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New cyclodextrin derivative 6-*O*-(2-hydroxyl-3-betainylpropyl)-*β*-cyclodextrin: preparation and its application for enantiomer separation of drugs by capillary electrophoresis

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Abstract A new cyclodextrin derivative 6-*O*-(2-hydroxyl-3-betainylpropyl)-*β*-cyclodextrin (6-HBP-*β*-CD) was prepared with a "synthesis-deprotection one pot" method and studied as an efficient chiral selector in the separation of racemic mixtures of drugs by capillary electrophoresis (CE). Compared with *β*-CD and 2-HP-*β*-CD, 6-HBP-*β*-CD could provide efficient separating capability about alkali racemic mixtures of drugs under suitable pH.

Keywords 6-*O*-(2-hydroxyl-3-betainylpropyl)-*β*-cyclodextrin · Inner-salt · Capillary electrophoresis · Chiral selector

Introduction

Torus-shaped cyclodextrins, which are cyclic oligosaccharides consisting of 6, 7 or 8 glucopyranosidic units (named α-, *β*- or *γ*-CD, respectively) linked by α-(1 → 4) glycosidic bonds have been widely used as host compounds in molecular recognition, enantioseparation and green organic chemistry due to their regiospecificity and stereospecificity abilities based on forming inclusion complexes [1–4]. Modification of native cyclodextrins could achieve a better fit and a stronger interaction (i.e., induced fit), which would further improve the molecular recognition ability of CDs greatly in the process of interaction with a guest molecule [5–9].

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Betaine, an inner-salt compound, is a natural product and found in most green plants. It could be a useful building block in synthesis for its special structure and physiological functions [10–12]. Auzély-Velty et al. prepared starch betainate by linking betaine to starch backbone with an ester bond and found that it might be a more biodegradable product than the traditional cationic starch ethers [13]. Granö et al. prepared starch betainate by reacting starch with betainyl chloride in refluxing 1,4-dioxane in the presence of pyridine as a nucleophilic catalyst and found that this compound could increase the strength of paper sheets and have beneficial influence on retention [14].

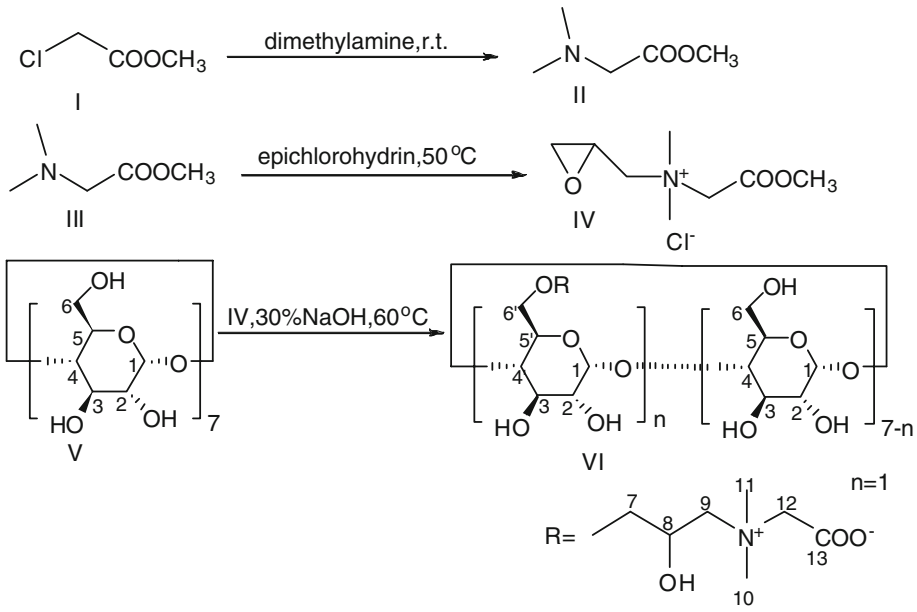
The linking of the inner-salt substituent to cyclodextrin might result in a new useful host compound. In this work, a new cyclodextrin derivative 6-*O*-(2-hydroxyl-3-betainylpropyl)-*β*-cyclodextrin (6-HBP-*β*-CD) was prepared by a "synthesis-deprotection one pot" method (Scheme 1) with the intention of producing useful functional models and enhanced performance characteristics. It was found that 6-HBP-*β*-CD, which has an inner-salt structure linked by the stable ether bond, was an efficient chiral selector in the separation of alkali racemic mixtures of drugs by capillary electrophoresis (CE).

Experimental

General method

Commercial cyclomaltoheptaose (*β*-CD) was recrystallized from water and dried at 100 °C in vacuum (2 torr) for 4 h. Methyl chloroacetate, dimethylamine, epichlorohydrin, sodium hydroxide and other reagents were of A.R. grade and used without further purification. NMR spectra were

Scheme 1 Synthesis of 6-*O*-(2-hydroxyl-3-betainylpropyl)-*β*-cyclodextrin



recorded on Bruker Avance-400 spectrometers. Capillary electrophoresis (CE) was run on an ACS-2000 capillary electrophoresis system (Beijing Cai-Lu Scientific Apparatus Co. Ltd. China). Mass spectrum was recorded on API-4000 spectrometers.

6-O-(2-hydroxyl-3-betainylpropyl)-β-cyclodextrin

Methyl chloroacetate (I) 0.3 mol was dropped into 100 mL dimethylamine (33%) water solution and stirred for 2 h at room temperature. The mixture was extracted with 40 mL ethyl acetate for three times. The extract was dried with anhydrous sodium sulfate for 3 h. Methyl 2-(dimethylamino) acetate (II) 18.7 g was received after distilling ethyl acetate [18].

Methyl 2-(dimethylamino) acetate 18.7 g and epichlorohydrin (1:1.2 in mole ratios) were dissolved in 60 mL methanol and stirred for 4 h under 52 °C. Pro-betaine compound (IV) 33.5 g was received after distilling methanol and epichlorohydrin [19].

β-cyclodextrin (10.0 g) was dissolved in 50 mL 25% NaOH and stirred for 1 h. Pro-betaine compound IV 5.0 g was dropped in the solution. The temperature of the solution heated to 60 °C and maintained for 8 h. Then, the solution was adjusted to pH 7, condensed and further purified by silica gel chromatography to give product (VI) (1.3 g) (eluent, MeOH: EtOAc: H_2O = 2:1:1(v/v)). TLC on silica gel showed the presence of the product VI (Rf 0.36 for VI, Rf 0.64 for V; developing agent, PrOH–EtOAc–H_2O–NH_3 (28%) = 5:3:2:3 (v/v)).

^{1}H-NMR (D_2O, 400 MHz): δ 4.90 ~ 5.13 (m, 7H, H-1, H-1′), δ 4.23 ~ 4.33 (s, 2H, H-12), δ 3.40 ~ 3.95 (m, ~47H, H-2, H-3, H-4, H-4′, H-5, H-6, H-5′, H-6′, H-7, H-8, H-9), δ 3.15 ~ 3.25 (m, 6H, H-10, H-11). ^{13}C-NMR (D_2O, 400 MHz): 164.78 (C-13), 101.91 (C-1), 80.93 ~ 81.48 (C-4,4′), 70.69 ~ 73.58 (C-2,3,5,5′,12), 66.48 (C-6′), 63.77 ~ 65.72 (C-7,8,9), 60.32 (C-6), 52.74 ~ 52.83 (C-10,11). [TurbocV] M^+: 1294.9. DS = 1.

Results and discussion

A “synthesis-deprotection one pot” method was introduced to link betaine substituent to cyclodextrin with the entire inner-salt structure (Scheme 1). The carboxyl group of betaine could result in many byproducts for its activity. In this method, the pro-betaine compound IV was prepared to protect the inner-salt structure with a stable methyl ester bond. The epoxide of pro-betaine compound IV would react with cyclodextrins under reaction conditions. Then, the ester bond would hydrolyze gradually catalyzed by sodium hydroxide to give 6-HBP-*β*-CD.

According to the elegant explanation from Ueno and Breslow [15], the substituent position of the product VI could be clearly indicated on the 6-OH of *β*-CD by the ^{13}C-NMR spectrum, which showed a large downfield chemical shift for C-6 (60.32 → 66.48), and nearly no change in the chemical shift of C-2, 3 of the unsubstituted glucose units. The ^{1}H-NMR spectrum of H-1 with nearly no chemical shift suggested that 6-OH of *β*-CD was substituted. The degree of substitution (DS) of VI could be obtained from the Mass spectrum. The M^+ of 6-HBP-*β*-CD is 1294.9. Thus, $DS = (M^+ - M_{\beta\text{-CD}})/M_R = (1{,}294.9 - 1{,}135)/159.2 = 1$.

Separation of enantiomers by CE has attracted much attention for its high efficiency, rapid method development, short analysis times and low consumption of reagents [16,

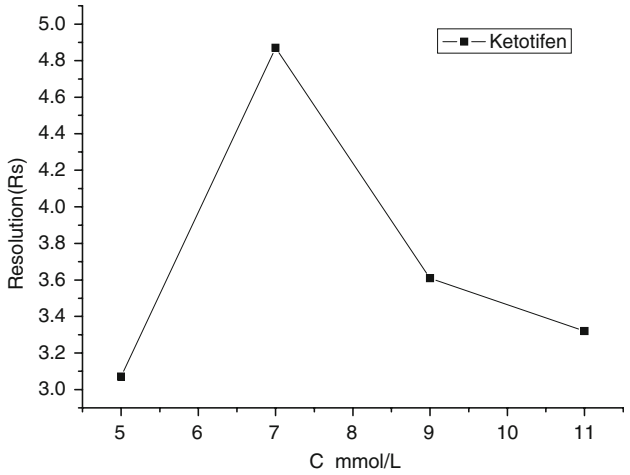


Fig. 1 The effect of the concentration of 6-HBP-β-CD on the resolution (Rs) of ketotifen. Conditions: Britton-Robinson buffer, pH = 3; ketotifen, 0.01 mg/mL; uncoated fused-silica capillary, 50 μm ID with 48 cm total and 40 cm effective length; applied voltage, 10 kV; 25 °C; electro-kinetic injection, 15.00 kV for 6 s; λ = 214 nm. The resolution of chlorphenamine, dioxopromethazine, terbutaline have a similar fashion with the resolution of ketotifen

17, 20]. CE with a background electrolyte containing modified CDs as the chiral selector has been widely used in the operating mode of CE for resolving enantiomers. When 6-HBP-β-CD was used as a chiral selector in CE, it was found that the enantiomers of chlorphenamine, dioxopromethazine, ketotifen and terbutaline could be separated effectively under the optimum conditions. The optimum conditions of 6-HBP-β-CD were obtained by a

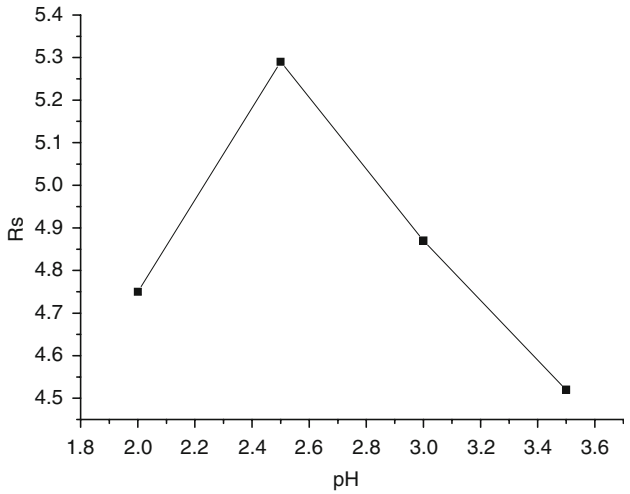


Fig. 2 The effect of pH of Britton-Robinson buffer on the resolution (Rs) of ketotifen. Conditions: Britton-Robinson buffer; 6-HBP-β-CD (7 mmol/L); ketotifen, 0.01 mg/mL; uncoated fused-silica capillary, 50 μm ID with 48 cm total and 40 cm effective length; applied voltage, 10 kV; 25 °C; electro-kinetic injection, 15.00 kV for 6 s; λ = 214 nm. The resolution of chlorphenamine, dioxopromethazine, terbutaline have a similar fashion with the resolution of ketotifen

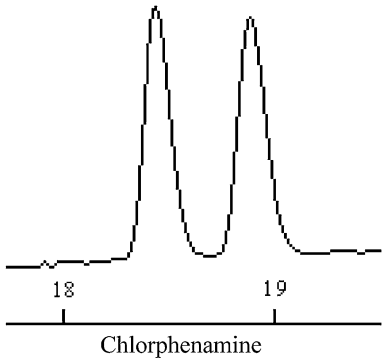


Fig. 3 The effect separation of chlorphenamine using 6-HBP-β-CD as the chiral selector. Conditions: Britton-Robinson buffer, pH = 2.5; 6-HBP-β-CD (7 mmol/L); chlorphenamine, 0.01 mg/mL; uncoated fused-silica capillary, 50 μm ID with 48 cm total and 40 cm effective length; applied voltage, 10 kV; 25 °C; electro-kinetic injection, 15.00 kV for 6 s; λ = 214 nm

concentration-sweep (Fig. 1) and pH-sweep (Fig. 2) method, the optimum concentration is 7 mmol/L and the optimum pH is 2.5. The electropherogram of chlorphenamine, which indicated the effect separation based on 6-HBP-β-CD as the chiral selector, is shown in Fig. 3. Compared with β-CD and 2-HP-β-CD, 6-HBP-β-CD showed better performance on the separation of these racemic alkali drugs (Table 1).

pH 2.5 was found to be the most suitable pH of the Britton-Robinson buffer (containing a mixture of acetic acid, phosphoric acid and boric acid) for the separation of the drug enantiomers (Fig. 2). It might relate to the nitrogen-containing drugs, which could cause enhanced complexation of the drugs with CDs by inhibiting the electroosmotic flow under acidic conditions. On the other hand, it might also relate to the stability of the inner-salt structure of the betainyl group. When pH increased, electrovalent interaction of the betainyl group was stable and 6-HBP-β-CD had no selectivity to the drugs. When pH

Table 1 The separation of racemic drugs by CE using β-CD derivatives as chiral selectors

Pharmaceutical	Resolution (Rs)		
	6-HBP-β-CD[a]	β-CD[b]	2-HP-β-CD[c]
Chlorphenamine	1.92	2.33	1.56
Dioxopromethazine	1.63	1.23	–
Ketotifen	5.29	3.94	2.89
Terbutaline	2.16	1.47	2.76

Conditions: Britton-Robinson buffer, chiral compounds, 0.01 mg/mL; uncoated fused-silica capillary, 50 μm ID with 48 cm total and 40 cm effective length; applied voltage, 10 kV; 25 °C; electro-kinetic injection, 15.00 kV for 6 s; λ = 214 nm

[a] 6-HBP-β-CD 7 mmol/L, pH = 2.5. [b] β-CD 12 mM/L, pH = 3.0. [c] 2-HP-β-CD 15 mM/L, pH = 3.0

decreased, electrovalent interaction of the betainyl group could be broken and the carboxylate group turned to be carboxyl group, which resulted in a decreased enantioselectivity of 6-HBP-β-CD to the drugs.

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