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Preparation and chiral recognition of (*S*)-binaphthol derivative-bonded phase for high-performance liquid chromatography

Yoshihisa Sudo[a,*], Tomohiko Yamaguchi[b], Toshio Shinbo[b]

[a]*Chemicals Inspection and Testing Institute, Division of Research and Development, 4-1-1 Higashimukojima, Sumida-ku, Tokyo 131, Japan*

[b]*National Institute of Materials and Chemical Research, 1-1 Higashi, Tsukuba, Ibaraki 305, Japan*

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Abstract

(*S*)-Binaphthol derivative-bonded phases were prepared for direct chiral separation by high-performance liquid chromatography. The bonded phases were prepared by methylation of the hydroxyl groups or introduction of aryl groups at 3,3′-positions in the binaphthol moiety. Methylation varied retention and enantioselectivity for amines, which clarified that the hydroxyl groups are essential for chiral recognition of amines. Substitution of phenyl or naphthyl groups at 3,3′-positions of the binaphthol moiety increased both hydrophobicity and steric hindrance, which also vary retention and enantioselectivity of analytes.

Keywords: Chiral stationary phases, LC; (*S*)-Binaphthol derivative; Amines

1. Introduction

Chiral stationary phases (CSPs) have been developed for direct separation of enantiomers by high-performance liquid chromatography (HPLC). Many CSPs have been prepared from synthetic selectors with an asymmetric center and their mechanisms of chiral recognition have been studied in detail [1–4], whereas only a few CSPs derived from axially asymmetric selectors have been reported. Tichy et al. [5] reported CSPs on which biphenyl-2,2′-dicarbonic acid or 2,2′-bipyridine-3,3′-dicarbonic acid was ionically bonded. Newcomb et al. [6] synthesized chiral crown ethers from binaphthol, which exhibited excellent enantioselectivity for amino acids. Enantiomers of amino acids have been separated with high resolution by HPLC on a CSP prepared from such chiral crown ethers [7,8]. Pirkle and Schreiner [9] predicted that a CSP derived from binaphthol would show chiral recognition for *N*-3,5-dinitrobenzoyl amino acids, since the enantiomers of binaphthol were separated by HPLC on a CSP derived from *N*-3,5-dinitrobenzoyl-α-phenylglycine. Oi and coworkers [10,11] prepared CSPs with binaphthalene or bianthracene derivatives for enantiomer resolution of 3,5-dinitrophenyl derivatives, and attributed its mechanism to the π–π interaction.

Previously, we prepared a binaphthol-bonded CSP (CSP-BN) that was designed to maintain two hydroxyl groups of binaphthol to separate basic enantiomers without the need for their prederivatization [12]. Enantiomers of secondary and tertiary amines

*Corresponding author. Address for correspondence: Chemicals Inspection and Testing Institute, Chemical Biotesting Center, 19–14 Chuo-machi, Kurume, Fukuoka 830, Japan.

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were separated on this CSP, suggesting that the two hydroxyl groups are important in the chiral recognition. Additives in the mobile phase seemed to give a clue for understanding the mechanism of enantiomer separation. Diethylamine (DEA) in the mobile phase significantly decreased the value of the retention factor (k') for basic analytes, which indicated that DEA competitively blocks the hydroxyl groups of binaphthol. Conveniently, DEA did not always decrease the enantioselectivity. On the other hand, trifluoroacetic acid (TFA) in the mobile phase increased the enantioselectivity for some amines. Protonation of amines in the presence of TFA may affect hydrogen-bonding interaction between analytes and the CSP.

In this study, we examine the role of the hydroxyl groups in binaphthol in greater detail by substituting its hydrogens with methyl groups. Since this methylation completely blocks the hydrogen bonding of X···H—O type, where X is nitrogen or oxygen of analytes, the importance of hydrogen-bonding interaction in both chiral recognition and retention will become more clear upon comparison between CSP-BN and methyl-substituted CSP-BN (CSP-DM). Another subject is the effect of substitution at the 3,3′-positions of binaphthol in CSP-BN. The enantioselectivity of Newcomb-type crown ethers is known to be improved by substitution at the 3,3′-positions of their binaphthyl moiety [13]. The best enantioselectivity is achieved for α-amino acids when the substituents are phenyl groups [14,15], by which steric interaction, as well as π−π interaction, function most effectively in chiral recognition. In a similar manner, the chiral recognition of CSP-BN is also expected to be influenced by the substituents at the 3,3′-positions of binaphthol.

2. Experimental

2.1. Reagents and materials

(*S*)-2,2′-Dihydroxy-1,1′-binaphthyl (100% e.e.) was provided by Mitsubishi Gas Chemical (Tokyo, Japan). 3-Aminopropyltrimethoxysilane (Shin-etsu, Tokyo, Japan), 1,3-dicyclohexylcarbodiimide (Aldrich, WI, USA) and 1-hydroxybenzotriazole (Dojin, Kumamoto, Japan) were used without further purification. DEA, TFA and heptafluorobutyric acid (HFB) were purchased from Wako (Tokyo, Japan) and used without further purification. Compounds that can be purchased commercially were used as testing analytes. (*R*)- and (*S*)-α-methyl-4-nitrobenzylamine hydrochloride, 1-(1-naphthyl)ethylamine, 1-(*p*-tolyl)ethylamine, propranolol hydrochloride, pindolol, butethamate citrate, chlorpheniramine maleate, disopyramide, meclizine hydrochloride, promethazine hydrochloride, tolperizone hydrochloride, trihexyphenidyl hydrochloride, verapamil hydrochloride, benzoin, ketoprofen, ibuprofen, α-carbetoxy-γ-phenyl-γ-butyrolactone and α-hydroxyethylbenzene were purchased from Wako as test analytes. (*R*)- and (*S*)-α-methylbenzylamine, 6-ethoxy-1,2,3,4-tetrahydro-2,2,4-trimethylquinoline, laudanosine and warfarin were also purchased from Aldrich as test analytes. The amines obtained in their salt forms were used after being transformed into free forms by shaking with aqueous ammonia and chloroform. The structures of the HPLC analytes were shown in a previous paper [12]. The silica gel used was M.S.GEL SIL (EP-DF grade, particle size 5 μm, pore size 120 Å and surface area 350 m^2/g) from Dohkai Chemical (Fukuoka, Japan).

2.2. Preparation of chiral stationary phases

Fig. 1 shows the preparation procedure of (*S*)-12.

2.2.1. (S)-6-(4-Carboxybutyryl)-3,3′-diphenyl-2,2′-dimethoxy-1,1′-binaphthyl [(S)-9]

To 10 ml of CH_2Cl_2 was added 9.8 g (73 mmol) of $AlCl_3$ under N_2 and cooled to 0°C. To the mixture stirred under N_2 were added 10 ml (72 mmol) of methyl 4-(chloroformyl)butyrate and the mixture was stirred for 1 h to give a homogeneous solution. To this stirred solution was added 8.5 g (18 mmol) of (*S*)-3,3′-diphenyl-2,2′-dimethoxy-1,1′-binaphthyl [(*S*)-8] [14] and 10 ml of CH_2Cl_2 and the solution was stirred for 7 h at 25°C. The solution was cooled to 0°C, and 100 ml of 1.2 *M* HCl was carefully added to the stirred solution. The suspension was shaken with 80 ml of chloroform. The organic layer was dried, evaporated and chromatographed (silica gel, benzene) to give 5.0 g (46%) of (*S*)-9.

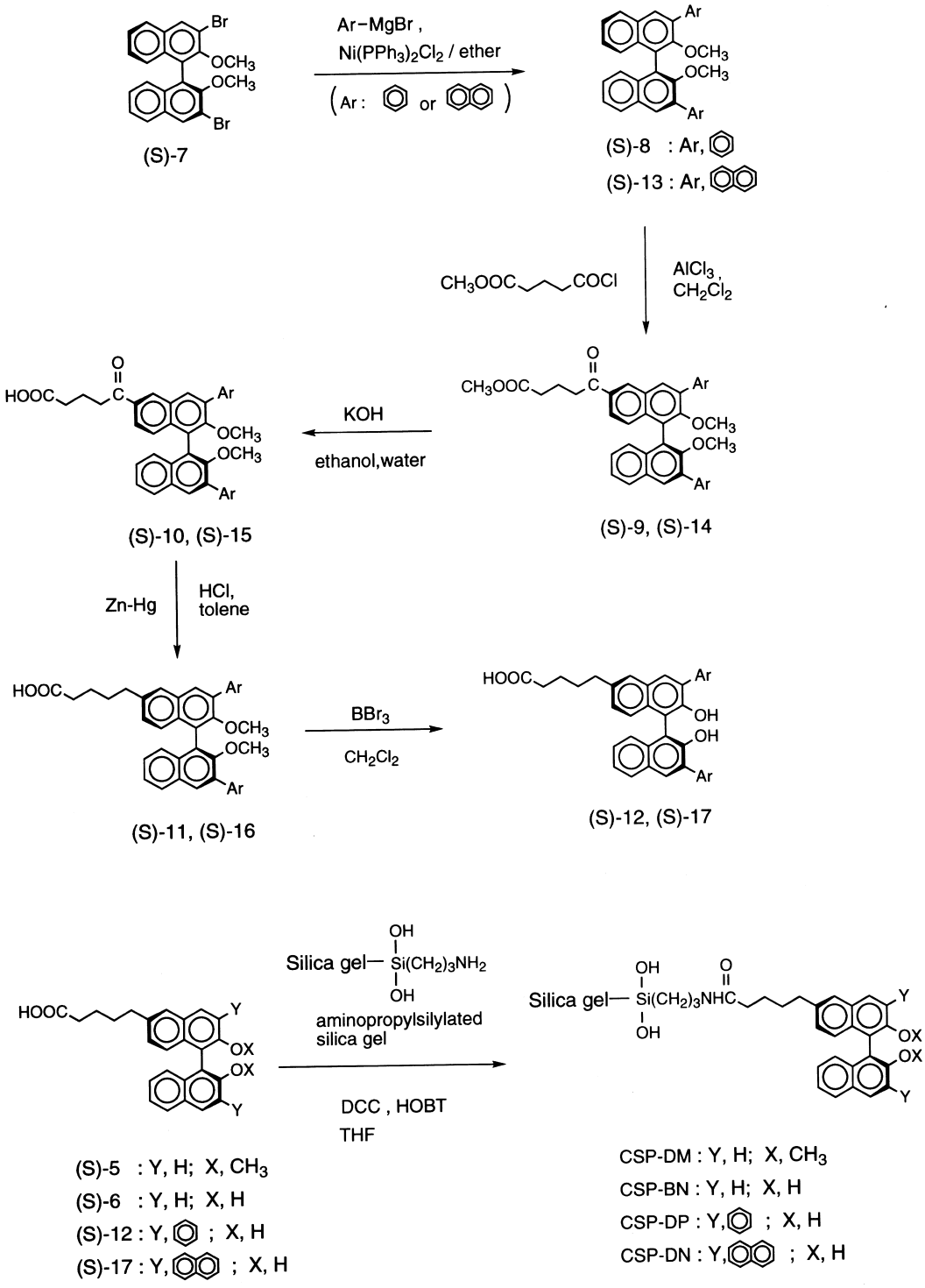

Fig. 1. Preparation procedure of CSPs.

2.2.2. (S)-6-(4-Carboxybutyl)-3,3′-diphenyl-2,2′-dimethoxy-1,1′-binaphthyl [(S)-11]

To a suspension of 5.0 g (8.4 mmol) of (*S*)-9 in 40 ml of ethanol was added 10 ml of water and 1.0 g (18 mmol) of KOH. The suspension was refluxed for 1 h, cooled to 25°C and shaken with a mixture of 50 ml of water, 2 ml of conc. HCl and chloroform. The organic layer was dried and evaporated to give (*S*)-6-(3-carboxybutyryl)-2, 2′-dimethoxy-1, 1′-binaphthyl [(*S*)-10].

(*S*)-11 was prepared by reduction of (*S*)-10 as follows. A mixture of 40 g of Zn, 4 g of $HgCl_2$, 60 ml of water and 2 ml of conc. HCl was stirred for 10 min, then the water was poured off. To the amalgamated Zn were added 15 ml of water, 35 ml of conc. HCl and the total amount of (*S*)-10 in 60 ml of toluene. The mixture was vigorously refluxed for 48 h, and 30 ml of conc. HCl was added three times during this period. The mixture was cooled to 25°C and shaken with 50 ml of water and 150 ml of $CHCl_3$. The organic layer was dried, evaporated and chromatographed (silica gel, benzene–ethyl acetate mixture) to give 2.2 g (46%) of (*S*)-11.

2.2.3. (S)-6-(4-Carboxybutyl)-3,3′-diphenyl-2,2′-dihydroxy-1,1′-binaphthyl [(S)-12]

To a solution of 2.0 g (3.5 mmol) of (*S*)-11 in 150 ml of CH_2Cl_2 was added 2.2 ml (23 mmol) of BBr_3 at 0°C. The solution was stirred for 15 min at 0°C and the excess of BBr_3 was decomposed by dropwise addition of water. The mixture was shaken with 100 ml of water. The organic layer was dried, evaporated and chromatographed (silica gel, benzene–ethyl acetate mixture) to give 1.5 g (79%) of (*S*)-12. Mass spectrum: *m*/*z* 538 (M^+). ^1H NMR (200 MHz): δ 1.69 (t, CH_2, 4H), 2.34 (t, $O{=}CCH_2$, 2H), 2.70 (t, $ArCH_2$, 2H), 7.6 (m, ArH, 19H). The enantiomeric purity of (*S*)-12 was determined to be 100% e.e. by HPLC under the following conditions: column, Chiralcel OD (250 mm×4.6 mm I.D.) (Daicel, Osaka, Japan); mobile phase, acetic acid–methanol–ethanol–hexane (1:50:50:100, v/v/v/v); flow-rate, 0.5 ml/min; temperature, 25°C; UV detection, 254 nm.

2.2.4. (S)-3,3-Dinaphthyl-2,2′-dimethoxy-1,1′-binaphthyl [(S)-13]

To a suspension of 16 g (34 mmol) of (*S*)-3,3′-dibromo-2,2′-dimethoxy-1,1′-binaphthyl [(*S*)-7] [14] and 1.4 g (3.3 mmol) of $Ni[P(C_6H_5)_3]_2Cl_2$ in 100 ml of ether stirred under N_2 was added 104 mmol of naphthylmagnesium bromide in 120 ml of ether. The mixture was refluxed for 20 h, cooled and shaken with 500 ml of 1.2 *M* HCl and 500 ml of $CHCl_3$. The organic layer was dried and evaporated, then chromatographed (silica gel, cyclohexane–benzene mixture) to give 12 g (62%) of (*S*)-13.

2.2.5. (S)-6-(4-Carboxybutyl)-3,3′-diphenyl-2,2′-dihydroxy-1,1′-binaphthyl [(S)-17]

(*S*)-17 was prepared from (*S*)-13 by the same procedure as the preparation of (*S*)-12 from (*S*)-8. Yield: 66%. Mass spectrum: *m*/*z* 638 (M^+). ^1H NMR (200 MHz): δ 1.66 (t, CH_2, 4H), 2.30 (t, $O{=}CCH_2$, 4H), 2.71 (t, $ArCH_2$, 2H), 7.7 (m, ArH, 23H).

2.3. Preparation of binaphthol derivatives-bonded silica gels

Selectors, (*S*)-5, (*S*)-6, (*S*)-12 and (*S*)-17, were bonded to aminopropylsilylated silica gel as follows to give CSP-DM, CSP-BN, CSP-DP and CSP-DN, respectively. Aminopropylsilylated silica gel was prepared as described in the previous paper [12]. Tetrahydrofuran (THF) (15 ml) was added to a mixture of 3.5 g of aminopropylsilylated silica gel dried at 120°C in vacuo for 5 h, 1.7 mmol of selector [(*S*)-5, (*S*)-6, (*S*)-12 or (*S*)-17], 0.54 g (2.6 mmol) of 1,3-dicyclohexylcarbodiimide and 0.35 g (2.6 mmol) of 1-hydroxybenzotriazole, and then the mixture was stirred for 24 h. To the mixture were added 0.54 g (2.6 mmol) of 1,3-dicyclohexylcarbodiimide, 0.35 g (2.6 mmol) of 1-hydroxybenzotriazole, 0.27 ml (4.8 mmol) of acetic acid and 5 ml of THF. The mixture was stirred for an additional 24 h to acetylate the residual amino groups. The particles were filtered, washed sequentially with THF, ethanol and hot ethanol, and dried at 60°C in vacuo for 4 h to give each CSP. Amount of selectors bonded per 1 g of aminopropylsilylated silica gel: 0.48 mmol/g CSP-DM, 0.47 mmol/g CSP-BN, 0.39 mmol/g CSP-DP, and 0.35 mmol/g CSP-DN.

2.4. Chromatographic measurements

The prepared CSPs were packed into a stainless

steel tube (150 mm×4.6 mm I.D.) by a high-pressure slurry-packing procedure.

The HPLC system consisted of a pump (LC-10AD, Shimadzu, Kyoto, Japan), a UV detector (SPD-10A, Shimadzu), a thermostatted chamber (TCO-10AC, Shimadzu), a data processor (C-R4A, Shimadzu) and an injector (Model 7125, Rheodyne, Cotati, CA, USA). HPLC conditions were as follows: mobile phase, a mixture of ethanol and hexane; flow-rate, 1 ml/min; temperature, 25°C; UV detection, 260 nm for chlorpheniramin; 270 nm for disopyramide and pindolol; 280 nm for verapamil; 254 nm for the others; sample volume, 0.2–0.5 μl; sample concentration, 2–10%. The dead time (t_0) of the column was determined from the retention time of 1,3,5-tri-*tert*.-butylbenzene [16,17]. The separation factor (α) between enantiomers was defined as

$$\alpha = k_2'/k_1'$$

where k_1' and k_2' are the retention factors of the first and second eluted enantiomers, respectively. The value of α is a measure of enantioselectivity.

2.5. Calculation of octanol–water partition coefficient of analytes

The hydrophobic parameter, log *P*, where *P* is the octanol–water partition coefficient of analytes, was calculated. The software used was ''Clog P for windows'' (Biobyte, CA, USA). The software calculates log *P* basically by Rekker's fragment method [18,19].

3. Results

Table 1 shows the results of HPLC of primary, secondary and tertiary amines and the other analytes without amino groups on CSP-DM, CSP-BN, CSP-DP and CSP-DN. Typical chromatograms on CSP-BN, CSP-DP and CSP-DN are given in Fig. 2 as examples. Methylation of the hydroxyl groups of binaphthol decreased the k' values of amines. It improved the enantioselectivity of primary amines, whereas it reduced that of secondary and tertiary amines. Substitution at the 3,3′-positions also decreased the k' values of amines, but the tendency of the chiral recognition is not so apparent in Table 1. From the point of view of enantioseparation, each amine examined here was separated on at least one of the CSPs.

The chirality of the analytes without amino groups were poorly recognized; among them the separated possessed at least one carbonyl or ester group. The enantioselectivity for these analytes tended to be reduced by addition of DEA in the mobile phase.

Table 2 summarizes the results of chiral recognition when the mobile phase contained TFA or DEA (''+'' indicates that the recognition took place and ''−'' indicates its absence). The analytes are listed in the order of hydrophobicity, and their molecular masses are also given. In the mobile phase containing TFA, hydrophobic CSP tended to show chiral recognition for hydrophilic tertiary amines, and hydrophilic CSP for hydrophobic tertiary amines. On the other hand, in the mobile phase containing DEA, CSP-DP and CSP-DN showed chiral recognition for the tertiary amines with relatively high molecular mass.

Table 3 shows the effect of size of acidic additives in the mobile phase, TFA and HFB, on the chiral recognition on CSP-BN and CSP-DP. The combination of CSP-DP and HFB tended to show low enantioselectivity.

4. Discussion

4.1. Retention on CSPs

The retention time of the analytes without amino groups increased with an increase of hydrophobicity of the selectors by substitution at the 3,3′-positions of binaphthol; the order of the k' values was CSP-BN<CSP-DP<CSP-DN (Table 1). This result indicates the existence of hydrophobic interaction between the analytes and the selectors. For amines, on the other hand, the 3,3′-substitution decreased the k' values of the secondary and the tertiary amines, which suggests that the aromatic substituents sterically hindered the hydrogen bonding between the hydroxyl groups of binaphthol and the amino group. It is noteworthy that the k' values of the tertiary amines tended to be larger on the naphthyl substituent (CSP-DN) than on the phenyl substituent (CSP-DP), indicating that hydrophobic interaction must also be taken into consideration for retention of amines. The substitution did not considerably de-

Table 1
Separation of enantiomers on CSPs

No.	Analyte	Mobile phase		CSP-BN		CSP-DM		CSP-DP		CSP-DN	
		Ethanol–hexane	Additive[a]	k'_1	α	k'_1	α	k'_1	α	k'_1	α
Primary amines											
1	α-Methyl-4-nitrobenzyl-amine	5:95	no	23.00	1	18.41	1	25.74	1	27.05	1.022
		5:95	TFA	34.03	1	16.30	1.033	21.18	1	17.65	1
		5:95	DEA	10.00	1	4.163	1	14.67	1	19.98	1
2	α-Methylbenzylamine	2:98	no	25.98	1	31.69	1	30.61	1	12.27	1
		2:98	TFA	27.75	1	21.25	1.034	23.98	1	19.17	1
		2:98	DEA	4.247	1	1.797	1	5.638	1	2.942	1
3	1-(*p*-Tolyl)ethylamine	2:98	no	23.19	1	26.65	1	26.84	1	15.49	1
		2:98	TFA	24.46	1	16.78	1.023	20.37	1	12.37	1.032
		2:98	DEA	4.052	1	0.589	1	1.217	1	–	–
4	1-(1-Naphthyl)ethyl amine	5:95	no	13.09	1	11.99	1.034	14.90	1	12.48	1
		5:95	TFA	13.57	1	10.09	1.041	11.02	1.028	8.362	1
		5:95	DEA	4.721	1	2.077	1	6.352	1	8.236	1
Secondary amines											
5	Pindolol	10:90	no	53.02	1.039	28.04	1	39.37	1.031	28.19	1.043
		10:90	TFA	49.37	1.044	22.45	1	28.81	1.031	24.51	1
		10:90	DEA	12.16	1.037	7.006	1	12.83	1.053	15.04	1.054
6	Propranolol	5:95	no	35.17	1.076	15.25	1	20.03	1.037	10.66	1
		5:95	TFA	34.76	1.056	11.03	1	16.40	1.033	15.63	1
		1:99	DEA	10.25	1.079	6.072	1	13.89	1.036	16.63	1
7	6-Ethoxy-1,2,3,4-tetrahydro-2,2,4-trimethylqunoline	5:95	no	0.715	1	0.594	1	0.786	1	0.908	1
		5:95	TFA	15.37	1	6.265	1.055	9.114	1.047	8.903	1.015
		1:99	DEA	0.760	1	0.736	1	0.873	1	0.992	1
Tertiary amines											
8	Disopyramide	20:80	no	47.32	1	4.794	1	19.78	1	7.682	1.149
		20:80	TFA	53.24	1	4.849	1	17.56	1	21.27	1.044
		5:95	DEA	8.839	1.058	2.409	1	6.538	1.110	10.12	1.112
9	Chlorpheniramine	20:80	no	22.55	1	1.851	1	10.92	1.109	7.422	1
		20:80	TFA	45.57	1	2.691	1	10.56	1.114	15.11	1.072
		1:99	DEA	5.272	1	1.694	1	9.818	1	13.49	1
10	Laudanosine	20:80	no	28.00	1	4.762	1	12.88	1	11.84	1
		20:80	TFA	52.55	1.036	6.879	1	18.07	1	24.71	1.039
		2:98	DEA	12.94	1	7.216	1	18.49	1	30.18	1
11	Verapamil	30:70	no	35.52	1.054	2.689	1	8.485	1	11.30	1.178
		30:70	TFA	43.06	1.068	3.576	1	9.452	1	15.72	1
		10:90	DEA	9.336	1.050	3.679	1	10.84	1.077	20.28	1.168
12	Butethamate	1:99	no	6.453	1	2.465	1	5.387	1	1.926	1
		5:95	TFA	86.87	1.045	7.151	1	45.21	1.022	30.00	1.040
		1:99	DEA	0.706	1	0.464	1	0.890	1	1.128	1
13	Tolperisone	10:90	no	9.312	1.078	1.206	1	3.852	1.060	1.675	1
		10:90	TFA	36.68	1.057	3.481	1.037	11.00	1.079	14.92	1.069
		1:99	DEA	0.990	1.079	0.589	1	1.217	1	1.679	1

Table 1. Continued

No.	Analyte	Mobile phase		CSP-BN		CSP-DM		CSP-DP		CSP-DN	
		Ethanol–hexane	Additive[a]	k'_1	α	k'_1	α	k'_1	α	k'_1	α
14	Trihexyphenidyl	10:90	no	6.338	1.108	1.183	1	2.375	1	0.669	1
		10:90	TFA	19.93	1.095	3.025	1	5.971	1	7.623	1
		1:99	DEA	0.679	1	0.364	1	0.523	1	0.693	1
15	Promethazine	10:90	no	9.258	1.118	2.500	1	5.764	1	4.125	1.051
		10:90	TFA	39.22	1.114	6.076	1.019	13.76	1	12.39	1
		1:99	DEA	2.189	1	1.391	1	3.695	1.065	5.074	1
16	Meclizine	1:99	no	3.052	1	1.088	1	2.405	1	2.968	1.048
		10:90	TFA	31.45	1	4.999	1	11.51	1	15.89	1
		1:99	DEA	0.974	1	0.933	1	1.373	1	1.877	1.033
Compounds without amino groups											
17	α-Carboxy-γ-phenyl-γ-butyrolacetone	1:99	no	7.527	1.036	7.078	1	11.11	1	13.83	1
		1:99	TFA	7.291	1.047	6.276	1.030	10.57	1	13.68	1
		1:99	DEA	5.686	1.036	6.878	1.029	9.017	1	11.01	1
18	Warfarin	5:95	no	27.59	1	16.86	1	19.57	1	–	–
		5:95	TFA	13.95	1	11.40	1.034	12.51	1.050	15.31	1.080
		20:80	DEA	38.64	1	17.62	1	–	–	19.90	1
19	α-Hydroxyethylbenzene	1:99	no	2.837	1	3.115	1	3.253	1	3.199	1
		1:99	TFA	2.613	1	2.534	1	2.851	1	2.874	1
		1:99	DEA	2.779	1	3.170	1	3.084	1	3.149	1
21	Benzoin	1:99	no	4.846	1	5.219	1	6.619	1	7.533	1.018
		1:99	TFA	4.558	1.016	4.473	1	6.144	1.018	7.333	1.021
		1:99	DEA	4.272	1	5.259	1	6.047	1.032	6.987	1.039
22	Ketoprofen	5:95	no	51.69	1	19.78	1	25.92	1	–	–
		1:99	TFA	19.37	1	19.93	1	27.04	1.017	32.35	1
		20:80	DEA	33.80	1	13.30	1	18.88	1	14.09	1
23	Ibuprofen	1:99	no	11.48	1	7.415	1	7.385	1	–	–
		1:99	TFA	2.284	1	2.554	1	2.731	1	2.739	1
		5:95	DEA	39.62	1	6.979	1	9.673	1	9.910	1

[a] TFA, containing 13 m*M* trifluoroacetic acid; DEA, containing 9.7 m*M* diethylamine.
[b] Capacity factor of first eluted enantiomer.

crease the k' values of the primary amines, indicating that the primary amines suffer little from the steric effect caused by the 3,3′-substitution because of their compactness around the amino group.

Methylation of the hydroxyl groups of binaphthol decreased the k' values of the amines considerably. The degree of decrease on CSP-DM was in the order of tertiary>secondary>primary amines. Apparently, the bulkiness around the nitrogen seems to play an important role for the strength of hydrogen bonding. Contrary, less bulkiness of primary amines allows them to form N—H···O-type hydrogen bonding, resulting in less decrease in their k' values on CSP-DM also. This idea is supported by the fact that the k' values of the primary amines decrease considerably by the addition of DEA in the mobile phase: DEA is known to restrict hydrogen bonding between CSP-BN and amines [12].

Thus k' is a measure for estimating the analyte–selector interaction at the molecular level. *O*-Methylation and 3,3′-substitution revealed the importance of hydrogen bonding and hydrophobic interaction for retention, respectively. 3,3′-Substituents simultaneously restricted the hydrogen bonding between

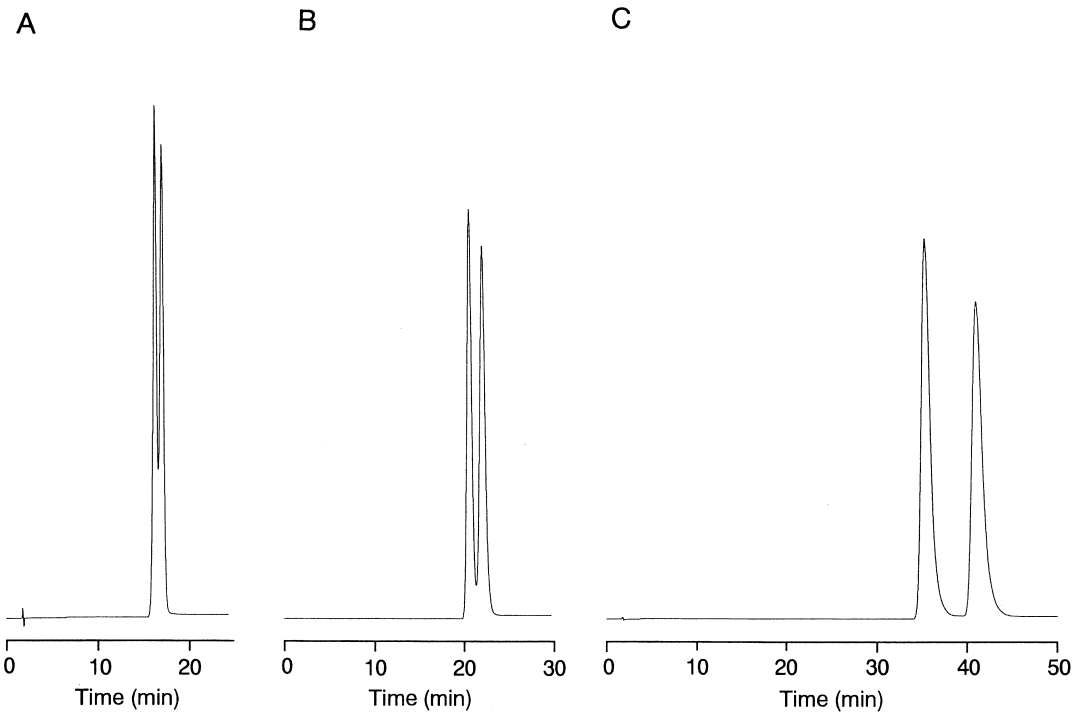

Fig. 2. Typical chromatogram on binaphthol derivative-bonded CSPs. HPLC conditions: analyte, verapermil; stationary phase, (A) CSP-BN; (B) CSP-DP; (C) CSP-DN; mobile phase, ethanol–hexane (10:90) containing 9.7 m*M* DEA; flow-rate, 1 ml/min; detection, UV 254 nm; temperature, 25°C.

analytes with high molecular mass and the selector because of their steric hindrance effect.

4.2. Enantioselectivity of CSPs

Chiral recognition for the primary amines was characteristically observed on CSP-DM. As mentioned above, primary amines are able to form the N—H···O-type hydrogen bonding with the methoxy groups of CSP-DM, so that the asymmetric carbon of primary amines bonded to the amino group can be settled near the methoxy groups of binaphthol. Here, most probably, a steric interaction introduced by the methoxy moieties solves the degeneration between free energy levels of diastereomeric complexes, resulting in chiral recognition for primary amines.

On the other hand, CSP-DM showed negligible chiral recognition for the secondary and tertiary amines. This is regarded to be caused by the steric hindrance against the hydrogen bonding between the methoxy groups and highly substituted amino group. This low enantioselectivity on CSP-DM clearly indicates the importance of the hydroxyl groups of binaphthol for chiral recognition for secondary and tertiary amines.

DEA added in the mobile phase also blocks the hydrogen bonding of binaphthol, but its effect is different from that caused by the covalent methylation of the hydroxy groups. The interaction between DEA and the hydroxyl group is in dynamic equilibrium, which allows amines to form hydrogen bonds with the CSPs in competition with DEA. Moreover, DEA blocks residual silanol groups on the surface of the CSPs and reduces the retention unrelated with chiral recognition, resulting in improvement of the enantioselectivity on the CSPs in general [20,21]. Therefore, enantioselectivity on the present CSPs was not necessarily decreased by this treatment.

Table 2 symbolically shows the influence of the

Table 2
Effect of hydrophobicity of CSPs and analytes on chiral recognition in the mobile phase with TFA or DEA

Analyte No.	"Clog P"[a]	M_r	Chiral recognition (TFA)				Chiral recognition (DEA)			
			CSP-BN	CSP-DM	CSP-DP	CSP-DN	CSP-BN	CSP-DM	CSP-DP	CSP-DN
Primary amines										
1	1.176	166	−	+	−	−	−	−	−	−
2	1.403	121	−	+	−	−	−	−	−	−
3	1.902	135	−	+	−	+	−	−	−	
4	2.577	171	−	+	+	−	−	−	−	−
Secondary amines										
5	1.671	248	+	−	+	−	+	−	+	+
6	2.753	259	+	−	+	−	+	−	+	−
7	4.374	219	−	+	+	+	−	−	−	−
Tertiary amines										
8	1.574	339	−	−	−	+	+	−	+	+
9	3.018	275	−	−	+	+	−	−	−	−
10	3.184	357	+	−	−	+	−	−	−	−
11	3.257	441	+	−	−	−	+	−	+	+
12	3.836	263	+	−	+	+	−	−	−	−
13	3.848	245	+	+	+	+	+	−	−	−
14	4.490	301	+	−	−	−	−	−	−	−
15	4.810	284	+	+	−	−	−	−	+	−
16	7.040	391	−	−	−	−	−	−	−	+
Compounds without amino groups										
17	0.843	220	+	+	−	−	+	+	−	−
18	1.324	310	−	+	+	+	−	−		−
19	1.413	122	−	−	−	−	−	−	−	−
20	2.130	212	+	−	+	+	−	−	+	+
21	2.761	254	−	−	+	−	−	−	−	−
22	3.500	206	−	−	−	−	−	−	−	−

[a] Log *P* calculated with a computer.

3,3′-substitution on binaphthol to chiral recognition for the tertiary amines, which is difficult to observe from Table 1. When the mobile phase contained TFA, hydrophobic CSP tended to show chiral recognition for hydrophilic analytes. This seems to imply the importance of hydrophobic interaction in chiral recognition as well as retention. However, there were several exceptions: CSP-DP did not exhibit chiral recognition for laudanosine (10) and verapamil (11), nor CSP-DN for (11). These exceptions are attributed to the steric hindrance discussed in Section 4.3, for the molecular masses of (10) and (11) are relatively high.

It has already been pointed out in Section 3 that carbonyl or ester group participates in chiral recognition for the analyses without amino groups. DEA added in the mobile phase reduced the enantioselectivity for these analytes, which is an evidence for contribution of C=O···H-O-type hydrogen bonding to their chiral recognition.

In summary, the chiral binaphthyl- and its derivative-bonded phases interact with analytes through hydrogen bonding, hydrophobic bonding and steric hindrance to exhibit enantiomer separation. This meets the well-known requirement of the three point rule [22] for chiral recognition.

4.3. Effect of bulkiness of the ion-pair

The tertiary amines were well separated into enantiomers in the mobile phase containing TFA

Table 3
Comparison of TFA and heptafluorobutyric acid (HFB) as an additive to the mobile phase

Analyte No.	Mobile phase (ethanol–hexane)	CSP-BN			CSP-DP		
		k'_{HFB} [a]/k'_{TFA} [b]	α_{TFA}	α_{HFB}	k'_{HFB} [a]/k'_{TFA} [b]	α_{TFA}	α_{HFB}
Primary amines							
1	5:95	0.567	1	1	0.361	1	1
2	2:98	0.406	1	1	0.266	1	1
3	2:98	0.424	1	1	0.263	1	1
4	5:95	0.480	1	1	0.349	1.028	1
Secondary amines							
5	10:90	0.626	1.044	1.048	0.357	1.031	1
6	5:95	0.492	1.056	1.062	0.473	1.033	1
7	5:95	0.486	1	1	0.322	1.047	1
Tertiary amines							
8	20:80	0.497	1	1	0.363	1	1
9	20:80	0.874	1	1	0.587	1.114	1.143
10	20:80	0.624	1.036	1.014	0.487	1	1
11	30:70	0.658	1.068	1.069	0.580	1	1
12	10:90	0.605	1.037	1.039	0.479	1	1
13	10:90	0.609	1.057	1.056	0.462	1.079	1.091
14	10:90	0.619	10.95	1.113	0.498	1	1
15	10:90	0.677	1.111	1.114	0.479	1	1
16	10:90	0.558	1	1	0.466	1	1
Compounds without amino groups							
17	1:99	0.954	1.047	1.048	0.888	1	1
18	5:95	0.954	1	1	0.918	1.050	1.053
19	1:99	1.06	1	1	0.934	1	1
20	1:99	1.00	1	1	0.924	1.018	1
21	1:99	0.968	1	1	0.914	1.017	1.016
22	1:99	1.07	1	1	0.934	1	1

[a] Retention factor of the first eluted enantiomer in adding HFB to the mobile phase.
[b] Retention factor of the first eluted enantiomer in adding TFA to the mobile phase.
[c] Separation factor of enantiomers in adding TFA to the mobile phase.
[d] Separation factor of enantiomers in adding HFB to the mobile phase.

(Table 2). Perfluorocarboxylic acids such as TFA are known to form an ion-pair with basic compounds [23–25]. In normal-phase HPLC, amines in their ion-pair forms are retained on stationary phases in normal-phase HPLC [26–28]. Therefore, under the present HPLC conditions containing TFA, amines are regarded to interact with the selectors in their ion-pair forms.

This idea was confirmed experimentally using bulky heptafluorobutyric acid (HFB) in place of TFA in the mobile phases. Table 3 shows the influence of counter ion size on enantioselectivity. On CSP-BN, each acid showed the same enantioselectivity. On CSP-DP, however, addition of HFB could not perform chiral separation of all the primary and secondary amines that were separated into enantiomers in the presence of TFA. Thus, not the size of amines itself but the bulkiness of the ion pair is to be considered to affect chiral recognition via steric interaction in the acidic mobile phases.

In contrast, amines in the mobile phases containing DEA are in their free forms in general, and are smaller in size than their ion-pair. This is advantageous for the present analytes to reach inside the chiral cavity of the selector composed of hydroxyl groups and a steric barrier of 3,3′-substituents. In the present of DEA, for example, both CSP-DP and CSP-DN showed chiral recognition for tertiary amines with even high molecular mass (Table 2).

5. Conclusions

New CSPs were prepared from binaphthol-derivatives, and all amines examined were separated into enantiomers on them or on previously prepared CSP-BN. Methylation of the hydroxyl groups of binaphthol improved the enantioselectivity for primary amines, but reduced it for secondary and tertiary amines. The results confirmed that the hydroxyl groups in binaphthol moiety play a dominant role in chiral recognition and retention for secondary and tertiary amines. The steric hindrance caused by this methyl group was regarded to contribute to the chiral recognition for the primary amines. Importance of steric effect on hydrogen bonding was pointed out by 3,3′-substituents on binaphthol, which changes the cavity size of selector, and by use of different additives to control the bulkiness of analytes. Hydrophobicity of selectors negatively correlated with that of analytes in enantioselectivity, suggesting that hydrophobic interaction also contributed to chiral recognition.

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