ELSEVIER

Journal of Chromatography A, 722 (1996) 199–209

JOURNAL OF CHROMATOGRAPHY A

Enantioseparation and recognition mechanisms of dinitrobenzoyl-derivatized amino acids and amino alcohols on chiral stationary phases consisting of cyanuric chloride with (*S* or *R*)-phenylalanyl-(*S* or *R*)-1-(1-naphthyl)ethylamide substituent

Ching-Erh Lin*, Fang-Kuo Li

Department of Chemistry, National Taiwan University, Roosevelt Road Section 4, Taipei 10764, Taiwan

Abstract

Chiral stationary phases (CSPs) derived from (*S* or *R*)-phenylalanyl-(*S* or *R*)-1-(1-naphthyl)ethylamide as a chiral selector were prepared by bonding a chiral moiety onto 3-aminopropylsilane-modified silica gels through the *s*-triazine ring. These phases provide satisfactory recognition ability to separate most enantiomers of methyl esters of N-(3,5-dinitrobenzoyl) amino acids except phenylglycine. However, only the CSP with the (*S,S*) configuration provides satisfactory recognition ability to separate most enantiomers of N-(3,5-dinitrobenzoyl) amino alcohols except phenylglycinol and phenylalaninol. The CSPs with either (*S,R*) or (*R,S*) configuration provide no enantioseparation for most amino alcohols, except phenylglycinol. The chromatographic results also show that alteration of the absolute configuration of the CSP from the (*S,S*) to the (*S,R*) form decreases enantioseparation of most amino acids and amino alcohols. With the aid of AM1 calculations, elution orders of enantiomers of amino acids on *SS*-CSP and *SR*-CSP can be predicted. The calculations reveal that two mechanisms of chiral recognition for CSP bearing two chiral centers are involved in the phenylalanyl and 1-(1-naphthyl)ethylamido moieties of CSP. In the case of *SS*-CSP, the phenylalanyl moiety of CSP plays the dominant role in chiral recognition; in the case of *SR*-CSP, the 1-(1-naphthyl)ethylamido moiety of the CSP plays the dominant role. These theoretical predictions are consistent with the experimental observations in a high-performance liquid chromatograph.

1. Introduction

Understanding the mechanisms of chiral recognition not only affords insight into the selection of an effective chiral stationary phase (CSP) to improve resolution of a given analyte but also aids in the design of a CSP with enhanced enantioselectivity. Thus, to elicit various data about the origins of chiral recognition, experimental [1–13] and computational [14–23] methods were applied.

In previous work [24], we described the preparation of CSPs consisting of disubstituted cyanuric chloride with an amino acid and a naphthylalkylamino substituent. Among them, the phase derived from (*R*)-phenylalanyl- and (*S*)-1-(1-naphthyl)ethylamino-disubstituted cyanuric chloride afforded satisfactory chiral separation for methyl esters of N-(3,5-dinitrobenzoyl) amino acids and N-(3,5-dinitrobenzoyl) amino

* Corresponding author.

0021-9673/96/$15.00
SSDI 0021-9673(95)00688-5

alcohols. When the absolute configuration of this CSP was altered from the (*R*,*S*) form to the (*S*,*S*) form, the chiral separability of amino acids deteriorated in most instances. On comparing results obtained from corresponding CSPs derived from (*R*)-alanyl- and (*S*)-1-(1-naphthyl)ethylamino-disubstituted cyanuric chloride, we conclude that the role in chiral recognition played by the phenyl ring in the phenylalanyl moiety has a steric origin, as Oliveros et al. also observed [25].

Cyanuric chloride (2,4,6-trichloro-*s*-triazine) is a good linking reagent as its three chlorine substituents can be replaced by suitable nucleophiles at varied reaction temperatures [26,27]. The inclusion of an *s*-triazine ring in the bonded stationary phase facilitates its modification so that functional groups which enhance the enantioselectivity can be introduced into the stationary phase [28].

Chiral stationary phases with two or more asymmetric centers are rarely reported. The chiral resolution of racemic mixtures is improved on CSPs with two or more asymmetric centers [29–33]. Pirkle and McCune [32] reported that a CSP derived from a β-amino acid with two chiral centers affords a larger separation factor than that derived from its α-amino acid analogue with one chiral center. They presumed that a greater degree of preorganization of the recognition site and conformational rigidity were responsible for the improved performance. Compared with the performance of a CSP derived from L-phenylalanyl- and pyrrolidinyl-disubstituted cyanuric chloride [34], we found that the enantioseparation of the CSP derived from (*R*)-phenylalanyl- and (*S*)-1-(1-naphthyl)ethylamine-disubstituted cyanuric chloride decreased for chiral resolution of N-(3,5-dinitrobenzoyl) amino acids and amino alcohols, most likely because of competition of two opposite chiral recognitions or conformational interference between two chiral moieties in the CSP.

In order to improve enantioseparation of the CSP derived from (*R*)-phenylalanyl- and (*S*)-1-(1-naphthyl)ethylamino-disubstituted cyanuric chloride prepared previously [24], and to improve our understanding of the mechanism of recognition, CSPs with varied structural designs were considered. By coupling (*S* or *R*)-phenylalanine with (*S* or *R*)-1-(1-naphthyl)ethylamine as a chiral selector, three CSPs were prepared by bonding the chiral selector to silane-modified silica gels through the linkage of the *s*-triazine ring. The chiral selectors of the CSPs prepared were similar to those reported by Oliveros et al. [35], but with another structural design of the spacer arm and conformational rigidity. In our CSPs, the *s*-triazine ring was selected as the spacer arm to link the chiral selector and the supports. The rigid and π-basic character of the *s*-triazine spacer would enable interaction with the π-acidic 3,5-dinitrobenzoyl ring.

The influence of altering the absolute configuration of CSPs from the (*S*,*S*) to the (*S*,*R*) form on the elution order and enantioseparation of N-(3,5-dinitrobenzoyl) amino acids and amino alcohols was investigated, and the effect of chiral centers on the enantioselectivity of amino acids and amino alcohols was examined, as a basis for discussing mechanisms of chiral recognition in a liquid chromatograph. We performed theoretical calculations using the AM1 (Austin Model 1) method [36] to predict the elution order of enantiomers of amino acids on these CSPs to complement the experimental results and improve our understanding of models of chiral recognition.

2. Experimental

2.1. Chemicals and reagents

N,N-dicyclohexylcarbodiimide and 3,5-dinitrobenzoyl chloride (Merck, Germany), the silica gel used (Nucleosil, pore size, 10 nm; particle size, 10 mm; surface area, 350 m^2/g, Macherey-Nagel, Germany), 3-aminopropyltriethoxysilane (APS) (Janssen, Belgium), (*R* or *S*)-1-(1-naphthyl)ethylamine and di-*tert*.-butyl dicarbonate (Tokyo Chemical, Japan), cyanuric chloride and N-hydroxysuccinimide (Aldrich, USA), amino acids (Sigma, USA), reagents for the chiral stationary phase and derivatives of chiral analytes obtained from various suppliers were used without further purification. 2-Propanol and *n*-hexane are of LC grade (Mallinckrodt, USA).

Water was purified with an ion exchanger and a Milli-Q water purification system (Millipore, USA).

2.2. Preparation of chiral stationary phases

tert.-Butyloxycarbonyl-(S or R)-phenylalanine

The preparation of *tert.*-butyloxycarbonyl-(*S* or *R*)-phenylalanine followed that in the literature [37] with slight modification. A solution of sodium carbonate (0.02 mol) and phenylalanine (0.01 mol) in water (30 ml) was stirred and kept in an ice-water bath. A solution of di-*tert.*-butyl dicarbonate (0.01 mol) in dioxane (20 ml) was added. After the mixed solution was reacted at 0°C for 1 h and at ambient temperature for another 1 h, it was concentrated in vacuo to about 20 ml, then covered with a layer of ethyl acetate (30 ml) and acidified with HCl (1 *M*) to pH 2–3. The aqueous phase was extracted with ethyl acetate (3×30 ml); the extracts were collected and washed with water, then dried over anhydrous Na_2SO_4 and evaporated in vacuo. The product yield was about 92%; m.p. 87–88°C; FAB-MS (fast atom bombardment-mass spectroscopy): *m/z* 266 ($M + H^+$), 210 ($M + H^+ - 56$), 166 ($M + H^+ - 100$), intense.

tert.-Butyloxycarbonyl-(S or R)-phenylalanine N-hydroxysuccinimide ester

A solution of *tert.*-butyloxycarbony-(*S* or *R*)-phenylalanine (0.01 mol) and N-hydroxysuccinimide (0.01 mol) dissolved in dry ethyl acetate (50 ml) was cooled in an ice-water bath, and dicyclohexylcarbodiimide (DCC) (0.01 mol) was added under stirring. The mixture was kept in the refrigerator (0–5°C) overnight. The N,N-dicyclohexylurea was separated and removed by filtration, and the filtrate was evaporated in vacuo. The product yield was about 85%; m.p. 152–154°C; FAB-MS: *m/z* 363 ($M + H^+$), 307 ($M + H^+ - 56$), 263 ($M + H^+ - 100$), intense.

tert.-Butyloxycarbonyl-(S or R)-phenylalanyl-(S or R)-1-(1-naphthyl)ethylamide

A solution of (*S* or *R*)-naphthylethylamine (0.01 mol) in ethyl acetate (10 ml) was added under agitation to a solution of *tert.*-butyloxycarbonyl-(*S* or *R*)-phenylalanine N-hydroxysuccinimide ester (0.01 mol) in ethyl acetate (50 ml). The mixture was reacted under agitation at ambient temperature for 2 h, then poured into distilled water (100 ml) and stirred for a further 24 h. The organic phase was extracted and solidified by evaporation. The pure product (yield 80%) was obtained after purification on a small silica column with appropriate solvents as eluent, m.p. 143–145°C for (*S,S*) product and 152–154°C for (*S,R*) product. FAB-MS: *m/z* 419 ($M + H^+$), 363 ($M + H^+ - 56$), 319 ($M + H^+ - 100$), intense. Elemental analysis: calculated for $C_{26}H_{30}N_2O_3$, C 74.64, H 7.18, N 6.70%; found C 74.32, H 7.09, N 6.58%.

N-[2-(4,6-dichloro-1,3,5-triazinyl)]-(S or R)-phenylalanyl-(S or R)-1-(1-naphthyl)ethylamine

tert.-Butyloxycarbonyl-(*S* or *R*)-phenylalanyl-(*S* or *R*)-1-(1-naphthyl)ethylamide (0.005 mol) was dissolved in trifluoroacetic acid (25 ml) and left standing at ambient temperature for 1 h. The solution was concentrated to dryness in vacuo; then diethyl ether (20 ml) was added to the residue. After drying in vacuo, the crude product was dissolved in acetone (20 ml) and added to the solution of cyanuric chloride (0.005 mol) and sodium carbonate (0.005 mol) dissolved in acetone (50 ml) and water (50 ml). The reaction temperature was kept at 0°C. After 1 h, the precipitate was collected by filtration and washed with methanol and water. Recrystallization from acetone gave a white solid (yield, 65%), m.p. 100–103°C for (*S,S*) product, 119–120°C for (*S,R*) product. FAB-MS: *m/z* 466 ($M + H^+$, ^{35}Cl–^{35}Cl), 468 ($M + H^+$, ^{35}Cl–^{37}Cl), 450 ($M + H^+$, ^{37}Cl–^{37}Cl), 319 ($M + H^+$–triazine ring), intense. Elemental analysis: calculated for $C_{24}H_{21}N_5OCl_2$, C 61.80, H 4.51, N 15.02%; found C 61.66, H 4.43, N 14.92%.

APS-modified silica gels

The preparation of silane-modified silica gels has been previously described [38]. The silane used is 3-aminopropylsilane (APS). The loading capacity determined from the nitrogen content obtained from elemental analyses for APS-modified silica gel was 0.91 mmol/g.

Chemically bonded chiral stationary phases

Sodium bicarbonate (0.002 mol) and N-[2-(4,6-dichloro-1,3,5-triazinyl)]-(*S*/*R*)-phenylalanyl-(*S*/*R*)-1-(1-naphthyl)ethylamine (0.002 mol) were dissolved in acetone (50 ml) and water (100 ml). The APS-modified silica gel (3 g) was added and suspended. The reaction proceeded under agitation at 50°C for 12 h. On filtration, the product was collected, washed thoroughly with acetone, methanol and water, and dried over P_2O_5 at reduced pressure. Loading capacities obtained from nitrogen contents were 0.51 mmol/g for *SS*-CSP, 0.49 mmol/g for *SR*-CSP and 0.40 mmol/g for *RS*-CSP. Fig. 1 presents the reaction schemes for the preparation of these chiral stationary phases.

2.3. Apparatus and chromatography

The chromatographic system and the apparatus of column packing have been described previously [38]. Mixtures of 2-propanol and *n*-hexane (20:80, v/v, typically) were used as the mobile phase and were filtered through a 0.45-mm membrane filter and degassed by ultrasonic vibration. The flow-rate was 1.0 ml/min. The void volume of the column was measured by injecting 1,3,5-tri-*tert.*-butylbenzene [39]. The detector was operated at 254 nm. Fast atom bombardment (FAB) was performed on a double-focusing mass spectrometer of reversed geometry (Jeol SX-102A). The FAB gun was operated at 6 kV using xenon as ionizing gas. Elemental analyses of the chiral stationary phase and its corresponding APS-modified silica gel were performed with an elemental analyzer (Perkin-Elmer Model 240C).

2.4. Computational method

The completely relaxed geometries of complexes of *SS*-CSP (or *SR*-CSP) with *S*- and *R*-enantiomers of methyl esters of N-(3,5-dinitrobenzoyl)valine were estimated according to the semi-empirical AM1 method [36], implemented in Gaussian 92. AM1 is a quantum mechanical molecular model based on the NDDO (neglect of diatomic differential overlap) approximation. In this method, the core repulsion function in MNDO (modified neglect of diatomic overlap)

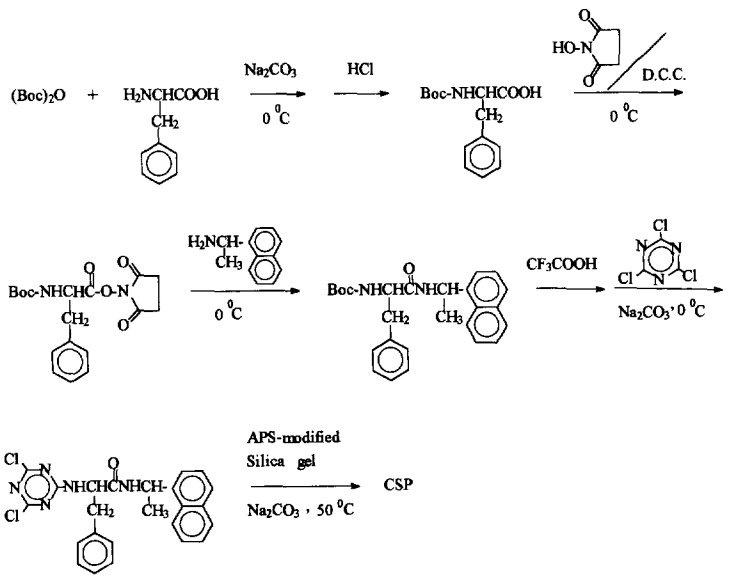


Fig. 1. Reaction schemes for preparation of chiral stationary phases.

[40] was modified so that the major weakness of MNDO, in particular failure to reproduce hydrogen bonds, was overcome. The AM1 energies of interaction were calculated for completely relaxed geometries of the complexes. The effect of the mobile phase was neglected, and the CSP was reasonably modified by replacing the propyl spacer and silica gel with the methyl group to simplify the system. The complexes of CSP with *S*- and *R*-enantiomers of the methyl ester of N-(3,5-dinitrobenzoyl)valine were assumed to have a ratio of 1:1.

3. Results and discussion

Fig. 2 depicts the structures of chiral stationary phases bearing the phenylalanyl-1-(1-naphthyl)ethylamido moiety as a chiral selector. As this chiral selector possesses a strong π-donor character, chiral selectands possessing a strong π-acceptor character were considered so that $\pi-\pi$ interaction became a favored interaction in chiral recognition. Hence, the analytes were converted into N-(3,5-dinitrobenzoyl) derivatives before chromatography.

3.1. Enantioseparation of amino acids

Table 1 presents results of enantiomeric separation of methyl esters of 3,5-dinitrobenzoyl amino acids on these three chiral stationary phases. Fig. 3 shows typical chromatograms of the enantiomeric separation of valine on these CSPs. Except for phenylglycine which shows no chiral selectivity, most amino acids listed in Table 1 are well resolved on these three CSPs with 2-propanol–hexane (20:80, v/v) as eluent.

As shown in Table 1, the capacity factors of enantiomers of amino acids having an alkyl

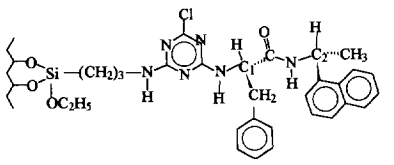


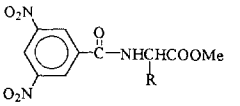
Fig. 2. Structures of chiral stationary phases prepared. *SS*-CSP = (1*S*,2*S*); *SR*-CSP = (1*S*,2*R*); *RS*-CSP = (1*R*,2*S*).

substituent attached to the chiral carbon decrease with increasing chain length or with increasing bulkiness of the alkyl group, but the enantioselectivity indicated by the α-values seems to vary irregularly on altering either factor. The trends in variation of capacity factors of these amino acids indicate that a steric interaction exists between the bulkier alkyl group of the chiral selectand and the chiral selector of the chiral stationary phase; hence glycine, the amino acid with least steric hindrance, interacted strongly with the CSP and exhibited the greatest retention. This steric interaction provides insignificant enhancement of a stereochemical contribution to chiral recognition, because better enantioseparation of these amino acids with a longer chain or bulkier alkyl group was not observed.

Chiral separation was achieved for enantiomers of phenylalanine and tryptophan on *SS*-CSP, but no enantioseparation was observed for phenylglycine. The α-values for phenylglycine, phenylalanine and tryptophan on *SS*-CSP were 1.00, 1.77 and 1.52, respectively. The remarkably varied enantioselectivity between phenylglycine and phenylalanine or tryptophan may be related to the changed rigidity of the conformation of chiral analytes with various aromatic substituents attached to the chiral center. Apparently, the preferred orientation of the phenyl substituent attached to the chiral center of phenylglycine is unfavorable for this enantioseparation.

Comparing chromatographic results of serine and threonine with those of alanine and valine on *SS*-CSP, we found the capacity factors of amino acids with a hydroxyl group to be larger than for the corresponding amino acids with an alkyl group. This effect likely reflects the formation of additional hydrogen bonding between the hydroxyl group of serine or threonine and the secondary amino group of the chiral stationary phase. However, our chromatographic results based on α-values reveal that the formation of this hydrogen bond produces more non-enantioselective interactions. The hydroxyl group of threonine or serine may be considered to form a hydrogen bond with the unreacted amino group of the APS-modified silica. This hydrogen bond

Table 1
Enantioseparation of methyl esters of N-(3,5-dinitrobenzoyl) amino acids on CSPs



Amino acid	*SS*-CSP			*SR*-CSP			*RS*-CSP		
	k'	α	*R/S*	*k'*	α	*R/S*	*k'*	α	*R/S*
Glycine	13.27			9.26			8.85		
Alanine	5.34	1.98	*R*	5.47	1.63	*S*	3.77	1.54	*R*
Aminobutyric acid	4.85	2.38	*R*	4.32	1.66	*S*	3.22	1.59	*R*
Norvaline	3.31	2.06	*R*	3.43	1.57	*S*	2.69	1.53	*R*
Norleucine	3.20	1.90	*R*	2.96	1.41	*S*	2.14	1.42	*R*
Valine	3.48	2.74	*R*	3.19	1.67	*S*	2.42	1.61	*R*
Isoleucine	2.92	2.16	*R*	2.97	1.52	*S*	2.33	1.50	*R*
Leucine	2.79	2.02	*R*	2.78	1.44	*S*	2.30	1.45	*R*
tert.-Leucine	2.34	2.04	*R*	2.09	1.47	*S*	1.98	1.43	*R*
Phenylglycine	9.06	1.00		7.76	1.00		6.03	1.00	
Phenylalanine	8.97	1.77	*R*	7.04	1.39	*S*	7.67	1.35	*R*
Tryptophan	13.85	1.52	*R*	16.48	1.23	*S*	19.36	1.26	*R*
Serine	7.74	1.40	*R*	9.50	1.25	*S*	4.72	1.27	*R*
Threonine	5.71	1.65	*R*	7.29	1.29	*S*	8.69	1.27	*R*
Aspartic acid	12.33	1.37	*R*	10.19	1.22	*S*	8.23	1.33	*R*
Methionine	7.98	2.20	*R*	7.07	1.65	*S*	6.87	1.68	*R*

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

is non-enantioselective and simply increases the retention of both enantiomers of threonine or serine. The *k'*- and α-values obtained were 5.34 and 1.98 for alanine, but 7.74 and 1.40 for serine; 3.48 and 2.74 for valine, but 5.71 and 1.65 for threonine, respectively. As a consequence, the separation factor of threonine is smaller than that of valine, whereas the capacity factor of threonine is larger than that of valine. As the α-value of threonine was smaller than that of valine, the presence of the hydroxyl group in threonine is unfavorable for chiral discrimination. A similar situation occurs between serine and alanine.

3.2. Enantioseparation of amino alcohols

Table 2 presents results of enantiomeric separation of 3,5-dinitrobenzoyl amino alcohols on these chiral stationary phases. The variations of capacity factors of enantiomers of N-(3,5-dinitrobenzoyl) amino alcohols on these CSPs were similar to those for amino acids. This again indicates that steric interaction exists between the alkyl group of the chiral selectand and that of the chiral selector. The α-values seem less affected by changes in the chain length or the bulkiness of the alkyl group attached to the chiral center of amino alcohols. These results further support our previous finding that steric interaction exists between a bulkier or longer alkyl group of the chiral selectand and the chiral selector of the chiral stationary phase.

Generally, the α-values for amino alcohols were much smaller than those of the corresponding amino acids on the same CSPs. For instance, the α-values of alanine and alaninol were 1.98 and 1.21 on *SS*-CSP, and 1.63 and 1.00

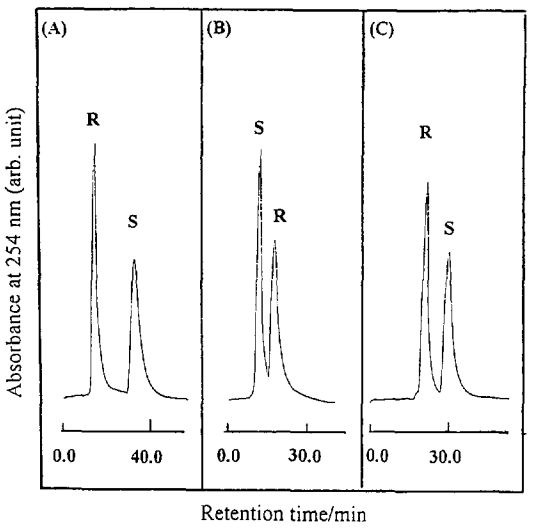


Fig. 3. Chromatograms of enantiomeric separation of methyl esters of N-(3,5-dinitrobenzoyl)valine on CSPs. (A) *SS*-CSP, (B) *SR*-CSP, and (C) *RS*-CSP. Eluent, 2-propanol–hexane (20:80, v/v); flow-rate, 1 ml/min.

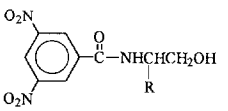
on *SR*-CSP, respectively. The results strongly indicate that the carboxyl group in amino acids plays an important role in chiral discrimination.

The enantiomers of amino alcohols with an aromatic substituent such as phenylglycinol or phenylalaninal were retained longer on these CSPs than those of amino alcohols with alkyl substituents. However, the values obtained for phenylglycinol on *SS*-CSP, *SR*-CSP and *RS*-CSP are 1.00, 1.28 and 1.38, respectively. This unexpected result is most likely related to the orientation of the phenyl substituent and the hydrogen-bonding interaction involving the hydroxyl group of phenylglycinol. Thus, structural rigidity between phenylglycinol and the CSP may play a determining factor in chiral separation.

3.3. Effect of absolute configuration of the CSP bearing two chiral centers

As shown in Tables 1 and 2, the enantioselectivity of amino acids and amino alcohols can vary greatly with the absolute configuration of the CSP bearing two chiral centers. When the absolute configuration of the CSP was altered from the (*S*,*S*) form to the (*S*,*R*) or (*R*,*S*) form, the chiral separability of amino acids and amino alcohols diminished in all instances. This significantly altered enantioselectity demonstrates that the absolute configuration of the CSP bear-

Table 2
Enantioseparation of N-(3,5-dinitrobenzoyl) amino alcohols on CSPs



Amino alcohol	*SS*-CSP			*SR*-CSP			*RS*-CSP		
	k'	*α*	*R/S*	*k'*	*α*	*R/S*	*k'*	*α*	*R/S*
Alaninol	3.07	1.21	*R*	5.98	1.00		4.16	1.00	
2-Amino-1-butanol	2.51	1.34	*R*	3.82	1.00		3.56	1.00	
2-Amino-1-pentanol	2.21	1.27	*R*	3.04	1.00		2.94	1.00	
2-Amino-1-hexanol	2.06	1.21	*R*	2.7	1.00		2.61	1.00	
Valinol	2.14	1.65	*R*	3.05	1.00		3.01	1.00	
Leucinol	2.06	1.24	*R*	2.64	1.00		2.62	1.00	
Phenylglycinol	4.90	1.00		6.31	1.28	*R*	5.77	1.39	*S*
Phenylalaninol	5.14	1.00		6.27	1.00		5.07	1.00	

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

ing two stereogenic centers may play a crucial role in chiral recognition. The loading capacity of *SS*-CSP is about the same as that of *SR*-CSP. Therefore, to discover the nature of the roles of these two chiral entities of a CSP, we performed semi-empirical AM1 calculations on diastereomeric complexes formed between chiral selectands and a chiral selector in order to predict the elution order of chiral analytes and to gain further insight into chiral recognition. The analyte that is more tightly bound to the CSP has a greater negative free energy and is retained longer. The energy difference between the *S–S* complex and the *R–S* complex determines the efficiency of chiral separation.

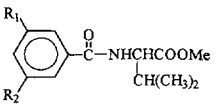
According to our computational results [42], the complex formed by the *S*-form of the methyl ester of N-(3,5-dinitrobenzoyl)valine and the *SS*-CSP is relatively more stable than the complex formed by the corresponding *R*-form of the chiral analyte on *SS*-CSP. This *S*-form analyte was retained longer than the *R*-form. The difference in interaction energy calculated for these two complexes is about 25 kJ/mol. The complex formed by the methylester of benzoyl-derivatized valine (*R*-form) and *SR*-CSP is relatively more stable than the complex formed by the corresponding *S*-form of the chiral analyte and *SR*-CSP. The energy difference between these two complexes is about 16 kJ/mol. Hence, *SS*-CSP has better enantioseparation than *SR*-CSP. These results are consistent with our chromatographic results.

3.4. Elution order and chiral recognition mechanism

As shown in Tables 1 and 2, the absolute configuration of the last-eluted analyte seems consistent with the configuration of the 1-(1-naphthyl)ethylamido moiety of the CSPs tested. For instance, the *R*-form of enantiomeric alanine was eluted before the *S*-form analyte on *SS*-CSP, whereas the *S*-form analyte was eluted before the *R*-form analyte on *SR*-CSP. Analogously, the *R*-form of enantiomeric alanine was eluted before the *S*-form analyte on *RS*-CSP. The results of AM1 calculations reveal that two mechanisms of chiral recognition are rationalized in the phenylalanyl and 1-(1-naphthyl)ethylamido moieties of CSP [42]. In the case of *SS*-CSP, chiral recognition depends predominantly on the phenylalanyl moiety. The interactions dominantly involved in chiral recognition are the $\pi-\pi$ interaction between the π-acidic 3,5-dinitrobenzoyl ring of the chiral selectands and the π-basic naphthyl ring of the selector, a hydrogen bonding between the acidic NH proton of the amide bond of the chiral selectand and the carbonyl group of the phenylalanyl moiety of CSP, and a hydrogen bonding between the NH proton of the phenylalanyl moiety of the CSP and the carboxyl group of amino acids or the hydroxyl group of amino alcohols. In the case of *SR*-CSP and *RS*-CSP, chiral recognition depends mostly on the naphthyl-ethylamido moiety. The discriminatory interactions involved in the chiral recognition are the $\pi-\pi$ interaction between the π-acidic 3,5-dinitro-benzoyl ring of the chiral selectands and the π-basic naphthyl ring of the selector, a hydrogen bond between the acidic NH proton of the amide bond of the chiral selectand and the carbonyl group of the phenylalanyl moiety of the chiral selector, and a hydrogen bonding between the NH proton of the amide bond of the naphthyl-ethylamido moiety of CSP and the carboxyl group of amino acids or the hydroxyl group of amino alcohols.

The role played by the 3,5-dinitrobenzoyl group of the chiral selectand in chiral recognition was evaluated by examining the effect of various π-acceptor substituents on the enantioseparation of methyl esters of benzoyl-derivatized amino acids. Table 3 presents such results for the dependence of the methyl ester of various valine derivatives on these CSPs. As shown in Table 3, the replacement of a 3,5-dinitrobenzoyl group with a 3-nitrobenzoyl group or a benzoyl group evidently decreases the enantioselectivity. For instance, the α-value on the *SS*-CSP decreased from 2.74 to 1.39, 1.00 and 1.00 for replacement by 3-nitrobenzoyl, 3-chlorobenzoyl and benzoyl groups, respectively. This effect is simply due to the decreased $\pi-\pi$ interaction between the chiral selector and the chiral selectand. Our chromatographic data thus confirm the importance of

Table 3
Effect of π-acceptor substituents of valine methyl ester on CSPs



R_1	R_2	*SS*-CSP		*SR*-CSP		*RS*-CSP	
		k'	α	k'	α	k'	α
NO_2	NO_2	3.48	2.74	3.19	1.67	2.42	1.61
NO_2	H	1.66	1.39	1.48	1.00	1.34	1.00
Cl	H	0.63	1.00	0.49	1.00	0.48	1.00
H	H	0.65	1.00	0.53	1.00	0.54	1.00

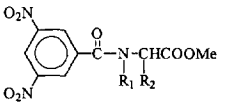
Eluent, 2-propanol–hexane (20:80, v/v); flow-rate, 1 ml/min.
k', capacity factor of the first-eluted enantiomer.

the interaction between a π-acceptor and π-donor group in the chiral discrimination process, consistent with the findings of Pirkle et al. [43].

On comparing chromatographic resolution of enantiomers of 3,5-dinitrobenzoyl amino acids with that of 3,5-dinitrobenzoyl amino alcohols, we obtained additional evidence to support the important role of the ester group in chiral recognition (Tables 1 and 2). As the absence of the ester group in 3,5-dinitrobenzoyl amino alcohols excludes the formation of a hydrogen bond between the carbonyl group of the chiral selectand and the secondary amino group of the chiral moiety of the chiral stationary phase, chiral discrimination deteriorated or was not possible on these CSPs for 3,5-dinitrobenzoyl amino alcohols.

The role of the secondary amino group in chiral analytes in chiral recognition was examined by comparing chromatographic results of enantioseparation of methyl esters of 3,5-dinitrobenzoyl alanine possessing an acidic NH

Table 4
Effect of N-methyl-substituted groups of N-(3,5-dinitribenzoyl) amino acids on CSPs



Compound	R_1	R_2	*SS*-CSP		*SR*-CSP		*RS*-CSP	
			k'	α	k'	α	k'	α
Alanine	H	CH	5.34	1.98	5.47	1.63	3.77	1.54
N-Methylalanine	CH_3	CH_3	4.56	1.00	2.81	1.00	2.85	1.00
Valine	H	$(CH_3)_2CH$	3.48	2.74	3.19	1.67	2.42	1.61
N-Methylvaline	CH_3	$(CH_3)_2CH$	3.04	1.00	3.10	1.00	3.02	1.00
Phenylalanine	H	$C_6H_5CH_2$	8.97	1.77	7.04	1.39	7.67	1.35
N-Methylphenylalanine	CH_3	$C_6H_5CH_2$	3.25	1.00	2.78	1.00	3.01	1.00

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

group with those of the N-methyl substituent of the methyl esters of 3,5-dinitrobenzoyl-alanine, -valine and -phenylalanine. The smaller capacity factors and the inability to chirally separate the N-methyl substituent of methyl esters of alanine, valine and phenylalanine are due mainly to the absence of the acidic NH group, because under these conditions, no hydrogen bond can be formed between the chiral analyte and the chiral stationary phase. Although this hydrogen bond is considered a third preferential interaction, it may not be essential for chiral discrimination, because no enantioseparation of phenylglycine was achieved on these CSPs, despite three major preferential interactions persisting between the CSP and the methyl ester of 3,5-dinitrobenzoyl phenylglycine. Hence, enantiomers of an amino acid may fail to be discriminated with only those three preferential interactions. Structural conformation and steric repulsion between a substitutional group attached to the chiral center of the chiral analyte and that of the chiral stationary phase needs to be taken into consideration in chiral recognition.

4. Conclusion

Chiral stationary phases derived from (*R*/*S*)-phenylalanyl-(*R*/*S*)-1-(1-naphthyl)ethylamine as a chiral selector were prepared by bonding a chiral moiety onto APS-modified silica gels through the *s*-triazine ring. The chromatographic results show that most amino acids, except for phenylglycine, are well resolved on these three CSPs. However, only *SS*-CSP provides excellent recognition ability to separate enantiomers of amino alcohols except phenylglycinol and phenylalaninol. In the case of *SS*-CSP, the *R*-enantiomer was eluted before the *S*-enantiomer, whereas in the case of *SR*-CSP, the *S*-enantiomer was eluted first. With the aid of AM1 calculations, the elution orders of enantiomers of amino acids on *SS*-CSP and *SR*-CSP were predicted theoretically, and the mechanisms of chiral recognition can be better understood. AM1 calculations reveal that two mechanisms of chiral recognition for CSP bearing two chiral centers are involved in the phenylalanyl and 1-(1-naphthyl)ethylamido moieties of CSP. In the case of *SS*-CSP, chiral recognition depends predominantly on the phenylalanyl moiety, whereas in the case of *SR*-CSP, the chiral recognition depends mostly on the 1-(1-naphthyl)ethylamido moiety. The theoretical predictions are consistent with experimental observations in a high-performance liquid chromatograph.

Acknowledgements

We thank the National Science Council of the Republic of China for financial support and the National Center for High-Performance Computing for generous allocations of Convex C3840 computer time.

References

[1] W.J. Lough (Editor), Chiral Liquid Chromatography, Chapman and Hall, New York, 1989.
[2] D. Stevenson and I.D. Wilson (Editors), Recent Advances in Chiral Separation, Plenum Press, New York, 1990.
[3] S. Ahuja (Editor), Chiral Separation by Liquid Chromatography, American Chemical Society, Washington, 1991.
[4] W.H. Pirkle, T.C. Pochapsky, G.S. Mahler, D.E. Corey, D.S. Reno and D.M. Alesi, J. Org. Chem., 51 (1986) 4991.
[5] W.H. Pirkle and T.C. Pochapsky, J. Am. Chem. Soc., 108 (1986) 352.
[6] W.H. Pirkle, K.C. Deming and J.A. Burke, Chirality, 3 (1991) 183.
[7] W.H. Pirkle and T.C. Pochapsky, J. Am. Chem. Soc., 108 (1986) 5627.
[8] W.H. Pirkle and T.C. Pochapsky, J. Am. Chem. Soc., 109 (1987) 5975.
[9] P. Shan, T.B. Hsu and L.B. Rogers, J. Chromatogr., 396 (1987) 31.
[10] W.H. Pirkle, J.A. Burke and R. Wilson, J. Am. Chem. Soc., 111 (1989) 9222.
[11] R. Dappen, G. Rihs and C.W. Mayer, Chirality, 2 (1990) 185.
[12] P. Salvadori, C. Pini, C. Rosini and G. Uccello-Barretta, J. Am. Chem. Soc., 112 (1990) 2707.
[13] X.-J. Lu, L.B. Rogers and J.A. deHaseth, Anal. Chem., 63 (1991) 2939.
[14] K.B. Lipkowitz, D.A. Demeter, R. Zegarra, R. Larter and T. Darden, J. Am. Chem. Soc., 110 (1988) 3446.

[15] K.B. Lipkowitz and R. Zegarra, J. Comput. Chem., 10 (1989) 595.
[16] R.E. Boehm, D.E. Martire and D.W. Armstrong, Anal. Chem., 60 (1988) 522.
[17] M.G. Still and L.B. Rogers, Talanta, 36 (1989) 35.
[18] S. Topiol and M.J. Sabio, J. Chromatogr., 461 (1989) 129.
[19] S. Topiol and M.J. Sabio, Chirality, 3 (1991) 56.
[20] K.B. Lipkowitz, R. Zegarra and B. Baker, J. Comput. Chem., 10 (1989) 718.
[21] K.B. Lipkowitz and B. Baker, Anal. Chem., 62 (1990) 770.
[22] U. Norinder and E.G. Sundholm, J. Liq. Chromatogr., 10 (1987) 2825.
[23] R. Dappen, H.R. Karfunkel and F.J.J. Leusen, J. Comput. Chem., 11 (1990) 181.
[24] C.E. Lin, F.K. Li and C.H. Lin, J. Chromatogr. A, in press.
[25] L. Oliveros, C. Minguillon and T. Gonzalez, J. Chromatogr. A, 672 (1994) 59.
[26] J.T. Thurston, J.R. Dudley, D.W. Kaiser, I. Hechenbleikner, F.C. Scheffer and D. Holm-Hansen, J. Am. Chem. Soc., 73 (1951) 2981.
[27] M.A. Ditzler, L.H. Melendez, T.J. Onofrey and K.A. Mills, Anal. Chim. Acta, 228 (1990) 235.
[28] C.E. Lin and C.H. Lin, J. Chromatogr. A, 676 (1994) 303.
[29] N. Oi and H. Kitahara, J. Chromatogr., 265 (1983) 117.
[30] N. Oi and H. Kitahara, J. Liq. Chromatogr., 9 (1986) 511.
[31] M.J.B. Lloyd, J. Chromatogr., 351 (1986) 219.
[32] W.H. Pirkle and J.E. McCune, J. Chromatogr., 471 (1989) 271.
[33] N. Bargmann-Leyder, A. Tambute, A. Begos and M. Caude, Chromatographia, 37 (1993) 433.
[34] C.E. Lin, C.H. Lin and F.K. Li, J. Chromatogr., (accepted) (Chromsymp 3373).
[35] L. Oliveros, C. Minguillon, B. Desmazieres and P.-L. Desbène, J. Chromatogr., 543 (1991) 277.
[36] M.J.E. Dewar, E.G. Zoebish, E.F. Healy and J.J.P. Stewart, J. Am. Chem. Soc., 107 (1985) 3902.
[37] M. Bodanszky and A. Bodanszky, The Practice of Peptide Synthesis, Springer, Berlin, 1984.
[38] C.E. Lin, C. Chen, C.H. Lin, M.H. Yang and J.C. Jiang, J. Chromatogr. Sci., 27 (1989) 665.
[39] W.H. Pirkle and L.J. Welch, J. Liq. Chromatogr., 14 (1991) 173
[40] M.J.S. Dewar and W.J. Thiel, J. Am. Chem. Soc., 99 (1977) 4899.
[41] M.J.S. Dewar and W.J. Thiel, J. Am. Chem. Soc., 99 (1977) 4907.
[42] C.E. Lin and F.K. Li, J. Comput. Chem., in preparation.
[43] W.H. Pirkle, K.C. Deming and J.A. Burke, Chirality, 3 (1991) 183.