HR-DOSY and sulfoxide enantiomeric discrimination by cyclodextrin

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Dedicated to the memory of Dr. Piet Leclercq

Abstract. Racemic and chiral ethyl-phenylsulfoxide (solute) and β -cyclodextrin (chiral selector) were used to compare two NMR methodologies to predict RP-HPLC enantiomeric resolution efficiency. One of them based on the classical approach involving apparent binding constants and complexation-induced chemical shifts at saturation and the other based on ^{13}C NMR signal splittings (solute and chiral selector in stoichiometric ratio) and HR-DOSY of the same solution. We have concluded that the latter methodology is rather efficient and though more elaborate from the NMR point of view, the results are promising and constitute an alternative method to investigate chiral recognition and other supramolecular phenomena.

1. Introduction

Resolution of racemic mixture into its enantiomers is an important issue that relies on the optimization of analytical methodologies. Nevertheless this procedure can be time consuming if one has to build HPLC and or GC columns to test the efficiency of chiral phases. Consequently methodologies that allow a rapid and inexpensive evaluation of the global efficiency of chiral selectors are particularly important. Therefore, investigation of chiral recognition by NMR methods to predict enantiomeric resolution is a field in expansion providing not only apparent binding constants and discrimination efficiency but also information about the topology of the solute-selector complexes [1–4]. Detailed informations of the chiral selector and solute interactions (stoichiometry and apparent binding constants) are obtained by complexation-induced ^1H NMR chemical shifts at different solute-chiral selector ratios while the topology of the complexes are mainly investigated by nuclear Overhauser effect (NOESY, ROESY) [1, 5]. One of the main goals of our group was to expand the plethora of NMR methods of enantiomeric recognition by applying Diffusion Ordered Spectroscopy (DOSY).

DOSY is a non invasive NMR tool that simultaneously determines the translational diffusion coefficients of components present in a mixture [6]. It has the further advantage of separating the signals belonging to each component without requiring chromatography which is particularly attractive when dealing with unstable complexes easily destroyed by light scattering or chromatographical processes. Simple diffusion experiments using NMR were first reported by Stejskal and Tanner [7] which were followed by DOSY a more elaborated NMR techniques applying field gradients [6]. Nowadays in HR-DOSY the use of field gradient compensated pulse sequence (DOSY-GCSTESL, Gradient Compensated

Stimulated Echo Spin Lock) with spin lock gives a well defined diffusion projection with no signals at intermediate diffusion values [8]. During a set of experiments using several natural and permethylated cyclodextrins and racemic mixtures we have concluded that diffusion ordered spectroscopy associated to ^{13}C NMR signals enantiomeric discrimination [9] was an alternative method to predict enantiomeric resolution by RP-HPLC.

We have therefore focused on the application to HR-DOSY improved by GCSTESL pulse sequence [8], to the enantiomeric discrimination of ethyl-phenylsulfoxide **1** by β -cyclodextrin (β -CD) with the purpose of comparing our method with the classical ^1H NMR complexation induced chemical shift approach [9] and also expanding the limits of the established methodology.

2. Experimental

2.1. Apparatus

2.1.1. Nuclear Magnetic Resonance Spectroscopy

^1H and ^{13}C NMR spectra were obtained on a Varian Inova-500 spectrometer with standard pulse sequences operating at 499.885 MHz and 125.695 MHz for ^1H and ^{13}C , respectively. The chemical shifts are reported in ppm using TMS (0 ppm) as external reference for ^1H and CCl_4 (96.0 ppm) for ^{13}C NMR spectra and pulses of 45° for ^1H and ^{13}C and delay times of 1 s and 2 s, respectively. The coupling constants (J) are in Hz. All data were obtained at $30 \pm 1^\circ\text{C}$.

The ROESY-1D experiments [10] were obtained starting with a selective 180° pulse inverting a selected hydrogen signal, followed by a non-selective 90° pulse and spin-lock time. The selective pulses were generated by pulse modulator which automatically attenuated the power and the pulse length to obtain the required selectivity. The subtraction of the on resonance and off-resonance acquisition furnished the ROESY-1D experiment.

HR-DOSY was carried out carefully choosing the correct pulse sequence and gradients for the experiments which were using a 5 mm inverse probe with z-gradient coil. A reliable diffusion data set was obtained with the GCSTESL (Gradient Compensated Stimulated Echo Spin Lock) DOSY sequence [8] and 15 mmol l^{-1} solutions of host and guest at $30 \pm 1^\circ\text{C}$. The amplitudes of the gradient pulses ranged from 0.0685 to 0.3427 T cm^{-1} , an approximately 100% decrease in the resonance intensity was achieved at the largest gradient amplitudes. For all experiments, 25 different gradient amplitudes were used in each experiment. The baselines of all arrayed spectra were corrected prior to processing. The data processing program (the macro DOSY in a Varian instrument) involves the determination of the peak heights of all signals above a pre-established threshold and fitting the decay curve for each peak to a Gaussian. The DOSY macro was run with data transformed using $fn = 64$ K. Very crowded spectra were processed in sections due to the limitation to handling only 512 lines at a time. The result of the DOSY method of analysis is a pseudo two-dimensional spectrum with NMR chemical shift (in Hz) along one axis and calculated diffusion coefficients ($\text{m}^2 \text{s}^{-1} \times 10^{-10}$) along the other.

The stoichiometry of host-guest complex was determined by the continuous variation method [11]. The total concentration of the interacting species in the solution was kept constant at 15 mmol l^{-1} and the molar fraction of the guest was varied in the range of 0.2–0.8 [12]. Apparent binding constants of the enantiomers of ($\pm$)-**1** with β -CD were calculated on the basis of Scott's modification of the Benesi-Hildebrand equation [13].

2.1.2. Chiral chromatography

HPLC chromatograms were obtained with a Varian 9050/9010 HPLC system, equipped with a variable wavelength UV–VIS detector, Rheodyne injector and Waters 374 integrator. The HPLC analyses were carried out using a Waters Nova Pack ODS ($4\ \mu\text{m}$, $150\ \text{mm} \times 3.9\ \text{mm}$ i.d) column eluted with chiral mobile phase (β -cyclodextrin $15\ \text{mmol l}^{-1}$) at a flow rate of the $0.5\ \text{ml min}^{-1}$ and a column temperature of 30°C . Injection volume, $0.2\ \mu\text{l}$ of a solution of about $10\ \text{mg ml}^{-1}$ of the mixture in water. The methyl-phenylsulfoxide was monitored at a wavelength of $234\ \text{nm}$.

GC-MS analyses were carried out using a HP-5990/5970 system equipped with HP-5 fused silica capillary column ($30\ \text{m} \times 0.25\ \text{mm} \times 0.25\ \mu\text{m}$); column temperatures were programmed from 80°C to 290°C at $15^\circ\text{C min}^{-1}$. GC/MS injector and detector temperatures were 220°C and 285°C , respectively, flow rate $1.16\ \text{ml min}^{-1}$, split mode. Injection volume, $0.5\ \mu\text{l}$ of a solution of about $10\ \text{mg ml}^{-1}$ of the mixture in ethyl acetate. Helium was used as the carrier gas. The MS were determined at $70\ \text{eV}$. Scanning speed was $0.84\ \text{scan sec}^{-1}$ from m/z 40 to 550.

GC analyses were carried out using a GC(FID) HP-5890 chromatograph, equipped with a chiral column Heptakis-(2,6-methyl-3-pentyl)- β -cyclodextrin ($25\ \text{m} \times 0.25\ \text{mm} \times 0.25\ \mu\text{m}$). Injector and detector temperatures were 200°C . Temperature program: 90°C -5 min- 3°C/min - 180°C -5min, pressure at the head of the column $0.6\ \text{bar}$.

2.2. Chemicals and reagents

Thiophenol (99%) and β -cyclodextrin (98%) were purchased from Aldrich and Merck, respectively. Racemic and *S*(-)-enantiomer of methyl-phenylsulfoxide were synthesized in our laboratory as described to follow. All solutions were prepared in D_2O , 99.9 atom% *d* (Aldrich). Because β -cyclodextrin is highly hydrated, the crystalline material was lyophilized prior to the preparation of solutions for NMR spectroscopy.

Synthesis of ethyl phenyl thioether:

To a round bottom flask (125 ml) equipped with a mechanical stirrer, thermometer and containing dichloromethane (10 ml), thiophenol (1.82 mmol), $(\text{CH}_3\text{CH}_2)_3\text{N}$ (2 mmol) and K_2CO_3 dried (5.45 mmol) were added. The mixture was stirred for 20 min at 0°C . $\text{CH}_3\text{CH}_2\text{I}$ (3.64 mmol) was slowly added and reaction mixture stirred overnight. Water was added to the reaction mixture and continually extracted with dichloromethane ($3 \times 20\ \text{ml}$). The organic layer was dried over Na_2SO_4 , filtered and evaporated under reduced pressure. The residue (250 mg) was purified by column chromatography, eluted with hexane, yielding ethyl phenyl thioether (240 mg; 96% yield).

IR. (film) ν_{max} (cm^{-1}) 3075, 1585, 779, 691; ^1H NMR (500 MHz, CDCl_3): δ (ppm) 1.29 (t, $^3J = 7.3\ \text{Hz}$, 3H, CH_3), 2.29 (q, $^3J = 7.3\ \text{Hz}$, 2H, CH_2), 7.14 (tt, $^3J = 7.5\ \text{Hz}$, $^4J = 1.3\ \text{Hz}$, 1H, CH), 7.25 (td, $^3J = 7.5\ \text{Hz}$, $^4J = 1.3\ \text{Hz}$, 2H, 2 CH), 7.31 (dd, $^3J = 7.5\ \text{Hz}$, $^4J = 1.3\ \text{Hz}$, 2H, 2 CH) ^{13}C NMR (125 MHz, CDCl_3): δ (ppm) 14.31 (CH_3), 27.60 (CH_2), 125.69 (CH), 128.75 (2CH), 129.00 (2CH), 136.62 (C_0). MS m/z (%): 138 (M^+ , 100), 123 (58), 110 (62), 77 (18), 65 (32), 51 (32), 45 (65).

Synthesis of (-)-ethyl-phenylsulfoxide:

The microorganism *Aspergillus terreus* CCT3320 were obtained from the Culture Collection, Fundação de Pesquisa e Tecnologia André Tosello (Brazil). The microorganism was grown at $48\ \text{h}/28^\circ\text{C}/120\ \text{rpm}$ in culture shaker-flasks in an appropriate medium (extract malt 2%) and cells were harvest by filtration. The oxidation of the ethyl phenyl thioether was performed in 125 ml bottles on rotatory shaker (120 rpm). The bottles containing phosphate buffer pH 7.0 (25 ml) and washed cells

(350 mg), the thioether was added (20 μ l). The mixture was shaken at 28°C and the reaction was monitored by chiral GC. Upon reaching the appropriate conversion degree, the cells were filtrated, the formed sulfoxide was extracted from the supernatants with acetate ethyl and dried (Na_2SO_4).

$[\alpha]_{\text{D}}^{25} = -104.5$ (c 0.51, CHCl_3) {lit. [14,15]: *R*-(+)-**1** $[\alpha]_{\text{D}}^{21} = +154$ and $[\alpha]_{\text{D}}^{24} = +176.2$ (c 1.3, acetone)}. IR. (film) ν_{max} (cm^{-1}): 3048, 1657, 1047, 742, 693; ^1H NMR (500 MHz, D_2O): δ (ppm) 0.94 (t, $^3J = 7.3$ Hz, 3H, CH_3), 2.82 (dq, $^2J = 13.6$ Hz, $^3J = 7.5$ Hz, Ha, methylene group), 2.88 (dq, $^2J = 13.6$ Hz, $^3J = 7.5$ Hz, 1Hb, methylene group), 7.43 (td, $^3J = 7.5$ Hz, $^4J = 1.3$ Hz, 2H, 2CH), 7.44 (tt, $^3J = 7.5$ Hz, $^4J = 1.3$ Hz, H, CH), 7.48 (dd, $^3J = 7.5$ Hz, $^4J = 1.3$ Hz, 2H, 2 CH) ^{13}C NMR (125 MHz, D_2O): δ (ppm) 5.32 (CH_3), 48.54 (CH_2), 124.46 (2CH), 129.35 (2CH), 131.84 (CH), 139.51 (C_0). MS m/z (%): 154 (M^+ , 13), 126 (46), 97 (14), 78 (100), 51 (38).

Enantiomeric excess was determined by chiral GC (ee > 99%).

Synthesis of ($\pm$)-ethyl-phenylsulfoxide:

To a stirred solution of ethyl phenyl thioether (0.72 mmol), in methanol/ H_2O 1 : 1 (10 ml) at room temperature, H_2O_2 30v (1.81 mmol) was slowly added. The reaction was monitored by TLC and as soon as the mercaptan was consumed. The methanol was evaporated and before the extraction with ethyl acetate was performed (5×10 ml). The organic layer was dried over Na_2SO_4 , filtered and evaporated under reduced pressure. The residue (110 mg) was purified by column flash chromatography, eluted with hexane-ethyl acetate 1 : 1, yielding ($\pm$)-ethyl-phenylsulfoxide (100 mg; 90% yield). Spectral data equal to enantiomerically pure (–)-**1**.

3. Results and discussions

The compound ($\pm$)-**1** was obtained in a two step synthesis from commercial thiophenol **2** and (–)-**1** was obtained by microbiological oxidation of **3** with *Aspergillus terreus* CCT3320 (Scheme 1).

Solutions of ($\pm$)-**1** and β -CD, (–)-**1** (ee = 33%) and β -CD, both in D_2O (1 : 1 ratio, 15 mmol l^{-1} of each) were investigated by ^1H and ^{13}C NMR. In the ^1H NMR spectrum of enantiomeric enriched (–)-**1** with β -CD all resonance signals are shifted due to the complexation with chiral selector. The different structures of the complexes formed by the enantiomeric forms and the CD induce chemical shift nonequivalence between the signals of the two enantiomeric forms: this difference of chemical shift

Scheme 1. Synthesis of methyl-phenylsulfoxide.

Fig. 1. Partial ^1H NMR spectra of (a) $(\pm)\text{-1}$ (15 mmol l^{-1}) without $\beta\text{-CD}$; (b) equimolar mixture (15 mmol l^{-1} each) of $(-)\text{-1}$ ($ee = 33\%$) and $\beta\text{-CD}$; (c) equimolar mixture (7.5 mmol l^{-1} each) of $(\pm)\text{-1}$ and $\beta\text{-CD}$.

was most pronounced for diastereotopic hydrogens alpha to the sulfoxide H-1'a and H-1'b (see Scheme 1). For the two diastereomeric complexes these signals were well resolved (Fig. 1).

The ^{13}C NMR signals of enantiomeric enriched $(-)\text{-1}$ ($ee\ 30\%$) also were separated into two sets of signals, for carbons 1 and 4 of the phenyl ring and for the carbons belonging to the ethyl moiety, (Fig. 2) while the signals corresponding to the $\beta\text{-CD}$ did not show any splitting nor signal broadening. We have therefore concluded that the complex formation was in the fast equilibrium range for the NMR time scale and adequate to our studies. We have also observed that the signals corresponding to $(+)\text{-1}$ were more deshielded than those of $(-)\text{-1}$ which could predict a larger binding constant for $(+)\text{-1}$.

Based on the Job plots [11,12] for the complexation-induced chemical shifts at different $(\pm)\text{-1}/\beta\text{-CD}$ ratios (Fig. 3) showing a maximum at the selector-solute molar fraction in solution 0.5–0.5 we have concluded that the complexes of 1 : 1 stoichiometry are predominant in solution (Fig. 4).

$^1\text{H}\text{-}^1\text{H}$ nuclear dipolar intermolecular interaction observed by ROESY 1D was a means to assess the topology of the adduct. The choice of rotating frame nOe arises from the fact that the $\omega\tau_c \approx 1$ and therefore rather small nOe are obtained in normal experiments [16]. Irradiation of the hydrogens H-2 of $(\pm)\text{-1}$ produced signal increments of H-3 (2%) and H-5 (1%) of the $\beta\text{-CD}$ as depicted in Fig. 5. This suggests that the guest is not deeply inserted into the cavity. The CD H-2 and H-4 increments were unexpected and they indicate that there is complexation from both sides but with a preference for the phenyl ring (Fig. 5).

Fig. 2. ^{13}C NMR spectra of (a) equimolar mixture (15 mmol l^{-1} each) of $(-)\text{-1}$ ($ee = 33\%$) and $\beta\text{-CD}$; (b) $(\pm)\text{-1}$ (15 mmol l^{-1}) without $\beta\text{-CD}$.

All the above experiments were performed with a racemic sample therefore the picture of the host-guest geometry is just a crude suggestion with no topological subtleties concerning the two diastereomeric complexes.

The latter was retrieved by calculating the apparent enantiomeric binding constant (K_{ap}) which can be obtained by applying the Benesi–Hildebrand method modified by Scott [13] binding constants, for the complex of known 1 : 1 stoichiometry. In Eq. (1) [17]:

$$\frac{[\beta\text{-CD}]_t}{\Delta\delta_{\text{obs}}} = \frac{[\beta\text{-CD}]_t}{\Delta\delta_c} + \frac{1}{K_{\text{ap}}\Delta\delta_c}. \quad (1)$$

$[\beta\text{-CD}]_t$ is molar concentration of the chiral selector, $\Delta\delta_{\text{obs}}$ is the observed chemical shift difference for a given $[\beta\text{-CD}]_t$ concentration, $\Delta\delta_c$ is the chemical shift of a pure sample of complex and the free component at the saturation. The slope of the plot of $[\beta\text{-CD}]/\Delta\delta_{\text{obs}}$ against $[\beta\text{-CD}]$ is thus equal to $1/\Delta\delta_c$ and the intercept with the vertical axis to $1/K_{\text{ap}}\Delta\delta_c$ allows the estimation of K_{ap} .

The binding constants and the chemical shift differences at saturation were calculated for both enantiomers of $(\pm)\text{-1}$ discriminated by the $\beta\text{-CD}$, measuring the complexation induced chemical shift in a 1 mmol l^{-1} solution of $(\pm)\text{-1}$ in D_2O and increasing amounts of CD from from 3 to 15 mmol l^{-1} . The ^1H NMR signals of the methylene group of $(\pm)\text{-1}$ at 2.82 and 2.88 ppm corresponding to H-1'a and H-1'b respectively, were used for the calculation of binding constants. The plot of $[\beta\text{-CD}]/\Delta\delta_{\text{obs}}$ versus $[\beta\text{-CD}]$ for the calculation of the binding parameters of $(\pm)\text{-1}$ with $\beta\text{-CD}$ is given on Fig. 6. The apparent binding constants (K_{ap}) calculated on the basis of these plots are given in Table 1. It is noteworthy to observe that the carbon-13 NMR signal splittings trend with deshielding of the $(+)\text{-1}$ signal were indeed indicative of a larger binding constant (Fig. 2).

Fig. 3. Partial ^1H NMR spectra of solutions of $(\pm)\text{-1}$ (H-1'a and H-1'b) and $\beta\text{-CD}$ at various ratios.

Fig. 4. Job plots for $(\pm)\text{-1}/\beta\text{-CD}$ complex.

Fig. 5. (A) Hydrogens of the sulfoxide which were perturbed by selective pulse and the observed intermolecular ROE (%) observed for β -CD hydrogens (a) H-2, (b) H-3, (c) H-4 and (d) H-5; (B) Suggested topology of the adduct.

Fig. 6. Scott plots of R-(+)-sulfoxide (■) and S-(-)-sulfoxide (▲) in solutions with β -CD.

Table 1

Apparent binding constants (K_{ap}), average K_{ap} and α for methyl-phenylsulfoxide **1** in solution with β -CD^a

Complex	$K_{\text{ap}(+)} \text{ (M}^{-1}\text{)}$		$K_{\text{ap}(-)} \text{ (M}^{-1}\text{)}$		$K_{\text{ap}(+)\text{av}} \text{ (M}^{-1}\text{)}$	$K_{\text{ap}(-)\text{av}} \text{ (M}^{-1}\text{)}$	α ($K_{\text{ap}(+)}/K_{\text{ap}(-)}$)
	H-1'a(+)	H-1'b(+)	H-1'a(-)	H-1'b(-)			
(±)- 1 / β -CD	172.4	340.4	166.4	254.0	256.4	210.2	1.22

^a All parameters were calculated on the basis of H-1'a and H-1'b ¹H NMR signals.

Based on previous experiments [9] we have observed that α values of 1.20–1.30 indicate that enantiomeric separation by RP-HPLC can be predicted using β -CD as mobile chiral selector.

Table 2
Diffusion coefficients of methyl-phenylsulfoxide free and complexed with β -CD by HR-DOSY^a

Compound	Diffusion coefficients ($10^{-10} \text{ m}^2 \text{ s}^{-1}$)	
	Free	Complex
($\pm$)- 1	6.28 ± 0.03	3.81 ± 0.02
β -CD	2.37 ± 0.01	2.31 ± 0.01

^a Data observed by GCSTESL DOSY sequence.

From the HR-DOSY spectra we have inferred, by applying the Eq. (2) [9,18] that the sulfoxide and β -CD inclusion complex in D_2O solution exist in a rapid equilibrium composed of 62% of the adduct.

$$D_{\text{obs}} = \chi_{\text{free}} D_{\text{free}} + \chi_{\text{bound}} D_{\text{bound}}, \quad \text{where } \chi_{\text{free}} + \chi_{\text{bound}} = 1. \quad (2)$$

The diffusion coefficient of the pure β -CD in D_2O was similar to that of a β -CD : **1** (1 : 1) solution (see Table 2) therefore small deviations are expected when it is assumed that the diffusion coefficient of a pure 100% β -CD : **1** complexed solution (D_{bound} of the 100% bound solute) is equal to that of a β -CD : **1** in a stoichiometric solution. The diffusion coefficient of the free solute (D_{free}) was obtained from a pure solute solution applying the same experimental conditions to all experiments (15 mmol l^{-1} in D_2O) (Fig. 6, Table 2).

In our previous publications we have observed that the experimental diffusion coefficient of a solute in a solute : chiral selector stoichiometric solution is not only directly proportional to the complexed population but also has a linear correlation to the average apparent binding constant value. Thus, large solute–chiral selector binding constants imply in high percentage of the complexed populations and also in large probabilities of efficient chiral discriminations by RP-HPLC with the same chiral selector. We have further observed that the presence of 60% of adduct in the solute : chiral selector in the solution was indicative of a medium value for binding constants and that in such examples the chiral discrimination could eventually be accomplished when rather different diastereomeric topologies of the adducts were present. The latter information was retrieved from the average ^{13}C NMR signal discrimination. The use of ^{13}C NMR signal complexation-induced chemical shifts enables a more precise evaluation of the enantiospecific binding parameter because they are more geometry dependent than the ^1H NMR signals which are largely influenced by anisotropy and not linearly dependent on the chiral recognition ability. According to these findings the average splitting of the enantiomeric ^{13}C NMR signals of the solute (above 4–5 Hz) in the presence of the chiral selector, used in our previous experiments [9] as a probe of the difference between the two enantiomeric binding constants, in connection with the diffusion coefficients (above 60% of complexed population) would predict an efficient enantiomeric resolution in RP-HPLC with the chiral selector under investigation.

The average ^{13}C NMR signal chemical shifts of ($\pm$)-**1** (solute) in the presence of β -CD (chiral selector) was 2,7 Hz, indicating that both diastereomeric complexes were not topologically very distinct and this datum together with the 62% of complexed population predicted an inefficient chiral recognition.

However the two methodologies (apparent binding constants + complexation-induced chemical shifts at saturation and HR-DOSY + ^{13}C NMR signal splittings) furnished two different predictions. RP-HPLC of ($\pm$)-**1** eluted with a 15 mmol l^{-1} β -CD solution did not show any enantiomeric resolution.

We finally conclude that the classical titration method using ^1H NMR complexation-induced chemical shifts [5,12,17] is not as efficient as the DOSY/ ^{13}C NMR complexation-induced chemical shift method here suggested.

Fig. 7. HR-DOSY spectra: (a) pure sulfoxide (±)-1 in D₂O (15 mmol l⁻¹); (b) complex (±)-1/β-CD (15 mmol l⁻¹).

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