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Chiral β-cyclodextrin-based polymer supports prepared via ring-opening metathesis graft-polymerization

Betina Mayr, Frank Sinner, Michael R. Buchmeiser*

Institute of Analytical Chemistry and Radiochemistry, University of Innsbruck, Innrain 52A, A-6020 Innsbruck, Austria

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Abstract

A series of norborn-2-ene-derivatized β-cyclodextrins (β-CDs), 6-*O*-(norborn-2-ene-5-carboxyl)-β-CD (**1**), tetrakis(6-*O*-norborn-2-ene-5-carboxyl)-β-CD (**2**), 6-*O*-(7-oxanorborn-2-ene-5-carboxyl)-β-CD (**3**), 6-*O*-(6-norborn-2-ene-5-carbonylaminohexoyl)-β-CD (**4**), 6-*O*-(norborn-2-ene-5-ylmethoxymethylsilyl)-β-CD (**5**), tris(6-*O*-norborn-2-ene-5-ylmethoxymethylsilyl)-β-CD (**6**), tetrakis(6-*O*-norborn-2-ene-5-ylmethoxymethylsilyl)-β-CD (**7**) and hexakis(6-*O*-norborn-2-ene-5-ylmethoxymethylsilyl)-β-CD (**8**), have been synthesized. Compounds **1**–**3** were prepared via reaction of β-CD with norborn-2-ene-5-carboxylic chloride and 7-oxanorborn-2-ene-5-carboxylic chloride, respectively; compounds **5**–**8** were synthesized from norborn-2-ene-5-yl-methyldichlorosilane and β-CD, respectively. Compound **4** was accessible by reaction of norborn-2-ene-5-carboxylaminohexoyl chloride with β-CD. Compounds **1**–**8** were surface grafted onto norborn-2-ene-derivatized silica-based supports using ring-opening metathesis polymerization employing the ruthenium-based initiator bis(tricyclohexylphosphino)benzylideneruthenium dichloride [$Cl_2Ru(CHC_6H_5)(PCy_3)_2$, Cy=cyclohexyl, **9**]. Generally speaking, the resulting chiral stationary phases (CSPs) **I**–**VIII** may be prepared with high reproducibility and may be used within a pH of 2–10. Thus, relative standard deviations (σ_{n-1}) of the mean resolution (R_s) are <7%. The CSPs were used for the enantioselective separation of β-blockers, *N*-dansyl-, *N*-3,5-dinitrobenzoyl- and Fmoc-protected amino acids and were characterized in terms of chemical stability, selectivity (α') and resolution (R_s). Additionally, the role of the spacer as well as influences of capacity and the degree of substitution of the β-CD moiety on the separation characteristics were determined.

Keywords: Chiral stationary phases, LC; Surface grafting; Ring-opening metathesis polymerization

1. Introduction

Chiral stationary phases (CSPs) represent important tools for analytical and preparative scale separations of enantiomers. While various routes for the enantioselective synthesis of chemical compounds, drugs and pharmaceuticals have been developed [1,2], a major part of chiral compounds is still produced as a racemate that needs to be separated carefully into both enantiomers by chiral high-performance liquid chromatography (HPLC). Despite the large variety of systems that are available so far [3–5], intense research still focuses on the economical development of more stable, more efficient and more selective chiral separation systems [6]. For practical reasons, CSPs consisting of surface modified supports are almost solely based on silica.

*Corresponding author. Tel.: +43-512-5075-184; fax: +43-512-5072-677.

E-mail address: michael.r.buchmeiser@uibk.ac.at (M.R. Buchmeiser).

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Taking advantage of the clean and straightforward surface chemistry of silica, a large variety of chiral selectors [7,8] including cyclodextrins (CDs) [9–16] may be attached onto its surface using simple anchors such as polyvinylimidazole, naphthylethylisocyanate [17], amides [18], urethanes [19] or tris(3,5-dimethylphenyl-carbamates) [20]. Consequently, numerous chiral supports based on native and derivatized CDs are now commercially available [21,22]. Representative examples are Cyclobond 2000, Chiraldex (both ASTEC), Nucleodex (Macherey-Nagel) or ChiraDex, ChiraDex GAMMA (both Merck).

Recently, we reported on the use of ring-opening metathesis graft polymerization (ROMP) for the preparation of surface-derivatized silica and polystyrene–divinylbenzene (PS–DVB) supports [23]. In contrast to coating procedures, this approach avoids any loss of specific surface area (σ) or pore-clogging. In this contribution we describe the preparation and properties of a large variety of different β-CD-based graft-type chiral supports.

2. Experimental

General experimental details and instrumentation are described elsewhere [23]. Stainless steel columns (150×2 mm) were used throughout. Silica material (Nucleosil 300-5, 5 μm, 300 Å, σ=100 m^2) was purchased from Merck (Darmstadt, Germany). 5-(Bicyclohept-2-ene-5-yl)methyldichlorosilane (4:1 *endo*/*exo* mixture) and norborn-2-ene-5-yltrichlorosilane (4:1 *endo*/*exo* mixture) were purchased from ABCR (Karlsruhe, Germany). *endo*-Norborn-2-ene-5-carboxylic acid chloride [38] and the initiator bis(tricyclohexylphosphino)benzylideneruthenium dichloride [$Cl_2Ru(PCy_3)_2(CHC_6H_5$, Cy=cyclohexyl, available from Fluka, Buchs, Switzerland] [39] were prepared according to literature procedures and checked for purity by means of nuclear magnetic resonance (NMR) spectroscopy. *endo–exo*-7-Oxanorborn-2-ene-5-carboxylic chloride (77% *endo*) was prepared from freshly distilled furan and acrylic chloride [34]. Purchased starting materials, HPLC solvents as well as the investigated chiral compounds were used without any further purification. DNB derivatives of amino acids were prepared as described in the literature [40]. Spectroscopic data of compounds **2**, **5**–**8** [37] as well as the synthesis of norborn-2-ene derivatized silica are described elsewhere [23]. The average numbers of norbornene units attached to a β-CD were determined by ^{1}H-NMR as well as by titration methods as described in the literature [41]. Elemental analyses were performed at the Institut für Physikalische Chemie, Universität Wien, Vienna, Austria. For HPLC experiments, a LC1 Modul Plus (Waters) and a C-R6A Chromatopac integrator (Shimadzu) were used. Stainless steel columns (GROM Analytik, Herrenberg, 150×2 mm) were used throughout. Injection volumes were 5 μl (80 ng).

2.1. Preparation of monomers

2.1.1. endo-6-O-(Norborn-2-ene-5-carboxyl)-β-CD (1) [42,43]

β-CD (1.55 g, 1.37 mmol), dried in refluxing toluene in a Dean–Stark apparatus, was dissolved in 35 ml dry pyridine and cooled to −20°C. Norborn-2-ene-5-carboxylic chloride (0.24 g, 1.53 mmol) was added. The reaction temperature was allowed to rise to room temperature and stirring was continued overnight. Pyridine was removed under reduced pressure at 40°C until a white residue remained. It was stirred for 10 min in 10 ml sodium hydrogencarbonate, filtered off, thoroughly washed with water and recrystallized from dimethylformamide (DMF)–water. Yield 73%. IR (KBr, cm^{-1}): 3341 $bs_{\nu(OH)}$, 2944s, 1736$_{\nu(C=O)}$, 1080s$_{\nu(C-O)}$; ^{1}H-NMR (DMSO-d_6) δ: 6.11 (d×d, 1H, J_1=5.8 Hz, J_2=2.3, H_2), 5.92 (d×d, 1H, J_1=5.84 Hz, J_2=2.5 Hz, H_3), 5.73 (d, J=4.5 Hz, 7H, H_{OH}), 5.68 [(m broad), 7H, $H_{OH\text{-}3'}$], 4.83 [s (broad), 7H, $H_{1'}$], 4.47 (t, 6H, $H_{OH\text{-}6'}$), 3.66 [m (broad), 12H, $H_{6'a,b}$], 3.64 [m (broad), 7H, $H_{3'}$], 5.56 [s (broad), 14H, $H_{2',4'}$], 1.27 (m, 2H, $H_{7a,7b}$), monosubstituted species (H^1: HC=CH and H^1: OH^6). Elemental analysis calculated for $C_{50}H_{78}O_{36}$ (M_r=1255.2 g/mol): C 47.84, H 6.26; found C 47.3, H 6.71. $\alpha 30/\lambda$=+123.9° (c=0.001 in *N*-methylformamide).

2.1.2. Tetrakis(endo-6-O-norborn-2-ene-5-carboxyl)-β-CD (2) [42,43]

β-CD (1.09 g, 0.96 mmol), dried in refluxing toluene in a Dean–Stark apparatus, was dissolved in

20 ml dry pyridine and cooled to −35°C. Norborn-2-ene-5-carboxylic chloride (1.0 g, 6.39 mmol) was added. The reaction temperature was allowed to rise to room temperature and stirring was continued overnight. Pyridine was removed under reduced pressure until a light yellow residue remained. It was stirred for 10 min in 20 ml acetone, filtered off and recrystallized from DMF–acetone. Yield: 60%. ^{1}H-NMR (DMSO-d_6): δ (selected) 6.0–5.7 (m, HC=CH).

2.1.3. endo–exo-6-O-(7-Oxanorborn-2-ene-5-carboxyl)-β-CD (3)

β-CD (2 g, 1.76 mmol), dried in refluxing toluene in a Dean–Stark apparatus, was dissolved in 35 ml dry pyridine and cooled to −35°C. 7-Oxanorborn-2-ene-5-carboxylic chloride (1.7 g, 11.2 mmol) was added. The reaction temperature was allowed to rise to room temperature and stirring was continued overnight. Pyridine was removed under reduced pressure until a light yellow residue remained. It was stirred for 10 min in 20 ml acetone, filtered off and recrystallized from DMF–acetone. Yield 79%. *endo*:*exo*=4:1. IR (KBr, cm^{-1}): 3300 $bs_{\nu(OH)}$; 2927 s, 1729 $m_{\nu(C=O)}$; 1659 s, 1541 m, 1490 m, 1156 m, 1079 $s_{\nu(C-O)}$, ^{1}H-NMR (DMSO-d_6) δ: 6.39 (s, $H_{2,3}$); 4.98 (s, H_4); 4.86 (s, 7H, H_1); 4.38 (s, broad, $H_{6'OH}$); 4.11 (s, broad, H_4); 3.64 (m, $H_{6'}$); 3.37 (m, $H_{4',2}$); 2.44 (s, broad, H_5); 1.91 (s, broad, H_{6exo}); 1.44 (s, broad, H_{6endo}); ^{13}C-NMR (DMSO-d_6) δ 173.1 (C_7); 145.7 (C_{3endo}); 145.2 (C_{3exo}); 144.4 (C_{3endo}); 143.1 (C_{3exo}); 101.9 ($C_{1'}$); 81.5 ($C_{4'}$); 80.4 (C_4); 77.2 (C_1); 72.9 ($C_{3'}$); 72.0 ($C_{5'}$); 68.9 ($C_{2'}$); 63.8 ($C_{6'+NBE}$); 59.8 ($C_{6'}$); 42.1 (C_5); 28.6 (C_6).

2.1.4. 6-O-(6-endo-Norborn-2-ene-5-carbonylaminohexoyl)-β-CD (4)

6-Aminocaproic acid (3.67 g, 28.0 mmol) was stirred with 56 ml CH_2Cl_2. Trimethylchlorosilane (6.19 g, 57.0 mmol) was added and heated under refluxing for 2 h. The solution was then cooled to 0°C, TEA (4.25 g, 42.0 mmol) and norborn-2-ene-5-carbonyl chloride (4.38 g, 28.0 mmol) was added. The reaction temperature was allowed to rise to room temperature and stirring was continued overnight. CH_2Cl_2 was removed under reduced pressure. The remaining residue was cooled to 0°C, dissolved in 100 ml saturated $NaHCO_3$ solution and stirred for 3 h. The pH was adjusted to 1 by hydrochloric acid and oil remained. The product was extracted with CH_2Cl_2 and dried with Na_2SO_4. CH_2Cl_2 was removed under reduced pressure and the remaining residue was purified by column chromatography (Silica 60, 220–440 mesh) using diethyl ether as the mobile phase. Yield: 41%. IR (KBr, cm^{-1}): $3355_{\nu(N-H)}$, $1700_{\nu(C=O)}$, $1650_{\nu(N-C=O)}$, $1520_{\nu(N-H)}$. ^{1}H-NMR (C^2HCl_3) δ: 6.23 (d×d, 1H, J_1=5.0 Hz, J_2=9.4 Hz, H_2), 5.96 (d×d, 1H, J_1=5.0 Hz, J_2=9.4 Hz, H_3), 5.51 [s (broad), 1H, H_{15}], 3.48 (m, 1H, H_5), 3.20 (d×t, 2H, J_1=11.5 Hz, J_2=10.0 Hz, H_{14}), 3.13 [s (broad), 1H, H_4], 2.91 [s (broad), 1H, H_1], 2.87 (m, 1H, H_{6exo}), 2.35 (t, 2H, J=11.9 Hz, H_{10}), 1.95 (m, 1H, H_{6endo}), 1.64 (m, 2H, H_{11}), 1.55 (m, 2H, H_{13}), 1.30 (m, 2H, H_{12}), 1.22 (d, 1H, J=12.0 Hz, H_{7a}), 1.20 (d, 1H, J=12.0 Hz, H_{7b}). ^{13}C-NMR (C^2HCl_3) δ: 178.6 C_9, 174.8 C_8, 138.0 C_2, 132.5 C_3, 50.2 C_6, 46.4 C_1, 45.1 C_4, 42.9 C_5, 39.4 C_{14}, 34.0 C_{10}, 30.2 C_{11}, 29.5 C_7, 26.4 C_{12}, 24.4 C_{13}. Elemental analysis calculated for $C_{14}H_{21}NO_3$ (M_r= 251.33 g/mol): C, 66.91; H, 8.42; N, 5.57. found: C, 66.86; H, 8.28; N, 5.76.

6-Norborn-2-ene-5-carbonylaminohexane acid (0.250 g, 0.99 mmol) was dissolved in 5 ml pyridine, DMF (23.5 mg, 0.32 mmol) was added and cooled to −35°C. Thionylchloride (0.165 g, 0.46 mmol) was added and the reaction temperature was allowed to rise to room temperature. The solution was cooled again to −35°C and β-CD (0.52 g, 0.46 mmol) dissolved in 10 ml pyridine was added. The mixture was stirred for further 2 h at 0°C and the reaction temperature was allowed to rise to room temperature and stirring was continued overnight. Methanol (10 ml) was added and stirred for 2 h. After removing the methanol under reduced pressure, the remaining residue was stirred for 10 min with acetone filtered off and recrystallized from DMF–acetone. Yield 53% of **4**. ^{1}H-NMR (DMSO-d_6): δ (selected) 6.05 (m, HC=CH), 5.85–5.50 (m, OH^2 and OH^3), 4.82 (H^1), 4.50–3.48 (m, OH^6, H^6, H^5, H^3), 3.45–3.20 (m, H^4, H^2, H_2O), 2.00–1.20 (5×CH_2, 2×CH of norbornene).

2.1.5. endo–exo-6-O-(Norborn-2-ene-5-ylmethoxymethylsilyl)-β-CD (5)

β-CD (1.09 g, 0.96 mmol), dried in refluxing toluene in a Dean–Stark apparatus, was dissolved in

20 ml dry pyridine and cooled to −35°C. Dichloromethylnorborn-2-ene-5-silane (0.58 g, 2.78 mmol) was added. The reaction temperature was allowed to rise to room temperature and stirring was continued overnight. Pyridine was removed under reduced pressure until a white residue remained. It was stirred for 15 min in 20 ml methanol. After removing the methanol under reduced pressure, the remaining residue was stirred for 10 min with acetone filtered off and recrystallized from DMF–acetone. Yield 55%. ^{1}H-NMR (DMSO-d_6): δ (selected) 6.1 (m, HC=CH). monosubstituted species (H^1: OH^6).

*2.1.6. Tris(endo–exo-6-O-norborn-2-ene-5-ylmethoxymethylsilyl)-β-CD (**6**)*

6 was prepared according to the procedure described for monomer **5** using a 4.4-fold excess of dichloromethylnorborn-2-ene-5-silane. Yield: 40%. ^{1}H-NMR (DMSO-d_6): δ (selected) 6.1 (m, HC=CH). trisubstituted species (H^1: OH^6).

*2.1.7. Tetrakis(endo–exo-6-O-norborn-2-ene-5-ylmethoxymethylsilyl)-β-CD (**7**)*

7 was prepared according to the procedure described for monomer **5** using a fivefold excess of dichloromethylnorborn-2-ene-5-silane. Yield: 40%. ^{1}H-NMR (DMSO-d_6): δ (selected) 6.0 (m, HC=CH), 5.80–5.30 (bs, OH^3, OH^2), 5.00–4.70 (s, H^1), 4.50 (m, OH^6), 4.00–3.40 (m, H^6, H^5, H^3), 3.40–3.20 (m, H^4, H^2), 1.80–0.80 (CH_2, CH of norbornene).

*2.1.8. Hexakis(endo–exo-6-O-norborn-2-ene-5-ylmethoxymethylsilyl)-β-CD (**8**)*

8 was prepared according to the procedure described for monomer **5** using a sixfold excess of dichloromethylnorborn-2-ene-5-silane. Yield: 40%. ^{1}H-NMR (DMSO-d_6): δ (selected) 6.2 (m, HC=CH), 6.15–5.90 (bs, OH^3, OH^2), 5.00–4.70 (s, H^1), 4.00–3.40 (m, H^6, H^5, H^3), 3.40–3.20 (m, H^4, H^2), 1.80–0.80 (CH_2, CH of norbornene).

2.1.9. Silanization of silica (NOR-Si-silica)

The corresponding silica was refluxed for 7 h in toluene using a Dean–Stark apparatus in order to remove all water. Toluene was removed, and silanization (6 g silica) was carried out under reflux in methylene chloride (150 ml) using trichloronorborn-2-ene-5-silane (1.25 g, 5.48 mmol) and triethylamine (2.98 g, 29.4 mmol). A reaction time of 6 h was found to be suitable. Elemental (carbon) analysis of an aliquot gave an average norborn-2-ene content of 0.25 mmol/g. Finally, a mixture of dichlorodimethylsilane (0.44 g, 3.4 mmol) and chlorotrimethylsilane (0.71 g, 6.57 mmol) was added, and stirring was continued overnight. After addition of 15 ml methanol stirring was continued 15 min. The silylated silica was filtered off, washed with methylene chloride and dried in vacuo.

2.1.10. Surface grafting of NOR-Si-silica

The initiator (**9**, 1% with respect to the support) was added to a suspension of NOR-Si-silica in dimethylformamide. The mixture was heated to 60°C. After 15 min, a monomer solution (10% with respect to the support) was added and stirring was continued overnight. Polymerization was terminated by addition of 1-hexene. Finally, the product was washed with DMF, methylene chloride, methanol and ether and dried in vacuo.

2.2. pH stability tests

The following procedure is representative. A poly-**1** grafted silica column (Nucleosil 300-5) was used. The mobile phase was adjusted to a pH between 2 and 11 using pH−1 increments and passed through the column at a flow-rate of 0.1 ml/min for 12 h at each pH. After and before each pH change the column was conditioned with the mobile phase used for the proglumide separation for at least 5 h. Separation efficiencies of the column for proglumide were tested after each pH change. Separations were carried out with CH_3CN–CH_3OH–NEt_3–CH_3COOH (90:10:0.1:0.1) as the mobile phase (flow=0.25 ml/min) with five repetitions for each pH change. Resolutions were stable (1.3–1.5) within a pH range of 2–10.

3. Results and discussion

3.1. Preparation of selectors

CDs and CD derivatives are extensively used in chiral chromatography [24,25]. Their structural fea-

tures [26] are well investigated, yet the principles of chiral recognition are still to be understood [3,19,27–32]. In order to extend the applicability of the ROMP concept to these types of selectors, eight polymerizable β-CD derivatives with different degrees of substitution (DSs) and different linkers have been prepared (Fig. 1). For this purpose, norborn-2-ene-5-carboxyl, 7-oxanorborn-2-ene-5-carboxyl, 7-oxanorborn-2-ene-5-carbonylaminohexoyl as well as norborn-2-ene-5-methoxymethylsilyl derivatives were prepared. In addition to the monosubstituted species **1**, **3**, **4** and **5**, the corresponding tris, tetrakis and hexakis derivatives were synthesized. The latter compounds were used to investigate the potential of preparing CSPs with enhanced pH stability by using chiral selectors that may be multiply attached to the support.

Briefly, compounds **1** and **2** were prepared by reaction of *endo*-norborn-2-ene-5-carboxylic chloride with β-CD (Scheme 1). Compound **3** may be prepared by the reaction of *endo*/*exo*-7-oxanorborn-2-ene-5-carboxylic chloride with β-CD. Compound **4** was accessible by reaction of *endo*-norborn-2-ene-5-carboxylaminohexoyl chloride with β-CD. Compounds **5**–**8** were synthesized from *endo*/*exo*-norborn-2-ene-5-yl-methyldichlorosilane with β-CD (Scheme 1B). Following the synthetic set-up, the DS may simply be varied from 1 to 6 by the stoichiometry of the reactants.

While the *average* DS (number of norborn-2-ene

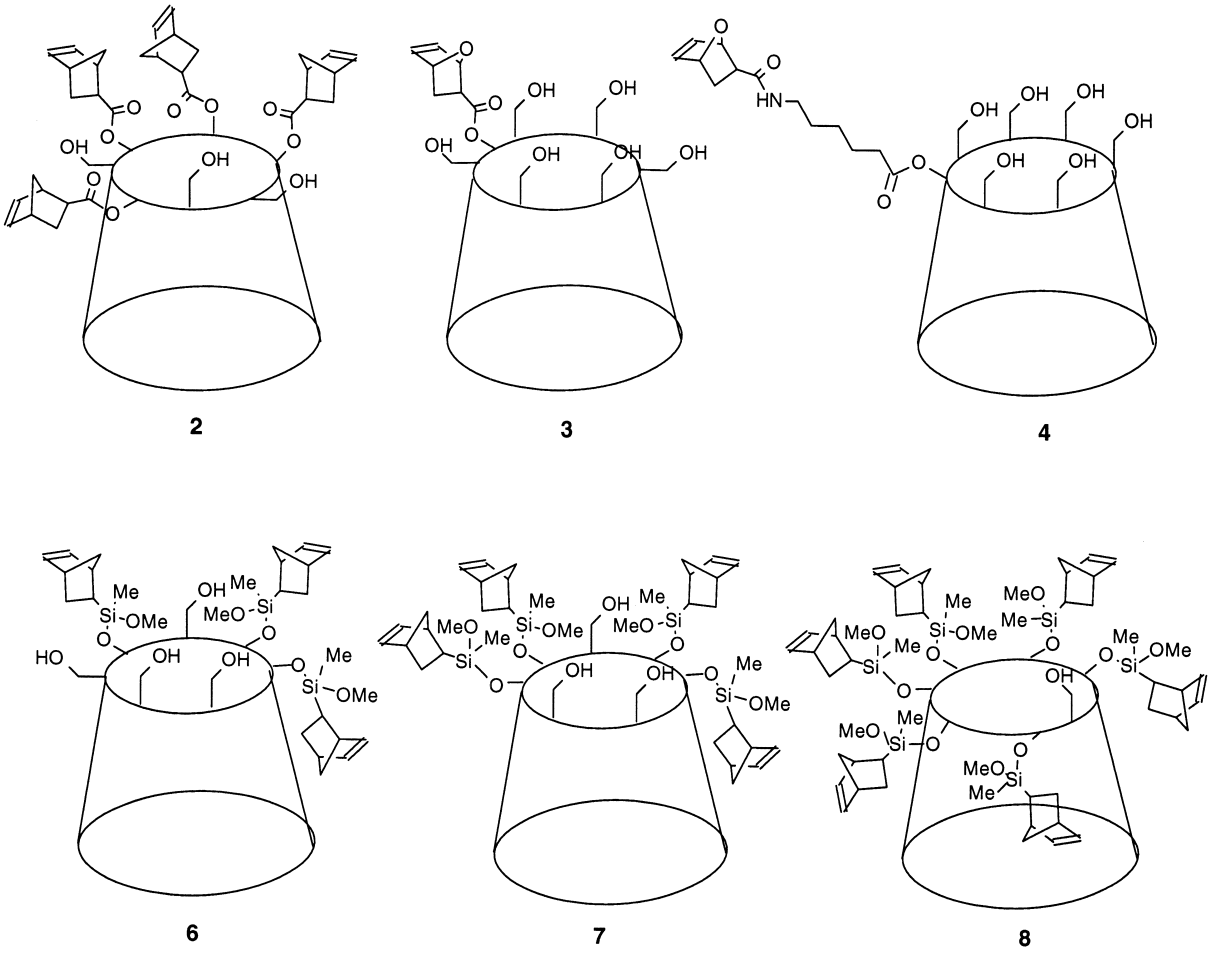
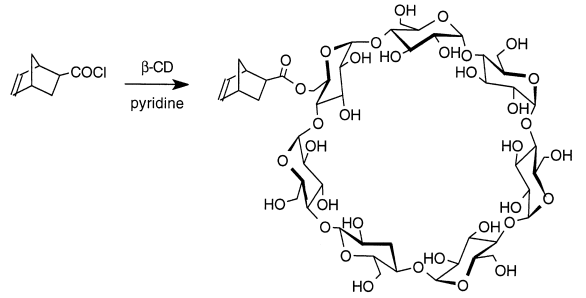
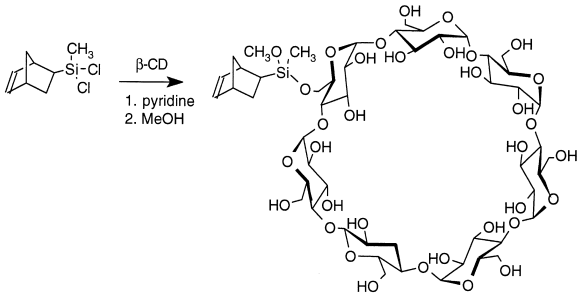


Fig. 1. Structures of compounds **2**–**4** and **6**–**8**. For structures of compounds **1** and **5** refer to Schemes 1 and 2.



A



B

Scheme 1. (A) Preparation of norborn-2-en-5-ylcarboxyl-β-CD (**1**) and (B) norborn-2-en-5-ylmethoxymethylsilyl-β-CD (**5**).

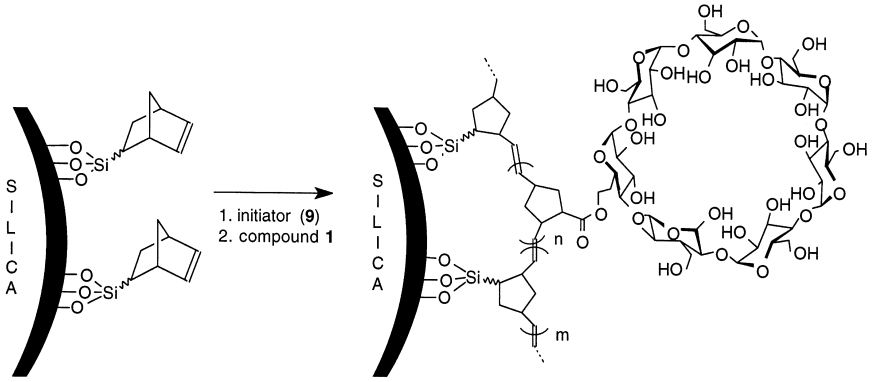
units per CD) may conveniently be determined via ^{1}H-NMR spectroscopy and titration methods (see Experimental), the problem of regioisomerism in particular in **2**, **6** and **7** remains. Thus, the substitution pattern (2-*O*, 3-*O* and 6-*O*) obtained from ^{1}H-NMR by integration must be regarded as an average pattern that does not provide any information about the exact distribution of regioisomers.

3.2. Surface grafting of silica supports

A detailed description of the grafting procedure is given elsewhere [23]. Generally, the synthesis is accomplished by a simple surface derivatization with copolymerizable anchoring groups followed by the use of a well-defined grafting chemistry for the attachment of the corresponding selector. Thus, silica was surface-derivatized with norborn-2-ene-5-yltrichlorosilane. Subsequent ''endcapping'' with a mixture of chlorotrimethylsilane and dichlorodimethylsilane lead to a sufficient derivatization of a major part of the surface silanol groups. In a second step, the initiator **9** was reacted with the support to become heterogenized. From the growing family of potentially useful ROMP initiators [33], initiator **9** [$Cl_2Ru(CHC_6H_5)(PCy_3)_2$] was chosen because of its high tolerance versus protic functionalities and its commercially availability. Finally, monomer was consecutively added to this heterogenized initiator and became grafted onto the surface (Scheme 2).

The resulting graft-polymer is multiply attached to the support via the norborn-2-ene groups and consequently provides a quite inert protective layer. Table 1 gives an overview over the CSPs that were prepared according to this procedure.

At this point, some features of the polymeric backbone need to be addressed. As for most norborn-2-ene- and 7-oxanorborn-2-ene-based ROMP poly-



Scheme 2. Surface-grafting of **1**.

Table 1
Surface-grafted CSPs based on Nucleosil-300-5

Column No.	Selector	Functional group (μmol/g)
I	Poly-**1**	26
II	Poly-**2**	34
III	Poly-**3**	30
IV	Poly-**4**	19
Va	Poly-**5**	32
Vb	Poly-**5**	11
VI	Poly-**6**	22
VIIa	Poly-**7**	28
VIIb	Poly-**7**	22
VIIc	Poly-**7**	25
VIII	Poly-**8**	19

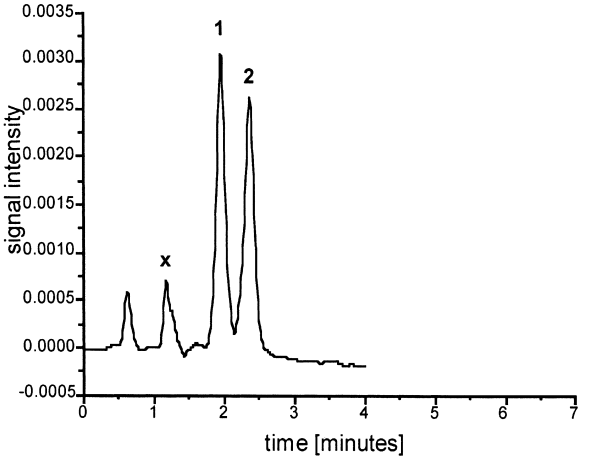


Fig. 2. Representative separation of proglumide on a poly-**8** grafted Nucleosil 300-5. Conditions: T=0°C; flow=0.5 ml/min; ACN–MeOH–AA–TEA (98:2:0.2:0.2). Elution order: x (degradation product of proglumide), 1, 2 (enantiomers of proglumide).

mers prepared from **9**, the configuration of the double bond which results from the metathesis approach displays roughly a 1:3 *cis*/*trans* mixture [34]. Additionally, the use of *exo*/*endo* isomers in the case of monomers **5**–**8** leads to a comparable ill-defined polymer backbone with respect to its overall configuration. Nevertheless, the pure carbon-based backbone is not believed to be involved into the chiral separation process anyway. Another important point is the reproducibility of any synthetic approach. Consequently, three identical columns (**Va**, **Vb**, **Vc**) based on selector **5** were prepared to check the reproducibility of the grafting procedure. Separation of proglumide (Fig. 2) was performed on these columns and the corresponding separation data were collected. Table 2 gives an overview over the determined standard deviations (σ_{n-1}).

As can be deduced from these data, standard deviations of resolution (R_s) were <7%. Another crucial point lies in the stability of any stationary phase. In order to obtain information on the pH stability, a poly-**1**-based column was exposed to pH values between 2 and 11 for several hours. Re-equilibration to separation conditions for proglumide was performed and the column was checked for its separation ability. Chiral separation of proglumide on a poly-**1** grafted silica remained constant within a pH of 2–10. Since chiral separations are drastically affected by the amount of chiral selector as well as by the presence of any free silanol groups that give raise to unspecific retention, any hydrolysis within this pH range may be ruled out. Despite the opportunity of being multiply attached to the support, CSPs based on compounds **2**, **6**, **7** and **8** did not show any differences in pH stability compared to the parent monosubstituted systems. Nevertheless, the substitution of more than one primary hydroxy group gave raise to changes in selectivity and resolution (see below).

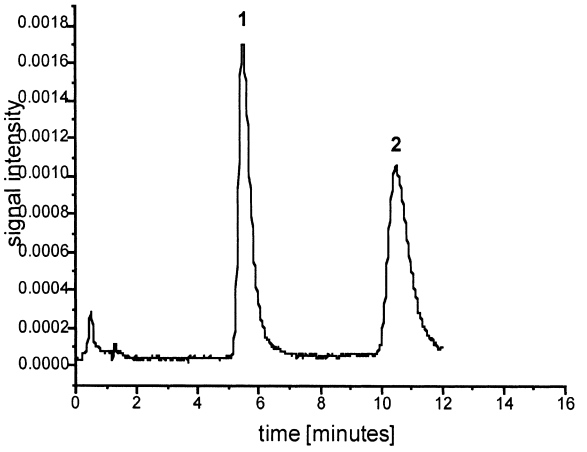


Fig. 3. Separation of DNS-valine on a poly-**8** grafted Nucleosil 300-5. Conditions: T=0°C; flow=0.5 ml/min; ACN–MeOH–AA–TEA (99.8:0.02:0.01:0.03). Elution order 1 (L-DNS-valine), 2 (D-DNS-valine).

Table 2
Reproducibility of derivatization

	Column **VIIa**	Column **VIIb**	Column **VIIc**	σ_{n-1}[a] (%)
Capacity (mequiv./g)	0.028	0.022	0.025	12
α	1.44	1.40	1.42	1.4
R_s	1.96	2.04	2.23	6.7
$N(1)$ (1/m)	6607	7667	7467	7.8
$N(2)$ (1/m)	8640	9013	9373	4.1

[a] Conditions for proglumide separation: T=25°C; flow=0.5 ml/min; ACN–MeOH–AA–TEA (90:10:0.1:0.1).

3.3. Chiral separations

Chiral separations were carried out on the β-CD grafted silica-supports with a large variety of β-blockers, polar *N*-3,5-dinitrobenzoyl (DNB)-protected amino acids as well as apolar dansyl (DNS)- and fluoren-9-ylmethoxycarbonyl (Fmoc)-protected amino acids. A representative separation is given in Fig. 3. Separations were performed in the polar organic mode, using the ''magic'' mobile phase [35,36].

The effect of capacity on the resolution is clearly illustrated by comparing columns **IVa** and **IVb**. A selection of DNS-, DNB- and Fmoc-protected amino acids as well as β-blockers was used for these purposes. As can be deduced from Table 3, R_s increases with increasing capacity.

Since the spacer that connects the β-CD moiety with the polymeric backbone may play a certain role, its potential influence on selectivity and resolution was investigated. In order to provide comparable conditions, the β-CD capacity that is known to show a significant influence on separation efficiency (see above) was kept constant. Columns **I**, **III**, **IV** and **Va** were prepared with an average selector loading of $2–3\cdot10^{-2}$ mmol/g from monomers **1**, **3**, **4** and **5**, respectively, and therefore only differed by the nature of the spacer that links the β-CD unit to the norborn-2-ene. Fourteen different chiral compounds were separated under identical conditions and the resulting data for selectivity and resolution are summarized in Table 4.

As expected, the spacer plays an important role in these chiral separations. Unfortunately, these effects produced by the spacer are different and vary from analyte to analyte. Consequently, it was not possible to make a general statement.

Another crucial point lies in the amount of

Table 3
Effects of capacity on chiral separation

	Column **IVa**				Column **IVb**			
	k_L	k_D	α	R_s	k_L	k_D	α	R_s
DNS-Val[a]	2.35	5.53	2.36	4.65	1.16	2.82	2.44	3.55
DNS-Trp[a]	3.41	5.50	1.61	2.57	1.82	2.76	1.52	1.68
DNS-Thr[a]	0.45	1.51	3.40	2.80	0.16	0.66	4.11	1.54
DNS-Ser[a]	0.77	1.66	2.16	1.63	0.37	0.75	2.02	0.75
DNS-Phe[a]	3.61	5.62	1.56	2.48	1.86	2.91	1.56	1.79
DNS-Met[a]	1.99	3.89	1.95	3.33	0.97	1.88	1.93	2.06
DNB-Val[a]	3.86	4.70	1.22	1.09	2.53	2.85	1.13	0.59
DNB-Trp[a]	5.43	6.32	1.16	0.84	3.63	4.05	1.12	0.54
DNB-Phe[a]	9.01	6.27	1.44	2.33	6.27	4.35	1.44	1.76
Fmoc-Phe[a]	11.04	9.57	1.15	0.88	6.71	5.70	1.18	0.85
Atenolol[b]	6.41	7.98	1.24	0.91	3.88	5.00	1.29	1.03
Propranolol[b]	2.31	2.31	1.00	0	1.26	1.26	1.00	0
Metoprolol[b]	2.21	2.92	1.32	1.05	1.21	1.50	1.24	0.65
Proglumid[b]	2.32	4.12	1.78	3.01	1.23	2.106	2.03	1.72

[a] T=0°C; flow=0.5 ml/min; ACN–MeOH–AA–TEA (99.8:0.2:0.01:0.03).
[b] T=0°C; flow=0.5 ml/min; ACN–MeOH–AA–TEA (98:2:0.2:0.2).

Table 4
Influence of spacers between the norborn-2-ene group and the β-CD moiety on selectivity and resolution

	Column **Va**				Column **I**				Column **III**				Column **IV**			
	k_L	k_D	α	R_s	k_L	k_D	α	R_s	k_L	k_D	α	R_s	k_L	k_D	α	R_s
DNS-Val[a]	2.35	5.53	2.36	4.65	2.03	5.07	2.50	4.75	2.59	7.03	2.71	5.00	0.75	1.49	1.98	2.70
DNS-Trp[a]	3.41	5.50	1.61	2.57	3.52	5.35	1.52	2.12	5.74	8.90	1.55	1.92	1.41	1.82	1.12	1.29
DNS-Thr[a]	0.45	1.51	3.40	2.80	0.47	1.44	3.08	2.38	0.82	2.27	2.77	2.33	0.12	0.33	2.76	1.09
DNS-Ser[a]	0.77	1.66	2.16	1.63	0.86	1.59	1.85	1.08	1.40	2.65	1.89	1.24	0.44	0.44	1.00	0
DNS-Phe[a]	3.61	5.62	1.56	2.48	3.10	5.02	1.62	2.50	4.02	6.59	1.64	2.22	0.72	1.13	1.57	1.49
DNS-Met[a]	1.99	3.89	1.95	3.33	1.72	3.30	1.92	2.71	2.38	4.68	1.97	2.65	1.12	1.59	1.42	1.36
DNB-Val[a]	3.86	4.70	1.22	1.09	4.32	5.50	1.27	1.22	4.93	4.93	1.00	0	1.89	3.04	1.61	2.31
DNB-Trp[a]	5.43	6.32	1.16	0.84	6.61	7.88	1.19	0.89	11.35	15.00	1.32	1.02	2.89	2.89	1.00	0
DNB-Phe[a]	9.01	6.27	1.44	2.33	10.21	6.84	1.49	2.36	12.65	9.75	1.30	1.14	3.65	2.48	1.47	2.06
Fmoc-Phe[a]	11.04	9.57	1.15	0.88	10.15	9.09	1.12	0.67	12.12	12.12	1.00	0	3.51	3.20	1.10	0.68
Atenolol[b]	6.41	7.98	1.24	0.91	9.09	11.68	1.28	1.05	9.57	12.12	1.27	0.91	1.44	1.44	1.00	0
Propranolol[b]	2.31	2.31	1.00	0	2.19	2.60	1.19	0.56	2.71	3.35	1.24	0.68	1.45	1.45	1.00	0
Metoprolol[b]	2.21	2.92	1.32	1.05	2.57	3.27	1.27	0.85	2.88	3.72	1.29	0.90	4.29	5.19	1.21	1.34
Proglumide[b]	2.32	4.12	1.78	3.01	2.08	3.54	1.70	2.57	2.17	3.78	1.74	2.44	0.66	1.00	1.53	1.65

[a] T=0°C; flow=0.5 ml/min; ACN–MeOH–AA–TEA (99.8:0.2:0.01:0.03).
[b] T=0°C; flow=0.5 ml/min; ACN–MeOH–AA–TEA (98:2:0.2:0.2).

norborn-2-ene groups linked to one β-CD unit. While higher DSs allow the synthesis of CSPs based on multiply attached β-CDs, the enhanced DS strongly influences the separation behavior due to changes in polarity and/or accessibility of the β-CD cavity. Columns **I**, **II**, **V–VIII** were prepared with an average selector loading of $2.5 \cdot 10^{-2}$ mmol/g from monomers **1**, **2**, **5**, **6**, **7** and **8**. As shown in Table 5, a higher DS leads to an enhanced resolution up to a DS of 4, while resolutions decrease with DS>4.

One explanation for these findings is, that substitution of a β-CD unit with five or six norborn-2-ene groups results in the deformation of the β-CD cavity. Complementarily, a high DS reduces the ability to form hydrogen bonds that are the most important interactions in the polar-organic mode that was chosen in these investigations. Unfortunately, and as might be expected, each class of compounds exhibits a different sensitivity towards any change in the DS of β-CD. Consequently, in view of the present set of

Table 5
Influence of the amount of norborn-2-ene groups linked to one β-CD unit on selectivity and resolution

	Column **I**				Column **II**				Column **V**				Column **VI**				Column **VII**				Column **VIII**			
	k_L	k_D	α	R_s	k_L	k_D	α	R_s	k_L	k_D	α	R_s	k_L	k_D	α	R_s	k_L	k_D	α	R_s	k_L	k_D	α	R_s
DNS-Val[a]	3.79	9.88	2.60	6.82	3.07	6.57	2.14	4.43	2.35	5.53	2.36	4.65	2.03	5.07	2.50	4.75	8.28	21.01	2.54	7.67	3.71	9.39	2.53	6.65
DNS-Trp[a]	6.47	10.5	1.63	3.45	4.74	6.89	1.45	2.00	3.41	5.50	1.61	2.57	3.52	5.35	1.52	2.12	15.33	23.84	1.56	3.02	6.10	9.73	1.60	3.37
DNS-Thr[a]	1.19	3.69	3.11	4.81	0.61	1.77	2.92	2.79	0.45	1.51	3.40	2.80	0.47	1.44	3.08	2.38	1.98	6.16	3.12	5.46	0.79	2.65	3.34	4.15
DNS-Ser[a]	1.73	3.71	2.15	2.46	0.96	1.94	2.01	1.42	0.77	1.66	2.16	1.63	0.86	1.59	1.85	1.08	2.79	6.37	2.28	3.24	1.17	2.69	2.29	2.20
DNS-Phe[a]	6.14	10.1	1.64	3.57	4.72	6.92	1.47	2.25	3.61	5.62	1.56	2.48	3.10	5.02	1.62	2.50	12.82	19.57	1.53	2.90	5.48	8.88	1.62	3.42
DNS-Met[a]	3.23	6.58	2.04	4.46	2.64	4.60	1.74	2.82	1.99	3.89	1.95	3.33	1.72	3.30	1.92	2.71	6.75	12.83	1.90	4.06	2.93	5.69	1.94	3.80
DNB-Val[a]	5.86	6.42	1.10	0.66	4.57	5.40	1.18	0.97	3.86	4.70	1.22	1.09	4.32	5.50	1.27	1.22	15.87	15.87	1.00	0	6.11	6.80	1.11	0.76
DNB-Trp[a]	10.53	12.82	1.22	1.34	8.29	8.29	1.00	0	5.43	6.32	1.16	0.84	6.61	7.88	1.19	0.89	27.79	36.94	1.33	1.87	10.76	12.70	1.18	1.11
DNB-Phe[a]	16.67	11.49	1.45	3.04	10.12	7.89	1.28	1.70	9.01	6.27	1.44	2.33	10.21	6.84	1.49	2.36	38.72	26.87	1.44	2.68	15.76	11.49	1.37	2.76
Fmoc-Phe[a]	17.52	15.43	1.14	0.93	14.33	13.23	1.08	0.57	11.04	9.57	1.15	0.88	10.15	9.09	1.12	0.67	39.17	36.90	1.06	0.50	18.3	16.44	1.12	0.83
Atenolol[b]	13.53	17.49	1.29	1.42	5.98	7.60	1.27	1.70	6.41	7.98	1.24	0.91	9.09	11.68	1.28	1.05	13.15	17.56	1.34	1.78	8.38	10.91	1.30	1.97
Propranolol[b]	3.14	3.79	1.21	0.79	2.20	2.63	1.19	0.81	2.31	2.31	1.00	0	2.19	2.60	1.19	0.56	4.75	6.11	1.28	1.28	2.46	3.06	1.24	1.03
Metoprolol[b]	4.05	5.45	1.35	1.61	2.44	3.31	1.35	1.61	2.21	2.92	1.32	1.05	2.57	3.27	1.27	0.85	5.39	7.87	1.46	2.19	3.00	4.09	1.36	1.73
Proglumide[b]	4.16	7.185	1.73	3.84	3.73	5.84	1.57	2.87	2.32	4.12	1.78	3.01	2.08	3.54	1.70	2.57	8.72	14.92	1.71	4.54	4.22	6.58	1.56	3.28

[a] T=0°C; flow=0.5 ml/min; ACN–MeOH–AA–TEA (99.8:0.2:0.01:0.03).
[b] T=0°C; flow=0.5 ml/min; ACN–MeOH–AA–TEA (98:2:0.2:0.2).

data, no exact and general correlation between resolution and the chemical nature of a selector may be performed so far. Nevertheless, the trends outlined here, which are also in accordance with data obtained from capillary electrophoresis (CEC) experiments [37], already facilitate the tailor-made synthesis of these types of β-CD-based CSPs.

4. Summary

A new approach for the surface modification of inorganic supports has been developed. ROMP was used for the controlled surface grafting of suitable modified polymer supports. The concept entails a surface modification with polymerizable groups, which become copolymerized with the corresponding functional monomer. Influences of the role of the spacer, capacity and the DS of the β-CD moiety on the separation characteristics have been studied.

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