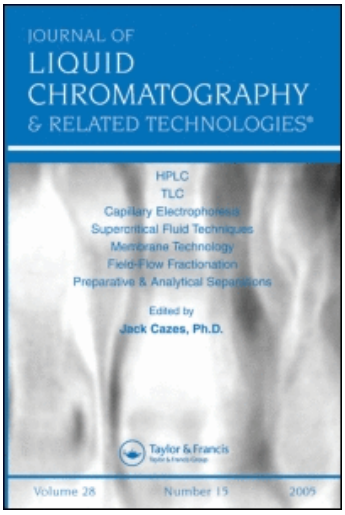

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Optical Resolution of Racemic α-Amino Acids on a Dynamic Chiral Stationary Phase by Ligand Exchange Chromatography

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OPTICAL RESOLUTION OF RACEMIC α-AMINO ACIDS ON A DYNAMIC CHIRAL STATIONARY PHASE BY LIGAND EXCHANGE CHROMATOGRAPHY

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ABSTRACT

A dynamic chiral stationary phase for the ligand exchange chiral liquid chromatography was prepared by tentatively loading (1S,2R)-N,N-carboxymethyl dodecylnorephedrine monosodium salt (**4**) prepared from (1S,2R)-norephedrine onto a commercial reverse phase octadecyl-silica gel column and successfully used for the resolution of various amino acids without derivatization. The retention of the two enantiomers of amino acids on the column was found to be significantly influenced by the organic modifier content , Cu(II) concentration and pH of the mobile phase. However, the enantioselectivity was found to be significantly influenced mainly by the organic modifier content in the mobile phase. Based on the resolution trends of two enantiomers, a chiral recognition model concerning the enantioselective formation of ternary complex from the fixed ligand, amino acids and Cu(II) was proposed.

INTRODUCTION

Chiral ligand exchange chromatography has been known to be a very useful method for the optical resolution of underivatized racemic α-amino acids.[1,2,3] It has been utilized by the use of chiral selectors, usually Cu(II) complexes of optically active α-amino acids which are used as chiral mobile phase additives or

chiral stationary phases after being bonded to solid column support such as silica gel or polymer.[1,2,3] Very few of other optically active materials have been used as chiral selectors for chiral ligand exchange chromatography.[3,4,5]

In recent years we have been interested in the use of optically active norephedrine as a chiral selector for chiral liquid chromatography because it is commercially available and inexpensive. The use of N-(3,5-dinitrobenzoyl)-(1S,2R)-norephedrine bound to silica gel as a π-π complex forming chiral stationary phase was reported previously.[6,7] Very recently, we reported the preliminary results of the use of the Cu(II)-complex of a (1S,2R)-norephedrine derivative tentatively adsorbed onto a commercial octadecyl-silica gel column for the resolution of underivatized racemic α-amino acids.[8]

In this paper, we report the detailed procedures for the preparation of the Cu(II)-complex of a (1S,2R)-norephedrine derivative tentatively adsorbed onto a commercial octadecyl-silica gel column and the studies concerning the effect of the composition, concentration of Cu(II), and pH of the mobile phase on the resolution behaviors.

EXPERIMENTAL

Instruments

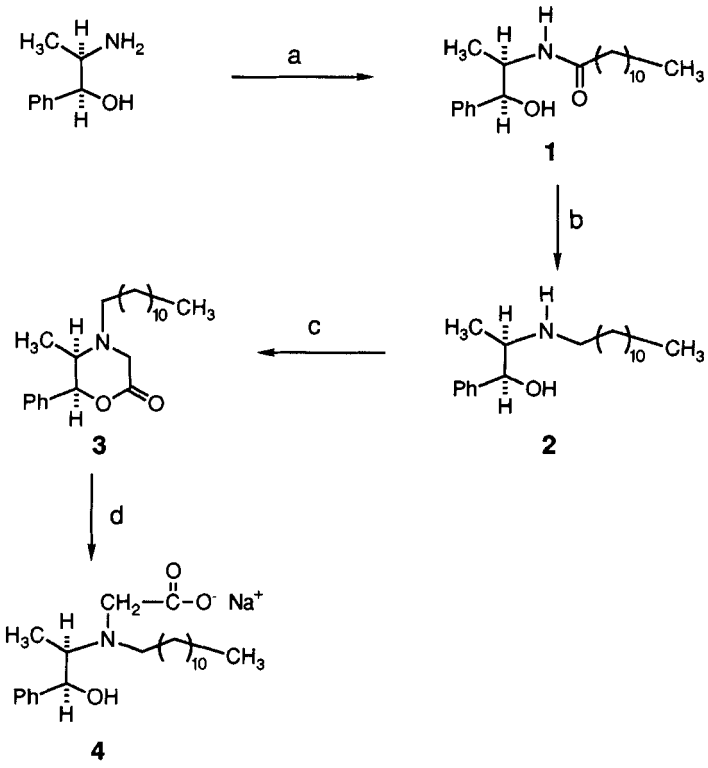
Melting point determination was performed by using Rigaku Thermal Analyzer TAS 100. ^{1}H NMR spectra were recorded on a Bruker AM-300 spectrometer or a Varian EM-360A spectrometer using tetramethylsilane as an internal standard. IR spectra were recorded on a Mattson Polaris FT-IR spectrometer. Mass data (EI or FAB) were obtained on a KRATOS MS 25RFA system.

Chromatography was performed on a HPLC system consisted 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 (1S,2R)-Norephedrine Derivative 4

(1S,2R)-N, N-Carboxymethyl dodecylnorephedrine monosodium salt (**4**) was prepared as shown in Scheme 1. The detailed procedures are as following.

(1S,2R)-N-Lauroyl norephedrine (**1**) : To a stirred solution of (1S,2R)-norephedrine (3.0 g, 16 mmole) and triethylamine (5 ml, 36 mmole) in 50 ml of dry



Scheme. (a) lauroyl chloride, triethylamine, methylene chloride, room temperature. (b) $LiAlH_4$, THF, reflux. (c) ethylbromoacetate, triethylamine, methylene chloride, room temperature. (d) 1 M NaOH solution in methyl alcohol, room temperature.

methylene chloride was added a solution of lauroyl chloride (3.8 ml, 16 mmole) in 10 ml of dry methylene chloride under nitrogen at room temperature. 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 petroleum ether to afford **1** as a white crystalline solid (5.75 g, 97 %). mp. : 73-75 °C, ^1H NMR ($CDCl_3$) δ 0.88(t, 3H), 1.03(d, 3H), 1.28(broad s, 16H), 1.62(m, 2H), 2.18(t, 2H), 3.60(broad s, 1H), 4.36(m, 1H),

4.84(d, 1H), 5.48(d, 1H), 7.33(s, 5H). IR(KBr) cm^{-1} 3300, 3080, 2915, 2840, 1645, 1555.

(1S,2R)-N-Dodecyl norephedrine (**2**) : A solution of $LiAlH_4$ (2.28 g, 60 mmole) in 50 ml of dry THF was added to a stirred solution of **1** (5 g, 15 mmole) in 15 ml of dry THF through dropping funnel over 30 min at 0 °C. The whole mixture was refluxed for 20 hr. 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 THF was removed under reduced pressure. The aqueous solution was extracted with methylene chloride. The methylene chloride solution was dried over anhydrous $MgSO_4$ and then methylene chloride was removed under reduced pressure. The residue was crystallized from the the mixed solvent of methylene chloride and petroleum ether to afford **2** as a white crystalline solid (3.93 g, 82 %). mp. : 52-54 °C, ^1H NMR ($CDCl_3$) δ 0.81(d, 3H), 0.88(t, 3H), 1.27(broad s, 18H), 1.48(m, 2H), 2.68(m, 2H), 2.91(m, 1H), 4.73(d, 1H), 7.30(m, 5H). IR(KBr) cm^{-1} 3300, 3070, 2920, 2860, 1450.

(5R,6S)-4-Dodecyl-5-methyl-6-phenyl-2,3,5,6-tetrahydro-4H-1,4-oxazin-2-one (**3**) : To a stirred solution of **2** (3.80 g, 11.9 mmole) in 30 ml of dry methylene chloride was added a solution of ethyl bromoacetate (1.46 ml, 13.1 mmole) in 10 ml of dry methylene chloride followed by addition of triethylamine (1.83 ml, 13.1 mmole) at room temperature. After being stirred for 24 hr, the reaction mixture was washed with water. The organic extract was dried over anhydrous $MgSO_4$, filtered and concentrated. The oily residue was purified by column chromatography on silica gel (ethylacetate:hexane = 5:95) to give **3** as a colorless oil (1.5 g, 41.8 %). ^1H NMR ($CDCl_3$) δ 0.71(d, 3H), 0.88(t, 3H), 1.30(broad s, 18H), 1.49(m, 2H), 2.50(m, 2H), 3.21(m, 1H), 3.33(d, 1H), 3.63(d, 1H), 5.67(d, 1H), 7.33(m, 5H). IR(KBr) cm^{-1} 3060, 3030, 2930, 2840, 1740, 1490, 1450. MS(EI) m/e : 359(M^+).

(1S,2R)-N,N-Carboxymethyl dodecylnorephedrine monosodium salt (**4**) : NaOH solution (1 M in CH_3OH, 3.45 ml) was added dropwise to a stirred solution of **3** (1.4 g, 3.90 mmole) in 20 ml of 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 in high vacuum for 10 hr to afford oily product (1.55 g, 99%). ^1H NMR (D_2O) δ 0.87(m, 6H), 1.05-1.50(broad m, 20H), 2.28(m, 2H), 2.96(m, 1H), 3.22(dd, 2H), 4.71(d, 1H), 7.20(m, 5H). IR(KBr) cm^{-1} 3292, 3020, 2925, 2854, 1635, 1595, 1455. MS(FAB) m/e : 400(M^++1).

Chromatography

Hydrophobic loading of **4** onto a commercial reverse phase octadecyl-silica gel column : The hydrophobic loading of **4** onto a commercial reverse phase octadecyl-silica gel column (Waters μ Bondapak^{TM} C_{18}, 3.9 x 300mm) was accomplished by eluting a solution of **4** (2.2 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 effort to figure out the loaded amount of **4** was not successful. However, the used amount of **4** (2.2 g) is assumed to be large enough to be fully loaded because the bleeding of the excess of **4** from the column was detected by the UV monitor.

Preparation of mobile phase : Mobile phase was prepared by dissolving $CuSO_4$ in deionized water or deionized water containing acetonitrile as a organic modifier. The pH adjusted mobile phase was prepared by dissolving $CuSO_4$ (2×10^{-4} mole), boric acid (2×10^{-4} mole) and acetic acid (4×10^{-4} mole) in the mixed solvent of acetonitrile (200 ml) and water (500 ml) followed by adding 0.01 N NaOH to adjust pH and then by diluting the whole mixture with water to 1000 ml.

Chromatography : To resolve racemic α-amino acids on the dynamic chiral stationary phase thus prepared, a mobile phase was eluted through the column until the baseline (UV monitor, 254 nm) became stable to equilibrate the column and then, a methanolic solution containing a racemic α-amino acid or α-amino acids was injected. Flow rate was 0.8 ml/min. The dynamic chiral stationary phase used in this study was found to be equally effective for the chiral separation of racemic amino acids after the use of three months.

RESULTS AND DISCUSSION

A dynamic chiral stationary phase which was prepared by tentatively loading **4** onto a commercial reverse phase octadecyl-silica gel column was successfully employed in resolving of the enantiomers of various underivatized α-amino acids. Figure 1 presents a typical chromatogram for such resolution, showing the excellent resolution and the good peak shapes.

In Table 1, the resolution trends with the variation of the organic modifier, acetonitrile, content in the aqueous mobile phase are summarized. Most of the investigated amino acids are resolved with reasonable or good separation factors

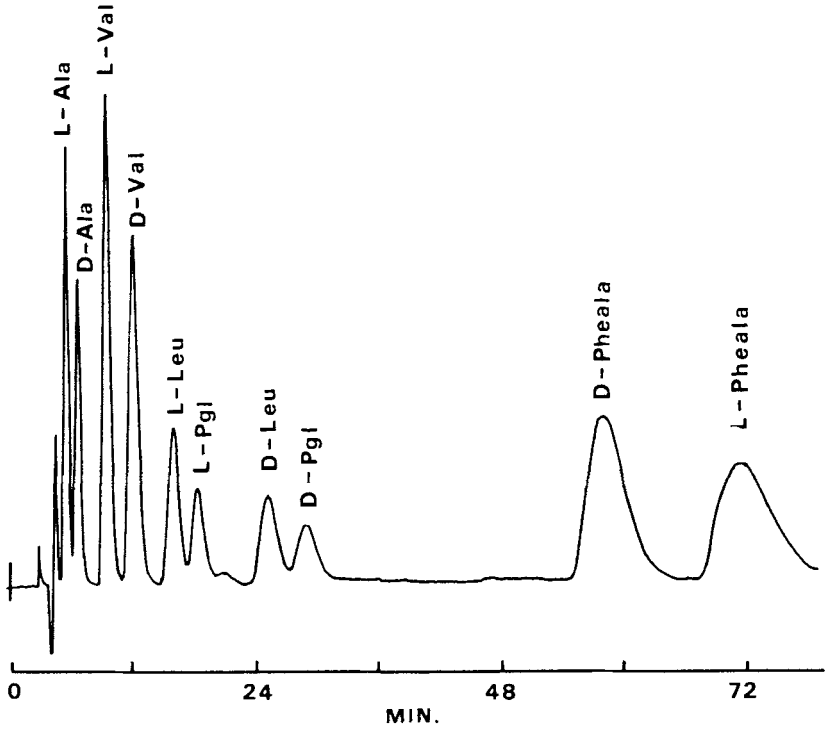


Figure 1. Separation of five racemic α-amino acids on a dynamic chiral stationary phase prepared by tentatively loading of (1S,2R)-norephedrine derivative **4** on a reverse phase octadecyl-silica gel column. Mobile phase : 20 % acetonitrile in water, 2 x 10^{-4} M $CuSO_4$, pH 4.6. See the experimental part for the other experimental conditions.

except cysteine as shown in Table 1. The elution orders are quite consistent. The (D)-enantiomers are retained longer on the column than the (L)-enantiomers for those α-amino acids which have a simple α-alkyl substituent such as alanine, valine, leucine, etc. except phenylalanine. However, for α-amino acids which have an extra hydrophilic group at the α-alkyl substituent (*e.g.*, tyrosine, asparagine, aspartic acid, histidine, serine and threonine), (L)-enantiomers elute last except glutamine, glutamic acid and arginine. As the concentration of organic modifier in the mobile phase increases, the retention of both enantiomers decreases.

Table 1. Resolution of amino acids on (1S,2R)-norephedrine derivative **4** loaded on a octadecyl-silica gel column with the variation of acetonitrile content in the mobile phase at the constant Cu(II) concentration (2×10^{-4} M).[a]

	20/80 (v/v)		10/90 (v/v)		5/95 (v/v)		100 % water	
AA[b]	k'[c]	α[d]	k'[c]	α[d]	k'[c]	α[d]	k'[c]	α[d]
ala	0.80(L)	1.61	1.58(L)	1.82	2.07(L)	2.00	3.14(L)	2.06
	1.29(D)		2.88(D)		4.13(D)		6.46(D)	
val	1.98(L)	1.43	3.77(L)	1.63	5.31(L)	1.93	7.91(L)	2.42
	2.83(D)		6.15(D)		10.27(D)		19.16(D)	
leu	3.27(L)	1.67	7.88(L)	2.16	11.63(L)	2.79	17.99(L)	3.92
	5.45(D)		17.00(D)		32.46(D)		70.56(D)	
pro	1.25(L)	1.56	2.33(L)	1.76	3.22(L)	1.94	4.70(L)	2.36
	1.95(D)		4.09(D)		6.23(D)		11.09(D)	
met	3.58(L)	1.29	7.25(L)	1.74	12.02(L)	2.16	22.86(L)	2.76
	4.63(D)		12.62(D)		25.95(D)		62.98(D)	
phe	13.13(D)	1.25						
	16.41(L)							
pgl	3.43(L)	1.65	8.89(L)	1.89	13.06(L)	2.63	19.14(L)	4.64
	5.66(D)		16.79(D)		34.39(D)		88.88(D)	
tyr	7.24(D)	1.21						
	8.75(L)							
asn			2.15(D)	1.29	3.60(D)	1.32	5.62(D)	1.36
			2.78(L)		4.75(L)		7.63(L)	
asp	1.71(D)	1.51	4.25(D)	1.62	8.90(D)	1.58	19.14(D)	1.45
	2.58(L)		6.88(L)		14.02(L)		27.81(L)	
his	2.13	1.00	4.01(D)	1.18	5.95(D)	1.12	10.03	1.00
	2.13		4.75(L)		6.67(L)		10.03	
gln	0.93(L)	1.57	2.86(L)	1.25	4.18(L)	1.41	6.65(L)	1.73
	1.46(D)		3.58(D)		5.91(D)		11.50(D)	
glu	1.89(L)	1.41	5.50(L)	1.63	11.22(L)	1.86	23.11(L)	2.25
	2.66(D)		8.94(D)		20.87(D)		52.06(D)	
arg	0.65(L)	1.37	2.20(L)	1.46	2.56(L)	1.49	3.33(L)	1.92
	0.89(D)		3.21(D)		3.81(D)		6.41(D)	
ser	0.74(D)	1.76	2.67	1.00	4.25	1.00	6.12	1.00
	1.30(L)		2.67		4.25		6.12	
thr	1.20(D)	1.22	3.15	1.00	5.13	1.00	7.01(D)	1.20
	1.46(L)		3.15		5.13		8.44(L)	
cys	0.11	1.00			3.96	1.00		
	0.11				3.96			

a: See text for the chromatographic conditions. For blanks, chromatography was not performed or data were not able to be collected because of the unreadability of chromatogram. b: See the foot note in Table 2 for the full name of amino acids. c: Capacity factors for the first and second eluted enantiomer. d: Separation factor.

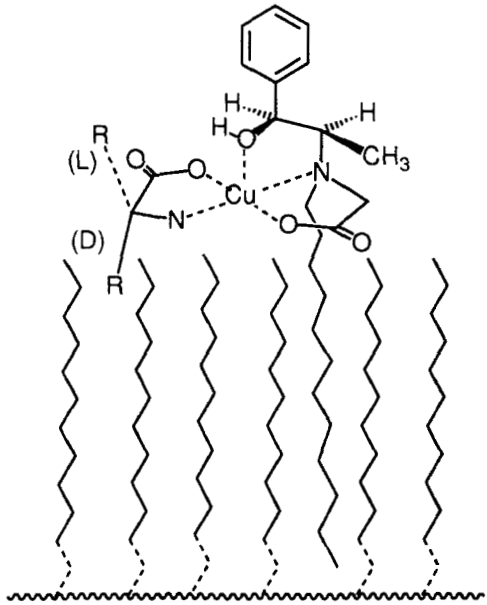


Figure 2. The proposed structure of the diastereomeric ternary complex formed from the fixed ligand, (D)- or (L)-amino acid and Cu(II).

To explain the resolution trends shown in Table 1, a chiral recognition model utilizing the enantioselective formation of ternary complex from the fixed ligand, (D)- or (L)-α-amino acid and Cu(II) is proposed from the study of chemical models as shown in Figure 2. In Figure 2, chiral selector **4** is bound to octadecyl-silica gel through the lipophilic interaction between the octadecyl chains of silica gel and the dodecyl alkyl chain of **4**. Bounded chiral selector **4** and α-amino acid coordinate around Cu(II) to form ternary complex with *trans* conformation which is known to be energetically more favorable than that with *cis* conformation.[9] Finally, in Figure 2, the hydroxy functionality of chiral selector **4** occupy the axial position in the coordination sphere of the Cu(II) ion of the square planar complex.

The chiral recognition model shown in Figure 2 indicates that the α-alkyl substituent of a (D)-α-amino acid is intercalated between the octadecyl chains of silica gel while that of a (L)-α-amino acid is directed into the bulk of mobile phase. In consequence, the ternary complex formed from a (D)-α-amino acid which has a

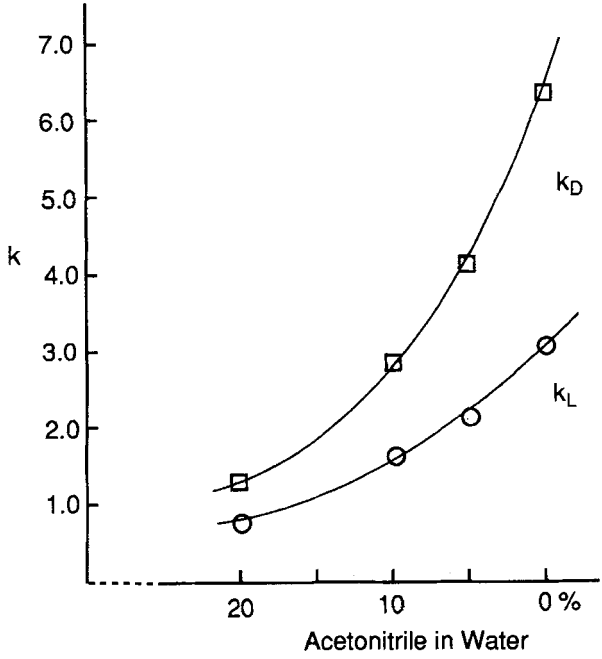


Figure 3. Dependence of retention of (D)- and (L)-alanine on the acetonitrile content in the aqueous mobile phase. Conditions are as in Table 1.

simple α-alkyl substituent is more stable than that from a (L)-α-amino acid because of the lipophilic interaction between the α-alkyl substituent of (D)-α-amino acid and the octadecyl chains of silica gel. In this event, (D)-amino acids are retained longer on the column than (L)-amino acids. However, this is not generalized in resolving the two enantiomers of phenylalanine because of the yet uncertain reason.

The polarity increase in the mobile phase is known to usually enhance the retention of analytes on the column in the reverse phase chromatography. This is indeed the case. As shown in Table 1, the retention of both enantiomers are enhanced continuously as the polarity of the mobile phase is increased by reducing the organic modifier content. However, the degree of the enhancement in the retention of the (D)-enantiomers of α-amino acids which have a simple α-alkyl substituent is greater than that of the (L)-enantiomers as shown in Figure 3 because of the favorable lipophilic interaction between the α-alkyl substituent of the (D)-

enantiomers and the octadecyl silica gel. Consequently, the enantioselectivity denoted by the separation factor, α, in Table 1 for the enantiomers of α-amino acids which have a simple α-alkyl substituent is improved as the polarity of the mobile phase is increased.

In resolving the two enantiomers of α-amino acids having a hydrophilic α-alkyl substituent, the (L)-enantiomers may form more stable complex than the (D)-enantiomers because the extra hydrophilic functionality of the α-alkyl substituent of the (L)-enantiomers can interact with the hydroxy group of the fixed ligand, for example, through the hydrogen bonding while the intercalation of the α-alkyl substituent of the (D)-enantiomers between the octadecyl chains of silica gel becomes less favorable. In this case, the (L)-enantiomers are retained longer on the column than the (D)-enantiomers. The relatively long hydrophilic α-alkyl substituents of α-amino acids such as glutamine, glutamic acid and arginine,however, seems to act as simple hydrophobic α-alkyl substituents from the resolution trends shown in Table 1. The terminal hydrophilic functionality of the relatively long hydrophilic α-alkyl substituents of these α-amino acids can also be thought to coordinate to the Cu(II) ion of the square planar complex at the axial position by replacing the hydroxy group of the fixed ligand. In this incident, the chiral recognition model shown in Figure 2 should be altered and the altered chiral recognition model may be responsible for the resolution trends of glutamine, glutamic acid and arginine.

The variation of the Cu(II) concentration at the constant composition of eluent (20 % acetonitrile in water) was found to change the resolution trends of α-amino acids. The data summarized in Table 2 show that the retention of the enantiomers of α-amino acids increases noticeably with decreasing the Cu(II) concentration in the mobile phase. However, the enantioselectivity seems not to be affected much by the variation of Cu(II) concentration. In general, the enantioselectivity at the Cu(II) concentration of 2×10^{-4} M seems to be slightly better than that at the other Cu(II) concentration. In the mobile phase, it is supposed that at least four species of complexes including Cu(II) ion are in equilibrium as shown in the following equation.[9] In this equation AA means amino acid analytes. The increase of the

$$[\text{Cu(AA)(Fixed Ligand)}] + \text{Cu(II)} \rightleftharpoons [\text{Cu(AA)}]^{+} + [\text{Cu(Fixed Ligand)}]^{+}$$

Table 2. Resolution of amino acids on (1S,2R)-norephedrine derivative **4** loaded on a octadecyl-silica gel column with the variation of Cu(II) concentration in acetonitrile/water of 20/80 (v/v).[a]

	5.0×10^{-4}M		2.0×10^{-4}M		1.0×10^{-4}M		0.5×10^{-4}M	
AA[b]	k'[c]	α[d]	k'[c]	α[d]	k'[c]	α[d]	k'[c]	α[d]
ala	0.79(L)	1.38	0.80(L)	1.61	1.53(L)	1.52	2.51(L)	1.39
	1.09(D)		1.29(D)		2.33(D)		3.48(D)	
val	1.57(L)	1.31	1.98(L)	1.43	3.49(L)	1.35	4.62(L)	1.34
	2.05(D)		2.83(D)		4.72(D)		6.19(D)	
leu	2.76(L)	1.57	3.27(L)	1.67	6.92(L)	1.59	8.46(L)	1.60
	4.32(D)		5.45(D)		11.02(D)		13.50(D)	
pro	1.02(L)	1.48	1.25(L)	1.56	2.30(L)	1.53	2.68(L)	1.54
	1.51(D)		1.95(D)		3.51(D)		4.13(D)	
met	2.30(L)	1.34	3.58(L)	1.29	5.53(L)	1.37	6.91(L)	1.36
	3.09(D)		4.63(D)		7.57(D)		9.41(D)	
phe	9.35(D)	1.20	13.13(D)	1.25			28.69(D)	1.24
	11.20(L)		16.41(L)				35.70(L)	
pgl	3.20(L)	1.53	3.43(L)	1.65	7.21(L)	1.58	8.97(L)	1.57
	4.88(D)		5.66(D)		11.41(D)		14.06(D)	
tyr	4.17(D)	1.20	7.24(D)	1.21	9.62(D)	1.24	11.84(D)	1.25
	5.02(L)		8.75(L)		11.96(L)		14.84(L)	
asp	1.26(D)	1.51	2.58(D)	1.51	1.63(D)	1.68	1.47(D)	1.65
	1.90(L)		1.71(L)		2.74(L)		2.42(L)	
gln	0.80(L)	1.23	0.93(L)	1.57	2.10(L)	1.16	2.87(L)	1.15
	0.98(D)		1.46(D)		2.43(D)		3.30(D)	
glu	1.46(L)	1.34	1.89(L)	1.41	2.16(L)	1.38	1.92(L)	1.37
	1.96(D)		2.66(D)		2.97(D)		2.63(D)	

a: See text for the chromatographic conditions. For blanks, chromatography was not performed or data was not able to be collected because of the unreadability of chromatogram. b: Full name of amino acids are as following. ala:alanine, val:valine, leu:leucine, pro:proline, met:methionine, phe:phenylalanine, pgl:phenylglycine, tyr:tyrocine, asn:asparagine, asp:aspartic acid, his:histidine, gln:glutamine, glu:glutamic acid, arg:arginine.HCl, ser:serine, thr:threonine, cys:cysteine. c: Capacity factors for the first and second eluted enantiomer. d: Separation factor.

Cu(II) concentration in the mobile phase may shift the equilibrium to the right side, increasing the formation of the mobile complex from Cu(II) and amino acids, and subsequently diminish the retention of amino acids on the column.

pH of the mobile phase was also found to affect the retention behaviors of the two enantiomers of α-amino acids on the column. The pH effects on the resolution trends at the constant Cu(II) concentration and the constant composition of mobile phase (20 % acetonitrile in water) are summarized in Table 3. At high

Table 3. Effect of pH on the resolution of amino acids on (1S,2R)-norephedrine derivative **4** loaded on a octadecyl-silica gel column at the constant Cu(II) concentration (2 x 10^{-4} M) and the costant composition of the mobile phase (acetonitrile/water of 20/80, v/v).[a]

	pH 4.0		pH 5.5		pH 6.5		pH 7.0	
AA[b]	k'[c]	α[d]	k'[c]	α[d]	k'[c]	α[d]	k'[c]	α[d]
ala	1.17(L)	1.30	1.28(L)	1.30	1.51(L)	1.38	1.65(L)	1.39
	1.52(D)		1.66(D)		2.09(D)		2.31(D)	
val	1.83(L)	1.25	2.09(L)	1.27	2.87(L)	1.27	3.39(L)	1.35
	2.29(D)		2.65(D)		3.64(D)		4.59(D)	
leu	3.21(L)	1.49	3.34(L)	1.50	4.89(L)	1.54	6.33(L)	1.60
	4.77(D)		5.02(D)		7.55(D)		10.14(D)	
pro	1.46(L)	1.43	1.53(L)	1.43	1.95(L)	1.40	1.98(L)	1.58
	2.09(D)		2.19(D)		2.73(D)		3.12(D)	
met	2.64(L)	1.30	2.68(L)	1.30	3.84(L)	1.30	5.35(L)	1.36
	3.44(D)		3.49(D)		5.00(D)		7.27(D)	
phe	9.38(D)	1.23	9.75(D)	1.22	17.11(D)	1.25	21.43(D)	1.27
	11.57(L)		11.94(L)		21.41(L)		27.18(L)	
pgl	3.44(L)	1.44	3.62(L)	1.44	5.08(L)	1.47	7.16(L)	1.58
	4.97(D)		5.22(D)		7.48(D)		11.28(D)	
tyr	4.28(D)	1.25	4.29(D)	1.26	5.80(D)	1.24	9.47(D)	1.24
	5.36(L)		5.42(L)		7.17(L)		11.74(L)	
asp	0.55(D)	1.51	0.56(D)	1.59	0.84(D)	1.61	1.56(D)	1.75
	0.83(L)		0.89(L)		1.35(L)		2.73(L)	
glu	0.66(L)	1.32	0.72(L)	1.32	1.03(L)	1.35	1.90(L)	1.39
	0.87(D)		0.95(D)		1.39(D)		2.65(D)	

a: See text for the chromatographic conditions. b: See the foot note of Table 2 for the full name of amino acids. c: Capacity factors for the first and second eluted enantiomer. d: Separation factor.

pH, α-amino acids can be easily deprotonated and favor the formation of the ternary complex shown in Figure 2. As results, the retention of the enantiomers of α-amino acids on the column becomes longer as pH of the mobile phase increases. However, as shown in Table 3, the effect of pH of the mobile phase on the enantioselectivity is not significant. The enatioselectivity appears to be slightly better at the higher pH.

In conclusion, in this report, we were able to show that (1S,2R)-norephedrine derivative **4** loaded tentatively on a commercial reverse phase octadecyl-silica gel column can be successfully used for the resolution of various α-amino acids without derivatization. The retention of the two enantiomers of α-amino acids on the column was found to be significantly influenced by the organic modifier

content, Cu(II) concentration and pH of the mobile phase. However, the enantioselectivity was found to be significantly influenced mainly by the organic modifier content in the mobile phase. Based on the resolution trends of two enantiomers, a chiral recognition model concerning the enantioselective formation of ternary complex from fixed ligand **4**, α-amino acids and Cu(II) was proposed.

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