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Immobilization and Chromatographic Evaluation of Novel Regioselectively Substituted Amylose-based Chiral Packing Materials for HPLC

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ABSTRACT The regioselectively substituted amylose derivatives bearing a 4-*tert*-butylbenzoate or 4-chlorobenzoate group at 2-position, and 3,5-dichlorophenylcarbamate and a small amount of 3-(triethoxysilyl)propylcarbamate groups at 3- and 6-positions were synthesized by a two-step process based on the esterification of 2-position of a glucose unit. The obtained derivatives were effectively immobilized onto macroporous silica gel by intermolecular polycondensation of triethoxysilyl groups. Their chiral recognition abilities were evaluated as chiral packing materials (CPMs) for high-performance liquid chromatography. These CPMs showed high chiral recognition as well as the conventional coated-type CPM, and can be used with the eluents-containing chloroform and tetrahydrofuran. With the extended use of these eluents, improvement of chiral recognition and reversed elution orders were realized. For some racemates, the immobilized CPM exhibited ability comparable or better to the commercial immobilized amylose- or cellulose-based columns, Chiralpak IA, IB, and IC. *Chirality 23:878–886, 2011.* © 2011 Wiley-Liss, Inc.

KEY WORDS: regioselective substitution; resolution; enantioseparation; chiral stationary phases; high-performance liquid chromatography; amylose; immobilization; chiral packing materials

INTRODUCTION

Polysaccharide-based derivatives, especially phenylcarbamate and benzoate derivatives of cellulose and amylose, are among the most efficient chiral stationary phase (CSP) for high-performance liquid chromatography (HPLC) and a broad range of chiral compounds have been sufficiently separated by the chiral packing materials (CPMs) based on these CSPs.[1–11] To derivatize the polysaccharide, the same substituents are usually introduced onto the three positions of the glucose unit. And the selective substitution have been only restricted between the 6-position and 2-, 3-positions.[12] The regioselective substitution at all three positions has not been realized until Dicke reported the method of selective esterification at 2-position of amylase.[13] Several series of polysaccharide derivatives bearing different substituents on 2-, 3-, and 6-positions have been successfully developed by our group recently.[14–16] These regioselective substituents at different position seem to change the structure and local polarity of the polysaccharide derivatives,[9,11] whose chiral recognition abilities will then be significantly influenced by the nature of the substituents on the aromatic moieties.[9,17–26] Among the coated-type CPM that we previously prepared, those bearing 4-*tert*-butylbenzoate and 4-chlorobenzoate groups at 2-position and 3,5-dichlorophenylcarbamate at 3- and 6-positions (**1** and **3**) (Fig. 1) exhibited a higher chiral recognition than the others with different substituents at three positions.[15] Some racemates could be more efficiently resolved on them than on the commercially available columns.

On the other hand, the coated-type CPMs prepared by the conventional coating procedure have much limitation in the usage of organic solvents, such as tetrahydrofuran (THF), chloroform, ethyl acetate, toluene, acetone, and so on, which can damage the packed columns by dissolving or swelling the polymer inside. These solvents may lead to a better solubility of racemates and more efficient enantioseparation in both analytical and large-scale preparative HPLC. Therefore, the immobilization of the regioselectively substituted polysaccharide derivatives is highly desired to enhance their solvent versatility and stability as well as resolution efficiency.

The goal of this study is to develop the efficient CPMs based on the novel regioselective polysaccharide derivatives with a high chiral recognition ability and universal solvent durability. In continuation of our previous study with coated CPMs prepared from the amylose derivatized by regioselectively substituted groups, amylose derivatives having 4-*tert*-butylbenzoate and 4-chlorobenzoate at 2-position and 3,5-dichlorophenylcarbamate and a controlled small amount

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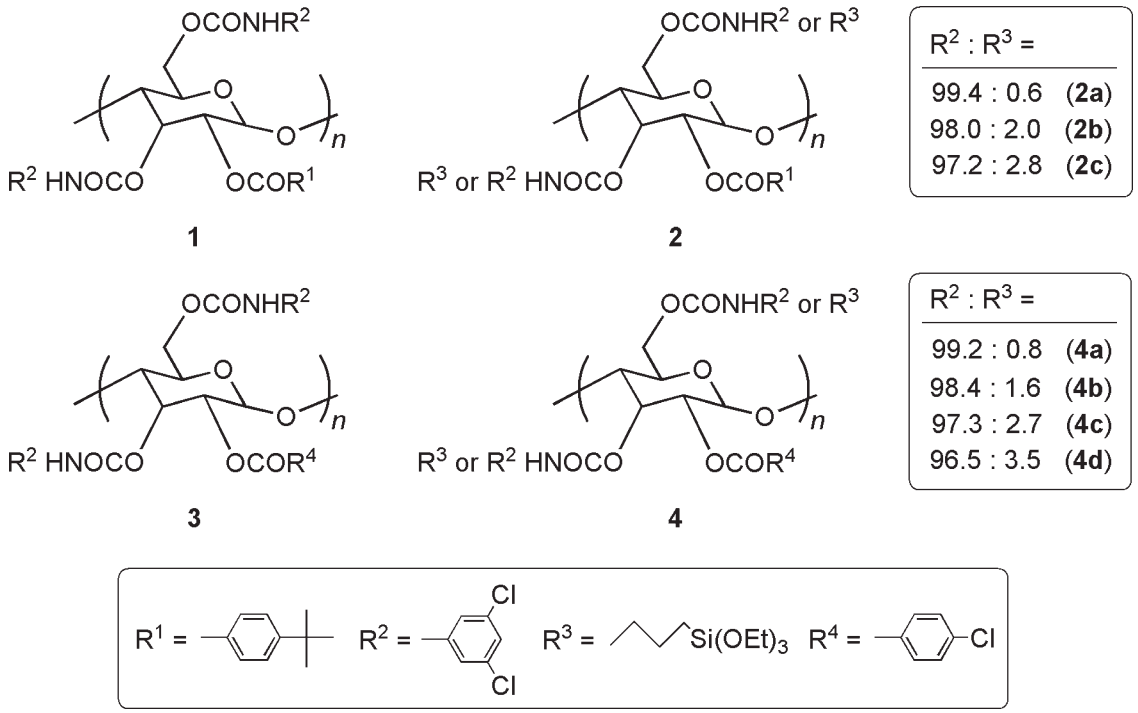

Fig. 1. Structures of amylose derivatives.

of 3-(triethoxysilyl)propyl residue as a crosslinkable group at 3- and 6-positions were prepared (Fig. 1). Then the immobilization of the amylose derivatives onto the surface of silica gel were efficiently performed via the intermolecular polycondensation of the triethoxysilyl groups as reported by our group.[16,27] The chiral recognition abilities of the coated-CPMs and immobilized-CPMs were evaluated by HPLC and compared with those of commercially available columns. The influence of the amount of 3-(triethoxysilyl)propyl residues introduced onto 3- and 6-position of the amylose derivatives on their chiral recognition abilities was also investigated.

EXPERIMENTAL

Materials

Amylose (DP-300) and 3,5-dichlorophenyl isocyanate were gifts from Daicel Chemical Industries (Tokyo, Japan). Vinyl acetate and dimethyl sulfoxide (DMSO) were purchased from Guangfu (Tianjin, China). Other solvents, such as acetonitrile, THF, and pyridine, were purchased from Kermel (Tianjin, China), which were dehydrated by distillation before use. Vinyl 4-*tert*-butylbenzoate and vinyl 4-chlorobenzoate were obtained from Wako (Tokyo, Japan). 3-(Triethoxysilyl)propyl isocyanate was obtained from Azmax (Chiba, Japan). Wide-pore silica gel (Daiso gel SP-1000) with a mean particle size of 7 μm and a mean pore diameter of 100 nm was kindly supplied by Daiso Chemical (Osaka, Japan). The solvents used in chromatographic experiments were of HPLC grade. The racemates were commercially available or prepared by the usual methods.

Apparatus and Chromatography

Chromatographic experiments were performed using a JASCO PU-2089 chromatograph equipped with UV/vis (JASCO UV-2070) and circular dichroism (JASCO CD-2095) detectors at room temperature. A solution of a racemate (3 mg/ml) was injected into the chromatographic system through an intelligent sampler (JASCO AS-2055). The ^{1}H NMR spectra (500 MHz) were recorded using a Bruker-500 spectrometer (Bruker). The thermogravimetric analyses (TGA) was performed using TGA Q 50 (TA).

Synthesis of Regioselective Amylose Derivatives Bearing 2-(4-Tert-butylbenzoate) and 2-(4-Chlorobenzoate) at 2-Position and 3,5-Dichlorophenylcarbamate/3-(Triethoxysilyl)propyl Residues at 3- and 6-Positions

The amylose derivatives **2a–c** bearing 4-*tert*-butylbenzoate at 2-position and 3,5-dichlorophenylcarbamate/3-(triethoxysilyl)propylcarbamate residues at 3- and 6-positions were synthesized by the regioselective esterification of 2-position of amylose using the method reported by Dicke,[13] followed by one-pot procedure reported by our group[27,28] (Fig. 2). The regioselective esterification of the 2-hydroxy group was realized by the following steps. First, the amylose (3.0 g) was dissolved in DMSO (60 ml) at 80°C. Second, vinyl 4-*tert*-butylbenzoate (2.3 equiv to 2-position) and Na_2HPO_4 (2 wt% of amylose, as the catalyst) were added to the solution at 40°C, and the reaction was continued with stirring for 21 days until the esterification of the 2-position was complete. The reaction mixture was added in a large excess of 2-propanol, and the product was recovered as an insoluble fraction in a 98% yield. The obtained monoester was then dissolved in a mixture of *N*,*N*-dimethylacetamide, lithium chloride, and pyridine. After completely dissolved, it was allowed to react with 3,5-dichlorophenyl isocyanate at 80°C, which not only converted most of the hydroxyl groups at 3- and 6-positions into carbamates but also eliminated the water in the reaction system at the same time. After reacted for 6 h, the calculated amount of 3-(triethoxysilyl)propyl isocyanate was then added, and the reaction was continued for 16 h at 80°C. Finally, an excess of 3,5-dichlorophenyl isocyanate was added into reaction mixture and reacted for 7 h at 80°C to complete the carbamoylation of the underivatized hydroxyl groups. Three amylose derivatives **2a–c** bearing different amounts of the 3-(triethoxysilyl)propylcarbamate residues at 3- and 6-positions were isolated as a methanol-insoluble fraction.

The amylose derivatives **4a–d** bearing 4-chlorobenzoate at 2-position and 3,5-dichlorophenylcarbamate/3-(triethoxysilyl)propylcarbamate residues at 3- and 6-positions were synthesized by similar procedure. The degree of substitution at three different positions and yields of **2a–c** and **4a–d** are summarized in Table 1, respectively.

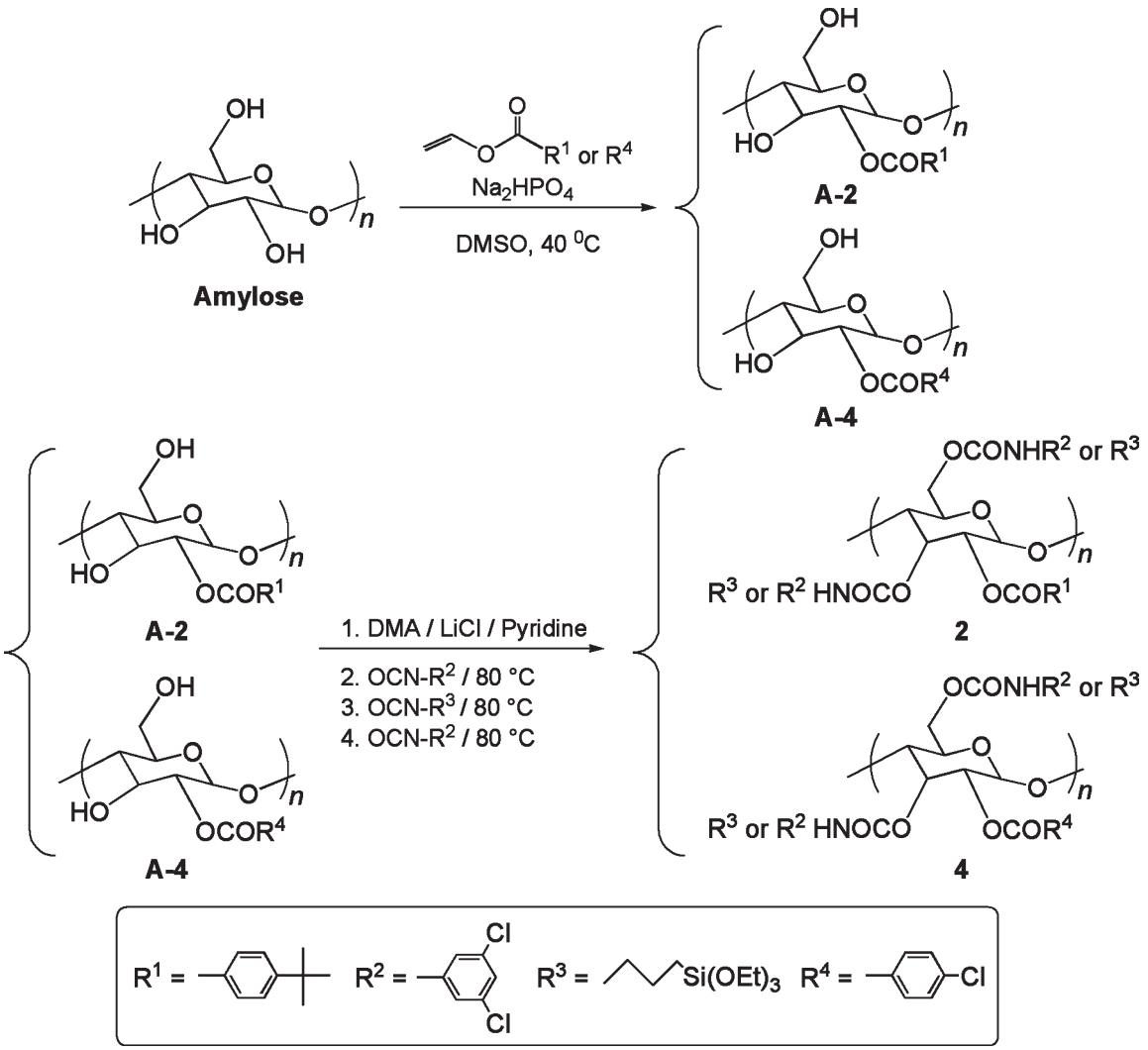

Fig. 2. Scheme of the synthesis of amylose derivatives **2** and **4**.

Immobilization of Amylose Derivatives by Intermolecular Polycondensation of Triethoxysilyl Groups

Seven amylose derivatives **2a–c** and **4a–d** (0.35 g) were first coated on the plain silica gel (1.40 g) after completely dissolving in THF (8 ml) according to the previous way.[9] Then the **2**-coated and **4**-coated silica gels (0.65 g) were added into a mixture of ethanol, water, and trimethylsilyl chloride (6/1.5/0.1, v/v/v). After stirring for 10 min at 110°C, the immobilized CPMs were sufficiently washed with THF and dried at 60°C in vacuo. The immobilization efficiency was estimated by thermogravimetric analysis with a TGA instrument.

Preparation and Evaluation of Packed Columns

The **2**-immobilized and **4**-immobilized silica gels were packed in a stainless-steel tube (25 × 0.20 cm i.d.) by a slurry technique. The plate numbers of the packed columns were 1800–2700 for benzene using hexane/2-propanol (90/10, v/v) mixture as the eluent at the flow rate of 0.1

TABLE 1. Synthesis of regioselective amylose derivatives 2a-c and 4a-d bearing a benzoate group at 2-position and 3,5-dichlorophenylcarbamate/3-(triethoxysilyl)propylcarbamate groups at 3- and 6-positions

Amylose derivatives	Amount of 3-(triethoxysilyl) propyl isocyanate (mol%)[a]	Degree of substitution (R^1)[b]	Degree of substitution (R^2/R^3)[b]	Yield (%)
2a	0.8	1.0	99.4/0.6	95
2b	2.3	1.0	98.0/2.0	98
2c	3.5	1.0	97.2/2.8	91
4a	1.0	1.0	99.2/0.8	88
4b	2.1	1.0	98.4/1.6	88
4c	3.4	1.0	97.3/2.7	84
4d	4.2	1.0	96.5/3.5	87

[a]On the basis of hydroxyl groups of amylose.
[b]See Figure 1.

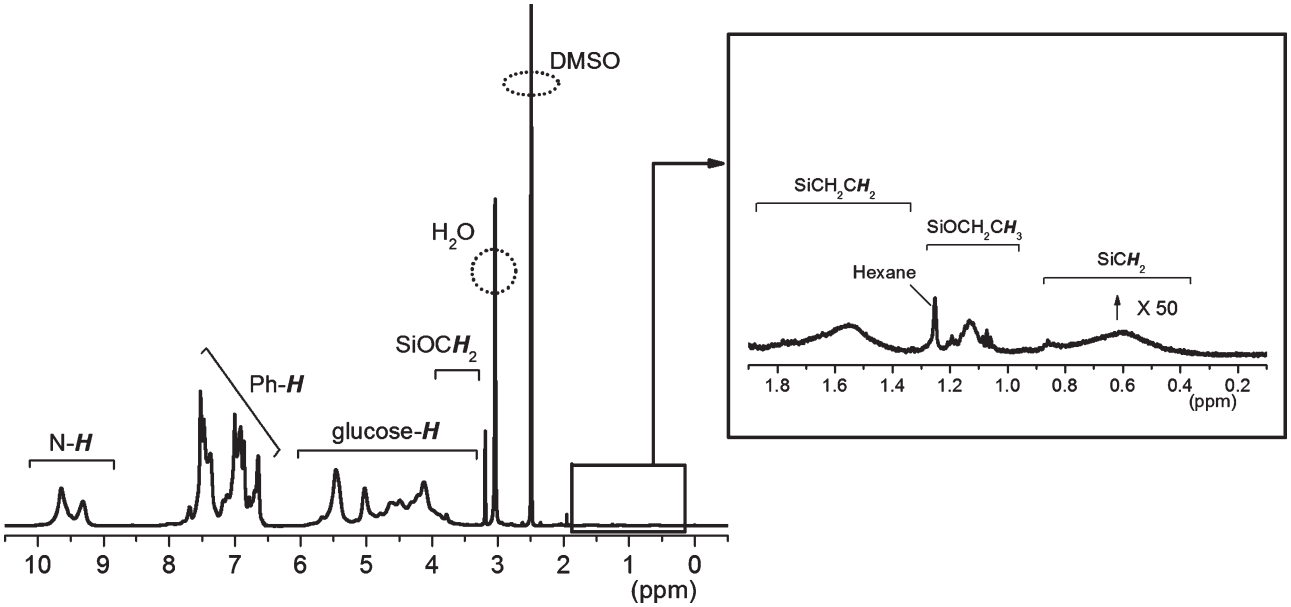

Fig. 3. ^{1}H NMR spectrum of the amylose derivative **4a** in DMSO-d_6 at 80°C.

ml/min. The dead time (t_0) was determined using 1,3,5-tri-*tert*-butylbenzene as a nonretained compound.[29]

RESULTS AND DISCUSSION

Synthesis of Regioselective Amylose Derivatives Bearing A Controlled Amount of 3-(Triethoxysilyl)propyl Residues at 3- and 6-Positions

The regioselectively substituted amylose derivatives **2a**–**c** were synthesized by a sequential procedure as shown in Figure 2. The vinyl 4-*tert*-butylbenzoate was first added into the amylose solution to selectively esterify only the hydroxyl group at 2-position of amylose. The monoester **A-2** was obtained with a high yield shown in Table 1. The 3,5-dichlorophenyl isocyanate was first added to partly convert 3- and 6-hydroxy groups of amylose into 3,5-dichlorophenylcarbamate residues and to eliminate water from the reaction system. A desired amount of 3-(triethoxysilyl)propyl isocyanate was then added, and finally, the underivatized hydroxy groups were treated with an excess of 3,5-dichlorophenyl isocyanate. The content ratio of the 3,5-dichlorophenylcarbamate to

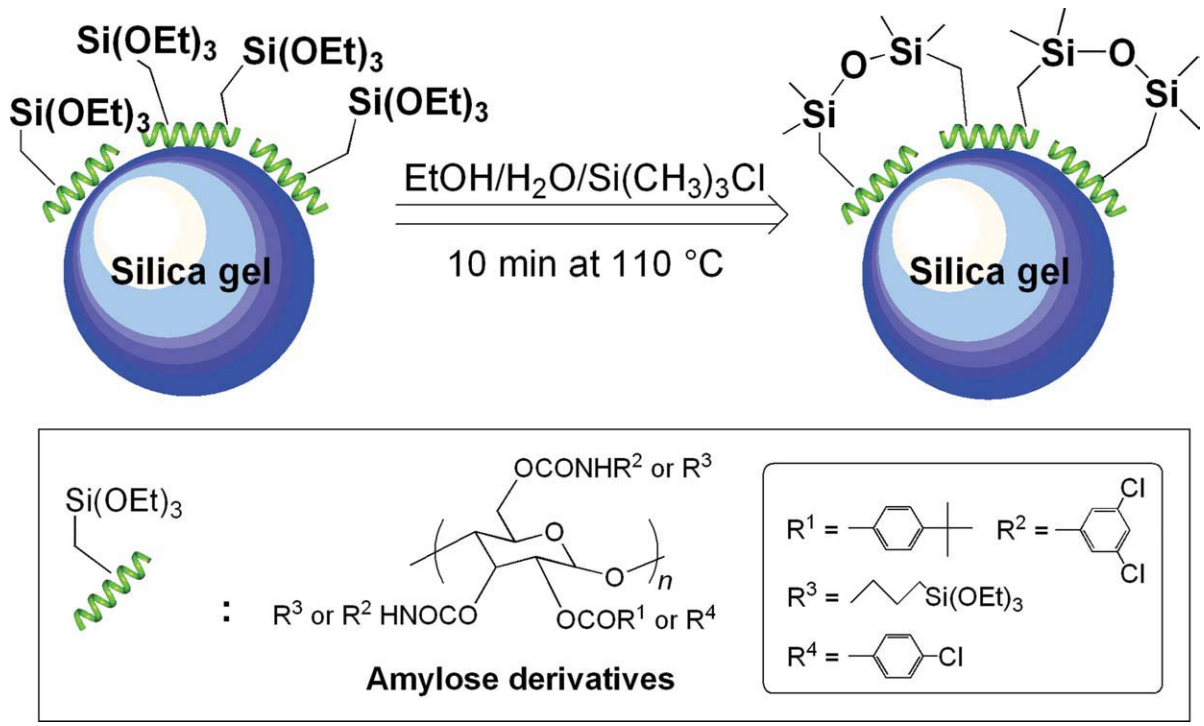

Fig. 4. Immobilization scheme of the amylose derivatives. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

TABLE 2. Immobilization of 2a-c and separation factors (α) on 2-immobilized CPMs[a], 1-coated CPM[b] and Daicel Chiralpak columns[c]

	CPMs				
	2aI	2bI[d]	2cI		
Racemates	(R^2/R^3=99.4/0.6) 32%[e]	(R^2/R^3=98.0/2.0) 89%[e]	(R^2/R^3=97.2/2.8) 97%[e]	1-Coated CPM[b]	Daicel chiralpak columns[c]
5	1.56 (+)	1.20 (+)	1.21 (+)	1.19 (+)	1.52 (+) IC
6	1.39 (−)	2.25 (−)	1.29 (−)	2.50 (−)	2.71 (+) IA
7	1.40 (−)	1.45 (−)	1.41 (−)	1.51 (−)	1.33 (+) IB
8	1.31 (+)	1.21 (+)	1.30 (+)	1.20 (+)	2.07 (+) IA
9	1.19 (−)	2.08 (−)	1.34 (−)	2.19 (−)	1.28 (−) IC
10	1.14 (+)	∼ 1 (+)	1.20 (+)	∼ 1	2.42 (−) IB
11	1.20 (−)	1.28 (−)	∼ 1 (−)	1.20 (−)	1.96 (+) IC
12	∼ 1 (+)	1.22 (+)	1.41 (+)	1.21 (+)	1.26 (−) IB
13	2.00 (+)	1.84 (+)	2.51 (+)	1.92 (+)	2.06 (+) IA
14	1.11 (−)	1.13 (−)	1.13 (−)	1.16 (−)	

[a]Column: 25 × 0.20 cm(i.d.); flow rate: 0.1 mL/min; eluent: hexane/2-propanol (90/10,v/v).
[b]Data taken from Ref. 15.
[c]Data of IA and IB taken from Ref. 27, data of IC was obtained under the same conditions as IA and IB. Column:25 × 0.46 cm(i.d.); flow rate: 0.5ml/min; eluent: hexane/2-propanol(90/10,v/v). The signs in parentheses indicate the optical rotation of the first-eluted enantiomer.
[d]Data taken from Ref. 16. The signs in parentheses indicate the CD detection at 254 nm of the first-eluted enantiomer.
[e]Immobilization efficiency.

3-(triethoxysilyl)propylcarbamate was controlled by the amount of 3-(triethoxysilyl)propyl isocyanate. The regioselective amylose derivatives **4a-d** were synthesized by similar procedure as shown in Figure 2. Figure 3 shows the ^{1}H NMR spectrum of the amylose derivative **4a**. The ratio of (3,5-dichlorophenylcarbamate)/(3-(triethoxysilyl)propylcarbamate) was estimated from (aromatic proton)/($SiCH_2$) intensity ratio.[27]

Immobilization of Regioselective Amylose Derivatives

The immobilization process is illustrated in Figure 4. The derivatives **2** and **4** were first coated on the plain silica gel and then was dispersed into a mixture of ethanol, water, and trimethylsilyl chloride to be immobilized onto the surface of silica gel by polycondensation of the triethoxysilyl groups at 3- and 6- positions. After the immobilization, the **2**-immobilized and **4**-immobilized silica gels were thoroughly washed and then dried. The immobilization efficiency is defined as the ratio of the (immobilized amylose derivative)/(coated amylose derivative), which was estimated from the organic content in the CPMs by thermogravimetric analysis.

Table 2 shows the immobilization efficiencies of derivatives **2a–c** on silica gel. Compared with the amylose derivatives **2b–c**, which have a relatively higher amount of the 3-(triethoxysilyl)propyl group (R^2/R^3 = 98.0/2.0 and 97.2/2.8, respectively) at 3- and 6-positions, less amount of **2a** was immobilized on the silica gel during the immobilization process. As shown in Table 3, similar phenomena can be observed when compared the immobilization efficiency of derivatives **4a** with those of **4b**−**d**. In **2a** and **4a**, some amy-

TABLE 3. Immobilization of 4a-d and separation factors (α) on 4-immobilized CPMs[a], 3-coated CPM, and Daicel Chiralpak columns[b]

	CPMs					
	4aI	4bI	4cI	4dI		
Racemates	(R^2/R^3 = 99.2/0.8) 53%[c]	(R^2/R^3 = 98.4/1.6) 64%[c]	(R^2/R^3 = 97.3/2.7) 70%[c]	(R^2/R^3 = 96.5/3.5) 73%[c]	3-Coated CPM	Daicel chiralpak columns[b]
5	1.54 (+)	1.37 (+)	1.29 (+)	1.23 (+)	1.91 (+)	1.52 (+) IC
6	1.90 (−)	1.73 (−)	1.30 (−)	1.27 (−)	2.15 (−)	2.71 (+) IA
7	1.84 (+)	1.42 (+)	1.07 (+)	1.07 (+)	2.97 (+)	1.33 (+) IB
8	1.31 (+)	1.32 (+)	1.32 (+)	1.25 (+)	1.60 (+)	2.07 (+) IA
9	∼ 1 (−)	∼ 1 (−)	∼ 1 (−)	∼ 1 (−)	∼ 1 (−)	1.28 (−) IC
10	1.14 (+)	1.23 (+)	1.34 (+)	1.28 (+)	1.0 (+)	2.42 (−) IB
11	1.18 (+)	∼ 1 (+)	∼ 1 (+)	∼ 1 (+)	1.68 (+)	1.96 (+) IC
12	1.92 (+)	1.76 (+)	1.82 (+)	1.61 (+)	1.32 (+)	1.26 (−) IB
13	1.71 (+)	2.05 (+)	2.03 (+)	1.97 (+)	2.21 (+)	2.06 (+) IA
14	1.04 (−)	1.04 (−)	1.10 (−)	1.06 (−)	2.17 (−)	

[a]Column: 25 × 0.20 cm (i.d.); flow rate: 0.1 ml/min; eluent: hexane/2-propanol (90/10, v/v).
[b]Data of IA and IB taken from Ref. 27, data of IC was obtained under the same conditions as IA and IB. Column: 25×i.d.); flow rate: 0.5 ml/min; eluent: hexane/2-propanol(90/10, v/v). The signsin parentheses indicate the optical rotation of the first-eluted enantiomer. Column: 25×i.d.); flow rate: 0.5ml/min; eluent: hexane/2-propanol(90/10, v/v). The signs in parentheses indicate the CD detection at 254 nm of the first-eluted enantiomer.
[c]Immobilization efficiency.

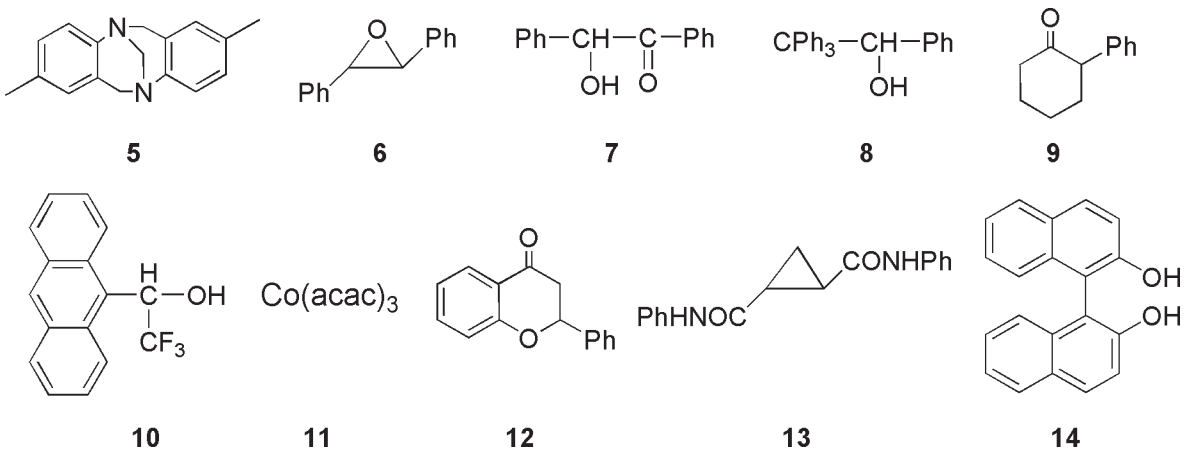

Fig. 5. Structures of racemates **5–14**.

lose chains may have no or only a few 3-(triethoxysilyl)propyl residues, which must make efficient immobilization difficult. On the other hand, the high immobilization efficiencies of **2b−c** and **4b−d** indicate that only a few percent of 3-(triethoxysilyl)propyl residues is sufficient for efficient immobilization.

The obtained immobilized CPMs were packed into HPLC columns, and their chiral recognition abilities were evaluated with 10 racemates (**5-14**, Fig. 5). Figure 6 shows the chromatogram of the resolution of racemic *trans*-stilbene oxide (**6**) on the immobilized CPM **4aI**. The enantiomers were eluted at the retention time t_1 and t_2, respectively. The dead time (t_0) was determined for 1,3,5-tri-*tert*-butylbenzene to be 8.65 min. After the retention factors, $k_1'((t_1 - t_0)/t_0)$ and $k_2'((t_2 - t_0)/t_0)$, were estimated to be 0.82 and 1.76, respectively, the separation factor α (k_2'/k_1') was then readily defined to be 1.90, and a baseline separation of racemate **6** was attained.

Evaluation of Chiral Recognition Ability of Immobilized CPMs

The results of chromatographic resolutions of 10 racemates (**5–14**) on the immobilized CPMs **2aI–2cI** are summarized in Table 2, which also included the resolution results on **1**-coated CPM. The **2**-immobilized CPMs exhibited similar or slightly different chiral recognition ability compared to **1**-coated one, and the elution orders of the enantiomers on them were the same. The α values on **2aI–2cI** were slightly larger for racemates **5**, **8**, **10**, **11**, **12**, and **13** and slightly smaller for **6**, **7**, **9**, and **14** than on **1**-coated CPM, implying that the procedure used in the study is efficient for the immobilization of amylose derivatives enabling to maintain their chiral recognition abilities and elution order of enantiomers. Among three immobilized CPMs, **2bI** CPM showed chiral recognition ability comparable to the **1**-coated CPM. The higher order structure of **2b** after the immobilization seems to be similar to that of **1**-coated on the silica gel. The slightly lower recognition of **2aI** may be ascribed to the low **2a** content on silica gel.

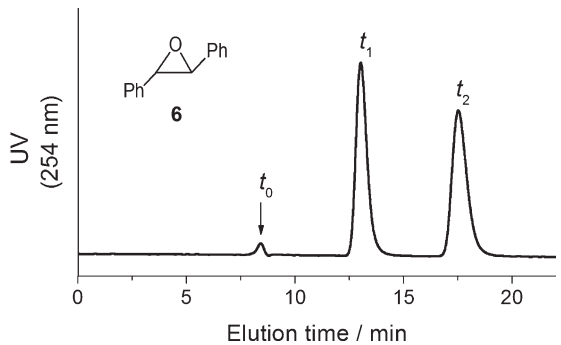

Fig. 6. Chromatogram for the resolution of **6** on **4aI** CPM. Column: 25 × 0.20 cm (i.d.); flow rate: 0.1 ml/min; eluent: hexane/2-propanol (90/10, v/v).

The highest α values for 10 racemates on three commercial immobilized-type chiral columns, Chiralpak IA, IB, and IC, consisting of amylose tris(3,5-dimethylphenylcarbamate), cellulose tris(3,5-dimethylphenylcarbamate), and cellulose tris(3,5-dichlorophenylcarbamate) as the CSPs, respectively, are selected and also included in Table 2 for comparison. In general, the derivatives **2aI-2cI** bearing a 4-*tert*-butylbenzoyl group at 2-position showed an equivalent or higher chiral recognition abilities for some racemates compared to Chiralpak IA, IB, and IC using the hexane-2-propanol (90/10, v/v) mixture as the standard eluent. Especially, **2aI–2cI** obtained much more efficient resolution for racemates **7** and **9**, which cannot be resolved efficiently on the three commercial immobilized columns. And the α value for racemate **9** on **2b**-immobilized CPM has appeared to be the highest one compared to several commercially available columns.[16] Racemates **12** and **13** were both better resolved on **2cI** than the three commercial columns.

The content of the R^3 residues introduced onto the glucose ring plays a critical role in the immobilization process. To efficiently prepare an immobilized CPM without losing its high chiral recognition, the content of the R^3 residues should be as low as possible, because the R^3 residues may disturb the regular structure of the polysaccharide derivative more or less. Nevertheless, the immobilization efficiency decreased when the R^3 content became lower, as shown in Table 2. Considering the two sides of its effect, the CPM obtained from **2b** bearing 2.0% R^3 seems to be preferable than those from the other two derivatives although the three **2**-immobilized CPMs have the same 4-*tert*-butylbenzoate group at 2-positioin and small difference in amounts of 3-(triethoxysilyl)propyl group at 3- and 6-positions, indicating that

TABLE 4. Separation factors (α) on 2bI CPM[a] and 4bI CPM

	2bI CPM				4bI CPM
Racemates	H/I (90/10)[b]	H/C/I(90/10/1)[b]	H/C (70/30)	H/T (70/30)	H/I (90/10)
5	1.20 (+)	1.51 (+)	1.22 (+)	1.17 (+)	1.37 (+)
6	2.25 (−)	1.28 (−)	∼ 1	1.36 (−)	1.73 (−)
7	1.45 (−)	1.24 (−)	1.56 (+)	1.17 (−)	1.42 (+)
8	1.21 (+)	1.21 (+)	1.22 (−)	1.57 (+)	1.32 (+)
9	2.08 (−)	1.29 (−)	∼ 1 (−)	1.38 (+)	∼ 1 (−)
10	∼ 1 (+)	∼ 1	1.13 (+)	1.00	1.23 (+)
11	1.28 (−)	1.12 (−)	∼ 1 (−)	∼ 1 (+)	∼ 1 (+)
12	1.22 (+)	1.15 (+)	1.11 (−)	∼ 1 (+)	1.76 (+)
13	1.84 (+)	∼ 1 (+)	∼ 1	1.09 (+)	2.05 (+)
14	1.13 (−)	1.12 (−)	1.13 (−)	1.06 (−)	1.04 (−)

[a]Column:25 ×0.20 cm (i.d.); flow rate: 0.1 ml/min; eluent: H, hexane; I,2-propanol; c,chloroform; T, tetrahydrofuran
[b]Data taken from Ref. 16 except for data of racemate 14.The signs in paretheses indicate CD detection at 254 nm of the first-eluted enantiomer.

the immobilization efficiency has a great effect on the chiral recognition abilities of these immobilized CPMs. As already mentioned, **2a** showed low immobilization efficiency (32 %), and therefore, its immobilized CPM **2aI** should have a rather high bare silica gel surface without immobilized **2a**, which must reduce its chiral recognition.

The chromatographic resolutions of 10 racemates (**5–14**) on the immobilized CPMs **4aI–4dI** are summarized in Table 3, which also includes the data on **3**-coated CPM and the commercial Chiralpak IA, IB, and IC. Using the hexane/2-propanol (90/10, v/v) as the eluent, four immobilized CPMs **4aI–4dI** showed similar or slight variation in chiral recognition abilities compared to those of **3**-coated one, such as the larger α values for racemates **10** and **12** and slightly smaller values for the others except for **9**. In contrast to Chiralpak IA, IB, and IC under the standard eluent, racemates **5** and **7** were equally or better resolved on **4aI** and **4bI**. And racemate **12** was more efficiently resolved on **4aI–4dI** than the three commercial immobilized columns. The immobilization efficiency gradually increased when the R^3 content increased from left (**4aI**, 53%) to right (**4dI**, 73%). The CPM obtained from **4a** bearing 0.8% R^3 content seems to be desirable than those from the other three derivatives. The immobilization efficiency (53 %) of **4a** is higher than that (32 %) of **2a**, which may be ascribed to the relatively high recognition of **4aI**.

Influence of Eluents on Chiral Recognition Ability of the Immobilized CPMs

The chiral recognition ability of **2b**-immobilized CPM under nonstandard eluents containing chloroform and THF was investigated, and the results are shown in Table 4. By using chloroform and THF, which are the prohibited eluents for **1**-coated CPM, the resolution for racemates **5**, **7**, **8**, and **10** was much improved on **2bI** in comparison with that on **1**-coated CPM in Table 2. Especially, the extended use of chloroform with different ratio can lead to different contribution to the improvement of the chiral recognition ability. For example, 10% chloroform containing eluent (H/C/I = 90/10/1, v/v/v) can lead to a more efficient enantioseparation for racemate **5**, and better resolution for **7**, **8**, and **10** could be obtained when increased chloroform ratio to 30% (H/C = 70/30, v/v). In the separation of **7**, **8**, and **12**, the sign of the CD detection was reversed by changing eluents from hexane/2-propanol (90/10, v/v) to hexane/chloroform (70/30, v/v) as shown in Table 4. This result may be ascribed to the reversed elution order of enantiomers, although the possibility of the reversal in the CD sign of some compounds caused by the change of solvents cannot completely excluded. The reverse elution order of **7** was actually confirmed using both pure enantiomers of **7** on **2bI** CPM. This indicates that the stereoselectivity can be very sensitive and varied depending on changes of the composition of the mobile phase, which is

TABLE 5. Separation factors (α) on 4aI CPM[a] and 2aI CPM

	4aI CPM (R^2/R^3=99.2/0.8)			2aI CPM (R^2/R^3=99.4/0.6)
Racemates	H/I (90/10)	H/C/I (90/10/1)	H/T/I (90/10/1)	H/I (90/10)
5	1.54 (+)	1.50 (+)	1.23 (+)	1.26 (+)
6	1.90 (−)	1.26 (−)	1.45 (+)	1.39 (−)
7	1.84 (+)	1.22 (+)	1.41 (−)	1.40 (−)
8	1.31 (+)	1.20 (+)	1.22 (+)	1.31 (+)
9	∼ 1 (−)	∼ 1 (−)	∼ 1	1.49 (−)
10	1.14 (+)	1.09 (+)	1.05 (+)	1.14 (+)
11	1.18 (+)	1.27 (+)	1.25 (−)	1.20 (−)
12	1.92 (+)	1.73 (+)	1.38 (+)	∼ 1 (+)
13	1.71 (+)	∼ 1 (+)	∼ 1 (+)	2.00 (+)
14	1.04 (−)	∼ 1 (−)	∼ 1 (−)	1.11 (−)

[a]Column: 25 × 0.20 cm (i.d.); flow rate: 0.1 ml/min; eluent: H, hexane; l, 2-propanol; c, chloroform; T, tetrahydrofuran. The signs in parentheses indicate the CD detection at 254 nm of the first eluted enantiomer.

in agreement with the previous results.[27] And the conformation of the amylose derivatives may be altered in these eluents, which can partially explain the improvement of chiral recognition and reversed elution orders. The better resolution for racemate **8** and reversed elution order for racemate **11** were also observed using a hexane-THF mixture (H/T = 70/30, v/v) as the eluent, whereas no obvious improvement was found under the eluent composition as H/T/I = 90/10/1, v/v/v.

The data for **4bI** CPM bearing a similar R^2/R^3 content ratio to that of **2bI** was also included in Table 4 for comparison. The only difference between the two CPMs is that **2bI** has an electron-donating substituent, 4-*tert*-butyl group, whereas **4bI** contains an electron-withdrawing group, 4-chloro, on the benzoate group at the 2-position of glucose unit. Five racemates, such as **6**, **7**, **9**, **11**, and **14**, were better resolved on the former under the same chromatographic condition using hexane and 2-propanol mixture as eluent. In particular, racemates **9** and **11** could not be separated on **4bI**, but baseline separation was attained on **2bI**. And racemate **10**, which was difficult to resolve on **2bI**, was sufficiently resolved on **4bI**. This implies that the two CPMs are complementary for each other for the efficient resolution of some racemates.

The influence of eluents on the chiral recognition abilities of **4aI** CPM was also explored. As shown in Table 5, the resolution for racemates **10** and **12** was effectively improved after adding 10% chloroform (H/C/I = 90/10/1, v/v/v) or THF (H/T/I = 90/10/1, v/v/v) to the standard eluent in comparison with the **3**-coated CPM in Table 3. However, more content of chloroform or THF, such as the eluent composition of H/C = 70/30 and H/T = 70/30, cannot contribute to the improvement of recognition ability. Moreover, the extended use of THF (H/T/I = 90/10/1, v/v/v) also reversed the elution orders of the enantiomers **6**, **7**, and **11** in comparison with the coated CPM. The complementary chiral recognition ability of **2aI** and **4aI** can also be observed, especially for the resolution of racemates **9** and **12**. This also implies that the chiral recognition and higher order structure of the amylose derivatives can be greatly influenced by the substituent at 2-position, which agreed with the previous results.[15]

CONCLUSIONS

Novel immobilized amylose derivatives bearing 4-*tert*-butylbenzoate and 4-chlorobenzoate at 2-position and 3,5-dichlorophenylcarbamate/3-(triethoxysilyl)propylcarbamate at 3- and 6-positions were prepared through the regioselective esterification of the 2-position of the glucose unit, followed by 3,5-dichlorophenylcarbamoylation and 3-(triethoxysilyl)propylcarbamoylation at 3- and 6-positions. After immobilized on silica gel via intermolecular polycondensation of triethoxysilyl groups introduced at 3- and 6-positions, their chiral recognition abilities were evaluated as CPMs by HPLC. For some racemates, the immobilized derivatives containing a certain amount of the 3-(triethoxysilyl)propyl residue showed high chiral recognition abilities that are comparable to the conventional-coated CPMs and the commercially available immobilized-type CPMs, Chiralpak IA, IB, and IC. The prohibited solvents for the coated-type CPMs, such as chloroform and THF, can be used for the novel immobilized ones. Particularly, by using these nonstandard solvents with suitable content, the novel immobilized CPMs could achieve more efficient resolution and reversed elution order for some racemates, which are difficult to be resolved on the coated CPMs or the commercially available columns, indicating that this method for immobilization of CPMs based on the regioselectively substituted amylose derivatives is useful to improve the chiral recognition ability and the solvent compatibility.

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