Chiral Recognition by Dissolution DNP NMR Spectroscopy of ^{13}C-Labeled DL-Methionine

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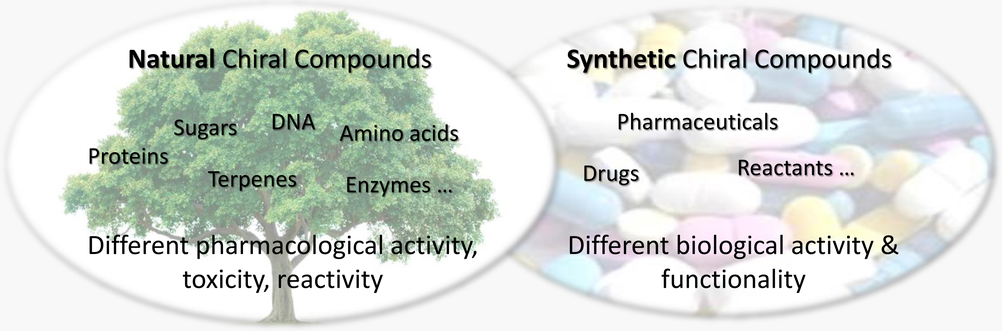
INTRODUCTION

Why differentiating enantiomeric molecules?



Enantiodifferentiation by :

^{1}H-NMR

- ✓ 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?

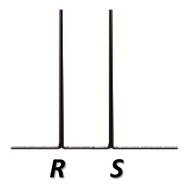
^{13}C-NMR[2]

- ✓ Simple signals (singlets)
- ✓ Larger chemical shift range
- ✖ Poor sensitivity
- ✖ Large acquisition times

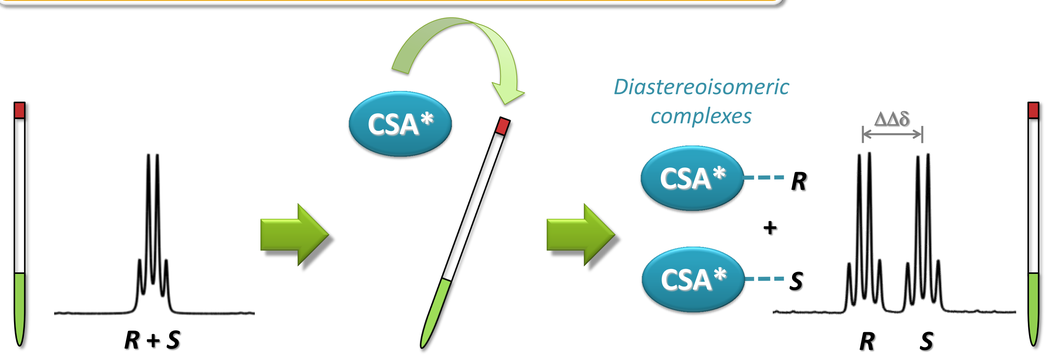
How to avoid ^{13}C NMR drawbacks?

dissolution ^{13}C DNP-NMR

- ✓ Enhanced signals
- ✓ Single scan ^{13}C NMR



CSA & NMR Spectroscopy for Enantiodifferentiation



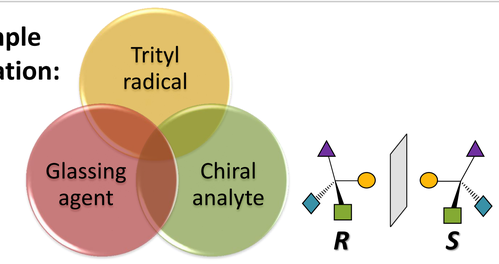
Example of enantiodifferentiation of a racemic mixture by ^{1}H NMR using a chiral solvating agent (CSA)

Applications

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

Method developed

i) Sample preparation:



sample cup

ii) Sample insertion

iii) Sample polarization

HyperSense® (Oxford Instruments)

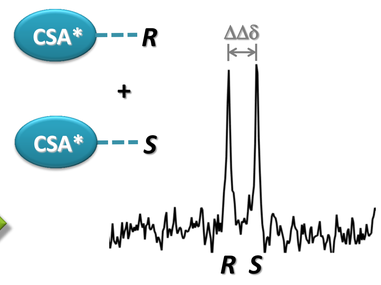
iv) Insert transfer solvent + CSA*

v) Sample dissolution

vi) Dissolution transfer

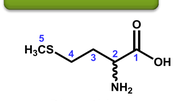
Bruker 600 MHz

vii) NMR Acquisition



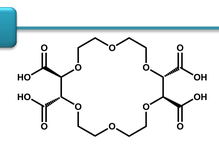
Proof of concept

Chiral analyte



DL-methionine

CSA



(-)-(18-crown-6)-2,3,11,12-tetracarboxylic acid (18C6H4)

Sample (50 µl)			Polarization		Dissolution	
Chiral analyte	Radical	Glassing Agent	MW freq.	Polarization time	Transf. solvent (5 ml)	Transf. time
DL-[1-^{13}C]-mMethionine (224 mM)	OX63 (15 mM)	H_2O:glycerol (1:1)	94.078 GHz	≈ 2,5 h	D_2O	5 s

^{13}C-NMR

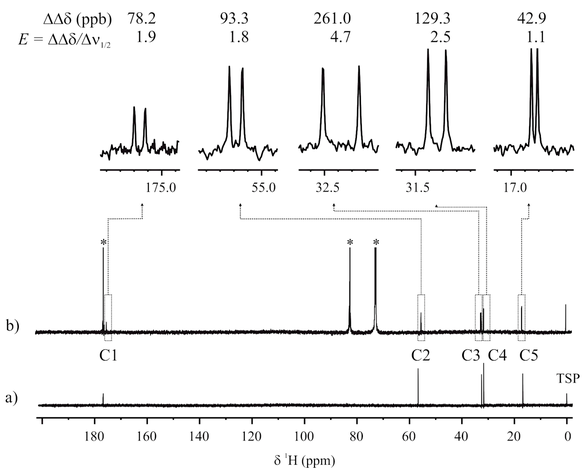
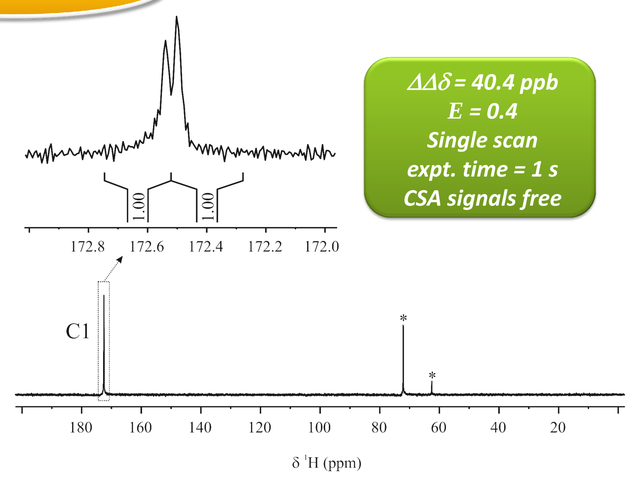


Figure 1. ^{13}C NMR spectra (150.92 MHz) of **(a)** DL-methionine (2.4 mM) in D_2O (expt 24 h 9 min) and **(b)** DL-methionine (2.4 mM) in D_2O after the addition of 19 equiv of (-)-18C6H4 (expt 24 h 9 min). Asterisks denote signals corresponding to the chiral auxiliary.

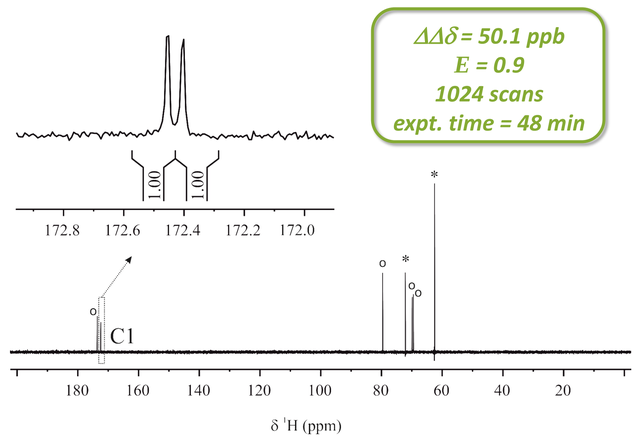
Dissolution ^{13}C DNP-NMR



ΔΔδ = 40.4 ppb
E = 0.4
Single scan
expt. time = 1 s
CSA signals free

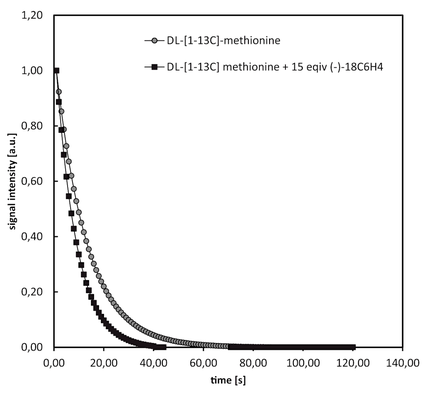
Figure 2. 150.92 MHz d-DNP ^{13}C NMR spectrum (single scan, expt 1 s) of hyperpolarized DL-[1-^{13}C]-methionine (2.2 mM) during the enantiodifferentiation experiment with CSA (-)-18C6H4 (15 equiv). Asterisks denote peaks corresponding to glycerol.

^{13}C- NMR at thermal equilibrium



ΔΔδ = 50.1 ppb
E = 0.9
1024 scans
expt. time = 48 min

Figure 3. ^{13}C NMR (150.92 MHz) spectrum (1024 scans, expt 43 min) of DL-[1-^{13}C]-methionine (2.2 mM) at thermal equilibrium with CSA (-)18C6H4 (15 equiv). The sample contains trityl radical, OX63, glycerol and H_2O. Asterisks and circles denote peaks corresponding to glycerol and CSA, respectively.



← **Figure 4.** ^{13}C NMR signal intensity decay curves of hyperpolarized C1 of DL-[1-^{13}C]-methionine without CSA (white circles, T_1(^{13}C) = 12.5 s) and with CSA (black squares, T_1(^{13}C) = 8.5 s). T_1(^{13}C) was obtained by fitting signal intensity values to a monoexponential decay curve.

SUMMARY & CONCLUSIONS:

- Chiral recognition by dissolution DNP ^{13}C NMR spectroscopy was demonstrated for the first time.[3]
- A method integrating d-DNP and ^{13}C NMR-aided enantiodifferentiation using chiral solvating agents was developed, in which only the chiral analyte was hyperpolarized and selectively observed by NMR spectroscopy.
- The described method enhances the sensitivity of the conventional NMR-based method and lightens the common problem of signal overlapping between analyte and CSA.
- Under hyperpolarization of the analyte, enantiodifferentiation $\Delta\Delta\delta$ and relative integration values split peaks were similar to those obtained at thermal equilibrium, whereas the enantioresolution quotient E decreased.

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

[2] Pérez-Trujillo, M., Monteagudo, E., Parella, T. *Anal. Chem*. **2013**, *85*, 10887-10894

[3] Monteagudo, E. ,Virgili, A., Parella, T., Pérez-Trujillo, M. *Anal. Chem.* **2017**, *89*, 4939-4944.

Acknowledgements

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