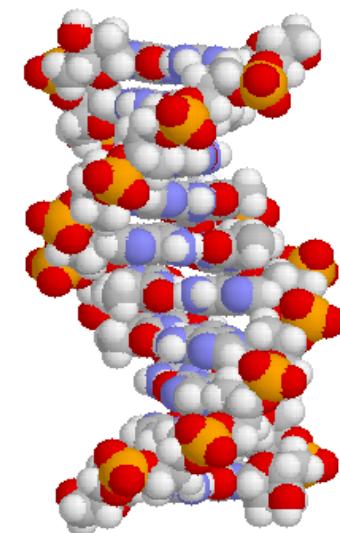
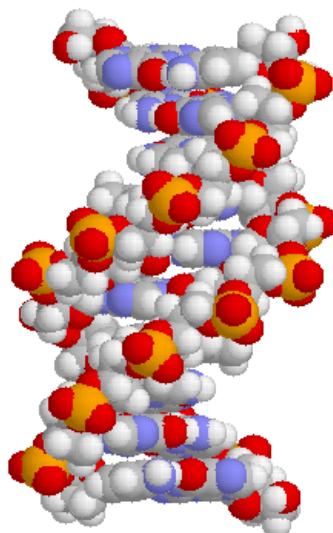


# Nucleic Acids

# NMR Spectroscopy

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Departments of Chemistry and Biology  
Georgia State University





**This is meant to give you a background in nucleic acid NMR  
--- with some tricks and practical hints**

# NMR of Nucleic Acids 1

- 1) Primary Structure of DNA and RNA
- 2) Resonance Assignment of DNA/RNA by Homonuclear NMR
  - A)  $^1\text{H}$  Chemical shifts
  - B) Assignment of exchangeable protons
  - C) Assignment of non-exchangeable proton
  - D) Typical NOEs in helical structures
  - E) Correlation between non-exchangeable and exchangeable protons

# NMR Spectroscopy is an Important Method for Structural Studies of Nucleic Acids:

PDB Holding, March 21, 2012

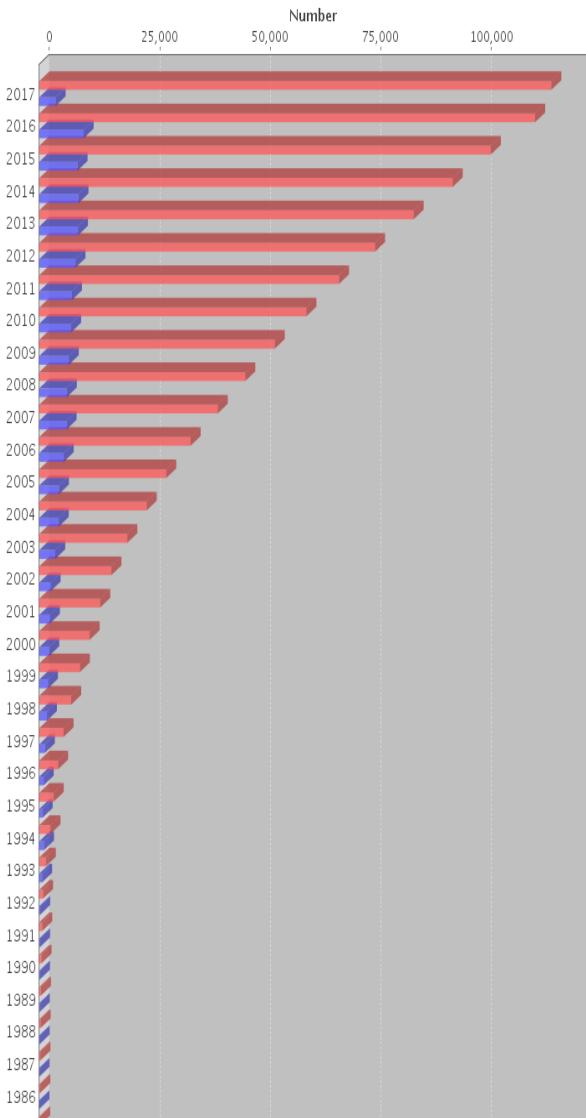
Technique	Molecule				
	Proteins	Nucleic Acids	Protein/Nucleic Acid Complexes	Other	
X-ray Diffraction	65'703	1'266	3'331	-	70'302
NMR	8'163	933	228	-	9'331
Other <sup>1)</sup>	430	24	122	-	492
total	74'294	2'223	3'681	-	80'264

1) EM, Hybrid, other

<http://www.rcsb.org/pdb>

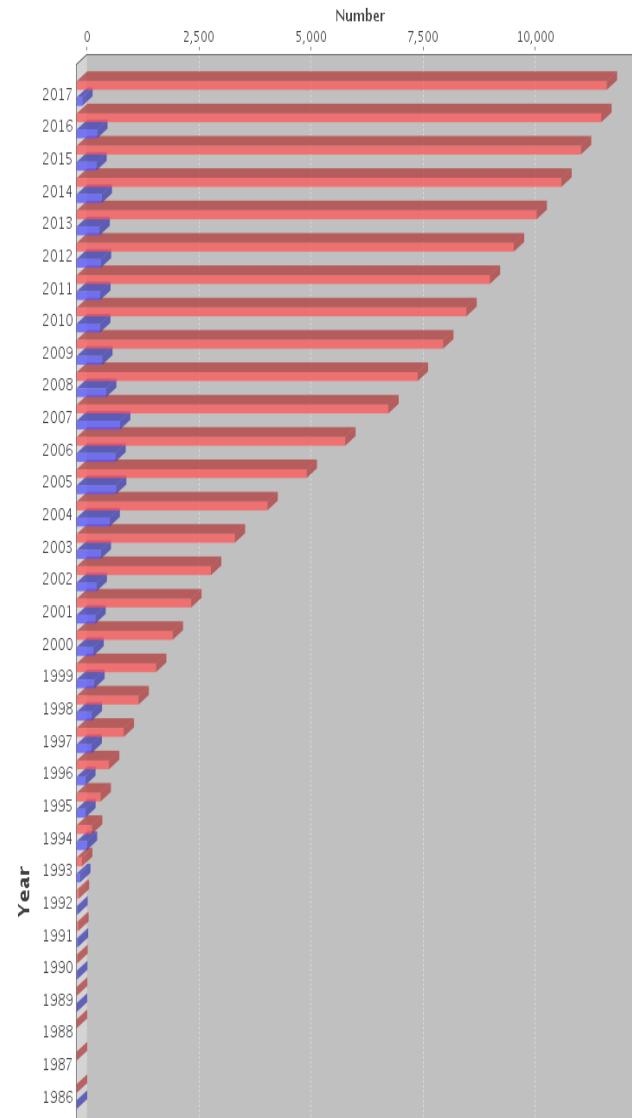
### Yearly Growth of Structures Solved By X-ray

number of structures can be viewed by hovering mouse over the bar



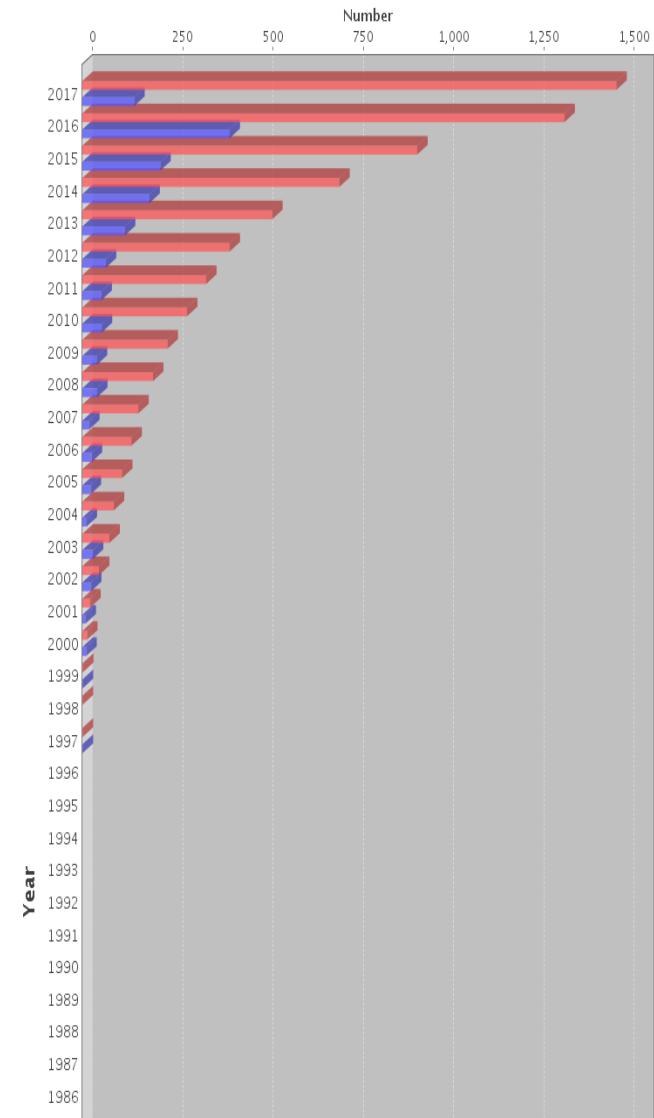
### Yearly Growth of Structures Solved By NMR

number of structures can be viewed by hovering mouse over the bar

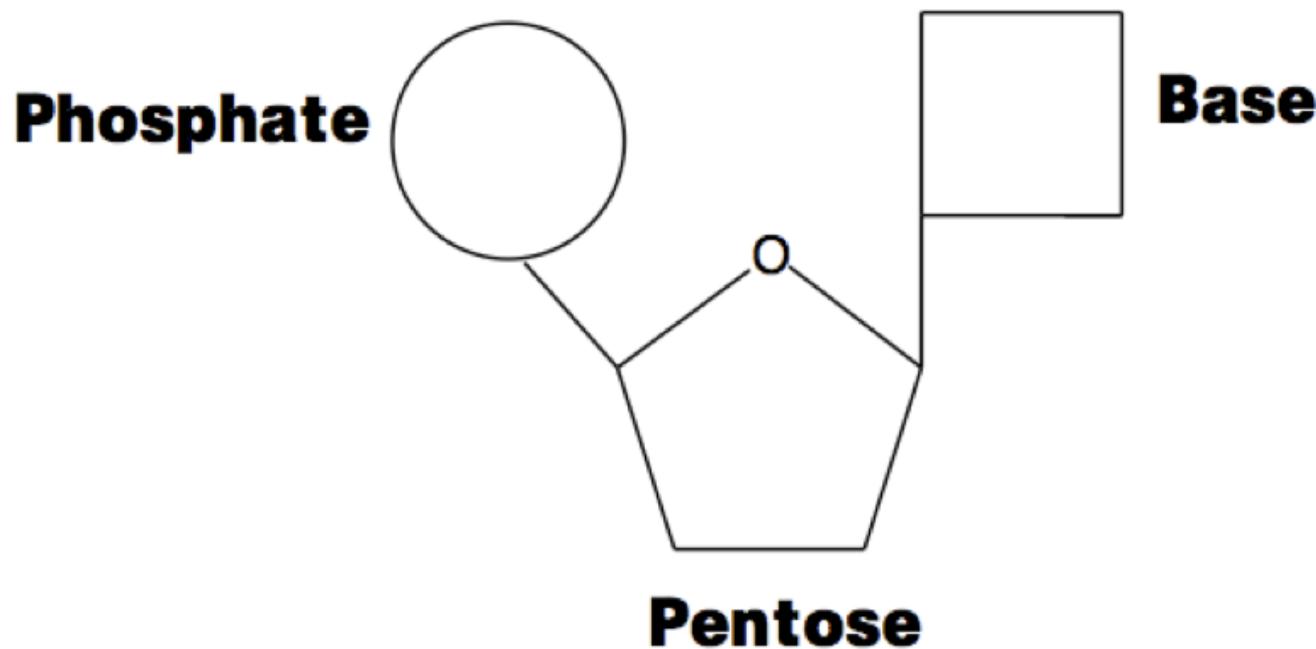


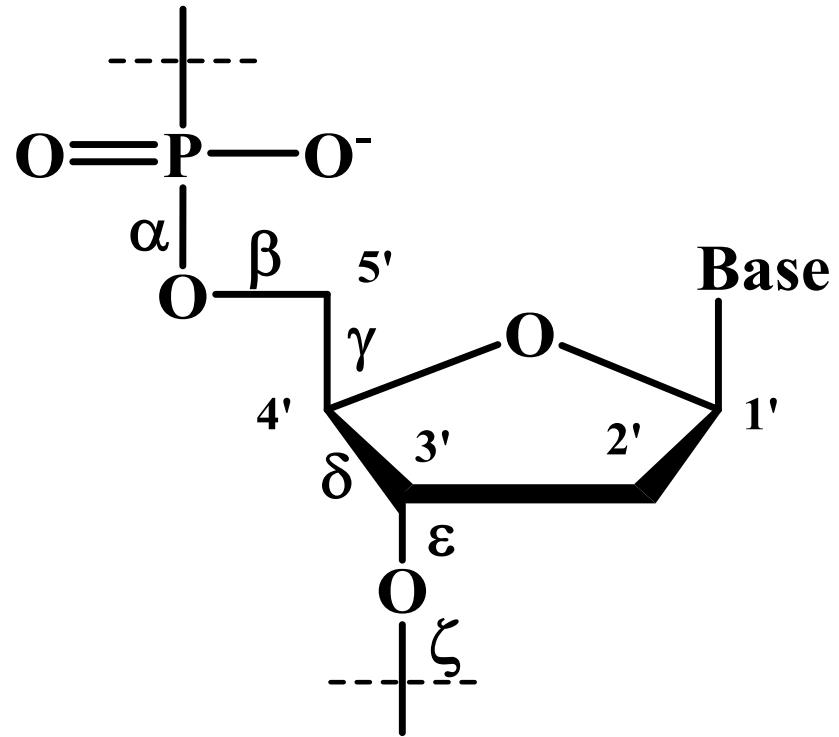
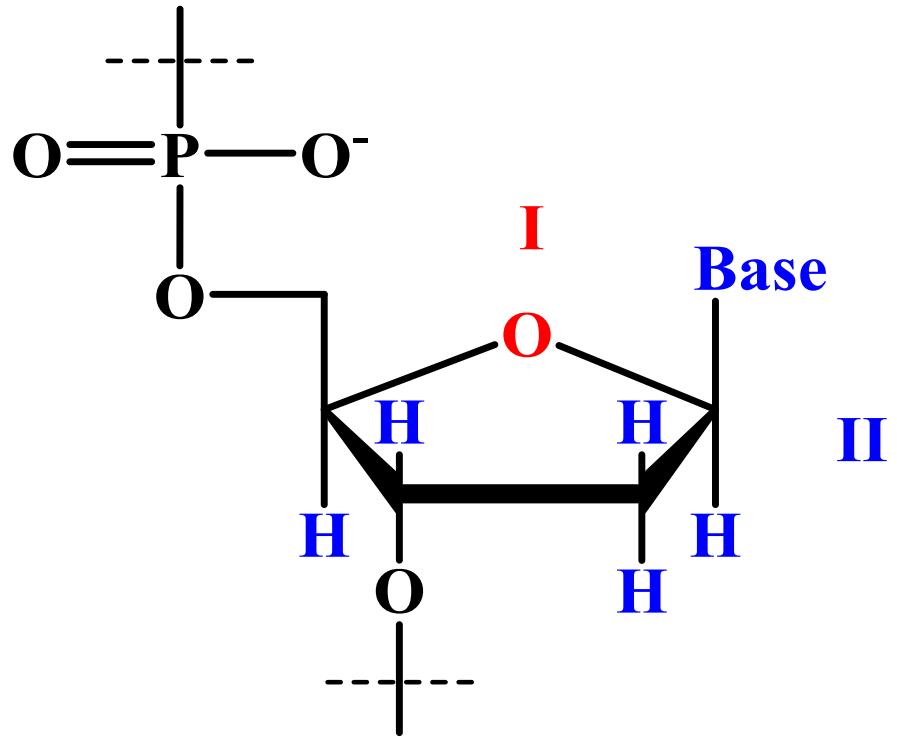
### Yearly Growth of Structures Solved By Electron Microscopy

number of structures can be viewed by hovering mouse over the bar

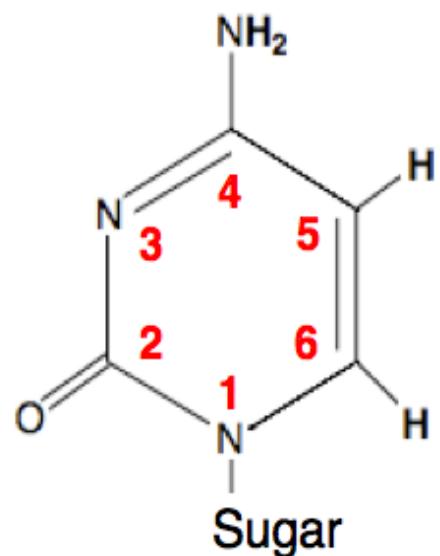


# Nucleic Acids are Polymers of Nucleotides

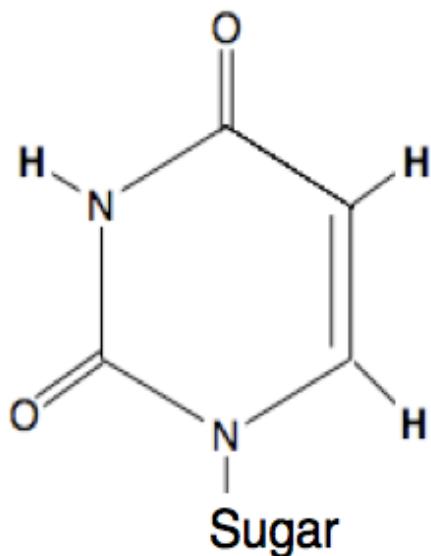




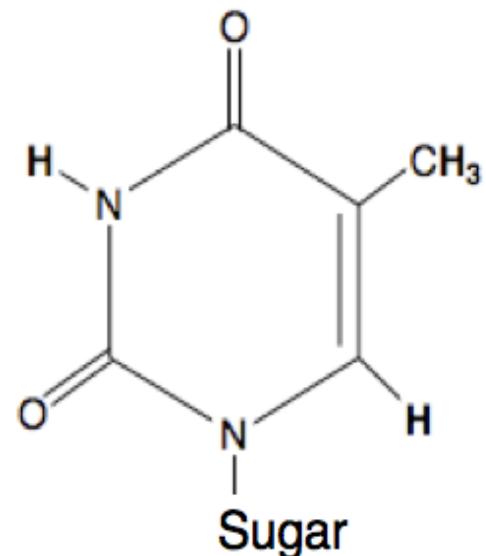
# Common Pyrimidine Bases



**Cytosine**



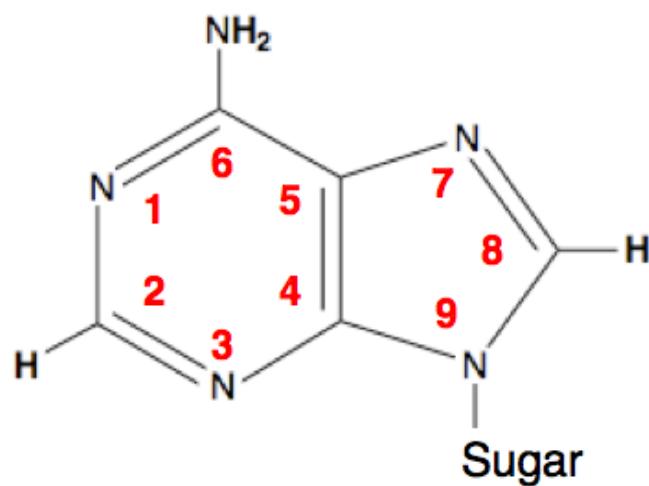
**Uracil  
(RNA)**



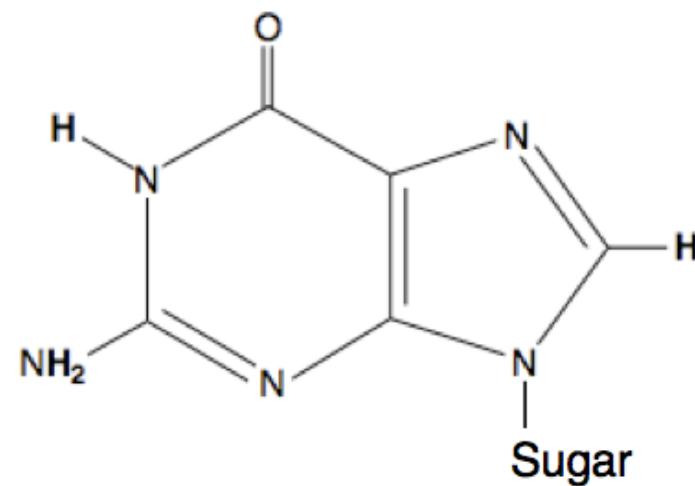
**Thymine  
(DNA)**

Numbering

# Common Purine Bases



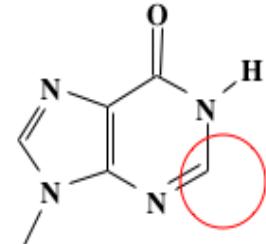
**Adenine**



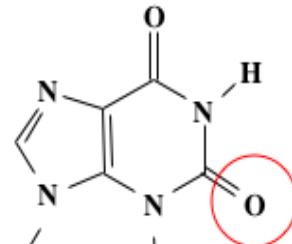
**Guanine**

Numbering

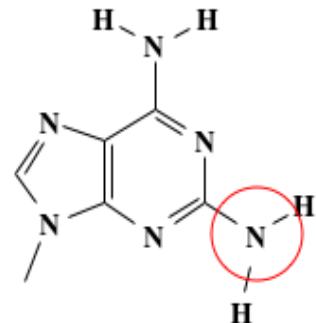
# Alternate Bases & Modifications (small selection):



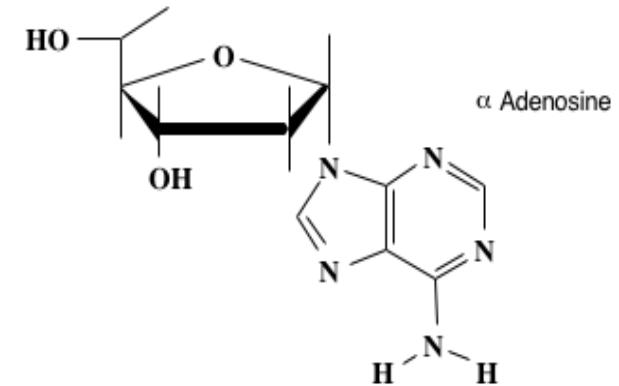
Inosine  
Base: Hypoxanthine



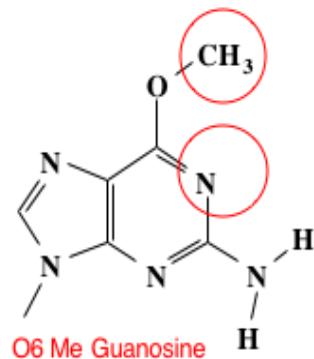
Xanthosine



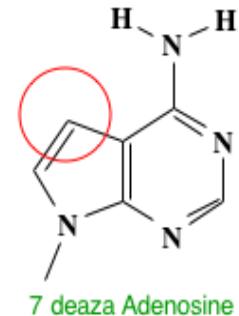
2Amino Adenosine



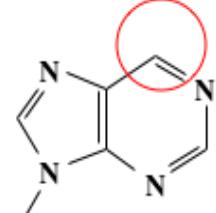
$\alpha$  Adenosine



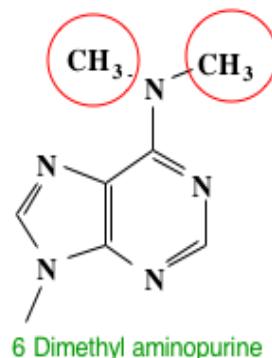
O6 Me Guanosine



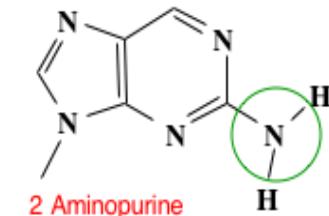
7 deaza Adenosine



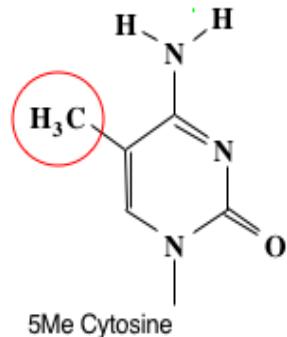
Nebularine



6 Dimethyl aminopurine

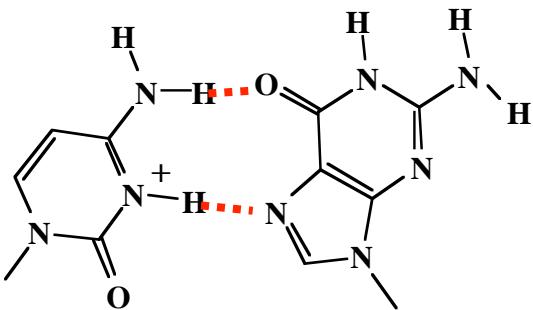
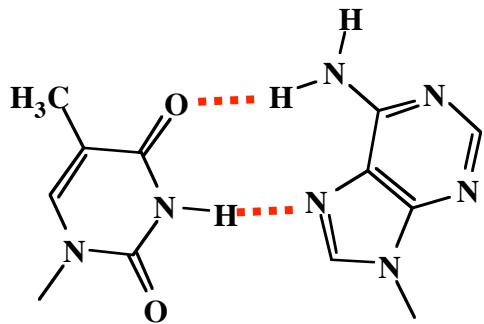


2 Aminopurine

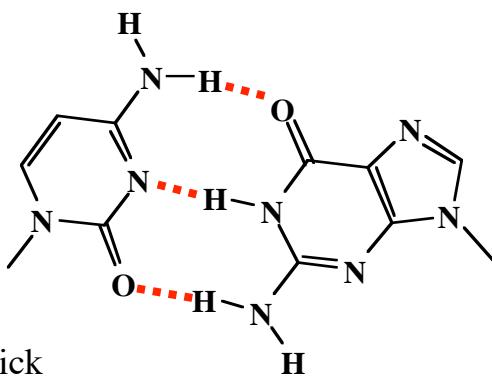
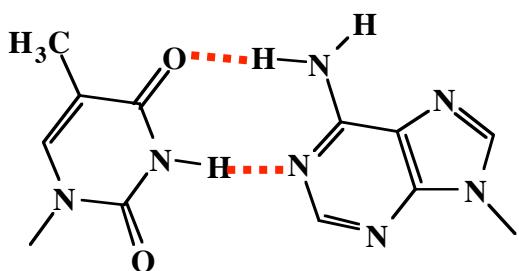


5Me Cytosine

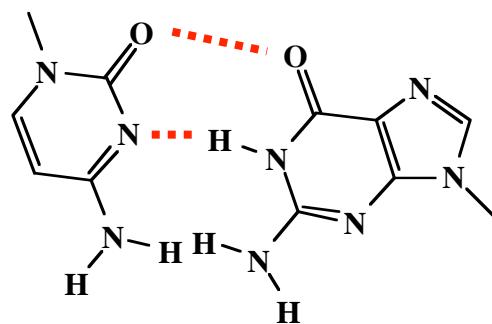
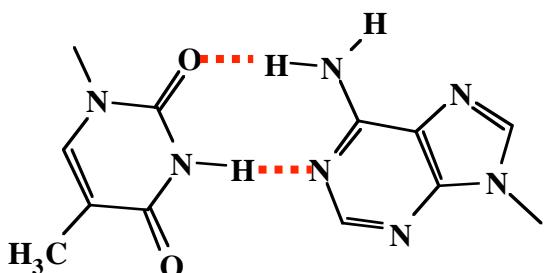
## HETERO BASE PAIRS



Hoogsteen



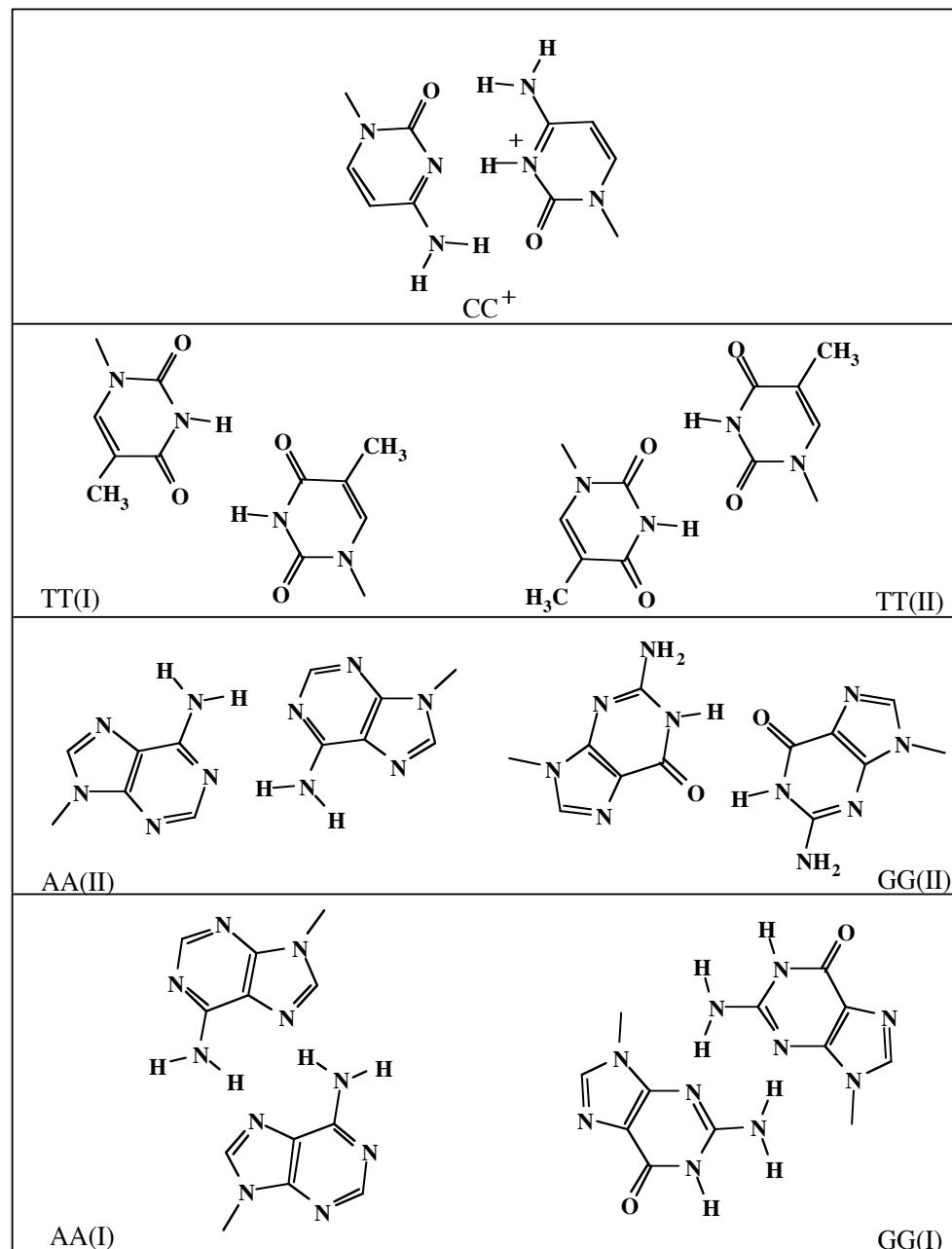
Watson-Crick



Reverse Watson-Crick

Germann et al., Methods in Enzymology (1995), 261, 207-225.  
Nucleic acids: structures, properties, and functions (2000) By Victor A. Bloomfield, Donald M. Crothers, Ignacio Tinoco

## HOMO BASE PAIRS



Germann et al., Methods in Enzymology (1995), 261, 207-225.  
 Nucleic acids: structures, properties, and functions (2000) By Victor A. Bloomfield, Donald M. Crothers, Ignacio Tinoco

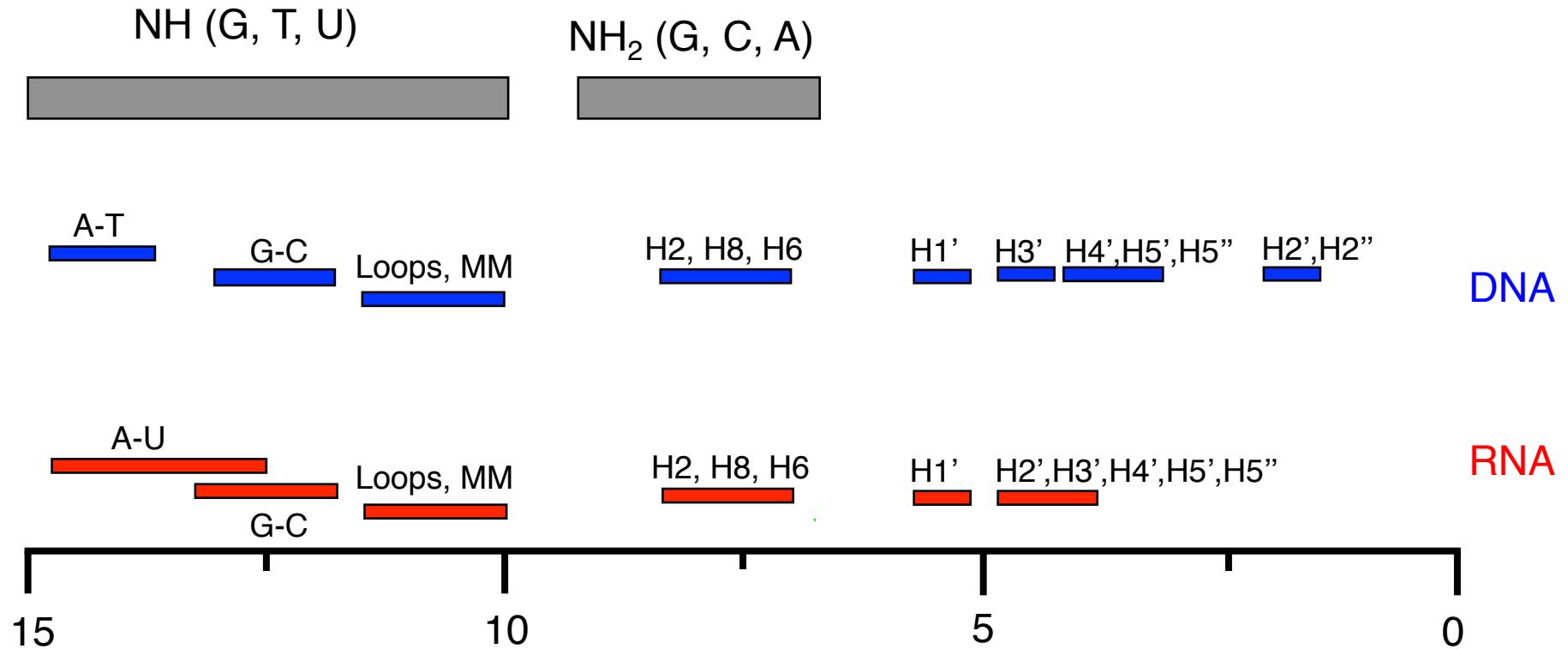
# Structure Determination:

- 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  $\alpha$  and  $\zeta$ .
- Dipolar couplings

# Chemical shift ranges in nucleic acids



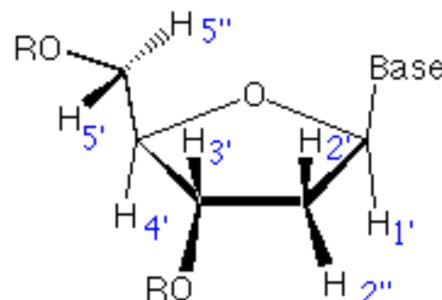
# DNA

H1'	5-6
H2'	2.3-2.9(A,G) 1.7-2.3(T,C)
H2''	2.4-3.1(A,G) 2.1-2.7(T,C)
H3'	4.4-5.2
H4'	3.8-4.3
H5'	3.8-4.3
H5''	3.8-4.3

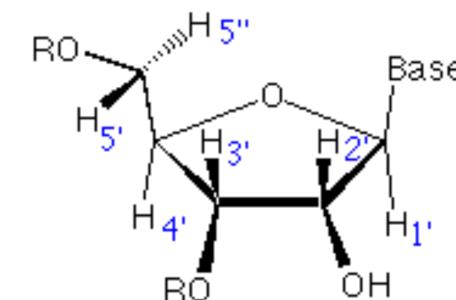
# RNA

H1'	5-6
H2'	4.4-5.0
H3'	4.4-5.2
H4'	3.8-4.3
H5'	3.8-4.3
H5''	3.8-4.3

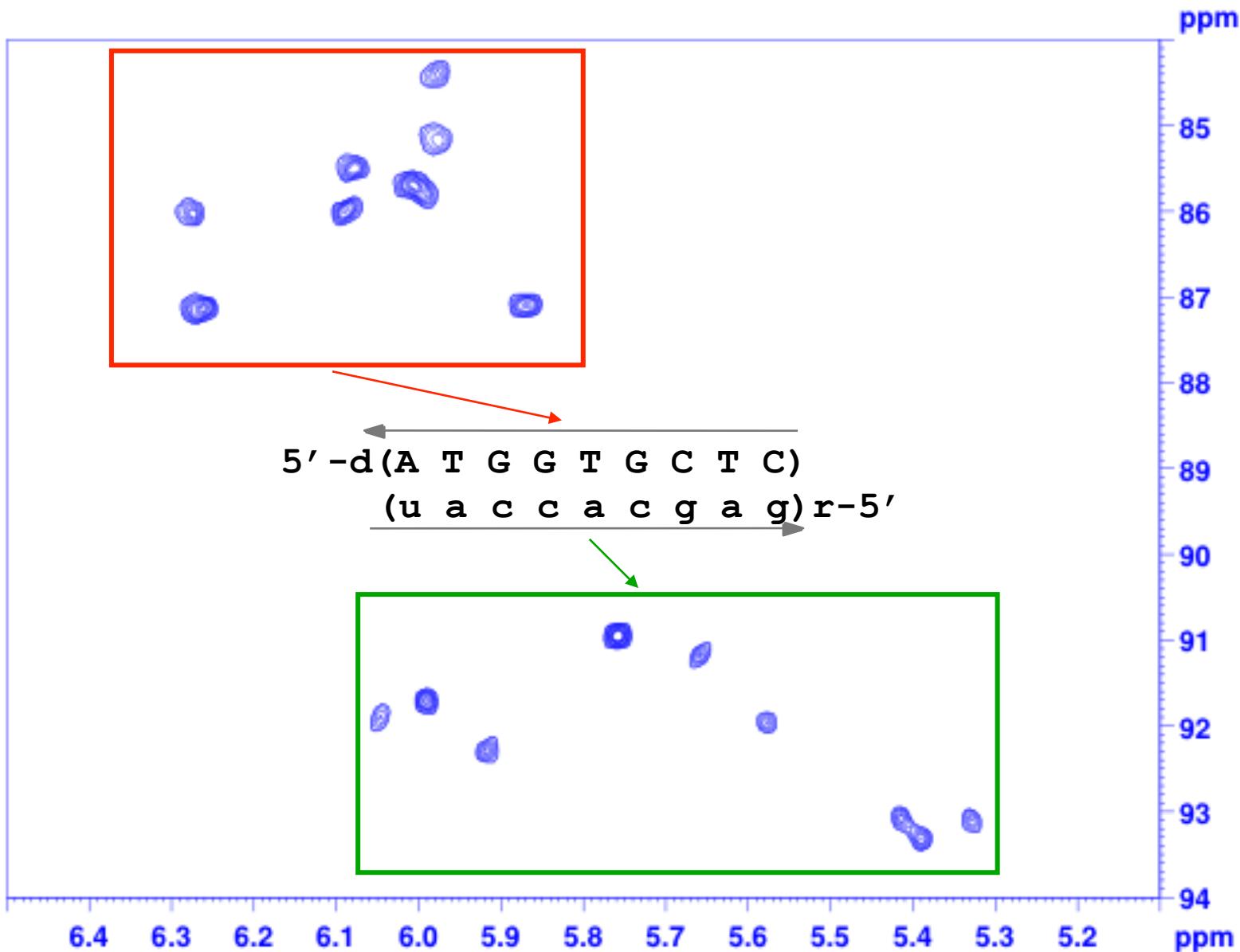
C1'	83-89
C2'	35-38
C3'	70-78
C4'	82-86
C5'	63-68



C1'	87-94
C2'	70-78
C3'	70-78
C4'	82-86
C5'	63-68



## 9R-Borano DNA•RNA H1'-C1' region



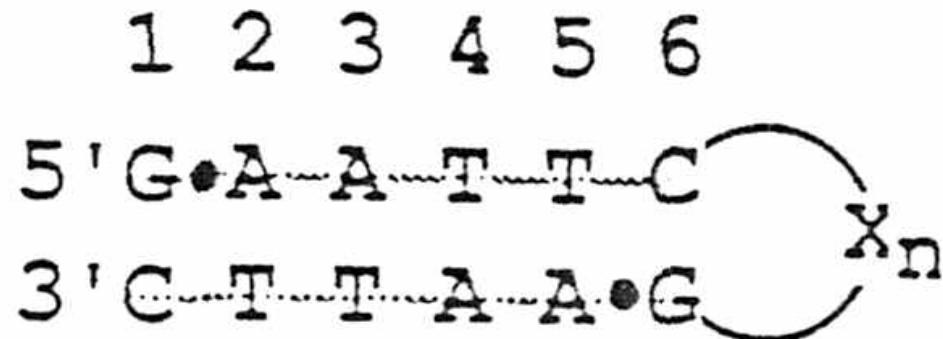
Adenine				Guanine			
H2	7.5-8	C2	152-156	-	-	C2	156
H8	7.7-8.5	C8	137-142	H8	7.5-8.3	C8	131-138
N6H	5-6/7-8	N6	82-84	N1H	12-13.6	N1	146-149
-	-	C4	149-151	N2H	5-6/8-9	N2	72-76
		C5	119-121			C4	152-154
		C6	157-158			C5	117-119
		N1	214-216			C6	161
		N3	220-226			N1	146-149
		N7	224-232			N3	167
		N9	166-172			N7	228-238
						N9	166-172

Thymidine				Uridine				Cytidine			
H6	6.9-7.9	C6	137-142	H6	6.9-7.9	C6	137-142	H6	6.9-7.9	C6	136-144
Me5	1.0-1.9	Me5	15-20	H5	5.0-6.0	C5	102-107	H5	5.0-6.0	C5	94-99
N3H	13-14	N3	156	N3H	13-14	N3	156-162	-	-	N3	210
-	-	-	-	-	-	-	-	N4H	6.7-7/81-8.8	N4	94-98
		C2	154			C2	154			C2	159
		C4	169			C4	169			C4	166-168
		C5	95-112			C5	102-107			C5	94-99
		N1	144			N1	142-146			N1	150-156

# No Structure Required!

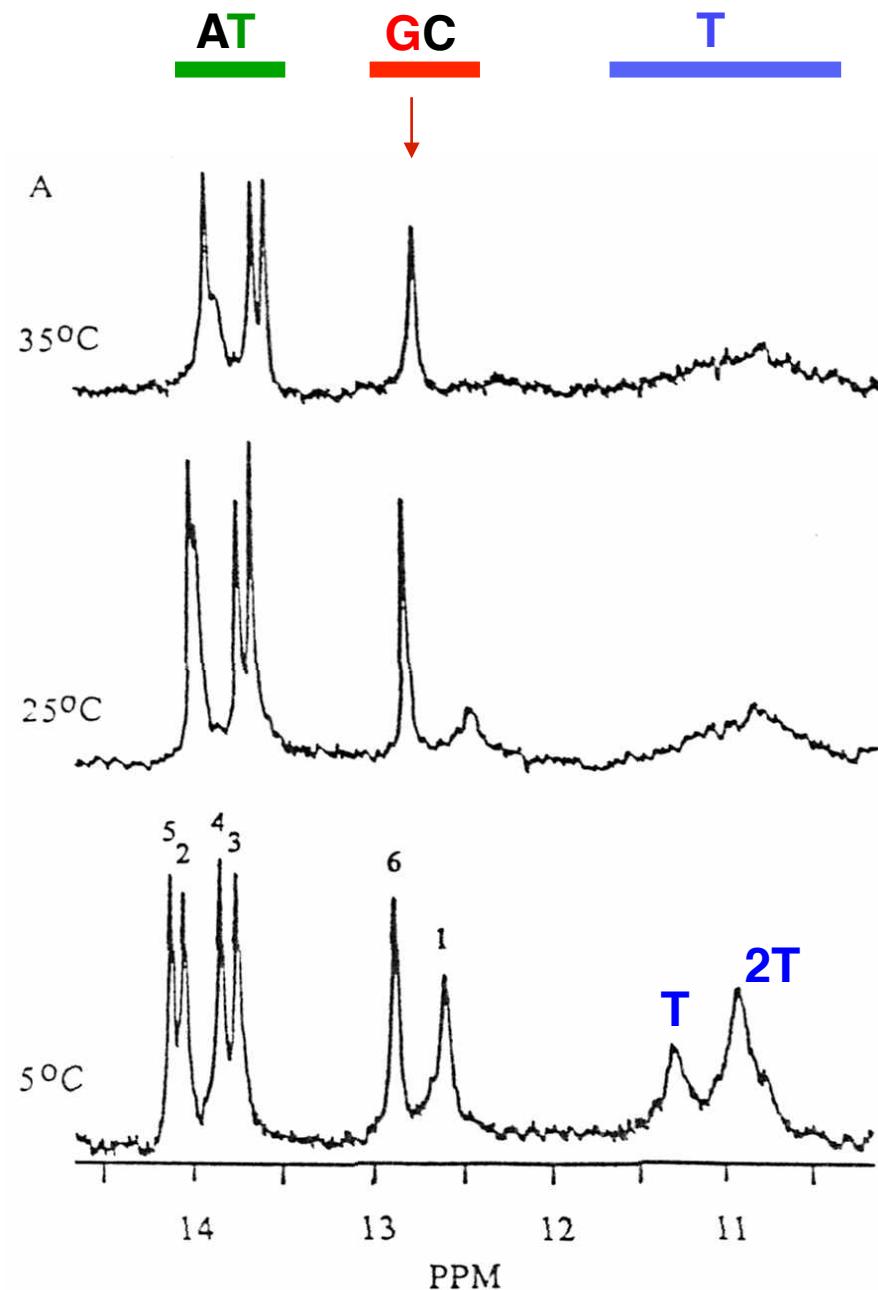
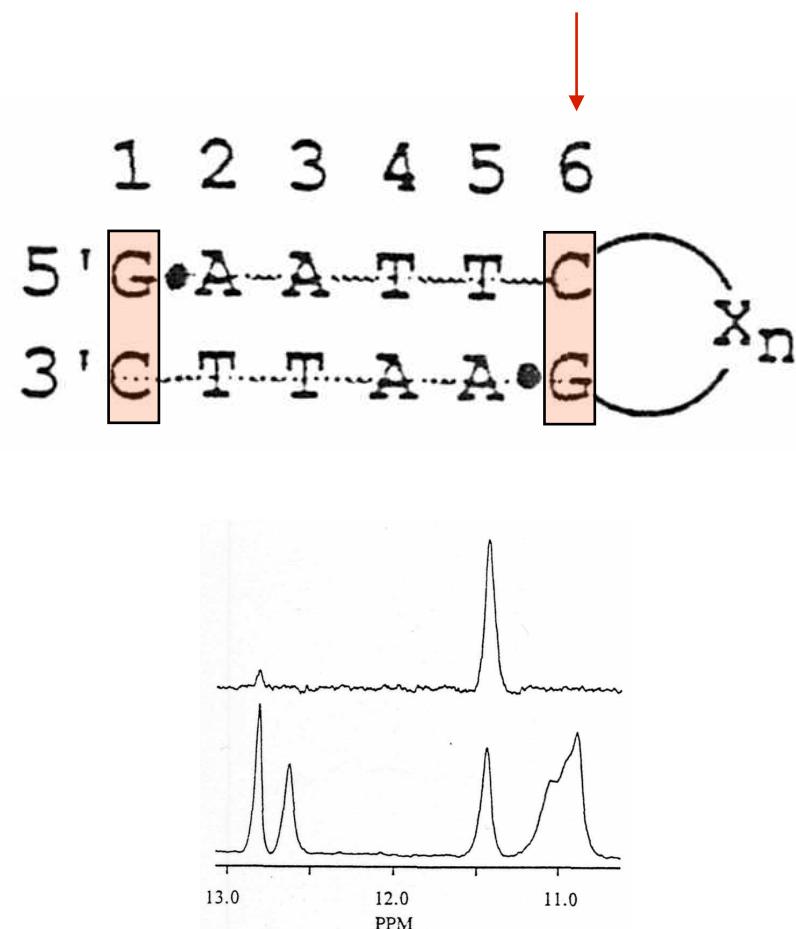
Often, depending on the question asked, a full structure determination is not required

- Does it form a duplex?
- Which base pairs are thermo labile?
- Which base pair is which... assignment?
- Is the loop structured?
- Structure



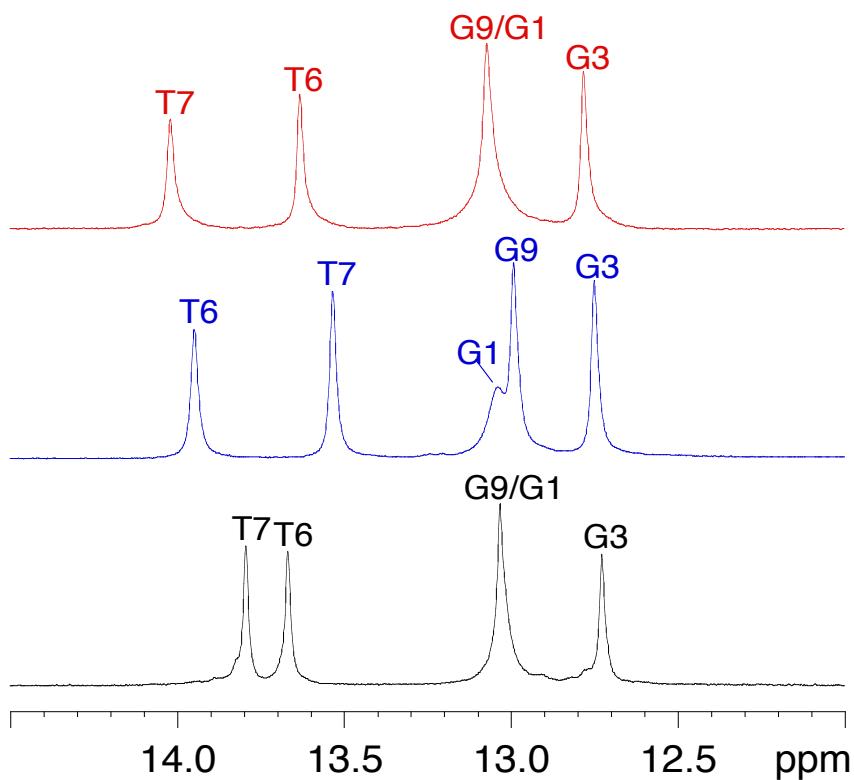
# DNA Hairpin

Thermal lability



# “New” DNA constructs

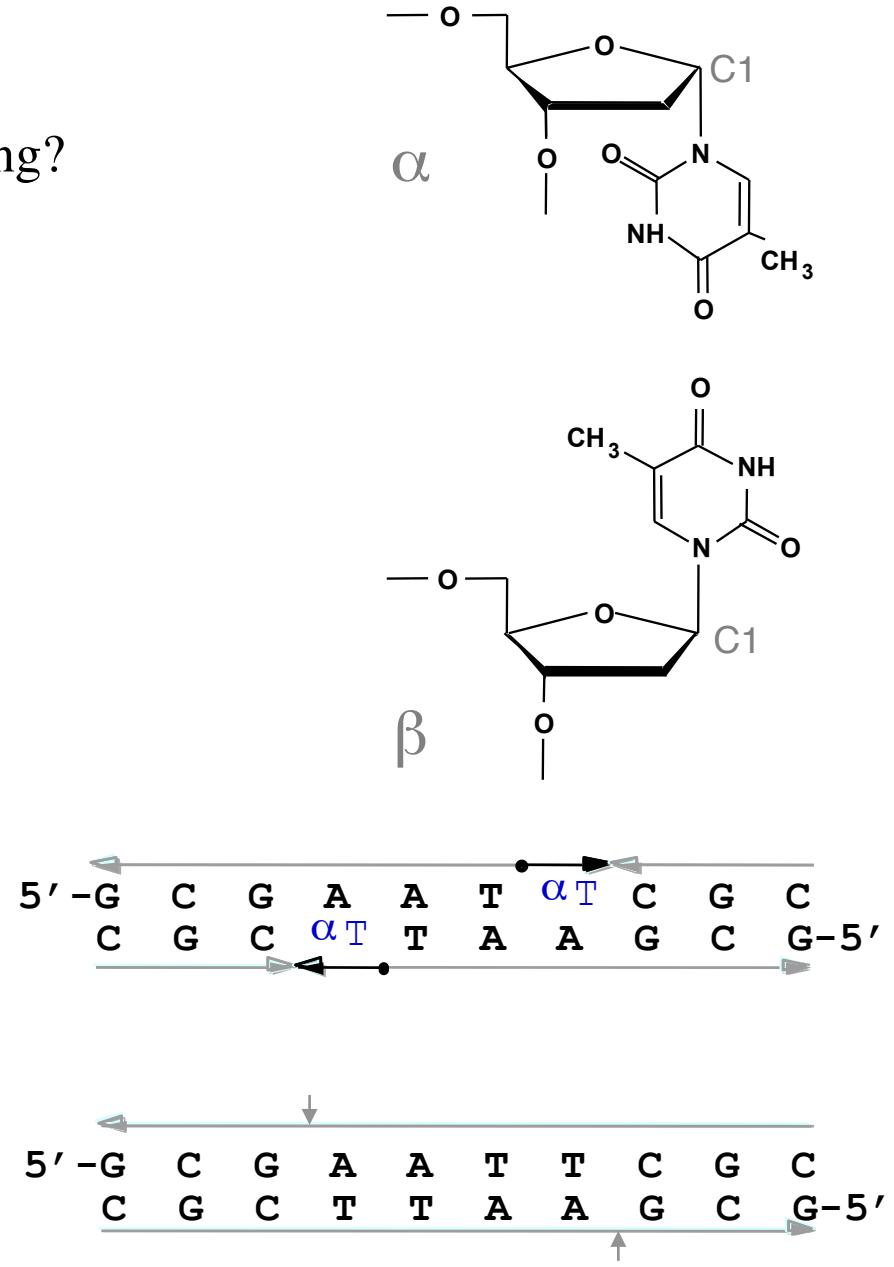
- Do the duplexes form, is there base pairing?
- Does the unusual base pair form?



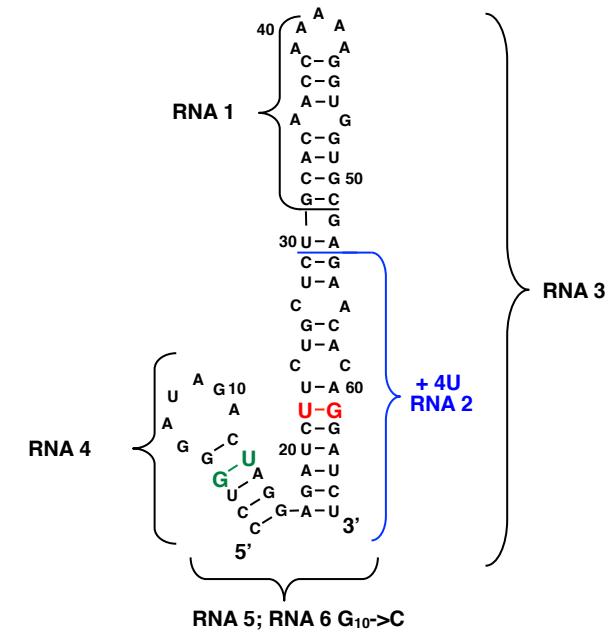
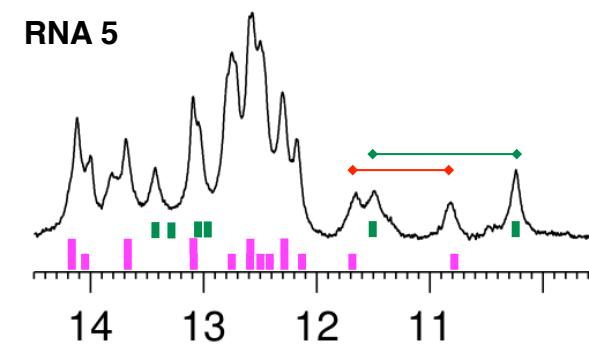
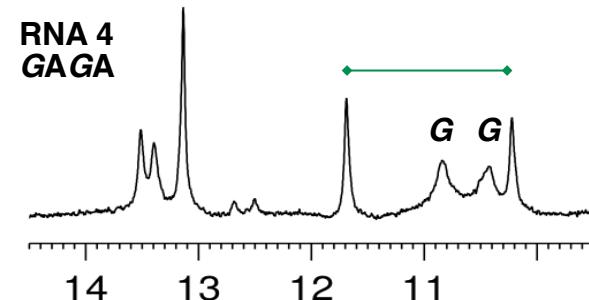
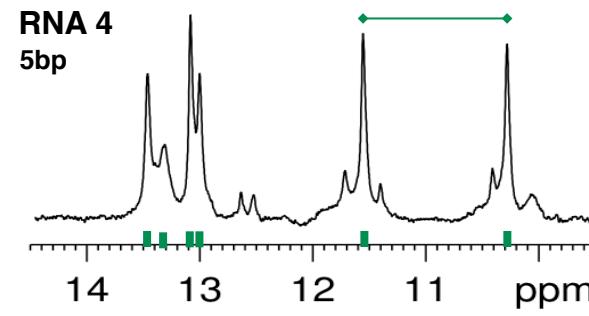
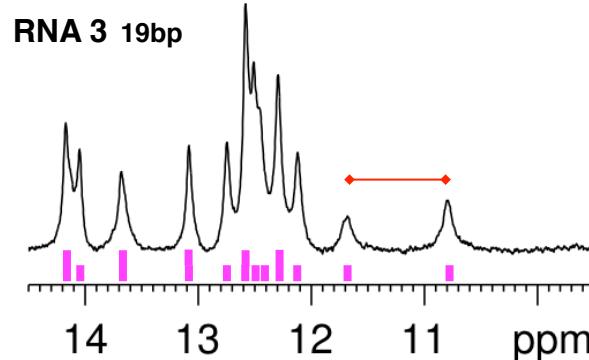
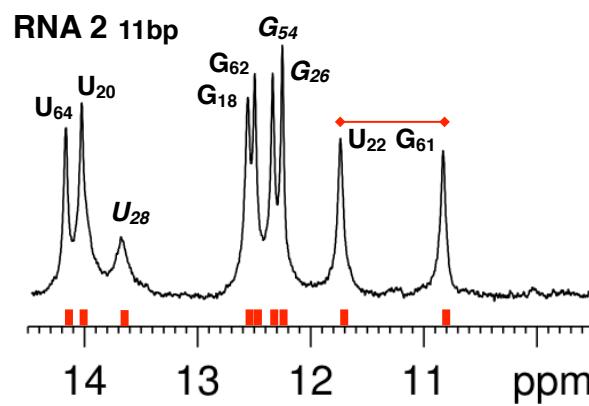
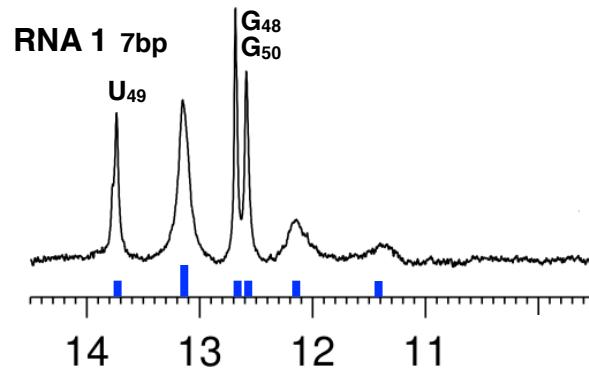
alpha C

alpha T

control



# WNV-RNA



**RNA 5 >23'000 g/mol**

# Fibrinogen Specific DNA Aptamer

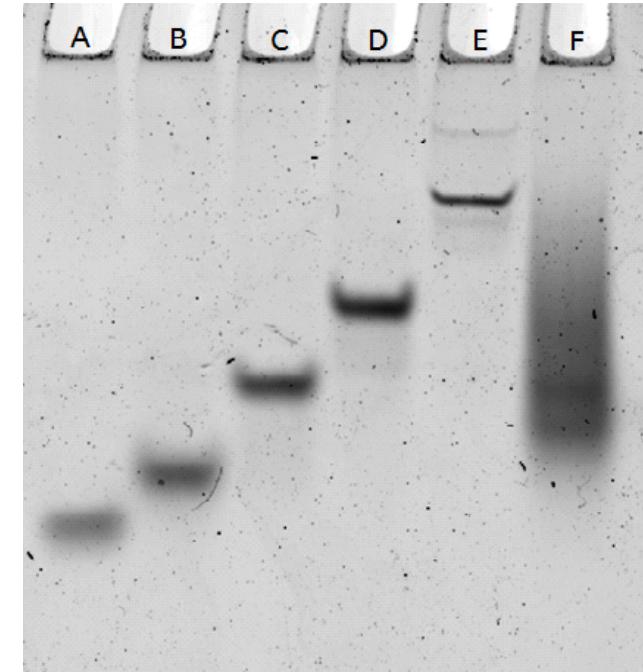
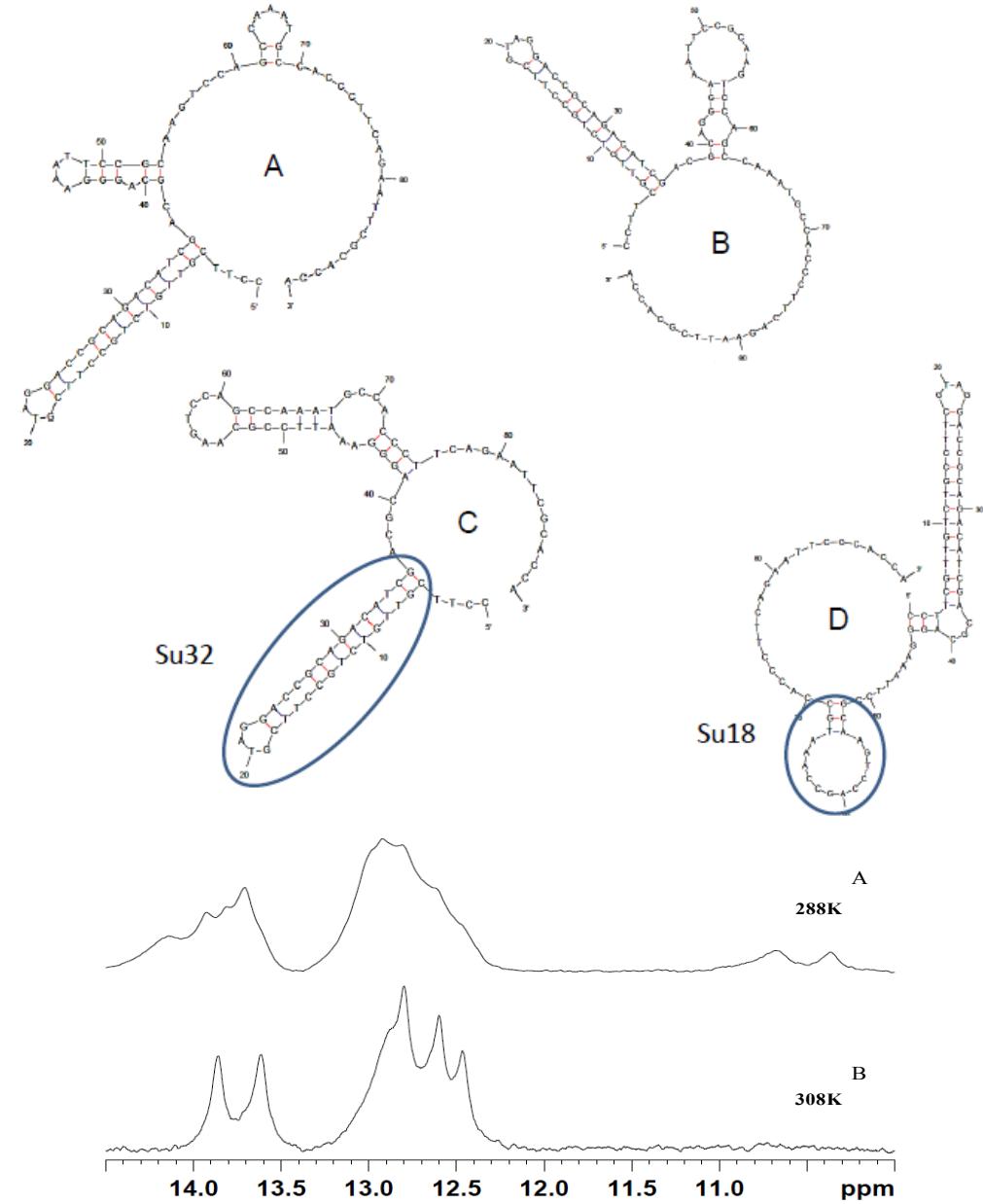


Figure 2: 12% Native PAGE to observe mobilities of Ap90. Ap90 is compared to single stranded oligomers of various lengths. The lanes were loaded from the smallest to the largest sequences with Lane A-E containing the 10-mer, 18-mer, 30-mer, 50-mer, and the 90-mer respectively. Lane F contains the aptamer Ap90. The smear in lane F encompassing a large range of DNA sizes (~90 nucleotides - ~30 nucleotides) indicates that the aptamer has multiple conformations.

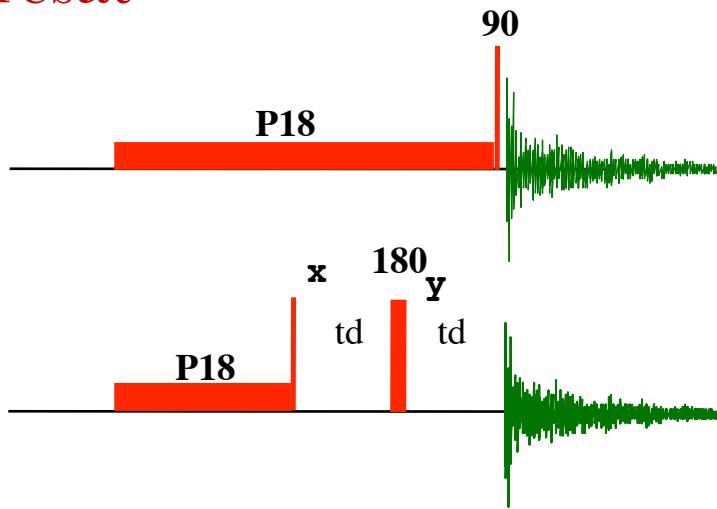
# Solvent Suppression

The presence of an intense solvent resonance necessitates an impractical high dynamic range. **110 M vs <1mM (down to 5-10 uM)**

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 field gradients 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 gradients to selectively dephase the solvent resonance (WATERGATE)
- 5c) Excitation sculpting

## Presat

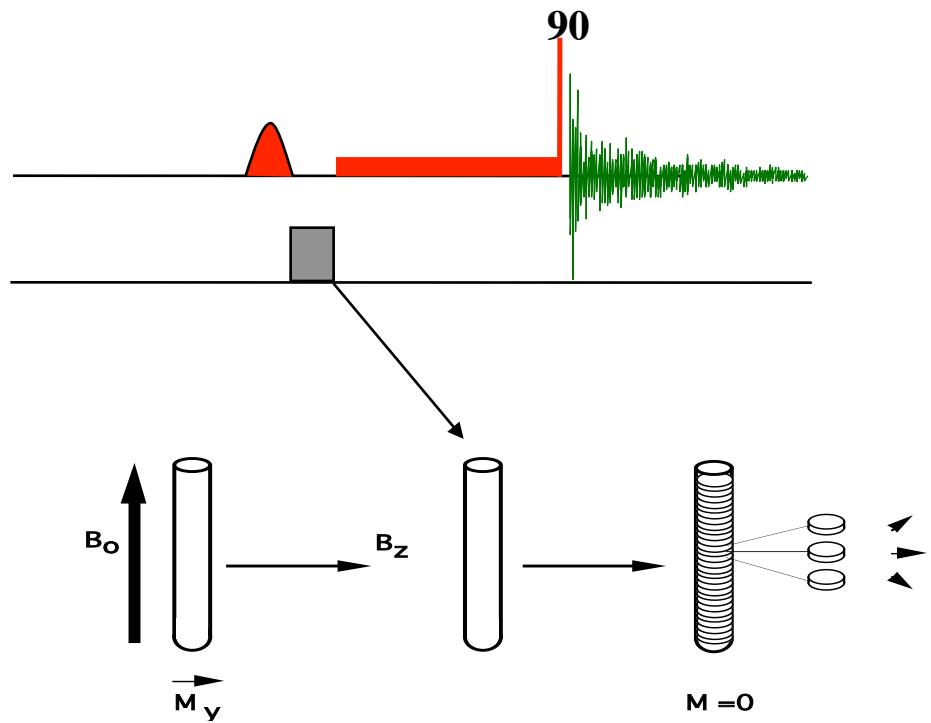


Presaturation field strength:

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)

## Selective Excitation

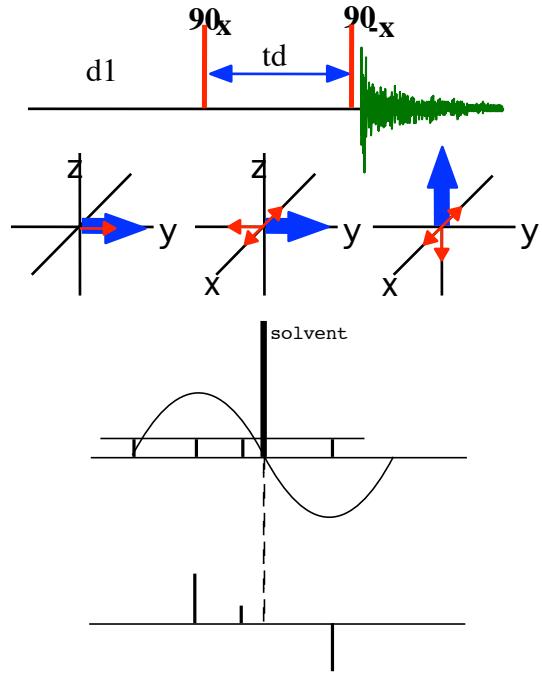


Selective rf pulse on solvent resonance followed by a gradient pulse to dephase the water signal.

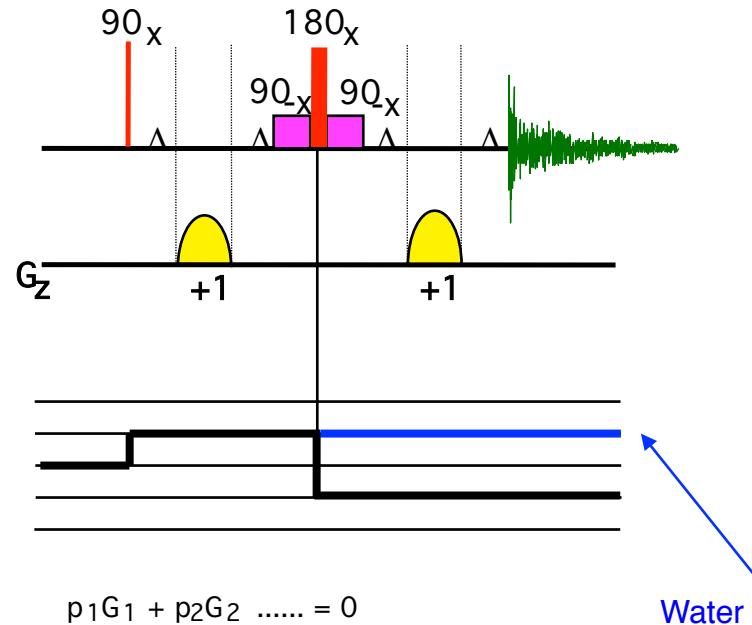
This could be followed by a mild presaturation field. The selective rf pulse (1-2ms, depending on width to be zeroed) is usually of the gauss type.

The selective rf pulse z-gradient constructs could be repeated (WET).

## Jump and return



## Watergate



Pros:

- Easy to set up
- Excellent water suppression  
(with proper setup as good as presat)
- Good for broad signals!**  $t_d$  is **VERY short!!**

Cons:

- Non uniform excitation Baseline not flat

Other sequences: 1331 etc

Pros:

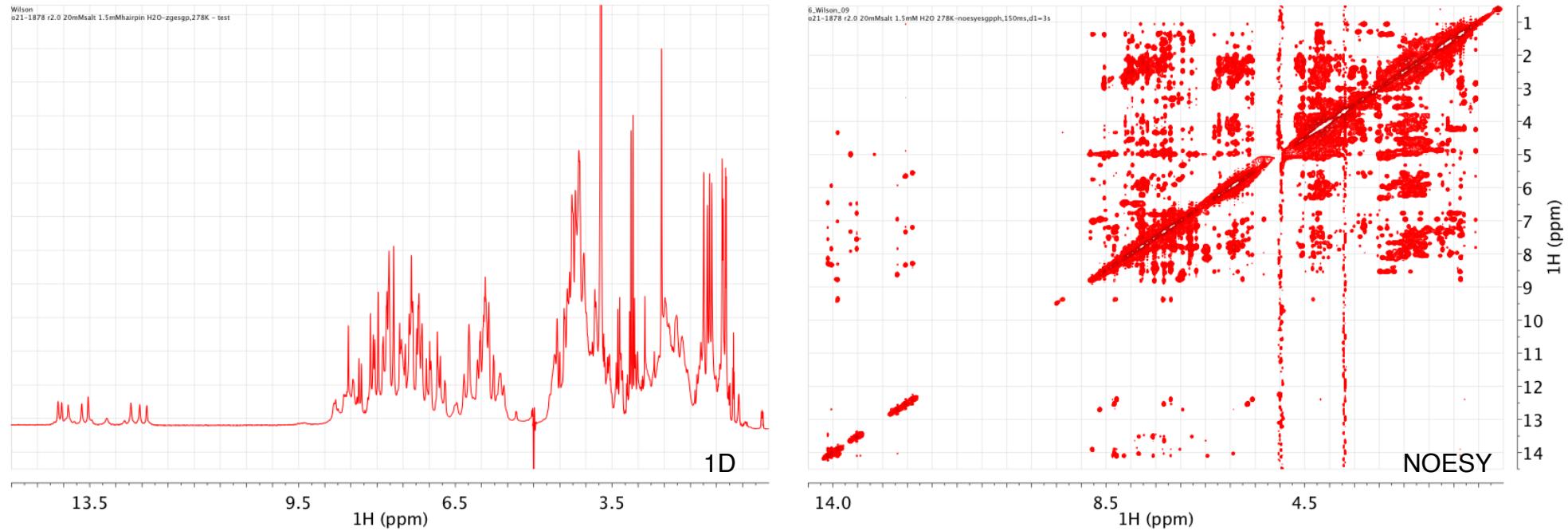
- Excellent water suppression
- Uniform excitation
- Baseline flat

Cons:

- May loose broad resonances

# Excitation Sculpting

T.-L. Hwang & A.J. Shaka, J. Mag. Res. (1995), 112 275–279



Pros:

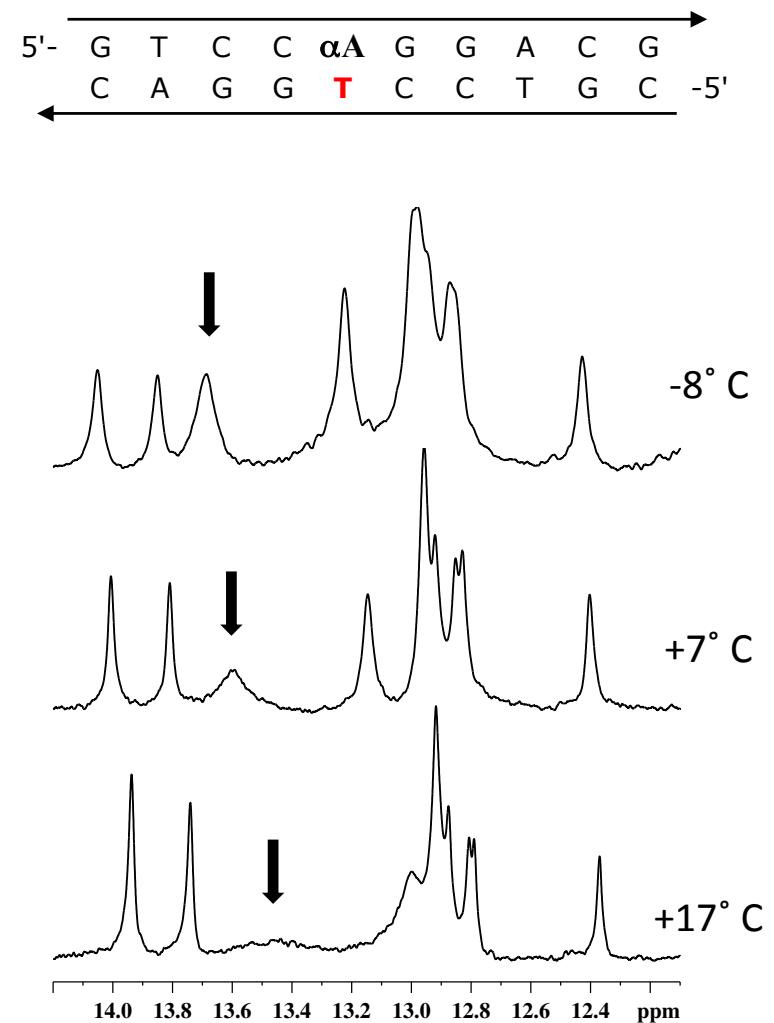
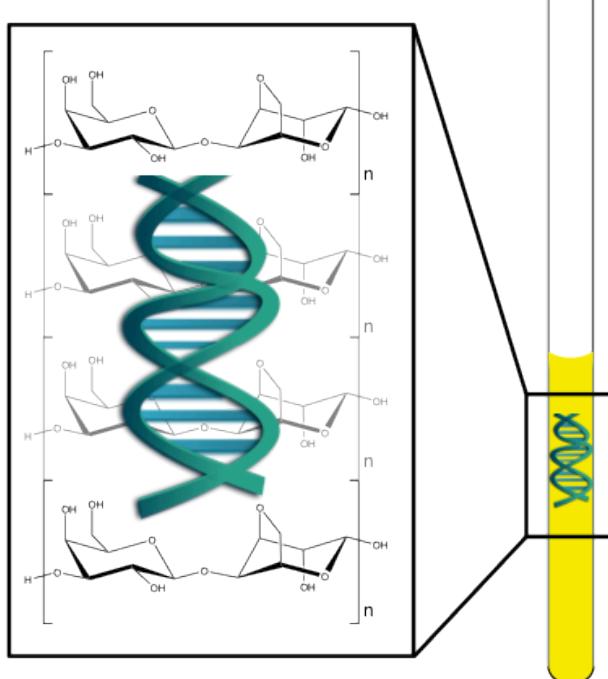
- Easy to set up
- Excellent water suppression
- “ok” for broad signals!
- Uniform excitation

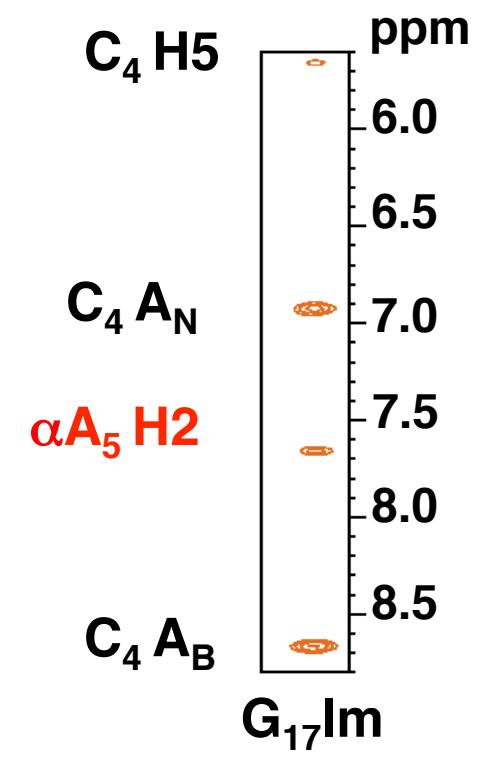
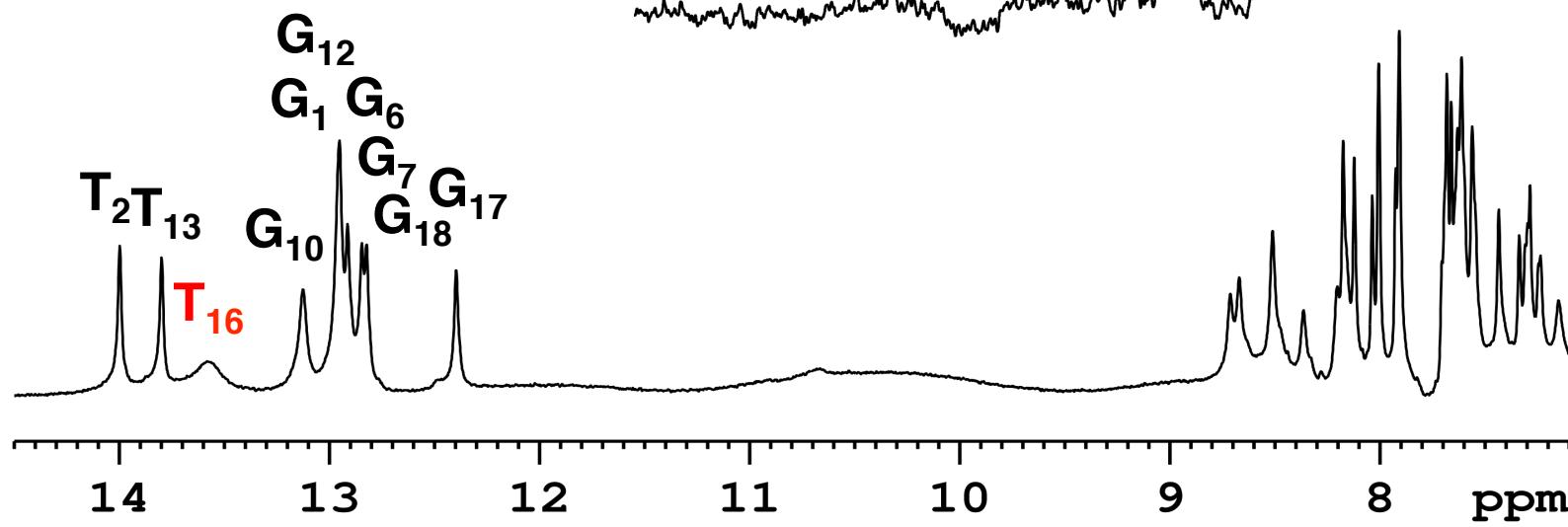
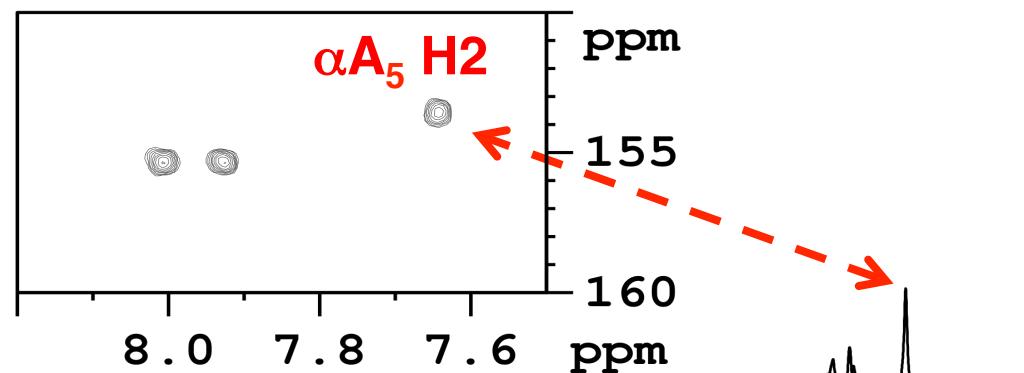
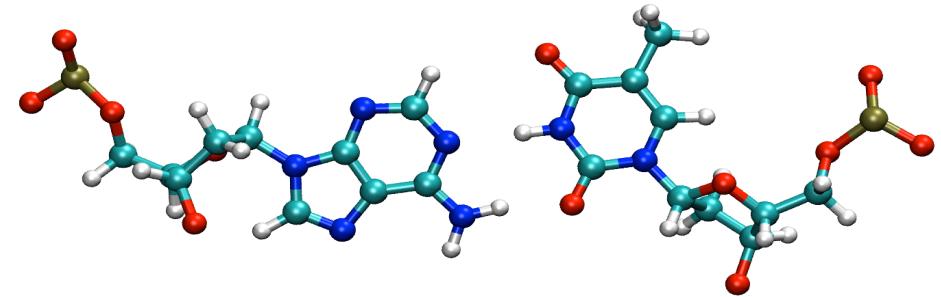
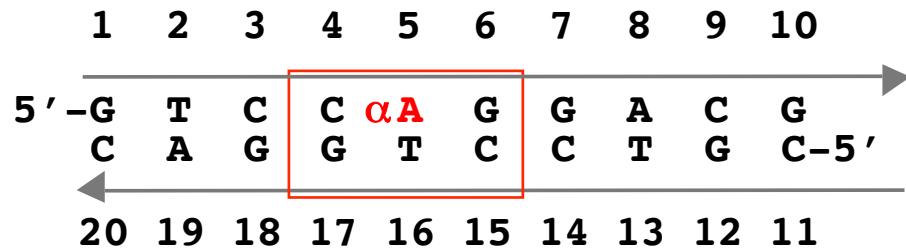
Cons:

- May lose some intensity on very broad signals

Interesting structures have often broad imino protons.  
→ Most modern techniques obliterate them.

Jump and return to the rescue  
+ supercooled conditions





# Structure Determination, NMR experiments:

## I) Assignment

NOESY, COSY, HSQC  
TOCSY.....

## II) Local Analysis

- glycosidic torsion angle
- sugar puckering
- backbone conformation
- base pairing

(NOE, COSY)  
(COSY, COSY, NOE, +)  
(COSY, CT NOESY +)  
(NOE, COSY)

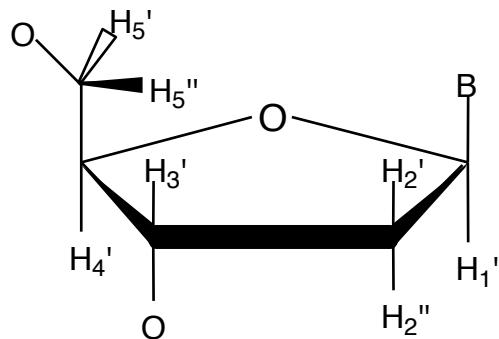
## III) Global Analysis

- sequential
- inter strand/cross strand
- dipolar coupling

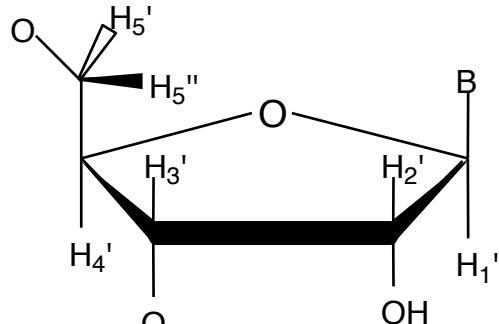
(NOE, HPCOR)  
(NOE, COSY)  
(HSQC, HSQC)

Black: unlabeled, Blue: labeled DNA or RNA

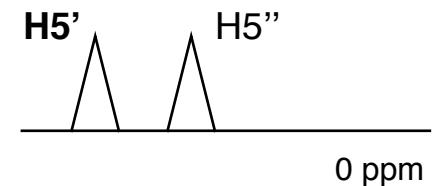
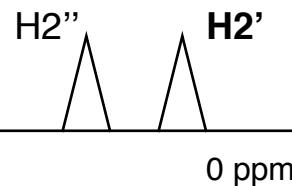
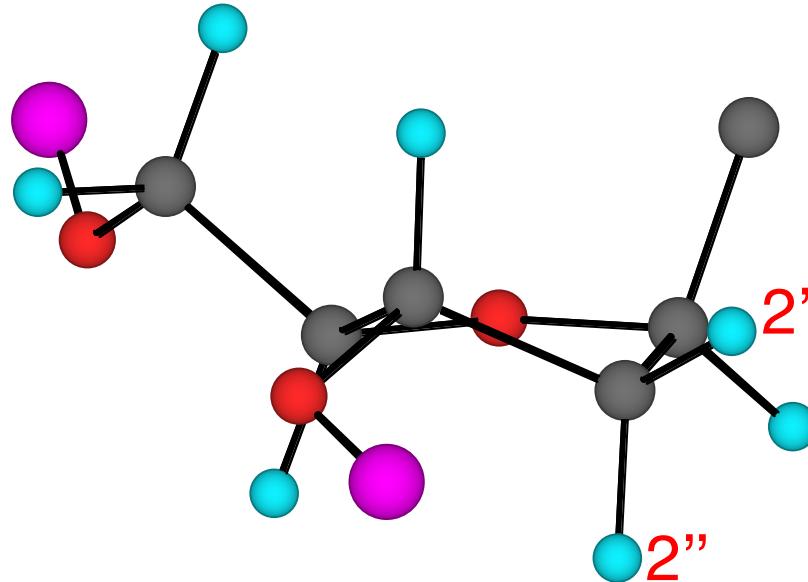
# Stereospecific Assignment



Deoxyribose



Ribose



How do we determine them?

a) Rule of Thumb (5' downfield of 5'')

Shugar and Remin BBRC (1972), 48, 636-642

b) Short mixing times NOESY

dH1'H2'' shorter than H1'H2'

-> Crosspeak H1'-H2'' > H1'H2'

# Structure Determination:

I) Assignment

II) Local Analysis

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

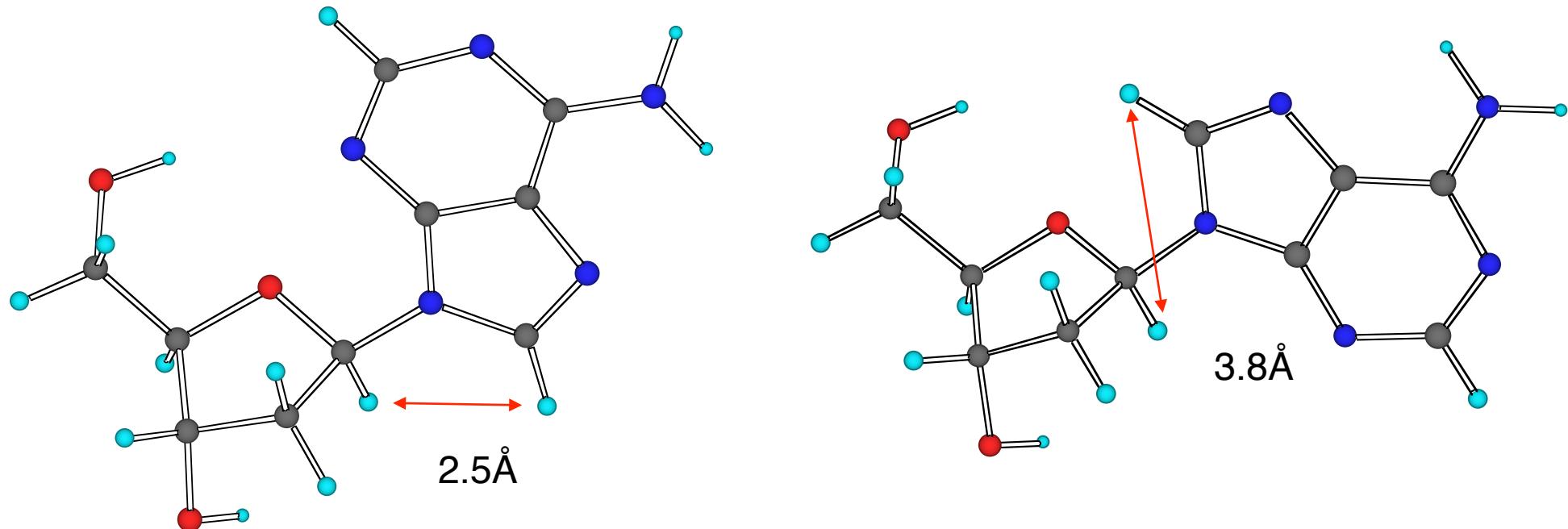
III) Global Analysis

- sequential, inter strand/cross strand, dipolar coupling

Nucleic Acids have few protons.....

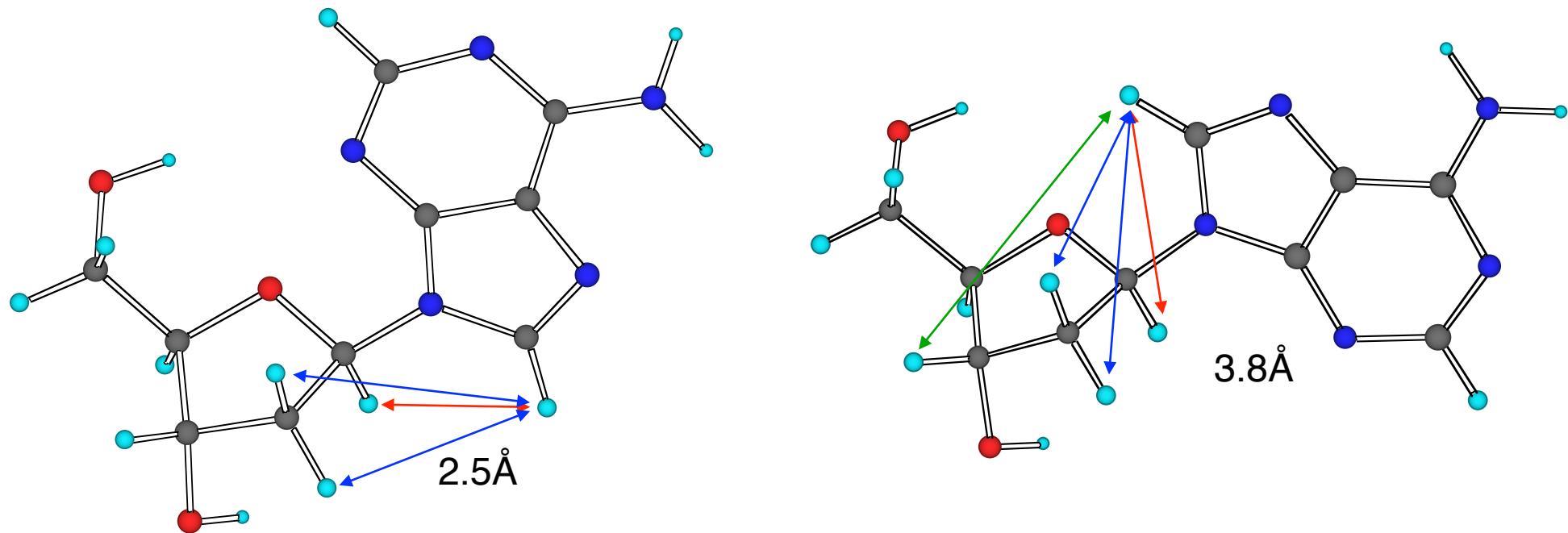
- NOE accuracy
  - > account for spin diffusion
- Backbone may be difficult to fully characterize
  - > especially  $\alpha$  and  $\zeta$ .
- Dipolar couplings

# Distance information determines the glycosidic torsion angle



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

# Distance information determines the glycosidic torsion angle



- How do we get distance information?
  - Nuclear Overhauser effect ( $< 6\text{\AA}$ )

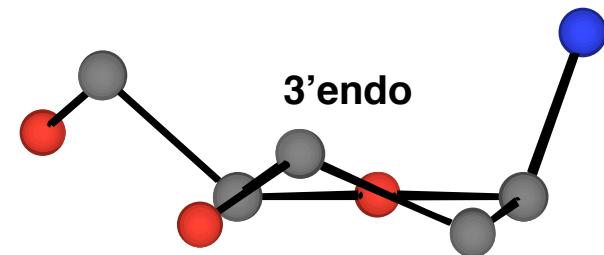
# Sugar puckering

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 ( $\Phi$ )**.

In general:

RNA (A type double helix) C3' endo.

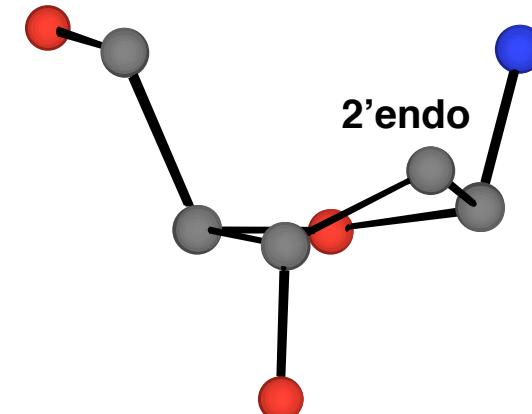
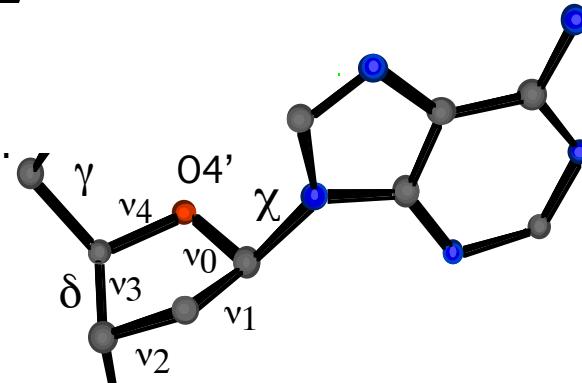
DNA (B type double helix) C2' endo.



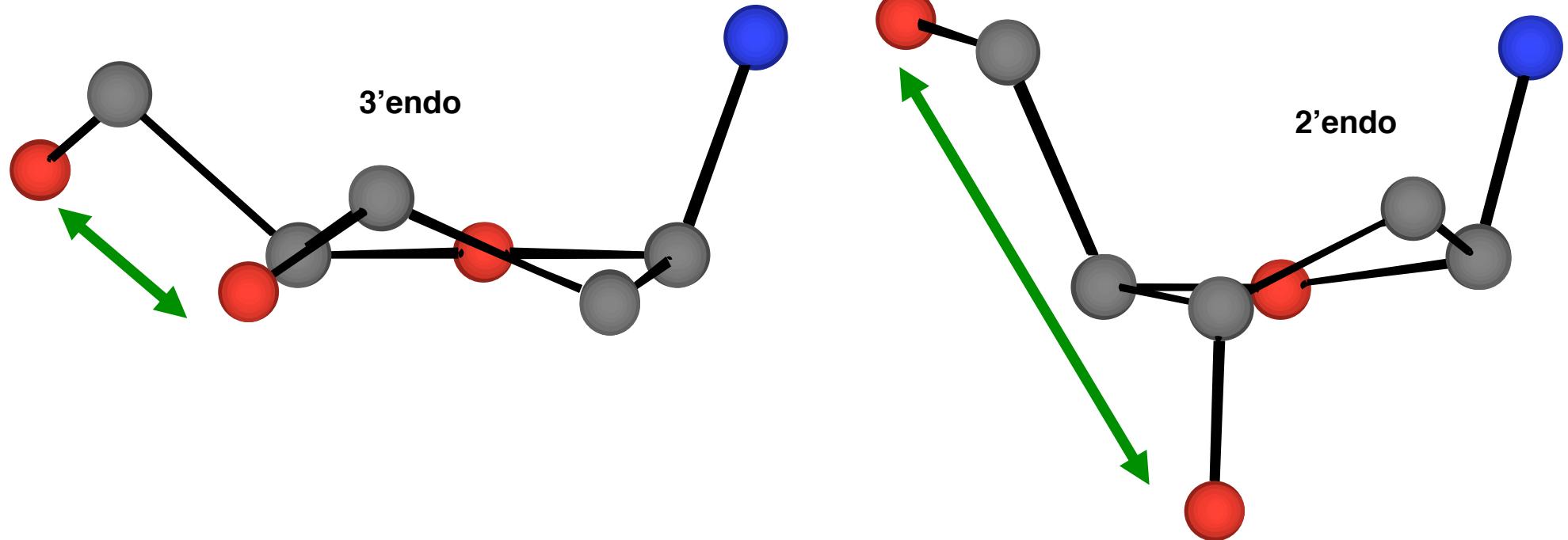
$$v_j = \Phi_m \cos (P + 144(j-2))$$

$\Phi_m$  range:  $34^\circ - 42^\circ$

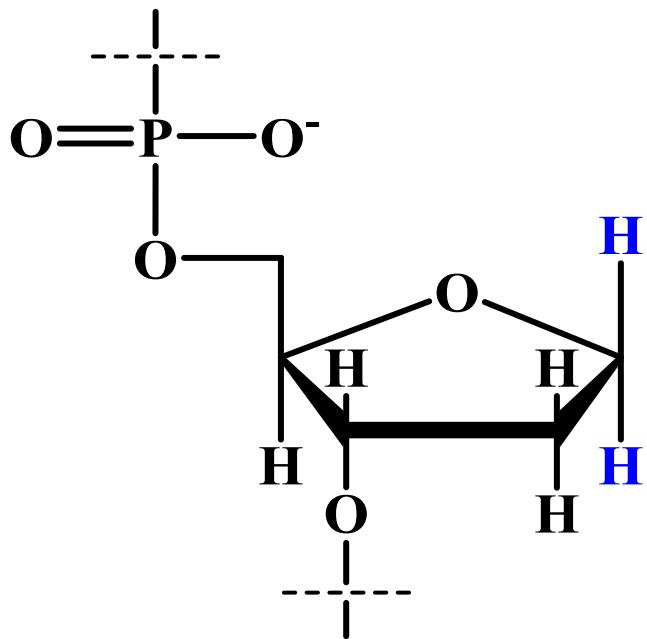
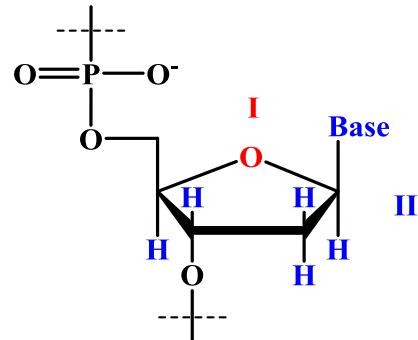
$$\delta = v_3 + 125^\circ$$



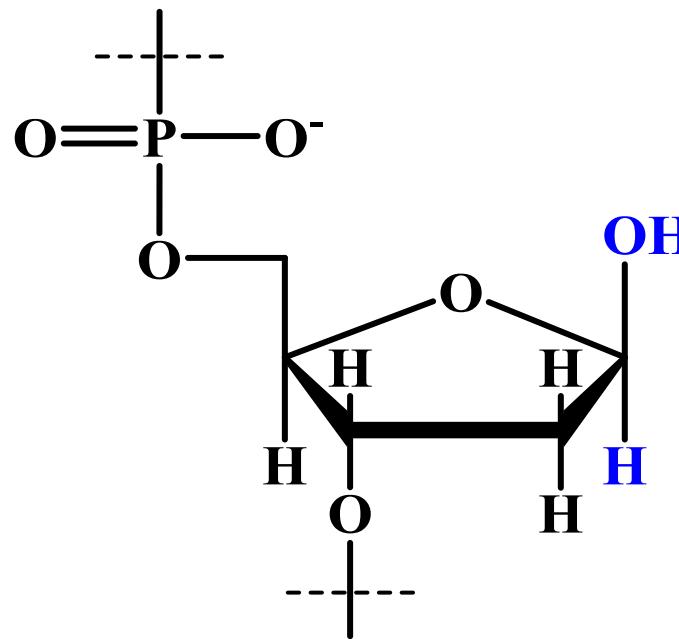
# Sugar puckering



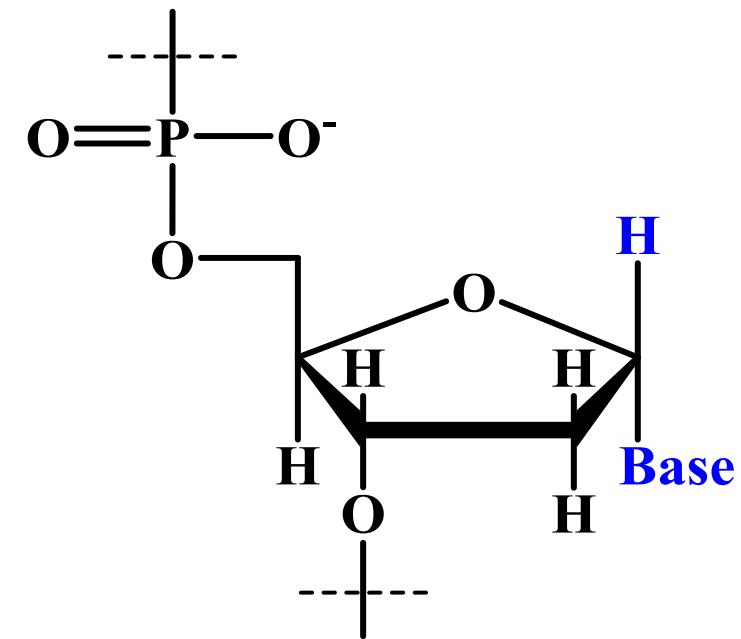
## Not just DNA and RNA



Tetrahydrofuran (THF) analogue

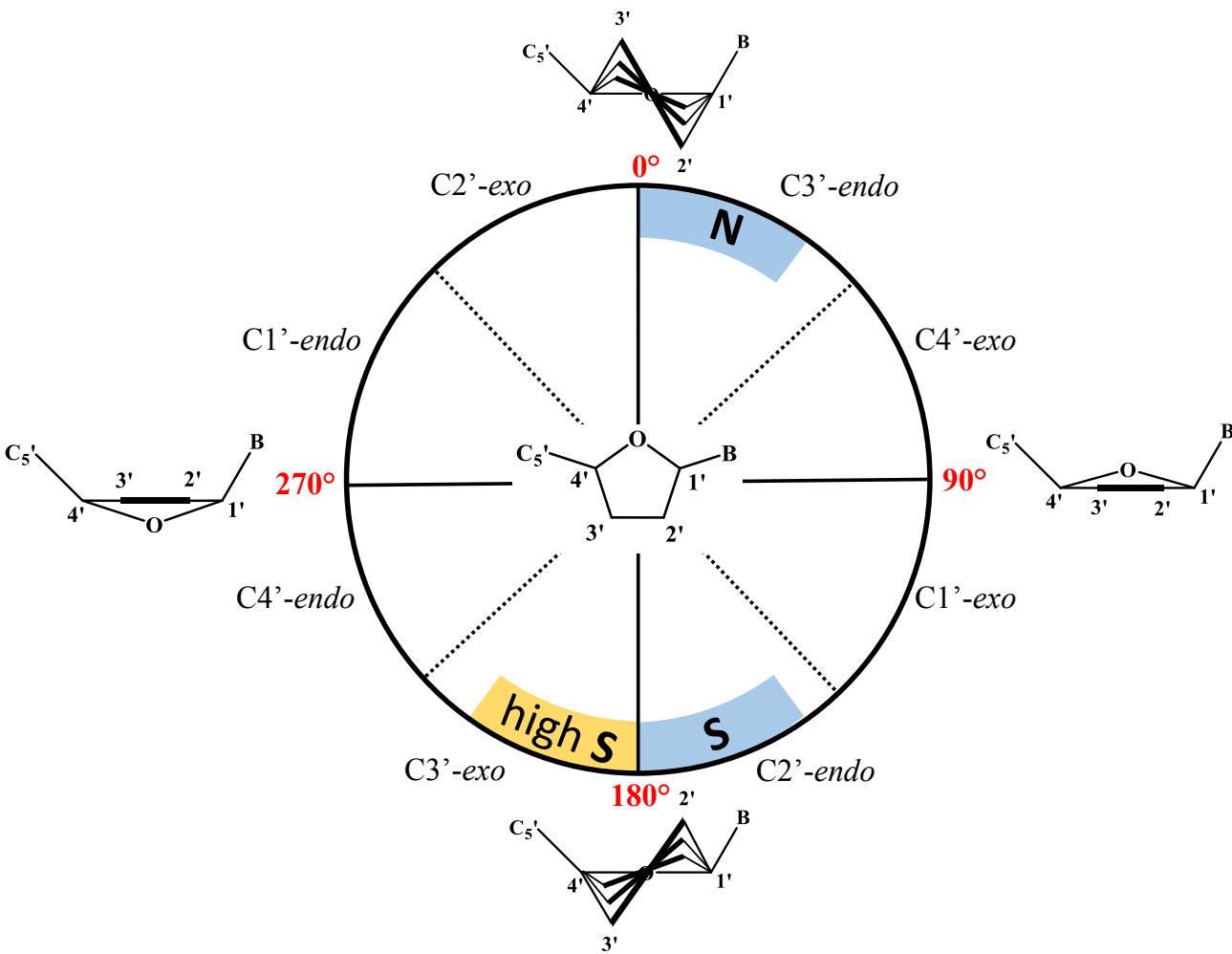


Apurinic/apyrimidinic (AP) site

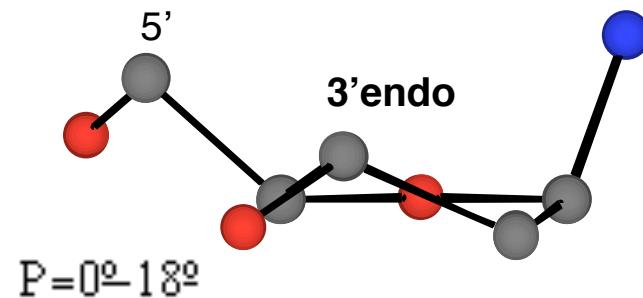


α-anomeric analogue

## N (Northern)

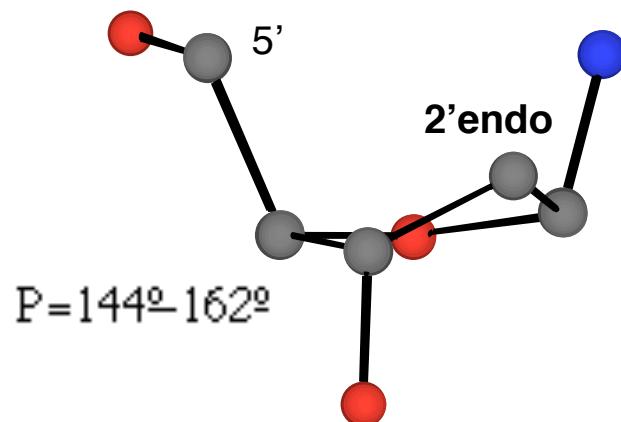


## (Southern)



Ribose:  ${}^3J_{H1'-H2'} \approx 1 \text{ Hz}$  (Angle ~ 90 deg)

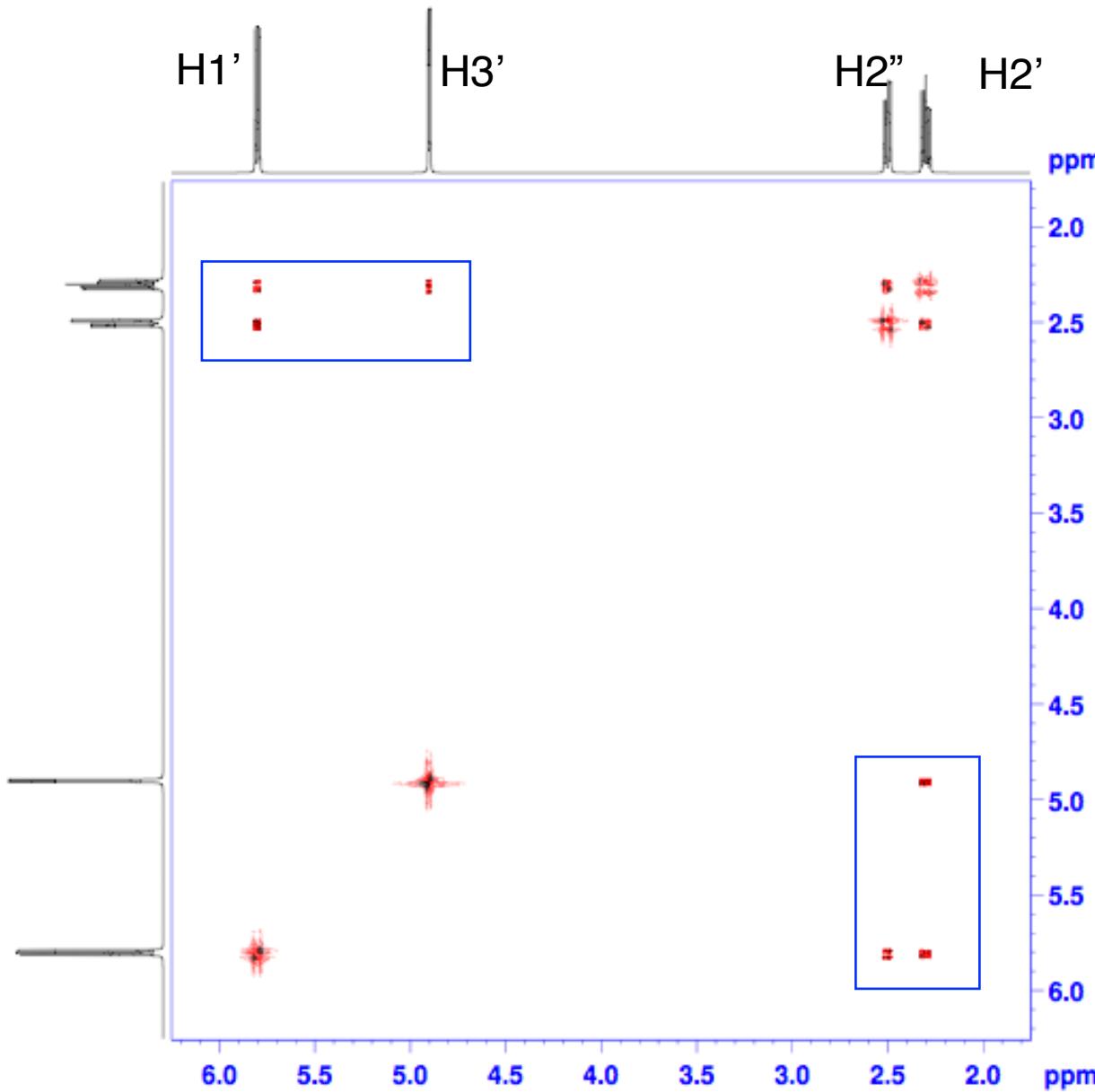
Deoxyribose:  ${}^3J_{H1'-H2'} \approx 1.8 \text{ Hz}$



Ribose:  ${}^3J_{H1'-H2'} \approx 7.9 \text{ Hz}$  (Angle ~ 170 deg)

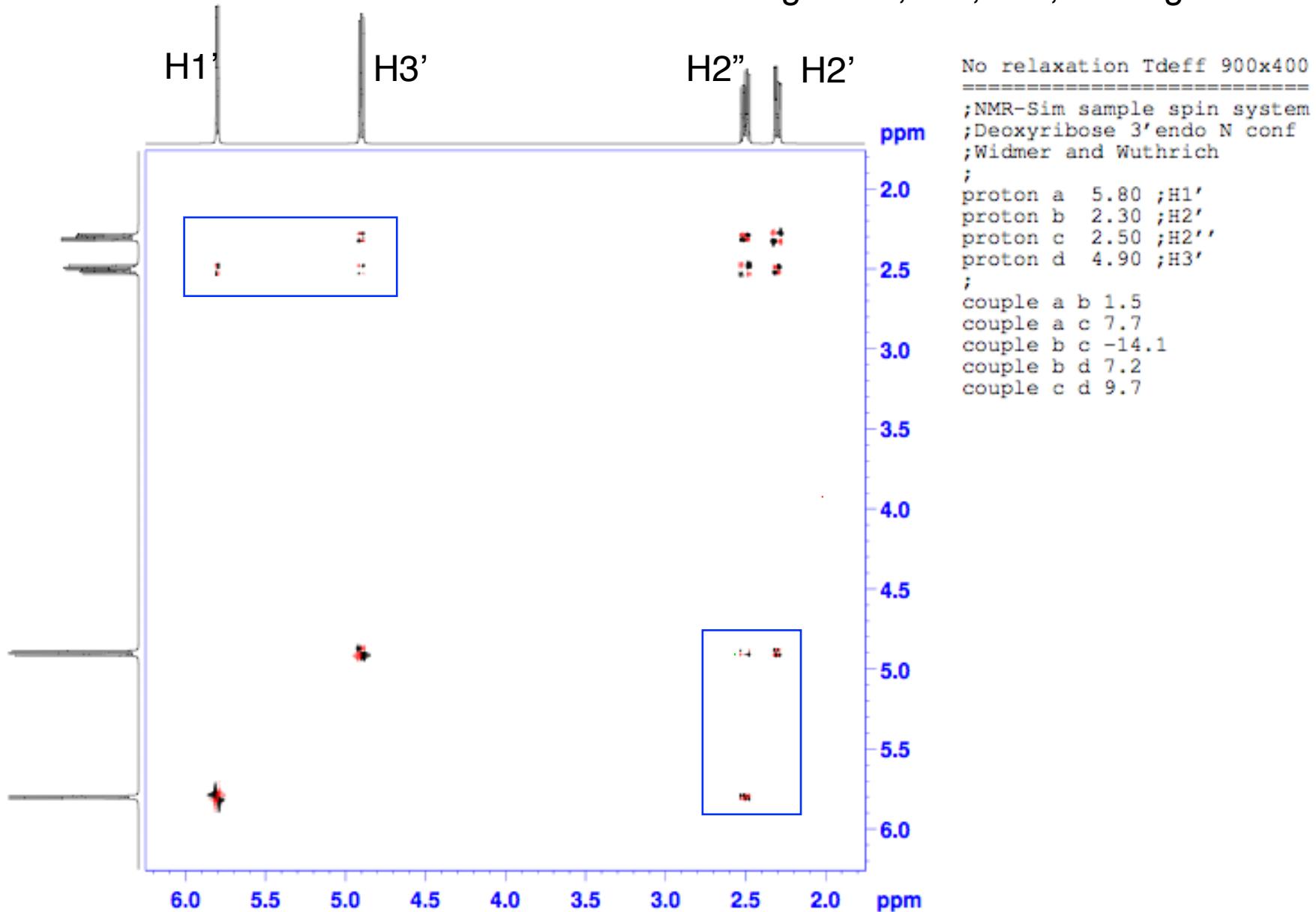
Deoxyribose:  ${}^3J_{H1'-H2'} \approx 10 \text{ Hz}$

## 2'endo sugar H1', H2', H2'', H3' region

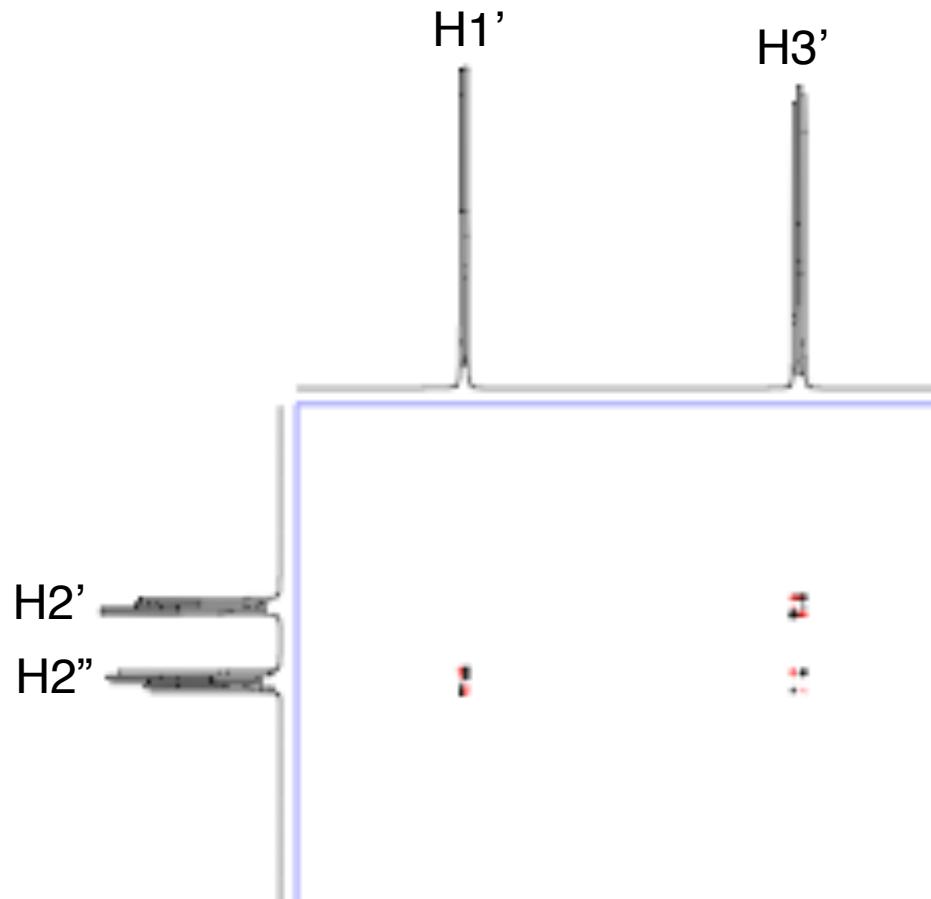


```
NO RELAXATION 1KX1K;TOPSPIN  
(Tdeff 900x400)  
=====  
;NMR-Sim sample spin system  
;Deoxyribose 2'endo S conf  
;from Widmer and Wuthrich  
;  
proton a 5.80 ;H1'  
proton b 2.30 ;H2'  
proton c 2.50 ;H2''  
proton d 4.90 ;H3'  
  
couple a b 9.5  
couple a c 5.8  
couple b c -14.1  
couple b d 5.5  
couple c d 1.3
```

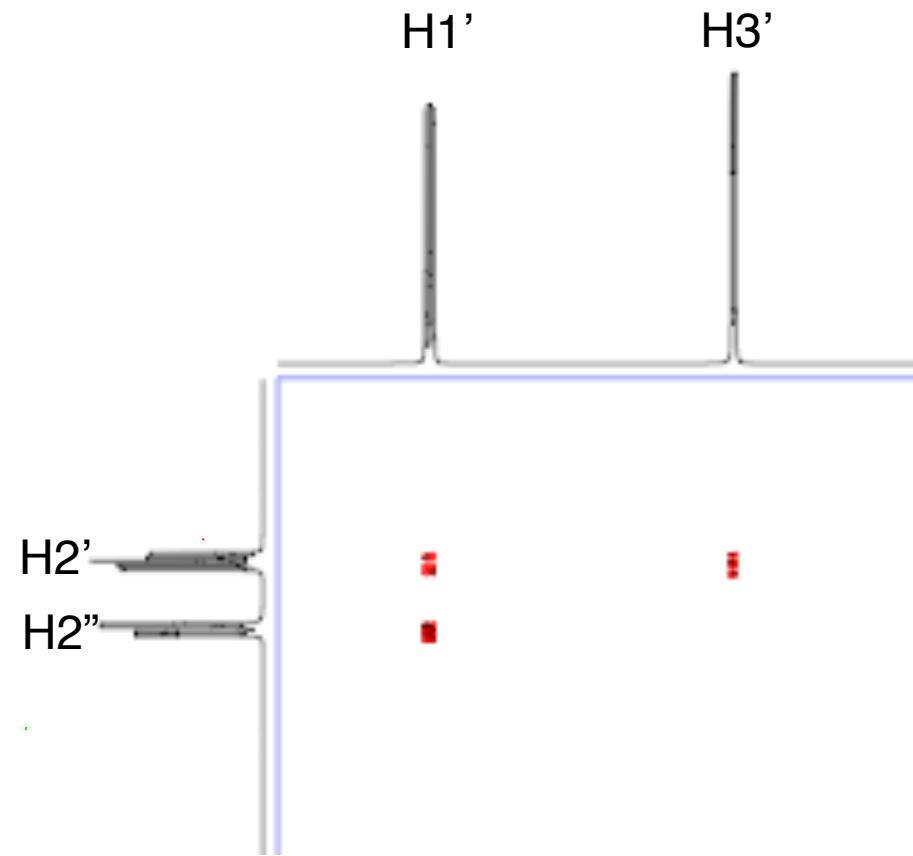
### 3'endo sugar H1', H2', H2'', H3' region



# Sugar puckering



3'endo sugar

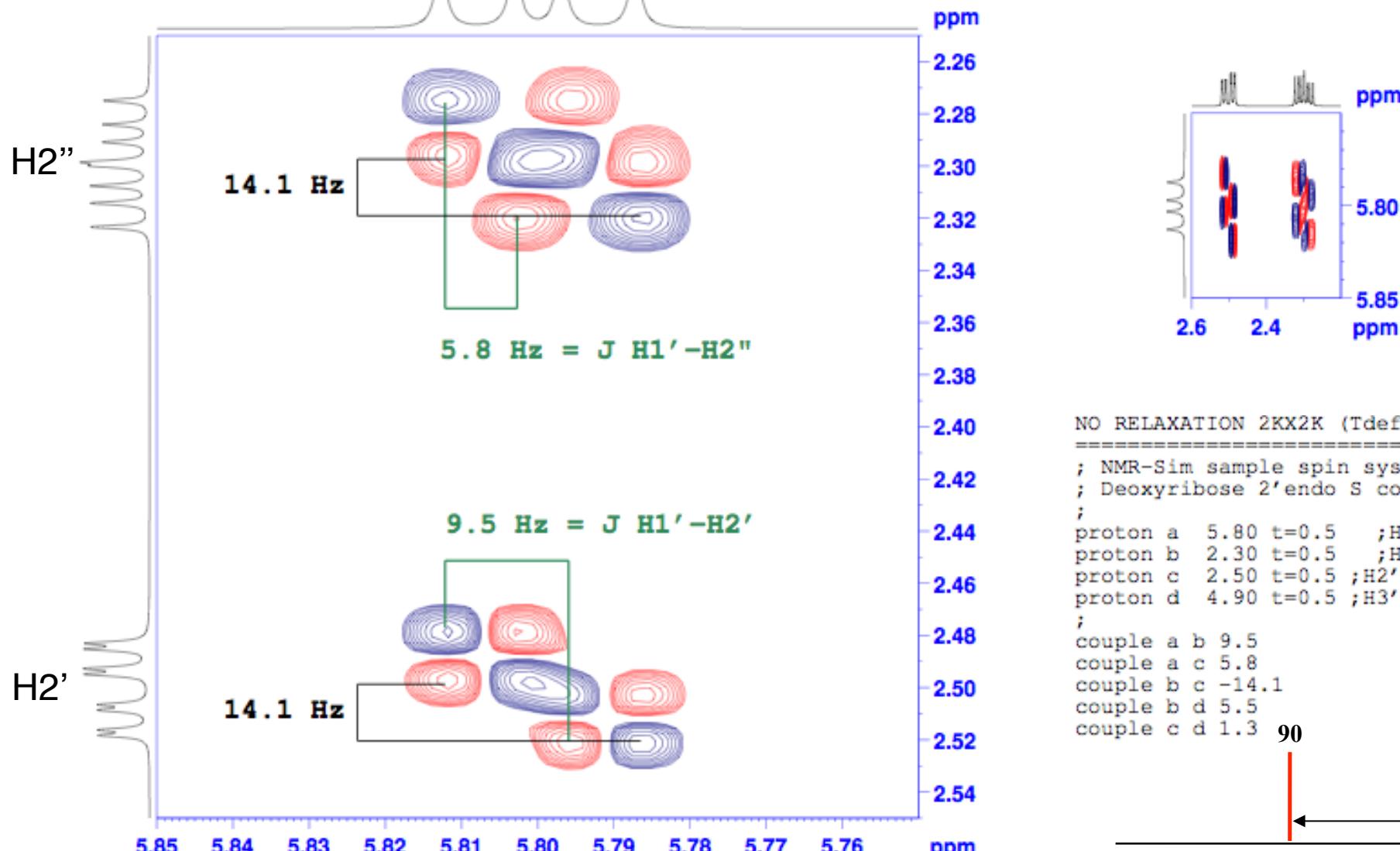


2'endo sugar

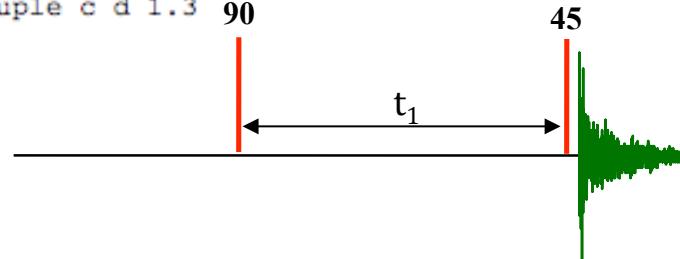
LFA- COSY

H1'

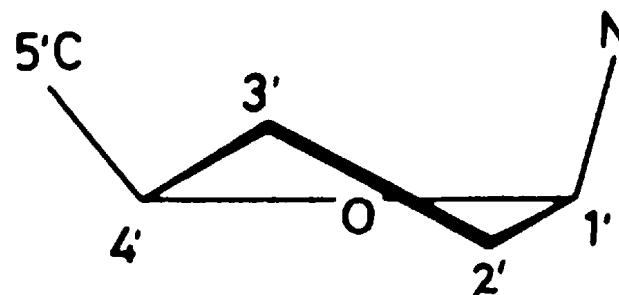
2'endo sugar H1', H2', H2" region



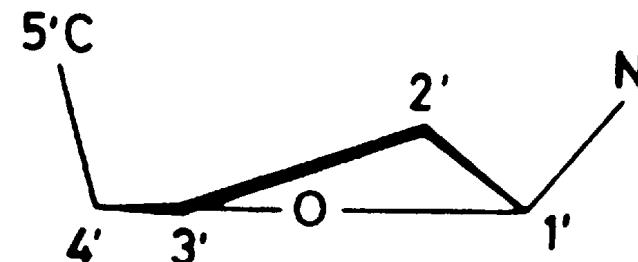
NO RELAXATION 2KX2K (Tdeff  
=====;  
; NMR-Sim sample spin syst  
; Deoxyribose 2'endo S con  
;  
proton a 5.80 t=0.5 ;H1  
proton b 2.30 t=0.5 ;H2  
proton c 2.50 t=0.5 ;H2''  
proton d 4.90 t=0.5 ;H3'  
;  
couple a b 9.5  
couple a c 5.8  
couple b c -14.1  
couple b d 5.5  
couple c d 1.3 90  
45



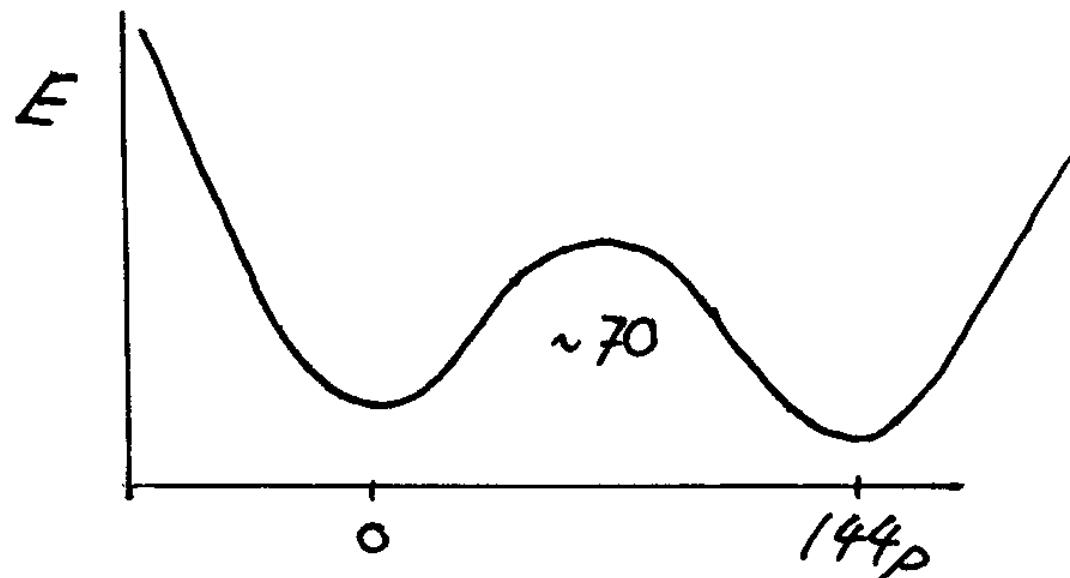
## Sugar puckering



C3'-endo C2'-exo:  $P = 0^\circ$   
 $J_{1'2'} 2 \text{ Hz}$  (with  $P = 9$ ) N



C2'-endo:  $P = 162^\circ$   
 $J_{1'2'} 9 \text{ Hz}$  (with  $P = 144$ ) S



In a 50/50 situation the measured  $J_{1'2'}$  is 5.5 Hz which would correspond to  $P$  of 70 degree.

## Sugar puckering

Usually (DNA) one observes **equilibrium** of the S and N forms sugar re-puckering. Unless one form greatly dominates the local analysis requires quite a few parameters:  $P_N$  ,  $P_S$  ,  $\Phi_N$  ,  $\Phi_S$  ,  $f_S$

Several methods for analysis exist, graphical and the more rigorous simulation. In practice the desired outcome determines the effort to be made. Sums of the coupling constants are often easier to obtain.

$$f_S = (\sum 1' - 9.8)/5.9$$

$$\sum 1' = J_{1'2'} + J_{1'2''}$$

$$\sum 2' = J_{1'2'} + J_{2'3'} + J_{2'2''}$$

$$\sum 2'' = J_{1'2''} + J_{2''3'} + J_{2'2''}$$

$$\sum 3' = J_{2'3'} + J_{2''3'} + J_{3'4'}$$

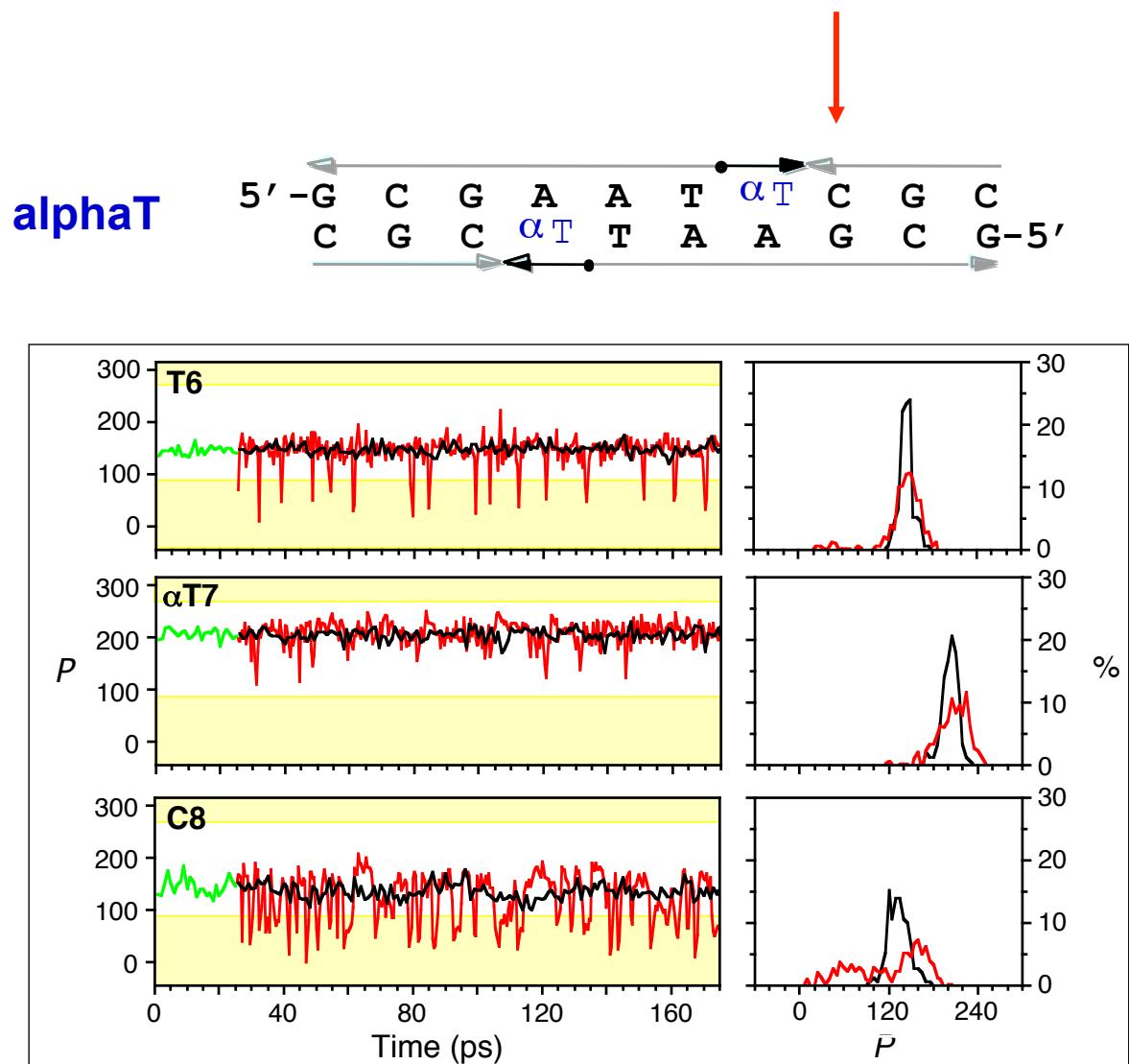
If  $f_S < 50\% \quad J_{1'2'} < J_{1'2''}$

If  $f_S \text{ ca } 0\% \quad J_{1'2'} \text{ very small}$

If  $f_S > 70\% \quad J_{1'2'} > J_{1'2''}$

# Sugar puckering

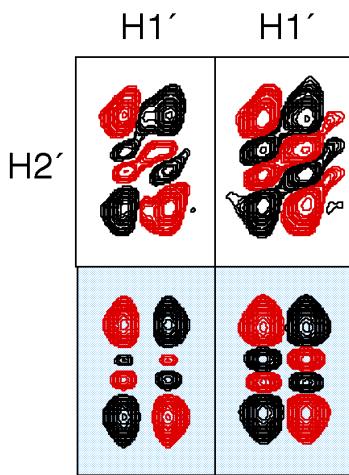
	control		alphaT	
Nt	$\Sigma 1'$	$f_s$	$\Sigma 1'$	$f_s$
G1	15.2	0.92	15.3	0.93
C2	15.1	0.90	14.7	0.83
G3	16.2	1.00	15.9	1.00
A4	16.2	1.00	15.3	0.93
A5	15.7	1.00	15.3	0.93
T6	15.1	0.90	15.3	0.93
T7	16.0	1.00	12.3	-
C8	15.1	0.90	12.9	0.53
G9	15.7	1.00	14.7	0.83
C10	(14)	(0.7)	(14)	(0.7)



MD calculation  
MD-Tar calculation

# Pseurot calculations

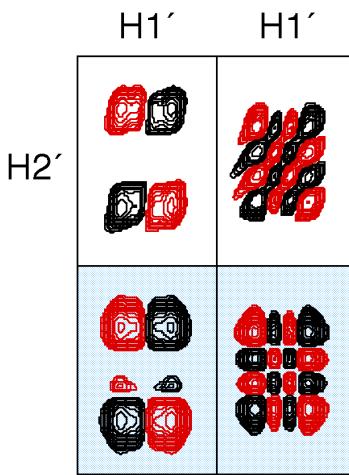
## alphaT C8



H2'  
H2''

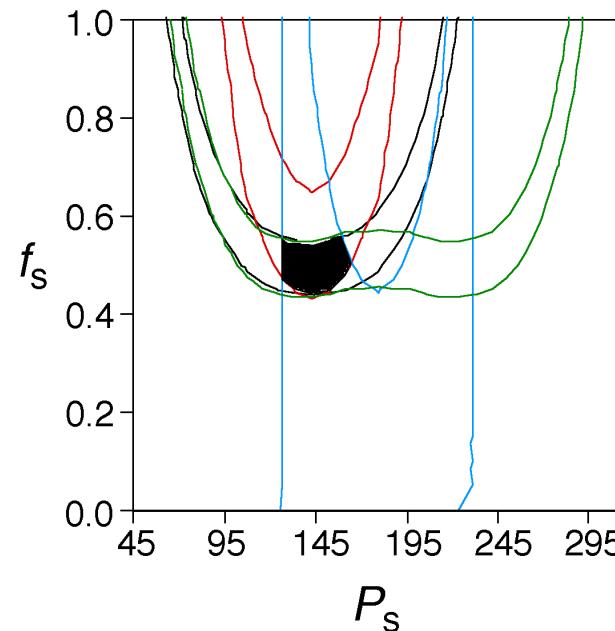
H1'  
H3'

## control C8

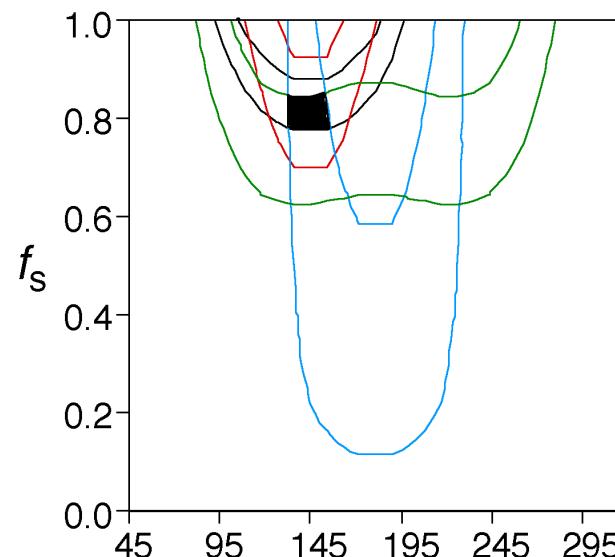
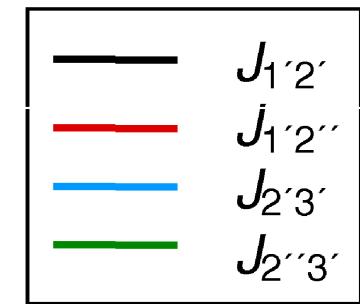


H2'  
H2''

H1'  
H3'

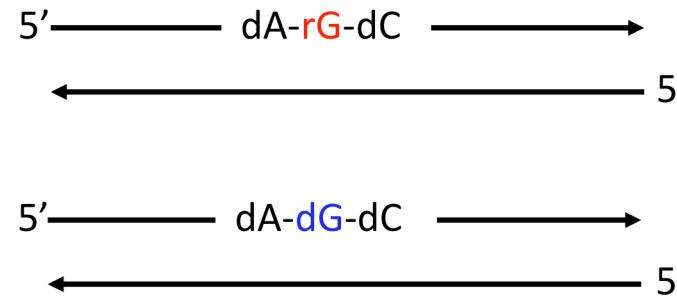
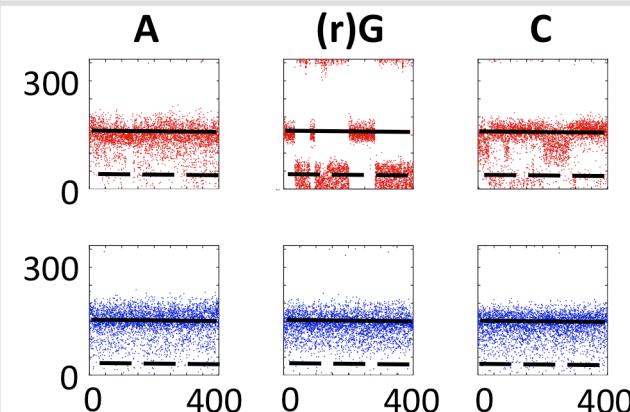
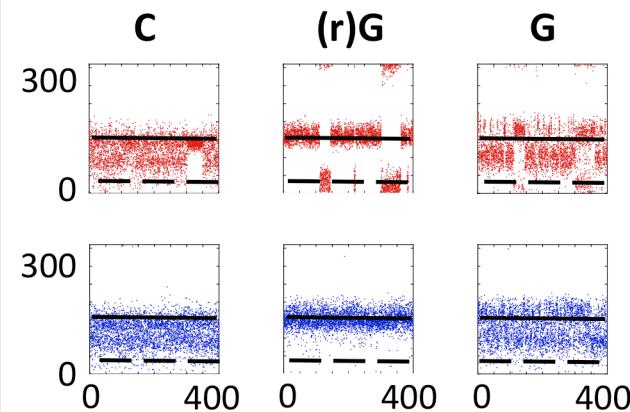
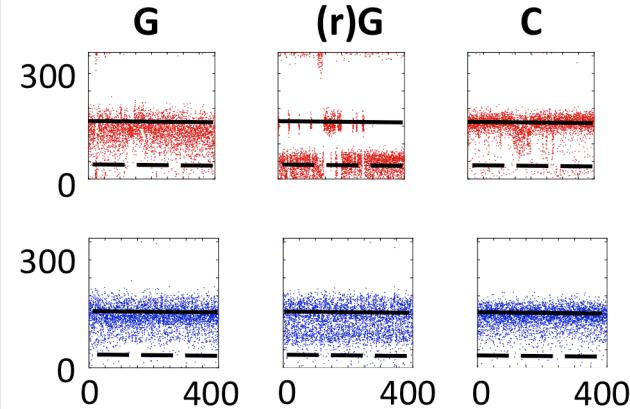


$$\begin{aligned}\Phi_{S,N} &= 37^\circ \\ P_S &= 125-165 \\ f_S &= 0.44-0.55\end{aligned}$$



$$\begin{aligned}\Phi_{S,N} &= 37^\circ \\ P_S &= 130-155 \\ f_S &= 0.78-0.86\end{aligned}$$

van Wijk,J., Haasnoot,K., de Leeuw,F.,  
Huckreide,D. and Altona,C. (1995) PSEUROT 6.2.  
A Program for the Conformational Analysis of Five  
Membered Rings. University of Leiden, The  
Netherlands



# Introduction to Cross-Correlated Relaxation

## Relaxation in NMR

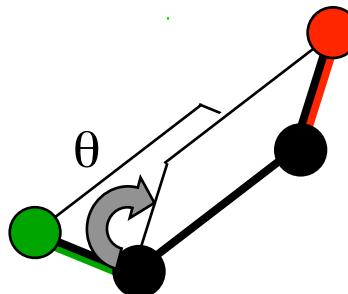
- determines experimental strategies and experiments
- dynamic and structural parameters

## Mechanisms

- Dipole -dipole
- CSA (e.g.  $^{31}\text{P}$  at higher fields; proportional to  $B^2$ )
- Scalar relaxation (first and second kind)
- paramagnetic, etc

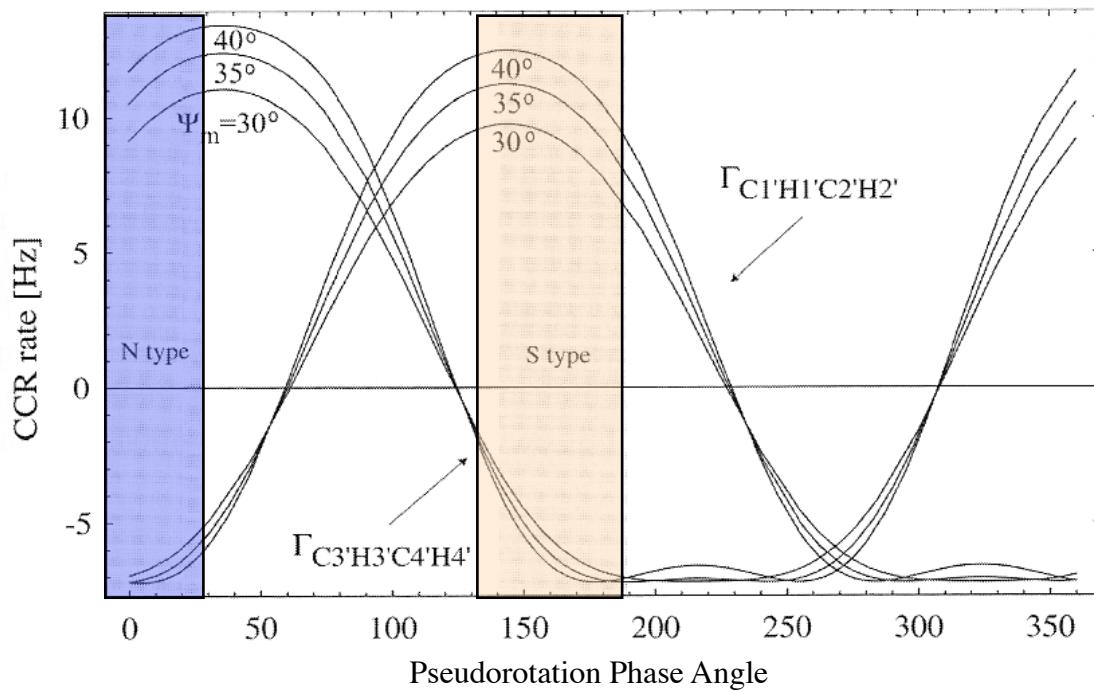
Recently it became possible to use cross correlated relaxation (CCR) to directly measure bond angles without using a calibration curve as is needed for J's.

- DD -DD
- DD -CSA



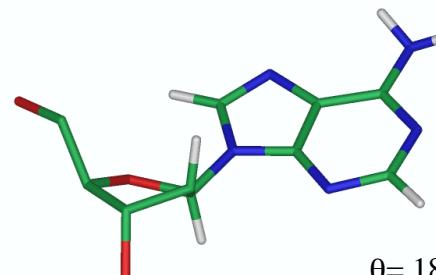
# Sugar Puckering from Cross-Correlated Relaxation

## $\Gamma_{\text{DD-DD}}$

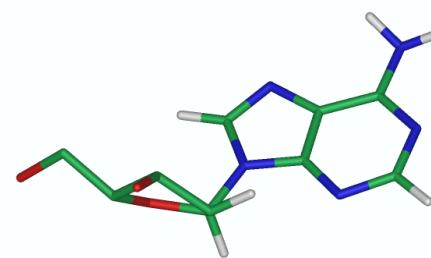


$$\theta_{1'2'} = 121.4^\circ + 1.03 \psi_m \cos(P - 144^\circ)$$

$$\Gamma_{C1'H1'-C2'H2'} = k (3\cos^2\theta - 1)\tau_c$$



$\theta = 180^\circ$ : for 2'endo (B form)  
Large and positive



$\theta = 90^\circ$ : for 3'endo (A form)  
Small and negative

# Sugar puckering: Summary

- Coupling constants: COSY, E.COSY, low flip angle COSY  
Homonuclear, Heteronuclear
- CT NOESY
- CSA-DD and DD-DD cross correlated data
- $^{13}\text{C}$  chemical shifts, in favorable cases

## Some references

Szyperski, T., et al. (1998). JACS. 120, 821- 822.

Measurement of Deoxyribose  $^3\text{JHH}$  Scalar Couplings Reveals Protein-Binding Induced Changes in the Sugar Puckers of the DNA.

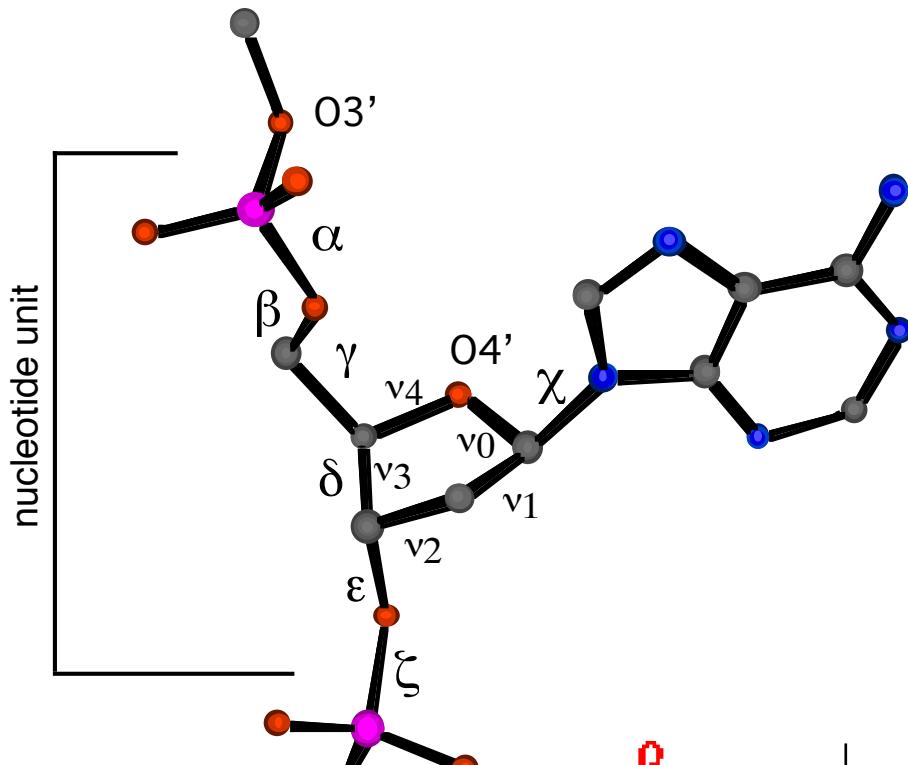
Iwahara J, et al. (2001), J. Mag Res. 2001, 153, 262

An efficient NMR experiment for analyzing sugar-puckering in unlabeled DNA:. Couplings via constant time NOESY.

J. Boisbouvier, B. Brutscher, A. Pardi, D. Marion, and J.-P. Simorre (2000), J. Am. Chem. Soc. 122, 6779–6780  
NMR determination of sugar-puckers in nucleic acids form CSA-dipolar cross correlated relaxation.

BioNMR in Drug Research 2003 Editor(s): Oliver Zerbe (Wiley-VCH)

Methods for the Measurement of Angle Restraints from Scalar, Dipolar Couplings and from Cross-Correlated Relaxation: Application to Biomacromolecules  
Chapter 7 p147-178. Christian Griesinger (also for  $\alpha$  and  $\zeta$ )



$\alpha$  and  $\zeta$  pose problems  
Determinants of  $^{31}\text{P}$  chem shift.

$\epsilon$  and  $\zeta$  correlate.  $\zeta = -317 - 1.23 \epsilon$

$\beta$

${}^3\text{J}_{\text{P}5'-\text{H}5'(\text{H}5'')}$   
 ${}^3\text{J}_{\text{P}5'-\text{C}4'}$

$\gamma$

${}^3\text{J}_{\text{H}4'-\text{H}5'(\text{H}5'')}$   
 ${}^3\text{J}_{\text{C}3'-\text{H}5'(\text{H}5'')}$

$\epsilon$

${}^3\text{J}_{\text{P}3'-\text{H}3'}$   
 ${}^3\text{J}_{\text{P}3'-\text{C}2'}$   
 ${}^3\text{J}_{\text{P}3'-\text{C}4'}$

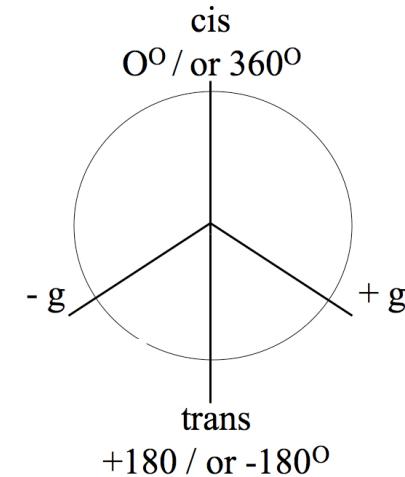
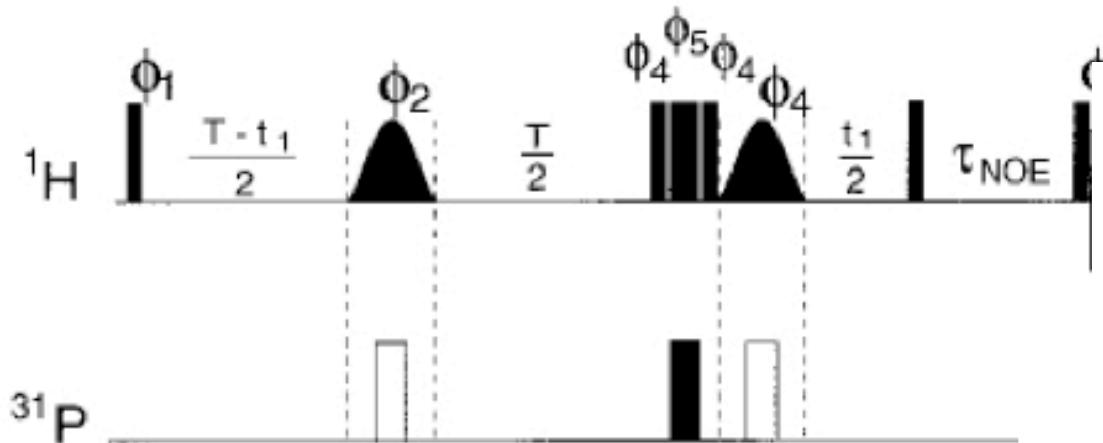
$\chi$

${}^3\text{J}_{\text{H}1'-\text{C}6} (\text{U,C,T})$   
 ${}^3\text{J}_{\text{H}1'-\text{C}2} (\text{U,C,T})$   
 ${}^3\text{J}_{\text{H}1'-\text{C}8} (\text{A,G})$   
 ${}^3\text{J}_{\text{H}1'-\text{C}4} (\text{A,G})$

+ NOE

+ NOE

# Backbone Experiments: CT-NOESY, CT-COSY



Bax, A., Tjandra, N., Zhengrong, W., ( 2001). Measurements of <sup>1</sup>H-<sup>31</sup>P dipolar couplings in a DNA oligonucleotide by constant time NOESY difference spectroscopy, *J. Mol. Biol.*, **19**, 367-270.

91.

