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Characterization of a Chiral Stationary Phase by HR/MAS NMR Spectroscopy and Investigation of Enantioselective Interaction with Chiral Ligates by Transferred NOE

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Abstract: The surface chemistry of a chiral stationary phase (CSP) with a (*tert*-butyl carbamoyl) quinine selector immobilized on thiol-modified silica has been characterized by ^{1}H HR/MAS NMR and ^{29}Si CP/MAS NMR spectroscopy. The mostly well-resolved ^{1}H signals could be assigned to stem from the surface-bound selector and the latter suggested a bi- and trifunctional silane linkage. Suspended-state NMR spectroscopy thus proved a well-characterized surface chemistry as proposed. To study chiral recognition phenomena in the presence of the CSP, ^{1}H HR/MAS 2D transfer NOESY investigations in methanol-d_4 have been undertaken with various solutes including *N*-3,5-dinitrobenzoyl derivatives of leucine (DNB-Leu) and *N*-acetyl phenylalanine (Ac-Phe). Both (*R*)- and (*S*)-enantiomers of DNB-Leu and Ac-Phe interacted with the tBuCQN-CSP as indicated by negative cross-peaks in the trNOESY spectra, while the 2D NOESY of the dissolved solutes in absence of the chiral stationary phase showed positive cross-peaks. The intensities of the trNOE cross-peaks were much stronger for the (*S*)-enantiomers. This stereoselectivity paralleled the experimental chromatographic behavior, where the (*S*)-enantiomers revealed stronger binding and retention on the tBuCQN-CSP as well. Hence, we were able to correlate the retention behavior to the trNOE NMR spectroscopic data in a qualitative manner.

Introduction

In the past years, the technology for the separation of stereoisomers by chromatographic methods has reached a high level of maturity in terms of availability of chiral separation materials. Thus, a large variety of chiral stationary phases, typically with synthetic, semi-synthetic, or naturally occurring enantiomeric selector molecules immobilized on a chromatographic support such as silica, are either commercially available or are described in the literature.[1,2] Each of them solves specific separation problems for a more or less broad application spectrum. Thereby, enantiomer separation relies on specific intermolecular interactions between analytes and selectors and requires a precise spatial and functional fit of the binding sites for both binding partners. Since both enantiomers are not able to match these demands equally, stereoselectivity may be observed as a result of tight binding of only one of the two opposite enantiomers.

Despite the long history in scientific discussions, the full understanding of chiral recognition phenomena on solid surfaces is still quite poor. One of the problems that appears to hamper the in-depth knowledge of molecular recognition of chiral stationary phases (CSPs) is the chemical heterogeneity of many CSPs and the poorly or improperly characterized surface chemistries.[3,4] This often originates from heterogeneous surfaces, mixtures of isomeric selectors, and undisclosed immobilization and linkage chemistries. On the other hand, the structural complexity of many selectors, in particular those of polymeric nature (proteins, polysaccharides, synthetic polymers) or macrocyclic nature (cyclodextrins, macrocyclic antibiotics) featuring a multiplicity of potential binding sites and interaction events, renders a detailed study more difficult, and causes the selector−analyte interactions as well as the chiral distinction mechanisms to remain largely imprecise and unknown for the majority of separations achieved nowadays. Only for a few model compounds has the mechanism been unveiled. This was accomplished by detailed FT-IR and NMR spectroscopic investigations, molecular modeling studies, or X-ray crystal structure analysis.[5−15] With few exceptions,[6,15] all of these studies have

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been performed merely with soluble analogues or precursors of the CSPs, i.e., with the chiral selector molecules only, while the support and usually also the spacer that links the selector to the support has been neglected. In these studies it is made use of the *tacit* assumption that the support and spacer (tether) do not influence the molecular recognition and chiral separation. This assumption, of course, does not always hold true. Hence, an enhanced understanding of chiral recognition mechanisms and processes on the surface of the CSP would be of utmost importance and of high interest. To generate a rational basis for design and construction of efficient molecular recognition tools would undoubtedly be a significant contribution.

Besides the study of chiral recognition phenomena on the surface of the separation materials, techniques that allow the fast detection of stereoselective interactions are also of interest, not only in the screening of various solutes for chiral recognition and separation by certain existing CSPs but also in the development and optimization of new selectors and CSPs. New chiral selectors and separation media are developed with the focus to solve individual enantiomer separation problems or as dedicated separation systems for preparative-scale applications. Moreover, for the elucidation of tentative selector−analyte binding models the design of new (more) active and even inactive analogues may be regarded as an invaluable approach to derive structure-resolution relationships.[16] Often, combinatorial chemistry concepts are pursued for this purpose[17] which requires the screening of libraries of potential selectors to select the most effective ones. The latter procedure is in fact the limiting step in terms of speed of discovery and development of new improved phases. To circumvent slow chromatographic testing, which implies also synthesis of selectors and CSPs in sufficient amounts to allow the packing of columns for their evaluation, there is an urgent demand for faster and more effective screening methods, which in addition require less material of precious CSPs.

For both screening and the study of molecular recognition processes on solid surfaces, NMR spectroscopy seems to be a suitable technique. For a long time NMR spectroscopy has been the method of choice to monitor intermolecular interactions and molecular recognition events, since several NMR parameters will change in a characteristic manner upon binding. A major benefit of NMR-based determinations is the ability to derive structural information, including conformational states, directly. Of particular interest for host−guest interaction studies are NMR methods such as those utilized in drug discovery which have been proposed for screening of binding activities.[18,19] These include T_2 filtering,[20] diffusion NMR,[21] transferred NOEs [22−24] or saturation transfer.[25] All of these methods may also be exploited to investigate stereoselectivity effects of selector−analyte binding.

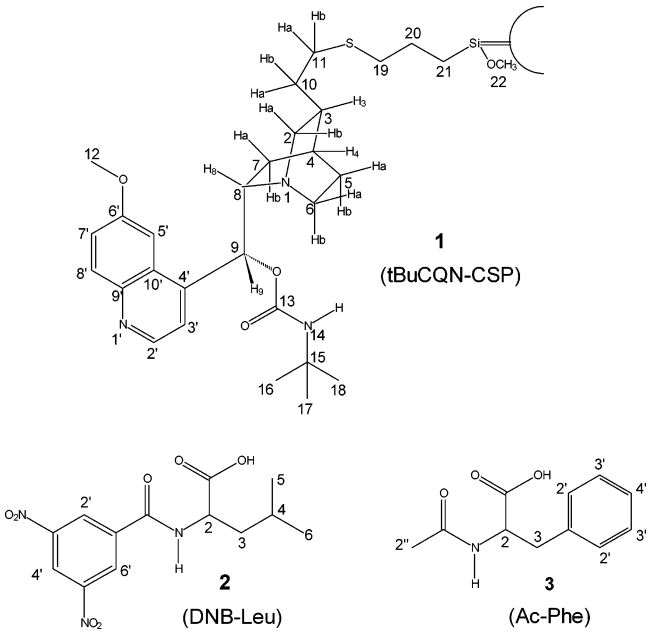

Figure 1. Structure of the *O*-9-(*tert*-butylcarbamoyl)quinine-based CSP **1** (tBuCQN-CSP) and the test solutes *N*-(3,5-dinitrobenzoyl)-leucine **2** (DNB-Leu) and *N*-acetyl phenylalanine **3** (Ac-Phe).

With the advent of high resolution/magic angle spinning NMR spectroscopy (HR/MAS NMR) the study of molecular recognition events directly at the binding sites on the surface of the stationary phase became possible and allowed the experiments to be performed under chromatographic conditions similar to those inside the column during the separation process.[26,27] The technique of HR/MAS NMR spectroscopy allows acquisition of spectra of adsorbates with high resolution in the presence of solid porous particles such as silica gels or bonded silica phases. The addition of a solvent to the solids increases the mobility of the surface structures or ligands and therewith minimizes susceptibility distortions and residual dipolar couplings between protons leading at least to better resolutions of the NMR spectra.

Herein, we report on the HR/MAS NMR spectroscopic characterization of a chiral stationary phase that has a quinine carbamate selector covalently bound to the surface of porous particulate silica gel (Figure 1). These studies should unequivocally prove the surface structure and type of covalent linkage that was predicted from the selector and immobilization chemistry. The main goal of this study focuses on the elucidation of the effectiveness and usefulness of HR/MAS 2D transfer NOESY spectroscopy in the study of chiral recognition directly on the surface of the solid chiral quinine carbamate-based CSP. The objective is to investigate if this technique could also be applied for a fast screening of new CSPs by correlation of

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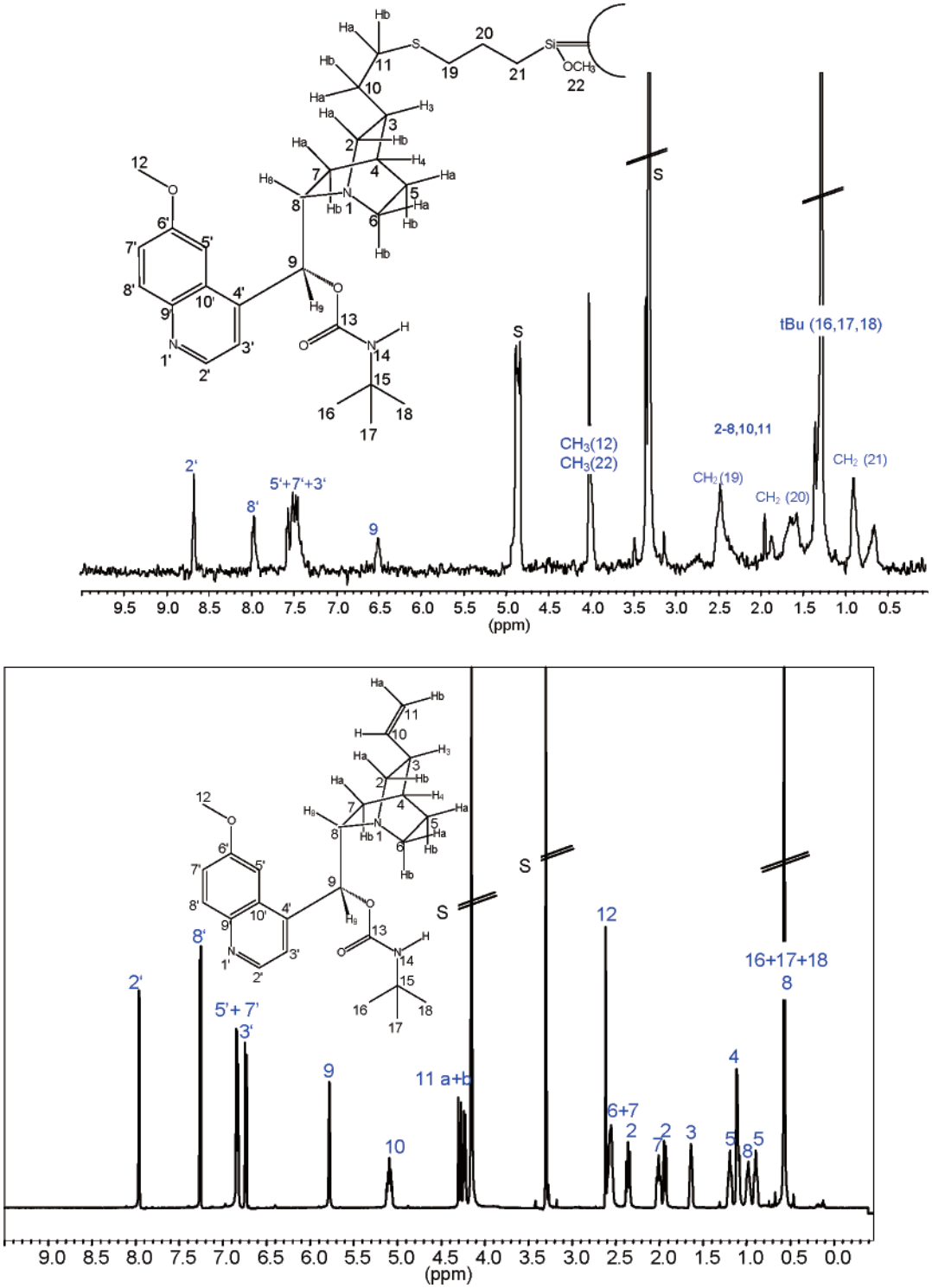

Figure 2. ^{1}H HR/MAS NMR spectra (400 MHz, spinning speed 4000 Hz) of the tBuCQN-CSP suspended in methanol-d_4 (top) and of the soluble precursor of the CSP, i.e., of the (*tert*-butylcarbamoyl)quinine selector dissolved in methanol-d_4 (bottom) under comparable conditions.

stereoselectively obtained effects, if any can be observed, with chromatographic data.

Results and Discussion

Characterization of the Chiral Stationary Phase by HR/MAS NMR in Suspended State. The presently investigated chiral stationary phase **1** (Figure 1) has been synthesized by two subsequent steps. First, the modification of silica particles with trifunctional 3-mercaptopropyl trimethoxysilane by a silylation reaction yields a modified silica material with reactive terminating thiol groups (Thiol-Kromasil). Second, the covalent binding of the (*tert*-butylcarbamoyl) quinine selector having a terminal vinyl group in position 3 of the quinuclidine, which stems from the native cinchona alkaloid quinine, by reaction with the thiol-modified silica in a radical addition reaction results in the structure as shown in Figure 1.

The surface structure proposed in Figure 1 is clearly validated by the NMR spectra shown in Figures 2 and 3. The ^{1}H HR/

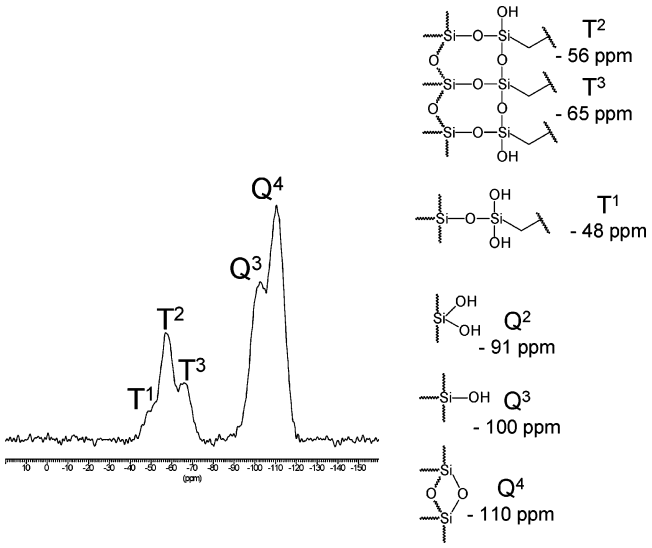

Figure 3. ^{29}Si CP/MAS NMR spectrum of the tBuCQN-CSP suspended in methanol-d_4.

MAS NMR spectrum of tBuCQN-thiopropyl-Kromasil (100, 5 μm) suspended in methanol-d_4 (Figure 2, top), demonstrates the excellent NMR resolution obtained in the suspension of the chemically modified inorganic support in the presence of the mobile phase equivalent (d_4-methanol) with a spinning speed of 4000 Hz that eliminates susceptibility distortions of the suspension. For comparison also the spectrum of the selector dissolved in methanol-d_4 and acquired under identical ^{1}H HR/MAS NMR conditions to facilitate the assignment of the signals of the CSP is shown (Figure 2, bottom). Most significant is the disappearance of the signals of the vinyl protons **10** and **11** between 5 and 6 ppm in the spectrum of the tBuCQN-CSP. This demonstrates that the selector molecules are covalently linked and that indeed the bonding completely and exclusively occurs at the vinyl group by the suggested mechanism. Furthermore, the HR/MAS NMR spectrum of the CSP renders all the signals of the aromatic quinoline protons (**2′**,**3′**,**5′**,**7′**,**8′**) as well as the proton of the stereogenic center C_9 (**9**) adequately resolved and readily assigned. The same is true for the protons at 4 ppm, which stem from the methoxy group of the quinoline (**12**) and the residual methoxy group of the silane after its mono- and difunctional bonding (**22**). The situation is more complex for the high-field shifted protons. The signals arising from the quinuclidine as well as from the spacer group are not fully resolved in the ^{1}H HR/MAS NMR spectrum of the CSP. However, the pattern seen in the solution NMR spectrum of the selector is, aside from the additional signals of the linker, largely reflected in the corresponding suspended-state spectrum of the bonded selector. Overall, these data suggest the existence of a well-defined surface, in particular when compared to other CSPs with more complex selector structure (e.g., polysaccharide CSPs; polysaccharide CSPs may contain a distribution of saccharide units in the chain and can therefore give a nonuniform three-dimensional as well as supramolecular structure of the CSP, which is known to affect the chiral discriminating ability. This was avoided here by immobilizing the monomeric chiral selector.).

Of interest from a chromatographic point of view is also the type of linkage and the presence of residual silanols in the bonded phase. In principle, the trimethoxy silane might be linked to the silica matrix by a mono-, bi-, or trifunctional bonding. The type of linkage will determine the stability of the resulting bonded phase, because the silica−silane linkage (siloxane bond) is prone to acid- as well as base-catalyzed hydrolysis. Valuable information about the chemical bonding may be derived from the ^{29}Si cross-polarization/magic angle spinning (CP/MAS) NMR spectrum (Figure 3). The transfer of the magnetization by heteronuclear dipolar coupling from the highly populated ^{1}H with large gyromagnetic constant to the insensitive and low-populated ^{29}Si improves the detection sensitivity of ^{29}Si nuclei significantly so that reasonable spectra can be acquired, which provide information about the silica surface and the siloxane bonding. Also, the cross-polarization allows an increase of the speed of the pulse repetition times due to shorter relaxation times. A drawback with the cross-polarization experiments is that no quantification of the signals from functional groups that differ significantly from each other can be done, since different functional groups within a molecule have different contact times.

The spectrum of Figure 3 shows two sets of signals. The signals denoted with Q^2, Q^3, and Q^4 correspond to Si atoms of the silica surface with geminal and free hydroxyl groups, as well as being part of a siloxane network, respectively. Only a number of free silanols (Q^3, −100 ppm), but no geminal silanols (Q^2, −91 ppm) are present, while the majority of the Si atoms are siloxane groups (Q^4, −110 ppm). The relative amounts of Q^2 and Q^3 can be estimated due to the similarities in contact times of the Q^2 and Q^3 groups. The T-group signals provide information about the connectivity of the silane to the silica support. Least stable and most prone to hydrolysis would be the monofunctional Si−O−Si− linkage (T^1 signal, −48 ppm), which is largely absent according to the ^{29}Si CP/MAS NMR spectrum. The T^2 and T^3 signals, on the other hand, correspond to the more stable bifunctional (T^2, −56 ppm) and trifunctional linkages (T^3, −65 ppm), respectively. A quantification of the T groups, possible due to the similarities in contact time, was performed by applying Gaussian curves over the corresponding peak.[28] From the results it can be observed that the content of T^1, T^2, T^3 were 14, 33, 53%, respectively. From these data, a low silanol activity as well as a stable bonding can be concluded for the investigated CSP. This has been additionally confirmed by chromatographic findings and experience gained over several years of application.

Interaction of DNB-Leu Enantiomers with the Chiral Stationary Phase Studied by HR/MAS 2D Transfer NOESY NMR in Suspended State. Upon addition of (*R*,*S*)-DNB-Leu to a *tert*-butylcarbamoyl quinine selector diastereomeric ion-pairs are formed with 1:1 stoichiometry as previously demonstrated by NMR titrations (Job plots) in solution.[10] Recently, the binding constants of these model analytes with the tBuCQN selector in methanol at 25 °C were found to be 2.2×10^2 and 3.7×10^3 M^{-1} (corresponding to K_D values of 4.5×10^{-3} and 2.7×10^{-4} M) for the weak (*R*)- and strong (*S*)-complexes, respectively, as determined by isothermal calorimetric titrations.[29] These binding constants are suitable for detection by transferred nuclear Overhauser effect (trNOE) assuming similar affinities with the surface-bound selector. Moreover, the reduc-

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tion of separation ability of the enantiomers of the immobilized selector, compared to that of the free selector in solution, can give valuable information of the nonselective interactions from the silica support.

Generally, trNOE studies have recently received great attention in drug discovery for the screening of libraries for effective binding ligands on various receptors. [23,24,30] Briefly, the trNOE NMR spectroscopy makes use of the observation that a weak positive intramolecular NOE of low-molecular weight ligands becomes a strong negative NOE in response to association with a macromolecular species, such as a protein. The strong negative NOE can be readily detected, quasi as memory of binding, in the free ligand after its dissociation from the macromolecular binding partner.[31] This phenomenon allows easy distinction between effectively binding ligands (negative cross-peaks) and nonbinding ones (positive cross-peaks). There is no upper limit given by the molecular mass of the macromolecular species so that even highly polymeric chromatographic supports are accessible for trNOE measurements. The application of trNOESY NMR spectroscopy to the study of interactions with chromatographic supports is, however, relatively new.[26,27] It has never before been applied to the study of stereoselective interactions with quinine-based CSPs representing a family of CSPs acting as enantioselective anion exchangers for chiral acids with a high success rate of discrimination.

Here we describe the application of HR/MAS 2D trNOESY NMR spectroscopy to stereoselectively distinguish between strong and weak binding enantiomers of DNB-Leu and the above characterized (*tert*-butylcarbamoyl)quinine-CSP. One of the favorable features of the HR/MAS 2D trNOESY NMR is that the NMR experiments can be performed under conditions that more or less resemble those of chromatography. Any results from such suspended-state NMR spectroscopy experiments should therefore be better correlated to observations from chromatographic evaluations compared to those from solution-state NMR. This is regarded to be of importance if such a technology is deemed applicable as a screening methodology for rapid CSP evaluation.

For sake of comparison and to facilitate the data processing of the spectra, we acquired 2D NOESY NMR spectra of (*R*)- and (*S*)-DNB-Leu, in addition to the trNOESY NMR spectra. To ensure similar conditions for NOESY and trNOESY spectra, the NOESY NMR experiments were performed for each enantiomer under an environment identical to that which was used for HR/MAS NMR, with DNB-Leu dissolved in methanol-d_4.

Figure 4 shows the HR/MAS 2D trNOESY spectrum of (*S*)-DNB-Leu **2** in the presence of 5 mg of the tBuCQN-CSP suspended in methanol-d_4 (bottom) in comparison to its 2D NOESY spectrum without the CSP (top) (details on the molar selector-to-solute ratio are discussed below). The 2D NOESY spectrum (Figure 4, top) reveals positive cross-peaks between the protons H(**3**+**4**) and the methyl groups H(**5**+**6**) as well as between the proton at the stereogenic center H(**2**) with protons H(**3**+**4**) and methyl protons H(**5**+**6**). The HR/MAS 2D trNOESY spectrum (Figure 4, bottom) exhibits negative cross-peaks between the protons H(**3**+**4**) and the methyl groups

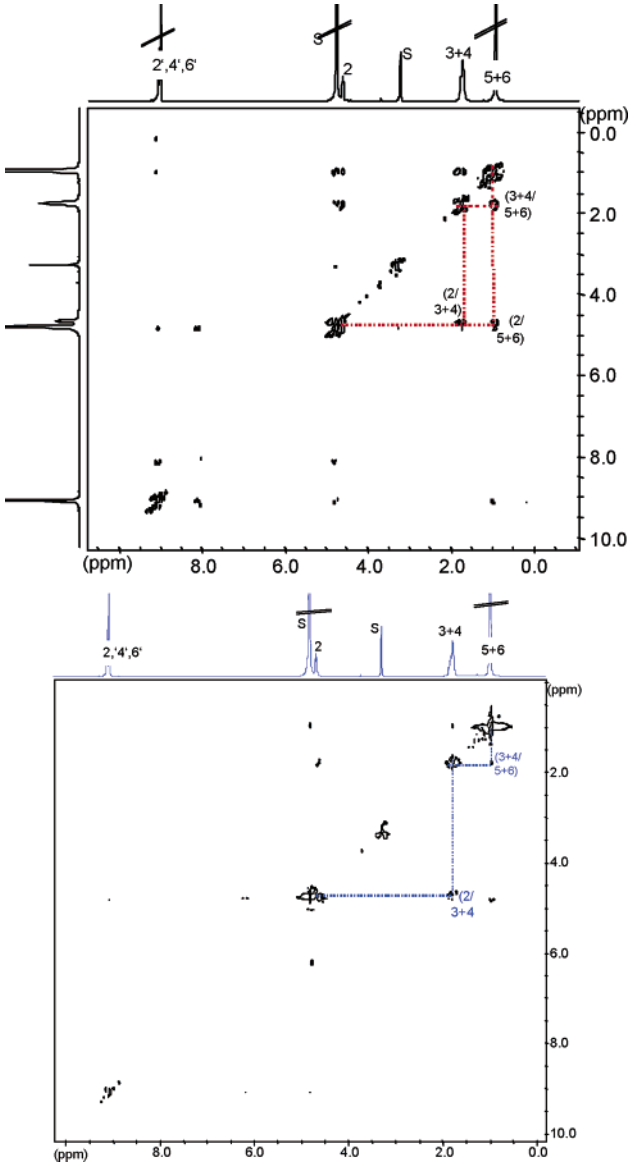

Figure 4. Comparison of 2D NOESY spectrum of (*S*)-DNB-Leu with positive intramolecular NOEs (top) and HR/MAS 2D trNOESY spectrum of (*S*)-DNB-Leu in the presence of the CSP with negative intramolecular cross-peaks indicating a transfer NOE (bottom). (Correlations highlighted in red for the positive intramolecular NOE and in blue for the negative transfer NOE; S = solvent signals)

H(**5**+**6**), as well as a negative cross-peak between proton H(**2**) and neighboring protons H(**3**+**4**). The existence of several negative cross-peaks clearly indicates binding of (*S*)-DNB-Leu to the CSP. The corresponding (*R*)-enantiomer binds to the stationary phase as is observed from the same pattern of negative cross-peaks in the HR/MAS 2D trNOESY spectrum (see Figure 5, top). However, comparison of integral values for the trNOE cross-peaks H(**2**/(**5**+**6**)) of the enantiomers shows a higher intensity of the trNOE cross signal by a factor of 7.4 for the (*S*)- over the (*R*)-enantiomer. Stereoselective binding of DNB-Leu enantiomers to the tBuCQN-CSP may be safely inferred from this difference.

To support this finding, analogous trNOESY NMR experiments have been performed with (*R*)- and (*S*)-DNB-Leu in the presence of unmodified Thiol-Kromasil as reference experiments. Since this stationary phase lacks a source of chiral discrimination, if any trNOE is detected, binding strengths and

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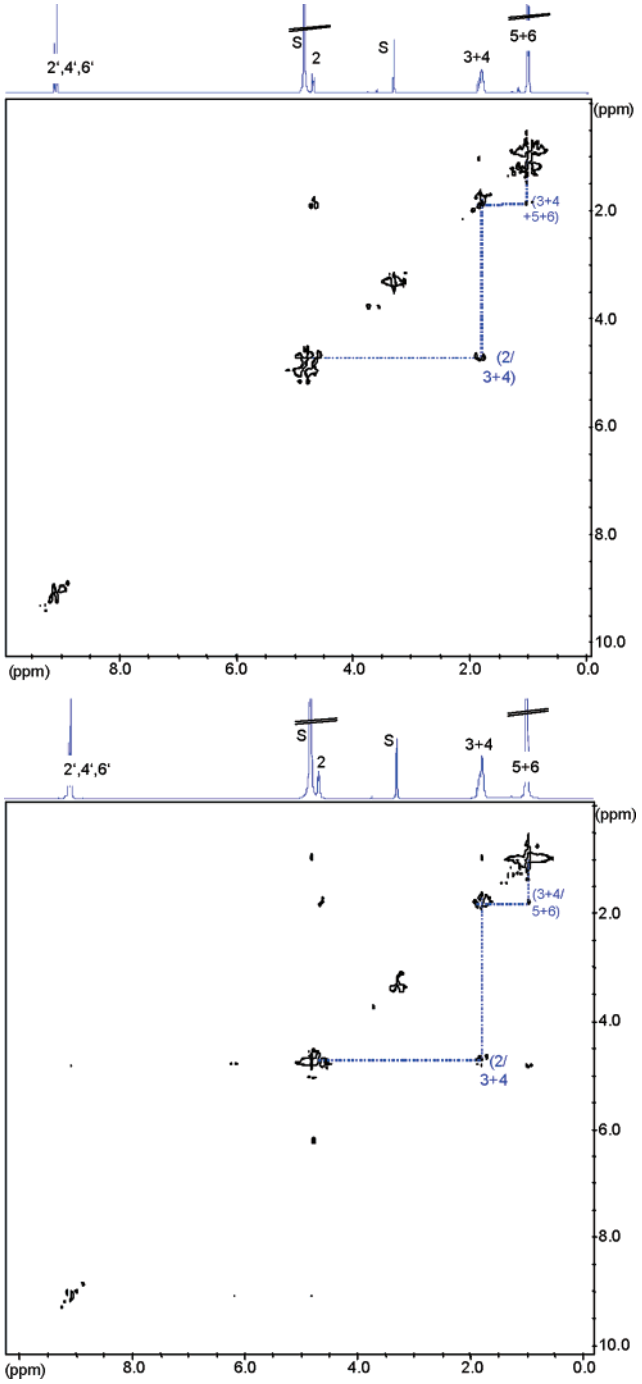

Figure 5. Comparison of the HR/MAS 2D trNOESY spectra of (*R*)-DNB-Leu (top) and (*S*)-DNB-Leu (bottom) in the presence of the CSP with negative intramolecular cross-peaks indicating the existence of a transfer NOE and binding of ligands to the CSP.

thus also cross-peak signal intensities are expected to be identical for both solute enantiomers. In fact, negative cross-peak signals were recorded for both (*R*)- and (*S*)-enantiomers of DNB-Leu on the achiral Thiol-Kromasil phase. The intensities of these cross-peak signals were very similar for both enantiomers and significantly weaker than for the interaction with the CSP. The significant difference in cross-peak intensity observed between the (*R*)- and (*S*)-DNB-Leu in the presence of the tBuCQN-CSP is in accordance with the chromatographic retention order where the (*R*)-enantiomer is eluted prior to the (*S*)-enantiomer. Herewith, it is proven that the difference of the negative cross-peak signal intensities in the trNOESY experiments with the CSP reflects stereoselective binding of the DNB-Leu enantiomers. Moreover, these results suggest that for the present tBuCQN-CSP the specific binding to the selector is superimposed by a weak nonspecific interaction of the DNB-Leu solutes to the thiol-modified support under the given conditions.

In addition to the above-described experiments, control experiments have been performed which include the variation of the selector−solute ratio. First, the amount of CSP in the rotor was decreased to 2 mg (corresponding to a molar selector-to-solute ratio of ca. 1:11). This showed a negligible effect on signal intensities compared to the experiments using 5 mg (corresponding to a molar selector-to-solute ratio of ca. 1:4). Concurrently, an increase to 15 mg of CSP (molar selector-to-solute ratio of ca. 1:1.4) also showed no improvement, but rather deterioration of the spectra due to stronger signals (noise) from the macromolecular species (sorbent) and more intense diagonal peaks. This increase in intensity also hampers the assignment of the signals of the cross-peaks lying close to the diagonal peaks. Hence, the amount of 5 mg of CSP turned out to be close to optimal conditions for the present trNOESY experiments.

The trNOESY experiment with 5 mg of CSP in methanol-d_4 was also repeated with racemic DNB-Leu instead of individual single enantiomers. An averaged response (i.e., trNOE intensities) was expected to be observed compared to that for the individual (*R*)- and (*S*)-enantiomers of DNB-Leu. The trNOESY spectrum shows the same pattern of negative cross-peaks as discussed above for the single enantiomers. Remarkably, the corresponding cross-peak signal intensities indeed revealed averaged values, namely only about 45% of the intensity of the (*S*)-enantiomer but higher intensity than that of the (*R*)-enantiomer.

Binding affinities and stereoselectivity of binding, as well, are strongly affected by the medium. Accordingly, the type of solvent (i.e., mobile phase) should also exert a significant effect on the measured negative cross-peak intensities in the trNOESY spectra. The experimental data confirm this assumption. The use of acetonitrile-d_3/D_2O (90:10, v/v) as liquid phase results in an interaction strength which is significantly diminished. This information can be derived from the weak negative cross-peak signal intensities (Figure 6). Such results are in agreement with chromatographic findings. Acetonitrile has much higher elution strength, leading to weaker binding of the DNB-Leu analytes. Solvophobic, van der Waals type, and $\pi-\pi$ (between the aromatic electron-rich quinoline moiety and the electron-poor dinitrobenzoyl group) interactions are all weakened by the presence of acetonitrile compared to methanol. Moreover, the aqueous component may interfere with electrostatic interactions (including ionic and hydrogen-bonding interactions). It was already shown that at neutral or slightly basic pH, which will be obtained with the above mobile phase, the ion-exchange capacity of the quinine carbamate CSP and hence the binding strength are reduced. At this point it is noted that for chromatography slightly different conditions are required compared to those employed in the present NMR experiments (for typical mobile phase in chromatography see Experimental Section). An optimum pH of 6 was established for such enantioselective anion-exchange chromatography. Also, to balance the otherwise very strong ionic interactions under such conditions, counterions

Table 1. Overview of Observed Intermolecular ROE Correlations Obtained by 2D-ROESY NMR Spectroscopy of 1:1 Complexes of (*R*)- and (*S*)-Enantiomers, Respectively, of DNB-Leu and *O*-9-(*tert*-Butylcarbamoyl) Quinine in Methanol-d_4 Solution

	tBuCQN															
DNB-Leu	**2′**	**8′**	**3′**	**5′**	**7′**	**9**	**10**	**11**	**2**	**12**	**6+8**	**3**	**7**	**5**	**4**	**16,17,18**
5+6	*S*/*R*	S	*R*	*S*/*R*	R	*S*/*R*	R	*S*/*R*		*S*/*R*		*R*	*S*			*S*/*R*
3+4			*R*	*R*						*S*/*R*		*S*/*R*				
2	S		*S*					*R*								
2′,4′,6′	*S*/*R*	*S*/*R*	*S*/*R*	*S*/*R*	*R*	*S*/*R*		*R*		*S*/*R*						*S*/*R*

[a] As evident from the Table, the 2D-ROESY NMR spectrum of the (*R*)-enantiomer showed more ROE cross-peaks. These, however, were approximately 50% weaker than the ones from the (*S*)-enantiomer.

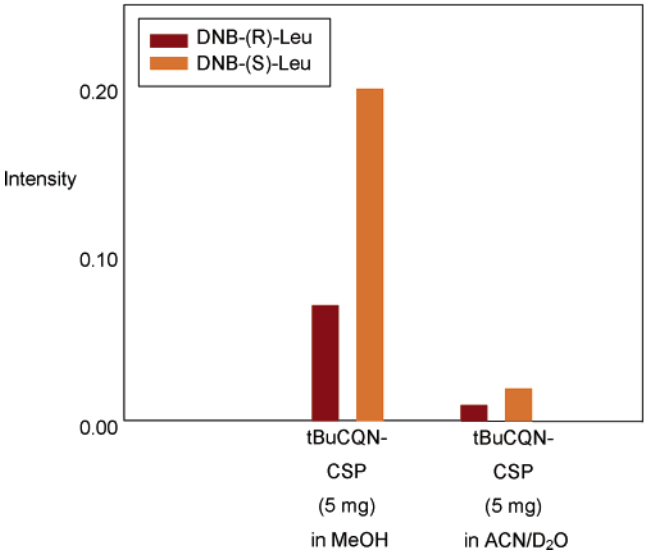

Figure 6. Negative cross-peak signal intensities of the trNOESY HR/MAS NMR spectra of (*R*)- and (*S*)-DNB-Leu in the presence of the CSP and in dependence of the mobile phase composition.

must be present in the eluent in sufficient amount. Otherwise, the solute will not be eluted from the column within reasonable run time.

Overall, all these findings provide conclusive evidence for the suitability of HR/MAS 2D trNOESY NMR spectroscopy for probing chiral recognition of CSPs and other macromolecular chiral media.

Comparison of Effects of HR/MAS 2D trNOESY NMR Spectroscopy with 2D ROESY NMR Spectroscopy in Solution. Recently, detailed NMR spectroscopic investigations of soluble structural analogues of the present selector and analyte pair (methoxy of the selector as well as DNB-amino acid residue replaced by a more bulky neopentyl group) have been presented. These investigations generated insight into the binding events of this system by exploiting information from complexation-induced shifts, job plots, and intermolecular NOEs detected by 2D-NOESY of the diastereomeric complexes.[10] Here, it was of interest to investigate whether the findings and stereoselective preference observed in solution can be directly extrapolated to the suspension state. In other words, it should be examined whether there are any unexpected differences in terms of stereoselectivity between suspended-state trNOESY experiments (with suspended CSP) and solution-state 2D ROESY (with soluble selector) of corresponding associates with DNB-Leu.

The ROESY experiments of 1:1 complexes of the chiral tBuCQN selector with either the (*R*)- or (*S*)-enantiomer of DNB-Leu have been acquired under high-resolution conditions at a magnetic field strength of 600 MHz which, due to the high

Table 2. Chromatographic Results Obtained for Various Chiral Amino Acid Derivatives on tBuCQN-CSP and Relative Signal Intensities (*I*) of Negative Cross-Peaks from the Transfer NOE Measurements of (*R*)- and (*S*)-Enantiomers of Various Amino Acid Derivatives in Presence of the CSP (tBuCQN-Thio-Kromasil)

analyte	k^a	α (*S*/*R*)[b]	$I_{(S)}/I_{(R)}$
(*R*)-Ac-Phe	4.45		
(*S*)-Ac-Phe	6.13	1.38	2.4
(*R*)-DNB-Leu	11.74		
(*S*)-DNB-Leu	186.34	15.88	7.4

[a] Retention factor, $k = (t_r - t_0)/t_0$, wherein t_r is the retention time and t_0 the elution time of an unretained void marker; [b] α $(S/R) = k_{(S)}/k_{(R)}$.

frequency, ensures high intensities of the ROEs that are measured in the rotating frame and therefore always yields positive cross-peaks. The results of the 2D-ROESY measurements are summarized in Table 1. It is seen that for the (*R*)-enantiomer significantly more ROE signals are detected than for the (*S*)-enantiomer. However, the ROE signal intensities for the (*R*)-complex appear to be very weak, while a comparison of the signal intensities with selected cross-peaks show again a higher intensity for (*S*)- than for the (*R*)-DNB-Leu of about 50%. A feasible explanation might be that more directed interactions and tighter binding are created with the (*S*)-enantiomer, whereas the (*R*)-enantiomer shows more, but nondirected interactions, thus less closely approaches the selector moiety. Hence, the outcome of the ROESY experiments corresponds with the above-described trNOESY NMR experiments for DNB-Leu enantiomer and tBuCQN-CSP.

Correlation of the Effects of trNOESY NMR with Chromatographic Data. In addition to the trNOESY investigations of (*R*)- and (*S*)-DNB-Leu **2** both (*R*)- and (*S*)-*N*-acetyl phenylalanine **3** (Ac-Phe) have been subjected to HR/MAS NMR measurement under identical conditions, to assess the general applicability of the procedure for the screening of binding strength and chiral recognition. Again, negative cross-peaks have been detected in the presence of the CSP for the (*R*)- and (*S*)-Ac-Phe, respectively, confirming that interactions do take place between the enantiomers and the tBuCQN-CSP. Since the trNOE cross-peaks of the different analytes were measured between different groups and because conformational changes can take place when analytes are binding to a selector, care must be taken in the interpretation of the data due to the differences in intramolecular distances. Although the relative binding strengths that might be inferred from the trNOESY experiments match the trend of retention factors in chromatography (see Table 2 and Figure 7), this aspect needs to be investigated in more depth and is therefore beyond the scope of this study. Here, the best comparison of trNOE and retention factors (k) is between the enantiomeric pairs, as evident from Table 2. It is clearly shown that the trNOESY cross-peak

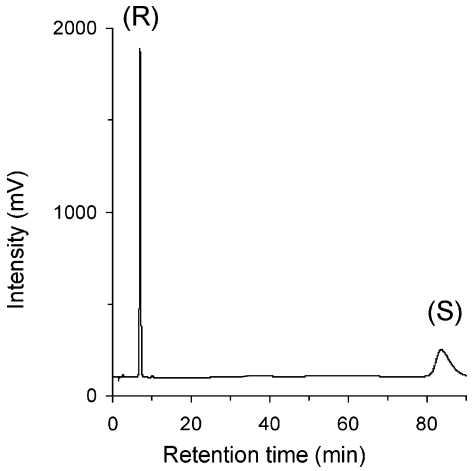

Figure 7. Representative chromatogram for the separation of (*R*)- and (S)-enantiomers of DNB-Leu on (*tert*-butylcarbamoyl)quinine-based CSP. Conditions, see Experimental Section.

intensity confirms the elution order of the analytes since the (*R*)-enantiomers always elute prior to the (*S*)-enantiomers and the magnitude of the (*S*)-enantiomer trNOESY cross-peak is always larger compared to that of the cross-peak obtained from the (*R*)-enantiomer in the presence of the tBuCQN selector. Even if slightly different solvent compositions were used in the NMR and the corresponding chromatographic experiments, it must be noted that the retention order of the enantiomeric analytes should remain the same in the chromatographic experiments with these two mobile phases. However, even if the differences in cross-peak magnitude are small between the enantiomers of (*R*)- and (*S*)-Ac-Phe, the trend is clear.

Conclusions

^{1}H HR/MAS NMR and ^{29}Si CP/MAS NMR spectroscopy have been successfully adopted for the characterization of the surface chemistry of a quinine carbamate-based chiral anion-exchange-type stationary phase. HR/MAS 2D transfer NOESY measurements of (*R*)- and (*S*)-DNB-Leu enantiomers in the presence of the above CSP based on porous silica particles revealed different negative cross-peak intensities, when detected in the free solute as a memory of binding to the CSP. Thus, the capability of HR/MAS trNOESY measurements to be used as a screening technique to probe chiral recognition and stereoselectivity of new CSPs seems plausible. A quantitative measure for stereoselectivity within the series of structurally comparable but distinct solutes with differing binding strengths could not be proven, because the trNOEs were detected on different spin systems. Overall, however, HR/MAS 2D trNOESY may be a valuable tool to rapidly determine the stereoselectivity of different CSPs, especially in light of current drawbacks with combinatorial screening methodologies.

Experimental Section

Materials. The CSP was synthesized from *tert*-butylcarbamoyl quinine and 3-mercaptopropyl-modified silica (Kromasil 100 - 5 μm, EKA Chemicals, Bohus, Sweden) by a procedure described in detail previously.[32] Elemental analysis yielded a coverage of 0.96 mmol thiol groups/g modified silica and a selector coverage of 0.28 mmol/g CSP. Racemic and (*S*)-*N*-(3,5-dinitrobenzoyl)-leucine (DNB-Leu) were supplied by Aldrich (Sigma-Aldrich, Vienna, Austria). The (*R*)-enantiomer of DNB-Leu was synthesized from (*R*)-leucine and 3,5-dinitrobenzoyl chloride (Aldrich). (*R*)- and (*S*)-enantiomers of *N*-acetyl phenylalanine (Ac-Phe) were generously provided by Degussa (Darmstadt, Germany).

For the 2D trNOESY NMR experiments the solutes were dissolved in methanol-d_4 at a concentration of 0.1 mol/L, and the rotor (4 mm ZrO_2 with a detection volume of ca. 60 μL) was filled with 60 μL of a suspension of 5 mg of the CSP in the 0.1 M solute solution.

NMR Spectroscopy. The NMR experiments were performed on Bruker ARX 400 MHz and AMX 600 MHz spectrometers.

All spectra were acquired at 300 K, and the signal of deuterated methanol was used as an internal reference (3.30 ppm). XWINNMR software (Bruker) was used for data acquisition and processing.

The suspended-state HR MAS 2D trNOESY and NOESY NMR experiments were performed at 400.13 MHz using TPPI and a spinning rate of 4500 Hz. A 4 mm HR MAS probe was used together with a rotor containing an inner bottom spacer so that spinning stability was improved. A total of 256 (t_1) $\times$ 4k (t_2) data points were recorded. A total of 256 scans and 16 dummy scans were performed. The relaxation delay was set at 2 s, and values of 900 ms for the 2D NOESY and 100 ms for the trNOESY NMR experiments were chosen for the mixing time. Suppression of the water signal in the methanol was achieved by presaturation with a weak rf field for 1.5 s during the relaxation delay and during the mixing time.

The 2D ROESY NMR spectra were performed at 600.13 MHz using TPPI without sample spinning. A total of 256 (t_1) $\times$ 4k (t_2) data points were recorded. A total of 64 scans and 16 dummy scans were performed. The relaxation delay was set at 2 s, and a value of 300 ms was chosen for the mixing time.

The water signal of the deuterated solvent methanol was suppressed by presaturation with a weak rf field for 1.5 s during the relaxation delay.

HPLC Method. The chromatographic column utilized for the HPLC method was of the dimension 150 mm $\times$ 4 mm i.d. and contained the above specified (*tert*-butylcarbamoyl)quinine-based chiral anion exchanger (tBuCQN-CSP) as stationary phase (see Figure 1). The mobile phase was composed of methanol and 0.1 M ammonium acetate buffer (80:20; v/v), and the pH of the mixture was adjusted to pH 6. The flow rate was set to 1 mL/min, and the temperature of the column was held constant at 25 °C during the separation. The chromatograms were monitored by UV detection at a wavelength of 254 nm.

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