Supplementary Material

A teicoplanin-cyclodextrin bilayer chiral stationary phase boosts chiral separation of native amino acids

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Chemicals and materials

Silica gel (3 μm) was obtained from Fuji silica (Fuji, Japan). The surface area of silica gel (SPS100-3) is 310 m^2/g. HPLC grade methanol (MeOH) and CH_3CN were supplied from Tianjin Concord Technology Company (Tianjin, China). All the other Chemicals were purchased from Heowns (Tianjin, China).

Fourier-transform infrared (FTIR) spectra analysis was carried out on an AVATR360 supplied by Thermo Nicolet (USA). ^1H NMR and Solid state ^{13}C NMR was performed on a Varian Infinityplus 600 NMR spectrometer (600 MHz, 7.0 T; USA). Thermal gravimetric analysis (TGA) was tested using a TG 209 F3 Tarsus (NETZSCH, Germany), which was conducted under N_2 circumstances, from 30 °C to 800 °C, at a rate of 10 ℃ per-minute. Elemental analysis was performed on a Vario EI CHNS elemental analyzer (Elementar Analysensysteme, Hanau, Germany). The enantioseparations were obtained on a shimadzu liquid chromatography analyzer.

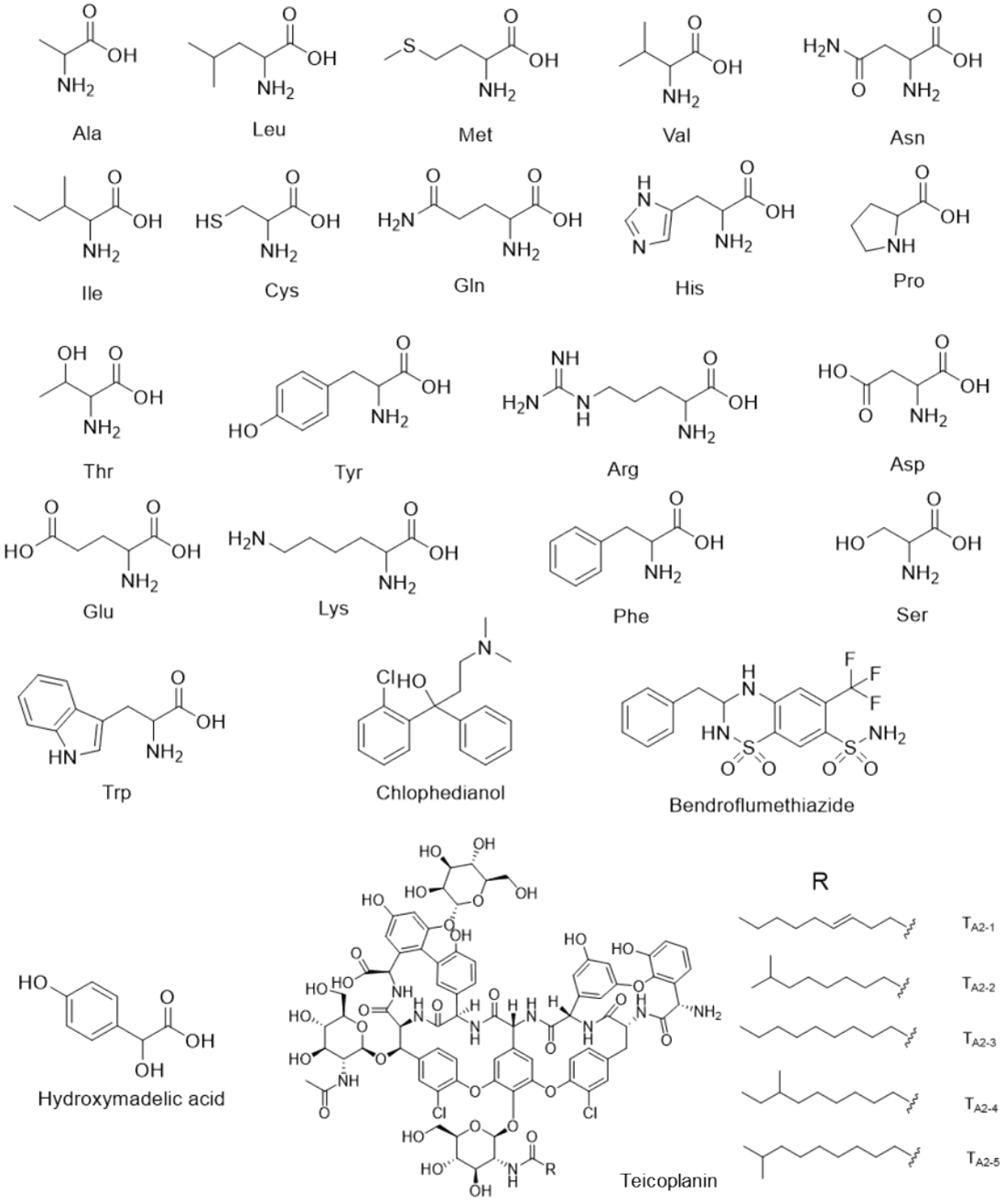
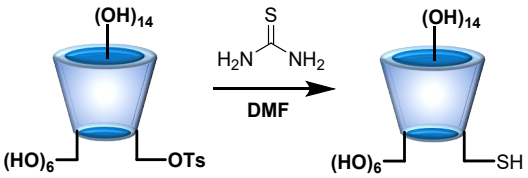


Fig. S1. Structures of the analytes and teicoplanin.

Note: Samples were prepared by weighing and mixing the chiral monomers. The ratio of D/L configuration in the chromatogram is not 1:1 due to the different purity and weighing errors of the two chiral monomers.

Synthesis of CD-SH.



CD-SH was synthesized according to the literature.[1]

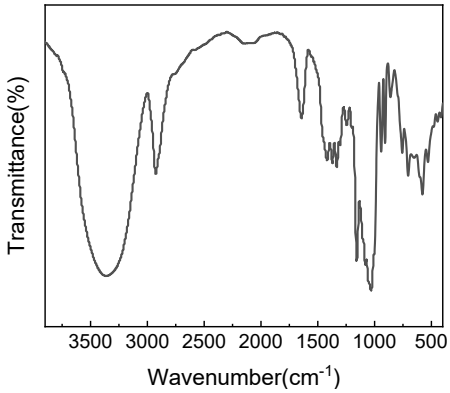
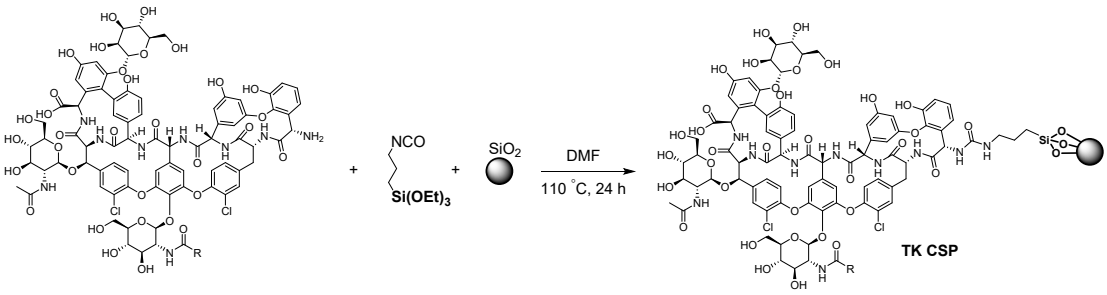


Fig. S2 IR of CD-SH

Synthesis of TK CSP.



TK (3.00 g, 1.58 mmol) was dissolved in dry DMF (50 mL) and dry pyridine (10 mL), then silane coupling agent (1.172 mL, about 3 equiv.) was added, followed by activated silica gel (6.00 g) was added. The reaction was refluxed at 110 °C for 24 h. The crude product was obtained by filtration and washed with DMF (10 mL) for three times. The final product was purified by Soxhlet extraction with methanol to give the product TK CSP. The IR data of the product is shown in Fig.1 and the solid ^{13}C NMR data is demonstrated in Fig. S3.

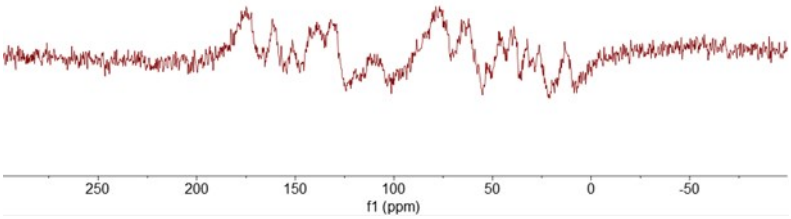
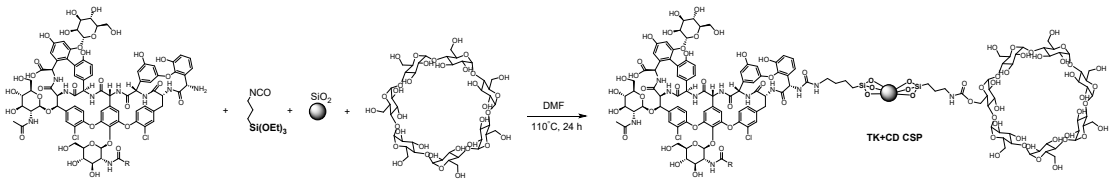


Fig. S3 Solid-state ^{13}C NMR of TK CSP

Synthesis of TK+CD CSP.



TK (1.50 g, 0.800 mmol) and β-CD (908 mg, 0.800 mmol) were dissolved in dry DMF (50 mL) and dry pyridine (10 mL), to which 0.6 mL of silane coupling agent (0.6 mL, about 3 equiv.) was added, followed by activated silica gel (3.00 g, dried at 120 °C for 8 h under vacuum). The reaction was refluxed at 110 °C for 24 h. The crude product of the synthesis was obtained by filtration and washed with DMF (10 mL) for three times. The final product was purified by Soxhlet extraction with methanol to give the end product TK+CD CSP. The IR data of the product are in Fig. 1 and the solid ^{13}C NMR data are in Fig. S4.

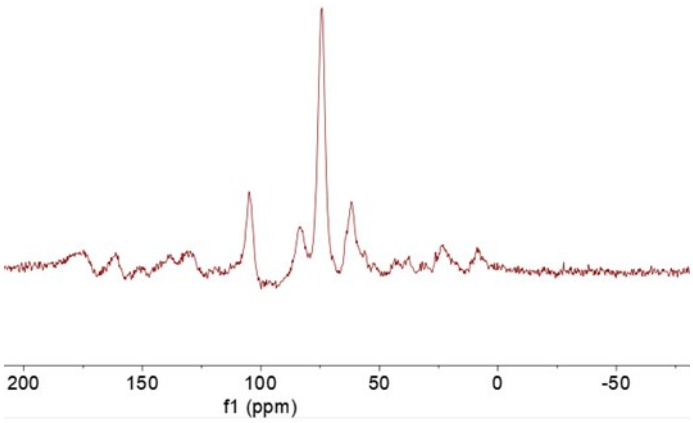
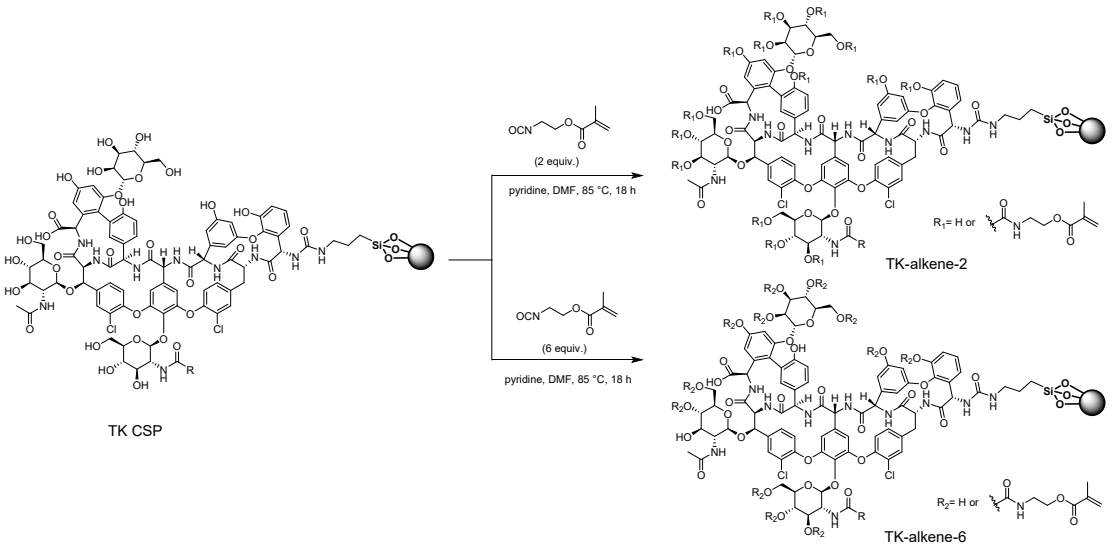


Fig. S4 Solid-state ^{13}C NMR of TK+CD CSP

Synthesis of TK-CD-2 CSP and TK-CD-6 CSP.



TK CSP (1.60 g, containing approximately 0.4 mmol TK), isocyanoethyl methacrylate (0.113 mL, about 2 equiv.), dry DMF (20 mL) and dry pyridine (20 mL) were added into a reaction bottle by syringe. The mixture was stirred at 85 °C under N_2 for 18 h. The pyridine was removed by distillation under reduced pressure and the solid was washed with ethyl acetate (10 mL × 3) and acetone (10 mL × 3), and finally TK-alkene-2 was dried in a vacuum oven to obtain the final TK-alkene-2. TK-alkene-6 was synthesized in the same way, and only the chemical equivalents of isocyanoethyl methacrylate added were different (6 equiv.). The IR data of the products are shown in Fig. S5.

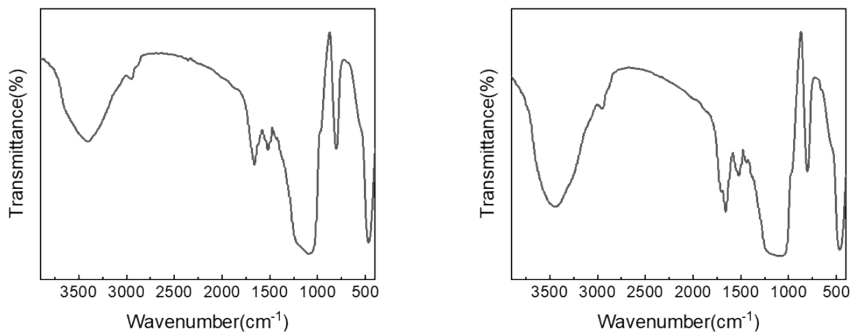
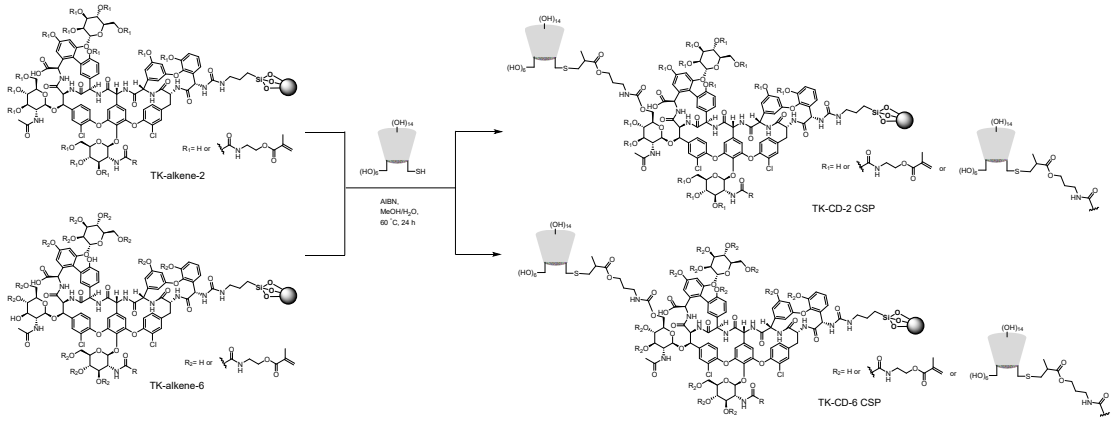


Fig. S5 The FTIR spectrum of TK-alkene-2 (left) and TK-alkene-6 (right).



CD-SH (1.50 g) was added to 30 mL of a mixture of water and methanol (1:1, v/v). The solution was stirred until clear, then TK-alkene-2 (3.00 g) and AIBN (70 mg) were added to the mixture and dispersed homogeneously by ultrasonication. The reaction system was stirred at 60 ℃ for 24 h. The crude product was filtered and washed with water (10 mL×3) and acetone (10 mL×3) sequentially. The final product was extracted by Soxhlet extraction using methanol as the extraction solution.TK-CD-2 CSP was obtained by vacuum drying. TK-CD-6 CSP was synthesized in the same way, except that TK-alkene-2 was replaced with an equivalent amount of TK-alkene-6. Infrared data for the products are in Fig.1 and solid-state NMR carbon spectral data are in Fig. S6 and Fig. S7.

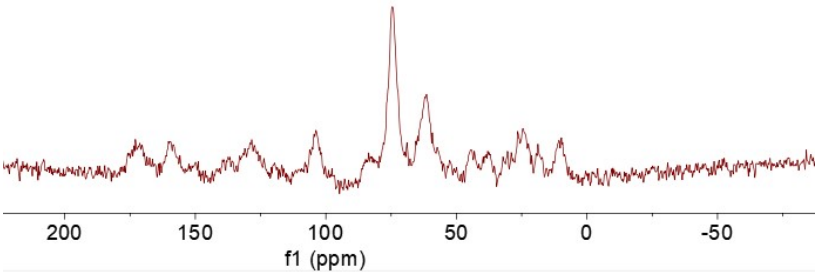


Fig. S6 Solid-state ^{13}C NMR of TK-CD-2 CSP

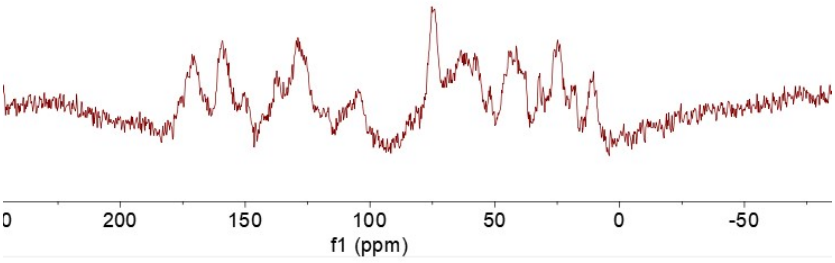


Fig. S7 Solid-state ^{13}C NMR of TK-CD-6 CSP

Note: the water content has a strong influence on the reaction during synthesis. All reagents (TK, DMF, etc.) should be treated anhydrous and the reaction needs to be carried out under anhydrous and N_2 conditions.

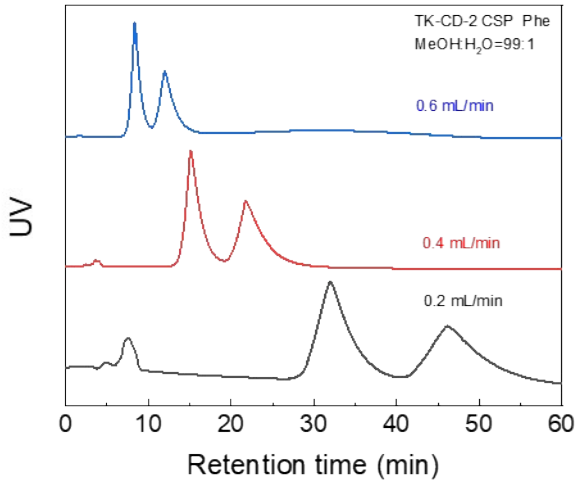


Fig. S8 Chromatograms on TK-CD-2 column of phenylalanine (Phe) at different flow rates.

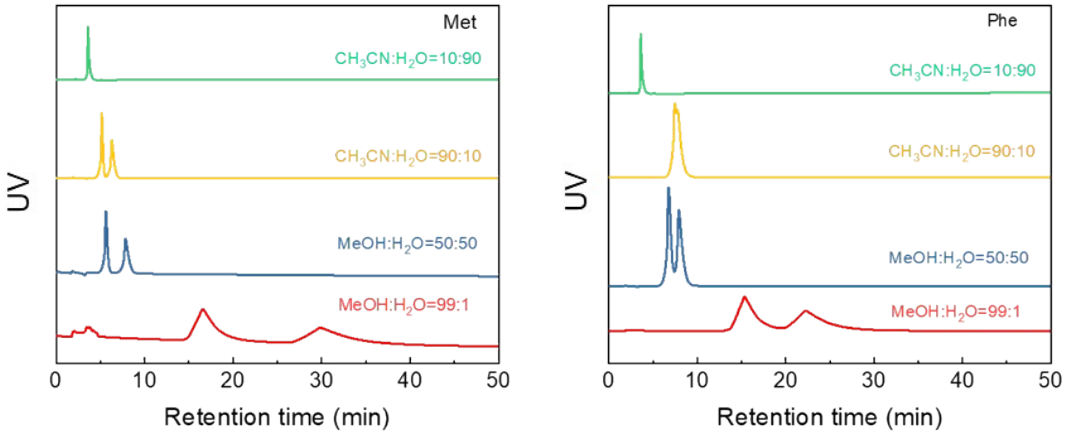


Fig. S9 Chromatograms on TK-CD-2 column of methionine (Met) and phenylalanine (Phe) in different mobile phases, 0.4 mL/min.

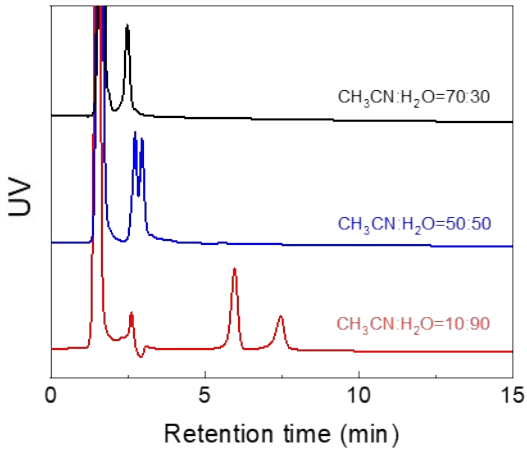


Fig. S10 Chromatograms on TK-CD-2 column of hydroxymadelic acid in different mobile phases, 0.4 mL/min.

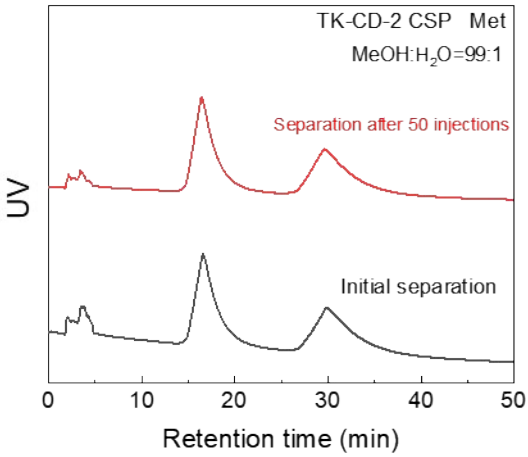


Fig. S11 Repeatability of TK-CD-2 column using Met as analyte.

Table S1 The elemental analyses of CSPs

CSPs	Average mass fraction (%)			
	N	C	H	S
TK CSP	2.4	14.29	2.43	0.00
TK+CD CSP	1.93	14.29	2.45	0.00
TK-CD-2 CSP	2.65	16.07	2.43	0.07
TK-CD-6 CSP	2.1	20.14	3.17	0.39

Table S2 Optimal separation results of analytes on TK-CD-6 column

Category	Analytes	k_1	k_2	*R*s	α	MP (*v*/*v*)
amino acids	Ala	4.823	9.802	2.99	2.033	MeOH/H_2O 99/1
	Arg	0	0	0	1	
	Asn	8.804	16.555	1.357	1.88	
	Asp	5.645	7.765	1.046	1.376	
	Cys	0	0	0	1	
	Gln	8.576	16.029	2.068	1.869	
	Glu	4.398	8.252	2.362	1.876	
	His	0	0	0	1	
	Ile	1.934	5.145	2.438	2.661	
	Leu	2.253	4.907	2.585	2.179	
	Lys	0	0	0	1	
	Met	4.163	9.66	2.364	2.321	
	Phe	4.255	8.434	1.916	1.982	
	Pro	0	0	0	1	
	Ser	4.609	6.67	1.02	1.447	
	Thr	2.967	4.389	0.777	1.479	
	Trp	5.441	10.879	1.957	2.011	
	Tyr	4.263	9.094	2.439	2.133	
	Val	2.281	4.821	2.253	2.114	
Neutral and acidic analytes	Bendroflumethiazide	8.37	10.714	2.269	1.28	ACN/H_2O 10/90
	Chlophedianol	0.838	1.653	3.219	1.972	
	Hydroxymadelic acid	2.982	3.982	3.269	1.335	

Table S3 Optimal separation results of analytes on TK column

Category	Analytes	k_1	k_2	*R*s	α	MP (*v*/*v*)
	Ala	3.723	7.208	2.201	1.936	MeOH/H_2O
	Arg	0	0	0	1	99/1
	Asn	7.371	13.2	2.011	1.791	
	Asp	3.556	5.035	1.169	1.416	
	Cys	3.84	7.335	2.673	1.91	
	Gln	6.085	11.026	2.223	1.812	
	Glu	0	0	0	1	
amino	His	2.829	2.829	0	1	
acids	Ile	1.619	4.048	3.049	2.489	
	Leu	4.081	8.231	2.553	2.017	
	Lys	0	0	0	1	
	Met	2.68	5.768	1.932	2.152	
	Phe	2.803	4.666	1.836	1.666	
	Pro	10.061	10.061	0	1	
	Ser	3.139	4.323	0.888	1.377	
	Thr	3.084	3.635	0.224	1.179	
	Trp	2.956	4.228	0.849	1.43	
	Tyr	2.766	4.813	1.953	1.74	
	Val	2.009	4.038	2.163	2.01	
Neutral	Bendroflumethiazide	4.835	4.835	0	1	ACN/H_2O
and	Chlophedianol	0	0	0	1	10/90
acidic	Hydroxymadelic acid	0.498	0.498	0	1	
analytes						

Table S4 Optimal separation results of analytes on TK+CD column

Category	Analytes	k_1	k_2	*R*s	α	MP (*v*/*v*)
amino acids	Ala	4.855	8.703	1.838	1.793	MeOH/H_2O 99/1
	Arg	0.287	0.287	0	1	
	Asn	9.682	17.197	2.419	1.776	
	Asp	4.427	4.427	0	1	
	Cys	0.1	0.1	0	1	
	Gln	7.926	14.331	2.793	1.808	
	Glu	3.041	4.833	1.147	1.589	
	His	0	0	0	1	
	Ile	2.001	4.593	1.61	2.296	
	Leu	2.204	4.521	2.1	2.051	
	Lys	0	0	0	1	
	Met	3.773	7.795	2.991	2.066	
	Phe	3.7	5.816	2.138	1.572	
	Pro	0	0	0	1	
	Ser	4.56	6.257	0.822	1.372	
	Thr	2.791	4.012	1.413	1.437	
	Trp	4.604	7.129	2.07	1.549	
	Tyr	3.76	6.139	2.558	1.633	
	Val	2.29	4.228	2.042	1.846	
Neutral and acidic analytes	Bendroflumethiazide	5.115	5.115	0	1	ACN/H_2O 10/90
	Chlophedianol	0.577	0.577	0	1	
	Hydroxymadelic acid	1.672	1.672	0	1	

Table S5 Optimal separation results of analytes on TK-CD-2 column

Category	Analytes	k_1	k_2	*R*s	α	MP (*v*/*v*)
	Ala	5.426	9.57	2.256	1.764	MeOH/H_2O
	Arg	0	0	0	1	99/1
	Asn	10.747	18.504	2.583	1.722	
	Asp	0	0	0	1	
	Cys	0	0	0	1	
	Gln	8.846	15.619	2.175	1.766	
	Glu	3.44	4.376	0.461	1.272	
amino	His	0	0	0	1	
acids	Ile	2.103	5.061	2.928	2.407	
	Leu	2.437	4.965	1.98	2.037	
	Lys	0	0	0	1	
	Met	4.205	8.48	2.381	2.017	
	Phe	4.148	6.573	1.83	1.585	
	Pro	0	0	0	1	
	Ser	4.96	6.615	1.009	1.334	
	Thr	3.147	4.433	1.082	1.409	
	Trp	5.105	7.788	1.701	1.526	
	Tyr	4.13	6.685	2.186	1.619	
	Val	2.574	4.831	1.998	1.877	
Neutral	Bendroflumethiazide	5.371	5.371	0	1	ACN/H_2O
and	Chlophedianol	1.624	1.624	0	1	10/90
acidic	Hydroxymadelic acid	1.565	3.54	6.232	2.263	
analytes						

References

1. J. Martinelli, K. Thangavel, L. Tei and M. Botta, *Chemistry – A European Journal*, 2014, **20**, 10944-10952.