Electronic Supplementary Information

A novel inorganic mesoporous material with a nematic structure derived from nanocrystalline cellulose as the stationary phase for high-performance liquid chromatography

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Experimental details

Preparation of nanocrystalline cellulose (NCC)

Suspensions of NCC were prepared by acid-catalyzed hydrolysis of Whatman-Xinhua filter paper as described previously.[S1-S2] In this case, the filter paper was first ground to a smaller than 20 mesh powder to increase its surface area, then hydrolyzed with sulfuric acid (64 wt%) at 60 °C for 2 h with continuous stirring. Typically, 1 g of filter paper was treated with 8.75 mL of sulfuric acid. Subsequently, the hydrolysis was terminated by dilution with a large amount of cold de-ionized (DI) water (ca. 10 times the volume of the acid solution used), and the mixture was allowed to settle overnight. The clear top layer was decanted and the remaining lower layer was centrifuged. The supernatant was decanted off and resulting thick white suspension was washed three times with DI water to remove soluble cellulose materials and sulfuric acid. After the third centrifugation, the thick white suspension was transferred to dialysis membrane tubes (12000–14000 molecular weight cut-off) and dialyzed against DI water with slow stirring for several days. The DI water was replaced periodically. The procedure was continued until the pH of the dialysate stayed constant over a period of 1 h. The suspension from the membrane tubes was dispersed with the aid of ultrasonication for 10 min at 60% power to obtain a colloidal NCC suspension, which was then diluted to the desired concentration.

Preparation of chiral nematic mesoporous silica (CNMS) films

CNMS films were prepared by addition of tetramethoxysilane (TMOS) to aqueous NCC suspension according to the method of MacLachlan.[S3] Typically, 10 mL of a 4.5 wt % NCC

aqueous suspension (pH = 2.4) was sonicated for 10 min, mixed with TMOS (0.60 mL, 4.05 mmol) and stirred at room temperature for 1 h to allow the formation of a homogeneous mixture. Then, portions of this homogeneous mixture were drop-casted into polystyrene dishes and allowed to slowly evaporate under ambient conditions for 48 h. Iridescent NCC-silica composite films showing chiral nematic organization were formed. For removal of NCC, the composite films were heated at a rate of 2 °C min^{-1} to 100 °C, held at 100 °C for 2 h, then heated to 540 °C at 2 °C min^{-1} and held at 540 °C for 6 h. After being cooled to room temperature, the free-standing CNMS films were recovered.

References

[S1] X. M. Dong, T. Kimura, J. F. Revol and D. G. Gray, *Langmuir,* 1996, **12**, 2076.

[S2] X. M. Dong and D. G. Gray, *Langmuir,* 1997, **13**, 2404.

[S3] K. E. Shopsowitz, H. Qi, W. Y. Hamad and M. J. MacLachlan, *Nature,* 2010, **468**, 422.

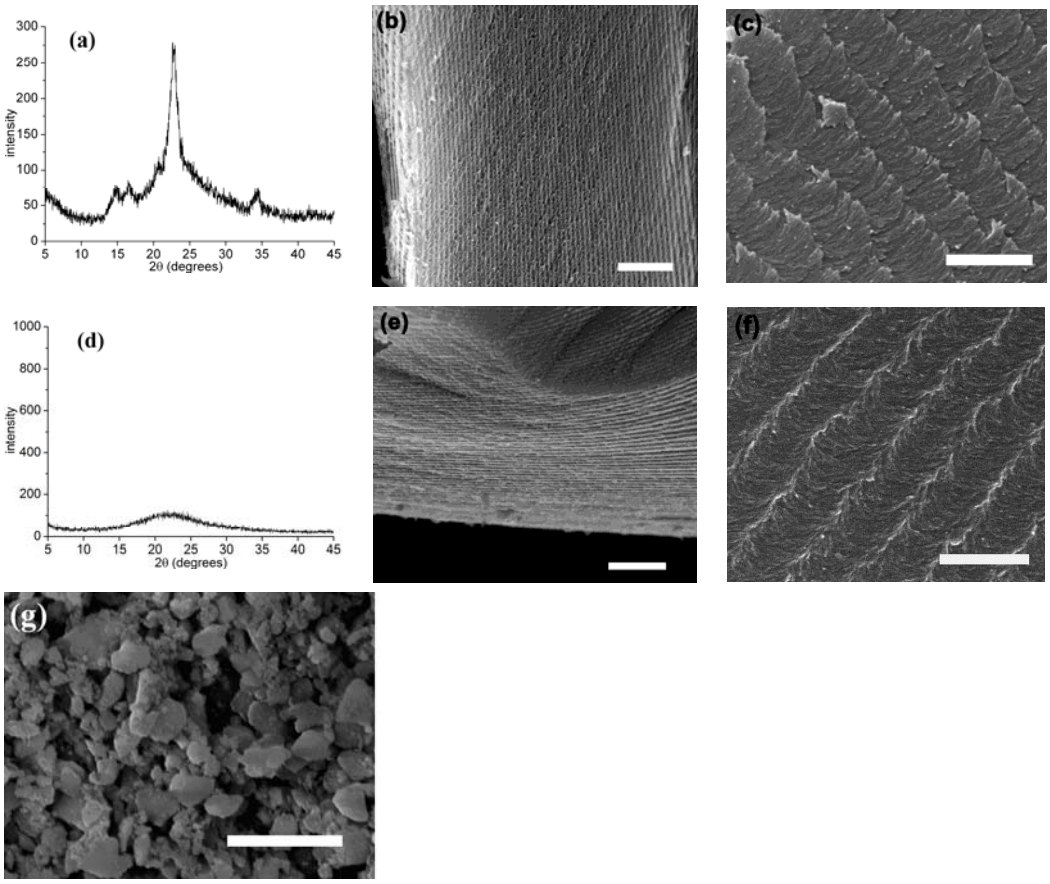

Fig. S1 PXRD patterns and SEM images of NCC-silica composite films and CNMS films. (a) PXRD of NCC-silica composite film shows two peaks characteristic of crystalline cellulose at 2θ≈16º and 24º. (b, c) Cross-section SEM images of NCC-silica composite film at different magnifications (scale bars = 10 μm in panel b; scale bar = 2 μm in panel c). (d) PXRD of CNMS after NCC removal by calcination shows only abroad peak characteristic of amorphous silica and no peaks associated with the NCC. (e, f) Cross-section SEM images of CNMS film at different magnifications (scale bar = 10 μm in panel e; scale bar = 2 μm in panel f). (g) SEM image of CNMS powder (scale bar = 10 μm).

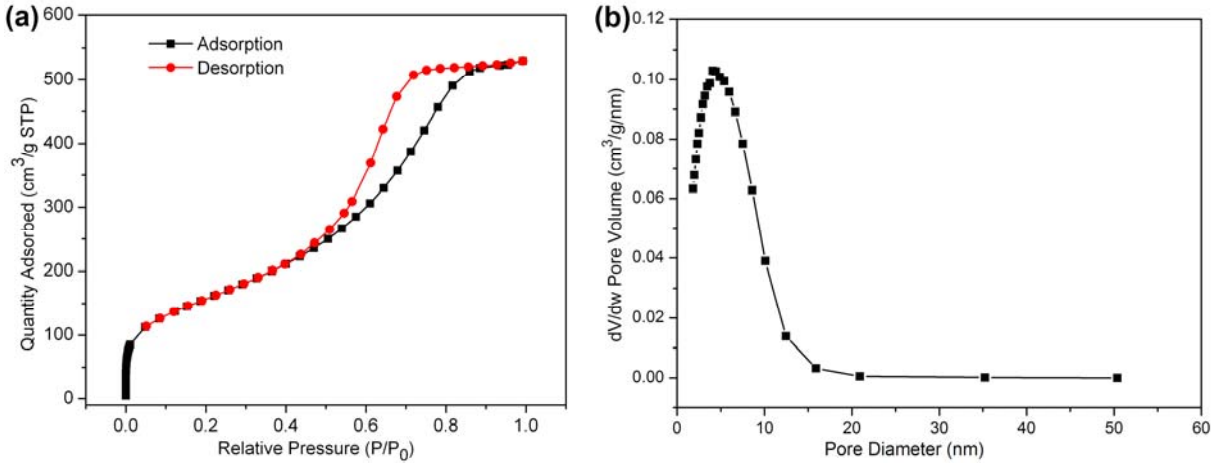

Fig. S2 (a) N_2 adsorption/desorption isotherms of CNMS; (b) Barret–Joyner–Halenda (BJH) pore size distribution for CNMS calculated from the adsorption branch of the isotherm.

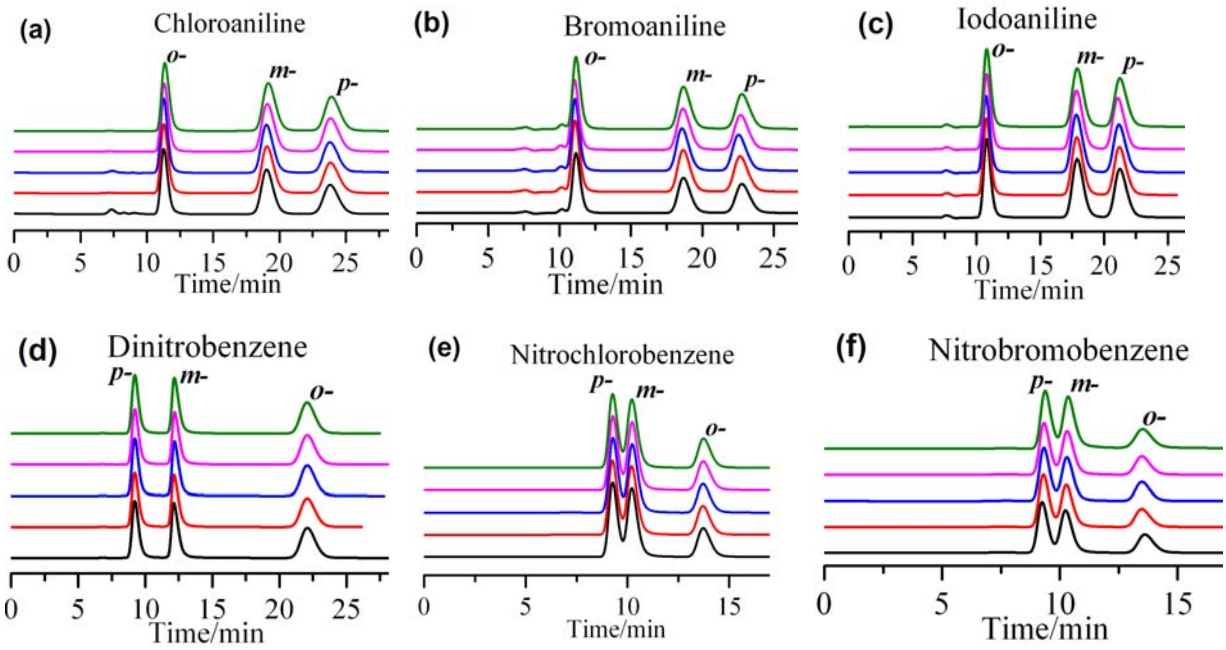

Fig. S3 HPLC chromatograms on the CNMS packed column for five replicate separations: (a) *o*-, *m*-, *p*-chloroaniline using hexane/isopropanol (95:5) as the mobile phase; (b) *o*-, *m*-, *p*-bromoaniline using hexane/isopropanol (95:5) as the mobile phase; (c) *o*-, *m*-, *p*-iodoaniline using hexane/isopropanol (95:5) as the mobile phase; (d) *o*-, *m*-, *p*-dinitrobenzene using hexane/isopropanol (85:15) as the mobile phase; (e) *o*-, *m*-, *p*-nitrochlorobenzene using hexane/isopropanol (99:1) as the mobile phase; (f) *o*-, *m*-, *p*-nitrobromobenzene using hexane/isopropanol (99:1) as the mobile phase. All the separations were performed with the mobile phase at a flow rate of 0.1 mL min^{-1} at 30 °C and monitored with a UV detector at 254 nm.

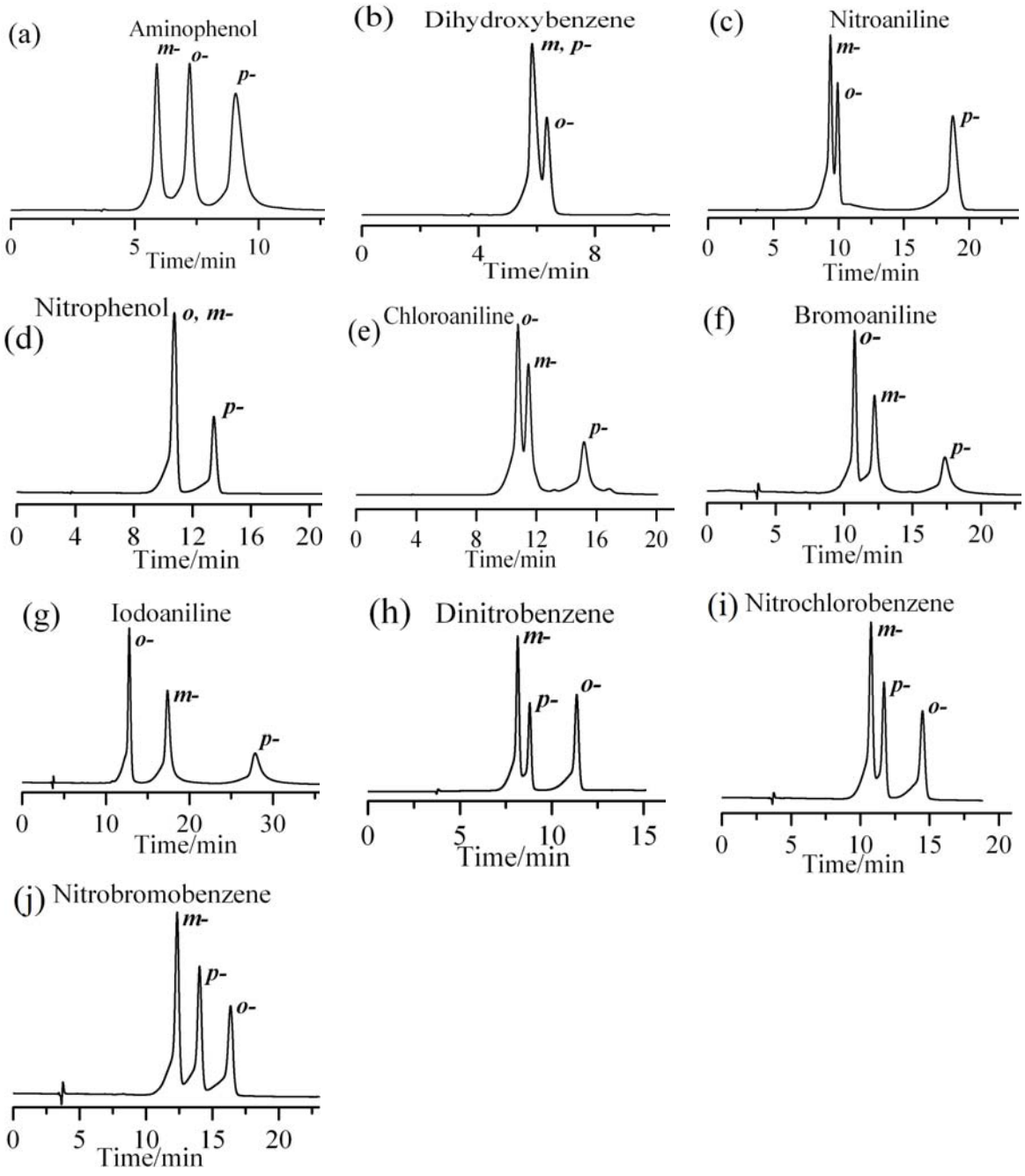

Fig. S4 HPLC chromatograms on the commercial β-cyclodextrin HPLC column (200 mm long × 4.0 mm i.d.) for the separation of aminophenol, dihydroxybenzene, nitroaniline, nitrophenol, chloroaniline, bromoaniline, iodoaniline, dinitrobenzene, nitrochlorobenzene and nitrobromobenzene isomers at 30 °C using MeOH/0.1% TEAA pH=4 (55:45, v/v) as the mobile phase (recommended by company of commercial β-cyclodextrin column) at a flow rate of 0.5mL min^{-1} with a UV detector at 254 nm.

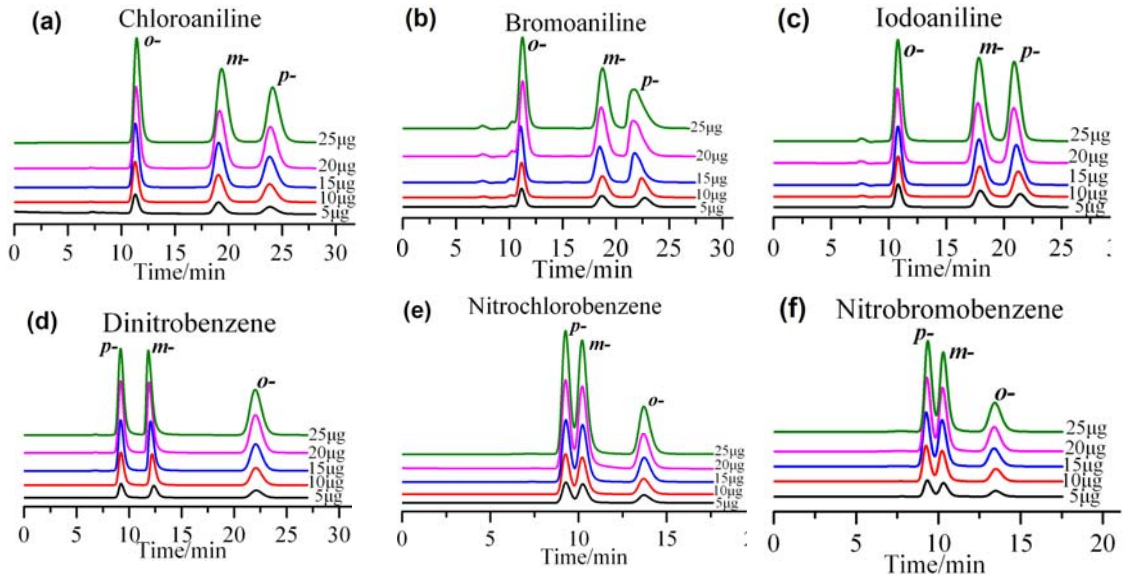

Fig. S5 HPLC chromatograms of positional isomers with different injected masses: (a) chloroaniline isomers; (b) bromoaniline isomers; (c) iodoaniline isomers; (d) dinitrobenzene isomers; (e) nitrochlorobenzene isomers; (f) nitrobromobenzene isomers. Separation conditions as shown in **Fig S3**.

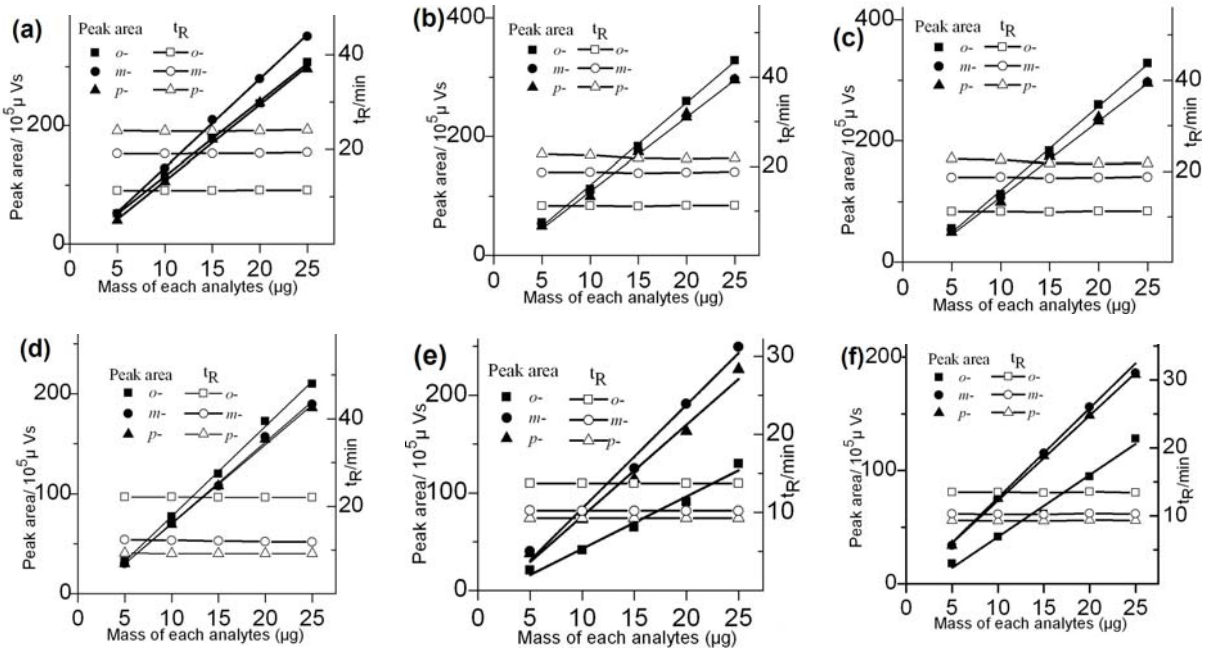

Fig. S6 Effects of injected mass on the peak area and retention time: (a) chloroaniline isomers; (b) bromoaniline isomers; (c) iodoaniline isomers; (d) dinitrobenzene isomers; (e) nitrochlorobenzene isomers; (f) nitrobromobenzene isomers. Separation conditions as shown in **Fig. S3**.

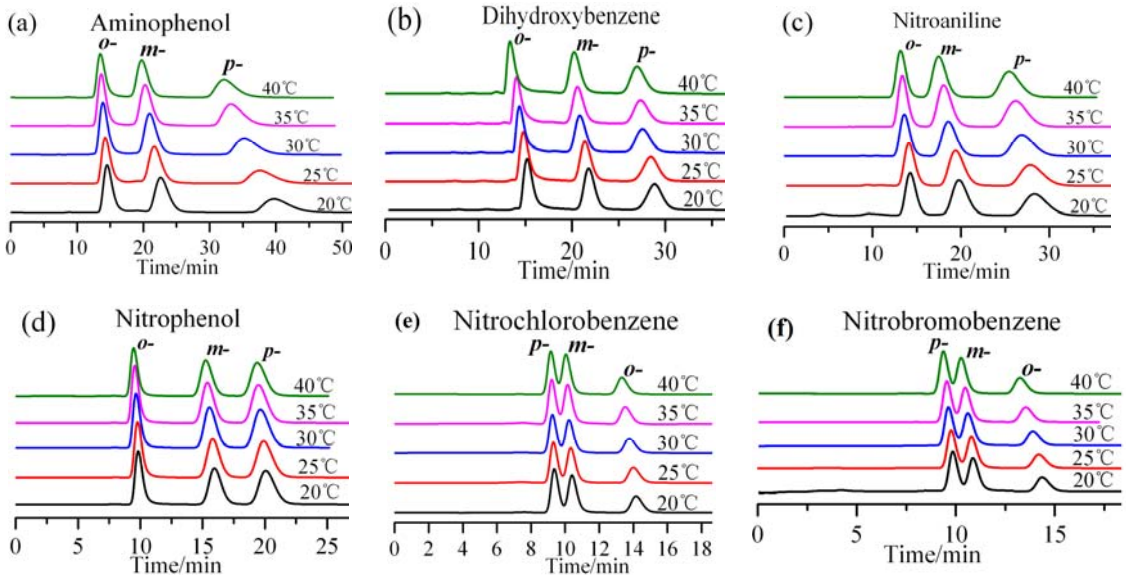

Fig. S7 HPLC Chromatograms on the CNMS packed column for the separation of isomers with the mobile phase at a flow rate of 0.1 mL min^{-1} at 20-40 °C. (a) aminophenol isomers; (b) dihydroxybenzene isomers; (c) nitroaniline isomers; (d) nitrophenol isomers; (e) nitrochlorobenzene isomers; (f) nitrobromobenzene isomers. Separation conditions as shown in **Fig. 2** and **Fig. S3**.

Table S1 Comparison of the CNMS packed column (B) with commercial β-cyclodextrin HPLC column (A) for the separation of positional isomers.

Isomers	A column [a]			B column [b]		
	Elution order	Separation factor (α)		Elution order	Separation factor (α)	
Aminophenol	*m* < *o* < *p*	*o/m* 1.62	*p/o* 1.54	*o* < *m* < *p*	*m/o* 2.01	*p/m* 2.01
Dihydroxybenzene	*m* = *p* < *o*	*p/m* 1.00	*o/m,p* 1.25	*o* < *m* < *p*	*m/o* 1.78	*p/m* 1.46
Nitroaniline	*m* < *o* < *p*	*o/m* 1.10	*p/o* 2.43	*o* < *m* < *p*	*m/o* 1.86	*p/m* 1.98
Nitrophenol	*o* = *m* < *p*	*o/m* 1.00	*p/o,m* 1.39	*o* < *m* < *p*	*m/o* 3.23	*p/m* 1.48
Chloroaniline	*o* < *m* < *p*	*m/o* 1.10	*p/m* 1.48	*o* < *m* < *p*	*m/o* 2.58	*p/m* 1.37
Bromoaniline	*o* < *m* < *p*	*m/o* 1.20	*p/m* 1.60	*o* < *m* < *p*	*m/o* 2.62	*p/m* 1.29
Iodoaniline	*o* < *m* < *p*	*m/o* 1.51	*p/m* 1.76	*o* < *m* < *p*	*m/o* 2.63	*p/m* 1.29
Dinitrobenzene	*m* < *p* < *o*	*p/m* 1.15	*o/p* 1.50	*p* < *m* < *o*	*m/p* 1.98	*o/m* 2.80
Nitrochlorobenzene	*m* < *p* < *o*	*p/m* 1.13	*o/p* 1.35	*p* < *m* < *o*	*m/p* 1.37	*o/m* 1.98
Nitrobromobenzene	*m* < *p* < *o*	*p/m* 1.20	*o/p* 1.23	*p* < *m* < *o*	*m/p* 1.33	*o/m* 1.84

[a] Separation conditions as shown in **Fig. S4**.

[b] Separation conditions as shown in **Fig. 2** and **Fig. S3**.

Table S2 Separation factor (α) of aminophenol, dihydroxybenzene, nitroaniline, nitrophenol, chloroaniline, bromoaniline, iodoaniline, dinitrobenzene, nitrochlorobenzene and nitrobromobenzene isomers on the CNMS packed column in the temperature range of 20-40 °C. Separation conditions as shown in **Fig. 2** and **Fig. S3**.

Isomers		Separation factor (α)				
		20°C	25°C	30°C	35°C	40°C
Aminophenol	*m/o*	2.06	2.01	2.01	1.99	1.94
	p/m	2.08	2.08	2.01	1.96	1.95
Dihydroxybenzene	*m/o*	1.77	1.77	1.78	1.80	1.82
	p/m	1.45	1.45	1.46	1.45	1.45
Nitroaniline	*m/o*	1.94	1.89	1.86	1.82	1.79
	p/m	1.98	1.97	1.98	1.99	2.00
Nitrophenol	*m/o*	3.16	3.18	3.23	3.28	3.30
	p/m	1.47	1.47	1.48	1.49	1.50
Chloroaniline	*m/o*	2.72	2.62	2.58	2.57	2.54
	p/m	1.39	1.38	1.37	1.37	1.37
Bromoaniline	*m/o*	2.68	2.65	2.62	2.57	2.53
	p/m	1.35	1.31	1.29	1.29	1.30
Iodoaniline	*m/o*	2.72	2.70	2.63	2.62	2.57
	p/m	1.33	1.30	1.29	1.29	1.29
Dinitrobenzene	*m/p*	2.02	2.00	1.98	1.98	2.00
	o/m	2.82	2.81	2.80	2.75	2.66
Nitrochlorobenzene	*m/p*	1.38	1.38	1.37	1.36	1.35
	o/m	1.99	1.99	1.98	1.96	1.96
Nitrobromobenzene	*m/p*	1.38	1.37	1.33	1.32	1.33
	o/m	1.92	1.89	1.84	1.81	1.83