NUCLEIC ACIDS

Basic terms and notions

Presentation by

Eva Fadrná

adapted by Radovan Fiala

Literature

Books

Saenger, W., Principles of Nucleic Acid Structure, Springer 1984.

Bloomfield, V. A., Crothers, D. M., Tinoco, I., Nucleic Acids, Structures, Properties, and Functions, Univ. Sci. Books, 2000.

Wuthrich, K., NMR of Proteins and Nucleic Acids, Wiley, 1986.

Review articles

Can be downloaded from https://web.ncbr.muni.cz/~fiala/

Bowater, R. P., Waller, Z. AE., DNA Structure, In: eLS. John Wiley & Sons, Chichester, 2014.

Wijmenga, S. S., van Buuren, B. N. M., The use of NMR methods for conformational studies of nucleic acids, Progr. NMR Spect. 32, (1998), 287-387.

Furtig, B. et al., NMR of RNA, ChemBioChem 4 (2003), 936-962.

equipment, and to Dr. G. E. R. Deacon and the captain and officers of R.R.S. *Discovery II* for their part in making the observations.

[1] Young, F. B., Gerrard, H., and Jevons, W., *Phil. Mag.*, **40**, 149 (1920).

[2] Longuet-Higgins, M. S., *Mon. Not. Roy. Astro. Soc., Geophys. Supp.*, **5**, 285 (1949).

[3] Von Arx, W. S., Woods Hole Papers in Phys. Oceanog. Meteor., **11** (3) (1950).

[4] Ekman, V. W., *Arkiv. Mat. Astron. Fysik.* (*Stockholm*), **2** (11) (1905).

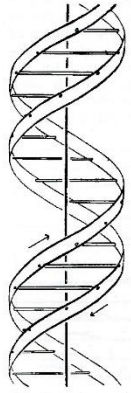
MOLECULAR STRUCTURE OF NUCLEIC ACIDS

A Structure for Deoxyribose Nucleic Acid

WE wish to suggest a structure for the salt of deoxyribose nucleic acid (D.N.A.). This structure has novel features which are of considerable biological interest.

A structure for nucleic acid has already been proposed by Pauling and Corey[1]. They kindly made their manuscript available to us in advance of publication. Their model consists of three inter-twined chains, with the phosphates near the fibre axis, and the bases on the outside. In our opinion, this structure is unsatisfactory for two reasons: (1) We believe that the material which gives the X-ray diagrams is the salt, not the free acid. Without the acidic hydrogen atoms it is not clear what forces would hold the structure together, especially as the negatively charged phosphates near the axis will repel each other. (2) Some of the van der Waals distances appear to be too small.

Another three-chain structure has also been suggested by Fraser (in the press). In his model the phosphates are on the outside and the bases on the inside, linked together by hydrogen bonds. This structure as described is rather ill-defined, and for this reason we shall not comment on it.



This figure is purely diagrammatic. The two ribbons symbolize the two phosphate—sugar chains, and the horizontal rods the pairs of bases holding the chains together. The vertical line marks the fibre axis

We wish to put forward a radically different structure for the salt of deoxyribose nucleic acid. This structure has two helical chains each coiled round the same axis (see diagram). We have made the usual chemical assumptions, namely, that each chain consists of phosphate di-ester groups joining β-D-deoxy-ribofuranose residues with 3′,5′ linkages. The two chains (but not their bases) are related by a dyad perpendicular to the fibre axis. Both chains follow right-handed helices, but owing to the dyad the sequences of the atoms in the two chains run in opposite directions. Each chain loosely resembles Furberg's[2] model No. 1; that is, the bases are on the inside of the helix and the phosphates on the outside. The configuration of the sugar and the atoms near it is close to Furberg's 'standard configuration', the sugar being roughly perpendicular to the attached base. There is a residue on each chain every 3·4 A. in the z-direction. We have assumed an angle of 36° between adjacent residues in the same chain, so that the structure repeats after 10 residues on each chain, that is, after 34 A. The distance of a phosphorus atom from the fibre axis is 10 A. As the phosphates are on the outside, cations have easy access to them.

The structure is an open one, and its water content is rather high. At lower water contents we would expect the bases to tilt so that the structure could become more compact.

The novel feature of the structure is the manner in which the two chains are held together by the purine and pyrimidine bases. The planes of the bases are perpendicular to the fibre axis. They are joined together in pairs, a single base from one chain being hydrogen-bonded to a single base from the other chain, so that the two lie side by side with identical z-co-ordinates. One of the pair must be a purine and the other a pyrimidine for bonding to occur. The hydrogen bonds are made as follows: purine position 1 to pyrimidine position 1; purine position 6 to pyrimidine position 6.

If it is assumed that the bases only occur in the structure in the most plausible tautomeric forms (that is, with the keto rather than the enol configurations) it is found that only specific pairs of bases can bond together. These pairs are: adenine (purine) with thymine (pyrimidine), and guanine (purine) with cytosine (pyrimidine).

In other words, if an adenine forms one member of a pair, on either chain, then on these assumptions the other member must be thymine; similarly for guanine and cytosine. The sequence of bases on a single chain does not appear to be restricted in any way. However, if only specific pairs of bases can be formed, it follows that if the sequence of bases on one chain is given, then the sequence on the other chain is automatically determined.

It has been found experimentally[3,4] that the ratio of the amounts of adenine to thymine, and the ratio of guanine to cytosine, are always very close to unity for deoxyribose nucleic acid.

It is probably impossible to build this structure with a ribose sugar in place of the deoxyribose, as the extra oxygen atom would make too close a van der Waals contact.

The previously published X-ray data[5,6] on deoxyribose nucleic acid are insufficient for a rigorous test of our structure. So far as we can tell, it is roughly compatible with the experimental data, but it must be regarded as unproved until it has been checked against more exact results. Some of these are given in the following communications. We were not aware of the details of the results presented there when we devised our structure, which rests mainly though not entirely on published experimental data and stereochemical arguments.

It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material.

Full details of the structure, including the conditions assumed in building it, together with a set of co-ordinates for the atoms, will be published elsewhere.

We are much indebted to Dr. Jerry Donohue for constant advice and criticism, especially on inter-atomic distances. We have also been stimulated by a knowledge of the general nature of the unpublished experimental results and ideas of Dr. M. H. F. Wilkins, Dr. R. E. Franklin and their co-workers at King's College, London. One of us (J. D. W.) has been aided by a fellowship from the National Foundation for Infantile Paralysis.

J. D. WATSON
F. H. C. CRICK

Medical Research Council Unit for the Study of the Molecular Structure of Biological Systems,
Cavendish Laboratory, Cambridge.
April 2.

[1] Pauling, L., and Corey, R. B., *Nature*, **171**, 346 (1953); *Proc. U.S. Nat. Acad. Sci.*, **39**, 84 (1953).

[2] Furberg, S., *Acta Chem. Scand.*, **6**, 634 (1952).

[3] Chargaff, E., for references see Zamenhof, S., Brawerman, G., and Chargaff, E., *Biochim. et Biophys. Acta*, **9**, 402 (1952).

[4] Wyatt, G. R., *J. Gen. Physiol.*, **36**, 201 (1952).

[5] Astbury, W. T., Symp. Soc. Exp. Biol. 1, Nucleic Acid, 66 (Camb. Univ. Press, 1947).

[6] Wilkins, M. H. F., and Randall, J. T., *Biochim. et Biophys. Acta*, **10**, 192 (1953).

Molecular Structure of Deoxypentose Nucleic Acids

WHILE the biological properties of deoxypentose nucleic acid suggest a molecular structure containing great complexity, X-ray diffraction studies described here (cf. Astbury[1]) show the basic molecular configuration has great simplicity. The purpose of this communication is to describe, in a preliminary way, some of the experimental evidence for the polynucleotide chain configuration being helical, and existing in this form when in the natural state. A fuller account of the work will be published shortly.

The structure of deoxypentose nucleic acid is the same in all species (although the nitrogen base ratios alter considerably) in nucleoprotein, extracted or in cells, and in purified nucleate. The same linear group of polynucleotide chains may pack together parallel in different ways to give crystalline[1-3], semi-crystalline or paracrystalline material. In all cases the X-ray diffraction photograph consists of two regions, one determined largely by the regular spacing of nucleotides along the chain, and the other by the longer spacings of the chain configuration. The sequence of different nitrogen bases along the chain is not made visible.

Oriented paracrystalline deoxypentose nucleic acid ('structure *B*' in the following communication by Franklin and Gosling) gives a fibre diagram as shown in Fig. 1 (cf. ref. 4). Astbury suggested that the strong 3·4-A. reflexion corresponded to the inter-nucleotide repeat along the fibre axis. The ~ 34 A. layer lines, however, are not due to a repeat of a polynucleotide composition, but to the chain configuration repeat, which causes strong diffraction as the nucleotide chains have higher density than the interstitial water. The absence of reflexions on or near the meridian immediately suggests a helical structure with axis parallel to fibre length.

Diffraction by Helices

It may be shown[5] (also Stokes, unpublished) that the intensity distribution in the diffraction pattern of a series of points equally spaced along a helix is given by the squares of Bessel functions. A uniform continuous helix gives a series of layer lines of spacing corresponding to the helix pitch, the intensity distribution along the nth layer line being proportional to the square of J_n, the nth order Bessel function. A straight line may be drawn approximately through

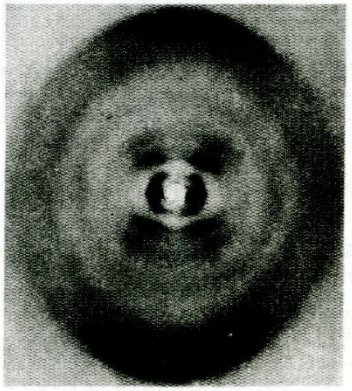


Fig. 1. Fibre diagram of deoxypentose nucleic acid from *B. coli*. Fibre axis vertical

the innermost maxima of each Bessel function and the origin. The angle this line makes with the equator is roughly equal to the angle between an element of the helix and the helix axis. If a unit repeats n times along the helix there will be a meridional reflexion (J_0^2) on the nth layer line. The helical configuration produces side-bands on this fundamental frequency, the effect[5] being to reproduce the intensity distribution about the origin around the new origin, on the nth layer line, corresponding to C in Fig. 2.

We will now briefly analyse in physical terms some of the effects of the shape and size of the repeat unit or nucleotide on the diffraction pattern. First, if the nucleotide consists of a unit having circular symmetry about an axis parallel to the helix axis, the whole diffraction pattern is modified by the form factor of the nucleotide. Second, if the nucleotide consists of a series of points on a radius at right-angles to the helix axis, the phases of radiation scattered by the helices of different diameter passing through each point are the same. Summation of the corresponding Bessel functions gives reinforcement for the inner-

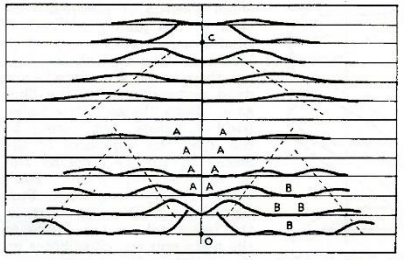
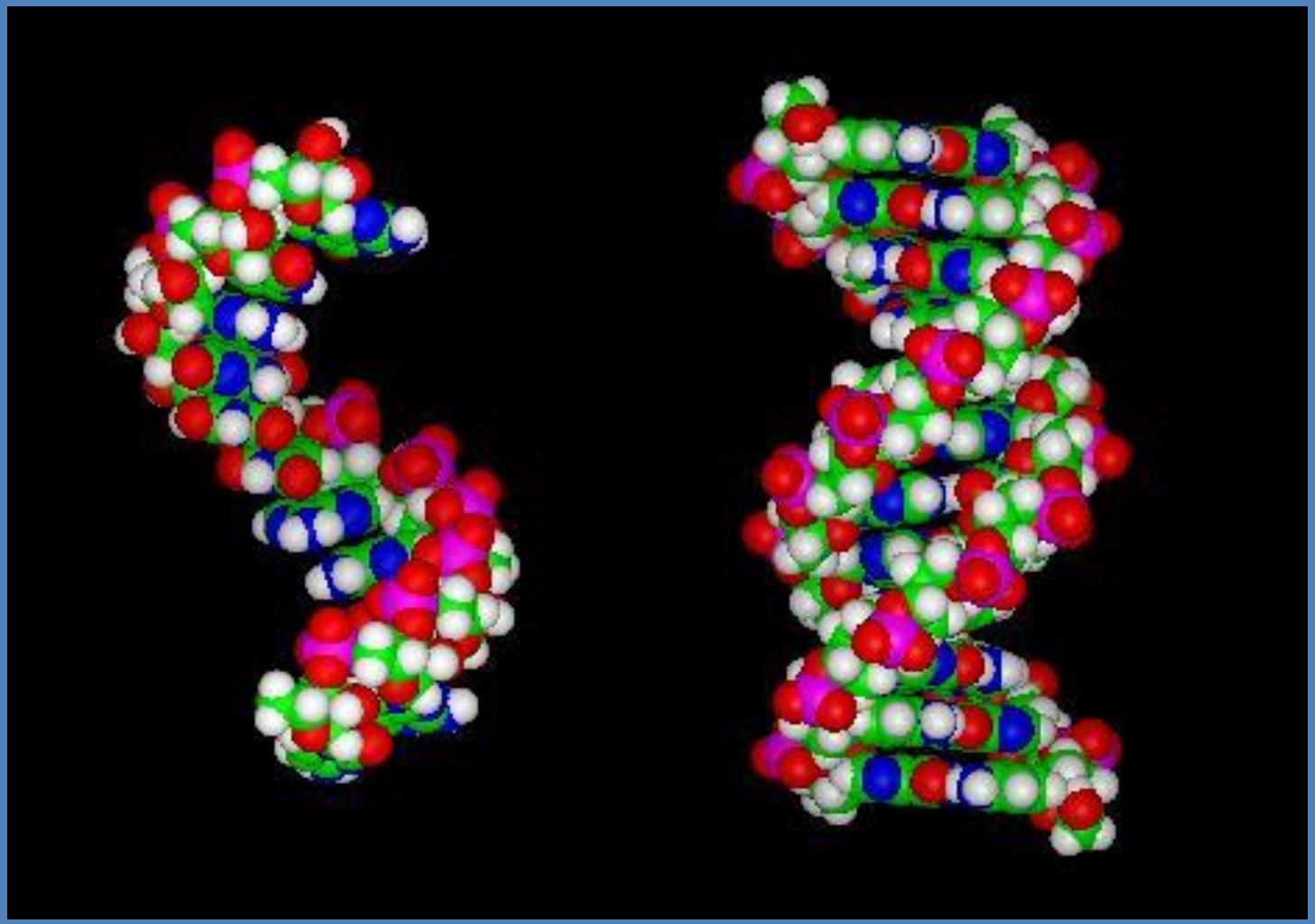


Fig. 2. Diffraction pattern of system of helices corresponding to structure of deoxypentose nucleic acid. The squares of Bessel functions are plotted about 0 on the equator and on the first, second, third and fifth layer lines for half of the nucleotide mass at 20 A. diameter and remainder distributed along a radius, the mass at a given radius being proportional to the radius. About C on the tenth layer line similar functions are plotted for an outer diameter of 12 A.

RNA vs DNA



Single strand A-RNA **B-DNA duplex**

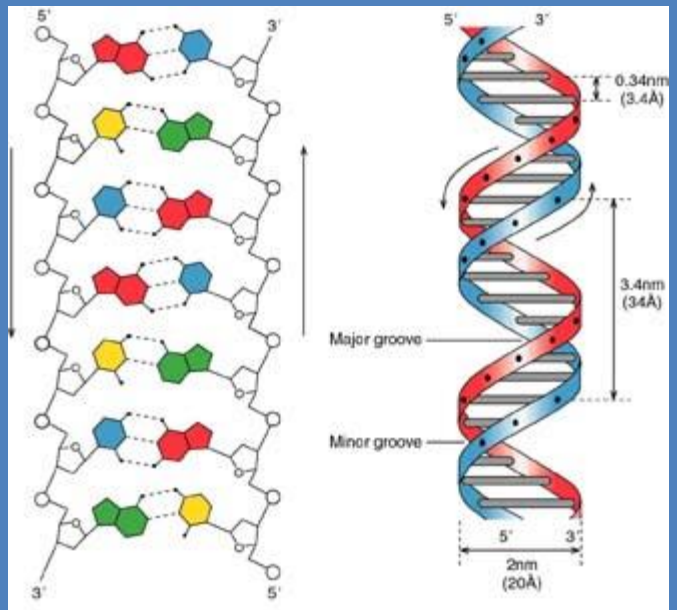
Length of NA

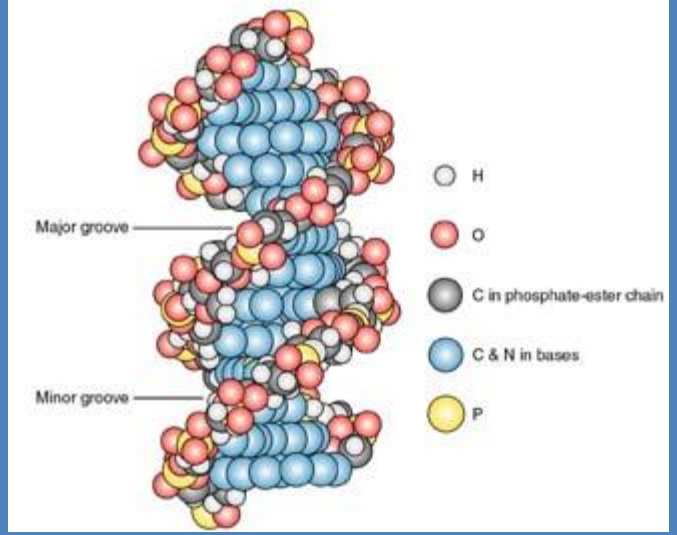
Total length of DNA in a human cell	1 m	(1000 km)
DNA in typical human chromozome	1 cm	(10 km)
DNA from bacterial chromozome	1 mm	
Diameter of typical human cell	0.01 mm	
Diameter of folded DNA	0.1 μm	(0.1 m)
Diameter of DNA fiber	1 nm	(1 mm)
Diameter of atom		1 Å

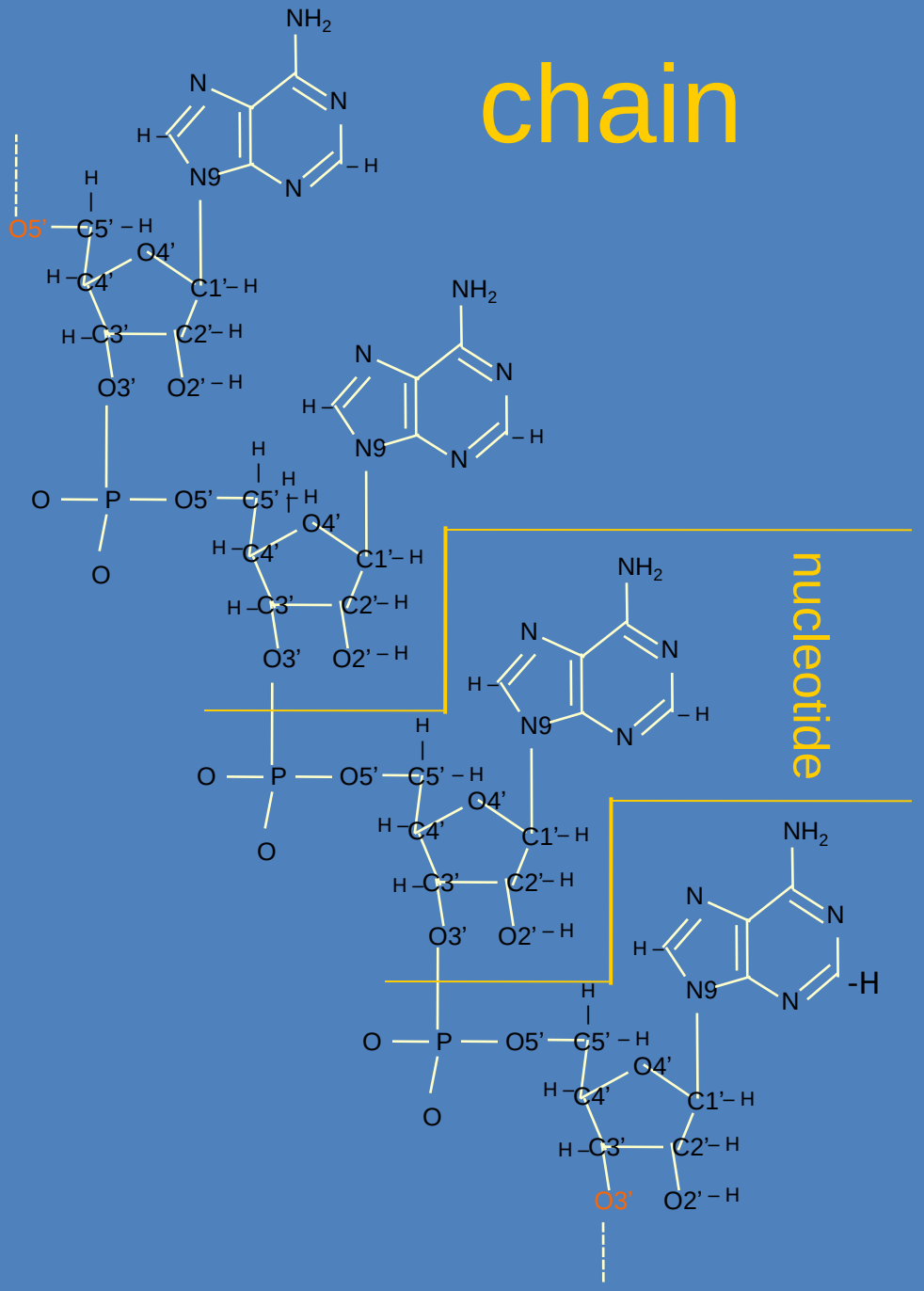
(multiplied by 10^6)

$\Rightarrow$ 1 chromozome would be 10km long with fiber diameter of 1 mm and it would fold into 10 cm diameter $\Rightarrow$ extraordinary DNA flexibility

Nucleotide chain

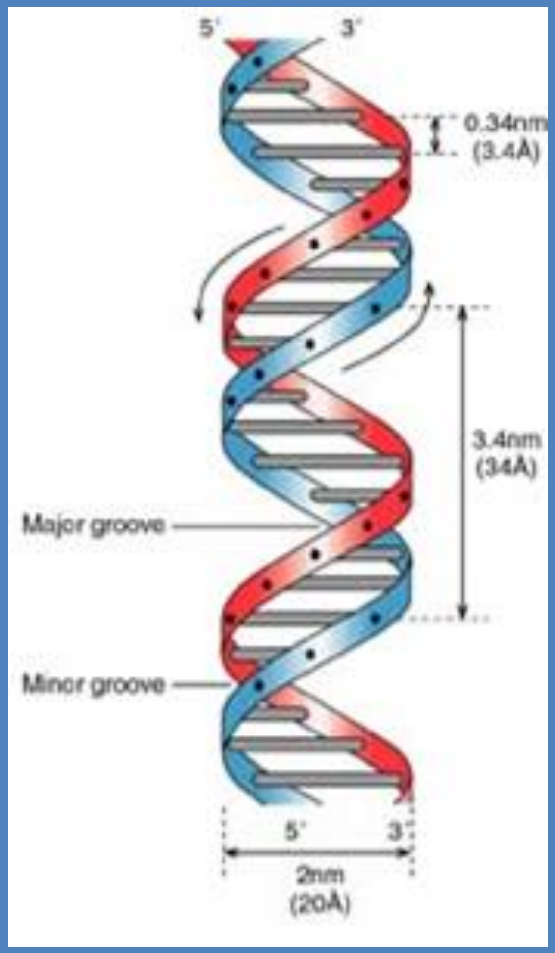


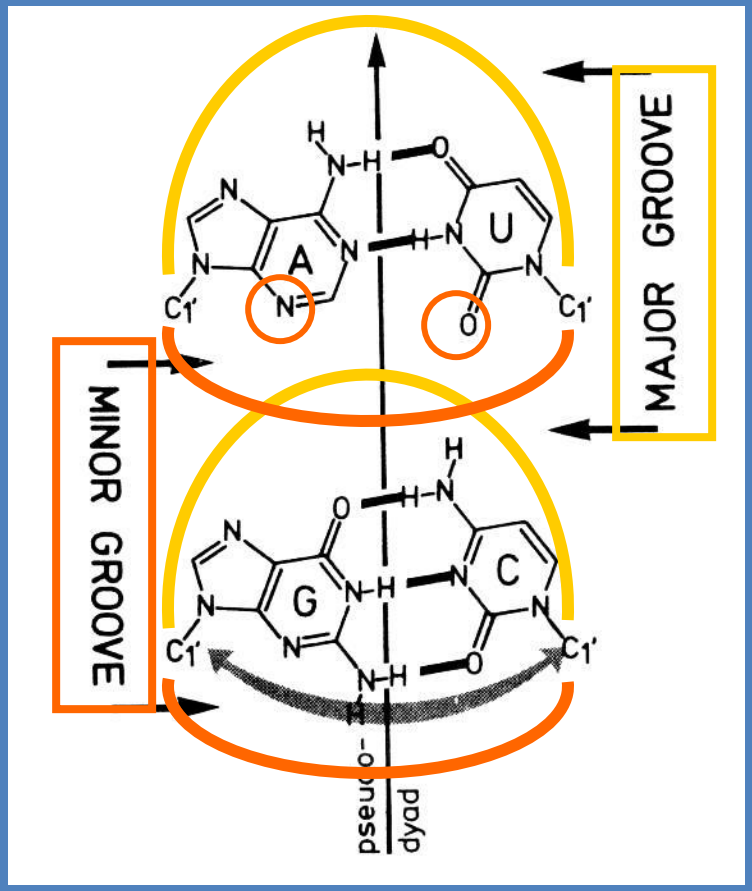




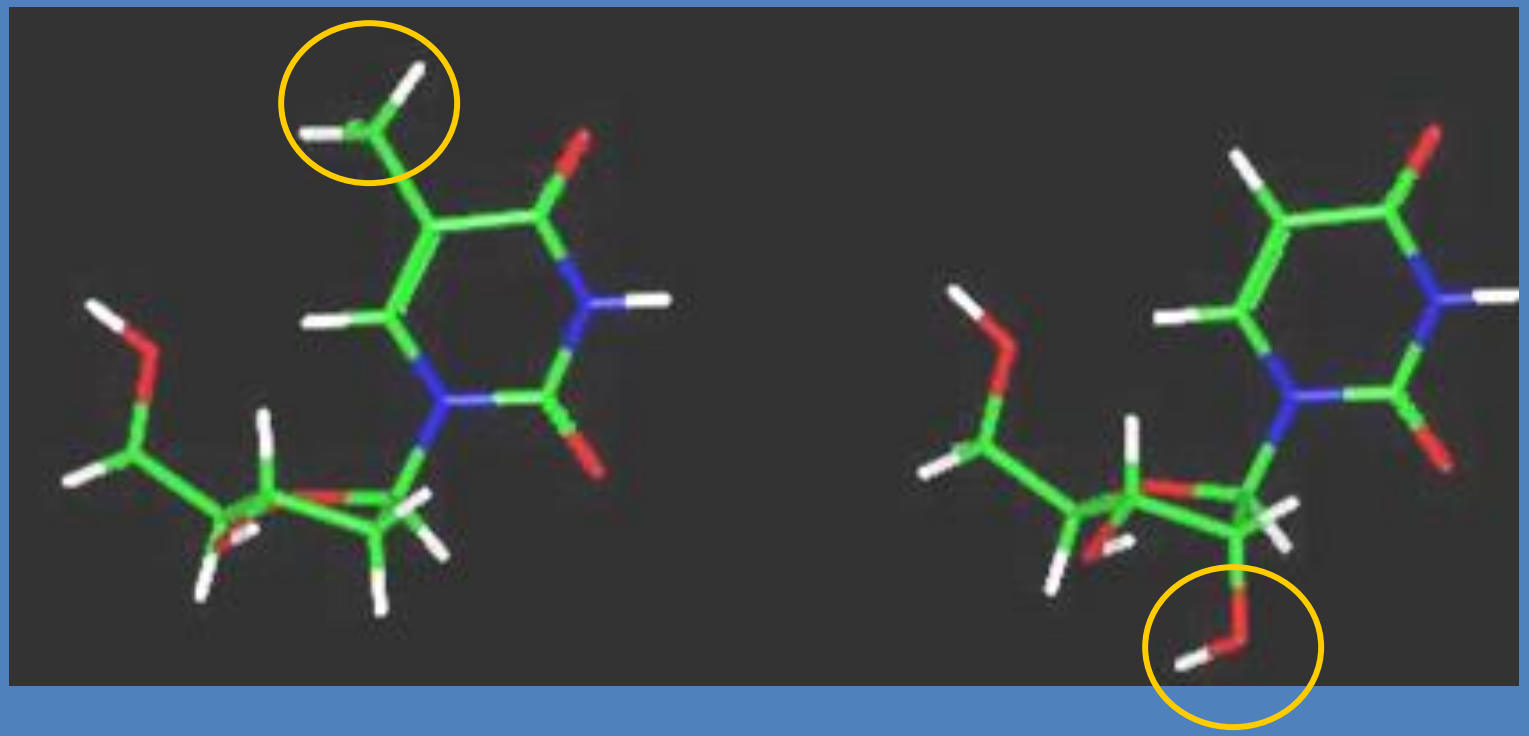
Grooves

major vs. minor





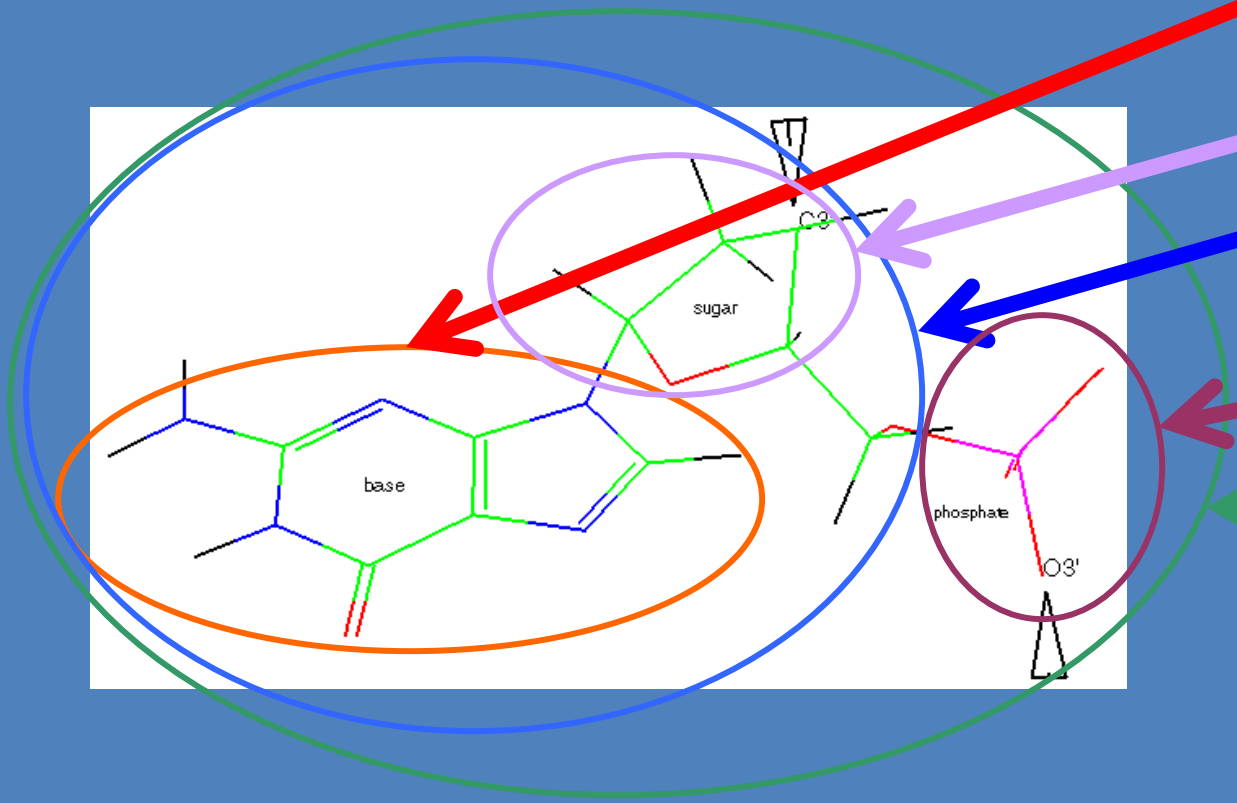
RNA vs DNA



deoxythymidine

uridine

Nukleotide/nukleoside



base
+
sugar (ribose/deoxyribose)

nukleoside
+
phosphate

nukleotide

Bases

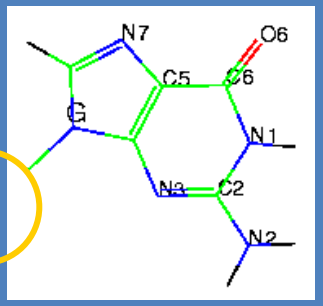
DNA

RNA

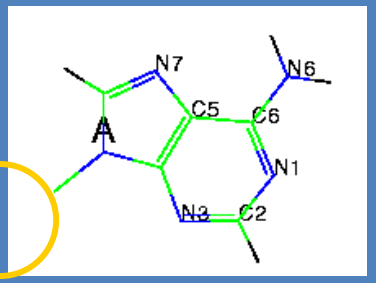
PURINES

PYRIMIDINES

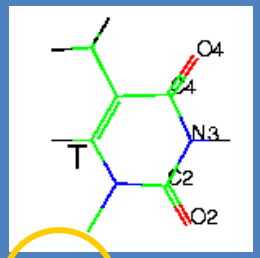
Guanin (Gua)



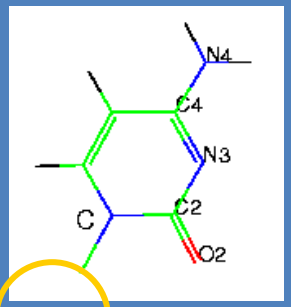
Adenin (Ade)



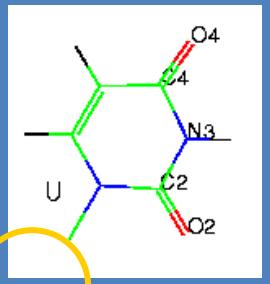
Thymin (Thy)



Cytosin (Cyt)

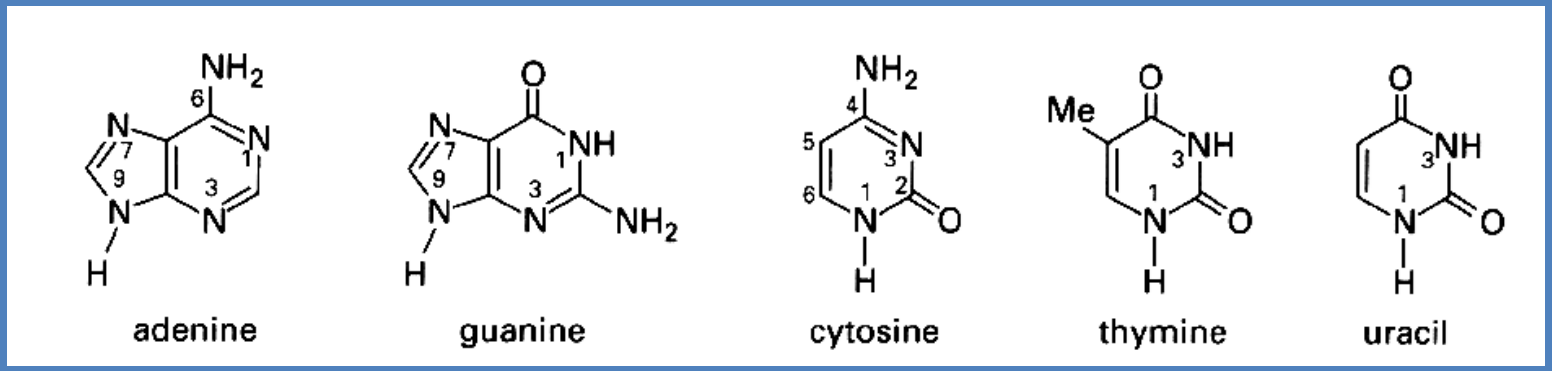


Uracil (Ura)

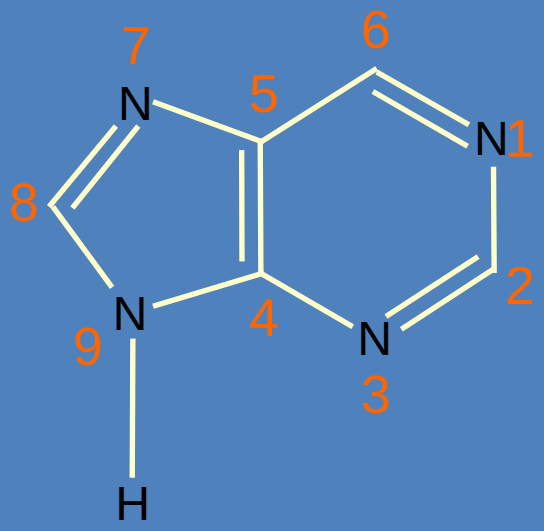


sugar

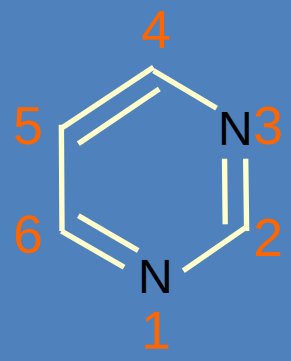
Base numbering



PURINES



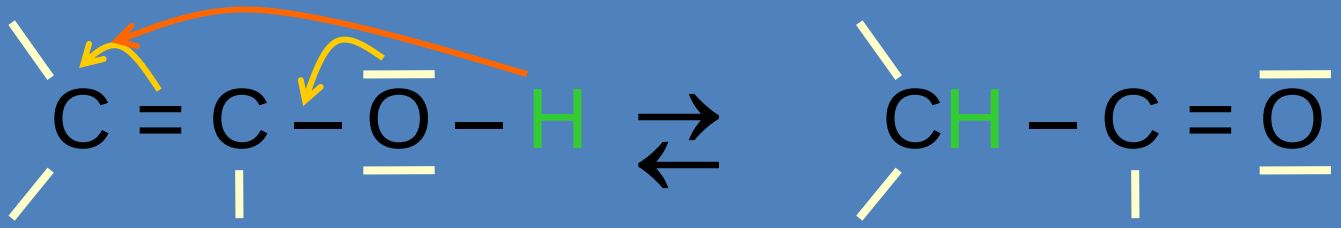
PYRIMIDINES



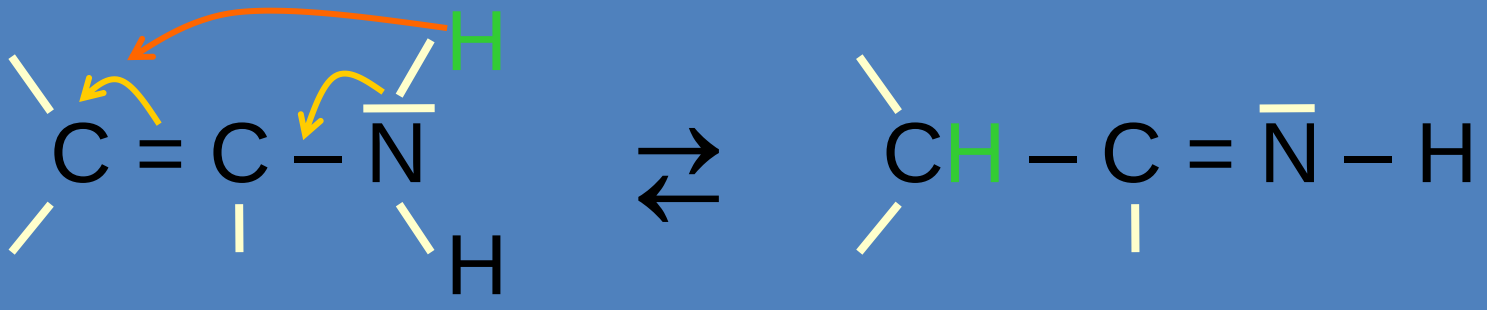
Base tautomerism

fysiolog. conditions

enol ↔ keto

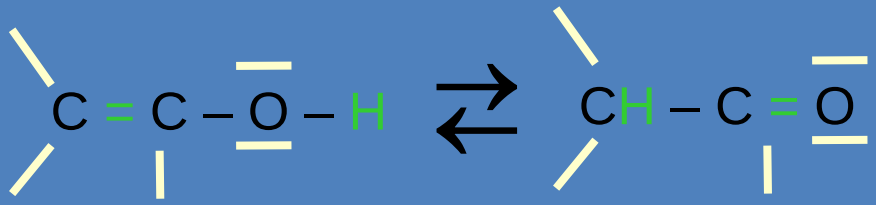


enamin ↔ imin

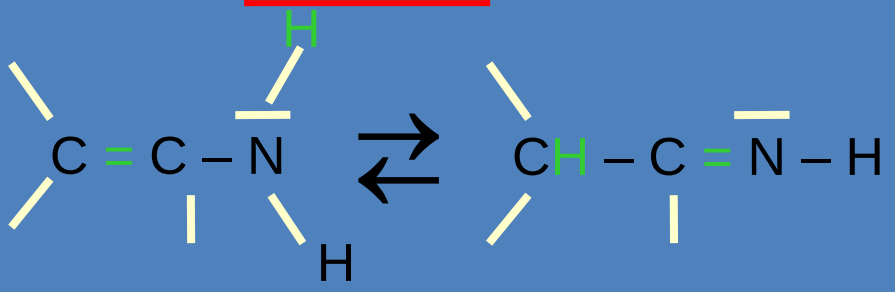


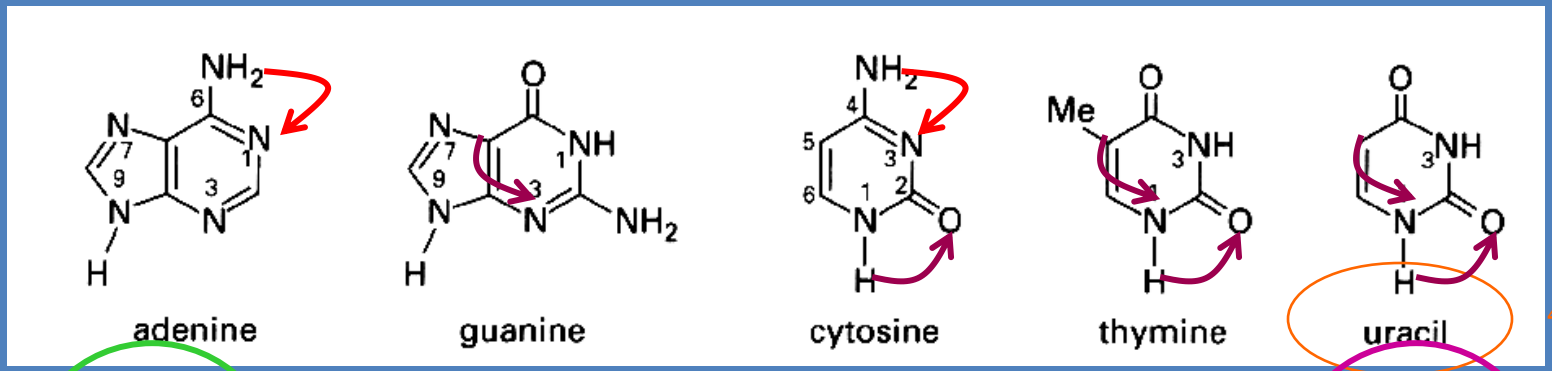
Base tautomerism

enol ↔ keto

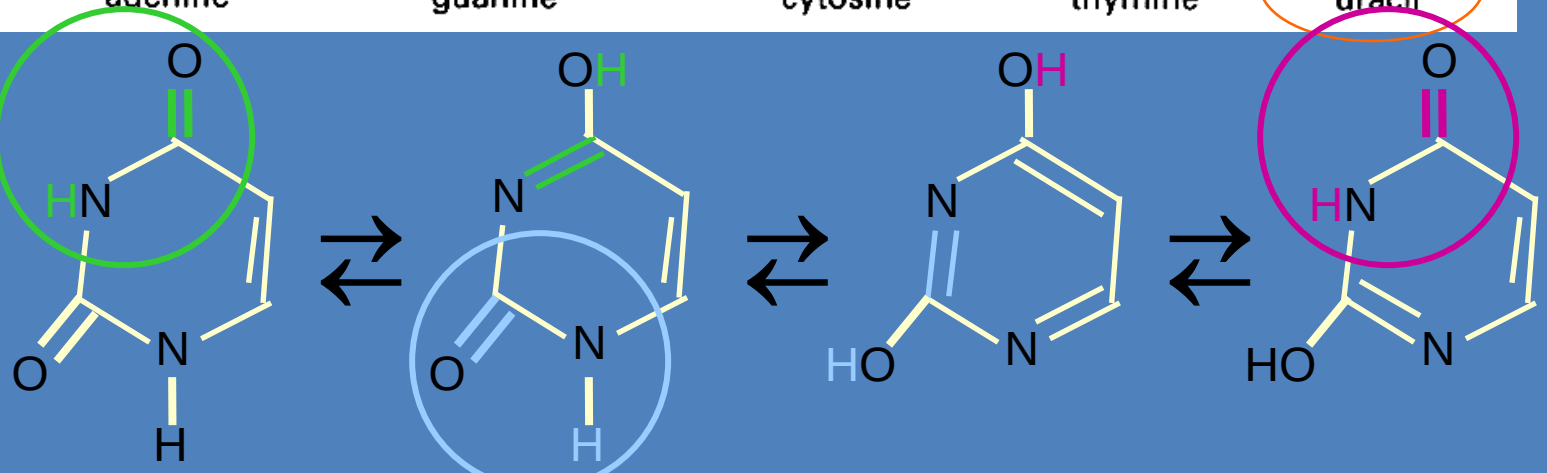


enamin ↔ imin

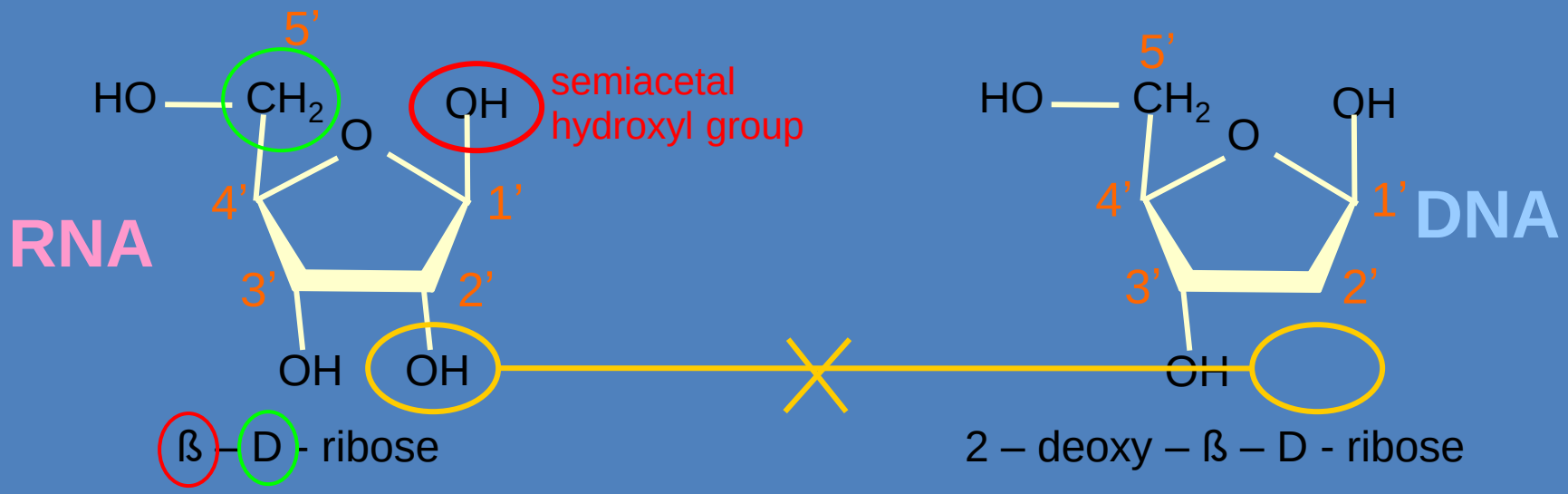




4 tautomers



Sugar - pentoses



Other aldopentoses: arabinose, xylose, lyxose

semiacetal hydroxyl group + base → N-glycosidic bond → nukleoside

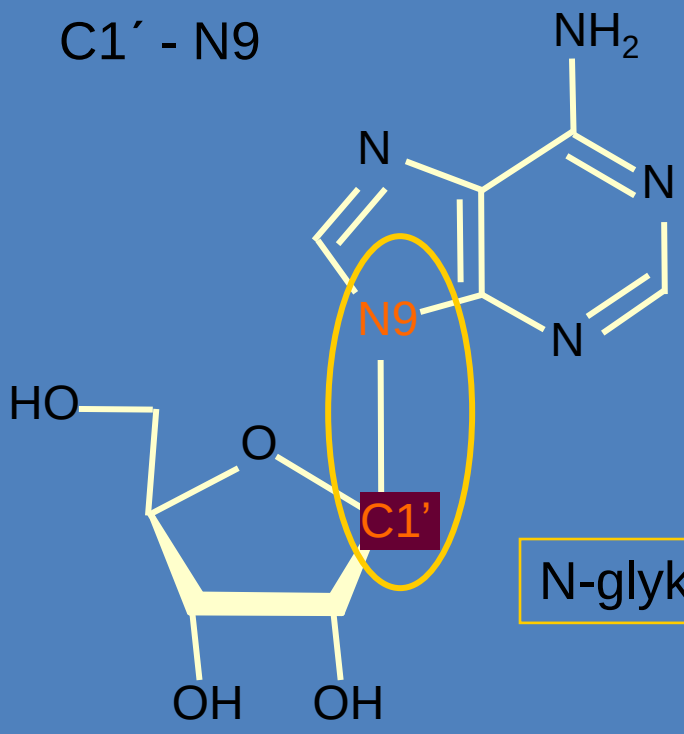
C1´ - N1 pyrimidines

C1´ - N9 purines

Nukleosides

purines

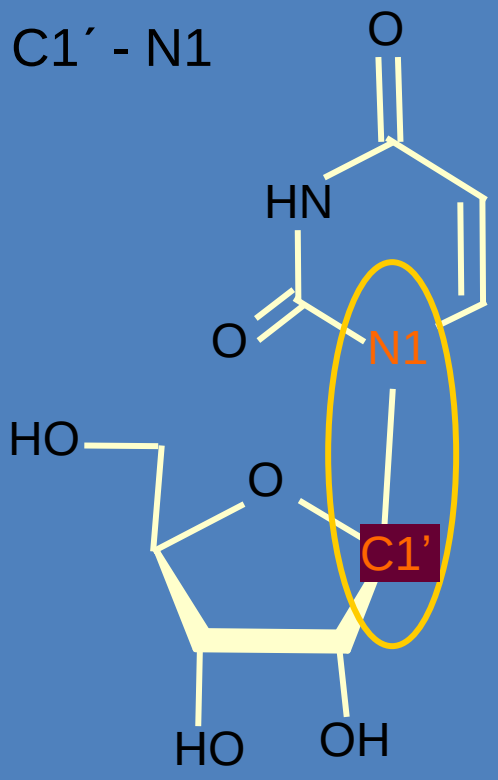
C1´ - N9



N-glykosidic bond

pyrimidines

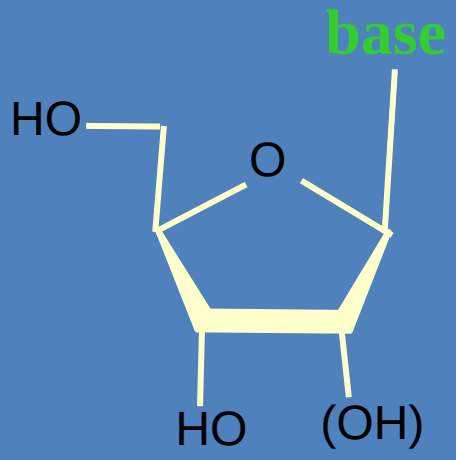
C1´ - N1



Nukleosides

Ribonukleosides

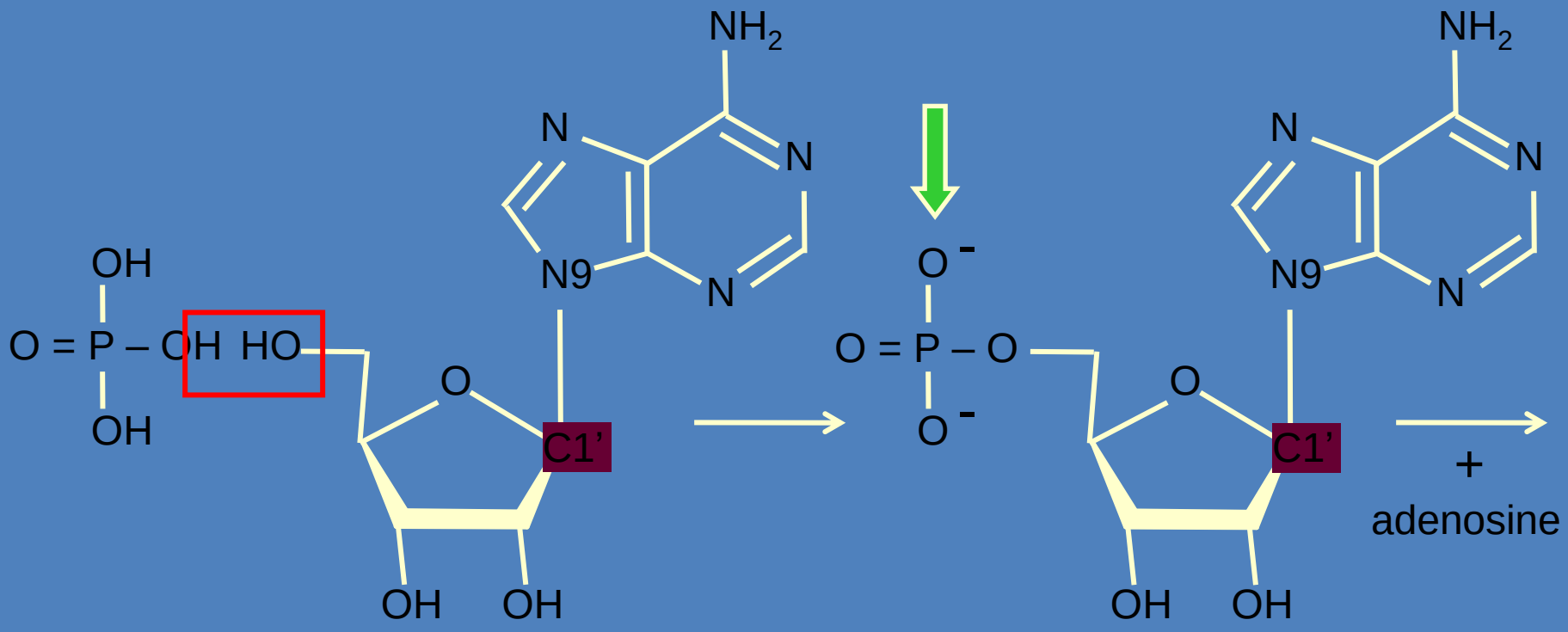
uridine	= U
cytidine	= C
adenosine	= A
guanosine	= G



Deoxyribonukleosides

deoxythymidine	= dT
deoxycytidine	= dC
deoxyadenosine	= dA
deoxyguanosine	= dG

Phosphate group



acid + alcohol → ester

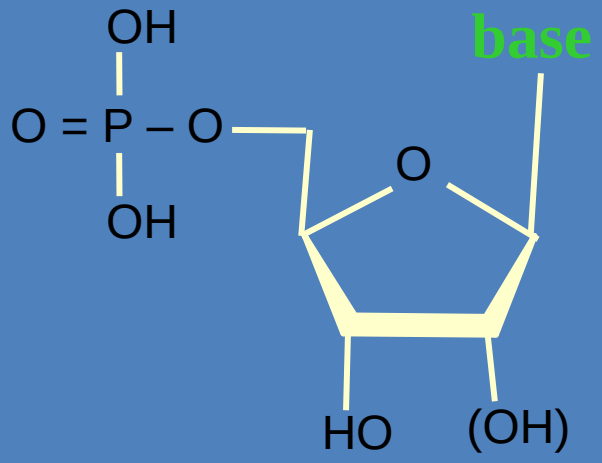
orthophosphoric acid

H_3PO_4

adenosine

adenosine(mono)phosphate (AMP)

Nukleotides



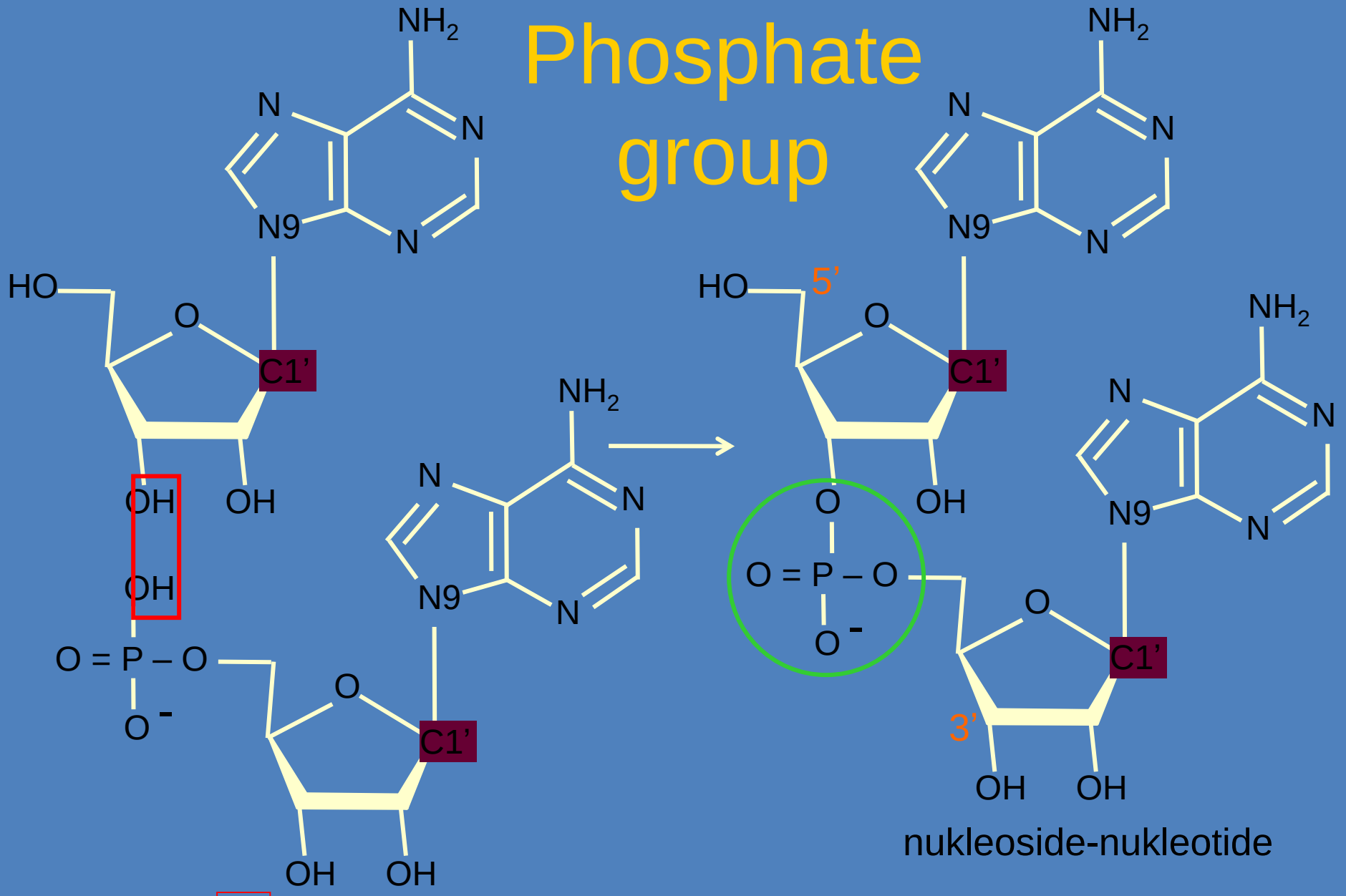
Ribonucleotides

uridyl acid	= uridine – 5´monophosphate		= UMP, pU
cytidyl acid	= cytidin	-“-	= CMP, pC
adenyl acid	= adenosin	-“-	= AMP, pA
guanyl acid	= guanosin	-“-	= GMP, pG

Deoxyribonucleotides

deoxytymidyl acid	= 2´deoxythymidine-5´-monophosphate			= dTMP, pdT
deoxycytidyl acid	= -“-	cytidin	-“-	= dCMP, pdC
deoxyadenyl acid	= -“-	adenosin	-“-	= dAMP, pdA
deoxyguanyl acid	= -“-	guanosin	-“-	= dGMP, pdG

Phosphate group



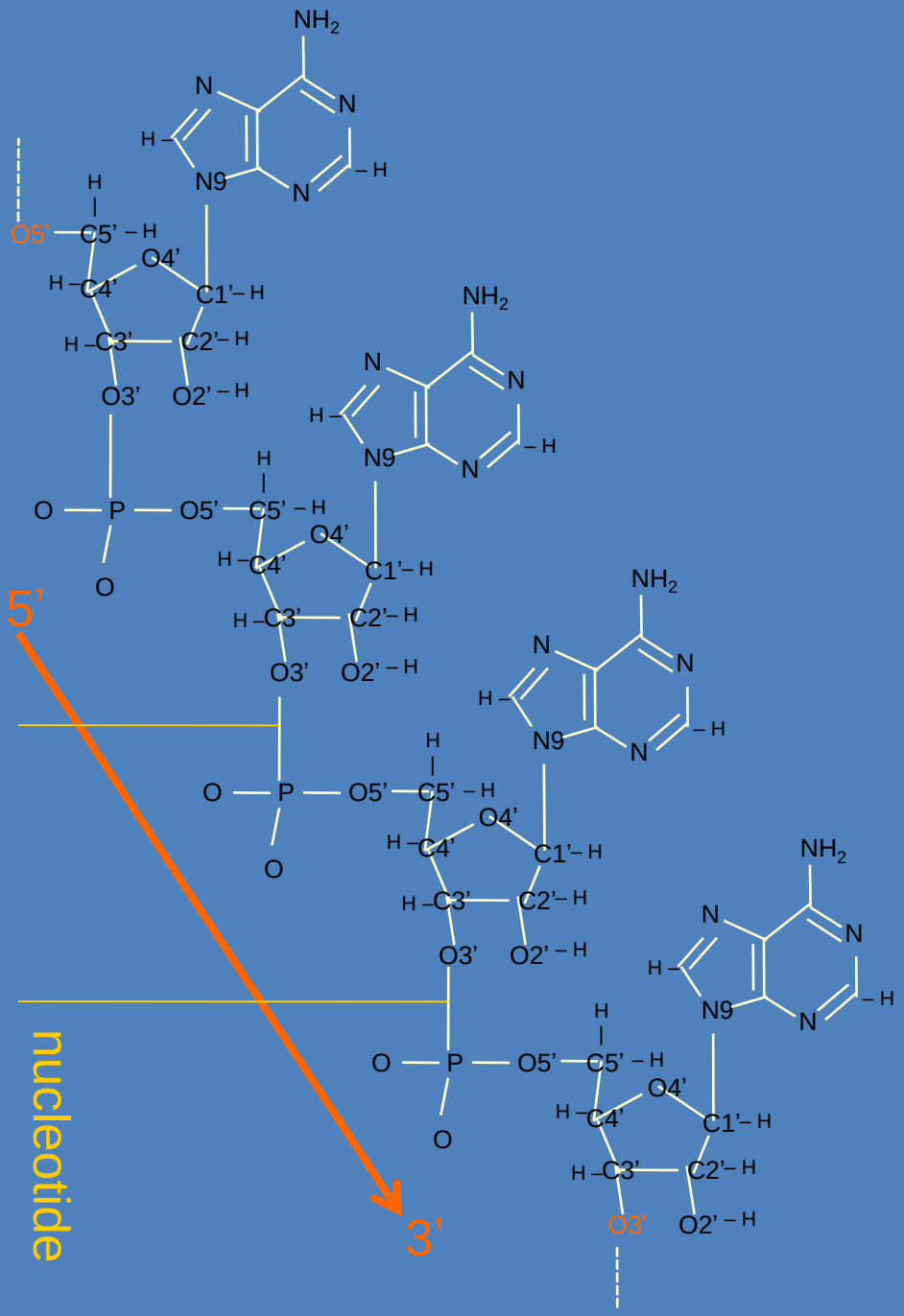
nukleoside-nukleotide

ApA

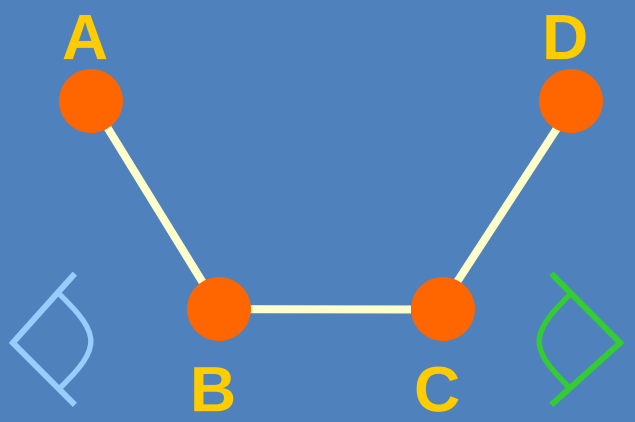
alcohol + acid → diester

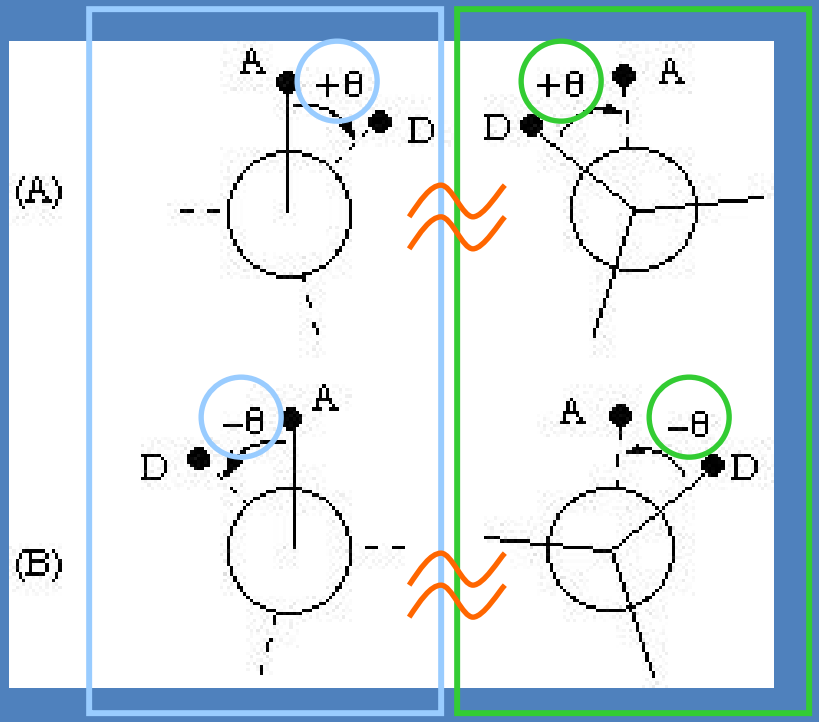
(ester)

Nucleotide chain



Torsion angle





<0º, 360º>

<-180º, 180º>

Torsion angle

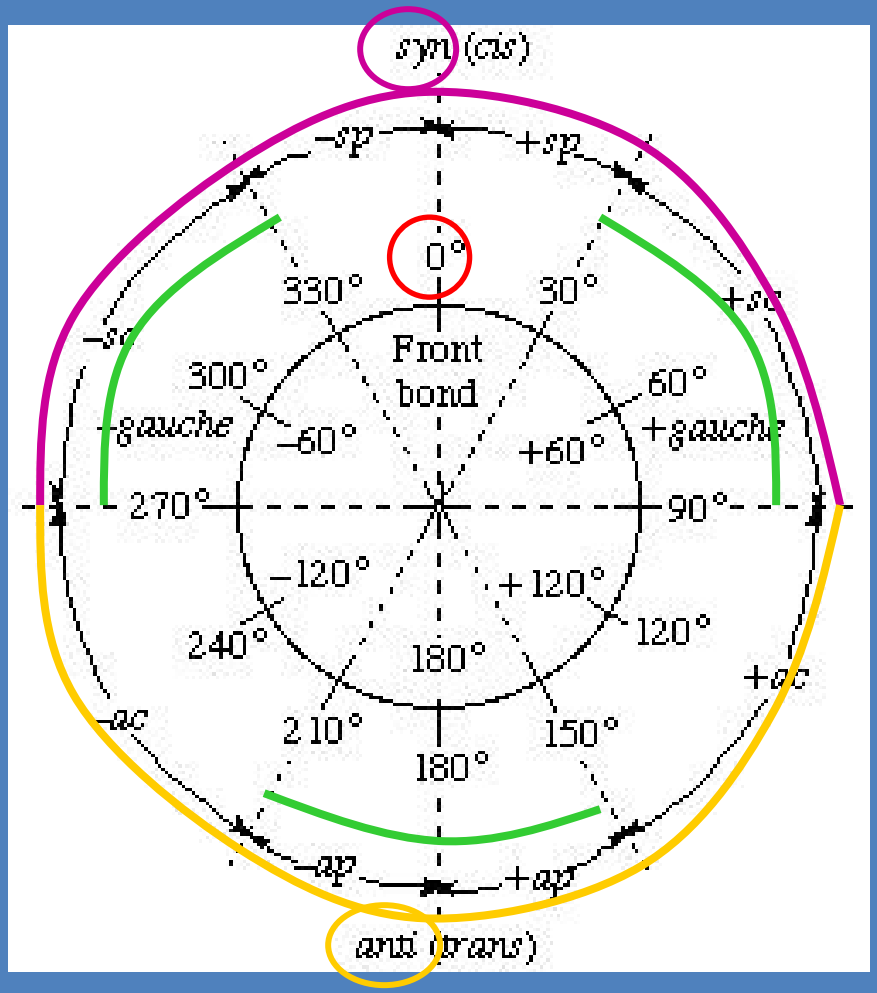
synperiplanar (sp)

-gauche (-g)

synclinal (sc)

anticlinal (ac)

antiperiplanar (ap)

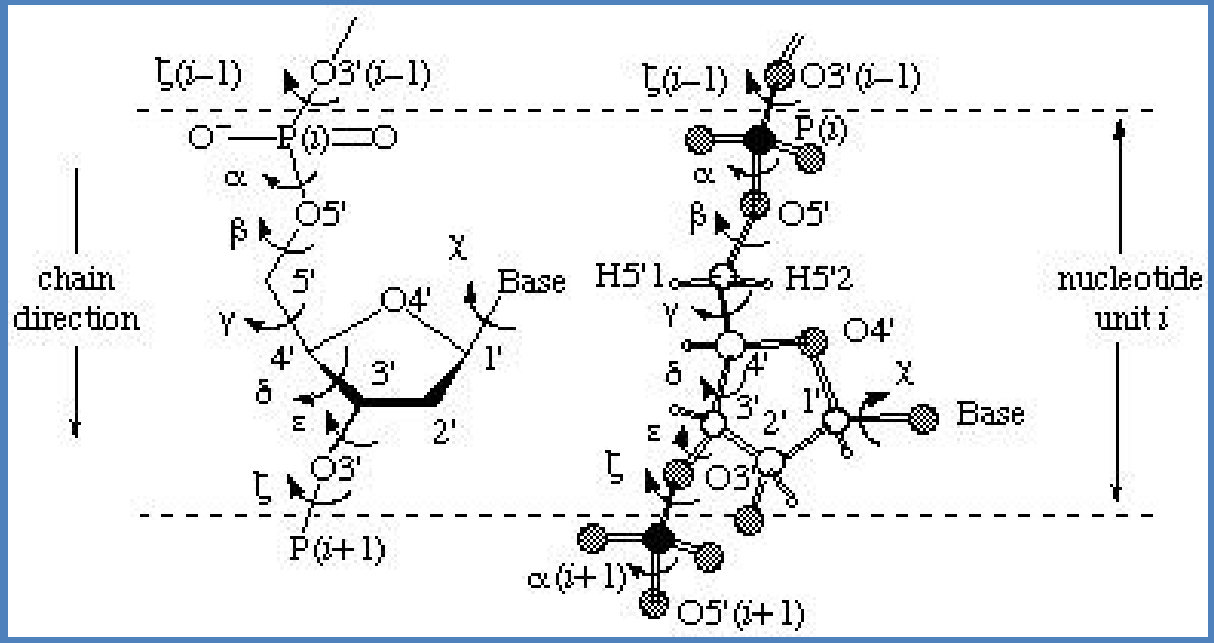


+gauche (+g)

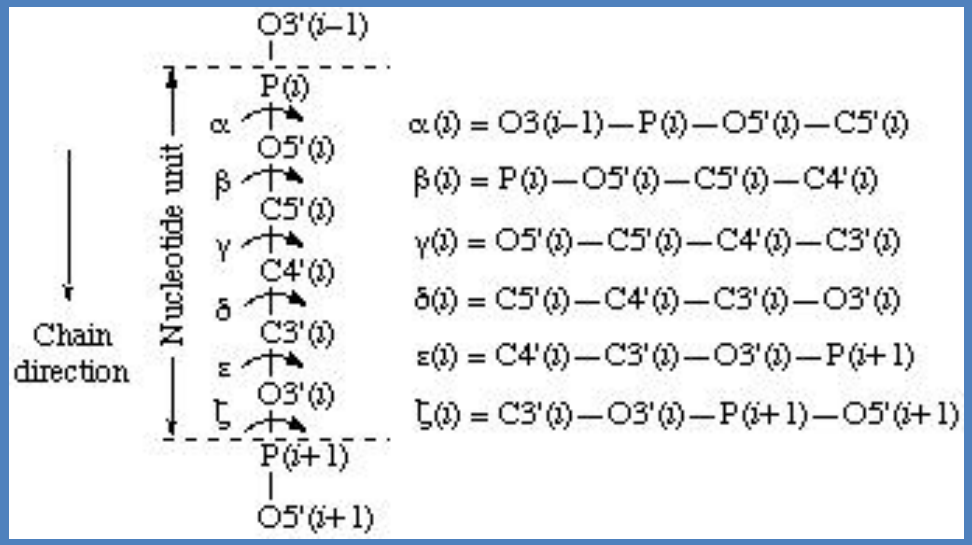
trans (t)

Torsion angles in NA

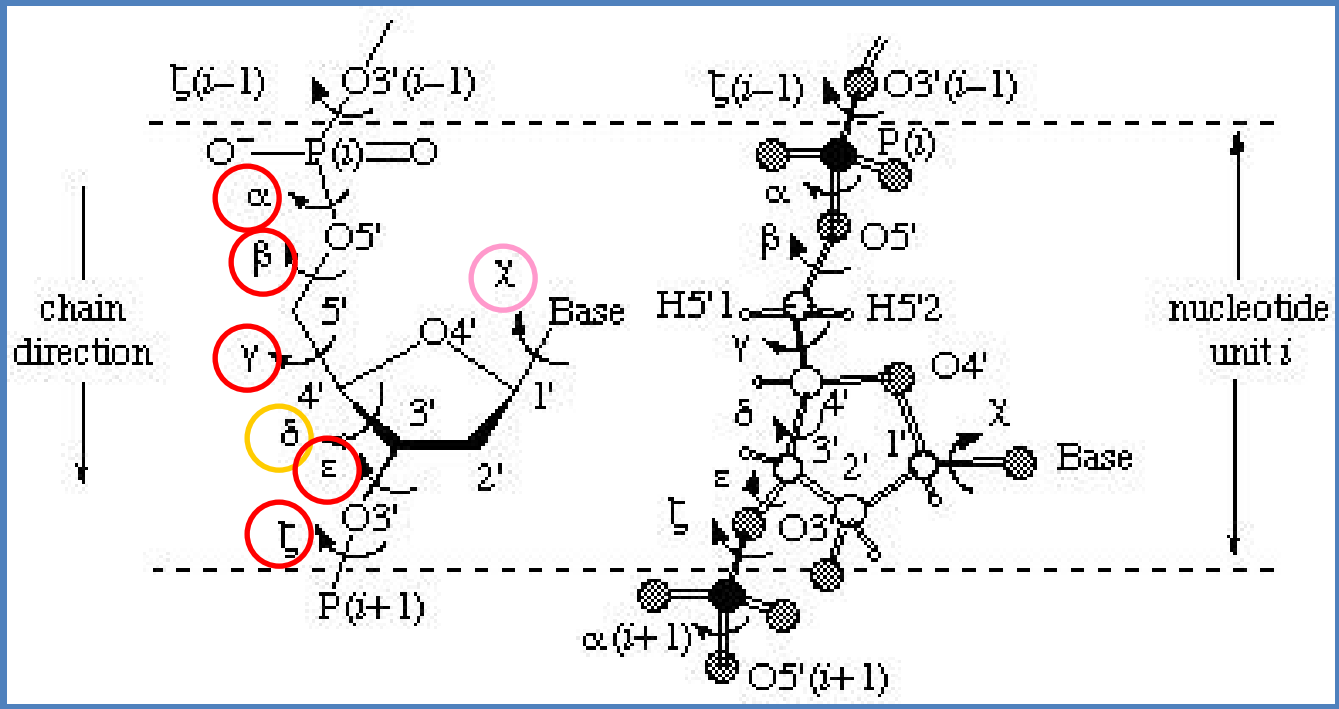
Sugar-phosphate backbone



Torsion angles cont.



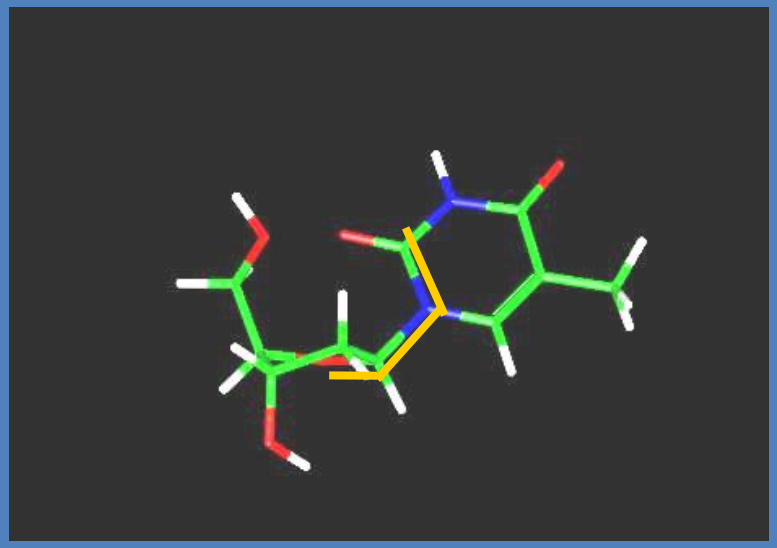
α
β
γ
δ
ε
ζ
χ

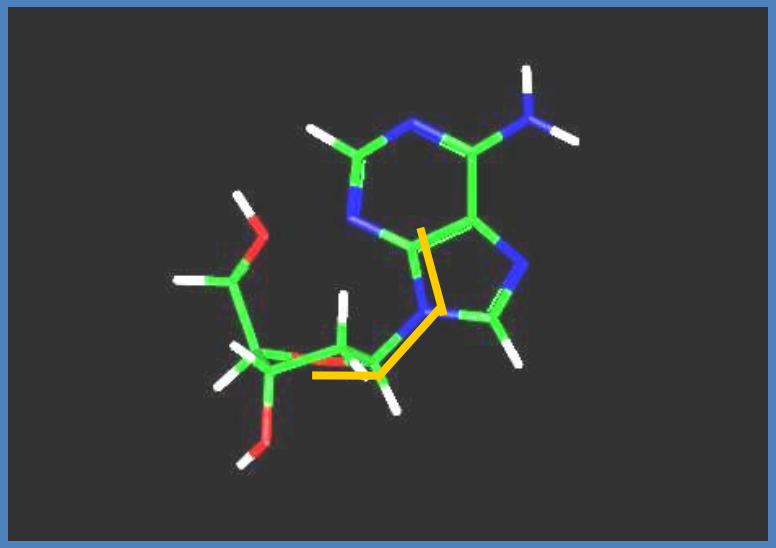


Torsion angle χ

SYN:

Pyrimidines: O2 above the sugar ring
Purines: 6-member purine ring above the sugar ring





Torsion angle χ

Orientation around the C1' – N glycosidic bond

O4' – C1' – N1 – C2 pyrimidines
O4' – C1' – N9 – C4 purines

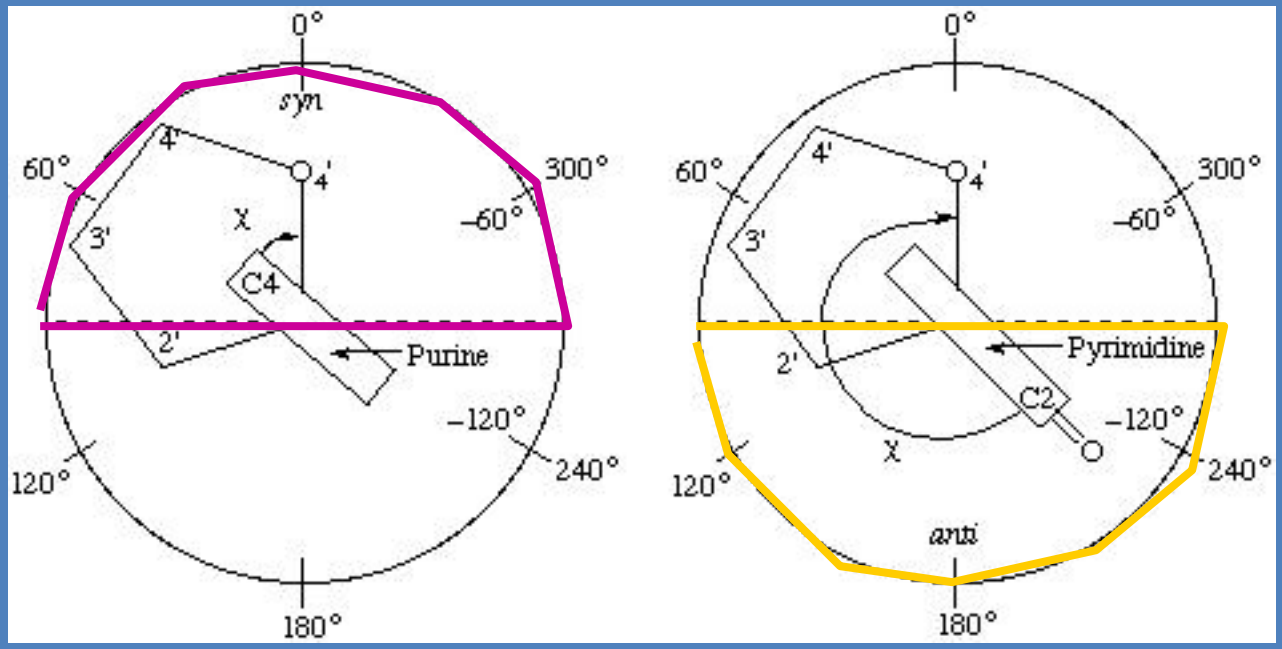
SYN

χ

<0°, 90°>

+

<270°, 360°>



ANTI

χ

<90°, 270°>

Torion χ – border intervals

high-syn (corresponds to +ac) … 90^o + intrudes into anti

high-anti (corresponds -sc) … 270^o + intrudes into syn

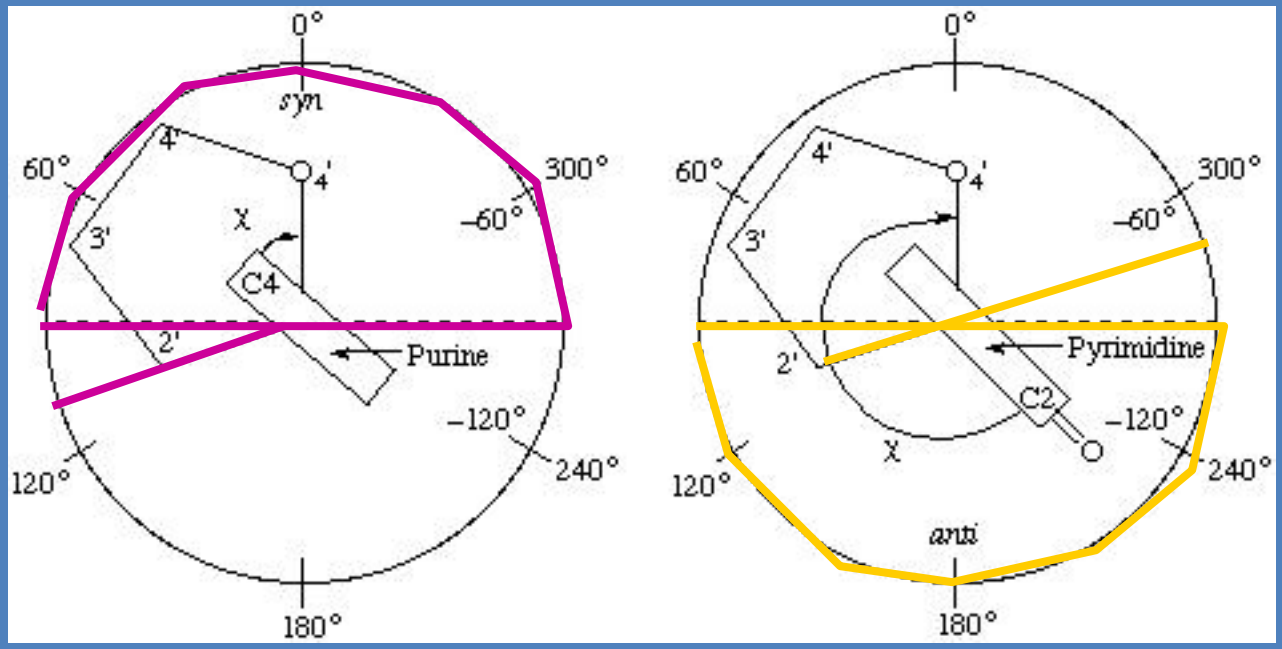
SYN

$\chi \in$

$<0^o, 90^o>$

+

$<270^o, 360^o>$



ANTI

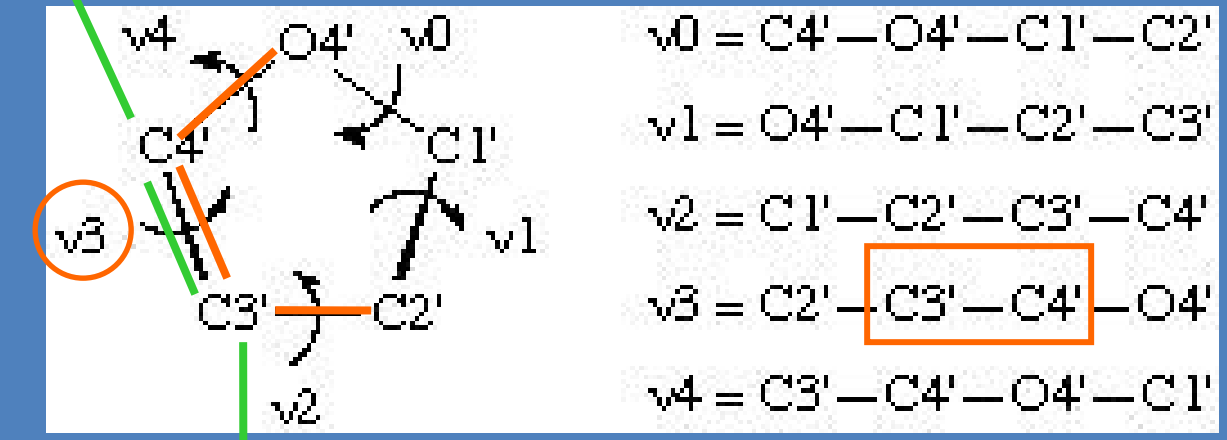
$\chi \in$

$<90^o, 270^o>$

Torsion angles in DNA

Angle	B-DNA	A-DNA
α	-40.7	-74.8
β	-135.6	-179.1
γ	-37.4	58.9
δ	139.5	78.2
ε	-133.2	-155.0
ζ	-156.9	-67.1
χ	-101.9	-158.9

Sugar conformation



C5'

δ

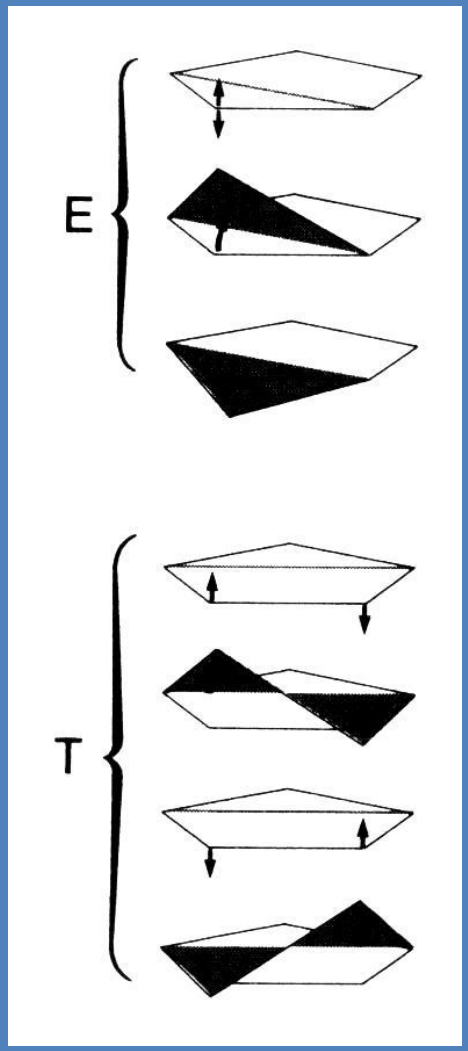
O3'

endocyclic

δ … C5' – C4' – C3' – O3' exocyclic

δ, ν3 relation

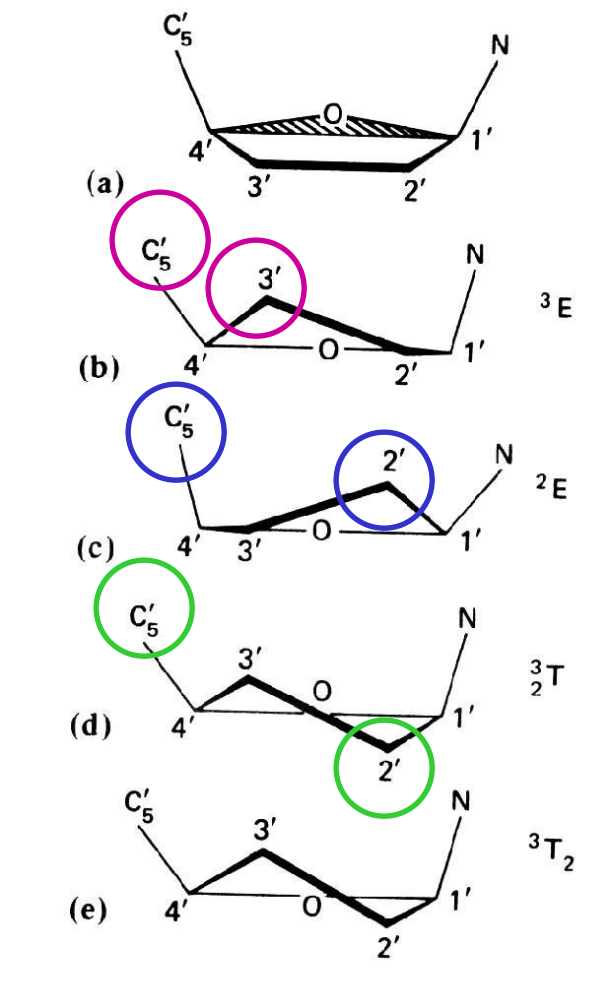
„Puckering“ of the sugar ring



Envelope — 4 atoms in a plane, the 5th above or below

Twist — 3 atoms in a plane, the 4th and the 5th on the oposite sides of the plane

Definition of the puckering modes



The sugar ring is not planar

C1’ – O4’ – C4’ plane

With respect to C5’
- endo
- exo

Envelope C3’-endo ^3E (prevalent in RNA)

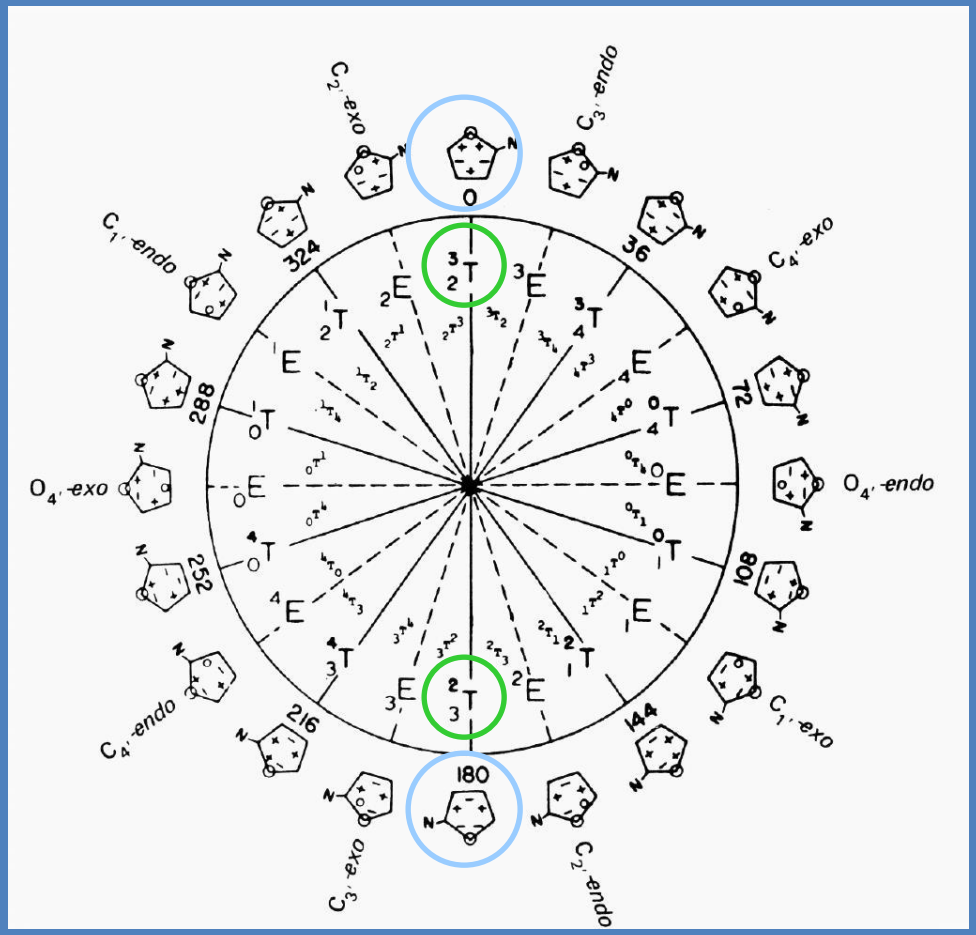
Envelope C2’-endo ^2E (prevalent in DNA)

symmetric Twist C2’-exo-C3’-endo ^3_2T

Non-symmetric Twist C3’-endo-C2’-exo $^3T_2$

Pseudorotation cycle

Theoretically – infinite number of conformations, can be characterized by maximum torsion angle (degree of pucker) and pseudorotation phase angle
Torsion angles are not independent (ring closed)



Pseudorotation phase angle P

$$\tan P = \frac{(\nu_4 + \nu_1) - (\nu_3 + \nu_0)}{2 \cdot \nu_2 \cdot (\sin 36^\circ + \sin 72^\circ)}$$

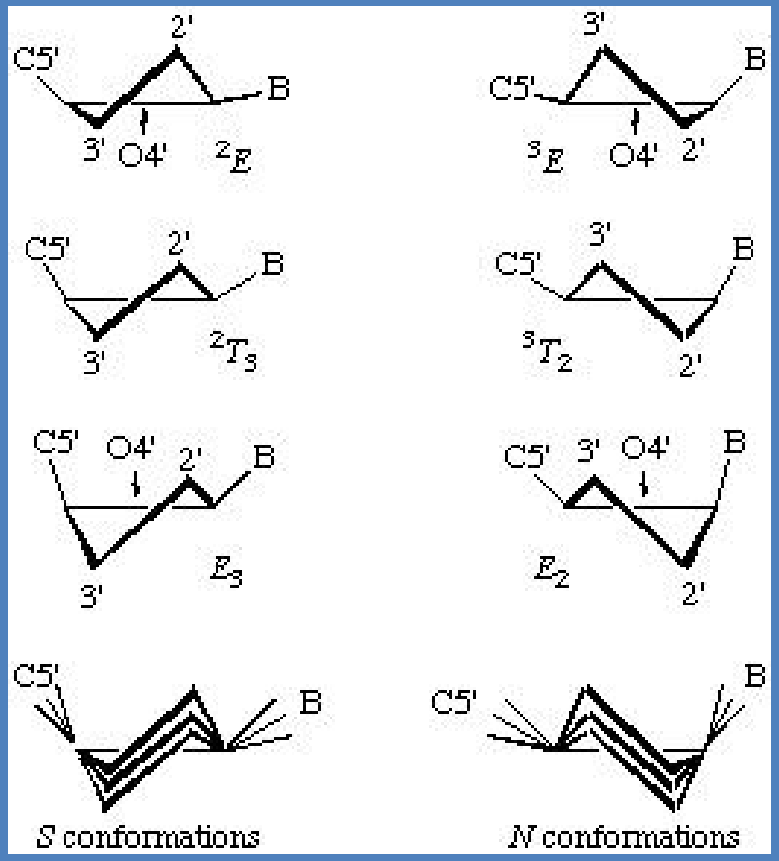
P = 0° :

symmetric Twist C2'-exo-C3'-endo $^{3}_{2}T$

P = 180° :

asymmetric Twist C2'-endo-C3'-exo $^{2}_{3}T$

ν_{max} amplitude



Maximum out-of-plane pucker

$$\nu_{max} = \nu_2 / \cos(P)$$

P, ν_j relation

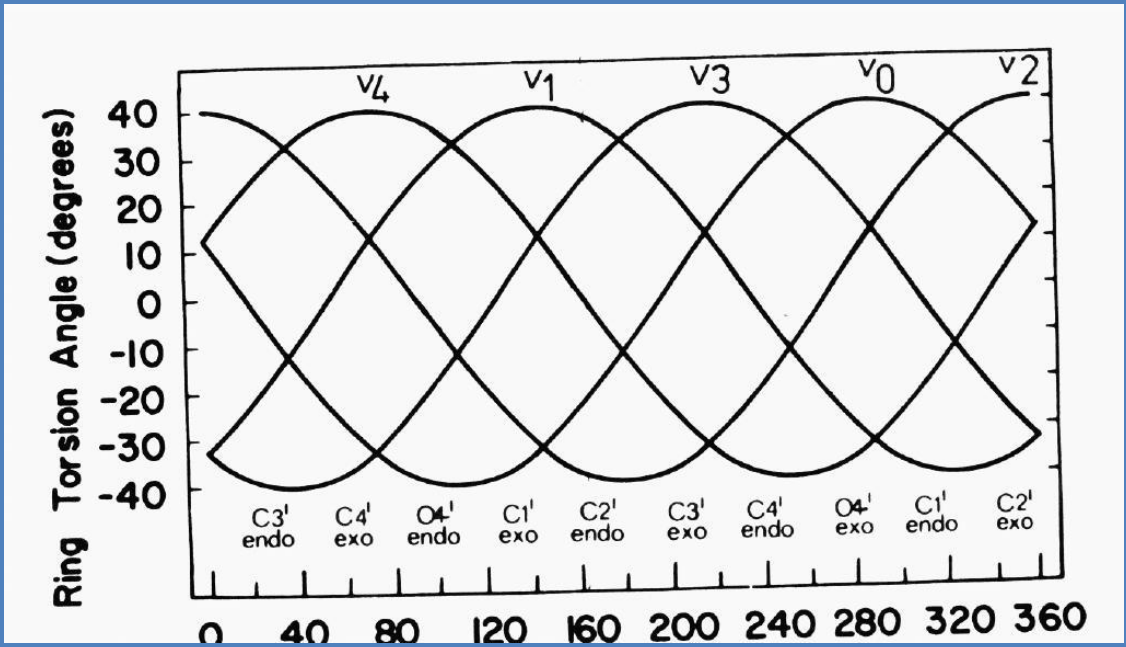
P value defines unambiguously all endocyclic torsion angles ν_0 to ν_4

$$\nu_2 = \nu_{max} \cdot \cos(P + (j - 2) \cdot 144°)$$

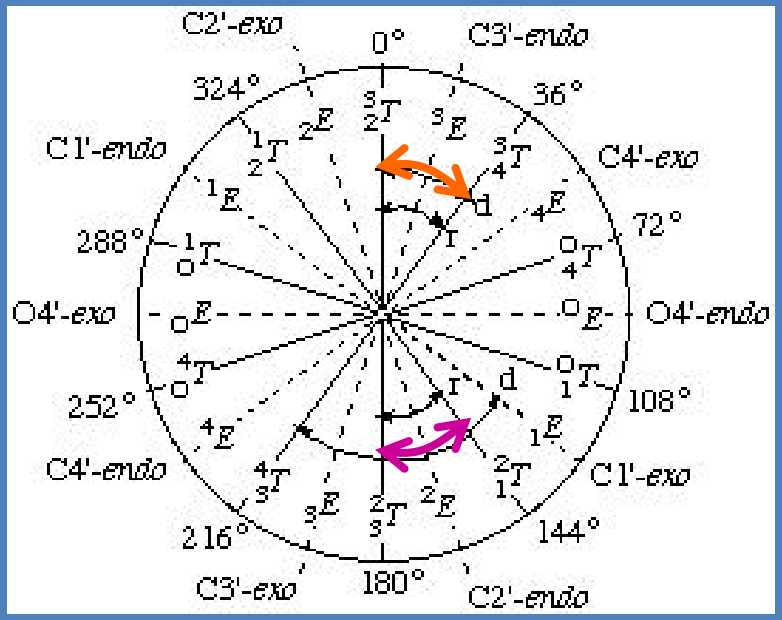
$$\nu_0 + \nu_1 + \nu_2 + \nu_3 + \nu_4 = 0$$

$j = 0 .. 4$

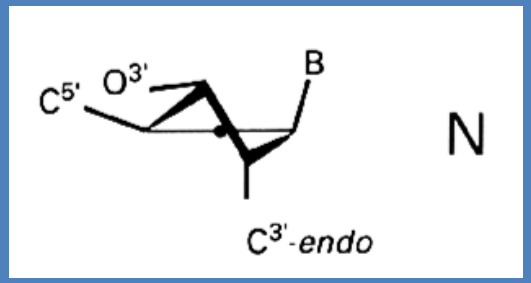
Sum of all 5 $\nu = 0$



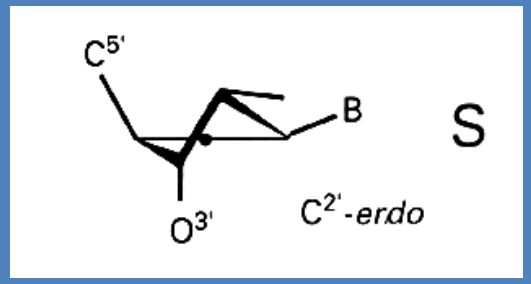
P in nucleic acids



NORTH



SOUTH



$0^o \leq P \leq 36^o$ north (prevalent in RNA)

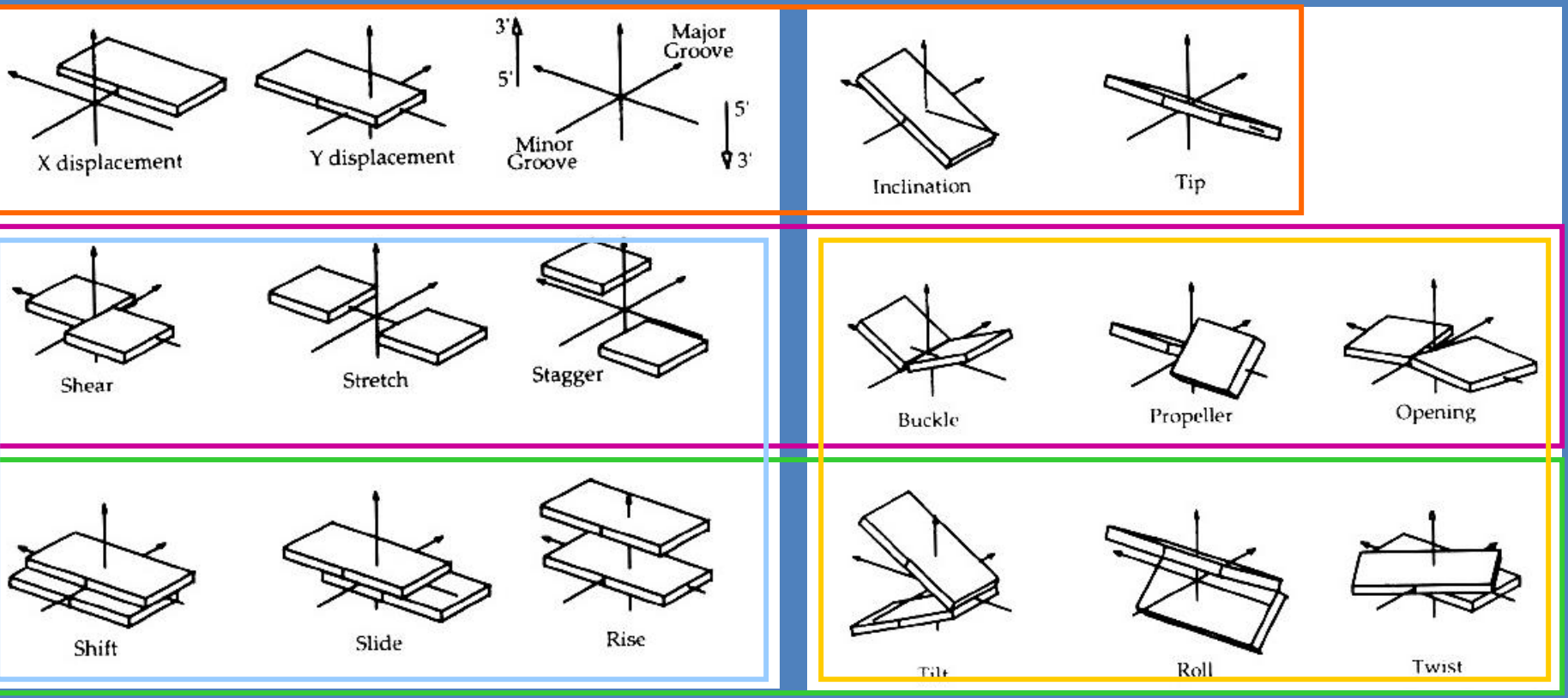
$144^o \leq P \leq 190^o$ south (prevalent in DNA)

Helical parameters

axis-base, axis-base pair

intra-base pair

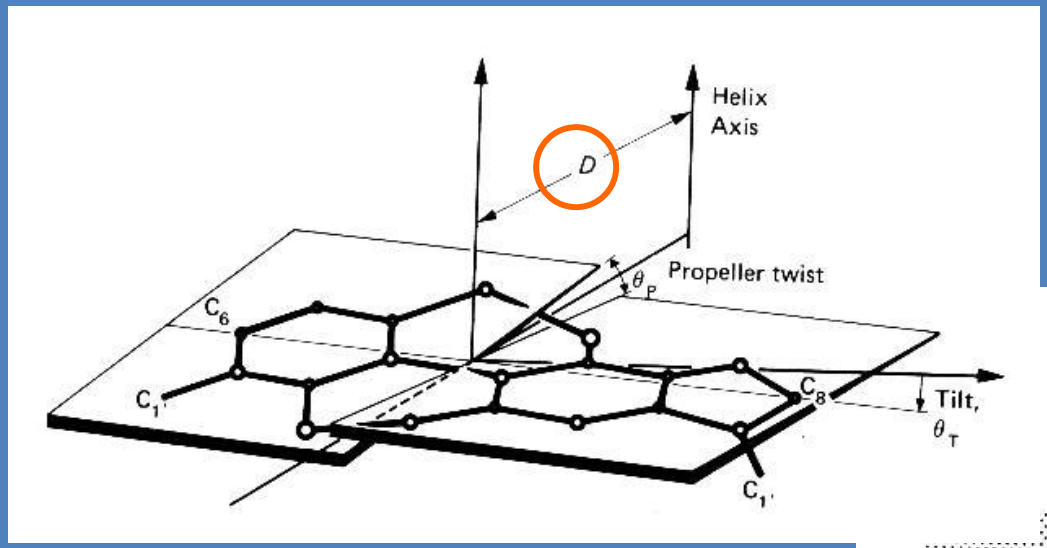
inter-base or inter-base pair



Distance/shift

Angle

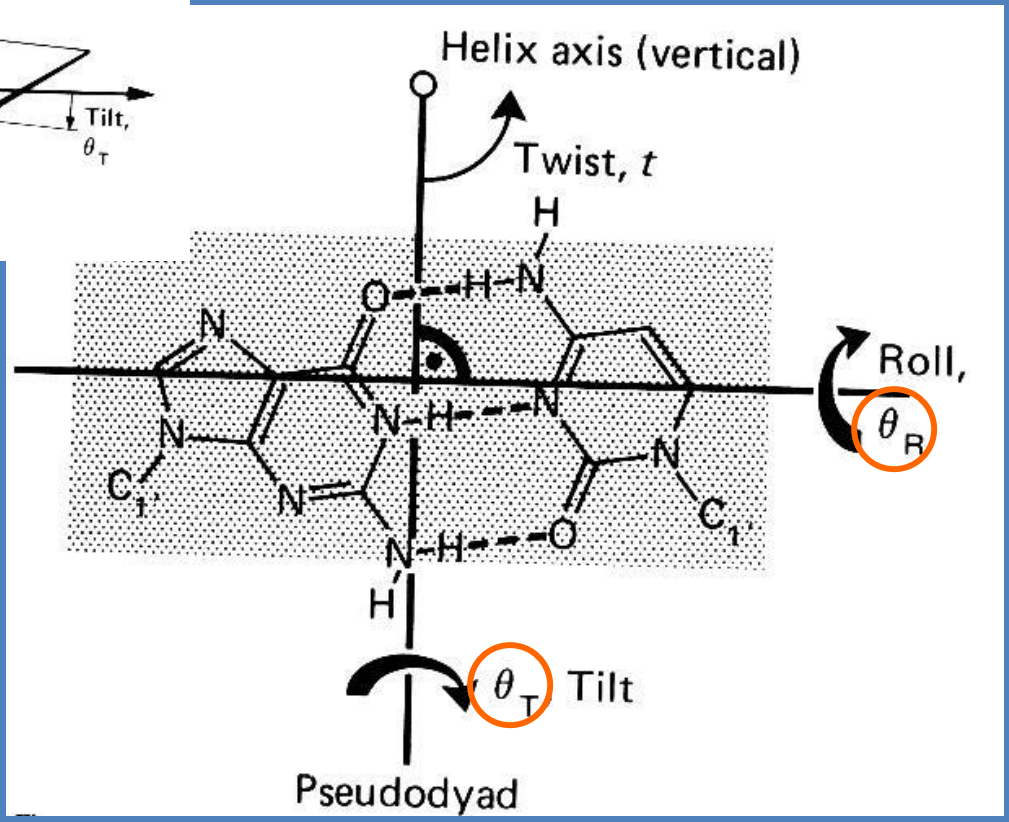
Helical…



D … displacement from helical axis

t …twist = 360° / n

θ_R … roll
θ_T … tilt

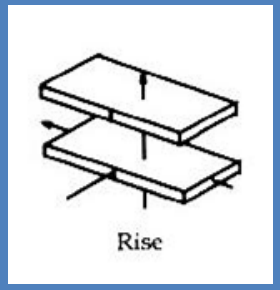


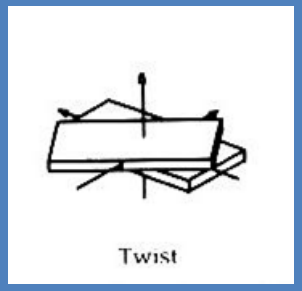
Helical parameters

for A and B DNA

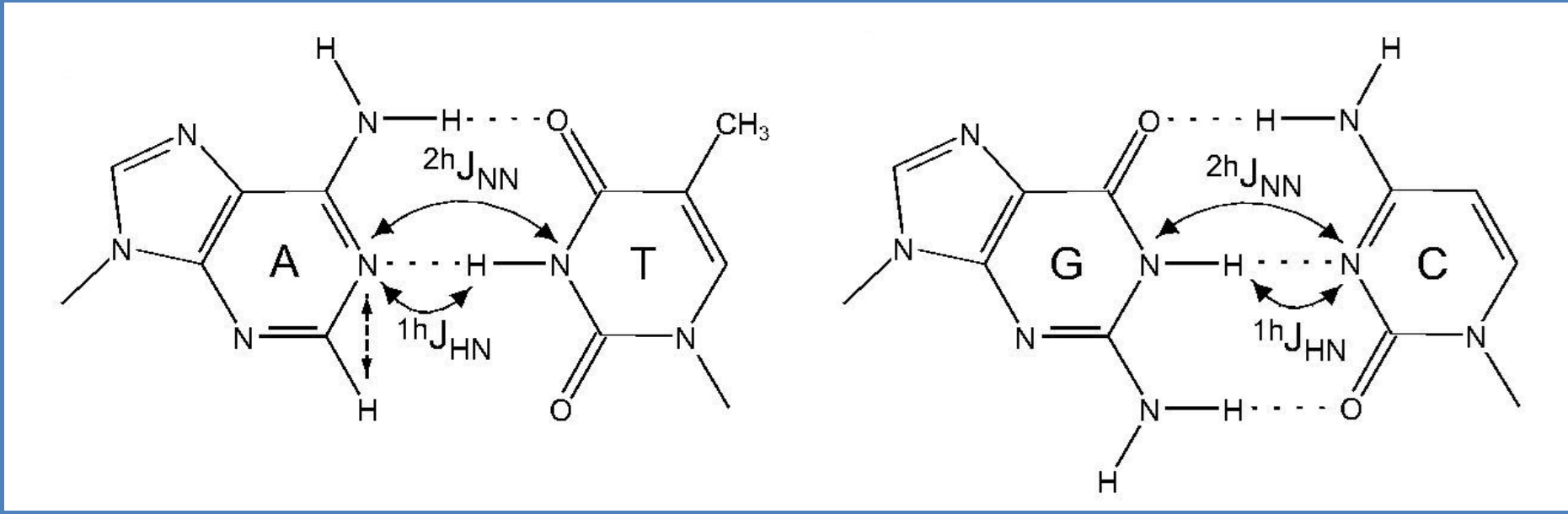
Global	B-DNA	A-DNA
X disp.	0.0	-5.28
Y disp.	0.0	0.0
Inclin	1.46	20.73
Tip	0.0	0.0
Shear	0.0	0.0
Stretch	0.0	0.01
Stagger	-0.08	-0.04
Buckle	0.0	0.0
Propeller	-13.3	-7.5
Openning	0.0	-0.02
Shift	0.0	0.0
Slide	0.0	0.0
Rise	3.38	2.56
Tilt	0.0	0.0
Roll	0.0	0.0
Twist	36.00	32.70
Bases per turn	360/36=10	360/32.7=11

Shifts in Å, angles in degrees



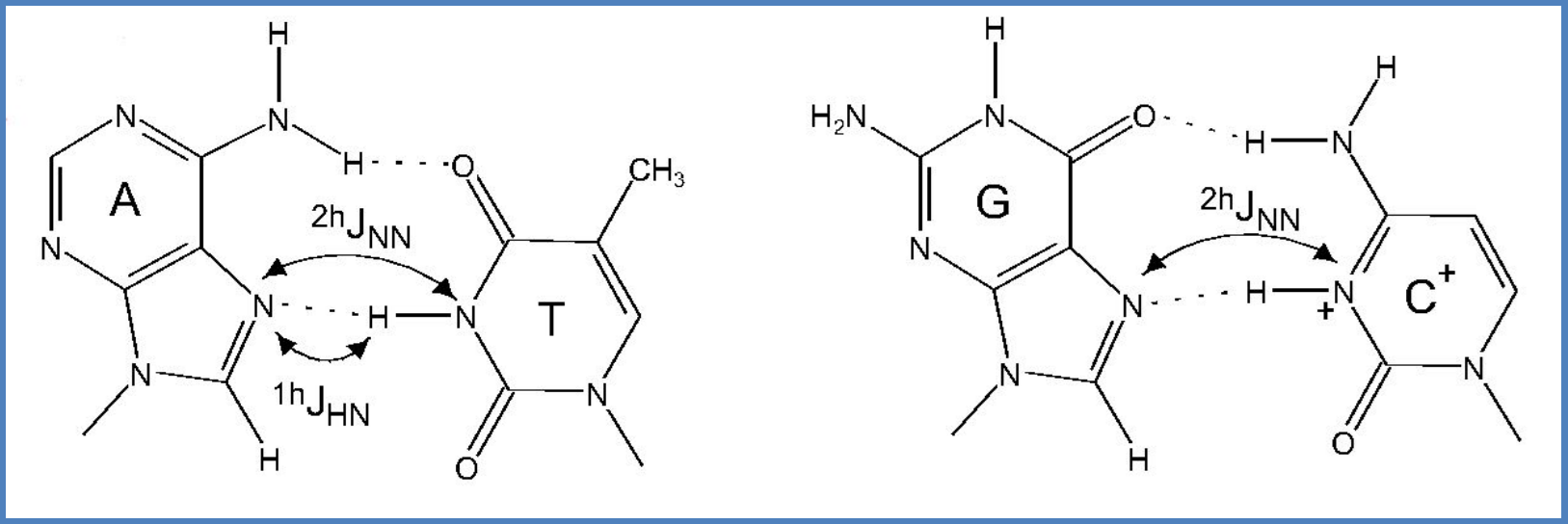


Base pairing

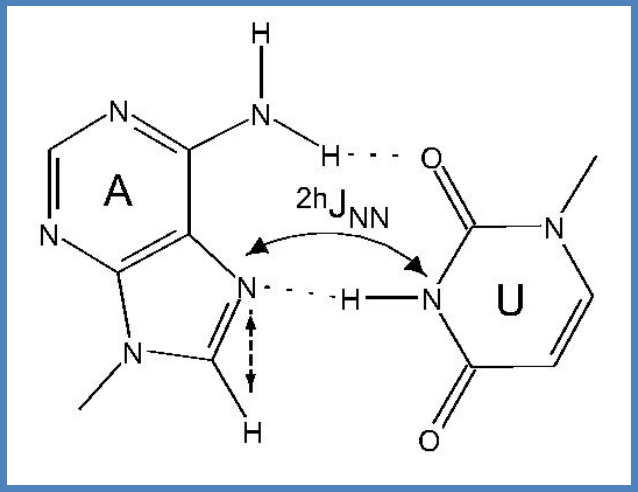


Watson-Crick pairs

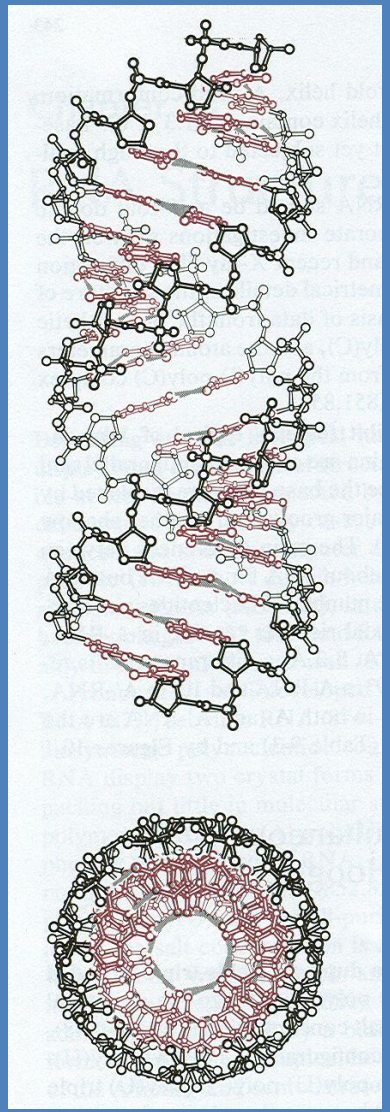
Base pairing



Hoogsteen and reverse Hoogsteen pairs



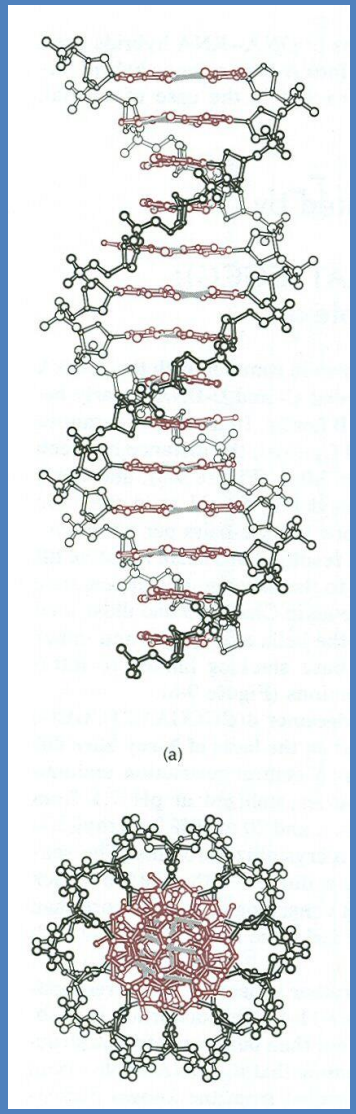
A and B double helix



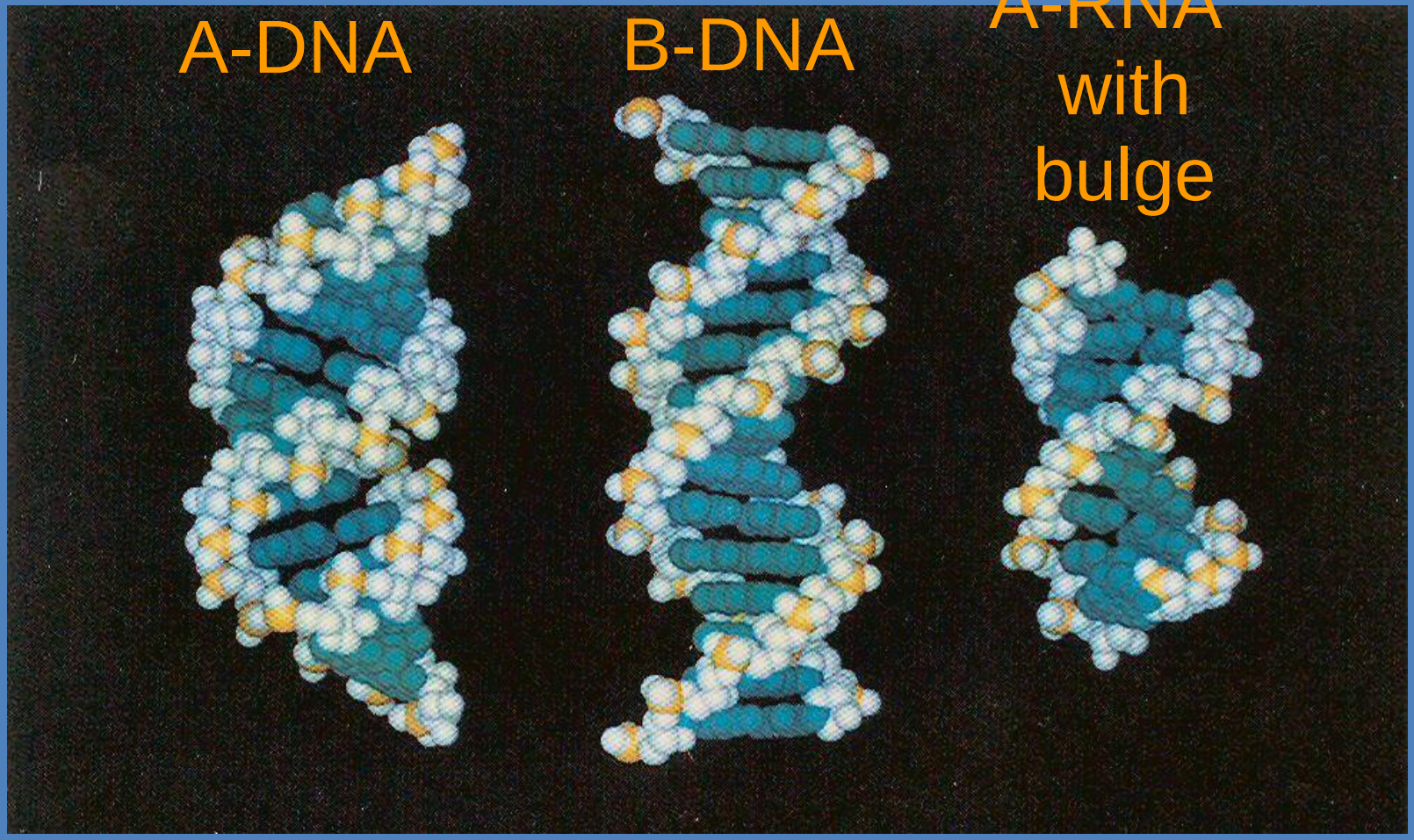
A-RNA

Ball and stick models

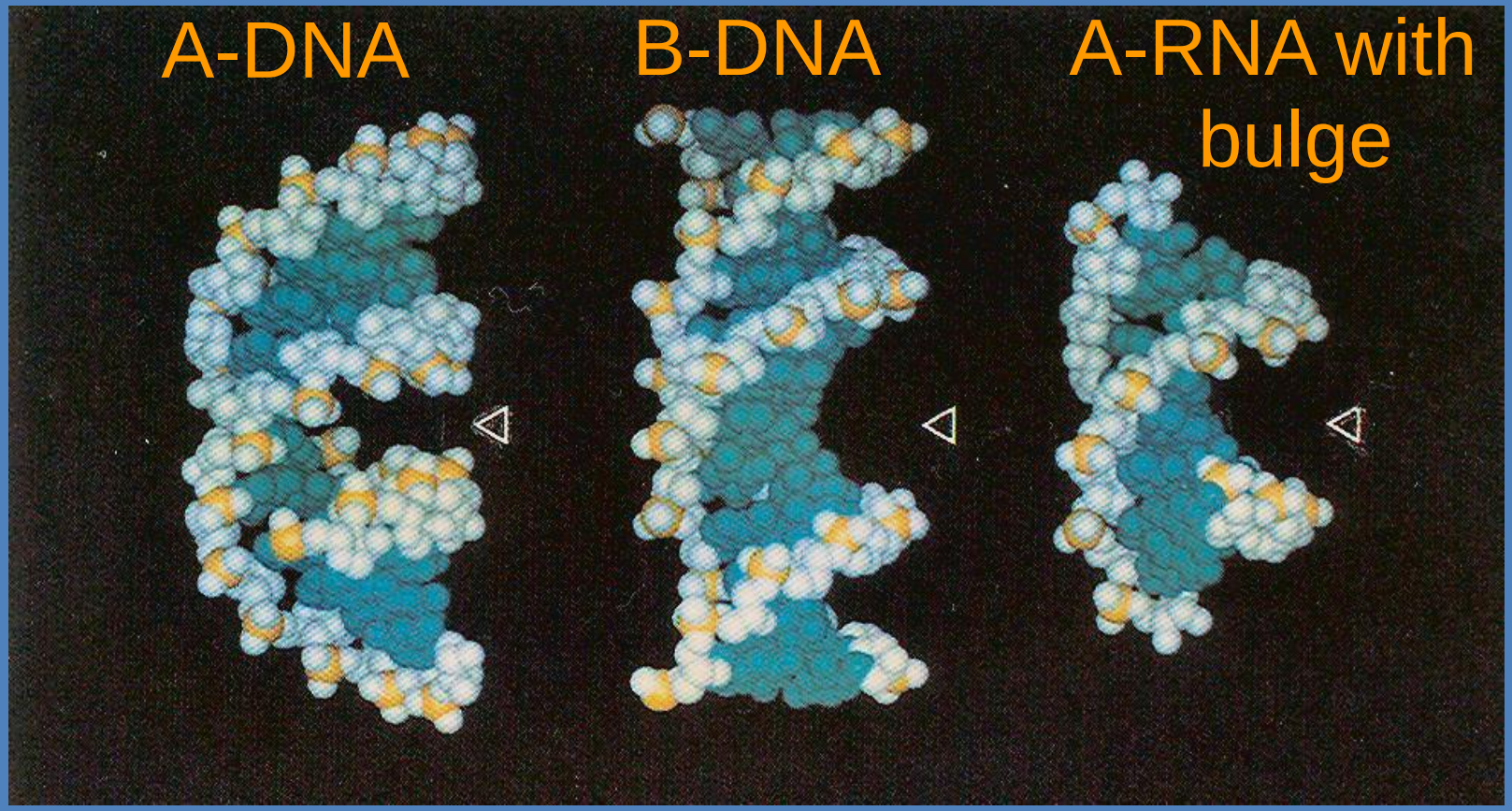
B-DNA



A and B helices



A and B helices



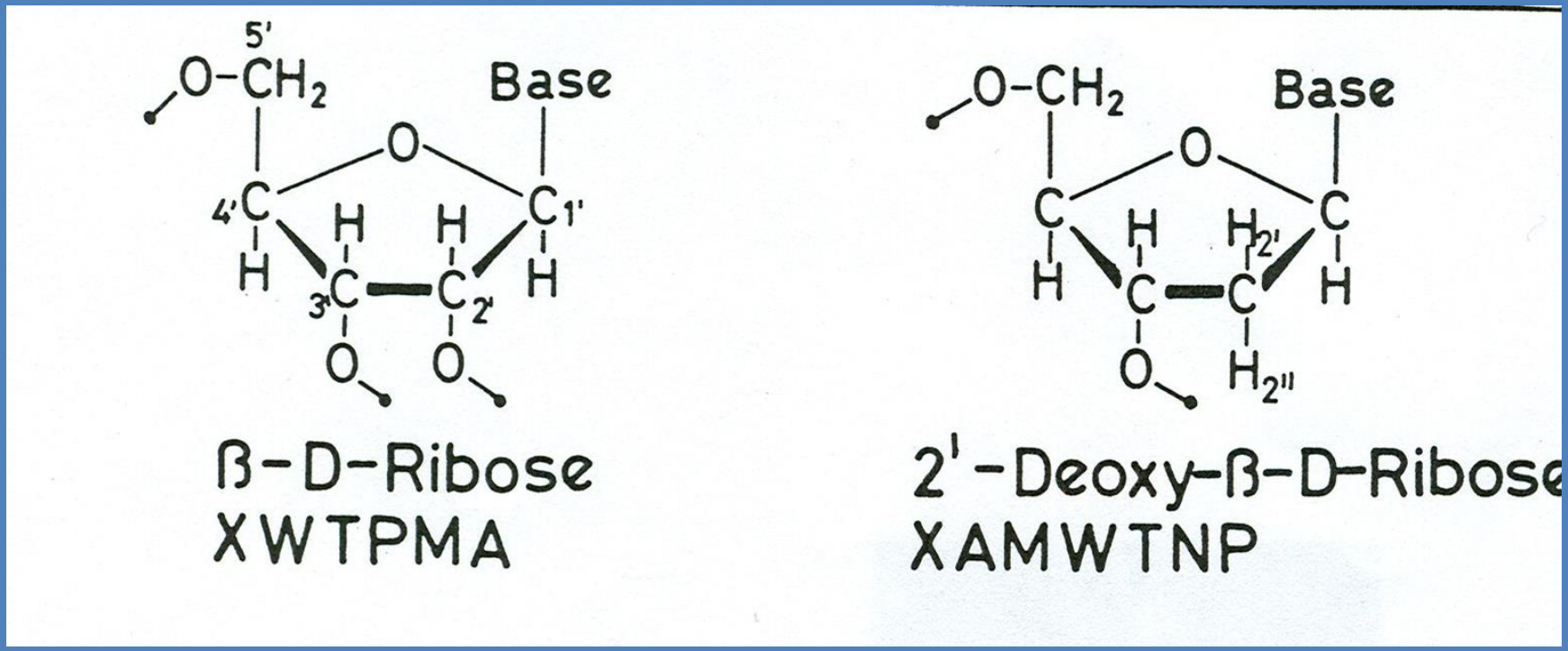
View tilted by 32° to show grooves

Nuclear properties of selected isotopes

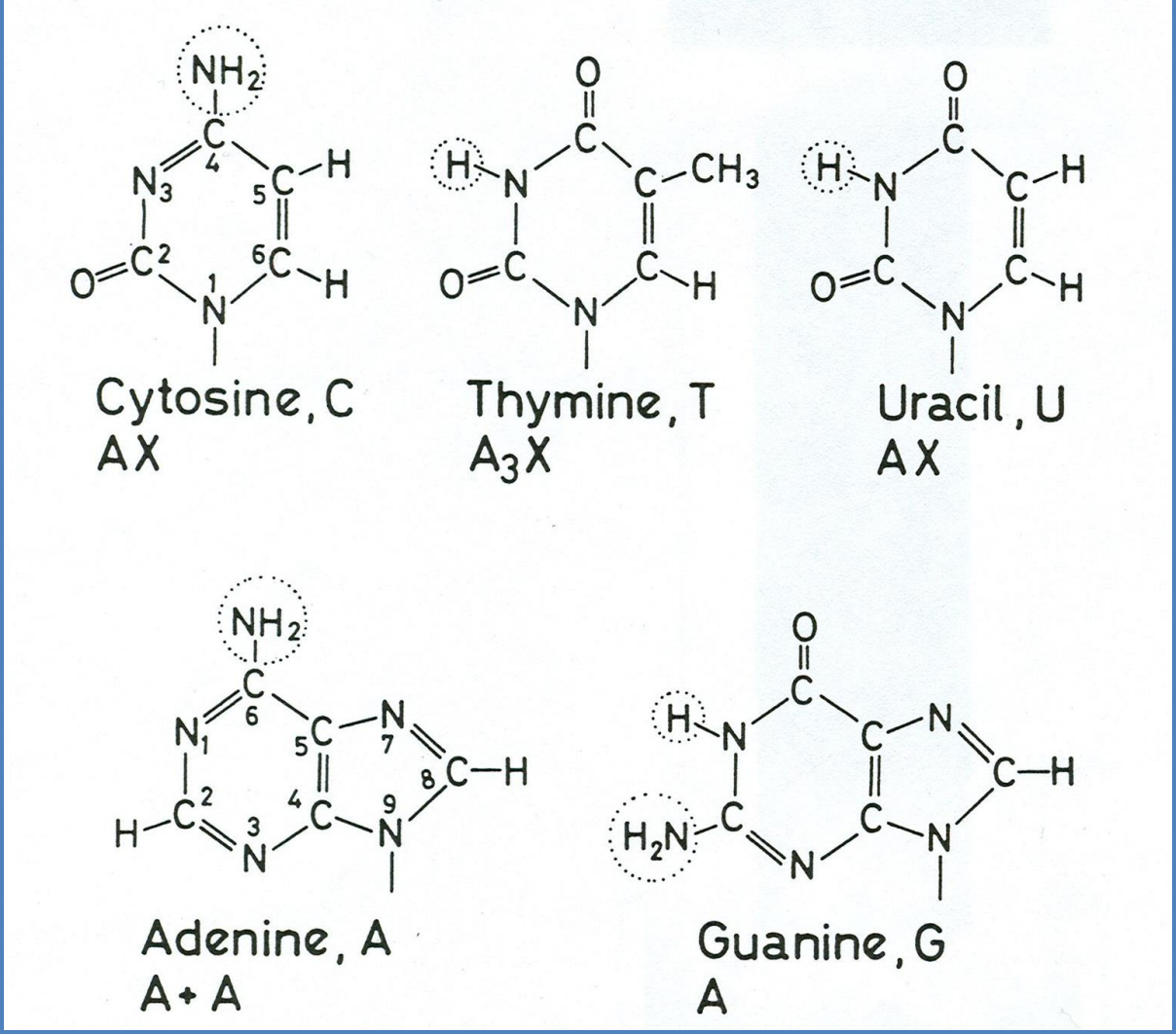
Isotope ($I=\frac{1}{2}$)	$\gamma \times 10^{-7}$ (rad $T^{-1}s^{-1}$)	ν at 11.74T (MHz)	Natural Abundance (%)	Sensitivity Rel.[a]	Sensitivity Abs.[b]
^{1}H	26.75	500.0	99.98	1.00	1.00
^{13}C	6.73	125.7	1.11	1.6×10^{-2}	1.8×10^{-4}
^{15}N	-2.71	50.7	0.37	1.0×10^{-3}	3.8×10^{-6}
^{31}P	10.83	202.4	100	6.6×10^{-2}	6.6×10^{-2}

[a] Relative sensitivity at constant field for equal number of nuclei.
[b] Product of relative sensitivity and natural abundance.

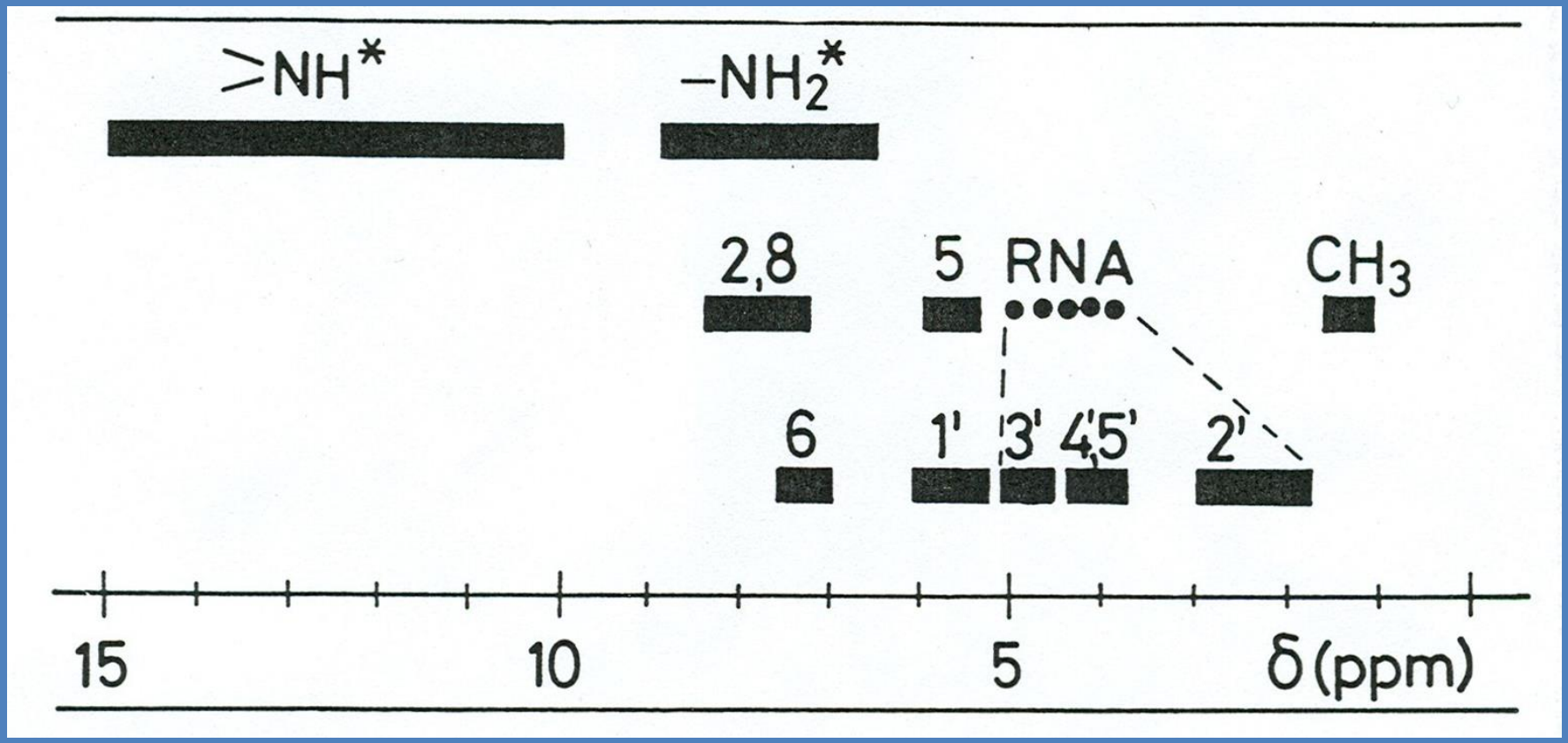
Spin systems in ribose and deoxyribose



Spin systems in nucleic acid bases



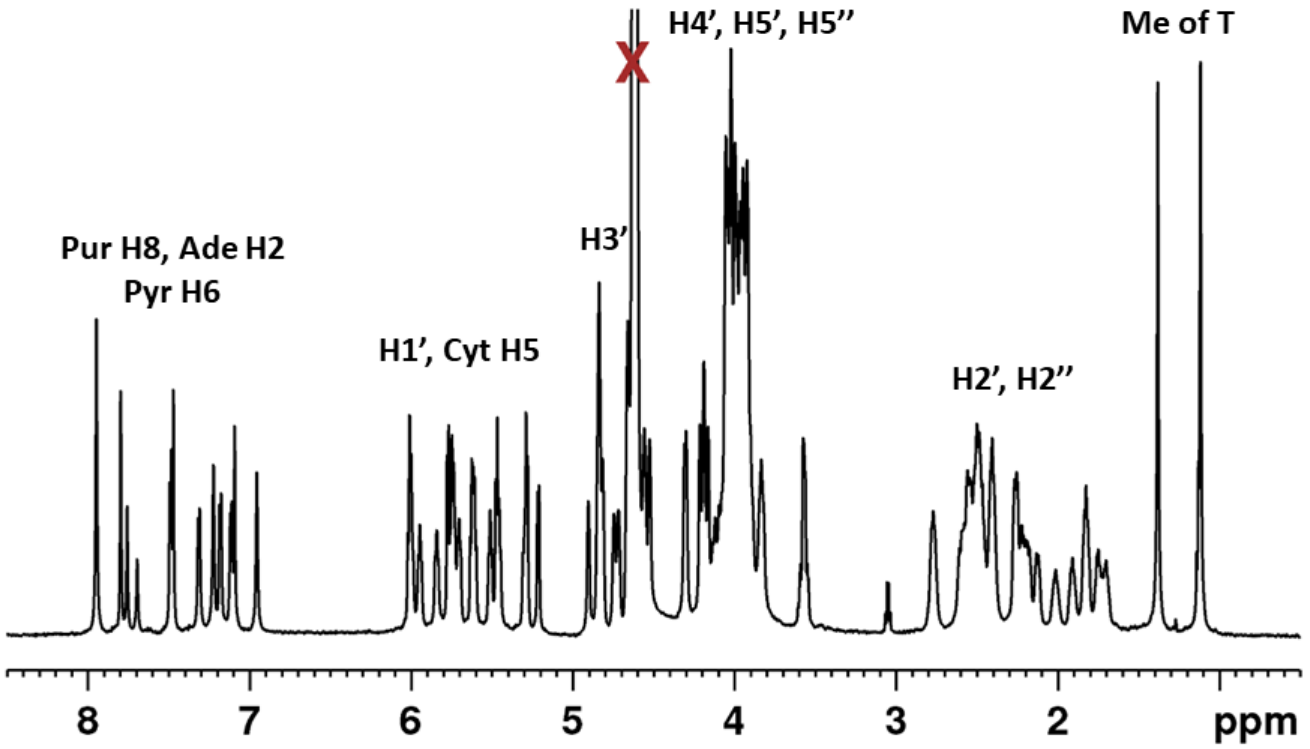
^{1}H chemical shift ranges in DNA and RNA



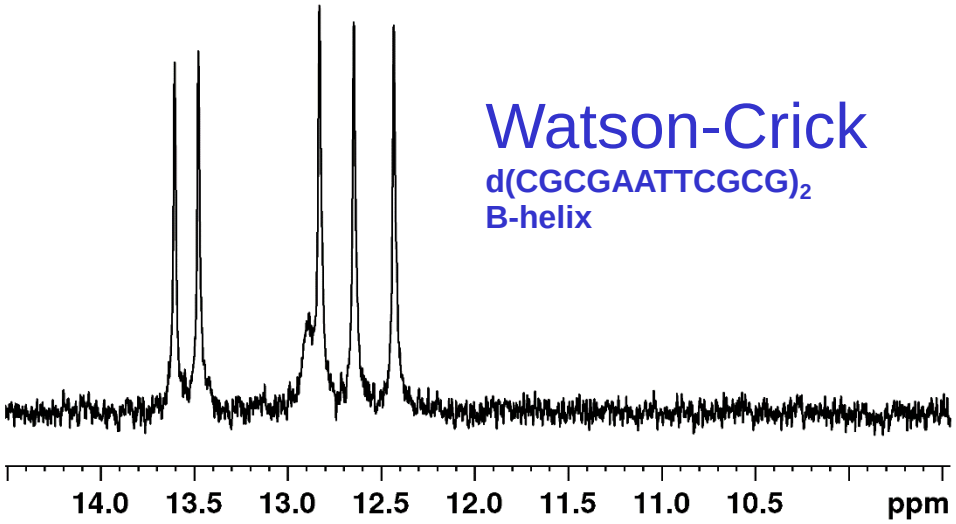
^{1}H chemical shift ranges in DNA and RNA

Code	δ (ppm)	Comments
2'	1.8-3.0	2'H, 2"H in DNA
4',5'	3.7-4.5	4'H, 5'H, 5"H in DNA
3'	4.4-5.2	3'H in DNA
•••••	3.7-5.2	2'H, 3'H, 4'H, 5'H, 5"H in RNA
1'	5.3-6.3	1'H
CH_3	1.2-1.6	CH_3 of T
5	5.3-6.0	5H of C and U
6	7.1-7.6	6H of C, T and U
2,8	7.3-8.4	8H of A and G, 2H of A
$-NH_2$*	6.6-9.0	NH_2 of A, C and G
>NH*	10 - 15	Ring NH of G, T and U

^{1}H NMR spectrum of d(CGCGAATTCGCG)



^{1}H NMR spectra in H_2O

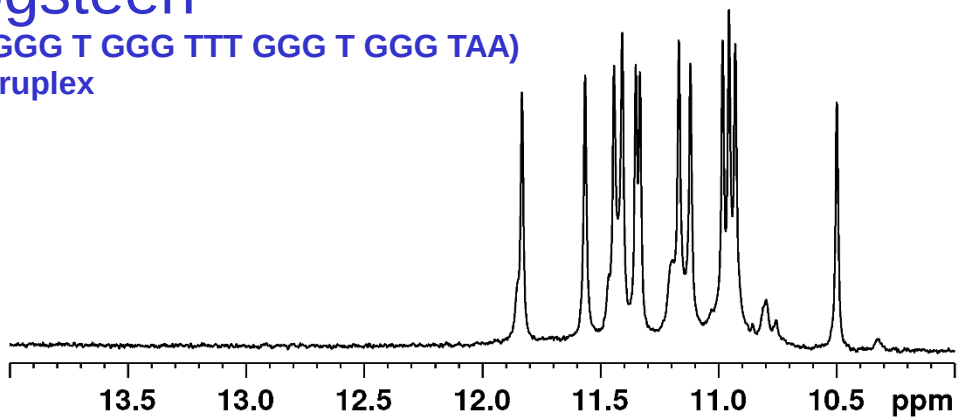


Watson-Crick

d(CGCGAATTCGCG)$_2$
B-helix

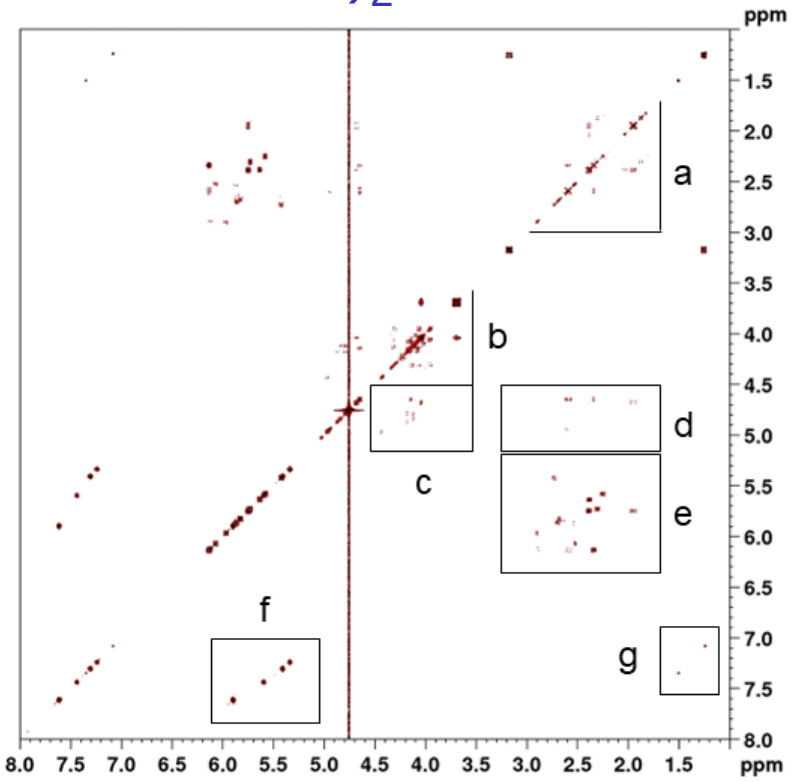
Hoogsteen

d(AAT GGG T GGG TTT GGG T GGG TAA)
G-quadruplex



^{1}H COSY spectrum of DNA

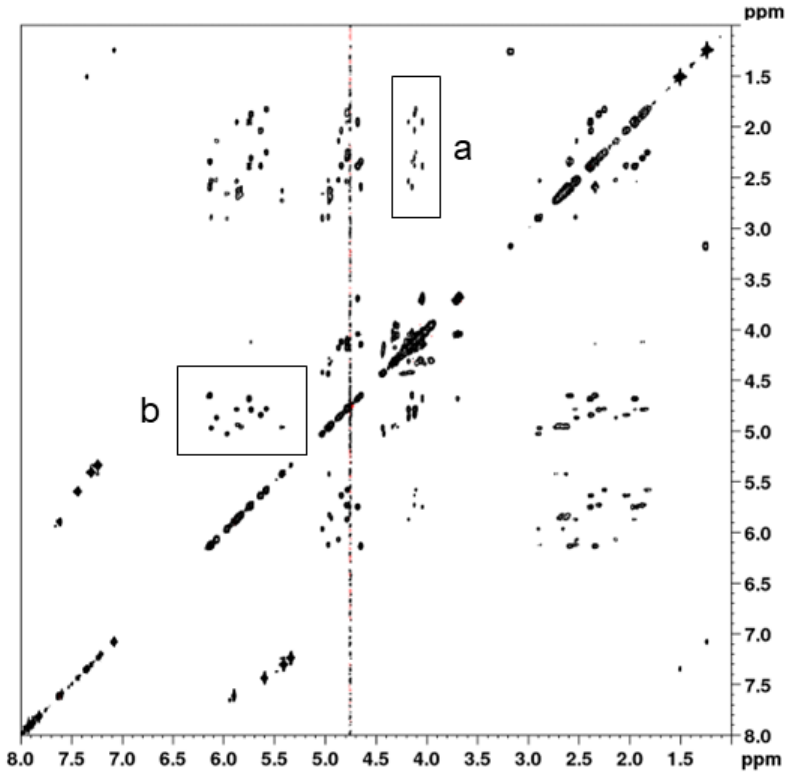
d(CGCGAATTCGCG)$_2$



a	H2'-H2''
b	H4'-H5',5''
	H5'-H5''
c	H3'-H4'
d	H2',2''-H3'
e	H1'-H2',2''
f	H5-H6 (Cyt)
g	CH_3-H6 (Thy)

^{1}H TOCSY spectrum of DNA

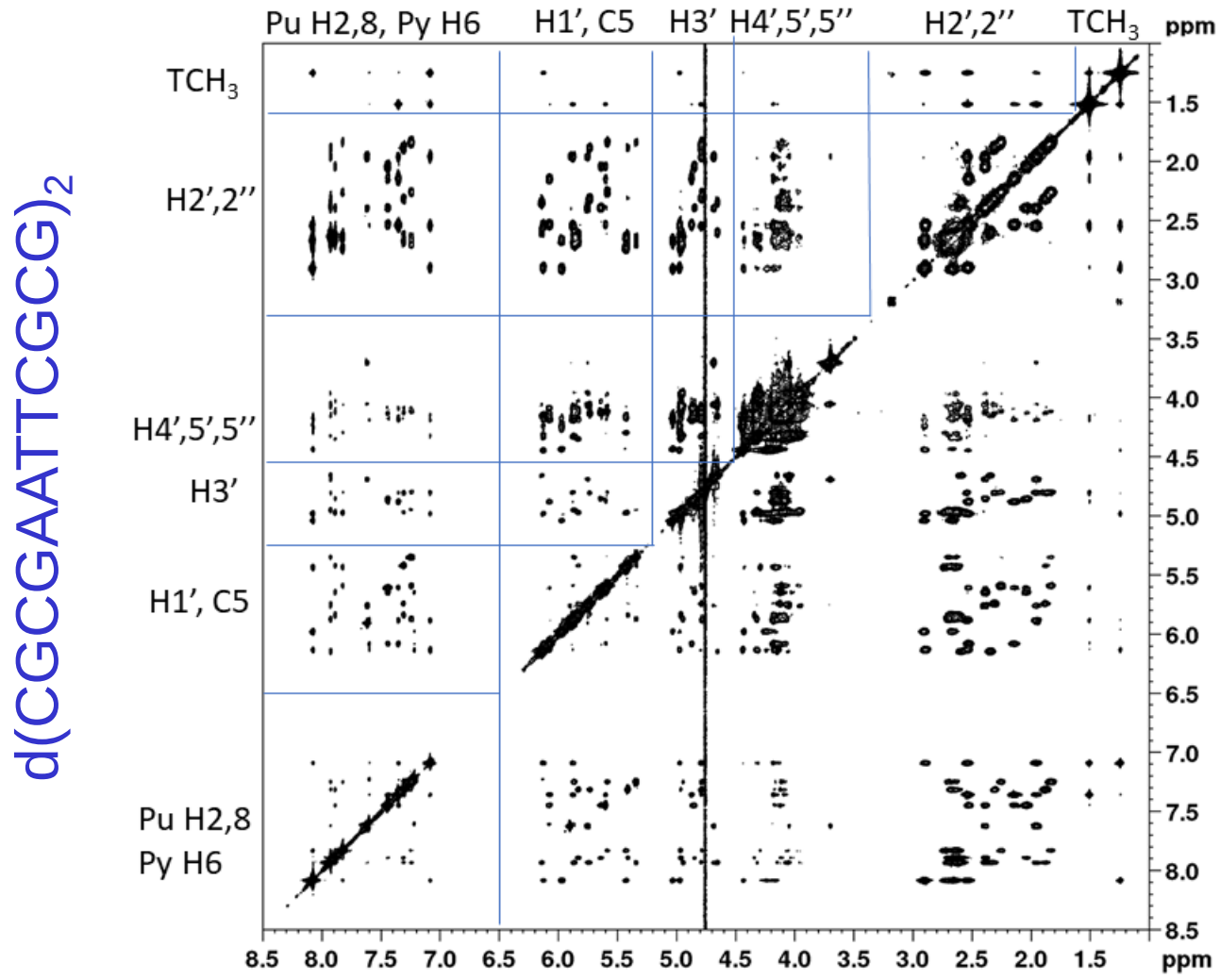
d(CGCGAATTCGCG)$_2$



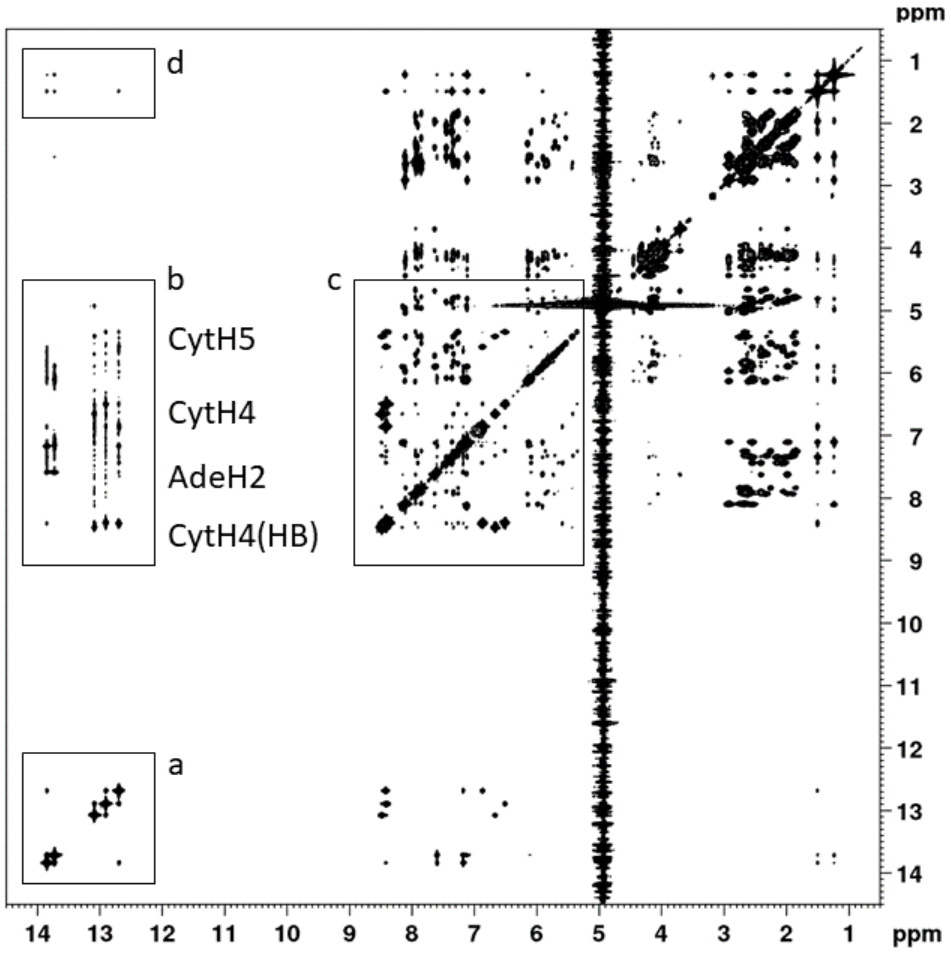
a H4'-H2',2''

b H1'-H3'

^{1}H NOESY spectrum of DNA in D_2O



^{1}H NOESY spectrum of DNA in H_2O



d(CGCGAATTCGCG)$_2$

a H imino - H imino

b H imino - H amino

H imino - AdeH2,CytH5

c H amino – H amino

H amino - AdeH2,CytH5,H6

d H imino – TCH_3

Water Suppression

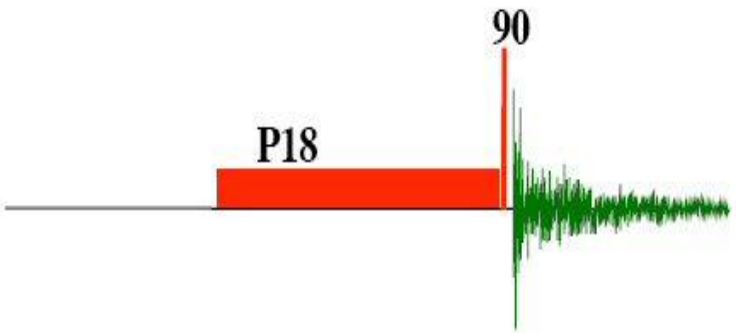
The presence of an intense solvent resonance necessitates an impractical high dynamic range. 110 M vs <1mM
To overcome this problem several methods are currently applied:

1) Presaturation.
2) Observing the FID when the water passes a null condition after a 180 degree pulse.
3) Suppression of broad lined based on their T_2 behavior.
4) Selectively excitation, with and without gradients

5a) Use of GRASP to select specific coherences thereby excluding the intense solvent signal. In this case the solvent signal never reaches the ADC. This allows the observation of resonances that are buried under the solvent peak.

5b) Use of GRASP to selectively dephase the solvent resonance (WATERGATE)

PRESAT



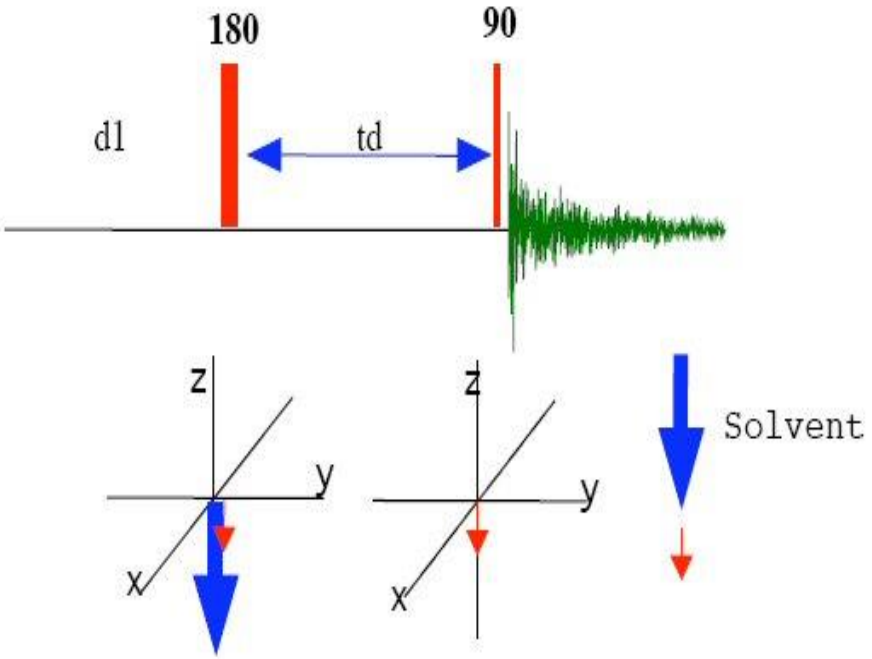
Presaturation field strength:

20-40 Hz corresponds to a
6-12ms 90deg pulse.

Pros: Easy to set up
Excellent water suppression

Cons: Resonances under water signal!
(T variation)
Labile protons not visible
(some GC pairs may be)

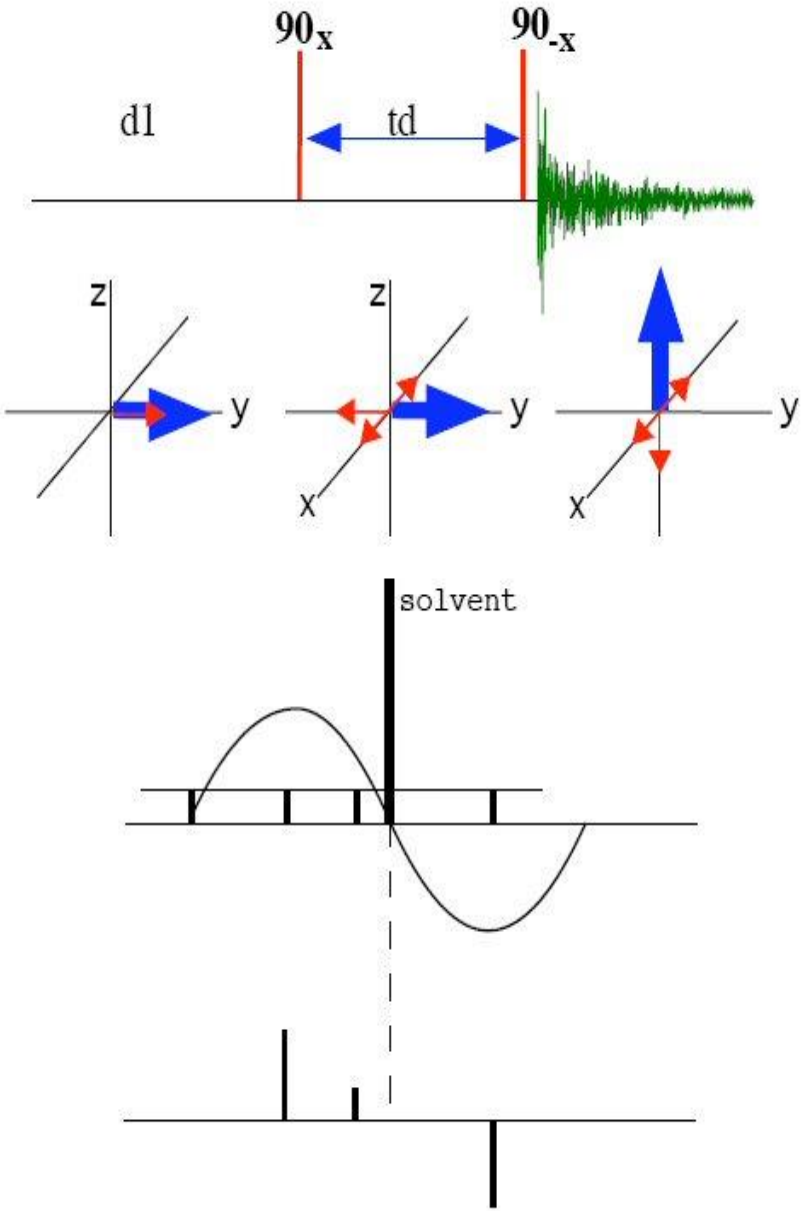
WEFT



Method relies on different T_1 values for water and solute.

It fails if the relaxation times are similar.
Intensity of the solute resonances may vary.
For a selective 180 degree pulse on the solvent these problems are largely avoided.

Jump and return

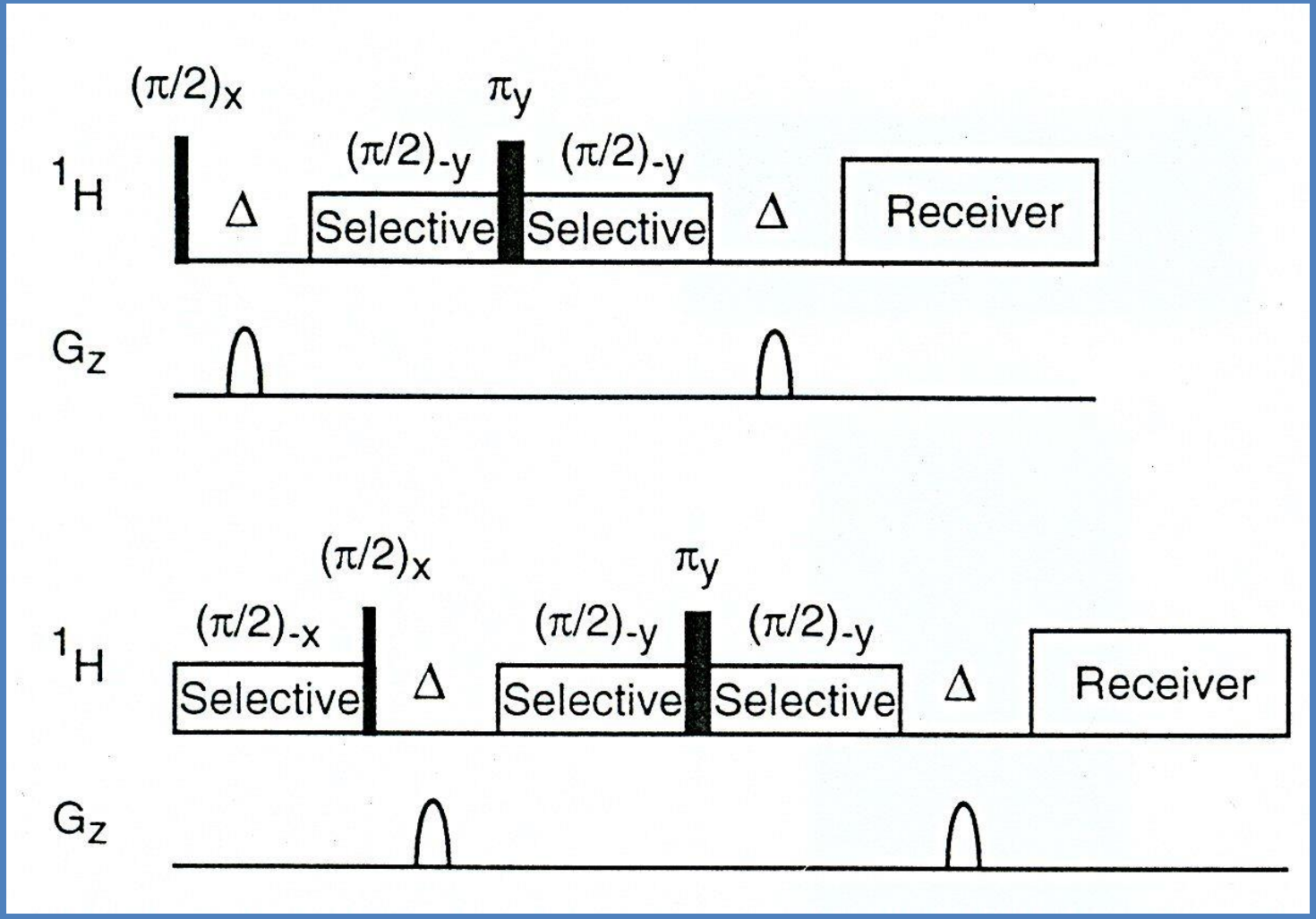


Pros: Easy to set up
Excellent water suppression
(with proper setup as good as presat)
Good for broad signals!

Cons: Non uniform excitation
Baseline not flat

Other sequences: 1331 etc

WATERGATE


(π/2)x
πy
(π/2)-y
(π/2)-y
1H
Δ
Selective
Selective
Δ
Receiver
Gz
(π/2)x
πy
(π/2)-x
(π/2)-y
(π/2)-y
1H
Selective
Δ
Selective
Selective
Δ
Receiver
Gz

Structure Determination Procedure

Structure Determination:

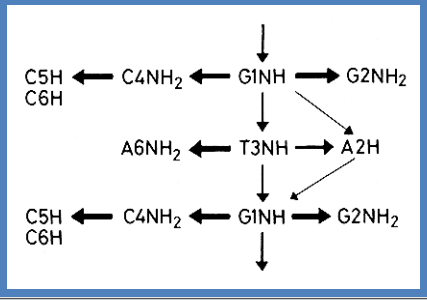
I)	Assignment	NOESY, COSY, HSQC TOCSY.....
II)	Local Analysis	
	•glycosidic torsion angle	(NOE, COSY)
	•sugar puckering	(COSY, NOE)
	•backbone conformation	(COSY)
	•base pairing	(NOE, COSY)
III)	Global Analysis	
	•sequential	(NOE, COSY)
	•inter strand/cross strand	(NOE, COSY)
	•dipolar coupling	(HSQC, HSQC)

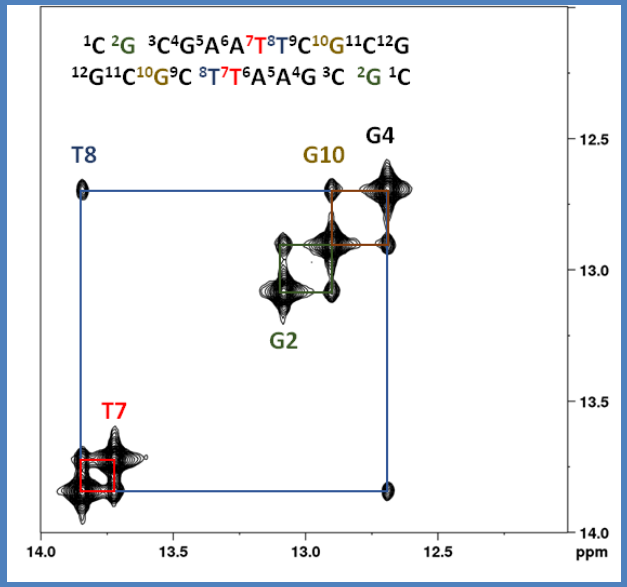
Resonance Assignment

A) Exchangeable protons:	1D ^{1}H, 2D NOESY
B) Non-exchangeable protons	
• Aromatic Spin Systems:	2D DQF-COSY (H5-H6), 2D NOESY
• Sugar Spin Systems:	2D DQF-COSY 2D TOCSY
• Sequential Assignment:	2D NOESY 2D (^{31}P, ^{1}H) HETCOR
C) Correlation of exchangeable and non-exchangeable protons:	2D NOESY

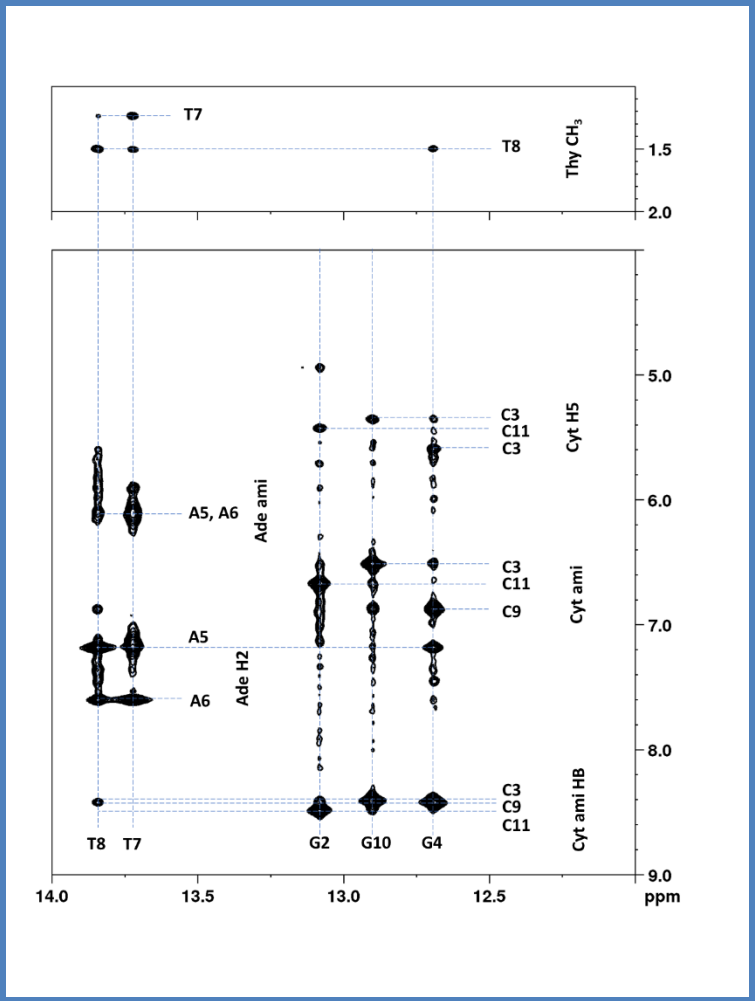
Sequential connectivities with exchangeable protons

Dickerson's dodecamer
d(CGCGAATTCGCG)$_2$



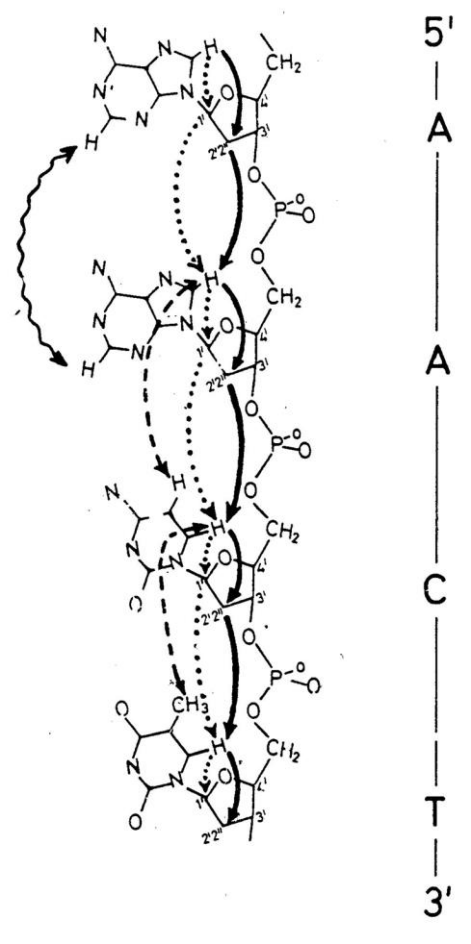


imino-imino

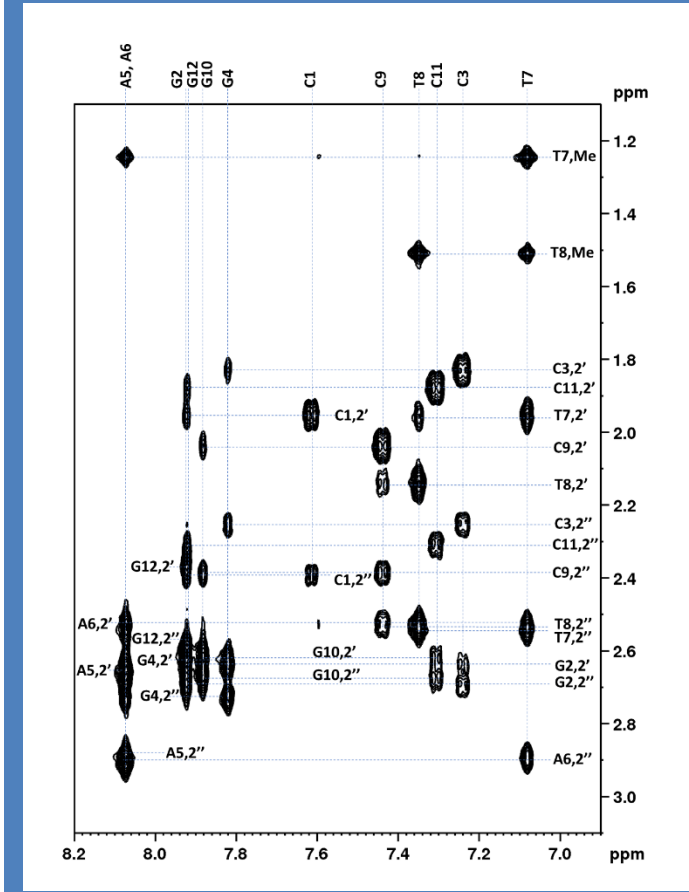


imino-amino

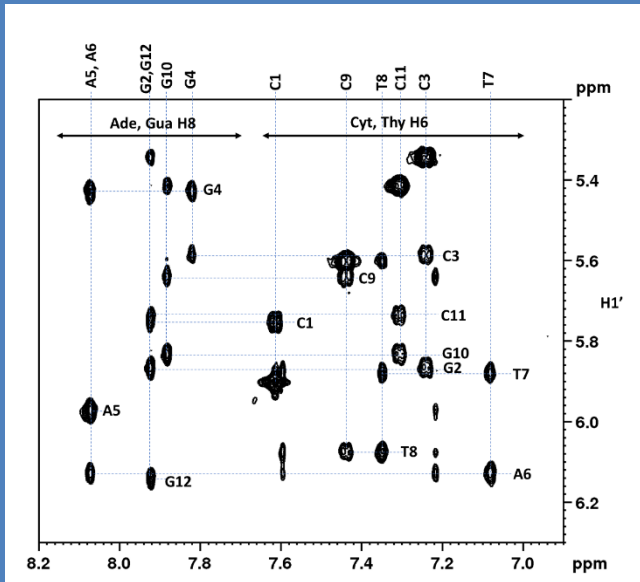
Sequential resonance assignments



H6/8-H2',2''/Me



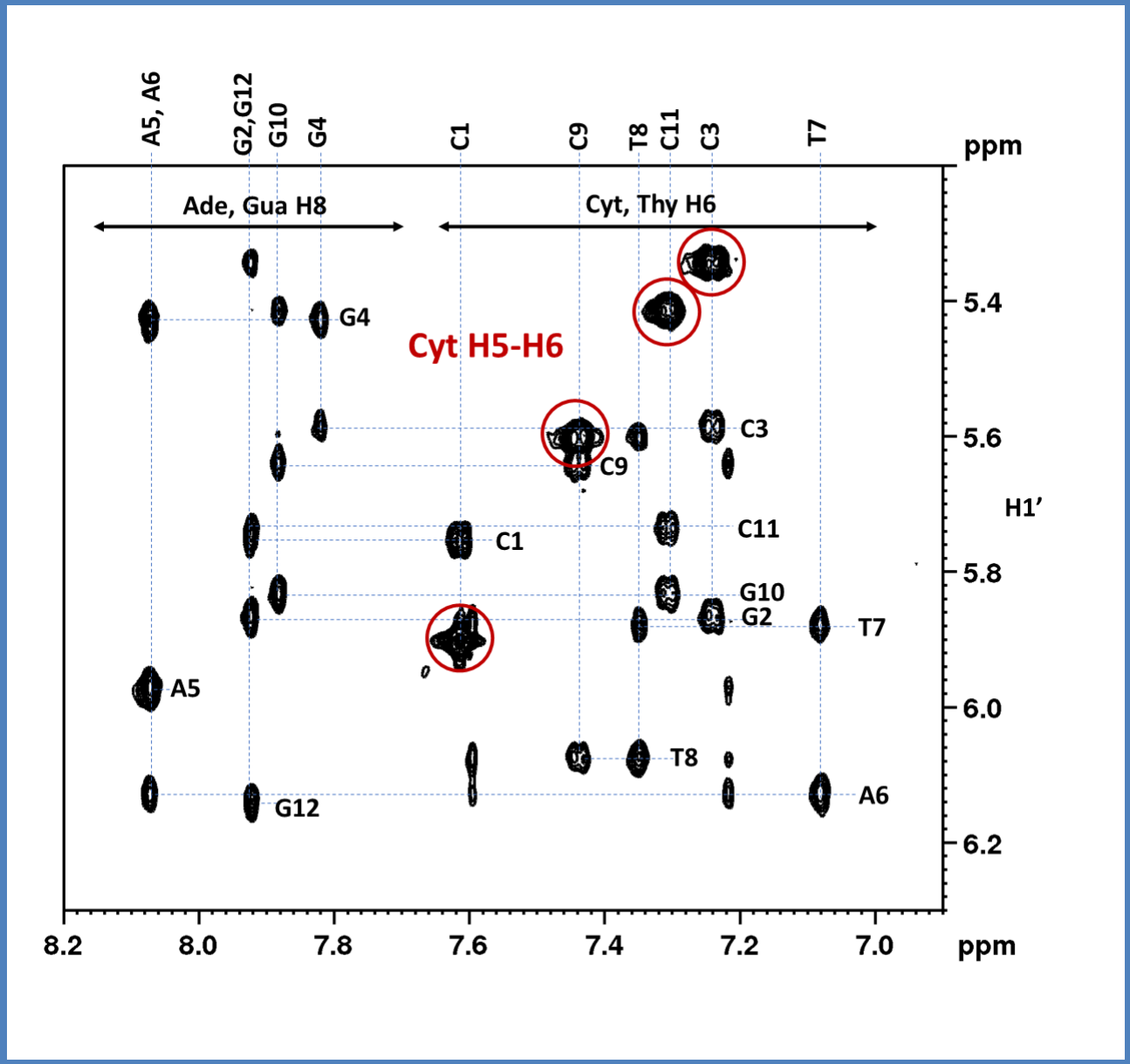
H6/8-H1'



$d(CGCGAATTCGCG)_2$

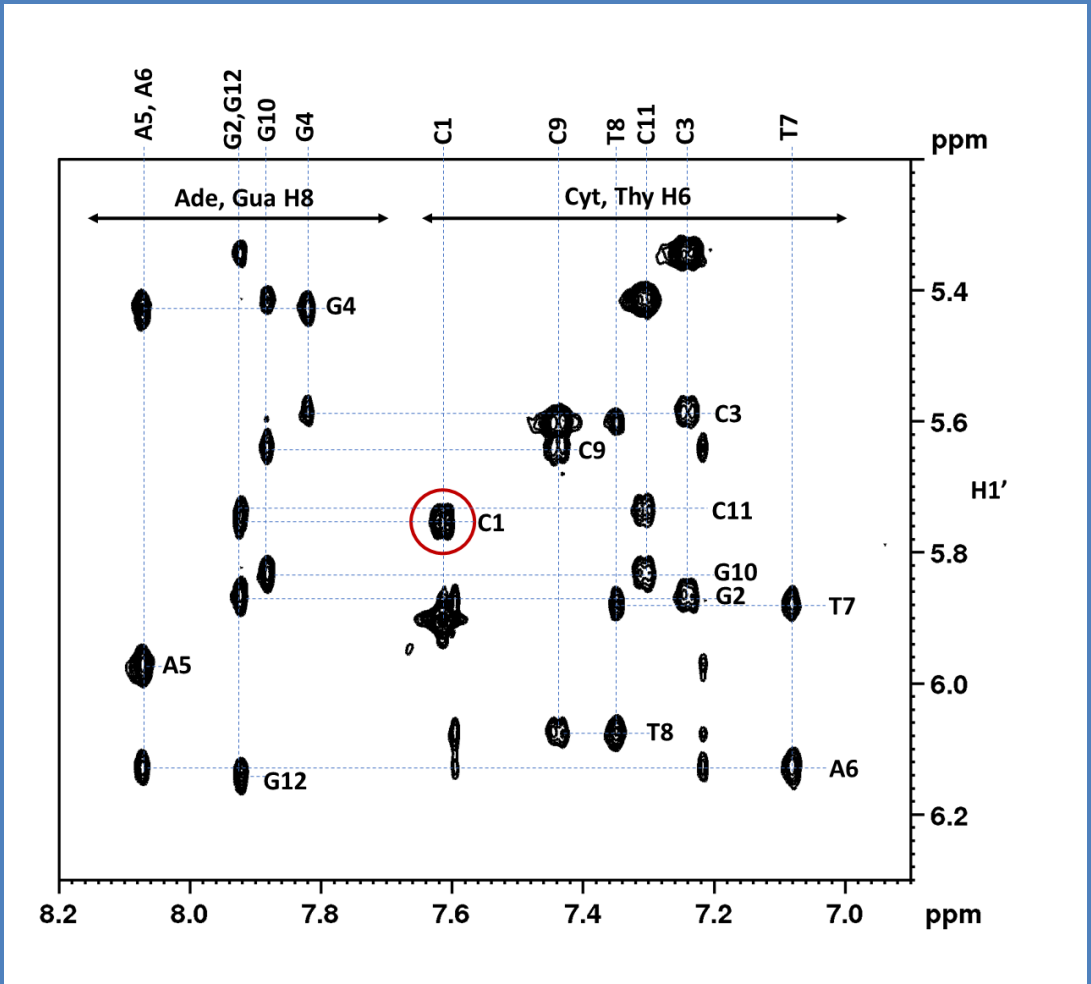
Assignment based on H6/8-H1’ connectivities

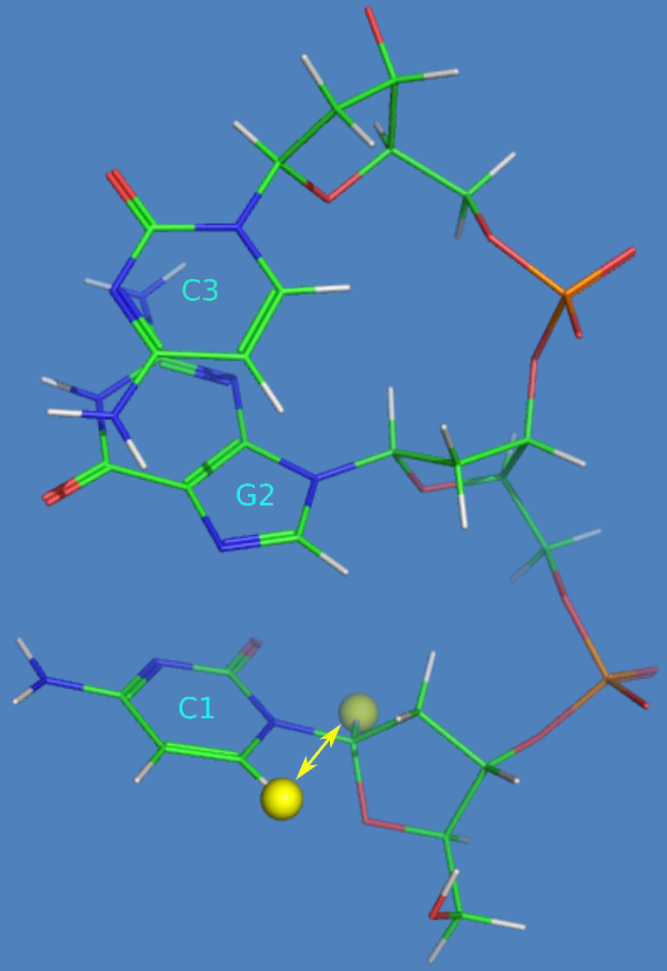
$d(CGCGAATTCGCG)_2$



Assignment based on H6/8-H1’ connectivities

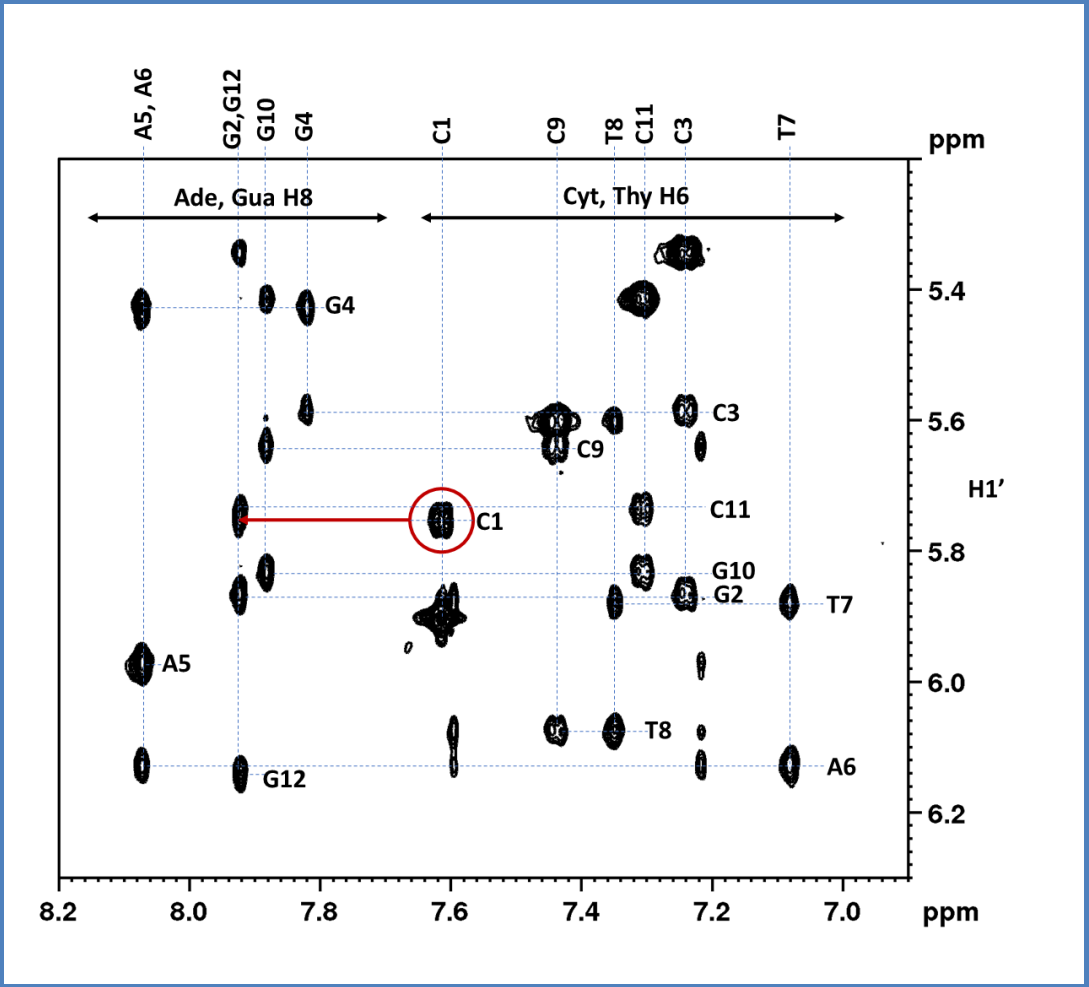
d(CGCGAATTCGCG)$_2$

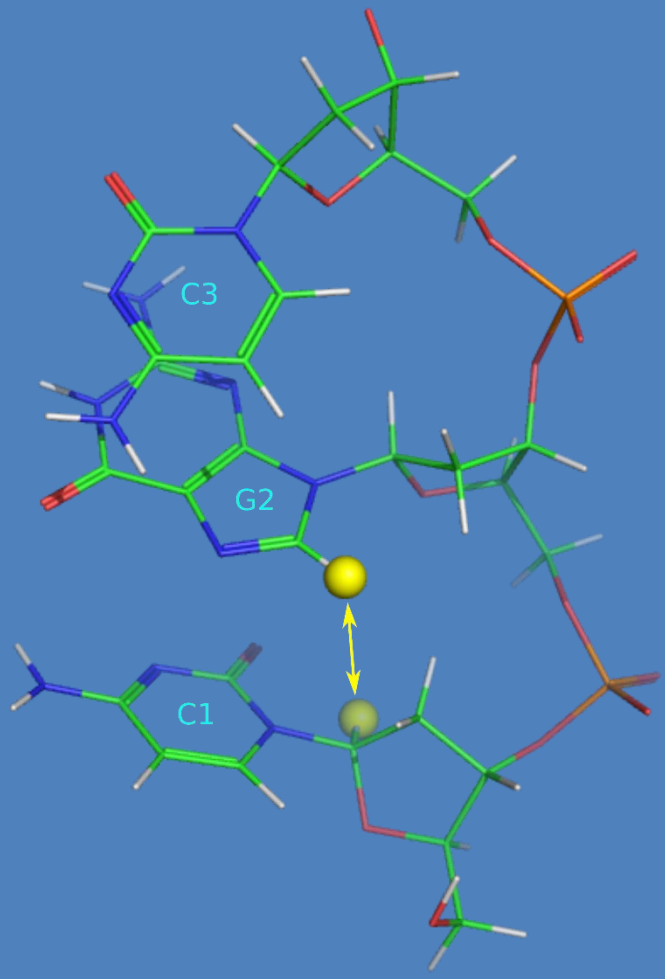




Assignment based on H6/8-H1’ connectivities

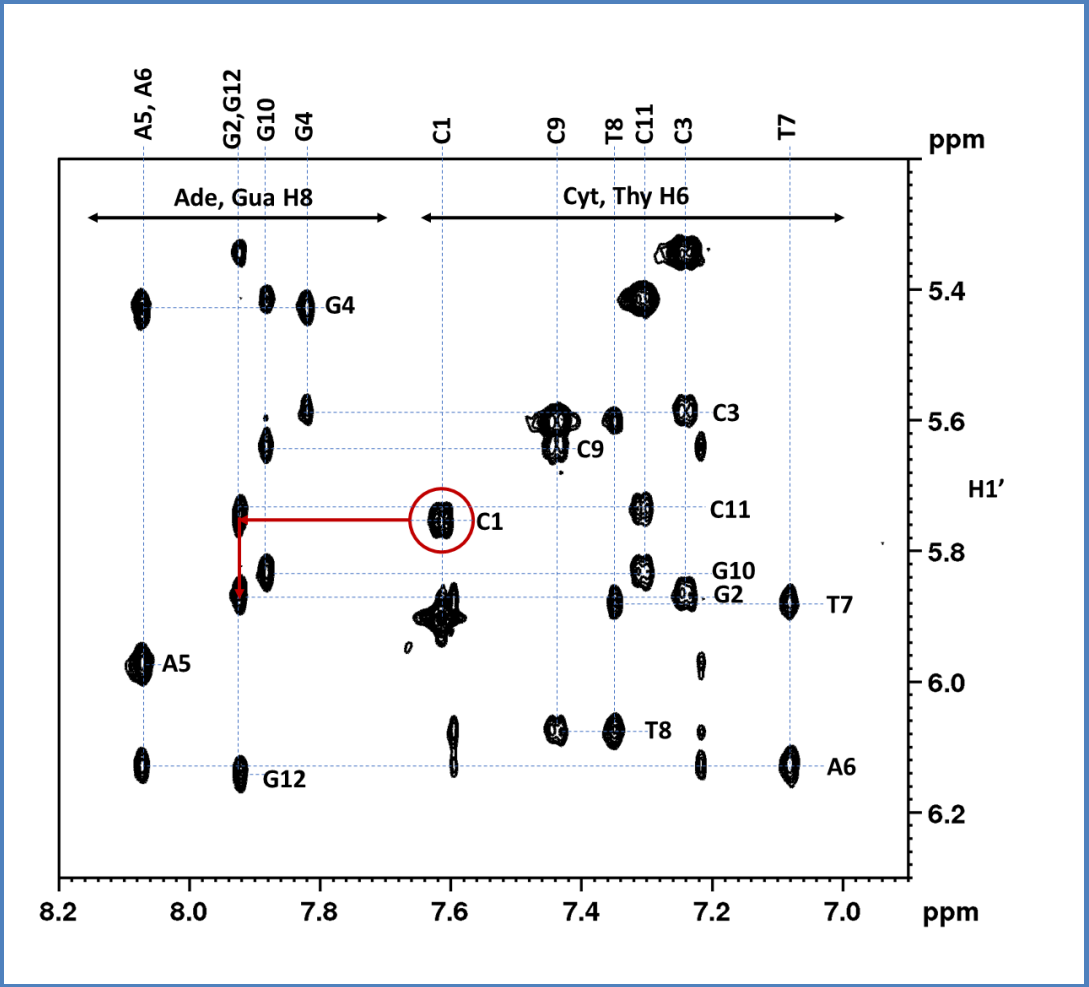
$d(CGCGAATTCGCG)_2$

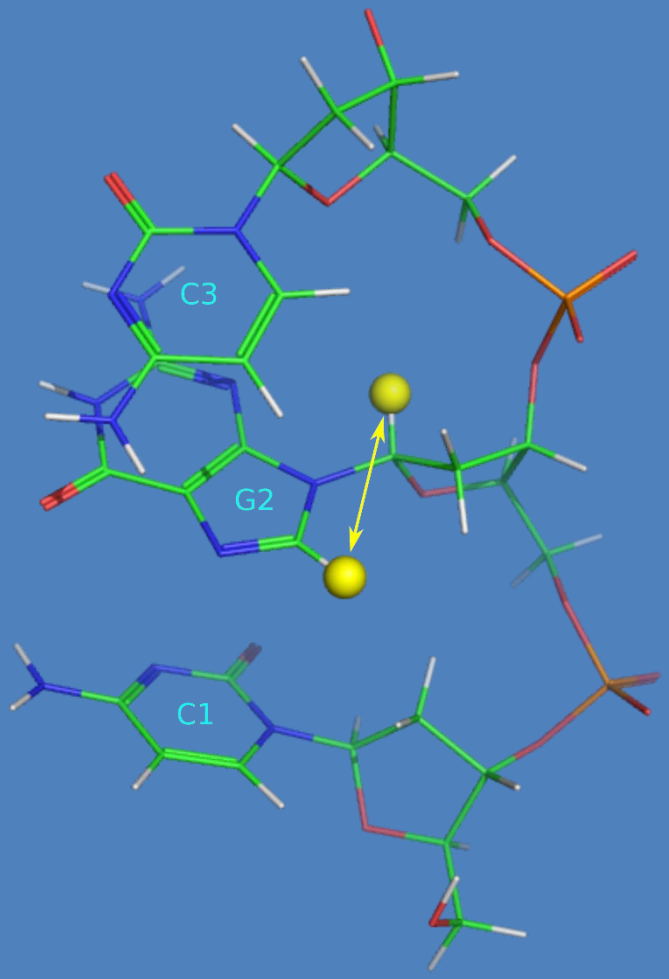




Assignment based on H6/8-H1’ connectivities

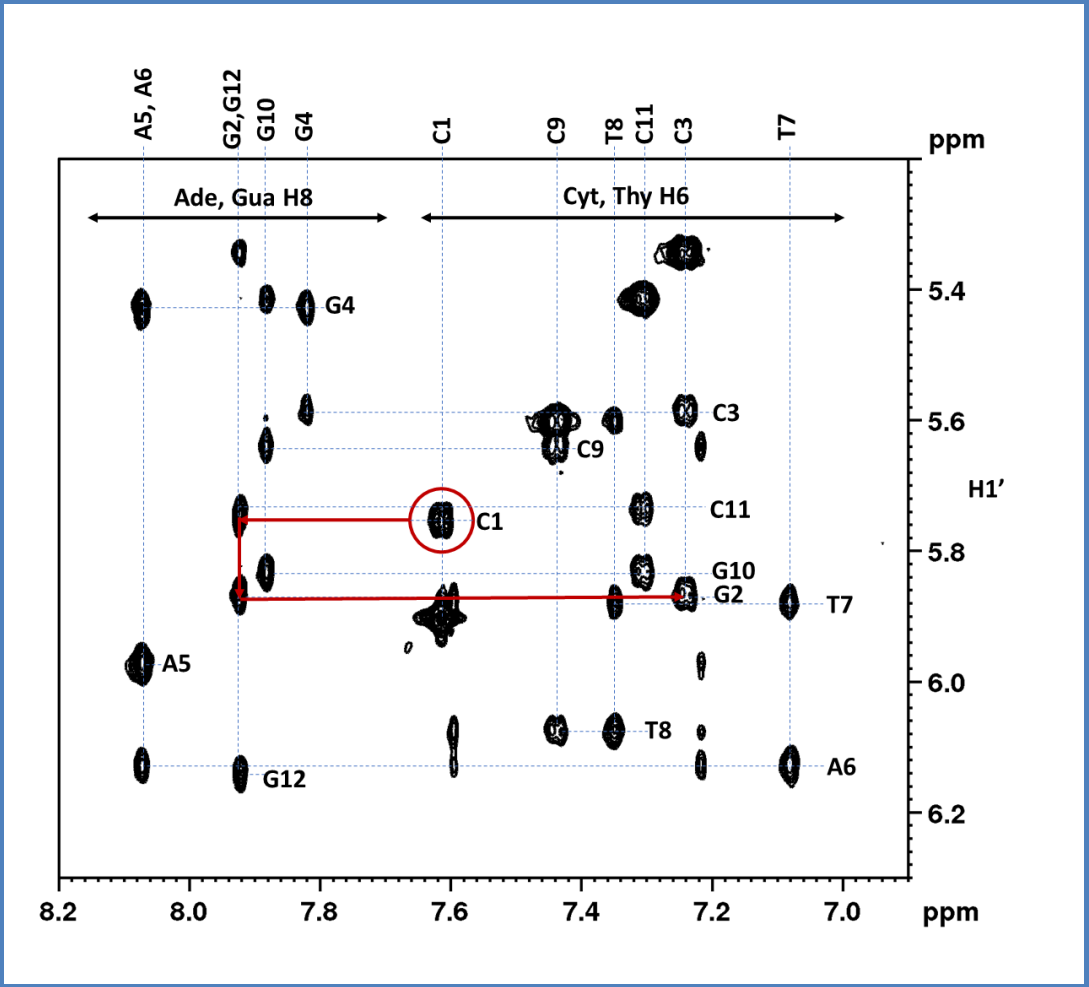
$d(CGCGAATTCGCG)_2$

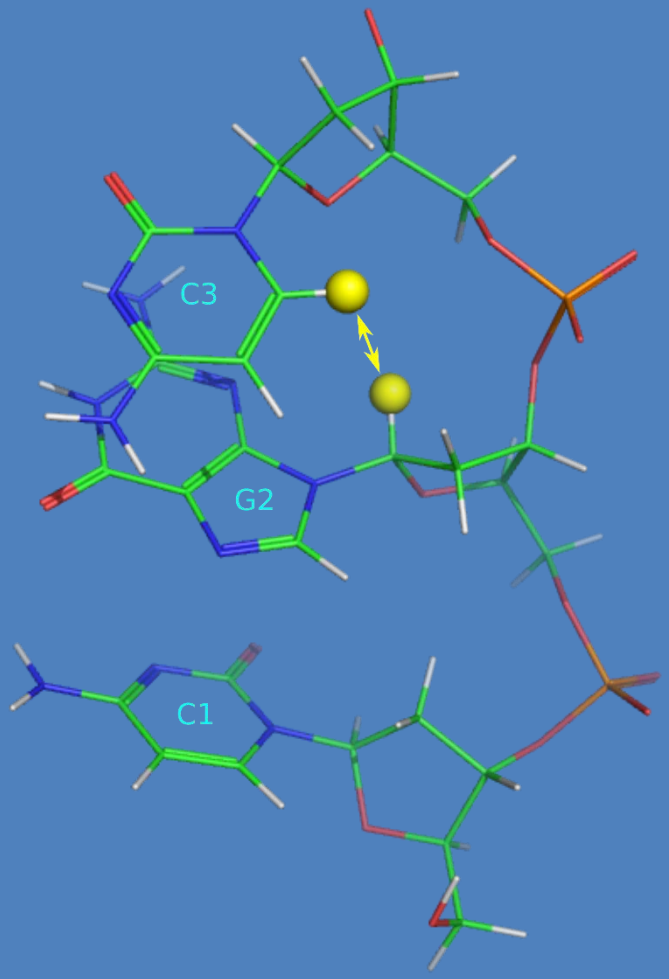




Assignment based on H6/8-H1’ connectivities

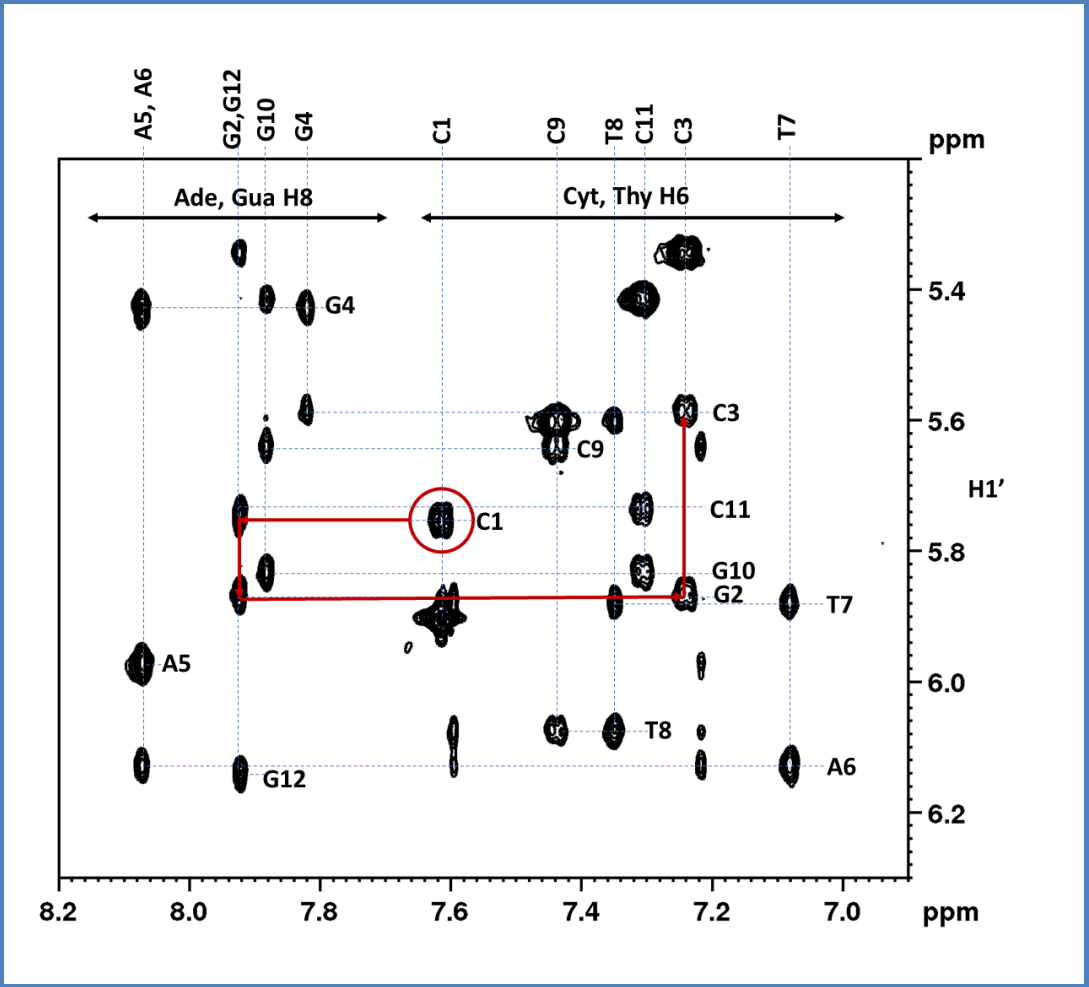
d(CGCGAATTCGCG)$_2$

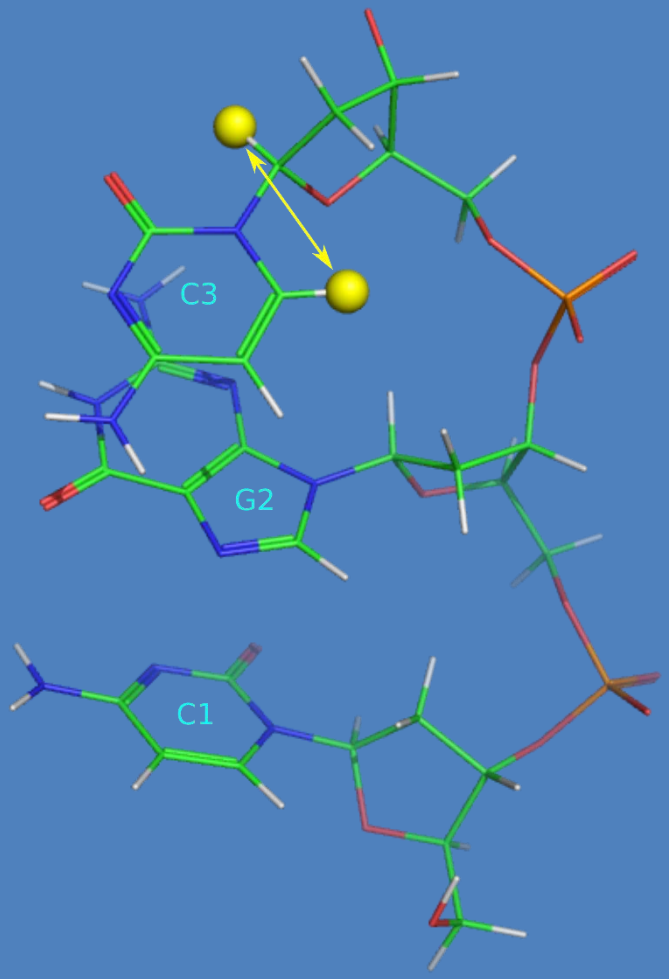




Assignment based on H6/8-H1’ connectivities

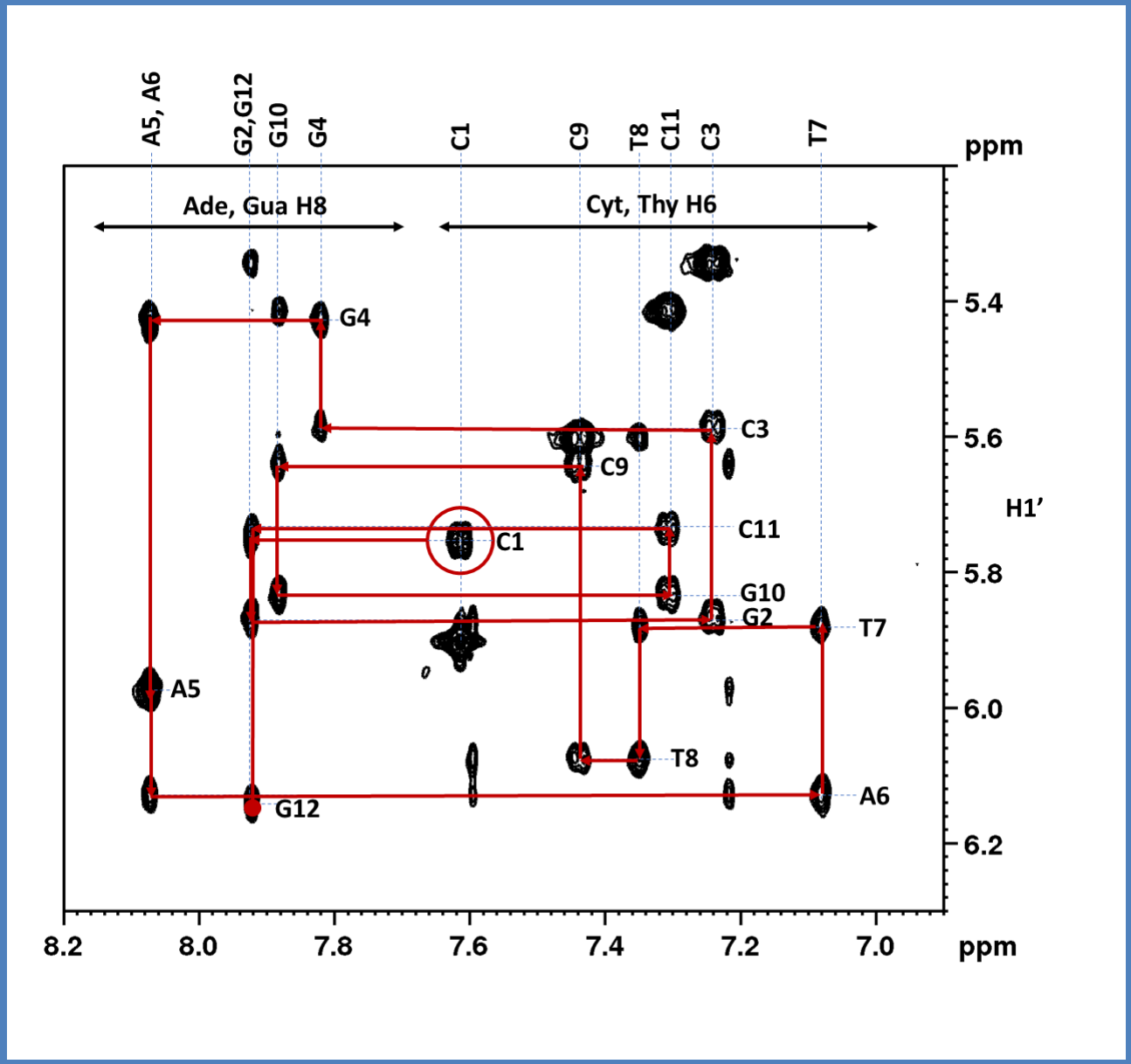
$d(CGCGAATTCGCG)_2$



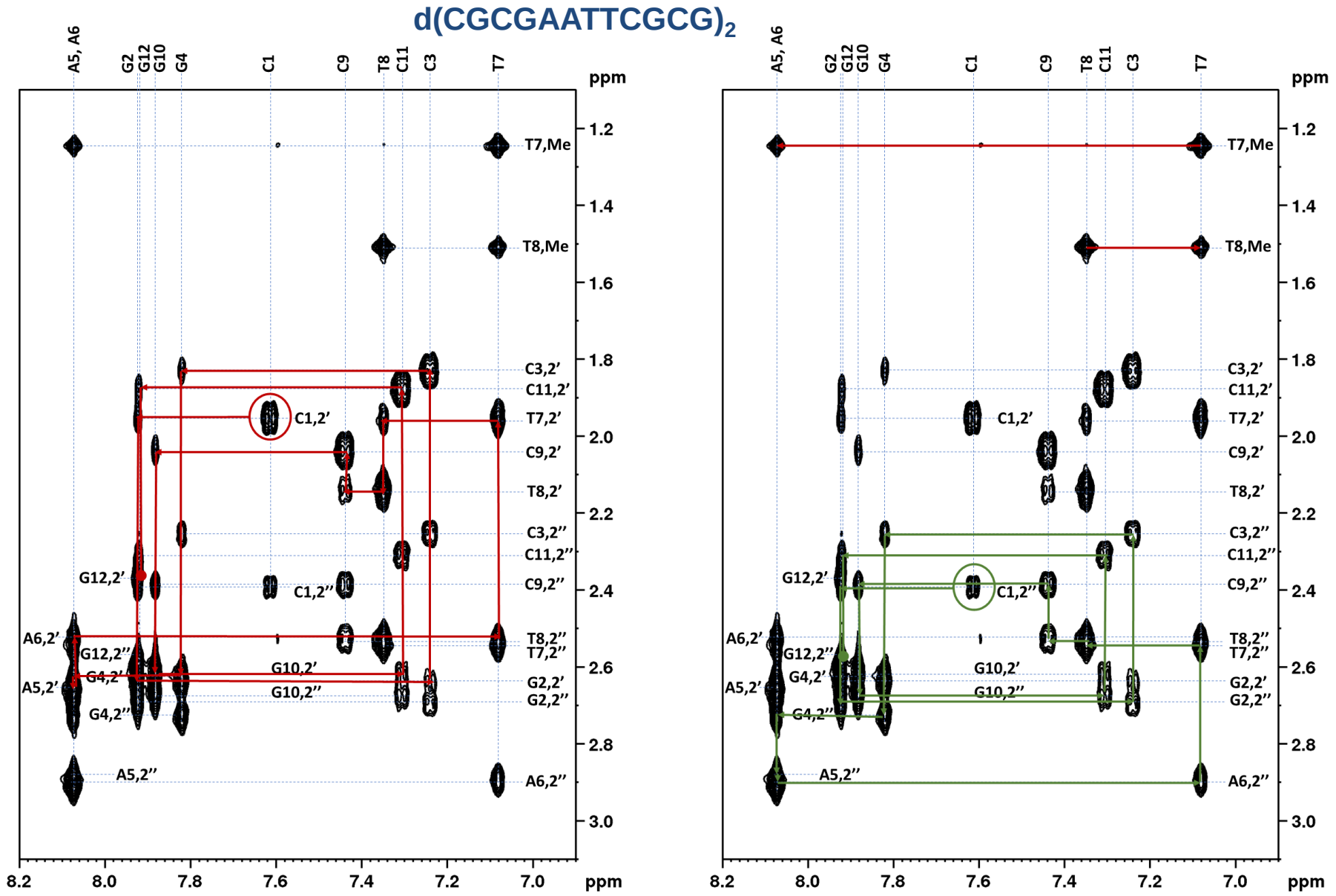


Assignment based on H6/8-H1’ connectivities

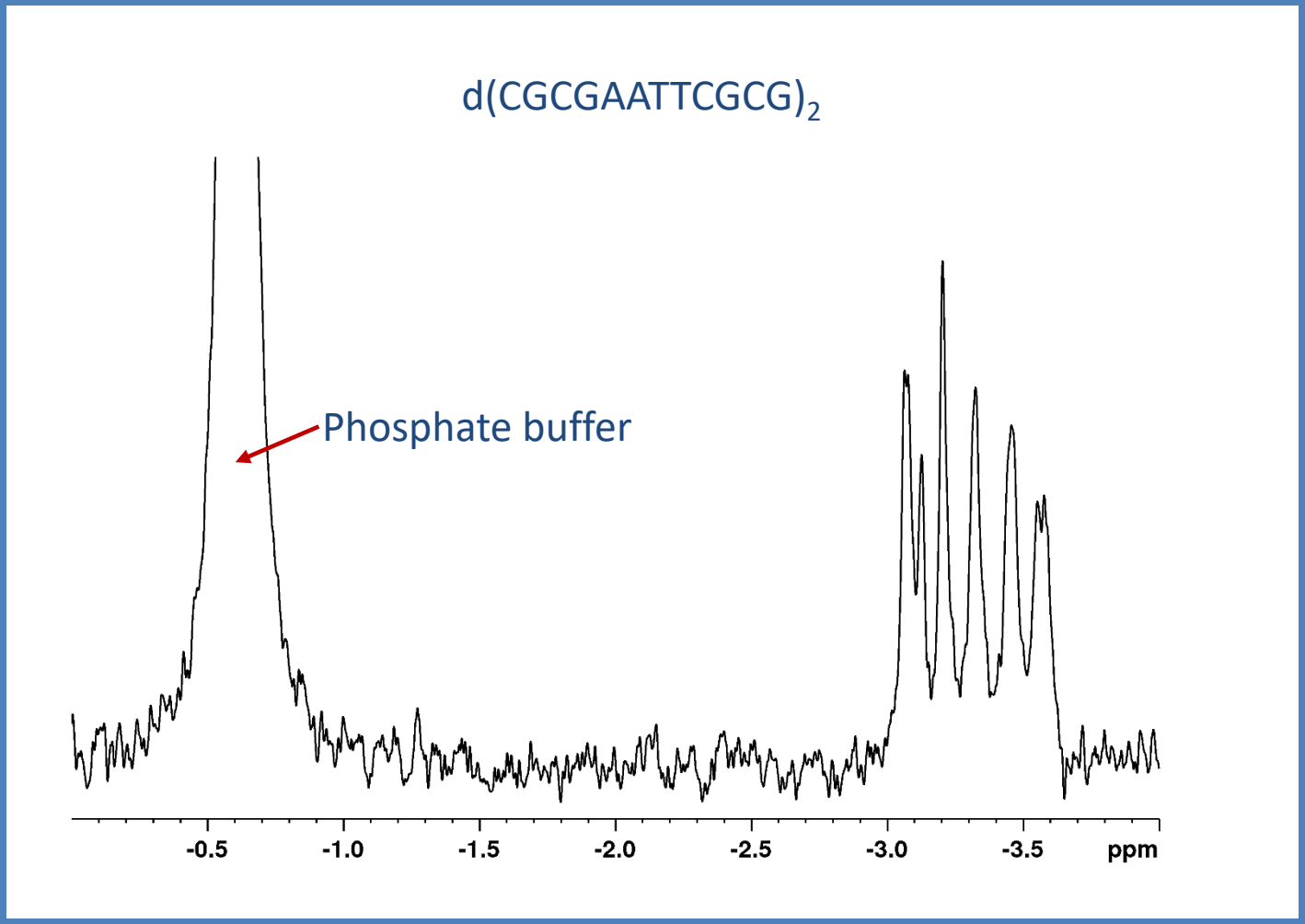
$d(CGCGAATTCGCG)_2$



Assignment based on H6/8-H2’2” connectivities



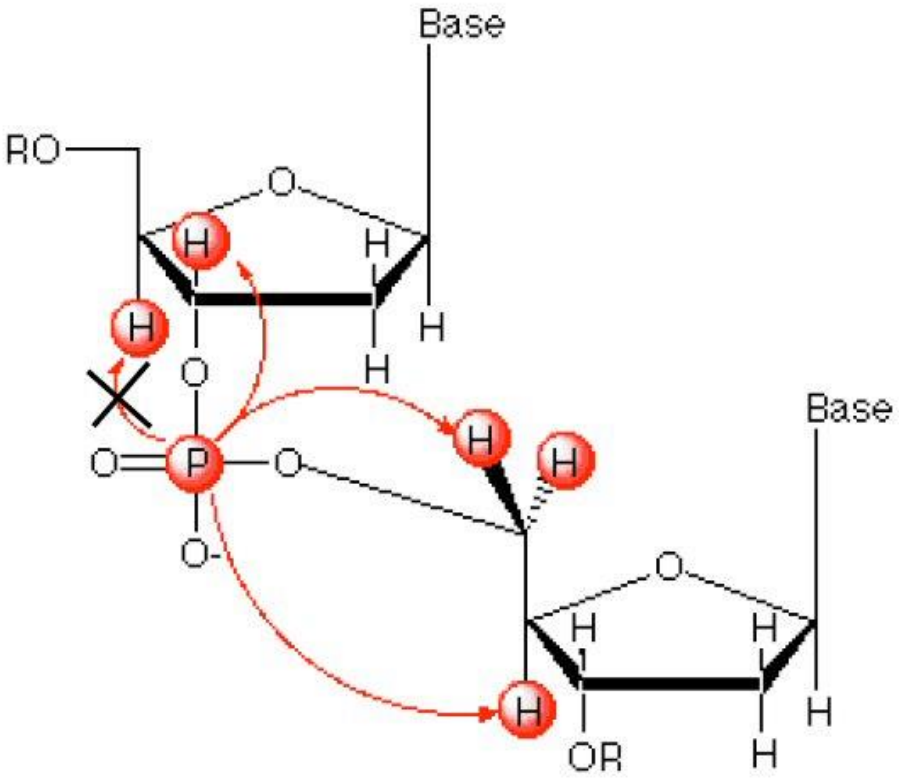
^{31}P spectrum of DNA



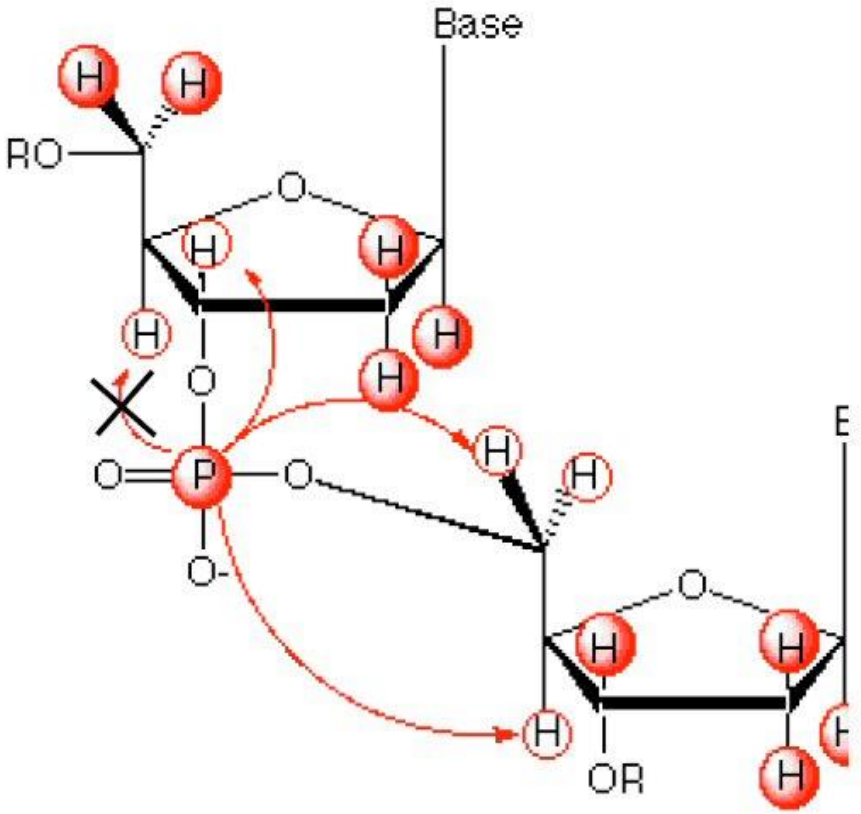
Assignment of Sugar-Phosphate Backbone

^{31}P NMR

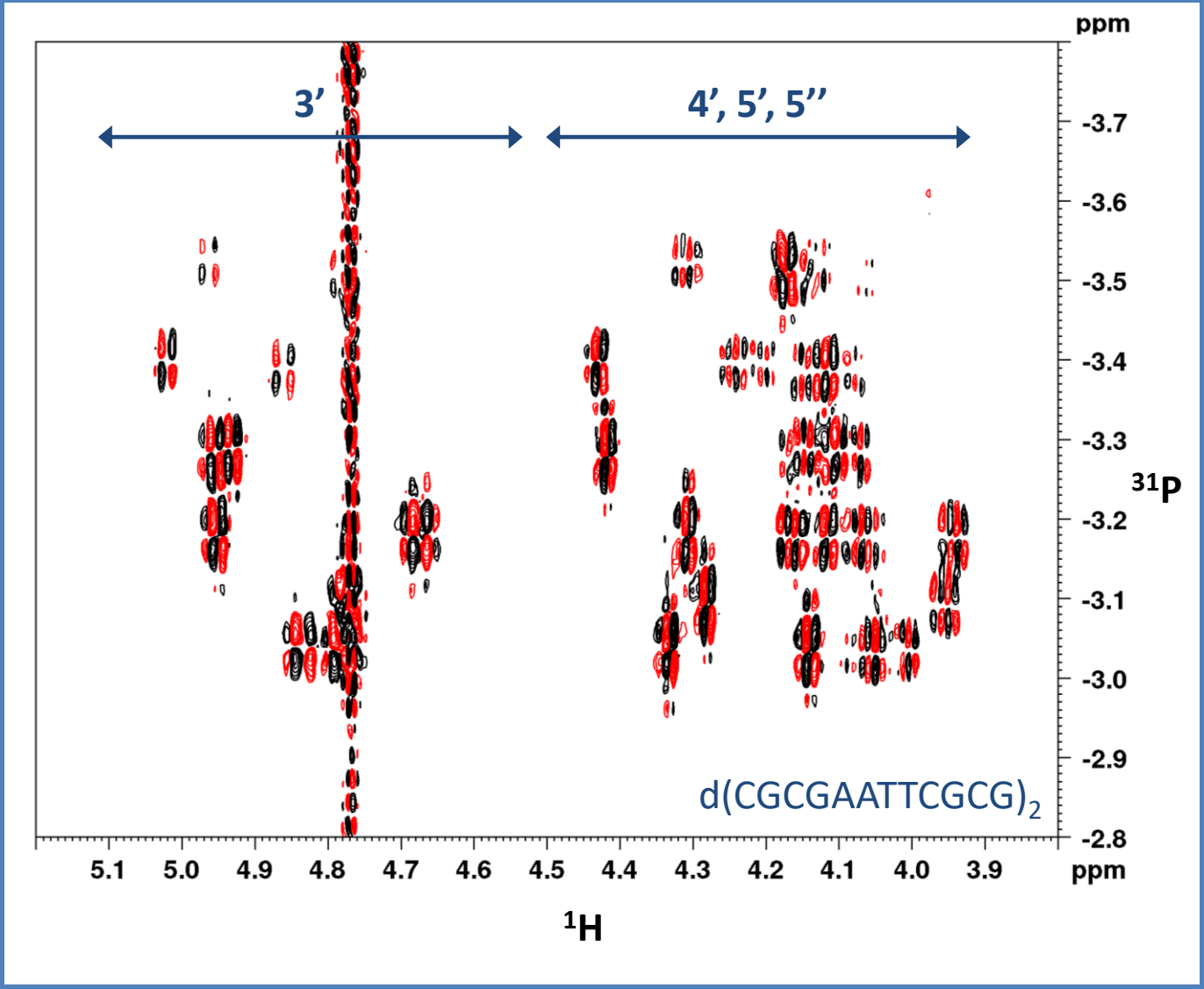
HP-COSY



HP-TOCSY



^{1}H - ^{31}P correlation spectrum



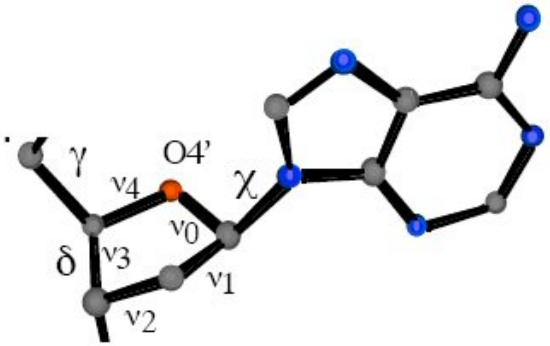
Sugar puckering

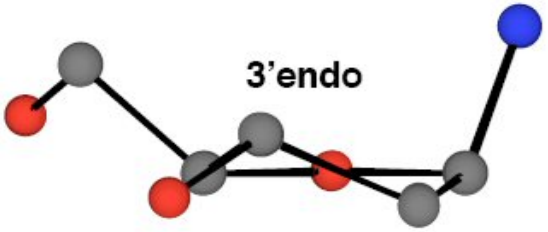
The five membered furanose ring is not planar. It can be puckered in an envelope form (E) with 4 atoms in a plane or it can be in a twist form. The geometry is defined by two parameters: **the pseudorotation phase angle (P)** and the **pucker amplitude** (Φ).

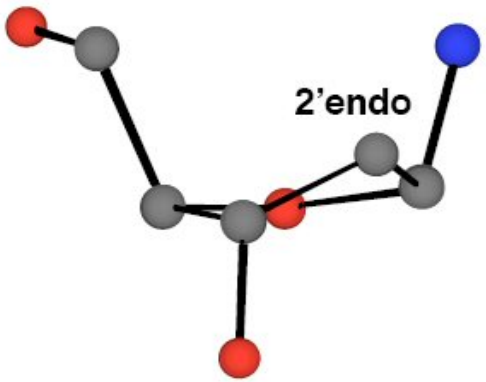
In general:
RNA (A type double helix) C3' endo.
DNA (B type double helix) C2' endo.

$$\nu_i = \Phi_m \cos (P + 144 (j-2))$$

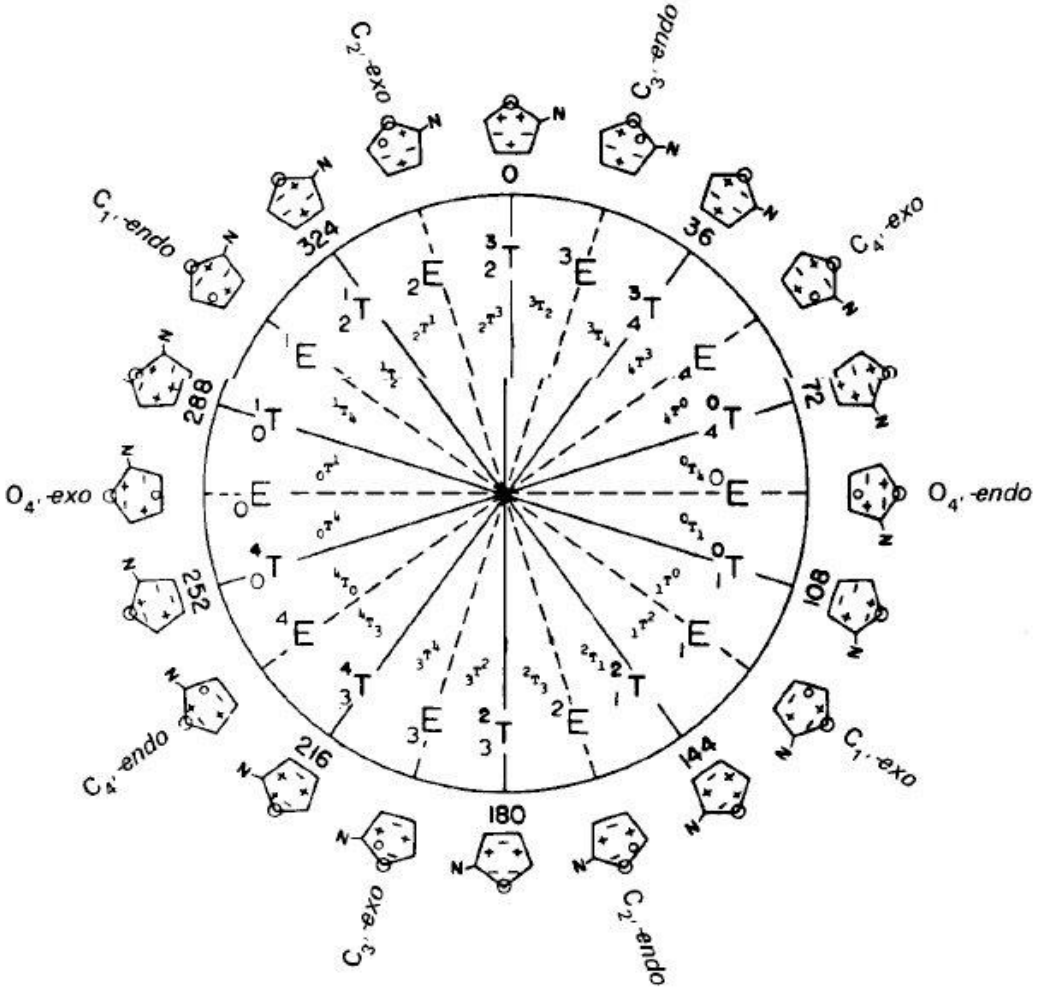
$$\delta = \nu_3 + 125^\circ$$



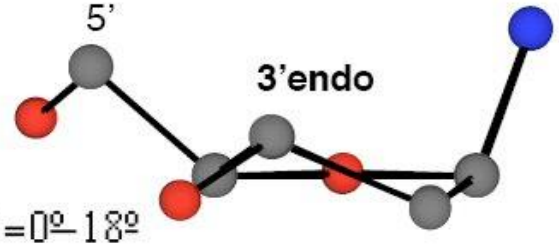




N (Northern)



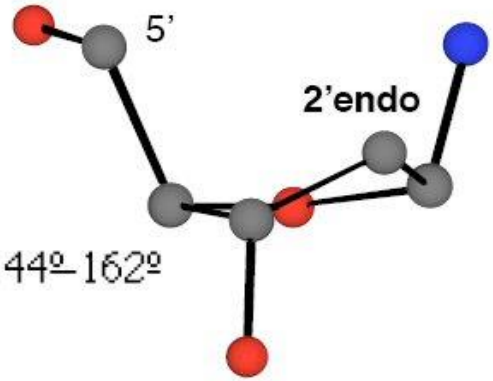
(Southern)



P=0º–18º

Ribose: $^{3}J_{H1'-H2'} \approx 1$ Hz

Deoxyribose: $^{3}J_{H1'-H2'} \approx 1.8$ Hz

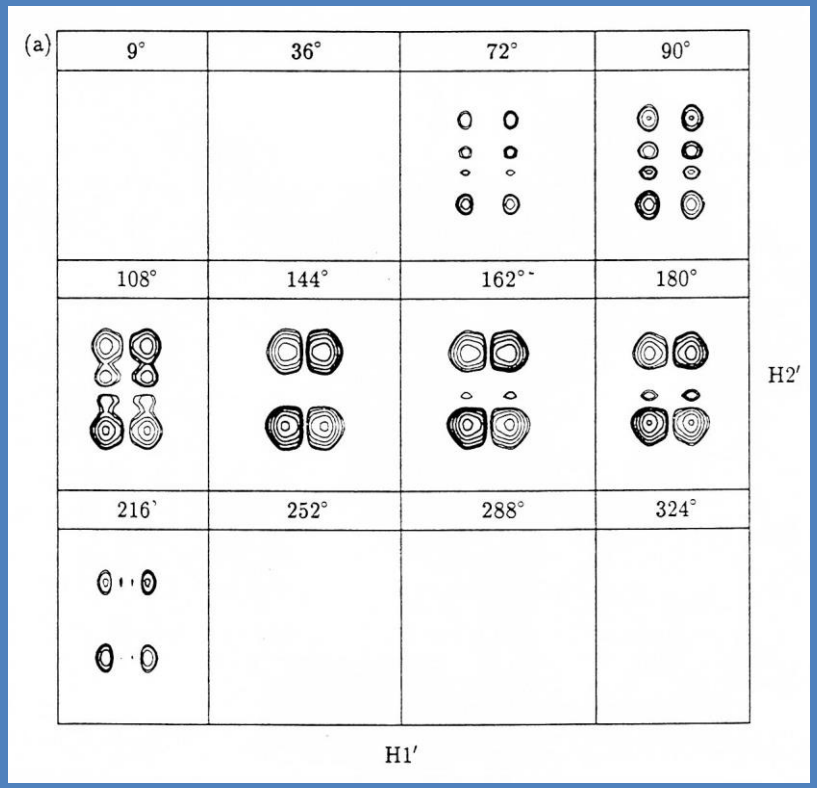


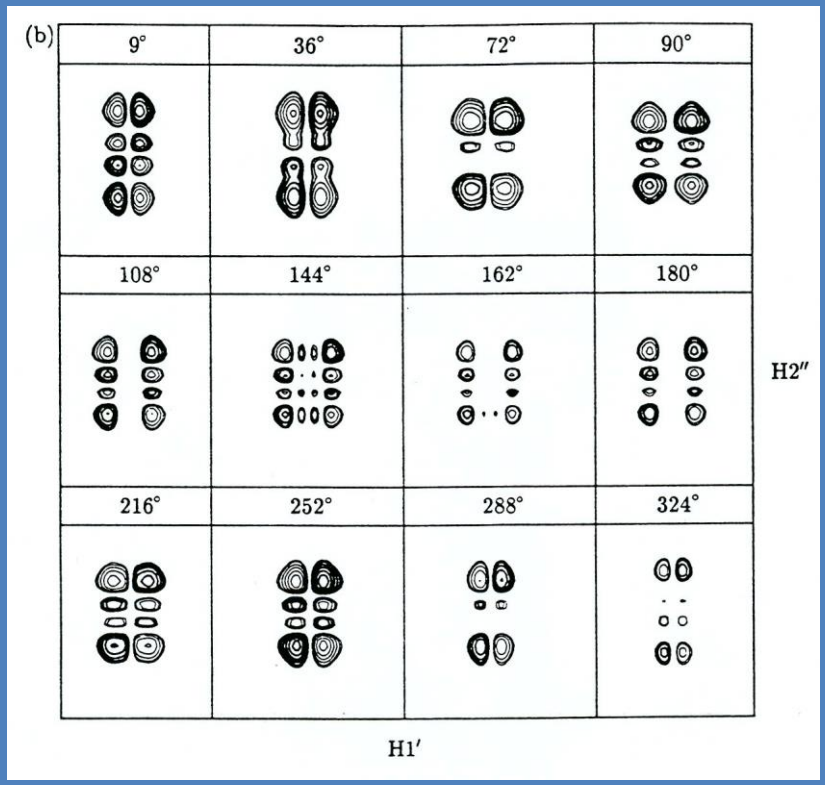
P=144º–162º

Ribose: $^{3}J_{H1'-H2'} \approx 7.9$ Hz

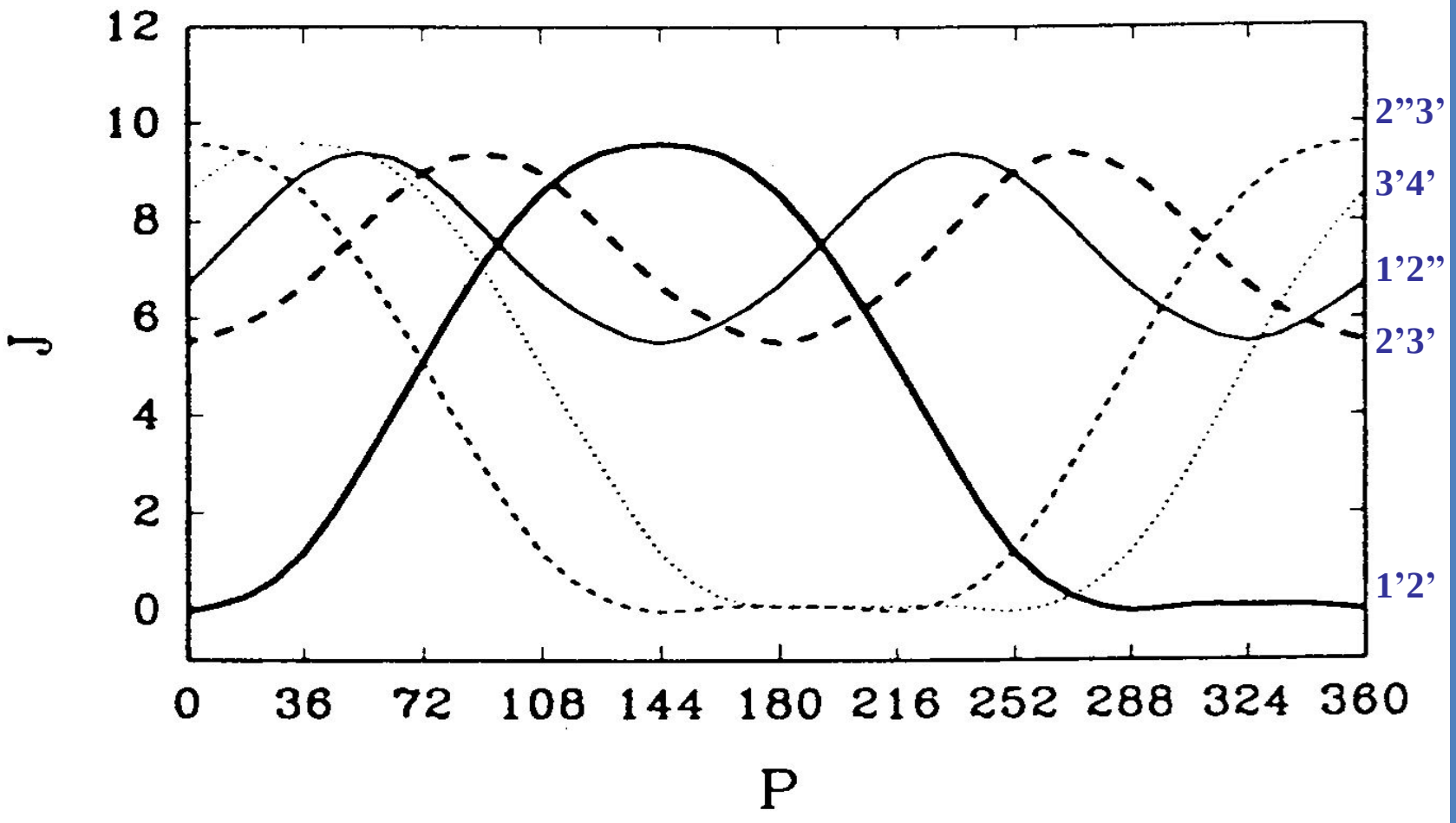
Deoxyribose: $^{3}J_{H1'-H2'} \approx 10$ Hz

J-couplings from COSY spectra

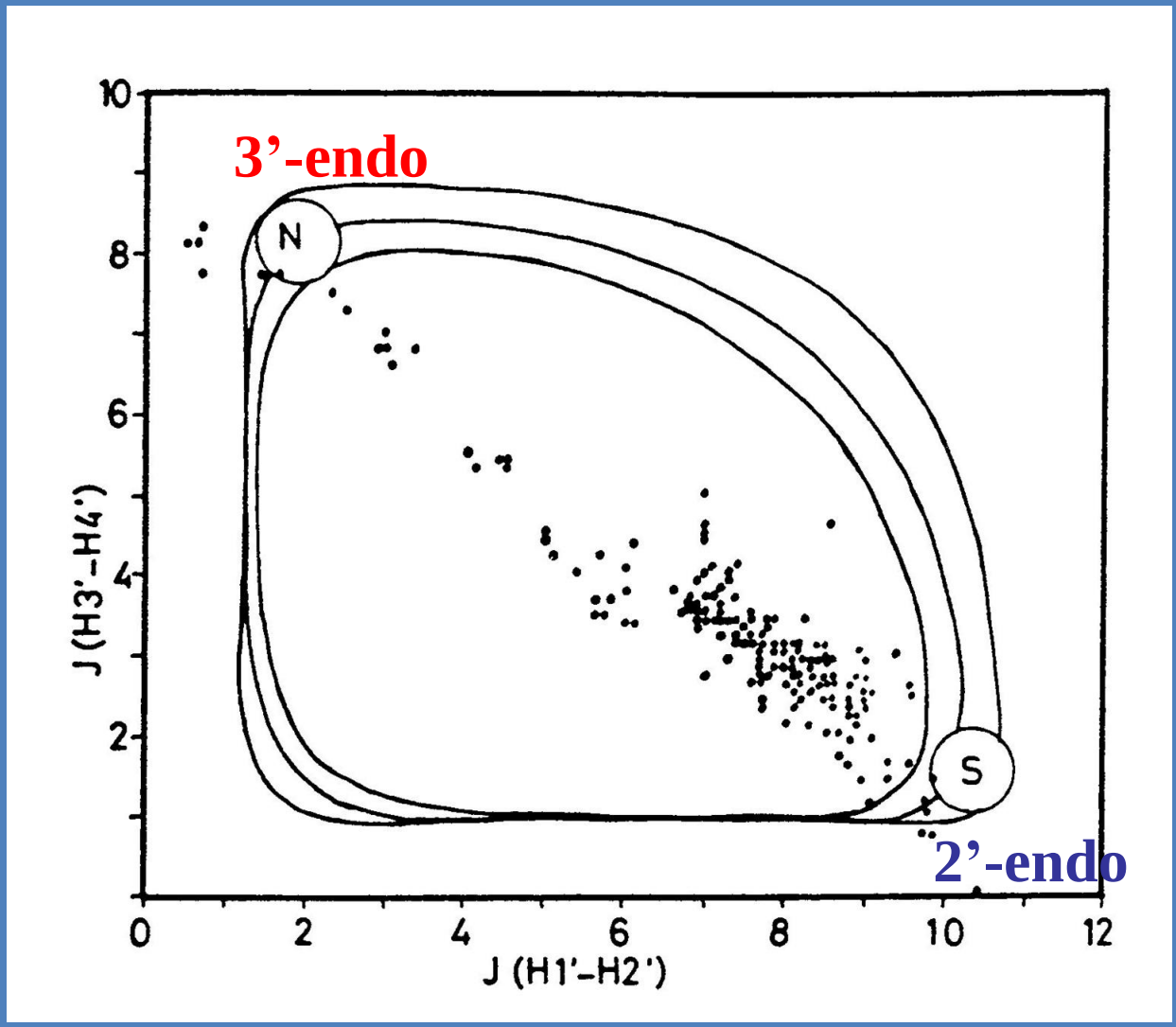




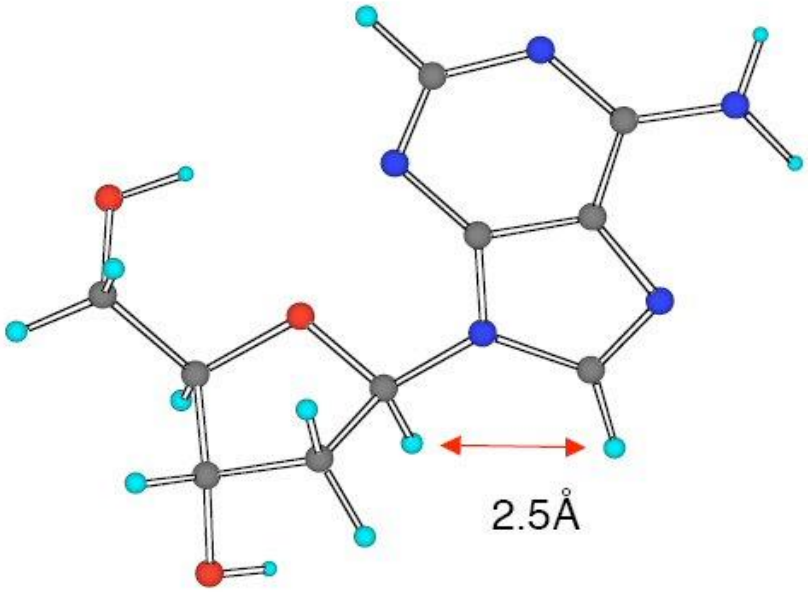
P determination from J-couplings

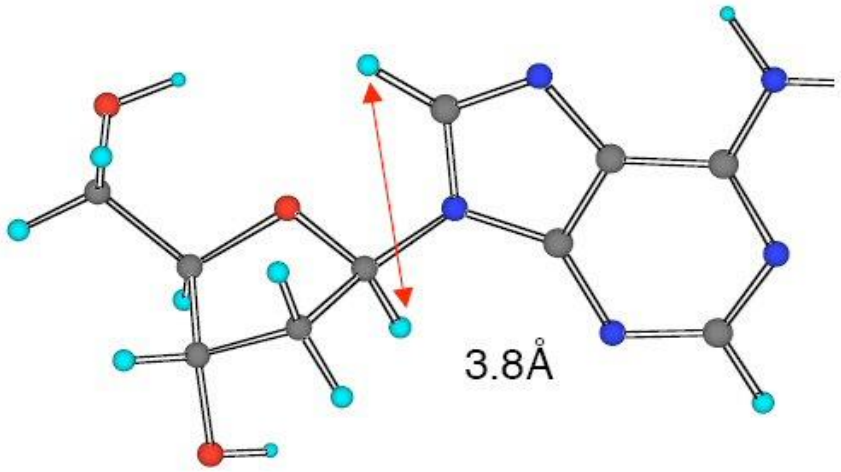


Equilibrium of N and S conformations

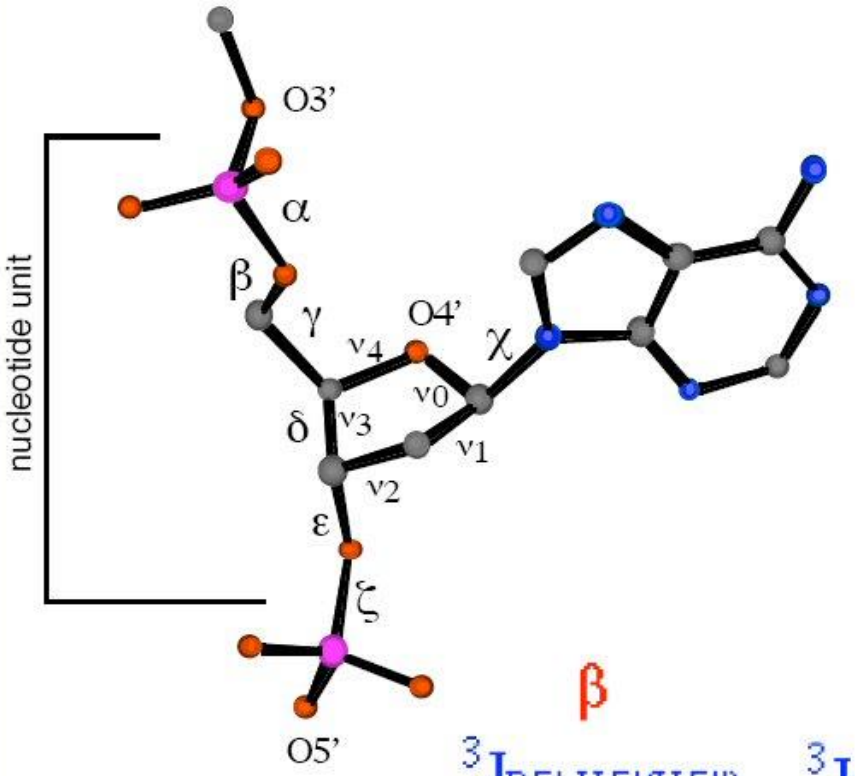


Distance information determines the glycosidic torsion angle





- How do we get distance information?
  - Nuclear Overhauser effect (< 6Å)



α and ζ pose problems
Determinants of ^{31}P chem shift.
ε and ζ correlate. ζ = -317-1.23 ε

β	γ	ε	χ
$^3J_{P5'-H5'(H5'')}$	$^3J_{H4'-H5'(H5'')}$	$^3J_{P3'-H3'}$	$^3J_{H1'-C6}$ (U,C,T)
$^3J_{P5'-C4'}$	$^3J_{C3'-H5'(H5'')}$	$^3J_{P3'-C2'}$	$^3J_{H1'-C2}$ (U,C,T)
		$^3J_{P3'-C4'}$	$^3J_{H1'-C8}$ (A,G)
			$^3J_{H1'-C4}$ (A,G)

Structure Determination:

I) Assignment

II) Local Analysis

•glycosidic torsion angle, sugar puckering,backbone conformation base pairing

III) Global Analysis

•sequential, inter strand/cross strand, dipolar coupling

Nucleic Acids have few protons.....

•NOE accuracy

> account for spin diffusion

•Backbone may be difficult to fully characterize

> especially α and ζ.

•Dipolar couplings

What do we know?
- Distance, Torsion, H-Bond constraints

What do we want?
- Low energy structures

Methods
- Distance Geometry
- Simulated annealing, rMD
- Torsion angle dynamics (DYANA)
- Mardigras/IRMA/Morass