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Synthesis of a Novel Chiral Stationary Phase by (R)-1,1′-Binaphthol and the Study on Mechanism of Chiral Recognition

Yongxi Wang [1,2], Dandan Liu [1], Yili Zhang [1], Yanmei Tang [3], Jingfeng Zhao [4,*] and Baochun Shen [1,*]

1 School of Pharmaceutical Sciences and Yunnan Key Laboratory of Pharmacology for Natural Products, Kunming Medical University, Kunming 650500, China; 18288961505@163.com (Y.W.); liudandan1017@sina.com (D.L.); yiliz74@hotmail.com (Y.Z.)
2 Walvax Biotechnology Co., Ltd., Dingyi Tiancheng Residential Quarter, Kunming 650500, China
3 Department of Pharmacy, Yunnan College of Business Management, Kunming 650106, China; zzjtym@163.com
4 Key Laboratory of Medicinal Chemistry for Natural Resource, Yunnan University, Ministry of Education, Yunnan University, Kunming 650091, China
* Correspondence: jfzhao@ynu.edu.cn (J.Z.); b.shen@kmmu.edu.cn (B.S.); Tel.: +86-136-0888-3075 (B.S.)

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Abstract: (R)-6-Acrylic-BINOL CSP, a novel chiral stationary phase was prepared by (R)-Binaphthol (R-BINOL) by introducing the acrylic group into the 6-position of (R)-BINOL before bonding it to the surface of silica gel. The structure of the CSP was characterized by IR, SEM, and element analysis. This new material was tested for its potential as a CSP for HPLC under normal phase conditions, especially for conjugated compounds. Six solutes were chosen to evaluate the chiral separation ability of the novel CSP. The effects of the mobile phase and temperature on enantioseparation were studied, and the chiral recognition mechanism was also discussed. The results showed that the space adaptability and π-π stacking between the solutes and the CSP affected the retention and enantioseparation. The Van't Hoff curve indicated that under the experimental conditions, the separation mechanism of six solutes did not change, which were all enthalpy driven.

Keywords: (R)-Binaphthol; chiral stationary phase; enantioseparation; conjugated compound; chiral recognition mechanism

1. Introduction

Stereospecific recognition of chiral molecules is an important matter in various aspects of chemistry and life sciences [1–3]. Enantioseparation of chiral compounds is attracting more and more interest, which partly contributes to the great differences of the biological activity, pharmacological action, and toxicity of different enatiomers [4,5]. Liquid chromatographic enantioseparations on chiral stationary phases (CSPs) are known to be a very attractive method used in the determination of the enantiomeric composition of chiral compounds for its advantages in terms of accuracy, speed, sensitivity, and reproducibility [6–10]. To date, several hundred CSPs have been synthesized, and approximately a hundred of them have become commercially available [11].

1,1′-Binaphthol (BINOL) is a C_2 symmetric molecule with a stable chiral structure; it contains two identical naphthol units [12–15]. BINOL and its derivatives are widely used in various applications such as molecular recognition, asymmetric catalysis, and new materials [16,17]. Since the late 1970s, the crown ether-based chiral stationary phase with BINOL became known to be quite effective for the separation of N-containing compounds in HPLC [18–20].

Our inspiration originated from some analogous CSPs based on (S)-BINOL (CSP-BIN, CSP-DM, CSP-DP, CSP-DN), which showed enantioselectivity for amines [21,22]. The hydroxyl groups of CSPs were confirmed to be crucial to the enantioseparation of chiral compounds [23]. In order to explore the conjugated effect to the enantioseparation, we considered the introduction of an acrylic group into the 6-position of (R)-BINOL and then bonded to the surface of silica gel, to finally prepare a novel CSP with the hydroxyl groups residue. We hope the conjugated effect among the acrylic group, carbonyl group, and 1,1′-binaphthyl unit play a part in the enantioseparation of conjugated solutes. Thus, 6 solutes with conjugated structures were chosen to evaluate the chiral ability of the novel CSP under normal phase. In order to discuss the mechanism of the chiral recognition, the effects of the mobile phase and the column temperature on the enantioseparation were studied. Apparent thermodynamic parameters were also calculated from the plots of $ln\alpha$ or lnk' versus 1/T [24].

And in our research, we improved the method of the preparation of (R)-BINOL derivative (mono-bromination (R)-3, yield 93% and methyl ester (R)-6, yield 86.4%) reported in the literature [25]. To the best of our knowledge, this new CSP has not yet been published in any other literature.

2. Materials and Methods

2.1. General Experimental

Proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on a Bruker Avance 300 or 400 spectrometer at 300 or 400 MHz. Carbon-13 nuclear magnetic resonance (^{13}C-NMR) was recorded on Bruker Avance 300 or 400 spectrometer at 75 or 100 MHz. Chemical shifts are reported as δ values in parts per million (ppm) relative to tetramethylsilane (TMS) for all recorded NMR spectra. Mass spectra were taken on a liquid chromatography mass spectrometer (LCMS-8040, Shiamdzu, Kyoto, Japan). Starting materials and reagents used in reactions were obtained commercially from Aldrich, Energy Chemical and other reagent companies, and were used without further purification, unless otherwise indicated. All reactions were conducted in dried glassware under a positive pressure of dry nitrogen. Silica gel (Qingdao, 200–300 mesh) was used for column chromatography. Silica gel (5 μm, specific surface area 260 $m^2 \cdot g^{-1}$) used for the preparation of chiral stationary phase was purchased from Qingdao Meigao Chemical Co. Ltd. (Qingdao, China). The structure of CSP was characterized using IR (Nicolet iS10, Thermo, Waltham, MA, USA), elemental analysis (Vario ELIII, Elementar, Langenselbold, Germany), and SEM (NOVA NANO SEM-450, FEI, OR, USA).

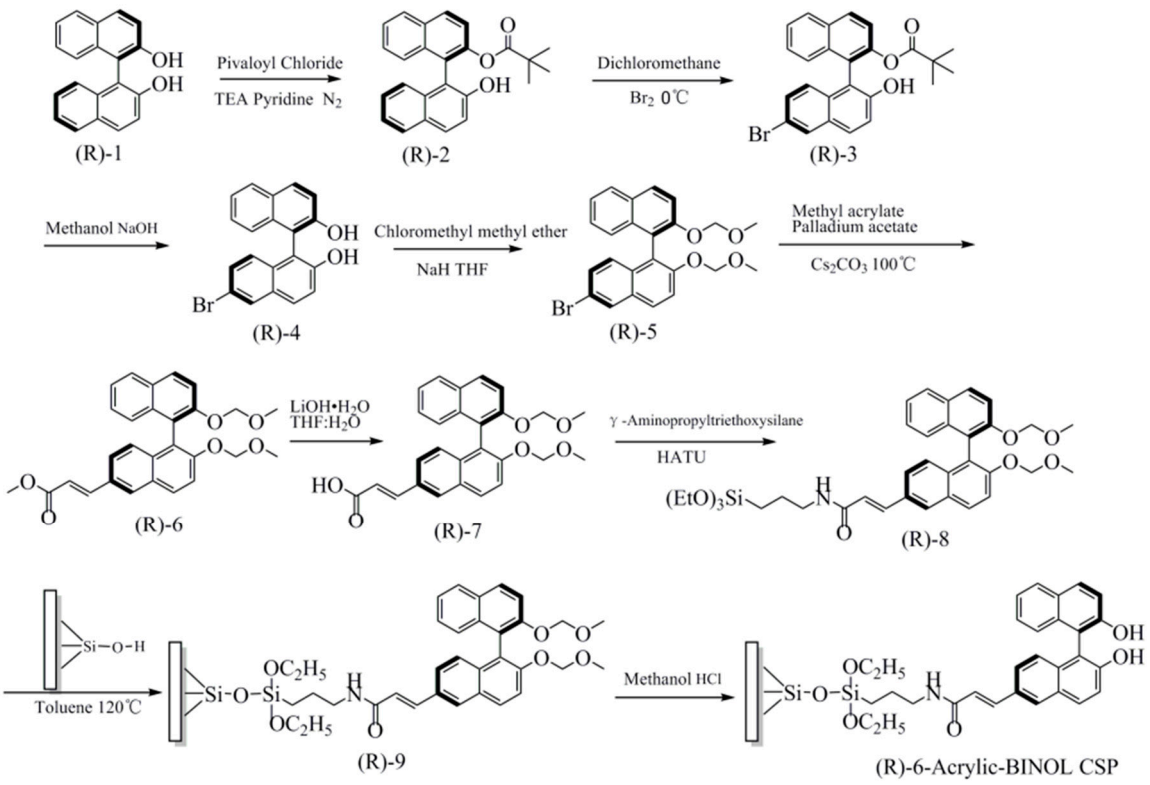
2.2. High Perfomance Liquid Chromatography Instrumentation and Conditions

Chromatography was performed on Agilent 1260 HPLC; the chiral column was a self-made column (R)-6-Acrylic-BINOL CSP. Chromatographic conditions: [a] mobile phase: hexane-ethanol-trifluoroacetic acid (99:1:0.1, *v*/*v*/*v*); [b] mobile phase: hexane-ethanol-trifluoroacetic acid (97:3:0.1, *v*/*v*/*v*); [c]mobile phase: hexane-ethanol-trifluoroacetic acid (90:10:0.1, *v*/*v*/*v*); flow rate: 1 mL/min; temperature: 0 °C, 5 °C, 10 °C, 15 °C, 20 °C, 25 °C, 30 °C, 35 °C, 40 °C, 45 °C; detection wavelength: 254 nm for rac-2,2,2-trifluoro-1-(9-anthryl)-ethanol, and 220 nm for other 5 solutes.

2.3. Synthesis

The synthetic route of the new CSP is illustrated in Scheme 1. Our journey started with mono-ester (R)-2, which could be prepared from (R)-BINOL (1) and pivaloyl chloride. The selective mono-bromination (R)-3 was obtained through the reaction of (R)-2 with bromine at low temperature ([25]. The momo-protected compound (R)-5 could be created from bromo ester (R)-3 by conducting ester hydrolysis and dihydroxy protection [26]. Methyl ester (R)-6, was produced from bromide (R)-5 by the Heck reaction using palladium acetate and methyl acrylate, which could yield acid (R)-7 with LiOH/KOH. Amide (R)-8 could be prepared from acid (R)-7 by the condensation reaction with HATU [27]. The key intermediate (R)-9 which was obtained through covalent bonding of (R)-8 with

acidified silica gel, and could then form a novel chiral stationary phase (R)-6-Acrylic-BINOL CSP by dihydroxy deprotection [28].



Scheme 1. The synthetic route to the (R)-6-Acrylic-BINOL CSP.

2.3.1. Synthesis of (R)-2

To a solution of (R)-1 (5.00 g, 17.46 mmol) in tetrahydrofuran (100 mL) at 0 °C was added triethylamine (3.46 mL, 24.97 mmol), pyridine (35.17 µL, 0.52 mmol), followed by pivaloyl chloride (2.35 mL, 19.21 mmol). The resulting solution was allowed to warm from 0 °C to room temperature and stirred for 3 h. The reaction mixture was quenched with water, pH adjusted to 1 with 1N HCl, extracted with ethyl acetate, and washed with brine. The organic solvent was dried (sodium sulfate) and evaporated. The residue was purified by flash chromatography (hexanes/ethyl acetate: 15/1) to give (R)-2 (5.62 g, 87%) as a white solid. $[\alpha]_D^{25} + 56.38°$ (C = 0.01, tetrahydrofuran).

^{1}H-NMR (300 MHz, $CDCl_3$), δ (ppm): 7.99 (1H, d, *J* = 8.82 Hz), 7.89 (1H, d, *J* = 8.16 Hz), 7.83 (1H, d, *J* = 8.88 Hz), 7.78 (1H, d, *J* = 7.5 Hz), 7.45–7.40 (1H, m), 7.36 (1H, s), 7.33 (1H, s), 7.30–7.17 (4H, m), 7.07 (1H, d, *J* = 8.22 Hz), 0.75 (9H, s).

^{13}C-NMR (75 MHz, $CDCl_3$), δ (ppm): 177.91, 151.94, 148.41, 133.79, 133.63, 132.30, 130.81, 130.42, 129.12, 128.47, 128.07, 127.55, 126.82, 126.32, 125.78, 124.72, 123.66, 123.22, 121.98, 118.40, 114.36, 38.86, 26.61 × 3.

2.3.2. Synthesis of (R)-3

To a solution of (R)-2 (500 mg, 1.34 mmol) in dichloromethane (50 mL) was added dropwise bromine (0.3 mL, 5.85 mmol) at 0 °C for 40 min. Then, the reaction mixture was quenched by sodium sulfite aqueous solution, extracted with ethyl acetate, and washed with brine. The organic solvent was dried (sodium sulfate) and evaporated. The residue was purified by flash chromatography (hexanes/ethyl acetate: 5/1) to give (R)-3 (565 mg, 93%) as a yellow solid. $[\alpha]_D^{25} - 6.35°$ (C = 0.01, tetrahydrofuran).

^{1}H-NMR (300 MHz, $CDCl_3$), δ (ppm): 8.04 (1H, d, *J* = 8.85 Hz), 7.97–7.93 (2H, m), 7.77 (1H, d, *J* = 8.94 Hz), 7.51–7.45 (1H, m), 7.37–7.25 (5H, m), 6.94 (1H, d, *J* = 8.97 Hz), 0.79 (9H, s).

^{13}C-NMR (75 MHz, $CDCl_3$), δ (ppm): 177.86, 152.21, 148.34, 133.39, 132.24 × 2, 131.06, 130.13, 129.96 × 2, 129.42, 128.48, 127.65, 126.53, 126.38, 125.45, 122.42, 121.90, 119.49, 117.37, 114.54, 77.58, 77.16, 76.73, 38.83, 26.57 × 3.

2.3.3. Synthesis of (R)-4

To a solution of (R)-3 (1.00 g, 2.18 mmol) in methanol (100 mL) was added saturated aqueous sodium hydroxide solution. After being stirred at room temperature for 3 h, the reaction mixture was acidified with HCl to pH = 1, extracted with ethyl acetate, and washed with brine. The organic solvent was dried (sodium sulfate) and evaporated. The residue was purified by flash chromatography (hexanes/ethyl acetate: 10/1) to give (R)-4 (0.77 g, 95%) as a yellow solid. $[\alpha]_D^{25} - 6.68^\circ$ (C = 0.01, tetrahydrofuran).

^{1}H-NMR (300 MHz, $CDCl_3$), δ (ppm): 8.05 (1H, d, *J* = 1.71 Hz), 7.98 (1H, d, *J* = 8.97 Hz), 7.90 (1H, d, *J* = 4.41 Hz), 7.87 (1H, d, *J* = 5.64 Hz), 7.41–7.32 (5H, m), 7.09 (1H, d, *J* = 8.52 Hz), 7.01 (1H, d, *J* = 8.97 Hz).

^{13}C-NMR (75 MHz, $CDCl_3$), δ (ppm): 153.02, 152.74, 133.30, 132.04, 131.73, 130.72, 130.59, 130.46, 130.39, 129.48, 128.52, 127.70, 126.14, 124.22, 124.03, 118.97, 117.82 × 2, 111.28, 110.22.

2.3.4. Synthesis of (R)-5

To a stirred suspension of sodium hydride (60% dispersion in mineral oil, 0.62 g, 25.73 mmol) in dry tetrahydrofuran (15 mL) at 0 °C was added a solution of R-(4) (3.11 g, 8.58 mmol) in dry tetrahydrofuran (50 mL), which was then allowed to warm to room temperature, and was stirred for 1 h. Then mixture was cooled to 0 °C and chloromethyl methyl ether (2.04 mL, 25.73 mmol) was added. The mixture was stirred for 3 h, and then quenched by saturated aqueous ammonium chloride, extracted with ethyl acetate, and washed with brine. The organic solvent was dried (sodium sulfate) and evaporated. The residue was purified by flash chromatography (hexanes/ethyl acetate: 10/1) to give (R)-5 (3.58 g, 93%) as a yellow solid. $[\alpha]_D^{25} + 59.84^\circ$ (C = 0.01, tetrahydrofuran).

^{1}H-NMR (300 MHz, $CDCl_3$), δ (ppm): 7.99 (1H, d, *J* = 1.89 Hz), 7.91 (1H, d, *J* = 9.03 Hz), 7.84–7.78 (2H, t, *J* = 9.18 Hz), 7.57 (1H, d, *J* = 4.65 Hz), 7.54 (1H, d, *J* = 4.62 Hz), 7.30–7.17 (3H, m), 7.11 (1H, d, *J* = 8.37 Hz), 7.03 (1H, d, *J* = 9.03 Hz), 5.07–5.03 (2H, m), 4.95 (1H, d, *J* = 2.94 Hz), 4.93 (1H, d, *J* = 2.97 Hz), 3.11 (6H, d, *J* = 2.40 Hz).

^{13}C-NMR (75 MHz, $CDCl_3$), δ (ppm): 153.02, 152.72, 133.95, 132.65, 132.49, 130.99, 129.91, 129.77, 129.66, 128.84, 128.58, 128.08, 127.54, 127.25, 126.58, 125.35, 124.25, 121.53, 120.71, 120.52, 95.11, 95.04, 55.95 × 2.

2.3.5. Synthesis of (R)-6

A mixture of palladium acetate (37.20 mg, 0.22 mmol), cesium carbonate (899 mg, 2.76 mmol), tetrabutylammonium bromide (356 mg, 1.103 mmol), lithium chloride (46.76 mg, 5.52 mmol), and methyl acrylate (0.2 mL, 2.21 mmol) in dry *N*,*N*-dimethylformamide (10 mL) was stirred for 30 min. After 30 min, the solution of (R)-5 (500 mg, 1.10 mmol) in dry *N*,*N*-dimethylformamide (10 mL) was added. The reaction mixture was placed under a nitrogen atmosphere. After being stirred at 100 °C for 12 h, the mixture was filtered and evaporated. The residue was purified by flash chromatography (hexanes/ethyl acetate: 5/1) to give (R)-5 (191 mg) and (R)-6 (270 mg, 86.4%) as a yellow solid. $[\alpha]_D^{25} + 8.82^\circ$ (C = 0.01, tetrahydrofuran).

^{1}H-NMR (300 MHz, $CDCl_3$), δ (ppm): 7.96–7.93 (3H, m), 7.88–7.80 (2H, t, *J* = 8.25 Hz), 7.61–7.56 (2H, t, *J* = 8.19 Hz), 7.42–7.38 (1H, dd, *J* = 8.88, 1.53 Hz), 7.36–7.31 (1H, t, *J* = 6.75 Hz), 7.25–7.19 (1H, m),

7.16–7.11 (2H, m), 6.44 (1H, d, *J* = 15.93 Hz), 5.10 (1H, d, *J* = 5.49 Hz), 5.08 (1H, d, *J* = 5.37 Hz), 4.99 (1H, d, *J* = 3.81 Hz), 4.97 (1H, d, *J* = 3.78 Hz), 3.79 (3H, s), 3.14 (6H, d, *J* = 2.73 Hz).

^{13}C-NMR (75 MHz, $CDCl_3$), δ (ppm): 167.66, 153.93, 152.67, 145.02, 135.07, 133.89, 130.24, 130.19 × 2, 129.89, 129.67, 129.55, 128.01, 126.49, 126.36, 125.30, 124.19, 123.93, 121.40, 120.67, 117.60, 117.17, 117.09, 95.16, 94.86, 55.93, 55.89, 51.72.

2.3.6. Synthesis of (R)-7

To a solution of (R)-6 (350 mg, 0.76 mmol) in tetrahydrofuran (20 mL) was added saturated mixed solution of lithium hydroxide and potassium hydroxide. After being stirred and refluxed for 12 h, the reaction mixture was extracted with ethyl acetate, and washed with brine. The organic solvent was dried (sodium sulfate) and evaporated to give (R)-7 (288 mg, 85%) as a yellow solid. $[\alpha]_D^{25} + 10.71^\circ$ (C = 0.01, tetrahydrofuran).

^{1}H-NMR (300 MHz, $CDCl_3$), δ (ppm): 7.96–7.84 (5H, m), 7.58 (2H, m), 7.43–7.39 (1H, dd, *J* = 9, 0.96 Hz), 7.35–7.30 (1H, m), 7.24–7.11 (3H, m), 6.44 (1H, d, *J* = 15.90 Hz), 5.10 (1H, d, *J* = 3.93 Hz), 5.08 (1H, d, *J* = 3.84 Hz), 4.99 (1H, d, *J* = 2.58 Hz), 4.97 (1H, d, *J* = 2.61 Hz), 3.14 (6H, d, *J* = 1.59 Hz).

2.3.7. Synthesis of (R)-8

A mixture of (R)-7 (500 mg, 1.13 mmol) and triethylamine (0.47 mL, 3.38 mmol) in dry dichloromethane (15 mL) was stirred at 0 °C for 5 min. Then HATU (470.25 mg, 1.24 mmol) was added. After being stirred at room temperature for 30 min, γ-aminopropyltriethoxysilane (0.26 mL, 1.13 mmol) was added, and the mixture was stirred at room temperature for 3 h. Then, the mixture was filtered and evaporated, yielding (R)-8 (320 mg, 91%) as a yellow solid.

^{1}H-NMR (300 MHz, $CDCl_3$), δ (ppm): 7.96–7.91 (3H, m), 7.87 (1H, d, *J* = 8.12 Hz), 7.74 (1H, d, *J* = 15.56 Hz), 7.58 (1H, d, *J* = 3.08 Hz), 7.56 (1H, d, *J* = 3.12 Hz), 7.37–7.32 (2H, m), 7.26–7.20 (1H, m), 7.15–7.10 (2H, t, *J* = 8.08 Hz), 6.38 (1H, d, *J* = 15.52 Hz), 6.12–6.09 (1H, t, *J* = 5.60 Hz), 5.09 (1H, d, *J* = 4.44 Hz), 5.07 (1H, d, *J* = 4.48 Hz), 4.98 (1H, d, *J* = 5.32 Hz), 4.96 (1H, d, *J* = 5.44 Hz), 3.84–3.79 (6H, dd, *J* = 14.00, 7.00 Hz), 3.40–3.35 (2H, dd, *J* = 12.92, 6.64 Hz), 3.14 (6H, d, *J* = 3.64 Hz), 1.71–1.67 (2H, m), 1.23–1.20 (9H, t, *J* = 7.00 Hz), 0.67–0.65 (2H, t, *J* = 8.16 Hz).

^{13}C-NMR (75 MHz, $CDCl_3$), δ (ppm): 166.00, 153.59, 152.64, 140.67, 134.61, 133.90, 130.68, 129.99, 129.87, 129.67, 129.56 × 2, 127.93, 126.42, 126.13, 125.36, 124.14, 123.82, 121.33, 120.87, 120.36, 117.56, 117.22, 95.20, 94.95, 77.40, 77.08, 76.76, 58.49 × 3, 55.87, 55.85, 42.06, 33.99, 25.65, 24.97, 22.94, 18.31 × 3, 7.86.

2.3.8. Preparation of the Chiral Stationary Phase

Four moles hydrochloric acid (100 mL) was added into a solution of silica gel (10 g). The reaction was refluxed for 5 h, and the mixture was filtered before being washed with water until the pH value became neutral. Then, the resulting solid substance was dried under a vacuum at 150 °C for 24 h. The solution of the (R)-8 and acidified silica gel (3.5 g) in toluene (100 mL) was refluxed for 12 h while being protected with nitrogen. Once the reaction was complete, the mixture was cooled to room temperature, filtered, and washed three times using toluene and methanol before being dried under vacuum for 4 h at 70 °C. The product obtained was (R)-9.

Hydrochloric acid (12 M; 2.0 mL) was added to the solution of (R)-9 in methanol (50 mL) and the mixture was stirred for 4 h. Afterwards, the mixture was filtered and washed with methanol for several times, and dried under vacuum for 4 h at 50 °C. The product obtained was (R)-6-Acrylic-BINOL CSP (3.64 g). Next, 3.30 g of the (R)-6-Acrylic-BINOL CSP was packed into the empty stainless-steel column (250 mm × 4.6 mm). Lastly, the chromatography was performed using Agilent 1260 HPLC.

3. Results and Discussion

3.1. Infrared Spectrum

The infrared spectrum results (Figure 1) provided the structural information of the prepared CSP. The strong peak at 3467.31 cm^{-1} was attributed to the stretching vibration absorption (N-H) and the residual silanol group (O-H). The peak at 2931.78 cm^{-1} represented the stretching vibration absorption (C-H) of benzene rings. And the peak at 1653.27 cm^{-1} was the C=O of the carbonyl group. The peaks at 1623.79 cm^{-1} represented the stretching vibration absorption (C=C) of benzene rings. The strong peak at 1109.93 cm^{-1} was related to the single bond absorption (Si-O-Si, C-O). The data up-mentioned indicated that (R)-6-Acrylic-BINOL has been bonded to the surface of the acidified silica gel.

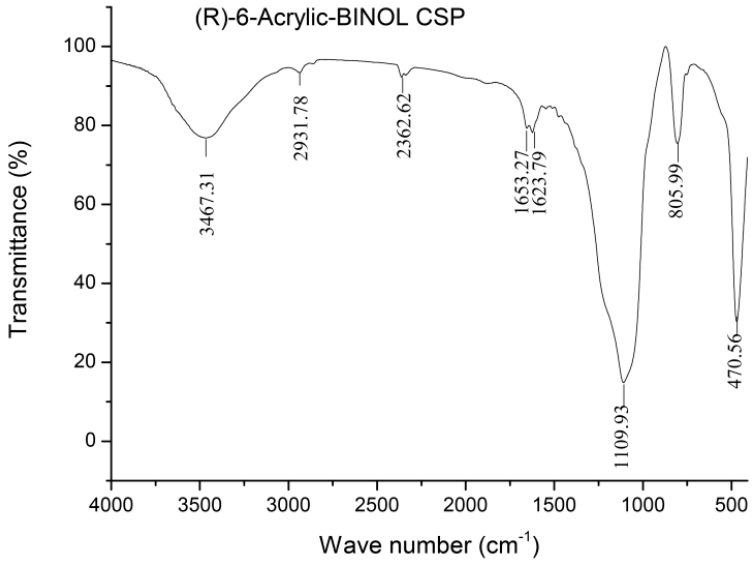


Figure 1. The infrared spectroscopy of (R)-6-Acrylic-BINOL CSP.

3.2. Elemental Analysis

The surface concentration of (R)-6-Acrylic-BINOL CSP was calculated based on the elemental analysis data of the CSP (found: C, 12.35%; H, 1.54%; N, 2.30%). Compared to acidified silica gel (found: C, 0.329%; H, 1.229%; N, 0.735%), the surface concentration of (R)-6-Acrylic-BINOL on the silica gel was 387.6 μmol/g, based on carbon.

3.3. Scanning Electron Microscope

As shown in the scanning electron microscopy data (Figure 2) of the acidified silica gel (A) and the (R)-6-Acrylic-BINOL CSP (B), there was a significant difference between (A) and (B) regarding the surface morphology of the silica particles. The surface of the acidified silica particles was relatively smooth before the bonding, but the particle surface became rough after being bonded with (R)-6-Acrylic-BINOL. This result indicated that the surface of the silica gel is covered with a layer of material.

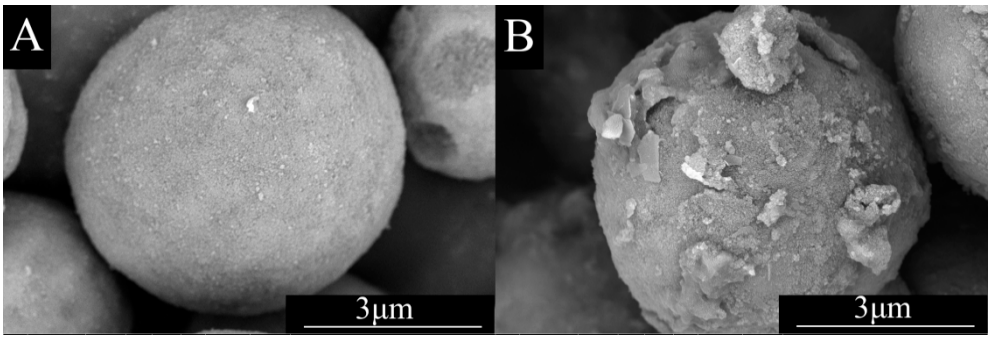


Figure 2. The scanning electron microscope of acidified silica gel (**A**) and (R)-6-Acrylic-BINOL CSP (**B**).

3.4. Enantioseparation

In this study, the following 6 solutes with the conjugated structure which, respectively, were rac-1,1′-Binaphthol (**1**), rac-3,5-dinitro–*N*-(1-phenylethyl) benzamide (**2**), rac-5-methoxy flavanone (**3**), rac-6-methoxy flavanone (**4**), rac-thalidomide (**5**), rac-2,2,2-trifluoro-1-(9-anthryl)-ethanol (**6**) (Figure 3), were chosen for the chiral ability evaluation of novel CSP. The separation results of enantiomers on (R)-6-Acrylic-BINOL CSP were listed in Table 1. The representative chromatogram data of (1) and (2) on the CSP were mentioned in Figure 4.

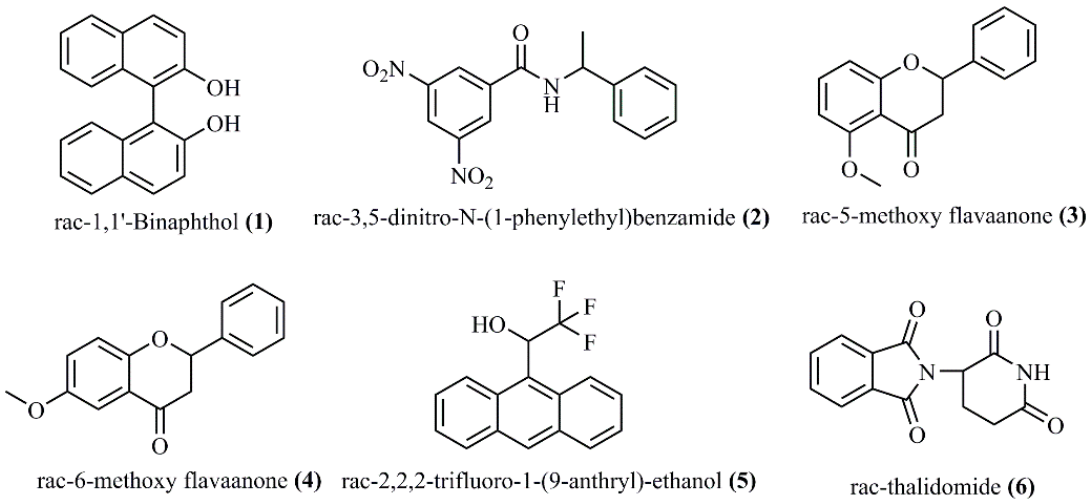


Figure 3. The structures of 6 solutes.

Table 1. The separation results of enantiomers on (R)-6-Acrylic-BINOL CSP.

Compound	k_1'	α
(**1**) [a]	5.96	1.111
(**2**) [b]	7.18	1.122
(**3**) [a]	9.09	1.057
(**4**) [a]	0.69	1.062
(**5**) [a]	2.41	1.033
(**6**) [c]	19.92	1.048

Conditions: [a] Mobile phase: hexane-ethanol-trifluoroacetic acid (99:1:0.1, *v*/*v*/*v*); [b] Mobile phase: hexane-ethanol-trifluoroacetic acid (97:3:0.1, *v*/*v*/*v*); [c] Mobile phase: hexane-ethanol-trifluoroacetic acid (90:10:0.1, *v*/*v*/*v*); Flow rate: 1 mL/min; Temperature: 5 °C; Detection wavelength: 254 nm for rac-2,2,2-trifluoro-1-(9-anthryl)-ethanol, 220 nm for other 5 solutes. k_1': retention factor of the first eluted enantiomer. α: separation factor.

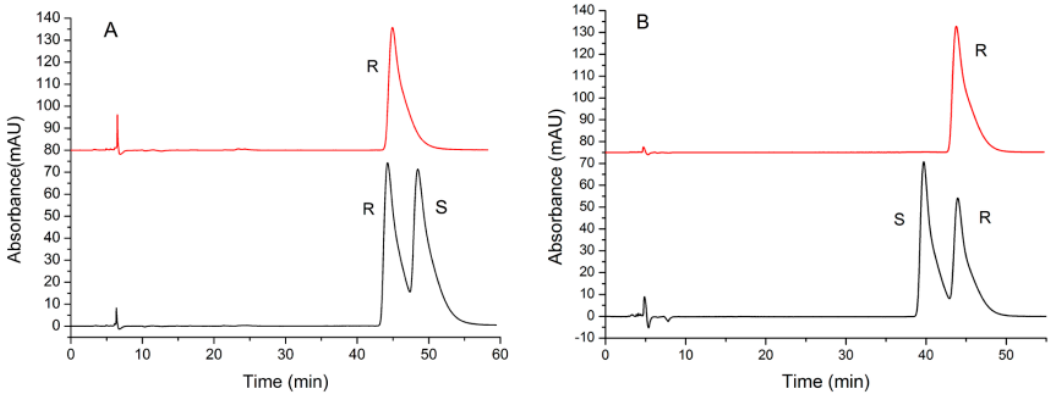


Figure 4. (**A**) Chromatogram of (1) on (R)-6-Acrylic-BINOL CSP. R: (R)-BINOL, S: (S)-BINOL. Mobile phase: hexane-ethanol-trifluoroacetic acid (99:1:0.1, $v/v/v$). Flow rate: 1 mL/min. Detection: 220 nm UV. Temperature: 5 °C. (**B**) Chromatogram of (2) on (R)-6-Acrylic-BINOL CSP. R: (R)-3,5-dinitro-*N*-(1-phenylethyl) benzamide, S: (S)-3,5-dinitro-*N*-(1-phenylethyl) benzamide. Mobile phase: hexane-ethanol-trifluoroacetic acid (97:3:0.1, $v/v/v$). Flow rate: 1 mL/min. Detection: 220 nm UV. Temperature: 5 °C.

Conventionally, there are three kinds of interactions between solute and chiral selectors, and at least one is near to the chiral center. The interactions are π-π stacking, hydrogen bonding interaction, dipole-dipole interaction, space adaptability etc. These interactions may influence the retention or/and selectivity. For example, because of two electron-withdrawing groups (-NO_2), the phenyl ring of (**2**) had low electron density, it could bring well π-π stacking with CSP, which contained two electron donating groups (-OH), and thus, achieved good enantioseparation. The results indicated that the π-π stacking between conjugated solutes and (R)-6-Acrylic-BINOL CSP contributed to the selectivity. Compared with the structure of (**3**) to (**4**), the unique difference was the position of –OCH_3, but the retention of (**3**) was much longer than that of (**4**), showing the space adaptability between the conjugated solutes and (R)-6-Acrylic-BINOL CSP affected the retention, but seemed to have little effect on enantioseparation.

3.4.1. Effect of the Mobile Phase

Without TFA in the mobile phase, all 6 solutes got little enantioseparation, indicating the TFA affected the enantioselective adsorption sites. The TFA concentration in ethanol increased from 0.1% to 0.15% then to 0.2%, while the retention factor decreased due to the eluting ability of mobile phase increasing. The retention factors were higher when a more branched alcohol was used as the polar modifier. Regardless of the alcohol modifier or TFA acidic modifier variation, the separation factor varied little. When the concentration of TFA in ethanol was kept at 0.1%, as the ethanol concentration increased, the retention of the solutes decreased, and so did the selectivity. The results indicated that the alcohol modifier also affected the enantioselective adsorption sites. The data are shown in supporting information.

3.4.2. Temperature Effect

In order to study the further chiral discrimination mechanism of novel CSP, the effect of temperature on six solutes were investigated.

Effect of Temperature on Retention Factor

The relationship between the solute retention factor (k') and temperature can be expressed by the Van't Hoff equation.

$$lnk\prime = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} + ln\varphi \quad (1)$$

where k' is the solute retention factor, ΔH and ΔS are the enthalpy change and entropy change, respectively, when the analyte transfers from the mobile phase to the stationary phase, and φ is the phase ratio of the column.

The plots of Van't Hoff of 6 solutes were obtained (Figure 5); the linear relationship were good, and linear correlation coefficients (R^2) were higher than 0.98. The Van't Hoff curve indicated that the separation mechanism of investigated solutes did not change during the temperature change. As can be seen in Figure 5, as the temperature increased, lnk' were gradually reduced. The reason may be that as the temperature rose, the structures of the CSP and solute did not change, while the viscosity of the mobile phase decreased and the mass transfer resistance of the components in the mobile phase decreased. In the Figure 5, 4 points of (**4**) were obtained because enantioseparation only occurred under 20 °C. In addition, the retention of (**4**) was short, so that the k was less than 1 and the lnk is negative.

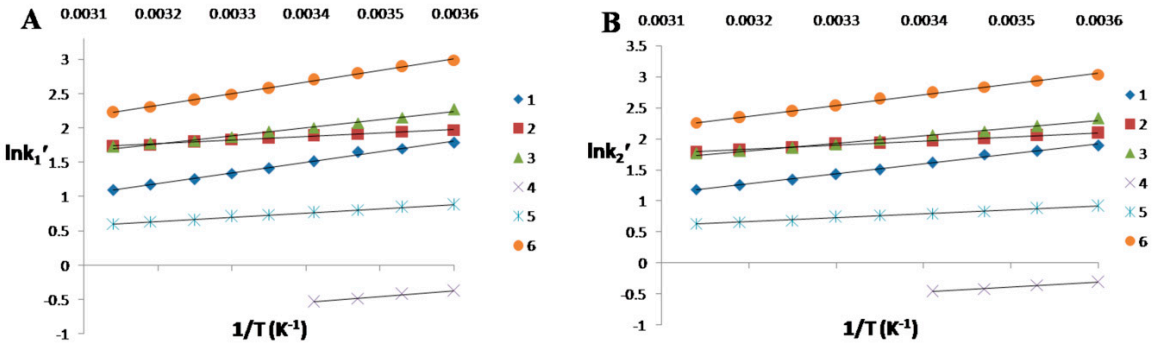


Figure 5. Van't Hoff Plots of lnk_1' vs. 1/T (**A**) and lnk_2' vs. 1/T (**B**). (1: 1,1′-Binaphthol; 2: 3,5-dinitro–*N*-(1-phenylethyl) benzamide; 3: 5-methoxy flavanone; 4: 6-methoxyflavanone; 5: thalidomide; 6: 2,2,2-trifluoro-1-(9-anthryl)-ethanol.

The ΔH and $\Delta S/R + ln\varphi$ values of the six solutes enantiomers were obtained from the slope and intercept of the Van't Hoff curve (Table 2). The thermodynamic parameter ΔH indicated the thermal effect of solute transferred from the mobile phase to the stationary phase. If it is negative, the solute adsorption on the stationary phase is an exothermic process. As can be seen from Table 2, the adsorption of 6 solutes on the stationary phase is an exothermic process ($\Delta H < 0$).

Table 2. ΔH and $\Delta S/R + ln\varphi$ values for 6 solutes.

Compound	ΔH (kJ/mol)	$\Delta S/R+ln\varphi$	$\Delta(\Delta H)$ (kJ/mol)	$\Delta(\Delta S)$ (J/mol * K)	T_{iso} (K)
(R)-1,1′-Binaphthol	−12.95	−3.80	−0.56	−1.13	495.57
(S)-1,1′-Binaphthol	−13.36	−3.87			
(R)-3,5-dinitro-*N*-(1-phenylethyl)benzamide	−5.58	−0.32	−1.17	−3.27	357.80
(S)-3,5-dinitro-*N*-(1-phenylethyl)benzamide	−4.41	0.07			
(1)-5-methoxy flavanone	−9.70	−1.97	−0.43	−1.08	398.15
(2)-5-methoxy flavanone	−10.17	−2.11			
(1)-6-methoxy flavanone	−7.14	−3.46	−0.96	−2.93	327.65
(2)-6-methoxy flavanone	−6.79	−3.25			
(1)-2,2,2-trifluoro-1-(9-anthryl)-ethanol	−5.10	−1.33	−0.12	−0.15	800
(2)-2,2,2-trifluoro-1-(9-anthryl)-ethanol	−5.21	−1.34			
(1)-thalidomide	−14.11	−3.11	−0.27	−0.57	473.68
(2)-thalidomide	−14.36	−3.16			

(1): the first eluted enantiomer; (2): the second eluted enantiomer.

Effect of Temperature on Separation Factor

According to Equation (1) and the selection factor ($\alpha = k_1'/k_2'$), the Equation (2) was obtained.

$$ln\alpha = -\frac{\Delta(\Delta H)}{RT} + \frac{\Delta(\Delta S)}{R} \quad (2)$$

where α is the solute separation factor, $\Delta(\Delta H)$ is the difference in enthalpy changes between the two enantiomers ($\Delta H_2 - \Delta H_1$), $\Delta(\Delta S)$ is the difference in entropy changes between the two enatiomers ($\Delta S_2 - \Delta S_1$).

The $ln\alpha$ vs. 1/T plot showed that the $ln\alpha$ decreased with increasing temperature (Figure 6) due to the different enantiomers of the six compounds interacting with the chiral stationary phase. $\Delta(\Delta H)$ and $\Delta(\Delta S)$ can be calculated separately from the slope and intercept of the straight line in Figure 6 (Table 2). The isoenantioselective temperature (T_{iso}) is defined as the temperature at which the enthalpy and entropy terms are balanced and $\alpha = 1$ ($T_{iso} = \frac{\Delta(\Delta H)}{\Delta(\Delta S)}$). That means the solute loses the enanioseparation as the temperature goes over T_{iso}. It may deviate a little from the actual value. For example, T_{iso} of (**4**) is 327.65 K (29.5 °C), but in the experimental conditions, it showed little enantioseparation above 25 °C.

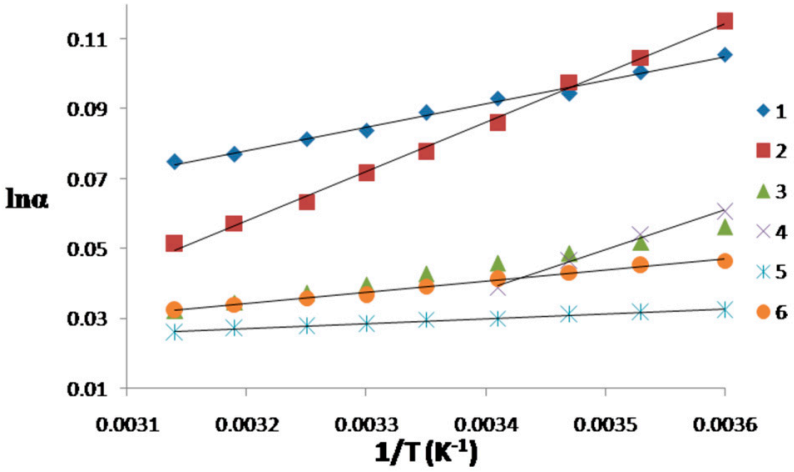


Figure 6. Plot of $ln\alpha$ vs. 1/T of 6 solutes. (1: 1,1′-Binaphthol; 2: 3,5-dinitro–*N*-(1-phenylethyl) benzamide; 3: 5-methoxy flavanone;4: 6-methoxyflavanone; 5: thalidomide; 6: 2,2,2-trifluoro-1-(9-anthryl)-ethanol.).

4. Conclusions

In order to explore the conjugated effect of BINOL CSP on enantioseparation, a novel CSP, (R)-6-Acrylic-BINOL, was prepared in this paper, introducing an acrylic group to the 6-position of (R)-Binaphthol, with hydroxyl group residues. The IR, SEM, and element analysis results showed that the (R)-6-Acrylic-BINOL was bonded to the surface of silica gel, and the surface concentration of (R)-6-Acrylic-BINOL on the silica gel was calculated to be 387.6 μmol/g(1.04 μmol/m^2), based on carbon. Six solutes with conjugated structures were chosen to evaluate the chiral ability of the novel CSP. The study on mechanism of chiral recognition was based on the variation of mobile phase and temperature. The results showed that the π-π stacking between the solutes and the CSP were beneficial for enantioseparation. And the space adaptability between the solutes and CSP contributed to the retention, but did not affect enantioseparation. Under the investigated temperature range, 6 solutes kept the separation mechanism unchanged; all were enthalpyprocesses. In a word, the novel (R)-BINOL derivative CSP can be used as a potential CSP for chiral separation processes under normal phase conditions, especially for conjugated compounds. More work on this CSP is needed in future work.

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