NUCLEIC ACIDS

Basic terms and notions

Presentation by

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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.

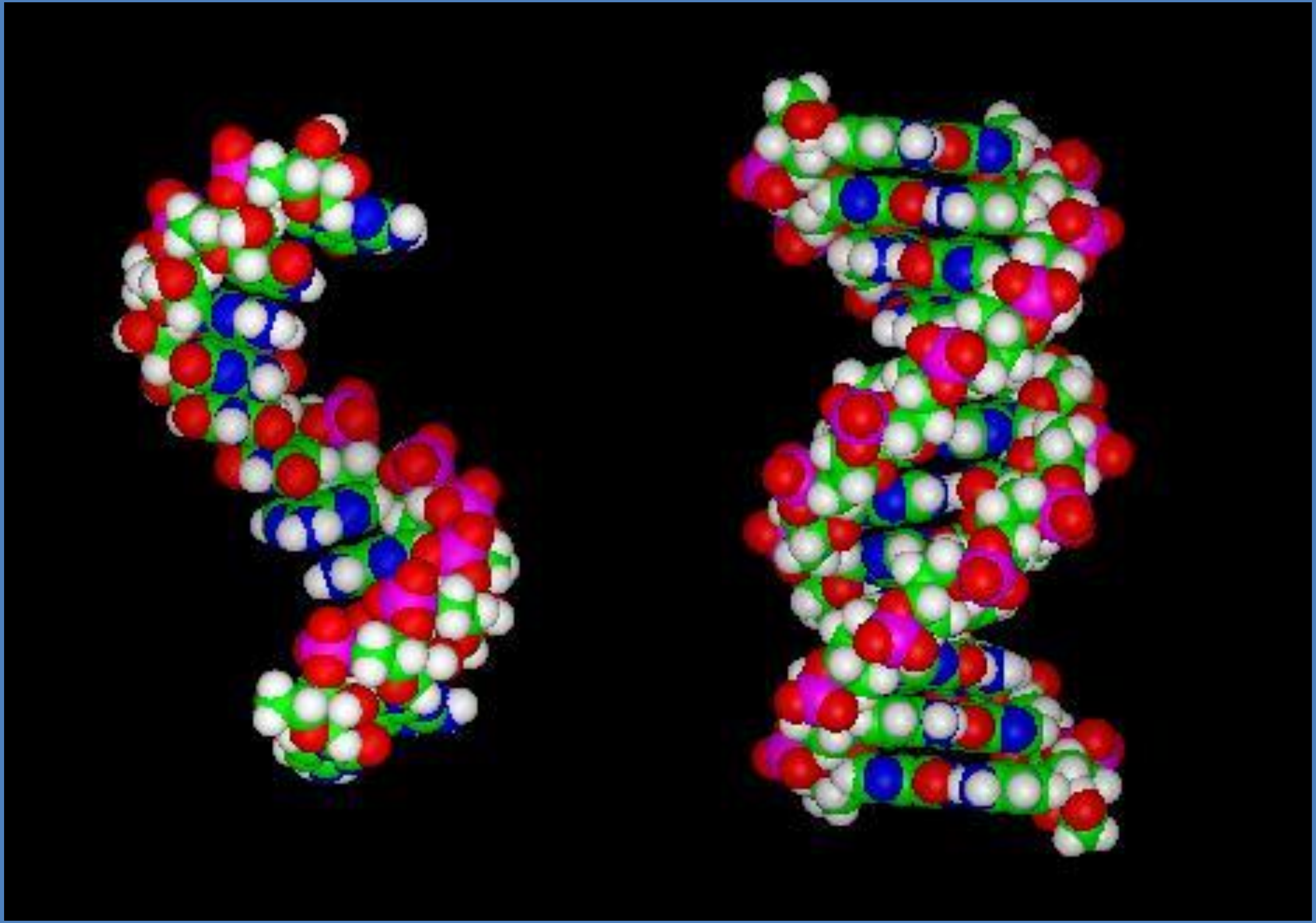
Review articles

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.

RNA vs DNA



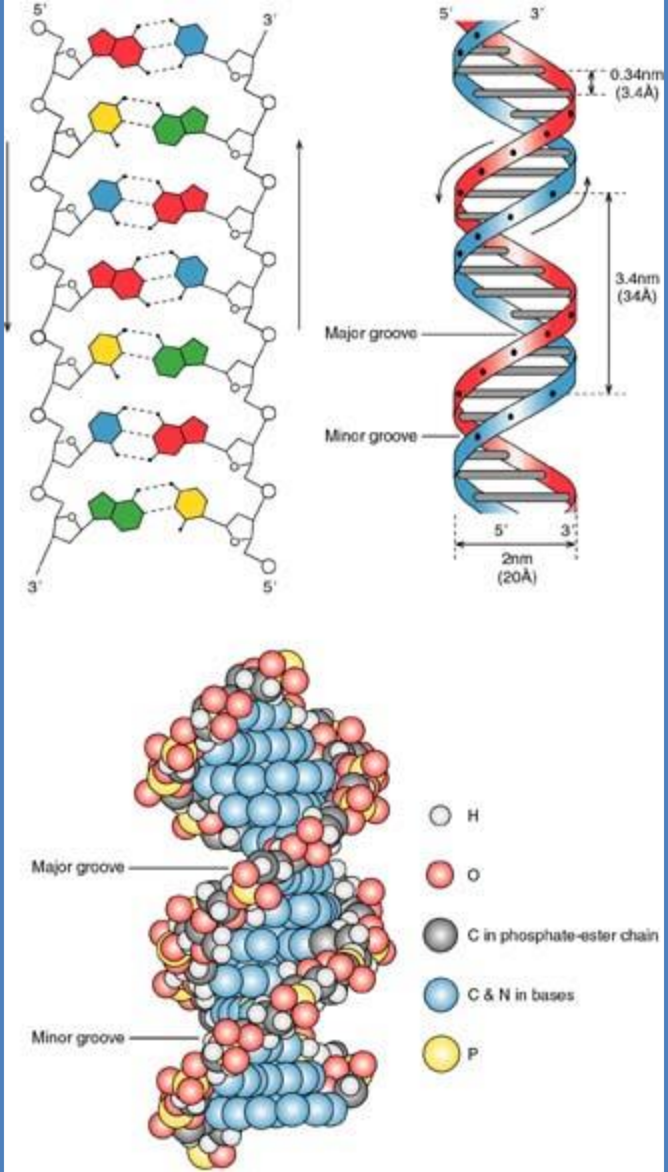
Single strand A-RNA **B-DNA duplex**

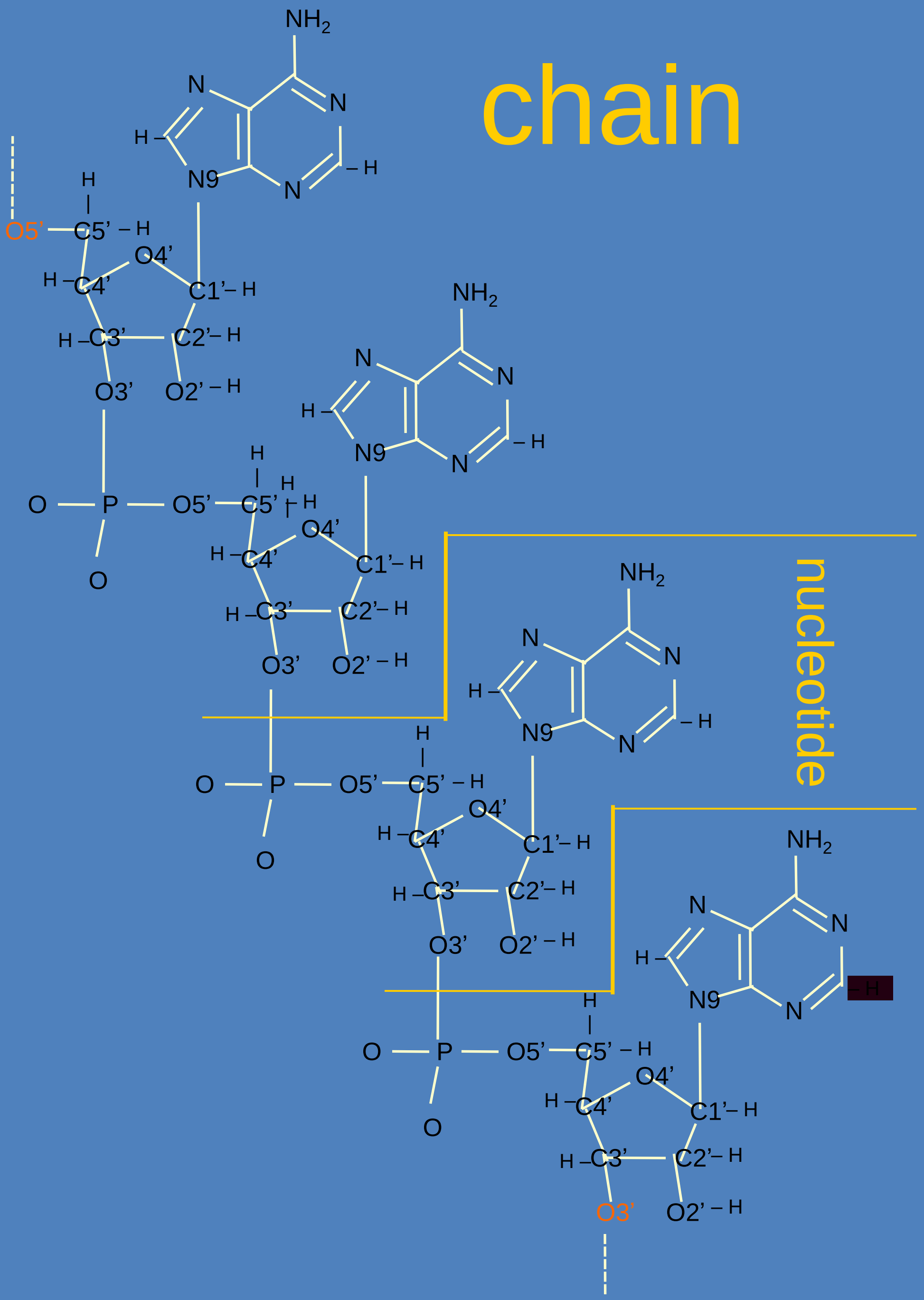
Length of NA

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

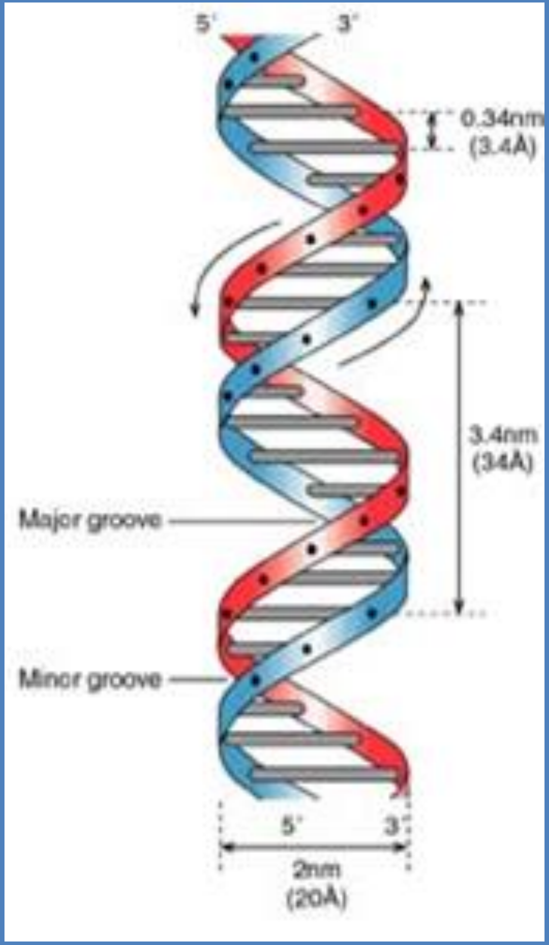
Nukleotide chain

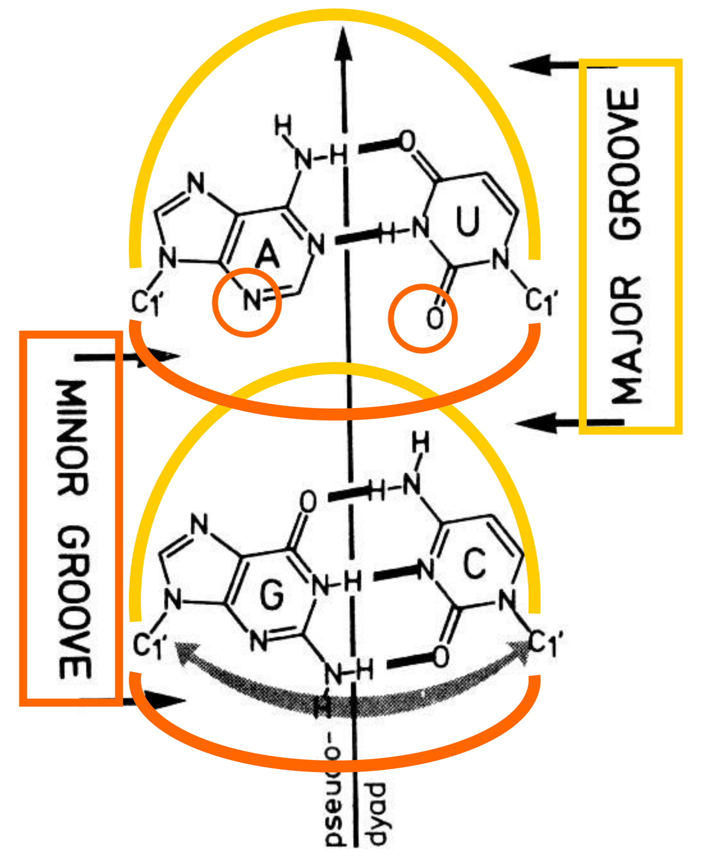




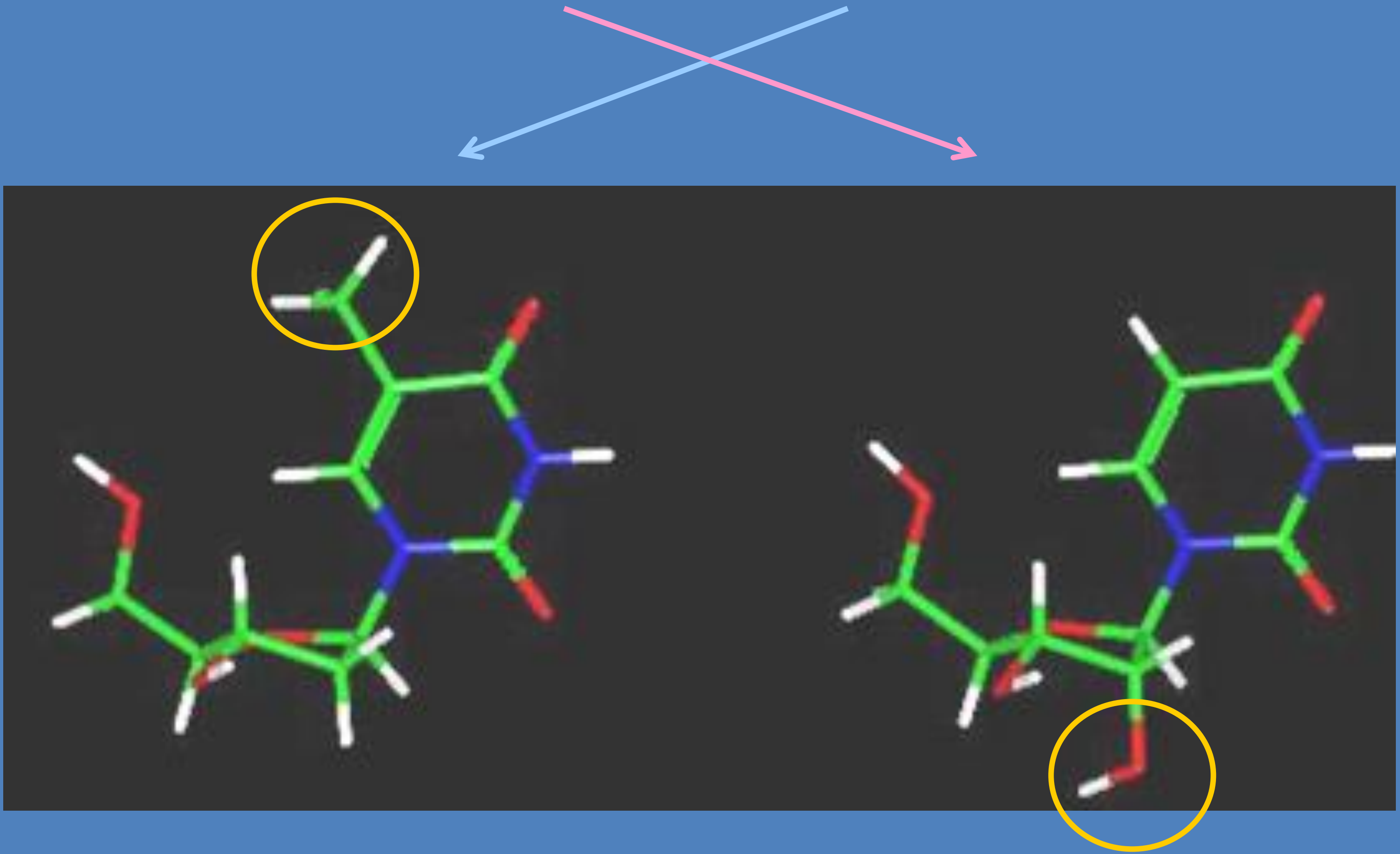
Grooves

major vs. minor





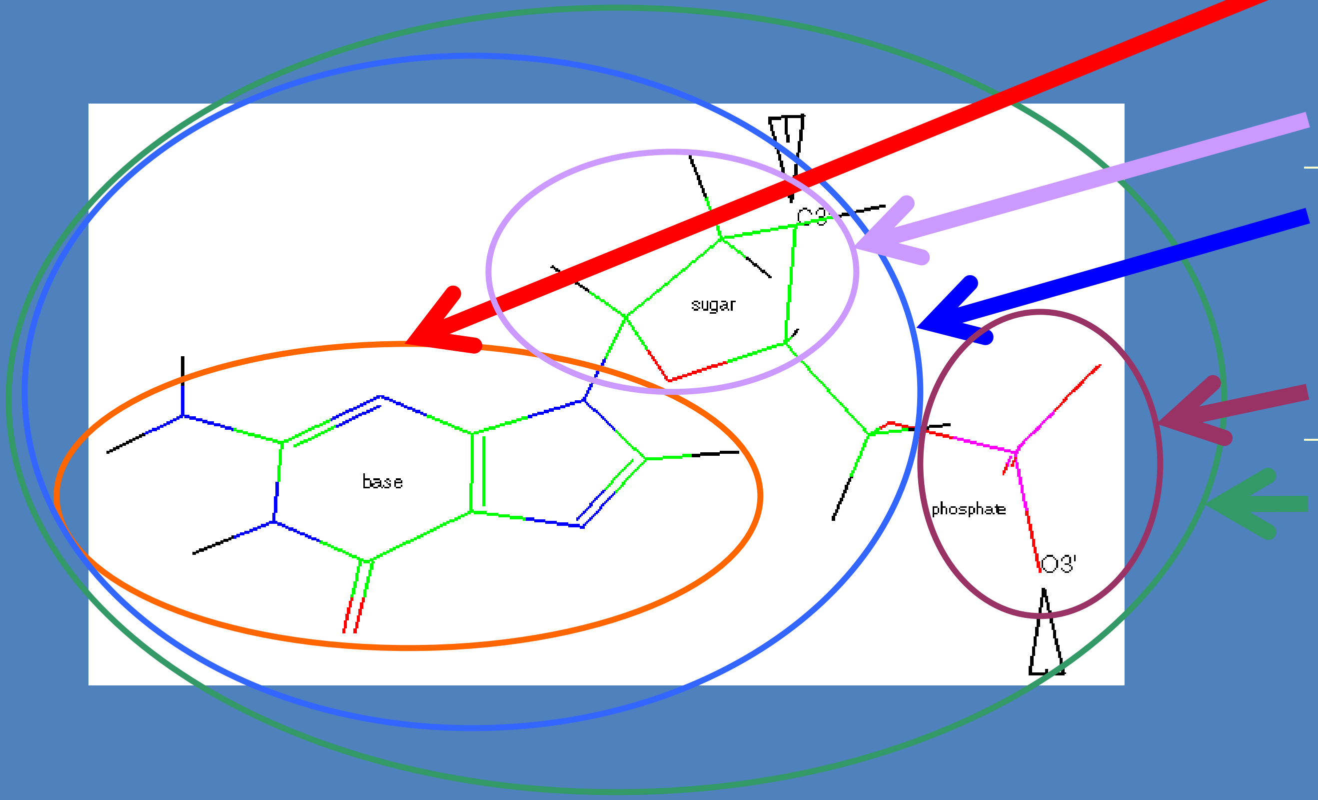
RNA vs DNA



deoxythymidine

uridine

Nukleotide/nukleoside



base

+

sugar (ribose/deoxyribose)

nukleoside

+

phosphate

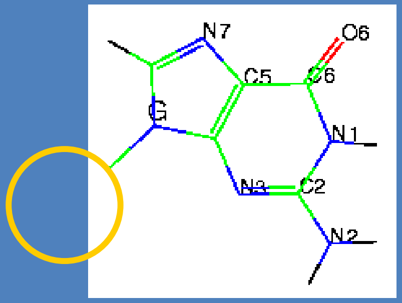
nukleotide

Bases

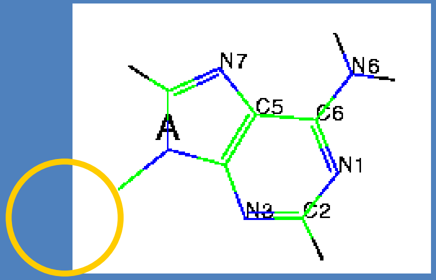
DNA

RNA

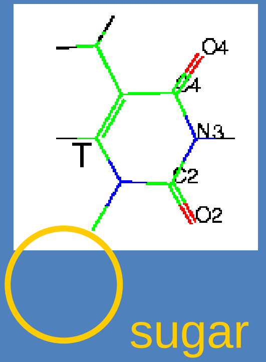
Guanin (Gua)



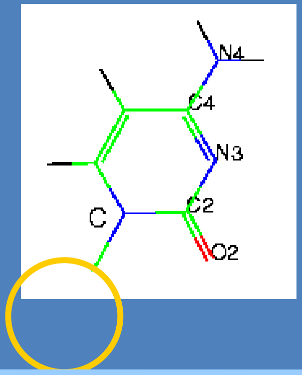
Adenin (Ade)



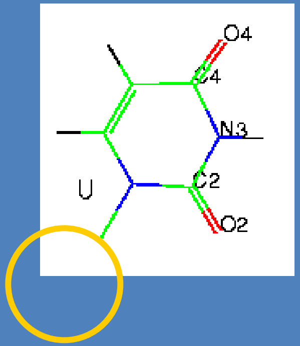
Thymin (Thy)



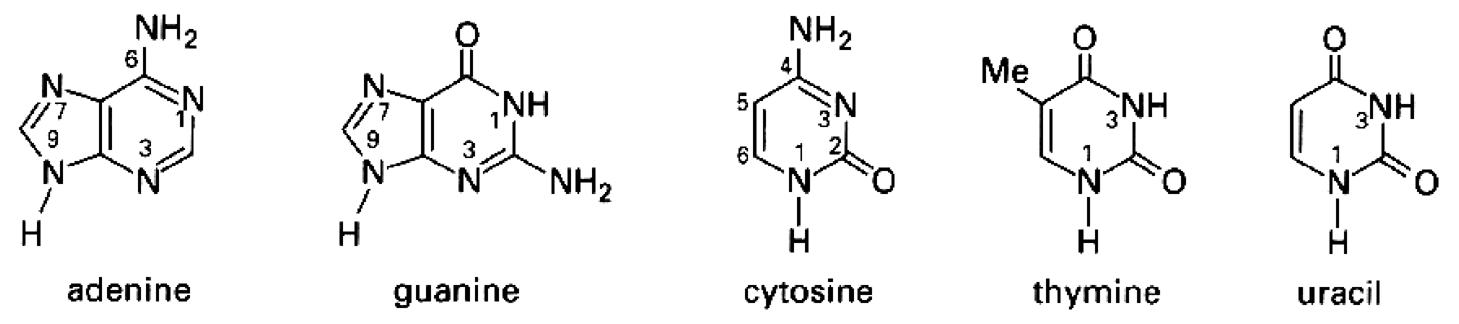
Cytosin (Cyt)



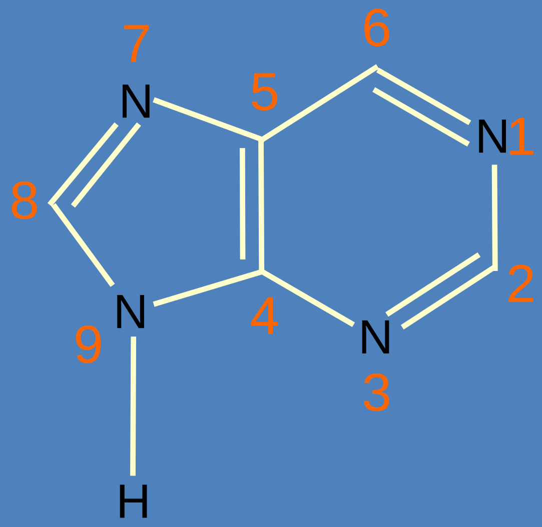
Uracil (Ura)



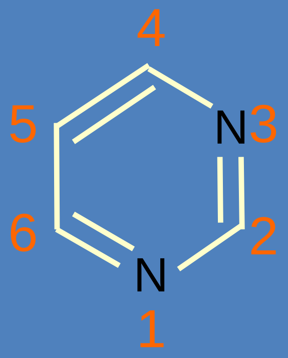
Base numbering



PURINES



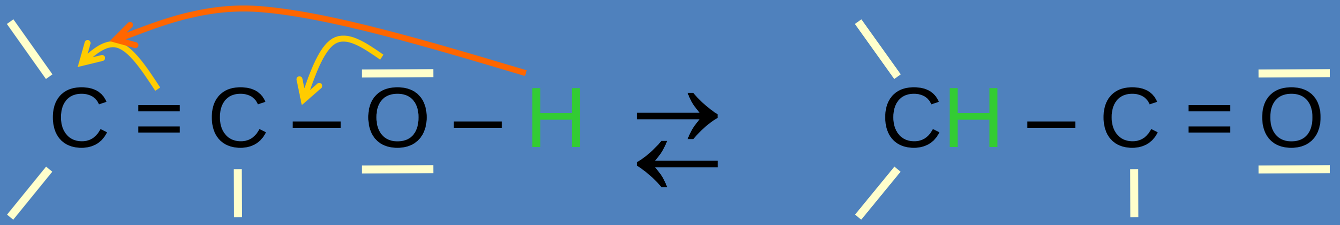
PYRIMIDINES



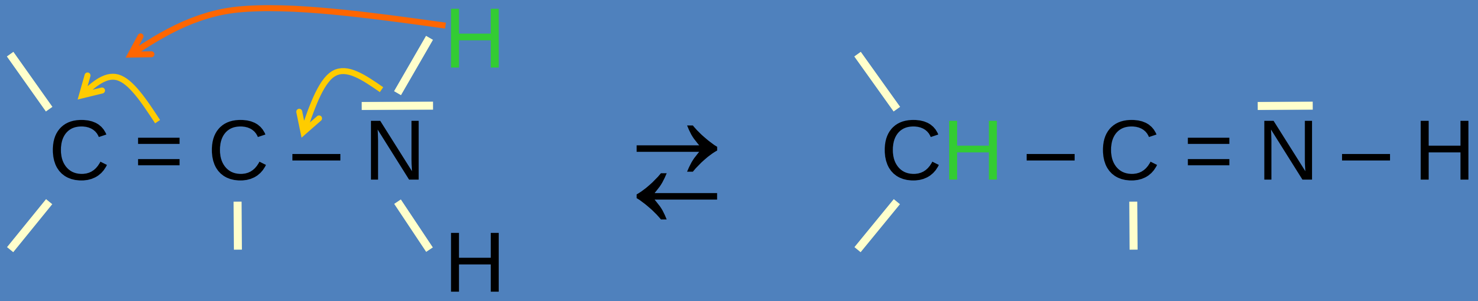
Base tautomerism

fysiolog. conditions

enol ↔ keto

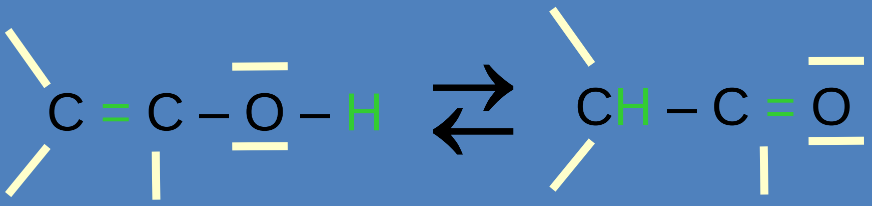


enamin ↔ imin

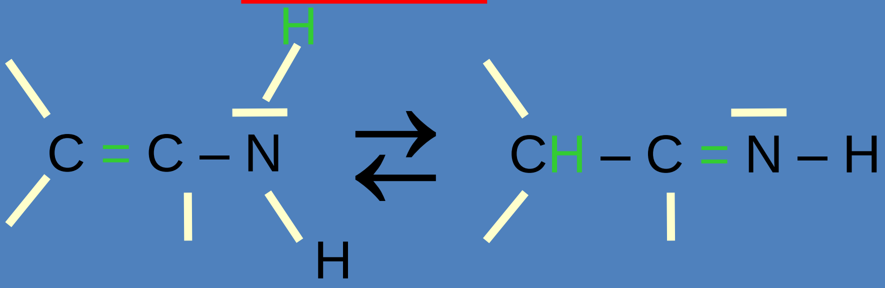


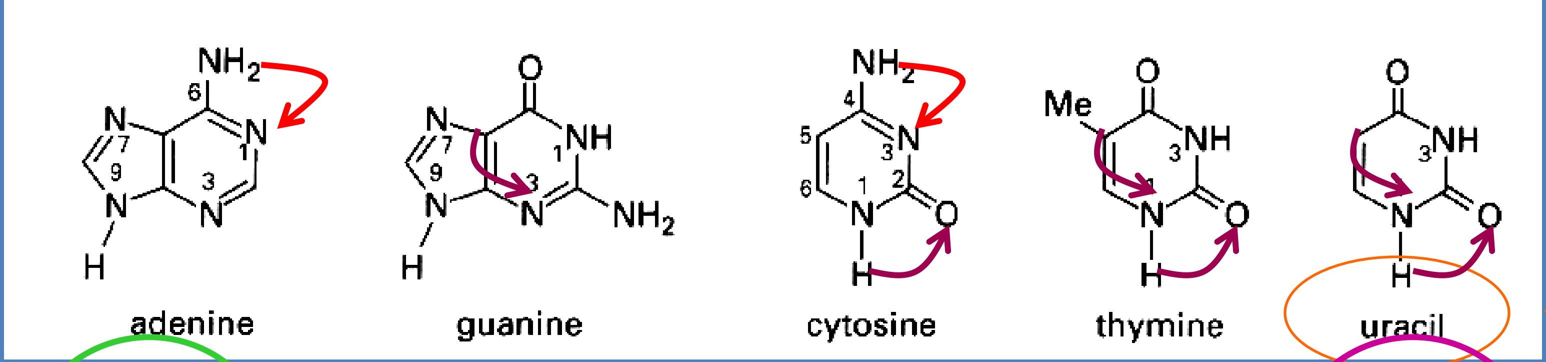
Base tautomerism

enol ↔ keto



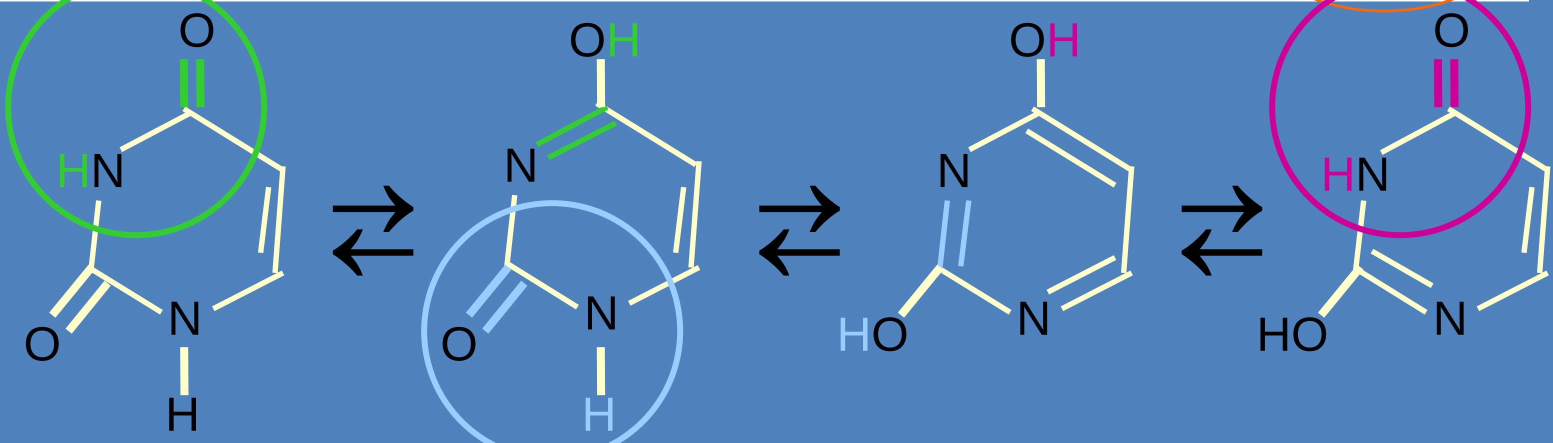
enamin ↔ imin



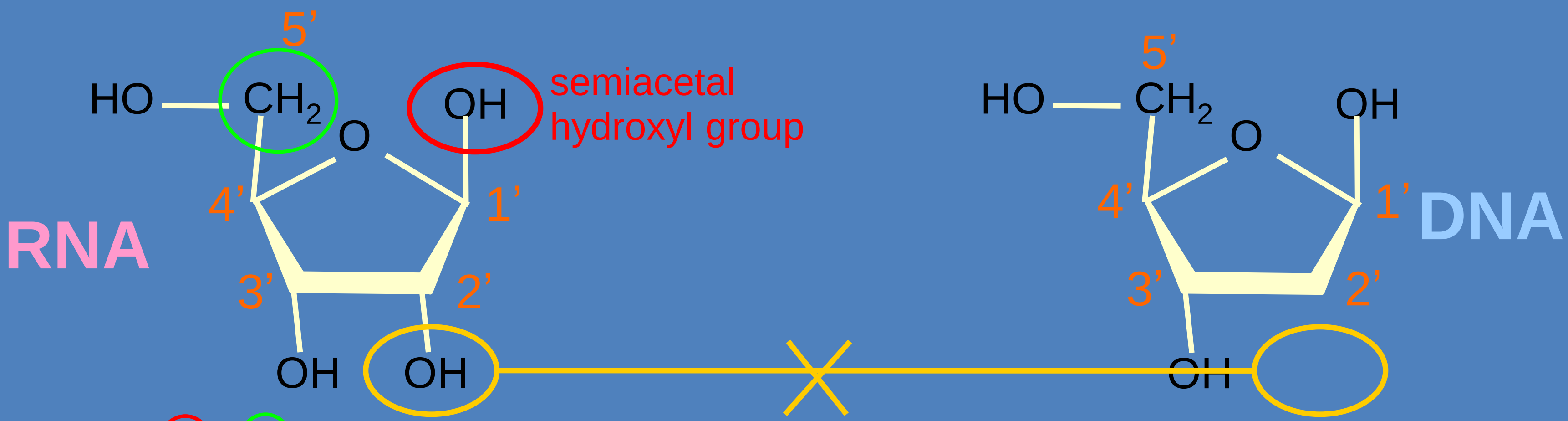


adenine guanine cytosine thymine uracil

4 tautomers



Sugar - pentoses



semiacetal hydroxyl group

ß – D - ribose

2 – deoxy – ß – D - ribose

Other aldopentoses: arabinose, xylose, lyxose

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

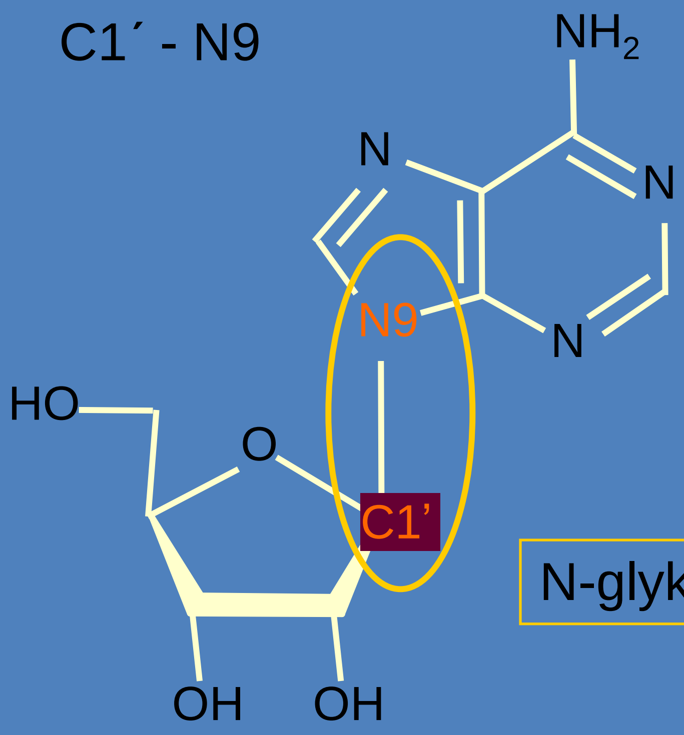
C1´ - N1 pyrimidines

C1´ - N9 purines

Nukleosides

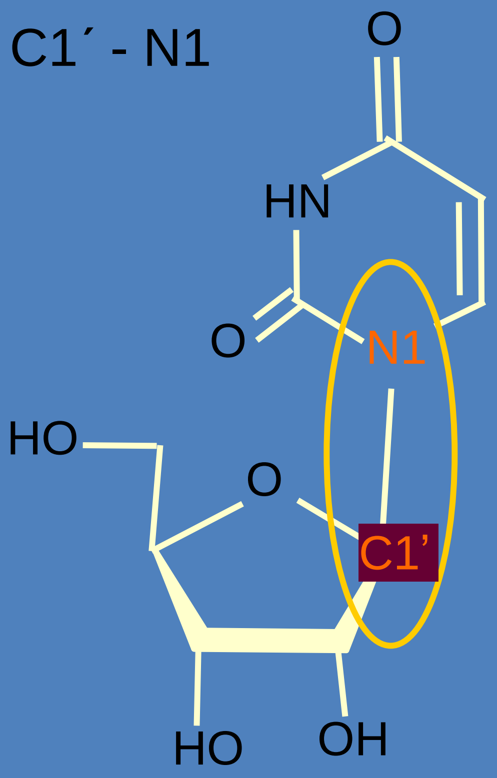
purines

C1´ - N9



pyrimidines

C1´ - N1



N-glykosidic bond

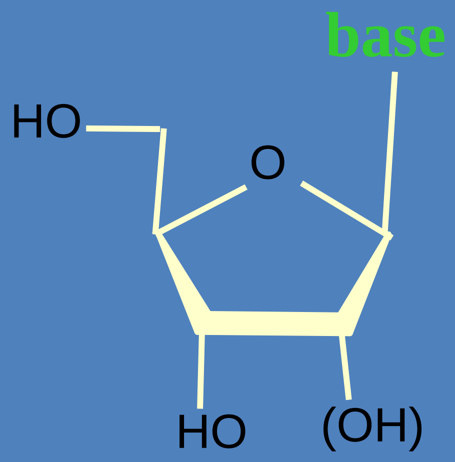
Nukleosides

Ribonukleosides

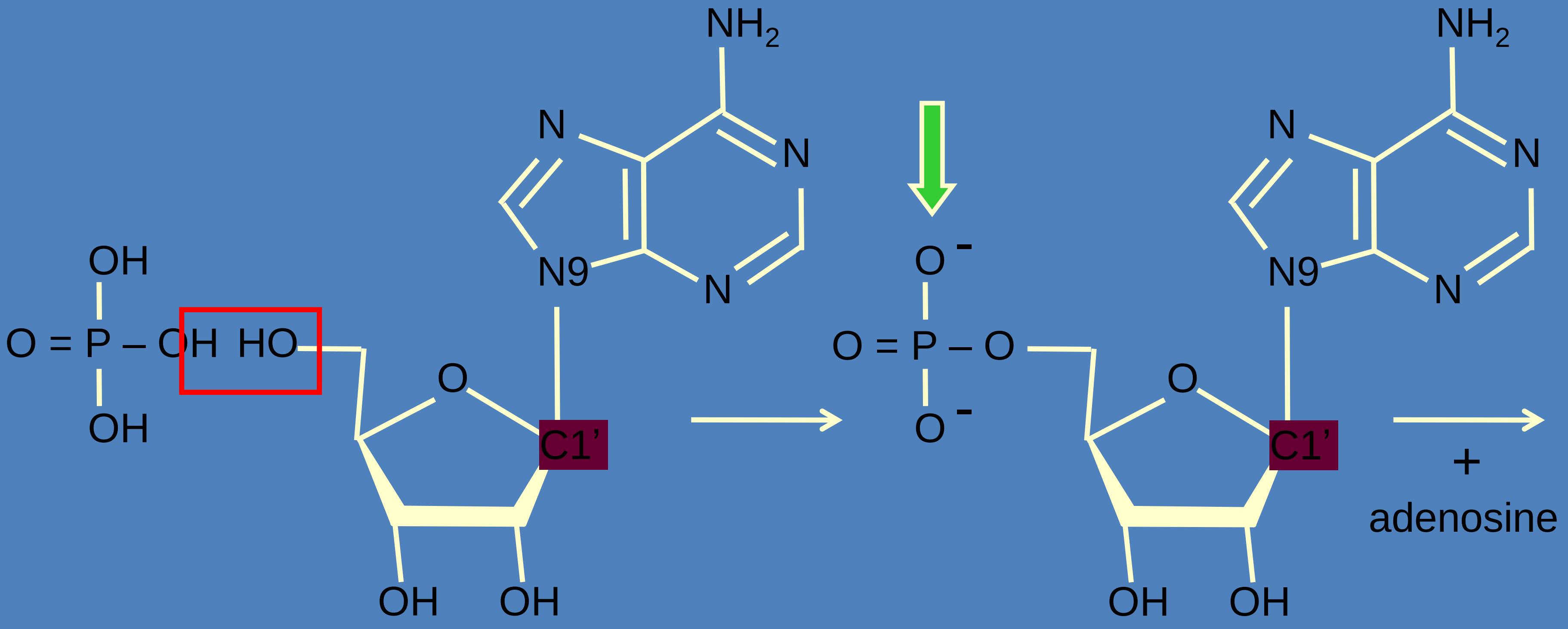
uridine = U
cytidine = C
adenosine = A
guanosine = G

Deoxyribonukleosides

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



Phosphate group



acid + alcohol → ester

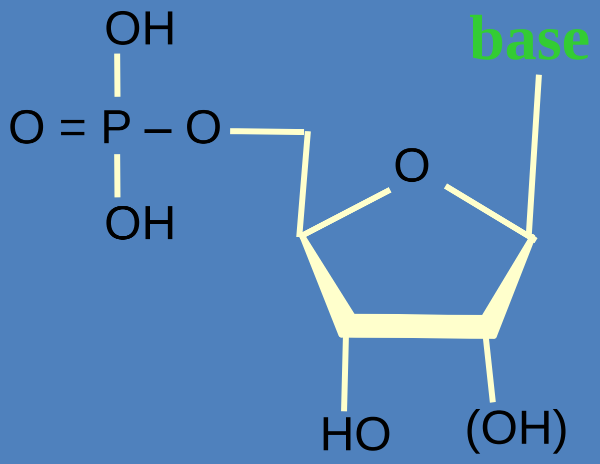
orthophosphoric acid

H_3PO_4

adenosine

adenosine(mono)phosphate (AMP)

Nukleotides



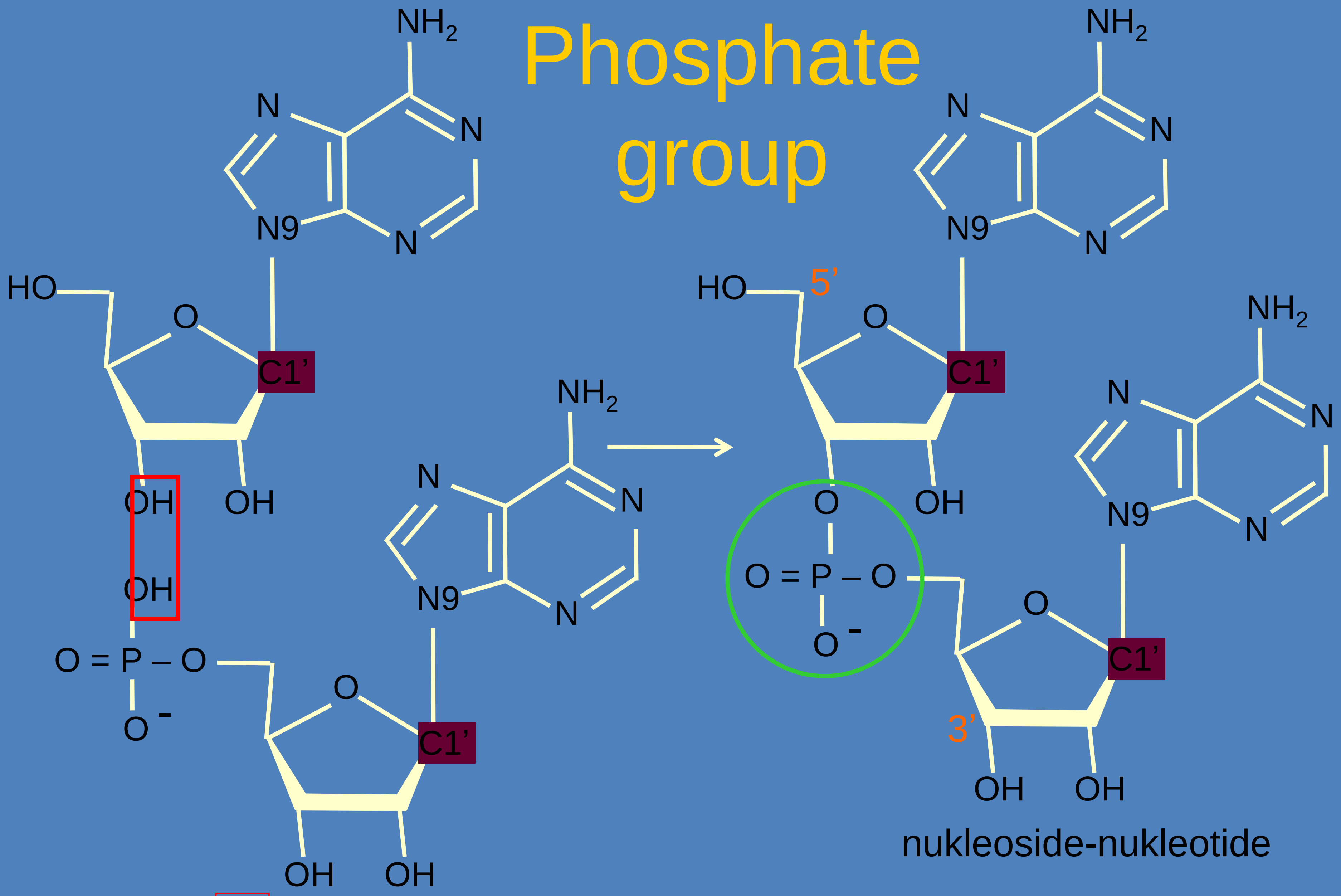
Ribonucleotides

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

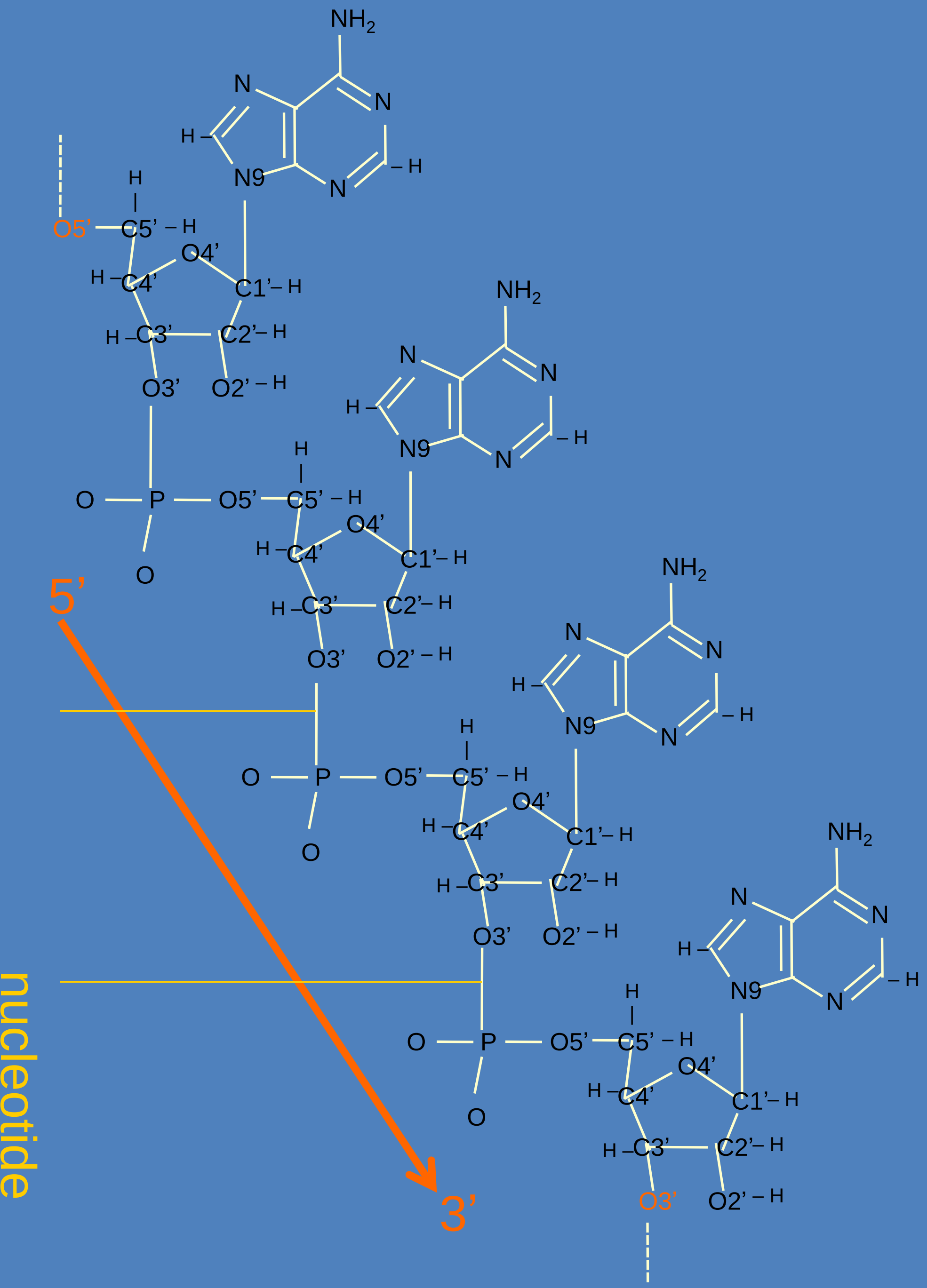


nukleoside-nukleotide

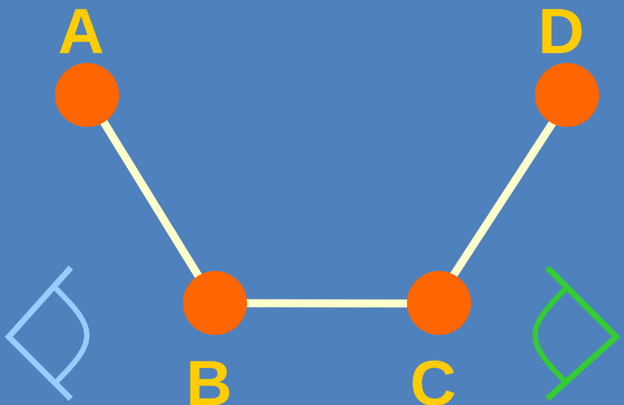
alcohol + acid (ester) → diester

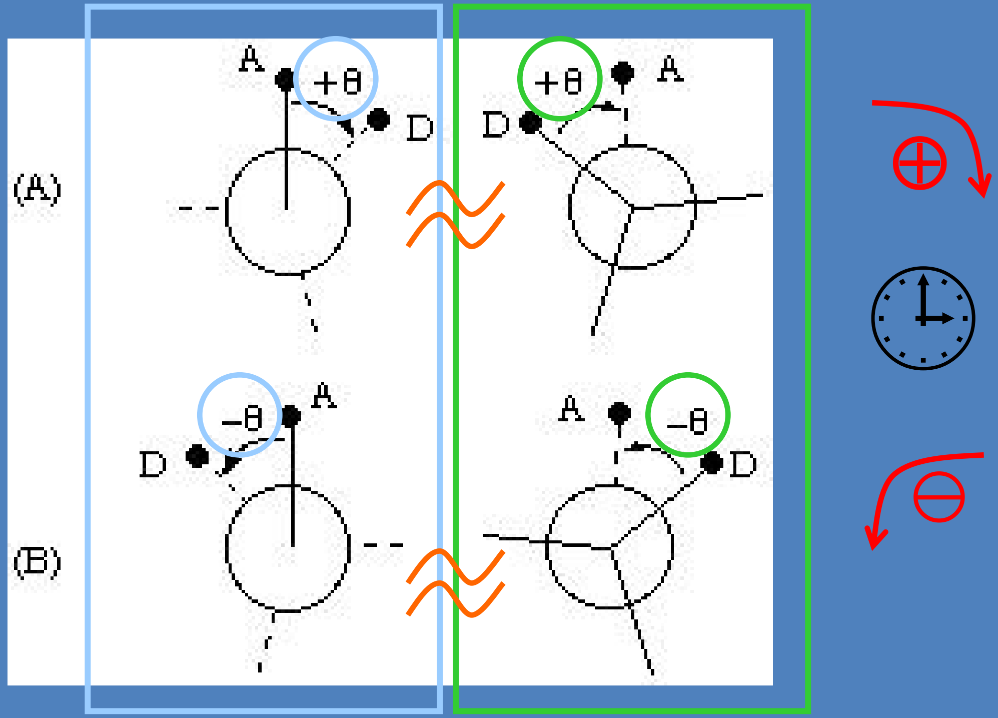
ApA

Nucleotide chain



Torsion angle





<0°, 360°>

<-180°, 180°>

Torsion angle

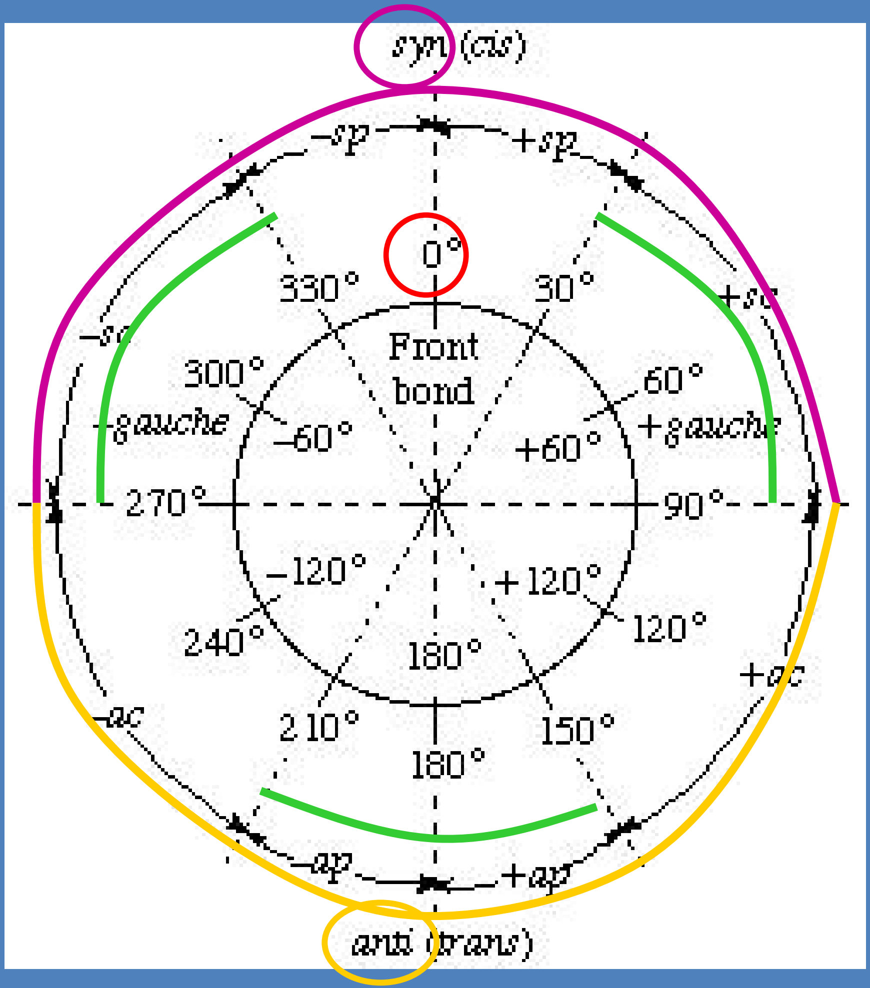
synperiplanar (sp)

synclinal (sc)

anticlinal (ac)

antiperiplanar (ap)

-gauche (-g)

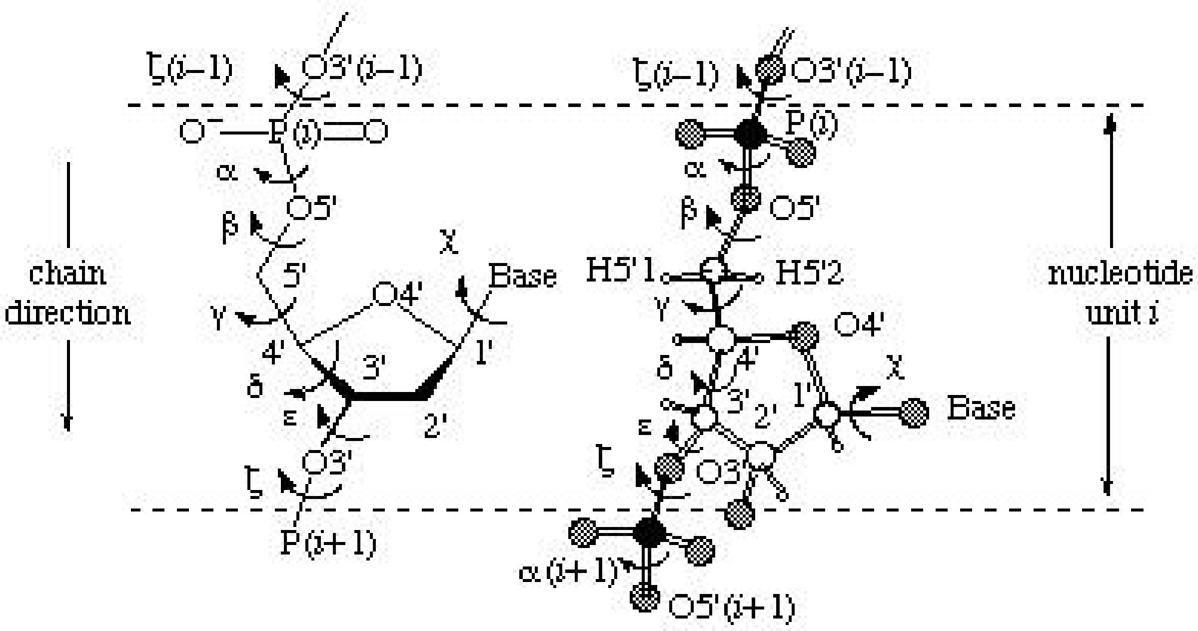


+gauche (+g)

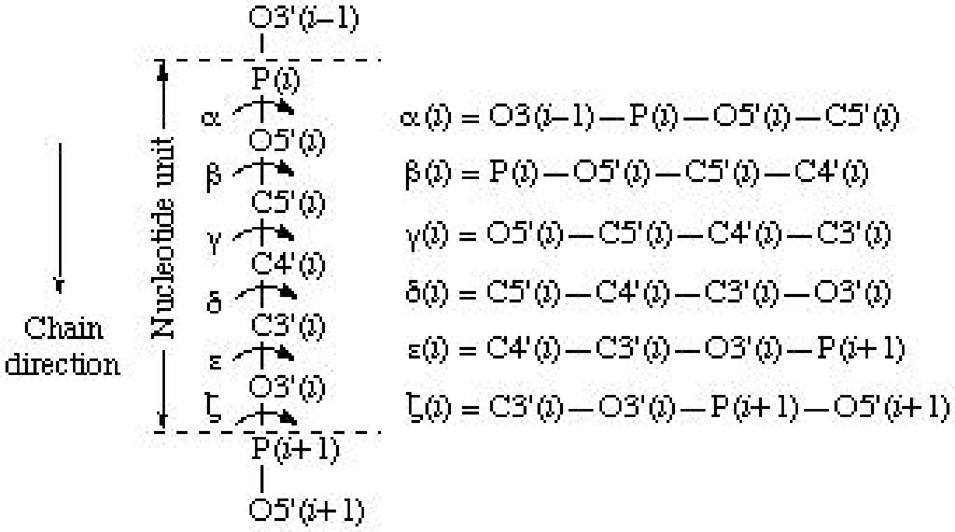
trans (t)

Torsion angles in NA

Sugar-phosphate backbone



Torsion angles cont.



α(i) = O3(i−1)—P(i)—O5'(i)—C5'(i)

β(i) = P(i)—O5'(i)—C5'(i)—C4'(i)

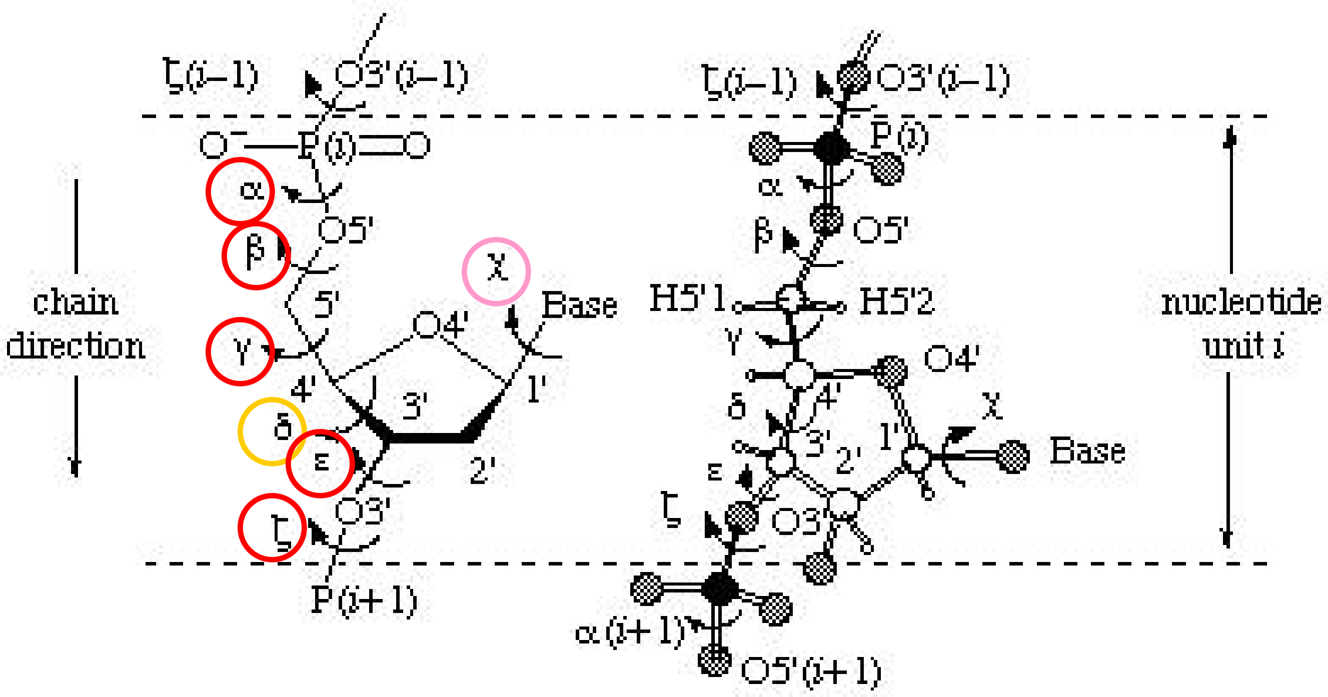
γ(i) = O5'(i)—C5'(i)—C4'(i)—C3'(i)

δ(i) = C5'(i)—C4'(i)—C3'(i)—O3'(i)

ε(i) = C4'(i)—C3'(i)—O3'(i)—P(i+1)

ζ(i) = C3'(i)—O3'(i)—P(i+1)—O5'(i+1)

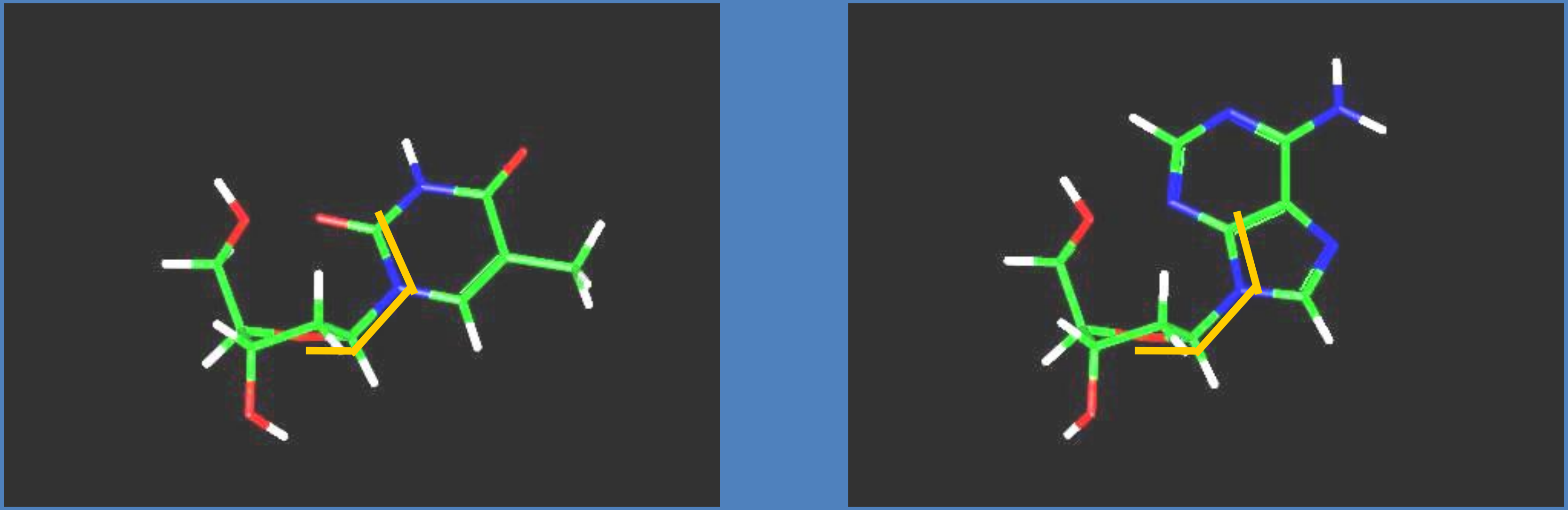
α
β
γ
δ
ε
ζ
χ



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

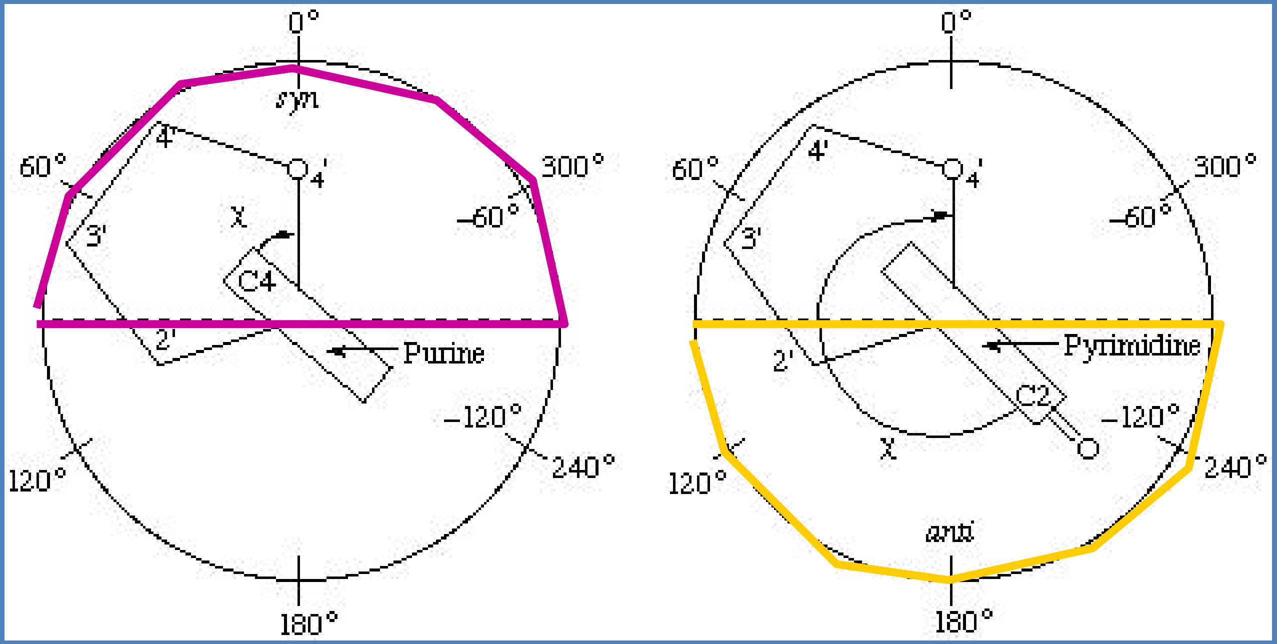
SYN

χ

<0^{o}, 90^{o}>

+

<270^{o}, 360^{o}>



ANTI

χ

<90^{o}, 270^{o}>

Torion χ – border intervals

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

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

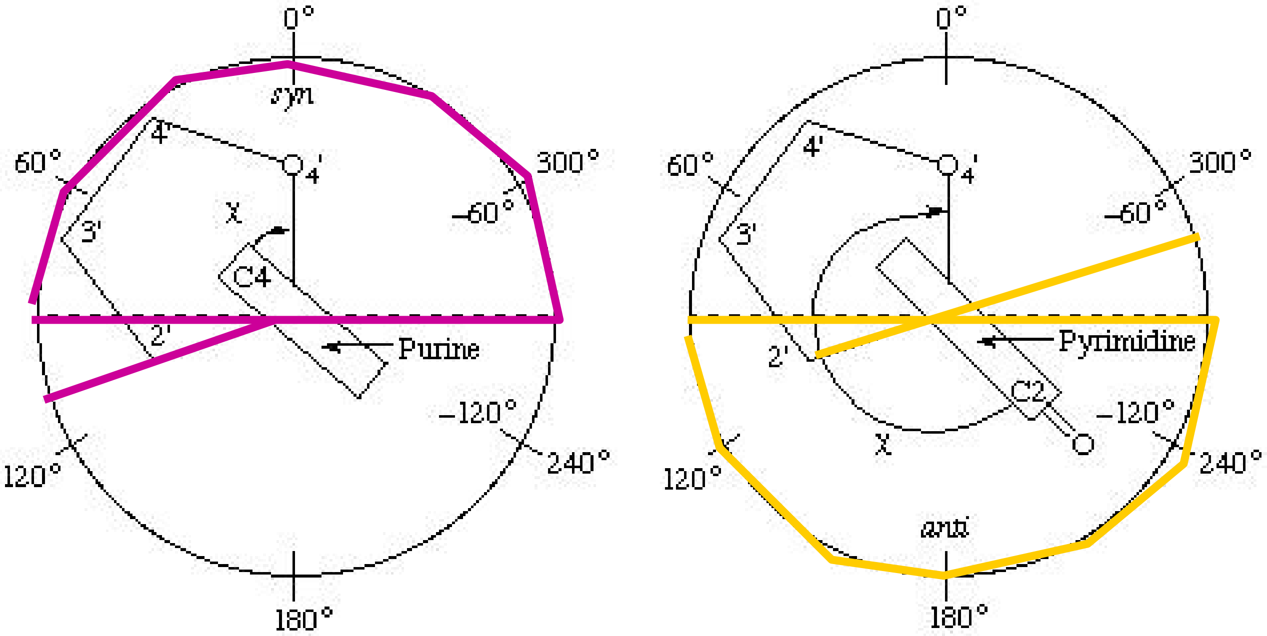
SYN

$\chi \in$

$\langle 0^{o}, 90^{o} \rangle$

$+$

$\langle 270^{o}, 360^{o} \rangle$



ANTI

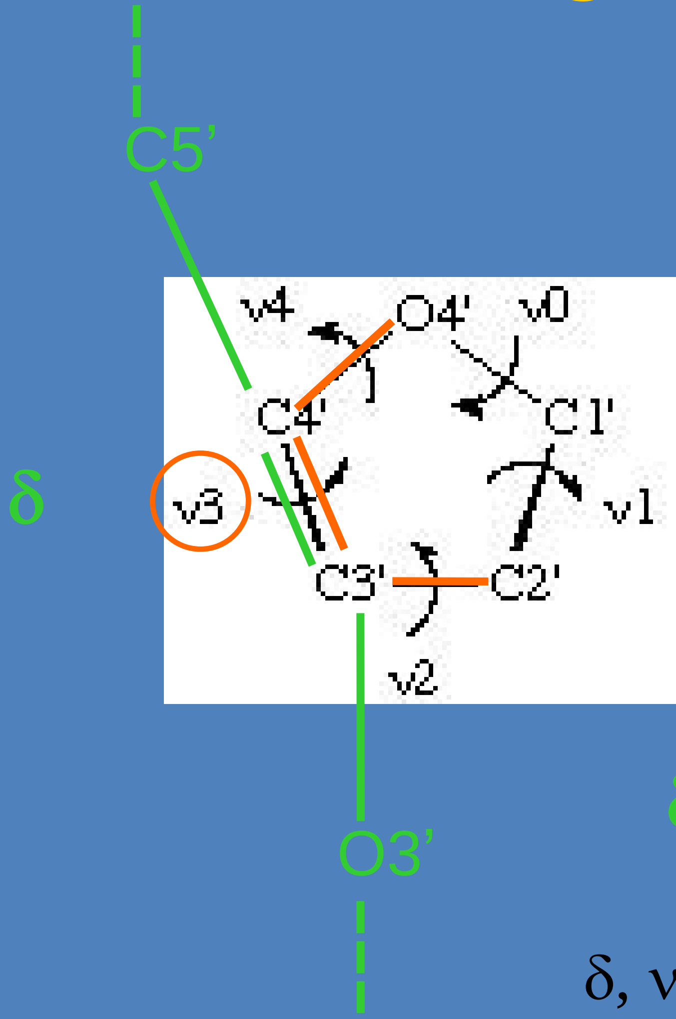
$\chi \in$

$\langle 90^{o}, 270^{o} \rangle$

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



ν0 = C4'—O4'—C1'—C2'

ν1 = O4'—C1'—C2'—C3'

ν2 = C1'—C2'—C3'—C4'

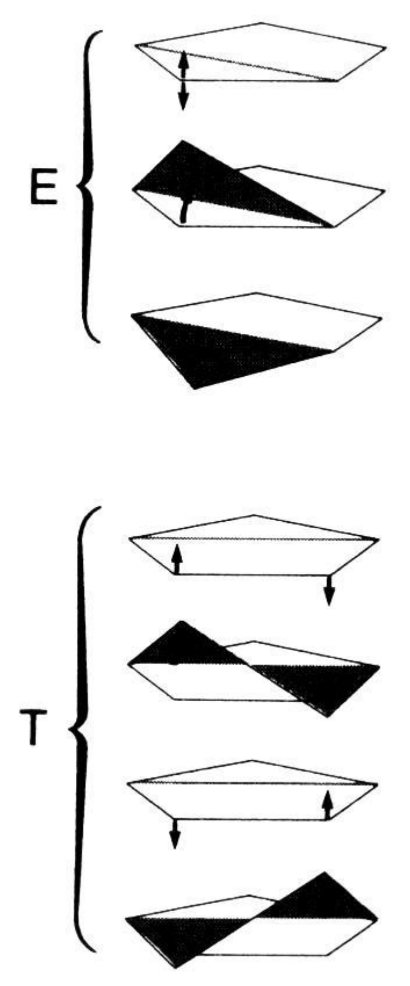
ν3 = C2'—C3'—C4'—O4' endocyclic

ν4 = C3'—C4'—O4'—C1'

δ … C5' – C4' – C3' – O3' exocyclic

δ, ν3 relation

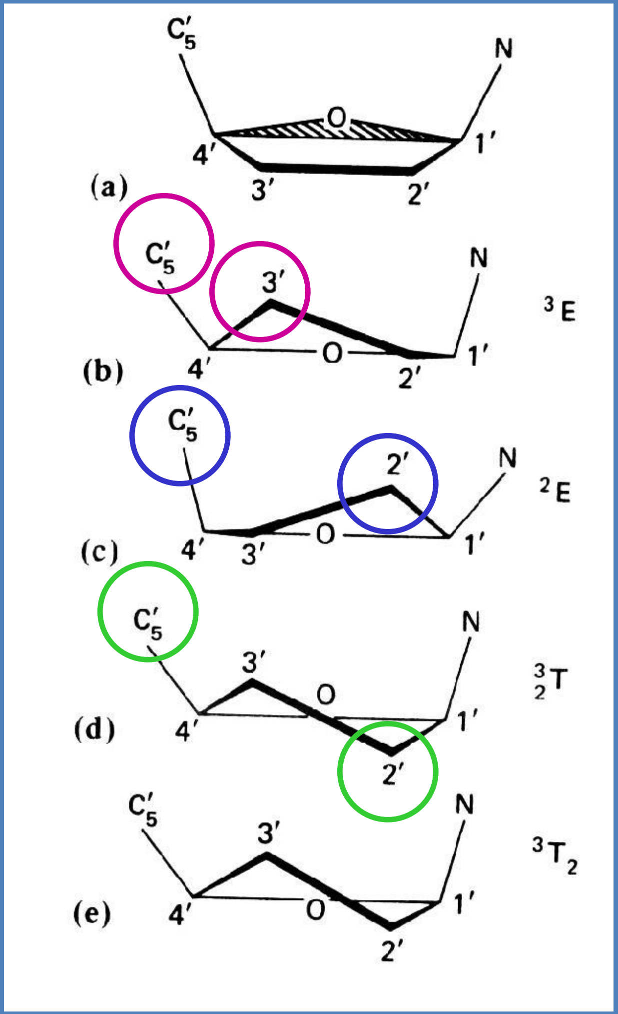
„Puckering“ of the sugar ring



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

With respect to C5’
- endo
- exo

C1’ – O4’ – C4’ plane

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

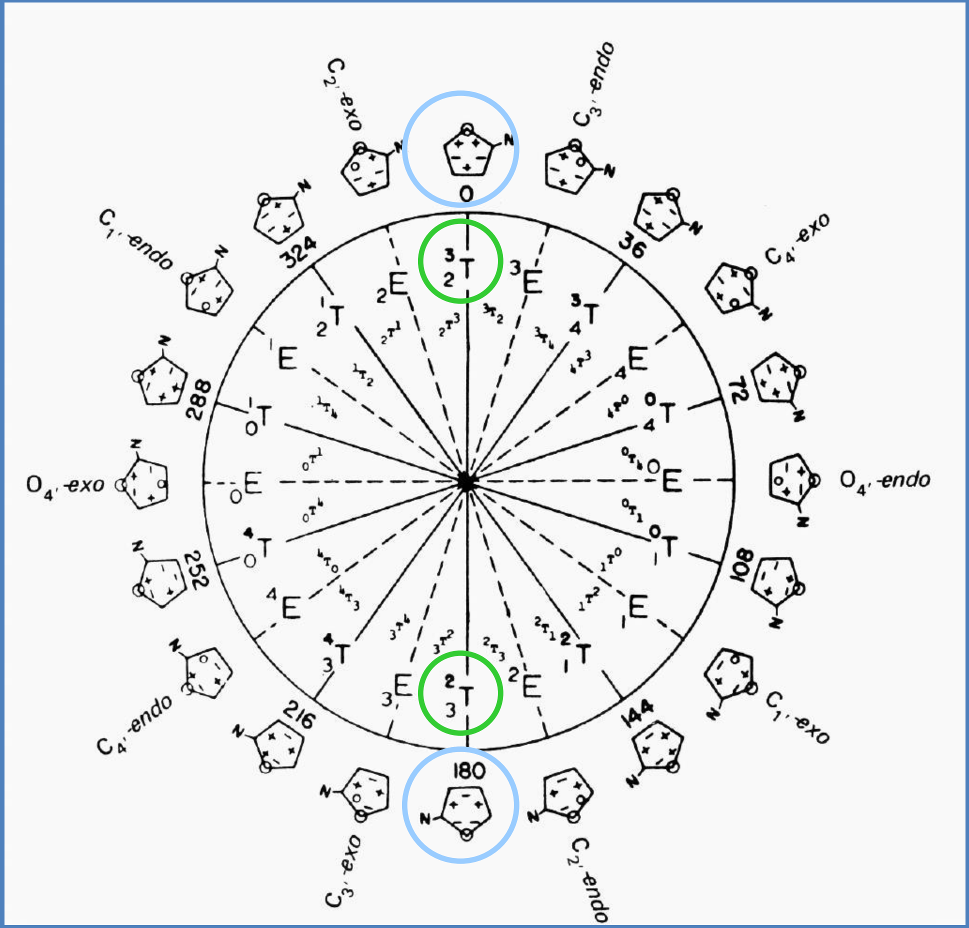
Envelope C2’-endo ^2E (prevalent in DNA)

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

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

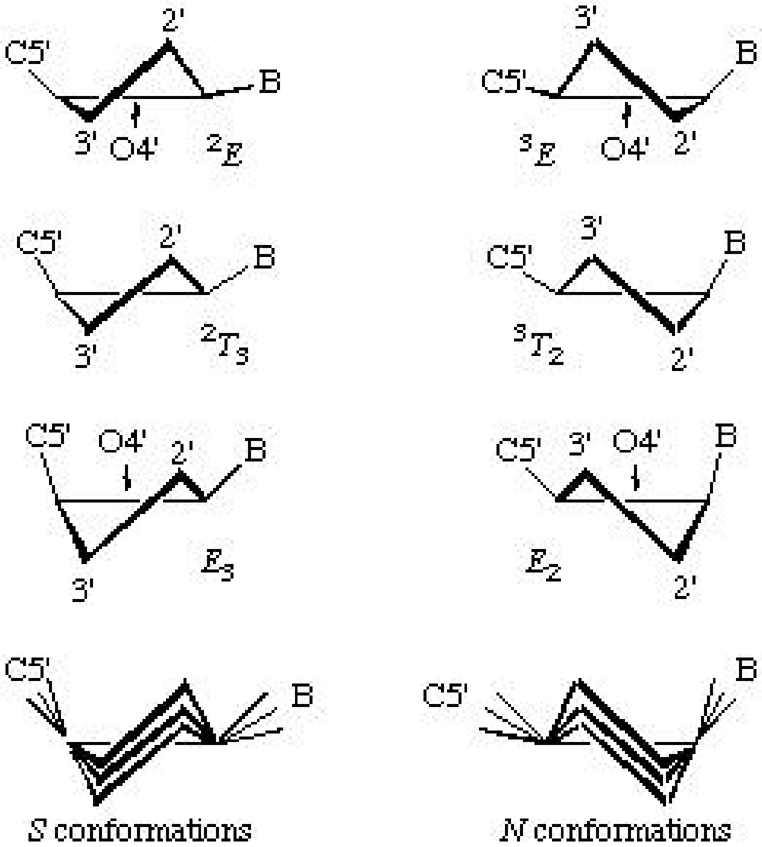
P = 0º :

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

P = 180º :

asymmetric Twist C2’-endo-C3’-exo ^2_3T

ν_{max} amplitude



Maximum out-of-plane pucker

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

P, ν_j relation

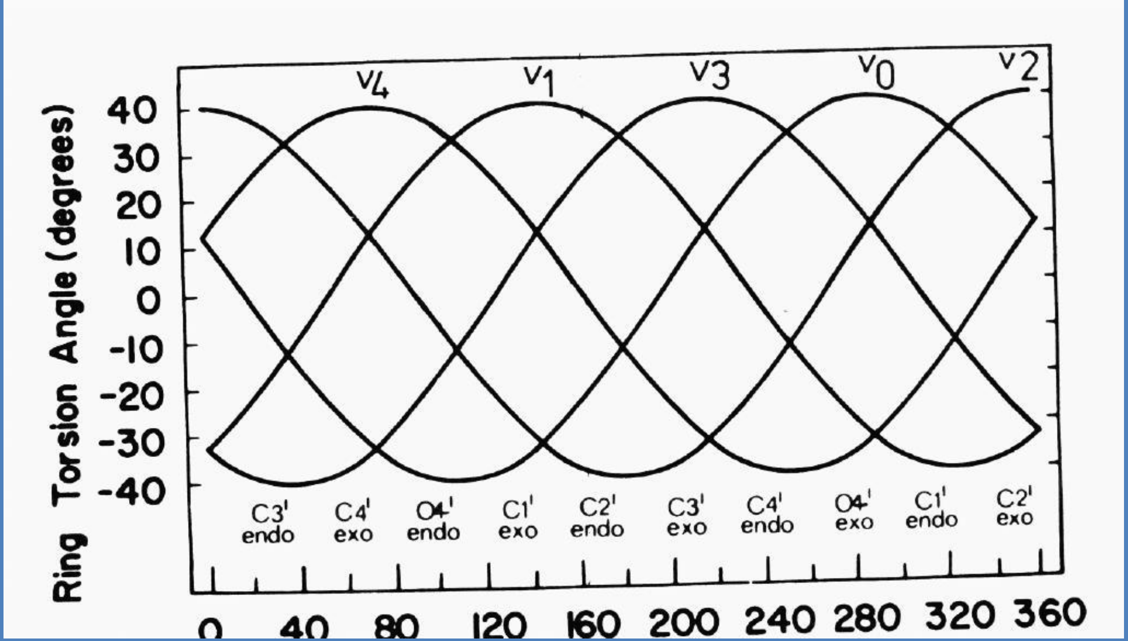
P value defines unambiguously all endocyclic torsion angles ν_0 to ν_4

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

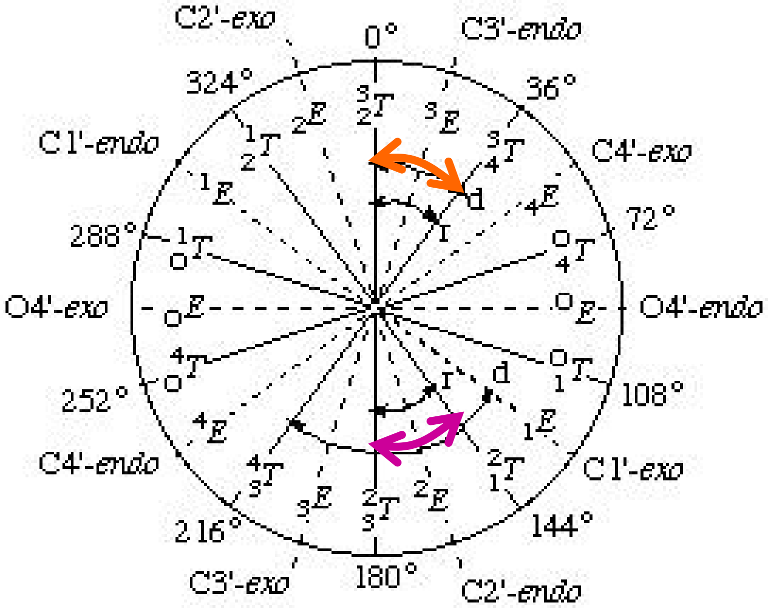
$$\nu_0 + \nu_1 + \nu_2 + \nu_3 + \nu_4 = 0$$

j = 0 .. 4

Sum of all 5 ν = 0

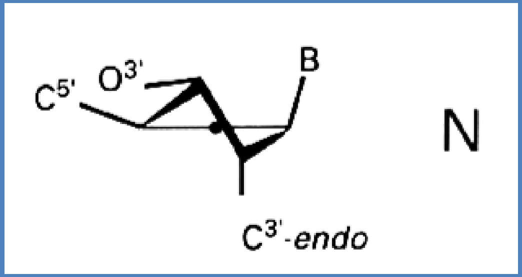


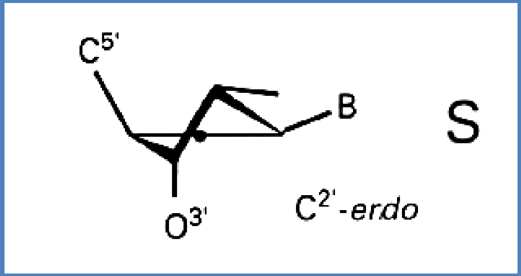
P in nucleic acids



NORTH

SOUTH





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

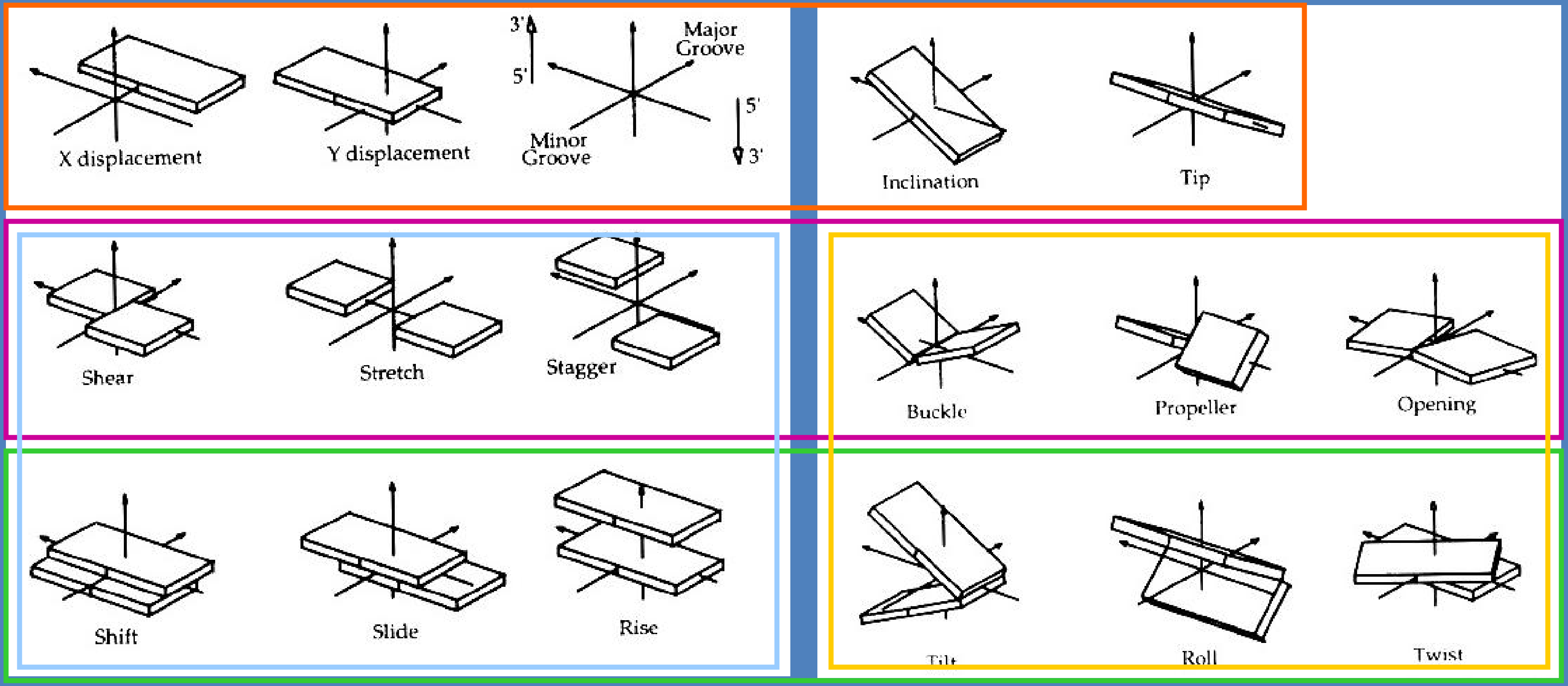
$144^{o} \leq P \leq 190^{o}$ south (prevalent in DNA)

Helical parameters

axis-base, axis-base pair

intra-base pair

inter-base or inter-base pair



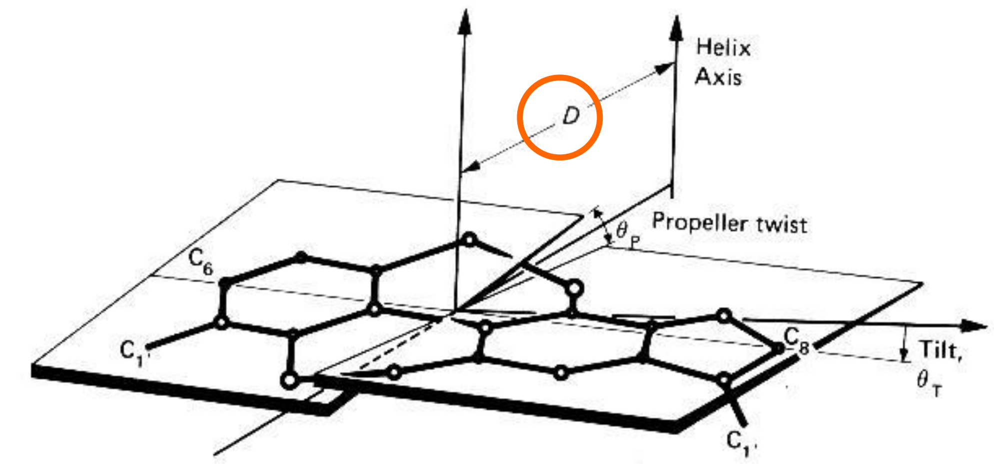
Distance/shift

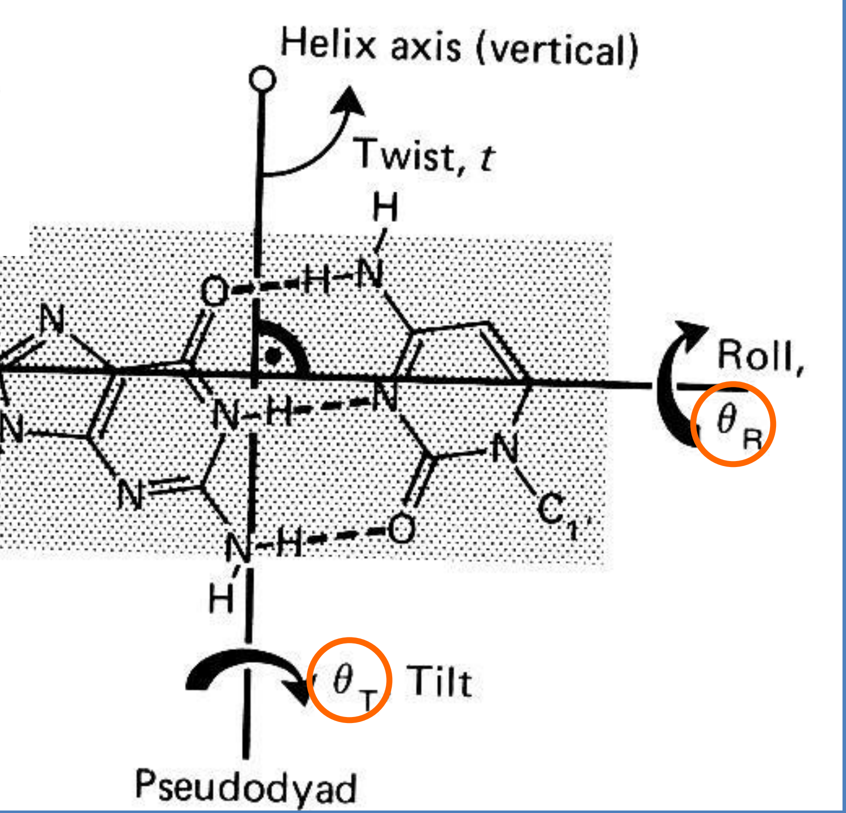
Angle

Helical…

D … displacement from helical axis

t …twist = 360º / n





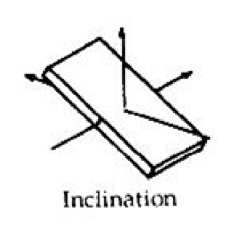
θ_R … roll
θ_T … tilt

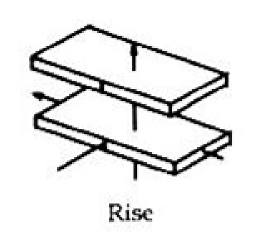
Helical parameters

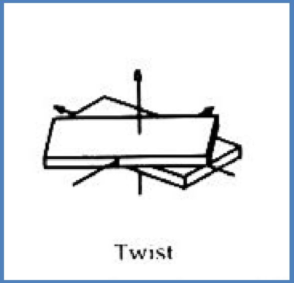
for A and B DNA

Shifts in Å, angles in degrees

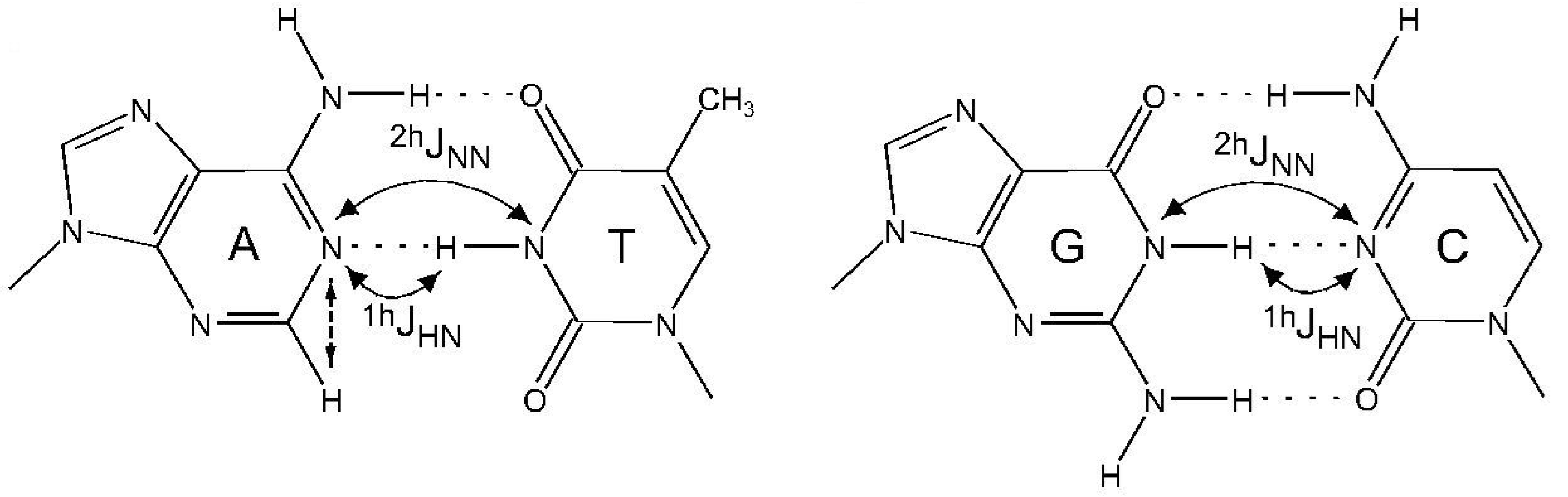
Global	B-DNA	A-DNA
X disp.	0.0	-5.28
Y disp.	0.0	0.0
Inclin	1.46	20.73
Tip	0.0	0.0
Shear	0.0	0.0
Stretch	0.0	0.01
Stagger	-0.08	-0.04
Buckle	0.0	0.0
Propeller	-13.3	-7.5
Openning	0.0	-0.02
Shift	0.0	0.0
Slide	0.0	0.0
Rise	3.38	2.56
Tilt	0.0	0.0
Roll	0.0	0.0
Twist	36.00	32.70
Bases per turn	360/36=10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





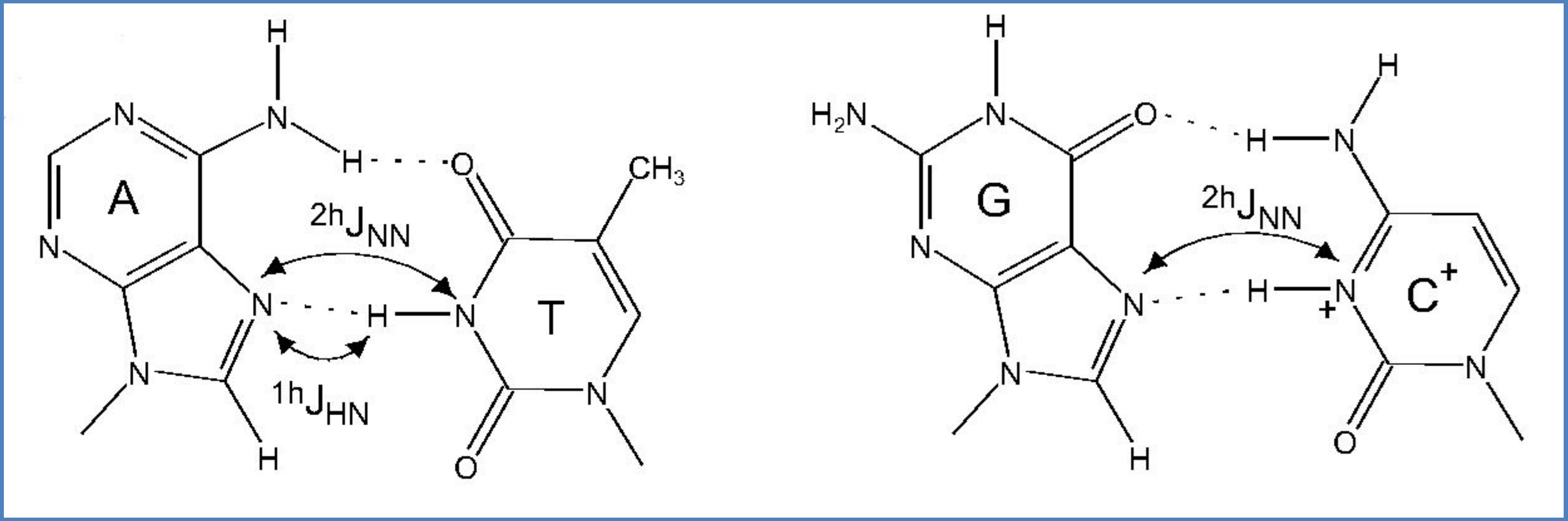


Base pairing

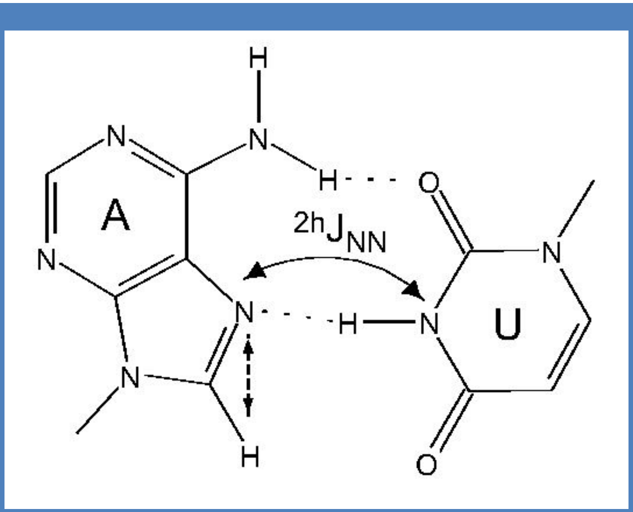


Watson-Crick pairs

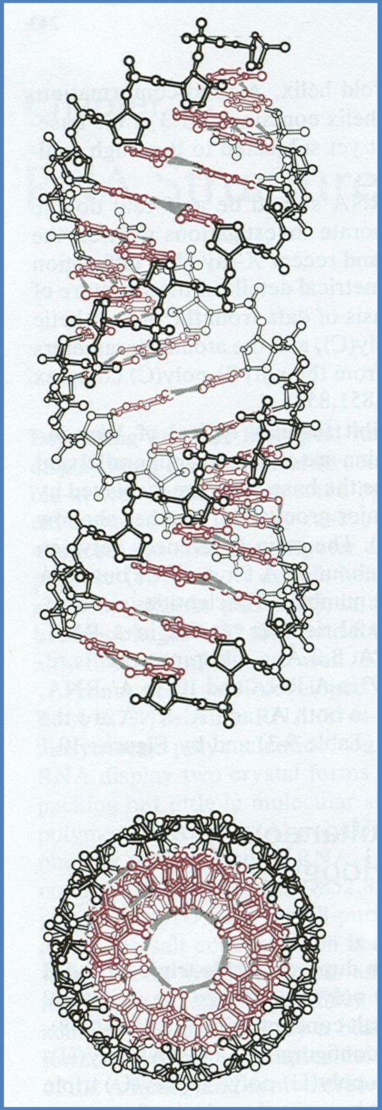
Base pairing



Hoogsteen and reverse Hoogsteen pairs



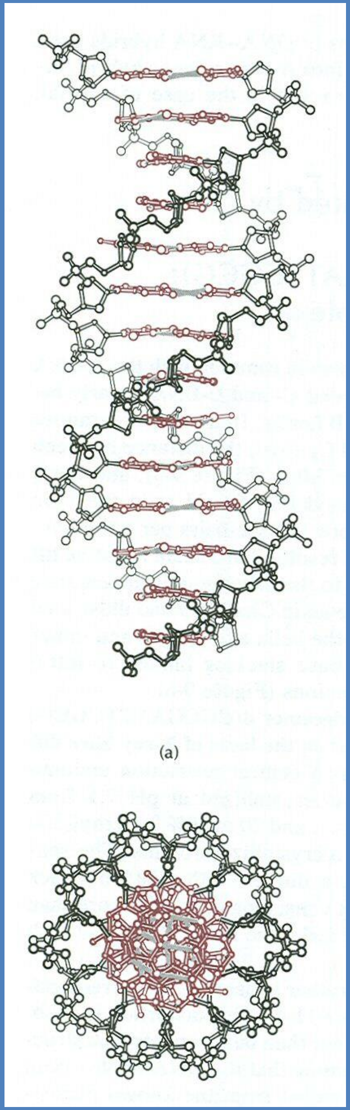
A and B double helix



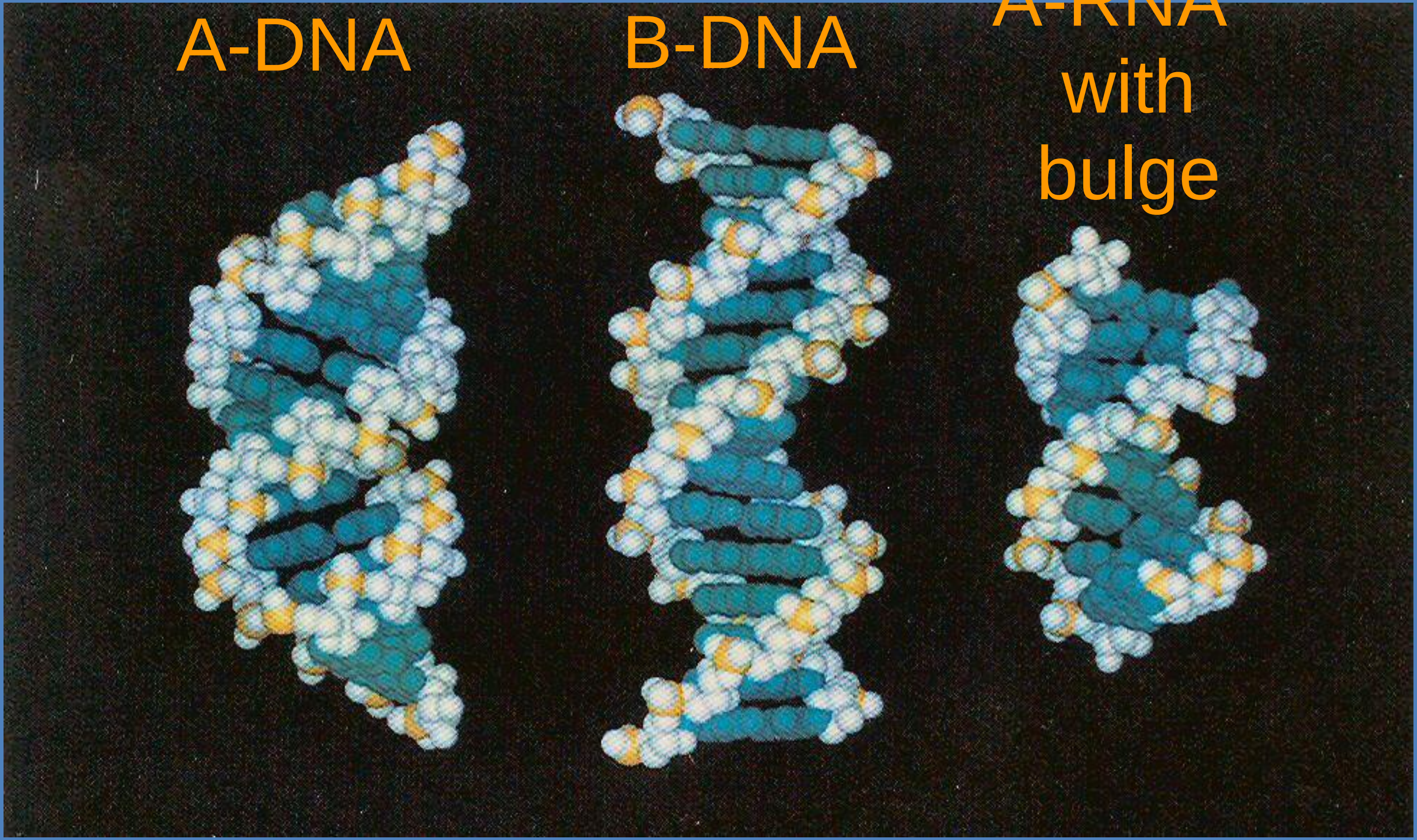
A-RNA

Ball and
stick
models

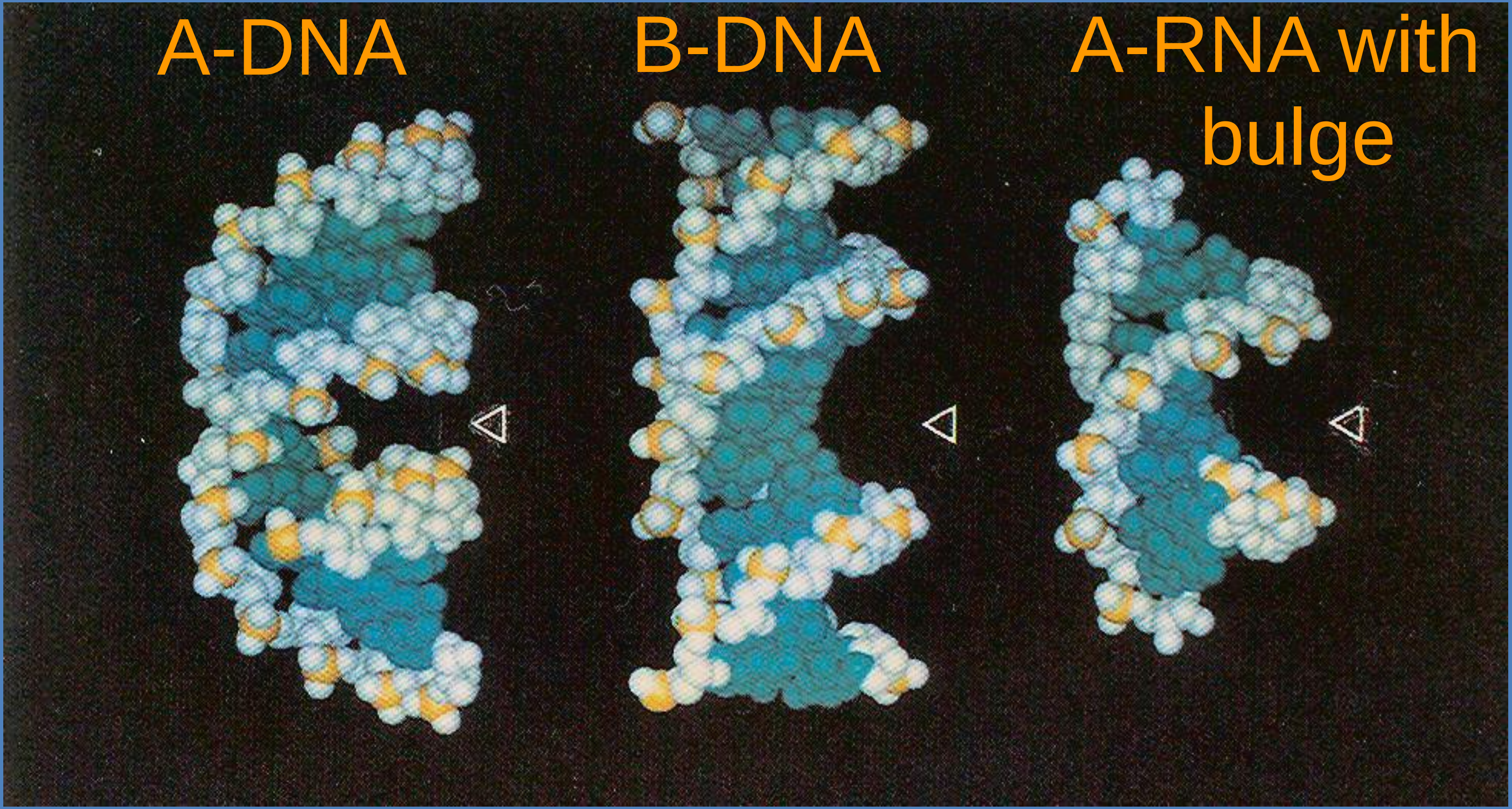
B-DNA



A and B helices

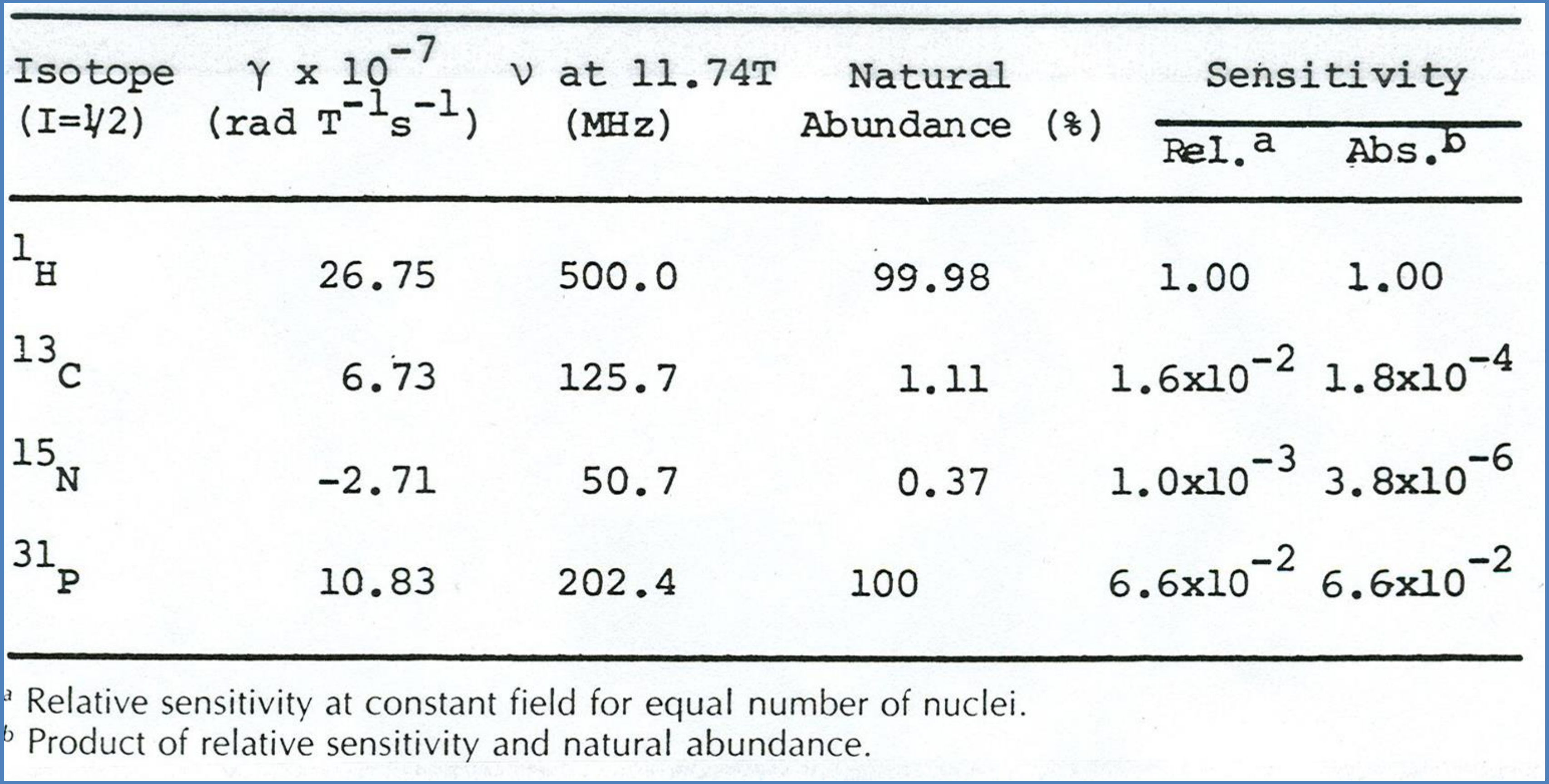


A and B helices



View tilted by 32° to show grooves

Nuclear properties of selected isotopes

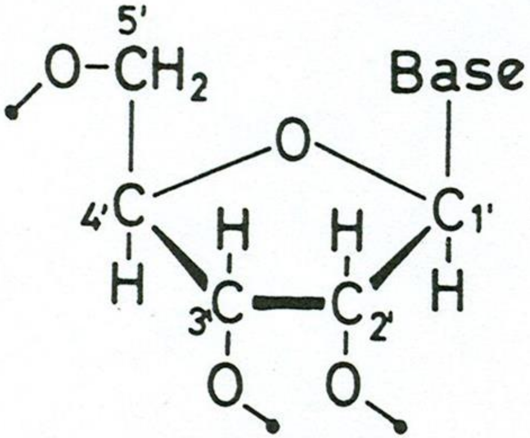


Isotope $(I=1/2)$	$\gamma \times 10^{-7}$ (rad $T^{-1}s^{-1}$)	ν at 11.74T (MHz)	Natural Abundance (%)	Sensitivity	
				Rel.[a]	Abs.[b]
^{1}H	26.75	500.0	99.98	1.00	1.00
^{13}C	6.73	125.7	1.11	1.6×10^{-2}	1.8×10^{-4}
^{15}N	-2.71	50.7	0.37	1.0×10^{-3}	3.8×10^{-6}
^{31}P	10.83	202.4	100	6.6×10^{-2}	6.6×10^{-2}

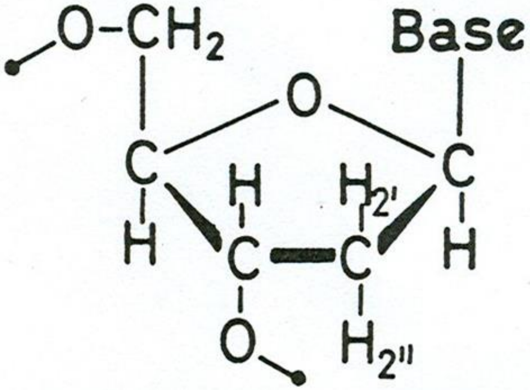
[a] Relative sensitivity at constant field for equal number of nuclei.

[b] Product of relative sensitivity and natural abundance.

Spin systems in ribose and deoxyribose

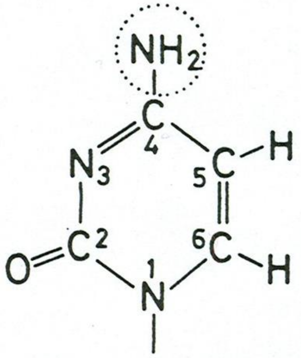


β-D-Ribose
XWTPMA



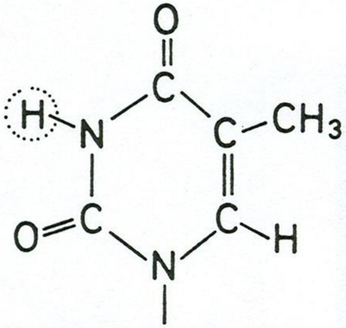
2'-Deoxy-β-D-Ribose
XAMWTNP

Spin systems in nucleic acid bases



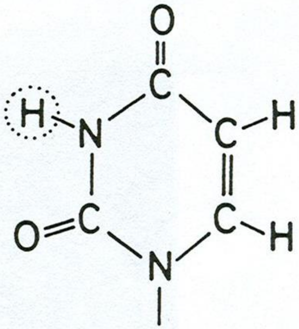
Cytosine, C

AX



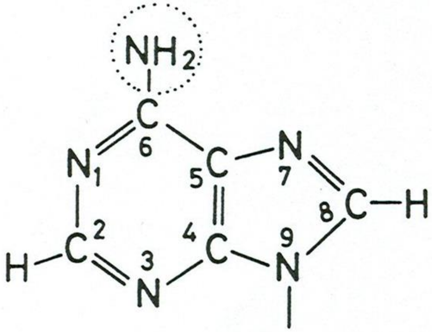
Thymine, T

A_3X



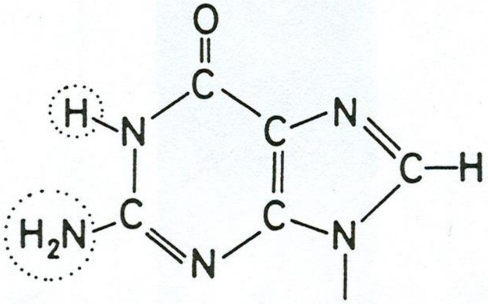
Uracil, U

AX



Adenine, A

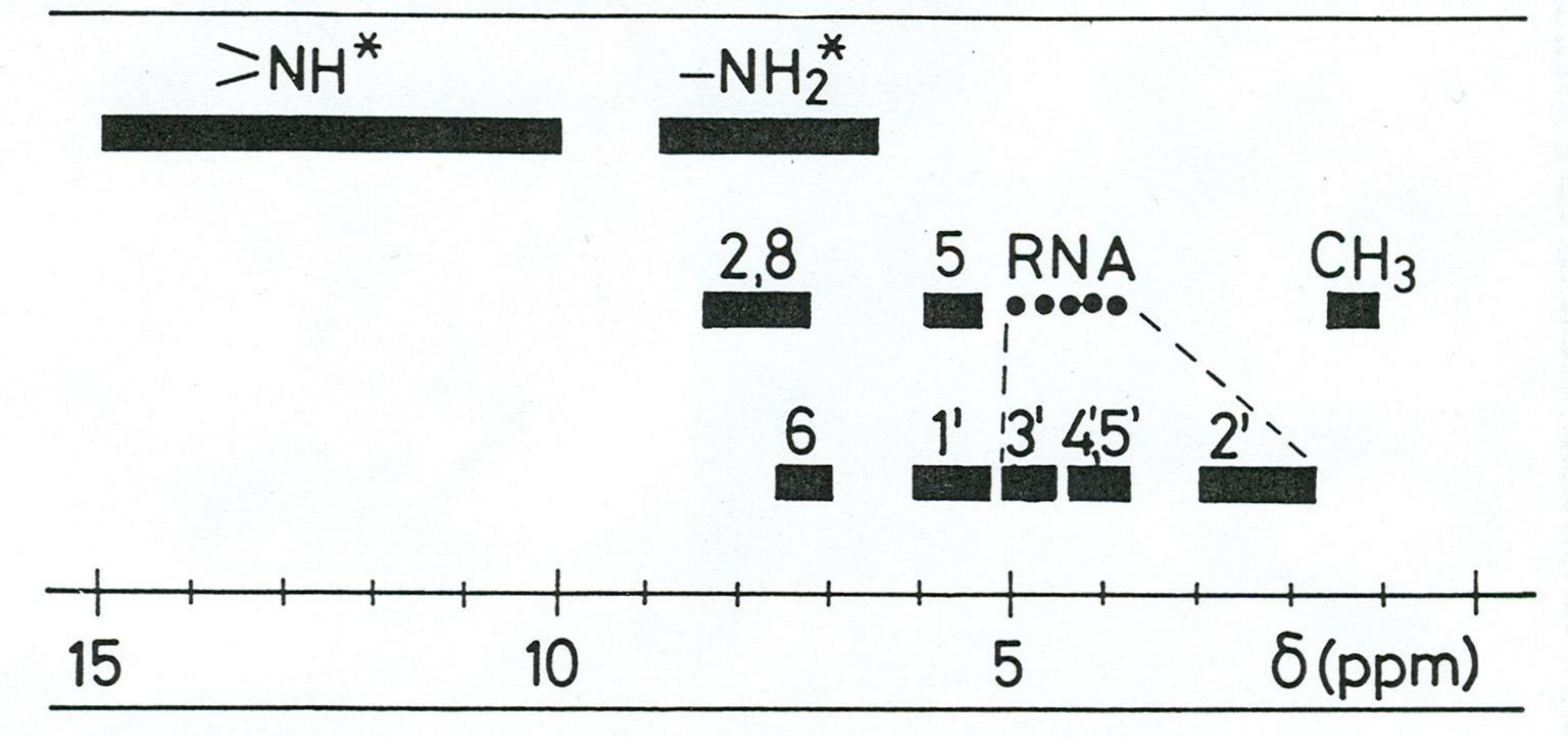
A + A



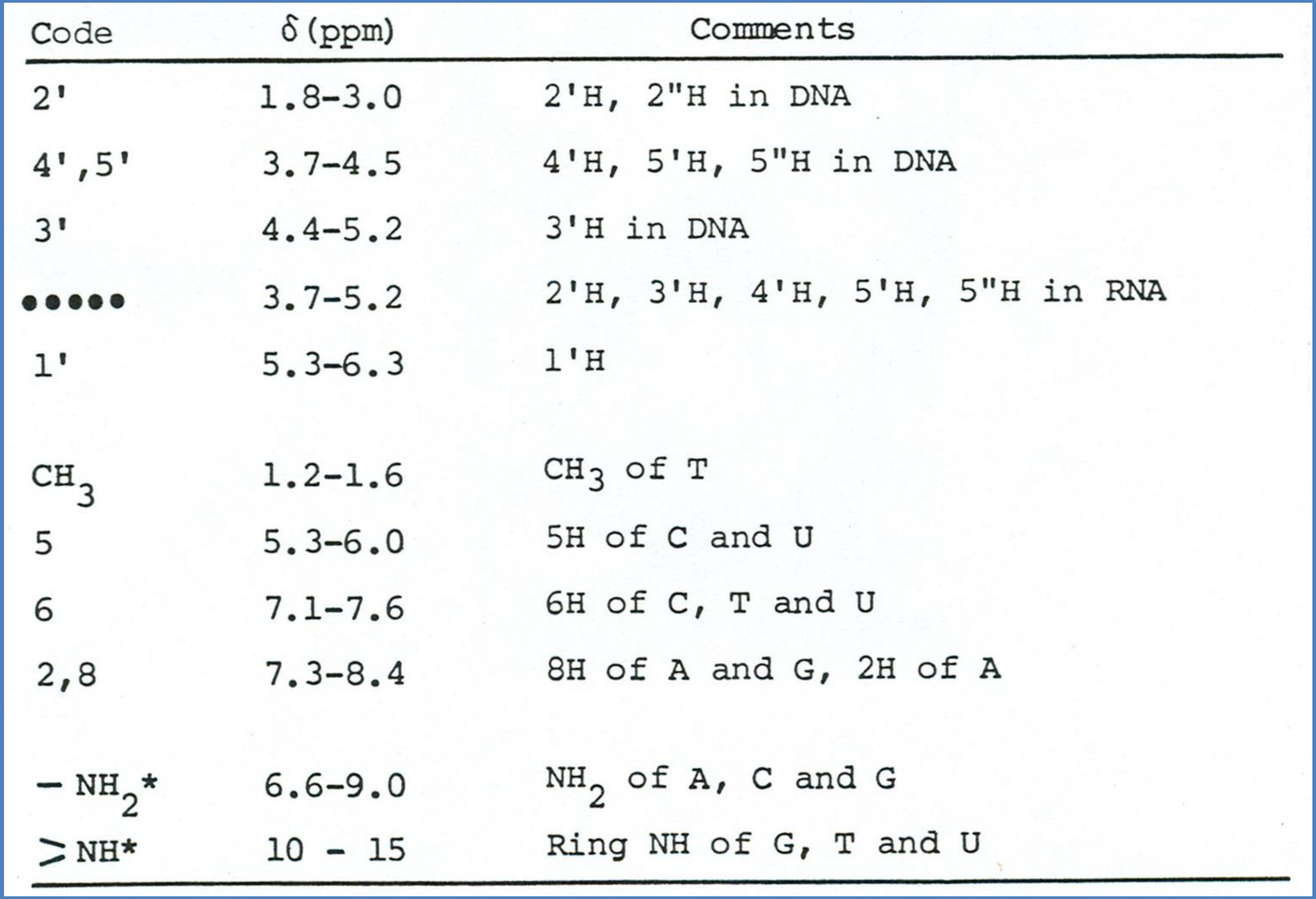
Guanine, G

A

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

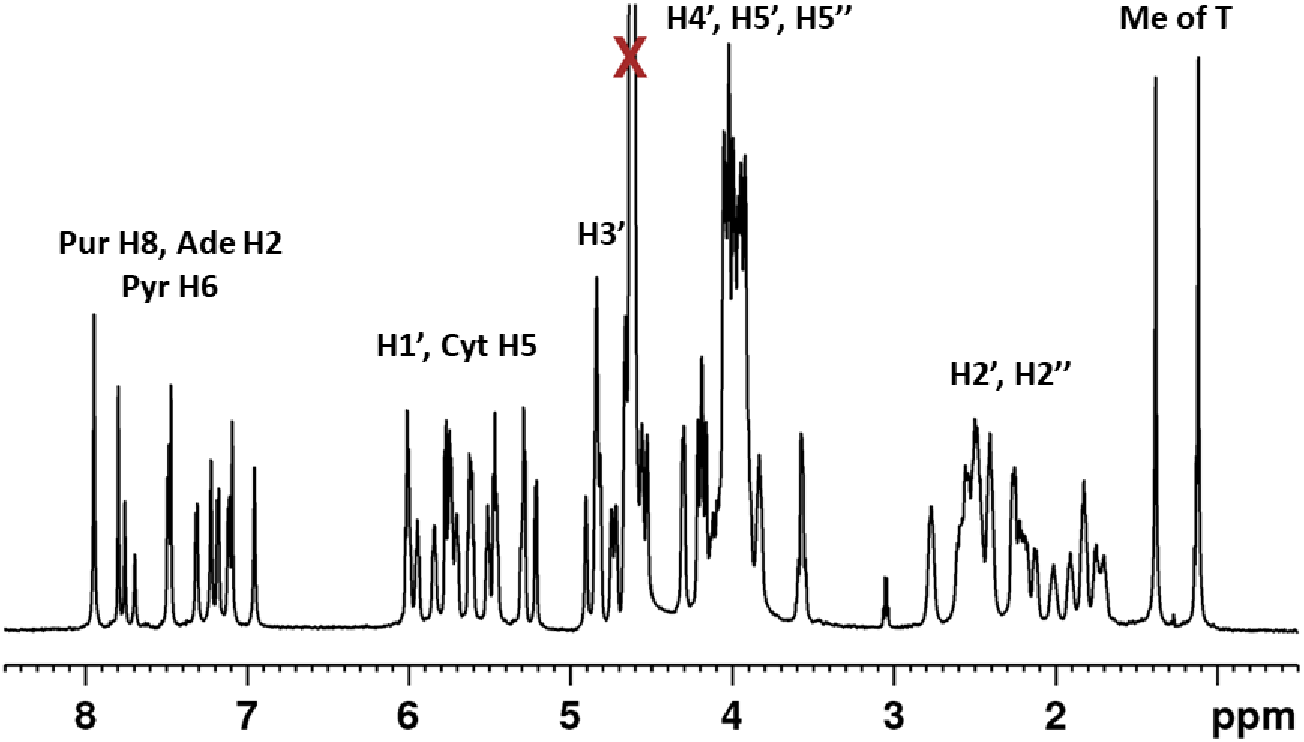


^{1}H chemical shift ranges in DNA and RNA

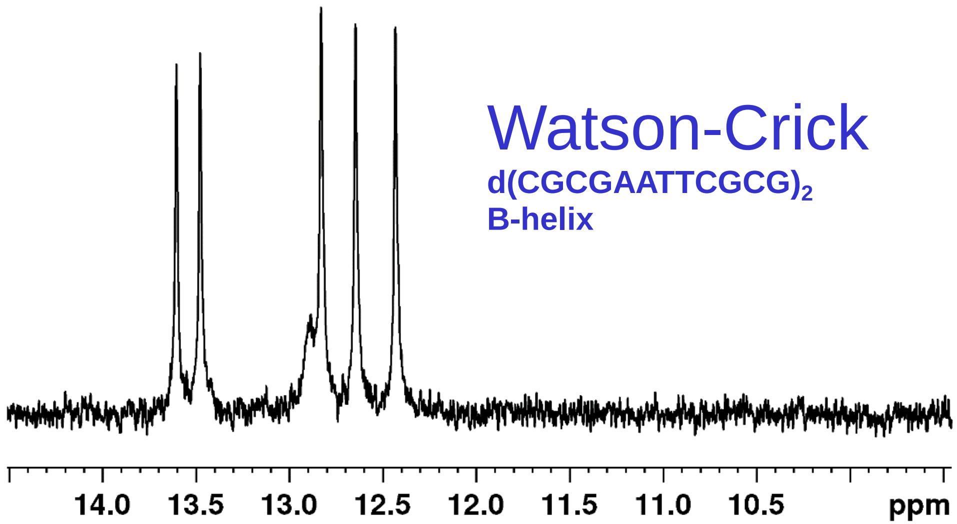


Code	δ (ppm)	Comments
2'	1.8-3.0	2'H, 2"H in DNA
4',5'	3.7-4.5	4'H, 5'H, 5"H in DNA
3'	4.4-5.2	3'H in DNA
●●●●●	3.7-5.2	2'H, 3'H, 4'H, 5'H, 5"H in RNA
1'	5.3-6.3	1'H
CH_3	1.2-1.6	CH_3 of T
5	5.3-6.0	5H of C and U
6	7.1-7.6	6H of C, T and U
2,8	7.3-8.4	8H of A and G, 2H of A
$-NH_2$*	6.6-9.0	NH_2 of A,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

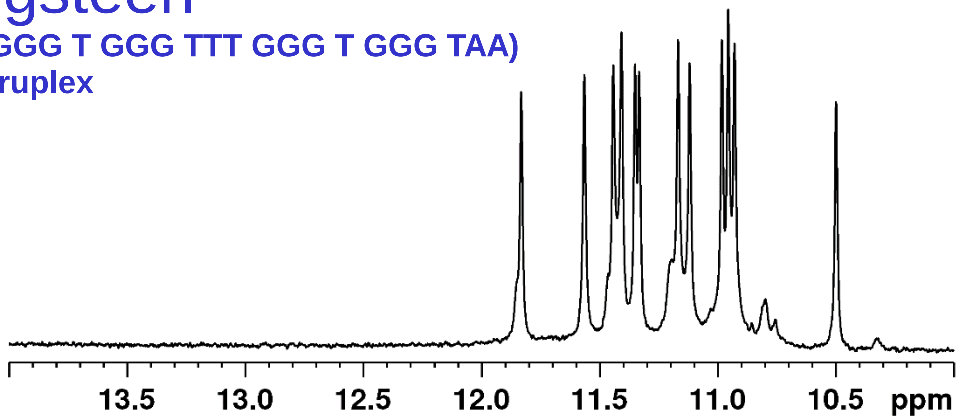
$d(CGCGAATTCGCG)_2$

B-helix

Hoogsteen

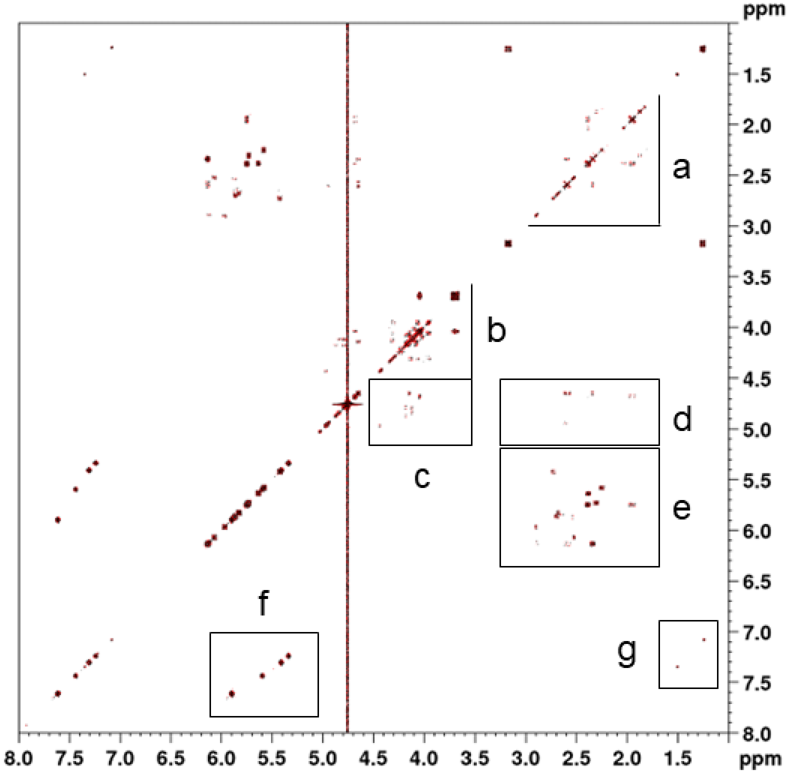
d(AAT GGG T GGG TTT GGG T GGG TAA)

G-quadruplex



^{1}H COSY spectrum of DNA

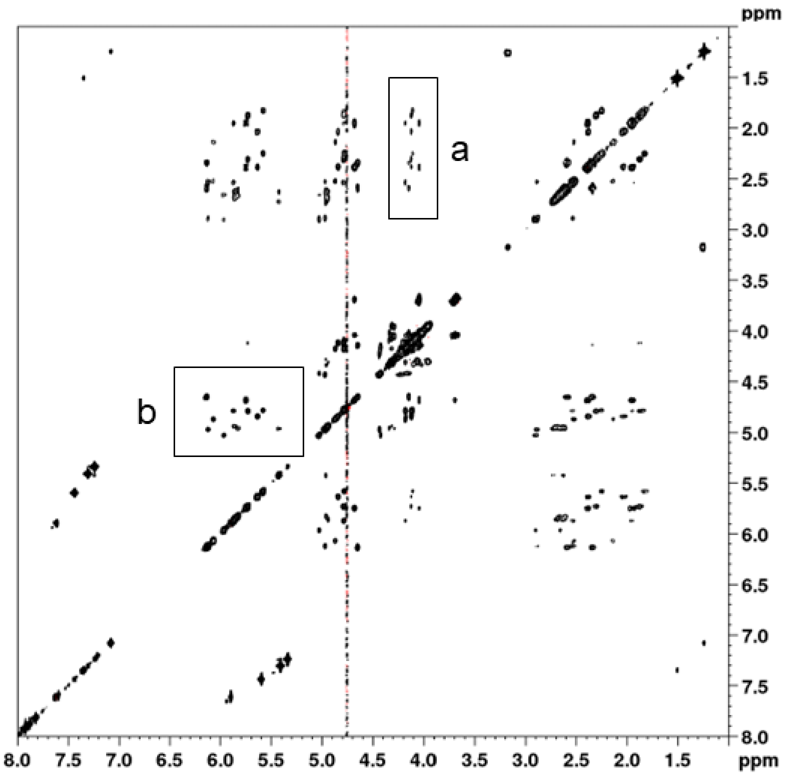
$d(CGCGAATTCGCG)_2$



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

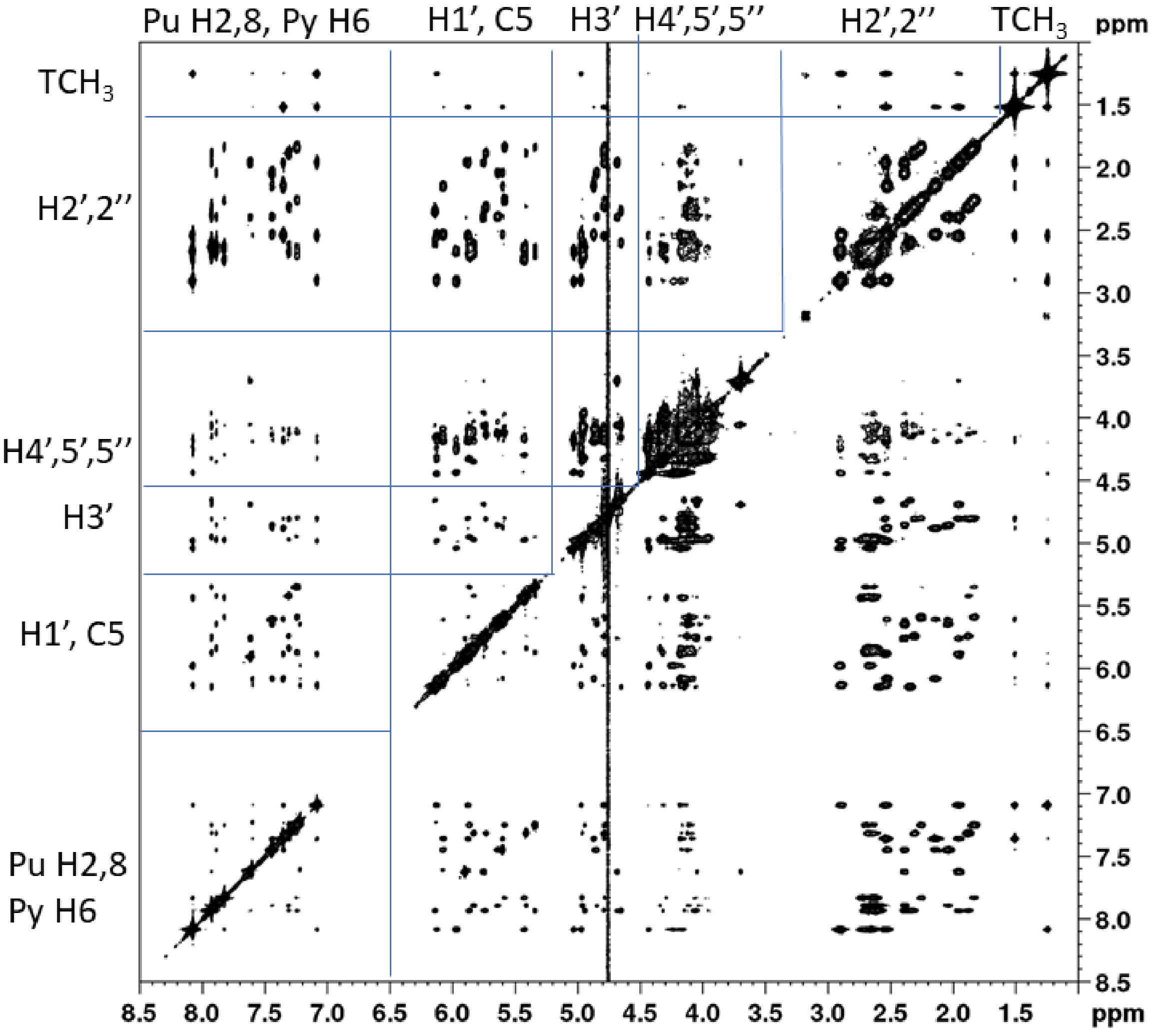
d(CGCGAATTCGCG)$_2$



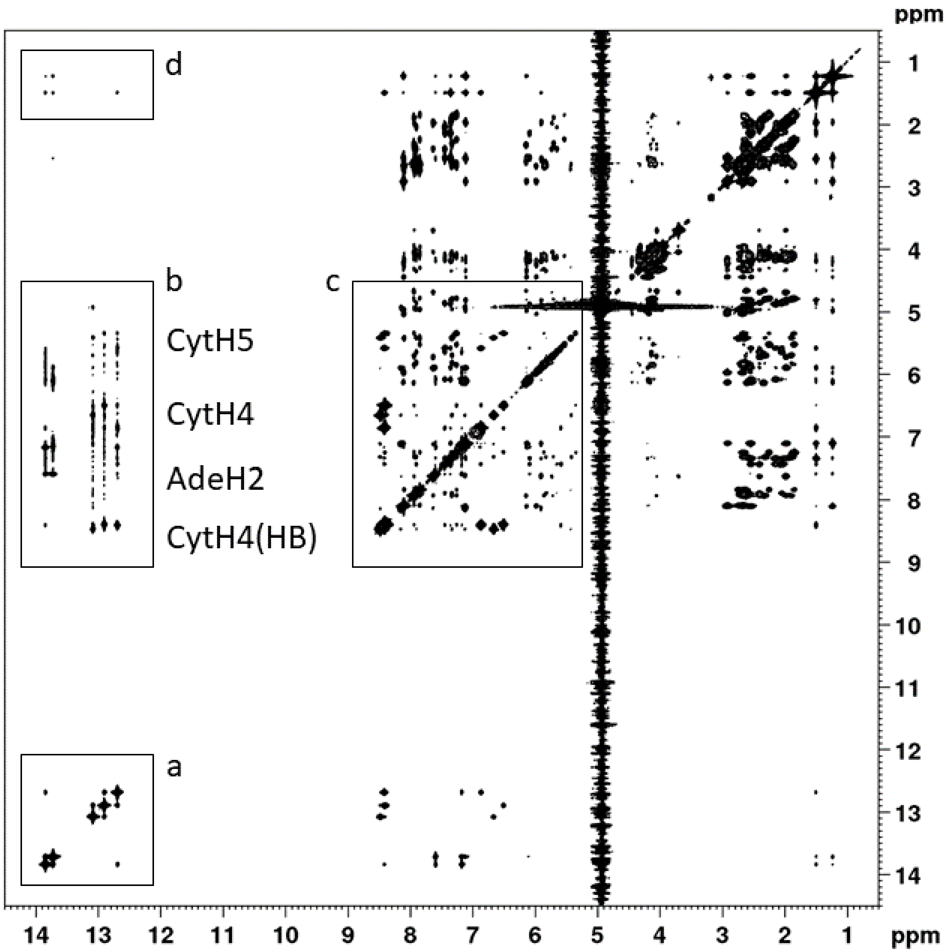
a	H4’-H2’,2”
b	H1’-H3’

^{1}H NOESY spectrum of DNA in D_2O

$d(CGCGAATTCGCG)_2$



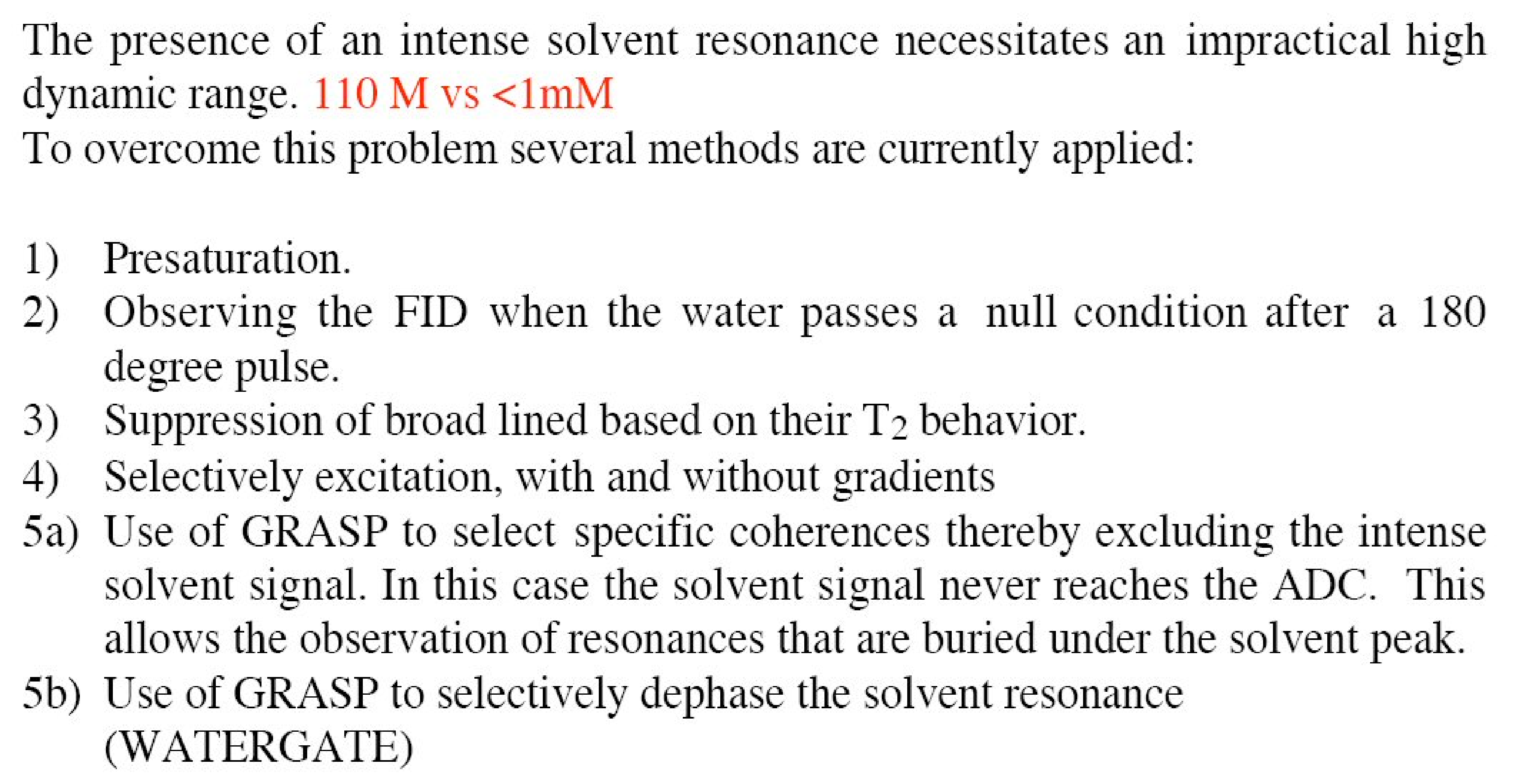
^1H NOESY spectrum of DNA in H_2O



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

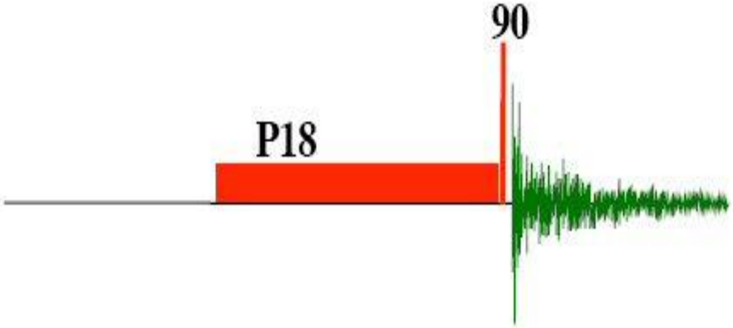


The presence of an intense solvent resonance necessitates an impractical high dynamic range. 110 M vs <1mM

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

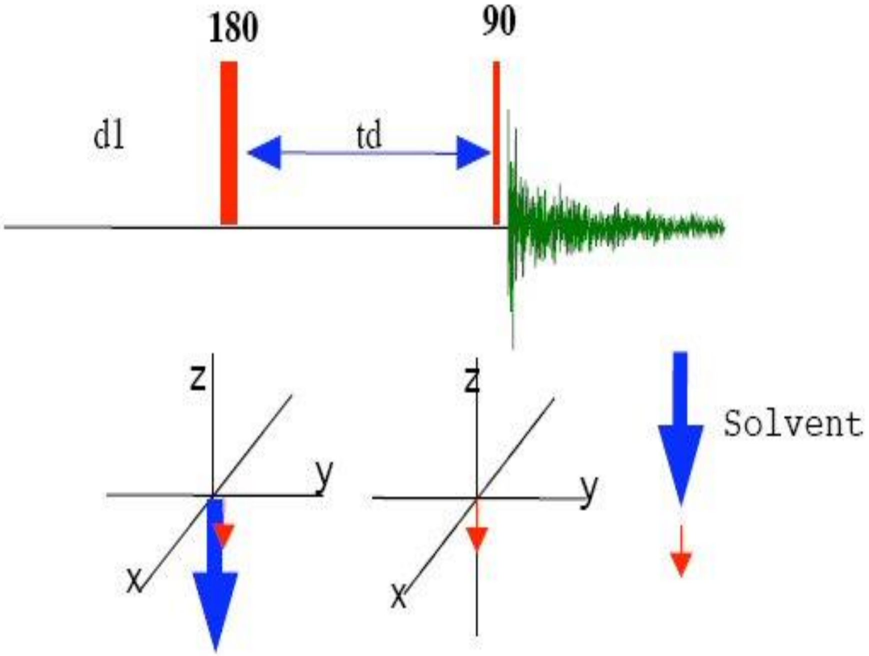


Presaturation field strength:
20-40 Hz corresponds to a
6-12ms 90deg pulse.

Pros: Easy to set up
Excellent water suppression

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

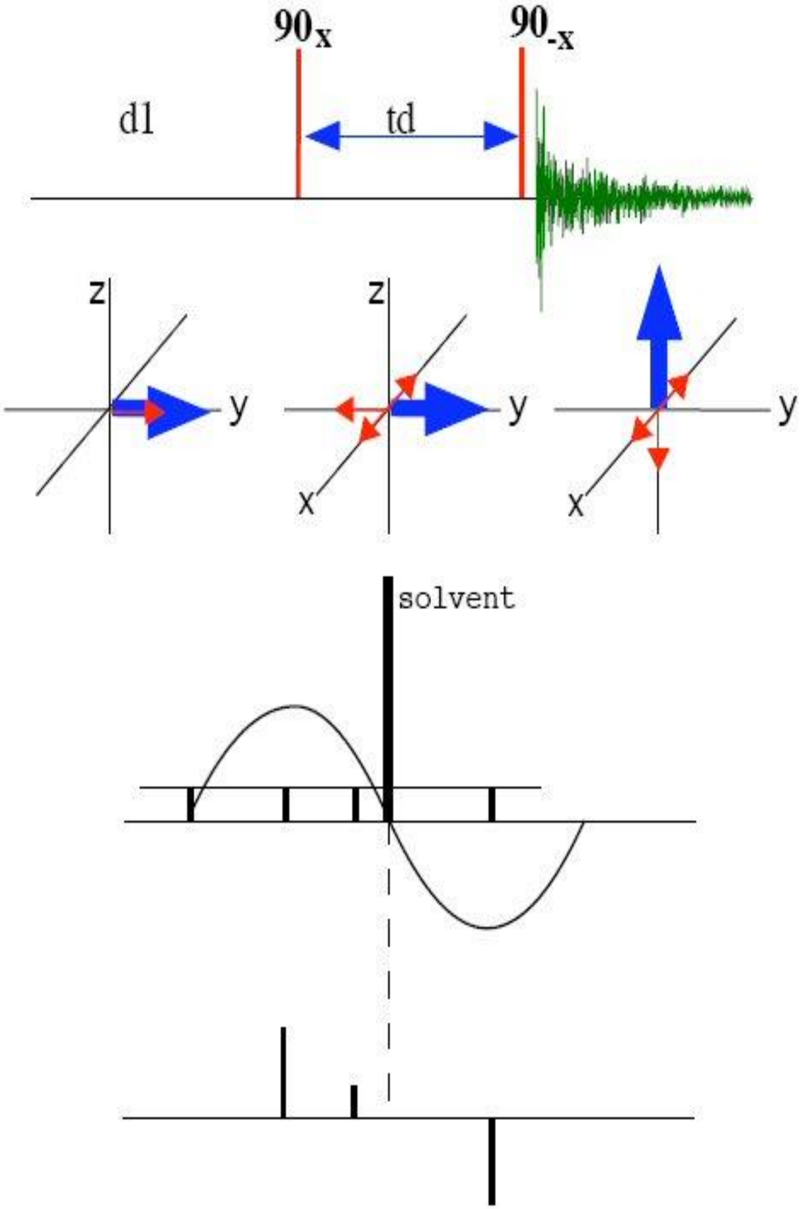
WEFT



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

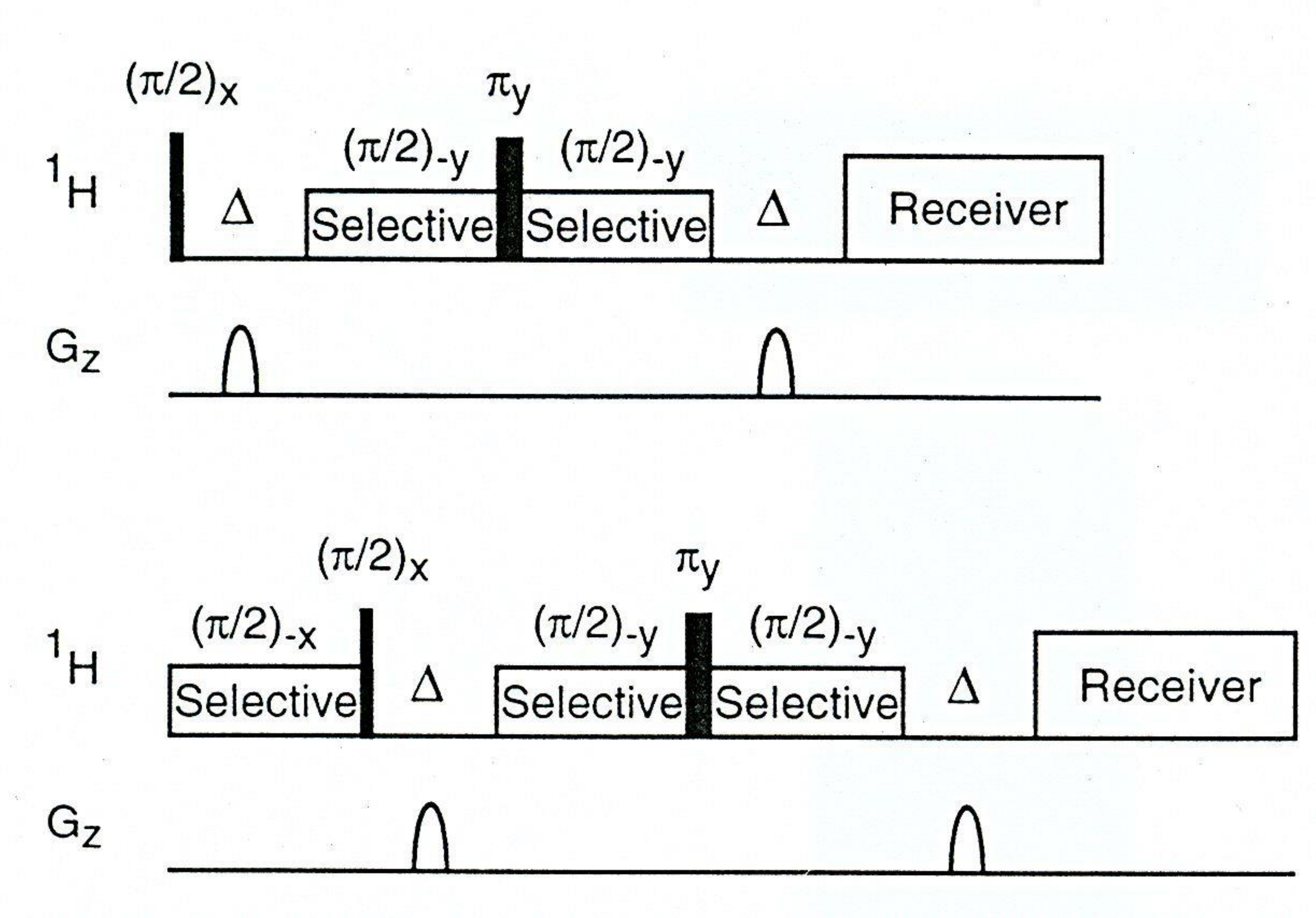


Pros: Easy to set up
Excellent water suppression
(with proper setup as good as presat)
Good for broad signals!

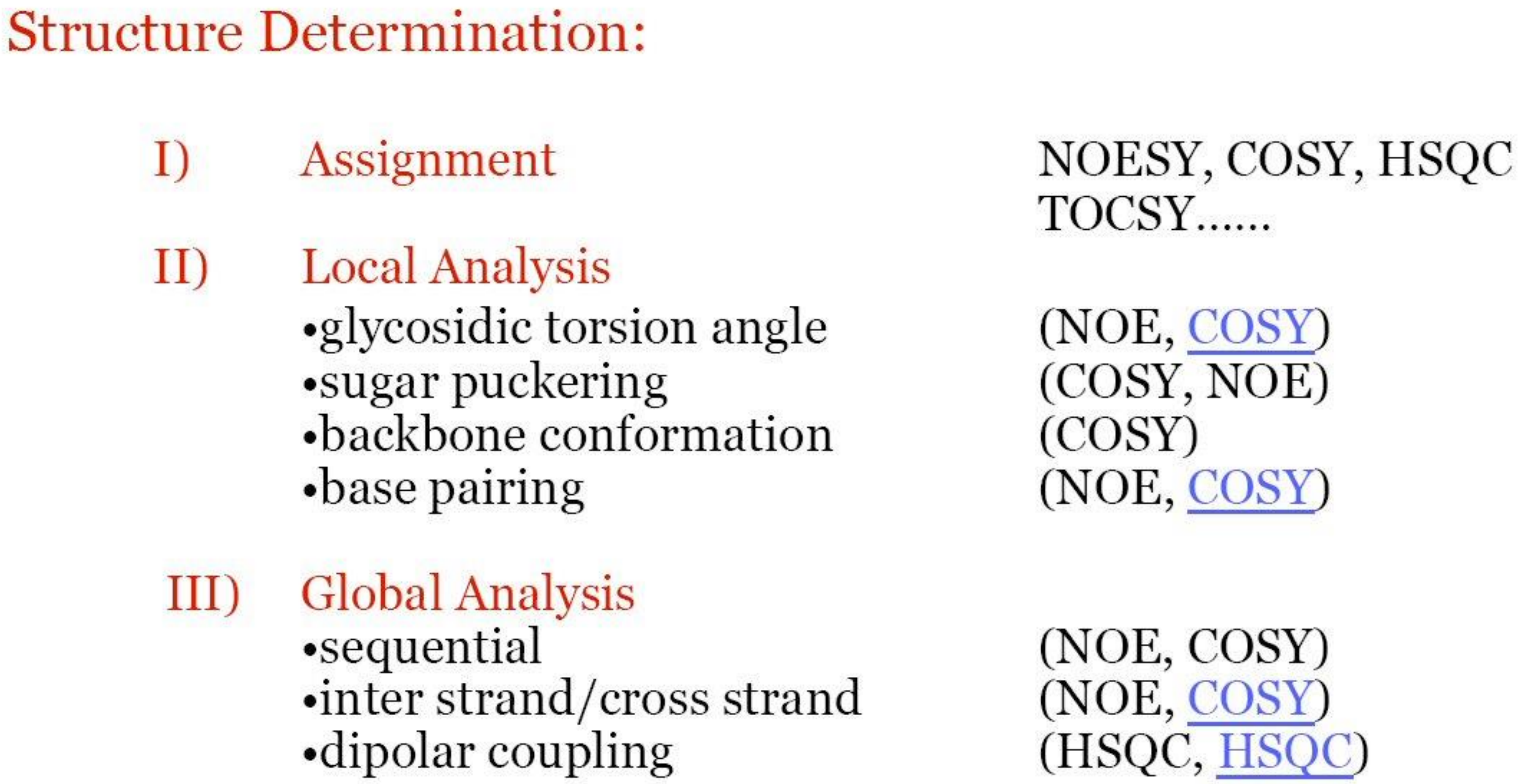
Cons: Non uniform excitation
Baseline not flat

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

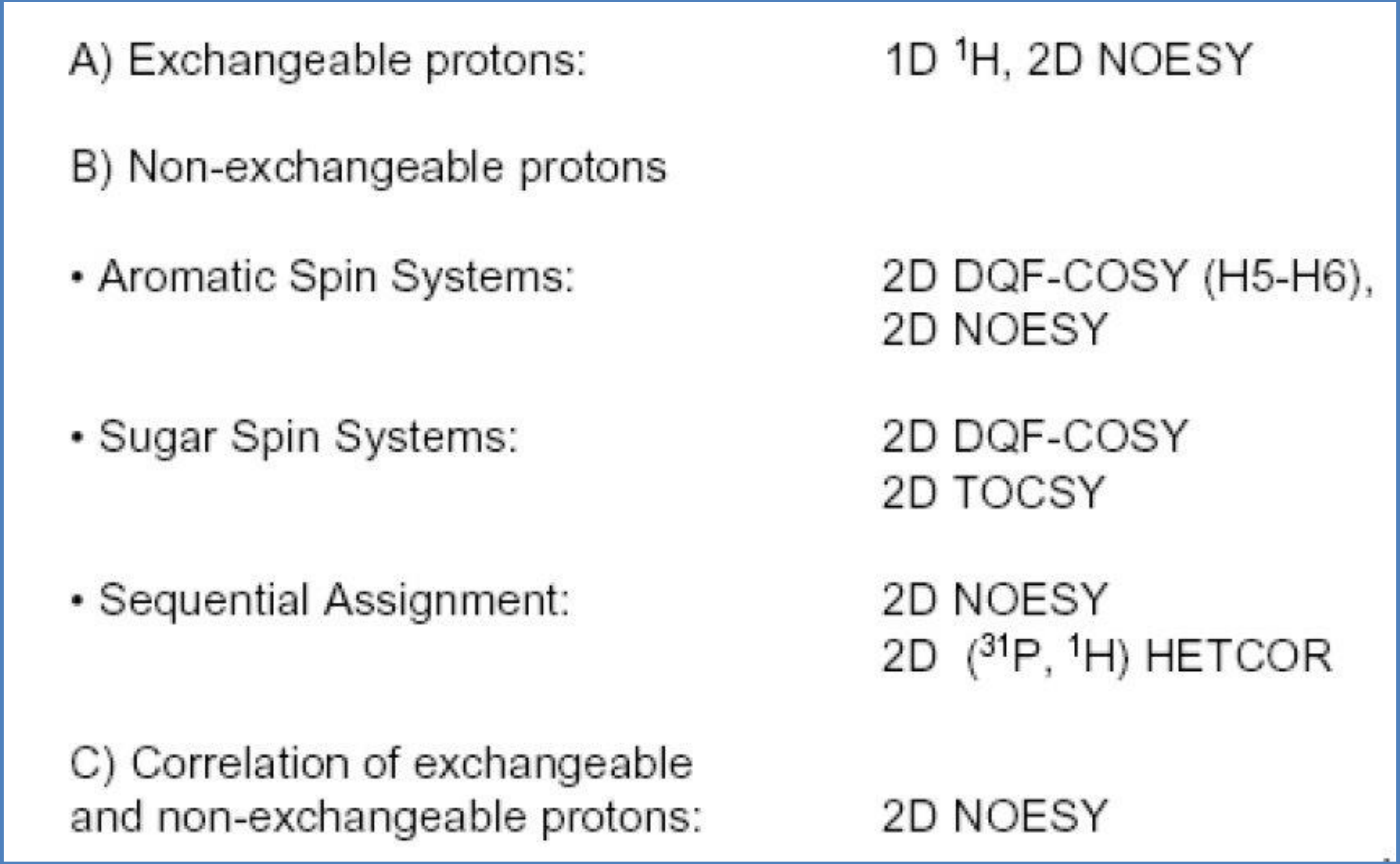
Structure Determination Procedure



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)

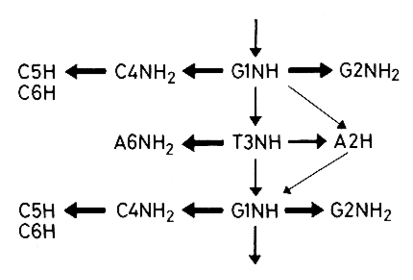
Resonance Assignment



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

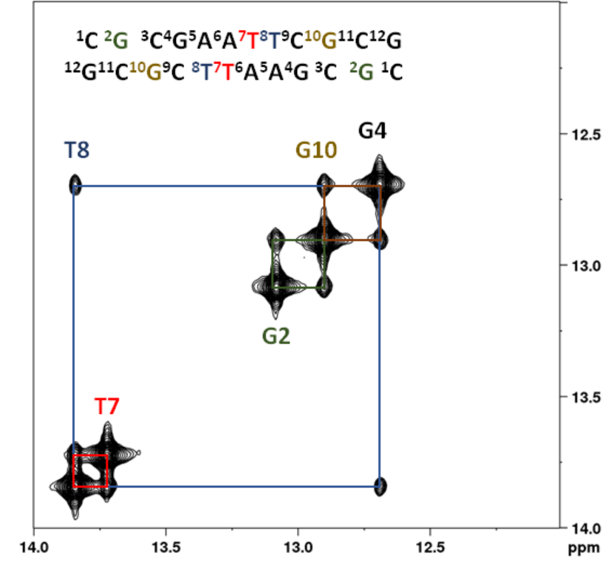
Dickerson's dodecamer
d(CGCGAATTCGCG)$_2$



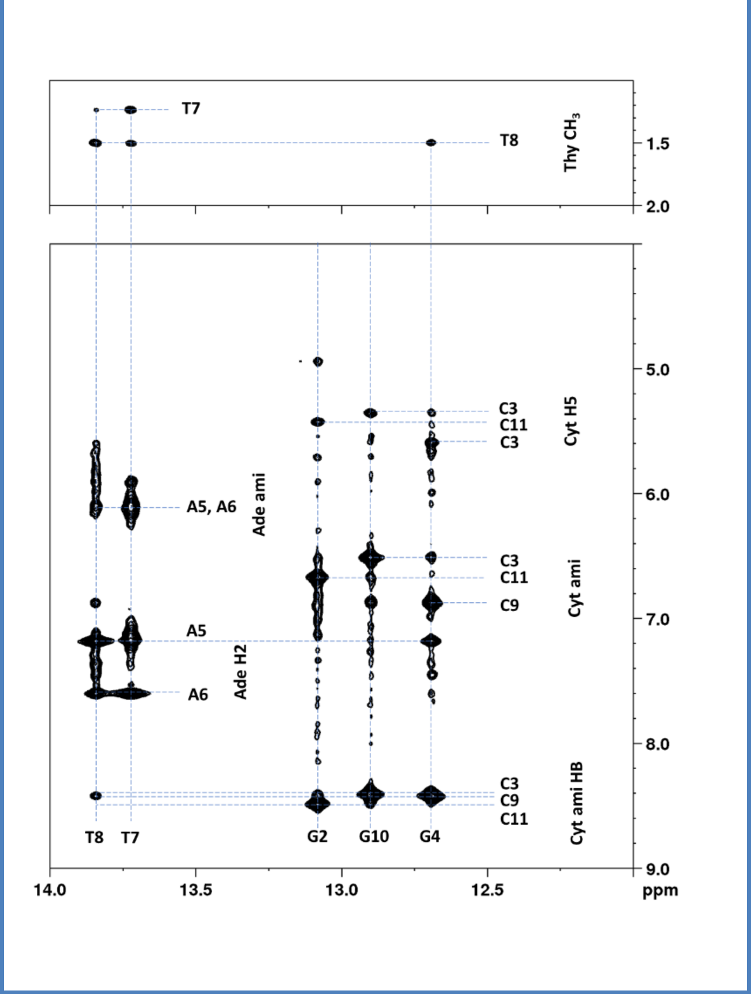
C5H C6H ← C4NH$_2$ ← G1NH → G2NH$_2$

A6NH$_2$ ← T3NH → A2H

C5H C6H ← C4NH$_2$ ← G1NH → G2NH$_2$

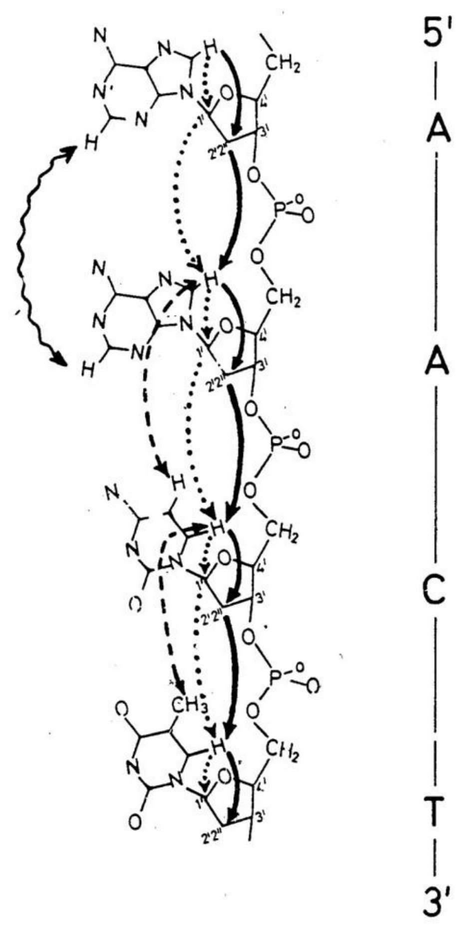


imino-imino

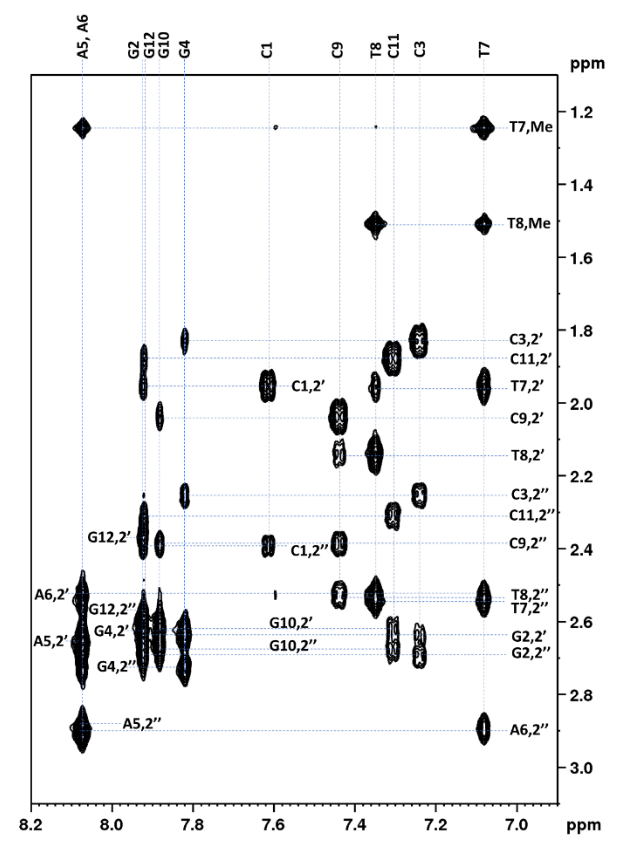


imino-amino

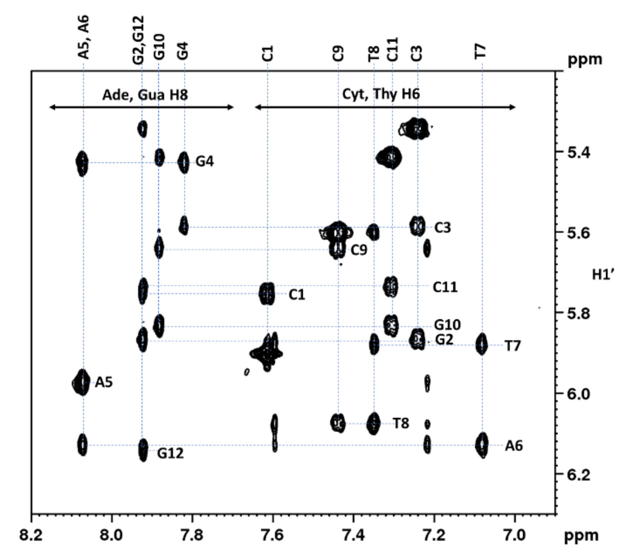
Sequential resonance assignments



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



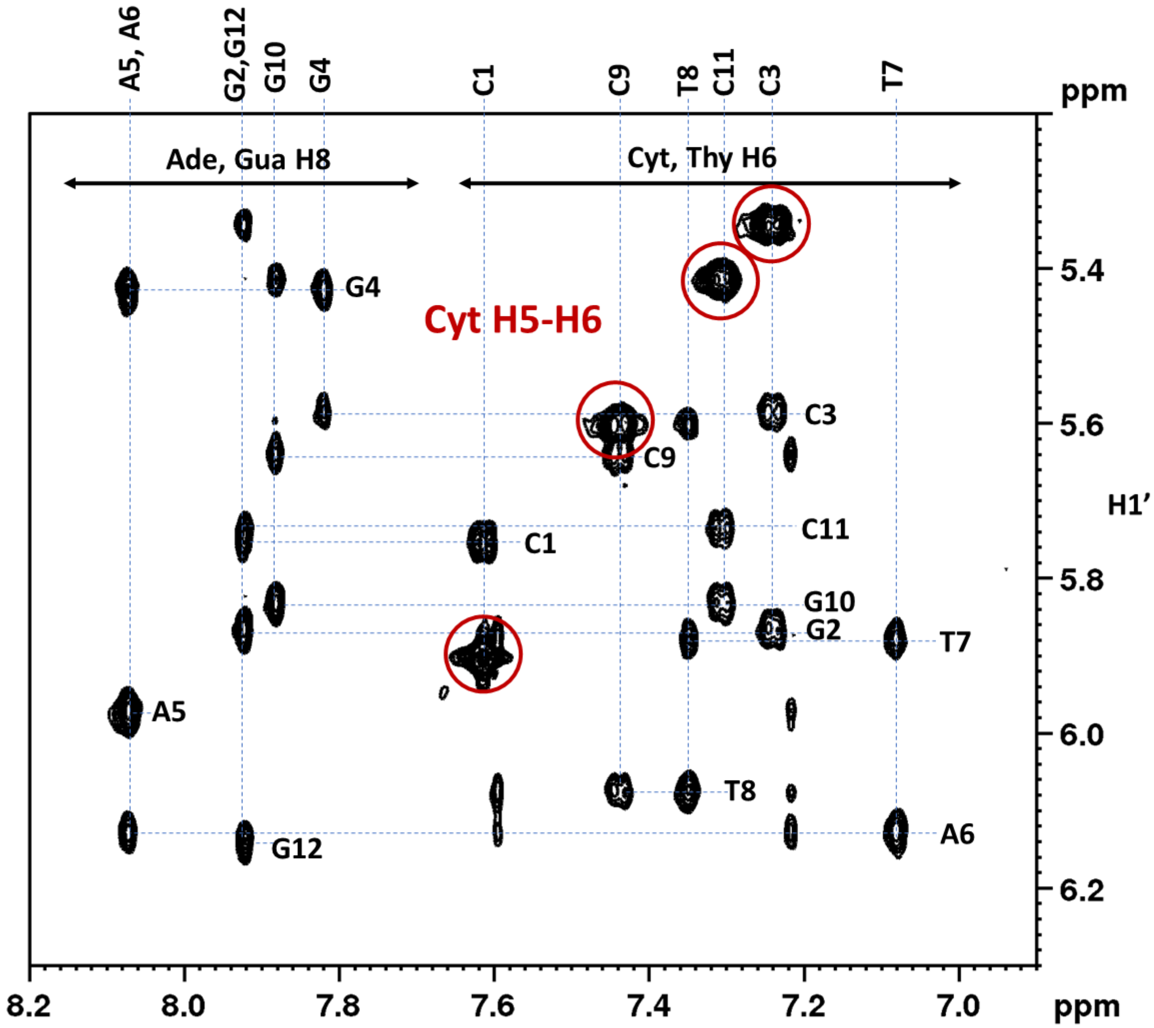
H6/8-H1'



d(CGCGAATTCGCG)$_2$

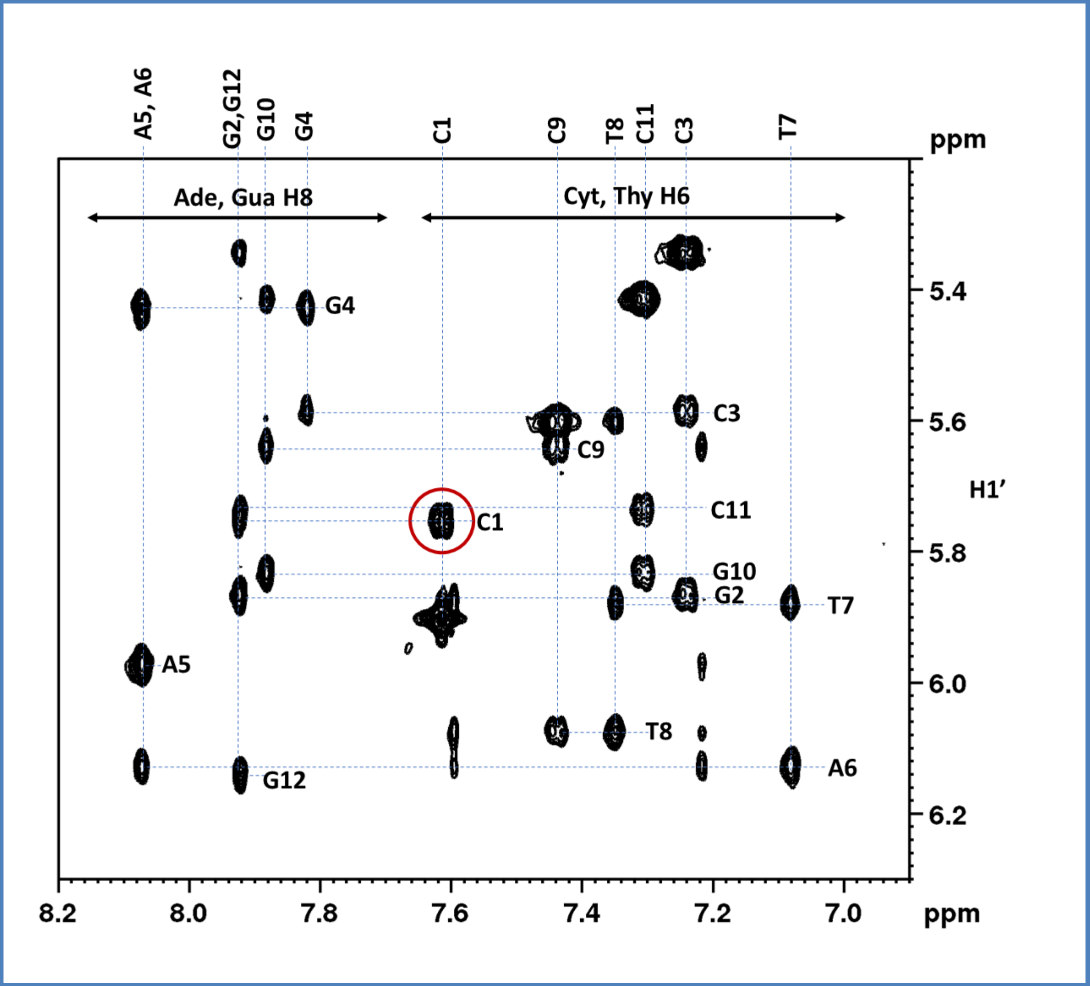
Assignment based on H6/8-H1’ connectivities

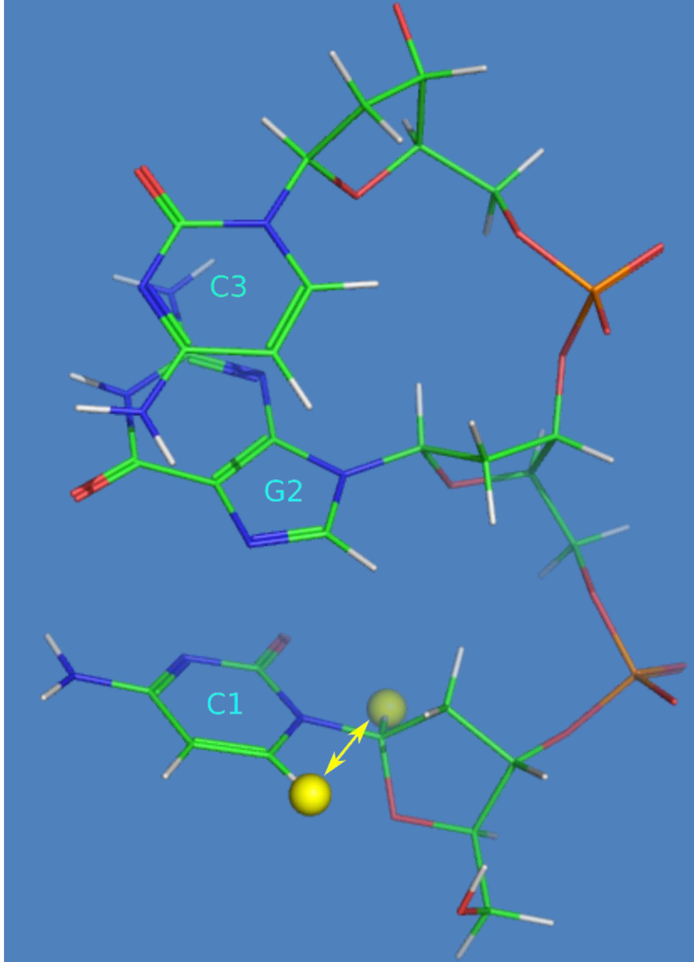
$d(CGCGAATTCGCG)_2$



Assignment based on H6/8-H1’ connectivities

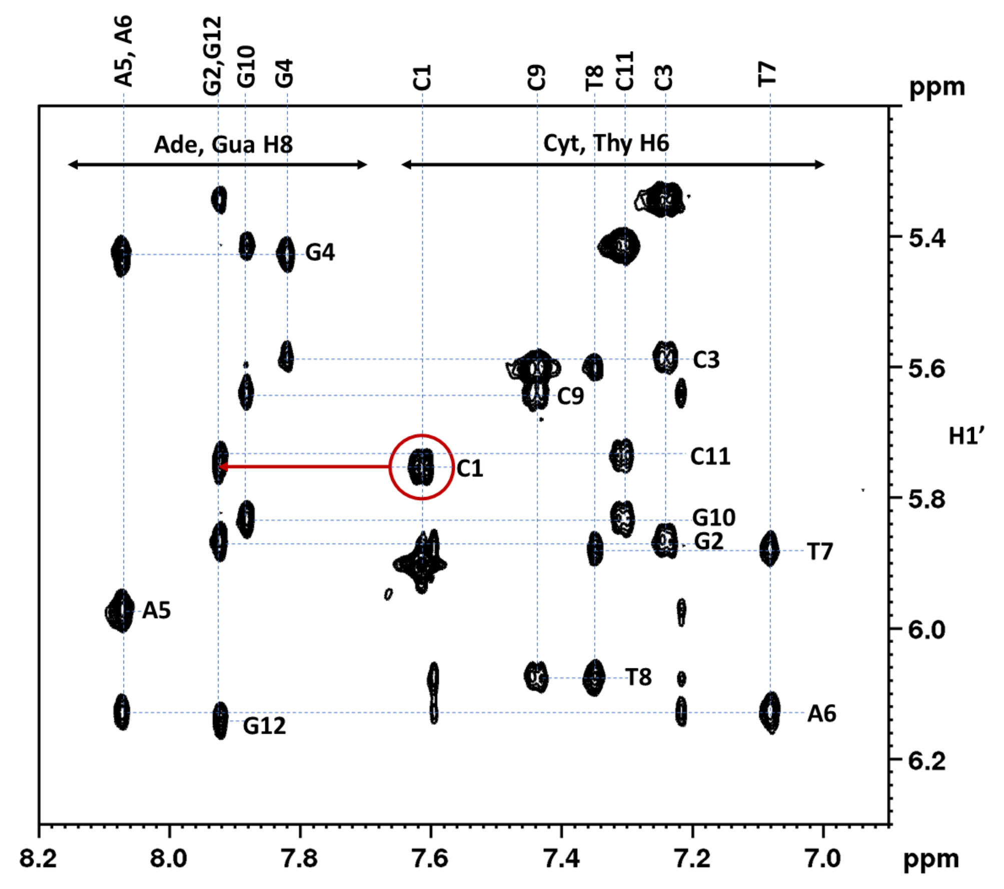
d(CGCGAATTCGCG)$_2$

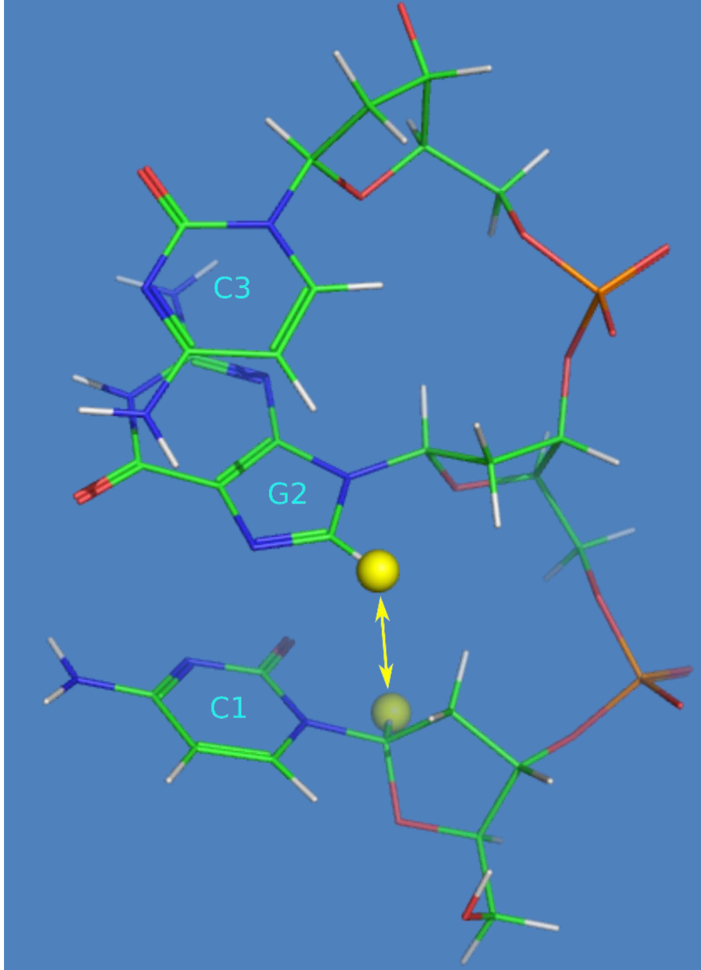




Assignment based on H6/8-H1’ connectivities

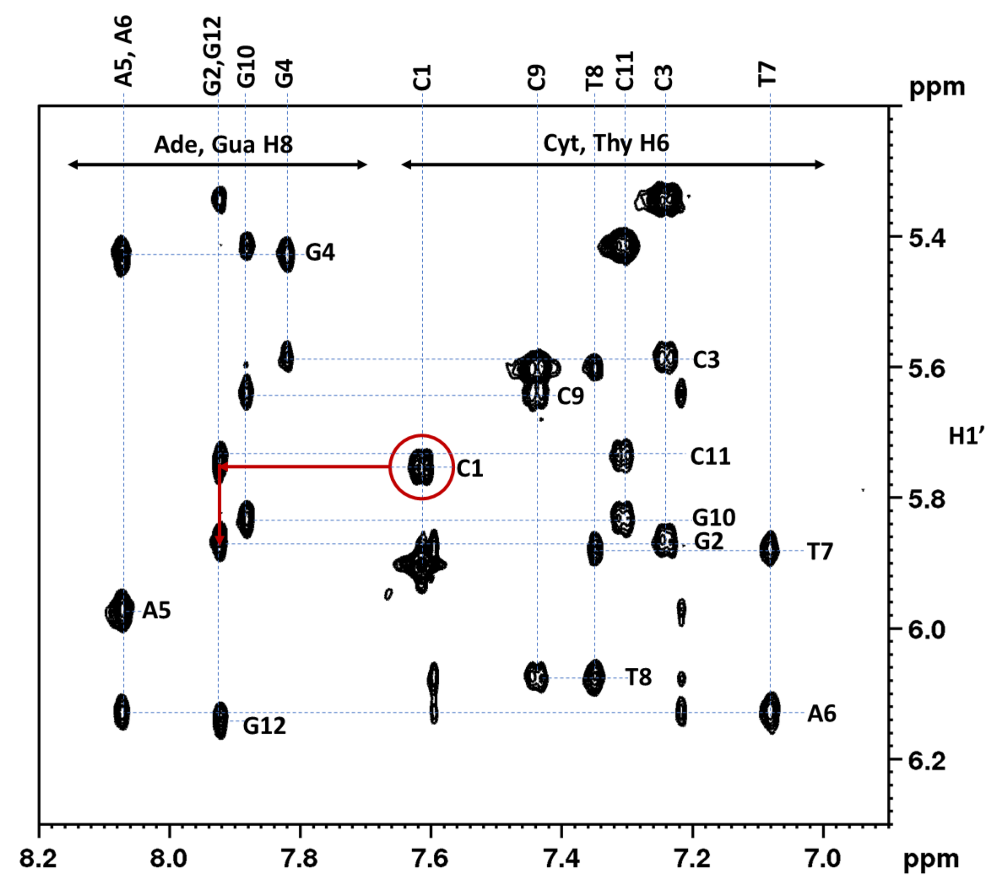
d(CGCGAATTCGCG)$_2$

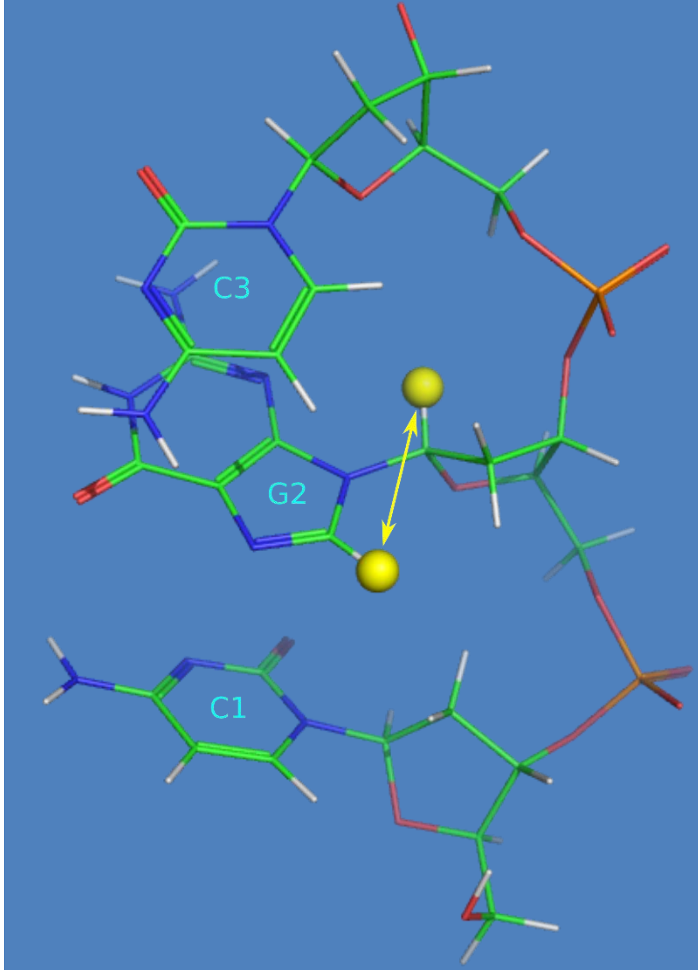




Assignment based on H6/8-H1’ connectivities

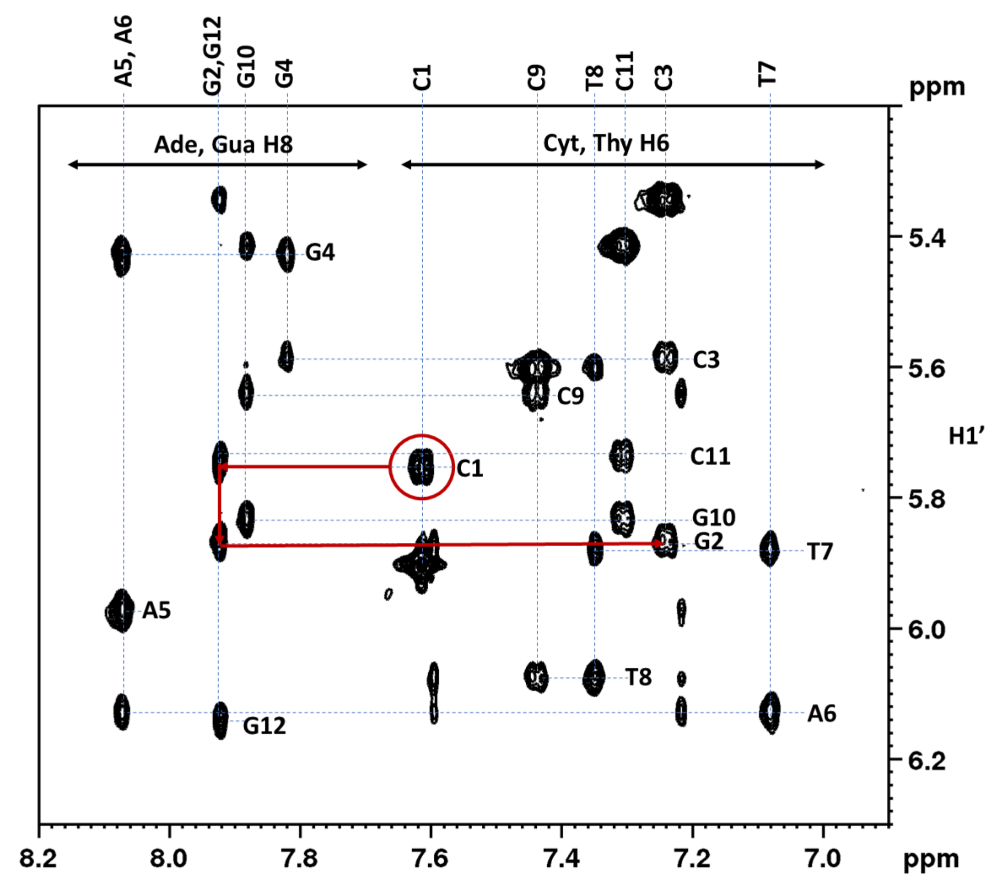
d(CGCGAATTCGCG)$_2$

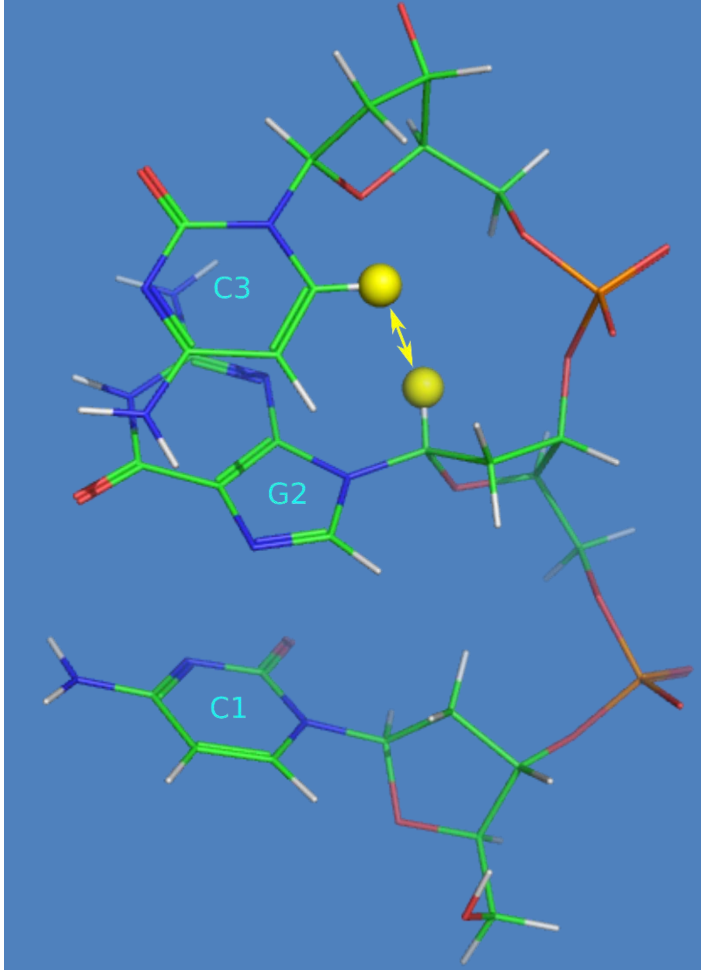




Assignment based on H6/8-H1’ connectivities

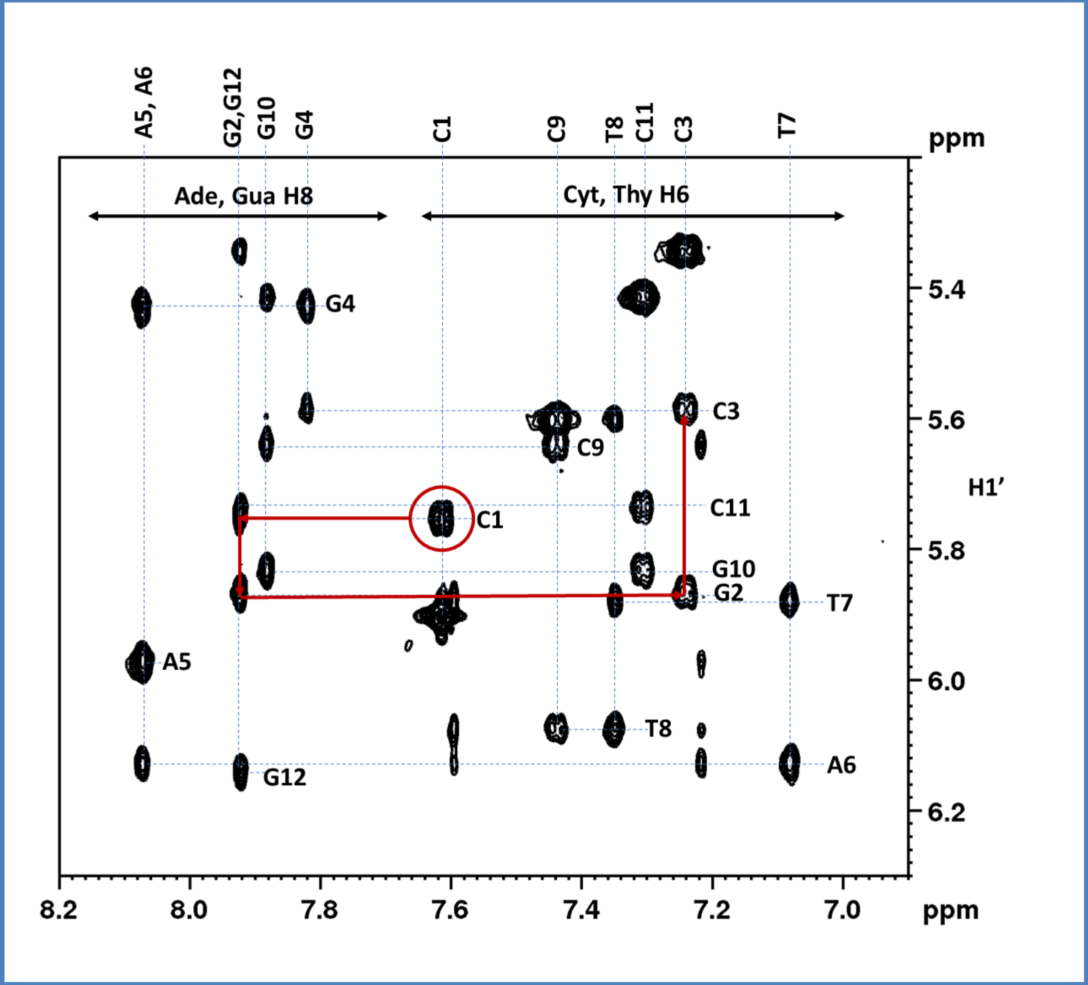
$d(CGCGAATTCGCG)_2$

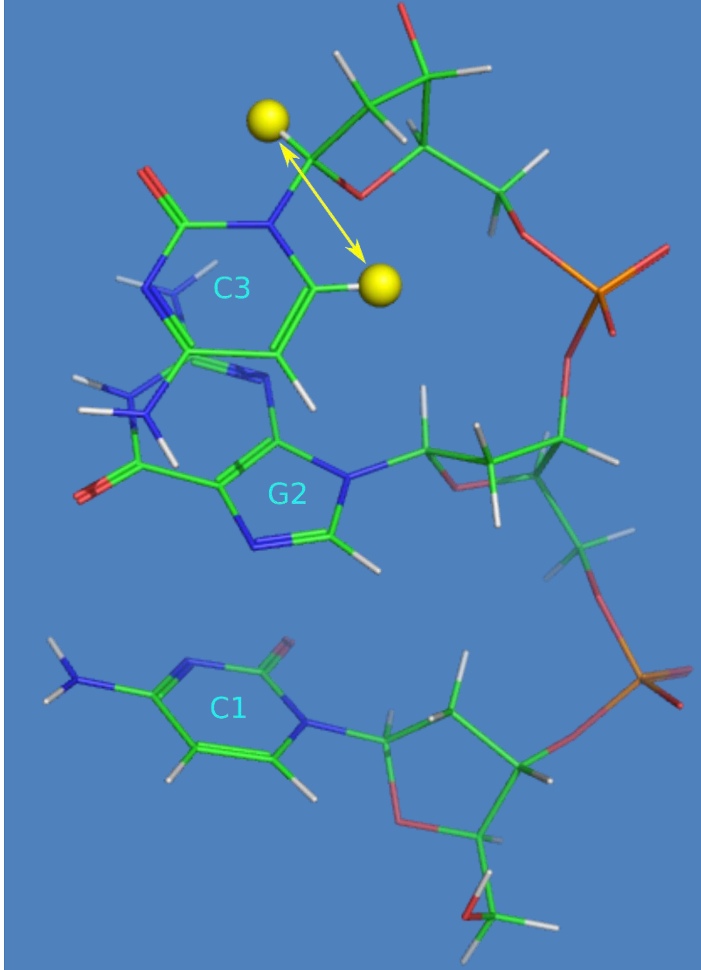




Assignment based on H6/8-H1’ connectivities

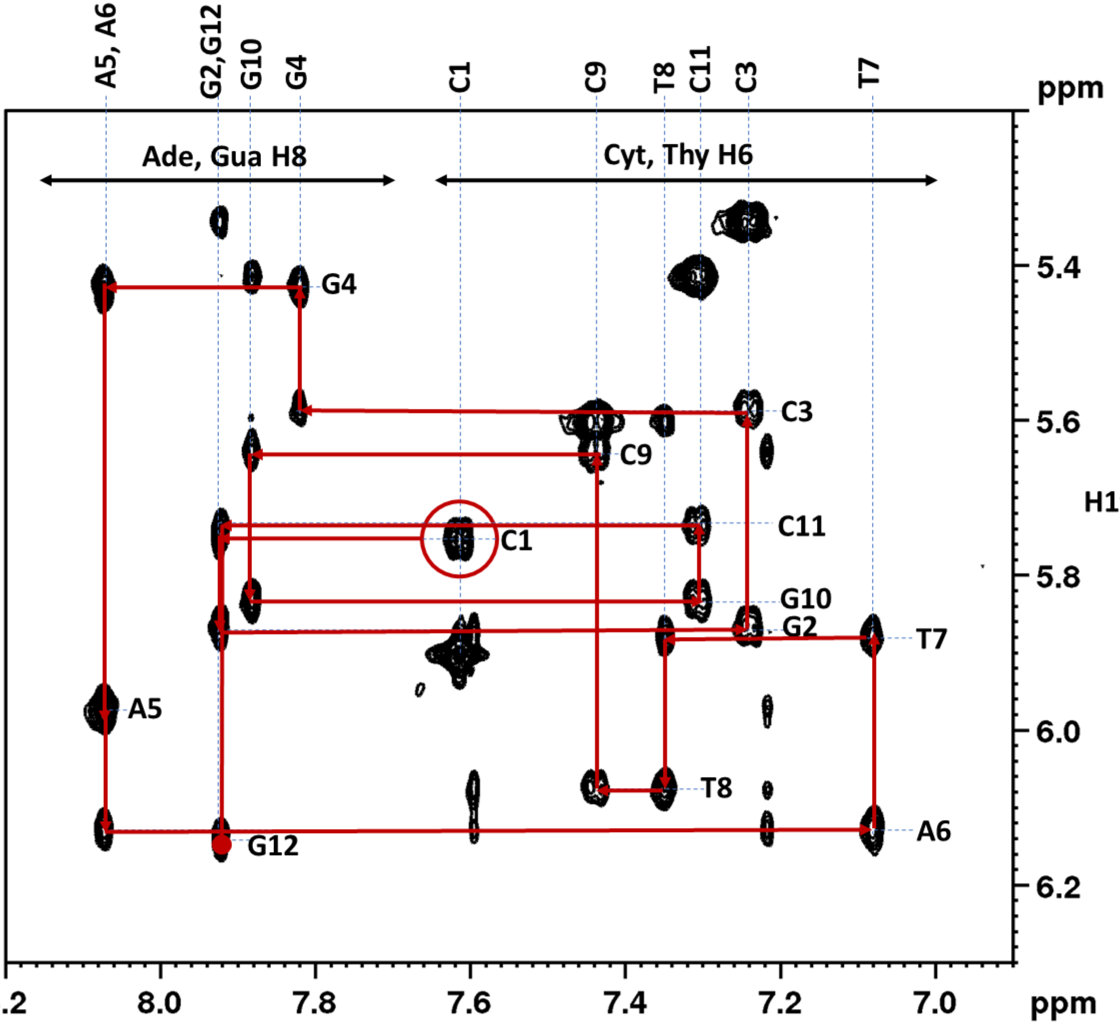
d(CGCGAATTCGCG)$_2$





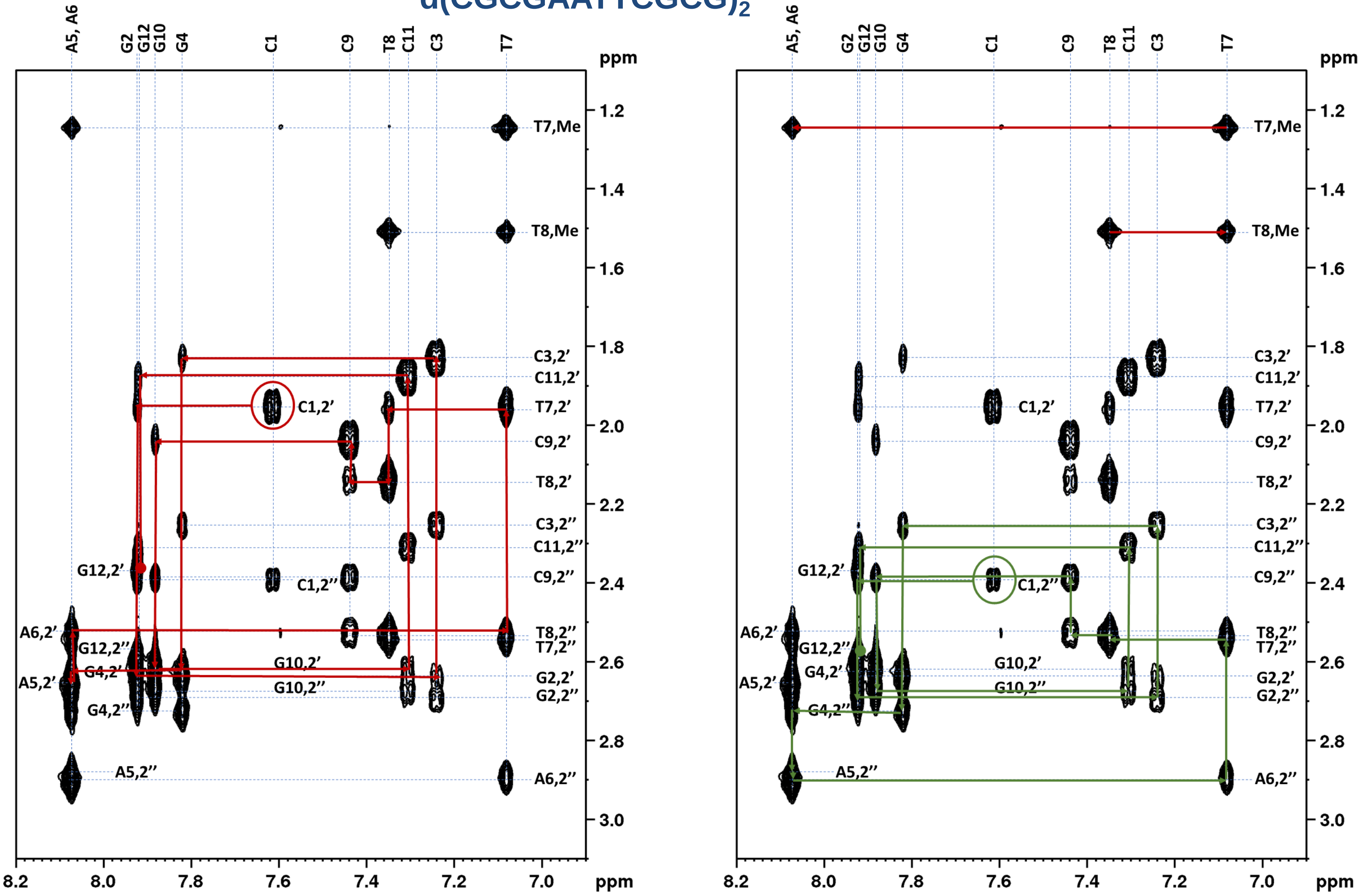
Assignment based on H6/8-H1’ connectivities

$d(CGCGAATTCGCG)_2$

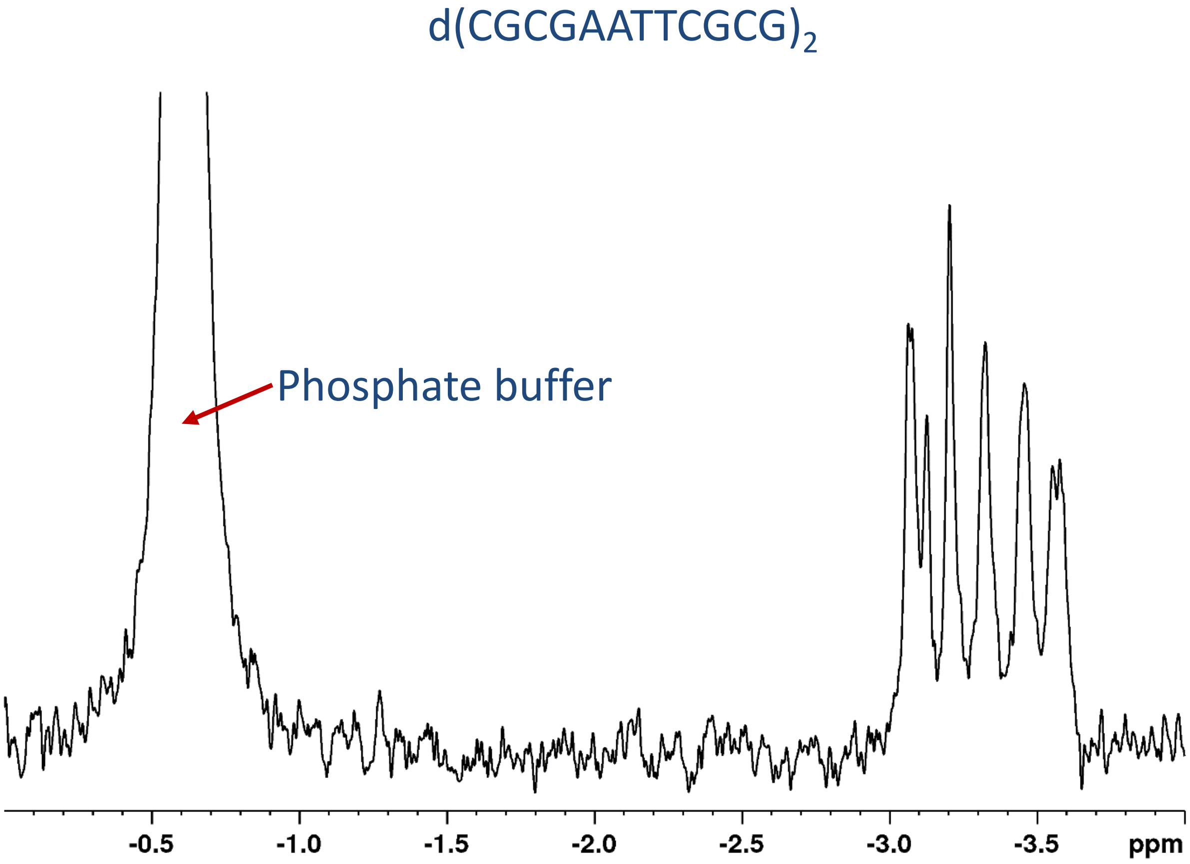


Assignment based on H6/8-H2'2" connectivities

d(CGCGAATTCGCG)$_2$



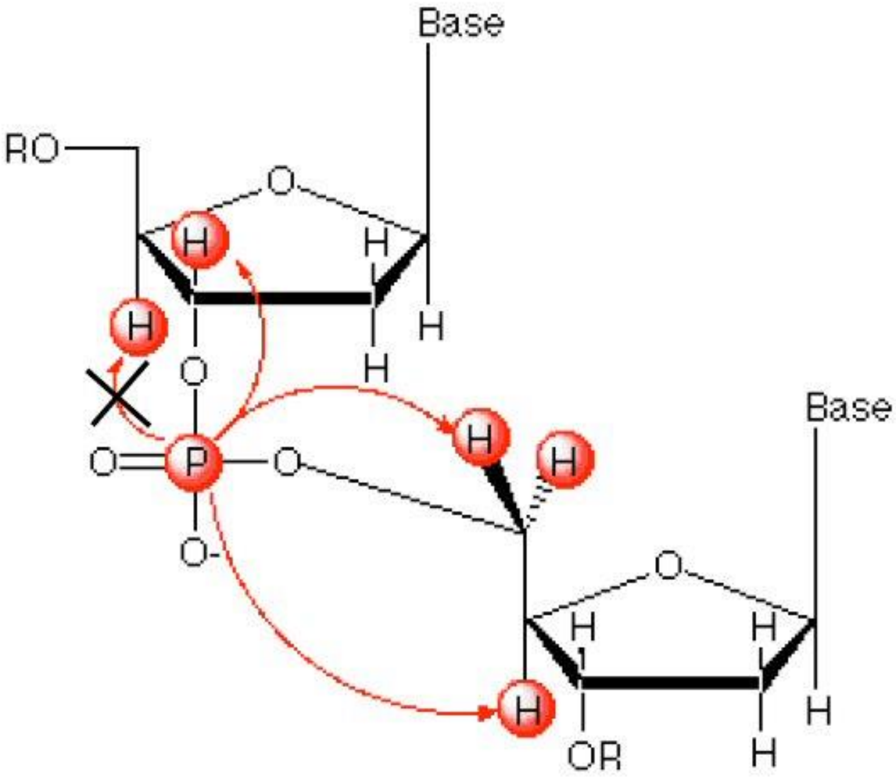
^{31}P spectrum of DNA



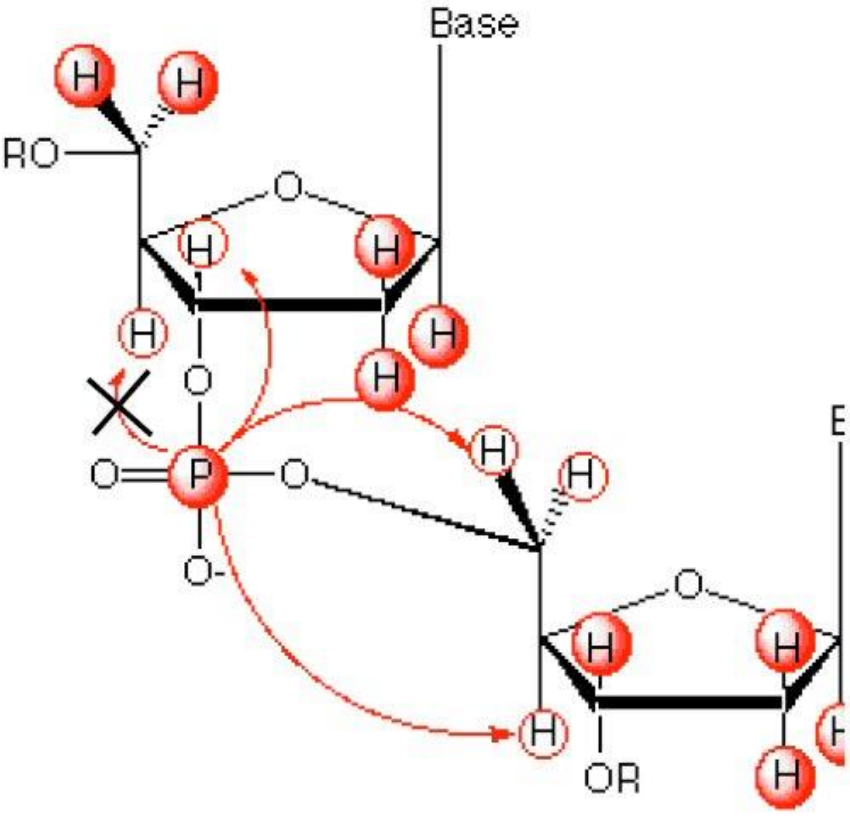
Assignment of Sugar-Phosphate Backbone

^{31}P NMR

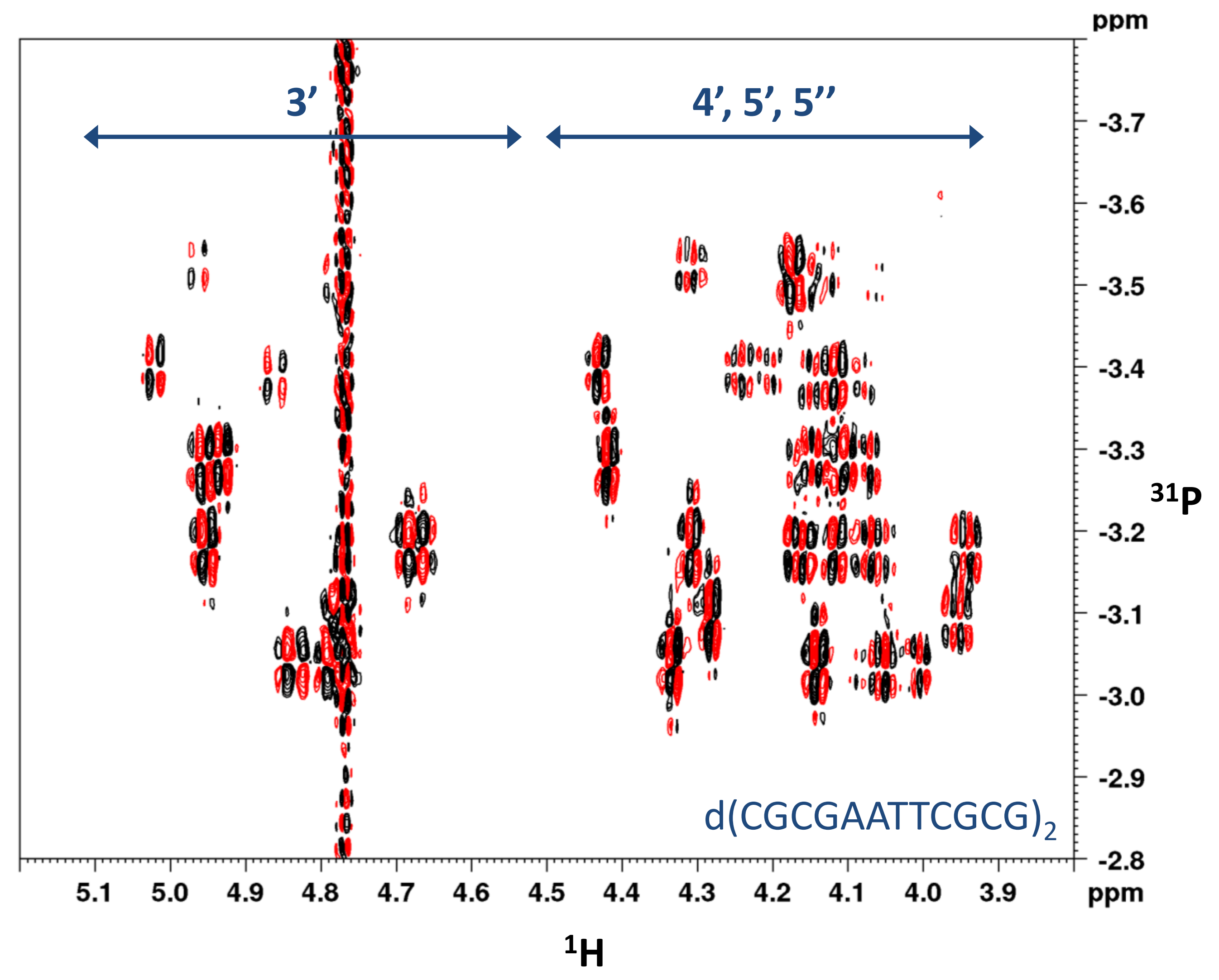
HP-COSY



HP-TOCSY



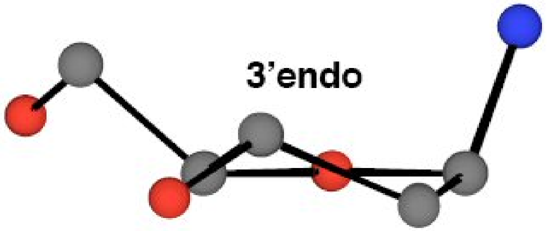
^{1}H - ^{31}P correlation spectrum



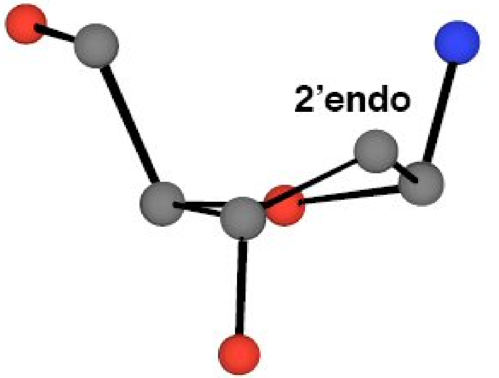
Sugar puckering

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).

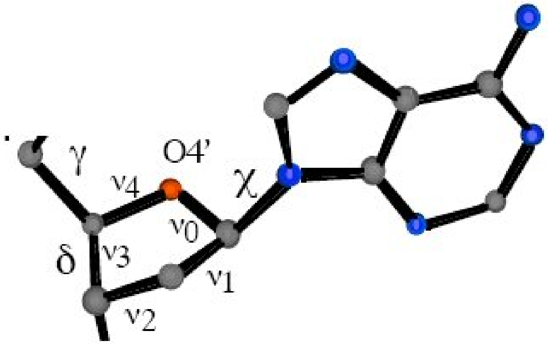
In general:
RNA (A type double helix) C3' endo.
DNA (B type double helix) C2' endo.



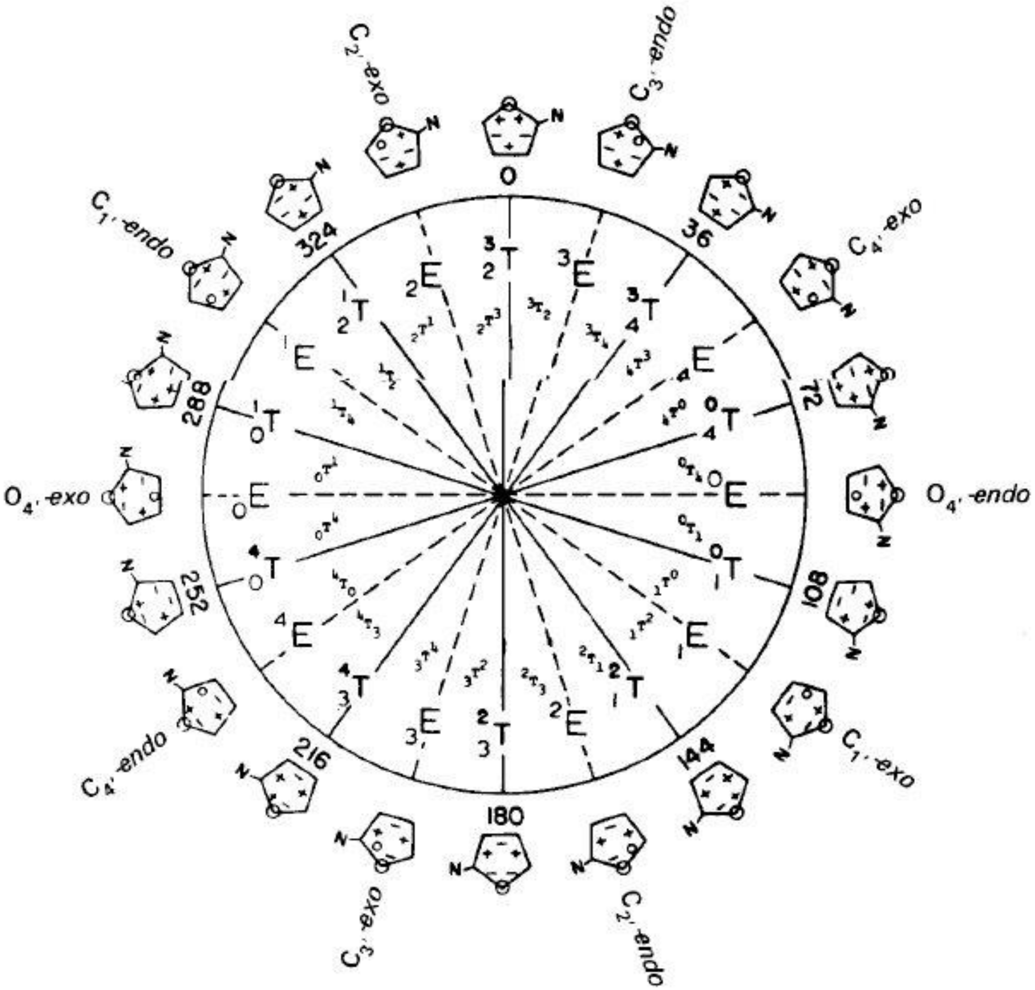
$$\nu_i = \Phi_m \cos (P + 144 (j-2))$$



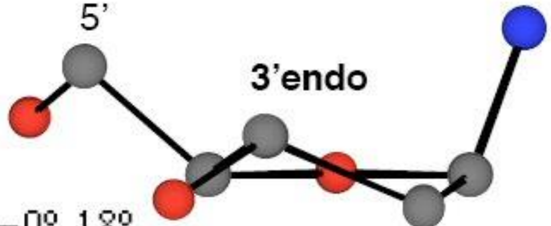
$$\delta = \nu_3 + 125^\circ$$



N (Northern)



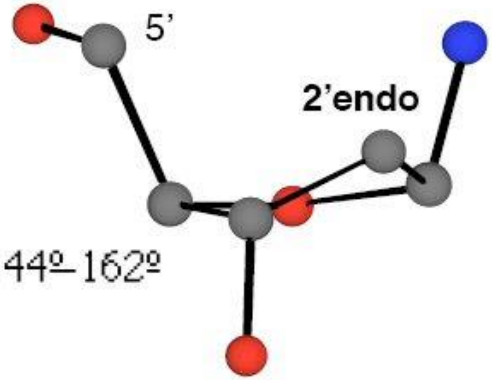
(Southern)



P=0º–18º

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

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

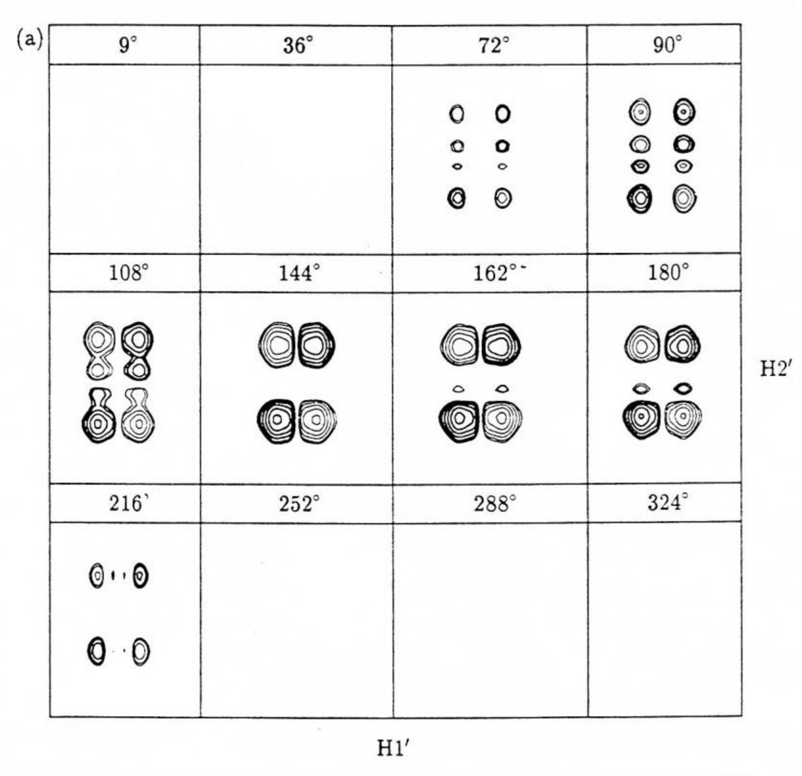


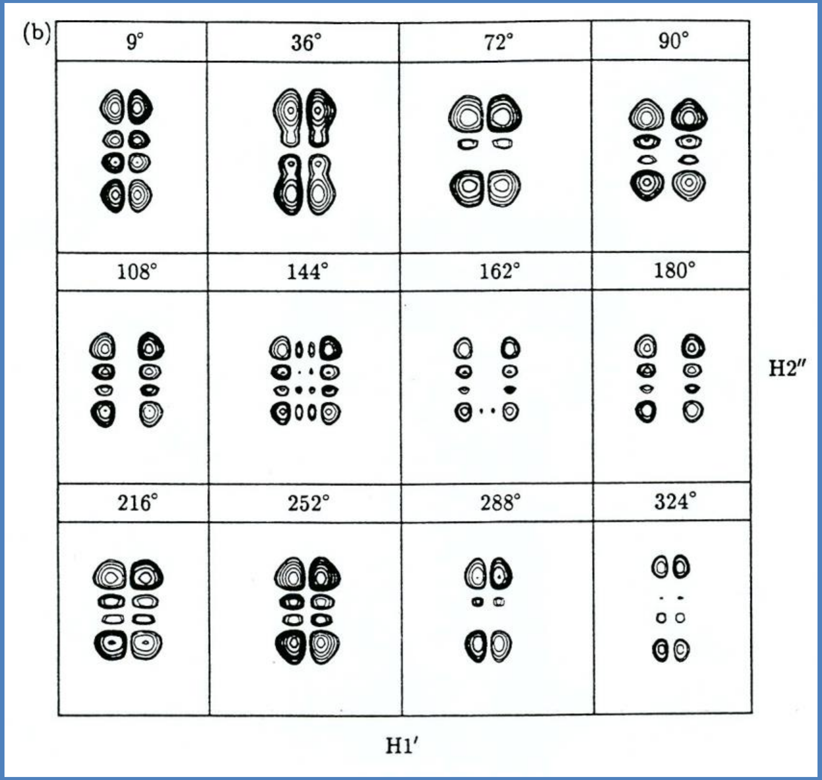
P=144º–162º

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

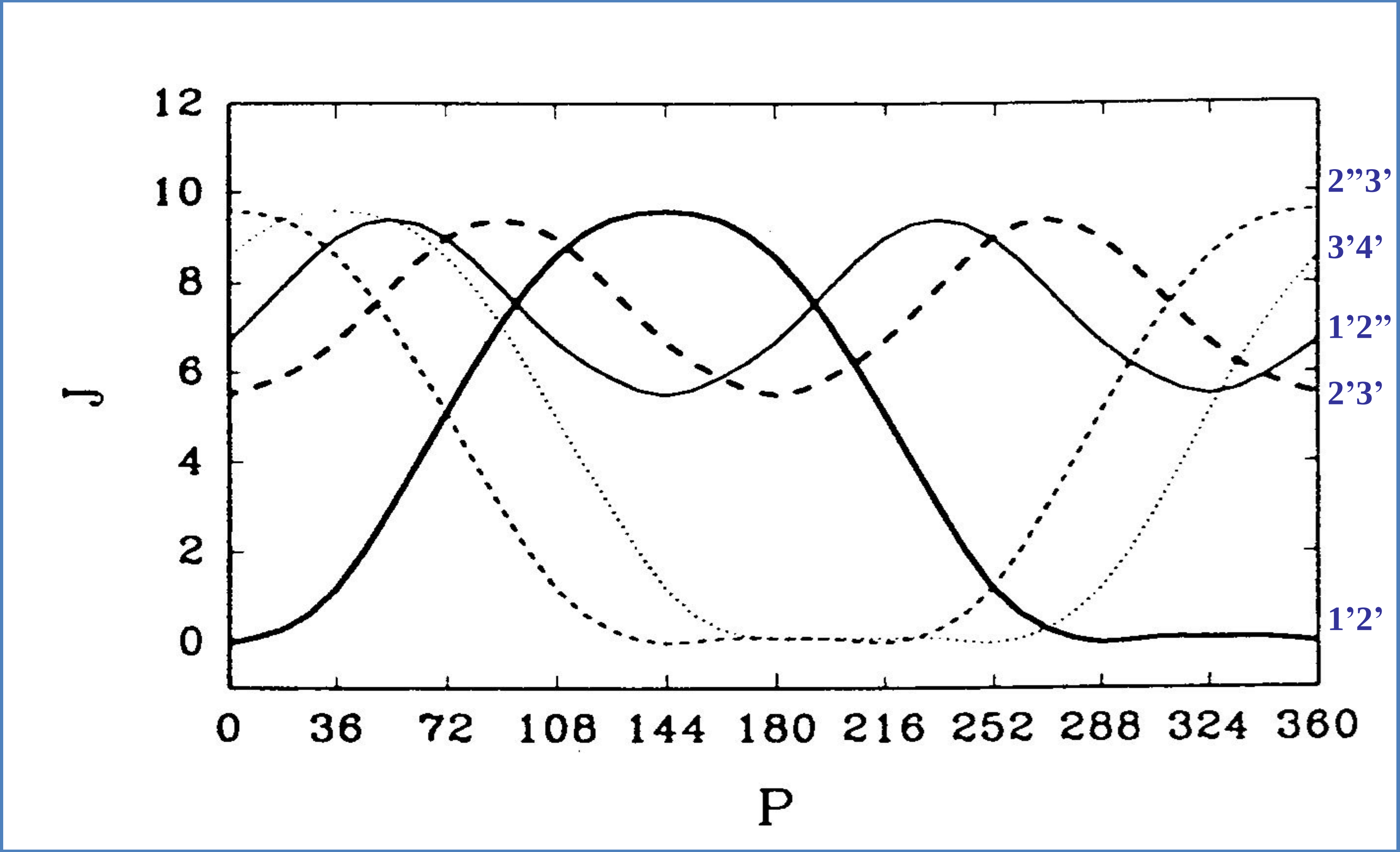
Deoxyribose: $^3J_{H1'-H2'} \approx 10$ Hz

J-couplings from COSY spectra

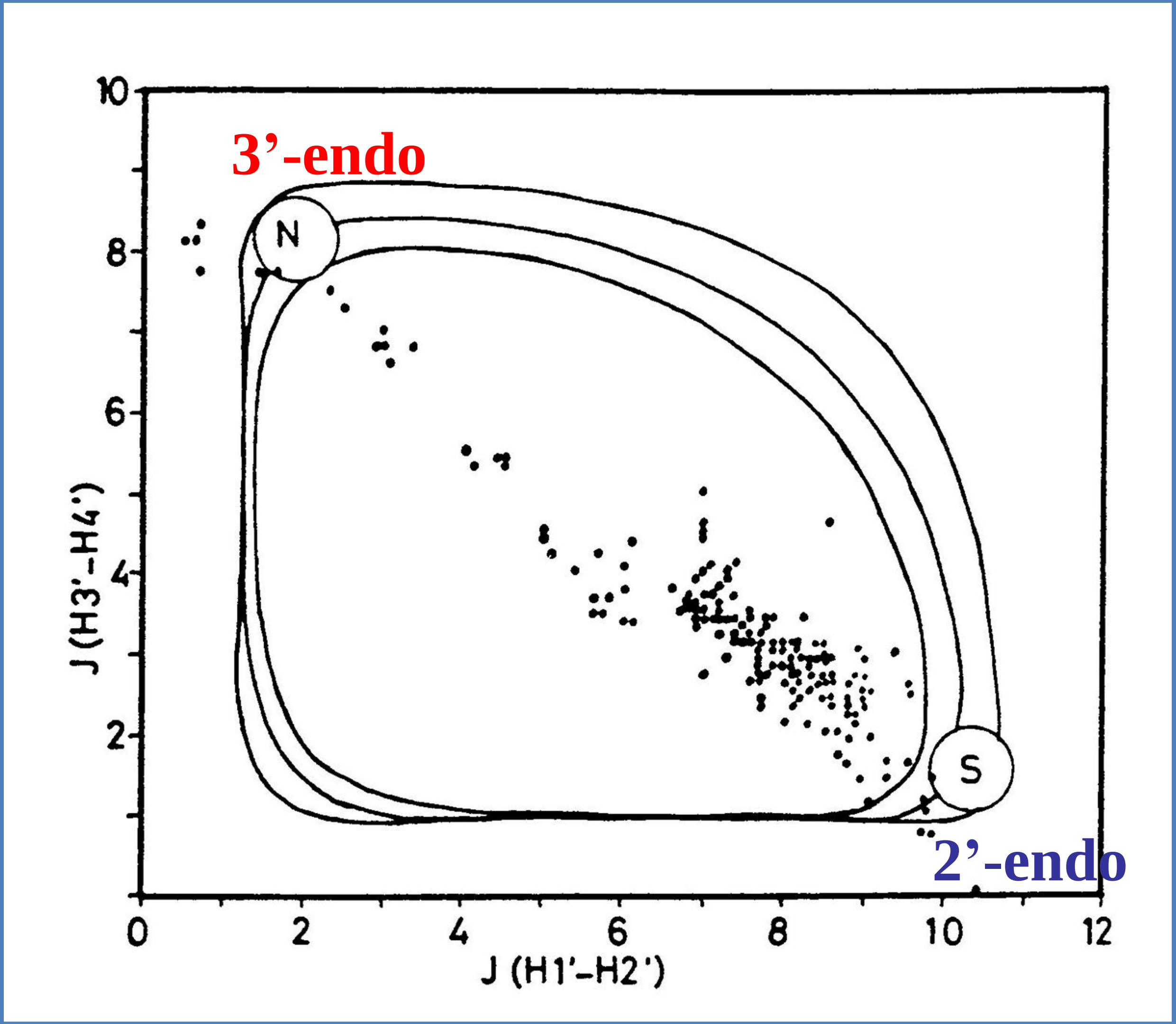




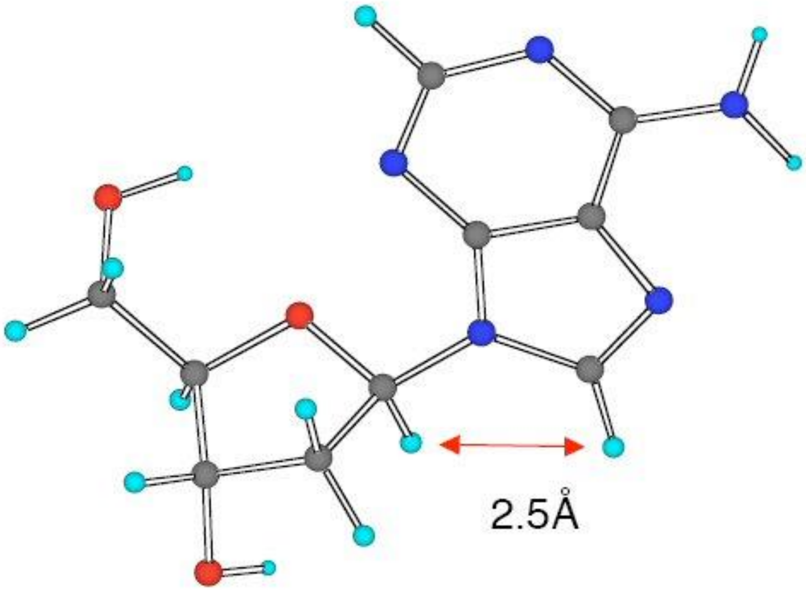
P determination from J-couplings

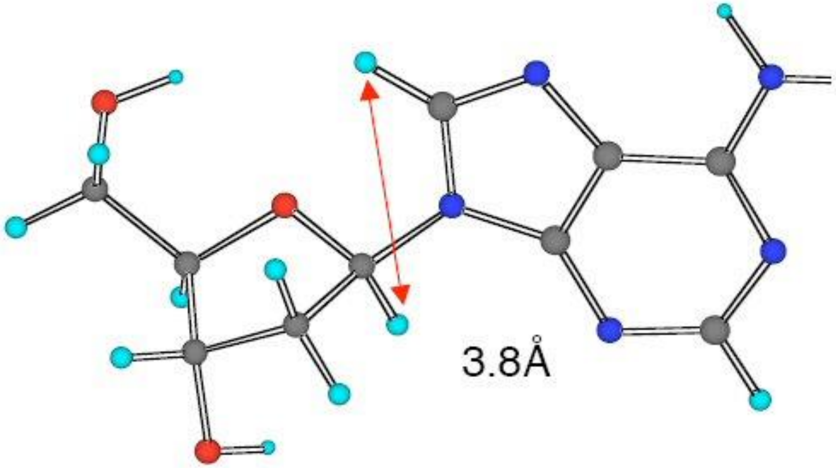


Equilibrium of N and S conformations

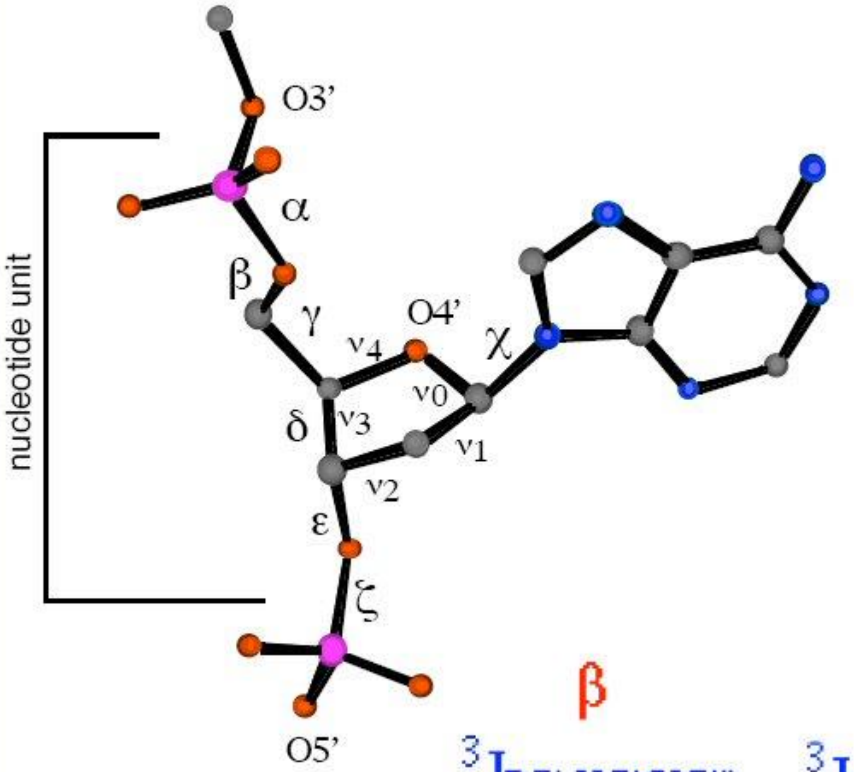


Distance information determines the glycosidic torsion angle





- 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. $\zeta = -317-1.23\,\varepsilon$

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