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Preparation and evaluation of a chiral stationary phase covalently bound with chiral pseudo-18-crown-6 ether having 1-phenyl-1,2-cyclohexanediol as a chiral unit

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Abstract

A chiral stationary phase (CSP) has been prepared by chemically bonding a chiral pseudo-18-crown-6 type host having a 1-phenyl-1,2-cyclohexanediol unit to 3-aminopropyl silica gel. The chiral column was prepared by the slurry-packing method in a stainless steel HPLC column. Normal mobile phases can be used with this CSP in contrast to conventional dynamic coating type CSPs. Enantiomers of 20 out of 30 amino compounds, including 20 amino acids, 2 amino acid methyl esters, 6 amino alcohols, and 2 lipophilic amines, were efficiently separated on columns with this CSP. It is noteworthy that 15 amino compounds out of 30 were separated with better separation factors and shorter retention times compared to the corresponding CSP having pseudo-18-crown-6 with 1-phenyl-1,2-ethanediol as a chiral unit. In view of the correlation between the enantiomer selectivities observed in chromatography and those obtained in gas phase FABMS-EL methods and solution phase titrations, chiral recognition in the host–guest interaction likely contributes to enantiomer separation.

Keywords: Chiral stationary phases; Crown ethers; Chemically bounded type; Enantiomer separation; ESI; LCMS; Amines; Amino acids; Amino alcohols

1. Introduction

Enantiomer separation by chromatography on chiral stationary phases (CSPs) has been an expanding field of research because of its effectiveness and convenience, and because of the increasing demand for the determination of enantiomeric purity and the resolution of enantiomeric mixtures. Chirality recognition and enantiomer separation of amines and protonated amino compounds are important since many of these compounds are known to possess potent biological activities, that differ from the two enantiomers. Among several types of compounds, chiral crown ethers have been recognized [1,2] and commercialized as promising selectors used in LC CSPs for the separation of amino compounds [3].

Regarding the CSPs based on crown ethers, although Cram and co-workers prepared for the first time those consisting of chiral crown ethers attached to polystyrene [1] or silica gel during the 1970s [2], these CSPs were not commercially available, presumably because of the low chromatographic performance. In 1987 Shinbo and co-workers reported the separation of amino acids using a CSP in which a hydrophobic chiral crown ether was dynamically coated on an ODS stationary phase [4,5]. This type of CSP was commercialized as CROWNPAK CR, which is the first commercialized crown ether-based CSP, and it has been proven useful for the resolution of chiral primary amines [6]. However, solvents that can be used as a mobile phase on this CSP are limited because of the dynamic coating process. In view of the better

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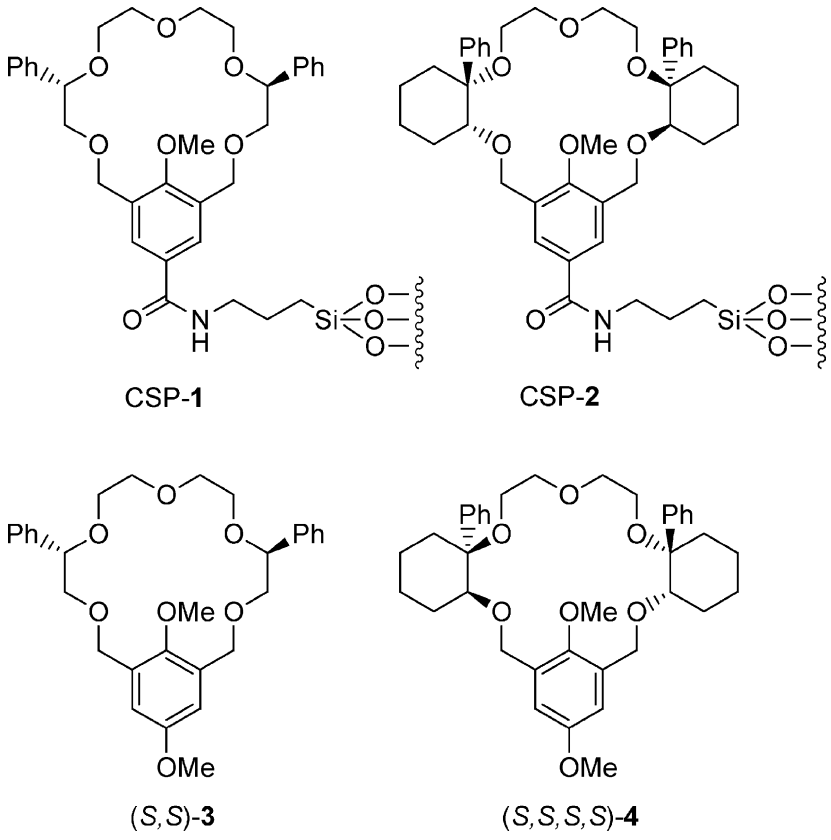


Fig. 1. The structure of chemically bonded type CSP-**1**, -**2** and corresponding crown ethers used for FABMS-EL method.

complexation ability of crown ethers in organic solvents than in aqueous media, the use of a normal mobile phase would be advantageous for the separation of chiral amino compounds. Therefore, it is desirable to develop CSPs that can be used with a normal mobile phase eluent. Indeed, since 1998, chemically bonded type CSPs containing chiral crown ethers have been actively developed [7–14]. Machida and co-workers reported this type of CSP using (+)-18-crown-6-2,3,11,12-tetracarboxylic acid as a chiral selector bound by an amide linkage to silica gel [7]. Hyun and co-workers also developed CSPs using the same crown ether as a selector, but a different bonding process that Machida's CSP [8,9]. Bradshow's group reported CSPs using selectors having a metacyclophane framework containing a pyridine moiety [11,12]. CSP-**1**, shown in Fig. 1, was developed by us and has a pseudo-18-crown-6 ether with a chiral 1-phenyl-1,2-ethanediol unit chemically bonded to silica gel [14,15]. CSP-**1** was commercialized as SUMICHIRAL OA-8000 in 2000 and was one of the earliest CSPs with a covalently bound chiral crown ether selector. In the same year, Hyun and co-workers commercialized a chemically bonded type CSP named Chirosil RCA in which (+)-18-crown-6-2,3,11,12-tetracarboxylic acid was used as a selector.

In order to respond to the increasing demand to separate chiral amino compounds including lipophilic examples, it is desirable to develop crown ether CSPs for which a normal mobile phase can be used as the eluent. In addition, it is also desirable to develop CSPs that provide short retention times but large differences in the separation of enantiomers [16]. Therefore, we designed CSP-**2**. In this paper, we report the preparation of CSP-**2** and its use for enantiomer separation of chiral amino compounds such as amines, amino alcohols, and amino acids.

2. Experimental

2.1. HPLC equipment and general procedure for chromatographic analysis

An HPLC system consisted of an LC-10ADvp high pressure pump, a DGU-14A degasser, an LCMS-2010 liquid chromatograph mass spectrometer, an SPD-10Avp variable-wavelength UV detector, and a CTO-10Avp column oven (Shimadzu, Kyoto, Japan) was employed. A solvent consisting of hexane/ethanol/TFA/water (85/15/0.5/0.2) was used as standard normal mobile phase for the evaluation of CSP-**2**, which is comparable to prior work with CSP-**1** [15]. Analyses of amino acids were carried out using a mass spectrometer as detector. ESI was used as a suitable ionization technique for all of the amino compounds. Injection samples were prepared by dissolving analytes in methanol with a few drops of TFA at a concentration of 10 mg/mL. Samples were applied to the column with a Rheodyne Model 7725i injector equipped with

a 5 mL sample loop. Column temperature was kept at 25 °C. The mass spectrometer was operated in the positive ion mode scan in a range of $M^+ - 5$ Da to $M^+ + 5$ Da at 4 amu/s sampling rate. The nebulizer nitrogen drying gas flow was set at 4.5 L/min. Vcap was set at 4.5 kV. Peak integration was carried out with a Shimadzu LCMS solution version 3.0 data processing system.

2.2. General procedure for the determination of binding constants

The titration experiment for complexation of host (*R*,*R*,*R*,*R*)-**6** with (*S*)-phenylglycinol is described here as an example for the determination of binding constants by ^{1}H NMR spectroscopy. A solution of (*R*,*R*,*R*,*R*)-**6** (7.76 μM) and a solution of the amine (302 μM) each in $CDCl_3/CD_3OD$ (7/3) were prepared. An initial ^{1}H NMR spectrum of (*R*,*R*,*R*,*R*)-**6** was recorded. Samples were made by adding the guest solutions to a 600 μL of the host solution. Namely, 10, 20, 30, 40, 50, 60, 80, 100, 130, 160, and 200 μL portions of the guest solution were added. Then, spectra of these samples were recorded. The association constant was calculated by the non-linear least-squares method [17] following the chemical shifts of one of the aromatic protons of (*R*,*R*,*R*,*R*)-**6** shown in Scheme 1 as H_a.

2.3. Reagents and materials

2.3.1. Materials

Solvents and reagents used were of reagent-grade purity. Enantiomers of amino acids, amino alcohols, and other amino compounds were purchased from Aldrich Chemicals, Japan (Tokyo, Japan), Katayama Kagaku Kogyo (Osaka, Japan), Nacalai Tesque (Kyoto, Japan), Tokyo Kasei Kogyo (Tokyo, Japan), and Wako Pure Chemicals (Tokyo, Japan).

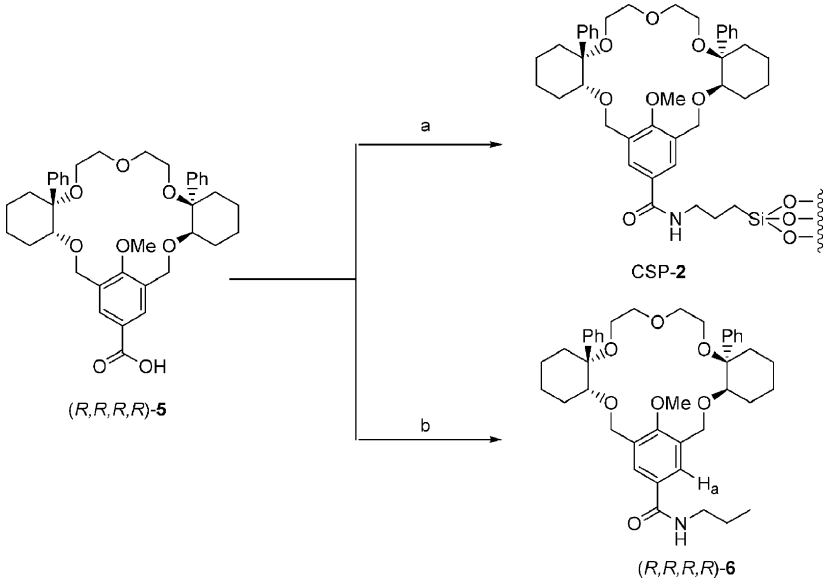
2.3.2. Apparatus.

^{1}H NMR spectra were recorded at 270 MHz on a JEOL JNM-MH-270 spectrometer or at 300 MHz on a Varian Mercury 300 spectrometer for solutions in $CDCl_3$ with $SiMe_4$ as an internal standard. Coupling constants are given in Hz. Mass spectra were recorded with 3-nitrobenzyl alcohol as a matrix on a JEOL-DX-303-HF spectrometer. IR spectra were measured on a JASCO FT/IR-410 spectrometer. Optical rotations were measured using a JASCO DIP-40 polarimeter and $[\alpha]_D$ values are given in unit of 10^{-1} degree $cm^2\ g^{-1}$. Elemental analysis was performed on a Perkin Elmer PE-2400II analyzer.

2.4. Preparation of the chiral stationary phase and its reference compound (*R*,*R*,*R*,*R*)-*6*

2.4.1. Chiral stationary phase CSP-*2*

A mixture of 3-aminopropyl silica gel (2.50 g, 5 μm, 120 Å, found: C 3.78%, N 1.16%), (*R*,*R*,*R*,*R*)-**5** (915 mg, 1.45 mmol) and 1-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (539 mg, 2.18 mmol) in toluene (30 mL) was stirred at ambient temperature for 1 h and at 110 °C for 4 h under nitrogen. The silica gel was collected by filtration and washed successively with toluene, methanol, chloroform, and hexane. After being dried under reduced pressure overnight, the silica gel was suspended in dry toluene (30 mL) containing acetic anhydride (1.42 mL, 13.9 mmol) and dry pyridine (2.43 mL, 30.7 mmol) at 0 °C. Then, the mixture was stirred at ambient temperature for 3 h. The silica gel was collected by filtration and washed



Scheme 1. Reagents and conditions: (a) (1) 3-amino-propylsilica-gel, 1 -ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline and (2) AC2O. The content of the selector bound to the aminopropyl group over the total amount of grafted aminopropyl group on silica gel was 23%. (b) *n*-Propylamine, 1-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline, 86%.

successively with toluene, methanol, chloroform, hexane, and dried overnight under reduced pressure to give CSP-**2** (2.90 g, found: C 12.49%, N 0.97%, grafted crown ether on silica gel is 0.19 mmol/g).

*2.4.2. N-Propyl (4R,9R,17R,22R)-29-methoxy-9,17-diphenyl-3,10,13,16,23-pentaoxatetracyclo [23.3.1.0^{4,9}.0^{17,22}]nonacosa-1(28),25(29),26-triene-27-carboxamide (R,R,R,R)-**6***

A solution of (*R*,*R*,*R*,*R*)-**5** (250 mg, 0.40 mmol), 1-propylamine (70 mg, 1.2 mmol), and 1-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (198 mg, 0.80 mmol) in THF (10 mL) was refluxed for 3 h. After cooling in an ice-bath to 0 °C, the reaction mixture was diluted with water and extracted with $CHCl_3$. The extract was washed successively with saturated $NaHCO_3$ aqueous solution, water, 0.5 N hydrochloric acid, and brine. After being dried over anhydrous $MgSO_4$, the solvent was removed under reduced pressure. The residue was purified by preparative HPLC to give (*R*,*R*,*R*,*R*)-**6** (230 mg, 86% yield) as colorless solid:

m.p. 84–85 °C; ^{1}H NMR (270 MHz, $CDCl_3$, 30 ° C) δ 7.52 (2H, s, H_a), 7.41 (4H, d, J = 7.3 Hz, *o*-Ph), 7.17–7.31 (6H, m, *m*-, *p*-Ph), 5.94 (1H, bs, NH), 4.37(2H, d, J = 10.3 Hz, benzyl CH_2), 4.76 (2H, d, J = 10.3 Hz, benzyl CH_2), 4.09 (3H, s, OCH_3), 3.94 (2H, dd, J = 2.1, 10.6 Hz, CH), 3.37–3.44 (2H, m, $NHCH_2$), 3.06–3.27 (8H, m, OCH_2), 1.26–1.96 (18H, m, cyclohexyl CH_2 and CH_2–CH_2–CH_3), 1.01 (3H, t, J = 7.4 Hz, CH_3); IR (KBr) 2937, 2865, 1637, 1542, 1480, 1323, 1233, 1091, 758, 701 cm^{-1}; MS (FAB) *m*/*z* 694 $(M + Na)^+$, 672 $(M + 1)^+$; $[\alpha]_D^{24} = -65.1$ (c = 0.66, $CHCl_3$); Anal. Calcd. for $C_{41}H_{53}NO_7$: C, 73.29; H, 7.95; N, 2.08. Found: C, 73.07; H, 8.11; N, 1.74.

3. Results and discussion

3.1. Design of CSP-2

In order to develop a CSP that has a better separation ability than CSP-**1**, we selected crown ether (*S*,*S*,*S*,*S*)-**4**. The relatively bulky chiral unit of (*S*,*S*,*S*,*S*)-**4**, reportedly exhibits better chiral recognition than the (*S*,*S*)-**3** unit of CSP-**1** [18]. CSP-**2**, which has the same pseudo-18-crown-6 moiety as that of (*S*,*S*,*S*,*S*)-**4** was designed with the expectation that it would not only provide good enantiomer separation for chiral primary amines, but also reduce the retention times for a faster analysis.

3.2. Preparation of CSP-2

The preparation of CSP-**2** is shown in Scheme 1. Crown ether (*R*,*R*,*R*,*R*)-**5** was prepared from optically pure *cis*-1-phenyl-1,2-cyclohexanediol with a known absolute configuration [19–24]. CSP-**2** was obtained by amidation of (*R*,*R*,*R*,*R*)-**5** with 3-aminopropyl silica gel, followed by treatment with acetic anhydride. The amount of selectors bound to the silica gel was 0.19 mmol/g calculated based on the elemental analyses of CSP-**2**. Then, CSP-**2** was packed into a 250 mm × 4.6 mm i.d. stainless steel column using a conventional slurry packing method.

3.3. Enantiomer separation of amino acids

Typical chromatograms of the enantiomer separation of selected amino acids with this normal mobile phase are shown in Fig. 2. The results for enantiomeric resolution of all of the amino acids used, including the common natural amino acids, phenylglycine, and 3-(2-naphthyl)alanine, are summarized in Table 1 together with the data obtained on CSP-**1**. No peak was detected for lysine because of its large retention. The separation factors (α) of isoleucine, phenylglycine, asparagine, threonine, and aspartic acid were relatively small (<1.2). However, the other 14 amino acids were reasonably well resolved on CSP-**2**. Compared to CSP-**1**, the separation performance of CSP-**2** was improved for 13 out of 20 amino acids. For example, the separation factors for alanine, methionine, glutamine (entries 1, 6, 11) were 1.23, 1.26, 1.31 on CSP-**1**, respectively. The corresponding values were improved to 1.66, 2.14 and 1.68 on CSP-**2**. Amino acids such as valine, isoleucine, phenylglycine and threonine (entries 2,

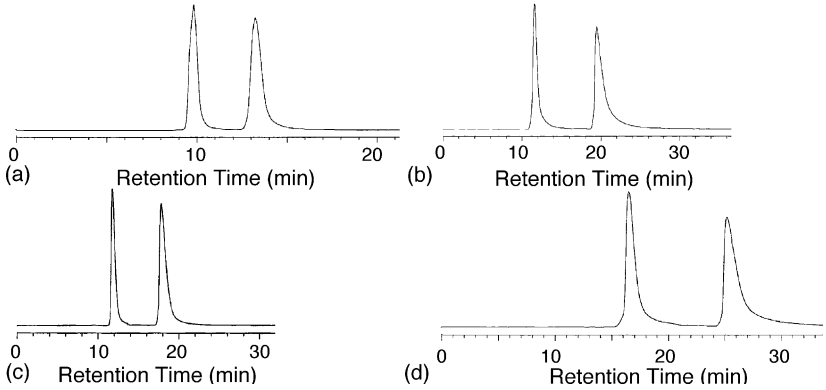


Fig. 2. Chromatograms of: (a) leucine, (b) methionine, (c) 3-(2-naphthyl)alanine, and (d) glutamic acid on CSP-**2** with hexane/ethanol/trifluoroacetic acid/water (85/15/0.5/0.2) with 0.7 mL/min flow rate detected by ESI-MS.

Table 1
Comparison of chromatographic resolution of amino acids between CSP-**1** and -**2**

Entry	Amino acid	CSP-**2**				CSP-**1**			
		t_1[a]	t_2[b]	α[c]	R_s[d]	t_1[a]	t_2[b]	α[c]	R_s[d]
1	Alanine	12.96	18.37	1.66	4.49	20.25	23.79	1.23	2.69
2	Valine	9.89	11.47	1.30	1.58	13.68	16.62	1.33	2.74
3	Leucine	9.83	13.25	1.67	3.23	13.35	14.53	1.14	1.64
4	Isoleucine[e]	8.42	8.42	1.00	–	11.85	14.80	1.41	3.07
5	Phenylalanine	10.96	16.43	1.87	4.19	17.08	20.45	1.27	2.68
6	Methionine	11.62	19.51	2.14	5.17	19.88	23.89	1.26	3.01
7	Tryptophan	17.62	25.42	1.60	4.70	43.00	52.92	1.26	4.47
8	Phenylglycine[e]	12.55	13.12	1.07	–	40.63	46.15	1.15	1.87
9	3-(2-Naphthyl)alanine	11.80	17.83	1.85	4.55	24.44	31.87	1.38	3.24
10	Asparagines	28.11	29.81	1.07	0.64	14.07	20.17	1.65	1.96
11	Glutamine	26.28	41.01	1.68	4.69	55.78	71.54	1.31	3.42
12	Cysteine	15.18	19.31	1.39	2.61	23.44	29.56	1.33	3.94
13	Serine	15.13	17.45	1.22	2.00	29.62	33.44	1.15	2.23
14	Threonine	11.01	11.64	1.10	0.37	22.66	27.24	1.26	3.27
15	Tyrosine	20.83	32.53	1.73	7.08	38.84	49.36	1.38	5.14
16	Aspartic acid	16.25	18.07	1.16	1.06	42.75	53.61	1.29	7.91
17	Glutamic acid	16.52	25.19	1.73	4.70	33.84	44.96	1.38	6.38
18	Histidine	28.17	51.41	1.99	3.31	30.90	35.48	1.17	2.09
19	Lysine[f]	–	–	–	–	56.23	67.16	1.21	2.04
20	Arginine	30.43	43.44	1.51	2.56	41.44	53.08	1.32	1.70

Mobile phase: hexane/ethanol/TFA/water = 85/l 5/0.5/0.2; flow rate: 0.7 mL/min; detection: ESl-MS.
[a] Retention time for the first eluted enantiomer.
[b] Retention time for the second eluted enantiomer.
[c] Separation factor.
[d] Resolution factor.
[e] Only one peak was detected on CSP-**2**.
[f] No peak was detected on CSP-**2**.

4, 8, 14) that have a branched or bulky group near the α-carbon were not well separated on CSP-**2**. Whereas CSP-**2** is not well suited for enantiomer separation of bulky amino acids, it works well for amino acids bearing a relatively small substituent group.

The retention times for amino acids having a bulky side chain are short on both CSP-**1** and -**2** (Table 1, entries 1–4), indicating that these amino acids do not strongly interact with the selector. Comparing the retention times on CSP-**2** with those on CSP-**1**, a notable reduction of retention times was observed for 17 of the 20 amino acids. Although, the amount of grafted crown ethers on silica gel of CSP-**2** (0.19 mmol/g) is smaller than that of CSP-**1** (0.23 mmol/g), it is noteworthy that 12 amino acids out of 20 were separated with better separation factors and shorter retention times.

3.4. Enantiomer separation of amino alcohols

CSP-**2** also exhibits good chiral separation ability for amino alcohols. As shown in Table 2, the aliphatic amino alcohols having a relatively small substituent at the α-position exhibited good separation factors (entries 1 and 2, $\alpha = 1.29$ and 1.52, respectively), whereas those having a bulky substituent group exhibited no or poor separation (entries 3 and 4, $\alpha = 1.00$ and 1.10, respectively). 1-Amino-2-propanol, which has a chiral center on the β-position, was separated well (entry 5, $\alpha = 1.25$). Moreover, the separation of (1*S*,2*R*)- and (1*R*,2*S*)-norephedrine, which have two chiral centers on the α- and β-positions, was substantially improved with a separation factor 3.82 (entry 6) compared to the case of CSP-**1** ($\alpha = 1.47$).

3.5. Enantiomer separation of other amines

Enantiomer separation of 1-phenylethylamine and 1-(1-naphthyl)ethylamine was examined with CSP-**2**. As shown in Table 2, while enantiomers of 1-phenylethylamine were separated well with a separation factor 1.26, those of 1-(1-naphthyl)ethylamine were not separated well (entries 7 and 8). In both cases, the retention times were shortened compared to those on CSP-**1**.

3.6. Enantiomer separation of methyl esters of amino acids and comparison between the selectivities in chromatographic resolution and in mass spectrometry

Crown ether (*S*,*S*,*S*,*S*)-**4**, which has the same pseudo-18-crown-6 moiety as that of CSP-**2**, but with opposite chirality, forms stable complexes with alanine methyl ester and phenylalanine methyl ester [18]. It was reported that (*S*,*S*,*S*,*S*)-**4** formed more stable complexes with (*S*)-enantiomers of the methyl esters than the corresponding (*R*)-enantiomers based on the peak intensity ratio (called *IRIS*) of the diastereomeric complex ions in the FABMS method, which used racemic esters where, one of the enantiomers was labeled with deuterium [25]. In order to examine the relationship between

Table 2
Comparison of chromatographic resolution of amino alcohols and amines between CSP-**1** and -**2**

Entry	Amino compound	CSP-**2**				CSP-**1**			
		t_1[a]	t_2[b]	α[c]	R_s[d]	t_1[a]	t_2[b]	α[c]	R_s[d]
1	2-Amino-1-propanol	17.77	21.54	1.29	1.37	21.05	22.95	1.12	0.39
2	2-Amino-l-butanol	13.75	18.49	1.52	3.47	16.33	17.53	1.10	0.66
3	2-Amino-3-methyl-1-butanol[f]	9.28	9.28	1.00	–	11.71	12.39	1.10	0.39
4	Phenylglycinol	10.68	11.27	1.10	0.42	18.95	20.57	1.11	0.98
5	1-Amino-2-propanol	17.20	20.29	1.25	3.60	24.36	25.27	1.05	0.15
6	Norephedrine[e]	9.77	24.07	3.82	7.36	8.69	10.56	1.47	2.30
7	1-Phenylethylamine	9.74	11.04	1.26	2.68	15.82	15.82	1.00	–
8	1-(1-Naphthy l)ethy-1-amine	9.75	10.66	1.17	1.66	19.91	23.69	1.25	3.13

Mobile phase: hexane/ethanol/TFA/water = 85/l 5/0.5/0.2; flow rate: 0.7 mL/min; detection: ESI-MS.
[a] Retention time for the first eluted enantiomer.
[b] Retention time for the second eluted enantiomer.
[c] Separation factor.
[d] Resolution factor.
[e] Mobile phase: hexane/ethanol/TFA/water = 70/30/0.5/0.2; flow rate: 0.7 mL/min; detection: UV (254 nm).
[f] Only one peak was detected on CSP-**2**.

Table 3
Chromatographic resolution of amino acid methyl esters on CSP-**2** and *R*/*S* values of the corresponding crown ether (*S*,*S*,*S*,*S*)-**4** by mass spectrometry

Entry	Methyl ester of amino acid	HPLC[a] on CSP-**2**					FAB-MS EL method[b]	
		t_1[c]	t_2[d]	α[c]	R_s[d]	Conf[e]	I_R/I_S[f]	Selectivity
1	Alanine	9.91	12.80	1.56	1.12	*R*	0.26	*S*
2	Phenylalanine	8.46	12.22	2.00	3.16	*R*	0.22	*S*

[a] Mobile phase: hexane/ethanol/trifluoroacetic acid/water = 85/15/0.5/0.2; flow rate: 0.7 mL/min; detection: ESI-MS.
[b] Reference [23].
[c] Separation factor.
[d] Resolution factor.
[e] Absolute configuration of the second eluted enantiomery.
[f] Peak intensity ratio of complex ions of the crown ether with (*R*)-and (*S*)-guest enantiomers, respectively, using enantiomer-labeled pseudo-racemic amino acid methyl esters (reference [16]).

the enantiomer selectivity observed for (*S*,*S*,*S*,*S*)-**4** by the FABMS method and that in chromatography using CSP-**2**, the enantiomer separation of these esters was carried out. The separation factors of alanine methyl ester (1.56) and phenylalanine methyl ester (2.00) were better than those with CSP-**1** (1.12 and 1.13, respectively). The configuration of the second eluted enantiomer turned out to be *R* for both amines (Table 3) indicating that CSP-**2** having an (*R*,*R*,*R*,*R*)-configuration interacted with the (*R*)-enantiomers more strongly than (*S*). Since (*S*,*S*,*S*,*S*)-**4** binds (*S*)-enantiomers of the corresponding ammonium ions of the esters [18], the selectivity of CSP-**2** is identical to that of (*S*,*S*,*S*,*S*)-**4**. These results suggest that the chiral separation on CSP-**2** is due in part to the host–guest interaction between the pseudo-18-crown-6 framework of CSP-**2** and the amines.

*3.7. Comparison between the enantiomer separation on CSP-**2** in chromatography and enantiomer selectivity of (R,R,R,R)-**6** in solution*

To further confirm the above observation, enantiomer selectivity of the model compound (*R*,*R*,*R*,*R*)-**6** was investigated in solution by ^{1}H NMR titration experiments. The hydrochloride salts of 1-phenylethylamine, 1-(1-naphthyl)ethylamine, and phenylglycinol were examined. However, the binding constants for the complexation of (*R*,*R*,*R*,*R*)-**6** with phenylethylamine and 1-(1-naphthyl)ethylamine enantiomers were too small to be determined ($K < 1\,M^{-1}$). While the binding constant with (*S*)-2-phenylglycinol was determined to be $18.4\,M^{-1}$, that with (*R*)-enantiomer was too small to be determined ($K < 1\,M^{-1}$). This means that (*R*,*R*,*R*,*R*)-**6** exhibits selective complexation with the (*S*)-enantiomer of 2-phenylglycinol hydrochloride in solution. The chromatographic retention time of (*S*)-2-phenylglycinol on CSP-**2** was longer than that of (*R*)-enantiomer. Therefore, enantiomer selectivities both in solution and in chromatography on CSP-**2** were (*S*)-selective.

In view of the correlation between the selectivity of chromatographic separations with CSP-**2** and solution-phase complexation of the model selector (*R*,*R*,*R*,*R*)-**6**, chiral separation likely arises from chiral recognition of the host–guest interactions [25].

4. Conclusion

In order to develop a CSP that has a better separation ability than CSP-**1**, chemically bonded type CSP-**2** having a rel-

atively bulky chiral 1-phenyl-1,2-cyclohexanediol unit was developed. Using normal phase chromatographic conditions, CSP-**2** exhibited short retention times and good resolution of the enantiomers of various amino compounds including amino acids, amino alcohols, and hydrophobic amino compounds. The separation of relatively bulky amino compounds was better on CSP-**1**, whereas small amino compounds were separated better on CSP-**2**. Overall, considerable improvements in the enantiomer separation were achieved for many amino compounds on CSP-**2** compared to CSP-**1**. CSP-**2** is not always superior to CSP-**1**. The two CSPs are complementary with each other in terms of chiral recognition. CSP-**2** and -**1** have little restriction in mobile phases, additives, and analytes because of chemically bonded nature, so that both phases have wide applicability for enantiomer separation.

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