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Preparation of Bonded Cellulose Tris(3,5-dimethylphenylcarbamate) Chiral Stationary Phases by Using Three Bifunctional Reagents

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Three di-acyl chlolide reagents, adipoyl chloride, terephthaloyl chloride and isophthaloyl chloride, were used as spacer reagents to prepare bonded type of three cellulose (3,5-dimethylphenyl)carbamate (CDMPC) chiral stationary phases (CSPs). The CDMPC CSPs were prepared using these three acid chlorides as spacer agents at the 6-position of the primary hydroxyl group on the glucose unit of cellulose regioselectively. The chiral recognition ability of the prepared CSPs for five racemates was evaluated by normal-phase high-performance liquid chromatography (HPLC) with the following mobile phases: hexane/isopropanol (IPA), hexane/IPA/tetrahydrofuran (THF) and hexane/IPA/chloroform. The result showed that these prepared CSPs can be used in THF and chloroform solutions and the chiral recognition abilities of the CSPs were improved depending on the eluents and chiral samples.

Key Words : Cellulose 3,5-dimethylphenylcarbamate, Chiral stationary phase, High performance liquid chromatography, Chiral separation

Introduction

Polysaccharide-based CSPs for high-performance liquid chromatography (HPLC) show broad applicability for the enantioseparation of a wide range of racemates.[1,2] Among the many polysaccharide derivatives, CDMPC exhibits high chiral recognition ability toward a wide range of chiral compounds, and has been recognized as the most powerful CSP for the direct separation of enantiomers by HPLC.[3,4]

Polysaccharide-based CSPs have been traditionally prepared by coating the derivatives onto macroporous silica gel.[5] Many solvents, such as THF and chloroform, which can dissolve or swell the polysaccharide derivatives, cannot be used as eluents because of their coated nature. Therefore, only a limited range of solvents, such as a hexane–alcohol mixture for normal phase separation and a water–acetonitrile mixture for reversed phase separation, can be used as eluents for these coated-type CSPs.[6]

Okamoto *et al.*[7,8] and Minguillón *et al.*[9] developed a bifunctional reagent method and radical copolymerization method to overcome this problem, respectively. Until now, diisocyanates, such as 3-(triethoxysilyl)propyl isocyanate,[10,11] and 3-epoxypropoxypropyltriethoxysilane,[12,13] have been the main bifunctional reagents used for the preparation of bonded-type polysaccharide CSPs. Chiralpak IA, Chiralpak IB, and Chiralpak IC (Daicel, Tokyo, Japan), which are composed of immobilized amylose 3,5-dimethylphenylcarbamate, CDMPC and cellulose 3,5-dichlorophenylcarbamate, respectively, have been commercialized as CSPs.[14-17]

Normally, macroporous APS (pore diameter of 500-4000 Å) is used as a matrix onto which polysaccharide derivatives are coated or chemically bonded. Some studies based on small pores (50-200 Å) have been also reported.[5,14]

To the best of the authors' knowledge, there are no reports of the preparation of CSPs based on polysaccharide derivatives using di-acid halides as cross-linkers. In this study, three di-acid halides, adipoyl chloride, terephthaloyl chloride and isophthaloyl chloride, were used as spacer reagents to prepare the bonded types of CSPs with CDMPC. CDMPC were bonded regioselectively to small pore APS at the 6-position of the primary hydroxyl group on the glucose unit of cellulose. The prepared CSPs were packed into stainless steel columns, and their chiral resolution was examined by HPLC using five racemates with a range of mobile phases, hexane/IPA, hexane/IPA/THF and hexane/IPA/chloroform. The results were compared with those of commercialized CDMPC CSP, Chiralpak IB.

Experimental

Materials. Microcrystalline cellulose (Avicel, DP 215-240) was purchased from Sigma-Aldrich Company (Gillingham, Dorset, U.K.). Spherical silica gel (Kromasil, 5 μm, 100 Å) was purchased from Akzo Noble (Nacka, Sweden). 2,2,2-trifluoro-1-(9-anthryl)ethanol (**S1**) was obtained from TCI Company (Tokyo, Japan). Triphenylmethyl chloride, adipoyl chloride, terephthaloyl chloride, isophthaloyl chloride, 3-aminopropyltriethoxysilane, 4-dimethylamiopryidine (DMAP), 3,5-dimethylphenyl isocyanate, benzoin (**S2**), Troger's base (**S3**), trans-stilbene oxide (**S4**), methyl mandelate (**S5**) were supplied by Sigma-Aldrich Company (Gillingham, Dorset, U.K.). Figure 1 shows the molecular

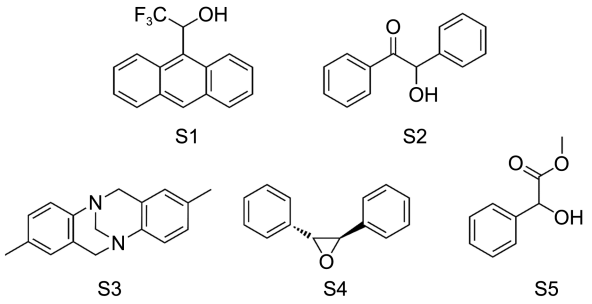

Figure 1. Molecular structures of five racemic samples used in this study. S1, 2,2,2-trifluoro-1-(9-anthryl)ethanol stilbene oxide; S2, benzoin; S3, Troger's base; S4, trans-Stilbene oxide; S5, methyl mandelate.

structures of the five test racemates.

All solvents used in the preparation of CSPs were of analytical reagent grade, dried carefully and distilled prior to use. Hexane, IPA, THF and chloroform were of HPLC grade. The other reagents were of analytical grade.

Preparation of Bonded CDMPC–CSPs (CSP1-3). The bonded CDMPC–CSPs were prepared according to Yashima's method with a slight modification, as shown in Figure 2.[8]

In detail, pre-dried microcrystalline cellulose (**1**) (4.0 g) and triphenylmethyl chloride (20.0 g) were added to a solution of anhydrous pyridine (100.0 mL) and reacted at 90 °C for 24 h under nitrogen. 3,5-dimethylphenyl isocyanate (20.0 mL) was then added to the solutions allowed to react for a further 24 h at 90 °C under nitrogen. The resulting solution was poured into a large flask with methanol (1000.0 mL) containing a small amount of hydrochloric acid (10.0 mL) and stirred for 24 h at room temperature. The suspended solution was filtered, washed with methanol and the solid substance of cellulose 2,3-bis(3,5-dimethylphenylcarbamate) (**2**) was collected after being dried under vacuum for 24 h at 80 °C.

Compound **2** (0.6 g) was dissolved in 10 mL of dried THF and coated onto 3.0 g of APS, which was prepared by a reaction of 3-aminopropyltriethoxysilane (20 mL) with spherical silica gel (10.0 g) in anhydrous toluene (200 mL) under reflux for 72 h in a nitrogen atmosphere. The coated silica gel was dispersed in a mixture of dried toluene (10.0 mL) and pyridine (2.0 mL) containing 5 mg of DMAP and acid chloride (10 mol % based on the 6-position hydroxy group in compound **2**), 24.0 mg of adipoyl chloride for **CSP 1**, or 27.0 mg of terephthaloyl chloride **CSP 2**, or 27.0 mg of isophthaloyl chloride for **CSP 3**, respectively, and was allowed to react at 100 °C for 24h under nitrogen. An excess of 3,5-dimethylphenyl isocyanate (2.0 mL) was added and allowed to react at 100 °C for a further 24 h under nitrogen. The products (**CSP 1-3**) were collected by filtration, treated with THF by Soxhlet extraction for 24 h, and washed with methanol. Finally, the prepared **CSP1-3** was dried under vacuum for 24 h at 80 °C.

The successful preparation of the CSPs was confirmed by Fourier transform infrared (FT-IR) spectroscopy, elemental analysis and thermal analysis.

Column Packing. The resulting **CSP 1-3** were suspended in a hexane/IPA (90/10, v/v) mixture and packed in a stainless steel column (25 cm long with an internal diameter of 0.46 cm) at 7000 psi using a slurry packing method, respectively.[18,19] The resulting packed columns were used in the subsequent HPLC experiments.

Apparatus and Chromatography. The FTIR spectra were recorded in the range, 400-4000 cm^{-1}, with 4 cm^{-1} resolution with a BRUKER TENSOR27 system (Bruker Scientific Technology Co. Ltd., Karlsruhe, Germany). The samples were examined by thermogravimetric analysis (TGA, SDT Q600, TA Instruments-Waters LLC, USA). Elemental analysis was performed using an automatic elemental analyzer (Vario EL, Elementar Analysensysteme GmbH, Germany).

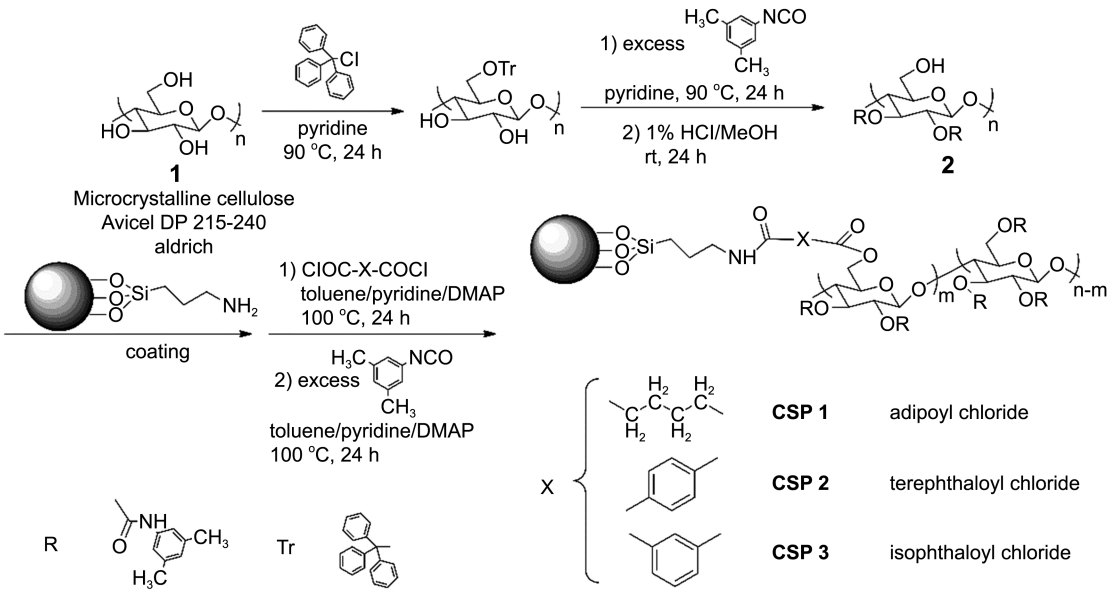

Figure 2. Synthetic procedure of three bonded CDMPCs, **CSP 1-3**.

HPLC was performed on a Agilent 1100 HPLC system (Agilent Technologies, Inc., Walbronn, Germany) equipped with a quaternary pump, vacuum degasser module, Rheodyne 7725i injector with a 20 μL sample loop, a temperature controlled column compartment and a variable wavelength UV detector. Chiralpak IB (250 mm L × 4.6 mm I.D., 5 μm) was purchased from the Daicel Chemical Company (Tokyo, Japan).

The sample solution was prepared by dissolving the racemates in a hexane/IPA (90/10, v/v) mixture. The column was 25 cm long with an internal diameter of 0.46 cm, hexane/IPA or hexane/IPA/THF or hexane/IPA/chloroform were used as the mobile phase, the flow rate was 1.0 or 0.5 mL/min at 20 °C and the detection wavelength was set to 214 or 254 nm for the test solutes. The mobile phases were filtered and sonicated prior to use. The void time of the columns was determined by 1,3,5-tri-*tert*-butylbenzene.

The retention factor (k') was calculated by $(t_R - t_0)/t_0$, where t_R is the retention time of the enantiomers and t_0 is the void time of the column. The separation factor (α) was calculated by $(t_2 - t_0)/(t_1 - t_0)$. The resolution (R_s) was obtained by the expression, $Rs = 2 \times (t_2 - t_1)/(w_1 + w_2)$, where w_1 and w_2 are the peak widths at the baseline of the enantiomers.

Results and Discussion

Preparation of Bonded CDMPC CSP 1-3. Figure 3 shows the TGA curves of the APS, **CSP 1-3** and CDMPC. A considerable change in the thermal behavior of the **CSP 1-3** was observed. The weight loss of CDMPC observed at 250 °C. The weight loss of **CSP 1-3** observed at 200 °C was assigned to the degradation of the grafted CDMPC. This suggests that the CDMPC were immobilized successfully on the surface of APS using the bifunctional reagents method. The bonded amounts of CDMPC (%) for **CSP 1**, **CSP 2** and **CSP 3** were calculated to be 14.11%, 13.65% and 16.57%, respectively, from the TGA data.

In the FTIR spectra of **CSP 1-3**, characteristic peak at 1730 cm^{-1} was found due to the carbonyl group of the bonded-CDMPC. Table 1 summarizes the results of elemental analysis of the APS and **CSP 1-3**. The content of CDMPC in the **CSP1-3** are estimated to approximately 14.27 wt %, 14.65 wt %, 18.39 wt % from elemental analysis (Table 1), respectively.

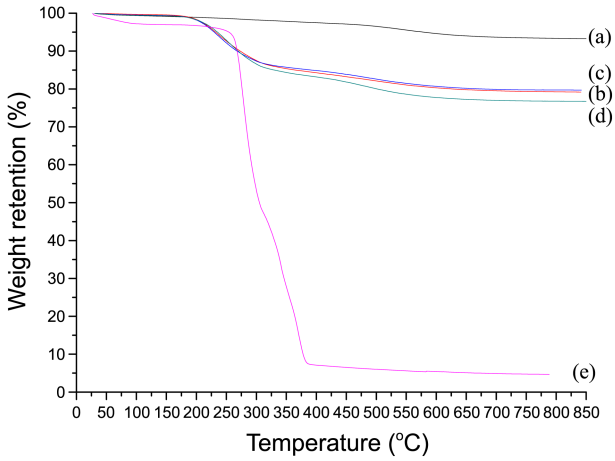

Figure 3. TGA curves for APS, **CSP 1-3** and CDMPC. a, APS; b, **CSP 1**; c, **CSP 2**; d, **CSP 3**; e, CDMPC.

Chiral Resolution of Five Racemic Samples on CSP 1-3. The prepared CSPs were packed in a stainless steel column and the resolution of 5 racemates was using hexane/IPA = 90/10 (v/v) mixture as mobile phase. As shown in Table 2, the chiral recognition ability of commercially available Chiralpak IB was much better than the **CSP 1**,

Table 1. Elemental analysis of APS and **CSP 1-3**

	Carbon (%)	Nitrogen (%)	Hydrogen (%)	CDMPC[a] (%)
APS	3.98	1.29	1.00	-
CSP 1	13.34	2.39	1.49	14.27
CSP 2	13.59	2.44	1.51	14.65
CSP 3	16.04	2.80	2.22	18.39

[a]Amount of CDMPC on APS calculated from the percentage of carbon in CSPs.

Table 2. Chiral resolution of five racemates on the **CSP 1-3** and Chiralpak IB using hexane/IPA = 90/10 (v/v) mixture as mobile phase

Flow rate	Sample	**CSP 1**			**CSP 2**			**CSP 3**			Chiralpak IB		
		k_1'	α	R_s	k_1'	α	R_s	k_1'	α	R_s	k_1'	α	R_s
1 mL/min	S1	2.43	1.79	4.47	2.31	1.81	3.03	2.78	1.63	2.45	1.58	2.33	14.55
	S2	2.49	1.65	2.45	2.58	1.19	1.13	3.33	1.10	0.54	2.13	1.38	5.81
	S3	1.13	1.18	0.91	1.08	1.12	0.50	1.17	1.18	0.63	0.91	1.31	2.50
	S4	0.64	1.31	1.38	0.60	1.31	0.89	0.73	1.00	0.00	0.61	1.73	6.67
	S5	1.99	1.12	0.55	2.10	1.17	0.83	2.25	1.11	0.55	1.45	1.54	7.55
0.5 mL/min	S1	2.39	1.80	4.72	2.77	1.83	3.46	2.69	1.63	2.43	1.54	2.43	17.45
	S2	2.58	1.19	1.42	2.29	1.19	0.98	2.96	1.11	0.56	2.10	1.38	6.44
	S3	1.12	1.18	0.98	0.94	1.12	0.49	1.07	1.19	0.64	0.92	1.28	2.75
	S4	0.63	1.31	1.41	0.50	1.38	1.07	0.70	1.00	0.00	0.59	1.71	6.67
	S5	2.13	1.19	1.39	2.10	1.18	0.91	2.27	1.11	0.59	0.98	1.25	2.48

Column size, 25 cm × 4.6 mm i.d.; eluent, hexane/IPA = 90/10, v/v; temperature, 20 °C; detection, 254 nm or 214 nm.

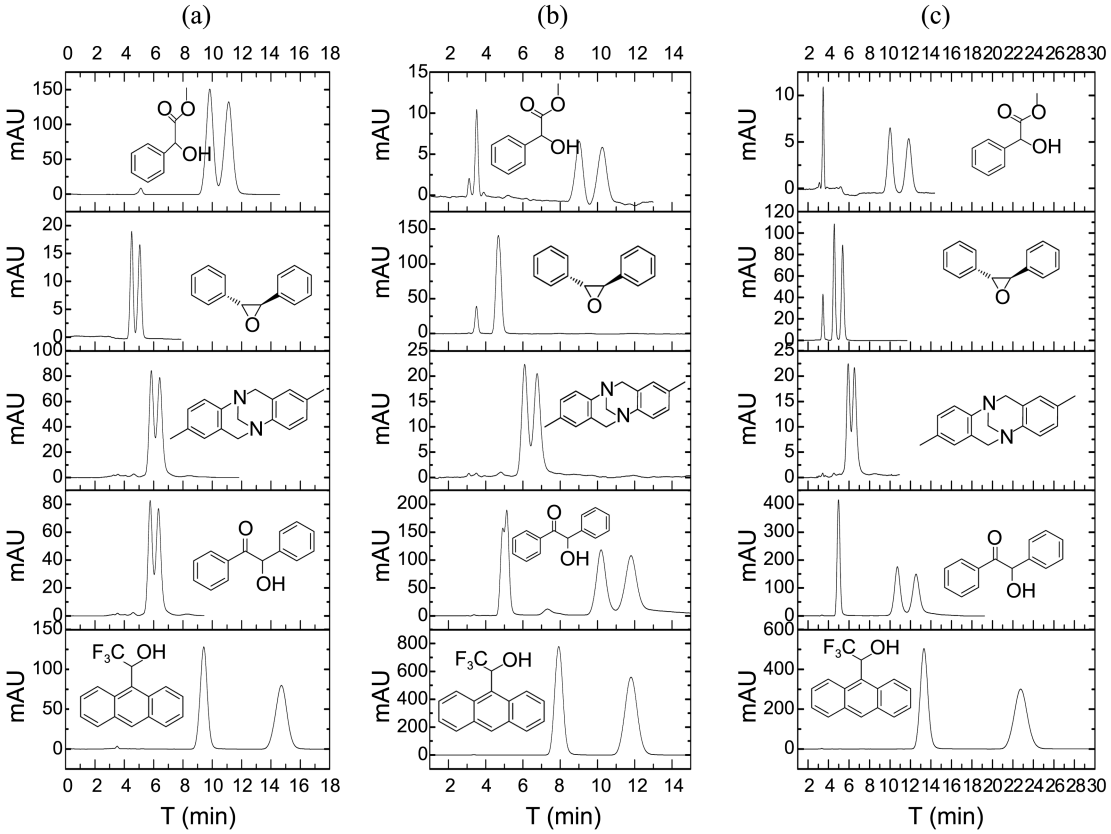

Figure 4. Chiral resolution of five racemates on **CSP 1** with different mobile phases. Flow rate, 1.0 mL/min; temperature, 20 °C; detectio, 254 nm or 214 nm. (a) Hexane/IPA = 90/10 (v/v), (b) hexane/IPA/THF = 90/6/4 (v/v/v), (c) hexane/IPA/Chloroform = 90/6/4 (v/v/v).

CSP 2 and **CSP 3** under the same chromatographic condition. Because the detail procedure and covalent bonding reagent for the synthesis of Chiralpak IB was not reported, we could not assumed the difference of enantioselectivity between Chiralpak IB and our columns. Adipoyl chloride has a flexible carbon chain, while terephthaloyl chloride and isophthaloyl chloride have a rigid benzene ring. It is assumed that the chiral selector can approach to racemic samples more easily because of the flexible adipoyl group. Therefore, **CSP 1** showed slightly better chiral recognition ability than **CSP 2** or **CSP 3**. The reason for the small differences of enantioselectivity between **CSP 1**, **CSP 2** and **CSP 3** could not clearly be verified at this time.

Figure 4(a) presents five chromatograms showing the resolution of 5 racemates on the **CSP 1**. The α of the 5 racemates were determined to be 1.79, 1.65, 1.18, 1.31 and 1.12, respectively.

Table 3 lists the chiral resolution of 5 racemates on **CSP 1-**

Table 3. Chiral resolution of five racemates on the **CSP 1-3** and Chiralpak IB using hexane/IPA/THF and hexane/IPA/Chloroform mixtures (90/8/2, v/v/v) as eluent

Mobile phase	Sample	**CSP 1**			**CSP 2**			**CSP 3**			Chiralpak IB		
		k_1'	α	R_s	k_1'	α	R_s	k_1'	α	R_s	k_1'	α	R_s
Hexane/IPA/THF 90/8/2 v/v/v	S1	2.05	1.82	4.04	2.03	1.83	3.32	2.30	1.66	2.43	1.18	2.29	13.85
	S2	2.71	1.22	1.57	2.56	1.22	1.12	3.04	1.13	0.58	1.97	1.37	5.65
	S3	1.21	1.20	0.98	1.13	1.15	0.56	1.12	1.22	0.65	0.86	1.32	2.84
	S4	0.66	1.19	0.81	0.62	1.22	0.61	0.73	1.00	0.00	0.61	1.35	3.89
	S5	2.23	1.22	1.57	2.18	1.21	1.11	2.18	1.14	0.64	0.89	1.30	2.73
Hexane/IPA/Chloroform 90/8/2 v/v/v	S1	3.04	1.91	4.78	3.07	1.90	3.72	3.48	1.71	2.73	1.83	2.44	16.45
	S2	2.80	1.23	1.62	2.73	1.23	1.15	3.19	1.13	0.62	2.08	1.39	5.93
	S3	1.17	1.18	0.78	1.13	1.14	0.53	1.06	1.21	0.64	0.86	1.28	2.45
	S4	0.64	1.43	1.86	0.61	1.50	1.11	0.70	1.22	0.63	0.60	1.79	7.99
	S5	2.35	1.25	1.79	2.36	1.24	1.20	2.33	1.14	0.65	1.48	1.54	7.94

Column size, 25 cm × 4.6 mm i.d.; flow rate, 1.0 mL/min; temperature, 20 °C; detection, 254 nm or 214 nm.

Table 4. Chiral resolution of five racemates on **CSP 1** using various hexane/IPA/THF mixture as mobile phase

Sample	Hexane/IPA/THF (v/v/v)														
	90/2/8			90/4/6			90/5/5			90/6/4			90/8/2		
	k_1'	α	R_s	k_1'	α	R_s	k_1'	α	R_s	k_1'	α	R_s	k_1'	α	R_s
S1	2.05	1.80	3.90	1.80	1.62	3.22	1.79	1.73	3.67	1.82	1.76	3.73	2.05	1.82	4.04
S2	2.66	1.22	1.56	2.62	1.21	1.53	2.67	1.21	1.50	2.63	1.22	1.55	2.71	1.22	1.57
S3	1.18	1.20	1.03	1.18	1.20	1.10	1.17	1.20	1.03	1.17	1.20	1.04	1.21	1.20	0.98
S4	0.66	1.14	0.62	0.64	1.00	0.00	0.65	1.00	0.00	0.67	1.00	0.00	0.66	1.19	0.81
S5	2.79	1.16	1.21	2.38	1.18	1.36	2.27	1.19	1.39	2.23	1.20	1.42	2.23	1.22	1.57

Column size, 25 cm × 4.6 mm i.d.; flow rate, 1.0 mL/min; temperature, 20 °C; detection, 254 nm or 214 nm.

Table 5. Chiral resolution of five racemates on **CSP 1** using various hexane/IPA/Chloroform mixture as mobile phase

Sample	Hexane/IPA/Chloroform (v/v/v)														
	90/2/8			90/4/6			90/5/5			90/6/4			90/8/2		
	k_1'	α	R_s	k_1'	α	R_s	k_1'	α	R_s	k_1'	α	R_s	k_1'	α	R_s
S1	8.72	2.00	5.99	4.92	1.94	5.31	4.28	1.94	5.29	3.76	1.90	4.96	3.04	1.91	4.78
S2	3.67	1.23	1.59	2.96	1.23	1.69	2.95	1.23	1.59	2.83	1.23	1.61	2.80	1.23	1.62
S3	1.37	1.17	0.83	1.13	1.19	0.87	1.11	1.19	0.83	1.11	1.19	0.87	1.17	1.18	0.78
S4	0.68	1.63	2.69	0.63	1.50	2.14	0.63	1.52	2.19	0.63	1.47	2.00	0.64	1.43	1.86
S5	3.82	1.24	1.91	2.86	1.25	1.87	2.68	1.26	1.87	2.58	1.25	1.86	2.35	1.25	1.79

Column size, 25 cm × 4.6 mm i.d.; flow rate, 1.0 mL/min; temperature, 20 °C; detection, 254 nm or 214 nm.

3 and Chiralpak IB using hexane/IPA/THF and hexane/IPA/chloroform mixtures (90/8/2, v/v/v) as eluent. As a result, the prepared CSPs can be used as eluents in THF and chloroform solutions. These results suggest that three bifunctional reagents, adipoyl chloride, terephthaloyl chloride and isophthaloyl chloride, are valuable for the efficient immobilization of CDMPC onto APS without losing their chiral recognition ability.

Tables 4 and 5 summarize the chiral resolution of the 5 racemates using various ratio of hexane/IPA/THF and hexane/IPA/chloroform mixtures as the mobile phase on **CSP 1**, respectively. As shown in Tables 4 and 5, when utilizing the eluents containing THF and chloroform, the R_s values of **S1**, **S4** and **S5** were increased significantly when the eluent was changed from hexane/IPA/THF to hexane/IPA/chloroform at the same ratio. Figure 4(b) presents a chromatogram showing chiral resolution of the 5 racemates on the **CSP 1** with hexane/IPA/THF as mobile phase. Figure 4(c) shows a chromatogram highlighting the chiral resolution of the 5 racemates on the **CSP 1** with hexane/IPA/chloroform as the mobile phase.

Conclusion

Regioselectively-bonded CDMPC **CSP 1-3** was prepared using the bifunctional reagents, adipoyl chloride, terephthaloyl chloride, isophthaloyl chloride as spacer reagents for effective enantioseparation. The prepared **CSP 1-3** exhibited effective chiral recognition for the tested racemic compounds with a range of mobile phases of hexane /IPA, hexane/IPA/THF and hexane/IPA/chloroform by HPLC. When these eluents were used, the chiral recognition ability of the CSPs prepared for the tested racemates were improved to some extent depending on the compounds.

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