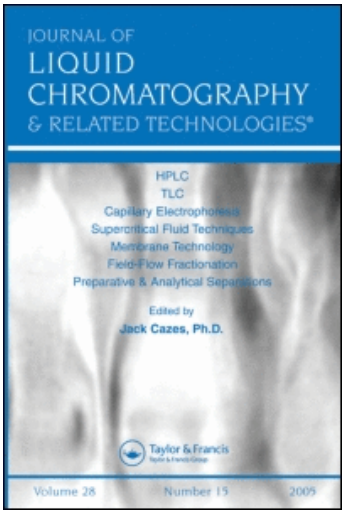

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Optical Resolution of Racemic α-Amino Acids on a Dynamic Chiral Stationary Phase Derived from (S)-Leucinol by Ligand Exchange Chromatography

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OPTICAL RESOLUTION OF RACEMIC α-AMINO ACIDS ON A DYNAMIC CHIRAL STATIONARY PHASE DERIVED FROM (S)-LEUCINOL BY LIGAND EXCHANGE CHROMATOGRAPHY

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ABSTRACT

A dynamic chiral stationary phase (CSP **7**) for resolving racemic α-amino acids have been prepared by hydrophobically loading (S)-N,N-carboxymethyl dodecyl leucinol monosodium salt onto a commercial reverse phase octadecyl silica gel column. CSP **7** was successfully employed in resolving various racemic α-amino acids. The chromatographic results for resolving various racemic α-amino acids on CSP **7** have been found to be generally better as expected from the chiral recognition model proposed than those on the previously reported dynamic CSP (**2**), (R)-N,N-carboxymethyl dodecyl alaninol monosodium salt which is hydrophobically bound onto a commercial reverse phase octadecyl silica gel column. Especially, CSP **7** seems to be more attractive than CSP **2** in that CSP **7** shows reasonably good resolving ability for the broad range of racemic α-amino acids at the high content of organic modifier (20 % CH_3CN) in the aqueous mobile phase while CSP **2** shows very poor resolving ability.

INTRODUCTION

Chiral ligand exchange chromatography has proven to be a very useful means for separating enantiomers of racemic α-amino acids as shown by Davankov and other workers.[1-5] For example, Cu (II) complexes of optically active α-amino acids and their derivatives have been successfully employed in resolving various racemic α-amino acids as chiral mobile phase additives[6,7] or chiral stationary phases (CSPs) after binding covalently[8,9] or hydrophobically[10-12] to solid column support.

In this area, our efforts have been focused on the use of Cu (II) complexes of optically active aminoalcohol derivatives hydrophobically bound to octadecyl-silica gel as dynamic CSPs in resolving various racemic α-amino acids. For example, two dynamic CSPs (CSP **1** and **2**) based on Cu (II) complexes of (1S,2R)-norephedrine derivative and (R)-alaninol derivative hydrophobically adsorbed on octadecyl-silica gel have been developed and used in resolving various racemic α-amino acids.[13-15]

Based on the chromatographic resolution trends of showing higher enantioselectivity on CSP **2** than on CSP **1** for the two enantiomers of α-amino acids having a simple hydrophobic α-alkyl substituent and the chiral recognition model concerning the formation of the energetically different two diastereomeric ternary complexes shown in Figure 1, we have concluded that the phenyl functionality at the first chiral center of CSP **1** is not essential in the chiral recognition and simply disturbs the axial coordination by the hydroxy group of the fixed ligand (chiral selector) in the square planar coordination sphere of the ternary complex.[15]

In the chiral recognition model shown in Figure 1, the α-alkyl substituent of the (D)-enantiomers is intercalated between the octadecyl chains of silica gel while that of the (L)-enantiomers is directed into the bulk of mobile phase and, in consequence, the (D)-enantiomers of α-amino acids having a simple α-alkyl substituent are retained longer than the (L)-enantiomers because of the greater lipophilic interaction between the α-alkyl substituent of the (D)-enantiomers and the octadecyl chains of silica gel. However, the retention mode of the (L)-enantiomers is not precisely explained in that model.

In this study, we propose a retention mode of the (L)-enantiomers, which also utilizes the lipophilic interaction between the α-alkyl substituent of the (L)-enantiomers and the octadecyl chains of silica gel. Based on the chiral recognition modes proposed for the retention of (D)- and (L)-enantiomers, we rationalize that a dynamic CSP derived from (S)-leucinol derivative may show greater enantioselectivity for the two enantiomers of racemic α-amino acids than CSP **1** or **2**.

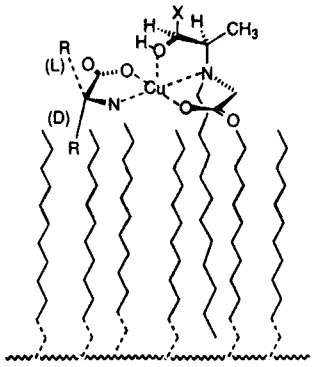


Figure 1. The proposed structure of the diastereomeric ternary complex formed (a) from the fixed ligand of CSP **1**, (D)- or (L)-amino acid and Cu(II) or (b) from the fixed ligand of CSP **2**, (D)- or (L)-amino acid and Cu(II).

EXPERIMENTAL

Instrumentation

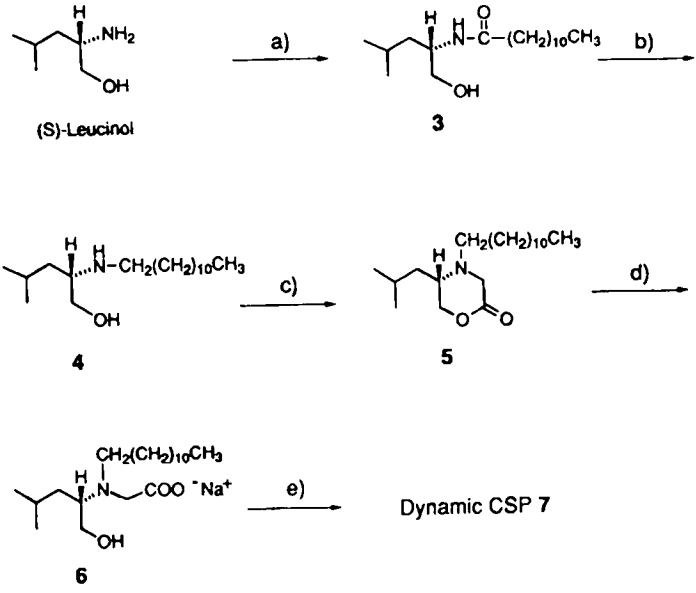
Melting point determination was performed by using a Rigaku Thermal Analyzer TAS 100. ^{1}H NMR spectra were recorded on a Varian Gemini 300 or a Varian EM-360A spectrometer using tetramethylsilane as an internal standard. IR spectra were recorded on a Mattson Galaxy 2000 FT-IR spectrometer. Mass data (EI) were obtained on a VG Trio 2000 GC/MS system.

Chromatographic resolution data were collected on an HPLC system consisting of Waters Model 510 pump, Waters Model U6k Universal Chromatographic Injector, Waters Model 441 Absorbance Detector with 254 nm UV filter and Waters Model 740 Data Module Recorder.

Preparation of Dynamic CSP 7 from (S)-Leucinol

Dynamic CSP 7 was prepared by the procedure shown in Scheme 1. The detailed synthetic procedures are as follows.

(S)-N-Lauroylleucinol 3: To a stirred solution of (S)-leucinol (3.0 g, 25.1 mmole) and triethylamine (5 mL, 36 mmole) in 50 mL of dry methylene chloride was added a solution of lauroyl chloride (3.8 mL, 25.1 mmole) in 10 mL of dry methylene chloride under nitrogen at room temperature.



Scheme 1. (a) lauroyl chloride, triethylamine, methylene chloride, room temperature, 40 min., 98 %. (b) $LiAlH_4$, THF, 48 hr., 97 %. (c) ethylbromoacetate, triethylamine, methylene chloride, room temperature, 48 hr., 42.5 %. (d) 1 N aq. NaOH, MeOH, room temperature, 6 hr. 99 %. (e) Hydrophobic loading onto a commercial reverse phase C_{18} column.

The reaction mixture was stirred at room temperature under nitrogen for 30 min and then washed successively with 0.5 N HCl, 0.5 N NaOH, and water. The organic solution was dried over anhydrous $MgSO_4$ and filtered, and the solvent was removed under reduced pressure. The residue was crystallized from the mixed solvent of methylene chloride and hexane at 0 °C to afford **3** as a white crystalline solid (7.35g, 98.0%). m.p. : 42.0-43.5 °C, ^{1}H NMR ($CDCl_3$) δ 0.87(t, 3H), 0.93(dd, 6H), 1.20-1.38(m, 18H), 1.58-1.67(m,3H), 2.19(t, 2H), 2.58(broad s, 1H), 3.52(dd, 1H), 3.67(dd,1H), 4.02-4.06(m, 1H), 5.60(broad s, 1H),. IR(KBr) cm^{-1} 3304, 2955, 2918, 2851, 1642, 1547.

(S)-N-Dodecyl leucinol 4: A solution of $LiAlH_4$ (2.28 g, 60 mmole) in 50 mL of dry tetrahydrofuran was added to a stirred solution of **3** (5 g, 16.7 mmole) in 15 mL of dry tetrahydrofuran through dropping funnel over 30 min at 0 °C. The whole mixture was refluxed for 2 days. The reaction mixture was cooled to 0 °C and then quenched by adding water. The whole mixture was passed through the bed of celite and then tetrahydrofuran was removed under reduced

pressure. The aqueous solution was extracted with methylene chloride. The methylene chloride solution was dried over anhydrous $MgSO_4$, and filtered and then methylene chloride was removed under reduced pressure. The residue was crystallized from the mixed solvent of methylene chloride and hexane to afford **4** as a white crystalline solid (4.65 g, 97.0 %). m.p. : 70.5-72.0 °C, ^{1}H NMR ($CDCl_3$) δ 0.87(t, 3H), 0.90(d, 6H), 1.19-1.35(m, 20H), 1.40-1.47(m, 2H), 1.57-1.66(m,1H), 2.03(broad s, 2H), 2.49-2.69(m, 3H), 3.21(dd, 1H), 3.60(dd, 1H) IR(KBr) cm^{-1} 3297, 3104, 2959, 2920, 2851, 1469.

(S)-4-Dodecyl-5-isobutyl-2,3,5,6-tetrahydro-4H-1,4-oxazin-2-one 5: To a stirred solution of **4** (4.50 g, 15.8 mmole) in 30 mL of dry benzene was added a solution of ethyl bromoacetate (1.94 mL, 17.3 mmole) in 10 mL of dry benzene. The reaction mixture was refluxed for 36 hr and then cooled to room temperature and concentrated. The white oily residue was purified by column chromatography on silica gel (ethylacetate:hexane:dichloromethane = 1:10:1, v/v/v) to give **5** as a colorless oil (2.18 g, 42.5 %). ^{1}H NMR ($CDCl_3$) 0.86(t, 3H), 0.92(dd, 6H), 1.21-1.32(m, 20H), 1.36-1.44(m, 2H), 1.57-1.66(m, 1H), 2.38-2.57(m, 2H), 3.33(d, 1H), 3.48(d, 1H), 4.12(dd, 1H), 4.39(dd, 1H). IR(KBr) cm^{-1} 2957, 2926, 2855, 1751, 1468. MS(EI) m/e : 325(M^+).

(S)-N,N-Carboxymethyl dodecyl leucinol monosodium salt 6 and hydrophobic loading onto a commercial reverse phase octadecyl-silica gel column (preparation of CSP 7): NaOH solution (1 M inH_2O, 6.20 mL) was added dropwise to a stirred solution of **5** (2.00 g, 6.15 mmole) in 30 mL CH_3OH at room temperature. After being stirred for 5 hr at room temperature, the solvent was evaporated under reduced pressure and the residue was dried under high vacuum for 10 hr to afford oily product (2.24 g, 99%). [IR(KBr) cm^{-1} 3296, 2955, 2926, 2855, 1678, 1595. MS(EI) m/e : 365(M^+)]. Hydrophobic loading of **6** onto a commercial reverse phase octadecyl-silica gel column (Waters μ-Bondapak™ C_{18}, 3.9 x 300mm) to afford dynamic CSP **7** was performed by eluting a solution of **6** (2.0 g) in 30 mL of methanol/water (1:2, v/v) through the column (flow rate : 0.5 mL/min) followed by washing with 150 mL of methanol/water (1:2, v/v, flow rate: 0.3 mL/min). The loaded amount of **6** was not able to be determined. However, the used amount of **6** (2.2 g) was assumed to be large enough to be fully loaded because the bleeding of the excess of **6** from the column was detected by the UV monitor.

Chromatography

Preparation of mobile phase: Mobile phase was prepared by dissolving specified amount of $CuSO_4$ in deionized water or deionized water containing acetonitrile or methanol as an organic modifier.

Chromatography: To resolve racemic α-amino acids on dynamic CSP **7**, a mobile phase was eluted through the column until the baseline (UV monitor, 254 nm) became stable to equilibrate the column and then, an aqueous solution containing a racemic α-amino acid was injected. Flow rate was 0.8 mL/min. Dynamic CSP **7** used in this study was found to be equally effective for the chiral separation of racemic α-amino acids for at least three months.

RESULTS AND DISCUSSION

The chiral recognition model shown in Figure 1 indicates that the lipophilic interaction between the α-alkyl substituent of the (D)-enantiomers and the octadecyl chains of silica gel plays an important role in retaining (D)-enantiomers. However, the chiral recognition mode retaining the (L)-enantiomers does not utilize the lipophilic interaction. Under the conditions of aqueous mobile phase, it seems to be unreasonable to leave out the possibility of the lipophilic interaction between the α-alkyl substituent of the (L)-enantiomers and the octadecyl chains of silica gel. Consequently, we tried to figure out from the study of chemical models any possible retention mode of the (L)-enantiomers, which utilizes the lipophilic interaction between the α-alkyl substituent of the (L)-enantiomers and the octadecyl chains of silica gel.

One possible retention mode of the (L)-enantiomers utilizing the lipophilic interaction between the α-alkyl substituent of the (L)-enantiomers and the octadecyl chains of silica gel with the trans (N,N)-configuration, which is known to be more stable than the cis (N,N)-configuration,[10] is observed from the molecular model study by reconstructing the ternary complex after switching the bonding positions of the N-carboxymethyl unit and the N-alkyl chain of the fixed ligand (chiral selector). (Note that the inversion of configuration at the nitrogen center is fast even at room temperature). Switching the bonding positions of the N-carboxymethyl unit and the N-alkyl chain of the fixed ligand reverses the direction of the N-carboxymethyl unit and, in consequence, the reconstructed ternary complex with the trans (N,N)-configuration allows the α-alkyl substituent of the (L)-enantiomer intercalate between the octadecyl chains of silica gel (the interested leaders are encouraged to construct the ternary complex using molecular models). The two chiral recognition modes for retaining (D)- and (L)-enantiomers selectively are compared in Figure 2. As shown in Figure 2, the two chiral recognition modes are quite similar except the orientation of the methyl group and the hydrogen at the chiral center of the fixed chiral selector of CSP **2**. The methyl group at the chiral center of the fixed chiral selector is positioned at the site of complex square planar ring in the retention mode of the (L)-enantiomer (Figure 2b) and, therefore, disturbs the complex formation. However, in the retention mode of the (D)-enantiomer (Figure 2a) the hydrogen at the chiral center of the fixed chiral selector is positioned at the site of complex square planar ring.

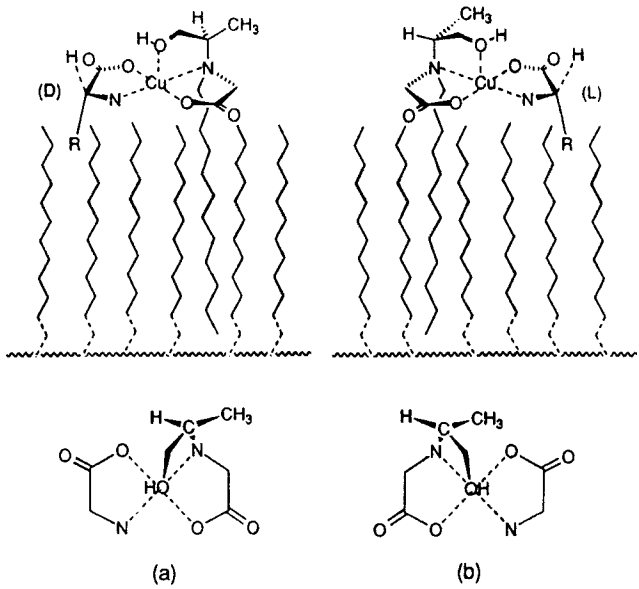


Figure 2. Top: the proposed structures of the ternary complex formed from the fixed ligand [(R)-chiral selector)] of CSP **2**, (a) (D)- and (b) (L)-amino acid and Cu(II). Bottom: schematic representation of the top ternary complexes viewed from above. The ternary complex (a) formed from the fixed ligand, (D)-amino acid and Cu(II) is more stable than the ternary complex (b).

Consequently, the complex shown in Figure 2a should be more stable than that shown in Figure 2b and the (D)-enantiomer should be retained longer. This is consistent with the observed elution orders on CSP **2** for the resolution of α-amino acids which have a simple a-alkyl substituent.[15]

From the chiral recognition model shown in Figure 2, the larger group than the methyl group at the chiral center of the fixed chiral selector, is expected to enhance the stability difference between the two diastereomeric complexes because the larger group may disturb more strongly, the formation of the less stable complex with the less retained enantiomer.

Based on this rationale, we planned to prepare new dynamic CSP **7** starting from (S)-leucinol. The large isobutyl group at the chiral center of CSP **7** instead of the methyl group at the chiral center of CSP **2** might improve the resolving ability of dynamic CSP **7** compared to that of CSP **2**.

Table 1

Resolution of α-amino Acids on (S)-leucinol Derivative 7 Adsorbed on an Octadecyl Silica Gel Column with the Variation of Organic Modifier Content in the Aqueous Mobile Phase at the Constant Cu(II) Concentration (2.5×10^{-4} M).[a]

	20% CH_3CN in Water		10% CH_3CN in Water		100% Water		10% MeOH in Water		20% MeOH in Water	
A[b]	k'[c]	α[d]	k'[c]	α[d]	k'[c]	α[d]	k'[c]	α[d]	k'[c]	α[d]
ala	3.94	1.26	5.29	1.33	8.77	1.72	7.15	1.55	6.60	1.45
	4.95		7.06		15.11		11.09		9.55	
val	4.81	3.30	8.08	3.50	14.69	6.27	10.57	5.06	9.32	4.22
	15.87		28.27		92.07		53.45		39.33	
leu	12.09	2.37	18.18	3.23			22.93	5.16	18.39	4.14
	28.67		58.86				118.43		76.22	
pro	6.73	1.54	9.61	1.92	18.22	3.62	14.30	2.47	13.48	1.99
	10.39		18.46		66.00		35.33		26.78	
met	8.95	1.96	13.60	2.50			19.68	3.31	15.05	2.93
	17.50		34.02				65.11		44.13	
phe	18.55	2.44	35.35	3.19					33.23	6.45
	45.27		112.84						214.25	
pgl	11.35	3.48	16.07	4.69			17.67	7.94	14.13	6.74
	39.47		75.38				140.22		95.20	
asp	10.81	1.27	11.99	1.54			18.30	1.79	16.39	1.59
	13.76		18.46				32.74		26.11	
his	12.67	1.68	14.41	1.71			14.95	1.54	11.55	1.73
	21.33		24.69				23.09		19.94	
gln			5.48	1.25	10.84	1.86	7.94	1.50	6.81	1.33
			6.85		10.19		11.89		9.07	
glu	21.96	1.51	23.93	2.01			35.16	2.47	32.31	2.16
	33.10		48.09				86.93		69.57	
ser	2.12	1.64	3.51	1.36	6.89	1.53	5.34	1.49	4.79	1.46
	3.47		4.76		10.56		7.94		6.99	
thr	2.33	1.53	3.92	1.35	8.26	1.73	7.73	1.46	5.05	1.54
	3.57		5.29		14.28		11.25		7.77	
arg					7.06	2.15	4.87	1.85	4.93	1.42
					15.20		9.02		7.01	

[a]: See text for the chromatographic conditions. For blanks, chromatography was not performed or data was not able to be collected because of the illegibility of chromatogam. In every case, (L)-enantiomer is retained longer than (D)-enantiomer except histidine. In the case of histidine, (D)-enantiomer is retained longer than the (L)-enantiomer. [b]: Full name of amino acids as following. ala:alanine, val:valine, leu:lecuine, pro:proline, met:methionine, phe:phenylalanine, pgl:phyenylglycine, asp:aspartic acid, his:histidine, gln:glutamine, glu:glutamic acid, ser:serine, thr:threonine, arg:arginine.HCl. [c]: Capacity factors for the first and second eluted enantiomer. [d]: Separation factor.

Chiral selector **6** was prepared starting from (S)-leucinol and then hydrophobically loaded onto a commercial octadecyl-silica gel column to afford dynamic CSP **7** as shown in Scheme 1. Dynamic CSP **7**, thus prepared, was very effective in resolving various racemic α-amino acids. Table 1 summarizes the resolution of various racemic α-amino acids on dynamic CSP **7** with varying organic modifier content in the aqueous mobile phase at constant Cu(II) concentration (2.5×10^{-4} M). For blanks in Table 1, data were not able to be collected because of the long retention times or the illegibility of chromatogram.

As shown in Table 1, most of the tested α-amino acids were resolved with reasonable or good separation factors. As expected from the proposed chiral recognition model shown in Figure 2, the separation factors on CSP **7** are found to be comparable to or better than those on dynamic CSP **2**.[15] Table 1 also shows that an increase in the content of the organic modifier in the aqueous mobile phase diminishes the retention of the two enantiomers as denoted by the capacity factors (k'). However, the retention of the more retained enantiomers is diminished more significantly than that of the less retained enantiomers. In consequence, the separation factors decrease as the content of the organic modifier in the aqueous mobile phase increases. The use of acetonitrile as an organic modifier decreases the retention of the more retained enantiomers more significantly than the use of methanol and, consequently, the separation factors decrease more rapidly when acetonitrile is used as an organic modifier. All of these are exactly consistent with those observed on dynamic CSP **2**.[15] These trends on dynamic CSP **2** were previously explained by the chiral recognition model shown in Figure 1.

According to the chiral recognition model shown in Figure 1, the lipophilic interaction between the α-alkyl substituent of the more retained enantiomer and the octadecyl chains of silica gel is expected to decrease as the polarity of the mobile phase decreases by increasing the content of organic modifier in the mobile phase. The reduction in the lipophilic interaction is justly more significant with less polar organic modifier (acetonitrile in this case), whereas the retention of the less retained enantiomers is not notably affected by the organic modifier in the aqueous mobile phase. In consequence, the separation factors decrease as the organic modifier content in the mobile phase increases and this is more significant with the use of less polar organic modifier.

However, the newly proposed chiral recognition model shown in Figure 2 utilizes the lipophilic interaction between both of (D)- and (L)-enantiomers and the octadecyl chains of silica gel. In consequence, the use of organic modifier in the aqueous mobile phase diminishes the retention of the less retained enantiomers as well as the retention of the more retained enantiomers. In this event, the more significant diminution in the retention of the more retained enantiomers than the less retained enantiomers by the use of organic modifier in

the aqueous mobile phase, may be rationalized on the basis that the ternary complex shown in Figure 2a is more stable and tighter than the ternary complex shown in Figure 2b.

In the previous study concerning the resolution of α-amino acids on CSP **2**, we already proposed that the reduction in the lipophilic interaction between the α-alkyl substituent of amino acids and the octadecyl chains of silica gel might be greater with the use of less polar organic modifier in the aqueous mobile and with tighter complexes.[15] Therefore, the retention of the more retained enantiomers is more significantly diminished than the retention of the less retained enantiomers, as the organic modifier content in the aqueous mobile phase increases and, in consequence, the separation factors decrease.

The same rationale can be applied for explaining the resolution trends of racemic α-amino acids on CSP **7** and the chiral recognition model, which utilizes the lipophilic interaction between the octadecyl chains of silica gel and both of (D)- and (L)-enantiomers, for resolving racemic α-amino acids on CSP **7** is shown in Figure 3.

As shown in Table 1, (L)-enantiomers are always retained longer on CSP **7** than the (D)-enantiomers, except histidine. In resolving histidine on CSP **7**, (D)-enantiomer is retained longer than the (L)-enantiomer. The elution orders on CSP **7** shown in Table 1, are opposite to those on CSP **2**.[15] However, the opposite elution orders on CSP **2** and **7** should be considered to be the same in the sense of chiral recognition because the absolute configuration of CSP **2** is opposite to that of CSP **7**. The exception in the elution order of the two enantiomers of histidine on CSP **7** might be explained by the exactly same rationale applied for the elution order on CSP **2**.[15]

It is quite interesting to note that dynamic CSP **7** shows reasonably good resolving ability for the two enantiomers of racemic α-amino acids even at the high content of organic modifier (20 % CH_3CN) in the aqueous mobile phase while dynamic CSP **2** shows very poor resolving ability under the same conditions of mobile phase.[15] Even though the resolutions on CSP **2** or **7** are very excellent with very polar aqueous mobile phase (for example 100 % water), the limitations in the use of CSP **2** or **7** as an analytical purpose with very polar aqueous mobile phase are the long retention times of the two enantiomers of relatively hydrophobic amino acids and consequently resolutions are limited to relatively less hydrophobic several amino acids.

To diminish the retention times of the two enantiomers (and consequently to extend the use of CSP **2** and **7** to the broad range of racemic α-amino acids), the polarity of the mobile phase should be decreased by increasing the content of organic modifier in the aqueous mobile phase.

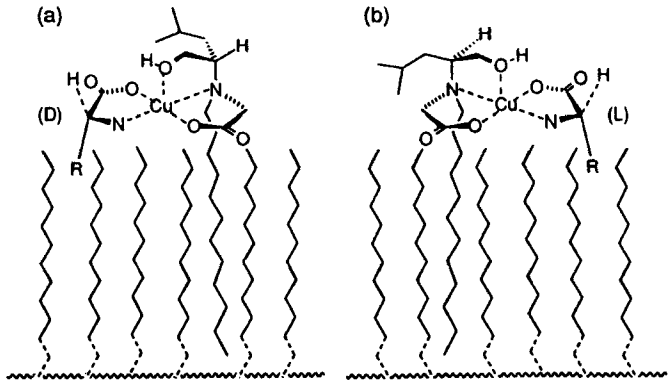


Figure 3. The proposed structures of the ternary complex formed from the fixed ligand [(S)-chiral selector)] of CSP **7**, (a) (D)- and (b) (L)-amino acid and Cu(II). The ternary complex (b) formed from the fixed ligand, (L)-amino acid and Cu(II) is more stable than the ternary complex (a).

In this context, CSP **7** is much more attractive than CSP **2** because CSP **7** shows reasonably good resolving ability for the broad range of racemic α-amino acids even at the high content of organic modifier (20 % CH_3CN) in the aqueous mobile phase, whereas CSP **2** shows very poor resolving ability.[15]

The discrepancy between the resolution behaviors of CSP **2** and CSP **7**, at high content of organic modifier (20 % CH_3CN) in the aqueous mobile phase, may be rationalized by considering that the ternary complex shown in Figure 3b, formed from the fixed ligand of CSP **7**, is less stable. Consequently, it is less compact than that shown in Figure 2a, formed from the fixed ligand of CSP **2**, because the large α-alkyl substituent (such as the isobutyl group in the fixed ligand of CSP **7**) somewhat disturbs the complex formation. As described above, the less compact complex might be less significantly influenced by the polarity of the aqueous mobile phase than the more compact complex.[15] Consequently, the retention time of the more retained enantiomer on CSP **7** decreases more slowly than the one on CSP **2** as the content of the organic modifier in the aqueous mobile phase increases and the resolving ability of CSP **7** is, to some extent, maintained; this is true even at the high content of organic modifier (20 % CH_3CN) in the aqueous mobile phase while that of CSP **2** is almost lost.[15]

The effect of the variation of the Cu(II) concentration in the mobile phase of constant composition [acetonitrile-water (20:80, v/v)] on the resolution trends for resolving racemic amino acids on CSP **7**, is summarized in Table 2.

Table 2

Resolution of α-amino Acids on (S)-leucinol Derivative 7 Adsorbed on an Octadecyl Silica Gel Column with the Variation of Cu(II) Concentration in Acetonitrile-Water (20:80 v/v)[a]

	5.0×10^{-4} M		2.5×10^{-4} M		2.0×10^{-4} M	
A[b]	k'[c]	α[d]	k'[c]	α[d]	k'[c]	α[d]
ala	2.96	1.24	3.94	1.26		
	3.67		4.95			
val	4.16	3.17	4.81	3.30	8.15	3.29
	13.17		15.87		26.82	
leu	8.66	2.45	12.09	2.37	21.15	2.34
	21.23		28.67		49.52	
pro	4.88	1.58	6.73	1.54	10.10	1.51
	7.68		10.39		15.22	
met	6.10	2.03	8.95	1.96	15.23	1.98
	12.36		17.50		30.75	
phe	14.38	2.53	18.66	2.44	35.98	2.47
	36.42		45.27		88.83	
pgl	7.96	3.60	11.35	3.48	19.48	3.38
	28.66		39.47		65.76	
asp	9.77	1.33	10.81	1.27	20.14	1.29
	13.00		13.76		25.99	
his	11.82	1.72	12.67	1.68	19.51	1.61
	20.31		21.33		31.44	
glu	19.80	1.53	21.96	1.51	37.63	1.44
	30.24		33.10		54.29	
ser			2.12	1.64	3.05	1.49
			3.47		4.54	
thr			2.33	1.53	3.81	1.27
			3.57		4.85	

As shown in Table 2, the retention of the two enantiomers decrease appreciably as the Cu(II) concentration increases. However, the enantioselectivity denoted by the separation factors, α, does not show any noticeable tendency. All of these observations are consistent with those on CSP **2**.[15] The appreciable diminution in the retention of the two enantiomers at the high concentration of Cu(II) in the mobile phase, may be explained by the fact, that the increase of the Cu(II) concentration in the mobile phase enhances the formation of the mobile binary complex from Cu(II) and α-amino acids, as described previously to explain the resolution trends on CSP **2**.[15]

In summary, in this study, we proposed an improved chiral recognition model for resolving racemic α-amino acids on CSP **2**, which utilizes the lipophilic interaction between the octadecyl chains of silica gel and both of (D)- and (L)-enantiomers. Based on the chiral recognition model proposed, we designed a new dynamic CSP (**7**) which is expected to show greater enantioselectivity for the two enantiomers of a-amino acids than CSP **2**. The designed CSP was prepared by tentatively loading (S)-N,N-carboxymethyl dodecylleucinol monosodium salt derived from (S)-leucinol onto a commercial reverse phase octadecyl silica gel column and, used in resolving various racemic α-amino acids. As expected, the resolving ability of CSP **7** for the two enantiomers of various racemic α-amino acids, was comparable to or greater than that of CSP **2**. Especially, CSP **7** seems to be more attractive as an analytical purpose than CSP **2** in that, CSP **7** shows reasonably good resolving ability for the broad range of racemic α-amino acids at the high content of organic modifier (20 % CH_3CN) in the aqueous mobile phase, while, CSP **2** shows very poor resolving ability.

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