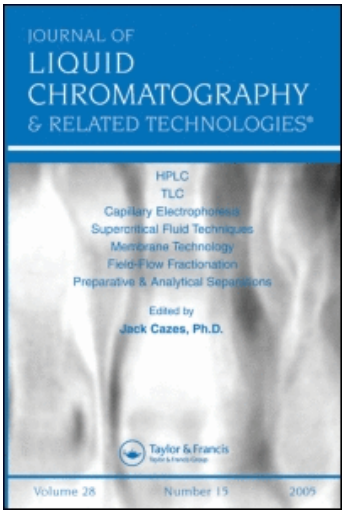

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Preparation and Enantioseparation Properties of Chiral Stationary Phases Derived from Arylcarbamoylated β-Cyclodextrin

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Preparation and Enantioseparation Properties of Chiral Stationary Phases Derived from Arylcarbamoylated β-Cyclodextrin

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Abstract: Four chiral stationary phases were prepared by the immobilization of arylcarbamoylated β-cyclodextrin onto functionalized silica gel via a long covalent linkage. Silica gel was glycidoxy-functionalized, followed by amino-functionalization with 1,6-diaminohexane for further reaction to yield a long chain spacer. The structures of all compounds prepared were characterized by FT-IR, ^{1}H NMR, ^{13}C NMR, and elemental analysis. From the data of elemental analysis, the surface concentrations of the spacer and the derivatized β-cyclodextrin on silica gel were determined, and the results showed that on average, of eight spacers on silica gel, only one was linked to β-cyclodextrin derivative. The enantioseparation capability of the four prepared chiral columns was evaluated by high performance liquid chromatography. The chiral stationary phase that was prepared from phenylcarbamoylated β-cyclodextrin showed the best enantioseparation capability towards a wide variety of racemic mixtures. The chiral stationary phase that was prepared from 3,5-dimethyl-phenylcarbmoylated β-cyclodextrin showed good enantioseparation capability for

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pyrimidine derivatives. The chiral stationary phases that were prepared, respectively, from 1-naphthylcarbamoylated β-cyclodextrin and 2-methoxyphenylcarbamoylated β-cyclodextrin were less effective for enantioseparation.

Keywords: High performance liquid chromatography, Enantioseparation, Chiral stationary phase, Arylcarbamate, β-Cyclodextrin

INTRODUCTION

It is well known that an effective chiral stationary phase (CSP) is the prerequisite for high performance liquid chromatographic (HPLC) technology to separate chiral compounds into their enantiomers. Materials that are employed to prepare CSPs include polysaccharides,[1,2] protein,[3] cyclodextrins (CDs), and small chiral molecules etc.[4,5] Among cyclodextrins, beta-cyclodextrin (β-CD) attracts more attention in the development of CSPs.

The structure of β-CD is featured with a hydrophobic cavity where an inclusion complexation may be most likely formed between guest molecules and its cavity.[6] This may lead to various applications of β-CD, such as enzyme-like catalysis and molecular recognition, etc.[7] Also, researches have revealed that the inclusion complexation between analyte and β-CD can be favorable to chiral discrimination.[8]

In initial attempts in the development of β-CD-based CSPs, native β-CD was applied as the chiral selector.[9,10] The resulting columns were effective mostly under reverse phase conditions.[11] In subsequent research works, β-CD was usually functionalized prior to being used as chiral selector. After derivatization, β-CD type CSPs showed satisfactory performance under a broader range of separation conditions.[11,12] The derivatization of β-CD includes carbamoylation,[13] alkylation,[14] and acylation, etc.[15] As a chiral selector, β-CD and/or its derivatives usually has been bonded to silica gel via a spacer. This allows the resulting CSPs to exhibit high anti-elution ability in wide varieties of mobile phases, such as polar organic solvents and buffer solution.[16]

In the reported study in which the investigation of the influence of spacer on immobilization was involved, Berthod et al. investigated the effect of spacers on chiral recognition of native β-CD type CSPs.[9] In their work, β-CD was immobilized, respectively, by 3-glycidoxypropyldimethylethoxysilane, 3-glycidoxypropyltrimethoxysilane, and trimethoxy[2-(7-oxabicyclo[4.1.0]hept-3-yl)ethyl]silane. However, no significant difference was found in terms of surface concentration and chiral recognition capability of these CSPs.

In this work, we aim at comparing the difference of enantioseparation capability of CSPs prepared from different carbamoylating agents. β-CD was first derivatized by four acryl isocyanates, followed by the immobilization via a long spacer, which was produced by two-step functionalization of silica

gel, i.e., glycidoxy-functionalization and amino-functionalization. The resulting CSPs were evaluated by the enantioseparations of pyrimidine compounds and other racemic mixtures. The utilization efficiency of the long functional groups on silica gel in the use of immobilization of β-CD derivatives was also discussed.

EXPERIMENTAL

Materials

β-CD, phenyl isocyanate, substituted phenyl isocyanates, naphthyl isocyanates, 1,6-diaminohexane, pyridine, and tetrahydrogenfuran (THF) were purchased from Fluka (Sweden). 3-Glycidoxypropyltrimethoxysilane and N, N-dimethylformamide (DMF) were purchased from Sigma-Aldrich (USA). Pyridine was treated with calcium hydride under reflux and was re-distilled prior to use. THF was refluxed with sodium and re-distilled before use. Other chemicals were used as received. Silica gel was obtained from Kromasil (Sweden), with a particle size of 5μ, pore size of 100Å, and surface area of $300\,m^2\,g^{-1}$.

Instrumentation

NMR spectra were performed on a Bruker DPX 400 NMR instrument (Switzerland) with tetramethylsilane as a reference. The frequency was set at 400 MHz for ^{1}H NMR, and 100 MHz for ^{13}C NMR. The solvents used were DMSO-d_6 for the detections of mono-(6-tosyl-6-dexoy)-β-CD and mono-(6-azido-6-deoxy)-β-CD; or $CDCl_3$ for the other compounds. FT-IR spectra were performed on a Bio-Rad FTS-165 FTIR spectrometer (USA) with KBr pellets. The IR spectra were recorded within a range of 4000–$400\,cm^{-1}$. Elemental analysis was performed on a Perkin-Elmer 2400 CHN analyzer (USA). Optical rotation was determined with an ADP 220 polarimeter (UK) at 25°C using sodium D line (598 nm) as the light source. The solutions were prepared with DMF for the optical rotation measurements of mono-(6-tosyl-6-dexoy)-β-CD and mono-(6-azido-6-deoxy)-β-CD; or acetone for the other compounds. All concentrations were of 0.5% ($g\,mL^{-1}$). Melting point was determined using a Büchi B-545 melting point apparatus (Germany). The HPLC system consisted of a Perkin Elmer series 200 LC pump (USA), a Perkin Elmer 785A UV/VIS detector (USA), a Hewlett Packard series 1100 autosampler (USA). The pump, the autosampler, and the detector were connected to a computer via a Perkin Elmer Nelson series 900 Interface (USA) and a Perkin Elmer Nelson series 600 Linker (USA). The prepared CSPs were packed into stainless steel columns

(Ø 4.6 mm × 250 mm) with an Alltech air pump (USA). Enantioseparation was fulfilled at room temperature (23°C). The wavelength of UV detector was set at 225 nm to run the separation.

Preparation of CSPa–d

The scheme for synthesis of β-CD derivatives and CSPs is shown in Figure 1.

Mono-(6-tosyl-6-dexoy)-β-cyclodextrin (1)

Monotosylated β-cyclodextrin was prepared according to the procedure described in literature with a yield of 41%.[17] Its melting point was measured as 180–181°C, and its specific rotation was determined as

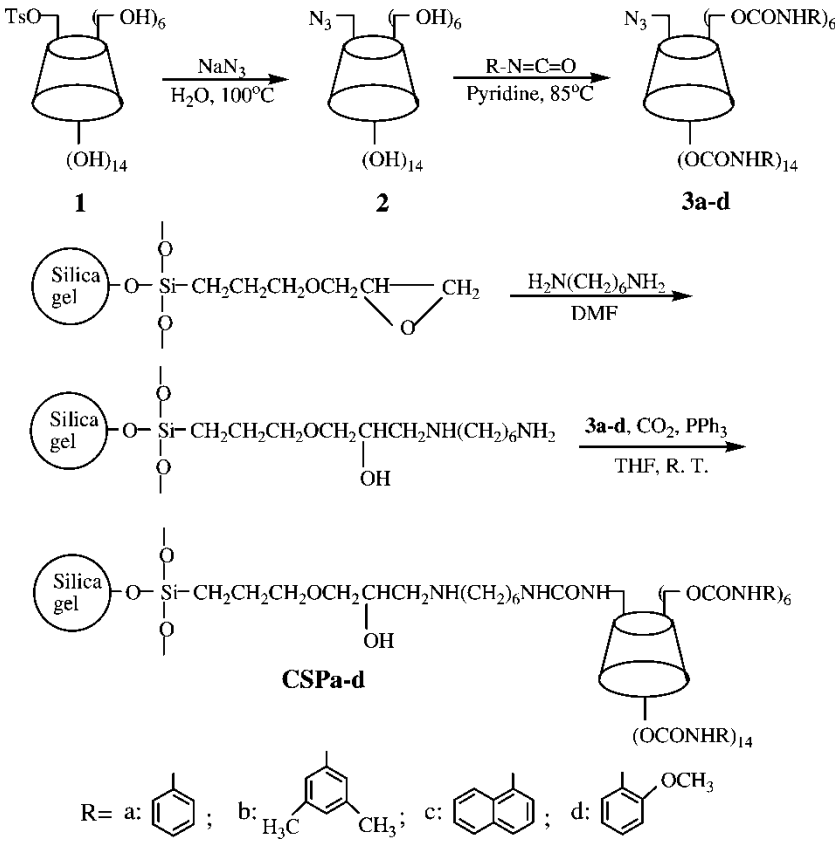


Figure 1. The synthetic scheme for β-cyclodextrin derivatives and CSPa–d.

+105.36°. The chemical shifts (ppm) of its ^{1}H NMR are shown as follows: 7.75 (d, 2H, J = 8.1), 7.43 (d, 2H, J = 8.1), 5.84–5.54 (br, s, 14H), 4.91–4.74 (m, 7H), 4.57–4.16 (m, 6H), 3.79–3.18 (overlapped with HDO, m, 42H), 2.48 (s, 3H). The chemical shifts (ppm) of its ^{13}C NMR are shown as follows: 144.9, 132.7, 129.9, 127.6, 102.0, 81.5–80.8 (m), 73.1–69.0 (m), 60.0–59.3 (m), 39.8–39.2 (m), 21.2. Its structure conformed to the absorbance occurred in FT-IR spectrum. The corresponding wavenumers of absorbance are shown as follows: 3683–3007 cm^{-1} (broad, arom C–H and OH overlapped), 2924 cm^{-1} (C–H), 1596 cm^{-1} (arom C=C), 1240 cm^{-1}, 1083 cm^{-1} (C–O–C).

Mono-(6-azido-6-deoxy)-β-cyclodextrin (2)

Compound **2** was prepared by following Melton's method[18] with a yield of 87%. Its melting point was measured as 219–220°C, while its specific rotation was determined as +141°. The chemical shifts (ppm) of its ^{1}H NMR are shown as follows: 5.81–5.62 (m, 14H), 4.91–4.73 (m, 7H), 4.59–4.14 (m, 6H), 3.76–3.22 (overlapped with HDO, 42H). The chemical shifts (ppm) of its ^{13}C NMR are given as follows: 102.2–101.5 (m); 82.9–81.3 (m), 73.0–70.1 (m), 59.8, 51.0, 40.1–38.4 (m). Its structure was confirmed by FT-IR, and the corresponding wavenumbers of absorbance are presented as follows: 3683–3215 cm^{-1} (OH), 2923 cm^{-1} (C–H), 2106 cm^{-1} ($-N_3$); 1232 cm^{-1}, 1068 cm^{-1} (C–O–C).

Mono-(6-azido-6-deoxy)-perphenylcarbamoylated-β-cyclodextrin (3a)

Vacuum dried compound **2** (2.80 g, 2.41 mmol) was dissolved in anhydrous pyridine (30 mL) and phenyl isocyanate (14.34 g, 0.12 mol) was added. The reaction mixture was stirred for 15 hours at 90°C under a nitrogen atmosphere. After the removal of pyridine and unreacted phenyl isocyanate under reduced pressure, the residue was dissolved in ethyl acetate (100 mL). The solution was washed with water (3 × 50 mL). The organic layer was combined and dried with anhydrous magnesium sulfate. The solution was filtered, concentrated, and the resulting yellowish residue was purified by column chromatography over silica gel, using n-hexane-chloroform (1:4) as eluent. After the removal of the eluent in vacuum, light yellow powder **3a** was obtained with a yield of 93%. Its melting point was measured as 194–198°C. Its specific rotation was determined as +56.57°. Compound **3a** was characterized by NMR. The chemical shifts (ppm) of its ^{1}H NMR are shown as follows: 7.62–6.57 (C_6H_5); 5.66–5.47, 5.32–4.84, 4.80–3.48 (β-CD CH_2, β-CD C*H*, CO–N*H*). The chemical shifts (ppm) of its ^{13}C NMR are shown as

follows: 153.9–152.7 (m), 138.2–136.8 (m), 128.7–128.4 (m), 123.4, 120.6–118.9 (m), 98.8–98.5 (m), 77.4–76.5 (m), 73.3–69.1 (m), 64.3, 31.2. The structure of compound **3a** was confirmed by FT-IR spectrum. The corresponding wavenumbers of absorbance are shown as follows: 3396–3310 cm^{-1} (amide N-H); 3064 cm^{-1} (arom C–H); 2956 cm^{-1} (C–H); 2108 cm^{-1} ($-N_3$); 1742 cm^{-1} (ester C=O); 1614 cm^{-1}, 1508 cm^{-1} (arom C=C); 1229 cm^{-1}, 1052 cm^{-1} (C–O–C).

Mono-(6-azido-6-deoxy)-perdimethylphenylcarbamoylated-β-cyclodextrin (3b)

By employing the similar procedure, **3b** (8.79 g) was prepared by using 2.80 g (2.41 mmol) compound **2** and 17.71 g (0.12 mol) 3,5-dimethylphenyl isocyanate. Its yield was 89%. Its melting point was measured as 223–231°C, and its specific rotation was determined as +32.70°. **3b** was characterized by NMR. The chemical shifts (ppm) of its ^1H NMR are shown as follows: 7.31–6.34 (C_6H_3); 5.70–5.48, 5.27–4.96, 4.72–4.34, 3.96–3.73 (β-CD CH_2, β-CD C*H*, CO-N*H*); 2.44–1.78 (CH_3). The chemical shifts (ppm) of its ^{13}C NMR are shown as follows: 153.6–152.7 (m), 138.9–136.7 (m), 125.5–124.9 (m), 118.3–116.7 (m), 98.6, 77.4–73.4 (m), 69.9, 63.3, 36.2, 21.1–20.8 (m). The structure of **3b** was also confirmed by FT-IR, and the corresponding wavenumbers of absorbance are shown as follows: 3526–3241 cm^{-1} (amide N-H); 3017 cm^{-1} (arom C–H); 2919 cm^{-1} (C–H); 2115 cm^{-1} ($-N_3$); 1736 cm^{-1} (ester C=O); 1620 cm^{-1}, 1541 cm^{-1} (arom C=C); 1229 cm^{-1}, 1095 cm^{-1} (C–O–C).

Mono-(6-azido-6-deoxy)-pernaphthylcarbamoylated-β-cyclodextrin (3c)

By employing the similar procedure, **3c** (9.52 g) was prepared with 2.80 g (2.41 mmol) compound **2** and 20.36 g (0.12 mol) 1-naphthyl isocyanate. Its yield was 87%. Its melting point was measured as 178–183°C. Its specific rotation was determined as +103.50°. **3c** was characterized by NMR. The chemical shifts (ppm) of its ^1H NMR are shown as follows: 8.43–6.37 ($C_{10}H_7$); 5.75–3.73, (broad overlapped, β-CD CH_2, β-CD C*H*, CO-N*H*). The chemical shifts (ppm) of its ^{13}C NMR are shown as follows: 154.5–153.9 (m), 133.7–124.9 (m), 121.8–120.0, 98.8, 77.4–76.6 (m), 72.3–70.4 (m), 64.1, 29.8, 17.7. The structure of 3c was confirmed by FT-IR, and the corresponding wavenumbers of absorbance are shown as follows: 3548–3217 cm^{-1} (amide N—H); 3060 cm^{-1} (arom C–H); 2937 cm^{-1} (C–H); 2114 cm^{-1} ($-N_3$); 1742 cm^{-1} (ester C=O); 1547 cm^{-1}, 1498 cm^{-1} (arom C=C); 1217 cm^{-1}, 1047 cm^{-1} (C–O–C).

Mono-(6-azido-6-deoxy)-permethoxyphenylcarbamoylated-β-cyclodextrin (3d)

By employing the similar procedure, **3d** (8.48) was prepared with 2.80 g (2.41 mmol) compound **2** and 17.95 g (0.12 mol) 2-methoxyphenyl isocyanate. Its yield was 85%. Its melting point was measured as 154–160°C. Its specific rotation was determined as +38.77°. **3d** was characterized by NMR. The chemical shifts (ppm) of its ^{1}H NMR are shown as follows: 8.17–6.32 (C_6H_4); 5.68–5.53, 5.37–5.16, 4.92–4.26, 4.11–3.64, 3.32–3.05 (β-CD CH_2, β-CD CH, CO-NH, OCH_3). The chemical shifts (ppm) of its ^{13}C NMR are shown as follows: 152.9–151.7 (m), 148.7–147.5 (m), 128.1–127.0 (m), 122.7–118.9 (m), 110.0–108.8 (m), 99.1, 78.5–76.5 (m), 72.2, 70.3, 63.4, 55.6–54.7 (m). Its structure was confirmed by FT-IR, and the corresponding wavenumbers of absorbance are shown as follows: 3564–3194 cm^{-1} (amide N-H); 3066 cm^{-1} (arom C–H); 2968 cm^{-1} (C–H); 2109 cm^{-1} ($-N_3$); 1736 cm^{-1} (ester C═O); 1602 cm^{-1}, 1541 cm^{-1} (arom C═C); 1242 cm^{-1}, 1052 cm^{-1} (C–O–C).

Functionalization of Silica Gel

Dry silica gel (15 g) was added to a solution of 7 mL 3-glycidoxypropyltrimethoxysilane in 40 mL toluene. The resulting mixture was stirred and refluxed for 8 h under a dry atmosphere. The silica gel was filtered and washed with acetone thoroughly. After the removal of acetone, 15.9 g glycidoxy-functionalized silical gel was obtained. By elemental analysis, the contents of carbon and hydrogen on glycidoxy-functionalized silical gel were found to be 7.48% and 1.16%, respectively.

Glycidoxy-functionalized silica gel (14 g) was added to a solution of 10 g 1,6-diaminohexane in 40 mL anhydrous DMF. The mixture was stirred at 50°C overnight. The solid was then filtered, and was continuously extracted with acetone in a soxhlet extractor for 8 h. Amino-functionalized silica gel (14.3 g) was obtained after drying to constant weight. By elemental analysis, the contents of carbon, hydrogen, and nitrogen on amino-functionalized silica gel were found to be 9.61%, 1.68%, and 1.64%, respectively.

Immobilization of 3a–d

Dry amino-functionalized silica gel (3.5 g) was added to 15 mL THF in which carbon dioxide was then bubbled. A solution of compound **3a** (2.0 g, 0.71 mmol) in 5 mL THF was added, followed by the addition of another solution of 1.40 g triphenylphosphine in 5 mL THF. The mixture was stirred for 12 h with continuous bubbling of carbon dioxide at room temperature.

The solid was filtered and extracted with acetone in a soxhlet extractor overnight. CSPa (3.4 g) was obtained, after the removal of acetone and drying to constant weight under vacuum.

CSPb, CSPc, and CSPd were prepared by using the same procedure.

RESULTS AND DISCUSSION

Besides the characterization by FT-IR and NMR, the β-CD derivatives were also characterized by elemental analysis shown in Table 1. These elemental analysis results demonstrate that the desired compounds have been prepared. The successful immobilization of β-CD derivatives on amino-functionalized silica gel are evidenced by weak but characteristic FT-IR spectra and elemental analysis data shown in Table 2.

However, it should be emphasized that there are two amino groups in each spacer anchored on the surface of silica gel in which the terminal one is primary amine, and another is secondary amine. The derivatized β-CD should selectively react with the terminal amine due to the less steric hindrance and its higher reactivity.

$$\mu\text{mol} \cdot \text{m}^{-2} = \frac{C_1\% - C_0\%}{W \times n \times S} \times 10^6 \quad \text{(Formula 1)}$$

The surface concentrations of long chain spacer and derivatized β-CDs were calculated according to Formula 1 based on the carbon contents. Where, W is atom weight of carbon, which is 12, S is surface area/gram of silica gel. When the surface concentration of the spacer is calculated, $C_1\%$ and $C_0\%$ are, respectively, carbon contents of amino-functionalized silica gel and glycidoxy-functionalized silica gel, where n equals 6, which is the number of carbon atoms contained in one 6-aminohexylamino moiety. The surface concentration of the spacer was calculated as 0.99 μmol m^{-2}. When surface concentration of derivatized β-CD is calculated, $C_1\%$ and $C_0\%$ are,

Table 1. The elemental analysis data of β-CD derivatives

		Elemental analysis (found/calculated, %)		
Compound	Molecular formula	C	H	N
1	$C_{49}H_{76}O_{37}S.6H_2O$	42.64/42.12	6.48/6.30	Nil/Nil
2	$C_{42}H_{69}N_3O_{34}.4H_2O$	40.93/40.94	6.28/6.26	3.20/3.41
3a	$C_{182}H_{169}N_{23}O_{54}$	61.53/61.71	4.61/4.78	8.87/9.10
3b	$C_{222}H_{249}N_{23}O_{54}$	65.26/64.99	6.36/6.07	7.80/7.86
3c	$C_{262}H_{209}N_{23}O_{54}$	69.61/69.27	4.63/4.60	7.04/7.09
3d	$C_{202}H_{209}N_{23}O_{74}$	58.93/58.56	5.25/5.05	7.88/7.78

Table 2. Characterization data of CSPa–d and efficiency of corresponding columns

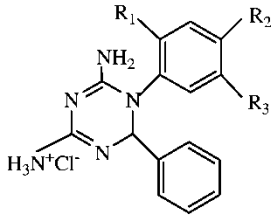
CSPs	Elemental analysis (%) C	H	N	Surface concentration ($\mu mol\,m^{-2}$)	Average number of spacers for one β-CD moiety	IR spectra (cm^{-1})	Efficiency (plates m^{-1})
CSPa	17.55	2.30	2.66	0.12	8.25	1,744, 1,630	42,196
CSPb	19.43	2.64	2.73	0.12	8.25	1,727, 1,647	39,552
CSPc	20.79	2.55	2.78	0.12	8.25	1,717, 1,647	40,073
CSPd	19.44	2.49	2.80	0.13	7.62	1,744, 1,641	42,314

respectively, carbon contents of a prepared CSP and amino-functionalized silica gel; n equals the number of carbon atom contained in one β-CD derivative plus one. The extra one carbon atom came from the carbonyl group that was introduced by the immobilization reaction.

From the results obtained, it was found that although four derivatizing agents had different sizes, the surface concentrations of resulting CSPs were very close in value. Comparing the surface concentrations of β-CD derivative and the spacer, it was also found that typically every eight spacers onto the silica gel, only one was covalently linked to β-CD derivative. However, the surface concentrations of β-CD derivatives in this work are lower than those reported by Berthod.[9] This is possibly due to the bulky size and higher steric hindrance of the β-CD derivatives compared to native β-CD in Berthod's work.

The column efficiencies of these four chiral columns are within a range of 39,552–42,314 plates/meter. These close values allow the comparison studies for enantioseparation properties of these chiral columns. The buffer used for separation was prepared from acetic acid and triethylamine with a concentration of 1% by weight. The enantioseparation capabilities of CSPa–d towards a wide variety of racemic mixtures (structures shown in Figures 2 and 3) were evaluated and the results are given in Tables 3 and 4. Table 3 summarizes the enantioseparation results of synthetic pyrimidine compounds that are the potential antimalaria drugs, while Table 4 summarizes the enantioseparation results of amines, beta-blockers, and alkaloid derivatives, etc. Figure 4 gives the representative chromatograms of enantioseparations of these racemic mixtures.

Thirty-two racemic compounds were separated on CSPa–d. The results show that CSPa was effective in separating pyrimidine derivatives and other chiral compounds, where baseline separations were easily achieved. CSPb



1. R_1=H; R_2=Cl; R_3=H
2. R_1=H; R_2=Br; R_3=H
3. R_1=H; R_2=CH_3; R_3=H
4. R_1=H; R_2=OCH_3; R_3=H
5. R_1=H; R_2=H; R_3=Cl
6. R_1=H; R_2=H; R_3=Br
7. R_1=H; R_2=H; R_3=CH_3
8. R_1=H; R_2=H; R_3=NO_2
9. R_1=H; R_2=H; R_3=CN
10. R_1=Cl; R_2=H; R_3=H
11. R_1=Br; R_2=H; R_3=H
12. R_1=CH_3; R_2=H; R_3=H
13. R_1=OCH_3; R_2=H; R_3=H
14. R_1=H; R_2=CH_3; R_3=Cl
15. R_1=H; R_2=OCH_3; R_3=Cl
16. R_1=H; R_2=CH_3; R_3=CH_3

Figure 2. The pyrimidine derivatives enantiomerically separated by CSPa–d.

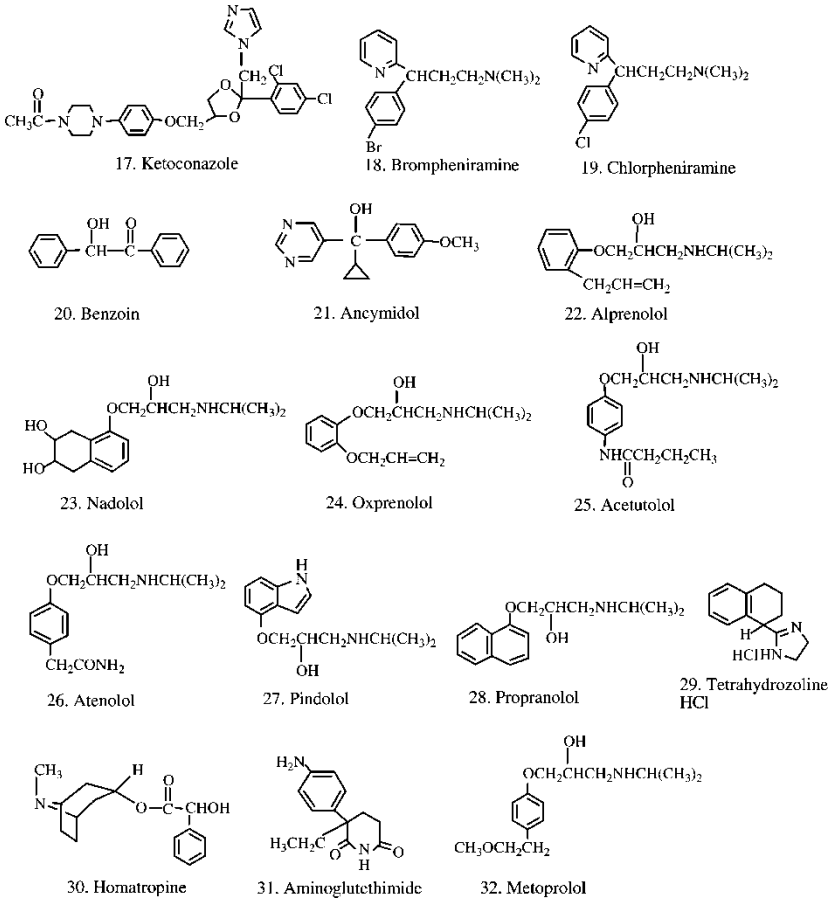


Figure 3. Chiral compounds enantiomerically separated by prepared CSPs.

Table 3. Enantioseparation results of pyrimidine derivatives on CSPa–d

Pyrimidine derivatives	CSPa	CSPb	CSPc	CSPd
1	$k_2 = 1.02$, $\alpha = 2.83$, $R_s = 0.91^b$	$k_2 = 4.41$, $\alpha = 1.68$, $R_s = 1.53^b$	$k_2 = 1.65$, $\alpha = 1.20$, $R_s = 0.61^a$	No separation
2	$k_2 = 2.12$, $\alpha = 1.61$, $R_s = 0.97^b$	$k_2 = 1.49$, $\alpha = 1.73$, $R_s = 1.08^a$	No separation	No separation
3	$k_2 = 1.53$, $\alpha = 1.58$, $R_s = 0.87^b$	$k_2 = 1.00$, $\alpha = 2.50$, $R_s = 1.23^a$	No separation	No separation
4	$k_2 = 3.10$, $\alpha = 1.37$, $R_s = 0.94^b$	$k_2 = 4.94$, $\alpha = 1.20$, $R_s = 0.87^b$	No separation	No separation
5	$k_2 = 0.39$, $\alpha = 2.44$, $R_s = 0.90^a$	$k_2 = 1.69$, $\alpha = 2.25$, $R_s = 0.77^a$	No separation	No separation
6	No separation	$k_2 = 2.91$, $\alpha = 1.20$, $R_s = 0.76^b$	No separation	No separation
7	No separation	$k_2 = 2.59$, $\alpha = 1.24$, $R_s = 0.82^b$	No separation	No separation
8	$k_2 = 1.79$, $\alpha = 2.36$, $R_s = 1.39^b$	$k_2 = 0.97$, $\alpha = 2.49$, $R_s = 1.22^a$	$k_2 = 0.83$, $\alpha = 1.57$, $R_s = 0.66^a$	No separation
9	$k_2 = 4.00$, $\alpha = 1.20$, $R_s = 0.76^b$	$k_2 = 1.98$, $\alpha = 1.37$, $R_s = 0.89^a$	No separation	$k_2 = 5.33$, $\alpha = 7.11$, $R_s = 3.42^a$
10	No separation	$k_2 = 5.49$, $\alpha = 1.17$, $R_s = 1.37^b$	No separation	$k_2 = 3.29$, $\alpha = 5.67$, $R_s = 3.03^a$
11	$k_2 = 2.10$, $\alpha = 1.51$, $R_s = 1.04^b$	$k_2 = 3.60$, $\alpha = 1.25$, $R_s = 0.92^b$	No separation	No separation
12	$k_2 = 0.74$, $\alpha = 1.48$, $R_s = 0.47^a$	$k_2 = 1.58$, $\alpha = 1.52$, $R_s = 1.00^a$	No separation	No separation
13	$k_2 = 5.00$, $\alpha = 1.90$, $R_s = 4.06^a$	No separation	No separation	No separation
14	No separation	$k_2 = 1.40$, $\alpha = 1.40$, $R_s = 0.63^b$	No separation	No separation
15	No separation	No separation	$k_2 = 3.80$, $\alpha = 3.09$, $R_s = 1.46^c$	No separation
16	$k_2 = 1.38$, $\alpha = 1.50$, $R_s = 0.73^b$	$k_2 = 2.15$, $\alpha = 1.41$, $R_s = 0.80^b$	No separation	No separation

Note: Separation conditions.

[a]Buffer (pH 4.65)-methanol, 65:35 (v/v); flow rate 0.5 mL min^{-1}.

[b]Buffer (pH 4.70)-methanol, 80:20 (v/v); flow rate 0.5 mL min^{-1}.

[c]Buffer (pH 4.65)-methanol, 80:20 (v/v); flow rate 0.5 mL min^{-1}.

Table 4. Enantioseparation results of chiral compounds on CSPa, CSPc, and CSPd

Chiral compounds	CSPa	CSPc	CSPd
17	$k_2 = 6.08$, $\alpha = 3.64$, $R_s = 10.82^a$	No separation	No separation
18	$k_2 = 0.87$, $\alpha = 1.26$, $R_s = 0.48^a$	No separation	No separation
19	$k_2 = 1.89$, $\alpha = 1.17$, $R_s = 0.86^b$	No separation	No separation
20	$k_2 = 2.18$, $\alpha = 1.63$, $R_s = 0.62^a$	No separation	No separation
21	$k_2 = 5.22$, $\alpha = 1.09$, $R_s = 0.66^a$	No separation	No separation
22	$k_2 = 2.65$, $\alpha = 1.68$, $R_s = 1.02^b$	No separation	No separation
23	$k_2 = 4.43$, $\alpha = 1.63$, $R_s = 0.83^a$	No separation	No separation
24	$k_2 = 4.45$, $\alpha = 2.00$, $R_s = 1.10^b$	No separation	No separation
25	No separation	No separation	$k_2 = 2.73$, $\alpha = 1.43$, $R_s = 1.02^c$
26	No separation	No separation	$k_2 = 0.63$, $\alpha = 1.70$, $R_s = 0.74^c$
27	No separation	$k_2 = 0.96$, $\alpha = 1.92$, $R_s = 0.74^a$	$k_2 = 1.38$, $\alpha = 1.23$, $R_s = 0.58^a$
28	No separation	No separation	$k_2 = 3.68$, $\alpha = 1.32$, $R_s = 0.75^a$
29	No separation	No separation	$k_2 = 1.85$, $\alpha = 1.29$, $R_s = 0.75^c$
30	$k_2 = 2.28$, $\alpha = 1.11$, $R_s = 0.81^b$	$k_2 = 1.54$, $\alpha = 1.12$, $R_s = 0.67^a$	No separation
31	$k_2 = 3.97$, $\alpha = 1.30$, $R_s = 1.33^b$	No separation	No separation
32	$k_2 = 0.62$, $\alpha = 2.07$, $R_s = 0.83^a$	$k_2 = 3.57$, $\alpha = 1.95$, $R_s = 1.05^b$	No separation

Note: Separation conditions.
[a]Buffer (pH 4.65)-methanol, 65:35 (v/v); flow rate 0.5 mL min^{-1}.
[b]Buffer (pH 4.70)-methanol, 80:20 (v/v); flow rate 0.5 mL min^{-1}.
[c]Buffer (pH 4.65)-methanol, 80:20 (v/v); flow rate 0.5 mL min^{-1}.

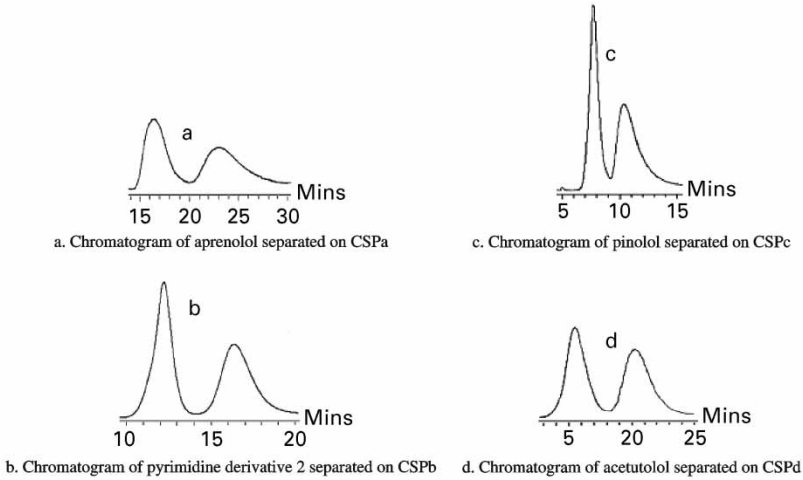


Figure 4. The representative chromatograms of enantioseparations on CSPa–d. (a) Chromatogram of aprenolol separated on CSPa. (b) Chromatogram of pyrimidine derivative 2 separated on CSPb. (c) Chromatogram of pinolol separated on CSPc. (d) Chromatogram of acetutolol separated on CSPd.

was most effective for the enantioseparation of pyrimidine compounds. However, for other tested chiral compounds, no enantionseparation was observed. Compared to CSPa and CSPb, CSPc and CSPd were less effective for enantioseparation. The low chiral recognition capacity of CSPc and CSPd is probably due to the different substitution groups at β-CD moieties. For CSPc and CSPd, there are, respectively, methoxy and benzo-rings located at the *o*-position of carbamoyl. These two groups may cause steric hindrance to the interaction between chiral compounds and carbamoyls in CSPs, thus leading to lower separation capacity.

We found that these separated chiral compounds were structurally similar. Namely, there is an aromatic ring contained in these chiral compounds and there is a trend that these compounds, with their aromatic rings adjacent to chiral centers, showed a more satisfactory resolution. It is possibly related to the π-π interaction of aromatic rings between analytes and CSPs.

In our previous work, a CSP was prepared by a similar procedure and had the same structure, except for the difference of length of linkage to silica gel.[19] Ideally, when the other environments are the same, it is easier to analyze the interaction of CSPs with the chiral selector. Experimental results showed that these two CSPs gave similar enantioseparation capabilities in terms of resolutions and selectivities of the analytes. In addition, the remaining shorter spacers that have not formed the linkages with silica gel surface are also detected. These may explain the decrease of enantioseparation capability, since the surface loading of the chiral selector is much less than for

the previous CSP.[20] We may draw the conclusion that, although the immobilization of chiral selector by a longer spacer is helpful to enantioseparation, this advantage was possibly offset by the impact of excess spacers on the surface of silica gel.

CONCLUSION

A series of mono-azido-functionalized β-CD derivatives were easily prepared. These β-CD derivatives were immobilized on silica gel through a long chain spacer that was prepared from glycidoxy and 1,6-diaminohexane. CSPs derived from phenylcarbamoylated β-CD and 3,5-dimethylphenylcarbamoylated β-CD were effective for enantioseparation of pyrimidine derivatives, and the former proved to be the most efficient for chiral separation among the CSPs prepared under the tested conditions. The CSPs prepared from 1-naphthylcarbamoylated β-CD and 2-methoxyphenyl- carbamoylated β-CD showed weak capabilities for chiral recognition compared to the former two CSPs.

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