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Comparisons of the interactions between two analytes and two structurally similar chiral stationary phases using high-performance liquid chromatography, suspended-state high-resolution magic angle spinning nuclear magnetic resonance and solid-state nuclear magnetic resonance spectroscopy

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Abstract

A study of the retention behaviour of the enantiomers (*R*)- and (*S*)-1,1′-binaphthyl-2,2′-diol as well as (+) and (−)-*O*,*O*′-dibenzoyl-tartaric acid was performed using the two chiral stationary phases (CSPs) Kromasil-DMB and Kromasil-TBB. Detailed information about the interactions between the analytes and the two CSPs was obtained from suspended-state HR/MAS transferred NOESY NMR experiments as well as suspended-state HR/MAS ^{1}H NMR titration experiments. Good correlation between the suspended-state HR/MAS NMR experiments and the corresponding HPLC experiments was obtained. This shows that suspended-state HR/MAS NMR as well as solid-state CP/MAS NMR spectroscopy can be used to investigate interactions between stationary phases and analytes under conditions that are similar to those used in HPLC.

Keywords: Nuclear magnetic resonance spectroscopy; Chiral stationary phases, LC; Retention mechanisms; Suspended-state high-resolution magic angle spinning nuclear magnetic resonance spectroscopy; Retention behaviour

1. Introduction

Chiral separation of enantiomers by use of a chiral stationary phase (CSP) is an important matter in pharmaceutical industry [1]. Even if a lot of enantioseparations are published the different retention mechanisms involved in the separation processes are not always well understood [2]. The interactions in a chromatographic system take place in the interphase between a solid phase and a mobile phase, hence both the solid structure of the stationary phase and the solvated structure of the analyte must be considered in order to describe the system properly [3]. A drawback of HPLC is that a chromatogram only shows the sum of interactions and not the different contributions to the interaction between a stationary phase and an analyte. However, NMR spectroscopy is nowadays an established method to obtain information about the proximities within a molecule as well as between molecules [4]. It is known that the interactions that take place between analytes and a stationary phase in an HPLC column and in an NMR tube can be described by the equilibrium constant (*K*) [5,6]. Therefore, in principle, a comparison of NMR data and retention factors (*k*) is possible. Several reports show that solution-state ^{1}H NMR spectroscopy can be used to investigate interactions between a CSP and an analyte and thereby extract valuable information of the retention behaviour of an analyte [7–10]. However, one drawback is that both the selector and the analyte are in solution during these measurements and hence a direct comparison to a chromatographic system is difficult to make. It is obvious that solution-state NMR investigations of the pure selector in presence of an analyte do not fully consider the complexity and the impact of the sorbents, for example, the

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problems associated with the immobilization technique and the non-selective silanol interactions from the silica support [11]. It has previously been shown that the use of solid-state and suspended-state HR/MAS NMR spectroscopy gives the possibility to yield reliable information about the interactions between an analyte in solution and a selector immobilised on a support [12–15]. These kinds of experiments are of great interest in the search for new tailored chromatography materials since they mimic a chromatographic situation better than the corresponding solution-state NMR experiment.

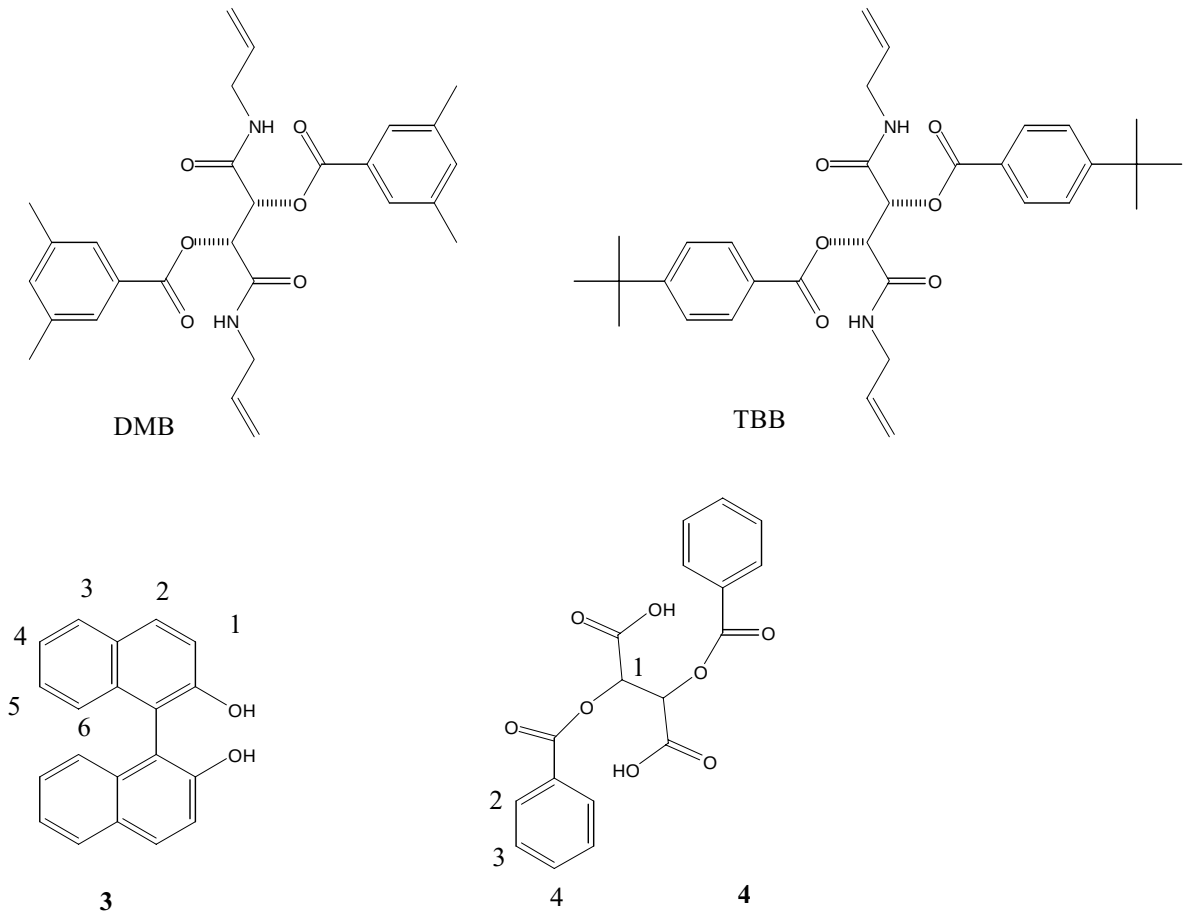
The two CSPs *N*,*N*′-diallyl-L-tartardiamide *bis*-(4-*tert*-butylbenzoate) (TBB) and *N*,*N*′-diallyl-L-tartardiamide *bis*-(3,5-dimethylbenzoate) (DMB), covalently bonded to Kromasil silica and crosslinked, have been used to separate enantiomers in analytical scale as well as in preparative scale [16,17]. It has been reported that the selectivity of (*R*)- and (*S*)-1,1′-binaphthyl-2,2′-diol obtained from Kromasil-DMB ($\alpha = 1.91$) is superior to the one obtained using Kromasil-TBB ($\alpha = 1.23$) during the same experimental conditions. It is apparent that the change from the two 3,5-dimethyl benzoyl groups to the two *tert*-butyl benzoyl groups on the selector has a significant effect on the chiral separation [16,17]. An investigation how the interactions take place and which interactions between the selectors and analytes that are important could reveal information useful for further developments of new CSPs.

Here, an extensive study of the different enantiodiscriminating abilities of Kromasil-TBB and Kromasil-DMB is presented, dealing with the retention behaviour of the enantiomers (*R*)- and (*S*)-1,1′-binaphthyl-2,2′-diol as well as (+) and (−)-*O*,*O*′-dibenzoyl-tartaric acid (see Scheme 1). Also, it is shown that by using a combination of HPLC and different suspended-state HR/MAS NMR experiments, a deeper understanding of the interactions between enantiomers and immobilized chiral selectors can be achieved.

2. Experimental

2.1. Materials

The Kromasil-DMB and Kromasil-TBB sorbents packed into HPLC columns as well as free for the suspended-state HR/MAS NMR experiments were a kind gift from EKA Chemicals AB (Bohus, Sweden). The amount of selector immobilized for Kromasil-DMB was 0.276 mmol/g silica gel and 0.270 mmol/g silica gel for Kromasil-TBB. (*R*)- and



Scheme 1.

(*S*)-1,1′-binaphthyl-2,2′-diol (>99%) were obtained from Merck and (+) and (−)-*O*,*O*′-dibenzoyl-tartaric acid (>99%) were obtained from Fluka.

All solvents used in the HPLC experiments were of HPLC grade (LiChrosolve, Merck). The NMR solvents used were $CDCl_3$ (99.95%), cyclohexane-d_{12} (C_6D_{12}) (>99%) and 2-propanol-d_8 (>99%), all obtained from Merck.

Stock solutions of (*R*)- and (*S*)-1,1′-binaphthyl-2,2′-diol for the suspended-state HR/MAS trNOESY experiments were prepared by dissolving the respective analyte (0.75 mg) in a solvent mixture (300 μl) composed of 2-propanol-d_8 (5%) in C_6D_{12}. Each of the enantiomer stock solutions (60 μl) was added with a syringe to Kromasil-DMB (9.9 mg) in a ZrO_2 suspended-state rotor. Also a suspended-state HR/MAS trNOESY NMR experiment using the (*R*)-1,1′-binaphthyl-2,2′-diol stock solution (60 μl) added with a syringe to vinyl silica (Kromasil) (9.9 mg) was performed. For the HR/MAS NOESY NMR experiment (*S*)-1,1′-binaphthyl-2,2′-diol stock solution (64 μl) was added to a ZrO_2 suspended-state rotor. In the suspended-state HR/MAS trNOESY experiments using the (*R*)- and (*S*)-1,1′-binaphthyl-2,2′-diol dissolved in 2-propanol-d_8 (5%) in C_6D_{12} the selector/analyte ratio (S/A) was 5.2. The S/A ratio was calculated with respect to the molar amounts, calculated from the degree of immobilized selector and the analyte stock solution concentration. The different S/A ratios in the experiments were adjusted by adding different amounts of the respective sorbent to the suspended-state rotor or dilution of the stock solutions.

Stock solutions for the suspended-state HR/MAS NMR titration experiments were prepared by dissolving (*R*)- and (*S*)-1,1′-binaphthyl-2,2′-diol (0.5 mg, respectively) in $CDCl_3$ (750 μl). The same concentrations of stock solutions were used for the NOESY and suspended-state HR/MAS trNOESY experiments with Kromasil-DMB (10.3 mg) in $CDCl_3$. After one suspended-state HR/MAS NMR titration experiment, with respective analyte and stationary phase, these stock solutions (590 μl) were further diluted with $CDCl_3$ (750 μl) in order to change the selector/analyte ratio.

A mixture of (+)-*O*,*O*′-dibenzoyl-tartaric acid (0.30 mg) and (−)-*O*,*O*′-dibenzoyl-tartaric acid (0.19 mg) was dissolved in $CDCl_3$ (0.75 ml). This stock solution (60 μl) was added to different amounts of Kromasil-TBB (2.4 and 5.9 mg, respectively) as well as to Kromasil-DMB (2.6 mg).

2.2. Chromatography

Analytical liquid chromatography was performed with the use of an equipment composed of a Merck-Hitachi 6200 A solvent delivery pump and a Merck-Hitachi L-4000 A variable wavelength UV detector. The columns used were of the dimensions 250 mm × 4.6 mm.

Samples were introduced via a Rheodyne injector equipped with a 20 μl loop. All solvents used were of HPLC grade. The void volume was determined from the solvent front. The mobile phases used during the separations were a mixture of 2-propanol (5%) in cyclohexane or neat chloroform, all analytes were dissolved in 2-propanol or chloroform. The flow used during the experiments was 1 ml/min.

2.3. Solid-state ^{13}C CP/MAS NMR

All ^{13}C CP/MAS NMR spectra have been recorded on a Bruker ASX 300 Spectrometer (7.05 T) at a spinning rate of 4000 Hz with 7 mm double bearing rotors of ZrO_2. The proton 90° pulse length was 6 μs and the temperature 295 K. The spectra were obtained with a cross-polarisation contact time of 3 ms. The pulse intervals were 1 s. Glycine was used as a reference and to adjust the Hartmann–Hahn condition. The number of scans recorded was 2048 in each experiment.

2.4. Suspended-state HR/MAS NMR

All suspended-state HR/MAS NMR spectra have been recorded on a Bruker 400 MHz spectrometer at a spinning rate of 4500 Hz with a 4 mm double bearing rotor of ZrO_2. For each spectrum a minimum of 512 scans were recorded, but in order to guarantee sufficient signal to noise when a lower concentration of analyte was used, up to 2048 scans were recorded. The 90° pulse was set to 8.5 μs. The analyte was dissolved in $CDCl_3$ to different concentrations and added to the sorbent with a syringe. Either the chloroform peak at 7.26 ppm or the cyclohexane peak at 1.38 ppm was used as a reference. All spectra were recorded at 300 K.

All suspended-state HR/MAS trNOESY spectra were recorded on a Bruker 400 MHz spectrometer at a spinning rate of 4500 Hz with a 4 mm double bearing rotor of ZrO_2. The total amounts of increments were 512 and in each increment 76 scans were recorded. The 90° pulse was set to 8.5 μs. A delay of 1.5 s was used before a new transient was recorded. The NOESY spectra with the analytes without any stationary phase were recorded with a mixing time of 900 ms. In the suspended-state HR/MAS trNOESY experiment the mixing time was set to 100 ms to ensure that mainly trNOE peaks were recorded. All NOESY spectra were processed with 2 K data points in the F2 and 512 increments in the F1 dimension and 512 data points zero filling.

3. Results and discussion

Fig. 1 shows that the separation of (*R*)- and (*S*)-**3** obtained by Kromasil-DMB is superior to the separation obtained by Kromasil-TBB when a mobile phase composition of 2-propanol (5%) in cyclohexane was used.

From a comparison of the resolutions, obtained in different mobile phase compositions, it is seen that an increase of the polarity of the mobile phase will reduce both the k and the α values (Table 1).

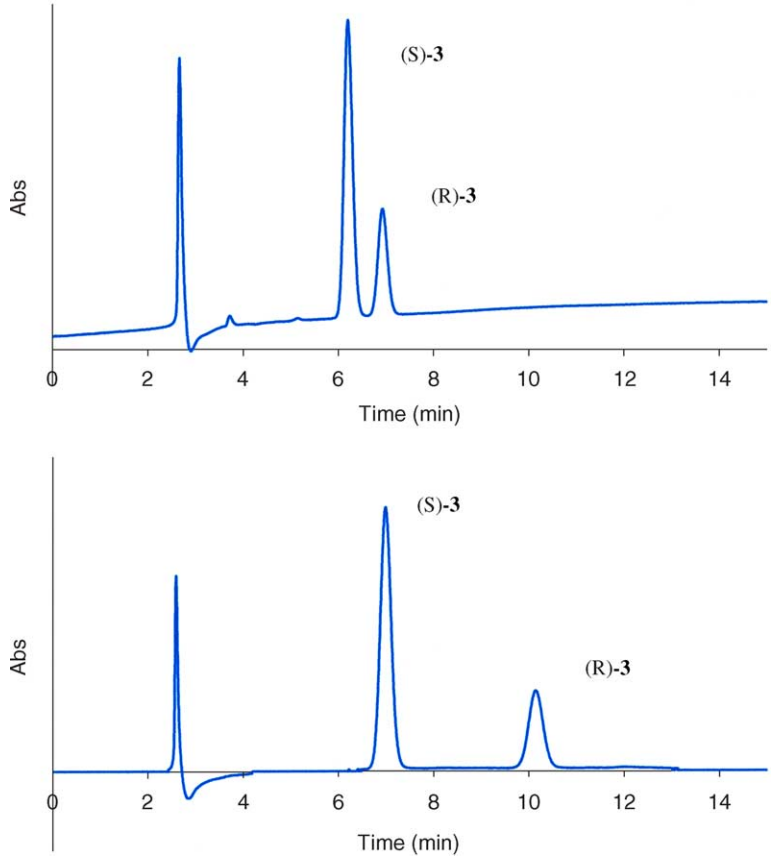


Fig. 1. Separation of (*R*)- and (*S*)-**3** when Kromasil-TBB (upper chromatogram) or Kromasil-DMB (lower chromatogram) are used as stationary phases with 2-propanol (5%) in cyclohexane as a mobile phase, flow rate = 1 ml/min.

It is evident, from Table 1, that Kromasil-DMB separates (*R*)- and (*S*)-**3** with a higher α-value than Kromasil-TBB under all the used mobile phase conditions. Due to stronger interactions, between (*R*)-**3** and the CSPs, (*S*)-**3** will always elute prior to (*R*)-**3**.

3.1. Suspended-state HR/MAS NOESY and trNOESY NMR spectroscopy

An extensive NMR investigation was done in order to examine the reasons for this significant difference in selectivity. The sorbents used in the suspended-state HR/MAS NMR experiments were from the same batches as the one used in the chromatographic experiments. Both TBB and DMB were immobilized to approximately the same degree on vinyl silica and therefore a direct comparison of the two sorbents, with respect to the *k* values from the HPLC experiments as well as the NMR data, are possible. Since the solvent polarity neither changed the elution order or the difference in magnitude of the resolutions between the two CSPs, both chloroform and 2-propanol/cyclohexane mixtures were used in the NMR and HPLC experiments.

Table 1
Retention and selectivity factors for (*R*)- and (*S*)-**3** eluted from Kromasil-DMB or Kromasil-TBB using different mobile phases, flow = 1 ml/min

Mobile phase	Kromasil-DMB			Kromasil-TBB		
	$k_{(S)}$	$k_{(R)}$	α	$k_{(S)}$	$k_{(R)}$	α
CSP						
2-Propanol (5%)/ hexane	2.22	4.33	1.95	2.95	3.64	1.23
2-Propanol (5%)/ cyclohexane	1.70	2.91	1.72	1.33	1.60	1.20
Chloroform	0.05	0.22	–[a]	0.20	0.30	–[a]

[a] The enantiomers were baseline separated but due to the low *k* the α values are not representative.

First, a complete signal assignment of analyte **3** was obtained by performing solution-state ^{1}H, ^{13}C, NOSEY and HSQC NMR experiments in both $CDCl_3$ as well as 2-propanol-d_8 (5%) in C_6D_{12}. A NOESY NMR experiment of (*S*)-**3** with a mixing time of 900 ms was recorded in a suspended-state NMR rotor. The spectra showed, as expected, only positive NOE crosspeaks between the aromatic signals in (*S*)-**3**. Also, a suspended-state HR/MAS trNOESY spectra with a mixing time of 100 ms of (*S*)-**3** dissolved in $CDCl_3$ in presence of vinyl silica was recorded. This experiment showed also only positive crosspeaks between the aromatic signals. It is known that a molecule with a low molecular weight (<1000 Da) will result in a positive NOE and a molecule with a high molecular weight (>1000 Da)

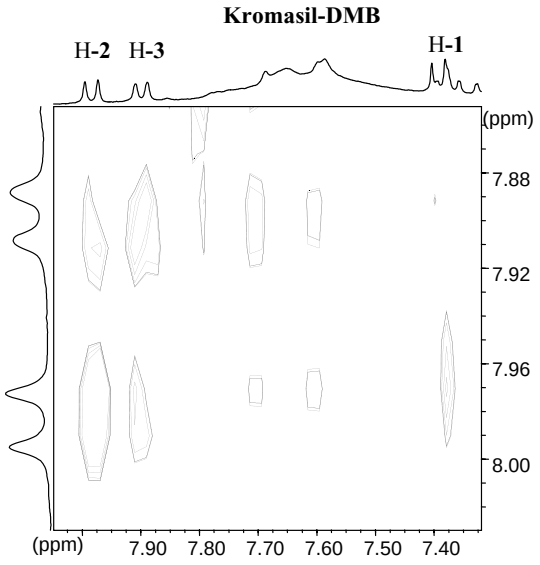


Fig. 2. Suspended-state HR/MAS trNOESY spectrum of (*S*)-**3** dissolved in $CDCl_3$ (2.47 mM) in presence of Kromasil-DMB (10.4 mg), S/A = 19.4.

will result in a negative NOE due to their differences in correlation times (τ_c). However, a small analyte that is interacting with a large molecule will obtain the correlation time of the large molecule and hence show a negative NOE [18–20]. Therefore, the observed positive NOE crosspeaks shows that no interactions are taking place between the vinyl silica and the analyte. Thereafter, a series of suspended-state HR/MAS trNOESY spectra were recorded to investigate how the analytes interacts with Kromasil-DMB. From the suspended-state HR/MAS trNOESY measurement of (*S*)-**3** dissolved in $CDCl_3$ significant negative trNOESY crosspeaks between H-**2** and H-**3** as well as H-**2** and H-**1** were observed (see Fig. 2). The change from positive crosspeaks, obtained in the experiment without Kromasil-DMB, to negative crosspeaks suggests that the analyte interacts with the stationary phase.

It can further be seen that the naphthyl groups in (*S*)-**3** have close proximities to the benzoyl groups in Kromasil-DMB due to the negative crosspeaks between H-**2** and H-*para* (7.60 ppm) as well as H-**2** and H-*ortho* (7.70 ppm). The same proximities, but stronger, are observed between H-**3** and the aromatic system in the selector as well. Therefore it is believed that either steric or π–π interactions are taking place between the aromatic system in analyte **3** and the aromatic groups in DMB. However, when (*R*)-**3** was examined in presence of Kromasil-DMB, only a week crosspeak between H-**1** and the CSP was observed, besides the negative intramolecular trNOESY crosspeaks.

A series of suspended-state HR/MAS trNOESY experiments with either (*R*)- or (*S*)-**3** dissolved in a mixture of 2-propanol-d_8 (5%) in C_6D_{12} using Kromasil-DMB or vinyl silica as sorbents were performed. The trNOESY crosspeak between H-**1** and H-**2** was of particular interest since no interfering signals were present to disturb the integration (see Fig. 3). The magnitude of the trNOESY crosspeak between H-**1** and H-**2** was normalised to the diagonal peak and then compared between the respective enantiomers. This showed that the (*R*)-**3** trNOESY crosspeak (−0.35) is significantly stronger than (*S*)-**3** (−0.27), showing that (*R*)-**3** interacts more strongly than (*S*)-**3** with Kromasil-DMB. These NMR data are consistent with the chromatographic elution order of (*R*)- and (*S*)-**3** eluted from the Kromasil-DMB HPLC column.

3.2. Suspended-state ^1H HR/MAS T_1 relaxation measurements

The T_1 relaxation process for protons is known to be faster when the numbers of relaxation pathways are increased. Therefore, ^1H T_1 measurements has often been used to map proximities between molecules [18]. The T_1 values for the NMR signals of (*R*)- and (*S*)-**3** (H-**1**, H-**2**, H-**3**, H-**4** and H-**6**) were measured using the inversion-recovery pulse sequence, the results are shown in Table 2. From a comparison

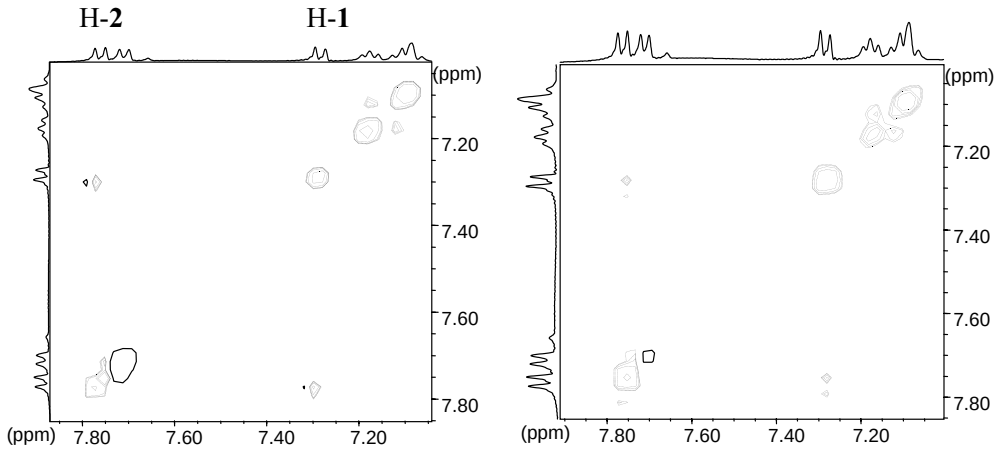


Fig. 3. Suspended-state HR/MAS trNOESY spectra of (*R*)-**3**, left and (*S*)-**3**, right, dissolved in 2-propanol-d_8 (5%) in C_6D_{12} (8.7 mM) with an addition of Kromasil-DMB (9.9 mg), S/A = 5.2.

Table 2
Suspended-state HR/MAS NMR T_1 measurements on (*R*)- or (*S*)-**3** in presence of Kromasil-DMB using 2-propanol-d_8 (5%) in C_6D_{12} as solvent, S/A = 5.2

Analyte signal	T_1 values for (*R*)-**3** (s)	T_1 values for (*S*)-**3** (s)
H-**1**	1.7	2.3
H-**2**	1.4	1.9
H-**3**	1.4	1.9
H-**4**	1.5	1.9
H-**6**	1.6	2.2

of the T_1 values it is seen that significantly shorter values are observed for the (*R*)-**3** than for (*S*)-**3** indicating closer proximities to Kromasil-DMB. Therefore a stronger interaction between (*R*)-**3** and Kromasil-DMB is taking place. It can further be seen that the longest T_1 values are for the signals H-**6** and H-**1** indicating less relaxation possibilities compared to the other signals, this is most certainly an effect of the nature of the probe.

3.3. Suspended-state ^1H HR/MAS NMR titration experiments

From the suspended-state ^{1}H HR/MAS NMR titration experiments, seen in Fig. 4, a severe broadening of the signal at 5 ppm, corresponding to the hydroxyl groups in analyte (*S*)-**3**, can be seen when Kromasil-DMB is added to the analyte solution. This broadening is due to a hydrogen bonding interaction between the selector molecule and the hydroxyl group in the analyte.

No significant broadening of the hydroxyl group in (*R*)-**3** (2.3 mM) was observed when vinyl silica (9.8 mg) was added to the analyte solution. This proves that no hydrogen bonding takes place between the support and the analyte. These results are in accordance with the suspended-state HR/MAS trNOESY NMR measurements of (*S*)-**3** in presence of vinyl silica. It is therefore suggested that the analyte and the stationary phase are interacting via a hydrogen bond.

The broadening of the hydroxyl group is observed in both (*S*)-**3** and (*R*)-**3** when Kromasil-DMB as well as Kromasil-TBB is added. The peak broadening is clearly increased when the selector/analyte ratio is increased (see Table 3), which most certainly is due to a higher amount of complexed analyte, i.e. a shift of the equilibrium. However, in the corresponding HPLC situation the S/A ratio will of course be even higher, due to the consecutive formation of new diastereomeric complexes when the analyte is passing through the column. It is also seen that the broadening of the hydroxyl peak is more pronounced when Kromasil-DMB is used compared to Kromasil-TBB. This strongly indicates that the hydrogen bonding interaction is stronger between the DMB selector and analyte **3** than between the TBB selector and analyte **3**. From Table 3 it can further be seen that the broadening caused by Kromasil-DMB seems

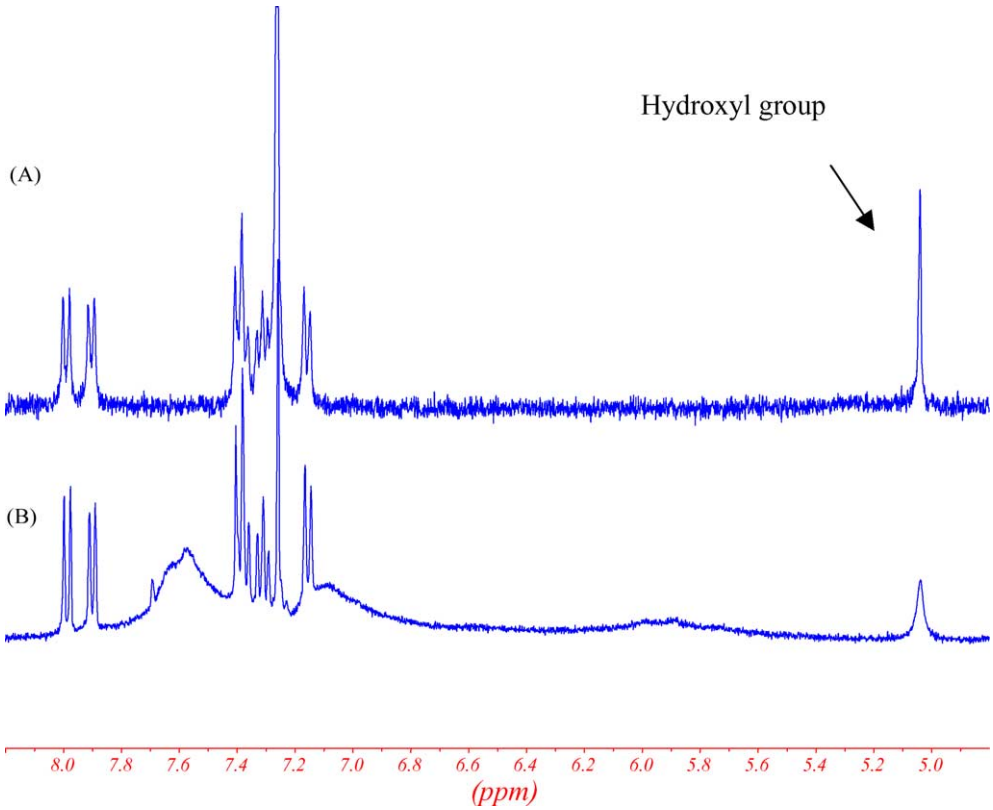


Fig. 4. Suspended-state ^{1}H HR/MAS NMR of (*S*)-**3** dissolved in $CDCl_3$ in presence of Kromasil-DMB at 300 K: (A) free (*S*)-**3** (2.8 mM); (B) (*S*)-**3** (2.8 mM) in presence of Kromasil-DMB (4.7 mg), S/A = 7.9.

Table 3
The half height peak broadening ($W_{1/2}$) of the hydroxyl group signal in (*S*)- or (*R*)-**3** obtained from suspended-state ^{1}H HR/MAS NMR measurements in presence of Kromasil-DMB or Kromasil-TBB, suspended in $CDCl_3$, at a given selector/analyte molar ratio

CSP	Analyte	S/A	$W_{1/2}$ (Hz)
–	**3**	–	3.5
Kromasil-DMB	(*S*)-**3**	8	11
Kromasil-DMB	(*S*)-**3**	20	16
Kromasil-DMB	(*S*)-**3**	91	[a]
Kromasil-DMB	(*R*)-**3**	8	15.3
Kromasil-DMB	(*R*)-**3**	31	19.7
Kromasil-DMB	(*R*)-**3**	79	[a]
Kromasil-TBB	(*S*)-**3**	9	5
Kromasil-TBB	(*S*)-**3**	24	6.2
Kromasil-TBB	(*S*)-**3**	72	9
Kromasil-TBB	(*R*)-**3**	8	5
Kromasil-TBB	(*R*)-**3**	25	5
Kromasil-TBB	(*R*)-**3**	82	13

[a] Not measurable due to its broadness.

to be slightly more pronounced for (*R*)-**3** than (*S*)-**3** at a given S/A ratio. This result is also in accordance with the chromatographic retention order.

^{1}H NMR titration experiments of a mixture of (±)-**4** in the presence of TBB or DMB either as free selectors, in solution, or immobilised on silica, in a suspension, was performed to investigate the role of the support. From previously published chromatographic separations of (±)-**4** on Kromasil-TBB a severe tailing was observed, this proves the analyte's sensitivity for silanol interactions with the sorbent [8]. In the suspended-state ^{1}H HR/MAS NMR spectra of (±)-**4** dissolved in $CDCl_3$ the methine signal (1) at 6.0 ppm is observed as a singlet (Fig. 5). However, in the presence of Kromasil-TBB a splitting of this signal is observed. This splitting of the methine signals is due to the formation of two different diastereomeric complexes between the enantiomer analytes and the selector [7].

Table 4
Chemical shift differences ($\Delta\delta$), observed at signal 1, of (±)-**4** in presence of TBB or DMB, both free and immobilized, obtained from ^{1}H NMR measurements using $CDCl_3$ as solvent, both in solution-state and suspended-state

CSP	S/A	$\Delta\delta$ (Hz)
Kromasil-TBB	5.9	6
Kromasil-TBB	14.6	14
TBB[a]	5.6	15.6
Kromasil-DMB	6.7	4
DMB[a]	5.8	6.8

[a] Previously published.

It is clearly shown that the difference in chemical shifts ($\Delta\delta$) between the two diastereomeric complexes increases when more selector is added (Table 4). This increase in $\Delta\delta$ is caused by a shift of the equilibrium, i.e. more diastereomeric complexes are formed. From a comparison of the free and immobilized selectors, for a comparable S/A ratio, it can be seen that the $\Delta\delta$ is reduced when the selectors are immobilized on silica. This is most certainly an effect of an increase in non-selective interactions between the analyte

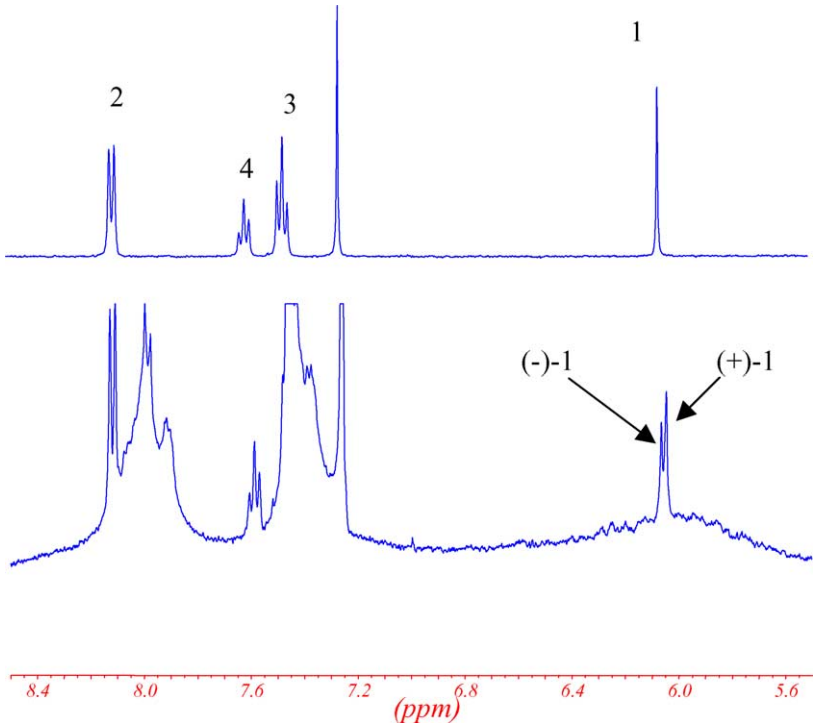
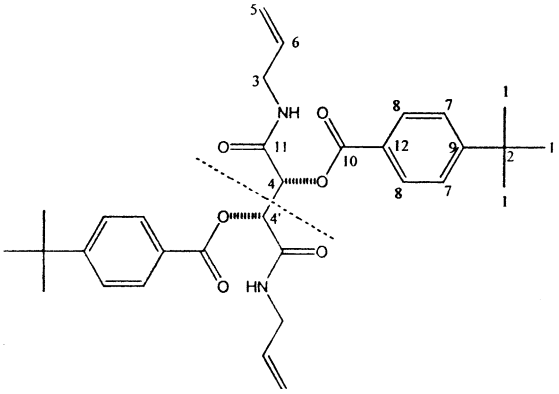


Fig. 5. Suspended-state ^{1}H HR/MAS NMR spectra of (±)-**4** in $CDCl_3$ (1.8 mM): (A) without Kromasil-TBB; (B) with addition of Kromasil-TBB (2.4 mg), S/A = 5.9.

Table 5
Comparison of the ^{13}C NMR chemical shifts of TBB obtained from solution-state and solid-state NMR measurements



Signal	Chemical shift in solution (ppm)	Chemical shift in solid-state (ppm)
1	31.1	31.2, 32.6
2	35.2	35.0
3	41.9	41.0, 42.7
4	73.1	74.1, 75.9
5	116.7	115.8, 117.8
6	133.4	134.0
7	125.7	124.6, 127.7
8	130.0	130.5, 131.7
9	157.7	157.1
10	165.0	165.8
11	166.3	165.8
12	126.0	126.8

and the silanol groups present in the sorbent. This conclusion is supported by previously performed chromatographic experiments [8].

3.4. Comparison of the NMR chemical shift data obtained in the solid-state and solution-state

From a comparison of the ^{13}C NMR chemical shift data obtained from the spectra of the monomer selectors, recorded in solution-state as well as in solid-state, differences in the selector structures were revealed (see Table 5). It can be seen that most of the signals in the TBB monomer structure are doublets when the solid-state NMR spectra is recorded.

This implies that different conformations may exist for the solid structure of TBB. This is most likely due to π–π stacking and/or restrictions in rotation around the C_2 symmetry axis [21]. The magnetic non-equivalence of signals 4 shows that the C_2 symmetry is broken close to the two chiral centres in TBB in the solid-state. No such splitting of the corresponding methine carbons can be observed in the solid-state ^{13}C NMR spectra of DMB, indicating that no or only small conformationally changes around the chiral centres are taking place. However, the aromatic signals are resolved in the solid-state ^{13}C NMR spectra of DMB as well as TBB. It can therefore not be neglected that the reduction of $\Delta\delta$ between the two diastereomeric complexes of (±)-**4** and TBB or DMB, observed in the titration experiments, could be caused by the conformational change around the chiral centres when the selector is immobilized on silica. Further suspended-state HR/MAS trNOESY measurements are in progress to perform a more detailed investigation of the reasons for these observations.

4. Conclusions

It has been proposed that the hydroxyl groups in (*R*)- and (*S*)-**3** interacts with both Kromasil-TBB and Kromasil-DMB through a hydrogen bonding. This hydrogen bonding is stronger with Kromasil-DMB than Kromasil-TBB. Further on, NOE revealed steric or π–π interactions between the naphthyl groups in analyte **3** and the different aromatic groups on the DMB selector. Both trNOESY experiments and T_1 measurements in the suspended-state of (*R*)- and (*S*)-**3** in presence of Kromasil-DMB confirmed the chromatographic elution order of the enantiomer analytes. It is shown that suspended-state HR/MAS NMR spectroscopy can provide valuable information about interactions between analytes and CSPs.

Suspended-state HR/MAS NMR titration experiments showed that different diastereomeric complexes are formed between the two sorbents and (±)-**4**.

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