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Evaluation of new chiral stationary phases of bonded cyanuric chloride with amino acid and naphthylalkylamine substituents for liquid chromatographic separation of amino acids and amino alcohols as dinitrobenzoyl derivatives

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Abstract

Chiral stationary phases (CSPs) with two chiral centers derived from (*R*/*S*)-phenylalanyl- and (*S*)-1-(1-naphthyl)ethylamino-disubstituted cyanuric chloride and the corresponding stationary phases containing one chiral center, by replacing a substituent group attached to the asymmetric center with hydrogen, were prepared for evaluating the enantioseparation of amino acid and amino alcohols as dinitrobenzoyl derivatives. Chiral stationary phases derived from (*R*)-alanyl- and (*S*)-1-(1-naphthyl)ethylamino-disubstituted cyanuric chloride were prepared for comparison. The phase (CSP-1) with two chiral centers provides barely satisfactory recognition ability to separate the enantiomers of dinitrobenzoyl (DNB) derivatives of amino acid methyl esters and amino alcohols by high-performance liquid chromatography (HPLC). The chromatographic results show that for a CSP bearing two chiral centers the phenylalanyl moiety dominates the chiral recognition and that the alteration of the absolute configuration of the phenylalanyl moiety from (*R*)- to (*S*)-configuration would worsen or even diminish the enantioseparation. The phenyl ring in the phenylalanyl moiety of the CSP seems to exert steric effects instead of acting as a π-interacting group in chiral recognition. Mechanisms for chiral recognition in liquid chromatography are discussed.

1. Introduction

The need to separate enantiomers from racemic mixtures has led to considerable advances in the field of chiral separation. Enantiomers can be effectively resolved using high-performance liquid chromatograph [1–4] with the utilization of chemically bonded chiral stationary phases (CSPs). The enantioseparation is greatly improved on a CSP with two or more chiral centers [5–10]. The effectiveness of chiral separation depends upon the structural design of the CSP. Hence, the absolute configurations of chiral moieties in a CSP are likely a determining factor for effective enantioseparation. The alteration of the absolute configuration of a chiral moiety in a CSP containing two chiral centers may increase or decrease the enantioselectivity depending upon whether the interactions between one chiral moiety and the chiral selectand re-enforce or interfere with those of the other chiral moiety in the same bonded phase [11].

The chiral stationary phase derived from (*S*)-1-(1-naphthyl)ethylamino-substituted cyanuric

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chloride enabled satisfactory enantioseparation of derivatized amino acids, alcohols and amines [12], whereas the chiral stationary phases containing a phenylalanyl moiety provide good recognition ability for derivatized amino acids and amino alcohols [13–15]. The combination of these two chiral moieties in a chiral stationary phase is expected to provide broader applications for various chiral solutes.

Cyanuric chloride (2,4,6-trichloro-*s*-triazine) is a good linking reagent; its three chlorine substituents can be replaced by a suitable and desirable nucleophile at varied reaction temperatures [16,17]. Besides, the π-donor character of the *s*-triazine ring is enhanced on substitution by a more strongly electron-donating group. The inclusion of an *s*-triazine ring in the bonded stationary phase provides the possibility of more versatile modifications of the bonded phase so that a favorable functional group capable of enhancing enantioselectivity can be introduced into the stationary phase [18–20]. For this purpose, the chiral phenylalanyl moiety and chiral 1-(1-naphthyl)ethylamino moiety were selected and CSPs of two structural types containing these two chiral moieties either linked to or linked through an *s*-triazine ring were considered.

In this work, CSPs containing the (*R*/*S*)-phenylalanyl and (*S*)-1-(1-naphthyl)ethylamino chiral moieties linked through an *s*-triazine ring were prepared. The enantioselectivity of the CSPs was examined with methyl esters of N-(3,5-dinitrobenzoyl) amino acids and N-(3,5-dinitrobenzoyl) amino alcohols. The effect of alteration of the absolute configuration of this CSP from (*R*,*S*) to (*S*,*S*) on enantioseparation was investigated. The CSP derived from (*R*)-alanyl- and (*S*)-1-(1-naphthyl)ethylamino-disubstituted cyanuric chloride was prepared for comparison in order to investigate the role played by the phenyl ring of the phenylalanyl moiety. Two additional CSPs, bearing only one chiral center, were also prepared, to elucidate the exact role of two chiral centers of a CSP in chiral recognition. One CSP was prepared by replacing the methyl group in the 1-(1-naphthyl)ethylamino group at the second asymmetric center with a hydrogen atom, the other by replacing the benzyl group with a hydrogen atom at the first asymmetric center. Mechanisms for chiral recognition in liquid chromatography are discussed. The results of this work enable improved insight into the model for chiral recognition.

2. Experimental

2.1. Chemicals and reagents

N,N′-Dicyclohexylcarbodiimide and 3,5-dinitrobenzoyl chloride were purchased from Merck (Germany). The silica gel used was Nucleosil (pore size, 10 nm; particle size, 10 μm; surface area, 350 m^2/g; Macherey-Nagel, Germany). 3-Aminopropyltriethoxysilane (APS, Janssen, Belgium), (*S*)-1-(1-naphthyl)ethylamine and N-hydroxysuccinimide (Tokyo Chemical Industry, Japan), cyanuric chloride and 1-(1-naphthyl)methylamine (Aldrich, USA), amino acids (Sigma, USA), reagents for the chiral stationary phase and derivatives of chiral analytes (from various suppliers) were used without further purification. Hexane and 2-propanol (Mallinckrodt, USA) are of LC grade. Water was purified with ion exchanger and a Milli-Q water purification system (Millipore, USA)

2.2. Preparation of chiral stationary phases

2,4-Dichloro-6-(S/R)-phenylalanyl-s-triazine

A solution of cyanuric chloride (0.01 mol) in acetone (20 ml) was added with agitation to a solution of sodium carbonate (0.02 mol) and (*S*/*R*)-phenylalanine (0.01 mol) in water (100 ml) kept in an ice-water bath. After the mixed solution had reacted at 0°C for 1 h, the solution was acidified with HCl (1 *M*). The white precipitate was collected on a filter and washed well with cold water several times and then dried over P_2O_5 in vacuo. The product yield is about 90%. Fast atom bombardment-mass spectroscopy (FAB-MS): *m*/*z* 313 ($[M+H]^+$, ^{35}Cl–^{35}Cl), 315

($[M+H]^+$, ^{35}Cl–^{37}Cl), 317 ($[M+H]^+$, ^{37}Cl–^{37}Cl) intense.

2,4-Dichloro-6-glycyl-s-triazine

A solution of cyanuric chloride (0.01 mol) in acetone (20 ml) was added with agitation to a solution of sodium carbonate (0.02 mol) and glycine (0.01 mol) in water (100 ml) kept in an ice-water bath. After the mixed solution had reacted at 0°C for 1 h, the solution was acidified with HCl (1 *M*). A precipitate formed on addition of saturated NaCl solution was collected on a filter, and dried over P_2O_5 in vacuo. FAB-MS: *m/z* 223 ($[M+H]^+$, ^{35}Cl–^{35}Cl), 225 ($[M+H]^+$, ^{35}Cl–^{37}Cl), 227 ($[M+H]^+$, ^{37}Cl–^{37}Cl) intense.

2,4-Dichloro-6-D-alanyl-s-triazine

The procedures were the same as for 2,4-dichloro-6-glycyl-*s*-triazine except that glycine was replaced with D-alanine.

2-Chloro-4-(S)-1-(1-naphthyl)ethylamino-6-(S/R)-phenylalanyl-s-triazine

A solution of (*S*)-1-(1-naphthyl)ethylamine (0.01 mol) in acetone (20 ml) was added with agitation to a solution of sodium carbonate (0.02 mol) and 2,4-dichloro-6-(*S*/*R*)-phenylalanyl-*s*-triazine (0.01 mol) in water (100 ml) kept in a water bath at 50°C. After the mixed solution had thus reacted for 2 h, the solution was acidified to precipitate with HCl (1 *M*). The precipitate was collected by filtration, washed well with cold water several times and dried over P_2O_5 in vacuo. The product yield of 2-chloro-4-(*S*)-1-(1-naphthyl)-ethylamino-6-(*S*/*R*)-phenylalanyl-*s*-triazine was about 75%. FAB-MS: *m/z* 448 ($[M+H]^+$, ^{35}Cl), 450 ($[M+H]^+$, ^{37}Cl) intense.

2-Chloro-4-(S)-1-(1-naphthyl)ethylamino-6-(R)-alanyl-s-triazine and 2-chloro-4-(S)-1-(1-naphthyl)ethylamino-6-glycyl-s-triazine

The procedures were the same as for 2-chloro-4-(*S*)-1-(1-naphthyl)-ethylamino-6-(*S*/*R*)-phenylalanyl-*s*-triazine except that phenylalanine was replaced with the corresponding alanine and glycine.

2-Chloro-4-1-(1-naphthyl)methylamino-6-(S)-phenylalanyl-s-triazine

The procedures were the same as for 2-chloro-4-(*S*)-1-(1-naphthyl)-ethylamino-6-(*S*/*R*)-phenylalanyl-*s*-triazine except that (*S*)-1-(1-naphthyl)-ethylamine was replaced with 1-(1-naphthyl)methylamine. The product yield is about 78%. FAB-MS: *m/z* 434 ($[M+H]^+$, ^{35}Cl), 436 ($[M+H]^+$, ^{37}Cl) intense.

Silane-modified silica gels

These were prepared as previously described [21]. The silane used was 3-aminopropylsilane (APS).

Chemically bonded chiral stationary phases

CSP-1 and CSP-2.

A solution of 2-chloro-4-(*S*)-1-(1-naphthyl)ethylamino - 6 - (*S*/*R*) - phenylalanyl - *s* - triazine(0.005 mol) and N-hydroxysuccinimide (0.005 mol) dissolved in dry tetrahydrofuran (THF, 100 ml) was cooled in an ice-water bath and N,N′-dicyclohexylcarbodiimide (DCC, 0.005 mol) was slowly added under stirring. The mixture solution was kept in a refrigerator (between 0 and 5°C) overnight. The precipitated N,N′-dicyclohexylurea was separated and removed by filtration and the filtrate was collected. The APS-modified silica gel (3 g) was added and suspended in the THF solution. The reaction proceeded with agitation at room temperature for 24 h. The product was collected on filtration, washed thoroughly with acetone, methanol and water, and dried over P_2O_5 in vacuo.

CSP-3, CSP-4 and CSP-5.

The procedures were the same as for CSP-1 except that 2-chloro-4-(*S*)-1-(1-naphthyl)ethylamino-6-(*R*)-phenylalanyl-*s*-triazine was replaced with the corresponding reagent, 2-chloro-4-(*S*)-1-(1-naphthyl)-ethylamino - 6 - (*R*)-alanyl-*s*-triazine, 2 - chloro - 4 - (*S*) - 1 - (1-naphthyl)ethylamino-6-glycyl-*s*-triazine or 2-chloro-4-1-(1-naphthyl)methylamino-6-(*S*)-phenylalanyl-*s*-triazine. Fig. 1 presents the reaction schemes for preparation of these chiral stationary phases. Fig. 2 depicts the structures of the CSPs prepared.

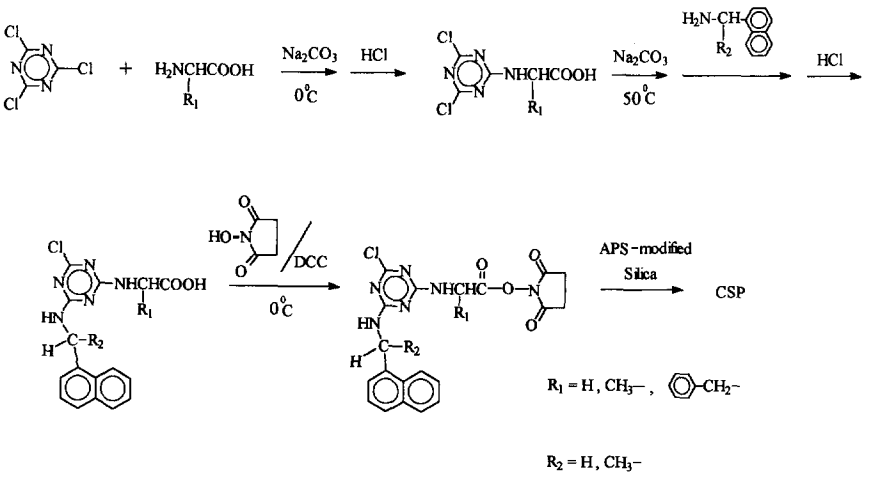
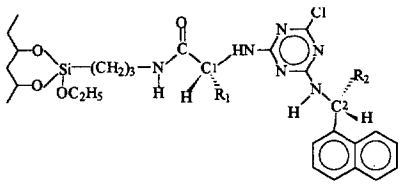


Fig. 1. Reaction schemes for the preparation of chiral stationary phases.

2.3. Derivatization of analytes

Dinitrobenzoyl-derivatized amino acids and amino alcohols were prepared as described elsewhere [21].



	R_1	R_2	Configuration
CSP-1	$-CH_2-C_6H_5$	$-CH_3$	(1R, 2S)
CSP-2	$-CH_2-C_6H_5$	$-CH_3$	(1S, 2S)
CSP-3	$-CH_3$	$-CH_3$	(1R, 2S)
CSP-4	$-CH_2-C_6H_5$	$-H$	(1R, –)
CSP-5	$-H$	$-CH_3$	(–, 2S)

Fig. 2. Structures of chiral stationary phases tested.

2.4. Apparatus and chromatography

The chromatographic system and the apparatus of column packing were described previously [22]. Mixtures of 2-propanol and *n*-hexane (20:80, v/v, typically) were used as the mobile phase and were degassed on ultrasonic vibration. The flow-rate was 1.0 ml/min. The detector was operated at 254 nm. The void volume was determined with tri-*tert.*-butylbenzene [23]. Fast atom bombardment (FAB) was performed on a double focusing mass spectrometry of reversed geometry (Jeol SX-102A). The FAB gun was operated at 6 kV using xenon as the ionizing gas. Elemental analyses of chiral stationary phases and the corresponding silane-modified silica gel were performed with an elemental analyzer (Perkin-Elmer Model 240C).

3. Results and discussion

As the chiral selectors investigated in this work possess π-donor character, chiral selectands possessing π-acceptor character are considered so that $\pi-\pi$ interaction becomes a preferential interaction for chiral recognition. Hence analytes were converted into dinitrobenzoyl derivatives

before chromatographic measurements were performed.

3.1. Enantioseparation of amino acids

Table 1 presents chromatographic results of enantiomeric separation of methyl esters of N-(3,5-dinitrobenzoyl) amino acids on five chiral stationary phases prepared using 2-propanol–*n*-hexane (20:80, v/v) as eluent. CSP-1, CSP-3 and CSP-4 provide recognition ability to separate enantiomers of methyl esters of N-(3,5-dinitrobenzoyl) amino acids. Fig. 3 shows typical chromatograms of methyl esters of N-(3,5-dinitrobenzoyl)leucine on these CSPs.

As demonstrated in Table 1, the capacity factors of the enantiomers of these DNB-derivatized amino acid methyl esters having an alkyl substituent attached to the chiral carbon decrease with increasing chain length of the alkyl group on CSP-1–CSP-5, but the enantioselectivity indicated by the α-values increases sig-

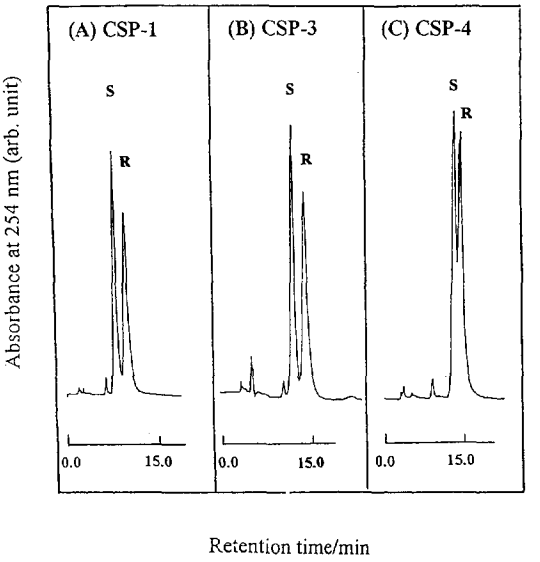
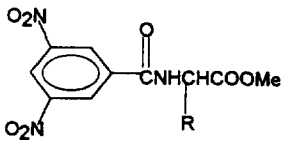


Fig. 3. Chromatograms of methyl ester of N-(3,5-dinitrobenzoyl)leucine on (A) CSP-1, (B) CSP-3 and (C) CSP-4. Eluent, 2-propanol–*n*-hexane (20:80, v/v); flow-rate, 1 ml/min.

Table 1
Capacity factors (k'_1), selectivity factors (α) and absolute configuration (R/S) of methyl esters of N-(3,5-dinitrobenzoyl)amino acids on CSP-1–CSP-5



Amino acids	CSP-1			CSP-2			CSP-3			CSP-4			CSP-5		
	k'_1	α	R/S	k'_1	α	R/S	k'_1	α	R/S	k'_1	α	R/S	k'_1	α	R/S
Alanine	6.03	1.13	*S*	5.88	1.00	–	4.60	1.08	*S*	4.33	1.07	*S*	3.19	1.00	–
2-Amino-butyric acid	5.11	1.18	*S*	4.16	1.00	–	3.81	1.13	*S*	3.73	1.10	*S*	2.43	1.00	–
Norvaline	4.38	1.21	*S*	3.28	1.00	–	3.08	1.17	*S*	3.38	1.11	*S*	1.89	1.00	–
Norleucine	3.72	1.22	*S*	2.97	1.03	–	2.64	1.17	*S*	3.12	1.10	*S*	1.67	1.00	–
Valine	4.35	1.15	*S*	3.20	1.03	–	2.94	1.14	*S*	3.25	1.12	*S*	1.84	1.00	–
Isoleucine	3.89	1.16	*S*	2.89	1.04	*R*	2.55	1.14	*S*	3.01	1.13	*S*	1.61	1.00	–
Leucine	3.58	1.29	*S*	2.83	1.00	–	2.57	1.23	*S*	3.06	1.13	*S*	1.61	1.10	*S*
Phenylglycine	7.63	1.21	*S*	5.81	1.00	–	8.12	1.24	*S*	6.61	1.01	–	2.97	1.00	–
Phenylalanine	7.55	1.18	*S*	6.06	1.00	–	7.63	1.17	*S*	5.91	1.10	*S*	3.25	1.09	*S*
Tryptophan	17.70	1.19	*S*	15.35	1.00	–	14.10	1.21	*S*	12.22	1.08	*S*	9.23	1.08	*S*

Eluent, 2-propanol–*n*-hexane (20:80, v/v); flow-rate, 1 ml/min; k'_1, capacity factor of the first-eluted enantiomer; (R/S), absolute configuration of the first-eluted enantiomer.

nificantly with increasing chain length and bulkiness of the alkyl group on CSP-1. These trends in the variation of capacity factors and enantioselectivities of these DNB-derivatized amino acids indicate that a steric interaction may exist between the chiral selectand with a longer or bulkier alkyl group and the chiral selector.

Since the loading capacity of CSP-1 (0.29 mmol/g) is about the same as that of CSP-3 (0.28 mmol/g), that the capacity factors and separation factors of DNB-derivatized amino acids with an alkyl substituent on CSP-1 exceed those on CSP-3 indicates that the benzyl group attached to the first chiral center of CSP-1 exhibits a steric effect in chiral recognition. This result is consistent with that obtained by Oliveros et al. [24]. The lack of $\pi-\pi$ interaction between the phenyl group in the phenylalanine moiety of CSP-1 and an aromatic substituent of chiral analytes was ascertained on comparison of chromatographic results of DNB-derivatized amino acids with an aromatic substituent such as phenylglycine, phenylalanine and tryptophan on CSP-1 with those on CSP-3. In all instances, enhanced enantioselectivity values were lacking with CSP-1. Thus the phenyl ring belonging to the phenylalanine moiety of CSP-1 seems to exert steric effects instead of acting as a π-interacting group in the mechanism of chiral recognition.

3.2. Effect of absolute configuration of CSP bearing two chiral centers

The enantioselectivity of DNB-derivatized amino acids can vary greatly with the absolute configuration of the CSP bearing two chiral centers. On altering the absolute configuration of the CSP-1 from the (*R*,*S*) form to the (*S*,*S*) form, the chiral separability of DNB-derivatized amino acids diminished in most instances. This significantly altered enantioselectivity demonstrates that the absolute configuration of the CSP bearing two stereogenic centers may play a crucial role in chiral recognition. Therefore, to discover the nature of the roles of the two chiral entities of a CSP is an important objective. For this purpose, chromatographic results of DNB-derivatized amino acids on CSP-4, that bears a phenylalanyl chiral moiety and a 1-(1-naphthyl)methylamino achiral moiety, and on CSP-5, which bears a glycyl achiral group and a (*S*)-1-(1-naphthyl)ethylamino chiral group, were examined.

On comparing chromatographic results obtained for DNB-derivatized amino acids on CSP-1 and CSP-3 with those on CSP-4, the greater capacity factors and separation factors obtained on CSP-1 and CSP-3 reveal that the presence of the secondary chiral center in the (*S*)-1-(1-naphthyl)ethylamino group of CSP-1 enhances significantly the enantioseparation of DNB- derivatized amino acids. The capacity factors of DNB-derivatized phenylglycine and phenylalanine are much greater than those of DNB-derivatized amino acids with an alkyl substituent on CSP-1, CSP-3 and CSP-4. This result indicates that the interaction between the substituent group attached to the first chiral center of the chiral stationary phase and chiral analytes is steric in origin and seems to be achiral in nature, because the enantioselectivity is not significantly enhanced.

The fact that capacity factors obtained for DNB-derivatized phenylglycine and phenylalanine on CSP-5 are as small as those of DNB-derivatized amino acids with alkyl substituents reveals that the existence of an additional $\pi-\pi$ interaction between the naphthyl group attached to the second chiral center of the CSP and the aromatic substituent of chiral analytes seems not very likely. However, the facts that only the DNB-derivatized leucine, phenylalanine and tryptophan were separable on CSP-5 and that capacity factors and separation factors of DNB-derivatized amino acids on both CSP-1 and CSP-3 are much greater than those on CSP-5 clearly demonstrate that enantioseparation is predominantly determined by the phenylalanyl chiral moiety. Hence a CSP with a 1-(1-naphthyl)ethylamino group alone cannot provide effective enantioseparation for DNB-derivatized amino acids. This effect obviously reflects insufficient preferential interactions between the methyl esters of DNB-derivatized amino acid and CSP-5. Our results appear to differ from

those reported by Oi et al. [12], despite that different mobile phases were used.

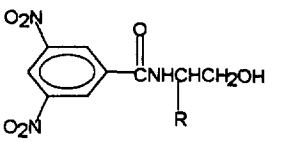
3.3. Elution order of enantiomers

The absolute configuration of the last eluted enantiomer on a CSP is generally consistent with the absolute configuration of the dominating chiral selector of the CSP, that is, the (*R*)-enantiomer would be more retained on an (*R*)-CSP. As CSP-1 and CSP-4 have (*R*)-phenylalanyl moiety as the dominating chiral selector, and CSP-3 has (*R*)-alanyl moiety as chiral selector, the (*R*)-enantiomer of DNB-derivatized amino acid would be retained longer than the corresponding (*S*)-enantiomer on CSP-1, CSP-3 and CSP-4 as expected. On this basis, the (*R*)-enantiomer of DNB-derivatized amino acid is expected to elute first, before the (*S*)-enantiomer on CSP-5, which possesses (*S*)-1-(1-naphthyl)ethylamino moiety as chiral selector. However, as indicated in Table 1, the elution order of DNB-derivatized amino acids on CSP-5 was found reversed. The (*S*)-enantiomers of DNB-derivatized leucine, phenylalanine and tryptophan eluted first, before the (*R*)-enantiomers. The reason is not clear at the present stage of the investigation, it seems that the steric effect due to the bulkiness of the naphthyl group in the (*S*)-1-(1-naphthyl)ethylamino moiety of CSP-5 has something to do with the reversal of the elution order of enantiomers. Further investigation on the mechanism of chiral recognition for amino acids on these CSPs using the semi-empirical AM1 method is being undertaken.

3.4. Enantioseparation of amino alcohols

Table 2 presents results of enantiomeric separation of 3,5-dinitrobenzoyl amino alcohols on CSP-1–CSP-5. Chiral analytes of DNB-derivatized amino alcohols were retained longer than their corresponding analytes of amino acids on CSP-3, but this situation was unclear on CSP-1 and CSP-4. The variations of the capacity factors and the separation factors of enantiomers of

Table 2
Capacity factors (k_1') and selectivity factors (α) and absolute configuration (R/S) of N-(3,5-dinitrobenzoyl) amino alcohols on CSP-1–CSP-5



Amino alcohols	CSP-1			CSP-2			CSP-3			CSP-4			CSP-5		
	k_1'	α	R/S	k_1'	α	R/S	k_1'	α	R/S	k_1'	α	R/S	k_1'	α	R/S
2-Amino-1-propanol	6.65	1.16	*S*	6.66	1.01	–	7.20	1.11	*S*	4.38	1.10	*S*	6.72	1.00	–
2-Amino-1-butanol	4.83	1.17	*S*	4.79	1.00	–	5.32	1.14	*S*	3.84	1.11	*S*	5.02	1.00	–
2-Amino-1-pentanol	3.85	1.24	*S*	3.52	1.01	–	4.18	1.19	*S*	2.88	1.12	*S*	3.59	1.00	–
Valinol	3.82	1.25	*S*	3.60	1.00	–				2.74	1.10	*S*	3.38	1.00	–
2-Amino-1-hexanol	3.26	1.27	*S*	2.92	1.00	–	3.35	1.21	*S*	2.39	1.12	*S*	2.89	1.07	*S*
Phenylglycinol	9.61	1.29	*S*	7.21	1.09	*S*	9.32	1.43	*S*	6.79	1.14	*S*	4.82	1.00	–
Phenylalaninol	5.81	1.00	–	6.14	1.00	–							6.86	1.15	*S*

Eluent, 2-propanol–*n*-hexane (20:80, v/v); flow-rate, 1 ml/min; k_1', capacity factor of the first-eluted enantiomer; (R/S), absolute configuration of the first-eluted enantiomer.

DNB-derivatized amino alcohols on these CSPs were similar to those for amino acids. The α-values obtained for DNB-derivatized amino alcohols on these CSPs are roughly the same as for DNB-derivatized amino acids. Better enantioseparation was achieved for phenylglycinol than for the corresponding phenylglycine on CSP-1, CSP-3 and CSP-4, but no resolution was obtained for phenylalaninol on CSP-1. As the case of amino acids, comparison of chromatographic results of phenylglycinol on CSP-1 with those on CSP-3 also reveals that the phenyl ring in the phenylalanyl chiral moiety of CSP-1 exhibits a steric effect instead of a $\pi-\pi$ interaction, because the presence of this group enabled no enhancement in enantioseparation.

3.5. Mechanism of chiral recognition

As the phenylalanyl moiety proves to be the dominating part of the CSP for effective enantioseparation of amino acids and amino alcohols, the mechanism of chiral recognition of the CSPs tested (i.e., CSP-1, CSP-3 and CSP-4) is essentially the same as for the CSP reported previously [18]. Accordingly, the following three interactions should be taken into consideration: $\pi-\pi$ interaction between the 3,5-dinitrobenzoyl group of the chiral selectand and the 1-(1-naphthyl)ethylamino-substituted *s*-triazine ring of the CSP, hydrogen bonding involving the carbonyl group in the carboxyl ester group of the chiral selectand and the secondary amino group belonging to the amino acid of the chiral selector, and hydrogen bonding between the secondary amino group in the amide linkage of the chiral selectand and the carbonyl group in the amide linkage of the chiral selector. However, steric interaction between the substituent attached to the chiral center of the chiral selectand and the substituent attached to the first chiral center of the chiral selector may also be involved to some significant extent in chiral discrimination.

The importance of $\pi-\pi$ interaction involving the 3,5-dinitrobenzoyl group in a chiral selectand and the pyrrolidinyl-substituted *s*-triazine ring in a chiral selector for recognition was examined previously [18]. To confirm an interaction of this sort, much supporting chromatographic data were provided [25], and the effect of the substituent, resulting from the varying π-basicity between a pyrrolidinyl- and a 1-(1-naphthyl)ethylamino-substituted *s*-triazine ring in the selector, on enantioseparation of methyl esters of DNB-derivatized amino acids was examined. On comparing chromatographic results for DNB-derivatized amino acids and amino alcohols on CSP-3 with results obtained previously [18], we found that replacement of a pyrrolidinyl group by a (1-naphthyl)ethylamino group decreased the enantioselectivity. This effect probably resulted from decreased $\pi-\pi$ interaction between the dinitrobenzoyl moiety of chiral analytes and the *s*-triazine ring of the chiral stationary phase due to the decreased π-basicity of the (1-naphthyl)ethylamino-substituted *s*-triazine ring.

The importance of the carbonyl group of the chiral analyte in chiral recognition was examined previously [18,26–27]. On comparing the chromatographic resolution of enantiomers of DNB-derivatized amino acids or the corresponding DNB-derivatized amino alcohols with that of the corresponding DNB-derivatized amine, we obtained additional evidence to support the importance of the role in chiral discrimination played by the hydrogen bond involving the carbonyl ester group of the chiral selectand and the secondary amino group belonging to the amino acid of the chiral selector. The absence of an ester group of an amino acid or a hydroxyl group of an amino alcohol precludes formation of a hydrogen bond between the ester group (or hydroxyl group) of chiral analytes and the secondary amino group of the chiral moiety in the CSP. Thus, elimination of this preferential interaction leads to failure of enantioseparation. For instance, the α-values obtained for alanine and alaninol on CSP-1 are 1.13 and 1.16, respectively, but 1.00 for the corresponding amine on CSP-1.

The role of the acidic NH group of chiral analytes in chiral recognition was examined on comparing the α-values of DNB-derivatized amino acids with those of corresponding DNB-derivatized N-methyl amino acids. For this pur-

pose, we tested N-(3,5-dinitrobenzoyl)-N-methylalanine methyl ester and N-(3,5-dinitrobenzoyl)-N-methylvaline methyl ester. The inability to resolve enantiomers of DNB-derivatized N-methylalanine and N-methylvaline methyl esters on CSP-1–CSP-5 appears to be due to lack of an acidic NH group. Under these conditions, no hydrogen bond was formed between the chiral analyte and the chiral selector. Thus, enantioseparation was not achieved.

It is of interest to note that no enantioseparation was achieved for phenylglycine on CSP-4 and phenylalaninol on CSP-1, despite the three preferential interactions responsible for chiral recognition still persisting between chiral analytes and CSP. Similar phenomena were also observed for phenylalaninol and phenylglycinol on ionically bonded CSPs consisting of cyanuric chloride with amino acid and dialkylamine substituents [21]. These results are contradictory to the expectation of the model of classical three-point interaction. Perhaps, steric interaction owing to the conformational rigidity of the aromatic substituent in chiral selectands and the substituent attached to the chiral center of the chiral selector has something to do with it. Further investigation is needed.

4. Conclusion

Silica-based chiral stationary phases derived from (*R*)-phenylalanyl- or ((*R*)-alanyl-) and (*S*)-1-(1-naphthyl)ethylamino-disubstituted cyanuric chloride provide barely satisfactory enantioselectivities for most racemates of DNB-derivatized amino acids and amino alcohols tested. Based on chromatographic results, the phenylalanyl chiral moiety dominates the recognition ability of the CSP bearing the two chiral centers considered. The phenyl ring in the phenylalanyl moiety of the CSP-1 exerts steric effects rather than showing an electronic role in chiral recognition. The alteration of absolute configuration of the phenylalanyl moiety of a CSP bearing two stereogenic centers from the (*R,S*) to the (*S,S*) configuration worsens or even diminishes enantioseparation. Mechanisms involved in chiral recognition are discussed

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