Journal of Chromatography A, 1467 (2016) 199–205

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Synthesis and chiral recognition of amylose derivatives bearing regioselective phenylcarbamate substituents at 2,6- and 3-positions for high-performance liquid chromatography

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ARTICLE INFO

Article history:
Received 2 April 2016
Received in revised form 12 July 2016
Accepted 14 July 2016
Available online 16 July 2016

Keywords:
Chiral recognition
Enantioseparation
Racemate
Chiral stationary phase
Polysaccharide
Phenylcarbamate
HPLC

ABSTRACT

Eighteen novel amylose derivatives bearing different phenylcarbamate substituents at 2,6- and 3-positions of a glucose ring were synthesized through the regioselective protection at 2- and 6-positions using a bulky trialkylsilyl chloride. Their chiral recognition abilities were then evaluated as the chiral stationary phases (CSPs) for high-performance liquid chromatography (HPLC) after coating them on the surface of macroporous silica gel. The chiral recognition abilities of these CSPs intricately depended on the nature, position and number of the substituents on the phenyl moieties. The introduction of substituents at *meta*-position of aromatic moieties at 2- and 6-positions of glucose unit was more attractive than other positions to improve the chiral recognition ability of these amylose derivatives. Each CSP seems to possess its own characteristic resolving power, and those based on amylose 3-(3,5-dichlorophenylcarbamate) showed comparatively better chiral recognition than others. For some racemates, the amylose derivatives with different phenylcarbamate substituents at 2,6- and 3-positions exhibited higher enantioselectivity than the amylose tris(3,5-dimethylphenylcarbamate), which is commercially available as Chiralpak AD, one of the most powerful CSPs. The structures of the obtained amylose derivatives were also investigated by circular dichroism spectroscopy.

1. Introduction

As a valuable tool to analyze and obtain optically pure enantiomers of various chiral compounds including drugs, agrochemicals, food additives, etc., high-performance liquid chromatography (HPLC) for enantioseparation has achieved significant advances with the development of chiral stationary phases (CSPs) in the recent decades [1,2]. Most of the enantiomeric excess (e.e.) determinations by HPLC have been performed using polysaccharide-derived CSPs [3–5]. Among these CSPs, carbamate or ester derivatives of cellulose and amylose appear to be some of the most useful CSPs and a wide range of racemic compounds can be resolved on them [6–13]. The polysaccharide derivatives have traditionally been prepared by the tris-substitution of the hydroxy groups at 2-,3- and 6-positions of a glucose ring with the same substituent, and the regioselective derivatization of polysaccharides has been restricted only between 2,3- and 6-position by the protection of 6-position using a bulky trityl group [14,15]. The regioselective introduction of different substituents between 2- and 3-positions of the glucose unit had been more difficult for many years mainly due to the similar reactivity between these two positions. In 2008, based on the esterification only at 2-position of amylose reported by Dicke [16], the regioselective introduction of different substituents at 2-, 3- and 6-positions has been successfully realized by our group [17], and a variety of polysaccharide derivatives bearing different combination of selective substituents at three positions have been thoroughly evaluated [18–21]. Recently, a new 2,6-protection method for cellulose using another bulky dimethyl-(1,1,2-trimethylpropyl)silyl (dimethylthexylsilyl) group has been reported by Klemm et al. [22], which makes it possible to prepare various functionalized cellulose derivatives with different substituents between 2,6- and 3-positions. However, the regioselective substitution between 2,6- and 3-positions of amylose has not been reported yet.

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http://dx.doi.org/10.1016/j.chroma.2016.07.035
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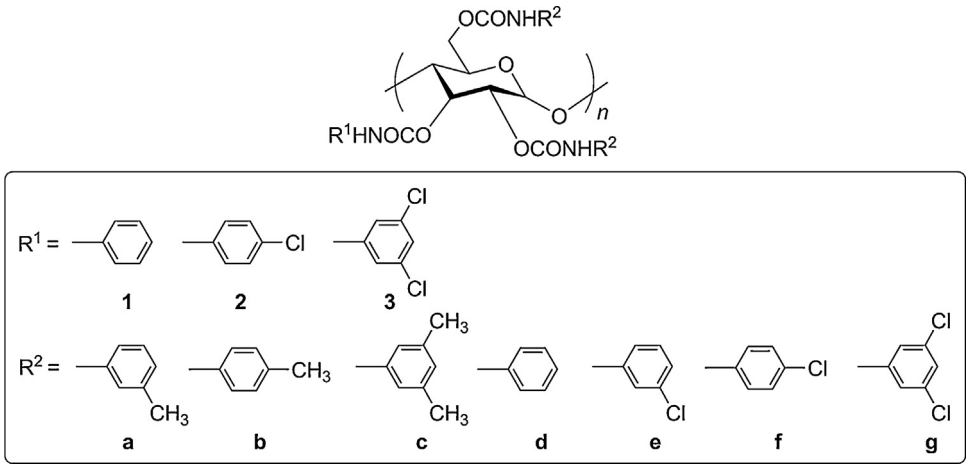

Fig. 1. Structures of novel amylose derivatives.

Systematical studies on the chiral recognition mechanism of the cellulose phenylcarbamate derivatives reveal that the electronic property and structure of the substituents on the aromatic moieties of the phenylcarbamates can significantly influence the chiral recognition ability of polysaccharide derivatives [23–34]. These substituents 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 [23,35]. Therefore, the polysaccharide derivatives bearing regioselective substituents at three positions must render valuable systems for elucidating the correlations between the structures of derivatives and their chiral recognition abilities.

In order to investigate the effect of regioselective substituents between 2,6- and 3-positions on the chiral recognition of the amylose phenylcarbamate derivatives as well as to develop the novel polysaccharide derivatives with a high chiral recognition ability, eighteen amylose derivatives bearing selective phenylcarbamate substituents at 2,6- and 3-positions were synthesized (Fig. 1) and their chiral recognition abilities were evaluated as CSPs by HPLC. The influence of the nature, position and number of the substituents on the chiral recognition of enantiomers was carefully examined. The enantioseparation abilities of the obtained amylose derivatives were compared with those of the commercially available Chiralpak AD, which contains tris-substituted 3,5-dimethylphenylcarbamate of amylose with a high recognition ability. The structures of the regioselectively substituted amylose derivatives were also investigated by circular dichroism spectroscopy.

2. Experimental

2.1. Chemicals

Amylose (DP = 300) was kind gift from Daicel Corporation (Tokyo, Japan). Imidazole, chlorodimethylthexylsilane (TDMS-Cl in Fig. 2), tetrabutylammonium fluoride (TBAF), dipotassium hydrogen phosphate (K_2HPO_4) and monopotassium phosphate (KH_2PO_4) were purchased from Energy Chemical (Shanghai, China). *N*,*N*-Dimethylacetamide (DMAc), lithium chloride (LiCl), THF and pyridine were purchased from Kermel (Tianjin, China). The phenyl isocyanate derivatives were obtained from Sigma-Aldrich (Shanghai, China). 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. All solvents used in the preparation of the amylose derivatives were of analytical reagent grade and dehydrated by fractional distillation before use. The solvents used in the chromatographic experiments were of HPLC grade. The racemates were commercially available or prepared by the usual methods.

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

The amylose derivatives (**1a**–**g**, **2a**–**g** and **3a**–**f**) bearing regioselective phenylcarbamate substituents at 2,6- and 3-positions, respectively, were synthesized based on the regioselective protection at 2- and 6-positions using a bulky trialkylsilyl chloride as reported by Klemm [22]. The sequential process used for synthesis of the amylose derivatives was shown in Fig. 2. To selectively protect the 2- and 6-positions, the amylose (3.0 g) was first dissolved in a mixture of DMAc and LiCl at 100 °C. Then, imidazole (2.4 equiv to the hydroxy groups at 2,6-positions) and TDMS-Cl (2 equiv to the hydroxy groups at 2,6-positions) were stepwise added to the solution at 100 °C, and the reaction was continued for 24 h to protect selectively 2,6-positions as dimethylthexylsilyl ethers. The reaction mixture was then added into a large excess of aqueous phosphate buffer solution (1.79 g K_2HPO_4 and 0.89 g KH_2PO_4 in 250 mL distilled water). The obtained precipitates were then sufficiently washed by ethanol and water, and the product was isolated as an insoluble fraction; yields were 80–100%. The obtained 2,6-di-*O*-dimethylthexylsilyl was then allowed to react with phenyl or 4-chlorophenyl or 3,5-dichlorophenyl isocyanate in pyridine at 80 °C to convert the hydroxy group at 3-position to the corresponding phenylcarbamate group. The product was isolated as a methanol-insoluble fraction; yields were 85–100%. Subsequently, the obtained 2,6-*O*-dimethylthexylsilyl-3-(phenylcarbamoyl or 4-chlorophenylcarbamoyl or 3,5-dichlorophenylcarbamoyl) amylose was suspended in THF, and the tetrabutylammonium fluoride trihydrate (TBAF) (20 wt% to THF as a catalyst) was then added, the mixture was stirred for 24 h at 50 °C to cleave the dimethylthexylsilyl group. Finally, the hydroxy groups at 2- and 6-positions of the regenerated amylose 3-(phenyl or 4-chlorophenyl or 3,5-dichlorophenyl)carbamate were treated with an excess of various phenyl isocyanates for 14 h at 80 °C. Eighteen amylose derivatives **1a**–**g**, **2a**–**g** and **3a**–**f** bearing two different phenylcarbamate substituents at 2,6- and 3-positions were isolated as a methanol-insoluble fraction; yields were 85–100%.