Electronic Supplementary Information for

Chiral pillar[5]arene-functionalized silica microspheres: synthesis, characterization and enantiomer separation

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Contents

1. Chemicals and reagents

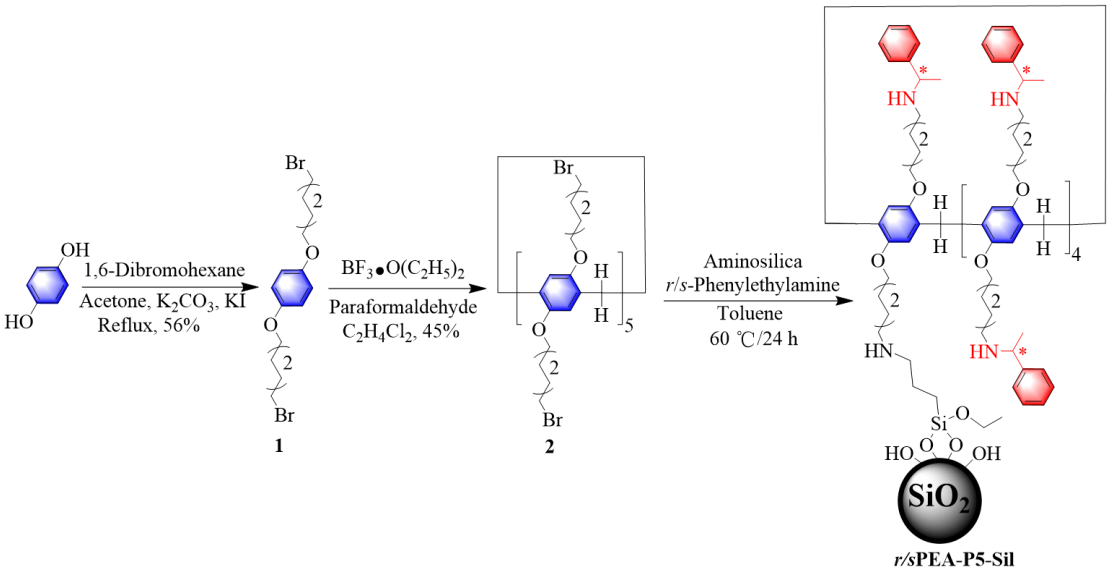
Enantiomers 1-phenyl-1-propanol, 1-(4-methylphenyl)-ethanol, 1-phenyl ethanol, 2-phenyl-1-propanol, benzoin, benzoin methyl ether, benzoin ethyl ether, triadimenol, tebuconazole, triadimefon and diniconazole were purchased from Sigma-Aldrich (Shanghai, China). Ninhydrin was obtained from Chaorui Biology Science and Technology Co., Ltd. (Shanghai, China). Chlorogenic acid were bought from Yuanye Biology Science and Technology Co., Ltd. (Shanghai, China). Flavanone was gotten from TCI Shanghai (China). Spherical porous silica (diameter: 5 μm, pore size: 10 nm, surface area: 306 m^2/g) was supplied by Fuji Silysia Chemical Ltd. (Aichi, Japan). Hydroquinone, acetone, $ClCH_2CH_2Cl$ and toluene were bought from Damao Chemical Reagent (Tianjin, China). 1,6-dibromohexane, were purchased from Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). K_2CO_3 and paraformaldehyde were provided by Shanghai Chemical Reagent Company of China Pharmaceutical Group (Shanghai, China). KI was gotten from Kelong Chemical Reagent Factory (Chengdu, China). $BF_3.OEt_2$ was purchased from Saen Chemistry Technology Co., Ltd. (Shanghai, China). Chiral phenethylamine (*r*PEA and *s*PEA) was obtained from Dibai Biology Science and Technology Co., Ltd. (Shanghai, China). Hexane, isopropanol (IPA), methanol (MeOH) and acetonitrile (ACN) all were HPLC-grade solvents bought from Sigma-Aldrich (Shanghai, China). Ultrapure water was produced on a PureLab Classic (DI, UK) water purification system. Chromatographic column stainless-steel empty tube (150 × 4.6 mm, I.D.) was purchased from Hanbon Technologies (China).

2. Instruments

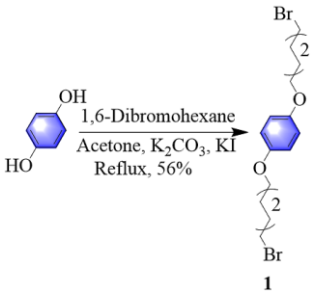
Alltech Slurry Packing Apparatus with air driver fluid pump imported from USA was 95,551 U type. HPLC system included a Shimadzu LC-10AT HPLC pump (Japan), equipped with 20 μL sample loop, and a Waters 2487 double λ absorbance detector (Waters, USA). Flow rate is 1.0 mL/min, detection wavelength is 254 nm and column temperature at 25 ℃. Chromatographic data were acquired and processed by Millennium 32 chromatography manager software (Waters, USA). Bruker 400MHz superconducting nuclear magnetic resonance spectrometer was AVANCE III HD 400MHz type, with Magnetic field intensity was 9.4 Tesla and frequency range was 6 ~ 430 MHz (Switzerland). FT-IR spectra were collected using a Nexus 870 infrared spectrometer (Nicolet, USA). Elemental analysis results were determined by Vario EL III elemental analyzer (Hanau, Germany). N_2 adsorption and desorption surface areas were measured at 77 K by the BET technique on a Micromeritics ASPS 2010 analyzer (USA). Thermogravimetric analysis (TGA) was performed on a 449F3 simultaneous thermal analyzer (Netzsch, Germany).

*3. Syntheses of **rPEA-P5-Sil** and **sPEA-P5-Sil***

Scheme S1.** Synthesis of **rPEA-P5-Sil** and **sPEA-P5-Sil



*3.1. Syntheses of compounds **1***



1,4-bis (6-bromohexoxy) benzene monomer, **1**, was prepared by 1,6-dibromohexane (18.3 mL, 120 mmol) reacted with hydroquinone (6.6 g, 60 mmol) in 400 mL acetone solution under the catalysis of K_2CO_3 (16.6 g, 120 mmol) and KI (39.8 g, 120 mmol). The product was collected by filtration with sand core funnel, thoroughly washed with dichloromethane. After purification by column chromatography on silica gel (petroleum ether: ethyl acetate, 100: 1 v/v), dried under high vacuum, the white powder product (6.5 g, 56%), was obtained. The ^{1}H NMR spectrum of **1** was shown in Figure S1. ^{1}H NMR (400 MHz, $CDCl_3$, room temperature) δ (ppm): 6.82 (s, 4H), 3.91 (t, J = 6.0 Hz, 4H), 3.24 (t, J = 8.0 Hz, 4H), 1.86 (m, J =8.0 Hz, 8H), 1.51 (m, J = 4.0 Hz, 8H).

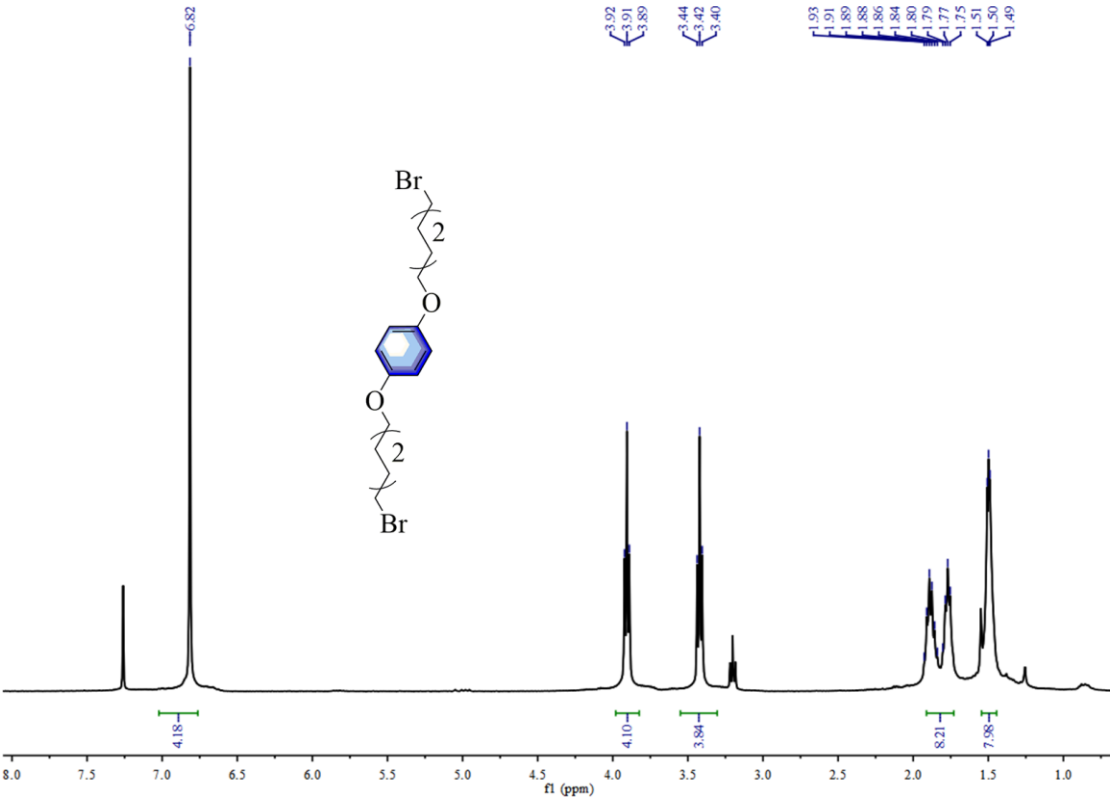
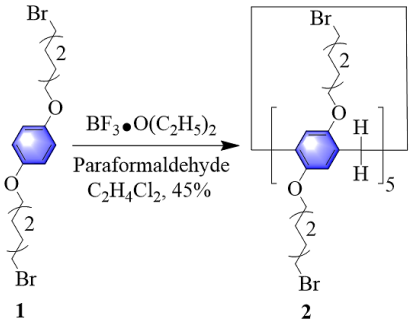


Fig. S1. ^{1}H NMR (400 MHz, $CDCl_3$, room temperature) of monomer **1.**

*3.2. Syntheses of compounds **2** [S1-S5]*



Using $BF_3.OEt_2$ (14 mL, 12 mmol) as catalyst, **1** (2.3 g, 12 mmol) and paraformaldehyde (0.4 g, 12 mmol) reacted in $ClCH_2CH_2Cl$ (200 mL) at room temperature for 2 h to prepare decabromohexyl substituted pillar[5]arene (P5) **2**. After the solvent was removed, a green solution was got. The obtained solid was purified by column chromatography on silica gel with petroleum ether: ethyl acetate (50: 1 v/v) as the eluent to get a white powder (2.4 g, 45%). The ^{1}H NMR spectrum of **2** was shown in Figure S2. ^{1}H NMR (400 MHz, $CDCl_3$, room temperature) δ (ppm): 6.85 (s, 4H), 3.87 (s, 20H), 3.74 (s, 10H), 3.26 (s, 20H), 1.80 (d, 40H), 1.33 (m, 40H). The ^{13}C NMR spectrum of **2** was shown in Figure S3. ^{13}C NMR (125 MHz, $CDCl_3$, room temperature) δ (ppm): 149.59, 128.19, 114.82, 67.89, 33.76, 32.63, 29.82, 28.16 and 25.49.

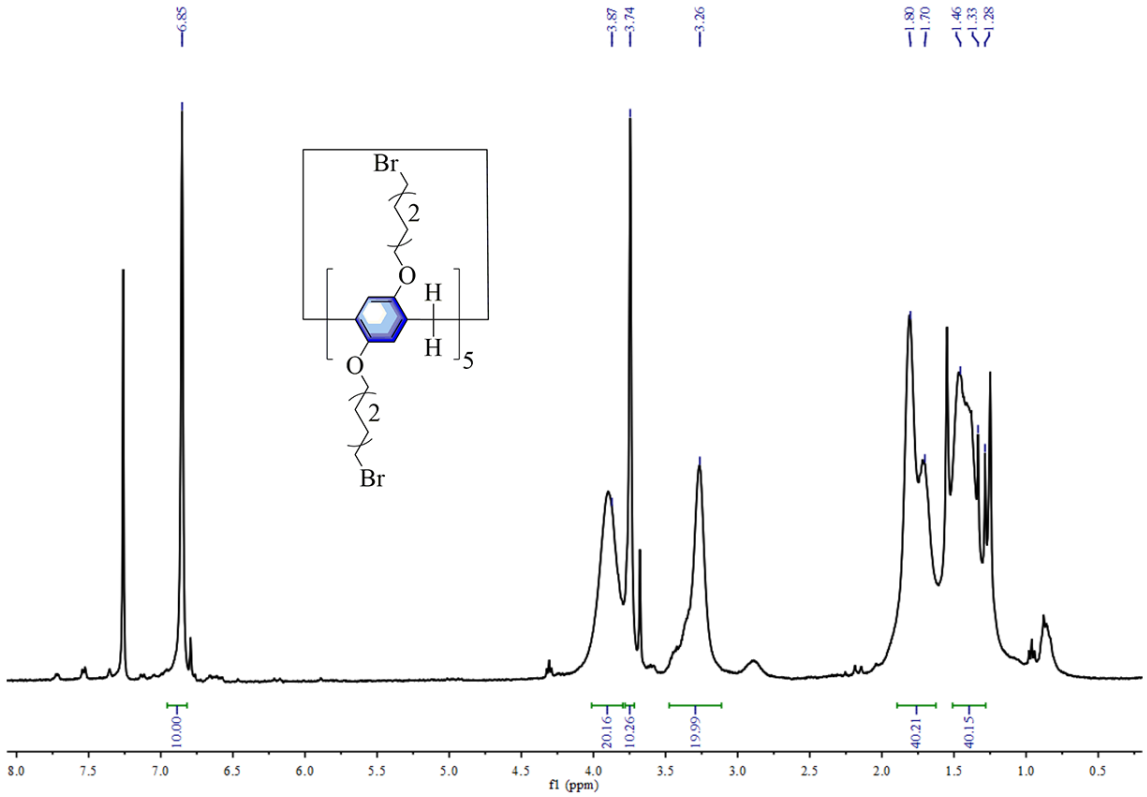


Fig. S2. ^{1}H NMR (400 MHz, $CDCl_3$, room temperature) of pillar[5]arene **2.**

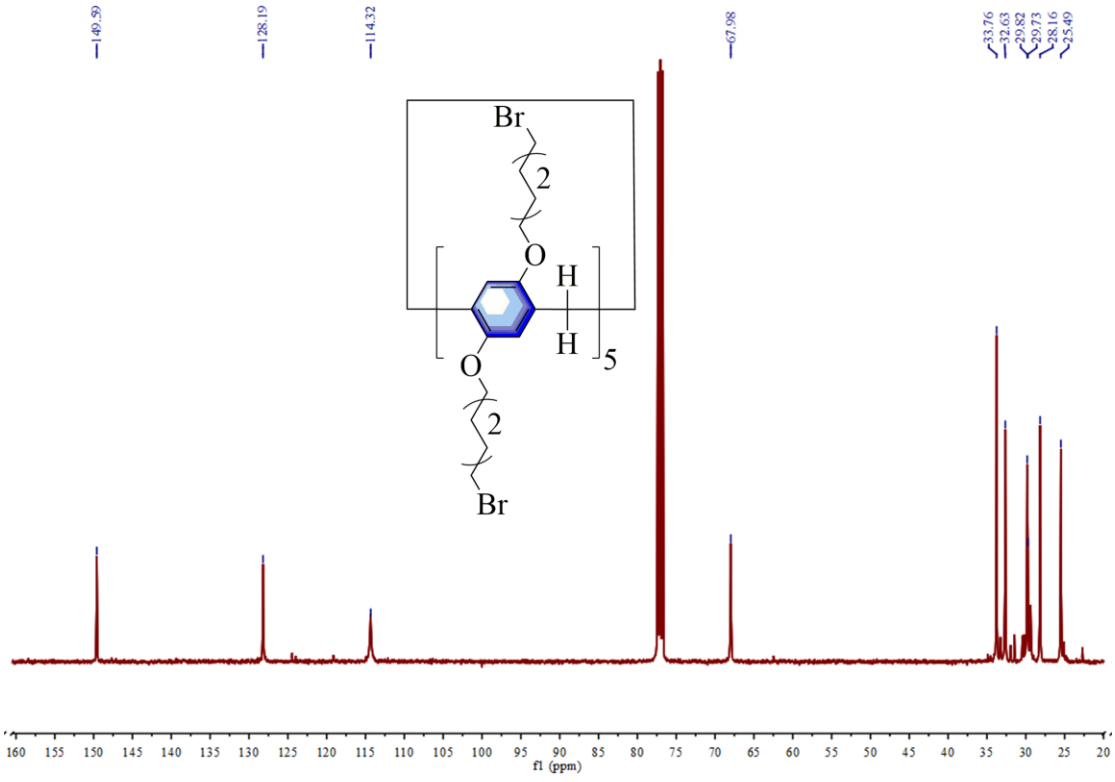
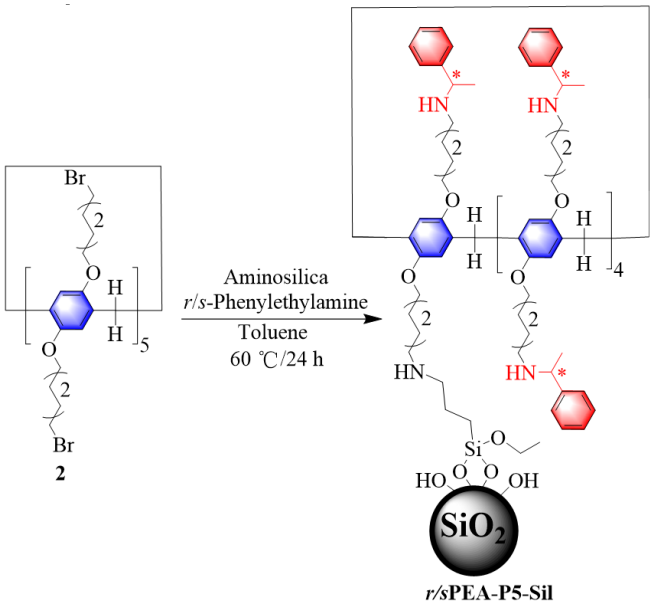


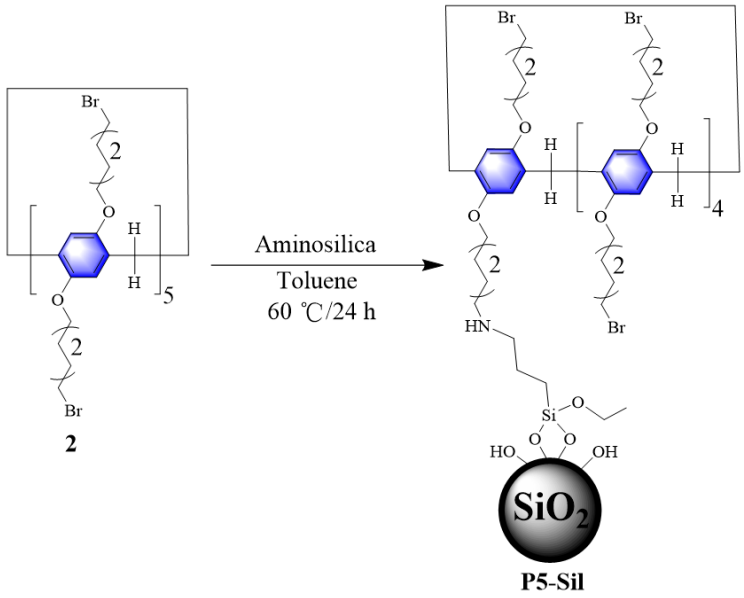
Fig. S3. ^{13}C NMR (400 MHz, $CDCl_3$, room temperature) of pillar[5]arene **2.**

*3.3. Syntheses of **rPEA-P5-Sil** and **sPEA-P5-Sil***



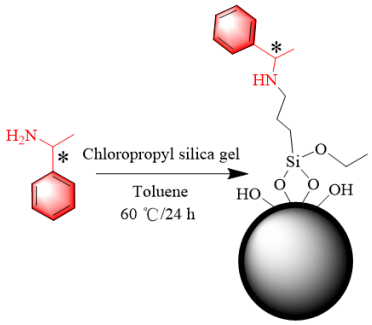
To a solution of **2** (1.12 g, 0.5 mmol) and Sil-NH_2 (4 g) were respectively reacted with *r*PEA and *s*PEA (0.7 mL, 5 mmol) for 24 h by one-pot method in analytical pure toluene (200 mL) at 60 ℃ to prepare chiral PEA substituted pillar[5]arenes bonded silica gel stationary phases ***r*PEA-P5-Sil** and ***s*PEA-P5-Sil**, respectively. The required khaki substances were dried under high vacuum 24 h at 50 ℃ (4.7 g, 90%).

*4. Syntheses of **P5-Sil***



By one-pot method, a solution of **2** (1.12 g, 0.5 mmol) reacted with Sil-NH_2 (4 g) for 24 h in analytical pure toluene (200 mL), at 60 ℃, we obtained pillar[5]arene bonded silica gel stationary phases **P5-Sil**. The required yellow substance was dried under high vacuum 24 h at 50 ℃ (4.6 g, 90%).

5. Syntheses of ***rPEA-Sil*** *and* ***sPEA-Sil*** [S6-S7]



In analytical pure toluene (200 mL), *r*PEA (0.7 mL, 5 mmol) reacted with chloropropyl silica (4 g) for 24 h at 60 ℃ by one-pot method. Then, we got *r*PEA bonded silica stationary phases ***r*PEA-Sil**. The required white material was dried under high vacuum 24 h at 50 ℃ (3.8 g, 95%). As for, ***s*PEA-Sil** was prepared by the same method.

6. Laser scanning confocal microscopy images of rPEA-P5-Sil and sPEA-P5-Sil, Elemental analysis and adsorption/desorption measurement results of Sil-NH_2, r/sPEA-P5-Sil columns

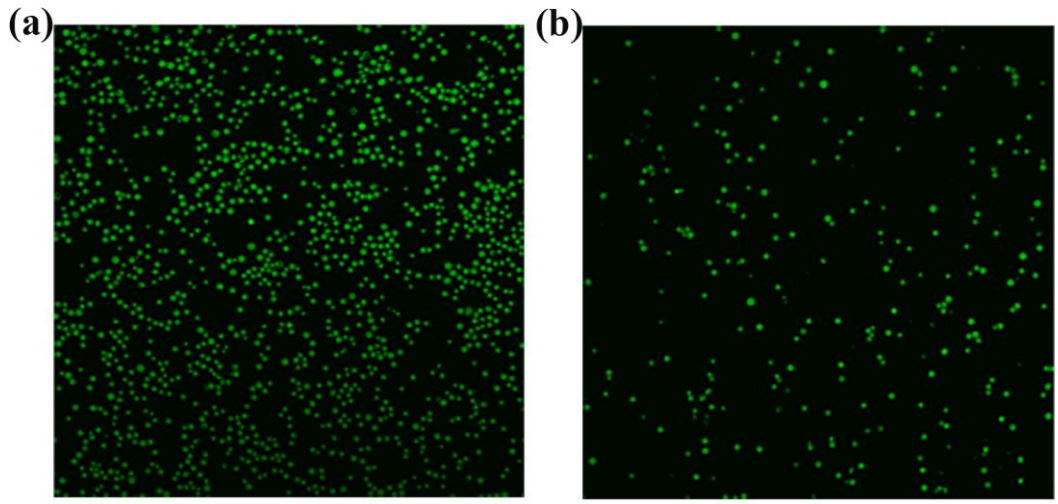


Fig. S4. (a) Laser scanning confocal microscopy images of *r*PEA-P5-Sil column and (b) *s*PEA-P5-Sil column.

Table S1. Elemental analysis of Sil-NH_2, P5-Sil and *r*/*s*PEA-P5-Sil chromatographic columns

Samples	N [%]	C [%]	H [%]	Surface coverage (μmol/m^2)
Sil-NH_2	1.45	4.56	0.93	/
P5-Sil	1.44	18.48	2.82	0.53
*r*PEA-P5-Sil	1.99	17.56	2.18	0.29
*s*PEA-P5-Sil	1.82	15.71	2.05	0.26

Table S2. The adsorption/desorption measurement results of Sil-NH_2, *r*/*s*PEA-P5-Sil columns

Samples	Specific surface area (m^2/g)	Pore volume (cm^3/g)	Pore size (nm)
Sil-NH_2	277	0.68	9.8
*r*PEA-P5-Sil	204	0.38	7.4
*s*PEA-P5-Sil	209	0.42	8.0

7. HPLC separation of racemic on the r/sPEA-P5-Sil packed columns in reversed phase mode.

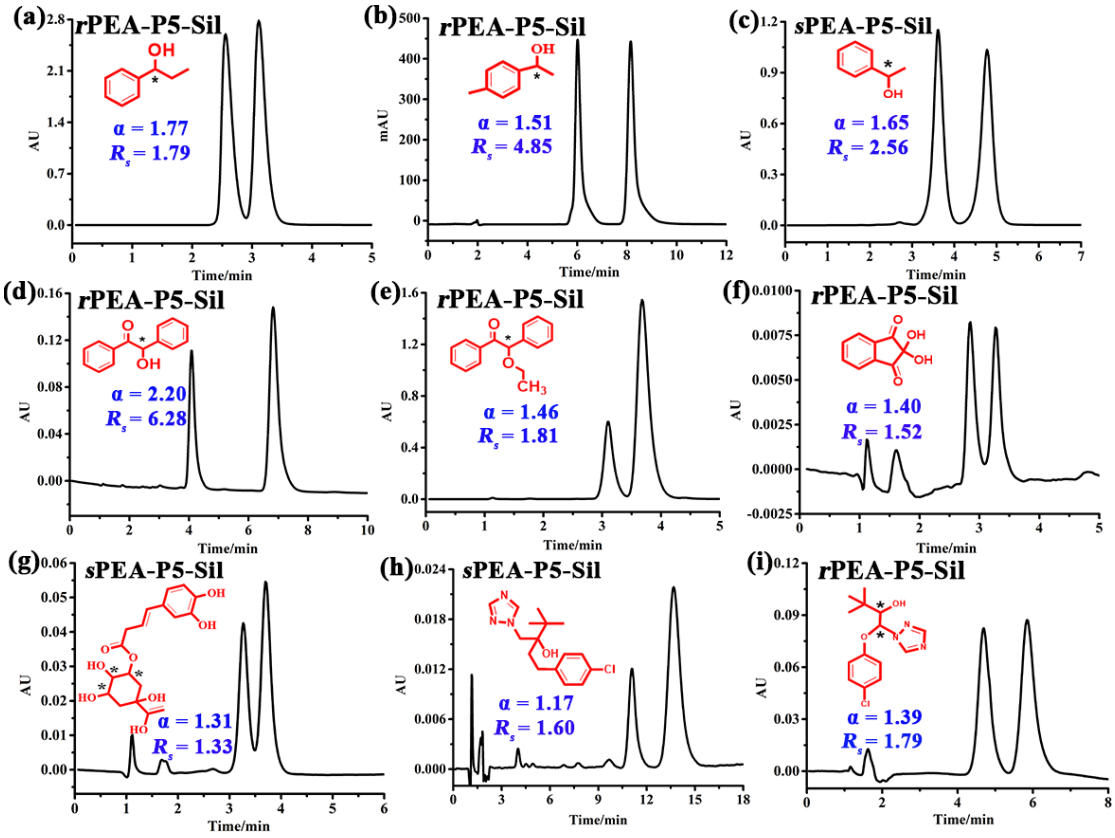


Fig. S5. HPLC separation of racemic on the *r*/*s*PEA-P5-Sil packed columns in reversed phase mode. Mobile phase: (a, b, d, e, g) Methanol/water (60/40, v/v). (c) Methanol/water (40/60, v/v). (h) Acetonitrile/water (25/75, v/v). (f, i) Acetonitrile/water (30/70, v/v). Flow rate at 1.0 mL/min. Detection wavelength, 254 nm. Temperature, 25 °C

8. Average retention factor, selectivity factor and resolution factor of different chiral enantiomers

Table S3. Average retention factor, selectivity factor and resolution factor of different chiral enantiomers by *r*/*s*PEA-P5-Sil in reversed phase mode

No.	Chiral samples	k_1	k_2	α	R_s
1	1-Phenyl-1-propanol [a]	0.41	0.73	1.77	1.79
2	1-(4-Methylphenyl)-ethanol [b]	2.34	3.52	1.51	4.85
3	1-Phenyl ethanol [c]	1.01	1.66	1.65	2.56
4	Benzoin [d]	1.27	2.79	2.20	6.28
5	Benzoin ethyl ether [e]	0.72	1.04	1.46	1.81
6	Ninhydrin [f]	0.58	0.82	1.40	1.52
7	Chlorogenic acid [g]	0.82	1.07	1.31	1.33
8	Tebuconazole [h]	3.47	4.07	1.17	1.60
9	Triadimenol [i]	1.61	2.24	1.39	1.79

[a] Acetonitrile/water (20/80, v/v). [b, d-e, g] Methanol/water (60/40, v/v). [c] Methanol/water (40/60, v/v). [h] Acetonitrile/water (25/75, v/v). [f, i] Acetonitrile/water (30/70, v/v)

Table S4. Average retention factor, selectivity factor and resolution factor of different chiral enantiomers by *r*/*s*PEA-P5-Sil in normal phase mode

No.	Chiral samples	k_1	k_2	α	R_s
1	1-Phenyl-1-propanol [a]	0.57	2.44	4.26	11.01
2	1-(4-Methylphenyl)-ethanol [b]	0.9	3.27	3.64	11.07
3	1-Phenyl ethanol [c]	0.99	3.49	3.53	9.32
4	2-Phenyl-1-propanol [d]	1.24	4.34	3.50	14.16
5	Benzoin [e]	1.18	6.19	5.24	12.97
6	Benzoin methyl ether [f]	0.43	1.62	3.76	8.52
7	Flavanone [g]	2.11	3.29	1.56	4.17
8	Triadimefon [h]	2.06	3.08	1.50	3.02
9	Diniconazole [i]	2.06	4.26	2.07	5.66

[a-j] Hexane/IPA (95/5, v/v). [h, i] Hexane/Ethanol (95/5, v/v)

9. Different volume fractions affected on separation

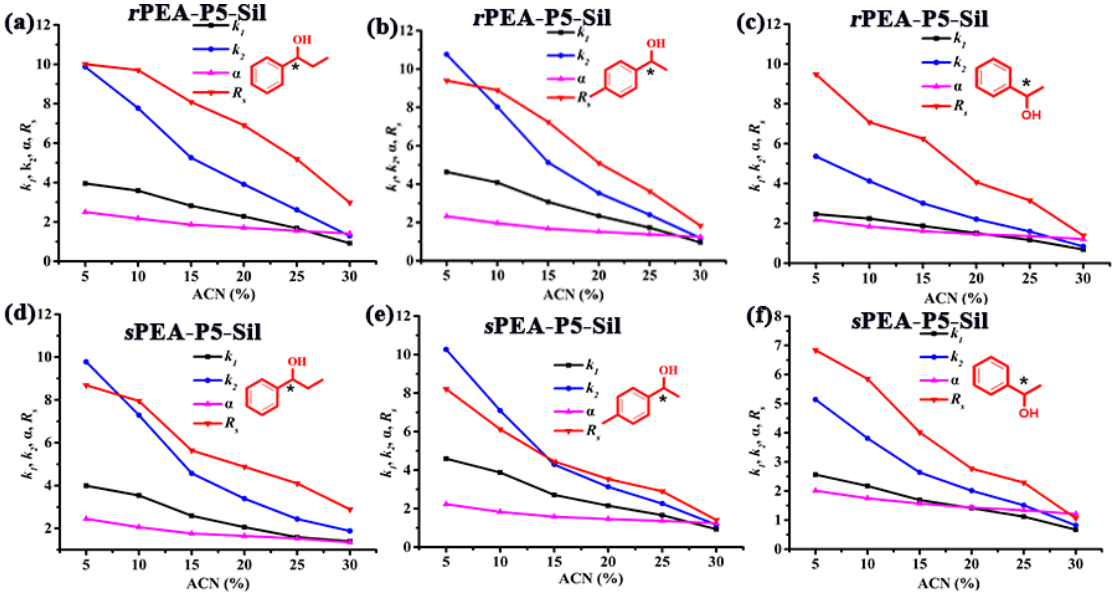


Fig. S6. Separation of 1-phenyl-1-propanol, 1-(4-methylphenyl)-ethanol and 1-phenyl ethanol on *r*PEA-P5-Sil and *s*PEA-P5-Sil columns under different acetonitrile (ACN) contents from 5% to 30%. Conditions: Flow rate at 1.0 mL/min. Detection wavelength, 254 nm. Temperature, 25 ℃

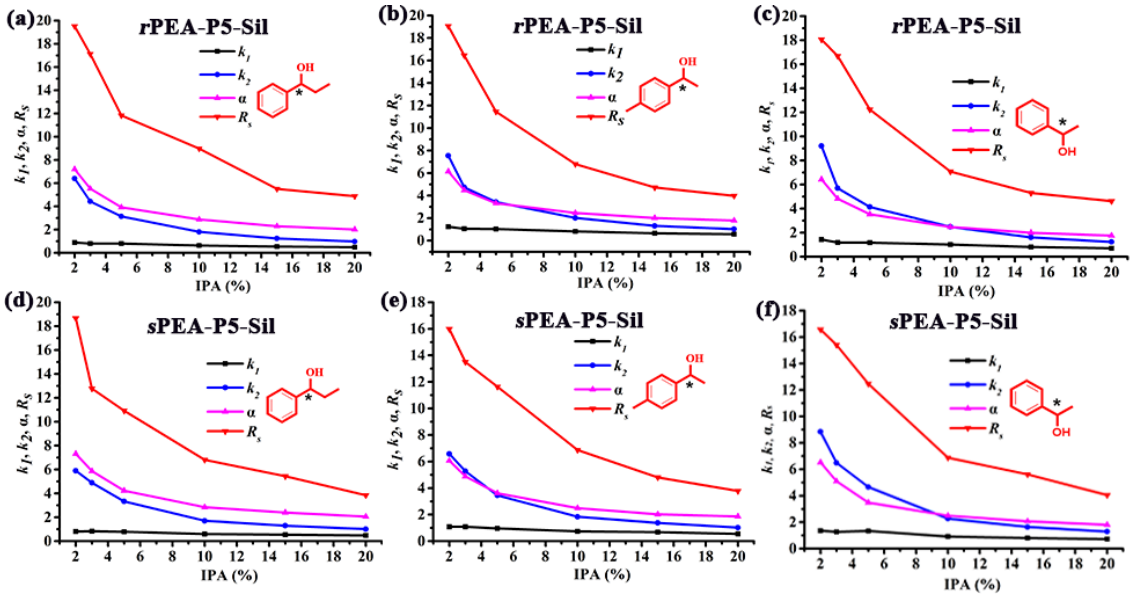


Fig. S7. Separation of 1-phenyl-1-propanol, 1-(4-methylphenyl)-ethanol and 1-phenyl ethanol on *r*PEA-P5-Sil and *s*PEA-P5-Sil columns under different isopropyl alcohol (IPA) contents from 2% to 20%. Conditions: Flow rate at 1.0 mL/min. Detection wavelength, 254 nm. Temperature, 25 ℃

10. Repeatability of rPEA-P5-Sil and sPEA-P5-Sil columns and its RSD

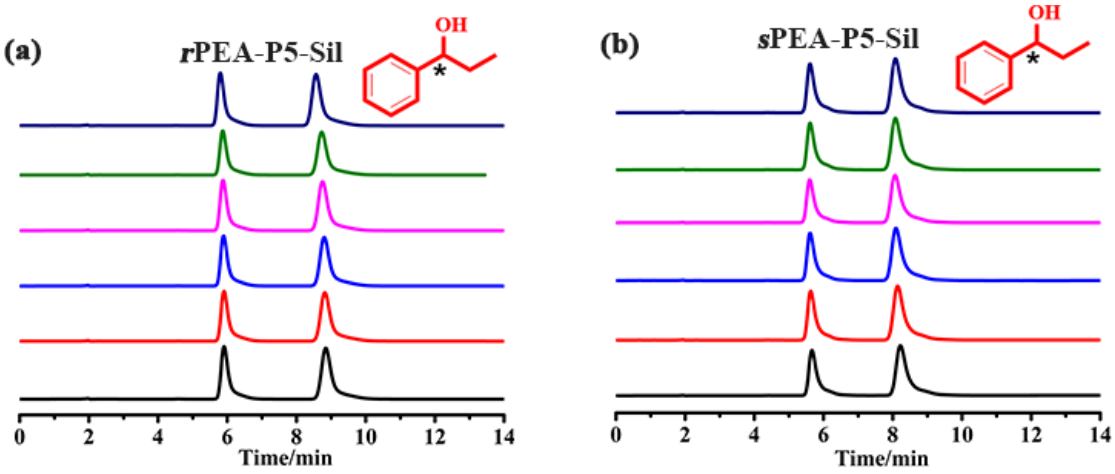


Fig. S8. Repeatability test of *r*PEA-P5-Sil column (a), and *s*PEA-P5-Sil column (b)). Conditions: Acetonitrile/water, 20/80, v/v. Flow rate at 1.0 mL/min. Detection wavelength, 254 nm. Temperature, 25 ℃

Table S5. RSD results for 1-phenyl-1-propanol separated on *r*PEA-P5-Sil column at acetonitrile/water (20/80, v/v)

	1	2	3	4	5	6	Avg.	SD	RSD
k_1	2.28	2.28	2.27	2.26	2.26	2.23	2.26	0.019	0.008
k_2	3.91	3.90	3.89	3.87	3.85	3.76	3.86	0.055	0.014
α	1.71	1.71	1.71	1.71	1.71	1.69	1.71	0.008	0.005
R_s	7.05	6.83	7.08	7.09	7.05	7.01	7.02	0.096	0.014

Table S6. RSD results for 1-phenyl-1-propanol separated on *s*PEA-P5-Sil column at acetonitrile/water (20/80, v/v)

	1	2	3	4	5	6	Avg.	SD	RSD
k_1	2.15	2.12	2.11	2.11	2.11	2.13	2.12	0.016	0.007
k_2	3.57	3.52	3.49	3.48	3.49	3.48	3.51	0.035	0.010
α	1.66	1.66	1.65	1.64	1.65	1.66	1.65	0.008	0.005
R_s	5.50	5.66	5.65	5.70	5.56	5.57	5.61	0.075	0.013

11. Enantiomeric resolution chromatograms of P5-Sil column and average retention factor, selectivity factor and resolution factor

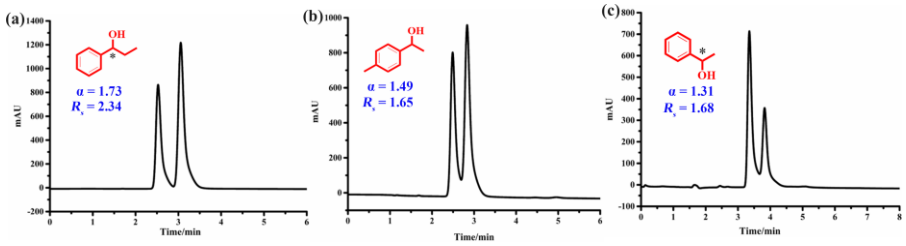


Fig. S9. Resolution of enantiomers by P5-Sil packed column. Mobile phase: (a-b) Methanol/water (60/40, v/v). (c) Acetonitrile/water (25/75, v/v). Flow rate at 1.0 mL/min. Detection wavelength, 254 nm. Temperature, 25 ℃

Table S7. Average retention factor, selectivity factor and resolution factor of different chiral enantiomers by P5-Sil packed column in different mode phases. Flow rate at 1.0 mL/min. Detection wavelength, 254 nm. Temperature, 25 ℃

No.	Chiral samples	P5-Sil			
		k_1	k_2	α	R_s
1	1-phenyl-1-propanol [a]	0.40	0.69	1.73	2.34
2	1-(4-methylphenyl)-ethanol [a]	0.38	0.57	1.49	1.65
3	1-phenyl ethanol [b]	0.85	1.12	1.31	1.68

[a] Methanol/water (60/40, v/v). [b] Acetonitrile/water (25/75, v/v)

12. Enantiomeric resolution chromatograms of rPEA-Sil and sPEA-Sil columns and average retention factor, selectivity factor and resolution factor

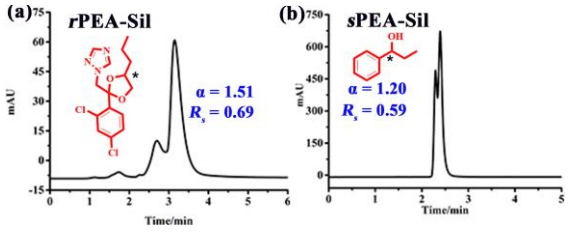


Fig. S10. Resolution of enantiomers by *r*PEA-Sil and *s*PEA-Sil packed columns. Mobile phase: (a) Acetonitrile/water (25/75, v/v). (b) Acetonitrile/water (35/65, v/v). Flow rate at 1.0 mL/min. Detection wavelength, 254 nm. Temperature, 25 ℃

Table S8. Average retention factor, selectivity factor and resolution factor of different chiral enantiomers by *r*/*s*PEA-Sil packed columns in different mobile phases. Flow rate at 1.0 mL/min. Detection wavelength, 254 nm. Temperature, 25 ℃

No.	Chiral samples	*r*PEA-Sil and *s*PEA-Sil			
		k_1	k_2	α	R_s
1	propiconazole [a]	0.49	0.74	1.51	0.69
2	1-phenyl-1-propanol [b]	0.27	0.33	1.20	0.59

[a] Acetonitrile/water (25/75, v/v). [b] Acetonitrile/water (35/65, v/v)

13. The AutoDockTools simulation binding energies of r and s 1-phenyl-1-propanol embedded in r/sPEA-P5

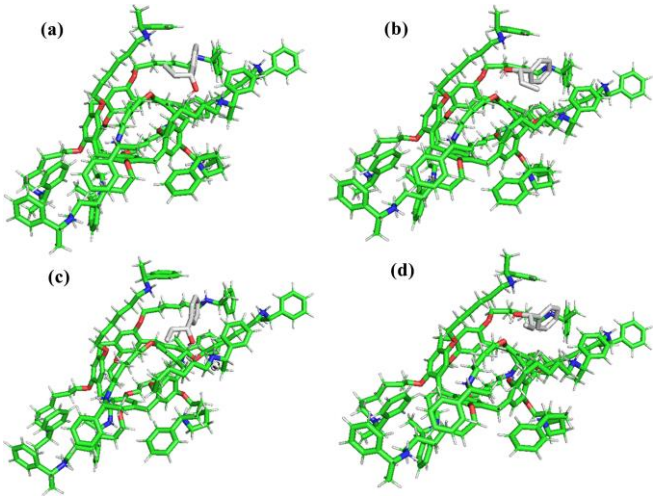


Fig. S11. The interaction between *r*PEA-P5 and *r*-1-phenyl-1-propanol (a), *r*PEA-P5 and *s*-1-phenyl-1-propanol (b), *s*PEA-P5 and *r*-1-phenyl-1-propanol (c), *s*PEA-P5 and *s*-1-phenyl-1-propanol (d). Simulation parameters: genetic algorithm.

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