

# Heteronuclear NMR of Nucleic Acids

In most cases, requires samples isotopically enriched by  $^{13}\text{C}$  and  $^{15}\text{N}$  (except for HSQC, HMQC)

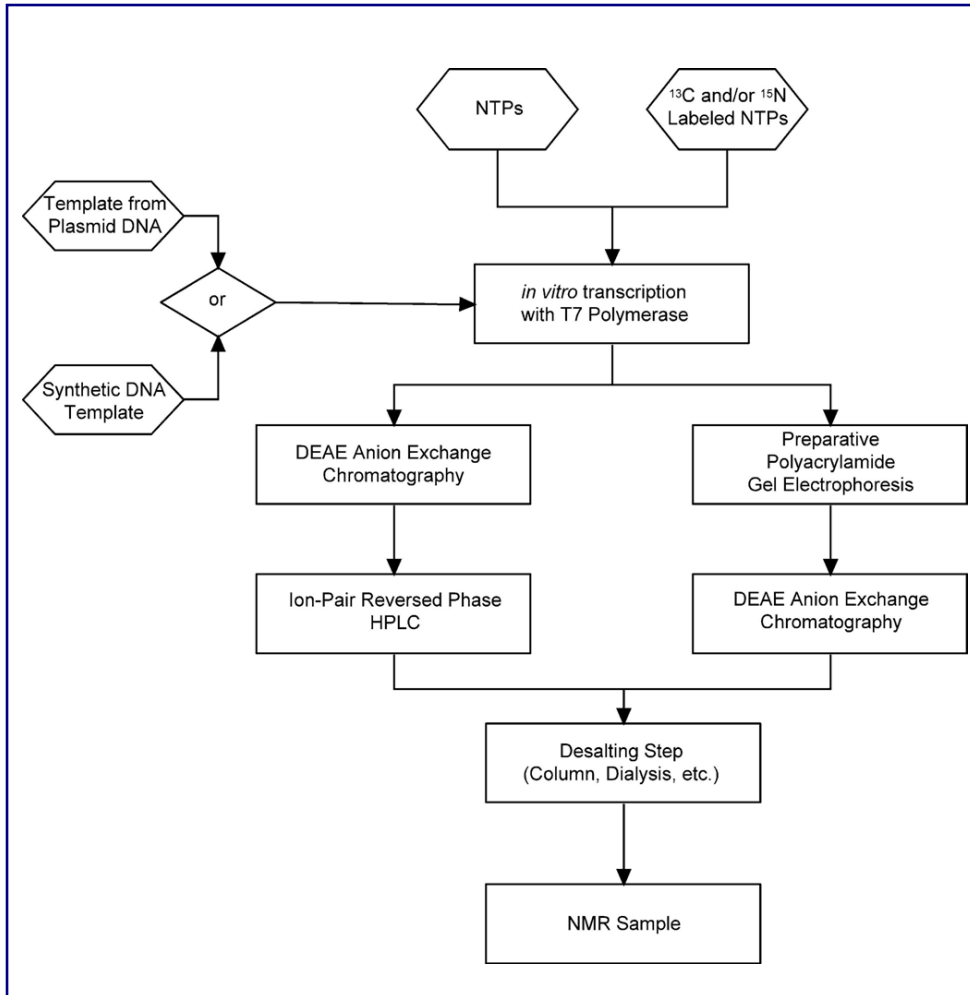
Assignment uses NOE or through-bond experiments

Traditional constraints (NOEs, J-couplings)

Novel constraints (RDCs, residual CSA)

Studies of intramolecular dynamics

# RNA preparation



**Labeled NTPs**

Available commercially

Can be grown in cells

E. Coli

<sup>13</sup>C-glucose, <sup>15</sup>N-ammonium

M. Methylotropus

<sup>13</sup>C-methanol, <sup>15</sup>N-ammonia

# Assignment procedure for labeled NA NOE based (I)

## I. (H<sub>2</sub>O)

### Correlation of exchangeable protons with <sup>15</sup>N

2D <sup>1</sup>H-<sup>15</sup>N HSQC NH imino optimized (Gua and Ura)

2D <sup>1</sup>H-<sup>15</sup>N HSQC NH<sub>2</sub> amino optimized (Cyt, Gua, Ade)

### Sequential assignment of exchangeable protons

3D NOESY-HSQC (<sup>1</sup>H-<sup>1</sup>H-<sup>15</sup>N, imino <sup>15</sup>N edited NOESY)

imino-imino and imino-amino interactions

3D NOESY-HSQC (<sup>1</sup>H-<sup>1</sup>H-<sup>15</sup>N, amino <sup>15</sup>N edited NOESY)

amino-imino interactions



## II. (H<sub>2</sub>O)

### Assignment of non-exchangeable protons with NOE connectivities to imino and amino protons

3D NOESY-HSQC (<sup>1</sup>H-<sup>1</sup>H-<sup>15</sup>N, imino/amino <sup>15</sup>N edited NOESY)

interactions of aromatic protons with imino and amino groups



# Assignment procedure for labeled NA NOE based (II)

**III. ( $^2\text{H}_2\text{O}$ ) Identification of hydrogen and carbon atoms in sugars**  
2D  $^1\text{H}$ - $^{13}\text{C}$  CT-HSQC identification of H-C pairs  
3D HCCH-COSY identification of neighboring C-H groups  
3D HCCH-RELAY H1'-C2'/C3' correlation  
3D HCCH-TOCSY H1'-C2'/C3'/C4'/C5' correlation

**Identification of hydrogen and carbon atoms in bases**  
2D  $^1\text{H}$ - $^{13}\text{C}$  CT-HSQC identification of H-C pairs  
2D/3D HCCH-COSY H5-H6 and C5-C6 correlations in pyrimidines

**Sequential assignment**  
3D NOESY-HSQC ( $^1\text{H}$ - $^1\text{H}$ - $^{13}\text{C}$ ), H6/8-H1', H6/8-H2' correlations

**IV. ( $^2\text{H}_2\text{O}$ ) Assignment of  $^{31}\text{P}$  resonances**  
 $^1\text{H}$ - $^{31}\text{P}$  HETCOR/heteroTOCSY

# Assignment procedure for labeled NA Through bond correlations (I)

I. (H<sub>2</sub>O) **Correlation of exchangeable protons with <sup>15</sup>N**  
2D <sup>1</sup>H-<sup>15</sup>N HSQC **NH imino optimized (Gua and Ura)**  
2D <sup>1</sup>H-<sup>15</sup>N HSQC **NH<sub>2</sub> amino optimized (Cyt, Gua, Ade)**

II. (H<sub>2</sub>O) **Correlation of imino and amino protons with non-exchangeable base protons**  
HCCNH-TOCSY / HNCCH-TOCSY

# Assignment procedure for labeled NA

## Through bond correlations (II)

### III. ( $^2\text{H}_2\text{O}$ )

#### Correlation of non-exchangeable protons with $^{13}\text{C}$

2D  $^1\text{H}$ - $^{13}\text{C}$  CT-HSQC      identification of H-C pairs  
3D HCCH-COSY      identification of neighboring C-H groups  
3D HCCH-TOCSY      H1'-C2'/C3'/C4'/C5' correlation

#### Identification of hydrogen and carbon atoms in bases

2D  $^1\text{H}$ - $^{13}\text{C}$  CT-HSQC      identification of H-C pairs  
2D/3D HCCH-COSY      H5-H6 and C5-C6 correlations in pyrimidines  
HCCH-TOCSY /  $^1\text{H}$ - $^{13}\text{C}$  HMBC      H2-H8 correlations in Ade

#### Sugar-base correlations

$\text{H}_s\text{C}_s\text{N}$  and  $\text{H}_b\text{C}_b\text{N}$   
 $\text{H}_s\text{C}_s\text{NC}_b\text{H}_b$  /  $\text{H}_s\text{C}_s\text{NH}_b$

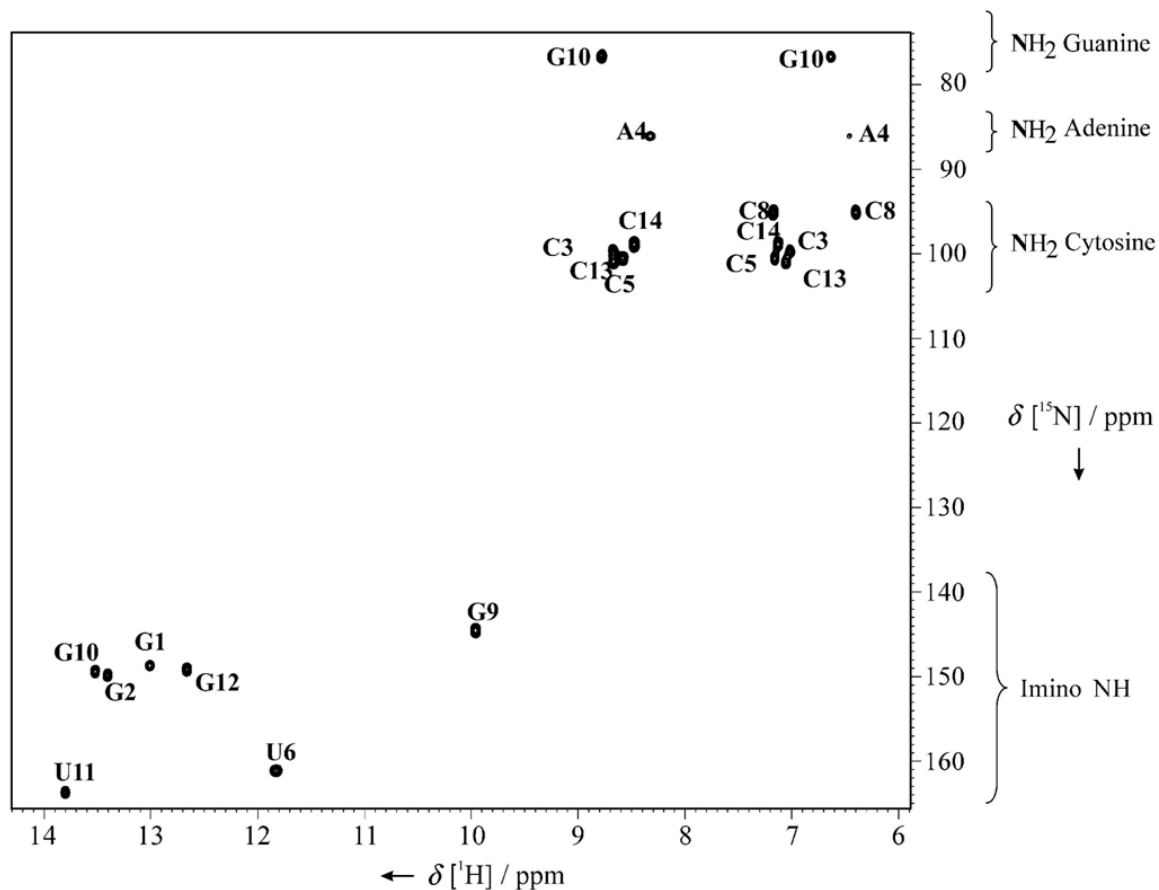
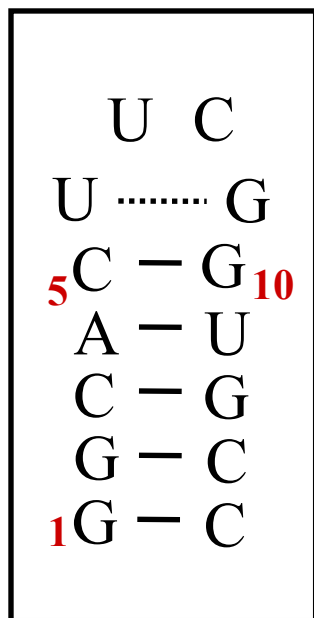


### IV. ( $^2\text{H}_2\text{O}$ )

#### Sequential assignment of $^{31}\text{P}$ resonances across the sugar-phosphate backbone

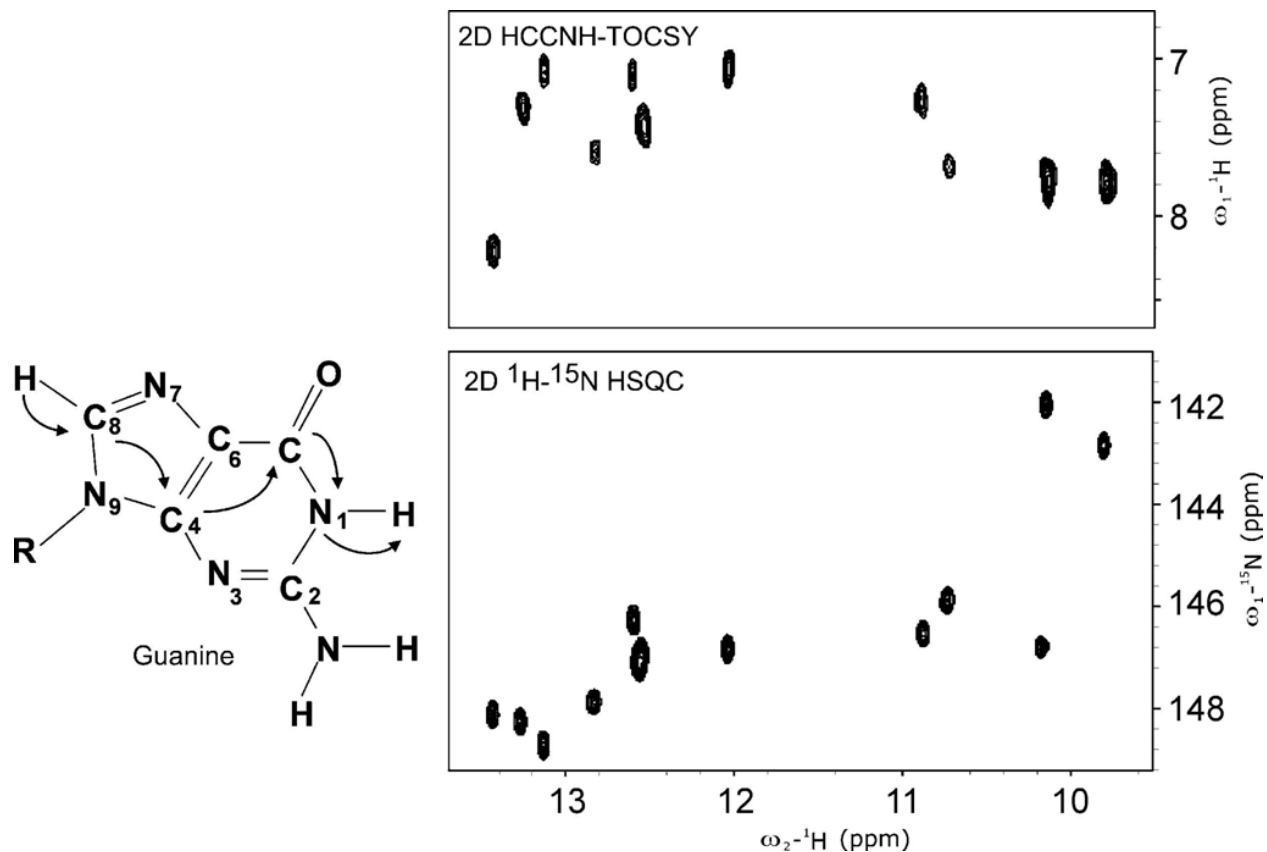
HCP / PCH / PCCH-TOCSY / HPHCH

# Correlation of exchangeable protons with $^{15}\text{N}$



Gradient sensitivity-enhanced HSQC  
 Kay, Keifer, Saarinen, JACS 1992.

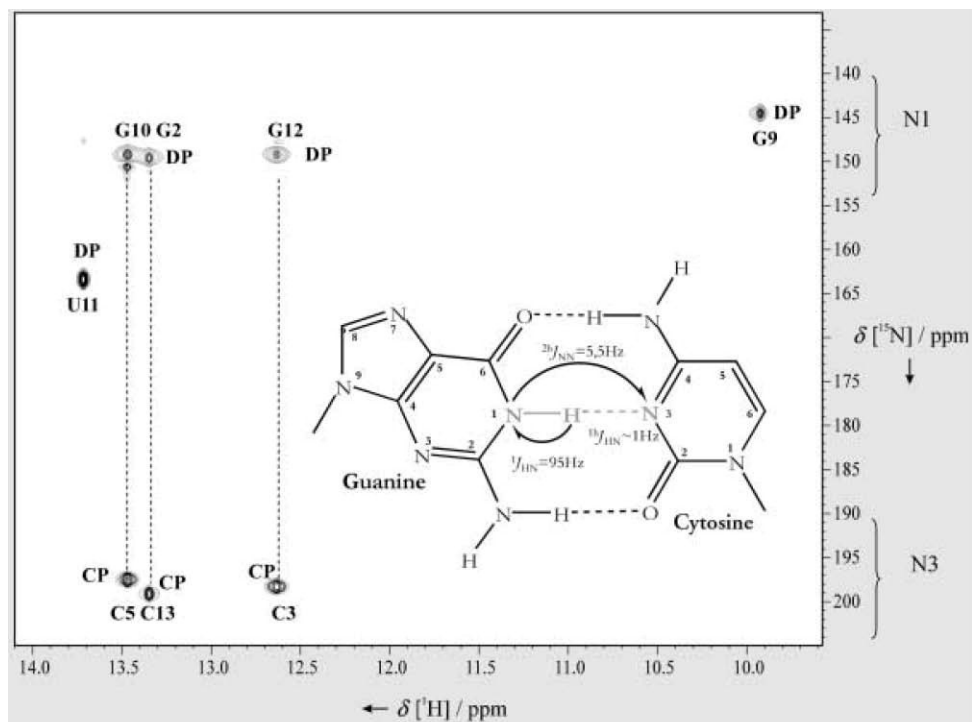
# Correlation of exchangeable and non-exchangeable protons



HCCNH-TOCSY, Fiala et al. JACS 1996, Sklenar et al. J. Biomol. NMR 1996.



# Correlation across the hydrogen bond HNN-COSY experiment

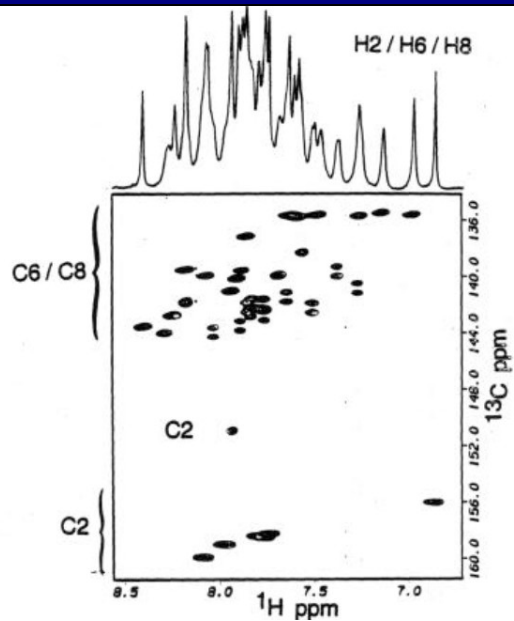


**Figure 11.** HNN-COSY experiment at 700 MHz and 298 K. On the right side, a Watson-Crick G:C base pair is depicted. The coupling constants are annotated. In this experiment, N1 of guanine can be correlated with the quaternary nitrogen atom of the cytosine residue.

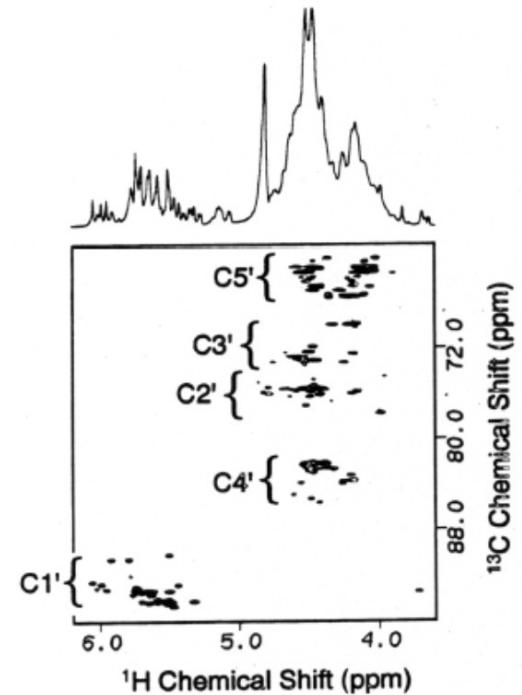
Dingley and Grzesiek, JACS 1998

# Identification of hydrogen and carbon atoms in bases and sugars

base

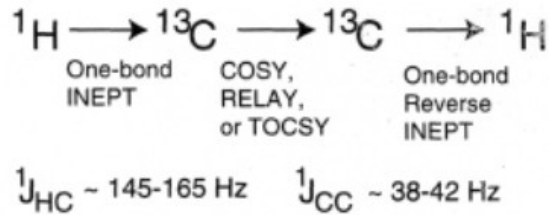


sugar

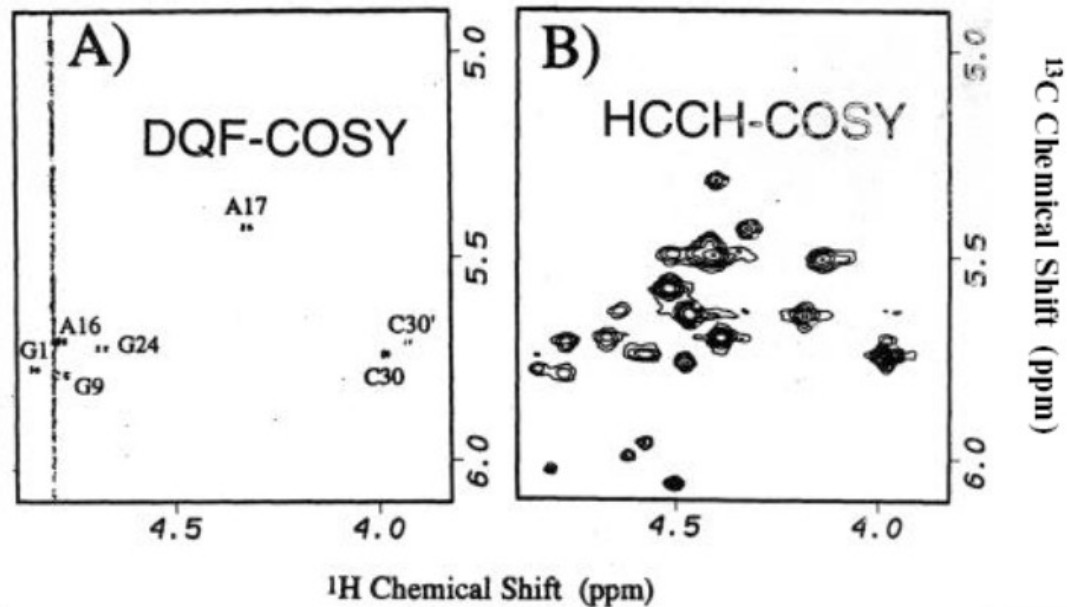


Constant-time  $^1\text{H}$ - $^{13}\text{C}$  HSQC experiment

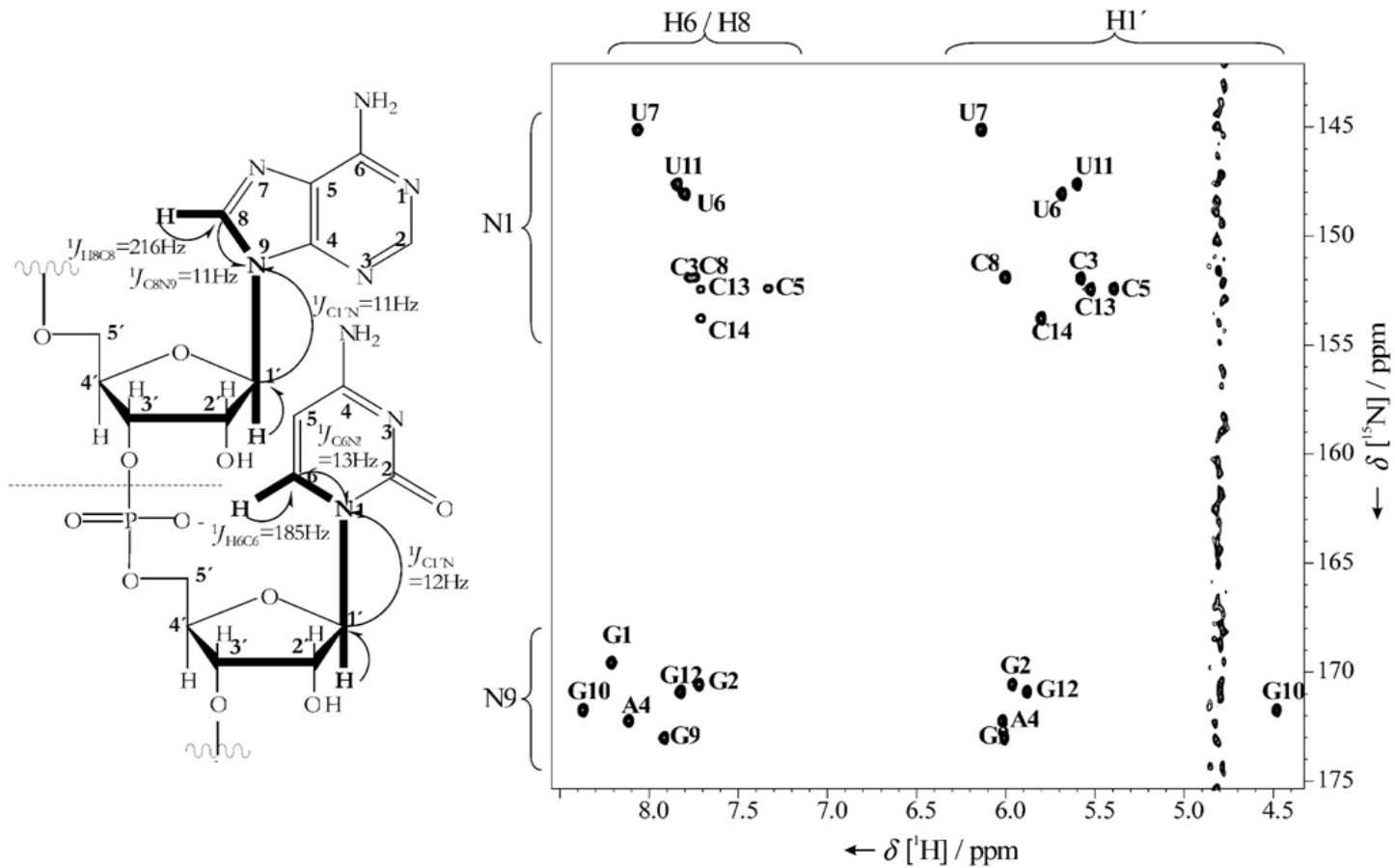
# Assignment of non-exchangeable protons: HCCH-type experiments



Allows for unambiguous assignments of  
 ${}^1\text{H}$  and  ${}^{13}\text{C}$  ribose as well as  
 $\text{H}_5\text{C}_5\text{C}_6\text{H}_6$  in pyrimidines

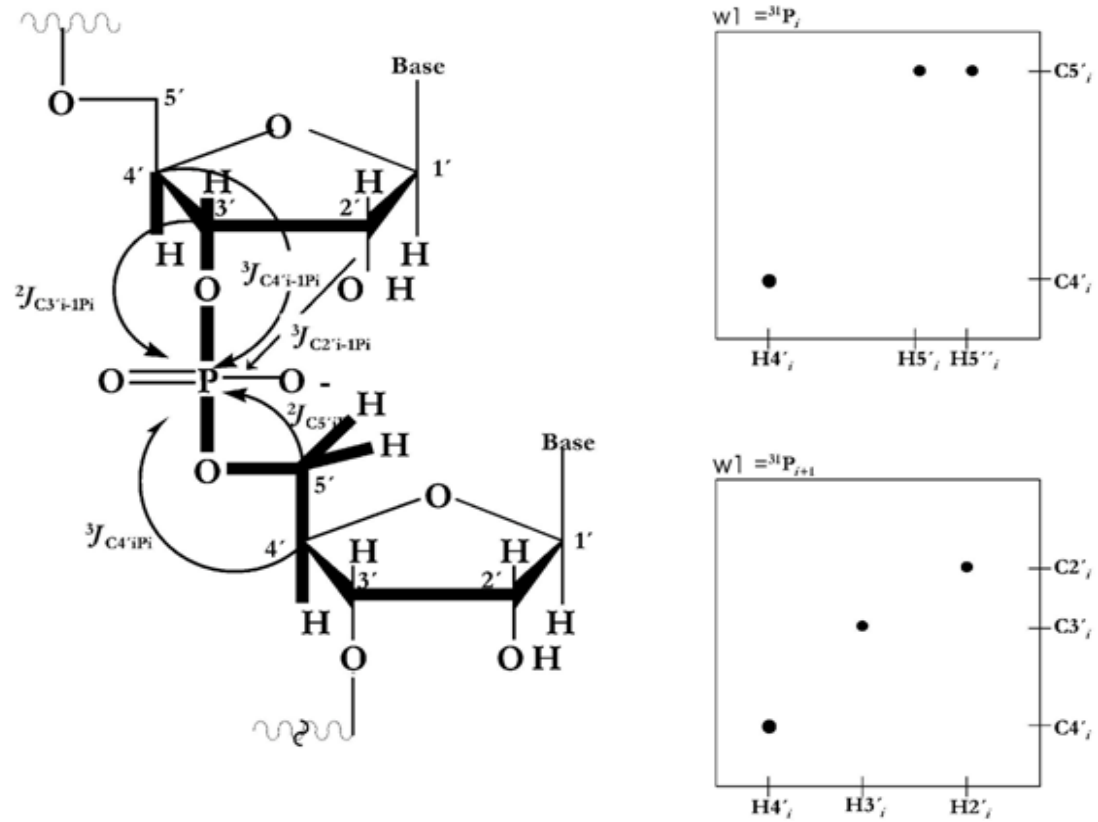


# Sugar to base correlation – the HCN experiment



Sklenar et al., J. Biomol. NMR 1993, 1994, Fiala et al., J. Biomol. NMR 1998, 2000.

# Sugar to phosphate correlation – the HCP experiment



# Dipolar couplings

- Dipolar couplings add to J couplings
- They show up as a field or alignment media dependence of the coupling
- If the overall orientation of the molecule is known the orientation of the vectors can be determined

