

Liquid Chromatographic Resolution of N-Protected α -Amino Acids as Their Anilide and 3,5-Dimethylanilide Derivatives on Chiral Stationary Phases Derived from (S)-Leucine[†]

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Various racemic N-protected α -amino acids such as N-*t*-BOC-(*tert*-butoxycarbonyl), N-CBZ-(benzyloxycarbonyl) and N-FMOC-(9-fluorenylmethyloxycarbonyl) α -amino acids were resolved as their anilide and 3,5-dimethylanilide derivatives on an HPLC chiral stationary phase (CSP) developed by modifying a commercial (S)-leucine CSP. The chromatographic resolution results were compared to those on the commercial (S)-leucine CSP. The resolutions were greater on the modified CSP than those on the commercial CSP with only one exception, the resolution of N-*t*-BOC-phenylglycine anilide. In addition, the chromatographic resolution behaviors were quite consistent except for the resolution of N-protected phenylglycine derivatives, the (S)-enantiomers being retained longer. Based on the chromatographic resolution behaviors and with the aid of CPK molecular model studies, we proposed a chiral recognition mechanism for the resolution of N-protected α -amino acid derivatives. However, for the resolution of N-protected phenylglycine derivatives, a second chiral recognition mechanism, which competes in the opposite sense with the first chiral recognition mechanism, was proposed. The two competing chiral recognition mechanisms were successfully used in the rationalization of the chromatographic behaviors for the resolution of N-protected phenylglycine derivatives.

Key Words : Liquid chromatography, Enantioseparation, Chiral stationary phase, α -Amino acids

Introduction

Optically active N-protected α -amino acids have been widely utilized in peptide synthesis.¹ Consequently, the demand for a method to determine the enantiomeric purity and to detect amounts of enantiomeric impurity in the N-protected α -amino acids has frequently arisen. Comparing various techniques, the chromatographic separation of enantiomers on chiral stationary phases (CSPs) might be the most accurate and convenient means to fulfill this purpose.² Actually, various CSPs have been successfully applied in the resolution of N-protected α -amino acids.³

CSP **1** (Figure 1) derived from (S)-leucine, which is commercially available (Regis Chemical Co., U.S.A.) has also been used in the resolution of N-protected α -amino acids such as N-*t*-BOC-(*tert*-butoxycarbonyl), N-CBZ-(benzyloxycarbonyl) and N-FMOC-(9-fluorenylmethyloxycarbonyl) α -amino acids as their anilide or 3,5-dimethylanilide derivatives.⁴ As an improved CSP, we recently developed CSP **2** (Figure 1) by simply replacing the superfluous adsorption site, the N-H hydrogen of the connecting tether of CSP **1**, with a phenyl group.⁵ CSP **2** has shown greater enantioselectivities for the enantiomers of π -acidic racemates such as N-(3,5-dinitrobenzoyl)- α -amino amides and esters⁵ and for the enantiomers of π -basic racemates such as N-(3,5-dimethoxy)- α -amino esters and amides⁶ and O-ethoxycarbonyl-2-hydroxy-

carboxylic anilides.⁷ In this study, we wish to elucidate that CSP **2** is also superior to CSP **1** in the resolution of N-*t*-BOC-, N-CBZ- and N-FMOC- α -amino acids as their anilide and 3,5-dimethylanilide derivatives (**3** and **4** in Figure 1) and wish to present a possible chiral recognition rationale.

Experimental Section

Chromatography was performed with an HPLC system

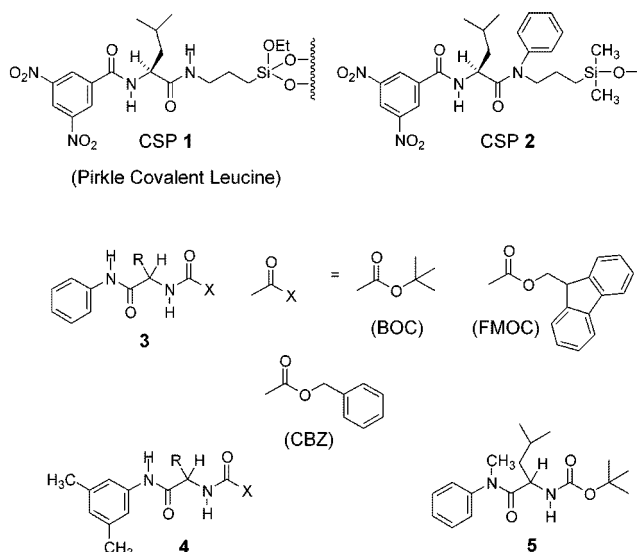


Figure 1. Structures of CSPs and the derivatives of N-protected α -amino acids used in this study.

[†]Dedicated to the late Professor Sang Chul Shim on his great achievements as a teacher and a scientist and on his invaluable contributions to the Korean Chemical Society.

consisting of a Waters Model 510 pump, a Rheodyne 7125 injector with a 20 μ L sample loop, a Younglin 710 absorbance detector with a 254 nm UV filter and a YoungLin Autochro data Module (Software: YoungLin Autochro-WIN 2.0 plus). All chromatographic data were collected using 20% isopropyl alcohol in hexane as a mobile phase with a flow rate of 2.0 mL/min at room temperature. The column void volumes were determined by injecting 1,3,5-tri-*tert*-butylbenzene. The elution orders were determined by injecting configurationally known samples.

Anilide (**3**) and 3,5-dimethylanilide derivatives (**4**) of *N*-protected- α -amino acids were prepared *via* a simple two-step procedure from α -amino acids obtained from Aldrich. As an example, leucine (260 mg, 2.0 mmole) was dissolved in 8 mL of dioxane and 8 mL of 0.5 N NaOH solution in a 50 mL round bottomed flask. The solution was stirred until clear and then di-*tert*-butyl dicarbonate (0.48 mL, 2.1 mmole) was added. After stirring the whole mixture for 5 hr at room temperature, the reaction mixture was diluted with 20 mL of water and extracted with diethyl ether. Then the aqueous layer was acidified with 6 N HCl and extracted with ethyl acetate. The organic layer was dried over anhydrous Na₂SO₄, filtered and evaporated. The residue was dissolved in 20 mL of methylene chloride and then aniline (0.19 mL, 2.1 mmole) and EEDQ (2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline, 544 mg, 2.2 mmole) were added. The whole mixture was stirred at room temperature for 5 hr, washed with 0.5 N HCl solution, 0.5 N NaOH solution and then brine. The organic solution was dried over anhydrous Na₂SO₄, filtered and evaporated. The residue was purified by silica gel column chromatography (ethyl acetate-hexane, 1 : 3, v/v) to afford the anilide derivative of *N*-*t*-BOC-leucine (477 mg, 78% yield). All anilide derivatives **3** of *N*-protected- α -amino acids thus prepared exhibited spectral data (¹H NMR) in accord with the assigned structures. The above procedure was used to prepare 3,5-dimethylanilide derivatives **4** of *N*-protected- α -amino acids except that 3,5-dimethylaniline was used instead of aniline. *N*-Methylanilide derivative **5** of *N*-*t*-BOC-leucine was also prepared by the procedure described above using *N*-methylaniline instead of aniline.

Results and Discussion

The chromatographic results for the resolution of *N*-*t*-BOC-, *N*-CBZ- and *N*-FMOC- α -amino acids as their anilide (**3**) and 3,5-dimethylanilide derivatives (**4**) on CSP **2** are summarized and compared with those on CSP **1** in Table 1 and the typical chromatograms are illustrated in Figure 2. The resolutions were excellent especially on CSP **2**.

As shown in Table 1, the enantioselectivities denoted by the separation factors (α) for the resolution of *N*-*t*-BOC-, *N*-CBZ- and *N*-FMOC- α -amino acids as their anilide (**3**) and 3,5-dimethylanilide derivatives (**4**) on CSP **2** are greater than those on CSP **1** except for the resolution of *N*-*t*-BOC-phenylglycine anilide, **3d**. In the resolution of *N*-*t*-BOC-phenylglycine anilide, **3d**, the separation factor (α) on CSP **1**

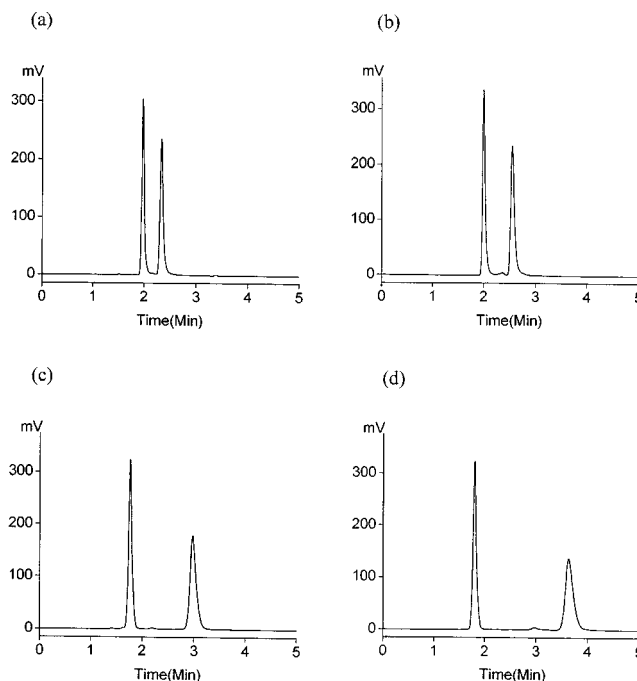


Figure 2. Comparison of the chromatograms for the resolution of (a) *N*-*t*-BOC-leucine anilide (**3c**) and (b) *N*-*t*-BOC-leucine 3,5-dimethylanilide (**4c**) on CSP **1** with those for the resolution of (c) *N*-*t*-BOC-leucine anilide (**3c**) and (d) *N*-*t*-BOC-leucine 3,5-dimethylanilide (**4c**) on CSP **2**.

is slightly greater than that on CSP **2**. The elution orders on CSP **1** and CSP **2** are generally consistent, the (*S*)-enantiomers being eluted second. Interestingly, however, the elution orders for the resolution of *N*-*t*-BOC-phenylglycine anilide, **3d**, and *N*-*t*-BOC-phenylglycine 3,5-dimethylanilide, **4d**, on CSP **1** are different from the others, the (*R*)-enantiomers being eluted second. In addition, the separation factors (α) for the resolution of *N*-protected phenylglycine derivatives on CSP **1** and CSP **2** are generally inferior to those for the resolution of other *N*-protected α -amino acid derivatives. From these results, the chiral recognition mechanism for the resolution of *N*-protected phenylglycine derivatives is expected to be somewhat different from that for the resolution of other *N*-protected α -amino acid derivatives.

Between derivatives **3** and **4** of *N*-protected α -amino acids, 3,5-dimethylanilides (**4**) are resolved with greater separation factors (α) than the corresponding anilides (**3**) except for the resolution of *N*-*t*-BOC-phenylglycine derivatives (**3d** and **4d**) on CSP **1**. The 3,5-dimethylanilide group is believed to be more π -basic than the anilide group because of the electron releasing nature of the two methyl groups on the phenyl ring of the 3,5-dimethylanilide group. In this instance, the greater enantioselectivities observed with 3,5-dimethylanilides might be expected to stem from the more effective π - π interaction between the π -basic 3,5-dimethylanilide group of analytes and the π -acidic 3,5-dinitrobenzoyl group of the CSP.

The role of the N-H hydrogen of the anilides or 3,5-dimethylanilides of *N*-protected- α -amino acids in chiral recognition has also been investigated by preparing the *N*-

Table 1. Resolution of the anilide (**3**) and 3,5-dimethylanilide derivatives (**4**) of *N*-protected α -amino acids on CSP **1** and CSP **2**^a

Analyte			CSP 1				CSP 2			
R	N-Pro ^b		k ₁ ' ^c	k ₂ ' ^d	α ^e	Conf. ^f	k ₁ ' ^c	k ₂ ' ^d	α ^e	Conf. ^f
3a	CH ₃ (alanine)	BOC	0.63	0.90	1.43	S	0.40	1.07	2.68	S
3b	CH(CH ₃) ₂ (valine)	BOC	0.37	0.53	1.43	S	0.27	0.93	3.44	S
3c	CH ₂ CH(CH ₃) ₂ (leucine)	BOC	0.35	0.60	1.71	S	0.28	1.15	4.11	S
3d	C ₆ H ₅ (phenylglycine)	BOC	0.81	1.12	1.38	R	0.95	1.27	1.34	S
3e	C ₆ H ₅ CH ₂ (phenylalanine)	BOC	0.57	0.85	1.49	S	0.44	1.54	3.50	S
3f	CH ₃ (alanine)	CBZ	1.44	2.85	1.98	S	0.85	4.28	5.04	S
3g	CH(CH ₃) ₂ (valine)	CBZ	0.85	1.61	1.89	S	0.61	3.62	5.93	S
3h	CH ₂ CH(CH ₃) ₂ (leucine)	CBZ	0.81	2.01	2.48	S	0.58	4.35	7.50	S
3i	C ₆ H ₅ (phenylglycine)	CBZ	2.40	2.40	1.00		1.87	4.34	2.32	S
3j	C ₆ H ₅ CH ₂ (phenylalanine)	CBZ	1.35	2.47	1.83	S	0.97	5.57	5.74	S
3k	CH ₃ (alanine)	FMOC	1.44	3.07	2.13	S	0.98	5.23	5.34	S
3l	CH(CH ₃) ₂ (valine)	FMOC	0.87	1.69	1.94	S	0.67	3.93	5.87	S
3m	CH ₂ CH(CH ₃) ₂ (leucine)	FMOC	0.82	2.14	2.61	S	0.66	4.96	7.52	S
3n	C ₆ H ₅ (phenylglycine)	FMOC	2.48	2.73	1.10	S	2.17	5.58	2.57	S
3o	C ₆ H ₅ CH ₂ (phenylalanine)	FMOC	1.34	2.71	2.02	S	1.04	6.25	6.01	S
4a	CH ₃ (alanine)	BOC	0.66	1.10	1.67	S	0.45	1.65	3.67	S
b	CH(CH ₃) ₂ (valine)	BOC	0.37	0.62	1.68	S	0.28	1.25	4.46	S
c	CH ₂ CH(CH ₃) ₂ (leucine)	BOC	0.37	0.76	2.05	S	0.30	1.63	5.43	S
d	C ₆ H ₅ (phenylglycine)	BOC	0.94	1.01	1.07	R	0.86	1.74	2.02	S
4e	C ₆ H ₅ CH ₂ (phenylalanine)	BOC	0.57	0.97	1.70	S	0.49	2.08	4.24	S
4f	CH ₃ (alanine)	CBZ	1.57	3.64	2.32	S	1.00	7.01	7.01	S
4g	CH(CH ₃) ₂ (valine)	CBZ	0.86	2.04	2.37	S	0.64	5.14	8.03	S
4h	CH ₂ CH(CH ₃) ₂ (leucine)	CBZ	0.84	2.53	3.01	S	0.67	6.87	10.25	S
4i	C ₆ H ₅ (phenylglycine)	CBZ	2.23	2.85	1.28	S	1.70	6.43	3.78	S
4j	C ₆ H ₅ CH ₂ (phenylalanine)	CBZ	1.37	3.01	2.20	S	1.12	8.01	7.15	S
4k	CH ₃ (alanine)	FMOC	1.51	3.90	2.58	S	1.07	7.96	7.44	S
4l	CH(CH ₃) ₂ (valine)	FMOC	0.83	2.09	2.52	S	0.69	5.42	7.86	S
4m	CH ₂ CH(CH ₃) ₂ (leucine)	FMOC	0.84	2.78	3.31	S	0.73	7.58	10.38	S
4n	C ₆ H ₅ (phenylglycine)	FMOC	2.23	3.37	1.51	S	2.02	8.79	4.35	S
4o	C ₆ H ₅ CH ₂ (phenylalanine)	FMOC	1.32	3.22	2.44	S	1.15	8.58	7.46	S
5			0.62	0.62	1.00		0.52	0.52	1.00	

^aSee the Experimental part for the chromatographic conditions. ^bN-Protecting group of α -amino acids. ^cRetention factor of the first eluted enantiomer. ^dRetention factor of the second eluted enantiomer. ^eSeparation factor. ^fAbsolute configuration of the second eluted enantiomer.

methylanilide derivative (**5**) of *N*-*t*-BOC-leucine, which does not contain the anilide N-H hydrogen, and resolving it on CSP **1** and CSP **2**. As shown in Table 1, *N*-methylanilide (**5**) of *N*-*t*-BOC-leucine was not resolved at all on CSP **1** and CSP **2**. Consequently, the N-H hydrogen of the anilides or the 3,5-dimethylanilides of *N*-protected α -amino acids is presumed to play an important role as a hydrogen bonding donor in the chiral recognition.

Based on the observed chromatographic resolution results on CSP **1** and CSP **2** and with the aid of CPK molecular model studies, we propose a chiral recognition mechanism for the resolution of *N*-protected α -amino acids (except for the resolution of *N*-protected phenylglycine as their anilide or 3,5-dimethylanilide derivatives) on CSP **2** as shown in Figure 3. In Figure 3, the model compound of the chiral selector of CSP **2**, (S)-*N*-(3,5-dinitrobenzoyl)leucine *N*-phenyl *N*-propylamide, interacts with (S)-*N*-*t*-BOC-leucine anilide, (S)-**3c**, through the face-to-face π - π interaction between the

π -acidic 3,5-dinitrophenyl group of the CSP and the π -basic anilide group of the analyte. Simultaneously, the model compound of the chiral selector of CSP **2** interacts with (S)-*N*-*t*-BOC-leucine anilide, (S)-**3c**, through the two hydrogen bonding interactions. One hydrogen bonding is presumed to be formed between the carbonyl oxygen of the amide tethering group of the model compound of the chiral selector of CSP **2** and the N-H hydrogen of the anilide group of the analyte. The other hydrogen bonding is formed between the only N-H hydrogen of the model compound of the chiral selector of CSP **2** and the carbonyl oxygen of the *t*-BOC group (or FMOC or CBZ group in the case of other *N*-protected groups) of the analyte. The three similar simultaneous interactions of the model compound of the chiral selector of CSP **2** with (R)-*N*-*t*-BOC-leucine anilide, (R)-**3c**, however, are energetically less favorable because of the inadequate three dimensional positions of the interaction sites. For this reason, the (S)-enantiomers are retained longer

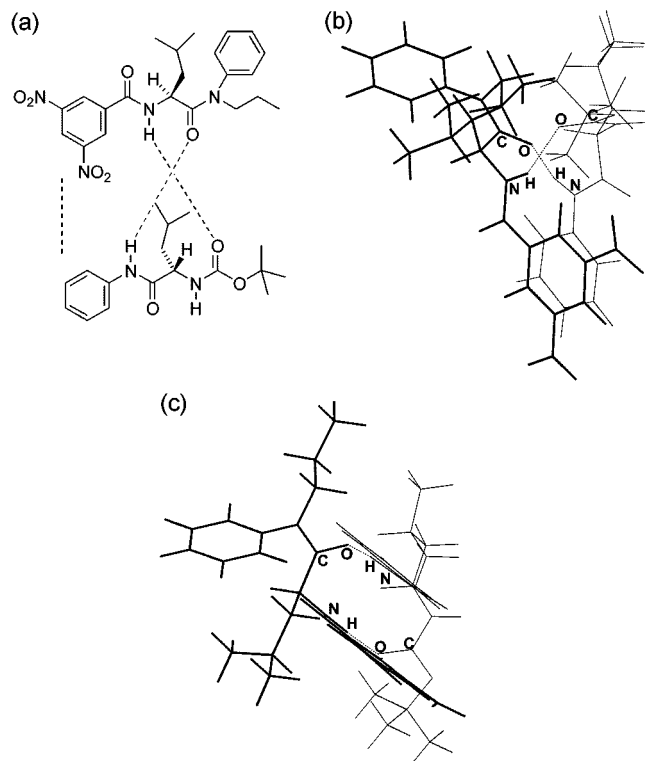


Figure 3. Proposed chiral recognition model for the more stable (S,S)-complex formed between the model compound (represented with thick lines) of the chiral selector of CSP 2, (S)-N-(3,5-dinitrobenzoyl)leucine N-phenyl N-propylamide and the (S)-enantiomer of an analyte (represented with thin lines), (S)-N-*t*-BOC-leucine anilide (**3c**). (a) Schematic presentation of the chiral recognition model showing the interaction sites of the chiral selector of CSP 2 and the analyte. (b) A computer generated (HyperChem 4.0) stick molecular models viewed from the angle showing the face-to-face π - π interaction between the 3,5-dinitrobenzoyl group of the CSP and the anilide phenyl group of the analyte and the two hydrogen bondings. (c) The same stick molecular model as in (b), but viewed from a different angle.

on the column and these are consistent with the elution orders shown in Table 1.

In the resolution of N-protected phenylglycines as their anilides or 3,5-dimethylanilides, the phenyl group at the chiral center of the analyte might play a role as a π -electron donor site for the face-to-face π - π interaction. In this instance, a second chiral recognition mechanism utilizing the phenyl group at the chiral center of the analyte as a π -electron donor site is expected to compete with the chiral recognition mechanism shown in Figure 3.

Fortunately, in elucidating the second competing chiral recognition mechanism, we are in the position of utilizing our previous research results concerning the resolution of N-butanoylphenylglycine propylamide on CSP **1**.⁸ N-Butanoylphenylglycine propylamide, which contains only one phenyl group as a π -electron donor site, was resolved quite well on CSP **1**, the (R)-enantiomer being retained longer on the chiral column and the chiral recognition mechanism involving π - π interaction between the 3,5-dinitrophenyl group of the CSP and the phenyl group of analyte was proposed in the previ-

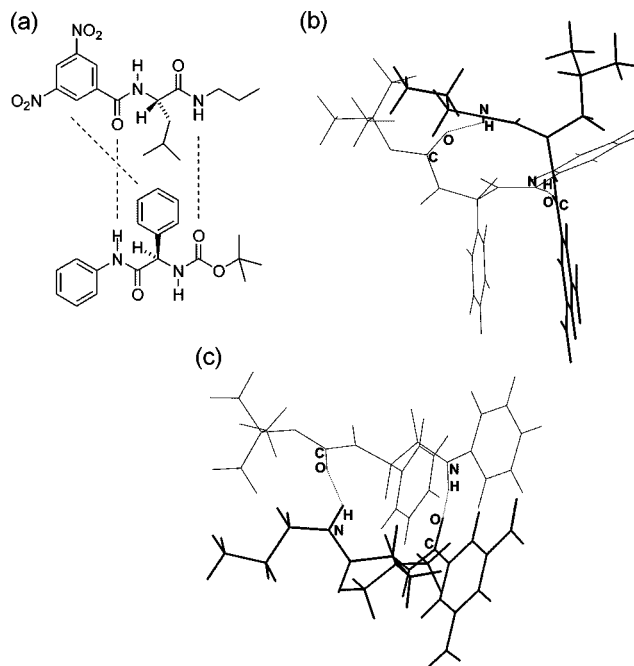


Figure 4. Proposed chiral recognition model for the stable (S,R)-complex formed between the model compound (represented with thick lines) of the chiral selector of CSP **1**, (S)-N-(3,5-dinitrobenzoyl)leucine propylamide and the (R)-enantiomer of an analyte (represented with thin lines), (R)-N-*t*-BOC-phenylglycine anilide (**3d**). (a) Schematic presentation of the chiral recognition model showing the interaction sites of the chiral selector of CSP **1** and the analyte. (b) A computer generated (HyperChem 4.0) stick molecular models viewed from the angle showing the face-to-face π - π interaction between the 3,5-dinitrobenzoyl group of the CSP and the phenyl group at the chiral center of the analyte and the two hydrogen bondings (side view). (c) The same stick molecular model as in (b), but viewed from a different angle (top view).

ous paper.⁸

Based on the previous study and from the study of CPK molecular models, a second chiral recognition mechanism, which is assumed to compete with that shown in Figure 3, for the resolution of N-*t*-BOC-phenylglycine anilide, **3d**, on CSP **1** is proposed in Figure 4. As shown in Figure 4, the face-to-face π - π interaction between the π -acidic 3,5-dinitrophenyl group of the model compound of the chiral selector of CSP **1**, (S)-N-(3,5-dinitrobenzoyl)leucine propylamide, and the π -basic phenyl group of N-*t*-BOC-(R)-phenylglycine anilide, (R)-**3d**, and the two hydrogen bonding interactions between the CSP and the analyte are involved in chiral recognition. It should be noted that the two hydrogen bonding interactions shown in Figure 4 are different from those shown in Figure 3 and one of the two hydrogen bonding interactions is not possible with CSP **2** because of the absence of the N-H amide hydrogen of the connecting tether. In addition, the chiral recognition mechanism shown in Figure 4 retains the (R)-enantiomer more strongly while that shown in Figure 3 retains the (S)-enantiomer more strongly. Consequently, it has been concluded that the two chiral recognition mechanisms compete in the opposite sense of chiral recognition.

Based on the two competing chiral recognition mechanisms shown in Figure 3 and Figure 4, the exceptional behaviors for the resolution of anilides and 3,5-dimethylanilides of N-protected phenylglycine on CSP 1 and CSP 2 can be rationalized. For example, in the resolution of N-*t*-BOC-phenylglycine anilide (**3d**) on CSP 1, the longer retention of the (R)-enantiomer on CSP 1 may be interpreted as the predominance of the chiral recognition mechanism shown in Figure 4 over that shown in Figure 3. However, in the resolution of N-*t*-BOC-phenylglycine anilide (**3d**) on CSP 2, the chiral recognition mechanism shown in Figure 4 is relatively less significant because of the lack of the N-H amide hydrogen of the connecting tether of the CSP and consequently the chiral recognition mechanism shown in Figure 3 is expected to be predominant, the (S)-enantiomer being eluted second. In the resolution of N-*t*-BOC-phenylglycine 3,5-dimethylanilide (**4d**), the resolution behaviors are similar to those for the resolution of N-*t*-BOC-phenylglycine anilide (**3d**). However, the separation factor (α) for the resolution of **4d** on CSP 1 decreased while that on CSP 2 increased significantly compared with that for the resolution of **3d** as shown in Table 1. The increased face-to-face π - π interaction between the 3,5-dinitrophenyl group of the CSP and the 3,5-dimethylanilide group of **4d** seems to favor the chiral recognition mechanism shown in Figure 3 while the chiral recognition mechanism shown in Figure 4 remains invariable. Consequently, the separation factor (α) for the resolution of **4d** on CSP 1 is decreased compared to that for the resolution of **3d**. However, the separation factor (α) for the resolution of **4d** on CSP 2 is increased compared to that for the resolution of **3d**.

In the resolution of the anilide derivatives (**3i** and **3n**) and the 3,5-dimethylanilide derivatives (**4i** and **4n**) of N-CBZ- and N-FMOC-phenylglycine on CSP 1 and CSP 2, the elution orders are consistent, the (S)-enantiomers being retained always longer. However, the separation factors (α) are relatively small compared to those for the resolution of other N-protected α -amino acid derivatives. All of these relatively small separation factors can also be rationalized by the two competing opposite sense chiral recognition mechanisms. Especially, in the resolution of N-CBZ-phenylglycine anilide (**3i**) on CSP 1, no resolution was observed. In general, enantioselectivities denoted by the separation factors (α) for the resolution of N-protected α -amino acid derivatives on CSP 1 and CSP 2 are increased as the amino acid N-protecting group changes from *t*-BOC to CBZ and to FMOC as shown in Table 1, indicating that the chiral recognition mechanism shown in Figure 3 becomes more favorable as the amino acid N-protecting group changes from *t*-BOC to CBZ and to FMOC. In this instance, the chiral recognition mechanism shown in Figure 3 for the resolution of N-CBZ-phenylglycine anilide (**3i**) on CSP 1 becomes more favorable compared to the resolution of N-*t*-BOC-phenylglycine anilide (**3d**) and finally the contribution of the chiral recognition mechanism shown in Figure 3 to the actual chiral recognition becomes equal to that of the chiral recognition mechanism shown in Figure 4. Consequently, the resolution

of N-CBZ-phenylglycine anilide (**3i**) on CSP 1 is not observed. However, in the resolution of N-FMOC-phenylglycine anilide (**3n**), N-CBZ-phenylglycine 3,5-dimethylanilide (**4i**) and N-FMOC-phenylglycine 3,5-dimethylanilide (**4n**) on CSP 1, the chiral recognition mechanism shown in Figure 3 seems to be predominant over that shown in Figure 4 and consequently the (S)-enantiomers are retained longer. In the resolution of N-protected phenylglycine derivatives (**3d**, **3i**, **3n**, **4d**, **4i** and **4n**) on CSP 2, the chiral recognition mechanism shown in Figure 3 seems to always predominate over that shown in Figure 4 because of the absence of the N-H amide hydrogen of the connecting tether and consequently the (S)-enantiomers are always retained longer and the separation factors (α) are greater than those on CSP 1.

In summary, in this study, we demonstrated that CSP 2 is more effective than CSP 1 in the resolution of N-protected α -amino acids as their anilide and 3,5-dimethylanilide derivatives. Between the two derivatives of N-protected α -amino acids, a greater resolution was obtained for the 3,5-dimethylanilide derivatives. The elution orders were consistent, the (S)-enantiomers being retained longer except for the resolution of N-*t*-BOC-phenylglycine anilide and 3,5-dimethylanilide on CSP 1. In order to rationalize the chromatographic behaviors for the resolution of N-protected α -amino acids as their anilide and 3,5-dimethylanilide derivatives except for the resolution of N-protected phenylglycine derivatives, we proposed a chiral recognition mechanism utilizing three simultaneous interactions such as the face-to-face π - π interaction and the two hydrogen bonding interactions between the (S)-CSP and the more retained (S)-enantiomer of the analytes. In the resolution of N-protected phenylglycine derivatives, we assumed that the phenyl group at the chiral center can be used as another π -electron acceptor site for the face-to-face π - π interaction with the CSP and consequently proposed another competing chiral recognition mechanism in which the (R)-enantiomer of analytes interacts more favorably with the (S)-CSP than the (S)-enantiomer does. The somewhat abnormal chromatographic behaviors for the resolution of N-protected phenylglycine derivatives were successfully interpreted as the competition of the two chiral recognition mechanisms proposed.

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