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Synthesis and chiral recognition of novel amylose derivatives containing regioselectively benzoate and phenylcarbamate groups

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ABSTRACT

A new class of regioselectively substituted amylose derivatives bearing three different substituents at 2-, 3- and 6-positions, and two different substituents at 2-position and 3-, 6-positions were synthesized by a sequential process based on the esterification of 2-position of a glucose unit. Their chiral recognition abilities were evaluated as chiral stationary phases (CSPs) for high-performance liquid chromatography (HPLC). Each derivative had its own characteristic recognition ability depending on the arrangement of side chains at the three positions. Among the derivatives, amylose 2-(4-*t*-butylbenzoate) and amylose 2-(4-chlorobenzoate) series exhibited high chiral recognition. Some racemates can be efficiently separated on these derivatives as well as on the amylose tris-3,5-dimethylphenylcarbamate, which is commercially available as Chiralpak AD and one of the most powerful CSPs. The structures of the amylose derivatives were also investigated by circular dichroism spectroscopy.

1. Introduction

Polysaccharides, such as cellulose and amylose, are among the most important and abundant natural biopolymers on the earth and are optically active. More than 90% of the enantiomeric excess determinations by high-performance liquid chromatography (HPLC), which is known to be the most attractive method, are performed using the polysaccharide-based chiral stationary phases (CSPs) [1,2]. Among them, phenylcarbamates and benzoate derivatives of cellulose and amylose appear to be some of the most useful CSPs [3–10]. These derivatives usually have the same substituents at the 2-, 3- and 6-positions of a glucose ring, and the regioselective derivatization of polysaccharides has been restricted only between 6-position and 2-, 3-positions [11]. However, the regioselective introduction of different substituents at 2- and 3-positions of a glucose ring had not been attained until recently. In 2004, Dicke reported the first regioselective esterification at 2-position of amylose [12]. On the basis of this method, the regioselective introduction of different substituents at 2-, 3- and 6-positions has been successfully performed in our group [13].

The amylose derivatives bearing different substituents regioselectively at three hydroxy groups must be valuable systems for studying the relationship between the structures of polysaccharide derivatives and their chiral recognition abilities. The recognition ability of polysaccharide derivatives, such as phenylcarbamates and benzoates, significantly depends on the nature and position of the substituents on the phenyl moieties [8,14–23]. These substituents may change the structure and local polarity of the polysaccharide derivatives [8,10].

This work was carried out in order to develop the efficient CSPs based on polysaccharide derivatives for HPLC and to elucidate the chiral recognition mechanism on the obtained CSPs. For this purpose, ten derivatives bearing two different substituents at 2-position and 3-, 6-positions, and ten amylose derivatives bearing three different substituents at 2-, 3- and 6-positions (Fig. 1) were prepared, and their chiral recognition abilities were evaluated as CSPs in HPLC. The effects of the nature of substituents at 2-position on chiral recognition abilities were studied. The structures of the amylose derivatives were also investigated by circular dichroism spectroscopy.

2. Experimental

2.1. Chemicals

Amylose (DP = 300) and 3,5-dimethylphenyl isocyanate were gifts from Daicel Chemical Industries (Tokyo, Japan). Vinyl acetate and dehydrated solvents, such as acetonitrile, tetrahydrofuran (THF) and pyridine, were purchased from Kanto (Tokyo, Japan). 4-Phenylbenzoic acid, 4-fluorobenzoic acid, 4-chlorobenzoic acid,

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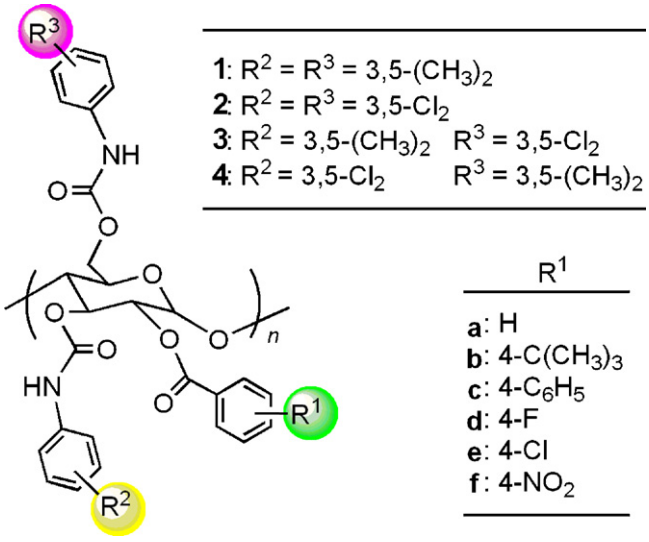

Fig. 1. Structure of amylose derivatives as CSPs for HPLC.

4-nitrobenzoic acid and 3,5-dichlorophenyl isocyanate were obtained from Tokyo Kasei (Tokyo, Japan). Palladium(II) chloride was obtained from Aldrich (USA), vinyl 4-*t*-butylbenzoate and dehydrated dimethyl sulfoxide (DMSO) from Wako (Tokyo, Japan), and anhydrous lithium chloride from Nacalai Tesque (Tokyo, Japan). Various vinyl 4-substituted benzoates were prepared by palladium(II) catalyzed exchange reactions of corresponding benzoic acids and vinyl acetate according to the literature method [24]. Wide-pore silica gel (Daiso gel SP-1000) with a mean particle size of 7 μm and a mean pore diameter of 100 nm, which was kindly supplied by Daiso Chemical (Osaka, Japan), was silanized using (3-aminopropyl)triethoxysilane in toluene at 80 °C. The solvents used in chromatographic experiments were of HPLC grade. The racemates were commercially available or prepared by the usual methods.

2.2. Synthesis of amylose derivatives bearing different substituents at 2-, 3- and 6-positions

The amylose derivatives **1** and **2** bearing two different substituents at 2-position and 3-, 6-positions were synthesized by the following procedure based on the regioselective esterification of 2-position reported by Dicke [12]. To regioselectively esterify only the 2-position, the amylose (3.0 g) was first dissolved in DMSO (60 mL) at 80 °C. Then, vinyl 4-substituted benzoate (2.3 equiv to 2-position) and Na_2HPO_4 (2 wt%, as the catalyst) were added to the solution at 40 °C, and the reaction was continued for enough time (4–200 h) to completely esterify the hydroxy group at 2-position depending on the substituents of the benzoate. The reaction mixture was then added into a large excess of 2-propanol, and the product was isolated as an insoluble fraction; yields were 70–100%. The hydroxy groups at 3- and 6-positions were then changed to the phenylcarbamates using either 3,5-dimethylphenyl or 3,5-dichlorophenyl isocyanate.

The amylose derivatives **3** and **4** bearing three regioselective substituents at 2-, 3- and 6-positions were prepared in a sequential process, as shown in Fig. 2. The hydroxy group at 2-position was first regioselectively esterified to the benzoate by the same method as that for **1** and **2**. The obtained mono ester was then allowed to react with 4-methoxytriphenylmethyl chloride in pyridine at 70 °C to protect selectively 6-position as trityl ether. After 24 h, an excess of either 3,5-dimethylphenyl or 3,5-dichlorophenyl isocyanate was added, and the reaction was

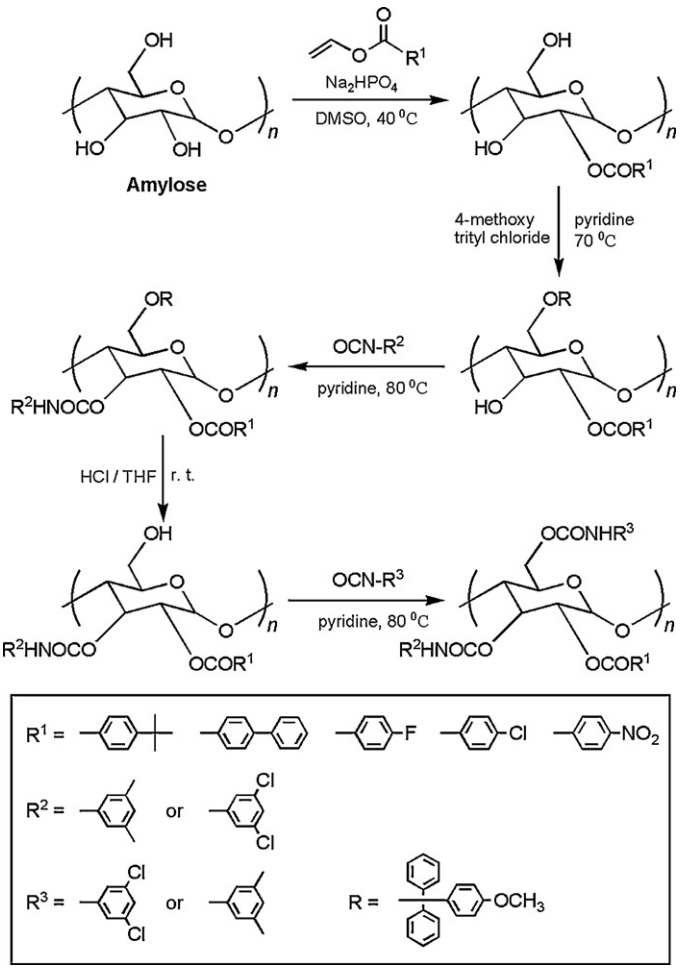

Fig. 2. Scheme of the synthesis of amylose derivatives (**3**–**4**).

continued for 14 h at 80 °C to convert the 3-hydroxy group to the corresponding phenylcarbamate group. The product was isolated as a methanol-insoluble fraction; yields were 75–100%. Subsequently, the obtained 2-benzoyl-3-(3,5-dimethylphenylcarbamoyl or 3,5-dichlorophenylcarbamoyl)-6-*O*-trityl amylose was suspended in THF containing a small amount of hydrochloric acid (1.8 vol% of THF) to cleave the triphenylmethyl group at room temperature. Finally, the hydroxyl group at 6-position of the recovered amylose 2-benzoyl-3-(3,5-dimethyl) or (3,5-dichloro)phenylcarbamate was treated with an excess of 3,5-dichlorophenyl or 3,5-dimethylphenyl isocyanate for 14 h at 80 °C. Ten amylose derivatives **3a**–**f** and **4a**–**f** bearing different substituents at 2-, 3- and 6-positions were isolated as a methanol-insoluble fraction. The elemental analysis data are summarized in Table 1.

2.3. Preparation of packed columns

The amylose derivatives **1**, **2**, **3** and **4** (0.35 g each) dissolved in THF (8 mL) were coated on aminopropyl silanized silica gel (1.40 g) according to the previous method [8]. The weight ratio of the derivatives to silica gel was approximately 1:4. The **1**-, **2**-, **3**- and **4**-coated silica gels were then packed in a stainless-steel tube (25 × 0.20 cm i.d.) by a slurry technique. The plate numbers of the packed columns was 1100–1800 for benzene using a hexane/2-propanol (90:10, v/v) mixture as the eluent at the flow rate of 0.1 mL/min. 1,3,5-Tri-*t*-butylbenzene was used as a non-retained compound to estimate the dead time (t_0) [25].

Table 1
Elemental analysis of amylose derivatives.

Derivatives	Calculated (%)[a]			Found (%)			Derivatives	Calculated (%)[a]			Found (%)		
	C	H	N	C	H	N		C	H	N	C	H	N
1b	68.17	6.54	4.54	68.04	6.32	4.72	**3b**	60.28	5.21	4.26	60.15	5.08	4.44
1c	69.80	5.70	4.40	69.59	5.81	4.61	**3c**	62.05	4.46	4.13	61.98	4.52	4.32
1d	64.35	5.40	4.84	64.36	5.47	5.06	**3d**	56.23	4.07	4.52	56.09	3.64	4.76
1e	62.57	5.25	4.71	62.31	5.02	4.94	**3e**	54.78	3.96	4.41	54.72	3.77	4.64
1f	62.35	5.87	6.61	62.21	5.08	6.92	**3f**	55.04	4.62	6.21	54.85	3.78	6.51
2b	53.31	4.04	4.01	53.09	3.97	4.31	**4b**	60.28	5.21	4.26	60.09	5.15	4.38
2c	55.17	3.37	3.90	55.04	3.31	4.14	**4c**	62.05	4.46	4.13	61.85	4.48	4.32
2d	49.12	2.90	4.24	49.02	2.80	4.38	**4d**	56.23	4.07	4.52	56.24	4.05	4.72
2e	47.92	2.83	4.14	47.68	2.67	4.42	**4e**	54.78	3.96	4.41	57.76	4.13	4.83
2f	48.56	3.51	5.86	48.40	2.81	5.97	**4f**	55.04	4.62	6.21	54.84	3.89	6.38

[a] Estimated based on a repeated glucose unit.

Table 2
Regioselective esterification at 2-position of amylose derivatives.

4-Substituent on benzoate group of amylose derivatives	Reaction time (days)	Degree of substitution
H	10	>0.99
t-Butyl-	21	0.95
Phenyl-	192	0.91
Fluoro-	69	0.99
Chloro-	11	>0.99
Nitro-	4	>0.99

2.4. Apparatus and chromatography

Chromatographic experiments were performed using a JASCO PU-980 chromatograph equipped with UV (JASCO MD-2010) and polarimetric (JASCO OR-990, Hg-Xe without filter) detectors at room temperature. A solution of a racemate (3 mg/ml) was injected into the chromatographic system through a Rheodyne Model 7125 injector. The IR analyses were carried out using a JASCO FT-IR-460 spectrometer as a KBr pellet. The circular dichroism spectra were measured in THF solutions in a 0.2 mm quartz cell using a JASCO J-720 L spectropolarimeter. The ^{1}H NMR spectra (500 MHz) were recorded using a Varian INOVA-500 spectrometer (Varian, USA).

3. Results and discussion

3.1. Synthesis of amylose derivatives bearing two different substituents at 2-position and 3-, 6-positions, and three different substituents at 2-, 3- and 6-positions

The amylose derivatives **1a**–**f** and **2a**–**f** bearing two different substituents at 2-position and 3-, 6-positions were synthesized as follows: the amylose was first dissolved in DMSO, and 4-substituted vinyl benzoate was added to regioselectively esterify only the hydroxyl group at 2-position of the glucose unit in the presence of Na_2HPO_4 catalyst at 40 °C [12]. The reactivity of the vinyl benzoates depended significantly on the substituents of the benzoates, as shown in Table 2. Either 3,5-dimethylphenyl isocyanate or 3,5-dichlorophenyl isocyanate was then added to convert the 3- and 6-hydroxy groups to the corresponding phenylcarbamate groups.

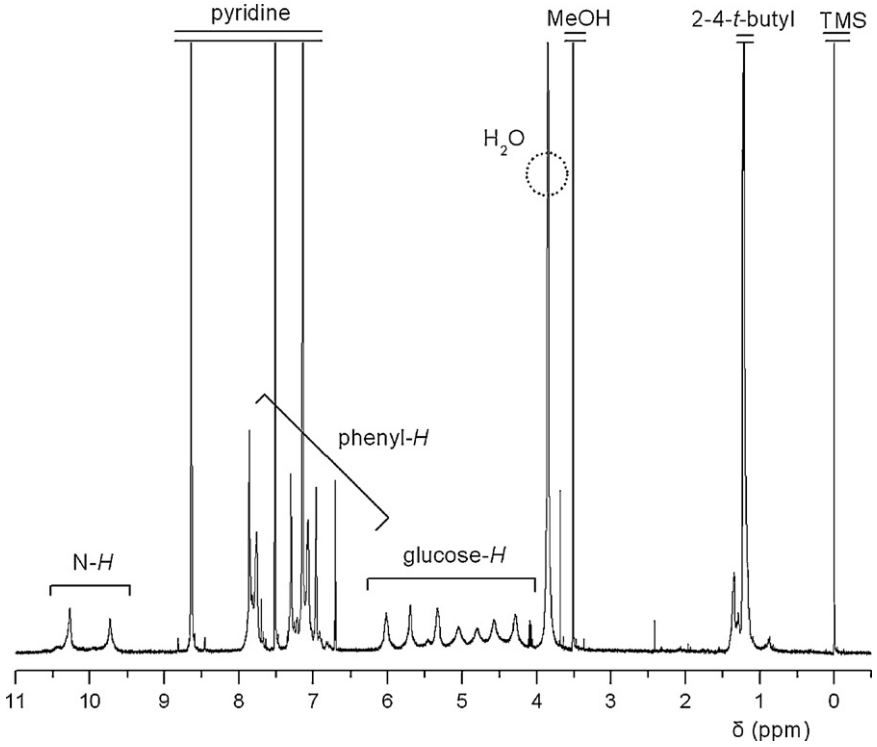

Fig. 3. ^{1}H NMR spectrum of amylose derivative **2b** in pyridine-d_5 at 80 °C.

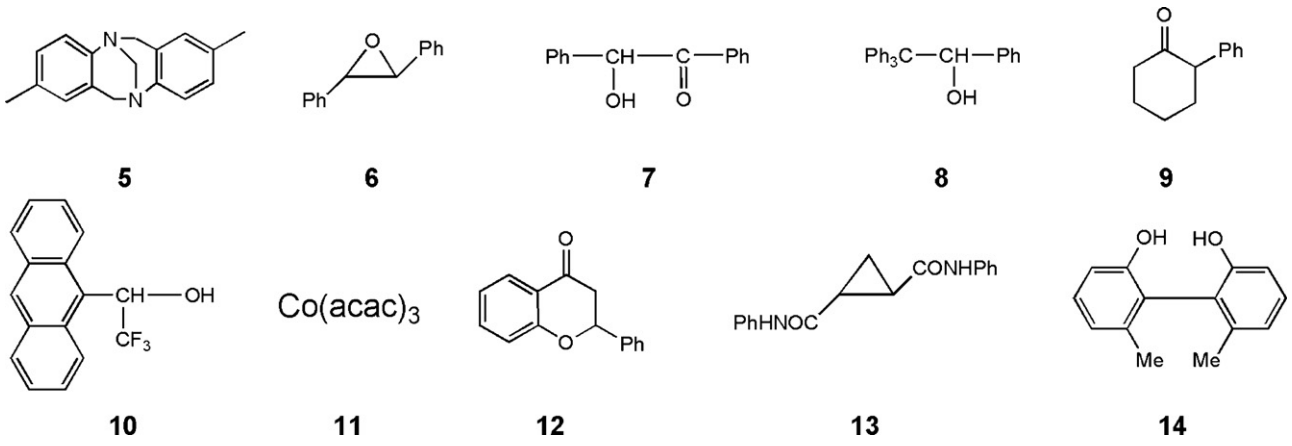

Fig. 4. Structures of racemates (**5**–**14**).

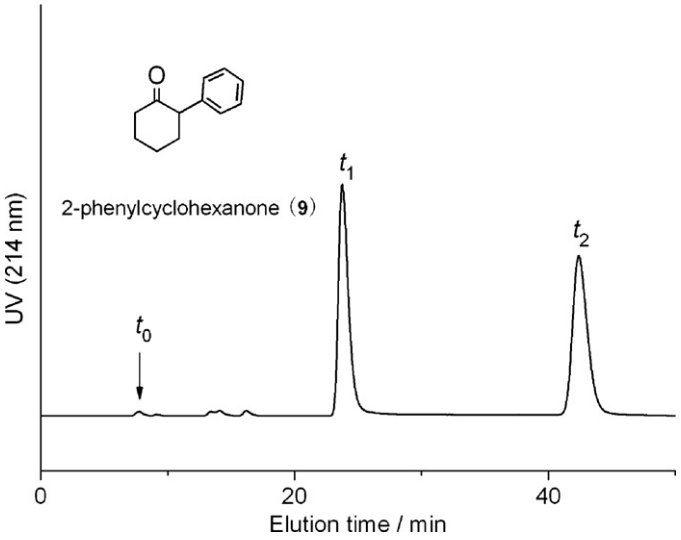

Fig. 5. Chromatogram for the resolution of **9** on amylose derivative **2b** coated CSP.

The derivatives **3a**–**f** and **4a**–**f** bearing three different substituents at 2-, 3- and 6-positions were prepared according to the process shown in Fig. 2. The hydroxyl group at 2-position was first regioselectively esterified to the benzoate by the same method as that for **1a**–**f** and **2a**–**f**. The 6-position was then selectively protected as trityl ether by the reaction with 4-methoxytriphenylmethyl chloride, and then either 3,5-dimethylphenyl isocyanate or 3,5-dichlorophenyl isocyanate was added to convert the 3-hydroxy group to the corresponding phenylcarbamate group. After the deprotection of the trityl group at 6-position with HCl/THF, the regenerated hydroxy group was converted to the carbamate using the corresponding isocyanate to obtain the new amylose derivatives. Fig. 3 shows the ^{1}H NMR spectrum of the obtained amylose derivative (**2b**). Characteristic peaks for the structure of **2b** can be assigned to the spectrum. The structures of other amylose derivatives were similarly confirmed by ^{1}H NMR and elemental analysis summarized in Table 1.

3.2. Chiral recognition ability of regioselective amylose derivatives bearing different groups at 2-, 3- and 6-positions

The amylose derivatives were coated on aminopropyl silanized macroporous silica gel, and the obtained chiral packing materials were packed into an HPLC column. Their chiral recognition abilities were evaluated with 10 racemates (**5**–**14**, Fig. 4).

Fig. 5 shows the chromatogram of the resolution of racemic 2-phenylcyclohexanone (**9**) 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 6.50 min. The retention factors, $k_1'((t_1-t_0)/t_0)$ and $k_2'((t_2-t_0)/t_0)$, were estimated to be 2.49 and 5.36, respectively, which resulted in the separation factor α (k_2'/k_1') to be 2.15.

The results of chromatographic resolutions of 10 racemates (**5**–**14**) on the CSPs **1a**–**f**, **2a**–**f**, **3a**–**f** and **4a**–**f** are shown in Tables 3–6, respectively. For comparison, the resolution results on the commercial amylose-based chiral column, Chiralpak AD, which is one of the most efficient CSPs consisting of the amylose tris-3,5-dimethylphenylcarbamate as the chiral selector, are

Table 3
Resolution of racemates (**5**–**14**) on amylose derivatives (**1a**–**f**)[a].

Racemates	1a		1b		1c		1d		1e		1f		Chiralpak AD[b]	
	k_1'	α	k_1'	α	k_1'	α	k_1'	α	k_1'	α	k_1'	α	k_1'	α
5	2.05 (+)	2.31	0.62 (+)	1.61	1.23 (+)	1.87	1.73 (+)	1.90	0.75 (+)	2.13	1.26 (+)	1.81	0.78 (+)	1.70
6	1.53 (−)	~1	0.43 (+)	~1	1.11 (+)	~1	1.45 (+)	1.15	0.71 (−)	~1	1.11 (−)	1.31	0.73 (+)	2.81
7	9.89 (−)	1.97	1.73	1.00	4.15 (−)	1.32	6.31 (−)	1.69	2.98 (−)	1.97	4.20 (−)	1.34	5.09 (−)	1.31
8	2.35 (+)	1.22	0.81 (+)	1.24	2.15 (+)	1.18	2.32 (+)	1.16	0.98 (+)	1.24	1.17 (+)	1.25	4.33 (+)	2.24
9	3.67 (+)	1.24	0.77 (−)	1.13	1.80 (−)	~1	2.23 (+)	1.27	1.16 (+)	1.23	1.94 (−)	~1	0.96 (−)	1.02
10	0.98 (+)	1.23	1.13 (+)	1.75	2.00 (+)	1.23	1.10 (+)	~1	0.71 (+)	~1	1.21 (+)	1.17	2.04 (+)	1.39
11	1.70 (−)	2.46	0.55 (−)	1.19	1.19 (−)	1.37	0.74 (−)	1.65	0.36 (−)	1.50	0.82 (−)	1.35	0.49	1.00
12	4.21 (+)	1.13	0.97 (+)	~1	2.84 (+)	~1	4.15 (+)	~1	1.81 (+)	~1	3.23 (+)	1.35	1.44 (+)	1.04
13	7.86 (+)	3.71	0.66 (+)	2.46	1.42 (+)	1.94	4.25 (+)	~1	1.46 (+)	2.66	1.21 (+)	1.39	3.38 (+)	1.59
14	3.45 (−)	1.12	1.24	1.00	2.25 (−)	~1			0.78	1.00	1.94 (−)	~1	3.35 (−)	2.22

[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. [13]. Column: 25 × 0.46 cm (i.d.); flow rate: 0.5 mL/min; eluent: hexane/2-propanol (90/10, v/v). The signs in parentheses inclicate the optical rotation of the first-elutecl enantiomer.

Table 4
Resolution of racemates (**5**–**14**) on amylose derivatives (**2a**–**f**)[a].

Racemates	**2a**		**2b**		**2c**		**2d**		**2e**		**2f**		Amylose tris-3.5-dichloro phenylcarbamate[b]	
	k_1'	α	k_1'	α	k_1'	α	k_1'	α	k_1'	α	k_1'	α	k_1'	α
5	1.92 (+)	2.21	3.09 (−)	1.53	1.29 (+)	1.41	1.71 (+)	1.39	1.02 (+)	1.70	1.49 (+)	1.59	0.98 (+)	1.34
6	1.32 (−)	1.44	0.80 (+)	1.16	0.76 (−)	1.50	0.90 (−)	1.83	0.55 (−)	2.02	1.05 (−)	1.87	0.59 (+)	1.32
7	8.75 (−)	1.89	3.09 (−)	1.54	5.66 (−)	1.92	5.29 (−)	2.04	3.33 (−)	~1	5.57 (−)	1.90	7.11 (+)	~1
8	1.79 (+)	1.34	0.67 (+)	1.21	1.17 (+)	1.42	1.02 (+)	2.21	0.70 (−)	1.56	0.91 (+)	~1	1.03 (+)	2.25
9	4.96 (+)	~1	2.49 (−)	2.15	3.53 (−)	1.07	2.21 (+)	1.22	0.94 (+)	1.63	3.09 (−)	~1	1.47 (−)	~1
10	0.73	1.00	0.51 (+)	~1	0.56 (+)	~1	0.49 (+)	~1	0.32 (−)	~1	0.71 (+)	~1	0.43	1.00
11	1.65 (−)	1.80	1.40 (−)	1.08	1.74 (−)	2.16	1.13 (+)	1.09	0.74 (+)	1.22	1.32 (−)	~1	0.74 (+)	~1
12	4.13 (−)	1.20	1.94 (+)	1.25	3.23 (+)	1.11	2.40 (+)	1.08	2.13 (−)	1.25	4.22 (+)	1.20	1.90 (+)	1.10
13	1.25 (+)	3.79	0.49 (+)	2.00	0.90 (+)	1.56	0.70 (+)	2.03	0.39 (+)	1.68	1.43 (+)	1.43	0.69 (−)	1.11
14	3.86	1.00	0.84 (+)	1.16	1.37 (−)	1.07	1.63 (+)	1.19	1.63 (+)	1.07	1.56 (−)	~1	1.29 (+)	ca.1

[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. [20]. Column: 25 × 0.46 cm (i.d.); flow rate: 0.5 mL/min; eluent: hexane/2-propanol (90/10, v/v). The signs in parentheses inclicate the optical rotation of the first-elutecl enantiomer.

Table 5
Resolution of racemates (**5**–**14**) on amylose derivatives (**3a**–**f**)[a].

Racemates	**3a**[b]		**3b**		**3c**		**3d**		**3e**		**3f**		Chiralpak AD[b]	
	k_1'	α	k_1'	α	k_1'	α	k_1'	α	k_1'	α	k_1'	α	k_1'	α
5	1.28 (+)	1.85	0.87 (+)	1.95	1.24 (+)	1.26	2.20 (+)	1.54	1.45 (+)	1.64	1.52 (+)	1.76	0.78 (+)	1.70
6	0.82 (−)	1.30	2.08 (+)	~1	0.91 (−)	1.11	1.42 (−)	1.50	1.13 (−)	1.45	1.35 (−)	1.48	0.73 (+)	2.81
7	3.84 (−)	1.73	3.94 (−)	1.45	4.28 (−)	1.35	7.41 (−)	1.83	5.10 (−)	~1	3.72 (−)	1.78	5.09 (−)	1.31
8	1.52 (+)	1.07	1.24 (−)	~1	1.53 (+)	1.22	2.34 (+)	1.61	1.42 (+)	1.17	1.45 (+)	1.14	4.33 (+)	2.24
9	2.27 (−)	1.15	4.27 (−)	~1	2.25 (−)	1.30	4.67 (+)	1.11	3.46 (−)	1.09	3.74 (−)	1.10	0.96 (−)	1.02
10	0.76 (−)	1.18	1.03 (+)	~1	1.37 (+)	1.11	0.99 (+)	~1	0.80 (+)	~1	0.99 (+)	~1	2.04 (+)	1.39
11	1.16 (−)	1.75	0.91 (−)	1.42	1.53 (−)	2.09	1.57 (−)	1.31	0.87 (−)	1.79	1.15 (−)	~1	0.49	1.00
12	2.73 (−)	1.17	5.56 (+)	1.23	2.61 (+)	1.12	4.54 (+)	1.13	3.57 (+)	1.28	4.50 (+)	1.22	1.44 (+)	1.04
13	1.53 (+)	3.21	1.33 (+)	1.80	1.49 (+)	1.27	1.33 (+)	2.13	1.98 (+)	1.72	1.52 (+)	1.51	3.38 (+)	1.59
14	2.36 (−)	1.11	2.09	1.00	2.19 (−)	1.07	2.43 (+)	1.06	1.81 (−)	1.14	1.63 (−)	~1	3.35 (−)	2.22

[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. [13]. Column: 25 × 0.46 cm (i.d.); Flow rate: 0.5 mL/min; eluent: hexane/2-propanol (90/10, v/v). The signs in parentheses inclicate the optical rotation of the first-elutecl enantiomer.

also included. Although the derivatives **1a**–**f** have the same 3,5-dimethylphenycarbamate group at 3- and 6-positions, their recognition abilities greatly varied depending on the substituents of the benzoate at 2-position, as shown in Table 3. Racemates **5**, **7**, **9**, **11** and **13** were better resolved on **1a**–**f** than on Chiralpak AD. Especially, racemate **11**, which cannot be resolved efficiently on Chiralpak AD and other commercially available columns, was completely resolved on **1a**–**f**. However, the chiral recognition powers of **1a**–**f** declined for racemates **6**, **8** and **14** with a polar group capable of hydrogen bonding to the carbamate residue of the phenylcarbamates. This result indicates that the substituent at 2-position is important for the chiral recognition of the amylose derivatives. The retention factors, k_1', for **5**, **9**, **11** and **12** were much larger than those for other racemates on the CSPs **1a**–**f**, suggesting that the interactions between **5**, **9**, **11** or **12** and the benzoate moieties of the derivatives **1a**–**f** are stronger than those of other derivatives and may not be significantly influenced by the substituents of the benzoate at 2-position.

The resolution results of 10 racemates on the CSPs **2a**–**f** are shown in Table 4, together with those on amylose tris-3,5-dichlorophenylcarbamate. Most racemates were better resolved on CSPs **2a**–**f** except **8**, and the retention factor k_1' also become longer for most of the racemates except **7** and **8** on CSPs **2a**–**f**, indicating that the interactions between enantiomeric pairs and the benzoate moieties of the amylose derivatives **2a**–**f** were stronger than those on amylose tris-3,5-dichlorophenylcarbamate. The elution orders

Table 6
Resolution of racemates **(5–14)** on amylose derivatives (**4a**–**f**)[a].

Racemates1	**4a**[b]		**4b**		**4c**		**4d**		**4e**		**4f**		Chiralpak AD[b]	
	k_1'	α	k_1'	α	k_1'	α	k_1'	α	k_1'	α	k_1'	α	k_1'	α
5	2.05 (+)	2.31	0.44 (+)	1.58	1.22 (+)	2.51	1.15 (+)	2.67	1.64 (+)	2.86	1.22 (+)	1.97	0.78 (+)	1.70
6	1.53 (−)	~1	0.33 (−)	2.42	0.94 (−)	1.38	1.13 (−)	~1	1.30 (−)	1.40	1.01 (−)	1.73	0.73 (+)	2.81
7	9.89 (−)	1.97	1.53 (−)	1.85	5.65 (−)	1.80	5.37 (−)	1.85	7.68 (−)	~1	6.04 (−)	1.62	5.09 (−)	1.31
8	2.35 (+)	1.22	0.48 (+)	1.48	1.64 (+)	1.34	1.25 (+)	1.27	1.37 (+)	1.31	0.94 (+)	1.15	4.33 (+)	2.24
9	3.67 (+)	1.24	1.08 (−)	1.40	3.16 (+)	~1	2.71 (+)	1.29	3.85 (−)	1.26	3.22 (−)	~1	0.96 (−)	1.02
10	0.98 (+)	1.23	0.40 (+)	~1	0.83 (+)	~1	0.51 (+)	~1	0.56 (+)	~1	0.74 (+)	~1	2.04 (+)	1.39
11	1.70 (−)	2.46	0.49 (−)	1.18	1.22 (−)	1.45	0.69 (−)	1.63	0.87 (−)	1.79	0.96 (−)	1.30	0.49	1.00
12	4.21 (+)	1.13	1.08 (+)	1.15	3.44 (−)	~1	3.00 (+)	1.04	3.73 (+)	1.11	3.24 (+)	1.20	1.44 (+)	1.04
13	7.86 (+)	3.71	0.34 (+)	1.99	0.98 (+)	1.73	4.87 (+)	1.17	2.69 (+)	1.30	0.92 (+)	1.41	3.38 (+)	1.59
14	3.45 (−)	1.12	0.47	1.00	2.30	1.00	1.85 (−)	~1	3.32 (−)	~1	1.63 (−)	~1	3.35 (−)	2.22

[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. [13]. Column: 25 × 0.46 cm (i.d.); flow rate: 0.5 mL/min; eluent: hexane/2-propanol (90/10, v/v). The signs in parentheses inclicate the optical rotation of the first-elutecl enantiomer.

Table 7
Resolution of racemates (**5**–**14**) on amylose derivatives (**1b**–**4b**)[a].

Racemates	**1b**		**2b**		**3b**		**4b**	
	k_1'	α	k_1'	α	k_1'	α	k_1'	α
5	0.62 (+)	1.61	3.09 (−)	1.53	0.87 (+)	1.95	0.44 (+)	1.58
6	0.43 (+)	~1	0.80 (+)	1.16	2.08 (+)	~1	0.33 (−)	2.42
7	1.73	1.00	3.09 (−)	1.54	3.94 (−)	1.45	1.53 (−)	1.85
8	0.81 (+)	1.24	0.67 (+)	1.21	1.24 (−)	~1	0.48 (+)	1.48
9	0.77 (−)	1.13	2.49 (−)	2.15	4.27 (−)	~1	1.08 (−)	1.40
10	1.13 (+)	1.75	0.51 (+)	~1	1.03 (+)	~1	0.40 (+)	~1
11	0.55 (−)	1.19	1.40 (−)	1.08	0.91 (−)	1.42	0.49 (−)	1.18
12	0.97 (+)	~1	1.94 (+)	1.25	5.56 (+)	1.23	1.08 (+)	1.15
13	0.66 (+)	2.46	0.49 (+)	2.00	1.33 (+)	1.80	0.34 (+)	1.99
14	1.24	1.00	0.84 (+)	1.16	2.09	1.00	0.47	1.00

[a] Column: 25 × 0.20 cm (i.d.); flow rate: 0.1 mL/min; eluent: hexane/2-propanol (90/10, v/v). The signs in parentheses indicate the optical rotation of the first-eluted enantiomer.

Table 8
Resolution of racemates (**5**–**14**) on amylose derivatives (**1e**–**4e**)[a].

Racemates	**1e**		**2e**		**3e**		**4e**	
	k_1'	α	k_1'	α	k_1'	α	k_1'	α
5	0.75 (+)	2.13	1.02 (+)	1.70	1.45 (+)	1.64	1.64 (+)	2.86
6	0.71 (−)	~1	0.55 (−)	2.02	1.13 (−)	1.45	1.30 (−)	1.40
7	2.98 (−)	1.97	3.33 (−)	~1	5.10 (−)	~1	7.68 (−)	~1
8	0.98 (+)	1.24	0.70 (−)	1.56	1.42 (+)	1.17	1.37 (+)	1.31
9	1.16 (+)	1.23	0.94 (+)	1.63	3.46 (−)	1.09	3.85 (−)	1.26
10	0.71 (+)	~1	0.32 (−)	~1	0.80 (+)	~1	0.56 (+)	~1
11	0.36 (−)	1.50	0.74 (+)	1.22	0.87 (−)	1.79	0.87 (−)	1.79
12	1.81 (+)	~1	2.13 (−)	1.25	3.57 (+)	1.28	3.73 (+)	1.11
13	1.46 (+)	2.66	0.39 (+)	1.68	1.98 (+)	1.72	2.69 (+)	1.30
14	0.78	1.00	1.63 (+)	1.07	1.81 (−)	1.14	3.32 (−)	~1

[a] Column: 25 × 0.20 cm (i.d.); flow rate: 0.1 mL/min; eluent: hexane/2-propanol (90/10, v/v). The signs in parentheses indicate the optical rotation of the first-eluted enantiomer.

of some enantiomeric pairs were reversed depending on the substituents of the benzoate at 2-position, suggesting that the higher order structures of the derivatives **2a**–**f** may be changed through the introduction of different substituents at 2-position.

As shown in Table 5, racemates **5**, **7**, **9**, **11**, **12** and **13** were better resolved on the CSPs **3a**–**f** than the other racemates. Similarly, the recognition ability of the regioselective derivatives **4a**–**f** was also high for these racemates (Table 6). Both **3a**–**f** and **4a**–**f** exhibit high chiral recognition for the racemates **5**, **7**, **12** and **13**, though these derivatives have reversed substituents at 3- and 6-positions. All 10 racemates can be resolved on the derivative **3c**, which appears to be a useful CSP with wide versatility. Retention factor, k_1', was much longer for **7**, **9** and **12** with a carbonyl group than other racemates on derivatives **3a**–**f**, and was not significantly influenced by the substituents on the benzoates. The hydrogen bond interaction between the carbonyl group and the NH groups of **3a**–**f** may play some role in this interaction.

3.3. Effect of three different substituents at 2-, 3- and 6-positions on recognition ability

The electronic properties, such as the electron withdrawing and electron donating powers, and the bulkiness of the substituents on the phenyl moiety may also affect the chiral recognition. As for **3d** and **3e** (Table 5), having halogen substituents on the benzoyl group at 2-position, showed better recognition ability than those having *t*-butyl and phenyl substituents, **3b** and **3c**, respectively. On the other hand, **3f** having the stronger electron withdrawer, nitro

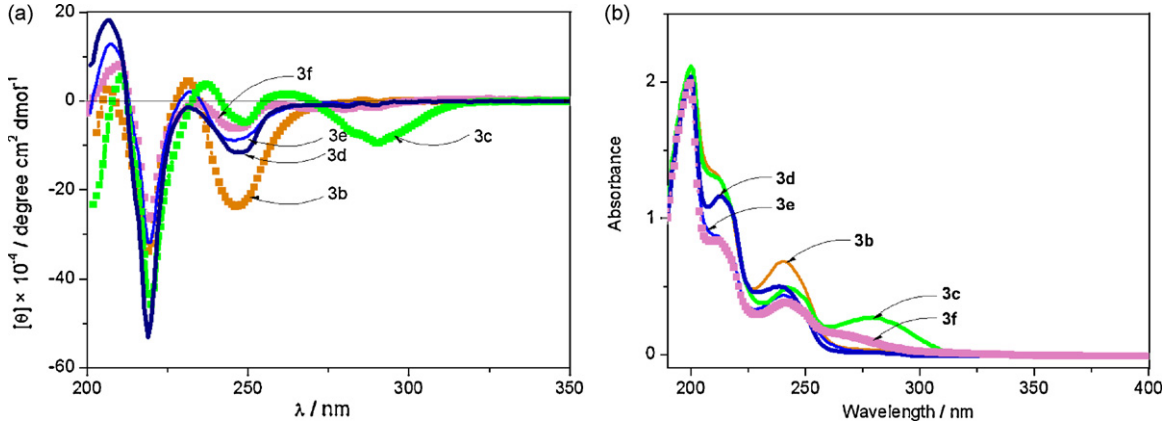

Fig. 6. (a) CD spectra of amylose derivatives (**3b**–**f**) in THF solutions. Cell length, 0.1 mm; concentration, 1.0×10^{-3} M. (b) UV spectra of amylose derivatives (**3b**–**f**) in THF solutions. Cell length, 0.1 mm; concentration, 1.0×10^{-3} M.

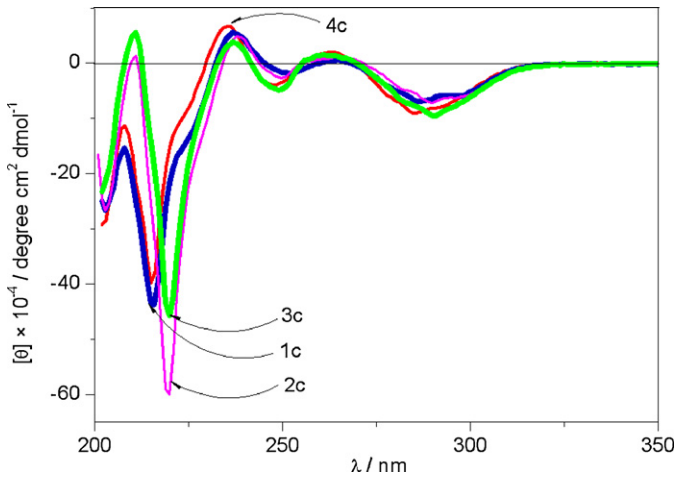

Fig. 7. CD spectra of the amylose derivatives (**1c**–**4c**) in THF solutions. Cell length, 0.1 mm; concentration, 1.0×10^{-3} M.

group, showed lower resolving ability due to the high polarity of the substituent itself. This effect of the substituents agrees with the observation in our pervious work [8].

Compared with **3a** and **4a** without a substituent on the benzoate, **3b**–**f** and **4b**–**f** show lower chiral recognition. Especially, racemate **10**, which can be resolved on **3a** and **4a**, cannot be separated on **3b**–**f** and **4b**–**f**, except for **3c**. In addition, the substituents of the benzoate significantly influence the chiral recognition ability and the elution orders of some enantiomeric pairs. These reversed elution orders have been attributed to the change of a higher order structure of the derivatives. On the contrary, derivatives **4b**–**f** show the same elution orders regardless of the substituents, except for racemates **9** and **12** on **4d** (Table 6). These results suggest that **4b**–**f** may have a similar higher order structure.

Among the novel derivatives, amylose 2-(4-*t*-butylbenzoate) **1b**–**4b** and amylose 2-(4-chlorobenzoate) series **1e**–**4e** exhibited relatively high recognition abilities. The resolving abilities for the 10 racemates are shown in Tables 7 and 8, respectively. The derivatives **1b**–**4b** with the same 4-(*t*-butyl) benzoyl group at 2-position exhibited almost the consistent elution orders except for racemates **5**, **6** and **8**. However, their chiral recognition abilities differ greatly depending on the substituents at 3- and 6-positions, and each derivative could show the highest α values for two or three racemates. For instance, **3b** showed the highest α values for compounds **5** and **11**. This means that all the amylose derivatives are valuable as CSPs. As for the derivatives **1e**–**4e** shown in Table 8, similar conclusion can be derived.

3.4. CD Spectra of regioselective amylose derivatives

The higher order structures of derivatives **3b**–**3f** were examined by CD spectroscopy (Fig. 6a)), referring to their UV spectra (Fig. 6(b)). The pattern of each CD spectrum is rather similar, though wavelengths of the peak tops and intensities depended on the benzoate substituents at 2-position. These derivatives seem basically to have similar structures, though the following differences were observed. Interestingly, **3b** with 4-*t*-butylbenzoate substituent shows a much higher intensity around 241 nm than others, indicating that **3b** may possess a slightly different higher-order structure from others. This different higher-order structure of **3b** may be associated with the reverse elution orders of the enantiomers of **6** and **8** between **3b** and other amylose derivatives **3c**–**3f**. On the other hand, CSP **3c** bearing 4-phenylbenzoate group shows the red shift of the wavelength, which may be related to its conjugated structure. And the resolution of racemate **10** on **3c**, which cannot be separated by other amylose derivatives **3b**, **3d**, **3e** and **3f**, indicates that the site around the biphenyl group may be important for the recognition of this kind of enantiomers.

On the other hand, the spectral patterns of the derivatives **1c**–**4c**, which have the same 4-phenylbenzoate at 2-position and different substituents at 3- and 6-positions are similar to each other, as shown in Fig. 7, though the wavelength and intensity of the peak tops depend slightly on the substituents. This result implies that though the higher order structures of the amylose derivatives are rather similar, the chiral recognition ability can be significantly different depending on the electronic and steric effects of the substituents at 3- and 6-positions.

4. Conclusions

Novel regioselectively substituted amylose derivatives were prepared through the selective esterification of the 2-position of the glucose unit, followed by 3,5-dimethyl- or 3,5-dichlorophenylcarbamoylation at 3- and 6-positions, and their chiral recognition abilities were evaluated as CSPs for HPLC. Each substituent at different positions plays a significant role in the enantioselectivity and the elution order of enantiomers. The chiral recognition abilities of the amylose derivatives were subtly influenced by the electronic properties and steric effect of substituents and also by the higher order structure of the derivatives. Some of the derivatives could efficiently resolve several racemates. Among the derivatives, amylose 2-(4-*t*-butylbenzoate) and amylose 2-(4-chlorobenzoate) series bearing the different phenylcarbamates at 3- and 6-positions exhibited relatively high chiral recognition.

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References

[1] Y. Okamoto, T. Ikai, Chem. Soc. Rev. 37 (2008) 2593.
[2] Y. Okamoto, J. Polym. Sci. Part A. Polym. Chem. 47 (2009) 1731.
[3] T. Ikai, C. Yamamoto, M. Kamigaito, Y. Okamoto, Chem. Asian J. 3 (2008) 1494.
[4] R.W. Stringham, Adv. Chromatogr. 44 (2006) 257.
[5] E. Francotte, J. Chromatogr. A 906 (2001) 379.
[6] T. Ikai, C. Yamamoto, M. Kamigaito, Y. Okamoto, Polym. J. 38 (2006) 91.
[7] X.M. Chen, C. Yamamoto, Y. Okamoto, Pure Appl. Chem. 79 (2007) 1561.
[8] Y. Okamoto, M. Kawashima, K. Hatada, J. Chromatogr. 363 (1986) 173.
[9] Y. Okamoto, E. Yashima, Angew. Chem. Int. Ed. 37 (1998) 1020.
[10] P. Mobian, C. Nicolas, E. Francotte, T. Burgi, J. Lacour, J. Am. Chem. Soc. 130 (2008) 6507.
[11] T. Kubota, C. Yamamoto, Y. Okamoto, J. Polym. Sci. Part A: Polym. Chem. 41 (2003) 3703.
[12] R. Dicke, Cellulose 11 (2004) 255.
[13] S. Kondo, C. Yamamoto, M. Kamigaito, Y. Okamoto, Chem. Lett. 37 (2008) 558.
[14] Y. Okamoto, Y. Kaida, H. Hayashida, K. Hatada, Chem. Lett. (1990) 909.
[15] Y. Kaida, Y. Okamoto, J. Chromatogr. 641 (1993) 267.
[16] T. Ikai, C. Yamamoto, M. Kamigaito, Y. Okamoto, Chirality 17 (2005) 299.
[17] B. Chankvetadze, E. Yashima, Y. Okamoto, J. Chromatogr. A 670 (1994) 40.
[18] E. Yashima, C. Yamamoto, Y. Okamoto, Polym. J. 27 (1995) 858.
[19] B. Chankvetadze, L. Chankvetadze, S. Sidamonidze, E. Kasashima, E. Yashima, Y. Okamoto, J. Chromatogr. A 787 (1997) 67.
[20] Y. Okamoto, R. Aburatani, T. Fukumoto, K. Hatada, Chem. Lett. (1987) 1857.
[21] Y. Okamoto, T. Ohashi, Y. Kaida, E. Yashima, Chirality 5 (1993) 618.
[22] B. Chankvetadze, E. Yashima, Y. Okamoto, J. Chromatogr. A 694 (1995) 102.
[23] C. Yamamoto, S. Inagaki, Y. Okamoto, J. Sep. Sci. 29 (2006) 915.
[24] R.-R. Parviz, P. Farideh, Molecules 4 (1999) 137.
[25] H. Koller, K.-H. Rimböck, A. Mannschreck, J. Chromatogr. 282 (1983) 89.