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N-Arylcarbamoyl derivatives of amino acids as chiral stationary phases for optical resolution by high-performance liquid chromatography

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ABSTRACT

A series of twelve chiral stationary phases (CSPs) with arylcarbamoyl derivatives of amino acids bonded either ionically or covalently to 3-aminopropyltriethoxysilane-modified silica gel were prepared. These CSPs, except covalent CSP containing phenylcarbamoyl-(*S*)-phenylglycine, provide recognition ability for the separation of enantiomeric amide derivatives of amino acids, amino alcohols, amines and acids by high-performance liquid chromatography. Generally, the ionic type of CSPs were observed to be more effective than the corresponding covalent type. Among the twelve CSPs, the ionic type of CSPs bearing (*S*)-phenylcarbamoyl-(*S*)- or -(*R*)-phenylglycine show the best chiral resolution performance for the four types of enantiomeric solutes. Especially for enantiomeric ibuprofen, the separation factor is the best among all reported values. Based on the chromatographic behaviour in this study, it is concluded that the stereoselectivity is due to the stereochemical elements of the CSPs, the hydrogen-bonding interaction on the urea linkage, and the π–π interactions of the aromatic rings. A chiral recognition model of these CSPs for enantiomer separations is proposed.

INTRODUCTION

The development of chiral stationary phases (CSPs) has grown considerably in recent years. Chemically bonded N-(3,5-dinitrobenzoyl)phenylglycine prepared by Pirkle *et al.* [1] is a typical CSP. Although the separation mechanism for this CSP is not clearly understood in all instances, interaction forces such as charge-transfer interactions, hydrogen-bonding interactions, dipole–dipole interactions and steric effects may be involved [2]. As the dinitrobenzoyl (DNB) group is a π-acceptor, possible π–π charge-transfer interactions may occur between this group and the aromatic ring of the solute in the envisioned chiral recognition models for the resolution of enantiomeric aryl-containing amides [3,4].

Several CSPs with naphthyl or phenyl groups linked to the asymmetric carbon atom of the chiral group have also been prepared and showed chiral recognition ability for the separation of aryl-containing enantiomers [5–8]. However, the role of a benzyl group linked to the asymmetric carbon atom of the CSP for chiral resolution is still not clear.

Chromatographic resolution on CSPs containing two asymmetric centres has also been studied [8–11]. Unfortunately, chiral resolution on a CSP containing two phenyl groups located on two chiral carbons separately has not been reported elsewhere. In addition, the comparison of chromatographic behaviours on ionic- and covalent-type CSPs containing two chiral moieties has seldom been discussed.

Owing to the conformational rigidity and polar nature of the urea linkage, the urea linkage was found to be a reasonable means of connecting a chiral moiety to the silica support [12–14]. However, the chromatographic behaviour of the ionic type of CSPs containing a urea linkage has not been reported. Moreover, the role of the phenyl group of the phenylcarbamoyl moiety in the chiral recogni-

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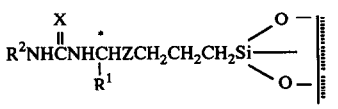
tion process has not yet been characterized as an active site for the charge-transfer interaction.

In this study, eleven of twelve CSPs (Fig. 1) were prepared by bonding N-arylcarbamoyl (CSP-7 was prepared by bonding N-phenylthiocarbamoyl) derivatives of optical active amino acids either covalently or ionically to 3-aminopropyltriethoxysilane (APS)-modified silica gel. We examined their chiral recognition ability and the contribution of the charge-transfer interaction provided by the phenyl groups of CSPs in chiral recognition. Various binding sites capable of π–π interactions were investigated on these CSPs.

CSPs containing two chiral moieties in which two phenyl groups attached separately to two different asymmetric centres were also studied. A comparative study was also made between these CSPs and the CSPs containing either one of these chiral moieties in order to shed more light on the chiral recognition process of enantiomers on these CSPs.



	R^2	X	R^1	Z	Configuration for C*	Configuration for C*
CSP-1		O	iPr	$-COO^-{}^+NH_3-$		S
CSP-2		O	iPr	$-CONH-$		S
CSP-3		O		$-COO^-{}^+NH_3-$		S
CSP-4		O		$-CONH-$		S
CSP-5		O		$-COO^-{}^+NH_3-$		S
CSP-6		O		$-CONH-$		S
CSP-7		S		$-COO^-{}^+NH_3-$		S
CSP-8		O		$-COO^-{}^+NH_3-$		S
CSP-9		O		$-COO^-{}^+NH_3-$		S
CSP-10		O		$-COO^-{}^+NH_3-$	S	S
CSP-11		O		$-CONH-$	S	S
CSP-12		O		$-COO^-{}^+NH_3-$	S	R

Fig. 1. Structures of CSPs.

These CSPs, except covalent CSP containing phenylcarbamoyl-(*S*)-phenylglycine (CSP-6), provide a recognition ability for the separation of enantiomeric amide derivatives of amino acids, amino alcohols, amines and acids by high-performance liquid chromatography (HPLC). However, the magnitude of the recognition ability varies with the detailed structures of these CSPs. A CSP containing a urea functional group has a better recognition ability than the corresponding CSP containing a thiourea functional group for the resolution of the same enantiomers. Generally, the ionic-type CSPs were observed to be more effective than the corresponding covalent-type CSPs for the chiral resolution of enantiomeric solutes. Among the nine CSPs containing one chiral moiety, the ionic-type CSP containing benzylcarbamoyl-(*S*)-phenylglycine (CSP-9) showed the best recognition ability for all types of test solutes. Moreover, the CSPs containing two chiral moieties were found to provide a better ability than the corresponding CSPs containing only one chiral moiety for the effective resolution of the four types of enantiomeric solutes.

Our results indicate that the chiral recognition model of these CSPs for enantiomer separation is based on steric interactions close to the chiral centres, the hydrogen-bonding interaction of the urea linkage and the π–π interaction of the aromatic moiety. Moreover, the chiral recognition ability of CSPs is apparently enchanced by an additional chiral centre attached to the other nitrogen atom of the carbamoyl group.

EXPERIMENTAL

Chemicals

All optically active amino acids, aryl isocyanates and (*S*)-phenylethyl isocyanate were obtained from Aldrich. The silica gel used was Nucleosil (pore size 100 Å; particle size 10 μm; surface area 350 m^2/g), obtained from Macherey–Nagel. APS was purchased from Chisso. The solutes used in the chromatographic experiments were of synthetic reagent grade (Merck).

Preparation of N-arylcarbamoyl derivatives of optically active amino acids

The amino acids used were L-phenylglycine, D-phenylglycine, L-valine and L-phenylalanine. To a

stirred solution of 0.02 mol of amino acid in 10 ml 2 *M* NaOH, a solution of 0.02 mol of aryl isocyanate in 4 ml of acetone was added. The mixed solution was reacted at room tempeature for 30 min. The product was precipitated by acidifying with 2.5 ml of 1 *M* HCl, filtered, washed with distilled water and dried at 100°C for 2 h.

Preparation of 3-aminopropylsilanized silica gel

Silica gel (3 g) dried at 180°C for 10 h, was suspended in 100 ml of dry toluene. After 1.5 ml of APS had been added, the reaction mixture was refluxed under nitrogen for 10 h with stirring. The silane-modified silica gel thus obtained was filtered, washed with methanol, water and acetone and then dried under vacuum overnight. Results of elemental analysis are given in Table I.

Preparation of ionic-type CSPs

To a solution of 0.01 mol of arylcarbamoyl derivative of optically active amino acid in 100 ml of tetrahydrofuran (THF), 2.5 g of silanized silica gel were added. The mixture was subjected to ultrasonic vibration for 10 min, followed by further stirring for 4 h. The product was filtered, washed with THF and dried under vacuum overnight.

Preparation of covalent-type CSPs

A solution of 0.01 mol of arylcarbamoyl derivative of L-amino acid in 100 ml of dimethylformamide was cooled in an ice-bath, 10 mmol 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 3-aminopropylsilanized silica 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 prepared CSPs are given in Table I.

Chromatographic studies

The chromatographic studies were carried out with a Kratos liquid chromatographic system which consisted of a Spectroflow 400 solvent-delivery system, a Spectroflow 480 injector and a Spectroflow 757 variable-wavelength UV detector. The recorder used was a Model 12 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 room temperature. Sample solutes, which were derivatized by a routine method, were dissolved in methanol and suitable amounts of the solutions were injected. Chromatographic peaks were assigned by injecting the corresponding derivative of enantiomeric enriched solute.

TABLE I

CHARACTERISTICS OF 3-AMINOPROPYLSILICA AND THE CSPs

Sample	Elemental analysis (%)			Loading capacity[a] (mmol/g)
	C	H	N	
3-Aminopropylsilica	3.74	1.00	1.31	0.93
CSP-1	11.12	1.80	2.58	0.47
CSP-2	11.52	1.82	2.33	0.38
CSP-3	13.66	1.98	2.35	0.39
CSP-4	13.60	1.90	2.32	0.37
CSP-5	10.00	1.48	2.20	0.34
CSP-6	9.97	1.50	1.91	0.33
CSP-7	11.91	1.95	2.15	0.32
CSP-8	19.93	3.24	2.33	0.38
CSP-9	11.29	1.82	2.35	0.39
CSP-10	13.85	1.57	2.63	0.48
CSP-11	11.21	1.54	2.24	0.35
CSP-12	13.76	1.98	2.54	0.46

[a] Based on the percentage of nitrogen.

RESULTS AND DISCUSSION

Twelve chiral stationary phases were successfully prepared by bonding N-arylcarbamoyl and N-phenylthiocarbamoyl derivatives of optically active amino acids either covalently or ionically, to APS-modified silica gel as shown in Fig. 2. Fig. 1 shows the structures of these CSPs. The presence of the

TABLE II

RESOLUTION OF DERIVATIZED ENANTIOMERS ON CSPs

Solute	α^a (configuation[b])											
	Ionic-type CSPs								Covalent-type CSPs			
	1	3	8	7	5	9	10	12	2	4	6	11
Amino acids[c]												
Valine	1.18 (*R*)	1.20 (*R*)	1	1.12 (*S*)	1.18 (*S*)	1.67 (*S*)	1.27 S	2.64 (*R*)	1.27 (*R*)	1.13 (*R*)	1	1.37 (*S*)
Leucine	1.26 (*R*)	1.28 (*R*)	1	1.24 (*S*)	1.26 (*S*)	1.58 (*S*)	1.41 S	1.82 (*R*)	1.32 (*R*)	1.14 (*R*)	1	1.18 (*S*)
Phenylalanine	1.18 (*R*)	1.18 (*R*)	1	1.07 (*S*)	1.06 (*S*)	1.39 (*S*)	1.22 S	1.71 (*R*)	1.26 (*R*)	1.02 (*R*)	1	1.17 (*S*)
Alanine	1.26 (*R*)	1.32 (*R*)	1	1	1.06 (*S*)	1.18 (*S*)	1	1.56 (*R*)	1.18 (*R*)	1.01 (*R*)	1	1.19 (*S*)
Methionine	1.31 (*R*)	1.31 (*R*)	1.47 (*S*)	1.08 (*S*)	1.06 (*S*)	1.30 (*S*)	1.18 S	1.91 (*R*)	1.36 (*R*)	1.11 (*R*)	1	1.21 (*S*)
Amino alcohols[d]												
2-Aminobutanol	1.17 (*R*)	1.12 (*R*)	1	1.08 (*S*)	1.13 (*S*)	1.41 (*S*)	1.57 S	1.74 (*R*)	1.08 (*R*)	1	1	1
2-Aminopropanol	1.11 (*R*)	1.13 (*R*)	1	1	1	1.24 (*S*)	1.32 S	1.41 (R)	1	1	1	1
Norephedrine	1.12	1.11	1	1	1.06	1.02	1.14	1.09	1	1	1	1
Amines[d]												
1,2-Diaminopropane	1.19	1.15	1		1.15	1.09	1.04	1.16	1	1	1	1.07
Phenylethylamine	1	1	1	1	1.04 (*R*)	1.09 (*R*)	1.37 R	1.18 (*R*)	1	1	1	1.18 (*R*)
1-Methylbutylamine	1	1	1	1.07	1.13	1.18	1.25	1.23	1	1	1	1
Acids[e]												
Ibuprofen	1	1	1.12 (*R*)[f]	1	1.14 (*R*)[f]	1.16 (*S*) 1.18 (*S*)[f]	1.93 S	1.20 (*S*)	1	1	1	1.32 (*S*)

[a] The separation factor of the enantiomers is the ratio of their capacity factors. Mobile phase: 2-propanol–hexane (10:90).
[b] Configuration of the first-eluted enantiomer.
[c] As N-dinitrobenzamide-O-methyl ester derivatives.
[d] As N-dinitrobenzamide derivatives.
[e] As dinitroanilide derivatives, except where indicated.
[f] As N-naphthylamide derivatives.

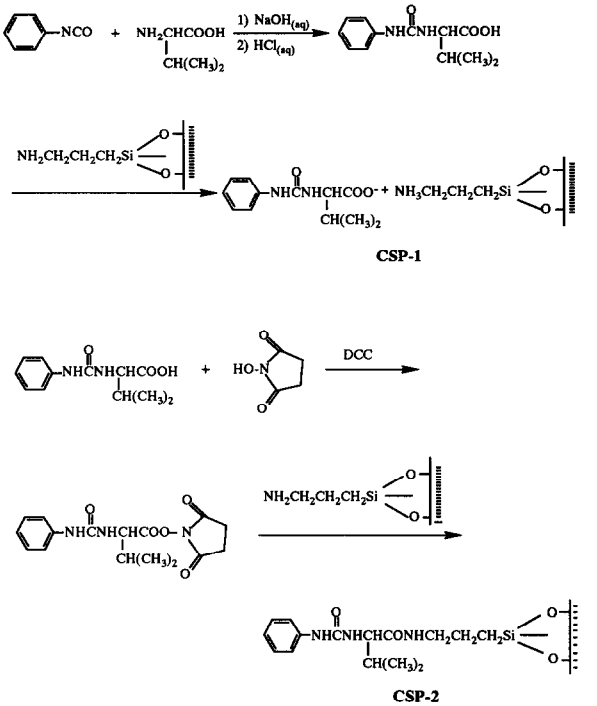


Fig. 2. Examples of the preparation of CSPs.

chiral ligands on the silica surface of the prepared CSPs was characterized by FT-IR spectrometry and combustion elemental analysis (Table I).

Chromatographic behaviours of CSPs

The chromatographic resolution ability of the amide derivatives of some representative racemic amino acids, amino alcohols, amines and carboxylic acids on these CSPs were examined and the results are summarized in Table II. Typical chromatograms are presented in Fig. 3. According to the separation factors (α), nearly all of the arylcarbamoyl-derived CSPs provide sufficient resolution for N,N-dinitrobenzamide (DNB) or N,N-dinitroanilide (DNA) derivatives. In general, the ionic-type CSPs were found to be more effective than the corresponding covalent-type CSPs for the resolution of enantiomeric amide derivatives. It is interesting that there were no significant resolutions on the covalent type of phenylcarbamoyl-(*S*)-phenylglycine (CSP-6) for any of the chosen solutes, whereas excellent resolutions for amino acids were obtained on the covalent type of phenylcarbamoyl-(*S*)-valine (CSP-2).

Contribution of the carbamoyl moiety

Compared with the ionic-type phenylcarbamoyl-(*S*)-phenylglycine-derived CSP-5, the corresponding thiourea linkage-containing CSP-7 showed a worse resolution ability and shorter solute retention. This indicates not only that did the thiourea linkage form weaker intermolecular complexes than the urea linkage with solutes, but also that the hydrogen bonding interaction contributed by the urea functional group is stereoselective in chiral recognition. Other than hydrogen bonding sites in CSPs, the hydrogen bonding interaction site of the solute also supported this assumption. Although the DNB derivative of 2-methylbutylamine with an amide group attached to an achiral carbon could not be resolved on all CSPs, the DNB derivative of 1-methylbutylamine could be effectively resolved on phenylglycine-derived CSPs (Table II).

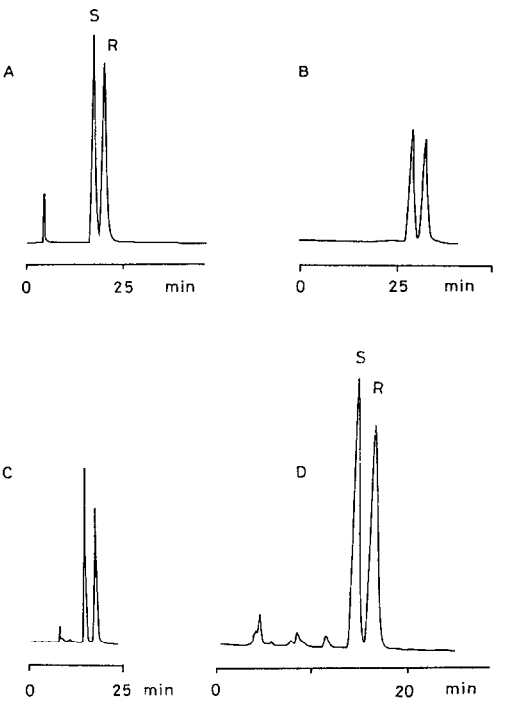


Fig. 3. Chromatographic separation of the enantiomers on CSPs. (A) Alanine ad DNB-O-methyl ester derivative on CSP-11; (B) norephedrine as DNB derivative on CSP-10; (C) 1-methylbutylamine as DNB derivative on CSP-10; (D) ibuprofen as N-naphthylamide derivative on CSP-9. Mobile phase: (A–C) 2-propanol–*n*-hexane (10:90); (D) 2-propanol–*n*-hexane (1:99).

Steric effect of the carbamoyl moiety

Naphthylcarbamoyl-(*S*)-phenylglycine-derived CSP-8, with a bulky R^2 group, showed significant resolution only for methionine DNB derivatives and ibuprofen DNA derivatives. However, benzylcarbamoyl-(*S*)-phenylglycine-derived CSP-9, with a less hinder R^2 group than the phenyl group on the carbamoyl moiety, showed the best chiral resolution performance for all test solutes among the nine CSPs that contained one chiral moiety. Hence the steric effect of the arylcarbamoyl moiety on the phenylglycine-derived CSPs was quite important.

Contribution of phenyl groups on the CSPs

According to the α values, the effects of the position of the phenyl group on the CSPs were different for the various solutes. For DNB derivatives of amino acids, amino alcohols and diamines, a phenyl group either attached to any of the chiral centres or as a substituent of the carbamoyl moiety of the CSPs will contribute an active π–π interaction site for chiral recognition. For the amide derivatives of amines and acids, the stereoselective π–π interaction site must be directly attached to the chiral centre on either side of the urea linkage. If the phenyl group is removed from this centre by even a urea linkage or a single methylene group, as in CSP-1 and CSP-3, there was no chiral resolution at all. As shown in Table II, an additional phenyl-substituted chiral moiety (as in CSP-10 *vs.* CSP-9) could provide a great improvement in the resolution of the amide derivatives of amines and acids.

The good resolution for the DNB derivatives of amino acids, amino alcohols and diamines on CSP-1 suggested that the phenyl group removed from the chiral centre by a urea linkage presumably contributed a stereoselective π–π interaction site for racemates. The enantiomeric elution order was determined by the amide synthesis either from L-amino acids or amino alcohols. The *R*-form enantiomer was first eluted on phenylcarbamoyl-(*S*)-valine-derived CSPs, whereas the *S*-form enantiomer was eluted first on arylcarbamoyl-(*S*)-phenylglycine-derived CSPs. Unlike CSP-1, both CSP-5 and CSP-3 provide an additional π–π interaction owing to the presence of phenyl and benzyl groups attached to the chiral centre of the L-amino acid moiety, respectively. However, the opposite elution order observed on CSP-5 suggests that the additional π–π interaction provided by a phenyl group attached to the chiral centre presumably results in an alterative chiral recognition model other than that on CSP-1, whereas the additional π–π interaction provided by a benzyl group attached to the chiral centre (CSP-3) does not. Further, it was also found that in CSP-10 and CSP-12, the change in the configuration on the chiral centre of the amino acid moiety on the CSP resulted in a change in the enantiomeric elution orders for the DNB derivatives of amino acids and amino alcohols. These facts reveal that, in the prepared CSPs, the π–π interaction provided by the phenyl group, but not the benzyl group, which is attached to the chiral centre of the amino acid moiety on the CSP acts as a stereoselective site and is important for the chiral discrimination of all the enantiomers in this work.

Advantage of two chiral moieties in the same CSP

Baseline separation was achieved for almost all of the enantiomeric solutes on the CSPs containing two chiral moieties. Especially the selectivity for enantiomeric ibuprofen, which is a non-stereoidal anti-inflammatory antipyretic analgesic, is the best among all values reported. In order to shed more light on the chiral recognition process of enantiomers on these CSPs, a comparative study between these CSPs and CSPs containing either one of these chiral moieties was made. The separation factors of amino acids, amino alcohols and amines on ionic- and covalent-type CSPs with two chiral centres (CSP-10 and CSP-11) were greater than those on the corresponding types with one chiral centre (CSP-5, -6 and -9). In addition, the chiral resolution on CSP-11 was also found to be more effective than that on Supelcosil LC-(*R*)-urea [13], in which the phenylethylamino chiral moiety was bonded to 3-aminopropylsilica through a urea functional group, for the enantiomeric separation of amino acids [4]. These results indicate that the chiral recognition ability can be improved by introducing an additional chiral moiety to the CSP backbones.

CONCLUSIONS

In the series CSPs derived from N-arylcarbamoyl derivatives of optically active amino acids, it is clear that in an arylcarbamoyl-derived CSP, owing to the

conformational rigidity and the hydrogen bonding available from the urea linkage, both the aryl group and the carbamoyl group of the carbamoyl moiety may interact with the racemates stereoselectively in the chiral recognition process. Ionic-type CSPs with the urea linkage always show better chiral recognition ability than the corresponding covalent-type CSPs. The elution orders of the analytes makes it clear that a phenyl group which is directly attached to the chiral centre of the CSPs provides stereoselective interaction, whereas a benzyl group does not. A phenyl group removed from the chiral centre by a urea linkage or a single methylene group shows no resolution ability for amide derivatives of amines and acids. Both chiral centres on either N atom of the urea linkage are stereoselective in the chiral recognition process. Therefore, CSPs containing two such chiral moieties provide the best chiral recognition ability and result in baseline separations for nearly all the chosen solutes.

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