Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1095 (2005) 40–49
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Synthesis and characterization of a novel resorcinarene-based stationary phase bearing polar headgroups for use in reversed-phase high-performance liquid chromatography

Alexander Ruderisch [a], Waldemar Iwanek [b], Jens Pfeiffer [c], Gert Fischer [a], Klaus Albert [a], Volker Schurig [a,*]

[a] *Institute of Organic Chemistry, Auf der Morgenstelle 18, D-72076 Tübingen, Germany*
[b] *Pedagogical University, Institute of Chemistry, Chęcińska 5, 25020 Kielce, Poland*
[c] *Macherey-Nagel GmbH, Neumann-Neander-Straße 6-8, D-52355 Düren, Germany*

Received 7 June 2004; received in revised form 31 May 2005; accepted 28 July 2005
Available online 25 August 2005

Abstract

A novel silica-bonded stationary phase containing a functionalized resorcinarene selector was prepared by a straightforward synthesis. The complete modification of all resorcinic hydroxyl groups was achieved by reaction with isopropyl isocyanate. The derivatized resorcinarene selector was subsequently immobilized via the four alkenyl chains containing a terminal double bond by a free radical-induced reaction on mercaptopropyl-functionalized silica. A comprehensive characterization of the resulting bonded stationary phase was carried out by solid state NMR, IR and elemental analysis. The resulting selector is defined as a "polar headed" reversed phase since the highly ordered polar carbamate groups of the new stationary phase are located, compared to conventional polar embedded stationary phases, at a greater distance from the silica surface. Thus a new concept is introduced in the field of polar modified reversed-phase HPLC. The properties of the novel stationary phase are demonstrated by comparison with commercially available reversed phases.

Keywords: HPLC; Resorcinarene; Polar headed stationary phase; Reversed phase

1. Introduction

A benefit of conventional reversed-phase (RP) HPLC is the large spectrum of selectivities covered by the commercially available stationary phases. Recent efforts of commercial suppliers toward higher hydrophobicity and a lower silanol activity at low pH via improved end-capping procedures resulted in a gradual loss of diversity of individual selectivities of the offered stationary phases. Consequently, stationary phases with additional polar selectivities were developed by modification of the classical alkyl reversed phases, exhibiting an increased retention for analytes with polar functionalities. Two approaches for the introduction of polarity in RP-HPLC stationary phases were used so far. The polar functionality, such as an amide or carbamate group, was directly inserted into the alkyl chain in close proximity to the silica surface (polar embedded phase) or attached to the silica surface by using an additional endcapping reagent bearing a polar functional group (polar endcapped phase) [1] (cf. Fig. 1). Compared to pure *n*-alkyl phases (C_8, C_{18}, C_{30}), the polar embedded phases offer certain advantages, such as stability under highly aqueous conditions, improved peak shape for basic compounds, efficiency for polar analytes and distinct selectivities and retentions. In addition, these phases exhibited enhanced selectivity toward low molecular weight acids. But the increased retention of polar analytes resulted in a loss of hydrophobicity in comparison to conventional *n*-alkyl phases with comparable length of the *n*-alkyl chain [1–3].

Previously, we described the first chiral stationary phase (CSP) containing polar chiral diamide moieties bonded to a resorcinarene matrix for use in enantioselective gas chro-

* Corresponding author. Tel.: +49 7071 29 76257; fax: +49 7071 29 5538.
E-mail address: volker.schurig@uni-tuebingen.de (V. Schurig).

0021-9673/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2005.07.109

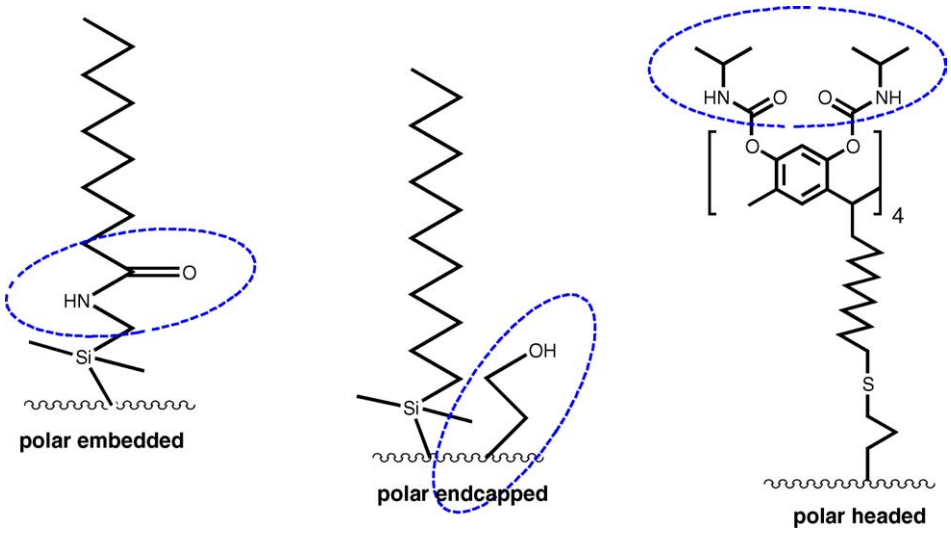

Fig. 1. Schematic representation of three concepts to introduce polar functionalities in RP-type stationary phases.

matography [4]. Resorcinarenes, consisting of four resorcinic units linked via alkylmethylene bridges [5], represent a supramolecular structure reminiscent to calixarenes, crown ethers and cyclodextrins. Due to the preformed cyclic arrangement of the resorcinic hydroxyl groups, the introduced polar headgroups are juxtaposed in a defined array at close proximity to each other on the upper rim of the resorcinarene entity away from the silica support ('polar headed' phase, cf. Fig. 1).

Whereas modified calixarenes have been employed as selectors in different modes of chromatography [6], the development of resorcinarene-based bonded stationary phases in HPLC is still at its infancy. Sokoließ et al. described a bonded *C*-decenylresorcinarene stationary phase for the separation of dibenzo[*b*,*e*]oxepin derivatives and *cis*/*trans* isomers of thioxanthene [7] while the separation of pyrimidine bases on an RP C-18 phase coated with *C*-undecenylresorcinarene was reported by Pietraszkiewicz and Pietraszkiewicz [8]. Recently the first synthesis of a CSP comprising of a nonracemic resorcinarene derivative [9,10] and its use in enantioselective HPLC has been described [10]. First investigations on the retention behaviour of steroids applying a calixarene-based stationary phase were carried out by Skogsberg et al. utilizing modern NMR spectroscopy [11].

In the present work a complementary approach for the introduction of additional polar functionalities in reversed phases is described based on an octakis-functionalized resorcinarene template. The preformed selector can exhaustively be purified before the attachment to silica. Therefore, the problem of column bleeding, which may occur in case of polar embedded phases due to the presence of unreacted isocyanate reagent, is negligible by using the 'polar headed' concept (cf. Fig. 1) involving a resorcinarene template. The absence of column bleeding is essential for the online hyphenation of HPLC with mass spectrometry. In contrast to the contemporarily used concepts of polar embedded and polar endcapped silica-based alkyl bonded phases, the polar groups are completely separated from the surface of the silica particles in the polar headed approach. Since elemental analysis and IR spectroscopy only afford limited information on the nature of the stationary phase, ^{13}C-, ^{29}Si- and ^{1}H-solid state CP/MAS-NMR spectroscopy was used to obtain additional information on the structure of the bonded stationary phase. The chromatographic evaluation showed a different selectivity pattern of the novel stationary phase toward different classes of compounds in comparison with commercially available RP-HPLC counterparts.

2. Experimental

2.1. General

^{1}H and ^{13}C NMR spectra were recorded on a Bruker WM 400 at 400 and 100 MHz, respectively. FAB mass spectra were obtained on a Finnigan MAT TSQ 70 mass spectrometer using 3-nitrobenzylalcohol (NOBA) as a matrix. Melting points were determined with a Büchi B-540 melting point apparatus and are uncorrected. Undec-10-enal was purchased from Sigma–Aldrich, Steinheim, Germany. Silica (Nucleosil 100-5) was obtained from Macherey-Nagel, Düren, Germany. Other reagents and solvents were purchased from Fluka, Buchs, Switzerland and were used without further purification.

The ATR-FTIR measurements of the bare and modified silica were carried out on a Biorad FTS 135 spectrometer coupled with a UMA 500 IR-microscope. The elemental composition was determined using a Carlo Erba elemental analyser 1104.

^{13}C-CP/MAS-NMR spectra were recorded on a Bruker ASX 300 spectrometer (7.05 T) at a spinning rate of 10000 Hz

with 4 mm double bearing rotors of ZrO_2. The proton 90° pulse length was 3.5 μs and the temperature was 295 K. The spectra were obtained with a cross-polarization contact time of 2 ms. The pulse intervals were 1 s. Glycine was used as reference and to adjust the Hartmann–Hahn condition. The number of transients used in the experiments were between 40,000 and 80,000 depending on signal-to-noise ratio.

^{29}Si-CP/MAS-NMR spectra were 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 5.5 μs and the temperature was 295 K. The spectra were obtained with a cross-polarisation contact time of 5 ms. The pulse interval was 1 s.

^{1}H-MAS-NMR spectra were obtained on a Bruker ASX 300 spectrometer (7.05 T). A 4 mm rotor was spun at 14 kHz, the recycle delay was 4 s and the 90° pulse length was 3.8 μs.

The chromatographic system used for the comparison of the different RP-type stationary phases consisted of an LC 6A fitted with a SPD 6A UV detector (Shimadzu, Japan) and a Rheodyne 7725i injector. Data analysis and chromatogram plotting were performed using the CHROMELEON software (Dionex). The resorcinarene-bonded RP was slurry-packed into a 250 mm × 4 mm stainless steel column. The column temperatures were controlled by applying a UC water bath (JULABO) [25 ± 0.1 °C]. The test compounds were purchased from Fluka (Buchs, Switzerland) at the highest available purity. HPLC-grade acetonitrile and water (Chromasolve) were purchased from Riedel-de Haen.

2.2. Syntheses

2.2.1. C-Dec-1-enylresorcinarene [1]

C-Dec-1-enyl-resorcinarene was derived from the ω-unsaturated aldehyde undec-10-enal and resorcinol according to a published method [12,13]. The crude product was recrystallized from acetonitrile. Yield: 45%; mp 290 °C, ^{1}H NMR (DMSO-d_6, 400 MHz, ppm): 1.05–1.35 (s, 48H), 1.90–2.03 (m, 16H), 4.20 (t, J = 7.58 Hz, 4H), 4.85–5.00 (m, 8H), 5.65–5.80 (m, 4H), 6.14 (s, 4H), 7.09 (s, 4H), 8.87 (s, 8H). ^{13}C NMR (DMSO-d_6, 100 MHz, ppm): 27.78, 28.39, 28.58, 29.02, 29.19, 29.27, 32.95, 33.25, 34.16, 102.39, 114.49, 123.01, 124.68, 139.69, 151.72. MS(FAB): 1043.0.

2.2.2. Octakis-O-(isopropylcarbamate)-C-dec-1-enyl-resorcinarene [2]

Isopropylisocyanate (3.4 ml, 34.5 mmol) and triethylamine (4.02 ml, 34.5 mmol) were added to a solution of 1 (3 g, 2.88 mmol) in toluene (50 ml). The reaction mixture was stirred for 24 h at 70 °C. Afterwards additional isopropylisocyanate (1.7 ml, 17.25 mmol) and triethylamine (2.01 ml, 17.25 mmol) were added. The mixture was stirred for further 12 h at 70 °C. After cooling, the solvent was evaporated and the residue was purified by flash chromatography (*n*-hexane/ethyl acetate 1:1). Yield: 3.2 g (65%); mp 152–154 °C; ^{1}H NMR (acetone-d_6, 400 MHz, ppm): 1.00–1.40 (m, 104H), 1.85 (m, 8H), 2.89 (s, 8H), 3.65–3.90 (m, 8H), 4.23 (t, J = 6.83 Hz, 4H), 4.85–5.00 (m, 8H), 5.70–5.85 (m, 4H), 6.62–6.76 (br. s, 4H), 6.95–7.15 (br. s, 4H). ^{13}C NMR (acetone-d_6, 100 MHz, ppm): 23.44, 29.64, 30.26, 30.43, 30.93, 31.12, 35.01, 35.75, 38.62, 44.47, 115.21, 127.10, 140.28, 149.03, 154.81. MS(FAB): 1745.1 $[M - Na]^+$, calc. (1722.1).

2.2.3. 3-Mercaptopropyl-silica [3]

In a 250 ml round-bottomed flask, 9 g of Nucleosil 100-5 were suspended in 100 ml of toluene. The flask was fitted with a rotatory evaporator and the solvent was slowly removed under reduced pressure. In an atmosphere of dinitrogen, 30 ml of toluene, followed by 18.8 ml pyridine and 31.3 ml of (3-mercaptopropyl)trimethoxysilane, were added to the silica. The mixture was heated to 80 °C and agitated for 28 h. After cooling, the silica was collected on a glass filter and washed sequentially with 100 ml each of acetone, diethylether, *n*-hexane and again diethylether, respectively, and finally dried under reduced pressure for few days. The degree of substitution of this functionalized silica was determined by elemental analysis to 0.688 mmol/g. Elemental analysis: S = 2.204%, C = 6.022%, H = 0.708%.

2.2.4. Octakis-O-(isopropylcarbamate)-C-dec-1-enyl-resorcinarene-bonded silica [4]

In an atmosphere of dinitrogen, 2.1 g of **2** and 0.9 g azobis-isobutyronitrile (AIBN) were added to 7 g of **3** in 100 ml of toluene in a 250 ml round-bottomed flask. The mixture was agitated by using a rotatory evaporator at 80 °C for 24 h. Afterwards additional 0.4 g AIBN was added. The mixture was allowed to agitate for further 24 h at 80 °C by using the rotatory evaporator. After cooling, the silica was collected on a glass filter and washed sequentially with 100 ml each of acetone, diethylether, *n*-hexane and (again) diethylether, respectively. It was finally dried under reduced pressure. Elemental analysis: N = 1.784%, C = 16.71%, S = 2.064%, H = 1.657%. ^{29}Si-CP/MAS-NMR: −50.1 (T_1), −58.2 (T_2), −66.5 (T_3), −101.6 (Q_3), −110.7 (Q_4). ^{13}C-CP/MAS-NMR: 154.2, 147.2, 126.6, 117.5, 51.2, 43.2, 36.1, 29.2, 22.2, 11.7.

3. Results and discussion

3.1. Synthesis of the octakis-O-(isopropylcarbamate) resorcinarene-bonded silica stationary phase

The carbamate group is a versatile entity for introducing polarity in polar functionalised RP-systems in HPLC [1]. Whereas the functionalization of cyclodextrin selectors with carbamate moieties is well established [14], the derivatization of resorcinarenes with only four carbamate residues using various reaction conditions has been described previously [15]. The here described resorcinarene selector represents the first example for a complete derivatization of all avail-

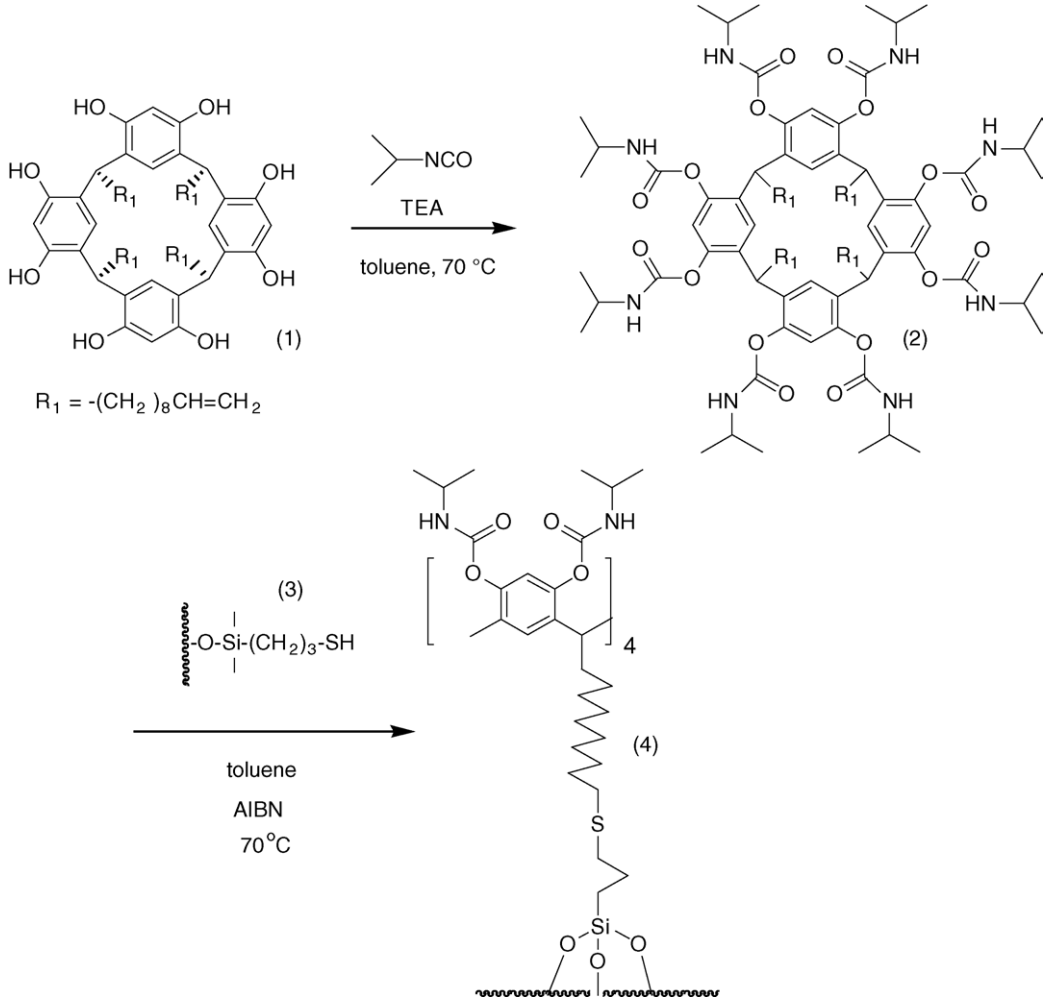

Fig. 2. Synthetic pathway towards the silica-bonded and polar functionalized resorcinarene RP-type stationary phase.

able resorcinic hydroxyl groups with carbamate moieties. The novel silica bonded resorcinarene stationary phase was synthesized according to the route described in Fig. 2. Starting from literature-known *C*-undecenyl-resorcinarene [12], eight polar residues were introduced by reaction of the resorcinic hydroxyl groups with isopropylisocyanate in the presence of triethylamine as organic base in toluene. The derivatizing reagent and base was used in large excess to ensure the complete derivatization of the hydroxy groups. The overall yield was 65% referred to the resorcinarene. The modification of silica was carried out by an established method [16] starting from Nucleosil 100-5 (Macherey-Nagel) and 3-(trimethoxysilyl)propanethiol in toluene in the presence of a small amount of pyridine as organic base. In order not to exert mechanical stress to the silica particles, the reaction suspension was agitated in a flask by employing an ordinary rotatory evaporator instead of using a mechanical stirrer. The loading of the silica was determined to 0.068 mmol/g silica by elemental analysis. The resorcinarene selector was chemically linked to mercaptopropyl-functionalized silica via a free radical-induced reaction. To initiate the reaction, azo-bis-isobutyronitrile (AIBN) was used. Again, the smooth agitating of silica was accomplished by using the rotatory evaporator. Subsequently, the silica was washed carefully by using different solvents.

The main advantage of this synthetic pathway compared to other procedures used to introduce polar groups in RP-systems, is the possibility of a proper workup of the selector before linkage to the silica surface. Column bleeding and ill-defined amounts of residual functionalities on the bonded-silica could be avoided by this strategy.

The surface concentration α_{exp} of the stationary phase was determined by using the method of Unger et al. [17] (cf. Eq. (1), where m is the used amount and M the molar mass of the selector).

$$\alpha_{exp} = \frac{m\,(\mathrm{g/g\,silica})}{M\,(\mathrm{g/mol}) \times s_{Bet}\,(m^2/g)} \quad (1)$$

According to data given by the manufacturer, the specific surface s_{Bet} of Nucleosil 100-5 is 350 m^2/g. Consequently, a surface concentration α_{exp} of 0.5 μmol/m^2 was calculated. The resorcinarene selector is bearing four *n*-alkenyl chains

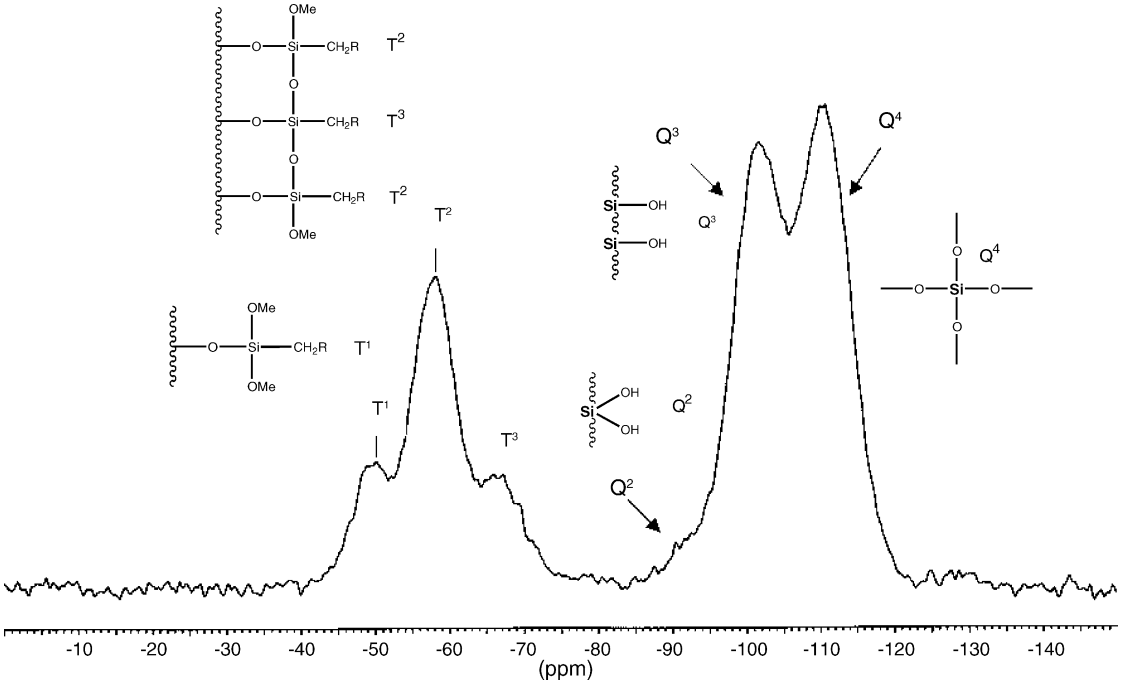

Fig. 3. ^{29}Si-CP/MAS-NMR spectra of the silica-bonded resorcinarene stationary phase.

for attachment to the silica. Hence, as applied to the *n*-alkyl chains, the surface concentration of 2 μmol/m^2 was obtained. This value is comparable to commonly used and commercially available *n*-alkyl-based RP systems. The carbon content of 16.7% determined by elemental analysis is also located in the range which normally is achieved by conventional C_{18}-phases. Therefore, a direct comparison of the selective chromatographic properties of the novel resorcinarene stationary phase and other RP counterparts is feasible.

3.2. Characterization of the stationary phase

3.2.1. CP/MAS-NMR spectroscopy

Solid state ^{29}Si-CP/MAS-NMR spectroscopy provides detailed information on the chemistry of surface modification by immobilizing the resorcinarene selector on silica. The used abbreviated terms of the different silyl species at the surface of the silica gel have been reviewed by Albert [18]. A major drawback in solid-state NMR is the rather poor signal-to-noise ratio due to extended cycle delays associated with long t_1-relaxation times. To overcome this disadvantage the cross-polarization technique has been used. As a result the delay times are much shorter and by transferring proton magnetization to other atoms (silicon, carbon) a characteristic change in signal intensity (depending on the surrounding protons) is achieved. Unfortunately the spectra can therefore no longer be quantitatively evaluated, since even similar groups as for example Q^2 and Q^4 may have different contact times. At the used contact time of 5 ms the Q^2 and Q^3 groups are enhanced to the same extend and therefore their intensity can be compared [19]. Also similar contact times have been measured for the T-groups. Therefore, it is possible to compare T^1, T^2 and T^3 groups to each other [20].

By consulting the Q groups in the obtained silica spectra (cf. Fig. 3) an extremely low quantity of Q^2 groups can be discerned although no additional endcapping step has been performed. The absence of this most active groups in the measured silica indicates a low degree of residual silanol activity. The area of the signals due to the T^2 group are approximately three times larger than the area of that of the T^1 or T^3 groups. Hence, 3-(trimethoxysilyl)propanethiol used to modify the silica mainly tethers via two of the available three binding sides to the silica surface. This represents an unusual high grade of cross-linking for trimethoxysilyl reagents and is usually only achieved by using the more reactive trichlorosilyl reagent [18] or an additional endcapping procedure. However, due to the presence of T^1 groups and the low concentration of T^3 groups an incomplete surface polymerization took place by using the 3-(trimethoxysilyl)propanethiol reagent. Thus, in future endeavours, an improvement of the surface polymerization is necessary to increase the stability of the resulting stationary phase in acidic and basic environments.

To get information on the resorcinarene selector itself, ^{1}H- or ^{13}C-CP/MAS-NMR spectroscopic measurements were applied. Unfortunately, the obtained ^{1}H solid-state-NMR spectra shows very broad lines. This is due to the strong homonuclear dipolar coupling of protons which could only be eliminated at a spinning rate of 100 kHz. Under applied spectrometer conditions, it is still possible to see the expected aromatic signal between 6 and 10 ppm and aliphatic signals between 2 and 0 ppm. The occurrence of both signals indicate the presence of the organic resorcinarene selector.

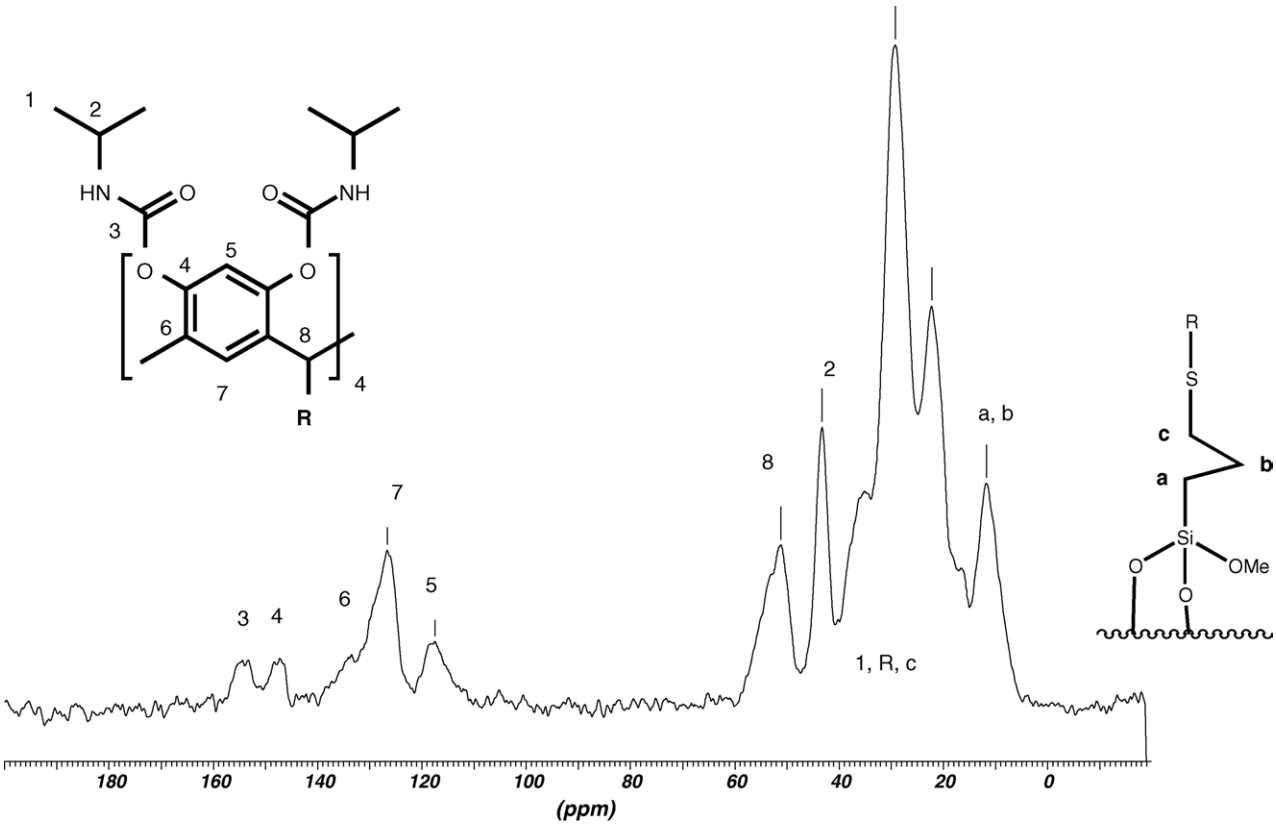

Fig. 4. ^{13}C-CP/MAS-NMR spectra of the silica-bonded resorcinarene stationary phase.

More information on the selector can be obtained from ^{13}C-CP/MAS-NMR (cf. Fig. 4). All signals can clearly be assigned to the organic selector as indicated in Fig. 4. Between 0 and 20 ppm the spacer signals *a*, *b* and *c* and between 20 and 60 ppm the aliphatic signals can be observed and assigned to the structure of the selector. The aromatic region exhibits most of the aromatic carbon atoms with yet different intensity as the result of the cross-polarization technique described above. Polarization is transferred from the protons to the carbon atoms. Therefore, every atom has a

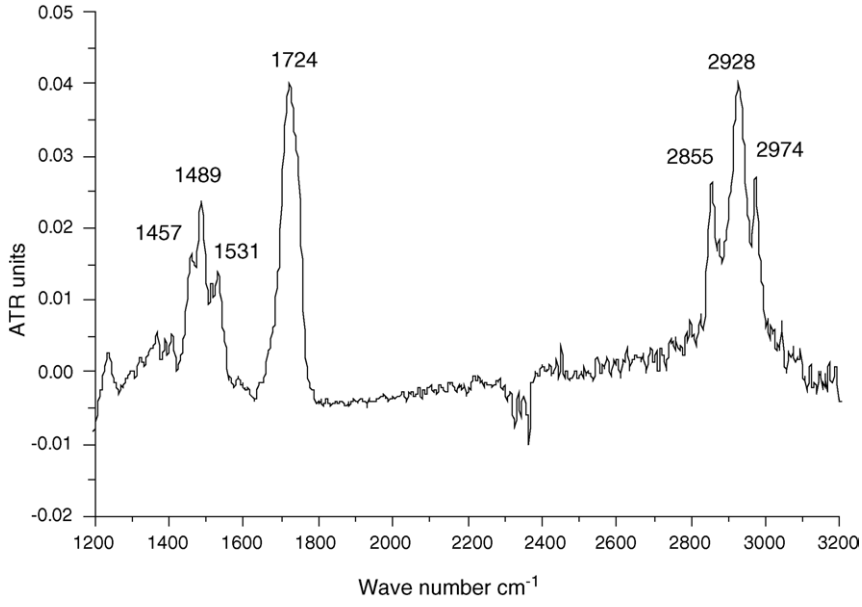

Fig. 5. ATR-FTIR spectra of the silica-bonded resorcinarene stationary phase.

different contact time. Carbon atoms with few or no protons attached to it are not as easily polarized as those in position 5 and 7. In addition to the performed elemental analysis, ^{13}C-CP/MAS-NMR spectroscopy verifies clearly the successful attachment of the resorcinarene selector to the silica particles.

3.2.2. IR-spectroscopy

Infrared spectroscopy is a useful tool to detect characteristic functional groups in organic molecules. For measuring the silica-bonded resorcinarene stationary phase, an IR microscope in the ATR mode was used. Sample preparation of the silica material is minimized compared to commonly used IR techniques. This procedure was recently applied successfully to characterize coatings, adhesives, sealants and resin materials for solid-phase synthesis [21]. The resorcinarene phase and the Nucleosil 100-5 were dried at 60 °C under reduced pressure overnight. The samples were measured and the spectra of the bare silica was subtracted mathematically from the spectra obtained from the novel stationary phase by using the program Origin® [22] resulting in the spectra shown in

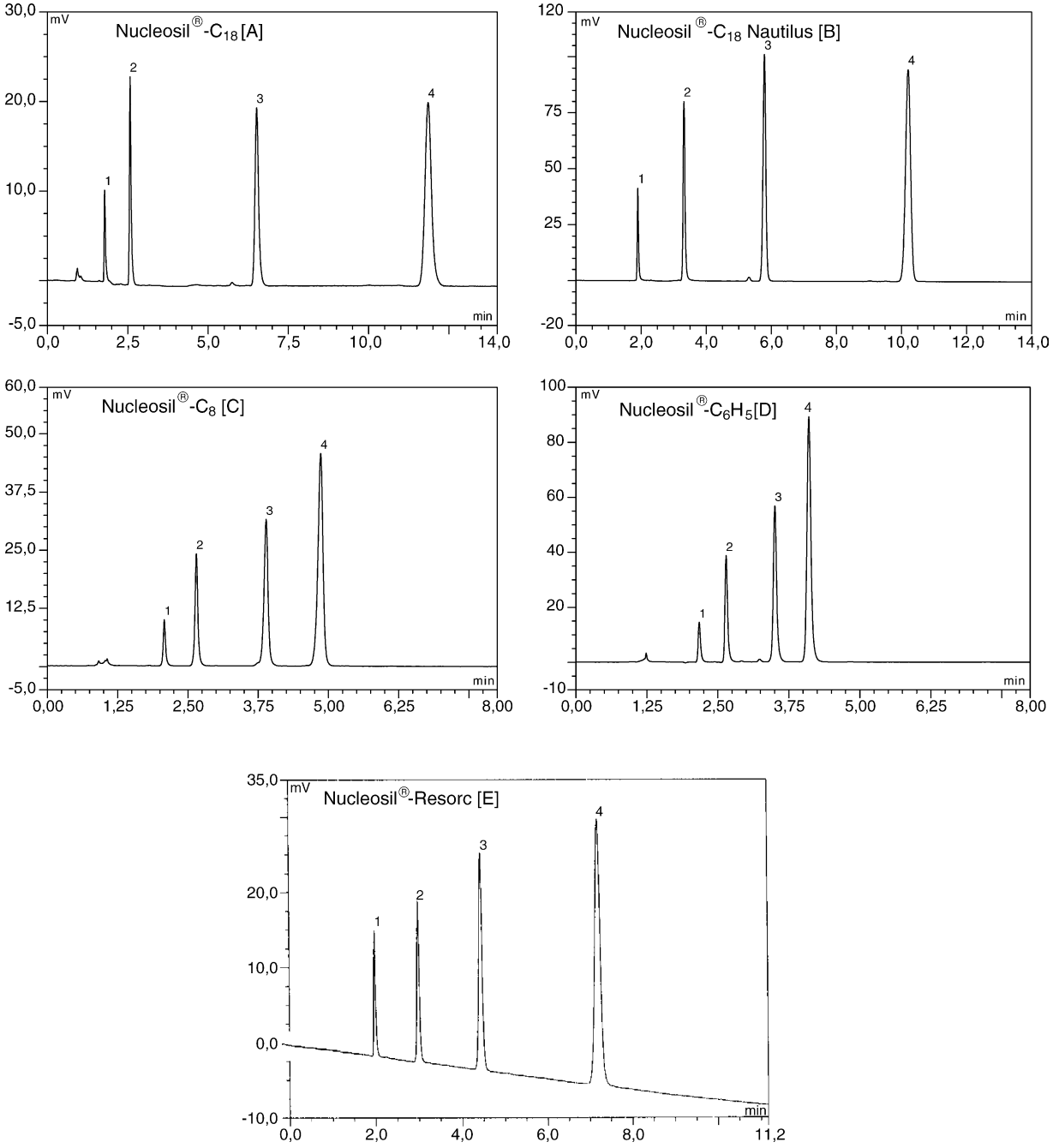

Fig. 6. Comparison of a test mixture on different commercially available RP-type stationary phases in columns [A]–[E] based on Nucleosil® 100-5 [temperature: 25 °C; mobile phase MeCN/water 70:30 (w/w); flow rate 1,0 ml/min.; detection: 254 nm; injection volume approx. 1,0 μl; column dimensions: 250 mm × 4 mm]. Test compounds: (1) uracil; (2) phenol; (3) naphthalene; and (4) anthracene.

Table 1
Chromatographic separation of the test mixture containing uracil, phenol, naphthalene and anthracene on the RP-type stationary phases: [A] Nucleosil® 100-5 C_{18}; [B] Nucleosil® 100-5 C_{18} Nautilus; [C] Nucleosil® 100-5 C_8; [D] Nucleosil® 100-5 C_6H_5 and [E] Nucleosil® 100-5 Resorcinarene [column temperature: 25 °C; mobile phase: acetonitrile/water 70:30 (w/w); flow: 1.0 ml/min; detection: UV at 254 nm]

Retention time (min)	N 100-5 C_{18} (A)	N 100-5 C_{18} Nautilus (B)	N 100-5 C_8 (C)	N 100-5 C_6H_5 (D)	N 100-5 Resorc (E)
t_{R1} uracil	1.78	1.90	2.08	2.17	1.98
t_{R2} phenol	2.57	3.32	2.65	2.64	2.95
t_{R3} naphthalene	6.51	5.79	3.89	3.50	4.44
t_{R4} anthracene	11.85	10.21	4.89	4.10	7.19
void time t_0 (KNO_3)	1.70	1.62	1.84	1.96	1.65
Retention factor k'	**N 100-5 C_{18} (A)**	**N 100-5 C_{18} Nautilus (B)**	**N 100-5 C_8 (C)**	**N 100-5 C_6H_5 (D)**	**N 100-5 Resorc (E)**
k'_1 uracil	0.04	0.17	0.13	0.11	0.20
k'_2 phenol	0.51	1.05	0.44	0.35	0.79
k'_3 naphthalene	2.83	2.57	1.11	0.79	1.69
k'_4 anthracene	5.97	5.30	1.66	1.09	3.36
Separation factor α	**N 100-5 C_{18} (A)**	**N 100-5 C_{18} Nautilus (B)**	**N 100-5 C_8 (C)**	**N 100-5 C_6H_5 (D)**	**N 100-5 Resorc (E)**
$\alpha_{2,3}$	5.55	2.45	2.52	2.26	2.14
$\alpha_{2,4}$	11.71	5.05	3.77	3.11	4.25
$\alpha_{3,4}$	2.11	2.06	1.50	1.38	1.99

Fig. 5. In the range from 2800 to 3000 cm^{-1} the spectral bands of the aliphatic and aromatic C–H valence vibration and at 1457 cm^{-1} of the C–H deformation vibration can be identified. The carbamate groups are represented by the strong C=O valence vibration at 1724 cm^{-1}. The presence of aromatic groups is confirmed by aromatic C=C valence vibrations in the range of 1480–1540 cm^{-1}. The turbulent baseline in the range of 2300–2400 cm^{-1} is due to the incomplete compensation of the CO_2 band in both spectra.

3.3. Chromatographic properties

The chromatographic behaviour of the novel resorcinarene stationary phase [E] was compared with four different commercially available packings [A–D]. From a practical point of view the most common and therefore most important surface modification is the *n*-octadecyl-type (C_{18} [A]). Additionally, the less common *n*-octyl- (C_8 [C]) and phenyl-(C_6H_5 [D]) type and finally, due to its different behaviour from the conventional C_{18} system, a polar embedded stationary phase type (C_{18}-system bearing a polar group within the alkyl chain [B]) were chosen to cover a maximum range of RP-HPLC phases for comparison. Additionally, all functionalities combined in the novel polar headed phase are present in these different commercial available phases. To eliminate the influence of the silica support each evaluated stationary phase was prepared from the same batch of Nucleosil 100-5. A comparison to commercial available resorcinarene-based stationary phase was not performed due to the fact that there was no such phase at the disposal of the present laboratories. Corresponding studies should be carried out in the future. A test mixture containing four different compounds each exhibiting either more hydrophilic or hydrophobic properties were chosen to cover a large range of polarity. The mixture was separated under identical chromatographic conditions. The resulting chromatograms show a characteristic pattern for each type of stationary phase (cf. Fig. 6). The chromatographic data obtained on the five RP-HPLC phases are shown in Table 1. The hold up time t_0 was measured by the retention time of potassium nitrate. Although uracil or thiourea are both commonly used as void time marker, interactions between these analytes and the stationary phases (especially the polar phases) are expected. The comparison of the obtained retention factors *k* (cf. Fig. 7, Table 1) and the separation factors α (cf. Fig. 8, Table 1) clearly shows that the chromatographic performance of the novel resorcinarene phase [E] is different from all commercial RP-phases [A–D] tested.

The main feature of RP-HPLC stationary phases containing additional polar groups is the enhanced retention for polar compounds like phenol compared to conventional C_{18}-systems while on the other hand the total hydrophobic

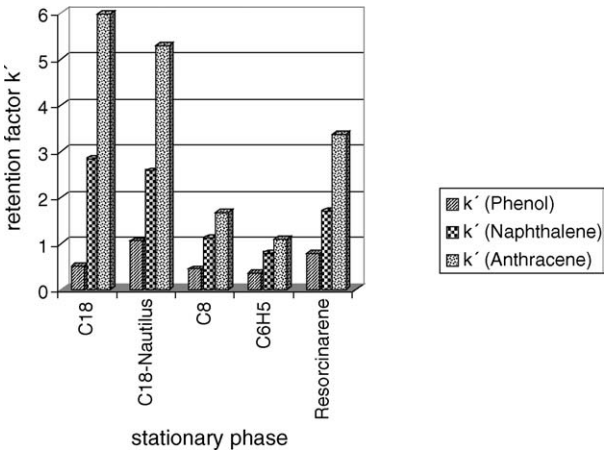

Fig. 7. Comparison of the retention factors *k* of the test compounds phenol, naphthalene and anthracene at 25 °C; acetonitrile/water 70:30; 1 ml/min; 254 nm.

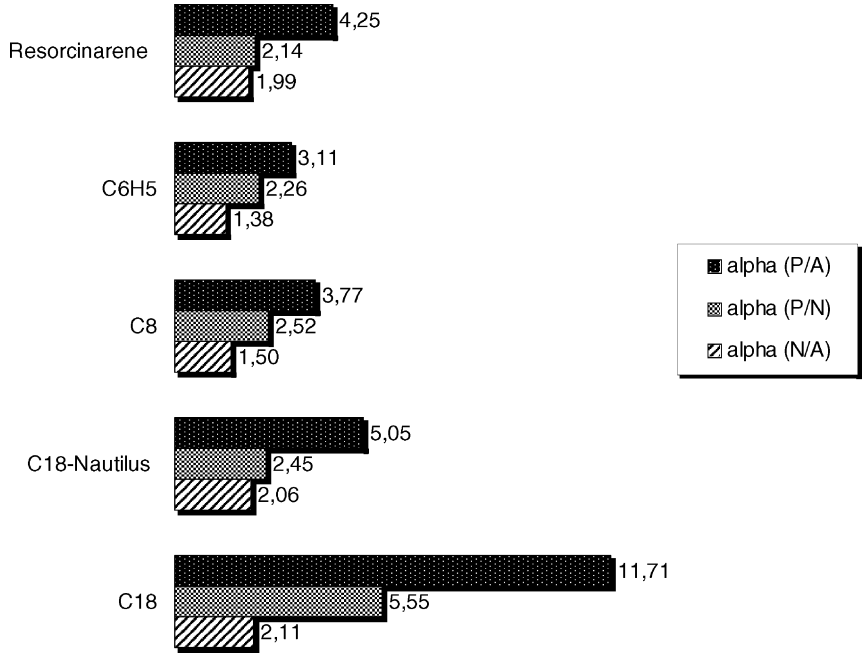

Fig. 8. Comparison of the separation factors α of the test compounds: P = phenol, N = naphthalene and A = anthracene at 25 °C; acetonitrile/water 70:30; 1 ml/min; 254 nm.

interactions are reduced. This effect is clearly demonstrated by the retention factor k of phenol on the C_{18} Nautilus [B] as well as for the new resorcinarene stationary phase [E] in comparison to the *n*-alkyl phase (C_{18} [A])). Therefore, it can be assumed that the retention of phenol is caused by the presence of the polar isopropylcarbamate head group of the resorcinarene selector.

The different retention factors k and the resulting separation factor α for the essentially hydrophobic analytes naphthalene and anthracene on the resorcinarene phase [E] seem to be governed by both the aromatic moieties and the *n*-alkyl chains of the resorcinarene selector. The retention factors k are in fact significantly higher compared to the C_8-phase [C] but lower as on the polar embedded C_{18} system [B]. The same separation factor α of the analytes naphthalene and anthracene on [A], [B] and [E] is diminished on the aryl system [D] in which π-interaction appears to be the predominant contribution to retention. Therefore, it can be assumed that the alkyl chains of the spacer play the major role for the hydrophobic retention. The overall molecular recognition mechanism may be determined by various structural sites of the resorcinarene selector [E]. This new kind of stationary phase offers different modes to govern selectivity via van der Waals-, π–π- or hydrogen bonding interactions. The participation of an inclusion mechanism can be firmly excluded due to the sterical hindrance at the upper rim of the resorcinarene selector. In case of the existence of inclusion phenomena a significant increase of the retention of the analyte should take place compared to the phenyl phase. This was not observed in the present evaluation. Further testing according to the procedures by Tanaka and coworkers [23] and Engelhardt et al. [24] will be necessary in the future to unravel the anticipated complex interaction mechanism in detail.

4. Conclusion

The novel concept of a polar headed RP-type stationary phase was realized by attaching a carbamate functionalized resorcinarene to silica particles. The resulting stationary phase exhibits different retention characteristics as compared to commercially available RP-counterparts. The obtained selectivity may be governed by a multitude of interaction mechanisms. Hence, in addition to the novel approach to introduce polarity in an RP-type stationary phase, the presence of groups of different polarity at the silica surface leads to a multifunctional phase (cf. Ref. [25]). Because of the steric overcrowding of the cavity by polar headgroups, molecular inclusion should be absent. The preliminary results using a prototype column should be completed by further studies concerned with silica deactivation for the analysis of basic compounds, an improved silica loading and possible variations in the carbamate moiety. The first results indicate that the novel concept of ‘polar headed’ stationary phases may complement hitherto applied strategies in the area of polar modified RP-counterparts.

Acknowledgments

The authors thank the ‘Deutsche Forschungsgemeinschaft’ and the ‘Graduiertenkolleg Analytische Chemie’ for financial support. We thank Michael Barth for measuring the ATR-IR spectra.

References

[1] J. Layne, J. Chromatogr. A 957 (2002) 149.
[2] U.D. Neue, Y.-F. Cheng, Z. Lu, B.A. Alden, P.C. Iraneta, C.H. Phoebe, K. van Tran, Chromatographia 54 (2001) 169.
[3] J.E. O'Gara, D.P. Walsh, B.A. Alden, P. Casellini, Th.H. Walter, Anal. Chem. 71 (1999) 2992.
[4] A. Ruderisch, J. Pfeiffer, V. Schurig, J. Chromatogr. A 994 (2003) 127.
[5] P. Timmerman, W. Verboom, D.N. Reinhoudt, Tetrahedron 52 (1996) 2663.
[6] R. Milbradt, V. Böhmer, Calixarenes 2001, Kluwer, Dordrecht, 2001, p. 663 (Chapter 36).
[7] T. Sokoließ, U. Menyes, U. Roth, T. Jira, J. Chomatogr. A 948 (2002) 309.
[8] O. Pietraszkiewicz, M. Pietraszkiewicz, J. Inclus. Phenom. Macro. 35 (1999) 261.
[9] J. Pfeiffer, Doctoral Thesis, University of Tübingen, 1999.
[10] T. Sokoließ, A. Opolka, U. Menyes, U. Roth, T. Jira, Pharmazie 57 (2002) 589.
[11] U. Skogsberg, H. Händel, E. Gesele, T. Sokoließ, U. Menyes, T. Jira, U. Roth, K. Albert, J. Sep. Sci. 26 (2003) 1119.
[12] E. van Velzen, J. Engberseb, D. Reinhoudt, Synthesis (1995) 989.
[13] A. Ruderisch, J. Pfeiffer, V. Schurig, Tetrahedron: Asymmetry 12 (2001) 2025.
[14] T. Takeichi, H. Toriyama, S. Shimura, Y. Takayama, J. High Resolut. Chromatogr. 18 (1995) 179.
[15] O. Middle, W. Verboom, D.N. Reinhoudt, Can. J. Chem. 79 (2001) 1525.
[16] C. Rosini, C. Bertucci, D. Pini, P. Altemura, P. Salvadori, Tetrahedron Lett. 26 (1985) 3361.
[17] K.K. Unger, N. Becker, P. Roumeliotis, J. Chromatogr. 125 (1976) 115.
[18] K. Albert, J. Sep. Sci. 26 (2003) 215.
[19] K. Albert, E. Bayer, J. Chromatogr. 544 (1991) 345.
[20] K. Albert, B. Pfleiderer, E. Bayer, in: D.E. Leyden, W.T. Collins (Eds.), Chemically Modified Surfaces, vol. 2, Gordon and Breach, New York, 1998, p. 287.
[21] H. Ishida, J. Adhesive Sealant Council (1996) 521.
[22] Origin® 6.0, © 1991–1999, Microcal Software, Northampton, MA, USA.
[23] K. Kimata, K. Iwaguci, S. Onishi, K. Jinno, R. Eksteen, K. Hosoya, M. Araki, N. Tanaka, J. Chromatogr. Sci. 27 (1989) 721.
[24] H. Engelhardt, H. Löw, W. Götzinger, J. Chromatogr. 544 (1991) 371.
[25] S. Kromidas, Nachrichten aus der Chemie 51 (2003) 1206.