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N-Alkylcarbamoyl derivatives of amino acids as chiral stationary phases for high-performance liquid chromatography I. An example of enhancing enantioselectivity by deleting the non-enantioselective $\pi-\pi$ interaction site on the chiral stationary phase

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Abstract

The role of the $\pi-\pi$ interaction provided by the benzyl group of N-benzylcarbamoyl-derived chiral stationary phases (CSPs) was studied. Based on deleting the non-enantioselective $\pi-\pi$ interaction site on an N-benzylcarbamoyl-(*S*)-phenylglycine-derived CSP, a series of seven CSPs were prepared by bonding N-alkylcarbamoyl derivatives of (*S*)-phenylglycine or (*S*)-phenylalanine either ionically or covalently on 3-aminopropyltriethoxysilane-modified silica gel. The chromatographic behaviour on the CSPs with regard to the resolution of dinitrobenzamide or dinitrobenzanilide derivatives of enantiomeric amino acids, amino alcohols, amines and carboxylic acid was studied. The best chromatographic results were found on ionic-type CSPs containing N-*n*-stearylcarbamoyl-(*S*)-phenylglycine or N-cyclohexylcarbamoyl-(*S*)-phenylglycine. It was also found that the chiral recognition abilities of all of the four ionic-type N-alkylcarbamoyl-(*S*)-phenylglycine CSPs were better than that of the ionic-type CSP containing N-benzylcarbamoyl-(*S*)-phenylglycine for the discrimination of the same enantiomeric analytes. The results clearly confirm that enhancement of enantioselectivity is achieved by deleting the non-enantioselective $\pi-\pi$ interaction site on the N-benzylcarbamoyl-(*S*)-phenylglycine-derived CSP.

1. Introduction

The study of the chiral recognition mechanism is important in that it not only affords insight into which chiral stationary phase (CSP) should be used for the effective resolution of a given analyte but also aids in the design of CSPs with enhanced enantioselectivity. Among the hundreds of chiral stationary phases, the chiral recognition mechanisms on π donor–acceptor phases have been studied intensively and reviewed [1]. It was proposed that the separation of enantiomers on these CSPs is dependent on the formation of diastereomeric complexes with different free energies between the CSP and the enantiomeric analyte. The formation of these complexes requires enantioselective interactions (chiral interactions), which are formed by an

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appropriate combination of suitably located functionalities on the CSP with complementary sites on the analyte. The enantioselective interactions are essential in recognizing the enantiomers, while non-enantioselective interactions are the interactions in excess of those required for chiral recognition. Däppen *et al.* [2] demonstrated that the retention of an analyte on a CSP can be described as the sum of non-chiral and chiral interactions. The enantioselective interactions are important in distinguishing the enantiomeric analyte and can affect the elution order of enantiomers.

However, the non-enantioselective interactions may also influence the magnitude of enantioselectivity of the chiral resolution by increasing retention. For example, the residual silanol groups on the underlying silica support often influence the enantioselectivity of the chiral resolution by the non-chiral interaction [3–6]. The chromatographic results on a tandem array of chiral and achiral columns also showed that a decrease in the magnitude of the enantioselectivity was observed owing to adsorption stemming from sites on the achiral packing [7]. Thus, the concept suggested by Pirkle and Welch [8] that the design of a CSP with enhanced enantioselectivity may be achieved by deleting the nonproductive interaction sites on the original CSPs is reasonable.

Owing to the conformational rigidity and polar nature of the urea functionality, many CSPs contain a urea linkage as their connection arm to silica gel [9–14]. In a previous study, the chiral recognition behaviour for the resolution of dinitrobenzamide or dinitroanilide derivatives of enantiomeric amino acids, amino alcohols, amines and carboxylic acid on N-arylcarbamoyl-derived CSPs containing either one or two chiral centres was studied [9]. Various magnitudes of enantioselectivities were observed, and in some instances CSPs containing two chiral centres provided better resolution than a commerically available CSP [Supelcosil LC-(*R*)-urea]. The chiral resolution on the N-benzylcarbamoyl-(*S*)-phenylglycine CSP (CSPA; Fig. 1) was found to be the best among the series of N-arylcarbamoyl-derived CSPs containing one chiral centre. It showed that the phenylglycine moiety of CSPA provides the enantioselective $\pi-\pi$ interaction that is important in chiral resolution and dominates the elution order of enantiomeric analytes. However, the role of the $\pi-\pi$ interaction provided by the N-benzylcarbamoyl moiety has not been reported.

This study was focused on the influence of the $\pi-\pi$ interaction provided by the benzyl group of CSPA (Fig. 1). A series of seven CSPs containing N-alkylcarbamoyl derivatives of (*S*)-phenylglycine and (*S*)-phenylalanine were prepared. The chromatographic results of this study show that the benzyl group of CSPA provides a non-enantioselective $\pi-\pi$ interaction and that the CSPs designed by deleting the non-enantioselective $\pi-\pi$ interaction site have a more effective recognition ability than the original CSPA.

2. Experimental

2.1. Chemicals

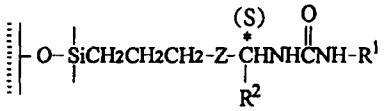
The silica gel (Nucleosil; pore size 100 Å, particle size 10 μm, surface area 350 m^2/g), was obtained from Macherey–Nagel. 3-Aminopropyltriethoxysilane (APS) was purchased from Chisso. The analytes used in the chromatographic experiments were of synthetic reagent grade (Merck). The N-alkylcarbamoyl derivatives of amino acids were obtained by the method described previously [9].

2.2. Preparation of APS-modified silica gel

The procedure for the preparation of APS-modified silica gel was the same as reported previously [9]. The results of elemental analysis are given in Table 1.

2.3. Preparation of ionic-type CSPs

To a solution of 0.01 mol of an N-alkylcarbamoyl derivative of an L-amino acid in 100 ml of tetrahydrofuran (THF), 2.5 g of APS-modified silica gel were added. The mixture was



CSP	-R¹	-R²	-Z-
CSPA	-CH_2-◎	-◎	-NH_3^+ $^-$OOC-
CSPB	-◎	-$CH(CH_3)_2$	-NH_3^+ $^-$OOC-
CSP1	-CH_2-◎	-$CH(CH_3)_2$	-NH_3^+ $^-$OOC-
CSP2	-⬡	-CH_2-◎	-NH_3^+ $^-$OOC-
CSP3	-⬡	-◎	-NH_3^+ $^-$OOC-
CSP4	-$CH_2CH_2CH_3$	-◎	-NH_3^+ $^-$OOC-
CSP5	-$CH(CH_3)_2$	-◎	-NH_3^+ $^-$OOC-
CSP6	-$CH_2(CH_2)_{16}CH_3$	-◎	-NH_3^+ $^-$OOC-
CSP7	-$CH_2CH_2CH_3$	-◎	-NHCO-
CSP8	-$CH(CH_3)_2$	-◎	-NHCO-

Fig. 1. Structures of chiral stationary phases.

subjected to ultrasonic vibration for 10 min followed by stirring for 4 h. The product was filtered, washed with THF and dried under vacuum overnight.

2.4. Preparation of covalent-type CSPs

A solution of 0.01 mol of an N-alkylcarbamoyl derivative of an L-amino acid in 100 ml of

Table 1
Characteristics of the 3-aminopropylsilica and the CSPs

Sample	Elemental analysis (%)			Loading capacity[a]	
	C	H	N	mmol/g	groups/nm²
3-Aminopropylsilica	3.71	1.06	1.27	0.91	1.59
CSPA[b]	11.29	1.82	2.35	0.39	0.67
CSPB[b]	11.12	1.80	2.58	0.47	0.81
CSP1	11.67	1.92	2.41	0.41	0.71
CSP2	12.25	1.62	2.33	0.38	0.65
CSP3	11.63	1.57	2.31	0.37	0.64
CSP4	10.68	1.77	2.36	0.39	0.67
CSP5	10.65	1.37	2.34	0.38	0.65
CSP6	19.26	1.99	2.28	0.36	0.62
CSP7	10.60	1.52	2.38	0.40	0.69
CSP8	10.64	1.27	2.34	0.38	0.65

[a] Based on the percentage of nitrogen.
[b] The results have been published in a previous paper [9].

dimethylformamide was cooled in an ice-bath. Next, 0.01 mol of N-hydroxysuccinimide and 0.01 mol of dicyclohexylcarbdiimide (DCC) were added to the above solution at 0°C and the mixture was stirred at room temperature for 24 h. After removal of the suspended solid dicyclohexylurea, 2.5 g of APS-modified silica gel were added and stirred for 48 h. After filtration, the product was suspended in 100 ml of toluene and end-capped by reaction with trimethylchlorosilane at 40°C for 4 h. The final product was filtered and washed with methanol, water and acetone and then dried under vacuum overnight.

The results of elemental analyses of all the CSPs prepared are given in Table 1.

2.5. Chromatographic studies

The chromatographic studies were carried out with a liquid chromatographic system consisting of an Alphatech solvent-delivery system and an Applied Biosystems Model 757 variable-wavelength UV detector. The recorder used was a Model 21 SIC Chromatocorder. Stainless-steel columns (300 mm × 4 mm I.D.) were packed by the balanced-density slurry method using an Econo-packing pump (Inpac International) at 400 kg/cm^2. Mixtures of *n*-hexane and 2-propanol (90:10–99:1, v/v) were used as the mobile phase, which was filtered through a 0.45-μm membrane filter and degassed by ultrasonic vibration prior to use. The flow-rate was 1.0 ml/min. The detector was operated at 254 nm. Experiments were carried out at 25°C. Analytes were dissolved in methanol and suitable amounts of the solutions were injected. Chromatographic peaks were assigned by injecting the corresponding derivative of enantiomeric enriched analyte.

3. Results and discussion

Fig. 1. shows the structures of the ten chiral stationary phases (CSPs) studied. The preparation and enantioselectivities of CSPA and CSPB have been reported previously [9]. Seven N-alkylcarbamoyl-derived CSPs and CSP1 were successfully prepared by bonding chiral entities either ionically or covalently to APS-modified silica gel, as shown in Fig. 2. In order to compare the enantioselectivities of the N-alkylcarbamoyl-derived CSPs with that of CSPA, the preparation and the packing of all these CSPs were carried out under identical conditions. The support of CSPA was 10-μm silica gel, so the same silica gel was used as the support of the other CSPs in this study, even though the small particle size of silica gel often increases the efficiency of chiral resolution. According to the results of elemental analyses (Table 1), the surface coverages of chiral ligands on these CSPs were found to range from 0.36 to 0.47 mmol/g (0.62–0.81 groups/nm^2).

The chiral recognition abilities of the amide derivatives of amino acids, amino alcohols, amines and carboxylic acid on these CSPs were examined, and the results are summarized in Tables 2 and 3. Typical chromatograms are shown in Figs. 3 and 4. According to the separation factors (α), nearly all of the seven N-alkylcarbamoyl-derived CSPs, except CSP2, provide sufficient resolution for the analytes chosen in this study. In general, the ionic-type CSPs were found to be more effective than the corresponding covalent-type CSPs for the separation of the enantiomeric analytes. The better results observed on the ionic-type CSP may be ascribed to the stronger chiral interaction provided by the ionic bond of the ionic-type CSP.

3.1. Non-enantioselective nature of the $\pi-\pi$ interaction provided by the N-benzylcarbamoyl moiety of CSPs

Although CSPA, CSPB and CSP1 contain a urea linkage, CSPA is derived from (*S*)-phenylglycine, whereas CSPB and CSP1 are derived from (*S*)-valine (Fig. 1). Further, CSPA and CSP1 contain an N-benzylcarbamoyl moiety, whereas CSPB contains an N-phenylcarbamoyl moiety. Hence two phenyl groups are present in CSPA and only one in CSPB and CSP1. Different chiral resolutions were observed on these CSPs (Table 2). CSPB provides chiral recogni-

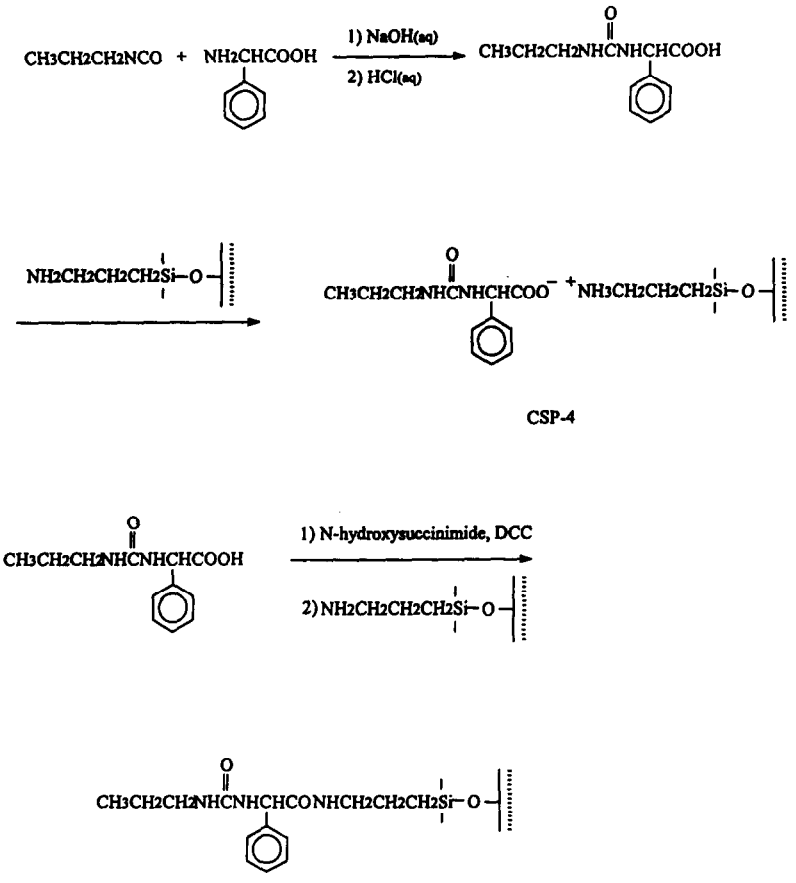


Fig. 2. Examples of the preparation of N-alkylcarbamoyl-derived chiral stationary phases.

tion ability for the separation of the DNB derivatives of amino acids, amino alcohols and diamine enantiomers. CSPA, having a better resolution ability than CSPB, could resolve all of the DNB derivatives of the chosen enantiomers. As expected, no significant chiral resolution was observed on CSP1. It is worth noting that opposite elution orders were observed on CSPA and CSPB. The *R*-enantiomer was first eluted on CSPB, whereas the *S*-enantiomer was eluted first on CSPA. The opposite elution order suggests that the $\pi-\pi$ interactions provided by the phenylcarbamoyl and phenylglycine dominate two different chiral recognition models. The results observed on CSP1 infer that the N-benzylcarbamoyl moiety provides only a non-enantioselective $\pi-\pi$ interaction.

3.2. Chromatographic behaviour of the N-alkylcarbamoyl-derived CSPs

Two amino acids, (*S*)-phenylglycine and (*S*)-phenylalanine, were used as chiral entities of the N-alkylcarbamoyl-derived CSPs in this study. As shown in Fig. 1, the structures of CSP2 and CSP3 are identical except that CSP3 contains (*S*)-phenylglycine and CSP2 contains (*S*)-phenylalanine. In a previous study [9], the benzyl group of phenylalanine was characterized as a non-enantioselective $\pi-\pi$ interaction site and the phenyl group of phenylglycine as an enantioselective $\pi-\pi$ interaction site. Comparing the chromatographic results on CSP2 with those on CSP3 for the separation of a series of twelve enantiomeric analytes under identical

Table 2
Resolution of derivatized enantiomers on CSPA, CAPB and CSP1

Analyte	CSPA			CSPB			CSP1		
	k'_1	α	M	k'_1	α	M	k'_1	α	M
Amino acids[a]									
Valine	3.23(*S*)	1.67	A	1.92(*R*)	1.18	A	2.49	1	A
Alanine	6.91(*S*)	1.18	A	3.50(*R*)	1.26	A	4.78	1	A
Leucine	3.60(*S*)	1.58	A	1.81(*R*)	1.26	A	2.92	1	A
Methionine	5.87(*S*)	1.30	A	6.54(*R*)	1.31	A	8.61	1	A
Phenylalanine	7.03(*S*)	1.39	A	3.07(*R*)	1.18	A	3.71	1	A
Amino alcohols[b]									
2-Aminobutanol	7.85(*S*)	1.41	A	4.30(*R*)	1.17	A	5.03	1	A
2-Aminopropanol	11.74(*S*)	1.24	A	7.26(*R*)	1.11	A	8.17	1	A
Norephedrine	9.63	1.02	A	5.12	1.12	A	6.04	1	A
Amines[b]									
Phenylethylamine	8.60(*R*)	1.09	A	4.35	1	C	5.52	1	C
1-Methylbutylamine	4.46	1.18	A	2.68	1	C	3.48	1	C
1,2-Diaminopropane	42.41	1.09	A	12.96	1.19	A	13.11	1	A
Carboxylic acid[c]									
Ibuprofen	6.82(*S*)	1.16	A	13.02	1	B	14.23	1	B

k'_1 is the capacity factor of the first-eluted enantiomer; k'_1 (*R*/*S*) indicates the absolute configuration of the first-eluted enantiomer. The separation factor (α) is the ratio of the capacity factors of the enantiomers. Mobile phase (M): A-*n*-hexane–2-propanol (90:10); B-*n*-hexane–2-propanol (97:3); C-*n*-hexane–2-propanol (99:1). Chromatographic results for CSPA and CSPB are from Ref. [9].

[a] As N-dinitrobenzamide-O-methyl ester derivatives.

[b] As N-dinitrobenzamide derivatives.

[c] As dinitroanilide derivative.

chromatographic conditions, it was found that there was no significant resolution on CSP2 for any chosen analytes, whereas good resolution was observed on CSP3. This illustrates that the enantioselective $\pi-\pi$ interaction provided by phenylglycine is necessary for enantiomer separation in the N-alkylcarbamoyl-derived CSPs.

Four different N-alkylcarbamoyl moieties, N-*n*-propyl-, N-isopropyl-, N-*n*-stearyl-, and N-cyclohexylcarbamoyl, were used as the terminus of the brush of CSPs. In order to investigate the influence of the N-alkylcarbamoyl moiety of CSPs on the chiral recognition, chromatographic results for the resolution of twelve enantiomeric analytes on these CSPs (CSP3–8) were compared (Table 3). In most instances, the enantioselectivities of these CSPs decrease with the order of the N-alkyl terminal groups N-*n*-stearyl, N-cyclohexyl > N-isopropyl > N-*n*-propyl, according to the separation factors of the chosen enantiomeric analytes. It is also worth noting that the retention of a given analyte on the ionic-type CSP6 is shorter than those on any other ionic-type CSPs (CSP3–5) according to the capacity factors of the more retained enantiomers. The shorter retention and higher separation factors on CSP6 can be illustrated by the non-polar character of the *n*-stearyl group in a normal-phase separation.

3.3. *Influence of the non-enantioselective $\pi-\pi$ interaction*

As shown in Fig. 1, both CSPA and CSP3-6 contain phenylglycine as a chiral entity. However, CSPA bears an additional benzyl group

Table 3
Resolution of derivatized enantiomers on CSP2–8

Analyte	CSP2		CSP3		CSP4		CSP5		CSP6		CSP7		CSP8	
	k_1'	α	k_1'	α	k_1'	α	k_1'	α	k_1'	α	k_1'	α	k_1'	α
Amino acids[a]														
Valine	2.79	1	2.14(*S*)	1.72	2.08(*S*)	1.69	2.02(*S*)	1.71	1.58(*S*)	1.77	2.88(*S*)	1.16	3.34(*S*)	1.29
Alanine	4.42	1	4.30(*S*)	1.18	4.31(*S*)	1.19	4.27(*S*)	1.19	3.67(*S*)	1.20	5.35(*S*)	1.06	5.19(*S*)	1.14
Leucine	2.58	1	2.37(*S*)	1.90	2.20(*S*)	1.76	2.19(*S*)	1.76	1.66(*S*)	1.93	3.02(*S*)	1.21	3.41(*S*)	1.31
Methionine	5.47	1	5.20(*S*)	1.57	5.39(*S*)	1.47	5.28(*S*)	1.47	4.29(*S*)	1.53	4.98(*S*)	1.13	7.40(*S*)	1.22
Phenylalanine	3.58	1	3.97(*S*)	1.70	3.91(*S*)	1.56	3.90(*S*)	1.57	3.15(*S*)	1.68	5.10(*S*)	1.15	7.14(*S*)	1.25
Amino alcohols[b]														
2-Aminobutanol	5.89	1	6.62(*S*)	1.43	5.81(*S*)	1.42	7.20(*S*)	1.42	5.73(*S*)	1.55	6.46(*S*)	1.10	6.22(*S*)	1.19
2-Aminopropanol	8.82	1	8.68(*S*)	1.26	9.58(*S*)	1.25	10.64(*S*)	1.25	8.38(*S*)	1.33	8.80(*S*)	1.06	9.00(*S*)	1.14
Norephedrine	8.01	1	9.05	1.04	8.59	1.03	8.73	1.05	7.85	1.06	7.99	1	7.32	1
Amines[b]														
Phenylethylamine	6.43	1	7.10	1.24	5.62(*R*)	1.18	5.35(*R*)	1.18	4.56(*R*)	1.25	5.89(*R*)	1.01	6.69	1
1-Methylbutylamine	3.71	1	3.90	1.28	3.24	1.24	3.07	1.24	2.27	1.28	3.19	1.04	3.70	1
1,2-Diaminopropane	28.22	1	26.92	1.11	24.50	1.10	24.34	1.10	23.77	1.22	21.48	1	21.53	1
Carboxylic acid[c]														
Ibuprofen	5.98	1	5.45(*S*)	1.17	5.56(*S*)	1.17	5.55(*S*)	1.17	4.34(*S*)	1.18	4.57	1	4.95	1

k_1' is the capacity factor of the first-eluted enantiomer; k_1' (R/S) indicates the absolute configuration of the first-eluted enantiomer. The separation factor (α) is the ratio of the capacity factors of enantiomers. Mobile phase: 2-propanol–*n*-hexane (10:90).

[a] As N-dinitrobenzamide-O-methyl ester derivatives.

[b] As N-dinitrobenzamide derivatives.

[c] As dinitroanilide derivative.

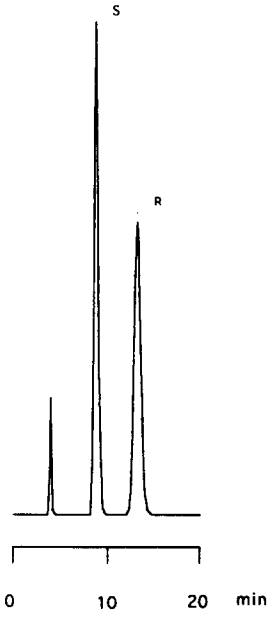


Fig. 3. Chromatogram for the resolution of N-dinitrobenzoyl-D,L-leucine-O-methyl ester on CSP6. Mobile phase, *n*-hexane–2-propanol (90:10); flow-rate, 1 ml/min.

which was characterized as a non-enantioselective $\pi-\pi$ interaction. Comparing the chiral resolution results on CSPA with those on CSP3–6, the capacity factor of a given analyte on CSPA is larger than any of those on CSP3–6 under the same chromatographic conditions. For example, the capacity factor of (*R*)-phenylalanine is 9.77 on CPSA, whereas that on CSP6 is only 5.29. Further, the separation factor of a given racemic analyte on CSPA was smaller than any of those on CSP3–6 under the same chromatographic conditions. For example, the separation factor of D,L-leucine on CSP6 is 1.93 whereas it is only 1.58 on CSPA.

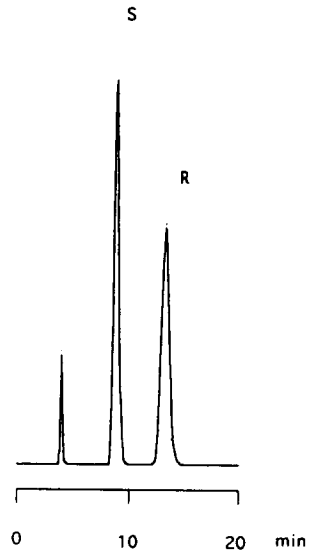


Fig. 4. Chromatogram for the resolution of N-dinitrobenzoyl-D,L-valine-O-methyl ester on CSP3. Mobile phase, *n*-hexane–2-propanol (90:10); flow-rate, 1 ml/min.

Because the silica supports are the same and the surface coverages are similar for CSPA and CSP3–6, the differences in the chromatographic results between CSPA and CSP3–6 may be ascribed to the additional non-enantioselective $\pi-\pi$ interaction provided by the benzyl group of CSPA. The longer retentions and smaller separation factors on CSPA can be illustrated by the non-enantioselective $\pi-\pi$ interaction. In other words, the enantioselectivity is enhanced by deleting this non-enantioselective $\pi-\pi$ interaction site on CSPA.

4. Conclusions

Based on deleting the non-enantioselective $\pi-\pi$ interaction site on the N-benzylcarbamoyl-(*S*)-phenylglycine, a series of seven chiral stationary phases containing N-alkylcarbamoyl-(*S*)-phenylglycine were prepared. It is found that (*S*)-phenylglycine is better than (*S*)-phenylalanine for acting as a chiral entity of the series of N-alkylcarbamoyl-drived CSPs. The best chromatographic results on the series of seven CSPs were found on the ionic-type N-*n*-stearylcarbamoyl-(*S*)-phenylglycine- and N-cyclohexylcarbamoyl-(*S*)-phenylglycine-derived CSPs. The chiral recognition abilities on all of the ionic-type CSPs derived from N-alkylcarbamoyl-(*S*)-phenylglycine are better than that on the ionic-type N-benzylcarbamoyl-(*S*)-phenylglycine CSP for the discrimination of the same enantiomers. The results clearly show that the enantioselectivity is enhanced by deleting the non-enantioselective $\pi-\pi$ interaction site on the N-benzylcarbamoyl-(*S*)-phenylglycine CSP.

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