Research Article

Magnetic Resonance in Chemistry

Received: 1 September 2009 Revised: 28 October 2009 Accepted: 30 October 2009 Published online in Wiley Interscience: 30 November 2009

(www.interscience.com) DOI 10.1002/mrc.2551

Characterization of binding affinities in a chromatographic system by suspended state HR/MAS NMR spectroscopy

Volker Friebolin, Silvia Marten and Klaus Albert*

In the current work a racemate of (*R*)- and (*S*)-benzylmandelate was separated with a stereoselective polysaccharide-based chiral stationary phase by HPLC. To elucidate the occurring chiral molecular recognition processes in the heterogeneous system used, NMR spectroscopy was chosen under high resolution/magic angle spinning (HR/MAS) NMR conditions in the suspended state. Therefore, and as a proof of concept, a combination of several NMR methods such as spin–lattice relaxation time (T_1) measurements (T_1), the saturation transfer difference, and the 2D experiment of the transferred nuclear overhauser enhancement spectroscopy technique were applied. With HR/MAS NMR it is feasible to combine NMR and chromatography to achieve further insights into the separation process.

Keywords: HPLC; chiral stationary phase; ^{1}H HR/MAS NMR; spin–lattice relaxation time (T_1); saturation transfer difference; transferred NOESY

Introduction

Throughout the last few decades, separations of racemic mixtures in liquid chromatography or in capillary electrophoresis[1] have been of increasing interest in the development of new drugs. Today, a large variety of chiral stationary phases (CSPs) with different synthetic, semi-synthetic, or naturally occurring chiral selectors exist.[2,3] For instance, macrocyclic antibiotics (e.g. vancomycin), pirkle-type selectors, molecular imprinted polymers, or polysaccharide derivatives (cellulose or amylose) are commonly used as CSPs selectors.[1,4]

The separation by HPLC is based on the formation of more or less stable diastereomeric complexes of the enantiomers and the CSP.[5] For the discovery of new enantioselective tailor-made column materials and to better understand the chromatographic retention mechanism, knowledge about intermolecular interactions forces like hydrogen bonding, $\pi-\pi$, dipole–dipole, or electrostatic (Coulomb) forces is crucial. To study such spatial interactions, several different NMR techniques have been applied. However, those experiments were commonly performed in the liquid state, resulting in narrow lines of the recorded resonances. In contrast, the investigated chromatographic system consists of a suspension of a solid CSP based on silica gel and an analyte solution, which is bound to the sorbent material or dissolved in an added solvent. Hence, NMR spectra of suspensions consist of broad signal resonances, due to e.g. chemical shift anisotropy or dipole–dipole interactions.

^{1}H high resolution NMR spectroscopy combined with magic angle spinning (^{1}H HR/MAS) reduces such line broadening effects thus resulting in better resolved spectra, possibly contributing to the elucidation of recognition processes at the binding site of the CSP surface.[6,7] Additionally, a solvent added to the sample mixture further decreases susceptibility effects leading to a better analysis of the recorded spectra.[8–12]

As stated previously, transient ligand–receptor complex formation results in a different dynamic and relaxation behavior of protons in the diastereomeric complex and free in solution.[6,7,13] In this complex, ligand protons that interact with the CSP material will probably gain the relaxation behavior of those of the receptor due to restricted molecular motions yielding in a shorter spin–lattice relaxation time T_1.[5,14] Hence, as an indication of a different binding affinity of both enantiomers toward the receptor molecules, the measurement of T_1 can be used for screening purposes.

To study intermolecular interactions in host–guest complexes, Meyer *et al*. used the saturation transfer difference (STD) NMR method.[15] In our approach we adopted this technique to HR/MAS and to the investigated suspension consisting of benzylmandelate dissolved in an added solvent together with a CSP material. After selective irradiation of CSP receptor protons the magnetization spreads by effective spin diffusion over the whole macromolecule. By cross-relaxation processes the saturation will be transferred in the diastereomeric complex to the analyte, in our case to the bound enantiomer. This results in a partial saturation of protons of the enantiomer decreasing the signal intensities, especially of those protons that are in close contact to the irradiated CSP receptor.[9,16,17]

In addition, the nuclear overhauser effect (NOE) can be used to detect through-space interactions. Principally, low molecular weight compounds show a different NOE behavior compared to macromolecules (e.g. CSP).[8,18–20] The NOE enhancement of large receptor molecules builds up more quickly compared to small an-

* *Correspondence to: Klaus Albert, University of Tuebingen, Institute of Organic Chemistry, Auf der Morgenstelle 18, 72076 Tuebingen, Germany. E-mail: klaus.albert@uni-tuebingen.de*

University of Tuebingen, Institute of Organic Chemistry, Auf der Morgenstelle 18, 72076 Tuebingen, Germany

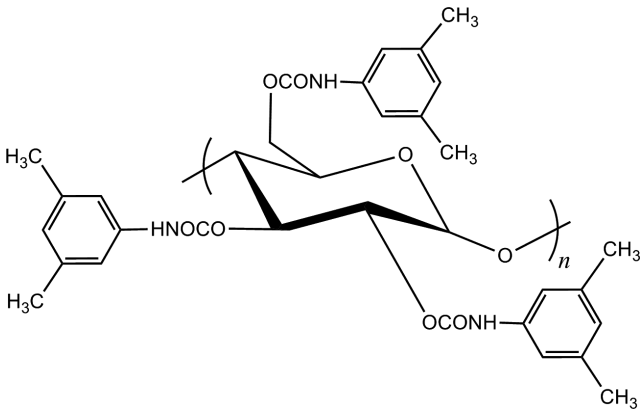

Figure 1. Structure of the chiral selector on the silica surface upon which the amylose tris (3,5-dimethylphenylcarbamate) CSP is based.

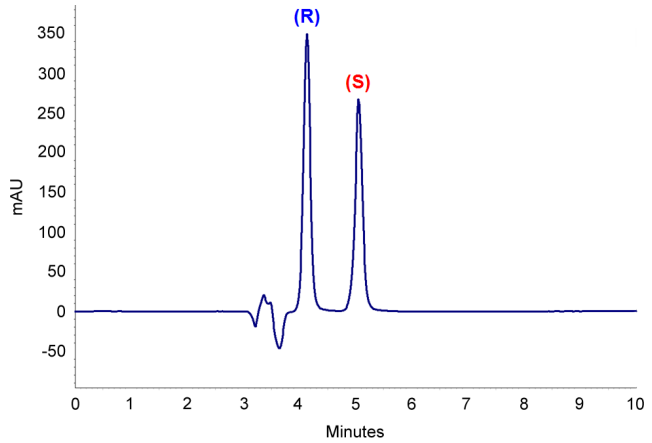

Figure 2. Chromatogram of the enantioseparation of (*R*)/(*S*)-benzylmandelate (benzyl-2-hydroxy-3-phenylpropionate) on the amylose tris (3,5-dimethylphenylcarbamate)-based CSP. For additional chromatographic conditions, see Experimental Section.

alytes due to fast cross-relaxation and short spin–spin relaxation. In the complex, the NOE effect of the bound analytes is dominated by the NOE behavior of the receptor resulting in different signs of the NOE cross-peaks in a 2D experiment. Although cross-peaks are positive or weak negative for free analytes in solution, they alter to strong negative by ligand–receptor complex formation. The resulting effects depend on the size and lifetime of the complex, the NOE buildup speed, represented by the mixing time τ_m in the NOE experiment, and the correlation time τ_c of both free and bound compounds.[20] Such changes of cross-peak signal intensities caused by complexation and intermolecular magnetization transfer can be measured in a 2D transferred nuclear overhauser enhancement spectroscopy (trNOESY) experiment and give valuable information about the binding process.[8]

In the present approach, we used a combination of T_1 measurements, STD, and the trNOESY NMR technique under HR/MAS NMR conditions to characterize molecular recognition processes in the investigated heterogeneous system consisting of a CSP with an amylose tris(3,5-dimethylphenylcarbamate) receptor and (*R*)- and (*S*)-benzylmandelate enantiomers.

Experimental Section

Materials

Both (*R*)- and (*S*)-benzylmandelate (benzyl-2-hydroxy-3-phenylpropionate) (Figs 3 and 4) with a purity of 99% as well as 1,3,5-tris-*tert*-butylbenzene (TTBB) as HPLC dead volume marker were obtained from Aldrich, Milwaukee, USA. Trifluoroacetic acid (TFA) was purchased from VWR, Darmstadt, Germany. Methanol was obtained from Merck, Darmstadt, Germany. The phenylcarbamate derivative of amylose was prepared by the reaction of hydroxyl groups of amylose with an excess of 3,5-dimethylphenylisocyanate in dry pyridine at 100 °C.[21,22] The preparation of the CSP consists of a coating process via adsorption of the chiral selector on a suitable silica phase.[23] The amylose tris(3,5-dimethylphenylcarbamate) and silica based CSP used had a particle size of 3 µm and a pore diameter of 1000 Å with an approximate surface area of 25 m^2/g. The coverage on the silica surface was about 20%. Figure 1 shows the chemical structure of the chiral selector immobilized on the stationary phase. The CSP was packed in a Vertex 250 mm × 4.6 mm (I.D.) stainless steel column from Knauer GmbH, Berlin, Germany.

For the NMR experiments, methanol-d_4 with a HDO content of less than 0.03% was received from Euriso-Top, France. TFA-d_1 with a purity of more than 99.5% was obtained from Fluka AG, Switzerland.

HPLC method

HPLC measurements were carried out on a Knauer Smartline System equipped with a Smartline Manager 5000 with degasser and low pressure gradient unit, a Smartline Pump 1000, Smartline PDA Detector 2800, and a Smartline Column Oven. The racemic sample solution was introduced via the Smartline autosampler 3950, and the chromatograms obtained were analyzed by ChromGate Software. A separation of the benzylmandelate enantiomers was performed isocratically by using the CSP with methanol acidified with 0.1% TFA at a flow rate of 1 ml/min. The separation was monitored by UV at 210 nm at 25 °C (Fig. 2). All analytes were preliminarily dissolved in methanol. A measured quantity of 10 µl of the sample was injected and peak identification in the chromatogram was realized by single enantiomer injection under the same HPLC conditions. Suppression of the benzylmandelate dissociation in the presence of TFA and the minimization of ionic interactions with active surface silanol groups of the CSP silica were detected due to a weaker retention compared to a nonmodified eluent. The retention factor k' that represents a measure of the migration rate of the two compounds was calculated by $k' = (t_r - t_0)/t_0$, wherein t_r represents the retention time of the analyte and t_0 the elution time of a nonretained compound. Determination of t_0 was realized by injecting TTBB, a suitable marker for the determination of the void volume.[24] TTBB has no retention on the investigated polysaccharide stationary phase in normal phase mode or in polar organic mode. The elution time observed with TTBB shows comparable results of void volume determined by the method of minor disturbance. The enantioselectivity α of the CSP, as a measure of the separation of the two enantiomers, was obtained by $\alpha = k'_{(S)}/k'_{(R)}$.[5]

HR/MAS NMR spectroscopy in the suspended state

^{1}H HR/MAS NMR spectra of the benzylmandelate/CSP suspension were recorded on a Bruker ARX 400 MHz spectrometer at a spinning rate of 6 kHz in 4 mm double-bearing ZrO_2 rotors. The spectrometer was equipped with a deuterium lock setup, which was set on the resonance frequency of methanol-d_4. The 90° pulse length was set to 10.5 µs. The spectra were recorded at 25 °C

and multiplied by an exponential line broadening function of lb = 0.5 Hz prior to Fourier transformation. Spectra processing was performed using Bruker TOPSPIN 2.0 software. Broad background signals of the stationary phase were reduced by using a weak spin lock pulse ($T_{1\rho}$ filter) with a duration of 200 ms and an attenuation of 30 dB to eliminate both transverse and longitudinal magnetization of the receptor macromolecule. Resonances of the free analyte were not affected considerably. Signal assignment in the recorded spectra was accomplished using a 2D $^{1}H-^{1}H$-COSY–NMR spectrum under HR/MAS conditions. The rotor was filled with 80 µl of the mobile phase with or without a 0.1 M solution of the isolated enantiomers in methanol-d_4 (with 0.1% deuterated TFA) and 10 mg of the CSP material. The ratio between the given analyte to receptor was determined using different amounts (5–15 mg) of the column material, but the results were not affected considerably.[8]

Spin–lattice relaxation times (T_1) were measured with the inversion recovery experiment. The time interval between the 90° and 180° pulses was varied from 0.01 to 20 s with a relaxation delay of at least 60 s between 16 experiments. The measurements were carried out several times and the deviation is estimated to be ±0.1 s.

To obtain 1D STD NMR spectra, the selective saturation of the target CSP material was achieved by a series of 40 shaped 270° Eburb-1 pulses of 50 ms length, separated from each other by a 30 ms delay yielding to a total saturation time of about 2 s.[9,25–27] The STD parameters, including the length and power attenuation of the $T_{1\rho}$ filter as well as the shape of the selective irradiation pulses used were optimized to fit to the investigated system. In total, 4k transients were recorded. Subtraction of a spectrum with receptor saturation (ON-resonance) from one without (OFF-resonance) leads to the STD spectrum only containing analyte signals with binding affinities to the CSP material. This subtraction was achieved internally by appropriate phase cycling after each scan. The irradiation in the ON-resonance spectrum with saturation of CSP signals was set to 340 Hz (~1 ppm). The OFF-experiment uses the same saturation pulses but with an irradiation more than 1700 Hz away from any ligand resonances, thus avoiding e.g. temperature differences due to different energy contents in the system between the ON- and OFF-experiments. This frequency was set to 32 kHz (80 ppm) yielding a direct magnetization of ligand resonances of less than 0.2%. Due to the low signal intensities of the STD spectrum (5–8% of the normal signal strength) and for a better comparability of the saturation efficiency, the ratio of the signal intensities $I_{STD}/I_{OFF-Res}$ was normalized to the strongest STD signal of the protons $H_{E/E'}$ to 100%.[28]

NOESY and trNOESY spectra were recorded with 2k (F2) × 512 (F1) data points for each experiment and a mixing time of 2 s and 200 ms, respectively. The mixing times τ_m for the NOESY/trNOESY spectra were determined by a series of experiments with different mixing times. The time at maximum cross-peak signal intensity of the analyte signals in the early phase of the NOE experiment was chosen as τ_m (200 ms) for the trNOESY. For the NOESY experiment, a ten times higher τ_m (2 s) was chosen. Between τ_m = 0.8 and 2 s, the cross-peak signal intensities did not differ significantly. In total, 16 transients and 16 dummy scans were performed. The relaxation delay was set to 2 s. The spectra were phased to pure absorption mode and the integration was performed in reference to the aromatic region in the 2D NOESY/trNOESY. In general, one can distinguish between intramolecular NOEs and intermolecular transferred NOEs, which can be built up fast during the mixing time of an NOESY experiment. The structure of benzylmandelate can be described in more detail by the intramolecular NOE, whereas the orientation of the analyte at the binding site of the stationary phase can be determined principally via intermolecular trNOEs between the analyte and the receptor. However, under HR/MAS NMR conditions, it is hard to figure out steric features of the bound analyte. In order to decide whether in our case the detected interactions were due to the chiral selector on the silica surface, experiments without selector were carried out. The error in the NOESY/trNOESY integral values is considered to be 10%. Occasionally, in an NOESY spectrum spin diffusion effects due to coherence transfer between coupled spin systems can occur, which lead to signal artifacts. To exclude this, an ROESY (rotating frame overhauser enhancement spectroscopy) experiment was performed. This experiment allows a relatively clear distinction between magnetization transfer through spin diffusion and chemical exchange effects. Spin diffusion processes lead to negative cross-peaks in the ROESY spectrum, whereas chemical exchange will result in positive cross-peaks in the same experiment.[19] Our experiment confirmed that the cross-peaks in the NOESY spectra have a negative signal intensity and consequently correspond to NOE effects.

Results and Discussion

The separation of the benzylmandelate racemate into its enantiomers by HPLC was accomplished with the derivatized polysaccharide-based CSP (Fig. 1) and a mobile phase consisting of methanol with 0.1% TFA. Due to the acidic nature of benzylmandelate, the addition of TFA as a modifier to the mobile phase in HPLC dramatically reduced the peak tailing for the polar organic mode investigated. Figure 2 shows the HPLC chromatogram of the separation in the polar organic mode in terms of resolution per time unit. This chromatogram shows that the (*R*)-enantiomer elutes prior to the (*S*)-enantiomer indicating that (*S*) interacts more strongly with the stationary phase than (*R*). The retention factor *k* which characterizes the interaction strength between a substance and the column packing material represents an independent value of the HPLC system. For the HPLC parameters listed, it was determined to be 0.28 for the (*R*)-enantiomer and to be 0.42 for the (*S*)-enantiomer. The quality of the separation of the racemic mixture is described by the separation factor α, in this case 1.5.

Despite performing HR/MAS at high rotation frequencies (6 kHz) on a 400 MHz NMR spectrometer, it was not possible to resolve signals of the single enantiomers in the racemic mixture in the presence of the CSP (Fig. 3). For binding affinity screening purposes, a comparison of spin–lattice relaxation times for all protons is promising because these values allow an indication of a different dynamic behavior of the (*R*)- or (*S*)-enantiomer bound to the stationary phase. The T_1 values obtained for each enantiomer are longer in solution than in suspension with CSP, especially for protons in the aromatic region. Probably, molecular motions in the diastereomeric complex of both enantiomers are limited, yielding to shorter correlation times τ_c (Table 1).

More detailed information about the specific binding site of the analyte molecule can be achieved by STD NMR experiments. For this purpose, the CSP material was selectively saturated at about 1 ppm in the aliphatic region. Ligand protons that closely interact with the irradiated receptor receive magnetization via the NOE, which will be transferred into solution after dissociation of the ligand yielding to decreased signal intensities in a 1D spectrum. Hence, the STD spectrum contains only signals corresponding to analyte protons that were bound to the sorbent material which permit the identification of protons or groups, which directly

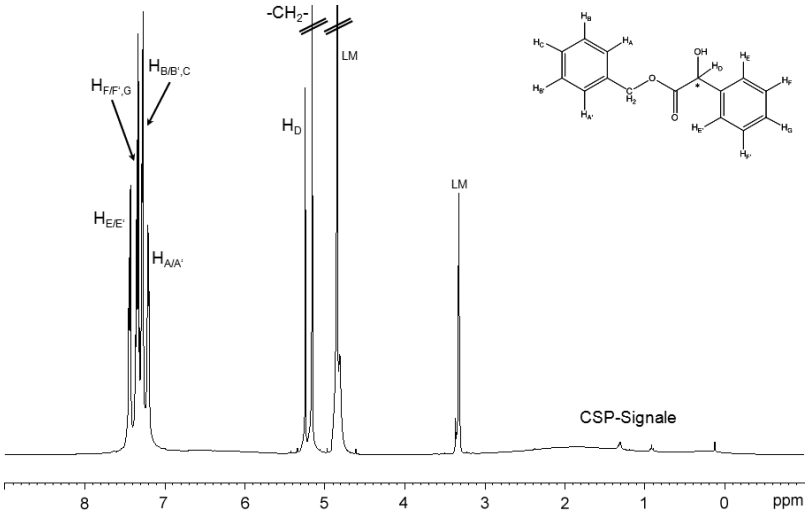

Figure 3. ^{1}H HR/MAS NMR spectrum of the racemic (*R*)/(*S*)-benzylmandelate together with the CSP recorded in acidified methanol-d_4 (+0.1% TFA). Signal assignment was performed with the help of 2D HR/MAS ^{1}H–^{1}H-COSY–NMR. S = solvent (methanol, water).

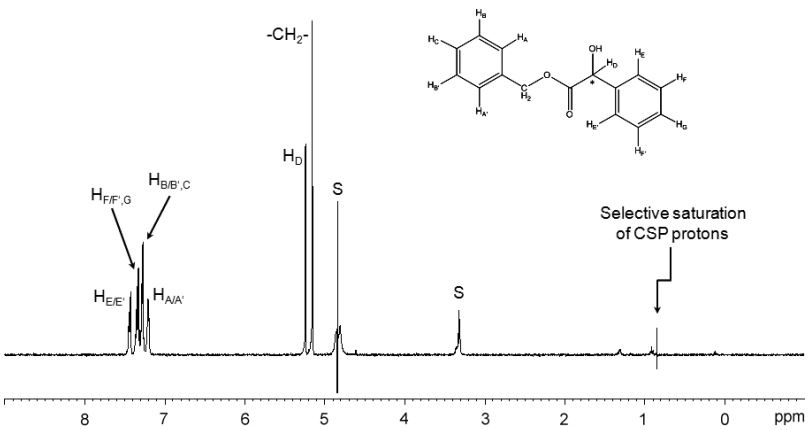

Figure 4. 1D ^{1}H STD HR/MAS NMR spectrum of (*S*)-benzylmandelate in suspension with the CSP. The signal intensities are decreased by approximately a factor of 10 compared to the OFF-resonance spectrum. S = solvent (methanol, water).

interact with the receptor. From the STD spectrum (Fig. 4), it follows that all signals of the analyte can be detected but with lower signal intensities (5–8%) compared to those of the OFF-resonance spectrum. This suggests that all protons of the analyte obtained saturation indicating binding affinities of all protons toward the receptors which was additionally supported by the decreased T_1 values. The signal intensities normalized to the strongest STD signal ($H_{E/E'}$, 100%) are presented in the bar diagram in Fig. 5. The values of the phenyl ring protons and of H_D of both benzylmandelate enantiomers are high, assuming that obviously these protons or groups are important for the molecular recognition process. Differences in the STD data for (*S*) and (*R*) suggest that the proton at the stereogenic centre H_D, the methylene group -CH_2-, and parts of the phenyl ring next to the -CH_2- group of the (*S*)-enantiomer interact stronger probably with the carbamoyl moiety of the CSP due to hydrogen bonding than those of to the (*R*)-enantiomer. The signal intensity for the methylene protons of the (*S*)-enantiomer is not as strong (60–66%) as most values of the phenyl protons or H_D. Hence, we propose that perhaps slight differences in the conformation at the stereogenic centre can be responsible for the different binding process of both enantiomers.

NOESY and trNOESY experiments were performed with (*R*)- and (*S*)-benzylmandelate once without (Fig. 6) and once with the CSP (Fig. 7) in order to confirm these assumptions. However, a quantitative analysis of the recorded spectra is very hard to achieve, therefore, only qualitative information about the bound ligands were treated.[29]

Without CSP selector, only weak intra-ligand NOE cross-peaks are detectable with positive intensity between the aromatic protons $H_{E/E'}$ and the proton H_D as well as between $H_{A/A'}$ and the methylene protons -CH_2-. In the presence of the CSP selector (Fig. 7 and Table 2), additional cross-peaks for both (*R*)- and (*S*)-enantiomers with negative sign between the aryl system ($H_{B/B',C}$ and $H_{F/F',G}$) and H_D and the -CH_2- protons occur respectively. This is expected for large molecules or receptor complexes with short correlation time. No differences between (*R*) and (*S*) were detectable, therefore, only NOESY spectra recorded with the (*S*)-enantiomer are shown. Figure 8 shows the trNOESY spectrum of the (*S*)-enantiomer/CSP mixture. The (*S*)- as well as the (*R*)-enantiomer (data not presented) shows in this spectrum an intense intermolecular negative cross-peak between the aromatic benzylmandelate protons and those of the aliphatic region of the stationary phase. The integral value of

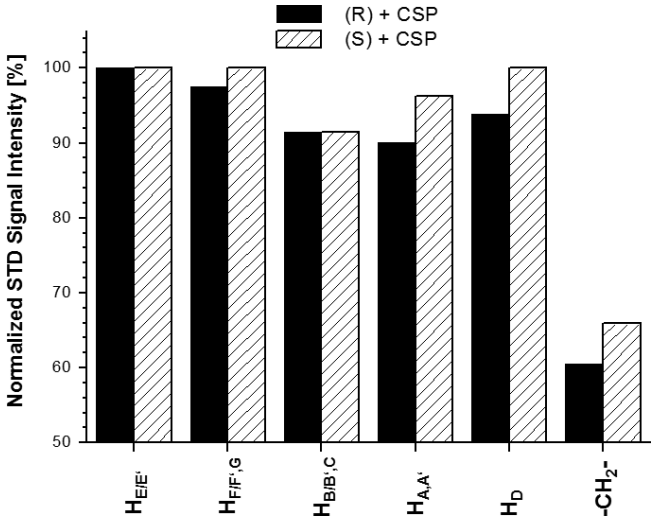

Figure 5. Bar diagram reflecting the normalized relative STD intensities for (*R*)- and (*S*)-benzylmandelate enantiomers in the presence of CSP recorded in methanol-d_4 (+0.1% deuterated TFA). The relative STD data (%) arise from the individual proton signal intensities of the STD spectrum in comparison to the OFF-resonance ^{1}H-HR/MAS NMR spectrum referenced to the methylene signal at 5.16 ppm.[16].

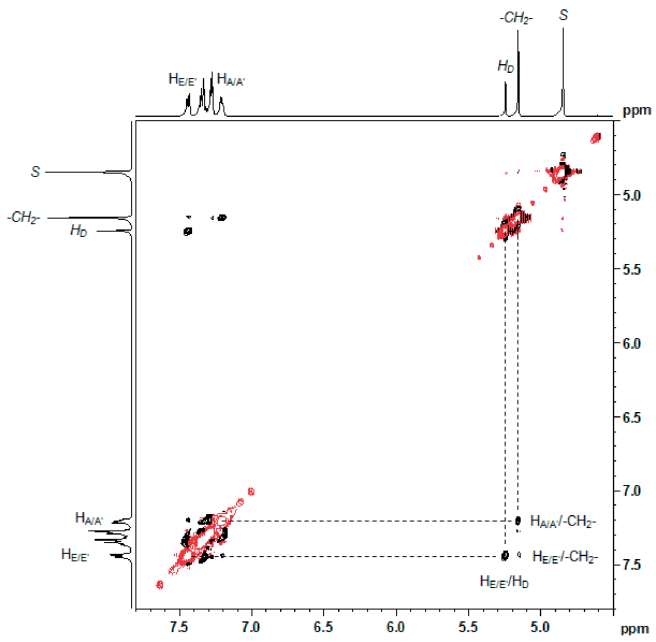

Figure 6. NOESY HR/MAS NMR spectrum of (*S*)-benzylmandelate in solution. Intramolecular cross-peaks occur with positive sign of the analyte protons $H_{E/E'}$ to the stereogenic centre (H_D) and to the -CH_2- group, and $H_{A/A'}$ to the methylene group. S = solvent (methanol, water).

this peak for (*S*) compared to (*R*) is stronger by approximately a factor of 2 (Table 2). In sum the NOE intensities of this system measured by trNOESY experiments are more negative compared to the NOESY suggesting interactions toward the CSP material (Table 2). The more negative integral values of (*S*) compared to (*R*) are probably due to stronger interaction forces of (*S*) with the CSP, which is in accordance with the other NMR measurements and with the retention order detected in chromatography.

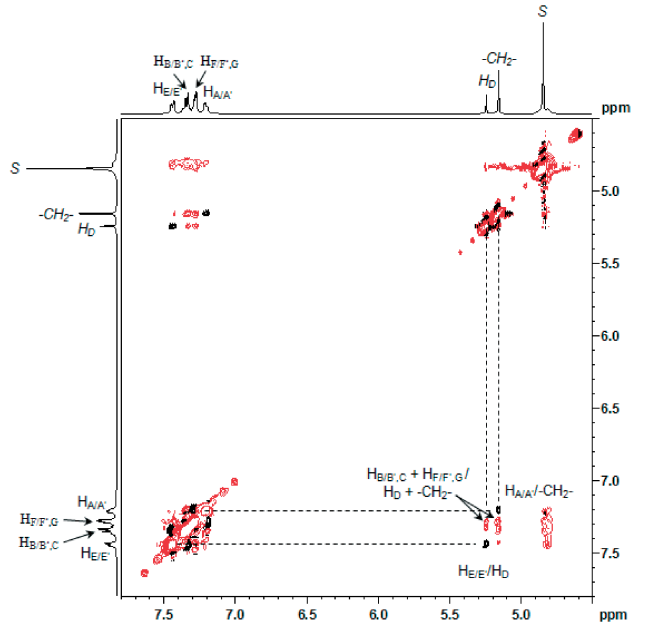

Figure 7. NOESY HR/MAS NMR spectrum of (*S*)-benzylmandelate in suspension with CSP material. The broken lines denote positive intramolecular cross-peaks of the ligands $H_{A/A'}$ and $H_{E/E'}$ to the -CH_2- group and to H_D, respectively. In addition, negative intramolecular cross-peaks from $H_{B/B',C'}$ and $H_{F/F',G'}$ to the methylene group and to the chiral proton occur. S = solvent (methanol, water).

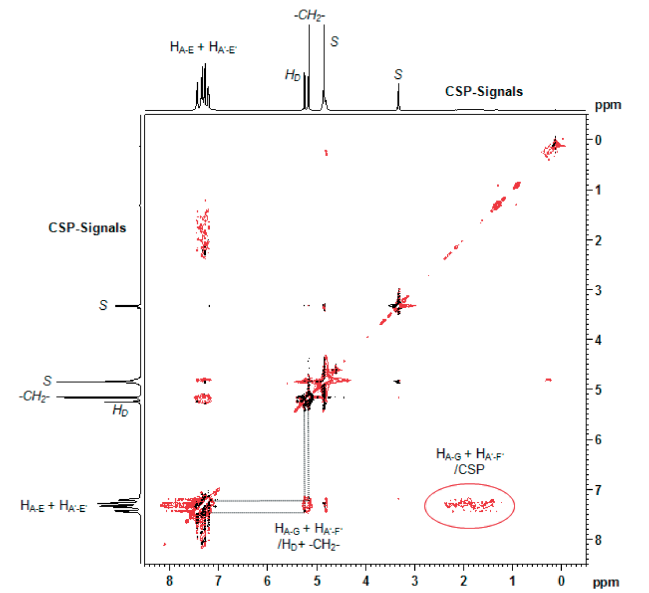

Figure 8. trNOESY HR/MAS NMR spectrum of (*S*)-benzylmandelate in the presence of CSP material. Besides the strong negative intra-ligand NOE effects indicated by the broken lines, a negative intermolecular cross-peak of the aromatic analyte moiety to the aliphatic region of the stationary phase material occurred (displayed within the circle). S = solvent (methanol, water).

Concluding Remarks

The separation of (*R*)- and (*S*)-benzylmandelate into its enantiomers can be achieved by HPLC using amylose tris(3,5-dimethylphenylcarbamate)-based silica CSP. The chiral recognition of both enantiomers relies on the formation of a transient

Table 1. Spin–lattice relaxation times (T_1) for (*R*)- and (*S*)-benzylmandelate enantiomers in solution and together with the CSP material

Signal assignment	Chemical shift (ppm)	T_1 (s)		
		R/S (in solution)	*R* & CSP	*S* & CSP
$H_{E/E'}$	7.44	3.15	3.00	2.52
$H_{F/F',G}$	7.34	2.99	2.99	2.47
$H_{B/B',C}$	7.28	3.60	3.10	2.69
$H_{A,A'}$	7.21	3.49	3.30	2.63
H_D	5.24	3.62	3.30	2.71
$-CH_{2}-$	5.16	2.00	2.08	1.80

The deviations are estimated to be ±0.1 s

diastereomeric ligand–receptor complex with different binding strengths of the ligands toward the CSP material. Due to the heterogeneity of the investigated system, suspended state HR/MAS NMR has to be used to obtain reasonable spectra. To further understand the retention behavior in chromatography of both enantiomers, a combination of different NMR experiments like spin–lattice relaxation time measurements T_1, the $T_{1\rho}$-filtered STD, and the transferred NOESY NMR technique were used allowing the detection of analyte fractions that were involved in molecular recognition processes. Although NMR in a rotor represents a functionally static system, a comparison between NMR data and dynamic HPLC values is feasible as it approximates in the best way a chromatographic-like environment.

The performed T_1 measurements showed that the spin–lattice relaxation times T_1 for the enantiomers in the diastereomeric complex are shorter, especially for H_D and for most of the aromatic protons compared to free in solution indicating a binding affinity toward the CSP. These interactions were also found in the STD and trNOESY spectra. The stronger detected (*S*)-enantiomer STD signal intensities of H_D and the methylene group as well as their cross-peak signal intensities in the transferred NOESY spectra give e.g. rise to hydrogen bonding toward the carbamoyl moiety of the CSP as possible binding forces on the modified silica surface. Probably solvophobic, van der Waals, and/or $\pi-\pi$ interactions between the phenyl protons moiety, and especially $H_{A/A'}$ and $H_{E/E'}$ of the enantiomers and the aromatic system of the derivatized polysaccharide stationary phase strongly influence the molecular recognition of (*R*)- and (*S*)-benzylmandelate.

Acknowledgements

Paul Schuler is acknowledged for valuable discussions and all technical assistance in performing HR/MAS NMR measurements in the suspended state. We would like to express our gratitude to Maria Campos for the support concerning this manuscript, and to Dr Ing. Herbert Knauer GmbH for the supply of the HPLC system. This work was financially supported through AL 298/14-1 provided by the Deutsche Forschungsgemeinschaft (DFG) and the National Science Foundation (NSF, CHE-0724218).

References

[1] D. Wistuba, V. Schurig, *Electrophoresis* **2000**, *21*, 4136.
[2] M. Lämmerhofer, W. Lindner, in *Recent Developments in Liquid Chromatographic Enantioseparation*, vol. 1, (Ed.: K. Valco), Elsevier: Amsterdam, **2000**.
[3] G. Subramanian, in *A Practical Approach to Chiral Separations by Liquid Chromatography*, Wiley-VCH: Weinheim, **1994**.
[4] (a) Y. Okamoto, E. Yashima, *Angew. Chem.* **1998**, *110*, 1072; (b) Y. Okamoto, E. Yashima, *Angew. Chem. Int. Edit.* **1998**, *37*, 1020.
[5] S. Kromidas, in *HPLC Made to Measure*, Wiley-VCH: Weinheim, **2006**.
[6] G. Pages, C. Delaurent, S. Caldarelli, *Anal. Chem.* **2006**, *78*, 561.
[7] C. Carrara, S. Viel, C. Delaurent, F. Ziarelli, G. Excoffier, S. Caldarelli, *J. Magn. Reson.* **2008**, *194*, 303.
[8] C. Hellriegel, U. Skogsberg, K. Albert, M. Lämmerhofer, N. M. Maier, W. Lindner, *J. Am. Chem. Soc.* **2004**, *126*, 3809.
[9] V. Friebolin, M. P. Bayer, M. T. Matyska, J. J. Pesek, K. Albert, *J. Sep. Sci.* **2009**, *32*, 1722.
[10] R. C. Anderson, M. A. Jarema, M. J. Shapiro, J. P. Stokes, M. Ziliox, *J. Org. Chem.* **1995**, *60*, 2650.
[11] P. A. Keifer, L. Baltusis, D. M. Rice, A. A. Tymiak, J. N. Shoolery, *J. Magn. Reson.* **1996**, *119*, 65.
[12] P. A. Keifer, *J. Org. Chem.* **1996**, *61*, 1558.
[13] E. Yashima, C. Yamamoto, Y. Okamoto, *J. Am. Chem. Soc.* **1996**, *118*, 4036.
[14] G. C. Roberts, *Drug Discov. Today* **2000**, *5*, 230.
[15] (a) B. Meyer, T. Peters, *Angew. Chem.* **2003**, *115/8*, 890; (b) B. Meyer, T. Peters, *Angew. Chem. Int. Ed.* **2003**, *42*, 864.
[16] S. Schauff, V. Friebolin, M. D. Grynbaum, C. Meyer, K. Albert, *Anal. Chem.* **2007**, *79*, 8323.
[17] K. Akasaka, *J. Magn. Reson.* **1979**, *36*, 135.
[18] M. Vogtherr, T. Peters, *J. Am. Chem. Soc.* **2000**, *122*, 6093.
[19] (a) H. Händel, E. Gesele, K. Gottschall, K. Albert, *Angew. Chem.* **2003**, *115*, 454; (b) H. Händel, E. Gesele, K. Gottschall, K. Albert, *Angew. Chem. Int. Ed.* **2003**, *42*, 438.
[20] D. Neuhaus, M. P. Williamson, in *The Nuclear Overhauser Effect in Structural and Conformational Analysis*, Wiley-VCH: New York, **2000**.
[21] E. Yashima, E. Kasashima, Y. Okamoto, *Chirality* **1997**, *9*, 63.
[22] B. Chankvetadze, E. Yashima, Y. Okamoto, *J. Chromatogr. A* **1994**, *670*, 39.
[23] Y. Okamoto, M. Kawashima, K. Hatada, *J. Chromatogr.* **1986**, *363*, 173.
[24] W. H. Pirkle, C. J. Welch, *J. Liq. Chromatogr. Relat. Technol.* **1991**, *14*, 1.
[25] T. Scherf, J. Anglister, *Biophys. J.* **1993**, *64*, 754.
[26] B. Cutting, S. V. Shelke, Z. Dragic, B. Wagner, H. Gathje, S. Kelm, B. Ernst, *Magn. Reson. Chem.* **2007**, *45*, 720.
[27] B. Claasen, M. Axmann, R. Meinecke, B. Meyer, *J. Am. Chem. Soc.* **2005**, *127*, 916.
[28] M. Mayer, B. Meyer, *J. Med. Chem.* **2000**, *43*, 2093.
[29] J. R. Cruz, B. A. Becker, K. F. Morris, C. K. Larive, *Magn. Reson. Chem.* **2008**, *46*, 838.

Table 2. Integral intensities of the observed NOESY (τ_m = 2 s) and trNOESY (τ_m = 200 ms) intra- and intermolecular NOE cross-peak signals of (*R*)- and (*S*)-benzylmandelate in the presence or absence of unmodified silica or CSP (*R/S* and silica) material

Cross-peaks	NOESY (integral $\times 10^{-2}$)				trNOESY (integral $\times 10^{-2}$)			
	R/S	*R/S* and silica	*R* and CSP	*S* and CSP	*R/S*	*R/S* and silica	*R* and CSP	*S* and CSP
$H_{E/E'} - H_D$	−0.06	+2.5	−0.8	−1.0	−0.3	+0.3	−3.1	−2.8
$H_{A/A'} - CH_2$	+2.2	+2.0	−2.4	−2.3	+0.2	−0.02	−2.6	−2.6
Phenyl-CSP	–	–	–	–	–	–	**−7.7**	**−15.4**