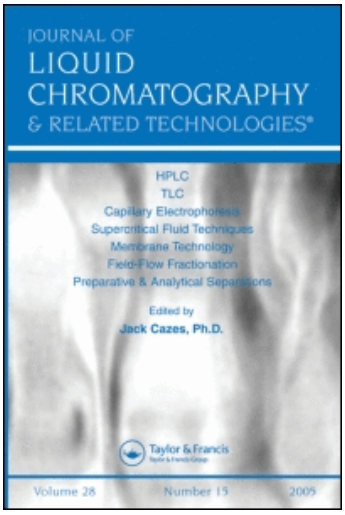


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# Preparation and Enantioseparation of Polymer-type Chiral Stationary Phases Derived from (1*S*,2*R*)-(+)-2-Amino-1,2-diphenylethanol

Shao-Hua Huang[a]; Jun-Yi Zhang[a]; Shi-Rong Li[a]; Chuan-Qi Yin[a]; Zhi-Quan Pan[a]; Zheng-Wu Bai[a]
[a] Key Laboratory of Green Chemical Process of Ministry of Education, Wuhan Institute of Technology, Wuhan, P.R. China

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# Preparation and Enantioseparation of Polymer-type Chiral Stationary Phases Derived from (1*S*,2*R*)-(+)-2-Amino-1,2-diphenylethanol

**Shao-Hua Huang, Jun-Yi Zhang, Shi-Rong Li, Chuan-Qi Yin, Zhi-Quan Pan, and Zheng-Wu Bai**

Key Laboratory of Green Chemical Process of Ministry of Education, Wuhan Institute of Technology, Wuhan, P.R. China



**Abstract:** Polymers 1 and 2 were synthesized, respectively, by the copolymerization of (1*S*,2*R*)-(+)-2-amino-1,2-diphenylethanol with 1,4-phenylene diisocyanate; and (1*S*,2*R*)-(+)-2-amino-1,2-diphenylethanol with 1,4-phenylene diisocyanate and terephthaloyl chloride. The corresponding chiral stationary phases, CSPs 1 and 2, were prepared by immobilizing these polymers on 3-aminopropyl silica gel. The enantioseparation ability of obtained chiral stationary phases was evaluated with a series of chiral analytes. The effects of organic additives, mobile phase composition, temperature, and substituents of chiral analytes on enantioseparation were investigated in high performance liquid chromatography. The preliminary studies demonstrated that the enantioseparation ability could be resumed, although the chiral stationary phase experienced acidic mobile phase.

**Keywords:** Additive, Chiral stationary phase, Co-polymer, Enantioseparation, High performance liquid chromatography

Correspondence: Prof. Zheng-Wu Bai, Key Laboratory of Green Chemical Process of Ministry of Education, Wuhan Institute of Technology, Wuhan 430073. P.R. China. E-mail: zhengwu_bai@yahoo.com

## INTRODUCTION

Recently, in the field of chiral liquid chromatography, polymer type chiral stationary phases (CSPs) have attracted much attention.[1–6] Commonly, polymer type CSPs are considered to have a potential large capacity for chiral compounds, which is essential for a CSP to be applied as an absorbent to separate chiral drugs in bulk by preparative high performance liquid chromatography (HPLC). In earlier reported works, polymer type CSPs were prepared by coating cellulose or amylose derivatives on silica gel. In this case, the CSPs were only used in limited mobile phases due to solubility or swelling of chiral selectors in mobile phases, such as chloroform, tetrahydrogenfuran, etc.[7–11] Afterwards, efforts were made to try to overcome this dilemma by covalently immobilizing the chiral selector on silica gel.[12–15] However, another problem arose; that was the enantioseparation ability decreased. Thus, up to date, many attempts have still been made to prepare polymer type CSPs by immobilizing synthetic polymers on silica gel.[4–6,16–20] The typical works are that the polymers are prepared by free radical polymerization; and then are immobilized by the reaction of two free radicals. Gasparrini and coworkers improved this technique, where the initiator was designated on the silica gel surface to avoid the polymerization of monomer in solvent that caused polymer not to be immobilized.[20] In addition, the polymers prepared by condensation polymerization are also employed as chiral selectors. Saigo and coworkers prepared a polymer type CSP by coating a polyamide on macroporous silica gel. This CSP exhibited satisfactory resolution for Tröger base, mandelamide, benzoin etc.[21] In our previous works, a novel approach was established to prepare polymer type CSPs.[22,23] The polymers, which were prepared by condensation polymerization or condensation like polymerization, were covalently immobilized on 3-aminopropyl silica gel by the residual reactive group, such as isocyanate or acyl chloride, occurring on the terminal of polymers. To investigate the relationship between polymer structures and its enantioseparation property, in this work two polymers were prepared with (1*S*,2*R*)-(+)-2-amino-1,2-diphenylethanol (ADPE) and 1,4-phenylene diisocyanate (PDI); and (1*S*,2*R*)-(+)-2-amino-1,2-diphenylethanol, 1,4-phenylene diisocyanate, and terephthaloyl chloride (TPC). The enantioseparation properties of obtained CSPs were also studied.

## EXPERIMENTAL

### Materials

ADPE was purchased from Chengdu Likai Chiral Tech. Co., Ltd. (China). PDI was purchased from Jiangsu Xingyi Pesticide Plant (China).

TPC was purchased from Jiangxi Nanchang Pesticide Plant (China). PDI and TPC were purified by recrystallization from toluene. 3-Aminopropyltriethoxysilane was obtained from Novel Organic Silicon Materials Co., Ltd. of Wuhan University (China), and redistilled before use. Silica gel (Lichrosorb Si 100) was purchased from Merck (Germany) with a particle size of 5 μm, a pore size of 100 Å, and a surface area of 300 $m^2/g$. N,N-Dimethylformamide (DMF) and triethylamine (TEA, used for CSP preparation) were dried over phosphorous pentoxide and redistilled. Toluene was refluxed with sodium and redistilled. All other chemicals used for the synthesis of CSPs were of analytical grade and used as received.

## Instrumentation

IR spectra were recorded on a Nicolet FT-IR instrument (USA) with KBr pellets. Elemental analysis (EA) was performed on an Elemental VarioEL III CHNOS apparatus (Germany). $^1H$ and $^{13}C$ nuclear magnetic resonance (NMR) spectra were recorded on a Varian INOVA 500 spectrometer (USA), setting at 500 MHz for $^1H$ spectra measurement and 125 MHz for $^{13}C$ spectra measurement, respectively. The solvent used was deuterated DMSO. Solid-state $^1H$ and $^{13}C$ NMR spectra were recorded on a Varian Infinity Plus 300 spectrometer (USA), setting at 300 MHz for $^1H$ spectra measurement and 75 MHz for $^{13}C$ spectra measurement, respectively. The number of average molecular weights of chiral polymers were determined by gel permeation chromatography (GPC) on an Agilent 1100 chromatograph (USA), equipped with an Agilent G1362A RID detector and an Agilent PLgel (5 μm 10E5Å) column (300 mm × 7.5 mm). The stainless steel HPLC empty columns (250 mm × 4.6 mm) were purchased from Hypsil (UK). The CSPs were packed into the empty columns with an Alltech model 1666 slurry packer (USA). The enantioseparation was run on a Waters chromatograph (USA), equipped with a Waters 996 photodiode array detector, a Waters 600E Quat Pump, a Waters Millenium 32 system controller, and Waters 717 plus autosampler.

## Preparation of CSPs

3-Aminopropyl silica gel was prepared according to Ihara's method by refluxing a mixture of dry silica gel and 3-aminopropyltriethoxysilane in dry toluene.[24]

ADPE, 3.02 g (14.2 mmol) and 2.50 g (15.6 mmol) PDI were dissolved in 20 mL DMF with stirring. The solution was stirred at ambient temperature for 1 h, and then was slowly heated to 60°C with stirring for 5 h. After the solution was cooled to ambient temperature, 150 mL dry

toluene was added. The solid that precipitated during the addition of toluene was collected by filtration in a nitrogen atmosphere. The solid was redissolved in DMF, and reprecipitated by the addition of toluene. The reprecipitation was repeated twice to give a pale yellow solid, polymer 1. A small portion of polymer 1 was kept for its characterization, and the rest was used in the sequent immobilization reaction without further drying. The number of average molecular weight of polymer 1 was determined as 3158 g/mol. Its specific rotation was measured as $-97.3^{\circ}$ (c 0.15, DMF). Polymer 1 was characterized by NMR at 25°C. The chemical shifts (ppm) of its $^{1}$H NMR are shown as follows: 4.91 (−CH−), 5.18–5.61 (−NHCONH−), 6.69–7.36 (aromatic H), 7.70–7.84 (aromatic H), 8.30, 8.37 (−CONH−);The chemical shifts (ppm) of its $^{13}$C NMR are shown as follows: 46.42 (−CH−), 120.45 (aromatic C), 129.88–130.37 (aromatic C), 136.87 (aromatic C), 143.35, 143.97 (aromatic C), 158.34 (−NHCONH−). Its structure was also confirmed by FT-IR, and the corresponding wave numbers of absorbance are presented as follows: 3374 $cm^{-1}$ (N−H), 3063 $cm^{-1}$, 3034 $cm^{-1}$ (C = C−H), 1720 $cm^{-1}$ ($-CO_2-$), 1654 $cm^{-1}$ (−NH−CO−), 1512 $cm^{-1}$ (−NH−CO−).

To the solution of polymer 1 in DMF, 3-aminopropyl silica gel (4.00 g) was added with gentle stirring. This mixture was heated to 75°C, and was gently stirred overnight. After the completion of immobilization, the silica gel was washed by DMF and acetone, respectively. Pale yellow CSP 1 (4.43 g) was obtained after complete removal of solvent. By element analysis, the contents of carbon, hydrogen, and nitrogen of CSP 1 were found to be 16.65%, 1.94%, and 3.31%, respectively. CSP 1 was characterized by solid-state NMR at 25°C. The chemical shifts (ppm) of its solid-state $^{1}$H NMR are shown as follows: 0.9 ($Si-CH_2$), 1.5 ($Si-CH_2-CH_2$), 2.9–3.6 ($CH_2-NH$, NH), 5.3 (−CH−), 6.8–7.8 (aromatic H). The chemical shifts (ppm) of its solid-state $^{13}$C NMR are presented as follows: 11.9 ($Si-CH_2$), 23.9 ($Si-CH_2-CH_2$), 42.5 ($CH_2-NH$), 57.7 (−CH−), 126.8–139.1 (aromatic C), 157.1, 168.3 (−CONH−). It was also detected by FT-IR, and the corresponding wave numbers of absorbance are displayed as follows: 3421 $cm^{-1}$ (N−H), 1705 $cm^{-1}$ ($-CO_2-$), 1655 $cm^{-1}$ (−NH−CO−), 1548 $cm^{-1}$, 1517 $cm^{-1}$ (−NH−CO−), 1102 $cm^{-1}$ (Si−O).

To the solution of 3.15 g (14.8 mmol) ADPE, 0.86 g (5.40 mmol) PDI, and 2.19 g (10.8 mmol) TPC in 25 mL DMF, 5 mL dry TEA was added dropwise in a nitrogen atmosphere. Then the resulting reaction solution was heated to 75°C for 10 h. Amine salt was removed by sucking filtration in a nitrogen atmosphere. The filtrate was added dropwise in a mixture of 80 mL dry toluene and 80 mL dry cyclohexane to yield a solid. The solid was reprecipitated twice using DMF as solvent, and the mixture of toluene and cyclohexane as precipitant to give pale yellow polymer 2. A small portion of polymer 2 was kept for its characterization. The rest was used for immobilization reaction. The number of average molecular weight of

polymer 2 was determined as 2898 g/mol. Its specific rotation was determined as $-131.2^{\circ}$ (c 0.05, DMF). Polymer 2 was characterized by NMR at 25°C. The chemical shifts (ppm) of its $^{1}H$ NMR are shown as follows: 5.11 (−CH−), 5.25–5.62 (−NHCONH−, −CH−), 7.07–7.48 (aromatic H), 7.75–7.81 (aromatic H), 8.29, 8.33 (−CONH−). The chemical shifts (ppm) of its $^{13}C$ NMR are shown as follows: 46.54 (−CH−), 121.33 (aromatic C), 129.72–130.79 (aromatic C), 137.30 (aromatic C), 143.42, 143.98 (aromatic C), 157.67 (−CONH−). Its structure was also determined by FT-IR, and the corresponding wave numbers of absorbance are exhibited as follows: 3399 $cm^{-1}$ (N−H); 3063 $cm^{-1}$, 3030 $cm^{-1}$ (C=C−H); 1720 $cm^{-1}$ ($-CO_2-$); 1653 $cm^{-1}$ (−NH−CO−); 1512 $cm^{-1}$ (−NH−CO−).

CSP 2 was prepared according to the procedure to prepare CSP 1, in which 40 mL DMF, 10 mL TEA, and 4.11 g 3-aminopropyl silica gel were used. After complete removal of solvent, 4.50 g CSP 2 was obtained. By element analysis, the contents of carbon, hydrogen, and nitrogen of CSP 2 were found to be 11.39%, 1.80%, and 1.93%, respectively. CSP 2 was characterized by solid-state NMR at 25°C. The chemical shifts (ppm) of its solid-state $^{1}H$ NMR are shown as follows: 0.8 ($Si-CH_2$), 1.6 ($Si-CH_2-CH_2$), 3.1–3.7 ($CH_2-NH$, NH), 5.7 (−CH−), 7.2–7.9 (aromatic H). The chemical shifts (ppm) of its solid-state $^{13}C$ NMR are given as follows: 8.4 ($Si-CH_2$), 18.5 ($Si-CH_2-CH_2$), 42.0 ($CH_2-NH$), 56.8 (−CH−), 126.6–134.2 (aromatic C), 158.1, 160.4 (−CONH−). It was also confirmed by FT-IR, and the corresponding wave numbers of absorbance are given as follows: 3449 $cm^{-1}$ (N−H); 1702 $cm^{-1}$ ($-CO_2-$); 1640 $cm^{-1}$ (−NH−CO−); 1545 $cm^{-1}$ (−NH−CO−); 1105 $cm^{-1}$ (Si−O).



### Column Packing and Enantioseparation

CSPs 1 and 2 were, respectively, packed into two columns by the balanced density slurry packing technique using acetone as packing solvent. The enantioseparation evaluation of CSPs 1 and 2 was conducted in a series of mobile phases. The flow rate of the mobile phases was set at 1.0 mL/min. The sample solutions were prepared by dissolving chiral analytes in methanol or actonitrile (ACN) and were filtered before injection.

## RESULTS AND DISCUSSION

### Synthesis of CSPs

The synthetic route is shown in Figure 1. Polymer 1 was prepared with ADPE and PDI, with a definite structure. Polymer 2 was prepared

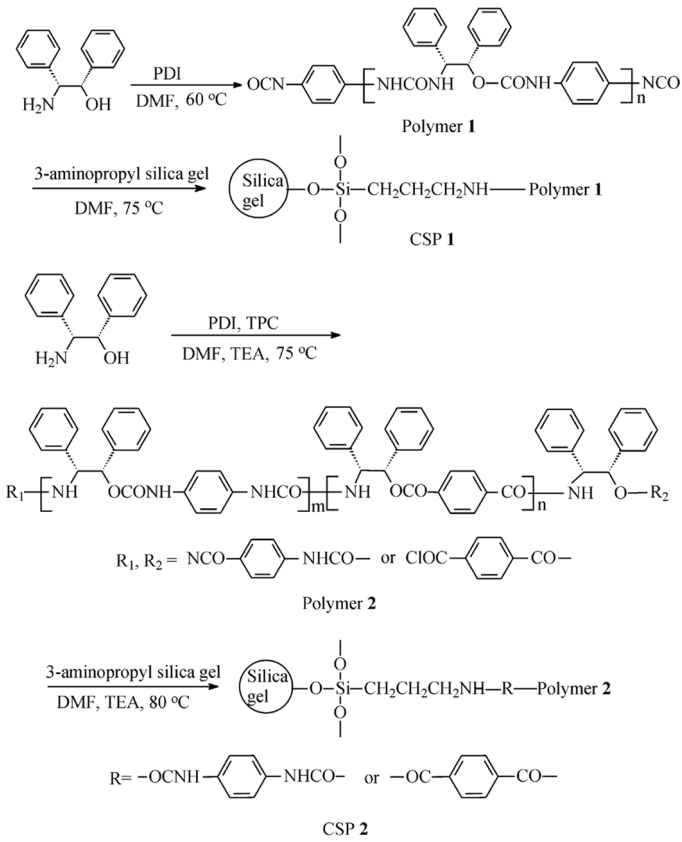



***Figure 1.*** Synthetic routes for chiral polymers and CSPs.

with ADPE, PDI, and TPC, and its structure was random. Polymer 1 was well precipitated by toluene. However, when the solution of polymer 2 in DMF was added dropwise in toluene, only a little solid precipitated. Therefore, a mixture of toluene and cyclohexane was used instead of toluene, for the purification of polymer 2 by reprecipitation. The reason for this is that the polarity of polymer 2 is comparatively lower than that of polymer 1, thus polymer 2 is precipitated in polarity lower solvent. The successful immobilization is evitable from Figure 2, where there is absorbance for amide and ester. The coverage of chiral units of these two CSPs was estimated on the basis of elemental analysis.

According to the formula 1, the coverages of a chiral unit of CSPs 1 and 2 were calculated as 40.1 mmol/g and 20.2 mmol/g, respectively. In formula 1, $C_1$ and $C_0$ are the carbon content of CSPs and 3-aminopropyl silica gel, respectively; *Mc* is the atom weight of carbon; *n* presents the carbon number of each chiral unit.

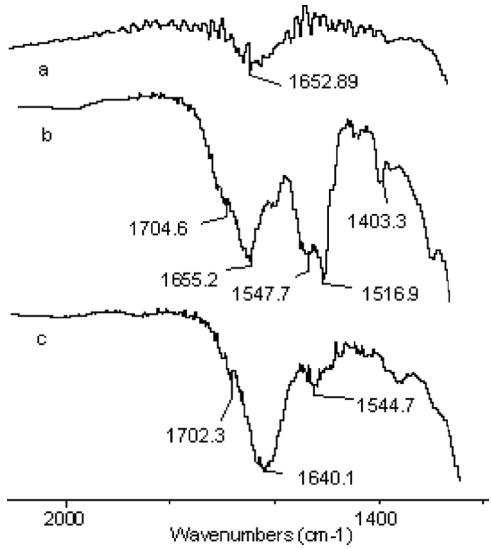



*Figure 2.* FT-IR spectra of 3-aminopropyl silica gel (a), CSP 1 (b), and CSP 2 (c).

$$\mu(\text{mmol}/g) = \frac{C_1 - C_0}{Mc \times n} \quad (1)$$

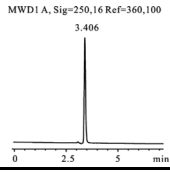
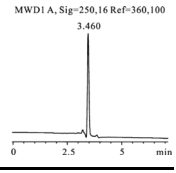
## Chromatographic Characters of Chiral Columns

The prepared CSPs were packed into columns to give the corresponding chiral columns. Their chromatograms, column pressures, and column efficiencies were investigated using biphenyl as a probe, and n-hexane/IPA (90/10), v/v as mobile phase (Table 1). These parameters demonstrate the prepared columns are suitable for the further investigation to separate chiral compounds.

## General Enantioseparation Evaluation of CSPs

The enantioseparation abilities of CSPs 1 and 2 were evaluated with various chiral compounds (Table 2). Of the chiral compounds listed in Table 2, thirteen of them were separated by CSP 1, while eleven of them were separated by CSP 2. With a view of the structures of CSPs 1 and 2, CSP 1 contains more amides, and CSP 2 contains more esters. More amides are advantageous for CSP 1 to form a hydrogen bond with chiral analytes, and as a result, the solvent of high polarity is needed to improve

***Table 1.*** Chromatographic properties of columns packed with CSPs 1 and 2

| Column | CSP 1 | CSP 2 |
|---|---|---|
| Column pressure | 39 bar | 42 bar |
| Column efficiency | 51 484 plates/m | 43 504 plates/m |
| Chromatogram |  |  |



*Character evaluation conditions*: sample: biphenyl; mobile phase: *n*-hexane/IPA (90/10), v/v; flow-rate: 1.0 mL/min.



the eluting power of mobile phases. Therefore, CSP 1 exhibited its separation ability mainly in reverse phases, and CSP 2 exhibited its separation ability in both normal phase and reverse phase. Figure 3 shows the chromatogram of compound 19 separated on CSP 1, where the peaks corresponding to *R* and *S* configuration were identified by comparing the peaks with those of pure *R* form and *S* form samples. These chromatograms are satisfactory. Thus, quantitative determination of a single isomer of a racemic mixture can be implemented if enough resolution is achieved under optimal separation condition.

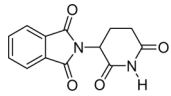
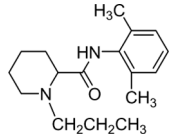
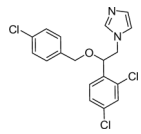
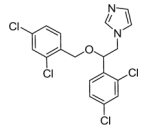
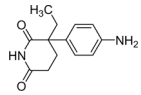
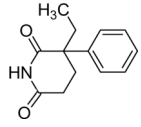
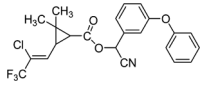
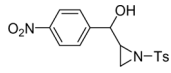
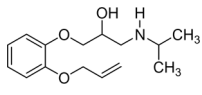
## Influence of Temperature on Enantioseparation

Usually temperature influences chiral recognition. Compounds 8, 15, and 22 were, respectively, separated on CSP 1 at different temperatures (Table 3). For compound 8, the resolution almost remained unchanged at the selected temperatures. For compounds 15 and 22, the resolution rose at the lower temperature, and then descended at the increase of the temperature. At lower temperature, the interaction between chiral selector and chiral analytes is strengthened and, on the contrary, it is weakened at higher temperature because of the molecular movement. It is believed that strengthening or weakening is variable for a pair of enantiomers, leading to different resolutions.

## Influence of Organic Additives on Enantioseparation

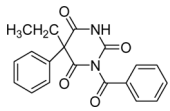
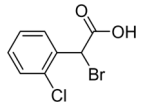
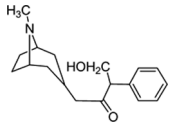
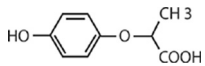
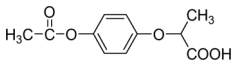
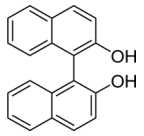
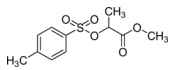
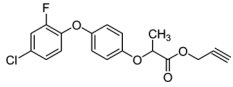
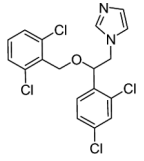
The influence of organic additives on chiral recognition was studied in many works.[25–28] Organic base or acid is usually used as additives.

***Table 2.*** Chromatographic resolution of chiral compounds on CSPs 1 and 2

| S/N | Chiral compound | Parameter | CSP 1 Separation result | CSP 1 Condition | CSP 2 Separation result | CSP 2 Condition |
|---|---|---|---|---|---|---|
| 1 |  | $k_1$ | 0.94 | | 0.63 | |
| | | $k_2$ | 1.38 | A | 0.63 | A |
| | | $\alpha$ | 1.47 | | 1.00 | |
| | | $R_s$ | 1.59 | | 0 | |
| 2 |  | $k_1$ | 1.52 | | 0.37 | |
| | | $k_2$ | 1.52 | B | 0.48 | B |
| | | $\alpha$ | 1.00 | | 1.30 | |
| | | $R_s$ | 0 | | 0.34 | |
| 3 |  | $k_1$ | 1.48 | | 3.30 | |
| | | $k_2$ | 1.48 | C | 4.73 | D |
| | | $\alpha$ | 1.00 | | 1.43 | |
| | | $R_s$ | 0 | | 0.91 | |
| 4 |  | $k_1$ | 2.48 | | 1.81 | |
| | | $k_2$ | 2.48 | C | 4.35 | D |
| | | $\alpha$ | 1.00 | | 2.41 | |
| | | $R_s$ | 0 | | 2.44 | |
| 5 |  | $k_1$ | 0.28 | | 0.44 | |
| | | $k_2$ | 0.39 | E | 0.44 | E |
| | | $\alpha$ | 1.39 | | 1.00 | |
| | | $R_s$ | 0.38 | | 0 | |
| 6 |  | $k_1$ | 0.23 | | 0.46 | |
| | | $k_2$ | 0.33 | F | 0.46 | F |
| | | $\alpha$ | 1.43 | | 1.00 | |
| | | $R_s$ | 0.33 | | 0 | |
| 7 |  | $k_1$ | 0.05 | | 0.76 | |
| | | $k_2$ | 0.21 | G | 1.02 | H |
| | | $\alpha$ | 4.20 | | 1.34 | |
| | | $R_s$ | 1.33 | | 0.99 | |
| 8 |  | $k_1$ | 0.56 | | 0.61 | |
| | | $k_2$ | 0.86 | I | 0.61 | I |
| | | $\alpha$ | 1.54 | | 1.00 | |
| | | $R_s$ | 2.31 | | 0 | |
| 9 |  | $k_1$ | 0.25 | | 0.89 | |
| | | $k_2$ | 0.28 | J | 1.45 | K |
| | | $\alpha$ | 1.12 | | 1.63 | |
| | | $R_s$ | 0.16 | | 1.73 | |

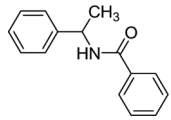
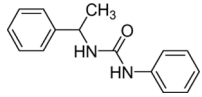
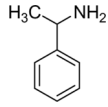
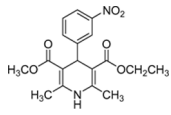
(*Continued*)

**Table 2.** Continued

| S/N | Chiral compound | Para-meter | CSP 1 Separation result | CSP 1 Condition | CSP 2 Separation result | CSP 2 Condition |
|---|---|---|---|---|---|---|
| 10 |  | $k_1$ | 2.10 | | 0.79 | |
| | | $k_2$ | 2.28 | L | 0.79 | L |
| | | $\alpha$ | 1.09 | | 1.00 | |
| | | $R_s$ | 0.47 | | 0 | |
| 11 |  | $k_1$ | 0.65 | | 0.51 | |
| | | $k_2$ | 0.65 | M | 0.83 | M |
| | | $\alpha$ | 1.00 | | 1.63 | |
| | | $R_s$ | 0 | | 1.50 | |
| 12 |  | $k_1$ | 0.18 | | 0.36 | |
| | | $k_2$ | 0.18 | F | 0.54 | F |
| | | $\alpha$ | 1.00 | | 1.50 | |
| | | $R_s$ | 0 | | 0.55 | |
| 13 |  | $k_1$ | | | 1.64 | |
| | | $k_2$ | – | | 1.64 | J |
| | | $\alpha$ | | | 1.00 | |
| | | $R_s$ | | | 0 | |
| 14 |  | $k_1$ | 0.64 | | 0.53 | |
| | | $k_2$ | 0.64 | A | 0.86 | A |
| | | $\alpha$ | 1.00 | | 1.62 | |
| | | $R_s$ | 0 | | 1.60 | |
| 15 |  | $k_1$ | 0.21 | | | |
| | | $k_2$ | 0.33 | N | - | |
| | | $\alpha$ | 1.57 | | | |
| | | $R_s$ | 0.49 | | | |
| 16 |  | $k_1$ | 0.57 | | 0.27 | |
| | | $k_2$ | 0.57 | L | 0.42 | L |
| | | $\alpha$ | 1.00 | | 1.56 | |
| | | $R_s$ | 0 | | 0.36 | |
| 17 |  | $k_1$ | 1.78 | | 0.72 | |
| | | $k_2$ | 1.95 | O | 0.72 | O |
| | | $\alpha$ | 1.10 | | 1.00 | |
| | | $R_s$ | 0.63 | | 0 | |
| 18 |  | $k_1$ | 1.08 | | 0.69 | |
| | | $k_2$ | 1.17 | P | 0.79 | C |
| | | $\alpha$ | 1.08 | | 1.14 | |
| | | $R_s$ | 0.18 | | 0.18 | |

(*Continued*)

**Table 2.** Continued

| S/N | Chiral compound | Parameter | CSP 1 Separation result | CSP 1 Condition | CSP 2 Separation result | CSP 2 Condition |
|---|---|---|---|---|---|---|
| 19 |  | $k_1$ | 8.86 | | 0.32 | |
| | | $k_2$ | 10.60 | Q | 0.32 | R |
| | | $\alpha$ | 1.20 | | 1.00 | |
| | | $R_s$ | 1.65 | | 0 | |
| 20 |  | $k_1$ | 1.00 | | 0.34 | |
| | | $k_2$ | 1.21 | R | 0.34 | R |
| | | $\alpha$ | 1.21 | | 1.00 | |
| | | $R_s$ | 1.15 | | 0 | |
| 21 |  | $k_1$ | 1.33 | | 0.48 | |
| | | $k_2$ | 1.33 | R | 0.48 | R |
| | | $\alpha$ | 1.00 | | 1.00 | |
| | | $R_s$ | 0 | | 0 | |
| 22 |  | $k_1$ | 0.20 | | 0.35 | |
| | | $k_2$ | 0.30 | N | 0.41 | N |
| | | $\alpha$ | 1.50 | | 1.17 | |
| | | $R_s$ | 0.51 | | 0.20 | |

Retention factor ($k_1$): $(t_1 - t_0)/t_0$, where $t_1$ is the retention time of the first-eluted enantiomer, and $t_0$ was determined by measuring the retention time of sodium nitrate; Retention factor ($k_2$): $(t_2 - t_0)/t_0$, where $t_2$ is the retention time of the second-eluted enantiomer; Separation factor ($\alpha$): $k_2/k_1$; Resolution ($R_s$): $2(t_2\text{-}t_1)/(w_1 + w_2)$, where $w_1$ and $w_2$ are, respectively, the bandwidth of the first-eluted enantiomer and the second-eluted enantiomer; *Chromatographic conditions*: A: eluent: methanol/water (70/30, v/v); flow-rate: 1.0 mL/min; UV detection: 225 nm; B: eluent: ACN/water (50/50, v/v); flow-rate: 1.0 mL/min; UV detection: 240 nm; C: eluent: hexane/IPA (50/50, v/v); flow-rate: 1.0 mL/min; UV detection: 230 nm; D: eluent: hexane/IPA (90/10, v/v); flow-rate: 1.0 mL/min; UV detection: 230 nm; E: eluent: ACN/water (90/10, v/v); flow-rate: 1.0 mL/min; UV detection: 245 nm; F: eluent: methanol/buffer (pH 5.43) (90/10, v/v); flow-rate: 1.0 mL/min; UV detection: 230 nm; G: eluent: methanol/water/TEA (90/10/0.1, v/v/v); flow-rate: 1.0 mL/min; UV detection: 205 nm; H: eluent: methanol/buffer (pH 5.43) (65/35, v/v); flow-rate: 1.0 mL/min; UV detection: 205 nm; I: eluent: methanol/water/TEA (70/30/0.1, v/v/v); flow-rate: 1.0 mL/min; UV detection: 254 nm; J: eluent: ACN/buffer (pH 5.35) (60/40, v/v); flow-rate: 1.0 mL/min; UV detection: 270 nm; K: eluent: methanol/water (70/30, v/v); flow-rate: 1.0 mL/min; UV detection: 270 nm; L: eluent: ACN/buffer (pH 7.03) (60/40, v/v); flow-rate: 1.0 mL/min; UV detection: 235 nm; M: eluent: hexane/IPA (80/20, v/v); flow-rate: 1.0 mL/min; UV detection: 210 nm; N: eluent: ACN/water (80/20, v/v); flow-rate: 1.0 mL/min; UV detection: 245 nm; O: eluent: methanol/water (50/50, v/v); flow-rate: 1.0 mL/min; UV detection: 254 nm; P: eluent: ACN/buffer (pH 5.43) (80/20, v/v); flow-rate: 1.0 mL/min; UV detection: 230 nm; Q: eluent: ACN/buffer (pH 5.43) (30/70, v/v); flow-rate: 1.0 mL/min; UV detection: 250 nm; R: eluent: ACN/water/TEA (60/40/0.1, v/v/v); flow-rate: 1.0 mL/min; UV detection: 250 nm

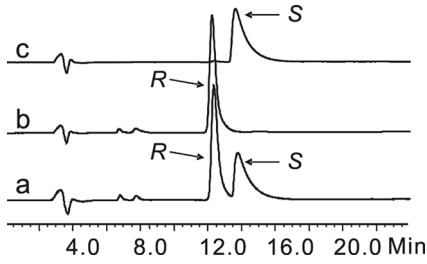
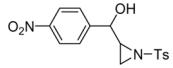
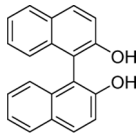
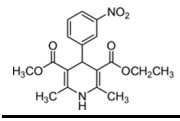



***Figure 3.*** Chromatogram of the enantioseparation of compound 19 on CSP 1. Chromatographic conditions: eluent: ACN/buffer (pH 5.43) (30/70, v/v); flow rate: 1.0 mL/min; UV detection: 250 nm.

Table 4 shows the influence of TEA on the enantioseparation of compounds 1, 7, and 8. For these three compounds, the all enantioseparations were improved in the presence of TEA. However, no satisfactory improvement was found when trichloroacetic acid (TCA) was used as additive. Meanwhile it was found that the compounds that were

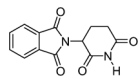
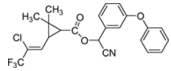
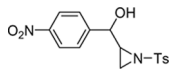


***Table 3.*** Effect of temperature on enantioseparation of compounds 8, 15, and 22 resolved by CSP 1

| Chiral compound | Parameter | Temperature 5°C | 20°C | 40°C |
|---|---|---|---|---|
| 8.  | $k_1$ | 0.82 | 0.83 | 0.63 |
| | $k_2$ | 0.88 | 0.89 | 0.67 |
| | α | 1.07 | 1.07 | 1.06 |
| | $R_s$ | 0.28 | 0.31 | 0.28 |
| 15.  | $k_1$ | 0.24 | 0.21 | 0.23 |
| | $k_2$ | 0.34 | 0.33 | 0.30 |
| | α | 1.42 | 1.57 | 1.30 |
| | $R_s$ | 0.29 | 0.49 | 0.27 |
| 22.  | $k_1$ | 0.21 | 0.20 | 0.20 |
| | $k_2$ | 0.32 | 0.30 | 0.25 |
| | α | 1.52 | 1.50 | 1.25 |
| | $R_s$ | 0.51 | 0.51 | 0.20 |

*Chromatographic conditions*: eluent: ACN/water (60/40, v/v); flow-rate: 1.0 mL/min; UV detection: 254 nm (compound 8). Eluent: ACN/water (80/20, v/v); flow-rate: 1.0 mL/min; UV detection: 245 nm (compounds 15 and 22).

***Table 4.*** Effect of TEA on enantioseparation of compounds 1, 7, and 8 resolved by CSP 1

| Chiral compound | Methanol/water (90/10, v/v) | | | | Methanol/water/TEA (90/10/0.1, v/v/v) | | | |
|---|---|---|---|---|---|---|---|---|
| | $k_1$ | $k_2$ | $\alpha$ | $R_s$ | $k_1$ | $k_2$ | $\alpha$ | $R_s$ |
| 1.  | 0.54 | 0.75 | 1.39 | 0.80 | 0.34 | 0.51 | 1.47 | 0.98 |
| 7.  | 0.75 | 1.08 | 1.44 | 0.33 | 0.05 | 0.21 | 4.19 | 1.06 |
| 8.  | 0.51 | 0.74 | 1.46 | 1.22 | 0.34 | 0.53 | 1.58 | 1.57 |

*Chromatographic conditions*: eluent: flow-rate: 1.0 mL/min; UV detection: 225 nm (compound 1), 205 nm (compound 7), 254 nm (compound 8).

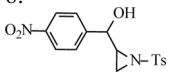
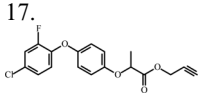
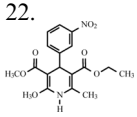


separated before use of TCA could not be separated any more after use of TCA. The reason possibly is that TCA reacts with the amine existing on the surface of silica gel, resulting in the change of CSP status. To resume the enantioseparation ability of acid experienced CSP, the column was flushed with diluted NaOH solution (pH 10) and pure water, respectively. Then the chiral compounds that were separated before use of TCA were tested again at the same condition. The chromatographic results showed these compounds could be separated again with almost the same resolution (Table 5). That means the CSP could be resumed from a changed status. The detailed investigation to resume CSPs from an acidic environment is undergoing, and the work will be published afterwards.

## Influence of the Composition of Mobile Phases on Enantioseparation

Generally, the composition of mobile phases affects the enantioseparation of chiral compounds. It is desired that a chiral compound be separated within a wide range of mobile phase composition. This investigation was performed in normal phase and reverse phase. The mobile phases of hexane/IPA, hexane/ethanol, and hexane/THF differently affected chiral recognition of compound 4 separated on CSP 2 (Table 6). This chiral compound was separated better in hexane/IPA than in hexnae/ethanol and in hexane/THF. The reason is that the polar organic solvent may be involved in the formation of the diastereomers between chiral analytes and chiral selector, which was discussed in our

***Table 5.*** Effect of TCA on enantioseparation of compounds 8, 17, and 22 resolved by CSP 2

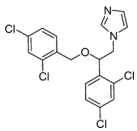
| Compound | Parameter | The status of chiral column | | |
|---|---|---|---|---|
| | | Before use of TCA | After use of TCA | After resuming |
| 8.  | $k_1$ | 0.35 | 0.32 | 0.28 |
| | $k_2$ | 0.43 | 0.32 | 0.38 |
| | $\alpha$ | 1.23 | 1.00 | 1.36 |
| | $R_s$ | 0.26 | 0 | 0.31 |
| 17.  | $k_1$ | 0.34 | 0.25 | 0.22 |
| | $k_2$ | 0.49 | 0.25 | 0.36 |
| | $\alpha$ | 1.44 | 1.00 | 1.64 |
| | $R_s$ | 0.44 | 0 | 0.59 |
| 22.  | $k_1$ | 0.35 | 0.32 | 0.28 |
| | $k_2$ | 0.45 | 0.32 | 0.39 |
| | $\alpha$ | 1.29 | 1.00 | 1.39 |
| | $R_s$ | 0.38 | 0 | 0.34 |

*Chromatographic conditions*: eluent: ACN/buffer (pH 7.95) (85/15, v/v); flow-rate: 1.0 mL/min; UV detection: 254 nm (compounds 8 and 17), 245 nm (compound 22).



previous work.[22,23] Figures 4, 5, and 6 show the influence of the content variation of mobile phase composition on the enantioseparation of compounds 7, 19, and 4, respectively. The features of the enantioseparation of these three compounds are different. For compound 7, the resolution reached maximum at the volume ratio of 0.05 when ethanol and hexane were used as mobile phase; for compound 19, the resolution became better as the increase of ACN content; when the volume ratio of ACN to

***Table 6.*** Effect of mobile phase composition on enantioseparation of compounds 4 resolved by CSP 2

| Chiral compound | Parameter | Polar solvent | | |
|---|---|---|---|---|
| | | IPA | Ethanol | THF |
|  | $k_1$ | 1.81 | 0.83 | 1.38 |
| | $k_2$ | 4.35 | 1.07 | 1.83 |
| | $\alpha$ | 2.41 | 1.27 | 1.32 |
| | $R_s$ | 2.44 | 0.47 | 0.87 |

*Chromatographic conditions*: eluent: a mixture of hexane and IPA, or ethanol, or THF with a ratio of 90/10 (v/v); flow-rate: 1.0 mL/min; UV detection: 225 nm.

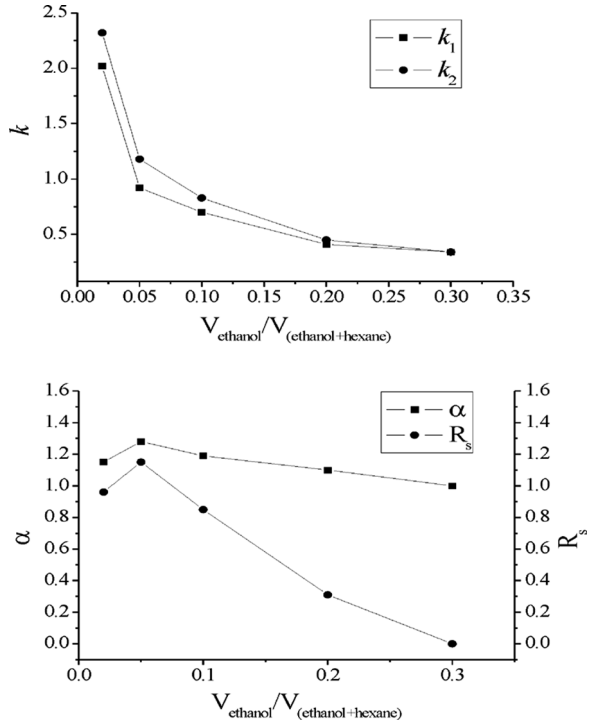



***Figure 4.*** Effect of the content variation of mobile phase composition on the enantioseparation of compound 7 resolved by CSP 1. The top: the relationship between retention factor ($k$) and mobile phase composition; below: the relationship between capacity factor ($\alpha$) and resolution ($R_s$), and mobile phase composition (the same definition hereafter). Flow rate: 1.0 mL/min; UV detection: 205 nm.

buffer went up to 0.7, the resolution was above 1.6, which was satisfactory for quantitative determination; for compound 4, the resolution descended first as the increase of IPA content, and then went up. Thus, the optimal composition of mobile phase should be investigated fully before the chiral columns are applied.

## Influence of Substituents of Chiral Compounds on Enantioseparation

The structure of chiral compounds is closely related to chiral recognition. Compounds 19, 20, and 21 are similar in structure. Compounds 19 and 20 were prepared by benzoylation and phenylcarbamoylation of 1-phenylethylamine, respectively. These two compounds bear more steric hindrance to the chiral center compared to 1-phenylethylamine. 1-Phenylethylamine was not resolved by CSP 1 or 2, while compounds

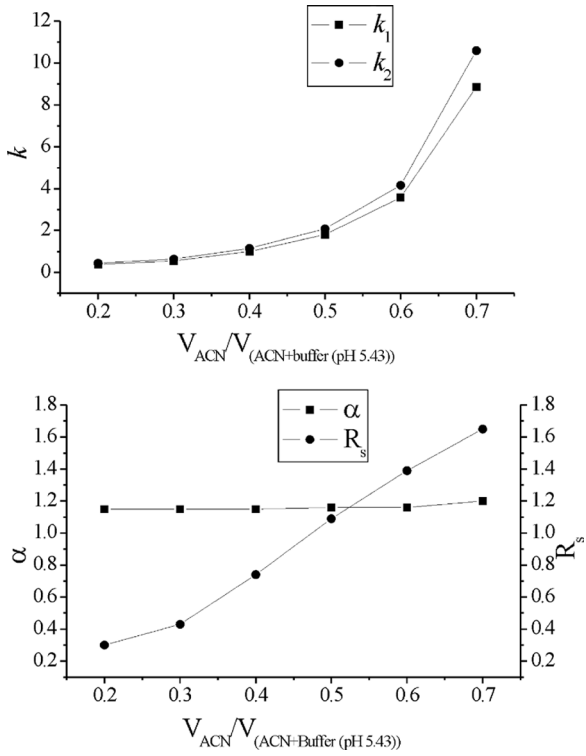



***Figure 5.*** Effect of the content variation of mobile phase composition on enantioseparation of compound 19 resolved by CSP 1. Flow rate: 1.0 mL/min.; UV detection: 250 nm.



19 and 20 were separated by CSP 1 (Table 2). The resolution difference is probably caused by different steric hindrance when chiral analytes interact with chiral selector. The same feature can be found in asymmetric synthesis, where chiral catalysts are usually used to produce stereo selectivity. These catalysts are often prepared with large steric hindrance ligands.[29] Therefore, steric hindrance is a necessary factor for chiral recognition.

## Influence of Injection Amount on Enantioseparation

As a common knowledge, chiral compounds are unsatisfactorily separated at a high injection amount. The relationship between injection amount of compound 19 and its corresponding resolution was investigated (Figure 7). At the injection amount of 15 μg, the resolution was

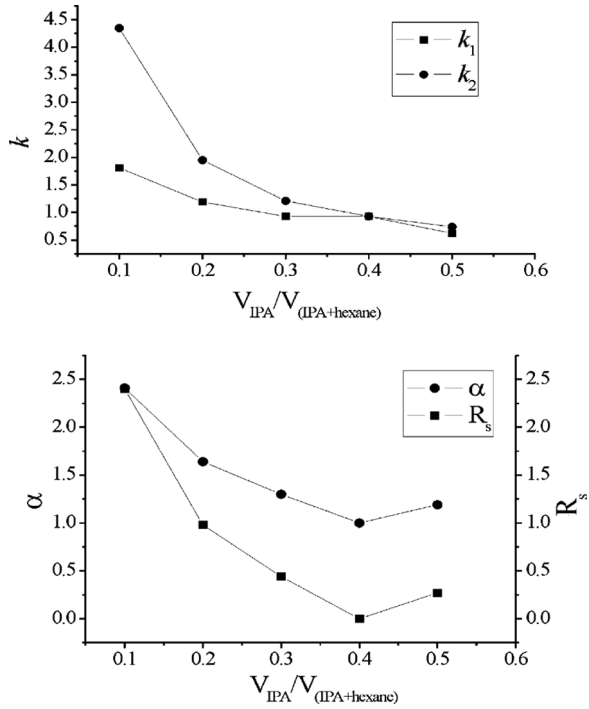



***Figure 6.*** Effect of content variation of mobile phase composition on enantioseparation of compound 4 resolved by CSP 2. Flow rate: 1.0 mL/min.; UV detection: 230 nm.



satisfactory. The resolution decreased to 0.5 when it was injected with 300 μg each time. When the injection amount was highly increased, however, the resolution did not decrease as sharply.

## CONCLUSION

A bipolymer and a terpolymer were, respectively, synthesized by the copolymerization of ADPE with PDI, and ADPE with PDI and TPC. These two polymers were immobilized on amino functionalized silica gel to produce two new CSPs. The CSP derived from ADPE and PDI, whose main chain is consisted of urea and carbamate, demonstrates enantioseparation ability mainly in reverse phase; and the CSP derived from ADPE, PDI, and TPC, whose main chain is consisted of urea, carbamate, amide, and ester, demonstrates enantioseparation ability in both normal phase and reverse phase. Many CSPs are prepared using aminated silica gel as the supporter, because the chiral selectors

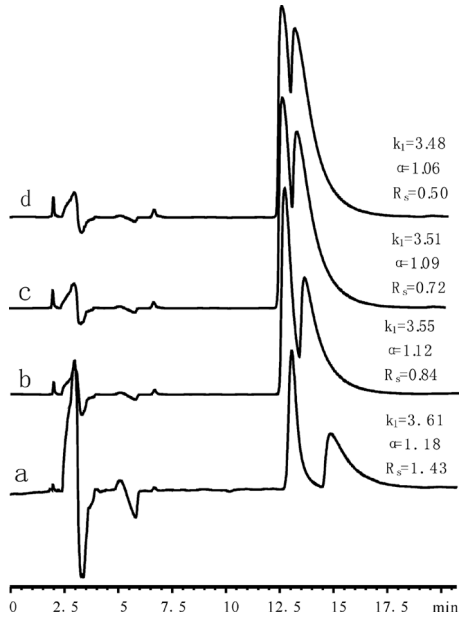



***Figure 7.*** Effect of injection amount of compound 19 on its enantioseparation resolved by CSP 1. Eluent: ACN/water (60/40, v/v); flow rate: 1.0 mL/min.; UV detection: 250 nm; injection amount: .15 μg (a), 75 μg (b), 150 μg (c), 300 μg (d).



are readily immobilized by the amino on silica gel. However, in this case, acidic organic additives that can possibly improve the resolution of chiral compounds cannot be used. In this work, it was found that the enantioseparation ability of the acid experienced CSP with the supporter of aminated silica gel could be resumed, after treating the CSP with a diluted NaOH solution. It suggests a methodological consideration to resume the enantioseparation ability of CSPs, which are prepared from amino fuctionalized materials.

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## REFERENCES

1. Chen, X.M.; Yamamoto, C.; Okamoto, Y. Influence of vinyl monomers and temperature on immobilization of cellulose 3,5-dimethylphenylcarbamate

onto silica gel as chiral stationary phases for high performance liquid chromatography. J. Chromatogr. A. **2006**, *1104*, 62–68.
2. Ghanem, A.; Hoenen, H.; Aboul-Enein, H.Y. Application and comparison of immobilized and coated amylose tris-(3,5-dimethylphenylcarbamate) chiral stationary phases for the enantioselective separation of beta-blockers enantiomers by liquid chromatography. Talanta. **2006**, *68*, 602–609.
3. Yang, G.S.; Vazquez, P.P.; Frenich, A.G.; Vidal, J.L.; Aboul-Enein, H.Y. Enantioseparation of organophosphonate derivatives on amylase tris(3,5-dimethylphenylcarbamate) chiral stationary phase by HPLC. J. Liq. Chromatogr. & Rel. Technol. **2003**, *26* (18), 3075–3084.
4. Lee, Y.K.; Nakashima, Y.; Onimura, K.; Tsutsumi, H.; Oishi, T. Free-radical polymerization of (*R*)-(−)-1-(1-naphthyl)ethyl(2- methacryloyloxyethyl)urea and chiral recognition ability. Macromolecules. **2003**, *36*, 4735–4742.
5. Kurata, K.; Shimoyama, T.; Dobashi, A. Enantiomeric separation using temperature-responsive chiral polymers composed of L-valine diamide derivatives in aqueous liquid chromatography. J. Chromatogr. A. **2003**, *1012*, 47–56.
6. Lee, Y.K.; Yamashita, K.; Eto, M.; Onimura, K.; Tsutsumi, H.; Oishi, T. Synthesis of novel chiral poly(methacrylate)s bearing urethane and cinchona alkaloid moieties in side chain and their chiral recognition abilities. Polymer. **2002**, *43*, 7539–7547.
7. Cirilli, R.; Ferretti, R.; Gallinella, B.; La Torre, F.; La Regina, G.; Silvestri, R. Comparative study between the polysaccharide-based Chiralcel OJ and Chiralcel OD CSPs in chromatographic enantioseparation of imidazole analogues of Fluoxetine and Miconazole. J. Sep. Sci. **2005**, *28*, 627–634.
8. Bielejewska, A.; Duszczyk, K.; Zukowski, J. HPLC separation of linezolid enantiomers using polysaccharide-based chiral stationary phases. Acta. Chromatogr. **2005**, *15*, 183–191.
9. Goossens, J.F.; Foulon, C.; Bailly, C. Chiral resolution of enantiomers of homocamptothecin derivatives, antitumor topoisomerase I inhibitors, using high performance liquid chromatography on polysaccharide-based chiral stationary phases. Chromatographia. **2004**, *59*, 305–313.
10. Okamoto, Y.; Yashima, E. Polysaccharide derivatives for chromatographic separation of enantiomers. Angew. Chem. Int. Ed. **1998**, *37* (8), 1020–1043.
11. Franco, P.; Senso, A.; Oliveros, L.; Minguillón, C. Covalently bonded polysaccharide derivatives as chiral stationary phases in high-performance liquid chromatography. J. Chromatogr. A **2001**, *906*, 155–170.
12. Zhang, T.; Nguyen, D.; Franco, P.; Murakami, T.; Ohnishi, A.; Kurosawa, H. Cellulose 3,5-dimethylphenylcarbamate immobilized on silica – A new chiral stationary phase for the analysis of enantiomers. Anal. Chim. Acta. **2006**, *557*, 221–228.
13. Ikai, T.; Yamamoto, C.; Kamigaito, M.; Okamoto, Y. Immobilized polysaccharide-based chiral stationary phases for HPLC. Polym. J. **2006**, *38*, 91–108.
14. Ghanem, A.; Naim, L. Immobilized versus coated amylose tris(3,5-dimethylphenylcarbamate) chiral stationary phases for the enantioselective separation

of cyclopropane derivatives by liquid chromatography. J. Chromatogr. A. **2006**, *1101*, 171–178.
15. Ghanem, A.; Aboul-Enein, H.Y. Comparison, applications, advantages, and limitations of immobilized and coated amylose tris-(3,5-dimethylphenylcarbamate)chiral stationary phases in HPLC. J. Liq. Chromatogr. & Rel. Technol. **2005**, *28* (17), 2669–2680.
16. Galli, B.; Gasparrini, F.; Misiti, D.; Pierini, M.; Viliani, C.; Bronzetti, M. HPLC resolution of atropoisomeric compounds on a CSP derived from (1*R*,2*R*)-diaminocyclohexane: Thermodynamic data from variable temperature chromatography. Chirality **1992**, *4*, 384–388.
17. Gasparrini, F.; Misiti, D.; Villani, C. Chromatographic optical resolution on trans-1,2-diaminocyclohexane derivatives: theory and applications. Chirality. **1992**, *4*, 447–458.
18. Thunberg, L.; Allenmark, S. Resolution studies on two regioisomeric chiral stationary phases: effects from reversed orientation of an amide group. J. Chromatogr. A. **2004**, *1026*, 65–76.
19. Janus, L.; Carbonnier, B.; Deratani, A.; Bacquet, M.; Crini, G.; Laureyns, J.; Morcellet, M. New HPLC stationary phases based on (methacryloyloxypropyl-$\beta$-cyclodextrin-co-N-vinylpyrrolidone) copolymers coated on silica. Preparation and characterisation. New J. Chem. **2003**, *27*, 307–312.
20. Gasparrini, F.; Misiti, D.; Rompietti, R.; Villani, C. New hybrid polymeric liquid chromatography chiral stationary phase prepared by surface-initiated polymerization. J. Chromatogr. A. **2005**, *1064*, 25–38.
21. Saigo, K.; Chen, Y.; Kubota, N.; Tachibana, K.; Yonezawa, N.; Hasegawa, M. New chiral stationary phases for the high-performance liquid chromatographic resolution of enantiomers. Chem. Lett. **1986**, *4*, 515–518.
22. Huang, S.H.; Bai, Z.W.; Yin, C.Q.; Li, S.R. Covalently bonding chiral polyurethane on aminated silica gel: a new strategy to prepare chiral stationary phase for high performance liquid chromatography. Chin. J. Polym. Sci. **2006**, *24*, 107–114.
23. Huang, S.H.; Bai, Z.W.; Yin, C.Q.; Li, S.R.; Pan, Z.Q. Synthesis of polymer type chiral stationary phases and their enantioseparation evaluation by high-performance liquid chromatography. Chirality **2007**, *19*, 141–147.
24. Ihara, T.; Sugimoto, Y.; Asada, M.; Nakagama, T.; Hobo, T. Influence of the method of preparation of chiral stationary phases on enantiomer separations in high-performance liquid chromatography. J. Chromatogr. A. **1995**, *694*, 49–56.
25. Ye, Y.K.; Stringham, R.W. The effect of acidic and basic additives on the enantioseparation of basic drugs using polysaccharide-based chiral stationary phases. Chirality. **2006**, *18*, 519–530.
26. Stringham, R.W.; Ye, Y.K. Chiral separation of amines by high-performance liquid chromatography using polysaccharide stationary phases and acidic additives. J. Chromatogr. A. **2006**, *1101*, 86–93.
27. Matthijsl, N. Chiral sparation strategy in polar organic solvent chromatography and performance comparison with normal-phase liquid and supercritical-fluid chromatography. J. Sep. Sci. **2006**, *29*, 1353–1362.

28. Tang, Y.B.; Reepmeyer, J.C.; Revelle, L.K.; Wilson, J.A. Enantioseparation of 3-phenylacetylamino-2,6-piperidinedione and related chiral compounds. J. Chromatogr. A. **1996**, *752*, 93–99.
29. Bolm, C.; Müller, P. Enantioselective trimethylsilylcyanation of aldehydes. Tetrahedron Lett. **1995**, *36*, 1625–1628.

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