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Novel methoxypropylimmidazolium β-cyclodextrin for improved enantioseparation of amino acids

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ABSTRACT

A new single-isomer cationic cyclodextrin, mono-6^A-[3-(3-methoxypropyl)imidazol-1-ium]-6^A-β-cyclodextrin chloride, has been synthesized and successfully used for the chiral separation of dansyl amino acids in capillary electrophoresis. With methoxy functionality, the new cationic cyclodextrin exhibits significantly improved enantioselectivities. Excellent enantioseparations for amino acids are obtained in chiral selector concentration range between 2.5 mM to 15 mM at pH 6.0. Chiral resolution as high as 7.3 was achieved for Dns-Aca with 5 mM chiral selector. Comparison study and theoretical calculation with Wren's model attribute the enhanced enantioseparation to the stronger inclusion complexation between amino acids and cyclodextrin. The binding constants for dansyl amino acids and the cationic cyclodextrins are calculated to be 173–253 M^{-1}, while the optimum cyclodextrin concentrations were estimated to be 4.1–7.6 mM.

1. Introduction

Over the last two decades, capillary electrophoresis (CE) has firmly established as a powerful tool for the analysis of chiral compounds, especially those with increased pharmaceutical and biochemical interest [1–4]. CE features as high efficiency, fast analysis and minimum consumption of chiral selectors and analytes. For chiral analysis with CE, cyclodextrins (CDs) and their derivatives are the most widely used chiral selectors, mainly attributed to their unique properties such as weak UV absorption, excellent chiral recognition and relatively low cost [5]. However, the use of neutral CDs is limited due to their low water solubility and lack of the ability to establish ion-pairing with uncharged analytes [6–8]. Accordingly, the development of charged CDs could afford the possibility to separate neutral racemates because the charged CDs can form ion-pairing interaction with guest analytes, and thus enhance the resolution of the enantiomeric analytes.

With charged CDs, improved enantioselectivities have also been achieved for oppositely charged analytes due to the synergistic effect of electrostatic interactions and inclusion complexation for chiral recognition [6–12]. For the concern of repeatability and reproducibility in synthesis and application, the strategy of structurally-defined CDs is recommended [12–17]. A library of imidazolium-substituted CDs have demonstrated good enantioselectivities for amino acids-like ampholytic racemates but limited for acidic racemates, while amine/ammonium-substituted CDs performed exactly opposite [18–21].

Recently, we reported on a versatile chiral selector, methoxypropylammonium monosubstituted CD (MPrAMCD) [16], which could provide potential hydrogen-bonding to enhance the chiral resolution of amino acids and hydroxyl acids in comparison with propylammonium monosubstituted one (PrAMCD) [22]. The extra interactions constructed between enantiomer and CD functionality effectively enhance the chiral recognition; resulting in improved enantioseparation of racemates in wider separation window. Inspired by this "extra interactions" enhancing chiral recognition concept, we wonder whether the incorporation of a methoxy functionality onto the alkylimmimidazolium-CD terminal will further enhance their chiral separation ability towards amino acids.

In this study, we report herein the development of a novel single-isomer cationic CD, mono-6^A-[3-(3-methoxypropyl)-imidazol-1-ium]-6^A-deoxy-β-cyclodextrin chloride (MPrIMCD). The chiral separation of MPrIMCD towards Dns-amino acids was evaluated by optimizing the buffer pH and CD concentration in CE. The impact of introduced methoxy functionality on the enhanced chiral recognition ability is revealed by Wren's model and comparison study. Enhanced enantioselectivities was observed with the existence of polar methoxy functionality.

2. Materials and methods

2.1. Materials and reagents

All chemicals were of analytical-reagent grade. All dansyl-DL-amino acids (Dns-amino acids) were purchased form Sigma

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(St. Louis, MO, USA) (see Fig. 1). The new chiral selector, MPrIMCD, was synthesized in the lab.

Background electrolytes (BGE) were of 50 mM NaH_2PO_4 buffer with varied pH values (5.0–8.0). Running buffers were prepared accordingly by dissolving appropriate chiral selector into phosphate BGEs. Stock solutions of 1 mg/mL racemic analytes were prepared with mixture solvent of methanol/water (50/50, v/v), which were stored at 4 °C and diluted to 50 μg/mL prior to use. All buffers and samples were filtrated through a 0.45 μm syringe type Millipore membrane and ultrasonicated before use.

2.2. Instrumentations

Both ^1H NMR and ^{13}C NMR spectra were obtained on Bruker AMX 500 (500 MHz). All CE separations were performed on a Beckman P/ACE™ MDQ CE unit (Fullerton, CA, USA), equipped with 59.2 cm × 50 μm I.D. uncoated fused silica capillary (49 cm to the detector. The capillary was mounted in a cartridge and thermostated at 25 ± 0.1 °C. Samples were injected into the capillary under a 0.5 psi pressure injection for 10 s. Detection of analytes was carried out at 214, 254 and 280 nm by using an online PDA (Photodiode Array, 190–300 nm) detector. CE runs were driven at 20 kV applied voltage. The whole instrument was controlled with the system 32 Karat Software (version 5) for data acquisition and system control.

2.3. CE procedures

The capillary was rinsed daily with 1.0 M NaOH (15 min), 0.1 M NaOH (30 min) and H_2O (30 min). Between sample runs, the capillary was rinsed with 1 M NaOH, 0.1 M NaOH, H_2O for 2 min each, and CD buffer for 5 min. The electroosmotic flow (EOF) was measured using MeOH as marker. The separation selectivity (α), was calculated as $\alpha = t_2/t_1$, where t_2 was assigned arbitrarily to the less mobile enantiomer in the CE separations. The peak resolution value R_s, were calculated by dividing the migration time difference of the two enantiomers with half of the sum of their peak widths at the baseline.

3. Results and discussion

3.1. Synthesis of MPrIMCD

The new chiral selector, MPrIMCD, was prepared via a three-step procedure (Scheme 1). Typically, imidazole was dissolved in freshly dried DMF. Towards the above solution was added with 1-bromo-3-methoxy propane. The mixture solution was reacted at 90 °C for 12 h under nitrogen. After removal of DMF via vacuum distillation, the intermediate compound 3-methoxy propylimidazole **1** was obtained by extraction with dichloromethane [10].

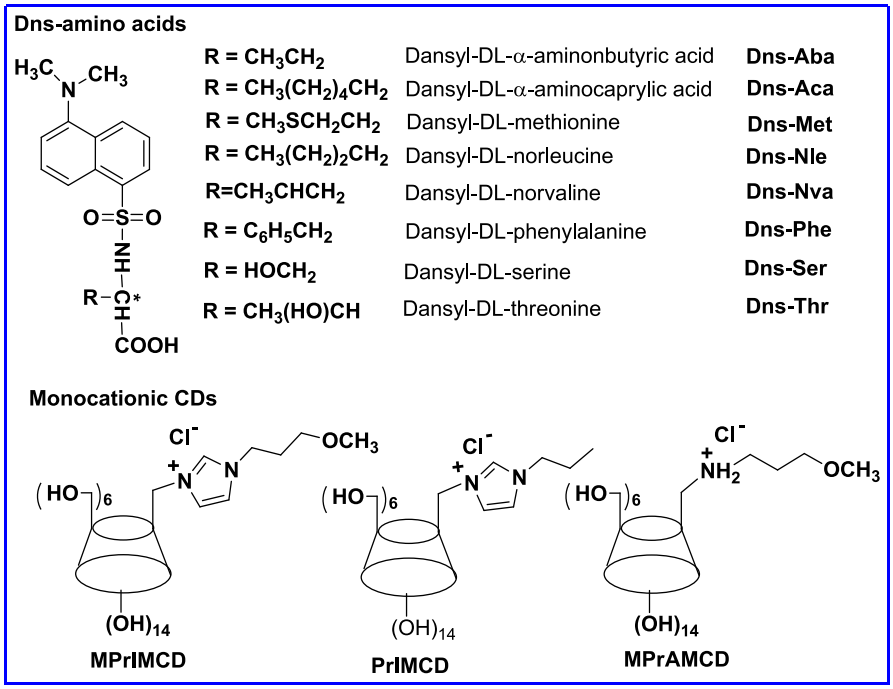
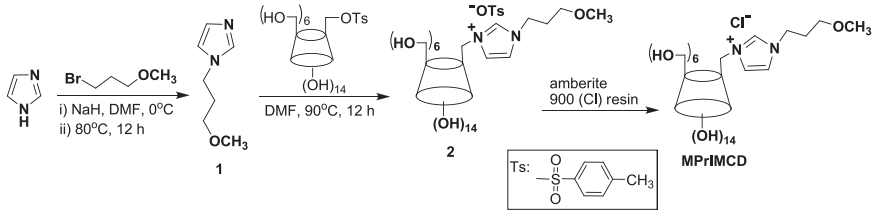


Fig. 1. Structures of Dns-amino acids and cationic CDs used in this study.



Scheme 1. Synthetic route of the chiral selector MPrIMCD.

A mixture solution of mono-6-tosyl-β-cyclodextrin [14] and 3-methoxy propylimidazole in DMF was then refluxed at 90 °C for 5 h under nitrogen. The reaction mixture was then added dropwise into acetone to precipitate out compound **2**. The aqueous solution of compound **2** was soaked in a bed of amberlite 900(Cl) resin for anion exchange, the eluent was dried to obtain the title product was obtained as a white solid (85% yield).

The characterization data of **1** are listed as follows: ^{1}H NMR (500 MHz, DMSO-d$_6$) δ: 8.13 (s, 1*H*, =CH–$_{2im}$), 7.77 (s, 1*H*, =CH–$_{4im}$), 7.64 (s, 1*H*, =CH$_{-5im}$), 3.98 (s, 2*H*, –CH$_2$–$_{im}$), 3.37 (t, 2*H*, –CH$_{2-OCH_3}$), 3.30 (s, 3*H*, –CH$_3$), 1.94 (m, 2*H*, –CH$_2$–CH$_2$); ^{13}C NMR (125 MHz, DMSO-d$_6$) δ: 132.84 (C–$_{2im}$), 122.96 (C–$_{4im}$), 126.61 (C–$_{5im}$), 68.9 (CH$_{2-OCH_3}$), 59.3 (CH$_3$), 46.2 (CH$_{2\text{-}im}$), 30.6 (CH$_2$–CH$_2$). The characterization data of **2** are listed as follows: FT-IR (KBr, cm^{-1}): 3398 (O–H, str), 2927 (C–H str), 1157 (S–O, str) 1080, 1030 (C–O–C, str); ^{1}H NMR (500 MHz, DMSO-d$_6$) δ: 7.48 (d, 2*H*, *J*=8.0 Hz, =CH–$_{ortho\text{-}OTs}$),7.45(s, 1*H*, CH–$_{N,N}$), 7.03(s, 1*H*, CH–$_N$), 7.12 (d, 2*H*, *J*=8.0 Hz, =CH–$_{meta\text{-}OTs}$), 6.89 (s, 1*H*, CH–$_{N\text{-}C}$), 5.80–5.63 (m, 14*H*, OH-2$_{CD}$ and OH-3$_{CD}$), 4.85 (s, 1*H*, H-1′$_{CD}$), 4.83 (s, 6*H*, H-1$_{CD}$), 4.49–4.44 (m, 6*H*, OH-6$_{CD}$), 4.04 (t, 2*H*, CH$_{2\text{-}N}$), 3.64–3.56 (m, 28*H*, H-5$_{CD}$, H-3$_{CD}$ and H-6$_{CD}$), 3.39–3.30 (m, 14*H*, H-2$_{CD}$ and H-4$_{CD}$), 3.37(t, 2*H*, CH$_{2-OCH_3}$),3.30 (s, 3*H*, –OCH$_3$), 2.29 (t, 3*H*, –CH$_{3\text{-}OTs}$), 1.94(m, 2*H*, CH$_2$–CH$_2$). The characterization data of MPrIMCD are as follows: FT-IR (KBr, cm^{-1}): 3390 (O–H str), 2920 (C–H str), 1032 (C–O–C str); ^{1}H NMR (500 MHz, DMSO-d$_6$) δ: 7.45 (s, 1*H*, CH–$_{N,N}$), 7.03 (s, 1*H*, CH–$_N$), 6.89 (s, 1*H*, CH–$_{N\text{-}C}$), 5.82–5.65 (m, 14*H*, OH-2$_{CD}$ and OH-3$_{CD}$), 4.88 (s, 7*H*, H-1$_{CD}$), 4.48 (m, 6*H*, OH-6$_{CD}$), 4.04 (t, 2*H*, CH$_{2\text{-}N}$), 3.65–3.55 (m, 28*H*, H-3′$_{CD}$, H-5$_{CD}$, H-3$_{CD}$ and H-6$_{CD}$), 3.38–3.29 (m, 14*H*, H-2$_{CD}$ and H-4$_{CD}$), 3.30 (s, 3*H*, –OCH$_3$), 3.07 (t, 2*H*, CH$_{2-OCH_3}$CH$_2$–$_{OCH3}$), 1.94(m, 2*H*, CH$_2$-CH$_2$); ^{13}C NMR (125 MHz, DMSO-d$_6$) δ: 137.5 (C$_{-N}$), 123.8 (C$_{-N}$), 123.1 (C$_{-N}$), 101.9 (C1$_{CD}$), 101.4 (C1′$_{CD}$), 83.5 (C4′$_{CD}$), 81.8–81.2 (C4$_{CD}$), 72.8 (C2$_{CD}$), 72.7 (C2′$_{CD}$), 72.4 (C3$_{CD}$), 72.2 (C3′$_{CD}$), 72.0 (C5$_{CD}$), 69.7 (C5′$_{CD}$), 60.2 (C6′$_{CD}$), 59.9 (C6$_{CD}$), 57.8 (CH$_{2\text{-}NH2}$), 48.4 (CH$_2$), 46.0 (CH$_2$), 30.6 (–OCH$_3$). The NMR spectra of MPrIMCD (Fig. 2) agree well with its chemical structure.

3.2. Effect of pH on enantioseparation

The pH of BGE is of great importance to the enantioseparation of charged analytes because the effective charge and mobility of analytes directly depend on the pH values [9,23]. Considering the p*I* values of most Dns-amino acids (< 6.0) [24], and the p*K*a of the imidazolyl nitrogen (5.81) [25] in the narrow rim of CD in MPrIMCD, the pH of BGE was varied from 5.0 to 8.0. In pH 5.0–7.0, the negatively charged Dns-amino acids would migrate to anode at the absence of CD. When the inclusion complexation between cationic CD and amino acids formed, they would move towards the cathode with the EOF. Under these conditions, the inclusion complexation coupled with ion pair interactions will facilitate the chiral recognition process. The enantioseparation data of Dns-amino acids with 5 mM MPrIMCD in various pH BGEs are summarized in Table 1.

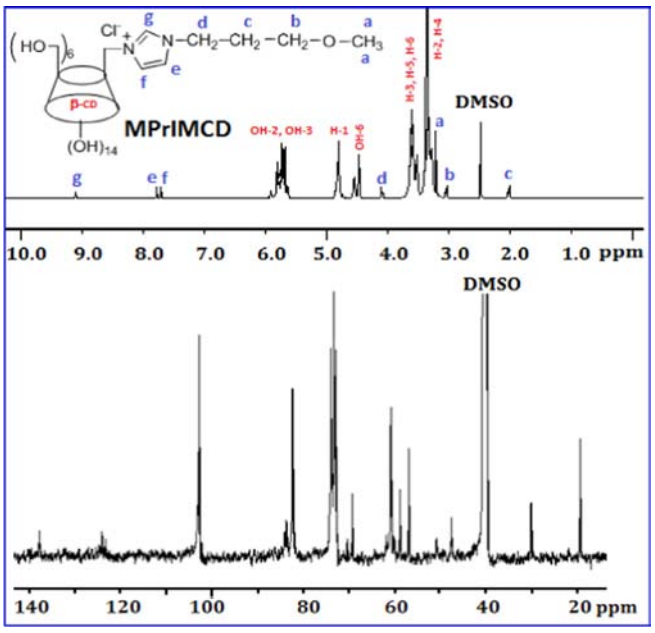


Fig. 2. ^{1}H NMR and ^{13}C NMR spectra of MPrIMCD.

Table 1
Enantioselectivity (α) and chiral resolutions (R_s) of Dns-amino acids with 5 mM MPrIMCD in various pH BGEs.

Analytes	pH 5.0		pH 6.0		pH 6.5		pH 7.0		pH 8.0	
	α	R_s	α	R_s	α	R_s	α	R_s	α	R_s
Dns-Aba	1.04	1.5	1.10	5.2	1.04	1.1	1.06	1.4	1.03	0.8
Dns-Aca	1.04	1.3	1.07	7.3	1.04	3.4	1.02	2.2	1.02	0.7
Dns-Met	1.08	6.4	1.06	2.3	1.06	2.8	1.04	3.0	1.04	0.6
Dns-Nle	1.04	1.1	1.04	2.8	1.03	1.9	1.04	2.1	1.01	0.7
Dns-Nva	1.02	1.6	1.04	2.2	1.02	1.9	1.02	1.5	1.01	0.6
Dns-Phe	1.09	2.3	1.14	3.7	1.10	2.7	1.11	2.3	1.06	1.7
Dns-Ser	1.02	1.0	1.02	2.1	1.02	1.1	1.06	3.2	1.03	0.9
Dns-Thr	1.08	3.1	1.15	7.1	1.05	1.7	1.06	2.5	1.06	1.3

As shown in Table 1, all Dns-amino acids can be well separated with MPrIMCD in pH rang 5.0–7.0. Except Dns-Met, all analytes achieved their highest resolution at pH 6.0, with an R_s as high as 7.3 and 7.1 obtained for Dns-Aca and Dns-Thr, respectively. The resolutions obtained for Dns-amino acids with MPrIMCD are better than those with histamine, alklyimmidazolium or alkylammonium modified CDs [11,20–22,26]. This indicated that MPrIMCD was a good complex agent for Dns-amino acids for the improved selectivity. At pH 8.0, the imidazolium moiety of CD may lose its protonated form, and its electrostatic interactions with negatively charged amino acids become inaccessible, resulting in weaker enantioselectivity. As expected, only Dns-Phe and Dns-Thr were baseline separated. The migration times of analytes increased with BGE pH, mainly due to the increased EOF. Considering both analysis time and enantioselectivity, the optimum pH 6.0 is selected for the further study.

3.3. Effect of CD concentration on enantioseparation

CD concentration is another key factor for enantioseparation in CE. Herein, MPrIMCD concentration was optimized by gradually increasing from 1 mM to 15 mM at the optimum pH 6.0. In order to reveal the contribution of methoxy functionality for enantioseparation of Dns-amino acids, the separation data of seven Dns-amino acids with MPrIMCD are compared with our previously reported PrIMCD at its optimized BGE pH 5.0 [21].

As shown in Table 2, the effective mobility of all racemates was found to decrease with the increment of CD concentration. This could be partially explained with the increased viscosity of BGE with increased CD concentration. The chiral resolutions of most Dns-amino acid analytes increased from 1 mM to 5 mM until, reaching a local maximum value, while further increase in concentration leads to a decrease in chiral resolution. All Dns-amino acids can be well separated ($R_s > 1.1$) at MPrIMCD concentration ranging from 2.5 mM to 15 mM. Even at 1 mM CD, most amino acids were baseline resolved except Dns-Aca and Dns-Phe.

All Dns-amino acids could achieve the best selectivities at 5 mM CD. For instance, the best selectivity of 1.15 and 1.14 was observed at 10 mM CD for Dns-Thr and Dns-Phe, respectively.

Table 2
Effective mobilities of the first mobile enantiomers (μ_{eff1}, in $\times 10^{-5}$ cm^2 V^{-1} s^{-1} units), separation selectivity (α) and chiral resolutions (R_s) of Dns-amino acids with various MPrIMCD concentration in pH 6.0 buffers.

Analytes	CD	1 mM			2.5 mM			5 mM			10 mM			15 mM		
		μ_{eff1}	α	R_s	μ_{eff1}	α	R_s	μ_{eff1}	A	R_s	μ_{eff1}	α	R_s	μ_{eff1}	α	R_s
Dns-Aba	MPrIMCD	−5.87	1.02	0.9	−5.35	1.05	1.6	−5.06	1.10	5.2	−4.50	1.05	2.1	−4.03	1.04	1.9
	PrIMCD	−7.11	1.01	0.6	−6.32	1.03	1.7	−5.58	1.03	1.1	−4.47	1.03	1.2	−3.89	1.02	1.7
Dns-Aca	MPrIMCD	−7.62	1.02	1.0	−6.71	1.06	2.8	−6.02	1.07	7.3	−5.11	1.04	3.1	−4.68	1.03	2.5
	PrIMCD	−7.94	1.01	0.7	−7.34	1.02	1.2	−6.59	1.04	1.8	−5.36	1.03	1.7	−4.72	1.03	1.4
Dns-Met	MPrIMCD	−8.19	1.02	1.4	−7.79	1.04	2.0	−7.39	1.06	2.3	−6.04	1.03	1.7	−5.13	1.02	1.5
Dns-Nle	MPrIMCD	−7.69	1.01	1.2	−7.19	1.03	1.4	−5.93	1.04	1.8	−4.73	1.03	1.6	−3.42	1.02	1.3
	PrIMCD	−6.92	1.00	0.6	−6.62	1.01	0.7	−5.83	1.02	0.9	−4.80	1.01	0.7	−4.09	1.01	0.6
Dns-Nva	MPrIMCD	−5.87	1.01	1.3	−5.74	1.03	1.7	−5.56	1.04	2.2	−5.42	1.02	1.2	−4.57	1.01	1.1
	PrIMCD	−6.85	1	< 0.5	−6.49	1.01	0.8	−5.49	1.01	0.9	−4.49	1.01	0.7	−3.72	1.01	0.7
Dns-Phe	MPrIMCD	−7.79	1.02	0.7	−7.09	1.06	1.7	−5.96	1.14	3.7	−4.35	1.10	3.2	−3.61	1.06	2.5
	PrIMCD	−5.62	1	< 0.5	−4.80	1.02	1.2	−3.68	1.04	1.7	−2.48	1.06	1.9	−2.12	1.04	1.6
Dns-Ser	MPrIMCD	−6.53	1.02	1.1	−6.08	1.02	1.3	−5.78	1.03	2.1	−4.41	1.02	1.4	−431	1.01	1.2
	PrIMCD	−7.36	1	< 0.5	−7.11	1.02	0.8	−6.26	1.02	0.8	−5.28	1.02	0.9	−4.71	1.01	0.8
Dns-Thr	MPrIMCD	−8.79	1.02	1.1	−7.87	1.08	7.0	−7.45	1.15	7.1	−5.05	1.07	2.9	−4.36	1.05	2.3
	PrIMCD	−7.24	1.00	0.6	−6.43	1.03	1.3	−5.56	1.03	1.7	−4.48	1.03	1.4	−3.82	1.03	1.6

According to Wren's model [27,28], the amino acids with branched alkyl chain and aryl ring such as Dns-Thra and Dns-Phe could fit better into the cavity of CD, resulting in larger binding constants [21]. These amino acids thus have different optimal CD concentrations for enantioseparations.

A close look at the enantioseparation data of seven Dns-amino acids obtained with MPrIMCD and PrIMCD at the different CD concentrations, MPrIMCD demonstrated better chiral resolution capability than PrIMCD in the whole studied CD concentration range, affording higher selectivities and chiral resolutions.

It is noteworthy that MPrIMCD could afford better selectivities as high as 7.3 for Dns-Aca, while PrIMCD could only partially resolved the racemate. Another good example is Dns-Ser, i.e., an R_s of 2.1 was achieved at 5 mM MPrIMCD while a maximum R_s of only 0.9 with PrIMCD. The enhanced selectivities and chiral resolution may be attributed to the higher polarity of methoxypropylimmidazolium moiety in MPrIMCD than propylimmidazolium one in PrIMCD.

The increase of resolution of Dns-amino acids with increment of CD concentration is depicted in Fig. 3 by taking the separation electropherograms of Dns-Thr as an example. Dns-Thr achieved the maximum R_s at 5.0 mM MPrIMCD. This can be explained with Wren's theoretical mode [27,28], where the optimum selector concentration for enantioseparation of a pair of enantiomers is related to the stability constants of the diastereomeric complexes. The theoretical determination of binding constants between MPrIMCD and Dns-amino acids is discussed in the following section.

3.4. Determination of binding constants

As discussed above, the methoxy functionalized MPrIMCD exhibited improved enantioselectivities towards Dns-amino acids than PrIMCD, which may be attributed to the different strength for inclusion complexation between CD and amino acids. The binding constants between MPrIMCD and analyte enantiomer were thus determined according to the *x*-reciprocal method proposed in Ref. [27–29], the binding constants (K) were calculated from the experimentally measured mobilities of each enantiomer when increasing the CD concentration from 1 mM to 15 mM at the optimum BGE pH 6.0 (Table 3). Representative example of fitting curves according to *x*-reciprocal method is shown in Fig. 4.

Considering the seven model analytes, the correction coefficients, r, of the fitting curves were at least 0.996 and 0.998 for Dns-Aca with MPrIMCD, respectively, whereas most of the correction

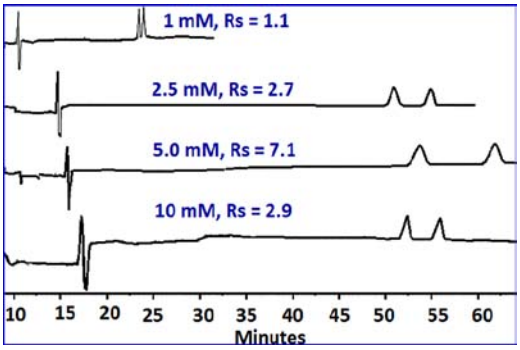


Fig. 3. Separation chromatogram of Dns-Thr with different MPrIMCD concentrations in pH 6.0 BGEs.

Table 3
Comparison of equilibrium constants (K_1, K_2) and the optimum concentration of MPrIMCD and PrIMCD[15].

Analytes	Chiral selector	K_1 (M^{-1})	K_2 (M^{-1})	C_{opt} (mM)
Dns-Aba	MPrIMCD	207	223	4.7
	PrIMCD	187	198	5.2
Dns-Aca	MPrIMCD	183	198	5.3
	PrIMCD	128	135	7.6
Dns-Nle	MPrIMCD	173	182	5.6
	PrIMCD	137	144	7.1
Dns-Nva	MPrIMCD	187	192	5.3
	PrIMCD	149	163	6.4
Dns-Phe	MPrIMCD	227	243	4.3
	PrIMCD	183	198	5.3
Dns-Ser	MPrIMCD	197	207	5.5
	PrIMCD	149	162	6.4
Dns-Thr	MPrIMCD	235	253	4.1
	PrIMCD	154	169	6.2

coefficients fell in the range from 0.992 to 0.998. The binding constants between these Dns-amino acids and MPrIMCD all fell in the range from 173 to 253. As clearly shown in Table 3, MPrIMCD exhibited higher binding constants with Dns-amino acids with shorter alkyl chains (i.e., Dns-Thr, Dns-Aba) or aryl ring (Dns-Phe). Hence, the R_s values of these Dns-amino acids should be higher than their analogues with long alkyl chains, which is consistent with the experiment results (Table 2). Dns-amino acids with increasing alkyl chain possessed lower K values with MPrIMCD, mainly due to the steric hindrance to prevent the inclusion

complexation with methoxypropylimidazolium β-CD from constructing. Moreover, the polarity of the side chain of the Dns-amino acids also affects the complex formation. Since repulsion will occur between the hydroxyl groups from the amino acids and CD, the stability of complex formed will decrease, which can be easily proved when comparing K values between Aba and Ser as well as Nva and Thr.

A close comparison of the binding constants between Dns-amino acids and MPrIMCD with those between Dans-amino acids and PrIMCD, one can conclude that MPrIMCD formed stronger inclusion complexation with Dns-amino acids than PrIMCD. This may be explained with the existence of higher polar methoxypropylimmidazolium moiety in MPrIMCD than propylimmidazolium one in PrIMCD. Similar behaviour was also observed for methoxylpropylammonium β-CD (MPrAMCD) and propylammonium β-CD (PrAMCD). The methoxy functionality enhanced chiral recognition was revealed with NMR techniques [16].

According to Wren's model [27,28], the optimum concentration of chiral selector (C_{opt}) for two enantiomeric analytes is dependent on the binding constants of inclusion complexes with the equation of $C_{opt}=1/(K_1K_2)^{1/2}$. In our case, the C_{opt} of MPrIMCD for seven analytes is close to 5 mM, which agree well with the CE results (Table 2). In comparison with PrIMCD, the C_{opt} is relatively low (Table 3). It thus could be concluded that MPrIMCD is more efficient chiral selector for Dns-amino acids than PrIMCD.

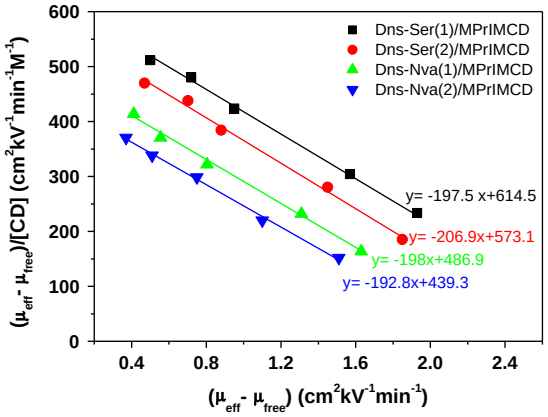


Fig. 4. x-Reciprocal plot for PrIMCD at 25 °C with Dns-Ser and Dns-Nva. μ_{eff}: the effective mobility; μ_{free}: the effective mobility of the free solute.

3.5. Comparison with PrIMCD and MPrAMCD

The above-mentioned calculation with Wren's model has revealed the higher enantioselectivity of MPrIMCD with the polar methoxy functionality. The superior resolution capability of MPrIMCD over PrIMCD is compared for seven racemates in Table 2. In order to further demonstrate the advantage of methoxylpropylimmidazolium functionality on CD, the chiral resolution ability of MPrIMCD is compared with that of PrIMCD [21] and MPrAMCD [16] for 8 Dans-amino acids (Fig. 5).

As shown in Fig. 5a, MPrIMCD could afford higher resolutions than PIMCD at the same concentration (5 mM). Especially, MPrIMCD could afford two–four fold R_s for Dns-amino acids than PrIMCD. The significant improvement could be attributed to the methoxy moiety at the terminal of propyl group, which may provide additional interactions or even form hydrogen bonding with analytes to enhance the chiral recognition. In comparison with MPrAMCD [16], MPrIMCD also affords higher enantioselectivities towards these analytes except Dns-Nle and Dns-Ser (Fig. 5b). This phenomenon could be attributed to the different cationic moiety. The imidazolium moiety may form π–π interactions with analytes in comparison with the ammonium based MPrAMCD.

4. Conclusions

A new single-isomer cationic CD, mono-6^A-[3-(3-methoxypropyl)imidazole-1-ium]-β-cyclodextrin chloride, has been synthesized and used for the chiral separation of 8 Dns-amino acids. Baseline separation of most analytes can be realized with CD concentration as low as 1 mM at the optimum pH 6.0. Excellent enantioseparations for amino acids in CD concentration range between 2.5 mM to 15 mM in pH 6.0 BGEs. Resolution as high as 7.3 was achieved for Dns-Aca with 10 mM CD. The comparison study of the enantioselectivity and the determination of binding constants of MPrIMCD with PrIMCD indicated the polar methoxypropylimmidazloium enhance the interactions between CD and analyte to afford better chiral resolutions toward amino acids. The development of this single-isomer cationic CD further demonstrates the role of structure design in constructing extra interactions between chiral selector and analytes for enhanced enantioselectivity, which may give insight for better understanding of chiral recognition mechanism with charged CDs.

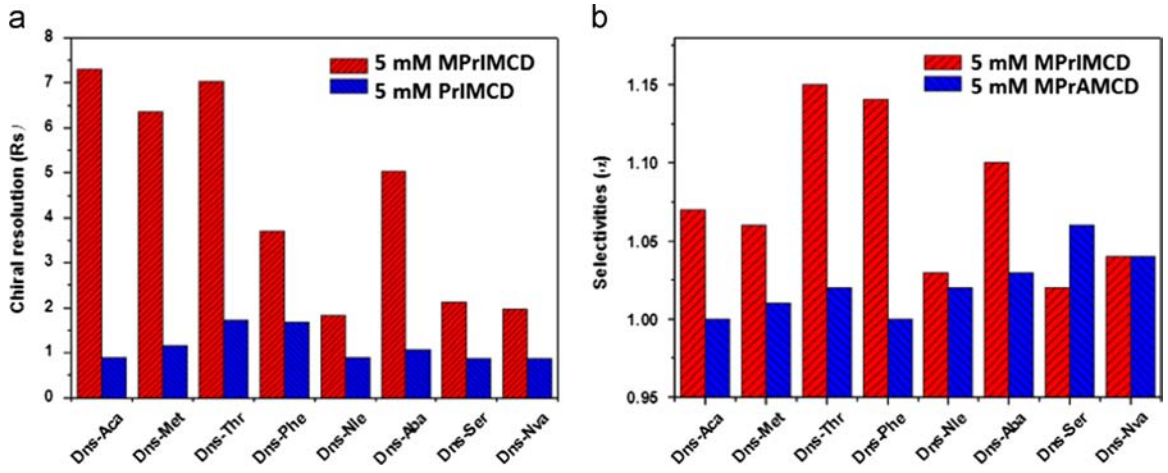


Fig. 5. Comparison of (a) chiral resolutions of 8 Dns-amino acids with MPrIMCD and PrIMCD, (b) selectivities of Dns-amino acids with MPrIMCD and MPrAMCD. Data adapted from Ref. [15] for PrIMCD and [10] for MPrAMCD.

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References

[1] V. Poinsot, M.A. Carpéné, J. Bouajila, P. Gavard, B. Feuere, F. Couderc, Electrophoresis 33 (2012) 14–35.
[2] M. Castro-Puyana, K. Lomsadze, A.L. Crego, M.L. Marina, B. Chankvetadze, Electrophoresis 28 (2007) 388–394.
[3] K. Klepárník, Electrophoresis 34 (2013) 70–85.
[4] M. Geiger, A.L. Hogerton, M.T. Bowser, Anal. Chem. 84 (2012) 577–596.
[5] G.K.E. Scriba, P. Jáč, in: G.K.E. Scriba (Ed.), Chiral Separations Methods in Molecular Biology, Springer, LLC, 2013, pp. 271–287.
[6] T. De Boer, R.A. De Zeeuw, K. Ensing, Electrophoresis 21 (2000) 3220–3239.
[7] J. Boonleang, J.F. Stobaugh, Electrophoresis 34 (2013) 1232–1240.
[8] E. Tutu, G. Vigh, Electrophoresis 32 (2011) 2655–2662.
[9] Y. Xiao, T.T.Y. Tan, S.C. Ng, Analyst 136 (2011) 1433–1439.
[10] W. Tang, S.C. Ng, Nat. Protoc. 2 (2007) 3195–3200.
[11] T.T. Ong, W. Tang, I.W. Muderawan, S.C. Ng, H.S.O. Chan, Electrophoresis 26 (2005) 3839–3848.
[12] V. Cucinotta, A. Contino, A. Giuffrida, G. Maccarrone, M. Messina, J. Chromatogr. A 1217 (2010) 953–967.
[13] R. Iványi, L. Jicsinszky, Z. Juvancz, N. Roos, K. Otta, J. Szejtli, Electrophoresis 25 (2004) 2675–2686.
[14] W. Tang, S.C. Ng, Nat. Protoc. 3 (2008) 691–695.
[15] A. Giuffrida, R. Caruso, M. Messina, G. Maccarrone, A. Continoa, A. Cifuentes, V. Cucinotta, J. Chromatogr. A 1269 (2012) 360–365.
[16] Y. Dai, S. Wang, J. Zhou, Y. Liu, D. Sun, J. Tang, W. Tang, J. Chromatogr. A 1246 (2012) 98–102.
[17] A. Rousseau, P. Chiap, R. Ivanyi, J. Crommen, M. Fillet, A.-C. Servais, J. Chromatogr. A 1204 (2008) 219–225.
[18] J. Zhou, Y. Dai, S. Wang, E. Zhu, J. Hai, Y. Liu, J. Tang, W. Tang, RSC Adv. 2 (2012) 5088–5093.
[19] G. Galaverna, R. Corradini, A. Dossena, R. Marchelli, Electrophoresis 20 (1999) 2619–2629.
[20] G. Galaverna, R. Corradini, A. Dossena, R. Marchelli, G. Vecchio, Electrophoresis 18 (1997) 905–911.
[21] W. Tang, T.T. Ong, I.W. Muderawan, S.C. Ng, Anal. Chim. Acta 585 (2007) 227–233.
[22] W. Tang, I.W. Muderawan, S.C. Ng, H.S.O. Chan, Anal. Chim. Acta 555 (2006) 63–67.
[23] M. Vaher, M. Koel, M. Kaljurand, Electrophoresis 23 (2002) 426–430.
[24] Z. Chen, K. Uchiyama, T. Hobo, Anal. Chim. Acta 523 (2004) 1–7.
[25] R.P. Bonomo, V. Cucinotta, F. D'Alessandro, G. Impellizzeri, G. Maccarrone, G. Vecchio, E. Rizzarelli, Inorg. Chem. 30 (1991) 2708–2713.
[26] W. Tang, I.W. Muderawan, T.T. Ong, S.C. Ng, Anal. Chim. Acta 546 (2005) 119–125.
[27] S.A.C. Wren, R.C. Rowe, J. Chromatogr. 609 (1992) 363–367.
[28] S.A.C. Wren, R.C. Rowe, J. Chromatogr. 603 (1992) 235–241.
[29] R. Ivanyi, L. Jicsinszky, Z. Juvancz, N. Roos, K. Otta, J. Szejtli, Electrophoresis 25 (2004) 2675–2686.