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Supporting information

Development of a capillary electrophoresis system with Mn(II) complexes and *β*-cyclodextrin as the dual chiral selectors for enantioseparation of dansyl amino acids and its application in screening enzyme inhibitors

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Characterization of AAILs

1. [EMIm][L-Ala]

^{1}H-NMR: ɗ= 8.43 (1H, s, CH), 7.41 (2H, d, CH_2), 7.35 (2H, d, CH_2), 4.70 (2H, s, CH_2), 3.47 (H, d, CH), 3.45 (H, d, CH), 1.43 (3H, t, CH_3), 1.28 (3H, d, CH_3) ppm.

^{13}C NMR: ɗ =178.4, 129.80, 123.38, 121.80, 50.98, 44.72, 35.51, 18.20, 14.41 ppm

2. [BMIm][L-Ala]

^{1}H-NMR : ɗ = 8.85 (1H, s, CH), 7.40 (1H, d, CH), 7.38 (1H, d, CH), 3.82 (3H, s, CH_3), 3.42 (3H, d, CH), 3.38 (3H, d, CH), 1.80 (2H, t, CH_2), 1.78 (2H, t, CH_2), 1.76 (2H, t, CH_2), 1.28 (2H, m, CH_2), 1.25 (2H, m, CH_2), 1.23 (2H, m, CH_2), 0.86 (3H, t, CH_3), 0.84 (3H, t, CH_3) ppm.

^{13}C NMR: ɗ =178.9, 130.23, 123.4, 122.15, 51.12, 49.22, 35.51, 31.21, 19.98, 18.71, 12.57 ppm

3. [HMIm][L-Ala]

^{1}H-NMR : ɗ = 8.41 (1H, s, CH), 7.41 (1H, d, CH), 7.36 (1H, d, CH), 3.83 (3H, s, CH_3), 3.35 (H, m, CH), 3.33 (H, m, CH), 1.80 (2H, m, CH_2), 1.24 (2H, m, CH_2), 1.22 (2H, m, CH_2), 1.21 (2H, t, CH_2), 0.81 (3H, t, CH_3) ppm.

^{13}C NMR: ɗ = 179.2, 131.23, 123.41, 122.15, 51.25, 49.52, 35.55, 30.29, 30.10, 24.94, 21.78, 19.47, 13.17 ppm

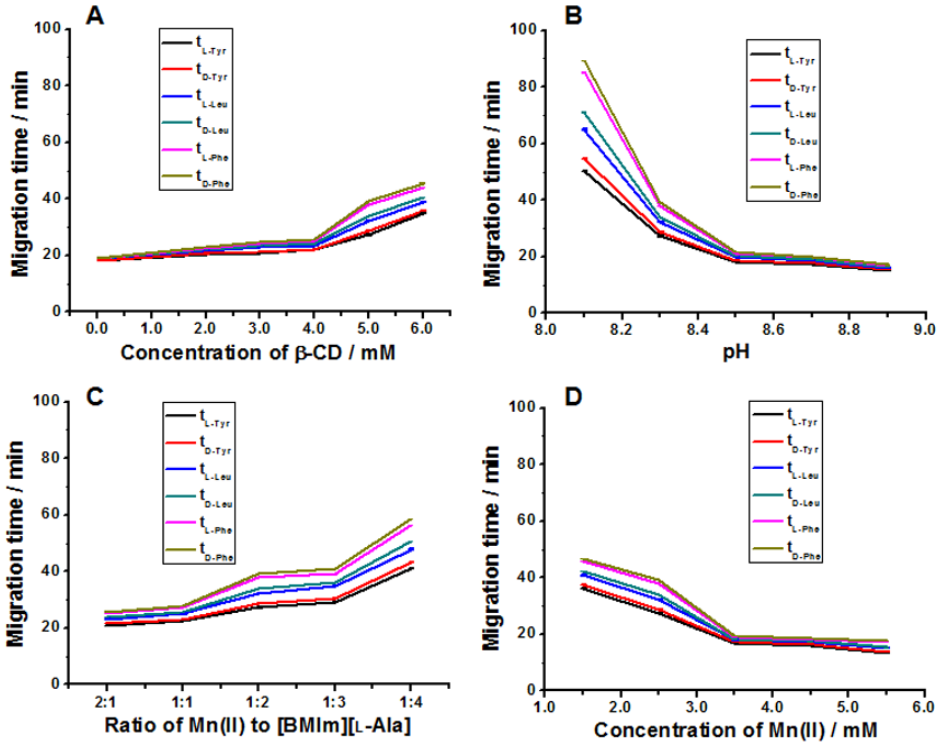

Fig. S1. **A.** Influence of *β*-CD concentrations on migration times. Buffer conditions: 100.0 mM boric acid, 5.0 mM NH_4AC, 2.5 mM Mn(II), 5.0 mM [BMIm][L-Ala], adjusted pH at 8.3, and different concentrations of *β*-CD ranging from 0-6.0 mM. **B.** Influence of pH on migration times. Buffer conditions: 100.0 mM boric acid, 5.0 mM NH_4AC, 2.5 mM Mn(II), 5.0 mM [BMIm][L-Ala] and 5.0 mM *β*-CD at different pH values. **C.** Influence of concentration ratio of Mn(II) to [BMIm][L-Ala] on migration times. Buffer conditions: 100.0 mM boric acid, 5.0 mM NH_4AC, 5.0 mM *β*-CD, the concentration ratio of Mn(II) to [BMIm][L-Ala] was from 2: 1 to 1: 4 with Mn(II) kept at 2.5 mM, adjusted pH to 8.3. **D.** Influence of complex concentrations on migration times. Buffer conditions: 100.0 mM boric acid, 5.0 mM NH_4AC, 5.0 mM *β*-CD and different concentrations of Mn(II) complex with [BMIm][L-Ala] in the range of 1.5-5.5 mM, the ratio of Mn(II) to [BMIm][L-Ala] was kept at 1:2, adjusted pH at 8.3. Capillary: 60 cm, 45 cm effective × 75 μm id; injection: siphoned for 8 s at 15 cm; voltage: -23 KV; UV detection: 254 nm; temperature: 25 ^oC.

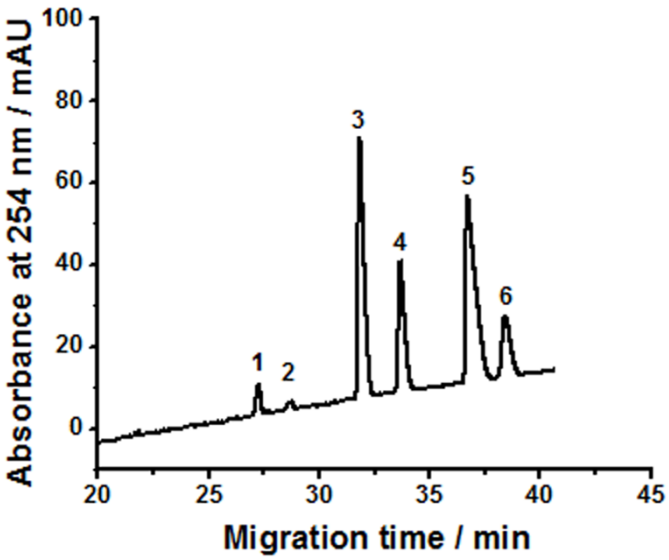

Fig. S2. Enantioseparation of Dns-D,L-Tyr, Dns--D,L-Leu, Dns-D,L-Phe. For the Dns-D,L-AAs, the concentration of L-AA is twice as large as D-AA. Buffer conditions: 100.0 mM boric acid, 5.0 mM ammonium acetate, 2.5 mM Mn(II), 5.0 mM [BMIm][L-Ala] and 5.0mM β-CD at pH 8.3. Other conditions are the same as **Fig. S1**.

Peaks: **1**. Dns-L-Tyr, **2**. Dns-D-Tyr, **3**. Dns-L-Leu, **4**. Dns-D-Leu, **5**. Dns-L-Phe, **6**. Dns-D-Phe.

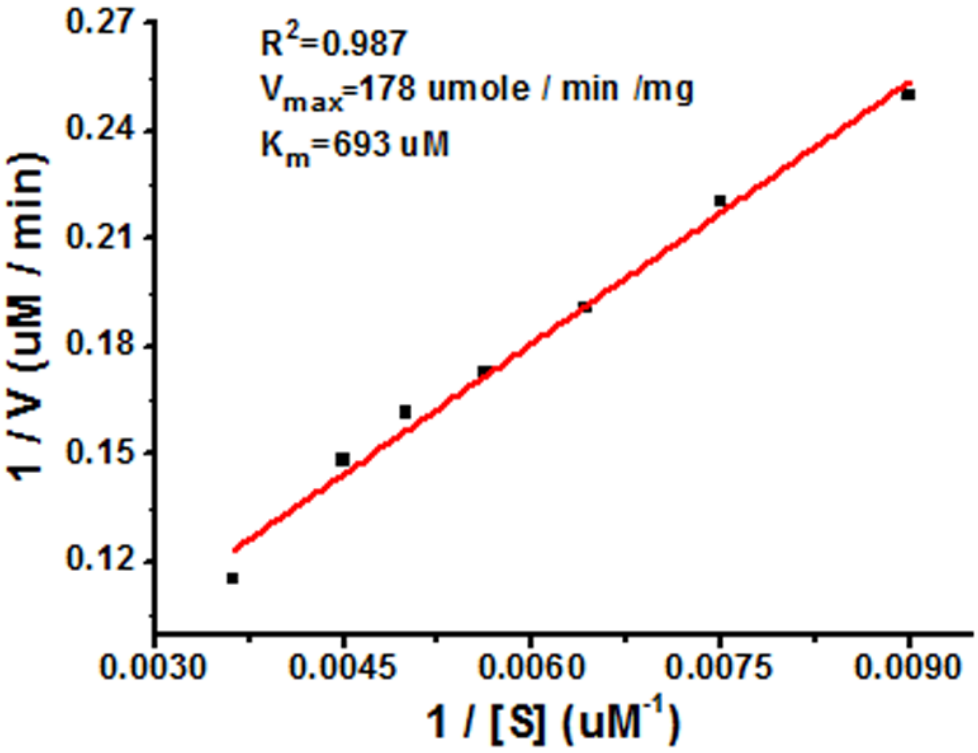

Fig. S3. Line weaver–Burk plot for the oxidation of L-Tyr catalyzed by tyrosinase (565 U/mg). The Michaelis–Menten equation was described as: $V = V_{max}$ [S] / (K_m+[S]), where [S] is the concentration of the substrate.

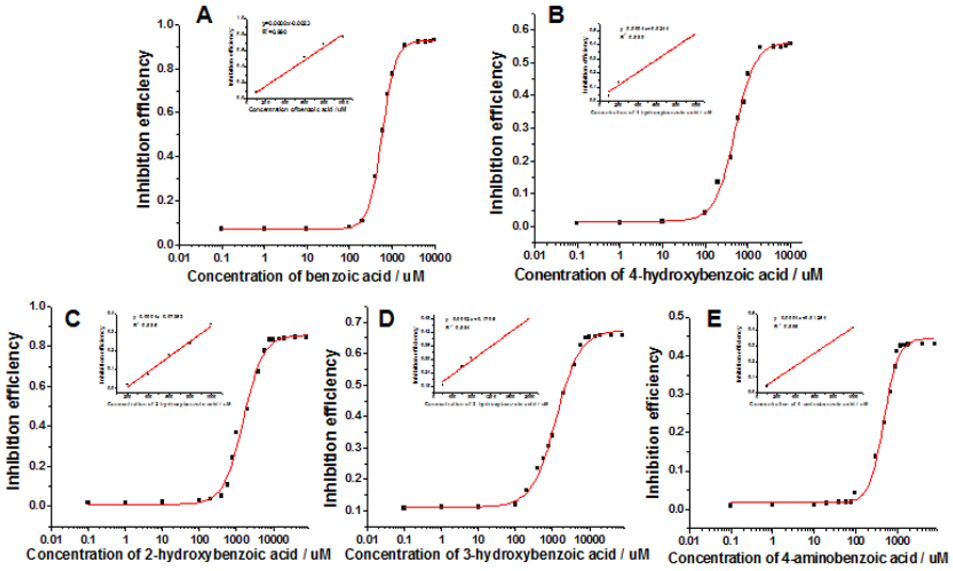

Fig. S4. Tyrosinase inhibition efficiency of different inhibitors: **A**. Benzoic acid; **B**. 4-hydroxybenzoic acid; **C**. 2-hydroxybenzoic acid; **D**. 3-hydroxybenzoic acid; **E**. 4-aminobenzoic acid. The insert graphs represent the linear plot. Incubation condition: 333 μM L-Tyr as the substrate and different concentration to incubate with tyrosinase.

Table S1. Enantioseparation of Dns-D,L-AAs with different running buffer solutions[a]

Dns-D,L-AAs	Mn-[BMIm][L-Ala]				β-CD			
	Rs[b]	t_L/min	t_D/min	α[c]	Rs[b]	t_L/min	t_D/min	α[c]
Dns-D,L- Thr	0	20.62	20.62	1.000	1.45	8.13	8.24	1.014
Dns-D,L-Val	0	20.58	20.58	1.000	1.17	7.53	7.62	1.012
Dns-D,L-Tyr	0	21.74	21.74	1.000	1.44	8.22	8.33	1.013
Dns-D,L-Leu	0	26.27	26.27	1.000	1.25	7.95	8.04	1.011
Dns-D,L-Ile	0	21.85	22.20	1.000	0.72	7.36	7.42	1.008
Dns-D,L-Pro	0	21.75	21.75	1.000	1.11	7.82	7.89	1.009
Dns-D,L-Met	0	22.58	22.58	1.000	0.63	8.33	8.40	1.008
Dns-D,L-Ser	0	20.31	20.31	1.000	0.39	9.92	9.99	1.007
Dns-D,L-His	0	29.81	29.81	1.000	0.66	7.34	7.38	1.005
Dns-D,L-Phe	0	28.02	28.02	1.000	0	8.35	8.35	1.000
Dns-D,L-Ala	0	18.05	18.05	1.000	0.62	8.24	8.28	1.005
Dns-D,L-Asn	0	20.80	20.80	1.000	0.45	7.94	7.99	1.006
Dns-D,L-Trp	0	24.88	24.88	1.000	0.63	7.56	7.60	1.005
Dns-D,L-Gln	0	24.34	24.34	1.000	0	8.36	8.36	1.000
Dns-D,L-Orn	0	33.94	33.94	1.000	0	9.40	9.40	1.000
Dns-D,L-Glu	0	19.49	19.49	1.000	0	9.17	9.17	1.000
Dns-D,L-Arg	0	16.05	16.05	1.000	0	9.05	9.05	1.000
Dns-D,L-Asp	0.43	14.61	14.73	1.008	0	9.18	9.18	1.000
Dns-D,L-Cys	0	15.52	15.52	1.000	0	8.83	8.83	1.000
Dns-D,L-Lys	0	33.32	33.32	1.000	1.30	5.54	5.70	1.029

[a] Running buffer: 100.0 mM boric acid, 5.0 mM NH_4 AC without *β*-CD or without Mn(II)-[BMIm][L-Ala] complex at pH 8.3. Other conditions are the same as in **Fig. S1**.

[b] $Rs = 2(t_D-t_L)/(W_D + W_L)$; t: migration time

[c] $\alpha = t_L / t_D$; t: migration time

Table S2. Enantioseparation of Dns-D,L-AAs with different kinds of CDs[a]

CDs	Dns-D,L-Tyr				Dns-D,L-Leu				Dns-D,L-Phe			
	***Rs*[b]**	**t_L/ min**	**t_D/ min**	**α[c]**	***Rs*[b]**	**t_L/ min**	**t_D/ min**	**α[c]**	***Rs*[b]**	**t_L/ min**	**t_D/ min**	**α[c]**
α-CD	0	18.99	18.99	1.000	0	22.32	22.32	1.000	0	26.10	26.10	1.000
β-CD	4.06	27.18	28.71	1.056	3.50	32.19	34.13	1.060	1.78	37.77	39.28	1.040
γ-CD	4.22	50.31	54.56	1.084	3.75	64.95	71.08	1.094	1.86	85.18	89.40	1.050

[a] Running buffer: 100.0 mM boric acid, 5.0 mM NH_4 AC, 2.5 mM Mn(II), 5.0 mM [BMIm][L-Ala], adjusted pH at 8.3, and different kinds of CDs. Other conditions are the same as in **Fig. S1**.

[b] *R*s = $2(t_D-t_L)/(W_D + W_L)$; t: migration time

[c] $\alpha = t_L / t_D$; t: migration time..

Table S3. Optimization of separation condition on α[a]

Conditions		α		
		Dns-D,L-Tyr	Dns-D,L-Leu	Dns-D,L-Phe
pH	8.1	1.084	1.090	1.050
	8.3	1.056	1.060	1.040
	8.5	1.028	1.027	1.017
	8.7	1.023	1.023	1.017
	8.9	1.017	1.018	1.015
ratio of Mn(II) to [BMIm][L-Ala]	2:1	1.033	1.031	1.017
	1:1	1.034	1.031	1.017
	1:2	1.056	1.060	1.040
	1:3	1.058	1.061	1.048
	1:4	1.060	1.063	1.050
concentrations of Mn(II)-[BMIm][L-Ala] complexes / mM	1.5	1.059	1.062	1.041
	2.5	1.056	1.060	1.040
	3.5	1.025	1.025	1.021
	4.5	1.024	1.024	1.016
	5.5	1.018	1.019	1.0150
concentration of β-CD / mM	0	0	0	0
	1.0	1.009	1.001	1.015
	2.0	1.009	1.011	1.017
	3.0	1.012	1.013	1.018
	4.0	1.014	1.024	1.022
	5.0	1.056	1.060	1.040
	6.0	1.067	1.065	1.042
Different AAILs	[EMIM][L-Ala]	1.033	1.031	1.018
	[BMIM][L-Ala]	1.056	1.060	1.040
	[HMIM][L-Ala]	1.084	1.094	1.050
	L-Ala	1.018	1.030	1.017
	L-Ala+[BMIm][Br]	1.038	1.041	1.018

[a] $\alpha = t_L / t_D$; t: migration time..