Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 992 (2003) 47–56
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

New ligand exchange chiral stationary phase for the liquid chromatographic resolution of α- and β-amino acids

Myung Ho Hyun*, Sang Cheol Han, Sung Hee Whangbo
Department of Chemistry and Chemistry Institute for Functional Materials, Pusan National University, Kuemjeong-Ku, Pusan 609-735, South Korea

Received 15 November 2002; received in revised form 28 January 2003; accepted 28 January 2003

Abstract

A new ligand exchange chiral stationary phase (CSP) has been developed by covalently bonding (*R*)-*N*,*N*-carboxymethyl undecyl phenylglycinol mono-sodium salt onto silica gel and applied in the resolution of α- and β-amino acids. In the resolution of α-amino acids, the new CSP was better in some cases than the old one, which was previously developed by covalently bonding (*S*)-*N*,*N*-carboxymethyl undecyl leucinol mono-sodium salt onto silica gel, but worse in some other cases than the old one in terms of the separation factors (α). However, the new CSP was always much better than the old one in terms of the resolution factors (R_s). In the resolution of β-amino acids, the new CSP was always much better than the old one in terms of both the separation and resolution factors. In an effort to characterize the new CSP, the chromatographic behaviors for the resolution of selected α- and β-amino acids were investigated with the variation of the content of organic modifier and Cu(II) concentration in aqueous mobile phase and the column temperature.

Keywords: Chiral stationary phases, LC; Enantiomer separation; Ligand exchange; Amino acids

1. Introduction

Since the pioneering work of Davankov and co-workers, ligand exchange chiral stationary phases (CSPs) have been extensively studied and utilized in the liquid chromatographic resolution of various racemic α-amino acids [1–3]. While Cu(II) complexes of α-amino acids and their derivatives have been generally employed in resolving various racemic α-amino acids as CSPs after binding covalently [4–8] or hydrophobically [9–11] to solid column support, we have devoted our efforts to the utilization of Cu(II) complexes of optically active amino alcohol derivatives hydrophobically bound to octadecyl silica gel as dynamic CSPs in resolving various racemic α-amino acids. Actually, Cu(II) complexes of (1*S*,2*R*)-norephedrine derivative, (*R*)-alaninol derivative and (*S*)-leucinol derivative hydrophobically adsorbed onto a commercial octadecyl silica gel column have been successfully utilized as dynamic chiral stationary phases in resolving various α-amino acids [12–14]. Especially, a ligand exchange CSP developed by binding (*S*)-leucinol derivative, (*S*)-*N*,*N*-carboxymethyl dodecyl leucinol monosodium salt, onto a commercial reverse phase octadecyl silica gel was quite effective in resolving various α-amino acids in the presence of Cu(II) ion in

*Corresponding author. Fax: +82-51-516-7421.
E-mail address: mhhyun@pusan.ac.kr (M.H. Hyun).

0021-9673/03/$ – see front matter
doi:10.1016/S0021-9673(03)00276-0

aqueous mobile phase [15]. However, the chiral selector, which is hydrophobically bound on octadecyl silica gel, was found to bleed from the column when an aqueous mobile phase containing more than 20% acetonitrile is used.

In order to overcome the drawback of the dynamically coated CSP, we recently developed a new CSP (CSP **1**, Fig. 1a) by covalently bonding (*S*)-leucinol derivative, (*S*)-*N*,*N*-carboxymethyl undecyl leucinol monosodium salt, on silica gel [16]. CSP **1** was very successful in the resolution of various α-amino acids. In an effort to improve the chiral recognition ability of the CSP, in this study, we prepared another ligand exchange CSP (CSP **2**, Fig. 1b) by covalently bonding (*R*)-phenylglycinol derivative, (*R*)-*N*,*N*-carboxymethyl undecyl phenylglycinol mono-sodium salt, to silica gel. The phenyl group at the chiral center of the chiral selector of CSP **2** is different in terms of steric bulkiness and electron density from the isobutyl group at the chiral center of the chiral selector of CSP **1** and consequently, CSP **2** is expected to show the chiral recognition ability somewhat different from CSP **1**. In this study, we wish to characterize the new CSP (CSP **2**) by resolving various α-amino acids and β-amino acids and by comparing the chromatographic resolution results with those on CSP **1**.

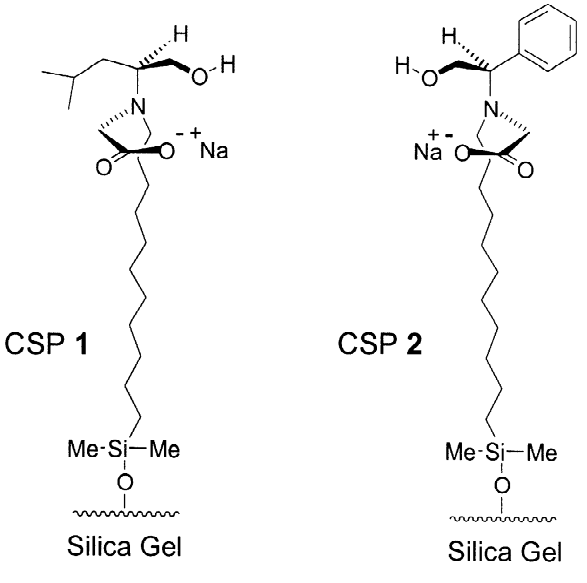

Fig. 1. The structures of CSP **1** and CSP **2**.

2. Experimental

2.1. Instrumentation

Chromatography was performed with a HPLC system consisting of a Waters Model 515 HPLC pump, a Rheodyne Model 7725i injector with a 20 μl sample loop, a YoungLin M720 Absorbance detector (variable wavelength) and a YoungLin Autochro Data Module (Software: YoungLin Autochro-WIN 2.0 plus). The temperature of the chiral column was controlled by using a Julabo F30 Ultratemp 2000 cooling circulator.

2.2. Preparation of CSP **2** and column packing

CSP **2** was prepared starting from (*R*)-phenylglycinol by covalently bonding its derivative, (*R*)-*N*,*N*-carboxymethyl undecyl phenylglycinol mono-sodium salt, to Kromasil silica gel (particle size: 5 μm, surface area: 340 m^2/g) via the exactly same procedure as that for the preparation of CSP **1** as shown in Fig. 2 [16]. Elemental analysis of modified silica gel **3** in Fig. 2 (C 5.05%, N 0.24%, H 0.92%) showed a loading of 0.18 mmol (based on C) of the chiral selector per gram of modified silica gel. The surface concentration of the chiral selector calculated based on the known equation [17] was 0.60 $\mu mol/m^2$ (based on C).

A mixture of modified silica gel **3** (4.2 g) suspended in 10 ml of methanol and 0.5 ml NaOH solution (1 *M* in water) in a 100-ml round bottom flask was stirred for 24 h at room temperature. The modified silica gel (CSP **2**) was filtered and washed with methanol. Elemental analysis of the modified silica gel (CSP **2**) did not show any notable change from that of modified silica gel **3** even after a treatment with NaOH solution. The modified silica gel (CSP **2**) thus prepared was slurried in methanol and packed into a 250×4.6 mm stainless steel HPLC column using a conventional method with an Alltech HPLC slurry packer.

2.3. Chromatography

All α-amino acids used in this study were available from previous study [13] or purchased from Aldrich. Both enantiomers of β-amino acids **4**–**13**

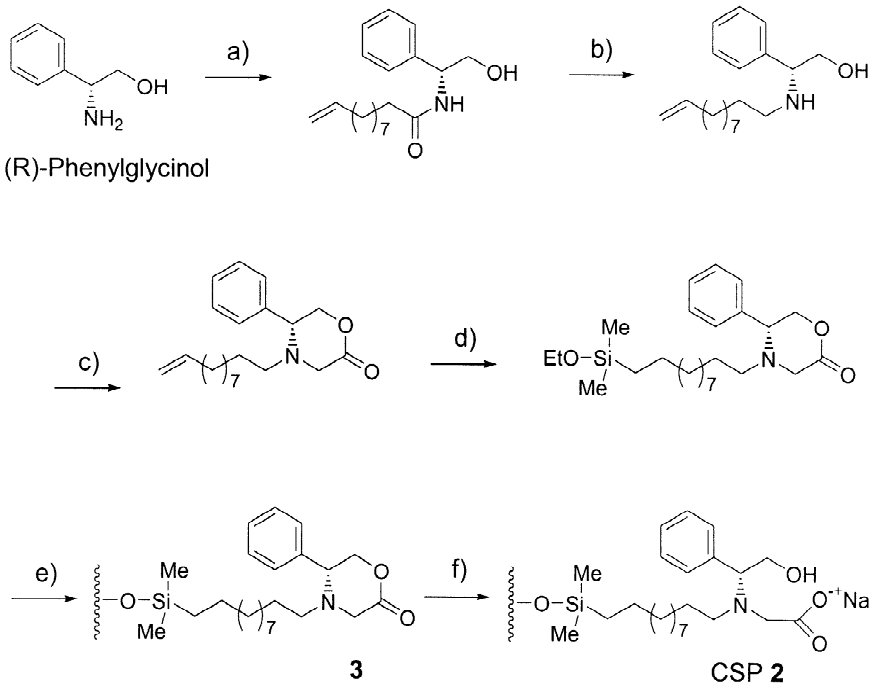

Fig. 2. Scheme for the preparation of CSP **2**. (a) 10-Undecenoyl chloride, triethylamine, methylene chloride, room temperature, 10 min, 70%. (b) $LiAlH_4$, tetrahydrofuran, 12 h, 94%. (c) Ethyl bromoacetate, triethylamine, methylene chloride, room temperature, 24 h, 48%. (d) (1) Chlorodimethylsilane, Pt/C (10%, w/w), methylene chloride, reflux, 18 h. (2) A mixture of absolute ethanol–triethylamine (1:1, v/v), methylene chloride, room temperature, 1 h, 78%. (e) 5 μm Kromasil silica gel, Dean-Stark trap, toluene, reflux, 72 h. (f) 1 *M* NaOH solution, methanol, room temperature, 24 h.

shown in Fig. 3 were purchased in an optically active form from Peptech (Cambridge, MA, USA). 3-Aminobutyric acid (**14**) and 3-aminoadipic acid (**15**) shown in Fig. 3 were available in racemic form from Aldrich.

To resolve racemic α-amino acids and β-amino acids on the chiral column packed with CSP **2**, a mobile phase, which was prepared by dissolving specified amount of $CuSO_4$ in deionized water or deionized water containing acetonitrile or methanol as an organic modifier, was passed through the column until the baseline (UV monitor, 254 nm) became stable to equilibrate the column and then, a methanolic solution (usually 3 μl) containing a racemic or optically enriched α-amino acid (usual concentration: 1.0 mg/ml) was injected. Column void volume (the elution time of an unretained solute) was measured by injecting aqueous $NaNO_3$ solution [18] or measured from the solvent front. The chiral column prepared in this study was found to be equally effective during the period of its use for more than 6 months.

3. Results and discussion

CSP **2** prepared via the procedure shown in Fig. 2 showed relatively low surface concentration of the chiral selector. However, CSP **2** showed excellent chiral recognition efficiencies for α- and β-amino acids. Ligand exchange chiral column packed with CSP **2** was first applied in the resolution of various α-amino acids. The chromatographic results for the resolution of various α-amino acids on CSP **2** are summarized and compared with those on CSP **1** in Table 1. All data in Table 1 were obtained using water containing Cu(II) ($2.0 \cdot 10^{-4}$ *M*) as a mobile phase with a flow-rate of 0.8 ml/min at 20 °C. The elution orders shown in Table 1 were determined by injecting configurationally known samples. The re-

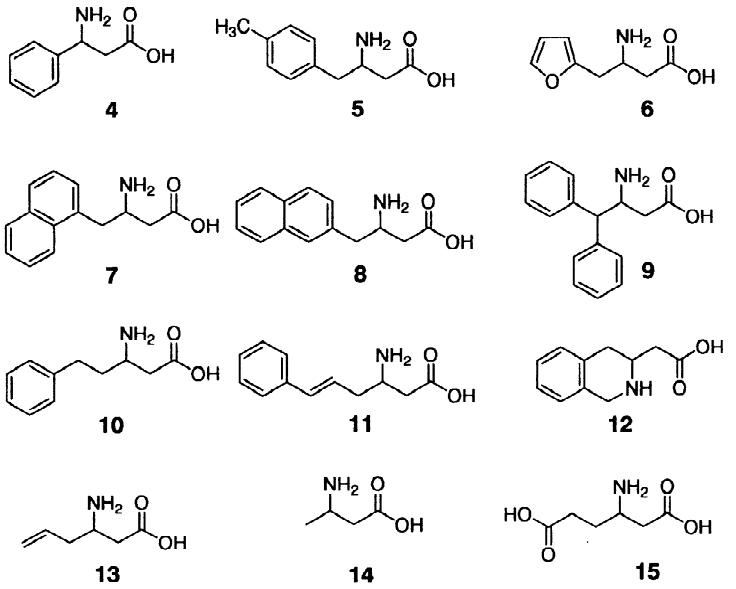

Fig. 3. The structures of β-amino acids used in this study. 3-Amino-3-phenylpropionic acid **4**, 3-amino-4-(4-methylphenyl)butyric acid **5**, 3-amino-4-(2-furyl)butyric acid **6**, 3-amino-4-(1-naphthyl)butyric acid **7**, 3-amino-4-(2-naphthyl)butyric acid **8**, 3-amino-4,4-diphenylbutyric acid **9**, 3-amino-5-phenylpentanoic acid **10**, 3-amino-6-phenyl-5-hexenoic acid **11**, 2-tetrahydroisoquinoline acetic acid **12**, 3-amino-5-hexenoic acid **13**, 3-aminobutyric acid **14** and 3-aminoadipic acid **15**.

Table 1
Comparison of the resolution of α-amino acids on CSP **1** and CSP **2** with the mobile phase of water containing $CuSO_4$ (0.2 m*M*) at 20 °C[a]

Amino acid	CSP **1**			CSP **2**		
	k_1	α	R_s	k_1	α	R_s
Alanine	2.03(D)	1.16	0.35	2.17(L)	1.17	1.05
2-Aminoadipic acid	4.42(D)	2.03	2.19	3.89(L)	2.12	4.18
Asparagine	2.29	1.00		1.82(L)	1.18	0.98
Aspartic acid	1.61(D)	1.69	0.88	1.73(L)	1.59	1.50
Glutamic acid	2.95(D)	2.23	1.74	3.75(L)	1.66	1.96
Glutamine	2.65(D)	1.26	0.75	2.73(L)	1.36	1.86
Leucine	5.03(D)	2.12	2.54	4.87(L)	1.73	2.64
iso-Leucine	4.48(D)	2.83	2.44	4.47(L)	2.36	4.12
nor-Leucine	5.24(D)	2.85	3.45	5.57(L)	2.20	3.74
tert.-Leucine	3.54	3.36	3.54	3.21	2.97	4.97
Methionine	5.05(D)	1.76	1.91	4.59(L)	1.61	3.70
Phenylalanine	9.38(D)	2.64	3.49	6.85(L)	2.20	4.49
Phenylglycine	4.51(D)	3.14	3.88	3.90(L)	2.43	5.64
Serine	1.98(D)	1.23	0.71	1.77(L)	1.40	2.03
Threonine	2.33(D)	1.21	0.60	2.30(L)	1.29	1.88
Tryptophan	24.88	2.85	3.35	13.65(L)	2.32	4.00
Tyrosine	5.10(D)	3.02	3.60	4.09(L)	2.62	4.55
α-Methyltyrosine	6.05	2.59	2.80	4.96(L)	2.70	4.57
Valine	3.07(D)	2.22	2.38	3.55(L)	1.97	3.98
nor-Valine	3.40(D)	2.10	2.43	3.61(L)	1.80	4.04

[a] The resolution data on CSP **1** were quoted from Ref. [13]. Flow-rate: 0.8 ml/min. Detection: 254 nm UV; k_1, retention factor of the first eluted enantiomer. Absolute configuration of the first eluted enantiomer is indicated in parentheses. For blanks, the elution order has not been determined; α, separation factor; R_s, resolution factor.

versed elution orders on CSP **1** and CSP **2** are rationalized to stem from the different absolute configuration of the chiral selectors of the two CSPs and consequently, the elution orders are concluded to be identical on the two CSPs in terms of chiral recognition mechanism.

As shown in Table 1, the separation factors (α values) are generally greater on CSP **1** than on CSP **2**. However, in the resolution of alanine, 2-aminoadipic acid, asparagine, glutamine, serine and threonine, the separation factors are slightly greater on CSP **2** than on CSP **1**. In addition, asparagine was resolved on CSP **2** with reasonable separation factor while it was not resolved at all on CSP **1**. In contrast to the separation factors, the resolution factors (R_s) are always much greater on CSP **2** than on CSP **1**. The greater resolution factors on CSP **2** were found to stem from the improved column efficiency. Especially, in the resolution of alanine, aspartic acid, glutamine, serine and threonine, clear baseline resolution was observed on CSP **2** while only none-baseline resolution was observed on CSP **1**. As an example, the typical chromatogram for the resolution of serine CSP **2** is compared with that on CSP **1** in Fig. 4.

CSP **2** was also applied to the resolution of β-amino acids. Optically active β-amino acids have attracted considerable attention due to their pharmacological activities and their usefulness as building blocks of many natural products [19,20]. However, the liquid chromatographic chiral resolution method of determining the enantiomeric composition of β-amino acids is not so common. Only limited number of papers has been reported for the liquid chromatographic resolution of β-amino acids on CSPs. For example, Pirkle-type CSPs were used for the direct resolution of β-amino acids with achiral derivatization [21,22]. CSPs based on macrocyclic antibiotics [23] and CSPs based on chiral crown ethers [23,24] were employed in the resolution of β-amino acids without derivatization. Liquid chromatographic resolution of β-amino acids on ligand exchange CSPs was also reported [25], but it was limited to only a few racemic samples. Very recently, we applied CSP **1** in the resolution of β-amino acids [26]. The resolution of β-amino acids on CSP **1** was generally successful. However, several β-amino acids were resolved on CSP **1** with only marginal separation factors and with non-baseline resolution and some β-amino acids were not resolved at all. In this instance, the very successful resolution of β-amino acids on CSP **2** is very interesting.

The resolution of β-amino acids on CSP **2** is summarized and compared with that on CSP **1** in Table 2. The resolution data shown in Table 2 were obtained under an identical resolution condition of 30% methanol in water containing 0.3 m*M* $CuSO_4$ as a mobile phase with a flow-rate of 0.8 ml/min at 20 °C. The elution orders obtained by injecting configurationally known samples are exactly opposite on the two CSPs and these opposite elution orders are rationalized to stem from the different absolute configuration of the chiral selectors of the two CSPs as described in rationalizing the reversed

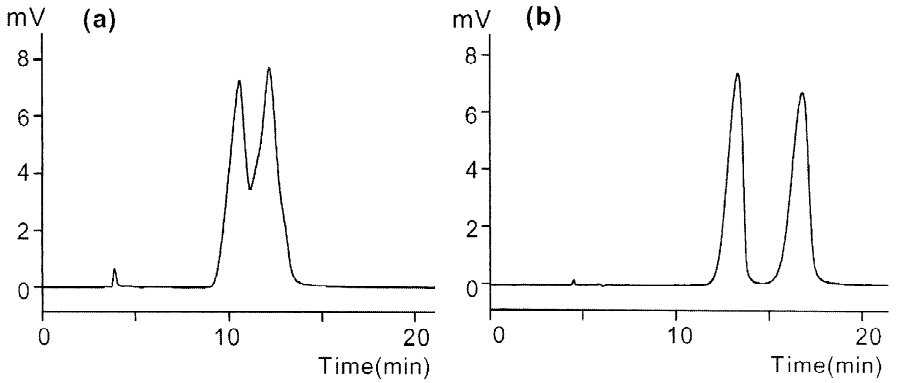

Fig. 4. Comparison of the chromatograms for the resolution of serine on (a) CSP **1** and (b) CSP **2**. Mobile phase: water + $CuSO_4$ (0.2 m*M*). Flow-rate: 0.8 ml/min. Detection: 254 nm UV. Temperature: 20 °C.

Table 2
Comparison of the resolution of 12 β-amino acids (**4**–**15**) on CSP **1** and CSP **2**[a]

β-Amino acid	CSP **1**			CSP **2**		
	k_1	α	R_s	k_1	α	R_s
4	1.46(*S*)	1.58	1.37	2.69(*R*)	2.03	3.05
5	3.62(*R*)	1.50	1.26	6.07(*S*)	1.85	2.91
6	1.55(*R*)	1.27	0.74	3.41(*S*)	1.53	1.82
7	12.68(*R*)	1.17	0.50	18.24(*S*)	1.47	1.92
8	12.41(*R*)	1.49	1.37	15.08(*S*)	1.95	3.00
9	10.08(*R*)	1.88	2.21	15.31(*S*)	2.39	4.88
10	2.95(*R*)	1.64	1.55	5.67(*S*)	1.92	3.16
11	4.92(*R*)	1.73	1.81	6.78(*S*)	2.11	3.52
12	1.84(*R*)	1.56	1.16	3.43(*S*)	2.14	2.69
13	0.86(*R*)	1.26	0.50	1.71(*S*)	1.57	1.53
14	0.48	1.00		0.83	1.25	0.45
15	1.65	1.00		5.75	1.16	0.56

[a] The resolution data on CSP **1** were quoted from Ref. [23]. Mobile phase: 30% CH_3OH in water + $CuSO_4$ (0.3 m*M*). Flow-rate: 0.8 ml/min. Detection: 254 nm UV. Temperature: 20 °C; k_1, retention factor of the first eluted enantiomer. Absolute configuration of the first eluted enantiomer is indicated in parentheses. For blanks, the elution order has not been determined; α, separation factor; R_s, resolution factor.

elution orders for the resolution of α-amino acids on the two CSPs. In addition, the elution orders for the resolution of 3-phenyl-3-aminopropanoic acid **3** on CSP **1** and CSP **2** are different from those for the resolution of other β-amino acids. These inconsistent elution orders have been rationalized to stem from the priority inversion of the substituents at the chiral center of β-amino acids according to the Cahn–Ingold–Prelog sequence rule [27]. The representative chromatograms for the comparison of the resolution of 3-amino-3-phenylpropionic acid **4** on CSP **1** and CSP **2** are presented in Fig. 5. As shown in Table 2 and in Fig. 5, CSP **2** is much better than CSP **1** in the resolution of β-amino acids in terms of both separation (α) and resolution factors (R_s).

As an effort to characterize CSP **2**, the effect of organic modifier and Cu(II) in aqueous mobile phase and the column temperature on the resolution of α- and β-amino acids was investigated with selected samples. The pH value of the aqueous mobile phase was also expected to affect the chromatographic behaviors for the resolution of α- and β-amino acids on CSP **2** from the earlier reports concerning the pH dependence of the chromatographic resolution behaviors [7,13]. However, the effort to improve further the chromatographic resolution behaviors on CSP **2** by optimizing pH is still under investigation and will be reported elsewhere. First of all, we selected three α-amino acids such as glutamic acid, leucine and phenylglycine and three β-amino acids such as 3-amino-3-phenylpropionic acid **4**, 3-amino-6-phenyl-5-hexenoic acid **11** and 3-amino-5-hexenoic acid **13** and resolved them on CSP **2** with the variation of the type and content of organic modifier and the Cu(II) concentration in aqueous mobile phase and the column temperature. The chromatographic resolution results are summarized in Table 3 and Table 4.

As shown in Table 3, an increase in the content of organic modifier such as methanol or acetonitrile in aqueous mobile phase improves the retention of α-amino acid analytes as denoted by the retention factors (k_1). These trends are exactly opposite to those on CSP **1**. Previously we reported that an increase in the content of organic modifier such as methanol or acetonitrile in aqueous mobile phase diminishes the retention of α-amino acid analytes [16]. In the resolution of β-amino acids on CSP **2**, an increase in the content of organic modifier such as methanol or acetonitrile in aqueous mobile phase also improves the retention of 3-amino-3-phenylpropionic acid **4** and 3-amino-5-hexenoic acid **13** as shown in Table 4. However, in the resolution of 3-amino-6-phenyl-5-hexenoic acid **11** on CSP **2**, the retention factor (k_1) diminishes as the content of organic modifier in aqueous mobile phase increases.

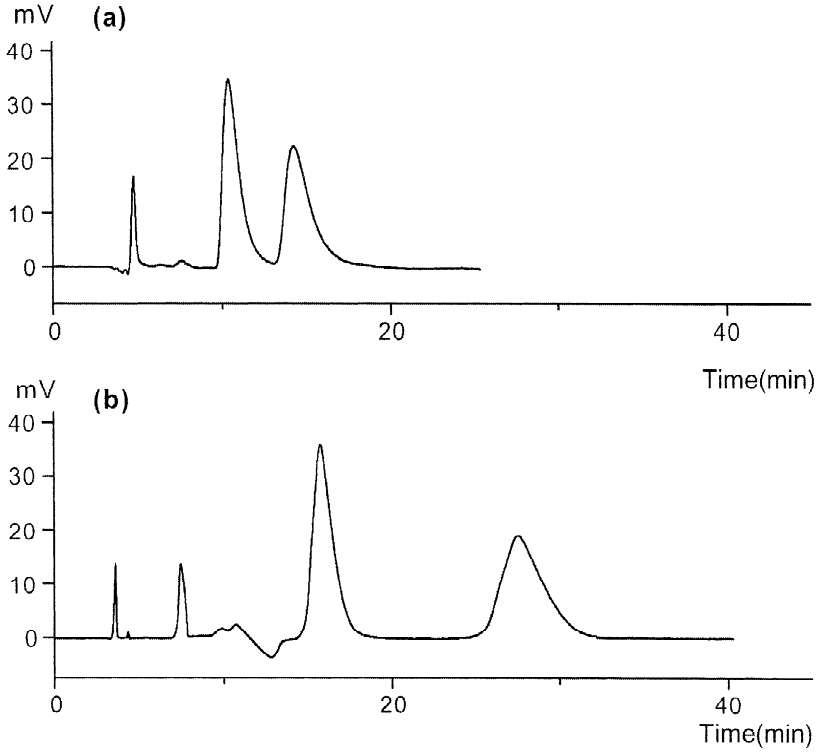

Fig. 5. Comparison of the chromatograms for the resolution of 3-amino-3-phenylpropionic acid **4** on (a) CSP **1** and (b) CSP **2**. Mobile phase: 30% CH_3OH in water + $CuSO_4$ (0.3 m*M*). Flow-rate: 0.8 ml/min. Detection: 254 nm UV. Temperature: 20 °C.

Table 3
Resolution of glutamic acid, leucine and phenylglycine on CSP **2** with the variation of the organic modifier (entry a) and Cu(II) concentration (entry b) in aqueous mobile phase and the column temperature (entry c)[a]

Entry	Variation	Glutamic acid			Leucine			Phenylglycine		
		k_1	α	R_s	k_1	α	R_s	k_1	α	R_s
a	10% CH_3OH in water + $CuSO_4$ (0.3 m*M*), 20 °C	5.02	1.53	1.84	3.41	1.89	3.07	3.51	1.99	4.65
	30% CH_3OH in water + $CuSO_4$ (0.3 m*M*), 20 °C	7.29	1.35	1.22	4.10	1.85	3.86	3.80	2.69	6.14
	50% CH_3OH in water + $CuSO_4$ (0.3 m*M*), 20 °C	10.14	1.23	0.80	4.30	1.73	3.04	4.00	2.58	5.88
	10% CH_3CN in water + $CuSO_4$ (0.3 m*M*), 20 °C	4.58	1.54	1.90	2.94	1.90	3.30	2.88	2.67	5.71
	30% CH_3CN in water + $CuSO_4$ (0.3 m*M*), 20 °C	5.44	1.09	0.30	3.55	1.60	3.09	3.29	2.33	5.68
	50% CH_3CN in water + $CuSO_4$ (0.3 m*M*), 20 °C	7.95	1.00		4.25	1.47	2.47	3.84	2.09	5.26
b	30% CH_3OH in water + $CuSO_4$ (0.2 m*M*), 20 °C	8.08	1.31	0.89	4.97	1.83	3.80	4.65	2.63	5.76
	30% CH_3OH in water + $CuSO_4$ (0.3 m*M*), 20 °C	7.29	1.35	1.22	4.10	1.85	3.86	3.80	2.69	6.14
	30% CH_3OH in water + $CuSO_4$ (0.4 m*M*), 20 °C	6.17	1.35	1.47	3.32	1.88	3.51	3.10	2.72	5.86
c	30% CH_3OH in water + $CuSO_4$ (0.3 m*M*), 15 °C	7.81	1.35	1.20	4.29	1.84	3.36	3.90	2.64	5.26
	30% CH_3OH in water + $CuSO_4$ (0.3 m*M*), 20 °C	7.29	1.35	1.22	4.10	1.85	3.86	3.80	2.69	6.14
	30% CH_3OH in water + $CuSO_4$ (0.3 m*M*), 25 °C	6.60	1.33	1.56	3.92	1.83	4.26	3.63	2.67	7.66

[a] Flow-rate: 0.8 ml/min. Detection: 254 nm UV; k_1, retention factor of the first eluted enantiomer; α, separation factor; R_s, resolution factor.

Table 4
Resolution of 3-amino-3-phenylpropionic acid **4**, 3-amino-6-phenyl-5-hexenoic acid **11** and 3-amino-5-hexenoic acid **13** on CSP **2** with the variation of the organic modifier (entry a) and Cu(II) concentration (entry b) in aqueous mobile phase and the column temperature (entry c)[a]

Entry	Mobile phase and column temperature	**4**			**11**			**13**		
		k_1	α	R_s	k_1	α	R_s	k_1	α	R_s
a	10% CH_3OH in water + $CuSO_4$ (0.3 m*M*), 20 °C	2.15	1.87	2.48	9.61	2.18	3.35	1.13	1.44	1.26
	30% CH_3OH in water + $CuSO_4$ (0.3 m*M*), 20 °C	2.69	2.03	3.05	6.78	2.11	3.52	1.71	1.57	1.53
	50% CH_3OH in water + $CuSO_4$ (0.3 m*M*), 20 °C	4.41	2.03	4.15	5.52	1.98	3.15	3.89	1.51	2.38
	30% CH_3CN in water + $CuSO_4$ (0.3 m*M*), 20 °C	5.15	1.77	4.84	7.78	1.60	3.86	2.90	1.38	1.85
	50% CH_3CN in water + $CuSO_4$ (0.3 m*M*), 20 °C	7.61	1.62	4.56	7.43	1.44	3.15	4.53	1.56	2.43
b	30% CH_3OH in water + $CuSO_4$ (0.2 m*M*), 20 °C	5.30	2.09	3.23	11.36	2.12	3.33	3.41	1.51	2.22
	30% CH_3OH in water + $CuSO_4$ (0.3 m*M*), 20 °C	2.69	2.03	3.05	6.78	2.11	3.52	1.71	1.57	1.53
	30% CH_3OH in water + $CuSO_4$ (0.4 m*M*), 20 °C	2.47	2.05	2.96	5.79	2.12	3.41	1.66	1.49	1.69
c	30% CH_3OH in water + $CuSO_4$ (0.3 m*M*), 15 °C	3.12	2.08	2.98	7.16	2.15	3.15	2.05	1.54	1.46
	30% CH_3OH in water + $CuSO_4$ (0.3 m*M*), 20 °C	2.69	2.03	3.05	6.78	2.11	3.52	1.71	1.57	1.53
	30% CH_3OH in water + $CuSO_4$ (0.3 m*M*), 25 °C	2.53	2.00	3.15	5.67	2.10	3.64	1.68	1.55	1.75

[a] Flow-rate: 0.8 ml/min. Detection: 254 nm UV; k_1, retention factor of the first eluted enantiomer; α, separation factor; R_s, resolution factor.

The retention trends for the resolution of 3-amino-3-phenylpropionic acid **4** and 3-amino-5-hexenoic acid **13** on CSP **2** are opposite to those on CSP **1**, but the trends for the resolution of 3-amino-6-phenyl-5-hexenoic acid **11** on CSP **2** are identical to those on CSP **1** [26].

In reversed-phase liquid chromatography, we assume that the retention of analytes is controlled by the balance of the two interactions such as the hydrophilic interaction of analytes with polar aqueous mobile phase and the lipophilic interaction of analytes with the stationary phase. In the resolution of α- or β-amino acids on CSP **1**, it seems from the resolution results that the lipophilic interaction of analytes with the CSP is predominant over the hydrophilic interaction of analytes with polar aqueous mobile phase presumably because of the relatively more lipophilic isobutyl group of the CSP. In this instance, an increase in the content of organic modifier in aqueous mobile phase reduces the lipophilic interaction of analytes with the CSP because the interaction between the organic modifier and the analyte competes with that between the analyte and the CSP and eventually reduces the retention of analytes. However, in the resolution of α- or β-amino acids on CSP **2**, it seems that the hydrophilic interaction of analytes with polar aqueous mobile phase is predominant over the lipophilic interaction of analytes with the CSP because of the less lipophilic phenyl group of the CSP. In this instance, an increase in the content of organic modifier in aqueous mobile phase diminishes the polarity of aqueous mobile phase and consequently, diminishes the hydrophilic interaction of analytes with the mobile phase. In this event, the retention of analytes increases as the content of organic modifier in aqueous mobile phase increases. However, in the resolution of highly lipophilic analyte such as 3-amino-6-phenyl-5-hexenoic acid **11** on CSP **2**, the lipophilic interaction of the analyte with the CSP seems to be predominant over the hydrophilic interaction of analytes with the polar aqueous mobile phase and consequently, the retention factor decrease as the content of organic modifier in aqueous mobile phase increases. In the resolution of more highly lipophilic β-amino acids such as 3-amino-4-(1-naphthyl)butyric acid **7**, 3-amino-4-(2-naphthyl)butyric acid **8**, 3-amino-4,4-diphenylbutyric acid **9** on CSP **2**, we also absorbed the same retention trends as those of 3-amino-6-phenyl-5-hexenoic acid **11** even though they are not included in Table 4.

The separation (α) and resolution factors (R_s) for the resolution of α- and β-amino acids on CSP **2** also depend on the content of organic modifier in aqueous mobile phase. As shown in Table 3, the separation (α) and resolution factors (R_s) for the resolution of

α-amino acids on CSP **2** generally decrease as the content of organic modifier in aqueous mobile phase increases. These trends, which are consistent with those on CSP **1** reported previously [16], are most significant in the resolution of glutamic acid on CSP **2**, but not so significant in the resolution of leucine and phenylglycine on CSP **2** with the mobile phase containing methanol as an organic modifier. In the resolution of 3-amino-3-phenylpropionic acid **4** and 3-amino-6-phenyl-5-hexenoic acid **11** on CSP **2**, notable resolution trends were not observed. However, in the resolution of 3-amino-5-hexenoic acid **1**, the resolution factor (R_s) was observed to increases as the content of methanol or acetonitrile in aqueous mobile phase increases while the separation factor (α) does not show significant trends as shown in Table 4.

An increase in Cu(II) concentration in aqueous mobile phase decreases the retention of both α- and β-amino acids continuously as shown in Table 3 (entry b) and Table 4 (entry b). In the resolution of α- and β-amino acids on CSP **1**, at least four species are proposed to be in equilibrium as shown in the following equation, where AA denotes α-amino acids [9]:

$$[\mathrm{Cu(AA)(Fixed\ ligand)}] + \mathrm{Cu(II)} \rightleftarrows [\mathrm{Cu(AA)}]^{+} + [\mathrm{Cu(Fixed\ Ligand)}]^{+}$$

The equilibrium shown above is also expected for the resolution of α- and β-amino acids on CSP **2**. In this instance, as the Cu(II) concentration in aqueous mobile phase increases, formation of mobile binary complex such as $[\mathrm{Cu(AA)}]^{+}$ improves and consequently, the retention decreases. However, the separation (α) and resolution factors (R_s) do not show significant trends in the resolution of α- and β-amino acids on CSP **2** with the variation of Cu(II) concentration in aqueous mobile phase.

The column temperature is another factor, which can influence the resolution of racemic analytes on CSP **2**. Resolution of α- and β-amino acids on CSP **2** with the variation of the column temperature is summarized in Table 3 (entry c) and Table 4 (entry c). As the column temperature increases, the retention of analytes decreases as denoted by the retention factors (k_1). At higher temperature, the formation of the complexes formed from the analyte, fixed chiral ligand and Cu(II) is expected to be less favorable. In this instance, the retention of analyte decreases as the column temperature increases.

As the column temperature increases, the resolution factors (R_s) for the resolution of α- and β-amino acids on CSP **2** increases as shown in Table 3 (entry c) and Table 4 (entry c). At higher temperature, the rate of equilibrium for the formation of ternary complexes is expected to be fast and consequently, the lifetime of the transient diastereomeric ternary complex is quite short. In this instance, the chromatographic peaks corresponding to the two enantiomers become sharp and large resolution factors (R_s) are observed at higher column temperature. However, the separation factors (α) do not show significant trends with the variation of the column temperature.

The chiral recognition mechanism for the resolution of α- and β-amino acids on CSP **1** has been proposed in the previous studies [16,26]. The identical chiral recognition mechanism is expected to be utilized for the resolution of α- and β-amino acids on CSP **2**. In that chiral recognition mechanism, the hydroxy group of the chiral selector of the CSP was proposed to play an important role in the chiral recognition by axially coordinating Cu(II) at the square planar coordination site.

In summary, a new ligand exchange CSP (CSP **2**) was developed in this study by covalently bonding (*R*)-*N*,*N*-carboxymethyl undecyl phenylglycinol mono-sodium salt onto silica gel and applied in the resolution of α- and β-amino acids. In the resolution of α-amino acids, CSP **2** was better in some cases than CSP **1**, which was previously developed by covalently bonding (*S*)-*N*,*N*-carboxymethyl undecyl leucinol mono-sodium salt onto silica gel, but worse in some other cases than CSP **1** in terms of the separation factors (α). However, CSP **2** was always much better than CSP **1** in terms of the resolution factors (R_s). In the resolution of β-amino acids, CSP **2** was always much better than CSP **1** in terms of both the separation (α) and resolution factors (R_s). The chromatographic behaviors for the resolution of α- and β-amino acids on CSP **2** were found to be dependent on the variation of the content of organic modifier, the Cu(II) concentration in aqueous mobile phase and the column temperature.

Acknowledgements

This work was supported by KISTEP (NRL program: M1-0104-00-0005-02-J0000-00310).

References

[1] V.A. Davankov, J.D. Navratil, H.F. Walton, Ligand Exchange Chromatography, CRC Press, Boca Raton, FL, 1988.
[2] V.A. Davankov, Enantiomer 5 (2000) 209.
[3] A. Kurganov, J. Chromatogr. A 906 (2001) 51.
[4] V.A. Davankov, Yu.A. Zolotarev, J. Chromatogr. 155 (1978) 285.
[5] P. Roumeliotis, K.K. Unger, A.A. Kurganov, V.A. Davankov, Angew. Chem. Int. Ed. Eng. 12 (1982) 930.
[6] G. Gubitz, F. Juffmann, W. Jellenz, Chromatographia 16 (1982) 103.
[7] P. Roumeliotis, K.K. Unger, A.A. Kurganov, V.A. Davankov, J. Chromatogr. 255 (1983) 51.
[8] G. Gubitz, J. Liq. Chromatogr. 9 (1986) 519.
[9] V.A. Davankov, A.S. Bochkov, A.A. Kurganov, P. Roumeliotis, K.K. Unger, Chromatographia 13 (1980) 677.
[10] V.A. Davankov, A.S. Bochkov, Yu.P. Belov, J. Chromatogr. 218 (1981) 547.
[11] M. Remelli, P. Fornasari, F. Pulidori, J. Chromatogr. A 761 (1997) 79.
[12] M.H. Hyun, N.-E. Lim, S.-N. Choi, Bull. Kor. Chem. Soc. 12 (1991) 594.
[13] M.H. Hyun, J.-J. Ryoo, N.-E. Lim, J. Liq. Chromatogr. 16 (1993) 3249.
[14] M.H. Hyun, D.H. Yang, H.J. Kim, J.-J. Ryoo, J. Chromatogr. A 684 (1994) 189.
[15] M.H. Hyun, J.-J. Ryoo, J. Liq. Chromatogr. Rel. Technol. 19 (1996) 2635.
[16] M.H. Hyun, S.C. Han, C.W. Lee, Y.K. Lee, J. Chromatogr. A 950 (2002) 55.
[17] J.S. Jin, A.M. Stalcup, M.H. Hyun, J. Chromatogr. A 933 (2001) 83.
[18] M.J.M. Wells, C.R. Clark, Anal. Chem. 53 (1981) 1341.
[19] M. Seki, K. Matsumoto, Tetrahedron Lett. 37 (1996) 3165.
[20] G. Cardillo, C. Tomasini, Chem. Soc. Rev. 25 (1996) 117.
[21] O.W. Griffith, E.B. Campbell, W.H. Pirkle, A. Tsipouras, M.H. Hyun, J. Chromatogr. 362 (1986) 345.
[22] F. Gasparrini, I. D'Acquarica, C. Villani, C. Cimarelli, G. Palmieri, Biomed. Chromatogr. 11 (1997) 317.
[23] A. Peter, L. Lazar, F. Fulop, D.W. Armstrong, J. Chromatogr. A 926 (2001) 229.
[24] M.H. Hyun, Y.J. Cho, J.S. Jin, J. Sep. Sci. 25 (2002) 648.
[25] S. Yamazaki, T. Takeuchi, T. Tanimura, J. Chromatogr. 540 (1991) 169.
[26] M.H. Hyun, S.C. Han, S.H. Whangbo, Biomed. Chromatogr., in press.
[27] E.L. Eliel, S.H. Wilen, Stereochemistry of Organic Compounds, Wiley, New York, 1994.