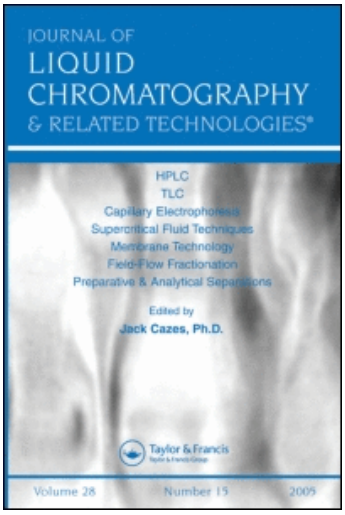

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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Online publication date: 17 April 2002

To cite this Article Hyun, Myung Ho , Cho, Young Ki , Han, Sang Cheol and Cho, Yoon Jae(2002) 'PREPARATION AND APPLICATION OF NEW ION-PAIRING CHIRAL STATIONARY PHASES FOR THE LIQUID CHROMATOGRAPHIC SEPARATION OF ENANTIOMERS', Journal of Liquid Chromatography & Related Technologies, 25: 4, 573 — 588
To link to this Article: DOI: 10.1081/JLC-120008812
URL: http://dx.doi.org/10.1081/JLC-120008812

J. LIQ. CHROM. & REL. TECHNOL., 25(4), 573–588 (2002)

PREPARATION AND APPLICATION OF NEW ION-PAIRING CHIRAL STATIONARY PHASES FOR THE LIQUID CHROMATOGRAPHIC SEPARATION OF ENANTIOMERS

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ABSTRACT

Two chiral stationary phases (CSP) containing a free primary amino group were prepared by bonding (S)-phenylglycine and (R)-4-methoxyphenylglycine to silica gel and applied in resolving various N-(3,5-dinitrobenzoyl)-α-amino acids. Among various mobile phases, methylene chloride containing 0.2% acetic acid and 0.1% triethylamine was found most useful as a mobile phase. Between the two CSPs, the one based on (R)-4-methoxyphenylglycine was more effective than the other based on (S)-phenylglycine in the resolution of N-(3,5-dinitrobenzoyl)-α-amino acids, indicating the importance of the π-basicity of the aromatic group of the CSP in the chiral recognition. The π-acid-

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ity of the N-(3,5-dinitrobenzoyl) group of analytes was also found important for the effective chiral recognition.

From these results, the π–π interaction between the CSP and the analytes was concluded to be the important factor for the chiral recognition. On the contrary, N-(3,5-dinitrobenzoyl)-leucine methyl ester was not resolved at all on the CSPs. Consequently, the free carboxylic acid group was expected to be absolutely necessary for the chiral recognition, indicating the importance of the ion-pairing or the electrostatic interaction in the chiral recognition. These two factors necessary for the chiral recognition were exactly consistent with the chiral recognition mechanism proposed previously, based on the relaxed scan calculation combined with a Monte Carlo conformation search in solution-phase.

INTRODUCTION

Liquid chromatographic separation of enantiomers on chiral stationary phases (CSPs) has demonstrated to be very favorable for the analysis of enantiomeric composition of chiral compounds and also for the preparative resolution of racemates (1–3). Consequently, extensive efforts have been devoted to the development of effective CSPs for the liquid chromatographic separation of enantiomers and hundreds of CSPs have been developed (1–3). All of those CSPs have been classified into five or six types, according to their manner of chiral recognition (4–5).

Especially, CSPs which discriminate two enantiomers through the enantioselective π–π interaction have been classified as Pirkle-type CSPs. In addition to π–π interactions, Pirkle-type CSPs utilize hydrogen bonding interactions and/or dipole-dipole interactions as attractive interactions. For example, CSP **1** (Figure 1) derived from *p*-hydroxyphenylglycine has been reported to utilize π–π interactions and two hydrogen bonding interactions in the resolution of N-(3,5-dinitrobenzoyl)-α-amino amides (6,7). However, ionic interactions have not generally been utilized as attractive forces for the chiral recognition.

Ionic interactions for the chiral recognition were first utilized by Baczuk and his coworkers in the resolution of racemic DOPA on an L-arginine-tailored Sephadex support (8). After Baczuk's work, cinchona alkaloids, especially quinine and quinidine covalently immobilized onto silica, have been most widely employed in ionic interactions in the chiral recognition (9–11). However, Pirkle-type CSPs based on α-amino acids have not utilized ionic interactions for the chiral recognition.

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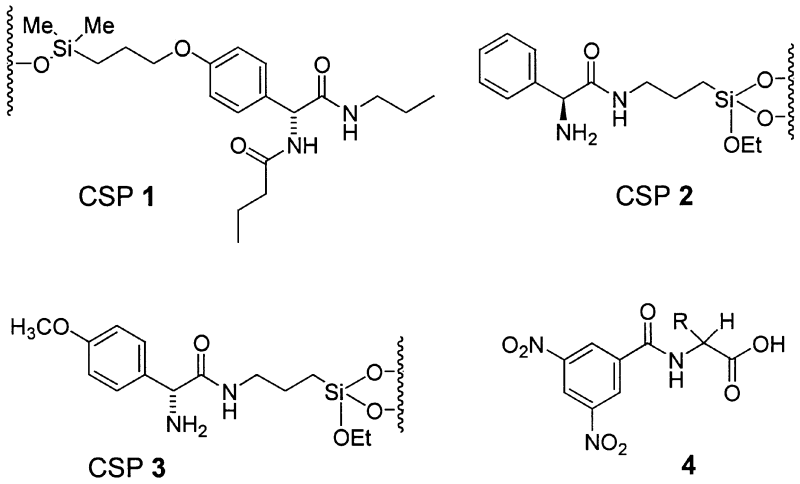

Figure 1. The structure of CSPs **1**, **2** and **3** and N-(3,5-dinitrobenzoyl)-α-amino acids **4**.

Very recently, in a short communication letter, we demonstrated experimentally and theoretically that ionic or electrostatic interactions play an important role in the chiral recognition for the resolution of N-(3,5-dinitrobenzoyl)leucine on a new CSP (CSP **2**, Figure 1) (12). However, the details of the preparation and application of CSP **2** have not been reported yet. In this study, we want to report the preparation and application of two CSPs (**2** and **3**, Figure 1) to demonstrate further that ionic or electrostatic interactions are useful as attractive interactions for the resolution of ionic racemates such as N-(3,5-dinitrobenzoyl)-α-amino acids **4** on Pirkle-type CSPs. CSPs **2** and **3** both contain a π-basic interaction site such as phenyl or *p*-methoxyphenyl group and an amide N-H hydrogen as a hydrogen bonding donor site, or an amide carbonyl oxygen as a hydrogen bonding acceptor site. In addition, they contain a primary amino functional group. The primary amino functional group might be utilized as an ionic interaction site after being protonated.

EXPERIMENTAL

General

^{1}H NMR spectra were obtained with a Varian Gemini 200 spectrometer (200 MHz). Chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane as the internal standard. IR spectra were recorded on a Mattson

Polaris FT IR spectrometer. Melting points were taken on a Rigaku TAS 100 thermal analyzer. Elemental analyses were performed at the Korea Basic Science Institute (Pusan Branch), Pusan, Korea.

Chromatography was performed with 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 with a 254 nm UV filter, and a Youngin D520B computing integrator. Analytes were prepared by simply stirring α-amino acids with desired benzoyl chloride in the presence of propylene oxide in tetrahydrofuran as reported previously (13).

Preparation of CSP 2

N-(t-Butyloxycarbonyl)-L-phenylglycine **5**

(L)-Phenylglycine (2.0 g, 13 mmole) and triethylamine (3.7 mL, 26 mmole) were dissolved in 30 mL of water. To the aqueous solution was added 30 mL of 1,4-dioxane with stirring. To the well-stirred solution was added ditert-butyldicarbonate (3.0 mL, 13 mmole). The whole mixture was stirred for 4 hrs at room temperature. To the reaction mixture was added 2 N HCl solution (100 mL) and the mixture was extracted with ethyl acetate (150 mL) twice. The combined ethyl acetate solution was dried over anhydrous $MgSO_4$, filtered, and then concentrated. The residue was dissolved in a minimal amount of acetone and then crystallized by adding n-hexane to the acetone solution to afford white crystalline product **5**(3.3 g, 98.1%).

The enantiomeric purity of **5** was more than 98% ee by HPLC analysis, as its anilide derivative on a CSP derived from (S)-(3,5-dinitrobenzoyl)leucine (14). m.p. 52-54°C. ^{1}H NMR ($CDCl_3$): δ 1.22(s, 9 H), 5.14(d, 1 H), 7.26–7.41(m, 5 H), 8.08(d, 1 H). IR (KBr): cm^{-1} 3425, 2978, 1718.

N-(t-Butyloxycarbonyl)-L-phenylglycine N-(3 triethoxysilylpropyl) Amide **6**

N-(t-Butyloxycarbonul)-L-phenylglycine **5** (2.8 g, 11 mmole) and EEDQ (2-ethoxycarbonyl-1,2-dihydroquinoline, 3.0 g, 12 mmole) were dissolved in 60 mL of methylene chloride. To the stirred solution was added 3-aminopropyl-triethoxysilane (2.9 mL, 12 mmole). The whole mixture was stirred for 6 hrs at room temperature and then concentrated. The residue was purified by silica gel column chromatography (ethyl acetate-hexane = 1 : 1, v/v) to afford triethoxysilyl compound **6** (2.5 g, 50%) as a white crystalline solid. The enantiomeric purity of **6** was more than 98% ee by HPLC analysis on a CSP derived from (S)-

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(3,5-dinitrobenzoyl)leucine (14). m.p. 53-55°C. ^{1}H NMR (CDCl3): δ 0.43–0.53 (m, 2H), 1.18 (t, 9H), 1.39 (s, 9 H), 1.46–1.58 (m, 2H), 3.16–3.26 (m, 2H), 3.75 (q, 6H), 5.10(d, 1 H), 5.85 (d, 1H), 6.08 (broad s, 1H), 7.27–7.36(m, 5 H). IR (KBr): cm^{-1} 3347, 3291, 1659.

Preparation of CSP **2** and Column Packing

A 200 mL three neck round bottom flask equipped with a Dean-Stark trap, a condenser, and a magnetic stirring bar was charged with Regis Rexchrom silica gel (5 μm, 4.5 g) and toluene (100 mL). The mixture was heated to reflux until the complete azeotropic removal of water. To the heterogeneous solution, was added silyl compound **6** (1.4 g, 3.1 mmole) dissolved in 10 mL of toluene. The whole mixture was heated to reflux for 72 hrs. The modified silica gel was filtered, washed successively with toluene, ethyl acetate, methanol, acetone, diethyl ether, and hexane and then dried. The modified silica gel mixed with 60 mL of methylene chloride in a 200 mL round bottom flask was treated with trifluoroacetic acid (1.4 mL, 18 mmole) for 48 hrs at room temperature. Finally, the modified silica gel was washed successively with toluene, ethyl acetate, methanol, acetone, diethyl ether, and hexane and then dried to afford CSP **2**. Elemental analysis of CSP **2** (found: C, 5.90%; H, 0.80%; N, 0.87%) showed a loading of 0.31 mmoles of selector (based on N), or 0.37 mmoles of selector (based on C) per gram of stationary phase. The modified silica gel was slurried in methanol and packed into a 250 mm × 4.6 mm I.D. stainless steel HPLC column using a conventional slurry packing method with an Alltech slurry packer.

Preparation of CSP 3

N-(t-Butyloxycarbonyl)-L-(4-methoxyphenylglycine) **7**

N-(t-Butyloxycarbonyl)-L-(4-hydroxyphenylglycine) (3.9 g, 15 mmole) prepared via the known procedure (7) was dissolved in 70 mL of DMF (dimethylformamide) and then the solution was cooled to 0°C. To the stirred solution, was added sodium hydride (1.5 g, 36 mmole, 60% dispersed in oil) in 20 mL of DMF via a cannula at 0°C. The mixture was warmed to 10°C and then stirred for 1 hr. After adding methyl iodide (0.8 mL, 13 mmole) to the stirred solution, the whole mixture was stirred for additional 3 hrs at 10°C. The reaction was quenched by adding 250 mL of water and the whole solution was extracted with 250 mL of ethyl acetate. The aqueous layer was acidified by adding 6 N HCl solution until the solution becomes turbid and then extracted with 200 mL of ethyl acetate. The ethyl acetate solutions were combined, dried over anhydrous

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$MgSO_4$, filtered, and concentrated. The residue was purified by silica gel column chromatography (ethyl acetate-hexane = 1 : 2, v/v) to afford compound **7** (1.3 g, 31.2%) as an oily material. The enantiomeric purity of **7** was more than 98% ee by HPLC analysis as its anilide derivative, on a CSP derived from (S)-N-(3,5-dinitrobenzoyl)leucine (14). ^{1}H NMR ($CDCl_3$): δ 1.42 (s, 9H), 3.80 (s, 3H), 5.06(d, 1 H), 6.88 (d, 2H), 7.31(d, 2H), 7.73 (d, 1H). IR (KBr): cm^{-1} 3423, 2978, 1718.

N-(t-Butyloxycarbonyl)-L-(4-methoxyphenylglycine)
N-(3-triethoxysilylpropyl) Amide **8**

Compound **8** was prepared as an oily material by treating compound **7** (1.2 g, 4.3 mmole) with 3-aminopropyltriethoxysilane (1.1 mL, 4.7 mmole) in the presence of EEDQ (2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline, 1.2 g, 4.7 mmole) as described in the preparation of compound **6**. The enantiomeric purity of **8** was more than 98% ee by HPLC analysis on a CSP derived from (S)-(3,5-dinitrobenzoyl)leucine (14). ^{1}H NMR ($CDCl_3$): δ 0.44–0.53 (m, 2H), 1.19 (t, 9H), 1.39 (s, 9 H), 1.46–1.58 (m, 2H), 3.17–3.27 (m, 2H), 3.74 (q, 6H), 3.76 (s, 3H), 4.99(broad s, 1H), 5.79 (broad s, 1H), 5.90 (broad s, 1H), 6.85(d, 2H), 7.27 (d, 2H). IR (KBr): cm^{-1} 3328, 1654.

Preparation of CSP **3** and Column Packing

CSP **3** was prepared by treating compound **8** (1.2 g, 2.5 mmole) with Rexchrom silica gel (5 μm, 4.5 g) as described in the preparation of CSP **2**. Elemental analysis of CSP **3** (Found: C, 5.51%; H, 0.90%; N, 0.85%) showed a loading of 0.30 mmoles of selector (based on N) or 0.33 mmoles of selector (based on C) per gram of stationary phase. CSP **3** was slurried in methanol and packed into a 250 mm × 4.6 mm I.D. stainless steel HPLC column using a conventional slurry packing method with an Alltech slurry packer.

RESULTS AND DISCUSSION

CSP **2** and CSP **3** were prepared by the procedure shown in Figure 2. Both CSPs contain a π-basic aromatic functional group as a π–π interaction site. However, the electron density of the 4-methoxyphenyl group of CSP **3** is greater than that of the phenyl group of CSP **2**. Consequently, the two CSPs expected to show different enantioselectivity in resolving racemic ionic compounds containing an π-acidic group.

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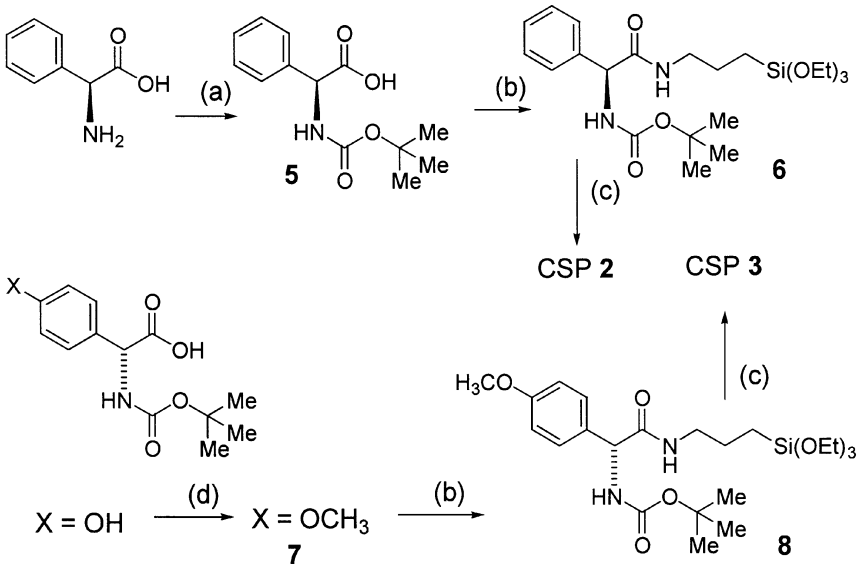

Figure 2. Scheme for preparation of CSPs **2** and **3**. (a) Di-tert-butyldicarbonate, triethylamine, dioxane/water. (b) 3-Aminopropyltriethoxysilane, EEDQ, methylene chloride. (c) (1) Silica gel (5 μm), toluene, Dean-Stark trap. (2) Trifluoroacetic acid, methylene chloride, room temperature. (d) CH_3I, NaH, DMF, 0–10°C.

CSP **2** and CSP **3** were applied in the resolution racemic N-(3,5-dinitrobenzoyl)-α-amino acids **4**. First of all, in order to find out the condition of mobile phase, we tried to resolve N-(3,5-dinitrobenzoyl)valine and N-(3,5-dinitrobenzoyl)phenylalanine on CSP **2** under various mobile phase conditions. Some of the mobile phases tried are summarized in Table 1. As shown in Table 1, all mobile phases tried contained a small quantity of acetic acid and triethylamine. Without acetic acid and triethylamine in the mobile phase, the separation of the two enantiomers was not absorbed. When water was contained in the mobile phase, the enantioselectivity for the two enantiomers denoted by the separation factor (α) and the resolution denoted by the resolution factor (R_S) were very low, as shown in Table 1 (entry a and b).

Use of isopropyl alcohol in hexane containing a small quantity of acetic acid and triethylamine as a mobile phase, was effective in the baseline resolution of N-(3,5-dinitrobenzoyl)valine and N-(3,5-dinitrobenzoyl)phenylalanine on CSP **2** (entry c in Table 1). However, the retention of the two enantiomers was quite long. Decreasing the retention times of the two enantiomers by increasing the content of isopropyl alcohol in the mobile phase was not successful, because high

Table 1. Resolution of N-(3,5-Dinitrobenzoyl)valine (A) and N-(3,5-Dinitrobenzoyl)-phenylalanine (B) on CSP 2 with Various Mobile Phases[a]

		Analyte A			Analyte B		
Entry	Mobile Phase	k_1,[b]	α[c]	R_S[d]	k_1,[b]	α[c]	R_S[d]
a	80% CH_3OH in H_2O + Additive[e]	8.71	1.00	0.51	9.44	1.00	
b	80% CH_3CN in H_2O + Additive[e]	2.53	1.04	0.52	2.54	1.00	
c	40% Isopropyl alcohol in Hexane + Additive[e]	22.64	1.16	1.07	33.64	1.15	1.23
d	40% Isopropyl alcohol + 20% CH_2Cl_2 in Hexane + Additive[e]	6.35	1.15	0.95	9.00	1.14	0.78
e	100% CH_2Cl_2 + Additive[e]	4.29	1.19	2.18	3.68	1.19	1.86

[a] In every case, the (R)-enantiomer was eluted second. Flow rate: 0.8 mL/min, Detection: 254 nm UV, Temperature: 20°C.
[b] Retention factor of the first eluted enantiomer.
[c] Separation factor.
[d] Resolution factor.
[e] 0.1% acetic acid + 0.1% triethylamine.

back pressure was experienced. Instead, addition of methylene chloride to the mobile phase of isopropyl alcohol in hexane containing a small quantity of acetic acid and triethylamine decreased the retention times appreciably (entry d in Table 1). However, baseline separation of the two enantiomers was lost. Finally, we found that methylene chloride containing a small quantity of acetic acid and triethylamine was quite good as a mobile phase for the resolution of N-(3,5-dinitrobenzoyl)valine and N-(3,5-dinitrobenzoyl) phenylalanine on CSP **2** in terms of retention time, separation factor (α), and resolution factor (R_S) (entry e in Table 1). Consequently, we utilized methylene chloride containing a small quantity of acetic acid and triethylamine as a mobile phase in the subsequent experiments.

In resolving N-(3,5-dinitrobenzoyl)-α-amino acids **4** on CSP **2** or CSP **3**, the ion-pairing or the electrostatic interaction between the ammonium ion of the CSP and the carboxylate ion of analytes is assumed to be an important factor for the successful resolution. The ion-pairing or the electrostatic interaction is expected to be dependent on the pH of the mobile phase, because the formation of the ammonium ion of the CSP and the carboxylate ion of analytes is dependent on the pH of the mobile phase. Consequently, the resolution of N-(3,5-

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dinitrobenzoyl)-α-amino acids **4** on CSP **2** and CSP **3** is expected to be dependent on the pH of the mobile phase.

In order to elucidate the dependence of the chromatographic behaviors for the resolution of N-(3,5-dinitrobenzoyl)-α-amino acids **4** on CSP **2** and CSP **3** on the pH of the mobile phase, we resolved two representative analytes, such as N-(3,5-dinitrobenzoyl)valine and N-(3,5-dinitrobenzoyl)phenylalanine on CSP **2** and CSP **3**, with the variation of the content of acetic acid and triethylamine in methylene chloride as the mobile phase. The chromatographic resolution behaviors with the variation of the mobile phase pH are illustrated in Figures 3, 4, and 5. The pH values shown in Figures 3, 4, and 5 were adjusted by changing the content of acetic acid or triethylamine in methylene chloride and measured simply with a pH meter. As shown in Figure 3, the retention factors for the resolution of analytes on CSP **2** and CSP **3** seem to increase up to pH 5.8. However, the separation factors, α, and resolution factors, R_S, generally show maximum value around pH 6.5, as shown in Figure 4 and Figure 5. From these results, the optimum pH of the mobile phase was concluded to be 6.5, which corresponds to 0.2% acetic acid and 0.1% triethylamine in methylene chloride. Methylene chloride containing 0.2% of acetic acid and 0.1% of triethylamine, seems to be acidic enough to protonate the free amino group of the CSP to afford an ammonium cation, and basic enough to deprotonate the free carboxylic acid group of analytes to afford a carboxylate anion.

Using methylene chloride containing 0.2% of acetic acid and 0.1% of triethylamine as a mobile phase, we resolved various N-(3,5-dinitrobenzoyl)-α-amino acids **4** on CSP **2** and CSP **3** and the chromatographic resolution results are summarized in Table 2. The representative chromatograms are shown in Figure 6. As shown in Table 2 and Figure 6, various N-(3,5-dinitrobenzoyl)-α-amino acids **4** are resolved with reasonable separation factors on CSP **2** and CSP **3**. The separation factors and the resolution factors are greater on CSP **3** than on CSP **2**, except for the resolution of the derivatives of threonine (entry f) and serine (entry g). However, the retention factors are always greater on CSP **3** than on CSP **2**, with no exception. One interesting observation to note, is that the retention of N-(3,5-dinitrobenzoyl)threonine and N-(3,5-dinitrobenzoyl)serine is considerably greater than other analytes and this is more significant on CSP **3** than on CSP **2**.

The long retention of N-(3,5-dinitrobenzoyl)threonine and N-(3,5-dinitrobenzoyl)serine on the CSPs perhaps, stems from the additional hydroxy group of analytes. In the presence of an additional hydroxy group of analytes, the non-stereoselective interaction between the CSP and analytes improves and consequently, the retention of analytes increases. The retention increased by the non-stereoselective interaction, in general, diminishes the enantioselectivity. In this instance, the diminished enantioselectivity on CSP **3** compared to that on CSP **2** for the resolution of N-(3,5-dinitrobenzoyl)threonine and N-(3,5-

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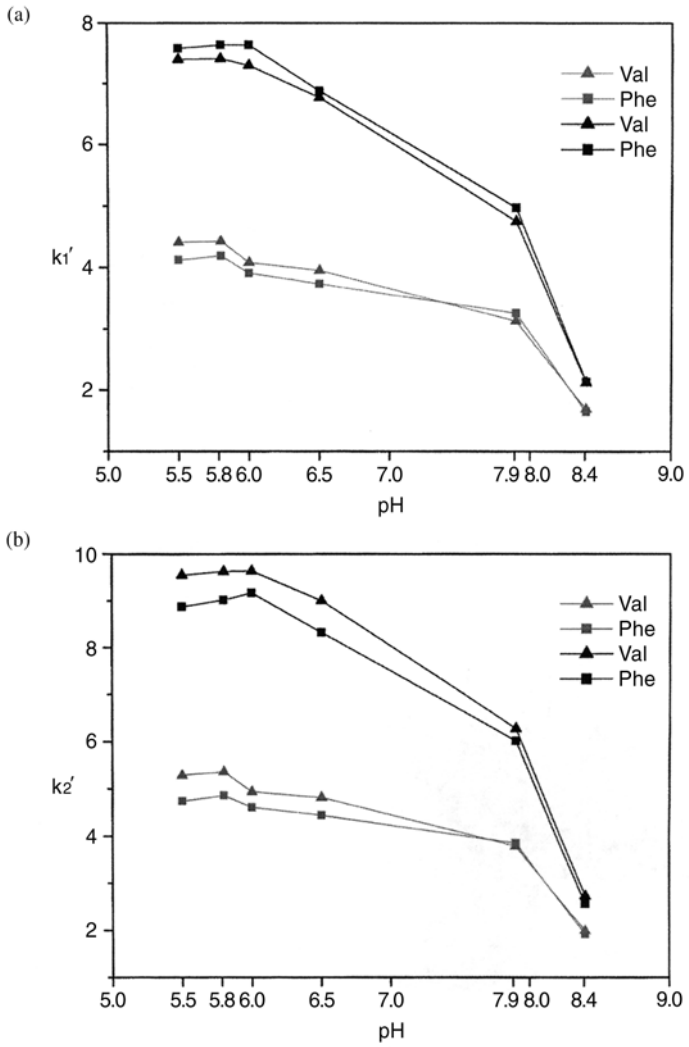

Figure 3. Trends of the retention factors (k_1' and k_2') for the resolution of N-(3,5-dinitrobenzoyl)valine (Val) and N-(3,5-dinitrobenzoyl)phenylalanine (Phe) on CSP **2** (represented with gray lines) and on CSP **3** (represented with black lines) with the variation of pH of mobile phase (methylene chloride). Mobile phase: methylene chloride with the following content of acetic acid and triethylamine: pH (8.4) = 0.1% acetic acid + 0.2% triethylamine, pH (7.9) = 0.1% acetic acid + 0.1% triethylamine, pH (6.5) = 0.2% acetic acid + 0.1% triethylamine, pH (6.0) = 0.3% acetic acid + 0.1% triethylamine, pH (5.8) = 0.4% acetic acid + 0.1% triethylamine and pH (5.5) = 0.5% acetic acid + 0.1% triethylamine. Flow rate: 1.2 mL/min. Detection: 254 nm UV. Temperature: 20°C.

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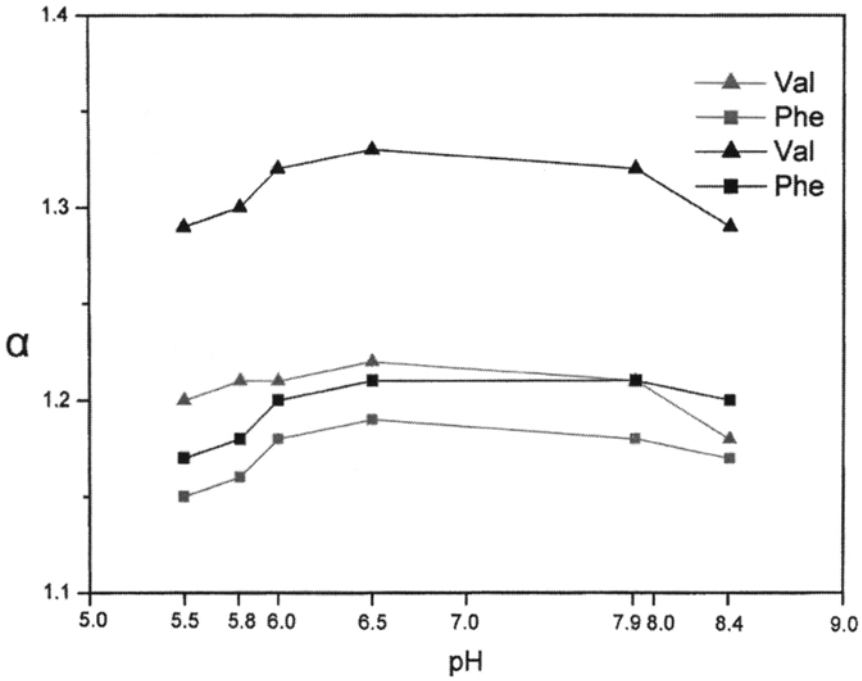

Figure 4. Trends of the separation factors (α) for the resolution of N-(3,5-dinitrobenzoyl)valine (Val) and N-(3,5-dinitrobenzoyl)phenylalanine (Phe) on CSP **2** (represented with gray lines) and on CSP **3** (represented with black lines) with the variation of pH of mobile phase (methylene chloride). See Figure 3 legend for the chromatographic conditions.

dinitrobenzoyl)serine might be rationalized as stemming from the more significant non-stereoselective interaction.

CSP **3**, which contains an additional methoxy group at the para position of the phenyl group of CSP **2**, seems to show two additional effects on the resolution of analytes. First, the oxygen of the 4-methoxy group at the phenyl ring of CSP **3** is expected to play a hydrogen-bonding acceptor site. In this instance, the non-stereoselective interaction between the CSP and analytes might improve and, consequently, the enantioselectivity diminishes while the retention increases. Second, the 4-methoxy group at the phenyl ring of CSP **3** improves the π-electron density of the phenyl ring and enhances the stereoselective π–π interaction between the CSP and analytes. In this instance, the enantioselectivity is expected to improve and the retention is also expected to improve. By combining the two

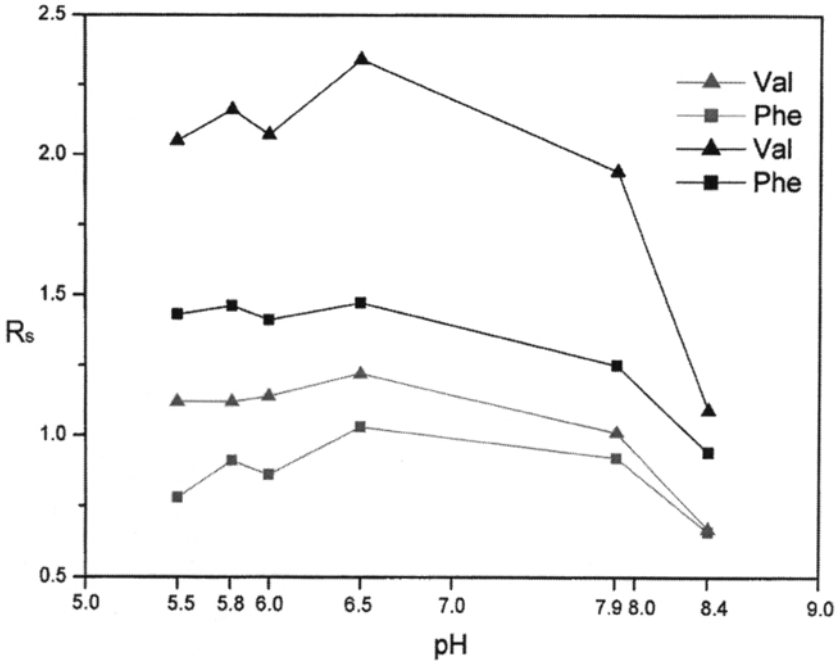

Figure 5. Trends of the resolution factors (R_S) for the resolution of N-(3,5-dinitrobenzoyl)valine (Val) and N-(3,5-dinitrobenzoyl)phenylalanine (Phe) on CSP **2** (represented with gray lines) and on CSP **3** (represented with black lines) with the variation of pH of mobile phase (methylene chloride). See Figure 3 legend for the chromatographic conditions.

effects of the **4**-methoxy group of the phenyl ring of CSP **3**, the retention of analytes is expected to improve significantly.

This expectation comes to be true as shown in Table 2 and Figure 3. However, the enantioselectivity seems to be determined by the valance of the two effects. In the resolution of N-(3,5-dinitrobenzoyl) derivatives of simple α-amino acids, the second effect related to the stereoselective π–π interaction between the CSP and analytes seems to be dominant and, consequently, the enantioselectivity improves on CSP **3**. On the contrary, in the resolution of N-(3,5-dinitrobenzoyl)-threonine and N-(3,5-dinitrobenzoyl)serine, which contain an additional hydroxy group, the first effect related to the non-stereoselective interaction between the CSP and analytes seems to be dominant, and consequently, the enantioselectivity diminishes on CSP **3**.

To obtain additional evidences for the stereoselective π–π interaction between the CSP and analytes, as an example, we prepared N-(3-nitrobenzoyl)-

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Table 2. Resolution of N-(3,5-Dinitrobenzoyl)α-amino Acids 4 on CSP 2 and CSP 3[a]

		CSP 2			CSP 3		
Entry	Analyte 4 (R)	k_1,[b]	α[c]	R_S[d]	k_1,[b]	α[c]	R_S[d]
a	CH_3 (alanine)	9.25	1.20	1.20	15.32	1.27	1.75
b	$(CH_3)_2CH$ (valine)	3.95	1.22	1.22	6.77	1.33	2.34
c	$(CS_3)_2CHCH_2$ (leucine)	4.29	1.23	1.27	8.61	1.25	1.79
d	C_6H_5 (phenylglycine)	3.34	1.14	1.04	5.68	1.23	1.87
e	$C_6H_5CH_2$ (phenylalanine)	3.73	1.19	1.03	6.88	1.21	1.47
f	$CH_3(OH)CH$ (threonine)	17.78	1.17	0.98	39.13	1.12	0.83
g	$HOCH_2$ (serine)	33.32	1.10	0.52	69.19	1.09	0.77

[a] Absolute configuration of the second eluted enantiomer for the resolution of N-(3,5-dinitrobenzoyl)valine and N-(3,5-dinitrobenzoyl)leucine was (R) on CSP **2** and (S) on CSP **3**. Mobile phase: methylene chloride + 0.2% acetic acid + 0.1% triethylamine, Flow rate: 1.2 mL/min, Detection: 254 nm UV, Temperature: 20°C.
[b] Retention factor of the first eluted enantiomer.
[c] Separation factor.
[d] Resolution factor.

valine and N-benzoylvaline and resolved them on CSP **3**. Comparison of the chromatograms for the resolution of N-(3,5-dinitrobenzoyl)valine, N-(3-nitrobenzoyl)valine, and N-benzoylvaline on CSP **3**, demonstrated that N-(3,5-dinitrobenzoyl)valine, which contains most π-acidic aromatic derivatizing groups, was resolved best ($\alpha = 1.33$, $R_S = 2.34$) and N-(3-nitrobenzoyl)valine was resolved better ($\alpha = 1.14$, $R_S = 1.13$) than N-benzoylvaline ($\alpha = 1.06$, $R_S = 0.55$). From these results, it is concluded that the π–π interaction between the CSP and analytes is the important factor in the chiral recognition.

The importance of the ion-pairing or the ionic interaction between the CSP and analytes in the chiral recognition was also investigated by resolving N-(3,5-dinitrobenzoyl)leucine methyl ester on CSP **2** or CSP **3**. The enantiomers of N-(3,5-dinitrobenzoyl)leucine methyl ester were not resolved at all on CSP **2** or CSP **3**. This result demonstrated that the free carboxylic acid group is absolutely necessary for the chiral recognition, indicating the importance of the ion pairing or the electrostatic interaction in the chiral recognition.

All of the chromatographic resolution behaviors described above, are consistent with the chiral recognition mechanism proposed previously, based on the relaxed scan calculation combined with a Monte Carlo conformation search in solution-phase (12). The calculated global minimum structure of the complex between (S)-phenylglycine (the chiral selector of CSP **2**, S-Host) and (R)-N-(3,5-dinitrobenzoyl)leucine (R-Guest), the free energy of which was 0.6 ± 0.4 kJ/mol

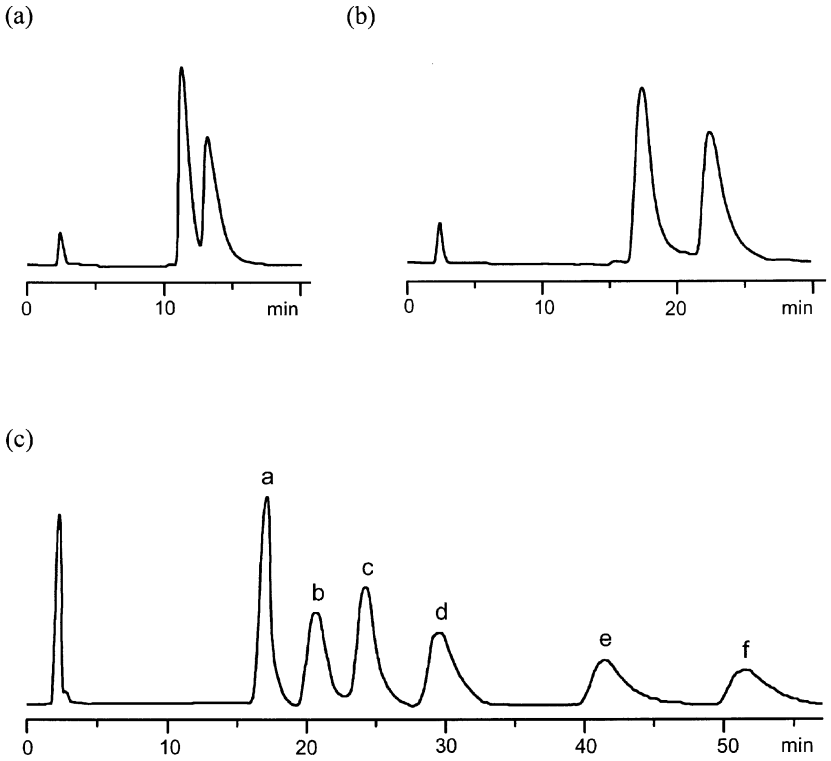

Figure 6. Representative chromatograms for the resolution of N-(3,5-dinitrobenzoyl)-valine (a) on CSP **2** and (b) on CSP **3**. Chromatogram (c) shows the simultaneous resolution of N-(3,5-dinitrobenzoyl)phenylglycine (a,b), N-(3,5-dinitrobenzoyl)valine (c,d) and N-(3,5-dinitrobenzoyl)alanine (e,f) on CSP **3**. Mobile phase: methylene chloride + 0.2% acetic acid + 0.1% triethylamine. Flow rate: 1.2 mL/min. for chromatograms (a) and (b), 0.8 mL/min for chromatogram (c). Detection: 254 nm UV. Temperature: 20°C.

lower than that of the complex between (S)-phenylglycine and (S)-N-(3,5-dinitrobenzoyl)leucine, clearly demonstrated the importance of the π–π interaction between the phenyl group of S-Host and the N-(3,5-dinitrobenzoyl) group of R-Guest, and the importance of the electrostatic interaction between the ammonium group of S-Host and the carboxylate group of R-Guest. In this instance, the chromatographic resolution results we have shown in this study might be additional evidences supporting the calculated chiral recognition mechanism.

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In summary, in this study, we demonstrated that CSP **2** and CSP **3**, which contain a free amino group, can be successfully applied in resolving N-(3,5-dinitrobenzoyl)-α-amino acids **4**, especially with the mobile phase of methylene chloride containing 0.2% acetic acid and 0.1% triethylamine. Between the two CSPs **2** and **3**, the latter was found more effective than the former in resolving N-(3,5-dinitrobenzoyl)-α-amino acids **4**, indicating the importance of the π-basicity of the aromatic group of the CSP. In addition, the π-acidity of N-(3,5-dinitrobenzoyl) group of analytes was also found to be important for the effective chiral recognition. From these results, we concluded that the π–π interaction between the CSP and analytes is the important factor for the chiral recognition.

On the contrary, N-(3,5-dinitrobenzoyl)leucine methyl ester was not resolved at all on CSP **2** or CSP **3**. Consequently, the free carboxylic acid group of analytes was expected to be absolutely necessary for the chiral recognition, indicating the importance of the ion-pairing or the electrostatic interaction in the chiral recognition. Finally, the chromatographic results obtained in this study for the resolution of N-(3,5-dinitrobenzoyl)-α-amino acids on CSP **2** and **3**, were consistent with the chiral recognition mechanism proposed previously, based on the relaxed scan calculation combined with a Monte Carlo conformation search in solution-phase.

ACKNOWLEDGMENT

This work has been supported by KISTEP (NRL Program : M1-0104-00-0005).

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Received September 13, 2001
Accepted September 30, 2001
Manuscript 5652

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