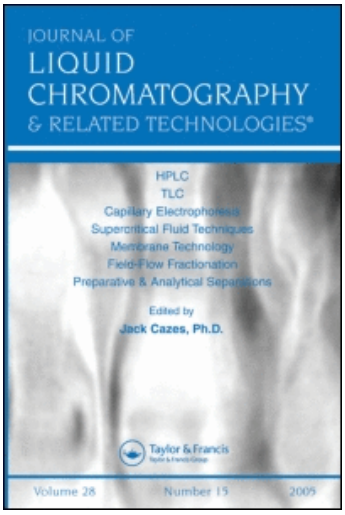

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ABSTRACT

A dynamic chiral stationary phase (CSP) prepared by hydrophobically bonding N-dodecyl diamide of (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid to octadecyl silica gel was successfully employed in resolving various racemic compounds containing a primary amino functional group. The racemic compounds resolved on the CSP include α-amino acids, α-amino esters, amino alcohols, amines, α-aminocarbonyl compounds, and fluoroquinolone antibacterial agents.

In order to see the effect of organic and acidic modifiers in the mobile phase and the temperature of the column on the enantioselectivity exerted by the CSP, five racemic compounds that resolved well on the CSP were selected and their resolutions were investigated with the variation of organic and acidic modifiers in the mobile phase and the temperature of the column. The results demonstrate that the chromatographic factors such as capacity

factors (k'), separation factors (α), and resolution factors (R_s) might be controlled, to some extent, by varying organic and acidic modifiers in the mobile phase and the temperature of the column.

INTRODUCTION

Liquid chromatographic separation of enantiomers on HPLC chiral stationary phases (CSPs) has been widely used as a powerful means in determining the enantiomeric composition of chiral compounds including chiral drugs.[1,2] Among others, CSPs based on chiral crown ethers have been known to be useful in separating the two enantiomers of primary amino compounds without derivatization. For example, CSPs, consisting of bis-(α,α'-binaphthyl)-22-crown-6 attached to silica gel or polystyrene, were reported in the late 1970s by Cram and his coworkers to be useful for the liquid chromatographic resolution of various racemic α-amino acids and α-amino esters.[3,4]

In the late 1980s and in the early 1990s, CSPs consisting of (substituted α,α'-binaphthyl)-20-crown-6 dynamically coated on octadecyl silica gel were also reported by Shinbo and his coworkers to be effective for the liquid chromatographic resolution of racemic primary amino compounds.[5,6]

Recently, we were also interested in developing CSPs based on chiral crown ether for the liquid chromatographic separation of racemic primary amino compounds and planned to utilize commercially available (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid **1** (Figure 1) as a chiral selector in high performance liquid chromatography. Previously, (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid **1** has been widely used as a chiral selector in electrophoretic resolution of racemic primary amino compounds.[7-10] However, the use of (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid **1** as a chiral selector in high performance liquid chromatography has been reported only in a few cases. Machida et. al. reported that a CSP based on (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid **1** can be utilized in resolving primary amino compounds.[11]

We were also successful in developing a new CSP by bonding (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid **1** to 3-aminopropyl silica gel. The new CSP developed by us was found to be very effective in resolving quinolone antibacterials,[12] α-amino acids, including their derivatives,[13] and racemic amines and amino alcohols.[14] However, the use of (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid **1** dynamically coated on octadecyl silica gel as a CSP is not known. As an effort to extend the use of (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid **1** as a chiral selector in high performance liquid chromatography, in this study, we report the preparation and the application of a new CSP (CSP **2**, Figure 1) based on (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid **1** dynamically coated on octadecyl silica gel.

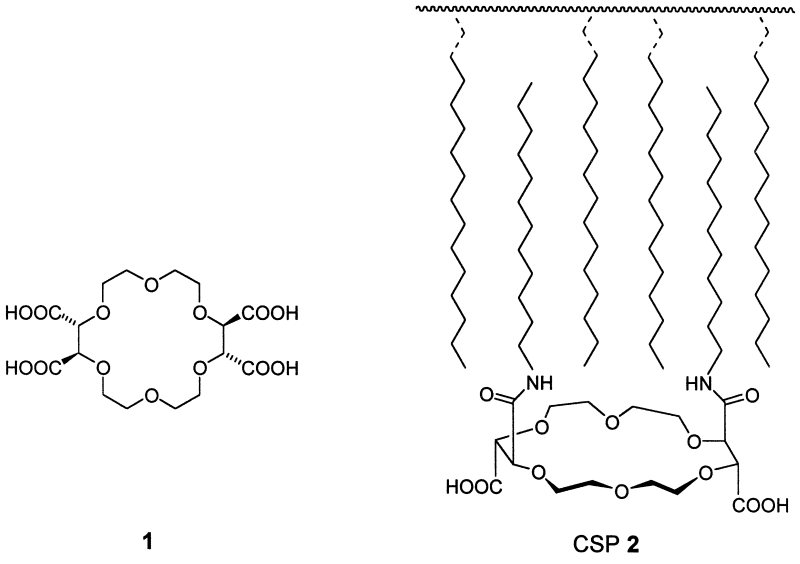

Figure 1. The structure of (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid **1** and CSP **2**.

EXPERIMENTAL

CSP **2** was prepared starting from (+)-(18-crown-6)-2,3,11,12-tetra-carboxylic acid **1** (available from Aldrich) as following. (+)-(18-Crown-6)-2,3,11,12-tetracarboxylic acid **1** (100 mg, 0.23 mmole) was refluxed in freshly distilled acetyl chloride (10 mL) for 12 hr and then excess acethyl chloride was removed under reduced pressure to afford white crystalline (+)-(18-crown-6)-2,3,11,12-tetracarboxylic dianhydride (93 mg, 100 % yield) as described previously.[15] (+)-(18-Crown-6)-2,3,11,12-tetracarboxylic dianhydride thus prepared (93 mg, 0.23 mmole) was dissolved in methylene chloride (5 mL) and the resulting solution was cooled to 0°C. A solution of dodecylamine (93 mg, 0.50 mmole) and triethylamine (0.075 mL, 0.54 mmole) in methylene chloride (3 mL) was slowly added at 0°C with stirring. The reaction mixture was stirred for an additional 12 hr at room temperature and then was washed with 1N HCl solution three times. The organic solution was dried over anhydrous Na_2SO_4 and then the solvent was evaporated to provide N-dodecyl diamide of (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid (170 mg, 100 % yield) as a white oily compound.

N-Dodecyl diamide of (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid thus prepared (170 mg, 0.23 mmole) was dissolved in 15 mL of the mixed sol-

vent consisting of methanol and water (10:90, v/v). The solution was circulated through a commercial octadecyl silica gel column (Waters Nova-Pak C_{18}, 150 x 3.9 mm) with a flow rate of 0.3 mL/min for 5 hr. Finally the column was washed with the mixed solvent of methanol and water (10:90, v/v) with a flow rate of 0.3 mL/min for 1 hr to afford a chiral column consisting of CSP **2.** N-Dodecyl diamide of (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid recovered from the loading experiment was 108 mg. Consequently, it is assumed that the amount of N-Dodecyl diamide of (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid loaded on octadecyl silica gel column is around 60 mg.

The chiral column consisting of CSP **2** was evaluated by using an HPLC system consisting of a Waters model 515 HPLC pump, a Rheodyne model 7125 injector with a 20 μL sample loop, a Younglin M720 Absorbance detector (variable wavelength), and a Youngin D520B computing integrator. The temperature of the column was controlled by using a Julabo F30 Ultratemp 2000 cooling circulator. Chromatography samples **3**-**10** were available from previous studies.[13,14] Chromatography sample **11** prepared by the reported method[16] was generously donated by Dr. C. Y. Hong at LG Biotec, Taejon, Korea.

RESULTS AND DISCUSSION

N-Dodecyl diamide of (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid which is used as a chiral selector of CSP **2** is believed to have the syn-diamide form based on the previous study concerning the stereoselective syn-opening of (+)-(18-crown-6)-2,3,11,12-tetracarboxylic dianhydride by primary amines in the presence of triethylamine.[17] In this instance, CSP **2** is presumed to have the structure shown in Figure 1. CSP **2** thus prepared, was applied in resolving various racemic compounds, including α-amino acids, α-amino esters, amino alcohols, amines, α-aminocarbonyl compound, and some fluoroquinolone derivatives. The structures of the racemic compounds used in this study are shown in Figure 2.

The chromatographic results for the resolution of α-amino acids **3** and α-amino esters **4** are summarized in Table 1 and Table 2, respectively. The chromatograms for the resolution of four α-amino acids and three α-amino esters are presented in Figure 3. All data shown in Table 1 and Table 2 and the chromatograms shown in Figure 3 were obtained by using water containing perchloric acid (1.0×10^{-2} M) as a mobile phase with a flow rate of 0.5 mL/min at 20°C. Perchloric acid added to the mobile phase is believed to protonate the primary amino functional group of racemic compounds and to enhance the diastereomeric complex formation of the primary ammonium group ($R\text{-}NH_3^+$) inside the cavity of 18-crown-6 ring of CSP **2,** as described previously.[8]

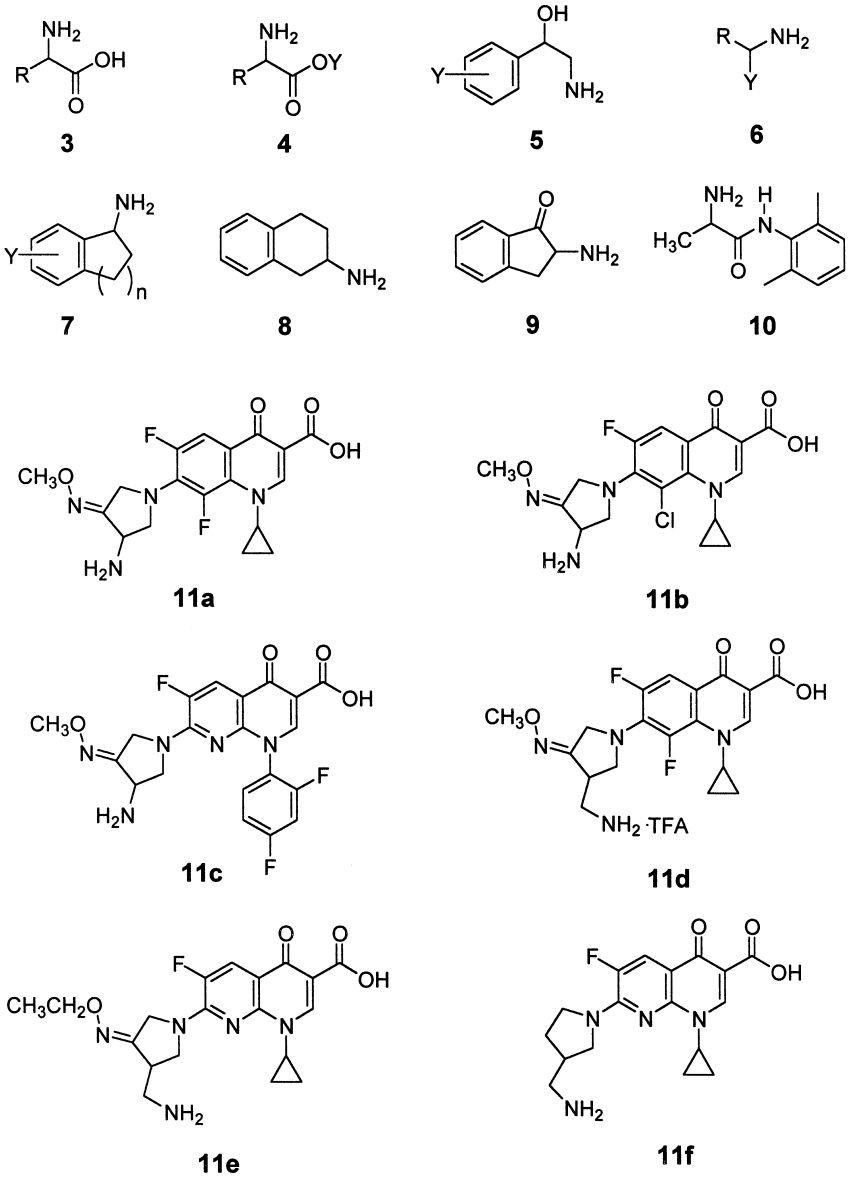

Figure 2. The structure of racemic compounds used in this study.

Table 1

Resolution of Various α-Amino Acids 3 on CSP 2[a]

	Amino Acid	k_1[b]	k_2[b]	α[c]	R_S[d]	Conf.[e]
3a	Alanine	0.19	0.50	2.62	1.37	D
3b	Arginine	0.34	0.48	1.42	0.56	D
3c	Aspartic acid	0.10	0.10	1.00	0.00	
3d	Cysteine	0.24	0.61	2.53	1.17	D
3e	Glutamic acid	0.09	0.09	1.00	0.00	
3f	Isoleucine	1.62	2.31	1.42	1.40	D
3g	Allo-isoleucine	1.09	3.35	3.08	3.96	D
3h	Leucine	2.37	6.42	2.71	3.40	D
3i	Lysine	1.21	1.66	1.37	0.44	
3j	Methionine	1.16	3.13	2.70	2.92	
3k	Phenylalanine	8.22	17.41	2.12	3.43	D
3l	Phenylglycine	3.17	12.79	4.03	4.08	D
3m	Proline	0.00	0.00	1.00	0.00	
3n	Serine	0.18	0.18	1.00	0.00	
3o	Tryptophan	17.60	25.12	1.43	2.04	D
3p	Tyrosine	1.89	2.90	1.53	1.55	D
3q	Valine	0.39	0.85	2.19	1.80	D
3r	3,4-Dihydroxypheny l- alanine (dopa)	2.91	3.96	1.36	1.45	
3s	2-Aminobutanoic acid	0.27	0.73	2.71	1.78	
3t	Norvaline	0.85	2.45	2.88	2.80	
3u	Norleucine	2.78	9.17	3.30	3.24	D

[a] Mobile phase : H_2O + perchloric acid (1.0×10^{-2} M), Flow rate : 0.5 mL/min, Detection: 210 nm UV, Temperature: 20 °C. [b] Capacity factor for the first and second eluted enantiomer. [c] Separation factor. [d] Resolution factor. [e] Absolute configuration of the second eluted enantiomer. For blanks, the elution orders were not determined.

Table 1 shows that various α-amino acids are resolved quite well on CSP **2** except for several α-amino acids such as aspartic acid, glutamic acid, proline, and serine. Among the four α-amino acids unresolved on CSP 2, proline does not contain a primary amino functional group. Consequently, formation of the primary ammonium ion ($R\text{-}NH_3^+$) is not possible and enantioselective diastere-

Table 2

Resolution of Various α-Amino Esters 4 on CSP 2[a]

	Amino Ester	k_1[b]	k_2[b]	α[c]	R_S[d]	Conf.[e]
4a	Ala-OMe	0.45	0.69	1.56	0.80	D
4b	Ala-OEt	0.92	1.46	1.58	0.82	D
4c	Asn-OMe	0.50	0.86	1.72	0.92	D
4d	Asp-OMe	0.49	0.81	1.66	1.30	D
4e	Glu-OEt	0.51	1.53	3.01	2.19	D
4f	Isoleu-OEt	1.44	2.99	2.08	2.50	D
4g	Met-OMe	2.76	4.11	1.49	1.53	D
4h	Met-OEt	1.13	3.07	2.71	2.76	D
4i	PheAla-OMe	18.02	24.84	1.38	1.42	D
4j	PheAla-OEt	5.17	11.63	2.25	3.24	D
4k	PheGly-OMe	7.48	18.51	2.47	3.98	D
4l	PheGly-OEt	4.76	10.75	2.26	3.25	D
4m	Ser-OMe	0.16	0.36	2.34	0.98	
4n	Val-OMe	1.00	1.40	1.40	0.82	D
4o	Val-OEt	3.00	4.16	1.39	1.31	D

[a, b, c, d, e] See the foot note to Table 1.

omeric complexation of the protonated proline inside the cavity of crown ether does not occur. In this instance, no resolution and no retention of proline on CSP **2,** shown in Table 1, is inferred.

Interestingly, aspartic acid, glutamic acid, and serine, which were not resolved on CSP **2**, were resolved quite well as their methyl or ethyl esters. From these results, it might be concluded, that all α-amino acids containing a primary amino functional group can be resolved on CSP **2** without derivatization and/or their ester derivatives.

Resolution of amino alcohols **5**, amines and other amino compounds **6**-**10**, and fluoroquinolone compounds **11,** are summarized in Table 3. As shown in Table 3, therapeutically active amino alcohols such as phenylethanolamine (**5a**), octopamine (**5b**), and norepinephrine (**5c**), are resolved on CSP **2**. In addition, amines, including cyclic amines (**6**-**8**) and other amino compounds (**9** and **10**) including tocainide (2,6-dimethylanilide derivative of alanine), which is known as a cardiac depressant, are resolved reasonably well. It is also very interesting to note that CSP **2** is very useful in resolving various fluoroquinolone derivatives **11**.

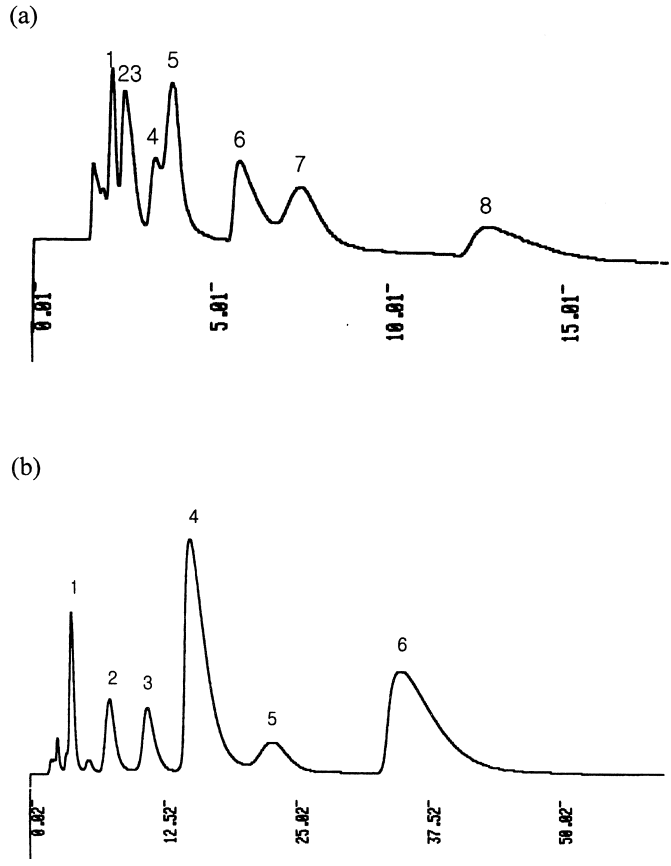

Figure 3. The chromatograms for the resolution of (a) four racemic α-amino acids (1: L-Alanine, 2: D-Alanine, 3: L-Valine, 4: D-Valine, 5: L-Methionine, 6: D-Methionine, 7: L-Leucine, 8: D-Leucine) and (b) three racemic α-amino esters (1: L-Met-OEt, 2: D-Met-OEt, 3: L-PheAla-OEt, 4: L-PheGly-OMe, 5: D-PheAla-OEt, 6: D-PheGly-OMe). Chromatograms were obtained by using 100 % water containing perchloric acid (1.0×10^{-2} M) with a flow rate of 0.5 mL/min at 20°C (210 nm UV). The abscissa represents the eluting time in min.

New fluoroquinolone derivatives (**11a-11e**), which bear an alkyloxime substituent in the 4-position and an amino or aminomethyl substituent in the 3-position of the pyrrolidine ring, were reported to show potent antibacterial activity against both Gram-negative and Gram-positive organisms.[16] All of these new fluoroquinolone derivatives **11a-11e** show base line resolution on

Table 3

Resolution of Amino Alcohols, Amines, α-Aminocarbonyl Compounds and Fluoroquinolone Antibacterial Agents on CSP 2[a]

A[b]	R	Y	n	k_1[c]	α[d]	R_s[e]	Mo[f]	Conf.[g]
5a		H		1.46	1.61	1.37	A	
5b		4-OH		0.33	1.61	0.80	A	
5c		3,4-Di-OH		0.22	1.61	0.67	A	
6a	C_6H_5	CH_3		1.48	1.14	0.43	A	
6b	1-$C_{10}H_7$	CH_2CH_3		12.31	1.13	1.03	A	R
6c	2-$CH_3OC_6H_4CH_2$	CH_3		5.48	1.17	0.82	A	
7a		H	1	1.47	1.24	1.49	A	
7b		H	2	3.07	1.10	0.45	A	
7c		6-CH_3O	2	7.32	1.13	0.87	A	
7d		5-CH_3O	2	8.42	1.69	1.00	A	
8				6.71	1.06	0.27	A	
9				0.52	2.21	1.78	A	
10				3.33	1.12	0.75	A	R
11a				51.71	1.48	2.46	B	
11b				52.49	1.55	3.35	B	
11c				68.38	1.99	8.10	B	
11d				164.50	1.70	7.77	B	
11e				102.10	1.75	4.41	B	
11f				37.87	1.28	0.77	B	

[a] All analytes were resolved at 25°C with a flow rate of 0.5 mL/min (254 nm UV). [b] Racemic analytes. [c] Capacity factor of the first eluted enantiomer. [d] Separation factor. [e] Resolution factor. [f] Mobile phase, A: 100 % water containing perchloric acid (1.0×10^{-2} M). B: 20 % methanol in water containing perchloric acid (1.0×10^{-2}M). [g] Absolute configuration of the second eluted enantiomer. For blanks, the elution orders have not been determined.

CSP **2**. In resolving fluoroquinolone derivatives, in order to reduce retention times of the two enantiomers, 20 % methanol in water containing perchloric acid (1.0×10^{-2} M) was used as a mobile phase. Retention times of the two enantiomers of fluoroquinolone derivatives are still quite long, as shown in Table 3. However, it must be noted that mobile phases containing more than 20 % methanol in water can not be used because N-dodecyl diamide of (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid, which is used as a chiral selector of CSP **2**, might be depleted from the column.

The effect of organic and acidic modifier in the mobile phase and the temperature of the column on the enantioselectivity exerted by CSP **2** was examined with five racemic compounds (**3k**, **3l**, **5b**, **7a**, and **9**) which are well resolved on CSP **2**. First of all, the effect of organic modifier in the mobile

phase on the enantioselectivity exerted by CSP **2** was investigated by changing the content of organic modifier in the mobile phase at the constant concentration of perchloric acid (1.0×10^{-2} M) at 20°C. The chromatographic resolution results with the variation of organic modifier in the mobile phase are summarized in Table 4.

Table 4

Resolution of Selected Racemic Compounds (3k, 3l, 5b, 7a, and 9) with the Variation of the Organic Modifier in the Aqueous Mobile Phase Containing Perchloric Acid (1.0×10^{-2} M) as an Acidic Modifier [a]

Organic Modifier(%) in H_2O		3k	3l	5b	7a	9
CH_3OH (0 %)	k_1'	8.22	3.17	8.50	19.79	12.91
	α	2.12	4.03	1.60	1.53	3.50
	R_s	3.43	4.08	1.41	1.06	5.72
CH_3OH (5%)	k_1'	6.39	2.72	7.10	14.42	10.72
	α	2.24	4.27	1.61	1.46	3.62
	R_s	3.01	5.37	1.40	2.03	6.29
CH_3OH (10%)	k_1'	5.94	2.60	7.02	11.48	10.00
	α	2.34	4.00	1.65	1.49	3.70
	R_s	3.58	4.39	1.66	2.20	5.46
CH_3CH_2OH (5%)	k_1'	6.09	2.52	6.99	11.75	10.05
	α	2.37	4.24	1.65	1.45	3.71
	R_s	3.07	7.02	1.41	1.92	5.67
CH_3CH_2OH (10 %)	k_1'	5.35	2.36	6.69	11.18	6.91
	α	2.40	3.51	1.65	1.30	4.94
	R_s	3.36	5.45	1.42	0.99	5.09
CH_3CN (5%)	k_1'	5.47	2.75	6.38	13.23	11.44
	α	2.27	3.42	1.55	1.16	4.02
	R_s	2.41	7.33	1.36	0.57	6.62

[a] The chromatography was performed with a flow rate of 0.5 mL/min at 20°C (254 nm UV).

As shown in Table 4, capacity factors (k'), in general, decrease as the content of organic modifier in the mobile phase increases. However, the separation factors (α) and the resolution factors (R_s) do not show significant trends with the variation of the content of organic modifier in the mobile phase. Among the three different organic modifiers tested in this study, methanol or ethanol seems to be slightly better than acetonitrile as shown in Table 4.

The effect of acidic modifier in the mobile phase was investigated with three different acids such as perchloric acid, trifluoroacetic acid, and sulfuric acid at the constant mobile phase condition (10 % ethanol in water) at 20°C, and the results are summarized in Table 5. As shown in Table 5, the separation factors (α) and resolution factors (R_s) do not show any significant trend. However, the capacity factors of the first eluted enantiomer (k_1') are strongly dependent on the type of acidic modifier in the mobile phase. From Table 5, it

Table 5

Resolution of Selected Racemic Compounds (3k, 3l, 5b, 7a, and 9) with the Variation of the Acidic Modifier in the Mobile Phase of 10 % Ethanol in Water[a]

Acidic Modifier(mM) in Mobile Phase		3k	3l	5b	7a	9
$HClO_4$ (10 mM)	k_1'	5.35	2.36	6.69	11.18	6.91
	α	2.40	3.51	1.65	1.30	4.94
	R_s	3.36	5.45	1.42	0.99	5.07
H_2SO_4 (5 mM))	k_1'	3.87	1.76	4.47	6.40	5.85
	α	2.60	4.55	1.66	1.67	3.78
	R_s	2.59	4.81	1.09	2.26	5.34
H_2SO_4 (10 mM)	k_1'	2.71	1.40	2.67	3.87	3.52
	α	2.55	4.32	1.67	1.45	3.73
	R_s	2.50	3.98	1.08	1.04	5.80
CF_3COOH (10 mM)	k_1'	4.23	2.02	4.93	7.59	6.48
	α	2.52	3.93	1.66	1.44	3.75
	R_s	2.54	6.10	1.11	1.85	5.10

[a] See the foot note to Table 4.

is clear that the largest capacity factors of the first eluted enantiomers (k_1') are observed when perchloric acid is used as an acidic modifier in the mobile phase.

The effect of the temperature of the column on the enantioselectivity exerted by CSP **2** is summarized in Table 6. As shown in Table 6, the capacity factors of the first eluted enantiomers (k_1') and the separation factors (α) increase continuously as the temperature decreases. However, the resolution factors (R_S) are, in general, observed best at 20°C. At lower temperature, it is expected that the two diastereomeric complexes formed by the two enantiomers inside the cavity of crown ether ring of CSP **2** become more favorable, and this is more significant with the more stable diastereomeric complex than the less stable diastereomeric complex. Consequently, the separation factors (α) increase continuously as the temperature decreases. The rate of equilibrium for the formation of diastereomeric complexes is also expected to be slower at lower temperature. Consequently, the chromatographic peaks for the two enan-

Table 6

Resolution of Selected Racemic Compounds (3k, 3l, 5b, 7a, and 9) with the Variation of the Temperature of the Column[a]

Temperature		3k	3l	5b	7a	9
10°C	k_1'	3.54	1.66	3.89	4.80	4.59
	α	2.63	4.79	1.86	1.51	3.96
	R_s	2.40	3.03	1.23	1.02	4.29
20°C	k_1'	2.71	1.40	2.67	3.87	3.52
	α	2.55	4.32	1.67	1.45	3.73
	R_s	2.50	3.98	1.08	1.04	5.80
25°C	k_1'	2.24	1.05	2.06	3.46	2.85
	α	2.51	4.04	1.62	1.41	3.59
	R_s	2.37	3.24	0.97	0.93	4.68
30°C	k_1'	1.78	0.91	1.66	2.92	2.38
	α	2.48	3.84	1.57	1.38	3.43
	R_s	2.32	3.07	0.87	0.93	4.06

[a] The chromatography was performed using a mobile phase of 10 % ethanol in water containing sulfuric acid (1.0×10^{-2} M) with a flow rate of 0.5 mL/min (254 nm UV).

tiomers become broader at lower temperature and the resolution factors (R_s) might be diminished, showing the best values, in general, at 20°C.

In summary, CSP **2,** prepared by hydrophobically bonding N-Dodecyl diamide of (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid on octadecyl silica gel, was quite successful in resolving various racemic compounds including α-amino acids, α-amino esters, amino alcohols, amines, α-amino carbonyl compounds, and fluoroquinolone derivatives. The effect of organic and acidic modifiers in the mobile phase and the temperature of the column on the enantioselectivity exerted by CSP **2** was examined with five selected racemic compounds.

The results demonstrate that the chromatographic factors such as capacity factors (k'), separation factors (α) and resolution factors (R_s) can be controlled, to some extent, by varying the content of organic and/or acidic modifiers in the mobile phase or varying the temperature of the column. The chiral recognition mechanism is not clear yet, except that, the enantioselective diastereomeric complex formation of the primary ammonium group ($R\text{-}NH_3^+$) inside the chiral cavity of 18-crown-6 crown ether ring of CSP **2** is essential for the chiral recognition.

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REFERENCES

1. **Chiral Separations by Liquid Chromatography**, S. Ahuja, ed., ACS Symposium Series 471, American Chemical Society, Washington, DC, 1991.

2. **A Practical Approach to Chiral Separations by Liquid Chromatography**, G. Subramanian, ed., VCH, Weinheim, 1994.

3. L. R. Sousa, G. D. Y. Sogah, D. H. Hoffman, D. J. Cram, J. Am. Chem. Soc., **100**, 4569-4576 (1978).

4. G. D. Y. Sogah, D. J. Cram, J. Am. Chem. Soc., **101**,3035-3042 (1979).

5. T. Shinbo, T. Yamaguchi, K. Nishimura, M. Sugiura, J. Chromatogr., **405**, 145-153 (1987).

6. T. Shinbo, T. Yamaguchi, H. Yanagishita, D. Kitamoto, K. Sakaki, M. Sugiura, J. Chromatogr., **625**, 101-108 (1992).

7. R. Kuhn, F. Erni, T. Bereuter, J. Hausler, Anal. Chem., **64**, 2815-2820 (1992).

8. R. Kuhn, C. Steinmetz, T. Bereuter, P. Haas, F. Erni, J. Chromatogr. A, **666**, 367-373 (1994).

9. M. G. Schmid, G. Gubitz, J. Chromatogr. A, **709**, 81-88 (1995).

10. Y. Mori, K. Ueno, T. Umeda, J. Chromatogr. A, **757**, 328-332 (1997).

11. Y. Machida, H. Nishi, K. Nakamura, H. Nakai, T. Sato, J. Chromatogr. A, **805**, 85-92 (1998).

12. M. H. Hyun, J. S. Jin, W. Lee, Bull. Kor. Chem. Soc., **19**, 819-821 (1998).

13. M. H. Hyun, J. S. Jin, W. Lee, J. Chromatogr. A, **822**, 155-161 (1998).

14. M. H. Hyun, J. S. Jin, H. J. Koo, W. Lee, J. Chromatogr. A, **837**, 75-82 (1999).

15. J.-P. Behr, J.-M. Girodeau, R. C. Hayward, J.-M. Lehn, J.-P. Sauvage, Helv. Chim. Acta, **63**, 2096-2111 (1980).

16. C. Y. Hong, Y. K. Kim, J. H. Chang, S. H. Kim, H. Choi, D. H. Nam, Y. Z. Kim, J. W. Kwak, J. Med. Chem., **40**, 3584-3593 (1997).

17. J.-P. Behr, J.-M. Lehn, D. Moras, J. C. Thierry, J. Am. Chem. Soc., **103**, 701-703 (1981).

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