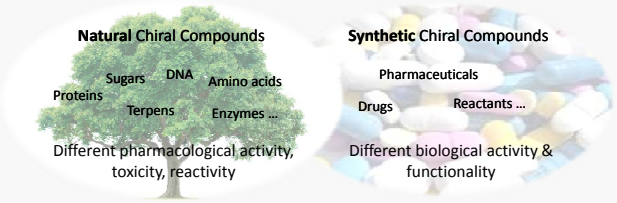
Enantiodiscrimination Studies by ^{13}C DNP-NMR Spectroscopy

Eva Monteagudo , Míriam Pérez-Trujillo, Teodor Parella

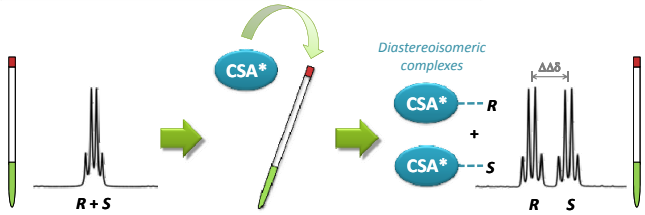
Servei de Ressonància Magnètica Nuclear, Universitat Autònoma de Barcelona, Bellaterra, Catalonia, Spain

INTRODUCTION

Why differentiating enantiomeric molecules?



CSA & ^{1}H NMR Spectroscopy for Enantiodifferentiation



Example of enantiodifferentiation of a racemic mixture by ^{1}H NMR using a chiral solvating agent (CSA)

Applications

- Organic synthesis
- Pharmacology
- Chiral Metabonomics[1]
- Natural Products
- Toxicity Studies
- ...

^{1}H

✓ Easy and fast enantiomeric excess measurement through signal integration

✖ Signal complexity (multiplets)

✖ Signal overlapping

Hamper the enantiodifferentiation study

How to avoid ^{1}H NMR drawbacks?

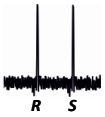
^{13}C

✓ Simple signals (singlets)

✓ Larger chemical shift range

✖ Poor sensitivity

✖ Large acquisition times



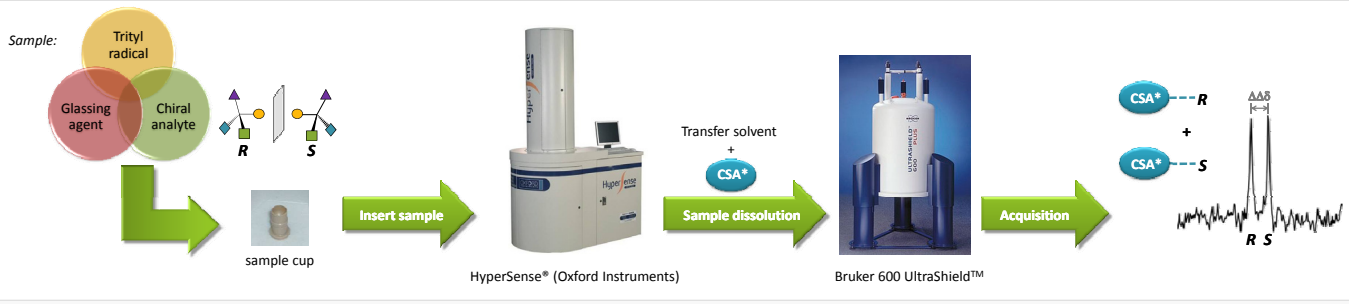
How to avoid ^{13}C NMR drawbacks?

Enantiodifferentiation by dissolution ^{13}C DNP-NMR

✓ Enhanced signals

✓ Single scan ^{13}C NMR

METHODOLOGY



Download the poster here

RESULTS

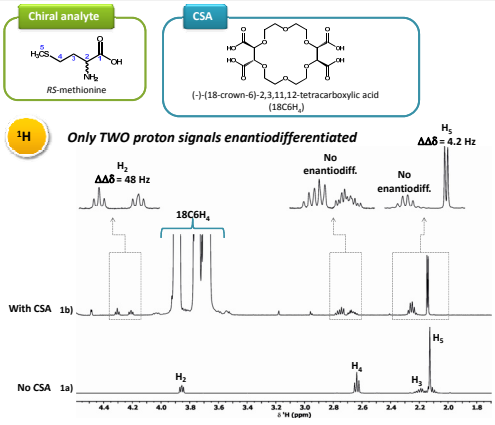


Figure 1. a) ^{1}H NMR 250 mM racemic methionine in D_2O; **b)** ^{1}H NMR 2.41 mM *RS*-methionine, 46 mM (19 eq.) $18C6H_4$ in D_2O. Experiments performed in a 500 MHz spectrometer equipped with TCI cryoprobe and using TSP as external reference.

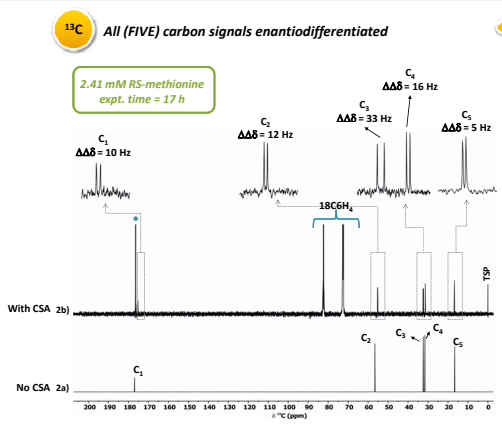
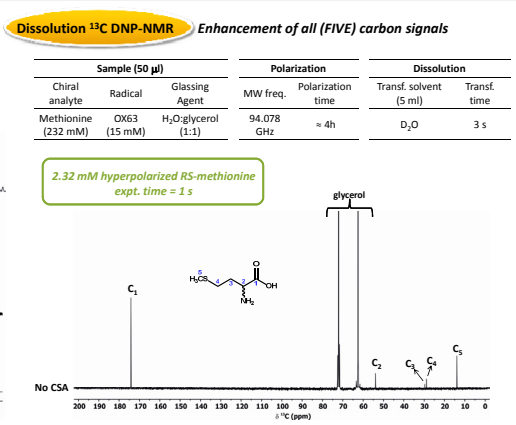
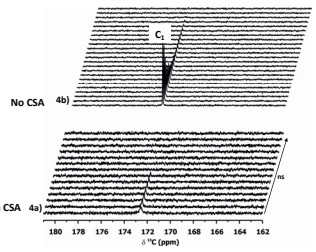


Figure 2. a) ^{13}C NMR 250 mM racemic methionine in D_2O; **b)** ^{13}C NMR 2.41 mM *RS*-methionine, 46 mM (19 eq.) $18C6H_4$ in D_2O. Experiments performed in a 500 MHz spectrometer equipped with TCI cryoprobe and using TSP as external reference.

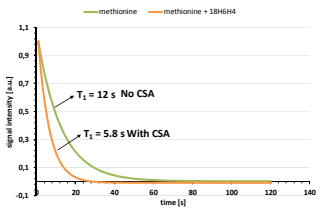


Sample (50 µl)			Polarization		Dissolution	
Chiral analyte	Radical	Glassing Agent	MW freq.	Polarization time	Transf. solvent (5 ml)	Transf. time
Methionine (232 mM)	OX63 (15 mM)	H_2O:glycerol (1:1)	94.078 GHz	≈ 4h	D_2O	3 s

Figure 3. ^{13}C DNP-NMR 600 MHz spectrum of a hyperpolarized dissolution of 2.32 mM natural abundance *RS*-methionine.



← **Figure 4.** C_1 hyperpolarized signal of *RS*-methionine (2.32 mM, nat. abu.) through SSFT/FLASH method[2] **a)** experiments with CSA (10 eq. $18C6H_4$) **b)** without CSA



→ **Figure 5.** Apparent T_1 relaxation time of C_1 hyperpolarized signal of *RS*-methionine from **Fig 4**. The T_1 for the other protonated carbons were too short to be accurately measured.

SUMMARY:

So far, no studies of chiral discrimination have been performed using dissolution ^{13}C DNP-NMR although this methodology overcomes the main drawbacks of both ^{1}H and ^{13}C NMR experiments.

- The *RS*-methionine sample preparation, polarization, dissolution and NMR experiment have been optimized in order to obtain its enhanced signals in a single scan ^{13}C DNP-NMR experiment.
- The formation of *RS*-methioine/$18C6H_4$ complex has been demonstrated by the decrease of the T_1 relaxation time value.
- Further work is being done on the resolution optimization of hyperpolarized racemic methionine + $18C6H_4$.

[1] Pérez-Trujillo, M. Lindon, J.C., Parella, T., Keun, H., Nicholson, J.K., Athersuch, T.J. *Anal. Chem.* **2012**, *84*, 2868-2874.

[2] Day, I.J. Mitchell, J.C. Snowden, M.J. Davis, A.L. *J. Magn. Reson.* **2007**, *187*, 216-224.

Acknowledgements

Financial support for this research provided by MICINN (project CTQ2012-32436) and Bruker Española S.A. are gratefully acknowledged. We also thank to the SeRMN, Universitat Autònoma de Barcelona, for allocating instrument time to this project.