doi:10.1246/cl.2010.442

Published on the web March 31, 2010

Synthesis and Immobilization of Amylose Derivatives Bearing a 4-*tert*-Butylbenzoate Group at the 2-Position and 3,5-Dichlorophenylcarbamate/3-(Triethoxysilyl)propylcarbamate Groups at 3- and 6-Positions as Chiral Packing Material for HPLC

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Two novel amylose derivatives **2a** and **2b** bearing 4-*tert*-butylbenzoate at the 2-position and 3,5-dichlorophenylcarbamate/3-(triethoxysilyl)propylcarbamate residues at 3- and 6-positions were successfully synthesized and immobilized onto silica gel, and their chiral recognition abilities were evaluated as chiral packing materials (CPMs) for high-performance liquid chromatography. These immobilized CPMs exhibited recognition ability similar to conventional coated CPM, and for some racemates, comparable or better resolutions were observed even compared to commercial immobilized amylose- or cellulose-based columns.

Polysaccharide-based CPMs for high-performance liquid chromatography (HPLC), especially those utilizing phenylcarbamate and benzoate derivatives of cellulose and amylose, are well-known to exhibit high chiral recognition to a wide range of compounds.[1,2] These derivatives traditionally have the same substituents at the 2-, 3-, and 6-positions of a glucose ring, and the regioselective derivatization of polysaccharides has been restricted to only the 6-position and 2-, 3-positions.[3] Based on a method of regioselective esterification at the 2-position of amylose reported by Dicke,[4] we have successfully introduced regioselectively different substituents onto 2-, 3-, and 6-positions, respectively.[5,6] These substituents may change the structure and local polarity of the polysaccharide derivatives, whose chiral recognition abilities will then be significantly influenced by the nature and position of the substituents on the aromatic moieties.[7,8]

Among the coated CPMs obtained using novel regioselective amylose derivatives which we previously prepared, those bearing a 4-*tert*-butylbenzoate group at the 2-position and 3,5-dichlorophenylcarbamate at 3- and 6-positions (**1**) (Figure 1) exhibited a higher chiral recognition than the others having different substituents at three positions, and some racemates were even more efficiently resolved on them than on the commercially available columns.[6] However, the coated CPMs are restricted in the use of organic eluents, such as chloroform, tetrahydrofuran (THF), acetone, toluene, and ethyl acetate, which dissolve or swell the derivatives and destroy their packed columns. These eluents may lead to efficient resolution of enantiomers both in analytical and preparative HPLC. To improve the stability of **1** in nonstandard solvents and its resolution ability, immobilization of the polysaccharide derivatives **1** on silica gel is highly recommended.

In the present study, in order to obtain efficient CPMs with a high chiral recognition and universal solvent durability, amylose derivatives bearing 4-*tert*-butylbenzoate at the 2-position and 3,5-dichlorophenylcarbamate and a controlled amount of 3-(triethoxysilyl)propylcarbamate residue as a cross-linkable group at 3- and 6-positions were prepared (Figure 1). The amylose derivatives were then efficiently immobilized onto silica gel via the intermolecular polycondensation of the triethoxysilyl groups as reported in a previous study by our group.[9]

The amylose derivatives **2a** and **2b** bearing 4-*tert*-butylbenzoate at the 2-position and 3,5-dichlorophenylcarbamate/3-(triethoxysilyl)propylcarbamate residues at 3- and 6-positions were synthesized by combination of a procedure based on the regioselective esterification of the 2-position reported by Dicke[4] and a one-pot method reported by our group (Figure 2).[9,10] To regioselectively esterify only the 2-position, amylose (3.0 g) was first dissolved in DMSO (60 mL) at 80 °C. Then, vinyl 4-*tert*-butylbenzoate (2.3 equiv to 2-position) and Na_2HPO_4 (2 wt %, as

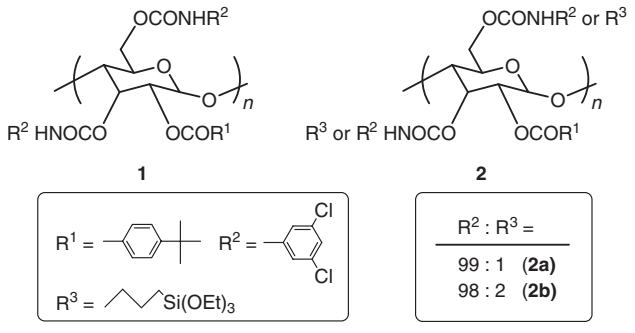

Figure 1. Structures of amylose derivatives **1** and **2** for CPMs.

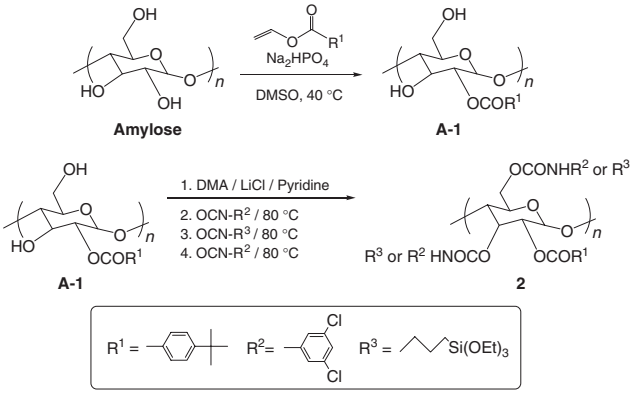

Figure 2. Scheme of the synthesis of amylose derivatives **2**.

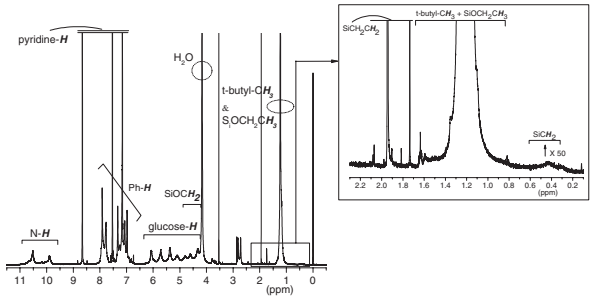

Figure 3. ^{1}H NMR (500 MHz) spectrum of the amylose derivative **2b** in pyridine-d_5 at 80 °C.

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-hydroxy group was complete. The reaction mixture was then dispersed into a large excess of 2-propanol, and the product (**A-1**) was recovered as an insoluble fraction in 98% yield. The obtained mono ester **A-1** was dissolved in a mixture of *N*,*N*-dimethylacetamide, lithium chloride, and pyridine. After being completely dissolved, 3,5-dichlorophenyl isocyanate was added and the reaction was continued for 6 h at 80 °C. A calculated amount of 3-(triethoxysilyl)propyl isocyanate was then added and allowed to react for 16 h at 80 °C. The ratio of the 3,5-dichlorophenylcarbamate to 3-(triethoxysilyl)propylcarbamate residues was controlled by the amount of 3-(triethoxysilyl)propyl isocyanate. Finally, the underivatized hydroxy groups were allowed to react with an excess of 3,5-dichlorophenyl isocyanate for 7 h at 80 °C. Two amylose derivatives **2a** and **2b** bearing different amounts of the 3-(triethoxysilyl)propylcarbamate residues at 3- and 6-positions were isolated as a methanol-insoluble fraction. The structures of the derivatives were confirmed by ^{1}H NMR and elemental analysis. The ^{1}H NMR spectrum of **2b** is shown in Figure 3, from which the ratio of (3,5-dichlorophenylcarbamate)/[3-(triethoxysilyl)propylcarbamate] could be estimated from (aromatic proton)/($SiCH_2$) intensity ratio.

For immobilization, two amylose derivatives **2a** and **2b** (0.35 g) were first dissolved in THF (8 mL) and coated onto silica gel (Daiso gel SP-1000, 7 μm, 1.40 g) in the previous manner.[7] The **2a**- and **2b**-coated silica gels (0.65 g) were then 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 (**2a**-IM and **2b**-IM) were extensively washed with THF and acetone and dried at 50 °C in vacuo. The immobilization efficiency was estimated by thermogravimetric (TG) analysis.

The **2a**-IM and **2b**-IM were packed in a stainless-steel tube (25 × 0.20 cm i.d.) as a slurry. The plate numbers of the packed columns were 1300–1800 for benzene using a hexane/2-propanol (90/10, v/v) mixture as the eluent at the flow rate of 0.1 mL min^{-1}. 1,3,5-Tri-*tert*-butylbenzene was used as a non-retained compound to estimate the dead time (t_0).[11]

The chiral recognition abilities of the new amylose derivatives were evaluated with 9 racamates **3**–**11** (Figure 4). Figure 5 shows the chromatogram of the resolution of racemic 2-phenylcyclohexanone (**7**) on the amylose derivative **2b**. The enantiomers were eluted at the retention time t_1 and t_2 with a baseline separation. The dead time (t_0) was determined to be 7.78 min. The retention factors, k_1' $[(t_1 - t_0)/t_0]$ and k_2'

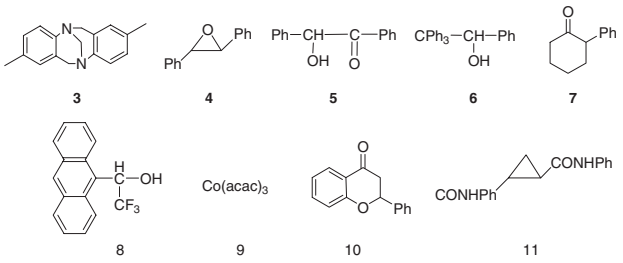

Figure 4. Structures of racemates **3**–**11**.

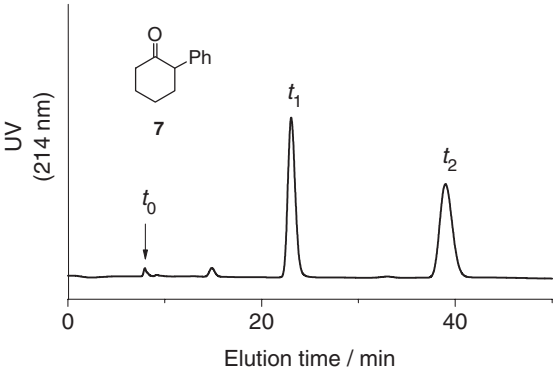

Figure 5. Chromatogram for the resolution of **7** on **2b**-IM.

$((t_2 - t_0)/t_0)$, were estimated to be 2.20 and 4.58, respectively, which resulted in the separation factor α (k_2'/k_1') to be 2.08.

The chromatographic resolution of 9 racemates **3**–**11** on **2a**-IM and **2b**-IM are summarized in Table 1.[12] For comparison, resolution on commercial polysaccharide-based chiral columns, Chiralpak IA, IB, and IC, which are immobilized CPMs consisting of amylose tris(3,5-dimethylphenylcarbamate), cellulose tris(3,5-dimethylphenylcarbamate) and cellulose tris(3,5-dichlorophenylcarbamate) as the chiral selectors, are also included. For some racemates, the derivatives **2a** and **2b** bearing a 4-*tert*-butylbenzoyl group at the 2-position showed equivalent or higher chiral recognition compared to Chiralpak IA, IB, and IC. Racemates **5** and **7**, which cannot be resolved efficiently on the three commercial immobilized columns, were better resolved on both **2a** and **2b**. Racemate **9**, which is rather difficult to resolve on Chiralpak IA and IB, was sufficiently resolved on both **2a** and **2b**, and these two CPMs both exhibited a much higher recognition ability for racemate **7** compared to other commercially available Daicel columns (Table 2). For preparation of the immobilized CPM with high chiral recognition as well as coated CPM, it is preferable for the R^3 residue content to be as low as possible. However, as shown in Table 2, the immobilization efficiency decreased as the R^3 content decreased. Therefore, the CPM obtained from **2b** bearing 2% R^3 at 3- and 6-positions seems to be better than that from **2a** bearing 1% R^3. If the immobilization efficiency is low, bare silica surfaces must reduce the chiral recognition through nonchiral interaction.

Under the same chromatographic conditions using a standard eluent consisting of hexane/2-propanol mixtures, **2b**-IM showed chiral recognition comparable to **1**-coated CPM (Table 3). The higher order structure of **2b** after the immobilization seems to be similar to that of **1**-coated on the silica gel.

Table 1. Resolution of racemates on **2**-immobilized CPMs,[a] Chiralpak IA, IB, and IC[b]

Racemates	CPMs				α		
	2a-IM ($R^2/R^3 = 99/1$), 70%[c]		**2b**-IM ($R^2/R^3 = 98/2$), 89%[c]		Chiralpak IA[b]	Chiralpak IB[b]	Chiralpak IC[b]
	k_1'	α	k_1'	α			
3	0.70	1.17 (+)	0.62	1.20 (+)	1.46 (+)	1.22 (+)	1.52 (+)
4	0.41	1.73 (−)	0.45	2.25 (−)	2.71 (+)	1.77 (−)	1.97 (+)
5	2.37	1.42 (−)	2.58	1.45 (−)	1.15 (−)	1.33 (+)	1.12 (−)
6	0.56	1.17 (+)	0.58	1.21 (+)	2.07 (+)	1.22 (+)	1.26 (+)
7	1.90	1.85 (−)	2.20	2.08 (−)	1.06 (−)	1.14 (−)	1.28 (−)
8	0.25	1.44 (+)	0.39	≈1 (+)	≈1 (+)	2.42 (−)	1.53 (−)
9	0.63	1.21 (−)	0.63	1.28 (−)	≈1 (−)	≈1 (+)	1.96 (+)
10	1.69	≈1	1.65	1.22 (+)	1.17 (+)	1.26 (−)	1.16 (−)
11	0.45	1.87 (+)	0.38	1.84 (+)	2.06 (+)	1.89 (+)	1.32 (+)

[a]Column: 25 × 0.20 cm (i.d.); Flow rate: 0.1 mL min^{-1}; Eluent: hexane/2-propanol (90/10, v/v). [b]Data taken from Ref. 9. Column: 25 × 0.46 cm (i.d.); Flow rate: 0.5 mL min^{-1}; Eluent: hexane/2-propanol (90/10, v/v). The signs in parentheses indicate the optical rotation of the first-eluted enantiomer. [c]Immobilization efficiency.

Table 2. Separation factors (α) of racemate **7** on Daicel columns,[a] **1**-coated CPM,[b] and immobilized **2a**-IM and **2b**-IM[b]

Daicel Chiralcel or Chiralpak							**1**-Coated	**2a**-IM	**2b**-IM
OD	AD	OB	OJ	IA	IB	IC			
1.13	1.03	1.65	1.33	1.06	1.14	1.28	2.15	1.85	2.08

[a]Data taken from Ref. 6 and Ref. 9. Column: 25 × 0.46 cm (i.d.); Flow rate: 0.5 mL min^{-1}. [b]Column: 25 × 0.20 cm (i.d.); Flow rate: 0.1 mL min^{-1}; Eluent: hexane/2-propanol = 9/1, v/v.

The development of the separation for racemate **3** was found by using chloroform and tetrahydrofuran (THF), which are prohibited eluents for **1**-coated CPM, and four racemates including **3**, **6**, **9**, and **10** could be slightly better resolved on the **2b**-IM than on the **1**-coated CPM.

For comparison, the results on a coated CPM consisting of amylose tris(3,5-dichlorophenylcarbamate) as the chiral selector,[13] are also shown in Table 3. The **2b**-IM CPM showed better chiral recognition for seven racemates except for **3** and **6** when compared with this 3,5-dichlorophenylcarbamate CPM at all three positions, and for the resolution of **4**, **5**, **9**, and **11**, the elution order of each enantiomer was reversed on the former. These results indicate that the substituent at 2-position may significantly affect the chiral recognition of the amylose CSPs and the elution order of the enantiomers. The extended use of THF in the eluents also reversed the elution orders of the enantiomers **3**, **4**, **7**, and **9** on the **2b**-IM in comparison with the **1**-coated CPM, which implied the high sensitivity of the stereoselectivity to the mobile phase composition and also agreed with previous reports.[9] This improvement of chiral recognition and reversed elution orders may partially be attributed to the conformation alteration of the amylose derivative in these eluents.

In this study, novel immobilized amylose derivatives bearing 4-*tert*-butylbenzoate at the 2-position and 3,5-dichlorophenylcarbamate/3-(triethoxysilyl)propylcarbamate at 3- and 6-positions were prepared and evaluated as CPMs for HPLC resolution of 9 racemates. The immobilized derivatives containing 1–2% of the 3-(triethoxysilyl)propylcarbamate residue showed high chiral recognition abilities comparable to the conventional coated CPMs and the Chiralpak IA, IB, and IC. Particularly, the novel immobilized CPM could efficiently resolve several racemates, which are rather difficult to resolve on commercially available columns.

Table 3. Separation factors (α) on **2b**-IM,[a] **1**-coated CPM,[b] and amylose tris(3,5-dichlorophenylcarbamate)[c]

Racemates	**2b**-IM CPM			**1**-Coated[b]	Amylose tris(3,5-dichlorophenyl carbamate)[c]
	H/I (90/10)	H/C/I (90/10/1)	H/T/I (90/10/1)	H/I (90/10)	H/I (90/10)
3	1.20 (+)	1.51 (+)	1.33 (−)	1.19 (+)	1.34 (+)
4	2.25 (−)	1.28 (−)	1.61 (+)	2.50 (−)	1.32 (+)
5	1.45 (−)	1.24 (−)	1.36 (−)	1.51 (−)	≈1 (+)
6	1.21 (+)	1.21 (+)	1.19 (+)	1.20 (+)	2.25 (+)
7	2.08 (−)	1.29 (−)	1.71 (+)	2.19 (−)	≈1 (−)
8	≈1 (+)	≈1	≈1	≈1	1.00
9	1.28 (−)	1.12 (−)	1.23 (+)	1.20 (−)	≈1 (+)
10	1.22 (+)	1.15 (+)	1.16 (+)	1.21 (+)	1.10 (+)
11	1.84 (+)	≈1 (+)	≈1	1.92 (+)	1.11 (−)

[a]Column: 25 × 0.20 cm (i.d.); Flow rate: 0.1 mL min^{-1}; Eluent: H, hexane; I, 2-propanol; C, chloroform; T, tetrahydrofuran. [b]Data taken from Ref. 6. [c]Data taken from Ref. 13. Column: 25 × 0.46 cm (i.d.); Flow rate: 0.5 mL min^{-1}. The signs in parentheses indicate the optical rotation of the first-eluted enantiomer.

This work was partially supported by Daicel Chemical Industries (Tokyo, Japan).

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