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Short communication

Synthesis and application of mono-6-ammonium-6-deoxy-β-cyclodextrin chloride as chiral selector for capillary electrophoresis

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Abstract

A facile synthetic approach for mono-6-amino-6-deoxy-β-cyclodextrin (β-CD-NH_2) was proposed. Its hydroxy chloride salt, mono-6-ammonium-6-deoxy-β-cyclodextrin chloride (β-CD-NH_3Cl) was further prepared and used for the enantioseparation of various anionic and ampholytic analytes by capillary electrophoresis (CE). The effect of background electrolyte (BGE) pH and selector concentration on the enantioseparation was studied. Results showed that β-CD-NH_3Cl displayed powerful chiral resolution ability towards anionic analytes. In addition, baseline separation of a standard mixture consisting of eight acids was achieved within 35 min.

Keywords: Enantioseparation; Single-isomer; Positively charged β-CD; Capillary electrophoresis; Mono-6-ammonium-6-deoxy-β-cyclodextrin chloride

1. Introduction

Chirality is a major concern in the modern pharmaceutical industry [1,2]. This interest can be attributed largely to a heightened awareness that enantiomers of racemic compounds may exhibit different physiological activities as well as different pharmacokinetic and pharmacodynamic effects [3,4]. Capillary electrophoresis (CE) has rapidly developed as one of the powerful techniques for chiral separation [5]. Chiral separation by CE occurs through different interactions between enantiomers and chiral selectors that are added into the running buffers. Native cyclodextrins (CDs) and their derivatives are most widely used chiral selectors. Charged CDs have been introduced to improve the chiral recognition ability for neutral and oppositely charged analytes. Until recently, negatively charged CDs, have been actively reported [6], while there are fewer reports using positively charged CDs.

The use of randomly multi-substituted CDs may provide higher enantioselectivity [5,7–10]. However, the resolutions are greatly affected by the degree and distribution of substitutions. Hence the use of single-isomer CDs was recommended by Armstrong and Nair [9] and Vigh and co-workers [11]. Mono-6-amino-6-deoxy-β-CD (β-CD-NH_2) has previously been synthesized [12–15] and employed in chiral CE for enantioseparation of anionic analytes [16,17]. This cationic CD demonstrated to be an effective chiral selector only when positively charged at pH lower than its pK_a (∼8.2). The objective of this paper is to present a more convenient synthetic approach for β-CD-NH_2 and also to assess the chiral resolution ability of β-CD-NH_3Cl by use of anionic and ampholytic racemates.

2. Experimental

2.1. Chemicals

Monobasic sodium phosphate (NaH_2PO_4), phosphoric acid and sodium hydroxide were purchased from Merck

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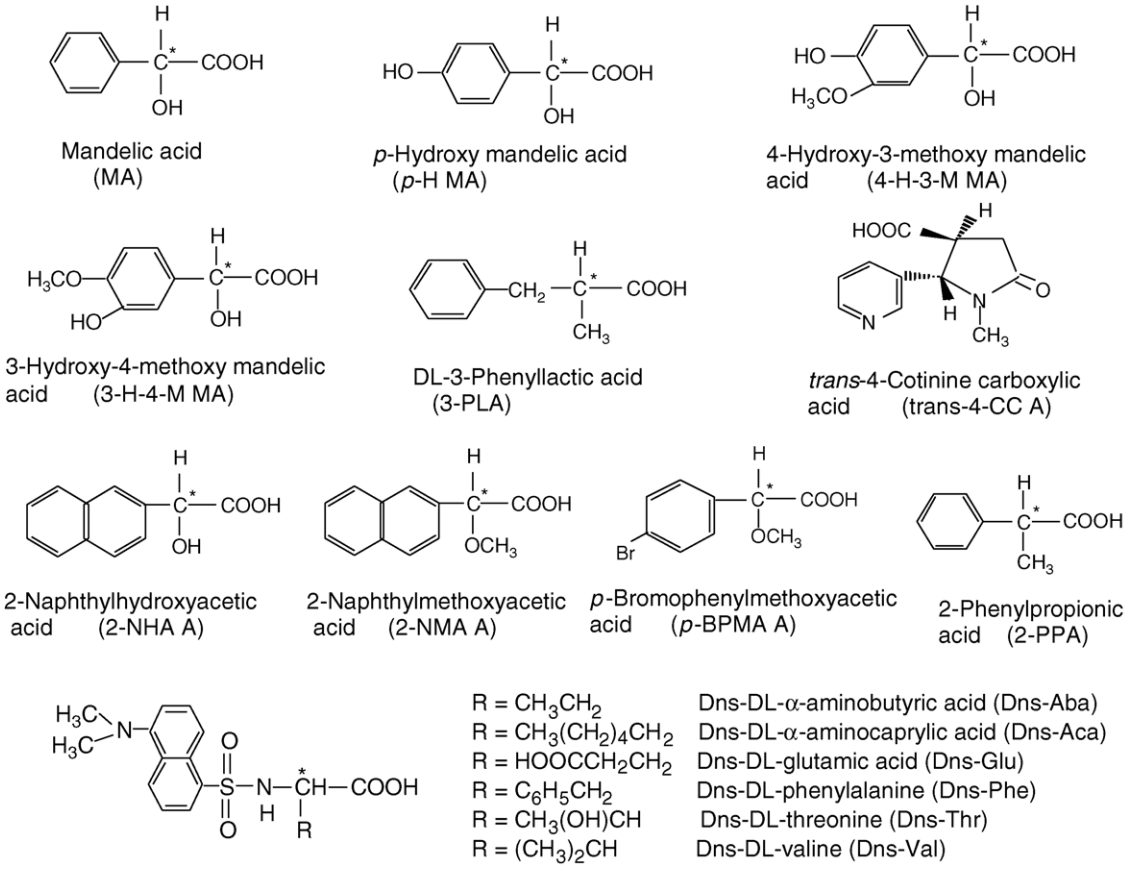

Fig. 1. Structures of anionic and ampholytic acids studied in this paper.

(Darmstadt, Germany). 2-Naphthylhydroxyacetic acid, 2-naphthylmethoxyacetic acid and *p*-bromophenylmethoxyacetic acid were synthesized according to the report [18]. Other chiral compounds were purchased from Aldrich (Steinheim, Germany) or Sigma (St. Louis, MO, USA). The structures of these racemic compounds are shown in Fig. 1.

2.2. Instrumentations

The NMR spectra were recorded on a Bruker ACF300 (300 MHz). Mass spectra were obtained from a Finnigan/MAT TSQ7000. All electrophoretic experiments were performed on a Beckman P/ACE MDQ CE unit (Fullerton, CA, USA) using 59.2 cm (effective length 49 cm) × 50 μm I.D. × 375 μm O.D., untreated fused-silica capillaries (Polymicro Technologies, Phoenix, AZ, USA). The cartridge coolant of the CE unit was thermostated at 25 °C. Samples were injected by 0.5 psi nitrogen (typically 10 s). The applied voltage was +15 kV. The variable-wavelength PDA detector was used for detection through three channels at 214, 254 and 280 nm.

2.3. Buffer and sample solutions

One hundred millimetres NaH_2PO_4 stock solutions were used as background electrolytes (BGEs). Running buffers were prepared by dissolving the appropriate amount of chiral selctor into BGEs and titrated with sodium hydroxide or phosphoric acid to the required pH (4.0–8.0). The 50 μg/mL stock solutions of racemic analytes were prepared and stored at 4 °C. All running buffers and sample solutions were filtrated with a 0.45 μm syringe type Millipore membrane and sonicated prior to use. The electroosmotic flow (EOF) was measured with MeOH as neutral marker.

3. Results and discussion

3.1. Synthesis of mono-6-ammonium-6-deoxy-β-cyclodextrin chloride (β-CD-NH_3Cl)

At the outset of our work, there are some reports on the synthesis of β-CD-NH_2 via either ammonia [12] or azide [13–15], starting from 6-monotosyl-β-CD **2** [19,20] (Fig. 2 as the dashed arrows). These previous routes are usually undesirable for large-scale production due to the demand of either longer reaction time or expensive catalyst and/or high pressure.

We modified the synthetic approach by the use of triphenyl phosphine (Fig. 2 as the solid arrow). Crude β-CD-NH_2 **4** was prepared by reacting mono-6-azido-6-deoxy-CD **3** with

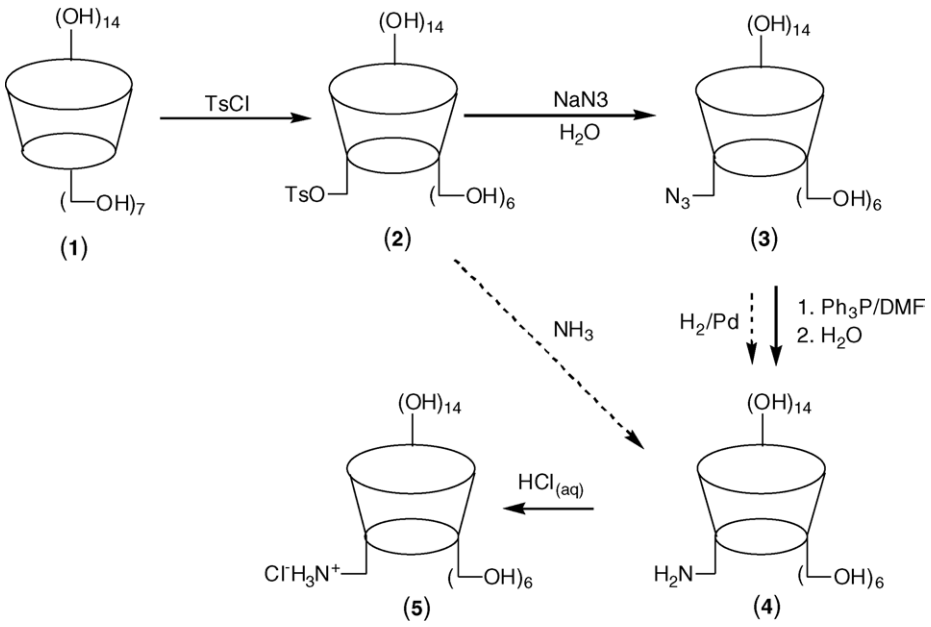

Fig. 2. Schematic synthesis for mono-6-ammonium-6-deoxy-β-cyclodextrin chloride.

triphenyl phosphine in DMF at ambient conditions for 2 h and following by hydrolysis. After precipitation in acetone, filtering and washing with acetone, pure amine **4** was obtained as white solid. β-CD-NH_3Cl **5** was further obtained by titration of aqueous amine **4** solution with hydrogen chloride. The spectral and physical properties of the compound **5** were consistent with those reported previously [15].

3.2. *Effect of pH on enantioseparation with β-CD-NH_3Cl*

The effect of pH is very complicated since the dissociation of anionic analytes and phosphate buffer varies with pH. In addition, β-CD-NH_3Cl may be deprotonated at basic pH ($pK_a \sim 8.2$). Therefore, pH affects both the EOF and the chiral recognition abilities of β-CD-NH_3Cl. The chiral separations of nine anionic analytes were examined by gradually increasing BGE pH from 4.0 to 8.0 (Table 1). Since all anionic analytes have a carboxylic function, which has a pK_a between 3.5 and 4, all nine anionic analytes are negatively charged at the BGE pH conditions.

As shown in Table 1, the migration times of all analytes decreased with increasing BGE pH (4.0–7.0). However, the reduction in the migration times with higher pH terminated as pH 7.0. From this very point, a further increase of buffer pH led to extension of the migration times. Meanwhile, since EOF increased with pH and achieved a maximum at pH 7.0, the magnitudes of effective mobilities (μ_{eff2}) of all analytes generally increased with pH before reaching their maximum at pH 7.0. This somewhat puzzling phenomenon was perhaps due to the diminution of EOF, flattening off around pH 7.0 [21].

The chiral α and R_s of mandelic acid and its derivatives also decreased with the increase of pH. Therefore, better resolutions were often obtained in the cost of longer migration time. For the case of carboxylic acids, however, the chiral α and R_s resolutions displayed a maximum at pH 5.0–6.0. For example, 3-PLA acid and trans-4-CC A achieved the

Table 1
Migration time of less mobile enantiomer (t_2, min), separation selectivity (α) and peak resolutions (R_s)of racemic acids in 5 mM β-CD-NH_3Cl BGEs with different pH value

Entry	4.0			5.0			6.0			6.5			7.0			8.0		
	t_2	α	R_s	t_2	α	R_s	t_2	α	R_s	t_2	α	R_s	t_2	α	R_s	t_2	α	R_s
MA	51.51	1.07	3.73	49.31	1.14	3.16	23.83	1.05	2.18	21.61	1.05	2.02	20.23	1.04	1.51	35.42	1.04	1.24
p-H MA	40.24	1.08	3.82	24.35	1.07	3.52	21.23	1.06	2.59	18.18	1.05	2.05	17.83	1.04	1.73	32.18	1.04	1.26
4-H-3-M MA	48.15	1.02	0.83	30.91	1.02	1.15	26.33	1.02	0.97	18.93	1.01	0.67	19.24	1.01	0.61	29.02	1.01	0.74
3-H-4-M MA	38.73	1.04	2.20	30.95	1.05	2.33	28.96	1.08	2.12	18.02	1.03	1.35	18.33	1.02	1.04	27.65	1.01	0.96
3-PLA	29.34	1.08	3.26	23.28	1.10	4.22	20.09	1.09	4.91	17.15	1.07	3.05	16.46	1.07	3.10	28.34	1.06	2.85
Trans-4-CC A	30.51	1.00	<0.5	24.02	1.01	<0.5	21.44	1.02	0.92	18.05	1.01	0.70	14.55	1.01	0.69	31.94	1.01	0.82
2-NHA A	26.85	1.05	0.85	16.32	1.14	1.65	12.91	1.05	1.52	12.13	1.05	1.48	8.71	1.04	1.46	20.97	1.05	1.29
2-NMA A	24.54	1.04	1.72	18.29	1.12	3.62	14.14	1.05	2.77	12.17	1.04	2.23	11.71	1.04	1.84	21.52	1.04	1.18
p-BPMA A	29.23	1.05	1.65	20.66	1.05	2.25	18.69	1.06	1.75	14.54	1.03	1.64	13.21	1.02	1.49	19.61	1.02	1.61

Table 2
Effective mobilities of the less mobile enantiomers (μ_{eff2}, in ($\times 10^{-5}$) $cm^2\ V^{-1}\ s^{-1}$ units), selectivities (α) and resolutions (R_s) of racemic acids in pH 6.0 β-CD-NH_3Cl BGEs

Entry	5 mM			10 mM			15 mM			20 mM		
	μ_{eff2}	α	R_s	μ_{eff2}	α	R_s	μ_{eff2}	α	R_s	μ_{eff2}	α	R_s
MA	−22.21	1.05	2.18	−20.22	1.19	4.33	−19.23	1.21	3.99	−16.01	1.35	7.99
p-H MA	−21.46	1.06	2.59	−20.74	1.13	4.46	−14.05	1.10	4.98	−11.87	1.14	7.23
4-H-3-M MA	−19.96	1.00	0.71	−18.82	1.03	1.35	−17.11	1.04	2.01	−15.79	1.15	4.31
3-H-4-M MA	−23.83	1.08	2.12	−22.50	1.03	2.39	−21.16	1.07	3.51	−20.18	1.16	4.39
3-PLA	−15.02	1.10	4.91	−10.36	1.07	4.61	−9.32	1.08	4.56	−7.68	1.09	5.01
Trans-4-CC A	−18.49	1.02	0.92	−16.81	1.03	1.34	−15.0	1.04	1.76	−13.90	1.08	2.48
2-NHA A	−8.24	1.05	1.05	−7.51	1.04	1.96	−4.34	1.04	0.88	−3.32	1.04	0.78
2-NMA A	−8.98	1.05	2.77	−6.92	1.07	4.27	−5.07	1.04	1.48	−4.99	1.05	1.42
p-BPMA A	−22.31	1.06	1.75	−11.60	1.04	2.50	−7.59	1.04	2.45	−5.82	1.03	2.41
2-PPA	−14.63	1.02	0.86	−12.15	1.02	1.24	-9.72	1.02	1.07	−7.84	1.02	1.06
Dns-Aba	−8.67	1.02	0.71	−6.37	1.03	1.34	−3.76	1.03	1.91	−2.80	1.04	2.07
Dns-Aca	−7.42	1.03	2.02	−6.58	1.03	1.88	−3.51	1.02	1.74	−2.61	1.01	1.17
Dns-Glu	−22.47	1.02	0.76	−16.86	1.04	1.99	−13.73	1.08	3.61	−12.16	1.09	4.27
Dns-Phe	−9.51	1.05	2.56	−8.02	1.04	1.58	−7.37	1.07	1.59	−5.76	1.02	1.30
Dns-Thr	−11.07	1.01	0.71	−9.38	1.01	0.61	−6.49	1.01	0.65	−5.73	1.00	<0.5
Dns-Val	−9.91	1.01	0.79	−9.61	1.01	0.74	−6.01	1.01	<0.5	−5.12	1.00	<0.5

Conditions: 50 mM phosphate buffer, uncoated capillary, +15 kV, 25 °C.

maximum in chiral α and R_s at pH 6.0 while 2-NHA A, 2-NMA A and *p*-BPMA A obtained at pH 5.0.

3.3. Effect of β-CD-NH_3Cl concentration on enantioseparation of anionic analytes

Taking both analysis speed and resolution of the enantiomers into consideration, pH 6.0 was chosen as optimal. Thus, the effect of selector concentration on enantioseparation of selected analytes was assessed with pH 6.0 BGEs. The separation results are summarized in Table 2.

The effective electrophoretic mobilities (μ_{eff2}) of all analytes decreased with increasing the concentration of β-CD-NH_3Cl. The decrease in the magnitudes of μ_{eff2} may be caused by the increased viscosity of BGE. Also the increase of CD concentration shifted the complexation equilibrium towards the forming of CD-analyte complexes. In such a case, because of the increased concentration of complexes, the net result was again a decreasing of the effective mobility of the enantiomeric compounds.

The chiral α and R_s of some anionic racemates continuously increased with increment in CD concentration. Examples such as MA and its derivatives, Dns-Aba and Dns-Glu presented better resolution as higher CD concentration. However, some other racemates displayed a maximum in the chiral α and R_s at 10 mM CD (ca. 2-NHA A, 2-NMA A, *p*-BPMA A and 2-PPA). For the rest Dns-amino acids, their chiral α and R_s deceased with increasing the concentration of β-CD-NH_3Cl.

3.4. Chiral separations of standard mixture of anionic analytes

In order to further demonstrate the chiral resolution power of β-CD-NH_3Cl for these anionic analytes, a standard mixture containing eight pairs of anionic and ampholytic analytes was baseline separated with 20 mM β-CD-NH_3Cl within 35 min (Fig. 3). The migration order of analyte enantiomers in the standard mixture was verified by injecting each racemate individually.

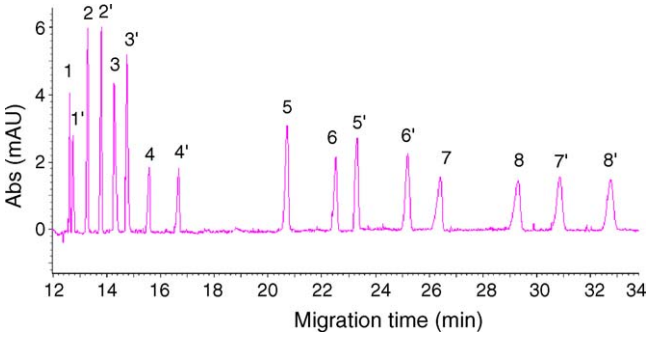

Fig. 3. Baseline separation of a mixture of eight acids by the use of β-CDNH_3Cl. *Conditions*: 20 mM CD, 50 mM phosphate buffer (pH 6.0). *Legends*: 1, 1′: Dns-Aca; 2, 2′: Dna-Aba; 3, 3′: 2-NHMA; 4, 4′: 3-PLA; 5, 5′: Dns-Glu; 6, 6′: 3-H-4-M MA; 7, 7′: MA; 8, 8′: 4-H-3-M MA.

4. Concluding remarks

In this paper, a convenient, synthetic approach of β-$CDNH_2$ was presented. The positively charged single-isomer CD, β-$CDNH_3Cl$ was further prepared and successfully used in the enantioseparation of anionic and ampholytic analytes by CE. The chiral resolution was strongly dependent upon BGE pH and CD concentration, as suggested by Wren and Rowe [22]. β-$CDNH_3Cl$ proved to be an effective chiral selector for the enantioseparation of the selected anionic analytes. In addition, an eight-acid mixture was baseline separated in a single run within 35 min.

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