# Nucleic Acids

# NMR Spectroscopy

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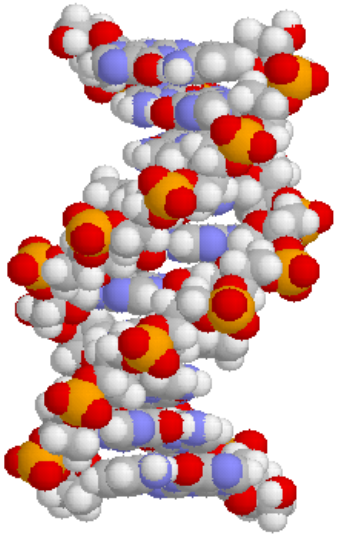
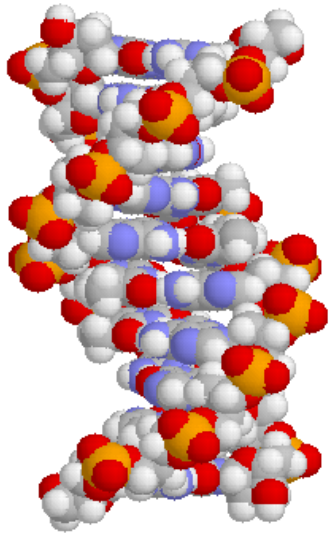




2014

# NMR of Nucleic Acids 1

1) Primary Structure of DNA and RNA

2) Resonance Assignment of DNA/RNA by Homonuclear NMR

   A) $^{1}$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:

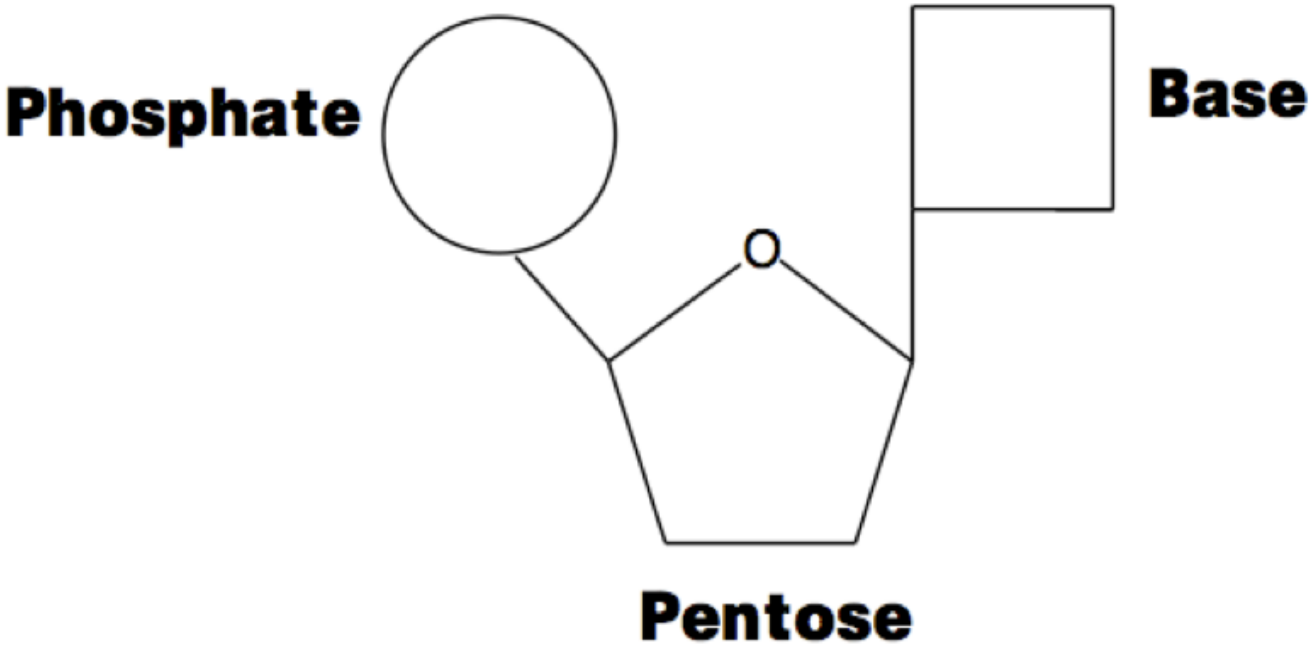
PDB Holding, March 21, 2012

| **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 [1)] | 430 | 24 | 122 | - | 492 |
| total | 74’294 | 2’223 | 3’681 | - | 80’264 |

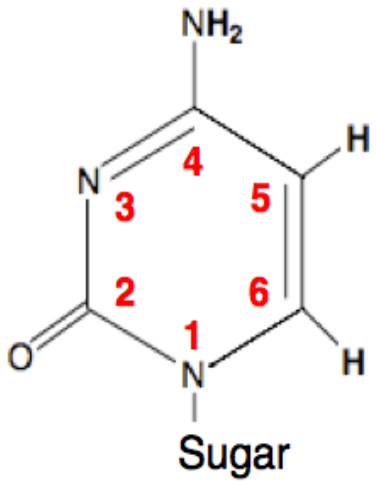
1) EM, Hybrid, other

http://www.rcsb.org/pdb

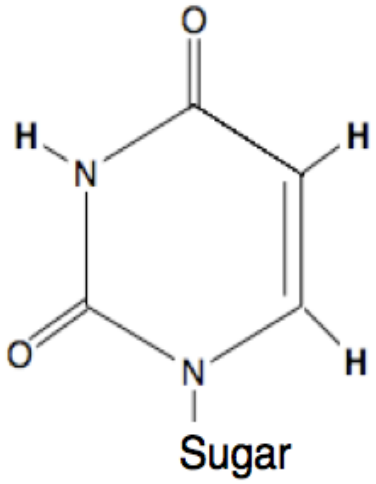
# Nucleic Acids are Polymers of Nucleotides



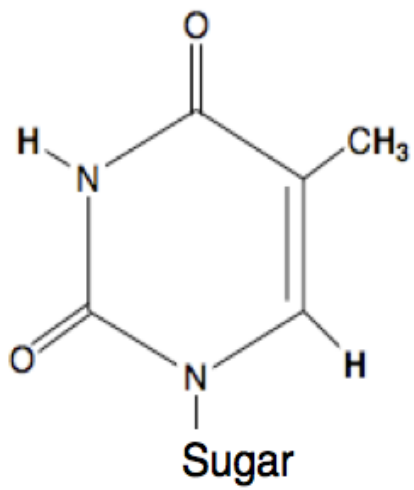
# Common Pyrimidine Bases




**Cytosine**



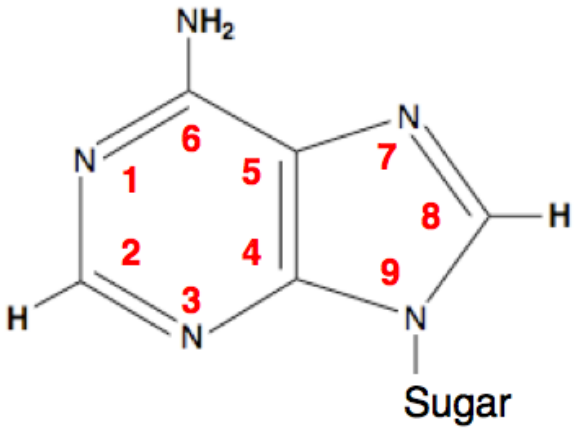

**Uracil (RNA)**



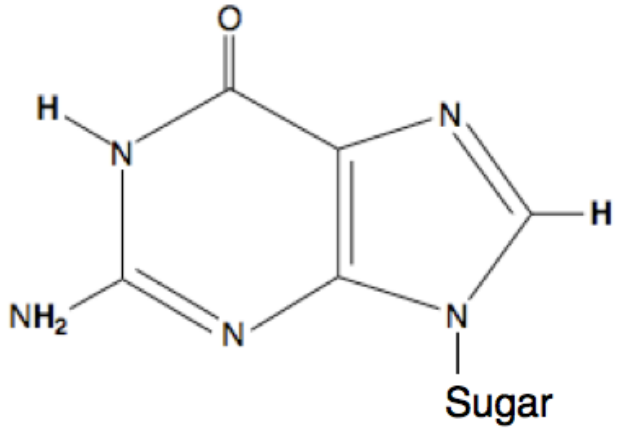

**Thymine (DNA)**

Numbering

# Common Purine Bases



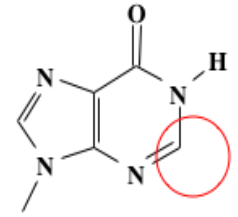

**Adenine**



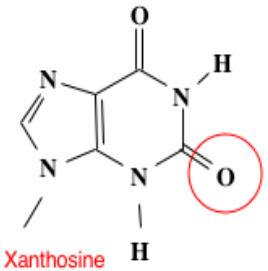

**Guanine**

Numbering

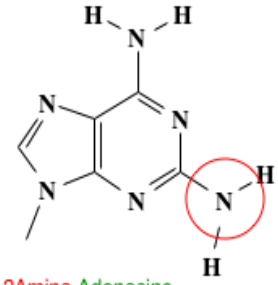
# Alternate Bases & Modifications (small selection):



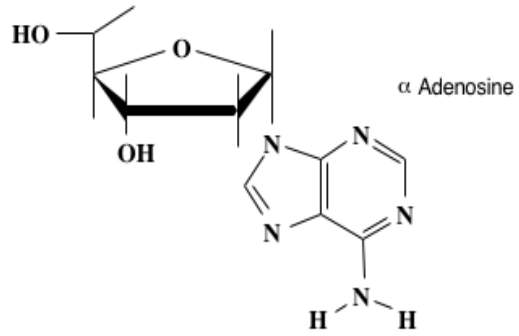
Inosine
Base: Hypoxanthine



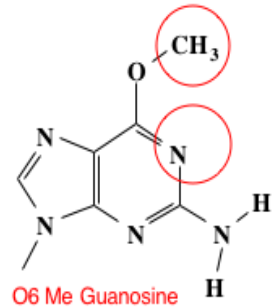
Xanthosine



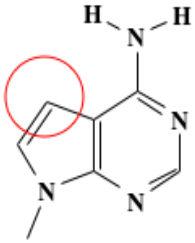
2Amino Adenosine



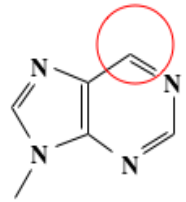
α Adenosine



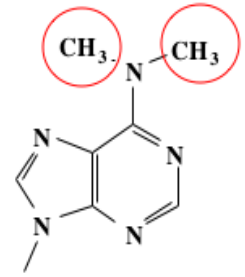
O6 Me Guanosine



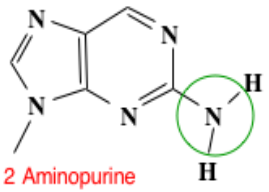
7 deaza Adenosine



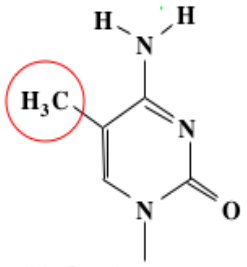
Nebularine



6 Dimethyl aminopurine

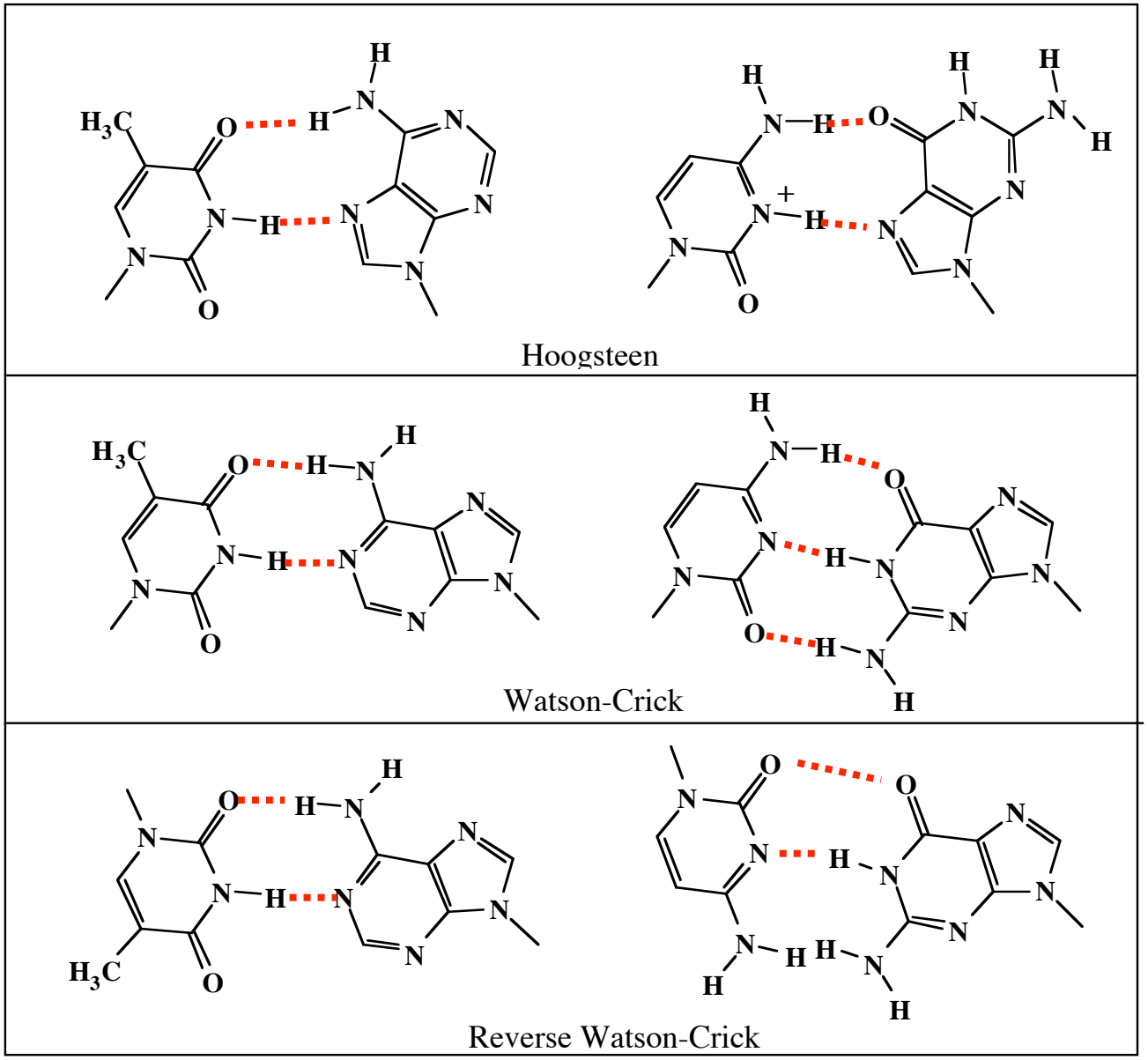


2 Aminopurine



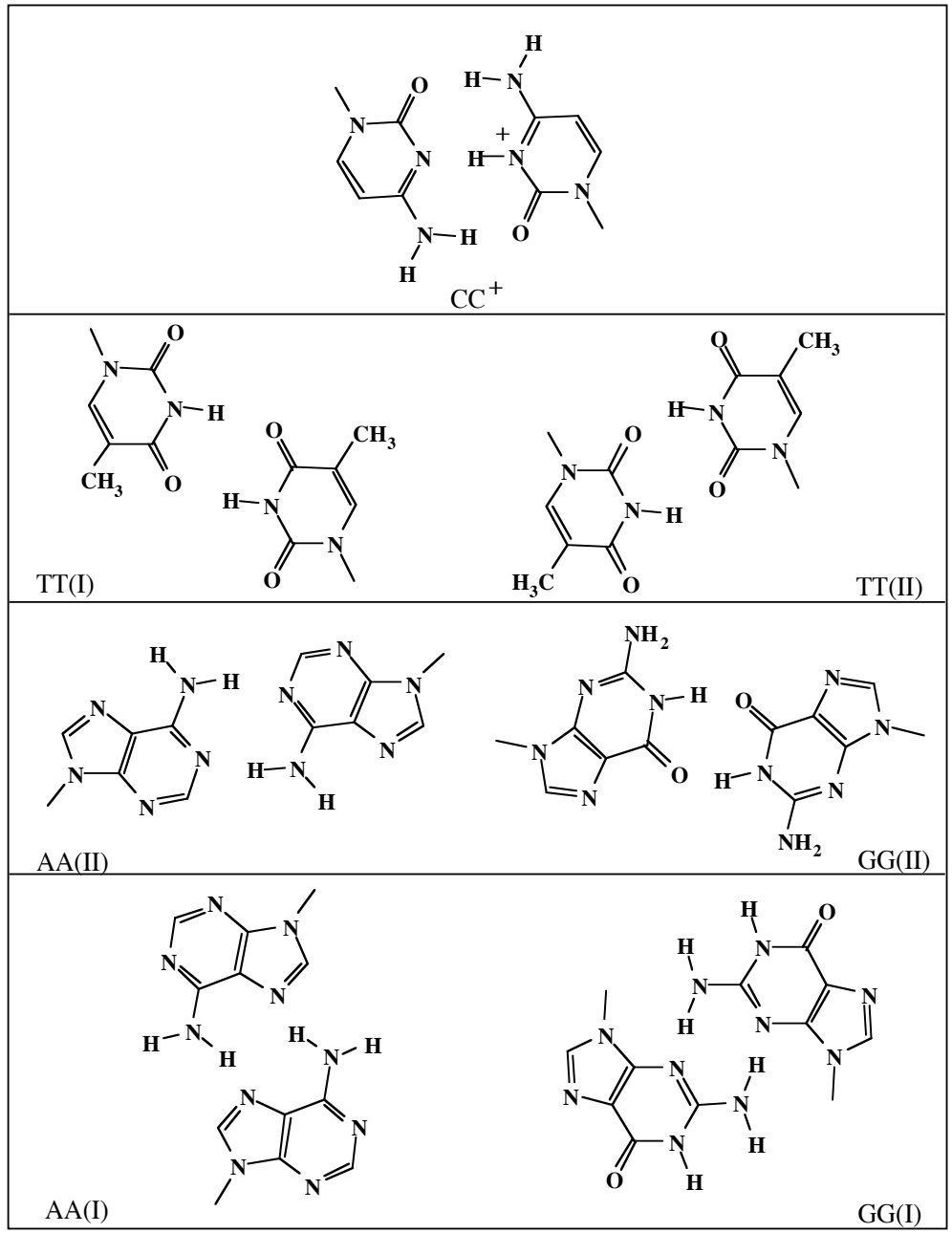
5Me Cytosine

# HETERO 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

# 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

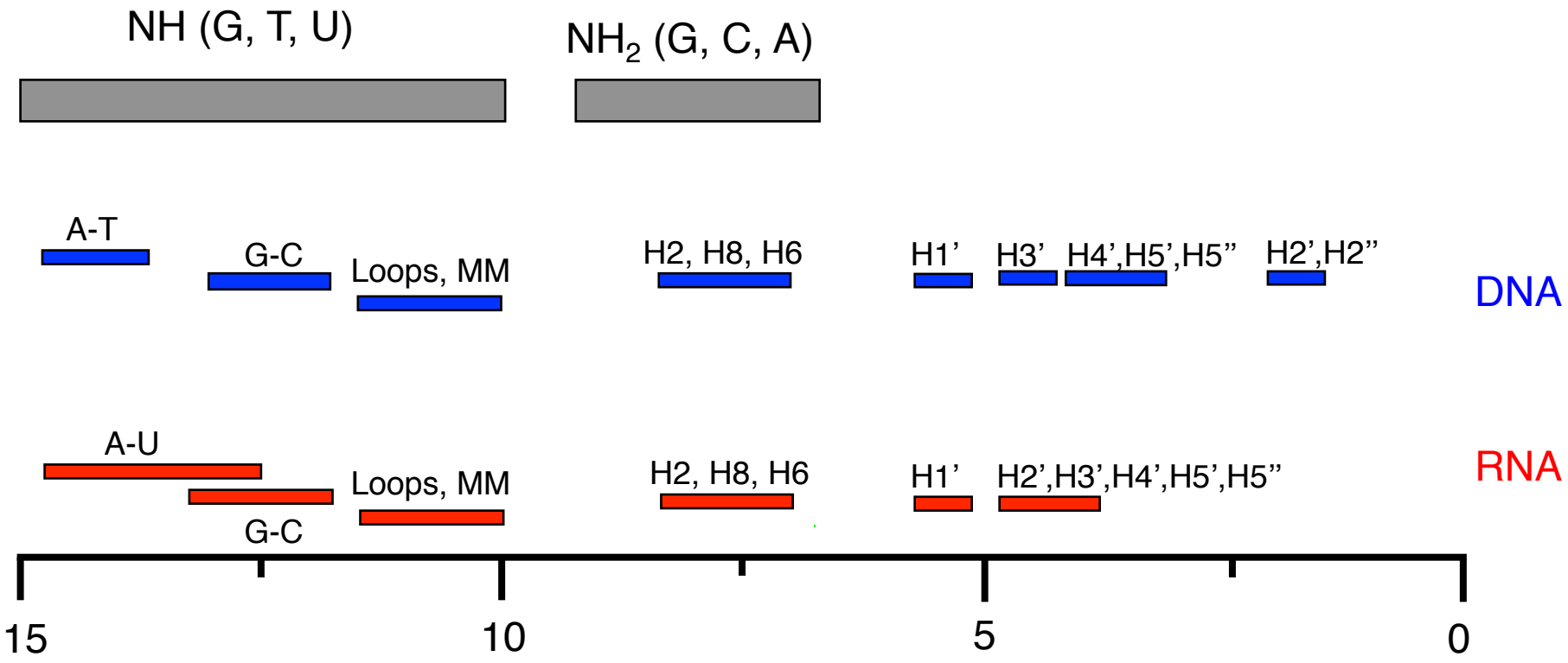
> account for spin diffusion

•Backbone may be difficult to fully characterize

> especially $\alpha$ and $\zeta$.

•Dipolar couplings

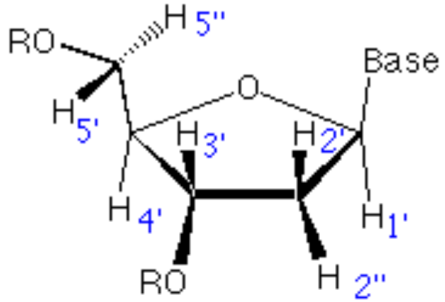
# Chemical shift ranges in nucleic acids



# 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 |

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

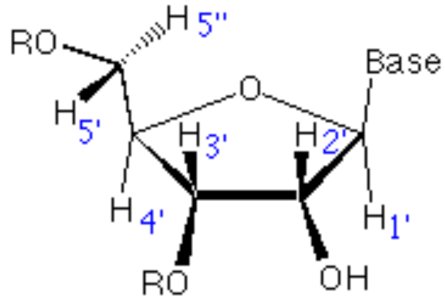



2''-Deoxy-β-D-Ribose

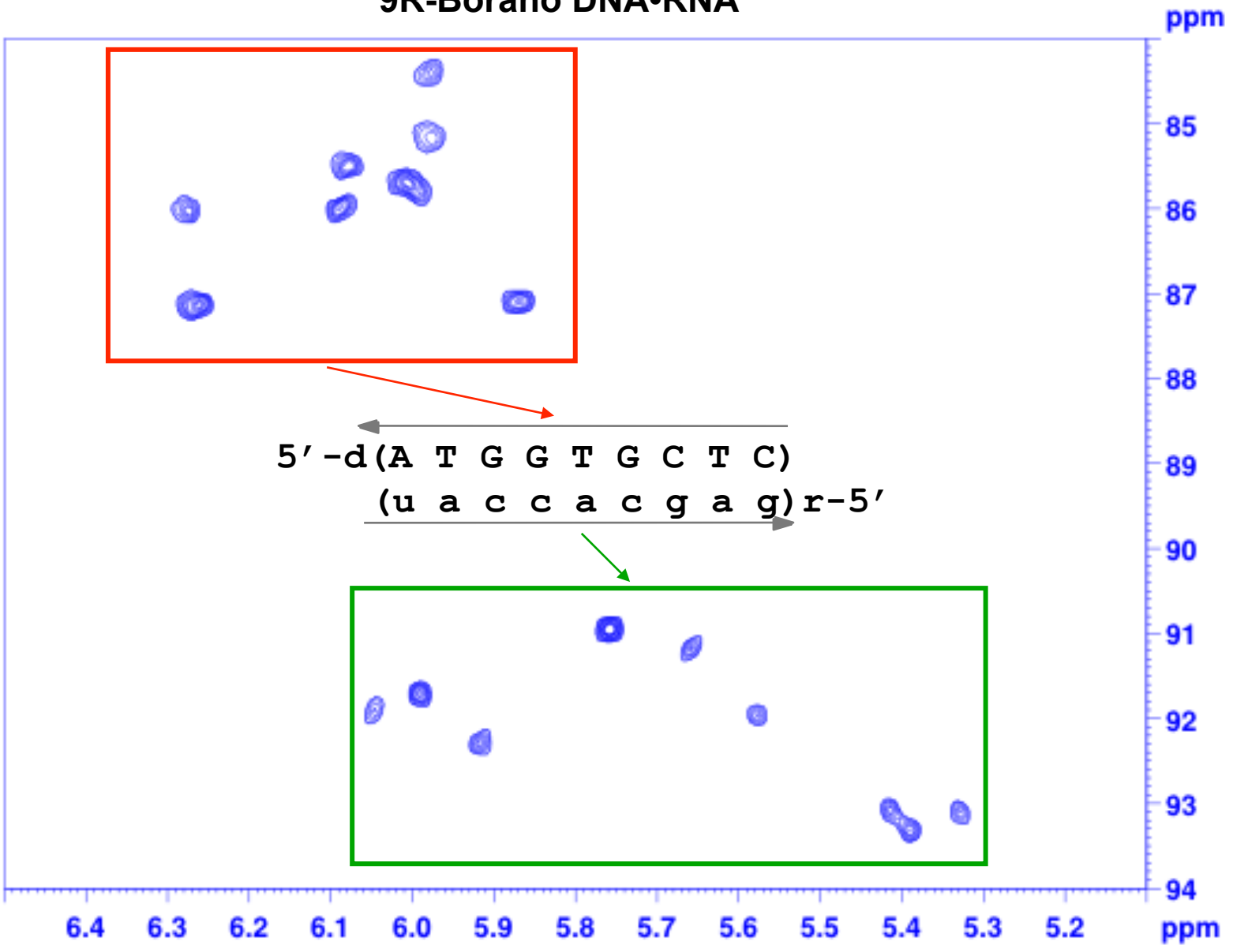
# RNA

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




β-D-Ribose


9R-Borano DNA•RNA
ppm
85
86
87
88
89
90
91
92
93
94
5′-d(A T G G T G C T C)
(u a c c a c g a g)r-5′
6.4
6.3
6.2
6.1
6.0
5.9
5.8
5.7
5.6
5.5
5.4
5.3
5.2
ppm

## Adenine

| | | | |
|---|---|---|---|
| H2 | 7.5-8 | C2 | 152-156 |
| H8 | 7.7-8.5 | C8 | 137-142 |
| N6H | 5-6/7-8 | N6 | 82-84 |
| - | - | | - |
| | | C4 | 149-151 |
| | | C5 | 119-121 |
| | | C6 | 157-158 |
| | | N1 | 214-216 |
| | | N3 | 220-226 |
| | | N7 | 224-232 |
| | | N9 | 166-172 |

## Guanine

| | | | |
|---|---|---|---|
| - | - | C2 | 156 |
| H8 | 7.5-8.3 | C8 | 131-138 |
| N1H | 12-13.6 | N1 | 146-149 |
| N2H | 5-6/8-9 | N2 | 72-76 |
| | | C4 | 152-154 |
| | | C5 | 117-119 |
| | | C6 | 161 |
| | | N1 | 146-149 |
| | | N3 | 167 |
| | | N7 | 228-238 |
| | | N9 | 166-172 |

## Thymidine

| | | | |
|---|---|---|---|
| H6 | 6.9-7.9 | C6 | 137-142 |
| Me5 | 1.0-1.9 | Me5 | 15-20 |
| N3H | 13-14 | N3 | 156 |
| - | - | - | - |
| | | C2 | 154 |
| | | C4 | 169 |
| | | C5 | 95-112 |
| | | N1 | 144 |

## Uridine

| | | | |
|---|---|---|---|
| H6 | 6.9-7.9 | C6 | 137-142 |
| H5 | 5.0-6.0 | C5 | 102-107 |
| N3H | 13-14 | N3 | 156-162 |
| - | - | - | |
| | | C2 | 154 |
| | | C4 | 169 |
| | | C5 | 102-107 |
| | | N1 | 142-146 |

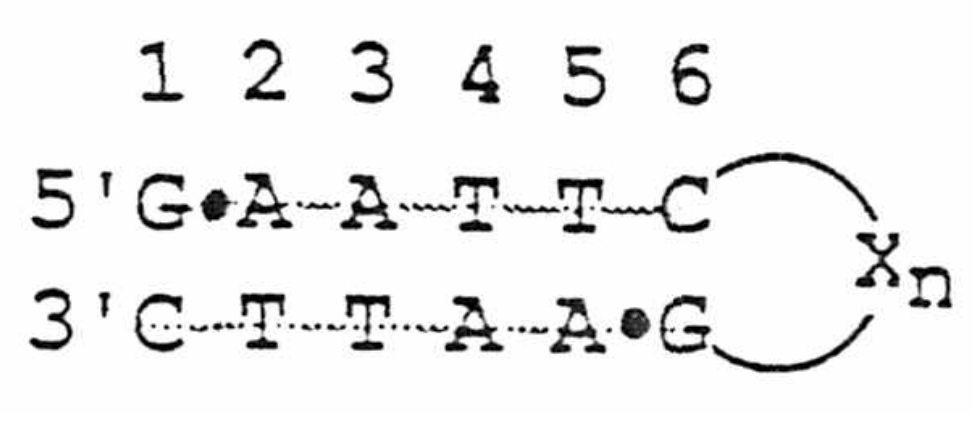
## Cytidine

| | | | |
|---|---|---|---|
| H6 | 6.9-7.9 | C6 | 136-144 |
| H5 | 5.0-6.0 | C5 | 94-99 |
| - | - | N3 | 210 |
| N4H | 6.7-7/81-8.8 | N4 | 94-98 |
| | | C2 | 159 |
| | | C4 | 166-168 |
| | | C5 | 94-99 |
| | | N1 | 150-156 |

# No Structure Required!

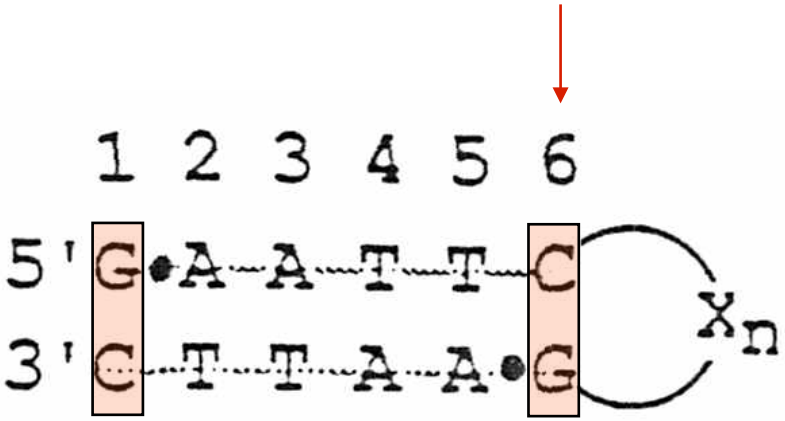
**Often, depending on the question asked, a full structure determination is not required**

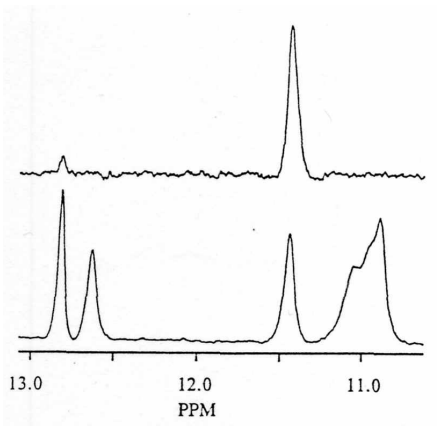
- Does it form a duplex?
- Which base pairs are thermo labile?
- Which base pair is which… assignment?
- Is the loop structured?
- Structure

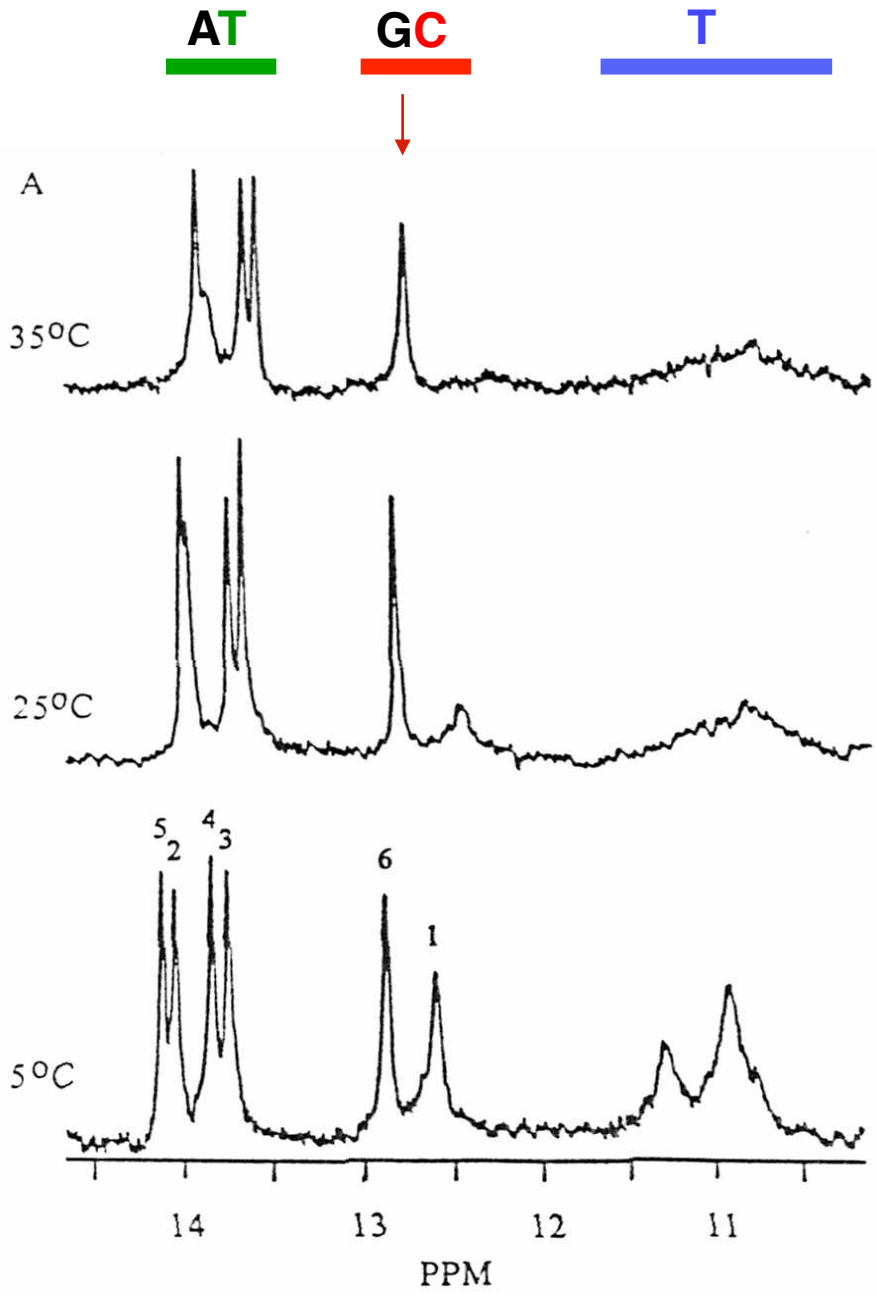


# DNA Hairpin

Thermal lability



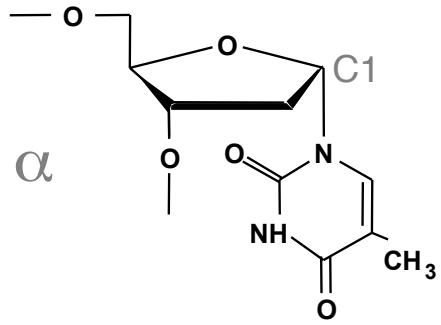



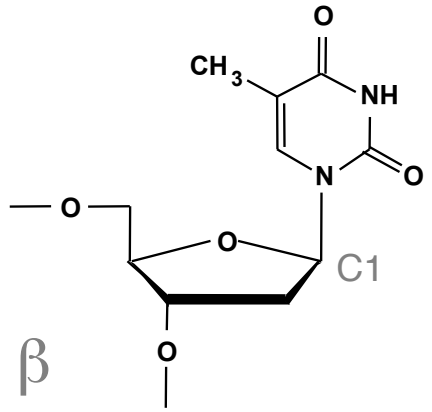



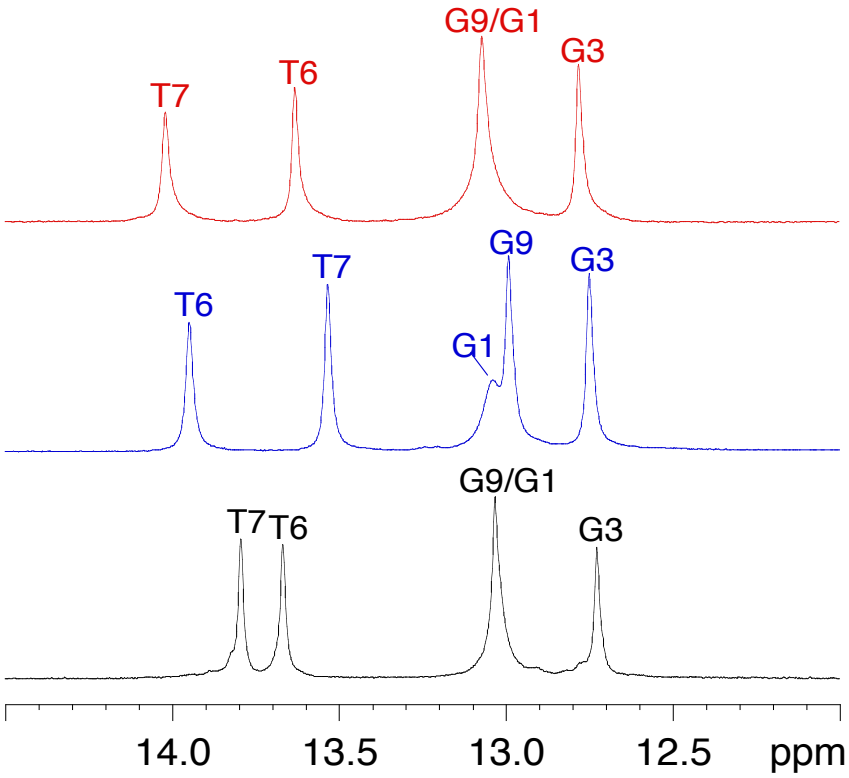

Germann et al., Nucl. Acids Res. 1990 18: 1489-1498

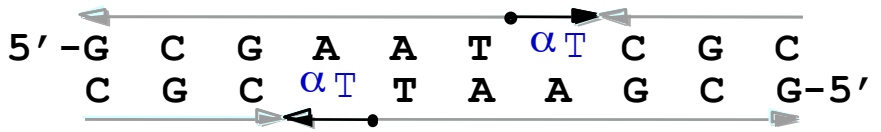
# “New” DNA constructs

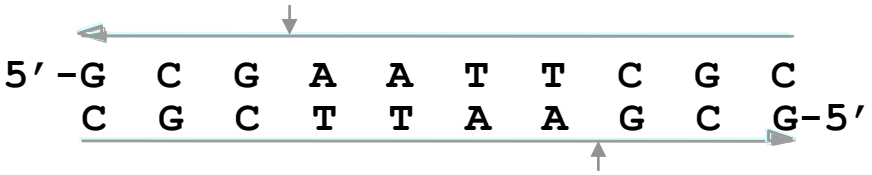
- Do the duplexes form, is there base pairing?
- Does the unusual base pair form?



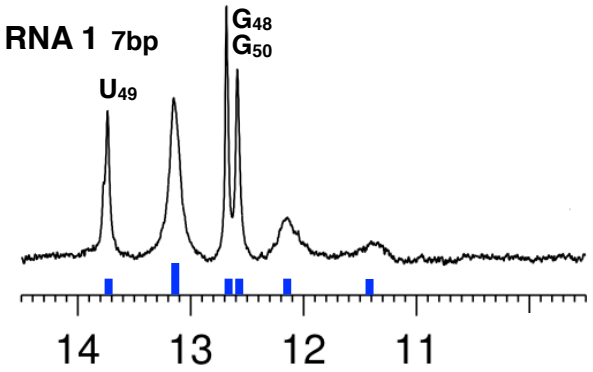



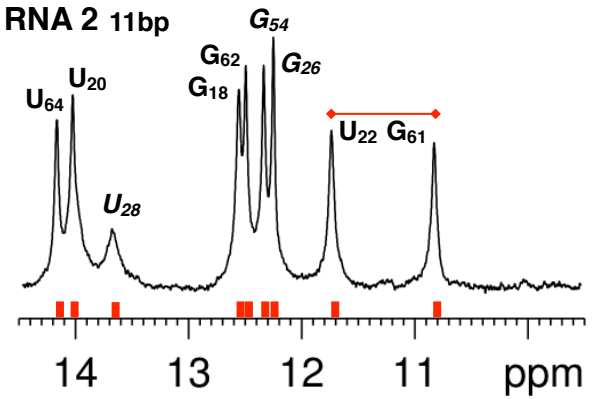



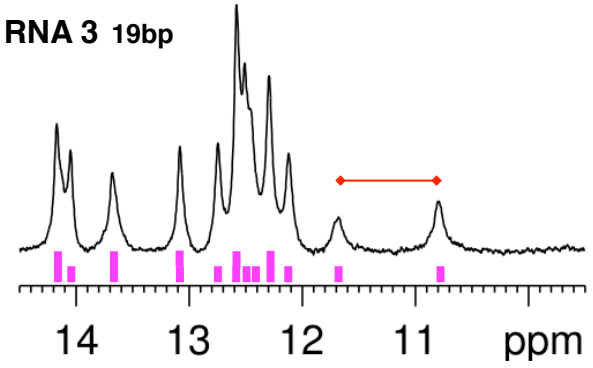



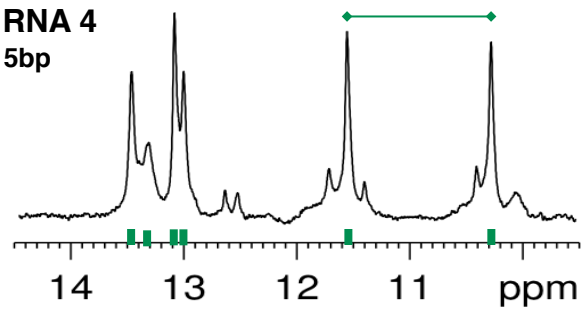



# WNV-RNA


RNA 1 7bp
G48
G50
U49
14
13
12
11


RNA 2 11bp
G54
G62
G18
G26
U20
U64
U28
U22 G61
14
13
12
11
ppm


RNA 3 19bp
14
13
12
11
ppm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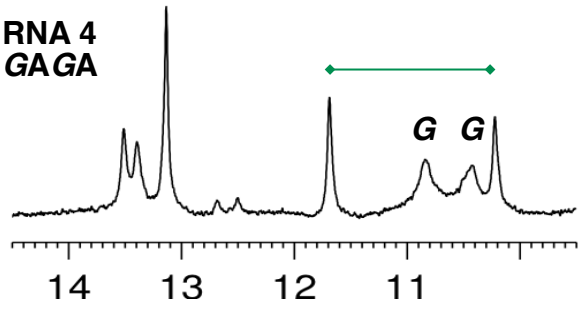
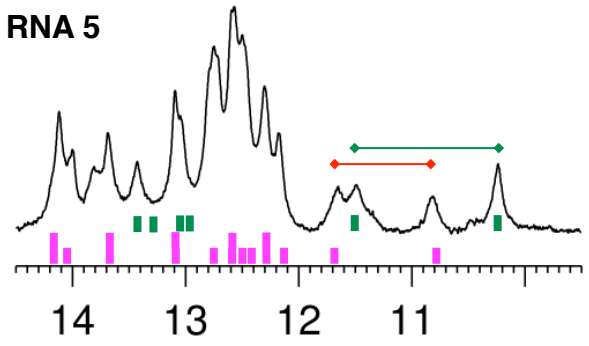
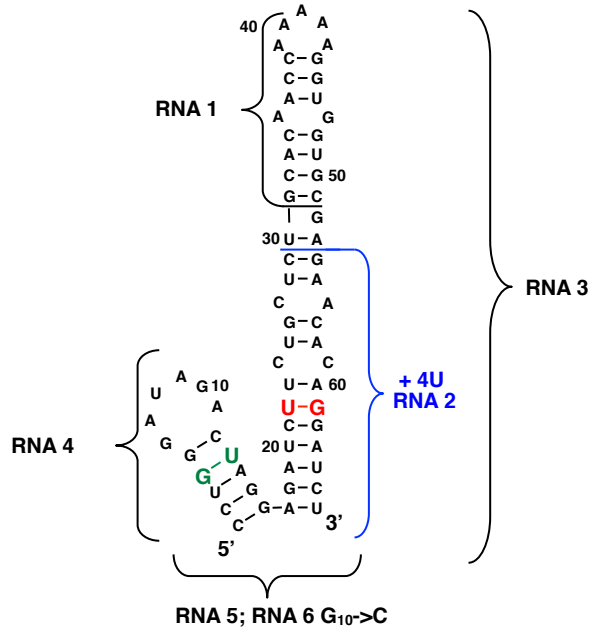
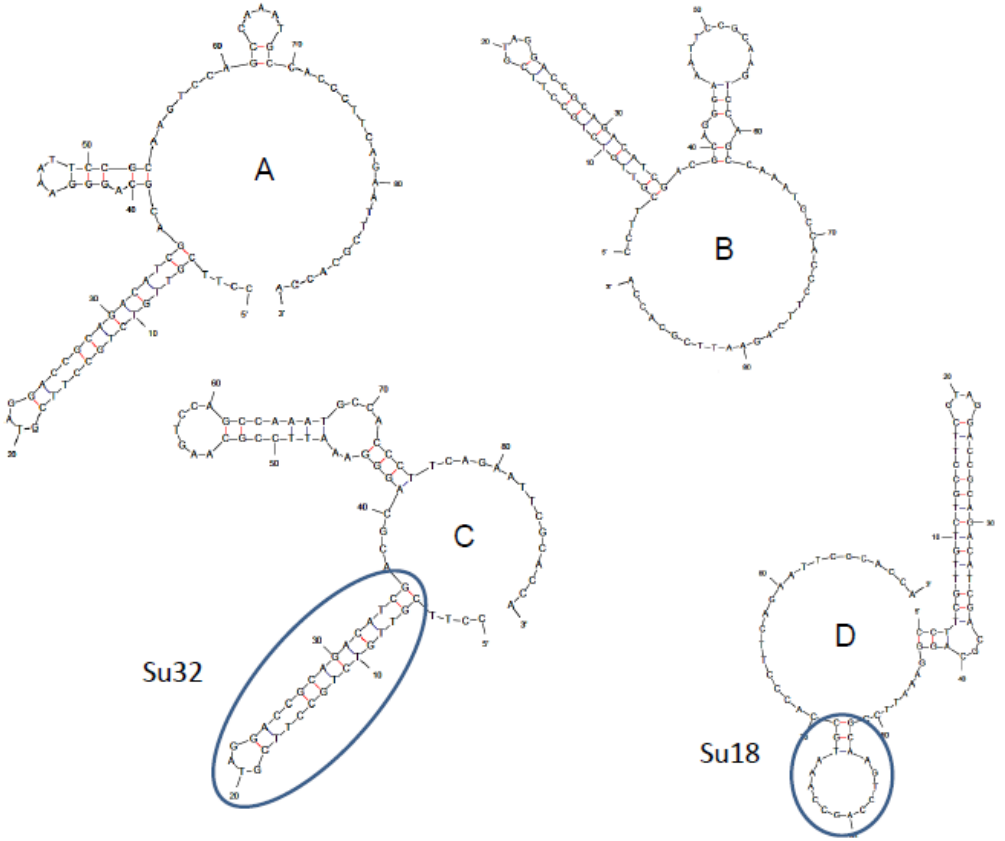
5bp
14
13
12
11
ppm

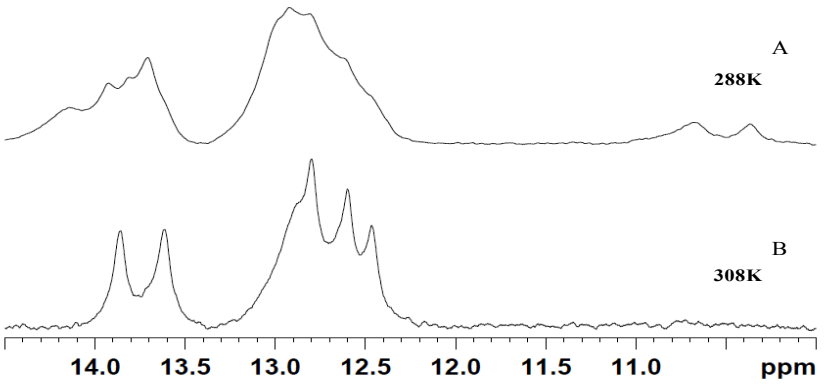

RNA 4
GAGA
G G
14
13
12
11


RNA 5
14
13
12
11


RNA 1
RNA 4
RNA 3
+ 4U
RNA 2
RNA 5; RNA 6 G10->C
5'
3'

# Fibrinogen Specific DNA Aptamer



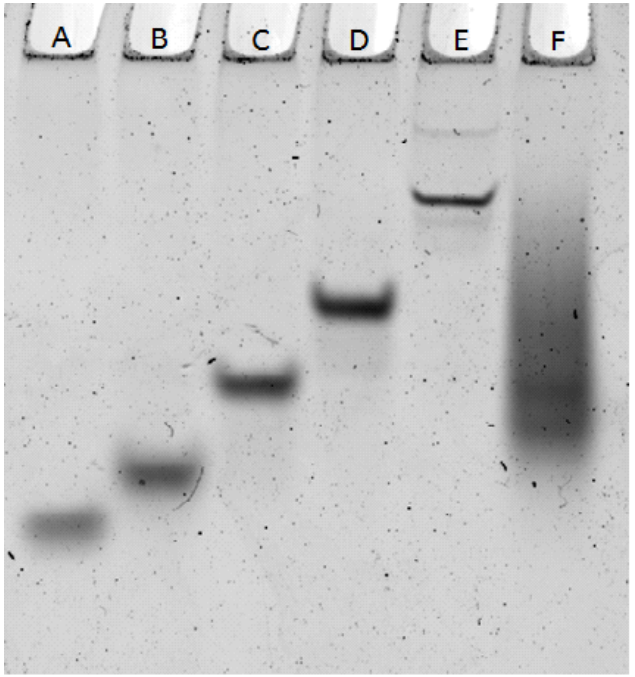




Figure 2: 12% Native PAGE to observe mobilities of Ap90. Ap90 is compared to single stranded oligomers of various lengths. The lanes were loaded from the smallest to the largest sequences with Lane A-E containing the 10-mer, 18-mer, 30-mer, 50-mer, and the 90-mer respectively. Lane F contains the aptamer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

Hamilton & Germann 2011

# 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:

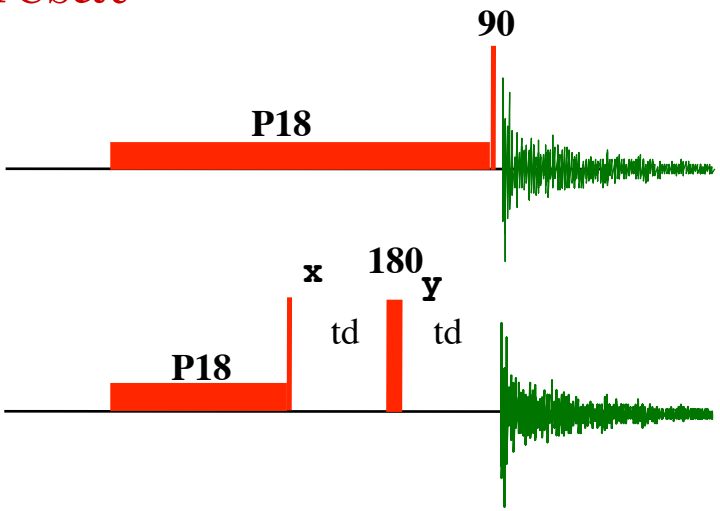
1) Presaturation
2) Observing the FID when the water passes a null condition after a 180 degree pulse.
3) Suppression of broad lined based on their $T_2$ behavior.
4) Selectively excitation, with and without gradients

5a) Use of 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)

5c) Excitation sculpting

# Presat

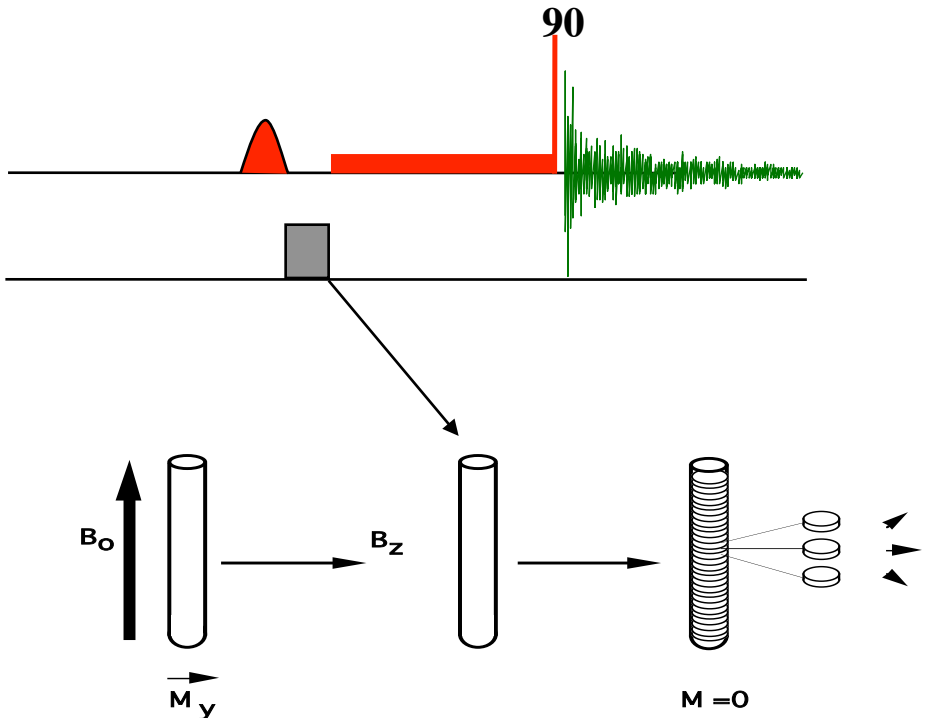



Presaturation field strength:
20-40 Hz corresponds to a
6-12ms 90deg pulse

Pros: Easy to set up
Excellent water suppression

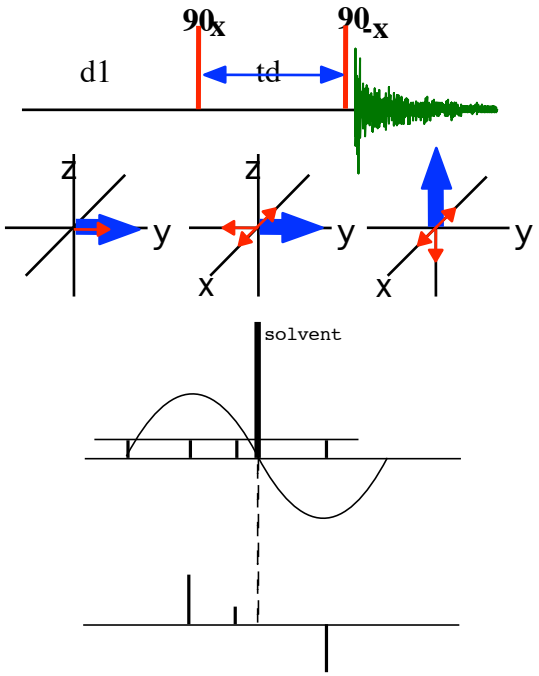
Cons: Resonances under water signal!
(T variation)
Labile protons not visible
(some GC pairs may be)

# 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

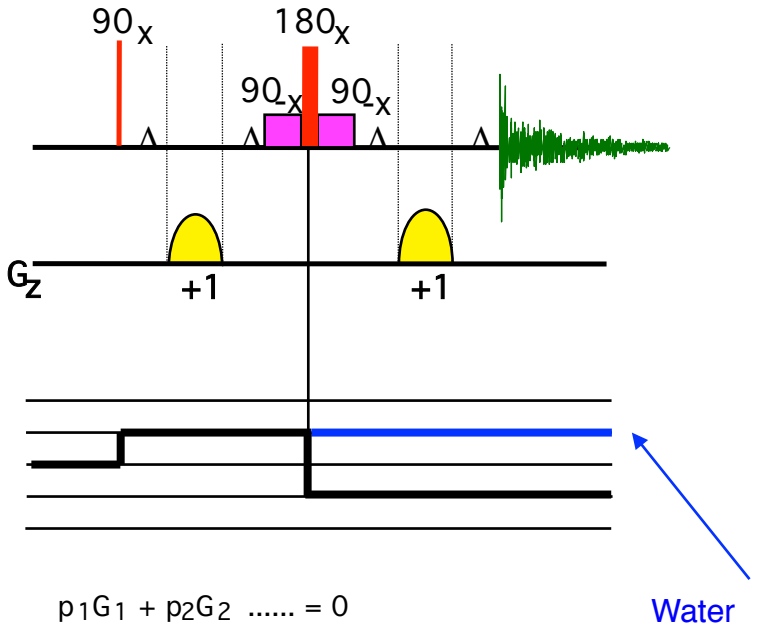



Pros: Easy to set up
Excellent water suppression
(with proper setup as good as presat)
Good for broad signals!

Cons: Non uniform excitation Baseline not flat

Other sequences: 1331 etc

# Watergate

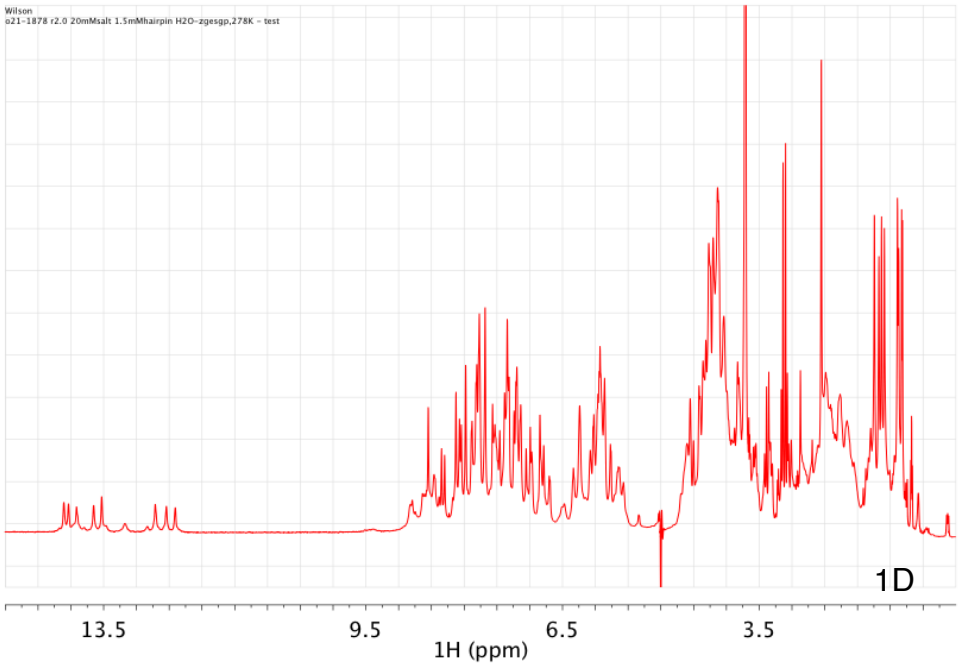


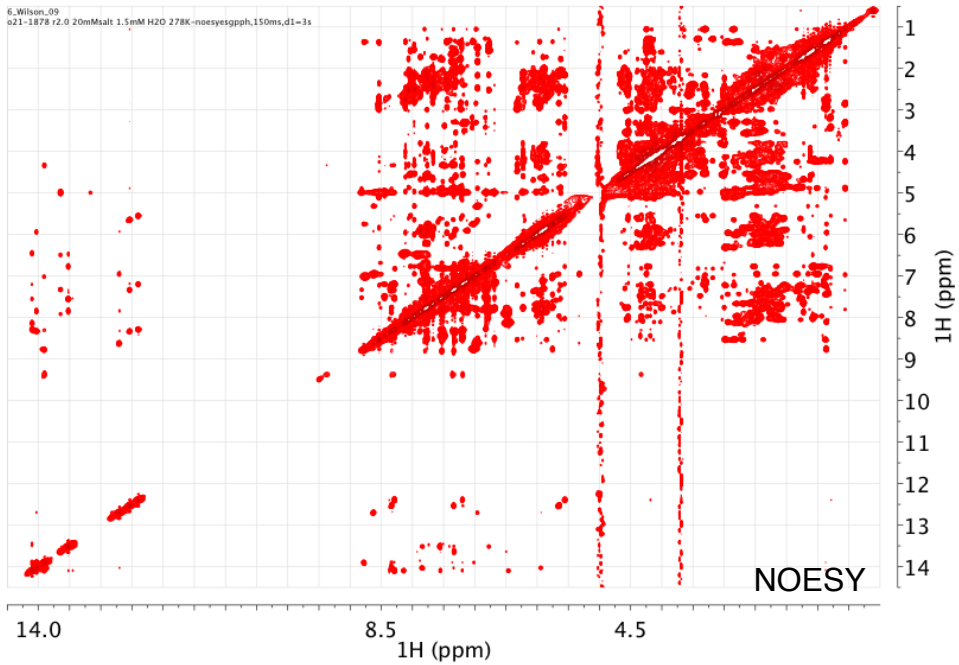

Pros: Excellent water suppression
Uniform excitation
Baseline flat

Cons: May loose broad resonances

# Exitation Sculpting

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



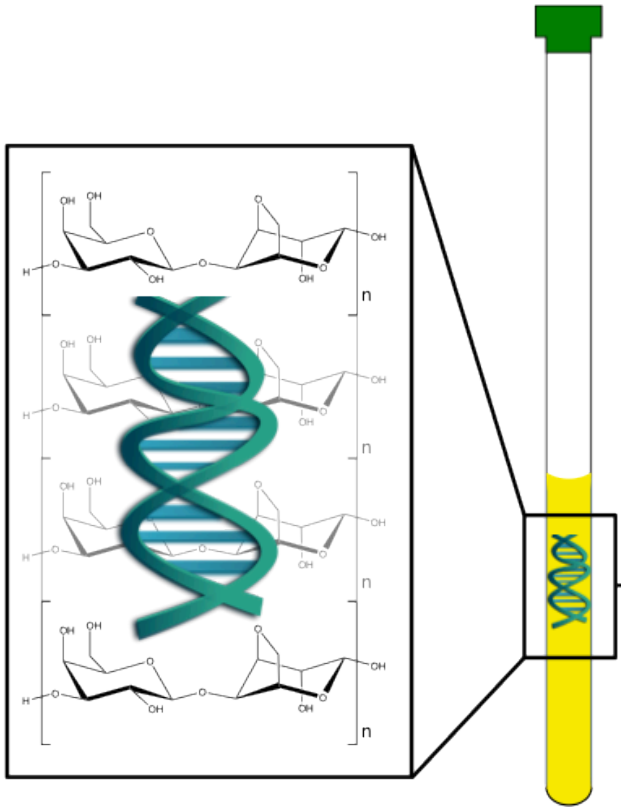

Pros: Easy to set up
Excellent water suppression
"ok" for broad signals!
Uniform excitation

Cons: May loose some intensity on very broad signals

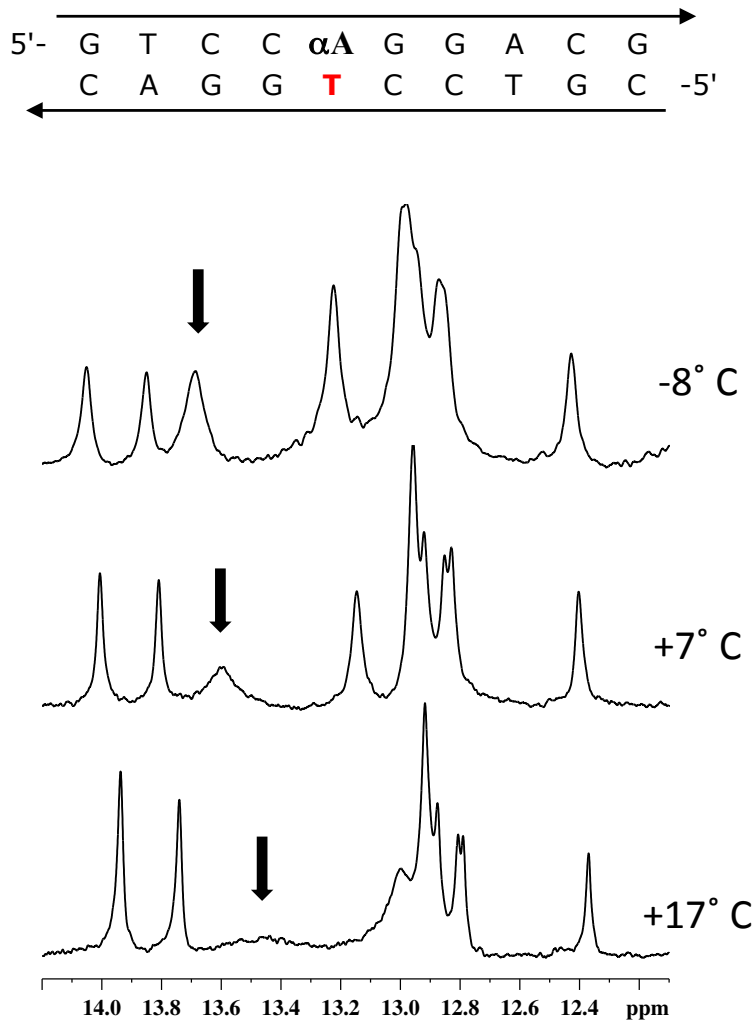
Spectra: 1.5mM DNA in Water, Nanjunda, Wilson and Germann unpublished

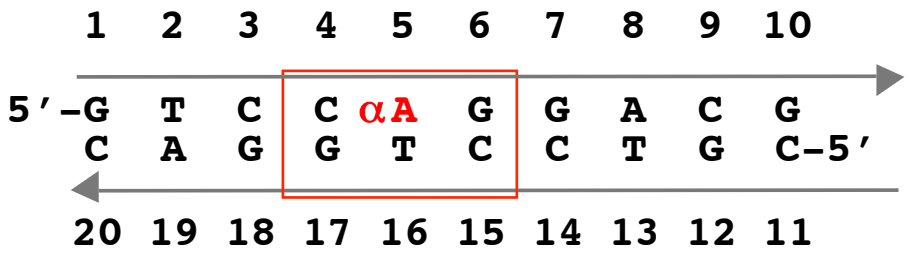
# Interesting structures have often broad imino protons.
# →Most modern techniques obliterate them.

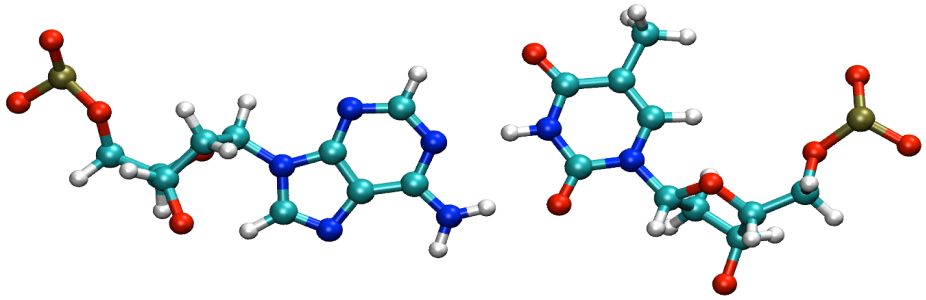
## Jump and return to the rescue
## + supercooled conditions

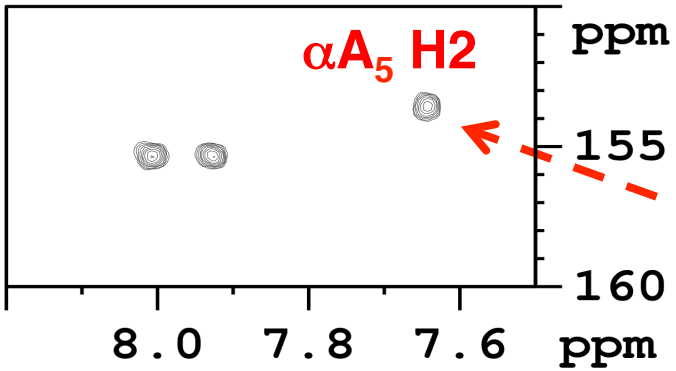


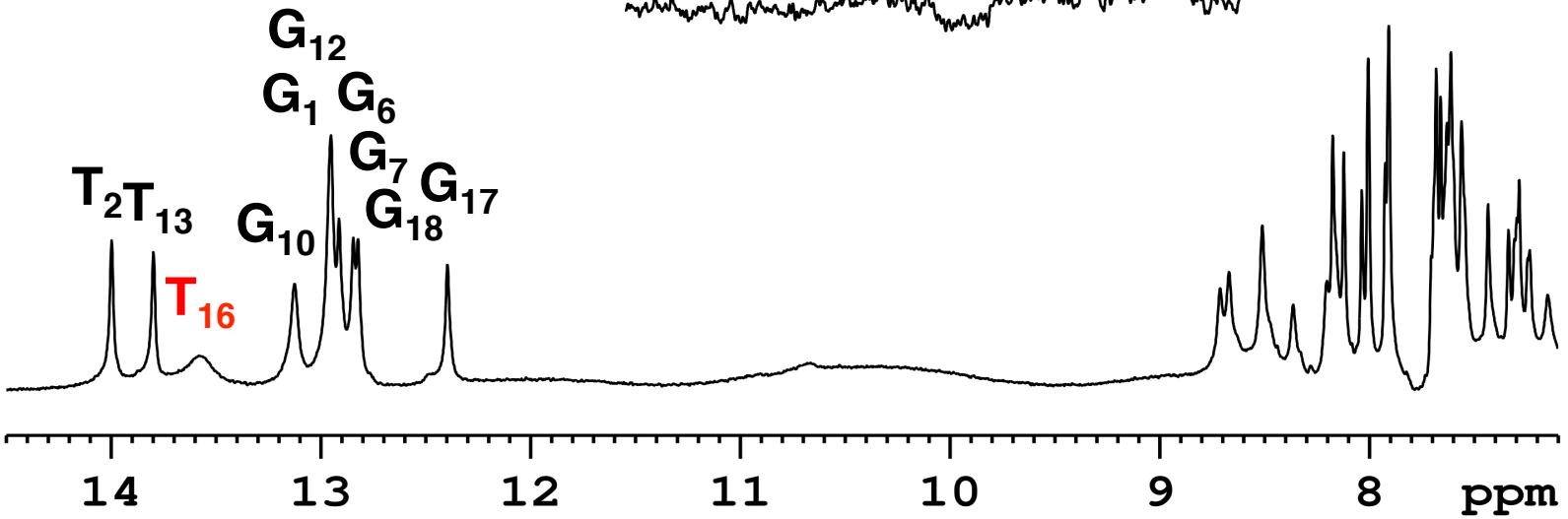
Spring, A.M. & Germann, M.W., *Anal. Biochem.*, 2012.

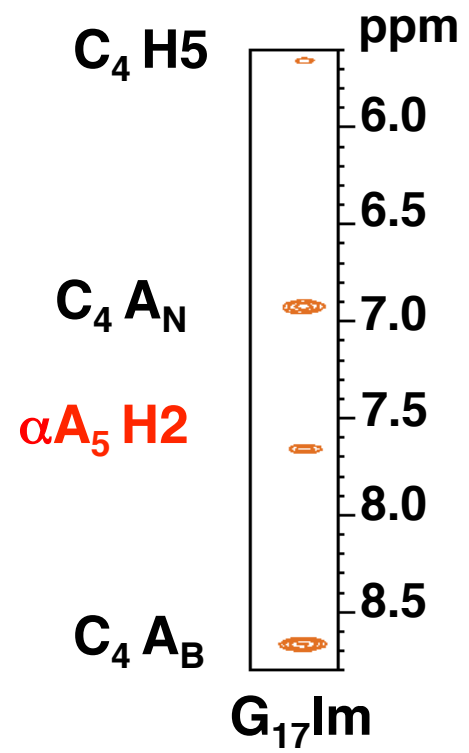



1 2 3 4 5 6 7 8 9 10
5′-G T C C αA G G A C G
C A G G T C C T G C-5′
20 19 18 17 16 15 14 13 12 11




αA5 H2
ppm
155
160
8.0 7.8 7.6 ppm


T2T13
T16
G10
G12
G1
G6
G7
G18
G17
14 13 12 11 10 9 8 ppm

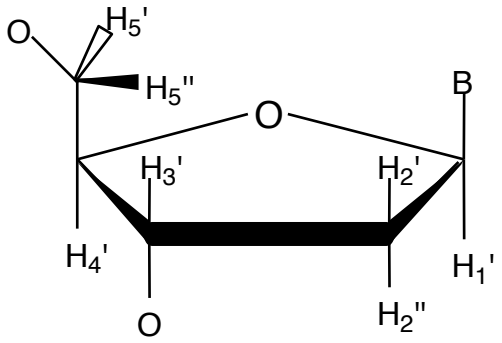

C4 H5
C4 AN
αA5 H2
C4 AB
ppm
6.0
6.5
7.0
7.5
8.0
8.5
G17Im

# Structure Determination, NMR experiments:

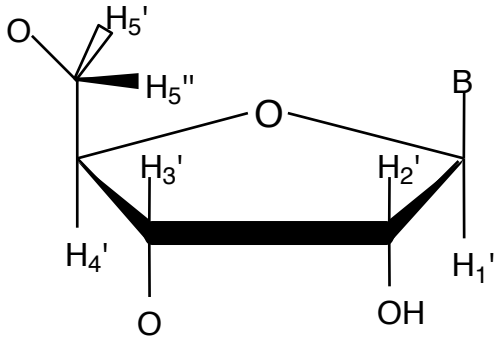
| | | |
|---|---|---|
| I) | Assignment | NOESY, COSY, HSQC TOCSY..... |
| II) | Local Analysis | |
| | •glycosidic torsion angle | (NOE, COSY) |
| | •sugar puckering | (COSY, COSY, NOE, +) |
| | •backbone conformation | (COSY, +) |
| | •base pairing | (NOE, COSY) |
| III) | Global Analysis | |
| | •sequential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

Black: unlabeled, Blue: labeled DNA or RNA

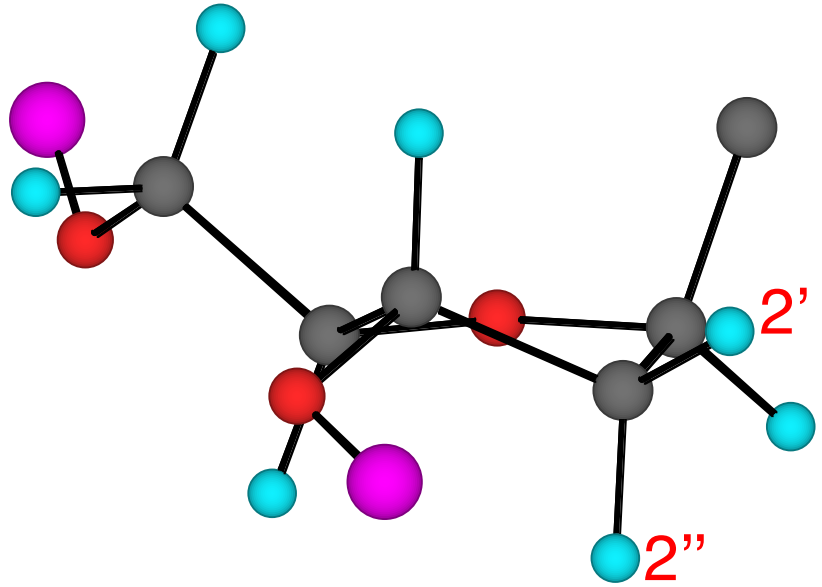
# Stereospecific Assignment

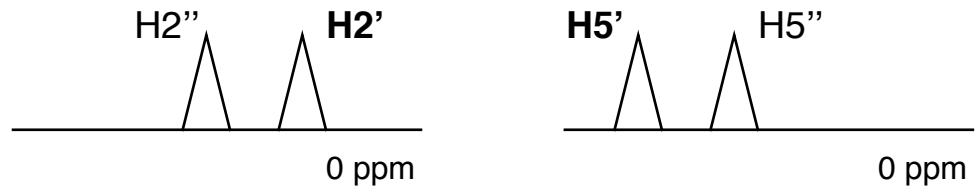



Deoxyribose




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

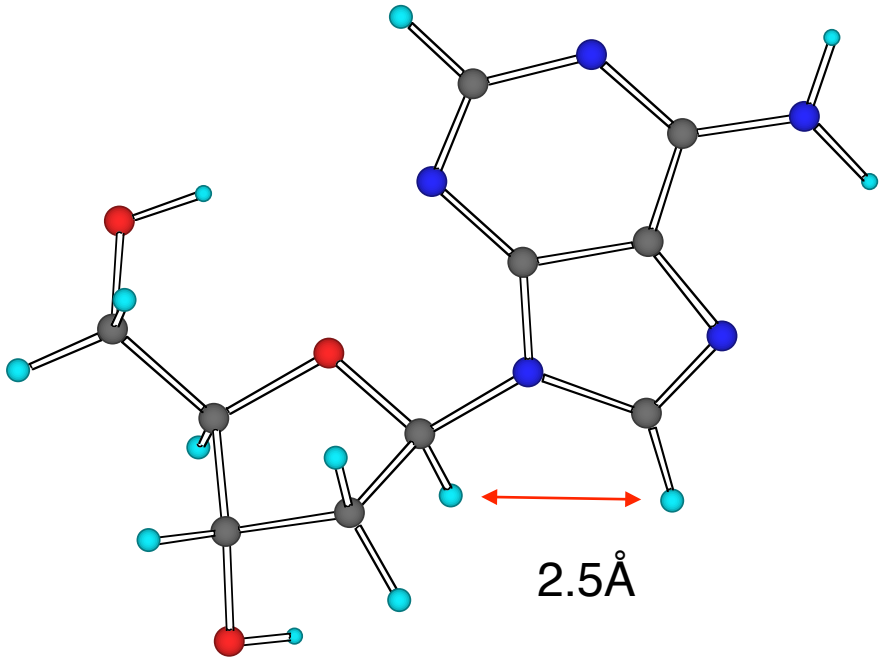
> account for spin diffusion

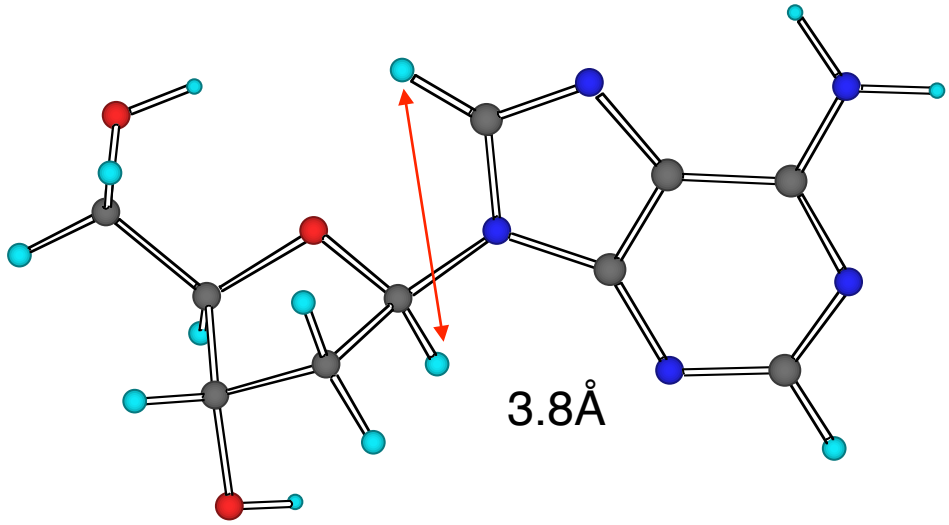
•Backbone may be difficult to fully characterize

> especially $\alpha$ and $\zeta$.

•Dipolar couplings

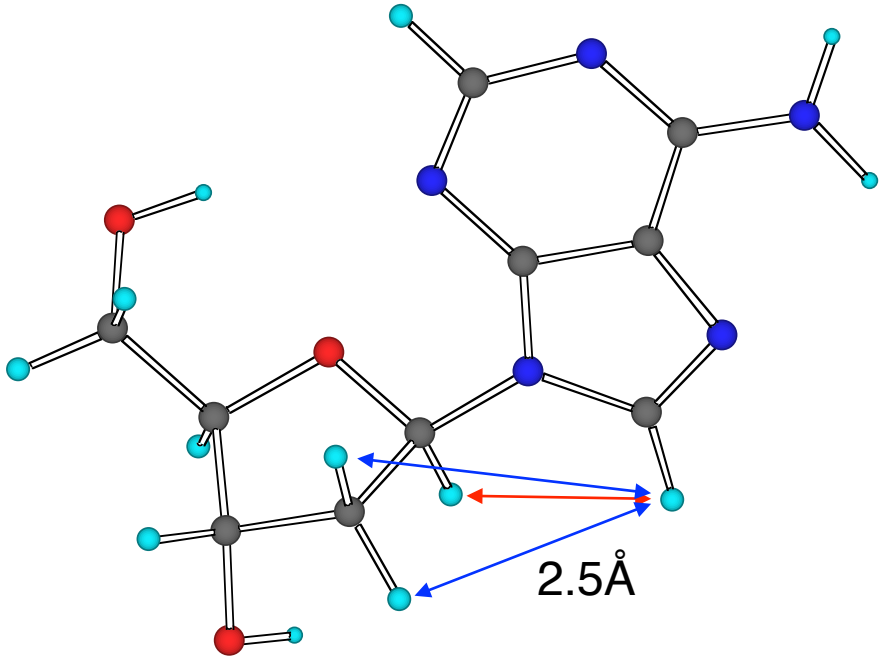
# Distance information determines the glycosidic torsion angle

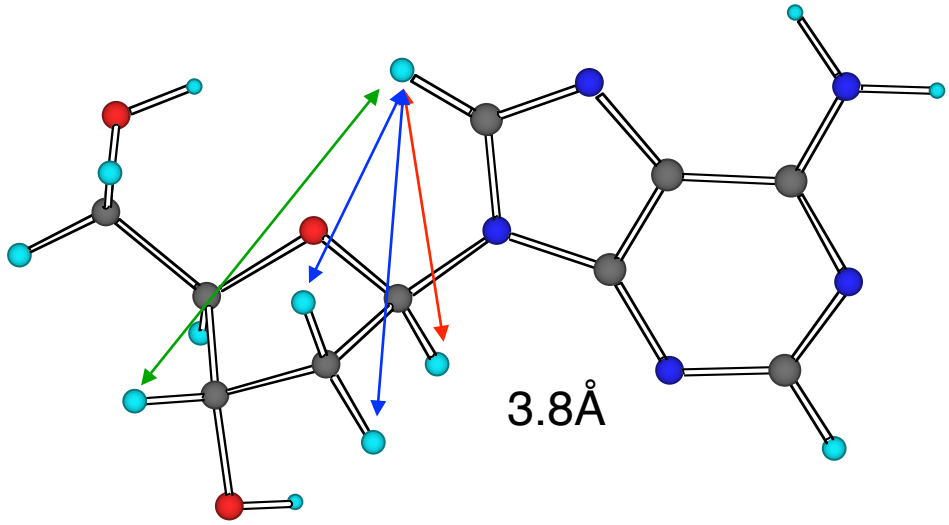



- How do we get distance information?
  - Nuclear Overhauser effect (< 6Å)

# Distance information determines the glycosidic torsion angle



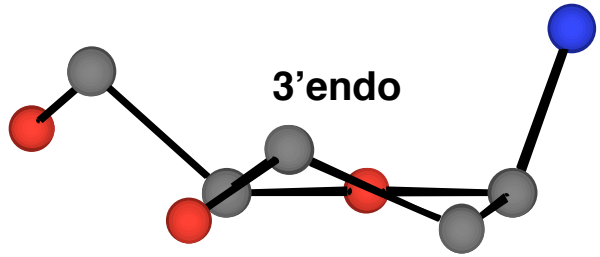




- How do we get distance information?
  - Nuclear Overhauser effect (< 6Å)

# 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).

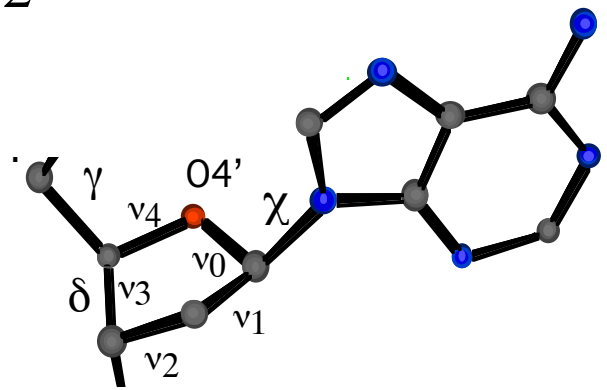
In general:
RNA (A type double helix) C3' endo.
DNA (B type double helix) C2' endo.

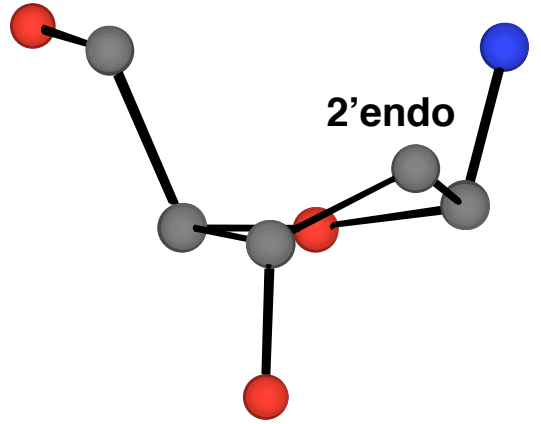



$$\nu_j = \Phi_m \cos(P + 144\,(j-2))$$

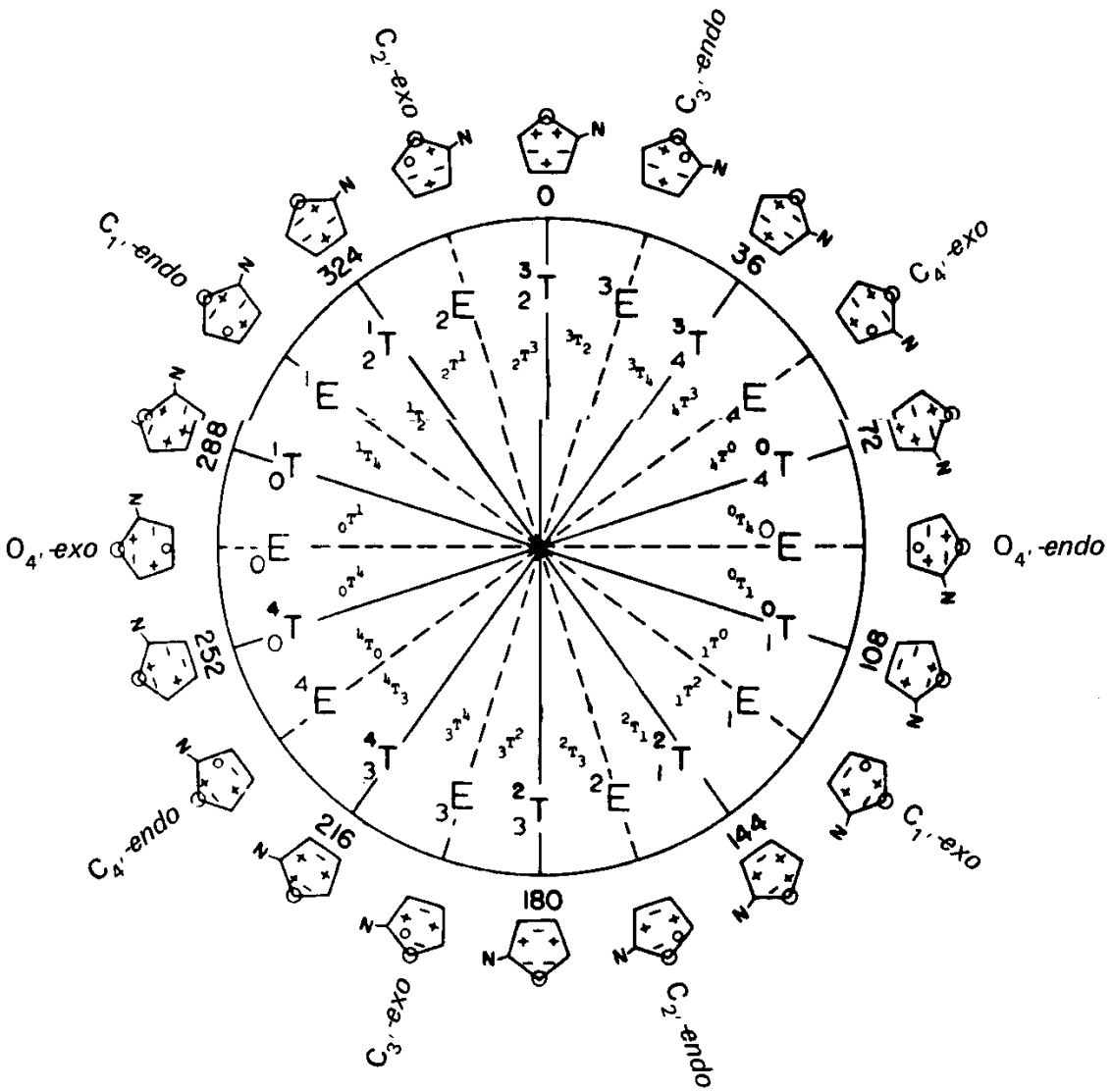
$\Phi_m$ range: 34° - 42°

$$\delta = \nu_3 + 125°$$

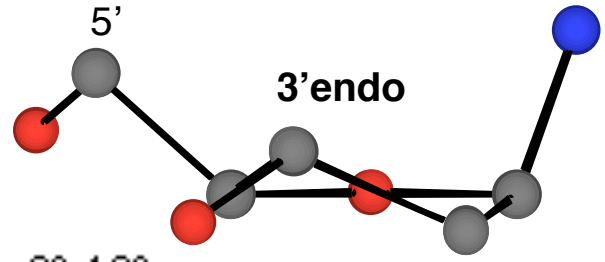


**N (Northern)**



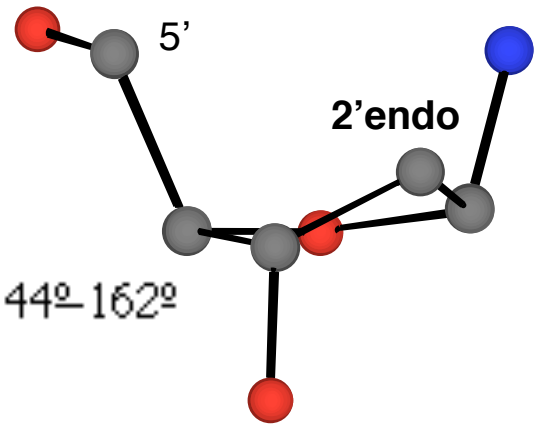

**(Southern)**




P=0º–18º

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

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

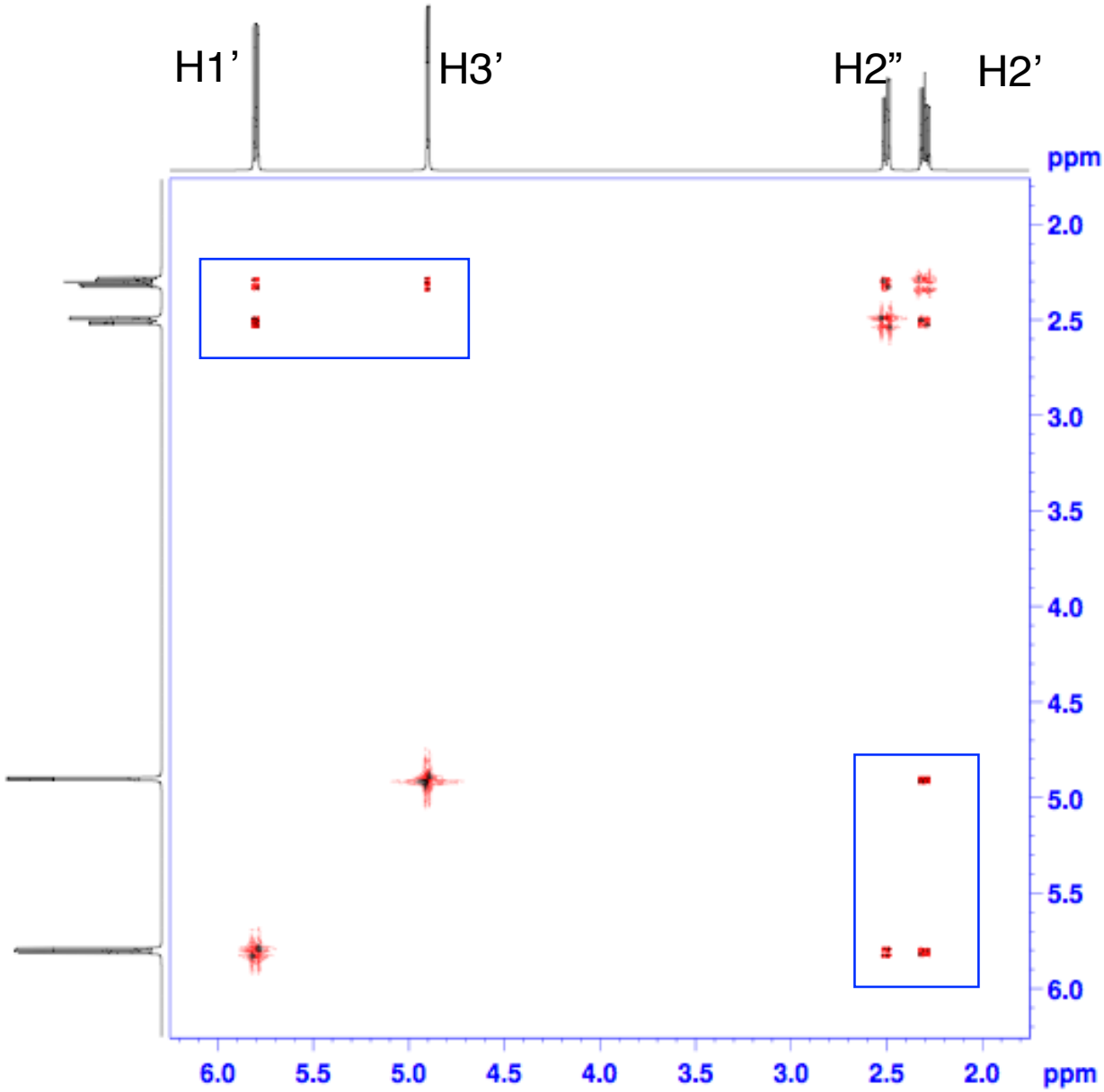



P=144º–162º

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

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

# 2’endo sugar H1’, H2’, H2”, H3’ region

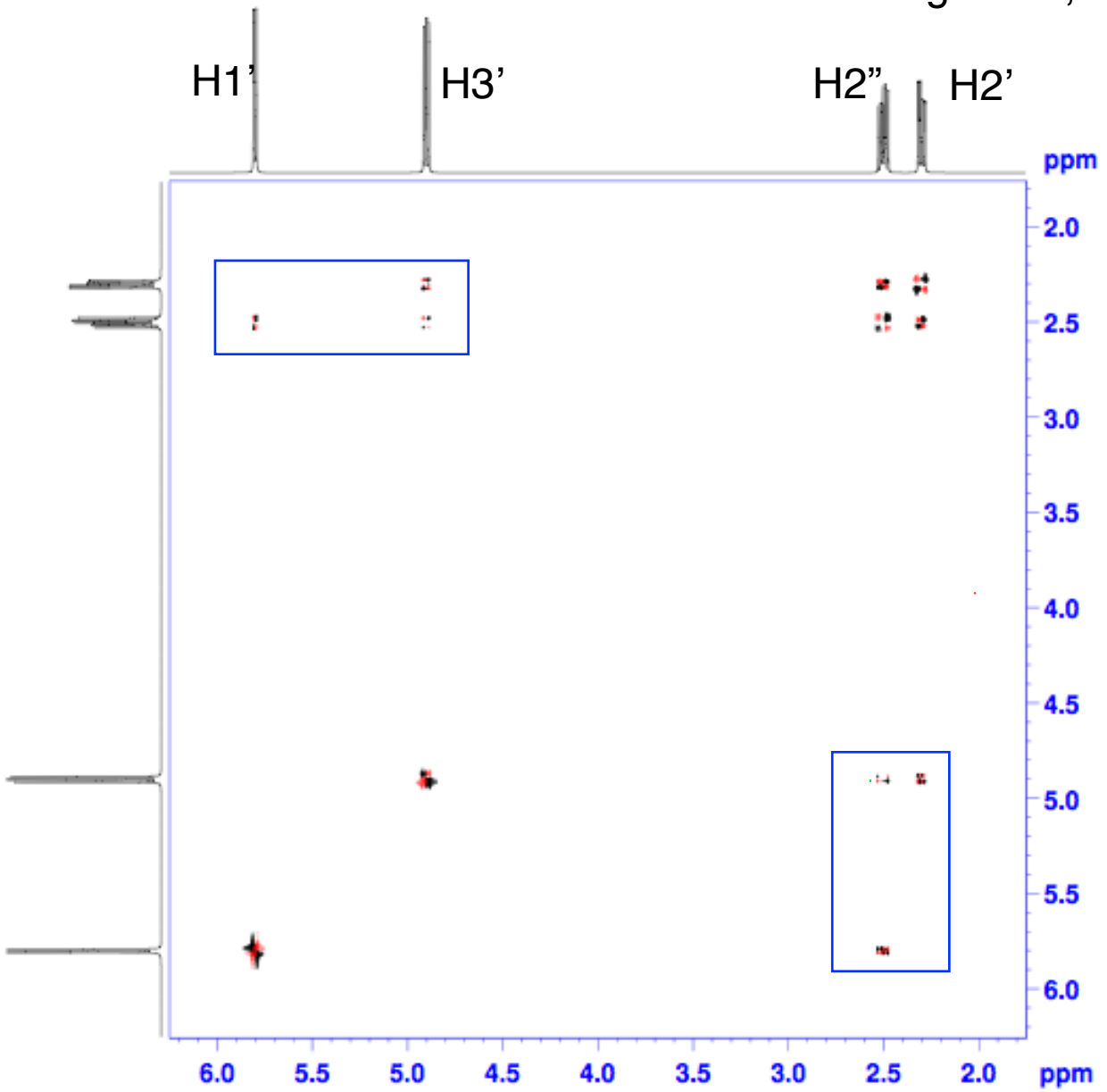



```
NO RELAXATION 1KX1K;TOPSPIN
(Tdeff 900x400)
============================
;NMR-Sim sample spin system
;Deoxyribose 2’endo S conf
;from Widmer and Wuthrich
;
proton a   5.80 ;H1’
proton b   2.30 ;H2’
proton c   2.50 ;H2’’
proton d   4.90 ;H3’

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
```

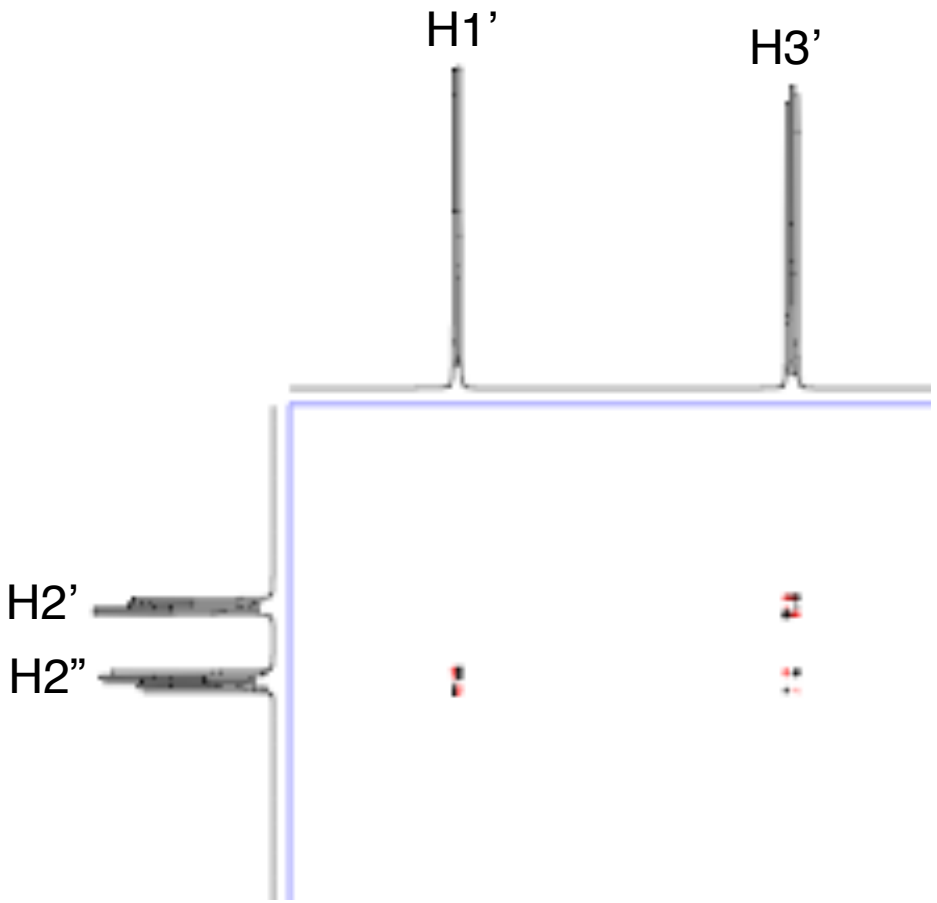
Widmer,H. and Wüthrich,K. (1987) J. Magn. Reson., 74, 316-336.

# 3’endo sugar H1’, H2’, H2”, H3’ region

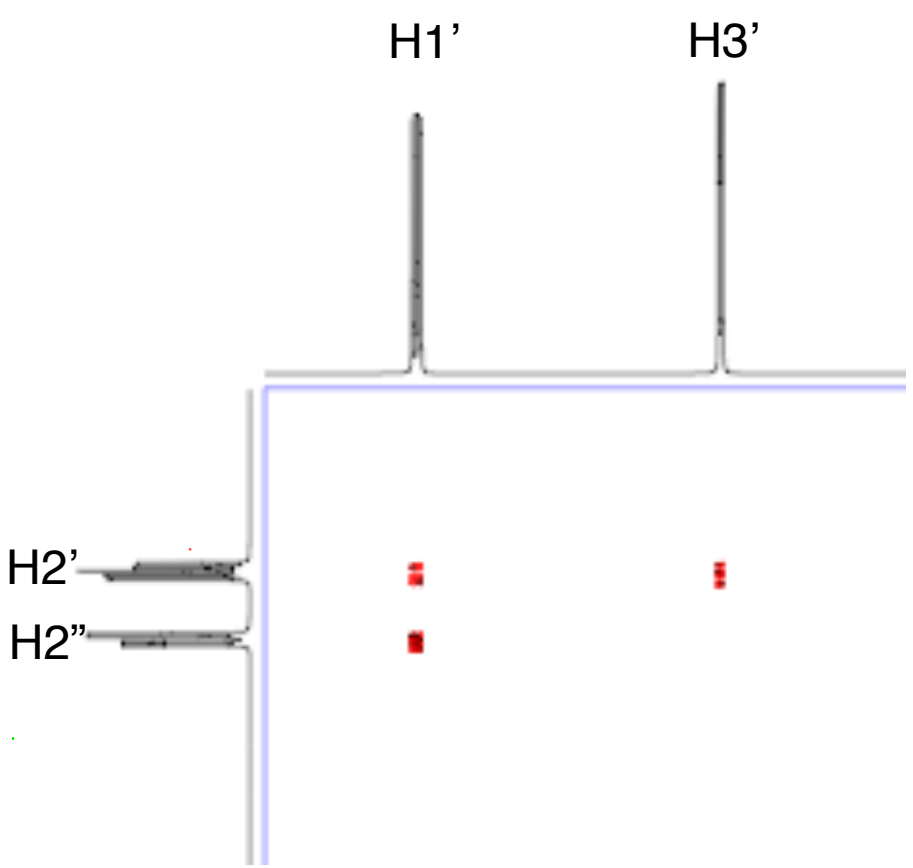



```
No relaxation Tdeff 900x400
===========================
;NMR-Sim sample spin system
;Deoxyribose 3’endo N conf
;Widmer and Wuthrich
;
proton a   5.80 ;H1’
proton b   2.30 ;H2’
proton c   2.50 ;H2’’
proton d   4.90 ;H3’
;
couple a b 1.5
couple a c 7.7
couple b c -14.1
couple b d 7.2
couple c d 9.7
```

# Sugar puckering



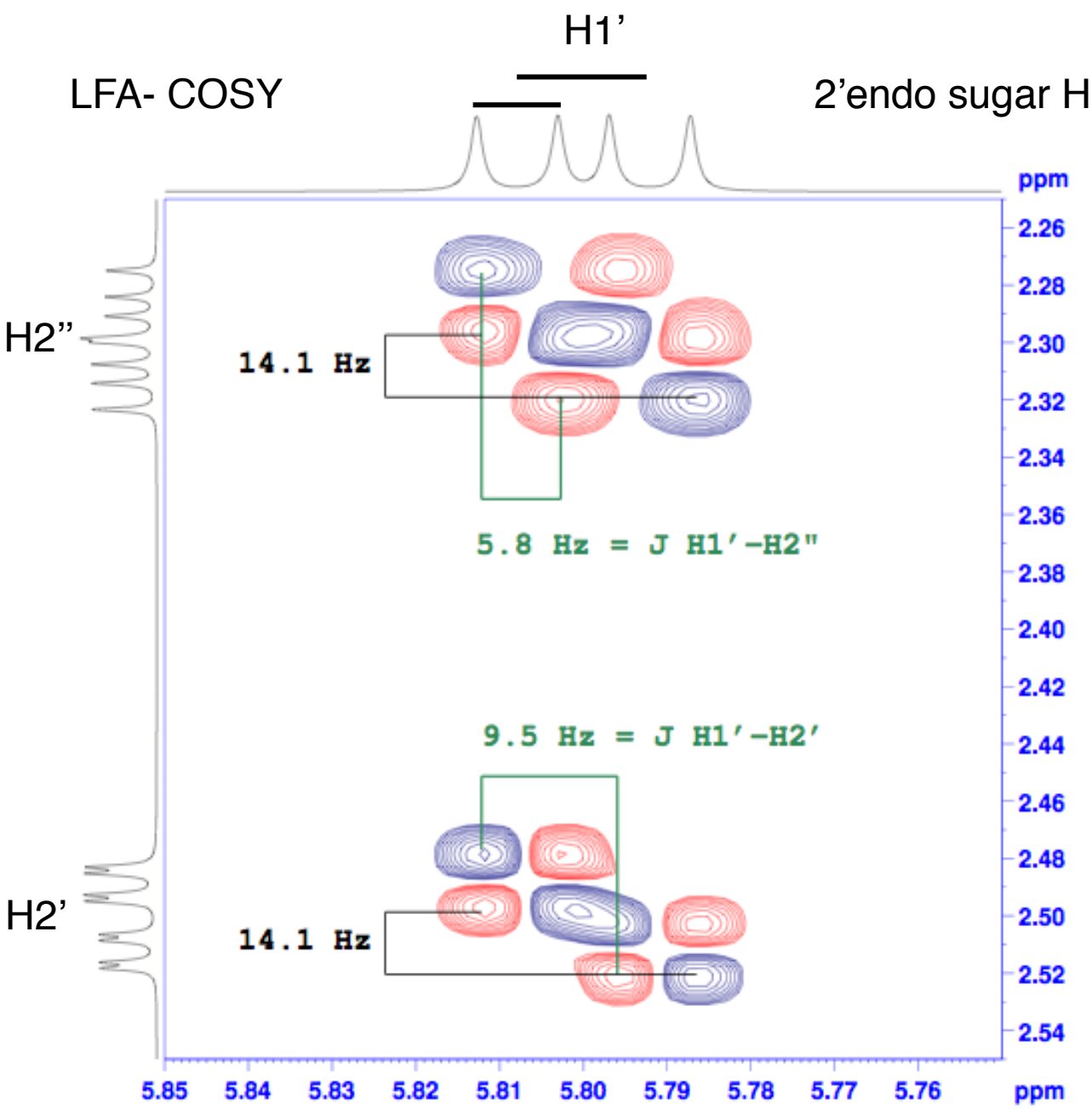

3’endo sugar

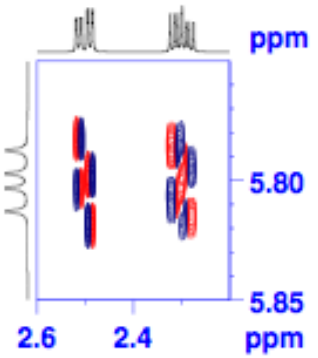



2’endo sugar

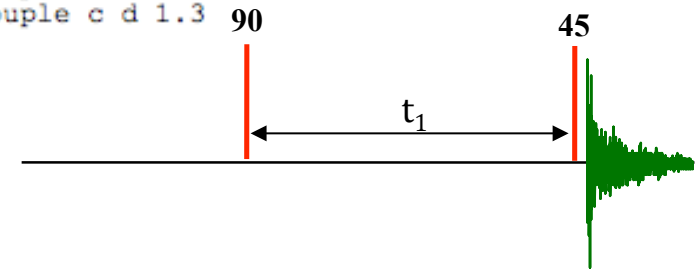
LFA- COSY

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

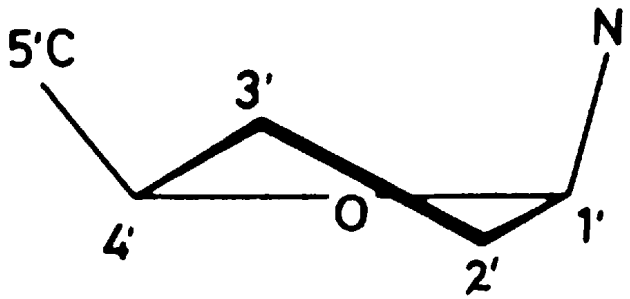



```
NO RELAXATION 2KX2K (Tdeff
==========================
; NMR-Sim sample spin syst
; Deoxyribose 2'endo S con
;
proton a   5.80 t=0.5    ;H1
proton b   2.30 t=0.5    ;H2
proton c   2.50 t=0.5 ;H2''
proton d   4.90 t=0.5 ;H3'
;
couple a b 9.5
couple a c 5.8
couple b c -14.1
couple b d 5.5
couple c d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

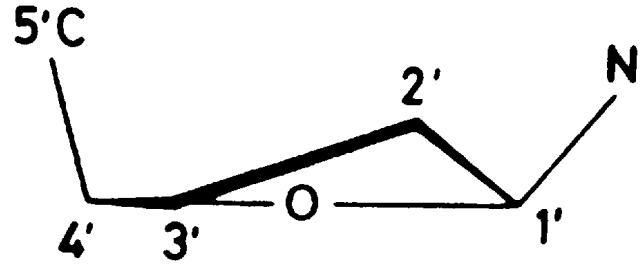


# Sugar puckering



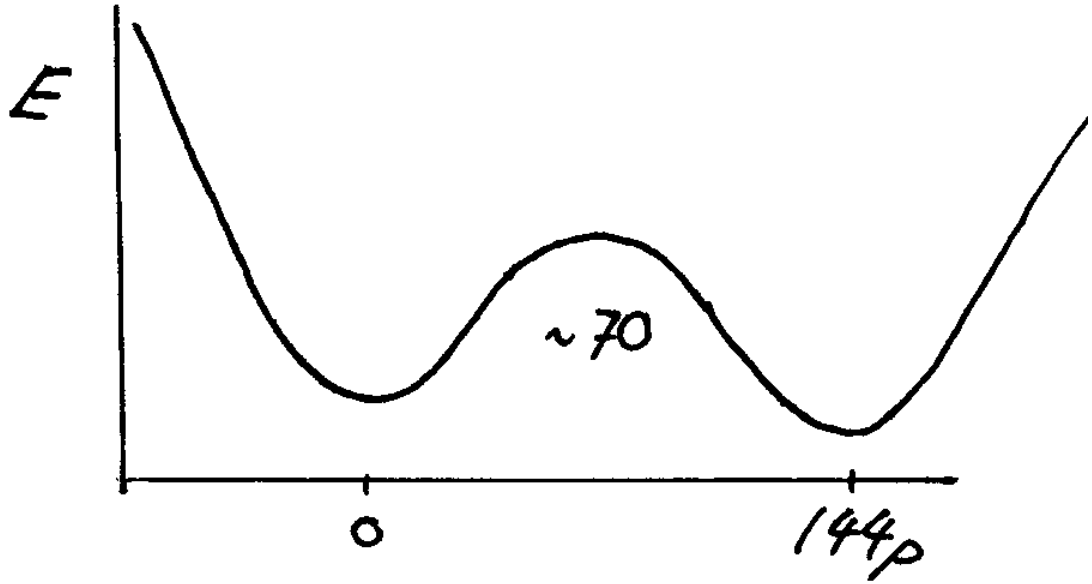

**C3´-endo C2´-exo: P = 0°**

$J_{1'2'}$ 2 Hz (with P = 9) N




**C2´-endo: P = 162°**

$J_{1'2'}$ 9 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.

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

See also our pure examples:
$f_S$=0 and ~1 respectively

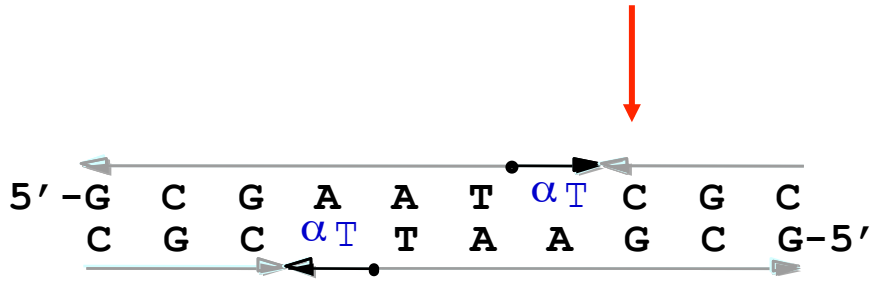
$$\Sigma\, 1' = J_{1'2'} + J_{1'2''}$$
$$\Sigma\, 2' = J_{1'2'} + J_{2'3'} + J_{2'2''}$$
$$\Sigma\, 2'' = J_{1'2''} + J_{2''3'} + J_{2'2''}$$
$$\Sigma\, 3' = J_{2'3'} + J_{2''3'} + J_{3'4'}$$

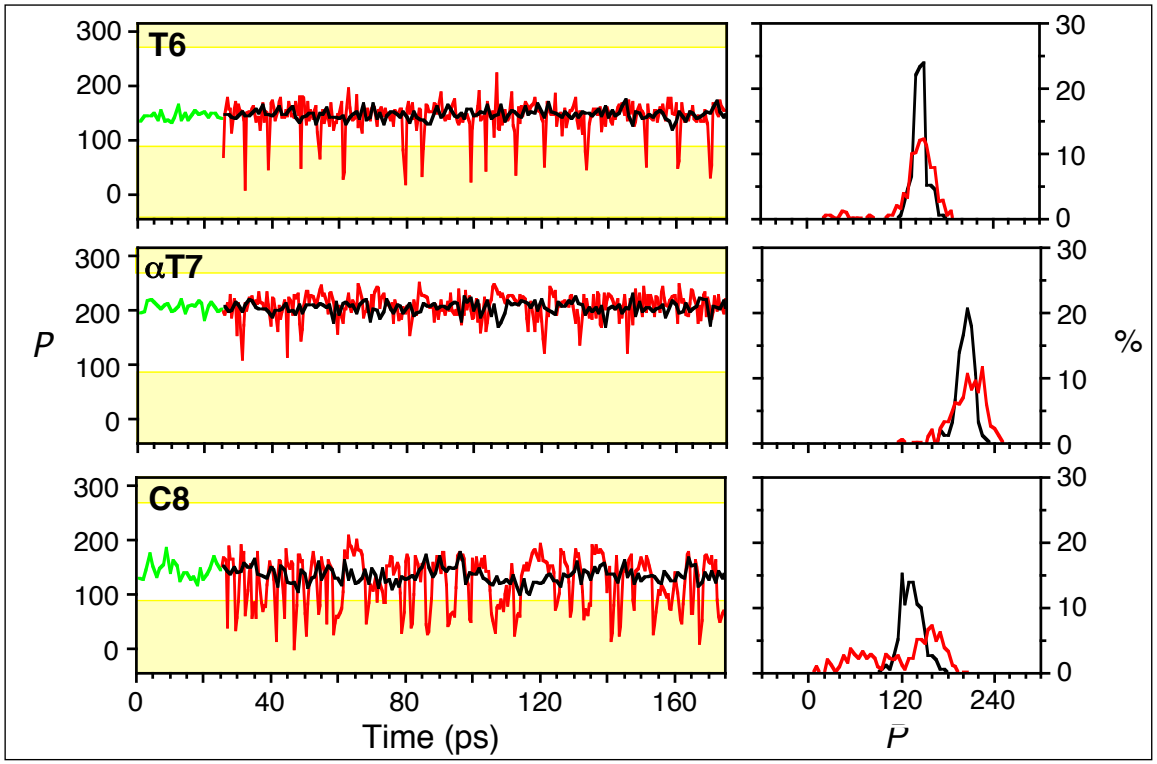
If fs < 50% $J_{1',2'} < J_{1',2''}$
If fs ca 0% $J_{1',2'}$ very small
If fs > 70% $J_{1',2'} > J_{1',2''}$

# Sugar puckering

| | control | | alphaT | |
|---|---|---|---|---|
| Nt | Σ1′ | *f*s | Σ1′ | *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) |

**alphaT**



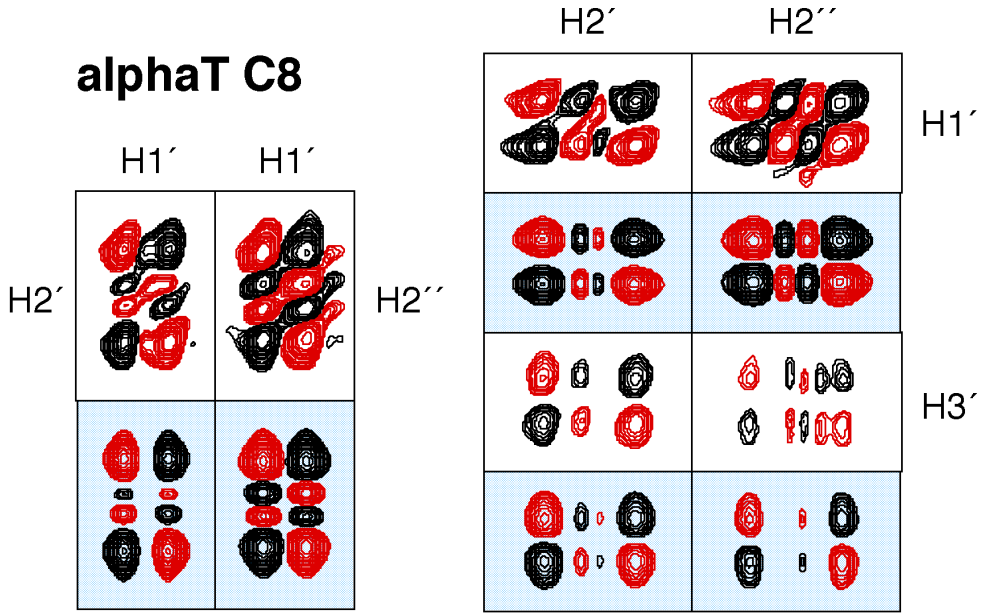



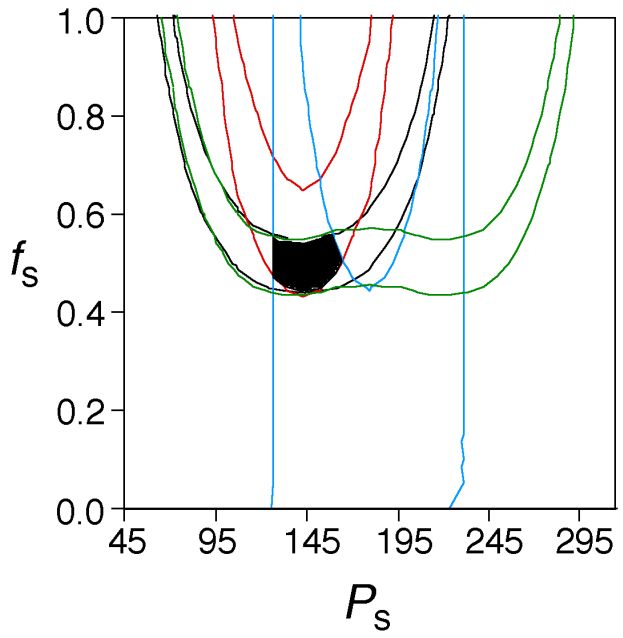

MD calculation
MD-Tar calculation

Aramini, 2000, J. Biomolecular NMR, 18, 287-302

# Pseurot calculations

## alphaT C8

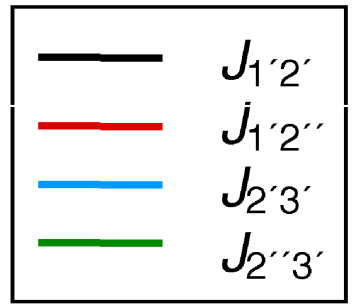


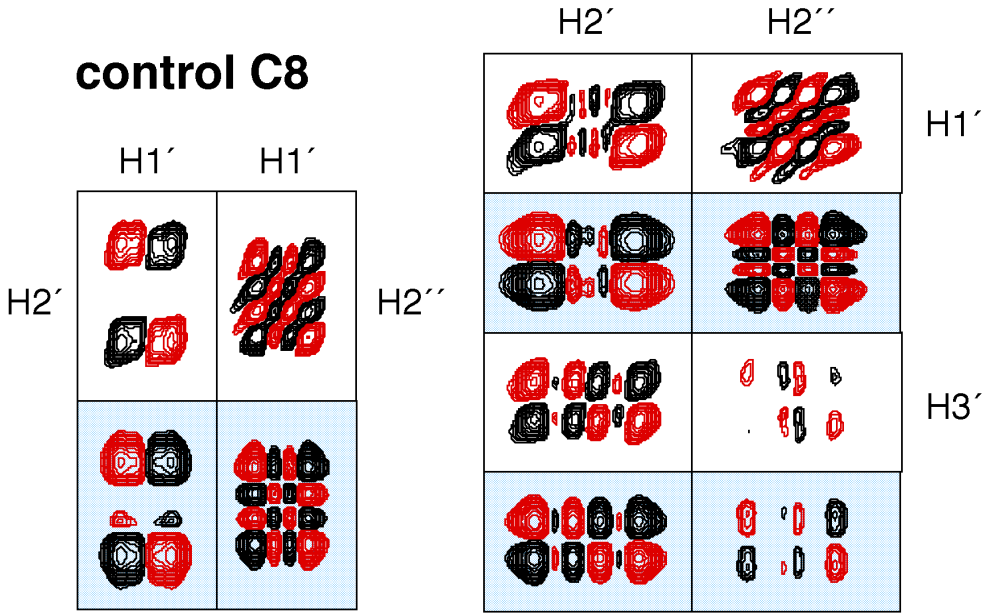

$\Phi_{S,N}$ = 37°

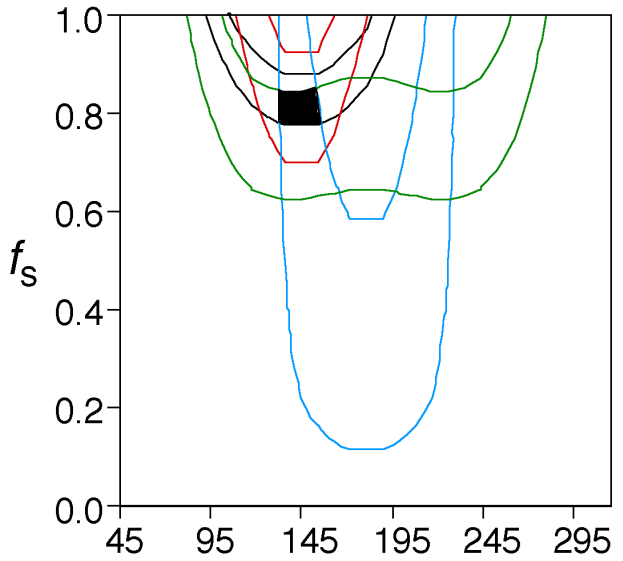
$P_S$ = 125-165

$f_S$ = 0.44-0.55




## control C8




$\Phi_{S,N}$ = 37°

$P_S$ = 130-155

$f_S$ = 0.78-0.86

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

Aramini, et al., 1998, Nucleic Acid Research, 26, 5644-5654

# Introduction to Cross-Correlated Relaxation

**Relaxation in NMR**

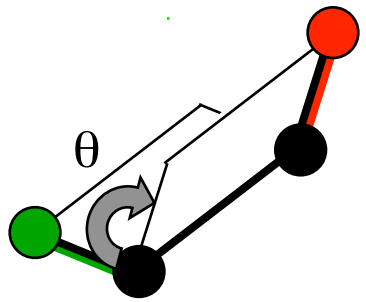
→ determines experimental strategies and experiments
→ dynamic and structural parameters

**Mechanisms**

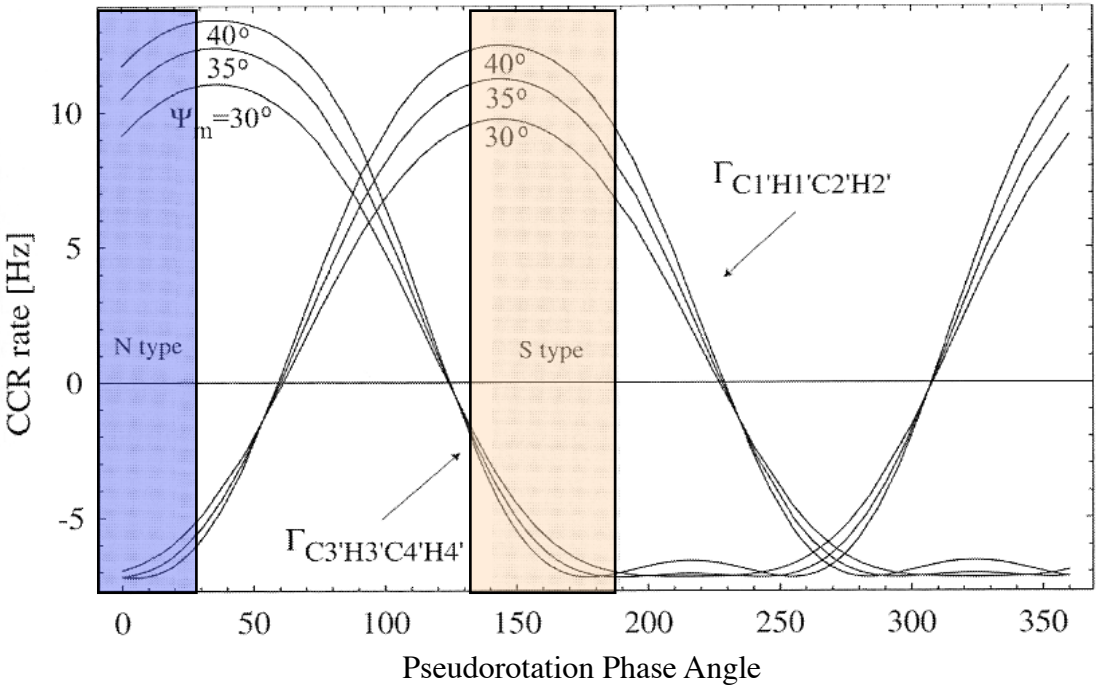
→ Dipole -dipole
→ CSA (e.g. $^{31}P$ at higher fields; proportional to $B^2$)
→ Scalar relaxation (first and second kind)
→ paramagnetic, etc

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

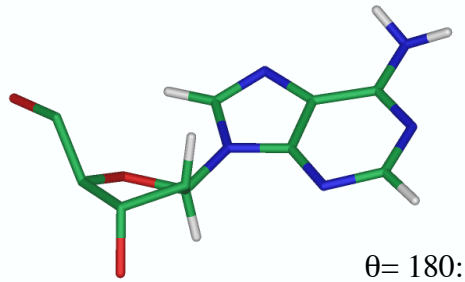


# Sugar Puckering from Cross-Correlated Relaxation $\Gamma_{DD\text{-}DD}$

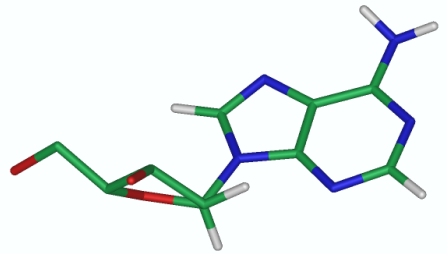



$$\theta_{1'2'} = 121.4° + 1.03\ \psi_m \cos(P - 144°)$$

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



θ= 180: for 2'endo (B form)
Large and positive



θ= 90: for 3'endo (A form)
Small and negative

BioNMR in Drug Research (2003) Chapter 7 p147-178. Christian Griesinger

# Sugar puckering: Summary

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

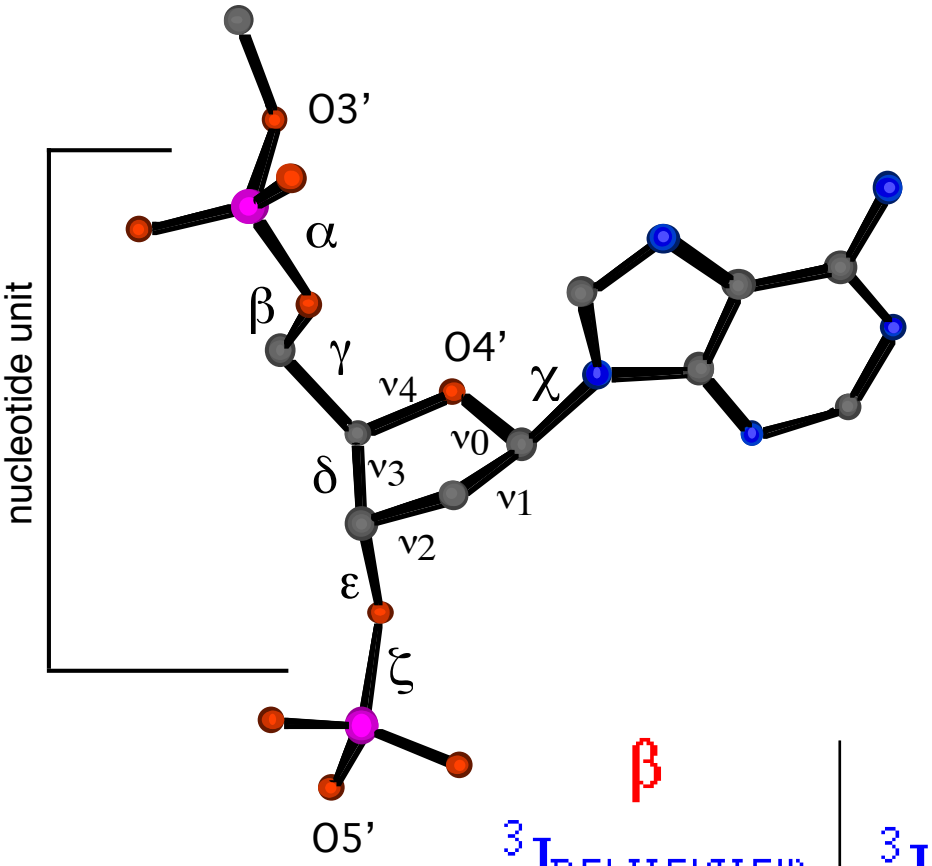
## Some references


Szyperski, T., et al. (1998). JACS. 120, 821- 822.
Measurement of Deoxyribose $^{3}JHH$ 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 α and ζ)

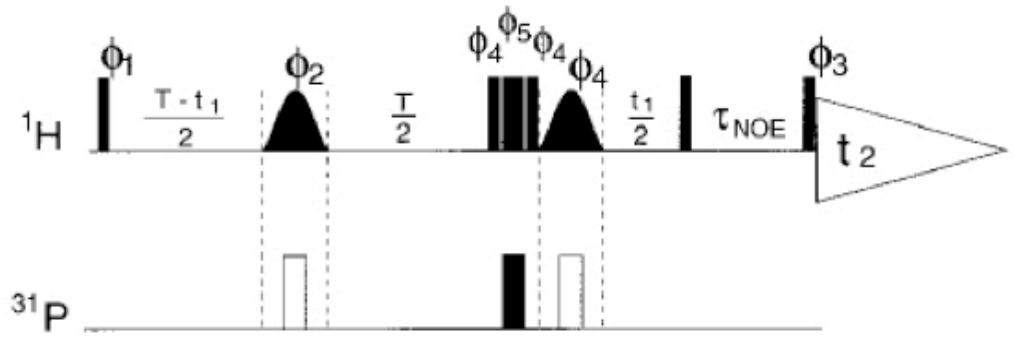


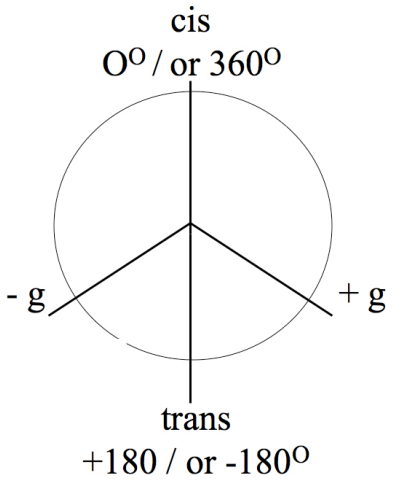

α and ζ pose problems
Determinants of $^{31}P$ chem shift.

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) |
| | **+ NOE** | | **+ NOE** |

# Backbone Experiments: CT-NOESY, CT-COSY




Bax, A., Tjandra, N., Zhengrong, W., ( 2001). Measurements of $^{1}H$-$^{31}P$ dipolar couplings in a DNA oligonucleotide by constant time NOESY difference spectroscopy, *J. Mol. Biol.,* **19,** 367-270. 91.