

Preparation and chiral separation of a novel immobilized cellulose-based chiral stationary phase in high-performance liquid chromatography

Zhang, Zhen Bin; Peng, G. M.; Wu, S. Q.; Fang, Z. L.; Wu, S. S.; Ng, S. C.; Zhang, Wei-Guang; Fan, Jun; Zheng, Sheng-Run

2012

Peng, G. M., Wu, S. Q., Fang, Z. L., Zhang, W. G., Zhang, B.Z., Fan, J., et al. (2012). Preparation and chiral separation of a novel immobilized cellulose-based chiral stationary phase in high-performance liquid chromatography. *Journal of chromatographic science*, 50(6), 516-522.

<https://hdl.handle.net/10356/105703>

<https://doi.org/10.1093/chromsci/bms047>

© 2012 The Authors (published by Oxford University Press). This paper was published in *Journal of Chromatographic Science* and is made available as an electronic reprint (preprint) with permission of Oxford University Press. The paper can be found at the following official DOI: [<http://dx.doi.org/10.1093/chromsci/bms047>]. One print or electronic copy may be made for personal use only. Systematic or multiple reproduction, distribution to multiple locations via electronic or other means, duplication of any material in this paper for a fee or for commercial purposes, or modification of the content of the paper is prohibited and is subject to penalties under law

Preparation and Chiral Separation of a Novel Immobilized Cellulose-Based Chiral Stationary Phase in High-Performance Liquid Chromatography

Gui Ming Peng¹, Su Qin Wu¹, Zhi Li Fang¹, Wei Guang Zhang^{1*}, Zhen Bin Zhang¹, Jun Fan¹, Sheng Run Zheng¹, Shang Sen Wu¹ and Siu Choon Ng^{1,2}

¹School of Chemistry and Environment, South China Normal University, Guangzhou, 510006, China, and ²Division of Chemical and Biomolecular Engineering, Nanyang Technological University, 637459, Singapore

*Author to whom correspondence should be addressed. Email: wgzhang@scnu.edu.cn

Received 15 July 2011; revised 13 September 2011

The chiral selector 6-azido-2, 3-di(*p*-chlorophenylcarbamoylated) cellulose was synthesized and further chemically immobilized onto 5- μ m amino functionalized spherical porous silica gel. It was used as chiral stationary phase in high-performance liquid chromatography. Thirty racemates were successfully separated into enantiomers in either normal phase mode or reversed-phase mode. Good reproducibility and stability of the chiral stationary phase have been demonstrated.

Introduction

Many drugs, natural products and food compounds are chiral, with their enantiomers often showing different or even opposing pharmacology, toxicity and metabolic activities (1). Currently, many top-selling drugs around the world are single enantiomers with the desired biological activity (2). Moreover, the obtainment of pure enantiomers from racemates is an important concern in the stereochemical area. Among many other methodologies, high-performance liquid chromatography (HPLC) using chiral stationary phase (CSP) affords one of the most direct and effective approaches for enantioseparation.

During the past two decades, CSPs have advanced rapidly. The majority of polysaccharide-based CSPs employed have been cellulose-based columns (3). Polysaccharide derivatives that were coated on silica gel as CSPs appeared in the 1980s (4, 5). However, solvents that can swell or dissolve the derivatives could not be used as mobile phases, because the chiral selectors were physically coated onto the surface of the silica gel. Accordingly, coated CSPs were only amenable for use in a limited range of eluents, which were usually mixtures of non-polar solvents and alcohols used in normal phase mode (6, 7). To overcome the solubility of the coated selectors, Okamoto *et al.* first bonded the polysaccharide to a γ -aminopropyl silica gel matrix using a diisocyanate as a spacer that was expected to react with the free amino groups on the matrix surface and the hydroxyl groups of the polysaccharide (8). Because polysaccharide derivatives were covalently bonded onto silica gel, the CSP could be applied with a much broader range of solvents as mobile phases (9–17), which would enhance the success rate in enantioseparation.

As for amylose-based CSPs, Chiralpak IA is a most successful immobilized CSP using amylose 3,5-dimethylphenylcarbamate as its chiral selector (18). Thus far, many functional groups have been used to modify cellulose and have been further

immobilized onto silica gel used as CSPs; for example, cellulose 3,5-dimethylphenylcarbamate (Chiralpak IB) (14), cellulose tris(3,5-dichlorophenylcarbamate) (Chiralpak IC) (19) and azido cellulose phenylcarbamate (20).

Cellulose tris(*p*-chlorophenylcarbamate) used as chiral selector that was coated onto silica gel was reported by Okamoto *et al.* (5). In this paper, cellulose *p*-chlorophenylcarbamate was first chemically immobilized onto silica gel by Staudinger reaction. The enantioseparation results showed that the CSP afforded high enantioseparation ability towards structurally diverse chiral compounds in either normal or reversed-phase mode.

Materials and Methods

Equipments

Nuclear magnetic resonance (NMR) was carried out on a Bruker ACF300FT-NMR spectrometer with tetramethylsilane as internal standard. Fourier transform infrared (FTIR) was performed on a Bio-Rad TFS156 instrument. Elemental analysis was determined on a PerkinElmer 2400CHN analyzer. The columns were packed using an Alltech pneumatic HPLC pump. Evaluation of the column was performed on an HPLC system, which comprised a Lib Alliance HPLC Series iii system, a Lib Alliance Model 201 ultraviolet-visible (UV-vis) detector and a 7725i injector equipped with a 20- μ L sample loop.

Chemicals and reagents

Microcrystalline cellulose [degree of polymerization (DP) \approx 200] and *p*-chlorophenyl isocyanate were purchased from Shanghai Hengxin Chemical Reagent Co. 3-Aminopropyltriethoxysilane and Compounds 9, 12–15, 20–22, 24, 29 and 30 were obtained from Alfa Aesar (Tianjin, China); Compounds 1–8 were provided by Professor Ding-Qiao Yang's lab, and the other compounds were obtained from Professor Zhao-Yang Wang's lab. HPLC-grade hexane, acetonitrile, isopropyl alcohol (IPA), methanol and ethanol were purchased from Tianjin Damao Chemical Reagent Co. Deionized and distilled water was used throughout the study. Silica gel (5 μ m, 500 Å, 300 m²/g) was purchased from Fuji Silysia Chemical Ltd. (Aichi, Japan).

Preparation of mobile phases and samples

Triethylammonium acetate buffer (TEAA) was prepared by adding acetic acid to a solution containing 0.1% (v/v) of

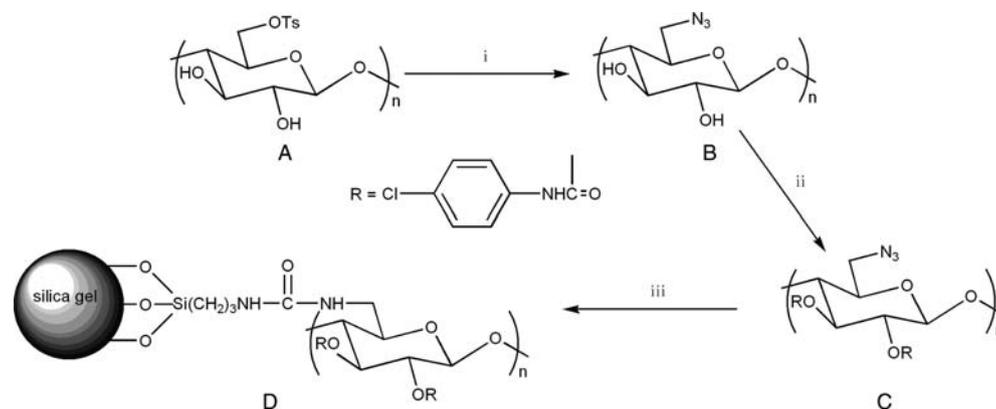


Figure 1. Synthetic procedures of the CSP. NaN_3 /dry DMSO/100°C (A); *p*-chlorophenyl isocyanate/dry pyridine/triethylamine/100°C (B); amino functionalized silica gel/PPh₃/THF/carbon dioxide (C).

triethylamine to adjust to the desired pH 4.0. NaClO_4 aqueous solution was controlled at the concentration 0.30 mol/L. All buffers were filtrated through a 0.45- μm membrane and degassed before use. The samples were prepared at a concentration of approximately 1 mg/mL. Ten microliters of the sample was injected. All chromatographic experiments were carried out at room temperature.

Preparation of CSP

Figure 1 shows the synthetic route to the CSP.

Preparation of 6-azido-deoxycellulose (Compound B)

Microcrystalline cellulose was functionalized with a *p*-toluene sulfonylchloride in *N,N*-dimethylacetamide/LiCl system to afford 6-tosyl-cellulose (Compound A) according to the reported method (21). FTIR (cm^{-1} , KBr): 3524 (OH), 3066 (C-H_{arom}), 1598, 1496, 1455 ($\text{C}=\text{C}_{\text{arom}}$), 1367, 1176 ($-\text{SO}_2-$), 1060 (C-O-C). Elemental analysis, found: C 49.85%, H 4.97%, S 10.39%. Degree of substitution was 1.06, which was calculated by using sulphur content. This indicated that almost all 6-OH and partial 2-OH or 3-OH of cellulose were converted to tosyl. Pre-dried Compound 1 (3.16 g) was dissolved in dry DMSO (100 mL). After addition of NaN_3 (3.25 g), the mixture was stirred at 100°C for 24 h in a nitrogen atmosphere. Then the product was cooled to room temperature and separated by precipitation in ice-cold water (1,500 mL), filtered off and washed with distilled water and ethanol until the filtrate was colourless. The product was dried at 50°C in vacuum overnight.

Yield 91%; FTIR (cm^{-1} , KBr): 3,468 (OH), 2,891 (CH), 2,108 (N_3), 1,060 (C-O-C). No peaks were found in the range from 1,600 to 1,400 cm^{-1} , which indicated that all 6-tosyl was substituted by azido group.

Preparation of 6-azido-2, 3-di(*p*-chlorophenylcarbamoylated) cellulose (Compound C)

Compound B (3.73 g) was dissolved in 47 mL pyridine and 47 mL TEA. *p*-Chlorophenyl isocyanate (0.16 mol) was added to the reaction flask and stirred in nitrogen atmosphere for 48 h at 100°C. The excess solvent was removed from the homogeneous brown solution using vacuum distillation. The dissolved cellulose derivative was recovered by precipitation from methanol, washed repeatedly with ethanol for 24 h, and finally dried at 50°C in vacuum overnight.

The yield was 87%; FTIR (cm^{-1} , KBr): 3,297 (NH), 3,073 (C-H_{arom}), 2,921 (CH), 2,108 (N_3), 1,722 ($\text{C}=\text{O}$), 1,591, 1,561, 1,491 ($\text{C}=\text{C}_{\text{arom}}$), 1,088 (C-O-C). ^1H NMR (CDCl_3 , TMS) δ (ppm): 8.82 (s, 2), 7.60–7.28 (m, 8H), 5.54 (d, 2H), 4.98–3.60 (m, 15H), 1.60–1.32 (m, 2H). Elemental analysis for $(\text{C}_{20}\text{H}_{17}\text{N}_5\text{O}_6\text{C}_{12})_n$, calculated: C 48.58%, H 3.44%, N 14.17%; found: C 50.09%, H 4.68% and N 13.83%.

Preparation of amino functionalized silica gel

Silica gel (20 g, dried at 180°C under 0.1 mm Hg for 4 h) was suspended in a mixture of 250 mL toluene in nitrogen atmosphere. Five millilitres of 3-aminopropyltriethoxysilane was added to the reaction flask. The mixture was refluxed at 110°C for 15 h. The resultant product was washed by soxhlet extraction with acetone for 24 h (22). Elemental analysis: C 5.21%, H 1.88%, N 2.18%.

Chemical immobilization of 6-azido-2, 3-di(*p*-chlorophenylcarbamoylated) cellulose onto amino functionalized silica gel

Amino functionalized silica gel (4.00 g) was stirred in anhydrous tetrahydrofuran (THF) (30 mL) through which a continuous carbon dioxide was bubbled. After 20 min, a solution of 6-azido-2, 3-di(*p*-chlorophenylcarbamoylated) cellulose (8.40 g) in anhydrous THF (30 mL) was added. Stirring was continued for another 5 min, then triphenylphosphine (2.00 g) in 25 mL THF was added. The mixture was stirred for 24 h with bubbling carbon dioxide at room temperature, and then filtrated. Excess non-bonded Compound C was washed with acetone overnight after the procedure (iii). The CSP was obtained, marked as CSP-1.

CSP-1, FTIR (cm^{-1} , KBr): 3,428 (NH), 1,634 ($\text{HN-C}=\text{O}$), 1,555, 1,496 ($\text{C}=\text{C}_{\text{arom}}$). Elemental analysis: C 8.72%, H 2.24%, N 2.82%. The surface concentration of cellulose derivative on the CSP-1 was determined to be $6.1 \times 10^{-3} \mu\text{mol}/\text{m}^2$ (DP = 200 was used) (23).

Evaluation of the column

Using the standard slurry method, the CSP was packed into a stainless steel column (250 \times 4.6 mm i.d.) at a pressure of 8,000 psi. CCl_4 -dioxane (2:1, *v/v*) was used as slurry solvent and methanol was used as packing solvent.

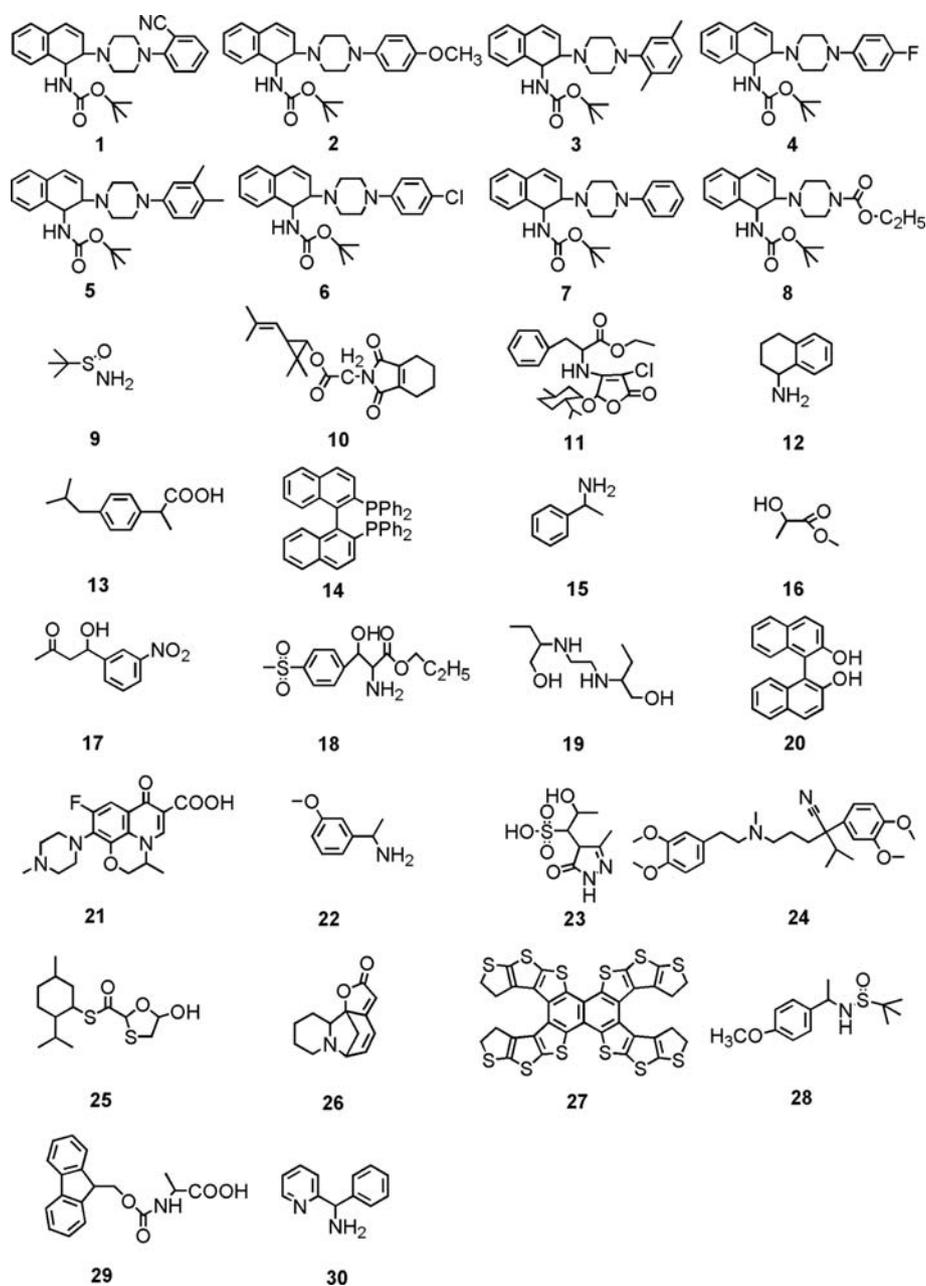


Figure 2. Structures of chiral compounds mentioned in this work.

The columns packed with CSP-1 afforded efficiency of Approximately 3.8×10^4 plates/m using biphenyl as test probe in normal phase mode [hexane-IPA (90:10, *v/v*)].

Calculations

The following resolution parameters were determined: k was calculated from the equation $k = (t_R - t_0)/t_0$, where t_R referred to the retention time and t_0 was the dead time. The t_0 for the analytical column was measured from the retention of sodium nitrate in reversed phase mode and 1,3,5-tri-*tert*-butylbenzene in normal phase mode. The void volume was 1.60 mL. The separation factors (α) were calculated using $\alpha = k_2/k_1$, where k_1 and k_2 were the retention factors for the first and second eluted enantiomers, respectively. The resolution (R_s) was

calculated from the equation $R_s = 2(t_2 - t_1)/(w_1 + w_2)$, where t_1 and t_2 were the retention times of the first and the second enantiomers, respectively, and w_1 and w_2 were the peak widths (based on USP standards).

Results and Discussion

Chiral separations were conducted in normal and reversed-phase mode. Structures of chiral compounds mentioned in this work are shown in Figure 2.

Enantiomeric separation in normal phase mode

Mixtures of hexane and alcohols were used in the enantioseparation. Fourteen analytes were effectively separated, with k_1

ranging from 1.04 to 4.13 and R_s ranging from 0.93 to 12.26. Mobile phase, retention factor (k), separation factor (α) and resolution (R_s) of all compounds separated in normal phase mode are shown in Table I.

The mechanism of enantioselectivity of polysaccharide derivatives has been studied by several research groups. Fukui *et al.* concluded that π - π interaction between the solute and CSPs played an important role in the chiral recognition process (24, 25). O'Brien reported that the difference in H-bonding of the two enantiomers with the polysaccharide derivatives was another factor that contributed to the enantioselectivity (26).

Table I

Chromatographic Enantioseparation Results of some Chiral Compounds in Normal Phase Mode*

Compound	Enantioseparation data			Mobile phase
	k_1	α	R_s	
1	2.77	1.18	1.50	Hexane-IPA (98:2, v/v)
2	2.95	1.16	1.58	Hexane-IPA (93:7, v/v)
3	3.18	1.15	1.49	Hexane-IPA (95:5, v/v)
4	3.26	1.15	1.30	Hexane-IPA (95:5, v/v)
9	4.13	1.28	2.54	Hexane-IPA (90:10, v/v)
10	1.86	1.39	2.78	Hexane-IPA (80:20, v/v)
13	2.71	1.44	5.13	Hexane-IPA (90:10, v/v)
14	1.34	1.37	1.58	Hexane-IPA (35:65, v/v)
15	1.93	1.41	0.93	Hexane-IPA (40:60, v/v)
16	1.04	1.18	1.41	Hexane-methanol (40:60, v/v)
27	1.14	1.38	2.69	Hexane-IPA (95:5, v/v)
28	2.36	1.68	12.26	Hexane-CHCl ₃ -ethanol (68:30:2, v/v/v)
29	1.62	1.34	1.88	Hexane-IPA-ethanol-DEA (70:15:15:0.1, v/v/v/v)
30	1.88	1.42	2.35	Hexane-IPA-ethanol-DEA (70:15:15:0.1, v/v/v/v)

*Separation conditions: flow rate, 1.0 mL/min; UV detector, $\lambda = 254$ nm; Compound 9, $\lambda = 228$ nm.

Accordingly, π - π interaction and H-bonding might be the intrinsic forces for chiral resolution of the polysaccharide in normal phase mode. Because there is no phenyl in the structure of Compound 9, H-bonding between S = O, -NH₂ of the analyte and N-H, C = O of CSP-1 might be the dominant factor contributing to its R_s to 2.54.

Chromatograms of several compounds separated on the CSP-1 in normal phase mode are presented in Figure 3.

Enantiomeric separation in reversed-phase mode

Methanol, ethanol, acetonitrile and IPA mixed with an appropriate proportion of H₂O or TEAA or NaClO₄ solution were used as mobile phases. Enantioseparation data of 16 solutes separated successfully in reversed phase mode are listed in Table II.

The helical backbone and side chain of the cellulose derivative keep the analyte in the chiral cavities environment, so it could be deduced that solutes would be wrapped into the heliced polymer cavity. In addition, the side chains were exposed to the outside, which would help to form π - π interaction with solutes (27).

Compound 19 is a racemate that possesses no aromatic moiety in its structure, hence there is no sorbent-solute π - π interaction in its chiral separation process. However, it afforded R_s up to 1.66 in reversed-phase mode. This may be due to H-bonding and steric effects. In contrast to Compound 14 separated in normal phase mode, Compound 20 was separated in reversed-phase. Compound 14 contained additional phenyl rings while Compound 20 embodied two free phenolic groups.

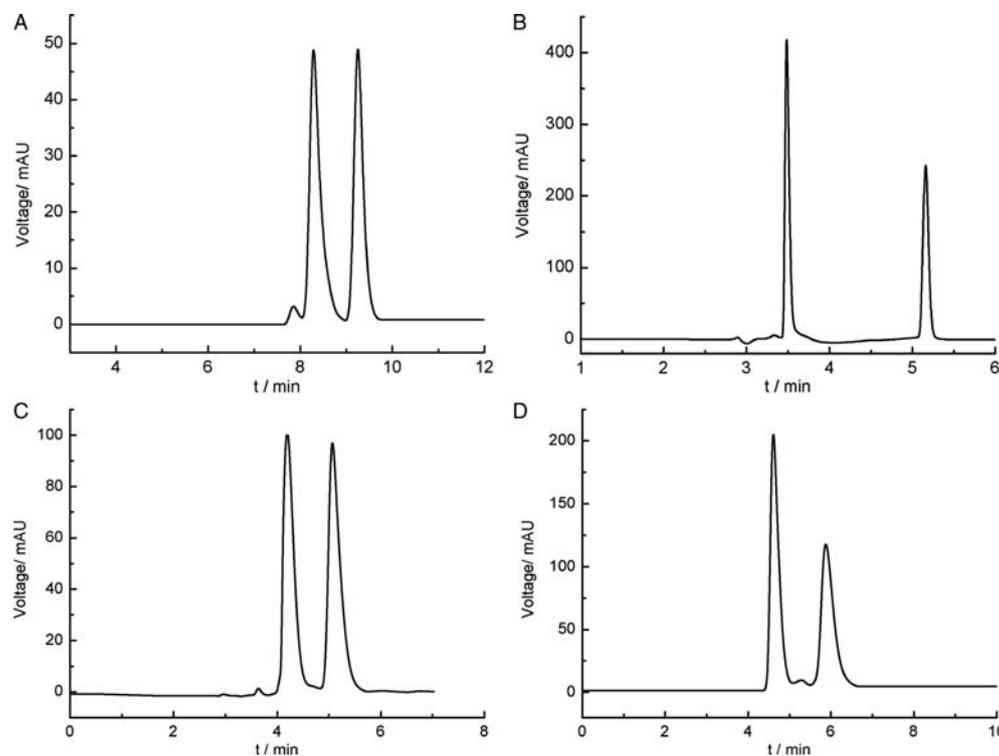


Figure 3. Chromatograms for some chiral compounds separated on the CSP-1 in normal phase mode (separation conditions and results are shown in Table I): Compound 9 (A); Compound 28 (B); Compound 29 (C); Compound 30 (D).

As for Compound 14, the additional phenyl groups might afford additional π - π interaction sites and steric hindrance. The two free phenyl hydroxyl groups of Compound 20 made H-bonding interaction with mobile phase and CSP-1 possible.

Chromatograms of several representative compounds separated on the CSP-1 in reversed phase mode are presented in Figure 4.

Table II
Chromatographic Enantioseparation Results of Some Chiral Compounds in Reversed-Phase Mode*

Compound	Enantioseparation data			Mobile phase
	k_1	α	R_s	
5	0.95	1.27	1.40	methanol-H ₂ O (80:20, v/v)
6	0.69	1.35	1.84	acetonitrile-TEAA (60:40, v/v)
7	0.97	1.27	1.52	acetonitrile-H ₂ O (60:40, v/v)
8	0.62	1.11	0.45	IPA-H ₂ O (85:15, v/v)
11	0.93	1.30	2.45	methanol-TEAA (80:20, v/v)
12	0.12	3.55	2.46	acetonitrile-TEAA (80:20)
17	2.23	1.42	2.20	ethanol-H ₂ O (50:50, v/v)
18	0.48	1.33	1.42	methanol-TEAA (80:20, v/v)
19	0.05	3.20	1.66	methanol-TEAA (80:20, v/v)
20	1.02	1.19	1.79	methanol
21	0.20	5.61	3.02	acetonitrile-TFA (100:0.1, v/v)
22	0.70	1.34	2.47	acetonitrile-H ₂ O (60:40, v/v)
23	2.21	1.53	4.13	methanol-NaClO ₄ (80:20, v/v)
24	0.70	1.17	0.69	IPA-H ₂ O (85:15, v/v)
25	0.97	2.05	3.46	methanol
26	0.88	1.25	1.39	acetonitrile-NaClO ₄ (98:2, v/v)

*Separation conditions: flow rate, 0.5 mL/min; UV detector, $\lambda = 254$ nm; Compound 19, $\lambda = 228$ nm.

Reproducibility of chiral stationary phase

To investigate the reproducibility of the CSP, several batches of the CSP have been synthesized. Standard sample Compound 3 was separated on different batches of CSP in the same condition. Three batches (marked as CSP-1, CSP-2 and CSP-3, respectively) were chosen as representatives. The results are listed in Table III. Chromatograms are shown in Figure 5.

Stability of the chiral stationary phase

The stability of the immobilized CSP would undoubtedly be a determining factor for the repeatability of separation methods and the column lifetime. Six hundred injections were carried out in both normal and reversed-phase modes within two months. Compound 1 was repeatedly injected on the CSP-1 with chromatographic data listed in Table IV and chromatograms presented in Figure 6.

Owing to its immobilized nature, non-standard solvents were tried to be used as eluents. Surprisingly, total separation of Compound 28 ($R_s = 12.26$) was achieved using hexane-CHCl₃-ethanol (68:30:2, v/v/v) which is shown in Figure 3B. Additionally, the last process of the preparation of the CSP is to wash the CSP with acetone overnight by soxhlet extraction, aiming at washing off the non-bonded 6-azido-2,3-di(*p*-chlorophenylcarbamoylated) cellulose. Consequently, the CSP is resistant to non-standard solvents such as acetone, CHCl₃.

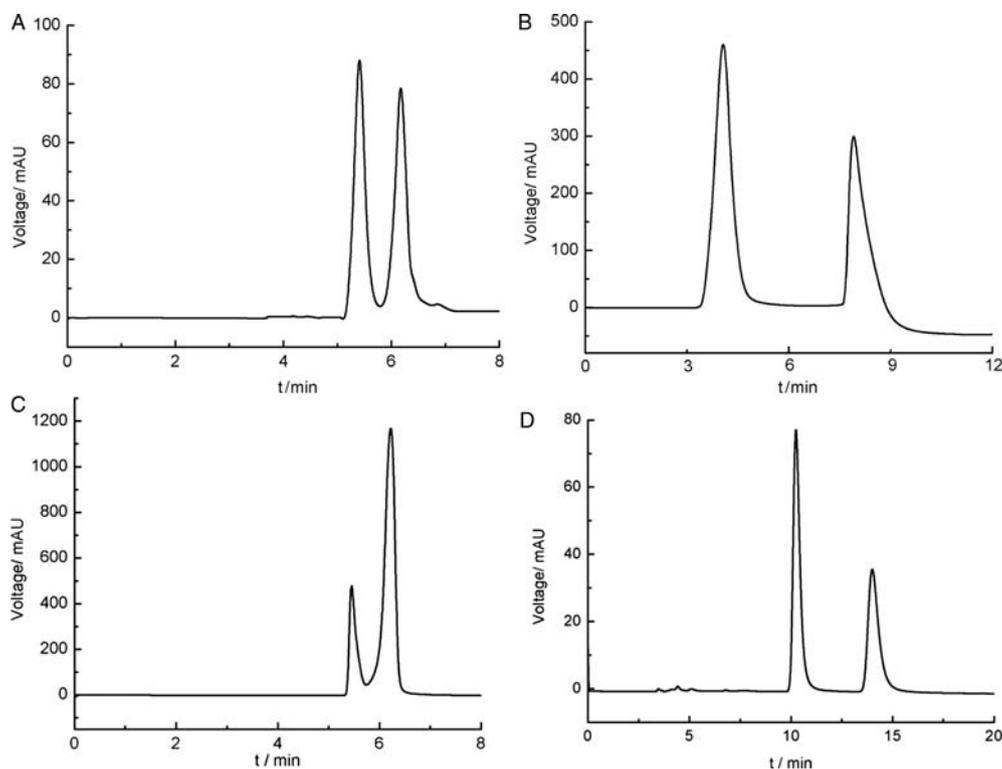


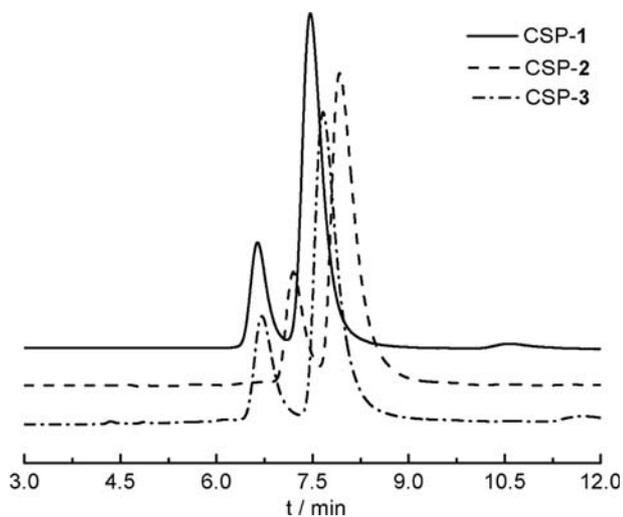
Figure 4. Chromatograms for some chiral compounds separated on the CSP-1 in reversed phase mode (separation conditions and results are shown in Table II): Compound 6 (A); Compound 21 (B); Compound 22 (C); Compound 23 (D).

Table III

Elemental Analysis Results and Enantioseparation Results of Compound 3 on Different Batches*

Batch	Elemental analysis			Enantioseparation data of Compound 3		
	%C	%H	%N	k_1	α	R_s
CSP-1	8.73	2.25	2.82	3.18	1.16	1.49
CSP-2	8.44	2.21	2.66	3.50	1.13	1.26
CSP-3	8.80	2.24	2.77	3.19	1.19	1.47

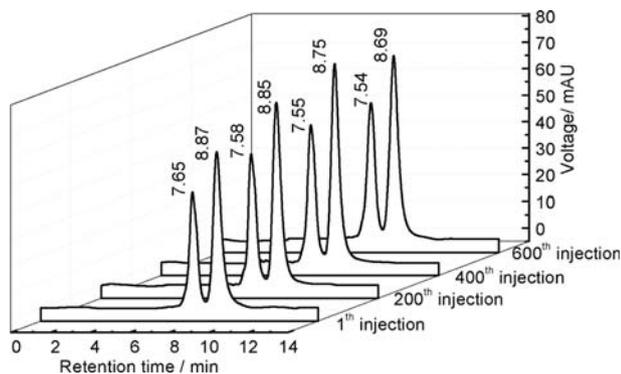
*Separation conditions: hexane-IPA (95:5, v/v); flow rate, 1.0 mL/min; UV detector: 254 nm.

**Figure 5.** Chromatograms for Compound 3 on different batches of the CSP (separation conditions are shown in Table I).**Table IV**

Enantioseparation Results of Repetitive Injection of Compound 1 on the CSP-1*

Injection order	Enantioseparation data		
	k_1	α	R_s
1st	3.78	1.20	1.55
200th	3.74	1.21	1.65
400th	3.72	1.20	1.57
600th	3.71	1.19	1.43

*Separation conditions: hexane-IPA (98:2, v/v); flow rate, 1.0 mL/min; UV detector: 254 nm.

**Figure 6.** Chromatograms of repetitive injections of Compound 1 on the CSP-1 (separation conditions are shown in Table I).

Conclusion

In summary, a novel chemically immobilized cellulose-based CSP has been prepared using Staudinger reaction between 6-azido-2,3-di(*p*-chlorophenylcarbamoylated) cellulose and amino functionalized silica gel. Chiral compounds were effectively enantioseparated in either normal phase mode or reversed-phase mode. After analysis of the enantioseparation data and structures of the chiral compounds, synergistic forces comprising inclusion, π - π interaction, H-bonding and steric effects may contribute to enantioseparation of racemates. Additionally, results revealed good reproducibility and stability of the CSP.

Acknowledgments

Support of funding from the Guangdong Science and Technology Department (No. 2008B050100017, 2009B090300081 and 2010B050300021) and the Ministry of Science and Technology of China (10C26214412704) are gratefully acknowledged.

References

- Maier, N.M., Franco, P., Lindner, W.; Separation of enantiomers: Needs, challenges, perspectives; *Journal of Chromatography A*, (2001); 906: 3–33.
- Caner, H., Groner, E., Levy, L., Agranat, I.; Trends in the development of chiral drugs; *Drug Discovery Today*; (2004); 9: 105–110.
- Timothy, J.W., Beth, A.B.; Chiral separations; *Analytical Chemistry*, (2008); 80: 4363–4372.
- Okamoto, Y., Kawashima, M., Hatada, K.; Useful chiral packing materials for high performance liquid chromatographic resolution of enantiomers: Phenylcarbamates of polysaccharides coated on silica gel; *Journal of the American Chemical Society*, (1984); 106: 5357–5359.
- Okamoto, Y., Kawashima, M., Hatada, K.; Controlled chiral recognition of cellulose triphenylcarbamate derivatives as stationary phases for HPLC; *Journal of Chromatography*; (1986); 363: 173–186.
- Okamoto, Y., Kaida, Y.; Resolution by high performance liquid chromatography using polysaccharide carbamates and benzoates as chiral stationary phases; *Journal of Chromatography A*, (1994); 666: 403–419.
- Yashima, E., Okamoto, Y.; Chiral discrimination on polysaccharides derivatives; *Bulletin of the Chemical Society of Japan*, (1995); 68: 3289–3307.
- Okamoto, Y., Aburatani, R., Miura, S., Hatada, K.; Chiral stationary phases for HPLC: Cellulose tris(3,5-dimethylphenylcarbamate) and tris(3,5-dichlorophenylcarbamate) chemically bonded to silica gel; *Journal of Liquid Chromatography and Related Technologies*, (1987); 10: 1613–1628.
- Yashima, E., Fukaya, H., Okamoto, Y.; 3,5-Dimethylphenylcarbamates of cellulose and amylose regioselectively bonded to silica gel as chiral stationary phases for high performance liquid chromatography; *Journal of Chromatography A*, (1994); 677: 11–19.
- Oliveros, L., Lopez, P., Minguillon, C., Franco, P.; Chiral chromatographic discrimination ability of a cellulose 3,5-dimethylphenylcarbamate/10-undecenoate mixed derivative fixed on several chromatographic matrices; *Journal of Liquid Chromatography and Related Technologies*, (1995); 18: 1521–1532.
- Franco, P., Senso, A., Oliveros, L., Minguillon, C.; Covalently bonded polysaccharide derivatives as chiral stationary phases in high performance liquid chromatography; *Journal of Chromatography A*, (2001); 906: 155–170.

12. Ikai, T., Yamamoto, C., Kamigaito, M., Okamoto, Y.; Immobilized polysaccharide derivatives onto silica gel: Facile synthesis of chiral packing materials by means of intermolecular polycondensation of triethoxysilyl groups; *Journal of Chromatography A*, (2007); 1157: 151–158.
13. Franco, P., Minguillón, C., Oliveros, L.; Solvent versatility of bonded cellulose-derived chiral stationary phases for high performance liquid chromatography and its consequences in column loadability; *Journal of Chromatography A*, (1998); 793: 239–247.
14. 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; *Analytica Chimica Acta*, (2006); 557: 221–228.
15. Zhang, T., Nguyen, D., Franco, P.; Enantiomer resolution screening strategy using multiple immobilised polysaccharide-based chiral stationary phases; *Journal of Chromatography A*, (2008); 1191: 214–222.
16. Zhang, T., Kientzy, C., Franco, P., Ohnishi, A., Kagamihara, Y., Kurosawa, H.; Solvent versatility of immobilized 3,5-dimethylphenylcarbamate of amylose in enantiomeric separations by HPLC; *Journal of Chromatography A*, (2005); 1075: 65–75.
17. Cirilli, R., Ferretti, R., Gallinella, B., Santis, E.D., Zanitti, L., Torre, F.L.; High-performance liquid chromatography enantioseparation of proton pump inhibitors using the immobilized amylose-based Chiralpak IA chiral stationary phase in normal phase, polar organic and reversed phase conditions; *Journal of Chromatography A*, (2008); 1177: 105–113.
18. Zhang, T., Kientzy, C., Franco, P., Ohnishi, A., Kagamihara, Y., Kurosawa, H.; Solvent versatility of immobilized 3,5-dimethylphenylcarbamate of amylose in enantiomeric separations by HPLC; *Journal of Chromatography A*, (2005); 1075: 65–75.
19. Zhang, T., Nguyen, D., Franco, P., Isobe, Y., Michishita, T., Murakami, T.; Cellulose tris(3,5-dichlorophenylcarbamate) immobilised on silica: A novel chiral stationary phase for resolution of enantiomers; *Journal of Pharmaceutical and Biomedical Analysis*, (2008); 46: 882–891.
20. Zhang, S., Ong, T.T., Ng, S.C., Chan, H.S.O.; Chemical immobilization of azido cellulose phenylcarbamate onto silica gel via Staudinger reaction and its application as a chiral stationary phase for HPLC; *Tetrahedron Letters*, (2007); 48: 5487–5490.
21. Rahn, K., Diamantoglou, M., Klemm, D., Berghmans, H., Heinze, T.; Homogeneous synthesis of cellulose *p*-toluenesulfonates in *N,N*-dimethylacetamide/LiCl solvent system; *Angewandte Makromolekulare Chemie*, (1996); 238: 143–163.
22. Zhang, Z.B., Zhang, W.G., Luo, W.J., Fan, J.; Preparation and enantio-separation characteristics of a novel chiral stationary phase based on mono (6A-azido-6A-deoxy)-per(*p*-chlorophenylcarbamoylated)-cyclodextrin; *Journal of Chromatography A*, (2008); 1213: 162–168.
23. Berthod, A., Chang, S.C., Armstrong, D.W.; β -Cyclodextrin chiral stationary phases for liquid chromatography. Effect of the spacer arm on chiral recognition; *Talanta*, (1993); 40: 1367–1373.
24. Fukui, Y., Ichida, A., Shibata, T., Mori, K.; Optical resolution of racemic compounds on chiral stationary phases of modified cellulose; *Journal of Chromatography*, (1990); 515: 85–90.
25. Aboul-Enein, H.Y., Abou-Basha, L.I., Bakr, S.A.; Direct enantioselective separation of some propranol analogs by HPLC on normal and reversed cellulose chiral stationary phases; *Chirality*, (1996); 8: 153–156.
26. O'Brien, T., Crocker, L., Thompson, R., Thompson, K., Toma, P.H., Conlon, D.A., etc.; Mechanistic aspects of chiral discrimination on modified cellulose; *Analytical Chemistry*, (1997); 69: 1999–2007.
27. Kasat, R.B., Wang, N.H.L., Franses, E.I.; Effects of backbone and side chain on the molecular environments of chiral cavities in polysaccharide-based biopolymers; *Biomacromolecules*, (2007); 8: 1676–1685.