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Immobilization and Chiral Recognition of 3,5-Dimethylphenylcarbamates of Cellulose and Amylose Bearing 4-(Trimethoxysilyl)phenylcarbamate Groups

SHOUWAN TANG,[1,2] TOMOYUKI IKAI,[1] MASASHI TSUJI,[1] AND YOSHIO OKAMOTO[1,3]*

[1]*EcoTopia Science Institute, Nagoya University, Furo-Cho, Chikusa-Ku, Nagoya, Japan*

[2]*Department of Chemistry, School of Pharmaceutics and Chemical Engineering, Taizhou College, Linhai, Zhejiang, People's Republic of China*

[3]*College of Material Science and Chemical Engineering, Harbin Engineering University, Harbin, People's Republic of China*

ABSTRACT A small amount of 4-(trimethoxysilyl)phenyl groups was randomly introduced onto the 3,5-dimethylphenylcarbamates of cellulose and amylose by a one-pot method. The obtained derivatives were then effectively immobilized onto silica gel as chiral packing materials (CPMs) for high-performance liquid chromatography through intermolecular polycondensation of the trimethoxysilyl groups. The effects of the amount of 4-(trimethoxysilyl)phenyl groups on immobilization and enantioseparation were investigated. Also, the solvent durability of the immobilized-type CPMs was examined with the eluents containing chloroform and tetrahydrofuran. When these eluents were used, the chiral recognition abilities of the CPMs for most of the tested racemates were improved to some extent depending on the compounds. *Chirality 22:165–172, 2010.*

KEY WORDS: chiral recognition; chiral separation; chiral stationary phase; chiral packing materials; high-performance liquid chromatography; HPLC; immobilization; polysaccharide derivative

INTRODUCTION

Polysaccharide derivatives-based chiral packing materials (CPMs), such as the phenylcarbamates, especially 3,5-dimethylphenylcarbamates **1** and **2** in Figure 1, and benzoates of cellulose and amylose, exhibit high chiral recognition abilities toward a wide range of chiral compounds and have been recognized as the most powerful CPMs for direct separation of enantiomers by high-performance liquid chromatography (HPLC).[1–6] These polysaccharide-based CPMs have been traditionally prepared by coating the derivatives onto macroporous silica gel. Because of their coated nature, solvents such as tetrahydrofuran (THF) and chloroform, which can dissolve or swell the polysaccharide derivatives, cannot be used as eluents. 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 the eluents for these coated-type CPMs. The strict limitation of eluent selection is sometimes a serious problem for the efficient analytical and preparative resolution of enantiomers.[7,8] On the other hand, better resolution,[9,10] reversed elution orders of enantiomers,[11] and higher sample solubility of some racemates[8,12–14] can be achieved by utilizing the prohibited eluents. To confer universal solvent compatibility upon these kinds of CPMs, synthesis of immobilized-type CPMs is a direct approach. This can significantly broaden the choice of solvents as eluents or sample dissolving reagents.

Till now, various interesting methods, such as the bifunctional reagent method using diisocyanate,[15,16] radical polymerization,[17–20] photoirradiation,[21] enzyme-catalyzed polymerization,[22] and so on, have been developed for the immobilization of polysaccharide derivatives onto silica gel. Moreover, Chiralpak IA, Chiralpak IB, and Chiralpak IC (Daicel, Tokyo, Japan), which are composed of immobilized amylose 3,5-dimethylphenylcarbamate, cellulose 3,5-dimethylphenylcarbamate, and cellulose 3,5-dichlorophenylcarbamate, respectively, have been commercialized as CPMs.[23–25]

Recently, we found that polysaccharide 3,5-dimethylphenylcarbamates bearing a small amount of alkoxysilyl groups could be effectively immobilized onto silica gel through intermolecular polycondensation of these groups under an acidic condition.[26–28] For instance, 89% of the cellulose derivative (**3** in Fig. 1) and 86% of the amylose derivative (**4** in Fig. 1) could be immobilized onto silica gel when only 2% and 1% of 3-(triethoxysilyl)propyl groups

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*Correspondence to: Prof. Yoshio Okamoto, EcoTopia Science Institute, Nagoya University, Furo-Cho, Chikusa-Ku, Nagoya 464-8603, Japan; College of Material Science and Chemical Engineering, Harbin Engineering University, 145 Nantong St., Harbin 150001, People's Republic of China. E-mail: okamoto@apchem.nagoya-u.ac.jp

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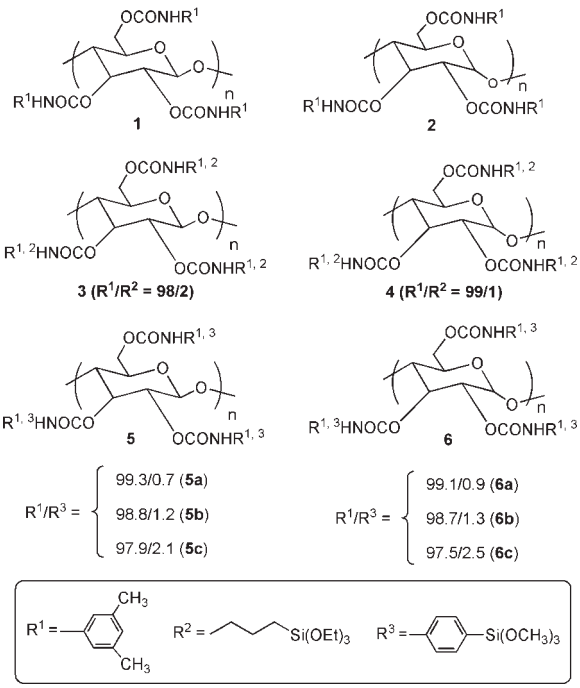

Fig. 1. Structures of cellulose (**1**, **3**, and **5**) and amylose (**2**, **4**, and **6**) derivatives.

were introduced on the derivatives, respectively. The obtained CPMs showed high chiral recognition abilities and universal solvent compatibility.

In the present study, to investigate the influence of the trialkoxysilyl groups on the performance of CPMs, the polysaccharide derivatives **5** and **6** bearing a small amount of 4-(trimethoxysilyl)phenyl groups in Figure 1 were synthesized by a one-pot method and immobilized onto silica gel by intermolecular polycondensation of the trimethoxysilyl groups. The relationships among the amount of 4-(trimethoxysilyl)phenyl groups introduced, immobilization, and enantioseparation of the obtained CPMs were investigated using a hexane/2-propanol mixture as eluent. Also, the chiral recognition abilities of the obtained CPMs were evaluated with the eluents containing chloroform and THF.

EXPERIMENTAL

Materials

Cellulose (Avicel, DP = 200) was purchased from Merck (Darmstadt, Germany). Amylose (DP = 300), 3,5-dimethylphenyl isocyanate, Chiralpak IA, Chiralpak IB, Chiralcel OD, and Chiralpak AD-H (25 × 0.46 cm i.d.) were kindly provided by Daicel Chemical Industries (Tokyo, Japan). The macroporous silica gel (Daisogel SP-1000) with a mean particle size of 7 μm and a mean pore diameter of 100 nm was a gift from Daiso Chemical (Osaka, Japan). The dehydrated solvents such as *N,N*-dimethylacetamide (DMA), pyridine, and THF were obtained from Kanto (Tokyo, Japan). Triethylamine was obtained from Kanto (Tokyo, Japan), anhydrous lithium chloride from Nakalai Tesque (Kyoto, Japan), and 4-aminophenyl trimethoxysilane from Gelest (PA, USA). Triphosgene and trimethylsilyl chloride were purchased from TCI (Tokyo, Japan).

Synthesis of 4-(Trimethoxysilyl)phenyl isocyanate

4-(Trimethoxysilyl)phenyl isocyanate was synthesized from 4-aminophenyl trimethoxysilane and triphosgene by a conventional method, as follows. Triphosgene (2.67 g, 9.0 mmol) was dissolved in 50 ml of dry THF. A mixture of 4-aminophenyl trimethoxysilane (3.84 g, 18 mmol) and triethylamine (5.6 ml, 40 mmol) in dry THF (40 ml) was slowly dropped into the aforementioned solution kept in an ice bath. After the dropping, the temperature was raised to 70°C, and the mixture was allowed to react at 70°C for 2.5 h. After the reaction, triethylhydrogenammonium chloride was removed by filtration, and THF was evaporated from the filtrate. After most of the THF was removed, 4-(trimethoxysilyl)phenyl isocyanate was then collected by distillation under reduced pressure. Yield 1.2 g (28% from 4-aminophenyl trimethoxysilane). IR (cm^{-1}), 2270 (N=C=O); ^{1}H NMR δ (ppm) (300 MHz, $CDCl_3$), 3.60 (9H, s, $Si(OCH_3)_3$), 7.05–7.13 (2H, d, 2 × Ar-*H*), 7.54–7.63 (2H, d, 2 × Ar-*H*). Anal. calcd for $C_{10}H_{13}NO_4Si$: C, 50.19; H, 5.48; N, 5.85; Found: C, 50.07; H, 5.46; N, 6.08.

One-Pot Synthesis of Polysaccharide Derivatives Bearing 4-(Trimethoxysilyl)phenyl Groups

According to the previous procedure,[27] the derivatives of cellulose **5** and amylose **6** bearing 4-(trimethoxysilyl)-phenyl groups were synthesized by a one-pot method as indicated in Figure 2. After cellulose was dissolved in a mixture of DMA/LiCl/pyridine, 3,5-dimethylphenyl isocyanate (0.83 equiv relative to the hydroxyl groups of cellulose) was added, and the mixture was allowed to react at 80°C for 8 h. Various amounts of 4-(trimethoxysilyl)phenyl isocyanate were then added and allowed to react at 80°C for 13 h. Finally, the residual hydroxyl groups of cellulose

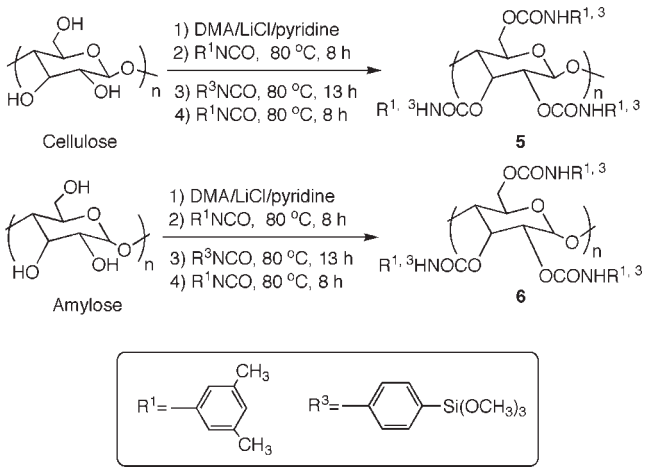

Fig. 2. Scheme of the synthesis of cellulose (**5**) and amylose (**6**) derivatives bearing 4-(trimethoxysilyl)phenyl groups.

were treated with an excess of 3,5-dimethylphenyl isocyanate (0.83 equiv relative to the hydroxyl groups of cellulose) at 80°C for 8 h. The cellulose derivatives **5a–c** were obtained as methanol-insoluble fractions. Also, the amylose derivatives **6a–c** were synthesized by a similar method.

Immobilization of Polysaccharide Derivatives onto Silica Gel

The immobilization of polysaccharide derivatives having 4-(trimethoxysilyl)phenyl groups onto bare silica gel was similarly performed as reported previously.[26–28] In brief, each polysaccharide derivative (0.35 g) in THF (8 ml) was coated onto bare silica gel (1.40 g). The **5**- or **6**-coated silica gel (0.65 g) was then suspended in a mixture of ethanol (6 ml), water (1.5 ml), and trimethylsilyl chloride (0.1 ml) and allowed to react at 110°C for 10 min. After being washed extensively with THF and dried, the **5**- and **6**-immobilized CPMs were obtained. The immobilization efficiency was calculated from the organic contents of the CPMs before and after immobilization estimated by thermogravimetric (TG) analysis.

Apparatus and Chromatography

The 300 and 500 MHz ^{1}H NMR spectra were measured on a Varian Mercury and a Varian INOVA-500 spectrometers, respectively. The TG analysis was performed using a Seiko EXSTRA 6000 system (Seiko, Chiba, Japan). The HPLC experiments were carried out on a Jasco chromatograph (Jasco, Tokyo, Japan) consisting of a multiwavelength detector (Jasco MD-910), a circular dichrosim detector (Jasco CD-1595 YS), an intelligent HPLC pump (Jasco PU-1580), and an intelligent column thermostat (Jasco CO-1560). The obtained CPMs were packed into stainless-steel columns (25 × 0.20 cm i.d.) by a slurry packing method. The dead time (t_0) was determined using 1,3,5-tri-*t*-butylbenzene as the nonretained compound.[29] The plate numbers of the packed columns were about 2000 for benzene using hexane/2-propanol (90/10, v/v) as eluent at a flow rate of 0.1 ml/min. The columns were maintained at 25°C by a column thermostat during the chromatographic experiments.

RESULTS AND DISSUSION

Synthesis of 3,5-Dimethylphenylcarbamates of Cellulose and Amylose Bearing 4-(Trimethoxysilyl)phenyl Groups

Polysaccharide 3,5-dimethylphenylcarbamates containing a small amount of 3-(triethoxysilyl)propyl groups have been prepared by a one-pot method.[26,27] In a similar way, the derivatives of cellulose **5a–c** and amylose **6a–c** bearing 4-(trimethoxysilyl)phenyl residues were synthesized by adding successively 3,5-dimethylphenyl isocyanate, 4-(trimethoxysilyl)phenyl isocyanate, and 3,5-dimethylphenyl isocyanate as indicated in Figure 2. The roles of the first addition of 3,5-dimethylphenyl isocyanate are not only to convert the hydroxyl groups of polysaccharide into phenylcarbamate residues but also to eliminate a small amount of water in the reaction system, which disturbs quantitative reaction of the 4-(trimethoxysilyl)phenyl isocyanate with the hydroxyl groups of polysaccharide. Through this procedure, the content of 4-(trimethoxysilyl)-phenyl groups introduced onto the polysaccharides can be controlled by the amount of isocyanate added.

Six polysaccharide derivatives (see Fig. 1) were synthesized using the one-pot method. The ^{1}H NMR spectra of the obtained cellulose (**5b**) and amylose (**6a**) derivatives in Figure 3 clearly indicate that the introduction of the 4-(trimethoxysilyl)phenyl groups was successful, as shown by the triplet signals (SiOCH_3) at about 3.55–3.70 ppm. The content of 4-(trimethoxysilyl)phenyl groups in **5a–c** and **6a–c** was estimated to be 0.7, 1.2, 2.1% and 0.9, 1.3, 2.5%, respectively. The ratio of (3,5-dimethylphenylcarbamate)/(4-(trimethoxysilyl)phenylcarbamate) (R^1/R^3) was determined from the ratio of (Ar-CH_3)/(SiOCH_3) in the ^{1}H NMR spectra. It should be pointed out that because the peaks of glucose units of the cellulose derivatives overlap those of SiOCH_3 to some extent (Fig. 3a), the R^3 contents of these derivatives calculated by this method may not be as accurate as those of the amylose derivatives. In fact, when the cellulose and amylose derivatives were prepared under the same conditions, the R^3 contents of the cellulose derivatives were always smaller than those of the amylose derivatives. However, the R^3 contents of amylose derivatives as well as those of cellulose derivatives determined by this method were found to be proportional to the amount of 4-(trimethoxysilyl)phenyl isocyanate added.

The derivatives were coated and immobilized onto bare silica gel to obtain the CPMs, and their chiral recognition abilities were evaluated by HPLC using 10 racemates shown in Figure 4.

Effect of 4-(Trimethoxysilyl)phenyl Groups on Chiral Recognition Abilities of Cellulose Derivatives

The 4-(trimethoxysilyl)phenyl groups introduced onto cellulose derivative **1** for the intermolecular polycondensation may significantly influence the chiral recognition ability of **1**. Therefore, 20 wt % of the derivatives **5a–c** were coated onto bare silica gel using a conventional method, and the resolution results of the racemates on these CPMs are shown in Table 1, together with those on the commercially available Chiralcel OD coated with cellulose derivative **1**, having no 4-(trimethoxysilyl)phenyl group. The elution orders for the enantiomers were the same on the **5**-coated CPMs, whereas the α values, which represent the chiral recognition ability, were slightly decreased with the increased content of R^3 in **5**. Compared with Chiralcel OD, slightly smaller α values were observed on the **5**-coated CPMs, except for racemates **7**, **11**, and **16**, whose values were larger. Because the R^3 content of **5a** was smaller than those of **5b** and **5c**, the α values on the **5a**-coated CPM were more similar to those on Chiralcel OD. One point should be mentioned here is that the particle sizes of the silica gels used for the commercially available columns are 10 μm for Chiralcel OD and 5 μm for Chiralpak IA and Chiralpak IB, which are different from that on the CPMs prepared in our experiments with 7 μm silica gels.

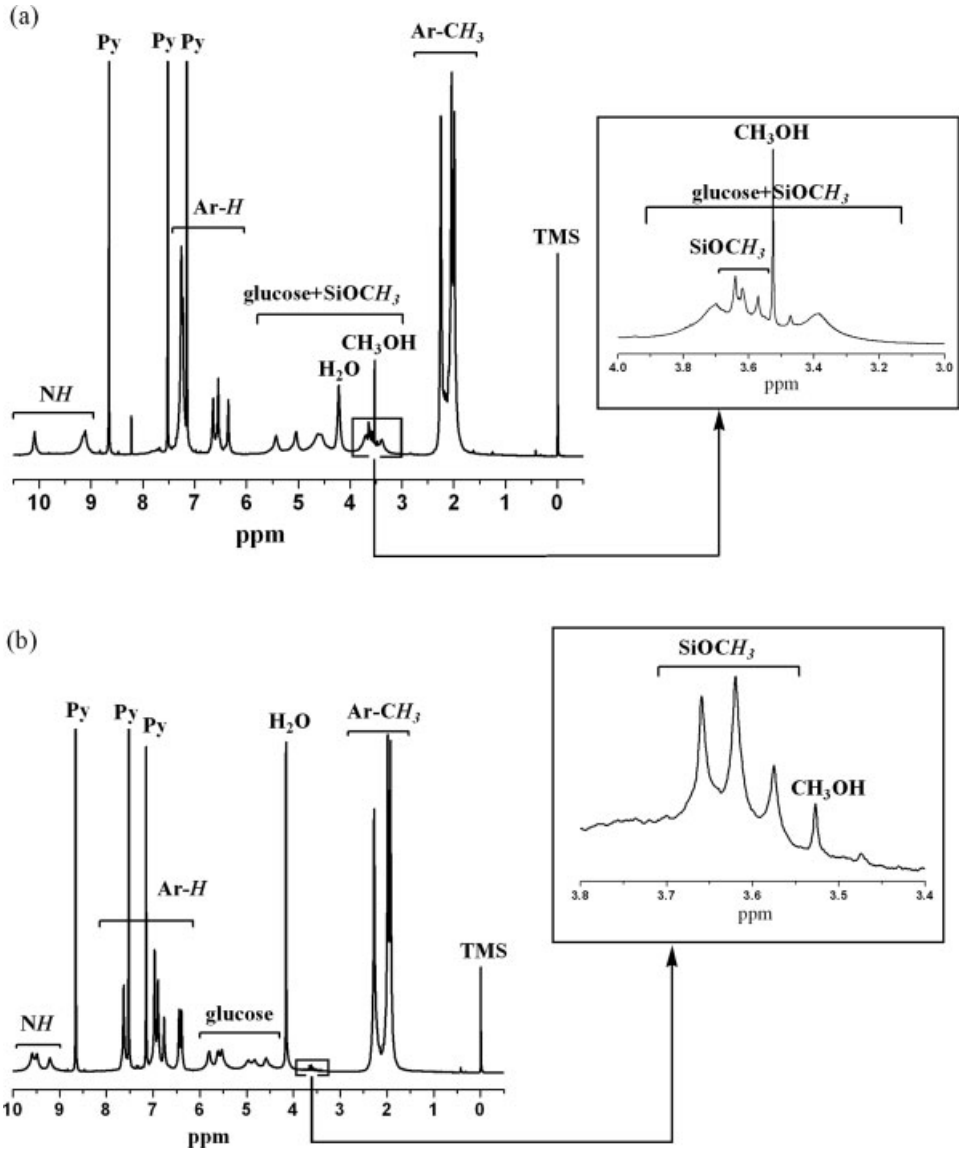

Fig. 3. ^{1}H NMR spectra of the derivatives of (**a**) cellulose **5b** and (**b**) amylose **6a** in pyridine-*d5* at 80°C. Py, pyridine.

Immobilization of Cellulose Derivatives and Their Chiral Recognition Abilities

Figure 5 shows a possible immobilization process. The **5**-coated silica gel was dispersed into a mixture of EtOH/H_2O/$(CH_3)_3SiCl$ and then **5** was immobilized onto the silica gel by heating at 110°C for 10 min.[26–28] After the immobilization, the obtained CPMs were extensively washed with THF to exclude the nonimmobilized derivatives and dried. The immobilization efficiency was estimated by TG analysis. In Table 2 are given the immobilization efficiencies of the cellulose derivatives. The immobilization efficiency increased with an increase in the R^3 content of **5**: 78% for **5a** with 0.7% R^3, 87% for **5b** with 1.2% R^3, and 93% for **5c** with 2.1% R^3. It can be seen that the immobilization method is very efficient; even if the R^3 content was reduced to 0.7% (**5a**), 78% of the cellulose derivative could be immobilized. The immobilization was mainly attained through the intermolecular polycondensation among the trimethoxysilyl groups of **5** as well as the previous study,[27,28] because the immobilization efficiency did not decrease when 3-aminopropyl silica gel was used instead of the bare silica gel. Because of the hydrolysis of $(CH_3)_3SiCl$, HCl was produced in the reaction system and

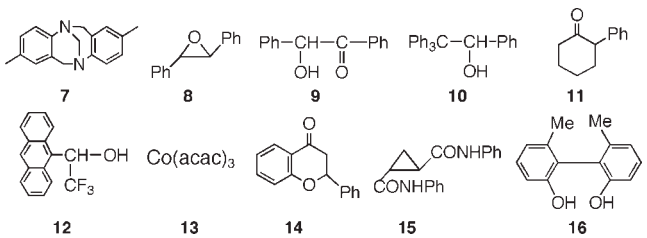

Fig. 4. Molecular structures of racemates.

TABLE 1. Separation results on the 5-coated CPMs and Chiralcel OD

Derivatives (R^1/R^3)[a] Racemates	**5a** (99.3/0.7)		**5b** (98.8/1.2)		**5c** (97.9/2.1)		Chiralcel OD[b,c]	
	k_1'	α	k_1'	α	k_1'	α	k_1'	α
7	0.66 (+)	1.37	0.79 (+)	1.35	0.66 (+)	1.40	1.05 (+)	1.26
8	0.44 (–)	1.76	0.50 (–)	1.67	0.48 (–)	1.44	0.79 (–)	2.11
9	1.46 (+)	1.41	1.71 (+)	1.37	1.52 (+)	1.35	2.44 (+)	1.57
10	0.91 (–)	1.20	1.00 (–)	1.23	0.96 (–)	1.14	1.50 (–)	1.27
11	0.74 (–)	1.21	0.84 (–)	1.21	0.67 (–)	1.22	1.22 (–)	1.14
12	1.08 (–)	2.73	1.26 (–)	2.54	1.09 (–)	2.43	2.29 (–)	2.87
13	0.66 (–)	~1	0.65 (–)	~1	0.64 (–)	~1	0.42 (–)	1.13
14	0.84 (–)	1.30	1.00 (–)	1.23	0.85 (–)	1.22	1.46 (–)	1.40
15	0.66 (+)	2.00	0.83 (+)	1.70	0.72 (+)	1.41	0.91 (+)	2.63
16	1.02 (–)	3.34	1.15 (–)	3.26	1.07 (–)	2.78	3.74 (–)	1.47

Eluent, hexane/2-propanol (90/10, v/v); column size, 25 × 0.20 cm i.d.; flow rate, 0.1 ml/min; temperature, 25°C. The signs in parentheses represent the CD detection (254 nm) of the first-eluted enantiomer.
[a]The ratio of R^1/R^3 was determined from the ratio of the (Ar-C*H*$_3$)/(SiOC*H*$_3$) in the ^{1}H NMR spectrum.
[b]Data from ref. 17.
[c]Column size, 25 × 0.46 cm i.d.; flow-rate, 0.5 ml/min.

the acidic condition was favorable for the immobilization.[27] In addition, the silanol groups on the silica gel surface are expected to be simultaneously end-capped with trimethylsilyl groups during the immobilization process.[26–28]

Figure 6 shows the chromatogram for the resolution of racemic Tröger's base **7** on the **5b**-immobilized CPM with hexane/2-propanol (90/10, v/v) as eluent. The dead time (t_0) was estimated to be 9.35 min. The enantiomers were eluted at retention times t_1 and t_2. The retention factors (k_1', k_2'), which are estimated as $(t_1 - t_0)/t_0$ and $(t_2 - t_0)/t_0$, were determined to be 0.73 and 1.09, respectively, which led the separation factor α (= k_2'/k_1') to be 1.49.

In Table 2 are summarized the resolution results of 10 racemates on the **5**-immobilized CPMs using hexane/2-propanol (90/10, v/v) as eluent. By comparing the resolution results on the **5**-coated and **5**-immobilized CPMs shown in Tables 1 and 2, we can easily recognize the effect of the immobilization process on the chiral recognition abilities of the cellulose derivative **5**. Generally, the α values on **5a**, **5b**, and **5c** were similar before and after immobilization, except for the slightly smaller α values for racemates **8**, **12**, and **15** after immobilization, and the larger values for racemates **7**, **13**, and **16**. These results indicate that this method is valuable for efficient immobilization of the cellulose derivatives onto silica gel without losing their chiral recognition abilities. Therefore, the effect of R^3 groups on the chiral recognition abilities of the immobilized-type CPMs was negligible, when its content was small.

The data for the commercially available Chiralpak IB consisting of the immobilized cellulose 3,5-dimethylphenylcarbamate as the chiral selector are included in Table 2 for comparison. Basically, for racemates **7**, **11**, **13**, and **16**, better resolution was attained on the **5**-immobilized CPMs than on Chiralpak IB. For the rest of the racemates, the **5a**-immobilized CPM showed α values comparable with those of Chiralpak IB, whereas the **5b**- and **5c**-immobilized CPMs showed slightly smaller values. In addition, the α values on CPM **3** immobilized using 3-(triethoxysilyl)propyl residue are listed in Table 2 for comparison with the **5b**-immobilized CPM. Both CPMs were prepared by intermolecular polycondensation of different amounts of trialkoxysilyl residues, and the immobilization efficiencies of the cellulose derivatives were close, being 89% and 87%

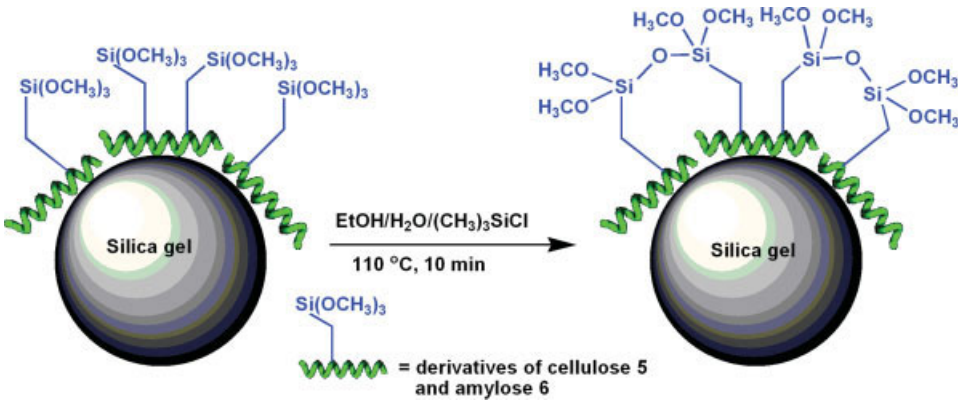

Fig. 5. Scheme of immobilization of polysaccharide derivatives onto silica gel. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

TABLE 2. Immobilization efficiencies and separation results on the 5-immobilized CPMs, Chiralpak IB, and 3-immobilized CPM

Cellulose derivative Racemate	**5a** (R^1/R^3 = 99.3/0.7) [a]78%[b]		**5b** (R^1/R^3 = 98.8/1.2) [a]87%[b]		**5c** (R^1/R^3 = 97.9/2.1) [a]93%[b]		Chiralpak IB[c,d]		**3**[e] (R^1/R^2 = 98/2) 89%[b]
	k_1'	α	k_1'	α	k_1'	α	k_1'	α	α
7	0.59 (+)	1.39	0.73 (+)	1.49	0.79 (+)	1.48	0.96 (+)	1.22	1.53 (+)
8	0.35 (–)	1.64	0.42 (–)	1.34	0.52 (–)	1.28	0.55 (–)	1.77	1.51 (–)
9	1.24 (+)	1.36	1.51 (+)	1.32	1.76 (+)	1.29	2.00 (+)	1.33	1.35 (+)
10	0.72 (–)	1.17	0.90 (–)	1.15	1.02 (–)	1.16	1.12 (–)	1.22	1.15 (–)
11	0.63 (–)	1.21	0.75 (–)	1.26	0.81 (–)	1.26	1.00 (–)	1.14	1.23 (–)
12	0.88 (–)	2.56	1.13 (–)	2.19	1.24 (–)	2.17	1.54 (–)	2.42	2.35 (–)
13	0.71 (–)	~1	0.57 (–)	1.15	0.70 (–)	1.14	3.15 (–)	~1	~1 (–)
14	0.70 (–)	1.24	0.90 (–)	1.20	1.03 (–)	1.17	1.13 (–)	1.26	1.21 (–)
15	0.57 (+)	1.92	0.86 (+)	1.32	0.98 (+)	1.28	0.86 (+)	1.89	1.65 (+)
16	0.74 (–)	4.16	1.16 (–)	3.78	1.28 (–)	3.71	1.48 (–)	2.72	3.52 (–)

Eluent, hexane/2-propanol (90/10, v/v); column size, 25 × 0.20 cm i.d.; flow rate, 0.1 ml/min; temperature, 25°C. The signs in parentheses represent the CD detection (254 nm) of the first-eluted enantiomer.
[a]The ratio of R^1/R^3 was determined from the ratio of the (Ar-CH_3)/(SiOCH_3) in the ^{1}H NMR spectrum.
[b]Immobilization efficiency estimated from TG analysis.
[c]Data from Ref. 17.
[d]Column size, 25 × 0.46 cm i.d.; flow-rate, 0.5 ml/min.
[e]Data from Ref. 27.

for the **3**- and **5b**-immobilized CPMs, respectively. The α values on the **3**- and **5b**-immobilized CPMs were similar except for the slightly smaller values for racemates **8**, **12**, and **15** on the **5b**-immobilized CPM, and slightly larger values for racemates **13** and **16**, indicating that the chiral recognition abilities of these two CPMs are rather similar. These results suggest that the method reported in this study is valuable for immobilizing cellulose derivatives onto silica gel without significantly reducing their chiral recognition abilities.

The effect of eluents on the chiral recognition ability of the **5a**-immobilized CPM was also investigated. The results are shown in Table 3. By utilizing the eluents containing chloroform and THF, which cannot be used for those coated-type CPMs, the α values for most of the racemates were improved to some extent depending on the racemates except for racemates **7**, **15**, and **16**. A typical example is the separation of racemate **12** with eluents containing chloroform. Its k_1' and α values were significantly increased when the eluent was changed from hexane/2-propanol (90/10, v/v; k_1' = 0.88, α = 2.56) to hexane/chloroform/2-propanol (90/10/1, by volume; k_1' = 7.27, α = 3.68). The increased k_1' value may be attributed to the increased H-bonding interactions between the enantiomers of **12** and the **5a**-immobilized CPM resulted from the decreased portion of the H-bonding solvent, 2-propa-

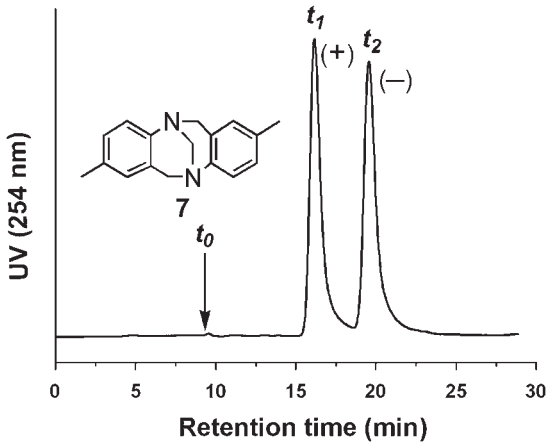

Fig. 6. Chromatographic resolution of **7** on the **5b**-immobilized CPM. Eluent, hexane/2-propanol (90/10, v/v); column size, 25 × 0.20 cm i.d.; flow-rate, 0.1 ml/min; temperature, 25°C. The signs in the parentheses represent the CD detection (254 nm) of the first-eluted enantiomer.

TABLE 3. Separation results on the 5a-immobilized CPM with the eluents containing chloroform and THF

	5a-Immobilized CPM					
	H/I (90/10)		H/C/I (90/10/1)		H/T/I (90/10/1)	
Racemates	k_1'	α	k_1'	α	k_1'	α
7	0.59 (+)	1.39	0.75 (+)	1.31	0.75 (+)	1.31
8	0.35 (–)	1.64	0.43 (–)	2.11	0.43 (+)	~1
9	1.24 (+)	1.36	1.98 (+)	1.41	1.62 (+)	1.34
10	0.72 (–)	1.17	1.30 (–)	1.22	0.74 (–)	1.29
11	0.63 (–)	1.21	0.73 (–)	1.24	0.65 (–)	1.19
12	0.88 (–)	2.56	7.27 (–)	3.68	0.73 (–)	2.71
13	0.71 (–)	~1	0.66 (–)	~1	1.76 (–)	~1
14	0.70 (–)	1.24	0.91(–)	1.29	0.98 (–)	1.27
15	0.57 (+)	1.92	–[a]	–	7.52 (+)	1.73
16	0.74 (–)	4.16	0.95 (–)	1.27	1.02 (–)	1.12

Column size, 25 × 0.20 cm i.d.; temperature, 25°C; flow rate, 0.1 ml/min. The signs in parentheses represent the CD detection (254 nm) of the first-eluted enantiomer. H, hexane; I, 2-propanol; C, chloroform; T, tetrahydrofuran.
[a]Enantiomers were not eluted in 4 h.

TABLE 4. Separation results on the 6-immobilized CPMs, Chiralpak IA, and 4-immobilized CPM

Amylose derivative Racemate	**6a** (R^1/R^3 = 99.1/0.9) [a]58%[b]		**6b** (R^1/R^3 = 98.7/1.3) [a]77%[b]		**6c** (R^1/R^3 = 97.5/2.5) [a] 95%[b]		Chiralpak IA[c]		**4**[d] (R^1/R^2 = 99/1) 86%[b]
	k_1'	α	k_1'	α	k_1'	α	k_1'	α	α
7	0.31 (+)	1.30	0.42 (+)	1.32	0.85 (+)	1.24	0.76 (+)	1.50	1.44 (+)
8	0.14 (+)	2.76	0.22 (+)	2.52	0.40 (+)	1.98	0.59 (+)	2.72	2.83 (+)
9	1.16 (–)	1.08	1.55 (–)	1.07	2.93 (+)	~1	3.82 (–)	1.26	1.18 (–)
10	0.86 (–)	2.09	1.09 (–)	2.04	1.59 (–)	1.80	2.99 (–)	2.40	2.11 (–)
11	0.30 (–)	~1	0.34 (–)	~1	0.73 (–)	~1	0.81 (–)	1.04	~1 (–)
12	0.50	1.0	0.64	1.0	1.04	1.0	1.70 (+)	1.19	1.0
13	0.34	1.0	0.43	1.0	0.95 (+)	~1	0.96 (+)	~1	1.0
14	0.38 (+)	~1	0.49 (+)	~1	0.96 (+)	1.16	1.18 (+)	1.09	1.08 (+)
15	1.37 (+)	2.79	1.78 (+)	1.88	3.52 (+)	1.18	3.14 (+)	1.65	3.49 (+)
16	0.90 (–)	2.14	1.21 (–)	2.10	1.87 (–)	1.84	2.92 (–)	2.21	2.27 (–)

Eluent, hexane/2-propanol (90/10, v/v); column size, 25 × 0.20 cm i.d.; flow rate, 0.1 ml/min; temperature, 25°C. The signs in parentheses represent the CD detection (254 nm) of the first-eluted enantiomer.
[a]The ratio of R^1/R^3 was determined from the ratio of the (Ar-CH_3)/(SiOCH_3) in the ^{1}H NMR spectrum.
[b]Immobilization efficiency estimated from TG analysis.
[c]Column size, 25 × 0.46 cm i.d.; flow-rate, 0.5 ml/min; temperature, 25°C.
[d]Data from Ref. 27.

nol. Moreover, the reversal elution orders of enantiomers for racemate **8** were observed by CD detection with a mixture of hexane/THF/2-propanol (90/10/1, by volume) as the eluent and confirmed by HPLC analysis of the pure enantiomers of **8** isolated using Chiralpak AD-H (25 × 0.46 cm i.d.) with hexane/2-propanol (90/10, v/v) as reported previously.[28] The conformational change in the cellulose derivative in these eluents may partially contribute to the above results.

Immobilization of Amylose Derivatives and Their Chiral Recognition Abilities

The amylose derivatives **6a–c** bearing 0.9, 1.3, and 2.5% of R^3, respectively, were prepared using the one-pot method shown in Figure 2 and immobilized onto silica gel by a similar method applied to the cellulose derivatives. The results of the immobilization and enantioseparation on the obtained CPMs are shown in Table 4. Similar to the cellulose derivatives, as the content of 4-(trimethoxysilyl)-phenyl groups in the amylose derivatives increased, the immobilization efficiency increased and the chiral recognition ability decreased. The reversal of elution orders of enantiomers for racemate **9** were observed by CD detection on the **6c**-immobilized CPM containing a higher content of R^3. In this case, same eluent was used and thus this is a true reversal of elution orders. The results on a commercial column, Chiralpak IA, composed of immobilized amylose 3,5-dimethylphenylcarbamate as the chiral selector are included in Table 4 for comparison. The α values on the **6a**-immobilized CPM were comparable with those on Chiralpak IA, whereas those on the **6b**- and **6c**-immobilized CPMs were somewhat lower. In Table 4 are also included the separation data for the **4**-immobilized CPM. Compared with the **4**-immobilized CPM (immobilization efficiency, 86%), the **6b**-immobilized CPM (immobilization efficiency, 77%) showed slightly smaller α values.

TABLE 5. Separation results on the 6a-immobilized CPM with the eluents containing chloroform and THF

	6a-Immobilized CPM					
	H/I (90/10)		H/C/I (90/10/1)		H/T/I (90/10/1)	
Racemate	k_1'	α	k_1'	α	k_1'	α
7	0.31 (+)	1.30	0.93 (+)	1.22	0.53 (+)	1.23
8	0.14 (+)	2.76	0.20 (+)	2.64	0.16 (+)	2.00
9	1.16 (–)	1.08	2.10 (–)	~1	1.41 (+)	1.20
10	0.86 (–)	2.09	1.62 (–)	1.60	0.85 (–)	1.75
11	0.30 (–)	~1	0.59 (+)	1.41	0.25	1.0
12	0.50	1.0	3.04 (–)	1.45	0.44	1.0
13	0.34	1.0	0.33 (+)	~1	0.73 (+)	~1
14	0.38 (+)	~1	0.54 (+)	1.39	0.44 (+)	1.16
15	1.37 (+)	2.79	–[a]	–	–[a]	–
16	0.90 (–)	2.14	3.60 (–)	2.10	1.18 (–)	1.85

Column size, 25 × 0.20 cm i.d.; temperature, 25°C; flow rate, 0.1 ml/min. The signs in parentheses represent the CD detection (254 nm) of the first-eluted enantiomer. H, hexane; I, 2-propanol; C, chloroform; T, tetrahydrofuran.
[a]Enantiomers were not eluted in 4 h.

The solvent compatibility of the **6a**-immobilized CPM was also investigated and the results are shown in Table 5. With the eluents containing chloroform and THF, racemates **9**, **11**, **12**, and **14** were better resolved. Additionally, with hexane/THF/2-propanol (90/10/1, by volume) as the eluent, the elution orders of the enantiomers for **9** were reversed by CD detection and confirmed by the HPLC analysis of the pure enantiomers of **9**.

CONCLUSION

The 3,5-dimethylphenylcarbamates of cellulose and amylose containing a small amount of 4-(trimethoxysilyl)-phenyl groups were synthesized by a one-pot method and immobilized onto silica gel via intermolecular polyconden-

sation of trimethoxysilyl residues under an acidic condition. When a suitable amount of 4-(trimethoxysilyl)phenyl groups was introduced, the obtained CPMs exhibited high chiral recognition ability, which are comparable with those of the commercially available immobilized-type CPMs. The solvents unusable with the conventional coated-type CPMs, such as chloroform and THF, could be used for the immobilized-type CPMs, and the chiral recognition abilities of these CPMs were improved for most of the racemates. As in the previous studies,[26–28] this immobilization method seems to be valuable.

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