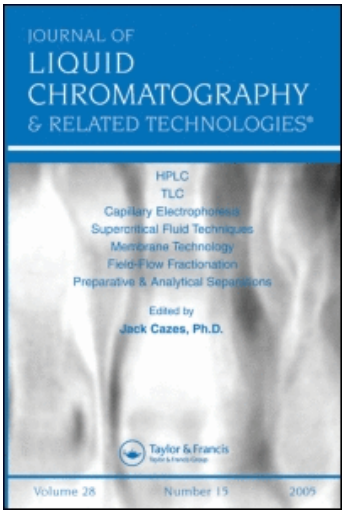

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An Improved Chiral Recognition Model for Resolving N-ACYL-α-arylalkylamines on Pirkle-Type π-Acidic Chiral Stationary Phases

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AN IMPROVED CHIRAL RECOGNITION MODEL FOR RESOLVING N-ACYL-α-ARYL-ALKYLAMINES ON PIRKLE-TYPE π-ACIDIC CHIRAL STATIONARY PHASES

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ABSTRACT

An improved chiral recognition model for resolving N-acyl-α-arylalkylamines on Pirkle-type π-acidic chiral stationary phases (CSPs) has been proposed based on the chiral resolution trends of a homologous series of N-acyl-α-(1-naphthyl)ethylamines on CSPs derived from N-(3,5-dinitrobenzoyl)-(R)-phenylglycine (CSP **1**) and N-(3,5-dinitrobenzoyl)-(S)-leucine (CSP **2**). The chiral recognition model proposed has been evidenced by its successful application to the explanation of the chromatographic resolution trends of a homologous series of N-acetyl-α-(1-naphthyl)alkylamines on CSP **1** and **2** and a homologous series of N-acyl-α-(1-naphthyl)ethylamines and N-acetyl-α-(1-naphthyl)alkylamines on CSP **5**, which has the same structure as CSP **1** except the tether direction.

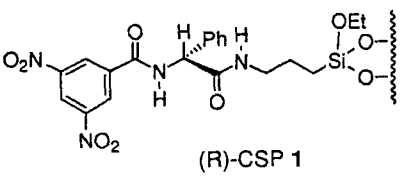
INTRODUCTION

Chiral recognition models proposed for the discrimination of the two enantiomers of racemates by certain liquid chromatographic chiral stationary phases (CSPs) have been often successfully utilized in developing improved CSPs and in predicting the possibility of resolving certain recemates on a given CSP.[1] For example, a CSP derived from α-(6,7-dimethyl-1-naphthyl)-isobutylamine has been designed based on the chiral recognition model proposed for resolving N-acyl-α-arylalkylamines on a CSP derived from the 3,5-dinitrobenzamide of (R)-phenylglycine (CSP **1**) and has been proved to be excellent in resolving various racemates including N-(3,5-dinitrobenzoyl)-α-amino esters.[2,3,4] However, it should be noted that chiral recognition models might be improved or modified as more chromatographic and/or spectroscopic data are accumulated.[5]

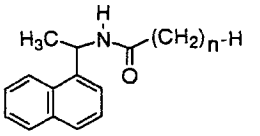
Recently, we had the chance of resolving N-acyl-α-(1-naphthyl)ethylamines **3** on a CSP derived from the 3,5-dinitrobenzamide of (S)-leucine (CSP **2**) and we expected that the chromatographic resolution behaviors of **3** on CSP **2** should be identical with those reported on CSP **1** based on the chiral recognition model proposed previously.[2] However, surprisingly, the elution orders and the resolution trends of **3** on CSP **2** were not consistent with those expected from the chiral recognition model proposed. Therefore, herein, we systematically reinvestigate the chromatographic resolution behaviors of a homologous series of **3** on CSP **1** and **2** and propose an improved chiral recognition model which can rationalize the discrepancies between the chromatographic resolution behaviors on CSP **1** and **2**. The chiral recognition model proposed is also tested by additional chromatographic resolution experiments.

EXPERIMENTAL

Chromatographic resolution data were collected with an HPLC system consisting of a Waters model 510 pump, a Rheodyne model 7125 injector with a



(R)-CSP **1**



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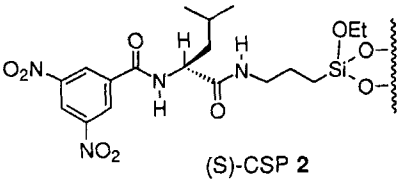
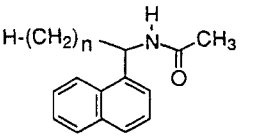


(S)-CSP **2**



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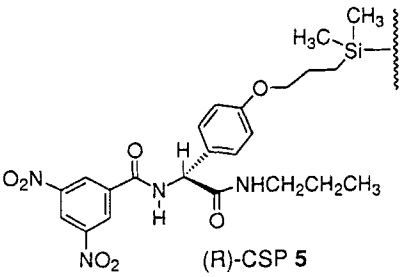

(R)-CSP **5**

20 μl sample loop, a Youngin model 710 absorbance detector with a 254 nm UV filter and a Youngin D520B computing integrator. The chiral columns packed with CSP **1** and **2** were available from the Regis Chemical Company, Morton Grove, Illinois, U.S.A. All chromatographic resolution experiments were performed using 2-propanol-hexane (10:90 or 20:80, v/v) as a mobile phase with a flow rate of 2 ml/min at 20 °C. Column void volume was measured by injecting 1,3,5-tri-*t*-butylbenzene.[6] All analytes used in this study were prepared by the procedures described previously.[2]

RESULTS AND DISCUSSION

The dipole-stacking chiral recognition model proposed previously for resolving N-acylated α-arylalkylamines on CSP **1** implies the π-π interaction

between the 3,5-dinitrobenzoyl group of the CSP and the α-aryl group of the analyte and the simultaneous dipole-stacking between the amide dipoles of the CSP and the analyte.[2,3] Another possible hydrogen-bonding chiral recognition model proposed includes the π-π interaction between the 3,5-dinitrobenzoyl group of the CSP and the α-aryl group of the analyte and the simultaneous hydrogen bonding interaction between the amide N-H hydrogen of the CSP and the carbonyl oxygen of the analyte.[2] In each model, the phenyl group of CSP **1** plays a role as a steric barrier which interferes with the analyte in its approaching to the CSP from the side of the phenyl group of the CSP. In this instance, the isobutyl group at the chiral center of CSP **2** can play a role similar to that of the phenyl group of CSP **1** and in consequence, the chromatographic resolution behaviors of N-acylated α-arylalkylamines on CSP **2** are expected to be identical with those on CSP **1** in the sense of chiral recognition. However, the chromatographic resolution behaviors of N-acyl-α-(1-naphthyl)-ethylamines **3** on CSP **2** are exactly opposite to those on CSP **1** as shown in Table 1 and Figures 1 and 2.

Table 1 summarizes the data concerning the resolution of N-acyl-α-(1-naphthyl)-ethylamines **3** on CSP **1** and **2**. The chromatographic resolution results shown in Table 1 are graphically illustrated in Figures 1 and 2. As shown in Figure 1, the capacity factor of the more retained (S)-enantiomer on CSP **1** decreases more rapidly than that of the (R)-enantiomer and, in consequence, the separation factor, which is defined as the ratio of the capacity factor of the second eluted enantiomer to that of the first eluted enantiomer, decreases continuously as the N-acyl chain of **3** increases in length. In contrast, the capacity factor of the less retained (R)-enantiomer on CSP **2** decreases more rapidly than that of the (S)-enantiomer and, in consequence, the separation factor on CSP **2** increases continuously as the N-acyl chain of **3** increases in length as shown in Figure 2. Based on the chiral recognition model proposed previosly,[2] CSP **1** and **2** are also expected to show opposite elution orders in resolving N-acyl-α-(1-naphthyl)-ethylamines **3** because their absolute configurations are opposite to each other. However, identical elution orders were observed in the resolution of N-acyl-α-(1-naphthyl)-ethylamines **3** on CSP **1** and **2** as shown in Table 1 and Figures 1 and 2.

TABLE 1
Resolution of N-Acyl-α-(1-naphthyl)-ethylamines **3** on CSP **1** and CSP **2**.[a]

		CSP **1**				CSP **2**			
3	n[b]	k_1'[c]	k_2'[d]	α[e]	Conf.[f]	k_1'[c]	k_2'[d]	α[e]	Conf.[f]
	1	8.44	17.00	2.01	S	5.51	5.51	1.00	
	2	6.51	12.38	1.90	S	3.67	4.65	1.27	S
	3	5.03	9.27	1.84	S	2.81	3.59	1.28	S
	4	4.53	7.97	1.76	S	2.29	3.16	1.38	S
	6	3.51	5.64	1.61	S	1.72	2.51	1.46	S
	7	3.35	5.28	1.58	S	1.58	2.39	1.51	S
	9	3.00	4.54	1.51	S	1.36	2.13	1.57	S
	11	2.69	3.94	1.46	S	1.22	1.96	1.61	S
	13	2.54	3.73	1.47	S	1.13	1.86	1.65	S
	15	2.35	3.42	1.46	S	1.05	1.74	1.66	S
	17	2.20	3.18	1.45	S	0.98	1.63	1.66	S

a: See the experimental section for the chromatographic conditions. Mobile phase was 20 % 2-propanol in hexane. b: Length of the N-Acyl alkyl chain [$(CH_2)_n$-H] of analyte **3**. c: Capacity factor of the first eluted enantiomer. d: Capacity factor of the second eluted enantiomer. e: Separation factor. f: Absolute configuration of the second eluted enantiomer.

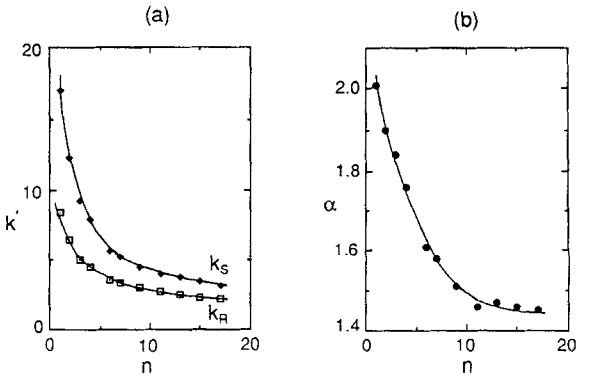

FIGURE 1. The trends of (a) capacity factors and (b) separation factors for resolving N-acyl-α-(1-naphthyl)-ethylamines **3** on CSP **1**. Chromatographic conditions are given in the experimental part.

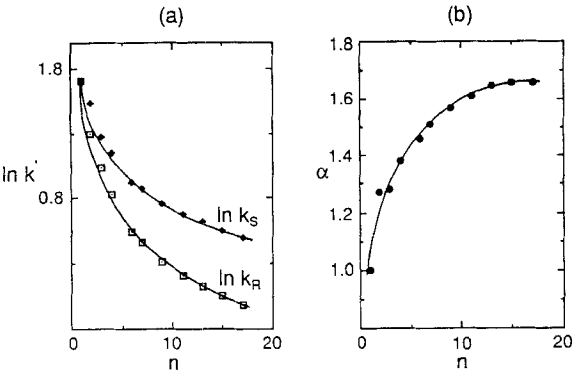

FIGURE 2. The trends of (a) capacity factors and (b) separation factors for resolving N-acyl-α-(1-naphthyl)-ethylamines **3** on CSP **2**. Chromatographic conditions are given in the experimental part.

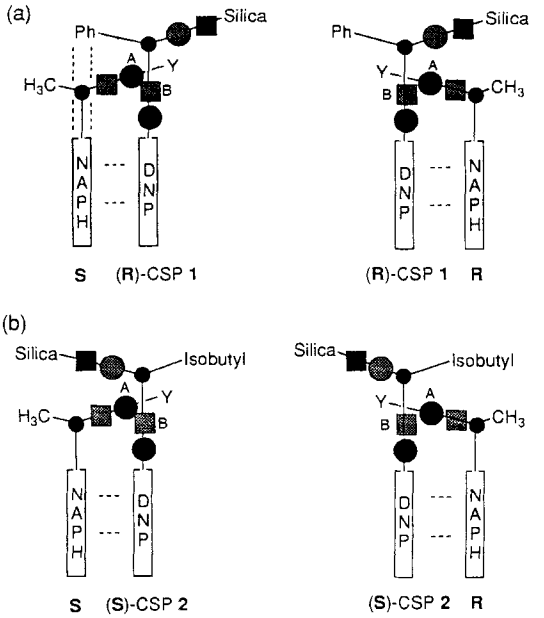

FIGURE 3. The proposed chiral recognition model for resolving N-acyl-α-(1-naphthyl)-ethylamine on (R)-CSP **1** or (S)-CSP **2**. Small solid circle : Methine hydrogen toward the viewer. Large solid circle : Carbonyl oxygen toward the viewer. Large gray circle : Carbonyl oxygen away from the viewer. Solid square : Amide hydrogen toward the viewer. Gray square : Amide hydrogen away from the viewer.

To rationalize those discrepancies between the chromatographic resolution behaviors on CSP **1** and **2**, we propose from study of the CPK molecular models a new chiral recognition model as is shown in Figure 3. In the model shown in Figure 3, (R)-CSP **1**, (S)-CSP **2** and N-acyl-α-(1-naphthyl)ethylamine are schematically represented in conformations which are presumed to be in their lowest energy and hence preferentially populated as described previously.[2] In Figure 3a, (R)-CSP **1** and the analyte are proposed to interact through the face-to-face π-π complexation between the 3,5-dinitrophenyl group (DNP) of the CSP and the 1-naphthyl group (NAPH) of the analyte and through the hydrogen bonding between the carbonyl oxygen (A) of the analyte and the amide N-H hydrogen (B) of the CSP. In this instance, the edge of the 1-naphthyl group (NAPH) of the (R)-analyte confronts the face of the phenyl group of the CSP, invoking the face-to-edge π-π interaction which has been considered as an associative force between aromatic rings.[7] Therefore, the transient diastereomeric (R,S)-complex formed between (R)-CSP **1** and the (S)-analyte might be more stable than the (R,R)-complex and consequently the (S)-enantiomer is retained longer than the (R)-enantiomer on CSP **1**. In this event, the N-acyl alkyl chain (denoted by Y in the model) of the (S)-analyte is oriented alongside the direction of the connecting tether of the CSP and eventually intercalates between the adjacent strands of the CSP. The intercalation of the N-acyl alkyl chain of the (S)-analyte might be evidenced by the trends in the capacity factors of the two enantiomers shown in Figure 1a. The more rapid decrease in the capacity factor of the (S)-analyte than that of the (R)-analyte, which is shown in Figure 1a, suggests in the normal phase chromatography that unfavorable steric interactions are occurring for the (S)-analyte. In consequence, the stability of the originally more stable (R,S)-complex decreases as the N-acyl alkyl chain increases in length and the separation factor decreases continuously as shown in Figure 1b.

The model of Figure 3b for the interaction between (S)-CSP **2** and the analyte shows the same face-to-face π-π complexation between the 3,5-dinitrophenyl group (DNP) of the CSP and the 1-naphthyl group (NAPH) of the analyte and the same hydrogen bonding between the carbonyl oxygen (A) of the analyte and the amide N-H hydrogen (B) of the CSP as that of Figure 3a. In

TABLE 2
Resolution of N-Acetyl-α-(1-naphthyl)-alkylamines **4** on CSP **1** and CSP **2**.[a]

		CSP **1**				CSP **2**			
4	n[b]	k_1'[c]	k_2'[d]	α[e]	Conf.[f]	k_1'[c]	k_2'[d]	α[e]	Conf.[f]
	2	5.76	10.73	1.86	S	7.36	8.29	1.13	R
	3	4.50	8.75	1.94		5.00	6.18	1.24	
	4	3.81	7.89	2.07		4.25	5.74	1.35	
	5	3.34	7.22	2.16		3.68	5.17	1.40	
	7	2.72	6.14	2.26		2.93	4.29	1.46	
	9	2.39	5.60	2.34		2.51	3.77	1.50	
	13	2.01	4.94	2.46		2.16	3.41	1.58	
	15	1.81	4.41	2.43	S	1.95	3.06	1.57	R

a: See the experimental section for the chromatographic conditions. Mobile phase was 20 % 2-propanol in hexane. b: Length of the alkyl chain [-$(CH_2)_n$-H] at the chiral center of analyte **4**. c: Capacity factor of the first eluted enantiomer. d: Capacity factor of the second eluted enantiomer. e: Separation factor. f: Absolute configuration of the second eluted enantiomer. For blanks, the elution orders have been determined to be the same as others in the series based on the TRAC technique.[3]

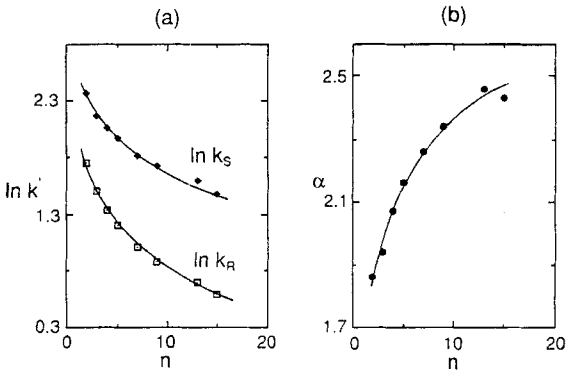

FIGURE 4. The trends of (a) capacity factors and (b) separation factors for resolving N-acetyl-α-(1-naphthyl)-alkylamines **4** on CSP **1**. Chromatographic conditions are given in the experimental part.

this instance, the N-acyl alkyl chain (denoted by Y in the model) of the (R)-analyte is directed between the adjacent strands of bonded phase and toward the silica support. When the N-acyl alkyl chain of the analyte is methyl, the stability of the (S,S)- and the (S,R)-complex is presumed to be identical from the separation factor (α = 1.00) for resolving N-acetyl-α-(1-naphthyl)-ethylamine (n = 1 in Table 1) on CSP **2**. As the N-acyl alkyl chain increases in length, however, the intercalation of the N-acyl alkyl chain of the (R)-enantiomer between adjacent strands of bonded phase experiences more difficulty and the retention of the (R)-analyte decreases more rapidly than that of the (S)-analyte (Figure 2a). In consequence, the stability of the (S,R)-complex decreases and the separation factor increases continuously with the (S)-enantiomer being retained longer on the column (Figure 2b).

The chiral recognition model proposed might be tested by the resolution trends of N-acetyl-α-(1-naphthyl)alkylamines **4** on CSP **1** and **2**. The chromatographic resolution results of N-acetyl-α-(1-naphthyl)alkylamines **4** on CSP **1** and **2** are summarized in Table 2 and the resolution trends are drawn in Figures 4 and 5. As shown in Figure 4, the resolution trends of N-acetyl-α-(1-naphthyl)alkylamines **4** on CSP **1** are exactly opposite to those (shown in Figure 1) of N-acyl-α-(1-naphthyl)ethylamines **3** on CSP **1**. The resolution trends of N-acetyl-α-(1-naphthyl)alkylamines **4** on CSP **2**, which is shown in Figure 5, are quite similar to those shown in Figure 2 for N-acyl-α-(1-naphthyl)ethylamines **3** on CSP **2** but the elution orders are opposite.

All of those resolution trends shown in Figure 4 and 5 can be rationalized based on the chiral recognition model proposed in Figure 3. In resolving N-acetyl-α-(1-naphthyl)alkylamines **4** on CSP **1** and **2**, the methyl group at the chiral center of the analyte in the chiral recognition model shown in Figure 3 is changed to varying alkyl group whereas the N-acyl group is fixed as acetyl. In this instance, the intercalation of the N-acyl alkyl chain of the analyte does not influence the enantioselectivity any more. Instead the alkyl group at the chiral center of the analyte is considered to influence the enantioselectivity based on the chiral recognition model shown in Figure 3. Namely, the alkyl group at the chiral center of the less retained (R)-analyte is directed alongside the connecting

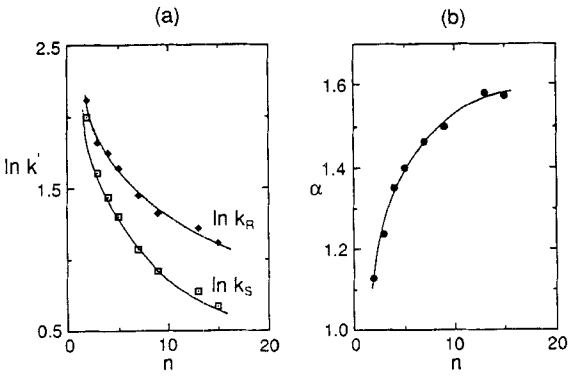

FIGURE 5. The trends of (a) capacity factors and (b) separation factors for resolving N-acetyl-α-(1-naphthyl)-alkylamines **4** on CSP **2**. Chromatographic conditions are given in the experimental part.

tether of CSP **1** and presumably intercalates between adjacent strands of bonded phase. In this instance, the capacity factor of the less retained (R)-analyte on CSP **1** should decrease more rapidly than that of the more retained (S)-analyte and the separation factor should increase continuously as the alkyl group at the chiral center of analyte **4** increases in length. These expectations are consistent with the trends shown in Figure 4.

Similarly, the alkyl group at the chiral center of the (S)-enantiomer of analyte **4** is oriented to the direction of the connecting tether of CSP **2** and probably intercalates between the connecting tethers. In this event, lengthening the alkyl chain at the chiral center of the analyte diminishes the retention of the (S)-enantiomer on CSP **2** more significantly than that of the (R)-enantiomer and consequently increases the separation factor continuously with the (R)-enantiomer being retained longer as is shown in Figure 5. Note again that the retention of the two enantiomers on CSP **2** is initially (when the alkyl group at the chiral center of the analyte is methyl *i.e.* n = 1) the same (see Table 1).

Resolution of N-acyl-α-(1-naphthyl)ethylamines **3** and N-acetyl-α-(1-naphthyl)alkylamines **4** on CSP **5**, the structure of which is identical with that of CSP **1** except the tether direction,[8] might be expected to show the reversed

TABLE 3
Resolution of N-Acyl-α-(1-naphthyl)-ethylamines **3** and N-Acetyl-α-(1-naphthyl)-alkylamines **4** on CSP **5**.[a]

analyte	n[b]	k_1'[c]	k_2'[d]	α[e]	Conf.[f]
3	1	6.55	8.11	1.24	S
	2	6.41	6.91	1.07	S
	3	5.23	5.82	1.11	
	4	4.89	5.58	1.14	S
	6	4.00	4.80	1.20	
	7	3.71	4.57	1.23	
	9	3.20	4.09	1.28	
	11	2.83	3.74	1.32	
	13	2.56	3.48	1.36	
	15	2.34	3.22	1.38	S
	17	2.17	3.02	1.39	S
4	2	2.13	2.51	1.18	S
	3	1.84	2.21	1.20	
	4	1.73	2.03	1.17	
	5	1.64	1.84	1.12	
	7	1.51	1.51	1.00	
	9	1.34	1.34	1.00	
	13	1.02	1.14	1.12	R
	15	0.90	1.05	1.17	R

a: See the experimental section for the chromatographic conditions. Mobile phase was 10 % 2-propanol in hexane for resolving **3** and 20 % 2-propanol in hexane for resolving **4**. b: Length of the N-Acyl alkyl chain [-$(CH_2)_n$-H] of analyte **3** or the alkyl chain [-$(CH_2)_n$-H] at the chiral center of analyte **4**. c, d, e, f: See the foot notes c, d, e and f of **TABLE 2**.

resolution trends compared to those on CSP **1** based on the chiral recognition model proposed in Figure 3. The tether direction of CSP **5** is exactly opposite to that of CSP **1**. Therefore, it is not difficult to imagine that the intercalation effects should be reversed.

Table 3 summarizes the resolution results of N-acyl-α-(1-naphthyl)-ethylamines **3** and N-acetyl-α-(1-naphthyl)alkylamines **4** on CSP **5**. The resolution trends are graphically shown in Figure 6 and 7. In resolving N-acyl-α-(1-naphthyl)ethylamines **3** on CSP **5**, the capacity factor of the less retained (R)-enantiomer decreases more rapidly than that of the more retained (S)-enantiomer and consequently, the separation factor increases continuously as is shown in Figure 6. These trends are exactly opposite to those on CSP **1** and are consistent with those expected from the chiral recognition model proposed in Figure 3. Note that the direction of the tether of the CSP in the chiral recognition model shown in Figure 3a is now reversed and the N-acyl alkyl chain (denoted by Y in the model) of the less retained (R)-enantiomer is oriented to the direction of the tether. In this instance, the N-acyl alkyl chain of the (R)-enantiomer intercalates between adjacent strands of bonded phase, the retention of the (R)-enantiomer decreases more rapidly than that of the (S)-enantiomer and the separation factor increases continuously as the N-acyl alkyl chain of the analyte increases in length. The minimum in the separation factor at n = 2 noted in Figure 6 might be a consequence of conformational factors as described previously.[9,10]

In resolving N-acetyl-α-(1-naphthyl)alkylamines **4** on CSP **5**, the alkyl group at the chiral center of the (S)-enantiomer is expected to be directed alongside the tether of the CSP and intercalate between adjacent strands of bonded phase based on the chiral recognition model proposed. In this instance, the retention of the more retained (S)-enantiomer should decrease more readily than that of the (R)-enantiomer and the separation factor should decrease continuously as the alkyl group at the chiral center of the analyte increases in length. As expected, the capacity factor of the (S)-enantiomer decreases more readily than that of the (R)-enantiomer as shown in Figure 7. In this case, however, the capacity factor of the initially more retained (S)-enantiomer becomes equal to that of the (R)-enantiomer when the alkyl chain at the chiral

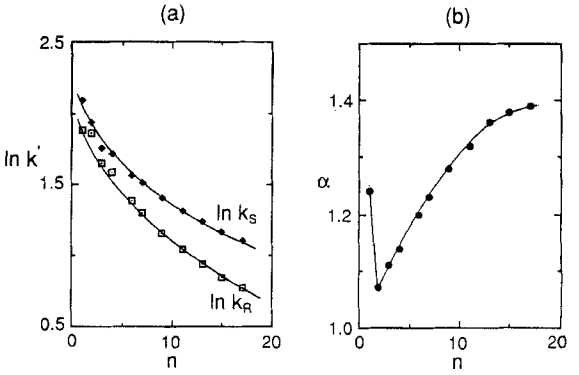

FIGURE 6. The trends of (a) capacity factors and (b) separation factors for resolving N-acyl-α-(1-naphthyl)-ethylamines **3** on CSP **5**. Chromatographic conditions are given in the experimental part.

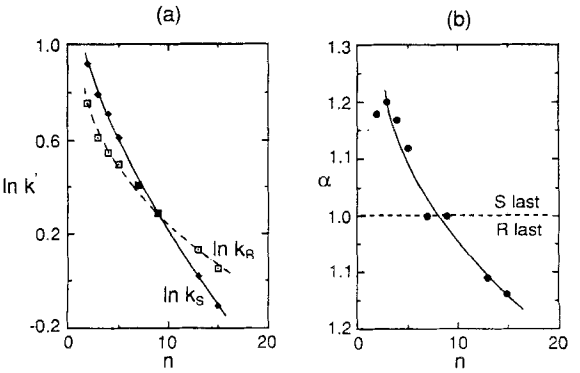

FIGURE 7. The trends of (a) capacity factors and (b) separation factors for resolving N-acetyl-α-(1-naphthyl)-alkylamines **4** on CSP **5**. Chromatographic conditions are given in the experimental part.

center of the analyte reaches at a certain length and after that (R)-enantiomer is retained longer than the (S)-enantiomer as the alkyl chain at the chiral center of the analyte increases in length further, resulting in the inversion of elution order. The overall resolution trends shown in Figure 7 are exactly consistent with those expected from the chiral recognition model proposed.

At this stage, it should be noted that the chromatographic trends described herein for resolving a series of N-acyl-α-(1-naphthyl)alkylamines on CSP **1** and **2** are closely related to earlier observations made for a series of N-acyl-α-(2-fluorenyl)alkylamines as a reviewer pointed out.[11] In the earlier paper, the chromatographic trends for a series of N-acyl-α-(2-fluorenyl)alkylamines have been explained by two competing, opposite sense chiral recognition mechanisms termed the "dipole-stacking" and "hydrogen-bonding" process. However, the two competing chiral recognition mechanisms can not explain the chromatographic resolution trends of N-acyl-α-(1-naphthyl)alkylamines on CSP **5**. In this instance, the chiral recognition model proposed in this study is thought to be more general.

In summary, in this study, we proposed a chiral recognition model which can rationalize the chromatographic trends of resolving N-acyl-α-(1-naphthyl)-ethylamines **3** on Pirkle-type π-acidic CSPs such as CSP **1** or **2**. Based on the chiral recognition model proposed, the chromatographic resolution behaviors of N-acetyl-α-(1-naphthyl)alkylamines **4** on CSP **1** and **2** and the chromatographic resolution behaviors of N-acyl-α-(1-naphthyl)ethylamines **3** and N-acetyl-α-(1-naphthyl)alkylamines **4** on CSP **5**, which has the same structure as CSP **1** except the tether direction, have been successfully rationalized. From these results, we conclude that the chiral recognition model proposed in Figure 3 is quite convincing. However, it should be reminded that mechanistic hypotheses can be disproven but not proven[1,5] and the chiral recognition model proposed in this study might be disproved or solidified as more chromatographic and/or spectroscopic data are accumulated. The efforts to acquire more solid evidences to support the chiral recognition model proposed are currently underway in our laboratory.

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