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Preparation and evaluation of regioselectively substituted amylose derivatives for chiral separations

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Abstract

Six novel regioselectively substituted amylose derivatives with a benzoate at 2-position and two different phenylcarbamates at 3- and 6-positions were synthesized and their structures were characterized by ^{1}H nuclear magnetic resonance (NMR) spectroscopy. Their enantioseparation abilities were then examined as chiral stationary phases (CSPs) for high-performance liquid chromatography (HPLC) after they were coated on 3-aminopropyl silica gels. Investigations indicated that the substituents at the 3- and 6-positions played an important role in chiral recognition of these amylose 2-benzoate serial derivatives. The derivatives demonstrated characteristic enantioseparation and some racemates were better resolved on these derivatives than on Chiralpak AD, which is one of the most efficient CSPs, utilizing coated amylose tris(3,5-dimethylphenylcarbamate) as the chiral selector. Among the derivatives prepared, amylose 2-benzoate-3-(phenylcarbamate/4-methylphenylcarbamate)-6-(3,5-dimethylphenylcarbamate) exhibited chiral recognition abilities comparable to that of Chiralpak AD and may be useful CSPs in the future. The effect of mobile phase on chiral recognition was also studied. In general, with the decreased concentration of 2-propanol, better resolutions were obtained with longer retention times. Moreover, when ethanol was used instead of 2-propanol, poorer resolutions were often achieved. However, in some cases, improved enantioselectivity was achieved with ethanol rather than 2-propanol as the mobile phase modifier.

KEYWORDS

amylose derivative, chiral separation, chiral stationary phase, HPLC, polysaccharide

1 | INTRODUCTION

For their distinguished chiral recognition abilities, polysaccharide derivatives, especially the benzoates and phenylcarbamates of cellulose and amylose, are recognized as the most powerful chiral stationary phases (CSPs) in high-performance liquid chromatography (HPLC).[1-10] Generally, these derivatives are homogeneously substituted, which means that they bear the same substituent at 2-, 3-, and 6-positions of a glucose ring. The heterosubstituted polysaccharide derivatives having different substituents at 2, 3-positions and 6-position has also been obtained.[11-17] However, the regioselective introduction of different substituents at 2- and 3-positions was not achieved until 2008.[18] Based on the regioselective esterification at 2-position of amylose reported by Dicke,[19] two novel amylose derivatives with three different substituents at 2-, 3-, and 6-positions have been successfully synthesized by the Okamoto group and they exhibited enantioseparation abilities comparable to or better than the commercial amylose-based column, Chiralpak AD.[18] In 2010, the Okamoto group extended the above work by synthesizing a series of amylose 2-(substituted benzoate) derivatives and found that amylose 2-(4-*tert*-butylbenzoate) and amylose 2-(4-chlorobenzoate) series

showed high enantioseparation abilities.[20] In 2012, Tang and coworkers prepared four novel regioselectively amylose derivatives bearing a chiral substituent, (S)-1-phenylethylcarbamate at 3- or 6-position, and found that increased enantioselectivity could be achieved by introducing a chiral substituent at the 6-achiral rather than the 3-chiral position.[21] Among the derivatives, amylose 2-benzoate-3-(3,5-dimethylphenylcarbamate/3,5-dichlorophenylcarbamate)-6-((S)-1-phenylethylcarbamate) exhibited chiral recognition abilities comparable to that of Chiralpak AD.[21] Recently, the regioselective introduction of substituents at 2-, 6-positions and 3-postion have been carried out through the regioselective protection at 2- and 6-positions using a bulky trialkylsilyl chloride.[22]

In order to investigate the regioselective substituents introduced between 3- and 6-positions on the enantioseparation abilities of 2-benzoyl amylose derivatives as well as to develop new polysaccharide derivatives-based CSPs with high chiral recognition, six novel amylose derivatives (Figure 1, **1a–f**) were synthesized. Their structures were characterized by ^{1}H nuclear magnetic resonance (NMR) spectroscopy. After being coated on 3-aminopropyl silica gels, they were then utilized as HPLC CSPs. Their chiral recognition abilities were evaluated and compared with commercially available Chiralpak AD, which is regarded as one of the most promising CSPs. The effects of the nature and the position of the substituents and mobile phase on enantioseparation abilities of the derivatives were examined.

2 | MATERIALS AND METHODS

2.1 | Chemicals and reagents

Vinyl benzoate was purchased from Alfa Aesar (Haverhill, MA, Germany). Amylose (DP, 300) was kindly supplied by Prof. Y. Okamoto. Anhydrous dimethyl sulfoxide (DMSO) was obtained from Acros (Somerville, NJ). 3,5-Dichlorophenyl isocyanate was bought from Zhejiang Tongfen Chemical and Pharmaceutical Enterprise (Zhejiang, China). Triphenylmethyl chloride, anhydrous lithium chloride, and triphosgene were purchased from Aladdin (Shanghai, China). 3,5-Dimethylphenyl, 4-methylphenyl, and phenyl isocyanates were prepared by reaction of the corresponding anilines with triphosgene in dry toluene utilizing the traditional method. Wide pore silica gel (Daiso gel, SP-7-1000) with a mean particle size of 7 μm and a mean pore diameter of 100 nm, purchased from Daiso Chemical (Osaka, Japan), was silanized using (3-aminopropyl)triethoxysilane in dry toluene at 80 °C, together with a small amount of pyridine as catalyst. All solvents used in preparation of amylose derivatives were of analytical reagent grade, carefully dried, and distilled before use.

2.2 | Synthesis of regioselectively substituted amylose derivatives

As represented in Figure 2, six amylose 2-benzoate derivatives, **1a–f**, bearing two different phenylcarbamate substituents at 3- and 6-positions, were prepared in a sequential procedure. To regioselectively esterify only 2-position,[19] amylose (9.0 g) was first dissolved in dry DMSO (180 ml) at 80 °C and then vinyl benzoate (2.3 equiv. to 2-position) and Na_2HPO_4 (2 wt%) as catalyst were added at 40 °C. The above mixture was magnetically stirred slowly at 40 °C for 216 h under N_2 atmosphere. 2-Benzoyl amylose was isolated as a 2-propanol-insoluble fraction. To selectively protect 6-position as trityl ether, the obtained monoester was allowed to react with triphenylmethyl chloride (2 equiv. to 6-position) in pyridine at 80 °C for 24 h. 2-Benzoyl-6-*O*-trityl amylose was obtained as a methanol-insoluble fraction. In order to convert 3-hydroxyl to the corresponding phenylcarbamate group, 2-benzoyl-6-*O*-trityl amylose was allowed to react

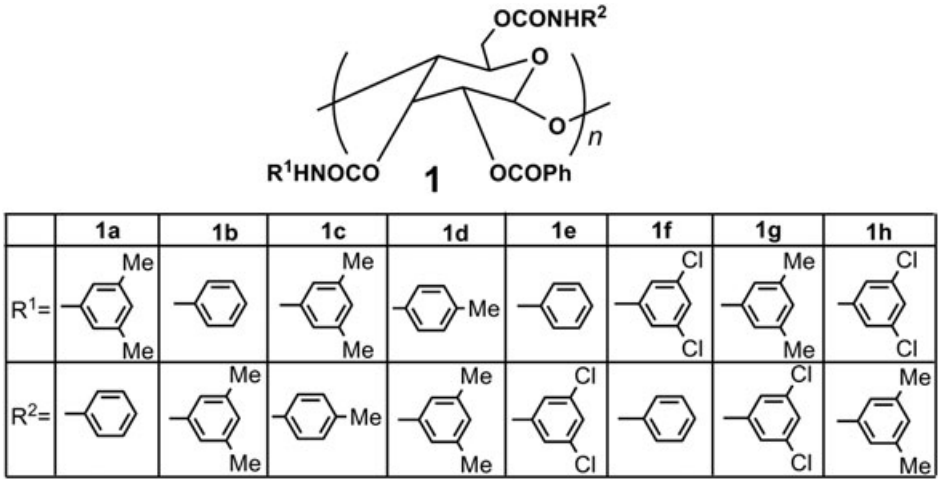

FIGURE 1 Structures of amylose derivatives

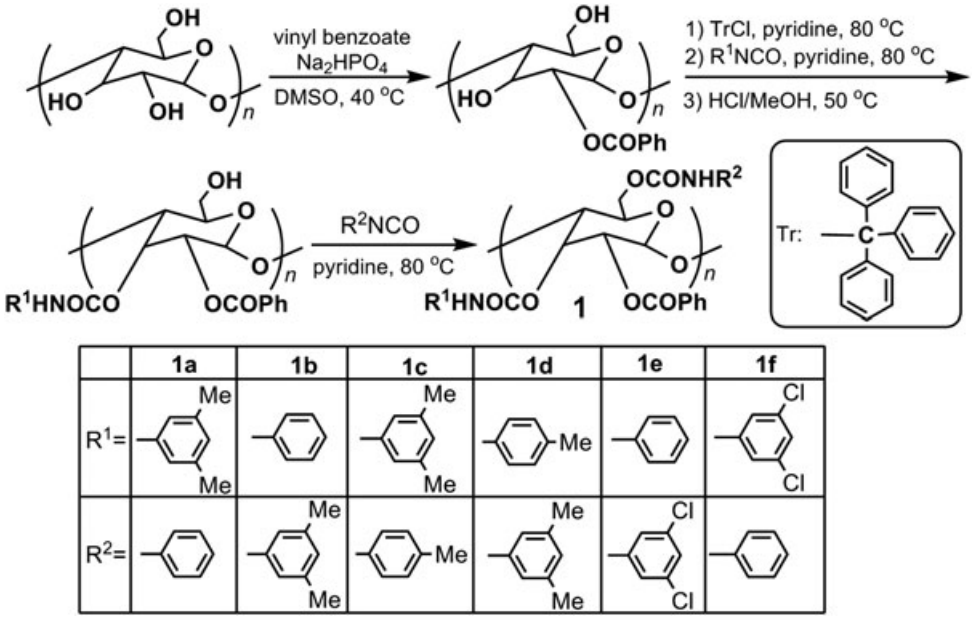

FIGURE 2 Schematic synthesis of amylose derivatives, **1a–f**

with an excess of R^1NCO in pyridine at 80 °C for 24 h. The product was isolated as an insoluble fraction in methanol. Subsequently, the obtained 2-benzoyl-3-carbamoyl-6-*O*-trityl amylose was suspended in methanol containing a small amount of hydrochloric acid (2%, *v*/v) at 50 °C for 24 h to cleave the triphenylmethyl group. Finally, the hydroxyl group at 6-position of the recovered 2-benzoyl-3-carbamoyl amylose was treated with an excess of R^2NCO in pyridine for 24 h at 80 °C. **1a–f** having different substituents at 2-, 3-, and 6-positions were isolated as methanol-insoluble fractions.

2.3 | Apparatus, preparation of packed columns, and chromatography

Chromatographic experiments were performed on an Agilent 1200 series liquid chromatography (Agilent Technologies, Palo Alto, CA) consisting of a degasser, a quaternary pump, and a UV detector at ambient temperature. In addition, a circular dichroism (CD) detector (CD 2095, Jasco, Tokyo, Japan) was equipped to monitor the signals of the eluted enantiomers. Fourier transform infrared (FTIR) spectra were measured using a Shimadzu (Kyoto, Japan) FTIR-8400 spectrophotometer as KBr pellets. ^1H and ^{13}CNMR spectra were carried out on a Bruker (Billerica, MA) Avance III spectrometer (500 MHz). Thermo gravimetric analysis (TGA) was performed on an SDT Q600 thermo analyzer (TA Instruments, New Castle, DE).

The amylose derivatives (0.15 g each) were dissolved in tetrahydrofuran (THF) (5 ml) and then coated on 3-aminopropyl silica gels (0.6 g) according to the method described elsewhere.[5] The chiral packing materials (CPMs) obtained above were then packed into stainless-steel tubes (25 × 0.20 cm i.d.) by a slurry method at 30–40 MPa.[5]

Solvents used in chromatographic experiments were of HPLC grade and mobile phases were filtered through a 0.22-μm pore diameter membrane and degassed by ultrasonication prior to use. 1,3,5-Tri-*tert*-butylbenzene was utilized as the nonretained compound to determine dead time (t_0).[23] HPLC enantioseparations were carried out at a flow rate of 0.1 ml min^{-1} and solutes were detected at 254 nm.

3 | RESULTS AND DISCUSSION

3.1 | Synthesis of regioselectively amylose derivatives

The amylose derivatives **1a–f** with three different substituents at 2-, 3-, and 6-positions were synthesized as previously described.[21] First, in order to esterify only 2-hydroxyl group of amylose, it was dissolved in DMSO and reacted with vinyl benzoate at 40 °C in the presence of a catalyst, Na_2HPO_4. Then, by reacting with triphenylmethyl chloride, the 6-hydroxyl group was selectively protected as trityl ether. Subsequently, the 3-hydroxyl group was converted to the corresponding carbamate group by reacting with an excess of R^1NCO. Finally, the 6-hydroxyl group was regenerated by the removal of the trityl group in a methanol/HCl mixture and reacted with R^2NCO to form the corresponding carbamate. It should be pointed out that in this study triphenylmethyl chloride was used instead of 4-methoxytriphenylmethyl chloride to selectively protect the 6-hydroxyl group and both of them were effective to protect the 6-hydroxyl group as trityl ether.[11-13,15,18,20,21]

Figure 3 demonstrated the ^{1}H and ^{13}C NMR spectra of 2-benzoyl amylose. In the ^{13}C NMR spectrum, only one carbonyl peak at 165 ppm occurred, which was assigned as the carbonyl peak at 2-position of amylose.[19] Thus, the 2-position of amylose was successfully regioselectively esterified and the degree of substitution was calculated to be 0.95 from the ^{1}H NMR spectrum. The ^{1}H NMR spectra of **1a** and **1b** with reversed substituents at 3- and 6-positions are shown in Figure 4. Based on the spectrum of amylose tris(3,5-dimethylyphenylcarbamate) reported by Kaida and Okamoto,[11] the peaks at about 2.0 and 2.4 ppm in Figure 4A, B, individually, were assigned to the methyl groups on the phenyl moieties at 3- and 6-positions, respectively. Thus, it confirmed that **1a** and **1b** had a 3,5-dimethylphenylcarbamate at 3- and 6-positions, individually. Minor methyl signals are also observed in Figure 4, which indicated that the regioselective substitution was not 100%. The structures of the other amylose derivatives were similarly confirmed by ^{1}H NMR. Moreover, the derivatives were characterized by elemental analysis and the data are listed in Table 1. The obtained CPMs were characterized by FTIR spectroscopy and TGA. Compared with the FTIR spectrum of 3-aminopropyl silica gel, when the amylose derivatives were coated onto 3-aminopropyl silica gel, new peaks at about 1730 (C = O) and 1630 ($-C_6H_5$) cm^{-1} occurred, demonstrating that the coating was successful.

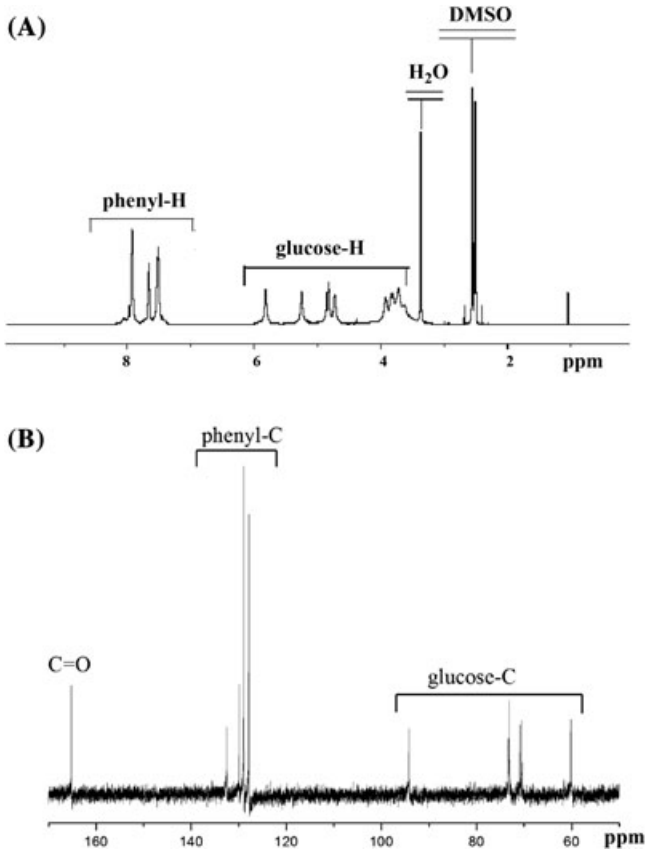

FIGURE 3 (**A**) ^{1}H and (**B**) ^{13}C NMR spectra of 2-benzoyl amylose in DMSO-d_6 at 80 °C

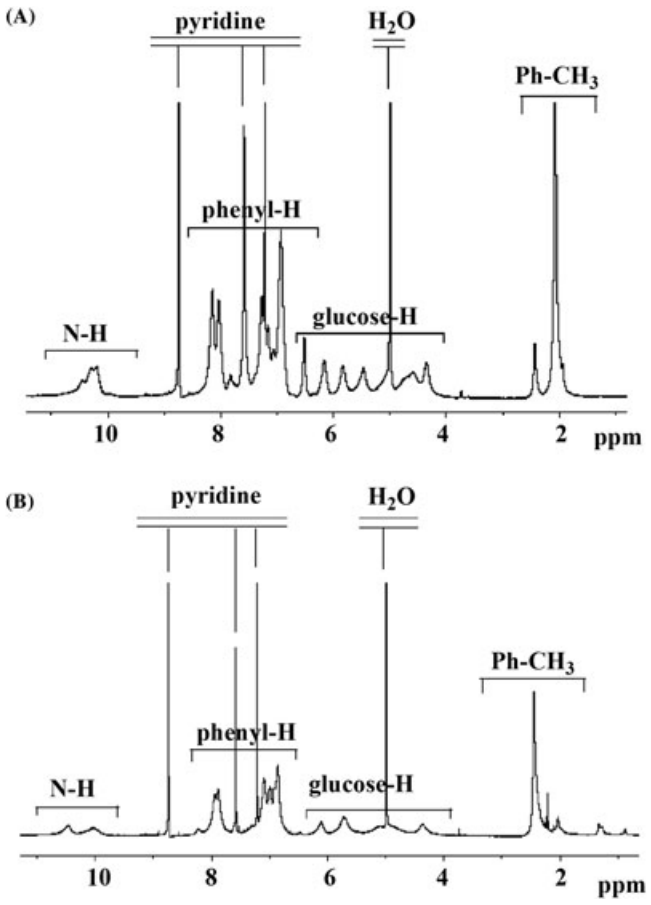

FIGURE 4 ^{1}H NMR spectra of amylose derivatives, (**A**) **1a** and (**B**) **1b** in pyridine-d_5 at 80 °C

TABLE 1 Element analysis results of amylose derivatives, **1a–f**

	Calculated (%)[a]			Found (%)		
Derivatives	**C**	**H**	**N**	**C**	**H**	**N**
1a	65.41	5.26	5.26	58.67	4.68	4.77
1b	65.41	5.26	5.26	63.48	5.28	5.20
1c	66.18	5.51	5.05	63.69	5.43	5.10
1d	66.18	5.51	5.05	64.68	5.52	5.09
1e	56.54	3.84	4.89	54.28	3.76	4.76
1f	56.54	3.84	4.89	55.09	3.94	4.73

[a]Estimated based on a repeated glucose unit.

Figure 5 shows the FTIR spectra of 3-aminopropyl silica gel before (Figure 5A) and after (Figure 5B) being coated with the amylose derivative, **1a**. The final polymer contents for CPMs **1a–f** estimated by TG were 18.8, 18.3, 18.6, 18.1, 19.0, and 18.6%, respectively.

3.2 | Chiral recognition of novel amylose derivatives

The resolving abilities of the obtained CSPs were evaluated using the racemates shown in Figure 6: Tröger's base (**2**),

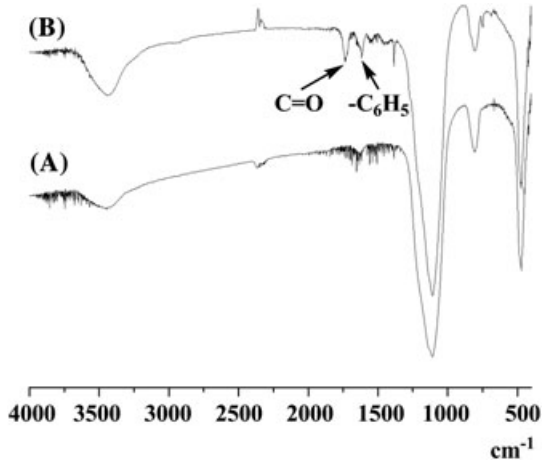

FIGURE 5 FTIR spectra of 3-aminopropyl silica gel before (**A**) and after (**B**) coated with the amylose derivative **1a**

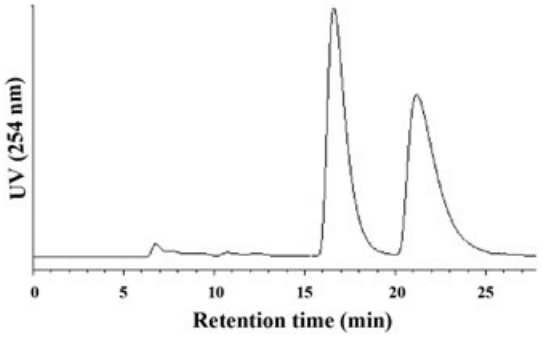

FIGURE 7 Chromatographic resolution of racemate **7** on **1a**. Column size, 25 × 0.20 cm i.D.; mobile phase, hexane/2-propanol (90:10, *v*/v); flow rate, 0.1 ml min^{-1}

trans-2,3-diphenyloxirane (**3**), benzoin (**4**), 2-phenylcyclohexanone (**5**), 1-(9-anthryl)-2,2,2-trifluoroethanol (**6**), cobalt (III) tris(acetylacetonate) (**7**), flavanone (**8**), and *trans*-cyclopropanedicarboxylic acid dianilide (**9**). The chromatogram for resolution of racemate **7** on **1a** is shown in Figure 7 with hexane/2-propanol (90:10, *v*/v) as mobile phase. The enantiomers were eluted at retention times of t_1 and t_2 with a baseline separation. The retention factors, k_1' [= $(t_1 - t_0)/t_0$] and k_2' [= $(t_2 - t_0)/t_0$], were obtained as 1.51 and 2.20, respectively, which led the separation factor α (= k_2'/k_1') to be 1.46.

In Table 2 are summarized the chromatographic resolution results of racemates **2**–**9** on **1a**–**f** using a conventional eluent, hexane/2-propanol (90:10, *v*/v). Moreover, the data on the commercially available chiral column, Chiralpak AD,[18] utilizing coated amylose tris(3,5-dimethylphenylcarbamate) as chiral selector, which is regarded as one of the most useful CSPs, are included. Compared with Chiralpak AD, **1a**–**f** showed higher chiral recognition abilities to racemates **2** and **7**. Especially, racemate **7,** which is usually rather difficult to resolve on commercially available columns, was completely resolved on these phases with α values larger than 1.20. Racemates **2**, **5**, **7**, and **9** were better resolved on **1b** and **1d** than on Chiralpak AD, while the opposite for racemates **3** and **6**. As for racemates **4** and **8**, very close α values were achieved on **1b**, **1d**, and Chiralpak AD. Thus, **1b** and **1d** demonstrated equivalent enantioseparation abilities in contrast with Chiralpak AD, which appeared to be useful CSPs with wide versatility. The other derivatives exhibited somewhat decreased but characteristic chiral recognition. For example, exhibiting a relatively lower enantioseparation, the highest α value, 1.33, for racemate **3** was obtained on **1e**. Another example is the separation of racemates **2** and **6**; the highest α values, 2.91 and 1.18, respectively, were achieved on **1f**. Especially racemate **6** was only discriminated on **1f**. The different substituents introduced at 3- and 6-positions are responsible for their different and unique chiral recognition since they are expected to change the 3D structure and local polarity of the polysaccharide derivatives, which are critical factors to keep a regular higher-order structure as well as the enantioselective interaction between enantiomeric pairs and derivatives for efficient chiral recognition.[5,24]

Investigations indicated that for regioselectively substituted polysaccharide derivatives, their chiral recognition abilities were affected by the nature of the substituent as well as its position on a glucose unit.[18,20,21] The 2-benzoyl amylose derivatives, **1a** and **1b**, **1c** and **1d**, and **1e** and **1f**, which had reversed substituents at 3-, and 6-positions, demonstrated somewhat different chiral recognition. For **1a** and **1b**, better resolutions were often achieved on **1b**. Especially

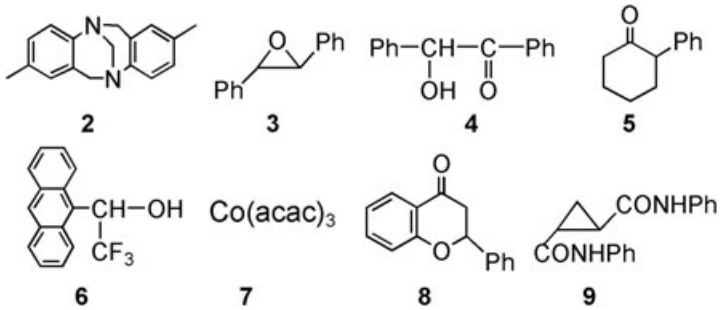

FIGURE 6 Molecular structures of racemates **2**–**9**

TABLE 2 Resolution results of racemates **2–9** on **1a–h** and Chiralpak AD

	1a [a, b]		1b [a, b]		1c [a, b]		1d [a, b]		1e [a, b]		1f [a, b]		1g [a, c]	1h [a, c]	Chiralpak AD[c, d]
	k_1'	α	k_1'	α	k_1'	α	k_1'	α	k_1'	α	k_1'	α	α	α	α
2	0.89 (+)	2.03	1.22 (+)	2.44	0.84 (+)	2.02	1.15 (+)	2.13	1.02 (+)	1.76	1.87 (+)	2.91	1.85 (+)	2.31 (+)	1.70 (+)
3	0.69 (−)	1.16	0.64	1.00	0.64	1.00	0.78 (+)	1.14	0.59 (−)	1.33	1.13	1.00	1.30 (−)	ca. 1 (−)	2.81 (+)
4	2.54 (−)	1.13	2.83 (−)	1.35	2.41 (−)	1.15	3.04 (−)	1.23	2.92 (−)	1.20	8.43 (−)	1.11	1.73 (−)	1.97 (−)	1.31 (−)
5	1.24	1.00	1.38 (+)	1.19	1.09	1.00	1.30 (+)	1.08	1.78 (−)	1.08	2.51	1.00	1.15 (−)	1.24 (+)	1.02 (−)
6	1.40	1.00	0.77	1.00	1.11	1.00	1.14	1.00	0.51	1.00	0.60 (+)	1.18	1.18 (−)	1.23 (+)	1.39 (+)
7	1.51 (+)	1.46	1.22 (+)	1.30	1.72 (+)	1.29	1.47 (+)	3.14	2.17 (+)	1.49	1.39 (+)	1.55	1.75 (−)	2.46 (−)	ca. 1 (−)
8	1.97	1.00	1.80 (−)	1.11	1.76	1.00	2.34 (+)	1.04	2.47	1.00	2.64	1.00	1.17 (−)	1.13 (+)	1.04 (+)
9	1.13 (+)	1.48	1.19 (+)	1.80	1.24 (+)	1.69	1.32 (+)	2.05	1.08 (+)	1.63	3.26 (+)	1.57	3.21 (+)	3.71 (+)	1.59 (+)

Mobile phase, hexane/2-propanol (90:10, *v*/v). The structures of **1a–h** are shown in Figure 1.

[a]Column size, 25 × 0.2 cm i.d.; flow rate, 0.1 ml/min.

[b]The signs in parentheses represent the CD detection of the first eluted enantiomer.

[c]Data taken from Ref. [18]. The signs in parentheses represent the optical rotation of the first eluted enantiome.

[d]Column size, 25 × 0.46 cm i.d.; flow rate, 0.5 ml min^{-1}.

racemates **5** and **8**, which were not resolved on **1a**, were separated on **1b**, with α values 1.19 and 1.11, respectively. However, larger α values for racemates **3** (α: **1a**, 1.16; **1b**, 1.00) and **7** (α: **1a**, 1.46; **1b**, 1.30) were obtained on **1a** rather than **1b**. The chromatographic resolutions of racemates **3** and **8** on **1a** and **1b** are shown in Figure 8. While for **1c** and **1d**, **1d** always showed a higher enantioselective ability except racemate **6,** which were not resolved on both derivatives, indicating that for 2-benzoyl amylose derivatives the combination of a 4-methylphenylcarbamate at 3-position and a 3,5-dimethylphenylcarbamate at 6-position is superior for chiral recognition of the tested racemates than that of reversed

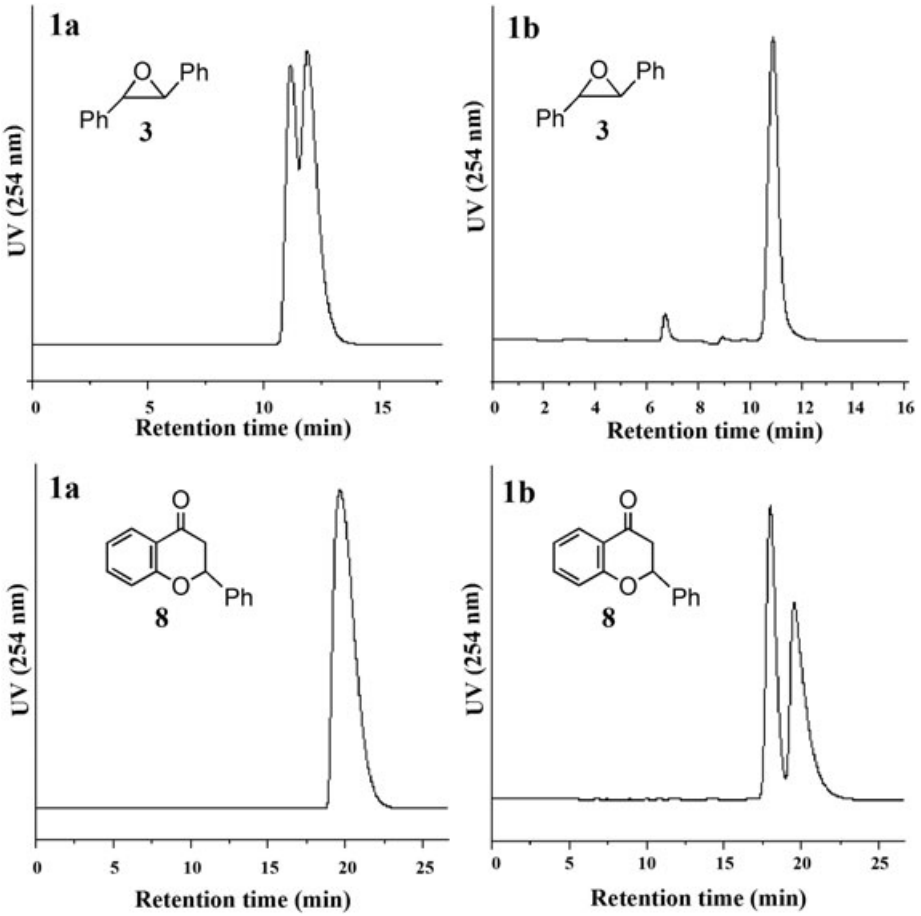

FIGURE 8 Chromatographic resolution of racemates **3** and **8** on **1a** and **1b**. Conditions are the same as in Figure 7

substituents at 3- and 6-positions. For example, racemates **3**, **5**, and **8**, which were not resolved on **1c**, were resolved on **1d** and their α values were 1.14, 1.08, and 1.04, individually. Another example is the resolution of racemate **7**: its α value was significantly larger on **1d** (3.14) than that on **1c** (1.29). As for **1e** and **1f**, **1e** exhibited a higher chiral recognition for most racemates, except racemates **2** (α: **1e**, 1.76; **1f**, 2.91) and **6** (α: **1e**, 1.00; **1f**, 1.18). Especially racemates **3** and **5** were realized on **1e** (α: **3**, 1.33; **5**, 1.08) rather than on **1f**. These results implied that, as well as the substituent at 2-position,[20] the substituents at 3- and 6-positions have a large influence on enantioseparation.[20,21]

The data[18] on **1g** and **1h** are also included in Table 2 for comparison. Having the same substituents at 2- and 3-positions, **1a**, **1c**, and **1g**, **1b** and **1e**, and **1f** and **1h** exhibited varied chiral recognition abilities depending on the substituent at 6-position. As indicated in Table 2, the k_1' values for all eight racemates were close on **1a** and **1c**, suggesting that the interactions between the racemates and the substituents at 6-position, being phenylcarbamate and 4-methylphenylcarbamate moieties, respectively, were similar, while their α values on **1a** and **1c** were somewhat different. For racemates **2**, **4**, **5**, **6**, and **8**, close or identical α values were achieved on these two derivatives. For racemates **3** and **7**, higher α values were realized on **1a** (α: **3**, 1.16; **7**, 1.46) than on **1c** (α: **3**, 1.00; **7**, 1.29). Especially racemate **3**, which was partially resolved on **1a**, was not resolved on **1c**. For racemate **9**, its α value on **1a** (α, 1.48) was smaller than on **1c** (α, 1.69). Thus, **1a** generally exhibited a higher chiral recognition ability than that of **1c**. Their different chiral recognition abilities may be ascribed to the different electronic effects of the substituents at 6-position, that is, phenylcarbamate is a less powerful electron-donating group compared with 4-methylphenylcarbamate. However, with the introduction of an electron-withdrawing 3,5-dichlorophenylcarbamate at 6-position instead of an electron-donating substituent being phenylcarbamate or 4-methylphenylcarbamate, the chiral recognition of **1g** significantly differed from those of **1a** and **1c**, indicating that the electronic effect of the chloro group changed the chiral recognition of **1g** dramatically, which might be due to the changed local polarity and even the higher-order structure of the polymer. All eight racemates were resolved on **1g**, while only 5 and 4 racemates were separated on **1a** and **1c**, respectively. Among the three derivatives, **1g** always exhibited the highest enantioseparations for the tested racemates, except racemates **2** and **9**, whose α values were slightly higher on **1a** and **1c**. Especially racemates **5**, **6**, and **8**, which could not be resolved on **1a** and **1c**, were resolved on **1g,** with α values, 1.15, 1.18, and 1.17, respectively, implying that the combination of an electron-donating 3,5-dimethylcarbamate at 3-position and an electron-withdrawing 3,5-dichlorophenylcarbamate at 6-position is favorable for chiral recognition of these three racemates. As for **1b** and **1e**, their chiral recognition abilities differed greatly. For racemate **2**, a much larger α value, 2.44, was obtained on **1b** than that on **1e**, 1.76. In addition, racemate **8**, which was not resolved on **1e**, was partially resolved on **1b** (α, 1.11). On the contrary, with α value, 1.33, **1e** rather than **1b** (α, 1.00) demonstrated a fairly good separation for racemate **3**, indicating that the introduction of an electron-withdrawing 3,5-dichlorophenylcarbamate instead of an electron-donating 3,5-dimethylphenylcarbamate at 6-position of 2-benzoyl-3-phenylcarbamoyl amylose is preferable for the chiral recognition of this racemate. Moreover, in the resolution of racemate **5**, reversed elution orders of the enantiomers were achieved on **1b** and **1e**, suggesting that the separation mechanisms of racemate **5** were different. It seems that the electronic effect of methyl and chloro groups are responsible for the significantly changed chiral recognition between **1b** and **1e**, which was the same for 3,5-dimethylphenylcarbamtes and 3,5-dichlorophenylcarbamates of cellulose[5] and amylose.[25] In the case of **1f** and **1h**, **1h** always exhibited a higher chiral recognition, except racemates **2** (α: **1f**, 2.91; **1h**, 2.31) and **3** (α: **1f**, 1.00; **1h**, ca. 1), demonstrating that for 2-benzoyl-3-(3,5-dichlorophenylcarbamoyl) amylose, the introduction of a more powerful electron-donating 3,5-dimethylphenylcarbamate rather than a less powerful phenylcarbamte at 6-position is favorable for enantioseparation. In addition, racemates **5** and **8**, which were realized on **1h** (α: **5**, 1.24; **8**, 1.13), but were not on **1f**. As indicated above, the substituent at 6-position influenced the chiral recognition of amylose derivatives with three different substituents at 2-, 3-, and 6-positions, and the extent depending on the nature of substituent.

Investigations indicated that the substituent at 3-position has a large influence on chiral recognition of regioselectively substituted amylose derivatives,[20,21] which is also the same in this study. Bearing the same substituents at 2- and 6-positions, **1b**, **1d** and **1h**, **1a** and **1f**, and **1e** and **1g**, demonstrated changed chiral recognition relying on the substituent at 3-position. Having different substituent at 3-position, being phenylcarbamte, 4-methylphenylcarbamate, and 3,5-dichlorophenylcarbamate, respectively, **1b**, **1d**, and **1h** showed different chiral recognition abilities. In general, **1b** and **1d** exhibited somewhat similar chiral recognition, while **1h** showed the highest chiral recognition ability to most racemates. Among these three derivatives, racemate **3** was separated only on **1d** and racemate **6** only on **1h**, demonstrating that the introduction of an electron-donating 4-methylphenylcarbamate and an electron-withdrawing 3,5-dichlorophenylcarbamate at 3-position was favorable for the chiral recognition of the corresponding racemates. The elution orders of racemate **8** on **1b** and **1d** were reversed, meaning that the separation mechanisms were different, which might be ascribed to the different higher-order structures of the derivatives due to the different substituent at 3-position.

The highest α values obtained on **1b**, **1d**, and **1h** were 1 (racemate **2**), 2 (racemates **3** and **7**), and 5 (racemates **4**, **5**, **6**, **8**, and **9**) racemates, respectively. Racemates that were not separated on **1b**, **1d**, and **1h** were 2 (racemates **3** and **6**), 1 (racemate **6**), and 1 (racemate **3**), individually. These results imply that for 2-benzoyl-6-(3,5-dimethylphenylcarbamoyl) amylose, the introduction of an electron-withdrawing 3,5-dichlorophenylcarbamate rather than an electron-donating phenylcarbamate or 4-methylphenylcarbamate at 3-position is more favorable for chiral recognition. For **1a** and **1f**, close α values for most racemates were realized on these two derivatives, except racemates **2** (α: **1a**, 2.03; **1f**, 2.91), **3** (α: **1a**, 1.16; **1f**, 1.00), and **6** (α: **1a**, 1.00; **1f**, 1.18). Thus, the introduction of an electron-donating 3,5-dimethyphenylcarbamate and an electron-withdrawing 3,5-dichlorophenylcarbamate at 3-position of 2-benzoyl-6-phenylcarbamoyl amylose is preferable for the chiral recognition of racemates **3** and **6**, respectively. As for **1e** and **1g**, higher α values were always achieved on **1g**, except a slightly decreased α value for racemate **3**. Especially racemates **6** and **8**, which were not resolved on **1e,** were partially resolved on **1g** (α: **6**, 1.18; **8**, 1.17). Also, **1g** demonstrated a significantly larger α value, 3.21, for racemate **9** than **1e** (α, 1.63). It seems that for **1e** and **1g**, a more powerful electron-donating 3,5-dimethylphenylcarbamate at 3-position is more suitable than a less powerful phenylcarbamate for chiral recognition.

The effect of mobile phase on chiral recognition was also investigated and the results are listed in Table 3. Generally, the k_1' and α values were increased with the decreased concentration of 2-propanol in mobile phase. A typical example is the resolution of racemate **7** on **1d**; its k_1' values were increased from 0.73 to 1.47 and 3.23, and α values from 2.74 to 3.14 and 3.25, when the concentration of 2-propanol decreased from 20 to 10 and 5%. The increased k_1' values are due to the increased H-bonding interactions between the racemates and the derivatives ascribed to the decreased H-bonding solvent, 2-propanol. When ethanol was used instead of 2-propanol, poorer separations were often obtained. A typical example is the resolution of racemate **3** on **1d**. As shown in Figure 9, when the eluent was switched from

TABLE 3 Influence of mobile phase on optical resolution of racemates **2–9** on **1a–f**

		1a		1b		1c		1d		1e		1f	
	Eluent	k_1'	α	k_1'	α	k_1'	α	k_1'	α	k_1'	α	k_1'	α
2	A	0.64 (+)	1.97	0.67 (+)	1.84	0.49 (+)	1.72	0.76 (+)	1.99	0.76 (+)	1.53	1.43 (+)	2.69
	B	0.89 (+)	2.03	1.22 (+)	2.44	0.84 (+)	2.02	1.15 (+)	2.13	1.02 (+)	1.76	1.87 (+)	2.91
	C	1.17 (+)	2.10	1.73 (+)	2.07	1.12 (+)	2.08	1.27 (+)	2.04	1.64 (+)	1.54	2.69 (+)	3.05
	D	0.83 (+)	1.98	1.13 (+)	2.02	0.79 (+)	1.93	0.87 (+)	1.88	0.91 (+)	1.50	1.79 (+)	2.85
3	A	0.56 (−)	1.17	0.59	1.00	0.41	1.00	0.63	1.00	0.56 (−)	1.33	1.07	1.00
	B	0.69 (−)	1.16	0.64	1.00	0.64	1.00	0.78 (+)	1.14	0.59 (−)	1.33	1.13	1.00
	C	0.80 (−)	1.17	0.88 (−)	1.09	0.76	1.00	0.81 (+)	1.21	0.60 (−)	1.33	1.32	1.00
	D	0.64 (−)	1.16	0.65	1.00	0.60	1.00	0.65	1.00	0.54 (−)	1.39	0.94 (−)	1.13
4	A	1.44 (−)	1.12	1.27 (−)	1.14	1.31 (−)	1.12	1.65 (−)	1.21	1.75 (−)	1.20	5.69	1.00
	B	2.54 (−)	1.13	2.83 (−)	1.35	2.41(−)	1.15	3.04 (−)	1.23	2.92 (−)	1.20	8.43 (−)	1.11
	C	3.57 (−)	1.15	5.28 (−)	1.24	3.65 (−)	1.16	6.19 (−)	1.28	4.57 (−)	1.20	13.86 (−)	1.17
	D	2.33 (−)	1.13	2.22 (−)	1.15	2.09 (−)	1.16	2.14 (−)	1.15	2.25 (−)	1.18	6.15 (−)	1.34
5	A	0.84	1.00	0.78 (+)	1.14	0.61	1.00	0.82 (+)	1.09	1.60 (−)	1.07	1.96	1.00
	B	1.24	1.00	1.38 (+)	1.19	1.09	1.00	1.30 (+)	1.08	1.78 (−)	1.08	2.51	1.00
	C	1.62	1.00	2.08 (+)	1.21	1.49	1.00	1.88 (+)	1.10	3.38 (−)	1.12	3.33	1.00
	D	1.18	1.00	1.11 (+)	1.14	1.00	1.00	1.00 (+)	1.13	1.74	1.00	2.49	1.00
6	A	0.60	1.00	0.38	1.00	0.48	1.00	0.39	1.00	0.33	1.00	0.29	1.00
	B	1.40	1.00	0.77	1.00	1.11	1.00	1.14	1.00	0.51	1.00	0.60 (+)	1.18
	C	2.62 (+)	1.07	1.73 (+)	1.11	2.30	1.00	2.45 (+)	1.10	1.56 (+)	1.17	1.01 (+)	1.28
	D	1.31	1.00	0.79	1.00	1.05	1.00	0.80	1.00	0.48 (+)	1.54	0.53 (+)	1.27
7	A	0.80 (+)	1.35	0.64 (+)	1.25	0.92 (+)	1.21	0.73 (+)	2.74	1.19 (+)	1.39	0.77 (+)	1.68
	B	1.51 (+)	1.46	1.22 (+)	1.30	1.72 (+)	1.29	1.47 (+)	3.14	2.17 (+)	1.49	1.39 (+)	1.55
	C	2.41 (+)	1.34	5.84 (+)	1.21	3.21 (+)	1.28	3.23 (+)	3.25	5.42 (+)	1.56	2.73 (+)	1.60
	D	1.45 (+)	1.39	1.13 (+)	1.30	1.54 (+)	1.26	0.97 (+)	1.95	1.74 (+)	1.11	1.28 (+)	1.52
8	A	1.42	1.00	1.32 (−)	1.13	1.08	1.00	1.73	1.00	1.77	1.00	2.20	1.00
	B	1.97	1.00	1.80 (−)	1.11	1.76	1.00	2.34 (+)	1.04	2.47	1.00	2.64	1.00
	C	2.41	1.00	3.09 (−)	1.15	2.26	1.00	3.23 (+)	1.07	4.47	1.00	3.74	1.00
	D	1.93	1.00	1.79 (+)	1.13	1.55	1.00	1.72 (+)	1.11	2.01 (+)	1.10	2.58	1.00
9	A	0.61 (+)	1.58	0.58 (+)	1.51	0.55 (+)	1.46	0.55 (+)	2.00	0.45 (+)	1.50	1.38 (+)	1.77
	B	1.13 (+)	1.48	1.19 (+)	1.80	1.24 (+)	1.69	1.32 (+)	2.05	1.08 (+)	1.63	3.26 (+)	1.57
	C	3.18 (+)	1.93	3.39 (+)	1.46	3.29 (+)	1.80	3.68 (+)	1.98	2.67 (+)	1.87	8.86 (+)	1.44
	D	1.02 (+)	1.37	1.15 (+)	1.57	1.21 (+)	1.62	1.08 (+)	1.64	0.95 (+)	1.27	2.18 (+)	1.60

Mobile phase: *A* hexane/2-propanol (80:20, *v*/v), *B* hexane/2-propanol (90:10, *v*/v), *C* hexane/2-propanol (95:5, *v*/v), *D* hexane/ethanol (90:10, *v*/v). The signs in parentheses represent the CD detection of the first eluted enantiomer. Column size, 25 × 0.2 cm i.d.; flow rate, 0.1 ml min^{-1}.

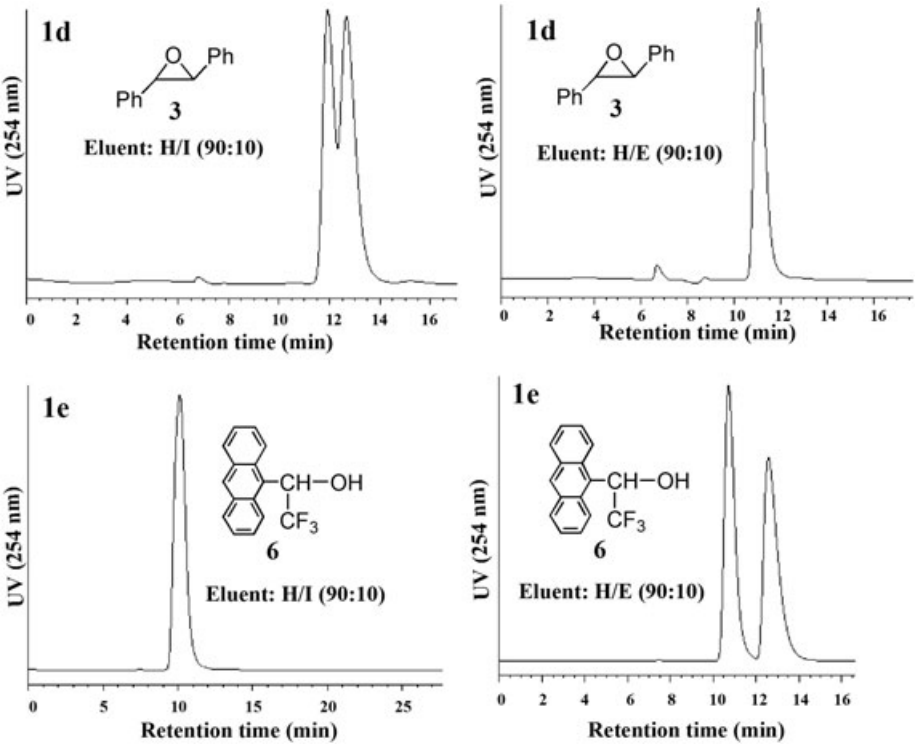

FIGURE 9 Chromatographic resolution of racemate **3** on **1d** and **6** on **1e**. Column size, 25 × 0.20 cm i.D.; flow rate, 0.1 ml min^{-1}. H, hexane; I, 2-propanol; E, ethanol

hexane/2-propanol (90:10, *v*/v) (α, 1.14) to hexane/ethanol (90:10, *v*/v), it could not be resolved. However, in the resolution of racemate **6** on **1e** (Figure 9) and **3** on **1f**, better resolutions were obtained when ethanol was used in place of 2-proppanol, which may be due to the fact that the changes of the stereo environment of the chiral cavities in the polymer chains caused by ethanol is favorable for the enantioseparations of the corresponding racemates.[26]

4 | CONCLUSION

In this study, six amylose derivatives having a benzoate at 2-postition and two different phenylcarbamates at 3- and 6-positions were synthesized and their structures were characterized by ^{1}H NMR spectroscopy. After being coated onto 3-aminopropyl silica gels, they were evaluated as CSPs for HPLC enantioseparations of eight racemates. The results indicated that the derivatives demonstrated characteristic enantioseparation abilities and some racemates were better resolved on these derivatives than on Chiralpak AD. The substituents at 3- and 6-positions have a large influence on their chiral recognition. Among the derivatives prepared, amylose 2-benzoate-3-(phenylcarbamate/4-methylphenylcarbamate)-6-(3,5-dimethylphenylcarbamate) exhibited chiral recognition abilities comparable to that of Chiralpak AD and may be potential useful CSPs in the future. The effect of mobile phase on chiral recognition was also investigated. In general, with the decreased concentration of 2-propanol in eluent, better resolutions were obtained. Moreover, when ethanol was used instead of 2-propanol, poorer resolutions were often achieved. However, in some cases, improved enantioselectivity was achieved with ethanol rather than 2-propanol as the mobile phase modifier.

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