^{13}C NMR Spectroscopy for the Differentiation of Enantiomers in Complex Systems using Chiral Solvating Agents (CSA)

SeRMN

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INTRODUCTION

Why differentiating enantiomeric molecules?

are

Natural Chiral Compounds
Amino Acids, Sugars, Terpenes, Hydroxy Acids …

show

Different biological activity & functionality

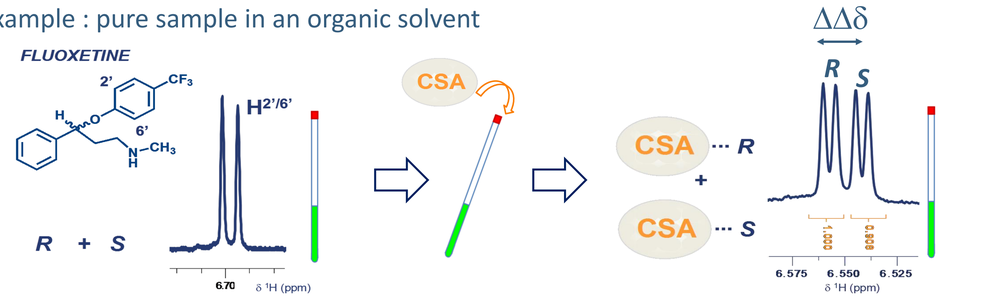
Synthetic Chiral Compounds
Pharmaceuticals, Drugs, Reactants …

show

Different pharmacological activity, toxicity, reactivity

CSA & ^{1}H NMR Spectroscopy for Enantiodifferentiation

Example : pure sample in an organic solvent



Enantiodifferentiation of racemic fluoxetine in $CDCl_3$ using ABTE-18 as CSA [2]

Applications

- ✓ Pharmacology
- ✓ Chiral Metabonomics [1]
- ✓ Natural Products
- ✓ Toxicity Studies
- ✓ …

^{1}H NMR Spectroscopy & Complex Systems

- Pure enantiomeric mixture with complex ^{1}H NMR spectrum
- Mixture of compounds , e.g. Chiral Metabonomics [1]

DRAWBACKS

- **Multiplicity of signals** → High ΔΔδ needed → Partial enantioresolution of signals → Inaccuracy of the *R/S* molar ratio measurement
- **Low chemical shift range** → Overlapping → Hampers the enantiodifferentiation study

Download the poster here

1D ^{1}H *vs* 1D ^{13}C NMR SPECTROSCOPY FOR THE STUDY OF COMPLEX SYSTEMS

Enantiodifferentiation

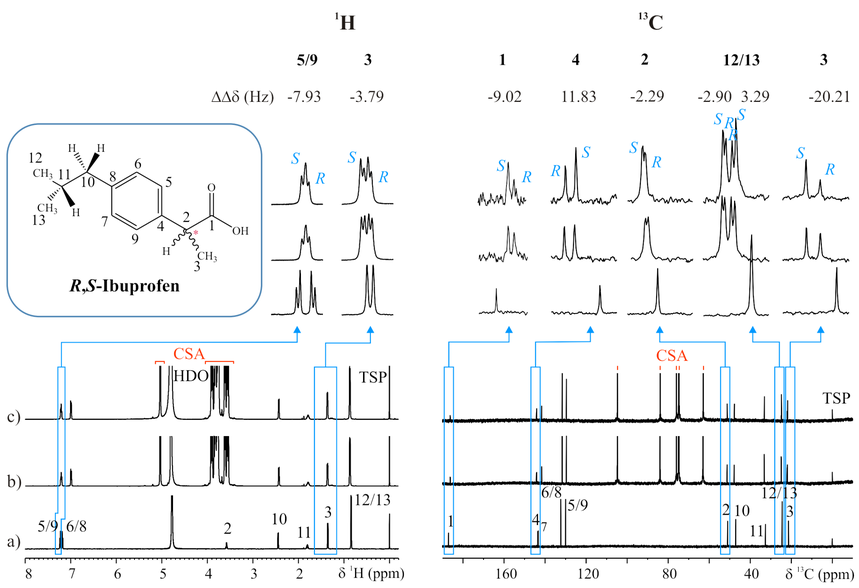


Figure 1. a) 2.2 mM Racemic IBU in D_2O; b) with 4.6 equivalents of βCD added and c) sample spiked with *S*-IBU. Experiments performed in a 500 MHz spectrometer equipped with a TCI cryoprobe.

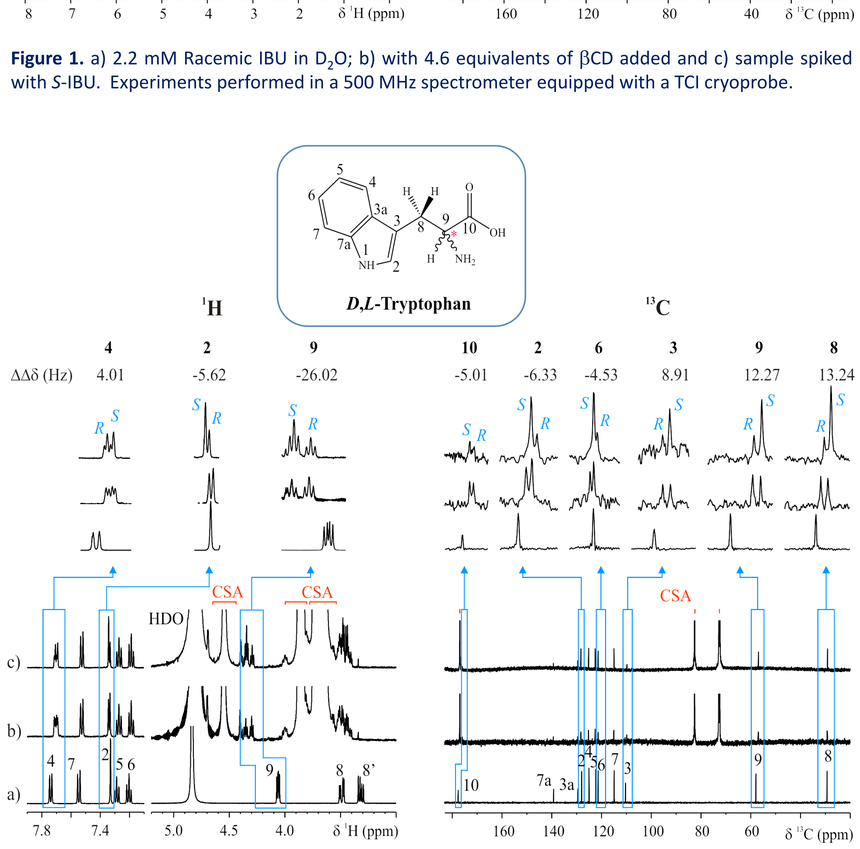


Figure 2. a) 2.3 mM Racemic TRP in D_2O; b) with 5.4 equivalents of 18C6TCA added and c) after spiking the sample with L-TRP. Experiments performed in a 500 MHz spectrometer equipped with a TCI cryoprobe.

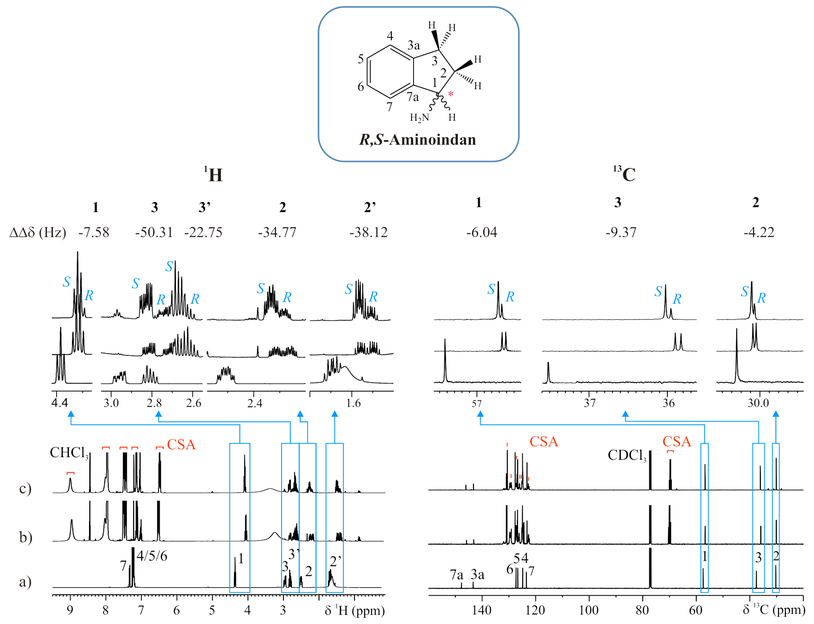


Figure 3. a) 50 mM Racemic AMI in $CDCl_3$; b) with 4.5 equivalents of *R*-PA added and c) after spiking the sample with *S*-AMI. Experiments performed in a 500 MHz spectrometer equipped with a TCI cryoprobe.

Sensitivity – Experimental Time

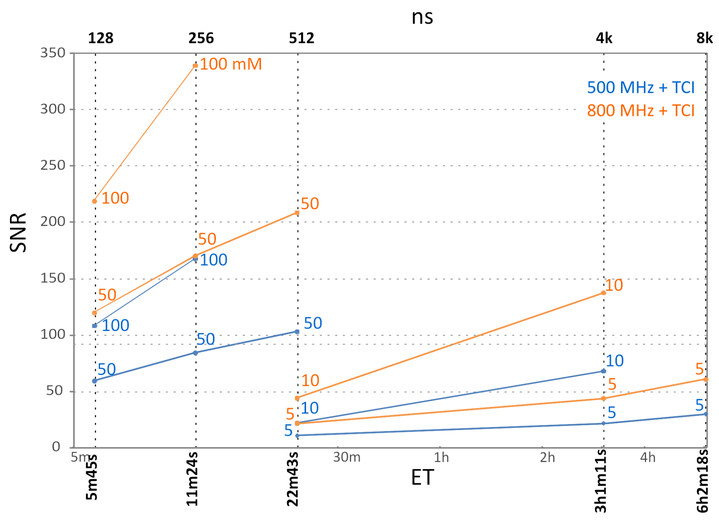


Figure 4. Plot of the SNR of split C2 signal of *R,S*-AMI in front of the ET and the number of transients (ns) of the 1D *pg* {^{1}H} ^{13}C NMR experiment. Results for concentrations of analyte of 100, 50, 10 and 5 mM are indicated. Experiments were carried out in $CDCl_3$, in the presence of 4.5 equivalents of *R*-AP, at 298.0 K of temperature and at a magnetic field of 500 MHz with a cryogenic probe spectrometer. Estimated values for analogous experiments under a magnetic field of 800 MHz are shown.

R/S Molar Ratio Measurement

Table 1. Theoretical and measured (by the ratio of the signal integrals) *R/S* molar ratio values of mixtures of *R,S*-AMI. Measured values correspond to three different experiments: 1D ^{1}H (zg), 1D ^{13}C with NOE contribution (zgpg) and 1D ^{13}C without NOE contribution (zgig). The observational error for each measurement in percentage is indicated. The three mixtures were prepared from a 50 mM racemic AMI solution and the CSA used was 4,5 equivalents of *R*-PA.

Theoretical *S/R* ratio[a] (*R*:*S*)	Measured *R/S* ratio and Error[b]							
	^{1}H			^{13}C				
				pg			*ig*	
	H atom	meas.	error (%)	C atom	meas.	error (%)	meas.	error (%)
1	H-1	0,98	1,62 *	C-1	1,01	1,25	1,00	0,11
(50:50)	H-2	1,01	1,28 *	C-2	1,00	0,35	1,01	0,99
	H-2'	0,97	2,92 *	C-3	1,00	0,31	0,99	0,71
	H-3	0,77	22,90 **p	C-3a	0,98	2,40	1,03	2,66
	H-3'	-	- */**					
3	H-1	3,08	2,50 *	C-1	3,00	0,14	3,00	0,03
(25:75)	H-2	4,34	44,57 *	C-2	3,00	0,05	3,03	1,00
	H-2'	3,38	12,73 *	C-3	3,06	1,98	3,02	0,62
	H-3	-	- **	C-3a	2,91	2,87	3,10	3,20
	H-3'	-	- */**					
9	H-1	6,98	22,44 *	C-1	9,05	0,57	8,81	2,10
(10:90)	H-2	11,11	23,41 *	C-2	8,92	0,90	8,95	0,58
	H-2'	10,11	12,30 *	C-3	8,97	0,29	9,01	0,11
	H-3	-	- **	C-3a	- *		- *	
	H-3'	12,58	39,80 *					

[a] From weighted values
[b] Observational error (fm-fr)*100/fr
*Signal partially split
**(p) Signal (partially) overlapped with another signal of the spectrum

CONCLUSIONS

Though experimental times are longer than using ^{1}H NMR spectroscopy, observing ^{13}C nuclei is a convenient information-rich alternative in many situations and particularly when studying complex systems.

The 1D {^{1}H} ^{13}C NMR experiment:

- provides valuable and complementary information to the 1D ^{1}H NMR experiment
- extends the possibilities of enantiodifferentiation to fully deuterated and nonproton containing compounds
- is a powerful alternative to the ^{1}H NMR experiment due to the intrinsic high dispersion of the ^{13}C and to the easiness of obtaining a proton decoupled spectrum; and that overcomes the main drawbacks of the 1D ^{1}H NMR experiment (see above)
- has a huge potential for the enantiomeric study of complex mixtures (Chiral Metabonomics)

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.; Virgili, A., Tetrahedron-Asymmetr. **2006**, *17*, 2842-2846.

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