



# Solid-State NMR Spectroscopy in Organic and Biological Chemistry

Timothy R. Ramadhar

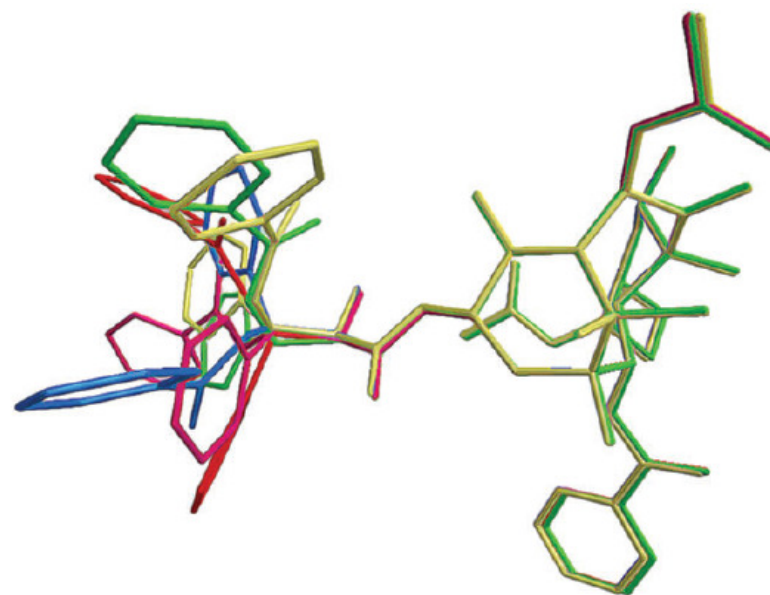
Davenport Research Laboratories  
Department of Chemistry  
University of Toronto

Monday Night Seminar – April 6, 2009



# Solid-State NMR Spectroscopy

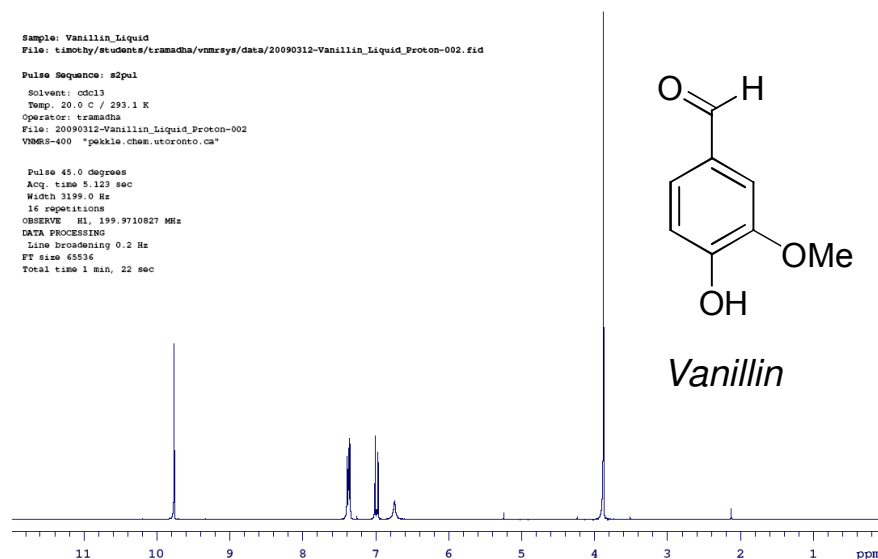
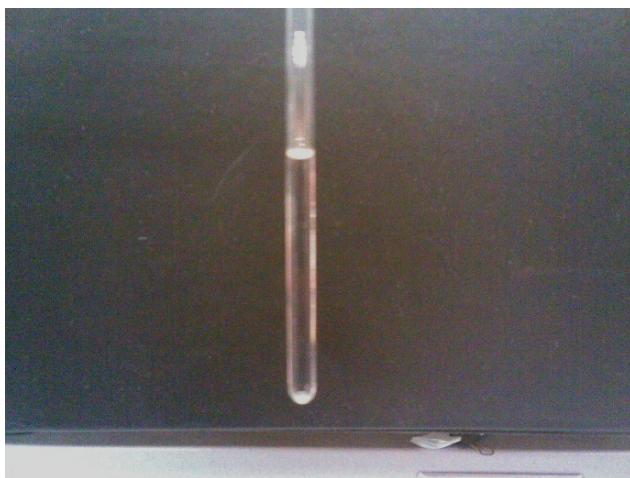
- SSNMR - for amorphous solids, powder crystalline samples
  - Useful when X-ray quality crystals unavailable, solution NMR data ambiguous
- Structure
- Chirality
- Enzyme mechanisms
- Polymorphism
  - **Different** crystal forms of *same* compound
    - Conformations, hydrates, etc.
- Solid-phase peptide synthesis



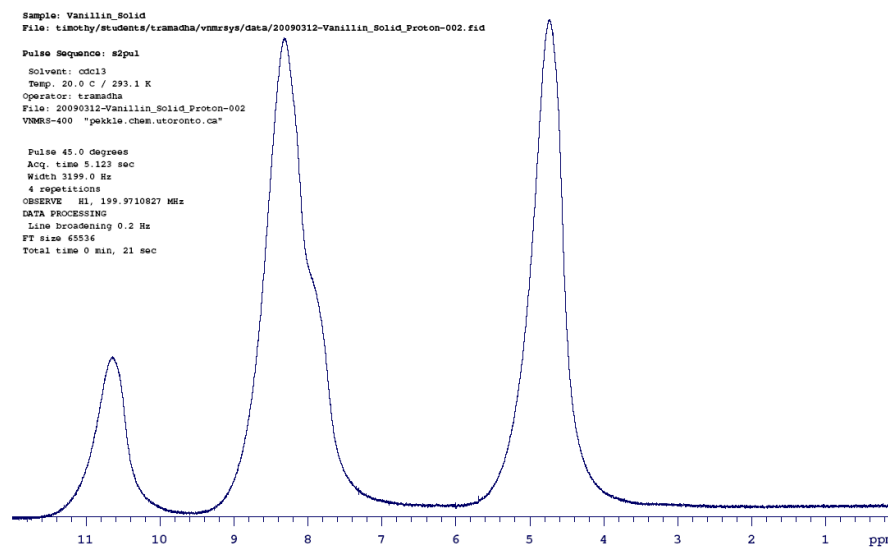
*5 possible conformers of 1 polymorph of Paclitaxel*

# Running Solid Samples on NMR Probes for Liquids

Liquid –  $^1\text{H}$  NMR (Mercury 200,  $\text{CDCl}_3$ )



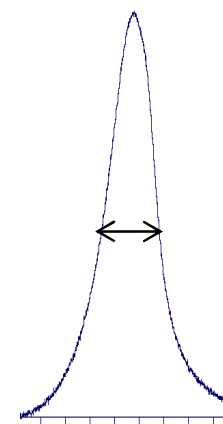
Solid (adsorbed with minimal  $\text{CDCl}_3$ )  
 $^1\text{H}$  NMR (Mercury 200) unlocked, FID shimmed



# Line-shape Broadening Factors for Solid Samples

- Direct Dipolar Coupling
  - Between at least two nuclear magnetic moments
  - *Heteronuclear and Homonuclear*
- Chemical Shift Anisotropy
  - Orientation dependence of molecule relative to  $B_0$
- Shorter Spin-Spin ( $T_2^*$ ) Relaxation
  - Larger linewidths at half-height
- Quadrupolar Interaction for Spin  $> \frac{1}{2}$  (discuss later)
  - Between nuclear charge distribution and electric field gradient in the solid
- Magnetic Susceptibility (discuss later)
  - Differences of  $H_0$  (mag. flux) at solid / liquid interface

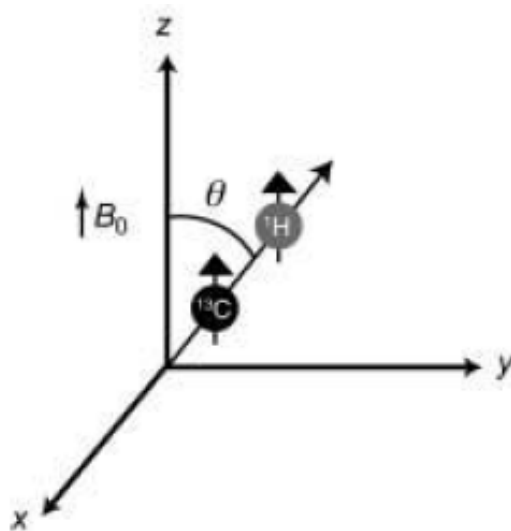
Linewidth at half-height



# Direct Dipolar (DD) Coupling

- When two spins (nuclei I and S) are close ( $\leq 10 \text{ \AA}$ ) in a magnetic field ...
  - One spin affects local magnetic field at another spin
  - Changes frequency of paired nuclei
  - Interaction depends on I-S distance and angle between I-S and  $B_0$

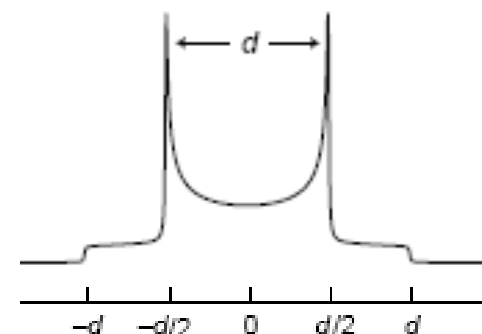
$\theta$  – Angle between I-S and  $B_0$



Dipolar Coupling Constant ( $d$ )

$$d = \left(\frac{\mu_0}{4\pi}\right) \left(\frac{\hbar\gamma_I\gamma_S}{r^3_{IS}}\right)$$

Theoretical Pake Doublet  
 $^{13}\text{C}$  NMR



$^{13}\text{C}$ - $^1\text{H}$   
pure dipolar coupling

- Solution-state: DD averages to 0...*molecules tumble*
  - **Not in solid-state powder samples!**

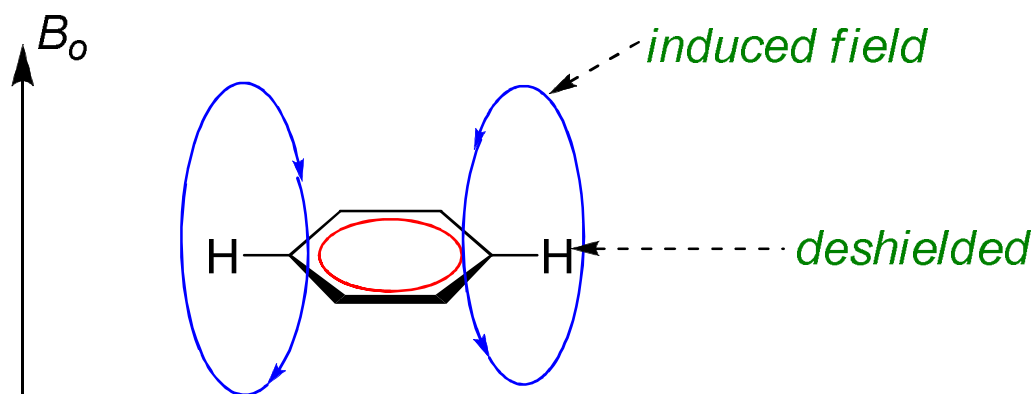
# Liquid-State: Diamagnetic Anisotropy

## Origin of chemical shifts

- In  $B_0$ , electron currents create mini magnetic fields that affect local  $B_0$  at nucleus

## For diamagnetic anisotropy ...

- Shielding and deshielding zones ... “ring current effects”

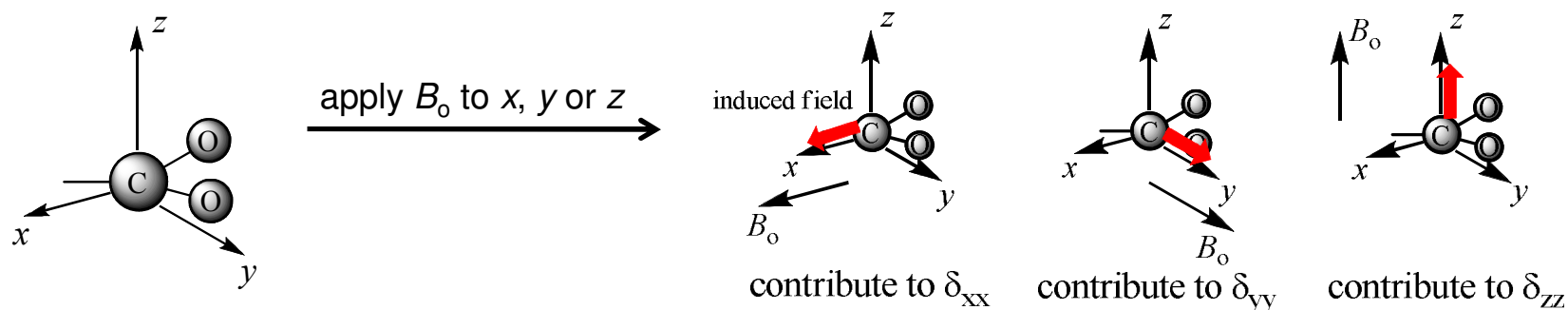


- Orientation dependence with  $B_0$  ... although molecules tumble in solution ...
  - But small amount of aligned molecules affect average  $\delta$  significantly
- This is **not** the same as chemical shift anisotropy (CSA) ...

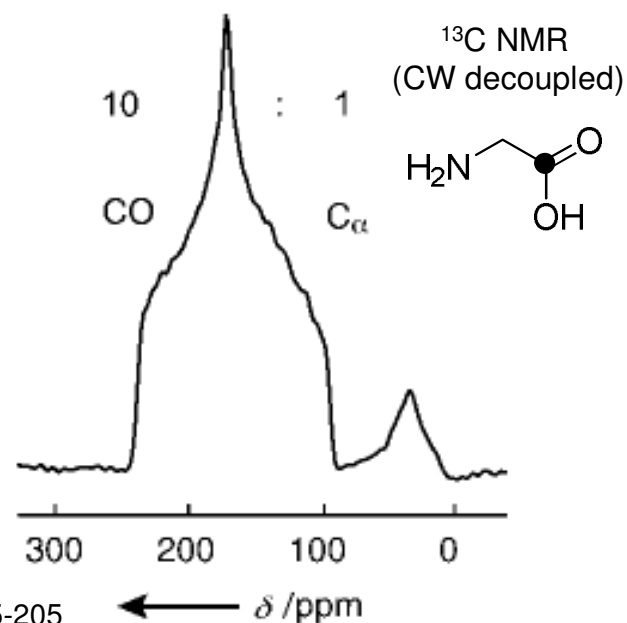
# Solid-State: Chemical Shift Anisotropy (CSA)

CSA ... observe *all* possible chemical shifts from **fixed** molecules

## Principal Axis System



- $\delta_{xx}$ ,  $\delta_{yy}$ ,  $\delta_{zz}$  - diagonal elements in the *chemical shift tensor* (3 x 3 matrix)
- Average = isotropic chemical shift ( $\delta_{iso}$ )
- **Solids: No averaging**
  - *Molecules cannot tumble like in solution*
- Broadening can be *extremely significant*



# Averaging Out Broadening Interactions

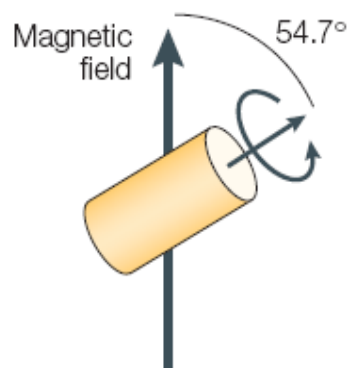
- Both DD and CSA are dependent on  $(3\cos^2(\theta) - 1)$ 
  - What if we made this equal 0?

$$3\cos^2(\theta) - 1 = 0$$

$$\cos(\theta) = \pm \frac{1}{\sqrt{3}}$$

$$\theta = \arccos\left(\frac{1}{\sqrt{3}}\right)$$

$$\theta \cong 54.74^\circ = \text{Magic Angle}$$



*Doty 4 mm OptiMAS with Gradients*

- Average DD and CSA to 0
- Good for single crystals...but not enough for powder samples

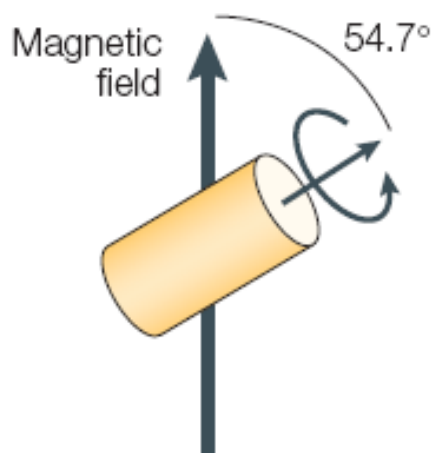
Review: A. Watts, *Nature Rev.* **2005**, *4*, 555-568.

Review: D. D. Laws, H. -M. L. Bitter, A. Jerschow, *Angew. Chem. Int. Ed.* **2002**, *41*, 3096-3129.

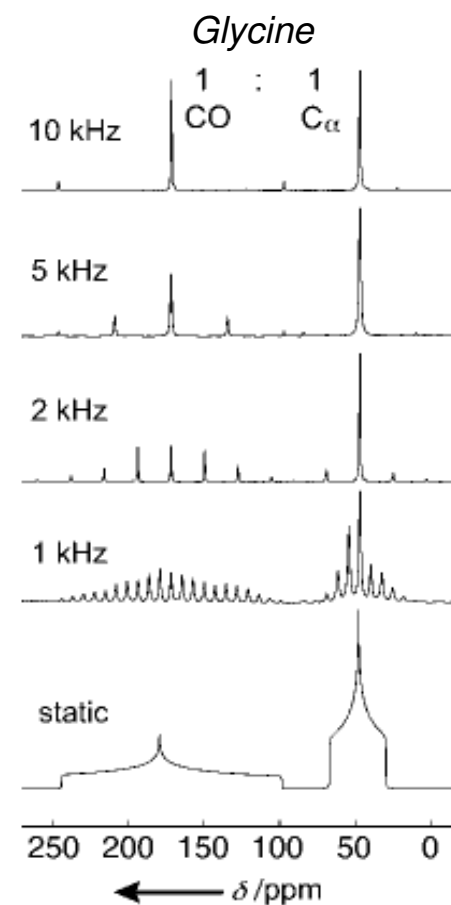
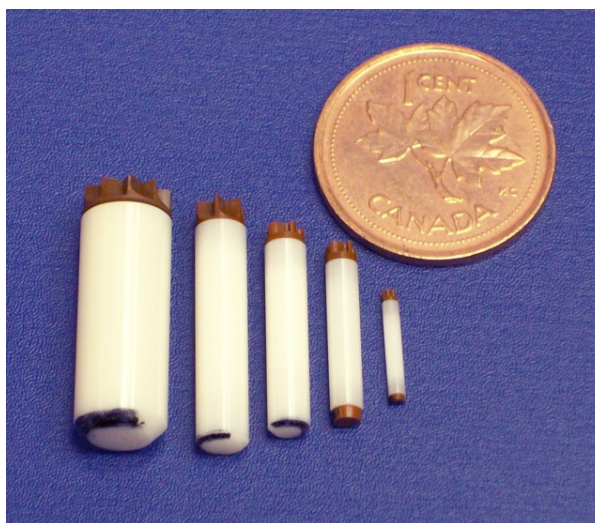
# Magic Angle Spinning (MAS)

- For powder samples...
  - Spin along the longitudinal rotor axis at high speed (kHz) ... *time averaging*

*Magic Angle Spinning*



*Bruker Rotors with Turbine Caps*



## Net result

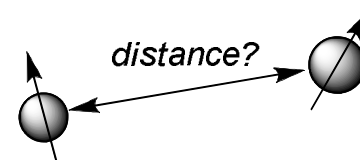
**Solution-like spectra for solid samples!**

Review: A. Watts, *Nature Rev.* **2005**, *4*, 555-568.

Review: D. D. Laws, H. -M. L. Bitter, A. Jerschow, *Angew. Chem. Int. Ed.* **2002**, *41*, 3096-3129.

# Reinstating Direct Dipolar Interactions

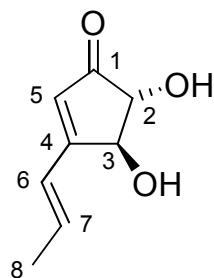
- Direct Dipolar (for **distances**)
  - Homonuclear
    - RR **Rotational Resonance**
    - DRAMA **Dipolar Recovery At the Magic Angle**
  - Heteronuclear
    - REDOR **Rotational Echo DOuble Resonance**
    - HORROR **double-quantum HOmonuclear ROTatory Resonance**
    - MELODRAMA **MElding of spin-LOcking and DRAMA**
    - C7 **C7 Rotor-Synchronized DD Recoupling Pulse Train**
- Are there accurate distance determining methods in solution state?
  - i.e. NOESY, ROESY, TROSY, HOESY
- **No!** Accurate distances can never be measured in solution state!
  - Nuclei distances are not the only variables in NOE/ROE equations...
- **SSNMR is the only way to measure accurate distances!**



# Reinstating Chemical Shift Anisotropy

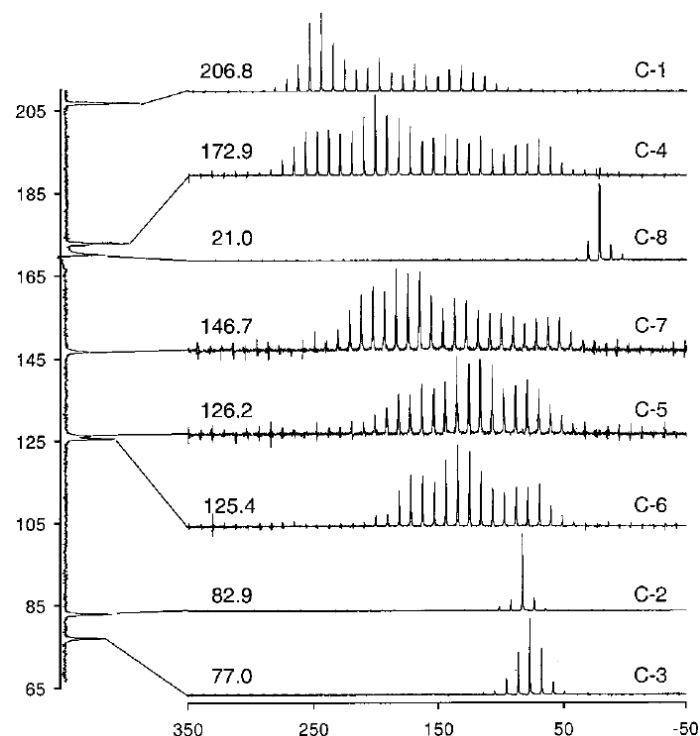
- Chemical Shift Anisotropy (for **electronic env.**, **H-bonding**, **structure**)
  - Not directly done
  - Perform spinning sideband analysis
  - 2D NMR correlation of iso and aniso chemical shifts
    - FIREMAT **Five  $\pi$ -RE**licated **M**agic **A**ngle **T**urning
    - VACSY **V**ariable **A**ngle **C**orrelated **S**pectroscop**Y**

- FIREMAT of Terrein



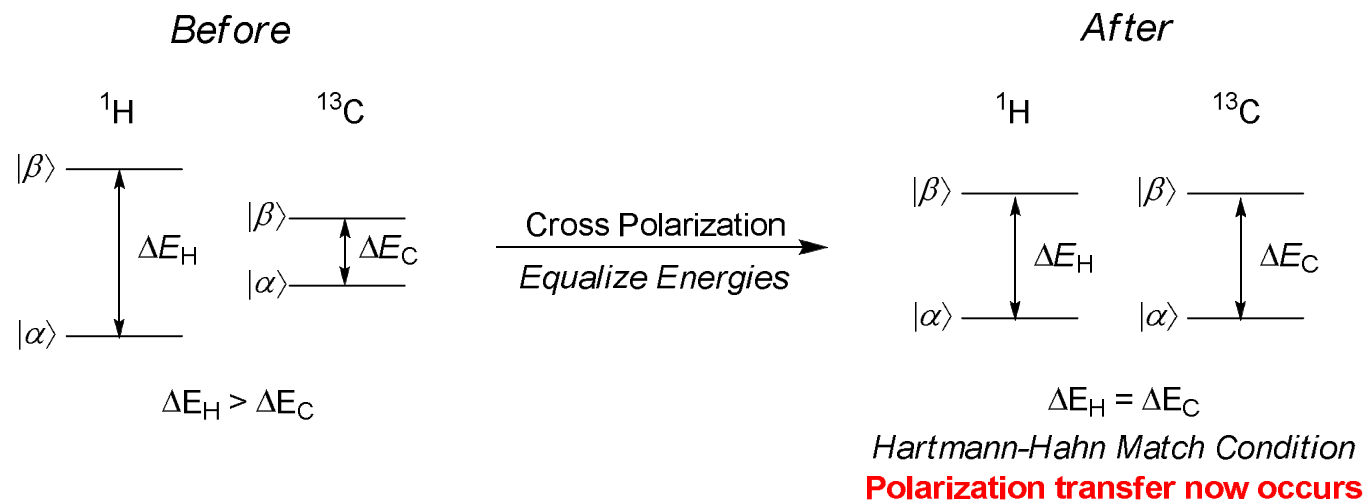
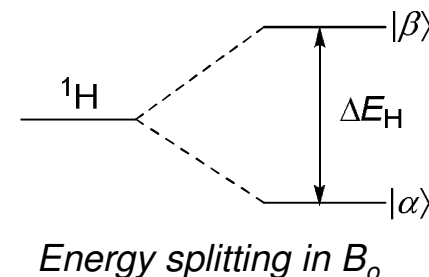
Terrein

Obtain CST values ( $\delta_{xx}$ ,  $\delta_{yy}$ ,  $\delta_{zz}$ )  
(analyze band patterns)



# Cross Polarization (CP) Theory

- SSNMR analysis directly of  $^1\text{H}$ ...usually not done
  - Direct dipolar coupling too large...still broad *after* MAS
  - Easiest to analyze other nuclei (i.e.,  $^{13}\text{C}$ )
- Cross polarization
  - Use sensitive nuclei (e.g.,  $^1\text{H}$ ) to *enhance* low sensitivity nuclei signals (e.g.,  $^{13}\text{C}$ )
  - Magnetization transfer *from DD coupled* “hot nucleus” to “cold nucleus”
    - Mostly true...except polarization transfer is *cosine modulated*
- Equalize energy levels of the two different nuclei to transfer polarization
  - Equalization = Hartmann-Hahn match condition



# Cross Polarization Theory and Example

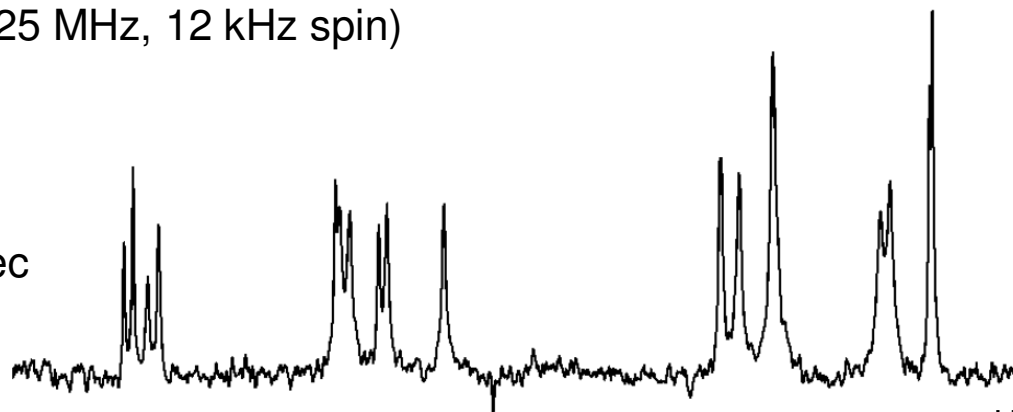
Also ... insensitive nuclei  $T_1$  relax at the same rate as the sensitive nuclei

$^{13}\text{C}$  MAS NMR (125 MHz, 12 kHz spin)

**With CP**

Relax. delay: 2 sec

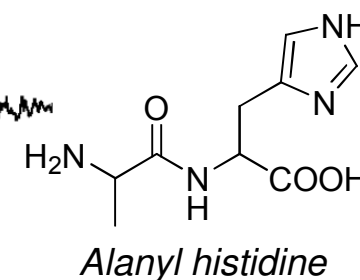
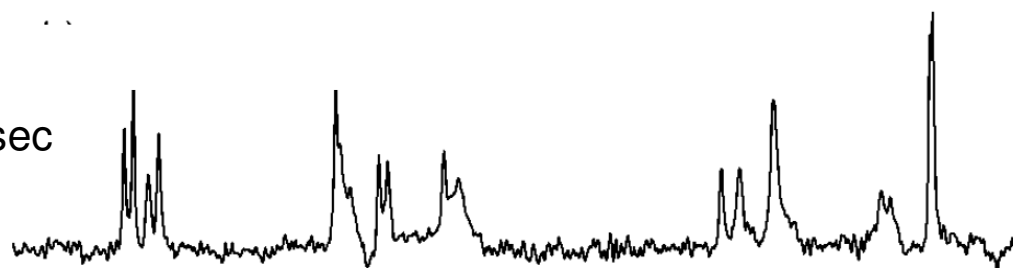
Time: ~ 4 min



**No CP**

Relax. delay: 60 sec

Time: ~ 128 min



In 1970's, original name for CP was **P**roton **E**nhanced **N**uclear **I**nduction **S**pectroscopy

# Applications of SSNMR

## 1. Chirality

- Discriminating racemate and enantiomers
- Determining relative stereochemistry

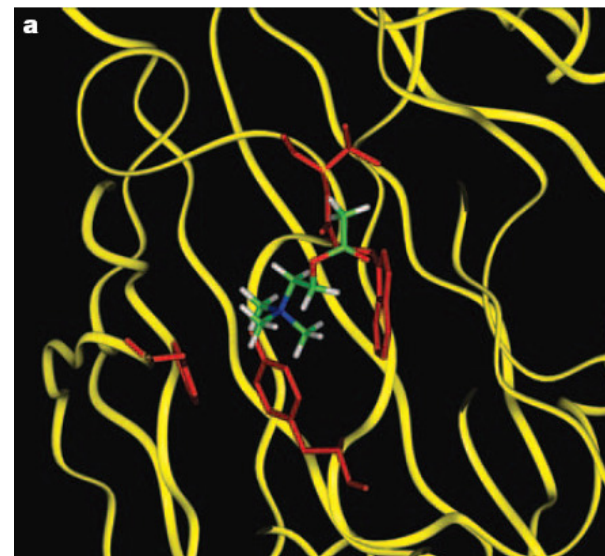
## 2. Enzyme mechanisms

- Human Carbonic Anhydrase Isozyme II (Zn-enzyme)
- FYI ... other examples not discussed ...
  - Triose Phosphate Isomerase (a.k.a. TIM enzyme)
  - Cu(II)-Zn(II) Superoxide Dismutase
  - Vanadium chloroperoxidase
  - Ligand / drug – receptor interactions
    - Especially for membrane receptors

## 3. Polymorphism

## 4. Solid-phase peptide synthesis

- Gel-phase High Resolution Magic Angle Spinning (HRMAS) NMR



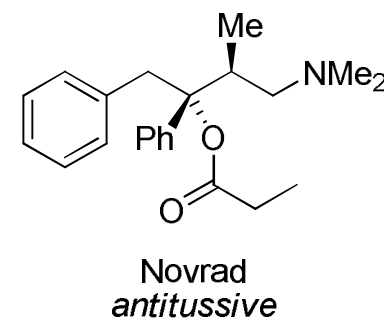
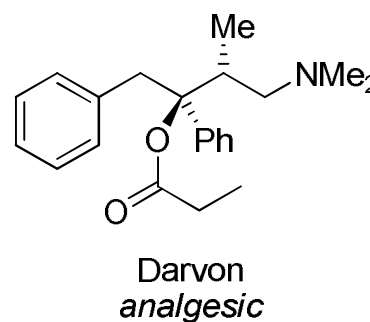
*Acetylcholine-nAChR interaction*

A. Watts, *Nature Rev.* **2005**, *4*, 555-568.

A. McDermott, T. Polenova, *Curr. Opin. Struct. Biol.* **2007**, *17*, 617-622.

# Chirality

- Worldwide revenue from chiral products: \$7B USD in 2002, c.a. \$15B USD in 2009
  - Pharmaceuticals (*Receptor active sites are chiral*)
  - Flavour and aroma chemicals
  - Agricultural sector
  - Specialty materials
- Chiral compound synthesis
  - 50% from chiral pool and separation
  - 35% by chemocatalysis
  - 15% by biocatalysis
- Chirality detection methods
  - Derivatization to form diastereomers
  - Polarimetry
  - Chiral chromatography
  - Chiral shift reagents (solution-state NMR)
  - **Solid-state NMR Spectroscopy**



# Crystals and Chirality

- Enantiomeric and racemic crystals: packing and symmetries not the same!

Compound	Chiral Xtal Space Group	Racemate Xtal Space Group
aspartic acid	$P2_1$	$C_2/c$
histidine	$P2_1$ or $P2_12_12_1$	$P2_1/c$
dichlorobis(2,2'-bipyridine)iron(III)	$P2_12_12_1$	$Pccn$

- Racemic crystals usually denser and more stable than enantiopure
- Differences:
  - Melting points
  - Solubilities
  - Intermolecular distances between chemically equivalent nuclei
- *Exploit crystal differences in SSNMR*

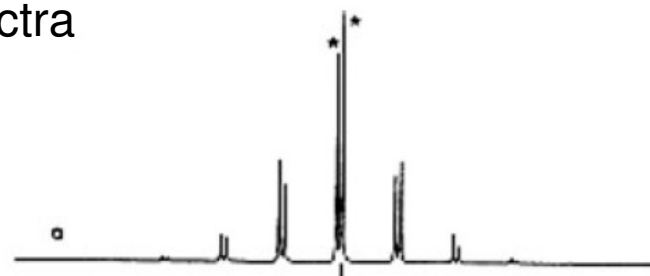
O. Wallach, *Liebigs Ann. Chem.* **1895**, 286, 90-143.

C. P. Brock, W. B. Schweizer, J. D. Dunitz, *J. Am. Chem. Soc.* **1991**, 113, 9811-9820.

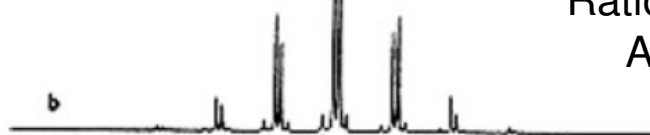
# Discriminating Racemates and Enantiomers via Isotropic Chemical Shift Comparison

$^{31}\text{P}$  CP/MAS NMR Spectra

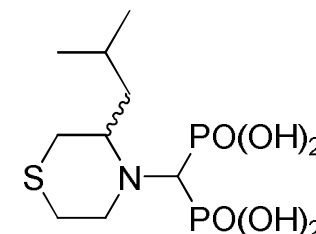
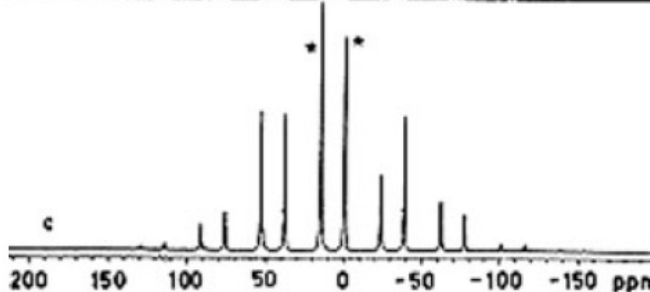
(-)-enantiomer



(+)-enantiomer  
(and some racemate)



racemate



Ratio of integrations yields e.e.  
Accuracy better than 1%

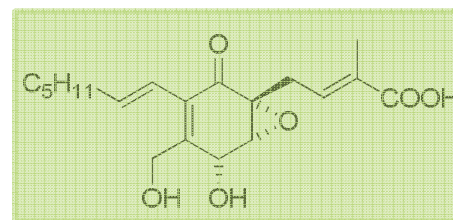
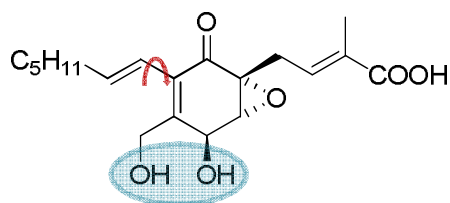
- When racemic and enantiopure  $\delta_{\text{iso}}$  are *accidentally equivalent*...
  - ODESSA – **O**ne-**D**imensional **E**xchange **S**pectroscopy by **S**ideband **A**lternation
  - Dependent on internuclear distances

K. V. Andersen, H. Bildsoe, H. J. Jacobsen, *Magn. Reson. Chem.* **1990**, *28*, S47-S51.

Review: M. J. Potrzebowski, A. Jeziorna, S. Kaźmierski, *Concepts in Magnetic Resonance Part A*, **2008**, *32A*, 201-218.

# Determination of Relative Stereochemistry

## Ambuic Acid



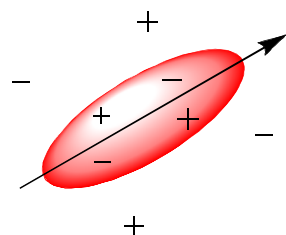
Which one?

- Use SSNMR to obtain chemical shift tensor (CST) values ( $\delta_{xx}$ ,  $\delta_{yy}$ ,  $\delta_{zz}$ )
  - Sensitive to environment
- 1. Obtain CST values for the stereocentres
  - FIREMAT
- 2. Generate *multiple* structures with *different* conformations and stereoconfiguration
- 3. Calculate the CST values of those stereocentres in the proposed structures
  - Gaussian '03
- 4. Match CST values between experiment and theory
  - However ... CST values **cannot** discriminate *absolute* stereochemistry

# Enzyme Mechanism Prelude...Quadrupolar NMR

Two severe line broadening factors for quadrupolar nuclei...

- For spin ( $I$ )  $> 1/2$  - more energy levels ( $2I + 1$  spin states)
  - $^1\text{H}$  ( $I = 1/2$ )  $\rightarrow |\alpha\rangle, |\beta\rangle$
  - $^2\text{H}$  ( $I = 1$ )  $\rightarrow |-1\rangle, |0\rangle, |+1\rangle$  *More complicated splitting patterns*
- Interaction of *nuclear charge distribution* and *electric field gradient* in solid



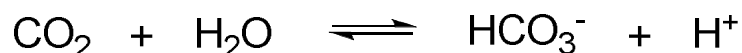
*Quadrupolar nuclei in electric field gradient  
(Nuclear charge distribution non-spherical)*

- **Must use SSNMR for accurate data**
  - Quadrupolar coupling constant ( $C_Q$ ) sensitive to local environment
- If needed: MAS averages out 1<sup>st</sup> order quadrupolar broadening
- Deuterium - useful for motion studies (proteins, lipids, drug partitioning)

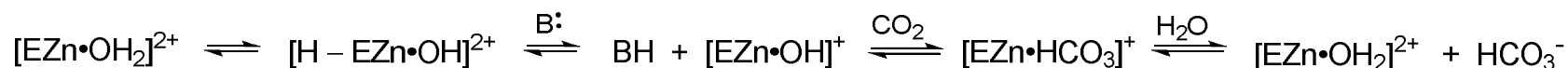
# Enzyme Mechanisms

## Case study

### Human Carbonic Anhydrase Isozyme II (Zn-metalloenzyme)



- Assumed mechanism



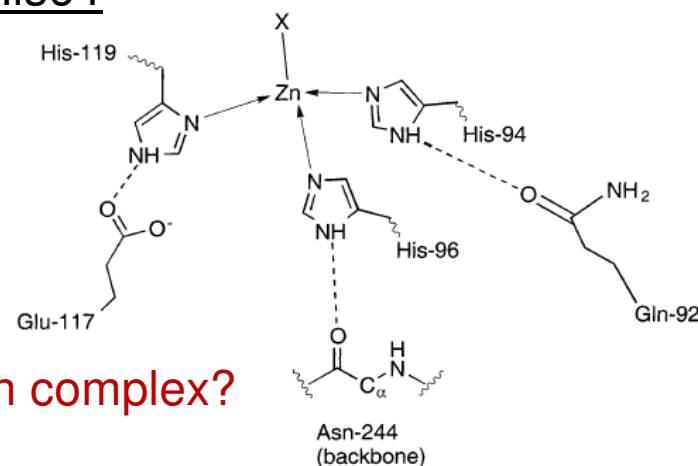
- Charge-cycling of Zn complex
- RDS -  $\text{H}^+$  transfer from activated  $\text{H}_2\text{O}$  to His64

- Ionization dependency of two above groups

- Believed for active site Zn:

- pH 5 binds  $\text{H}_2\text{O}$  ...  $[\text{EZn}\cdot\text{OH}_2]^{2+}$
- pH 9 binds  $^-\text{OH}$  ...  $[\text{EZn}\cdot\text{OH}]^{1+}$

- **Is the above true? What is the nature of Zn complex?**



## What to Look for in Solid-State $^{67}\text{Zn}$ NMR of CAII?

- Obtain  $C_Q$  (Quadrupolar Coupling Constant) for Zn

From Carbonic Anhydrase II active site Zn calculations ...

- *via* Gaussian

- $[\text{EZn}\cdot\text{OH}]^{1+}$

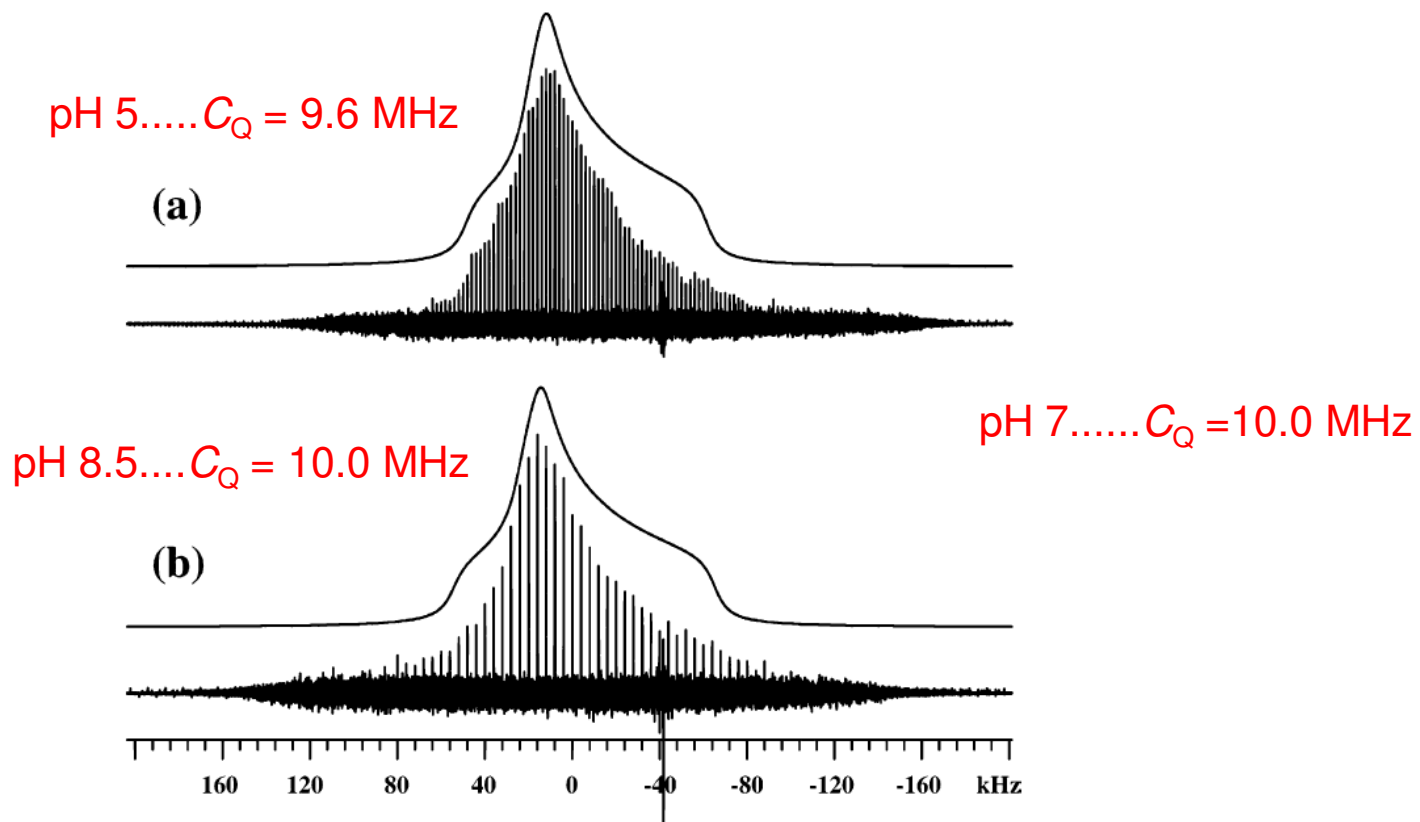
$$C_Q \approx 8 - 10 \text{ MHz}$$

- $[\text{EZn}\cdot\text{OH}_2]^{2+}$

$$C_Q \approx 25 - 35 \text{ MHz}$$

# $^{67}\text{Zn}$ QCPMG NMR Spectra of CAII

- Samples equilibrated in desired pH, lyophilized and sparingly hydrated

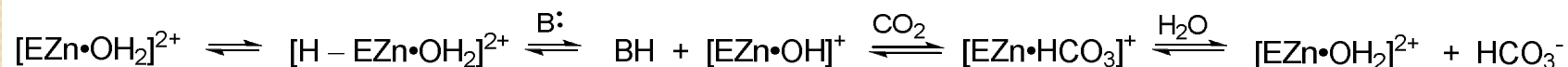


- $C_Q$  values similar for broad pH range
- Zn insensitivity to environment or something else ... ?

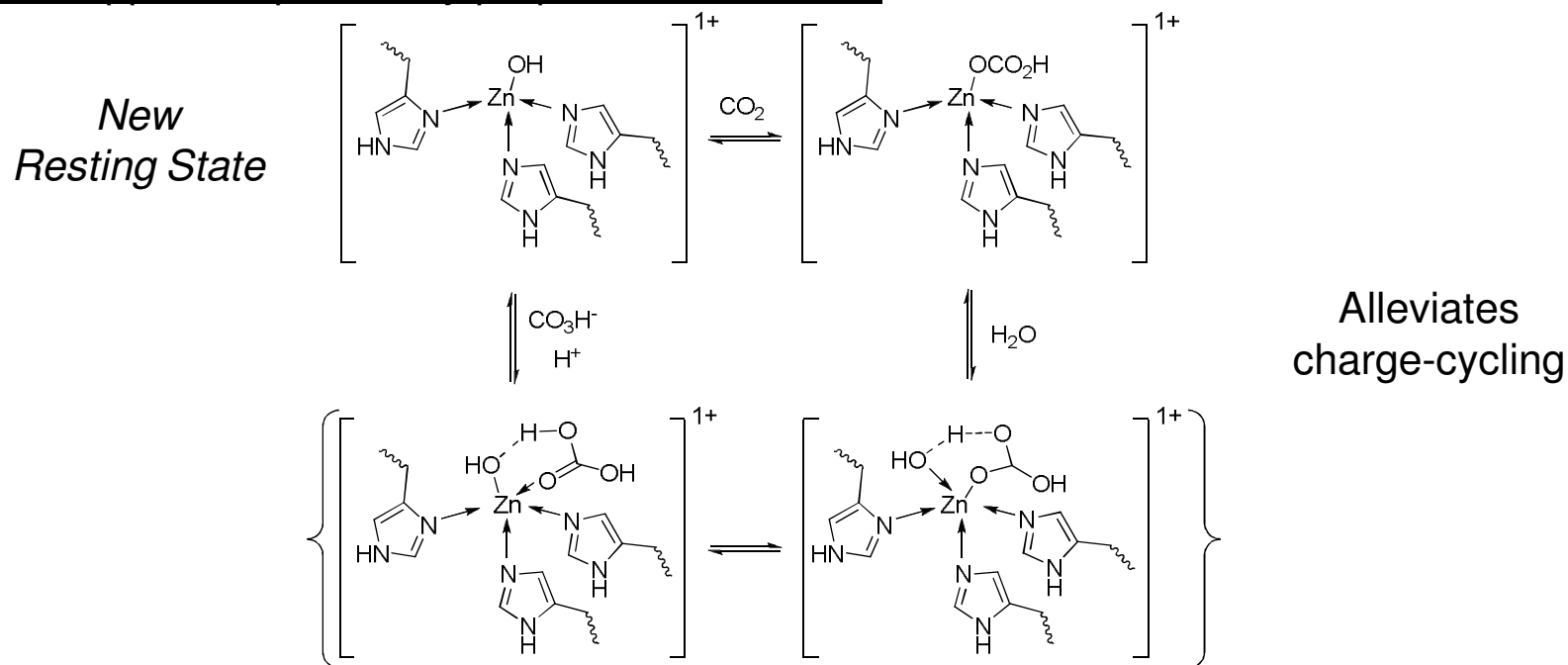
# Findings and Alternate Mechanistic Proposal

- Computational models made: calculate  $C_Q$  and electric field gradient tensor
  - Zn sensitive to ligands
  - Expt  $C_Q \approx$  calc  $C_Q \approx 9$  MHz ... Zn always binds  $^-OH$  between pH 5 – 8.5!

## Old “accepted” mechanism



## New support for previously proposed mechanism



A. S. Lipton, R. W. Heck, P. D. Ellis *J. Am. Chem. Soc.* **2004**, *126*, 4735-4739.

K. M. Merz, *et al. J. Am. Chem. Soc.* **1989**, *111*, 5636-5649. | M. Mauksch, *et al. ChemBioChem* **2001**, *2*, 190-198.

# Polymorphism

## Case Study

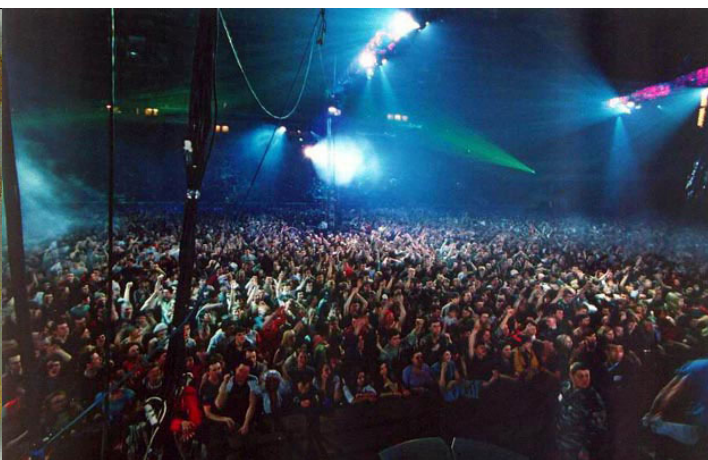
### Ecstasy

E, XTC, “Scooby Snack”, “Hug Drug”

- Addictive, illegal hallucinogen



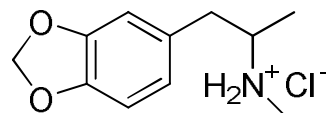
*Ecstasy pills*



*Rave*



*Creative smuggling*

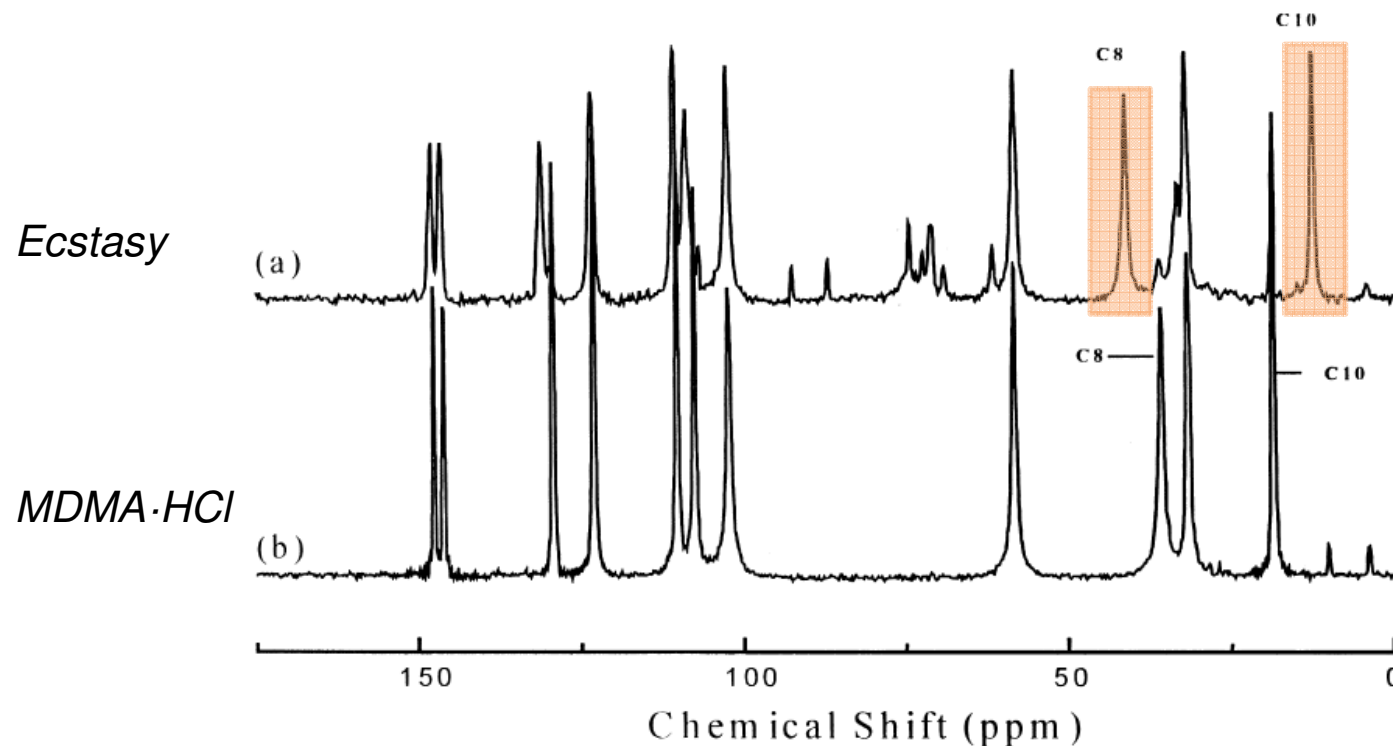
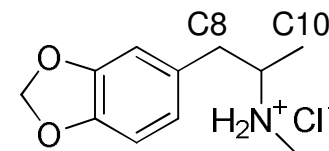


Methylenedioxyamphetamine hydrochloride (MDMA·HCl)

- Solid MDMA·HCl has *low pharmacological efficacy*
- Ecstasy = MDMA·HCl co-crystallized with lactose. Much more active.

# Compare Ecstasy and Solid MDMA·HCl

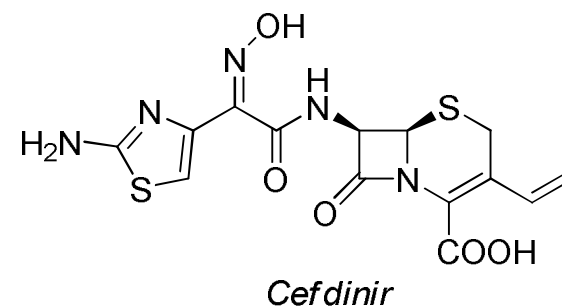
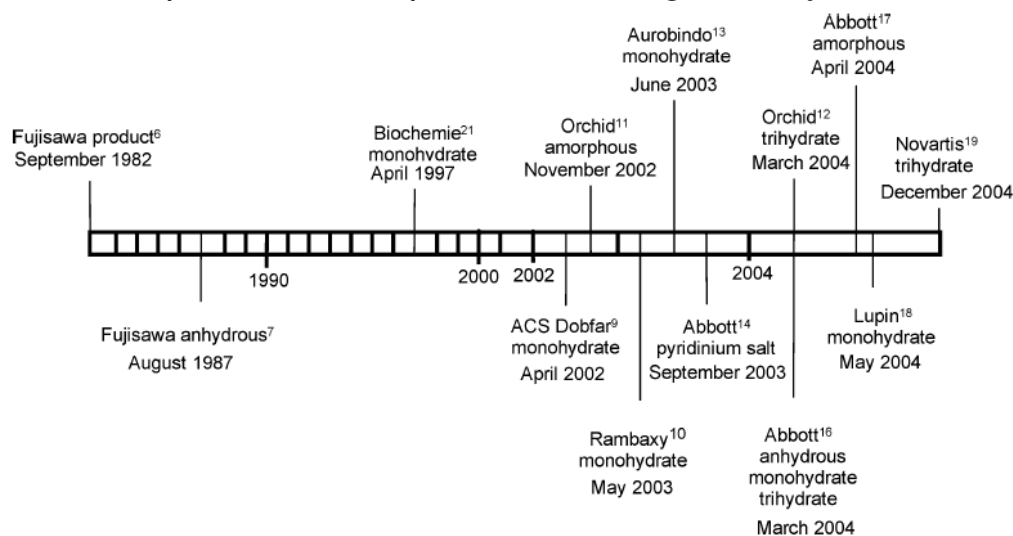
$^{13}\text{C}$  CP/MAS SSNMR (10 kHz spin rate)



- Ecstasy structure resembles solution-state conformation of MDMA·HCl
- Greater aqueous solubility (bioavailability) than solid MDMA·HCl

# Polymorphism and the Pharmaceutical Industry

- Cefdinir “Patent Tangle”
  - 8 companies file 11 patents relating to 5 crystalline forms

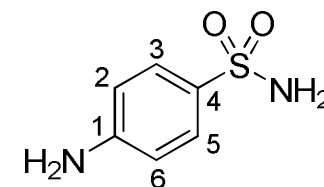


- With polymorphism ...
  - Extend original patents on existing pharmaceuticals
  - Generic company creates different polymorph and files patent ... *marketing rights!*
  - Ensure one polymorph doesn't change to another and infringe another patent ...
    - Or decompose drug ...
- XRPD and IR are not enough ... **SSNMR most comprehensive method**
  - Abbott's "new polymorph" in 2003 ... not a polymorph, but a pyridinium salt!

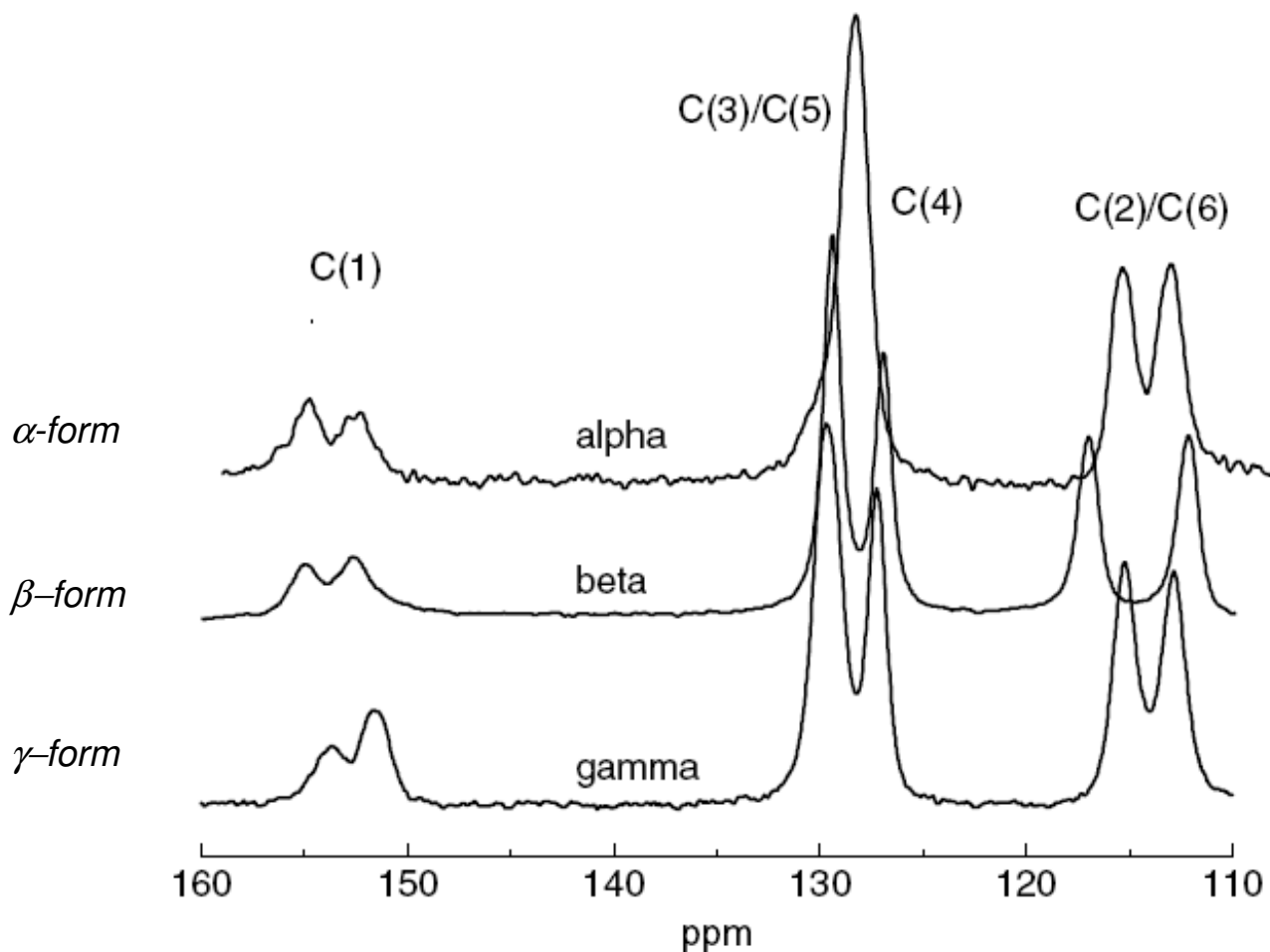
# Sulfanilamide, Polymorphism and SSNMR

- Antibacterial agent

$^{13}\text{C}$  CP/MAS SSNMR (50.3 MHz, 4 kHz spin)



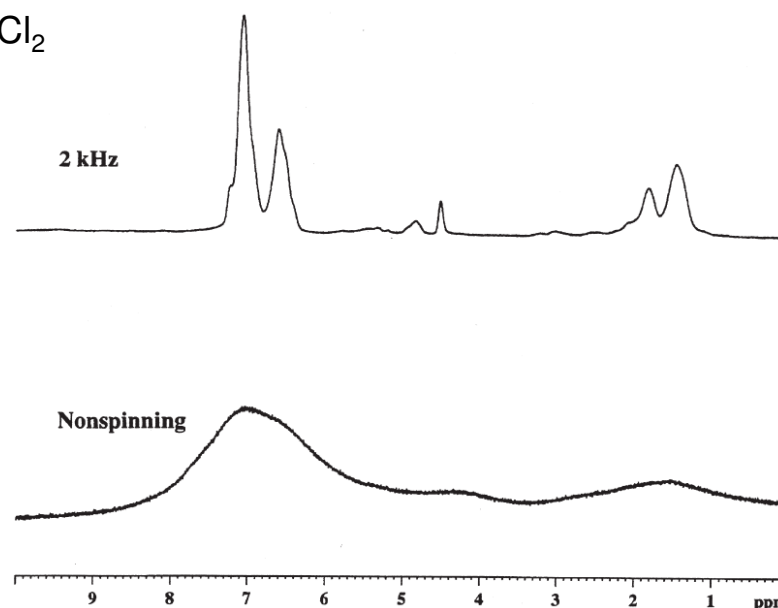
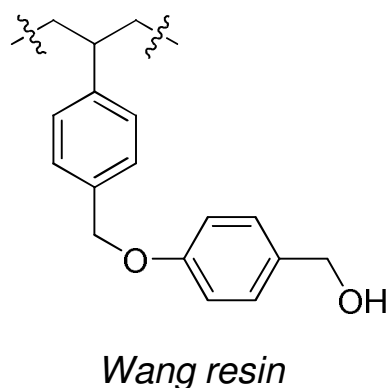
Sulfanilamide



# Gel-Phase High-Resolution MAS (HRMAS)

- Technique for obtaining solution-like spectra of semi-solids / gels
  - Synthetic resins swollen in organic solvents, organic tissues, etc.
  - More freedom in motion of sample...reduced DD values
    - Can get good  $^1\text{H}$  NMR spectra!
- MAS averages DD, CSA, Magnetic Susceptibility ( $\chi$ ) to 0
  - $\chi$ : magnetic flux differences at solid/liquid interface – field inhomogeneities
  - Slower spin rates (2 – 5 kHz), lower RF power needed

$^1\text{H}$  NMR HRMAS (500 MHz) of Wang resin in  $\text{CD}_2\text{Cl}_2$



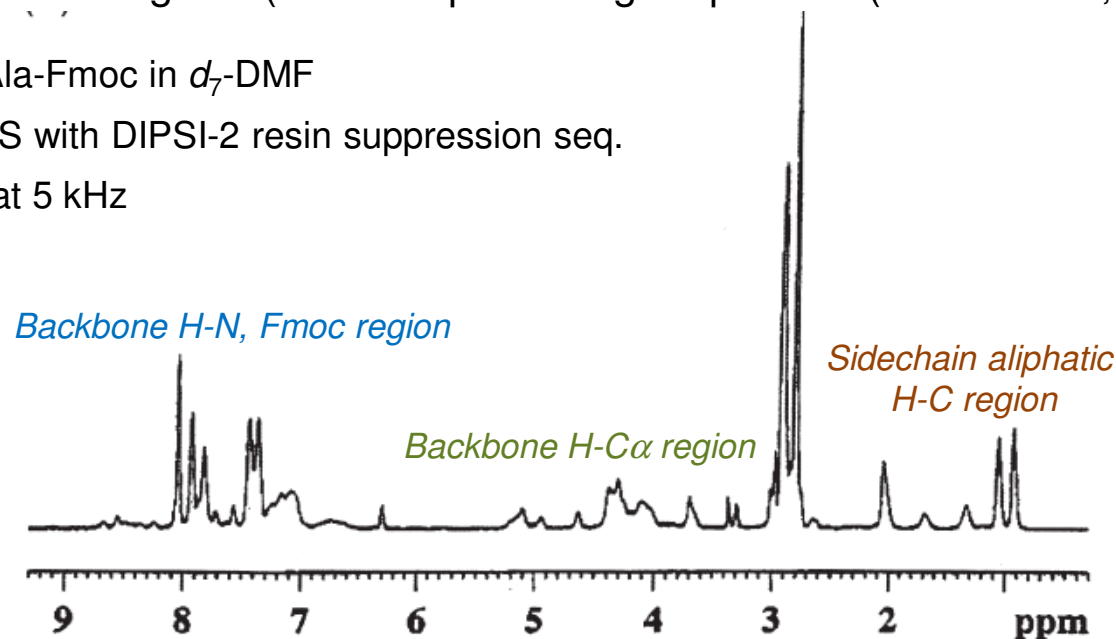
# HRMAS NMR and Solid-Phase Peptide Synthesis

- Analyze structure of peptide on resin
  - NMR: Non-destructive characterization
  - IR: Not much info
  - MS: Destructive - requires cleavage from the resin
- Determine correct structure, success of coupling, byproducts
  - Determine conformation ... may account for difficult couplings
- Attenuate resin signals (*via* isotropic mixing sequences (i.e. DIPSI-2, FLOPSY-8))

Wang-Asp-Ile-Ala-Fmoc in  $d_7$ -DMF

$^1\text{H}$  NMR HRMAS with DIPSI-2 resin suppression seq.

500 MHz, spin at 5 kHz

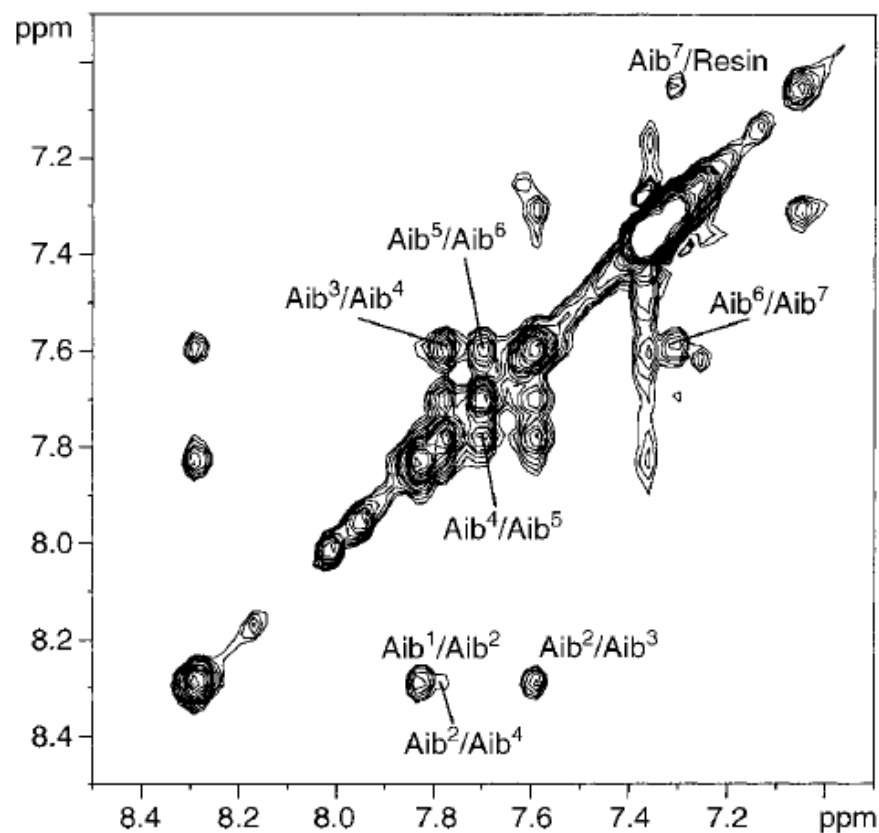


Review: W. P. Power *Annu. Rep. NMR. Spec.* **2003**, 51, 261-295.

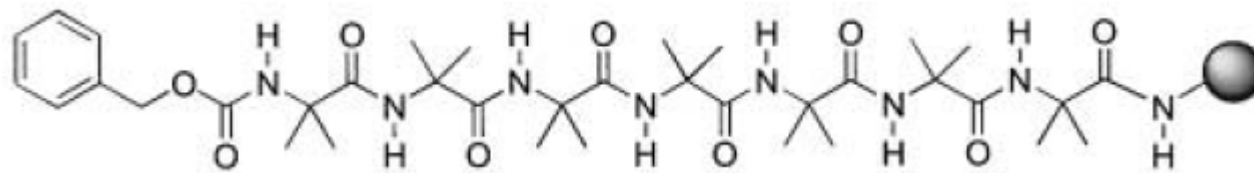
M. J. T. Ditty, H. N. Hunter, R. M. E. Mainville, W. P. Power, *Magn. Reson. Chem.* **2001**, 39, 241-248.

# Characterization of $3_{10}$ -Helix of Model Peptides

$^1\text{H}$  NOESY HRMAS NMR (500 MHz,  $\tau_m = 500$  ms, 8 kHz spin,  $d_6$ -DMSO)



Z-Aib<sub>7</sub>-POEPOP



# Summary, UofT SSNMR Equipment

- Invaluable, powerful structure elucidation technique of amorphous solids
- Importance in pharmaceutical / organic / biological applications
- SSNMR – actively researched field...stay tuned for new developments!

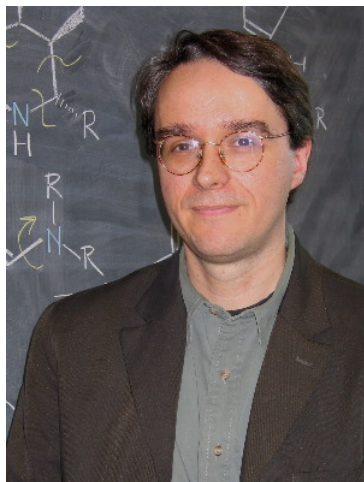
## UofT SSNMR Equipment

- Bruker DSX 200
  - High power amp stack
  - Variable temperature
  - 4 mm and 7 mm MAS BB probe (H, X channels)
  - 10mm BB probe (8 – 85 MHz)
- Solids equipment also at UTM and UTSC
  - UTM contacts: Prof. Peter Macdonald
  - UTSC contacts: Prof. Andre Simpson, Prof. Myrna Simpson



*Bruker DSX 200*

# Acknowledgements



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