

Heteronuclear NMR of Nucleic Acids

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

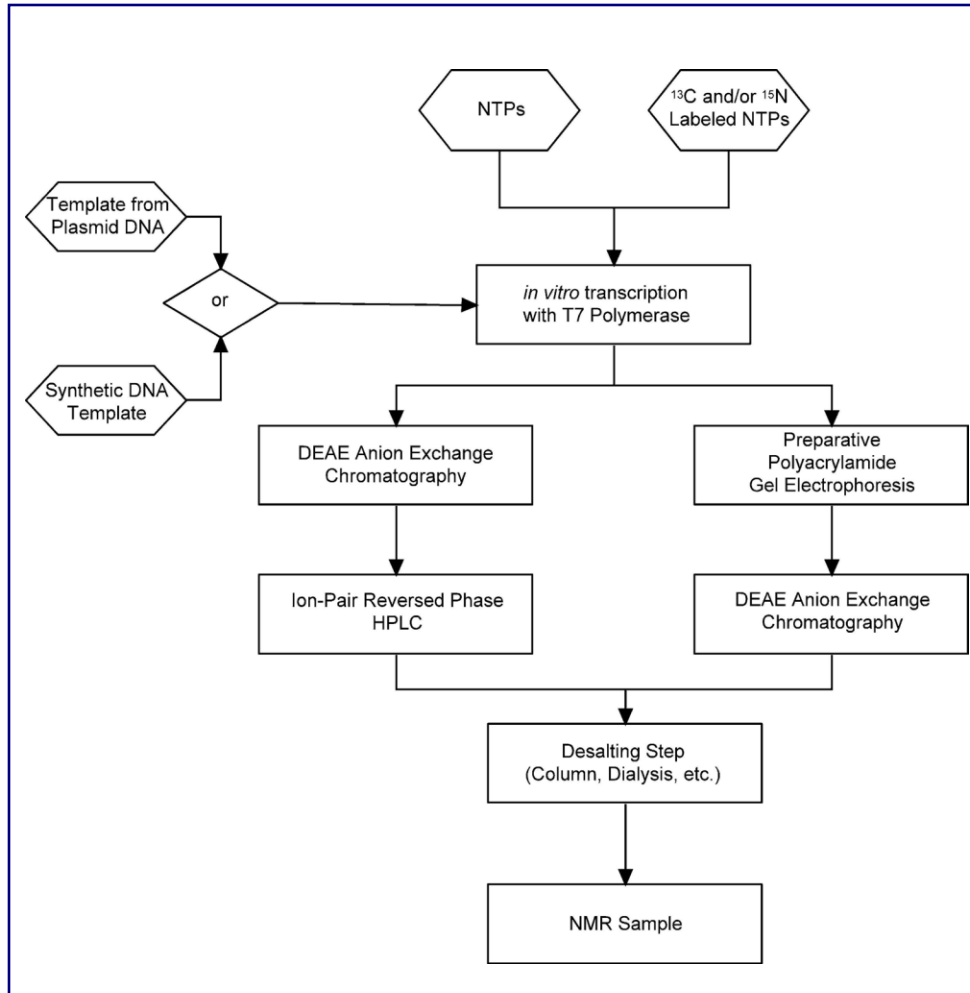
Assignment uses NOE or through-bond experiments

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

¹³C-glucose, ¹⁵N-ammonium

M. Methylotropus

¹³C-methanol, ¹⁵N-ammonia



Assignment procedure for labeled NA NOE based (I)

I. (H₂O)

Correlation of exchangeable protons with ¹⁵N

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

2D ¹H-¹⁵N HSQC NH₂ amino optimized (Cyt, Gua, Ade)

Sequential assignment of exchangeable protons

3D NOESY-HSQC (¹H-¹H-¹⁵N, imino ¹⁵N edited NOESY)

imino-imino and imino-amino interactions

3D NOESY-HSQC (¹H-¹H-¹⁵N, amino ¹⁵N edited NOESY)

amino-imino interactions



II. (H₂O)

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

3D NOESY-HSQC (¹H-¹H-¹⁵N, imino/amino ¹⁵N 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 ^1H - ^{13}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 ^1H - ^{13}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 (^1H - ^1H - ^{13}C), H6/8-H1', H6/8-H2' correlations

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

^1H - ^{31}P HETCOR/heteroTOCSY

Assignment procedure for labeled NA Through bond correlations (I)

I. (H₂O) **Correlation of exchangeable protons with ¹⁵N**
2D ¹H-¹⁵N HSQC **NH imino optimized (Gua and Ura)**
2D ¹H-¹⁵N HSQC **NH₂ amino optimized (Cyt, Gua, Ade)**

II. (H₂O) **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}C

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

Sugar-base correlations

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

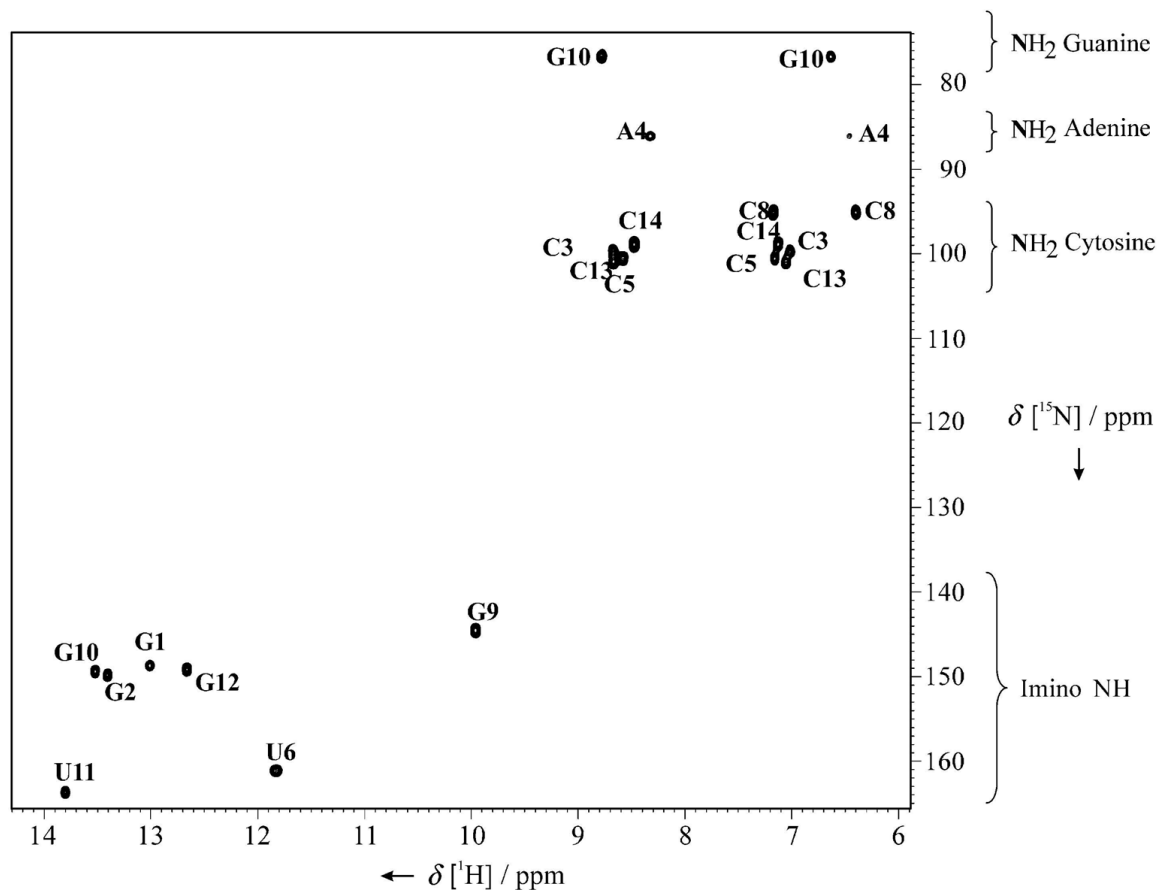
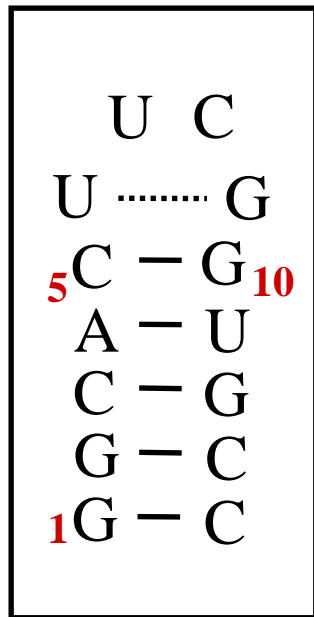


IV. ($^2\text{H}_2\text{O}$)

Sequential assignment of ^{31}P resonances across the sugar-phosphate backbone

HCP / PCH / PCCH-TOCSY / HPHCH

Correlation of exchangeable protons with ^{15}N

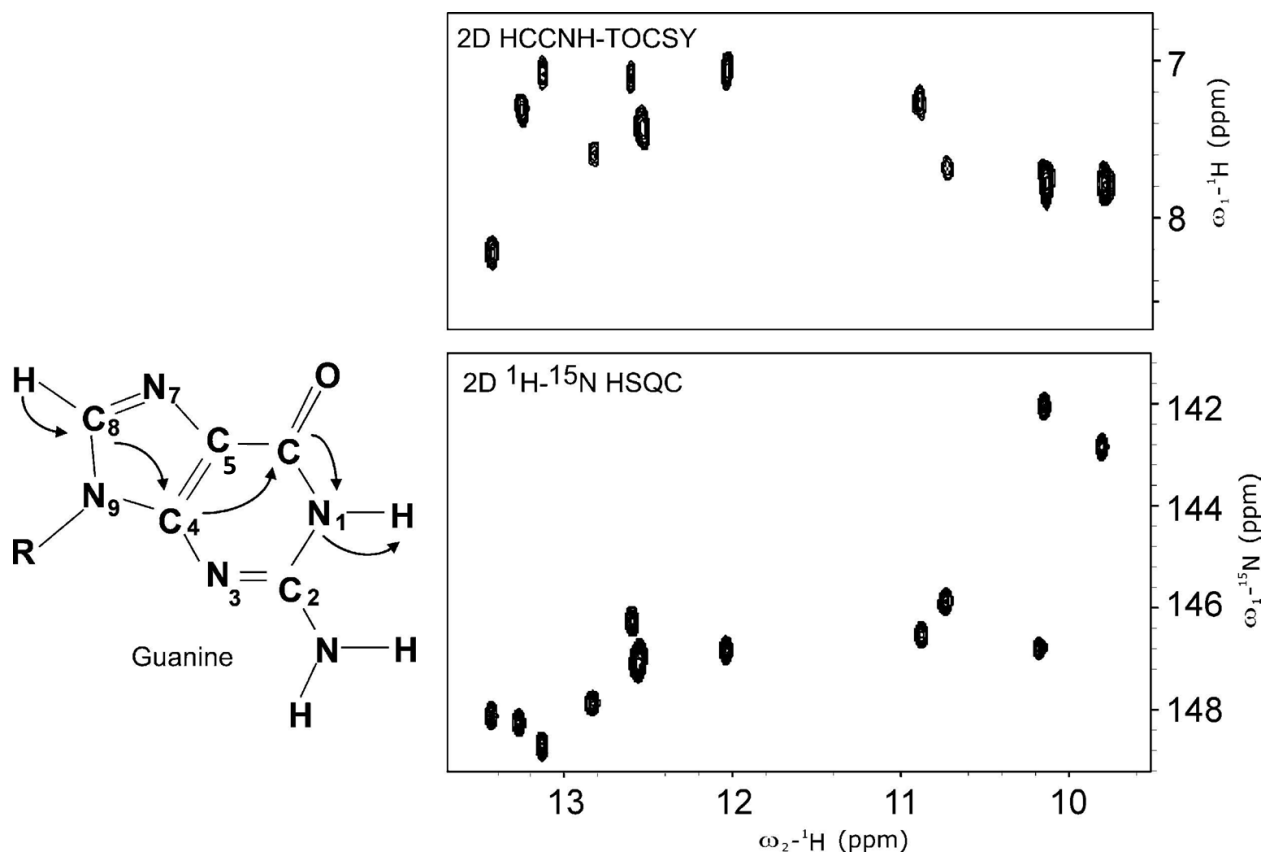


Gradient sensitivity-enhanced HSQC
 Kay, Keifer, Saarinen, JACS 1992.

Assignment of imino protons in loops

Correlation of exchangeable and non-exchangeable protons

Labeled samples – HCCNH-TOSCY

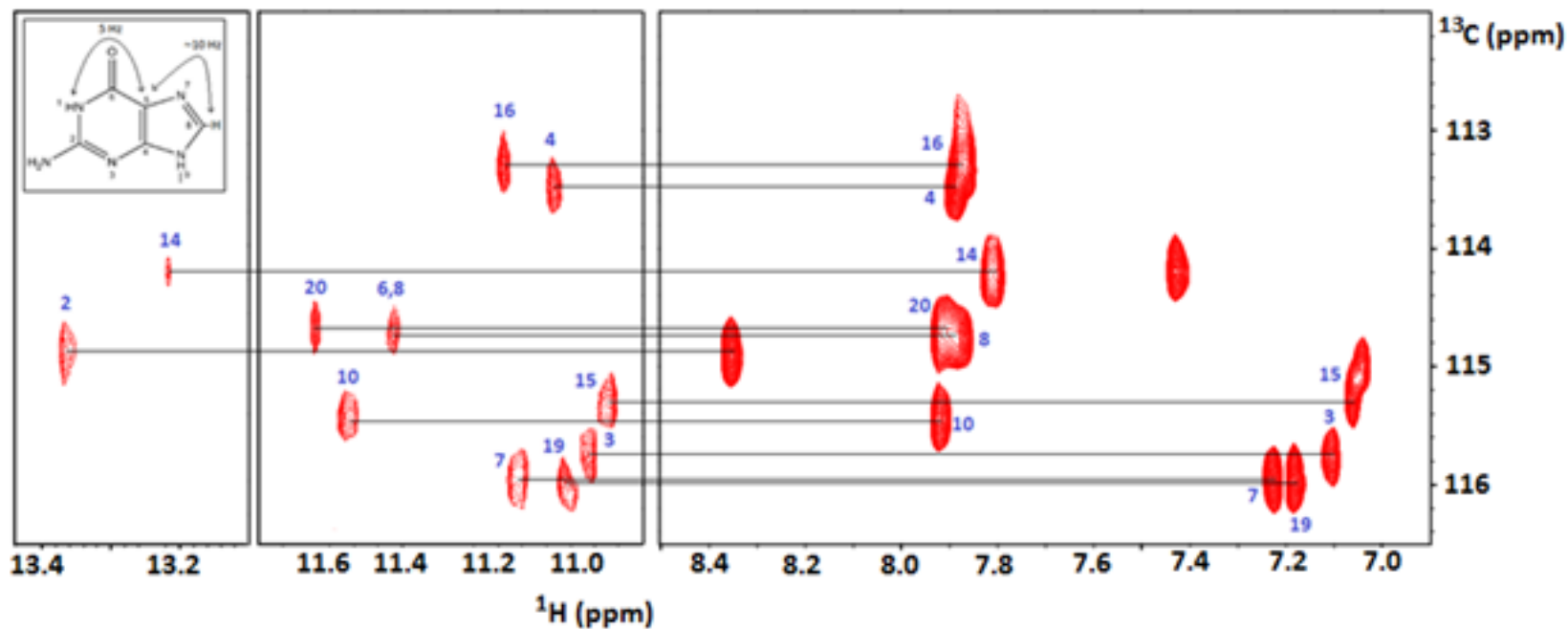


Fiala et al. JACS 1996, Sklenar et al. J. Biomol. NMR 1996.

Assignment of imino protons in loops

Correlation of exchangeable and non-exchangeable protons

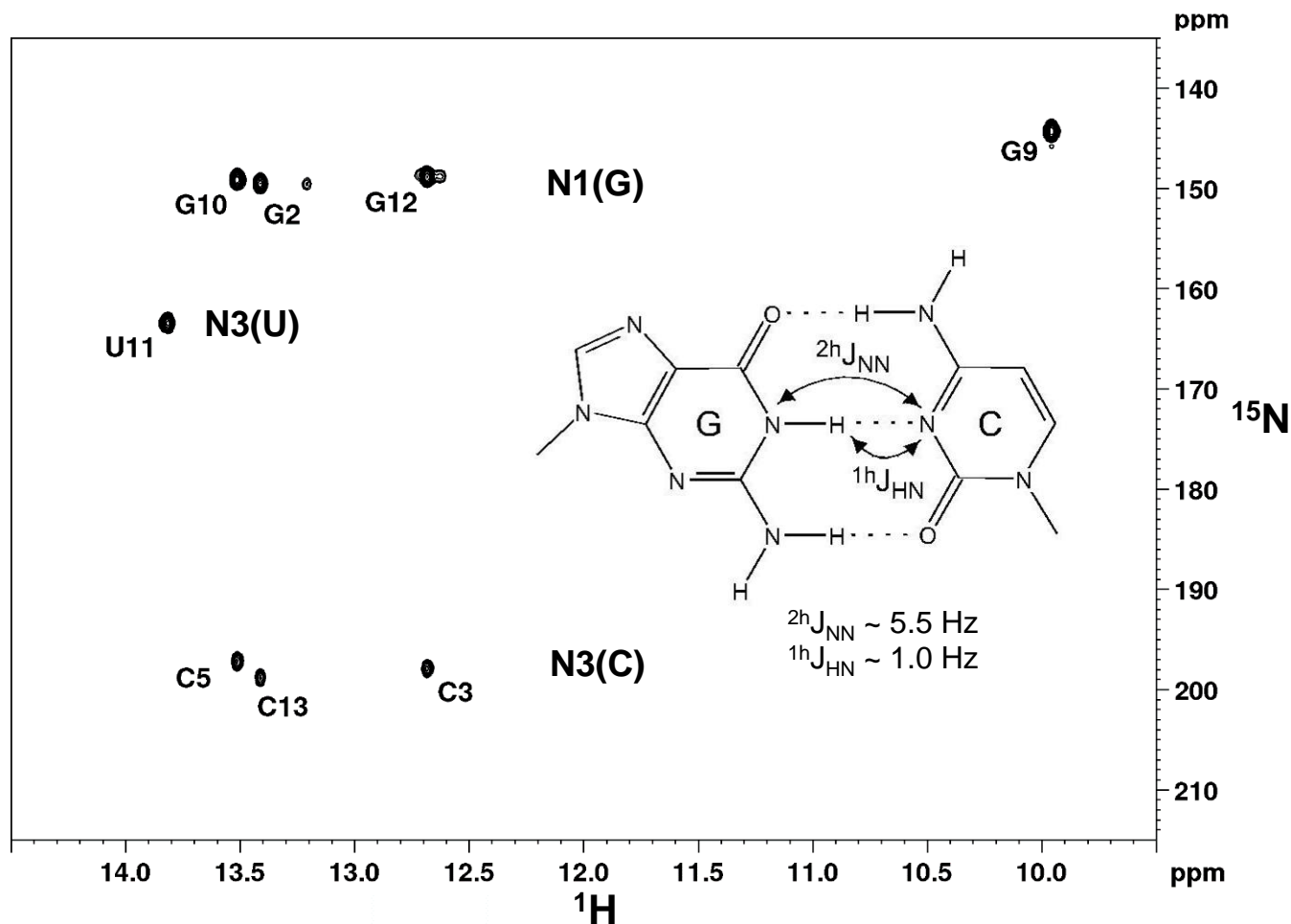
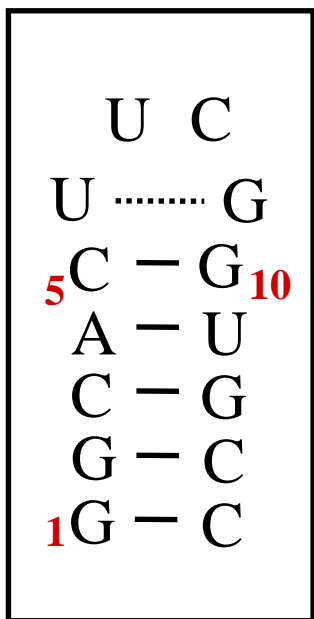
Unlabeled samples – JRHMBC



“Green Monkey” 5'-CGGGCGGGCGCGAGGGAGGGC-3' (21 nt)

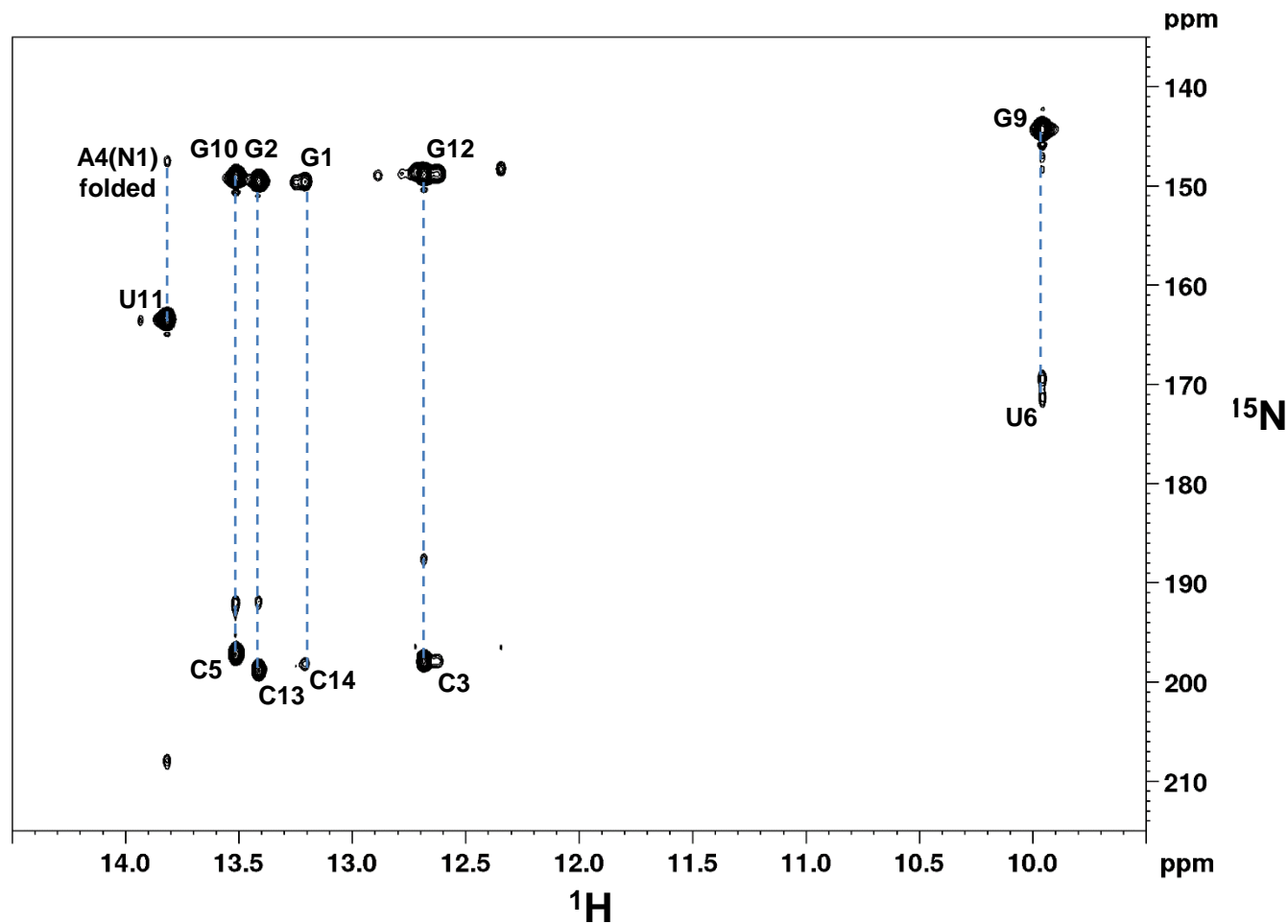
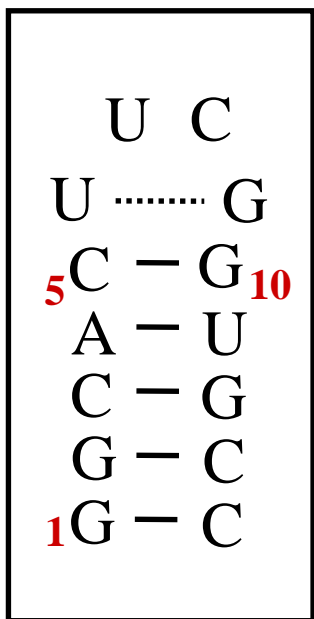
Phan, Journal of Biomolecular NMR, 16: 175–178, 2000.

Correlation across the hydrogen bond HNN-COSY experiment



Dingley and Grzesiek, JACS 1998

Correlation across the hydrogen bond HNN-COSY experiment

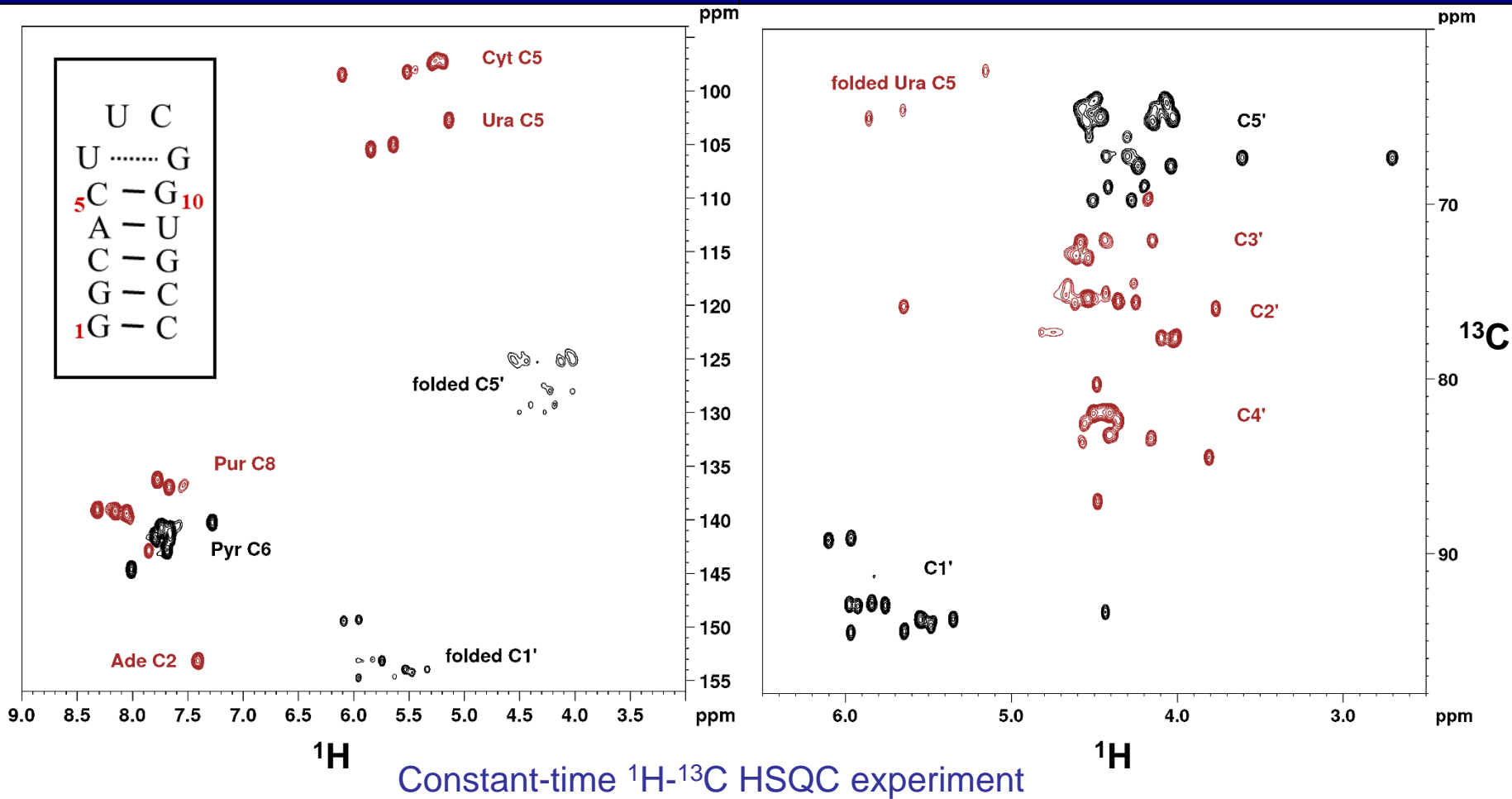


Dingley and Grzesiek, JACS 1998

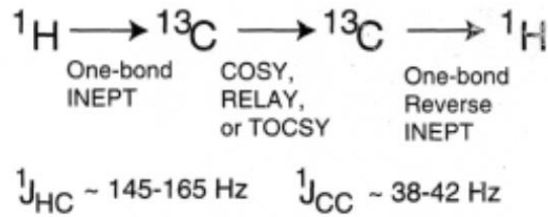
Identification of hydrogen and carbon atoms in bases and sugars

base

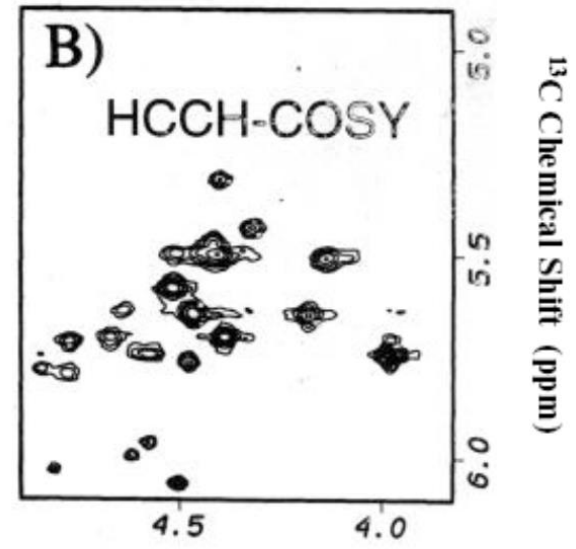
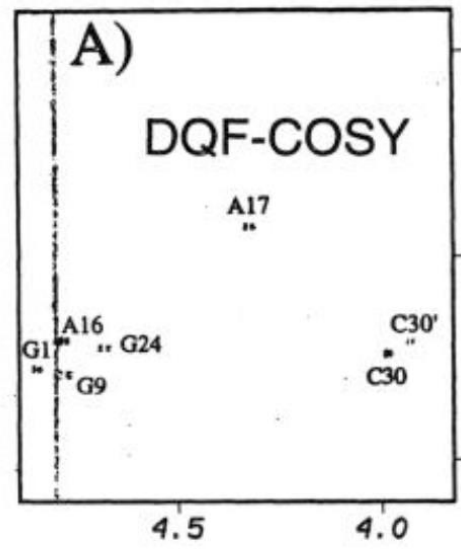
sugar



Assignment of non-exchangeable protons: HCCH-type experiments



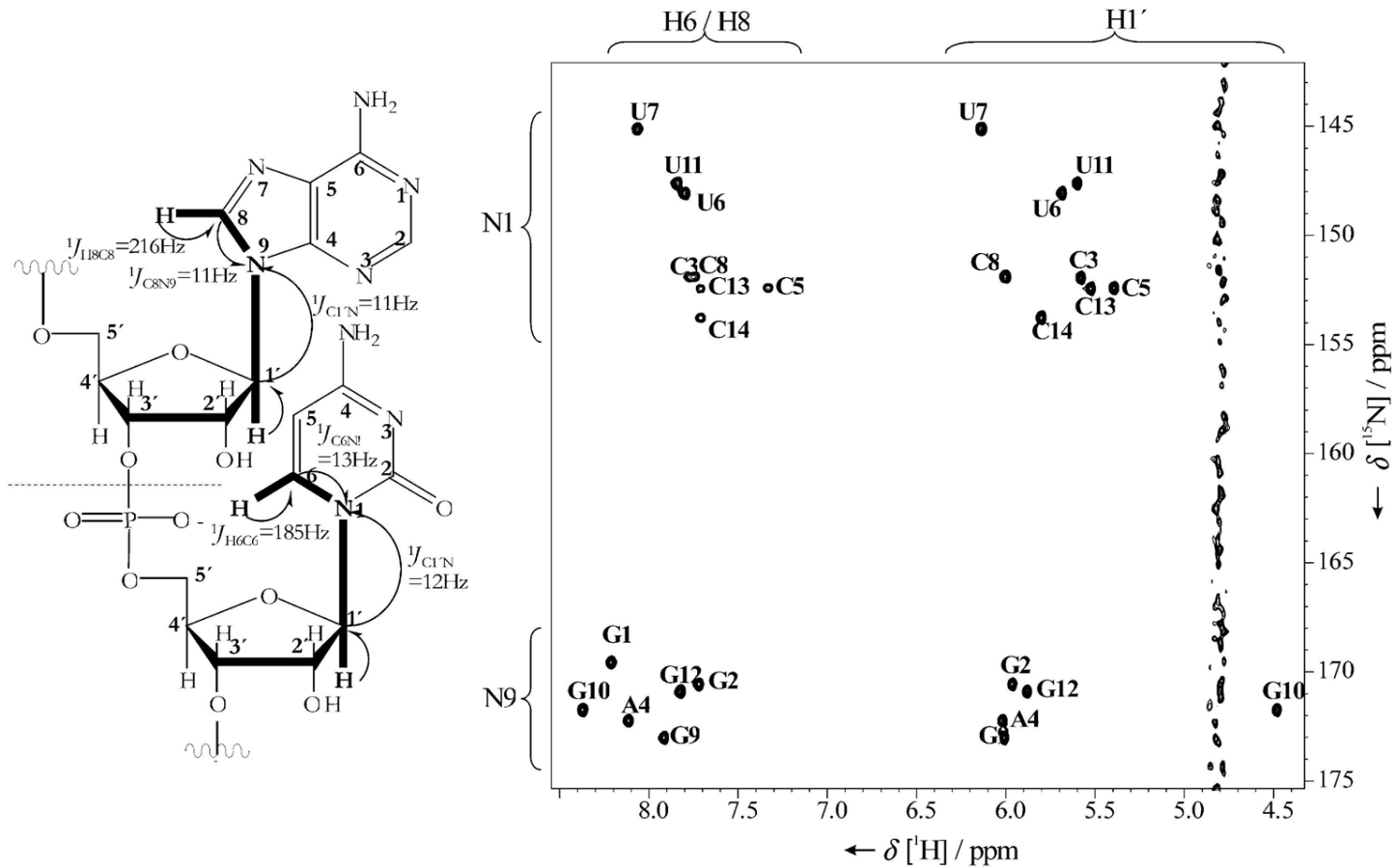
Allows for unambiguous assignments of ${}^1\text{H}$ and ${}^{13}\text{C}$ ribose as well as $\text{H}_5\text{C}_5\text{C}_6\text{H}_6$ in pyrimidines



${}^1\text{H}$ Chemical Shift (ppm)

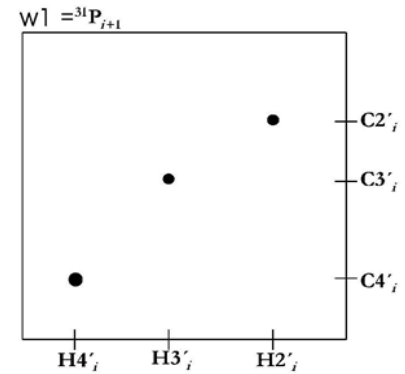
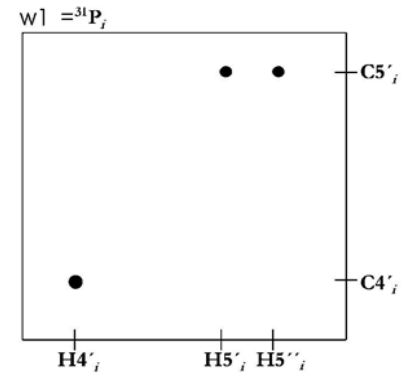
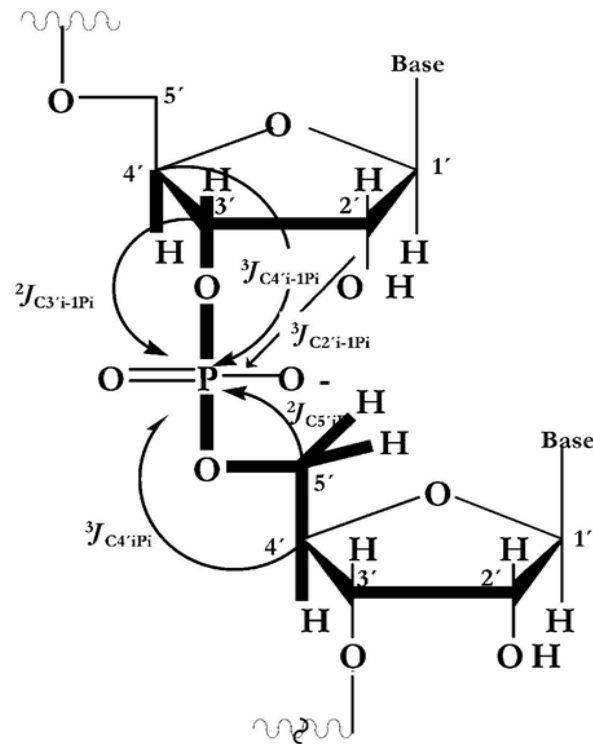
${}^{13}\text{C}$ Chemical Shift (ppm)

Sugar to base correlation – the HCN experiment



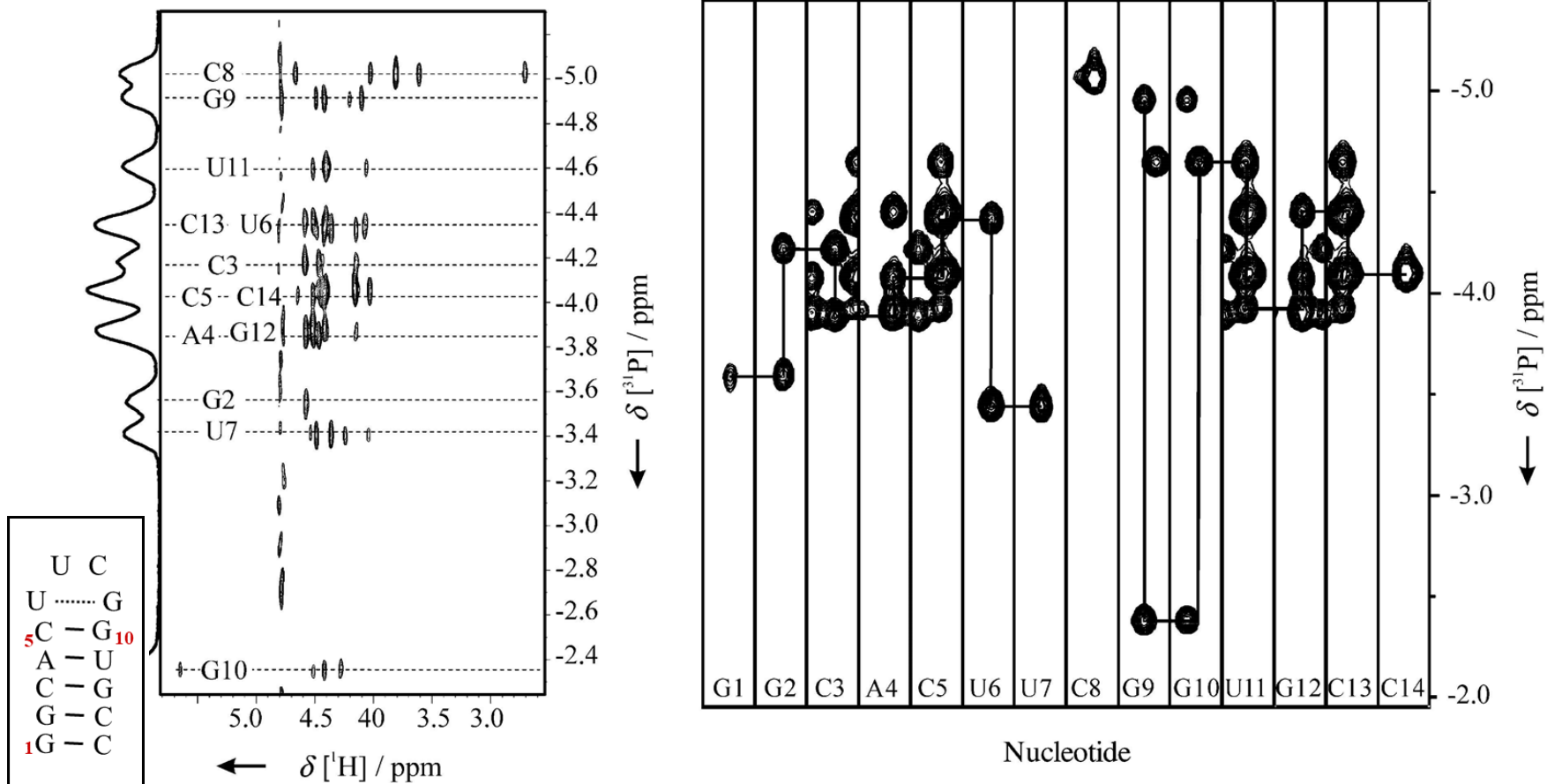
Sklenar et al., J. Biomol. NMR 1993, 1994, Fiala et al., J. Biomol. NMR 1998, 2000.

Sugar to phosphate correlation – the HCP experiment

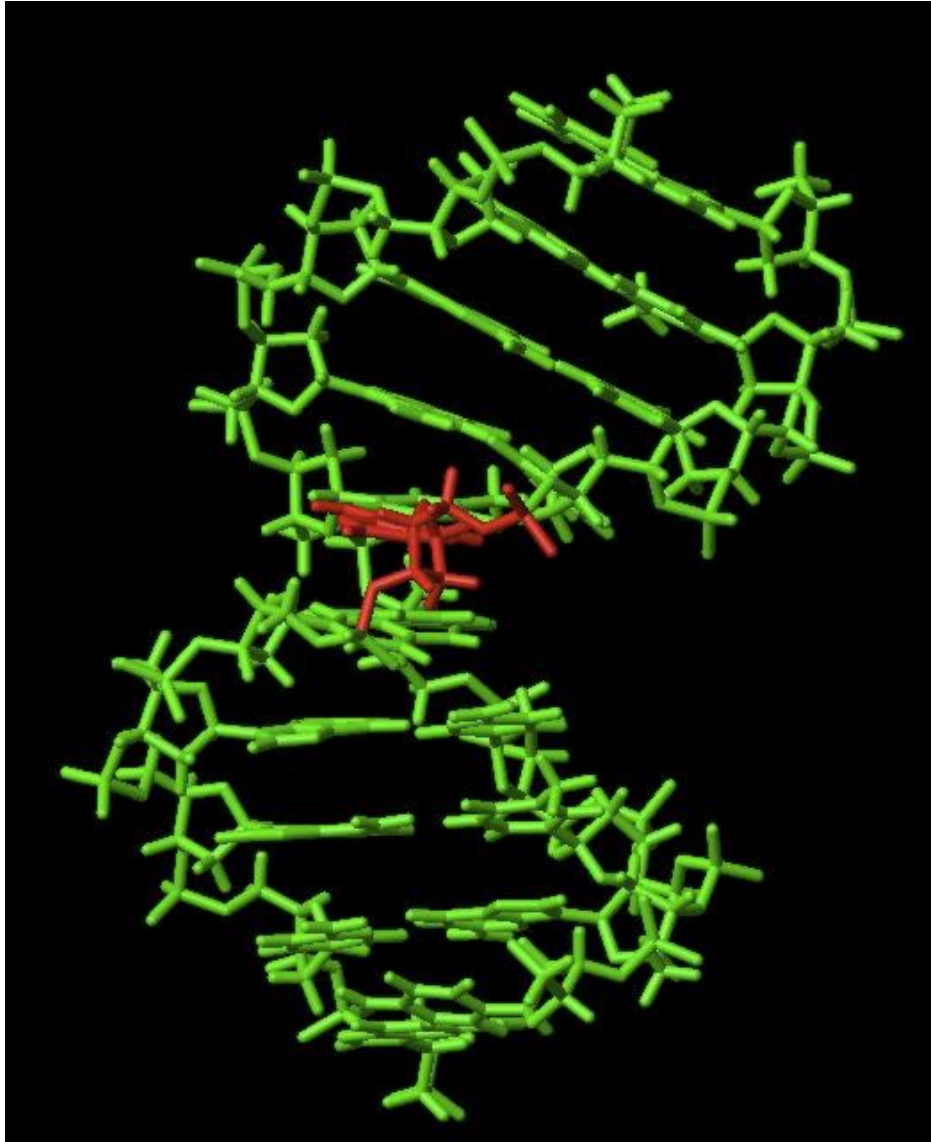


Sugar to phosphate correlation – the HCP experiment

real spectra – nt-14 RNA with UUCG loop



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

