Synthesis and Characterization of Macroporous Silica Modified with Optically Active Poly[*N*-(oxazolinylphenyl)acrylamide] Derivatives for Potential Application as Chiral Stationary Phases

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ABSTRACT: Enantiopure acrylamide derivatives, (*S*)-*N*-[*o*-(4-methyl-4,5-dihydro-1,3-oxazol-2-yl)phenyl]acrylamide and (*R*)-*N*-[*o*-(4-phenyl-4,5-dihydro-1,3-oxazol-2-yl)phenyl]acrylamide, were synthesized through the acylation of chiral 2-oxazolinylanilines. The radical polymerization of the chiral monomers was carried out with (3-mercaptopropyl)trimethoxysilane as a chain-transfer agent to obtain the corresponding optically active prepolymers with a trimethoxysilyl group. By immobilizing the prepolymers on porous silica gel via the grafting-to method, we prepared a new chiral stationary phase (CSP) and characterized it by elemental analysis, thermogravimetry, and Fourier transform infrared spectroscopy. The enantioseparation capacities of the CSPs were evaluated with high-performance liquid chromatography toward several racemic compounds, including 1,1′-bi-2-naphthol, benzoin, 2-amino-1-butanol, and loxoprofen sodium under the normal-phase mode. The results indicate that the CSPs exhibited improved chromatographic performances compared to their brush-type analogs obtained by the alternative grafting-from approach. Also, the column packed with poly{(*R*)-*N*-[*o*-(4-phenyl-4,5-dihydro-1,3-oxazol-2-yl)phenyl]acrylamide}-bonded silica was found to have an extent of enantioselectivity in the chiral resolution of some unmodified amino acids with reversed-phase eluents. J Appl Polym Sci 115: 999–1007, 2010

Key words: chiral; high performance liquid chromatography; molecular recognition; radical polymerization; synthesis

INTRODUCTION

The chromatographic separation of enantiomers of chiral stationary phases (CSPs) has attracted great attention as one of the most efficient separation techniques.[1,2] In addition to brush-type CSPs based on low-molecular-mass selectors,[3–8] a large number of CSPs containing surface-adsorbed polysaccharide derivatives, especially cellulose and amylose carbamates, have been used extensively at the analytical and preparative levels because they can resolve the enantiomers of a wide range of racemic compounds by high-performance liquid chromatography (HPLC).[9–17] At the same time, research on CSPs based on synthetic, optically active polymers has also been rapidly evolving.[18–26] Some columns packed with polymeric CSPs have been commercialized. Attractive features of this type of CSPs are the large chemical and structural variability that can be exploited in the preparation of the chiral selectors, the possibility of having two enantiomeric versions of the CSP, and the chemical and thermal inertness of the packing material that is derived from the covalent attachment of the chiral polymer to the solid support.

Two strategies, namely, the grafting-to and grafting-from approaches, are available for the preparation of silica-type HPLC separation materials, where a chiral selector is end-grafted to the porous support. For example, Lindner's group developed a series of cinchona-alkaloid-derivative-based CSPs with these methods and applied them to the chiral separation of enantiomers under aqueous mobile-phase conditions. The spectrum of successfully resolved racemates contained chiral aryl-, aryloxy-, and arylthiocarboxylic acids, *N*-derivatized amino acids, and many other chiral acids, including also sulfonic, phosphonic, and phosphoric acids.[3] Recently, new hybrid organic/inorganic CSPs were synthesized by surface-initiated polymerization on porous azo-activated silica. The chiral packing material was proven to have high enantioselectivity and chemical and thermal inertness.[19]

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In general, the optical resolution capacity of polymeric CSPs is significantly dependent on the chiral recognition ability of the pendant group used and/or the stable higher ordered structures of the polymers. Miyano et al.[27] reported optically active polymethacrylates bearing binaphthol moiety in the side chain obtained by radical polymerization of the corresponding monomer. They found that the chiral recognition ability of the polymer toward racemic 3,5-dinitrophenylcarbamate derivatives was mainly based on the interaction between the individual 1,1′-binaphthalene units and the racemates. However, for chiral columns packed with optically active polymethacrylamide-modified silica gel, the prominent enantioseparation power was ascribed to the chain conformation controlled by hydrogen bonds between amide groups.[28–31]

We previously reported a new type of optically active polymer based on *N*-phenylmaleimide- and *N*-phenylmethacrylamide derivatives, in which a chiral oxazoline lateral group was connected to the backbone through the benzene ring.[32–38] It was demonstrated that the maleimide-type polymers and their metal-complexes had a moderate enantioselectivity in fluorescent response toward some chiral compounds.[39] More recently, we found that polyacrylamides bearing a chiral oxazoline pendant presented a favorable chiral recognition ability, as evidenced by ^{1}H-NMR study, where the characteristic signal of the hydroxyl group in 1,1′-bi-2-naphthol (BINOL) was split into two peaks, ascribed, respectively, to the levo and dextro isomers due to the asymmetric induction of the polymer.[40] These findings encouraged us to test the possibility of these polymers for HPLC enantioseparation as chiral macromolecular selectors.

In this study, we synthesized chiral *N*-(oxazolinylphenyl)acrylamides (OPAMs) and covalently immobilized them onto porous silica using two different grafting methods, thus obtaining CSPs. The enantioselectivities of the prepared packing materials were briefly examined against some racemic compounds by HPLC in the normal-phase and the reversed-phase modes.

EXPERIMENTAL

Materials

Acryloyl chloride was distilled before use. Triethylamine was dried over KOH and distilled. 2,2′-Azobisisobutyronitrile (AIBN), purchased from Shanghai Chemical Reagent Co. (China), was purified by recrystallization from methanol. BINOL, (3-mercaptopropyl)trimethoxysilane (MPS; Aldrich), and other chemicals were used as received. Solvents for the polymerization and CSP preparation were treated over a benzophenone-sodium complex for 3 days and distilled before use. Macroporous silica gel as an HPLC support was purchased from Lanzhou Institute of Chemistry & Physics (Chinese Academy of Sciences) with a mean particle size of 5 μm, an average pore diameter of 5.2 nm, and a specific surface area of 220 m^2/g.

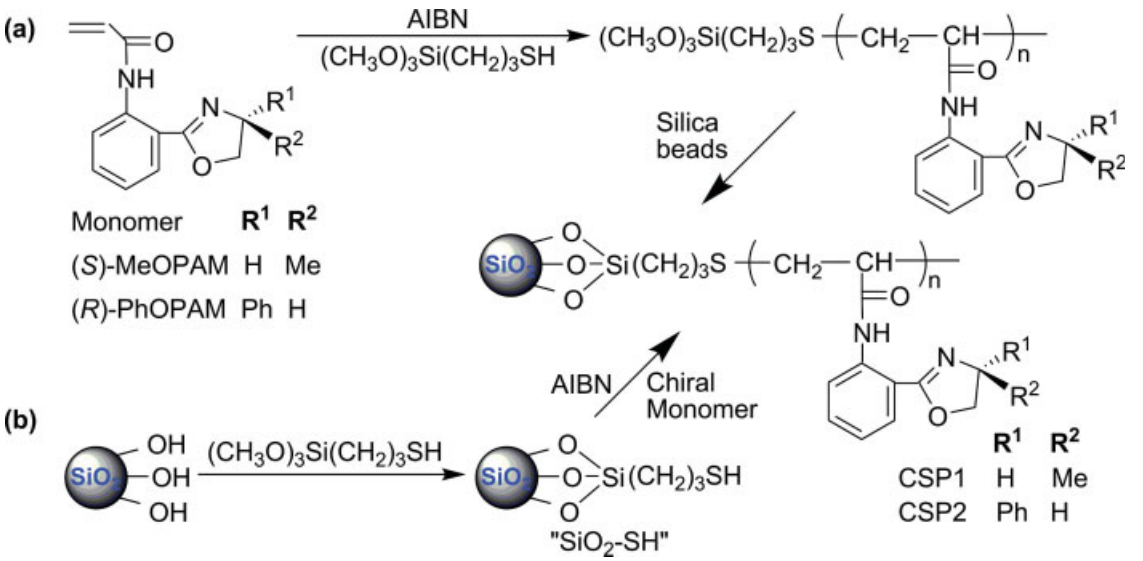
Measurements

A Bruker Avance AMX-500 NMR spectrometer (Rheinstetten, Germany) was applied to record the ^{1}H-NMR spectra. The IR spectra in KBr pellets were recorded with a Bruker Vector 22 Fourier transform infrared (FTIR) spectrometer (Ettlingen, Germany). Elemental analysis was performed on a Thermo-Finnigan Flash EA 1112 analyzer (Italy). The thermogravimetric analysis was carried out in N_2 with a Perkin Elmer Pyris 6 thermogravimetric analyzer (USA) at a heating rate of 10°C/min. The measurement of melting points (uncorrected) were taken with a Büchi 535 instrument (Flawil, Switzerland) at a heating rate of 1°C/min. Optical rotation was measured in tetrahydrofuran (THF) at 25°C with a Wzz-2s automatic digital polarimeter (Shanghai Pudong Optics Apparatus Co., China). The molecular weights of the polymers were determined by gel permeation chromatography (GPC) with a Waters 2414 GPC apparatus (USA) equipped with a set of Styragel columns (HT-1, HT-3, and HT-4) and a differential refractometer (eluent, THF; flow rate = 0.5 mL/min). The GPC chromatogram was calibrated against standard polystyrene samples. The chromatographic measurements were performed with a DIONEX P-680 HPLC apparatus (USA) with a UV detector at ambient temperature. Scanning electron microscopy (SEM) micrographs were taken of the surface of silica beads with a Hitachi S4800 instrument (Japan) at accelerated potentials of 3 and 5 kV; the samples were gold-sputtered to reduce charge effects.

Monomer synthesis

Enantiopure *ortho*-oxazolinyl-substituted anilines were synthesized according to a previously reported method.[32,33] Then, the compounds were submitted to acylation with acryloyl chloride, which led to production of the desired monomers. With the synthesis of (*R*)-*N*-[*o*-(4-phenyl-4,5-dihydro-1,3-oxazol-2-yl)phenyl]acrylamide [(*R*)-PhOPAM] as an example, the general procedure for the target compounds is described as follows.

To a THF solution (20 mL) containing (*R*)-2-(4′-phenyloxazolinyl)aniline (5.0 g, 0.021 mol) and triethylamine (4.0 mL, 0.028 mol), acryloyl chloride (2.15 mL, 0.026 mol) was added dropwise over a period of 10 min at 0°C. After it was stirred at 0°C for 3 h under a nitrogen atmosphere, the reaction



Scheme 1 Synthetic strategy for the preparation of CSPs: (a) grafting-to method and (b) grafting-from method. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

mixture was poured into a large amount of deionized water. The precipitate was collected and recrystallized from anhydrous ethanol to yield the title compound as a colorless crystal (4.8 g, yield = 85%).

mp = 127.5–128.0°C. $[\alpha]_D^{25}$: +223.0° (c = 1.0 g/dL, l = 10 cm, THF). FTIR (KBr): 3408 (N—H); 1687 (C=O); 1539 (CONH); 1635, 1065, 957 (oxazoline ring); 1589, 1493 (phenyl); 761, 678 cm^{-1}. ^{1}H-NMR ($CDCl_3$, δ): 12.56 (s, 1H, NH), 8.87 (d, 1H, —C_6H_5), 7.94 (d, 1H, —C_6H_5), 7.52 (t, 1H, —C_6H_5), 7.33 (m, 5H, —C_6H_5), 7.13 (t, 1H, —C_6H_5), 6.33 (d, 1H, =CH), 6.20 (m, 1H, =CHCO), 5.64 (d, 2H, =CH_2), 5.53 (t, 1H, OCH_2), 4.77 (t, 1H, =NCH), 4.20 (t, 1H, OCH_2). ANAL. Calcd for $C_{18}H_{16}N_2O_2$: C, 74.21%; H, 5.21%; N, 9.62%. Found: C, 74.31%; H, 5.47%; N, 9.64%.

The same procedure gave (*S*)-*N*-[*o*-(4-methyl-4,5-dihydro-1,3-oxazol-2-yl)phenyl]acrylamide [(*S*)-MeOPAM] at a yield of 66% (colorless crystal).

mp = 56.5–57.5°C. $[\alpha]_D^{25}$: +6.2° (c = 1.0 g/dL, l = 10 cm, THF). FTIR (KBr): 3420 (N—H); 1682 (C=O); 1544 (CONH); 1619, 1064, 960 (oxazoline ring); 1604, 1449 (phenyl); 769, 679 cm^{-1}. ^{1}H-NMR ($CDCl_3$, δ): 12.56 (s, 1H, NH), 8.83 (d, 1H, —C_6H_5), 7.85 (d, 1H, —C_6H_5), 7.47 (t, 1H, —C_6H_5), 7.08 (t, 1H, —C_6H_5), 6.45 (d, 1H, =CH), 6.30 (m, 1H, =CHCO), 5.75 (d, 1H, =CH_2), 4.48 (m, 2H, OCH_2), 3.91 (m, 1H, =NCH), 1.37 (d, 3H, —CH_3). ANAL. Calcd for $C_{13}H_{14}N_2O_2$: C, 68.11%; H, 5.72%; N, 12.22%. Found: C, 68.44%; H, 5.99%; N, 12.01%.

Preparation of the CSP

The column packing materials were prepared via the grafting-to approach in two steps (Scheme 1). First, the macromolecular chiral selector [i.e., the polymer of (*R*)-PhOPAM or (*S*)-MeOPAM] was synthesized by conventional radical polymerization in the presence of MPS with the Schlenk technique. MPS (1.85 mL, 10 mmol) and AIBN (82 mg, 0.5 mmol) were added to a THF solution (25 mL) of the monomer (25 mmol) and stirred at 60°C for 24 h under an N_2 atmosphere. The reaction mixture was then poured into a large amount of *n*-hexane to precipitate the product. For purification, the crude product was dissolved in THF and then recovered by precipitation in excess methanol to remove the unreacted monomer and MPS. After repeating this process several times, we subjected the dried product to GPC and elemental analysis. The results obtained were as follows: number-average molecular weight (M_n) = 4.14×10^3 and 0.40% S for poly[(*R*)-PhOPAM] and $M_n = 5.59 \times 10^3$ and 0.87% S for poly[(*S*)-MeOPAM]. In the next step, a 3.0-g amount of dried silica and the prepared poly[(*R*)-PhOPAM] and poly[(*S*)-MeOPAM] (~ 2.0 mmol monomer units), respectively, were refluxed in toluene (30 mL) for 24 h with stirring under an N_2 atmosphere. The modified silica was collected by filtration, washed with dichloromethane in a Soxhlet extractor for 24 h to remove the unattached polymer, and dried at reduced pressure to a constant weight (5 mmHg, temperature = 60°C). Elemental analysis gave 6.77% C, 0.52% H, and 0.81% N for CSP1; this corresponded to a selector coverage of 289 μmol/g silica. Elemental analysis gave 5.41% C, 0.47% H, and 0.96% N for CSP2; this corresponded to a selector coverage of 343 μmol/g silica.

The preparation of CSPs by the grafting-from approach was carried out in the presence of chiral

TABLE I
Synthesis of CSPs and Their Characterization

No.	Method[a]	Chiral selector[b]	Loading amount	
			wt %[c]	Monomer units (μmol/g)[d]
CSP1	Grafting-to	Poly[(*R*)-PhOPAM]	8.7	289 (298)
CSP2	Grafting-to	Poly[(*S*)-MeOPAM]	7.6	343 (330)
CSP3	Grafting-from	(*R*)-PhOPAM	2.3	75 (79)
CSP4	Grafting-from	(*S*)-MeOPAM	1.9	93 (83)

[a] For the preparation of the CSPs, see the Experimental section.
[b] $M_n = 4.14 \times 10^3$ and $M_w/M_n = 1.37$ for poly[(*R*)-PhOPAM]; $M_n = 5.59 \times 10^3$ and $M_w/M_n = 1.45$ for poly[(*S*)-MeOPAM].
[c] Determined by thermogravimetric analysis.
[d] The corresponding monomer units per gram of matrix as estimated by elemental analysis. The data in parentheses were obtained from thermogravimetric analysis.

monomers, AIBN, and silica gel containing 3-mercaptopropyl groups on the surface. Dried silica (10.0 g) was placed in a 500-mL, three-necked, round-bottom flask equipped with a Dean–Stark trap, a reflux condenser, and an inert gas inlet, and 200 mL of toluene was added. The slurry was heated to reflux temperature with violent stirring under an N_2 atmosphere, and 25 mL of distillate was collected over a period of 1 h. After the mixture was cooled to room temperature, 5.0 mL of MPS (27 mmol) was added, and the slurry was heated to reflux temperature for 8 h under an N_2 atmosphere. The mercapto-activated silica (SiO_2—SH) was collected by filtration and washed with 100-mL portions of toluene, methanol, and dichloromethane and dried at reduced pressure (5 mmHg, temperature = 60°C) to a constant weight. The modified material had a calculated coverage of about 350 μmol of thiol groups/g of silica on the basis of elemental analysis (C, 1.26%; H, 0.61%; S, 1.04%). To toluene solutions (5×10^{-2} *M*, 100 mL) of (*R*)-PhOPMA and (*S*)-MeOPAM, respectively, were added both SiO_2—SH (6.0 g) and AIBN (16.4 mg, 0.1 mmol); the resulting slurry was then heated at 60°C for 24 h under an N_2 atmosphere with stirring. The toluene was removed, and the modified silica was extracted with dichloromethane in a Soxhlet extractor for 24 h and dried at reduced pressure (5 mmHg, temperature = 60°C) to a constant weight. Elemental analysis gave 2.97% C, 0.13% H, and 0.21% N for CSP3; this corresponded to a selector coverage of 75 μmol/g silica. Elemental analysis gave 2.55% C, 0.36% H, and 0.26% N for CSP4; this corresponded to a selector coverage of 93 μmol/g silica.

Chromatographic procedures

The analytical stainless-steel columns (150 mm × 4.6 mm I.D.), supplied by Yingpu Co. (Dalian, China), were packed by the slurry technique with a VFD-M packer pump (Dalian Kerui Instrument Co., Dalian, China). Chromatographic evaluation of the columns was performed with the conventional mobile-phase modes: normal (*n*-hexane/chloroform/2-propanol) and reversed mode (a dilute aqueous solution of copper acetate) at room temperature with a flow rate of 0.5–1.0 mL/min. Each analysis was repeated in triplicate. The column dead time was determined with 1,3,5-tri-*tert*-butylbenzene and sodium nitrate for the normal-phase mode and reversed-phase mode, respectively. All of the tested analytes were prepared to about a 1-mg/mL concentration with methanol/water (1 : 1 v/v). The injected volume was 2 μL. The chromatograms were recorded at a wavelength of 254 or 223 nm.

RESULTS AND DISCUSSION

Preparation of the CSPs

In this study, two different types of grafting modes of the selectors to silica were examined first with regard to the loadings (see Scheme 1). In the grafting-from procedure, the expected graft reaction on the solid support surface was achieved in the presence of mercapto-activated silica (SiO_2-SH), where the chiral monomer and the radical initiator were both kept in solution. As shown in Table I, the CSP3 and CSP4 thus prepared had calculated coverages of about 75 and 93 μmol of monomeric units/g of silica, respectively. With a loading of 350 μmol of thiol groups/g of SiO_2—SH, it appeared that only partial silica-supported thiol groups captured a single chiral monomer via the radically promoted thiol-ene reaction, which thus produced the stoichiometric 1 : 1 addition product rather than extended polymeric grafts. In other words, this procedure resulted in the formation of brush-type CSPs, and the polymer was mainly formed in solution. A probable reason for the low mass loadings may be the large hindrance of silica gel.

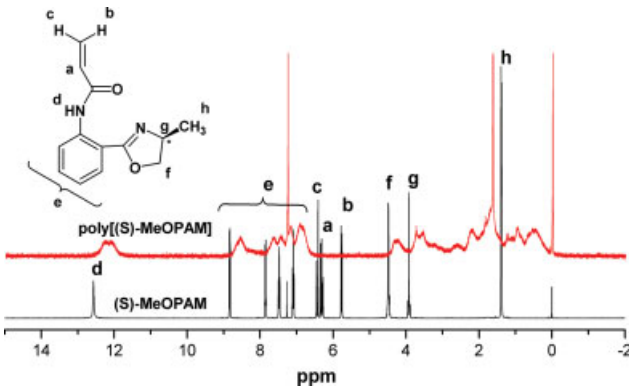


Figure 1 ^{1}H-NMR spectra of (*S*)-MeOPAM and poly[(*S*)-MeOPAM]. The polymer sample was obtained by radical polymerization of the corresponding monomer in the absence of MPS. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Comparatively, the grafting-to approach was more effective in our study; this method is based on the condensation reaction of prepolymers bearing trimethoxysilyl groups at the chain end with the silanol groups from the silica surface. The procedure had the advantage of easier control of the chemical structure of the polymeric selectors and, in turn, of the properties of the final CSPs. That is, the chiral prepolymers to be grafted to the surface of silica gel could be largely altered in both molecular weight and chiroptical properties by a change in the feed ratios at their preparation step. For example, when the original feed composition was monomer/AIBN/MPS = 50 : 1 : 20 (molar ratio), M_n values of 4.14×10^3 and 5.59×10^3 could be obtained for poly[(*R*)-PhOPAM] and poly[(*S*)-MeOPAM], respectively. Thus, with such a prepolymer,

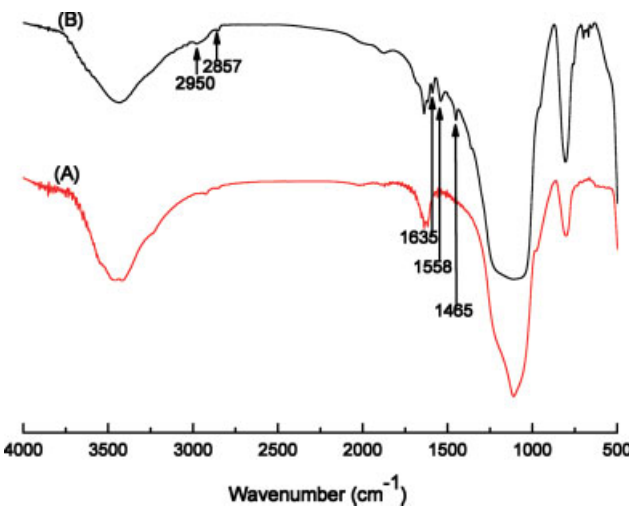


Figure 2 FTIR spectra of (A) the silica gel and (B) the poly[(*S*)-MeOPAM]-bonded silica gel (CSP2). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

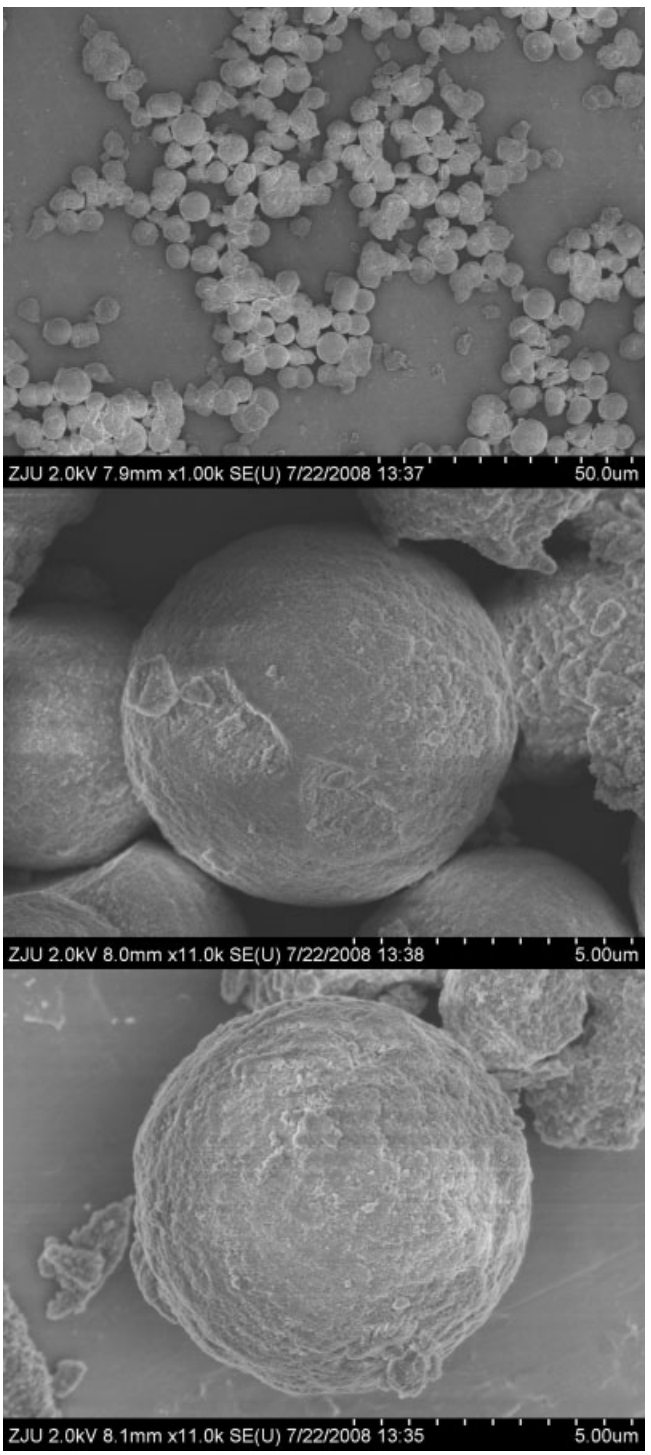


Figure 3 SEM images of CSP1 [top (magnification = 1.0×10^3) and middle (magnification = 11.0×10^3)] and the unmodified silica gel [bottom (magnification = 11.0×10^3)].

higher mass loadings were available for CSP1 and CSP2, which data, respectively, contained 289 and 343 μmol of monomer units/g of matrix on the basis of elemental analysis. The data were very consistent with the results obtained from thermogravimetric analysis (see Table I). However, the

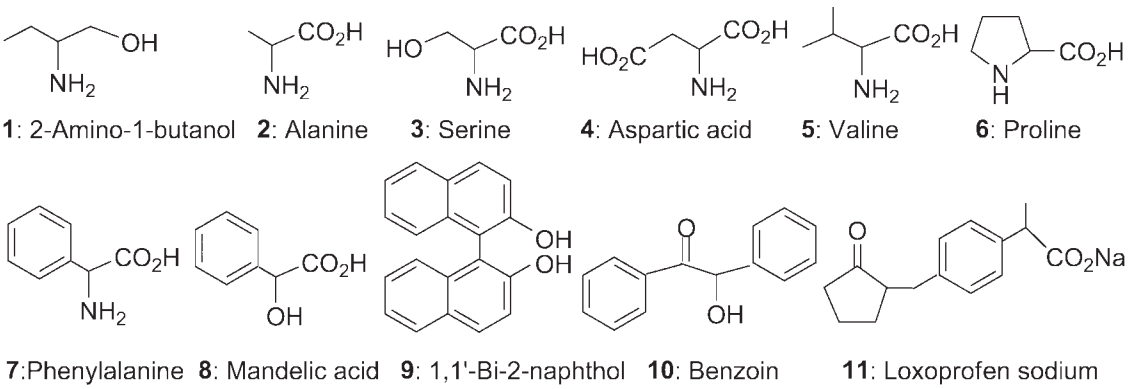


Figure 4 Molecular structures of the test racemates.

polymerization product comprised the desired macromolecular selector with a trimethoxysilyl group and its analog without the end-group. Also, attempted detection of the relative sulfur content was unsuccessful for the grafting-to materials because the content was too low. So, we failed to obtain the grafting efficiency of the approach.

Characterization of both the polymeric chiral selector and CSPs

^{1}H-NMR characterization of the pure prepolymer with a trimethoxysilyl group was unachievable because its separation from the polymerization product was very difficult. Nevertheless, some structural information was obtained from the spectrum of poly[(*S*)-MeOPAM] without the end group. As shown in Figure 1, the disappearance of acrylic CH_2=CH protons (peaks a, b, and c) indicated that the polymerization took place in a common way. Furthermore, the characteristic signals of oxazoline residue (peaks f, g, and h) evidently shifted upfield in the polymer, and such a change was fairly much for the protons located away from C=C bond. These facts suggest that in this system, some strong intermolecular and/or intramolecular forces should have existed via the lateral groups. Similar spectral variation was also observed in our previous studies.[36]

Figure 2 gives the FTIR spectrum of poly[(*S*)-MeOPAM]-bonded silica gel (CSP2). The absorptions arising from poly[(*S*)-MeOPAM] were clearly visible in the amide-stretching region (1635 and 1558 cm^{-1}) and in the aliphatic stretching and bending regions (2857–2950, 1465 cm^{-1}); this indicated that the macromolecular chiral selector was immobilized on the carrier successfully.

Surface characterization of CSP2 was also carried out with SEM. Under low-magnification conditions, the modified silica appeared to consist of micrometer-sized particles with some interparticle aggregation (Fig. 3, top). The comparison of SEM images of CSP2 and the starting silica beads at a higher magnification taken under identical conditions, showed that the surface morphology was not greatly affected by the polymer grafting process (Fig. 3, middle and bottom).

Enantioseparation on the chiral polymer bonded silica gel

The chromatographic evaluation of the polymeric CSPs was performed under normal-phase and reversed-phase modes with an *n*-hexane/

TABLE II
Chromatographic Parameters Obtained in the Normal Phase Mode for Compounds 1 and 9–11 Resolved on the Prepared CSPs

	CSP1				CSP2			
Racemate[a]	k_1'	k_2'	α	R_s	k_1'	k_2'	α	R_s
1	0.42	0.51	1.21	0.56	1.78	2.10	1.18	0.63
9	3.51	3.51	1.00	0	5.67	5.67	1.00	0
10	0.88	0.93	1.06	0.37	1.81	3.11	1.72	0.97
11	1.17	1.17	1.00	0	2.14	2.14	1.00	0

k_1', k_2': capacity factor of the less and more retained enantiomer, respectively; α, separation factor; R_s, resolution factor. Conditions: eluent = *n*-hexane/chloroform/2-propanol (89 : 10 : 1); flow rate = 0.5 mL/min; column size = 150 mm × 4.6 mm I.D.; temperature = 25°C; UV detection = 254 nm.

[a] See Figure 4.

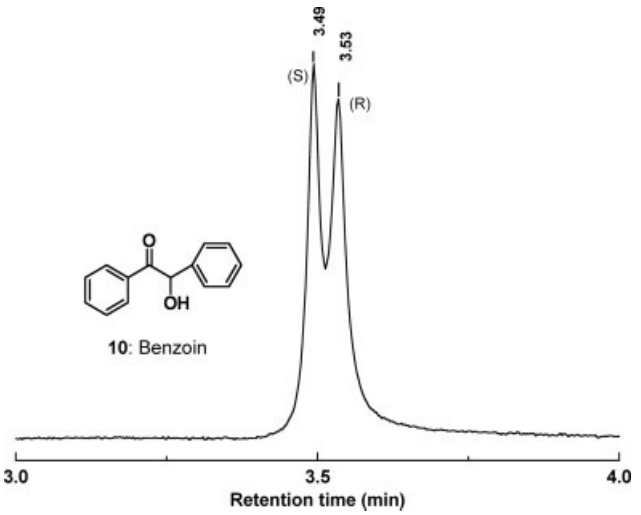


Figure 5 Resolution of benzoin on CSP2 by HPLC [eluent = *n*-hexane/chloroform/2-propanol (8 : 1 : 1), flow rate = 0.5 mL/min, column size = 150 mm × 4.6 mm (inside diameter), UV detection = 254 nm]. The elution order was confirmed by the injection of an enantiomerically enriched sample under the same conditions.

chloroform/2-propanol mixture and aqueous solution copper acetate as an eluent, respectively. The test compounds included several unprotected amino acids and hydroxyl-containing substrates, as depicted in Figure 4.

Table II summarizes the results of the screening of CSP1 and CSP2 under the normal-phase mode. The column packed with CSP2 was more efficient for the chiral separation of the solutes compared to CSP1, where benzoin reached the largest resolution with a partial resolution with R_s of 0.97. The corresponding chromatogram is shown in Figure 5. Also, in all cases, the CSP2-packed column exhibited a higher retention than that of another one. This fact suggested that poly[(*S*)-MeOPAM] on the CSP2 had a greater tendency to form hydrogen bonding with an analyte, which was probably because of its smaller steric constraint from the pendant groups. On the other hand, the retention of the enantiomers seemed to be greatly affected by the presence of H-bonding sites in the analytes: BINOL, with two phenolic OH's available to complementary H-bonding sites on the CSP, showed the highest retention (see Table II). However, BINOL could not be resolved on the CSPs, although the corresponding polymeric selectors exhibited a favorable chiral recognition to this compound in a relevant ^{1}H-NMR study with $CDCl_3$ as solvent.[40] The exact reason for this is still unclear. Nevertheless, we assumed that the chiral recognition mechanism under the chromatographic condition should have differed from the enantioselective interaction between the polymeric selector and the solute in the case of ^{1}H-NMR measurement.

A set of amino acids and hydroxyl acids (**2–8;** Fig. 4) was used to evaluate the enantioselectivity of the poly[(*R*)-PhOPAM]-bonded silica (CSP1) in reversed-phase mode (Table III). The data showed that the capacity factor decreased for compounds **2–5** with an increase in the concentration of the salt at pH 7.0. Except for serine (**3**), the capacity factor values obtained in mobile phase I (pH 4.5) were lower than that in mobile phase II (pH 7) under the same concentration of $Cu(OAc)_2$. Among these solutes, aspartic acid (**4**) showed a relatively large separation factor (α = 1.18; see Fig. 6). This indicated that hydrogen-bonding may have played an important role in the chiral discrimination of the polymeric selector, and the enantioseparation probably stemmed from the difference in the intensity of this interaction. Interestingly, no resolution was observed on CSP1 with the aqueous mobile phase for some acidic substrates (compounds **6–8**) with cyclic or

TABLE III
Chromatographic Parameters Obtained in the Reversed-Phase Mode for Amino and Hydroxyl Acids Resolved on CSP1

	Mobile phase I[b]			Mobile phase II[c]			Mobile phase III[d]		
Racemate[a]	k'_1	k'_2	α	k'_1	k'_2	α	k'_1	k'_2	α
2	4.07	4.35	1.07	6.23	6.92	1.11	5.49	6.10	1.11
3	3.37	3.54	1.05	3.03	3.32	1.10	2.67	2.84	1.06
4	1.83	2.00	1.09	2.43	2.86	1.18	1.22	1.36	1.11
5	7.09	7.35	1.04	8.23	8.89	1.08	6.73	7.20	1.07
6	4.96	4.96	1.00	4.52	4.52	1.00	4.27	4.27	1.00
7	5.23	5.33	1.02	5.46	5.57	1.02	5.21	5.31	1.02
8	6.13	6.18	1.01	6.88	7.01	1.02	6.69	6.81	1.02

Conditions: flow rate = 1.0 mL/min; column size = 150 mm × 4.6 mm I.D.; temperature = 25°C; UV detection = 223 nm.
[a] See Figure 4.
[b] Eluent = aqueous $Cu(OAc)_2$ (0.1 mmol/L) + KH_2PO_4 (0.05 mol/L); pH = 4.5.
[c] Eluent = aqueous $Cu(OAc)_2$ (0.1 mmol/L); pH = 7.0.
[d] Eluent = aqueous $Cu(OAc)_2$ (1.0 mmol/L); pH = 7.0.

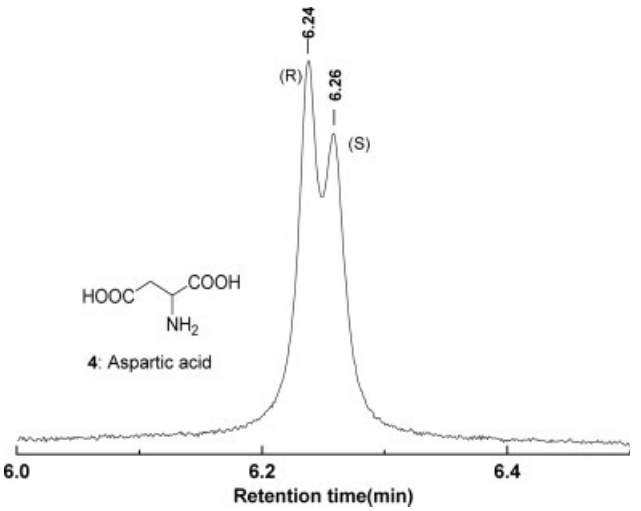


Figure 6 Typical HPLC chromatogram of racemic aspartic acid with CSP1 [eluent = aqueous $Cu(OAc)_2$ (0.1 mmol/L), CSP1 = poly[(*R*)-PhOPAM]-bonded silica gel, column size = 150 mm × 4.6 mm I.D., flow rate = 0.5 mL/min, UV detection = 223 nm]. The elution order was confirmed by the injection of an enantiomerically enriched sample under the same conditions.

aromatic fragment in their structure; this suggested that this structural feature seemed to be deleterious for the enantioselectivity.

From the aspect of the recognition mechanism, the chromatographic process in an aqueous mobile phase was similar to the case of chiral ligand exchange chromatography.[41,42] According to this mechanism, the enantiomeric separation of unmodified amino acids was achieved in HPLC by means of chiral copper(II) complexes dissolved in the eluent with achiral reversed-phase columns.[43,44] α-Amino acids in many cases chelate with copper(II) by coordinating through the amino and carboxylic groups. For this system, polymeric selectors containing both amide linkages and chiral oxazoline residues provided potential copper(II) binding sites. Thus, the amino acids in the mobile phase should have undergone dynamic ligand exchange on the polymeric Cu(II) complexes bonded covalently to the support surface.

In contrast to CSP1 and CSP2, no enantioseparation was observed for the columns packed with CSP3 and CSP4 under the same chromatographic conditions as a result of the very low loading amount of chiral selectors on the silica gel.

CONCLUSIONS

New CSPs for HPLC were prepared by the covalent attachment of optically active polyacrylamide derivatives (poly[(*R*)-PhOPAM] and poly[(*S*)-MeOPAM]) to macroporous silica gel via the grafting-to procedure. Under the normal-phase mode, racemic benzoin and 2-amino-1-butanol were partially resolved on the CSPs. The chiral recognition was probably due to the difference in hydrogen-bonding interaction between the polymeric selector and the enantiomers. Also, the new chiral packing materials exhibited an extent of enantioseparation capability toward some unmodified amino acids in the reversed-phase mode.

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