25	4.30	8.06	–	12.26	n.d.
dT_{14}	6.09	2.12	2.54	5.00	4.33	4.27	4.27	7.36	1.72	13.59	–
dG_{15}	6.32	2.78	2.54	4.83	4.31	2.77	2.55	8.06	–	n.d.	n.d.

TABLE 3.1 *Chemical Shifts of the DNA protons; all the value are measured at 313 K except {^} at 273 K. $^{(*)}$ - degenerated chemical shifts; (n.d.) - not detectable resonance.*

^{13}C Chemical Shift Assigments (ppm) of the DNA Stem-Loop							
Residue	C1'	C2'	C3'	C4'	C5'	C6/8	C2/5/Me
dC_1	85.64	37.90	75.32	85.94	61.34	141.19	96.70
dA_2	82.90	37.53	77.41	85.35	65.87	139.23	152.76
dC_3	83.85	37.28	74.44	83.20	n.a.	140.02	n.d.
dA_4	82.67	38.36	77.12	85.01	n.a.	139.15	151.46
dG_5	81.75	38.59	75.17	83.84	n.a.	134.60	–
dC_6	83.55	35.25	76.33	83.64	65.08	139.35	95.23
dG_7	81.41	36.87	76.92	84.69	n.a.	136.67	–
dA_8	82.27	40.46	77.12	84.32	65.12	139.99	152.35
dA_9	84.23	33.93	74.54	86.33	67.31	139.40	152.28
dG_{10}	81.85	38.05	76.26	84.88	65.70	136.01	–
dC_{11}	84.67	37.77	75.47	83.85	65.64	140.51	n.d.
dT_{12}	83.32	36.83	75.46	83.35	n.a.	136.85	12.40
dG_{13}	82.87	37.93	76.62	84.70	n.a.	136.02	–
dT_{14}	83.24	36.87	77.41	83.34	64.57	136.16	11.97
dG_{15}	82.58	39.36	70.57	85.38	n.a.	137.05	–

TABELLE 3.2 *Chemical Shift of the DNA carbons measured at 313 K. (n.a.) - not assignable due to scarce dispersion; (n.d.) - not detectable resonance.*

^{31}P Chemical Shift Assignments (ppm) of the DNA Stem-Loop	
Residue	P
dC_1	–
dA_2	– 0.746
dC_3	– 0.991
dA_4	– 0.644
dG_5	– 0.509
dC_6	– 0.882
dG_7	– 1.512
dA_8	– 1.253
dA_9	– 1.300
dG_{10}	– 1.726
dC_{11}	– 0.375
dT_{12}	– 1.029
dG_{13}	– 0.829
dT_{14}	– 0.732
dG_{15}	– 0.901

TABELLE 3.3 *Chemical Shift of the DNA phosphorus measured at 313 K.*

3.5 Structure calculation

With the full assignment of the NOESY spectrum in hand, it is possible to derive distance restraints based on NOE-intensities. For the precision of NMR derived structures, generally the number of structurally relevant distance constraints is more important than the precision of constraints. The number of structurally relevant constraints should as far as possible be uniformly spread through the chemical structure of the molecule. Constraints should be derived so that each category is represented (intranucleotide, sequential and cross-strand inter-nucleotide) [148]. Structure determination requires that the accuracy of the experimental NOESY spectrum has a flat base line and is free of spectral artifact which generate dramatic imprecision on the cross-peak volume determination.

3.5.1 Extraction of NOE restrictions

The dipolar cross-relaxation rate constant is proportional to the inverse sixth power of the distance between two interacting ^{1}H spins (see section 2.2.1). The NOE cross-peaks are proportional to the cross-relaxation rate constant. If one interproton distance, r_{ref} is known from covalent geometry, it is possible to derive the other unknown interproton distances (see eq. 2.6). A typical reference proton pair suitable for that purpose in nucleic acids is represented by H5 and H6 in cytosines (r_{HH} = 2.45 Å). The oligomer em43 includes four cytosine residues and all of them give very well defined NOE cross peaks (see next figure).

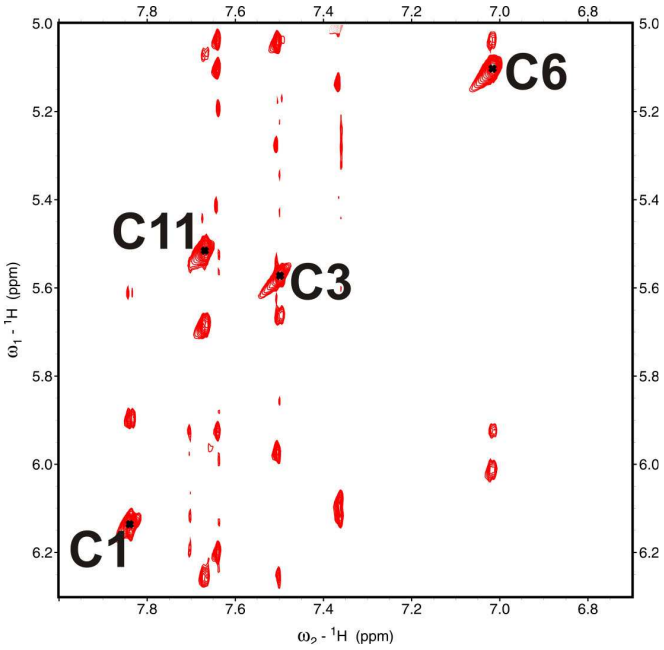


FIGURE 3.15 *NOE cross-peaks between H5 and H6 of the cytosine residues in em43 measured in H_20/D_2O 90:10 and T = 313 K. All four peaks are very well defined and isolated; they can be taken as reference cross peaksfor the calibration of NOE signal intensities.*

Residue	A – Integral (10^7)
C1	4.74
C3	6.47
C6	7.66
C11	7.25

TABLE 3.4 *The integrals are calculated in Sparky via "sum over box" method.*

From the integrals relative to H5-H6 interproton distance in the cytosine residues derived experimentally (see table 3.4) it is possible to calculate the average integral value of <A> = $6.53 \cdot 10^7$. With the known interproton distance r_{ref} = 2.45 Å [153] and the relation:

$$\langle A \rangle = \frac{K}{r^6} \qquad \text{(eq. 3.1)}$$

the proportionally constant K is consequently calculated to be K = 1.41 10^{10}. From this value the distance restraints relative to the NOE connectivities to be used in the structure calculation can be easily backcalculated (see relative table in appendix 1). From this procedure it emerges clearly how crucial is the estimation of the H5-H6 cross-peaks in the cytosine residues. The peaks must be well defined and isolated. The ones which cannot satisfy these strictly necessary conditions must be ruled out.

3.5.2 Definition of the starting structure

Qualitative and quantitative analysis of NOE distance values of the oligomer are consistent with a B-form helix along the stem. It is possible to suppose that each sugar pucker is C2'-endo and the torsion angle χ adopts *anti* conformation because the NOE correlations between H2'/H2" and H6/H8 are of medium strength and the peaks between H1' and H6/8 are clearly visible. The only exception is the apical deoxynucleoside of the loop. For A8H2'/H2" and A9H8 no NOE's are observable but connectivities between A8H5'/H5" and A9H8 have been reported (see *"DNA_NOE.tbl"* in appendix 1). Moreover, the A8H4' signal is moved 2.5 ppm upfield as compared with H4' resonances of other residues (see HSQC aliphatic spectrum 3.8.a and tab. 3.1). That is typical for the apical adenosine residue of the GAA triloop. The experimental evidence suggests that the conformation around the C5'-O5' (torsion angle β) does not adopt *trans* form and the conformation of the fragment is locally folded back between A8 and A9 [173,175,178].

3.5.3 Structure determination of the hairpin "em43"

The structure of the oligomere em43 is determined performing a series of simulated annealing calculations using XPLOR NIH 2.9.4a, one of the most popular programs for this purpose [187, 188]. The global procedure of structure determination consists on:

1) generating an extended set of random structures (protocol *generate_template. inp*); this ensure that the conformational space is explored as fully as possible. An arbitrary extended conformation with ideal geometry with respect to the specified force fields is produced for which the only input needed are the oligomer sequence and relative constitution of each deoxy-nucleoside.

2) the second step consists on the definition of the Starting Structure (the file *MODELL_FINAL.pdb* is produced) which simulates the annealing refinement with H-bonds (*plane.inp*, *dna_dna_pairs_03.setup*, *hbonds_assumed.tbl*), van der Waals interaction (*reset_force_constants.inp*) and theoretical intra-residue distance restrains of an ideal B-form DNA structure for the stem and the experimentally determined –GAA– loop structure (PDB ID 1KR, model 7; [178]) (*DNA_NOE_intra.tbl*) as explained in section 3.5.2.

3) in the final stage the previous derived structure is refined using the NOE derived distance restraints (*DNA_NOE.tbl*).

The structure of em43 has been calculated with 336 NOE restraints (205 intra-residual, 131 inter-residual, see tab. 3.5) uniformly distributed along the sequence. Distances were given a variable error range to cover the inaccuracy of NOE intensity due to peak overlaps, noise, peak spread from scalar coupling and spin diffusion (see section 2.2.1.2). In particular to take in account this latter – 0.8/+1.2 Å are the lower and upper limits for strong peaks (integral $\geq 10^7$), – 0.8/+1.5 Å for peaks of medium strength ($10^6 \leq$ integral $\geq 10^7$) and – 0.8/+2.0 Å for peaks of weak strength (integral $\leq 10^5$). The upper limit of the afore mentioned sets of error range has been doubled for non-distinguishable geminal protons. Special cases are also considered e.g., the connectivities involving the exchangeable protons which are determined at very different condition compared to all other NOE's. To check whether the experimental data are consistent with the derived structure, each calculated structure is characterized by the number of violations of experimental constraints (distance, dihedral angle, etc.) and their energy contributions originating from various structural parameters (bond, bond angles, dihedral, restraint dihedrals, impropers, van der Waals) and the distance and dipolar-coupling restraints (see *"violations"* in appendix 1). The energies provide an indication of whether the structure is strained to an unusual extent by the experimental data and allow a ranking with respect to the feasibility of the calculated structure. For a more detailed overview of the XPLOR protocols and values used for the structure determination of the oligomere em43, see appendix 1.

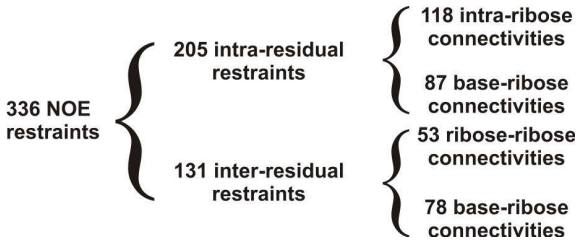


TABELLE 3.5 *Number and type of NOE restraints used in the structural calculation*

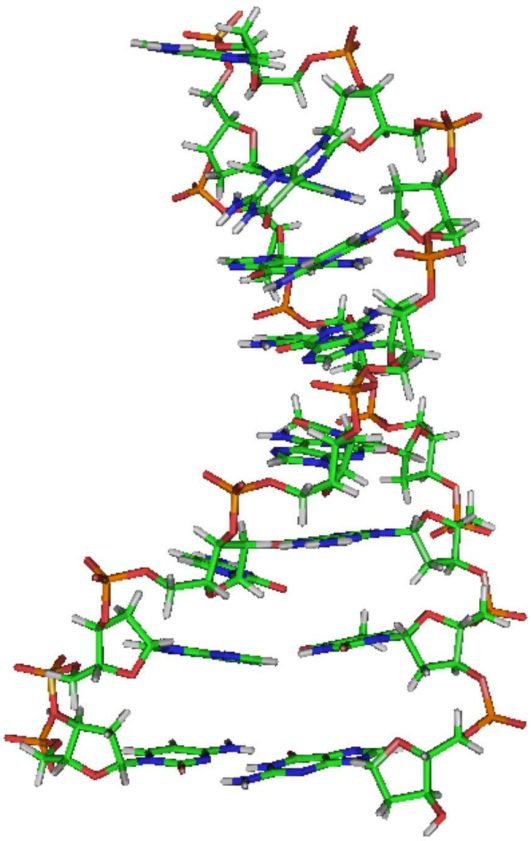


FIGURE 3.16 *Structure of oligomere em43 obtained as described in 3.5.3*

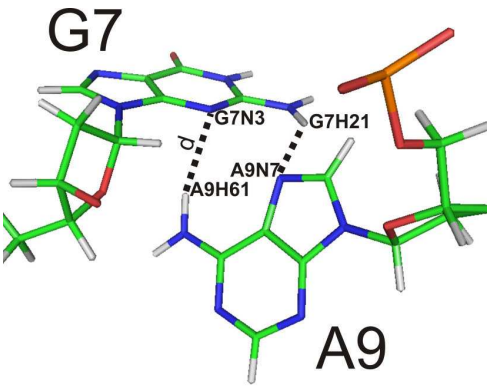


FIGURE 3.17 *Detail of the triloop: the two non-conventional hydrogen bonded Watson-Crick base pairs between G7 and A9 are evident. The distance d between the nuclei being involved in hydrogen bonding is about 2.7 Å.*

As evident in fig. 3.15, em43 nicely adopts a regular B-form duplex with the tight triloop at one end of the helix. With the knowledge of the structure in hand, we are now prepared for studying the structural influences of chemically modified bases on regular B-form DNA.

"Ma mettiamo che... cioè se si decide cà... cioè, comme 'o chiammassimo?"

Marta rincuorata risponde "Mah, io non ci avevo pensato; Massimiliano!"

- "no no no! cioè se si decide cà... io avevo pensato Ugo, cosi viene più educato"..

- "Ma perchè Massimiliano?"

- No, Massimiliano viene scostumato! Io sento a volte per esempio, 'a creatura sta vicino 'a mamma, s'alluntana e a mamma 'o chiama: Ma ssi mi li a no! Chillo 'o guaglione chissà addò sta che sta facenno, invece Ugo, appena se move a mamma: Ugo! e nun se pò alluntanà pecchè Ugo! Nun tene 'o tiempo, al massimo 'o putimme chiammà Ciro, nu poco cchiù luongo, giusto pè nun 'o fa venì troppo represso, almeno Ciro tene 'o tiempo 'e piglià nu poco d'aria..."

"Let's assume that… I mean, we decide it… How would we call him?"

Marta comforted answers "Well, I didn't think about; Massimiliano!"

- "No no no! If we decide that, I thought Ugo, so he grew up more well-mannered"
-
- "Why not Massimiliano?"
-
- "No, Massimiliano grows up into a rude guy! I hear sometime the child being close to the mother and he moves away and the mother calls him: Ma ssi mi li a no! Who knows where the boy is and what he is doing. Ugo, instead, he just moves and the mother immediately: Ugo! And he can't go too away; he has no time. We could call him Ciro, a bit longer – to not repress him so much. Ciro has at least time to breath a little…"

From "ricomincio da tre" (I'm starting from three) by Massimo Troisi

4. Structure of a stable DNA stem-loop with Py-dU incorporation

4.1 Synthesis of Py-dU-modified oligonucleotide

The uridine analog 5-(1-pyrenyl)-2'-deoxyuridine (Py–dU) is incorporated in an oligonucleotide on a 1 µmol scale by standard phosphoramidite chemistry [189] and subsequently HPLC purified by Dr. E. Mayer-Enthart in the laboratory of Prof. H. A. Wagenknecht at the TU München. The pyrenil group can be selectively excited (λ_{ex} = 340 nm) and used to induce a reductive electron transfer (ET) process along the stem (see section 1.2.4).

4.1.1 Synthesis of 5-(1-pyrenyl)-2'-deoxyuridine (Py–dU)

The modified nucleoside 5-(1-pyrenyl)-2'-deoxyuridine (PydU) (**1**) was prepared by a Suzuki-Miyaura type cross coupling [190-194] (see fig. 4.1) of pyren-1-ylboronic acid

(**2**) to 5-iodo-2'-deoxyuridine in THF/H_2O/MeOH (2:2:1) at 80 °C for 30 hours (see fig. 4.2); the used catalyst is tetrakis-(triphenylphosphine)palladium(0) [$Pd(PPh_3)_4$] and the reaction yield is about 80%. Pyren-1-ylboronic acid [196, 197] is achieved by the bromation of pyrene [195] with the addition of cupric bromide in chlorobenzene and left to reflux for 2 hours under argon atmosphere (see fig. 4.3). The yield of this reaction step is of about 70 %. Subsequently the 1-bromopyrene (**3**) was dissolved in anhydrous diethyl ether and to this solution is added n-butyllithium (BuLi) solution in hexane at 0 °C. The content is poured into another flask containing trimethyl borate [$B(OCH_3)_3$] in anhydrous diethyl ether at –78 °C over 30 min. The product PydU as well as the intermediate (**2**) and (**3**) are light sensitive and avoiding the direct exposition to light is required in order to prevent the photo-decomposition of the molecules.

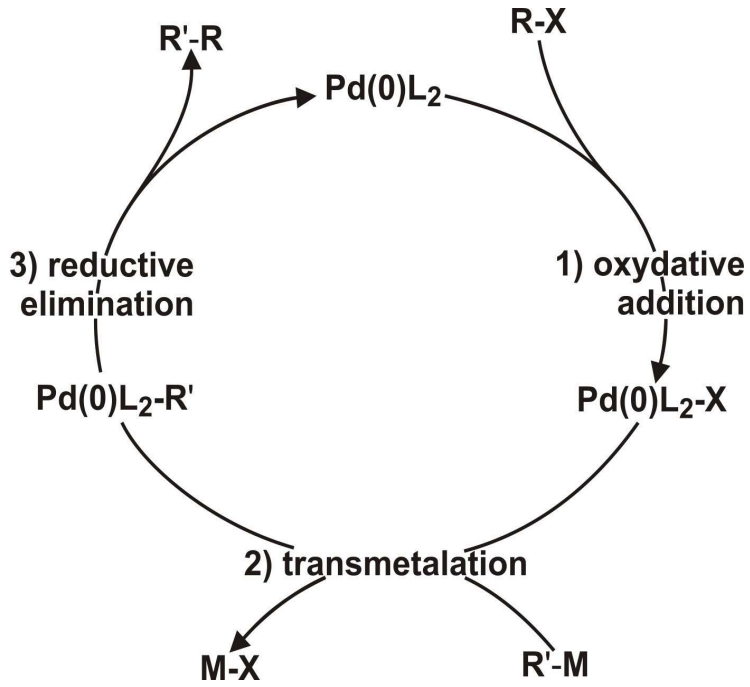


FIGURE 4.1 *schematic view of the typical palladium catalyzed Suzuki-Miyaura type cross coupling reaction (X = halogenide and L = ligand e.g. PPh_3).*

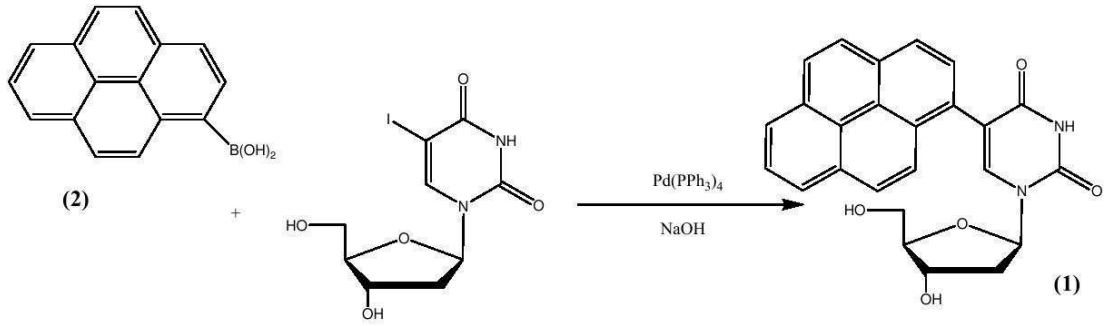


FIGURE 4.2 *scheme of the synthesis of 5-(1-pyrenyl)-2'-deoxyuridine (Py–dU) by Suzuki-Miyaura type cross coupling reaction.*

The 5'-OH group of the modified 2'-deoxyribose is protected with 4,4'-dimethoxytrityl (DMT) and the 3'-OH group with β-cyanoethyl-N,N-diisopropyl-phosphoramidite (see fig. 4.4). The so obtained phosphoramidite (**5**) is used as building block in the automized DNA solid-phase synthesis to finally obtain the oligonucleotide 5'-CACAGCGAAGC(PydU)GTG-3' which we call "Hugo" in the following after the naming given in the movie "ricomincio da tre" by "Massimo Troisi". The process takes place according to the standard methods described in the literature [194,198]. This oligomer is similar to em43 with the exception of T12 which has been replaced by PydU.

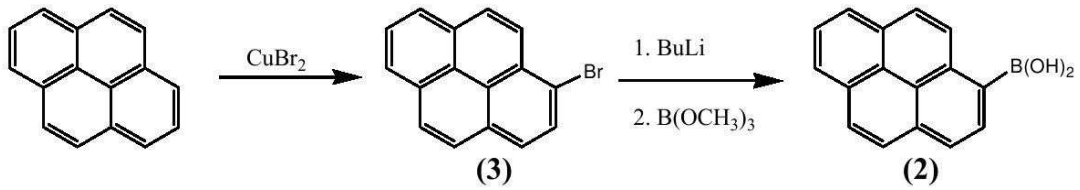


FIGURE 4.3 *the synthesis of pyren-1-ylboronic acid (2) runs in two steps. The bromation of pyrene with the addition of cupric bromide in chlorobenzene; the obtained 1-bromopyrene (**3**) is purified per crystallization. The lithiation of (**3**) takes place in hexane at 0 °C and the subsequent boration in anhydrous diethyl ether at –78 °C for 30 min.*

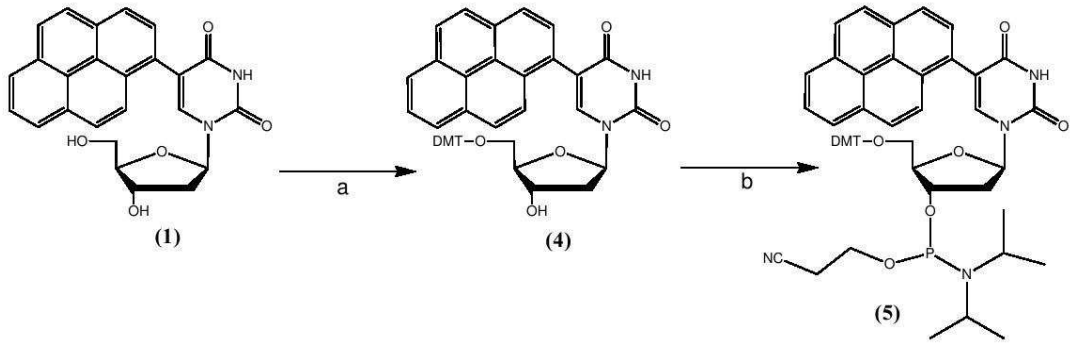


FIGURE 4.4 *the phosphoramidite (**5**) is obtained by addition of 4,4'-dimethoxytrityl chloride (DMT-Cl, a) – reaction time 20 h with yield of 65%. At the modified 2'-deoxyribose with the 5'-OH group protected (**4**) is added β-cyanoethyl-N,N-diisopropyl-phosphoramidite (b) in DCM – reaction time 2 h with yield of 100%.*

4.2 Imino Spectra and Base Pairing

Fig. 4.5 shows the temperature dependence of the DNA hairpin with PydU incorporated. Differently than the oligonucleotide em43 where six well defined imino proton peaks are visible at low temperature (see fig. 3.2), Hugo shows four main imino protons peaks and between 10.9 and 11.5 ppm a plethora of several weak and broad peaks corresponding to different conformations; they undergo coalescence and partially fast exchange as the temperature is raised. The behavior of the imino proton peaks in relation to the rise of temperature is not easily rationalizable but it reveals the high structural flexibility and conformational variability of the molecule. The addition of counterion Na^+ produces evident effects on the peaks in term of intensity especially on the two ones with resonances between 10.5 and 11.0 (see fig. 4.6.a) but even the two downfield peaks (δ = 12.6 and 13.5) don't remain indifferent. For these latter ones it is evident the appearance of shoulders not visible before the addition of Na^+. Further additions of Mg^{2+} as well as EDTA don't produce any remarkable variation in the imino protons spectra (see fig. 4.6.b and c)

4.3 Assignment strategy

4.3.1 Assignment of exchangeable protons

The assignment of the exchangeable amino and imino protons of Hugo oligomer is not feasible. From the NOESY spectrum recorded at 273 K in H_2O/D_2O 90:10 (τ_m = 80 ms) not any single connectivity is evident; this is a further sign of the exceptional structural dynamics of the oligomer. The triloop –GAA– is the part of the structure which is locally less influenced by the presence of the PydU and the behavior of the imino protons of the guanine residue in the sheared G•A base pair in em43 and in Hugo is qualitatively comparable. Therefore, it can be taken as reference to roughly estimate the strength of the respective imino peaks located along the stem for the natural and modified oligomers (see fig. 4.7). The larger flexibility of the modified oligomer determines the facts that the

imino protons even being involved in base-pairing are easily accessible for exchange with the solvent and/or experiencing line broadening by conformational exchange dynamics. The intensity of the imino proton peaks along the stem is consequently weaker in Hugo than in em43.

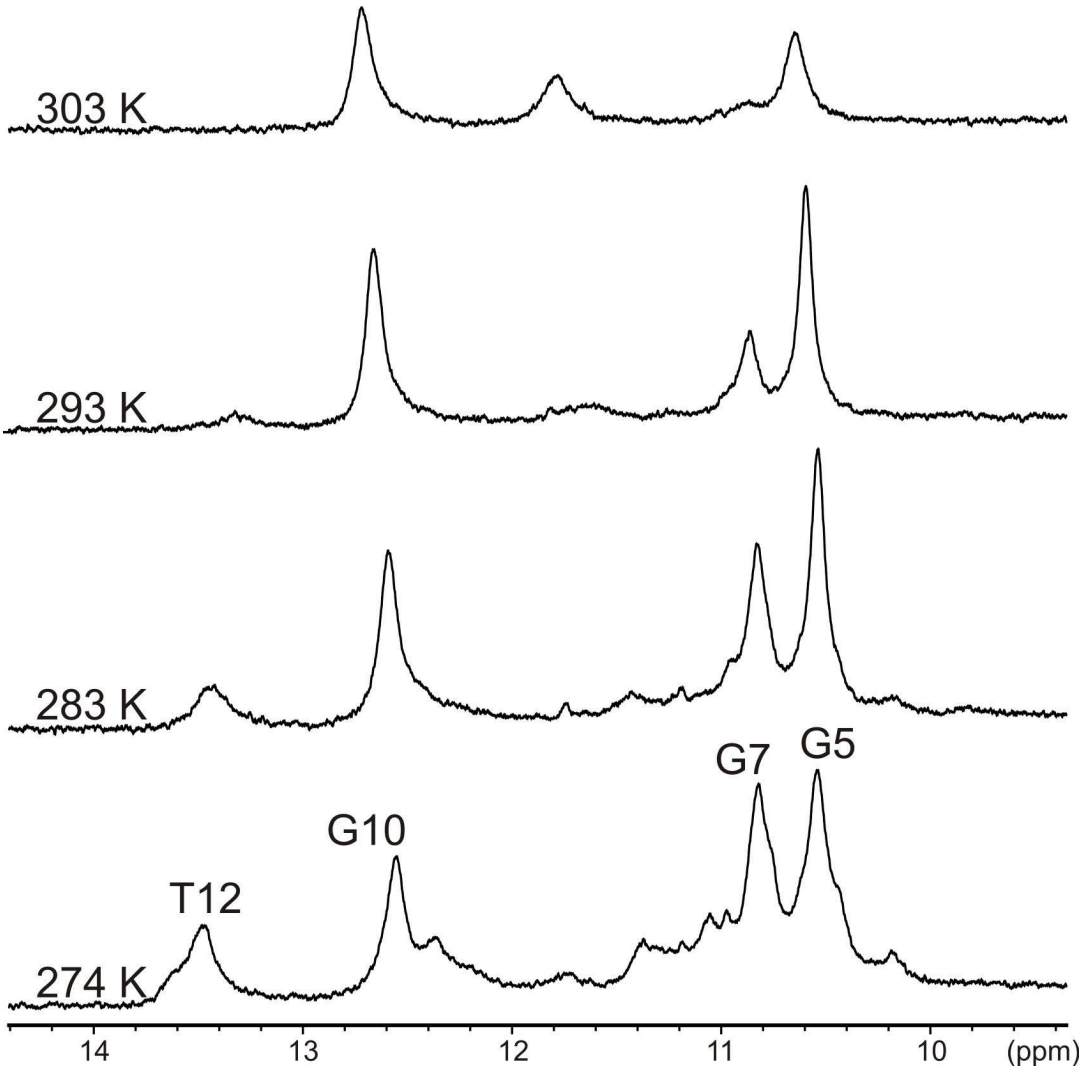


FIGURE 4.5 *temperature dependence of the DNA hairpin with PydU incorporated. At low temperatures several different conformations can be seen which undergo coalescence and partially fast exchange at elevated temperatures. Spectra measured in H_2O/D_2O 90:10, [Hugo] = 1.94 mM, [Cacodilate] = 1 mM, pH = 6.5, [NaCl] = 50 mM.*

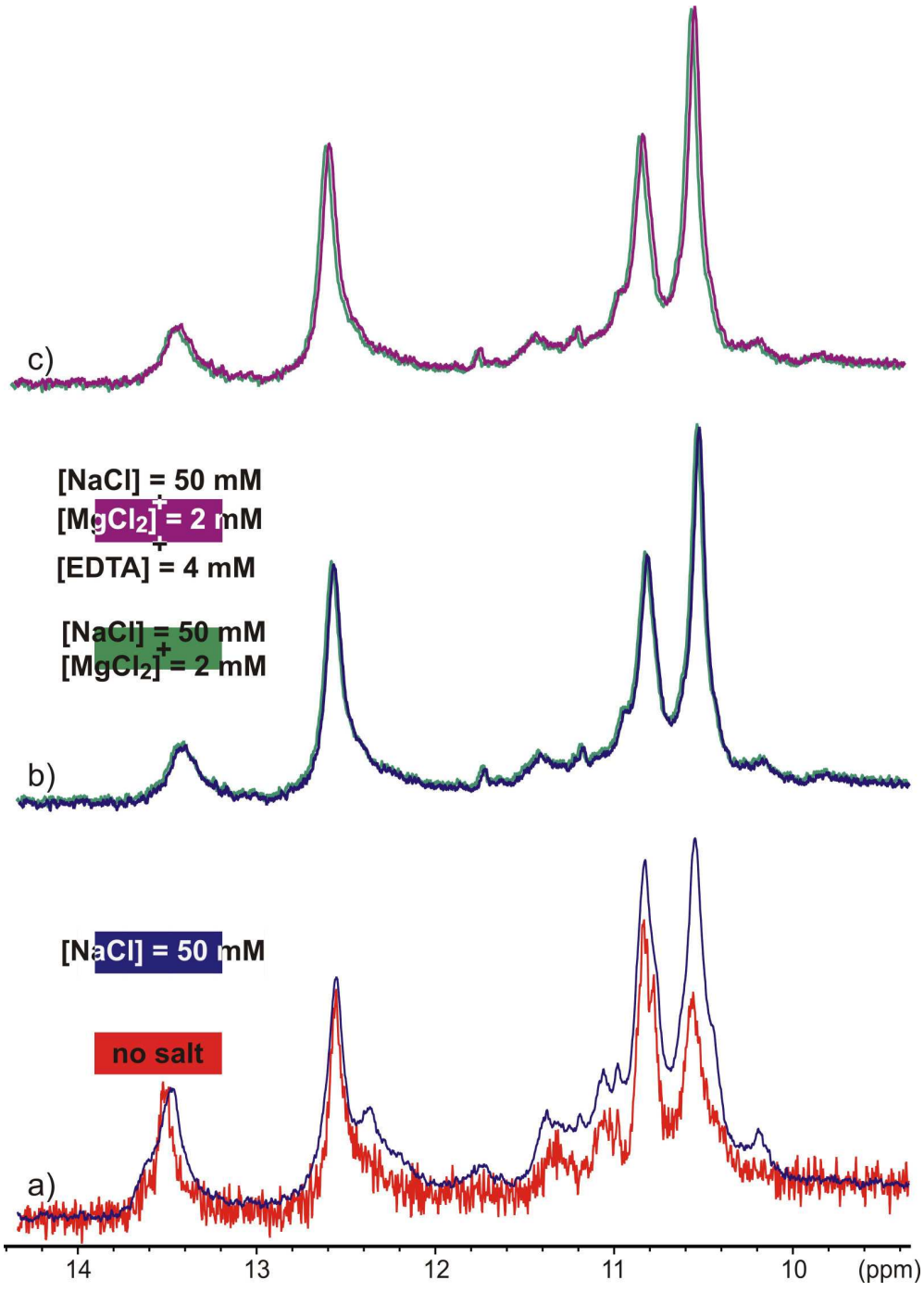


FIGURE 4.6 *Effect of the counterion on the imino protons of Hugo. a) comparison with no salt (red) and with NaCl added (blue); the different signal-to-noise ratio depends on the different number of scans (NS) used in the two acquisitions (128 with salt, 16 without salt) b) comparison after the addition of* Mg^{2+} *(green). c) comparison after the addition of EDTA (purple). Spectra measured in* H_2O/D_2O *90:10, [Hugo] = 1.94 mM, [Cacodilate] = 1 mM, pH = 6.5, T = 273 K.*

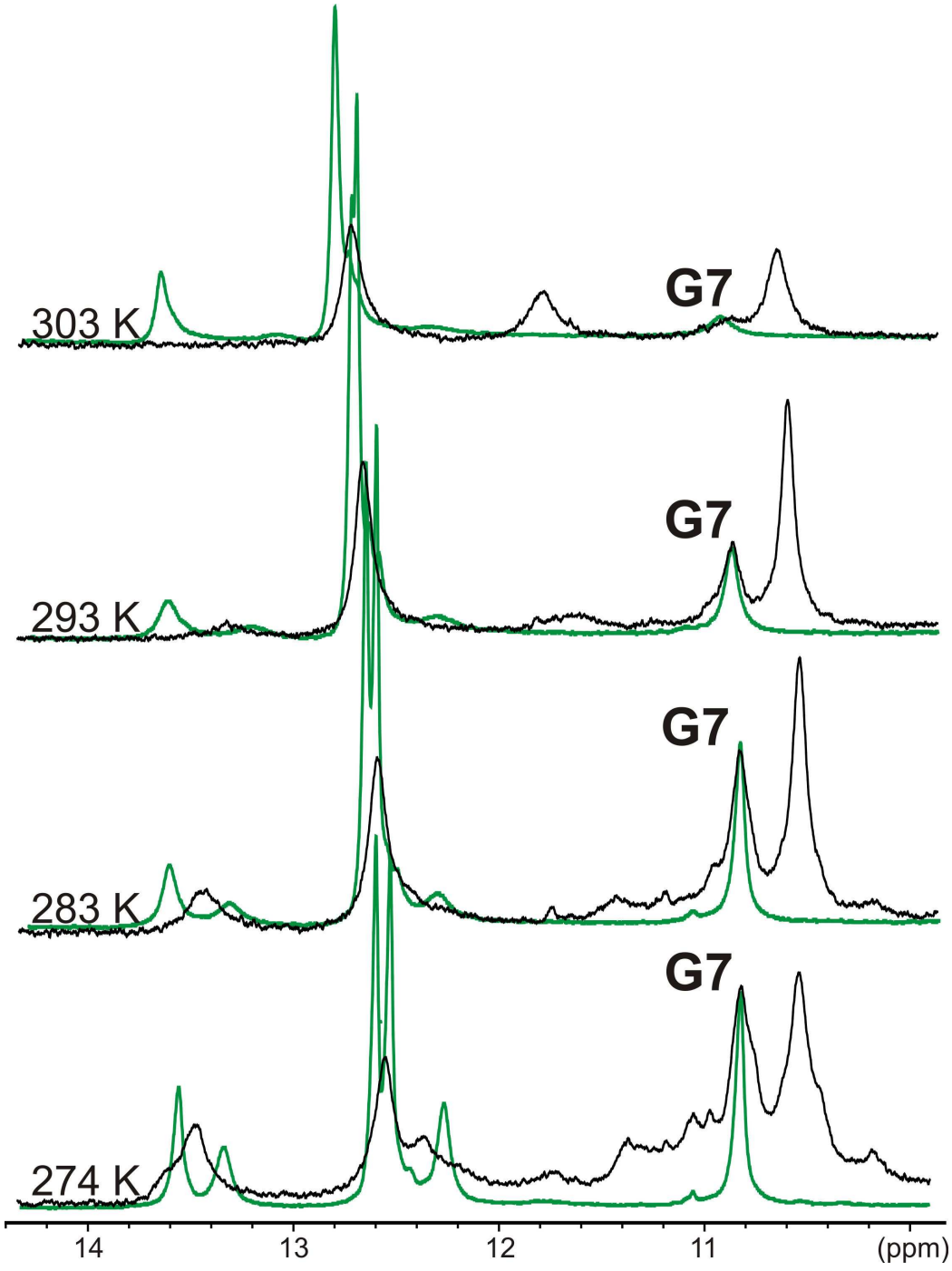


FIGURE 4.7 *comparison of the imino protons of em43 (green) and Hugo (black) at different temperature. The spectra have been acquired at different instrumental condition and they have been resized in order to compare the relative intensity of the peaks. It is evident how the peak of G7 behaves similarly in both the cases while the peaks along the stem of Hugo are significatively weaker than the corresponding in em43 and decidedly less persistent as the temperature raises.*

4.3.2 Assignment of nonexchangeable protons

4.3.2.1 Tuning of the temperature

As seen in section 3.4.2.1, temperature is an important variable to modulate since it affects the line width and the conformational dynamics of the oligomer. The appropriate temperature can be chosen in order to achieve the favorable peak sharpness for the assignment; this is for Hugo even more important than for em43 because the aromatic non-exchangeable protons of the bases have similar resonance than the additive nine protons of the pyrenil group (see fig. 4.8). The variation on the peak width in relation to the temperature modification is evident; this attests the remarkable flexibility of the modified oligomer. Although the dispersion at 313 K is not optimal, this is the temperature at which the 2D experiments have been acquired. If on the one hand a higher temperature below the melting point could provide a better resolution, on the other hand technical drawbacks can easily rise, e.g. the rapid formation of air bubble in the Shigemi tube during the acquisition of long run experiments (typically HSQC for unlabeled molecules) which spoils the shimming and determines spectral distortion and artifacts. Moreover, acquisition at 313 K allows meaningful comparisons of the spectra of Hugo and em43.

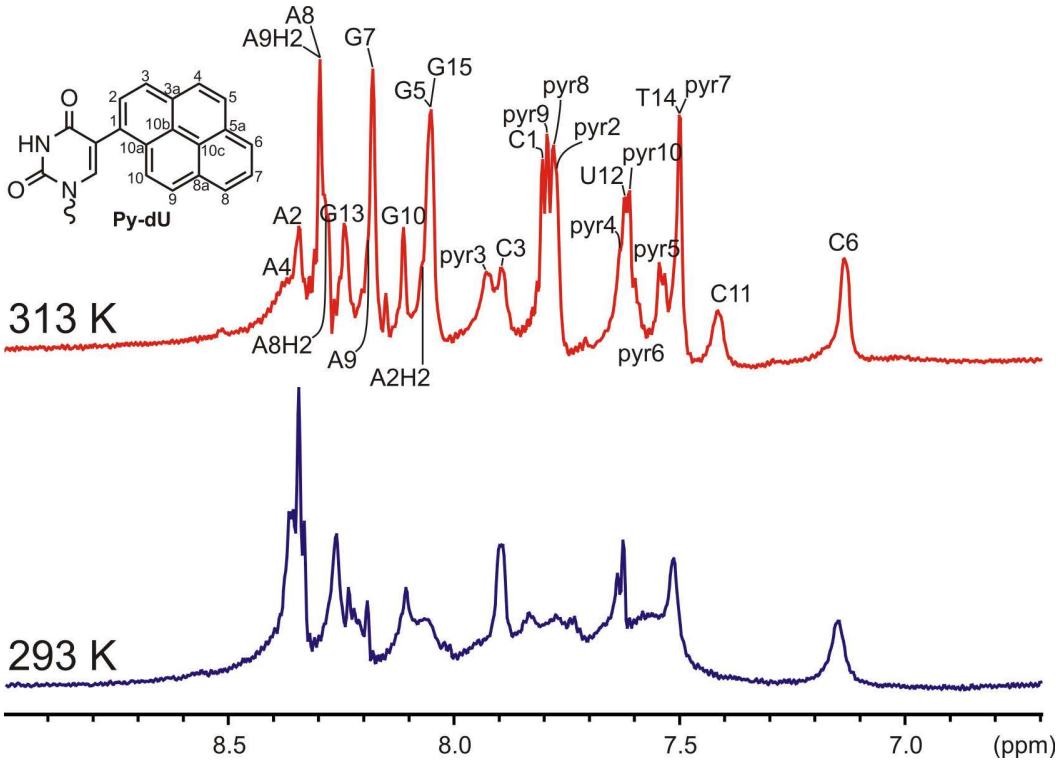


FIGURE 4.8 *aromatic proton region of Hugo in solution (H_2O/D_2O 90:10, [NaCl] = 50 mM, [Cacodilate] = 1 mM, pH = 6.5) at different temperatures. Raising the temperature narrows the peak width and improves the resonance resolution.*

4.3.2.2 Base and sugar protons

The assignment strategy for nonexchangeable protons is analogous to the already explained approach used for the oligomer em43 (see section 3.4.2.2). The assignment of the nonexchangeable base and sugar protons is accomplished at 313 K temperature in D_2O. Natural abundance (^1H,^{13}C) HSQC spectra of the oligomer in the aromatic and aliphatic regions are acquired (see fig. 4.9.a and .b respectively). The different spectral regions for different carbon proton correlations are indicated in boxed regions. The sugar proton spin systems and the pyrimidine proton spin systems (H6 to H5/5M) are identified in (^1H,^1H) TOCSY spectrum (see fig. 4.11). The cross peaks between cytosine H6 and H5 resonances are a direct way to identify these residues; noteworthy is the absence of the H5-H6 connectivity for C3. Cross peaks between H6 and methyl resonances H5M provide the identification of thymine residues. Interestingly for the only one thymidine present in the modified oligomer three different peaks are detected (see fig. 4.9.a inset and 4.11). The TOCSY cross peaks of pyrimidines allow the discrimination between them and purinic bases as well. In the acquired TOCSY spectrum the partial identification of the sugar proton spin systems i.e., H1', H2', H2" and H3' is possible. Just few H4's are identified and no cross peak for H5' and H5" has been detected. The identification of these spin systems aids in the subsequent sequential assignment of the non-exchangeable base and sugar proton resonances via sequential NOE contacts. Interestingly enough, other than the oligomer em43, there' s not a perfect correspondence between the peaks detected in TOCSY and NOESY spectrum (see fig. 4.12 and 4.13). This is yet another indication for the high flexibility of the Hugo oligomer compared to em43, as the difference in chemical shifts between NOESY and TOCSY spectra can only be explained by slightly different effective temperatures due to the different amount of rf irradiation applied during the experiments. As Hugo is highly dynamics, the equilibrium conformations are strongly temperature dependent, leading to the observed inconsistency of chemical shifts.

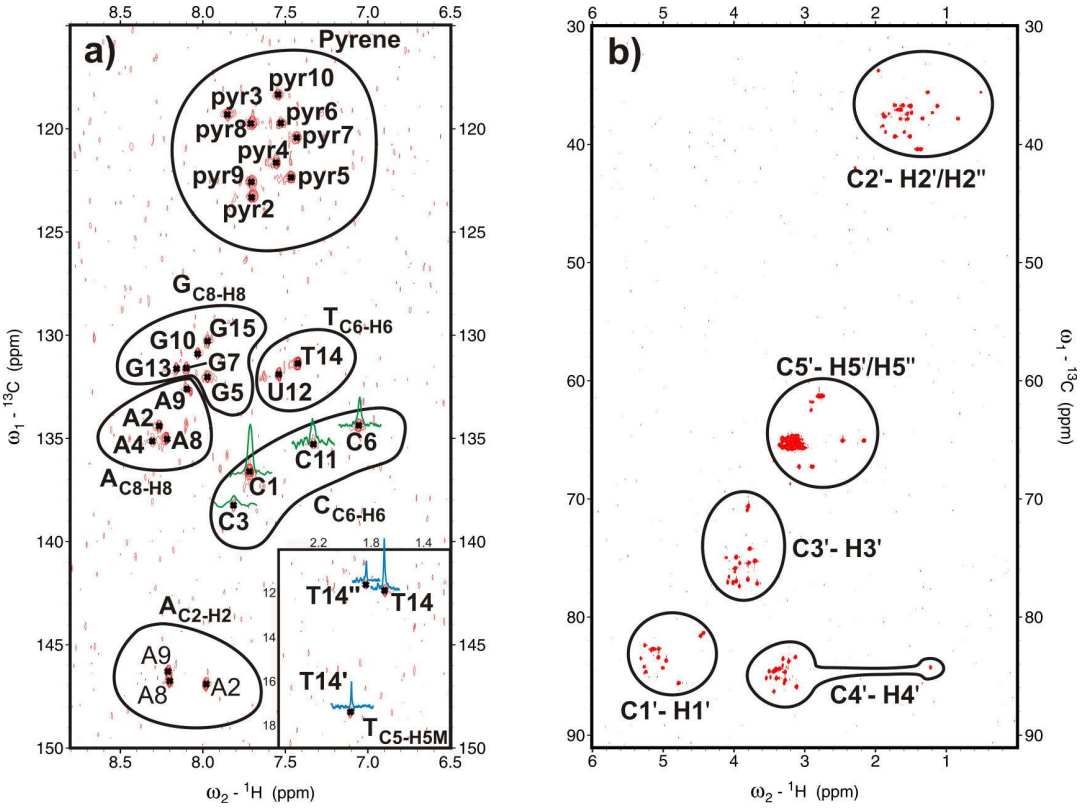


FIGURE 4.9 *Natural abundance HSQC spectra of aromatic (a) and aliphatic (b) regions of Hugo ([Hugo] = 1.94 mM, D_2O, [NaCl] = 50 mM, [Cacodilate] = 1 mM, pH = 6.5, T = 313 K); the different spectral regions for different carbon-protons correlations are circled in black. In the boxed area, H5M of the thimidine. Worth to note: in spectrum b) the classical 2.5 ppm upfielded chemical shift of A8 H4' is indicative for the triloop. On the spectrum a) it is possible to see in detail the three signals relative to the different conformation of T14C5-H5M and the corresponding H5M slice (blue); the peak of A4C2-H2 is not observable as well as just over the detection threshold are A4C8-H8 and C3C6-H6. Remarkably, A4 and C3 are the bases on the other side of the stem direclyt facing PydU.*

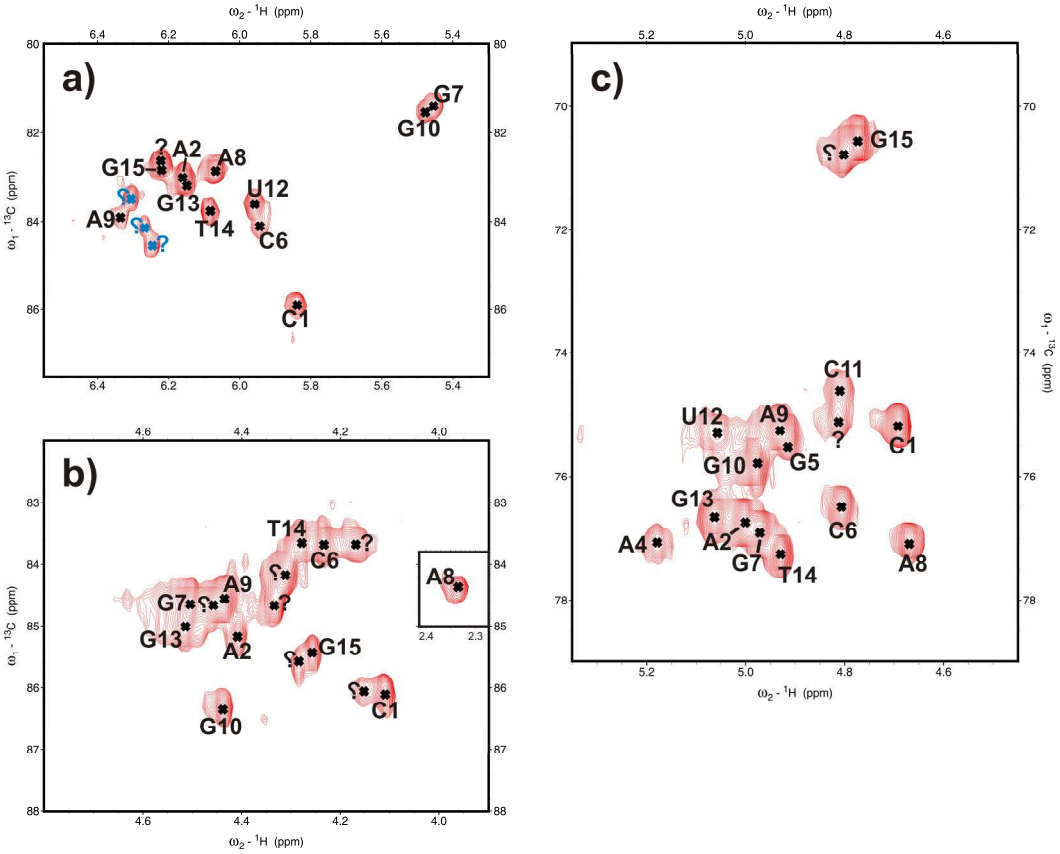


FIGURE 4.10 *Detail of natural abundance HSQC spectra in aliphatic region of Hugo; a) C1'-H1', b) C3'-H3', c) C4'-H4'. A complete assignment is not possible. Exemplary is the assignment of the C1'-H1' peaks (a); fourteen signals out of fifteen have been assigned. The three leftover signals (marked in blue) are most probably relative to three different conformation of C3C1'-H1'.*

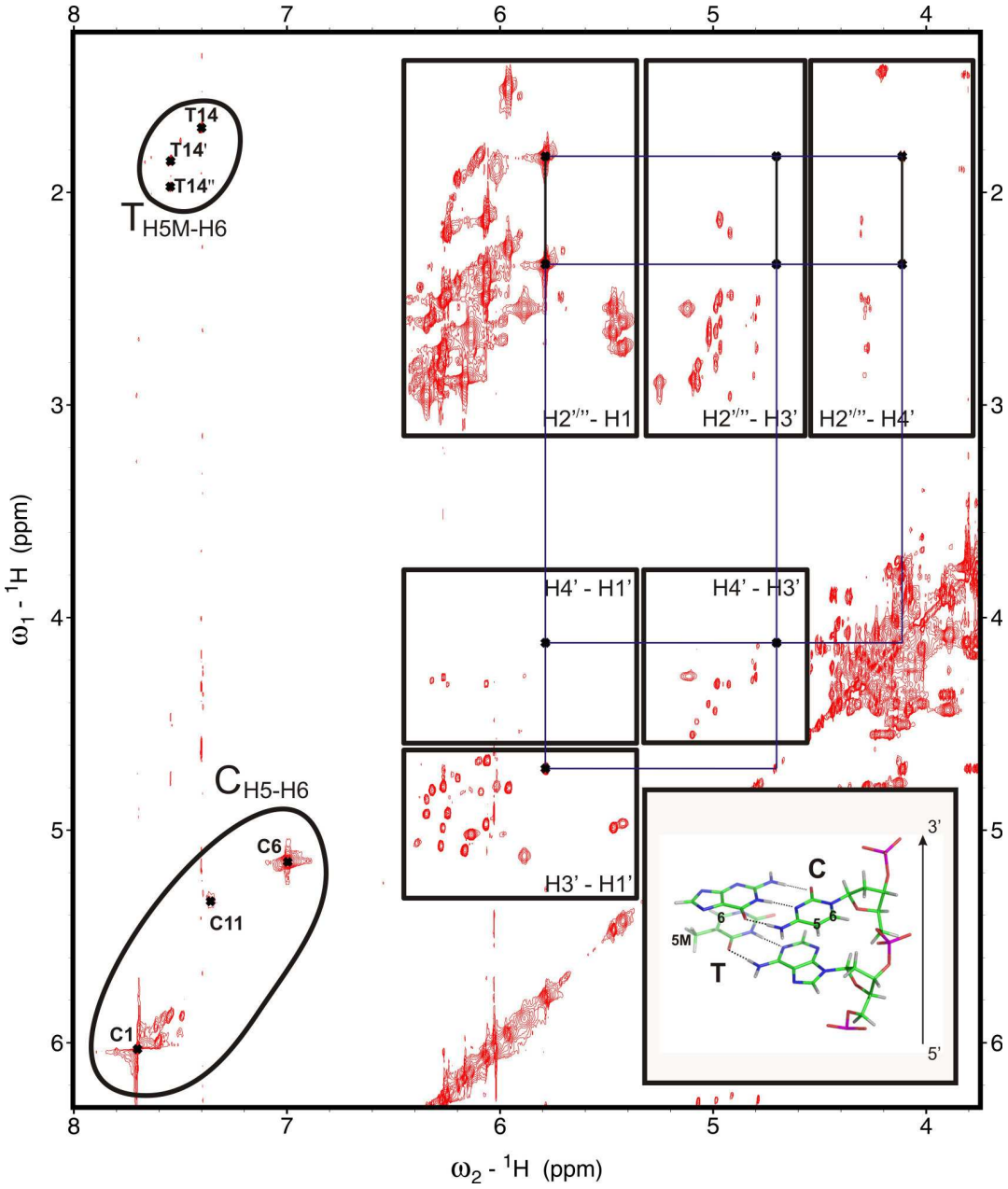


FIGURE 4.11 *TOCSY spectrum of Hugo; the cross peaks between cytosine H6 and H5 and between thymidine H6 and H5M resonances are circled in black; noteworthy absence of the H5-H6 connectivity for C3. The different spectral regions for different proton-protons correlations of sugar hydrogens are black boxed. In the spectrum it is possible the partial identification of the sugar proton spin systems. H1', H2', H2'' and H3' can be determined for each of the fifteen spin systems while the H4's are hardly identified and no cross peak for H5' and H5'' has been detected. The violet lines identify the spin system of C1; the H5' and H5'' are not detectable.*

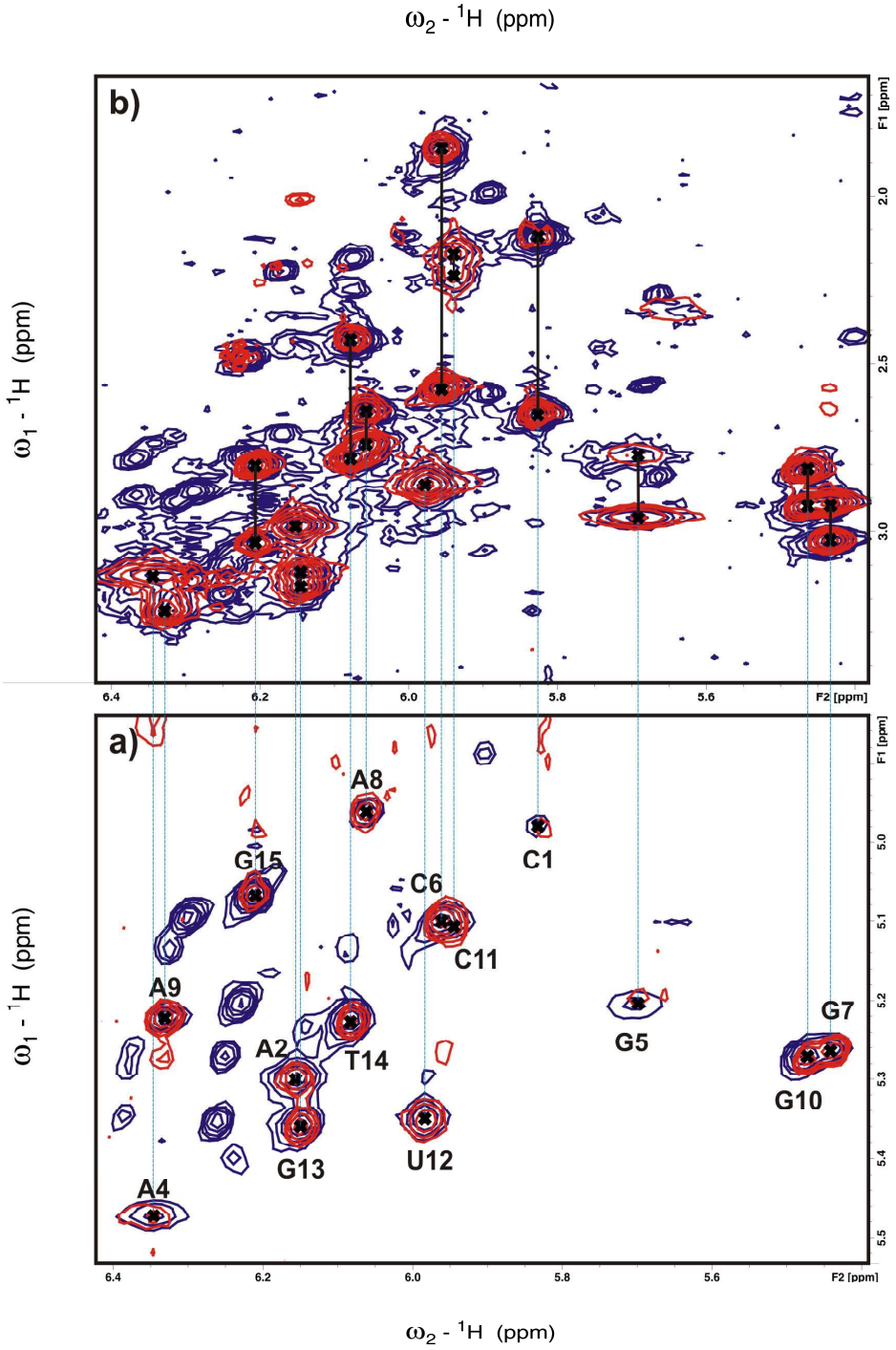


FIGURE 4.12 *Overlap of TOCSY (blue) and NOESY (red) spectra in the H3'-H1' (a) and H2'/''-H1' regions. Slight chemical shift changes between peaks found in the two different spectra are evident.*

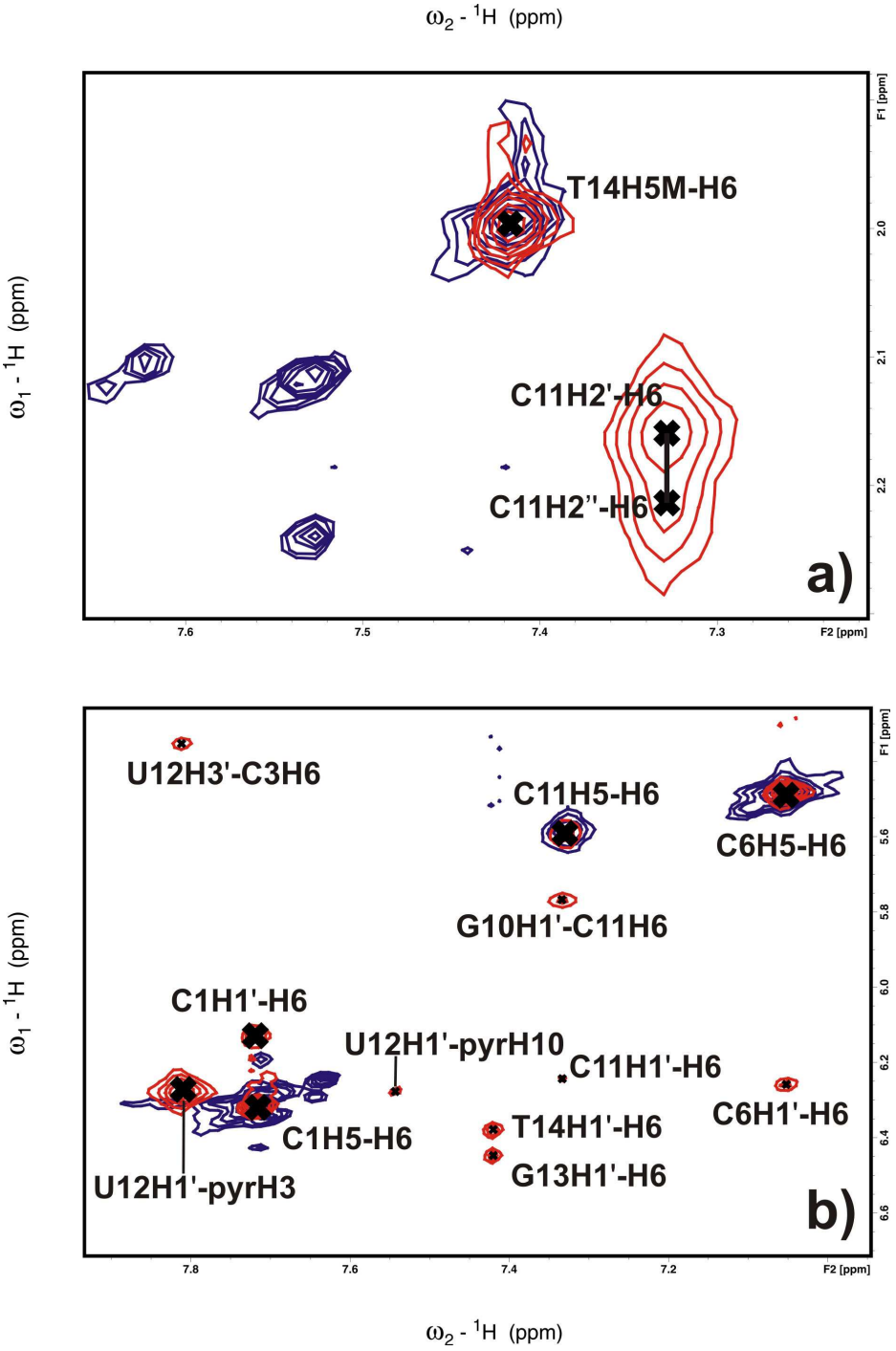


FIGURE 4.13 *Overlap of TOCSY (blue) and NOESY (red) spectra for the T14H5M-H6 peak (a) and H5-H6 region of cytosine residue. The non complete correspondence between peaks found in the two different spectra is evident*

4.2.3.3 Sequential assignment

The sequential assignment strategy of oligomer Hugo proceeds in a similar way to the already explained approach applied for oligomer em43 (see section 3.4.2.3). The signals of a particular sugar and those of its corresponding base (and similarly sugar protons of nucleotide *i* and base protons of the contiguous nucleotide *i+1*) are connected by NOEs (see excerpt of fig. 4.14.a). The connectivity pathway used is that given by the cross-peaks between H6/8 and H2'/H2". Once the connectivity patterns of individual nucleotides have been identified, the following step is to connect these patterns so as to obtain sequential assignments. From the TOCSY experiment the resonances of the aromatic protons of three out of the four cytosine residues as well as the aromatic and methyl protons of thymidine are known. Differently than all the others, the last nucleotide (G15) will give just intranucleotide NOEs connectivities between H8 and H2'/H2" but not connectivities between G15H8 and H2'/H2" of the following nucleotide. Similarly, the first nucleotide (C1) will give just intranucleotide NOEs connectivities between H8 and H2'/H2". These prerogatives are used to identify the resonances of C1 and G15 and they act as stepping-stones in the process of sequential assignment. The main problem of the sequential assignment of Hugo consists in the absence of detected connectivities relative to C3 in NOESY spectrum; the connectivities A2H8-C3H2'/", C3H2'/"-H6 and C3H6-A4H2' necessary for the complete sequential assignment of the oligomer are not visible (see fig. 4.14.b).

4.2.3.4 Assignment of PydU

The full assignment of the protons of the pyrenil group is accomplished from the connectivity between U12H6 and PyrH10. Proceeding backwards all the remaining protons of the group are assigned (see fig. 4.15). The resonance of U12H6 and PyrH10 are very similar (see fig. 4.9.a and tab. 4.1 and 4.3) but this doesn't result to be an insurmountable problem since the NOESY interactions between the remaining pyrenil protons are quite strong (distance between contiguous protons is on the order of 2.6 Å) and sufficiently dispersed.

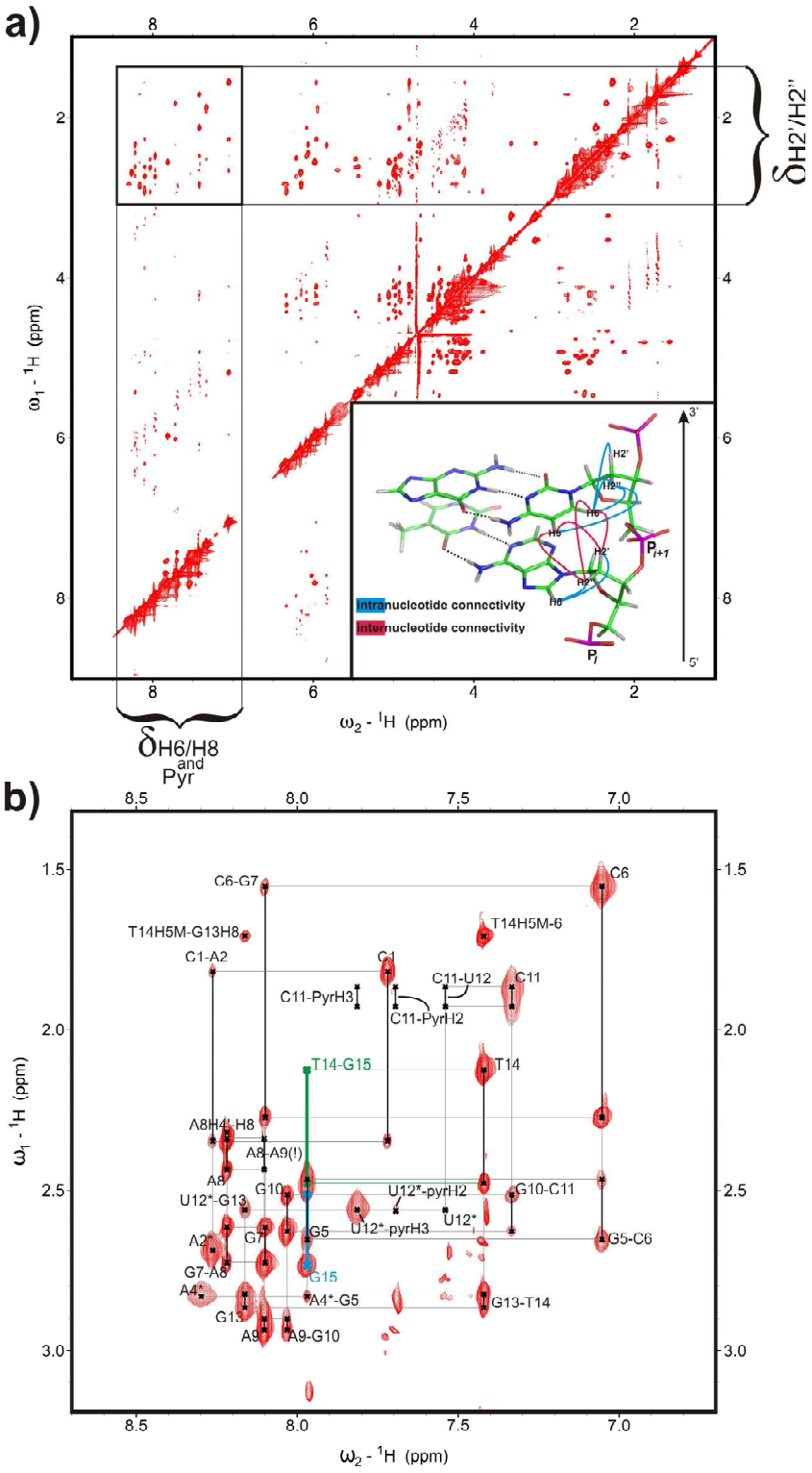


FIGURE 4.14 *a) black boxed the NOESY cross-peaks between H6/8, Pyr and H2'/H2'' b) full sequential assignment of the oligomer Hugo. Similarly to em43 the A8H2'/''-A9H8 is not detected as well as all the expected peaks involving C3.*

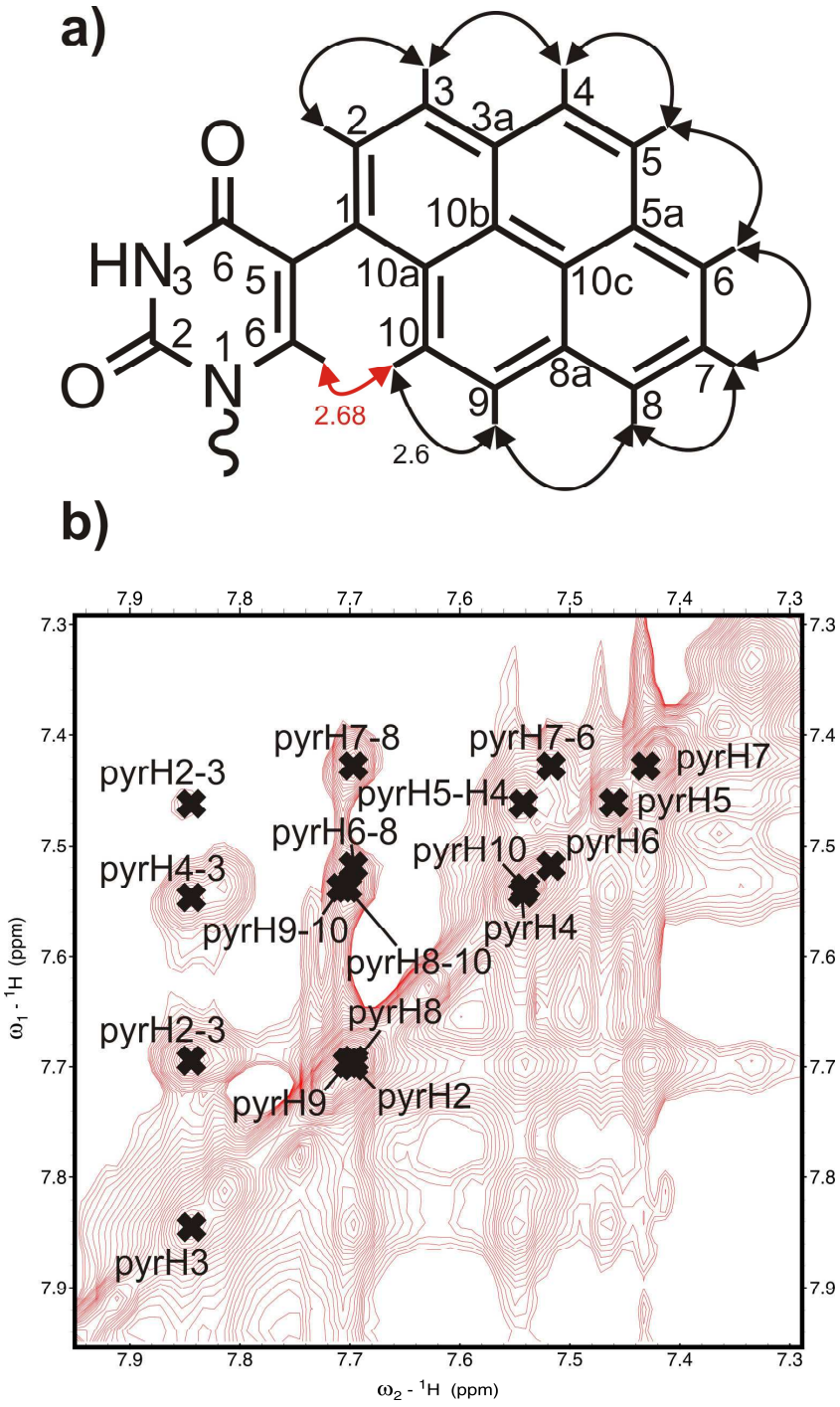


FIGURE 4.15 *The distance between U12H6 and PyrH10 is 2.62 Å a) Once known, the resonance of PyrH10 is straightforwardly traced back to the remaining protons of the Pyrenil group because the various resonances are sufficiently dispersed and the NOESY peaks rather strong due to the reduced interatomic distance (in the order of 2.6 Å, b).*

^{1}H Chemical Shift Assignments (ppm) of the Hugo DNA Stem-Loop									
Residue	H1’	H2’	H2’’	H3’	H4’	H5’	H5’’	H6/8	H2/5/Me
dC_1	5.84	1.82	2.35	4.69	4.11	3.76	3.74	7.72	6.03
dA_2	6.16	2.69$^{(*)}$	2.69$^{(*)}$	5.00	4.40	4.04	4.13	8.26	7.98
dC_3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	7.82	n.d.
dA_4	6.35	2.83$^{(*)}$	2.83$^{(*)}$	5.18	4.54	4.27	4.22	8.31	n.d.
dG_5	5.70	2.47	2.66	4.90	4.46	4.30	4.27	7.97	–
dC_6	5.96	1.56	2.27	4.80	4.23	4.11$^{(*)}$	4.11$^{(*)}$	7.05	5.15
dG_7	5.44	2.73	2.62	4.97	4.49	n.d.	n.d.	8.10	–
dA_8	6.06	2.34	2.44	4.66	2.22	3.23	3.54	8.22	8.20
dA_9	6.33	2.90	2.93	4.92	4.44	3.89	4.06	8.10	8.21
dG_{10}	5.48	2.63	2.52	4.98	4.44	4.34	4.22	8.03	–
dC_{11}	5.95	1.87	1.93	4.80	4.30	4.17	4.08	7.34	5.33
dU_{12}	5.99	2.56$^{(*)}$	2.56$^{(*)}$	5.05	4.31	4.19$^{(*)}$	4.19$^{(*)}$	7.54	–
dG_{13}	6.15	2.86	2.83	5.06	4.50	4.19	4.22	8.16	–
dT_{14}	6.09	2.13	2.45	4.93	4.28	4.19$^{(*)}$	4.19$^{(*)}$	7.42	1.69
dG_{15}	6.21	2.74	2.51	4.77	4.28	4.16	4.19	7.97	–

TABLE 4.1 *^{1}H Chemical Shifts of Hugo measured at 313 K. $^{(*)}$ - degenerated chemical shifts; (n.d.) - not detectable resonance.*

^{13}C Chemical Shift Assigments (ppm) of the Hugo Stem-Loop							
Residue	C1’	C2’	C3’	C4’	C5’	C6/8	C2/5/Me
dC_1	85.86	37.87	75.19	85.91	61.15	141.60	96.21
dA_2	82.96	37.36	76.74	84.39	n.a.	139.39	151.89
dC_3	n.d.	n.d.	n.d.	n.d.	n.d.	143.25	n.d.
dA_4	n.u.a.	38.35	77.08	85.16	n.a.	140.13	n.d.
dG_5	n.u.a.	37.58	75.52	84.65	n.a.	137.02	–
dC_6	n.u.a.	35.66	76.51	83.76	n.a.	139.36	95.27
dG_7	81.28	36.66	76.88	85.07	n.a.	136.59	–
dA_8	82.83	40.26	77.07	84.27	65.16	139.99	151.79
dA_9	83.91	33.91	75.24	84.57	67.05	137.61	151.27
dG_{10}	81.50	37.78	75.78	84.32	n.a.	135.88	–
dC_{11}	n.u.a.	37.49	74.61	83.53	n.a.	140.27	96.19
dU_{12}	83.62	37.24	75.31	84.10	n.a.	136.90	–
dG_{13}	83.22	37.60	76.66	84.59	n.a.	136.62	–
dT_{14}	83.77	36.94	77.27	85.35	n.a.	136.37	11.74
dG_{15}	82.82	39.04	70.55	84.67	n.a.	135.29	–

TABLE 4.2 *^{13}C Chemical Shifts of Hugo oligomer measured at 313 K.. (n.a.) - not assignable due to scarce dispersion; (n.d.) - not detectable resonance;(n.u.a.) not unambiguously assignable.*

Pyrenil group	^{13}C (ppm)	^{1}H (ppm)
2	128.25	7.70
3	124.28	7.85
4	126.65	7.55
5	127.34	7.47
6	124.71	7.52
7	125.43	7.43
8	124.70	7.70
9	127.58	7.70
10	123.34	7.54

TABELLE 4.3 *^{13}C and ^{1}H Chemical Shifts of the of the pyrenil group of PydU measured at 313 K.*

4.4 Structure calculation

4.4.1 Extraction of NOE restrictions

The dipolar cross-relaxation rate constant is proportional to the inverse sixth power of the distance between two interacting ^{1}H spins (see section 2.2.1). If one interproton distance (r_{ref}) is known from covalent geometry, then it is possible to derive the other unknown interproton distances (see eq. 2.6). The reference proton pair suitable to derive the unknown interproton distances is represented by H5 and H6 in cytosine (r_{HH} = 2.45 Å). The oligomer Hugo include four cytosine residues but just three give NOE connectivities, C3 is missing and moreover, the peak of C11H5-H6 is weak and affected by a baseline phase imperfection. The reason of this latter artifact is the rather long measurement time; at 313 K about 12 hours are necessary to obtain a NOESY spectrum with an acceptable S/N ratio. Meanwhile, air bubbles are generated; they are responsible of spoiling the shimming which subsequently causes distortions in the baseline. The peaks relative to C6 and C1 emerge over a flat base line; both are isolated and give very well defined NOE cross peaks. These latter ones are suitable reference to back calculate the NOE restrictions. From the integrals relative to H5-H6 interproton distance in the cytosine residues derived experimentally it is possible to calculate the average integral value of $\langle A \rangle = 1.54 \cdot 10^{7}$ (see eq. 3.1) and known interproton distance being 2.45 Å [153], the proportional constant K is consequently extracted to be $K = 3.33 \cdot 10^{9}$. From this value the distance restraints relative to the NOE connectivities to be used in the structure

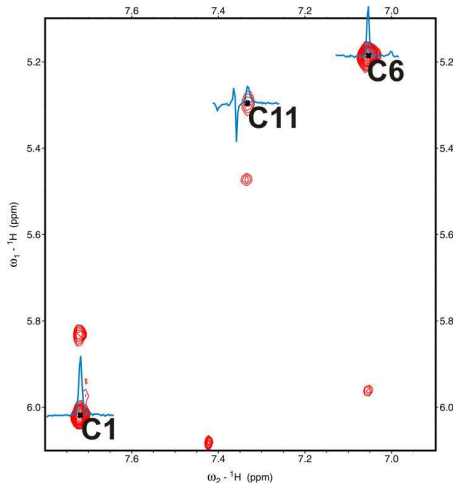


FIGURE 4.16 *NOE cross-peaks between H5 and H6 of the cytosine residues in Hugo measured in H_20/D_2O 90:10 and T = 313 K. C3 is not visible and C11 is affected by a baseline imperfection; the peaks C1 and C6 are very well defined and isolated; they are appropriate references to the calculation of the NOE derived distance restraints.*

Residue	A – Integral (10^7)
C1	1.70
C6	1.38

TABELLE 4.4 *The integrals are calculated in Sparky via "sum over box" method.*

calculation have been back calculated (see relative table in appendix 2).

4.4.2 Definition of the starting structure

With the exception of C3, the peaks of whom are not visible, the NOEs correlations between H2'/H2" and H6/H8 are of medium strength and the peaks between H1' and H6/8 are clearly visible for the deoxynucleotides along the stem. For them, qualitative and quantitative analysis of NOE distance values of the oligomer are consistent with B-form helix. The apical deoxynucleosides –GAA– give NOE correlations typical of the loop structure. For A8H2'/H2" and A9H8 no NOE's are observable but connectivities between A8H5'/H5" and A9H8 has been reported. Moreover, the A8H4' signal is moved 2.5 ppm upfield as compared with H4' resonances of other residues (see HSQC aliphatic spectrum 4.9.b and tab. 4.1). This is typical of the apical adenosine residue of the GAA triloop. These experimental evidences suggest how the conformation around the C5'-O5' (torsion angle β) doesn't adopt *trans* form and the conformation of the fragment is locally folded back between A8 and A9 [173,175,178].

4.4.3 Structure determination

The structure of the oligomer Hugo is determined performing a series of simulated annealing calculations using XPLOR NIH 2.9.4a [187, 188] with the üprotocol as described in section 3.5.3. The lacking of NOE restriction relative to C3 is taken in account in the *hbonds_assumed.tbl* where no hydrogen bond involving C3 is defined and in *plane.inp* where no planarity restriction can be settled. No hydrogen bond and no planar restriction is defined for PydU as well (see relative section in appendix 2). The structure is calculated with 467 NOE restraints (281 intra-residual, 186 inter-residual, see tab. 4.5) uniformly distributed along the sequence. Distances were given a variable error range to cover the inaccuracy of NOE intensity due to peak overlaps, noise, peak spread from scalar coupling and spin diffusion (see section 2.2.1.2) similarly as already explained in section 3.5.3.

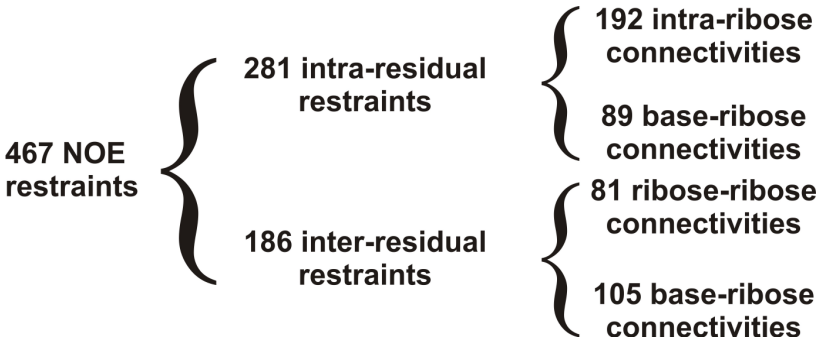


TABELLE 4.5 *Number and type of NOE restraints used in the structural calculation of Hugo.*

Among the whole set of connectivities found, a decisive role for the final structural determination is played by the NOE restraints involving the protons of the pyrenil group and protons of the deoxynucleosides. A total of 19 NOEs have been found between protons of the pyrenil group and protons of C11, upstreamed with respect to PydU (8 restraints), G13 downstreamed (5 restraints) and A4H5$^{1/}$" (6 restraints) (see fig. 4.17). In particular these latter NOEs between pyrenil and ribose protons of the deoxynucleoside directly facing PydU in the duplex state unequivocally how the hydrocarbon is intercalated in the helix and how the structure in fig. 4.18 is consistent with experimental data.

4.4.4 Structural comparison of conventional duplex DNA and duplex DNA with PydU incorporated

Em43 shows a well-ordered B-form double helix capped by the GAA-motif that forms a triloop used for stability reasons. All the deoxynucleosides along the stem form conventional Watson-Crick base pairs (fig. 4.19.a and .c). For DNA hairpin counterpart with PydU incorporated (Hugo) PydU makes A4 flip out and introduces slow dynamics in the G5-C11 base pair. The NMR-signals of C3 are so strongly broadened that most of

its resonances could not be assigned. While C1-G15 and A2-T14 are still base-paired, the intercalation of the pyrenil group into the duplex prevents the formation of hydrogen bonds between PydU and A4 and between C3 and G13 (see fig. 4.5 and fig. 4.19.b and .d). Hugo shows four imino proton peaks compared to six of em43. The enhanced flexibility of the stem in proximity of the intercaled pyrene group makes the base pairs G5-C11 and A2-T14 accessible to the solvent; exchange of the relative imino protons can easily take place and this results in weak peaks scarcely persistent as the temperature rises (see fig. 4.7). Consequence of that is the undetectability of NOE connectivities between imino protons even at 273 K (see section 4.3.1). The local structural dynamics of the loop region and its closing base pair C6-G11 is not substantially affected by the intercalation of the hydrocarbon. This is expressed by the strength and persistence of the peak G10H1 and with the behavior of G7H1 comparable to the equivalent imino proton of em43. Other evidence of the augmented local flexibility brought into Hugo by the pyrenil group intercalation are the non uniform linewidths of the non-exchangeable protons. In the 300 ms NOESY spectrum of em43 the linewidths are rather homogeneous while for Hugo a variety of linewidths can be observed. H1' resonances for G5 and C11 show for example broadened lines as well as A4 and PydU. The last two central residues also have degenerate chemical shifts for H2' and H2", a hint for faster motion of the unpaired nucleotides (see fig. 4.20).

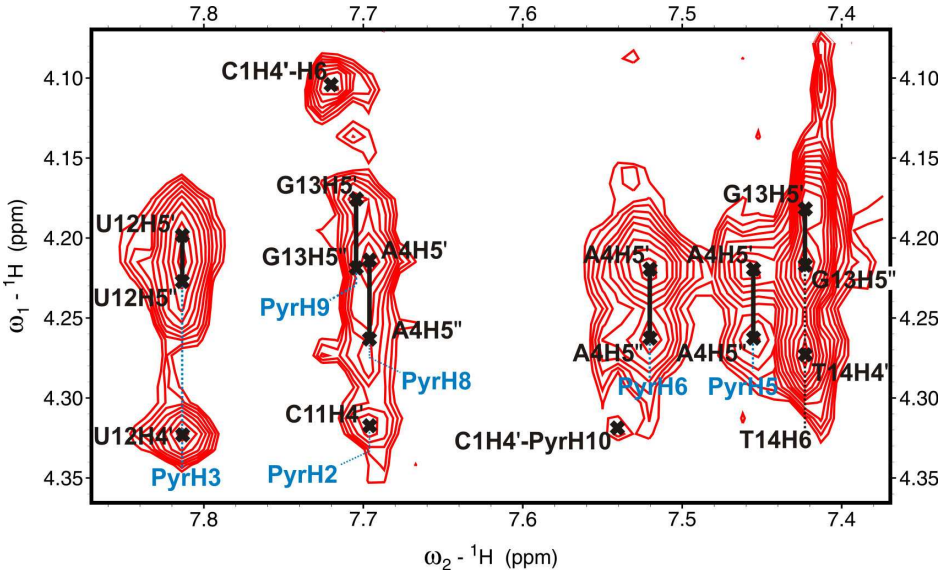


FIGURE 4.17 *Structurally decisive NOE connectivities between protons of the pyrenil group and of the deoxynucleosides; very significative are the peaks relative to A4H5'/" and PyrH5, PyrH6, PyrH8. They state beyond a reasonable doubt that the pyrenil group is stacked in the helix and the opposite base A4 is flipped out.*

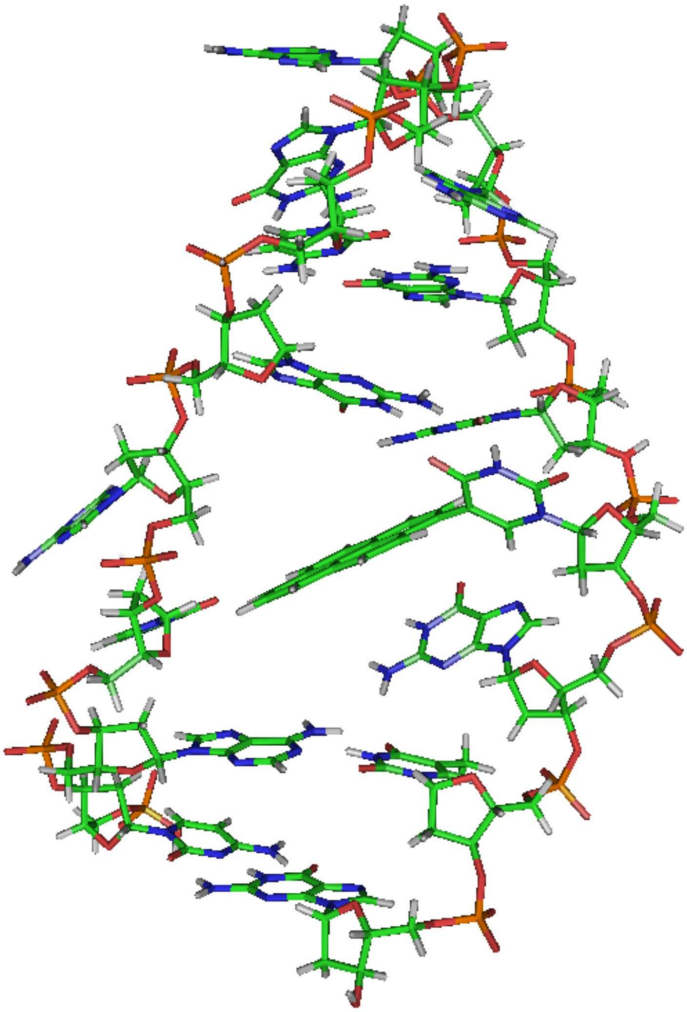


FIGURE 4.18 *Structure of oligomer Hugo obtained as described in 4.4.3. As the pyrenil group is stuck into the duplex, the deoxynucleosides C3 and A4 are forced to swing out.*

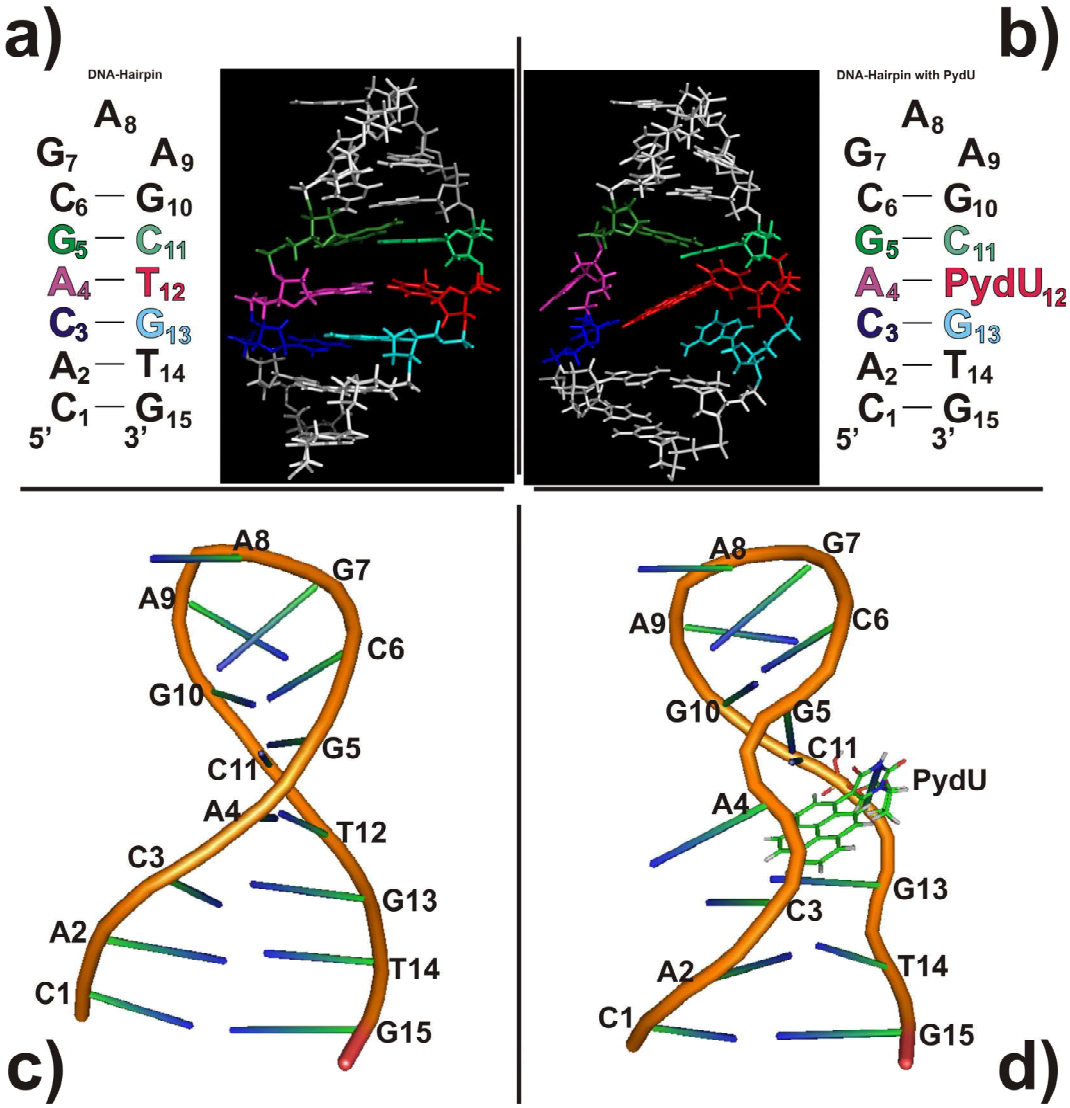


FIGURE 4.19 *DNA hairpin of conventional duplex DNA (em43, a) and duplex DNA with PydU incorporated (Hugo, b). Phosphodiester backbone for em43 and Hugo are shown in c) and d) respectively: the dramatic structural change brought by the intercalation of the pyrenil group into the stem is evident.*

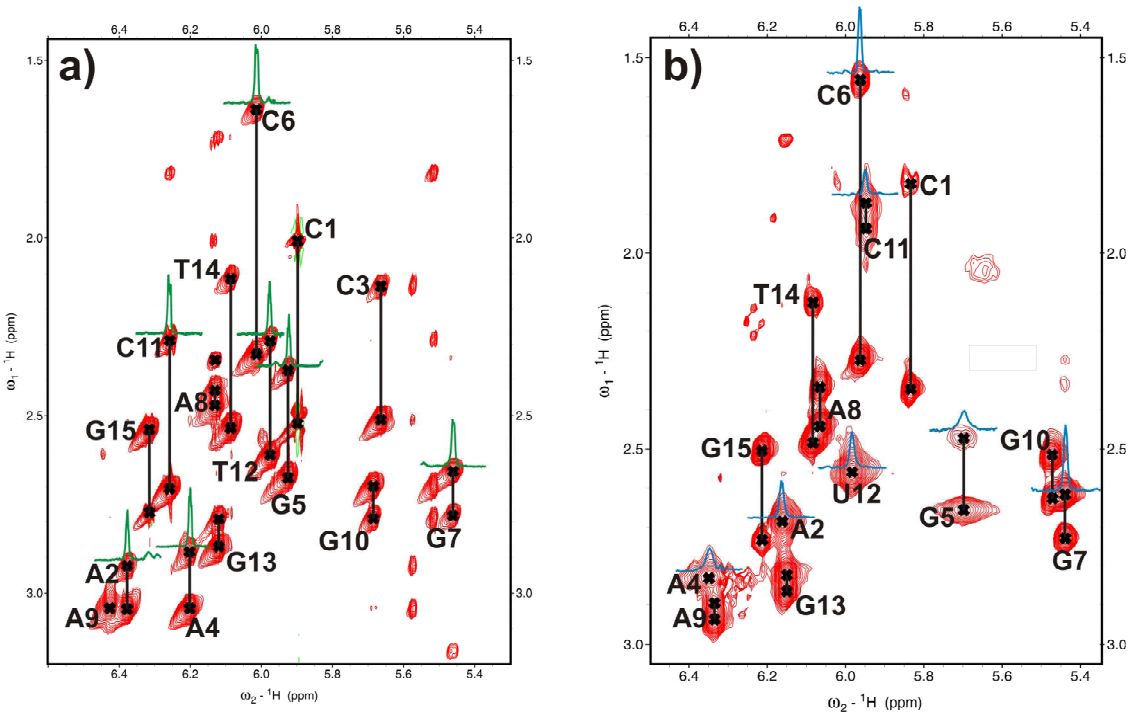


FIGURE 4.20 *Comparison of H2'''-H1' region of the 300 ms NOESY spectra for em43 (a) and Hugo (b). It is there evident how the linewidths of H1' resonances are generally very homogeneous for em43 and how for Hugo a variety of linewidths can be observed. This expresses the conformation stability of em43 (a) and on the other hand the enhanced local flexibility of Hugo induced by the intercaled pyrenil group (b).*

As visible in fig. 4.12 and 4.13 there's not a perfect correspondence of the NOESY and TOCSY spectra. Peaks ascribable to C3 are not detected in NOESY while a plethora of peaks not assignable are found in TOCSY. Moreover, HSQC-cross peaks for the only methyl group in T14 are a clear indicator for a minimum of three minor conformations beside one major for which the structure determination has been accomplished (see fig. 4.9.a inset).

4.5 Conclusions

- PydU changes duplex structure significantly;
- Clear evidence for stacking of PydU;
- A4 flipped out;
- C3 also flipped out according to structure calculation although almost no resonance assigned. Most likely this effect is due to induced slow dynamics of flipp-in/flipp-out of C3;

- Dynamics only affect neighboring nucleotides, i.e. C3, A4, G5, C11, G13;

- Although slightly broadened, A2-T14 is clearlz conventionally base-paired, as well as C6-G11.

5. Summary

In the presented thesis the structures of the control and modified oligomere has been determined by NMR spectroscopy at natural abundance. The goal was to investigate the possible structural perturbations induced by the incorporated non-natural nucleotide. Although modified bases are frequently used in modern biochemical approaches to study properties of nucleic acids and their complexes, very little is known about the structural changes of the DNA duplex formation caused by artificial nucleosides. In particular fluorescent chromophores with optimized luminescent properties are incorporated in oligomeres since the intrinsic fluorescence of the naturally occurring nucleotide bases in nucleic acids is extremely weak. Fluorescence spectroscopy can profitably be employed to measure excited state lifetimes of redox probes within the DNA π-stack and to characterize charge transfer quenching of these photo excited reagents. After decades of controversial debate about, reductive as well as oxidative hole transfer chemistry through DNA are now widely accepted and the discussion has moved to the decisive question of its mechanism and most recently, to its biological relevance and biotechnical application. CT in DNA is extremely sensitive to the π–stacking of the intervening DNA structure; perturbations of the base stacking caused by base mismatches, DNA lesions or DNA-protein interactions are effectively reported by altered CT. From that perspective, it is intuitive to understand that elucidation of the structural modifications induced by the incorporation in the oligomere of a chromophore, e.g. the pyrenil group, could help to provide new insight into the still open question of the mechanism of electron transfer between stacked planes of a duplex. In chapter 2 useful NMR-spectroscopical aspects to the structure determination of a generic oligomere has been introduced. In particular, assignment of resonances is the first essential step in an NMR study of biomolecules targeted toward three-dimensional structural elucidation of a natural oligomere. In chapter 3 it is described how once accomplished a full assignment, it is possible to accurately extract valuable information about internuclear distance from the integral of the cross relaxation signals of a two-dimensional NOESY experiments. The intensity of NOE connectivity is inversely proportional to the sixth power of the distances between the nuclei. The whole assignment strategy is based on NOE cross-peaks between H6/8 and H2'/H2" which enable to connect sugar protons of nucleotide *i* with base proton of the same and base protons of the contiguous nucleotide. That approach of assignment and distance restraints extraction is completely based on NOEs connectivities and could give rise to ambiguities. As a matter of fact, the NOE intensities are themselves dependent on the structure of the molecule. In order to get rid of any possible source of equivocacy and validate the NOE-based assignment approach, two additional experiments are performed:

- HMBC experiment to correlate the aromatic carbons C6,8 to the H1' ribose proton of the same nucleoside;

- (^{1}H-^{31}P) CPMG-correlated experiment to accomplish the sequential assignment.

Subsequently, the extracted interproton distance restraints are used to perform a series of simulated annealing calculations using XPLOR NIH 2.9.4a: one of the most popular programs for this purpose.

A total of 336 NOE-derived distance restraints have been used to calculate the NMR-structure of the hairpin. The control oligomere exhibits a well-ordered B-form double helix capped by the GAA-motif which forms a triloop.

The uridine analog 5-(1-pyrenyl)-2'-deoxyuridine is incorporated in a DNA hairpin counterpart. The pyrenil group can be selectively excited and used to induce a reductive electron transfer (ET) process along the stem (see section 1.2.4). In chapter 4 it is described how to accomplish the assignment of the non-exchangeable protons. The assignment of the exchangeable protons is not feasible because the imino proton peaks are too weak and not any single connectivity involving iminos is detectable in two-dimensional NOESY spectrum at 273 K in H_2O. In this case the assignment and distance restraints extraction, including the pyrenil protons, are completely based on NOEs connectivities according to the aforementioned strategy. The 467 interproton distance restraints extracted from the two-dimensional NOESY experiment are used to calculated the structure of the modified oligomere. A series of simulated annealing calculations are performed using XPLOR NIH 2.9.4a. The lacking of NOE restriction relative to C3 is taken in account in the relative protocols as well as no hydrogen bond and no planar restriction is defined for PydU and A4. A total of 19 NOEs have been found between protons of the pyrenil group and protons of C11, upstreamed with respect to PydU, G13 downstreamed and A4H5$^{'/''}$. These NOEs state unequivocally how the hydrocarbon is intercalated into the helix. From the structural determination it is evident how C1-G15 and A2-T14 are still base-paired, PydU makes A4 flip out and introduces slow dynamics in the G5-C11 base pair. The structure is consistent with the experimental data e.g.,:

- the NMR-signals of C3 are strongly broadened so that not a single resonance could be assigned to it;

- only four imino protons are detectable vs. six of the control oligomere. The peaks are very weak and scarcely persistent as the temperature raises;

- In the 300 ms NOESY spectrum a variety of linewidths can be observed. H1' resonances for G5 and C11show broadened lines as well as A4 and PydU;

- A4 and A2 residues have degenerate chemical shifts for H2' and H2", a hint for faster motion of the unpaired nucleotides.

Appendix 1

"XPLOR NIH 2.9.4a simulated annealing" protocol:

```
remarks file nmr/refine_gentle.inp  -- Gentle simulated annealing
refinement
remarks                                  for NMR structure determination
remarks DNA refinement with dipolar couplings, H-bonds, NOEs, and
dihedral remarks angles
evaluate ($seed=93869099)
{* This uses database-derived information for the DNA. The force is
initially *}
{* set to 1.0 and then the scale is changed to change the force
constant.     *}
{* This is the same method used with other experimental restraints,
such as   *}
{* NOEs and dihedral angles. *}
set seed $seed  end

{====>}
{====>}
{* ====> is the symbol for things that are likely to need to be
changed.  *}

{* in order to use dipolar couplings, it is necessary to define the
axis. *}
{* Using a parameter file, structure file and PDB file.
*}
{* These files are par_axis_3.pro, axis_new.psf, and axis.pdb.
*}


parameter                                        {* Read the parameter
files.*}
{====>}
   @TOPPAR:parnah1er1_mod_new.inp
   @TOPPAR:par_axis_3.pro
end



{====>} structure @DNA.psf @axis_new.psf end           {*Read the
structure file.*}


{====>}
coor @StartingStructures/MODELL_FINAL.pdb
{====>}
coor @axis.pdb

flags exclude * include bonds angles impr end

mini powell nstep=100 step=1.0 end
```

```
{* minimize structure with respect to covalent constraints. *}
{* use this as a starting structure for all generated structures. *}

coor copy end

{====>}
@plane.inp
{* This sets up basepair planarity constraints. If residue numbers
change, this needs to be changed. *}

!ncs restraints
!{* Set the two strands symmetric to one another. Again residue number-
specific *}
!   group
!{====>}         equi ((resid  1:12) )
!{====>}         equi ((resid 13:24) )
!         weight=50
!  !       sigb=2.0
!   end
!   ?   {* print the NCS relations when starting *}
!
!end


{* This (dna_dna_pairs_03.setup) sets up some a priori constraints.
*}
{* The restraints are orientation restraints between pairs of DNA
nucleotides.*}
{* This file would need to be edited if the nucleotide numbers change.
*}
{* See dna_dna_pairs_03.setup for details about matching Watson-Crick
*}
{* base partners.The script dna_dna_pairs_03.setup takes care of
setting      *}
{* different SEGIDs for the two DNA strands to correctly implement the
*}
{* database potential.
*}

@dna_dna_pairs_03.setup


evaluate ($knoe =1.0)           {* to make the scaling later more
foolproof, *}
                                {* this initial force constant is set
to 1.0 *}
                                {* The scale command is used to adjust
the *}
                                {* effective force. *}

noe
{====>}
   nres=3000             {*Estimate greater than the actual number of
NOEs.*}
   class all
{====>}
```

```
   @Restraints/DNA_NOE_intra.tbl                        {*Read NOE
distance ranges.*}
   class hb
   @Restraints/hbonds_assumed.tbl
end

{====>}
evaluate ($kcdi = 1.0)
!restraints dihed
!  scale $kcdi
!  nass = 5000
!  set message on echo on end
!    @dihedral.tbl    {* dihedral angle restraints. *}
!  set message on echo on end
!end


!evaluate ($knoe = 1.0)
evaluate ($knoe = 0.1)  !==> this is the original value
noe                              {*Parameters for NOE effective energy
term.*}
   ceiling=1000
   averaging  all sum
   potential  all square
   sqconstant all 1.
   sqexponent all 2
   scale      all $knoe           {*Constant NOE scale throughout the
protocol.*}

   averaging  hb sum
   potential  hb biharmonic
   sqconstant hb 1.
   sqexponent hb 2
   scale      hb $knoe           {*Constant NOE scale throughout the
protocol.*}

end


evaluate ($knuc=1.0)
  {* for nucleic acid gaussians the force *}
{* constant is $knuc. *}
rama
nres=10000
  {* Nucleic acid database
*}
  {* The torsion angles that are being correlated are mostly
*}
  {*intranucleotide; they include the sugar torsion angles, and the
chi1   *}
  {* for the base with respect to the sugar. Actually two torsion
angles   *}
  {* from adjacent nucleotides are included.
*}
  @GAUSSIANS:all_na_gaussians.tbl
  @GAUSSIANS:force_nucleic.tbl
end
```

```
  set message on echo on end
  @GAUSSIANS:setup_nucleic_new.tbl
{* Set up of a priori restraints. *}


evaluate ($ini_sani = 0.01)
evaluate ($ksani  = $ini_sani)
evaluate ($ksanib = $ksani)
evaluate ($ksanin = $ksani*1.0)
evaluate ($ksanim = $ksani*1.0)
evaluate ($ksaddr = $ksani*1/5)
evaluate ($ksaddb = $ksani*1/8)

!@read_dipolars.inp


parameter                        {*Parameters for the repulsive energy
term.*}
   nbonds
      cutnb=6.5
      rcon=4.0 nbxmod=4 repel=0.8 rexp=2 irex=2 {*With internal
dynamics and *}
                                                {* the database
potential,   *}
                                                {* nbxmod should be 4.
*}
      wmin=0.01 tolerance=0.5
   end
end



@setup_int_dynamics.inp


evaluate ($cool_steps = 3000)
evaluate ($init_t  = 3000.01)
evaluate ($tol = $init_t/1000)

vector do (mass = 100.0) (all)  {* all atom masses are set the same so
that *}
                                {* motion of each atom is equally
likely in *}
                                {* the dynamics. Note that the dynamics
is  *}
                                {* not a realistic model of the motion
of   *}
                                {* the system; it is a method of
minimizing *}
                                {* a highly complicated function.
*}
vector do (fbeta = 10.0) (all)


{====>}
evaluate ($end_count=1)            {*Loop through a family of 2
structures.*}
```

```
evaluate ($count = 0)
while ($count < 2 ) loop structure

   evaluate ($count=$count+1)



   vector do (x=xcomp) (all)
   vector do (y=ycomp) (all)
   vector do (z=zcomp) (all)


   {* Set values for scaling parameters while the temperature changes
in the
         cooling step. *}
   evaluate ($ini_rad  = 0.9)        evaluate ($fin_rad  = 0.78)
   evaluate ($ini_con=  0.004)       evaluate ($fin_con=  4.0)
   evaluate ($ini_ang = 0.4)         evaluate ($fin_ang = 1.0)
   evaluate ($ini_imp = 0.1)         evaluate ($fin_imp = 1.0)
   evaluate ($ini_noe = 2.0)         evaluate ($fin_noe = 30.0)
   evaluate ($knoe  = $ini_noe)
   evaluate ($ini_nuc = 1.000)       evaluate ($fin_nuc = 1.0)
   evaluate ($knuc = $ini_nuc)
   evaluate ($ini_sani = 0.01)       evaluate ($fin_sani = 1.0)
   evaluate ($ksani  = $ini_sani)

   evaluate ($kcdi  = 10.0)   ! torsion angles


   noe scale * $knoe end
   {* The scale command after a type of restraint, allows you to change
the *}
   {* force constant of that particular restraint. This is usually seen
in  *}
   {* the cooling steps. *}

   restraints dihed
      scale $kcdi
   end

   rama
      scale $knuc
   end


   evaluate ($ksanib = $ksani)
   evaluate ($ksanin = $ksani*1.0)
   evaluate ($ksanim = $ksani*1.0)
   evaluate ($ksaddr = $ksani*1/5)
   evaluate ($ksaddb = $ksani*1/8)

   xdip class JCH force $ksani end
   xdip class JCHB force $ksanib end
   xdip class JCNB force $ksanin end
   xdip class JCMT force $ksanim end
   xdip class JADR force $ksaddr end
```

```
   xdip class JADB force $ksaddb end
   xdip class HABS force $ksani end
   xdip class HSIG force $ksani end


   evaluate ($rcon  = 1.0)

   parameters
      nbonds
       atom
       nbxmod 4        {* This is 4, because we are using database
(delphic) *}
                       {* potentials.
*}
       wmin  =   0.01  {* warning off
*}
       cutnb =   100   {* nonbonded cutoff
*}
       tolerance 45
       repel=    1.2   {* scale factor for vdW radii = 1 ( L-J radii)
*}
                       {* This is so large, because it is only applied
to CA *}
                       {* atoms.
*}
                       {* Of course, in DNA, there aren't any CA atoms.
*}
       rexp   =  2     {* exponents in (r^irex - R0^irex)^rexp
*}
       irex   =  2
       rcon=$rcon      {* actually set the vdW force constant
*}
      end
   end

   flags
      exclude *
      include bond angl impr vdw noe cdih  ncs  orie rama xdip plan
   end




   constraints

        interaction (not name ca ) (all)

        weights * 1 angl 0.4 impr 0.1 vdw 0 elec 0 end

        interaction (name ca) (name ca)

        weights * 1 angl 0.4 impr 0.1 vdw 1.0 end

      {* Due to this set-up, van der Waals forces are turned off for
the first
          5000 steps of refinement. *}
```

```
        {* This allows atoms to pass though one another in the high
temperature
           phase of annealing. *}
      {* This increases the conformational space that can be sampled in
the                  high temperature phase. *}
      {* However, the choice of dynamics type (in this case torsional)
will                 also affect the conformational space accessible.
*}
   end


   vector do (vx = maxwell($init_t)) (all)
   vector do (vy = maxwell($init_t)) (all)
   vector do (vz = maxwell($init_t)) (all)


   dynamics internal
      itype=pc6
      etol=$tol
      tbath=$init_t
      response= 5
      nprint=25
      nstep=0          {* This being zero, guarentees that endtime is
the  *}
                       {* controlling variable.
*}
      timestep=  0.002 {* This is the starting value of timestep,
adjustTS *}
                       {* is the default, so this will change over the
run *}
      endtime=10       {* 10 picoseconds, if nstep is not reached
first.   *}
      cloop=true
   end




   parameters
    nbonds
     atom
     nbxmod 4          {* This is 4, because we are using database
(delphic) *}
                     {* potentials (on the torsion angles).
*}
     wmin  =   0.01  {* warning off
*}
     cutnb =   4.5   {* nonbonded cutoff
*}
     tolerance 0.5
     repel=    0.9   {* scale factor for vdW radii = 1 ( L-J radii).
All   *}
                     {* atoms are subject to the van der Waals force.
*}
     rexp   =  2     {* exponents in (r^irex - R0^irex)^rexp
*}
```

```
     irex   =  2
     rcon = 1.0      {* actually set the vdW weight
*}
    end
   end


   evaluate ($kcdi = 200)
   restraints dihed
     scale $kcdi
   end

   {* Set up of changing scale of energy terms with the cooling steps.
*}
   evaluate ($final_t = 25)     { K }
   evaluate ($tempstep = 25)     { K }

   evaluate ($ncycle = ($init_t-$final_t)/$tempstep)
   evaluate ($nstep = int($cool_steps*4.0/$ncycle))
   evaluate ($endtime = $nstep*0.002)

   evaluate ($bath  = $init_t)
   evaluate ($k_vdw = $ini_con)
   evaluate ($k_vdwfact = ($fin_con/$ini_con)^(1/$ncycle))
   evaluate ($radius=    $ini_rad)
   evaluate ($radfact = ($fin_rad/$ini_rad)^(1/$ncycle))
   evaluate ($k_ang = $ini_ang)
   evaluate ($ang_fac = ($fin_ang/$ini_ang)^(1/$ncycle))
   evaluate ($k_imp = $ini_imp)
   evaluate ($imp_fac = ($fin_imp/$ini_imp)^(1/$ncycle))
   evaluate ($noe_fac = ($fin_noe/$ini_noe)^(1/$ncycle))
   evaluate ($knoe = $ini_noe)
   evaluate ($nuc_fac = ($fin_nuc/$ini_nuc)^(1/$ncycle))
   evaluate ($knuc = $ini_nuc)

   evaluate ($sani_fac = ($fin_sani/$ini_sani)^(1/$ncycle))
   evaluate ($ksani = $ini_sani)
   evaluate ($ksanib = $ksani)
   evaluate ($ksanin = $ksani*1.0)
   evaluate ($ksanim = $ksani*1.0)
   evaluate ($ksaddr = $ksani*1/5)
   evaluate ($ksaddb = $ksani*1/8)



   xdip class JCH force $ksani end
   xdip class JCHB force $ksanib end
   xdip class JCNB force $ksanin end
   xdip class JCMT force $ksanim end
   xdip class JADR force $ksaddr end
   xdip class JADB force $ksaddb end
   xdip class HABS force $ksani end
   xdip class HSIG force $ksani end

   flags
       exclude *
       include bond angl impr vdw noe cdih ncs orie rama xdip plan
```

```
   end


   vector do (vx = maxwell($bath)) (all)
   vector do (vy = maxwell($bath)) (all)
   vector do (vz = maxwell($bath)) (all)

   evaluate ($i_cool = 0)
   while ($i_cool < $ncycle) loop cool
       evaluate ($i_cool=$i_cool+1)

       @reset_force_constants.inp
       {* In this file the constraints are set back to apply van der
Waals *}
       {* to all atoms. As well, as scaling the force constants for all
*}
       {* energy terms.
*}

       dynamics internal
          itype=pc6
          etol=$tol
          tbath=$bath
          response= 5
          nprint=25
          nstep=0     {* with this set to zero, endtime is the
controlling *}
                      {* variable of how long these dynamics will run.
*}
          endtime=$endtime
          timestep=  0.002
        end

    end loop cool

   {* Final minimization. *}
   dynamics internal
      itype=powell
      nstep=20000
      maxcalls=20000
      nprint=1
      etol=1e-7
      gtol=0.01
      depred=0.001
   end

   @write_output.inp

end loop structure

stop
```

"Generate template" protocol:

```
!+++++++++++++++++++++++++ Generate_template.inp ++++++++++++++++++++++++++

!      Generates a "regularised random structure" as a start structure
!      for simulated annealing. Another script (regularise.inp) is
!      designed for regularising average structures etc under restrints.

!      Remarks from the original version:

remarks  file  nmr/generate_template.inp
remarks  Generates a "template" coordinate set.  This produces
remarks  an arbitrary extended conformation with ideal geometry.
remarks
remarks  Author: Axel T. Brunger

!================================ Input ===================================

!      The only input needed are the structure and output filenames.


       structure @DNA.psf end

       evaluate ($outfile="DNA_template.pdb")


!============================================================================
=

!      Below this line nothing need be changed

!============================================================================
=


       set seed = 2917830993 end

!      The peptide bond force constant is high

       evaluate ($kpept=500)

!      Floating chirality for methylenes and floating proline isomers
!      are both irrelevant here, but values are needed for the parameter file.

       evaluate ($float=0 )
       evaluate ($cispro=0)


       parameter
          @/AK_Util/xplor-nih-2.9.9/Examples/DNA_REFI/parnah1er1_mod_new.inp
! Read parameters
!         @Topologies/BMT_ins.par
           @XP:/sani/axis.param
       end


!      Set the initial coordinates.

!      atoms are given x coordinates acording to their atom number!! Then y and
```

```
z
!      coordinates at random between 0 and 0.5.

       vector ident (x) ( all )
       vector do (x=x/10.) ( all )
       vector do (y=random(0.5) ) ( all )
       vector do (z=random(0.5) ) ( all )

       vector do (fbeta=50) (all)   ! Friction coefficient, in 1/ps.
       vector do (mass=100) (all)   ! Uniform heavy masses for all atoms

!      Non-bonded parameters

       parameter
          nbonds
             cutnb=5.5 rcon=20. nbxmod=-2 repel=0.9  wmin=0.1 tolerance=1.
             rexp=2 irexp=2 inhibit=0.25
          end
       end


!      ======================= Minimisation and dynamics ======================


              flags exclude * include bond angle vdw end

       minimize powell nstep=50  nprint=10 end

              flags include impr end

       minimize powell nstep=50 nprint=10 end

       dynamics  verlet
          nstep=50  timestep=0.001 iasvel=maxwell  firsttemp= 300.
          tcoupling = true  tbath = 300.   nprint=50  iprfrq=0
       end

              parameter
                 nbonds
                    rcon=2. nbxmod=-3 repel=0.75
                 end
              end

       minimize powell nstep=100 nprint=25 end

       dynamics  verlet
          nstep=1000  timestep=0.005 iasvel=maxwell  firsttemp= 300.
          tcoupling = true  tbath = 300.   nprint=100  iprfrq=0
       end

              flags exclude vdw elec end
              vector do (mass=1.) ( name h* )
              hbuild selection=( name h* ) phistep=360 end
              flags include vdw elec end

       minimize powell nstep=1000 nprint=50 end


!      ======================= Write output file ======================

       remarks produced by nmr/generate_template.inp
       write coordinates output=$outfile end
```

```
stop
```

"plane.inp":

```
restraints plane
 group
  select= ((resid 15 and (name n1 or name c6 or name c2)) or
              (resid 1 and (name n3)))
  weight=80.0
 end

group
  select= ((resid 2 and (name n1 or name c6 or name c2)) or
              (resid 14 and (name n3)))
  weight=50.0
 end

group
  select= ((resid 13 and (name n1 or name c6 or name c2)) or
              (resid 3 and (name n3)))
  weight=20.0
 end

group
  select= ((resid 4 and (name n1 or name c6 or name c2)) or
              (resid 12 and (name n3)))
  weight=20.0
 end

group
  select= ((resid 5 and (name n1 or name c6 or name c2)) or
              (resid 11 and (name n3)))
  weight=20.0
 end

group
  select= ((resid 10 and (name n1 or name c6 or name c2)) or
              (resid 6 and (name n3)))
  weight=20.0
 end

!group
!  select= ((resid 9 and (name n1 or name c6 or name c2)) or
!               (resid 7 and (name n3)))
!  weight=20.0
! end


group
  select= ((resid 15 and (name n1)) or
              (resid 1 and (name n3 or name c2 or name c4)))
  weight=80.0
 end
```

```
group
  select= ((resid 2 and (name n1)) or
              (resid 14 and (name n3 or name c2 or name c4)))
  weight=50.0
 end


group
  select= ((resid 13 and (name n1)) or
              (resid 3 and (name n3 or name c2 or name c4)))
  weight=20.0
 end


group
  select= ((resid 4 and (name n1)) or
              (resid 12 and (name n3 or name c2 or name c4)))
  weight=20.0
 end


group
  select= ((resid 5 and (name n1)) or
              (resid 11 and (name n3 or name c2 or name c4)))
  weight=20.0
 end


group
  select= ((resid 10 and (name n1)) or
              (resid 6 and (name n3 or name c2 or name c4)))
  weight=20.0
 end


!group
!  select= ((resid 9 and (name n1)) or
!              (resid 7 and (name n3 or name c2 or name c4)))
!  weight=20.0
! end



!planarity for end base pairs
group
  select= ((resid 15 and (name n1 or name c6 or name c2)) or
              (resid 1 and (name n3 or name c2 or name c4)))
  weight=40.0
 end



end
```

"dna_dna_pairs_03.setup"

```
{* Setting the two strands to different SEGIDs is necessary to use the
*}
{* DNA oriented pairs potential correctly. If the SEGID is the same for
*}
{* both strands (including both set to "") and the bases are numbered
in *}
{* a consecutive manner (i.e. strand 1 is residues 1 to 12 and strand 2
*}
{* is residues 13 to 24, the database will interprete residue 13 as 3'
*}
{* residue 12 and expect adjacent interactions between them. This
problem*}
{* is solved by setting the SEGIDs different.
*}


!vector identify (store9) (segid DNA1 or segid DNA2)

vector do (ustring = "") (all)
vector do (ustring = "DNA2") (segid DNA1)
vector do (ustring = "DNA1") (segid DNA2)

vector do (store4 = 0) (all)

{* Each Watson-Crick basepair is identified here. This is necessary for
the  *}
{* Oriented pairs database.
*}
vector do (store4 = 15) (resid 1)
vector do (store4 = 14) (resid 2)
vector do (store4 = 13) (resid 3)
vector do (store4 = 12) (resid 4)
vector do (store4 = 11) (resid 5)
vector do (store4 = 10) (resid 6)

vector do (store4 = 6) (resid 10)
vector do (store4 = 5) (resid 11)
vector do (store4 = 4) (resid 12)
vector do (store4 = 3) (resid 13)
vector do (store4 = 2) (resid 14)
vector do (store4 = 1) (resid 15)


set echo off message off end
orient
   nres 10000
   residues 10000
   maxgau 128
   {* shape quartic *}
   {* Both options for database refinement. In this case, gaussians
seem to *}
   {* work better. *}
   shape gaussian
```

```
   scale 0.3
   {* @DNA_DNA_PAIRS:dna_dna_quarts_combo_res23.tbl   *}

   @DNA_DNA_PAIRS:dna_gaussians.tbl
end
set echo on message on end

@DNA_DNA_PAIRS:dna_gaussians_bases_setup.tbl
{* This setup script appears to be OK for both the quartic potential
and the  *}
{* Gaussian potential. *}

!vector do (segid = "") (segid DNA1)
!vector do (segid = "") (segid DNA2)
```

"hbonds_assumed.tbl"

```
!  These restraints are derived from high-resolution X-ray structures
of the
!  individual basepairs. The use of these restraints is described in
Huang et
!  al. EMBO (2000) 19(11):2615-2628.  Two of these restraints (per
basepair)
!  are cross-hydrogen bond in order to prevent shearing of the
basepair. These
!  are O6-N3 and N2-N3 for G:C basepairs and N1-O4 and N1-O2 for A:T
basepairs.
!  ---------------------------------

!GC basepairs
assign (resid 15 and name n1) (resid 1 and name n3) 2.87 .2 .2
assign (resid 15 and name h1) (resid 1 and name n3) 1.86 0.2 0.2
assign (resid 15 and name o6) (resid 1 and name n4) 2.81 .2 .2
!assign (resid 15 and name o6) (resid 1 and name HN') 1.80 .2 .2
assign (resid 15 and name n2) (resid 1 and name o2) 2.81 .2 .2
!assign (resid 15 and name HN'') (resid 1 and name o2) 1.79 0.2 0.2
assign (resid 15 and name n2) (resid 1 and name n3) 3.58 0.2 0.2
assign (resid 15 and name o6) (resid 1 and name n3) 3.63 0.2 0.2

assign (resid 13 and name n1) (resid 3 and name n3) 2.87 .2 .2
assign (resid 13 and name h1) (resid 3 and name n3) 1.86 0.2 0.2
assign (resid 13 and name o6) (resid 3 and name n4) 2.81 .2 .2
!assign (resid 13 and name o6) (resid 3 and name HN') 1.80 .2 .2
assign (resid 13 and name n2) (resid 3 and name o2) 2.81 .2 .2
!assign (resid 13 and name HN'') (resid 3 and name o2) 1.79 0.2 0.2
assign (resid 13 and name n2) (resid 3 and name n3) 3.58 0.2 0.2
assign (resid 13 and name o6) (resid 3 and name n3) 3.63 0.2 0.2

assign (resid 5 and name n1) (resid 11 and name n3) 2.87 .2 .2
assign (resid 5 and name h1) (resid 11 and name n3) 1.86 0.2 0.2
assign (resid 5 and name o6) (resid 11 and name n4) 2.81 .2 .2
!assign (resid 5 and name o6) (resid 11 and name HN') 1.80 .2 .2
assign (resid 5 and name n2) (resid 11 and name o2) 2.81 .2 .2
!assign (resid 5 and name HN'') (resid 11 and name o2) 1.79 0.2 0.2
assign (resid 5 and name n2) (resid 11 and name n3) 3.58 0.2 0.2
assign (resid 5 and name o6) (resid 11 and name n3) 3.63 0.2 0.2

assign (resid 10 and name n1) (resid 6 and name n3) 2.87 .2 .2
assign (resid 10 and name h1) (resid 6 and name n3) 1.86 0.2 0.2
assign (resid 10 and name o6) (resid 6 and name n4) 2.81 .2 .2
!assign (resid 10 and name o6) (resid 6 and name HN') 1.80 .2 .2
assign (resid 10 and name n2) (resid 6 and name o2) 2.81 .2 .2
!assign (resid 10 and name HN'') (resid 6 and name o2) 1.79 0.2 0.2
assign (resid 10 and name n2) (resid 6 and name n3) 3.58 0.2 0.2
assign (resid 10 and name o6) (resid 6 and name n3) 3.63 0.2 0.2


!AT bastpairs
assign (resid 4 and name n1) (resid 12 and name n3) 2.92 .2 .2
assign (resid 4 and name n1) (resid 12 and name h3) 1.81 .2 .2
```

```
assign (resid 4 and name n6) (resid 12 and name o4) 2.89 .2 .2
assign (resid 4 and name HN'') (resid 12 and name o4) 1.87 0.2 0.2
assign (resid 4 and name h2) (resid 12 and name o2) 2.94 0.2 0.2
assign (resid 4 and name n1) (resid 12 and name o4) 3.69 0.2 0.2
assign (resid 4 and name n1) (resid 12 and name o2) 3.67 0.2 0.2

assign (resid 2 and name n1) (resid 14 and name n3) 2.92 .2 .2
assign (resid 2 and name n1) (resid 14 and name h3) 1.81 .2 .2
assign (resid 2 and name n6) (resid 14 and name o4) 2.89 .2 .2
assign (resid 2 and name HN'') (resid 14 and name o4) 1.87 0.2 0.2
assign (resid 2 and name h2) (resid 14 and name o2) 2.94 0.2 0.2
assign (resid 2 and name n1) (resid 14 and name o4) 3.69 0.2 0.2
assign (resid 2 and name n1) (resid 14 and name o2) 3.67 0.2 0.2


!!GC pairs additional restraints
!assign (resid 15 and name H1') (resid 1 and name H1') 9.5 .1 .1
!assign (resid 15 and name H1') (resid 1 and name O2) 7.43 .1 .1
!assign (resid 15 and name H1) (resid 1 and name H1') 5.1 .1 .1
!assign (resid 15 and name HN'') (resid 1 and name H1') 4.05 .1 .1
!assign (resid 13 and name H1') (resid 3 and name H1') 9.5 .1 .1
!assign (resid 13 and name H1') (resid 3 and name O2) 7.43 .1 .1
!assign (resid 13 and name H1) (resid 3 and name H1') 5.1 .1 .1
!assign (resid 13 and name HN'') (resid 3 and name H1') 4.05 .1 .1
!assign (resid 5 and name H1') (resid 11 and name H1') 9.5 .1 .1
!assign (resid 5 and name H1') (resid 11 and name O2) 7.43 .1 .1
!assign (resid 5 and name H1) (resid 11 and name H1') 5.1 .1 .1
!assign (resid 5 and name HN'') (resid 11 and name H1') 4.05 .1 .1
!assign (resid 10 and name H1') (resid 6 and name H1') 9.5 .1 .1
!assign (resid 10 and name H1') (resid 6 and name O2) 7.43 .1 .1
!assign (resid 10 and name H1) (resid 6 and name H1') 5.1 .1 .1
!assign (resid 10 and name HN'') (resid 6 and name H1') 4.05 .1 .1
!
!!AT pairs additional restraints
!assign (resid 14 and name H1') (resid 2 and name H1') 9.25 .1 .1
!assign (resid 14 and name H1') (resid 2 and name H2) 5.05 .1 .1
!assign (resid 14 and name H3) (resid 2 and name H1') 6.65 .1 .1
!assign (resid 14 and name O2) (resid 2 and name H1') 7.22 .1 .1
!assign (resid 14 and name H1') (resid 2 and name N1) 5.81 .1 .1
!assign (resid 12 and name H1') (resid 4 and name H1') 9.25 .1 .1
!assign (resid 12 and name H1') (resid 4 and name H2) 5.05 .1 .1
!assign (resid 12 and name H3) (resid 4 and name H1') 6.65 .1 .1
!assign (resid 12 and name O2) (resid 4 and name H1') 7.22 .1 .1
!assign (resid 12 and name H1') (resid 4 and name N1) 5.81 .1 .1
```

"reset_force_constants.inp"

```
evaluate ($bath  = $bath  - $tempstep)
evaluate ($k_vdw=min($fin_con,$k_vdw*$k_vdwfact))
evaluate ($radius=max($fin_rad,$radius*$radfact))  {* This is the radius of *}
                                                   {* atoms in the van der  *}
                                                   {* Waals constraint.     *}
evaluate ($k_ang = $k_ang*$ang_fac)
evaluate ($k_imp = $k_imp*$imp_fac)

evaluate ($knoe  = $knoe*$noe_fac)
evaluate ($knuc  = $knuc*$nuc_fac)

evaluate ($ksani  = $ksani*$sani_fac)
evaluate ($ksanib = $ksani)
evaluate ($ksanin = $ksani*1.0)
evaluate ($ksanim = $ksani*1.0)
evaluate ($ksaddr = $ksani*1/5)
evaluate ($ksaddb = $ksani*1/8)


{* reset force constants of the dipolar couplings. *}
xdip class JCH force $ksani end
xdip class JCHB force $ksanib end
xdip class JCNB force $ksanin end
xdip class JCMT force $ksanim end
xdip class JADR force $ksaddr end
xdip class JADB force $ksaddb end
xdip class HABS force $ksani end
xdip class HSIG force $ksani end



constraints
        interaction (all) (all)
        weights * 1 angles $k_ang improper $k_imp
       {* This restores the Van der Waal repulsion energy and prevents atoms *}
       {* from passing though each other during the cooling phase. It also *}
        {* changes the scaling on the angles and impropers. *}
    end
end

{* Reset van der Waals parameters. *}
parameter
        nbonds
        cutnb=4.5 rcon=$k_vdw nbxmod=4 repel=$radius
        {* nbxmod is set to 4, because we are using database *}
        {* (delphic) potentials for torsion angles.          *}
  end
end

noe
   scale * $knoe
end

rama
   scale $knuc
end
```

"DNA_NOE.tbl"

```
!Intraresidual test
!
!H1'-H2'
assign ( resid 1   and name H1'   )  ( resid 1   and name H2'   )  2.97
0.801  1.202
assign ( resid 2   and name H1'   )  ( resid 2   and name H2'   )  2.88
0.801  1.202
assign ( resid 3   and name H1'   )  ( resid 3   and name H2'   )  2.96
0.801  1.202
assign ( resid 4   and name H1'   )  ( resid 4   and name H2'   )  2.77
0.801  1.202
assign ( resid 5   and name H1'   )  ( resid 5   and name H2'   )  2.91
0.801  1.202
assign ( resid 6   and name H1'   )  ( resid 6   and name H2'   )  3.10
0.801  1.202
assign ( resid 7   and name H1'   )  ( resid 7   and name H2'   )  3.17
0.801  1.202
assign ( resid 8   and name H1'   )  ( resid 8   and name H2'   )  2.48
0.801  1.202
assign ( resid 9   and name H1'   )  ( resid 9   and name H2'   )
2.30 0.801  1.8
assign ( resid 10  and name H1'   )  ( resid 10  and name H2'   )  2.97
0.801  1.202
assign ( resid 11  and name H1'   )  ( resid 11  and name H2'   )  2.98
0.801  1.202
assign ( resid 12  and name H1'   )  ( resid 12  and name H2'   )  2.96
0.801  1.202
assign ( resid 13  and name H1'   )  ( resid 13  and name H2'   )  2.83
0.801  1.202
assign ( resid 14  and name H1'   )  ( resid 14  and name H2'   )  2.99
0.801  1.202
assign ( resid 15  and name H1'   )  ( resid 15  and name H2'   )  2.98
0.801  1.202
!H1'-H2''
assign ( resid 1   and name H1'   )  ( resid 1   and name H2''  )  2.43
0.801  1.202
assign ( resid 2   and name H1'   )  ( resid 2   and name H2''  )  2.41
0.801  1.202
assign ( resid 3   and name H1'   )  ( resid 3   and name H2''  )  2.43
0.801  1.202
assign ( resid 4   and name H1'   )  ( resid 4   and name H2''  )  2.36
0.801  1.202
assign ( resid 5   and name H1'   )  ( resid 5   and name H2''  )  2.44
0.801  1.202
assign ( resid 6   and name H1'   )  ( resid 6   and name H2''  )  2.80
0.801  1.202
assign ( resid 7   and name H1'   )  ( resid 7   and name H2''  )  2.61
0.801  1.202
assign ( resid 8   and name H1'   )  ( resid 8   and name H2''  )  3.12
0.801  1.202
assign ( resid 9   and name H1'   )  ( resid 9   and name H2''  )
2.30 0.801  1.8
assign ( resid 10  and name H1'   )  ( resid 10  and name H2''  )  2.49
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```
0.801  1.202
assign ( resid 11  and name H1'   )  ( resid 11  and name H2''  )  2.35
0.801  1.202
assign ( resid 12  and name H1'   )  ( resid 12  and name H2''  )  2.44
0.801  1.202
assign ( resid 13  and name H1'   )  ( resid 13  and name H2''  )  2.45
0.801  1.202
assign ( resid 14  and name H1'   )  ( resid 14  and name H2''  )  2.37
0.801  1.202
assign ( resid 15  and name H1'   )  ( resid 15  and name H2''  )  2.40
0.801  1.202
!H1'-H3'
assign ( resid 1   and name H1'   )  ( resid 1   and name H3'   )
4.49  2.0  1.202
assign ( resid 2   and name H1'   )  ( resid 2   and name H3'   )  3.70
0.801  1.202
assign ( resid 3   and name H1'   )  ( resid 3   and name H3'   )  3.66
0.801  1.202
assign ( resid 4   and name H1'   )  ( resid 4   and name H3'   )  3.58
0.801  1.202
assign ( resid 5   and name H1'   )  ( resid 5   and name H3'   )  3.58
0.801  1.202
assign ( resid 6   and name H1'   )  ( resid 6   and name H3'   )  3.78
0.801  1.202
assign ( resid 7   and name H1'   )  ( resid 7   and name H3'   )
3.99  2.0  1.202
assign ( resid 8   and name H1'   )  ( resid 8   and name H3'   )  3.63
0.801  1.202
assign ( resid 9   and name H1'   )  ( resid 9   and name H3'   )  3.56
0.801  1.202
assign ( resid 10  and name H1'   )  ( resid 10  and name H3'   )  3.77
0.801  1.202
assign ( resid 11  and name H1'   )  ( resid 11  and name H3'   )  3.68
0.801  1.202
assign ( resid 12  and name H1'   )  ( resid 12  and name H3'   )  3.61
0.801  1.202
assign ( resid 13  and name H1'   )  ( resid 13  and name H3'   )  3.60
0.801  1.202
assign ( resid 14  and name H1'   )  ( resid 14  and name H3'   )  3.75
0.801  1.202
assign ( resid 15  and name H1'   )  ( resid 15  and name H3'   )  3.82
0.801  1.202
!H1'-H4'
assign ( resid 1   and name H1'   )  ( resid 1   and name H4'   )  3.43
0.801  1.202
assign ( resid 2   and name H1'   )  ( resid 2   and name H4'   )  3.35
0.801  1.202
assign ( resid 3   and name H1'   )  ( resid 3   and name H4'   )  3.26
0.801  1.202
assign ( resid 4   and name H1'   )  ( resid 4   and name H4'   )  3.26
0.801  1.202
assign ( resid 5   and name H1'   )  ( resid 5   and name H4'   )  3.20
0.801  1.202
assign ( resid 6   and name H1'   )  ( resid 6   and name H4'   )  2.95
0.801  1.202
assign ( resid 7   and name H1'   )  ( resid 7   and name H4'   )  3.61
0.801  1.202
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assign ( resid 8   and name H1'   )  ( resid 8   and name H4'   )  3.45
0.801  1.202
assign ( resid 9   and name H1'   )  ( resid 9   and name H4'   )  3.50
0.801  1.202
assign ( resid 10  and name H1'   )  ( resid 10  and name H4'   )  3.28
0.801  1.202
assign ( resid 11  and name H1'   )  ( resid 11  and name H4'   )  3.33
0.801  1.202
assign ( resid 12  and name H1'   )  ( resid 12  and name H4'   )  3.56
0.801  1.202
assign ( resid 13  and name H1'   )  ( resid 13  and name H4'   )  3.38
0.801  1.202
assign ( resid 14  and name H1'   )  ( resid 14  and name H4'   )  3.26
0.801  1.202
assign ( resid 15  and name H1'   )  ( resid 15  and name H4'   )  3.55
0.801  1.202
!H1'-H5'
assign ( resid 2   and name H1'   )  ( resid 2   and name H5'   )  4.65
0.801  1.202
assign ( resid 8   and name H1'   )  ( resid 8   and name H5'   )  4.45
0.801  1.202
assign ( resid 9   and name H1'   )  ( resid 9   and name H5'   )  4.35
0.801  1.202
!H1'-H5''
assign ( resid 8   and name H1'   )  ( resid 8   and name H5''  )  4.61
0.801  1.202
assign ( resid 9   and name H1'   )  ( resid 9   and name H5''  )  4.88
0.801  1.202
!H2'-H3'
assign ( resid 1   and name H2'   )  ( resid 1   and name H3'   )
2.90 2.0    1.202
assign ( resid 2   and name H2'   )  ( resid 2   and name H3'   )  2.63
0.801  1.202
assign ( resid 3   and name H2'   )  ( resid 3   and name H3'   )  2.69
0.801  1.202
assign ( resid 4   and name H2'   )  ( resid 4   and name H3'   )  2.54
0.801  1.202
assign ( resid 5   and name H2'   )  ( resid 5   and name H3'   )  2.59
0.801  1.202
assign ( resid 6   and name H2'   )  ( resid 6   and name H3'   )  2.57
0.801  1.202
assign ( resid 7   and name H2'   )  ( resid 7   and name H3'   )  2.69
0.801  1.202
assign ( resid 8   and name H2'   )  ( resid 8   and name H3'   )  2.63
0.801  1.202
assign ( resid 9   and name H2'   )  ( resid 9   and name H3'   )
2.32 0.801  1.8
assign ( resid 10  and name H2'   )  ( resid 10  and name H3'   )  2.52
0.801  1.202
assign ( resid 11  and name H2'   )  ( resid 11  and name H3'   )  2.60
0.801  1.202
assign ( resid 12  and name H2'   )  ( resid 12  and name H3'   )  2.69
0.801  1.202
assign ( resid 13  and name H2'   )  ( resid 13  and name H3'   )  2.67
0.801  1.202
assign ( resid 14  and name H2'   )  ( resid 14  and name H3'   )  2.65
0.801  1.202
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```
assign ( resid 15  and name H2'   )  ( resid 15  and name H3'   )
3.00 2.0    1.202
!H2''-H3'
assign ( resid 1   and name H2''  )  ( resid 1   and name H3'   )
3.43 2.0    1.202
assign ( resid 2   and name H2''  )  ( resid 2   and name H3'   )  2.70
0.801  1.202
assign ( resid 3   and name H2''  )  ( resid 3   and name H3'   )  2.97
0.801  1.202
assign ( resid 4   and name H2''  )  ( resid 4   and name H3'   )  2.96
0.801  1.202
assign ( resid 5   and name H2''  )  ( resid 5   and name H3'   )  2.86
0.801  1.202
assign ( resid 6   and name H2''  )  ( resid 6   and name H3'   )  2.77
0.801  1.202
assign ( resid 7   and name H2''  )  ( resid 7   and name H3'   )  2.82
0.801  1.202
assign ( resid 8   and name H2''  )  ( resid 8   and name H3'   )  2.75
0.801  1.202
assign ( resid 9   and name H2''  )  ( resid 9   and name H3'   )
2.32 0.801  1.8
assign ( resid 10  and name H2''  )  ( resid 10  and name H3'   )  2.88
0.801  1.202
assign ( resid 11  and name H2''  )  ( resid 11  and name H3'   )  3.02
0.801  1.202
assign ( resid 12  and name H2''  )  ( resid 12  and name H3'   )  3.03
0.801  1.202
assign ( resid 13  and name H2''  )  ( resid 13  and name H3'   )  2.76
0.801  1.202
assign ( resid 14  and name H2''  )  ( resid 14  and name H3'   )  3.00
0.801  1.202
assign ( resid 15  and name H2''  )  ( resid 15  and name H3'   )
3.13 2.0    1.202
!H2'-H5'
assign ( resid 1   and name H2'   )  ( resid 1   and name H5'   )  4.44
0.801  1.202
!assign ( resid 6   and name H2'   )  ( resid 6   and name H5'   )
4.02 0.801  1.8
assign ( resid 8   and name H2'   )  ( resid 8   and name H5'   )  4.67
0.801  1.202
assign ( resid 9   and name H2'   )  ( resid 9   and name H5'   )
3.38 0.801  1.8
!H2''-H5'
!assign ( resid 6   and name H2''  )  ( resid 6   and name H5'   )
3.05 0.801  1.8
assign ( resid 8   and name H2''  )  ( resid 8   and name H5'   )  4.67
0.801  1.202
assign ( resid 9   and name H2''  )  ( resid 9   and name H5'   )
3.38 0.801  1.8
!H2''-H5''
!assign ( resid 6   and name H2''  )  ( resid 6   and name H5''  )
3.05 0.801  1.8
assign ( resid 8   and name H2''  )  ( resid 8   and name H5''  )  4.20
0.801  1.202
assign ( resid 9   and name H2''  )  ( resid 9   and name H5''  )
2.76 0.801  1.8
!H2'-H5''
```

```
assign ( resid 1   and name H2'   )  ( resid 1   and name H5''  )  4.90
0.801  1.202
!assign ( resid 6   and name H2'   )  ( resid 6   and name H5''  )
4.02 0.801  1.8
assign ( resid 8   and name H2'   )  ( resid 8   and name H5''  )  4.18
0.801  1.202
assign ( resid 9   and name H2'   )  ( resid 9   and name H5''  )
2.76 0.801  1.8
!H3'-H4'
assign ( resid 8   and name H3'   )  ( resid 8   and name H4'   )  2.76
0.801  1.202
!H3'-H5'
assign ( resid 8   and name H3'   )  ( resid 8   and name H5'   )  3.24
0.801  1.202
assign ( resid 9   and name H3'   )  ( resid 9   and name H5'   )  3.15
0.801  1.202
!H3'-H5''
assign ( resid 8   and name H3'   )  ( resid 8   and name H5''  )  2.83
0.801  1.202
assign ( resid 9   and name H3'   )  ( resid 9   and name H5''  )  3.38
0.801  1.202
!H4'-H5'
assign ( resid 8   and name H4'   )  ( resid 8   and name H5'   )  2.47
0.801  1.202
assign ( resid 9   and name H4'   )  ( resid 9   and name H5'   )  3.44
0.801  1.202
!H4'-H5''
assign ( resid 8   and name H4'   )  ( resid 8   and name H5''  )  2.41
0.801  1.202
assign ( resid 9   and name H4'   )  ( resid 9   and name H5''  )  3.86
0.801  1.202
!Interesidual test-sugar/sugar interactions
assign ( resid 3   and name H3'   )  ( resid 2   and name H1'   )  4.83
0.801  1.202
assign ( resid 7   and name H1'   )  ( resid 6   and name H1'   )  5.66
0.801  1.5
assign ( resid 2   and name H3'   )  ( resid 3   and name H5    )  5.83
0.801  1.5
assign ( resid 6   and name H5    )  ( resid 5   and name H1'   )  5.83
0.801  1.5
assign ( resid 6   and name H5    )  ( resid 6   and name H1'   )  5.50
0.801  1.0
assign ( resid 10  and name H3'   )  ( resid 9   and name H1'   )  4.76
0.801  1.0
assign ( resid 5   and name H3'   )  ( resid 4   and name H1'   )  5.97
0.801  1.5
assign ( resid 7   and name H3'   )  ( resid 6   and name H1'   )  5.34
0.801  1.0
assign ( resid 11  and name H3'   )  ( resid 10  and name H1'   )  5.12
0.801  1.0
assign ( resid 15  and name H3'   )  ( resid 14  and name H1'   )  5.57
0.801  1.5
assign ( resid 7   and name H4'   )  ( resid 6   and name H1'   )  3.75
0.801  1.0
assign ( resid 10  and name H4'   )  ( resid 9   and name H1'   )  3.37
0.801  0.5
assign ( resid 10  and name H5'   )  ( resid 9   and name H1'   )  2.52
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```
0.801  0.5
assign ( resid 10  and name H5''  )  ( resid 9   and name H1'   )  3.32
0.801  0.5
assign ( resid 5   and name H4'   )  ( resid 4   and name H1'   )  4.77
0.801  1.0
assign ( resid 11  and name H5''  )  ( resid 10  and name H1'   )  4.80
0.801  0.5
assign ( resid 11  and name H5'   )  ( resid 10  and name H1'   )  4.35
0.801  1.0
assign ( resid 5   and name H5'   )  ( resid 4   and name H1'   )  4.06
0.801  1.0
assign ( resid 13  and name H5''  )  ( resid 12  and name H1'   )  3.48
0.801  0.5
assign ( resid 13  and name H5'   )  ( resid 12  and name H1'   )  3.06
0.801  0.5
assign ( resid 2   and name H5''  )  ( resid 1   and name H1'   )  6.35
0.801  1.0
assign ( resid 2   and name H5'   )  ( resid 1   and name H1'   )  3.83
0.801  1.0
assign ( resid 4   and name H5'   )  ( resid 3   and name H1'   )  3.80
0.801  1.0
!assign ( resid 7   and name H5+   )  ( resid 7   and name H1'   )
4.37 0.801  2.5
!assign ( resid 7   and name H5+   )  ( resid 7   and name H4'   )
2.57 0.801  2.0
!assign ( resid 7   and name H5+   )  ( resid 7   and name H3'   )
3.00 0.801  2.0
!assign ( resid 6   and name H5'   )  ( resid 5   and name H1'   )
3.09 0.801  2.5
!assign ( resid 6   and name H5''  )  ( resid 5   and name H1'   )
3.09 0.801  2.5
assign ( resid 8   and name H5''  )  ( resid 7   and name H1'   )  5.09
0.801  1.0
assign ( resid 8   and name H5'   )  ( resid 7   and name H1'   )  3.57
0.801  0.5
assign ( resid 9   and name H2'   )  ( resid 10  and name H1'   )  4.39
0.801  2.0
assign ( resid 9   and name H2''  )  ( resid 10  and name H1'   )  4.39
0.801  2.0
assign ( resid 4   and name H2''  )  ( resid 5   and name H1'   )  5.21
0.801  1.5
assign ( resid 13  and name H2'   )  ( resid 12  and name H1'   )  5.27
0.801  1.0
assign ( resid 10  and name H2'   )  ( resid 9   and name H1'   )  4.51
0.801  1.0
assign ( resid 10  and name H2''  )  ( resid 9   and name H1'   )  5.24
0.801  1.0
assign ( resid 3   and name H2''  )  ( resid 2   and name H1'   )  4.59
0.801  1.0
assign ( resid 3   and name H2'   )  ( resid 2   and name H1'   )  4.34
0.801  1.0
assign ( resid 8   and name H4'   )  ( resid 9   and name H1'   )  5.28
0.801  1.0
assign ( resid 6   and name H2''  )  ( resid 7   and name H1'   )  4.47
0.801  1.0
assign ( resid 6   and name H2'   )  ( resid 7   and name H1'   )  5.92
0.801  1.5
```

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assign ( resid 11  and name H2'   )  ( resid 10  and name H1'   )  4.64
0.801  1.0
assign ( resid 14  and name H2'   )  ( resid 15  and name H1'   )  5.50
0.801  1.5
assign ( resid 6   and name H1'   )  ( resid 5   and name H1'   )  4.71
0.801  1.0
assign ( resid 7   and name H4'   )  ( resid 8   and name H5''  )  4.32
0.801  0.5
assign ( resid 7   and name H4'   )  ( resid 8   and name H5'   )  3.68
0.801  1.5
assign ( resid 4   and name H4'   )  ( resid 3   and name H2'   )  4.27
0.801  1.0
assign ( resid 4   and name H4'   )  ( resid 3   and name H2''  )  4.84
0.801  1.0
assign ( resid 10  and name H5'   )  ( resid 9   and name H2'   )  3.25
0.801  2.0
assign ( resid 10  and name H5'   )  ( resid 9   and name H2''  )  3.25
0.801  2.0
assign ( resid 7   and name H5+   )  ( resid 7   and name H2'   )  3.43
0.801  2.0
assign ( resid 2   and name H5'   )  ( resid 1   and name H2'   )  4.79
0.801  1.0
assign ( resid 2   and name H5'   )  ( resid 1   and name H2''  )  3.35
0.801  0.5
!Interesidual test-base/sugar interactions
assign ( resid 2   and name H2''  )  ( resid 3   and name H5    )  3.41
0.801  0.5
assign ( resid 2   and name H2'   )  ( resid 3   and name H5    )  3.67
0.801  1.0
assign ( resid 10  and name H2'   )  ( resid 11  and name H5    )  3.63
0.801  0.5
assign ( resid 10  and name H2''  )  ( resid 11  and name H5    )  2.89
0.801  0.5
assign ( resid 3   and name H2''  )  ( resid 3   and name H5    )  4.98
0.801  1.0
assign ( resid 3   and name H2'   )  ( resid 3   and name H5    )  3.59
0.801  1.0
assign ( resid 11  and name H2'   )  ( resid 11  and name H5    )  4.02
0.801  1.0
assign ( resid 1   and name H2'   )  ( resid 1   and name H5    )  3.95
0.801  1.0
assign ( resid 12  and name H5    )  ( resid 11  and name H5    )  3.43
0.801  0.5
assign ( resid 12  and name H5    )  ( resid 11  and name H1'   )  3.90
0.801  1.0
assign ( resid 12  and name H5    )  ( resid 12  and name H1'   )  4.97
0.801  1.0
assign ( resid 14  and name H5    )  ( resid 13  and name H3'   )  5.45
0.801  1.5
assign ( resid 14  and name H5    )  ( resid 14  and name H1'   )  5.00
0.801  1.0
assign ( resid 14  and name H5    )  ( resid 13  and name H1'   )  4.27
0.801  1.0
assign ( resid 6   and name H5    )  ( resid 6   and name H2''  )  5.31
0.801  1.0
assign ( resid 6   and name H5    )  ( resid 6   and name H2'   )  4.20
0.801  1.0
```

```
assign ( resid 11  and name H5    )  ( resid 10  and name H1'   )  3.53
0.801  0.5
assign ( resid 11  and name H5    )  ( resid 11  and name H1'   )  6.03
0.801  1.5
assign ( resid 3   and name H5    )  ( resid 2   and name H1'   )  4.96
0.801  1.0
assign ( resid 3   and name H1'   )  ( resid 2   and name H1'   )  4.28
0.801  1.0
assign ( resid 10  and name H1'   )  ( resid 9   and name H1'   )  4.17
0.801  1.0
assign ( resid 10  and name H1'   )  ( resid 11  and name H1'   )  4.28
0.801  1.0
assign ( resid 1   and name H1'   )  ( resid 1   and name H5    )  5.16
0.801  1.0
assign ( resid 6   and name H2'   )  ( resid 6   and name H6    )  2.10
0.801  1.202
assign ( resid 6   and name H2''  )  ( resid 6   and name H6    )  2.65
0.801  1.202
assign ( resid 5   and name H2'   )  ( resid 6   and name H6    )  2.74
0.801  1.202
assign ( resid 5   and name H2''  )  ( resid 6   and name H6    )  2.62
0.801  1.202
assign ( resid 13  and name H2''  )  ( resid 14  and name H6    )  2.75
0.801  1.202
assign ( resid 13  and name H2'   )  ( resid 14  and name H6    )  3.14
0.801  1.202
assign ( resid 14  and name H2''  )  ( resid 14  and name H6    )  2.86
0.801  1.202
assign ( resid 14  and name H2'   )  ( resid 14  and name H6    )  2.22
0.801  1.202
assign ( resid 14  and name H5    )  ( resid 14  and name H6    )  2.47
0.801  1.202
assign ( resid 12  and name H5    )  ( resid 12  and name H6    )  2.48
0.801  1.202
assign ( resid 3   and name H2'   )  ( resid 3   and name H6    )  2.25
0.801  1.202
assign ( resid 3   and name H2''  )  ( resid 3   and name H6    )  2.86
0.801  1.202
assign ( resid 2   and name H2'   )  ( resid 3   and name H6    )  3.19
0.801  1.202
assign ( resid 2   and name H2''  )  ( resid 3   and name H6    )  2.67
0.801  1.202
assign ( resid 10  and name H2'   )  ( resid 11  and name H6    )  3.26
0.801  1.202
assign ( resid 11  and name H2'   )  ( resid 11  and name H6    )  2.28
0.801  1.202
assign ( resid 12  and name H5    )  ( resid 11  and name H6    )  2.28
0.801  1.202
assign ( resid 1   and name H2'   )  ( resid 1   and name H6    )  2.48
0.801  1.202
assign ( resid 1   and name H2''  )  ( resid 1   and name H6    )  3.47
0.801  1.202
assign ( resid 13  and name H2''  )  ( resid 13  and name H8    )  3.21
0.801  1.202
assign ( resid 12  and name H2''  )  ( resid 13  and name H8    )  3.00
0.801  1.202
assign ( resid 12  and name H2'   )  ( resid 13  and name H8    )  3.29
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0.801  1.202
assign ( resid 14  and name H2'   )  ( resid 15  and name H8    )  3.44
0.801  1.202
assign ( resid 14  and name H5    )  ( resid 13  and name H8    )  3.01
0.801  1.202
!Interesidual test-resid7 aromatic interactions
assign ( resid 6   and name H2'   )  ( resid 7   and name H8    )  3.36
0.801  1.202
assign ( resid 6   and name H2''  )  ( resid 7   and name H8    )  2.97
0.801  1.202
assign ( resid 7   and name H2''  )  ( resid 7   and name H8    )  3.50
0.801  1.202
!Interesidual test-resid9 aromatic interactions
!assign ( resid 8   and name H2''  )  ( resid 9   and name H8    )
5.52 0.801  1.5
!assign ( resid 8   and name H2'   )  ( resid 9   and name H8    )
5.71 0.801  1.5
!Interesidual test-resid10 aromatic interactions
assign ( resid 10  and name H2''  )  ( resid 10  and name H8    )  2.85
0.801  1.202
!Interesidual test-resid4 aromatic interactions
assign ( resid 3   and name H2'   )  ( resid 4   and name H8    )  3.24
0.801  1.202
assign ( resid 3   and name H2''  )  ( resid 4   and name H8    )  2.96
0.801  1.202
assign ( resid 4   and name H2'   )  ( resid 4   and name H8    )  2.24
0.801  1.202
assign ( resid 4   and name H2''  )  ( resid 4   and name H8    )  2.96
0.801  1.202
!Interesidual test-resid2 aromatic interactions
assign ( resid 2   and name H2''  )  ( resid 2   and name H8    )  2.94
0.801  1.202
assign ( resid 2   and name H2'   )  ( resid 2   and name H8    )  2.33
0.801  1.202
assign ( resid 1   and name H2''  )  ( resid 2   and name H8    )  3.16
0.801  1.202
assign ( resid 1   and name H2'   )  ( resid 2   and name H8    )  3.24
0.801  1.202
!Interesidual test-resid6 aromatic interactions
assign ( resid 6   and name H1'   )  ( resid 6   and name H6    )  3.76
0.801  1.202
assign ( resid 5   and name H1'   )  ( resid 6   and name H6    )  4.04
0.801  1.0
!assign ( resid 6   and name H5    )  ( resid 6   and name H6    )
2.64 0.801  1.202
assign ( resid 5   and name H3'   )  ( resid 6   and name H6    )  3.73
0.801  1.202
assign ( resid 6   and name H3'   )  ( resid 6   and name H6    )  3.25
0.801  1.202
assign ( resid 5   and name H4'   )  ( resid 6   and name H6    )  6.74
0.801  2.0
assign ( resid 6   and name H4'   )  ( resid 6   and name H6    )  4.77
0.801  1.0
!assign (( resid 6   and name H5+) or ( resid 7   and name H5+)    )  (
resid 6   and name H6    )  3.66 0.801  1.8
!Interesidual test-resid14 aromatic interactions
assign ( resid 13  and name H1'   )  ( resid 14  and name H6    )  3.69
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0.801  1.0
assign ( resid 14  and name H1'   )  ( resid 14  and name H6    )  3.65
0.801  1.0
assign ( resid 13  and name H3'   )  ( resid 14  and name H6    )  4.06
0.801  1.0
assign ( resid 14  and name H3'   )  ( resid 14  and name H6    )  3.35
0.801  1.202
!Interesidual test-resid3 aromatic interactions
assign ( resid 2   and name H1'   )  ( resid 3   and name H3    )  3.60
0.801  1.202
assign ( resid 3   and name H1'   )  ( resid 3   and name H3    )  3.67
0.801  1.202
assign ( resid 3   and name H5    )  ( resid 3   and name H3    )  2.69
0.801  1.202
assign ( resid 2   and name H3'   )  ( resid 3   and name H3    )  4.43
0.801  1.0
!Interesidual test-resid12 aromatic interactions
assign ( resid 11  and name H1'   )  ( resid 12  and name H6    )  4.11
0.801  1.0
assign ( resid 12  and name H1'   )  ( resid 12  and name H6    )  3.63
0.801  1.202
assign ( resid 12  and name H3'   )  ( resid 12  and name H6    )  3.34
0.801  1.202
!Interesidual test-resid15 aromatic interactions
assign ( resid 15  and name H1'   )  ( resid 15  and name H8    )  4.04
0.801  1.0
assign ( resid 14  and name H3'   )  ( resid 15  and name H8    )  4.69
0.801  1.0
assign ( resid 15  and name H3'   )  ( resid 15  and name H8    )  3.78
0.801  1.202
!Interesidual test-resid9 aromatic interactions
assign ( resid 9   and name H1'   )  ( resid 9   and name H8    )  2.92
0.801  1.202
!assign ( resid 8   and name H1'   )  ( resid 9   and name H8    )
4.75 0.801  1.0
assign ( resid 9   and name H3'   )  ( resid 9   and name H8    )  3.83
0.801  1.0
assign ( resid 9   and name H4'   )  ( resid 9   and name H8    )  5.24
0.801  1.0
assign ( resid 10  and name H5'   )  ( resid 9   and name H8    )  4.62
0.801  1.0
assign ( resid 10  and name H5''  )  ( resid 9   and name H8    )  6.32
0.801  1.5
assign ( resid 9   and name H5'   )  ( resid 9   and name H8    )  4.38
0.801  1.0
assign ( resid 9   and name H5''  )  ( resid 9   and name H8    )  3.35
0.801  1.0
assign ( resid 8   and name H5''  )  ( resid 9   and name H8    )  5.10
0.801  1.0
assign ( resid 8   and name H5'   )  ( resid 9   and name H8    )  4.64
0.801  1.0
assign ( resid 8   and name H4'   )  ( resid 9   and name H8    )  3.81
0.801  1.0
!Interesidual test-resid7 aromatic interactions
assign ( resid 6   and name H1'   )  ( resid 7   and name H8    )  4.93
0.801  1.0
assign ( resid 6   and name H6    )  ( resid 7   and name H8    )  4.94
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0.801  1.0
assign ( resid 7   and name H1'   )  ( resid 7   and name H8    )  3.80
0.801  1.0
assign ( resid 7   and name H3'   )  ( resid 7   and name H8    )  3.69
0.801  1.0
assign ( resid 6   and name H3'   )  ( resid 7   and name H8    )  4.84
0.801  1.0
!assign ( resid 7   and name H5+   )  ( resid 7   and name H8    )
3.94 0.801  1.8
!Interesidual test-resid10 aromatic interactions
assign ( resid 11  and name H6    )  ( resid 10  and name H8    )  5.07
0.801  1.202
assign ( resid 10  and name H1'   )  ( resid 10  and name H8    )  4.30
0.801  1.0
assign ( resid 11  and name H5    )  ( resid 10  and name H8    )  4.64
0.801  1.0
assign ( resid 10  and name H3'   )  ( resid 10  and name H8    )  3.99
0.801  1.0
!Interesidual test-resid8 aromatic interactions
assign ( resid 7   and name H2'   )  ( resid 8   and name H8    )  3.14
0.801  1.202
assign ( resid 7   and name H2''  )  ( resid 8   and name H8    )  2.67
0.801  1.202
assign ( resid 8   and name H2''  )  ( resid 8   and name H8    )  3.47
0.801  1.202
assign ( resid 8   and name H2'   )  ( resid 8   and name H8    )  2.82
0.801  1.202
assign ( resid 8   and name H1'   )  ( resid 8   and name H8    )  3.52
0.801  1.202
assign ( resid 7   and name H1'   )  ( resid 8   and name H8    )  3.29
0.801  1.202
assign ( resid 7   and name H3'   )  ( resid 8   and name H8    )  4.38
0.801  1.0
assign ( resid 8   and name H3'   )  ( resid 8   and name H8    )  4.50
0.801  1.0
assign ( resid 7   and name H4'   )  ( resid 9   and name H2    )  5.00
0.801  1.5
assign ( resid 8   and name H4'   )  ( resid 8   and name H8    )  4.43
0.801  1.0
assign ( resid 8   and name H5''  )  ( resid 8   and name H8    )  4.36
0.801  1.202
assign ( resid 8   and name H5'   )  ( resid 8   and name H8    )  4.01
0.801  1.202
!Interesidual test-resid5 aromatic interactions
assign ( resid 6   and name H6    )  ( resid 5   and name H8    )  4.63
0.801  1.0
assign ( resid 4   and name H1'   )  ( resid 5   and name H8    )  3.57
0.801  1.202
assign ( resid 5   and name H1'   )  ( resid 5   and name H8    )  3.91
0.801  1.0
assign ( resid 4   and name H3'   )  ( resid 5   and name H8    )  4.30
0.801  1.0
assign ( resid 6   and name H5    )  ( resid 5   and name H8    )  3.53
0.801  1.202
assign ( resid 5   and name H3'   )  ( resid 5   and name H8    )  3.49
0.801  1.202
assign ( resid 5   and name H4'   )  ( resid 5   and name H8    )  4.41
```

```
0.801  1.0
assign ( resid 5   and name H5''  )  ( resid 5   and name H8    )  4.59
0.801  1.0
assign ( resid 5   and name H5'   )  ( resid 5   and name H8    )  4.01
0.801  1.0
!Interesidual test-resid11 aromatic interactions
assign ( resid 12  and name H6    )  ( resid 11  and name H6    )  4.24
0.801  1.0
assign ( resid 11  and name H1'   )  ( resid 11  and name H6    )  3.42
0.801  1.202
assign ( resid 10  and name H1'   )  ( resid 11  and name H6    )  3.09
0.801  1.202
assign ( resid 11  and name H5    )  ( resid 11  and name H6    )  2.62
0.801  1.202
assign ( resid 10  and name H3'   )  ( resid 11  and name H6    )  3.97
0.801  1.202
assign ( resid 11  and name H3'   )  ( resid 11  and name H6    )  3.40
0.801  1.202
assign ( resid 10  and name H4'   )  ( resid 11  and name H6    )  5.27
0.801  1.0
assign ( resid 11  and name H4'   )  ( resid 11  and name H6    )  4.02
0.801  1.0
assign ( resid 11  and name H5''  )  ( resid 11  and name H6    )  3.63
0.801  1.202
assign ( resid 11  and name H5'   )  ( resid 11  and name H6    )  3.40
0.801  1.202
!Interesidual test-resid1 aromatic interactions
assign ( resid 1   and name H5    )  ( resid 1   and name H6    )  2.83
0.801  1.202
assign ( resid 1   and name H1'   )  ( resid 1   and name H6    )  3.50
0.801  1.202
assign ( resid 1   and name H3'   )  ( resid 1   and name H6    )  3.57
0.801  1.202
assign ( resid 1   and name H4'   )  ( resid 1   and name H6    )  4.26
0.801  1.0
assign ( resid 1   and name H5''  )  ( resid 1   and name H6    )  3.67
0.801  1.202
assign ( resid 1   and name H5'   )  ( resid 1   and name H6    )  3.60
0.801  1.202
!Interesidual test-resid13 aromatic interactions
assign ( resid 13  and name H1'   )  ( resid 13  and name H8    )  3.91
0.801  1.0
assign ( resid 12  and name H1'   )  ( resid 13  and name H8    )  4.15
0.801  1.0
assign ( resid 13  and name H3'   )  ( resid 13  and name H8    )  3.67
0.801  1.202
assign ( resid 12  and name H1'   )  ( resid 13  and name H8    )  4.16
0.801  1.0
assign ( resid 13  and name H4'   )  ( resid 13  and name H8    )  5.15
0.801  1.0
!Interesidual test-resid4 aromatic interactions
assign ( resid 4   and name H1'   )  ( resid 4   and name H8    )  3.91
0.801  1.0
assign ( resid 3   and name H1'   )  ( resid 4   and name H8    )  4.24
0.801  1.0
assign ( resid 4   and name H3'   )  ( resid 4   and name H8    )  3.55
0.801  1.202
```

```
assign ( resid 3   and name H3'   )  ( resid 4   and name H8    )  4.15
0.801  1.0
assign ( resid 4   and name H4'   )  ( resid 4   and name H8    )  5.01
0.801  1.0
assign ( resid 4   and name H5''  )  ( resid 4   and name H8    )  4.08
0.801  1.202
assign ( resid 4   and name H5'   )  ( resid 4   and name H8    )  3.92
0.801  1.202
!Interesidual test-resid2 aromatic interactions
assign ( resid 3   and name H6    )  ( resid 2   and name H8    )  4.52
0.801  1.0
assign ( resid 2   and name H1'   )  ( resid 2   and name H8    )  4.04
0.801  1.0
assign ( resid 1   and name H1'   )  ( resid 2   and name H8    )  5.00
0.801  1.0
assign ( resid 3   and name H5    )  ( resid 2   and name H8    )  4.29
0.801  1.0
assign ( resid 2   and name H3'   )  ( resid 2   and name H8    )  3.81
0.801  1.0
assign ( resid 1   and name H3'   )  ( resid 2   and name H8    )  4.31
0.801  1.0
assign ( resid 2   and name H4'   )  ( resid 2   and name H8    )  5.03
0.801  1.0
assign ( resid 2   and name H5''  )  ( resid 2   and name H8    )  4.25
0.801  1.0
assign ( resid 2   and name H5'   )  ( resid 2   and name H8    )  4.28
0.801  1.0
assign ( resid 1   and name H4'   )  ( resid 2   and name H8    )  6.41
0.801  1.5
!Interesidual test-attempts
assign ( resid 7   and name HN'   )  ( resid 9   and name HN''  )  2.60
0.801  1.0
assign ( resid 7   and name HN'   )  ( resid 9   and name H8    )  3.20
0.801  1.0
```

Appendix 2

"pydu.par":

```
nonbonded C10              0.0903       3.43273      0.0903       3.43273
nonbonded H13              0.0498       2.13273      0.0498       2.13273
nonbonded N13              0.1592       2.73273      0.1592       2.73273
nonbonded C16              0.0903       3.43273      0.0903       3.43273
nonbonded C14              0.0903       3.43273      0.0903       3.43273
nonbonded N12              0.1592       2.73273      0.1592       2.73273
nonbonded H11              0.0498       2.13273      0.0498       2.13273
nonbonded O10              0.2342       2.63273      0.2342       2.63273
nonbonded O15              0.2342       2.63273      0.2342       2.63273
nonbonded H18              0.0498       2.13273      0.0498       2.13273
nonbonded O19              0.2342       2.63273      0.2342       2.63273
nonbonded P12              0.1    3.53273      0.1    3.53273
nonbonded O13              0.2342       2.63273      0.2342       2.63273
```

"plane.inp":

```
restraints plane
 group
  select= ((resid 15 and (name n1 or name c6 or name c2)) or
              (resid 1 and (name n3)))
  weight=80.0
 end

group
  select= ((resid 2 and (name n1 or name c6 or name c2)) or
             (resid 14 and (name n3)))
  weight=50.0
 end

!group
!  select= ((resid 13 and (name n1 or name c6 or name c2)) or
!              (resid 3 and (name n3)))
!  weight=20.0
! end

!group
!  select= ((resid 4 and (name n1 or name c6 or name c2)) or
!              (resid 12 and (name n3)))
!  weight=20.0
! end

group
  select= ((resid 5 and (name n1 or name c6 or name c2)) or
             (resid 11 and (name n3)))
  weight=20.0
 end

group
  select= ((resid 10 and (name n1 or name c6 or name c2)) or
             (resid 6 and (name n3)))
  weight=20.0
```

```
 end

!group
!  select= ((resid 9 and (name n1 or name c6 or name c2)) or
!              (resid 7 and (name n3)))
!  weight=20.0
! end


group
  select= ((resid 15 and (name n1)) or
              (resid 1 and (name n3 or name c2 or name c4)))
  weight=80.0
 end


group
  select= ((resid 2 and (name n1)) or
              (resid 14 and (name n3 or name c2 or name c4)))
  weight=50.0
 end


!group
!  select= ((resid 13 and (name n1)) or
!              (resid 3 and (name n3 or name c2 or name c4)))
!  weight=20.0
! end


!group
!  select= ((resid 4 and (name n1)) or
!              (resid 12 and (name n3 or name c2 or name c4)))
!  weight=20.0
! end


group
  select= ((resid 5 and (name n1)) or
              (resid 11 and (name n3 or name c2 or name c4)))
  weight=20.0
 end

group
  select= ((resid 10 and (name n1)) or
              (resid 6 and (name n3 or name c2 or name c4)))
  weight=20.0
 end

!group
!  select= ((resid 9 and (name n1)) or
!              (resid 7 and (name n3 or name c2 or name c4)))
!  weight=20.0
! end



!planarity for end base pairs
group
  select= ((resid 15 and (name n1 or name c6 or name c2)) or
              (resid 1 and (name n3 or name c2 or name c4)))
  weight=40.0
 end
```

"dna_dna_pairs_03.setup":

```
{* Setting the two strands to different SEGIDs is necessary to use the   *}
{* DNA oriented pairs potential correctly. If the SEGID is the same for  *}
{* both strands (including both set to "") and the bases are numbered in *}
{* a consecutive manner (i.e. strand 1 is residues 1 to 12 and strand 2  *}
{* is residues 13 to 24, the database will interprete residue 13 as 3'   *}
{* residue 12 and expect adjacent interactions between them. This problem*}
{* is solved by setting the SEGIDs different.                            *}


!vector identify (store9) (segid DNA1 or segid DNA2)

vector do (ustring = "") (all)
vector do (ustring = "DNA2") (segid DNA1)
vector do (ustring = "DNA1") (segid DNA2)

vector do (store4 = 0) (all)

{* Each Watson-Crick basepair is identified here. This is necessary for the  *}
{* Oriented pairs database.                                                  *}
vector do (store4 = 15) (resid 1)
vector do (store4 = 14) (resid 2)
!vector do (store4 = 13) (resid 3)
!vector do (store4 = 12) (resid 4)
vector do (store4 = 11) (resid 5)
vector do (store4 = 10) (resid 6)

vector do (store4 = 6) (resid 10)
vector do (store4 = 5) (resid 11)
!vector do (store4 = 4) (resid 12)
!vector do (store4 = 3) (resid 13)
vector do (store4 = 2) (resid 14)
vector do (store4 = 1) (resid 15)


set echo off message off end
orient
   nres 10000
   residues 10000
   maxgau 128
   {* shape quartic *}
   {* Both options for database refinement. In this case, gaussians seem to *}
   {* work better. *}
   shape gaussian
   scale 0.3
   {* @DNA_DNA_PAIRS:dna_dna_quarts_combo_res23.tbl   *}

   @DNA_DNA_PAIRS:dna_gaussians.tbl
end
set echo on message on end

@DNA_DNA_PAIRS:dna_gaussians_bases_setup.tbl
{* This setup script appears to be OK for both the quartic potential and the
*}
{* Gaussian potential. *}

!vector do (segid = "") (segid DNA1)
!vector do (segid = "") (segid DNA2)
```

"hbonds_assumed.tbl":

```
!  These restraints are derived from high-resolution X-ray structures of the
!  individual basepairs. The use of these restraints is described in Huang et
!  al. EMBO (2000) 19(11):2615-2628.  Two of these restraints (per basepair)
!  are cross-hydrogen bond in order to prevent shearing of the basepair. These
!  are O6-N3 and N2-N3 for G:C basepairs and N1-O4 and N1-O2 for A:T basepairs.
!  ----------------------------------

!GC basepairs
assign (resid 15 and name n1) (resid 1 and name n3) 2.87 .2 .2
assign (resid 15 and name h1) (resid 1 and name n3) 1.86 0.2 0.2
assign (resid 15 and name o6) (resid 1 and name n4) 2.81 .2 .2
!assign (resid 15 and name o6) (resid 1 and name HN') 1.80 .2 .2
assign (resid 15 and name n2) (resid 1 and name o2) 2.81 .2 .2
!assign (resid 15 and name HN'') (resid 1 and name o2) 1.79 0.2 0.2
assign (resid 15 and name n2) (resid 1 and name n3) 3.58 0.2 0.2
assign (resid 15 and name o6) (resid 1 and name n3) 3.63 0.2 0.2

!assign (resid 13 and name n1) (resid 3 and name n3) 2.87 .2 .2
!assign (resid 13 and name h1) (resid 3 and name n3) 1.86 0.2 0.2
!assign (resid 13 and name o6) (resid 3 and name n4) 2.81 .2 .2
!!assign (resid 13 and name o6) (resid 3 and name HN') 1.80 .2 .2
!assign (resid 13 and name n2) (resid 3 and name o2) 2.81 .2 .2
!!assign (resid 13 and name HN'') (resid 3 and name o2) 1.79 0.2 0.2
!assign (resid 13 and name n2) (resid 3 and name n3) 3.58 0.2 0.2
!assign (resid 13 and name o6) (resid 3 and name n3) 3.63 0.2 0.2

assign (resid 5 and name n1) (resid 11 and name n3) 2.87 .2 .2
assign (resid 5 and name h1) (resid 11 and name n3) 1.86 0.2 0.2
assign (resid 5 and name o6) (resid 11 and name n4) 2.81 .2 .2
!assign (resid 5 and name o6) (resid 11 and name HN') 1.80 .2 .2
assign (resid 5 and name n2) (resid 11 and name o2) 2.81 .2 .2
!assign (resid 5 and name HN'') (resid 11 and name o2) 1.79 0.2 0.2
assign (resid 5 and name n2) (resid 11 and name n3) 3.58 0.2 0.2
assign (resid 5 and name o6) (resid 11 and name n3) 3.63 0.2 0.2

assign (resid 10 and name n1) (resid 6 and name n3) 2.87 .2 .2
assign (resid 10 and name h1) (resid 6 and name n3) 1.86 0.2 0.2
assign (resid 10 and name o6) (resid 6 and name n4) 2.81 .2 .2
!assign (resid 10 and name o6) (resid 6 and name HN') 1.80 .2 .2
assign (resid 10 and name n2) (resid 6 and name o2) 2.81 .2 .2
!assign (resid 10 and name HN'') (resid 6 and name o2) 1.79 0.2 0.2
assign (resid 10 and name n2) (resid 6 and name n3) 3.58 0.2 0.2
assign (resid 10 and name o6) (resid 6 and name n3) 3.63 0.2 0.2

!!AT bastpairs
!assign (resid 4 and name n1) (resid 12 and name n3) 2.92 .2 .2
!assign (resid 4 and name n1) (resid 12 and name h3) 1.81 .2 .2
!assign (resid 4 and name n6) (resid 12 and name o4) 2.89 .2 .2
!assign (resid 4 and name HN'') (resid 12 and name o4) 1.87 0.2 0.2
!assign (resid 4 and name h2) (resid 12 and name o2) 2.94 0.2 0.2
!assign (resid 4 and name n1) (resid 12 and name o4) 3.69 0.2 0.2
!assign (resid 4 and name n1) (resid 12 and name o2) 3.67 0.2 0.2

assign (resid 2 and name n1) (resid 14 and name n3) 2.92 .2 .2
assign (resid 2 and name n1) (resid 14 and name h3) 1.81 .2 .2
assign (resid 2 and name n6) (resid 14 and name o4) 2.89 .2 .2
assign (resid 2 and name HN'') (resid 14 and name o4) 1.87 0.2 0.2
assign (resid 2 and name h2) (resid 14 and name o2) 2.94 0.2 0.2
assign (resid 2 and name n1) (resid 14 and name o4) 3.69 0.2 0.2
assign (resid 2 and name n1) (resid 14 and name o2) 3.67 0.2 0.2
```

"DNA_NOE.tbl":

```
!Intraresidual test
!
!H1'-H2'
assign ( resid 1   and name H1'   )  ( resid 1   and name H2'   )  3.20
0.801  1.202
assign ( resid 2   and name H1'   )  ( resid 2   and name H2'   )   2.34
0.801  1.8
assign ( resid 4   and name H1'   )  ( resid 4   and name H2'   )   2.57
0.801  1.8
assign ( resid 5   and name H1'   )  ( resid 5   and name H2'   )  3.20
0.801  1.202
assign ( resid 6   and name H1'   )  ( resid 6   and name H2'   )  2.86
0.801  1.202
assign ( resid 7   and name H1'   )  ( resid 7   and name H2'   )  2.75
0.801  1.202
assign ( resid 8   and name H1'   )  ( resid 8   and name H2'   )  3.11
0.801  1.202
assign ( resid 9   and name H1'   )  ( resid 9   and name H2'   )  3.04
0.801  1.202
assign ( resid 10  and name H1'   )  ( resid 10  and name H2'   )  2.62
0.801  1.202
assign ( resid 11  and name H1'   )  ( resid 11  and name H2'   )  2.94
0.801  1.202
assign ( resid 12  and name H1'   )  ( resid 12  and name H2'   )   2.25
0.801  1.8
assign ( resid 13  and name H1'   )  ( resid 13  and name H2'   )  2.81
0.801  1.202
assign ( resid 14  and name H1'   )  ( resid 14  and name H2'   )  2.81
0.801  1.202
assign ( resid 15  and name H1'   )  ( resid 15  and name H2'   )  2.92
0.801  1.202
!H1'-H2''
assign ( resid 1   and name H1'   )  ( resid 1   and name H2''  )  2.69
0.801  1.202
assign ( resid 2   and name H1'   )  ( resid 2   and name H2''  )   2.34
0.801  1.8
assign ( resid 4   and name H1'   )  ( resid 4   and name H2''  )   2.58
0.801  1.8
assign ( resid 5   and name H1'   )  ( resid 5   and name H2''  )  2.85
0.801  1.202
assign ( resid 6   and name H1'   )  ( resid 6   and name H2''  )  2.36
0.801  1.202
assign ( resid 7   and name H1'   )  ( resid 7   and name H2''  )  2.34
0.801  1.202
assign ( resid 8   and name H1'   )  ( resid 8   and name H2''  )  2.40
0.801  1.202
assign ( resid 9   and name H1'   )  ( resid 9   and name H2''  )  2.65
0.801  1.202
assign ( resid 10  and name H1'   )  ( resid 10  and name H2''  )  2.44
0.801  1.202
assign ( resid 11  and name H1'   )  ( resid 11  and name H2''  )  2.83
0.801  1.202
assign ( resid 12  and name H1'   )  ( resid 12  and name H2''  )   2.25
0.801  1.8
assign ( resid 13  and name H1'   )  ( resid 13  and name H2''  )  2.46
0.801  1.202
assign ( resid 14  and name H1'   )  ( resid 14  and name H2''  )  2.40
```

```
0.801  1.202
assign ( resid 15  and name H1'   )  ( resid 15  and name H2''  )  2.39
0.801  1.202
!H1'-H3'
assign ( resid 1   and name H1'   )  ( resid 1   and name H3'   )   5.16
2.0  1.202
assign ( resid 2   and name H1'   )  ( resid 2   and name H3'   )  3.85
0.801  1.202
assign ( resid 4   and name H1'   )  ( resid 4   and name H3'   )  3.87
0.801  1.202
assign ( resid 5   and name H1'   )  ( resid 5   and name H3'   )  3.31
0.801  1.202
assign ( resid 6   and name H1'   )  ( resid 6   and name H3'   )  3.81
0.801  1.202
assign ( resid 7   and name H1'   )  ( resid 7   and name H3'   )  3.83
0.801  1.202
assign ( resid 8   and name H1'   )  ( resid 8   and name H3'   )  3.83
0.801  1.202
assign ( resid 9   and name H1'   )  ( resid 9   and name H3'   )  3.33
0.801  1.202
assign ( resid 10  and name H1'   )  ( resid 10  and name H3'   )  3.68
0.801  1.202
assign ( resid 11  and name H1'   )  ( resid 11  and name H3'   )   4.95
2.0  1.202
assign ( resid 12  and name H1'   )  ( resid 12  and name H3'   )  3.57
0.801  1.202
assign ( resid 13  and name H1'   )  ( resid 13  and name H3'   )  3.52
0.801  1.202
assign ( resid 14  and name H1'   )  ( resid 14  and name H3'   )  3.81
0.801  1.202
assign ( resid 15  and name H1'   )  ( resid 15  and name H3'   )  3.87
0.801  1.202
!H1'-H4'
assign ( resid 1   and name H1'   )  ( resid 1   and name H4'   )  3.29
0.801  1.202
assign ( resid 2   and name H1'   )  ( resid 2   and name H4'   )  3.09
0.801  1.202
assign ( resid 4   and name H1'   )  ( resid 4   and name H4'   )   4.12
2.0  1.202
assign ( resid 5   and name H1'   )  ( resid 5   and name H4'   )  3.72
0.801  1.202
assign ( resid 6   and name H1'   )  ( resid 6   and name H4'   )  2.83
0.801  1.202
assign ( resid 7   and name H1'   )  ( resid 7   and name H4'   )  3.05
0.801  1.202
assign ( resid 8   and name H1'   )  ( resid 8   and name H4'   )  3.26
0.801  1.202
assign ( resid 9   and name H1'   )  ( resid 9   and name H4'   )  2.98
0.801  1.202
assign ( resid 11  and name H1'   )  ( resid 11  and name H4'   )  3.08
0.801  1.202
assign ( resid 12  and name H1'   )  ( resid 12  and name H4'   )  3.05
0.801  1.202
assign ( resid 13  and name H1'   )  ( resid 13  and name H4'   )  3.10
0.801  1.202
assign ( resid 14  and name H1'   )  ( resid 14  and name H4'   )  3.21
0.801  1.202
assign ( resid 15  and name H1'   )  ( resid 15  and name H4'   )  3.25
0.801  1.202
!H1'-H5'
```

```
assign ( resid 4   and name H1'   )  ( resid 4   and name H5'   )  4.31
0.801  1.202
assign ( resid 6   and name H1'   )  ( resid 6   and name H5'   )   2.40
3.2  1.202
assign ( resid 8   and name H1'   )  ( resid 8   and name H5'   )  3.09
0.801  1.202
assign ( resid 9   and name H1'   )  ( resid 9   and name H5'   )  4.08
0.801  1.202
assign ( resid 12  and name H1'   )  ( resid 12  and name H5'   )   4.95
2.0  1.202
!H1'-H5''
assign ( resid 4   and name H1'   )  ( resid 4   and name H5''  )  5.51
0.801  2.202
assign ( resid 6   and name H1'   )  ( resid 6   and name H5''  )   2.40
3.2  2.202
assign ( resid 8   and name H1'   )  ( resid 8   and name H5''  )  3.11
0.801  2.202
assign ( resid 9   and name H1'   )  ( resid 9   and name H5''  )  4.26
0.801  2.202
assign ( resid 12  and name H1'   )  ( resid 12  and name H5''  )   4.95
2.0  1.202
!H2'-H3'
assign ( resid 1   and name H2'   )  ( resid 1   and name H3'   )  2.58
0.801  1.202
assign ( resid 2   and name H2'   )  ( resid 2   and name H3'   )   2.37
0.801  1.8
assign ( resid 4   and name H2'   )  ( resid 4   and name H3'   )   2.29
0.801  1.8
assign ( resid 5   and name H2'   )  ( resid 5   and name H3'   )  2.93
0.801  1.202
assign ( resid 6   and name H2'   )  ( resid 6   and name H3'   )  2.53
0.801  1.202
assign ( resid 7   and name H2'   )  ( resid 7   and name H3'   )  2.34
0.801  1.202
assign ( resid 8   and name H2'   )  ( resid 8   and name H3'   )  2.68
0.801  1.202
assign ( resid 9   and name H2'   )  ( resid 9   and name H3'   )  2.59
0.801  1.202
assign ( resid 10  and name H2'   )  ( resid 10  and name H3'   )  2.48
0.801  1.202
assign ( resid 11  and name H2'   )  ( resid 11  and name H3'   )  3.07
0.801  1.202
assign ( resid 12  and name H2'   )  ( resid 12  and name H3'   )   2.50
0.801  1.8
assign ( resid 13  and name H2'   )  ( resid 13  and name H3'   )  2.70
0.801  1.202
assign ( resid 14  and name H2'   )  ( resid 14  and name H3'   )  2.71
0.801  1.202
assign ( resid 15  and name H2'   )  ( resid 15  and name H3'   )  2.97 2.0
1.202
!H2''-H3'
assign ( resid 1   and name H2''  )  ( resid 1   and name H3'   )  2.68
0.801  1.202
assign ( resid 2   and name H2''  )  ( resid 2   and name H3'   )   2.37
0.801  1.8
assign ( resid 4   and name H2''  )  ( resid 4   and name H3'   )   2.29
0.801  1.8
assign ( resid 5   and name H2''  )  ( resid 5   and name H3'   )  2.99
0.801  1.202
assign ( resid 6   and name H2''  )  ( resid 6   and name H3'   )  2.78
```

```
0.801  1.202
assign ( resid 7   and name H2''  )  ( resid 7   and name H3'   )  2.46
0.801  1.202
assign ( resid 8   and name H2''  )  ( resid 8   and name H3'   )  3.00
0.801  1.202
assign ( resid 9   and name H2''  )  ( resid 9   and name H3'   )   3.94
1.2   1.8
assign ( resid 10  and name H2''  )  ( resid 10  and name H3'   )  2.57
0.801  1.202
assign ( resid 11  and name H2''  )  ( resid 11  and name H3'   )  3.30
0.801  1.202
assign ( resid 12  and name H2''  )  ( resid 12  and name H3'   )   2.50
0.801  1.8
assign ( resid 13  and name H2''  )  ( resid 13  and name H3'   )  2.77
0.801  1.202
assign ( resid 14  and name H2''  )  ( resid 14  and name H3'   )  2.78
0.801  1.202
assign ( resid 15  and name H2''  )  ( resid 15  and name H3'   )  3.48
1.2   1.202
!H2'-H4'
assign ( resid 2   and name H2'   )  ( resid 2   and name H4'   )   3.65
0.801  1.8
assign ( resid 4   and name H2'   )  ( resid 4   and name H4'   )   4.71
1.2  1.8
assign ( resid 5   and name H2'   )  ( resid 5   and name H4'   )  3.88
0.801  1.202
assign ( resid 6   and name H2'   )  ( resid 6   and name H4'   )  3.84
0.801  1.202
assign ( resid 7   and name H2'   )  ( resid 7   and name H4'   )  3.56
0.801  1.202
assign ( resid 9   and name H2'   )  ( resid 9   and name H4'   )  3.86
0.801  1.202
assign ( resid 11  and name H2'   )  ( resid 11  and name H4'   )  4.12
0.801  1.202
assign ( resid 12  and name H2'   )  ( resid 12  and name H4'   )   2.93
0.801  1.8
assign ( resid 13  and name H2'   )  ( resid 13  and name H4'   )  3.56
0.801  1.202
assign ( resid 14  and name H2'   )  ( resid 14  and name H4'   )  3.53
0.801  1.202
assign ( resid 15  and name H2'   )  ( resid 15  and name H4'   )  3.70
0.801  1.202
!H2''-H4'
assign ( resid 2   and name H2''  )  ( resid 2   and name H4'   )   3.65
0.801  1.8
assign ( resid 4   and name H2''  )  ( resid 4   and name H4'   )   4.71
1.2  1.8
assign ( resid 5   and name H2''  )  ( resid 5   and name H4'   )  4.03
0.801  1.202
assign ( resid 6   and name H2''  )  ( resid 6   and name H4'   )  4.01
0.801  1.202
assign ( resid 7   and name H2''  )  ( resid 7   and name H4'   )  3.84
0.801  1.202
assign ( resid 9   and name H2''  )  ( resid 9   and name H4'   )  3.85
0.801  1.202
assign ( resid 11  and name H2''  )  ( resid 11  and name H4'   )  4.32
0.801  1.202
assign ( resid 12  and name H2''  )  ( resid 12  and name H4'   )   2.93
0.801  1.8
assign ( resid 13  and name H2''  )  ( resid 13  and name H4'   )  3.87
```

```
0.801  1.202
assign ( resid 14  and name H2''  )  ( resid 14  and name H4'   )  3.67
0.801  1.202
assign ( resid 15  and name H2''  )  ( resid 15  and name H4'   )  3.89
0.801  1.202
!H2'-H5'
assign ( resid 4   and name H2'   )  ( resid 4   and name H5'   )   3.59
0.801  1.8
assign ( resid 6   and name H2'   )  ( resid 6   and name H5'   )   3.04
0.801  1.8
assign ( resid 8   and name H2'   )  ( resid 8   and name H5'   )  3.92
0.801  1.202
assign ( resid 9   and name H2'   )  ( resid 9   and name H5'   )   3.11
0.801  1.8
assign ( resid 10  and name H2'   )  ( resid 10  and name H5'   )  3.86
0.801  1.202
assign ( resid 13  and name H2'   )  ( resid 13  and name H5'   )  3.97
0.801  1.202
!H2''-H5'
assign ( resid 1   and name H2''  )  ( resid 1   and name H5'   )  5.03
0.801  1.202
assign ( resid 4   and name H2''  )  ( resid 4   and name H5'   )   3.59
0.801  1.8
assign ( resid 5   and name H2''  )  ( resid 5   and name H5'   )  4.82
0.801  1.202
assign ( resid 6   and name H2''  )  ( resid 6   and name H5'   )   2.98
0.801  2.8
assign ( resid 8   and name H2''  )  ( resid 8   and name H5'   )  4.65
0.801  1.202
assign ( resid 9   and name H2''  )  ( resid 9   and name H5'   )   2.95
0.801  2.8
assign ( resid 9   and name H2''  )  ( resid 9   and name H5'   )   3.96
0.801  1.8
!H2''-H5'
assign ( resid 4   and name H2''  )  ( resid 4   and name H5'   )   3.35
0.801  2.8
assign ( resid 5   and name H2''  )  ( resid 5   and name H5'   )  4.61
0.801  1.202
assign ( resid 6   and name H2''  )  ( resid 6   and name H5'   )   2.98
0.801  2.8
assign ( resid 8   and name H2''  )  ( resid 8   and name H5'   )   3.94
0.801  1.8
assign ( resid 9   and name H2''  )  ( resid 9   and name H5'   )   3.41
0.801  1.5
!H2'-H5''
assign ( resid 4   and name H2'   )  ( resid 4   and name H5''  )   3.35
0.801  2.8
assign ( resid 6   and name H2'   )  ( resid 6   and name H5''  )   3.04
0.801  1.8
assign ( resid 8   and name H2'   )  ( resid 8   and name H5''  )   3.15
0.801  1.8
assign ( resid 9   and name H2'   )  ( resid 9   and name H5''  )  3.56
0.801  1.202
assign ( resid 10  and name H2'   )  ( resid 10  and name H5''  )  3.45
0.801  1.202
assign ( resid 13  and name H2'   )  ( resid 13  and name H5''  )   3.11
0.801  1.8
!H3'-H4'
assign ( resid 2   and name H3'   )  ( resid 2   and name H4'   )  2.87
0.801  1.202
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assign ( resid 4   and name H3'   )  ( resid 4   and name H4'   )  3.57
0.801  1.202
assign ( resid 5   and name H3'   )  ( resid 5   and name H4'   )  3.09
0.801  1.202
assign ( resid 6   and name H3'   )  ( resid 6   and name H4'   )  2.99
0.801  1.202
assign ( resid 7   and name H3'   )  ( resid 7   and name H4'   )  2.75
0.801  1.202
assign ( resid 8   and name H3'   )  ( resid 8   and name H4'   )  2.48
0.801  1.202
assign ( resid 9   and name H3'   )  ( resid 9   and name H4'   )  3.00
0.801  1.202
assign ( resid 11  and name H3'   )  ( resid 11  and name H4'   )  3.04
0.801  1.202
assign ( resid 12  and name H3'   )  ( resid 12  and name H4'   )  2.86
0.801  1.202
assign ( resid 13  and name H3'   )  ( resid 13  and name H4'   )  2.80
0.801  1.202
assign ( resid 14  and name H3'   )  ( resid 14  and name H4'   )  2.80
0.801  1.202
assign ( resid 15  and name H3'   )  ( resid 15  and name H4'   )  3.07
0.801  1.202
!H3'-H5'
assign ( resid 1   and name H3'   )  ( resid 1   and name H5'   )   4.79
2.0   1.202
assign ( resid 2   and name H3'   )  ( resid 2   and name H5'   )  3.04
0.801  1.202
assign ( resid 4   and name H3'   )  ( resid 4   and name H5'   )  3.29
0.801  1.202
assign ( resid 5   and name H3'   )  ( resid 5   and name H5'   )  3.21
0.801  1.202
assign ( resid 6   and name H3'   )  ( resid 6   and name H5'   )   2.54
0.801  1.8
assign ( resid 8   and name H3'   )  ( resid 8   and name H5'   )   2.98
0.801  1.503
assign ( resid 9   and name H3'   )  ( resid 9   and name H5'   )   2.60
0.801  1.503
assign ( resid 10  and name H3'   )  ( resid 10  and name H5'   )   2.77
0.801  1.503
assign ( resid 11  and name H3'   )  ( resid 11  and name H5'   )   2.48
0.801  1.503
assign ( resid 13  and name H3'   )  ( resid 13  and name H5'   )   2.57
0.801  1.503
assign ( resid 15  and name H3'   )  ( resid 15  and name H5'   )  3.24
0.801  1.202
!H3'-H5''
assign ( resid 1   and name H3'   )  ( resid 1   and name H5''  )   3.67
2.0   1.202
assign ( resid 2   and name H3'   )  ( resid 2   and name H5''  )  2.67
0.801  1.202
assign ( resid 4   and name H3'   )  ( resid 4   and name H5''  )  2.74
0.801  1.202
assign ( resid 5   and name H3'   )  ( resid 5   and name H5''  )  2.98
0.801  1.202
assign ( resid 6   and name H3'   )  ( resid 6   and name H5''  )   2.54
0.801  1.8
assign ( resid 8   and name H3'   )  ( resid 8   and name H5''  )  2.72
0.801  1.202
assign ( resid 9   and name H3'   )  ( resid 9   and name H5''  )  2.46
0.801  1.202
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assign ( resid 10  and name H3'   )  ( resid 10  and name H5''  )  2.66
0.801  1.202
assign ( resid 11  and name H3'   )  ( resid 11  and name H5''  )  2.39
0.801  1.202
assign ( resid 13  and name H3'   )  ( resid 13  and name H5''  )  2.45
0.801  1.202
assign ( resid 15  and name H3'   )  ( resid 15  and name H5''  )  2.87
0.801  1.202
!H4'-H5'
assign ( resid 1   and name H4'   )  ( resid 8   and name H5'   )  2.82
0.801  1.202
assign ( resid 2   and name H4'   )  ( resid 2   and name H5'   )  2.46
0.801  1.202
assign ( resid 4   and name H4'   )  ( resid 4   and name H5'   )  3.06
0.801  1.202
assign ( resid 5   and name H4'   )  ( resid 5   and name H5'   )  2.66
0.801  1.202
assign ( resid 6   and name H4'   )  ( resid 6   and name H5'   )   2.02
0.801  1.8
assign ( resid 8   and name H4'   )  ( resid 8   and name H5'   )  3.03
0.801  1.202
assign ( resid 9   and name H4'   )  ( resid 9   and name H5'   )  2.63
0.801  1.202
assign ( resid 11  and name H4'   )  ( resid 11  and name H5'   )  2.58
0.801  1.202
assign ( resid 13  and name H4'   )  ( resid 13  and name H5'   )  2.49
0.801  1.202
!H4'-H5''
assign ( resid 1   and name H4'   )  ( resid 8   and name H5''  )  2.73
0.801  1.202
assign ( resid 2   and name H4'   )  ( resid 2   and name H5''  )  2.51
0.801  1.202
assign ( resid 4   and name H4'   )  ( resid 4   and name H5''  )  2.80
0.801  1.202
assign ( resid 5   and name H4'   )  ( resid 5   and name H5''  )  2.43
0.801  1.202
assign ( resid 6   and name H4'   )  ( resid 6   and name H5''  )   2.02
0.801  1.8
assign ( resid 8   and name H4'   )  ( resid 8   and name H5''  )  2.89
0.801  1.202
assign ( resid 9   and name H4'   )  ( resid 9   and name H5''  )  2.48
0.801  1.202
assign ( resid 11  and name H4'   )  ( resid 11  and name H5''  )  2.47
0.801  1.202
assign ( resid 13  and name H4'   )  ( resid 13  and name H5''  )  2.41
0.801  1.202
!Interesidual test
assign ( resid 10  and name H1'   )  ( resid 9   and name H1'   )  4.56
0.803  2.202
assign ( resid 7   and name H3'   )  ( resid 6   and name H1'   )  6.25
0.803  2.202
assign ( resid 10  and name H3'   )  ( resid 9   and name H1'   )  4.71
0.803  2.202
assign ( resid 7   and name H4'   )  ( resid 6   and name H1'   )  3.53
0.802  1.702
assign ( resid 9   and name H4'   )  ( resid 10  and name H1'   )  3.15
0.802  1.702
assign ( resid 10  and name H5'   )  ( resid 9   and name H1'   )  2.47
0.801  1.202
assign ( resid 10  and name H5''  )  ( resid 9   and name H1'   )  3.09
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0.802  1.702
assign ( resid 14  and name H5'   )  ( resid 14  and name H1'   )   3.02
0.802  2.502
assign ( resid 14  and name H5''  )  ( resid 14  and name H1'   )   3.02
0.802  2.502
assign ( resid 6   and name H5'   )  ( resid 5   and name H1'   )   3.95
0.802  2.502
assign ( resid 6   and name H5''  )  ( resid 5   and name H1'   )   3.95
0.802  2.502
assign ( resid 11  and name H5'   )  ( resid 10  and name H1'   )  5.06
0.803  2.202
assign ( resid 11  and name H5''  )  ( resid 10  and name H1'   )  5.17
0.803  2.202
assign ( resid 8   and name H5'   )  ( resid 7   and name H1'   )  3.17
0.802  1.702
assign ( resid 8   and name H5''  )  ( resid 7   and name H1'   )  5.18
0.803  2.202
assign ( resid 8   and name H5''  )  ( resid 7   and name H3'   )  6.99
0.803  2.202
assign ( resid 8   and name H5'   )  ( resid 7   and name H4'   )  3.89
0.802  1.702
assign ( resid 8   and name H5''  )  ( resid 7   and name H4'   )  4.33
0.803  2.202
assign ( resid 9   and name H2''  )  ( resid 10  and name H1'   )  4.64
0.803  2.202
assign ( resid 10  and name H2'   )  ( resid 11  and name H5    )  3.58
0.802  1.702
assign ( resid 10  and name H2''  )  ( resid 11  and name H5    )  3.33
0.802  1.702
assign ( resid 8   and name H2'   )  ( resid 7   and name H1'   )  4.06
0.802  1.702
assign ( resid 8   and name H2''  )  ( resid 7   and name H1'   )  4.66
0.803  2.202
assign ( resid 6   and name H2'   )  ( resid 7   and name H1'   )  4.72
0.803  2.202
assign ( resid 6   and name H2''  )  ( resid 7   and name H1'   )  3.99
0.802  1.702
assign ( resid 6   and name H2'   )  ( resid 6   and name H5    )  3.57
0.802  1.702
assign ( resid 6   and name H2''  )  ( resid 6   and name H5    )  4.30
0.803  2.202
assign ( resid 6   and name H2'   )  ( resid 7   and name H3'   )  4.89
0.803  2.202
assign ( resid 6   and name H2''  )  ( resid 7   and name H3'   )  4.02
0.802  1.702
assign ( resid 14  and name H5    )  ( resid 13  and name H1'   )  3.51
0.802  1.702
assign ( resid 4   and name H2'   )  ( resid 5   and name H3'   )  3.91
0.802  2.502
assign ( resid 4   and name H2''  )  ( resid 5   and name H3'   )  3.91
0.802  2.502
assign ( resid 4   and name H2'   )  ( resid 5   and name H4'   )   4.40
0.803  4.402
assign ( resid 4   and name H2''  )  ( resid 5   and name H4'   )   4.40
0.803  4.402
assign ( resid 5   and name H2'   )  ( resid 6   and name H5    )  3.81
0.802  1.702
assign ( resid 5   and name H2''  )  ( resid 6   and name H5    )  3.67
0.802  1.702
assign ( resid 6   and name H1'   )  ( resid 7   and name H1'   )  3.93
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0.802  1.702
!C1
assign ( resid 1   and name H1'   )  ( resid 1   and name H6    )  3.07
0.802  1.702
assign ( resid 1   and name H1'   )  ( resid 2   and name H8    )  3.08
0.802  1.702
assign ( resid 1   and name H2'   )  ( resid 1   and name H6    )  2.53
0.801  1.202
assign ( resid 1   and name H2''  )  ( resid 1   and name H6    )  3.46
0.802  1.702
assign ( resid 1   and name H2'   )  ( resid 2   and name H8    )  3.64
0.802  1.702
assign ( resid 1   and name H2''  )  ( resid 2   and name H8    )  3.83
0.802  1.702
assign ( resid 1   and name H3'   )  ( resid 1   and name H6    )  6.91
0.803  2.202
assign ( resid 1   and name H3'   )  ( resid 2   and name H8    )  5.73
0.803  2.202
assign ( resid 1   and name H4'   )  ( resid 1   and name H6    )  6.06
0.803  2.202
assign ( resid 1   and name H5'   )  ( resid 1   and name H6    )  3.75
0.802  1.702
assign ( resid 1   and name H5''  )  ( resid 1   and name H6    )  3.70
0.802  1.702
!A2
assign ( resid 2   and name H1'   )  ( resid 2   and name H8    )  3.82
0.802  1.702
assign ( resid 2   and name H2'   )  ( resid 2   and name H8    )  2.27
0.801  2.502
assign ( resid 2   and name H2''  )  ( resid 2   and name H8    )  2.27
0.801  2.502
assign ( resid 2   and name H3'   )  ( resid 2   and name H8    )  3.57
0.802  1.702
assign ( resid 2   and name H4'   )  ( resid 2   and name H8    )  5.16
0.803  2.202
assign ( resid 2   and name H5'   )  ( resid 2   and name H8    )  4.13
0.803  2.202
assign ( resid 2   and name H5''  )  ( resid 2   and name H8    )  4.38
0.803  2.202
!A4
assign ( resid 4   and name H1'   )  ( resid 4   and name H8    )  4.77
0.803  2.202
assign ( resid 4   and name H2'   )  ( resid 4   and name H8    )  2.76
0.801  2.502
assign ( resid 4   and name H2''  )  ( resid 4   and name H8    )  2.76
0.801  2.502
assign ( resid 4   and name H2'   )  ( resid 5   and name H8    )  3.73
0.802  2.702
assign ( resid 4   and name H2''  )  ( resid 5   and name H8    )  3.73
0.802  2.702
assign ( resid 4   and name H3'   )  ( resid 4   and name H8    )  4.24
0.803  2.202
assign ( resid 4   and name H3'   )  ( resid 5   and name H8    )   3.34
0.803  3.202
assign ( resid 4   and name H4'   )  ( resid 5   and name H8    )  5.63
0.803  2.202
assign ( resid 4   and name H5'   )  ( resid 12  and name H11   )  4.11
0.803  2.202
assign ( resid 4   and name H5''  )  ( resid 12  and name H11   )  4.00
0.802  1.702
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assign ( resid 4   and name H5'   )  ( resid 12  and name H14   )  4.29
0.803  2.202
assign ( resid 4   and name H5''  )  ( resid 12  and name H14   )  4.79
0.803  2.202
assign ( resid 4   and name H5'   )  ( resid 12  and name H15   )  3.65
0.802  1.702
assign ( resid 4   and name H5''  )  ( resid 12  and name H15   )  3.91
0.802  1.702
assign ( resid 4   and name H5'   )  ( resid 4   and name H8    )  5.11
0.803  2.202
assign ( resid 4   and name H5''  )  ( resid 4   and name H8    )  5.72
0.803  2.202
assign ( resid 4   and name H8    )  ( resid 11  and name H3'   )  4.72
0.803  2.202
!G5
assign ( resid 5   and name H1'   )  ( resid 5   and name H8    )  4.02
0.802  1.702
assign ( resid 5   and name H1'   )  ( resid 6   and name H6    )  4.29
0.803  2.202
assign ( resid 5   and name H2'   )  ( resid 5   and name H8    )  3.20
0.802  2.702
assign ( resid 5   and name H2''  )  ( resid 5   and name H8    )  3.48
0.802  1.702
assign ( resid 5   and name H2'   )  ( resid 6   and name H6    )  3.42
0.802  1.702
assign ( resid 5   and name H2''  )  ( resid 6   and name H6    )  3.01
0.802  1.702
assign ( resid 5   and name H3'   )  ( resid 5   and name H8    )  3.56
0.802  1.702
assign ( resid 5   and name H3'   )  ( resid 6   and name H6    )  3.99
0.802  1.702
assign ( resid 5   and name H4'   )  ( resid 5   and name H8    )  4.49
0.803  2.202
assign ( resid 5   and name H4'   )  ( resid 6   and name H6    )  5.68
0.803  2.202
assign ( resid 5   and name H5'   )  ( resid 5   and name H8    )  4.07
0.803  2.202
assign ( resid 5   and name H5''  )  ( resid 5   and name H8    )   4.36
0.803  3.202
assign ( resid 5   and name H5''  )  ( resid 6   and name H6    )  5.08
0.803  2.202
assign ( resid 5   and name H8    )  ( resid 6   and name H5    )   3.50
0.801  2.702
assign ( resid 5   and name H8    )  ( resid 6   and name H5    )  4.55
0.803  2.302
!C6
assign ( resid 6   and name H1'   )  ( resid 6   and name H6    )  3.44
0.802  1.702
assign ( resid 6   and name H2'   )  ( resid 6   and name H6    )  2.31
0.801  1.202
assign ( resid 6   and name H2''  )  ( resid 6   and name H6    )  2.54
0.801  1.202
assign ( resid 6   and name H2''  )  ( resid 7   and name H2'   )  3.33
0.802  1.702
assign ( resid 6   and name H2''  )  ( resid 7   and name H2''  )  3.32
0.802  1.702
assign ( resid 6   and name H2'   )  ( resid 7   and name H2''  )  4.95
0.803  2.202
assign ( resid 6   and name H2''  )  ( resid 7   and name H4'   )  3.93
0.802  1.702
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assign ( resid 6   and name H2'   )  ( resid 7   and name H8    )  3.27
0.802  1.702
assign ( resid 6   and name H2''  )  ( resid 7   and name H8    )  3.02
0.802  1.702
assign ( resid 6   and name H3'   )  ( resid 6   and name H6    )  3.29
0.802  1.702
assign ( resid 6   and name H3'   )  ( resid 7   and name H8    )  4.77
0.803  2.202
assign ( resid 6   and name H4'   )  ( resid 6   and name H6    )  4.45
0.803  2.202
assign ( resid 6   and name H5    )  ( resid 5   and name H8    )   3.35
0.802  2.702
assign ( resid 6   and name H5'   )  ( resid 6   and name H6    )   3.49
0.802  2.502
assign ( resid 6   and name H5''  )  ( resid 6   and name H6    )   3.49
0.802  2.502
assign ( resid 6   and name H5'   )  ( resid 7   and name H3'   )   2.38
0.801  2.202
assign ( resid 6   and name H5''  )  ( resid 7   and name H3'   )   2.38
0.801  2.202
assign ( resid 6   and name H5'   )  ( resid 7   and name H2'   )  3.31
0.802  2.502
assign ( resid 6   and name H5''  )  ( resid 7   and name H2'   )  3.31
0.802  2.502
assign ( resid 6   and name H5'   )  ( resid 7   and name H2'   )  3.46
0.802  2.502
assign ( resid 6   and name H5'   )  ( resid 7   and name H2''  )  3.46
0.802  2.502
assign ( resid 6   and name H5'   )  ( resid 7   and name H4'   )   2.31
0.801  2.202
assign ( resid 6   and name H5''  )  ( resid 7   and name H4'   )   2.31
0.801  2.202
assign ( resid 6   and name H5'   )  ( resid 7   and name H8    )  5.07
0.803  2.203
assign ( resid 6   and name H5''  )  ( resid 7   and name H8    )  5.07
0.803  2.203
assign ( resid 6   and name H6    )  ( resid 7   and name H8    )  4.18
0.803  3.203
!G7
assign ( resid 7   and name H1'   )  ( resid 7   and name H8    )  3.91
0.802  1.202
assign ( resid 7   and name H1'   )  ( resid 8   and name H8    )  3.07
0.802  1.202
assign ( resid 7   and name H2'   )  ( resid 7   and name H8    )  2.14
0.801  1.201
assign ( resid 7   and name H2''  )  ( resid 7   and name H8    )  2.80
0.802  1.202
assign ( resid 7   and name H2'   )  ( resid 8   and name H8    )  3.01
0.802  1.702
assign ( resid 7   and name H2''  )  ( resid 8   and name H8    )  2.95
0.802  1.702
assign ( resid 7   and name H3'   )  ( resid 7   and name H8    )  3.50
0.802  1.702
assign ( resid 7   and name H3'   )  ( resid 8   and name H8    )  4.01
0.802  1.702
assign ( resid 7   and name H4'   )  ( resid 7   and name H8    )  4.87
0.803  2.202
assign ( resid 7   and name H4'   )  ( resid 8   and name H8    )  4.69
0.803  2.202
!A8
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assign ( resid 8   and name H1'   )  ( resid 8   and name H8    )  3.93
0.802  1.202
assign ( resid 8   and name H1'   )  ( resid 9   and name H8    )  3.59
0.802  1.202
assign ( resid 8   and name H2'   )  ( resid 8   and name H8    )   2.48
0.801  2.501
assign ( resid 8   and name H2''  )  ( resid 8   and name H8    )  2.87
0.802  1.702
assign ( resid 8   and name H2'   )  ( resid 9   and name H5'   )  3.77
0.802  1.702
assign ( resid 8   and name H2'   )  ( resid 9   and name H5''  )  3.74
0.802  1.702
assign ( resid 8   and name H2''  )  ( resid 9   and name H5'   )  5.57
0.803  2.203
assign ( resid 8   and name H2''  )  ( resid 9   and name H5''  )  5.91
0.803  2.203
assign ( resid 8   and name H2'   )  ( resid 9   and name H8    )  4.93
0.803  2.203
assign ( resid 8   and name H2''  )  ( resid 9   and name H8    )  5.23
0.803  2.203
assign ( resid 8   and name H3'   )  ( resid 8   and name H8    )  2.89
0.802  1.702
assign ( resid 8   and name H3'   )  ( resid 9   and name H8    )  3.89
0.803  2.203
assign ( resid 8   and name H4'   )  ( resid 8   and name H8    )   2.61
0.801  2.501
assign ( resid 8   and name H4'   )  ( resid 9   and name H5'   )  4.20
0.803  2.203
assign ( resid 8   and name H4'   )  ( resid 9   and name H5''  )  7.77
0.803  2.203
assign ( resid 8   and name H5'   )  ( resid 7   and name H2'   )  4.59
0.803  2.203
assign ( resid 8   and name H5'   )  ( resid 7   and name H2''  )  4.32
0.803  2.203
assign ( resid 8   and name H5''  )  ( resid 7   and name H2'   )  4.91
0.803  2.203
assign ( resid 8   and name H5''  )  ( resid 7   and name H2''  )  4.81
0.803  2.203
assign ( resid 8   and name H5'   )  ( resid 8   and name H8    )  3.94
0.802  1.702
assign ( resid 8   and name H5''  )  ( resid 8   and name H8    )  3.67
0.802  1.702
assign ( resid 8   and name H5''  )  ( resid 9   and name H8    )  6.06
0.803  2.203
!A9
assign ( resid 9   and name H1'   )  ( resid 9   and name H8    )  3.14
0.802  1.702
assign ( resid 9   and name H1'   )  ( resid 10  and name H8    )  4.06
0.803  2.203
assign ( resid 9   and name H2'   )  ( resid 9   and name H8    )  2.78
0.802  1.702
assign ( resid 9   and name H2''  )  ( resid 9   and name H8    )  2.50
0.802  1.702
assign ( resid 9   and name H2'   )  ( resid 10  and name H5'   )  4.23
0.803  2.203
assign ( resid 9   and name H2'   )  ( resid 10  and name H5''  )  6.30
0.803  2.203
assign ( resid 9   and name H2''  )  ( resid 10  and name H5'   )  2.79
0.802  1.702
assign ( resid 9   and name H2''  )  ( resid 10  and name H5''  )  3.63
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0.802  1.702
assign ( resid 9   and name H2'   )  ( resid 10  and name H8    )  3.78
0.802  1.702
assign ( resid 9   and name H2''  )  ( resid 10  and name H8    )  3.06
0.802  1.702
assign ( resid 9   and name H3'   )  ( resid 9   and name H8    )  3.47
0.802  1.702
assign ( resid 9   and name H3'   )  ( resid 10  and name H8    )  4.50
0.803  2.203
assign ( resid 9   and name H4'   )  ( resid 9   and name H8    )  4.48
0.803  2.203
assign ( resid 9   and name H4'   )  ( resid 10  and name H2'   )  4.22
0.803  2.203
assign ( resid 9   and name H4'   )  ( resid 10  and name H2''  )  3.53
0.802  1.702
assign ( resid 9   and name H4'   )  ( resid 10  and name H3'   )  2.82
0.802  1.702
assign ( resid 9   and name H4'   )  ( resid 10  and name H5'   )  2.46
0.801  1.201
assign ( resid 9   and name H4'   )  ( resid 10  and name H5''  )  2.55
0.801  1.201
assign ( resid 9   and name H4'   )  ( resid 10  and name H8    )  5.01
0.803  2.203
assign ( resid 9   and name H5'   )  ( resid 8   and name H5'   )  5.51
0.803  2.203
assign ( resid 9   and name H5'   )  ( resid 8   and name H5''  )  4.14
0.803  2.203
assign ( resid 9   and name H5''  )  ( resid 8   and name H5'   )  6.05
0.803  2.203
assign ( resid 9   and name H5''  )  ( resid 8   and name H5''  )  4.64
0.803  2.203
assign ( resid 9   and name H5'   )  ( resid 9   and name H8    )  3.53
0.802  1.702
assign ( resid 9   and name H5''  )  ( resid 9   and name H8    )  4.57
0.803  2.203
assign ( resid 9   and name H8    )  ( resid 10  and name H1'   )  5.12
0.803  2.203
!G10
assign ( resid 10   and name H1'   )  ( resid 10  and name H8    )  3.86
0.802  1.702
assign ( resid 10   and name H1'   )  ( resid 11  and name H6    )  3.38
0.802  1.702
assign ( resid 10   and name H2'   )  ( resid 10  and name H8    )  2.45
0.801  1.201
assign ( resid 10   and name H2''  )  ( resid 10  and name H8    )  2.92
0.802  1.702
assign ( resid 10   and name H2'   )  ( resid 11  and name H6    )  4.53
0.803  2.203
assign ( resid 10   and name H2''  )  ( resid 11  and name H6    )  2.50
0.802  1.702
assign ( resid 10   and name H3'   )  ( resid 10  and name H8    )  3.58
0.802  1.702
assign ( resid 10   and name H3'   )  ( resid 11  and name H6    )  4.56
0.803  2.203
assign ( resid 10   and name H5'   )  ( resid 10  and name H8    )  4.82
0.803  2.203
assign ( resid 10   and name H5''  )  ( resid 10  and name H8    )  4.69
0.803  2.203
assign ( resid 10   and name H5''  )  ( resid 11  and name H6    )  4.27
0.803  2.203
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assign ( resid 10   and name H8    )  ( resid 11  and name H6    )  4.87
0.803  2.203
!C11
!assign ( resid 11   and name H1'   )  ( resid 12  and name H11   )  4.09
0.803  2.203
!assign ( resid 11   and name H1'   )  ( resid 12  and name H19   )  4.15
0.803  2.203
assign ( resid 11   and name H1'   )  ( resid 10  and name H1'   )  3.74
0.802  1.702
assign ( resid 11   and name H1'   )  ( resid 11  and name H6    )  3.94
0.802  1.702
!assign ( resid 11   and name H2'   )  ( resid 12  and name H19   )  5.66
0.803  2.203
assign ( resid 11   and name H2'   )  ( resid 12  and name H11   )  3.78
0.802  1.702
assign ( resid 11   and name H2''  )  ( resid 12  and name H11   )  4.69
0.803  2.203
assign ( resid 11   and name H2'   )  ( resid 12  and name H6    )  5.16
0.803  2.203
assign ( resid 11   and name H2''  )  ( resid 12  and name H6    )  4.97
0.803  2.203
assign ( resid 11   and name H2'   )  ( resid 11  and name H6    )  2.80
0.802  1.702
assign ( resid 11   and name H2''  )  ( resid 11  and name H6    )  3.01
0.802  1.702
!assign ( resid 11   and name H3'   )  ( resid 12  and name H11   )  4.63
0.803  2.203
!assign ( resid 11   and name H3'   )  ( resid 4   and name H8    )  4.22
0.803  2.203
assign ( resid 11   and name H3'   )  ( resid 11  and name H6    )  3.61
0.802  1.702
!assign ( resid 11   and name H3'   )  ( resid 12  and name H6    )  5.11
0.803  2.203
!assign ( resid 11   and name H4'   )  ( resid 12  and name H19   )  4.86
0.803  2.203
assign ( resid 11   and name H4'   )  ( resid 12  and name H11   )  4.25
0.803  2.203
assign ( resid 11   and name H4'   )  ( resid 11  and name H6    )  4.32
0.803  2.203
assign ( resid 11   and name H4'   )  ( resid 12  and name H6    )  5.12
0.802  3.303
assign ( resid 11   and name H5'   )  ( resid 11  and name H6    )  4.70
0.803  2.203
assign ( resid 11   and name H5''  )  ( resid 11  and name H6    )  3.48
0.803  2.203
!U12
!assign ( resid 12   and name H1'   )  ( resid 12  and name H19   )  3.67
0.802  1.702
!assign ( resid 12   and name H1'   )  ( resid 12  and name H11   )  3.91
0.802  1.702
!assign ( resid 12   and name H2'   )  ( resid 12  and name H11   )   3.78
0.802  2.502
!assign ( resid 12   and name H2''  )  ( resid 12  and name H11   )   3.78
0.802  2.502
assign ( resid 12   and name H1'   )  ( resid 12  and name H6    )  2.34
0.801  1.201
assign ( resid 12   and name H2'   )  ( resid 12  and name H19   )  3.97
0.802  2.502
assign ( resid 12   and name H2''  )  ( resid 12  and name H19   )  3.97
0.802  2.502
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assign ( resid 12   and name H2'   )  ( resid 12  and name H6    )  2.49
0.801  2.201
assign ( resid 12   and name H2''  )  ( resid 12  and name H6    )  2.49
0.801  2.201
assign ( resid 12   and name H2'   )  ( resid 13  and name H4'   )  4.10
0.803  3.203
assign ( resid 12   and name H2''  )  ( resid 13  and name H4'   )  4.10
0.803  3.203
assign ( resid 12   and name H2'   )  ( resid 13  and name H5'   )  3.34
0.802  2.502
assign ( resid 12   and name H2''  )  ( resid 13  and name H5'   )  3.34
0.802  2.502
assign ( resid 12   and name H2'   )  ( resid 13  and name H5''  )  3.09
0.802  2.502
assign ( resid 12   and name H2''  )  ( resid 13  and name H5''  )  3.09
0.802  2.502
assign ( resid 12   and name H2'   )  ( resid 13  and name H8    )  3.52
0.802  2.502
assign ( resid 12   and name H2''  )  ( resid 13  and name H8    )  3.52
0.802  2.502
assign ( resid 12   and name H3'   )  ( resid 11  and name H5''  )  4.76
0.803  2.203
assign ( resid 12   and name H3'   )  ( resid 12  and name H6    )   3.31
0.802  3.202
assign ( resid 12   and name H4'   )  ( resid 12  and name H6    )  4.59
0.803  2.203
assign ( resid 12   and name H4'   )  ( resid 13  and name H8    )  6.21
0.803  2.203
assign ( resid 12   and name H6    )  ( resid 11  and name H6    )  4.15
0.803  2.203
!G13
!assign ( resid 13   and name H1'   )  ( resid 12  and name H19   )  4.97
0.803  2.203
!assign ( resid 13   and name H1'   )  ( resid 12  and name H11   )  4.70
0.803  2.203
assign ( resid 13   and name H1'   )  ( resid 12  and name H6    )  4.66
0.803  2.203
assign ( resid 13   and name H1'   )  ( resid 13  and name H8    )  3.67
0.802  1.702
assign ( resid 13   and name H1'   )  ( resid 14  and name H6    )  3.41
0.802  1.702
assign ( resid 13   and name H2'   )  ( resid 13  and name H8    )  2.68
0.801  1.201
assign ( resid 13   and name H2''  )  ( resid 13  and name H8    )  2.70
0.801  1.201
assign ( resid 13   and name H2'   )  ( resid 14  and name H6    )  3.46
0.801  1.201
assign ( resid 13   and name H2''  )  ( resid 14  and name H6    )  2.81
0.801  1.201
assign ( resid 13   and name H2''  )  ( resid 14  and name H2'   )  3.92
0.802  1.702
assign ( resid 13   and name H3'   )  ( resid 12  and name H11   )  4.47
0.803  2.203
assign ( resid 13   and name H3'   )  ( resid 12  and name H6    )  3.31
0.802  1.702
assign ( resid 13   and name H3'   )  ( resid 13  and name H8    )  3.50
0.802  1.702
assign ( resid 13   and name H3'   )  ( resid 14  and name H6    )  3.81
0.802  1.702
assign ( resid 13   and name H4'   )  ( resid 12  and name H6    )  5.95
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0.803  2.203
assign ( resid 13   and name H4'   )  ( resid 13  and name H8    )  4.79
0.803  2.203
assign ( resid 13   and name H4'   )  ( resid 14  and name H6    )  4.43
0.803  2.203
!assign ( resid 13   and name H5'   )  ( resid 12  and name H11   )  4.83
0.803  2.203
!assign ( resid 13   and name H5''  )  ( resid 12  and name H11   )  4.49
0.803  2.203
assign ( resid 13   and name H5'   )  ( resid 12  and name H6    )  4.13
0.803  2.203
assign ( resid 13   and name H5''  )  ( resid 12  and name H6    )  4.61
0.803  2.203
assign ( resid 13   and name H5'   )  ( resid 13  and name H8    )  5.00
0.803  2.203
assign ( resid 13   and name H5''  )  ( resid 13  and name H8    )  4.10
0.803  2.203
assign ( resid 13   and name H5'   )  ( resid 14  and name H4'   )  2.79
0.802  1.702
assign ( resid 13   and name H5''  )  ( resid 14  and name H4'   )  3.20
0.802  1.702
assign ( resid 13   and name H5'   )  ( resid 14  and name H6    )  3.90
0.802  1.702
assign ( resid 13   and name H5''  )  ( resid 14  and name H6    )  3.92
0.802  1.702
assign ( resid 13   and name H8    )  ( resid 12  and name H6    )  4.63
0.803  2.203
!T14
assign ( resid 14   and name H1'   )  ( resid 14  and name H6    )  3.54
0.802  1.702
assign ( resid 14   and name H2'   )  ( resid 14  and name H6    )  2.29
0.801  1.201
assign ( resid 14   and name H2''  )  ( resid 14  and name H6    )  2.97
0.802  1.702
assign ( resid 14   and name H2'   )  ( resid 15  and name H8    )  3.95
0.802  1.702
assign ( resid 14   and name H2''  )  ( resid 15  and name H8    )   3.20
0.802  3.002
assign ( resid 14   and name H3'   )  ( resid 14  and name H6    )  3.39
0.802  1.702
assign ( resid 14   and name H4'   )  ( resid 14  and name H6    )  4.16
0.803  2.203
assign ( resid 14   and name H5    )  ( resid 13  and name H2'   )  3.79
0.802  1.702
assign ( resid 14   and name H5    )  ( resid 13  and name H2''  )  3.12
0.802  1.702
assign ( resid 14   and name H5    )  ( resid 13  and name H4'   )  4.52
0.803  2.203
assign ( resid 14   and name H5    )  ( resid 13  and name H5'   )  4.05
0.802  1.702
assign ( resid 14   and name H5    )  ( resid 13  and name H5''  )  4.40
0.803  2.203
assign ( resid 14   and name H5    )  ( resid 13  and name H8    )  3.54
0.802  1.702
assign ( resid 14   and name H6    )  ( resid 13  and name H8    )  7.16
0.803  2.203
!G15
assign ( resid 15   and name H1'   )  ( resid 15  and name H8    )  3.51
0.802  1.702
assign ( resid 15   and name H2'   )  ( resid 15  and name H8    )  2.68
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0.801  1.201
assign ( resid 15   and name H2''  )  ( resid 15  and name H8    )  3.27
0.802  1.702
assign ( resid 15   and name H3'   )  ( resid 15  and name H8    )  3.85
0.802  1.702
assign ( resid 15   and name H4'   )  ( resid 15  and name H8    )  4.29
0.803  2.203
assign ( resid 15   and name H2'   )  ( resid 15  and name H8    )  4.97
0.803  2.203
assign ( resid 15   and name H2''  )  ( resid 15  and name H8    )  4.07
0.803  2.203
```

Appendix 3

```
REMARK FILENAME="DNA_Structure1/run1_1.pdb"
REMARK =============================================================
REMARK
overall,bonds,angles,improper,vdw,noe,cdih,xdip,rama,ncs,orie,plan
REMARK energies: -2063.31, 11.4227, 176.221, 16.7232, 14.4753, 58.418,
0, 0, -2345.79, 0, 0, 5.22216
REMARK =============================================================
REMARK            bonds,angles,impropers,noe,cdih,xdipo
REMARK rms-d: 4.682359E-03,1.10576,0.631482,6.767463E-02,0,0
REMARK =============================================================
REMARK               noe,  cdih
REMARK violations.: 0, 0
REMARK =============================================================
REMARK R-factor: JCH JCHB JCMT JADR JADB JCNB
REMARK R-factor xdip: 0 0 0 0 0 0
REMARK rms xdip: 0 0 0 0 0 0
REMARK =============================================================
REMARK rms for 1H-1H dipo: HABS HSIG
REMARK RMS xdip: 0 0
REMARK =============================================================
REMARK DATE:21-Oct-05  18:32:57       created by user: [unknown]
ATOM      1  P   CYT     1      20.421  -2.495  -2.183  0.00  0.00
ATOM      2  O1P CYT     1      20.872  -1.215  -2.774  0.00  0.00
ATOM      3  O2P CYT     1      21.070  -3.764  -2.580  0.00  0.00
ATOM      4  O5' CYT     1      18.840  -2.640  -2.435  0.00  0.00
ATOM      5  H5T CYT     1      21.426  -2.479  -0.338  1.00  0.00
ATOM      6  O5T CYT     1      20.505  -2.366  -0.583  1.00  0.00
ATOM      7  C5' CYT     1      18.272  -2.191  -3.666  0.00  0.00
ATOM      8  H5' CYT     1      18.797  -2.662  -4.497  1.00  0.00
ATOM      9 H5'' CYT     1      18.394  -1.110  -3.737  1.00  0.00
ATOM     10  C4' CYT     1      16.790  -2.525  -3.757  0.00  0.00
ATOM     11  H4' CYT     1      16.413  -2.194  -4.724  1.00  0.00
ATOM     12  O4' CYT     1      16.591  -3.924  -3.653  0.00  0.00
ATOM     13  C1' CYT     1      15.260  -4.152  -3.153  0.00  0.00
ATOM     14  H1' CYT     1      14.609  -4.445  -3.977  1.00  0.00
ATOM     15  N1  CYT     1      15.329  -5.243  -2.167  0.00  0.00
ATOM     16  C6  CYT     1      16.454  -5.446  -1.423  0.00  0.00
ATOM     17  H6  CYT     1      17.324  -4.807  -1.575  1.00  0.00
ATOM     18  C2  CYT     1      14.201  -6.031  -2.006  0.00  0.00
ATOM     19  O2  CYT     1      13.201  -5.820  -2.691  0.00  0.00
ATOM     20  N3  CYT     1      14.235  -7.025  -1.076  0.00  0.00
ATOM     21  C4  CYT     1      15.334  -7.235  -0.336  0.00  0.00
ATOM     22  N4  CYT     1      15.341  -8.215   0.568  0.00  0.00
ATOM     23  HN' CYT     1      16.166  -8.377   1.129  1.00  0.00
ATOM     24 HN'' CYT     1      14.523  -8.794   0.694  1.00  0.00
ATOM     25  C5  CYT     1      16.498  -6.424  -0.501  0.00  0.00
ATOM     26  H5  CYT     1      17.391  -6.591   0.102  1.00  0.00
ATOM     27  C2' CYT     1      14.750  -2.844  -2.552  0.00  0.00
ATOM     28  H2' CYT     1      14.472  -2.977  -1.507  1.00  0.00
ATOM     29 H2'' CYT     1      13.916  -2.450  -3.132  1.00  0.00
ATOM     30  C3' CYT     1      15.938  -1.911  -2.662  0.00  0.00
ATOM     31  H3' CYT     1      16.500  -1.879  -1.727  1.00  0.00
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ATOM     32  O3' CYT     1      15.512  -0.607  -3.058  0.00  0.00
ATOM     33  P   ADE     2      15.151   0.496  -1.942  0.00  0.00
ATOM     34  O1P ADE     2      15.860   0.142  -0.691  0.00  0.00
ATOM     35  O2P ADE     2      15.337   1.837  -2.540  0.00  0.00
ATOM     36  O5' ADE     2      13.574   0.256  -1.711  0.00  0.00
ATOM     37  C5' ADE     2      12.644   0.454  -2.780  0.00  0.00
ATOM     38  H5' ADE     2      12.854  -0.264  -3.573  1.00  0.00
ATOM     39 H5'' ADE     2      12.769   1.463  -3.174  1.00  0.00
ATOM     40  C4' ADE     2      11.199   0.279  -2.318  0.00  0.00
ATOM     41  H4' ADE     2      10.540   0.401  -3.177  1.00  0.00
ATOM     42  O4' ADE     2      11.028  -1.058  -1.796  0.00  0.00
ATOM     43  C1' ADE     2      10.637  -0.995  -0.412  0.00  0.00
ATOM     44  H1' ADE     2       9.584  -1.261  -0.318  1.00  0.00
ATOM     45  N9  ADE     2      11.458  -1.968   0.333  0.00  0.00
ATOM     46  C4  ADE     2      11.017  -3.149   0.880  0.00  0.00
ATOM     47  N3  ADE     2       9.764  -3.644   0.811  0.00  0.00
ATOM     48  C2  ADE     2       9.667  -4.807   1.447  0.00  0.00
ATOM     49  H2  ADE     2       8.686  -5.282   1.443  1.00  0.00
ATOM     50  N1  ADE     2      10.639  -5.456   2.090  0.00  0.00
ATOM     51  C6  ADE     2      11.879  -4.939   2.143  0.00  0.00
ATOM     52  N6  ADE     2      12.836  -5.601   2.793  0.00  0.00
ATOM     53  HN' ADE     2      13.771  -5.221   2.839  1.00  0.00
ATOM     54 HN'' ADE     2      12.627  -6.483   3.239  1.00  0.00
ATOM     55  C5  ADE     2      12.094  -3.712   1.504  0.00  0.00
ATOM     56  N7  ADE     2      13.222  -2.907   1.361  0.00  0.00
ATOM     57  C8  ADE     2      12.780  -1.902   0.665  0.00  0.00
ATOM     58  H8  ADE     2      13.416  -1.067   0.373  1.00  0.00
ATOM     59  C2' ADE     2      10.838   0.440   0.061  0.00  0.00
ATOM     60  H2' ADE     2      11.815   0.563   0.528  1.00  0.00
ATOM     61 H2'' ADE     2      10.038   0.740   0.740  1.00  0.00
ATOM     62  C3' ADE     2      10.762   1.241  -1.228  0.00  0.00
ATOM     63  H3' ADE     2      11.438   2.095  -1.197  1.00  0.00
ATOM     64  O3' ADE     2       9.419   1.654  -1.475  0.00  0.00
ATOM     65  P   CYT     3       8.706   2.681  -0.465  0.00  0.00
ATOM     66  O1P CYT     3       9.759   3.396   0.290  0.00  0.00
ATOM     67  O2P CYT     3       7.685   3.444  -1.216  0.00  0.00
ATOM     68  O5' CYT     3       7.951   1.687   0.549  0.00  0.00
ATOM     69  C5' CYT     3       6.910   0.828   0.081  0.00  0.00
ATOM     70  H5' CYT     3       7.225   0.355  -0.849  1.00  0.00
ATOM     71 H5'' CYT     3       6.017   1.424  -0.106  1.00  0.00
ATOM     72  C4' CYT     3       6.583  -0.248   1.105  0.00  0.00
ATOM     73  H4' CYT     3       5.796  -0.889   0.706  1.00  0.00
ATOM     74  O4' CYT     3       7.723  -1.035   1.386  0.00  0.00
ATOM     75  C1' CYT     3       7.471  -1.689   2.644  0.00  0.00
ATOM     76  H1' CYT     3       7.043  -2.674   2.460  1.00  0.00
ATOM     77  N1  CYT     3       8.760  -1.838   3.345  0.00  0.00
ATOM     78  C6  CYT     3       9.681  -0.831   3.352  0.00  0.00
ATOM     79  H6  CYT     3       9.475   0.094   2.813  1.00  0.00
ATOM     80  C2  CYT     3       8.983  -3.029   4.017  0.00  0.00
ATOM     81  O2  CYT     3       8.133  -3.917   3.992  0.00  0.00
ATOM     82  N3  CYT     3      10.152  -3.178   4.696  0.00  0.00
ATOM     83  C4  CYT     3      11.067  -2.200   4.715  0.00  0.00
ATOM     84  N4  CYT     3      12.200  -2.379   5.395  0.00  0.00
ATOM     85  HN' CYT     3      12.895  -1.647   5.419  1.00  0.00
ATOM     86 HN'' CYT     3      12.362  -3.247   5.885  1.00  0.00
ATOM     87  C5  CYT     3      10.842  -0.969   4.022  0.00  0.00
ATOM     88  H5  CYT     3      11.583  -0.170   4.036  1.00  0.00
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ATOM     89  C2' CYT     3       6.467  -0.835   3.410  0.00  0.00
ATOM     90  H2' CYT     3       6.911  -0.434   4.320  1.00  0.00
ATOM     91 H2'' CYT     3       5.564  -1.403   3.631  1.00  0.00
ATOM     92  C3' CYT     3       6.143   0.294   2.451  0.00  0.00
ATOM     93  H3' CYT     3       6.706   1.193   2.701  1.00  0.00
ATOM     94  O3' CYT     3       4.740   0.550   2.440  0.00  0.00
ATOM     95  P   ADE     4       4.099   1.517   3.553  0.00  0.00
ATOM     96  O1P ADE     4       5.110   2.532   3.925  0.00  0.00
ATOM     97  O2P ADE     4       2.761   1.940   3.086  0.00  0.00
ATOM     98  O5' ADE     4       3.908   0.521   4.802  0.00  0.00
ATOM     99  C5' ADE     4       3.004  -0.582   4.716  0.00  0.00
ATOM    100  H5' ADE     4       3.307  -1.227   3.890  1.00  0.00
ATOM    101 H5'' ADE     4       2.001  -0.204   4.521  1.00  0.00
ATOM    102  C4' ADE     4       2.989  -1.396   6.004  0.00  0.00
ATOM    103  H4' ADE     4       2.297  -2.229   5.886  1.00  0.00
ATOM    104  O4' ADE     4       4.278  -1.924   6.288  0.00  0.00
ATOM    105  C1' ADE     4       4.363  -2.169   7.702  0.00  0.00
ATOM    106  H1' ADE     4       4.268  -3.239   7.890  1.00  0.00
ATOM    107  N9  ADE     4       5.683  -1.708   8.168  0.00  0.00
ATOM    108  C4  ADE     4       6.405  -2.252   9.200  0.00  0.00
ATOM    109  N3  ADE     4       6.052  -3.322   9.940  0.00  0.00
ATOM    110  C2  ADE     4       6.967  -3.595  10.864  0.00  0.00
ATOM    111  H2  ADE     4       6.763  -4.448  11.511  1.00  0.00
ATOM    112  N1  ADE     4       8.105  -2.938  11.086  0.00  0.00
ATOM    113  C6  ADE     4       8.432  -1.874  10.331  0.00  0.00
ATOM    114  N6  ADE     4       9.575  -1.230  10.568  0.00  0.00
ATOM    115  HN' ADE     4       9.829  -0.429  10.009  1.00  0.00
ATOM    116 HN'' ADE     4      10.187  -1.544  11.308  1.00  0.00
ATOM    117  C5  ADE     4       7.536  -1.497   9.325  0.00  0.00
ATOM    118  N7  ADE     4       7.550  -0.472   8.380  0.00  0.00
ATOM    119  C8  ADE     4       6.435  -0.653   7.734  0.00  0.00
ATOM    120  H8  ADE     4       6.122  -0.007   6.914  1.00  0.00
ATOM    121  C2' ADE     4       3.209  -1.435   8.371  0.00  0.00
ATOM    122  H2' ADE     4       3.572  -0.783   9.164  1.00  0.00
ATOM    123 H2'' ADE     4       2.471  -2.141   8.752  1.00  0.00
ATOM    124  C3' ADE     4       2.606  -0.609   7.251  0.00  0.00
ATOM    125  H3' ADE     4       3.040   0.391   7.224  1.00  0.00
ATOM    126  O3' ADE     4       1.185  -0.553   7.395  0.00  0.00
ATOM    127  P   GUA     5       0.499   0.694   8.156  0.00  0.00
ATOM    128  O1P GUA     5       1.197   1.932   7.741  0.00  0.00
ATOM    129  O2P GUA     5      -0.968   0.586   7.990  0.00  0.00
ATOM    130  O5' GUA     5       0.858   0.410   9.702  0.00  0.00
ATOM    131  C5' GUA     5       0.325  -0.739  10.364  0.00  0.00
ATOM    132  H5' GUA     5       0.523  -1.622   9.759  1.00  0.00
ATOM    133 H5'' GUA     5      -0.753  -0.616  10.468  1.00  0.00
ATOM    134  C4' GUA     5       0.928  -0.946  11.747  0.00  0.00
ATOM    135  H4' GUA     5       0.482  -1.842  12.178  1.00  0.00
ATOM    136  O4' GUA     5       2.342  -1.120  11.658  0.00  0.00
ATOM    137  C1' GUA     5       2.887  -0.804  12.944  0.00  0.00
ATOM    138  H1' GUA     5       3.035  -1.727  13.506  1.00  0.00
ATOM    139  N9  GUA     5       4.202  -0.176  12.702  0.00  0.00
ATOM    140  C4  GUA     5       5.289  -0.214  13.543  0.00  0.00
ATOM    141  N3  GUA     5       5.328  -0.804  14.758  0.00  0.00
ATOM    142  C2  GUA     5       6.522  -0.682  15.343  0.00  0.00
ATOM    143  N2  GUA     5       6.732  -1.218  16.544  0.00  0.00
ATOM    144  HN' GUA     5       5.986  -1.716  17.010  1.00  0.00
ATOM    145 HN'' GUA     5       7.634  -1.129  16.988  1.00  0.00
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ATOM    146  N1  GUA     5       7.591  -0.023  14.768  0.00  0.00
ATOM    147  H1  GUA     5       8.464   0.027  15.275  1.00  0.00
ATOM    148  C6  GUA     5       7.573   0.590  13.522  0.00  0.00
ATOM    149  O6  GUA     5       8.580   1.152  13.094  0.00  0.00
ATOM    150  C5  GUA     5       6.303   0.459  12.896  0.00  0.00
ATOM    151  N7  GUA     5       5.868   0.921  11.656  0.00  0.00
ATOM    152  C8  GUA     5       4.633   0.517  11.606  0.00  0.00
ATOM    153  H8  GUA     5       3.992   0.706  10.745  1.00  0.00
ATOM    154  C2' GUA     5       1.876   0.079  13.689  0.00  0.00
ATOM    155  H2' GUA     5       2.304   1.057  13.904  1.00  0.00
ATOM    156 H2'' GUA     5       1.549  -0.407  14.610  1.00  0.00
ATOM    157  C3' GUA     5       0.707   0.209  12.710  0.00  0.00
ATOM    158  H3' GUA     5       0.728   1.170  12.195  1.00  0.00
ATOM    159  O3' GUA     5      -0.550   0.009  13.358  0.00  0.00
ATOM    160  P   CYT     6      -1.338   1.180  14.122  1.00  0.00
ATOM    161  O1P CYT     6      -2.683   0.676  14.470  1.00  0.00
ATOM    162  O2P CYT     6      -1.202   2.417  13.314  1.00  0.00
ATOM    163  O5' CYT     6      -0.546   1.383  15.508  1.00  0.00
ATOM    164  C5' CYT     6      -0.535   0.287  16.427  1.00  0.00
ATOM    165  H5' CYT     6      -0.263  -0.619  15.890  1.00  0.00
ATOM    166 H5'' CYT     6      -1.539   0.163  16.829  1.00  0.00
ATOM    167  C4' CYT     6       0.440   0.491  17.577  1.00  0.00
ATOM    168  H4' CYT     6       0.352  -0.357  18.257  1.00  0.00
ATOM    169  O4' CYT     6       1.776   0.533  17.045  1.00  0.00
ATOM    170  C1' CYT     6       2.468   1.625  17.636  1.00  0.00
ATOM    171  H1' CYT     6       2.949   1.283  18.552  1.00  0.00
ATOM    172  N1  CYT     6       3.507   2.082  16.695  1.00  0.00
ATOM    173  C6  CYT     6       3.195   2.737  15.537  1.00  0.00
ATOM    174  H6  CYT     6       2.151   2.934  15.289  1.00  0.00
ATOM    175  C2  CYT     6       4.819   1.814  17.045  1.00  0.00
ATOM    176  O2  CYT     6       5.064   1.221  18.092  1.00  0.00
ATOM    177  N3  CYT     6       5.808   2.220  16.208  1.00  0.00
ATOM    178  C4  CYT     6       5.528   2.863  15.070  1.00  0.00
ATOM    179  N4  CYT     6       6.532   3.247  14.280  1.00  0.00
ATOM    180  HN' CYT     6       6.340   3.736  13.418  1.00  0.00
ATOM    181 HN'' CYT     6       7.488   3.046  14.546  1.00  0.00
ATOM    182  C5  CYT     6       4.172   3.144  14.698  1.00  0.00
ATOM    183  H5  CYT     6       3.939   3.665  13.769  1.00  0.00
ATOM    184  C2' CYT     6       1.414   2.656  17.996  1.00  0.00
ATOM    185  H2' CYT     6       1.169   3.281  17.137  1.00  0.00
ATOM    186 H2'' CYT     6       1.735   3.261  18.845  1.00  0.00
ATOM    187  C3' CYT     6       0.225   1.781  18.380  1.00  0.00
ATOM    188  H3' CYT     6      -0.721   2.260  18.123  1.00  0.00
ATOM    189  O3' CYT     6       0.288   1.473  19.775  1.00  0.00
ATOM    190  P   GUA     7      -0.146   2.563  20.877  1.00  0.00
ATOM    191  O1P GUA     7      -0.458   1.840  22.133  1.00  0.00
ATOM    192  O2P GUA     7      -1.153   3.456  20.268  1.00  0.00
ATOM    193  O5' GUA     7       1.211   3.402  21.101  1.00  0.00
ATOM    194  C5' GUA     7       2.368   2.756  21.633  1.00  0.00
ATOM    195  H5' GUA     7       2.615   1.904  20.998  1.00  0.00
ATOM    196 H5'' GUA     7       2.135   2.393  22.634  1.00  0.00
ATOM    197  C4' GUA     7       3.576   3.688  21.709  1.00  0.00
ATOM    198  H4' GUA     7       4.380   3.161  22.222  1.00  0.00
ATOM    199  O4' GUA     7       4.003   3.995  20.370  1.00  0.00
ATOM    200  C1' GUA     7       4.118   5.417  20.227  1.00  0.00
ATOM    201  H1' GUA     7       5.158   5.706  20.382  1.00  0.00
ATOM    202  N9  GUA     7       3.744   5.708  18.828  1.00  0.00
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ATOM    203  C4  GUA     7       4.668   5.838  17.813  1.00  0.00
ATOM    204  N3  GUA     7       6.001   5.960  17.988  1.00  0.00
ATOM    205  C2  GUA     7       6.663   6.043  16.834  1.00  0.00
ATOM    206  N2  GUA     7       7.995   6.154  16.843  1.00  0.00
ATOM    207  HN' GUA     7       8.494   6.172  17.726  1.00  0.00
ATOM    208 HN'' GUA     7       8.505   6.225  15.976  1.00  0.00
ATOM    209  N1  GUA     7       6.052   6.008  15.601  1.00  0.00
ATOM    210  H1  GUA     7       6.619   6.050  14.775  1.00  0.00
ATOM    211  C6  GUA     7       4.682   5.887  15.397  1.00  0.00
ATOM    212  O6  GUA     7       4.236   5.809  14.256  1.00  0.00
ATOM    213  C5  GUA     7       3.970   5.803  16.634  1.00  0.00
ATOM    214  N7  GUA     7       2.599   5.673  16.880  1.00  0.00
ATOM    215  C8  GUA     7       2.532   5.629  18.183  1.00  0.00
ATOM    216  H8  GUA     7       1.596   5.537  18.720  1.00  0.00
ATOM    217  C2' GUA     7       3.281   6.057  21.322  1.00  0.00
ATOM    218  H2' GUA     7       2.255   6.213  20.989  1.00  0.00
ATOM    219 H2'' GUA     7       3.733   6.991  21.661  1.00  0.00
ATOM    220  C3' GUA     7       3.326   5.006  22.439  1.00  0.00
ATOM    221  H3' GUA     7       2.393   4.974  22.998  1.00  0.00
ATOM    222  O3' GUA     7       4.454   5.206  23.304  1.00  0.00
ATOM    223  P   ADE     8       4.567   6.480  24.266  1.00  0.00
ATOM    224  O1P ADE     8       5.219   6.064  25.527  1.00  0.00
ATOM    225  O2P ADE     8       3.248   7.151  24.300  1.00  0.00
ATOM    226  O5' ADE     8       5.569   7.431  23.459  1.00  0.00
ATOM    227  C5' ADE     8       6.920   7.064  23.125  1.00  0.00
ATOM    228  H5' ADE     8       6.924   6.178  22.488  1.00  0.00
ATOM    229 H5'' ADE     8       7.480   6.862  24.041  1.00  0.00
ATOM    230  C4' ADE     8       7.548   8.247  22.388  1.00  0.00
ATOM    231  H4' ADE     8       8.571   8.031  22.100  1.00  0.00
ATOM    232  O4' ADE     8       6.770   8.536  21.242  1.00  0.00
ATOM    233  C1' ADE     8       5.714   9.388  21.664  1.00  0.00
ATOM    234  H1' ADE     8       4.947   8.776  22.124  1.00  0.00
ATOM    235  N9  ADE     8       5.088  10.134  20.572  1.00  0.00
ATOM    236  C4  ADE     8       5.520  11.279  19.935  1.00  0.00
ATOM    237  N3  ADE     8       6.667  11.982  20.150  1.00  0.00
ATOM    238  C2  ADE     8       6.746  13.044  19.327  1.00  0.00
ATOM    239  H2  ADE     8       7.645  13.676  19.407  1.00  0.00
ATOM    240  N1  ADE     8       5.848  13.412  18.402  1.00  0.00
ATOM    241  C6  ADE     8       4.721  12.690  18.226  1.00  0.00
ATOM    242  N6  ADE     8       3.777  13.112  17.413  1.00  0.00
ATOM    243  HN' ADE     8       3.916  13.930  16.838  1.00  0.00
ATOM    244 HN'' ADE     8       2.906  12.632  17.404  1.00  0.00
ATOM    245  C5  ADE     8       4.540  11.569  19.019  1.00  0.00
ATOM    246  N7  ADE     8       3.513  10.643  19.061  1.00  0.00
ATOM    247  C8  ADE     8       3.897   9.835  19.982  1.00  0.00
ATOM    248  H8  ADE     8       3.337   8.934  20.245  1.00  0.00
ATOM    249  C2' ADE     8       6.301  10.304  22.729  1.00  0.00
ATOM    250  H2' ADE     8       5.578  10.494  23.510  1.00  0.00
ATOM    251 H2'' ADE     8       6.635  11.230  22.294  1.00  0.00
ATOM    252  C3' ADE     8       7.479   9.498  23.271  1.00  0.00
ATOM    253  H3' ADE     8       7.290   9.214  24.309  1.00  0.00
ATOM    254  O3' ADE     8       8.677  10.254  23.167  1.00  0.00
ATOM    255  P   ADE     9       9.735  10.213  24.377  1.00  0.00
ATOM    256  O1P ADE     9       8.988  10.146  25.651  1.00  0.00
ATOM    257  O2P ADE     9      10.730  11.286  24.155  1.00  0.00
ATOM    258  O5' ADE     9      10.458   8.792  24.149  1.00  0.00
ATOM    259  C5' ADE     9      11.368   8.624  23.067  1.00  0.00
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ATOM    260  H5' ADE     9      12.189   9.333  23.178  1.00  0.00
ATOM    261 H5'' ADE     9      10.849   8.821  22.133  1.00  0.00
ATOM    262  C4' ADE     9      11.934   7.213  23.032  1.00  0.00
ATOM    263  H4' ADE     9      12.336   6.979  24.017  1.00  0.00
ATOM    264  O4' ADE     9      10.879   6.282  22.740  1.00  0.00
ATOM    265  C1' ADE     9      11.296   5.414  21.682  1.00  0.00
ATOM    266  H1' ADE     9      11.699   4.502  22.116  1.00  0.00
ATOM    267  N9  ADE     9      10.089   5.079  20.899  1.00  0.00
ATOM    268  C4  ADE     9       9.345   3.933  21.055  1.00  0.00
ATOM    269  N3  ADE     9       9.649   2.888  21.857  1.00  0.00
ATOM    270  C2  ADE     9       8.721   1.940  21.796  1.00  0.00
ATOM    271  H2  ADE     9       8.887   1.054  22.414  1.00  0.00
ATOM    272  N1  ADE     9       7.609   1.956  21.062  1.00  0.00
ATOM    273  C6  ADE     9       7.325   3.010  20.271  1.00  0.00
ATOM    274  N6  ADE     9       6.190   3.017  19.573  1.00  0.00
ATOM    275  HN' ADE     9       5.565   2.225  19.624  1.00  0.00
ATOM    276 HN'' ADE     9       5.953   3.809  18.992  1.00  0.00
ATOM    277  C5  ADE     9       8.245   4.068  20.257  1.00  0.00
ATOM    278  N7  ADE     9       8.277   5.285  19.576  1.00  0.00
ATOM    279  C8  ADE     9       9.384   5.827  19.993  1.00  0.00
ATOM    280  H8  ADE     9       9.719   6.804  19.651  1.00  0.00
ATOM    281  C2' ADE     9      12.408   6.123  20.917  1.00  0.00
ATOM    282  H2' ADE     9      11.993   6.727  20.110  1.00  0.00
ATOM    283 H2'' ADE     9      13.145   5.410  20.534  1.00  0.00
ATOM    284  C3' ADE     9      13.035   7.016  21.998  1.00  0.00
ATOM    285  H3' ADE     9      13.357   7.975  21.591  1.00  0.00
ATOM    286  O3' ADE     9      14.119   6.334  22.636  1.00  0.00
ATOM    287  P   GUA    10      15.342   5.751  21.775  1.00  0.00
ATOM    288  O1P GUA    10      16.532   5.675  22.648  1.00  0.00
ATOM    289  O2P GUA    10      15.403   6.504  20.503  1.00  0.00
ATOM    290  O5' GUA    10      14.863   4.251  21.447  1.00  0.00
ATOM    291  C5' GUA    10      14.412   3.367  22.479  1.00  0.00
ATOM    292  H5' GUA    10      13.637   3.859  23.065  1.00  0.00
ATOM    293 H5'' GUA    10      15.250   3.115  23.132  1.00  0.00
ATOM    294  C4' GUA    10      13.853   2.085  21.872  1.00  0.00
ATOM    295  H4' GUA    10      13.582   1.397  22.674  1.00  0.00
ATOM    296  O4' GUA    10      12.680   2.399  21.123  1.00  0.00
ATOM    297  C1' GUA    10      12.667   1.648  19.913  1.00  0.00
ATOM    298  H1' GUA    10      12.121   0.718  20.079  1.00  0.00
ATOM    299  N9  GUA    10      12.000   2.408  18.850  1.00  0.00
ATOM    300  C4  GUA    10      10.749   2.154  18.354  1.00  0.00
ATOM    301  N3  GUA    10       9.871   1.259  18.859  1.00  0.00
ATOM    302  C2  GUA    10       8.736   1.218  18.162  1.00  0.00
ATOM    303  N2  GUA    10       7.764   0.382  18.523  1.00  0.00
ATOM    304  HN' GUA    10       7.891  -0.220  19.326  1.00  0.00
ATOM    305 HN'' GUA    10       6.905   0.345  17.995  1.00  0.00
ATOM    306  N1  GUA    10       8.493   2.004  17.053  1.00  0.00
ATOM    307  H1  GUA    10       7.607   1.914  16.576  1.00  0.00
ATOM    308  C6  GUA    10       9.382   2.928  16.518  1.00  0.00
ATOM    309  O6  GUA    10       9.082   3.566  15.513  1.00  0.00
ATOM    310  C5  GUA    10      10.593   2.973  17.260  1.00  0.00
ATOM    311  N7  GUA    10      11.732   3.753  17.065  1.00  0.00
ATOM    312  C8  GUA    10      12.515   3.373  18.031  1.00  0.00
ATOM    313  H8  GUA    10      13.515   3.783  18.170  1.00  0.00
ATOM    314  C2' GUA    10      14.110   1.330  19.578  1.00  0.00
ATOM    315  H2' GUA    10      14.516   2.073  18.892  1.00  0.00
ATOM    316 H2'' GUA    10      14.189   0.331  19.151  1.00  0.00
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ATOM    317  C3' GUA    10      14.827   1.389  20.921  1.00  0.00
ATOM    318  H3' GUA    10      15.757   1.953  20.842  1.00  0.00
ATOM    319  O3' GUA    10      15.074   0.050  21.330  1.00  0.00
ATOM    320  P   CYT    11      16.012  -0.859  20.388  0.00  0.00
ATOM    321  O1P CYT    11      16.470  -0.013  19.260  0.00  0.00
ATOM    322  O2P CYT    11      17.001  -1.560  21.233  0.00  0.00
ATOM    323  O5' CYT    11      14.975  -1.940  19.793  0.00  0.00
ATOM    324  C5' CYT    11      14.294  -2.869  20.641  0.00  0.00
ATOM    325  H5' CYT    11      13.976  -2.360  21.550  1.00  0.00
ATOM    326 H5'' CYT    11      14.975  -3.680  20.903  1.00  0.00
ATOM    327  C4' CYT    11      13.074  -3.453  19.938  0.00  0.00
ATOM    328  H4' CYT    11      12.553  -4.121  20.625  1.00  0.00
ATOM    329  O4' CYT    11      12.200  -2.410  19.536  0.00  0.00
ATOM    330  C1' CYT    11      11.423  -2.893  18.421  0.00  0.00
ATOM    331  H1' CYT    11      10.413  -3.129  18.757  1.00  0.00
ATOM    332  N1  CYT    11      11.363  -1.822  17.405  0.00  0.00
ATOM    333  C6  CYT    11      12.424  -0.991  17.192  0.00  0.00
ATOM    334  H6  CYT    11      13.326  -1.103  17.792  1.00  0.00
ATOM    335  C2  CYT    11      10.196  -1.713  16.664  0.00  0.00
ATOM    336  O2  CYT    11       9.257  -2.476  16.878  0.00  0.00
ATOM    337  N3  CYT    11      10.128  -0.752  15.703  0.00  0.00
ATOM    338  C4  CYT    11      11.160   0.071  15.479  0.00  0.00
ATOM    339  N4  CYT    11      11.059   0.997  14.525  0.00  0.00
ATOM    340  HN' CYT    11      10.209   1.075  13.985  1.00  0.00
ATOM    341 HN'' CYT    11      11.832   1.621  14.342  1.00  0.00
ATOM    342  C5  CYT    11      12.367  -0.036  16.242  0.00  0.00
ATOM    343  H5  CYT    11      13.209   0.631  16.062  1.00  0.00
ATOM    344  C2' CYT    11      12.091  -4.166  17.907  0.00  0.00
ATOM    345  H2' CYT    11      12.282  -4.101  16.836  1.00  0.00
ATOM    346 H2'' CYT    11      11.489  -5.043  18.146  1.00  0.00
ATOM    347  C3' CYT    11      13.403  -4.210  18.670  0.00  0.00
ATOM    348  H3' CYT    11      14.194  -3.706  18.115  1.00  0.00
ATOM    349  O3' CYT    11      13.767  -5.554  18.980  0.00  0.00
ATOM    350  P   THY    12      14.479  -6.462  17.864  0.00  0.00
ATOM    351  O1P THY    12      15.249  -5.577  16.960  0.00  0.00
ATOM    352  O2P THY    12      15.147  -7.596  18.539  0.00  0.00
ATOM    353  O5' THY    12      13.219  -7.030  17.052  0.00  0.00
ATOM    354  C5' THY    12      12.235  -7.821  17.715  0.00  0.00
ATOM    355  H5' THY    12      11.780  -7.238  18.515  1.00  0.00
ATOM    356 H5'' THY    12      12.716  -8.701  18.142  1.00  0.00
ATOM    357  C4' THY    12      11.160  -8.267  16.743  0.00  0.00
ATOM    358  H4' THY    12      10.417  -8.859  17.279  1.00  0.00
ATOM    359  O4' THY    12      10.495  -7.112  16.190  0.00  0.00
ATOM    360  C1' THY    12      10.552  -7.167  14.754  0.00  0.00
ATOM    361  H1' THY    12       9.604  -7.542  14.369  1.00  0.00
ATOM    362  N1  THY    12      10.776  -5.797  14.251  0.00  0.00
ATOM    363  C6  THY    12      11.934  -5.117  14.524  0.00  0.00
ATOM    364  H6  THY    12      12.697  -5.588  15.143  1.00  0.00
ATOM    365  C2  THY    12       9.779  -5.242  13.472  0.00  0.00
ATOM    366  O2  THY    12       8.737  -5.843  13.220  0.00  0.00
ATOM    367  N3  THY    12      10.022  -3.970  12.988  0.00  0.00
ATOM    368  H3  THY    12       9.303  -3.553  12.415  1.00  0.00
ATOM    369  C4  THY    12      11.160  -3.216  13.213  0.00  0.00
ATOM    370  O4  THY    12      11.273  -2.092  12.730  0.00  0.00
ATOM    371  C5  THY    12      12.147  -3.875  14.039  0.00  0.00
ATOM    372  CM  THY    12      13.452  -3.153  14.362  0.00  0.00
ATOM    373  H51 THY    12      13.885  -2.756  13.444  1.00  0.00
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ATOM    374  H52 THY    12      14.150  -3.852  14.821  1.00  0.00
ATOM    375  H53 THY    12      13.251  -2.333  15.052  1.00  0.00
ATOM    376  C2' THY    12      11.671  -8.137  14.388  0.00  0.00
ATOM    377  H2' THY    12      12.623  -7.616  14.291  1.00  0.00
ATOM    378 H2'' THY    12      11.428  -8.683  13.476  1.00  0.00
ATOM    379  C3' THY    12      11.693  -9.075  15.579  0.00  0.00
ATOM    380  H3' THY    12      12.710  -9.399  15.800  1.00  0.00
ATOM    381  O3' THY    12      10.833 -10.190  15.357  0.00  0.00
ATOM    382  P   GUA    13      11.140 -11.198  14.144  0.00  0.00
ATOM    383  O1P GUA    13      12.560 -11.040  13.754  0.00  0.00
ATOM    384  O2P GUA    13      10.614 -12.533  14.501  0.00  0.00
ATOM    385  O5' GUA    13      10.228 -10.591  12.970  0.00  0.00
ATOM    386  C5' GUA    13       8.817 -10.462  13.147  0.00  0.00
ATOM    387  H5' GUA    13       8.618  -9.883  14.048  1.00  0.00
ATOM    388 H5'' GUA    13       8.382 -11.455  13.257  1.00  0.00
ATOM    389  C4' GUA    13       8.176  -9.771  11.954  0.00  0.00
ATOM    390  H4' GUA    13       7.103  -9.691  12.125  1.00  0.00
ATOM    391  O4' GUA    13       8.704  -8.467  11.780  0.00  0.00
ATOM    392  C1' GUA    13       8.425  -8.093  10.419  0.00  0.00
ATOM    393  H1' GUA    13       7.492  -7.531  10.381  1.00  0.00
ATOM    394  N9  GUA    13       9.531  -7.238   9.947  0.00  0.00
ATOM    395  C4  GUA    13       9.440  -6.269   8.978  0.00  0.00
ATOM    396  N3  GUA    13       8.314  -5.914   8.319  0.00  0.00
ATOM    397  C2  GUA    13       8.533  -4.941   7.430  0.00  0.00
ATOM    398  N2  GUA    13       7.527  -4.474   6.692  0.00  0.00
ATOM    399  HN' GUA    13       7.693  -3.738   6.020  1.00  0.00
ATOM    400 HN'' GUA    13       6.598  -4.855   6.804  1.00  0.00
ATOM    401  N1  GUA    13       9.769  -4.366   7.217  0.00  0.00
ATOM    402  H1  GUA    13       9.854  -3.635   6.526  1.00  0.00
ATOM    403  C6  GUA    13      10.937  -4.716   7.883  0.00  0.00
ATOM    404  O6  GUA    13      11.993  -4.145   7.618  0.00  0.00
ATOM    405  C5  GUA    13      10.707  -5.751   8.829  0.00  0.00
ATOM    406  N7  GUA    13      11.597  -6.385   9.695  0.00  0.00
ATOM    407  C8  GUA    13      10.845  -7.241  10.319  0.00  0.00
ATOM    408  H8  GUA    13      11.235  -7.917  11.080  1.00  0.00
ATOM    409  C2' GUA    13       8.273  -9.373   9.605  0.00  0.00
ATOM    410  H2' GUA    13       9.063  -9.455   8.858  1.00  0.00
ATOM    411 H2'' GUA    13       7.288  -9.422   9.140  1.00  0.00
ATOM    412  C3' GUA    13       8.415 -10.477  10.635  0.00  0.00
ATOM    413  H3' GUA    13       9.417 -10.906  10.612  1.00  0.00
ATOM    414  O3' GUA    13       7.421 -11.479  10.437  0.00  0.00
ATOM    415  P   THY    14       7.651 -12.615   9.326  0.00  0.00
ATOM    416  O1P THY    14       9.102 -12.900   9.245  0.00  0.00
ATOM    417  O2P THY    14       6.693 -13.714   9.575  0.00  0.00
ATOM    418  O5' THY    14       7.214 -11.857   7.979  0.00  0.00
ATOM    419  C5' THY    14       5.866 -11.429   7.796  0.00  0.00
ATOM    420  H5' THY    14       5.566 -10.807   8.641  1.00  0.00
ATOM    421 H5'' THY    14       5.218 -12.305   7.750  1.00  0.00
ATOM    422  C4' THY    14       5.715 -10.633   6.510  0.00  0.00
ATOM    423  H4' THY    14       4.678 -10.312   6.410  1.00  0.00
ATOM    424  O4' THY    14       6.553  -9.483   6.531  0.00  0.00
ATOM    425  C1' THY    14       6.823  -9.108   5.169  0.00  0.00
ATOM    426  H1' THY    14       6.205  -8.251   4.901  1.00  0.00
ATOM    427  N1  THY    14       8.247  -8.733   5.071  0.00  0.00
ATOM    428  C6  THY    14       9.191  -9.307   5.882  0.00  0.00
ATOM    429  H6  THY    14       8.881 -10.038   6.628  1.00  0.00
ATOM    430  C2  THY    14       8.590  -7.801   4.111  0.00  0.00
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ATOM    431  O2  THY    14       7.752  -7.281   3.377  0.00  0.00
ATOM    432  N3  THY    14       9.933  -7.488   4.020  0.00  0.00
ATOM    433  H3  THY    14      10.199  -6.810   3.320  1.00  0.00
ATOM    434  C4  THY    14      10.948  -8.020   4.795  0.00  0.00
ATOM    435  O4  THY    14      12.114  -7.670   4.627  0.00  0.00
ATOM    436  C5  THY    14      10.497  -8.984   5.773  0.00  0.00
ATOM    437  CM  THY    14      11.517  -9.649   6.692  0.00  0.00
ATOM    438  H51 THY    14      11.470  -9.190   7.679  1.00  0.00
ATOM    439  H52 THY    14      12.517  -9.521   6.278  1.00  0.00
ATOM    440  H53 THY    14      11.291 -10.713   6.775  1.00  0.00
ATOM    441  C2' THY    14       6.455 -10.291   4.280  0.00  0.00
ATOM    442  H2' THY    14       7.298 -10.589   3.658  1.00  0.00
ATOM    443 H2'' THY    14       5.581 -10.060   3.671  1.00  0.00
ATOM    444  C3' THY    14       6.119 -11.395   5.264  0.00  0.00
ATOM    445  H3' THY    14       6.991 -12.019   5.462  1.00  0.00
ATOM    446  O3' THY    14       5.026 -12.175   4.785  0.00  0.00
ATOM    447  P   GUA    15       5.312 -13.476   3.884  0.00  0.00
ATOM    448  O1P GUA    15       6.529 -14.141   4.401  0.00  0.00
ATOM    449  O2P GUA    15       4.051 -14.238   3.752  0.00  0.00
ATOM    450  O5' GUA    15       5.663 -12.827   2.456  0.00  0.00
ATOM    451  C5' GUA    15       4.663 -12.123   1.719  0.00  0.00
ATOM    452  H5' GUA    15       4.239 -11.339   2.347  1.00  0.00
ATOM    453 H5'' GUA    15       3.874 -12.820   1.437  1.00  0.00
ATOM    454  C4' GUA    15       5.242 -11.494   0.459  0.00  0.00
ATOM    455  H4' GUA    15       4.452 -10.947  -0.056  1.00  0.00
ATOM    456  O4' GUA    15       6.283 -10.593   0.803  0.00  0.00
ATOM    457  C1' GUA    15       7.198 -10.509  -0.299  0.00  0.00
ATOM    458  H1' GUA    15       7.047  -9.565  -0.823  1.00  0.00
ATOM    459  N9  GUA    15       8.566 -10.548   0.249  0.00  0.00
ATOM    460  C4  GUA    15       9.638  -9.825  -0.212  0.00  0.00
ATOM    461  N3  GUA    15       9.613  -8.939  -1.234  0.00  0.00
ATOM    462  C2  GUA    15      10.809  -8.390  -1.456  0.00  0.00
ATOM    463  N2  GUA    15      10.961  -7.493  -2.431  0.00  0.00
ATOM    464  HN' GUA    15      11.865  -7.078  -2.599  1.00  0.00
ATOM    465 HN'' GUA    15      10.170  -7.230  -3.002  1.00  0.00
ATOM    466  N1  GUA    15      11.937  -8.693  -0.721  0.00  0.00
ATOM    467  H1  GUA    15      12.807  -8.234  -0.951  1.00  0.00
ATOM    468  C6  GUA    15      11.985  -9.598   0.331  0.00  0.00
ATOM    469  O6  GUA    15      13.042  -9.802   0.924  0.00  0.00
ATOM    470  C5  GUA    15      10.712 -10.185   0.570  0.00  0.00
ATOM    471  N7  GUA    15      10.328 -11.130   1.520  0.00  0.00
ATOM    472  C8  GUA    15       9.062 -11.297   1.278  0.00  0.00
ATOM    473  H8  GUA    15       8.443 -11.986   1.852  1.00  0.00
ATOM    474  C2' GUA    15       6.892 -11.665  -1.248  0.00  0.00
ATOM    475  H2' GUA    15       7.786 -12.261  -1.432  1.00  0.00
ATOM    476 H2'' GUA    15       6.471 -11.295  -2.183  1.00  0.00
ATOM    477  C3' GUA    15       5.856 -12.494  -0.507  0.00  0.00
ATOM    478  H3' GUA    15       6.346 -13.291   0.054  1.00  0.00
ATOM    479  O3' GUA    15       4.872 -13.041  -1.387  0.00  0.00
ATOM    480  H3T GUA    15       5.159 -12.852  -2.284  1.00  0.00
END
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++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++
+++++++
 ++++++++++++++++++++++++++++++ CLASS ALL
++++++++++++++++++++++++++++++++++
 for this class: SCALe=  30.690 AVERage=sum    POTEntial=square-well
                 SQCOnstant=   1.000 SQEXponent=    2 SQOFfsets(+/-)=
0.000   0.000

 ========== spectrum     1 restraint    62 ==========
 set-i-atoms
               8    ADE  H1'
 set-j-atoms
               8    ADE  H5'
 R<average>=   3.285 NOE= 4.45 (- 0.80/+ 1.20) Delta=   0.364  E(NOE)=
4.067
 ========== spectrum     1 restraint   133 ==========
 set-i-atoms
               13   GUA  H5''
 set-j-atoms
               12   THY  H1'
 R<average>=   4.248 NOE= 3.48 (- 0.80/+ 0.50) Delta=  -0.268  E(NOE)=
2.205
 ========== spectrum     1 restraint   154 ==========
 set-i-atoms
               7    GUA  H4'
 set-j-atoms
               8    ADE  H5''
 R<average>=   5.159 NOE= 4.32 (- 0.80/+ 0.50) Delta=  -0.339  E(NOE)=
3.519
 ========== spectrum     1 restraint   161 ==========
 set-i-atoms
               2    ADE  H5'
 set-j-atoms
               1    CYT  H2'
 R<average>=   3.775 NOE= 4.79 (- 0.80/+ 1.00) Delta=   0.214  E(NOE)=
1.411
 ========== spectrum     1 restraint   192 ==========
 set-i-atoms
               14   THY  H2''
 set-j-atoms
               14   THY  H6
 R<average>=   4.432 NOE= 2.86 (- 0.80/+ 1.20) Delta=  -0.370  E(NOE)=
4.202
 ========== spectrum     1 restraint   197 ==========
 set-i-atoms
               3    CYT  H2''
 set-j-atoms
               3    CYT  H6
 R<average>=   4.267 NOE= 2.86 (- 0.80/+ 1.20) Delta=  -0.205  E(NOE)=
1.284
 ========== spectrum     1 restraint   205 ==========
 set-i-atoms
               13   GUA  H2''
```

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 set-j-atoms
               13   GUA  H8
 R<average>=   4.648 NOE= 3.21 (- 0.80/+ 1.20) Delta=  -0.236  E(NOE)=
1.714
 ========== spectrum     1 restraint   217 ==========
 set-i-atoms
               4    ADE  H2''
 set-j-atoms
               4    ADE  H8
 R<average>=   4.611 NOE= 2.96 (- 0.80/+ 1.20) Delta=  -0.449  E(NOE)=
6.182
 ========== spectrum     1 restraint   245 ==========
 set-i-atoms
               10   GUA  H5'
 set-j-atoms
               9    ADE  H8
 R<average>=   5.973 NOE= 4.62 (- 0.80/+ 1.00) Delta=  -0.353  E(NOE)=
3.820
 ========== spectrum     1 restraint   264 ==========
 set-i-atoms
               8    ADE  H2'
 set-j-atoms
               8    ADE  H8
 R<average>=   4.256 NOE= 2.82 (- 0.80/+ 1.20) Delta=  -0.234  E(NOE)=
1.687
 ========== spectrum     1 restraint   265 ==========
 set-i-atoms
               8    ADE  H1'
 set-j-atoms
               8    ADE  H8
 R<average>=   2.480 NOE= 3.52 (- 0.80/+ 1.20) Delta=   0.239  E(NOE)=
1.758
 ========== spectrum     1 restraint   271 ==========
 set-i-atoms
               8    ADE  H5''
 set-j-atoms
               8    ADE  H8
 R<average>=   5.989 NOE= 4.36 (- 0.80/+ 1.20) Delta=  -0.427  E(NOE)=
5.600

 NOEPRI: RMS diff. =   0.068,  #(violat.> 0.2)=    12 of    344 NOEs
 NOEPRI: RMS diff. class ALL  =   0.071,  #(viol.> 0.2)=    12 of
306 NOEs
 NOEPRI: RMS diff. class HB   =   0.037,  #(viol.> 0.2)=     0 of
38 NOEs
 (atom-i        |atom-j        )    dist.   equil.   delta    energy
const.

 (atom-i        |atom-j        |atom-k        )  angle    equil.
delta    energy  const.

 (     8    C5' |     8    C4' |     8    C3' )  109.626  114.700   -
5.074    3.922  500.000
 (     8    O4' |     8    C1' |     8    N9  )  113.711  107.800
5.911    5.322  500.000
 (atom-i        |atom-j        |atom-k        |atom-L        )    angle
equil.   delta    energy   const.   period
```

```
 (     7    C1' |     7    C4  |     7    C8  |     7    N9  )    6.406
0.000   -6.406    6.251  500.000   0


++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++
+++++++
 ++++++++++++++++++++++++++++++ CLASS ALL
++++++++++++++++++++++++++++++++
 for this class: SCALe=  30.690 AVERage=sum    POTEntial=square-well
                 SQCOnstant=   1.000 SQEXponent=    2 SQOFfsets(+/-)=
0.000   0.000

 ========== spectrum     1 restraint   264 ==========
 set-i-atoms
               8    ADE  H2'
 set-j-atoms
               8    ADE  H8
 R<average>=   4.882 NOE= 2.82 (- 0.80/+ 1.20) Delta=  -0.860  E(NOE)=
22.697

 NOEPRI: RMS diff. =   0.076,  #(violat.> 0.5)=     1 of    344 NOEs
 NOEPRI: RMS diff. class ALL  =   0.080,  #(viol.> 0.5)=     1 of
306 NOEs
 NOEPRI: RMS diff. class HB   =   0.035,  #(viol.> 0.5)=     0 of
38 NOEs
 (atom-i        |atom-j        )    dist.   equil.   delta    energy
const.

 (atom-i        |atom-j        |atom-k        )  angle    equil.
delta    energy  const.

 (     8    C5' |     8    C4' |     8    C3' )  109.626  114.700   -
5.074    3.922  500.000
 (     8    O4' |     8    C1' |     8    N9  )  113.711  107.800
5.911    5.322  500.000
 (atom-i        |atom-j        |atom-k        |atom-L        )    angle
equil.   delta    energy   const.   period

 (     7    C1' |     7    C4  |     7    C8  |     7    N9  )    6.406
0.000   -6.406    6.251  500.000   0
The following dipolar couplings have
delta XDIPlar greater than or
equal to the cutoff:
class JCH
The following dipolar couplings have
delta XDIPlar greater than or
equal to the cutoff:
class JCH
The following dipolar couplings have
delta XDIPlar greater than or
equal to the cutoff:
The following dipolar couplings have
delta XDIPlar greater than or
equal to the cutoff:
The following dipolar couplings have
delta XDIPlar greater than or
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equal to the cutoff:
The following dipolar couplings have
delta XDIPlar greater than or
equal to the cutoff:
The following dipolar couplings have
delta XDIPlar greater than or
equal to the cutoff:
The following dipolar couplings have
delta XDIPlar greater than or
equal to the cutoff:
The following dipolar couplings have
delta XDIPlar greater than or
equal to the cutoff:
```

Appendix 4

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REMARK FILENAME="DNA_Structure2/run1_67.pdb"
REMARK =============================================================
REMARK
overall,bonds,angles,improper,vdw,noe,cdih,xdip,rama,ncs,orie,plan
REMARK energies: -1722.1, 14.934, 200.864, 20.9281, 52.2324, 131.46, 0,
0, -2145.48, 0, 0, 2.9574
REMARK =============================================================
REMARK            bonds,angles,impropers,noe,cdih,xdipo
REMARK rms-d: 5.713473E-03,1.15265,0.671221,9.700697E-02,0,0
REMARK =============================================================
REMARK               noe,  cdih
REMARK violations.: 6, 0
REMARK =============================================================
REMARK R-factor: JCH JCHB JCMT JADR JADB JCNB
REMARK R-factor xdip: 0 0 0 0 0 0
REMARK rms xdip: 0 0 0 0 0 0
REMARK =============================================================
REMARK rms for 1H-1H dipo: HABS HSIG
REMARK RMS xdip: 0 0
REMARK =============================================================
REMARK DATE:23-Apr-06  15:35:33       created by user: [unknown]
ATOM      1  P   CYT     1      12.492  11.611   2.099  0.00  0.00
ATOM      2  O1P CYT     1      12.112  10.190   2.269  0.00  0.00
ATOM      3  O2P CYT     1      13.911  11.973   1.885  0.00  0.00
ATOM      4  O5' CYT     1      11.955  12.435   3.375  0.00  0.00
ATOM      5  H5T CYT     1      12.233  12.671   0.293  1.00  0.00
ATOM      6  O5T CYT     1      11.623  12.224   0.887  1.00  0.00
ATOM      7  C5' CYT     1      12.846  13.284   4.104  0.00  0.00
ATOM      8  H5' CYT     1      13.308  13.992   3.416  1.00  0.00
ATOM      9 H5'' CYT     1      13.624  12.670   4.556  1.00  0.00
ATOM     10  C4' CYT     1      12.131  14.057   5.205  0.00  0.00
ATOM     11  H4' CYT     1      12.862  14.662   5.741  1.00  0.00
ATOM     12  O4' CYT     1      11.184  14.942   4.591  0.00  0.00
ATOM     13  C1' CYT     1       9.849  14.570   4.980  0.00  0.00
ATOM     14  H1' CYT     1       9.430  15.344   5.623  1.00  0.00
ATOM     15  N1  CYT     1       9.040  14.453   3.755  0.00  0.00
ATOM     16  C6  CYT     1       9.303  13.487   2.830  0.00  0.00
ATOM     17  H6  CYT     1      10.137  12.803   2.986  1.00  0.00
ATOM     18  C2  CYT     1       7.989  15.342   3.597  0.00  0.00
ATOM     19  O2  CYT     1       7.775  16.201   4.451  0.00  0.00
ATOM     20  N3  CYT     1       7.210  15.230   2.484  0.00  0.00
ATOM     21  C4  CYT     1       7.456  14.284   1.567  0.00  0.00
ATOM     22  N4  CYT     1       6.679  14.195   0.487  0.00  0.00
ATOM     23  HN' CYT     1       6.861  13.483  -0.206  1.00  0.00
ATOM     24 HN'' CYT     1       5.910  14.837   0.363  1.00  0.00
ATOM     25  C5  CYT     1       8.540  13.367   1.729  0.00  0.00
ATOM     26  H5  CYT     1       8.739  12.594   0.986  1.00  0.00
ATOM     27  C2' CYT     1       9.935  13.260   5.760  0.00  0.00
ATOM     28  H2' CYT     1       9.702  12.409   5.122  1.00  0.00
ATOM     29 H2'' CYT     1       9.285  13.285   6.635  1.00  0.00
ATOM     30  C3' CYT     1      11.385  13.194   6.201  0.00  0.00
ATOM     31  H3' CYT     1      11.763  12.173   6.153  1.00  0.00
ATOM     32  O3' CYT     1      11.533  13.743   7.511  0.00  0.00
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ATOM     33  P   ADE     2      11.048  12.902   8.796  0.00  0.00
ATOM     34  O1P ADE     2      11.173  11.460   8.483  0.00  0.00
ATOM     35  O2P ADE     2      11.718  13.456   9.993  0.00  0.00
ATOM     36  O5' ADE     2       9.479  13.263   8.878  0.00  0.00
ATOM     37  C5' ADE     2       9.054  14.613   9.089  0.00  0.00
ATOM     38  H5' ADE     2       9.537  15.257   8.354  1.00  0.00
ATOM     39 H5'' ADE     2       9.356  14.926  10.088  1.00  0.00
ATOM     40  C4' ADE     2       7.539  14.760   8.960  0.00  0.00
ATOM     41  H4' ADE     2       7.275  15.807   9.110  1.00  0.00
ATOM     42  O4' ADE     2       7.084  14.374   7.676  0.00  0.00
ATOM     43  C1' ADE     2       5.655  14.307   7.838  0.00  0.00
ATOM     44  H1' ADE     2       5.237  15.313   7.799  1.00  0.00
ATOM     45  N9  ADE     2       5.112  13.507   6.724  0.00  0.00
ATOM     46  C4  ADE     2       3.859  13.631   6.173  0.00  0.00
ATOM     47  N3  ADE     2       2.912  14.521   6.532  0.00  0.00
ATOM     48  C2  ADE     2       1.810  14.376   5.804  0.00  0.00
ATOM     49  H2  ADE     2       0.988  15.057   6.026  1.00  0.00
ATOM     50  N1  ADE     2       1.599  13.489   4.832  0.00  0.00
ATOM     51  C6  ADE     2       2.561  12.611   4.494  0.00  0.00
ATOM     52  N6  ADE     2       2.331  11.730   3.521  0.00  0.00
ATOM     53  HN' ADE     2       3.046  11.065   3.263  1.00  0.00
ATOM     54 HN'' ADE     2       1.441  11.727   3.043  1.00  0.00
ATOM     55  C5  ADE     2       3.768  12.676   5.200  0.00  0.00
ATOM     56  N7  ADE     2       4.950  11.942   5.119  0.00  0.00
ATOM     57  C8  ADE     2       5.694  12.481   6.038  0.00  0.00
ATOM     58  H8  ADE     2       6.706  12.135   6.250  1.00  0.00
ATOM     59  C2' ADE     2       5.416  13.693   9.212  0.00  0.00
ATOM     60  H2' ADE     2       5.202  12.627   9.131  1.00  0.00
ATOM     61 H2'' ADE     2       4.613  14.214   9.735  1.00  0.00
ATOM     62  C3' ADE     2       6.737  13.911   9.931  0.00  0.00
ATOM     63  H3' ADE     2       7.242  12.963  10.114  1.00  0.00
ATOM     64  O3' ADE     2       6.528  14.628  11.146  0.00  0.00
ATOM     65  P   CYT     3       6.425  13.823  12.534  0.00  0.00
ATOM     66  O1P CYT     3       7.411  12.719  12.504  0.00  0.00
ATOM     67  O2P CYT     3       6.441  14.800  13.645  0.00  0.00
ATOM     68  O5' CYT     3       4.954  13.183  12.443  0.00  0.00
ATOM     69  C5' CYT     3       3.799  14.023  12.424  0.00  0.00
ATOM     70  H5' CYT     3       3.900  14.755  11.622  1.00  0.00
ATOM     71 H5'' CYT     3       3.726  14.547  13.377  1.00  0.00
ATOM     72  C4' CYT     3       2.532  13.211  12.205  0.00  0.00
ATOM     73  H4' CYT     3       1.688  13.892  12.096  1.00  0.00
ATOM     74  O4' CYT     3       2.669  12.452  10.997  0.00  0.00
ATOM     75  C1' CYT     3       2.727  11.047  11.308  0.00  0.00
ATOM     76  H1' CYT     3       1.824  10.558  10.943  1.00  0.00
ATOM     77  N1  CYT     3       3.903  10.478  10.622  0.00  0.00
ATOM     78  C6  CYT     3       5.170  10.839  10.975  0.00  0.00
ATOM     79  H6  CYT     3       5.323  11.577  11.762  1.00  0.00
ATOM     80  C2  CYT     3       3.666   9.544   9.626  0.00  0.00
ATOM     81  O2  CYT     3       2.513   9.239   9.331  0.00  0.00
ATOM     82  N3  CYT     3       4.735   8.983   8.998  0.00  0.00
ATOM     83  C4  CYT     3       5.986   9.325   9.332  0.00  0.00
ATOM     84  N4  CYT     3       7.010   8.752   8.700  0.00  0.00
ATOM     85  HN' CYT     3       7.958   8.999   8.945  1.00  0.00
ATOM     86 HN'' CYT     3       6.837   8.070   7.974  1.00  0.00
ATOM     87  C5  CYT     3       6.236  10.290  10.360  0.00  0.00
ATOM     88  H5  CYT     3       7.253  10.569  10.636  1.00  0.00
ATOM     89  C2' CYT     3       2.797  10.908  12.824  0.00  0.00
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ATOM     90  H2' CYT     3       3.828  10.790  13.158  1.00  0.00
ATOM     91 H2'' CYT     3       2.177  10.080  13.167  1.00  0.00
ATOM     92  C3' CYT     3       2.231  12.226  13.318  0.00  0.00
ATOM     93  H3' CYT     3       2.725  12.543  14.237  1.00  0.00
ATOM     94  O3' CYT     3       0.820  12.125  13.499  0.00  0.00
ATOM     95  P   ADE     4       0.233  11.590  14.897  0.00  0.00
ATOM     96  O1P ADE     4       1.104  12.085  15.986  0.00  0.00
ATOM     97  O2P ADE     4      -1.220  11.864  14.928  0.00  0.00
ATOM     98  O5' ADE     4       0.446  10.001  14.768  0.00  0.00
ATOM     99  C5' ADE     4      -0.262   9.255  13.778  0.00  0.00
ATOM    100  H5' ADE     4      -0.009   9.642  12.790  1.00  0.00
ATOM    101 H5'' ADE     4      -1.333   9.374  13.942  1.00  0.00
ATOM    102  C4' ADE     4       0.089   7.773  13.837  0.00  0.00
ATOM    103  H4' ADE     4      -0.582   7.225  13.176  1.00  0.00
ATOM    104  O4' ADE     4       1.432   7.611  13.356  0.00  0.00
ATOM    105  C1' ADE     4       2.220   6.935  14.351  0.00  0.00
ATOM    106  H1' ADE     4       2.307   5.881  14.087  1.00  0.00
ATOM    107  N9  ADE     4       3.560   7.547  14.367  0.00  0.00
ATOM    108  C4  ADE     4       4.699   7.001  13.829  0.00  0.00
ATOM    109  N3  ADE     4       4.782   5.832  13.163  0.00  0.00
ATOM    110  C2  ADE     4       6.027   5.586  12.772  0.00  0.00
ATOM    111  H2  ADE     4       6.189   4.657  12.224  1.00  0.00
ATOM    112  N1  ADE     4       7.101   6.350  12.978  0.00  0.00
ATOM    113  C6  ADE     4       6.988   7.511  13.646  0.00  0.00
ATOM    114  N6  ADE     4       8.072   8.261  13.842  0.00  0.00
ATOM    115  HN' ADE     4       7.998   9.135  14.342  1.00  0.00
ATOM    116 HN'' ADE     4       8.968   7.955  13.490  1.00  0.00
ATOM    117  C5  ADE     4       5.715   7.870  14.105  0.00  0.00
ATOM    118  N7  ADE     4       5.241   8.973  14.815  0.00  0.00
ATOM    119  C8  ADE     4       3.971   8.720  14.934  0.00  0.00
ATOM    120  H8  ADE     4       3.287   9.390  15.455  1.00  0.00
ATOM    121  C2' ADE     4       1.472   7.078  15.670  0.00  0.00
ATOM    122  H2' ADE     4       1.766   7.991  16.186  1.00  0.00
ATOM    123 H2'' ADE     4       1.631   6.203  16.301  1.00  0.00
ATOM    124  C3' ADE     4       0.022   7.157  15.229  0.00  0.00
ATOM    125  H3' ADE     4      -0.554   7.800  15.894  1.00  0.00
ATOM    126  O3' ADE     4      -0.542   5.846  15.158  0.00  0.00
ATOM    127  P   GUA     5      -0.835   5.019  16.513  0.00  0.00
ATOM    128  O1P GUA     5      -0.566   3.587  16.250  0.00  0.00
ATOM    129  O2P GUA     5      -0.148   5.703  17.632  0.00  0.00
ATOM    130  O5' GUA     5      -2.426   5.203  16.705  0.00  0.00
ATOM    131  C5' GUA     5      -2.964   6.476  17.071  0.00  0.00
ATOM    132  H5' GUA     5      -2.696   7.207  16.310  1.00  0.00
ATOM    133 H5'' GUA     5      -2.529   6.780  18.022  1.00  0.00
ATOM    134  C4' GUA     5      -4.480   6.450  17.214  0.00  0.00
ATOM    135  H4' GUA     5      -4.786   7.365  17.718  1.00  0.00
ATOM    136  O4' GUA     5      -5.082   6.438  15.920  0.00  0.00
ATOM    137  C1' GUA     5      -5.584   5.127  15.640  0.00  0.00
ATOM    138  H1' GUA     5      -6.629   5.199  15.334  1.00  0.00
ATOM    139  N9  GUA     5      -4.790   4.606  14.509  0.00  0.00
ATOM    140  C4  GUA     5      -5.204   4.513  13.202  0.00  0.00
ATOM    141  N3  GUA     5      -6.426   4.851  12.734  0.00  0.00
ATOM    142  C2  GUA     5      -6.537   4.655  11.419  0.00  0.00
ATOM    143  N2  GUA     5      -7.679   4.948  10.798  0.00  0.00
ATOM    144  HN' GUA     5      -8.458   5.319  11.325  1.00  0.00
ATOM    145 HN'' GUA     5      -7.766   4.802   9.803  1.00  0.00
ATOM    146  N1  GUA     5      -5.518   4.159  10.630  0.00  0.00
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ATOM    147  H1  GUA     5      -5.680   4.040   9.640  1.00  0.00
ATOM    148  C6  GUA     5      -4.255   3.805  11.090  0.00  0.00
ATOM    149  O6  GUA     5      -3.412   3.373  10.307  0.00  0.00
ATOM    150  C5  GUA     5      -4.134   4.015  12.490  0.00  0.00
ATOM    151  N7  GUA     5      -3.048   3.794  13.335  0.00  0.00
ATOM    152  C8  GUA     5      -3.499   4.159  14.499  0.00  0.00
ATOM    153  H8  GUA     5      -2.887   4.122  15.400  1.00  0.00
ATOM    154  C2' GUA     5      -5.497   4.295  16.928  0.00  0.00
ATOM    155  H2' GUA     5      -4.776   3.487  16.814  1.00  0.00
ATOM    156 H2'' GUA     5      -6.478   3.900  17.192  1.00  0.00
ATOM    157  C3' GUA     5      -5.022   5.270  18.006  0.00  0.00
ATOM    158  H3' GUA     5      -4.248   4.823  18.631  1.00  0.00
ATOM    159  O3' GUA     5      -6.109   5.742  18.806  0.00  0.00
ATOM    160  P   CYT     6      -6.710   4.931  20.055  1.00  0.00
ATOM    161  O1P CYT     6      -7.565   5.838  20.840  1.00  0.00
ATOM    162  O2P CYT     6      -5.582   4.253  20.724  1.00  0.00
ATOM    163  O5' CYT     6      -7.669   3.809  19.414  1.00  0.00
ATOM    164  C5' CYT     6      -8.834   4.248  18.714  1.00  0.00
ATOM    165  H5' CYT     6      -8.546   5.030  18.017  1.00  0.00
ATOM    166 H5'' CYT     6      -9.541   4.663  19.433  1.00  0.00
ATOM    167  C4' CYT     6      -9.509   3.123  17.944  1.00  0.00
ATOM    168  H4' CYT     6     -10.450   3.499  17.538  1.00  0.00
ATOM    169  O4' CYT     6      -8.651   2.726  16.861  1.00  0.00
ATOM    170  C1' CYT     6      -8.567   1.307  16.849  1.00  0.00
ATOM    171  H1' CYT     6      -9.377   0.912  16.238  1.00  0.00
ATOM    172  N1  CYT     6      -7.278   0.923  16.241  1.00  0.00
ATOM    173  C6  CYT     6      -6.092   1.093  16.898  1.00  0.00
ATOM    174  H6  CYT     6      -6.082   1.525  17.898  1.00  0.00
ATOM    175  C2  CYT     6      -7.329   0.377  14.970  1.00  0.00
ATOM    176  O2  CYT     6      -8.412   0.240  14.404  1.00  0.00
ATOM    177  N3  CYT     6      -6.166   0.009  14.375  1.00  0.00
ATOM    178  C4  CYT     6      -4.994   0.167  14.997  1.00  0.00
ATOM    179  N4  CYT     6      -3.878  -0.212  14.375  1.00  0.00
ATOM    180  HN' CYT     6      -2.982  -0.104  14.829  1.00  0.00
ATOM    181 HN'' CYT     6      -3.929  -0.608  13.445  1.00  0.00
ATOM    182  C5  CYT     6      -4.928   0.730  16.313  1.00  0.00
ATOM    183  H5  CYT     6      -3.974   0.863  16.824  1.00  0.00
ATOM    184  C2' CYT     6      -8.774   0.854  18.282  1.00  0.00
ATOM    185  H2' CYT     6      -7.849   0.930  18.854  1.00  0.00
ATOM    186 H2'' CYT     6      -9.176  -0.159  18.315  1.00  0.00
ATOM    187  C3' CYT     6      -9.804   1.867  18.773  1.00  0.00
ATOM    188  H3' CYT     6      -9.696   2.057  19.842  1.00  0.00
ATOM    189  O3' CYT     6     -11.121   1.402  18.460  1.00  0.00
ATOM    190  P   GUA     7     -11.732   0.107  19.196  1.00  0.00
ATOM    191  O1P GUA     7     -13.209   0.214  19.151  1.00  0.00
ATOM    192  O2P GUA     7     -11.041  -0.055  20.492  1.00  0.00
ATOM    193  O5' GUA     7     -11.286  -1.096  18.224  1.00  0.00
ATOM    194  C5' GUA     7     -11.682  -1.088  16.850  1.00  0.00
ATOM    195  H5' GUA     7     -11.403  -0.130  16.413  1.00  0.00
ATOM    196 H5'' GUA     7     -12.765  -1.198  16.798  1.00  0.00
ATOM    197  C4' GUA     7     -11.025  -2.206  16.047  1.00  0.00
ATOM    198  H4' GUA     7     -11.386  -2.147  15.022  1.00  0.00
ATOM    199  O4' GUA     7      -9.593  -2.009  16.057  1.00  0.00
ATOM    200  C1' GUA     7      -8.976  -3.254  16.421  1.00  0.00
ATOM    201  H1' GUA     7      -8.764  -3.821  15.519  1.00  0.00
ATOM    202  N9  GUA     7      -7.700  -2.895  17.060  1.00  0.00
ATOM    203  C4  GUA     7      -6.494  -2.890  16.387  1.00  0.00
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ATOM    204  N3  GUA     7      -6.284  -3.441  15.169  1.00  0.00
ATOM    205  C2  GUA     7      -5.038  -3.263  14.738  1.00  0.00
ATOM    206  N2  GUA     7      -4.681  -3.743  13.544  1.00  0.00
ATOM    207  HN' GUA     7      -5.358  -4.237  12.972  1.00  0.00
ATOM    208 HN'' GUA     7      -3.732  -3.645  13.220  1.00  0.00
ATOM    209  N1  GUA     7      -4.075  -2.587  15.448  1.00  0.00
ATOM    210  H1  GUA     7      -3.187  -2.429  15.020  1.00  0.00
ATOM    211  C6  GUA     7      -4.265  -2.017  16.711  1.00  0.00
ATOM    212  O6  GUA     7      -3.354  -1.418  17.271  1.00  0.00
ATOM    213  C5  GUA     7      -5.600  -2.211  17.176  1.00  0.00
ATOM    214  N7  GUA     7      -6.208  -1.803  18.365  1.00  0.00
ATOM    215  C8  GUA     7      -7.434  -2.237  18.242  1.00  0.00
ATOM    216  H8  GUA     7      -8.183  -2.093  19.020  1.00  0.00
ATOM    217  C2' GUA     7      -9.986  -4.018  17.251  1.00  0.00
ATOM    218  H2' GUA     7      -9.966  -3.684  18.290  1.00  0.00
ATOM    219 H2'' GUA     7      -9.814  -5.092  17.178  1.00  0.00
ATOM    220  C3' GUA     7     -11.296  -3.615  16.568  1.00  0.00
ATOM    221  H3' GUA     7     -12.133  -3.627  17.264  1.00  0.00
ATOM    222  O3' GUA     7     -11.556  -4.431  15.415  1.00  0.00
ATOM    223  P   ADE     8     -11.868  -5.996  15.538  1.00  0.00
ATOM    224  O1P ADE     8     -12.974  -6.333  14.615  1.00  0.00
ATOM    225  O2P ADE     8     -11.977  -6.332  16.977  1.00  0.00
ATOM    226  O5' ADE     8     -10.518  -6.660  14.985  1.00  0.00
ATOM    227  C5' ADE     8     -10.047  -6.486  13.637  1.00  0.00
ATOM    228  H5' ADE     8      -9.883  -5.427  13.427  1.00  0.00
ATOM    229 H5'' ADE     8     -10.778  -6.891  12.935  1.00  0.00
ATOM    230  C4' ADE     8      -8.738  -7.253  13.504  1.00  0.00
ATOM    231  H4' ADE     8      -8.364  -7.217  12.482  1.00  0.00
ATOM    232  O4' ADE     8      -7.767  -6.690  14.367  1.00  0.00
ATOM    233  C1' ADE     8      -7.930  -7.302  15.641  1.00  0.00
ATOM    234  H1' ADE     8      -8.727  -6.799  16.159  1.00  0.00
ATOM    235  N9  ADE     8      -6.747  -7.250  16.496  1.00  0.00
ATOM    236  C4  ADE     8      -5.627  -8.045  16.486  1.00  0.00
ATOM    237  N3  ADE     8      -5.325  -9.058  15.631  1.00  0.00
ATOM    238  C2  ADE     8      -4.126  -9.589  15.923  1.00  0.00
ATOM    239  H2  ADE     8      -3.769 -10.400  15.267  1.00  0.00
ATOM    240  N1  ADE     8      -3.304  -9.216  16.918  1.00  0.00
ATOM    241  C6  ADE     8      -3.654  -8.211  17.754  1.00  0.00
ATOM    242  N6  ADE     8      -2.892  -7.915  18.807  1.00  0.00
ATOM    243  HN' ADE     8      -2.010  -8.381  18.951  1.00  0.00
ATOM    244 HN'' ADE     8      -3.216  -7.249  19.471  1.00  0.00
ATOM    245  C5  ADE     8      -4.863  -7.588  17.536  1.00  0.00
ATOM    246  N7  ADE     8      -5.469  -6.530  18.196  1.00  0.00
ATOM    247  C8  ADE     8      -6.559  -6.384  17.532  1.00  0.00
ATOM    248  H8  ADE     8      -7.270  -5.581  17.749  1.00  0.00
ATOM    249  C2' ADE     8      -8.380  -8.735  15.378  1.00  0.00
ATOM    250  H2' ADE     8      -9.148  -9.028  16.082  1.00  0.00
ATOM    251 H2'' ADE     8      -7.547  -9.419  15.439  1.00  0.00
ATOM    252  C3' ADE     8      -8.938  -8.697  13.958  1.00  0.00
ATOM    253  H3' ADE     8     -10.006  -8.934  13.964  1.00  0.00
ATOM    254  O3' ADE     8      -8.224  -9.603  13.121  1.00  0.00
ATOM    255  P   ADE     9      -6.755  -9.203  12.591  1.00  0.00
ATOM    256  O1P ADE     9      -6.018 -10.442  12.261  1.00  0.00
ATOM    257  O2P ADE     9      -6.170  -8.224  13.536  1.00  0.00
ATOM    258  O5' ADE     9      -7.086  -8.443  11.213  1.00  0.00
ATOM    259  C5' ADE     9      -7.586  -9.176  10.100  1.00  0.00
ATOM    260  H5' ADE     9      -8.508  -9.680  10.391  1.00  0.00
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ATOM    261 H5'' ADE     9      -6.850  -9.920   9.803  1.00  0.00
ATOM    262  C4' ADE     9      -7.877  -8.256   8.919  1.00  0.00
ATOM    263  H4' ADE     9      -8.452  -8.816   8.185  1.00  0.00
ATOM    264  O4' ADE     9      -8.636  -7.117   9.397  1.00  0.00
ATOM    265  C1' ADE     9      -7.931  -5.909   9.097  1.00  0.00
ATOM    266  H1' ADE     9      -8.375  -5.446   8.222  1.00  0.00
ATOM    267  N9  ADE     9      -8.096  -5.008  10.260  1.00  0.00
ATOM    268  C4  ADE     9      -9.039  -4.014  10.355  1.00  0.00
ATOM    269  N3  ADE     9      -9.876  -3.622   9.372  1.00  0.00
ATOM    270  C2  ADE     9     -10.676  -2.645   9.776  1.00  0.00
ATOM    271  H2  ADE     9     -11.388  -2.267   9.041  1.00  0.00
ATOM    272  N1  ADE     9     -10.705  -2.079  10.982  1.00  0.00
ATOM    273  C6  ADE     9      -9.859  -2.491  11.946  1.00  0.00
ATOM    274  N6  ADE     9      -9.932  -1.940  13.153  1.00  0.00
ATOM    275  HN' ADE     9     -10.598  -1.202  13.330  1.00  0.00
ATOM    276 HN'' ADE     9      -9.321  -2.256  13.891  1.00  0.00
ATOM    277  C5  ADE     9      -8.965  -3.519  11.622  1.00  0.00
ATOM    278  N7  ADE     9      -7.972  -4.176  12.350  1.00  0.00
ATOM    279  C8  ADE     9      -7.499  -5.036  11.492  1.00  0.00
ATOM    280  H8  ADE     9      -6.696  -5.725  11.739  1.00  0.00
ATOM    281  C2' ADE     9      -6.500  -6.268   8.745  1.00  0.00
ATOM    282  H2' ADE     9      -5.866  -6.224   9.630  1.00  0.00
ATOM    283 H2'' ADE     9      -6.117  -5.619   7.954  1.00  0.00
ATOM    284  C3' ADE     9      -6.628  -7.708   8.244  1.00  0.00
ATOM    285  H3' ADE     9      -5.757  -8.297   8.522  1.00  0.00
ATOM    286  O3' ADE     9      -6.842  -7.732   6.829  1.00  0.00
ATOM    287  P   GUA    10      -5.780  -7.065   5.825  1.00  0.00
ATOM    288  O1P GUA    10      -5.844  -7.780   4.531  1.00  0.00
ATOM    289  O2P GUA    10      -4.491  -6.939   6.537  1.00  0.00
ATOM    290  O5' GUA    10      -6.381  -5.589   5.614  1.00  0.00
ATOM    291  C5' GUA    10      -7.713  -5.387   5.129  1.00  0.00
ATOM    292  H5' GUA    10      -8.387  -6.094   5.610  1.00  0.00
ATOM    293 H5'' GUA    10      -7.731  -5.549   4.049  1.00  0.00
ATOM    294  C4' GUA    10      -8.170  -3.965   5.425  1.00  0.00
ATOM    295  H4' GUA    10      -9.234  -3.872   5.198  1.00  0.00
ATOM    296  O4' GUA    10      -7.968  -3.687   6.807  1.00  0.00
ATOM    297  C1' GUA    10      -7.456  -2.369   6.982  1.00  0.00
ATOM    298  H1' GUA    10      -8.270  -1.713   7.297  1.00  0.00
ATOM    299  N9  GUA    10      -6.411  -2.362   8.016  1.00  0.00
ATOM    300  C4  GUA    10      -6.511  -1.762   9.243  1.00  0.00
ATOM    301  N3  GUA    10      -7.630  -1.204   9.757  1.00  0.00
ATOM    302  C2  GUA    10      -7.426  -0.689  10.971  1.00  0.00
ATOM    303  N2  GUA    10      -8.426  -0.090  11.615  1.00  0.00
ATOM    304  HN' GUA    10      -9.338  -0.026  11.182  1.00  0.00
ATOM    305 HN'' GUA    10      -8.276   0.295  12.536  1.00  0.00
ATOM    306  N1  GUA    10      -6.210  -0.727  11.621  1.00  0.00
ATOM    307  H1  GUA    10      -6.135  -0.312  12.539  1.00  0.00
ATOM    308  C6  GUA    10      -5.048  -1.295  11.112  1.00  0.00
ATOM    309  O6  GUA    10      -4.006  -1.262  11.762  1.00  0.00
ATOM    310  C5  GUA    10      -5.263  -1.847   9.821  1.00  0.00
ATOM    311  N7  GUA    10      -4.374  -2.501   8.969  1.00  0.00
ATOM    312  C8  GUA    10      -5.111  -2.776   7.932  1.00  0.00
ATOM    313  H8  GUA    10      -4.719  -3.286   7.055  1.00  0.00
ATOM    314  C2' GUA    10      -6.929  -1.873   5.650  1.00  0.00
ATOM    315  H2' GUA    10      -5.841  -1.817   5.665  1.00  0.00
ATOM    316 H2'' GUA    10      -7.359  -0.901   5.410  1.00  0.00
ATOM    317  C3' GUA    10      -7.391  -2.914   4.636  1.00  0.00
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ATOM    318  H3' GUA    10      -6.540  -3.373   4.137  1.00  0.00
ATOM    319  O3' GUA    10      -8.233  -2.257   3.703  1.00  0.00
ATOM    320  P   CYT    11      -7.572  -1.090   2.821  0.00  0.00
ATOM    321  O1P CYT    11      -6.130  -1.031   3.162  0.00  0.00
ATOM    322  O2P CYT    11      -7.997  -1.246   1.413  0.00  0.00
ATOM    323  O5' CYT    11      -8.267   0.221   3.436  0.00  0.00
ATOM    324  C5' CYT    11      -9.684   0.380   3.439  0.00  0.00
ATOM    325  H5' CYT    11     -10.153  -0.504   3.867  1.00  0.00
ATOM    326 H5'' CYT    11     -10.031   0.513   2.413  1.00  0.00
ATOM    327  C4' CYT    11     -10.071   1.601   4.250  0.00  0.00
ATOM    328  H4' CYT    11     -11.157   1.638   4.336  1.00  0.00
ATOM    329  O4' CYT    11      -9.512   1.509   5.554  0.00  0.00
ATOM    330  C1' CYT    11      -9.162   2.836   6.017  0.00  0.00
ATOM    331  H1' CYT    11      -9.803   3.118   6.853  1.00  0.00
ATOM    332  N1  CYT    11      -7.744   2.811   6.449  0.00  0.00
ATOM    333  C6  CYT    11      -6.813   2.062   5.781  0.00  0.00
ATOM    334  H6  CYT    11      -7.128   1.405   4.970  1.00  0.00
ATOM    335  C2  CYT    11      -7.383   3.636   7.503  0.00  0.00
ATOM    336  O2  CYT    11      -8.241   4.280   8.101  0.00  0.00
ATOM    337  N3  CYT    11      -6.068   3.701   7.855  0.00  0.00
ATOM    338  C4  CYT    11      -5.142   2.985   7.205  0.00  0.00
ATOM    339  N4  CYT    11      -3.859   3.101   7.559  0.00  0.00
ATOM    340  HN' CYT    11      -3.599   3.710   8.322  1.00  0.00
ATOM    341 HN'' CYT    11      -3.147   2.583   7.062  1.00  0.00
ATOM    342  C5  CYT    11      -5.507   2.122   6.123  0.00  0.00
ATOM    343  H5  CYT    11      -4.756   1.537   5.590  1.00  0.00
ATOM    344  C2' CYT    11      -9.390   3.808   4.852  0.00  0.00
ATOM    345  H2' CYT    11      -8.521   4.445   4.683  1.00  0.00
ATOM    346 H2'' CYT    11     -10.298   4.395   5.000  1.00  0.00
ATOM    347  C3' CYT    11      -9.586   2.897   3.659  0.00  0.00
ATOM    348  H3' CYT    11      -8.637   2.709   3.154  1.00  0.00
ATOM    349  O3' CYT    11     -10.587   3.321   2.736  0.00  0.00
ATOM    350  P   PDU    12     -10.339   4.412   1.619  1.00  0.00
ATOM    351  O1P PDU    12     -11.300   4.159   0.520  1.00  0.00
ATOM    352  O2P PDU    12      -8.955   4.276   1.109  1.00  0.00
ATOM    353  O5' PDU    12     -10.576   5.882   2.223  1.00  0.00
ATOM    354  C1' PDU    12     -11.054   8.368   5.326  1.00  0.00
ATOM    355  H1' PDU    12     -11.896   8.997   5.659  1.00  0.00
ATOM    356  N1  PDU    12     -10.210   8.120   6.498  1.00  0.00
ATOM    357  C6  PDU    12      -8.682   8.054   6.501  1.00  0.00
ATOM    358  H6  PDU    12      -8.112   8.213   5.528  1.00  0.00
ATOM    359  C5  PDU    12      -7.926   7.783   7.755  1.00  0.00
ATOM    360  C4  PDU    12      -8.629   7.584   9.004  1.00  0.00
ATOM    361  N3  PDU    12     -10.102   7.661   8.965  1.00  0.00
ATOM    362  H3  PDU    12     -10.616   7.527   9.825  1.00  0.00
ATOM    363  C2  PDU    12     -10.857   7.929   7.725  1.00  0.00
ATOM    364  O2  PDU    12     -12.181   7.979   7.817  1.00  0.00
ATOM    365  O4  PDU    12      -8.096   7.333  10.196  1.00  0.00
ATOM    366  O4' PDU    12     -11.612   7.168   4.762  1.00  0.00
ATOM    367  C4' PDU    12     -11.912   7.489   3.436  1.00  0.00
ATOM    368  H4' PDU    12     -12.882   8.011   3.406  1.00  0.00
ATOM    369  C3' PDU    12     -10.805   8.357   2.944  1.00  0.00
ATOM    370  H3' PDU    12     -10.006   7.773   2.459  1.00  0.00
ATOM    371  C2' PDU    12     -10.299   9.031   4.198  1.00  0.00
ATOM    372 H2'' PDU    12     -10.491  10.118   4.182  1.00  0.00
ATOM    373  H2' PDU    12      -9.214   8.902   4.317  1.00  0.00
ATOM    374 HCAP PDU    12      24.819  -8.454  -4.219  1.00  0.00
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ATOM    375  C5' PDU    12     -11.914   6.283   2.547  1.00  0.00
ATOM    376 H5'' PDU    12     -12.432   5.441   3.035  1.00  0.00
ATOM    377  H5' PDU    12     -12.467   6.491   1.616  1.00  0.00
ATOM    378  C10 PDU    12      -6.455   7.720   7.750  1.00  0.00
ATOM    379  C20 PDU    12      -5.678   8.676   8.553  1.00  0.00
ATOM    380  C19 PDU    12      -6.373   9.670   9.389  1.00  0.00
ATOM    381  C18 PDU    12      -5.598  10.609  10.216  1.00  0.00
ATOM    382  H18 PDU    12      -6.135  11.357  10.883  1.00  0.00
ATOM    383  C25 PDU    12      -4.125  10.580  10.180  1.00  0.00
ATOM    384  C17 PDU    12      -3.349  11.525  11.001  1.00  0.00
ATOM    385  C16 PDU    12      -1.877  11.530  10.926  1.00  0.00
ATOM    386  H16 PDU    12      -1.279  12.276  11.541  1.00  0.00
ATOM    387  C15 PDU    12      -1.180  10.567  10.054  1.00  0.00
ATOM    388  H15 PDU    12      -0.045  10.580   9.986  1.00  0.00
ATOM    389  C24 PDU    12      -1.954   9.587   9.271  1.00  0.00
ATOM    390  C14 PDU    12      -1.256   8.586   8.444  1.00  0.00
ATOM    391  H14 PDU    12      -0.119   8.564   8.417  1.00  0.00
ATOM    392  C13 PDU    12      -2.031   7.611   7.656  1.00  0.00
ATOM    393  H13 PDU    12      -1.493   6.840   7.017  1.00  0.00
ATOM    394  C22 PDU    12      -3.504   7.639   7.693  1.00  0.00
ATOM    395  C12 PDU    12      -4.280   6.664   6.906  1.00  0.00
ATOM    396  H12 PDU    12      -3.743   5.888   6.271  1.00  0.00
ATOM    397  C11 PDU    12      -5.753   6.697   6.939  1.00  0.00
ATOM    398  H11 PDU    12      -6.353   5.936   6.343  1.00  0.00
ATOM    399  C21 PDU    12      -4.202   8.639   8.521  1.00  0.00
ATOM    400  C23 PDU    12      -3.427   9.598   9.327  1.00  0.00
ATOM    401  H17 PDU    12      -3.886  12.251  11.692  1.00  0.00
ATOM    402  H19 PDU    12      -7.510   9.712   9.395  1.00  0.00
ATOM    403  H5T PDU    12     -11.487   3.033   2.781  1.00  0.00
ATOM    404  O5T PDU    12     -10.565   3.326   2.798  1.00  0.00
ATOM    405  O2' PDU    12     -22.834 -64.921  48.486  1.00  0.00
ATOM    406  O3' PDU    12     -11.296   9.309   2.040  1.00  0.00
ATOM    407  P   GUA    13     -10.684   9.396   0.557  0.00  0.00
ATOM    408  O1P GUA    13     -10.493   8.020   0.044  0.00  0.00
ATOM    409  O2P GUA    13     -11.480  10.374  -0.222  0.00  0.00
ATOM    410  O5' GUA    13      -9.245  10.038   0.843  0.00  0.00
ATOM    411  C5' GUA    13      -9.152  11.381   1.318  0.00  0.00
ATOM    412  H5' GUA    13      -9.670  11.462   2.275  1.00  0.00
ATOM    413 H5'' GUA    13      -9.634  12.042   0.598  1.00  0.00
ATOM    414  C4' GUA    13      -7.704  11.815   1.496  0.00  0.00
ATOM    415  H4' GUA    13      -7.688  12.812   1.934  1.00  0.00
ATOM    416  O4' GUA    13      -7.025  10.920   2.363  0.00  0.00
ATOM    417  C1' GUA    13      -5.627  10.934   2.018  0.00  0.00
ATOM    418  H1' GUA    13      -5.063  11.437   2.804  1.00  0.00
ATOM    419  N9  GUA    13      -5.178   9.536   1.913  0.00  0.00
ATOM    420  C4  GUA    13      -3.902   9.086   2.137  0.00  0.00
ATOM    421  N3  GUA    13      -2.856   9.849   2.519  0.00  0.00
ATOM    422  C2  GUA    13      -1.736   9.138   2.659  0.00  0.00
ATOM    423  N2  GUA    13      -0.610   9.743   3.033  0.00  0.00
ATOM    424  HN' GUA    13       0.239   9.209   3.141  1.00  0.00
ATOM    425 HN'' GUA    13      -0.608  10.738   3.210  1.00  0.00
ATOM    426  N1  GUA    13      -1.663   7.778   2.438  0.00  0.00
ATOM    427  H1  GUA    13      -0.777   7.309   2.565  1.00  0.00
ATOM    428  C6  GUA    13      -2.727   6.975   2.044  0.00  0.00
ATOM    429  O6  GUA    13      -2.561   5.769   1.870  0.00  0.00
ATOM    430  C5  GUA    13      -3.921   7.732   1.895  0.00  0.00
ATOM    431  N7  GUA    13      -5.201   7.322   1.521  0.00  0.00
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ATOM    432  C8  GUA    13      -5.885   8.427   1.552  0.00  0.00
ATOM    433  H8  GUA    13      -6.946   8.466   1.306  1.00  0.00
ATOM    434  C2' GUA    13      -5.470  11.708   0.712  0.00  0.00
ATOM    435  H2' GUA    13      -4.864  11.149  -0.002  1.00  0.00
ATOM    436 H2'' GUA    13      -5.046  12.696   0.896  1.00  0.00
ATOM    437  C3' GUA    13      -6.900  11.832   0.215  0.00  0.00
ATOM    438  H3' GUA    13      -7.166  10.986  -0.420  1.00  0.00
ATOM    439  O3' GUA    13      -7.101  13.071  -0.460  0.00  0.00
ATOM    440  P   THY    14      -5.858  13.815  -1.145  0.00  0.00
ATOM    441  O1P THY    14      -4.806  12.811  -1.424  0.00  0.00
ATOM    442  O2P THY    14      -6.369  14.677  -2.233  0.00  0.00
ATOM    443  O5' THY    14      -5.350  14.756   0.047  0.00  0.00
ATOM    444  C5' THY    14      -6.246  15.669   0.674  0.00  0.00
ATOM    445  H5' THY    14      -7.146  15.139   0.987  1.00  0.00
ATOM    446 H5'' THY    14      -6.519  16.447  -0.041  1.00  0.00
ATOM    447  C4' THY    14      -5.595  16.312   1.881  0.00  0.00
ATOM    448  H4' THY    14      -6.331  16.925   2.402  1.00  0.00
ATOM    449  O4' THY    14      -5.137  15.305   2.771  0.00  0.00
ATOM    450  C1' THY    14      -3.866  15.705   3.313  0.00  0.00
ATOM    451  H1' THY    14      -3.925  15.712   4.401  1.00  0.00
ATOM    452  N1  THY    14      -2.853  14.721   2.884  0.00  0.00
ATOM    453  C6  THY    14      -2.963  14.056   1.691  0.00  0.00
ATOM    454  H6  THY    14      -3.827  14.242   1.052  1.00  0.00
ATOM    455  C2  THY    14      -1.777  14.518   3.727  0.00  0.00
ATOM    456  O2  THY    14      -1.667  15.109   4.799  0.00  0.00
ATOM    457  N3  THY    14      -0.826  13.614   3.293  0.00  0.00
ATOM    458  H3  THY    14      -0.033  13.458   3.898  1.00  0.00
ATOM    459  C4  THY    14      -0.859  12.904   2.106  0.00  0.00
ATOM    460  O4  THY    14       0.045  12.123   1.818  0.00  0.00
ATOM    461  C5  THY    14      -2.019  13.177   1.290  0.00  0.00
ATOM    462  CM  THY    14      -2.174  12.460  -0.048  0.00  0.00
ATOM    463  H51 THY    14      -1.336  12.716  -0.696  1.00  0.00
ATOM    464  H52 THY    14      -3.107  12.768  -0.519  1.00  0.00
ATOM    465  H53 THY    14      -2.189  11.383   0.119  1.00  0.00
ATOM    466  C2' THY    14      -3.579  17.118   2.815  0.00  0.00
ATOM    467  H2' THY    14      -2.517  17.251   2.609  1.00  0.00
ATOM    468 H2'' THY    14      -3.936  17.861   3.529  1.00  0.00
ATOM    469  C3' THY    14      -4.391  17.163   1.536  0.00  0.00
ATOM    470  H3' THY    14      -3.835  16.727   0.706  1.00  0.00
ATOM    471  O3' THY    14      -4.793  18.499   1.241  0.00  0.00
ATOM    472  P   GUA    15      -3.936  19.373   0.201  0.00  0.00
ATOM    473  O1P GUA    15      -3.529  18.498  -0.921  0.00  0.00
ATOM    474  O2P GUA    15      -4.669  20.630  -0.069  0.00  0.00
ATOM    475  O5' GUA    15      -2.627  19.727   1.062  0.00  0.00
ATOM    476  C5' GUA    15      -2.739  20.489   2.263  0.00  0.00
ATOM    477  H5' GUA    15      -3.379  19.957   2.968  1.00  0.00
ATOM    478 H5'' GUA    15      -3.191  21.453   2.030  1.00  0.00
ATOM    479  C4' GUA    15      -1.378  20.721   2.902  0.00  0.00
ATOM    480  H4' GUA    15      -1.519  21.221   3.860  1.00  0.00
ATOM    481  O4' GUA    15      -0.721  19.475   3.113  0.00  0.00
ATOM    482  C1' GUA    15       0.697  19.690   3.069  0.00  0.00
ATOM    483  H1' GUA    15       1.102  19.628   4.079  1.00  0.00
ATOM    484  N9  GUA    15       1.288  18.623   2.241  0.00  0.00
ATOM    485  C4  GUA    15       2.531  18.066   2.414  0.00  0.00
ATOM    486  N3  GUA    15       3.413  18.390   3.387  0.00  0.00
ATOM    487  C2  GUA    15       4.542  17.682   3.303  0.00  0.00
ATOM    488  N2  GUA    15       5.519  17.878   4.187  0.00  0.00
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ATOM    489  HN' GUA    15       6.373  17.342   4.124  1.00  0.00
ATOM    490 HN'' GUA    15       5.406  18.563   4.921  1.00  0.00
ATOM    491  N1  GUA    15       4.774  16.728   2.333  0.00  0.00
ATOM    492  H1  GUA    15       5.655  16.234   2.337  1.00  0.00
ATOM    493  C6  GUA    15       3.883  16.380   1.326  0.00  0.00
ATOM    494  O6  GUA    15       4.185  15.516   0.506  0.00  0.00
ATOM    495  C5  GUA    15       2.680  17.133   1.413  0.00  0.00
ATOM    496  N7  GUA    15       1.542  17.095   0.608  0.00  0.00
ATOM    497  C8  GUA    15       0.768  17.990   1.147  0.00  0.00
ATOM    498  H8  GUA    15      -0.222  18.220   0.754  1.00  0.00
ATOM    499  C2' GUA    15       0.945  21.090   2.516  0.00  0.00
ATOM    500  H2' GUA    15       1.635  21.057   1.672  1.00  0.00
ATOM    501 H2'' GUA    15       1.317  21.754   3.297  1.00  0.00
ATOM    502  C3' GUA    15      -0.428  21.549   2.052  0.00  0.00
ATOM    503  H3' GUA    15      -0.569  21.300   0.999  1.00  0.00
ATOM    504  O3' GUA    15      -0.630  22.947   2.261  0.00  0.00
ATOM    505  H3T GUA    15      -1.069  23.047   3.110  1.00  0.00
ATOM    506  X   ANI   500     101.090   1.322  -0.815  1.00  0.09
ATOM    507  Y   ANI   500      99.001  -2.119   0.525  1.00  0.09
ATOM    508  Z   ANI   500      96.857   1.180  -1.064  1.00  0.09
ATOM    509  OO  ANI   500      99.099  -0.560  -2.037  1.00  0.08
END
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