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Direct enantiomer separations by high-performance liquid chromatography with chiral urea derivatives as stationary phases

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Abstract

The chromatographic properties of urea derivatives derived from (*S*)- and (*R*)-1-(α-naphthyl)ethylamine with (*S*)-valine, (*S*)-*tert.*-leucine, (*S*)-proline and (*S*)-indoline-2-carboxylic acid bonded to 3-aminopropylsilica gel as chiral stationary phases were examined by HPLC to investigate the effect of the structure of the amino acid moiety. The specific enantiomer separation of various racemic compounds including alcohols, esters, amines, amino alcohols, carboxylic acids and amino acids was accomplished on these phases using normal mobile phases. The effect of the stereochemical structure in these urea derivatives on chiral recognition is discussed.

1. Introduction

It is well known that high-performance liquid chromatography (HPLC) using chiral stationary phases (CSPs) is a powerful tool for the direct enantiomer separation [1,2]. One of the most important classes of CSPs is the "brush-type" CSP, containing various low-molecular-mass chiral selectors. Pioneering work in this area was done by Pirkle et al. [3], and many useful CSPs have subsequently been developed. It is believed that chiral recognition on this type of CSP is based on at least three simultaneous interactions, hydrogen bond formation, $\pi-\pi$ interactions and dipole–dipole stacking. We have found [4,5] that CSPs derived from (*S*)- and (*R*)-1-(α-naphthyl)-ethylamine with (*S*)-valine or (*R*)-phenylglycine chemically bonded to 3-aminopropylsilanized silica (CSPs 1, 2, 3 and 4) showed excellent enantioselectivity. Accordingly, it was expected that similar urea derivatives containing another amino acid moieties would provide characteristic enantioselectivities. Recently we have developed six CSPs (CSPs 5–10) derived from (*S*)- and (*R*)-1-(α-naphthyl)ethylamine with (*S*)-*tert.*-leucine, (*S*)-proline and (*S*)-indoline-2-carboxylic acid covalently bonded to 3-aminopropylsilanized silica. These CSPs are now commercially available, and some application data are given in brochures.

In this study, we examined their chromatographic properties as CSPs to investigate the effect of the structure of the amino acid moiety. CSPs 1 and 2 were also used to compare the enantioselectivity with these CSPs.

2. Experimental

2.1. Chiral stationary phases

The structures of the CSPs are shown in

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Fig. 1. CSPs 5–10 were synthesized using (*S*)-*tert.*-leucine, (*S*)-proline and (*S*)-indoline-2-carboxylic acid instead of (*S*)-valine in CSPs 1 and 2 by the procedures described previously [4]. Develosil-NH_2 (5 μm) (Nomura Chemical, Seto, Japan) was used as the starting material. Grafting rates were calculated according to the C and N elemental analysis for each CSP: CSP 5, 0.43; CSP 6, 0.41; CSP 7, 0.48; CSP 8, 0.49; CSP 9, 0.44; and CSP 10, 0.43 mmol/g.

2.2. Liquid chromatography

The experiments were carried out using a Waters Model 510 high-performance liquid chromatograph, equipped with a variable-wavelength UV detector operated at 230, 254 and 280 nm. Stainless-steel columns (250 × 4.6 mm I.D.) were slurry packed with these CSPs using a conventional technique. These columns are available from Sumika Chemical Analysis Service (Osaka, Japan), as Sumichiral OA-4000 (CSP 1), OA-4100 (CSP 2), OA-4600 (CSP 5), OA-4700 (CSP 6), OA-4400 (CSP 7), OA-4500 (CSP 8), OA-4800 (CSP 9) and OA-4900 (CSP 10). Solutes and solvents of analytical-reagent grade were purchased from Wako (Osaka, Japan). Some compounds were kindly provided by Sumitomo Chemical (Osaka, Japan). The structures of the racemic compounds used are shown in Fig. 2. The chromatographic conditions are given in Table 1.

3. Results and discussion

The chromatographic results are summarized in Table 1. The direct separation of a wide range of racemic compounds was accomplished on these CSPs using normal mobile phases.

The separation of racemic pyrethroid insecticide esters (**1** and **2**) was achieved on CSP 5

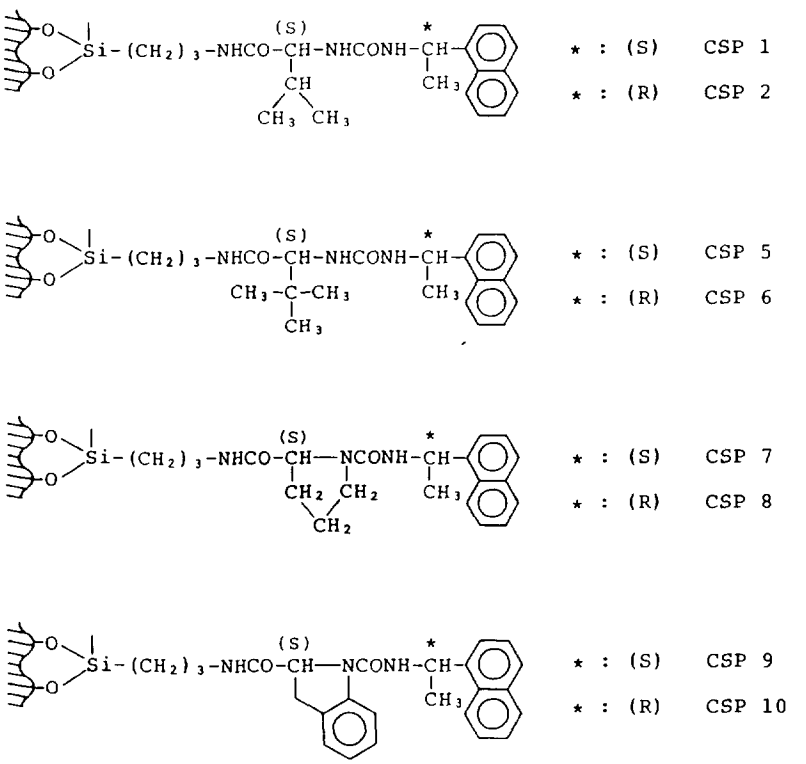

Fig. 1. Structures of the CSPs.

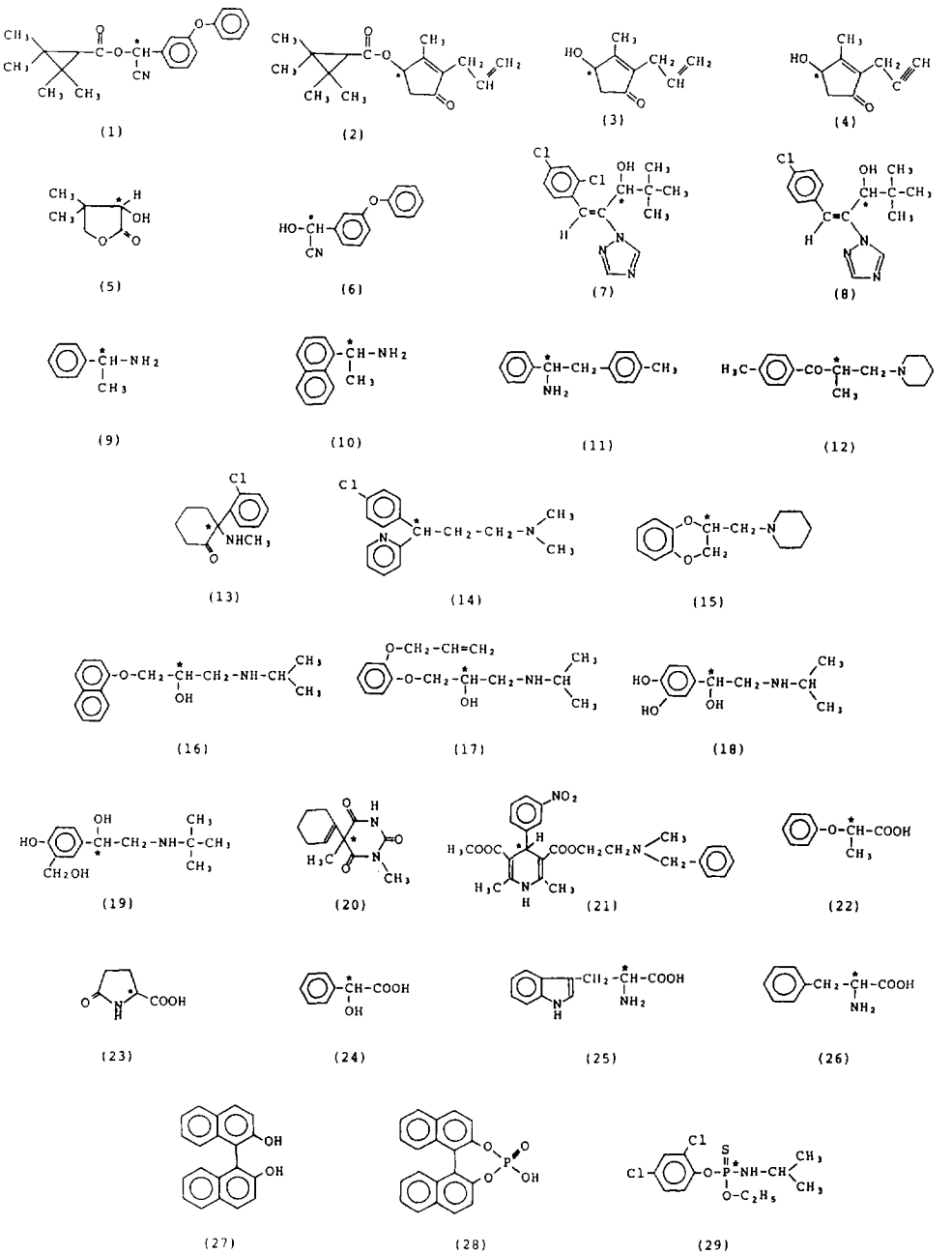

Fig. 2. Structures of racemic compounds in Table 1.

and also on CSP 1 as reported previously [6], and approximate values of the separation factors showed that the enantioselectivity was not remarkably influenced by the bulky *tert.*-butyl group in place of the isopropyl group attached to the asymmetric carbon atom in the amino acid moiety. On the other hand, various racemic alcohols, amines, amino alcohols and carboxylic acids were well resolved on CSPs 6 and 2. Typical chromatograms are shown in Figs. 3 and

Table 1
HPLC separation of enantiomers on chiral stationary phases

No.	Compound	CSP 1			CSP 2			CSP 5			CSP 6			CSP 7			CSP 8			CSP 9			CSP 10		
		k'_1	α	M	k'_1	α	M	k'_1	α	M	k'_1	α	M	k'_1	α	M	k'_1	α	M	k'_1	α	M	k'_1	α	M
1	Fenpropathrin	4.22	1.11	A	3.03	1.04	A	2.90	1.26	A	2.37	1.11	A	3.91	1.00	A	2.10	1.09	A	2.38	1.00	A	2.57	1.00	A
2	Terallethrin	5.32	1.16	B	3.22	1.00	B	7.48	1.18	B	3.21	1.02	B	5.57	1.08	A	2.76	1.03	A	4.66	1.05	A	6.99	1.00	A
3	Allethrolone	12.21	1.05	C	11.86	1.10	C	11.13	1.08	C	10.71	1.12	C	9.99	1.00	C	8.17	1.08	C	12.07	1.03	C	6.25	1.00	C
4	Propargyllone	15.69	1.04	C	17.19	1.09	C	14.06	1.06	C	16.78	1.10	C	17.15	1.00	C	13.46	1.07	C	21.55	1.04	C	11.09	1.00	C
5	Pantolactone	4.38	1.04	D	4.32	1.10	D	4.61	1.00	D	3.72	1.06	D	7.26	1.07	C	5.47	1.08	C	3.47	1.08	C	4.40	1.03	C
6	α-Cyano-3-phenoxybenzyl alcohol	4.80	1.00	E	4.46	1.08	E	3.89	1.00	E	3.66	1.09	E	12.93	1.08	E	11.22	1.05	E	4.61	1.03	E	4.38	1.03	E
7	Diniconazole	3.99	1.22	C	3.32	1.27	C	2.99	1.32	C	2.53	1.36	C	3.42	1.00	C	2.99	1.02	C	4.48	1.05	C	2.70	1.00	C
8	Uniconazole	5.45	1.15	C	5.04	1.18	C	4.28	1.21	C	3.46	1.29	C	4.46	1.00	C	3.54	1.00	C	5.83	1.07	C	3.39	1.02	C
9	1-Phenylethylamine	7.19	1.05	F	8.77	1.07	F	5.63	1.04	F	10.53	1.08	F	22.10	1.00	F	11.51	1.00	F	12.55	1.00	F	10.31	1.06	F
10	1-(α-Naphthyl)ethylamine	7.97	1.04	F	8.61	1.08	F	8.32	1.03	F	9.08	1.16	F	24.77	1.03	F	12.93	1.04	F	13.15	1.08	F	12.03	1.00	F
11	1-Phenyl-2-(*p*-tolyl)ethylamine	5.13	1.00	F	5.47	1.06	F	4.89	1.04	F	5.63	1.09	F	15.21	1.09	F	7.77	1.09	F	10.78	1.09	F	9.39	1.00	F
12	Tolperisone	6.72	1.15	F	6.14	1.33	F	5.00	1.15	F	2.96	1.36	F	4.78	1.22	F	6.07	2.10	F	19.34	1.00	F	10.22	1.44	F
13	Ketamine	1.84	1.00	G	2.07	1.00	G	2.02	1.00	G	1.68	1.00	G	5.21	1.03	G	3.10	1.05	G	13.11	1.00	G	7.78	1.12	G
14	Chlorpheniramine	5.71	1.00	H	6.58	1.21	H	6.09	1.03	H	3.26	1.16	H	5.44	1.11	H	13.55	1.37	H	20.59	1.19	H	10.37	1.05	H
15	Piperoxane	1.50	1.07	I	2.30	1.11	I	1.75	1.06	I	1.26	1.00	I	4.80	1.22	I	2.13	1.07	I	6.72	1.33	I	6.70	1.00	I
16	Propranolol	9.50	1.00	J	9.90	1.08	J	8.09	1.00	J	9.41	1.10	J	7.71	1.00	J	11.81	1.00	J	14.83	1.00	J	10.54	1.13	J
17	Oxprenolol	5.54	1.00	J	6.04	1.07	J	5.17	1.00	J	4.97	1.06	J	5.51	1.00	J	6.41	1.05	J	10.44	1.00	J	6.25	1.06	J
18	Isoproterenol	7.97	1.03	K	5.54	1.09	K	5.59	1.09	K	6.41	1.10	K	8.84	1.00	K	12.38	1.03	K	7.15	1.00	K	8.53	1.17	K
19	Salbutamol	8.89	1.05	K	5.91	1.09	K	7.02	1.13	K	4.99	1.17	K	5.89	1.00	K	7.82	1.00	K	5.11	1.08	K	5.41	1.33	K
20	Hexobarbital	4.58	1.00	L	5.13	1.00	L	5.76	1.00	L	5.56	1.07	L	6.12	1.00	L	6.24	1.00	L	6.94	1.08	L	6.37	1.03	L
21	Nicardipine	4.51	1.03	I	4.52	1.00	I	6.76	1.03	I	3.69	1.00	I	5.67	1.00	I	8.20	1.06	I	11.03	1.00	I	9.87	1.00	I
22	2-Phenoxypropionic acid	4.17	1.03	L	4.08	1.00	L	4.45	1.03	L	4.34	1.03	L	5.57	1.11	L	5.43	1.05	L	4.58	1.04	L	3.56	1.09	M
23	Pyroglutamic acid	6.44	1.07	M	6.91	1.07	M	6.95	1.08	M	7.04	1.11	M	8.84	1.09	M	9.46	1.07	M	8.77	1.00	M	8.45	1.06	M
24	Mandelic acid	7.32	1.03	N	7.75	1.03	N	5.80	1.04	N	4.76	1.04	N	7.82	1.05	N	10.17	1.06	N	6.59	1.03	N	7.57	1.04	N
25	Tryptophan	8.00	1.00	O	5.99	1.09	O	6.77	1.00	O	5.04	1.08	O	16.62	1.07	O	11.15	1.06	O	6.94	1.21	O	6.90	1.00	O
26	Phenylalanine	4.78	1.00	O	3.62	1.03	O	4.05	1.00	O	3.24	1.00	O	5.41	1.00	O	3.80	1.06	O	3.19	1.34	O	3.41	1.00	O
27	1,1′-Bi-2-naphthol	1.84	1.34	D	1.87	1.22	D	1.96	1.35	D	1.90	1.27	D	12.90	1.10	D	11.16	1.14	D	2.70	1.10	D	2.63	1.00	D
28	1,1′-Binaphthyl-2,2′-diyl hydrogenphosphate	3.70	1.14	P	4.01	1.13	P	5.04	1.07	P	3.06	1.21	P	4.22	1.03	P	1.85	1.00	P	5.08	1.05	P	6.72	1.09	P
29	O-Ethyl-O-2,4-dichlorophenyl-N-isopropylphosphoramidothioate	0.50	1.00	B	0.56	1.14	B	0.74	1.00	B	1.14	1.24	B	0.86	1.00	B	1.05	1.00	B	1.35	1.00	B	0.96	1.00	B

The separation factor of the enantiomers, α, is the ratio of their capacity factors; k'_1 is the capacity factor of the initially eluted enantiomer. Mobile phase (M): A = *n*-hexane–1,2-dichloroethane–ethanol (500:10:0.05); B = *n*-hexane–1,2-dichloroethane–ethanol (500:30:0.15); C = *n*-hexane–1,2-dichloroethane–ethanol (100:20:1); D = *n*-hexane–1,2-dichloroethane–ethanol (50:10:1); E = *n*-hexane–1,2-dichloroethane–ethanol–acetic acid (500:150:5:0.6); F = *n*-hexane–ethanol–trifluoroacetic acid (240:10:1); G = *n*-hexane–ethanol–trifluoroacetic acid (200:40:0.6); H = *n*-hexane–1,2-dichloroethane–methanol–trifluoroacetic acid (250:140:10:1); I = *n*-hexane–1,2-dichloroethane–ethanol–trifluoroacetic acid (240:140:20:1); J = *n*-hexane–1,2-dichloroethane–ethanol–trifluoroacetic acid (250:140:10:1); K = *n*-hexane–1,2-dichloroethane–methanol–trifluoroacetic acid (240:140:20:1); L = *n*-hexane–ethanol–trifluoroacetic acid (500:5:0.6); M = *n*-hexane–ethanol–trifluoroacetic acid (90:10:0.2); N = *n*-hexane–1,2-dichloroethane–ethanol–trifluoroacetic acid (400:90:10:1); O = *n*-hexane–1,2-dichloroethane–methanol–trifluoroacetic acid (50:45:5:0.2), P = 0.03 *M* ammonium acetate in methanol. A flow-rate of 1.0 ml/min was used for the 250 × 4.6 mm I.D. column at room temperature.

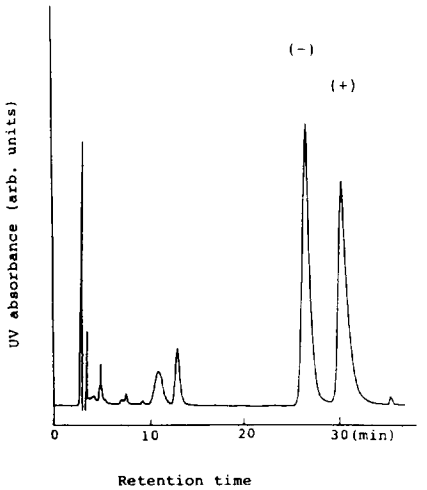

Fig. 3. Enantiomer separation of racemic 1-(α-naphthyl)-ethylamine on CSP 6. Chromatographic conditions as in Table 1.

4. Judging from the separation factors obtained on CSPs 6 and 2, the steric effect of the *tert.*-butyl group may be weak.

Racemic fenpropathrin (**1**) and terallethrin (**2**) were resolved on CSPs 5 and 1 but poorly or not resolved on CSPs 6 and 2. In contrast, racemic propranolol (**16**) and oxprenolol (**17**) were resolved on CSPs 6 and 2, but not at all on CSPs 5 and 1. Although CSPs 5 and 6 contain (*S*)-*tert.*-leucine and CSPs 1 and 2 contain (*S*)-valine, the configuration of the 1-(α-naphthyl)ethylamine moiety is reversed between CSPs 5 and 6 and between CSPs 1 and 2. The results clearly show that the combination of the configuration of two chiral centres in these urea derivatives may play an important role in chiral recognition [7].

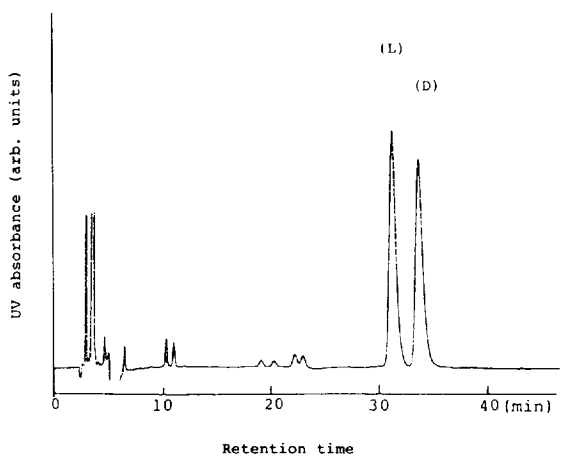

Fig. 4. Enantiomer separation of racemic pyroglutamic acid on CSP 6. Chromatographic conditions as in Table 1.

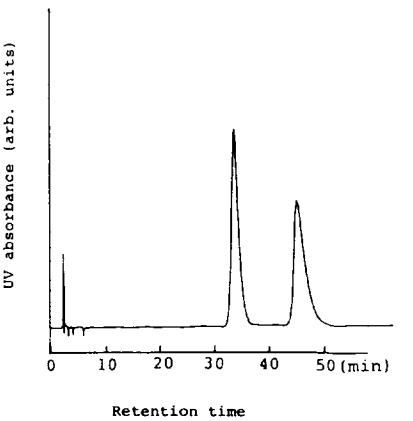

Fig. 5. Enantiomer separation of racemic chlorpheniramine on CSP 8. Chromatographic conditions as in Table 1.

A number of racemic amines, amino alcohols, carboxylic acids, amino acids and miscellaneous compounds of pharmaceutical interest were resolved specifically into their antipodes on CSPs 7, 8, 9 and 10 using normal mobile phases containing a small amount of trifluoroacetic acid. Typical examples are shown in Figs. 5–7. It should be emphasized that the enantioselectivities of CSPs 9 and 10 were different. Enantiomers of **10**, **11** and **15** were resolved on CSP 9

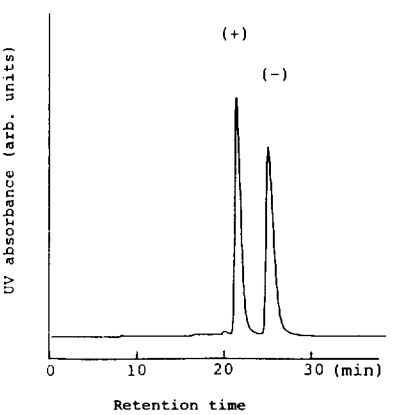

Fig. 6. Enantiomer separation of racemic isoproterenol on CSP 10. Chromatographic conditions as in Table 1.

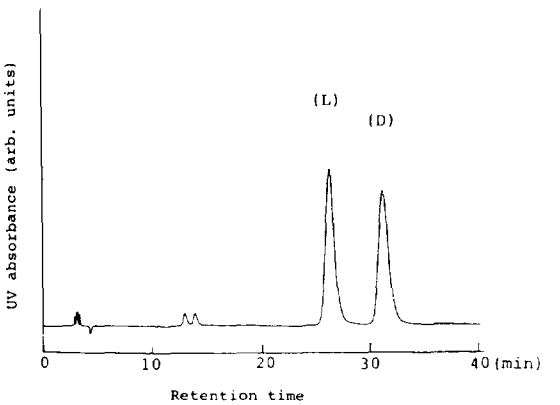

Fig. 7. Enantiomer separation of racemic tryptophan on CSP 9. Chromatographic conditions as in Table 1.

but not at all on CSP 10. In contrast, **12**, **13**, **16** and **18** enantiomers were resolved on CSP 10, but not at all on CSP 9. These results again showed that chiral recognition was controlled by the combination of configurations of the two chiral centres in the amino acid and amine moieties of these urea derivatives.

Amino acid enantiomers **25** and **26** were directly resolved on CSP 9 using normal mobile phases. Usually the direct separation of racemic amino acids can be accomplished with copper(II) complexes of chiral ligands as CSPs using aqueous mobile phases [8,9], and they are resolved in the form of derivatives such as N-acyl O-esters on brush-type CSPs using normal mobile phases [3,4].

The specific separation of various racemic compounds on these CSPs showed that the enantioselectivity of urea derivatives may depend on the structure of the amino acid moiety. However, the detailed effect of structure on chiral recognition is still unclear, and additional work is in progress.

The durability of columns packed with these CSPs was excellent; more than 300 analyses of some racemic compounds did not cause any change in their retention parameters, enantioselectivity or efficiency using the normal mobile phases described in Table 1.

4. Conclusions

Urea derivatives derived from (*S*)- or (*R*)-1-(α-naphthyl)ethylamine with (*S*)-valine, (*S*)-*tert.*-leucine, (*S*)-proline and (*S*)-indoline-2-carboxylic acid bonded to 3-aminopropylsilica gel are very promising as CSPs for the specific separation of various racemic compounds by HPLC. It is emphasized that the stereochemical structure of these CSPs may play an important role in the formation of interactions for chiral recognition.

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