Performance of HPLC Chiral Stationary Phases with Two Chiral Units and the Effect of the Stereochemistry of the Second Chiral Unit on the Chiral Recognition

Myung Ho Hyun,* Seung-Ryul Hwang, and Sang Cheol Han

Department of Chemistry and Chemistry Institute for Functional Materials, Pusan National University, Pusan 609-735, Korea
Received August 30, 1999

Two chiral stationary phases (CSPs) derived from two diastereomers consisting of (R)- or (S)-α-naphthylethylamine and (S)-naproxen were found to show different chromatographic behaviors in resolving N-(3,5-dinitrobenzoyl)-α-arylalkylamines and N-(3,5-dinitrobenzoyl)-α- or β-amino amides and esters. From the different chromatographic resolution behaviors on the two CSPs, the chiral recognition is proposed to be controlled mainly by the (R)- or (S)-α-naphthylethylamine part of the CSP. In contrast, the (S)-naproxen part of the two CSPs is proposed to exert some subordinate effects on the chiral recognition.

Introduction

Pirkle-type chiral stationary phases (CSPs) have been known to resolve racemates through enantioselective π-π donor acceptor interaction between the CSP and the two enantiomers of racemic analytes.[1] For the effective π-π donor acceptor interaction with racemic analytes, Pirkle-type CSPs have been usually designed to contain π-acidic and/or π-basic aromatic groups.[2] In this context, (R)-α-naphthylethylamine and (S)-naproxen which are readily available as optically active forms are attractive candidates for chiral selectors of Pirkle-type CSPs because they contain a strong π-basic aromatic group such as α-naphthyl or 6-methoxy-2-naphthyl group. Indeed, CSPs based on (R)-α-naphthylethylamine or (S)-naproxen have been developed and demonstrated to be useful in resolving various racemic compounds.[3]

As an extension of the utility of CSPs based on (R)-α-naphthylethylamine or (S)-naproxen, we were interested in designing CSPs based on both (R)-α-naphthylethylamine and (S)-naproxen and finally developed CSP 1 (see Figure 1 for the structures of the CSPs and analytes used in this study). The combination of (R)- or (S)-α-naphthylethylamine and (S)-naproxen as a chiral selector of CSP **1** is quite interesting in that the CSP contains two chiral units and two strong π-basic aromatic groups. In this instance, which chiral unit is more important in the chiral recognition and what is the effect of the stereochemistry of the second chiral unit on the chiral recognition are our great concern.

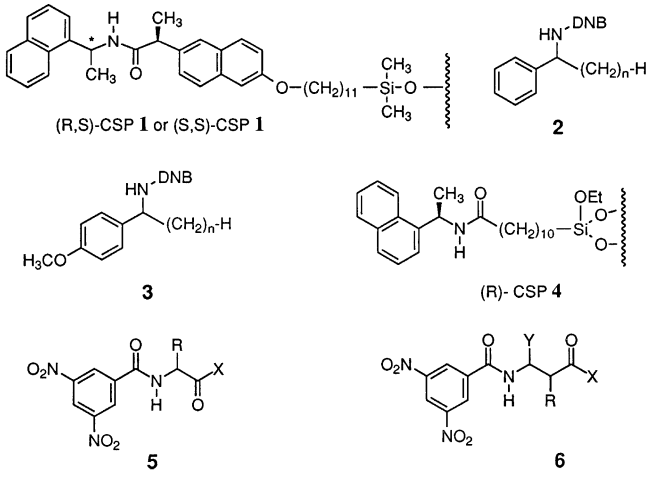

Figure 1. Structures of CSPs and analytes used in this study. DNB means 3,5-dinitrobenzoyl group.

Previously, preparation of CSP **1** and its application to the resolution of the derivatives of racemic nonsteroidal anti-inflammatory drugs (NSAIDs) related to α-arylpropionic acids were briefly reported.[4] However, application of CSP **1** to the resolution of other analytes is not studied yet. In this study, we wish to extend the use of CSP **1** to the resolution of other racemic compounds and find out which chiral unit is more important in the chiral recognition. In addition, we wish to explore the effect of the stereochemistry of the second chiral unit on the chiral recognition.

Experimental Section

The chromatographic resolution data were collected on an HPLC system consisting of a Waters Model 510 pump, a Rheodyne Model 7125 Injector with a 20 μL sample loop, a Youngin Model 710 Absorbance detector and a Youngin D520B Computing Integrator. Chiral columns packed with (R,S)-CSP **1** or (S,S)-CSP **1** were available from the previous study.[4] All chromatographic samples were available from previous studies or prepared by general methods.[5]

Results and Discussion

Chiral columns packed with (R,S)-CSP **1** or (S,S)-CSP **1** were applied in resolving the two enantiomers of N-(3,5-dinitrobenzoyl)-α-arylalkylamines **2** and **3**. The chromatographic resolution results are summarized in Table 1. The elution orders shown in Table 1 for the resolution of N-(3,5-dinitrobenzoyl)-α-phenylethylamine **2a** and N-(3,5-dinitrobenzoyl)-α-(4-methoxyphenyl)ethylamine **3a** were determined by injecting configurationally known samples. Configurationally known samples for other analytes are not available. However, the elution orders not indicated in Table 1 for

Table 1. Chromatographic resolution of N-(3,5-dinitrobenzoyl)-α-arylalkylamines **2** and **3** [alkyl = $(CH_2)_n$-H] on (R,S)- and (S,S)-CSP **1**[a]

Analyte	n	(R,S)-CSP **1**				(S,S)-CSP **1**			
		k_1'[b]	k_2'[c]	α'[d]	Conf.[e]	k_1'[b]	k_2'[c]	α'[d]	Conf.[e]
2a	1	16.90	38.10	2.25	R	7.67	10.83	1.41	S
b	2	16.00	43.23	2.71		8.28	11.80	1.43	
c	3	17.85	41.44	2.32		8.25	11.73	1.42	
d	4	18.94	42.63	2.25		8.44	11.83	1.40	
e	5	18.94	42.63	2.25		8.53	11.89	1.40	
f	6	18.31	41.48	2.26		8.16	11.57	1.42	
g	7	17.88	40.73	2.28		8.03	11.31	1.41	
h	8	17.90	40.63	2.27		7.95	11.09	1.40	
i	9	17.53	39.88	2.28		7.77	10.88	1.40	
j	11	17.25	39.13	2.27		7.60	10.56	1.39	
k	13	16.38	36.50	2.23		7.20	9.97	1.39	
l	15	15.50	33.88	2.19		6.80	9.44	1.39	
m	17	14.44	31.28	2.17		6.43	8.84	1.38	
3a	1	22.00	52.25	2.38	R	9.47	13.60	1.44	S
b	2	22.88	64.89	2.84		9.48	14.67	1.55	
c	3	24.10	60.48	2.51		10.20	14.69	1.44	
d	4	25.56	61.63	2.41		10.40	14.73	1.42	
e	5	25.25	61.50	2.44		10.40	14.73	1.42	
f	6	24.40	59.50	2.44		10.13	14.77	1.43	
g	7	24.06	58.28	2.42		10.00	14.17	1.42	
h	8	23.53	57.66	2.45		9.87	13.95	1.41	
i	9	22.98	56.20	2.45		9.57	13.53	1.41	
j	11	21.56	52.49	2.43		9.12	12.91	1.42	
k	13	20.53	48.85	2.38		8.44	11.92	1.41	
l	15	19.53	45.54	2.33		7.95	11.13	1.40	
m	17	17.80	42.25	2.38		7.73	10.83	1.40	

[a]All data were collected by using 20% isopropyl alcohol in hexane as a mobile phase with a flow rate of 2 mL/min at room temperature. [b]Capacity factor of the first eluted enantiomer. [c]Capacity factor of the second eluted enantiomer. [d]Separation factor. [e]Absolute configuration of the second eluted enantiomer.

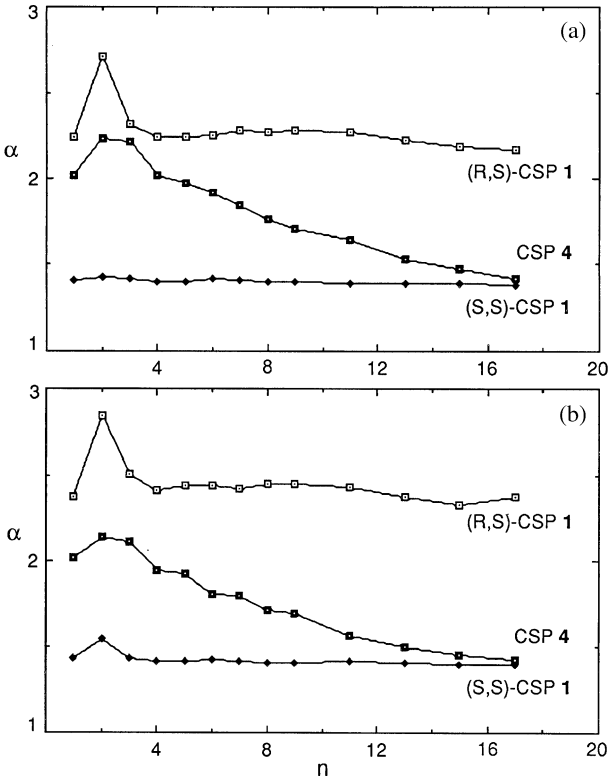

Figure 2. Trends of the enantioselectivities, α, for resolving (a) N-(3,5-dinitrobenzoyl)-α-phenylalkylamines **2** [alkyl = $(CH_2)_n$-H] and (b) N-(3,5-dinitrobenzoyl)-α-(4-hydroxyphenyl)alkylamines **3** [alkyl = $(CH_2)_n$-H] on (R,S)-CSP **1**, (S,S)-CSP **1** and CSP **4**.

other analytes could be presumed to be identical with those for analytes **2a** and **3a** from the technique termed "tracking of absolute configuration (TRAC)".[6] These results indicate that the elution orders of the two enantiomers are dependent on the absolute stereochemistry of the (R)- or (S)-α-naphthylethylamine part of the CSPs. Consequently, it is concluded that the (R)- or (S)-α-naphthylethylamine part of the CSPs plays the major role in the chiral recognition.

Previously, a CSP derived from (R)-α-naphthylethylamine (CSP **4**) was reported to resolve N-(3,5-dinitrobenzoyl)-α-arylalkylamines **2** and **3**.[3a] In this instance, comparison of the resolution results on (R,S)- and (S,S)-CSP **1** with those on (R)-CSP **4** is expected to explore the effect of the stereochemistry of the (S)-naproxen part of (R,S)- and (S,S)-CSP **1** on the resolution of N-(3,5-dinitrobenzoyl)-α-arylalkylamines **2** and **3**. Based on this rationale, the separation factors, α, for the resolution of N-(3,5-dinitrobenzoyl)-α-arylalkylamines **2** and **3** on (R,S)- and (S,S)-CSP **1** shown in Table 1 are graphically compared in Figure 2 with those on CSP **4**.[3a] From Figure 2, two points emerge. One point is that the enantioselectivity exerted by (R,S)-CSP **1** is greater than that by CSP **4** while the enantioselectivity exerted by (S,S)-CSP **1** is worse than that by CSP **4**. Another point is that the separation factors on (R,S)- and (S,S)-CSP **1** remain almost constant while those on CSP **4** decrease continuously after the maximum resolution at the position of n = 2 which corresponds to the ethyl group of the alkyl chain at the chiral center of analytes as the length of the alkyl chain at the chiral center of analytes increases in length. The maximum resolutions at n = 2 shown in Figure 2 might be originated from conformational reasons. As the alkyl substituent at the chiral center of the analyte changes from methyl to ethyl, a significant change in steric bulk is experienced and consequently, the conformational preferences should be altered. However, the conformational preferences may not be altered by further lengthening the alkyl chain because the changes in the structure occur at sites remote from the stereogenic center. This point was described previously.[7] The discrepancies between the chiral resolution behaviors on (R,S)- and (S,S)-CSP **1** and those on CSP **4** shown in Figure 2 are expected to stem from the stereochemistry of the second chiral unit consisting of the (S)-naproxen part of (R,S)- and (S,S)-CSP **1**.

The decreasing trends of the separation factors for the resolution of N-(3,5-dinitrobenzoyl)-α-arylalkylamines **2** and **3** on CSP **4** shown in Figure 2 might be rationalized by the chiral recognition model shown in Figure 3, which was originally proposed to rationalize the resolution of N-(3,5-dinitrobenzoyl)-α-arylalkylamines on CSPs derived from α-

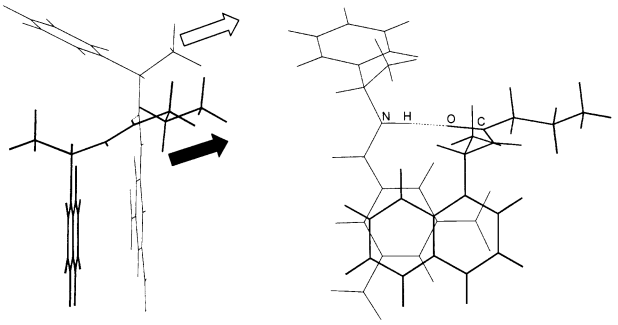

Figure 3. The proposed chiral recognition model viewed from the front side (left) and from the right side (right) for resolving N-(3,5-dinitrobenzoyl)-α-phenylethylamine **2a** on CSP **4**. The conformations of the model compound of CSP **4**, (R)-N-propanoyl-α-naphthylethylamine (represented with thick lines), and (R)-N-(3,5-dinitrobenzoyl)-α-phenylethylamine (represented with thin lines) shown in the drawing have been proposed in the previous studies.[8] The solid arrow indicates the direction of the connecting tether of the CSP. The empty arrow indicates the direction of the alkyl chain at the chiral center of the (R)-analyte.

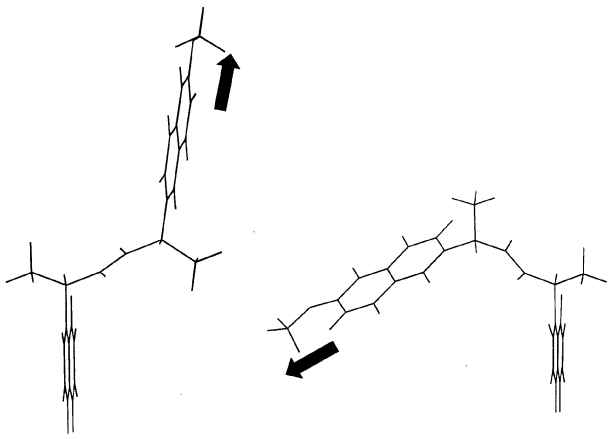

Figure 4. The conformations of the model compounds of (R,S)-CSP 1 (left) and (S,S)-CSP 1 (right). The conformations shown are consistent with those for (R)- or (S)-N-acyl-α-naphthylethylamine and (S)-naproxen amide proposed previously.[10,3e] The solid arrows indicate the direction of the connecting tether of the CSPs.

(6,7-dimethyl-1-naphthyl)alkylamines.[8] In Figure 3, CSP 4 and the analyte are proposed to interact each other through the face-to-face π-π interaction between the 1-naphthyl group of the CSP and the 3,5-dinitrobenzoyl group of the analyte and through the hydrogen bonding interaction between the carbonyl oxygen of the connecting amide tether of the CSP and the amide N-H hydrogen of the analyte. In addition, the face of the phenyl group (in general α-aryl group at the chiral center) of the more retained (R)-enantiomer of the analyte confronts the edge of the 1-naphthyl group of the CSP, invoking the face-to-edge π-π interaction which has attracted considerable attention as an associative force between aromatic rings recently.[9] In this instance, the alkyl chain at the chiral center of the (R)-enantiomer of the analyte intercalates between the strands of the connecting tether of the CSP. The intercalation process is expected to experience difficulties as the length of the alkyl chain at the chiral center of the (R)-enantiomer of the analyte increases. Consequently, the enantioselectivities denoted by separation factors, α, decrease continuously as the length of the alkyl chain at the chiral center of the analyte increases. However, the direction of the connecting tether of (R,S)- and (S,S)-CSP **1** is altered from that of CSP **4** because of the presence of the second chiral unit as shown in Figure 4. In this instance, the alkyl chain at the chiral center of the (R)-enantiomer of the analyte does not intercalate between the strands of the connecting tether of (R,S)- or (S,S)-CSP **1** and consequently, the separation factors for the resolution of N-(3,5-dinitrobenzoyl)-α-arylalkylamines **2** and **3** on (R,S)- and (S,S)-CSP **1** remain almost constant as shown in Figure 2.

Previously, we reported that CSPs based on (S)-naproxen can also resolve N-(3,5-dinitrobenzoyl)-α-arylalkylamines **2** and **3**, the (R)-enantiomers being retained longer.[5] In this instance, the second chiral unit consisting of (S)-naproxen part of (R,S)-CSP **1** is expected to exert somewhat added effect on the chiral recognition while that of (S,S)-CSP **1** is presumed to exert somewhat subtractive effect on the chiral recognition even though the exact modes for the added and the subtractive effects are not clear yet. Consequently, the added and the subtractive effect of the second chiral unit of (R,S)- and (S,S)-CSP **1** on the chiral recognition might rationalize the different sizes of the separation factors on the three CSPs shown in Figure 2.

(R,S)- and (S,S)-CSP **1** were also applied in resolving N-(3,5-dinitrobenzoyl)-α- and β-amino amides and esters **5** and **6**. The results for the chromatographic resolution of N-(3,5-dinitrobenzoyl)-α-amino amides and esters **5** and **6** on (R,S)- and (S,S)-CSP **1** are summarized in Table 2. Among others, the resolution of N-(3,5-dinitrobenzoyl) derivative of tocainide (**5p**), a antiarrhythmic agent, is quite interesting in that CSP **1** can be successfully used in resolving racemic drugs. As shown in Table 2, the resolution behaviors for N-(3,5-dinitrobenzoyl)-α-amino amides and esters **5** and **6** are quite similar to those for N-(3,5-dinitrobenzoyl)-α-arylalkylamines **2** and **3**. The elution orders on the two CSPs are opposite. Consequently, the (R)- or (S)-α-naphthylethylamine part of the CSP are again presumed to play the major role in the chiral recognition. In addition, the enantioselectivities exerted by (R,S)-CSP **1** are greater than those on (S,S)-CSP **1**. Previously, CSPs based on (S)-naproxen were also reported to resolve N-(3,5-dinitrobenzoyl)-α-amino esters, the (R)-enantiomers being retained longer.[3d] Consequently, the greater enantioselectivities for the resolution of N-(3,5-dinitrobenzoyl)-α- or β-amino amides and esters 5 and **6** on (R,S)-CSP **1** can be rationalized again by the added and the subtractive effect of the (S)-naproxen part of the CSP.

In summary, in this study, we were able to show that the chromatographic behaviors for the resolution of N-(3,5-dinitrobenzoyl)-α-arylalkylamines **2** and **3** and N-(3,5-dinitrobenzoyl)-α-amino amides and esters **5** and **6** on (R,S)- and (S,S)-CSP **1** which are derived from two diastereomers consisting of (R)- or (S)-α-naphthylethylamine and (S)-naproxen are controlled mainly by the (R)- or (S)-α-naphthylethyl-

Table 2. Chromatographic resolution of N-(3,5-dinitrobenzoyl)-α- and β-amino amides and esters **5** and **6** on (R,S)- and (S,S)-CSP **1**[a]

Analyte	R	X	Y	(R,S)-CSP **1**				(S,S)-CSP **1**				
				k_1'[b]	k_2'[c]	α[d]	Conf.[e]	k_1'[b]	k_2'[c]	α[d]	Conf.[e]	Eluant
5a	CH_3	OCH_3		6.54	18.45	2.82	R	4.56	5.37	1.18	S	A
b	$(CH_3)_2CH$	OCH_3		5.38	22.13	4.12		3.73	5.13	1.38		A
c	$(CH_3)_2CHCH_2$	OCH_3		6.60	19.73	2.99		4.91	6.03	1.23		A
d	$(CH_3)_2CHCH_2$	OCH_2CH_3		5.50	16.20	2.95	R	3.47	4.40	1.27	S	A
e	$(CH_3)_2CHCH_2$	$O(CH_2)_3CH_3$		4.85	13.63	2.81		3.20	4.00	1.25		A
f	$(CH_3)_2CHCH_2$	$O(CH_2)_7CH_3$		4.25	11.94	2.80						A
g	$C_6H_5CH_2$	OCH_3		15.06	35.46	2.35	R					A
h	$C_6H_5CH_2$	OCH_2CH_3		10.38	28.98	2.79						A
i	CH_3	$NHCH_3$		4.67	7.60	1.63	R	2.84	3.59	1.26	S	B
j	CH_3	$NH(CH_2)_3CH_3$		2.67	4.79	1.80	R	1.76	2.31	1.31	S	B
k	CH_3	$NH(CH_2)_7CH_3$		2.56	4.55	1.78	R	1.72	2.19	1.27	S	B
l	$(CH_3)_2CH$	$NHCH_2CH_3$		1.60	4.67	2.92	R	1.31	2.13	1.63	S	B
m	$(CH_3)_2CH$	$NH(CH_2)_3CH_3$		1.33	4.21	3.16	R	1.13	2.01	1.78	S	B
n	$(CH_3)_2CHCH_2$	$NHCH_3$		5.53	10.24	1.85	R	3.33	4.33	1.30	S	B
o	$(CH_3)_2CHCH_2$	$NH(CH_2)_3CH_3$		1.68	3.80	2.26	R	1.23	1.91	1.55	S	B
p	CH_3	NH-(2,6-dimethylphenyl)		5.25	8.33	1.59		2.38	3.41	1.44		B
6a	CH_3	$O(CH_2)_7CH_3$	Phenyl	12.20	41.50	3.40		7.25	10.11	1.39		A
b	H	OCH_3	Cyclohexyl	23.77	34.03	1.43						B
c	H	OCH_3	Isobutyl	18.80	39.40	2.10		6.63	7.58	1.14		B
d	H	$O(CH_2)_7CH_3$	Isobutyl	11.33	21.93	1.94		4.13	4.56	1.11		B
e	H	OCH_3	Ethyl	16.12	25.25	1.57		7.17	7.64	1.07		B

[a]All data were collected by using (A) 20% isopropyl alcohol in hexane or (B) 10% isopropyl alcohol in hexane as a mobile phase with a flow rate of 2 mL/min at room temperature. [b]Capacity factor of the first eluted enantiomer. [c]Capacity factor of the second eluted enantiomer. [d]Separation factor. e Absolute configuration of the second eluted enantiomer.

amine part of the CSPs. In contrast, the (S)-naproxen part of (R,S)- or (S,S)-CSP **1** is proposed to exert some secondary effects on the chiral recognition. First of all, the direction of the connecting tether of the CSPs altered by the stereochemistry of the naproxen part of the CSPs was proposed to handle the chromatographic behaviors for the resolution of N-(3,5-dinitrobenzoyl)-α-arylalkylamines **2** and **3**. Second, the different enantioselectivities exerted by (R,S)- or (S,S)-CSP **1** were rationalized by the added and the subtractive effect of the (S)-naproxen part of the CSPs on the chiral recognition. However, the modes for the added and the subtractive effects of the (S)-naproxen part of the CSPs are not clear yet. In order to explore the role of the two chiral units of diastereomeric CSPs, further studies are needed.

Acknowledgment. This work was supported by grants from the Basic Science Research Program, Ministry of Education, Korea (BSRI-98-3410) and from the Korea Science and Engineering Foundation (96-0501-08-03-3).

References

1. (a) Pirkle, W. H.; Pochapsky, T. C. *Chem. Rev.* **1989**, *89*, 347. (b) Taylor, D. R.; Maher, K. *J. Chromatogr. Sci.* **1992**, *30*, 67.
2. (a) Hyun, M. H.; Kim, M. H. *J. Liq. Chromatogr.* **1990**, *13*, 3229. (b) Hyun, M. H.; Min, C.-S. *Chem. Lett.* **1994**, 1463. (c) Hyun, M. H.; Min, C.-S. *Bull. Korean Chem. Soc.* **1996**, *17*, 1117. (d) Hyun, M. H.; Min, C.-S. *Tetrahedron Lett.* **1997**, *38*, 1943.
3. (a) Hyun, M. H. *Ph. D. Dissertation*; University of Illinois: Urbana, Illinois, 1984. (b) Hyun, M. H.; Jin, J. S.; Ryoo, J.-J.; Jyung, K. K. *Bull. Korean Chem. Soc.* **1994**, *15*, 497. (c) Hyun, M. H.; Cho, Y. J.; Ryoo, J.-J.; Jyung, K. K.; Heo, G. S. *J. Chromatogr. A* **1995**, *696*, 173. (d) Hyun, M. H.; Na, M. S.; Min, C.-S. *J. Chromatogr. A* **1996**, *732*, 209. (e) Hyun, M. H.; Na, M. S.; Jin, J. S. *J. Chromatogr. A* **1996**, *752*, 77.
4. Hyun, M. H.; Hwang, S.-R.; Ryoo, J. J. *Chem. Lett.* **1994**, 1021.
5. Hyun, M. H.; Kim, K. J.; Jyung, K. K. *Bull. Korean Chem. Soc.* **1997**, *18*, 1085.
6. Pirkle, W. H.; Hyun, M. H.; Bank, B. *J. Chromatogr.* **1984**, *316*, 585.
7. Hyun, M. H.; Cho, S. M.; Ryoo, J.-J.; Kim, M. S. *J. Liq. Chromatogr.* **1994**, *17*, 317.
8. Pirkle, W. H.; Welch, C. J.; Hyun, M. H. *J. Chromatogr.* **1992**, *607*, 126.
9. (a) Grossel, M. C.; Cheetham, A. K.; Hope, D. A. O.; Weston, S. C. *J. Org. Chem.* **1993**, *58*, 6654. (b) Hong, B. H.; Lee, Y. J.; Cho, S. J.; Yun, S.; Kim, K. S. *J. Org. Chem.* **1999**, *64*, 5661
10. Hyun, N. H.; Min, C. S.; Cho, Y. J.; Na, M. S. *J. Liq. Chromatogr.* **1995**, *18*, 2527.