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Resolution of Tocainide and Its Analogues on a Doubly Tethered N-CH_3 Amide Chiral Stationary Phase Based on (+)-(18-Crown-6)-2,3,11,12-tetracarboxylic Acid

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A doubly tethered N-CH_3 amide chiral stationary phase (CSP **4**) based on (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid was applied to the resolution of an antiarrythmic agent, tocainide, and its analogues and the chromatographic resolution results were compared with those on a singly tethered N-H amide CSP (CSP **1**), a singly tethered N-CH_3 amide CSP (CSP **2**) and a doubly tethered N-H amide CSP (CSP **3**) under an identical aqueous mobile phase condition. CSP **4** was found to be generally better than other CSPs in terms of the separation factors (α) and resolutions (R_S). The retention times of analytes denoted by the retention factors (k_1) on CSP **4** were quite long compared to those on other CSPs because of the improved lipophilicity of CSP **4**. The long retention times of analytes on CSP **4** were successfully controlled by the addition of a small amount of ammonium acetate to aqueous mobile phase without hurting the chiral recognition efficiency. The variation of the content and type of organic and acidic modifier in aqueous mobile phase was found not to change the chiral recognition efficiency significantly.

Key Words : Chiral stationary phase, Enantioselective separation, Liquid chromatography, Tocainide

Introduction

Enantioselective separation of enantiomers with liquid chromatographic chiral stationary phases (CSPs) has been widely utilized for the resolution of racemic chiral compounds including chiral drugs.[1] For the successful enantioselective separation, various efforts have been devoted to the development of effective CSPs. In our laboratory, we have been interested in the development of CSPs based on chiral crown ethers.[2] Especially, CSP **1** (Fig. 1) was prepared by bonding (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid to aminopropylsilica gel and successfully applied to the resolution of various racemic primary amino compounds[3] and non-primary amino compouns.[4]

CSP **1** was also applied to the separation of the two enantiomers of tocainide, an antiarrythmic agent.[5] The (*R*)-enantiomer of tocainide was reported to be three times more potent than the (*S*)-enantiomer.[6] In addition, the (*R*)-enantiomer of tocainide was reported to be more rapidly eliminated from plasma than the (*S*)-enantiomer.[7] Consequently, the enantiomeric separation of the two enantiomers of tocainide is important. However, the base-line separation of the two enantiomers of tocainide was not obtained on CSP **1**.[5] On the other hand, CSP **2** (Fig. 1) showed the base-line separation of the two enantiomers of tocainide with the separation factor (α) of 1.25 and the resolution (R_S) of 2.66.[5] By replacing the N-H hydrogens of the tethering amide groups of CSP **1** with methyl groups in CSP **2**, the possible intramolecular hydrogen bonds between the N-H hydrogens of CSP **1** and the crown ether oxygens of the CSP, which might hinder the complexation between the crown ether ring of the CSP and the ammonium ions (R-NH_3^+) of analytes, was eliminated and, consequently, tocainide was resolved better on CSP **2** than on CSP **1**.[5] Doubly tethered CSP **3** (Fig. 1), which was developed to improve the CSP stability under acidic mobile phase condition,[2h] also showed the better chiral recognition for the two enantiomers of tocainide than CSP **1**.[8] The improved lipophilicity of CSP **3** compared to that of CSP **1** might be responsible for the improved chiral recognition. In this event, doubly tethered CSP **4** (Fig. 1) having N-CH_3 amide tethering groups is quite interesting in that CSP **4** can show the characteristics of both CSP **2** and CSP **3**, and, consequently, can show better chiral recognition than CSP **2** and CSP **3**. Indeed, CSP **4** was found to be better than **2** and CSP **3** in the resolution of α-amino acids, amines and amino alcohols especially in terms of the resolutions.[9] However, CSP **4** have not been applied to the resolution of tocainide and its analogues.

In this study, CSP **4** was applied to the resolution of tocainide and its analogues. By comparing the chromatographic results for the resolution of tocainide and its analogues on CSP **4** with those on CSP **1**, CSP **2** and CSP **3**, the effect of both of the double tethering groups and N-CH_3 amide groups of CSP **4** on the chiral recognition is expected to be elucidated.

Experimental Section

An HPLC system consisting of a Waters model 510 HPLC Pump, a Rheodyne model 7725i injector with a 20 μL sample loop, a Waters 486 Absorbance Detector and a YoungLin Autochro Data Module (Software: YoungLin Autochro-2000) was used for chromatographic separation experiment. The chiral column temperature was controlled

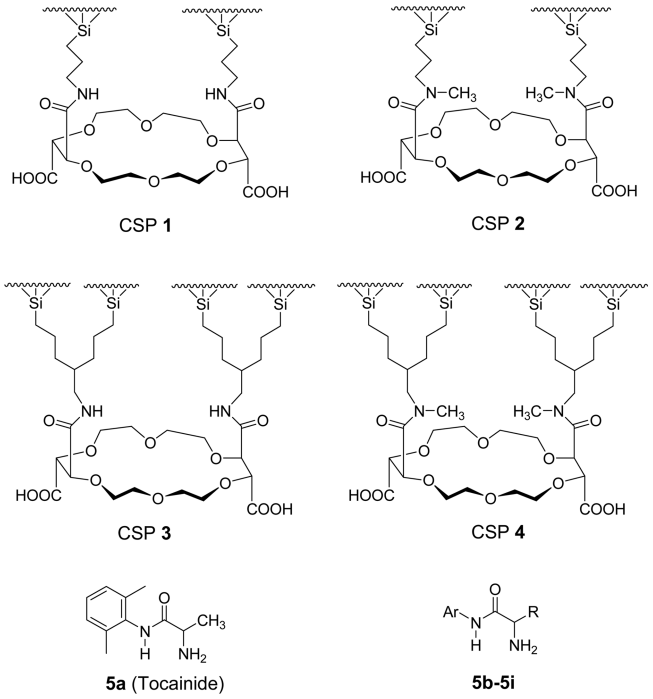

Figure 1. Structures of CSP **1**, CSP **2**, CSP **3**, CSP **4**, tocainide (**5a**) and tocainide analogues (**5b-5i**).

by using a JEIO TECH VTRC-620 Circulator (Seoul, Korea). The chiral column (150 mm × 4.6 mm I.D.) packed with CSP **4** was available from the previous study.[9] Analytes such as racemic and optically active tocainide (**5a**) and its analogues (**5b-5i**) shown in Figure 1 prepared from the corresponding racemic and optically active α-amino acids in this laboratory were available from the prior study.[8] Sample solution was prepared by dissolving each of racemic and optically active tocainide (**5a**) and its analogues (**5b-5i**) in water (usually 2.5 mg/mL). The usual injection volume was 0.1 μL.

Results and Discussion

Tocainide (**5a**) and its analogues (**5b-5i**) were resolved on CSP **4** with the use of 80% methanol in water containing 10 mM sulfuric acid as a mobile phase and the chromatographic resolution results were compared with those on CSP **1**, CSP **2** and CSP **3** in Table 1. To minimize the effect of mobile phase composition on the chiral recognition, the mobile phase condition for the resolution on CSP **4** was maintained to be identical with that for the resolution on CSP **1**, CSP **2** and CSP **3**. The elution orders were identical on the four CSPs, the (*S*)-enantiomer being eluted first.

CSP **2** containing N-CH_3 amide methyl groups instead of N-H amide hydrogens of CSP **1** shows the increased retention factors (k_1) compared to those on CSP **1** as shown in Table 1. Doubly tethered CSP **3** also shows the quite increased retention factors compared to those on CSP **1**. The improved lipophilicity has been proposed to be responsible for the increased retention factors (k_1) on CSP **2** and CSP **3**.[5,8] Doubly tethered CSP **4** containing N-CH_3 amide groups shows even much more increased retention factors (k_1). CSP **4** is expected to be even more lipophilic than CSP **2** or CSP **3** because of the double tethering groups and the N-CH_3 amide methyl groups. The quite improved lipophilicity of CSP **4** might be responsible for the much more increased retention factors (k_1) on CSP **4**.

The separation factors (α) and resolutions (R_S) for the resolution of tocainide (**5a**) and its analogues (**5b-5i**) on CSP **4** shown in Table 1 were graphically compared in Figure 2. In the resolution of tocainide (**5a**) and its analogues (**5b-5i**), the separation factors (α) on CSP **2** are always greater than those on CSP **1** except for the resolution of **5i** and the resolutions (R_S) on CSP **2** are always greater than those on CSP **1** without any exception, indicating the advantage of the N-CH_3 amide methyl groups of CSP **2** over the N-H amide hydrogens of CSP **1**. The separation factors (α) on CSP **3** are also greater than those on CSP **1** for **5a**, **5d**, **5g** and **5h** and the resolutions (R_S) on CSP **3** are also always greater than those on CSP **1**. However, the resolutions (R_S) on CSP **2** are generally greater than those on CSP **3** except for **5a**, **5g** and **5h**. From these results, it is concluded that the effect of the N-CH_3 amide methyl groups of CSP **2** on the chiral recognition efficiency is concluded to be generally

Table 1. Comparison of the resolution of tocainide (**5a**) and its analogues (**5b-5i**) on CSP **1**, CSP **2**, CSP **3** and CSP **4**[a]

	Analytes		CSP **1**			CSP **2**			CSP **3**			CSP **4**		
	R	Ar	k_1	α	R_S	k_1	α	R_S	k_1	α	R_S	k_1	α	R_S
5a	Methyl	2,6-Dimethylphenyl	1.05	1.17	0.92	4.93	1.25	2.66	5.46	1.35	3.03	15.56	1.45	3.76
5b	Methyl	Phenyl	1.82	1.73	2.52	8.39	1.80	6.33	8.02	1.63	4.52	31.92	1.59	4.34
5c	Methyl	Benzyl	1.38	1.44	2.10	6.23	1.81	6.45	6.87	1.39	3.10	25.81	1.84	5.59
5d	Isopropyl	2,6-Dimethylphenyl	0.10	1.00		0.38	1.66	2.56	1.03	1.24	1.34	1.47	1.83	5.31
5e	Isopropyl	Phenyl	0.34	2.10	2.56	1.81	2.37	8.90	1.88	1.81	5.33	6.69	2.49	9.77
5f	Isopropyl	Benzyl	0.30	1.42	1.00	1.43	2.00	6.81	1.85	1.18	1.55	5.54	2.01	7.73
5g	Isobutyl	2,6-Dimethylphenyl	0.25	1.17	0.25	1.17	1.24	1.71	1.33	1.29	2.09	4.80	1.46	3.47
5h	Benzyl	2,6-Dimethylphenyl	0.58	1.00		2.28	1.08	0.69	3.06	1.15	1.13	8.23	1.11	0.99
5i	Phenyl	2,6-Dimethylphenyl	1.49	2.05	3.52	9.27	1.86	5.32	6.85	1.88	5.44	32.48	2.12	6.06

[a]Chromatographic data for CSP **1**, CSP **2** and CSP **3** are quoted from Ref. 5 and 8. Mobile phase: 80% methanol in water + sulfuric acid (10 mM). Flow rate: 0.5 mL/min. Detection: 210 nm UV. Temperature: 20 °C. k_1: Retention factor of the first eluted enantiomer. a: Separation factor. R_S: Resolution. In every case, the (*S*)-enantiomer was eluted first.

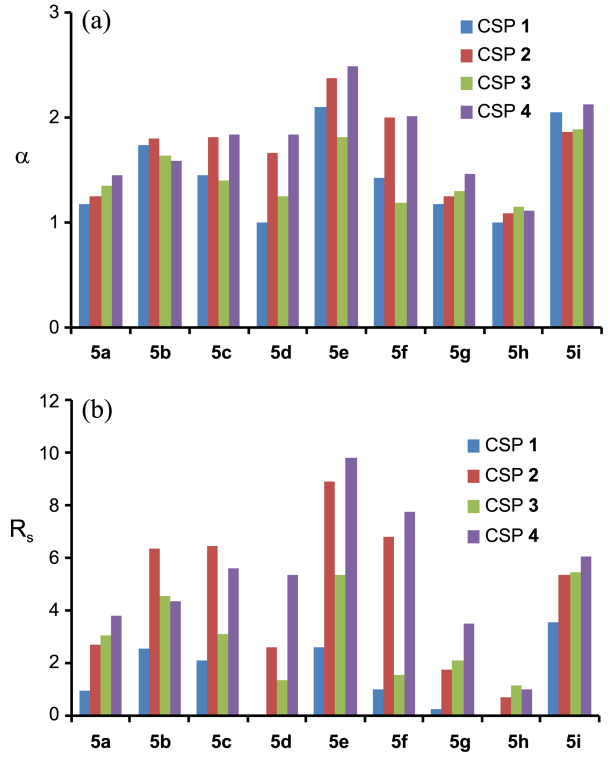

Figure 2. Comparison of (a) the separation factors (α) and (b) resolutions (R_S) for the resolution of tocainide (**5a**) and its analogues (**5b-5i**) on CSP **1**, CSP **2**, CSP **3** and CSP **4**. For the chromatographic conditions, see the footnote to Table 1.

greater than that of the double tethering groups of CSP **3**. The separation factors (α) on CSP **4** are greater than those on CSP **1**, CSP **2** and CSP **3** except for the resolution of **5b** and **5h**. The resolutions (R_S) on CSP **4** are also greater than those on CSP **1**, CSP **2** and CSP **3** except for the resolution of **5b**, **5c** and **5h**. These results clearly indicate that the advantage of both the N-CH_3 amide methyl groups of CSP **2** and the double tethering groups of CSP **3** is well realized in CSP **4** for the resolution of tocainide and its analogues.

As shown in Table 1, the retention times denoted by the retention factors (k_1) for the resolution of tocainide and its analogues on CSP **4** are quite long. For example, it took more than 70 min to complete the tocainide resolution. The long retention times of the two enantiomers are sometimes attractive for the preparative scale resolution because the loading amount of racemic substrate can be increased. However, the long retention times become disadvantages for the analytical scale resolution because of the long analytical time and large amount of mobile phase. In order to reduce the retention times on crown ether-based CSPs, addition of ammonium acetate to aqueous mobile phase has been known to be quite effective.[2g,2j,21] The competition between the primary ammonium ions (R-NH_3^+) of analytes and ammonium ions (NH_4^+) added to mobile phase for the complexation inside the cavity of the crown ether ring of the stationary phase has been proposed to be responsible for the reduced retention times of analytes.[2g,2j,21] The long retention times for the resolution of tocainide and its analogues on CSP **4** were also expected to be controlled by the addition of ammonium acetate. In order to see the effect of ammonium acetate concentration in aqueous mobile phase on the resolution of tocainide (**5a**) and its analogues (**5b-5i**) on CSP **4**, we selected two analytes including tocainide (**5a**) and one (**5i**) of its analogues, which shows long retention time, and resolved them on CSP **4** with the variation of ammonium acetate concentration in aqueous mobile phase. In addition, we also resolved the selected two analytes (**5a** and **5i**) on

Table 2. Resolution of tocainide (**5a**) and its analogue (**5i**) on CSP **4** with the variation of the content and the type of organic and acidic modifiers in aqueous mobile phase without or with ammonium acetate (NH_4OAc) at 20 °C[a]

	Mobile phase	5a			5i		
		k_1	α	R_S	k_1	α	R_S
a	80% CH_3OH + H_2SO_4 (10 mM)	15.56 (S)	1.45	3.76	32.48 (S)	2.12	6.06
	80% CH_3OH + H_2SO_4 (10 mM) + NH_4OAc (1 mM)	4.40 (S)	1.38	3.46	13.36 (S)	2.10	5.12
	80% CH_3OH + H_2SO_4 (10 mM) + NH_4OAc (2 mM)	2.02 (S)	1.35	2.02	4.81 (S)	2.06	5.86
	80% CH_3OH + H_2SO_4 (10 mM) + NH_4OAc (5 mM)	1.12 (S)	1.35	2.12	2.50 (S)	2.09	6.18
b	80% CH_3OH + H_2SO_4 (10 mM) + NH_4OAc (1 mM)	4.40 (S)	1.38	3.46	13.36 (S)	2.10	5.12
	50% CH_3OH + H_2SO_4 (10 mM) + NH_4OAc (1 mM)	5.11 (S)	1.33	2.67	23.80 (S)	1.94	5.17
	30% CH_3OH + H_2SO_4 (10 mM) + NH_4OAc (1 mM)	5.95 (S)	1.27	2.07	28.41 (S)	1.84	4.95
c	80% CH_3OH + H_2SO_4 (10 mM) + NH_4OAc (1 mM)	4.40 (S)	1.38	3.76	13.36 (S)	2.10	5.12
	80% CH_3CH_2OH + H_2SO_4 (10 mM) + NH_4OAc (1 mM)	4.14 (S)	1.41	2.91	11.69 (S)	2.32	5.83
	80% CH_3CN + H_2SO_4 (10 mM) + NH_4OAc (1 mM)	3.47 (S)	1.30	3.10	5.19 (S)	1.67	5.43
d	80% CH_3OH + H_2SO_4 (1 mM) + NH_4OAc (1 mM)	5.00 (S)	1.42	3.10	10.35 (S)	2.24	5.51
	80% CH_3OH + H_2SO_4 (5 mM) + NH_4OAc (1 mM)	4.02 (S)	1.37	2.86	9.10 (S)	2.04	4.63
	80% CH_3OH + H_2SO_4 (10 mM) + NH_4OAc (1 mM)	4.40 (S)	1.38	3.46	13.36 (S)	2.10	5.12
	80% CH_3OH + $HClO_4$ (10 mM) + NH_4OAc (1 mM)	4.62 (S)	1.36	2.88	10.53 (S)	2.01	3.88
	80% CH_3OH + CF_3CO_2H (10 mM) + NH_4OAc (1 mM)	2.86 (S)	1.39	2.43	5.81(S)	2.21	4.30

[a]Flow rate: 0.5 mL/min. Detection: 210 nm UV. k_1: Retention factors of the first eluted enantiomer. In the parenthesis, the absolute configurations of the first eluted enantiomers are presented. α: Separation factor. R_S: Resolution.

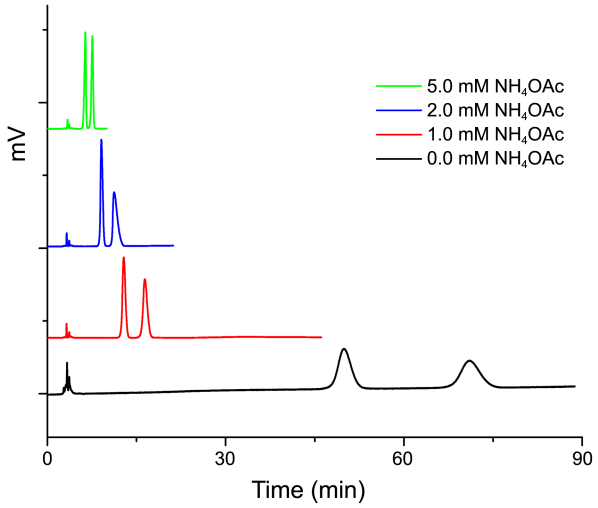

Figure 3. Comparison of the representative chromatograms for the resolution of tocainide (**5a**) on CSP **4** with the variation of the content of ammonium acetate in aqueous mobile phase consisting of 80% methanol in water containing sulfuric acid (10 mM). For the other chromatographic conditions, see the footnote to Table 1.

CSP **4** with the variation of the content and type of organic and acidic modifier in aqueous mobile phase. The chromatographic resolution results are summarized in Table 2.

As shown in Table 2 (entry a), the retention times denoted by the retention factors (k_1) for the resolution of **5a** and **5i** on CSP **4** were reduced significantly when the concentration of ammonium acetate in aqueous mobile phase was increased from 0 to 5 mM. The representative chromatograms for the resolution of tocainide on CSP **4** with the use of 80% methanol in water containing sulfuric acid (10 mM) and ammonium acetate are presented in Figure 3. The separation factors (α) and resolutions (R_S) were reduced only slightly or remained almost constant while the retention factors (k_1) were reduced quite much by the addition of ammonium acetate. Consequently, it is concluded that the addition of an appropriate amount of ammonium acetate to aqueous mobile phase is very effective to control the retention times of analytes without hurting the chiral recognition efficiency significantly.

Effect of methanol content in aqueous mobile phase on the chiral recognition efficiency for the resolution of **5a** and **5i** on CSP **4** were also investigated as shown in Table 2 (entry b). The retention factors (k_1) were found to increase as the methanol content in aqueous mobile phase is decreased. As the methanol content in aqueous mobile phase is decreased, the mobile phase polarity is expected to increase. In this instance, the lipophilic interaction between the analytes and the stationary phase is expected to increase and, consequently, the retention factors (k_1) are expected to increase. The separation (α) and resolutions (R_S) were found to be reduced slightly as the methanol content in aqueous mobile phase is decreased.

The use of ethanol or acetonitrile as an organic modifier in aqueous mobile phase was found to be as effective as the use of methanol for the resolution of **5a** and **5i** on CSP **4** as shown in Table 2 (entry c). The use of ethanol instead of methanol as an organic modifier in aqueous mobile phase decreased the retention times. The separation factors (α) were found to increase slightly for both analytes with the use of ethanol as an organic modifier in aqueous mobile phase. However, the resolution (R_S) was found to decrease for the resolution of **5a** whereas it was found to increase for the resolution of **5i** with the use of ethanol as an organic modifier in aqueous mobile phase. The use of acetonitrile instead of methanol as an organic modifier in aqueous mobile phase decreased the retention factors (k_1) more significantly than the use of ethanol.

The effect of the variation of the content and type of acidic modifier in aqueous mobile phase on the chiral recognition efficiency for the resolution of **5a** and **5i** on CSP **4** was summarized in Table 2 (entry d). The acidic modifier added to aqueous mobile phase has been known to protonate the primary amino group of analytes and the resulting primary ammonium ions ($R\text{-}NH_3^+$) of analytes have been known to form enantioselective host-guest complex inside the cavity of the crown ether ring of the stationary phase.[2g,2j,2l] The effect of the sulfuric acid concentration on the chiral recognition efficiency for the resolution of **5a** and **5i** on CSP **4** was not significant. The use of perchloric acid or trifluoroacetic acid as an acidic modifier in aqueous mobile phase was found to be also effective even though the resolutions (R_S) decreased compared with those obtained with the use of sulfuric acid as an acidic modifier in aqueous mobile phase.

In summary, doubly tethered N-CH_3 amide CSP **4** was found to be generally better than singly tethered N-CH_3 amide CSP **2** or doubly tethered N-H amide CSP **3** for the resolution of tocainide and its analogues. Considering that CSP **2** and CSP **3** are also better than singly tethered N-H amide CSP **1** for the resolution of tocainide and its analogues, the chromatographic results for the resolution of tocainide and its analogues on CSP **4** clearly indicate that both of the advantage of the N-CH_3 methyl groups of CSP **2** over the N-H hydrogens of CSP **1** and the advantage of the double tethering groups of CSP **3** over the single tethering groups of CSP **1** are well realized in CSP **4**. The long retentions times of analytes for the resolution of tocainide and its analogues on CSP **4**, which were originated from the improved lipophilicity of the stationary phase, were elucidated to be effectively controlled by adding a small amount of ammonium acetate to aqueous mobile phase. The effect of the variation of the content and type of organic and acidic modifier in aqueous mobile phase on the resolution of tocainide and its analogues on CSP **4** was not significant. The use of methanol, ethanol or acetonitrile as an organic modifier and the use of sulfuric, perchloric or trifluoroacetic acid as an acidic modifier in aqueous mobile phase were found not to hurt the chiral recognition efficiency significantly for the resolution of tocainide and its analogues on CSP **4** even though each of organic and acidic modifiers showed somewhat different effect on the chiral recognition efficiency. From these results, it is concluded that CSP **4** is quite compatible with various mobile phase conditions for the resolu-

tion of tocainide and its analogues.

References

1. (a) Francotte, E. R. *J. Chromatogr. A* **2001**, *906*, 379. (b) Andersson, S.; Allenmark, S. G. *J. Biochem. Biophys. Methods* **2002**, *54*, 11. (c) Lammerhofer, M. *J. Chromatogr. A* **2010**, *1217*, 814.
2. (a) Hyun, M. H.; Han, S. C.; Lipshutz, B. H.; Shin, Y.-J.; Welch, C. J. *J. Chromatogr. A* **2001**, *910*, 359. (b) Hyun, M. H.; Cho, Y. J.; Kim, J. A.; Jin, J. S. *J. Chromatogr. A* **2003**, *984*, 163. (c) Hyun, M. H. *J. Sep. Sci.* **2003**, *26*, 242. (d) Hyun, M. H.; Kim, D. H. *Chirality* **2004**, *16*, 294. (e) Hyun, M. H.; Cho, Y. J. *J. Sep. Sci.* **2005**, *28*, 31. (f) Hyun, M. H.; Kim, D. H.; Cho, Y. J.; Jin, J. S. *J. Sep. Sci.* **2005**, *28*, 421. (g) Hyun, M. H. *Bull. Kor. Chem. Soc.* **2005**, *26*, 1153. (h) Hyun, M. H.; Song, Y.; Cho, Y. J.; Kim, D. H. *J. Chromatogr. A* **2006**, *1108*, 208. (i) Hyun, M. H. *J. Sep. Sci.* **2006**, *29*, 750. (j) Choi, H. J.; Hyun, M. H. *J. Liq. Chromatogr. Rel. Technol.* **2007**, *30*, 853. (k) Hyun, M. H.; Han, S. C.; Choi, H. J.; Kang, B. S.; Ha, H. J. *J. Chromatogr. A* **2007**, *1138*, 169. (l) Hyun, M. H. In *Comprehensive Chirality*, Carreira, E. M.; Yamamoto, H., Eds.; Elsevier: Amsterdam, Netherlands, 2012; Vol. 8, p 263.
3. (a) Hyun, M. H.; Jin, J. S.; Lee, W. J. *Chromatogr. A* **1998**, *822*, 155. (b) Hyun, M. H.; Jin, J. S.; Koo, H. J.; Lee, W. *J. Chromatogr. A* **1999**, *837*, 75. (c) Hyun, M. H.; Han, S. C.; Koo, H. J.; Lee, W. *Chromatographia* **2000**, *52*, 473. (d) Hyun, M. H.; Cho, Y. J.; Jin, J. S. *J. Sep. Sci.* **2002**, *25*, 648. (e) Hyun, M. H.; Tan, T.; Cho, Y. J. *J. Liq. Chromatogr. Rel. Technol*. **2004**, *27*, 1671. (g) Conrad, U.; Chankvetadze, B.; Scriba, G. K. E. *J. Sep. Sci.* **2005**, *28*, 2275. (h) Berkecz, R.; Sztojkov-Ivanov, A.; Ilisz, I.; Forro, E.; Fulop, F.; Hyun, M. H.; Peter, A. *J. Chromatogr. A* **2006**, *1125*, 138. (i) Lee, S. J.; Cho, H. S.; Choi, H. J.; Hyun, M. H. *J. Chromatogr. A* **2008**, *1188*, 318.
4. (a) Steffeck, R. J.; Zelechonok, Y.; Gahm, K. H. *J. Chromatogr. A* **2002**, *947*, 301. (b) Zhang, D.; Famei, L; Kim, D. H.; Choi, H. J. Hyun, M. H. *J. Chromatogr. A* **2005**, *1083*, 89. (c) Hyun, M. H.; Tan, G.; Xue, J. Y. *J. Chromatogr. A* **2005**, *1097*, 188. (d) Tan, G.; Xue, J. Y.; Hyun, M. H. *J. Sep. Sci.* **2006**, *29*, 1407. (e) Choi, H. J.; Park, Y. J.; Hyun, M. H. *J. Chromatogr. A* **2007**, *1164*, 235. (f) Lee, A.; Choi, H. J.; Hyun, M. H. *Chirality* **2010**, *22*, 693. (g) Lee, A.; Choi, H. J.; Jin, K. B.; Hyun, M. H. *J. Chromatogr. A* **2011**, *1218*, 4071.
5. Hyun, M. H.; Min, H. J.; Cho, Y. J. *Bull. Korean Chem. Soc.* **2003**, *24*, 911.
6. Jamali, F.; Mehvar, R.; Pasutto, F. M. *J. Pharm. Sci.* **1989**, *78*, 695.
7. Antonsson, A.-M.; Gyllenhaal, O.; Kylberg-Hanssen, K.; Johansson, L.; Vessman, J. *J. Chromatogr. Biomed. Appl.* **1984**, *308*, 181.
8. Kim, H. J.; Choi, H. J.; Hyun, M. H. *Bull. Korean Chem. Soc.* **2010**, *31*, 678.
9. Hyun, M. .; Cho, Y. J.; Song, Y.; Choi, H. J.; Kang, B. S. *Chirality* **2007**, *19*, 74.