Chiral Stationary Phases Derived from (−)-*anti* Head-to-Head Coumarin Dimer: Preparation, Characterization, and Chiral Recognition Ability

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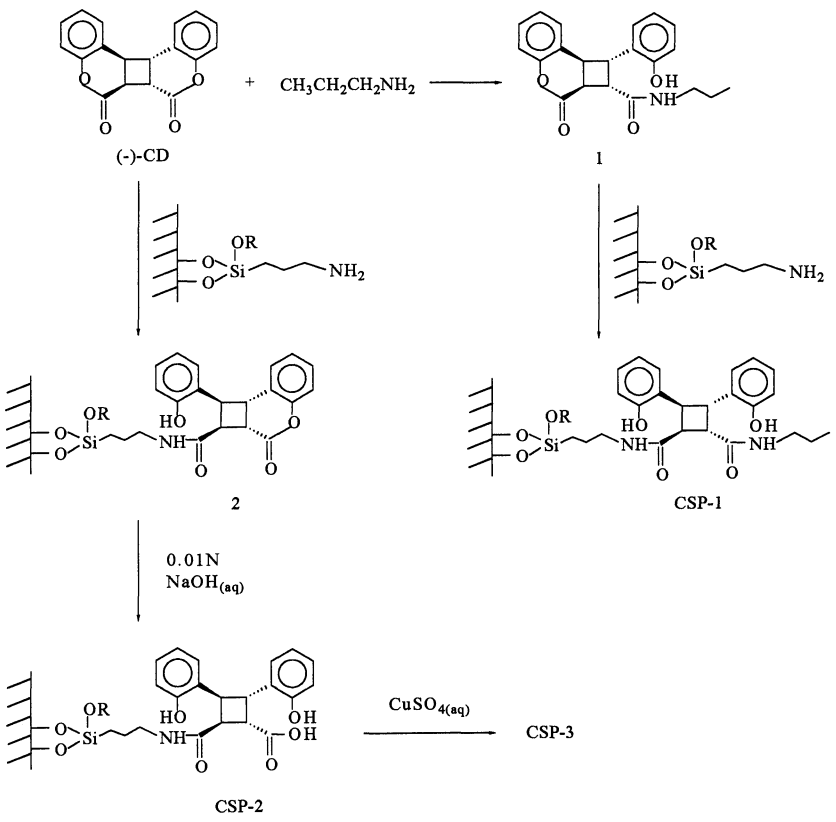
A monoamide derived from (−)-*anti* head-to-head coumarin dimer [(−)-CD] with propylamine has been successfully bonded to silica gel (Lichrospher 100 NH_2: Merck) through amide linkage to prepare CSP-1. Another monoamide derivative CSP-2 was prepared by direct nucleophilic opening of one lactone ring of (−)-CD by Lichospher 100 NH_2, followed by hydrolysis of the other lactone. CSP-1 and CSP-2 showed high enantioselectivity to *trans*-stilbene oxide with separation factors (α) at 2.42 and 2.46, respectively, and the (−)-isomer eluted first. The capacity factor (k') and α decrease with increasing polarity of the eluent (hexane/2-propanol), indicating that hydrogen bonding plays important role in chiral recognition. CSP-3, obtained by ligand exchange of CSP-2 with $CuSO_{4(aq)}$, resolved DL-tryptophan, DL-tyrosine, and DL-phenylalanine ($\alpha=k_D'/k_L'=1.14$—1.33), which all contain aromatic chromophore. The stability difference of the ternary complexes formed with D-isomer and L-isomer is explained by steric hindrance and aromatic π–π interactions.

Several types of chiral stationary phase (CSP) for high-performance liquid chromatography (HPLC) have been developed and successfully employed in the direct resolution of racemic compounds.[1–5] Low molecular weight CSPs, obtained by bonding small chiral molecules onto a silica surface, exhibit many unique features. Such as highly efficient column can be obtained, each enantiomeric form is available, and the chemical structure of the CSPs can be easily modified to improve and/or broaden chiral recognition ability.

anti Head-to-head coumarin dimer (CD), synthesized by photodimerization of coumarin in the presence of benzophenone,[6] has been successfully resolved by diastereomeric method.[7,8] Optically active polyamides have been prepared by ring-opening polyaddition reaction of the chiral coumarin dimer with various diamines.[9,10] Some of them showed high chiral recognition ability to racemates containing aromatic chromo-



Scheme 1.

phores.[11-13] Diastereomeric diamide derivatives of CD with (−)-1-phenylethylamine have also been directly employed in the synthesis of optically active polyurethanes which showed effective resolution to racemates with *trans*-phenyl groups.[14] The chiral discrimination ability of these optically active polymers has been attributed to three simultaneous interactions (π–π aromatic packing, hydrogen bonding, steric) with racemate or the ordered conformation.[11-13] The former mechanism implies that the ring-opened derivatives of the chiral CD would be an effective CSPs if σ-bonded to silica gels.

Chiral ligand exchange chromatography (CLEC) has been developed for direct optical resolution of amino acids using liquid chromatography.[15-17] Diastereomeric ternary complexation works as the driving force for the chiral discrimination in the chromatographic process. Hydrolysis of the lactone rings of (−)-CD result in simultaneous formation of carboxyl and phenolic hydroxyl groups, which can be effective ligands for ternary complexation. Therefore, silica gel-bonded (−)-CD would be potential stationary phases for CLEC after hydrolysis of the lactone rings.

We report here the preparation, characterization, and chiral recognition ability of silica gel-bonded derivatives of (−)-CD (CSP-1 and CSP-2 as shown in Scheme 1). Resolution results of seven amino acids by CSP-3, using chiral ligand-exchange chromatography (CLEC), are also presented.

Experimental

Materials. (−)-*anti* Head-to-head coumarin dimer [(−)-CD] was prepared by fractional crystallization of the diastereomeric diamides of (±)-CD with (−)-1-phenylethylamine.[7,8] The (±)-CD was obtained by photodimerization (>310 nm) of coumarin in benzene using benzophenone as photosensitizer.[6] The melting point was 165—166 °C and $[\alpha]_D^{25}$ =−9.0° (*c* 0.1, DMAc). Propylamine was purchased from Wako Chemical Co. and used as received. Remaining chemicals and solvents were of reagent grade and HPLC grade, respectively.

Macroporous silica gels (Lichrospher 100 NH_2 of Merck) was used as stationary phase onto which the derivatives of (−)-CD was bonded. The average particle size was 10 μm with average pore diameter and pore volume of 100 Å and 1.25 ml g^{-1}, respectively. The specific surface area was 350 $m^2 g^{-1}$ as measured by the BET method. The concentration of surface NH_2 groups was 0.695 mmol g^{-1} as determined by acid-base titration.

Synthesis of Monoamide 1. To a solution of (−)-CD (1.023 g, 3.5 mmol) in 30 ml of 1,4-dioxane was added dropwise a solution of propylamine (0.207 g, 3.5 mmol) in 50 ml of 1,4-dioxane. The mixture was allowed to react for 27 h at room temperature, and then the solvents were removed by vaccuum evaporation. The residues were dissolved in tetrahydrofuran (THF) and then reprecipitated in toluene to obtain 0.888 g of **1**. Yield 72.2%; mp 186 °C; $[\alpha]_D^{25}$ =+217° (*c* 0.245, DMAc); IR (KBr) ν 1750 (C=O), 1640 (amide I), 1535 (amide II), 745 cm^{-1} (phenyl); ^1H NMR (DMSO-d_6) δ=0.501 (t, 3H, $-CH_3$), 0.942 (h, 2H, $-CH_2-$), 2.4—3.2 (m, 2H, $-CH_2-N$), 3.5—4.6 (m, 4H, cyclobutane), 6.7—7.6 (m, 8H, phenyl), 9.4 (s, 1H, φ-OH).

Found: C, 71.43; H, 6.12; N, 3.98%. Calcd for ($C_{21}H_{21}NO_4$): C, 71.58; H, 6.02; N, 3.99%.

Preparation of CSP-1 and CSP-2. A general procedure for the synthesis of the chiral stationary phases is shown in Scheme 1. Into a solution of **1** (0.804 g, 2.29 mmol) in 20 ml of THF was added 2.53 g of Lichrospher 100 NH_2 (1.76 mmol NH_2) all at once. The mixture was allowed to react at room temperature for 24 h. Then the solids were collected by filtration and washed successively with THF, methanol, and diethyl ether to obtain 2.976 g of CSP-1.

Found for CSP-1: C, 13.52; H, 2.13; N, 1.75%. Found for Lichrospher 100 NH_2: C, 4.30; H, 1.35; N, 1.59%.

The CSP-2 was prepared by the opening of one lactone ring in (−)-CD by the reaction with amino group on silica gel, followed by hydrolysis of the other lactone ring (Scheme 1). To a solution of (−)-CD (4.684 g, 16.03 mmol) in 20 ml of THF was added slowly the mixture of Lichrospher 100 NH_2 (2.517 g) in 100 ml THF. The solids were collected and dried after reacting for 72 h to obtain 2.932 g of **2**.

Found for **2**: C, 14.32; H, 2.02; N, 1.26%.

The hydrolysis of **2** (2.714 g) was conducted in NaOH aqueous solution (20 ml, 0.02 equiv) for 8 h at room temperature. After neutralizing with 0.01 equiv HCl aqueous solution, the solids were collected by filtration and dried in vacuo at 40 °C for 48 h to obtain CSP-2.

Found for CSP-2: C, 13.39; H, 1.88; N, 1.21%.

Chromatographic Resolution of 4—17 by CSP-1 and CSP-2. The stainless steel column [250 mm×4.0 mm (i.d.)] for HPLC was packed with CSP-1 and CSP-2 using the slurry method. For example, 2.1 g of CSP-1 was dispersed in 20 ml of hexane and then treated ultrasonically for 5 min. The slurry was packed with a Hitachi L-6000 pump using hexane/2-propanol (v/v=9/1) as pressuring solvent. The packing pressure was kept at 200 kg cm^{-2} and the packing time was ca. 30 min.

Analytical liquid chromatography was performed with a Hitachi L-6000 pump, a D-2000 chromato-integrator, and a L-4000 variable-wavelength detector monitored at 254 nm. A JASCO digital polarimeter, model DIP-370, equipped with a flow-through cell [50 mm×1.0 mm (i.d.)] was employed for measuring the optical rotations of separated enantiomers. The pressures of the columns bearing CSP-1 and CSP-2 were 81 kg cm^{-2} and 96 kg cm^{-2}, respectively, at a flow rate of 0.5 ml min^{-1}.

Results and Dicussion

Preparation of CSP-1. Ring-opening addition of (−)-CD with equimolar propylamine in dioxane resulted in good yield (72%) of monoamide derivative (**1**) of (−)-CD. The leftover lactone rings of **1** are reactive to nucleophilic reagents such as amino groups on the surface of Lichrospher 100 NH_2. The grafting ratio can be estimated by the following equation:

$$\text{GR (\%)} = \text{grafted mmole of } \mathbf{1}/\text{mmole of } NH_2 \text{ on silica gel}$$
$$= [(W_1 - W_s)/(N_s \times W_s \times M_1)] \times 100\% \quad (1)$$

where W_s and W_1 represent the weight of silica gel (Lichrospher 100 NH_2) before and after grafting reaction, respectively. N_s and M_1 are the quantity of surface NH_2 (0.695 mmol g^{-1} silica) and moleclular weight of the grafted **1** (351.304), respectively. The calculated

grafting ratio is 72.3%, which is a little higher than the value 64.8% estimated from the elemental analysis data by the following equation:

$$GR\ (\%) = [m/[N_s \times (W_2 - m) \times M_1]] \times 100\% \qquad (2)$$

where W_2 is the sampling weight and m is the total grafted weight of **1** estimated from carbon percent of elemental analysis data.

Preparation of CSP-2. Direct grafting of coumarin dimer to Lichrospher 100 NH_2 was attained by using an excess of (−)-CD. The grafting ratio of **2** was 81.2% as calculated by Eq. 1, which is similar to the value of 82.7% as estimated by Eq. 2.

The leftover lactone rings are readily hydrolyzed in basic aqueous solution. However, the found contents from elemental analysis data are lower than those of **2**, indicating that the grafted (−)-CD are partially cleavaged by the hydrolysis. Therefore, the grafting ratio is only 68.3% as estimated from elemental analysis data by using Eq. 2.

Chiral Separation of 4—17 (Fig. 1) by CSP-1 and CSP-2. Polyamide derived from (−)-CD and 1,6-hexanediamine has been proven to be an effective CSP for the resolution of **10**, **12**, and **16** (α=1.46—1.64).[11−13] The chiral recognition ability has been attributed to both silmultaneous interactions and ordered conformation (or morphology).[12,13] The CSP-1 possesses similar interaction sites for chiral recognition, such as two phenyl groups, amide linkage, and phenolic hydroxyl groups. The CSP-2 also contains many functional groups suitable for chiral recognition, although it contains an extra carboxyl group. Therefore, it is reasonable to predict that CSP-1 and CSP-2 can resolve similar racemates as the polyamide.

The capacity factor (k'), separation factor (α) and resolution (R_s) of the racemates on column bearings CSP-1 and CSP-2 are given in Table 1. Both CSP-1 and CSP-2 show selective resolution to only *trans*-stilbene oxide (**10**) with α value at 2.42 and 2.46, respectively, and the (−)-isomer elutes first. The chromatograms of **10** by CSP-1 and CSP-2 with optical rotations of the eluted enantiomers are shown in Fig. 2. However, racemates **12** and **16**, which have been resolved by the polyamide mentioned above, can not be separated by CSP-1 and CSP-2. These results also support the previous conclusion that ordered conformation plays an important role in chiral recognition on the polyamide derived from (−)-CD and 1,6-

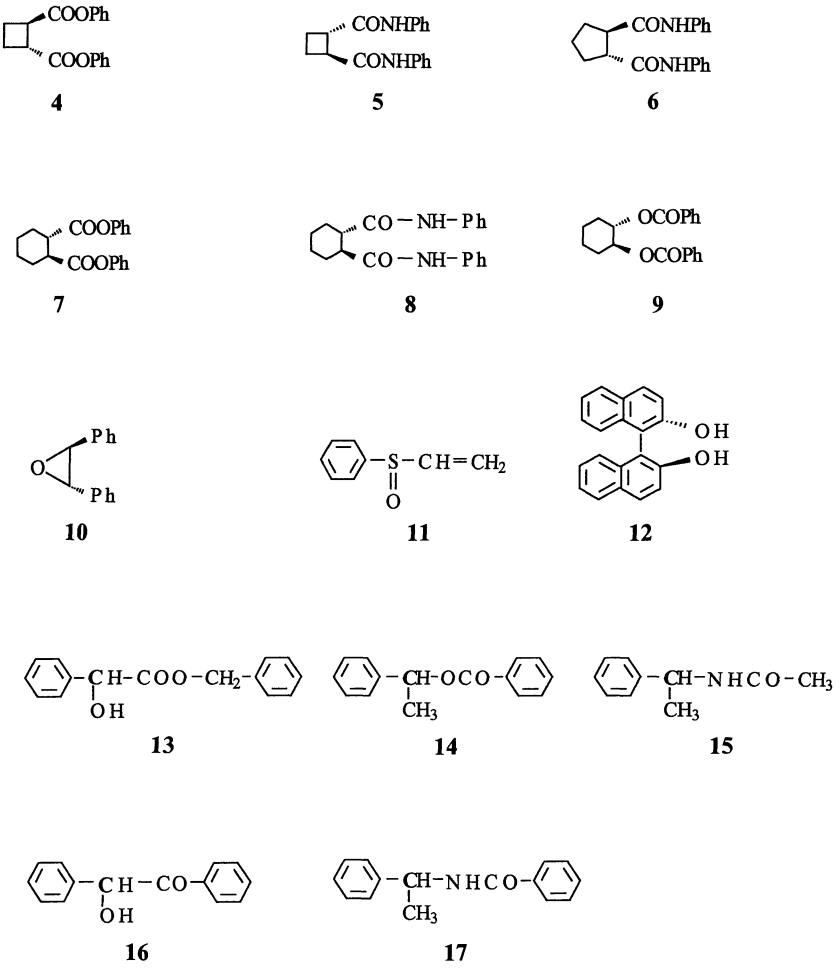


Fig. 1. Structural formulas of racemates **4**—**17**.

Table 1. Chromatographic Separation of Racemates **4**—**17** on CSP-1 and CSP-2[a)]

Racemates	CSP-1			CSP-2		
	k_1'[b)]	α[c)]	R_s[d)]	k_1'	α	R_s
4	0.48	1	—	0.33	1	—
5	0.81	1	—	0.96	1	—
6	0.53	1	—	0.58	1	—
7	0.47	1	—	0.29	1	—
8	0.44	1	—	0.67	1	—
9	0.37	1	—	0.27	1	—
10	0.17 (−)	2.42	2.00	0.14 (−)	2.46	1.68
11	2.75	1	—	2.30	1	—
12	3.78	1	—	—	—	—
13	1.28	1	—	1.35	1	—
14	0.07	1	—	0.02	1	—
15	0.74	1	—	0.63	1	—
16	1.40	1	—	3.56	1	—
17	3.42	1	—	3.19	1	—

a) The eluent was hexane/IPA (v/v=9/1) and the flow rate was 0.5 ml min^{-1} at room temperature. The column pressures of CSP-1 and CSP-2 were 81 kg cm^{-2} and 96 kg cm^{-2}, respectively. b) Capacity factor $k'=(V-V_0)/V_0$, where V_0 and V are the dead volume and elution volume, respectively. c) Separation factor $\alpha=k_2'/k_1'$. d) Resolution $R_s=2(V_2-V_1)/(W_1+W_2)$, V and W are the elution volume and band width, respectively.

Table 2. Effect of Eluent Composition (Hexane/2-Propanol) on the Resolution of *trans*-Stilbene Oxide (**10**) by CSP-1

Composition (v/v)	Solubility parameter (cal $ml^{-1})^{1/2}$[a)]	k_1'[b)]	k_2'[b)]	α
97:3	7.426	0.21	0.54	2.57
95:5	7.510	0.19	0.48	2.53
90:10	7.720	0.17	0.41	2.42
85:15	7.938	0.16	0.37	2.31
87:13	8.014	0.16	0.36	2.25
80:20	8.140	0.15	0.33	2.20
75:25	8.350	0.15	0.31	2.07
70:30	8.568	0.15	0.29	1.93

a) Solubility parameters are calculated by $[x_1v_1(sp)_1+x_2v_2(sp)_2]/(x_1v_1+x_2v_2)$, where x, v, and (sp) represent molal fraction, molar volume, and solubility parameter, respectively. b) k_1' and k_2' are the capacity factor of (−)- and (+)-*trans* stilbene oxide, respectively.

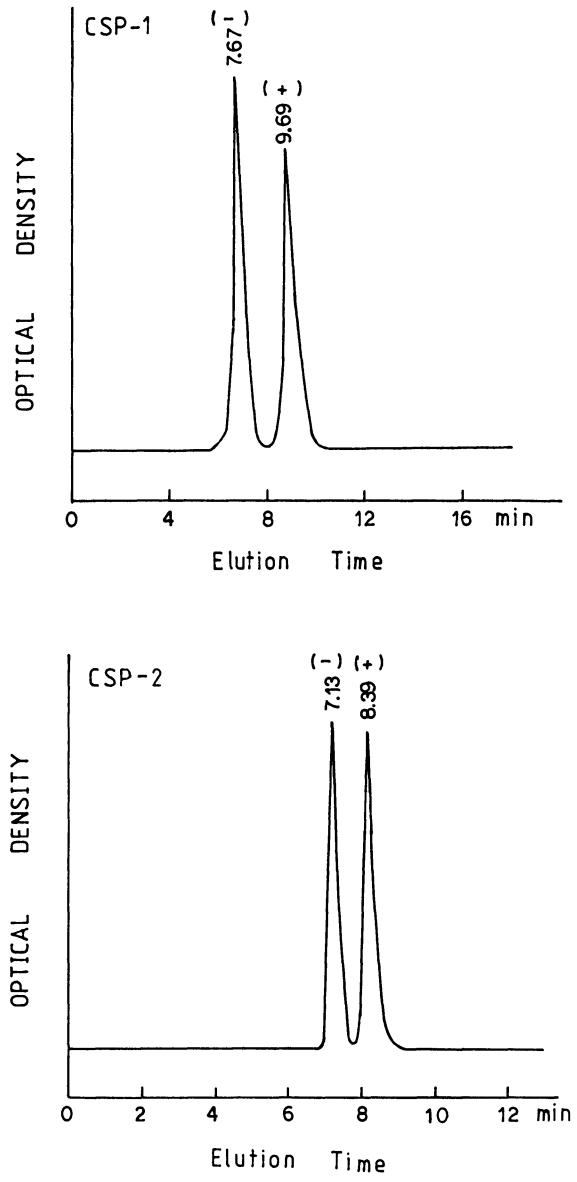


Fig. 2. Resolution chromatograms and optical rotations of *trans*-stilbene (**10**) by CSP-1 and CSP-2. Eluent: hexane/2-propanol (v/v=9/1); flow rate: 0.5 ml min^{-1}.

hexanediamine.

Chiral Recognition Mechanism. The silica gel-bonded chiral stationary phases (CSP-1 and CSP-2) interact with solute molecules through a number of different interactions, depending on the functionality of the solute and solvent molecules. The strength of these interactions determines the degree of retention, as seen by the different elution time of the racemates. The difference in stability of the transient diastereomeric complexes, formed between the CSP and solute enantiomers, determines the degree of chiral recognition. When *trans*-stilbene oxide is used as the solute mixture, the CSP-solute interactions consist of a combination of two π-π packing and a hydrogen-bonding interactions between the solute and the CSPs.

Furthermore, the solvent-CSP interactions depend on the chemical properties of the solvent used. Mixtures of hexane/2-propanol were used as eluent to study solvent effect. Hexane is nonpolar, nonselective solvent, serving only to adjust the strength of the mobile phases. 2-Propanol interacts with the CSP through reciprocal hydrogen bonding at the amide group, which is capable of reducing the strength of the solute-CSP interactions.

The influences of the solubility parameter of mobile phase on the retention and enantioselectivity (α) are shown in Tables 2 and 3 and Figs. 3 and 4. An increase in 2-propanol concentration (increase hydrogen-bonding capability of the mobile phase) decreases the

Table 3. Effect of Eluent Composition (Hexane/2-Propanol) on the Resolution of *trans*-Stilbene Oxide (**10**) by CSP-2

Composition (v/v)	Solubility parameter $(\text{cal ml}^{-1})^{1/2}$ a)	k_1' b)	k_2' b)	α
97:3	7.426	0.17	0.46	2.71
95:5	7.510	0.16	0.41	2.56
90:10	7.720	0.14	0.35	2.46
85:15	7.938	0.13	0.37	2.38
87:20	8.148	0.13	0.29	2.23
70:30	8.568	0.11	0.22	2.00

a) Solubility parameters are calculated by $[x_1v_1(\text{sp})_1 + x_2v_2(\text{sp})_2]/(x_1v_1+x_2v_2)$, where x, v, and (sp) represent molal fraction, molar volume, and solubility parameter, respectively. b) k_1' and k_2' are the capacity factor of (−)- and (+)-*trans* stilbene oxide, respectively.

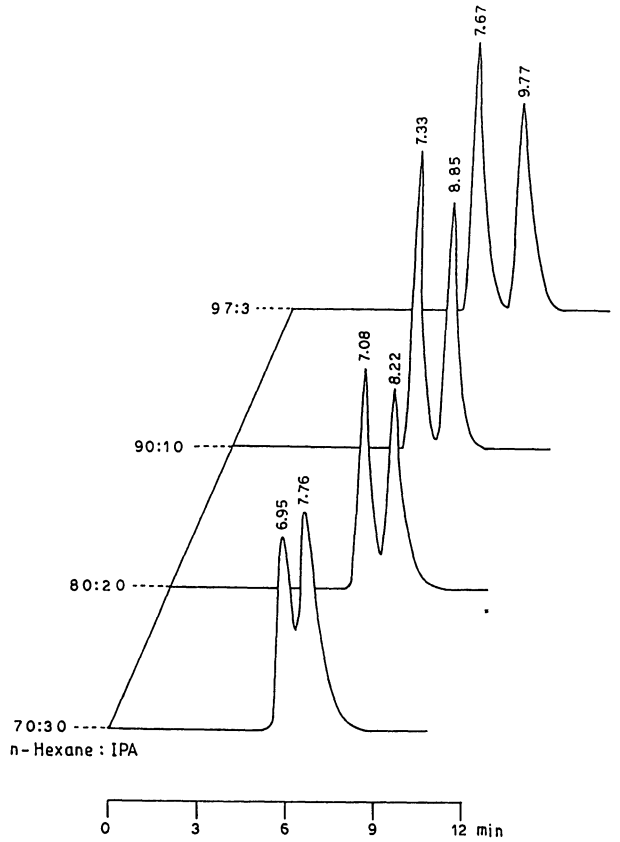


Fig. 3. Resolution chromatograms of **10** by CSP-1 at different eluent composition (hexane/2-propanol= v/v).

retention of both CSP-1 and CSP-2. This indicates that 2-propanol competes with *trans*-stilbene oxide for the polar sites on the CSPs, causing a more rapid displacement of the latter. Moreover, the selectivity (α) also decreases with the solubility parameter, suggesting that the difference in stability of the transient diastereomeric complexes has been reduced. This should be due to the solvation of the solute by 2-propanol. These results lead to the conclusion that, in addition to π–π

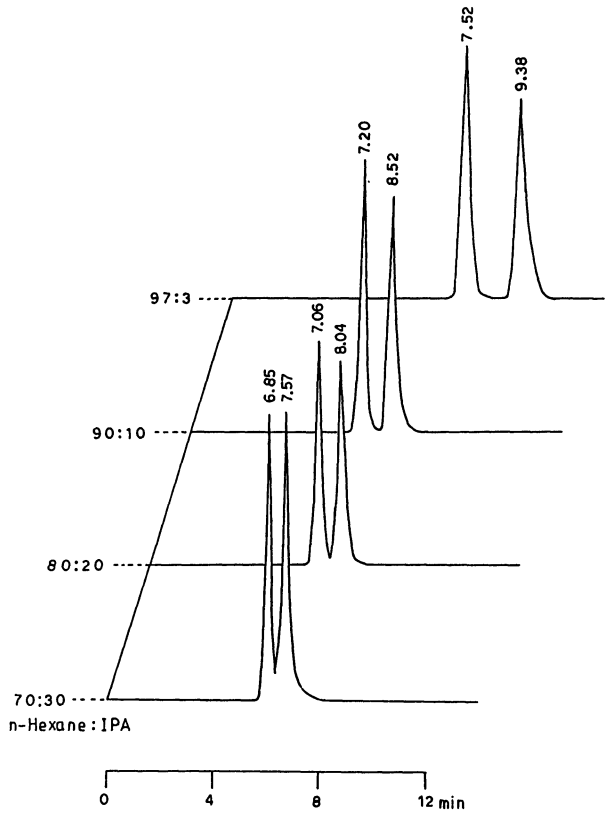


Fig. 4. Resolution chromatograms of **10** by CSP-2 at different eluent composition (hexane/2-propanol= v/v).

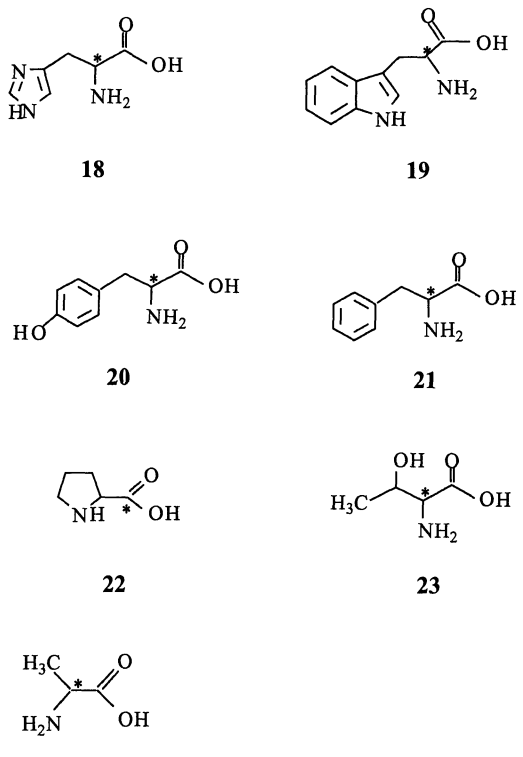


Fig. 5. Structural formulas of amino acids (**18**—**24**) used for CLEC resolution.

packing, hydrogen-bonding also plays important role in the chiral recognition process.

Resolution of DL-Amino Acids (18—24) by Chiral Ligand Exchange Liquid Chromatography. Direct optical resolution of seven amino acids (Fig. 5) by CLEC, using CSP-3 as chiral stationary phase, was performed with the same apparatus described above. However, the mobile phase used was 5×10^{-3} M $CuSO_4$ aqueous solution (1 M=1 mol dm^{-3}) and the flow rate was 0.3 ml min^{-1} due to very high pressure of the column (320 kg cm^{-2}). The capacity factors (k_D', k_L') and separation factor (α) are presented in Table 4, and the resolution chromatograms of DL-tyrosine and DL-phenylalanine are shown in Fig. 6.

Table 4. The CLEC Resolution of DL-Amino Acids (18—24) by CSP-3[a)]

Amino acid	CSP-3		
	k_L'	k_D'	α
DL-Histidine (18)	2.064	—	1
DL-Tryptophan (19)	3.857	4.407	1.14
DL-Tyrosine (20)	1.281	1.465	1.14
DL-Phenylalanine (21)	2.241	2.981	1.33
DL-Proline (22)	0.573	—	1
DL-Threonine (23)	0.376	—	1
DL-Alanine (24)	0.288	—	1

a) Eluent: 5×10^{-3} M $CuSO_4$ aqueous solution; flow rate: 0.3 ml min^{-1}.

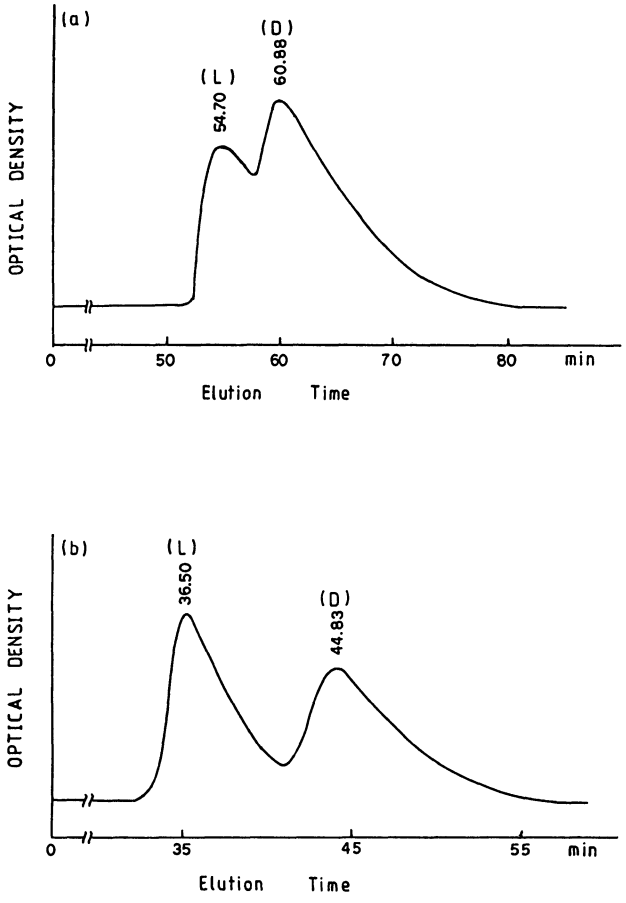


Fig. 6. Resolution chromatograms of (a) DL-tyrosine and (b) DL-phenylalanine on CSP-3. Eluent: 5×10^{-3} M $CuSO_4$ aqueous solution; flow rate: 0.3 ml min^{-1}.

The CSP-3 shows good resolution ability to DL-tryptophan, DL-tyrosine, and DL-phenylalanine (α= 1.14—1.33), which all contain an aromatic chromophore. From the k' value, clearly the retention of the amino acids containing aromatic or imidazole chromophores is much longer. The π–π interactions of the aromatic chromophores between amino acid and CSP-3 results in delayed elution of tryptophan, tyrosine, and phenylalanine. The higher retention of histidine, however, seems due to the extra complexation of imidazolyl groups with the copper ions.

The first-eluted enantiomer was L-isomer for all the three resolved amino acids. However, contrary to those discussed in CSP-1 and CSP-2, the elution order of the amino acids was determined by comparing their chromatograms with that of the corresponding L-isomer. Because the resolved amino acids (19—21) have so limited a solubility that they could not be detected by a polarimeter. From the resolution results and elution order, it is evident that π–π interaction of aromatic chromophores should be one of the key chiral discrimination force in this CLEC system. As an illustration of the recognition mechanism we propose the possible model as shown in Fig. 7. For L-isomer, the steric hindrance between phenyl group and cyclobutane ring prevents the formation of stable ternary complexes. On the contrary, for D-isomer not only this steric hindrance are lacking but also possesses an extra aromatic π–π interaction with CSP-3. In this way, the energy difference, between the ternary complexes from D- and

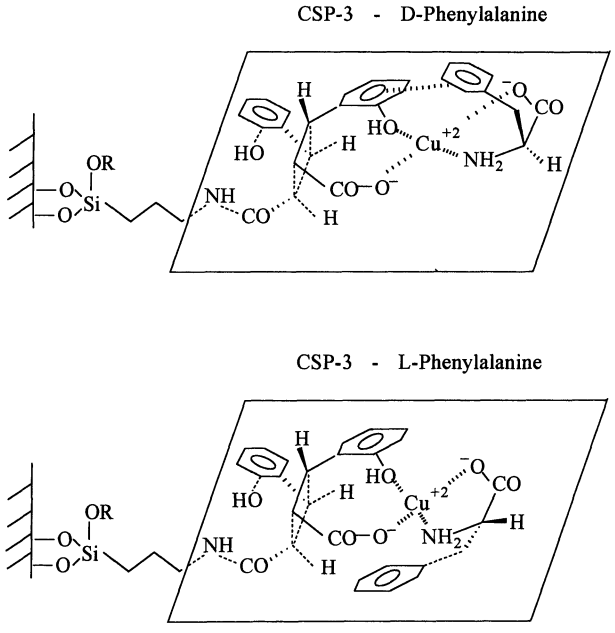


Fig. 7. Possible chiral recognition mechanism of DL-phenylalanine on CSP-3.

L-isomer, increases to such a degree that chiral discrimination can be attained.

Conclusions

Monoamide derivative of (−)-*anti* head-to-head coumarin dimer [(−)-CD] with propylamine has been successfully bonded to silica gel, Lichrospher 100 NH_2, through amide linkage to prepare CSP-1. On the other hand, CSP-2 was prepared by direct nucleophilic opening of a lactone ring of (−)-CD by Lichospher 100 NH_2, followed by hydrolysis of the other lactone ring. Both CSP-1 and CSP-2, after packing as columns of HPLC, showed high selective resolution to *trans*-stilbene oxide with α value of 2.42 and 2.46, respectively. Chiral recognition seems mainly due to π–π packing and hydrogen bonding between *trans*-stilbene oxide and the CSPs. CSP-3, obtained by ligand exchange of CSP-2 with $CuSO_{4(aq)}$, resolved DL-trypyophan, DL-tyrosine, and DL-phenylalanine (α=1.14—1.33), which all contain aromatic chromophore. From the elution order (L-isomer first) and delayed retention of the amino acids with aromatic groups, a ternary complex model was proposed for the chiral recognition mechanism.

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