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Synthesis of chitosan 3,6-diphenylcarbamate-2-urea derivatives and their applications as chiral stationary phases for high-performance liquid chromatography

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ABSTRACT

Fourteen chitosan 3,6-diphenylcarbamate-2-urea derivatives were synthesized using well-deacetylated chitosan and the corresponding phenyl isocyanates. After coating them on silica gel, their chiral recognition abilities were evaluated as the chiral stationary phases (CSPs) for high-performance liquid chromatography. These coated-type CSPs exhibited different chiral recognitions depending on the position, nature, and number of the substituents introduced on the phenyl group, and the introduction of either an electron-withdrawing or an electron-donating substituent improved the chiral recognition of the CSPs. Among the CSPs, the 2-substituted CSPs showed low chiral recognition abilities, while those with 3,5-dimethyl and 3,5-dichloro substituents showed relatively higher chiral recognition abilities, which enabled the baseline separation of some racemates. The CSPs could be used with some eluents containing chloroform, which cannot be used for other polysaccharide-based CSPs. Some racemates were more efficiently resolved with these nonstandard eluents. The correlation between the chiral recognition ability and the chemical shifts of the N-H protons in the ^{1}H NMR spectra of the chitosan derivatives or the N-H frequencies in the IR spectra of the carbamate moieties was discussed.

1. Introduction

Enantioseparation by high-performance liquid chromatography (HPLC) provides a more promising technique for analyzing chiral compounds and obtaining them in their pure enantiomer form. The preparation of chiral stationary phases capable of effective chiral recognition is the key to the HPLC enantioseparation technique and various CSPs have been developed for this purpose [1–4]. Among the many kinds of CSPs so far developed for HPLC, the phenylcarbamates and benzoates of polysaccharides, cellulose and amylose, are well known to exhibit a high chiral recognition to a wide range of compounds [5–7]. A significant number of research studies have shown that the chiral recognition abilities of the cellulose and amylose derivatives with various substituents on the phenyl groups are greatly influenced by the nature, position, and number of the substituents [8–12].

Chitosan is also one of the abundant biopolymers derived by the deacetylation of chitin, a major component of the shells of crustaceans [13–17]. As a renewable resource from nature, chitosan has many unique properties, such as an antimicrobial activity [18–20], biodegradability [21,22], and hypocholesterolemic functions [23,24], which attract scientific and industrial interest in such fields as food science, biomedical applications, and as a support of metals and biocatalysts. To improve the physicochemical and biological properties of chitosan, several chemical modifications of chitosan have been reported [25–31]. The structure of chitosan is similar to those of chitin and cellulose, except for the groups at the 2-position. Besides a significant number of research studies regarding the cellulose phenylcarbamates, various carbamates of chitin were also evaluated as the CSPs for HPLC and some of them showed relatively high chiral recognition abilities [32,33]. However, much less attention has been paid to the study of the enantioseparations by HPLC on the chitosan derivatives [34–37].

Recently, we reported the chiral separation on the chitosan 3,6-di(4-chlorophenylcarbamate)-2-urea derivatives bearing small amounts of biuret and allophanate structures [38]. These derivatives exhibited different recognition abilities depending on their structures, and could be used with a few nonstandard solvents as

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the eluents, which efficiently improved the resolution for some racemates. However, a systematic study of the position, number, and nature of the substituent on the phenyl group has not yet been reported.

In this study, we synthesized fourteen chitosan 3,6-diphenylcarbamate-2-urea derivatives in order to gain a better understanding of the effect of the substituents at different positions on the chiral recognition of these phenylcarbamate–urea derivatives and to correlate their chiral recognition with the characteristics of the substituents on the phenyl rings. Their chiral recognition abilities were evaluated as CSPs for HPLC. The correlations between the chiral recognition abilities and the chemical shifts of the N-H protons in the ^{1}H NMR spectra and the N-H frequencies in the IR spectra of the carbamate moieties of these derivatives are also discussed.

2. Experimental

2.1. Chemicals

The chitosan was purchased from Aldrich (USA). 4-Nitrophenyl isocyanate, 4-methoxyphenyl isocyanate, 4-bromophenyl isocyanate, 4-fluorophenyl isocyanate, 3,5-dichlorophenyl isocyanate, and 3,5-dimethylphenyl isocyanate were purchased from Aldrich (USA). The 2-chloro-, 3-chloro-, and 4-chlorophenyl isocyanates were purchased from Bailingwei (Beijing, China). The 3-methyl- and 4-methylphenyl isocyanates were obtained from TCI (Tokyo, Japan). The 4-ethyl- and 2-methylphenyl isocyanates were purchased from Alfa Aesar (Tianjin, China). Phenyl isocyanate was obtained from Lubang (Dezhou, China). The 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. Dimethyl sulfoxide (DMSO), methanol (MeOH), ethyl acetate, and sodium hydroxide (NaOH) were purchased from Kemiou Chemical (Tianjin, China). All the solvents used in the preparation of the chitosan 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 enantiomers were commercially available or were prepared by the usual methods.

2.2. Deacetylation of chitosan

The deacetylation was performed by the following method reported by Mima et al. [39]. First, the commercial chitosan powder containing ca. 15% of the *N*-acetyl group was suspended in 50% aqueous NaOH and stirred at 125 °C for 2–3 h. After cooling down, the chitosan was filtered off and thoroughly washed with water. This alkali treatment was then repeated three times. Finally, the chitosan product obtained by the alkali treatment was washed with water to neutrality and dried at 60 °C *in vacuo*. The complete deacetylation of the chitosan was then confirmed by comparing the ^{1}H NMR spectra of the chitosan before and after the deacetylation.

2.3. Synthesis of chitosan 3,6-diphenylcarbamate-2-urea derivatives

The chitosan derivatives (Fig. 1, **a**–**n**) were synthesized by the following procedure. The completely deacetylated chitosan was dried at 80 °C under vacuum for 4 h and suspended in DMSO at 80 °C. A corresponding phenyl isocyanate (2–2.5 equiv. of the total hydroxyl and amino groups) was added to the suspension at 70–100 °C and kept for 1–12 h with stirring. Most of the products were then isolated as the methanol-insoluble fraction, while

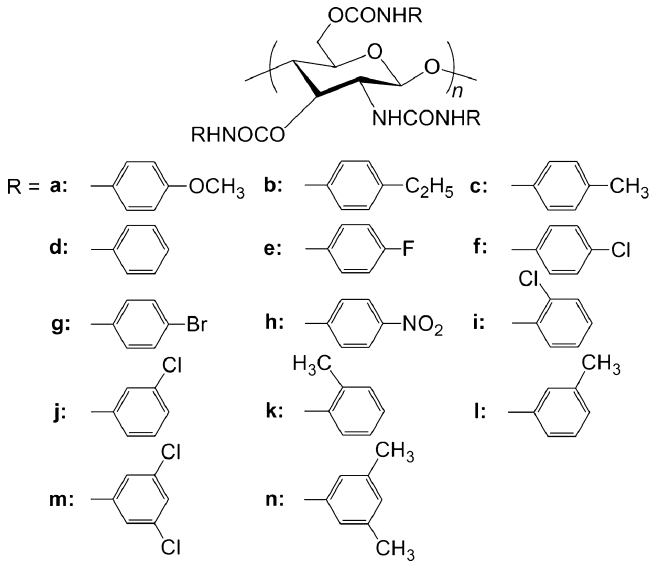

Fig. 1. Structures of chitosan phenylcarbamate–urea derivatives.

only the chitosan 3,6-di(4-nitrophenylcarbamate)-2-urea derivative was isolated as the ethyl acetate-insoluble fraction; the yields were 60–80%.

2.4. Preparation of chiral stationary phases (CSPs)

The chitosan derivatives **a**, **h** and **j** (0.2 g) were dissolved in DMSO (5 mL) due to their low solubility in THF, then coated on the aminopropylsilanized silica gel (0.8 g) [8]. The other chitosan derivatives (0.2 g) were dissolved in THF (5 mL) for coating on the silica gel. The weight ratio of the derivatives to the silica gel was approximately 1:4. The derivative-coated silica gels were then packed in a stainless-steel tube (25 cm × 0.20 cm i.d.) by a slurry method. The plate numbers of the packed columns were 1400–3700 for benzene with a hexane-2-propanol (90/10, v/v) mixture as the eluent at a flow rate of 0.1 mL/min at 25 °C. The dead time (t_0) of the columns was determined using 1,3,5-tri-*tert*-butylbenzene as the non-retained compound [40].

2.5. Apparatus and chromatography

The ^{1}H NMR spectra of the chitosan derivatives were obtained at 80 °C by a Bruker-500 MHz instrument. The samples (20 mg) were dissolved in DMSO-d_6 (0.5 mL). The IR analyses of the chitosan derivatives were carried out in the solid state using a PE FT-IR spectrometer (Spectrum 100) with a KBr pellet.

The chromatographic experiments were performed using a JASCO PU-2089 Intelligent HPLC pump equipped with a UV (JASCO UV-2070) detector and a circular dichroism (JASCO CD-2095) detector at room temperature. A solution of the racemate (2 mg/mL, 5 μL) was injected into the chromatographic system through an intelligent sampler (JASCO AS-2055). The flow rate of the eluent (hexane-2-propanol (90/10, v/v)) was 0.1 mL/min.

3. Results and discussion

3.1. Characterization of the chitosan derivatives

The structures of the chitosan derivatives were confirmed by ^{1}H NMR and elemental analysis. As shown in the ^{1}H NMR spectra of all derivatives in Supplementary data, all the peaks are well assigned to the structures for all derivatives. The elemental analysis data are summarized for some derivatives in Table S1 (see supplementary