

Recent Advances in the Development of Heteronuclear Correlation Experiments

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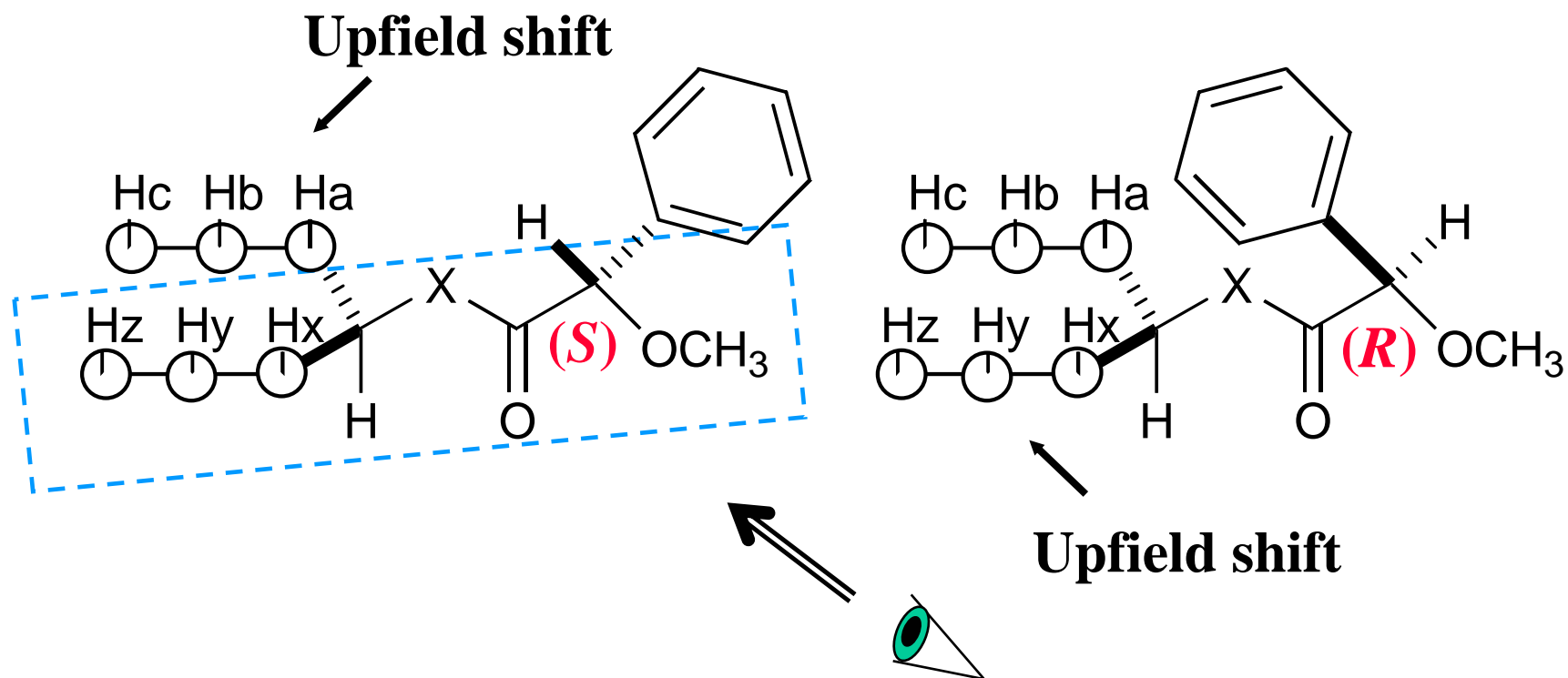
Outline of Presentation

- Stereochemistry by NMR
 - Mosher Method
 - Overview of *J*-based configuration analysis.
- Development of the GBIRD-CPMG-HSQMBC
- Measuring the heteronuclear coupling constants
- Application of the *J*-based configuration analysis
- Determination of the relative stereochemistry of several cyclopentane derivatives

Introduction to the Mosher Method

- The Mosher method involves derivatization of a substrate of unknown stereochemical configuration with two enantiomers (*R* and *S*) of a chiral derivatizing reagent (CDR), such as MPA.
- The proton NMR spectrum of the resulting diastereomers are compared in order to obtain a difference in chemical shifts.
- These chemical shift differences arise due to the selective shielding effect of the aryl group of the CDR on the nuclei of the substrate part of the obtained diastereomer.

Overview of Mosher Method



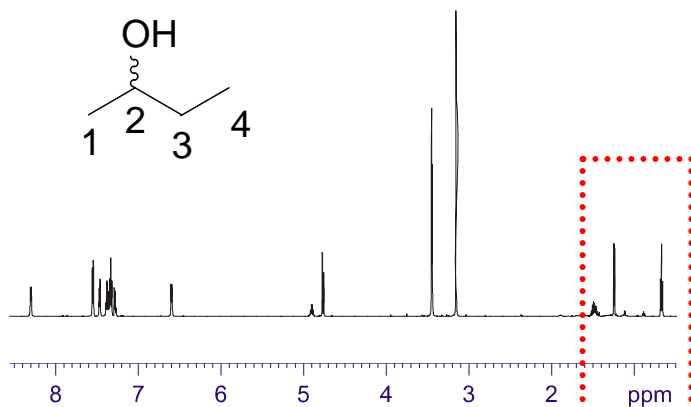
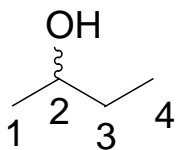
Determine Configuration using $\Delta = \delta S - \delta R$

Δ = positive values for “left” side of ester

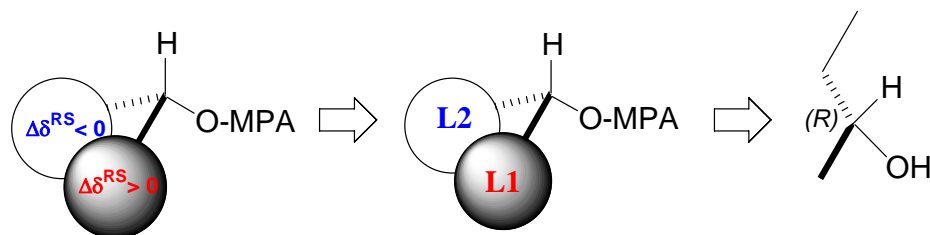
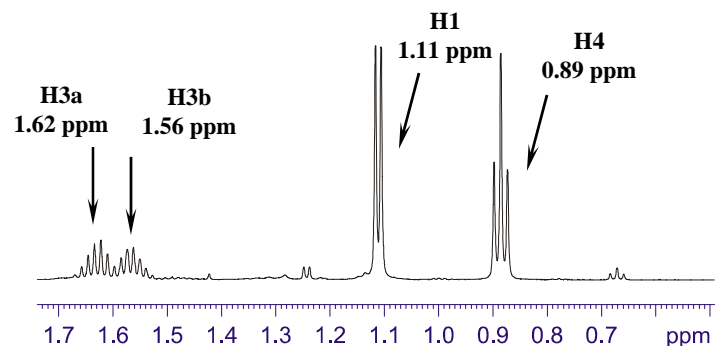
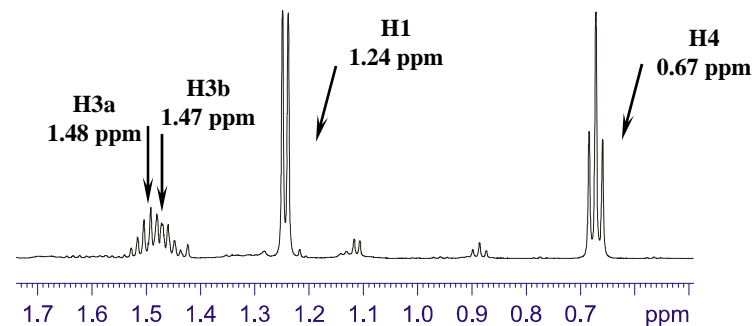
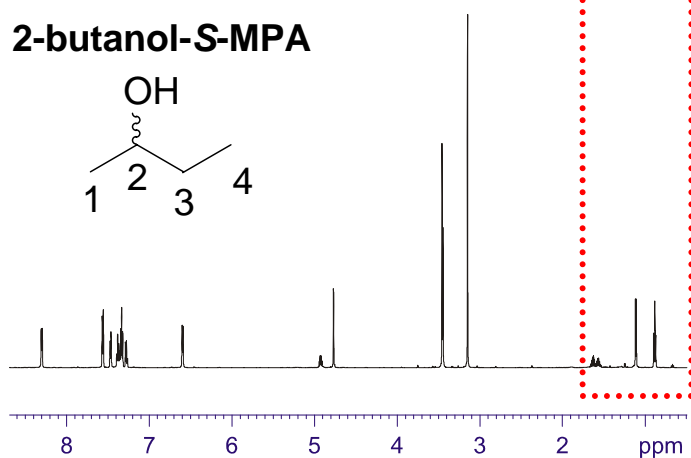
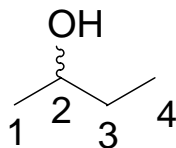
Δ = negative values for “right” side of ester

Example of Mosher Method Using 2-butanol

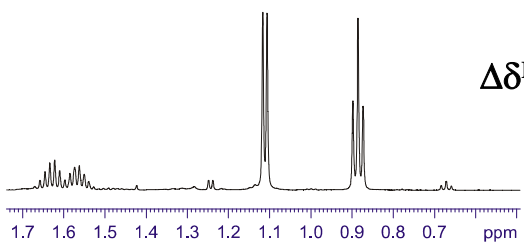
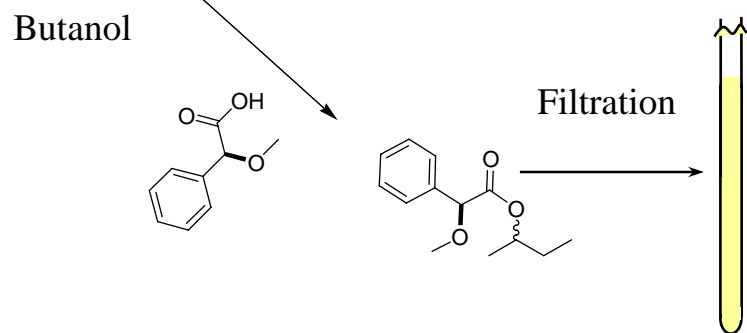
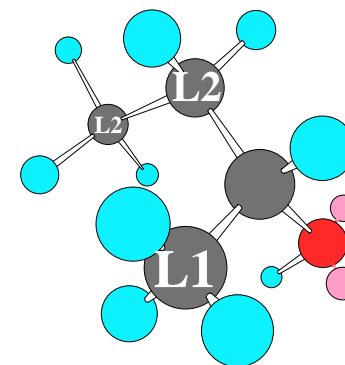
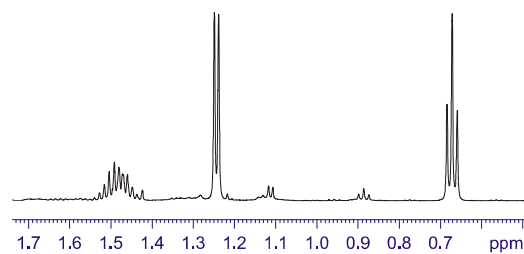
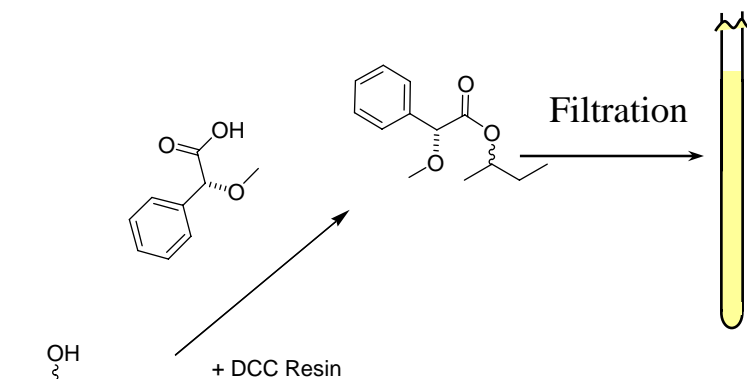
2-butanol-*R*-MPA



2-butanol-*S*-MPA

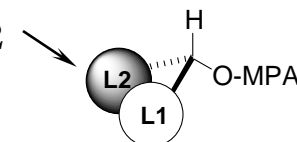


DCC resin method



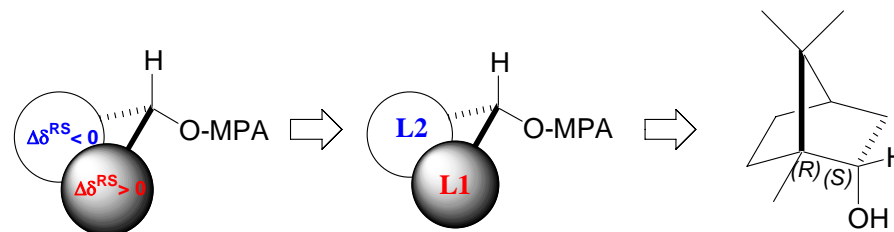
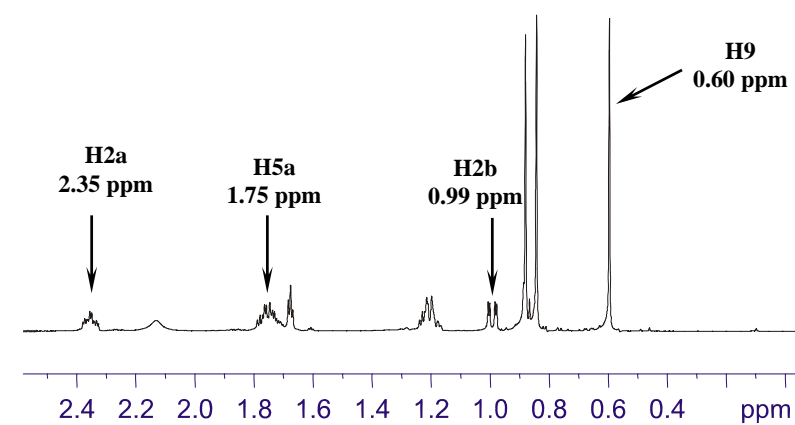
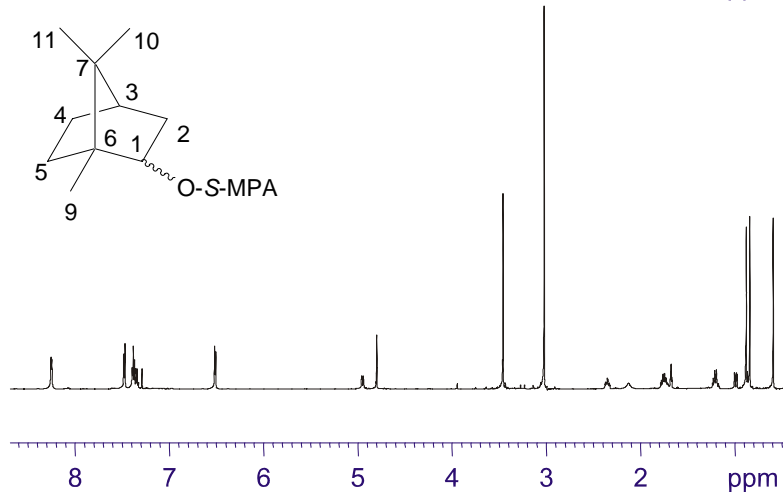
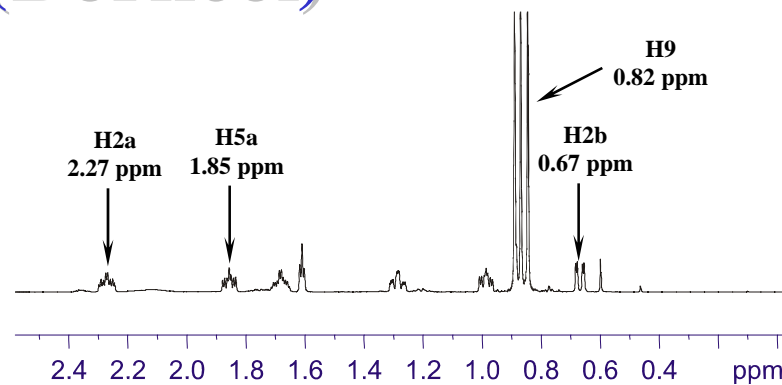
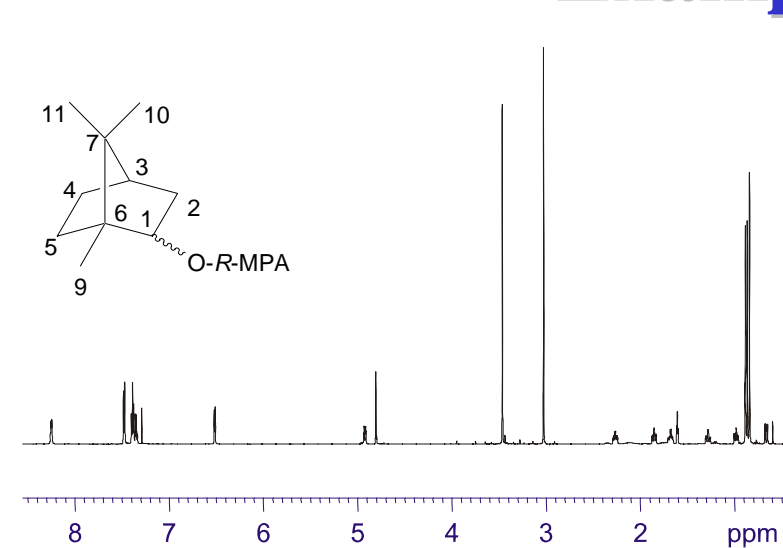
$$\Delta\delta^{RS}L2 = \delta^{RL2} - \delta^{SL2}$$

$$\Delta\delta^{RS}L1 = \delta^{RL1} - \delta^{SL1}$$



Absolute Stereochemistry

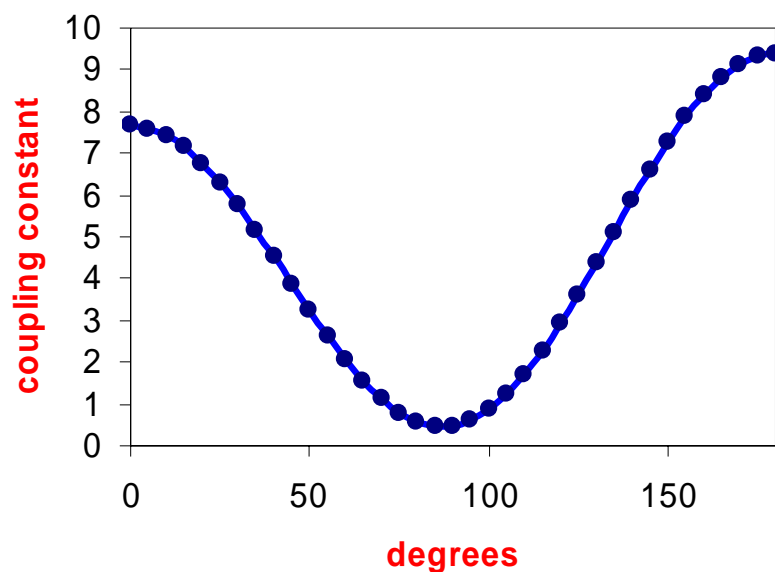
Example (Borneol)



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J-Based Configuration Analysis

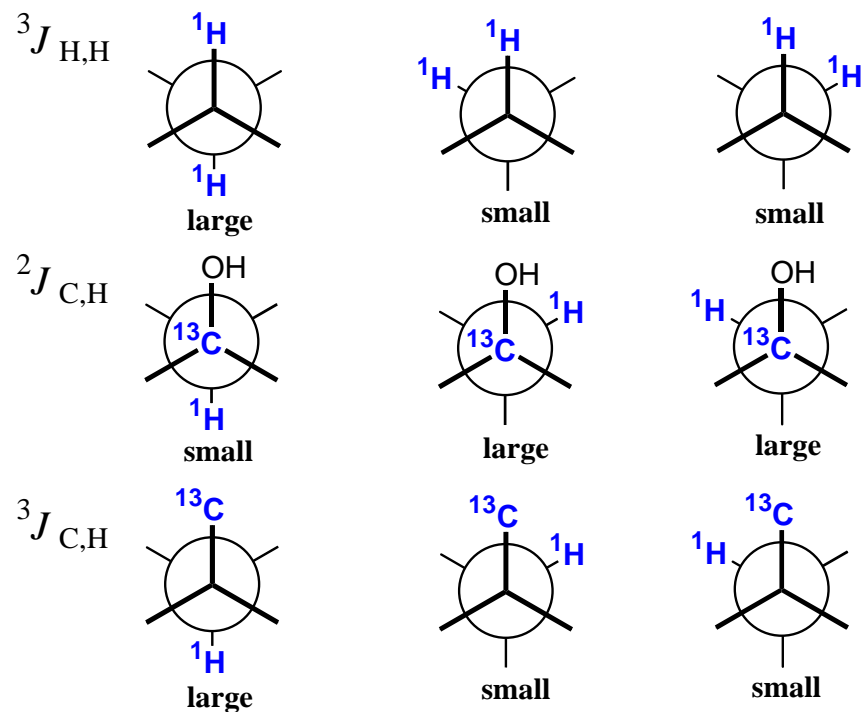


- Utilizes 2 and 3 bond heteronuclear coupling constants ($^2J_{\text{CH}}$ and $^3J_{\text{CH}}$)
- Basic principle is the same that applies for ^1H - ^1H coupling constants- Hz value is directly correlated to torsion angle.
- Yields relative stereochemistry unless a stereocenter is known within the molecule.

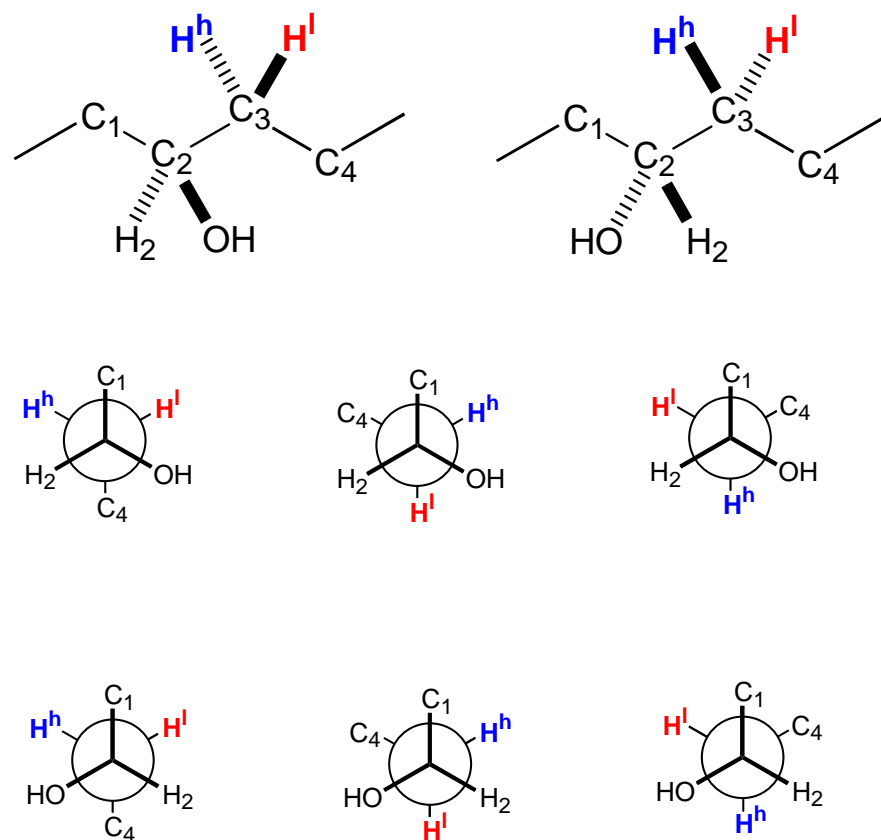
Aydin, R.; Günther, H. *Magn. Reson. Chem.* **1990**, 28, 448-457.

Basic Theory of the *J*-Based Configuration Analysis

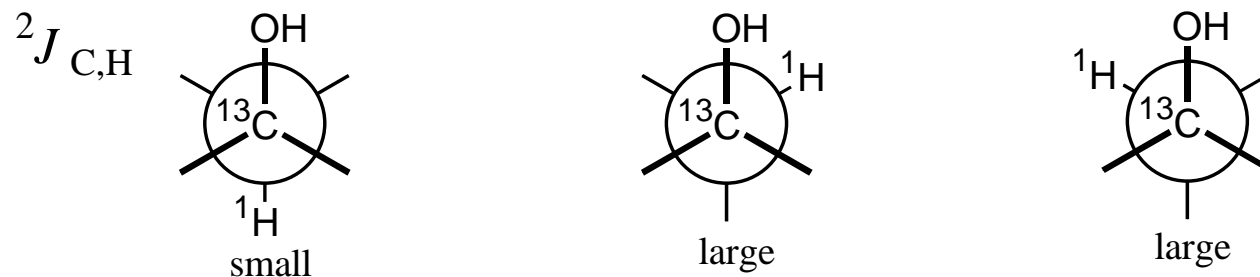
Basic Theory of the *J*-Configuration Analysis



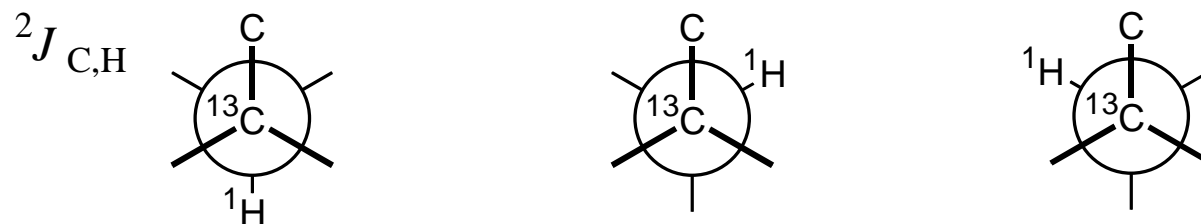
Illustrative Example of the *J*-Configuration analysis



$^2J_{\text{CH}}$ Coupling Constants

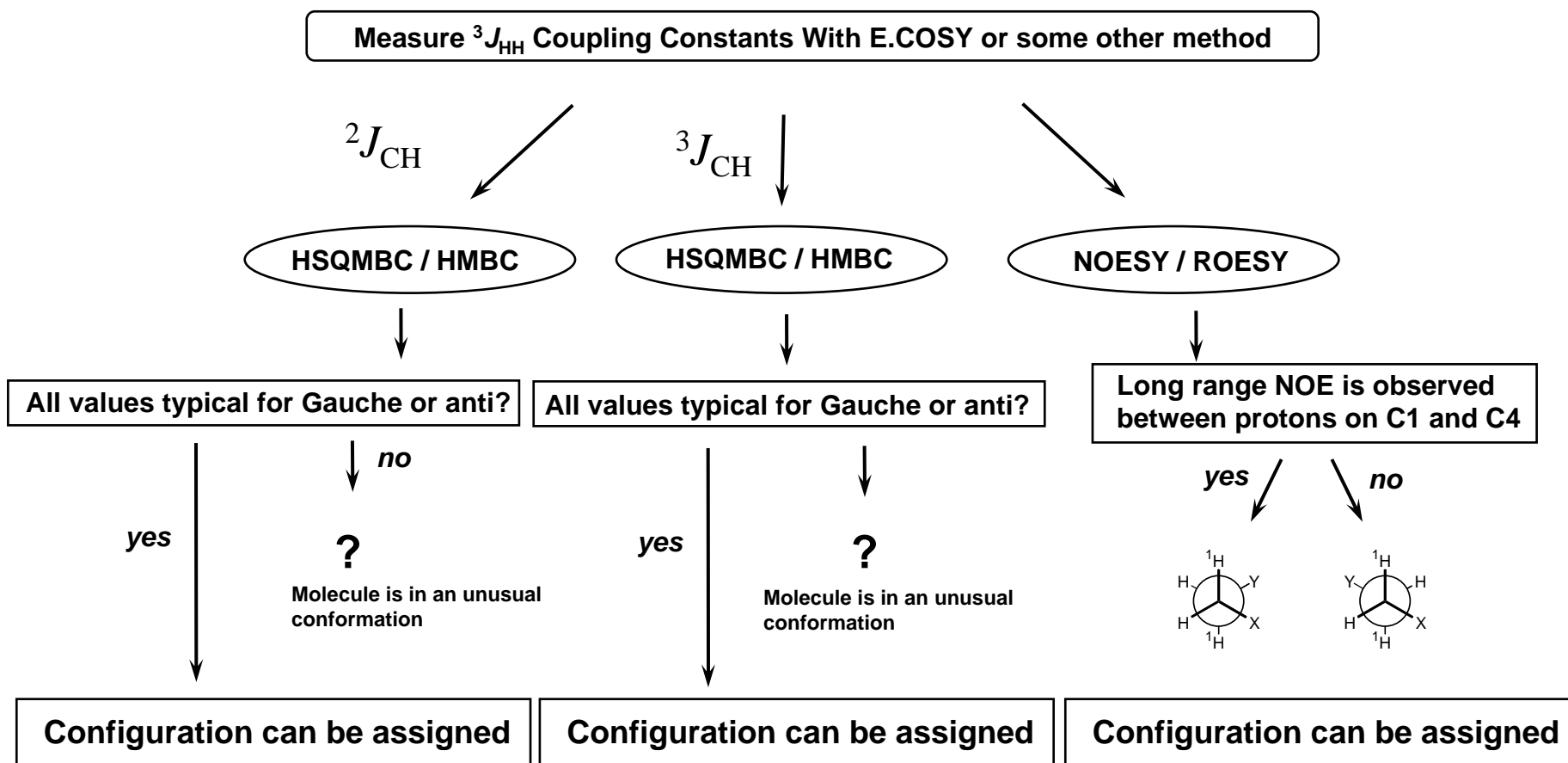


Magnitude of $^2J_{\text{CH}}$ Scalar Coupling Constants Depends on the Torsion Angle of the Neighboring **ELECTRONEGATIVE** Substituent.

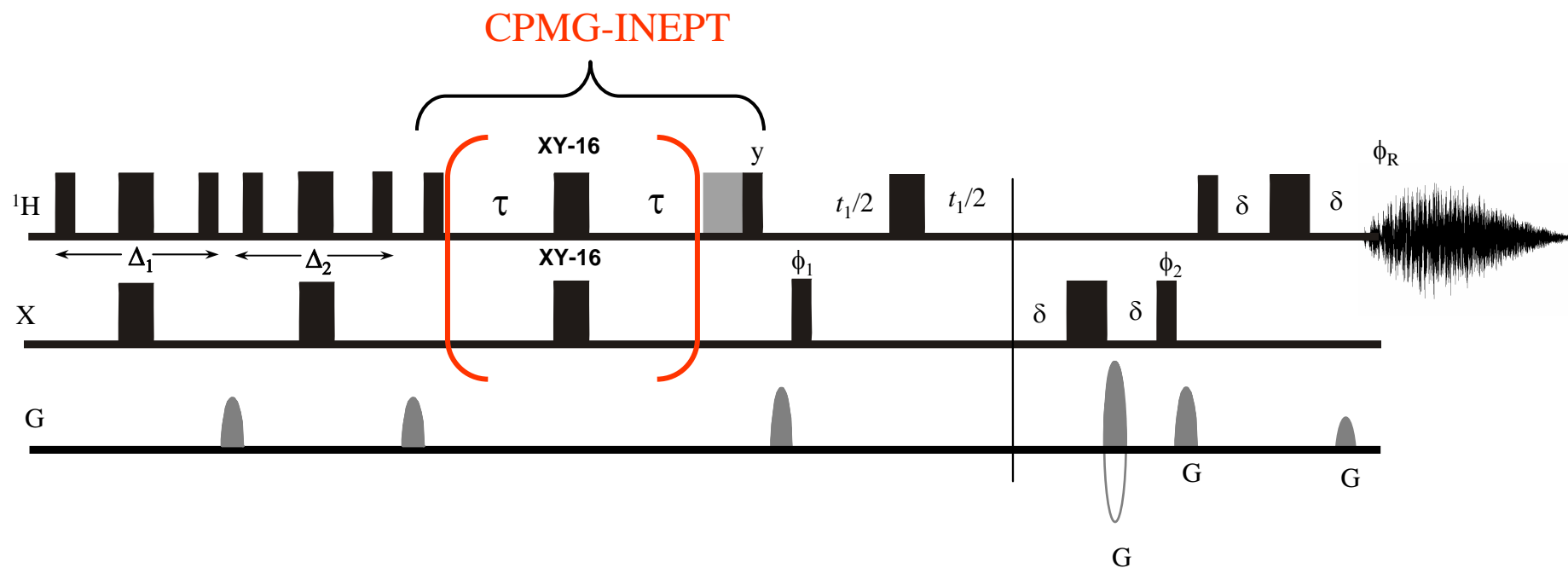


If there are no adjacent electronegative substituents then the magnitude of $^2J_{\text{CH}}$ coupling constants can not usually be predicted.

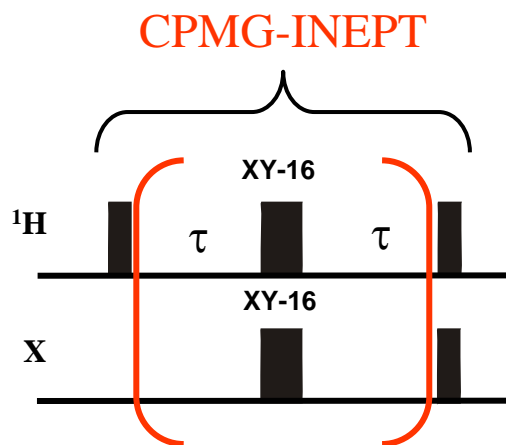
Flow Chart for the *J*-Configuration Analysis method



LR-CAHSQC



CPMG-INEPT

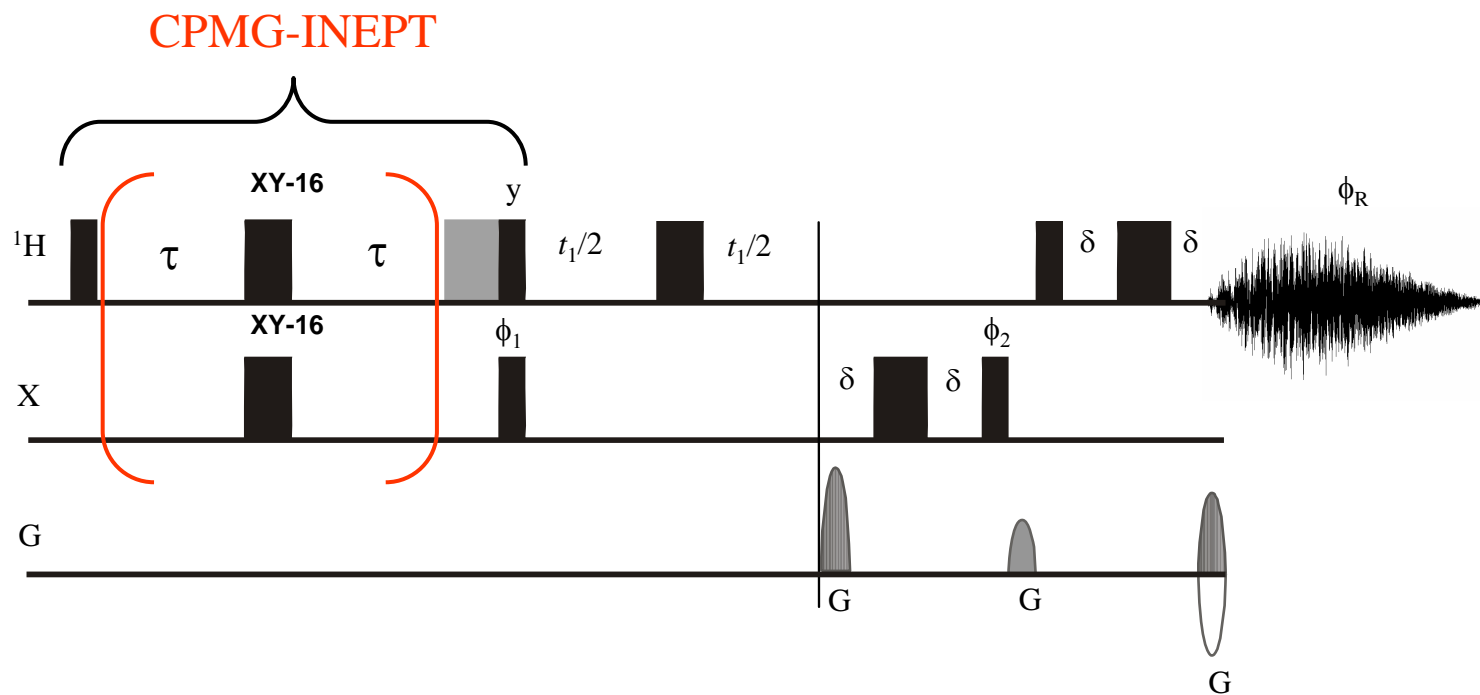


- XY-16 first published by Gullion et. al. *J. Magn. Reson.* 89 (1990) 479
- Employed for the improved detection of fast-exchange protons by Mueller et. al. *J. Am. Chem. Soc.* 117 (1995) 11043 and Mulder et. al. *J. Biomol. NMR* 8 (1996) 223.
- Applied to ^1H - ^{31}P HSQC experiments by Luy et. al. *J. Am. Chem. Soc.* 123 (2001) 11306.
- Applied to the measurement of long-range heteronuclear coupling constants by Koskela et. al. *J. Magn. Reson.* 164, (2003) 228-232.

- The use of the CPMG-INEPT creates isotropic mixing conditions for homonuclear spins providing in-phase magnetization, suppressing the anti-phase evolution of homonuclear coupling.
- To obtain this condition the delay between the successive 180 pulses must be kept short --

$$\ll 1/\left(2\sqrt{J^2 + \Delta\nu^2}\right) \quad \text{which simplifies to} \quad 1/\left(2\Delta\nu_{\max}\right)$$

CPMG-HSQMBC



Phase-Cycle

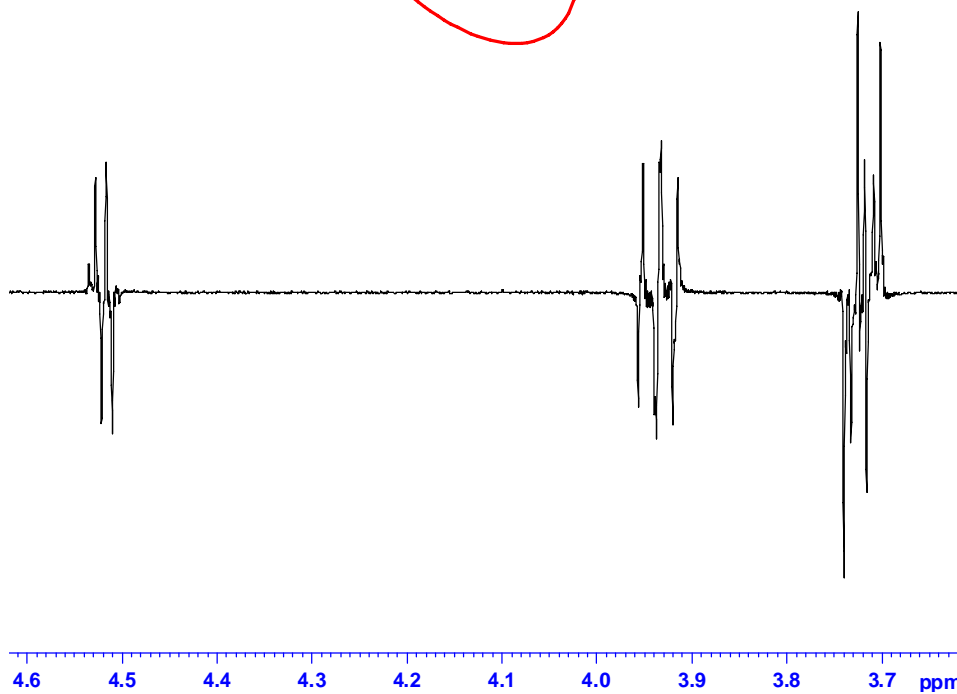
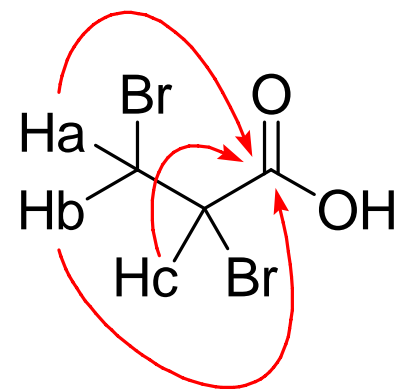
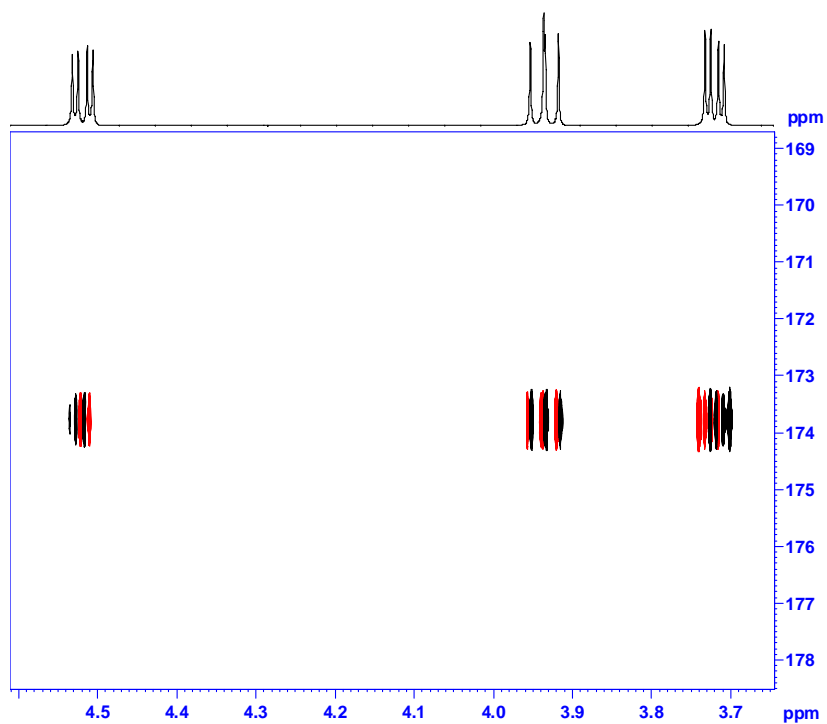
$$\phi_1 = \mathbf{x, -x}$$

$$\phi_2 = \mathbf{x, x, -x, -x}$$

$$\phi_R = \mathbf{x, -x, -x, x}$$

$$\text{XY-16} = \mathbf{x, y, x, y, y, x, y, x, -x, -y, -x, -y, -y, -x, -y, -x}$$

CPMG-HSQMBC Data on 1,2-dibromopropionic Acid

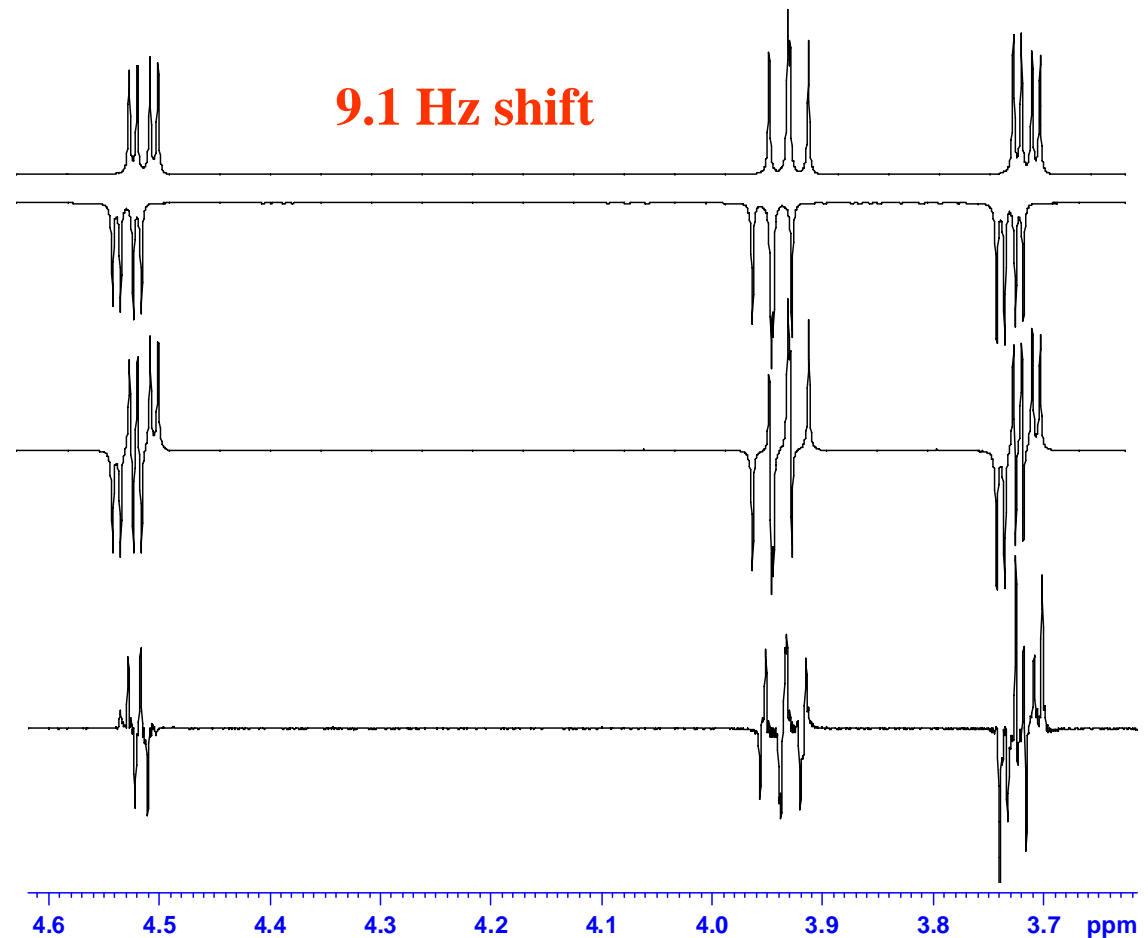


Measuring Coupling Constant

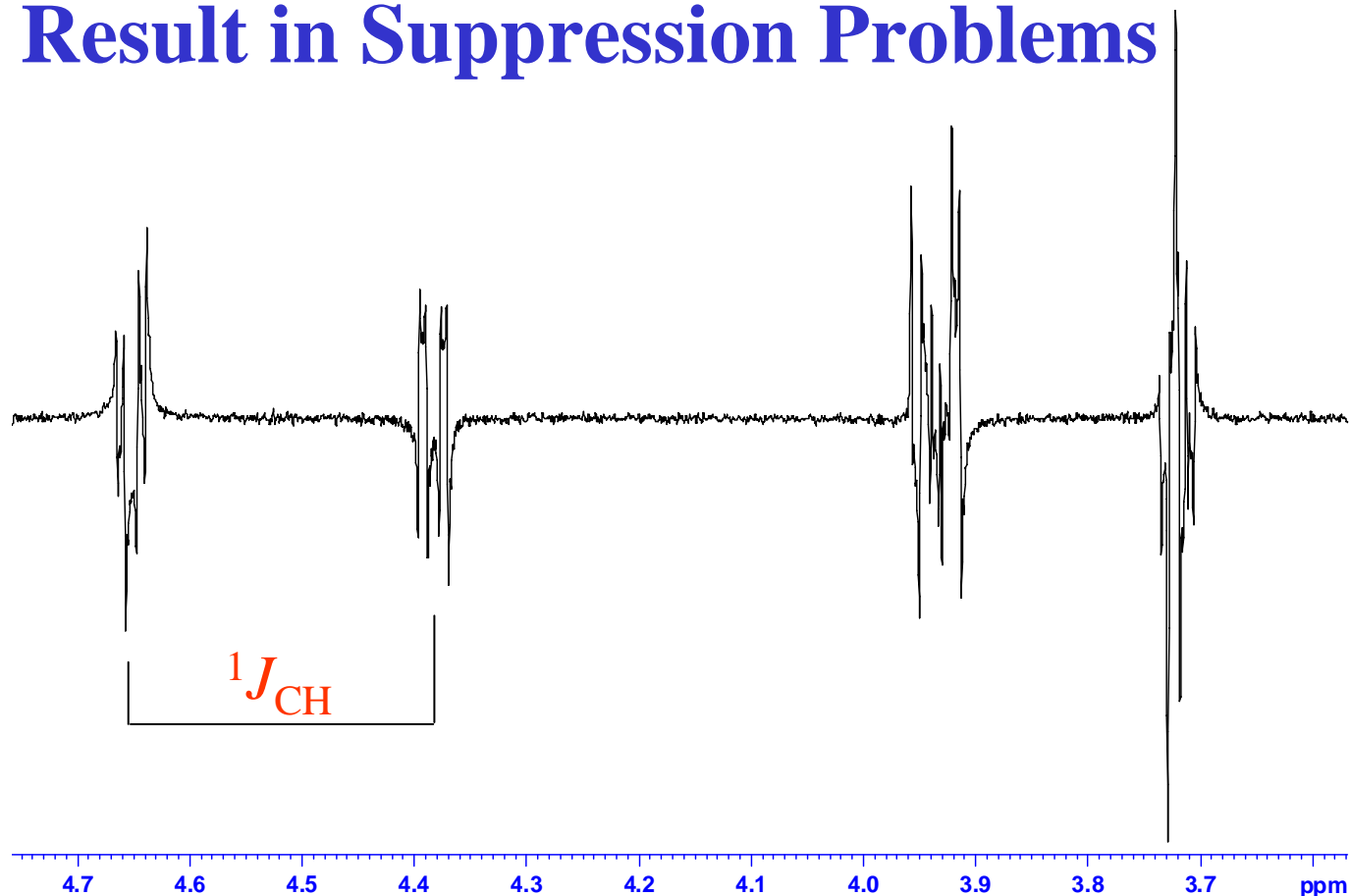
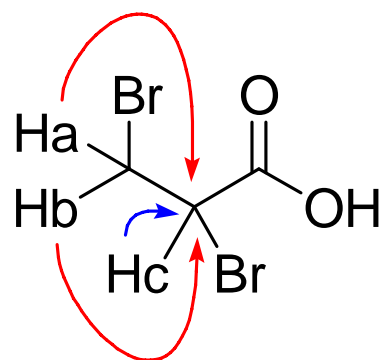
**^1H Spectra 180°
out of phase**

Peak Fit

CPMG-HSQMBC

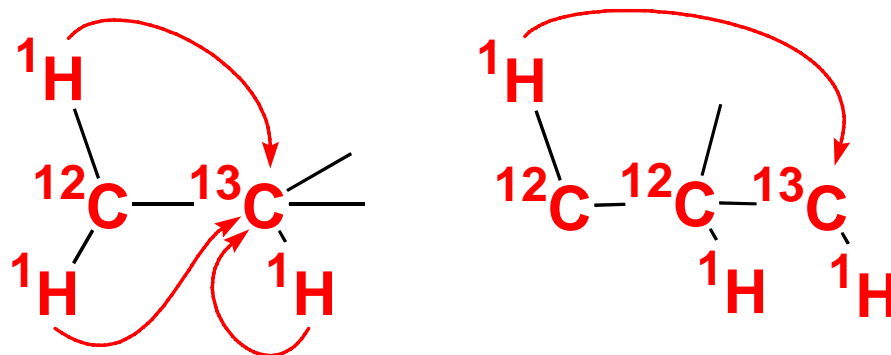


$^2J_{\text{CH}}$ Result in Suppression Problems

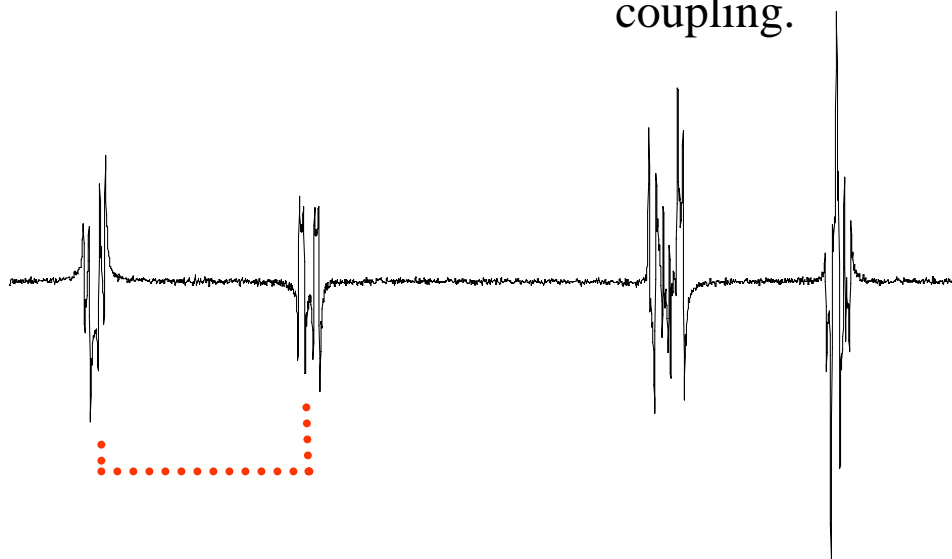


Correlations in which ^1H 's long-range bound to ^{13}C with $^1J_{\text{CH}}$ give rise to inefficient suppression of homonuclear coupling and significant line-shape distortion.

LR-CAHSQC/CPMG-HSQMBC

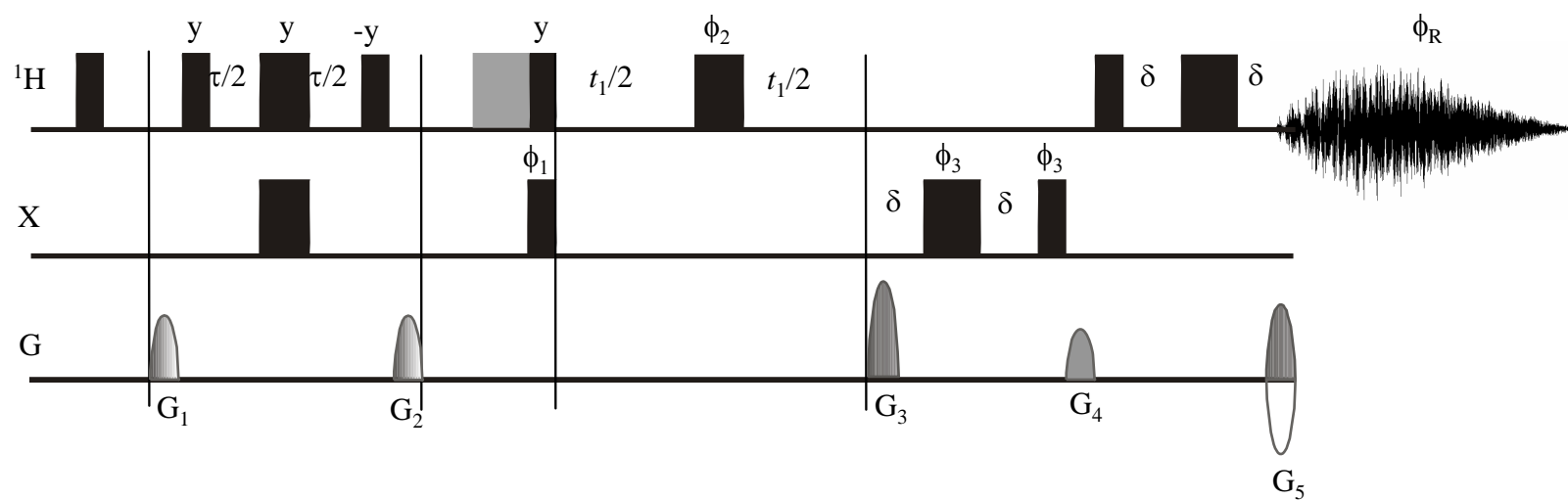


If the CPMG-HSQMBC correlation (i.e. $^2J_{CH}$ to protonated carbon) is to a carbon with a homonuclear coupled proton, CPMG does not decouple homonuclear coupling.



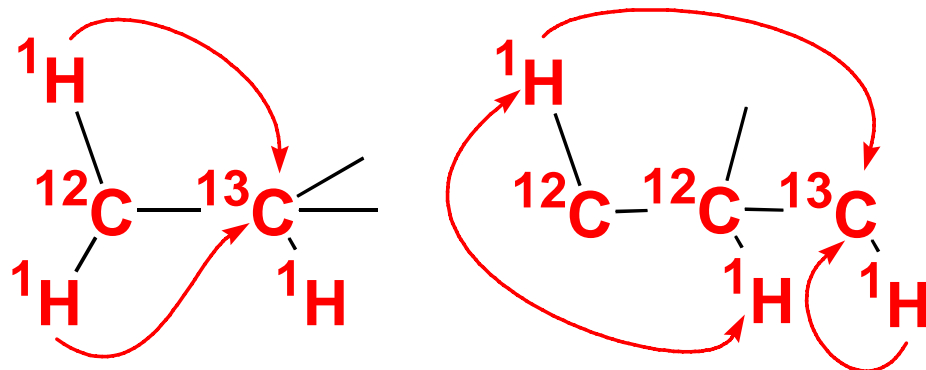
2 bond J_{CH}

G-BIRD_R-HSQMBC

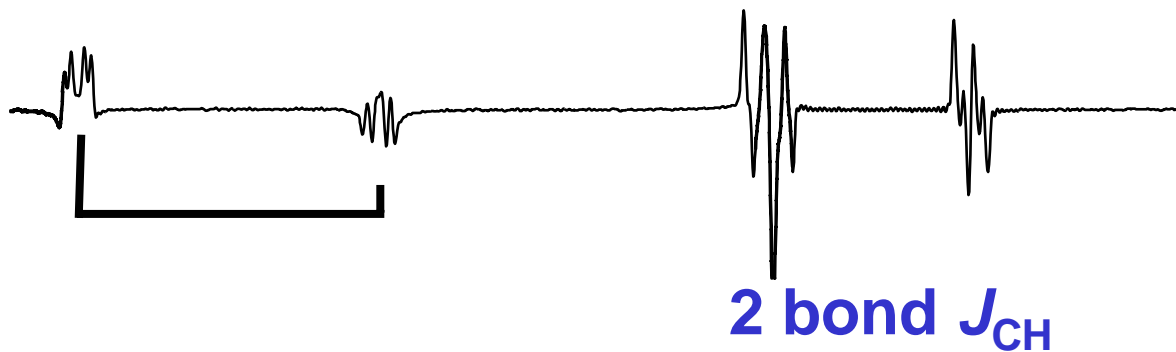


Marquez, B. M.; Williamson, R. T.; Gerwick, W. H. *Magn. Reson. Chem.* 2001, 39, 499-530.

G-BIRD_R-HSQMBC



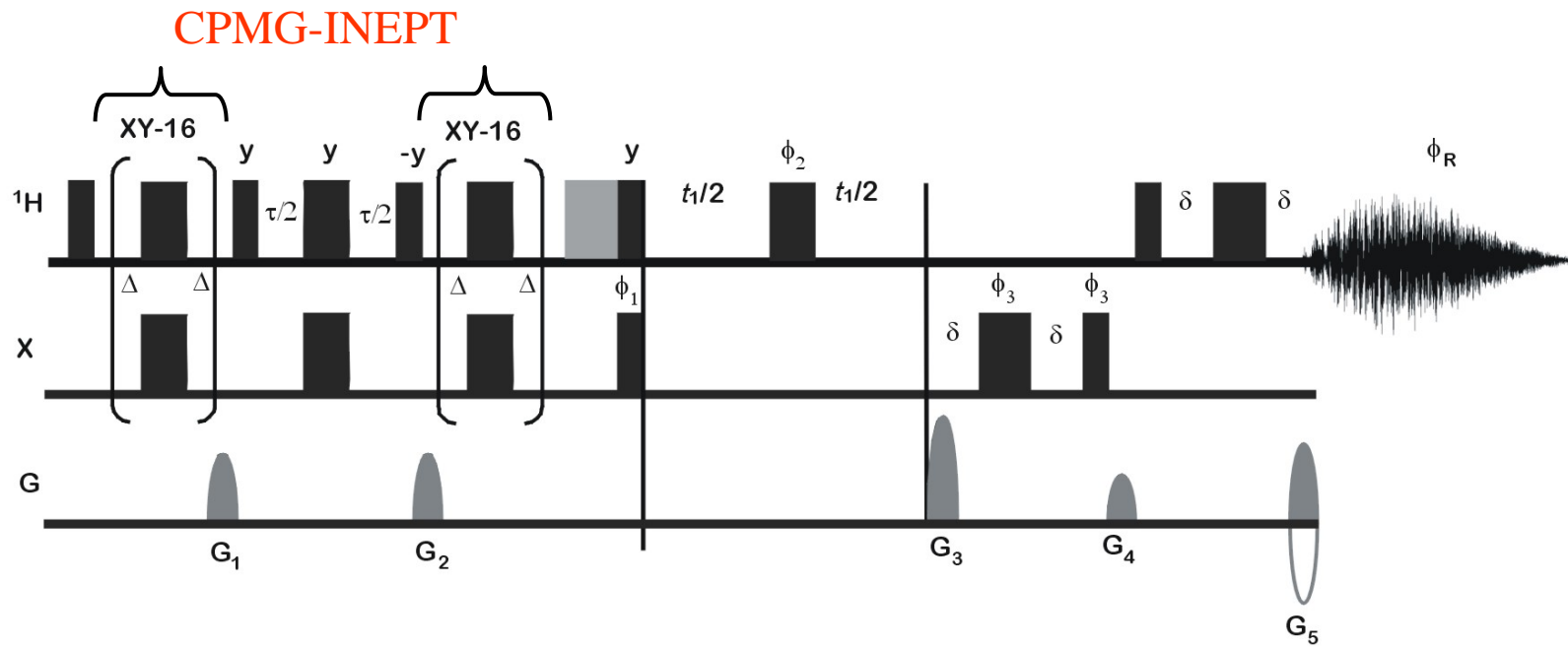
G-BIRD decouples remote protons from carbons bound to ^{13}C but has no effect on carbons bound to ^{12}C (i.e. $^3J_{\text{CH}}$ correlations).



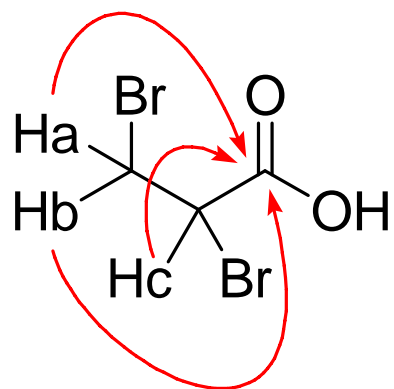
G-BIRD_{R,X}-CPMG-HSQMBC

G-BIRD_{R,X}-CPMG-HSQMBC NMR pulse sequence provides excellent phase properties in heteronuclear long-range correlation data, thereby in most cases obviating the need for any peak fitting protocol.

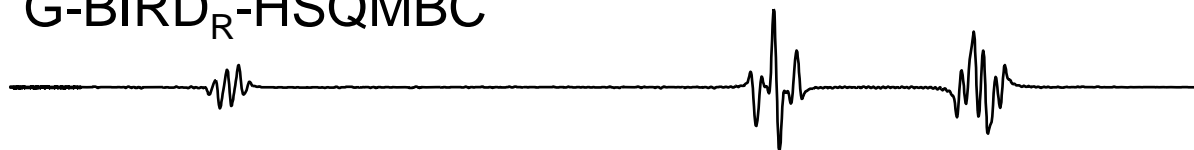
The inclusion of the CPMG effectively suppresses J_{HH} evolution during the polarization transfer. This element in tandem with the G-BIRD provides lineshape with very little dispersive contribution.



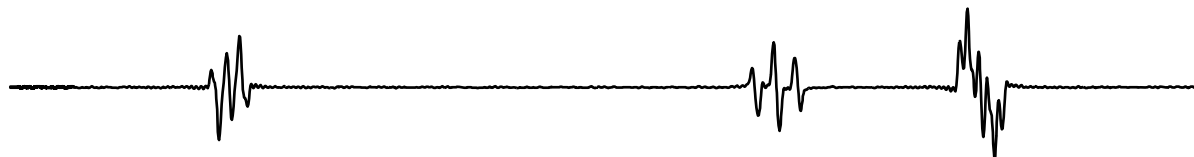
HSQMBC variants



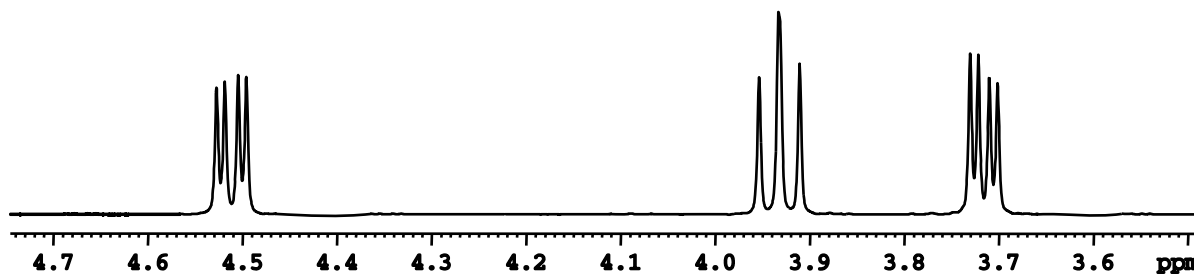
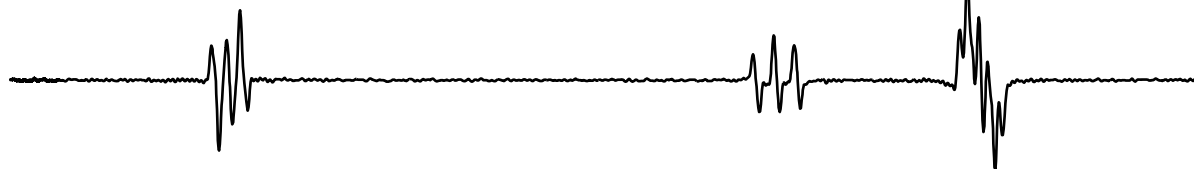
G-BIRD_R-HSQMBC



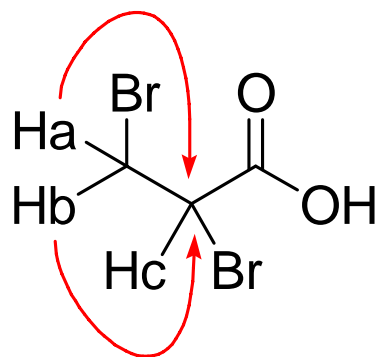
CPMG-HSQMBC



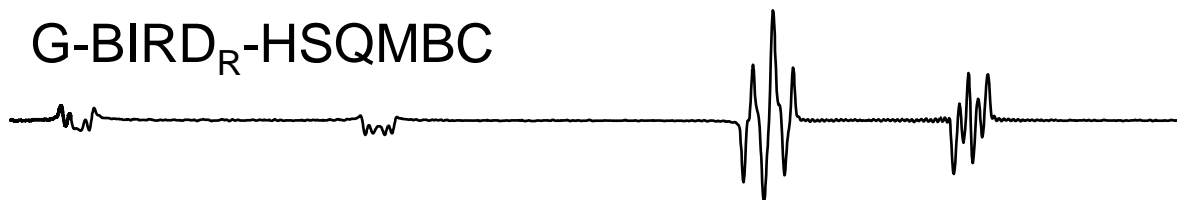
G-BIRD_R-CPMG-HSQMBC



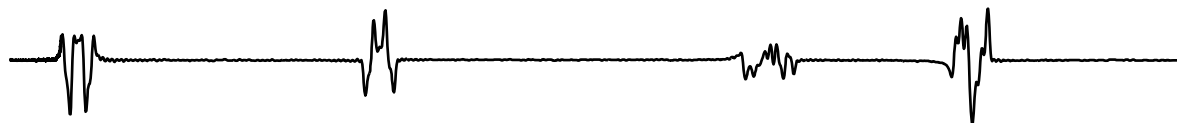
HSQMBC variants



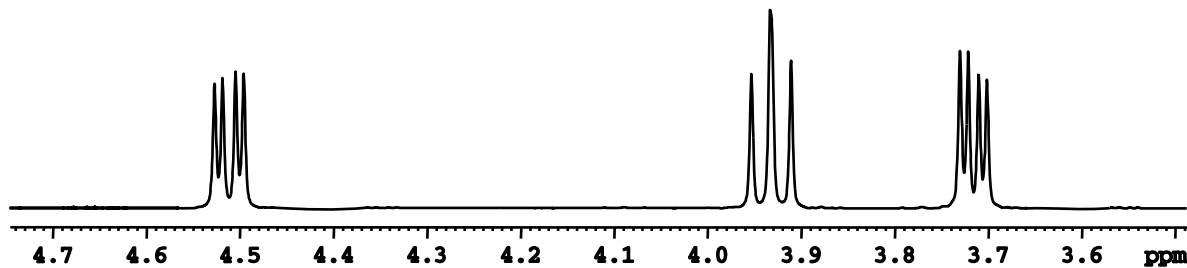
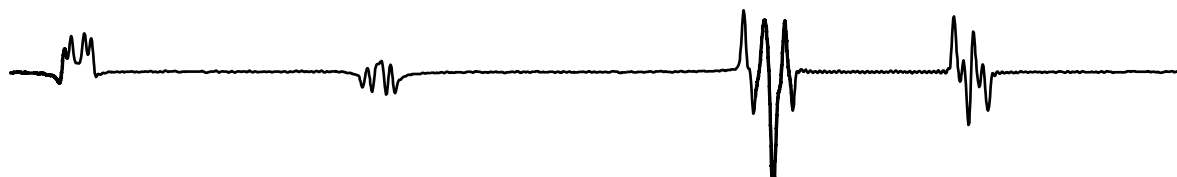
G-BIRD_R-HSQMBC



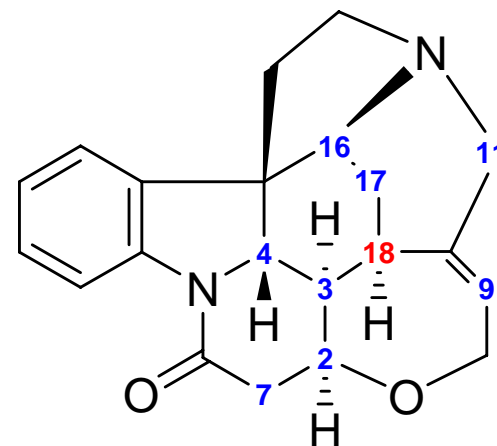
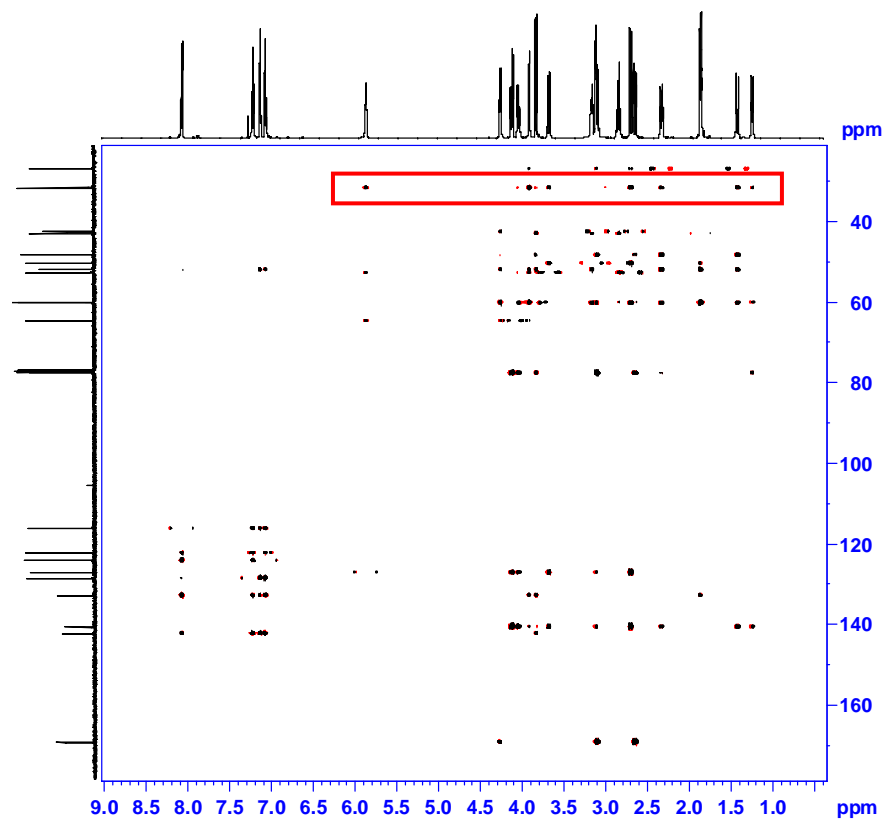
CPMG-HSQMBC



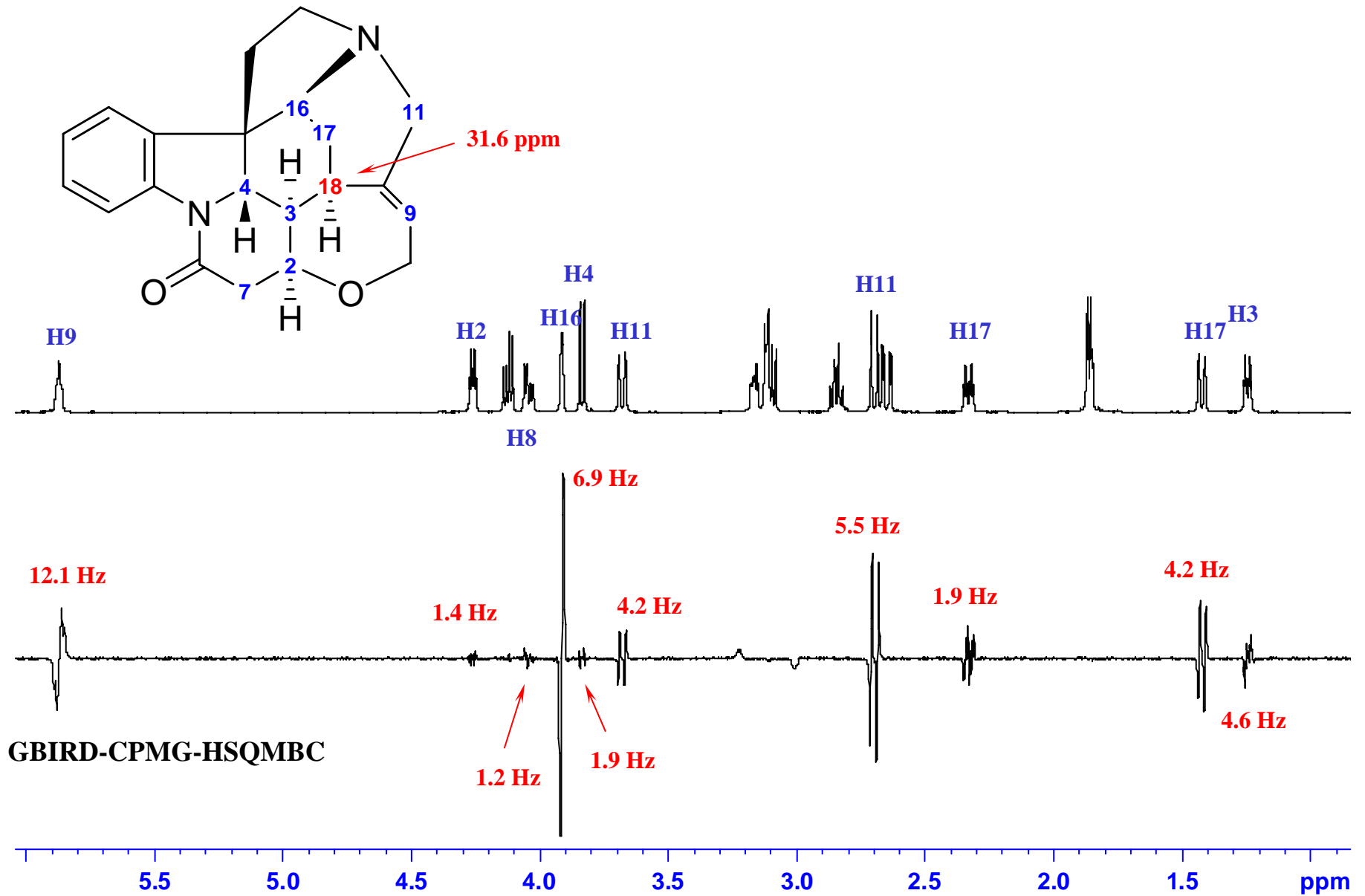
G-BIRD_R-CPMG-HSQMBC



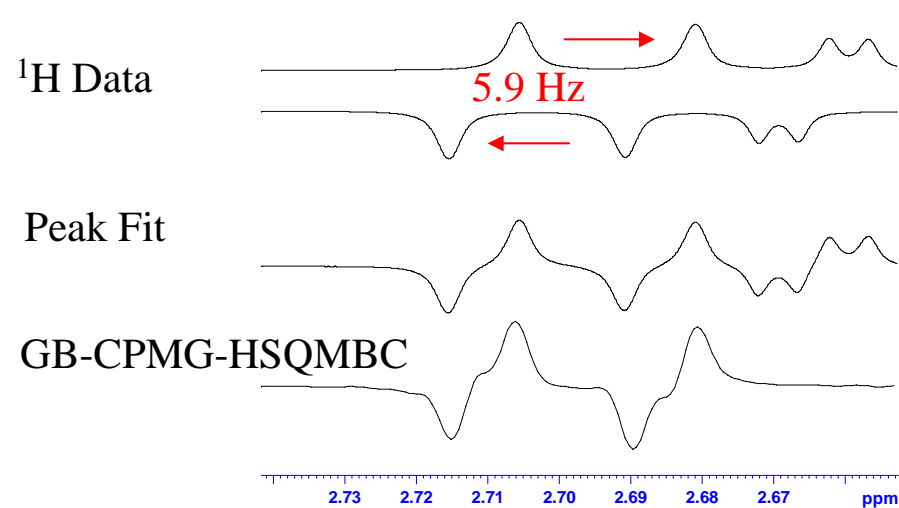
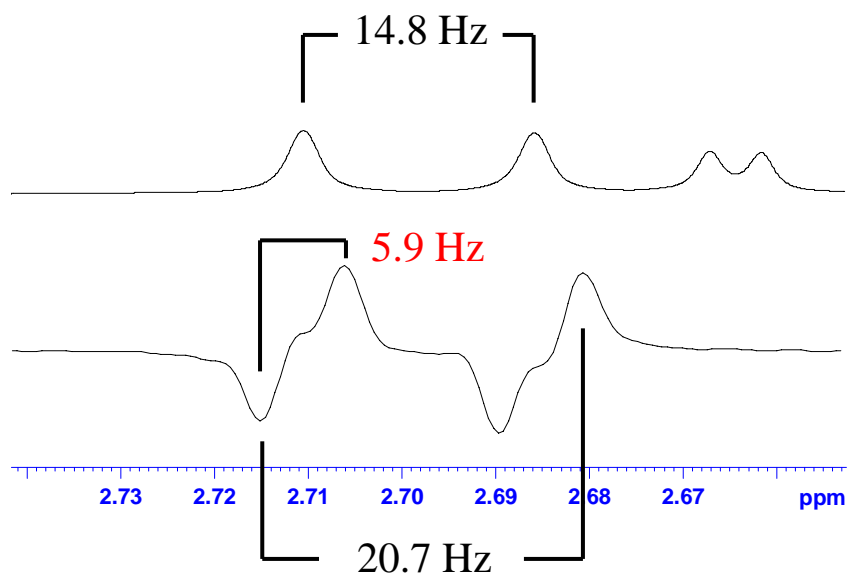
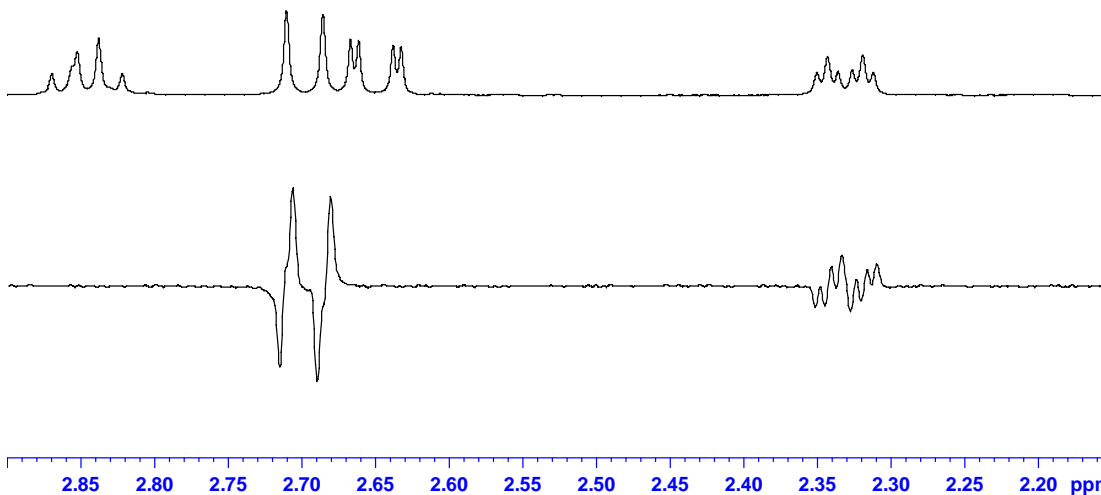
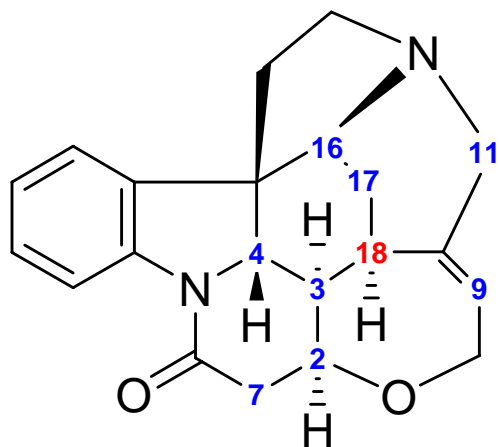
GBIRD-CPMG-HSQMBC of Strychnine



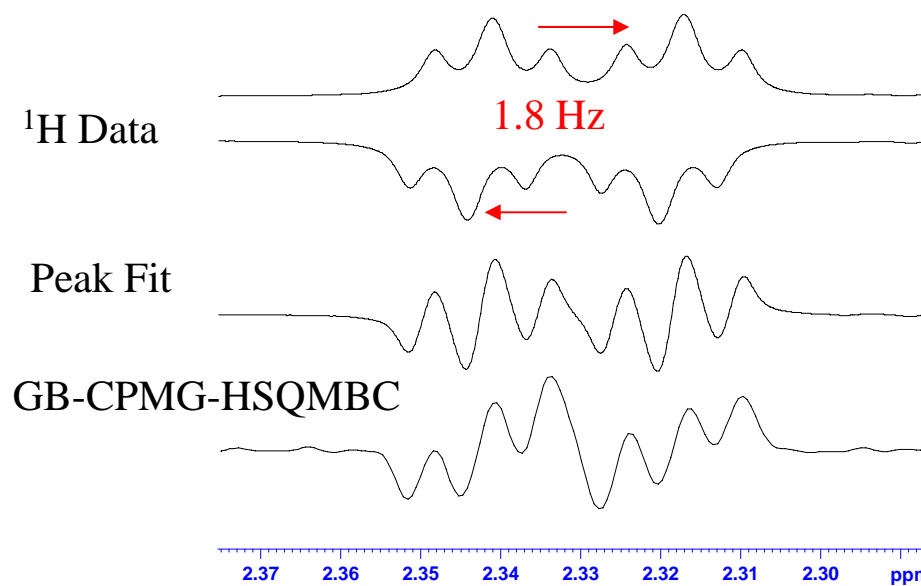
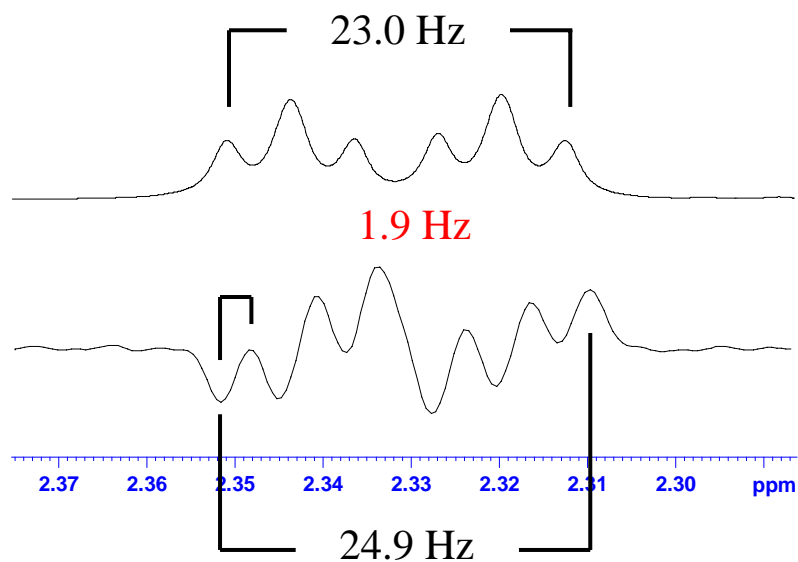
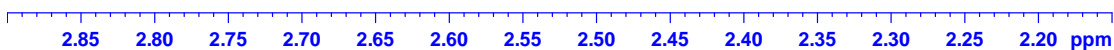
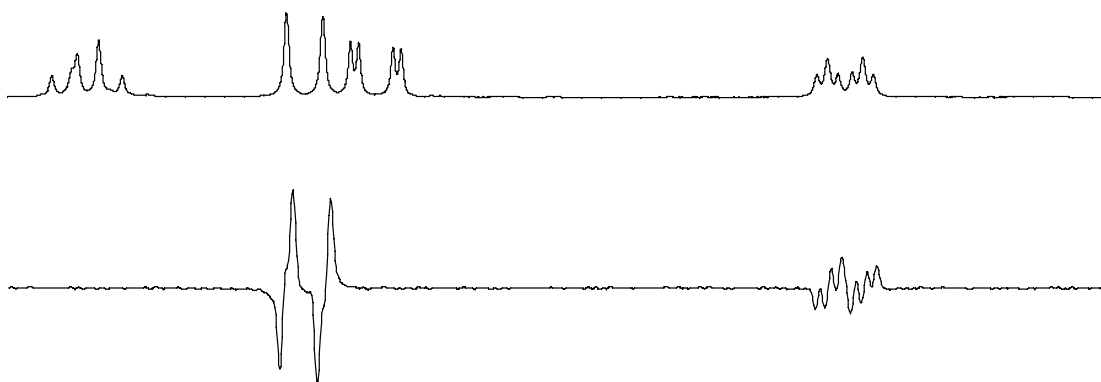
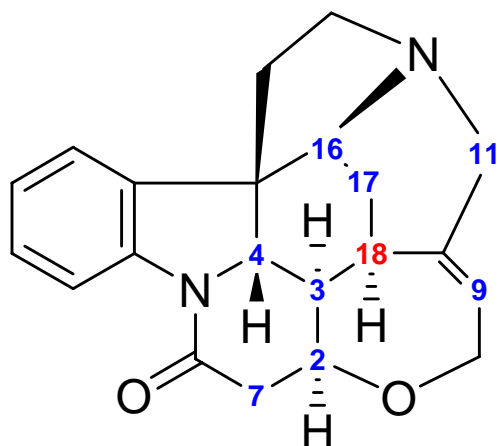
Example using Strychnine



Fitting Peak Width and Direct Measure

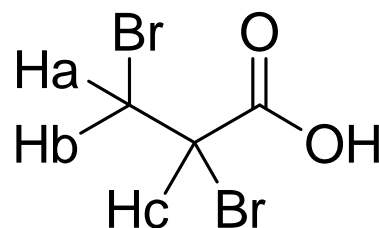


Fitting Peak Width and Direct Measure

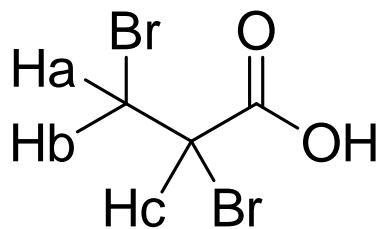


DBA: A Nice Example

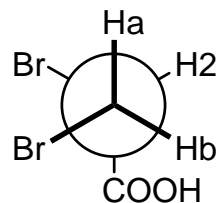
- The next few slides will detail how one will utilize the needed NMR data.
- 2,3 dibromopropionic acid will be used as an example on how to extrapolate the needed coupling information.



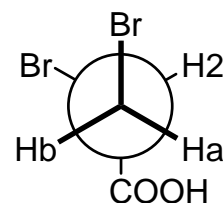
Rotameric Possibilities for 2,3 Dibromopropionic Acid



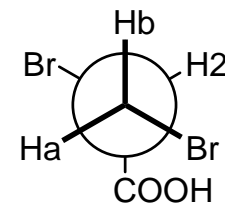
Ha-H2
Hb-H2
Ha-COOH
Hb-COOH



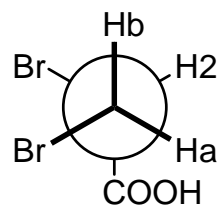
SM
SM
LG
SM



SM
LG
SM
SM

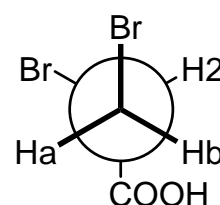


LG
SM
SM
LG

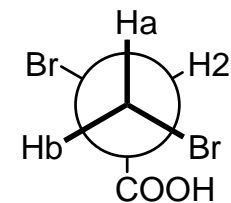


Ha-H2
Hb-H2
Ha-COOH
Hb-COOH

SM
SM
SM
LG

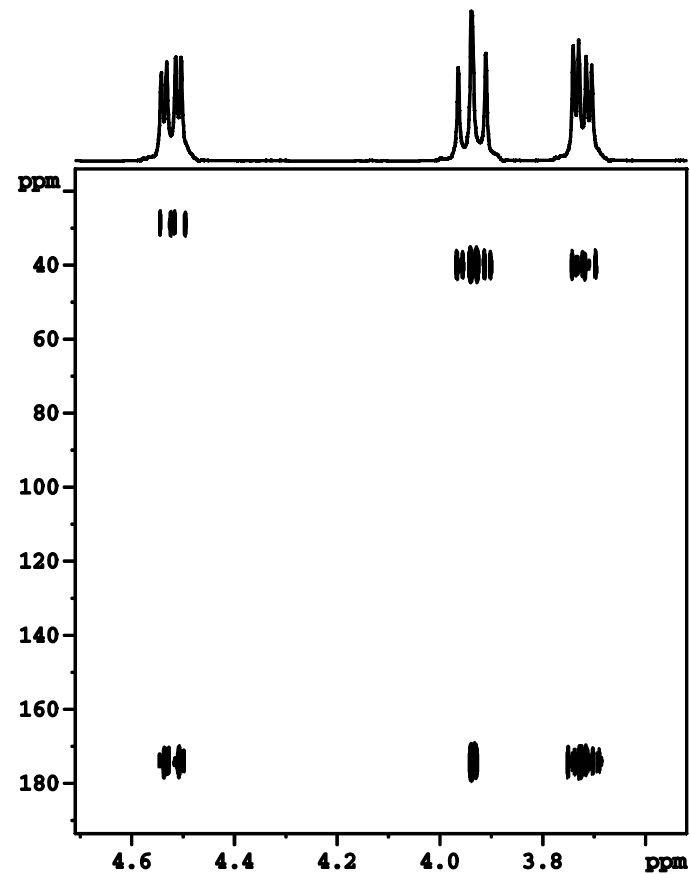
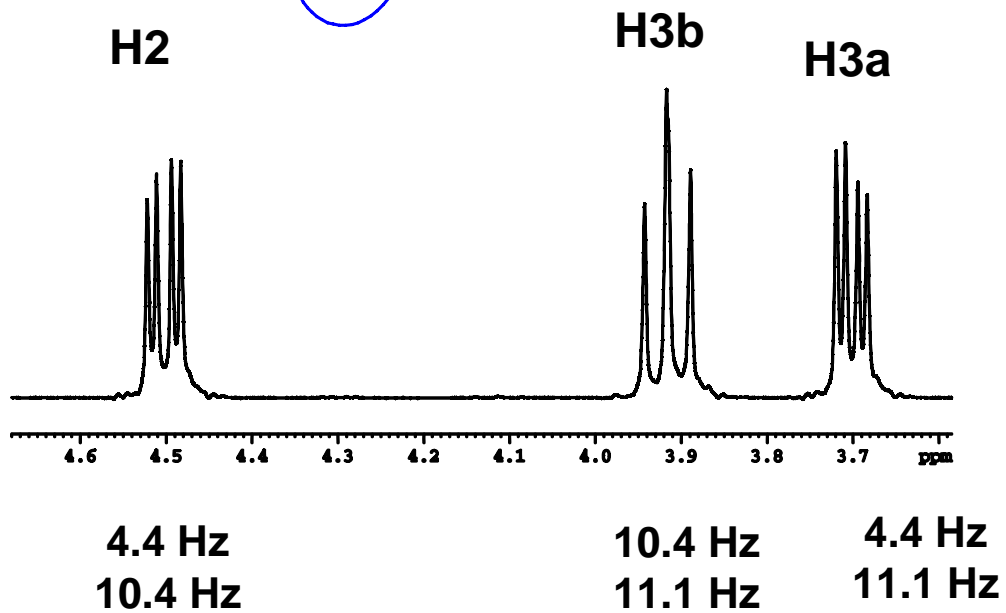
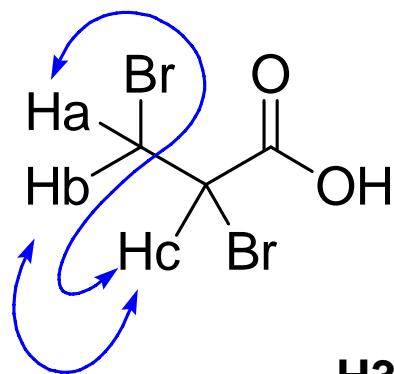


LG
SM
SM
SM

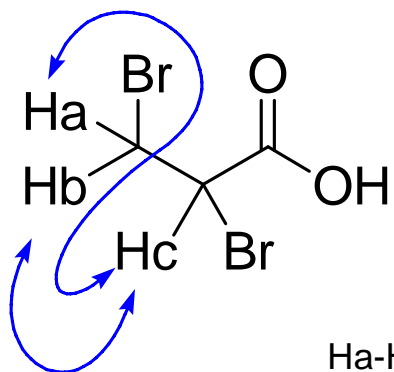


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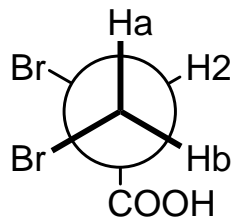
HSQMBC data for DBA



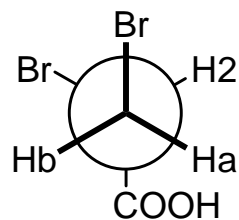
Reduction in Possible Rotamers



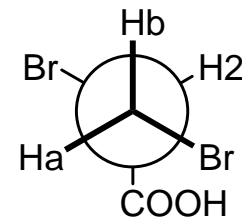
Ha-H2
Hb-H2
Ha-COOH
Hb-COOH



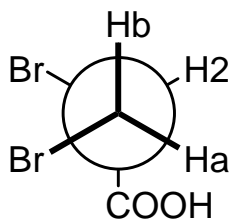
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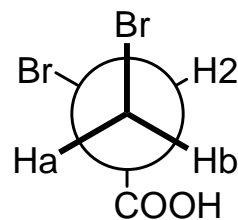


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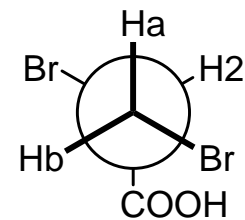


Ha-H2
Hb-H2
Ha-COOH
Hb-COOH

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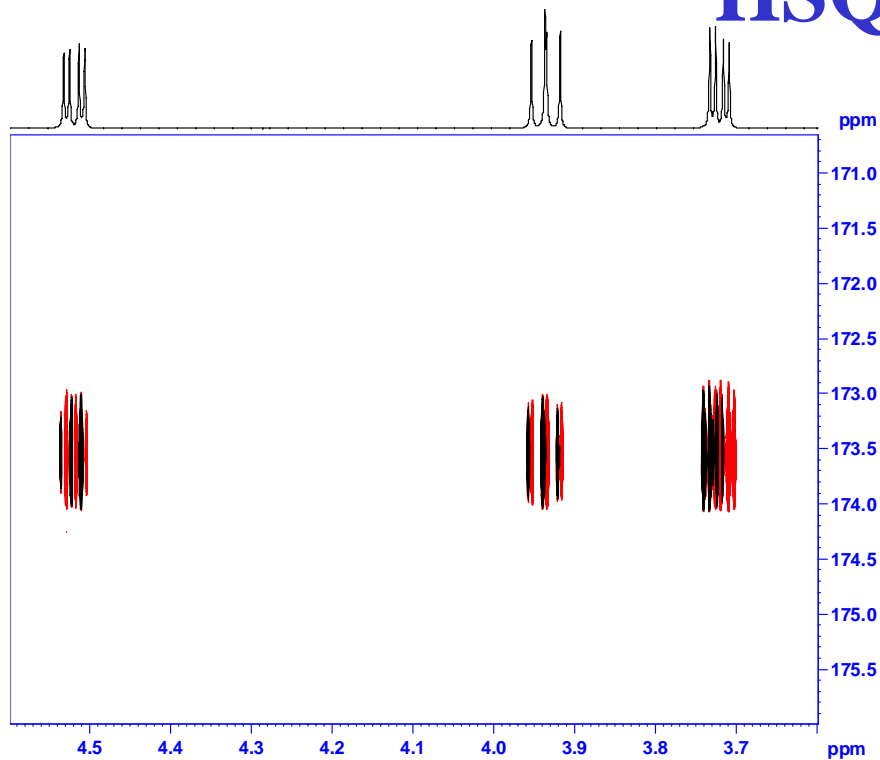


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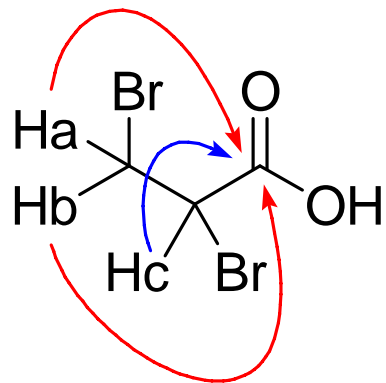
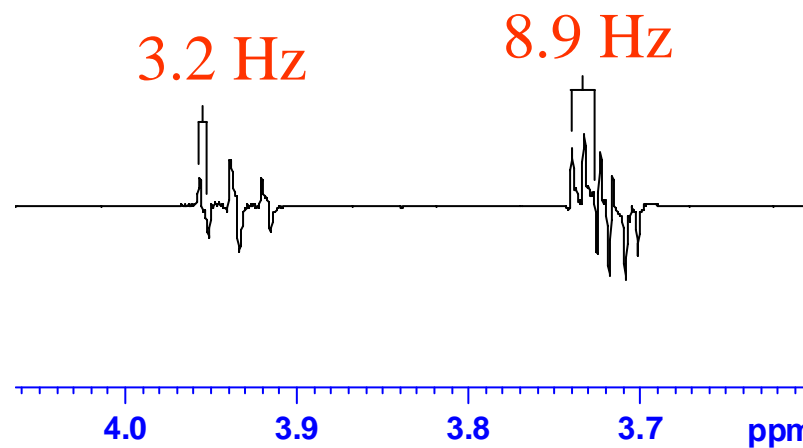


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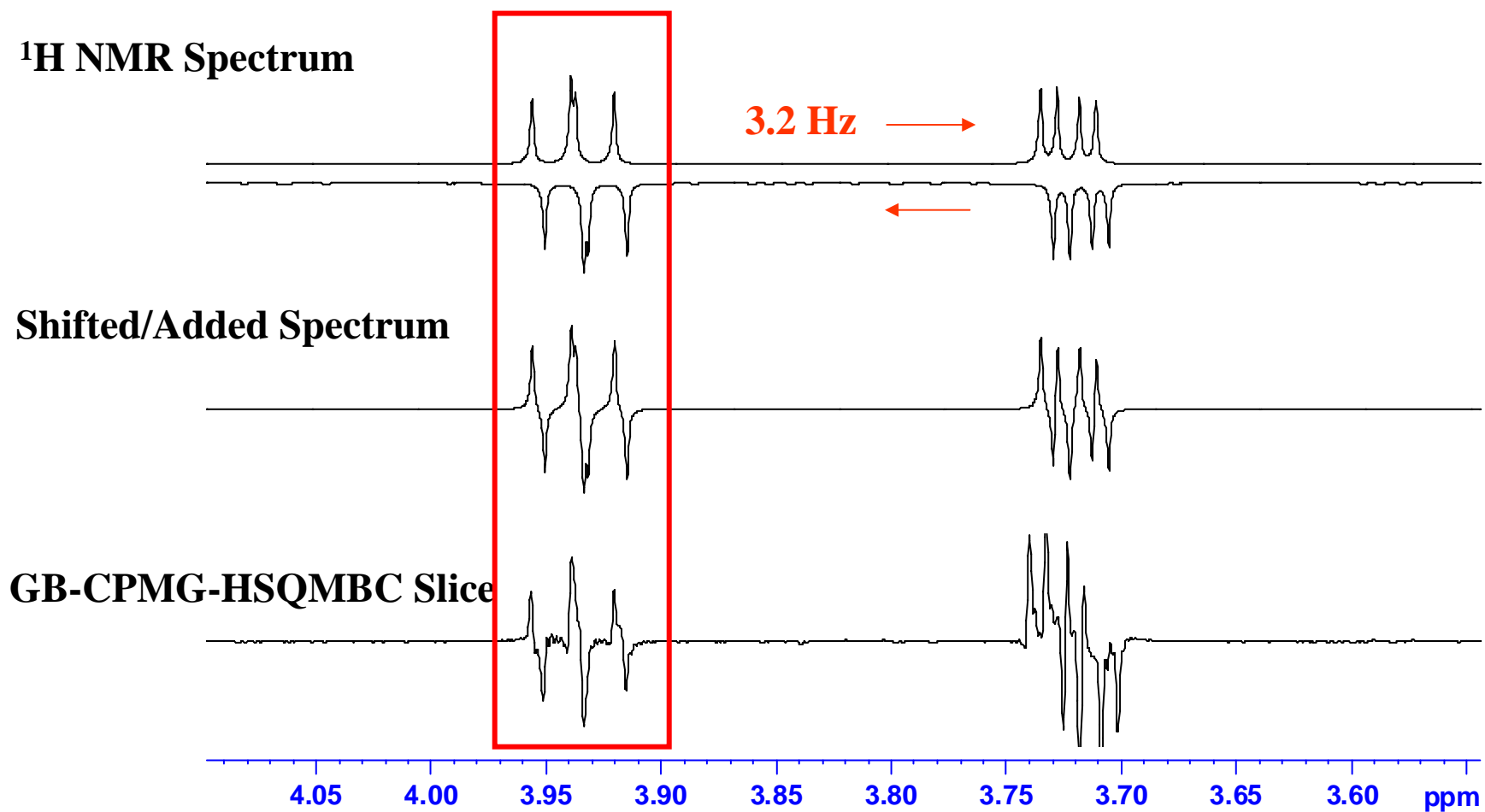
Extracted *F2* Slice of GBIRD-CPMG-HSQMBC



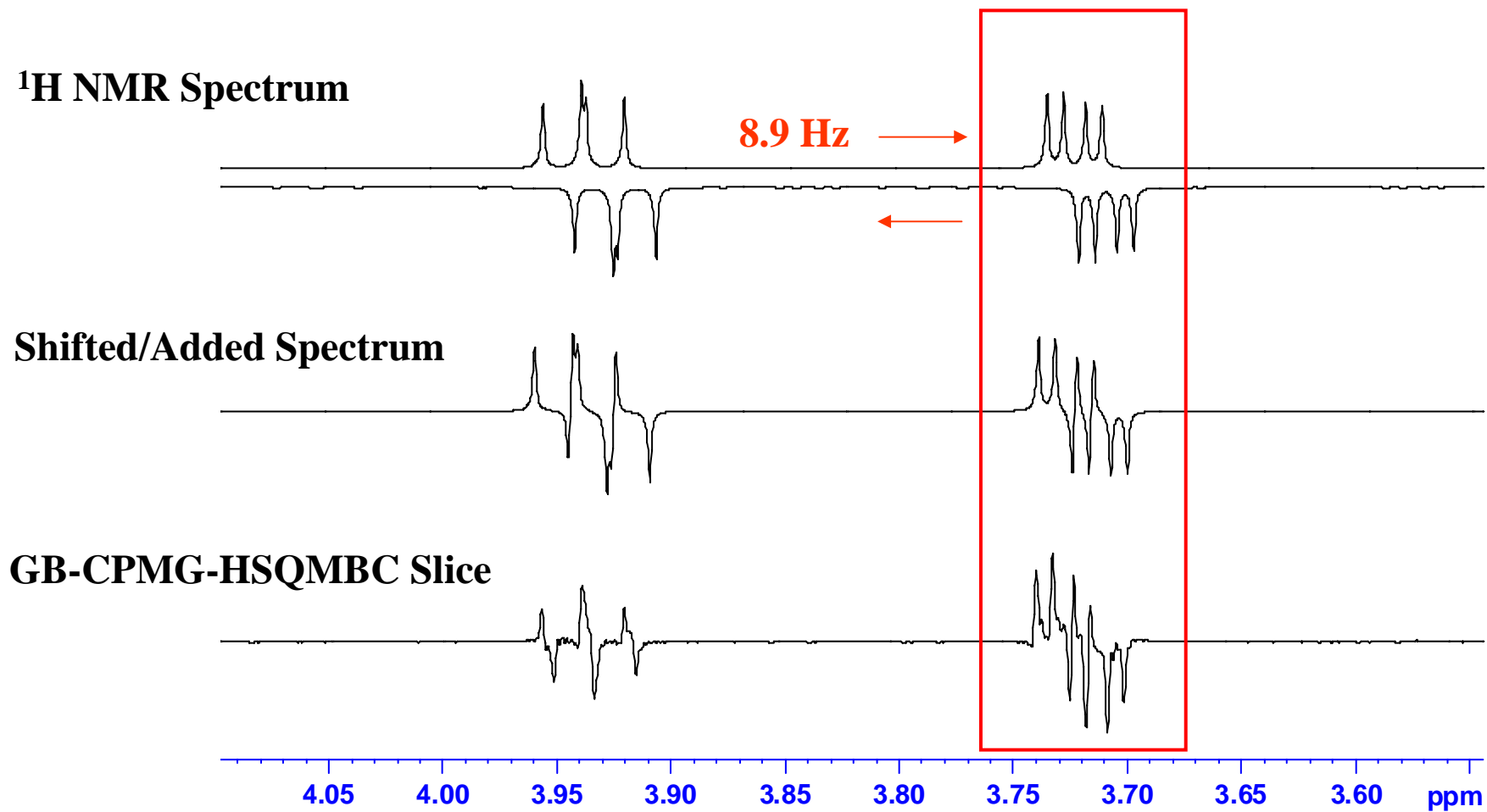
Direct Measure



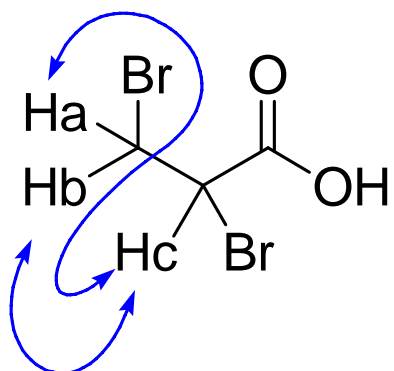
Peak Fitting Analysis



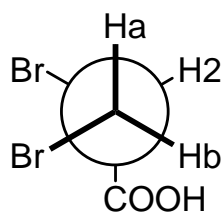
Continued Peak Fitting Analysis



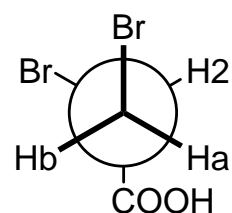
Assignment of Rotamer



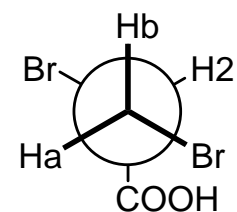
Ha-H2
Hb-H2
Ha-COOH
Hb-COOH



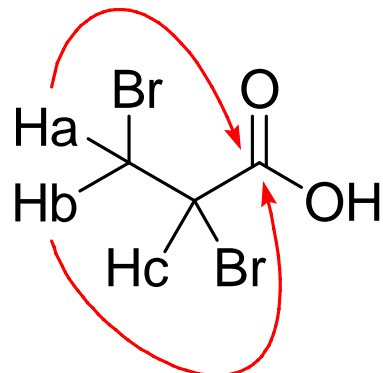
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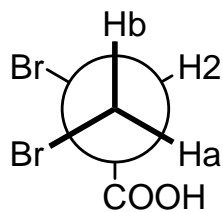
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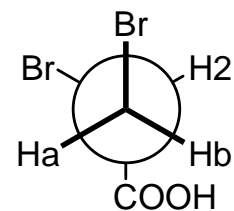
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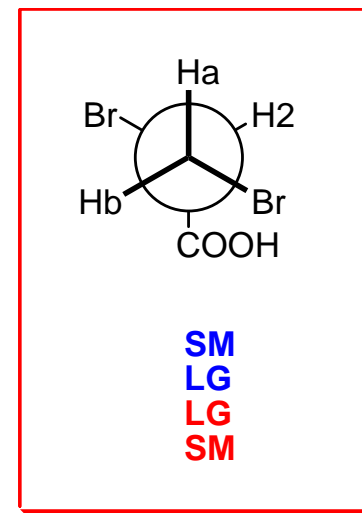
Ha-H2
Hb-H2
Ha-COOH
Hb-COOH



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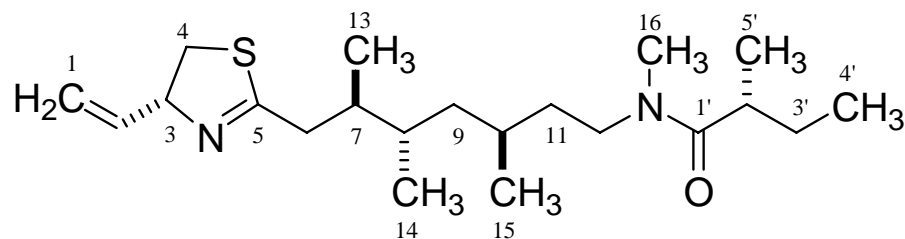
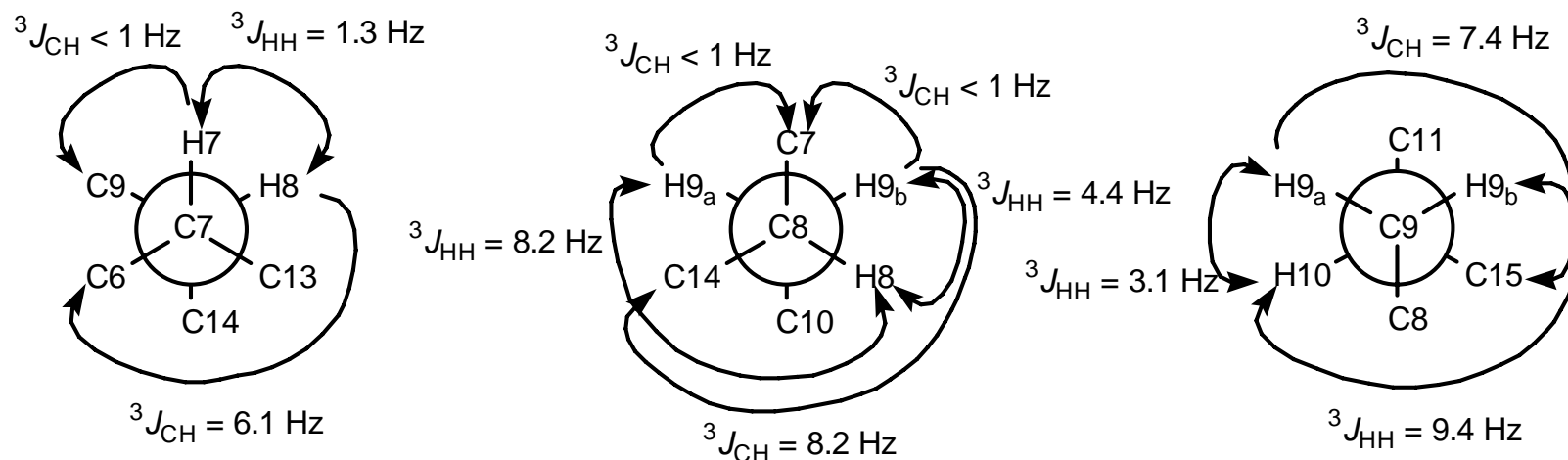


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Examples of Complex Systems

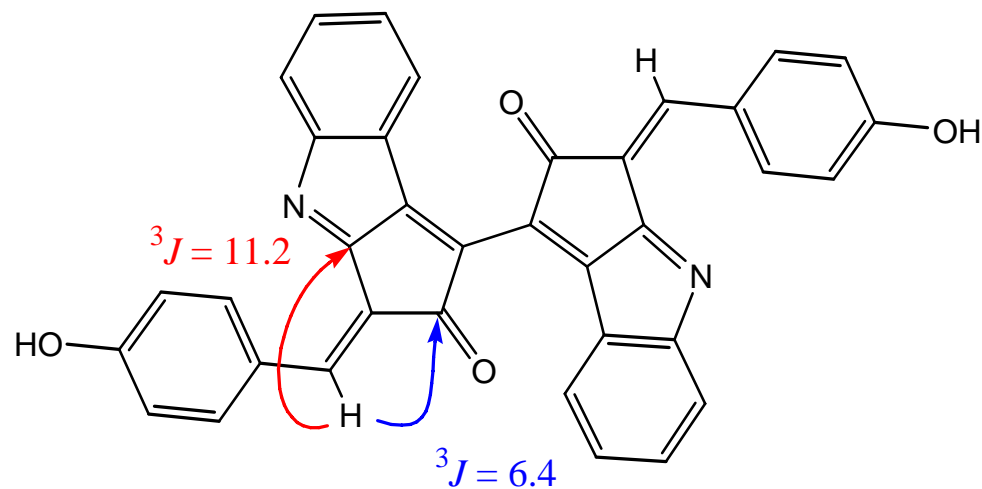


Kalkitoxin

Predicted the relative stereochemistry of 7R,8S,10S

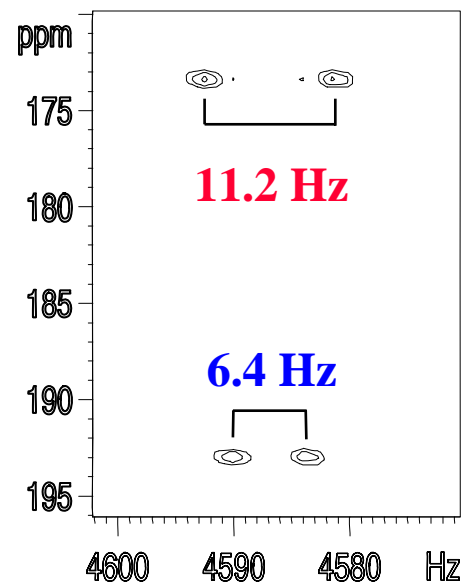
Wu, M.; Okino, T.; Nogle, L. M.; Marquez, B. L.; Williamson, R. T.; Sitachitta, N.; Berman, F. W.; Murray, T. F.; McGough, K.; Jacobs, R.; Colsen, K.; Asano, T.; Yokokawa, F.; Shioiri T.; Gerwick, W. H. *J. Am. Chem. Soc.*, **2000**, 122(48), 12041-12042.

Regiochemistry of Proton Deficient Systems



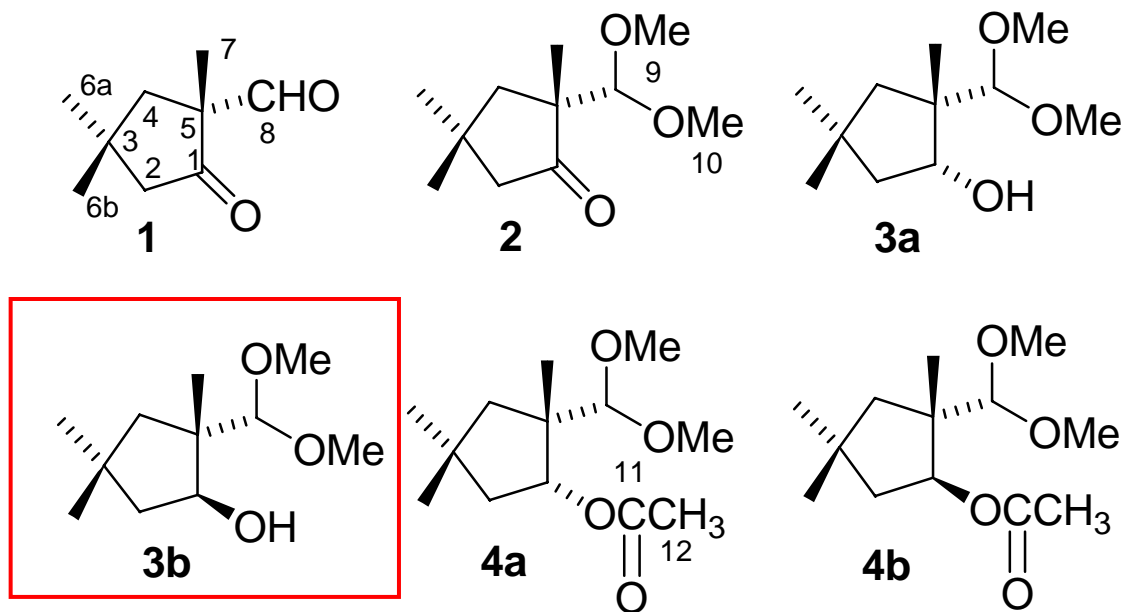
Scytonemin

HSQMBC



Williamson, R. T.; Marquez, B. M.; Gerwick, W. H.; Kover, K.
Magn. Reson. Chem. **2000**, *38*, 265-273.

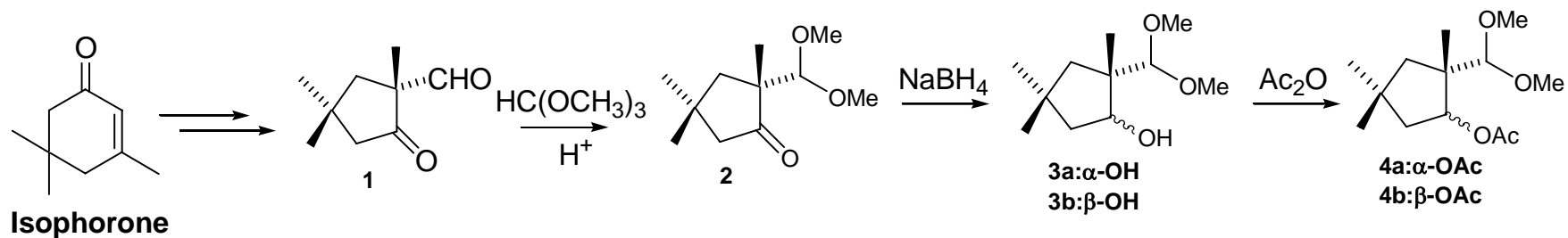
Relative Stereochemistry of Cyclopentane Derivatives



Cyclopentanes prepared and used in NMR studies

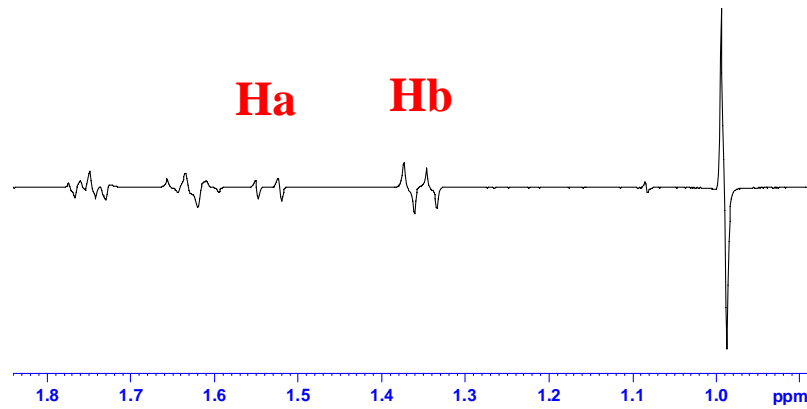
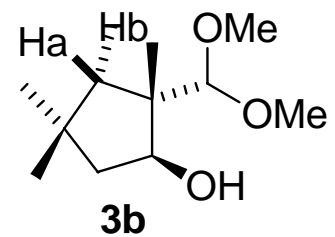
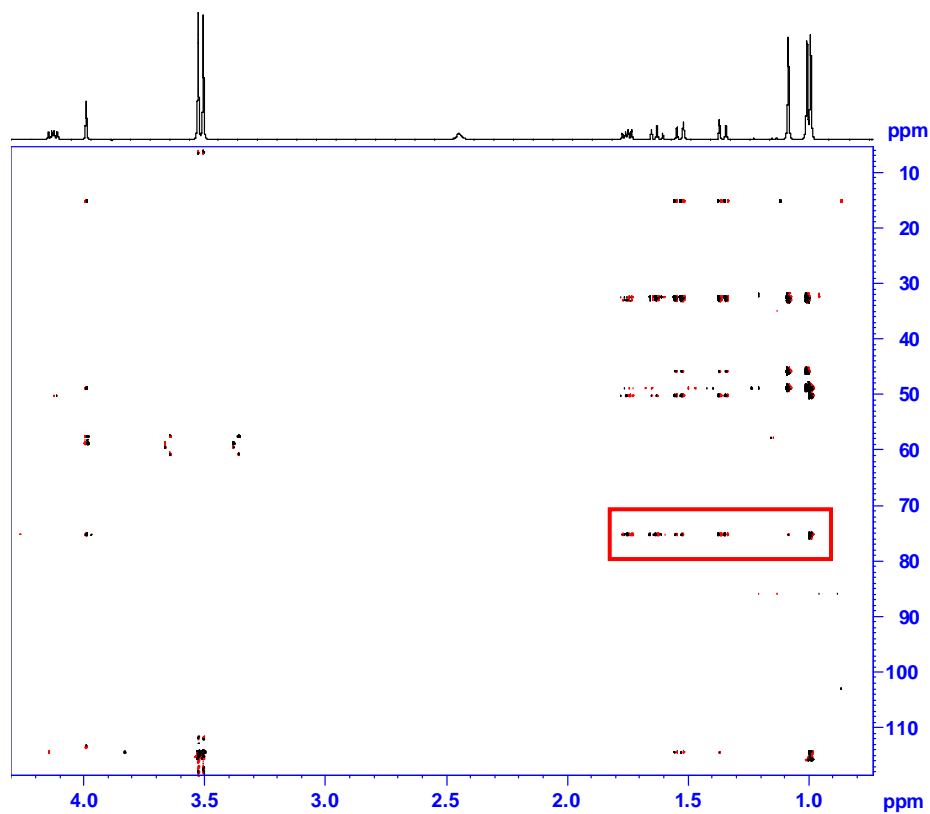
Lacerda Jr., V.* da Silva, G. V. J. Constantino M. G., Tormena C. F., Williamson R. T., and Marquez B. L. *Magn. Reson. Chem.* Accepted

Synthetic Scheme of Cyclopentane Derivatives

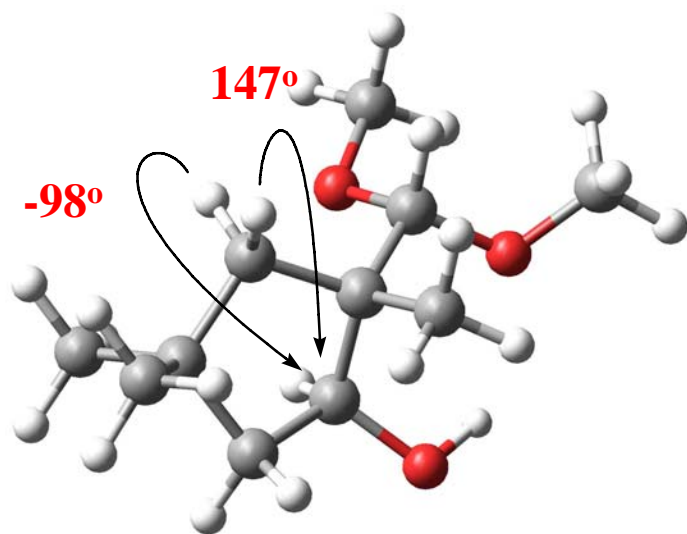


Constantino MG, Lacerda Jr. V, da Silva GVJ *Magn. Reson. Chem.* 2003; 41: 641-643.

GB-CPMG-HSQMBC of 3b



Coupling Constants Fit the Model



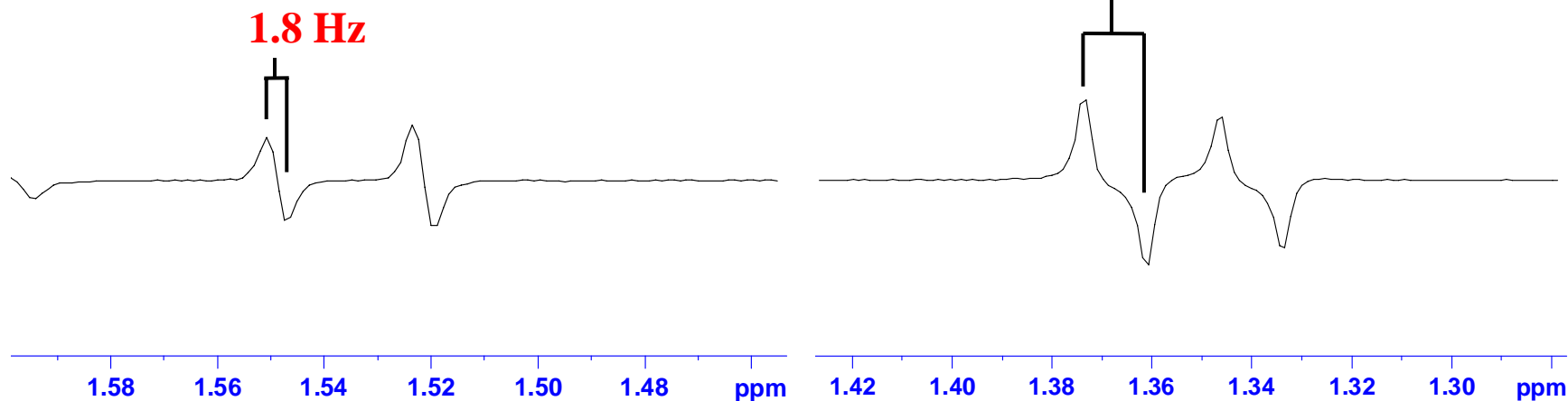
$$J = 8.06 \cos^2(q) - 0.87 \cos(q) + 0.47$$

**Calculated values
(from coupling constants):**

-109

143

6.3 Hz



Conclusions

- The use of heteronuclear coupling constants is instrumental in many elements of structure elucidation.
- The combination of the CPMG pulse train and the G-BIRD during polarization transfer give rise to excellent lineshape providing a very straight forward analysis.
- Using the peak width to determine the heteronuclear coupling constant is extremely simple and fast.
- The determination of the relative stereochemistry of cyclopentane derivatives is straight forward when using long-range heteronuclear coupling constants.

Acknowledgements

Universidade de Sao Paulo

- Valdemar Lacerda Jr.
- Gil V. J. da Silva
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- Claudio F. Tormena

Pfizer

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- Stephen Brown
- Chris Sinko
- Michael Motto