

New Developments in In-vitro DNP NMR

Dr. Damir Blazina

Applications Chemist

Oxford Instruments Molecular Biotools Ltd.

17th September 2006

Overview

- The *in vitro* method
- Improved dissolution process
 - HyperSense™ instrument
- Optimal solid state polarisation
 - Solid state diagnostics
- Understanding DNP NMR
 - ^{13}C : minor isomers and chemical kinetics
 - Other nuclei
 - Complex pulse sequences (2D)

Manual *In-vitro* DNP NMR

- Uses GE approach
- Sample size:
up to 200 μL
- Solvent volume:
typically 4-5 mL
- Transfer time:
 - Manual process
 - Distance between magnets

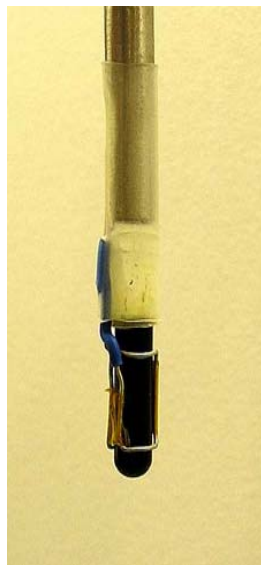


HyperSense™



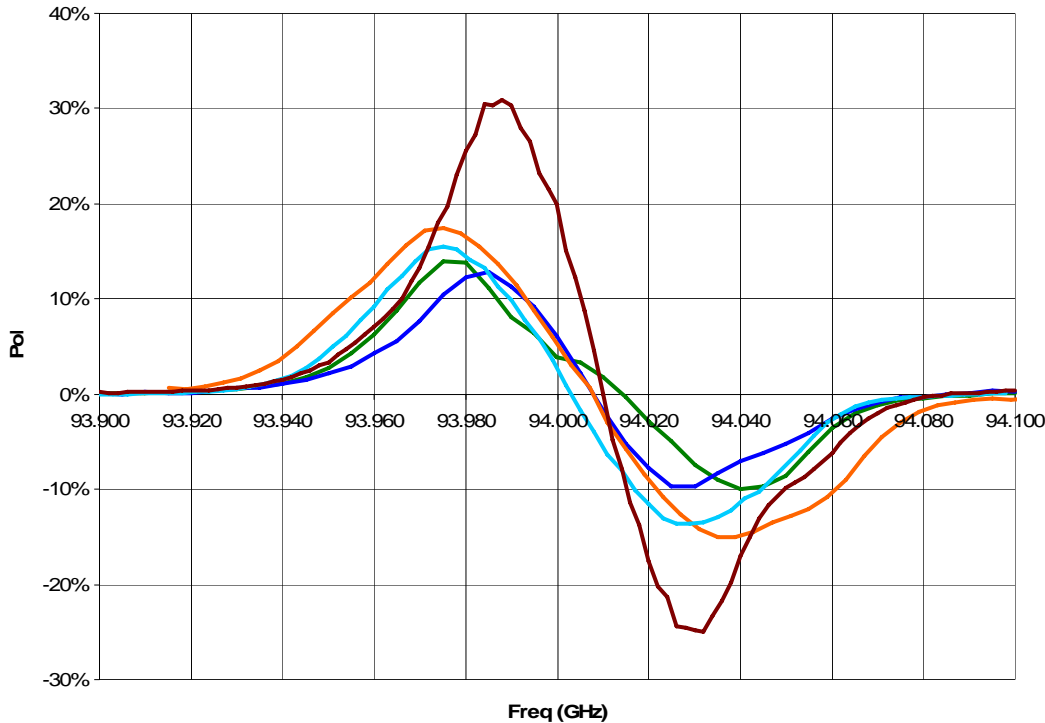
- Automated stand-alone DNP polarizer
- Dedicated, point and click software
- Compatible with any current NMR system
- Dissolution solvents: water and methanol
- Transfer time: <1 s

Solid-state Diagnostic Probe



- Solid-state DNP research at high sensitivity
- ^{13}C and ^{15}N nuclei tested
- Designed to be easy to modify
- Potential for ^{29}Si , ^{31}P and others

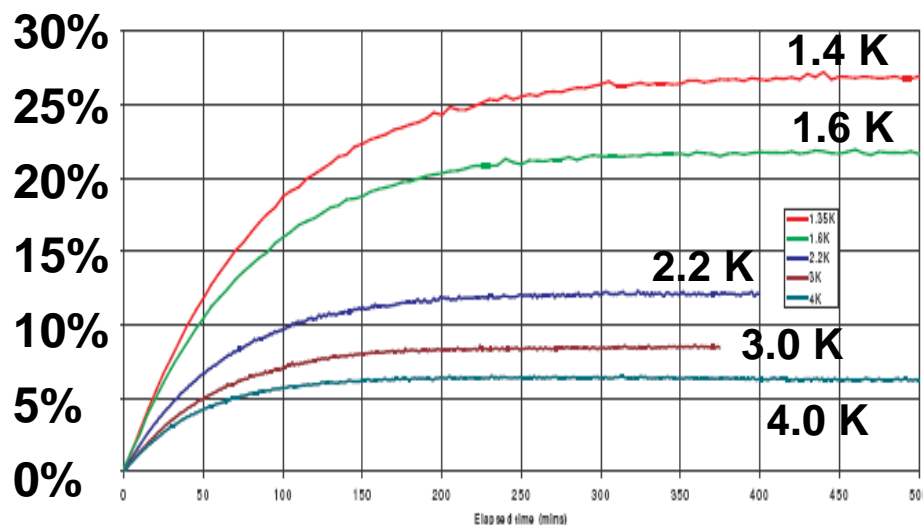
^{13}C Microwave Sweeps



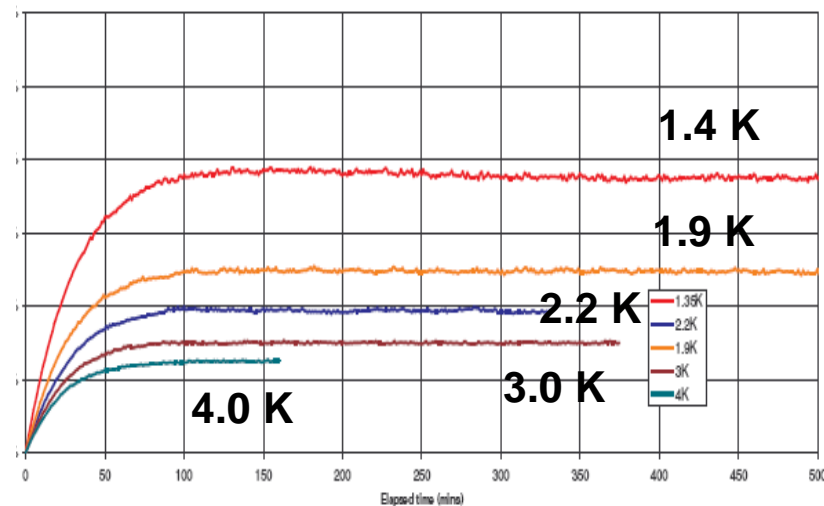
^{13}C -alanine
 ^{15}N -pyridine
 $^{15}\text{N}_2$ - ^{13}C -urea
 $^{13}\text{C}_2$ -DMSO
 $^{15}\text{N}_2$ - ^{13}C -urea and ^{15}N -pyridine

- High S/N – probe dedicated to solid state work
- Optimum frequency depends on compound

Polarisation Build-up: ^{13}C Urea



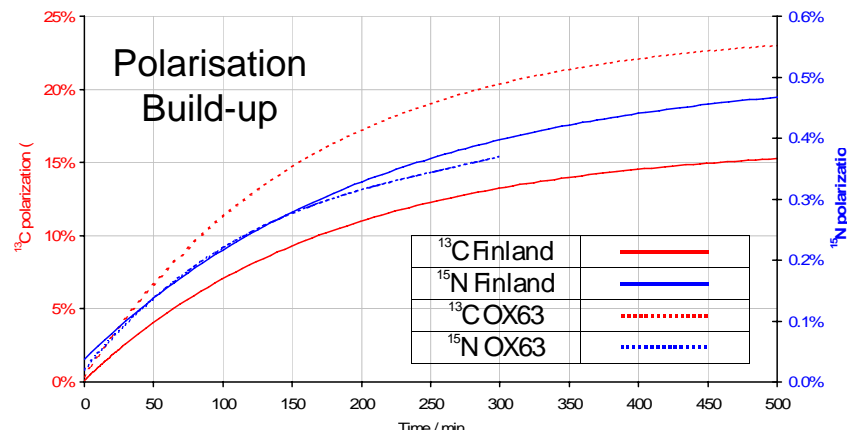
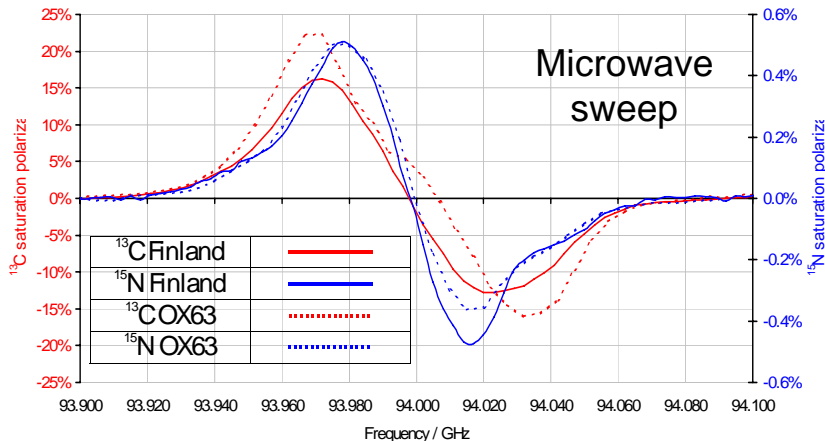
Urea with 15 mM trityl



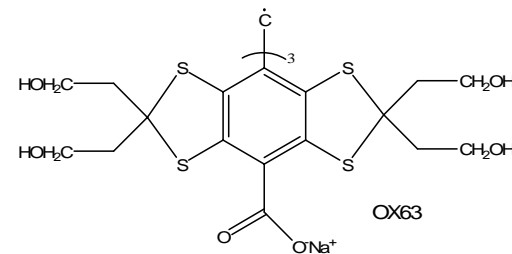
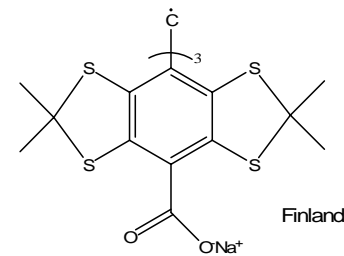
Urea with 30 mM trityl

- Higher radical concentration leads to lower maximum polarization, but faster build-up
- Polarisation inversely dependent upon temperature

Comparison of Trityl Radicals



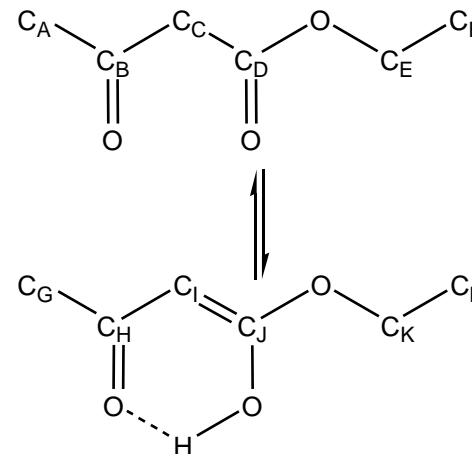
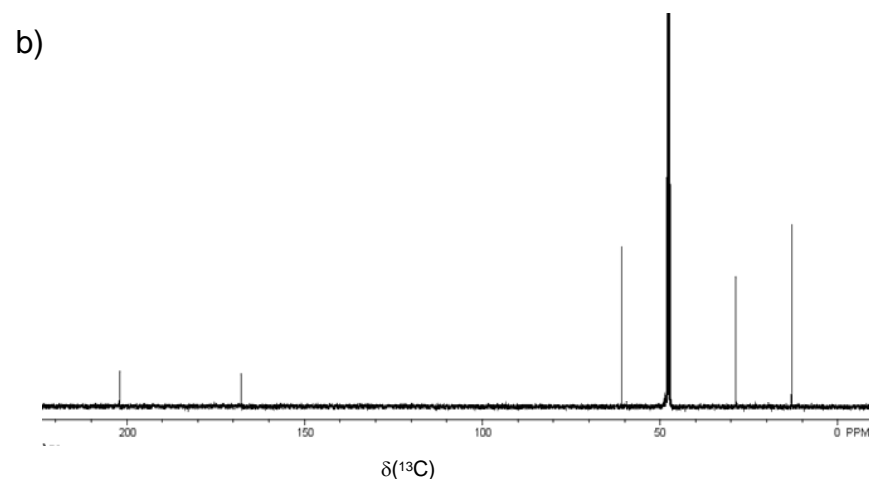
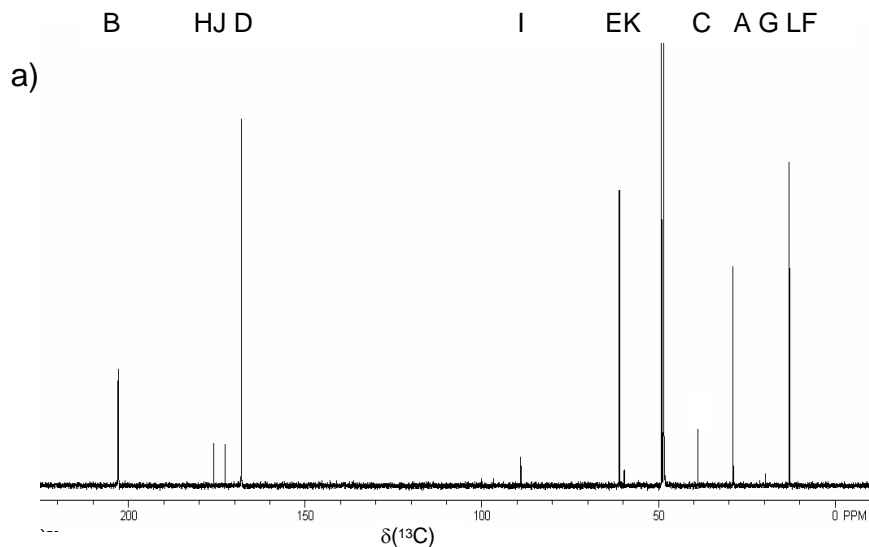
- Radicals differ in hydrophilicity
- DL urea: microwave sweep and polarisation build up studies
- May correspond to urea polarity



Understanding DNP NMR

- Ongoing applications development
- ^{13}C DNP
 - Minor isomers and chemical kinetics
 - Complex pulse sequences (NOESY, 2D)
- DNP of other nuclei (^{15}N , ^{29}Si)

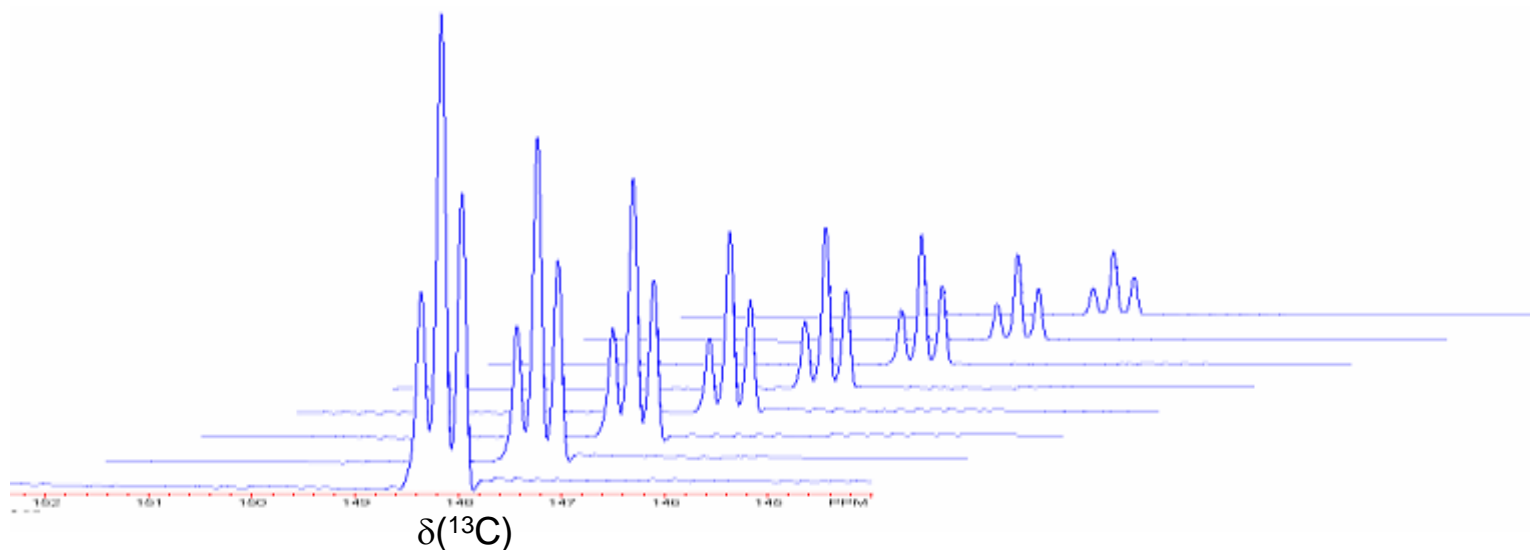
Detection of Minor Isomers



^{13}C NMR spectra of ethyl acetoacetate (60 μmol):

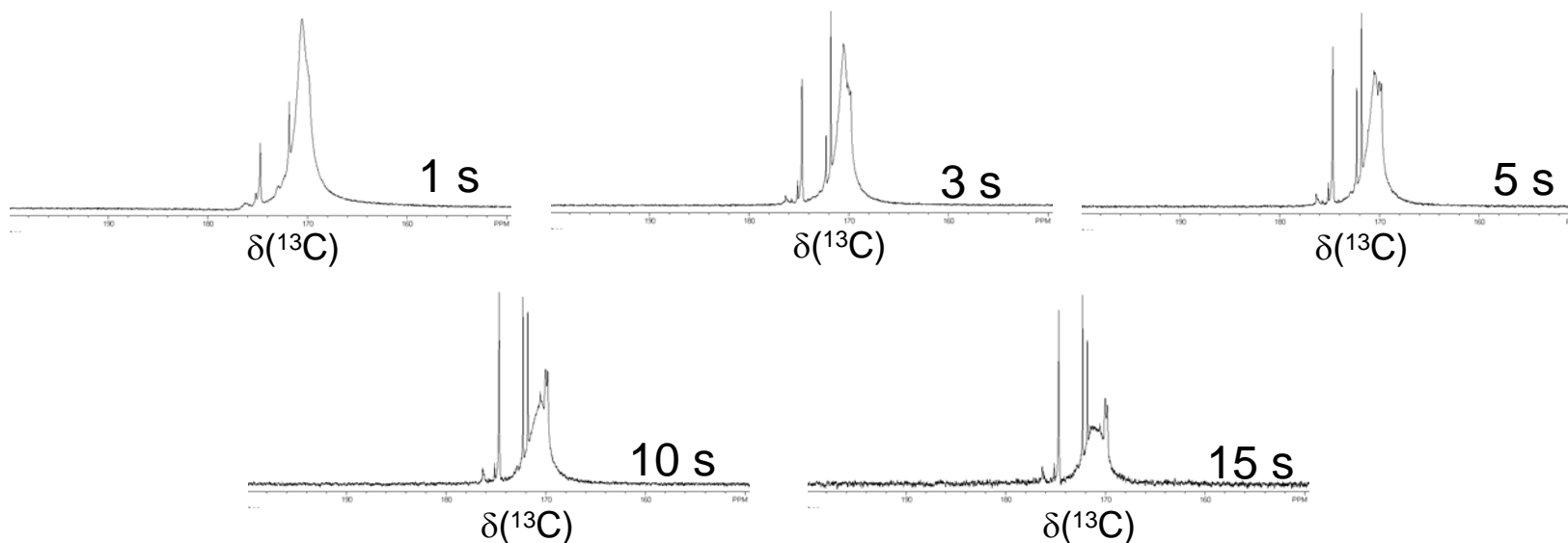
- (A) One-scan DNP NMR after 3 hours polarisation.
- (B) Conventional NMR spectrum recorded over 3 hours.

Acquisition of Multiple Spectra



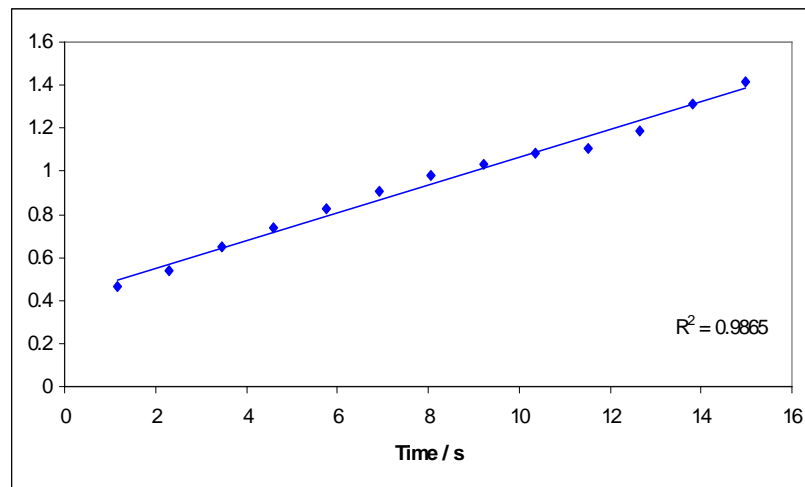
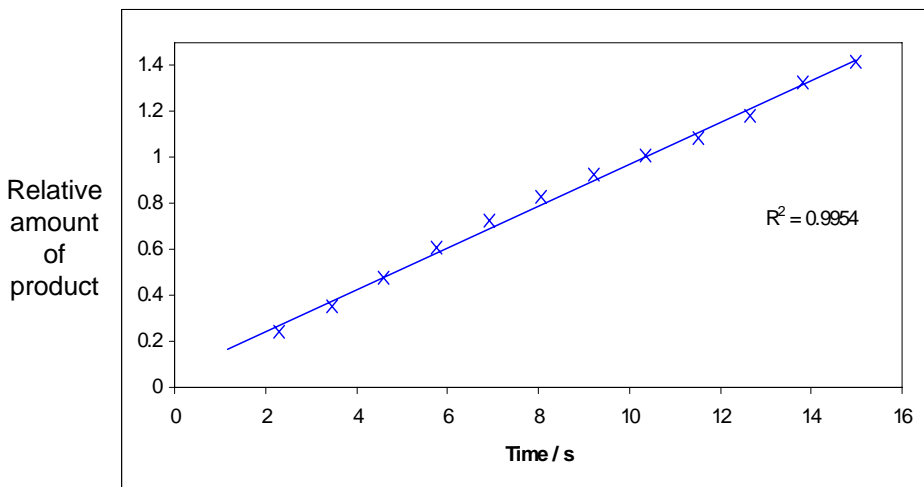
- Eight spectra from one DNP sample with 90° pulses
- >150 spectra possible with 30° pulses
- Reaction kinetics, dynamic processes, 2D DNP NMR

Reaction Kinetics



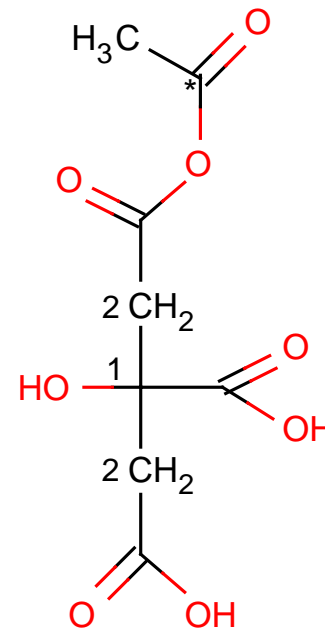
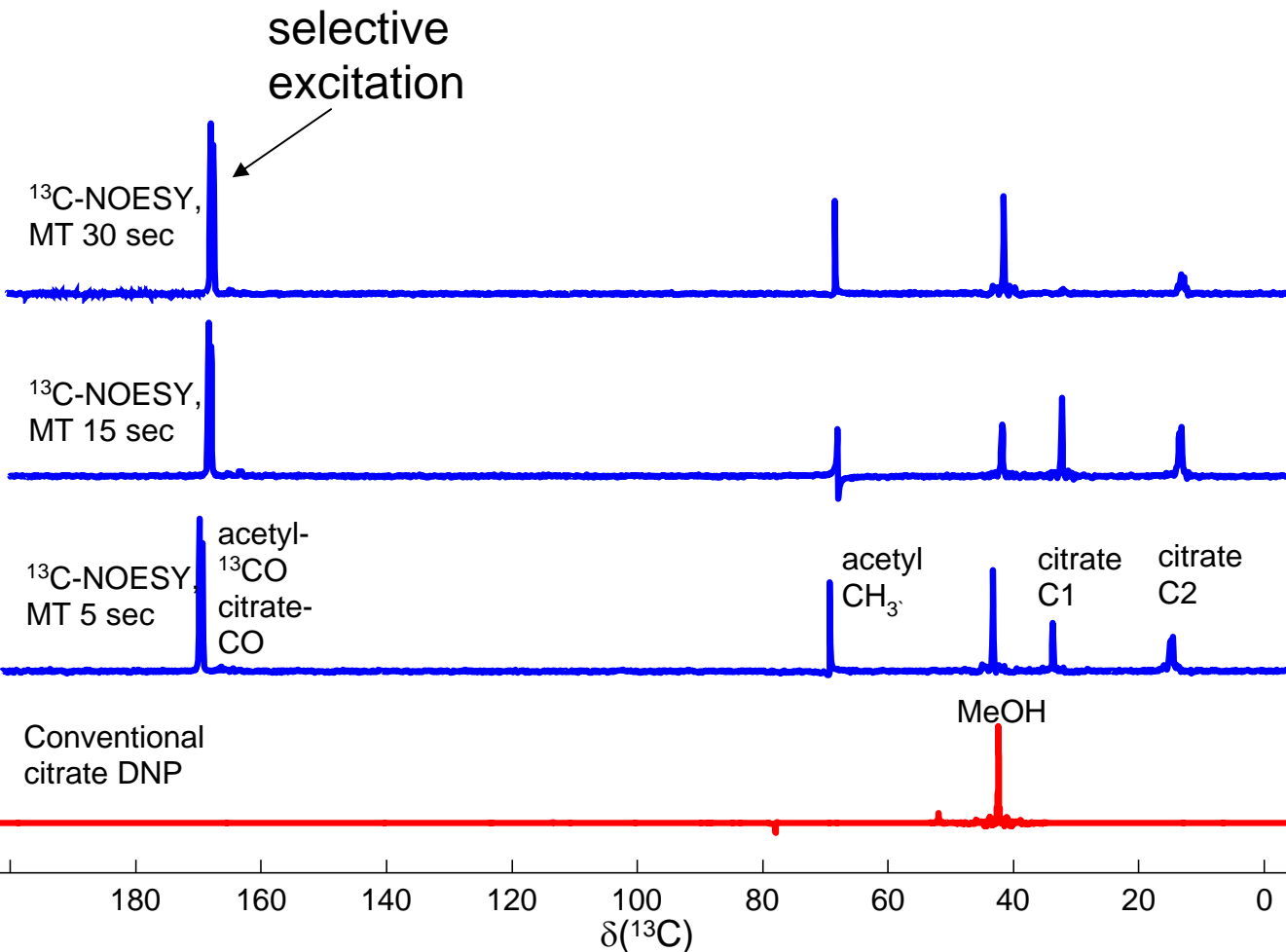
- Base-catalysed hydrolysis of ethyl pyruvate
- Pyruvic acid and methyl pyruvate formed
- Identity of broad resonance?

Reaction Kinetics (2)

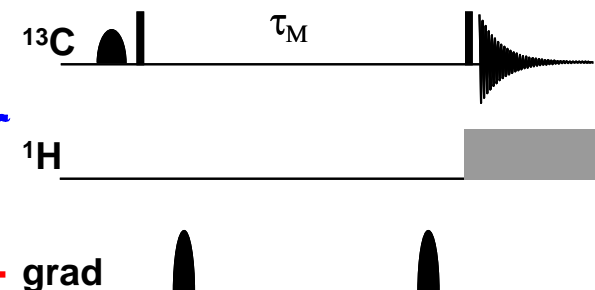


- Plot relative amount of product vs. time
- Identical T_1 values in all three carbonyls
- Rate of formation: 0.09 and 0.06 s^{-1}

¹³C DNP NOESY

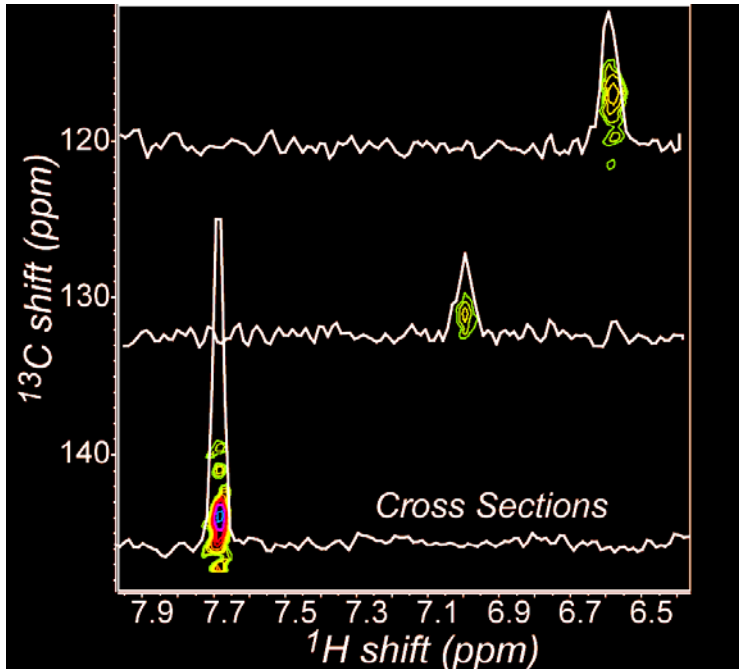


Selective C-NOESY

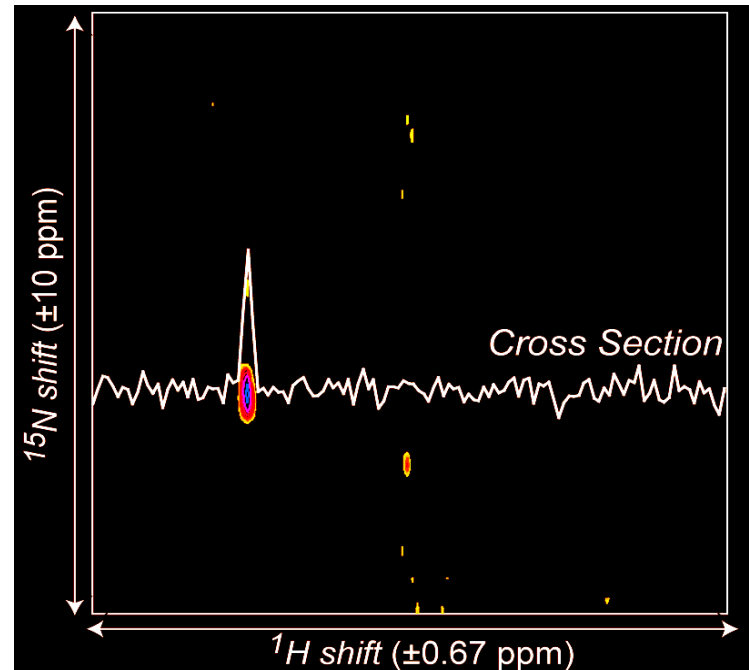


Dr. Ulrich Guenther,
University of Birmingham, UK

2D DNP NMR



Single-scan (0.15 s) 2D
 ^1H - ^{13}C HSQC DNP NMR
 [pyridine] = 0.675 mM
 Natural abundance

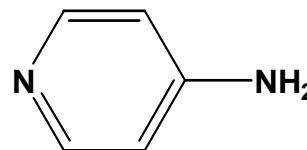


Single-scan (0.13 s) 2D
 ^1H - ^{15}N HSQC DNP
 [$^{15}\text{N}_2$ -urea] = 500 nM

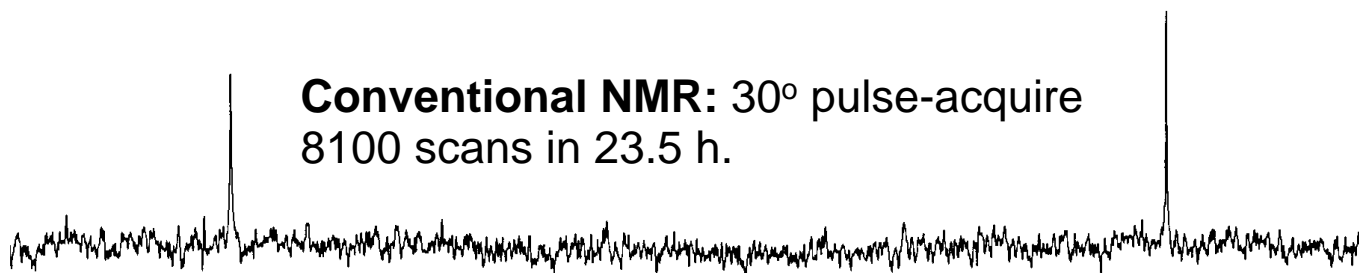
Collaboration with Prof. Lucio Frydman, Weizmann Institute of Science, Israel

^{15}N DNP NMR

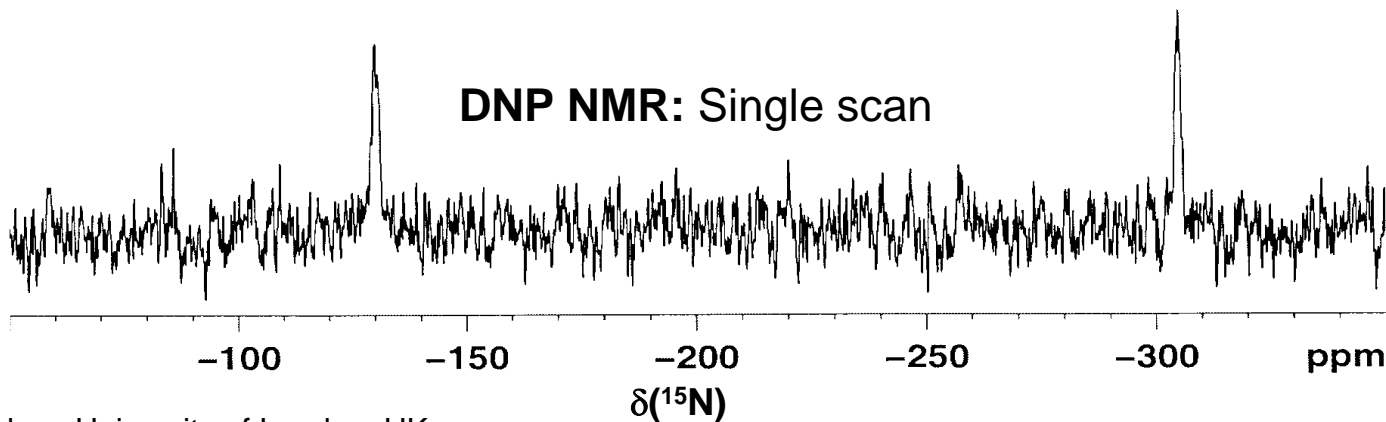
4-Aminopyridine



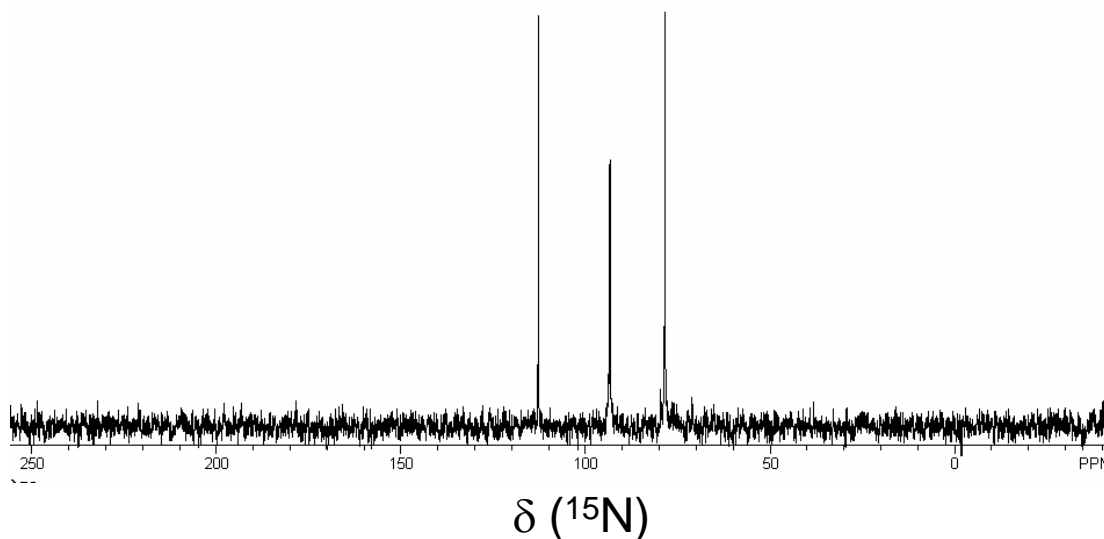
Conventional NMR: 30° pulse-acquire
8100 scans in 23.5 h.



DNP NMR: Single scan



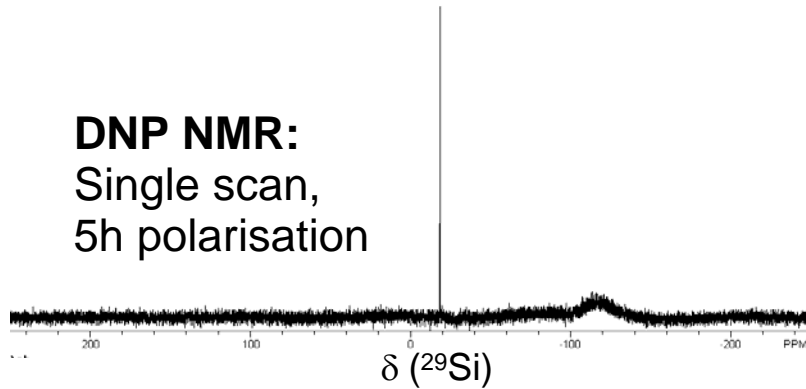
^{15}N DNP NMR (2)



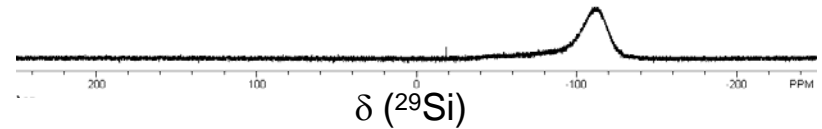
- Mixture of L-alanine- t BOC, glycine- t BOC and N-acetyl glycine, 10 mg each
- Polarised for 5 hours

^{29}Si DNP NMR

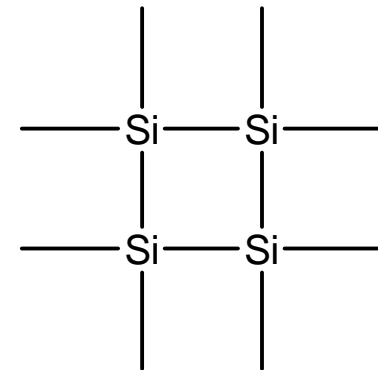
DNP NMR:
Single scan,
5h polarisation



Conventional NMR:
1600 scans, overnight



- ^{29}Si DNP of Volasil-244
- Enhancement ≈ 1100 -fold



Conclusions

- HyperSense™ instrument
 - Automated, reliable, reproducible DNP
- Solid state DNP
 - Understand polarisation conditions
 - Optimise polarisation process for any sample
- Solution DNP NMR
 - ^{13}C : minor isomers and chemical kinetics
 - Complex pulse sequences
 - ^{15}N and ^{29}Si

Facilities at Tubney

- Purpose-built demonstration laboratory
- Equipment:
 - Solid-state DNP
 - HyperSense™
 - 3 integrated spectrometers
- Happy to run demonstrations with your samples



Acknowledgements

- Beta users
 - Geoff Hawkes (QMUL)
 - Ulrich Günther (Birmingham)
 - Adrian Davies and Iain Day (Pfizer)
- Collaborators
 - Lucio Frydman
 - Dusan Uhrin
 - Jonathan Boyd
 - Tim Claridge
- OIMBL-DNP team
 - Steve Reynolds
 - Rob Slade