Synthesis of Chemically Bonded Cellulose Trisphenylcarbamate Chiral Stationary Phases for Enantiomeric Separation

Xiaoming Chen, Hanfa Zou*, Qiang Zhang, Jianyi Ni, and **Zengzi Zhang**

National Chromatographic Research & Analysis Center, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian 116011, China

Abstract

Cellulose trisphenylcarbamate is regioselectively bonded to 3-aminopropyl silica gel and underivatized silica gel, respectively, at the 6-position of the primary hydroxyl group on the glucose unit of cellulose with 4,4'-diphenylmethane diisocyanate (DPDI) as a spacer. Enantioseparations are evaluated on these prepared chiral stationary phases (CSPs) with several organic acids as the modifiers in the mobile phase by high-performance liquid chromatography. The influence of the amount of DPDI used on chiral resolution is investigated. Also, the corresponding coated-type phase is also prepared for the aim of comparison. It is observed that the bonded-type phase shows a lower chiral recognition power but a better column efficiency than the coated-type phase under the liquid chromatographic mobile phase with hexane–alcohol. However, the bonded-type CSPs are compatible with a wider number of solvents such as tetrahydrofuran (THF) or chloroform, which generally result in the solubility or swelling of the cellulose derivatives on the coated-type CSPs. The results obtained from this study indicate that the bonded-type CSP may provide complementary enantioselectivity over the coated-type phase by adopting THF as a component in the mobile phase.

Introduction

The optical resolution of enantiomers has been attracting much attention in various fields in the past decades, and liquid chromatography (LC) for enantioseparations has developed rapidly during the past years either for determining optical purity or for the purification of optical isomers (1). Cellulose derivatives have been known and used as one of the most popular chiral stationary phases (CSPs) for the chromatographic separation of optical isomers (2,3), with three different forms in LC. Initially, cellulose derivatives were used directly with a polymeric form after being swollen in boiling alcohols, but their chiral recognition abilities were substantially reduced once they were dissolved in a solvent (4). Then, Okamoto et al. (5) first developed a method to coat the cellulose derivatives on macroporous silica gel, which showed a higher chiral recognition and a good column performance for the separation of enantiomers. These kinds of coated-type phases were developed very quickly, and many of them are commercially available. Recently, we have coated the cellulose derivatives on underivatized silica gel in place of derivatized silica as CSPs for separating a series of α-alkyl phenyl acetonitriles (6,7). Although a very large range of racemates were resolved on the coated-type phases, the solubility of cellulose derivatives in a number of solvents limited the application of coated-type phases. Thus, a third form was developed for the synthesis of bonded-type phases by chemically bonding cellulose derivatives to silica gel, which has been reported by Okamoto et al. (8,9) and other researchers (10–12). In principle, the advantage of the bonded-type phases over the coated-type phases is to eliminate the solubility of cellulose derivatives in the mobile phases, and the number of solvents used for the mobile phases is greatly extended, which may provide better enantioselectivity on a bonded-type phase than the corresponding coated-type CSP (8). In our previous report (13), we have successfully synthesized a bonded cellulose trisphenylcarbamate (CTPC), CSP, for the separation of enantiomers by means of a bisfunctional reagent originally developed by Okamoto et al. (9). According to our results, it was found that the CTPC that was regioselectively bonded to silica gel at the 6-position possessed a better chiral recognition than that that was either regioselectively bonded at the 2- and 3-positions or nonregioselectively bonded at the 2-, 3-, and 6-positions.

In this study, CTPC was chemically bonded to 3-aminopropyl silica gel (APS) and underivatized silica gel (SI), respectively, at the 6-position. The influence of the amount of 4,4'-diphenylmethane diisocyanate (DPDI) used on enantioseparations was investigated. Also, the chiral discrimination on the bonded-type phases with ASP and SI supports and the coated-type phases was also discussed.

* Author to whom correspondence should be addressed: email zouhfa@mail.dlptt.ln.cn.

Experimental

Chemicals

Microcrystalline cellulose was obtained from Serva (Heidelberg, Germany). Spherical silica gel (5 µm, 300 Å) was purchased from Chrom Expert Company (Sacramento, CA). Triphenylmethyl chloride, 3-aminopropyltriethoxysilane, and phenyl isocyanate were purchased from Sigma-Aldrich Company (Gillingham, Dorset, U.K.). DPDI was obtained from TCI Company (Tokyo, Japan). Other reagents were of analytical grade. The racemic compounds of *trans*-stilbene oxide (a), benzoin (b), warfarin (c), and praziquantel (d) were purchased from Sigma Company (St. Louis, MO).

Preparation of the bonded type of CTPC–CSPs at the 6-position

The APS-bonded phases were prepared according to the following procedures. Dried microcrystalline cellulose (3.0 g) and triphenylmethyl chloride (10.0 g) were added into the solution of pyridine (60.0 mL) and reacted at 90°C for 30 h. Then, phenyl isocyanate (10.0 mL) was added to the carbamate residue and was allowed to react for another 30 h, and the solution was poured into a large flask with methanol (300 mL) containing a small amount of hydrochloric acid and stirred for 24 h. The suspending solution was filtered, and the solid substance was collected after being dried. The solid substance (0.48 g) was taken to dissolve in tetrahydrofuran (THF) and coated onto APS (2.0 g) as described previously (13), which was prepared by the reaction of 3-aminopropyltriethoxysilane (15 mL) with silica gel (10 g) in dry toluene (50 mL) refluxed for 12 h. Then, the cellulose derivative on silica gel was dispersed in a mixture of dried toluene (10.0 mL) and pyridine (2.0 mL) containing different amounts of DPDI (40 mg of CSP1, 25 mg of CSP2, and 14 mg of CSP3), and was allowed to react at 90°C for 5 h. Then, an excess of phenyl isocyanate (3.0 mL) was added and allowed to react at 100°C for another 24 h. The product was collected by centrifugation and was washed by THF completely. Thus, CSP1, CSP2, and CSP3 were obtained, respectively, with different amounts of DPDI. The scheme for the synthesis of the chemically bonded CSPs is presented in Figure 1.

The SI-bonded stationary phase (CSP4) was prepared by the same procedures for the preparation of CSP3 as described previously, except that only SI was used instead of APS.

Preparation of the coated type of CTPC–CSP (CSP5)

The CTPC derivative was synthesized according to previous reports (13,14). The dried microcrystalline cellulose was refluxed for 12 h in dry pyridine. Then, 3.5 equivalents of phenyl isocyanate were added and the mixture was refluxed for a further 48 h. The cooled solution was washed completely by methanol and the solid was dried under vacuum, thus CTPC was acquired. Then, the synthesized CTPC was dissolved in THF and coated onto SI at a level of 20% (w/w) by evaporation, thus the coated type of CSP5 was achieved.

Apparatus and chromatographic conditions

The LC experiments were performed with a Waters 510 pump (Waters, Milford), a Spectra-200 UV detector (Spectra-

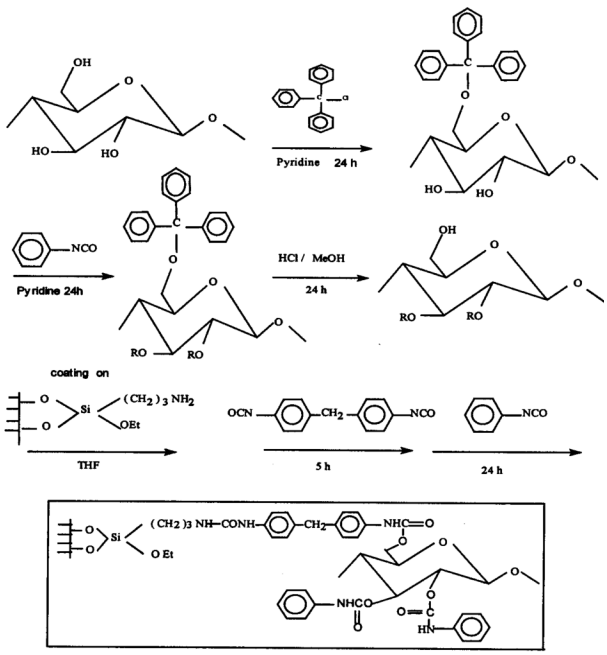

Figure 1. Scheme for the synthesis of a cellulose derivative regioselectively bonded at the 6-position to APS.

Table I. Preparation and Characterization of CSPs

Stationary phase*	Matrix	DPDI (mg)	Ratio† (%)	Void time‡ (min)	Elemental analyses			Surface coverage§
					C (%)	N (%)	H (%)	
CSP1	APS	40	8.3	3.41	6.14	1.02	0.91	1.65
CSP2	APS	25	5.2	3.24	5.58	0.97	0.89	1.46
CSP3	APS	14	2.9	3.58	5.04	0.91	0.78	1.37
CSP4	SI	14	2.9	3.52	2.95	0.67	0.41	1.04
CSP5	SI	–	–	3.56	9.92	1.15	0.82	–
APS	–	–	–	–	1.76	0.75	0.38	3.40

* Nonend-capped.
† Weight percentage of DPDI used based on the weight of CBPC.
‡ A flow rate of 0.5 mL/min was used.
§ Based on the percentage of nitrogen (µmol/m²).

Physics, San Jose, CA), and a WDL-95 workstation (National Chromatographic R&A Center, Dalian, China). The CSPs were dispersed in a solution of hexane–ethanol (50:50, v/v) and packed into stainless-steel columns (150- × 4.6-mm i.d.) by a slurry packing technique, respectively.

All of the separations were performed at ambient temperature, the sample solution was prepared by dissolving racemates in 2-propanal, and 10 µL of the sample solution was injected for the separation runs, respectively. The detection wavelength was set at 254 nm for the test solutes, with the exception of solute c at 280 nm. The mobile phases were filtered and sonicated prior to use. The void times of the prepared columns were determined by the marker of 1,3,5-tri-*tert*-butylbenzene; the data are listed in Table I (a flow rate of 0.5 mL/min was typically employed).

Chromatographic calculations

The retention factors (k'_1 and k'_2) of all of the solutes were calculated with $(t_1 - t_0) / t_0$ and $(t_2 - t_0) / t_0$, the separation factors (α) as k_2/k_1, and the resolution (Rs) as $2(t_2 - t_1) / (w_{1/2(1)} + w_{1/2(2)})$, respectively, in which t_1 and t_2 refer to the retention times for the first and second eluting enantiomers, t_0 refers to the void time of the column, $w_{1/2(1)}$ and $w_{1/2(2)}$ are the peak width at half height for the first and second eluting enantiomers, and N_1 is the plate number of the first eluting peak of the enantiomer.

Results and Discussion

Characterization of the prepared CSPs

Yashima et al. (8) have investigated the influence of the positions of chemical bonding on enantioselectivity when the cellulose 3,5-dimethylphenylcarbamate (CDMPC) was bonded to the APS. It was found that the polysaccharide derivatives that were regioselectively bonded to silica gel at the 6-position of the glucose unit possessed a higher chiral recognition than that that was either regioselectively bonded at the 2- or 3-positions or nonregioselectively bonded at the 2-, 3-, or 6-positions. In our experiments, CTPC was chemically bonded to APS and SI at the 6-position by using the similar procedures developed by Yashima et al. (8,9). The obtained products were characterized by an FT-IR spectrum. For the peaks resulting from the carbonyl group at approximately 1710 cm^{-1} and the phenyl group at approximately 1550 cm^{-1}, 1615 cm^{-1} was observed, which indicated that the cellulose derivatives were successfully fixed to the silica surface. Elemental analyses of the base aminopropyl material and the prepared CSPs were carried out, and the obtained results as well as the crude estimation of the chiral moiety are shown in Table I. For the material APS, the amino groups bound to the silica surface was approximately at 3.4 µmol/m^2, which was less than half of the silanol groups on the silica gel (approximately 8 µmol/m^2) according to the report by Feibush (15). Therefore, more than half of the free silanol groups on the silica gel remained after the aminopropylation step. The surface coverage of the cellulose derivative on the bonded-type phases was less than 1.7 µmol/m^2, which suggests that only half of the free primary amino groups on ASP reacted with the cellulose derivative after the chiral-bonding step.

Effect of DPDI on Rs

Different amounts of DPDI (from 14 to 40 mg) were used for the preparation of the APS-bonded CSPs (as listed in Table I). The chromatographic data for the enantiomeric separation of solutes a and c are presented in Table II. It can be seen that the k and α values of enantiomers solutes a and c increase with the reduction of DPDI used, which indicates that the amount of DPDI used for the synthesis of CSPs may play an important role on enantioseparation. As is known, the principal reaction in this bisfunctional method was that DPDI reacted with the hydroxyl group of cellulose and the silanol group, which was expected to be responsible for the enantioseparation. However, according to our results, the higher the amount of DPDI used, the lower the enantioselectivities and k values were achieved (as shown in Table I). This may be caused from the side reactions such as cross-linking between cellulose (via hydroxyl groups) as well as between silica gel (via amino groups). These reactions might be competitive up to the actual experimental circumstance, especially the amount of DPDI adopted. With the increment of the amount of DPDI used, however, side reactions might occur, which was disadvantageous for cellulose derivative bonded to the silica. Although the amount of DPDI was increased, the real amount of CTPC that was bonded to silica gel might decrease because of the side reactions, thus the capacity factor and selectivity decreased instead. However, it should be taken into account that the possible by-products would be adsorbed on the synthesized CSPs because of their insolubilization in solvents, thus they might affect the molecular recognition interaction between the solutes and CSPs.

Chromatographic discrimination between APS-bonded (CSP3) and SI-bonded (CSP4) stationary phases

The chromatographic data of the separation of the four solutes on CSP3 and CSP4 under different chromatographic conditions are presented in Table III. It can be found that a good Rs of the test racemates was obtained on both phases. The

Table II. Effect of the Amount of DPDI Used for the Synthesis of the Bonded Phase on the Rs of Solutes a and c*

Stationary phase	Solute a			Solute c		
	k'_1	k'_2	α	k'_1	k'_2	α
CSP1	0.66	0.86	1.29	0.84	1.17	1.41
CSP2	0.87	1.14	1.31	1.03	1.45	1.42
CSP3	0.90	1.22	1.36	1.07	1.55	1.45

* The mobile phase for solute a was hexane–2-propanol (99:1, v/v) and for solute c hexane–1-propanol–trifluoroacetic (85:15:1, v/v/v). The flow rate was 0.5 mL/min.

α values of the neutral compounds (solutes a, b, and d) on CSP3 and CSP4 were very close under the corresponding mobile phases. However, for the weak acidic solute c, the α values were quite different; that is to say, the α and Rs values of solute c on CSP3 were higher than on CSP4 when the C1 in Table III was used as the mobile phase. This may be caused from the nonstereoselective interaction between the acidic solute and the acidic silanol groups on CSP4, which is stronger than that on CSP3 because CSP4 has a shorter spacer and higher density of the residual silanol groups. Thus, a lower enantioselectivity of solute c on CSP4 resulted. However, for the neutral solutes the nonstereoselective interaction on both CSPs was weak, thus no significant chiral discrimination was observed on CSP3 and CSP4.

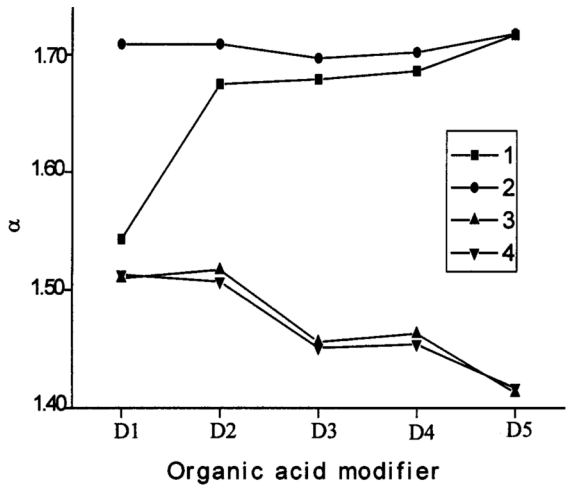

Figure 2. Effect of organic acid modifiers on the α values of solutes c and d on CSP3 and CSP4: trifluoroacetic, D1; *n*-hexoic acid, D2; trichloroacetic acid, D3; acetic acid, D4; and formic acid, D5. The α values of solute b on CSP3 and CSP4 are shown as 1 and 2, and the α values of solute d on CSP3 and CSP4 are shown as 3 and 4, respectively. Other chromatographic conditions are the same as described in Table II.

However, when a small amount of organic acid was employed as mobile phase modifiers from D1 to D5 (see Table III), respectively, the values of the α and Rs of solute c on the two kinds of CSPs generally showed a reverse trend, especially when trichloroacetic acid (D1) was used as a mobile phase modifier. The effect of organic acids in the mobile phases on the separation of solutes c and d are presented in Figure 2. It is very clear that by the addition of a small amount of organic acids to the mobile phase, the separation of solute c on CSP4 was better than on CSP3. However, for solute d no significant change was observed between CSP3 and CSP4 under the corresponding mobile phase modifiers from D1 to D5, which may suggest that the organic acids provide a more effective contribution to the chiral Rs of the acidic solute than to the neutral solute. It may be explained by the possibility that the acidic modifiers may depress the dissociation of acidic solute and weaken the adsorption between the acidic solute and the remaining acidic silanol groups on the SI-bonded phase, thus decreasing the nonstereoselective interactions between the acidic solutes and the CSP (better Rs may then result). However, from the experimental data it could be said that the SI is also a suitable material for synthesizing the CTPC-bonded stationary phase in place of derivatized silica traditionally used. In addition, it shows more simply to be prepared because the silanization of silica gel is avoided.

Table III. Chromatographic Data from the Rs of Four Solutes on the Prepared CSP3 and CSP4

		Stationary phases							
		CSP3				CSP4			
Sample	Mobile phase*	k'_1	k'_2	α	Rs	k'_1	k'_2	α	Rs
a	A1	1.11	1.56	1.41	5.05	0.67	0.95	1.41	3.67
	A2	0.90	1.19	1.32	4.04	0.50	0.69	1.37	3.62
b	B1	6.58	7.39	1.12	2.75	4.05	4.49	1.11	2.48
	B2	4.96	5.38	1.09	–	3.39	3.63	1.07	–
c	C1	3.05	4.52	1.48	7.48	2.32	3.20	1.38	6.26
	D1	1.35	2.08	1.54	2.34	0.85	1.45	1.71	2.48
	D2	1.39	2.32	1.68	4.21	0.87	1.48	1.71	4.31
	D3	1.33	2.23	1.68	2.97	0.81	1.38	1.70	3.28
	D4	1.36	2.29	1.69	3.84	0.83	1.41	1.70	4.67
	D5	1.31	2.25	1.72	4.43	0.79	1.35	1.72	4.37
d	D1	6.49	9.81	1.51	3.39	4.27	6.46	1.51	3.52
	D2	5.97	9.06	1.52	3.90	3.83	5.77	1.51	3.78
	D3	5.78	8.42	1.47	3.60	3.71	5.39	1.45	3.55
	D4	6.19	9.06	1.46	3.34	3.88	5.65	1.45	3.66
	D5	5.73	8.10	1.41	3.50	3.37	4.77	1.42	3.22

* A1 is hexane–2-propanol (100:1, v/v); A2 is hexane–2-propanol–formic acid (96:4:0.1, v/v); B1 is hexane–tetrahydrofuran–2-propanol (95:5:1, v/v); B2 is hexane–tetrahydrofuran–2-propanol–formic acid (95:5:1:1, v/v); C1 is hexane–1-propanol–acetonitrile (82:15:3, v/v); and D1–D5 are hexane–2-propanol–tetrahydrofuran–organic acids (70:30:4:1, v/v) (D1 is triflouroacetic acid, D2 is *n*-hexoic acid, D3 is trichloroacetic acid, D4 is acetic acid, and D5 is formic acid). The flow rate is 0.5 mL/min.

Comparison of chiral separation between the coated and APS-bonded phases

Table IV summarizes the results for the Rs of racemic compounds (solutes a and c) on the bonded-type (CSP3) and coated-type phase (CSP5), respectively. It can be seen from Table IV that the k and α values of the two racemates on the coated-type phase are higher than those on the bond-type phase with the mobile phases designated in Table IV as A and B, and the values of Rs and N_1 show a reverse order. This means that the coated stationary phase possesses a higher chiral recognition ability but a lower column efficiency than the bonded phase. In order to compare the column efficiency between CSP3 and CSP5 with similar k values for each of the test solutes, the mobile phases designated in Table IV as C and D were also employed on CSP5 for the enantioseparation of solutes a and c, respectively. In this case, the k values of

solutes a and c were close on the two types, and again a higher column efficiency was observed on the bonded-type phase (CSP3) (as described in Table IV).

It has also been reported that the bonded type of cellulose CDMPC (CDMPC–CSP) showed a lower chiral recognition than the coated-type phases (8). This is explained by the fact that the cellulose carbamate derivative possesses the conformation of a left-handed threefold (3/2) helix, which plays an important role for the separation of chiral compounds. During the synthesizing procedures of chemical binding, however, the helix structure might be damaged because of the intervening of the chemical bonding. Therefore, the steric environment of the chiral cavities in CSP might be changed disadvantageously and the chiral recognition ability of CSP decrease to some extent.

However, as is known, cellulose derivatives can be swollen or dissolved in some solvents, such as $CHCl_3$ and THF. Therefore, these solvents cannot be used as a component of the mobile phase on coated-type phases, although bonded-type phases do not have this limitation and these solvents might affect enantioseparations dramatically once they are added to a mobile phase. For example, Yashima et al. (8) have reported that 2-phenylcyclohexanone and lavanone, which could not be separated on the coated amylose CDMPC (ADMPC-CSP) under normal-phase conditions, were almost completely resolved on the ADMPC-bonded-type column when a small amount of $CHCl_3$ was added into the mobile phase. In our study, similar phenomena were also found on the CTPC-bonded phase. For example, Figure 3 shows the chromatograms for the separation of solute b. It can be seen that solute b could not be separated on either the CSP5 (I) or CSP3 (II) under normal-phase conditions with hexane–2-

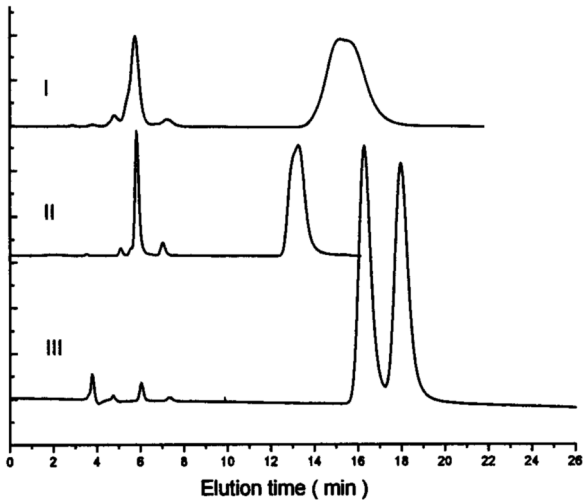

Figure 3. Chromatograms for the optical resolution of solute b on the prepared CSPs: (I) a CSP5 column with a hexane–2-propanol (90:10, v/v) mobile phase; (II) a CSP3 column with a hexane–2-propanol (90:10, v/v) mobile phase; and (III) a CSP3 column with a hexane–tetrahydrofuran–2-propanol (95:5:1,v/v/v) mobile phase. The flow rate was 0.6 mL/min.

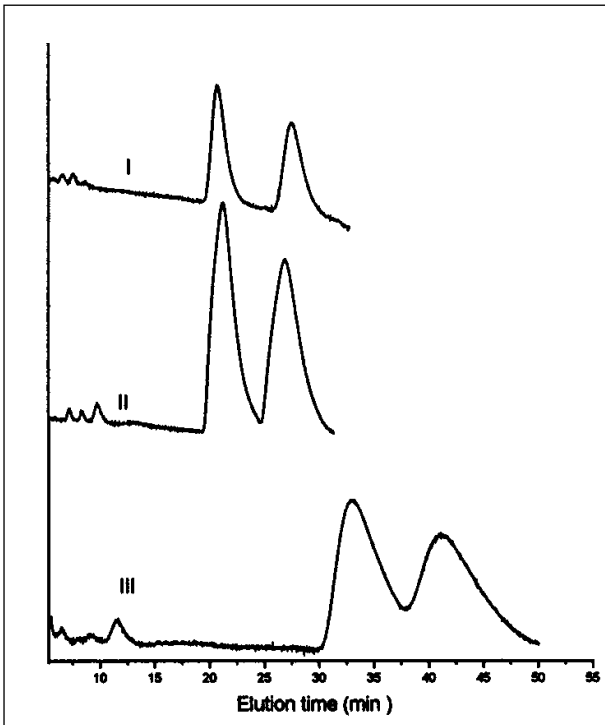

Figure 4. Chromatograms for the optical resolution of solute d on the prepared CSPs: (I) a CSP3 column with a hexane–2-propanol–tetrahydrofuran–trifluoroacetic acid (70:30:4:1, v/v/v) mobile phase and (II) a CSP3 column and (III) a CSP5 column with a hexane–2-propanol–trifluoroacetic acid (70:30:1, v/v/v) mobile phase. The flow rate was 0.6 mL/min.

Table IV. Chromatographic Data from Optical Resolution of Solutes a and c on the Prepared CSP3 and CSP5

CSP	Solute	Mobile phase*	k'_1	k'_2	α	Rs	N_1†
CSP3	a	A	0.81	1.06	1.31	3.90	31,080
	c	B	1.86	2.47	1.33	5.18	10,913
CSP5	a	A	1.44	2.02	1.40	3.11	8,360
	c	B	2.53	3.70	1.46	3.85	6,593
CSP5	a	C	0.87	1.20	1.38	2.98	10,860
	c	D	1.91	2.69	1.41	3.67	8,700

* A is hexane–2-propanol (98:2, v/v); B is hexane–1-propanol–acetonitrile (85:15:3, v/v); C is hexane–2-propanol (90:10, v/v); D is hexane–1-propanol–acetonitrile (75:25:3, v/v/v). The flow rate is 0.5 mL/min.
† Plates/m.

propanol as the mobile phase, but it almost completely resolved on the CSP3 (III) when hexane–THF–2-propanol (95:5:1,v/v/v) was used as the mobile phase. Another example was the Rs of solute d on CSP5 (III) and CSP3 (II) with a mobile phase of hexane–2-propanol, respectively (the obtained chromatograms are shown in Figure 4). It can be observed that solute d was partially resolved with low column efficiency under normal-phase LC conditions. However, it is completely separated with much higher column efficiency on the CSP3 (I) by using THF as a component of the mobile phase. These results clearly indicate that THF added into the mobile phase plays a role for the Rs of racemates on the bonded-type phase. This might be ascribed to the change of CTPC conformation in some degree, because CTPC will be swollen by THF. In addition, dichloromethane, chloroform, and *N*,*N*-dimethylacetamide have also been used as a component of the mobile phases to investigate their effect on the Rs of enantiomers, respectively, but no significant results were obtained.

Conclusion

CTPC was successfully fixed to the APS and SI with DPDI as a spacer reagent. The amount of the spacer reagent used played a role in the performance of bonded-type CSPs for enantioseparations. The CSPs with CTPC bonding to both the underivatized and APS showed similar chiral recognition, but the former showed even better chiral recognition for the acidic solute by the addition of a small amount of organic acidic modifier into the mobile phase. It was also observed that the bonded-type phase showed higher column efficiency but a lower chiral recognition ability than those on the coated-type phase. However, some racemates that were not or poorly separated on the coated CTPC–CSP under the normal-phase LC conditions might be more efficiently resolved on the bonded-type phase by using some polar solvents (such as THF) as a component of the mobile phase.

Acknowledgments

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