The Comparison in Enantioseparation Ability of the Chiral Stationary Phases with Single and Mixed Selector—The Selectors Derived from Two D-Tartrates

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ABSTRACT (2*S*,3*S*)-2,3-Bis(3,5-dimethylphenylcarbonyloxy)-3-(benzyloxycarbonyl)-propanoic acid and (2*S*,3*S*)-2,3-bis(1-naphthalenecarbonyloxy)-3-(benzyloxycarbonyl)-propanoic acid were synthesized from D-tartaric acid. These two compounds were chlorinated to afford two chiral selectors for chiral stationary phases (CSPs). The selectors were separately immobilized on aminated silica gel to give two single selector CSPs; and were simultaneously immobilized to obtain a mixed selector CSP. Comparing to the single selector CSPs, the mixed selector CSP bears the enhanced enantioseparation ability, suggesting that the two selectors in the mixed selector CSP are consistent for chiral recognition in most mobile phase conditions. *Chirality 23:228–236, 2011.*

KEY WORDS: single selector; mixed selector; chiral stationary phase; enantioseparation; high-performance liquid chromatography

INTRODUCTION

Chiral high-performance liquid chromatography (HPLC) has been proved to be a very effective technique to separate racemates into their enantiomers thus being widely used for the enantioseparation of drugs, especially for the development of new drugs.[1–4] Chiral stationary phases (CSPs) are essential absorbent materials for chiral HPLC columns.[5] In this technique, enantioseparation results from the difference of the interactions between a pair of enantiomers and CSPs.[6] Many authors discussed the mechanism of chiral recognition during enantioseparation.[7–10] Generally, it is believed that temporary diastereoisomers are formed when chiral selectors interact with chiral analytes through H bonding, π-π interaction, dipole-dipole interaction, or van der Waals force.[6,10] In addition, the stereo-hindrance in the interaction between chiral selectors and chiral analytes is another element for chiral discrimination.[11] Under the guidance of these principles, various types of CSPs were developed. To improve the enantioseparation ability, some biselector CSPs were reported.[12–16] However, these biselector CSPs do not always show enhanced enantioseparation ability. In the reported works, two chiral compounds were connected with a cross-linker of multiple reactive groups to form a biselector, which was then immobilized on a support to afford a biselector CSP. Kraak and coworkers[17] first prepared a mixed selector CSP by immobilizing two derivatives of phenylglycine on aminated silica gel through acid–base reaction. The enantioseparation ability of this CSP was tested only in limited mobile phases with few chiral analytes, because the selector will disassociate from the CSP when the acidity of mobile phases changes dramatically. The CSP cannot be subjected to the enantioseparation of the chiral analytes whose acidity is stronger than that of carboxylic acid or whose basicity is stronger than that of amine. Otherwise, the disassociation also takes place. To establish a method to prepare mixed selector CSPs by covalent immobilization, in our previous work, a mixed selector CSP was synthesized. The two chiral selectors were prepared from L-dibenzoyl tartaric acid and (1*R*,2*R*)-1,2-diphenylethylenediamine.[18] However, its enantioseparation ability is lower than that of single selector CSPs. The reason probably is these two selectors may reversely contribute the chiral recognition and as a result, the enantioseparation ability of the CSP is impaired. To investigate the fact causing the impair in enantioseparation ability of the mixed selector CSP, in this work, another mixed selector CSP was synthesized, where the two selectors were both prepared from D-tartaric acid and they are close in their structures. The enantioseparation ability of the relevant single selector and mixed selector CSPs was evaluated.

MATERIALS AND METHODS

Materials and Chemicals

D-Tartaric acid was purchased from Chengdu Likai Chiral Tech. (China). 3,5-Dimethylbenzoic acid and 1-naphthalenecarbonyl chloride were, respectively, available from Shanghai Zhuorui and Changzhou Wujin Chemical (China). 3-Aminopropyltriethoxysilane (APTES) was obtained from Novel Organic Silicon Materials of Wuhan University (China) and redistilled before use. Silica gel (Lichrosorb Si 100) was obtained from Merck (Germany) with a particle size of 5 μm, a pore size of 100 Å, and a surface area of 300 $m^2\ g^{-1}$. Triethylamine (TEA) was dried over phosphorous pentoxide and redistilled. Pyridine was dried with NaOH and CaH_2 in sequence and redistilled. All other chemicals used for the CSPs synthesis were of analytical grade and used as received.

Instruments and Measurements

IR spectra were recorded on a Nicolet FTIR instrument (USA) with KBr pellets. Elemental analysis (EA) measurement was conducted on an

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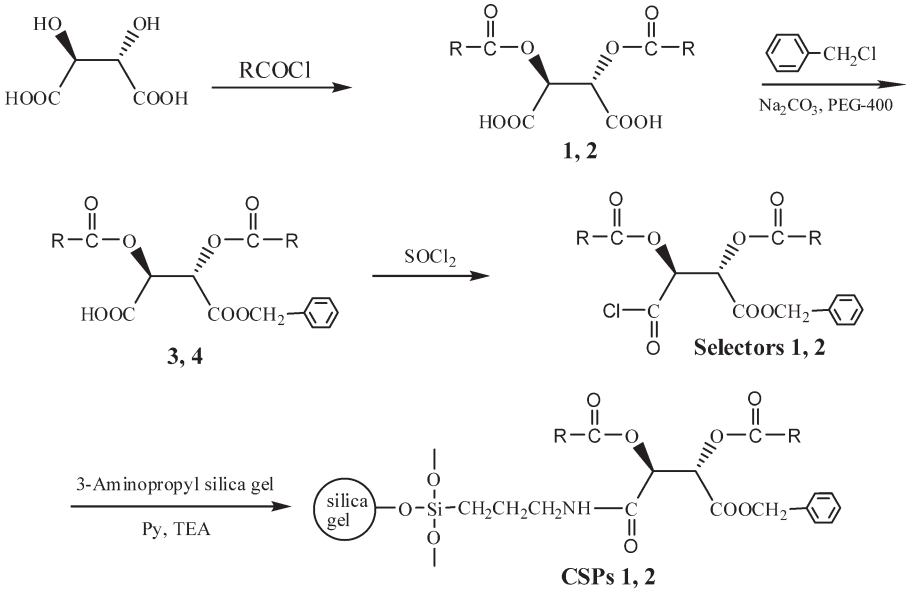

1, 3, selector **1**, and CSP **1**: R: 3,5-dimethylphenyl; 2, 4, selector **2**, and CSP **2**: R: 1-naphthyl.

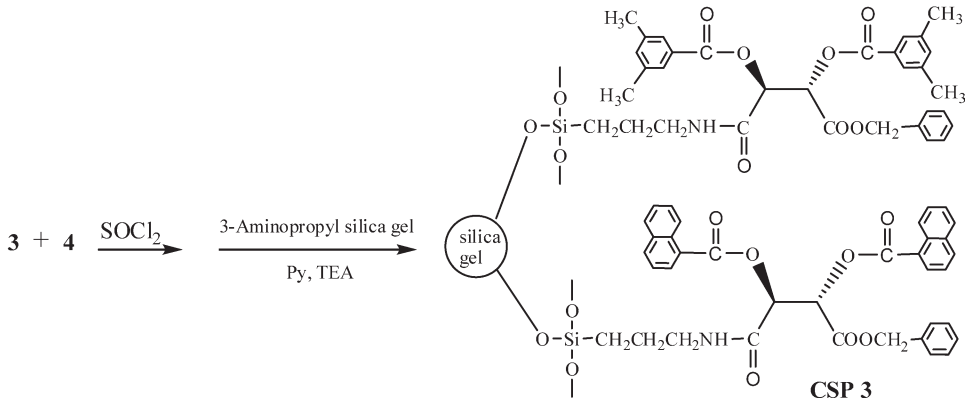

Fig. 1. The synthetic scheme of CSPs **1–3**.

Elementar VarioEL III CHNOS apparatus (Germany). ^{1}H NMR spectra were performed on a Varian INOVA 500 spectrometer (USA). The stainless steel HPLC empty columns (250 mm × 4.6 mm) were purchased from Hypersil (US). The CSPs were packed into the empty columns with an Alltech Model 1666 slurry packer (USA). The enantioseparation was run on a Waters chromatograph (USA) equipped with a Waters 996 photodiode array detector, a Waters 600E Quat Pump, a Waters Millenium 32 system controller, a Waters 717 plus autosampler.

Preparation of Chiral Stationary Phases

$SOCl_2$ (18 ml, 0.25 mol) was added dropwise to 3,5-dimethylbenzoic acid (90.8 g, 60.5 mmol) with stir. The resulting solution was then stirred at 60°C for 3 h to ensure the completion of the reaction. The excess $SOCl_2$ was removed by distillation in vacuo to afford yellow oil, in which D-tartaric acid (3.03 g, 20.2 mmol) was added. The mixture was stirred at 110°C for 6 h. After cooling to ambient temperature, the mixture solidified. The solid was suspended in 100 ml water, and the suspension was stirred at 100°C for 1 h. The solid was filtered and washed with benzene thoroughly. Compound **1** (6.36 g) was white powder after drying in vacuo. Yield: 76%; m.p: 206–208°C;$[\alpha]_D^{20}$: +44.8° (C 1.0, methanol); IR (KBr, cm^{-1}): 3672–3079 ($-CO_2H$, aromatic C—H), 2944, 2913 (C—H), 1764 ($-CO_2-$), 1714 ($-CO_2H$), 1453, 1378 (C—H); EA ($C_{22}H_{22}O_8 \cdot H_2O$, %): Calcd C 61.11, H 5.59; Found C 61.31, H 5.57; ^{1}H NMR (DMSO, 25°C) δ: 2.3 (12H, CH_3), 5.8 (2H, C—H), 7.3–7.6 (m, 6H, Ar—H); ^{13}C NMR (DMSO, 25°C) δ: 72 (CH), 125–135 (m, Ar—C), 160 ($-CO_2-$), 166, 168 ($-CO_2H$).

Compound **2** was prepared in a similar manner to prepare Compound **1**, with 1-naphthalenecarbonyl chloride (10.59 g, 55.6 mmol) and tartaric acid (2.79 g, 18.6 mmol). The reaction mixture was stirred overnight at 100°C. The crude product was collected by heat-filtration and washed thoroughly with chloroform. Compound **2** (6.48 g) was obtained as a white powder after drying in vacuo. Yield: 76%; m.p: 179–181°C; $[\alpha]_D^{20}$: +42.8° (C 1.0, methanol); IR (KBr, cm^{-1}): 3651–3141 ($-CO_2H$), 3091, 3046 (aromatic C—H), 2917, 2842 (C—H), 1764 ($-CO_2-$), 1727 ($-CO_2H$), 1453, 1378 (C—H); EA ($C_{26}H_{18}O_8 \cdot 5H_2O$, %): Calcd C 56.93, H 5.15; Found C 57.11, H 5.23; ^{1}H NMR (DMSO, 25°C) δ: 6.2 (2H, CH), 7.6–8.9 (m, 14H, Ar—H); ^{13}C NMR (DMSO, 25°C) δ: 72 (C—H), 125–135 (m, Ar—C), 160 ($-CO_2-$), 166—168 ($-CO_2H$).

Compound **1** (14.49 g, 35.0 mmol) and Na_2CO_3 (3.14 g, 29.6 mmol) were mixed in water (100 ml). To this solution, toluene (80 ml), benzyl chloride (3.69 g, 29.8 mmol), and catalytic amount of PEG-400 were added. After stirring at 85°C for 18 h, the reaction mixture was cooled and separated into two phases. The toluene layer was neutralized with diluted hydrochloric acid under vigorous stir and was then washed with water till aqueous phase became neutral in pH. The organic phase was dried over anhydrous $MgSO_4$. Yellow oil was given after toluene was removed in vacuo. (2*S*,3*S*)-2,3-Bis(3,5-dimethylphenylcarbonyloxy)-3-

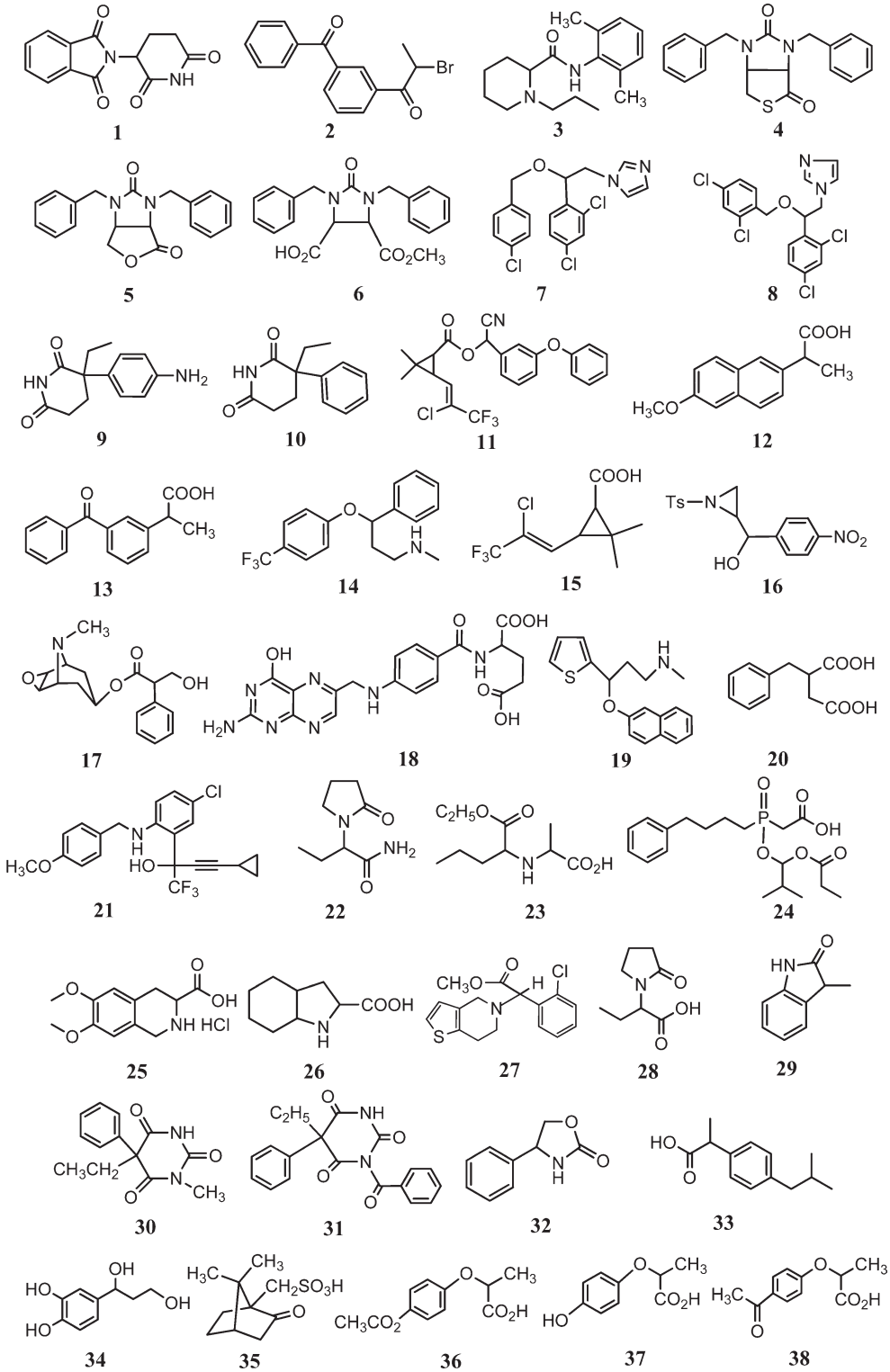

Fig. 2. The structures of chiral analytes resolved on CSPs **1–3** (1–38).

(benzyloxycarbonyl)-propanoic acid (Compound **3**) (5.71 g) was recrystallized from cyclohexane as a white solid. Yield: 38%; m.p: 128–130°C; $[\alpha]_D^{20}$: +18.4° (C 1.0, methanol), IR (KBr, cm^{-1}) 3676–3108 (−CO_2H, aromatic C−H), 2958, 2912 (C−H), 1752, 1742 (−CO_2−), 1727 (−CO_2H), 1457, 1374 (C−H), EA ($C_{29}H_{28}O_8$, %): Calcd C 69.04, H 5.59; Found C 69.46, H 5.42; ^{1}H NMR (DMSO, 25°C) δ: 2.4 (12H, CH_3), 5.2 (m, 2H, CH_2), 5.9 (1H, CH), 6.0 (1H, CH), 7.1–7.6 (m, 11H, Ar−H); ^{13}C NMR (DMSO, 25°C) δ: 21 (CH_3), 52 (CH_2), 68 (CH), 72 (CH), 127–139 (Ar−C), 160 (−CO_2−), 165–167 (−CO_2H).

(2*S*,3*S*)-2,3-Bis(1-naphthalenecarbonyloxy)-3-(benzyloxycarbonyl)-propanoic acid (Compound **4**) was prepared in the same manner to prepare Compound **3**, with Compound **2** (10.19 g, 22.2 mmol), Na_2CO_3 (2.37 g, 22.4 mmol) and benzyl chloride (2.80 g, 22.2 mmol). It was purified with column chromatography packed with silica gel and eluted with a mixture of cyclohexane/ethyl acetate/acetic acid (2/1/0.02, v/v/v). Yield: 43%; m.p: 46–48°C; $[\alpha]_D^{20}$: +2.8° (C 1.0, methanol); IR (KBr, cm^{-1}): 3680–3150 (−CO_2H), 3089, 3057 (aromatic C−H), 2955, 2848 (C−H), 1763 (−CO_2−), 1726 (−CO_2H), 1464, 1383 (C−H), EA ($C_{33}H_{24}O_8$, %): Calcd

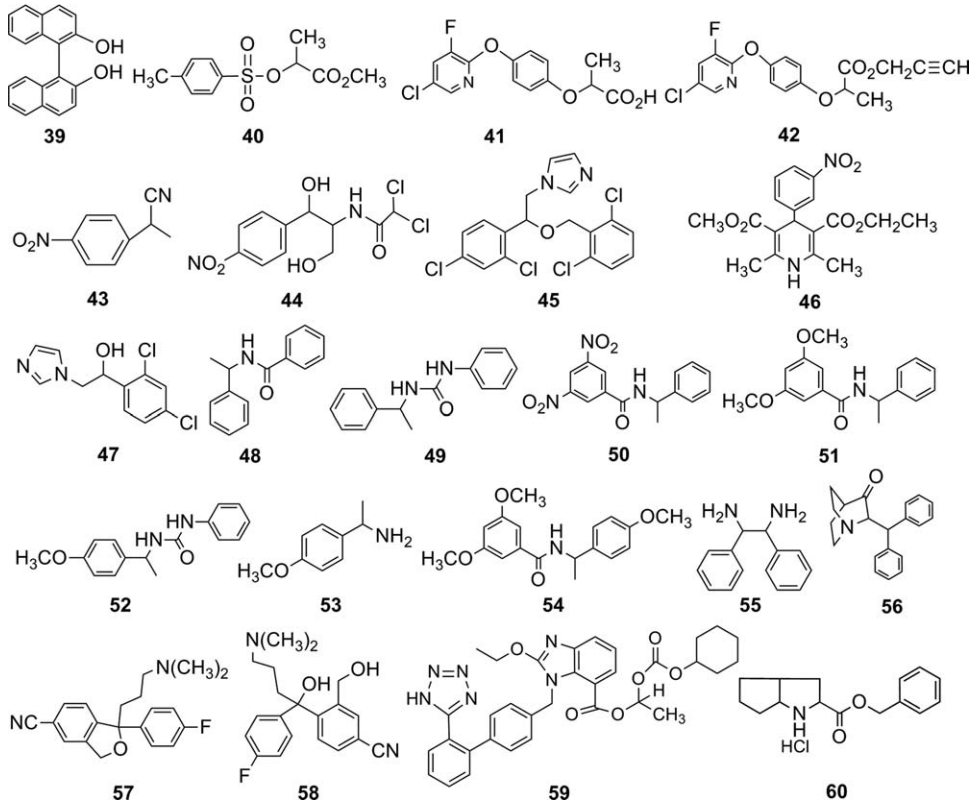

Fig. 3. The structures of chiral analytes resolved on CSPs **1–3** (39–60).

C 72.26, H 4.41; Found C 72.38, H 4.86; ^{1}H NMR ($CHCl_3$, 25°C) δ: 4.9–5.0 (m, 2H, CH_2), 5.9 (2H, CH), 6.7–8.8 (m, 19H, Ar—H); ^{13}C NMR ($CHCl_3$, 25°C) δ: 68–72 (m, CH_2), 76–77 (CH), 124–134 (m, Ar—C), 165–166 (m, $-CO_2-$), 171 ($-CO_2-$), 172 ($-CO_2H$).

3-Aminopropyl silica gel was prepared with dried silica gel and APTES.[19] EA (%): C 5.58, H 1.50.

Compound **3** (2.56 g, 5.1 mmol) was placed in a 50 ml three-necked round bottom flask, in which $SOCl_2$ (10 ml) was added dropwise with stir. The resulting solution was heated to 75°C and stirred for 8 h. The excess $SOCl_2$ was thoroughly removed in vacuo to give Selector **1** as viscous oil, which was mixed with 3-aminopropyl silica gel (3.65 g), pyridine (15 ml), and TEA (3 ml). The suspension was gently stirred overnight at 80°C. The solid was collected by filtration and washed with acetone in a Soxhlet apparatus. CSP **1** (4.03 g) was a pale yellow solid after the removal of the solvent. IR (KBr, cm^{-1}): 3461, 1544 (—NH—), 2980, 2938 (—CH—), 1603, 1646 (—NH—CO—), 1111 (Si—O); EA (%): C 12.60, H 2.05; Suspension-state ^{1}H NMR (D_2O, 25°C) δ: 0.8 ($Si—CH_2$), 1.30 ($SiCH_2CH_2CH_2$), 2.4 (CH_3, NH_2), 3.3 ($SiCH_2CH_2CH_2$), 5.4 (CH, CH_2), 5.8 (CH), 7.2–8.9 (m, Ar—H).

CSP **2** (3.88 g) as a brown solid was prepared in the same manner to prepare CSP **1** using Compound **4** (3.87 g, 7.1 mmol), $SOCl_2$ (10 ml), and 3-aminopropyl silica gel (3.50 g). IR (KBr, cm^{-1}): 3461, 1545 (—NH—), 1646 (—NH—CO—), 1111 (Si—O); EA (%): C 12.63, H 1.94; Suspension-state ^{1}H NMR (D_2O, 25°C) δ: 0.8 ($Si—CH_2$), 1.3 ($SiCH_2CH_2CH_2$), 2.3 (NH_2), 2.8–3.3 (m, $SiCH_2CH_2CH_2$), 5.3–5.6 (m, CH, CH_2), 5.9 (CH), 7.3–8.9 (m, Ar—H).

CSP **3** (3.86 g) as a brown solid was prepared in the same manner to prepare CSP **1** using a mixture of Compound **3** (1.83 g, 3.6 mmol) and Compound **4** (2.40 g, 4.0 mmol), $SOCl_2$ (10 ml), and 3-aminopropyl silica gel (3.44 g). IR (KBr, cm^{-1}): 3457, 1548 (—NH—), 2944 (—CH—), 1731 ($-CO_2-$), 1641 (—NH—CO—), 1111 (Si—O); EA (%): C 14.03, H 2.05; Suspension-state ^{1}H NMR (D_2O, 25°C) δ: 0.8 ($Si—CH_2$), 1.3 ($SiCH_2CH_2CH_2$), 2.1 (NH_2), 2.9–3.3 (m, $SiCH_2CH_2CH_2$), 5.0–5.6 (m, CH, CH_2), 6.8–7.8, 8.6–9.1 (m, Ar—H).

Column Packing and Enantioseparation

The columns were packed using a pressurized slurry technique. CSPs **1–3** (~3.3 g) were added in chloroform (30 ml), subsequently followed by sonication for 8 min to form slurries. These slurries were packed into empty HPLC columns with methanol as displacer solvent, under the pressure no more than 50 Mpa. The packed columns were flushed with isopropanol and isopropanol/*n*-hexane in turn. The column efficiency was measured by biphenyl, with isopropanol/*n*-hexane (10/90, v/v) as the mobile phase. The sample solutions (1 mg ml^{-1}) were prepared by dissolving the chiral compounds in acetonitrile and were filtered through 0.2-μm membrane. The injection volume was 15 μl. All mobile phases were filtered and degassed before use. The flow rates were set at 1 ml min^{-1}. Column temperature was set at 25°C.

The retention factors (k_1 and k_2) were calculated according to the formulas of $(t_1 - t_0)/t_0$ and $(t_2 - t_0)/t_0$, where t_1 and t_2 are, respectively, the retention time of the first and the second-eluted enantiomers, and t_0 is determined by measuring the retention time of $NaNO_2$ solution. The separation factor (α) was calculated from the formula of k_2/k_1. The resolution (R_s) was calculated from the formula of $2(t_2 - t_1)/(w_1 + w_2)$, where w_1 and w_2 are the bandwidth of the first and the second-eluted enantiomers, respectively.

RESULTS AND DISCUSSION

Preparation and Characterization of CSPs 1–3

Figure 1 shows the synthetic scheme of CSPs **1–3**. Because the molecular size of Selector **2** is larger than that of Selector **1**, in the preparation of CSP **3**, the fed amount of Compound **4** is a little more than that of Compound **3** in molar number to ensure both selectors are sufficiently immobilized on aminated silica gel. There are many residual amino groups on the surface of aminated silica gel. In the FTIR spectra of CSPs **1–3**, the absorbance approximately at

TABLE 1. The chromatographic data of chiral compounds resolved by CSPs 1–3

	CSP **1**				CSP **2**				CSP **3**			
S/N	k_1	α	R_s	Eluent	k_1	α	R_s	Eluent	k_1	α	R_s	Eluent
1	1.96	1.15	0.42	A(70/30)[a]								
2	2.14	1.20	0.43	B(40/60)[b]	0.44	1.18	0.51	C(20/80)[c]	1.39	1.31	3.06	B(90/10)[c]
3					0.35	1.27	0.39	D(80/20)[d]	0.27	3.14	1.35	A(10/90)[e]
4	1.95	1.22	0.34	A(10/90)[g]	0.24	1.18	0.28	E(70/30)[f]	0.50	3.64	4.38	E(50/50)[e]
5	1.92	1.22	0.35	A(10/90)[g]	0.31	1.46	0.92	D(90/10)[h]	0.39	1.32	1.00	D(90/10)[g]
6	2.07	1.22	0.44	E(90/10)[h]	0.64	1.24	0.30	C(10/90)[c]	0.30	2.52	0.77	E(60/40)[e]
7									0.20	1.17	0.60	B(90/10)[e]
8									0.74	1.87	0.38	B(90/10)[g]
9	1.90	1.20	0.50	A(10/90)[b]					0.80	2.01	0.58	B(90/10)[g]
10	1.88	1.30	0.77	C(10/90)[c]	0.24	1.90	0.33	B(30/70)[f]	0.35	1.21	0.36	F(30/35/35)[c]
11	3.10	1.08	0.43	B(90/10)[g]	0.65	1.11	0.52	C(80/20)[i]	0.78	1.16	0.90	B(90/10)[i]
12									0.70	1.83	0.85	E(50/50)[i]
13	0.55	1.76	0.73	E(50/50)[j]	0.27	1.64	0.65	E(70/30)[i]	0.32	1.45	0.86	C(20/80)[c]
14									0.63	0.45	0.65	C(20/80)[c]
15	1.92	1.37	0.37	E(40/60)[i]	0.20	1.38	0.59	E(60/40)[i]	0.20	2.94	0.61	E(90/10)[i]
16	2.25	1.11	0.28	A(30/70)[i]								
17					0.75	1.18	0.19	C(10/90)[i]	0.25	1.37	0.42	A(30/70)[i]
18					0.33	1.52	0.53	C(10/90)[f]	0.28	1.29	0.32	D(90/10)[h]
19					0.91	1.24	0.50	B(60/40)[f]	0.25	1.39	0.59	C(30/70)[b]
20					0.28	1.48	0.25	D(90/10)[f]	0.52	1.19	0.38	E(70/30)[f]
21	2.00	1.11	0.30	A(30/70)[h]					0.27	1.19	0.31	E(90/10)[e]
22	1.91	1.15	0.46	D(90/10)[f]					0.31	1.30	0.42	E(60/40)[i]
23	2.19	1.08	0.19	D(90/10)[g]	0.23	1.98	1.10	C(10/90)[f]	0.27	1.17	0.19	C(10/90)[f]
24					0.25	1.27	0.17	A(50/50)[f]	0.25	1.25	0.27	A(30/70)[h]
25					0.21	1.32	0.25	C(10/90)[f]	1.81	1.29	0.25	A(50/50)[f]
26									0.23	1.74	1.08	A(50/50)[f]
27	1.91	1.10	0.27	A(30/70)[h]					0.28	1.21	0.42	E(90/10)[g]
28					0.24	1.73	0.51	A(50/50)[f]	0.17	1.49	0.82	D(80/20)[i]
29	1.92	1.09	0.23	A(50/50)[c]								
30	1.84	1.14	0.38	A(30/70)[h]	0.23	1.34	0.51	E(90/10)[h]				
31	1.93	1.30	0.65	C(20/80)[c]	0.27	1.19	0.31	E(90/10)[g]	0.42	1.18	0.56	F(30/35/35)[f]
32	1.90	1.15	0.35	A(30/70)[g]	0.26	1.23	0.41	E(90/10)[g]	0.21	1.67	0.48	B(90/10)[f]
33	1.08	1.16	0.17	E(70/30)[a]					0.99	1.72	0.80	D(70/30)[i]
34									0.38	1.10	0.15	E(50/50)[e]
35	1.99	1.11	0.30	D(90/10)[f]								
36					0.13	1.39	0.14	A(70/30)[h]	0.38	1.58	1.06	E(50/50)[b]
37					0.22	1.49	0.23	A(70/30)[f]	0.28	1.43	0.45	B(30/70)[f]
38	1.70	1.10	0.36	A(70/30)[g]	0.26	1.74	0.49	A(30/70)[f]	0.44	1.89	1.91	E(40/60)[i]
39	2.33	1.17	0.48	C(10/90)[i]								
40	1.75	1.18	0.52	A(70/30)[a]	0.16	1.77	0.85	E(90/10)[g]	0.76	2.15	1.06	B(90/10)[i]
41					0.24	1.31	0.33	E(70/30)[f]	0.99	1.35	0.35	D(70/30)[i]
42	1.90	1.10	0.27	A(70/30)[a]	0.18	1.92	1.01	E(90/10)[g]				
43	1.89	1.13	0.32	A(70/30)[a]	0.29	1.16	0.21	E(90/10)[d]				
44	2.11	1.19	0.47	C(10/90)[c]					1.32	1.10	0.18	E(40/60)[i]
45	3.45	1.08	0.33	E(70/30)[a]								
46	2.18	1.18	0.38	B(40/60)[b]					0.29	1.14	0.26	E(90/10)[d]
47									1.06	1.15	0.68	D(70/30)[i]
48	1.77	1.13	0.46	A(70/30)[i]								
49	1.97	1.21	0.61	C(30/70)[b]					0.26	1.30	0.66	A(50/50)[c]
50	2.44	1.15	0.50	C(10/90)[c]	0.29	1.16	0.31	E(90/10)[g]	1.21	1.07	0.42	C(80/20)[b]
51	1.90	1.12	0.38	A(50/50)[c]	0.24	1.26	0.38	E(90/10)[i]				
52	2.09	1.19	0.51	C(30/70)[b]	0.10	4.12	0.75	B(70/30)[i]				
53					0.27	1.26	0.40	E(90/10)[g]	0.28	1.20	0.22	C(10/90)[i]
54	1.89	1.15	0.43	A(10/90)[b]								
55	2.03	1.10	0.19	E(90/10)[h]	0.22	1.40	0.42	A(70/30)[g]	0.86	1.70	2.67	D(80/20)[f]
56					0.27	1.28	0.44	E(90/10)[g]	0.29	1.23	0.27	C(10/90)[g]
57	2.13	1.17	0.58	B(70/30)[h]	0.24	1.79	0.85	A(30/70)[g]	0.25	1.29	0.28	A(30/70)[f]
58									2.76	2.14	1.47	A(10/90)[i]
59					0.06	3.53	0.30	A(70/30)[g]	0.26	2.69	0.65	C(10/90)[g]
60									0.24	3.24	1.26	F(30/35/35)[f]

Eluent: A: Methanol/ethanol; B: *n*-Hexane/isopropanol; C: *n*-Hexane/ethanol; D: Methanol/water; E: Acetonitrile/water; F: *n*-Hexane/ethanol/methanol. [a]The wavelength of UV detection 230 nm.
[b]The wavelength of UV detection 245 nm.
[c]The wavelength of UV detection 254 nm.
[d]The wavelength of UV detection 200 nm.
[e]The wavelength of UV detection 215 nm.
[f]The wavelength of UV detection 210 nm.
[g]The wavelength of UV detection 220 nm.
[h]The wavelength of UV detection 225 nm.
[i]The wavelength of UV detection 235 nm.
[j]The wavelength of UV detection 270 nm.

TABLE 2. The representative chromatographic resolution of eight chiral compounds on the CSPs under the same separation conditions

	CSP **1**				CSP **2**				CSP **3**				
S/N	k_1	α	R_s	λ	k_1	α	R_s	λ	k_1	α	R_s	λ	Eluent
2	2.10	1.21	0.38	254	No separation				0.69	1.11	0.51	254	A(30/70)
	No separation				0.75	1.08	0.39	254	0.62	1.16	0.71	254	B(70/15/15)
3	No separation				0.22	1.85	0.73	210	0.29	2.44	1.56	225	C(10/90)
					0.35	1.27	0.39	200	0.60	1.24	0.31	235	D(80/20)
6	No separation				0.77	1.49	0.90	210	0.69	1.14	0.45	210	A(40/60)
10	1.98	1.19	0.45	254	No separation				0.35	1.21	0.36	254	B(30/35/35)
11	3.10	1.08	0.43	220	No separation				0.78	1.16	0.90	235	A(90/10)
13	No separation				0.27	2.66	1.22	210	0.32	8.20	1.99	210	E(70/30)
	0.55	1.76	0.73	270	0.70	1.39	0.62	220	0.50	2.12	0.78	254	F(50/50)
15	No separation				0.20	1.38	0.59	235	0.44	1.17	0.28	245	F(60/40)
38	No separation				No separation				0.44	2.80	2.74	235	F(60/40)
41	No separation				0.45	1.58	0.48	254	0.26	1.24	0.35	215	E(70/30)
					0.51	1.20	0.23	210	0.46	3.14	1.11	235	F(50/50)
44	1.95	1.15	0.36	240	No separation				0.24	1.34	0.64	220	E(50/50)
53	No separation				0.27	1.26	0.40	220	0.27	1.38	0.66	215	F(90/10)
55	2.37	1.25	1.21	210	No separation				0.41	1.39	1.25	220	C(80/20)
	No separation				0.23	1.35	0.30	220	0.33	1.15	0.12	275	E(50/50)

Eluent: A: *n*-Hexane/isopropanol; B: *n*-Hexane/ethanol/methanol; C: *n*-Hexane/ethanol; D: Methanol/water; E: Ethanol/methanol; F: Acetonitrile/water. UV detection: λ (nm).

1650 cm^{-1} designating to the amine is much stronger than that approximately at 1720 cm^{-1} designating to the esters in the selectors, which are much less than amino groups in amount. The absorbance peaks of esters are partially overlapped by those of amino groups, thus not separately appearing in the spectra. On the basis of the increment of the contents of carbon, the capacities of the two selectors on CSPs **1** and **2** are estimated as 0.67 μmol m^{-2} and 0.60 μmol m^{-2}, respectively.[20] The capacities of Selectors **1** and **2** on CSP **3** are calculated as follows: Suppose x and y present the capacities (μmol m^{-2}) of Selectors **1** and **2** on CSP **3**; n_C^1 and n_C^2 are carbon atom numbers contained in each molecule of Selectors **1** and **2**; n_H^1 and n_H^2 present hydrogen atom numbers contained in each molecule of Selectors **1** and **2**. After the immobilization of the two selectors, the content of carbon and hydrogen on aminated silica gel increases and the increments are defined as ΔC_C (%) and ΔC_H (%). The carbon content increment that comes from Selectors **1** and **2** are defined as ΔC_C^1 and ΔC_C^2; the hydrogen content increment are defined as ΔC_H^1 and ΔC_H^2. The relationship between the selector capacities and the carbon content increment is expressed by eqs. 1 and 2:

$$10 \times \Delta C_C^1 = 12 \times n_C^1 \times x..... \quad (1)$$

$$10 \times \Delta C_C^2 = 12 \times n_C^2 \times y..... \quad (2)$$

Equation 1 adds with eq. 2 to give eq. 3:

$$12n_C^1 x + 12n_C^2 y = 10(\Delta C_C^1 + \Delta C_C^2) = 10\Delta C_C..... \quad (3)$$

Similarly, based on the hydrogen content increment, eq. 4 is obtained:

$$1\times(n_H^1 - 1)x + 1\times(n_H^2 - 1)y = 10(\Delta C_H^1 + \Delta C_H^2) = 10\Delta C_H \quad (4)$$

In eqs. 1 and 2, 12 is the relative atom mass of carbon. In eq. 4, $(n_H^1 - 1)$ refers to the actual numbers of hydrogen atom introduced into CSP **3** during the immobilization of the Selector **1**, where one hydrogen atom left amianted silica gel yielding hydrogen chloride when Selector **1** was immobilized. The same case is for $(n_H^2 - 1)$ during the immobilization of Selector **2**. ΔC_C and ΔC_H are available from the EA of CSP **3** and the aminated silica gel. By resolving the equation group consisting of eqs. 3 and 4, the capacities of Selectors **1** and **2** on CSP **3** were estimated as 0.43 μmol m^{-2} and 0.30 μmol m^{-2}, respectively.

General Enantioseparation Evaluation of CSPs 1–3

The efficiency of the columns packed with CSPs **1–3** is determined as 21,900, 20,500, and 15,100 plates per meter.

TABLE 3. The influence of the alcohols content on the enantioseparation of the chiral analytes resolved by CSP 1 in normal phase mode and polar organic mode

S/N	k_1	α	R_s	UV detection (nm)	Eluent
4	2.06	1.24	0.52	225	Ethanol/*n*-hexane: 70/30
	2.02	1.24	0.44	220	Ethanol/*n*-hexane: 80/20
	2.02	1.22	0.41	235	Ethanol/*n*-hexane: 90/10
5	2.10	1.36	0.78	220	Ethanol/*n*-hexane: 60/40
	2.10	1.27	0.66	220	Ethanol/*n*-hexane: 70/30
	2.00	1.16	0.38	220	Ethanol/*n*-hexane: 80/20
9	2.13	1.36	0.91	245	Ethanol/*n*-hexane: 70/30
	2.07	1.29	0.62	254	Ethanol/*n*-hexane: 80/20
	2.05	1.19	0.37	254	Ethanol/*n*-hexane: 90/10
21	1.89	1.17	0.39	254	Methanol/ethanol: 10/90
	1.93	1.11	0.35	230	Methanol/ethanol: 50/50
	1.92	1.12	0.34	254	Methanol/ethanol: 70/30
31	1.58	1.68	1.24	245	Ethanol/*n*-hexane: 60/40
	1.91	1.33	0.86	254	Ethanol/*n*-hexane: 70/30
32	1.86	1.37	0.85	220	Ethanol/*n*-hexane: 70/30
	1.83	1.33	0.75	215	Ethanol/*n*-hexane: 80/20
	1.88	1.26	0.58	220	Ethanol/*n*-hexane: 90/10
49	1.97	1.21	0.61	245	Ethanol/*n*-hexane: 70/30
	1.99	1.17	0.42	245	Ethanol/*n*-hexane: 80/20
	1.97	1.17	0.42	245	Ethanol/*n*-hexane: 90/10

TABLE 4. The influence of the alcohols content on the enantioseparation of the chiral analytes resolved by CSP 2 in normal phase mode and polar organic mode

S/N	k_1	α	R_s	UV detection (nm)	Eluent
3	0.22	1.95	0.91	210	Ethanol/*n*-hexane: 80/20
	0.22	1.85	0.73	210	Ethanol/*n*-hexane: 90/10
	0.22	1.44	0.40	210	Methanol/ethanol: 30/70
	0.23	1.33	0.31	220	Methanol/ethanol: 50/50
23	0.22	2.86	1.78	210	Ethanol/*n*-hexane: 70/30
	0.24	1.71	0.98	220	Ethanol/methanol: 90/10
	0.23	1.61	0.86	220	Ethanol/methanol: 70/30
38	0.30	3.39	1.42	220	Methanol/ethanol/*n*-hexane: 35/35/30
	0.11	2.21	0.47	254	Methanol/ethanol/*n*-hexane: 45/45/10
	0.11	1.90	0.25	225	Methanol/ethanol: 50/50
41	0.45	1.58	0.48	254	Methanol/ethanol: 30/70
	0.13	1.71	0.20	225	Methanol/ethanol: 50/50
	0.27	3.40	1.40	235	Methanol/ethanol/*n*-hexane: 35/35/30
	0.12	1.93	0.35	225	Methanol/ethanol/*n*-hexane: 45/45/10
55	0.20	1.53	0.61	220	Methanol/ethanol/*n*-hexane: 25/25/50
	0.18	1.50	0.42	220	Methanol/ethanol/*n*-hexane: 35/35/30
	0.23	1.78	1.00	220	Ethanol/*n*-hexane: 50/50
	0.21	1.54	0.42	210	Ethanol/*n*-hexane: 70/30
59	0.30	2.50	1.03	210	Ethanol/*n*-hexane: 80/20
	0.25	1.99	0.76	235	Ethanol/*n*-hexane: 90/10
	0.08	2.79	0.32	210	Ethanol/methanol: 50/50

Under normal and reversed phase modes and polar organic mode, and in the linear separation conditions, CSPs **1–3** were undergone enantioseparation evaluation toward structurally various chiral compounds (Figs. **2** and **3**). The enantioseparation was confirmed by the same UV absorbance feature of a pair of two enantiomers. The chromatographic results are tabulated in Tables 1 and 2. CSPs **1** and **2**, respectively, separated 37 and 35 chiral compounds, whereas 47 chiral compounds were separated by CSP **3**. Because the selectors are close in their capacities and structures, CSPs **1** and **2** have the equivalent enantioseparation ability. In the view of the numbers of separated chiral compounds, CSP **3** has the best enantioseparation ability. Under most tested conditions, the resolutions on CSP **3** are better than those on CSPs **1** and **2**.

The difference in enantioseparation ability between CSP **3** and the two single selector CSPs is related to the structural similarity of the two selectors in CSP **3**. Based on the theory of forming diastereoisomers during chiral recognition,[6,7] two enantiomers of a racemate interact with a selector through stereoselective complexation to give two diastereoisomers. The stability difference of the two diastereoisomers leads to enantioseparation. The enantiomer forms the less stable diastereoisomer is eluted out early, and another one is eluted out late. There are two selectors in CSP **3**, with which a pair of enantiomers of a chiral analyte interact to produce two group diastereoisomers. If the diastereoisomers results from *R* (or *S*)-form isomer and the two selectors are both more stable than the ones results from *S* (or *R*)-form isomer and the two selectors, the effects of Selectors **1** and **2** on enantioseparation enhance each other. In the event that these diastereoisomers are not both more stable than the ones formed between the *S* (or *R*)-form isomer and the two selectors, the effects of Selectors **1** and **2** on enantioseparation are just slightly enhanced, or not enhanced, or even impaired. Because Selectors **1** and **2** were both prepared from tartaric acid, except the derivatization with different substituents (Fig. 1), they have the same steric configurations, which can match that of an enantiomer forming stable diastereoisomers. Relatively, the steric configuration of another enantiomer cannot well fit those of Selectors **1** and **2** forming less stable diastereoisomers, or not forming diastereoisomers. Further, the substituents, i.e., 3,5-dimethylbenzoyl and 1-naphthalenecarbonyl, are complementary in their structures or electronic effects for the diastereoisomers formation by the complexation between the two selectors with an enantiomer. Accordingly, the two selectors in CSP **3** consistently contribute enantioseparation resulting in the better enantioseparation ability, although the capacity of individual selectors in CSP **3** is lower in comparison with CSPs **1** and **2**.

In the previous work, a mixed selector CSP was prepared from L-dibenzoyl tartaric acid and (1*R*,2*R*)-1,2-diphenylethylenediamine, where the two selectors are not so close in their structures as those in this work. An enantiomer cannot simultaneously well fit the structures of the two selectors. Thus, the mixed selector CSP did not show enhanced enantioseparation ability.

Influence of Alcohols Content of Mobile Phases on Enantioseparation

Mobile phases not only elute chiral analytes out but also impact enantioseparation. Alcohols are usually used as the compositions for chiral HPLC. The influence of alcohols upon enantioseparation is caused by modulating the polarity of mobile phases or by involving in the interaction between chiral analytes and selectors. Under normal phase mode and polar organic mode, the effect of the content of methanol, ethanol, and isopropanol on the enantioseparation was investigated (Tables 3–5). With the content increment of alcohols or higher-polarity alcohols, the overall polarity of mobile phase increases, however, the resolution decreases. The reason for the observed trend is related to the following fact: the functional groups in Selectors **1** and **2** are ester, while only one amide bond is employed to link the selectors and the

TABLE 5. The influence of the alcohols content on the enantioseparation of the chiral analytes resolved by CSP 3 in normal phase mode and polar organic mode

S/N	k_1	α	R_s	UV detection (nm)	Eluent
6	0.60	1.13	0.50	210	Ethanol/*n*-hexane: 40/60
	0.42	1.18	0.42	220	Ethanol/*n*-hexane: 70/30
	0.40	1.17	0.30	215	Ethanol/*n*-hexane: 80/20
	0.39	1.14	0.15	215	Ethanol/*n*-hexane: 90/10
	0.34	1.16	0.26	210	Methanol/ethanol: 50/50
	0.33	1.16	0.22	205	Methanol/ethanol: 70/30
11	0.23	1.32	0.41	215	Methanol/ethanol: 10/90
	0.22	1.27	0.27	225	Methanol/ethanol: 30/70
	0.51	1.16	0.69	210	Methanol/ethanol/*n*-hexane: 25/25/50
	0.21	1.26	0.32	220	Methanol/ethanol/*n*-hexane: 45/45/10
23	0.26	1.22	0.20	235	Methanol/ethanol: 10/90
	0.25	1.20	0.23	220	Methanol/ethanol: 30/70
	0.24	1.19	0.25	205	Methanol/ethanol: 50/50
	0.29	1.49	0.49	220	Ethanol/*n*-hexane: 60/40
	0.28	1.34	0.38	225	Ethanol/*n*-hexane: 70/30
	0.28	1.24	0.25	215	Ethanol/*n*-hexane: 80/20
	0.75	1.11	0.65	220	Methanol/ethanol/*n*-hexane: 15/15/70
	0.25	1.28	0.35	220	Methanol/ethanol/*n*-hexane: 35/35/30
	0.24	1.21	0.26	205	Methanol/ethanol/*n*-hexane: 45/45/10
41	0.26	1.29	0.43	235	Methanol/ethanol: 10/90
	0.26	1.24	0.35	215	Methanol/ethanol: 30/70
	0.22	1.58	0.91	210	Methanol/ethanol/*n*-hexane: 25/25/50
	0.32	1.30	0.60	235	Methanol/ethanol/*n*-hexane: 45/45/10
55	0.28	1.46	0.72	210	Ethanol/*n*-hexane: 40/60
	0.26	1.40	0.56	220	Ethanol/*n*-hexane: 50/50
	0.26	1.30	0.39	235	Ethanol/*n*-hexane: 60/40
	0.31	1.73	1.02	220	Isopropanol/*n*-hexane: 40/60
	0.30	1.68	0.91	220	Isopropanol/*n*-hexane: 50/50
	0.30	1.52	0.58	210	Isopropanol/*n*-hexane: 60/40
60	0.23	1.61	0.76	210	Ethanol/*n*-hexane: 40/60
	0.26	3.14	0.62	215	Ethanol/*n*-hexane: 80/20
	0.25	3.48	1.01	215	Methanol/ethanol: 30/70
	0.28	1.96	0.50	220	Methanol/ethanol: 50/50
	0.29	2.08	0.49	210	Methanol/ethanol: 70/30

support. In addition to the π-π interaction between the aromatic rings in the selectors and in the chiral analytes, the complexation between the selectors and the analytes is mainly owed to the dipole-dipole interaction and H bonding between the esters in the selectors and the functional groups in analytes, such as carbonyl, carboxyl, etc. These interactions will be weakened if the content of the alcohols or higher-polarity alcohol contained in mobile phase increases, because there is a competition for the analytes to interact with alcohols and with the selectors.

CONCLUSIONS

Contrary to the previous work, in which the two selectors in a mixed selector CSP were prepared from different chiral compounds, the selectors in the present work were both derived from the identical chiral compound. The corresponding mixed selector CSP shows enhanced enantioseparation ability. It remains unknown whether all mixed selector CSPs whose selectors are very close in their structures show improved enantioseparation ability comparing with the corresponding single selector CSPs. Therefore, for mixed selector CSPs, the relationship between the selector structures and the enantioseparation ability is worth wider and deeper investigations that possibly offers new considerations for the development of chiral packing materials.

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